SYNTHESIS AND EVALUATION OF SAFE ANTIFUNGAL COMPOUNDS

RAMAKRISHNA I. ANEGUNDI

DR. ARVIND A. NATU
(Research Guide)

DIVISION OF ORGANIC CHEMISTRY: SYNTHESIS
NATIONAL CHEMICAL LABORATORY
DR. HOMI BHABHA ROAD
PUNE - 411008 (INDIA).

JANUARY 2007

SYNTHESIS AND EVALUATION OF SAFE ANTIFUNGAL COMPOUNDS

A THESIS

SUBMITTED TO

UNIVERSITY OF PUNE

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

(IN CHEMISTRY)

BY

RAMAKRISHNA I. ANEGUNDI

DIVISION OF ORGANIC CHEMISTRY: SYNTHESIS

NATIONAL CHEMICAL LABORATORY

DR. HOMI BHABHA ROAD

PUNE - 411 008 (INDIA).

JANUARY 2007

Dedicated

To

My wife Padmaja for taking care of me and my son

Roshan through out this achievement

NATIONAL CHEMICAL LABORATORY

Dr.Homi Bhabha Road, Pune. 411008, INDIA.

Dr. Arvind A. Natu
Sc. F
Division of Organic Chemistry: Synthesis

Telephone: +91-20-25902071
Telefax : +91-20-25902624
E mail: aa.natu@ncl.res.in

CERTIFICATE

This is to certify that the work presented in this thesis entitled "Synthesis and Evaluation of Safe Antifungal Compounds" submitted by Mr. Ramakrishna I. Anegundi, has been carried out by the candidate at National Chemical Laboratory, Pune, India, under my supervision. Such materials as obtained from other sources have been duly acknowledged in the thesis. This work is original and has not been submitted for any other degree or diploma of this or any other university.

January 2007

Dr. Arvind A Natu
(Research Guide)

DECLARATION

I here by declare that the research work presented in this thesis was carried out by me at the National Chemical Laboratory, Pune, India, under the supervision of Dr. Arvind A. Natu, Sc.F. Division of Organic Chemistry: Synthesis, National Chemical Laboratory, Pune, India, submitted for the degree of Doctor of Philosophy in Chemistry to the University of Pune. This work is original and has not been submitted in part or full by me for any other degree or diploma of this or any other university.

January 2007 Ramakrishna I. Anegundi

Acknowledgements

*It gives me great pleasure to express my deep sense of gratitude to my mentor and guide **Dr. Arvind Natu** for his all constant and generous support and guidance. I am grateful to him for giving me a great opportunity to flow in such an ocean of chemistry, just words of appreciations are not enough.*

*I am fortunate enough to have **Dr. Srinivas Hotha** in achieving this entire endeavor. I am very much grateful to him for motivating me in carbohydrate chemistry and appreciate his able guidance, wholeheartedly. Working with him was really a great pleasure and fetched me a lot of learning experience.*

It is really a great help and guidance rendered by Dr. M. V Deshpande, and Mr. Fazal Shiraji, Ms. Pradnya and Mrs. Medha, in screening for biological activity of new molecules, without their help it would be a incomplete research. I express my gratitude to all of them.

I am grateful to Dr. V. R. Pedireddi, Dr.(Mrs) V. G. Puranik, and Mr. Kapil Arora, it is really worth in appreciating their help in X-ray crystallographic studies.

I wholeheartedly thank and give acknowledgements to entire NMR and Elemental analysis group. I also thank Dr. P. L. Joshi and Dr.(Mrs) V. S. Joshi, for their help in spectroscopic studies and Mrs. Shantakumari for her timely help in mass spectroscopic analysis.

Most importantly, I am very much thankful to my labmates Girish Kulkarni, Vidya Gham, Panjab Wagmare, Sudhir Kashyap, Sushil Maurya, Ashish Tripathi, G. Suresh Kumar, Dr.(Mrs) Aruna Sattar, who helped and supported me during every walk of life in the laboratory to achieve this goal.

I am thankful to Dr. Suresh Iyer, Dr. I. Shivakumar, Dr. Ramana, Dr. U. R. Kalkote, Dr. S. S. Bosale, Dr.(Mrs) S. R. Deshpande, Dr.(Mrs) S. P. Mayabate, Dr.(Mrs) A. P. Likhite, Dr.(Mrs) Anita Gunjal, Dr. M. N. Deshmukh, Dr. R. A. Joshi. Dr. M. V. Badiger, Dr. M. G. Kulkarni, Dr. S. S. Mirji, Dr. S. Toke for their help, guidance and moral support.

I also extend my thanks to other colleagues, Khirud, Umashankar, Gourishankar, Balakrishna, Kesari, Swaroop, Amol, Pranjal, Prasanna, Nagendra, Satyendra, Puspesh, Srinivas Rao, Sandesh, Sunil, Sudarshan.

I wholeheartedly thank my friends Vijayanand, Jayant and Krishnmurthy for their unconditional help, encouragement and moral support throughout my carrier.

I dedicate this achievement to my wife Padmaja and my son Roshan, who walked along with me, sharing every ups and downs and I am highly indebted to

them for their sacrifice, help and encouragement. I also thank my parents, father in law, mother in law, all other relatives and friends for their moral support.

I thank Dr. K. N. Ganesh and Dr. M. K. Gurjar for their valuable guidance and support. Director, NCL, Pune for allowing and providing all the facilities and infrastructure to complete this endeavor.

Lastly, I sincerely thank CSIR, New Delhi, for a Senior Research Fellowship.

Ramakrishna I. Anegundi

Table Of Contents

Chapter 3: "Click Chemistry" guided diversity oriented synthesis of glycoconjugates containing 1,2,3-triazole.

Chapter 4: Evaluation of antifungal activity of the new molecules.

General Remarks

1. All the melting points were recorded on the Celsius scale using Buchi B 540 melting point apparatus and are uncorrected.
2. IR spectra were recorded as neat or Chloroform solution or Nujol mull, on a Schimadzu FT-IR spectrophotometer, using NaCl optics. IR bands are expressed in frequency (cm^{-1}).
3. Nuclear Magnetic Resonance spectra were recorded on Bruker AV 200 (200 MHz for ^{1}H and 50 MHz for ^{13}C NMR) or Bruker MSL 300 (300 MHz for ^{1}H and 75 MHz for ^{13}C NMR) or Bruker AV 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C NMR) spectrometers or Bruker DRX 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C NMR). Chemical shifts (δ_H) are quoted in ppm and are referenced to tetramethylsilane (internal).
4. Mass spectra were recorded on Applied Biosystems API QSTAR Pulsar Mass Spectrometer (Electro spray ionization, direct infusion method, solvents used acetonitrile/methanol)
5. Elemental analysis was carried out on Thermo Finnigan Flash EA 1112 series analyzer.
6. Optical rotations were measured on a JASCO-181 digital polarimeter, using D line (589.3 nm).
7. All reactions were monitored by thin-layer chromatography (TLC) using pre-coated silica plates (Merck F_{254}, 0.25 mm thickness) and compounds were visualized by UV, I_2 and Anisaldehyde reagent.
8. All evaporations were carried out under reduced pressure using Buchi rotary evaporator below 50 °C.
9. All solvents and reagents were purified and dried by following the procedures given in the book "Purification Of Laboratory Chemicals" by Armarego and Perrin (3^{rd} edition).
10. Silica gel (100-200) and (230-400) used for column chromatography was purchased from Spectrochem company.
11. Continuous numbering has been given to compounds from chapter 1 to chapter 3, except figures, schemes and tables.

Abbreviations

Ac	Acetyl/Acetate	MsCl	Methanesulphonyl chloride
Ac_2O	Acetic anhydride	Ms	Methanesulphonyl
AcCN	Acetonitrile	ND	Not detected
Ar	Aryl	A	Absorbance
BAIB	Bisacetoxy iodobenzene	PTSA	*p*-toluene sulfonic acid
Bn	Benzyl	Ph	Phenyl
Bz	Benzoyl	Py	Pyridine
$CuOTf.C_6H_6$	Coppertriflate.benzene	TBAF	Tetrabutylammonium fluoride
DCM	Dichloromethane	TBDPS	*tert*-butyldiphenylsilyl
DIPEA	*N,N*-diisopropylethylamine	TBS	*tert*-butyldimethylsilyl
DMAP	4-(dimethylamino)pyridine	TBTA	*tris*-(benzyltriazolylmethyl) amine
DMF	*N,N*-dimethylformamide	TCEP	*tris*-(carboxyethyl)phosphine
DMSO	Dimethyl sulfoxide	TEMPO	2,2,6,6-Tetramethyl piperidinyl oxy free radical
Fmoc	9-Flourenylmethoxy carbonyl	TfOH	Triflic acid
GlcNAc	*N*-acetyl-D-glucosamine	TFA	Trifluoroacetic acid
HBTU	*O*-(1H-Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyl uron ium hexafluoro phosphate	THF	Tetrahydrofuran
		TLC	Thin layer chromatography
HMDS	Hexamethyl disilazane	TMS	Trimethylsilyl
HOBt	1-Hydroxybenzotriazole hydrate	TMSCl	Trimethylsilyl chloride
		$TMSN_3$	Trimethylsilyl azide
Im	Imidazole	TEA	Triethyl amine
KHMDS	Potassium hexamethyl disilazane	TsOH	*p*-toluene sulfonic acid
		TsCl	p-toluene sulfonic chloride
M	Molar	Ts	*p*-toluene sulfonyl
ml	Milliliter	UDP	Uridine diphosphate
mol	Mole	$Yb(OTf)_3$	Ytterbium triflate
mmol (mM)	Millimole		
m.p.	Melting point		
MPM	4-methoxyphenyl methyl		

Abstract Of The Thesis

(Compound and scheme numbers in the abstract are different from those in the thesis)

Abstract Of The Thesis

The thesis entitled “Synthesis and Evaluation of Safe Antifungal Compounds” is divided into four chapters.

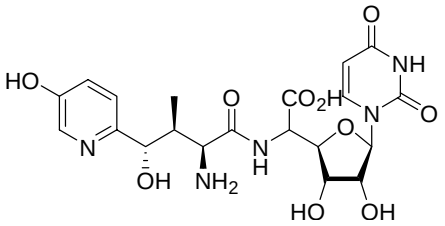
The first chapter highlights the current status of antifungals and the rationale behind its design and synthesis, the second chapter comprises “Click Chemistry” guided Diversity Oriented Synthesis of 1,2,3-triazoles and 1,2,3,4-tetrazole fused carbohydrate derived polycyclic compounds, the third chapter describes the “Click Chemistry” guided diversity oriented synthesis of glycoconjugates containing 1,2,3-triazole and the fourth chapter highlights evaluation of antifungal activity of the new molecules.

Chapter 1: The Current Status Of Antifungals And The Rationale Behind Its Design And Synthesis:

Compared to antiviral and antibacterial drug development, less attention has been given to the safer antifungal development. Control of eukaryotic pathogens is difficult because most of the targets are shared with the host. Currently, clinically used antifungal agents suffer from severe side effects and develop the resistance after prolonged use. Thus, development of fungal resistance to existing drugs, especially in immuno compromised patients and emergence of resistant strains, a “newer challenge” prompted us to under take the development of novel antifungals.

Peptidyl nucleosides are the naturally occurring antibiotics, such as polyoxins and nikkomycins. These are the competitive inhibitors of Chitin synthase, an enzyme essential for survival of fungi and is absent in mammals, plants and bacteria. Thus peptidyl nucleosides are selectively toxic to fungi while nontoxic to mammals, plants and bacteria. Peptidyl nucleosides are more potent against filamentous fungi compared to *Candida albicans*, an opportunistic pathogenic fungus. However, none of

the compounds from this class are in clinical treatment of mycosis, mainly due to their poor cell wall penetration and hydrolytic liability. Nikkomycin Z, the most potent of the class against *C. albicans* with MIC of 14 μg/ml. Hence the peptidyl nucleosides are the suitable lead structures for the development of safe and potent antifungals.



Nikkomycin Z

The detailed account on development of antifungals and petidyl nucleosides is described in this chapter.

Chapter 2: "Click Chemistry" Guided Diversity Oriented Synthesis Of 1,2,3-Triazole And 1,2,3,4-Tetrazole Fused Carbohydrate Derived Polycyclic Compounds:

In medicinal chemistry, several compounds containing 1,2,3-triazole heterocycle, proved useful for having a range of biological properties including fungicidal activity. 1,2,3-triazoles can be synthesized by Huisgen 1,3-dipolar cycloaddition reaction between the azide and alkyne species and the corresponding reaction catalyzed by Cu(I), which gives regioselectivelly 1,4-disubstituted triazole and produce no significant side products is referred as the "click reaction".

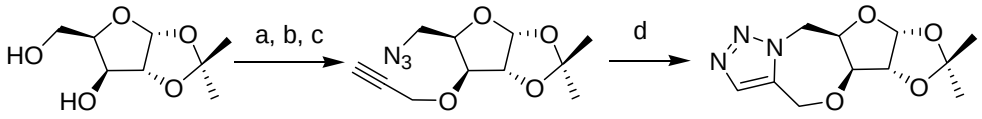
It was planned to design and synthesize novel analogues of polyoxins and nikkomycins. As all these compounds contain furanose sugar as common structural feature, 1,2,3-triazole and 1,2,3,4-tetrazole fused polycyclic compounds were designed from pentofuranoses and hexofuranoses. We have exploited "Click Chemistry" on carbohydrate templates to obtain a library of chiral small polycyclic molecules such as 1,2,3-triazole and 1,2,3,4-tetrazole class of compounds. These were

synthesized by an intramolecular "Click Chemistry" process on azido-alkynes and azido-nitrile respectively, derived from carbohydrate sources.

A. Synthesis Of 1,2,3-Triazole Fused Carbohydrate Derived Polycyclic Compounds:

In our effort, carbohydrate derived azido substrates for intramolecular click reactions, were synthesized by displacement of tosyl group from corresponding tosylates in the presence of NaN_3 (Scheme 1).The *C-3* hydroxyl group was converted to propargyl ether (**2**) using sodium hydride and propargyl bromide to afford the required azido-alkyne (**3**). The 1,3-dipolar cycloaddition reactions were carried out by heating azido alkynes in Toluene at 80 ^oC for 2-6 h. It is interesting to note that the resultant multicyclic 1,2,3-triazole for e.g. (**3**), from α-D-xylofuranose derived azido-alkyne (**2**), came out as a white solid upon cooling to room temperature (Scheme 1).

Scheme 1:



1 **2** **3**

Reagents: a) *p*-TsCl, Py., 0 ^oC-rt, 10 h; b) NaN_3, DMF, 90 ^oC, 8 h; c) NaH, Propargyl bromide, DMF, 0 ^oC-rt, 2 h; d) Toluene, 80 ^oC, 1.5 h.

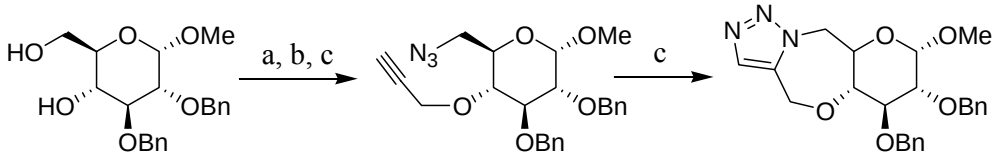
The same procedure was also extended on other pentoses such as of *ribofuranose* and *arabinofuranose* derived azido alkynes so that stereochemical diversity can be achieved in the resultant products (**4** and **5**).

4 **5**

In addition to these, hexoses such as *glucopyranose, glucofuranose* and *allofuranose* derived azido alkynes were also used to obtain skeletally diverse

molecules. G*lucopyranose* derived compound (Scheme 2, **8**) was synthesized by following the protocol as above (Scheme 1).

Scheme 2:

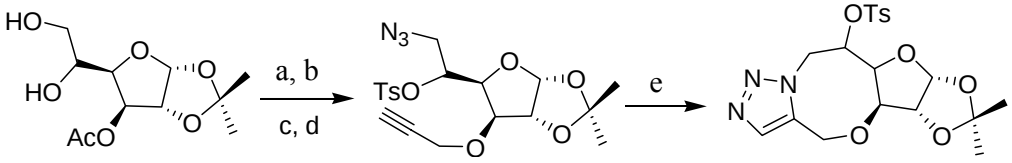


6 **7** **8**

Reagents: a) *p*-TsCl, Py., 0 ^{o}C-rt, 10 h; b) NaN_3, DMF, 90 ^{o}C, 8 h; c) NaH, Propargyl bromide, DMF, 0 ^{o}C-rt, 2 h; d) Toluene, 90 ^{o}C, 3 h.

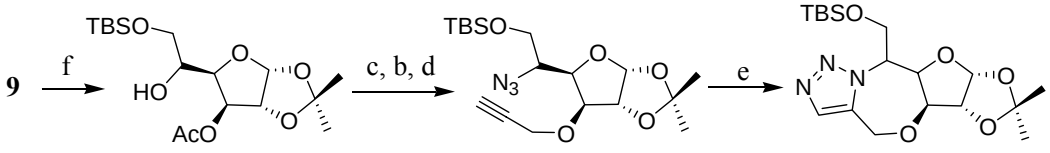
In case of *glucofuranose* derived compounds, the primary alcohol of compound (**9**) was converted to corresponding p-toluenesulfonate using *p*-TsCl, reacted with NaN_3 at 120 ^{o}C for 8 h, treated with *p*-TsCl to obtain the azido tosylate that was intern converted to the required azido alkyne (**10**). The azido alkyne (**10**) was heated to 90 ^{o}C in toluene for 6.5 h to yield triazole fused tetracyclic compound (**11**) (Scheme 3).

Scheme 3:



9 **10** **11**

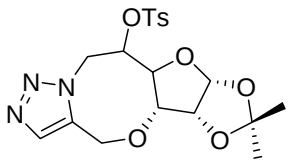
Scheme 4:



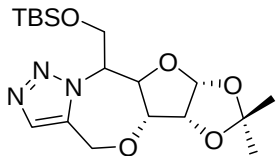
12 **13** **14**

Reagents: a) *p* TsCl, Py., 0 ^{o}C-rt, 15 h; b) NaN_3, DMF, 120 ^{o}C, 8 h; c) *p*-TsCl, Py., 100 ^{o}C, 6 h or MsCl, Et_3N, DCM, 0 ^{o}C-rt, 5 h; d) (i) NaOMe, MeOH, rt, 0.5 h; (ii) NaH, Propargyl Br, DMF, 0 ^{o}C-rt, 2 h; e) $C_6H_5CH_3$, 60 ^{o}C, 6.5 h; f) TBDMSCl, Im., DMF, 1 h.

In another set of reactions, the secondary hydroxyl group of (**9)** was converted to azido derivative and which was subsequently transformed to azido alkyne (**13**), using the reagents as shown in the scheme and the same was also heated in toluene to get the triazole derivative (**14)** (Scheme 4). Similar set of reactions were also carried out on *allofuranose* derived azido alkynes to yield tetracyclic triazole with different stereochemistry (**15** and **16**). All the newly synthesized molecules were well characterized by ^{1}H and ^{13}C NMR.



15



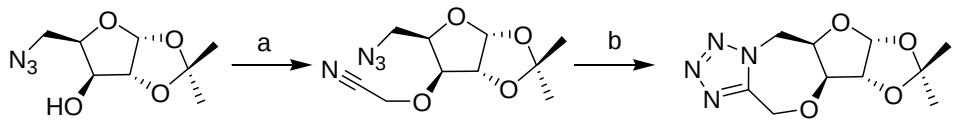
16

B. Synthesis Of 1,2,3,4-Tetrazole Fused Carbohydrate Derived Polycyclic Compound:

Tetrazoles have been studied extensively since they were first described and have been used in a variety of synthetic and medicinal chemistry applications. Although many intermolecular, substituted 1*H*-1,2,3,4-tetrazoles are known, only a few number of intramolecular 1,2,3,4-tetrazoles have been described. In continuation of our endeavor in Click Chemistry, we also report the synthesis of intramolecular 1,2,3,4-tetrazole fused tetracyclic compound from azido-nitrile derived from carbohydrate template.

Accordingly, in our effort, (Scheme 5) 3-hydroxy-5-deoxyazido-xylo furanoside (**17**) was treated with Sodium hydride and Chloro acetonitrile in DMF to afford required azido-nitrile (**18**). The resultant azido-nitrile was heated in toluene to reflux for 24 h, a ”Click” chemistry method to yield 1,2,3,4-tetrazole fused tetracyclic compound (**19**) as colorless solid and was characterized by ^{1}H and ^{13}C NMR.

Scheme 5:



17 **18** **19**

Reagents: a) NaH, Chloro acetonitrile, DMF, 0 °C, 3 h. b) Toluene, reflux, 24 h.

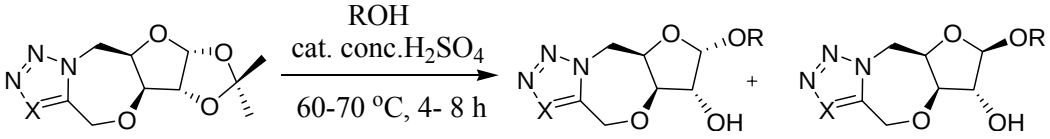
C. Diversity Oriented Synthesis:

Diversity Oriented Synthesis (DOS) is an algorithm that facilitates the synthesis of a collection of small molecules capable of perturbing any disease related biological pathways, leading to the identification of therapeutically useful small molecule inhibitors for protein targets. In the view of diversification of 1,2,3-triazoles and 1,2,3,4-tetrazoles, we attempted to transform them into nucleosides and glycosides.

1. Synthesis Of 1,2,3-Triazole / 1,2,3,4-Tetrazole Fused Tricyclic Glycosides By Fischer's Method:

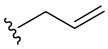
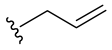
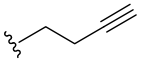
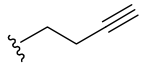
In an attempt to diversify the 1,2,3-triazole and 1,2,3,4-tetrazole fused tetracyclic compounds, we carried out the glycosidation by Fischer method, using alcohols such as isopropyl alcohol, allyl alcohol and homopropargyl alcohol. The xylofuranoside derived 1,2,3-Triazole (**3**) and 1,2,3,4-Tetrazole (**19**) fused tetracyclic compound were selected and were treated with respective alcohol at 60-70 °C for 4-8 h (Scheme 6). The glycosidation resulted in a mixture of α,β-anomers (table 1) and both of were isolated and well characterized by ^{1}H , ^{13}C NMR and MS.

Scheme 6:



3. X = CH, **19,** X = N **α : β** -glycosides

Table 1

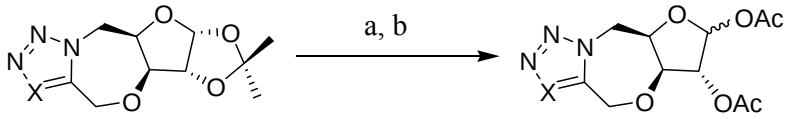
	R	X	Time (h)	Yield (%)	α : β [a]		R	X	Time (h)	Yield (%)	α : β [a]
20.		CH	6	85	1 : 2	**23.**		N	4	90	2 : 3
21.		CH	8	75	2 : 3	**24.**		N	5	93	1 : 2
22.		CH	8	80	1 : 2	**25.**		N	6	95	1 : 3

a = isolated ratios

2. Synthesis Of 1,2,3-Triazole / 1,2,3,4-Tetrazole Fused Tricyclic Nucleosides By Vorbruggen's One Pot Method:

Xylofuranose derived 1,2,3-Triazole (**3**) and 1,2,3,4-Tetrazole (**19**) fused tetracyclic compound were selected for nucleoside synthesis. The synthesis involves the deprotection of 1,2-*O*-isopropylidine group using catalytic amount of sulfuric acid in dioxane-water mixture to yield 1,2-dihydroxy derivative in 80% yield. 1,2-diol was then transformed into diacetate (**26/27**) by treating with acetic anhydride in pyridine (Scheme 7).

Scheme 7:

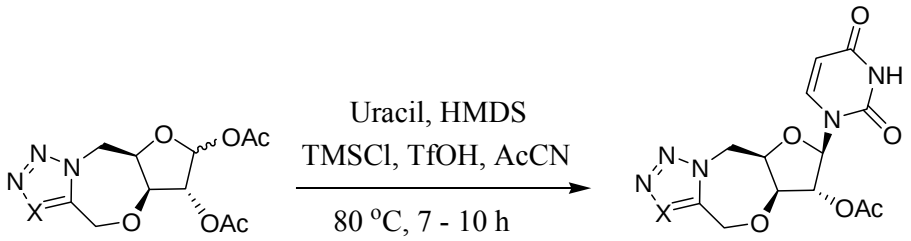


3. X = CH, **19,** X = N **26.** X = CH, **27,** X = N

Reagents: a) cat. H_2SO_4, Dioxane-Water (3:2), 60 ^oC, 24 h; b) Ac_2O, Py, 0 ^oC – rt, 5 h, (X = CH, 75%; X = N, 70% in two steps).

The diacetate (**26/27**) was concomitantly converted into nucleosides by Vorbruggen's one pot method using uracil (Scheme 8, **28** and **29**), exclusively with β-configuration.

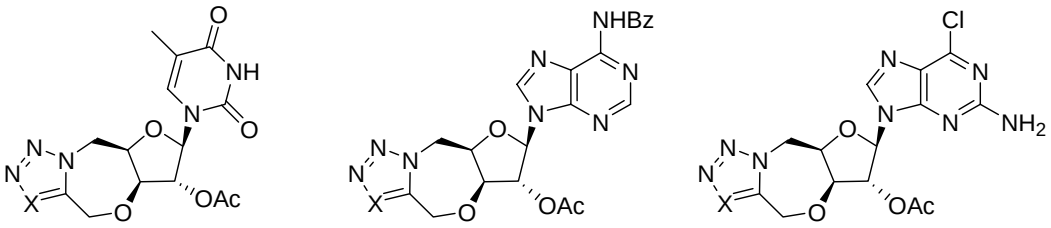
Scheme 8:



β-Nucleoside

26. X = CH, **27,** X = N **28.** X = CH, **29,** X = N

Thymine (**30, 31**), N^6-Benzoyl adenine (**32, 33**) and 6-Cloroguanine (**34, 35**) were also used and all were characterized by ^1H, ^{13}C NMR and MS.



30. X = CH, **31,** X = N **32.** X = CH, **33,** X = N **34.** X = CH, **35,** X = N

In conclusion, we have successfully exploited click chemistry and diversity oriented synthesis in synthesizing intramolecular 1,2,3-triazole / 1,2,3,4-tetrazole fused tetracyclic compounds from carbohydrate templates and their facile transformation to diverse structures such as glycosides and nucleosides.

The detailed experimental procedure for the synthesis of all the new molecules and their analytical data along with spectra are described in the experimental section of this chapter.

Chapter 3: "Click Chemistry" Guided Diversity Oriented Synthesis Of Glycoconjugates Containing 1,2,3-Triazole:

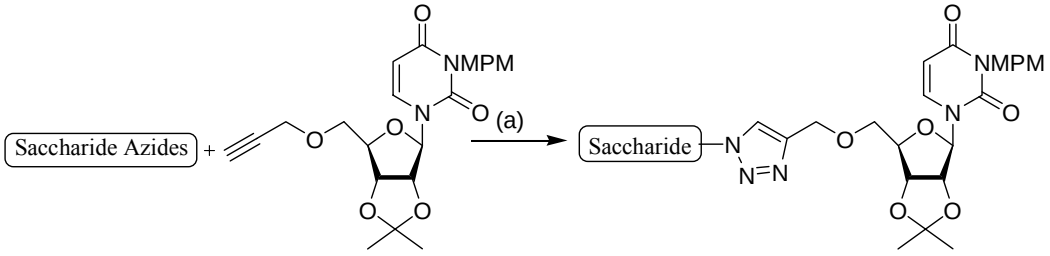
In view of studying the interaction between carbohydrate and peptide / carbohydrate and nucleic acid and exploiting the advantage of 1,2,3-triazole being bioisoster of phosphate and amide group, it was proposed to synthesize

glycoconjugates with nucleic acids containing 1,2,3-triazole. On the other hand, we planned to design the analogs of UDP-*N*-acetyl glucosamine, a precursor for the Chitin synthesis in fungal cell wall with the anticipation of potent antifungal activity.

A. Synthesis Of Glycoconjugates By Ligation Of Carbohydrate Derived Azides With Propargyl Ether Of Nucleosides By "Click Chemistry":

Glycoconjugates were synthesized using Cu(I) catalyzed 1,3-dipolar cycloaddition reacton, a Click Chemistry process.

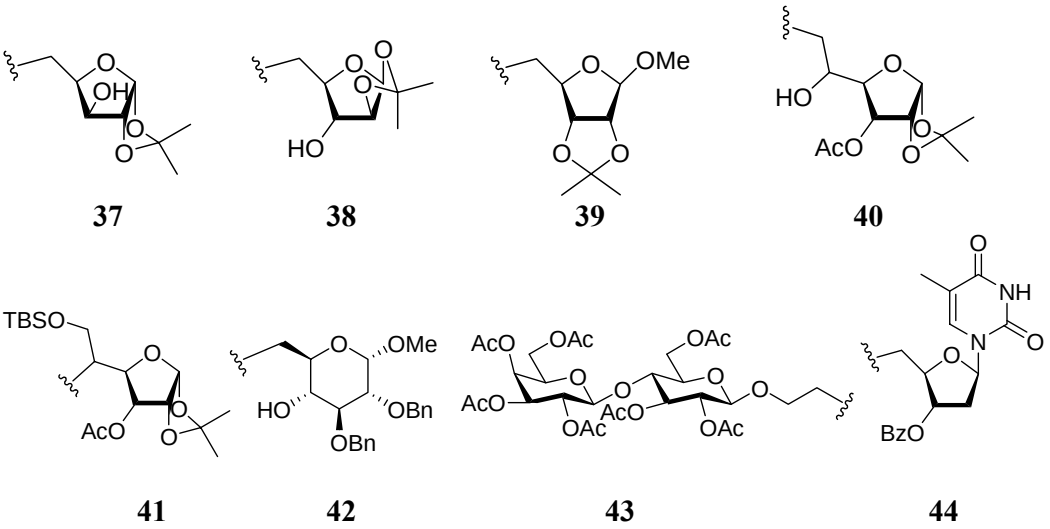
Scheme 9:



36 **Compounds 37-44**

Reagents: a) 1.0 mol % $CuSO_4.5H_2O$, 10.0 mol % Sod.ascorbate, *t*-BuOH:H_2O (1:1), rt, 8-12h.

Sacharides:



37 **38** **39** **40**

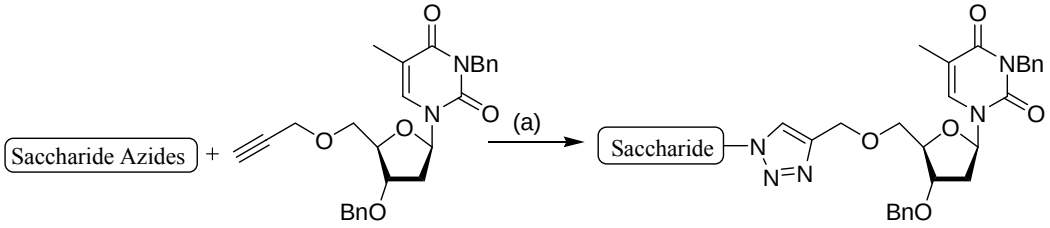
41 **42** **43** **44**

Propargyl ether of uridine (**36**) (Scheme 9) and azido compounds derived from diverse sugars such as *xylose, arabinose, ribose, glucofuranose, glucopyranose, lactose* and nucleosides such as uridine /thymidine were dissolved in 1:1 mixture of

t-butanol - water and to the mixture was added 1 mole % of copper sulphate pentahydrate and 10 mole % of sodium ascorbate. The reaction mixture was stirred for 8-12 h at room temperature and usual workup yields diverse glycoconjugates (**37-44**) with nucleoside containing 1,2,3-triazole in quantitative amount from respective nucleoside and carbohydrate derived azides.

The success of this endeavor led to the extension of glycoconjugation with other nucleosides like thymidine (**45**) using the same protocol (Scheme 10, Compounds **46-51**). All the newly synthesized glycoconjugates were well characterized by ^{1}H, ^{13}C NMR and MS.

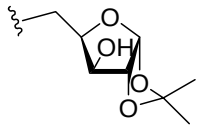
Scheme 10:



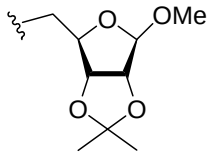
45 **Compounds 46-51**

Reagents: a) 1.0 mol % $CuSO_4.5H_2O$, 10.0 mol % Sod.ascorbate, *t*-BuOH:H_2O (1:1), rt, 8-12h.

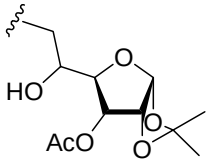
Saccharides:



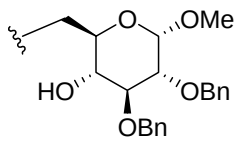
46



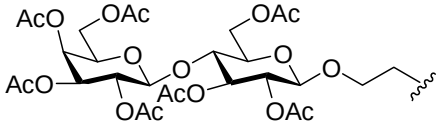
47



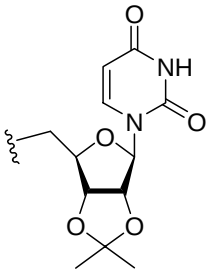
48



49



50



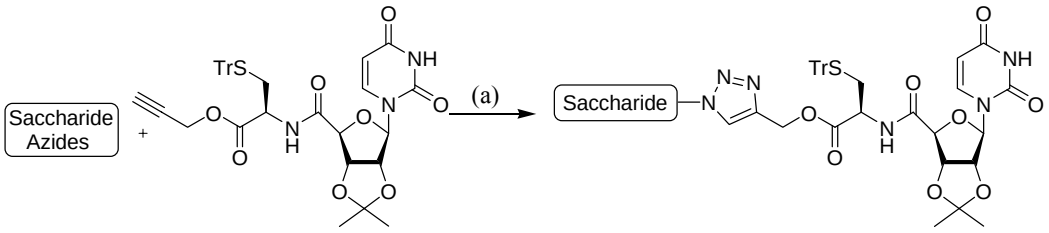
51

B. Synthesis Of Glycoconjugates By Ligation Of Carbohydrate Derived Azides With Propargyl Ether Of Nucleosides By "Click Chemistry" Containing Amino Acid Linkage:

Incorporation of amino acids in synthetic biologically useful molecules can enhance the target protein binding of that molecule, so that to elicit the biological activity. According to a recent report an amino acid such as *S*-trityl-L-cysteine itself exhibited protein binding property. Thus in our next endeavor it was planned to incorporate *S*-trityl-L-cysteine in the glycoconjugates along with 1,2,3-triazole linker.

The protocol involves the preparation of a pseudodipeptides from propargyl ester of *S*-trityl-L-cysteine(NH_2) and 5'-carboxylic acid of nucleosides such as uridine(**52**)/thymidine (**58**). Then the propargyl compound (**52**)/(**58**) was conjugated with various azido compounds derived from diverse monosaccharides and a nucleoside itself by Cu(I) mediated 1,3-dipolar cycloaddition, a Click Chemistry process to obtain diverse glycoconnjugates (Scheme 11, compounds **53-57**/Scheme 12, compounds **59-63**).

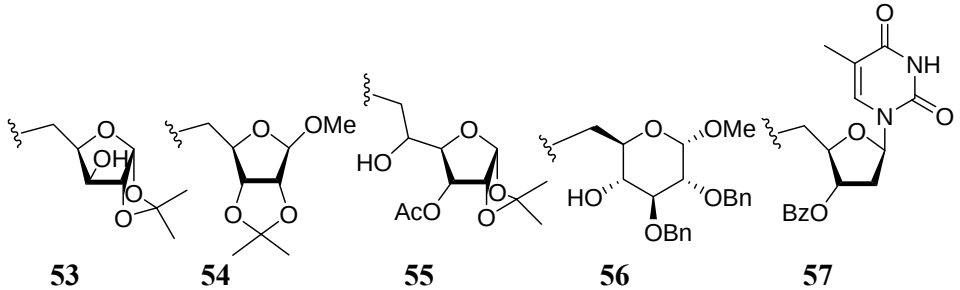
Scheme 11: Conjugation of saccharide azides and uridine.



52 **Compounds 53-57**

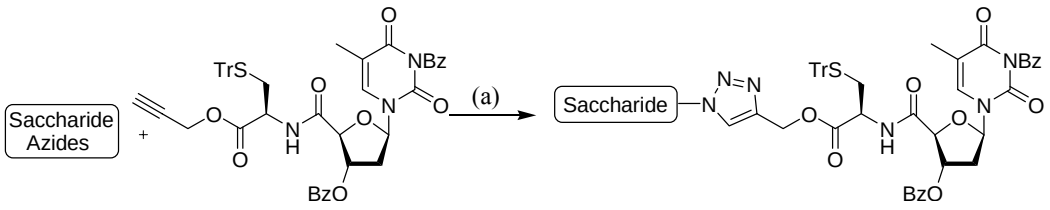
Reagents: a) 2.0 eq CuI, 5.0 eq DIPEA, Acetonitrile, rt.

Saccharides:



53 **54** **55** **56** **57**

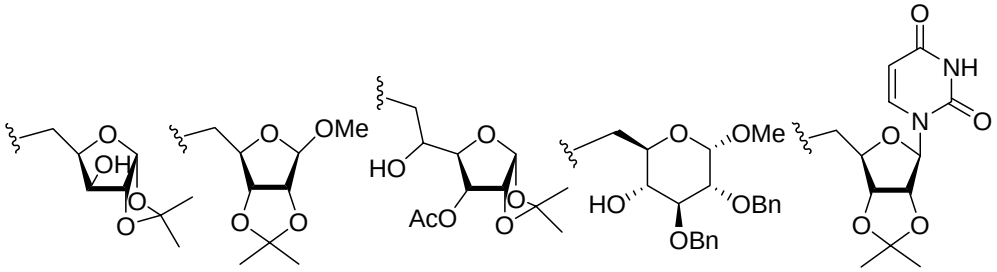
Scheme 12: Conjugation of saccharide azides and thymidine.



58 **Compounds 59-63**

Reagents: a) 2.0 eq CuI, 5.0 eq DIPEA, Acetonitrile, rt.

Saccharides:



59 **60** **61** **62** **63**

All the new glycoconjugates synthesized were well characterized by ^{1}H, ^{13}C NMR and MS.

In conclusion we have demonstrated the application of click chemistry to synthesize the diverse glycoconjugates from carbohydrate and nucleoside substrates, bridged by 1,2,3-triazole, a metabolically stable linkage and amino acid, *S*-trityl-L-cysteine, which can enhance the protein binding.

The experimental section gives detailed procedure for the synthesis of new molecules and also the analytical data including ^{1}H and ^{13}C spectra for new molecules.

Chapter 4: Evaluation Of Antifungal Activity Of New Molecules:

The new molecules were evaluated for their antifungal activity using whole cell based plate assay, haploinsufficiency assay and Chitin synthase non-radioactive

assay. These molecules were divided into four groups according to their structural features. The most active of these groups in plate assay *in vivo* against *C. albicans* and *F. oxysporum,* and Chitin synthase *in vitro* assay are summarized in table 1.

Table 1

Test Compounds	**Plate assay (9-10 mm)**	**Chitin synthase assay (*in vitro*)**
Group 1	3, 11, 14 and 19	3, 11, 14 and 19
Group 2	24β	24β
Group 3	32 and 29	32 and 29
Group 4	38, 39 and 41	38, 39 and 41

All the evaluation methods and their results and discussions will be described in detail in this chapter.

Chapter 1: The Current Status Of Antifungals And Rationale Behind Its Design And Synthesis

1.1. Introduction

Fungi are plant-like organisms that lack chlorophyll and are one of the five kingdoms of life. There are over 1,500,000 species of fungi, only 72,000 are known.[1] Fungal infections have emerged as a significant clinical problem in recent years. Both the number of fungal infections and the number of fungal species causing them are increasing, as a result of the exponential increasing number of immunosuppressed and immunocompromised patients.[2] Due to the increasing frequency of fungal infections and development of resistance to the current treatment,[3] mycology is today undergoing a true renaissance. Fungi cause a range of illnesses (mycoses) ranging from the chronic to the serious. Invasive fungal infections are nowadays a major cause of morbidity and mortality in patients such as with neutropenic, AIDS, organ transplantation, etc.[4]

The Term **mycosis** (plural: *mycoses*) refers to conditions in which fungi invade the resistance barriers of the human body and establish infections. These mycoses can manifest themselves in a variety of ways. Infections can be superficial, that is situated at or close to the surface of the skin, or systemic, which means they can affect the body as a whole rather than individual parts or organs.[5]

Mycoses are classified according to the tissue levels initially colonized[5,6]:

1. Superficial mycoses.
2. Cutaneous mycoses.
3. Subcutaneous mycoses.
4. Systemic mycosis due to primary pathogens.
5. Systemic mycosis due to opportunistic pathogens.

Risk Factors Responsible for Development of Fungal Infections[1, 4, 7]:

- ❖ Use of drugs that suppreess the immune system, ex. Anticancer drugs, Corticosteroids.
- ❖ Diseases and conditions, such as AIDS, Kidney failure, Diabetes, Lung diseases, Leukemia, Organ transplantation etc.
- ❖ Fungal infections are extremely difficult to diagnose and therefore delays in initiation of treatment.

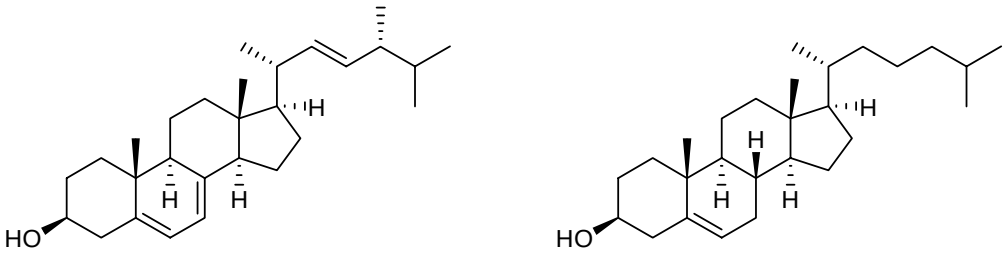
1.2. The Fungal Cell[5,6]

Knowledge of fungal cell structure and function is essential for understanding the pharmacology of antifungal agents. Like mammalian cells and unlike bacteria, fungi are eukaryotes with chromosomes within the cell nucleus and have distinct cytoplasmic organelles including endoplasmic reticulum, Golgi apparatus, mitochondria, and storage vacuoles (Figure 1). This homology to mammalian cells also extends to biosynthetic pathways, where fungi share similar mechanisms for DNA replication and protein synthesis. The similarity of fungal and mammalian cells creates a number of problems for designing drugs that are selectively toxic to fungal cells but not to the human host. Thus the issue of selectivity predominates in the search for safe and effective chemotherapeutic remedies for mycoses.

The Cell Membrane:

Fungi and mammalian cells both contain a cell membrane that plays vital role in cell structure, division, and metabolism. It is composed of complex lipids such as sterols, which account for approximately 25% of the weight of the cell membrane. In all pathogenic fungi, the principle sterol is Ergosterol, whereas the mammalian cell membranes contain primarily cholesterol. Although the sterols are different, the principle route for the biosynthesis of ergosterol parallels with the biosynthesis of

cholesterol, therefore it is difficult to design drugs that are selectively toxic to fungal cells but not the mammalian host.



Ergosterol Cholesterol

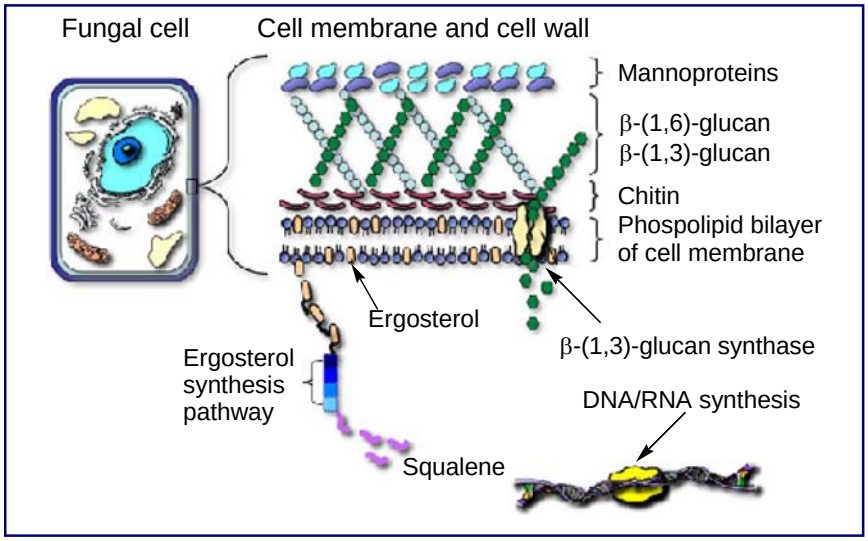


Figure 1: Fungal Cell

The Fungal Cell Wall: A unique organelle

The fungal cell wall is a unique organelle, required for the growth, for the maintenance of osmotic stability of the cell and fungal viability as well as for its pathogenicity. The only difference between mammalian cell and of fungal is the absence of cell wall in mammalian cells. The fungal cell wall is composed of Chitin, β-(1,3) and β-(1,6)-glucan and mannoproteins.

Chitin is a linear homopolymer of *N*-acetyl glucosamine (GlcNAc) units that are linked by β-(1,4) linkage, that found in most fungal cell wall. Chitin is biosynthesized on the cytoplasmic surface of the plasma membrane from uridine

diphosphate *N*-acetyl glucosamine (UDP-GlcNAc) and the synthesis is catalyzed by membrane bound enzymes called Chitin synthase (there are three isoenzymes Chs 1, 2 and 3) (Figure 2 and 3). It is a minor but essential cell wall component and the proportion varies among the species, ranging from less than 1% in the yeast form of *C. albicans* to 10-20% for the pathogenic forms of the dimorphic fungi *Blastomyces dermatidis, C. immitis* and the mycelial form *C. albicans*.

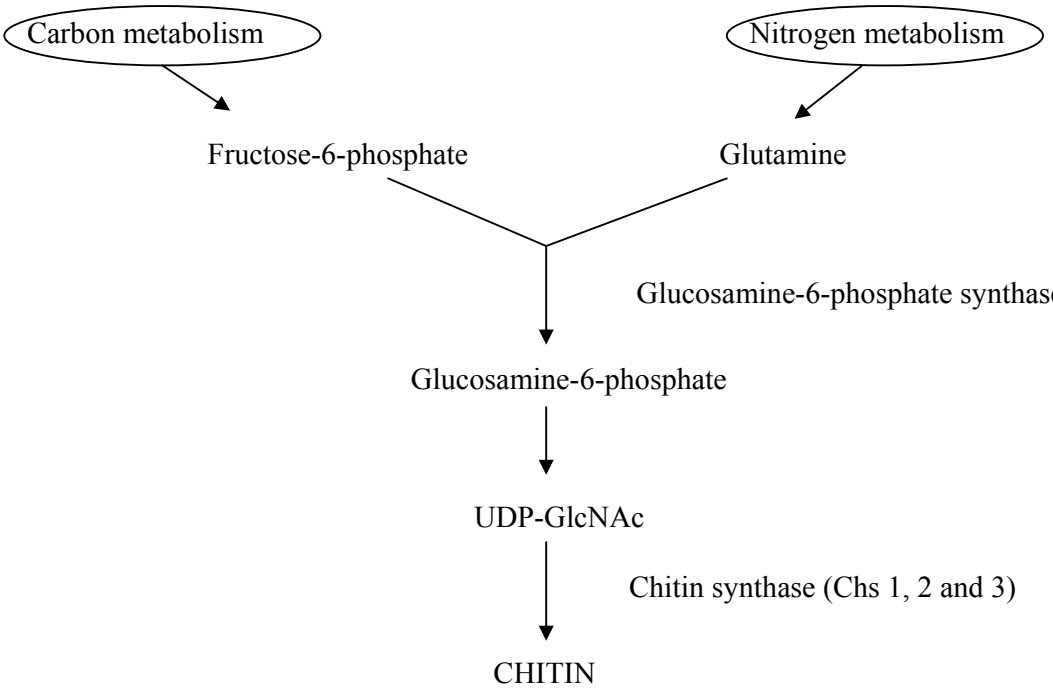


Figure 2: Chitin biosynthesis pathway

Glucans are the major components that strengthen the cell wall. The glucosyl subunits in these glucans are linked by β-(1,3) linkages as a long coiling chain, with occasional side chains having β-(1,6) linkages, produced by glucan synthase enzyme and three β-(1,3) chains run in parallel to form triple helix. These β-(1,3)-glucans are required to maintain the integrity and form of the cell wall.

However, mannoproteins are unlikely to be either unique or essential as *O*-and *N*-glycosylated proteins are universal in eukaryotes. These constitute approximately

40% of the cell wall and are linked at their manno-oligosaccharide to β-(1,3)-glucan by phosphodiester linkage.

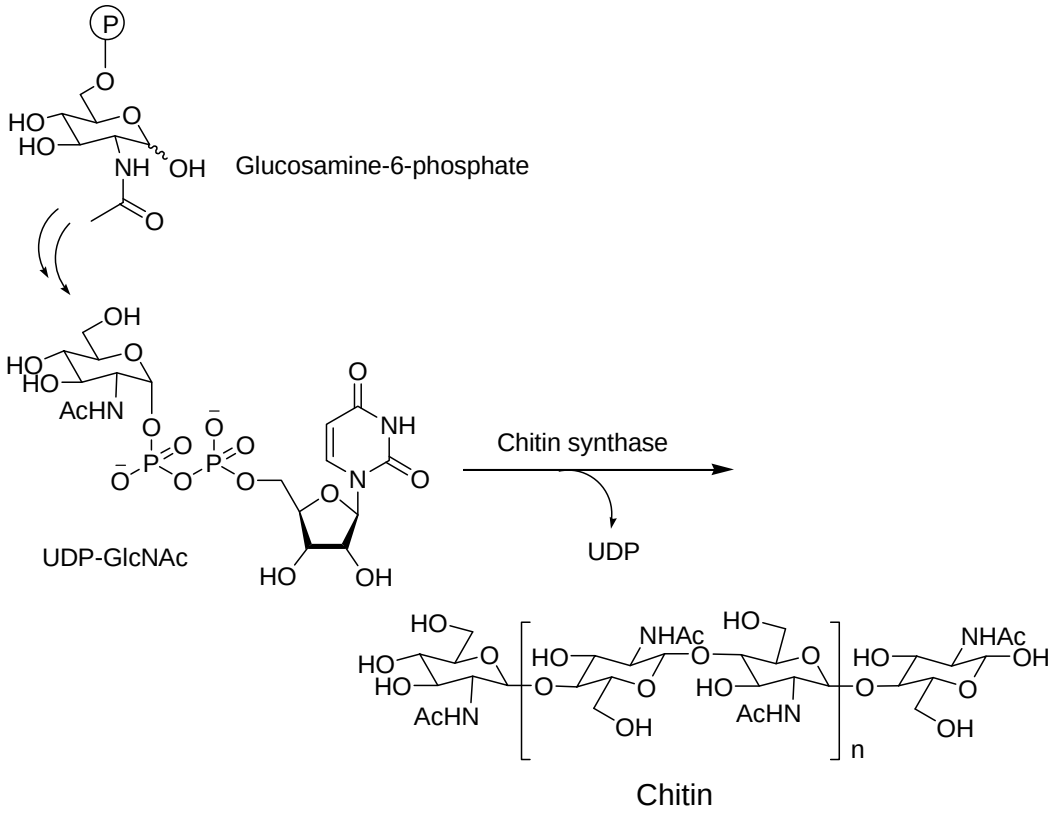


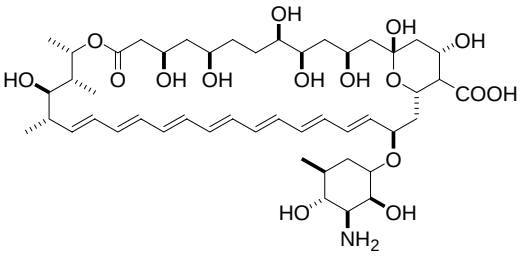
Figure 3: Chitin synthase catalyzed reaction.

Drugs in Clinical Use, Their Targets in Fungal Cell and Toxicities[5a]:

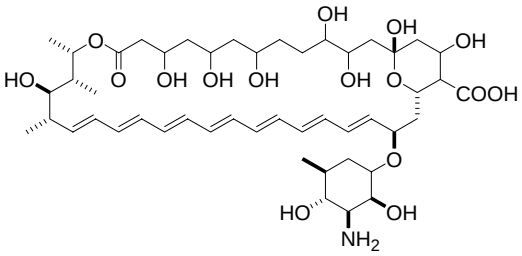
Antifungal agents currently utilized for the treatment of mycoses and their targets in fungal pathogens are as follows.

Polyene Antifungals: Amphotericin B and Nystatin

Amphotericin B is produced by *Streptomyces nodusus,* was discovered in 1955 by Gold and coworkers. The polyenes act by binding to ergosterol in the fungal cell membrane. The binding results in depolarization of the membrane and formation of pores that increases permeability to proteins and monovalent and divalent cations, eventually leading to cell death.



Amphotericin B



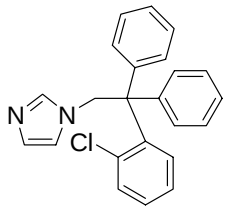
Nystatin

Although Amphotericin B binds approximately 10 times more strongly to fungal cell membrane components than mammalian cell membrane cholesterol, it definitely disrupts mammalian cell giving rise to adverse side effects. Therefore ployenes have greater toxicities for mammalian cells and causes nephrotoxicity that limits the clinical use of polyenes. Resistant strains have also been isolated under laboratory conditions with alteration in the nature and amount of sterols present in the membrane.

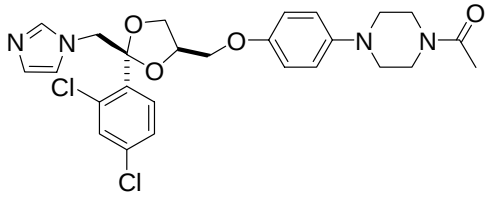
Azole Antifungals:

Azole antifungals are the major class of drugs which are widely used clinically. Miconazole, Clortrimazole and Econazole are the topical agents and

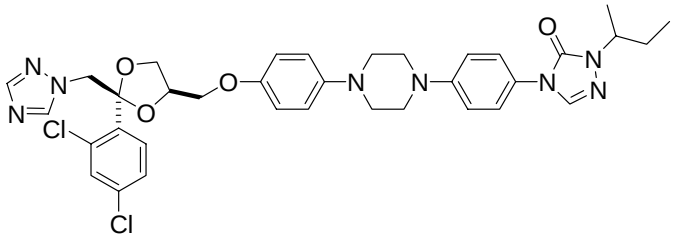
Ketoconazole, Itraconazole and Fluconazole are useful in the treatment of systemic mycoses. The mode of action of azole antifungals is inhibition of ergosterol biosynthesis by inhibiting the fungal cytochrome P-450 3-A dependent enzyme, lanosterol 14-α-demethylase, thereby interrupting the synthesis of ergosterol. Inhibition of the enzyme leads to the depletion of ergosterol in the cell membrane and accumulation of toxic intermediate sterols, causing increased membrane permeability and inhibition of fungal growth.



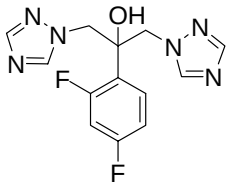
Clotrimazole



Ketoconazole



Itraconazole



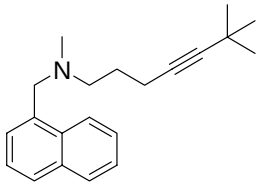
Fluconazole

Azole antifungals can also inhibit mammalian cytochrome P450-dependent enzymes involved in hormone synthesis or drug metabolism. Therefore, azole antifungals cause hepatoxicity. Azoles are only fungistatic[5b]. Due to the increased administration of Azole antifungals for the treatment of systemic fungal infections, pathogenic yeasts are developing resistance to these drugs. Target modification is a common factor contributing to clinical resistance to azole therapy. However, azole moiety itself has been proved to be effective pharmcophore.

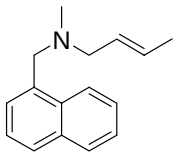
Allylamines as Antifungals:

Allylamines are the other class of antifungals which also work in a similar fashion i.e. by inhibiting the synthesis of ergosterol. However, allylamines act at an

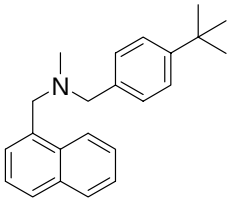
earlier step in the ergosterol synthesis pathway by inhibiting the enzyme squalene epoxidase leading to the accumulation of intracellular squalene that causes fungicidal effect upon exposer to the drug. Like the azoles, terbinafine causes hepatic toxicity and has the potential for drug interaction with other medications metabolized through the mammalian cytochrome P-450 pathway.



Terbinafine



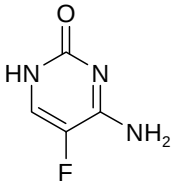
Naftifine



Butenafine

Antimetabolites: Flucytosine

Flucytosine or 5-fluorocytosine (5-FC) was originally developed in the 1957 as a potential antineoplastic agent. It was found to have antifungal activity in 1968 to treat candida and cryptococal infections in human. Flucytosine inhibits DNA synthesis by blocking the functions of a key enzyme thymidylate synthetase in the DNA replication. Flucytosine is also incorporated in fungal RNA, thereby disrupting transcription and translation. Selectivity is achieved because mammalian cells are unable to convert flucytosine to fluorouracil. But flucytosine can be converted to 5-fluorouracil (5-FU) by bacteria residing in the gastrointestinal tract.



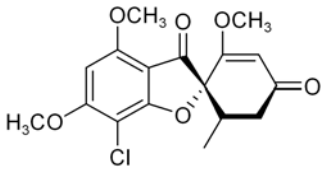
Flucytosine

The most common adverse effects seen with flucytosine are similar to 5-FU chemotherapy (diarrhea, nausea and vomiting, bone marrow suppression) however

with reduced intensity. The serious side effects associated with flucytosine are hematological, manifested as leucopenia and thrombocytopenia.

Griseofulvin:

Griseofulvin is a natural product first isolated in 1939 from *Penicillium griseofulvum*. It inhibits fungal cell mitosis by disrupting mitotic spindle formation a critical step in cellular division. Griseofulvin served as first line drug for treatment of dermatophytosis for many years. Because of its limited efficacy and untoward side effects, it is recently being replaced by itraconazole and terbinafine.



Other Class of Medicinal Interest:

Besides above class of antifungals there are few other classes of antifungals such as N-Miristoyl Transferase Inhibitors, Fungal Efflux inhibitors, etc.

Although the number of antifungal drugs that are available for clinical use has grown, there are still weakness in both their spectrum and potency. Both the number of fungal infections and the number of fungal species causing them are increasing, as a result of the increased number of immunosuppressed and immunocompromised patients. In addition to these facts, the currently administered drugs are only fungistatic (azoles) and causes sever side effects such as Nephrotoxicity (polyenes) and hepatotoxicity (azole).

1.4. Need for Further Research in Antifungal Agents

There are mainly three challenging problems for antifungal researchers in development of an effective drug in combating severely invasive mycosis.

1. Toxicity of currently used antifungal agents:

The currently administered drugs are only fungistatic and causes sever side effects such as Nephrotoxicity (polyenes) and hepatotoxicity (azole), as the fungi shares similar cellular components and mechanism, as that of mammalian cell.

2. Resistance of yeasts to clinically useful antifungal agents:

The molecular basis of resistance to azole antifungals, there are three different resistance mechanisms are known in pathogenic yeasts.[9]

- First, the reduced access of the agents to the target cytochrome P450 enzyme because of increased efflux of antifungals, caused by the action of resistance gene products.
- Second, the over production of cytochrome P450 enzyme, possibly by gene amplification.
- Third resistance mechanism a structural alteration in cytochrome P450 enzyme which results in lower susceptibility to azole antifungals.

3. Emergence of newer strains by mutation:

The treatment of immunosuppressed and immunocompromised patients such as in Cancer and AIDS patient needs long term administration of antifungal drugs to treat the invasive infection caused by opportunistic pathogenic fungi. The consequence leads to the development of resistance of fungi to these drugs by mutation in the genes leading to the birth of newer resistant strains.

Novel Inhibitors of Cell Wall Biosynthesis[3b, 6]:

The fungal cell wall is critical for the cell viability and pathogenicity. Beyond serving as a protective shell and providing cell morphology, the fungal cell wall is a critical site for exchange and filtration of ions and proteins, as well as metabolism and catabolism of complex nutrients. The fungal cell wall is a unique target because mammalian cells lack a cell wall, it represents an ideal, safe and specific target for antifungal therapy. Cell wall is present in all fungi, therefore cell wall biosynthesis inhibitors would exhibit broad spectrum of antifungal activity.

Structurally, the fungal cell wall is composed of a complex network of mannoproteins and polysaccharides such as Chitin and β-(1,3) and β-(1,6)-glucan. Disruption of this protein/carbohydrate matrix results in a structurally-defective cell wall, rendering the fungal cell sensitive to osmotic lysis.

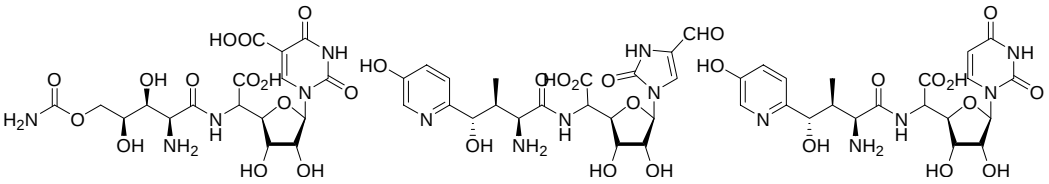
Inhibitors of Chitin Synthesis:

Chitin synthase is the enzyme that plays an essential role in the chitin biosynthesis pathway (Figure 2 and 3, page No. 5 and 6). The enzyme is unique target for antifungal compounds as it is essential for the survival of fungi, present in all fungi and absent in mammalian as well as plant cells. Therefore the Chitin synthase inhibitors can emerge as fungicidal (lethal only to fungi), broad spectrum, specific and less toxic to mammals and plants.

The naturally occurring peptidyl nucleoside antibiotics, such as polyoxins and nikkomycins are the competitive inhibitors of Chitin synthase enzyme with inhibition constant in the range of about 0.1 – 1 μM. The inhibitory activity is due to the structural resemblance of these agents with that of the UDP-GlcNAc, a substrate for

Chitin biosynthesis (Figure 2 and 3). Nikkomycins also synergize with azoles and glucan synthase inhibitors.

The extent of inhibition by these inhibitors varies in the different isoenzymes of Chitin synthase. For ex. in *S. cerevisiae*, Chs1 and Chs3 are more sensitive to nikkomycin derivatives than in Chs 2. However *C. albicance* and other clinically important species are resistant to polyoxin antibiotics because of their poor transport across the cell membrane.



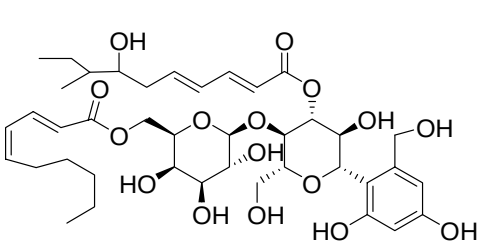
Polyoxin D Nikkomycin X Nikkomycin Z

Although the naturally occurring polyoxins and nikkomycins showed promising activity their use as clinical agents have been a great failure due to several reasons. The reasons and synthetic modifications are described in the latter part.

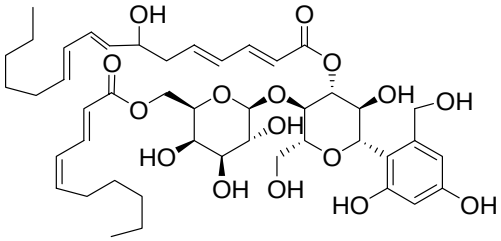
Inhibitors of β-Glucan synthesis:

The echinocandins and the glycolipid populacandins are the two groups of anticandidal antibiotics, which interfere with the biosynthesis of cell wall glucan. These antibiotics inhibit β-(1,3) glucan formation by acting on glucan synthase enzyme non-competitively. The inhibition leads to cessation of growth and cell death. Populacandins are acylated derivatives of the disaccharide β-(1,4)-galactosylglucose, such as populacandin A, B & C, corynecandin, fusacandin, (Figure 4a) etc. The efficacy of populacandins against pathogenic fungi is very poor. Any synthetic effort to improve the potency and to expand antifungal spectrum have been unsuccessful. The echinocandins are acylated cyclic hexapeptides such as echinocandin B, anidulafungin, and caspofungin, etc (Figure 4b). As per the mechanism of action, these agents appear to be well-tolerated and have relatively less toxicity than polyene

or azole-class antifungals. Echinocandins are fungicidal for *C. albicance* and other *Candida* spp., but the antifungal spectrum is similar to that of populacandins. The semisynthetic derivatives of these families are therefore currently potential candidates for antifungal drug development.

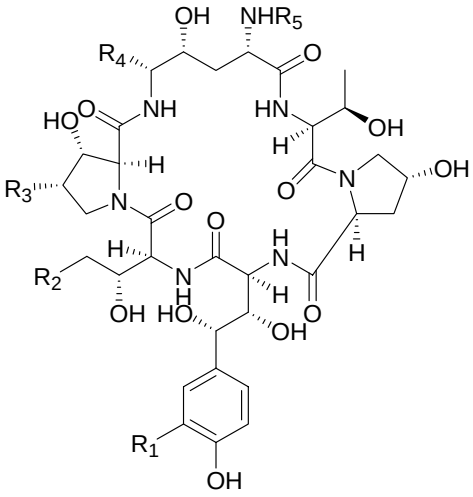


Corynecandin



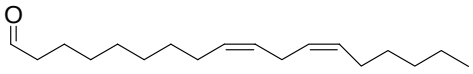
Fusacandin

Figure 4a: Populacandins



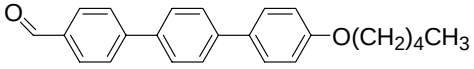
Echinocandin B: R_1,R_2= H, R_3= CH_3, R_4= OH

R5=



Anidulafungin : R_1, R_2, R_3= H, R_4= OH

R5=



Caspofungin : R_1,R_3= H, R_2= CH_2NH_2

R_4= $NH(CH_2)_2NH_2$

R5=

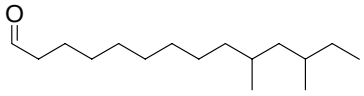
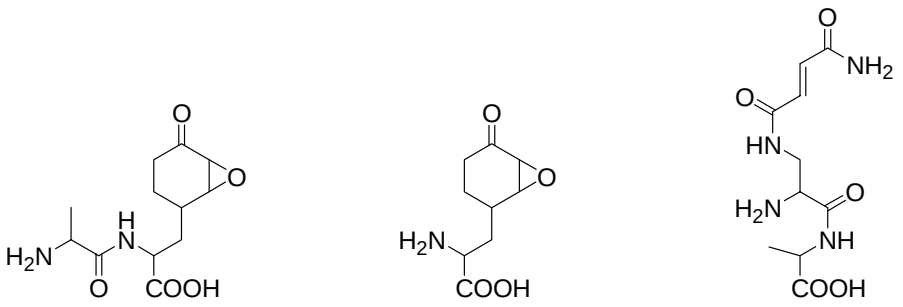


Figure 4b: Echinocandins

Inhibitors of Glucosamine-6-Phosphate Biosynthesis:

The building block needed for the biosynthesis of Chitin is glucosamine-6-Phosphate. It is produced by the enzyme L-glutamine:fructose-6-phosphate amidotransferase (glucosamine-6-Phosphate synthase) (Figure 2 and 3, page No. 5 and 6). The enzyme is target of the dipeptide antibiotic bacilysin (tetaine, bacillin). It is actually a prodrug, which is cleaved by intracellular dipeptidase to bioactive epoxyamino acid anticapsin. Another antibiotic having similar activity is A 19009

(*N*3-fumaroyl-L-2,3-diaminopropanoyl-L-alanine) (Figure 5). Both of these agents are antagonized by glutamine and are non-selective because they inhibit both the prokaryotic and eukaryotic amidotransferase enzyme.

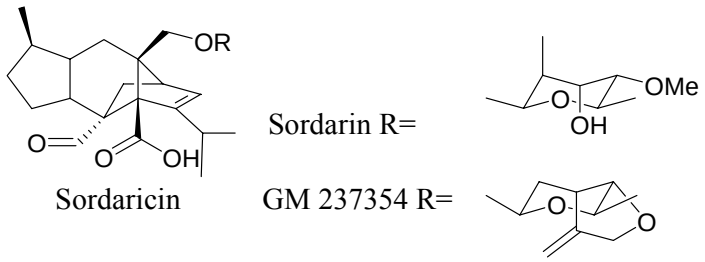


Bacilysin (Tetain) Anticapsin A 19009

Figure 5

Inhibitors of Fungal Protein and Amino Acid Synthesis:

Compounds that inhibit fungal protein synthesis selectively can emerge as useful antifungal agents. Eukaryotic cells need two proteins, elongation factor 1 (EF1) and elongation factor 2 (EF2) for ribosomal translocation during protein synthesis. In addition to these, fungi also have a third elongation factor, (EF3), which is not present in mammalian cells. It is a unique fungal ribosomal factor essential for protein synthesis, obviously a selective target for antifungal development.



Sordaricin

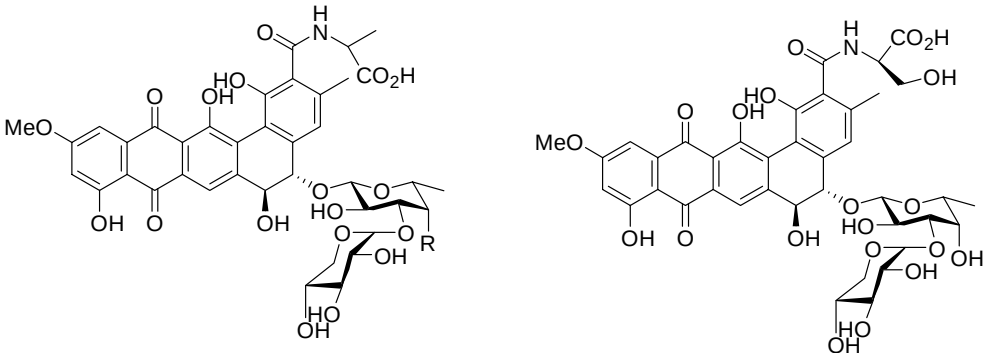
Figure 6: Sordarin and its derivatives

Sordarin and its analogues, (Figure 6) a family of selective EF2 inhibitors are the antifungal agents bearing the tetracyclic diterpene aglycon Sordaricin, shown activity against a wide range of pathogenic fungi, such as *Candida spp*., *Cryptococcus*

neoforrnans, and *P. carinii*. Toxicological properties of these compounds after preclinical studies have shown no evidence of genetotoxicity.

Pradimicin and Benanomicin:

The Pradimicin and Benanomicin are dihydrobenzonaphthacene quinone antibiotics conjugated with D-amino acids such as D-alanine, D-serine or D-glycine and a disaccharide side chain (Figure 7). The mode of action of these agents is, they bind to cell wall mannoproteins in a calcium dependent manner. The binding causes disruption of cell membrane and leakage of intracellular potassium. Pradimicins are active against cryptococcosis, candidiasis and aspergillosis. BMS 181184 is a water soluble derivative having activity against *Aspergillus* spp. *in vitro*, but with less potency than itraconazole or ampotericin B.



Pradimicin A R = $NHCH_3$ BMS 181184

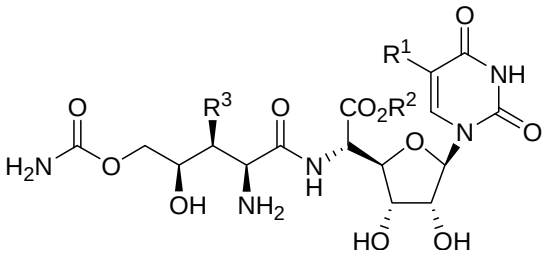
Benanomicin A R = OH

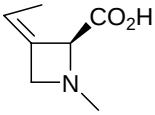
Figure 7

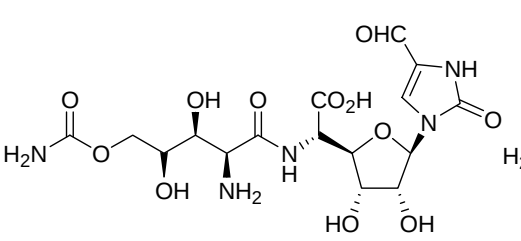
The extensive review of current antifungals showed the efforts to enhance the potency and spectrum, at the same time to reduce the untoward side effects of various classes of antifungals. The study also showed the unsuccessful efforts to bring newer antifungals with novel mode of action for clinical treatment of life threatening systemic fungal infections. However at the same time intensive research resulted in promising targets and lead structures.

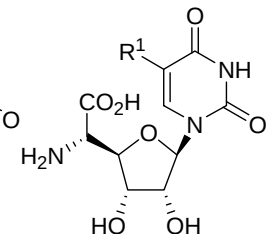
1.6. An Account on Peptidyl Nucleoside Antifungals

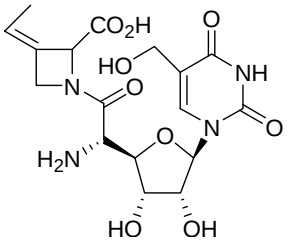
Peptidyl Nucleosides[11], the naturally occurring nucleoside antibiotics are secondary microbial metabolites that have broad biological activities. Polyoxins (Figure 8) and Nikkomycins are representatives of nucleoside antibiotics. A series of polyoxins were isolated from *Streptomyces cacaoi* by Isono and coworkers during 1960s. Polyoxins exhibited a very selective antimicrobial activity against some phytopathogenic fungal species. These have been widely used as agricultural fungicides with no side effects.



Polyoxins	R^1	R^2	R^3
A	CH_2OH		OH
B	CH_2OH	OH	OH







Polyoxin N

Polyoxin C $R^1 = CH_2OH$

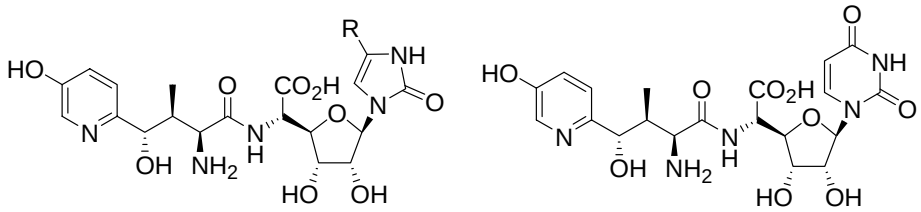
Thymine polyoxin C $R^1 = CH_3$

Uracil polyoxin C $R^1 = H$

Polyoxin I

Figure 8: Polyoxins

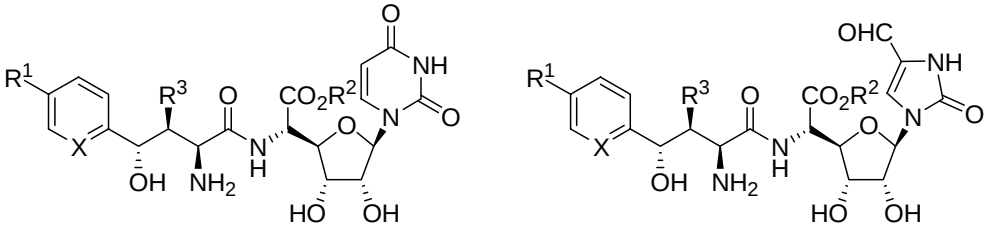
Isono and coworkers also isolated during 1980s, some three new antibiotics named neopolyoxins A, B and C (Figure 9) from *Streptomyces cacaoi.* Neopolyoxins also displayed selective inhibitory activity against phytopathogenic fungi. Neopolyoxins A and C showed higher inhibitory activity against the human pathogenic fungus *Candida albicans.*



Neopolyoxin A R= CHO, Neopolyoxin B R= CO_2H Neopolyoxin C

Figure 9: Neopolyoxins

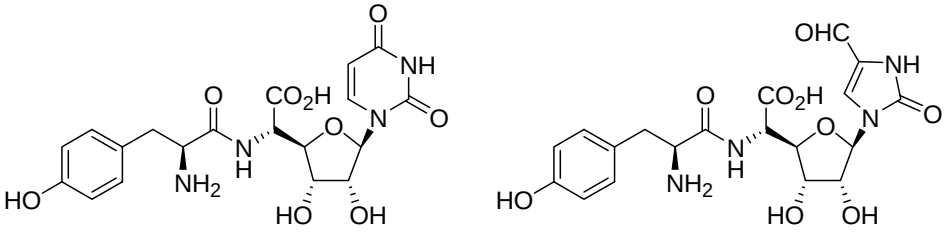
Nikkomycins are another family of nucleoside antibiotics isolated from *Streptomyces tendae.* The isolation of these compounds was reported by the Zahner and Konig groups. Nikkomycin Z and X (Figure 10) were thought to possess identical structure to neopolyoxins C and A, respectively.



Nikkomycins	X	R^1	R^2	R^3	Nikkomycins	X	R^1	R^2	R^3
Z	N	OH	OH	CH_3	X	N	OH	OH	CH_3
J	N	OH	Glu	CH_3	I	N	OH	Glu	CH_3

Figure 10: Nikkomycins

Nikkomycin W_Z and W_X are the some other biologically active compounds (Figure 11) isolated from wild type or mutant *Streptomyces tendae.* These compounds have shown acaricidal and insecticidal activity.



Nikkomycin W_Z Nikkomycin W_X

Figure 11

. Polyoxins and nikkomycins are known for their distinct activity against phytopathogenic fungi while being non-toxic to bacteria, plants and animals. The antifungal activity is due to their strong competitive inhibitory activity on Chitin synthase enzymes from fungi and yeast. The structural similarity between these antibiotics and UDP-GlcNAc, a substrate for chitin syntheses was thought to responsible for their antifungal activity (Figure 3, page No. 6). Inhibition of chitin synthesis could be lethal in fungi cells, because chitin is an essential structural component of fungal cell wall. Polyoxins and nikkomycins are more potent against filamentous fungi than against *C. albicans.* Comparatively nikkomycins are more potent than polyoxin C against *C. albicans,* with nikkomycin Z being the most potent of the class with MIC of 14μg/ml.

Although polyoxins and nikkomycins exhibit activity against a variety of fungi, remain unsuccessful for clinical applications. The factors which make them unsuccessful are due to their hydrolytic liability by dipeptidases and inefficient fungal cell wall permeability. In other words *C. albicans* and other clinically important species are resistant to polyoxin antibiotics because of their poor transportation across the cell membrane.

As the mammalian cells lack cell wall, these antibiotics are therefore selectively lethal to fungi and yeasts with a novel mode of action. Thus these antibiotics can be potentially useful models for development of effective agents against opportunistic fungi, as well as safer, nontoxic antifungal agents.

Several efforts have been made to modify the peptidyl nucleosides since its initial isolation. Extensive efforts are made with the hope of establishing useful structure activity relationship (SAR) for the development of newer antifungal agents. The review of synthetic efforts and subsequent biological studies reveals that,

although much has been accomplished, ideal antifungal agents have not yet been developed from the natural peptidyl nucleoside leads. Several synthetic efforts to improve their transport across the fungal cell have not produced drug candidates of any clinical value.

1.7. Brief Review on Efforts to Discover Synthetic Peptidyl Nucleosides

Review of literature showed the focus on two major research areas in the discovery of polyoxins and nikkomycins. One is mainly on synthetic methodology development to produce these molecules in an efficient and enantioselective manner. The second focused on the search for more potent and safer polyoxin based antifungal agents. In this area several synthetic analogues have been synthesized and their antifungal activity evaluated.

The major shortcomings of these polyoxins and nikkomycins antifungals are hydrolytic liability to dipeptidase and poor cell wall penetration. Several attempts were carried out to overcome these challenges. The common structural component present in most of the polyoxins and nikkomycins is uracil polyoxin C (UPOC). The structural feature of these agents presents several interesting sites for modification and diversification (Figure 12).

Some of the major types of modifications are,

- Modification of the *N*- terminal amino acid side chain.
- Substitution of carboxylic acid group of the nucleoside amino acid.
- Base modification.
- Substitution of oxygen atom of furanose ring with other atoms.
- Modification of hydroxyl groups of furanose ring.
- Modifications leading to increase the hydrophobicity.

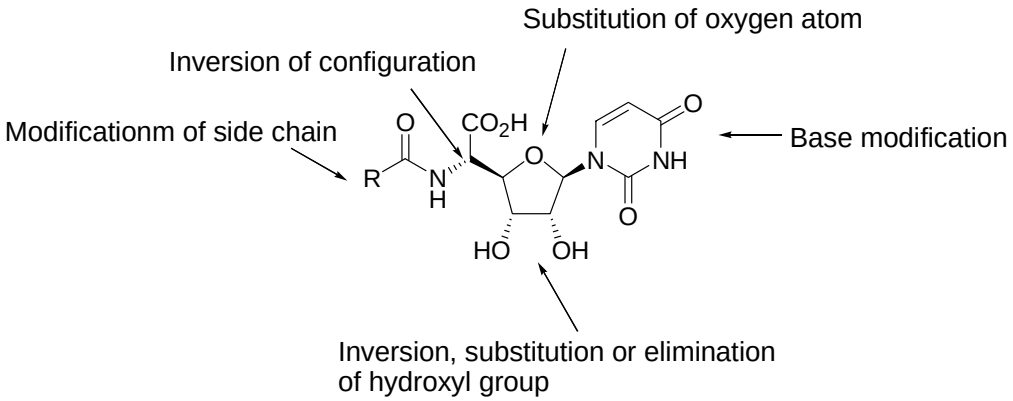
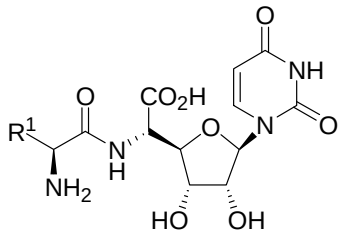


Figure 12: Sites of Diversification

A series of amino acyl derivatives of polyoxin C and L were synthesized and evaluated for chitin synthase inhibitory activity by Isono[12] and Hori.[13] They used amino acids such as glycine, alanine, ornithine and norvaline as the side chains. *In vitro* whole cell assay and *in vitro* chitin synthase inhibitory assays showed that: 1) the α-L-amino group and 5'-carboxyl group are essential for the activity. 2) the alkyl chain length and the nature of ω-substituent affect the activity, polar groups such as ureido or carbamoyl increase activity, whereas ionic groups such as amino or carboxyl groups depress activity. These synthetic modifications did not showed broader spectrum than the natural polyoxins.

Naider and coworkers[14] attempted to alter the polyoxin molecules so as to enhance the penetration into fungal cells like *C. albicans* and inhibit chitin synthase. They synthesized polyoxin analogues containing hydrophobic amino acids in the side chain (Figure 13). The most active derivatives (**1, 4** and **5**) amongst these modifications were approximately equal in activity to ployoxin D (ID_{50} 10^{-6} M). Derivatives (**2**) and (**6**) showed 50% inhibition *in vitro* against *C. albicans* at concentrations similar to polyoxin D. Unfortunately further studies showed that these dipeptide analogues were rapidly metabolized to inactive UPOC and corresponding amino acid. In order to improve metabolic stability, N^{ϵ}-Oct-Lys-UPOC and N^{ϵ}-Oct-

Gln-UPOC (**7** and **8)** were synthesized and were found to be more resistant to dipeptidase than previous derivatives. But there efficacy against *C. albicans* was still less than that of polyoxin D.[15]



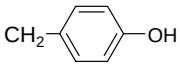
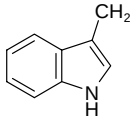
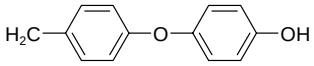
1. $R^1 = CH_2CH_2Ph$
2. $R^1 = CH_2(CH_2)_2CH_3$
3. $R^1 = CH_2(CH_2)_2NHCONH_2$
4. $R^1 =$ 
5. $R^1 =$ 
6. $R^1 =$ 
7. $R^1 = (CH_2)_4NHCO(CH_2)_6CH_3$
8. $R^1 = (CH_2)_2CONH(CH_2)_7CH_3$

Figure 13

Further Naider and coworkers[16] reported the synthesis and testing of polyoxins containing α-amino fatty acids. Although most of them were not better than that of natural polyoxins, compound (Figure 14, **9**) showed more activity with a ID_{50} of 0.3 μg/ml and MIC of 40 μg/ml against *C. albicans 124.*

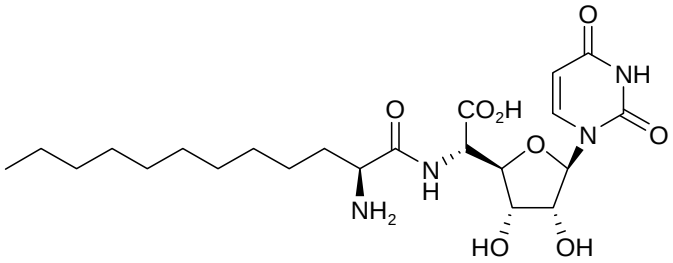


Figure 14: 9

Grassberger and coworker[17] synthesized two L-alanine-UPOC analogs (Figure 15, **10** and **11**) in order to obtain resistant analogs to peptidase hydrolysis. But the modifications resulted in total loss of activity against chitin synthase.

Figure 15: 10 and **11**

Ugi four component reaction strategy is a very interesting reaction to design diverse molecules. Boehm and Kingsbury[18] utilized this strategy to synthesize *N*-methylated di- and tripeptidyl polyoxin analogs (Figure 16, **12** and **13**).

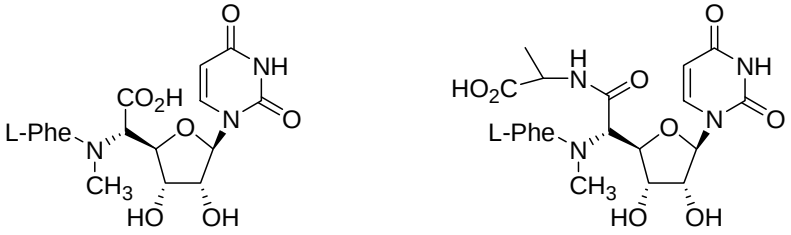


Figure 16: 12 and **13**

As the polyoxin analogs are regarded as nucleotide analogs, an attempt was made to replace uracil by adenine. Isono and Azuma[19] developed an adenine analog of UPOC (Figure 17, **14**) which may be regarded as AMP analogs, but found to be biologically inactive.

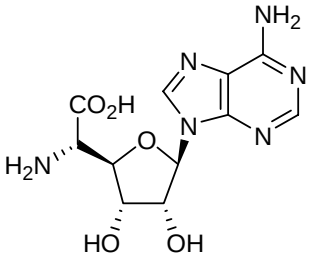


Figure 17: 14

Cooper and coworkers[20] reported a synthetic study of njikkomycin Z containing different amino acyl groups. The activity of these derivatives was found to be much lower than that of natural analogs (Figure 18, **15-20**).

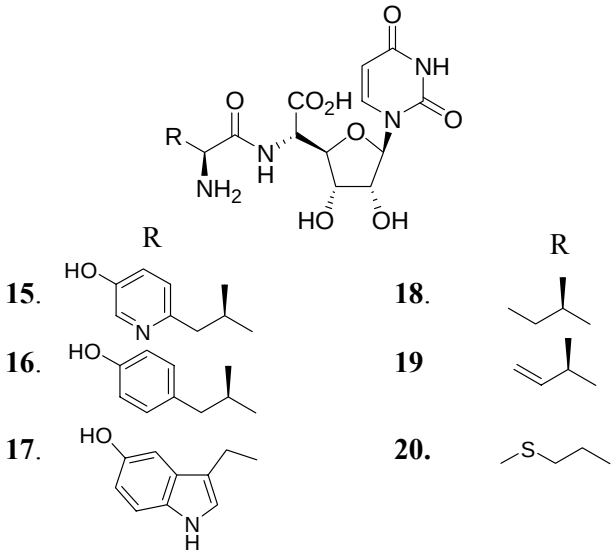


Figure 18: 15-20

Recently Obi and coworkers[21] discovered some Chs inhibitors having activity comparable to that of nikkomycin Z (NZ). The active analogs in this series contain hydrophobic groups in the side chain, which are summarized below (Figure 19, **21-26**). Compound **26** was the most active inhibitor amongst all the analogs against Chs enzyme with IC_{50} about 0.31 μg/ml (IC_{50} NZ 0.393 μg/ml).

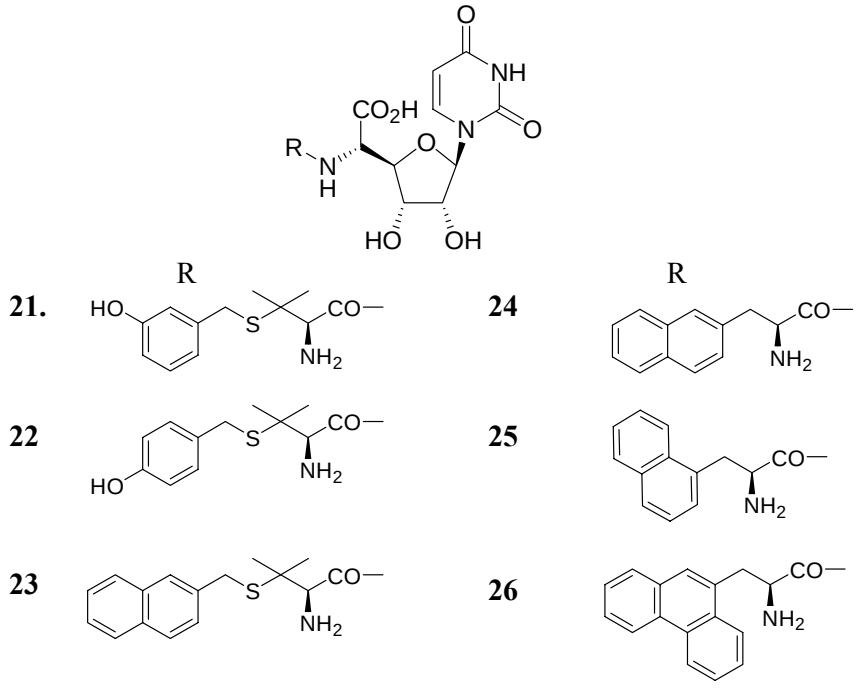


Figure 19: 21-26

Tsukuda and coworkers[22] employed Ugi 4 Component Coupling reaction to explore the active antifungal analogs of polyoxins using combinatorial concept. All the Ugi products obtained were a mixture of diastereomers at C5' position (Figure 20, **27-29**). The IC_{50} value of compounds **27, 28** and **29** against *C. albicans* chitin synthase 1 were determined to be 6.07 μM, 15.0 μM and 16.8 μM (NZ 9.49 μM). Where as only compound **27** was showed inhibitory activity against *C. albicans* chitin synthase 2 with IC_{50} 4.78 μM (NZ 0.06 μM).

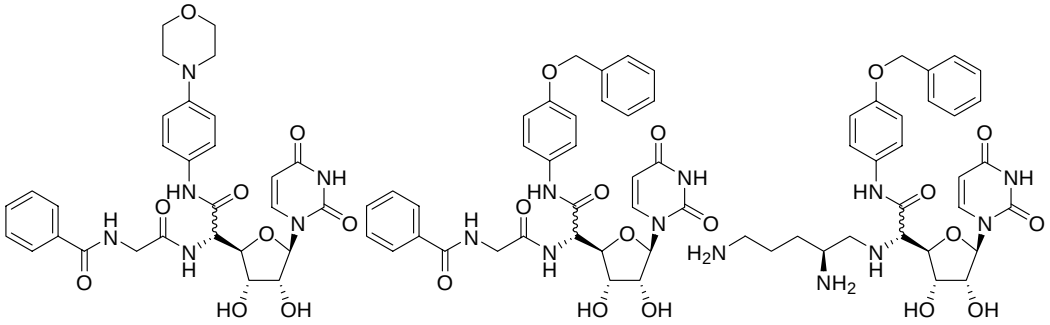
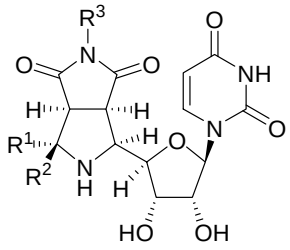


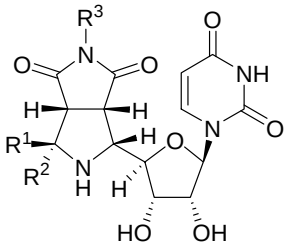
Figure 20: 27, 28, 29.

An entirely distinct modification of nikkomycins was reported by Grigg and coworkers[23]. They report the cascade thermal and decorboxylative 1,3-dipolar cycloaddition reaction of uracil polyoxin C (figure 21).



30

	R^1	R^2	R^3
a.	Me	CO_2Me	Me
b.	Me	CO_2Me	Ph
c.	Me	Ph	Me
d.	H		Me



31

	R^1	R^2	R^3
a.	Me	CO_2Me	Me
b.	Me	CO_2Me	Ph
c.	Me	Ph	Me
d.	H		Me

Figure 21

The reaction was carried with mono- and dicarbonyl compounds in the presence of a dipolarophile, via an intermediate azomethine ylide, to a series of polyoxin cycloadducts in excellent yield and high diastereoselectivity (Figure 21, **30** and **31**). However, the biological activity of these analogs is not reported.

In conclusion, review of the literature on the discovery of potent and broad spectrum peptidyl nucleoside antifungals against opportunistic fungi revealed that none of the efforts led to the successful candidate for the clinical treatment. Although peptidyl nucleosides are non toxic to human beings, remained out of reach of therapeutic applications. The modification of the natural analogs such as polyoxin D and nikkomycin Z either resulted in lower activity against Chitin synthetase enzymes or complete loss of activity. Every effort to make them resistant to dipeptidase and to enhance fungal cell uptake remains challenging. Thus, the current status of peptidyl nucleoside discovery prompted us to undertake further development of peptidyl nucleosides which will be safer and effective antifungals.

Chapter 1: References

1.8. References

1. Hawksworth, D. L. *Studies in Mycology,* **2004,** *50,* 9.
2. Hoepelmn, I. M. Inter. *J. Antimicrob. Agents,* **1996,** *6,*129.
3. (a) Turner, W. W.; Rodriguez, M. *J. Curr. Pharm. Des.,* **1996,** *2,* 209. (b) Fostel, J. M.; Lartey, P. A. *Drug Discov. Today*, **2000,** *5,* 25. (c) Hossain, M. A.; Ghannom, M, A. *Exp. Opin. Invest. Drugs,* **2000,** *9,* 1797.
4. Meunier, F. Inter. *J. Antimicrob. Agents,* **1996,** *6,* 135.
5. (a) Watkins, W. J.; Renau, T. E. Antifungal agents in *Burger's Medicinal Chemistry and Drug Discovery*, 6th Edition, *vol. 5*, p 881. (b) Frost, D. J.; Brandt, K. D.; Cugier, D.; Goldman, R. *J. Antibiotics,* **1995,** *48,* 306.
6. Georgopapadakou, N. H.; Tkacz, J. S. *Trends in Microbiology,* **1995,** *3,* 98.
7. Barrett, D. *Biochimica et Biophysica Acta,* **2002,** *1587*, 224.
8. Muehlbauer, M. S.; Birgit Willinger, B.; Egner, R.; Ecker, G.; Karl Kuchler, K. *Inter. J. Antimicrob. Agents,* 2003**,** 22, 291.
9. Odds, F. C. *Inter. J. Antimicrob. Agents*, **1996,** *6*, 145.
10. Moir, D. T.; Shaw, K. J.; Hare, R. S.; Vovis, G. F. *Antimicrob. Agents Chemother*., ***43***, 1999, 439.
11. Zang, D.; Miller, M. J. *Currrent Pharmaceutical design,* **1999,** *5,* 73.
12. (a) Isono, K.; Azuma, T.; Suzuki, S. *Chem. Pharm. Bull.,* **1971,** *19,* 505. (b) Isono, K.; Suzuki, S.; Azuma, T. *Agr. Biol. Chem.,* **1971,** *35*, 1986. (c) Azuma, T.; Saita, T,; Isono, K. *Chem. Pharm. Bull.,* **1977,** *25,* 1740.
13. (a) Hori, M.; Kakiki, K.; Misato, T. *Agr. Biol. Chem.,* **1974,** *38,* 691. (b) Hori, M.; Kakiki, K.; Misato, T. *Agr. Biol. Chem.,* **1974,** *38,* 699.
14. Shenbagmurthy, P.; Smith, H.A.; Becker, J. M.; Steifeld, A.; Naider, F. *J. Med. Chem.,* **1983,** *26,* 1518.
15. Smith, H.A.; Shenbagmurthy, P.; Naider, F.; Kundu, B.; Becker, J. M. *J. Antimicrob. Chemother.,* **1986,** *29,* 33.
16. Khare, R. K.; Becker, J. M.; Naider, F. *J. Med. Chem.,* **1988,** *31,* 650.
17. Emmer, G.; Ryder, N. S.; Grassberger, M.A. *J. Med. Chem.,* **1985,** *28,* 278.
18. Boehm, J.; Kingsbury, W. D. *J. Org. Chem.,* **1986,** *51,* 2307.
19. (a). Azuma, T.; Isono, K. *J. Chem.Soc. Chem. Comm.,* **1977,** 159. (b) Azuma, T.; Isono, K. *Chem. Pharm. Bull.,* **1977,** *25,* 3347.

20. Cooper, A. B.; Desai, J.; Lovey, R. G.; Sanksena, A. K.; Girijavallabhan, V. M.; Ganguly, A. K.; Loebenberg, D.; Parmegiani, R.;Cacciapuoti,A. *Bioorg. Med. Chem. Lett.,* **1993,** *3,* 1079.
21. Obi, K.; Uda, J.; Iwase, K.; Sugimoto, O.; Ebisu, H.; Matsuda, A. *Bioorg. Med. Chem. Lett.,* **2000,** *10,* 1451.
22. Suda, A.; Ohta, A.; Sudoh, M.; Tsukuda, T.; Shimma, N. *Heterocycles.* **2001,** *55,* 1023.
23. Dondas, H. A.; Fishwick, C. W. G.; Grigg, R.; Kilner, C. *Tetrahedron,* **2004,** *60,* 3473.

****

Chapter 2: “Click Chemistry” Guided Diversity Oriented Synthesis Of 1,2,3-Triazole And 1,2,3,4-Tetrazole Fused Carbohydrate Derived Polycyclic Compounds

2.1. Introduction

Click Chemistry:

"Click Chemistry" is a modern concept in synthetic chemistry that facilitates the construction of newer chemical substances. The approach makes use of a few near perfect chemical reactions i.e., the most facile and selective chemical transformations, for the synthesis of designed molecules. The resultant molecules have a high built energy content that makes the chemical reaction spontaneous and irreversible, therefore highly stable product. The strategy is useful for the exploration of the novel molecules in lead discovery. Lead optimization is also faster through analog library generation by using "Click Chemistry". In addition to this, the concept works well in conjunction with structure based design and combinatorial chemistry. The process utilizes several appropriate building blocks to provide variety of useful chemical substances such as mimics of pharmacophores, drugs, natural products, etc. [1a, b]

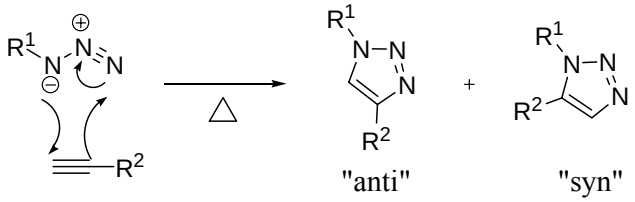
Scope of a click reaction must be wide with high yield both in small and large scale, without producing any offensive byproducts. It must be easy to perform, insensitive to oxygen or water, use only readily available reagents, the use of benign solvents or easily removed solvent or no solvent. The reaction workup and product isolation must be simple by using non-chromatographic methods, such as crystallization or distillation.[1a]

"Click Chemistry" uses carbon-heteroatom bond forming reactions from carbon-carbon multiple bonds. Olefins and acetylenes are regarded as the best and most energetic building blocks. Kolb and coworkers[1b] framed some reliable processes for the synthesis of building blocks and compound libraries. The most common examples include the reactions such as,

1. Cycloaddition reactions for ex. 1,3-dipolar class and hetero Diels-Alder reactions, nucleophilic substitution reaction, for ex. ring-opening reactions of strained heterocyclic electrophiles like epoxides, aziridines, etc.
2. Reactions such as formation of ureas, aromatic heterocycles, oxime, amides etc.
3. Additions to carbon-carbon multiple bonds, for ex. oxidative reactions such as epoxidation, dihydroxylation, aziridination and also Michael addition reactions.

1,3-Dipolar Cycloaddition Reaction: Formation Of 1,2,3-Triazoles.

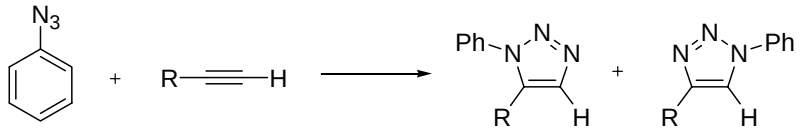
One of the most consistent examples of click reaction is Huisgen's[2] 1,3-dipolar cycloaddition reaction of alkynes and azides yielding 1,2,3-triazole heterocyclic compound. Alkynes and azides are known as least reactive groups in organic chemistry, but they are most easy to add among the energetic species known. The stability of these species is responsible for the slow nature of cycloaddition reaction. However, the desired triazole-forming cycloaddition was carried out at elevated temperature and usually results in a mixture of the 1,4 and 1,5 regioisomers (Figure 1).



(Ca 1:1)

Figure 1: Huisgens concerted [2+3] cycloaddition reaction

Rolf Huisgen reported a 1,3-dipolar cycloaddition reaction in his paper where the outcome of reaction shown the dependence of electronic and steric effects (Scheme 1, **32** and **33**).[3]

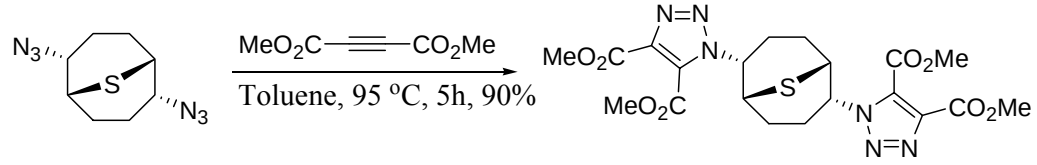


32. R = C_6H_5 52% 43%

33. R = $CH(OC_2H_5)_2$ 23% 52%

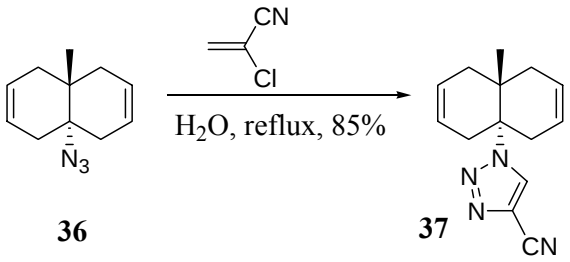
Scheme 1

Sharpless and coworkers[1a] reported azide cycloaddition where azido molecule readily adds to alkyne (Scheme 2a and 2b, **35** and **37**). In these cases the alkyne bonds are substituted by electron withdrawing groups, which make the alkyne group more reactive. Azide **36** reacts with the cyanoacetylene equivalent, 2-chloroacrylonitrile, to give only one regioisomer of triazole **37**.



34 **35**

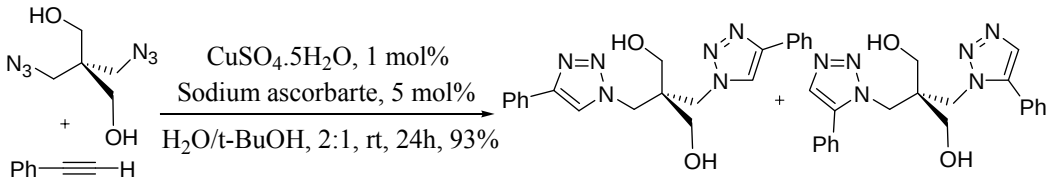
Scheme 2a



36 **37**

Scheme 2b

Recently Sharpless[4a] reported copper-(I) catalyzed reaction sequence which regiospecifically couples azides and terminal acetylenes to give only 1,4-disubstituted 1,2,3-triazoles (Scheme 3, **38**). For comparison, the thermal reaction (neat, 92 ^oC, 18 h) between these substrates gives both regioisomers in a ratio of 1.6:1 in favor of the 1,4 isomer (Scheme 3, **38** and **39**). The process is experimentally simple and appears to have enormous scope.



38 (Only product) **39**

Scheme 3

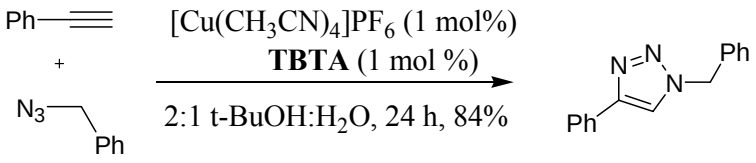
The use of Cu(I) catalyst dramatically accelerated the rate of the azido-alkyne coupling. Another advantage of this procedure is use of water as solvent which is benign and product can be just filtered, rendering purification unnecessary.

Sharpless and coworkers found that the catalyst is better prepared in situ by reduction of Cu(II) salts using sodium ascorbate as reductant. The procedure was found to be high yielding and purity at catalytic amount of Cu(II) salts. The reaction conditions were very simple. The reaction proceeds at ambient temperature, wide choice of solvent, independent of pH, reaction completes in 6 to 36 hours. In other words, this is a very robust catalytic process, which is so insensitive to the usual reaction parameters. Another scope of this copper-catalyzed triazole synthesis is the lack of functional group interference.

Copper(I) salts, for example, CuI, $CuOTf.C_6H_6$, and $[Cu(NCCH_3)_4][PF_6]$, can also be used directly in the absence of a reducing agent. These reactions uses acetonitrile as co-solvent and one equivalent of a nitrogen base (for example, 2,6-lutidine, triethylamine, *N,N*-diisopropylethylamine, or pyridine). However, formation of undesired byproducts, such as diacetylenes, bis-triazoles, and 5-hydroxy-triazoles, was often observed. Therefore Cu(II)/sodium ascorbate catalyst system found more reliable in aqueous condition.

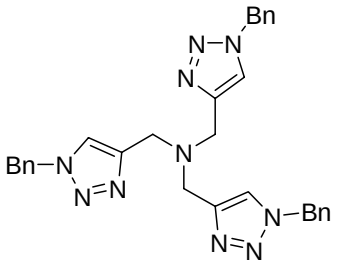
In the latter studies Fokin group[4b] developed oligotriazole ligands such as tris-(benzyltriazolylmethyl)amine (TBTA) for protecting Copper(I) catalyst under aerobic

aqueous conditions and promoting Cu(I) catalysed transformations (Scheme 4). The TBTA has also been successfully applied in bioconjugation studies.



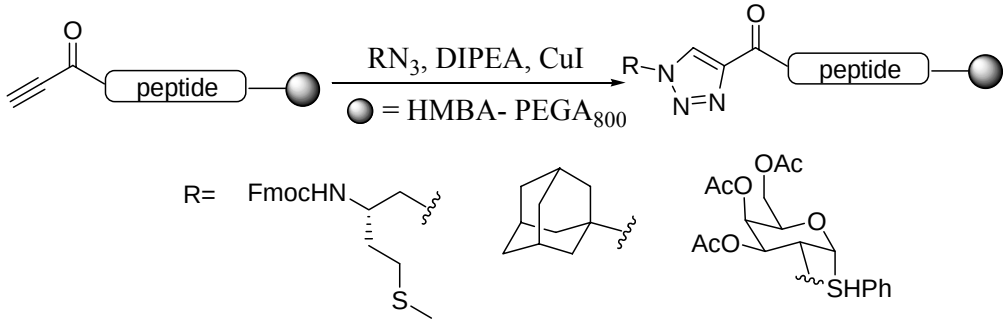
40

TBTA =



Scheme 4

Meldal[5] reported peptidotriazole synthesis on solid phase using copper iodide and *N,N*-diisopropylethylamine (Scheme 5) obtained regiospecifically 1,4-disubstituted products in quantitative yields.



41 **42** **43**

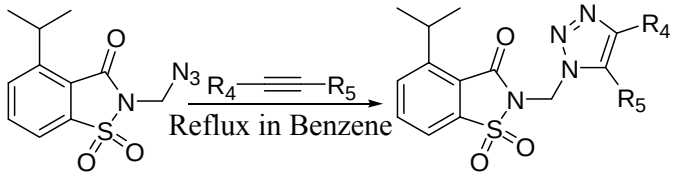
Scheme 5

This kind of transformation is significantly useful for drug discovery, because of not only its reliability, but also due to the favorable physicochemical properties of triazoles. 1,4-Disubstituted 1,2,3-triazoles are regarded as amide surrogates because of their rigid linkage (Figure 2). Unlike amides, triazoles cannot be cleaved by

hydrolysis. They are impossible to oxidize or reduce as in the case of benzenoid and related aromatic heterocycles. These facts demonstrates the metabolic stability of 1,2,3-triazoles. 1,4-Disubstituted 1,2,3- triazoles also possess a large dipole moment of about 5 Debye and interestingly nitrogen atoms two and three (Figure 2) function as weak hydrogen bond acceptor.[1b] Thus 1,2,3-triazoles can participate in the hydrogen bonding which can enhance the affinity of drug molecules to the target receptors and also modify the solubility.

Figure 2: Triazole as amide surrogate: electronic similarities.

In contrast to the Cu(I) catalyzed regeospecific 1,3-dipolar cycloaddition reaction, Hlasta and coworker[6] reported synthesis of a new class of human leukocyte elastase inhibitors by 1,3-dipolar cycloaddition reaction of an (azidomethyl) benzisothiazolone with trimethylsilyl substituted acetylenes (Scheme 6). He reported the trimethylsilyl controlled regeoselectivity over the electron deficient sulfonyl group. The influence of trimethylsilyl group is attributed to their steric effect and stabilization of a partial positive charge on the acetylene β carbon.



	R_4	R_5	Yield %
44.	TMS	H	81
45.	TMS	SO_2Ph	73

Scheme 5

Mechanism of Cu(I) Catalysed 1,3-Dipolar Cycloaddition[4, 7]:

It is known that copper(I) readily inserts into terminal alkynes in the presence of base, e.g. the Sonogashira coupling. The polarization of the terminal triple bond by the covalently bound copper(I) catalyzes the cycloaddition which probably changes

from a concerted reaction into a stepwise addition. Sharpless and coworkers proposed a catalytic cycle for Cu(I) catalyzed 1,3-dipolar cycloaddition reaction (Figure 3). It begins with formation of the copper(I) acetylide **I**. According to density functional theory calculations copper catalysed reaction disfavors the concerted [2+3] cycloaddition and favors to a stepwise, annealing sequence, hence the term "ligation", which proceeds via the six membered copper-containing intermediate **II**.

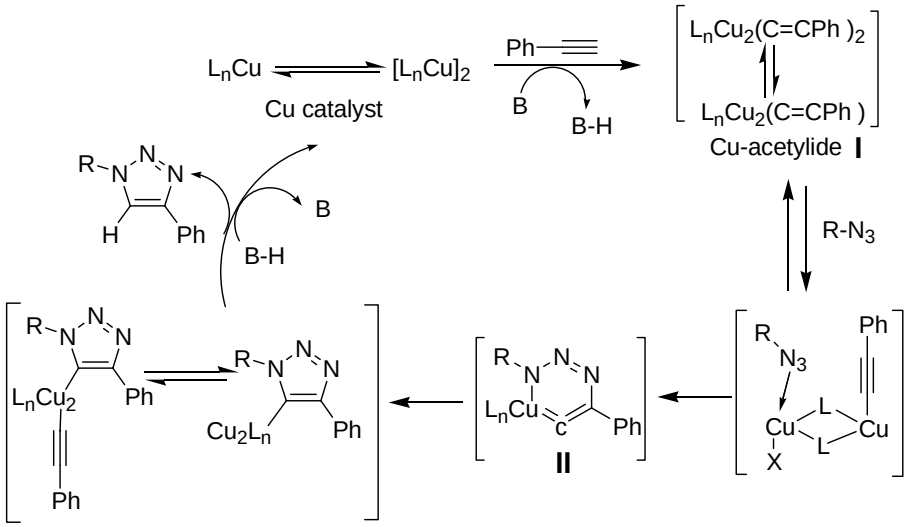


Figure 3: Mechanism of Cu(I) Catalysed 1,3-Dipolar Cycloaddition

Intramolecular 1,3-Dipolar Cycloaddition Reaction: Another Perfect Reaction.

"Click Chemistry" process is designed on any perfect or near perfect chemical reactions. Like Cu(I) catalyzed intermolecular 1,3-dipolar cycloaddition reaction, the intramolecular 1,3-dipolar cycloaddition reaction (Figure 4) is also a perfect reaction for "Click Chemistry" concept.

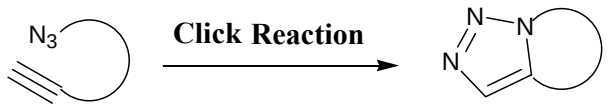
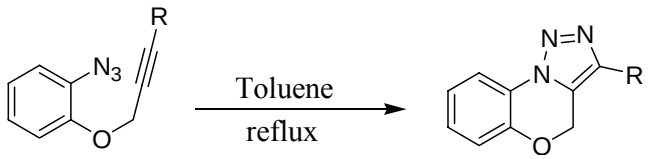


Figure 4: Intramolecular 1,3-dipolar cycloaddition reaction

The reaction requires no catalyst, activator or any other reagent. The built in azido-alkyne substrates when subjected to "Click Chemistry" conditions such as reaction as neat or in benign solvents like water, toluene etc. can result in perfect

reaction product. As expected azido-alkyne substrates are converted into 1,3-dipolar cycloaddition reaction product that is fused ring 1,2,3-triazoles as single product.

Fusco and coworkers[8] carried out the study of intramolecular 1,3-dipolar cycloaddition reactions of aryl azides bearing alkenyl, alkynyl and nitrile groups. They conducted the decomposition of these substrates in aromatic hydrocarbon solvents. While refluxing aryl azides bearing *O*-propargyl substituent in toluene, they observed the formation of triazole as only decomposition product (Scheme 6) and reported the first example of intramolecular 1,3-dipolar cycloaddition of the azido group to the acetylinic functional group.

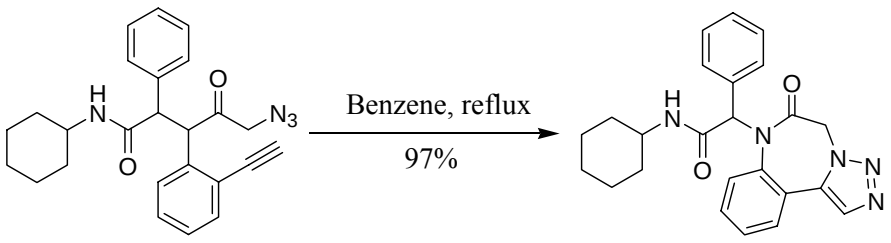


46a. R = H
46b. R = Ph

47a. R = H (3 h)
47b. R = Ph (15 min)

Scheme 6

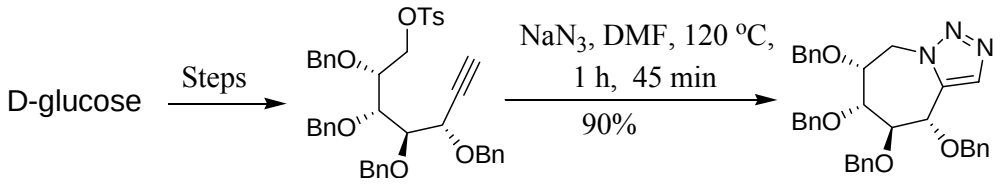
Fused ring or multicyclic 1,2,3-triazoles can also be designed by planning multicomponent and tandem reactions. Zanze *et. al*.[9] synthesized bicyclic and tricyclic 1,2,3-triazoles derivatives by sequential Ugi/allkyne-azide cycloaddition reactions. The allkyne-azido substrates were synthesized from Ugi four component condensation reactions. The resultant Ugi products were refluxed in benzene for about 4-18 hours to yield 1,2,3-triazoles fused multicyclic compounds in 86-98% (Scheme 7).



48 **49**

Scheme 7

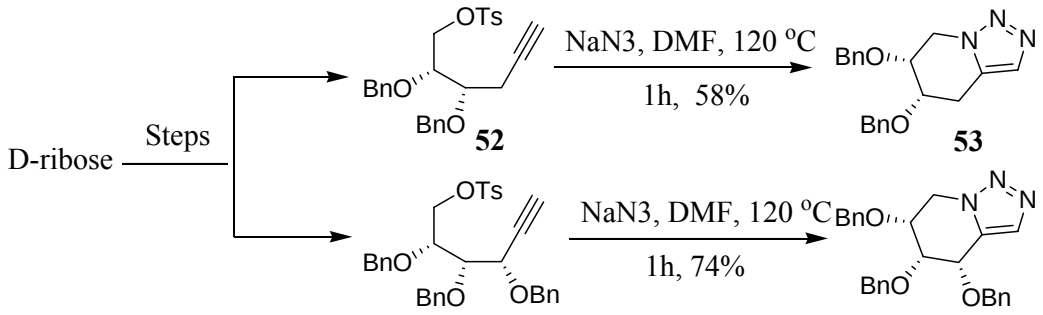
Lievre and coworkers[10] reported the synthesis of glyco-1,2,3-triazoles prepared by intramolecular 1,3-dipolar cycloadditions of carbohydrate derivatives from aldohexoses (Scheme 8) and aldopentoses (Scheme 9) and such carbohydrate mimics belongs to the family of imino sugars, which have been shown to be potent inhibitors of glycosidases and glycosyltransferases.



50 **51**

Scheme 8

Above reports are the some representative examples of intramolecular cycloaddition reactions. In some of the cases cycloaddition was affected by refluxing in benzene and in other heating the *O*-tosyl substrate in dimethyl formamide with sodium azide. The yields obtained are moderate to good. Most of these reactions represent the near perfect reactions for "Click Chemistry".



Scheme 9

1,3-Dipolar Cycloaddition Reaction: Formation of 1,2,3,4-Tetrazoles.

Like triazoles, tetrazoles also have been used in a variety of synthetic and medicinal chemistry applications. The new applications of these heterocycles are emerging in both materials science and pharmaceutical applications. Tetrazoles have

been investigated for many medicinal applications including antibacterials,[11a] cancer,[11b] heart disease,[11C] neurodegenerative disease,[11d] etc. Tetrazoles are stable in strongly acidic and basic media, as well as to oxidizing and reducing conditions and tolerate a wide range of chemical environments[12]. More importantly tetrazole can serve as metabolically stable surrogates for a carboxylic acid and *cis*-amide group (Figure 5). The bioisosteric replacement of a carboxylic acid group by tetrazole results in molecule with greater lipophilicity that enables the drug to pass through biological membrane.[13]

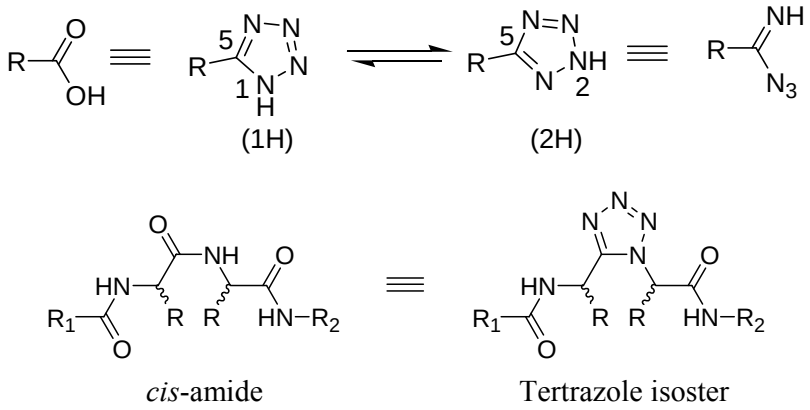
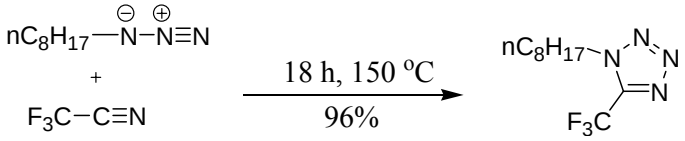


Figure 5: Tetrazole as carboxylic acid and *cis*-amide surrogate

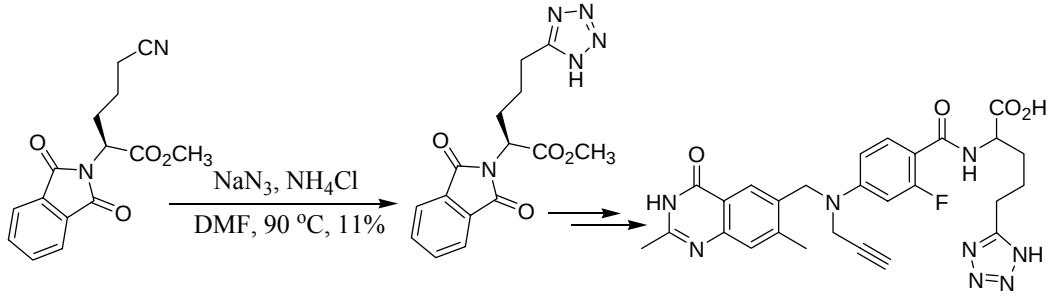
Tetrazole synthesis is another example of Huisgen's 1,3-dipolar cycloaddition reaction. [2, 5] The early preparation of tetrazole was from hydrogen cyanide or organic cyanide[13] and hydrazoic acid requires heating for several days at 100 ^{o}C. On the other hand, when cyanamide used that yields 5-aminotetrazole smoothly even at room temperature. Aliphatic nitriles must be heated with HN_3 at 150 ^{o}C in order to obtain 5-alkyltetrazoles; it is advantageous to use lithium or ammonium azide in this case. The addition of hydrazoic acid onto imido chlorides offers a useful route to 1,5-disubstitutcd tetrazoles. And only the electron-poor C-N triple bonds of ethyl cyanoformate or of perfluoroalkyl cyanides are capable of adding organic azides (Scheme 10, **56**).



56

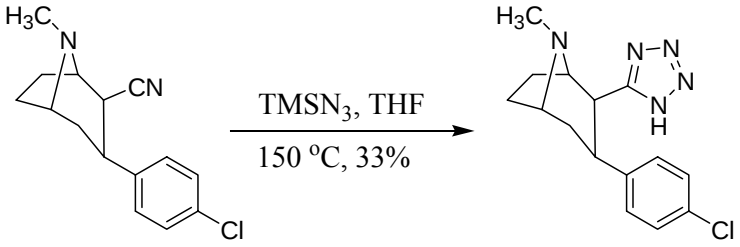
Scheme 10

The use of hydrazoic acid is very disadvantageous because it is highly toxic and extremely explosive in organic solutions. Alternately tetrazoles are synthesized from metal salts like sodium azide (Scheme 11), trimethyl silyl azide (Scheme 12) and tri-(n-butyl)tin azide (Scheme 13).[13]



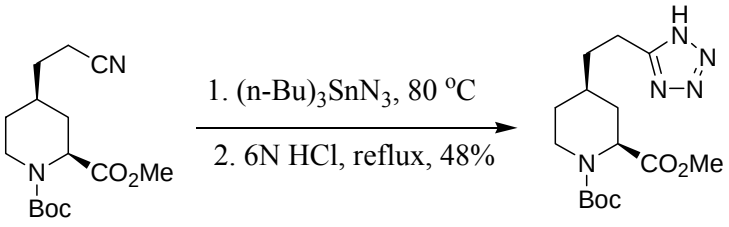
57 **58** **59**

Scheme 11



60 **61**

Scheme 12

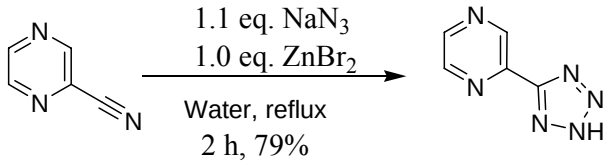


62 **63**

Scheme 13

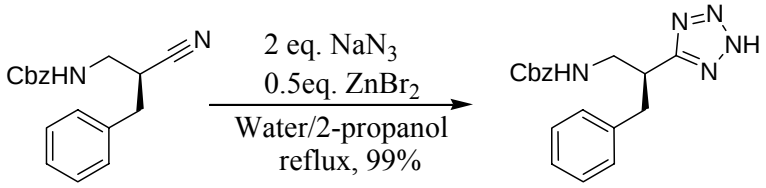
All the above methods used hydrazoic acid or release of hydrazoic acid, excess of sodium azide, tin or silicon azides or strong Lewis acids and use dimethyl formamide as organic solvent. All of these have one or the other drawbacks such as use of expensive and toxic metals, water sensitivity, hydrazoic acid is toxic and explosive as well as volatile.

Sharpless and coworkers[14, 15] reported one of the efficient, high yielding and safer condition that run in water for transformation of nitriles into tetrazoles. The procedure involves a simple heating or reflux (80 ^{o}C) of nitrile substrate in water or a mixture of water/2-propanol in the presence of sodium azide and zinc bromide to affect cycloaddition in excellent yield (Scheme 14 and 15). The procedure is safer as the release of toxic hydrazoic acid is nil or very minimum.



64 **65**

Scheme 14

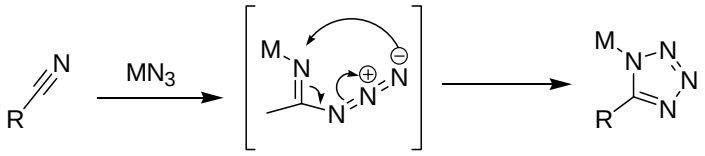


66 **67**

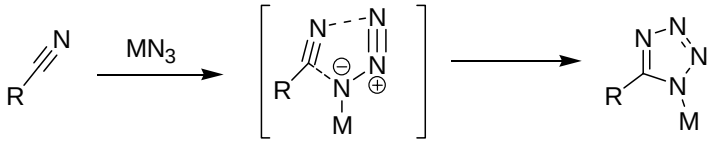
Scheme 15

The addition of hydrazoic acid or azide ion to a nitrile to give a tetrazole follows two types of mechanisms. One a two step mechanism and the other is a concerted [2+3] cycloaddition (Figure 6 a and b).[14]

a. Two step mechanism:



b. Concerted mechanism:

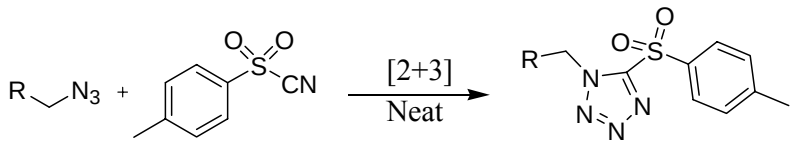


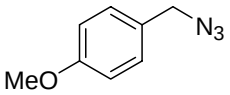
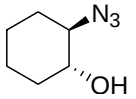
M= H, L_nZn, other metals

Figure 6: Mechanism for tetrazole formation

Although above methods are high yielding but employ metal azide and zinc bromide for the transformation. In most of the cases, reactions require vigorous conditions and prolonged time, except electron poor aromatic nitriles.

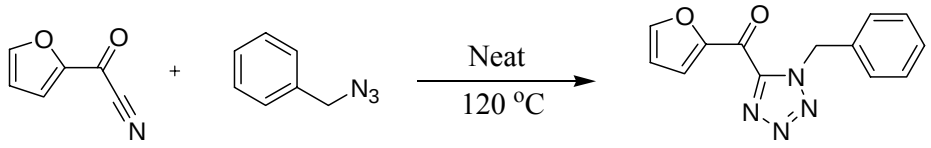
Sharpless *et. al*. thought that the use of organic azide would be better and powerful alternative for such kind of transformations. Recently they proposed two "Click Chemistry" approaches to tetrazole formation by direct [2+3] cycloaddition rout. In one of the report synthesized 5-sulfonyl tetrazoles[12] by simple heating of neat organic azides and sulfonyl cyanides. They observed high regioselectivity and quantitative conversion to the 1-alkyl-5-sulfonyl tetrazole with no side products and no purification in all the cases, therefore truly a "click reaction" (Scheme 16).



		Temp [^oC]	Time [h]	Yield [%]
68.		80	16	99
69.		80	16	76

Scheme 16

In another report Sharpless *et. al.*[16] reported one more "Click Chemistry" approach to tetrazoles and produced similar results. They synthesized 1-substituted-5-acyl tetrazoles using various types of acyl cyanides and aliphatic azides (Scheme 17).



70 **71** **72**

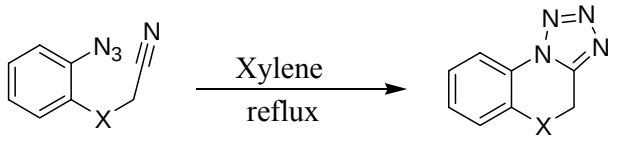
Scheme 17

The reactions were high yielding, regioselective and involved simple product isolation. Sharpless reported this protocol as another perfect reaction to "Click Chemistry".

Intramolecular 1,3-Dipolar Cycloaddition Reaction: Formation Of 1,2,3,4-Tetrazoles, Another Near Perfect Reaction.

The intramolecular [2+3] rout to tetrazoles is very much reliable compared to intermolecular reaction. Although nitriles are poor dipolarophiles,[17] when both azide and nitrile groups are in the same molecule as neighboring groups or if two groups are in the proper spatial relationship, rates of cycloaddition can be greatly increased.[18] Therefore intramolecular 1,3-dipolar cycloaddition reaction leading to fused or polycyclic tetrazole is also a suitable reaction for click reaction.

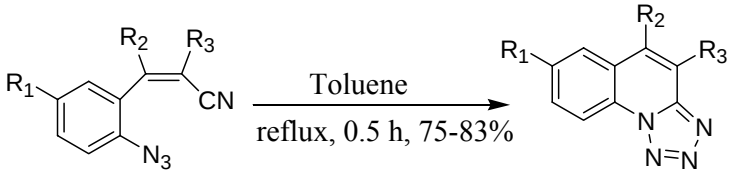
While performing the decomposition and stability studies of aryl azides bearing alkynyl and nitrile groups Fusco and coworkers[8] found that, azido nitrile were more stable, as it required higher temperature for decomposition. After analyzing the decomposition product they found the formation of fused ring tetrazole and interpreted the formation of tetrazole as the result of intramolecular 1,3-dipolar cycloaddition reaction (Scheme 18).



73a. X = O**, 73b.** X = CH_2 **74a.** X = O (4 h), **74b.** X = CH_2 (17 h)

Scheme 18

In another report Garanti *et. al.*[18] studied the thermochemical behavior of *o*-azido cinnamonitrile. Reaction of **75** (Scheme 19) in boiling toluene resulted in the rapid formation of tetrazole(1,5-a)quinolines (**76**) in high yields. They report the involvement of intramolecular 1,3-dipolar cycloaddition in the formation of fused ring tetrazoles.

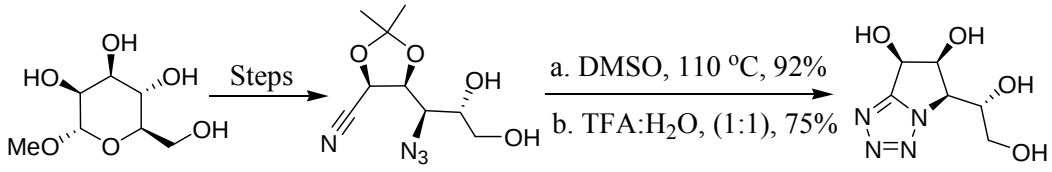


75 **76**

a. R_1, R_2, R_3 = H; b. R_1 = Cl, R_2 = Ph, R_3 = H: c. R_1, R_2 = H, R_3 = Me

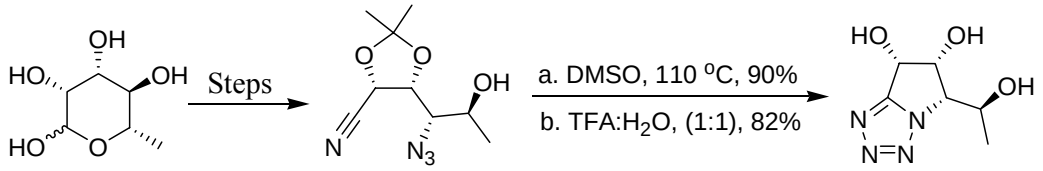
Scheme 19

It is interesting to report here the synthesis of fused ring tetrazoles from carbohydrate precursors by intramolecular 1,3-dipolar cycloaddition from Davis group.[19] They synthesized manno- and L-rhamnofuranotetrazoles from methyl mannopyranoside (Scheme 20, **77**) and L-rhamnose (Scheme 21, **80**) respectively and manno- and rhamnopyranotetrazoles from L-gulonolactone (Scheme 22, **83**) possessing glycosidase activity. The intramolecular 1,3-dipolar cycloaddition was affected by heating azido-nitrile substrate in dimethyl sulfoxide at 110 ^oC.



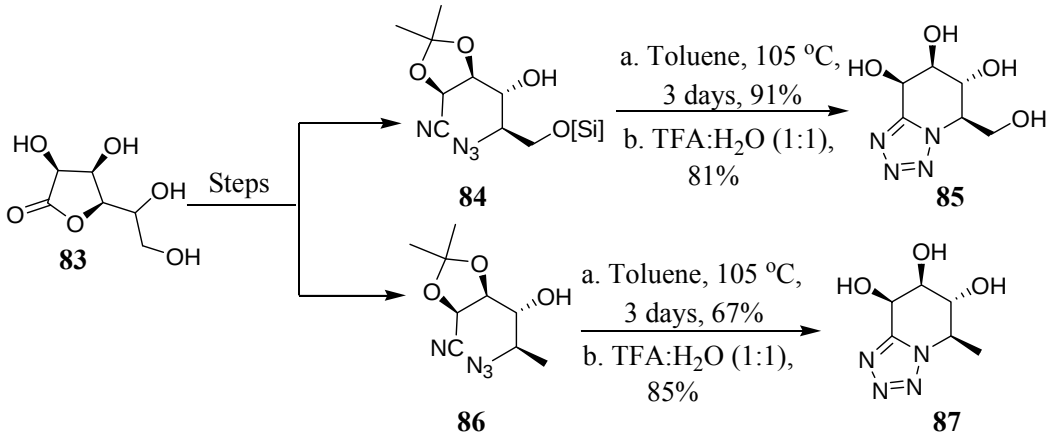
77 **78** **79**

Scheme 20



80 **81** **82**

Scheme 21



83 **84** **85** **86** **87**

Scheme 22

In all the above cases the nitrile group was found attached to carbon atom led to the efficient synthesis of polycyclic fused tetrazoles. In the similar manner Sharpless group[20] showed that nitrile function attached to heteroatoms are also efficient for intramolecular cycloaddition reaction with organic azides (Figure 7).

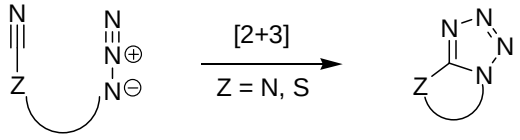
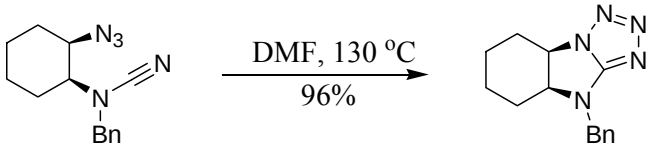


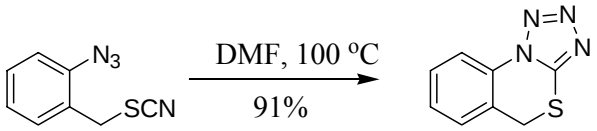
Figure 7

Simple heating for example, of azido cyanamides (Scheme 23, **88**) and azido thiocyanates (Scheme 24, **90**) in dimethyl formamide solution at 130-140 °C resulted in pure tetrazoles with higher yields.



88 **89**

Scheme 23



90 **91**

Scheme 24

In conclusion after analyzing above literature for intramolecular cycloaddition reactions leading to fused ring tetrazoles, one can regard these set of reactions also as nearly perfect and facile reactions. Although nitrile functionality is poor dipolarophile, intramolecular cycloaddition of azido-nitrile is also best fit for "Click Chemistry". While in some of the cases very high temperature was used to affect the cycloaddition and aprotic polar solvents such as dimethyl formamide and dimethyl sulfoxides were used as reaction medium. Despite these disadvantages, in all the cases yields are excellent and considering the biological significance of tetrazoles, it is noteworthy in the discovery of novel tetrazoles.

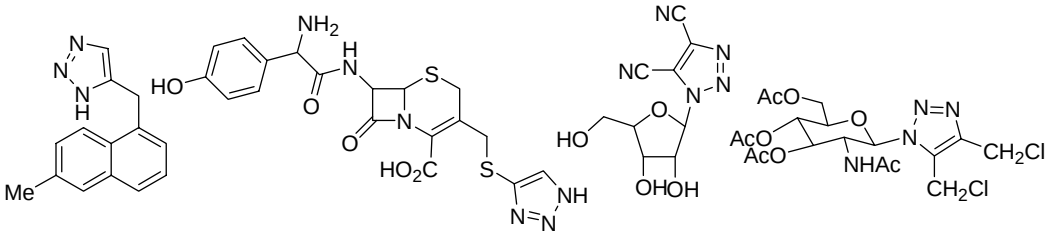
2.2. Biological Profiles of 1,2,3-Triazoles and 1,2,3,4-Tetrazoles

Majority of therapeutically useful medicinal agents and naturally occurring biologically active substances contains one or more heterocyclic rings. In this universe of heterocyclic substances, the presence of 1,2,3-triazole and 1,2,3,4-tetrazole nucleus is significant. The motifs containing these ring systems have shown enormous, range of biological activities. Both triazole and tetrazole containing compounds exhibited anti HIV, anticancer, antibacterial, antiallergic, fungicidal, glycosidase inhibitory activities, etc. The azido and terminal alkyne compounds which form 1,2,3-triazole are regarded as bioorthogonal chemical reporters[21a, b] and used in designing bioconjugates.

The significant profiles of triazoles and tetrazoles are not only limited to biology or therapeutics. The applications of these motifs are also extended to other face of the chemical science. These are explored and utilized as ligands for Pd catalyst, in material science such as cyclic peptides, nanoparticles, functionalized polymers, dendrimers, molecular electronic devices, etc.

In this section it is difficult to uncover every application of these chemical entities, as they are widely utilized. Here in this part highlighted some medicinal profiles of triazoles and tetrazoles by reviewing literature. Following are the examples of triazoles which are reported early to "Click Chemistry".

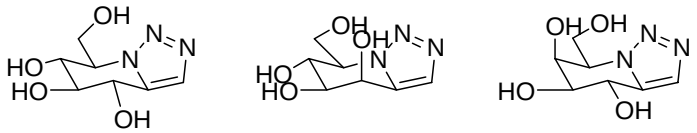
1,2,3-triazole-1,8-naphthyridine derivatives (**92**) have been synthesized and reported to have analgesic, sedative and fungicidal activity. Cifatrizin (**93**) is cephalosporin antibiotic which has activity against 342 different germs. 1,2,3-triazole-nucleosides such as (**94**) found to possess antibacterial and *in vitro* antiviral activity against herpes and measles virus and *N*-glycosyl(halomethyl)- 1,2,3-triazole (**95**) have been detected as alkylating agent showing cytostatic activity (Figure 8).[22]



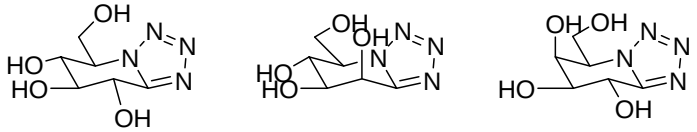
92 **93** **94** **95**

Figure 8

Vasella group[23] reported the synthesis of triazole and tetrazole fused bicyclic compounds derived from carbohydrate substrates. These compounds have shown strong β-glycosidase and glycogen phosphorylase b inhibitory activities (Figure 9, **96– 101**).



96 **97** **98**



99 **100** **101**

Figure 9

Camarasa *et.al.*[24] reported a series of 1,2,3-triazole spirocyclic ribofuranosyl nucleosides derivatives were synthesized and evaluated for their inhibitory activity on HIV-1 and HIV-2 induced cytopathicity in MT-4 cells and syncytium formation in CEM Cell cultures. The unsubstituted 1,2,3-triazoleTSAO (Figure 10, **102**) found active and had an EC50 for HIV-1 in MT-4 cells and CEM cells of 3.7 and 3.4pM respectively.

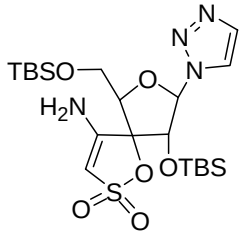


Figure 10: 102

Buckle and coworkers[25] reported the H1-antihistamine activity for 1,2,,3-triazole containing benzopyranone derivatives. Compound (Figure 11, **103**) was found to have potent H1-antihistamine activity comparable to that of mepyramine.

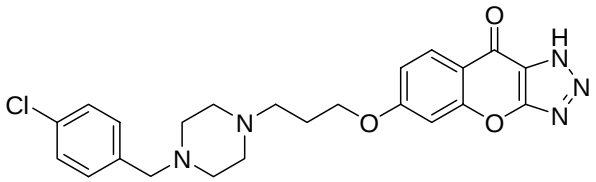
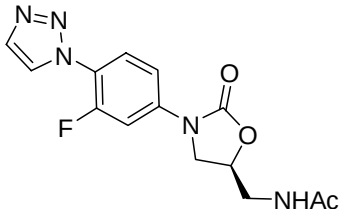
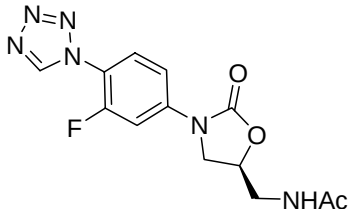


Figure 11: 103

Genin and coworkers[26] reported the synthesis of oxazolidinones containing 1,2,,3-triazole and tetrazole ring (Figure 12) and these possess antibacterial activity of against both gram positive and gram negative organisms including *S. aureus, H. influenzae, S. pneumoniae* etc.



104



105

Figure 12

Kalman and coworkers[11b] synthesized tetrazole isoster of antimetabolite agents methotrexate (**106**) and aminopterin (**107**) (Figure 13) and these have shown potent inhibition of growth of CCRF-CEM and K562 human leukemia cell lines.

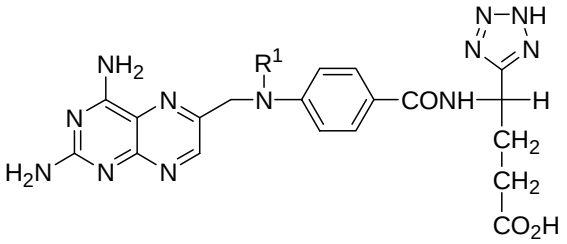


Figure 13: (106) Methotrexate R^1= Me

(107) Aminoptein R^1= H

Kolb and coworkers[27] discovered potent acetylcholine esterase inhibitors by in situ screening using acetylcholine esterase enzyme as a test system. The in situ products *syn*-**TZ2PA6 (108)** and *syn*-**TA2PZ5 (109)** (Figure 14) are the some examples derived from tacrine and phenylphenanthridinium acetylene and azide respectively.

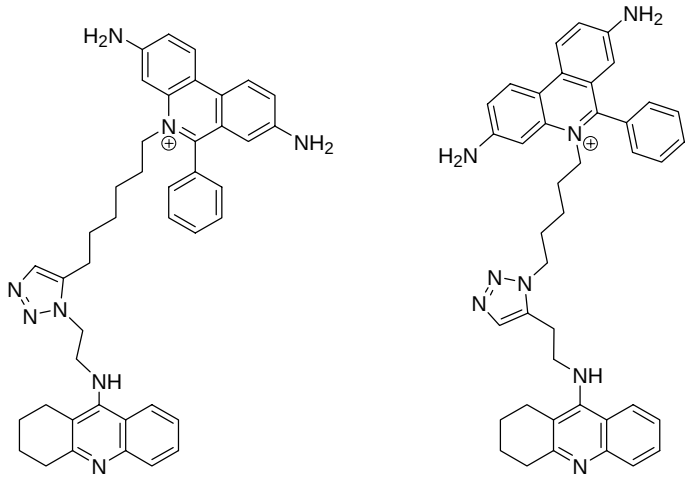


Figure 14: *syn*-**TZ2PA6** (**108**) and *syn*-**TA2PZ5** (**109**)

2.3. Diversity Oriented Synthesis of Small Molecules[28]

When we reveal the evolution of organic chemistry from the date Wohler's first laboratory synthesis of urea in 1828 and the synthesis of complex molecules such as vincristine and vinablastin by Kobayashi and the small complex structural features of most of the bioactive natural organic molecules, show the substantial importance of small complex molecules.

Small molecules can exert powerful effects on the functions of macromolecules that include living systems. They are useful having macromolecule perturbing properties. These are useful both as research tools for understanding life processes and as pharmacological agents for promoting and restoring health.

Structural complexity is very significant because many of the small molecules known to disrupt protein-protein interactions are complex natural products or natural product-like compounds. Many small molecules are used as probes to explore biology for ex. Cholchicine probe for tubulin, Spidamine used to study glutamate receptor function, Phorbal used to study a family of protein kinase etc. The potential for therapeutic development depends however not only upon structural complexity but also upon synthetic accessibility and scalability. To achieve this difficult balance it is required to develop innovative chemistry.

Diversity Oriented Synthesis (DOS):

The traditional drug discovery uses target oriented synthesis (TOS) approach that is to synthesize a single target molecule having known target protein. TOS is carried mainly by performing retrosynthetic analysis. Diversity oriented synthesis is a novel approach that is intended to synthesize effectively a collection of small molecules capable of perturbing any disease related biological pathway, leading eventually to the identification of therapeutic protein targets capable of being modulated by small molecules. DOS are used in efforts to identify simultaneously therapeutic protein targets (therapeutic validation) and their small molecule regulators that can modulate the function of these therapeutic targets (Chemical validation).

Employing diversity-oriented synthesis, one can achieve more structural complexity than in the traditional chemistry, by tackling compounds with multiple stereocenters and complex, natural product-like libraries. Preparation of structurally complex and diverse compounds results in a broader population of chemical space and facilitates effective probing of biological space. DOS in conjunction with combinatorial chemistry was considered as a tool for drug discovery compound

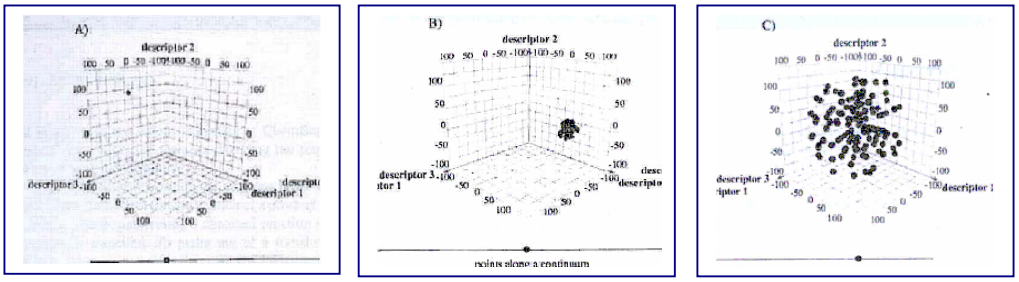
collections in order to populate new areas of the chemical space that was not covered by historical libraries.

Comparison of TOS (A), Medicinal & Combinatorial Chemistry (B), and DOS (C):

A. The aim of TOS is to synthesize a single target structure having known or predicted properties.

B. The goal in Medicinal & Combinatorial Chemistry is to synthesize a collection of analogues of a target structure having known or predicted properties.

C. The aim in DOS is to populate chemistry space broadly with complex and diverse structures having known properties as a first step in the small molecule discovery process (Figure 15a & 15b and Table 1).



A. TOS B. Med. & Comb. Chem. C. DOS

Figure 15a: Schematic of TOS and DOS

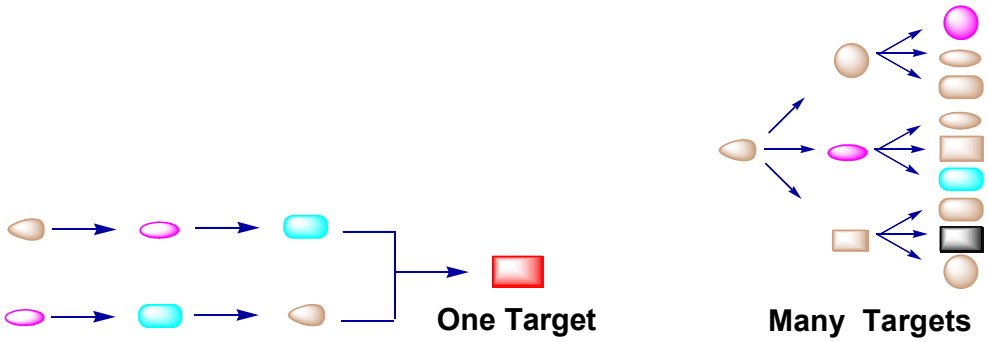


Figure 15b: Schematic of TOS and DOS

The planning of DOS to achieve diverse chemical libraries requires designing a series of products-equals-substrate relationships in the forward direction, i.e. pairs of reactions, in which the product of first diversity generating step become substrate for the second. In other words it is required to design complexity generating reactions at each step to achieve structural complexity and diversity.

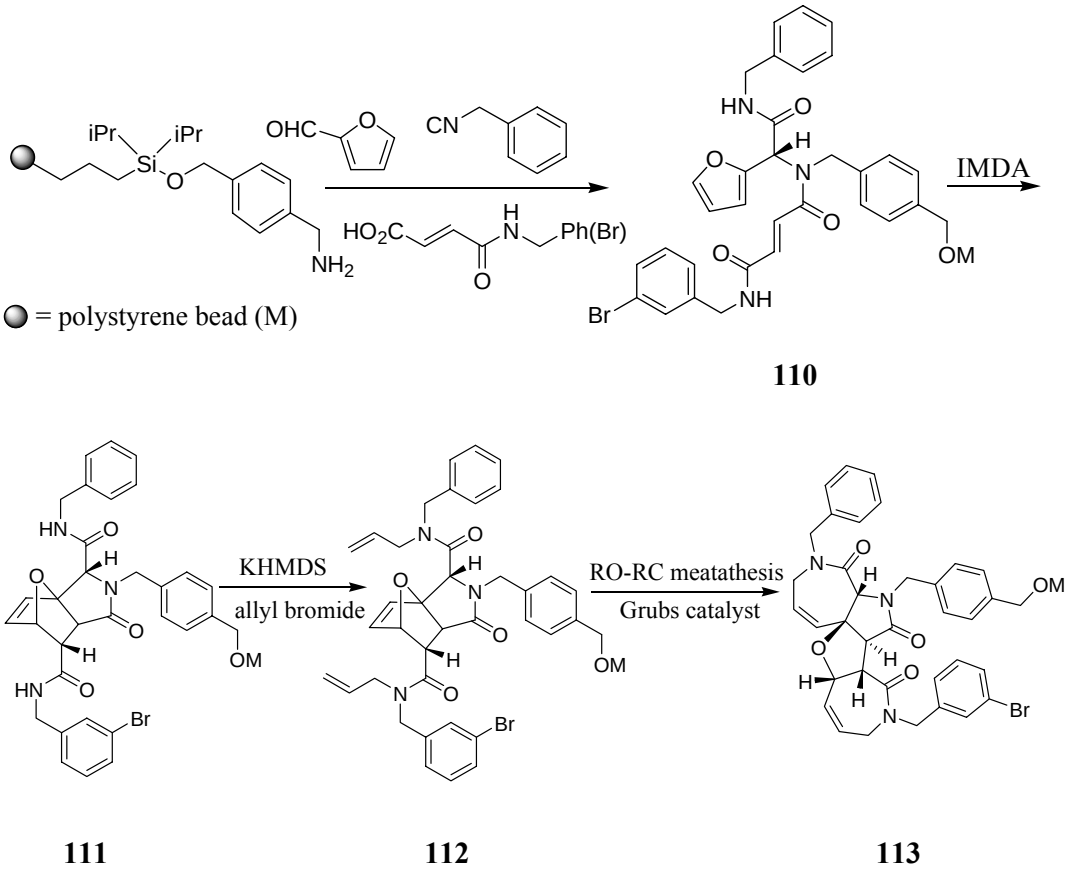
Target Oriented Synthesis (TOS)	**Diversity Oriented Synthesis (DOS)**
❖ Traditional method ❖ Convergent approach ❖ Retro synthetic approach (complex to simple) ❖ Fragment coupling reactions ❖ Linear ❖ Target is preselected protein	❖ New millennium method ❖ Divergent approach ❖ Forward synthetic approach (simple & similar to complex & divers) ❖ Diversity at every step ❖ Branching ❖ Not any particular target

Table1: Comparison of TOS & DOS

These kinds of reactions are also regarded as tandem reactions, for ex. Ugi 4CC reaction coupled with intramolecular Diels-Alder reaction or ring opening/ ring closing metathesis reactions. One can consider three different diversity elements while performing diversity oriented synthesis. These include; appendages, stereochemistry and skeletons. To depict the DOS by complexity generating reactions a simple example is summarized here.

In this example the first complexity generation step is the Ugi 4 component condensation reaction. The building blocks are selected in a manner that the resultant Ugi adduct should bear some reactive functionality so that it can be further diversified. Here in this reaction the Ugi adduct is suitable substrate for intramolecular Diels-Alder reaction, intern the Diels-Alder product undergo ring

opening and ring closing metathesis reaction simultaneously (Scheme 25). These set of reactions shows how one can achieve complexity and diversity at each step. If these reactions are planned combinatorially a library of complex and diverse small molecules can be reached.



110

111 **112** **113**

Scheme 25: Ugi 4 CC reaction/IMDA/RO-RC metasthesis

Chapter 2: Present Work

2.4. Present Work

Intense analysis of current status and development of antifungal agents, as well as in search of antifungals acting with novel and safer mode of action demands further research in this area. The growing number of immunosuppressed and immunocompromised patients, toxicity of clinically used drugs, development of resistance to existing drugs and emergence of newer pathogenic fungal strains are the major challenges. 1,2,3-triazole and 1,2,3,4-tetrazole bearing molecules possess range of therapeutic values including antimicrobial property. Thanks to advent of "Click Chemistry" which made lead discovery and lead optimization easier and faster particularly in azido-alkyne ligation processes. A modern approach, diversity oriented synthesis is very well complimented in the library generation of small complex molecules, so that therapeutic and chemical validation can be carried out efficiently.

In the area of 1,2,3-triazole and 1,2,3,4-tetrazole containing molecule development is mainly concentrated on intermolecular 1,3-dipolar cycloaddition reactions. A very few number of intramolecular 1,3-dipolar cycloadditions to 1,2,3-triazole and 1,2,3,4-tetrazole are revealed. The intramolecular cycloadditions can create very interesting and useful motifs such as small and complex fused polycyclic compounds.

Here in this chapter, an effort to synthesize 1,2,3-triazole and 1,2,3,4-tetrazole fused polycyclic compounds from carbohydrate templates is presented. Carbohydrates are exploited as very well known substrates with diverse chirality and are densely distributed in nature. With the application of "Click Chemistry" and utilizing the nature gifted carbohydrate templates, we planned to generate a library of chiral, oxygen rich, 1,2,3-triazole and 1,2,3,4-tetrazole fused tetracyclic class of compounds

via intramolecular 1,3-dipolar cycloaddition strategy following “Click Chemistry” conditions. Further we planned to diversify these tetracyclic motifs into glycosides and nucleosides which mimic like naturally occurring biologically active compounds. Nucleosides are aimed to mimic the structural requirements of naturally occurring peptidyl nucleosides,[29] which contain nucleobase and furanose skeletons in their molecular structures.

"Click Chemistry" Guided Diversity Oriented Synthesis Of 1,2,3-Triazole And 1,2,3,4-Tetrazole Fused Carbohydrate Derived Polycyclic Compounds

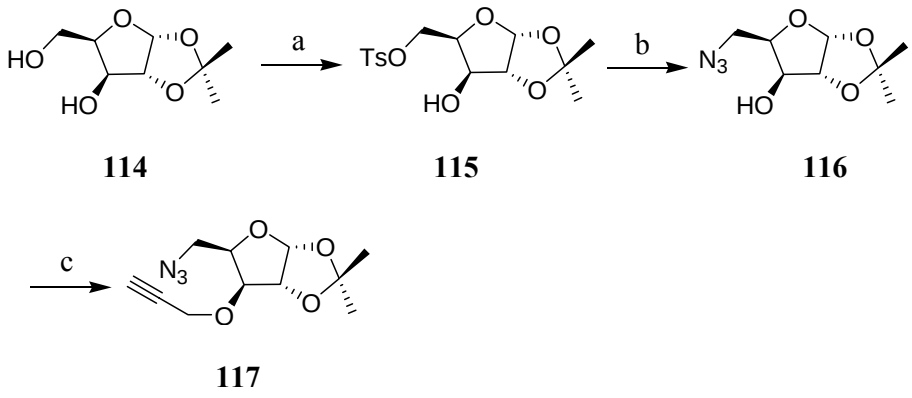
A. Synthesis Of 1,2,3-Triazole Fused Carbohydrate Derived Tetracyclic Compounds:

Carbohydrate derived 1,2,3-triazole tetracyclic compounds are synthesized from azido-alkyne substrates of respective pentoses and hexoses, by heating in toluene at 80 to 90 ^{o}C. In most of the cases the product was separated as colorless solid upon cooling to room temperature in excellent yields.

1. α-D-Xylofuranose derived 1,2,3-triazole fused tetracyclic compound:

In the early effort, we synthesized α-D-xylofuranose derived 1,2,3-triazole fused tetracyclic compounds (**118**). It involves synthesis of α-D-xylofuranose derived 5-*O*-tosylate (**115**) by treating 1,2-*O*-isopropylidene-α-D-xylofuranose[30] (**114**) with p-tosyl chloride in pyridine at 0 ^{o}C for 6 h in 90% yield. Xylofuranose tosylate (**115**) was transformed into 5-deoxyazidoxylofuranoside (**116**) by S_N2 displacement of tosyl group in the presence of NaN_3 in 93% yield. The azido substrate (**116**) showed characteristic stretching peak for azide group in IR spectrum at frequency 2104.19 cm^{-1}. Intern the azido-alkyne substrate for intramolecular click reaction was obtained by conversion of C-*3* hydroxyl group of azido compound (**116**) to propargyl ether (**117**) using sodium hydride and propargyl bromide in 88% yield (Scheme 1). The formation azido-alkyne was confirmed by IR and proton NMR. In the IR spectrum peaks at frequency 3282.62 cm^{-1} and 2102.26 cm^{-1} were observed corresponding to alkyne *C-H* and azide stretching respectively. The proton NMR showed resonances corresponding to alkyne *CH* at δ 2.48 ppm (t, J = 2.40 Hz) and anomeric proton at δ 5.89 (d, J = 3.79 Hz).

Scheme 1:



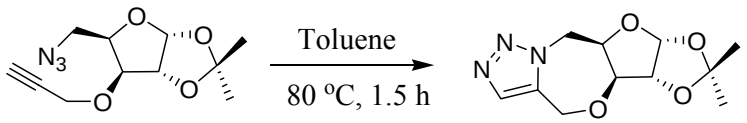
114 **115** **116**

117

Reagents: a) *p*-TsCl, Py., 0 ^{o}C - rt, 6 h, 90%; b) NaN_3, DMF, 90 ^{o}C, 8 h, 93%; c) NaH, Propargyl bromide, DMF, 0 ^{o}C - rt, 2 h, 88%.

The 1,3-dipolar cycloaddition reaction was carried out by heating azido-alkyne substrate in toluene to 80 ^{o}C for 1.5 h. It is interesting to note that the resultant tetracyclic 1,2,3-triazole (**118**) was separated as a white solid upon cooling to room temperature in quantitative yield (95%) (Scheme 2).

Scheme 2:



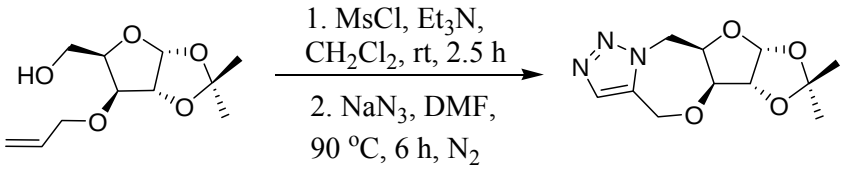
117 **118**

The high resolution proton NMR spectrum of 1,2,3-triazole fused tetracyclic compound (**118**) confirmed the presence of an olefinic proton of triazole ring at δ 7.54 ppm as a singlet and anomeric proton at δ 5.80 ppm (d, J = 3.67 Hz). In the ^{13}C NMR spectrum resonances characteristic of two olefinic carbons were observed at δ 134.98 and 131.93 ppm and rest of the spectrum is in complete agreement with that of the assigned structure.

During the progress of this endeavor we come across with the formation of 1,2,3-triazole compound (**118**) from 3-*O*-allyl ether of 1,2-*O*-isopropylidene-α-D-

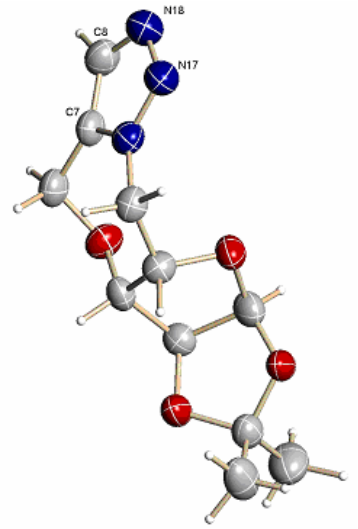
xylofuranose (**119**) reported by Tripati and coworkers.[31] They reported that, the reaction involves initial formation of triazoline from (**119**) which had a *trans* ring fusion and was therefore oxidized by atmospheric oxygen to compound (**118**) during the reaction (Scheme 3). However the spectral data of the triazole (**118**) derived by us from 3-*O*-propargyl ether (**117**) did not match with their data reported.

Scheme 3:



119 **118**

However our structure was confirmed by X-ray crystallographic studies without any ambiguity (Figure17). 1,2,3-triazole compound (**118**) was crystallized by slow evaporation in light petroleum (b.p. 60-80 °C) and dichloromethane. The crystals were subjected to X-ray analysis and proved the structural authenticity of tetracyclic triazole (**118**) beyond any ambiguity (Figure 17). The bond lengths between C_7,C_8 and N_{17},N_{18} were found to be 1.363 A° and 1.324 A° respectively, confirming the presence of double bonds in the fused 1,2,3-triazole moiety.



Crystal data: $C_{11}H_{15}N_3O_4$, M = 253.26, orthorhombic, space group $P2_12_12_1$, a = 5.495(1), b = 10.054(2), c = 21.330(4) *Å*, V = 1178.4(4) *Å*^{3}, Z = 4, D_{calc} = 1.428 g cm^{-1}, T = 298(2) K, μ = 0.110 mm^{-1}, *F(000)* = 536, λ = MoKα = 0.7107 Å, 5138 reflections measured, 1686 unique, observed with I > 2σ(I), final R_1 = 0.0283, wR_2 = 0.0306.

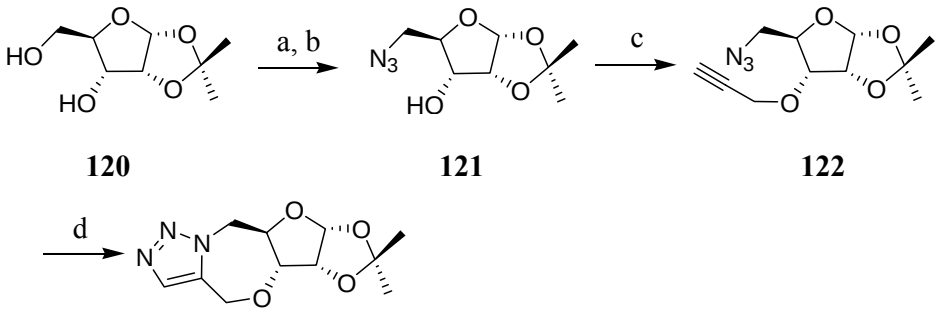
Figure 17: ORTEP diagram of compound **118**

The successful formation of tetracyclic 1,2,3-triazole (**118**) proved intramolecular 1,3-dipolar cycloaddition reaction to be a perfect example of "Click Chemistry" reactions. This prompted us to carryout the same protocol on other pentoses such as of *D-ribose* and *D-arabinose* derived azido alkynes (Scheme 4 and 5) so that to bring "stereochemical diversity" in the tetracyclic products.

2. α-D-Ribofuranose derived **1,2,3-triazole fused tetracyclic compound:**

In continuation of above stated protocol the ribofuranosyl diol (**120**)[32] was transformed into 1,2-*O*-isopropylidene-5-deoxyazido-α-D-ribofuranose (**121**) in 95% yield which intern to azido-alkyne substrate (**122**) in 91% yield in the similar manner as described in scheme 1. Finally the azido-alkyne was dissolved in toluene and heated to 90 ^{o}C to affect the 1,3-dipolar cycloaddition reaction to afford tetracyclic 1,2,3-triazole (Scheme 4, **123**) which was seen as solid upon cooling to room temperature in 90% yield.

Scheme 4:



120 **121** **122**

123

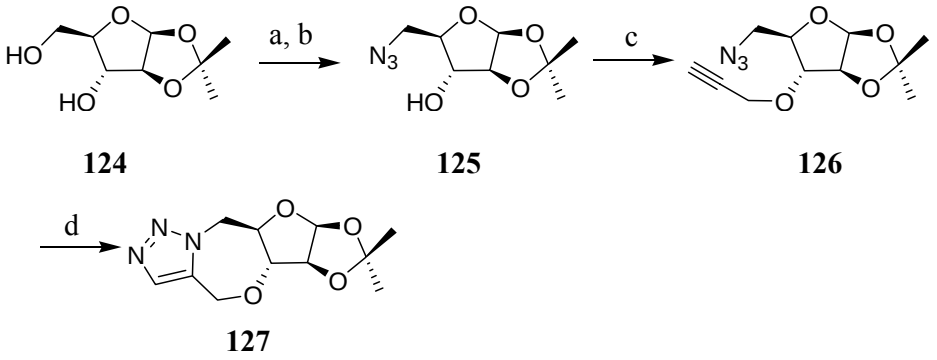
Reagents: a) *p*-TsCl, Py., 0 ^{o}C - rt, 6 h, 85%; b) NaN_3, DMF, 90 ^{o}C, 8 h, 92%; c) NaH, Propargyl bromide, DMF, 0 ^{o}C-rt, 2 h, 95%; d) Toluene, 80 ^{o}C, 2 h, 90%.

3. β-D-Arabinofuranose derived **1,2,3-triazole fused tetracyclic compound:**

In the similar manner arabinofuranosyl diol (**124**)[33] was also transformed into corresponding tetracyclic 1,2,3-triazole (Scheme 5, **127**), very successfully with the

separation of white solid in 92% yield by heating toluene solution of azido-alkyne precursor (**126**) over the period of 3.5 h.

Scheme 5:



124 **125** **126**

127

Reagents: a) *p*-TsCl, Py., 0 ^{o}C - rt, 6 h, 78%; b) NaN_3, DMF, 90 ^{o}C, 8 h, 96%; c) NaH, Propargyl bromide, DMF, 0 ^{o}C-rt, 2 h, 88%; d) Toluene, 90 ^{o}C, 3.5 h, 92%.

According to the crystallographic study and the use of different substrates, demonstrated the reliability of the protocol framed. Therefore above 1,3-dipolar cycloaddition transformations on pentofuranose azido-alkynes to tetracyclic 1,2,3-triazole in toluene was found to be very efficient and consistent, without employing any kind of catalysts or activators.

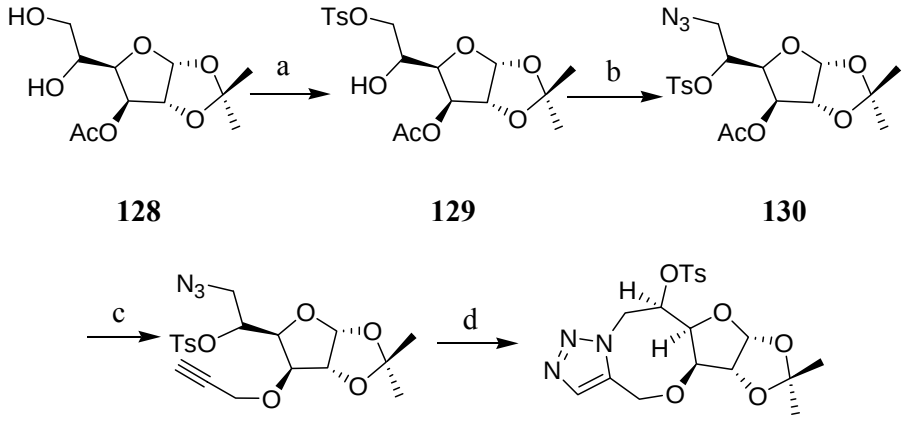
Enthusiastically we designed and extended the same protocol to hexoses such as *glucofuranose, allofuranose* and *glucopyranose* derived substrates so that the "skeletal diversity" can be achieved in the tetracyclic 1,2,3-triazoles, which can also enable the structure for further diversification.

4. α-D-Glucofuranose derived **1,2,3-triazole fused tetracyclic compounds:**

The primary alcohol of α-D-glucose derived compound (**128**)[34] was converted to corresponding p-toluenesulfonate (90% yield, **129**) using *p*-TsCl. The compound (**129**) was then treated with NaN_3 at 120 ^{o}C for 8 h and the C-5 hydroxyl group was protected with *p*-TsCl in pyridine at 100 ^{o}C to obtain the 5-*O*-tosyl-6-deoxyazido derivative (**130**) with 85% yield in two steps. Deprotection of 3-*O*-acetyl group by

sodium methoxide and subsequent treatment with NaH and propargyl bromide at 0 ^{o}C afforded the required 6-azido-3-*O*-propargyl compound (**131**). IR spectrum of compound (**131**) revealed the presence of peaks at 3298.05 cm^{-1} (alkyne *C-H* stretch), 2098.41 cm^{-1}, (azide stretch) and 1375cm^{-1} & 1178 cm^{-1} (S(=O)$_2$ stretch). In the proton NMR resonances of alkyne *C-H* at δ 2.49 ppm (t, *J* = 2.51 Hz), tosyl *CH$_3$* at δ 2.48 ppm (s) were observed. The azido-alkyne (**131**) was heated in toluene to 80 ^{o}C in toluene for 6 h to yield triazole fused tetracyclic compound (**132**) in 80% yield (Scheme 6). The proton NMR of the compound (**132**) showed the characteristic resonance of triazole ring olefinic proton at δ 7.47 ppm as a singlet, anomeric *CH* at δ 6.01 ppm (d, *J* = 3.79), tosyl *CH$_3$* at δ 2.49 as a singlet and in the ^{13}C NMR spectrum resonances characteristic of two olefinic carbons were observed at δ 130.15 ppm and 128.00 ppm. Remaining part of the spectrum is in agreement with the structure assigned.

Scheme 6:



128 **129** **130**

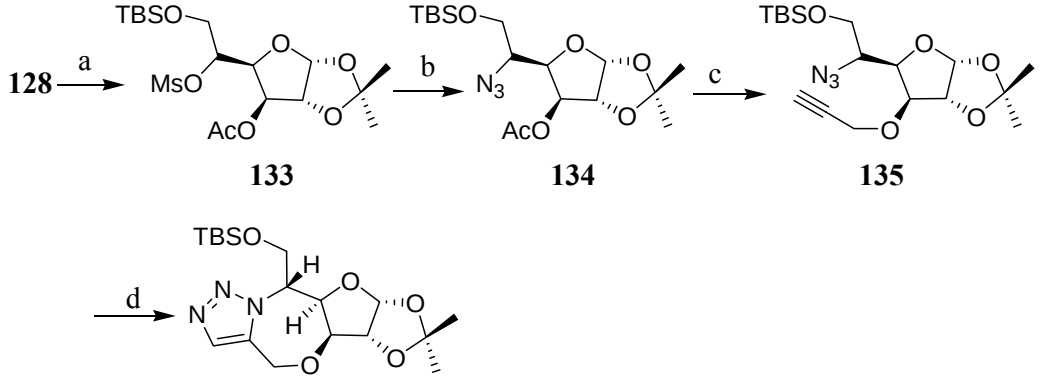
131 **132**

Reagents: a) *p* TsCl, Py., 0 ^{o}C - rt, 12 h, 90%; b) (i) NaN_3, DMF, 120 ^{o}C, 8 h; (ii) *p*-TsCl, Py., 100 ^{o}C, 6 h, 85% (in two steps); c) (i) NaOMe, MeOH, rt, 0.5 h; (ii) NaH, Propargyl bromide, DMF, 0^{o}C - rt, 2 h, 87% (in two steps); d) toluene, 90 ^{o}C, 6 h, 80%.

In another set of reactions with the intention to obtain skeletally different triazole from α-D-glucose, the secondary hydroxyl group of (**128**) was converted to the azido derivative (**134**) after protecting C-6 hydroxyl group of (**128**) with TBS group (Scheme 7). The azido compound (**134**) was subsequently transformed to 3-*O*-propargyl-5-deoxyazido derivative (**135**) after deacetylation, using the reagents as shown in the scheme and the same was also heated in toluene to get the triazole derivative (**136**) in 91% yield. The derived structure is completely in agreement with ^{1}H and ^{13}C NMR spectrum.

The consistency of the protocol designed for tetracyclic triazole was once again proved when hexoses were used. The continued success prompted to extend the procedure on different isomer. In the similar manner, the above protocol was continued using other isomer α-D-allose.

Scheme 7:



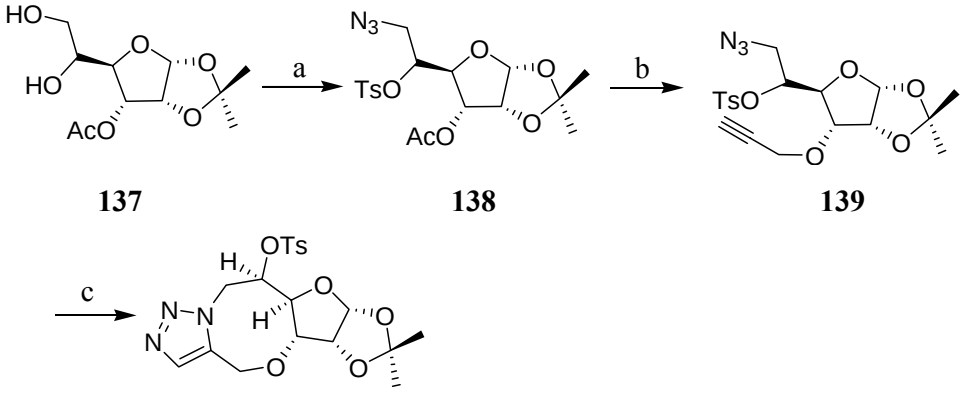
Reagents: a) (i) TBSCl, Im., DMF, 1 h, 84%; (ii) MsCl, Et_3N, DCM, 0 ^{o}C - rt, 5 h, 96%; b) NaN_3, DMF, 120 ^{o}C, 6 h, 82%; c) (i) NaOMe, MeOH, rt, 0.5 h; (ii) NaH, Propargyl Br, DMF, 0 ^{o}C - rt, 2 h, 75% (in two steps); d) toluene, 90 ^{o}C, 5 h, 90%.

5. α-D-Allofuranose derived **1,2,3-triazole fused tetracyclic compounds:**

The starting material α-D-allofuranose derivative (**137**) was prepared by known method.[35] α-D-allofuranose derivative (**137**) was transformed to 3-*O*-

propargyl-6-deoxyazido (**139**) and 3-*O*-propargyl-5-deoxyazido (**143**) derivatives using the reagents mentioned in scheme 8 and 9. These azido-alkynes (**139**) and (**143**) were finally heated in toluene to accomplish corresponding1,3-dipolar cycloaddition products i.e. tetracyclic triazoles in good yields (**140,** Scheme 8) & (**144,** Scheme 9).

Scheme 8:

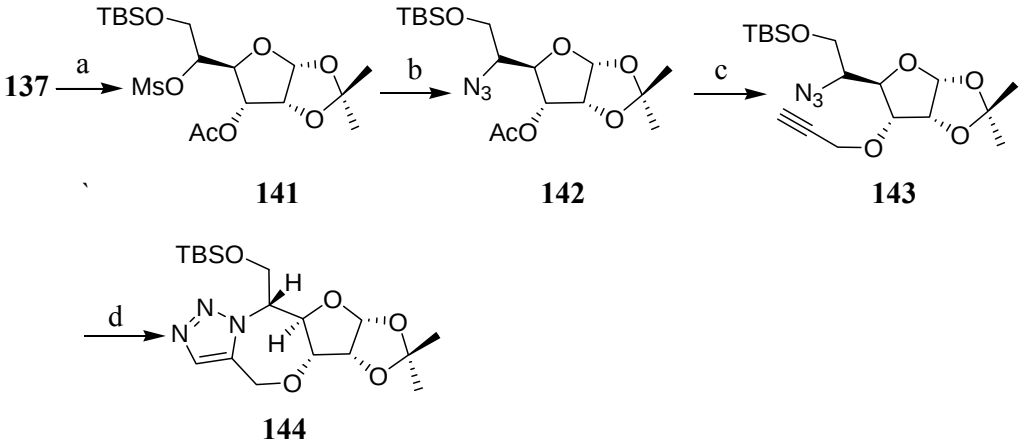


137 **138** **139**

140

Reagents: a) (i) *p* TsCl, Py., 0 ^{o}C - rt, 12 h, 90%; (ii) NaN_3, DMF, 120 ^{o}C, 8 h, 79%; (iii) *p*-TsCl, Py., 100 ^{o}C, 6 h, 70%; b) (i) NaOMe, MeOH, rt, 0.5 h; (ii) NaH, Propargyl bromide, DMF, 0^{o}C - rt, 2 h, 78%; c) toluene, 90 ^{o}C, 6.5 h, 92%.

Scheme 9:



141 **142** **143**

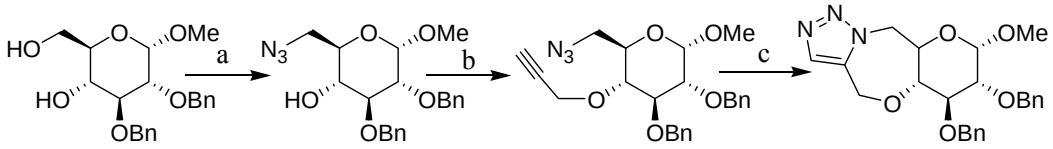
144

Reagents: a) (i) TBSCl, Im., DMF, 1 h; (ii) MsCl, Et_3N, DCM, 0 ^{o}C - rt, 5 h, 85% (in two steps); b) NaN_3, DMF, 120 ^{o}C, 5 h, 92%; c) (i) NaOMe, MeOH, rt, 0.5 h; (ii) NaH, Propargyl Br, DMF, 0 ^{o}C - rt, 2 h, 87%; d) toluene, 90 ^{o}C, 5 h, 85%.

6. Methyl α-D-Glucopyranose derived 1,2,3-triazole fused tetracyclic compound:

As an example of pyranose and to check the feasibility of the method, methyl α-D-glucopyranoside was also used to synthesize the 1,2,3-triazole, whereas in this case a tricyclic triazole was obtained. Employing the same protocol which was framed for xylofuranose derived triazole, the tricyclic triazole (**148**, Scheme 10) was accomplished excellently in 91% yield from methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside (**145**)[36].

Scheme 10:



145 **146** **147** **148**

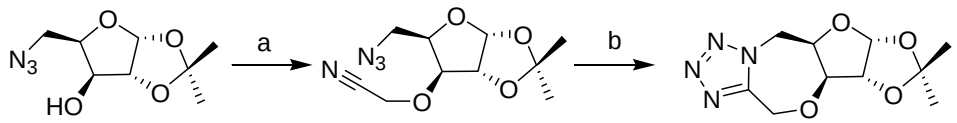
Reagents: a) (i) *p*-TsCl, Py., 0 ^{o}C - rt, 6 h; NaN_3, (ii) DMF, 90 ^{o}C, 8 h, 90% (in two steps); b) NaH, Propargyl bromide, DMF, 0 ^{o}C - rt, 2 h, 85%; c) Toluene, 90 ^{o}C, 3 h, 91%.

The formation of compound (**148**) was confirmed by both proton and ^{13}C NMR. Some important resonances observed in ^{1}H NMR are at δ 7.58 ppm (s, olefinic 1 H), δ 4.58 ppm (d, J = 3.54 Hz, anomeric 1 H) and δ 3.4 ppm (s, 3 H of OCH_3) and in case of ^{13}C are at δ 136.51 ppm and 132.74 ppm (two olefinic carbons), δ 98.47 ppm (anomeric carbon), δ 55.80 ppm (OCH_3).

B. Synthesis Of 1,2,3,4-Tetrazole Fused Carbohydrate Derived Polycyclic Compound (150):

In continuation of our successful endeavor in "Click Chemistry", we also report the synthesis of intramolecular 1,2,3,4-tetrazole fused tetracyclic compound from azido-nitrile derived from carbohydrate template.

Scheme 11:



116 **149** **150**

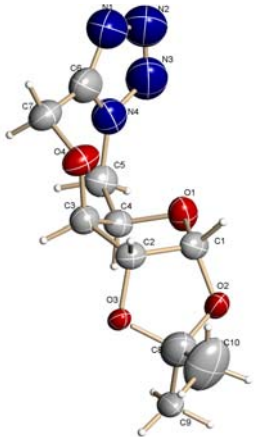
Reagents: a) NaH, Chloroacetonitrile, DMF, 0°C, 3 h, 60%; b) Toluene, reflux, 24h, 65%.

Accordingly, 5-deoxyazidoxylofuranoside (**116**) was treated with Sodium hydride and 5 equivalents of Chloroacetonitrile in DMF to afford required azido-nitrile (**149**) in 60% yield. The formation of compound (**149**) was confirmed by IR and NMR analysis. In the IR spectrum observed the characteristic peaks for nitrile and azido functions at frequencies 2254.63 cm^{-1} and 2104.19 cm^{-1} respectively. The proton NMR shows resonances at δ 5.93 ppm (d, 1 H, J = 3.79 Hz) for anomeric proton and δ 4.36 ppm (s, 2 H of O-***CH*$_2$**-CN) and in the ^{13}C spectrum resonances corresponding to nitrile carbon and nitrile methylene carbon appeared at δ 127.48 ppm and 55.47 ppm respectively. The resultant azido-nitrile was heated in toluene to reflux for 24 h, a ”Click” chemistry method to yield 1,2,3,4-tetrazole fused tetracyclic compound (**150**) (Scheme 11) which upon cooling to room temperature separated as colorless solid.

The ^{1}H NMR of tetracyclic tetrazole showed typically two doublets for methylene group attached to C-3 oxygen one at δ 5.22 ppm (d, 1 H, J = 15.66) and other at δ 4.91 ppm (d, 1 H, J = 15.79) and in the ^{13}C NMR lonely quaternary olefinic carbon of tetrazole ring is observed at δ 154.56 ppm. Rest of the spectrum is in accordance with the structure assigned.

Tetrazole (**150**) was crystallized from chloroform and subjected to X-ray crystallographic studies. The bond lengths between N_1, C6, and N_2, N_3 were found to

be 1.316 A^{o} and 1.292 A^{o} respectively, confirming the presence of double bonds in the fused 1,2,3,4-tetrazole moiety.



Crystal Data: $C_{10}H_{14}N_4O_4$, M = 254.25. Crystals belong to orthorhombic, space group $P2_12_12_1$, a = 5.6672(5), b = 9.8214(9), c = 21.2188(19) Å, V = 1181.03(18) Å^3, Z = 4, D_c = 1.430 mg m^{-3}, μ (Mo–K$_{\square}$) = 0.112mm^{-1}, 5801 reflections measured, 2080 unique [I>2σ(I)], R value 0.0406, wR2 = 0.0900. Largest diff. peak and hole 0.144 and -0.148 e. Å $^{-3}$.

Figure 18: ORTEP diagram of Compound **150**

C. Diversity Oriented Synthesis:

After keen analysis of the structural features of tetracyclic triazole and tetrazole, the molecules exhibited number of facile sites for structural diversification. These sites can facilitate synthesis of a library of small and complex bioactive molecules to explore the lead molecule. Figure 19 depicts the possible sites or points for diversification of tetracyclic triazole and tetrazole.

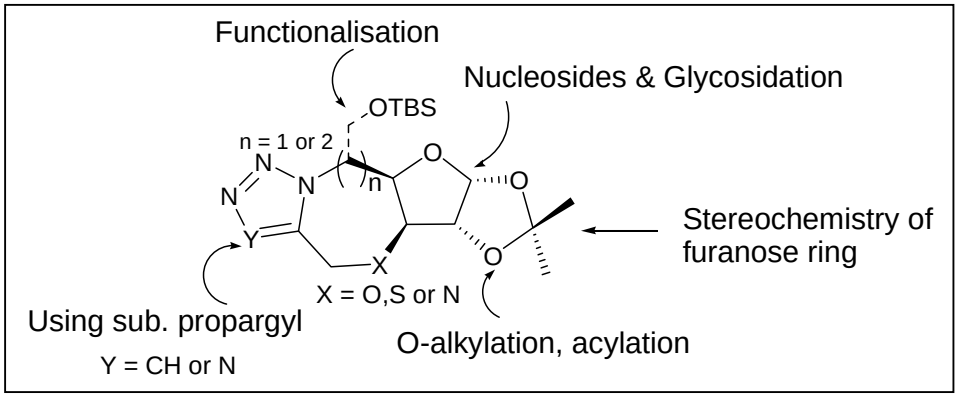


Figure 19: Points of diversification

In the view of diversity oriented synthesis of 1,2,3-Triazoles and 1,2,3,4-Tetrazole, in one of the effort, we planed to transform these molecules into

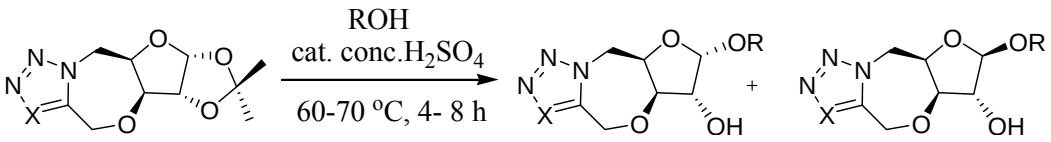
nucleosides and glycosides. Our attempt worked very well in transforming triazole and tetrazole into diverse molecules. To set an example to demonstrate the possibilities of diversity oriented synthesis on triazole and tetrazole molecules we have selected α-D-xylofuranose derived 1,2,3-triazole (**118**) and 1,2,3,4-tetrazole (**150**) molecules as a model.

1. Synthesis of 1,2,3-Triazole / 1,2,3,4-Tetrazole Fused Tricyclic Glycosides by Fischer's method:

The glycosidation of α-D-Xylofuranose derived 1,2,3-Triazole (**118**) / 1,2,3,4-tetrazole (**150**) fused polycyclic compounds were carried out intentionally by Fischer glycosidation, so that to generate stereochemically diverse glycosides. The glycosidation was performed by using alcohols such as isopropyl alcohol, allyl alcohol and homopropargyl alcohol (Scheme 12, Table 2).

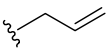
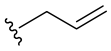
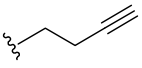
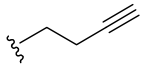
The glycosidaton was carried out by heating the 1,2,3-Triazole (**118**) / 1,2,3,4-tetrazole (**150**)substrates in either neat alcohol (in case of 2-propanol) or using two equivalent of alcohol (in case of allyl alcohol and propargyl alcohol) in 1,4-dioxane as solvent at 60-70 ^{o}C for 4 to 8 hours. Simple aqueous work up and silica gel column chromatography of the mixture by gradient elution using mixture of ethyl acetate and petroleum ether, accomplished α- and β-glycosides of tricyclic triazole and tetrazole compounds in excellent yields.

Scheme 12:



118. X = CH, **150,** X = N α **:** β **-**glycosides

Table 2

	R	X	Time (h)	Yield (%)	α : β [a]		R	X	Time (h)	Yield (%)	α : β [a]
151.		CH	6	85	1 : 2	**154.**		N	4	90	2 : 3
152.		CH	8	75	2 : 3	**155.**		N	5	93	1 : 2
153.		CH	8	80	1 : 2	**156.**		N	6	95	1 : 3

a = isolated ratios

Isopropyl glycoside of 1,2,3-Triazole tricyclic compound (151):

The 1,2,3-triazole tricyclic α- and β-glycosides were obtained with 85% yield in the ratio of 1 : 2 respectively. The proton NMR of α-glycoside showed resonance of anomeric proton at δ 5.07 ppm (d, 1 H, *J* = 3.79 Hz) and two doublets for isopropyl methyl- groups at δ 1.11 ppm (d, 3 H, *J* = 6.06 Hz) and 1.16 ppm (d, 3 H, *J* = 6.32 Hz). In the ^{13}C NMR spectrum the olefinic carbons resonances appeared at δ 134.12 ppm and 130.58 ppm and the anomeric carbon at δ 99.32 ppm.

Whereas the proton NMR of β-glycoside showed resonance of anomeric proton at δ 5.02 ppm (d, 1 H, *J* = 0.76 Hz) and two doublets for isopropyl methyl groups at δ 1.15 ppm (d, 3 H, *J* = 6.32 Hz) and δ 1.12 ppm (d, 3 H, *J* = 6.31 Hz). In the ^{13}C NMR spectrum the olefinic carbons resonances appeared at δ 134.10 ppm and 130.16 ppm and the anomeric carbon at δ 106.52 ppm.

Isopropyl glycoside of 1,2,3,4-tetrazole tricyclic compound (154):

Similarly, the 1,2,3,4-tetrazole tricyclic α- and β-glycosides were obtained in the ratio of 2 : 3 respectively. The proton NMR of α-glycoside showed resonance of anomeric proton at δ 5.15 ppm (d, 1 H, *J* = 3.91 Hz) and two doublets for isopropyl methyl groups at δ 1.24 ppm (d, 3 H, *J* = 6.32 Hz) and δ 1.20 ppm (d, 3 H, *J* = 6.19 Hz). In the ^{13}C NMR spectrum the olefinic carbons resonances appeared at δ 152.72 ppm and the anomeric carbon at δ 99.22 ppm.

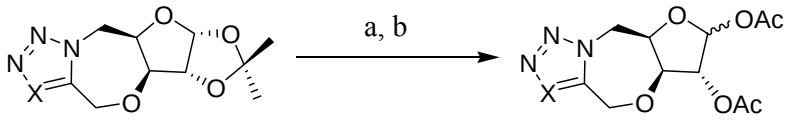
Whereas the proton NMR of β-glycoside showed resonance of anomeric proton at δ 5.10 ppm (d, 1 H, J = 0.76 Hz) and for isopropyl methyl groups at δ 1.18 ppm (m, 6 H). In the ^{13}C NMR spectrum the olefinic carbons resonances appeared at δ 152.71 ppm and the anomeric carbon at δ 106.53 ppm.

2. Synthesis of 1,2,3-Triazole / 1,2,3,4-Tetrazole Fused Tricyclic Nucleosides by Vorbruggen's One Pot Method:

The synthesis of nucleosides was intended to bring structural feature of peptidyl nucleoside antifungals into triazole and tetrazole fused polycyclic compounds. We anticipated designing novel analogs of peptidyl nucleosides which are known to be safer antifungals.[29]

The synthesis involves the preparation of the substrate, 1,2-*O*-diacetate of triazole and tetrazole fused polycyclic compounds (**157** & **158**). Diacetate derivatives were obtained by deprotection of 1,2-*O*-isopropylidine group of (**118** / **150**) using catalytic amount of sulfuric acid and heating at 60 °C for 24 h in 1,4-dioxane-water (3:2) mixture to yield 1,2-dihydroxy compound which was subsequently acetylated by treating with acetic anhydride in pyridine at 0 °C to accomplish diacetate (Scheme 13, **157** / **158**) in a mixture of α- and β-anomers.

Scheme 13:



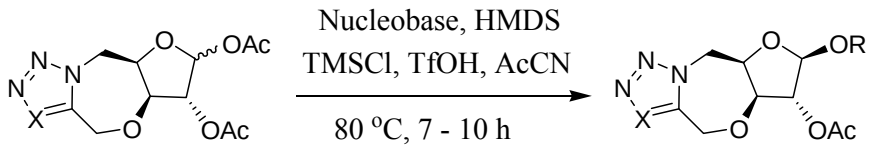
118. X = CH, **150,** X = N **157.** X = CH, **158,** X = N

Reagents: a) cat. H_2SO_4, Dioxane-Water (3:2), 60 °C, 24 h; b) Ac_2O, Py, 0 °C – rt, 5 h, (X = CH, 75%; X = N, 70% in two steps).

The substrate, diacetate in α,β-mixture (**157** / **158**) was concomitantly subjected to Vorbruggen's[37] one pot method to obtain nucleosides (Scheme 14). The

nucleosides were synthesized by using uracil, thymine, N^6-benzoyl adenine and 6-chloroguanine as nucleobase.

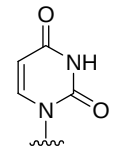
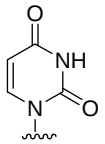
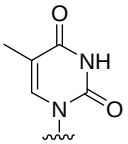
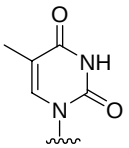
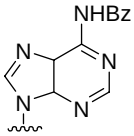
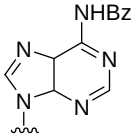
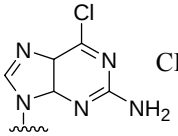
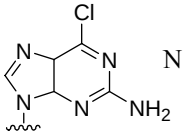
Scheme 14:



157. X = CH, **158,** X = N

β-Nucleosides

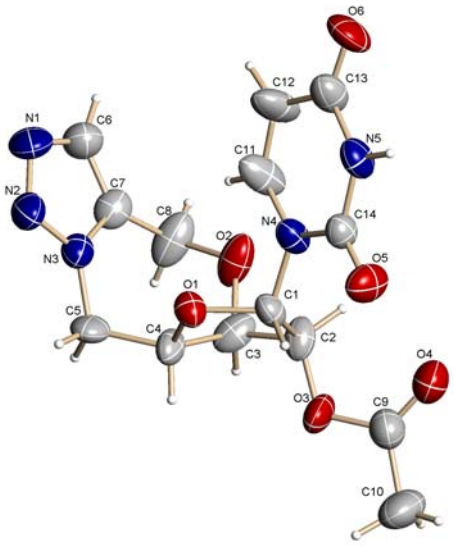
Table 3

	R	X	Time (h)	Yield (%)		R	X	Time (h)	Yield (%)
159.		CH	8	45	**163.**		N	8	35
160.		CH	10	40	**164.**		N	8	45
161.		CH	8	50	**165.**		N	10	45
162.		CH	8	35	**166.**		N	7	38

The method involves treatment of the solution of diacetate (mixture) and nucleobase in dry acetonitrile with the addition of HMDS, TMSCl and TfOH at room temperature. The mixture was refluxed at 80-90 ^oC for 7-10 h after stirring at room temperature for 30 min. After workup and chromatographic purification accomplished nucleoside, exclusively with β-configuration in 30 – 50% yield (Table 3).

Nucleoside (**159**) is the uracil nucleoside of tricyclic 1,2,3-triazole in 40% yield. The formation of nucleoside was proved by its proton and ^{13}C NMR, the resonances in pNMR spectrum are at δ 11.02 ppm (s, 1H, -NH), δ 8.01 ppm (s, 1H,

triazole proton), olefinic protons of uracil at δ 7.04 ppm (d, 1H J = 8.09 Hz) and δ 5.80 ppm (d, 1H J = 8. 21 Hz), anomeric proton at δ 6.30 ppm (d, 1H, J = 1.26 Hz) and the ^{13}C spectrum showed resonances of triazole olefinic carbons at δ 134.58 ppm and 131.15 ppm, anomeric carbon 86.53 ppm, acetate CH_3 carbon at δ 19.41ppm. The rest of the spectrum is in full agreement with the structure assigned.



Crystal Data: $C_{14}H_{15}N_5O_6$, M = 349.31. Crystals belong to orthorhombic, space group $P2_12_12_1$, a = 5.9329(8), b = 14.5318(19), c = 18.898(3) Å, V = 1629.3(4) $Å^3$, Z = 4, D_c = 1.424 mg m^{-3}, μ (Mo–K□) = 0.114mm^{-1}, 8097 reflections measured, 2861unique [I>2σ(I)], R value 0.0607, wR2 = 0.1060. Largest diff. peak and hole 0.189 and -0.179 e. $Å^{-3}$.

Figure 20: ORTEP diagram of nucleoside **159**

Further the nucleoside (**159**) was crystallized by slow evaporation of acetonitrile solution and was subjected to X-ray crystallographic studies. According to the X-ray analysis the configuration at anomeric carbon (C_1) is found to be **β-** configuration**.** The bond lengths between C_6,C_7 and N_1,N_2 were respectively found to be 1.364 A^o and 1.332 A^o confirms the presence of double bonds in the fused 1,2,3-triazole moiety. Figure 20 shows the ORTEP diagram of the nucleoside derived from 1,2,3-triazole tetracyclic compound and uracil.

Nucleosides (**164**), a thymine nucleoside of tricyclic tetrazole also had β–configuration. The proton NMR spectrum showed the resonances responsible for thymine NH at δ 11.16 ppm (s, 1H), anomeric proton at δ 6.73 (d, 1H, J = 1.01 Hz), 2'-O-acetate at δ 2.14 ppm (s, 3H), thymine CH_3 at δ 1.73 ppm (s, 3H), etc. The ^{13}C

NMR spectrum showed resonances corresponding to tetrazole ring olefinic carbon at δ 155.51 ppm, anomeric carbon at δ 89.09 ppm acetate CH_3 at δ 20.75 ppm and thymine CH_3 at δ 12.64 ppm, etc. Rest of the spectrum is in agreement with structure assigned.

2.5. Conclusion

In conclusion, we have demonstrated the application of "Click Chemistry" in designing polycyclic molecules, employing intramolecular 1,3-dipolar azido-alkyne cycloaddition reactions. The attempt to exploit carbohydrate substrates as templates to develop strategy in obtaining stereochemically diverse 1,2,3-triazole and 1,2,3,4-tetrazole fused polycyclic compounds was very fruitful. The strategy resulted in a series of chiral, oxygen rich, fused tetracyclic molecules (**118, 123, 127, 132, 136, 140, 144, 148** and **150**) from diverse pentoses and hexoses. The X-ray crystallographic study of compound (**118** and **150**) proved the assigned structures without ambiguity. Hence the strategy found to be very much consistent.

In the next endeavor after well equipped strategy for 1,2,3-triazole and 1,2,3,4-tetrazole fused polycyclic compounds, we have carried out the diversity oriented synthesis of glycosides and nucleosides from xylofuranose derived 1,2,3-triazole and 1,2,3,4-tetrazole fused polycyclic compounds. We have reported the facile route to tricyclic glycosides fused to triazole and tetrazole rings (**151**–**156**). Fischer glycosidation generated a small library of glycosides having stereochemical diversity. In the similar manner tetracyclic compounds were diversified in to tricyclic nucleosides fused to triazole and tetrazole rings that mimic peptidyl nucleoside structural features. Compounds (**159**–**166**) are the β-nucleosides resulted from xylofuranose derived 1,2,3-triazole and 1,2,3,4-tetrazole fused polycyclic compounds via Vorbruggens one pot method. Thus the DOS was very efficient in producing diverse chemical structures which are thought to be natural mimics. All the new molecules were subjected to evaluation of antifungal activity and are described in chapter 4 in detail.

Chapter 2: Experimental

A. Synthesis Of 1,2,3-Triazole Fused Tetracyclic Compounds Derived From Carbohydrates (Pentoses And Hexoses):

General procedure for 1,2-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-α-D-pentofuranose and methyl 2,3-di-*O*-benzyl-6-*O*-*p*-toluenesulfonyl-α-D-glucopyranoside:

To a solution of 1,2-*O*-isopropylidene-α-D-pentofuranose / methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside (5 mmol) in 10 ml of dry pyridine at 0 ^{o}C was added *p*-toluenesulfonyl chloride (5.5 mmol) under argon atmosphere. The reaction mixture was allowed to stir for 6 hours. After completion of the reaction monitored by TLC, the pyridine solution was concentrated in vacuo by forming pyridine-toluene azeotrope. The residue was dissolved in 150 ml of DCM and washed sequentially with 2 x 25 ml of dilute HCl, 25 ml of water, 25 ml of brine solution, then the organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield crude product. The crude tosylate was purified by silica gel column chromatography using ethyl acetate-petroleum ether mixture as eluent.

Compound No. 115: 1,2-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-α-D-xylofuranose:

Colorless solid: **m.p.:** 106 ^{o}C.

$[\alpha]_D^{25}$= -7.84 (c, 1.02, $CHCl_3$).

IR (cm^{-1}): 3423.41, 2989.46, 2935.46, 1598.88, 1359.72, 1176.5.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.30, 1.46 (2s, 6 H), 2.14 (s, 1H), 2.46 (s, 3H), 4.13 (d, 1 H, *J* = 4.68 Hz), 4.30-4.36 (m, 3 H), 4.51 (d, 1 H, *J* = 3.54 Hz), 5.88 (d, 1 H, *J* = 3.66 Hz), 7.36 (d, 2 H, *J* = 7.96 Hz). 7.81 (d, 2H, *J* = 8.33 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 21.65, 26.18, 26.75, 66.99, 74.27, 77.78, 85.07, 104.99, 112.04, 128.02, 130.01, 132.31, 145.26.

CHNS Anal.: Calculated for $C_{15}H_{20}O_7S$: C, 52.31; H, 5.85; S, 9.31.

Found. C, 52.29; H, 5.80; S, 9.35.

MS: m/z = 367.32 ($M + Na^+$).

General procedure for 1,2-*O*-isopropylidene-5-azido-5-deoxy-α-D-pentofuranose and methyl 2,3-di-*O*-benzyl-6-azido-6-deoxy-α-D-glucopyranoside:

To a solution of 1,2-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-α-D-pentofuranose/methyl 2,3-di-*O*-benzyl-6-*O*-*p*-toluenesulfonyl-α-D-glucopyranoside (4 mmol) in 15 ml of dry DMF was added sodium azide (20 mmol). The reaction mixture was stirred at 90 ^oC for 8 hours under argon atmosphere. After completion of the reaction monitored by TLC, was cooled and diluted with 250 ml of ethyl acetate and it was washed sequentially with 4 x 25 ml of water, 25 ml of brine solution and the aqueous extracts were extracted with another 25 ml of ethyl acetate, then the pooled organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo to yield crude product. The crude azido product was purified by silica gel column chromatography using ethyl acetate-petroleum ether mixture as eluent.

<u>Compound No. 116</u>: 1,2-*O*-isopropylidene-5-azido-5-deoxy-α-D-xylofuranose:

Colorless solid: **m.p.:** 60.5 ^oC.

$[\alpha]_D^{25}$= -35.18 (c, 1.08, $CHCl_3$).

IR (cm^{-1}): 3460.06, 2987.53, 2937.28, 2104.19, 1377.08.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.30, 1.48 (2s, 6 H), 2.82 (bs, 1H), 3.57 (s, 1 H), 3.60 (d, 1 H, *J* = 0.88 Hz), 4.21-4.30 (m, 2 H), 4.50 (d, 1 H, *J* = 3.66 Hz), 5.93 (d, 1 H, *J* = 3.66 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 26.05, 26.60, 49.05, 74.87, 78.40, 85.22, 104.68, 111.90.

CHNS Anal.: Calculated for $C_8H_{13}N_3O_4$: C, 44.65; H, 6.09; N, 19.53.

Found. C, 44.58; H, 6.07; N, 19.45.

MS: m/z = 215.11 (M^+).

<u>Compound No. 121</u>: 1,2-*O*-isopropylidene-5-azido-5-deoxy-α-D-ribofuranose:

Viscous liquid.

$[\alpha]_D^{25}$= +61.76 (c, 1.318, $CHCl_3$).

IR (cm^{-1}): 3461.99, 2987.53, 2937.38, 2104.19, 1375.15.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.38, 1.57 (2s, 6 H), 2.24 (bs, 1H), 3.35-3.44 (m, 1 H), 3.71 (dd, 1 H, *J* = 1.26, 12.88 Hz), 3.90-3.93 (m, 2H), 4.59 (t, 1H, *J* = 3.92), 5.85 (d, 1 H, *J* = 3.79 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 26.50, 26.54, 50.76, 72.16, 78.45, 79.41, 104.03, 112.95.

CHNS Anal.: Calculated for $C_8H_{13}N_3O_4$: C, 44.65; H, 6.09; N, 19.53.

Found. C, 44.70; H, 6.15; N, 19.49.

MS: m/z = 238.05 ($M+Na^+$).

<u>Compound No. 125</u>: 1,2-*O*-isopropylidene-5-azido-5-deoxy-β-D-arabinofuranose:

Viscous liquid.

$[\alpha]_D^{25}$= +65.75 (c, 1.165, $CHCl_3$).

IR (cm^{-1}): 3448.49, 2989.46, 2939.31, 2102.26, 1377.08.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.34, 1.55 (2s, 6 H), 3.43 (dd, 1 H, *J* = 6.32, 12.63 Hz), 3.62 (dd, 1 H, *J* = 6.95, 12.64 Hz), 4.06-4.14 (m, 1H), 4.23 (d, 1H, *J* = 2.15), 4.57 (d, 1H, *J* = 3.92), 5.95 (d, 1 H, *J* = 3.92 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 26.03, 26.91, 52.23, 76.43, 85.97, 86.89, 105.84, 112.97.

CHNS Anal.: Calculated for $C_8H_{13}N_3O_4$: C, 44.65; H, 6.09; N, 19.53.

Found. C, 44.59; H, 6.02; N, 19.47.

MS: m/z = 238.02 ($M+Na^+$).

<u>Compound No. 146</u>: Methyl 2,3-di-*O*-benzyl-6-azido-6-deoxy-α-D-glucopyranoside:

Viscous liquid.

$[\alpha]_D^{25}$= +22.77 (c, 1.01, $CHCl_3$).

IR (cm^{-1}): 3481.27, 3029.96, 2921.95, 2100.34, 1496.66, 1454.23, 1361.65.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.95 (bs, 1H), 3.41 (s, 3 H), 3.34-3.44 (m, 2 H), 3.46 (t, 1 H, *J* = 1.39 Hz), 3.53 (dd, 1 H, *J* = 3.53, 9.47 Hz), 3.68-3.80 (m, 2 H), 4.64 (d, 2 H, *J* = 3.03 Hz), 4.69 (d, 1 H, *J* = 2.52 Hz), 4.77 (d, 1 H, *J* = 12 Hz), 5.04 (d, 1 H, *J* = 11.62 Hz), 7.29-7.39 (m, 10 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 51.51, 55.38, 70.27, 70.64, 73.10, 75.36, 79.77, 81.07, 98.06, 127.97-128.66, 137.88, 138.60.

CHNS Anal.: Calculated for $C_{21}H_{25}N_3O_5$: C, 63.14; H, 6.31; N, 10.52.

Found: C, 62.85; H, 6.39; N, 10.60.

MS: m/z = 422.12 ($M+Na^+$).

General procedure for 1,2-*O*-isopropylidene-3-*O*-propargyl-5-azido-5-deoxy-α-D-pento furanose/methyl 2,3-di-*O*-benzyl-4-*O*-propargyl-6-azido-6-deoxy-α-D-gluco pyranoside:

To a solution of 1,2-*O*-isopropylidene-5-azido-5-deoxy-α-D-pentofuranose / methyl 2,3-di-*O*-benzyl-6-azido-6-deoxy-α-D-glucopyranoside (2 mmol) in 10 ml of dry DMF was added sodium hydride (3 mmol of 60% NaH in paraffin) under argon atmosphere at 0 ^oC. The reaction mixture was stirred for 30 min at room temperature and the resultant dark red solution was added propargyl bromide (0.24 ml, 2.6 mmol) at 0 ^oC. The reaction mixture was stirred for another 2 hours monitored by TLC and the reaction mixture was cooled and quenched with 5 ml of methanol, diluted with 100 ml of ether and washed sequentially with 3 x 25 ml of water, 25 ml of brine solution and the aqueous extracts were extracted with another 25 ml of ether, then the pooled ether layers were dried over anhydrous Na_2SO_4 and concentrated under

reduced pressure to yield crude product. The crude azido product was purified by silica gel column chromatography using ethyl acetate-petroleum ether mixture as eluent to yield 1,2-*O*-isopropylidene-3-*O*-propargyl-5-azido-5-deoxy-α-D-pento-furanose.

Compound No. 117: 1,2-*O*-isopropylidene-3-*O*-propargyl-5-azido-5-deoxy-α-D-xylofuranose:

Viscous liquid.

$[\alpha]_D^{25}$= -44.32 (c, 1.038, $CHCl_3$).

IR (cm^{-1}): 3282.62, 2967.53, 2937.38, 2102.26, 1450.37, 1375.15.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.30, 1.48 (2s, 6 H), 2.48 (t, 1H, *J* = 2.40 Hz), 3.51 (dt, 2 H, *J* = 6.77, 12.65 Hz), 4.07 (d, 1 H, *J* = 3.29 Hz), 4.22 (t, 2 H, *J* = 2.91 Hz), 4.26-4.34 (m, 1H), 4.62 (d, 1H, *J* = 3.79 Hz), 5.89 (d, 1 H, *J* = 3.79 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 26.27, 26.80, 49.26, 52.46, 75.47, 78.70, 78.77, 81.30, 82.05, 105.10, 112.02.

CHNS Anal.: Calculated for $C_{11}H_{15}N_3O_4$: C, 52.17; H, 5.97; N, 16.59.

Found. C, 52.20; H, 6.08; N, 16.52.

MS: m/z = 254.67 (M+1).

Compound No. 122: 1,2-*O*-isopropylidene-3-*O*-propargyl-5-azido-5-deoxy-α-D-ribofuranose:

Colorless solid: **m.p.:** 74 ^oC.

$[\alpha]_D^{25}$= -181.73 (c, 1.5, $CHCl_3$).

IR (cm^{-1}): 3301.91, 2989.46, 2935.46, 2104.19, 1448.44, 1375.15.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.37, 1.58 (2s, 6 H), 2.55 (t, 1H, *J* = 2.27 Hz), 3.36 (dd, 1 H, *J* = 3.92, 13.52 Hz), 3.73 (dd, 1 H, *J* = 2.40, 13.52 Hz), 4.02 (dd, 1 H, *J* = 4.16, 8.96), 4.13-4.22 (m, 1H), 4.31 (dd, 2H, *J* = 2.28, 8.47 Hz), 4.71 (t, 1H, *J* = 3.79 Hz) 5.82 (d, 1 H, *J* = 3.53 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 26.49, 26.77, 50.53, 57.69, 75.77, 77.06, 77.25, 77.41, 78.82, 104.17, 113.39.

CHNS Anal.: Calculated for $C_{11}H_{15}N_3O_4$: C, 52.17; H, 5.97; N, 16.59.

Found. C, 52.13; H, 5.90; N, 16.54.

MS: m/z = 253.36 (M^+).

<u>Compound No. 126</u>: 1,2-*O*-isopropylidene-3-*O*-propargyl-5-azido-5-deoxy-β-D-arabinofuranose:

Viscous liquid.

$[\alpha]_D^{25}$= +70.39 (c, 1.165, $CHCl_3$).

IR (cm^{-1}): 3288.40, 2939.31, 2866.02, 2102.26, 1375.15.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.35, 1.57 (2s, 6 H), 2.51 (t, 1 H, *J* = 2.40 Hz), 3.44 (dd, 1 H, *J* = 6.06, 12.88 Hz), 3.60 (dd, 1 H, *J* = 6.45, 12.76 Hz), 4.08-4.18 (m, 2 H), 4.24 (d, 2 H, *J* = 2.40 Hz), 4.64 (d, 1 H, *J* = 3.92 Hz), 5.89 (d, 1 H, *J* = 4.04 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 26.21, 27.10, 52.26, 57.34, 75.47, 78.61, 82.77, 83.15, 84.62, 105.77, 113.20.

CHNS Anal.: Calculated for $C_{11}H_{15}N_3O_4$: C, 52.17; H, 5.97; N, 16.59.

Found. C, 51.99; H, 5.92; N, 16.50.

MS: m/z = 254.06 (M+1).

<u>Compound No. 147</u>: Methyl 2,3-di-*O*-benzyl-4-*O*-propargyl-6-azido-6-deoxy-α-D-glucopyranoside:

Viscous liquid.

$[\alpha]_D^{25}$= +83.45 (c, 1.08, $CHCl_3$).

IR (cm^{-1}): 3303.83, 3014.53, 2927.74, 2100.34, 1496.66, 1454.23, 1357.79.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 2.46 (t, 1H, *J* = 2.40), 3.41 (s, 3 H), 3.35-3.56 (m, 4 H), 3.61-3.77 (m, 1 H), 3.93 (t, 1 H, *J* = 9.34 Hz), 4.36 (t, 2 H, *J* = 2.15 Hz), 4.60 (d, 1 H, *J* = 3.79 Hz), 4.64 (d, 1 H, *J* = 12.25 Hz), 4.77 (q, 2 H, *J* = 5.05 Hz), 4.97 (d, 1 H,

J = 10.87 Hz), 7.29-7.37 (m, 10 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 51.40, 55.39, 59.89, 69.63, 73.34, 74.77, 75.71, 77.41, 79.83, 79.89, 81.69, 97.93, 127.75-128.51, 137.94, 138.44.

CHNS Anal.: Calculated for $C_{24}H_{27}N_3O_5$: C, 65.89; H, 6.22; N, 9.60.

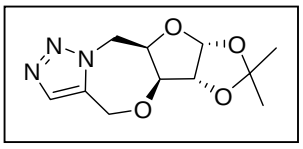
Found: C, 65.84; H, 6.30; N, 9.68.

MS: m/z = 460.12 ($M+Na^+$).

General procedure for 1,2-*O*-isopropylidene-α-D-pentofuranose / methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside derived 1,2,3-triazole fused tetracyclic compounds:

For 1,3-dipolar cycloaddition, 1 mmol of the respective azido-alkyne was dissolved in 2.5 ml of toluene and the solution was heated to 80-90 ^oC for about 1.5 – 3.5 h. After completion of the reaction monitored by TLC, the reaction mixture was cooled to room temperature whereupon a colorless solid was separated. The product was washed with 3 x 10ml of petroleum ether. For the analytical purpose the products were purified by silica gel column chromatography using ethyl acetate-petroleum ether mixture as eluent.

<u>Compound No. 118</u>:



Colorless solid: **m.p.:** 200 ^oC.

$[\alpha]_D^{25}$= -7.32 (c, 1.285, $CHCl_3$).

IR (cm^{-1}): 1461, 1379, 1215.07, 1093.56.

^{1}H NMR [$CDCl_3$, 500 MHz]: δ 1.29, 1.48 (2s, 6 H), 4.23 (d, 1 H, *J* = 1.38 Hz), 4.42-4.43 (m, 1 H), 4.51 (d, 1 H, *J* = 3.67 Hz), 4.62 (d, 1 H, *J* = 15.12 Hz), 4.74 (d, 1 H, *J* = 13.74 Hz), 4.94 (d, 1 H, *J* = 14.66), 5.12 (dd, 1 H, *J* = 5.04, 14.66 Hz), 5.80 (d, 1 H, *J* = 3.67 Hz), 7.54 (s, 1 H).

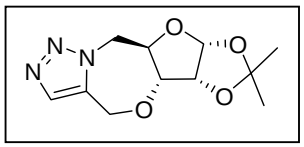
^{13}C NMR [$CDCl_3$, 125 MHz]: δ 26.05, 26.65, 48.20, 60.68, 74.31, 83.88, 84.43, 104.76, 112.03, 131.93, 134.98.

CHNS Anal.: Calculated for $C_{11}H_{15}N_3O_4$: C, 52.17; H, 5.97; N, 16.59.

Found. C, 51.97; H, 5.46; N, 16.67.

MS: m/z = 254.14 (M+1).

Compound No. 123:



Colorless solid: **m.p.:** 183 ^oC.

$[\alpha]_D^{25}$= -6.64 (c, 1.325, $CHCl_3$).

IR (cm^{-1}): 1465.80, 1377.08, 1105.14.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.34, 1.52 (2s, 6H), 3.71 (dd, 1H, *J* = 4.30, 8.72, Hz), 3.99-4.10 (m, 1H), 4.22 (dd, 1 H, *J* = 10.74, 13.26 Hz), 4.54 (d, 1H, *J* = 14.78 Hz), 4.80 (t, 1H, *J* = 3.79 Hz), 5.14 (d, 1H, *J* = 14.66 Hz), 5.31 (dd, 1H, *J* = 3.16, 13.27 Hz), 5.82 (d, 1 H, J = 3.53 Hz), 7.59 (s, 1 H).

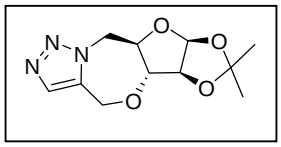
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 26.00, 26.22, 51.61, 62.93, 72.12, 78.53, 87.68, 103.40, 113.99, 133.92, 135.48.

CHNS Anal.: Calculated for $C_{11}H_{15}N_3O_4$: C, 52.17; H, 5.97; N, 16.59.

Found: C, 52.44; H, 5.90; N, 16.34.

MS: m/z = 254.07 (M+1).

Compound No. 127:



Colorless solid: **m.p.:** 214 ^oC.

$[\alpha]_D^{25}$= -66.86 (c, 1.05, $CHCl_3$).

IR (cm^{-1}): 2941.24, 1463.87, 1436.87, 1384.79.

^{1}H NMR [$CDCl_3$, 300 MHz]: δ 1.46, 1.63 (2s, 6 H), 3.56, 3.62 (2dd, 1 H, *J* = 3.16, 8.97, 3.16, 8.84 Hz), 3.97 (dd, 1 H, *J* = 4.42, 8.84, Hz), 4.39 (dd, 1 H, *J* = 10.48, 13.26, Hz), 4.54 (d, 1 H, *J* = 14.78 Hz), 4.76 (t, 1 H, *J* = 4.67 Hz), 5.11 (d, 1 H, *J* = 14.77 Hz), 5.33 (dd, 1 H, *J* = 3.16, 13.39, Hz), 5.77 (d, 1 H, *J* = 4.92 Hz), 7.58 (s, 1 H).

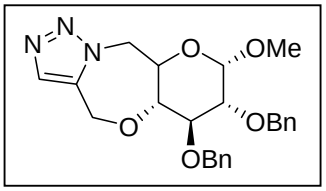
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 27.79, 28.05, 52.31, 62.84, 74.55, 85.46, 93.22, 103.57, 115.79, 133.88, 135.82.

CHNS Anal.: Calculated for $C_{11}H_{15}N_3O_4$: C, 52.17; H, 5.97; N, 16.59.

Found: C, 52.00; H, 5.99; N, 16.61.

MS: m/z = 254.06 (M+1).

<u>Compound No. 148</u>: 1,2,3-triazole derived from methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside:



Colorless solid: **m.p.:** 153 °C.

$[\alpha]_D^{25}$= -2.33 (c, 1.03, $CHCl_3$).

IR (cm^{-1}): 3060.82, 3010.67, 2900.74, 1496.66, 1444.58, 1355.86.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 3.40 (s, 3 H), 3.49-3.58 (m, 2 H), 3.69-3.91 (m, 2 H), 4.23 (dd, 1 H, *J* = 10.49, 14.02 Hz), 4.47 (d, 1 H, *J* = 14.90 Hz), 4.57 (d, 1 H, *J* = 3.54 Hz), 4.67 (d, 1 H, *J* = 12.13 Hz), 4.84 (d, 1 H, *J* = 12.12 Hz), 4.85 (s, 2 H), 5.01-5.11 (m, 2 H), 7.29-7.42 (m, 10 H), 7.58 (s, 1 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 52.98, 55.80, 62.06, 65.95, 73.62, 76.19, 78.65, 79.44, 88.50, 98.47, 127.68-128.49, 132.74, 136.51, 138.02, 138.79.

CHNS Anal.: Calculated for $C_{24}H_{27}N_3O_5$: C, 65.89; H, 6.22; N, 9.60).

Found: C, 65.82; H, 6.19; N, 9.58.

MS: m/z = 438.15 (M+1).

General procedure for 1,2-*O*-isopropylidene-3-*O*-acetyl-6-O-*p*-toluenesulfonyl-α-D-gluco/allofuranose:

To a solution of 1,2-*O*-isopropylidene-3-*O*-acetyl-α-D-glucofuranose (1.5 gm, 5.72 mmol) in 15 ml of dry pyridine at 0 °C was added *p*-tosyl chloride (1.2 gm, 6.3 mmol) under inert atmosphere. The reaction mixture was allowed to stir for 21 hours. After completion of the reaction monitored by TLC, the pyridine solution was

concentrated under reduced pressure by forming pyridine-toluene azeotrope. The residue was dissolved in 250 ml of DCM and it was washed sequentially with 3 x 25 ml of dilute HCl, 25 ml of water, 25 ml of brine solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield crude product. The crude tosylate (**129**) was purified by silica gel column chromatography using ethyl acetate-petroleum ether mixture as eluent.

Compound No. 129: 1,2-*O*-isopropylidene-3-*O*-acetyl-6-*O*-*p*-toluenesulfonyl-α-D-glucofuranose:

Sticky solid.

$[\alpha]_D^{25}$= +4.48 (c, 1.16, $CHCl_3$).

IR (cm^{-1}): 3519.85, 3022.25, 2987.53, 1745.46, 1598.88.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.30, 1.46 (2s, 6 H), 1.87 (s, 3 H), 2.08 (s,1 H), 2.45 (s, 3 H), 4.15 (d, 1 H, *J* = 5.68 Hz), 4.23 (dd, 1 H, *J* = 2.15, 9.35 Hz), 4.31 (dd, 1 H, *J* = 2.28, 12.76 Hz), 4.37 (d,1 H, 2.15), 4.56 (d, 1 H, 3.15 Hz), 4.89-4.98 (m, 1 H), 5.88 (d, 1 H, *J* = 3.54 Hz), 7.36 (d, 2 H, *J* = 7.96 Hz), 7.80 (d, 2 H, *J* = 8.34 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 20.37, 21.56, 26.13, 26.74, 63.13, 73.52, 75.37, 78.32, 84.40, 105.10, 112.10, 127.94, 129.88, 132.75, 145.54, 170.26.

CHNS Anal.: Calculated for $C_{18}H_{24}O_9S$: C, 51.91; H, 5.81; S, 7.70.

Found. C, 51.85; H, 5.75; S, 7.66.

MS: m/z = 417.03 (M+1).

General procedure for 1,2-*O*-isopropylidene-3-*O*-acetyl-5-*O*-*p*-toluenesulfonyl-6-azido-6-deoxy-α-D-gluco/allofuranose (130/138):

To a solution of 1,2-*O*-sopropylidene-3-*O*-acetyl-6-*O*-*p*-toluenesulfonyl-α-D-gluco/allofuranose (1.0 gm, 2.40 mmol) in 10 ml dry DMF was added sodium azide (780 mg, 12.00 mmol). The reaction mixture was stirred at 120-130 ^oC for 8 hours under argon atmosphere. After completion of the reaction monitored by TLC, the

reaction mixture was cooled and diluted with 250 ml of ethyl acetate and washed sequentially with 4 x 25 ml of water, 25 ml of brine solution and the aqueous extracts were extracted with another 25 ml of ethyl acetate, the pooled organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield crude product and was purified by silica gel column chromatography using ethyl acetate-petroleum ether mixture as eluent to obtain 1,2-*O*-isopropylidene-3-*O*-acetyl-6-azido-6-deoxy-α-D-gluco/allofuranose.

1,2-*O*-isopropylidene-3-*O*-acetyl-6-azido-6-deoxy-α-D-gluco/allofuranose (600 mg, 2,1 mmol) was dissolved in 10 ml of dry pyridine and was added tosyl chloide (440 mg, 2.31 mmol). The reaction mixture was stirred at 100 ^oC for 6 hours protected from moisture with the help of calcium chloride guard. After completion of the reaction indicated by TLC, the reaction mixture was cooled and concentrated under reduced pressure by making pyridine-toluene azeotrope. The residue was dissolved in 200 ml of DCM and washed sequentially with 2 x 25 ml of dilute HCl, 25 ml of water, 25 ml of brine solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield crude product and was purified by silica gel column chromatography using ethyl acetate-petroleum ether mixture as eluent to afford 1,2-*O*-isopropylidene-3-*O*-acetyl-5-*O*-*p*-toluenesulfonyl-6-azido-6-deoxy-α-D-gluco/allo-furanose (**130/138**).

<u>Compound No. 130</u>: 1,2-*O*-isopropylidene-3-*O*-acetyl-5-*O*-*p*-toluenesulfonyl-6-azido-6-deoxy-α-D-glucofuranose:

Viscous liquid.

$[\alpha]_D^{25}$= -12.26 (c, 1.06, $CHCl_3$).

IR (cm^{-1}): 3022.25, 2989.46, 2935.46, 2106.12, 1751.24, 1596.95, 1375.15.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.29, 1.48 (2s, 6 H), 2.08 (s, 3 H), 2.48 (s, 3H), 3.77-3.84 (m, 1 H), 3.87-3.97 (m, 2 H), 4.23 (dd, 1 H, *J* = 2.78, 8.21 Hz), 4.69 (d, 1 H, *J* =

3.79 Hz), 4.85 (d, 1 H, *J* = 2.90 Hz), 5.93 (d, 1 H, *J* = 3.67 Hz), 7.39 (d, 2 H, *J* = 7.96 Hz), 7.83 (d, 2 H, *J* = 8.47 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 20.61, 21.73, 26.23, 26.56, 58.84, 62.67, 78.34, 81.22, 83.17, 104.33, 112.78, 127.94, 130.26, 132.42, 146.14, 170.15.

CHNS Anal.: Calculated for $C_{18}H_{23}N_3O_8S$: C, 48.97; H, 5.25; N, 9.52; S, 7.26.

Found. C, 48.92; H, 5.30; N, 9.60; S, 7.20.

MS: m/z = 442.04 (M+1).

<u>Compound No. 138</u>: 1,2-*O*-isopropylidene-3-*O*-acetyl-5-*O*-*p*-toluenesulfonyl-6-azido-6-deoxy-α-D-allofuranose:

Gum.

$[\alpha]_D^{25}$= +88.21 (c, 1.17, $CHCl_3$).

IR (cm^{-1}): 2989.46, 2939.31, 2104.19, 1747.39, 1598.88.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.25, 1.50 (2s, 6 H), 2.09 (s, 3 H), 2.46 (s, 3 H), 3.36 (d, 1 H, *J* = 1.14 Hz), 3.39 (s, 1 H), 4.22 (dd, 1 H, *J* = 5.55, 8.59 Hz), 4.52 (dd, 1 H, *J* = 4.42, 8.21 Hz), 4.65 (dd, 1 H, *J* = 4.55, 8.59 Hz), 5.13 (q, 1 H, *J* = 5.43 Hz), 5.70 (d, 1 H, *J* = 3.41), 7.37 (d, 2 H, *J* = 7.96 Hz), 7.87 (d, 2 H, *J* = 8.34 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 20.80, 21.71, 26.51, 26.64, 50.55, 70.81, 75.50, 77.32, 77.51, 103.85, 113.84, 128.25, 129.81, 133.16, 145.53, 169.92.

CHNS Anal.: Calculated for $C_{18}H_{23}N_3O_8S$: C, 48.97; H, 5.25; N, 9.52; S, 7.26.

Found. C, 48.90; H, 5.35; N, 9.48; S, 7.32.

MS: m/z = 464.02 ($M+Na^+$).

General procedure for 1,2-*O*-isopropylidene-3-*O*-propargyl-5-*O*-*p*-toluene-sulfonyl-6-azido-6-deoxy-α-D-gluco/allofuranose (131/139):

To a solution of 1,2-*O*-isopropylidene-3-*O*-acetyl-5-*O*-*p*-toluenesulfonyl-6-azido-6-deoxy-α-D-gluco/allofuranose (800 mg, 1.81 mmol) in 10 ml of methanol was added sodium metal (40mg, 5% w/w) and stirred for 30 min at room temperature.

The reaction mixture was concentrated after completion monitored by TLC and after usual aqueous workup, ethyl acetate (200 ml) extract was washed with 25 ml water, 25 ml brine solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield crude product. The crude product was purified by silica gel column chromatography using ethyl acetate-petroleum ether mixture as eluent to yield 1,2-*O*-isopropyledine-5-*O*-*p*-toluenesulfonyl-6-azido-6-deoxy-α-D-gluco/allofuranose.

To a solution of 1,2-*O*-isopropyledine-5-*O*-*p*-toluenesulfonyl-6-azido-6-deoxy-α-D-gluco/allo furanose (700 mg, 1.75 mmol) in 10 ml of dry DMF was added sodium hydride (102 mg of 60% NaH in paraffin, 2.65 mmol) under argon atmosphere at 0 ^oC. After 30 min of stirring at room temperature to the resultant dark red solution was added propargyl bromide (0.18 ml, 1.93 mmol) at 0 ^oC. The reaction mixture was stirred for another 2 hours and monitored by TLC, the reaction mixture was cooled and quenched with 5 ml of methanol, diluted with 200 ml of ether and was washed sequentially with 3 x 25 ml of water, 25 ml of brine solution and the aqueous extracts were extracted with another 25 ml of ether, then the pooled organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield the crude product. The crude azido product was purified by silica gel column chromatography using ethyl acetate-petroleum ether mixture as eluent to obtain 1,2-*O*-isopropylidene-3-*O*-propargyl-5-*O*-*p*-toluenesulfonyl-6-azido-6-deoxy-α-D-gluco-/allofuranose (**131/139**).

<u>Compound No. 131</u>: 1,2-*O*-isopropylidene-3-*O*-propargyl-5-*O*-*p*-toluenesulfonyl-6-azido-6-deoxy-α-D-glucofuranose:

Viscous liquid.

$[\alpha]_D^{25}$= -7.73 (c, 1.035, $CHCl_3$).

IR (cm^{-1}): 3298.05, 2989.46, 2098.41, 1596.95, 1375.15, 1093.56.

^{1}H NMR [$CDCl_3$, 400 MHz]: δ 1.28, 1.49 (2s, 6 H), 2.48 (s, 3H), 2.49 (t, 1 H, *J* = 2.51 Hz), 3.47 (dd, 1 H, *J* = 5.52, 10.29 Hz), 3.55 (dd, 1 H, *J* = 3.26, 10.29 Hz), 3.69-3.74 (m, 1 H), 4.09 (d, 2 H, *J* = 2.51 Hz), 4.34 (dd, 1 H, *J* = 2.76, 8.54 Hz), 4.66 (d, 1 H, *J* = 3.76 Hz), 4.94 (d, 1 H, *J* = 3.01 Hz), 5.91 (d, 1 H, *J* = 3.77 Hz), 7.40 (d, 2 H, *J* = 8.03 Hz), 7.83 (d, 2 H, *J* = 8.54 Hz).

^{13}C NMR [$CDCl_3$, 100 MHz]: δ 21.72, 26.28, 26.60, 58.62, 59.72, 68.41, 75.42, 78.58, 78.85, 81.45, 83.11, 104.33, 112.73, 127.97, 130.21, 132.95, 145.84.

CHNS Anal.: Calculated for $C_{19}H_{23}N_3O_7S$: C, 52.16; H, 5.30; N, 9.61; 7.33.

Found. C, 52.21; H, 5.38; N, 9.70; S, 7.40.

MS: m/z = 438.06 (M+1).

General procedure for 1,2-*O*-isopropylidene-3-*O*-acetyl-5-azido-5-deoxy-6-*O*-*tert*-butyldimethylsilyl-α-D-gluco/allofuranose (134/142):

1,2-*O*-isopropylidene-3-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-α-D-gluco/allo-furanose were prepared from 1,2,5,6-*O*-diisopropylidene-3-*O*-acetyl-α-D-gluco-furanose according to the procedure described in Tripati et. al. report[31].

To a solution of 1,2-*O*-isopropylidene-3-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-α-D-gluco/allofuranose (1.5 gm, 3.99 mmol) and triethylamine (1.11 ml, 7.98 mmol) in 15 ml dry DCM was added methanesulfonyl chloride (0.37 ml, 4.8 mmol) dropwise at 0 ^oC. After completion of the reaction monitored by TLC, the reaction mixture was concentrated under reduced pressure and the residue was redissolved in 200 ml of DCM and washed with 3 x 25 ml of water, 25 ml of brine solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The product was purified by silica gel column chromatography using ethyl acetate-petroleum ether as eluent to afford 1,2-*O*-isopropylidene-3-*O*-acetyl-5-*O*-methanesulfonyl-6-*O*-*tert*-butyldimethylsilyl-α-D-gluco/allofuranose (**133**/**141**).

To a solution of mesyl derivative (**133/141**) (1.0 gm, 2.20 mmol) in 10 ml dry DMF was added sodium azide (715 mg, 11.00 mmol). The reaction mixture was stirred at 120-130 ^{o}C for 5-6 hours under argon atmosphere. After completion of the reaction monitored by TLC, reaction mixture was cooled and diluted with 250 ml of ethyl acetate and washed sequentially with 4 x 25 ml of water, 25 ml of brine solution and the aqueous extracts were extracted with another 25 ml of ethyl acetate, then the pooled organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield crude product. The crude product was purified by silica gel column chromatography using ethyl acetate-petroleum ether mixture as eluent to obtain 1,2-*O*-isopropylidene-3-*O*-acetyl-5-azido-5-deoxy-6-*O*-*tert*-butyldimethylsilyl-α-D-gluco/allofuranose (**134/142**).

Compound No. 134: 1,2-*O*-isopropylidene-3-*O*-acetyl-5-azido-5-deoxy-6-*O*-*tert*-butyldimethylsilyl-α-D-glucofuranose:

Viscous liquid.

$[\alpha]_D^{25}$= -16.16 (c, 1.25, $CHCl_3$).

IR (cm^{-1}): 2954.74, 2931.60, 2106.12, 1747.39, 1373.22, 1080.06.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 0.12, 0.17 (2s, 6 H), 0.90 (s, 9 H), 1.32, 1.50 (2s, 6-H), 2.11 (s, 3 H), 2.16 (d, 1 H, *J* = 7.70 Hz), 4.03-4.21 (m, 3 H), 4.34 (dd, 1 H, *J* = 3.03, 8.21 Hz), 4.39 (t, 1 H, *J* = 3.41 Hz), 5.94 (d, 1 H, *J* = 3.91 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ -5.17, -4.19, 17.90, 20.63, 25.63, 26.44, 26.94, 60.03, 63.42, 75.79, 80.34, 85.43, 104.68, 112.17, 170.48.

CHNS Anal.: Calculated for $C_{17}H_{31}N_3O_6Si$: C, 50.85; H, 7.78; N, 10.46.

Found. C, 51.00; H, 7.85; N, 10.35.

MS: m/z = 402.13 (M+1).

<u>Compound No. 142</u>: 1,2-*O*-isopropylidene-3-*O*-acetyl-5-azido-5-deoxy-6-*O*-*tert*-butyldimethylsilyl-α-D-allofuranose:

Viscous liquid.

$[\alpha]_D^{25}$= +102.48 (c, 1.21, $CHCl_3$).

IR (cm^{-1}): 2954.74, 2931.60, 2106.12, 1741.60, 1465.80, 1375.15.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 0.10, 0.11 (2s, 6 H), 0.91 (s, 9 H), 1.34, 1.53 (2s, 6 -H), 2.15 (s, 3 H), 3.50-3.58 (m, 1 H), 3.83-3.99 (m, 2 H), 4.17 (dd, 1 H, *J* = 2.15, 8.59 Hz), 4.80-4.93 (m, 2 H), 5.82 (d, 1 H, *J* = 3.53 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ -5.55, -5.55, 18.18, 20.67, 25.77, 26.62, 26.62, 61.89, 63.86, 72.75, 76.43, 77.22, 104.40, 113.26, 170.12.

CHNS Anal.: Calculated for $C_{17}H_{31}N_3O_6Si$: C, 50.85; H, 7.78; N, 10.46.

Found. C, 51.06; H, 7.72; N, 10.52.

MS: m/z = 402.15 (M+1).

General procedure for 1,2-*O*-isopropylidene-3-*O*-propargyl-5-azido-5-deoxy-6-*O*-*tert*-butyldimethylsilyl-α-D-gluco/allofuranose (135/143):

To a solution of 1,2-*O*-isopropylidene-3-*O*-acetyl-5-azido-5-deoxy-6-*O*-*tert*-butyldimethylsilyl-α-D-gluco/allofuranose (**134/142**) (750 mg, 1.87 mmol) in 10 ml of methanol was added sodium metal (40mg, 5% w/w) and stirred for 30 min at room temperature. After completion of the reaction, the reaction mixture was diluted with ethyl acetate (150 ml) and the organic layer was washed with 25 ml water, 25 ml brine solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield the crude product and was purified chromatographically on silica gel column using ethyl acetate-petroleum ether mixture as eluent to yield 1,2-*O*-isopropylidene-5-azido-5-deoxy-6-*O*-*tert*-butyldimethylsilyl-α-D-gluco/allofuranose.

To a solution of above azido derivative (500 mg, 1.39 mmol) in 10 ml of dry DMF was added sodium hydride (80 mg of 60% NaH in paraffin, 2.09 mmol) at 0 ^oC.

The reaction mixture was stirred for 30 min at room temperature under argon atmosphere and resultant dark red solution was added propargyl bromide (0.152 ml, 1.67 mmol) at 0 ^{o}C. After completion of the reaction monitored by TLC, the reaction mixture was cooled and quenched with 5 ml of methanol, diluted with 200 ml of ether and was washed sequentially with 3 x 25 ml of water, 25 ml of brine solution and the aqueous extracts were re-extracted with another 25 ml of ether, then the pooled ether layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield crude product. The crude product was purified by silica gel column chromatography using ethyl acetate-petroleum ether mixture as eluent 1,2-*O*-isopropylidene-3-*O*-propargyl-5-azido-5-deoxy-6-*O*-*tert*-butyldimethylsilyl-α-D-gluco/allo-furanose (**135/143**).

Compound No. 135: 1,2-*O*-isopropylidene-3-*O*-propargyl-5-azido-5-deoxy-6-*O*-*tert*-butyldimethylsilyl-α-D-glucofuranose:

Viscous liquid.

$[\alpha]_D^{25}$= -36.80 (c, 1.00, $CHCl_3$).

IR (cm^{-1}): 3307.69, 2954.74, 2931.60, 2102.26, 1471.59, 1375.15, 1080.06.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 0.09, 0.10 (2s, 6 H), 0.91 (s, 9 H), 1.33, 1.50 (2s, 6 H), 2.48 (t, 1 H, *J* = 2.40 Hz), 3.61-3.70 (m, 1 H), 3.74-3.81 (m, 1 H), 3.86 (d, 1 H, *J* = 3.03 Hz), 4.10 (d, 1 H, *J* = 3.29 Hz), 4.21 (d, 1 H, *J* = 2.40 Hz), 4.28 (d, 1 H, *J* = 2.40 Hz), 4.34 (dd, 1 H, *J* = 3.16, 9.98 Hz), 4.61 (d, 1 H, *J* = .3.91 Hz), 5.95 (d, 1 H, *J* = 3.79 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ -5.45, -5.45, 18.18, 25.77, 26.37, 26.70, 56.50, 61.97, 63.05, 75.68, 78.56, 78.80, 80.42, 81.46, 104.67, 111.98.

CHNS Anal.: Calculated for $C_{18}H_{31}N_3O_5Si$: C, 54.38; H, 7.86; N, 10.57.

Found. C, 54.30; H, 7.97; N, 10.45.

MS: m/z = 398.12 (M+1).

Compound No. 143: 1,2-*O*-isopropylidene-3-*O*-propargyl-5-azido-5-deoxy-6-*O*-*tert*-butyldimethylsilyl-α-D-allofuranose:

Viscous liquid.

$[\alpha]_D^{25}$= +80.65 (c, 1.24, $CHCl_3$).

IR (cm^{-1}): 3305.76, 2954.74, 2929.67, 2858.31, 2100.34, 1471.59, 1384.79.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 0.09, 0.10 (2s, 6 H), 0.91 (s, 9 H), 1.36, 1.56 (2s, 6 -H), 2.57 (t, 1 H, *J* = 2.40 Hz), 3.65, 3.70 (2dd, 1 H, *J* = 2.02, 4.80, 2.15, 4.80 Hz), 3.88 (d, 1 H, *J* = 2.90 Hz), 3.91 (d, 1 H, *J* = 6.82 Hz), 4.02 (dd, 1 H, *J* = 2.15, 8.97 Hz), 4.18 (dd, 1 H, *J* = 4.17, 8.84 Hz), 4.26 (dd, 1 H, *J* = 2.40, 16.17 Hz), 4.40 (dd, 1 H, *J* = 2.40, 16.17 Hz), 4.68 (t, 1 H, *J* = .3.91 Hz), 5.78 (d, 1 H, *J* = 3.54 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ -5.54, -5.54, 18.18, 25.79, 26.45, 26.77, 57.74, 62.17, 64.31, 75.68, 76.75, 76.82, 77.59, 78.82, 104.41, 113.33.

CHNS Anal.: Calculated for $C_{18}H_{31}N_3O_5Si$: C, 54.38; H, 7.86; N, 10.57.

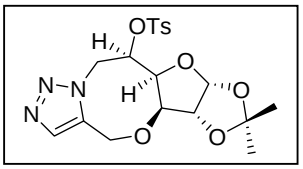
Found. C, 54.32; H, 7.90; N, 10.62.

MS: m/z = 398.14 (M+1).

General procedure for 1,2-*O*-isopropylidene-α-D-gluco/allofuranose derived 1,2,3-triazole fused tetracyclic compounds:

For 1,3-dipolar cycloaddition, 1 mmol of the respective azido-alkyne was dissolved in 2.5 ml of toluene and heated to 80-90 ^oC for about 5 – 6 h. After completion of the reaction monitored by TLC, the reaction mixture was cooled to room temperature and concentrate under reduced pressure. The crude products were purified by silica gel column chromatography using ethyl acetate-petroleum ether mixture as eluent.

Compound No. 132:



Colorless solid: **m.p.:** 149 ^oC.

$[\alpha]_D^{25}$= -34.73 (c, 1.10, $CHCl_3$).

IR (cm^{-1}): 1731.96, 1596.95, 1461.94, 1454.23, 1377.08, 1176.50, 1091.63.

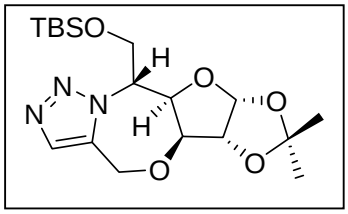
^{1}H NMR [$CDCl_3$, 300 MHz]: δ 1.25, 1.43 (2s, 6 H), 2.49 (s, 3 H), 3.85 (dd, 1 H, *J* = 3.53, 12.88 Hz), 4.06-4.14 (m, 1 H), 4.60 (d, 1 H, *J* = 3.79 Hz), 4.74 (dd, 1 H, *J* = 2.65, 9.22 Hz), 4.80-5.02 (m, 3 H), 5.12 (d, 1 H, *J* = 2.65 Hz), 6.01 (d, 1 H, *J* = 3.79 Hz), 7.39 (d, 2 H, *J* = 8.33 Hz), 7.47 (s, 1 H), 7.77 (d, 2 H, *J* = 8.21 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: 21.76, 26.09, 26.43, 54.59, 62.51, 64.54, 77.53, 81.39, 82.91, 104.84, 112.59, 127.78, 128.00, 130.15, 130.34, 132.66, 146.05.

CHNS Anal.: Calculated for $C_{19}H_{23}N_3O_7S$: C, 52.16; H, 5.30; N, 9.61; S, 7.33. Found: C, 52.21; H, 5.47; N, 9.65; S, 7.26.

MS: m/z = 438.06 (M+1).

<u>Compound No. 136</u>:



Thick syrup.

$[\alpha]_D^{25}$= +17.70 (c, 1.13, $CHCl_3$).

IR (cm^{-1}): 2954.74, 2931.60, 2856.38, 1461.94, 1375.15.

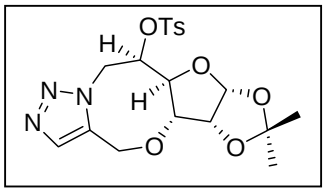
^{1}H NMR [$CDCl_3$, 200 MHz]: δ 0.08, 0.13 (2s, 6H), 0.91 (s, 9 H), 1.29, 1.49 (2s, 6H), 4.31 (d, 1 H, *J* = 1.90 Hz), 4.40-4.61 (m, 4 H), 4.73-4.94 (m, 3 H), 5.72 (d, 1 H, *J* = 3.67 Hz), 7.61 (s, 1 H).

^{13}C NMR [$CDCl_3$, 75 MHz]: δ -5.40, -5.59, 18.16, 25.57, 25.82, 26.03, 26.55, 59.24, 59.97, 72.61, 83.74, 85.03, 104.38, 111.76, 131.90, 135.08.

CHNS Anal.: Calculated for $C_{18}H_{31}N_3O_5Si$: C, 54.38; H, 7.86; N, 10.57; Si, 7.06. Found: C, 54.16; H, 7.48; N, 10.33.

MS: m/z = 398.16 (M+1).

Compound No. 140:



Colorless solid: **m.p.:** 187 ^{o}C.

$[\alpha]_D^{25}$= +117.70 (c, 1.045, $CHCl_3$).

IR (cm^{-1}): 1739, 1595, 1456, 1375.

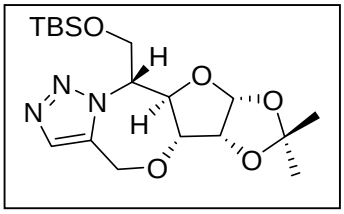
^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.35, 1.58 (2s, 6 H), 2.34 (s, 3 H), 3.80-4.01 (m, 2 H), 4.23-4.33 (m, 2 H), 4.66 (d, 1 H, *J* = 15.41 Hz), 4.73 (dd, 1 H, *J* = 4.42, 7.96 Hz), 4.80 (t, 1 H, *J* = 3.92 Hz), 4.88 (d, 1 H, *J* = 14.91 Hz), 5.79 (d, 1 H, *J* = 3.28 Hz), 7.20 -(d, 2 H, *J* = 8.08 Hz), 7.47 (s, 1 H), 7.80 (d, 2 H, *J* = 8.21 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 21.63, 26.45, 26.65, 45.46, 61.89, 72.04, 75.76, 76.12, 77.80, 103.85, 114.0, 127.79, 128.01, 129.80, 129.99, 132.61, 145.71

CHNS Anal.: Calculated for $C_{19}H_{23}N_3O_7S$: C, 52.16; H, 5.30; N, 9.61; S, 7.33. Found: C, 51.06; H, 5.28; N, 9.78; S, 7.38.

MS: m/z = 438.06 (M+1).

Compound No. 144:



Foamy solid: **m.p.:** 60 ^{o}C.

$[\alpha]_D^{25}$= +14.31 (c, 1.02, $CHCl_3$).

IR (cm^{-1}): 1461.94, 1375.15, 1257.50, 1027.99.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ -0.07, -0.05 (2s, 6 H), 0.81 (s, 9 H), 1,37, 1.55 (2s, 6 H), 4.09 (dd, 1 H, *J* = 3.66, 11.24 Hz), 4.25 (dd, 1 H, *J* = 4.80, 9.47 Hz), 4.31 (dd, 1 H, *J* = 2.15, 11.11 Hz), 4.56 (dd, 1 H, *J* = 4.42, 9.48 Hz), 4.79 (t, 1 H, *J* = 3.79 Hz), 4.96 (ABq, 2H, *J* = 13.52 Hz), 5.32-5.37 (m, 1 H), 5.88 (d, 1 H, *J* = 3.53 Hz), 7.54 (s, 1 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ -5.78, -5.50, 18.34, 25.86, 26.11, 26.37, 61.12, 61.63, 62.97, 74.24, 78.72, 81.06, 103.30, 113.88, 133.33, 137.07.

CHNS Anal.: Calculated for $C_{18}H_{31}N_3O_5Si$: C, 54.38; H, 7.86; N, 10.57; Si, 7.06.

Found: C, 54.47; H, 7.76; N, 10.70.

MS: m/z = 398.14 (M+1).

B. Synthesis Of 1,2,3,4-Tetrazole Fused Carbohydrate Derived Tetracyclic Compound:

Procedure for 1,2-*O*-isopropylidene-3-*O*-cyanomethylene-5-azido-5-deoxy-α-D-xylofuranose (149):

To a solution of 1,2-*O*-isopropylidene-5-azido-5-deoxy-α-D-xylofuranose (**116**) (1.0 gm, 4.70 mmol) in 10 ml of dry DMF was added sodium hydride (0.282 gm of 60% NaH in paraffin, 7.05 mmol) at 0 ^{o}C. The reaction mixture was stirred for 30 min at room temperature under argon and the resultant dark brown solution was added chloroacetonitrile (1.5 ml, 23.50 mmol) at 0 ^{o}C. After completion of the reaction monitored by TLC, the reaction mixture was cooled and quenched with 5 ml of methanol, diluted with 250 ml of ether and washed sequentially with 3 X 25 ml of water, 25 ml of brine solution and the aqueous extracts were extracted with another 25 ml of ether, then the pooled organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield crude product. The crude azido product was purified by silica gel column chromatography using ethyl acetate-petroleum ether mixture as eluent to afford 65% of azido-ntrile.

Compound No. 149:

Viscous liquid.

$[\alpha]_D^{25}$ = -83.17 (c, 1.01, $CHCl_3$).

IR (cm^{-1}): 2989.46, 2254.63, 2104.19.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.33, 1.51 (2s, 6 H), 3.54 (ddd, 2 H, *J* = 6.70, 12.51, 19.08 Hz), 4.05 (d, 1 H, *J* = 3.29 Hz), 4.31-4.39 (m, 3 H), 4.65 (d, 1 H, *J* = 3.79 Hz), 5.93 (d, 1 H, *J* = 3.79 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 26.11, 26.58, 48.67, 55.47, 78.09, 81.43, 83.28, 104.90, 112.30, 127.48.

CHNS Anal.: Calculated for $C_{10}H_{14}N_4O_4$: C, 47.24; H, 5.55; N, 22.04.

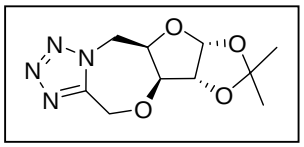
Found. C, 47.19; H, 5.60; N, 22.00.

MS: m/z = 278.21 ($M+Na^+$).

Procedure for 1,2-*O*-isopropylidene-α-D-xylofuranose derived 1,2,3,4-tetrazole fused tetracyclic compounds (150):

For 1,3-dipolar cycloaddition reaction, 1 mmol of the azido-nitrile (**149**) was dissolved in 5 ml of toluene and the solution was refluxed at 125-130 ^oC for about 24 h. After completion of the reaction monitored by TLC, the reaction mixture was cooled to room temperature whereupon a white solid was separated. The solid product was washed with 3 x 10 ml of petroleum ether. For the analytical purpose the product was purified on silica gel column chromatography using ethyl acetate-petroleum ether mixture as eluent, obtained 65% of tetrazole.

<u>Compound No. 150</u>:



Colorless solid: **m.p.:** 208 ^oC.

$[\alpha]_D^{25}$ = -12.24 (c, 1.16, $CHCl_3$).

IR (cm^{-1}): 3018.39, 2981.74, 1481.23, 1382.87.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.33, 1.51 (2s, 6 H), 4.27 (d, 1 H, *J* = 2.78 Hz), 4.61 (dd, 1 H, *J* = 3.16, 6.19 Hz), 4.65 (d, 1 H, *J* = 3.79 Hz), 4.79 (dd, 1 H, *J* = 3.42, 14.66 Hz), 4.91 (d, 1 H, *J* = 15.79 Hz), 5.03 (dd, 1 H, *J* = 6.19, 14.65 Hz), 5.22 (d, 1 H, *J* = 15.66 Hz), 5.90 (d, 1 H, *J* = 3.66 Hz).

^{13}C NMR [$DMSO_6$, 50 MHz]: δ 26.14, 26.55, 46.38, 59.64, 72.92, 83.38, 83.96, 104.09, 111.38, 154.56.

CHNS Anal.: Calculated for $C_{10}H_{14}N_4O_4$: C, 47.24; H, 5.55; N, 22.04.

Found. C, 47.35; H, 5.64; N, 22.18. **MS:** m/z = 255.97 (M+1).

C. Diversity Oriented Synthesis:

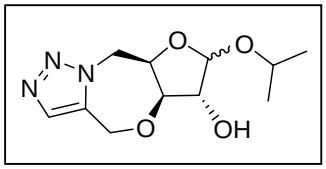
1. General procedure for Fischer's syntheses of 1,2,3-triazole / 1,2,3,4-tetrazole fused tricyclic glycosides:

Procedure A: To a solution of 1.5 mmol (380 mg) of α-D-xylofuranose derived 1,2,3-triazole (**118**) in 8 ml of isopropyl alcohol was added catalytic amount of concentrated sulfuric acid and the reaction mixture was stirred at 60-70 ^{o}C for specified time. The reaction completion was monitored by TLC and was neutralized with saturated solution of sodium bicarbonate and it is diluted with 200ml of ethyl acetate. The organic layer was washed with 2 x 25 ml of water, 25 ml of brine solution, dried over anhydrous sodium sulfate and concentrated under reduced pressure. After flash silica gel column chromatography accomplished α and β glycosides.

Procedure B: To a solution of 1.5 mmol (380 mg) of α-D-xylofuranose derived 1,2,3-triazole (**118**) and 3.0 mmol of allyl alcohol (0..210 ml) / 3.0 mmol of homopropargyl alcohol (0..230 ml) in 8 ml of anhydrous 1,4-dioxane was added catalytic amount of concentrated sulfuric acid and the reaction mixture was stirred at 60-70 ^{o}C for specified time. Remaining procedure is same as in method A.

Similarly, 1,2,3,4-tetrazole fused tricyclic glycosides were prepared from α-D-xylofuranose derived 1,2,3,4-tetrazole (**150**) by following procedure A and procedure B.

<u>Compound No. 151</u>:



<u>151α-glycoside</u>:

Colorless solid:

m.p.: 94 ^{o}C.

$[\alpha]_D^{25}$ = +52.84 (c, 1.09, $CHCl_3$)

IR (cm^{-1}): 3344.34, 2974.03, 1666.38.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.11 (d, 3 H, *J* = 6.06 Hz), 1.16 (d, 3 H, *J* = 6.32 Hz), 2.91 (bs, 1 H), 3.82-4.01 (m, 1 H), 4.07-4.16 (m, 2 H), 4.36-4.59 (m, 2 H), 4.78 (dd, 1 H, *J* = 3.79, 13.01 Hz), 4.92 (ABq, 2 H, *J* = 16.01 Hz), 5.07 (d, 1 H, *J* = 3.79), 7.39 (s, 1 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 21.67, 23.25, 48.50, 64.00, 70.98, 75.05, 76.77, 86.61, 99.32, 130.58, 134.12.

CHNS Anal.: Calculated for $C_{11}H_{17}N_3O_4$: C, 51.76; H, 6.71; N, 16.46.

Found. C, 51.68; H, 6.87; N, 16.58.

MS: m/z = 256.13 (M+1).

151β-glycoside:

Colorless solid:

m.p.: 110 °C.

$[\alpha]_D^{25}$ = -86.39 (c, 1.22, $CHCl_3$).

IR (cm^{-1}): 3359.77, 2972.10, 1654.81.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.15-1.21 (m, 6 H), 2.80 (bs, 1 H), 3.83-3.99 (m, 1 H), 4.13 (d, 1 H, *J* = 5.18 Hz), 4.31 (s, 1 H), 4.57-4.93 (m, 3 H), 4.94 (ABq, 2 H, *J* = 16.01 Hz), 5.06 (bs, 1 H), 7.42 (s, 1 H).

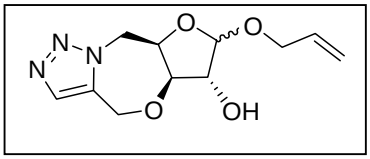
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 21.27, 23.19, 49.62, 64.41, 70.00, 78.72, 80.81, 86.82, 106.52, 130.16, 134.10.

CHNS Anal.: Calculated for $C_{11}H_{17}N_3O_4$: C, 51.76; H, 6.71; N, 16.46.

Found. C, 51.58; H, 6.67; N, 16.38.

MS: m/z = 256.14 (M+1).

Compound No. 152:



152α-glycoside:

Thick syrup.

$[\alpha]_D^{25}$ = +43.24 (c, 1.11, $CHCl_3$).

IR (cm^{-1}): 3332.76, 2987.53, 2933.53, 1731.96, 1647.10.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 2.50 (bs, 1 H), 4.05-4.35 (m, 4 H), 4.50-4.68 (m, 2 H), 4.76 (d, 1 H, *J* = 15.92 Hz), 4.87 (dd, 1 H, *J* = 3.28, 12.38 Hz), 5.09 (d, 1 H, *J* = 4.42 Hz), 5.20 (s, 1 H), 5.24-5.35 (m, 2 H), 5.80-5.99 (m, 1 H), 7.47 (s, 1 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 48.42, 63.94, 68.77, 75.10, 76.69, 86.29, 99.96, 117.82, 130.50, 133.25, 134.13.

CHNS Anal.: Calculated for $C_{11}H_{15}N_3O_4$: C, 52.17; H, 5.97; N, 16.59.

Found. C, 52.27; H, 6.08; N, 16.48.

MS: m/z = 254.12 (M+1).

152β-glycoside:

Colorless solid:

m.p.: 110 °C.

$[\alpha]_D^{25}$ = -79.82 (c, 1.17, $CHCl_3$)

IR (cm^{-1}): 3330.84, 2931.60, 1731.96, 1650.95, 1454.23.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 3.29 (bs, 1 H), 4.00-4.11 (m, 1 H), 4.15-4.28 (m, 2 H), 4.42 (s, 1 H), 4.69-4.94 (m, 4 H), 5.06 (s, 1 H), 5.18-5.21 (m, 1 H), 5.23-5.36 (m, 2 H), 5.80-6.00 (m, 1 H), 7.44 (s, 1 H).

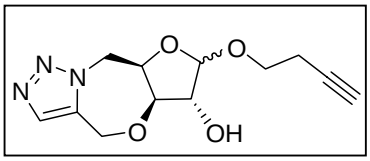
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 49.50, 64.55, 68.52, 79.23, 80.78, 86.83, 107.52, 117.62, 130.32, 133.58, 134.01.

CHNS Anal.: Calculated for $C_{11}H_{15}N_3O_4$: C, 52.17; H, 5.97; N, 16.59.

Found. C, 51.98; H, 6.03; N, 16.69.

MS: m/z = 254.12 (M+1).

Compound No. 153:



153α-glycoside:

Thick syrup.

$[\alpha]_D^{25}$ = +43.53 (c, 1.02, $CHCl_3$).

IR (cm^{-1}): 3450.41, 3292.26, 2929.67, 1652.88.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.99 (t, 1 H, *J* = 2.65 Hz), 2.38 (bs, 1 H), 2.51 (dt, 2 H, *J* = 2.52, 6.44 Hz), 3.63-3.75 (m, 1 H), 3.85-3.96 (m, 1 H), 4.15-4.29 (m, 2 H), 4.53-4.67 (m, 2 H), 4.87 (dd, 1 H, *J* = 2.65, 11.75 Hz), 5.00 (ABq, 2 H, *J* = 15.92 Hz), 5.09 (d, 1 H, *J* = 4.30 Hz), 7.47 (s, 1 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 19.83, 48.50, 64.10, 66.36, 69.76, 75.44, 77.02, 80.56, 86.39, 101.00, 130.67, 134.12.

CHNS Anal.: Calculated for $C_{12}H_{15}N_3O_4$: C, 54.33; H, 5.70; N, 15.84.

Found. C, 54.27; H, 5.78; N, 15.78.

MS: m/z = 266.12 (M+1).

153β-glycoside:

Thick syrup.

$[\alpha]_D^{25}$ = -67.85 (c, 1.07, $CHCl_3$).

IR (cm^{-1}): 3450.41, 3286.48, 2925.81, 1662.52, 1454.23, 1365.51.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 2.00 (t, 1 H, *J* = 2.65 Hz), 2.51 (dt, 2 H, *J* = 2.53, 6.69 Hz), 2.55 (bs, 1 H), 3.59-3.71 (m, 1 H), 3.79-3.93 (m, 1 H), 4.15 (dd, 1 H, *J* =

1.14, 5.31 Hz), 4.42 (s, 1 H), 4.69-4.85 (m, 3 H), 4.94 (dd, 1 H, *J* = 4.04, 11.87 Hz), 5.06 (s, 1 H), 5.26 (d, 1 H, *J* = 16.04 Hz), 7.45 (s, 1 H).

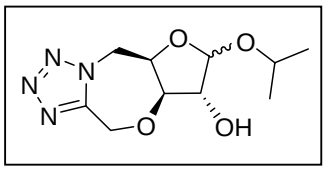
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 19.71, 49.50, 64.62, 66.20, 69.73, 79.35, 80.76, 80.92, 86.66, 108.55, 130.40, 133.97.

CHNS Anal.: Calculated for $C_{12}H_{15}N_3O_4$: C, 54.33; H, 5.70; N, 15.84.

Found. C, 54.38; H, 5.63; N, 15.68.

MS: m/z = 266.11 (M+1).

Compound No. 154:



154α-glycoside:

Gum.

$[\alpha]_D^{25}$ = +86.28 (c, 1.05, $CHCl_3$).

IR (cm^{-1}): 3562.28, 3018.39, 2977.8, 1731.96, 1525.59, 1469.66, 1384.79.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.20 (d, 3 H, *J* = 6.19 Hz), 1.24 (d, 3 H, *J* = 6.32 Hz), 2.20 (bs, 1 H), 3.90-4.09 (m, 1 H), 4.16-4.27 (m, 2 H), 4.38-4.54 (m, 2 H), 4.87-4.92 (m, 1 H), 5.15 (d, 1 H, *J* = 4.04), 5.20 (ABq, 2 H, *J* = 16.92 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 21.82, 23.37, 47.06, 65.20, 71.42, 75.15, 76.92, 87.22, 99.22, 152.72.

CHNS Anal.: Calculated for $C_{10}H_{16}N_4O_4$: C, 46.87; H, 6.29; N, 21.86.

Found. C, 46.93; H, 6.25; N, 21.98.

MS: m/z = 257.36 (M+1).

154β-glycoside:

Gum

$[\alpha]_D^{25}$ = -100.00 (c, 1.24, $CHCl_3$).

IR (cm^{-1}): 3402.20, 2975.96, 2975.96, 1604.66, 1525.59, 1467.73, 1384.79.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.18-1.26 (m, 6 H), 3.23 (bs, 1 H), 3.86-4.04 (m, 1 H), 4.23 (m, 1 H), 4.37 (s, 1 H), 4.66-4.93 (m, 3 H), 5.10 (s, 1 H), 5.19 (ABq, 2 H, *J* = 16.75 Hz).

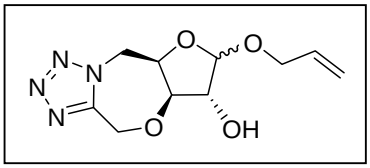
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 21.40, 23.27, 48.05, 65.35, 70.53, 78.71, 81.12, 87.53, 106.53, 152.71.

CHNS Anal.: Calculated for $C_{10}H_{16}N_4O_4$: C, 46.87; H, 6.29; N, 21.86.

Found. C, 46.80; H, 6.35; N, 21.68.

MS: m/z = 257.35 (M+1).

<u>Compound No. 155</u>:



<u>155α-glycoside</u>:

Gum.

$[\alpha]_D^{25}$ = +71.24 (c, 1.21, MeOH).

IR (cm^{-1}): 3425.34, 3016.46, 2927.74, 1649.02, 1527.52, 1442.65, 1352.01.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 2.98 (bs, 1 H), 3.98 (m, 1 H), 4.13-4.28 (m, 3 H), 4.32-4.57 (m, 2 H), 4.77-4.95 (m, 2 H), 5.00 (d, 1 H, *J* = 3.41 Hz), 5.07-5.45 (m, 3 H), 5.65-5.95 (m, 1H).

^{13}C NMR [$CDCl_3$, 125 MHz]: δ 46.93, 64.91, 68.85, 75.03, 76.73, 86.60, 99.74, 118.09, 133.14, 152.75.

CHNS Anal.: Calculated for $C_{10}H_{14}N_4O_4$: C, 47.24; H, 5.55; N, 22.04.

Found. C, 47.34; H, 5.35; N, 22.10.

MS: m/z = 255.29 (M+1).

<u>155β-glycoside</u>:

Gum

$[\alpha]_D^{25}$ = -86.29 (c, 1.05, MeOH).

IR (cm^{-1}): 3415.70, 2931.60, 1650.95, 1469.66, 1352.01.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 2.63 (bs, 1 H), 3.95-4.01 (m, 1 H), 4.16-4.26 (m, 2 H), 4.42 (s, 1 H), 4.50-4.82 (m, 2 H), 4.79-5.08 (m, 2 H), 5.03 (s, 1 H), 5.15-5.50 (m, 3 H), 5.75-5.98 (m, 1 H).

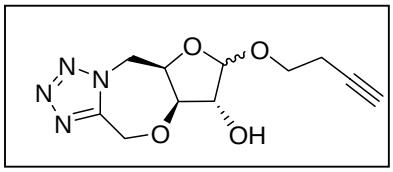
^{13}C NMR [$CDCl_3$, 125 MHz]: δ 47.86, 65.43, 66.62, 79.17, 80.83, 87.38, 107.33, 118.03, 133.26, 152.74.

CHNS Anal.: Calculated for $C_{10}H_{14}N_4O_4$: C, 47.24; H, 5.55; N, 22.04.

Found. C, 47.15; H, 5.45; N, 22.15.

MS: m/z = 255.26 (M+1).

<u>Compound No. 156</u>:



156(α+ β)-glycoside: Gum (inseparable mixture).

IR (cm^{-1}): 3423.41, 3305.76, 3016.46, 2929.67, 1622.02, 1527.52, 1440.73, 1352.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 2.01 (m, 1 H), 2.43-2.58 (m, 2 H), 3.01 (m, 1 H), 3.55-3.96 (m, 2 H), 4.20 (m, 1 H), 4.41-4.95 (m, 2 H), 4.70-5.15 (m, 4 H), 5.38-5.52 (m, 1 H).

^{13}C NMR [$CDCl_3$, 125 MHz]: δ 19.6, 19.7, 46.9, 47.8, 64.8, 65.2, 66.2, 66.3, 75.1, 76.8, 79.1, 80.8, 80.6, 80.9, 86.4, 87.2, 100.7, 108.4, 152.7, 152.8.

MS: m/z = 267.26 (M+1).

2. Syntheses of 1,2,3-triazole / 1,2,3,4-tetrazole Fused tricyclic nucleosides by Vorbruggen's One Pot method:

General procedure for the syntheses of 1,2-di-*O*-acetate of 1,2,3-triazole (157)/ 1,2,3,4-tetrazole (158) fused tricyclic compounds:

To a solution of (6.0 mmol) of α-D-xylofuranose derived 1,2,3-triazole (**118**) /1,2,3,4-tetrazole (**150**) in a mixture of 10 ml of 1,4-dioxane and 6 ml of water was added concentrated sulfuric acid (4%) and the reaction mixture was heated to 60-70 ^{o}C for specified time. The reaction mixture was neutralized with saturated solution of sodium bicarbonate and extracted repeatedly with ethyl acetate (5 x 75 ml). The pooled organic layer washed with 30 ml of brine solution, dried over anhydrous sodium sulfate and concentrated under reduced pressure to obtain the crude dihydroxy derivative.

The crude dihydroxy compound was dissolved in 10 ml of dry pyridine, cooled to 0 ^{o}C and was added acetic anhydride (1.9 ml, 5 mmol) and stirred for 5 hours. After completion of the reaction, the pyridine solution was concentrated under reduced pressure, the residue was dissolved in 200 ml of ethyl acetate and washed with 3 x 25 ml of dilute hydrochloric acid, 35 ml of water, 30 ml of brine solution and concentrated under reduced pressure. The crude diacetate was purified by silica gel column chromatography using ethyl acetate-petroleum mixture as eluent.

<u>Compound No. 157</u>: (α/β)-D-1,2-di-*O*-acetyl derivative of α-D-xylofuranose derived 1,2,3-triazole:

α/β-anomeric mixture: sticky solid (inseparable).

IR (cm^{-1}): 3016.49, 2962.64, 1755.10, 1436.87, 1373.22.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 2.03, 2.05, 2.08, 2.09 (4s, 6 H), 4.39-5.02 (m, 5 H), 5.20-5.34 (m, 2H), 6.10-6.49 (m, 1 H), 7.42, 7.44 (2s, 1H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 20.43, 20.70, 20.86, 21.10, 48.24, 49.01, 64.61, 64.85, 76.25, 76.56, 80.79, 81.54, 82.40, 83.48, 93.30, 99.27, 130.75, 133.83, 169.25, 169.81.

MS: m/z = 298.35 (M+1).

Compound No. 158: (α/β)-D-1,2-di-*O*-acetyl derivative of α-D-xylofuranose derived 1,2,3,4-tetrazole:

α/β-anomeric mixture: sticky solid (inseparable).

IR (cm^{-1}): 1745.46, 1525.59, 1461.94, 1377.08.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 2.09, 2.13, 2.14, 2.15 (4s, 6 H), 4.29 (m, 1 H), 4.48-4.75 (m, 2 H), 4.78-5.10 (m, 2 H), 5.20-5.63 (m, 2 H), 6.21-6.45 (m, 1 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 20.3, 20.6, 20.7, 21.0, 46.6, 47.3, 65.3, 65.8, 75.8, 76.1, 80.6, 81.5, 82.6, 84.4, 92.8, 99.2, 152.1, 152.3, 168.9, 169.0, 169.3, 169.7.

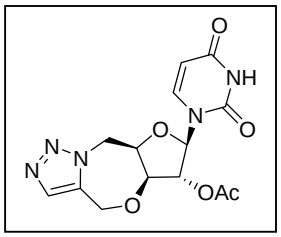
MS: m/z = 299.26 (M+1).

General procedure for Vorbruggen's One Pot synthesis of nucleosides:

A solution of diacetate (anomeric mixture) (2 mmol) and corresponding nucleobase (2 mmol) in 15 ml of anhydrous acetonitrile was charged consecutively with HMDS (0.5 ml, 2.4 mmol), TMSCl (0.35 ml, 2.8 mmol) and TfOH (0.21 ml, 2. 4 mmol) under argon. The reaction mixture was stirred at room temperature for 30 min whereupon the solution becomes turbid. Then the reaction mixture was refluxed under argon atmosphere and after completion of the reaction monitored by TLC, the acetonitrile suspension was cooled, diluted with 200 ml of ethyl acetate and the mixture was extracted with 25 ml of saturated solution of sodium bicarbonate. After reextracting the aqueous phase with another 25 ml of ethyl acetate, the combined extracts were washed with 25 ml of brine solution, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude nucleosides were purified

on silica gel column chromatography using ethyl acetate-petroleum ether mixture as eluent.

Compound No. 159:



Colorless solid: **m.p.:** 225 ^{o}C.

$[\alpha]_D^{25}$ = +72.88 (c, 1.18, MeOH).

IR (cm^{-1}): 2923.88, 1714.60, 1693.38, 1681.81, 1461.94.

^{1}H NMR [DMSO-d$_6$+ CDCl$_3$, 200 MHz]: δ 2.50 (s, 3 H), 4.76 (s, 2 H), 5.24 (ABq, 2 H), 5.27 (dd, 1 H, *J* = 0.96, 14.91 Hz), 5.44 (s, 1 H), 5.64-5.73 (m, 1 H), 5.80 (d, 1 H, *J* = 8.21 Hz), 6.29 (d, 1 H, *J* = 1.26 Hz), 7.04 (d, 1 H, *J* = 8.21 Hz), 8.01 (s, 1 H), 11.02 (s, 1 H).

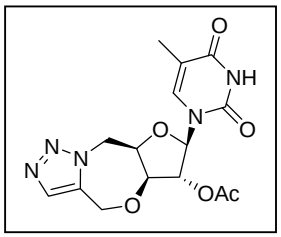
^{13}C NMR [DMSO-d$_6$+CDCl$_3$, 50 MHz]: δ 19.41, 46.20, 58.30, 74.47, 79.37, 81.09, 86.53, 100.85, 131.15, 134.58, 137.70, 149.06, 162.09, 167.74.

CHNS Anal.: Calculated for $C_{14}H_{15}N_5O_6$: C, 48.14; H, 4.33; N, 20.05.

Found. C, 48.05; H, 4.38; N, 19.79.

MS: m/z = 350.69 (M+1).

Compound No. 160:



Colorless solid: **m.p.:** 180 ^{o}C.

$[\alpha]_D^{25}$ = +6.19 (c, 1.68, MeOH).

IR (cm^{-1}): 1755.10, 1693.38, 1681.81, 1467.73, 1377.08.

^{1}H NMR [DMSO-d$_6$, 200 MHz]: δ 1.53 (s, 3 H), 2.07 (s, 3 H), 4.28-4.52 (m, 1 H), 4.74 (d, 1 H, *J* = 14.78 Hz), 4.87-5.05 (m, 3 H), 5.24 (dd, 1 H, *J* = 4.55, 15.54 Hz), 5.75 (d, 1 H, *J* = 0.76 Hz), 6.28 (d, 1 H, *J* = 0.63 Hz), 7.61 (s, 1 H), 7.85 (s, 1 H), 11.17 (s, 1 H).

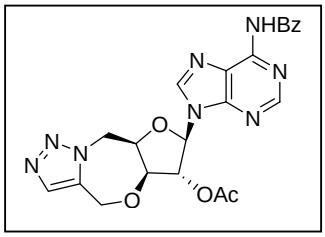
^{13}C NMR [DMSO-d_6+$CDCl_3$, 50 MHz]: δ 12.47, 20.84, 47.44, 58.97, 75.93, 80.66, 82.01, 87.63, 109.18, 132.72, 134.87, 136.56, 150.39, 163.94, 169.46.

CHNS Anal.: Calculated for $C_{15}H_{17}N_5O_6$: C, 49.59; H, 4.72; N, 19.28.

Found. C, 48.77; H, 4.60; N, 18.97.

MS: m/z = 386.11 ($M+Na^+$).

Compound No. 161:



Colorless solid: **m.p.:** 130 °C.

$[\alpha]_D^{25}$ = -3.04 (c, 1.315, MeOH).

IR (cm^{-1}): 3404.13, 1757.03, 1701.10, 1612.38, 1454.23.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 2.20 (s, 3 H), 4.46 (dd, 1 H, *J* = 0.88, 2.52 Hz), 4.56-4.62 (m, 2 H), 4.89 (dd, 1 H, *J* = 2.52, 15.03 Hz), 4.95 (ABq, 2 H, *J* = 15.16 Hz), 5.30 (dd, 1 H, *J* = 5.81, 15.16 Hz), 5.48 (t, 1 H, *J* = 1.28 Hz), 6.34 (d, 1 H, *J* = 1.39 Hz), 7.48-7.61 (m, 3 H), 7.65 (s, 1 H), 7.80 (s, 1 H), 8.00-8.05 (m, 2 H), 8.77 (s, 1 H).

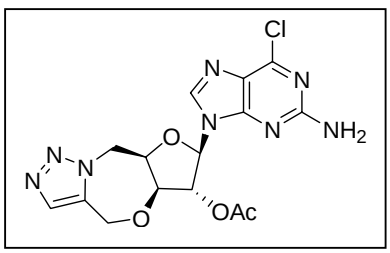
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 20.69, 47.79, 60.92, 76.79, 80.89, 83.01, 87.02, 122.79, 127.99, 128.79, 132.53, 132.79, 133.53, 134.61, 141.02, 149.61, 151.51, 152.73, 165.05, 169.18.

CHNS Anal.: Calculated for $C_{22}H_{20}N_8O_5$: C, 55.46; H, 4.23; N, 23.52.

Found. C, 55.50; H, 4.43; N, 23.45.

MS: m/z = 477.71 (M+1).

Compound No. 162:



Colorless solid: **m.p.:** 200 °C.

$[\alpha]_D^{25}$ = +28.14 (c, 1.13, MeOH).

IR (cm^{-1}): 3321.19, 3203.54, 1741.60, 1614.31, 1564.87, 1463.87, 1375.15.

^{1}H NMR [MeOH-D_4 + CDCl3, 200 MHz]: δ 2.19 (s, 3 H), 4.54-4.65 (m, 4 H), 4.98 (ABq, 2 H, *J* = 14.90 Hz), 4.99 (dd, 1 H, *J* = 1.89, 15.41 Hz), 5.27-5.40 (m, 2 H), 6.08 (d, 1 H, *J* = 1.27 Hz), 7.47 (s, 1 H), 7.69 (s, 1 H).

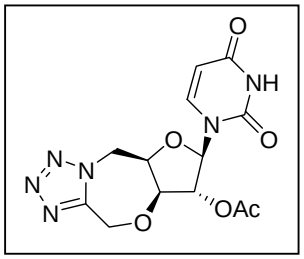
^{13}C NMR [MeOH-D4 + CDCl3, 50 MHz]: δ 20.64, 48.15, 60.47, 76.90, 81.25, 83.30, 87.25, 124.32, 132.71, 136.32, 140.82, 151.29, 153.69, 160.42, 170.18.

CHNS Anal.: Calculated for $C_{15}H_{15}ClN_8O_4$: C, 44.29; H, 3.72; Cl, 8.72; N, 27.55.

Found. C, 43.98; H, 3.83; Cl, 8.68; N, 26.99.

MS: m/z = 407.00 (M+1).

<u>Compound No. 163</u>:



Colorless solid: **m.p.:** 126 °C.

$[\alpha]_D^{25}$ = +13.27 (c, 1.1, MeOH).

IR (cm^{-1}): 1731.96, 1693.38, 1681.81, 1461.94, 1377.08.

^{1}H NMR [MeOH-d_4, 200 MHz]: δ 2.13 (s, 3 H), 4.50-4.56 (m, 1 H), 4.62 (dd, 1 H, *J* = 1.89, 3.16 Hz), 4.96-5.08 (m, 2 H), 5.18-5.38 (m, 3 H), 5.58 (d, 1 H, *J* = 8.09 Hz), 5.95 (d, 1 H, *J* = 2.78 Hz), 7.10 (d, 1 H, *J* = 8.09 Hz).

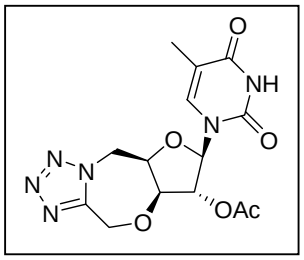
^{13}C NMR [MeOH-d_4 + $CDCl_3$, 50 MHz]: δ 18.67, 45.32, 60.26, 74.53, 79.36, 82.13, 87.00, 101.43, 139.15, 149.70, 153.29, 163.45, 169.03.

CHNS Anal.: Calculated for $C_{13}H_{14}N_6O_6$: C, 44.57; H, 4.03; N, 23.99.

Found. C, 44.68; H, 4.18; N, 23.85.

MS: m/z = 351.83 (M+1).

<u>Compound No. 164</u>:



Colorless solid: **m.p.:** 123 °C.

$[\alpha]_D^{25}$ = +4.38 (c, 1.14, MeOH).

IR (cm^{-1}): 1745.46, 1693.38, 1681.81, 1461.94, 1377.08.

^{1}H NMR [DMSO-d_6+$CDCl_3$, 200 MHz]: δ 1.73 (s, 3 H), 2.14 (s, 3 H), 4.30-4.45 (m, 1 H), 4.47-4.66 (m, 2 H), 5.06 (d, 1 H, *J* = 15.91 Hz), 5.17-2.21 (m, 2 H), 5.34 (d, 1 H, *J* = 15.41 Hz), 5.96 (d, 1 H, *J* = 2.90 Hz), 6.72 (d, 1 H, *J* = 1.01 Hz), 11.16 (s, 1 H).

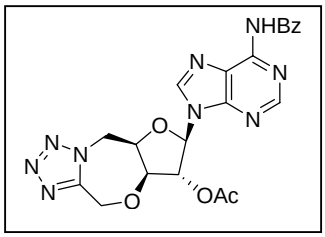
^{13}C NMR [MeOH-d_4+$CDCl_3$, 50 MHz]: δ 12.64, 20.75, 47.38, 61.87, 76.58, 81.44, 84.11, 89.09, 111.80, 136.53, 151.91, 155.51, 165.85, 170.99.

CHNS Anal.: Calculated for $C_{14}H_{16}N_6O_6$: C, 46.16; H, 4.43; N, 23.07.

Found. C, 46.10; H, 4.33; N, 23.15.

MS: m/z = 365.03 (M+1).

<u>Compound No. 165</u>:



Gum.

$[\alpha]_D^{25}$ = -12.45 (c, 1.06, MeOH).

IR (cm^{-1}): 1747.39, 1681.81, 1614.31, 1454.23, 1377.08.

^{1}H NMR [MeOH-d_4+$CDCl_3$, 200 MHz]: δ 2.18 (s, 3 H), 4.69-4.86 (m, 2 H), 5.05 (dd, 1 H, *J* = 2.78, 14.78 Hz), 5.28 (ABq, 2 H, *J* = 15.66 Hz), 5.29 (dd, 1 H, *J* = 6.19, 14.78 Hz), 5.74 (t, 1 H, *J* = 2.40 Hz), 6.33 (d, 1 H, *J* = 2.77 Hz), 7.50-7.75 (m, 4 H), 8.05-8.08 (m, 2 H), 8.12 (s, 1 H), 8.73 (s, 1 H).

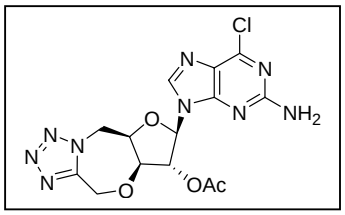
^{13}C NMR [MeOH-d_4+$CDCl_3$, 50 MHz]: δ 18.76, 45.61, 60.85, 75.59, 79.28, 82.41, 85.93, 127.37-127.80, 131.89, 141.21, 150.98, 151.53, 153.17, 162.01, 168.99.

CHNS Anal.: Calculated for $C_{21}H_{19}N_9O_5$: C, 52.83; H, 4.01; N, 26.40.

Found. C, 52.76; H, 4.10; N, 26.35.

MS: m/z = 478.84 (M+1).

Compound No. 166:



Colorless solid: **m.p.:** 170 ^{o}C (decomposed).

$[\alpha]_D^{25}$ = -11.00 (c, 1.60, MeOH).

IR (cm^{-1}): 3330.84, 3205.47, 1745.46, 1650.95, 1614.31, 1566.09, 1461.94.

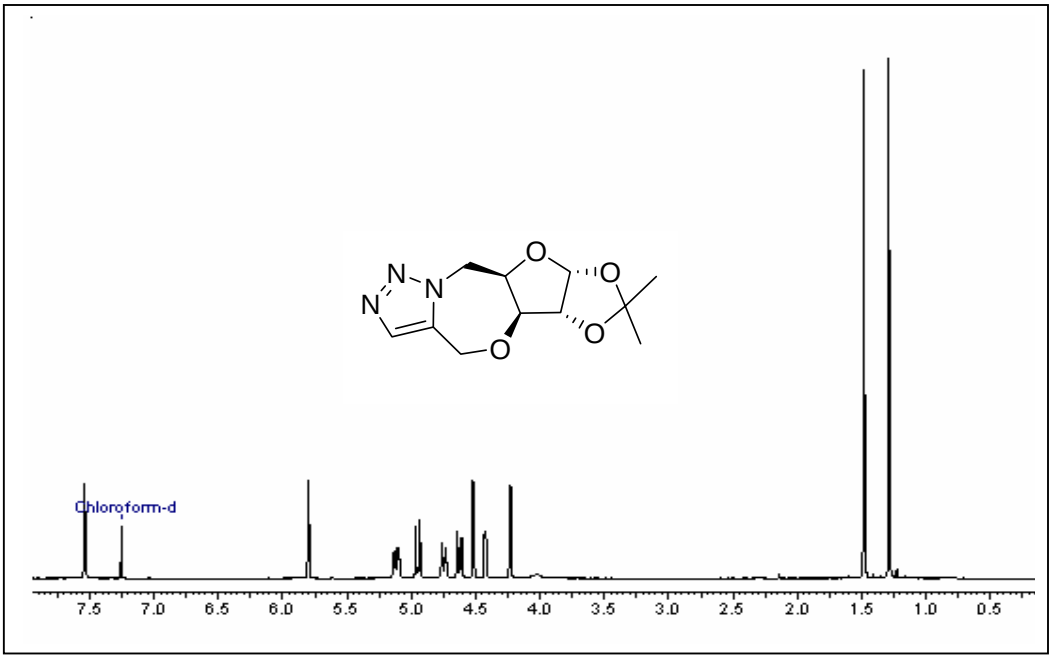
^{1}H NMR [MeOH-D$_4$+CDCl$_3$, 200 MHz]: δ 2.17 (s, 3 H), 4.64-4.74 (m, 4 H), 5.00 (dd, 1 H, *J* = 1.64, 13.89 Hz), 5.23 (dd, 1 H, *J* = 6.04, 14.40 Hz), 5.28 (ABq, 2 H, *J* = 15.79 Hz), 5.80 (m, 1 H), 6.08 (d, 1 H, *J* = 3.41 Hz), 7.82 (s, 1 H).

^{13}C NMR [MeOH-D4+CDCl3, 50 MHz]: δ 20.59, 46.48, 61.70, 75.62, 78.45, 82.53, 85.41, 120.16, 127.62, 150.24, 153.78, 159.97, 160.03, 169.36.

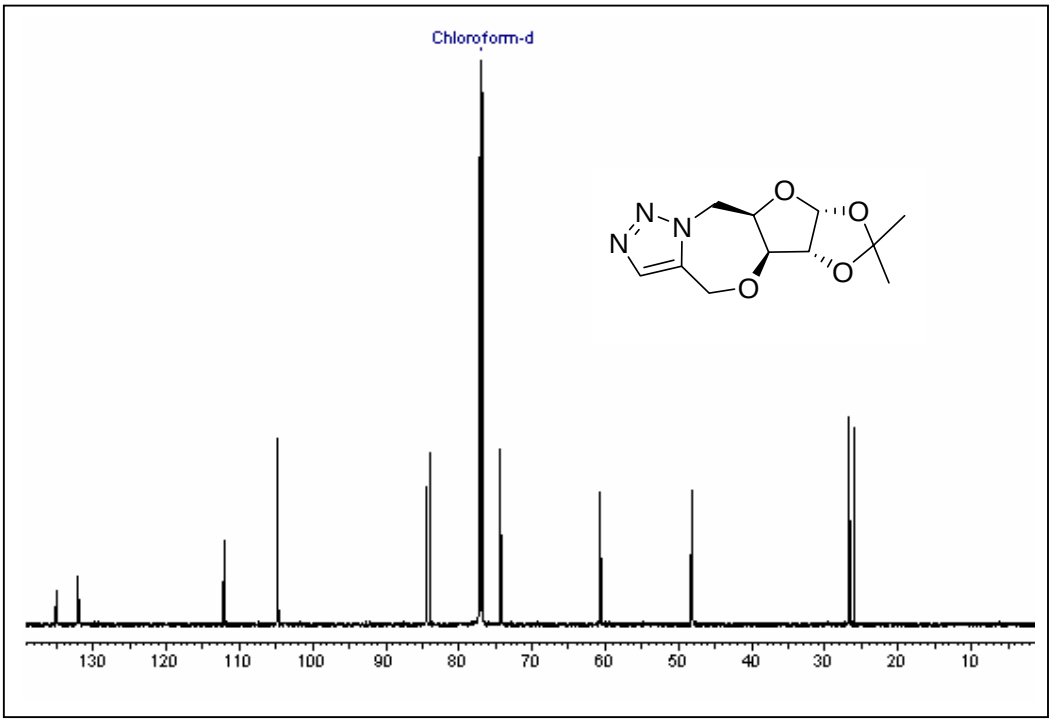
CHNS Anal.: Calculated for $C_{14}H_{14}ClN_9O_4$: C, 41.24; H, 3.46; Cl, 8.69; N, 30.91. Found. C, 41.15; H, 3.52; Cl, 8.72; N, 30.94.

MS: m/z = 408.99 (M+1).

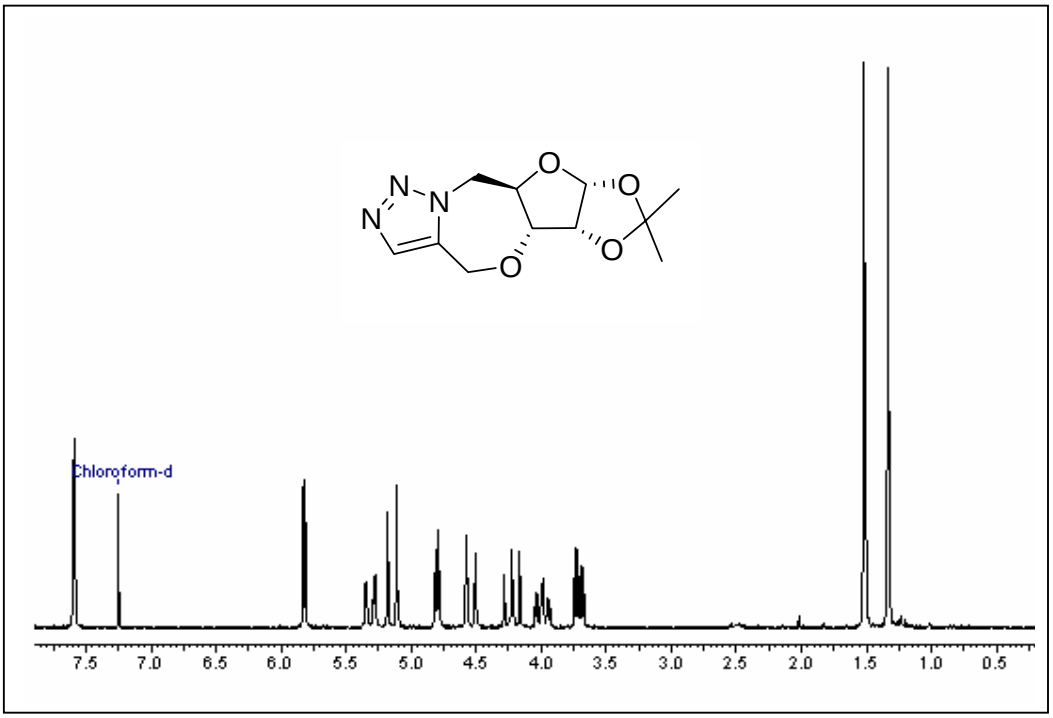
Chapter 2: Spectra



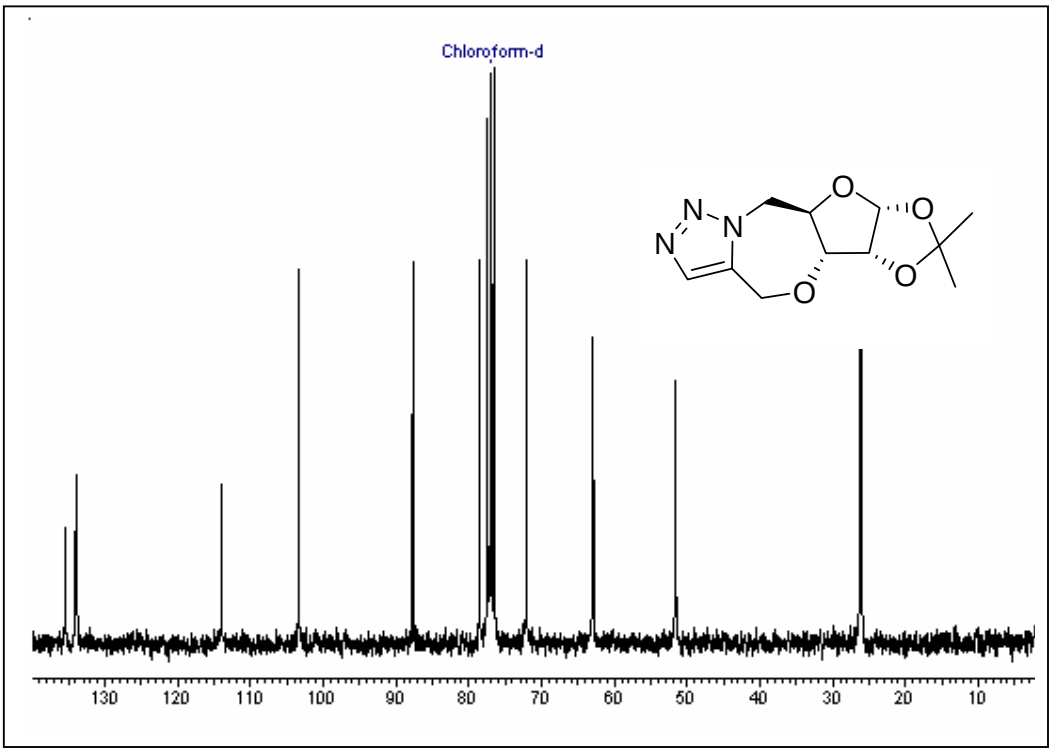
^{1}H NMR spectrum of compound **118** in $CDCl_3$



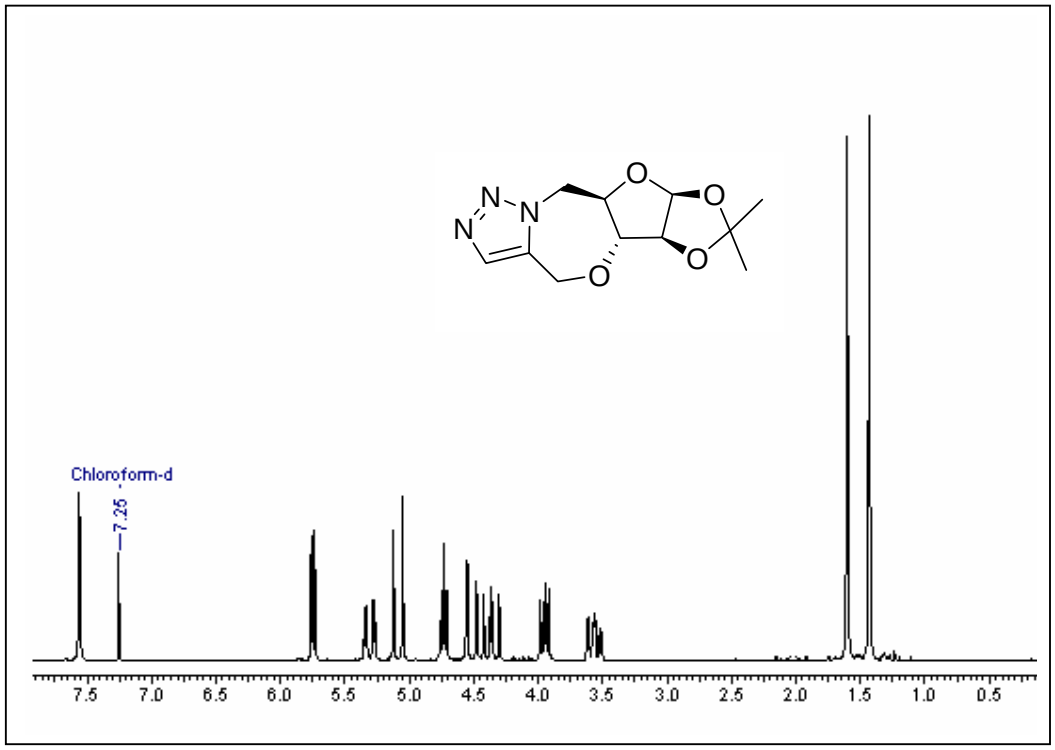
^{13}C NMR spectrum of compound **118** in $CDCl_3$



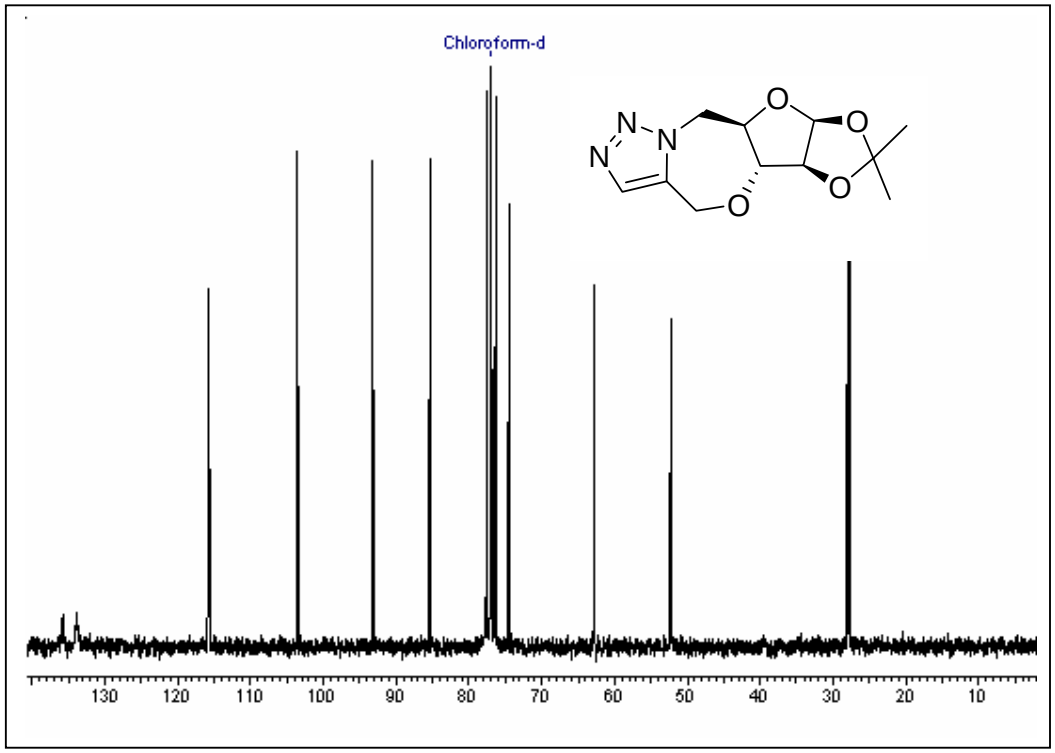
^{1}H NMR spectrum of compound **123** in $CDCl_3$



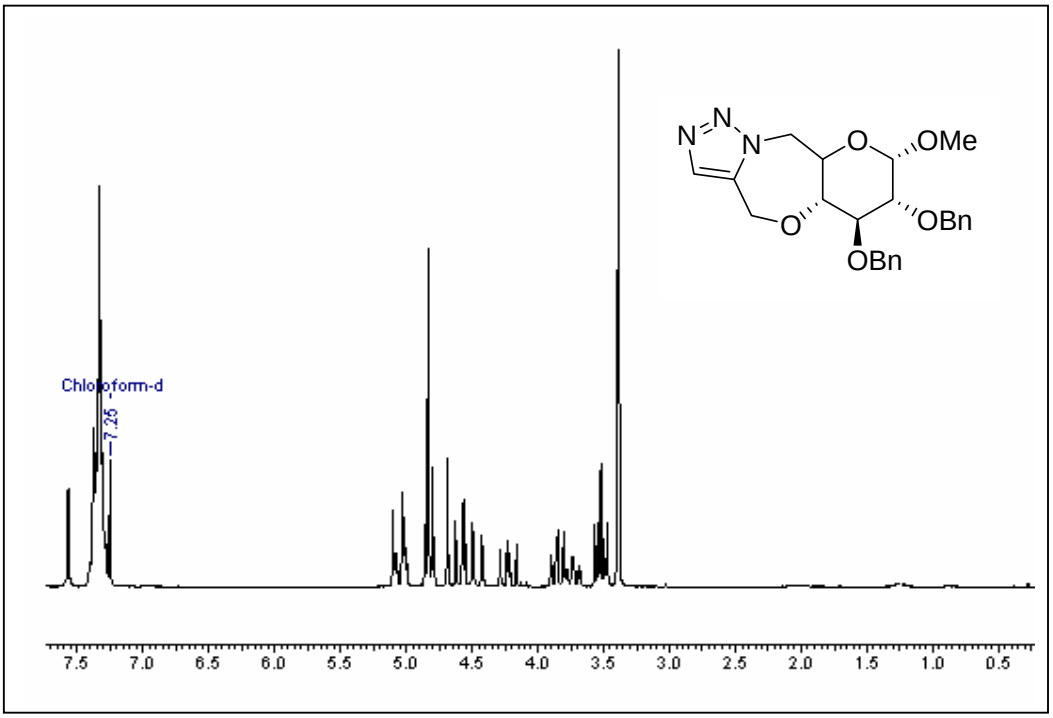
^{13}C NMR spectrum of compound **123** in $CDCl_3$



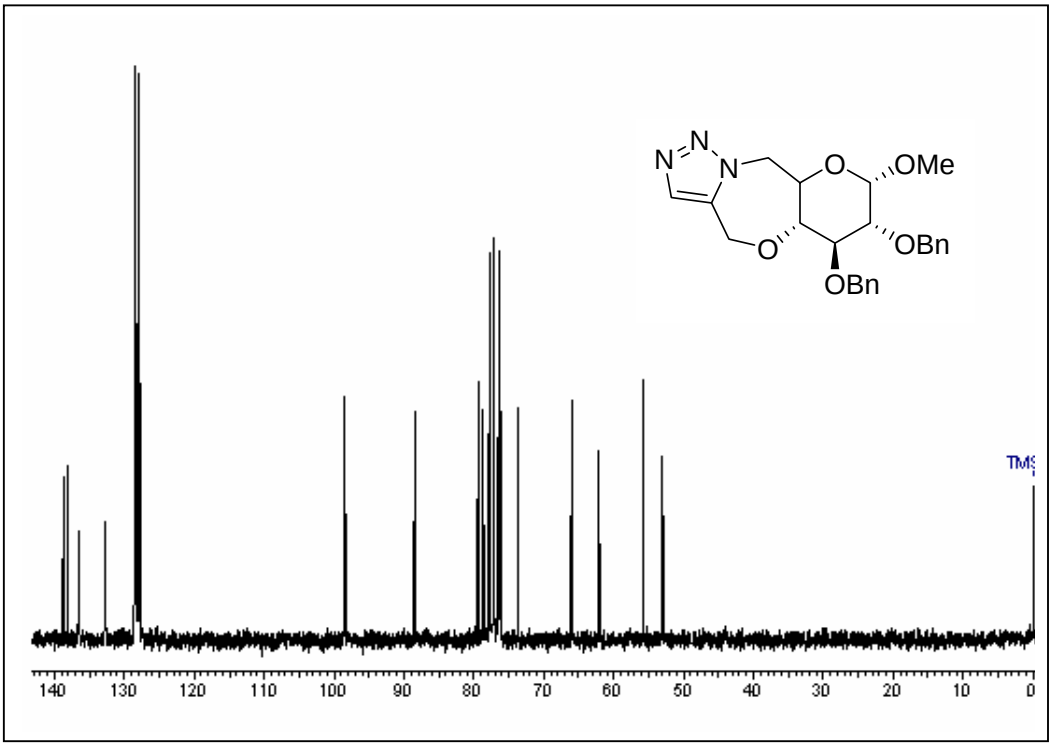
^{1}H NMR spectrum of compound **127** in $CDCl_3$



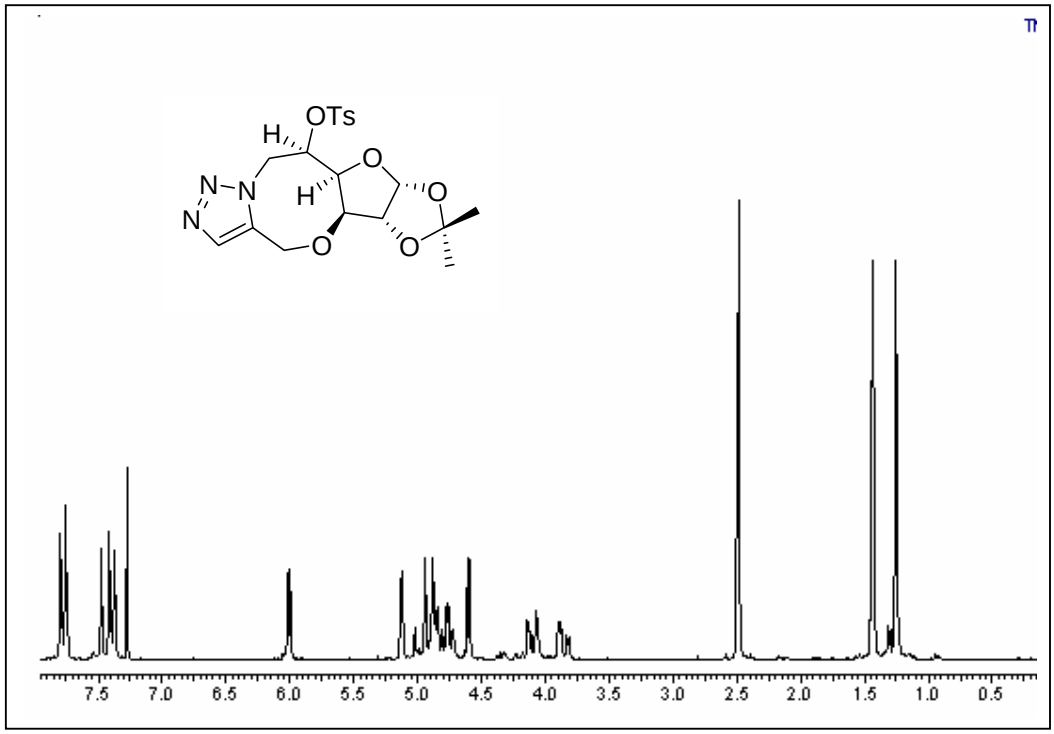
^{13}C NMR spectrum of compound **127** in $CDCl_3$



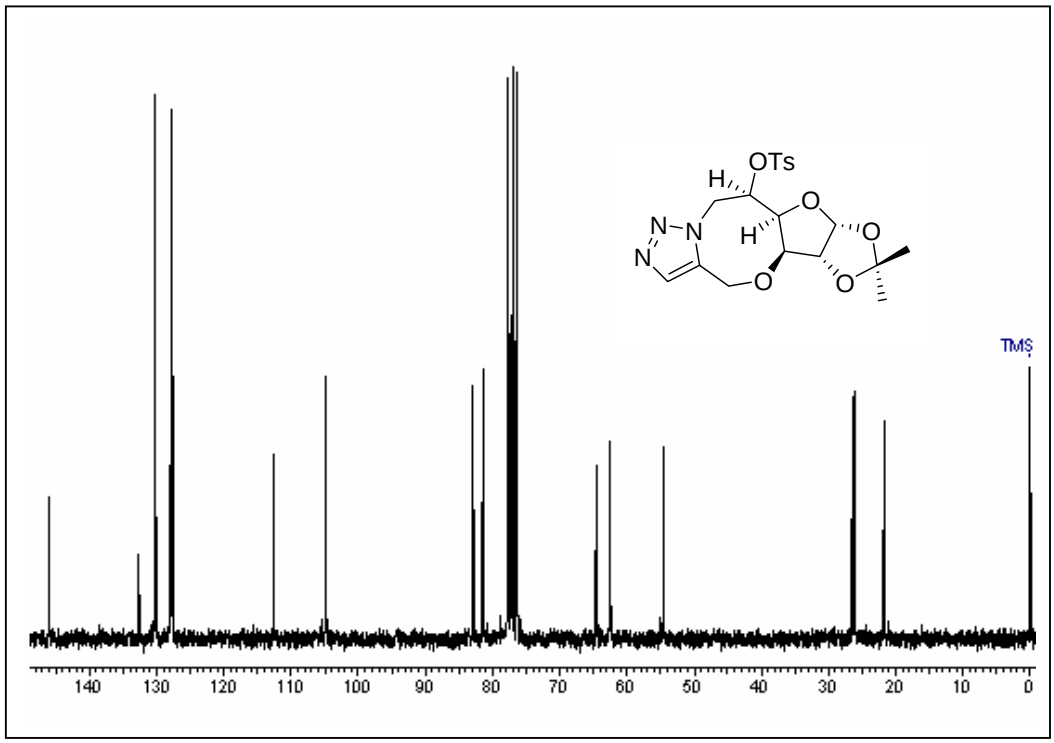
^{1}H NMR spectrum of compound **148** in $CDCl_3$



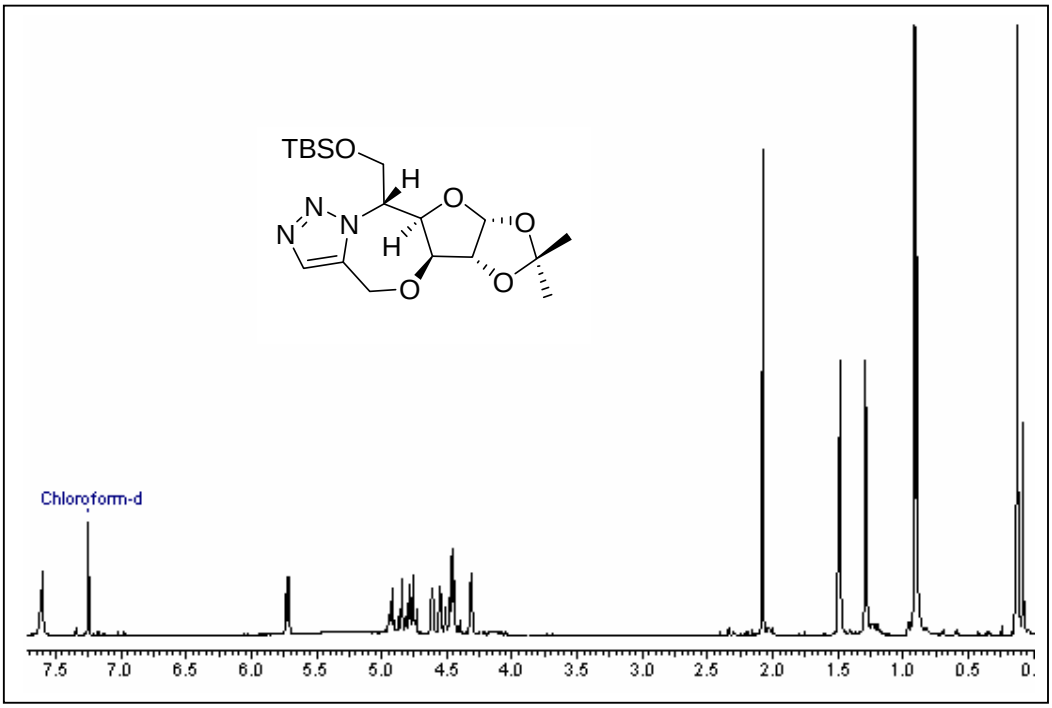
^{13}C NMR spectrum of compound **148** in $CDCl_3$



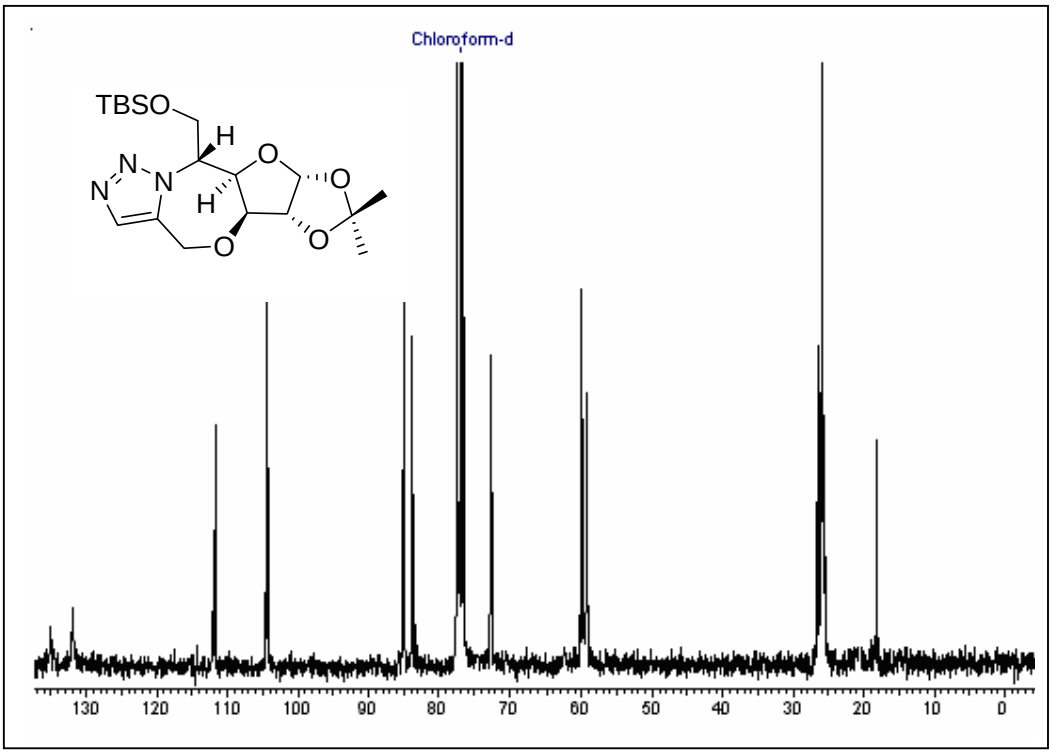
^{1}H NMR spectrum of compound **132** in $CDCl_3$



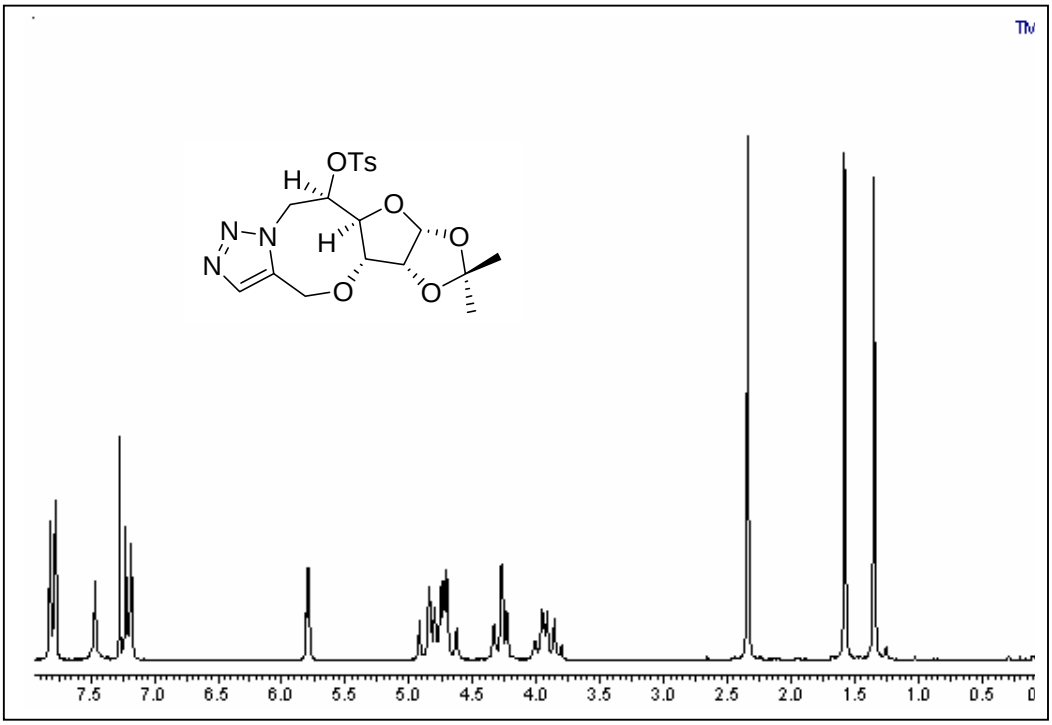
^{13}C NMR spectrum of compound **132** in $CDCl_3$



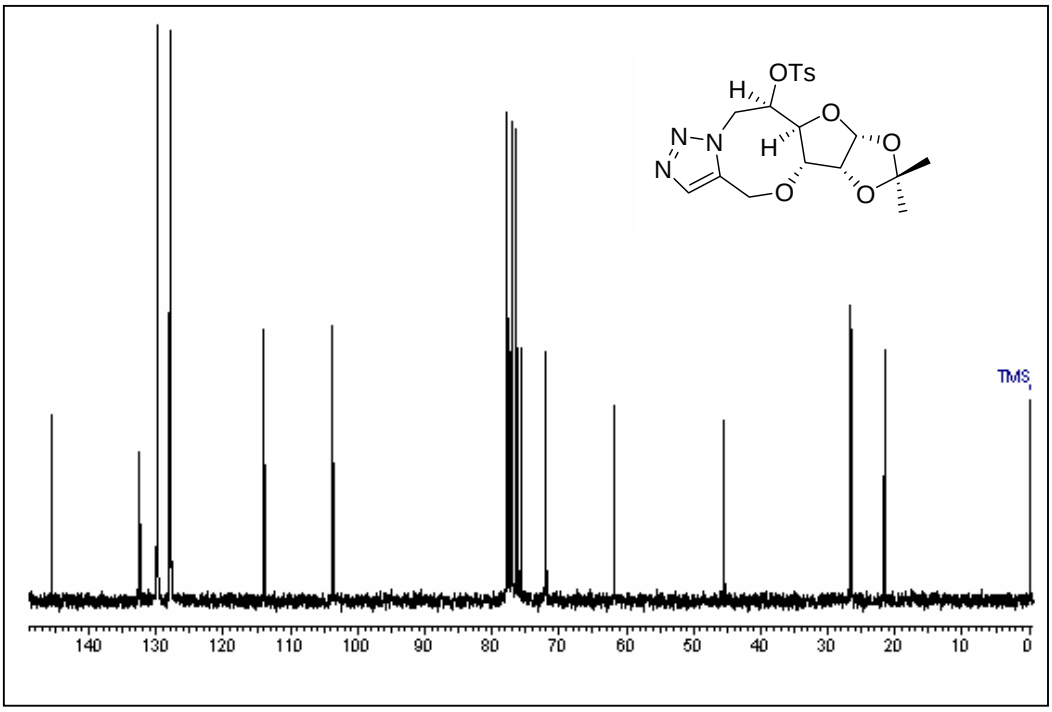
^{1}H NMR spectrum of compound **136** in $CDCl_3$



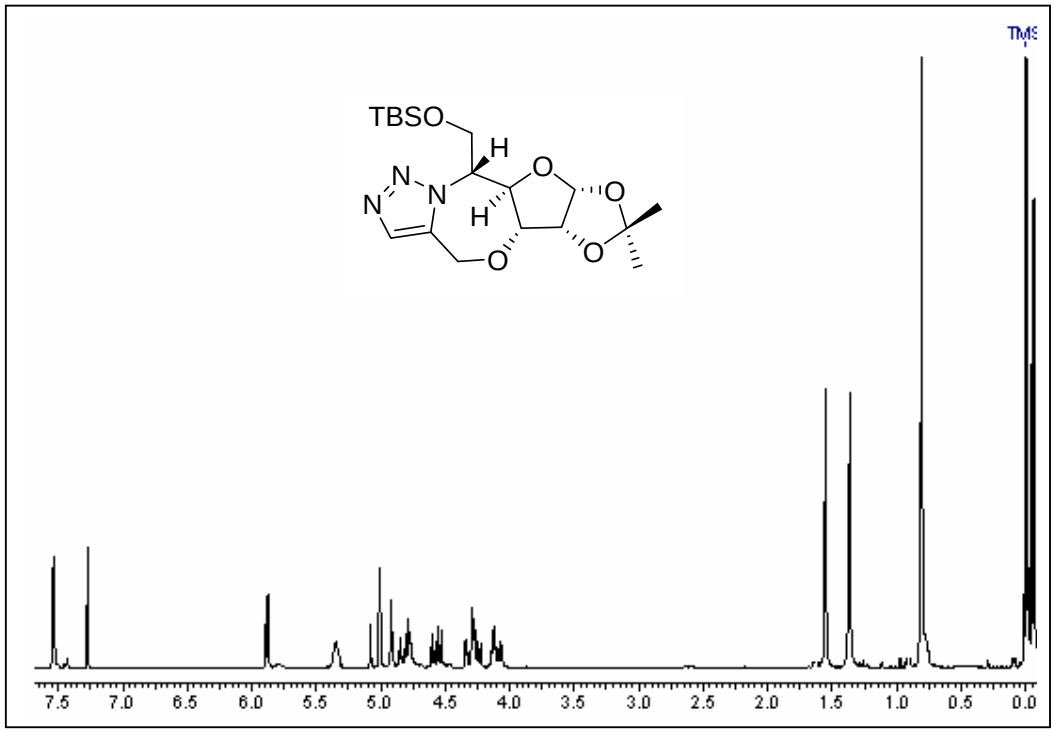
^{13}C NMR spectrum of compound **136** in $CDCl_3$



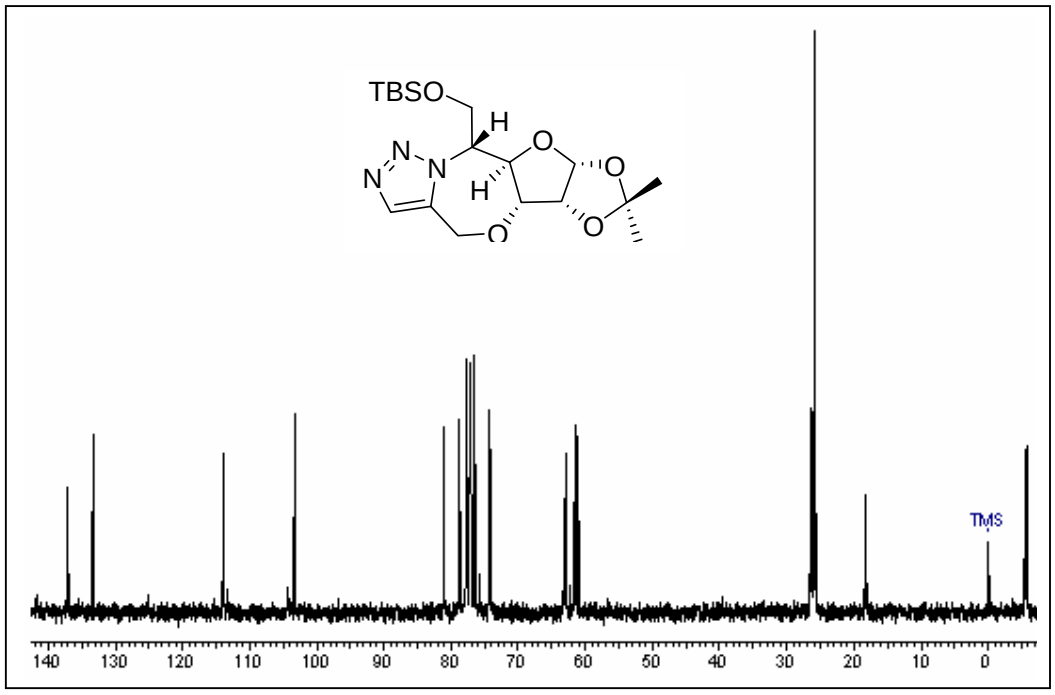
^{1}H NMR spectrum of compound **140** in $CDCl_3$



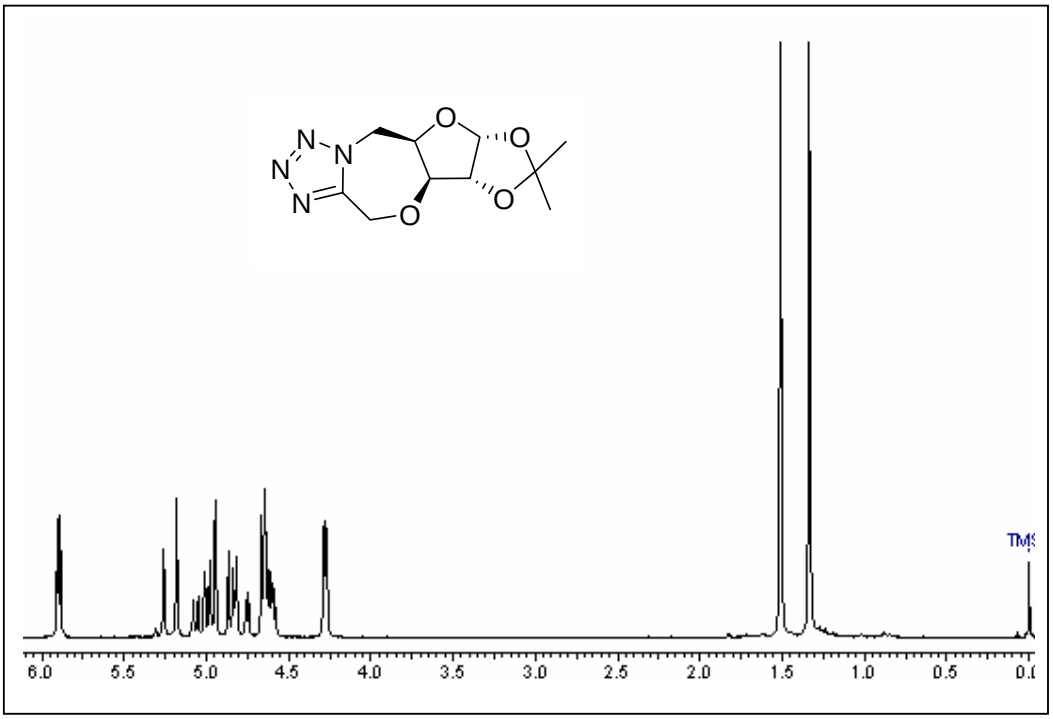
^{13}C NMR spectrum of compound **140** in $CDCl_3$



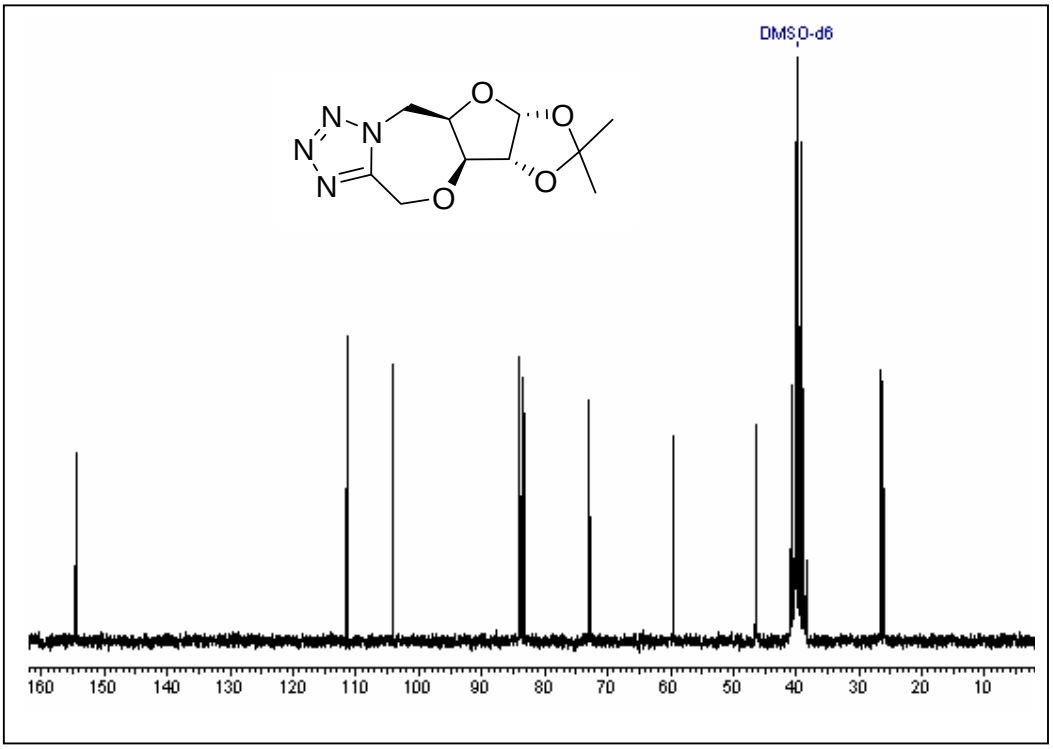
^{1}H NMR spectrum of compound **144** in $CDCl_3$



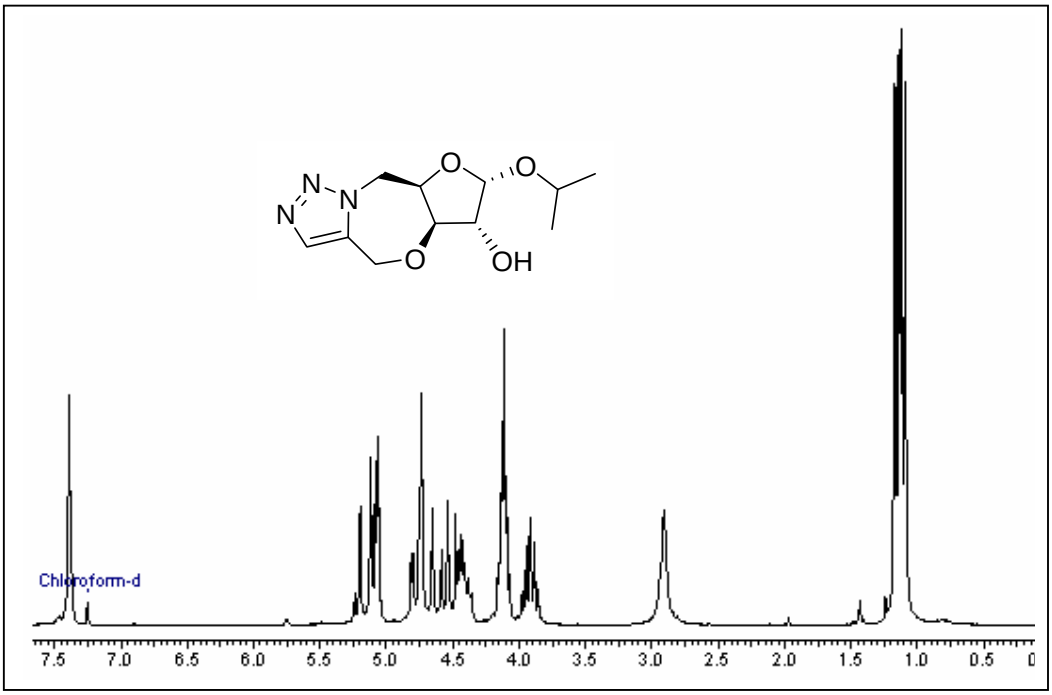
^{13}C NMR spectrum of compound **144** in $CDCl_3$



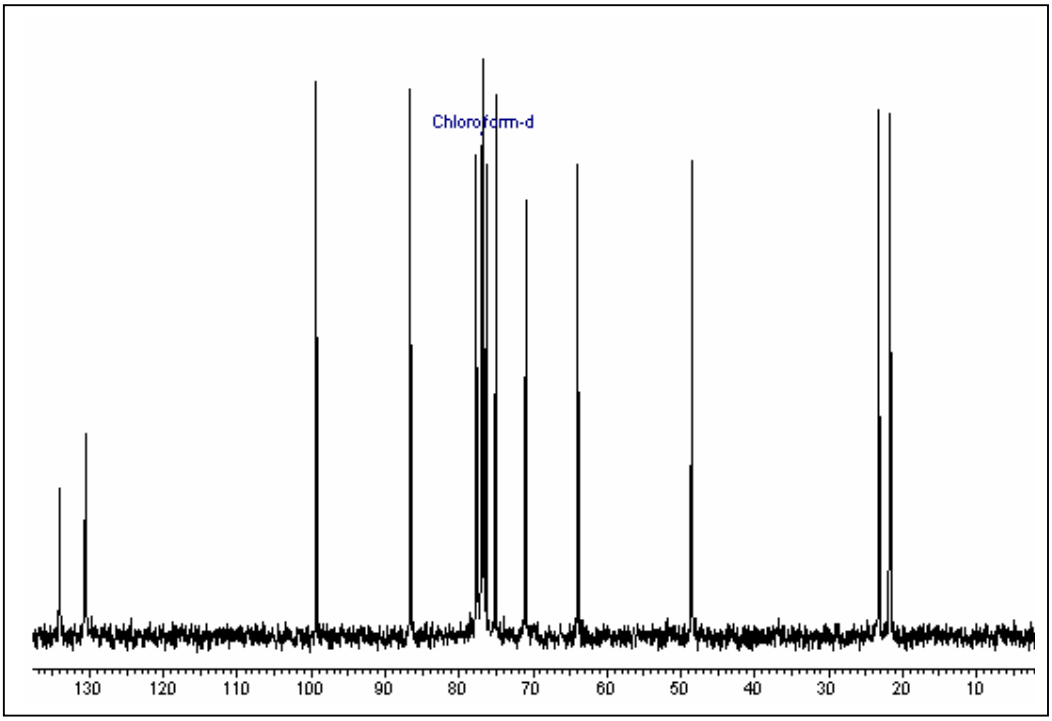
^{1}H spectra of compound **150** in $CDCl_3$



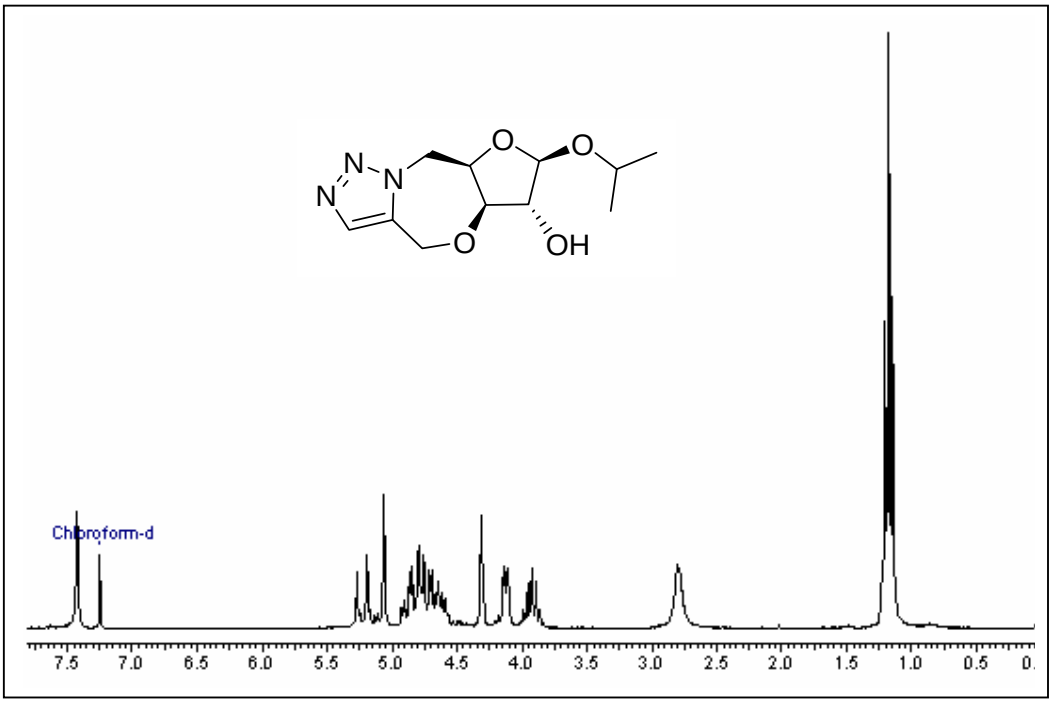
^{13}C NMR spectra of compound **150** in $DMSO_6$



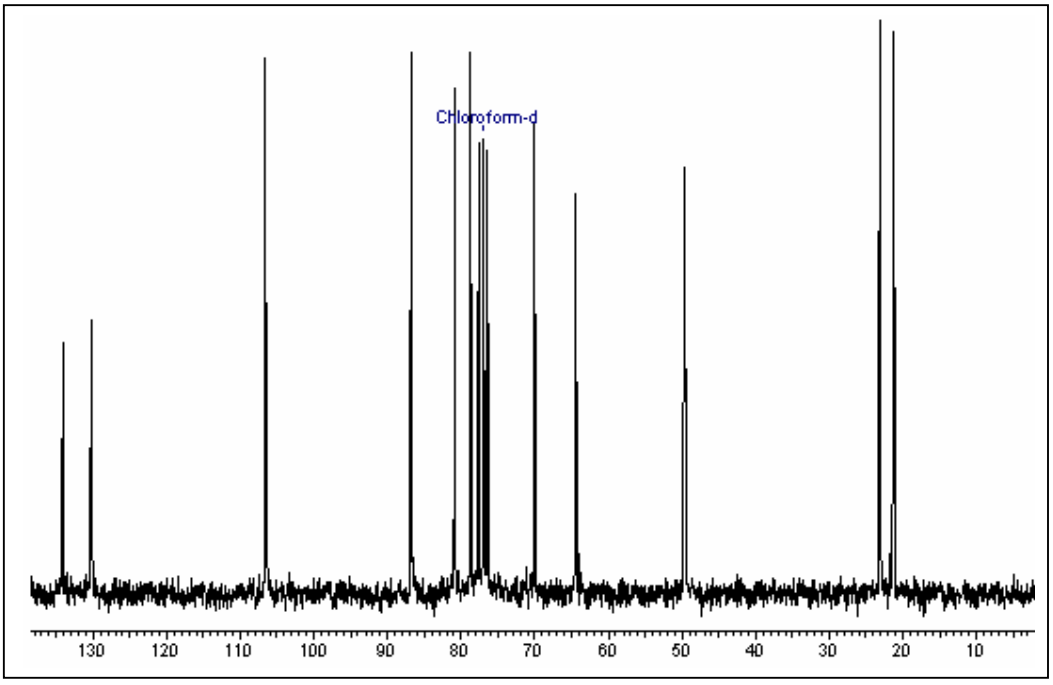
^{1}H NMR spectrum of compound **151 (α-glycoside)** in $CDCl_3$



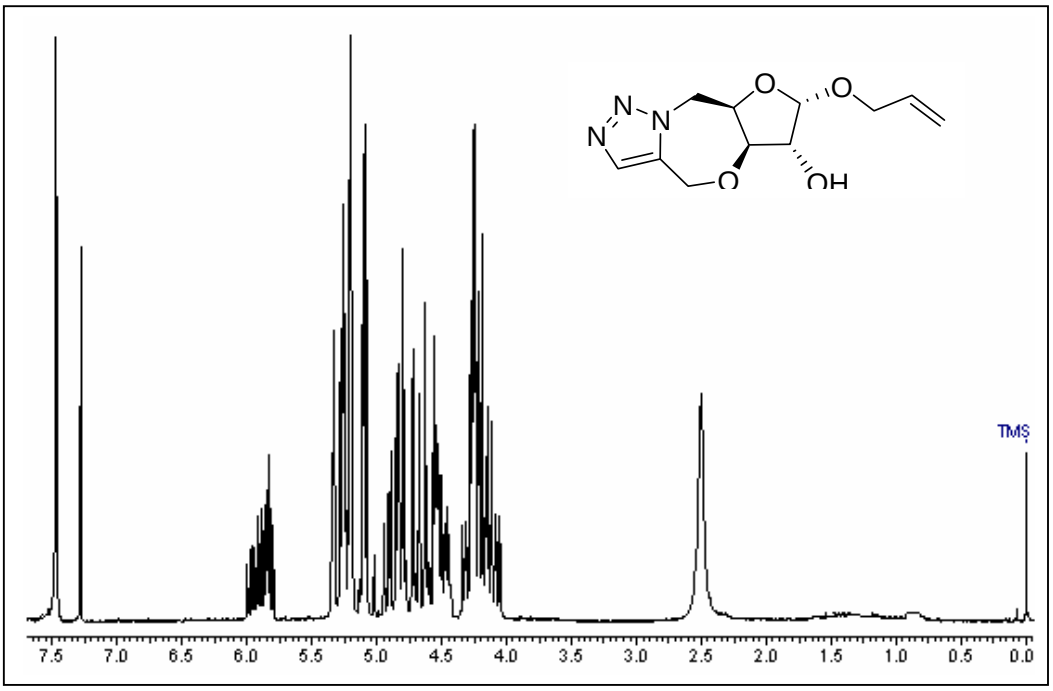
^{13}C NMR spectrum of compound **151 (α-glycoside)** in $CDCl_3$



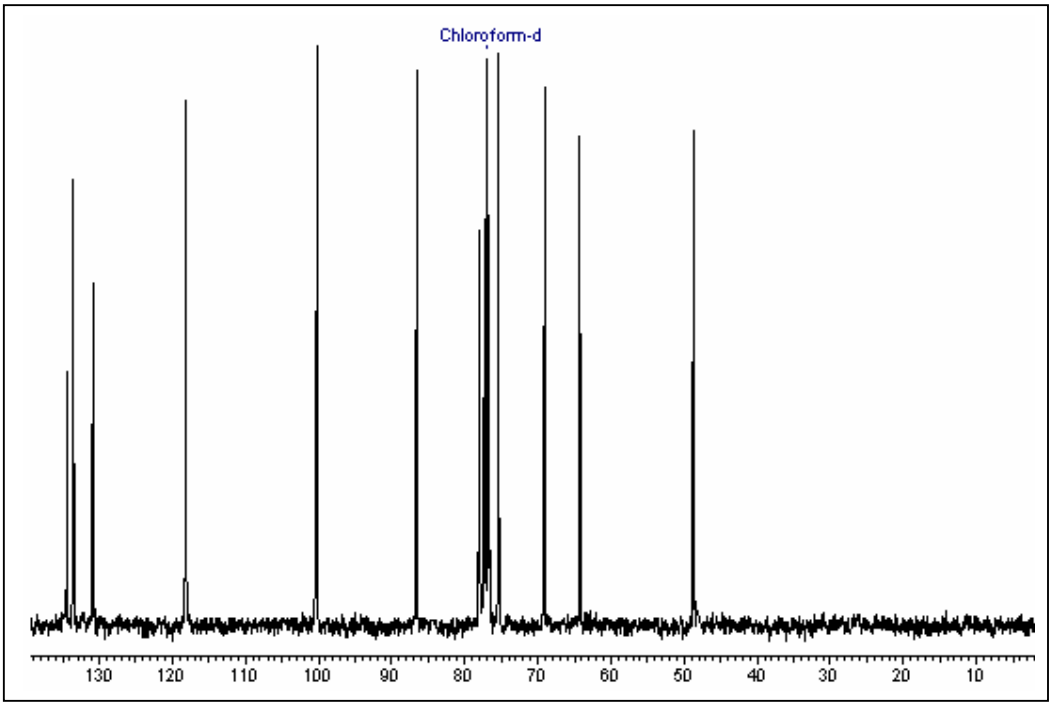
^{1}H NMR spectrum of compound **151 (β-glycoside)** in $CDCl_3$



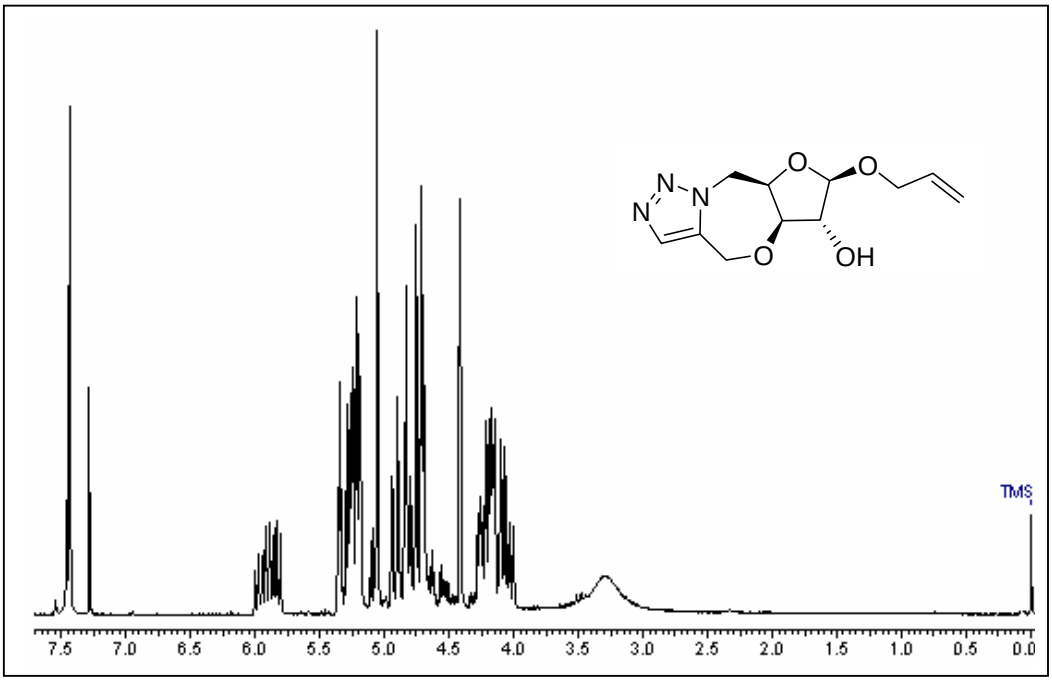
^{13}C NMR spectrum of compound **151 (β-glycoside)** in $CDCl_3$



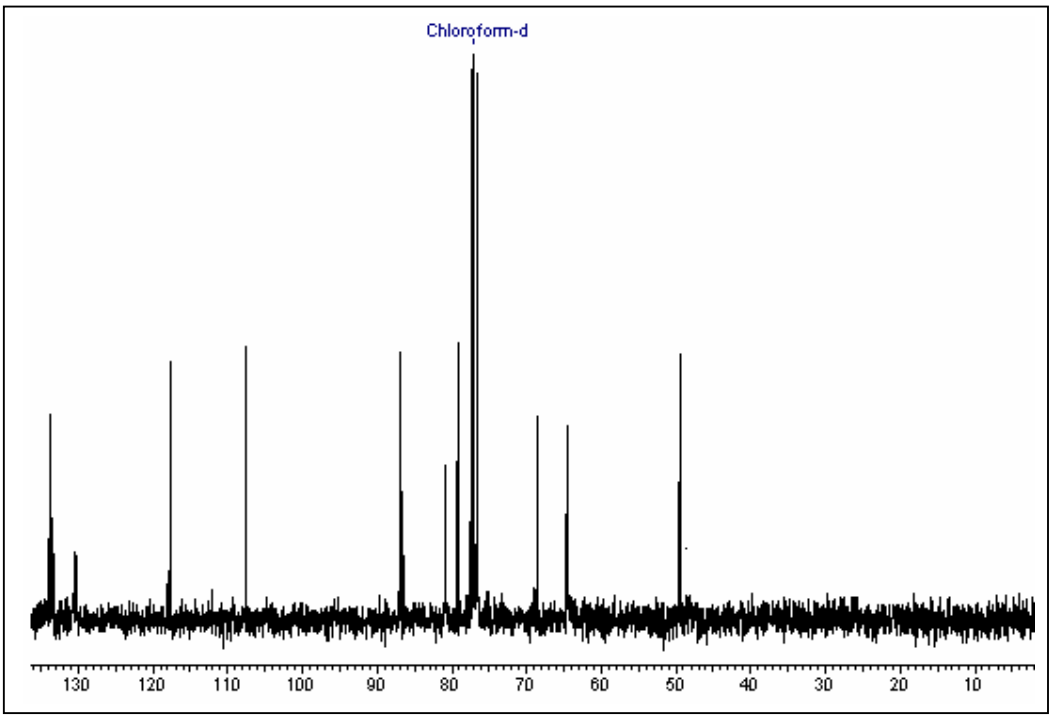
^{1}H NMR spectrum of compound **152 (α-glycoside)** in $CDCl_3$



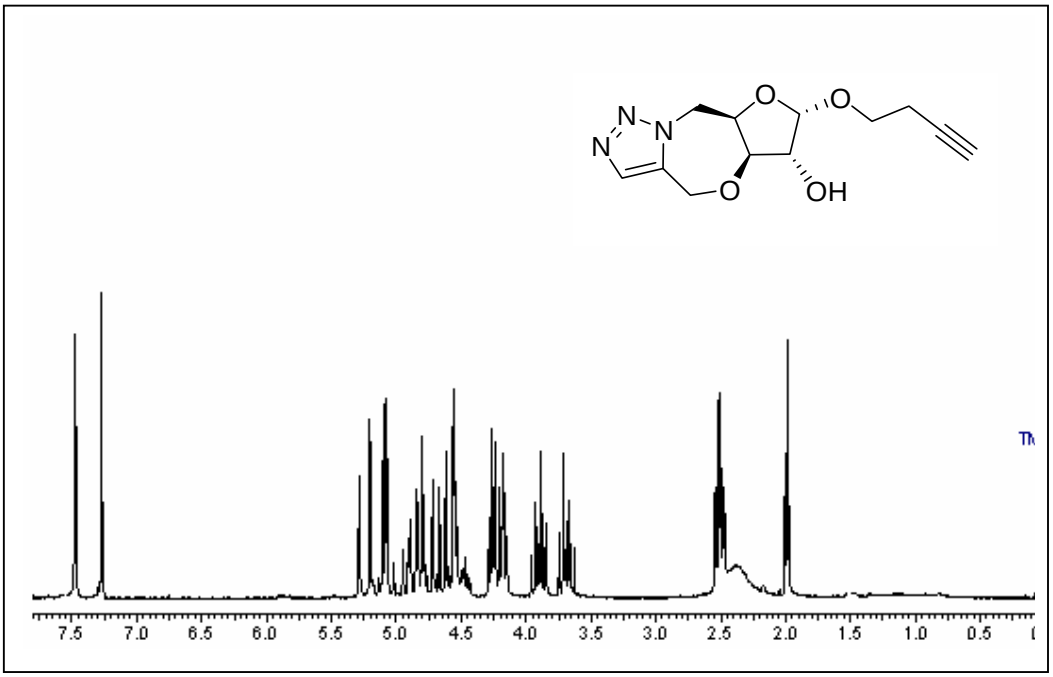
^{13}C NMR spectrum of compound **152 (α-glycoside)** in $CDCl_3$



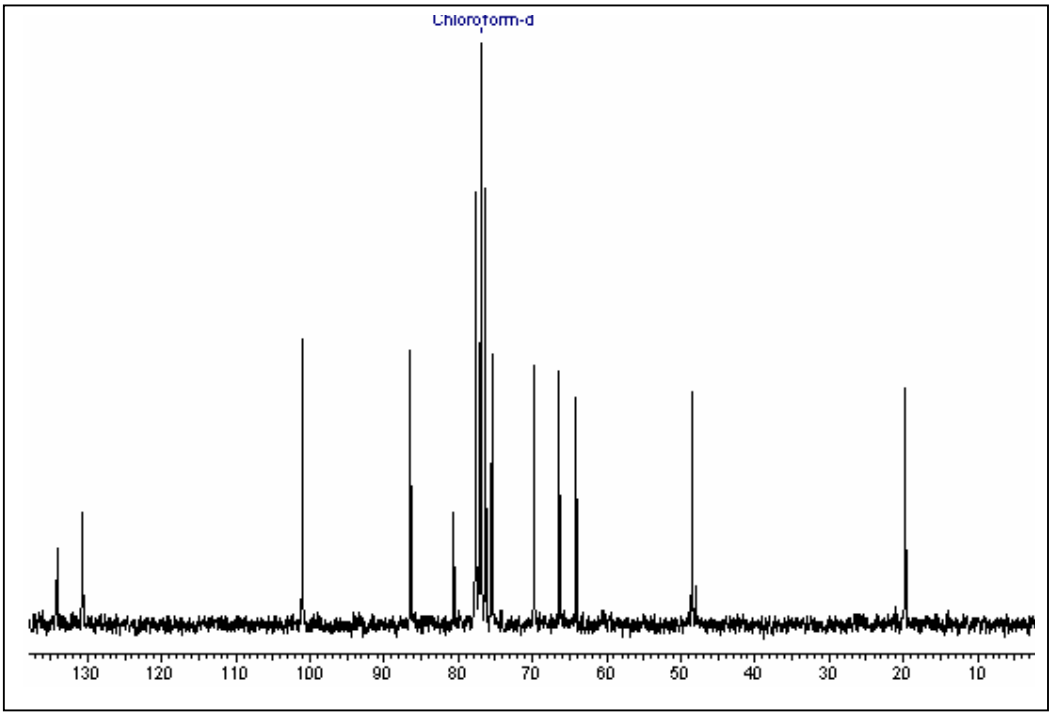
^{1}H NMR spectrum of compound **152 (β-glycoside)** in $CDCl_3$



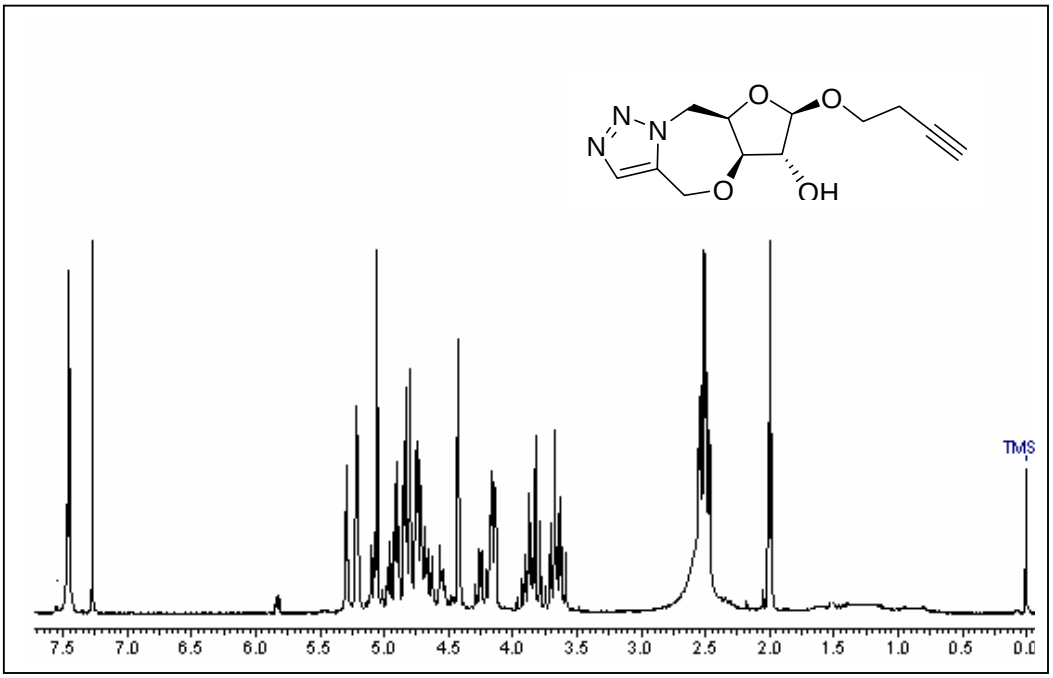
^{13}C NMR spectrum of compound **152 (β-glycoside)** in $CDCl_3$



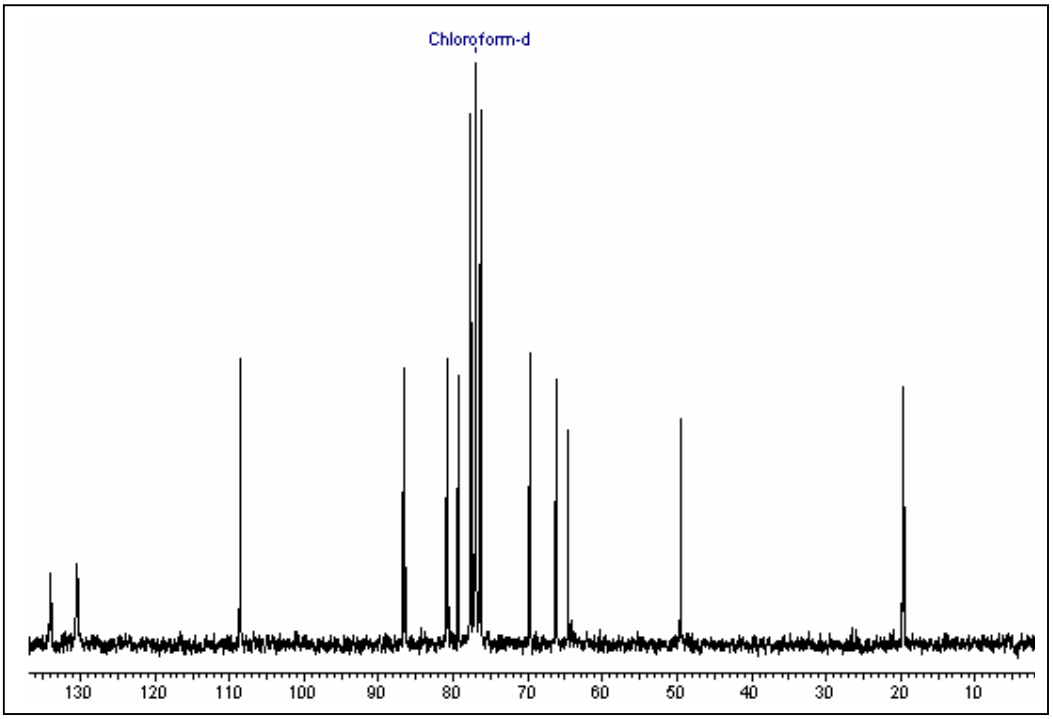
^{1}H NMR spectrum of compound **153 (α-glycoside)** in $CDCl_3$



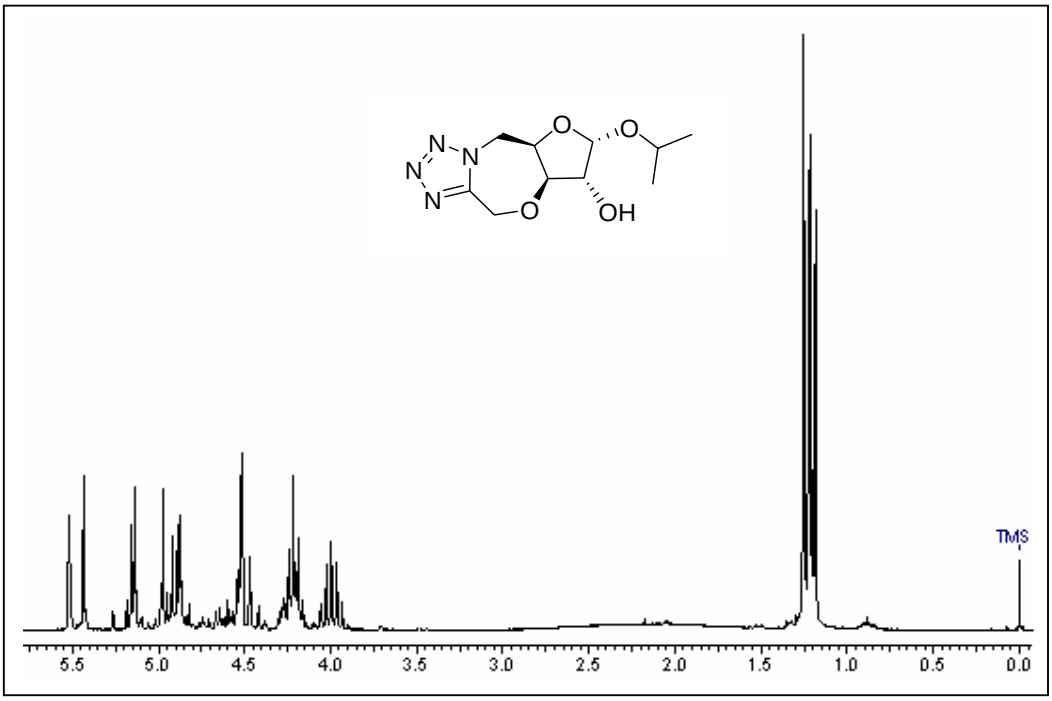
^{13}C NMR spectrum of compound **153 (α-glycoside)** in $CDCl_3$



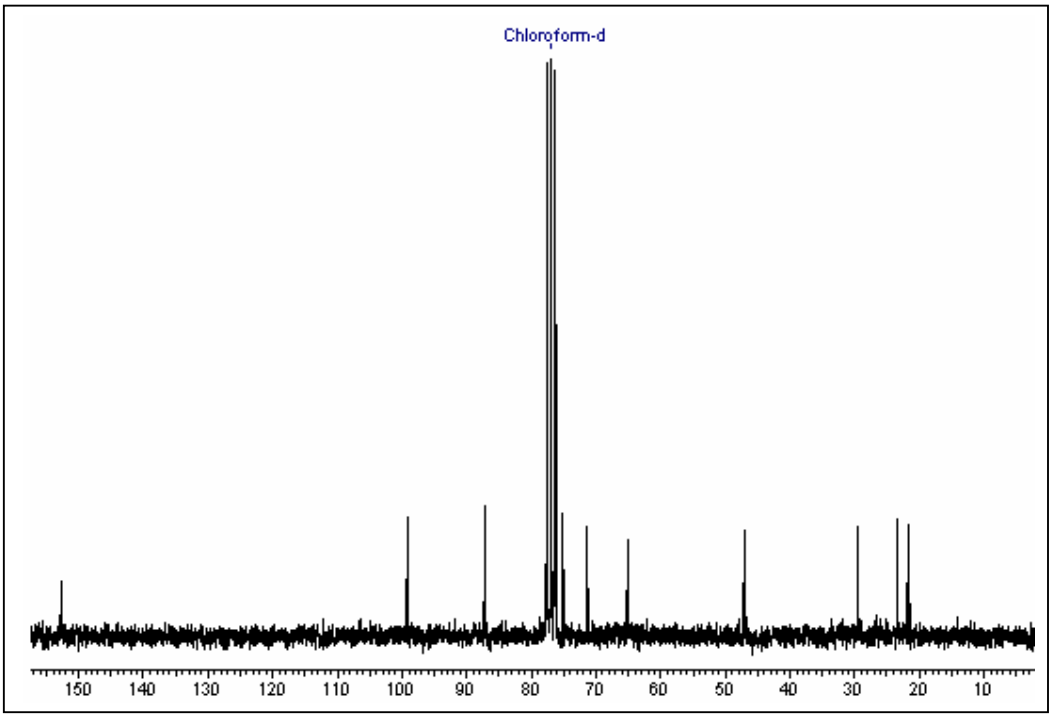
^{1}H NMR spectrum of compound **153 (β-glycoside)** in $CDCl_3$



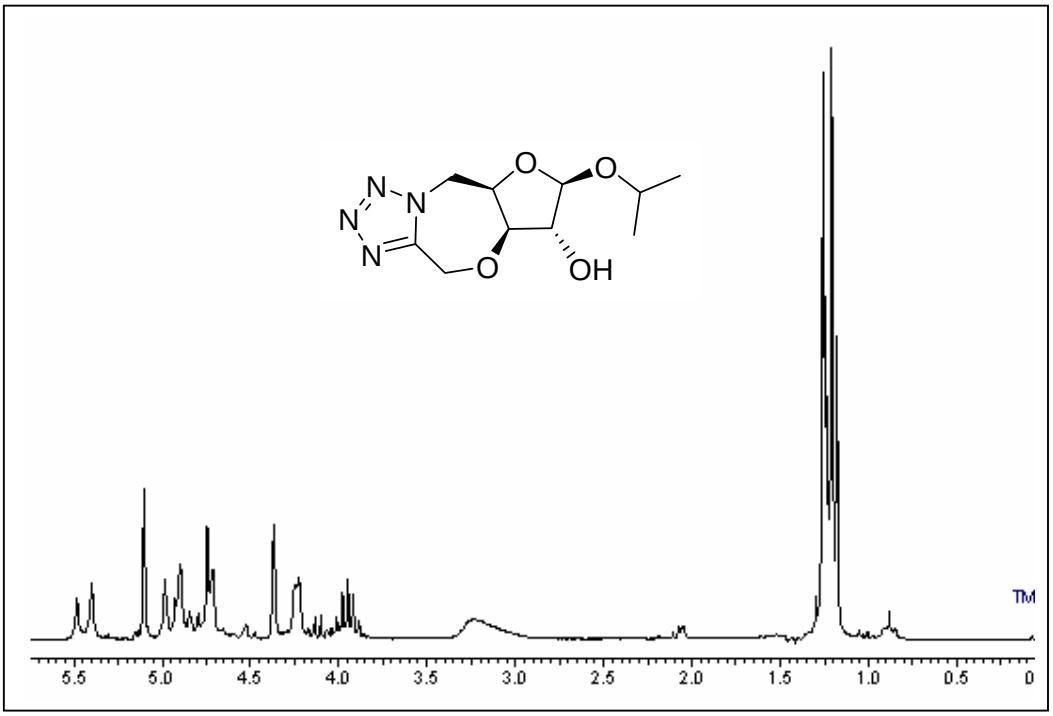
^{13}C NMR spectrum of compound **153 (β-glycoside)** in $CDCl_3$



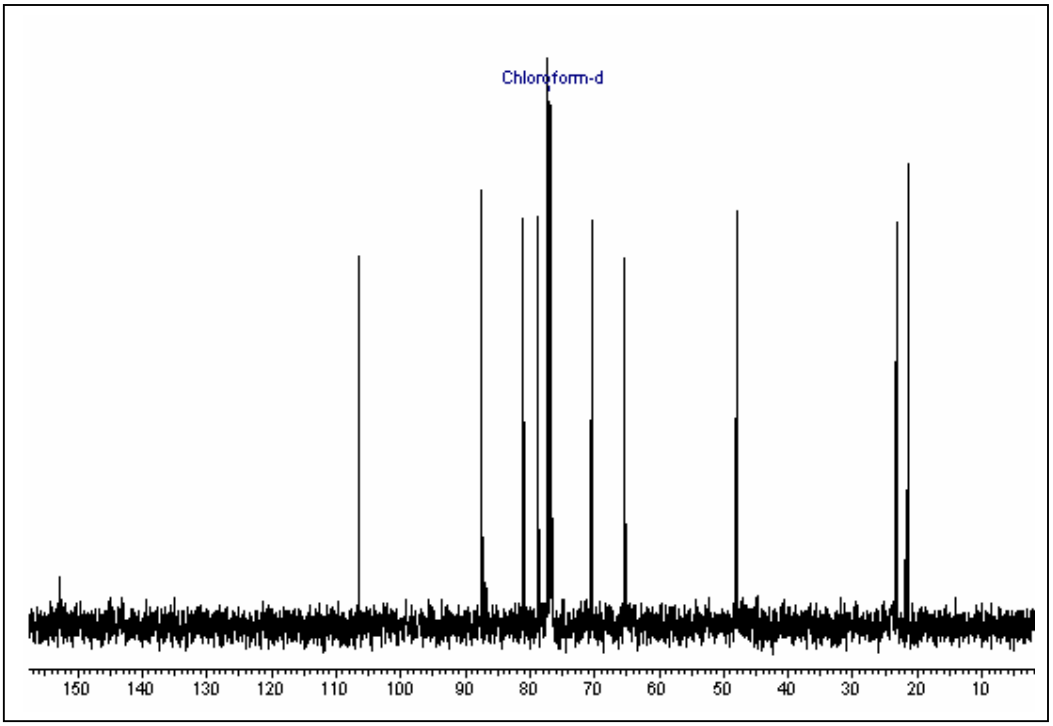
^{1}H NMR spectrum of compound **154 (α-glycoside)** in $CDCl_3$



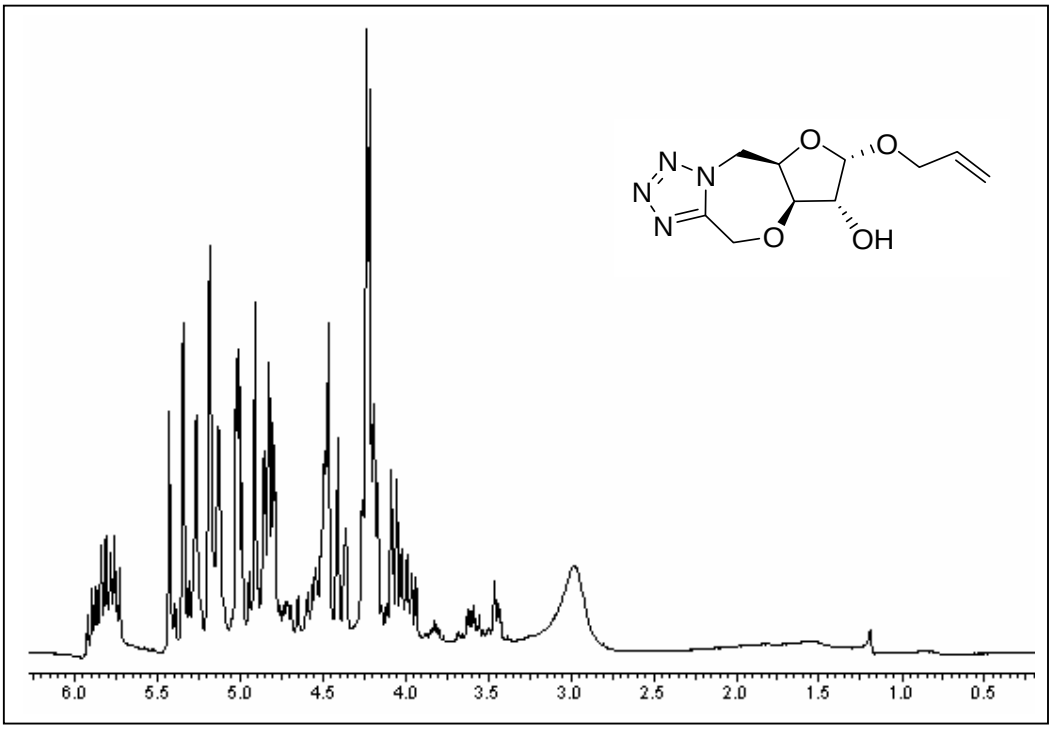
^{13}C NMR spectrum of compound **154 (α-glycoside)** in $CDCl_3$



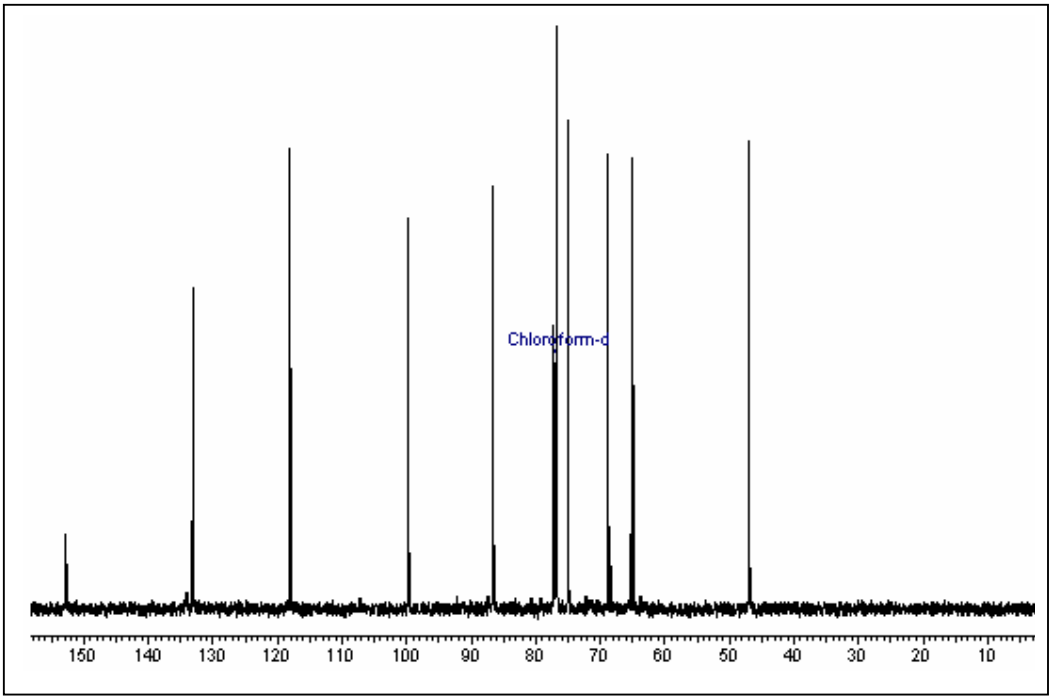
^{1}H NMR spectrum of compound **154 (β-glycoside)** in $CDCl_3$



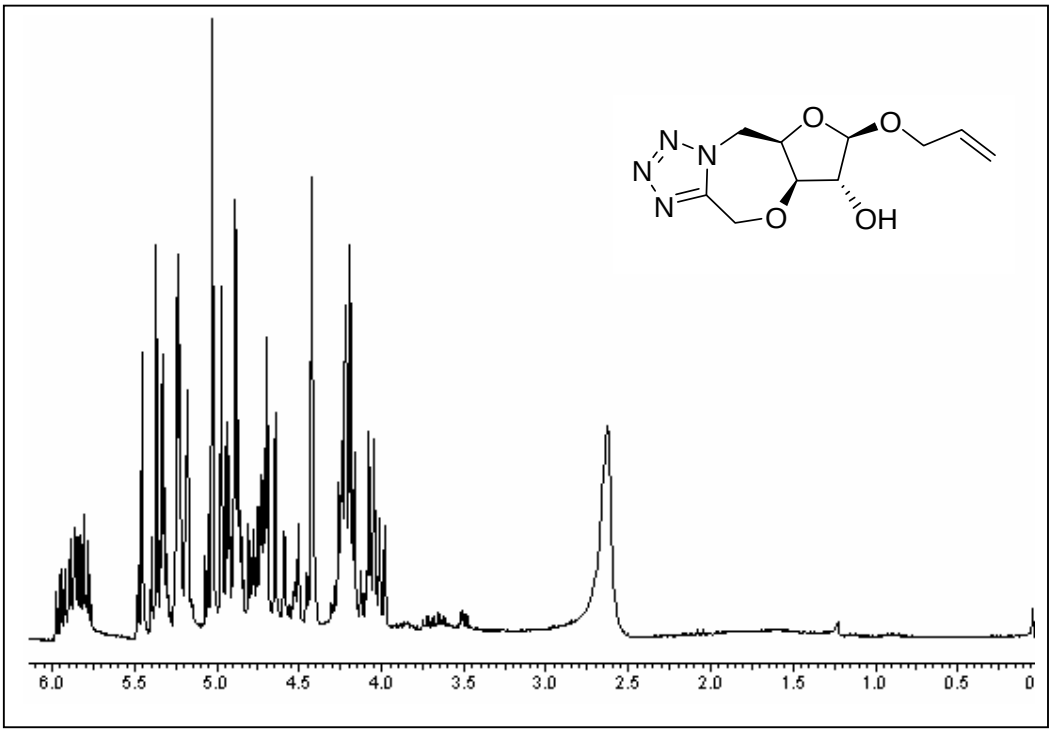
^{13}C NMR spectrum of compound **154 (β-glycoside)** in $CDCl_3$



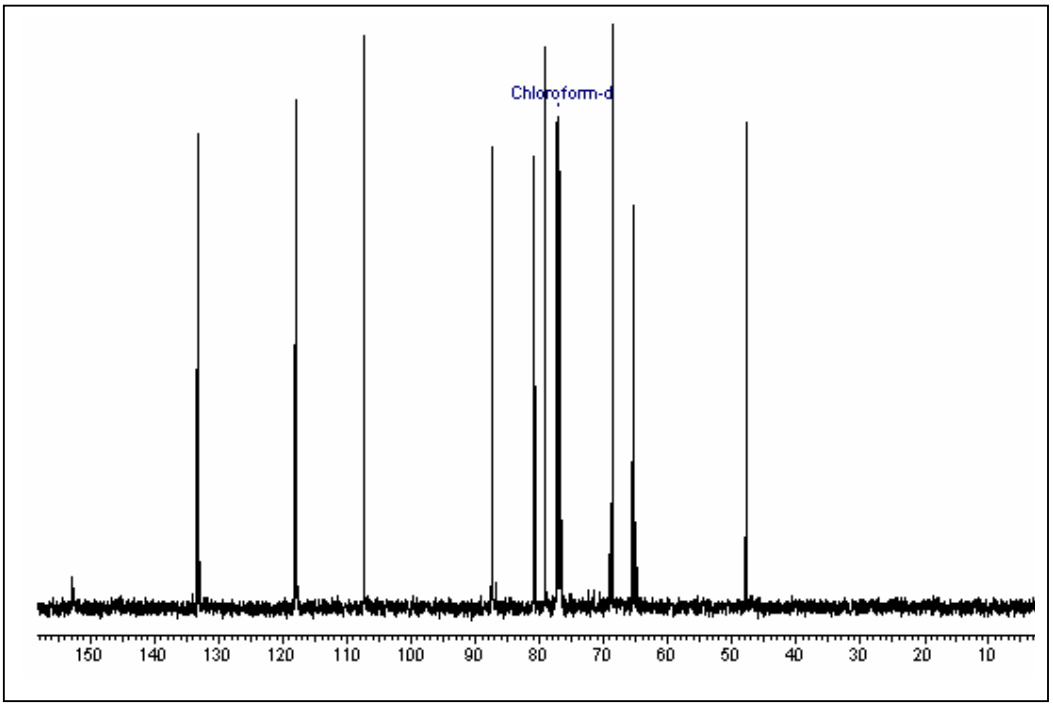
^{1}H NMR spectrum of compound **155 (α-glycoside)** in $CDCl_3$



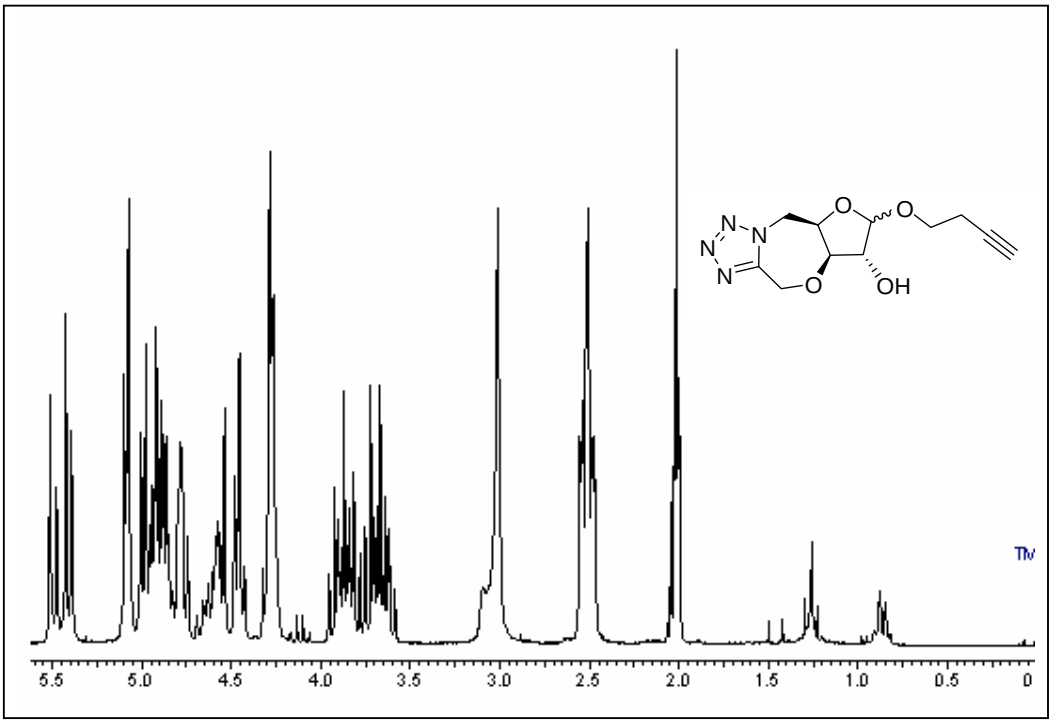
^{13}C NMR spectrum of compound **155 (α-glycoside)** in $CDCl_3$



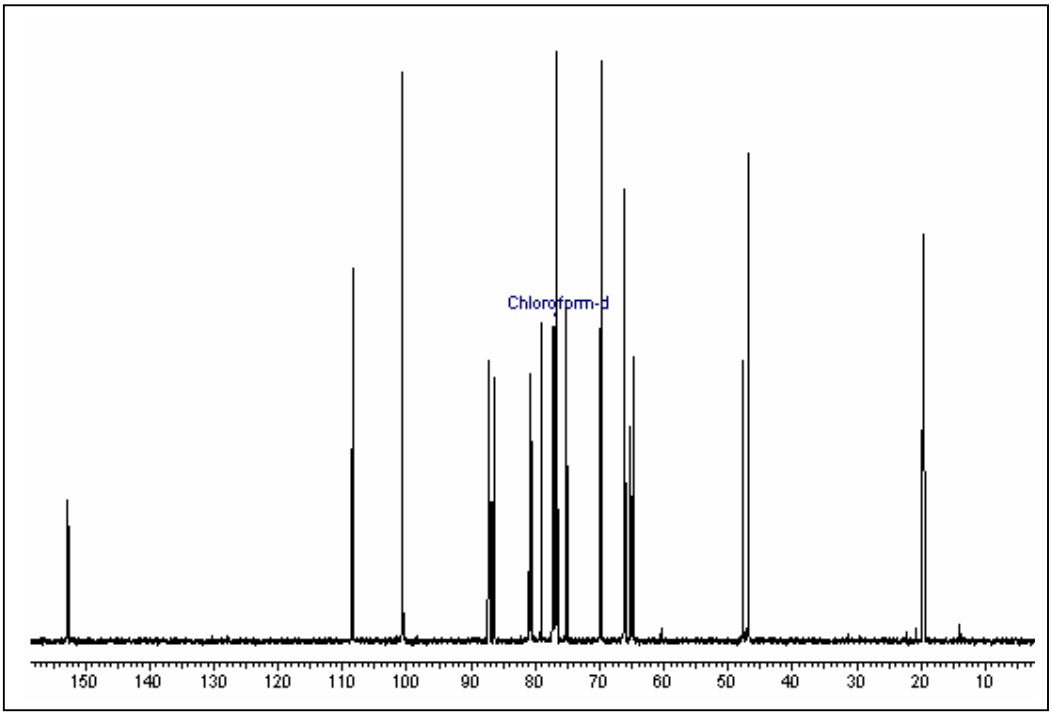
^{1}H NMR spectrum of compound **155 (β-glycoside)** in $CDCl_3$



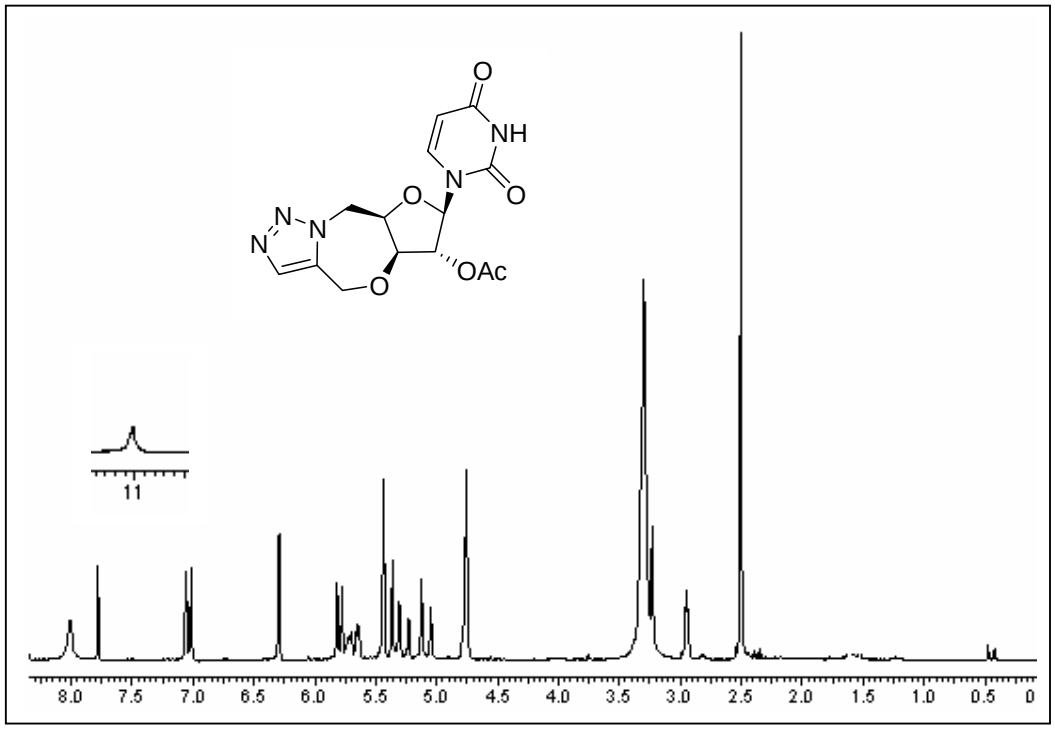
^{13}C NMR spectrum of compound **155 (β-glycoside)** in $CDCl_3$



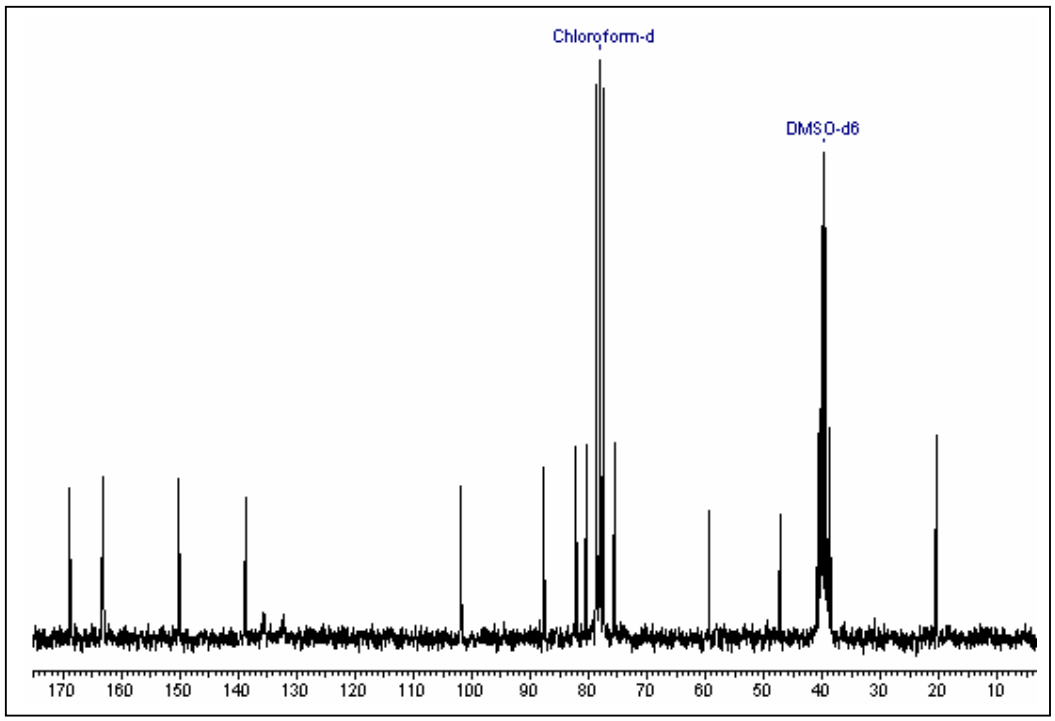
^{1}H NMR spectrum of compound **156 (α/β-glycoside mixture)** in $CDCl_3$



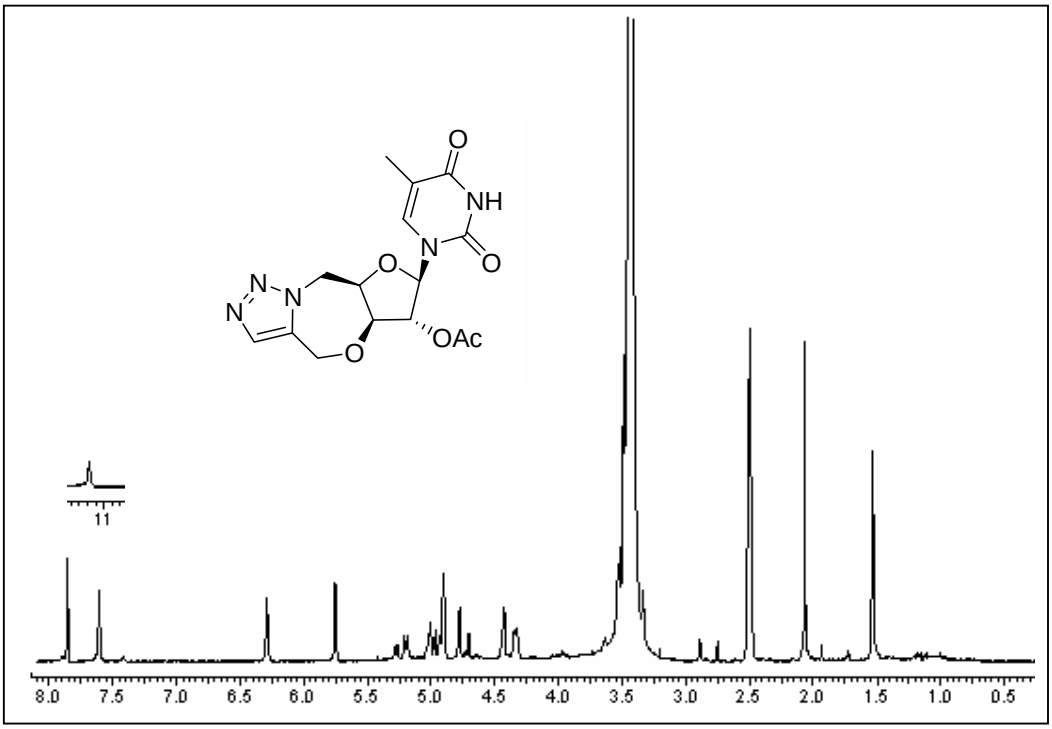
^{13}C NMR spectrum of compound **156 (α/β-glycoside mixture)** in $CDCl_3$



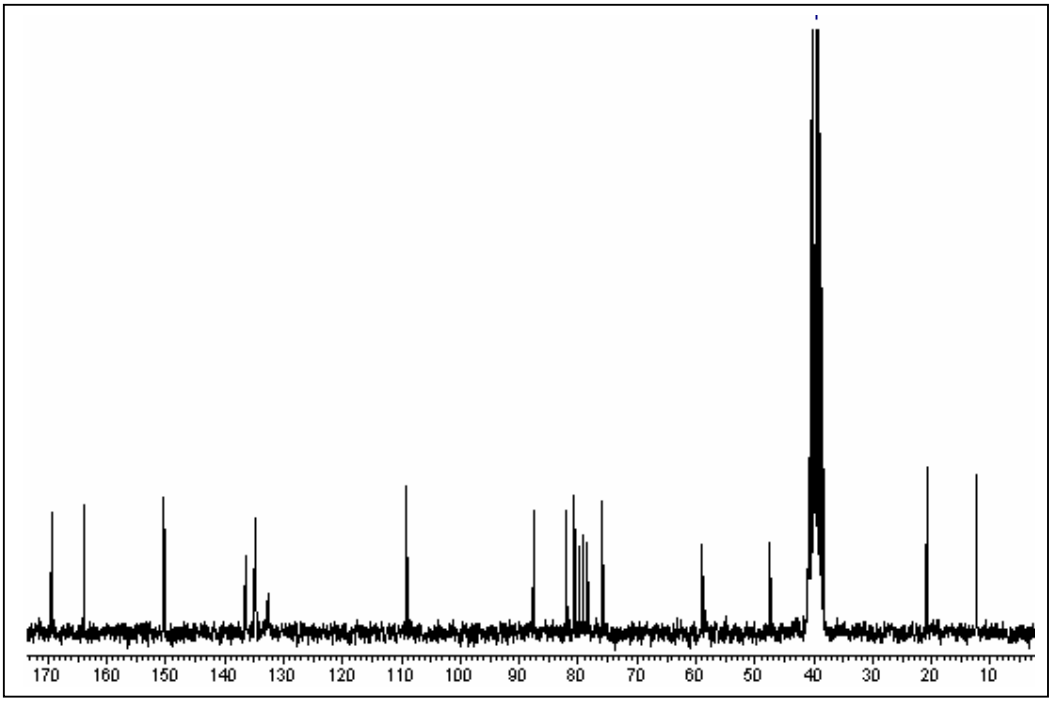
^{1}H NMR spectrum of compound **159 (β-nucleoside)** in DMSO-d_6+$CDCl_3$



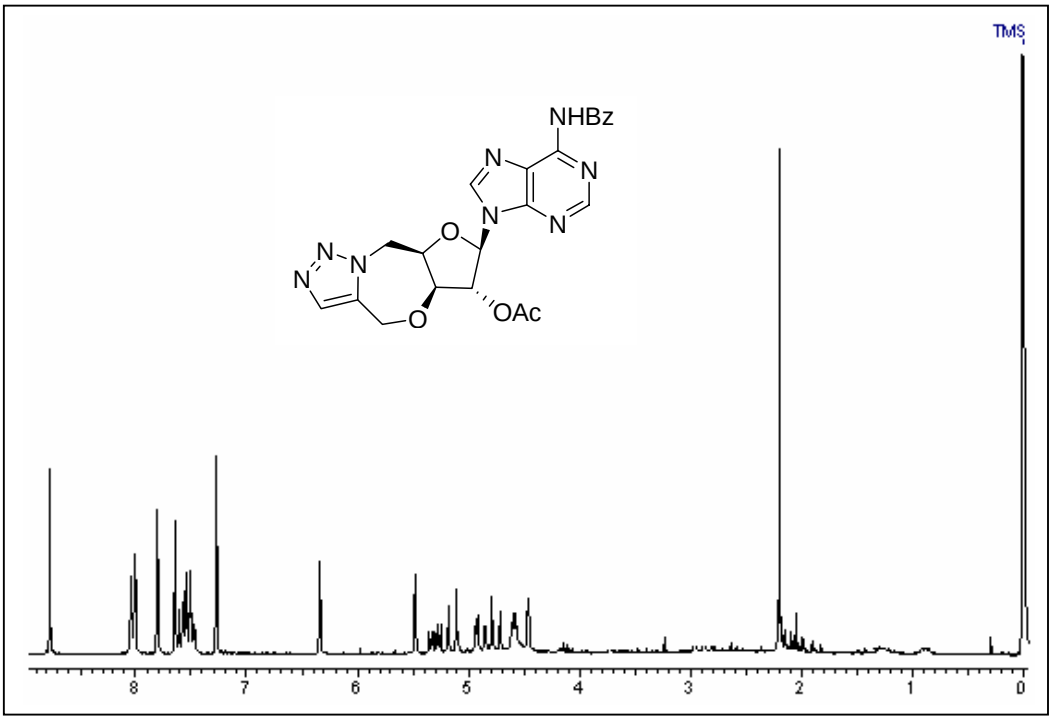
^{13}C NMR spectrum of compound **159 (β-nucleoside)** in DMSO-d_6+$CDCl_3$



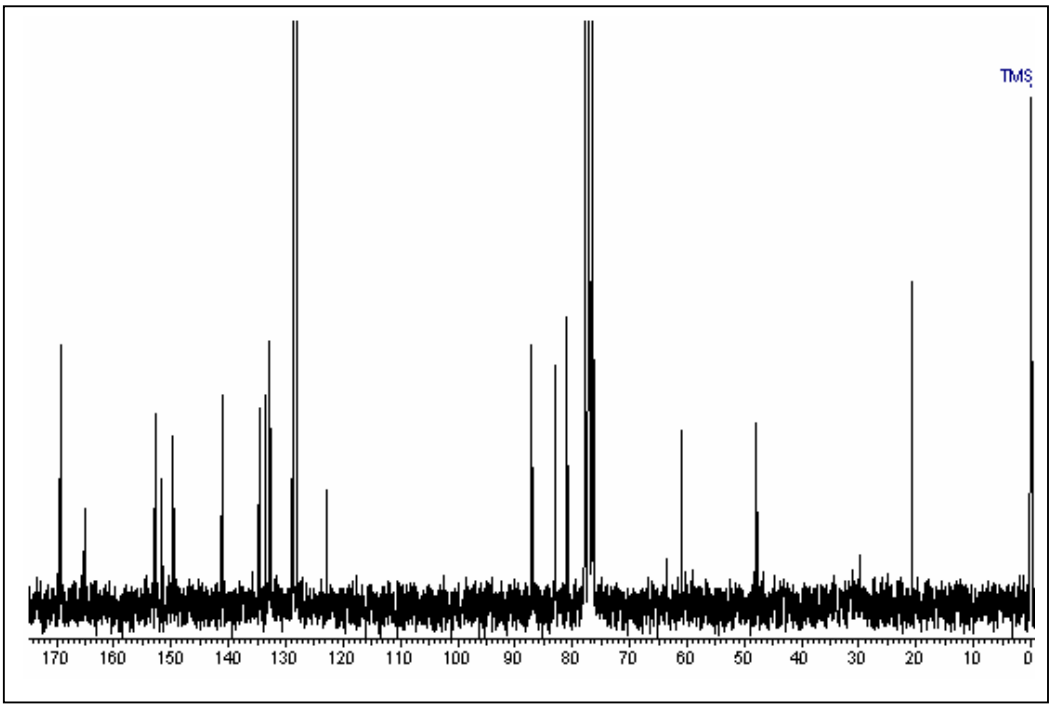
^{1}H NMR spectrum of compound **160 (β-nucleoside)** in DMSO-d_6+$CDCl_3$



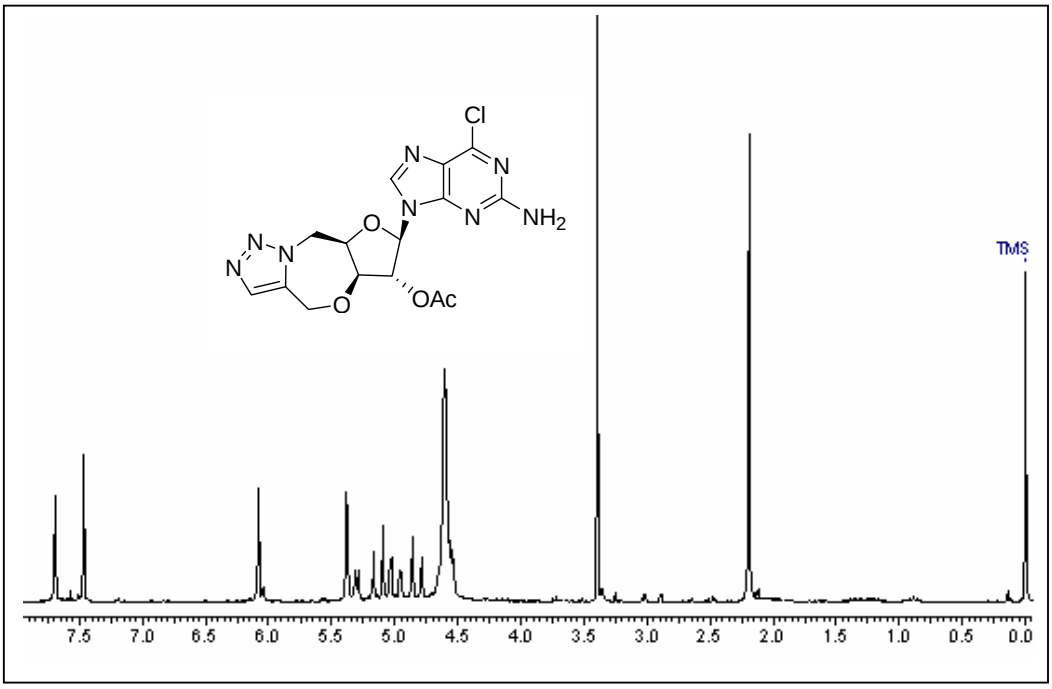
^{13}C NMR spectrum of compound **160 (β-nucleoside)** in DMSO-d_6+$CDCl_3$



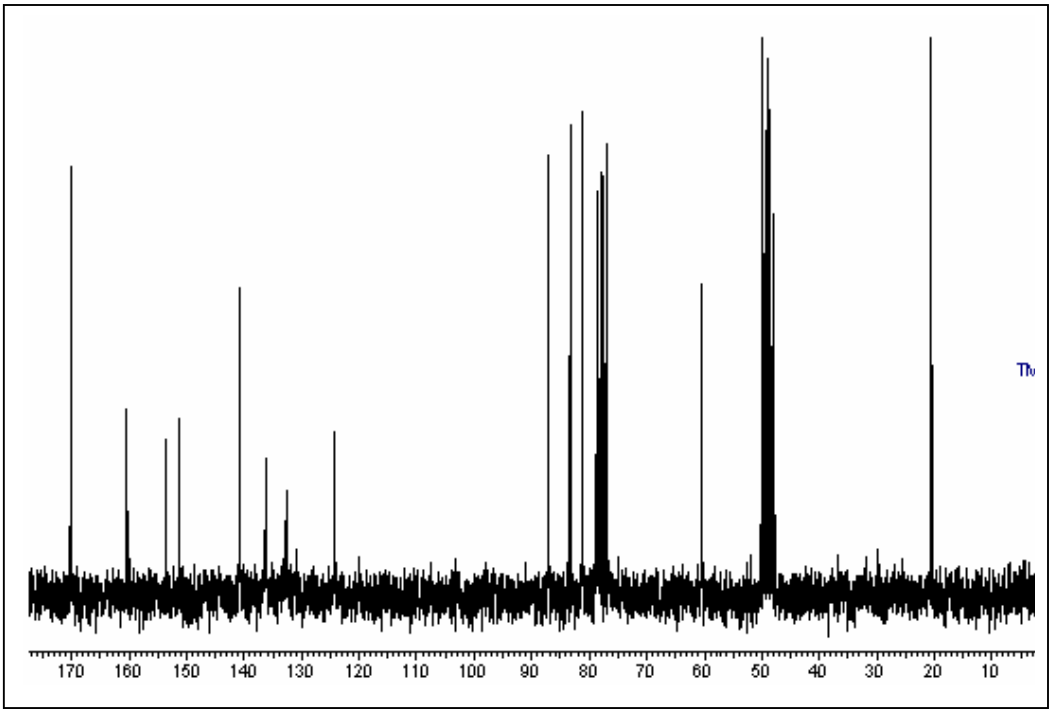
^{1}H NMR spectrum of compound **161 (β-nucleoside)** in $CDCl_3$



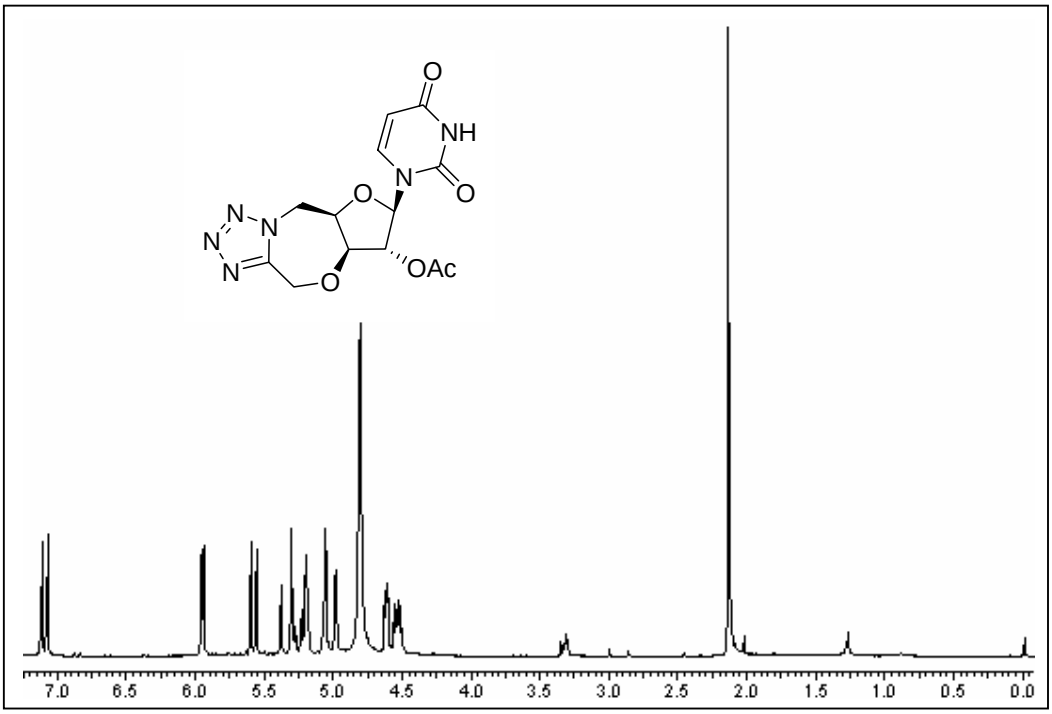
^{13}C NMR spectrum of compound **161 (β-nucleoside)** in $CDCl_3$



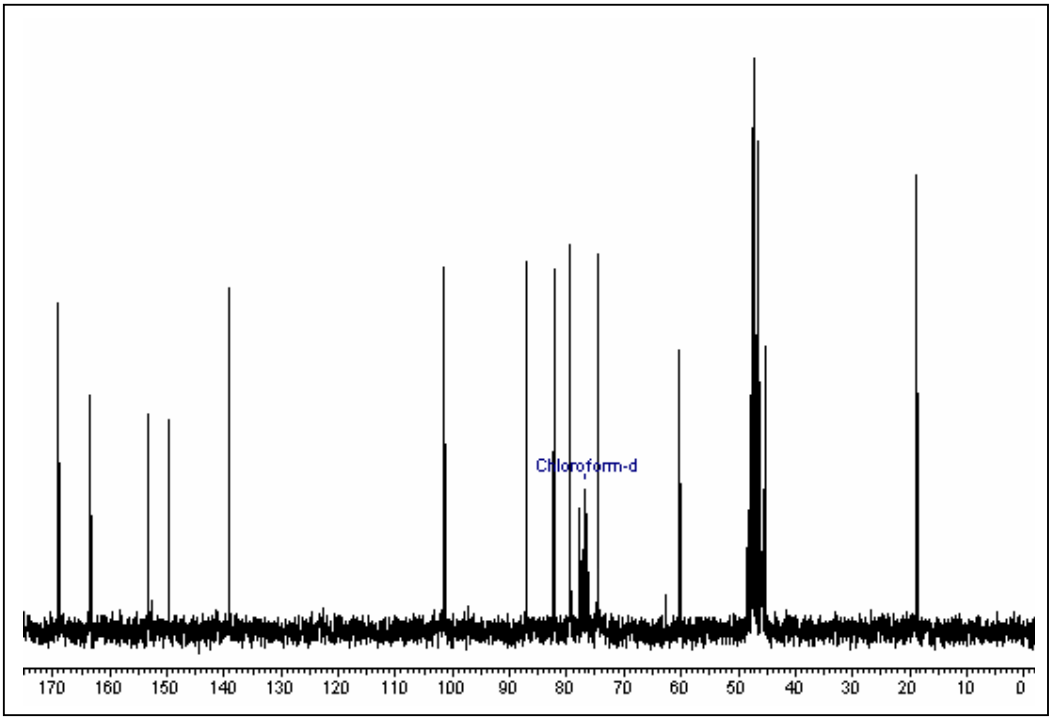
^{1}H NMR spectrum of compound **162 (β-nucleoside)** in MeOH-d_4+$CDCl_3$



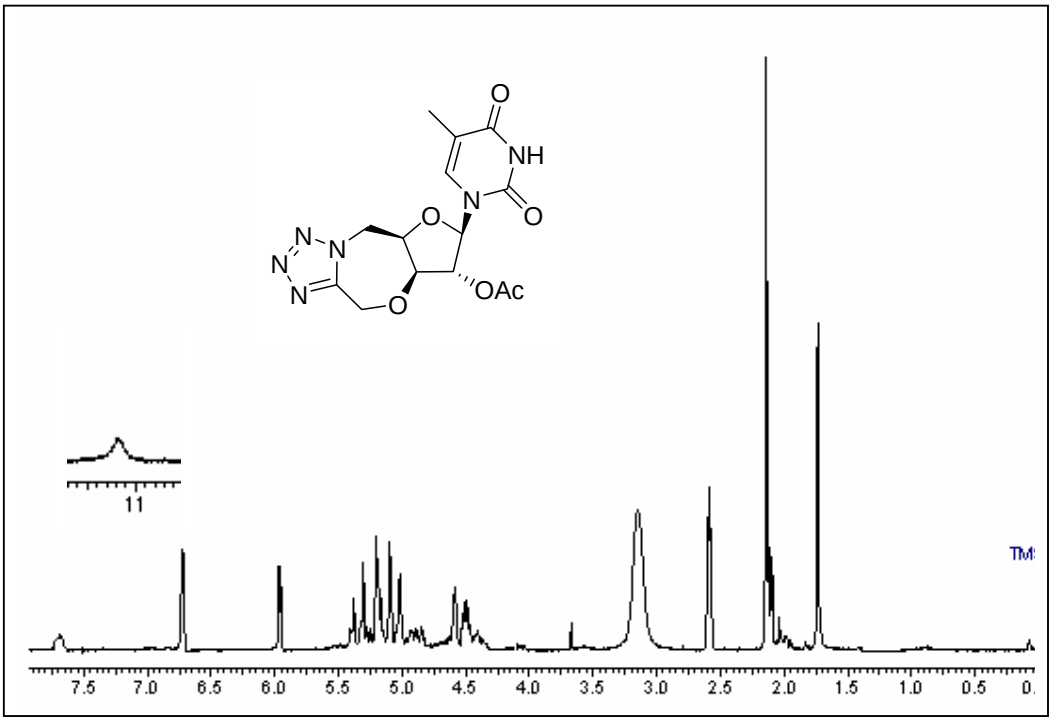
^{13}C NMR spectrum of compound **162 (β-nucleoside)** in MeOH-d_4+$CDCl_3$



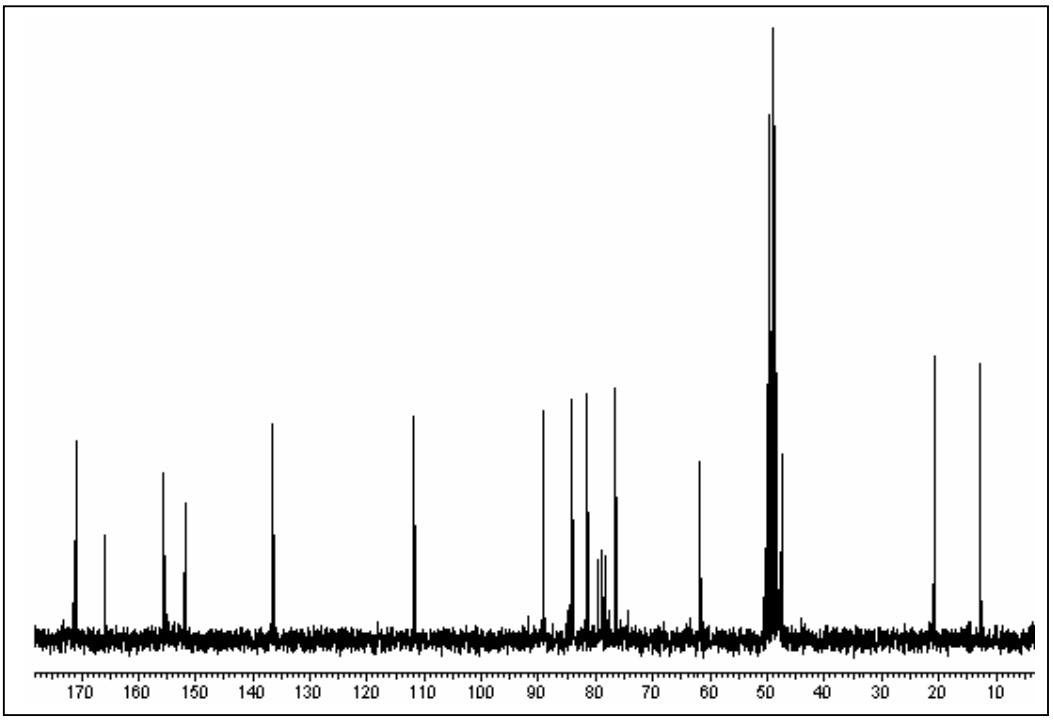
^{1}H spectra of compound **163 (β-nucleoside)** in MeOH-d_4



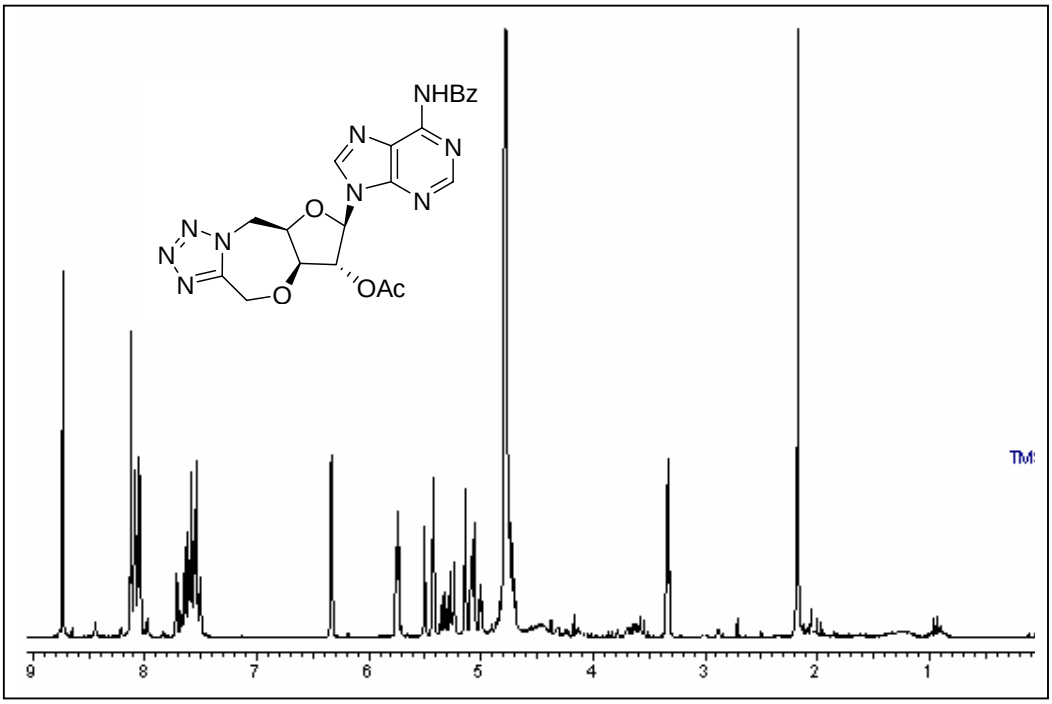
^{13}C NMR spectra of compound **163 (β-nucleoside)** in MeOH-d_4+$CDCl_3$



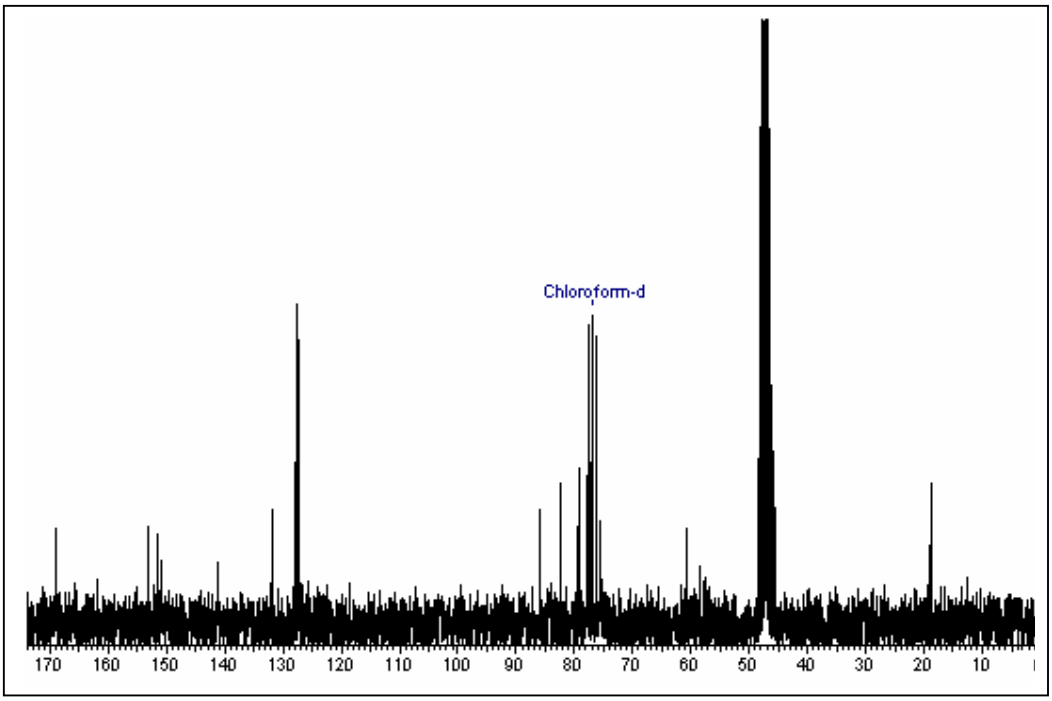
^{1}H NMR spectra of compound **164 (β-nucleoside)** in DMSO-d_6+$CDCl_3$



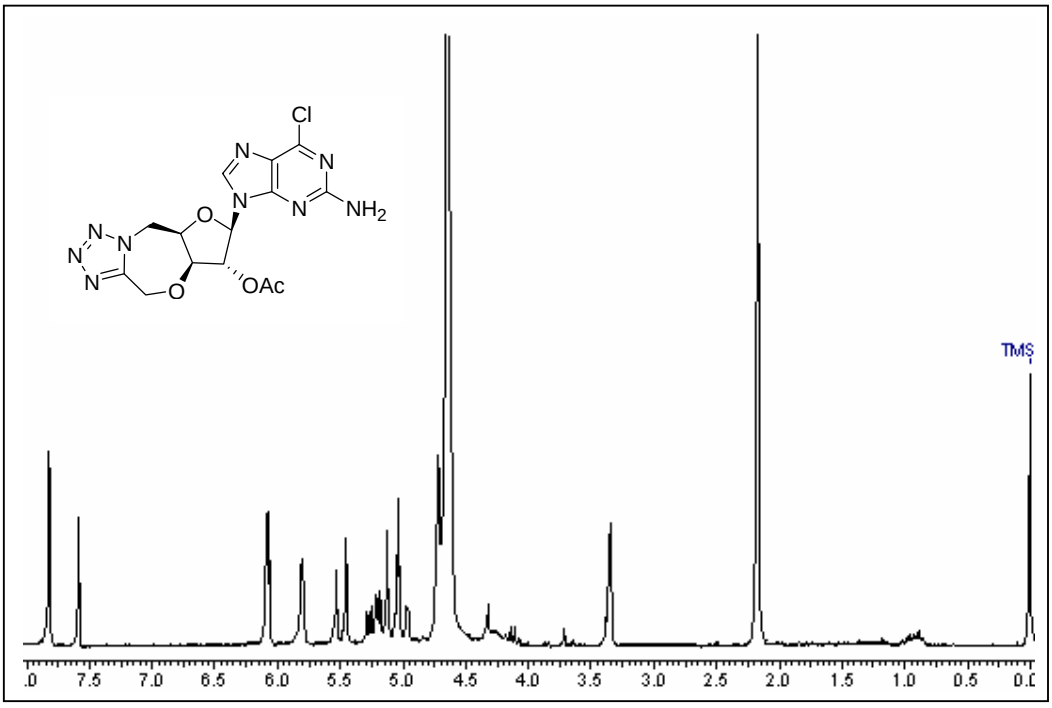
^{13}C NMR spectra of compound **164 (β-nucleoside)** in MeOH-d_4+$CDCl_3$



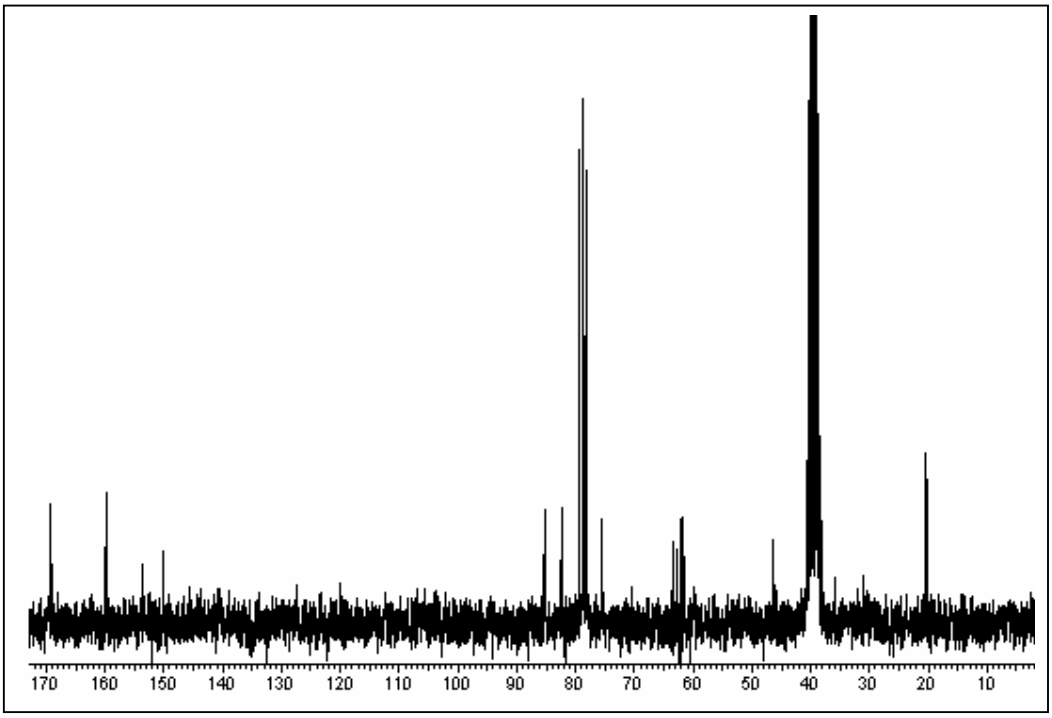
^{1}H NMR spectrum of compound **165 (β-nucleoside)** in MeOH-d_4+$CDCl_3$



^{13}C NMR spectrum of compound **165 (β-nucleoside)** in MeOH-d_4+$CDCl_3$



^{1}H NMR spectrum of compound **166 (β-nucleoside)** in MeOH-d_4+$CDCl_3$



^{13}C NMR spectrum of compound **166 (β-nucleoside)** in MeOH-d_4+$CDCl_3$

Chapter 2: References

2.8. References

1. (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Edn.,* **2001.** *40,* 2004. (b) Kolb, H. C,; Sharpless, K. B. *Drug Discovery Today,* **2003,** *8,* 1128.
2. Huisgen, R. In *1,3-Dipolar Cycloaddition Chemistry,* **1984,** *vol 1,* Padwa, A., ed., pp. 1-176, Wiley New York.
3. Huisgen, R. *Angew. Chern. Int. Ed., **1963,** 2,* 565.
4. (a) Rostovtsev, V. V.; Green, L. G,; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed*., **2002**, *41,* 2596. (b) Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V.V. *Org. Lett.,* **2004,** *6,* 2853.
5. Tornoe, C. W,; Christensen, C.; Meldal, M. *J. Org. Chem.,* **2002,** *67,* 3057.
6. (a) Hlasta, D. J,; James H. Ackerman, J. H. *J. Org. Chem*., **1994,** *59,* 6184. (b) Coat, S. J.; Link, J. S.; Gauthier, D.; Hlasta, D. J. *Org. Lett.,* **2005,** *7,* 1469.
7. Rodionov, V. O.; Valery V. Fokin, V. V.; Finn, M. G. *Angew. Chem. Int. Ed.,* **2005,** *44*, 2210.
8. Fusco, R.; Garanti, L.; Zecchi, G. *J. Org. Chem.,* **1975,** *40,* 1906.
9. Zanze, I. A.; Gracias, V.; Djuric, S.W. *Tetrahedron Lett.,* **2004,** *45,* 8439.
10. Dolhem, F.; Tahli, F. A.; Lievre, C.; Demailly, G. *Eur. J. Org. Chem.,* **2005,** 5019.
11. (a) Smissman, E. E.; Terada, A.; El-antably, S. *J. Med. Chem.* **1976**,*19,* 165.; (b) McGuire, J. J.; Russell, C. A.; Bolanowska, W. E.; Freitag, C. M.; Jones, C. S.; Kalman, T. I. *Cancer Res.,* **1990**, *50,* 1726. (c) Zablocky, J. A.; Miyano, M.; Sashidhar, N. R.; Panzer-Knodle, S.; Nicholson, N.; Feigen, L. *J. Med. Chem.,* **1992**, 35, 4914.; (d) Lunn, W. H.; Schoepp, D. D.; Calligaro, D. O.; Vasileff, R. T.; Heinz, L. J.; Salhoff, C. R.; O'Malley, P. J. *J. Med. Chem.,* **1992**, *35*, 4608.
12. Zachary P. Demko, Z. P.; Sharpless, K. B. *Angew. Chem. Int. Ed*., **2002**, *41,* 2110.
13. Herr, R. J. *Bioorg. & Med. Chem*., **2002,** *10,* 3379 and references there in.
14. Demko, Z. P.; Sharpless, K. B. *J. Org. Chem.,* **2001,** *66*, 7945.
15. Demko, Z. P.; Sharpless, K. B. *Org. Lett.,* **2002,** *4,* 2525.
16. Demko, Z. P.; Sharpless, K. B. *Angew. Chem. Int. Ed*., **2002**, *41,* 2113.
17. L'Abbe, G. *Chem. Rev.*, **1969,** *69,* 345.
18. Garanti, L.; Zecchi, G. *J. Org. Chem.,* **1980,** *45,* 4767.

19. (a) Davis, B.; Brandstetter, T. W.; Smith, C.; Hackett, L.; Winchester,B. G.; Fleet, G. W. J. *Tetrahedron Lett.,* **1995,** *36,* 7507. (b) Brandstetter, T. W.; Davis, B.; Hyett, D.; Smith, C.; Hackett, L.; Winchester,B. G.; Fleet, G. W. J. *Tetrahedron Lett.,* **1995,** *36,* 7511; (c) Davis, B. G.; Nash, R. J.; Watson, A. A.; Smith, C.; Fleet, G. W. J. *Tetrahedron,* **1999,** *55*, 4501.
20. Demko, Z. P.; Sharpless, K. B. *Org. Lett*. **2001,** *3,* 4091.
21. (a) Prescher, J. A.; Bertozzi, C. R. *Nature Chem. Biol.,* **2005,** *1,* 13; (b) Rabuka, D.; Hubbard, S. C.; Laughlin, S. T.; Argade, S. P.; Bertozzi, C. R. *J. Am. Chem. Soc.,* ***2006,*** *128,* 12078.
22. Wamhoff, H. In *Comprehensive Heterocyclic Chemistry*; *Katritzky*, A. R.; Rees, C. W. Eds.; Pergamon: Oxford, **1984**; *Vol. 5*, pp 669.
23. (a) Granie, T.; Panday, N.; Vasella, A. *Helv. Chem. Acta,* **1997,** *80,* 979. (b) Heightman, T. D.; Vasella, A.; Tsitsanou, K. E.; Zographos, S. E.; Skamnaki, V. T. Oikonomakos, N. G. *Helv. Chem. Acta,* **1998,** *81,* 853; c. Mitchell, E. P.; Withers, S. G.; Ermert, P.; Vasella, A.; Garman, E. F.; Oikonomakos, N. G.; Johnson, L. N. *Biochemistry,* **1996,** *35,* 7341.
24. Alvarez, R.; Velazquez, S.; San-Felix, A.; Aquaro, S.;De Clercq, E.; Perno, C. F.; Karlsson, A.; Balzarini, J.; Camarasa, M. J. *J. Med. Chem.,* **1994,** *37*, 4185.
25. Buckle, D. R.; Rockell, C. J. M.; Smith, H.; Spicer, B. A. *J. Med. Chem.,* **1986,** *29,* 2262.
26. Genin, M. J.; Allwine, D. A.; Anderson, D. J.; Barbachyn, M. R.; Emmert, D. E.; Garmon, S. A.; Graber, D. R.; Grega, K. C.; Hester, J. B.; Hutchinson, D. K.; Morris, J.; Reischer, R. J.; Ford, C. W.; Zurenko, G. E.; Hamel, J. C.; Schaadt, R. D.; Stper, D.; Yagi, B. H. *J. Med. Chem*., **2000,** *43,* 953.
27. Manetsch, R.; Krasinski, A,; Radic, Z.; Raushel, J.; Taylor, P.; Sharpless, K. B.; Kolb, H. C. *J. Am. Chem. Soc.,* ***2004,*** *126,* 12809.
28. (a) Schreiber, S. L. *Science,* **2000,** *287,* 1964. (b) Schreiber, S. L.. *Chem. & Eng. News,* **2003,** 51. (c) Burke, M. D.; Schreiber, S. L. *Angew. Chem. Int. Ed.,* **2004,** *43,* 46.
29. Zhang, D.; Miller, M. J.; *Current Pharmaceutical Design,* **1999,** *5,* 73.
30. Prepared according to the method given in Hasegawa, A.; Kisho, M. *Carbohydr. Res*., **1980**, *79*, 265.
31. Tripathi, S.; Singha, K.; Achari, B.; Mandal, S. B. *Tetrahedron*, **2004**, *60*, 4959.

32. Prepared according to the method given in Brimacombe, J. S.; Mofti, A. M. *Carbohydr. Res*., **1971**, *16*, 167.
33. Prepared according to the method given in Kawazu, M.; Kannos, T.; Yamamura, S.; Mizoguchi, T.; Saito, S. *J. Org. Chem.,* **1973,** *38,* 2887.
34. Prepared according to the method given in Somanthan, R.; Helberg, L. H. *Org. Prep. Proced. Int.,* **1984,** *16,* 388.
35. Prepared according to the method given in Sasai, H.; Matsuno, K.; Suami, T. *J. Carbohydr. Res.,* **1985,** *4,* 99.
36. Prepared according to the method given in Richtmyer, N. K. *Methods. Carbohydr. Chem.,* **1962,** *1,* 107.
37. Vorbruggen, H,; Bennua, B. *Chem. Ber.,* **1981,** *114,* 1279.

****

Chapter 3: “Click Chemistry” Guided Diversity Oriented Synthesis Of Glycoconjugates Containing 1,2,3-Triazole

3.1. Glycobiology / Glycomics:

The basic principle of modern molecular biology is that the biological information flows from **DNA to RNA to protein to cell**. In other words molecular biology is mainly concerned with the study of interrelationship of DNA, RNA and protein synthesis, including the study of how these interactions are regulated.

Nucleic acids, proteins and glycoconjugates are the three repeating biopolymers, responsible for the signal transduction processes in living organisms. In chemical biology the role of nucleic acids and proteins has been studied extensively.

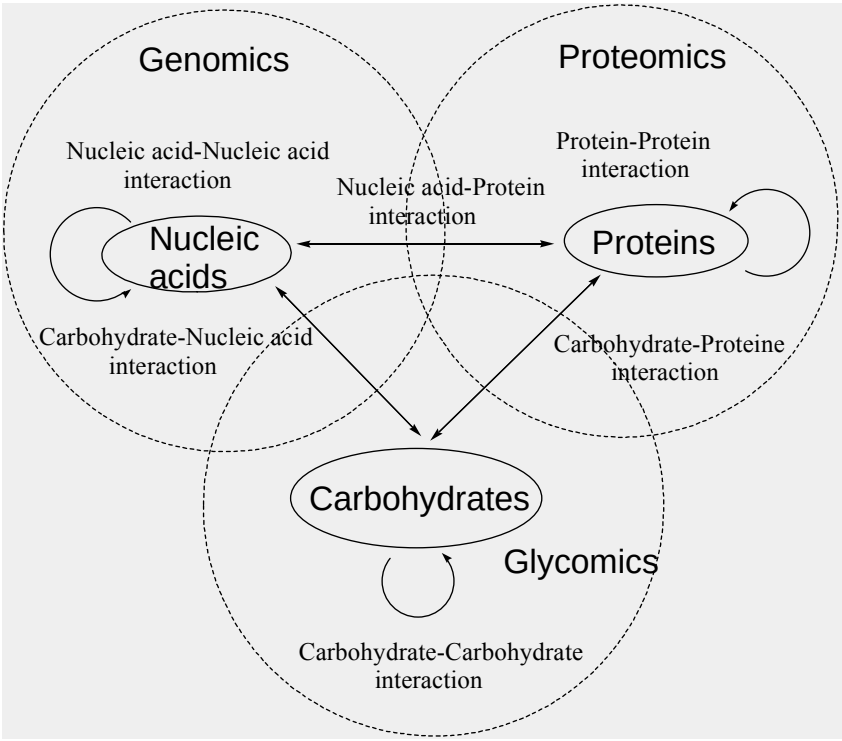


Figure 1: Interactions of three main biopolymers.

The interactions between protein-protein, protein-nucleic acid and nucleic acid-nucleic acid have been emerged in the recent years and are comparatively well defined (Figure 1). These interactions are well exploited to modify, enhance or disrupt

in a number of therapeutic approaches. However carbohydrates, the third major class of biopolymers are only recently driving attention in the interest of drug development. As compared to genomics and proteomics, fundamental glycobiology is less well established and is responsible for less interest from carbohydrates as drug development.[1]

"Glycobiology" is a branch of biology that deals with the structure, biosynthesis and function of oligosaccharides that are abundantly distributed in nature. The term "Glycomics" is recently introduced and derived from the chemical prefix for sweetness or a sugar, "glyco-". The identity of the total of carbohydrates in an organism is collectively referred to as the glycome. Glycomics describes glycobiology and the interaction of carbohydrates with other two major classes of biopolymers i.e. nucleic acids and proteins. Carbohydrate molecules can serve as intermediates in generating energy, as signaling molecules, or as structural components. Carbohydrates are highly complex and diverse class of compounds commonly found in nature as glycoconjugates such as glycoproteins and glycolipids.[1] A complete paradigm of molecular biology can now thus be considered as follows (Figure 2): [2]

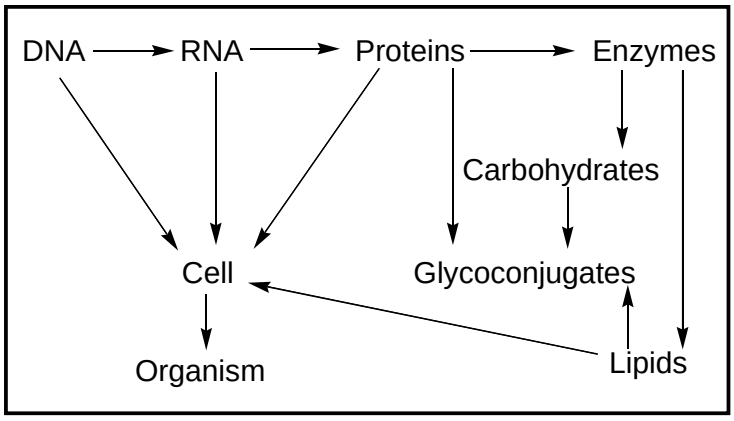


Figure 2: Paradigm of molecular biology

3.1. Glycoconjugates:

A glycoconjugate is a compound in which one or more monosaccharide or oligosaccharide units called the glycone are covalently linked to a noncarbohydrate moiety called the aglycone such as peptide, protein, lipid or any other biological or nonbiological molecules. The portion of the glycoconjugate molecule containing the glycans can vary from as small as a single monosaccharide to as large as a high-molecular-weight polysaccharide. The surfaces of most types of cells are greatly covered with a dense coating of sugars (Figure 3). The most commonly occurring monosaccharides in glycoconjugates are such as N-acetyl neuraminic acid, glucose (Glc), galactose (Gal), mannose (Man). *N*-acetylglucosamine (GlcNAc), xylose (Xyl).glucuronic acid (GlcA) and iduronic acid (IdA), etc.[2]

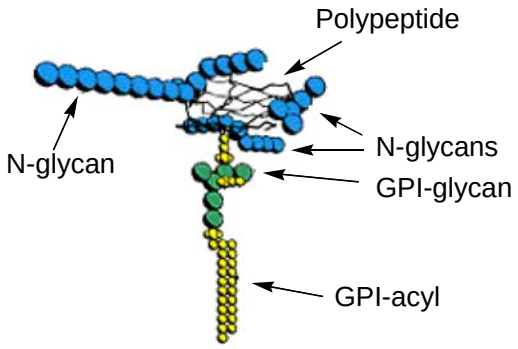


Figure 3: Schematic representation of the Thy-1 glycoprotein.

3.2. Biological role of glycoconjugates

In the earlier days of biology, the primary role of sugars in living systems was considered only as source of energy (glucose and glycogen) or in cellular structure (Chitin). However, over the last decades it has become important that carbohydrates bound to proteins or lipids play very essentials roles as communication molecules in many intercellular and intracellular processes. Carbohydrates are important mediators of cell-cell recognition events and related processes such as cell signaling regulation,

cellular differentiation and immune response.[3a] For e.g. sialic aid containing glycoconjugates play a vital role in a number of physiologically and pathologically important processes. These include embryogenesis, organ development, immune defense, migration and homing of leukocytes, metastasis of neoplastic cells, inflammation and infection caused by a variety of pathogens.[3b]

3.3. Therapeutic potential of glycoconjugates

Oligosaccharides linked to proteins and lipids via *N*- or *O*-glycosidic linkages (glycoconjugates) have been shown to govern crucial life processes and disease states.[4] Glycoconjugates are biosynthesized from glycosylation of proteins and lipids, an intracellular process. Glycosylation reactions conducted by glycosyltransferases are one of the essential biological processes for the post translational modification of protein and lipid functions. These processes greatly influence various molecular recognition processes, these include bacterial and viral infections, cell adhesion in inflammation, immune response, cellular differentiation, development, regulation and other intracellular communication and signal transductions. Therefore selective inhibitors of glycosyltransferase enzymes may lead to the development of novel and potential therapeutic reagents.[5] For ex. inhibitors of the biosynthesis of sialic acids may prove to be useful as anti-inflammatory, immunosuppressive and antimetastatic agents.[3b] Many glycomimetic compounds are developing that are approaching clinical evaluation. Synthetic glycoconjugates have exhibited range of therapeutic properties such as anti-inflammatory, antivirals, anticancer, antidiabetic agents, vaccines such as influenza vaccine, anti-HIV vaccines, antimalarial vaccines, tumor vaccines, immuno-modulators, etc.[3a,]

3.4. Glycoconjugates and Click Chemistry

The Cu(I) catalyzed, stepwise, Huisgen's 1,3-dipolar cycloaddition process is a best example of "Click Chemistry". The reaction involves the regioselective formation of 1,4-disubstituted 1,2,3-triazoles. Triazole heterocycles are thermally as well as enzymatically highly stable. They are also thought to be amide surrogates. The detailed account of Click Chemistry has already been discussed in chapter 2. With the help of click chemistry the facile formation of triazole process is emerging as useful tool for ligating wide variety of organic and inorganic molecules in the interest of development of novel therapeutic agents and in the material science.

The1,3-dipolar cycloaddition reactions have been widely used in the synthesis of glycoconjugates and the bioconjugation study of glycosides.[7] Several conjugates of nucleoside-carbohydrate are reported where these two molecules were linked by 1,2,3-triazole or phosphodiester bridge. Nucleoside-carbohydrate conjugates are playing role in development of nucleosides and their prodrugs with improved cellular permeability and enhanced antiviral or anticancer activity.[8] Herewith it is tried to put a concise report on glycoconjugates of therapeutic value, particularly nucleoside-carbohydrate conjugates and containing triazole heterocyclic bridge from the recent literature.

Giannis and coworker[3b] reported the synthesis of a transition state analogue (Figure 1, **167**) as the first potent inhibitor of sialyltransferase enzymes. Compound (**167**) is a conjugate of cytidine and N-acetyl neuraminic acid analogue. It has exhibited an inhibition constant of k_i of 40 nM to rat liver α-(2-6)-sialyltransferase [EC2.4.9.1].

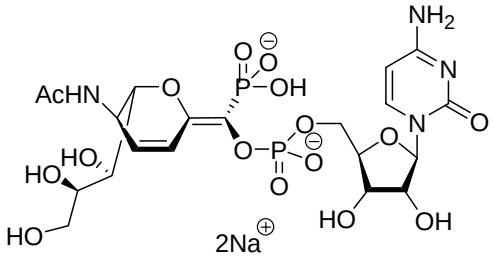
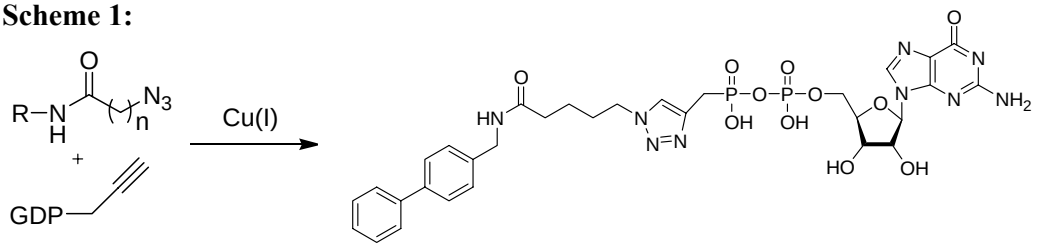


Figure 1: 167

After the successful discovery of Click chemistry process Wong and group[9] published a rapid synthesis of GDP-triazole library of 85 compounds via Click Chemistry. One of the compound in the library was found to have potent and highly selective inhibitory activity against human α-1,3-fucosyltransferase enzyme (Scheme 1, **168**).

Scheme 1:



168

Guillerm and coworker[10] synthesized hetaryl nucleoside derivatives as inhibitors of Chitin synthase (Figure 2, **169** & **170**). They designed new hetaryl nucleosides to mimic the sugar-nucleoside donor at the transition state during the glycosyl transfer process. In their study they have used glucose linkage as non hydrolyzable analogues of the pyrophosphate portion of UDP-GlcNAc, mimicking the probable six membered ring phosphate-Mg^{2+} complex formed in the active site. The attempt was failed to improve the binding affinity and so the activity. Compound (16**9**) showed weak activity Chs (IC_{50}, 3.2 mM) on *S. cerevisiae* (X2180 strain) when compared to nikkomycin Z (IC_{50}, 0.50 μM, K_i = 0.34 μM). Growth inhibition for (**9**) was also reported at 250 μg/ml against *Cryptococcus neoformans* (80%), *C. albicans* (30%), *S. cerevisiae* (30%) and *Aspergillus fumigatus* (30%).

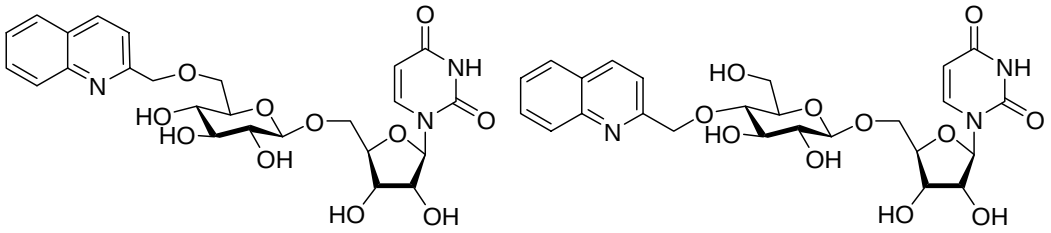
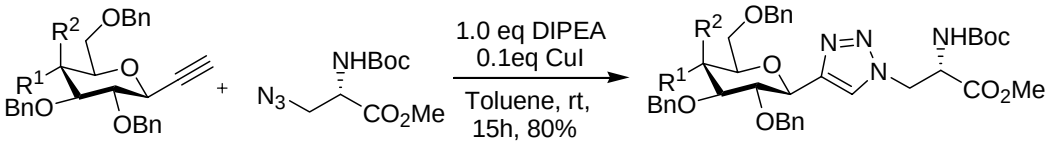


Figure 2: 169 & 170

The1,3-dipolar cycloaddition reaction was exploited to synthesize a series of *C*-glycosyl α-amino acids tethered through rigid five membered heterocycles like triazole. Dandoni *et. al.*[11] reported the synthesis of a class of glycosyl-amino acid conjugates containing 1,2,3-triazole ring as building blocks for new glycopeptides. Employing Cu(I) catalyzed click reaction, conjugated the sugar alkynes (**171a** & **171b**) and amino acid derived azide (**6**) in good yields (Scheme 2, **173a** & **173b**).

Scheme 2:



171a. (R^1= H, R^2= OBn) **172** **173a/173b**

171b. (R^1= OBn, R^2= H)

Finney and coworker[12] proposed a first novel two active site mechanism for Chitin synthase enzyme inhibition. In their attempt they have synthesized uridine derived dimeric inhibitors, as Chitin synthase polymerizes UDP-GlcNAc to form Chitin which is essential for fungal cell wall biosynthesis. Two of the dimeric inhibitors have shown to exhibit 10 fold greater inhibition than monomeric control (Figure 3, **174** & **175**). This observation shows the important implications for the development of antifungal agents. Dimers **174** and **175** exhibited significant inhibition of Chitin synthase activity at 1 mM concentration (32 & 45% respectively).

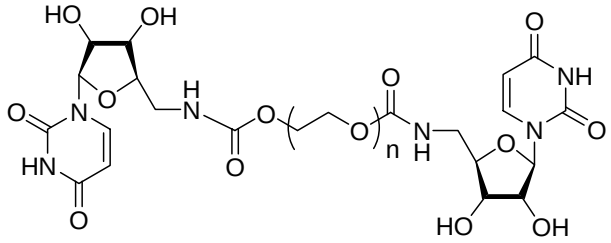


Figure 3: 174. n = 1; **175.** n = 2

Schmidt and coworkers[13] designed and synthesized several novel glycocojugates such as UDP-*exo*-glycal derivatives and *C*-glycosidic derivatives of 2-acetamidoglucal (Figure 4, e.g. **176 & 177**) as transition state analogue inhibitors of UDp-GlcNAc 2-epimerase, an enzyme essential in the biosynthesis of sialic acids. This enzyme catalyses the epimerization of UDP-GlcNAc to ManNAc.

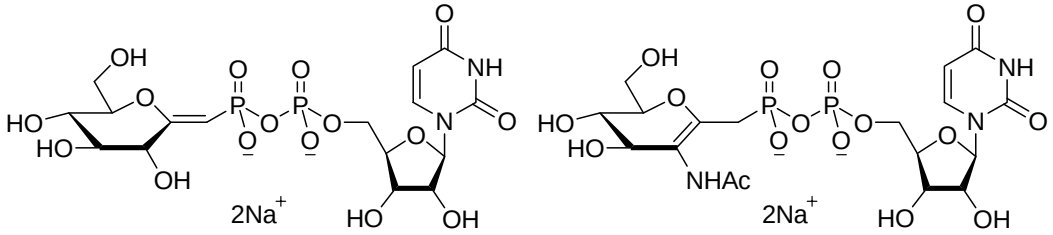


Figure 4: 176 and **177**

Nishimura group[5] designed and synthesized novel inhibitor of human β-1,4-galactosyltransferase (βGal-T1) enzyme on the basis of the affinity labeling of the Trp310 residue located in the small flexible loop near the active site. Among UDP-Gal analogues studied, compound **178** (Figure 5) showed the highest inhibitory activity (K*i* = 1.86 μM).

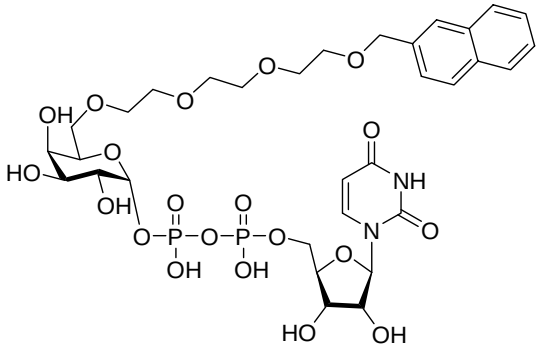


Figure 5: 178

Parang and coworker[8] proposed the first short route solid phase synthesis of dinucleoside and nucleoside-carbohydrate phosphodiesters (Figure 6) from unprotected starting materials. These molecules are playing role in development of nucleosides and their prodrugs with improved cellular permeability and enhanced antiviral or anticancer activity.

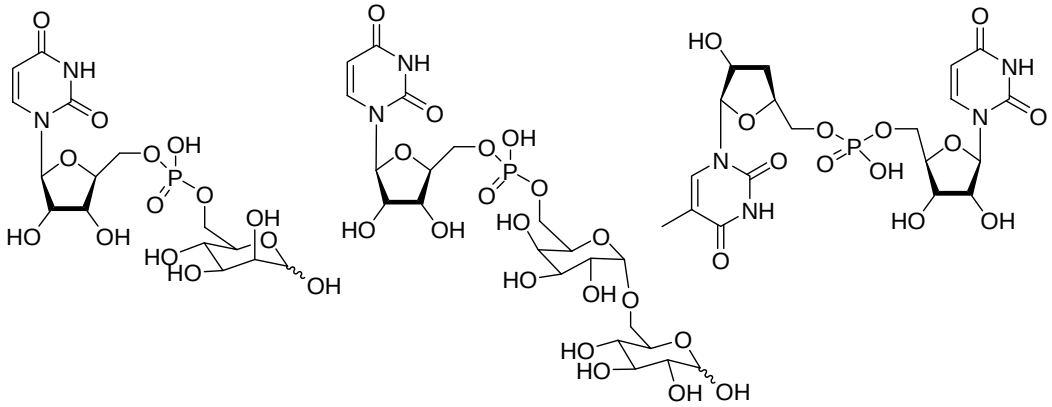


Figure 6: 179, 180 & 181

Queneau[14] group synthesized new amide linked pseudodisaccharides and sugar/nucleoside adduct by the carboxymethyl glycoside lactone strategy. These molecules were synthesized by the opening of carboxymethyl 3,4,6-tri-*O*-acetyl- α-D-glucopyranoside-2-*O*-lactone with deoxyamino sugars and 5'-deoxyamino uridine (Figure 7). Compound **183** potentially mimic the glucosyltransferases substrate (UDP-GlcNAc).

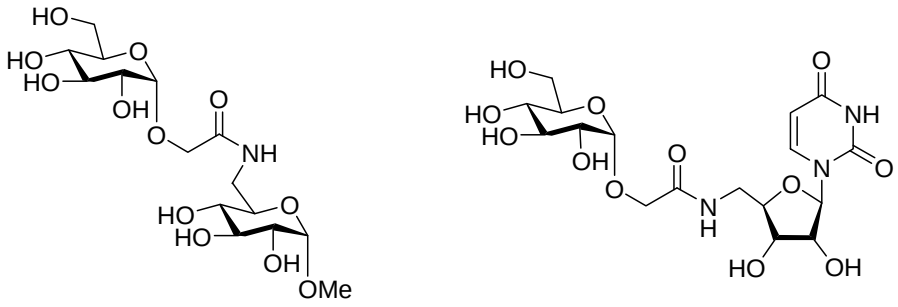
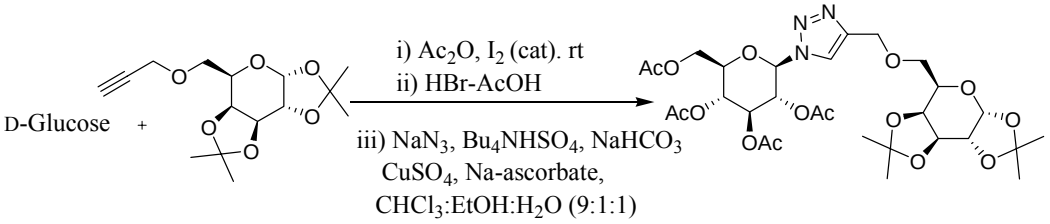


Figure 7: 182 & 183

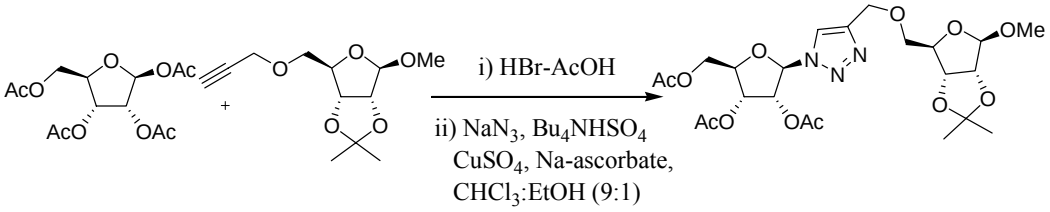
Wang and coworkers[7] reported the one pot synthesis of 1,2,3-triazole containing glycoconjugates involving a Cu(I) catalyzed 1,3-dipolar cycloaddition as the key step directly from unprotected sugars and also from saccharide acetates (Scheme 3 & 4). The method found to be very efficient to access the glycoconjugates in short route.

Scheme 3:



184 **185** **186**

Scheme 4:



187 **188** **189**

Houston and coworkers[15] synthesized a series of glycosyl triazoles from glucosyl azide and diverse acetylene functionalized molecules via click chemistry method in excellent yields under mild conditions (Figure 8, **190** & **191**). This proved the reliability of click chemistry process in triazole tethered glycoconjugates.

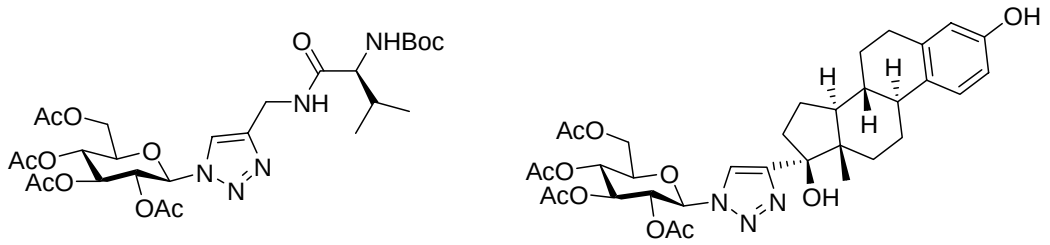


Figure 8: 190 & **191**

Hotha and Kashyap[16] recently reported synthesis of a series of pseudo-oligosaccharides and amino acid glycoconjugates via an intermolecular 1,3-dipolar cycloaddition by Cu(I) catalyzed reaction from various saccharide and amino acid derived alkynes and azides (Figure 9). This report sets a model for the designing of novel glycoconjugates bridged with metabolically stable amide surrogate, i.e. 1,2,3-triazole heterocycle.

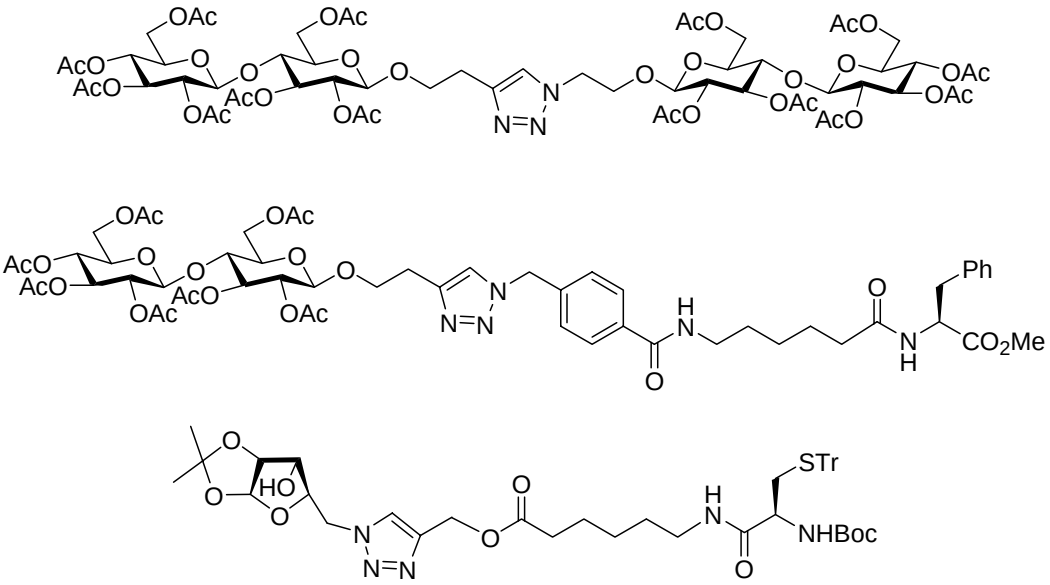


Figure 9: 192, 193 & 194

Aldrich and coworkers[17] synthesized and evaluated a series of rationally designed nucleoside antibiotics as bisubstrate inhibitors of the adenylate forming enzyme MbtA. The enzyme plays an important role in the biosynthesis of siderphore such as mycobactin-T and carboxymycobactin, inhibition of the enzyme leads to the inhibitors of *Mycobacterium tuberculosis* growth. In their studies they focused on analogous incorporation of stable linkers as bioisosters of the labile acyl phosphate group which provided potent enzyme inhibitors. And they classified these inhibitors as bisubstrate inhibitors or intermediate mimics. The stable bioisosteric functionalities are depicted in following figure (Figure 10). 1,4-disubstitute triazole was also a linker

in their investigations, but the resultant analogue (Figure 10, **195**) was found to be inactive, because, the model studies showed that there is loss of hydrogen bonding as exhibited by the phosphate group of the natural substrate.

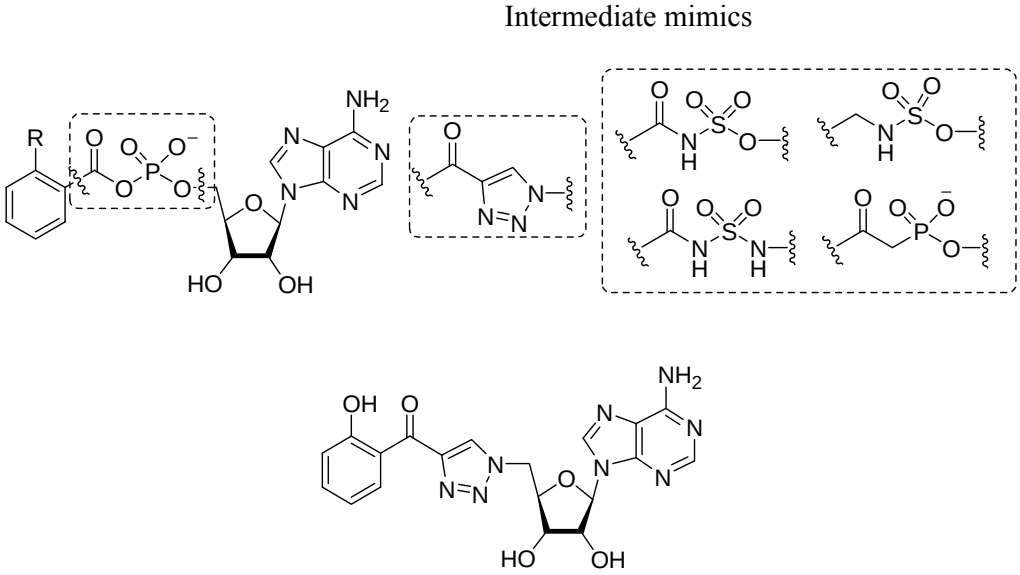


Figure 10: 195

In conclusion, overall literature review on the development of glycomimetics or synthetic glycoconjugates reflects the following observations:

1. Designing novel glycoconjugates were aimed to mimic the natural substrates for glycosyltrasferases, so that the glycosyltrasferases are inhibited, which could halt the biosyntheses of several glycoconjugates responsible for pathological circumstances.

2. Number of attempts were made to improve the physicochemical properties such as to enhance the cellular uptake by incorporating lipophilic substituents.

3. Attempts were also made to enhance the receptor affinity by substituting bioisosteric linkers like sulfamate, 1,2,3-triazole etc. and by incorporating lipophilic spacers so that to obtain maximum receptor fit.

4. Modified Huisgen's 1.3-dipolar cycloaddition reaction a perfect model of "Click Chemistry" was very well exploited in designing new type of glycoconjugates so that metabolically stable 1,2,3-triazole moiety can be incorporated as a linker.

5. A novel two active site mechanism for Chitin synthase enzyme inhibition was proposed by designing dimeric nucleoside inhibitors.

An overall observation indicates that there is a creed in the discovery of newer glycomimetics or synthetic glycoconjugates so that new therapeutic reagents can be obtained.

Chapter 3: Present work

3.5. Present Work

The role of carbohydrates in the biological system is very significant in the form of glvoconjugates. The study of chemical biology reveals the participation of conjugates of carbohydrates and peptides/proteins/lipids in many physiological and pathological conditions. The extent of research in genomics and proteomics is very large in comparison with glycomics. The interactions between two major biopolymers i.e. nucleosides and proteins are much well established compared to interaction of carbohydrates, the third major class of biopolymers with these two cell components. The nucleoside and protein interactions are frequently exploited for the development of therapeutic reagents. As the study of glycobiology is not much developed the development of carbohydrates in therapeutics is lagging behind.

A number of synthetic glycoconjugates are emerging as glycomimetics which are to be regarded as mimics of natural substrates for e.g. UDP-GlcNAc for glycosyltransferases. Chitin synthases (Chs 1, 2 & 3) are the enzymes which catalyze the biosynthesis of chitin, an essential component of the fungal cell wall and required for its survival. Chitin is a linear homopolymer of N-acetyl glucosamine units that are linked by β-(1,4) linkage.

Polyoxins and Nikkomycins are the naturally occurring peptidyl nucleoside antibiotics and are the competitive inhibitors of Chitin synthase. Polyoxins and nikkomycins lack mammalian toxicity as the Chitin synthase enzymes are absent in the mammalian cells. However these peptidyl nucleosides are ineffective *in vivo* as potent antifungals because of their poor cell wall penetration and hydrolytic liability to dipeptidases. Due to these shortcomings, peptidyl nucleosides and their analogues are unsuccessful in the clinical treatment of infections caused by opportunistic fungi, although they are safe to mammalian counterpart.[18]

Keeping the interest in the hope of developing an entirely different and a new class of peptidyl nucleoside mimics, here in this section, the synthesis of conjugates of nucleosides and diverse saccharide molecules containing a metabolically stable 1,2,3-triazole heterocyclic linkage is presented. As 1,2,3-triazole heterocyclic ring is regarded as a surrogate of amide linkage and phosphate group, it is expected that the resultant analogues would exhibit metabolic stability and enhanced antifungal activity. We have also planned to synthesize some dinucleotide aducts linked by 1,2,3-triazole heterocycle, keeping the reports of Parang[8] and Finney[12] in mind to make an effort by which the cellular uptake of the molecules may be enhanced.

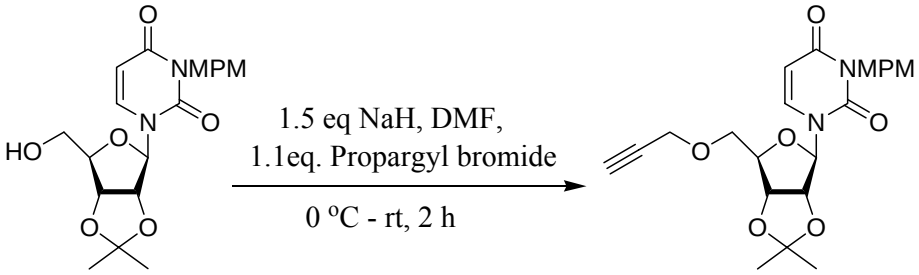
We planned to synthesize nucleoside and saccharide conjugates containing 1,2,3-triazole linkage by means of modified Huisgen's reaction, a "Click Chemistry" process, from alkyne functionalized nucleosides and diverse azido saccharide. Glycoconjugates were synthesized from 1,3-dipolar cycloaddition reaction of propargyl ethers of nucleosides like uridine and thymidine with azides derived from various saccharide molecules. In another part the reaction of propargyl ethers of nucleosides containing amino acid linkage with saccharide azides was reported.

Synthesis of Glycoconjugates by Ligation of Carbohydrate Derived Azides with Propargyl ether of Nucleosides by "Click Chemistry"

A. Glycoconjugates of nucleoside derived alkyne and saccharide azides:

The substrate N^3-*p*-methoxybenzyl-2',3'-*O*-isopropylidene uridine (**196**) was prepared by known method[19] and was transformed into propargyl ether [Scheme 1, (**197**)] upon treatment with sodium hydride and propargyl bromide at 0 ^oC for 2 hours. The IR spectrum of the propargyl ether showed characteristic stretching frequencies at 3272.98 cm^{-1} of alkyne *C-H* and at 1712.96 & 1666.38 cm^{-1} two carbonyl groups of uridine. The proton NMR depicts the resonances responsible for alkyne *C-H* proton at δ 2.46 ppm (t, *J* = 2.40 Hz), anomeric proton at δ 5.86 (d, *J* = 2.03 Hz) and in the ^{13}C spectrum resonances at δ 75.32 and 78.49 ppm were observed for two acetylinic carbons.

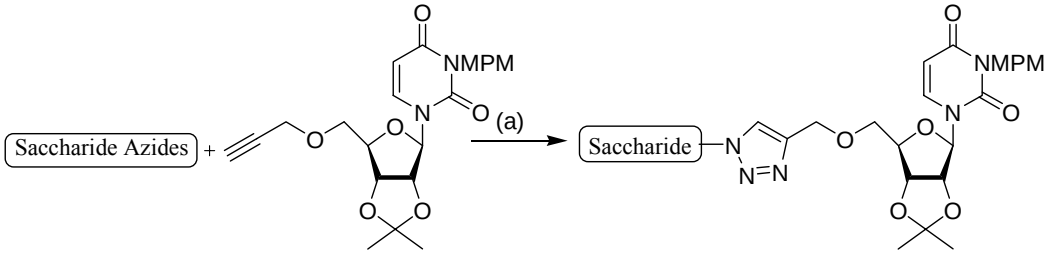
Scheme 1:



(**196**) (**197**)

Once ready with the propargyl ether of uridine, 1,3-dipolar cycloaddition reaction was carried with several saccharides azides. Most of the saccharide azides were reported in chapter 2 and others were synthesized by known methods. The cycloaddition reaction was carried out by following modified Huisgen's reaction i.e. copper(I) catalyzed reaction, reported by Sharpless and coworkers[20] as a much reliable Click Chemistry process, which yields very efficiently and regioselctively 1,4-disubstituted 1,2,3-triazoles. Scheme 2 and table 1 describes the "Click" reactions of propargyl ether of uridine and saccharide azides.

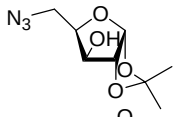
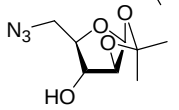
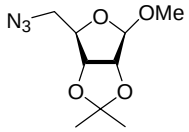
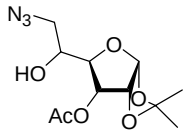
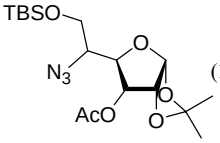
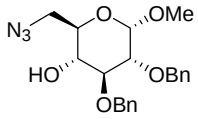
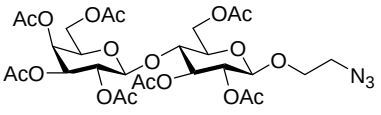
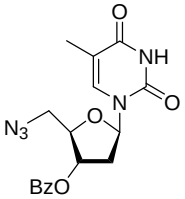
Scheme 2:



(**197**) **Compounds 203 – 210**

Reagents: (a) 0.01 mol% $CuSO_4.5H_2O$, 0.1 mol% Sod.ascorbate, *t*-BuOH:H_2O (1:1), rt, 8-12h.

Table 1

Compound No.	Saccharide Azides	Time (h)	Yield (%)
203.	 (**116**)	8	95
204.	 (**125**)	8	93
205.	 (**198**)	8	94
206.	 (**199**)	10	90
207.	 (**134**)	11	89
208.	 (**146**)	8	95
209.	 (**200**)	12	85
210.	 (**201**)	10	90

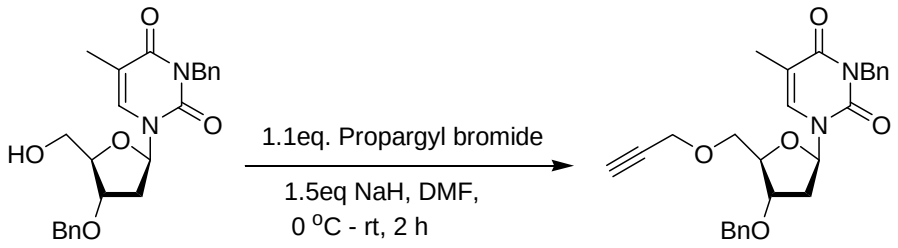
For example as a model of these reactions (Compound **205**, Table 1) 1.0 equivalent of propargyl ether of uridine (**197**) and 1.0 equivalent 5-deoxyazido derivative of methyl β-D-ribofuranoside (**198**) were dissolved in 1:1 mixture of *t*-butanol and water (8 ml) and the solution was treated with 1 mol% $CuSO_4.5H_2O$ and 10 mol% Sodium ascorbate. The reaction mixture was allowed stir at room temperature and took about 8 hours for the completion of cycloaddition. Glycoconjugate containing triazole linker was obtained in quantitative yield. In the similar manner rest of the transformations were carried out very efficiently (Compounds **203 – 210**).

All the glycoconjugates were characterized by IR, ^{1}H and ^{13}C NMR, MS etc. Methyl 5-deoxyazido-β-D-ribofuranoside derived glycoconjugate for example (Entry **205,** table 1) showed following observations in the IR and NMR spectrum. The IR spectrum showed characteristic stretching frequencies at 3018.39, 1710.74, 1666.38 cm^{-1}etc. The proton NMR spectrum showed resonances at δ 5.86 ppm (d, J = 2.15 Hz) and 5.00 ppm (s) for two anomeric protons, 7.59 ppm (s) for a olefinic proton of triazole ring and the ^{13}C NMR spectrum showed resonances at δ 93.48 & 109.83 ppm for two anomeric carbon atoms, 122.73 & 143.91 ppm for two olefinic carbons of triazole ring, rest of the spectrum is in complete agreement with the assigned structure.

Similar set of reactions as described above were also conducted on propargyl ether derived from dibenzyl thymidine [Scheme 3, (**212**)] as a different building block in the interest of generating diverse conjugates. Propargyl ether was prepared similarly as described in the case of propargyl ether of uridine, from N^3,3'-*O*-dibenzyl thymidine (**211**). IR spectrum of compound (**212**) showed characteristic frequencies at 3305.76 cm^{-1} (alkyne *C-H*) and at 1697.24 & 1666.38 cm^{-1} (two carbonyl groups of

thymidine). The proton NMR spectrum depicts the resonances responsible for alkyne *C-H* proton at δ 2.46 ppm (t, *J* = 2.40 Hz) anomeric proton at δ 6.40 ppm (dd, J = 5.94, 7.83 Hz) and in the ^{13}C spectrum the resonances observed at δ 75.23 & 78.79 ppm for two acetylinic carbons.

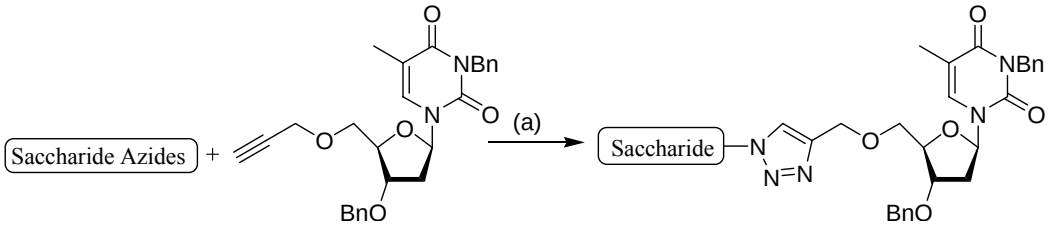
Scheme 3:



(**211**) (**212**)

After the synthesis of propargyl ether of thymidine, it was subjected to "Click" reaction with saccharide azides (Table 2) following the above described method. Glycoconjugates were obtained in good yields and are described in table 2 (Scheme 4, Compounds **213 – 218**).

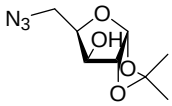
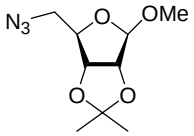
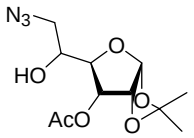
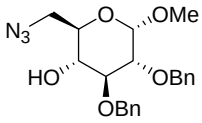
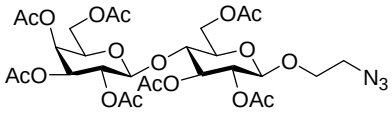
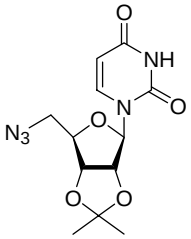
Scheme 4:



(**212**) **Compounds 213 - 218**

Reagent: a) 1 mol% $CuSO_4.5H_2O$, 10 mol% Sod.ascorbate, *t*-BuOH:H_2O (1:1), rt,8-12h.

Table 2

Compound No.	Saccharide Azides	Time (h)	Yield (%)
213.	 (**116**)	8	92
214.	 (**198**)	8.5	90
215.	 (**199**)	10	88
216.	 (**146**)	9	93
217.	 (**200**)	12	85
218.	 (**202**)	10	89

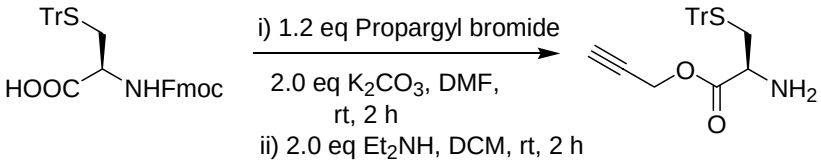
Compound **213** (Table 2) for instance is a glycoconjugate derived from 5-deoxyazido derived from α-D-xylofuranose (**116**) and propargyl ether of dibenzyl thymidine (**212**). IR spectrum of this conjugate showed stretching frequencies at 3409.91 cm^{-1}, 3018.39 cm^{-1}, 1697.24 cm^{-1}, 1666.38 cm^{-1}, etc. The proton NMR spectrum revealed the resonances at δ 5.96 ppm (d, 3.54 Hz) & 6.37 ppm (dd, J = 6.19, 7.20 Hz) two anomeric protons, 7.61 ppm (s) a olefinic proton, and the ^{13}C NMR spectrum showed the resonances at δ 85.83 ppm & 105.08 ppm of two anomeric carbon atoms, 123.88 ppm of a olefinic carbon of triazole,. Rest of the spectrum is in accordance with the structure assigned.

B. Glycoconjugates of nucleoside derived alkyne and saccharide azides containing amino acid and 1,2,3-triazole linkers:

After the successful completion of "Click" reactions of saccharide azides and nucleoside alkynes, it was thought to modify the linkage between these two components. In this context, the nucleoside alkynes were modified to contain an amino acid linkage. The amino acid incorporation can enhance the binding affinity of the drug molecule to the target protein. For example the amino acid, *S*-trityl-L-cysteine itself is a new potent inhibitor of the mitotic kinesin Eg5, this activity shows the protein binding property of *S*-trityl-L-cysteine.[21]

It was thought to incorporate *S*-trityl-L-cysteine in nucleoside alkynes in the form of pseudodipeptide. The resultant pseudodipeptide may be stable to hydrolytic activity by dipeptidases. Accordingly, commercially available *N*-Fmoc-*S*-trityl-L-cysteine (**219**) was modified to propargyl ester (Scheme 5, **220**). *N*-Fmoc-*S*-trityl-L-cysteine (**219**) upon treatment with propargyl bromide and potassium carbonate in DMF resulted in the propargyl ester, which was then subjected to *N*-Fmoc deprotection by treating it with diethyl amine in DCM to obtain free amine (**220**).

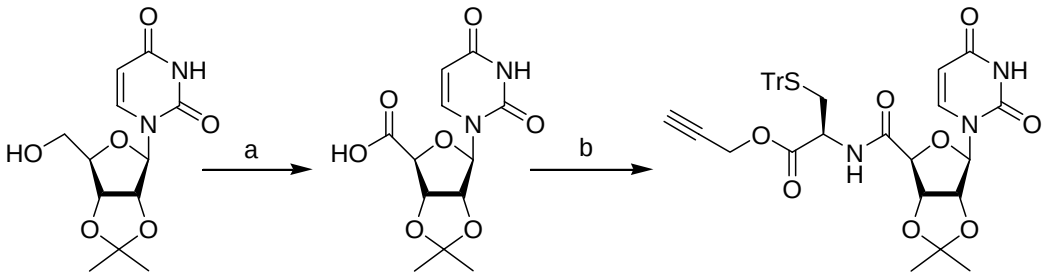
Scheme 5:



(**219**) (**220**)

The propargyl ester of *S*-trityl-L-cysteine(NH_2) was then coupled with nucleoside 5'-carboxylic acids. Nucleoside 5'-carboxylic acids were synthesized by following reported method using BAIB and TEMPO.[22] The effort was initiated with the preparation of 5'-carboxylic acid of 2',3'-*O*-isopropylidene uridine (**222**) from (**221**), which subsequently coupled with propargyl ester of *S*-trityl-L-cysteine (NH_2) (**220**) using HBTU as coupling agent in excellent yield (Scheme 6, **223**).

Scheme 6:



(**221**) (**222**) (**223**)

Reagents: a) 2.2 eq BAIB, 0.2 eq TEMPO, AcCN:H_2O (1:1), rt, 3 h. 70%; b) 1.0 eq of (**220**), 1.0 eq HOBt, 2.0 eq DIPEA, 1.1 eq HBTU, DMF, rt, 2 h.

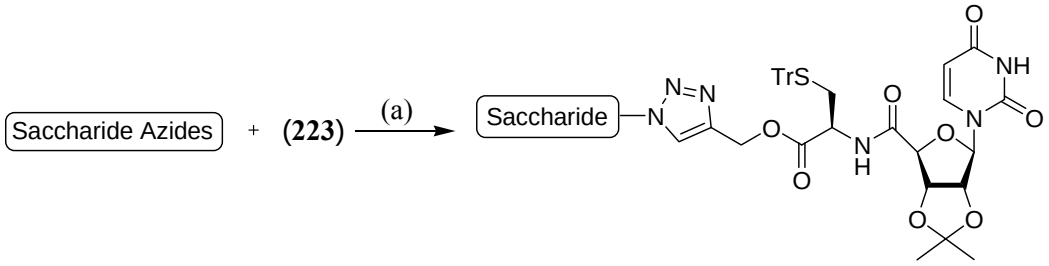
The formation of conjugate of uridine and *S*-trityl-L-cysteine (**223**) was confirmed by IR and NMR. The IR spectrum has shown the stretching frequencies at 3388.70 cm^{-1}, 1745.46 cm^{-1}, 1714.60 $cm^{-}1$, 1693.38 cm^{-1}, etc. The proton NMR spectrum revealed the resonance responsible for alkyne *C-H* proton at δ 2.48 ppm (t, *J* = 2.53 Hz), for anomeric proton at δ 5.63 ppm (d, *J* = 2.15 Hz) and in the ^{13}C spectrum resonances responsible for acetylinic carbons observed at δ 75.65 & 76.80 ppm.

The alkyne functionalized conjugate of uridine and *S*-trityl-L-cysteine (**223**) was then conjugated with diverse saccharide azides (Table 3) by using reported "Click" reaction methodology.[16] The method involves the treatment of acetonitrile solution alkyne conjugate (**223**) and selected saccharide azide with 2 equivalent of copper iodide and slight excess of *N,N*-diisopropyl ethylamine at room temperature for the specified period of time (Scheme 7). The glycoconjugates installed with 1,2,3-triazole and L-cysteine linkage were obtained in moderate yields (Compounds **224-228,** Table 3).

The copper sulfate-sodium ascorbate combination as catalyst for the click reaction of alkyne conjugate containing *S*-trityl-L-cysteine (**223**) and saccharide azide

did not gave satisfactory result. The reaction resulted in complex mixture and poor yields. Therefore the click reaction of these alkynes and azides was carried out using copper iodide and *N,N*-diisopropyl ethylamine in acetonitrile.

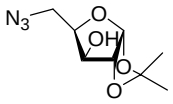
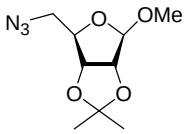
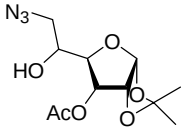
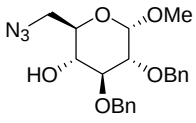
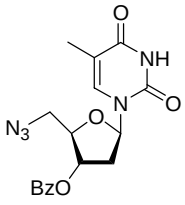
Scheme 7:



Compounds 224 – 228

Reagents: (a) 2.0 eq CuI, 5.0 eq DIPEA, Acetonitrile, rt.

Table 3

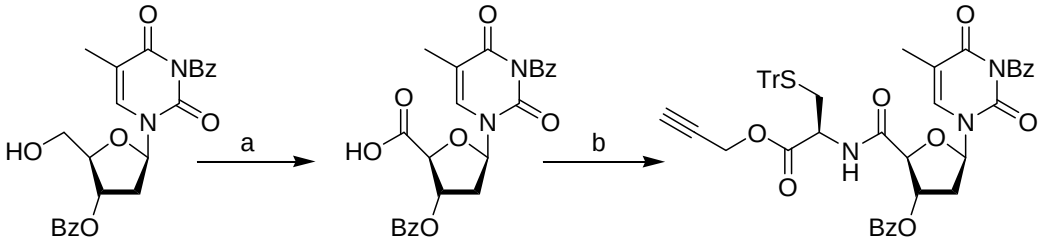
Compound No.	Saccharide Azides		Time (min)	Yield (%)
224.		(**116**)	15	65
225.		(**198**)	15	70
226.		(**199**)	15	50
227.		(**146**)	15	70
228.		(**201**)	15	60

Compound **227** (Table 3) was derived from "Click" reaction of alkyne conjugate (**223**) and 6-deoxyazido derivative of methyl α-D-glucopyranoside (**146**).

The IR spectrum of this compound showed frequencies at 3647.14 cm^{-1}, 1747.39 cm^{-1},1693.38 cm^{-1} etc. The proton NMR revealed the resonances at δ 5.62 (d, 1 H, J = 2.15 Hz, anomeric), 7.86 ppm (s, 1 H, amide), 8.48 ppm (s, 1 H, triazole), and ^{13}C NMR spectrum has shown resonances at δ 125.71 & 141.72 ppm for two olefinic carbon atoms of triazole ring.

In the similar manner, the above protocols were extended now with the alkyne conjugate (Scheme 8) (**231**) derived from 5'-carboxylic acid of N^3,3'-*O*-dibenzoyl thymidine (**230**) and propargyl ester of *S*-trityl-L-cysteine (NH_2) (**220**).

Scheme 8:

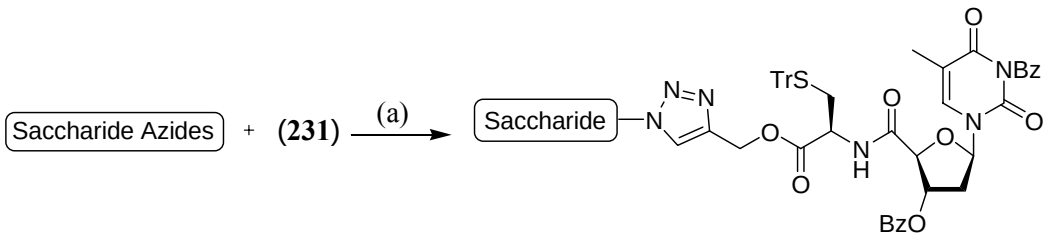


(**229**) (**230**) (**231**)

Reagents: a) 2.2 eq BAIB, 0.2 eq TEMPO, AcCN:H_2O (1:1), rt, 3 h. 72%; b) 1.0 eq of (**220**), 1.1 eq HOBt, 2.0 eq DIPEA, 1.1 eq HBTU, DMF, rt, 1.5 h.

The "Click" reactions of the alkyne conjugate (**231**) with the specified saccharide azides (Scheme 9, Table 4) was also carried out using the same protocol as described above in moderate to good yields. Table 4 gives the details of thymidine conjugates containing triazole ring and *S*-trityl-L-cysteine (Compounds **232 – 236**).

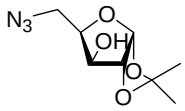
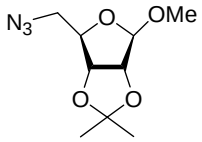
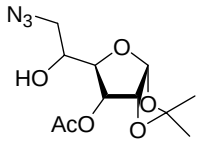
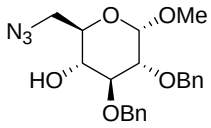
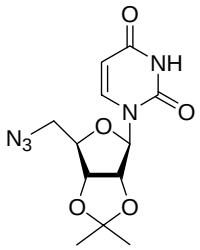
Scheme 9:



Compounds 232 - 236

Reagents: (a) 2.0 eq CuI, 5.0 eq DIPEA, Acetonitrile, rt.

Table 4

Compound No.	Saccharide Azides	Time (min)	Yield (%)
232.	 (**116**)	10	91
233.	 (**198**)	10	85
234.	 (**199**)	10	80
235.	 (**146**)	10	75
236.	 (**201**)	10	70

Compound (**232**) (Table 4) a glycoconjugate obtained from "Click" reaction of alkyne conjugate of dibenzoyl thymidine (**231**) and 5-deoxyazido compound derived from α-D-xylofuranose (**116**). The IR spectrum of this compound has shown frequencies at 3645.21 cm^{-1}, 1747.39 cm^{-1},1650.95 cm^{-1} etc. The proton NMR revealed the resonances at δ 6.58 (dd, 1 H, J = 5.31, 9.60 Hz, anomeric), 7.72 ppm (s, 1 H, triazole), 8.11 ppm (s, 1 H, amide), and ^{13}C NMR spectrum has shown resonances at δ 125.26 & 142.07 ppm for two olefinic carbon atoms of triazole ring. Rest of the spectrum is in agreement with the structure assigned.

3.6. Conclusion

In conclusion, herewith we have presented the efficient synthesis of glycoconjugates from saccharide molecules and nucleosides. We employed modified Huisgen's1,3-dipolar cycloaddition reaction. Both the "Click" reaction processes i.e. use of copper sulfate pentahydrate and copper iodide as Cu(I) catalyst were worked very well in obtaining glycoconjugates regioselectively and efficiently. The resultant glycoconjugates were incorporated with metabolically stable 1,2,3-triazole system and also contained *S*-trityl-L-cysteine linkage in the form of pseudodipeptide, with the anticipation of increased hydrolytic stability and augmented protein binding of resultant glycoconjugates so that the biological activity can be enhanced.

We employed different types of alkyne functionalized nucleoside and diverse azido carbohydrate building blocks and thus diversity was achieved in the resultant glycoconjugates. Compounds **203** – **210** (Table 1) and **213** - **218** (Table 2) are the glycoconjugates obtained from "Click" reaction of sacccharide azides with the alkyne functionalized uridine and thymidine derivatives respectively. In both the cases we obtained very excellent yields and are single regioisomers.

After this successful endeavor, we synthesized glycoconjugates which contained both 1,2,3-triazole and *S*-trityl-L-cysteine linkage following the same strategy. Compounds **224** – **228** (Table 3) and **232** - **236** (Table 4) are the glycoconjugates obtained from "Click" reaction of sacccharide azides with the alkyne functionalized *S*-trityl-L-cysteine coupled uridine and thymidine derivatives respectively. The yields obtained are moderate to good and are also single regioisomers.

Very interestingly, the compounds **210/218** and **228/236** are the dimers of nucleosides linked through triazole, and triazole with *S*-trityl-L-cysteine linkage

respectively. According to the literature[8], the dimeric modification of nucleosides would result in enhanced cellular uptake and so the biological activity. Some of the selected glycoconjugates were evaluated for antifungal activity and are revealed in chapter 4 in detail.

Chapter 3: Experimental

A. Glycoconjugates Of Nucleoside Derived Alkyne And Saccharide Azides:

Synthesis of N^3-*p*-methoxybenzyl-2',3'-*O*-isopropylidene-5'-*O*-propargyluridine (197):

To a solution of N^3-*p*-methoxybenzyl-2',3'-*O*-isopropylidene uridine (**196**) (1.2 gm, 2.96 mmol) in 15 ml of dry DMF was added sodium hydride (0.180 gm of 60% paraffin suspension, 4.44 mmol) at 0 ^oC under argon atmosphere. The reaction mixture was allowed to stir for an hour at room temperature. After one stirring at room temperature, the reaction mixture was again cooled to 0 ^oC and propargyl bromide (0.29 ml, 3.26 mmol) was added drop wise under argon. After completion of the reaction, monitored by TLC, reaction mixture was quenched with 5 ml of methanol and diluted with 300 ml of diethyl ether in a separating funnel. The ether layer was washed subsequently with 3 x 35 ml of water, 35 ml of brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Finally the crude propargyl ether was purified by silica gel column chromatography using ethyl acetate and petroleum ether as eluent to obtain 1.18 gm (90%) of (**197**).

Compound No. 197:

Thick syrup.

IR (cm^{-1}): 3272.98, 2987.53, 2937.38, 1712.67, 1666.38, 1514.02, 1454.23.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.36, 1.59 (2s, 6 H), 2.46 (t, 1 H, *J* = 2.40 Hz), 3.75 (ddd, 2 H, *J* = 3.91, 10.48, 14.27 Hz), 3.77 (s, 3 H), 4.15 (t, 2 H, *J* = 2.27 Hz), 4.41-4.45 (m, 1 H), 4.74-4.83 (m, 2 H), 5.04 (q, 2 H, *J* = 13.65 Hz), 5.75 (d, 1 H, *J* = 8.08 Hz), 5.86 (d, 1 H, *J* = 2.03 Hz), 6.82 (d, 2 H, *J* = 8.84 Hz), 7.46 (d, 2 H, *J* = 8.84 Hz), 7.49 (d, 1 H, *J* = 8.09 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 25.16, 27.04, 43.35, 55.07, 58.45, 69.63, 75.32, 78.49, 80.96, 85.34, 85.58, 94.03, 101.47, 113.53, 113.86, 128.87, 130.65, 138.71, 150.76, 158.96, 162.56.

CHNS Anal.: Calculated for $C_{23}H_{26}N_2O_7$: C, 62.43; H, 5.92; N, 6.33.

Found: C, 62.49; H, 6.00; N, 6.29.

MS: m/z = 443.49 (M + 1).

Synthesis of *N*^{3},3'-*O*-dibenzyl thymidine (211):

A solution of 5'-*O*-tert-butyldiphenylsilyl thymidine[23] (1.2 gm, 2.5 mmol) in 15 ml of dry DMF was cooled to 0 ^oC and was added sodium hydride (0.30 gm of 60% in paraffin, 7.5 mmol) under argon atmosphere. After 30 min of stirring, the reaction mixture was again cooled to 0 ^oC and was added benzyl bromide (0.65 ml, 5.5 mmol) drop wise. Then the reaction mixture was stirred for 2 hours at room temperature and quenched by adding 5ml of methanol. The DMF solution was diluted with 250 ml of ethyl and was washed subsequently with 3 x 30 ml of water, 30 ml of brine solution and dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using ethyl acetate and petroleum ether as eluent to obtain 1.76 gm of *N*^{3},3'-*O*-dibenzyl-5'-*O*-tert-butyldiphenylsilyl thymidine (92%).

To a solution of *N*^{3},3'-*O*-dibenzyl-5'-*O*-tert-butyldiphenylsilyl thymidine (1.62 gm, 2.45 mmol) in tetrahydrofuran was added tetra-butylammoniumfluoride (2.95 ml of 1 M solution in THF, 2.94 mmol) and was stirred at room temperature for 2.5 hours. After completion of the reaction, monitored by TLC, the solution was concentrated under reduced pressure, silica gel column chromatographic purification of the residue yielded 0.98 gm of (**211**) (94.6%).

Thick syrup.

IR (cm^{-1}): 3473.56, 3066.61, 2929.67, 1697.24, 1666.38, 1469.66.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.93 (d, 3 H, *J* = 1.14 Hz), 2.00 (bs, 1 H), 2.28-2.48 (m, 2 H), 3.83 (ddd, 2 H, *J* = 2.91, 11.88, 14.91Hz), 4.13-4.17 (m, 1 H), 4.25-4.31 (m, 1 H), 4.54 (q, 2 H, *J* = 11.75 Hz), 5.11 (s, 2 H), 6.15 (t, 1 H, *J* = 6.57 Hz), 7.27-7.34 (m, 9 H), 7.45-7.50 (m, 2 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 13.24, 37.19, 44.44, 62.77, 71.54, 78.57, 85.05, 87.91, 110.34, 127.55, 127.61, 127.92, 128.32, 128.51, 129.51, 134.95, 136.77, 137.48, 150.95, 163.31.

CHNS Anal.: Calculated for $C_{24}H_{26}N_2O_5$: C, 68.23; H, 6.20; N, 6.63.

Found: C, 68.20; H, 6.18; N, 6.59.

MS: m/z = 423.18 (M + 1).

Synthesis of *N^3*,3'-*O*-dibenzyl-5'-*O*-propargyl thymidine (212):

Following the same procedure as above, *N^3*,3'-*O*-dibenzyl thymidine (**211**) (1.0 gm, 2.37 mmol) was transformed into its propargyl ether (**212**). After column chromatographic purification obtained 0.98 gm of thick syrup (89%).

<u>Compound No. 212</u>:

Thick syrup.

IR (cm^{-1}): 3305.76, 2929.74, 2864.09, 1697.24, 1666.38, 1496.66, 1467.73, 1352.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.95 (d, 3 H, *J* = 1.14 Hz), 2.02-2.12 (m, 1 H), 2.43-2.54 (m, 1 H), 2.46 (t, 1 H, *J* = 2.40 Hz), 3.74 (ddd, 2 H, *J* = 2.40, 10.36, 12.76 Hz), 4.19 (dd, 2 H, *J* = 1.01, 2.40 Hz), 4.21-4.24 (m, 2 H), 4.53 (q, 2 H, *J* = 11.75 Hz), 5.12 (s, 2 H), 6.40 (dd, 1 H, *J* = 5.94, 7.83 Hz), 7.26-7.36 (m, 8 H), 7.46-750 (m, 2 H), 7.58 (d, 1 H, *J* = 1.26 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 13.30, 37.79, 44.34, 58.46, 69.85, 71.25, 75.23, 78.79, 79.04, 83.53, 85.80, 110.08, 127.41, 127.50, 127.79, 128.24, 128.42, 129.03, 133.82, 136.90, 137.43, 150.90, 163.37.

CHNS Anal.: Calculated for $C_{27}H_{28}N_2O_5$: C, 70.42; H, 6.13; N, 6.08.

Found: C, 70.37; H, 6.08; N, 6.15.

MS: m/z = 461.57 (M + 1).

Synthesis of Saccharide azides (azido sugars):

The synthesis of most of the saccharide azides i.e. azides derived from *xylose* (**116**), *arabinose* (**125**) and *glucose* (**199**) (**134**) **&** (**146**) employed for the synthesis of glycoconjugates were described in detail in chapter 2. The azide derived from *lactose* (**200**) was prepared by following reported method.[16]

Synthesis of methyl 2,3-*O*-isopropylidene-5-azido-5-deoxy-β-D-ribofuranoside (198):

Methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside[24] was transformed into 5-deoxy azido derivative by following the same method as employed for the synthesis of saccharide azides described in chapter 2.

Liquid.

IR (cm^{-1}): 2991.39, 2939.31, 2104.19, 1456.16, 1382.87.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.32, 1.49 (2s, 6 H), 3.36 (ddd, 2 H, *J* = 6.82, 12.50, 19.32 Hz), 3.38 (s, 3 H), 4.30 (t, 1 H, *J* = 7.20 Hz), 4.61 (s, 2 H), 5.00 (s, 1 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 24.85, 26.33, 53.71, 55.13, 82.00, 85.08, 85.33, 109.78, 112.61.

CHNS Anal.: Calculated for $C_9H_{15}N_3O_4$: C, 47.16; H, 6.60; N, 18.33.

Found: C, 47.09; H, 6.58; N, 18.41.

MS: m/z = 252.43 ($M + Na^+$).

1,2-*O*-isopropylidene-3-*O*-acetyl-6-azido-6-deoxy -α-D-glucofuranoside (199):

Thick syrup.

IR (cm^{-1}): 3467.77, 2989.46, 2937.38, 2108.05, 1743.53, 1375.15.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.32, 1.50 (2s, 6 H), 2.13 (s, 3 H), 3.96 (dd, 1 H, *J* = 3.41, 7.58 Hz), 4.09-4.23 (m, 3 H), 4.33 (dd, 1 H, *J* = 3.29, 11.63 Hz), 4.53 (d, 1 H, *J* = 3.66 Hz), 5.98 (d, 1 H, *J* = 3.66 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 20.78, 26.21, 26.76, 60.00, 63.79, 74.77, 79.96, 85.45, 104.61, 112.07, 171.23.

CHNS Anal.: Calculated for $C_{11}H_{17}N_3O_6$: C, 45.99; H, 5.96; N, 14.63.

Found: C, 45.89; H, 6.09; N, 14.55.

MS: m/z = 288.05 (M + 1).

Synthesis of 3'-*O*- benzoyl-5'-azido-5'-deoxy thymidine (201):

3'-*O*-benzoyl-5'-azido-5'-deoxy thymidine (**201**) was synthesized from N^3,3'-*O*-dibenzoyl thymidine by following the same method as employed for the synthesis of saccharide azides described in chapter 2. During the transformation of tosylate to azido derivative, N^3–benzoyl group was cleaved therefore obtained only mono benzoyl derivative (**10**).

Colorless solid.

IR (cm^{-1}): 3191.97, 2106.12, 1714.60, 1693.38, 1681.81.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.98 (d, 3 H, *J* = 1.01 Hz), 2.29-2.44 (m, 1 H), 2.55-2.65 (m, 1 H), 3.84 (d, 2 H, *J* = 2.90 Hz), 4.27 (q, 1 H, *J* = 2.90 Hz), 5.42-5.48 (m, 1 H), 6.45 (dd, 1 H, *J* = 5.56, 8.84 Hz), 7.44-7.62 (m, 4 H), 8.02-8.86 (m. 2 H), 8.87 (s, 1 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 12.56, 37.14, 52.58, 75.07, 82.73, 84.47, 111.80, 128.48, 128.87, 129.66, 133.60, 134.79, 150.58, 163.75, 166.06.

CHNS Anal.: Calculated for $C_{17}H_{17}N_5O_5$: C, 54.98; H, 4.61; N, 18.86.

Found: C, 54.79; H, 4.58; N, 18.71.

MS: m/z = 372.53 (M + 1).

Synthesis of 2',3'-*O*-isopropylidene-5'-azido-5'-deoxy uridine (202):

2',3'-*O*-isopropylidene uridine[19] was transformed into 5'-deoxyazido derivative by following the same method as employed for the synthesis of saccharide azides described in chapter 2.

Colorless solid.

IR (cm^{-1}): 2106.12, 1693.38, 1666.38, 1666.38, 1461.94, 1384.79.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.36, 1.57 (2s, 6 H), 3.63 (d, 2 H, *J* = 5.18 Hz), 4.25 (q, 1 H, *J* = 5.28 Hz), 4.82 (dd, 1 H, *J* = 4.04, 6.44 Hz), 5.01 (dd, 1 H, *J* = 2.15, 6.44 Hz), 5.66 (d, 1 H, *J* = 2.15 Hz), 5.78 (d, 1 H, 7.96 Hz), 7.31 (d, 1 H, *J* = 8.09 Hz), 9.62 (s, 1 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 25.16, 27.01, 52.28, 81.47, 84.25, 85.79, 94.76, 102.79, 114.71, 142.50, 150.14, 163.63.

CHNS Anal.: Calculated for $C_{12}H_{15}N_5O_5$: C, 46.60; H, 4.89; N, 22.64.

Found: C, 46.49; H, 4.98; N, 22.78.

MS: m/z = 310.06 (M + 1).

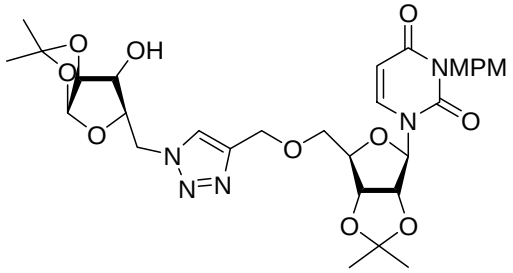
General procedure for "Click" reaction of nucleoside alkynes and saccharide azides:

"Click" reaction reported by Sharpless *et. al.*[20] was employed to conjugate the nucleoside alkynes and specified saccharide azides. The reaction was performed as follows. To a solution of 1 equivalent of nucleoside alkyne and 1 equivalent of specified saccharide azide in 1:1 mixture of *t*-butanol and water was added 1 mol% of copper sulfate pentahydrate and 10 mol% of sodium ascorbate. The reaction mixture was stirred at room temperature for specified period of time, after completion

monitored by TLC, it was diluted with 200ml of ethyl acetate. The organic layer was subsequently washed with 2 x 25 ml of water, 25 ml of brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude glycoconjugates were purified by silica gel column chromatography using ethyl acetate and petroleum ether to obtain glycoconjugates containing 1,2,3-triazole. The reactions are described in Scheme 2 and 4, and results are displayed in table 1 and 2.

"Click" reaction of N^3-*p*-methoxybenzyl-2',3'-*O*-isopropylidene-5'-*O*-propargyl uridine (197) and saccharide azides (Compounds 203- 210, Table 1):

Compound 203: (197) + 1,2-*O*-isopropylidene-5-azido-5-deoxy-α-D-xylofuranose (116):



Colorless solid:

m.p.: 97 ^oC.

$[\alpha]_D^{25}$ = -20.73 (c, 1.09, $CHCl_3$).

IR (cm^{-1}): 3398.34, 3018.39, 1706.88, 1666.38, 1512.09, 1454.23, 1384.79.

^1H NMR [$CDCl_3$, 200 MHz]: δ 1.30, 1.35, 1.45, 1.57 (4s, 12 H), 2.84 (bs, 1 H), 3.69-3.89 (m, 2 H), 3.77 (s, 3 H), 4.22 (d, 1 H, *J* = 1.76 Hz), 4.41 (d, 1 H, *J* = 2.40 Hz), 4.51-4.63 (m, 5 H), 4.71-4.84 (m, 3 H), 5.02 (q, 2 H, *J* = 13.64 Hz), 5.65 (d, 1 H, *J* = 8.08 Hz), 5.89 (d, 1 H, *J* = 2.14 Hz), 5.99 (d, 1 H, *J* = 3.53 Hz), 6.82 (d, 2 H, *J* = 8.71 Hz), 7.43 (d, 2 H, *J* = 8.72 Hz), 7.52 (d, 1 H, *J* = 8.08 Hz), 7.63 (s, 1 H).

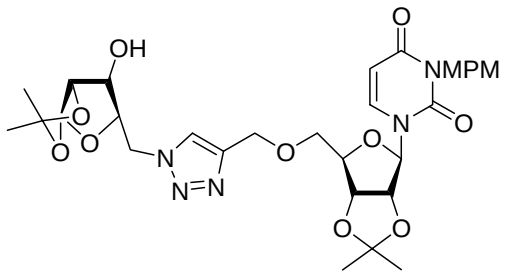
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 25.27, 26.10, 26.72, 27.12, 43.54, 48.82, 55.18, 64.48, 70.49, 74.43, 78.97, 80.82, 85.33, 85.37, 85.76, 93.65, 101.28, 105.02, 111.97, 113.66, 114.00, 128.78, 130.61, 139.15, 150.73, 159.04, 163.09.

CHNS Anal.: Calculated for $C_{31}H_{39}N_5O_{11}$: C, 56.61; H, 5.98; N, 10.65.

Found: C, 56.55; H, 6.10; N, 10.57.

MS: m/z = 657.95 (M^+).

Compound 204: (197) + 1,2-*O*-isopropylidene-5-azido-5-deoxy-β-D-arabino-furanose (125):



Colorless solid:

m.p.: 89 ^oC.

$[\alpha]_D^{25}$ = +33.08 (c, 1.07, $CHCl_3$).

IR (cm^{-1}): 3379.05, 2939.31, 1703.03, 1666.38, 1514.02, 1454.23, 1384.79.

^1H NMR [$CDCl_3$, 200 MHz]: δ 1.34, 1.35, 1.54, 1.57 (4s, 12 H), 3.01 (bs, 1 H), 3.68-3.90 (m, 2 H), 3.77 (s, 3 H), 4.32-4.38 (m, 2 H), 4.42 (d, 1 H, *J* = 2.40 Hz), 4.49-4.65 (m, 4 H), 4.69-4.77 (m, 2 H), 4.81-4.84 (m, 1 H), 5.00 (q, 2 H, *J* = 13.64 Hz), 5.56 (d, 1 H, *J* = 8.08 Hz), 5.92 (d, 1 H, *J* = 2.28 Hz), 5.99 (d, 1 H, *J* = 3.79 Hz), 6.81 (d, 2 H, *J* = 8.72 Hz), 7.42 (d, 2 H, *J* = 8.71 Hz), 7.53 (d, 1 H, *J* = 8.08 Hz), 7.74 (s, 1 H).

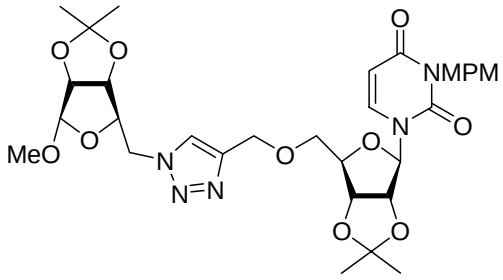
^{13}C NMR [$CDCl_3$, 125 MHz]: δ 25.26, 25.49, 26.63, 27.12, 43.53, 52.12, 55.16, 64.30, 70.44, 75.96, 80.77, 85.39, 85.68, 86.23, 86.78, 93.32, 101.16, 106.43, 112.18, 113.65, 113.95, 124.25, 128.83, 130.60, 138.94, 143.64, 150.78, 159.02, 163.07.

CHNS Anal.: Calculated for $C_{31}H_{39}N_5O_{11}$: C, 56.61; H, 5.98; N, 10.65.

Found: C, 56.57; H, 5.88; N, 10.50.

MS: m/z = 657.36 (M^+).

Compound 205: (197) + Methyl 2,3-*O*-isopropylidene-5-azido-5-deoxy-β-D-ribo-furanoside (198):



Gum.

$[\alpha]_D^{25}$ = -13.19 (c, 1.38, $CHCl_3$).

IR (cm^{-1}): 3018.39, 2937.38, 1710.74, 1666.38.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.31, 1.34, 1.46, 1.57 (4s, 12 H), 3.38 (s, 3 H), 3.68-3.85 (m, 2 H), 3.77 (s, 3 H), 4.38-4.59 (m, 4 H), 4.64-4.69 (m, 3 H), 4.72-4.81 (m, 3 H), 5.02 (q, 2 H, *J* = 13.64 Hz), 5.03 (s, 1 H), 5.71 (d, 1 H, *J* = 8.08 Hz), 5.87 (d, 1 H, *J* = 2.15 Hz), 6.82 (d, 2 H, *J* = 8.72 Hz), 7.44 (d, 2 H, *J* = 8.59 Hz), 7.48 (d, 1 H, *J* = 7.33 Hz), 7.59 (s, 1 H).

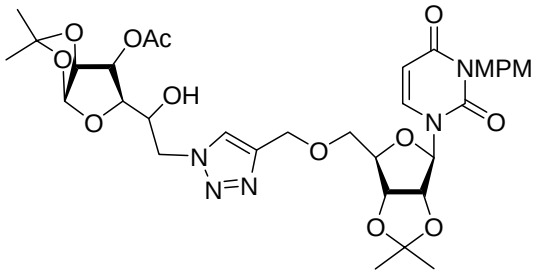
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 24.67, 25.09, 26.13, 26.96, 43.22, 52.93, 54.95, 55.30, 64.26, 70.16, 80.74, 81.52, 84.68, 84.95, 85.41, 93.48, 101.38, 109.83, 112.67, 113.44, 113.72, 122.73, 128.78, 130.42, 138.74, 143.91, 150.65, 158.82, 162.34.

CHNS Anal.: Calculated for $C_{32}H_{41}N_5O_{11}$: C, 57.22; H, 6.15; N, 10.43.

Found: C, 57.10; H, 6.18; N, 10.50.

MS: m/z = 671.23 (M^+).

Compound 206: (197) + 1,2-*O*-isopropylidene-3-*O*-acetyl-6-azido-6-deoxy-α-D-glucofuranose (199):



Gum.

$[\alpha]_D^{25}$ = -29.82 (c, 1.14, $CHCl_3$).

IR (cm^{-1}): 3433.06, 2962.46. 1722.31, 1714.60, 1666.38, 1514.02, 1454.23, 1384.79.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.28, 1.34, 1.45, 1.57 (4s, 12 H), 2.00 (s, 3 H), 2.88 (bs, 1 H), 3.67-3.89 (m, 2 H), 3.77 (s, 3 H), 4.35-4.39 (m, 2 H), 4.55-4.83 (m, 8 H), 4.94-5.10 (m, 3 H), 5.71 (d, 1 H, *J* = 7.95 Hz), 5.89 (d, 1 H, *J* = 3.16 Hz), 5.95 (d, 1 H, *J* = 2.15 Hz), 6.82 (d, 2 H, *J* = 8.71 Hz), 7.43 (d, 2 H, *J* = 8.59 Hz), 7.52 (d, 1 H, *J* = 7.95 Hz), 7.67 (s, 1 H).

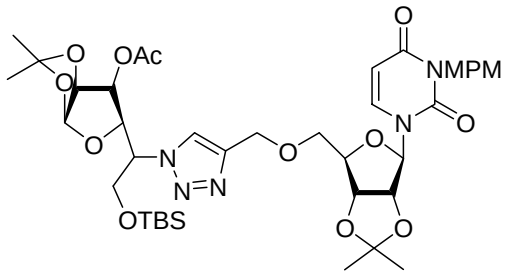
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 20.43 25.17, 26.03, 26.65, 27.04, 43.44, 55.05, 59.96, 60.24, 62.69, 70.27, 74.21, 78.89, 80.70, 85.10, 85.30, 85.47, 93.12, 101.42, 104.43, 111.89, 113.56, 113.88, 128.75, 130.44, 139.01, 150.71, 158.94, 162.91, 170.32.

CHNS Anal.: Calculated for $C_{34}H_{43}N_5O_{13}$: C, 55.96; H, 5.94; N, 9.60.

Found: C, 56.02; H, 6.10; N, 9.55.

MS: m/z = 730.83 (M+1).

Compound 207: (197) + 1,2-*O*-isopropylidene-3-*O*-acetyl-5-azido-5-deoxy-6-*O*-t-butyldimethylsilyl-α-D-glucofuranose (134):



Colorless solid:

m.p.: 99 °C.

$[\alpha]_D^{25}$ = -9.36 (c, 1.09, $CHCl_3$).

IR (cm^{-1}): 2954.74, 2933.53, 1747.39, 1708.81, 1666.38, 1512.09, 1454.23.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ -0.06, -0.03, (2s, 6 H), 0.82 (s, 9 H), 1.28, 1.32, 1.48, 1.56 (4s, 12 H), 2.02 (s, 3 H), 3.66-3.83 (m, 2 H), 3.75 (s, 3 H), 3.90-3.92 (m, 2H), 4.36 (d, 1 H, *J* = 2.91 Hz), 4.49 (d, 1 H, *J* = 3.79 Hz), 4.64 (s, 2 H), 4.69 (dd, 1 H, *J* =

2.65, 6.32 Hz), 4.75-4.84 (m, 2 H), 4.88-5.10 (m, 3 H), 5.32 (d, 1 H, *J* = 2.78 Hz), 5.71 (d, 1 H, *J* = 8.08 Hz), 5.87 (d, 1 H, *J* = 3.79 Hz), 5.92 (d, 1 H, *J* = 2.53 Hz), 6.80 (d, 2 H, *J* = 8.72 Hz), 7.43 (d, 2 H, *J* = 8.71 Hz), 7.50 (d, 1 H, *J* = 8.08 Hz), 7.68 (s, 1 H).

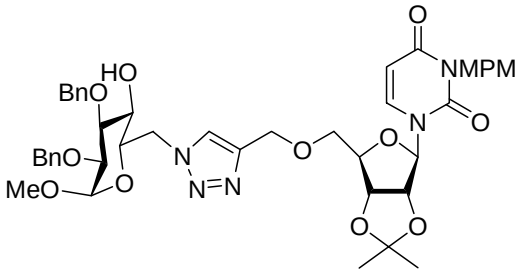
^{13}C NMR [$CDCl_3$, 125 MHz]: δ -5.82, -5.82, 17.96, 20.69, 25.26, 25.58, 26.08, 26.51, 27.14, 43.44, 55.13, 61.31, 62.87, 64.48, 70.18, 75.90, 75.96, 80.80, 83.35, 85.18, 85.39, 93.19, 101.76, 104.23, 112.29, 113.59, 113.98, 123.20, 128.96, 130.68, 138.66, 143.23, 150.87, 158.98, 162.58, 169.54.

CHNS Anal.: Calculated for $C_{40}H_{57}N_5O_{13}Si$: C, 56.92; H, 6.81; N, 8.30.

Found: C, 56.88; H, 6.80; N, 8.36.

MS: m/z = 844.83 (M+1).

Compound 208: (197) + Methyl 2,3-di-*O*-benzyl-6-azido-6-deoxy-α-D-gluco-pyranoside (146):



Colorless solid:

m.p.: 86 ^{o}C.

$[\alpha]_D^{25}$ = +15.67 (c, 1.2, $CHCl_3$).

IR (cm^{-1}): 3431.13, 2935.46. 1706.88, 1666.38, 1512.09, 1454.23, 1384.79.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.33, 1.57 (2s, 6 H), 2.38 (bs, 1 H), 3.11 (t, 1 H, 9.22 Hz), 3.23 (s, 3 H), 3.39 (dd, 1 H, 3.41, 9.47 Hz), 3.64-3.83 (m, 6 H), 3.86-3.95 (m, 1 H), 4.38 (d, 1 H, *J* = 2.27 Hz), 4.58-4.77 (m, 10 H), 4.92-5.09 (m, 3 H), 5.66 (d, 1 H, *J* = 7.96 Hz), 5.86 (d, 1 H, *J* = 2.15 Hz), 6.81 (d, 2 H, *J* = 8.71 Hz), 7.32-7.34 (m, 10 H), 7.41-7.50 (m, 3 H), 7.56 (s, 1 H).

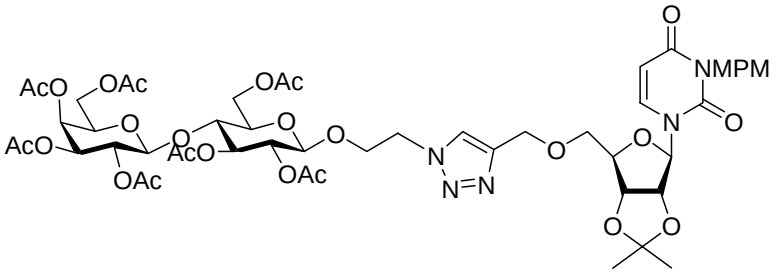
^{13}C NMR [$CDCl_3$, 125 MHz]: δ 25.23, 27.09, 43.39, 50.74, 55.11, 55.28, 64.37, 69.33, 70.19, 70.52, 73.04, 75.43, 79.48, 80.80, 85.29, 85.65, 93.65, 98.10, 101.32, 113.58, 113.89, 124.04, 127.81-127.91, 128.37, 128.48, 128.83, 130.63, 137.78, 138.39, 138.82, 141.73, 150.72, 158.96, 162.65.

CHNS Anal.: Calculated for $C_{44}H_{51}N_5O_{12}$: C, 62.77; H, 6.11; N, 8.32.

Found: C, 62.68; H, 6.15; N, 8.28.

MS: m/z = 842.89 (M+1).

Compound 209: (197) + (2-azido)ethylene *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galacto-pyranosyl)-(1-4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (200):



Colorless solid:

m.p.: 94 ^{o}C.

$[\alpha]_D^{25}$ = -7.00 (c, 1.0, $CHCl_3$).

IR (cm^{-1}): 2935.46. 1755.10, 1747.39, 1666.38, 1514.02, 1454.23, 1371.29.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.35, 1.57 (2s, 6 H), 1.97, 1.98, 2.05, 2.06, 2.07, 2.12, 2.15 (7s, 21 H), 3.57-3.91 (m, 9 H), 4.07-4.18 (m, 5 H), 4.40-4.63 (m, 7 H), 4.77-5.30 (m, 7 H), 5.35 (s, 1 H), 5.65 (d, 1 H, *J* = 7.20 Hz), 5.91 (s, 1 H), 6.82 (d, 2 H, *J* = 8.09 Hz), 7.41-7.53 (m, 3 H).

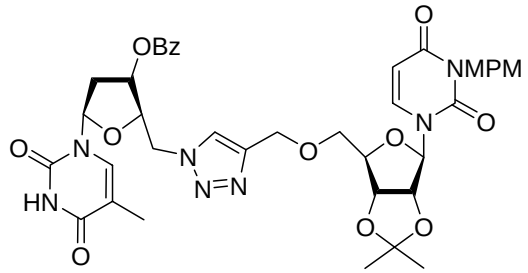
^{13}C NMR [$CDCl_3$, 125 MHz]: δ 20.37-20.72, 25.24, 27.10, 43.44, 55.13, 60.27, 60.65, 61.68, 64.44, 66.53, 67.63, 69.05, 70.28, 70.62, 70.86, 71.31, 72.33, 72.85, 75.96, 80.80, 85.23, 85.56, 93.38, 100.34, 100.95, 101.45, 113.63, 113.94, 128.91, 130.56, 138.86, 150.81, 158.99, 162.60, 168.95, 169.46, 169.53, 169.92, 170.01, 170.20.

CHNS Anal.: Calculated for $C_{51}H_{65}N_5O_{25}$: C, 53.35; H, 5.71; N, 6.10.

Found: C, 53.29; H, 5.80; N, 6.06.

MS: m/z = 1149.39 (M+1).

Compound 210: (197) + 3'-*O*-benzoyl-5'-azido-5'-deoxy thymidine (201):



Colorless solid.

$[\alpha]_D^{25}$ = +9.90 (c, 1.01, $CHCl_3$).

IR (cm^{-1}): 1712.67, 1666.38, 1512.09, 1454.23, 1384.79.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.34, 1.57 (2s, 6 H), 1.90 (s, 3 H), 2.18-2.74 (m, 2 H), 3.67-3.86 (m, 5 H), 4.41-4.55 (m, 2 H), 4.61-4.86 (m, 6 H), 4.94-5.11 (m, 2 H), 5.59 (s, 1 H), 5.74 (d, 1 H, *J* = 8.21 Hz), 5.89 (s, 1 H), 6.10-6.17 (m, 1 H), 6.81 (d, 2 H, *J* = 7.71 Hz), 7.06 (s, 1 H), 7.42-7.51 (m, 5 H), 7.61 (d, 1 H, *J* = 6.70 Hz), 7.67 (s, 1 H), 8.04 (d, 2 H, *J* = 7.20 Hz), 9.15 (s, 1 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 12.23, 25.18, 27.05, 35.93, 43.42, 51.51, 55.08, 60.24, 70.37, 74.85, 80.85, 82.61, 85.20, 85.62, 87.57, 93.56, 101.27, 111.37, 113.57, 113.89, 124.39, 128.48, 128.75, 128.81, 129.65, 130.53, 133.63, 137.04, 139.01, 143.77, 150.32, 150.72, 158.95, 162.79, 163.75, 165.99.

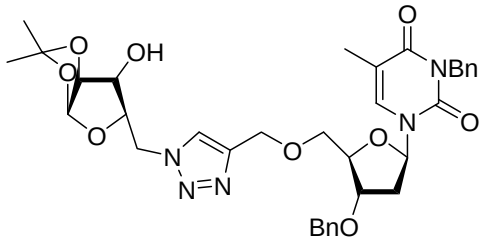
CHNS Anal.: Calculated for $C_{40}H_{43}N_7O_{12}$: C, 59.03; H, 5.33; N, 12.05.

Found: C, 58.99; H, 5.40; N, 12.12.

MS: m/z = 814.55 (M+1).

"Click" reaction of N^3,3'-*O*-dibenzyl-5'-*O*-propargyl thymidine (212) and saccharide azides (Compounds 213 - 218, and Table 2):

Compound 213: (212) + 1,2-*O*-isopropylidene-5-azido-5-deoxy-α-D-xylofuranose (116):



Gum.

$[\alpha]_D^{25}$ = +7.64 (c, 1.65, $CHCl_3$).

IR (cm^{-1}): 3409.91, 2948.96, 2869.88, 1697.24, 1666.38, 1467.73, 1375.15.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.29, 1.43 (2s, 6 H), 1.83 (d, 3 H, *J* = 0.50 Hz), 2.10 (bs, 1 H), 2.22-2.33 (m, 1 H), 2.40-2.51 (m, 1 H), 3.65-3.85 (m, 2 H), 4.19-4.20 (m, 3 H), 4.43-4.47 (m, 1 H), 4.49 (s, 1 H), 4.54-4.61 (m, 3 H), 4.68 (d, 2 H, *J* = 2.14 Hz), 4.75 (dd, 1 H, *J* = 5.68, 13.13 Hz), 5.10 (s, 2 H), 5.96 (d, 1 H, *J* = 3.54 Hz), 6.37 (dd, 1 H, *J* = 6.19, 7.46 Hz), 7.26-7.35 (m, 8 H), 7.44-7.48 (m, 2 H), 7.51 (d, 1 H, *J* = 0.88 Hz), 7.61 (s, 1 H).

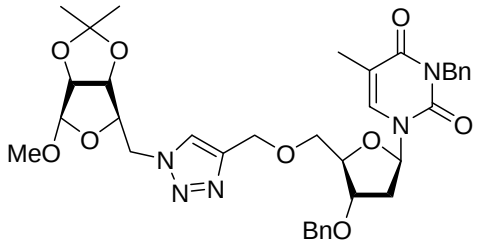
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 13.18, 26.08, 26.73, 37.83, 44.44, 48.92, 64.62, 70.49, 71.39, 74.67, 78.91, 79.08, 83.63, 85.26, 85.83, 105.08, 110.08, 112.03, 123.88, 127.50, 127.57, 127.88, 128.32, 128.48, 128.97, 134.04, 136.84, 137.48, 150.91, 163.54.

CHNS Anal.: Calculated for $C_{35}H_{41}N_5O_9$: C, 62.21; H, 6.12; N, 10.36.

Found: C, 62.18; H, 6.17; N, 10.38.

MS: m/z = 676.42 (M+1).

Compound 214: (212) + Methyl 2,3-*O*-isopropylidene-5-azido-5-deoxy-β-D-ribo-furanoside (198):



Gum.

$[\alpha]_D^{25}$ = +6.73 (c, 1.10, $CHCl_3$).

IR (cm^{-1}): 2933.53, 1697.24, 1666.38, 1469.66, 1454.23, 1373.22.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.30, 1.45 (2s, 6 H), 1.85 (d, 3 H, *J* = 1.01 Hz), 1.99-2.12 (m, 1 H), 2.39-2.50 (m, 1 H), 3.36 (s, 3 H), 3.75 (ddd, 2 H, *J* = 2.28, 10.61, 13.14 Hz), 4.18-4.20 (m, 2 H), 4.43-4.59 (m, 5 H), 4.62-4.76 (m, 4 H), 5.01 (s, 1 H), 5.11 (s, 2 H), 6.38 (dd, 1 H, *J* = 6.06, 7.70 Hz), 7.27-7.55 (m, 8 H), 7.45-7.49 (m, 2 H), 7.52 (d, 1 H, *J* = 1.26 Hz), 7.58 (s, 1 H).

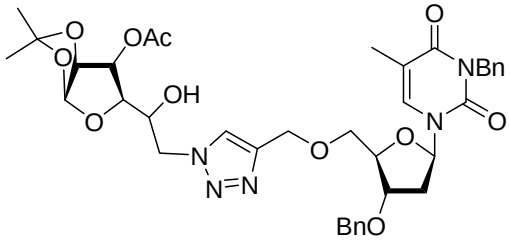
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 13.15, 24.85, 26.32, 37.78, 44.39, 53.19, 55.51, 64.59, 70.41, 71.35, 78.94, 81.73, 83.60, 84.88, 85.18, 85.70, 110.09, 110.16, 112.98, 122.68, 127.45, 127.53, 127.83, 128.28, 128.45, 129.07, 133.83, 136.94, 137.51, 144.37, 150.94, 163.34.

CHNS Anal.: Calculated for $C_{36}H_{43}N_5O_9$: C, 62.69; H, 6.28; N, 10.15.

Found: C, 62.65; H, 6.24; N, 10.10.

MS: m/z = 691.03 (M+1).

Compound 215: (212) + 1,2-*O*-isopropylidene-3-*O*-acetyl-6-azido-6-deoxy-α-D-glucofuranose (199):



Gum.

$[\alpha]_D^{25}$ = -13.93 (c, 1.22, $CHCl_3$).

IR (cm^{-1}): 3436.91, 2929.67, 1745.46, 1697.24, 1666.38, 1469.66, 1375.15.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.27, 1.43 (2s, 6 H), 1.84 (s, 3 H), 1.99 (s, 3 H), 2.03-2.28 (m, 2 H), 2.40-2.51 (m, 1 H), 3.65-3.86 (m, 2 H), 4.21 (s, 2 H), 4.35 (d, 1 H, *J* = 2.53 Hz), 4.44-4.59 (m, 4 H), 4.62-4.76 (m, 4 H), 5.00 (dt, 1 H, *J* = 3.79, 7.45 Hz), 5.10 (s, 2 H), 5.85 (d, 1 H, *J* = 3.54 Hz), 6.39 (dd, 1 H, *J* = 6.06, 7.58 Hz), 7.26-7.33 (m, 8 H), 7.43-7.48 (m, 2 H), 7.54 (d, 1 H, *J* = 0.63 Hz), 7.62 (s, 1 H).

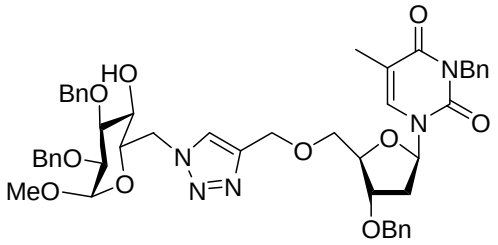
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 13.18, 20.51, 26.09, 26.74, 37.78, 44.42, 60.06, 62.79, 64.70, 70.46, 71.35, 74.39, 78.96, 79.08, 83.63, 85.28, 85.77, 104.53, 110.13, 112.06, 123.59, 127.46, 127.55, 127.84, 128.29, 128.44, 128.91, 134.06, 136.82, 137.49, 143.66, 150.91, 163.55, 170.56.

CHNS Anal.: Calculated for $C_{38}H_{45}N_5O_{11}$: C, 61.03; H, 6.07; N, 9.37.

Found: C, 61.12; H, 6.00; N, 9.29.

MS: m/z = 748.47 (M+1).

Compound 216: (212) + Methyl 2,3-di-*O*-benzyl-6-azido-6-deoxy-α-D-gluco-pyranoside (146):



Gum.

$[\alpha]_D^{25}$ = +31.35 (c, 1.41, $CHCl_3$).

IR (cm^{-1}): 3498.63, 2921.95, 1666.38, 1643.24, 1469.66, 1454.23, 1359.72.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.85 (d, 3 H, *J* = 0.76 Hz), 1.96-2.17 (m, 2 H), 2.38-2.48 (m, 1 H), 3.06-3.23 (m, 4 H), 3.37 (dd, 1 H, *J* = 3.54, 9.60 Hz), 3.64 (dd, 1 H, *J* = 2.02, 10.23 Hz), 3.72-3.81 (m, 2 H), 3.84-3.94 (m, 1 H), 4.15-4.23 (m, 2 H), 4.41-4.57

(m, 5 H), 4.61-4.76 (m, 5 H), 4.98 (d, 1 H, *J* = 11.36 Hz), 5.10 (s, 2 H), 6.37 (dd, 1 H, *J* = 6.07, 7.58 Hz), 7.26-7.34 (m,18 H), 7.44-7.49 (m, 3 H), 7.55 (s, 1 H).

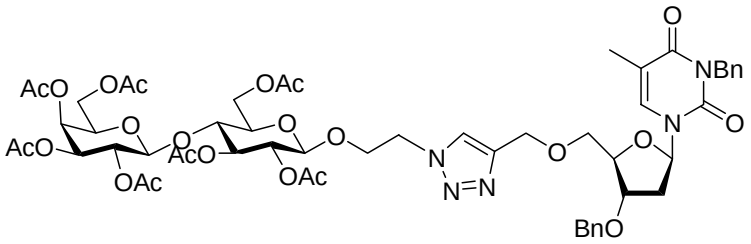
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 13.16, 37.70, 44.36, 50.84, 55.27, 64.53, 69.38, 70.27, 70.61, 71.28, 73.10, 75.43, 78.96, 79.55, 80.75, 83.57, 85.70, 98.18, 110.05, 127.44, 127.48, 127.79, 127.89, 127.93, 128.25, 128.42, 128.56, 129.01, 133.84, 136.87, 137.45, 137.78, 138.38, 150.88, 163.34.

CHNS Anal.: Calculated for $C_{48}H_{53}N_5O_{10}$: C, 67.04; H, 6.21; N, 8.14.

Found: C, 67.10; H, 6.25; N, 8.09.

MS: m/z = 860.58 (M+1).

Compound 217: (212) + (2-azido)ethylene *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galacto-pyranosyl)-(1-4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (200):



Colorless solid.

$[\alpha]_D^{25}$ =+5.08 (c, 1.22, $CHCl_3$).

IR (cm^{-1}): 2960.53. 2929.67, 1755.10, 1699.17, 1666.38, 1467.73, 1371.29.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.83 (d, 3 H, *J* = 0.63 Hz), 1.93, 1.97, 2.04, 2.05, 2.06, 2.11, 2.15 (7s, 21 H), 2.26-2.33 (m, 1 H), 2.40-2.50 (m, 1 H), 3.56-3.90 (m, 6 H), 4.01-4.21 (m, 6 H), 4.42-4.59 (m, 7 H), 4.66 (d, 2 H, *J* = 1.76 Hz), 4.83-4.99 (m, 2 H), 5.05-5.22 (m, 4 H), 5.35 (d, 1 H, *J* = 2.52 Hz), 6.38 (dd, 1 H, *J* = 6.06, 7.33 Hz), 7.27-7.33 (m, 8 H), 7.44-7.49 (m, 2 H), 7.53 (d, 1 H, *J* = 1.01 Hz), 7.56 (s, 1 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 13.05, 20.36-20.70, 37.73, 44.33, 49.98, 60.64, 61.62, 64.48, 66.51, 67.66, 69.04, 70.35, 70.62, 70.83, 71.19, 71.26, 72.32, 72.87, 75.89, 78.91, 83.56, 85.66, 100.37, 100.94, 101.01, 123.75, 127.39, 127.47, 127.76, 128.24,

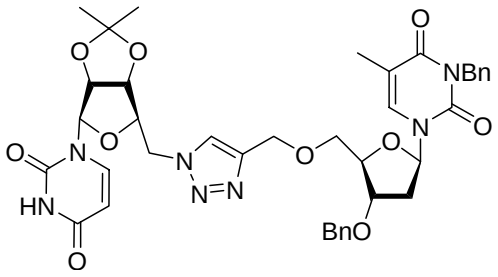
128.39, 128.96, 133.91, 136.89, 137.51, 143.91, 150.88, 163.34, 168.94, 169.42, 169.45, 169.91, 169.98, 170.13, 170.19.

CHNS Anal.: Calculated for $C_{55}H_{67}N_5O_{23}$: C, 56.65; H, 5.79; N, 6.01.

Found: C, 56.57; H, 5.80; N, 5.98.

MS: m/z = 1166.73 (M+1).

Compound 218: (212) + 2',3'-*O*-isopropylidene-5'-azido-5'-deoxy uridine (202):



Colorless solid.

$[\alpha]_D^{25}$ = +52.48 (c, 1.09, $CHCl_3$).

IR (cm^{-1}): 2927.74, 1693.38, 1666.38, 1643.24, 1469.66, 1454.23, 1380.94.

^{1}H NMR [$CDCl_3$, 200 MHz]: δ 1.33, 1.52 (2s, 6 H), 1.81 (s, 3 H), 2.26-2.33 (m, 1 H), 2.40-2.50 (m, 1 H), 3.63-3.85 (m, 2 H), 4.21 (s, 2 H), 4.43-4.52 (m, 3 H), 4.58-4.71 (m, 4 H), 4.91-4.96 (m, 1 H), 5.08-5.10 (m, 3 H), 5.41 (s, 1 H), 5.69 (d, 1 H, *J* = 7.96 Hz), 6.37 (dd, 1 H, *J* = 6.19, 7.07 Hz), 7.09 (d, 1 H, *J* = 8.08 Hz), 7.25-7.34 (m, 8 H), 7.43-7.48 (m, 2 H), 7.50 (s, 1 H), 7.54 (s, 1 H), 9.62 (s, 1 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 13.17, 25.16, 27.01, 37.77, 44.42, 51.83, 64.50, 70.40, 71.36, 78.99, 81.88, 83.64, 84.24, 85.81, 86.51, 96.96, 102.77, 110.02, 114.78, 127.47, 127.56, 127.85, 128.29, 128.46, 129.02, 134.03, 136.90, 137.49, 143.64, 150.07, 150.93, 163.19, 163.46.

CHNS Anal.: Calculated for $C_{39}H_{43}N_7O_{10}$: C, 60.85; H, 5.63; N, 12.74.

Found: C, 60.75; H, 5.59; N, 12.85.

MS: m/z = 770.55 (M+1).

B. Glycoconjugates of nucleoside derived alkynes and saccharide azides containing amino acid and 1,2,3-triazole linkers:

Synthesis of *S*-trityl-L-cysteine propargyl ester (NH_2) (220):

To a solution of *N*-Fmoc-*S*-trityl-L-cysteine (CO_2H) (**219**) (1.50 gm, 2.56 mmol) in 15 ml of dry DMF was added potassium carbonate (0.720 gm 5.12 mmol) and propargyl bromide (0.27 ml, 3.00 mmol). The reaction was stirred for 2 hours and diluted with 250 ml of ethyl acetate and was subsequenttly washed with 4 x 25 ml of water, 30 ml of brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain the crude propargyl ester.

The crude propargyl ester was dissolved in 20 ml of DCM and was added slight excess of diethyl amine (1 ml). The reaction mixture was stirred for 2 hours, concentrated under reduced pressure and the residue was dissolved in 200 ml of ethyl acetate. The organic layer washed with 2 x 25 ml of water, 30 ml of brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel chromatography using ethyl acetate and petroleum ether as eluent to obtain 0.92 gm (89% in two steps) *S*-trityl-L-cysteine propargyl ester (NH_2) (**220**).

Thick syrup.

IR (cm^{-1}): 3380.98, 3288.40, 3056.96, 1743.53, 1593.09, 1488.94, 1444.58.

^{1}H NMR [$CDCl_3$ 200 MHz]: δ 1.66 (s, 2 H), 2.47 (t, 1 H, *J* = 2.53 Hz), 2.55 (dd, 2 H, *J* = 7.58, 8.72 Hz), 3.22 (dd, 1 H, 5.05, 7.45 Hz), 4.65 (dd, 2 H, *J* = 2.40. 3.41 Hz), 7.21-7.33 (m, 9 H), 7.41-7.46 (m, 6 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 36.53, 52.44, 53.68, 66.82, 75.20, 77.14, 126.71, 127.89, 129.47, 144.39, 172.88.

CHNS Anal.: Calculated for $C_{25}H_{23}NO_2S$: C, 74.78; H, 5.77; N, 3.49, S, 7.99.

Found: C, 74.82; H, 5.76; N, 3.45; S, 7.89. **MS:** m/z = 402.54 (M+1).

Synthesis of 5'-carboxylic acid of nucleosides (222 & 230):

Nucleoside 5'-carboxylic acids, 2',3'-O-isopropylidene-5'-carboxylic acid uridine (**222**) from 2',3'-O-isopropylidene uridine (**221**) and N^3,3'-*O*-dibenzoyl-5'-carboxylic acid thymidine (**230**) from N^3,3'-*O*-dibenzoyl thymidine (**229**) were synthesized by following the method reported by Widlanski *et. al.*[21]

2',3'-O-isopropylidene-5'-carboxylic acid uridine (222):

Colorless solid.

IR (cm^{-1}): 3506-3000 (broad peak), 1745.46, 1697.24, 1681.81, 1461.94, 1377.08.

^{1}H NMR [$DMSO_6$ + $CDCl_3$ 200 MHz]: δ 1.36, 1.53 (2s, 6 H), 4.62 (s, 1 H), 5.17 (d, 1 H, *J* = 5.81 Hz), 5.33-5.35 (m, 1 H), 5.62 (d, 1 H, *J* = 7.83 Hz), 5.70 (s, 1 H), 7.58-7.75 (m, 1 H), 11.27 (s, 1 H).

^{13}C NMR [$DMSO_6$ + $CDCl_3$, 50 MHz]: δ 24.79, 26.52, 84.04, 84.25, 87.21, 96.23, 101.72, 112.44, 144.50, 150.97, 163.67, 171.08.

CHNS Anal.: Calculated for $C_{12}H_{14}N_2O_7$: C, 48.32; H, 4.73; N, 9.39.

Found: C, 48.40; H, 4.76; N, 9.32.

MS: m/z = 299.54 (M+1).

Synthesis of N^3,3'-*O*-dibenzoyl thymidine (229):

Benzoyl chloride (0.58 ml, 5 mmol) was added to a solution of 5'-*O*-tert-butyldiphenylsilyl thymidine[23] (1.0 gm, 2.08 mmol) in pyridine (20 ml) at 0 ^oC. The mixture was stirred at room temperature for 5 hours. The solvent was co evaporated with toluene and this was repeated twice. The residue was purified by silica gel column chromatography using ethyl acetate and petroleum ether as eluent to accomplish 1.33 gm (92.70%) of N^3,3'-*O*-dibenzoyl-5'-*O*-tert-butyldiphenylsilyl thymidine.

To a solution of N^3,3'-*O*-dibenzoyl-5'-*O*-tert-butyldiphenylsilyl thymidine (1.2 gm, 1.74 mmol) in tetrahydrofuran was added tetra-butylammoniumfluoride (2.11 ml of 1 M solution in THF, 2.10 mmol) and was stirred at room temperature for 2.5 hours. After completion of the reaction, the solution was concentrated under reduced pressure, silica gel column chromatographic purification of the residue afforded 0.76 gm of (**229**) (97.44%).

Colorless solid.

IR (cm^{-1}): 3417.63, 2923.88, 2854.45, 1758.96, 1714.60, 1660.60, 1650.95, 1446.51.

^{1}H NMR [$CDCl_3$ 200 MHz]: δ 1.98 (d, 3 H, *J* = 1.13 Hz), 2.47-2.66 (m, 3 H), 4.02 (d, 2 H, *J* = 2.53 Hz), 4.27 (d, 1 H), *J* = 2.27 Hz), 5.58-5.63 (m, 1 H), 6.40 (dd, 1 H, *J* = 6.32, 7.71 Hz), 7.41-7.65 (m, 6 H), 7.76 (d, 1 H, *J* = 1.13 Hz), 7.92-8.05 (m, 4 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 12.54, 37.60, 62.43, 75.35, 85.28, 85.66, 111.26, 128.45, 129.08, 129.58, 130.34, 130.88, 131.44, 133.52, 135.04, 136.11, 149.43, 162.84, 166.07, 168.85.

CHNS Anal.: Calculated for $C_{24}H_{22}N_2O_7$: C, 63.99; H, 4.92; N, 6.22.

Found: C, 64.00; H, 5.00; N, 6.18.

MS: m/z = 451.55 (M+1).

***N^3*,3'-*O*-dibenzoyl-5'-carboxylic acid thymidine (230):**

Colorless solid.

IR (cm^{-1}): 3645.21 (broad peak), 1747.39, 1693.38, 1650.95, 1446.51, 1398.30.

^{1}H NMR [$CDCl_3$ 200 MHz]: δ 2.02 (s, 3 H), 2.31-2.45 (m, 1 H), 2.73 (dd, 1 H, *J* = 5.30, 14.27 Hz), 4.81 (s, 1 H), 5.78 (d, 1 H, *J* = 4.80 Hz), 6.55 (dd, 1 H, *J* = 5.31, 8.84 Hz), 7.42-7.66 (m, 6 H), 7.92-8.09 (m, 5 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 12.73, 36.53, 76.77, 82.02, 87.15, 111.71, 128.56, 128.62, 129.17, 129.81, 130.45, 131.38, 133.95, 135.17, 136.11, 149.56, 162.89,-

165.70, 168.66, 172.14.

CHNS Anal.: Calculated for $C_{24}H_{20}N_2O_8$: C, 62.07; H, 4.34; N, 6.03.

Found: C, 62.15; H, 4.40; N, 5.99.

MS: m/z = 465.36 (M+1).

Coupling of 2',3'-*O*-isopropylidene-5'-carboxylic acid uridine (222) and *S*-trityl-L-cysteine propargyl ester (NH_2) (220):

To a solution of 2',3'-O-isopropylidene-5'-carboxylic acid uridine (**222**) (0.66 gm, 2.2 mmol) and *S*-trityl-L-cysteine propargyl ester (NH_2) (**220**) (0.89 gm, 2.22 mmol) in dry DMF (10 ml) was added sequentially HOBt (0.298 gm, 2.2 mmol), DIPEA (0.76 ml, 4.4 mmol) and HOBT (0.918 gm, 2.42 mmol) at room temperature. The reaction mixture was stirred for 2 hours and monitored by TLC. After completion, then the reaction mixture was diluted with 250 ml of ethyl acetate and was subsequently washed with 2 x 25 ml of dilute HCl, 25 ml of saturated $NaHCO_3$, 30 ml of brine and dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure and the crude product was purified by silica gel column chromatography using ethyl acetate-petroleum ether as eluent to obtain 1.45 gm of (**223**) in 96% yield.

Colorless solid.

IR (cm^{-1}): 3388.70, 3307.69, 1745.46, 1714.60, 1693.38, 1681.81, 1454.23, 1377.08.

^{1}H NMR [$CDCl_3$ 200 MHz]: δ 1.35, 1.56 (2s, 6 H), 2.48 (t, 1 H, *J* = 2.53 Hz), 2.66 (ddd, 2 H, *J* = 6.95, 12.38, 19.33 Hz), 4.45 (dt, 1 H, *J* = 4.93, 6.95 Hz), 4.57 (d, 1 H, *J* = 2.78 Hz), 4.66 (t, 2 H, *J* = 2.15 Hz), 5.02 (dd, 1 H, *J* = 1.76, 6.44 Hz), 5.22 (dd, 1 H, *J* = 2.66, 6.32 Hz), 5.58-5.64 (m, 2 H), 6.76 (d, 1 H, *J* = 7.45 Hz), 7.20-7.38 (m, 15 H), 7.91 (s, 1 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 24.99, 26.83, 33.32, 51.31, 53.05, 67.08, 75.65, 76.80, 83.13, 83.60, 87.01, 96.72, 102.87, 114.24, 126.97, 128.08, 129.44, 142.67, -

144.07, 149.81, 162.49, 168.88, 169.25.

CHNS Anal.: Calculated for $C_{37}H_{35}N_3O_8S$: C, 65.18; H, 5.17; N, 6.16; S, 4.70.

Found: C, 65.21; H, 5.14; N, 6.20; S, 4.65.

MS: m/z = 705.24 ($M+Na^+$).

Coupling of N^3,3'-*O*-dibenzoyl-5'-carboxylic acid thymidine (230) and *S*-trityl-L-cysteine propargyl ester (NH_2) (220):

Similarly as described above, N^3,3'-*O*-dibenzoyl-5'-carboxylic acid thymidine (0.79 gm, 1.70 mmol) (**230**) and *S*-trityl-L-cysteine propargyl ester (NH_2) (**220**) (0.685gm, 1.70 mmol) were used to obtain (**231**) (1.37 gm, 95%).

Colorless solid.

IR (cm^{-1}): 3305.75, 3062.75, 1747.39, 1693.38, 1681.81, 1666.38, 1515.94, 1444.58.

^{1}H NMR [$CDCl_3$ 200 MHz]: δ 1.99 (d, 3 H, *J* = 1.01 Hz), 2.22-2.37 (m, 1 H), 2.52 (t, 1 H, 2.40 Hz), 2.54-2.93 (m, 3 H), 4.51-4.60 (m,1 H), 4.62 (s, 1 H), 4.74 (d, 2 H, *J* = 2.53 Hz), 5.59 (d, 1 H, *J* = 5.18 Hz), 6.63 (dd, 1 H, *J* = 5.05, 9.12 Hz), 6.70 (d, 1 H, *J* = 7.83 Hz), 7.23-7.65 (m, 21 H), 7.92-8.06 (m, 4 H), 8.25 (d, 1 H, *J* = 1.14 Hz).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 12.62, 32.97, 35.58, 51.56, 53.34, 67.26, 75.84, 76.62, 78.25, 82.97, 86.28, 111.97, 126.99, 128.07, 128.12, 128.59, 129.08, 129.34, 129.43, 129.80, 130.39, 131.57, 133.90, 134.94, 135.76, 144.05, 149.59, 162.66, 166.52, 168.78, 169.13.

CHNS Anal.: Calculated for $C_{49}H_{41}N_3O_9S$: C, 69.41; H, 4.87; N, 4.96; S, 3.78.

Found: C, 69.46; H, 4.82; N, 5.01; S, 3.71.

MS: m/z = 870.64 ($M+Na^+$).

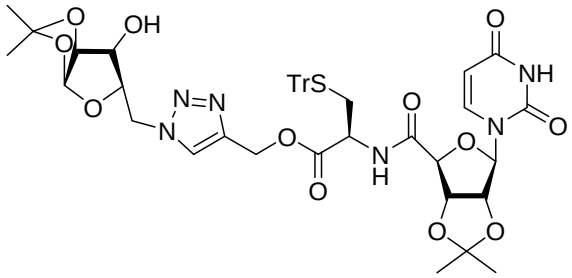
General procedure for "Click" reaction of nucleoside-*S*-trityl-L-cysteine alkynes (223)/(231) and saccharide azides:

To a solution of nucleoside-*S*-trityl-L-cysteine alkyne (1 mmol) and saccharide azide (1 mmol) in acetonitrile were added *N,N*-diisopropylethylamine (3

mmol) and copper iodide at room temperature, and the mixture was stirred for specified period of time. The reaction completion was monitored by TLC and then the mixture was quenched with 25 ml of water and 10 ml of saturated solution of NH_4Cl. The aqueous layer was extracted with 3x 50 ml of ethyl acetate and the combined extracts were washed with 2 x 25 ml of brine solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude products were purified by silica gel column chromatography using ethyl acetate and petroleum ether as eluent to obtain the glycoconjugartes containing 1,2,3-triazole with *S*-trityl-L-cysteine linker.

"Click" reaction of uridine-*S*-trityl-L-cysteine alkyne (223) and saccharide azides (Compounds 224 - 228, Table 3):

Compound 224: (223) + 1,2-*O*-isopropylidene-5-azido-5-deoxy-α-D-xylofuranose (116):



Colorless solid.

IR (cm^{-1}): 3411.84, 1745.46, 1712.67, 1693.38, 1681.81, 1517.87, 1448.44.

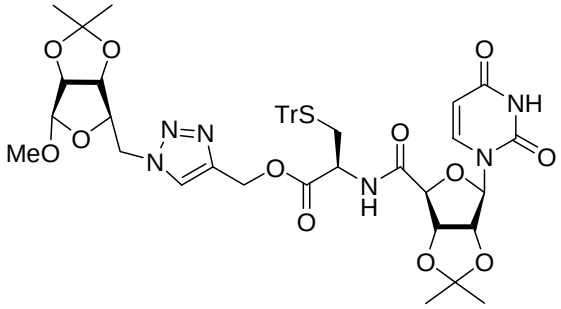
^{1}H NMR [$CDCl_3$ 200 MHz]: δ 1.26, 1.34, 1.48, 1.54 (4s, 12 H), 2.55-2.84 (m, 3 H), 3.67-3.76 (m, 1 H), 4.09 (s, 1 H), 4.52-4.60 (m, 5 H), 5.04-5.20 (m, 3 H), 5.41 (d, 1 H, *J* = 12.63 Hz), 5.50 (s, 1 H), 5.57 (d, 1 H, *J* = 7.20 Hz), 5.97 (d, 1 H, *J* = 3.41 Hz), -6.83 (d, 1 H, *J* = 5.81 Hz), 7.14-7.36 (m, 15 H), 7.75 (s, 1 H), 8.96 (s, 1 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 24.93, 26.18, 26.75, 26.80, 32.42, 48.30, 52.15, 58.49, 67.33, 73.61, 78.77, 83.17, 83.80, 85.41, 87.74, 97.58, 102.91, 105.17, 111.92, 114.07, 126.03, 127.01, 127.17, 127.87, 128.13, 129.40, 141.98, 143.12, 144.04, 146.85, 149.99, 162.89, 169.65, 169.84.

CHNS Anal.: Calculated for $C_{45}H_{48}N_6O_{12}S$: C, 60.26; H, 5.39; N, 9.37; S, 3.57.

Found: C, 60.30; H, 5.35; N, 9.33; S, 3.49.

Compound 225: (223) + methyl 2,3-*O*-isopropylidene5-azido-5-deoxy-β-D-ribo-furanoside (198):



Colorless solid.

$[\alpha]_D^{25}$= -13.23 (c, 1.24, $CHCl_3$)

IR (cm^{-1}): 2997.17, 2939.31, 1745.46, 1714.60, 1693.38, 1681.81, 1517.87.

^{1}H NMR [$CDCl_3$ 200 MHz]: δ 1.31, 1.35, 1.45, 1.55 (4s, 12 H), 2.65 (ddd, 2 H, *J* = 7.08, 12.38, 19.46 Hz), 3.36 (s, 3 H), 4.22-4.32 (m, 1 H), 4.34-4.49 (m, 2 H), 4.54 (d, 2 H, *J*= 2.53 Hz), 4.65-4.74 (m, 2 H), 5.01 (s, 1 H), 5.06 (d, 1 H, *J* = 7.58 Hz), 5.18-5.30 (m, 3 H), 5.55 (s, 1 H), 5.62 (d, 1 H, *J* = 7.83 Hz), 6.82 (d, 1 H, *J* = 6.94 Hz), 7.19-7.31 (m, 15 H), 7.70 (s, 1 H), 8.39 (s, 1 H).

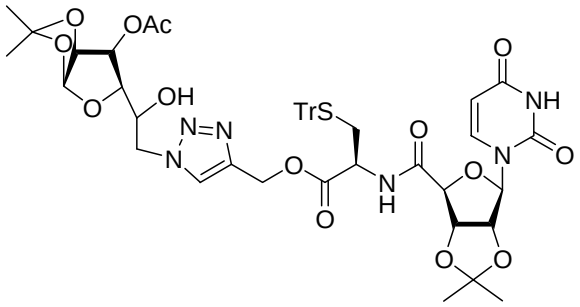
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 24.85, 25.00, 26.29, 26.82, 33.29, 51.60, 53.10, 55.58, 58.60, 66.97, 81.68, 83.20, 83.62, 84.90, 84.97, 87.33, 97.16, 102.92, 109.99, 112.88, 114.17, 124.36, 126.97, 128.09, 129.41, 142.83, 144.10, 149.87, 162.46, 168.92, 169.74.

CHNS Anal.: Calculated for $C_{46}H_{50}N_6O_{12}S$: C, 60.65; H, 5.53; N, 9.23; S, 3.52.

Found: C, 60.61; H, 5.49; N, 9.19; S, 3.49.

MS: m/z = 934.03 ($M+Na^+$).

Compound 226: (223) + 1,2-*O*-isopropylidene-3-O-acetyl-6-azido-6-deoxy-α-D-glucofuranose (199):



Colorless solid.

IR (cm^{-1}): 3415.70, 2927.74, 1745.46, 1712.67, 1693.38, 1681.81, 1446.51.

^{1}H NMR [$CDCl_3$ 200 MHz]: δ 1.29, 1.34, 1.44, 1.55 (4s, 12 H), 1.97 (s, 3 H), 2.49-2.96 (m, 3 H), 4.23 (s, 1 H), 4.47-4.82 (m, 6 H), 5.05 (s, 1 H), 5.10 (d, 1 H, *J* = 4.17 Hz), 5.17-5.24 (m, 2 H), 5.37 (d, 1 H, *J* = 12.76 Hz), 5.50 (s, 1 H), 5.57 (d, 1 H, *J* = 7.96 Hz), 5.89 (d, 1 H, *J* = 3.41 Hz), 7.19-7.35 (m, 15 H), 7.87 (s, 1 H), 8.84 (s, 1 H).

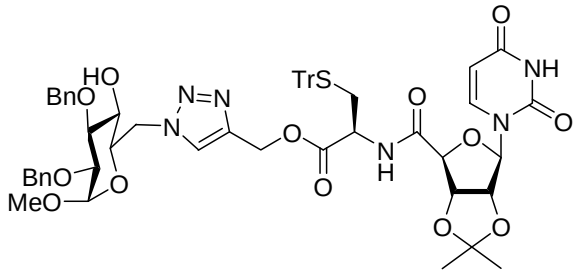
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 20.67, 25.02, 26.24, 26.78, 26.85, 32.64, 52.29, 58.85, 59.97, 62.77, 67.32, 74.74, 77.20, 83.16, 83.83, 85.32, 102.80, 104.58, 112.14, 114.11, 122.86, 126.96-127.22, 127.89-128.12, 129.46, 136.33, 141.64, 143.32, 144.14, 146.87, 149.90, 161.09, 162.86, 169.73, 170.67.

CHNS Anal.: Calculated for $C_{48}H_{52}N_6O_{14}S$: C, 59.49; H, 5.41; N, 8.67; S, 3.31.

Found: C, 59.45; H, 5.38; N, 8.65; S, 3.28.

MS: m/z = 970.05 (M+1).

Compound 227: (223) + methyl 2,3-di-*O*-benzyl-6-azido-6-deoxy-α-D-gluco-pyranoside (146):



Colorless solid.
$[\alpha]_D^{25}$= +8.40 (c, 1.19, $CHCl_3$).

IR (cm^{-1}): 3647.14, 1747.39, 1693.38, 1681.81, 1519.80, 1456.16, 1379.01.

^{1}H NMR [$CDCl_3$ 200 MHz]: δ 1.34, 1.54 (2s, 6 H), 2.07 (bs, 1 H), 2.51-2.70 (m, 2 H), 3.04-3.17 (m, 1 H), 3.21 (s, 3 H), 3.38-3.43 (m, 1 H), 3.77-3.85 (m, 2 H), 4.11-4.19 (m, 2 H), 4.52 (s, 2 H), 4.59-4.75 (m, 4 H), 4.95-5.05 (m, 2 H), 5.17 (s, 1 H), 5.22 (d, 1 H, *J* = 5.44 Hz), 5.51 (s, 1 H), 5.61 (d, 1 H, *J* = 7.95 Hz), 6.83 (d, 1 H, *J* = 7.96 Hz), 7.15-7.40 (m, 25 H), 7.68 (s, 1 H), 8.48 (s, 1 H).

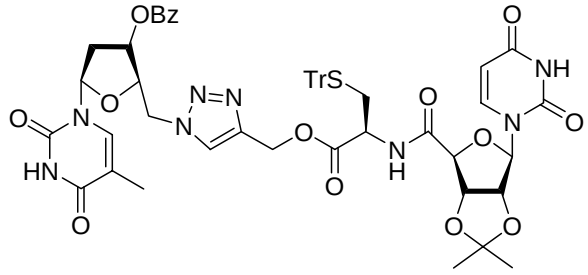
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 24.97, 26.77, 33.06, 50.96, 51.70, 55.34, 58.27, 60.34, 67.03, 69.24, 70.43, 73.12, 75.42, 79.52, 80.89, 83.17, 83.57, 87.31, 97.19, 98.10, 102.86, 114.14, 125.71, 126.95, 127.79, 127.82, 127.87, 128.08, 128.42, 128.46, 129.38, 137.83, 138.47, 141.72, 142.98, 144.08, 149.91, 162.75, 169.09, 169.66.

CHNS Anal.: Calculated for $C_{58}H_{60}N_6O_{13}S$: C, 64.43; H, 5.59; N, 7.77; S, 2.97.

Found: C, 64.46; H, 5.52; N, 7.72; S, 2.91.

MS: m/z = 1082.51 (M+1).

Compound 228: (223) + 3'-benzoyl-5'-azido-5'-deoxy thymidine (201):



Colorless solid.

$[\alpha]_D^{25}$= -16.73 (c, 1.10, $CHCl_3$).

IR (cm^{-1}): 1720.39, 1712.67, 1693.38, 1681.81, 1517.87, 1452.30, 1382.87.

^{1}H NMR [$CDCl_3$ 200 MHz]: δ 1.32, 1.53 (2s, 6 H), 1.93 (s, 3 H), 2.23-2.59 (m, 3 H), 2.76 (dd, 1 H, *J* = 4.92, 12.63 Hz), 4.39-4.41 (m, 1 H), 5,53 (s, 1 H), 4.64-4.88 (m, 2 H), 5.08-5.33 (m, 4 H), 5.51 (s, 1 H), 5.56 (d, 1 H, *J* = 7.95 Hz), 6.20-6.27 (m, 1 H),

6.81 (d, 1 H, *J* = 4.93 Hz), 7.24-7.29 (m, 15 H), 7.44-7.66 (m, 4 H), 7.82 (s, 1 H), 8.02 (d, 2 H, *J* = 7.20), 9.15 (s, 1 H).

^{13}C NMR [$CDCl_3$, 50 MHz]: δ 12.46, 24.93, 26.73, 33.00, 35.98, 51.77, 55.58, 58.58, 60.36, 64.50, 67.15, 74.64, 81.90, 83.26, 83.62, 87.53, 97.57, 102.97, 111.87, 114.06, 123.92, 126.99, 128.11, 128.58, 128.74, 129.41, 129.76, 133.77, 143.12, 144.05, 150.51, 162.92, 163.74, 166.11, 169.07, 169.82, 172.22.

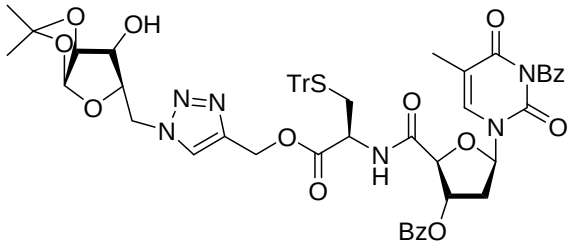
CHNS Anal.: Calculated for $C_{54}H_{52}N_8O_{13}S$: C, 61.59; H, 4.98; N, 10.64; S, 3.04.

Found: C, 61.62; H, 4.92; N, 10.60; S, 2.99.

MS: m/z = 1053.12 (M+1).

"Click" reaction of thymidine-*S*-trityl-L-cysteine alkyne (231) and saccharide azides (Compounds 232 - 236, Table 4):

Compound 232: (231) + 1,2-*O*-isopropylidene5-azido-5-deoxy-α-D-xylofuranose (116):



Colorless solid.

$[\alpha]_D^{25}$= -5.69 (c, 1.09, $CHCl_3$).

IR (cm^{-1}): 3645.21, 1747.39, 1712.67, 1681.81, 1650.95, 1446.51.

^{1}H NMR [$CDCl_3$ 200 MHz]: δ 1.27, 1.42 (2s, 6 H), 1.98 (s, 3 H), 2.27-2.41 (m, 1 H), 2.60-2.90 (m, 3 H), 3.30 (bs, 1 H), 4.09 (s, 1 H), 4.33 (dd, 1 H, *J* = 6.19, 11.75 Hz), 4.43-4.70 (m, 5 H), 5.20 (d, 1 H, *J* = 12.70 Hz), 5.37 (d, 1 H, 12.63 Hz), 5.54 (d, 1 H, 5.18 Hz), 5.91 (d, 1 H, *J* = 3.41 Hz), 6.58 (dd, 1 H, 5.31, 9.60 Hz), 7.08 (d, 1 H, *J* = 7.71 Hz), 7.22-7.65 (m, 21 H), 7.93-8.05 (m, 4 H), 7.72 (s, 1 H), 8.11 (s, 1 H).

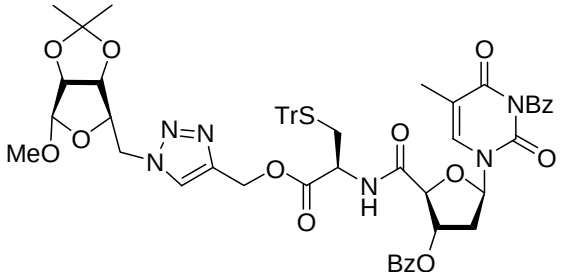
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 12.62, 26.07, 26.72, 32.86, 35.58, 48.43, 51.89, 58.91, 67.31, 74.27, 78.05, 78.85, 83,.00, 85.23, 86.52, 105.07, 111.98, 125.26, 127.02, 128.10, 128.57, 128.65, 129.14, 129.32, 129.43, 129.81, 130.44, 131.47, 133.99, 135.08, 135.92, 142.07, 144.04, 149.59, 162.70, 166.56, 168.81, 169.27, 169.63.

CHNS Anal.: Calculated for $C_{57}H_{54}N_6O_{13}S$: C, 64.40; H, 5.12; N, 7.90; S, 3.02.

Found: C, 64.45; H, 5.10; N, 7.88; S, 2.99.

MS: m/z = 1086.35 ($M+Na^+$).

Compound 233: (231) + methyl 2,3-*O*-isopropylidene-5-azido-5-deoxy-β-D-ribo-furanoside (198):



Colorless solid.

$[\alpha]_D^{25}$= -6.94 (c, 1.21, $CHCl_3$).

IR (cm^{-1}): 1747.39, 1703.03, 1666.38, 1517.87, 1444.58, 1384.79.

^{1}H NMR [$CDCl_3$ 200 MHz]: δ 1.30, 1.44, (2s, 6 H), 2.00 (d, 3 H, *J* = 1.01 Hz), 2.22-2.37 (m, 1 H), 2.60-2.90 (m, 3 H), 3.35 (s, 3 H), 3.38 (d, 1 H, 2.02 Hz), 4.39 (q, 1 H, 5.06 Hz), 4.44 (d, 1 H, 5.56 Hz), 4.51 (d, 1 H, *J* = 2.02 Hz), 4.59 (s, 1 H), 4.67 (s, 1 H), 4.73 (d, 1 H, *J* = 6.06 Hz), 4.99 (s, 1 H), 5.30 (s, 1 H), 5.32 (d, 1 H, *J* = 4.42 Hz), 5.51 (d, 1 H, *J* = 5.05 Hz), 6.63 (dd, 1 H, *J* = 5.18, 9.73 Hz), 6.99 (d, 1 H, *J* = 7.83 Hz), 7.22-7.65 (m, 21 H), 7.93-8.06 (m, 4 H), 7.71 (s, 1 H), 8.25 (s, 1 H).

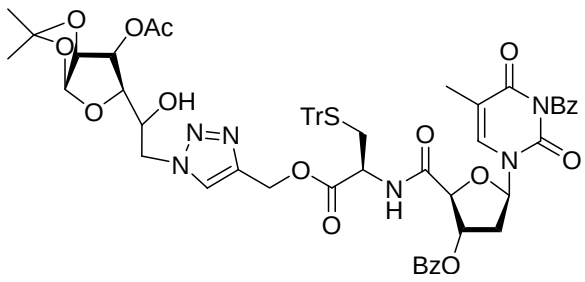
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 12.67, 24.85, 26.32, 33.16, 35.58, 51.56, 53.15, 55.61, 58.93, 67.20, 78.25, 81.70, 83.01, 84.90, 85.04, 86.28, 110.06, 111.97, 112.94,

124.29, 126.99, 127.88, 128.09, 128.64, 129.11, 129.43, 129.81, 130.43, 131.57, 133.94, 134.97, 135.83, 142.05, 144.06, 149.61, 162.67, 166.55, 168.80, 169.05, 169.67.

CHNS Anal.: Calculated for $C_{58}H_{56}N_6O_{13}S$: C, 64.67; H, 5.24; N, 7.80; S, 2.98.

Found: C, 64.62; H, 5.20; N, 7.84; S, 2.95.

Compound 234: (231) + 1,2-*O*-isopropylidene-3-*O*-acetyl-6-azido-6-deoxy-α-D-glucofuranose (199):



Colorless solid.

$[\alpha]_D^{25}$= -16.19 (c, 1.05, $CHCl_3$).

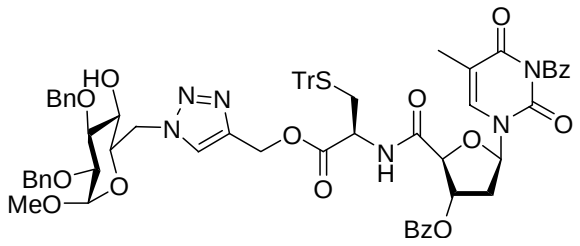
IR (cm^{-1}): 3643.28, 3062.75, 2986.46, 1747.39, 1703.03, 1666.38, 1446.51, 1375.15.

^{1}H NMR [$CDCl_3$ 200 MHz]: δ 1.25, 1.43 (2s, 6 H), 1.97 (s, 3 H), 1.99 (s, 3 H), 2.21 (bs, 2 H), 2.59-2.95 (m, 3 H), 4.30 (s, 1 H), 4.36-4.44 (m, 1 H), 4.51-4.64 (m, 5 H), 4.95-5.05 (m, 1 H), 5.21-5.37 (m, 2 H), 5.52 (d, 1 H, *J* = 2.78 Hz), 5.81 (s, 1 H), 6.57-6.63 (m, 1 H), 7.06 (d, 1 H, *J* = 7.20 Hz), 7.22-7.66 (m, 21 H), 7.75 (s, 1 H), 7.93-8.04 (m, 4 H), 8.24 (s, 1 H).

^{13}C NMR [$CDCl_3$, 125 MHz]: δ 12.65, 20.63, 26.11, 26.75, 32.90, 35.53, 51.83, 59.05, 60.23, 62.85, 67.34, 74.60, 78.24, 78.94, 83.01, 85.20, 86.45, 104.58, 111.92, 112.17, 124.90, 127.00, 127.87, 128.10, 128.64, 129.14, 129.46, 129.83, 130.47, 131.50, 133.96, 135.08, 136.02, 141.53, 144.07, 149.61, 162.76, 166.63, 168.98, 169.27, 169.62, 170.62.

CHNS Anal.: Calculated for $C_{60}H_{58}N_6O_{15}S$: C, 63.48; H, 5.15; N, 7.40; S, 2.82.
Found: C, 63.46; H, 5.12; N, 7.38; S, 2.79.

Compound 235: (231) + methyl 2,3-di-*O*-benzyl-6-azido-6-deoxy-α-D-gluco-pyranoside (146):



Colorless solid.

$[\alpha]_D^{25}$= +5.15 (c, 1.32, $CHCl_3$).

IR (cm^{-1}): 3643.28, 1747.39, 1703.03, 1666.38, 1446.51, 1265.22.

^{1}H NMR [$CDCl_3$ 200 MHz]: δ 1.99 (s, 3 H), 2.02 (bs, 1 H), 2.23-2.39 (m, 1 H), 2.59-2.78 (m, 3 H), 3.06 (t, 1 H, *J* = 9.85 Hz), 3.22 (s, 3 H), 3.38 (dd, 1 H, *J* = 3.16, 9.48 Hz), 3.74 (d, 1 H, *J* = 9.22 Hz), 3.85 (dd, 1 H, *J* = 6.69, 10.86 Hz), 4.50-4.53 (m, 2 H), 4.57-4.66 (m, 3 H), 4.70 (s, 2 H), 4.96 (s, 1 H), 5.01 (s, 1 H), 5.28 (s, 2 H), 5.53 (d, 1 H, *J* = 5.05 Hz), 6.62 (dd, 1 H, *J* = 4.55, 9.47 Hz), 6.97 (d, 1 H, *J* = 7.07 Hz), 7.29-7.765 (m, 31 H), 7.70 (s, 1 H), 7.94-8.04 (m, 4 H), 8.21 (s, 1 H).

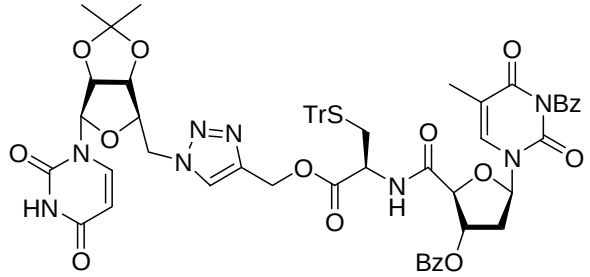
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 12.68, 33.07, 35.53, 50.79, 51.62, 55.40, 58.84, 67.16, 69.21, 70.45, 73.15, 75.46, 78.19, 79.58, 80.77, 82.95, 86.26, 98.19, 111.97, 125.63, 127.00, 127.00-128.16, 128.50, 128.62, 129.12, 129.41, 129.81, 130.44, 131.55, 133.95, 135.00, 135.81, 137.78, 138.40, 141.67, 144.05, 149.62, 162.69, 166.55, 168.79, 169.01, 169.68.

CHNS Anal.: Calculated for $C_{70}H_{66}N_6O_{14}S$: C, 67.40; H, 5.33; N, 6.74; S, 2.57.

Found: C, 67.45; H, 5.28; N, 6.70; S, 2.50.

MS: m/z = 1265.54 ($M+NH_3$).

Compound 236: (231) + 2',3'-*O*-isopropylidene-5'-azido-5'-deoxy uridine (202):



Colorless solid.

$[\alpha]_D^{25}$= +22.37 (c, 1.18, $CHCl_3$).

IR (cm^{-1}): 1714.60, 1693.38, 1681.81, 1666.38, 1519.80, 1454.23, 1380.94.

^{1}H NMR [$CDCl_3$ 200 MHz]: δ 1.32, 1.52 (2s, 6 H), 1.97 (s, 3 H), 2.00-2.20 (m, 1 H), 2.28-2.50 (m, 1 H), 2.58-2.90 (m, 2 H), 4.45-4.48 (m, 2 H), 4.63-4.69 (m, 3 H), 4.83-4.89 (m, 1 H), 5.11 (d, 1 H, *J* = 6.07 Hz), 5.29 (s, 2 H), 5.42 (s, 1 H), 5.56 (d, 1 H, *J* = 4.29 Hz), 5.66 (d, 1 H, 7.20 Hz), 6.62 (dd, 1 H, *J* = 5.17, 9.72 Hz), 7.02 (d, 1 H, *J* = 7.45 Hz), 7.13 (d, 1 H, *J* = 8.08 Hz), 7.26-7.61 (m, 21 H), 7.65 (s, 1 H), 7.96-8.04 (m, 4 H), 8.21 (s, 1 H), 9.28 (s, 1 H).

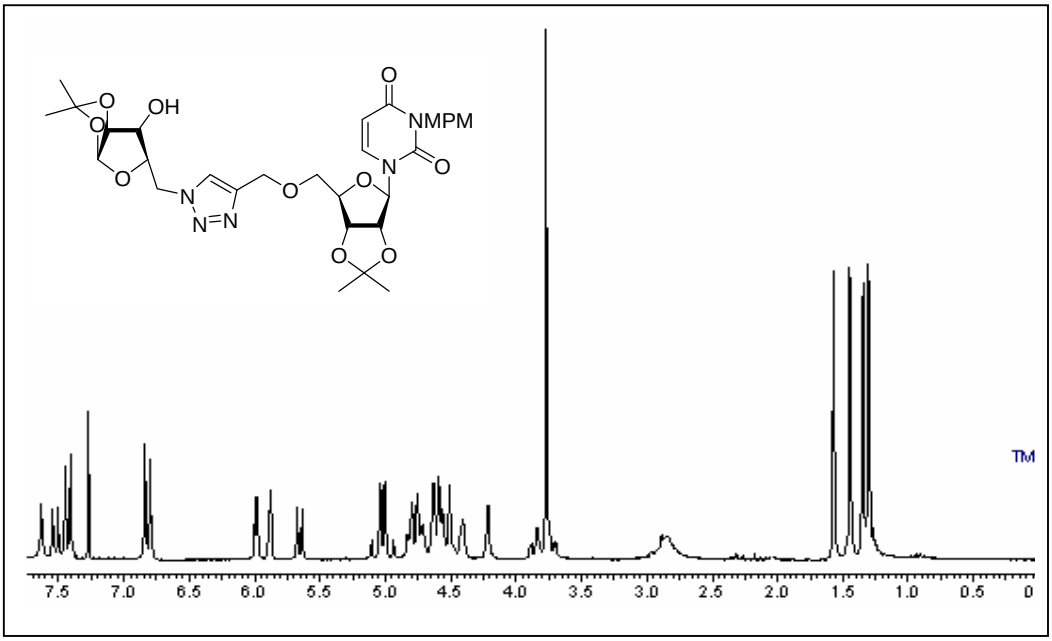
^{13}C NMR [$CDCl_3$, 50 MHz]: δ 12.62, 25.15, 27.01, 33.07, 35.58, 51.53, 51.65, 58.78, 67.21, 71.54, 78.06, 81.52, 82.99, 83.94, 85.86, 86.31, 96.58, 102.77, 111.84, 114.79, 125.28, 126.98, 127.85, 128.07, 128.15, 128.61, 128.67, 129.10, 129.29, 129.43, 129.78, 130.42, 131.53, 133.89, 135.00, 136.01, 141.88, 143.55, 144..04, 149.63, 149.91, 162.72, 163.31, 166.45, 168.83, 169.18, 169.73.

CHNS Anal.: Calculated for $C_{61}H_{56}N_8O_{14}S$: C, 63.31; H, 4.88; N, 9.68; S, 2.77.

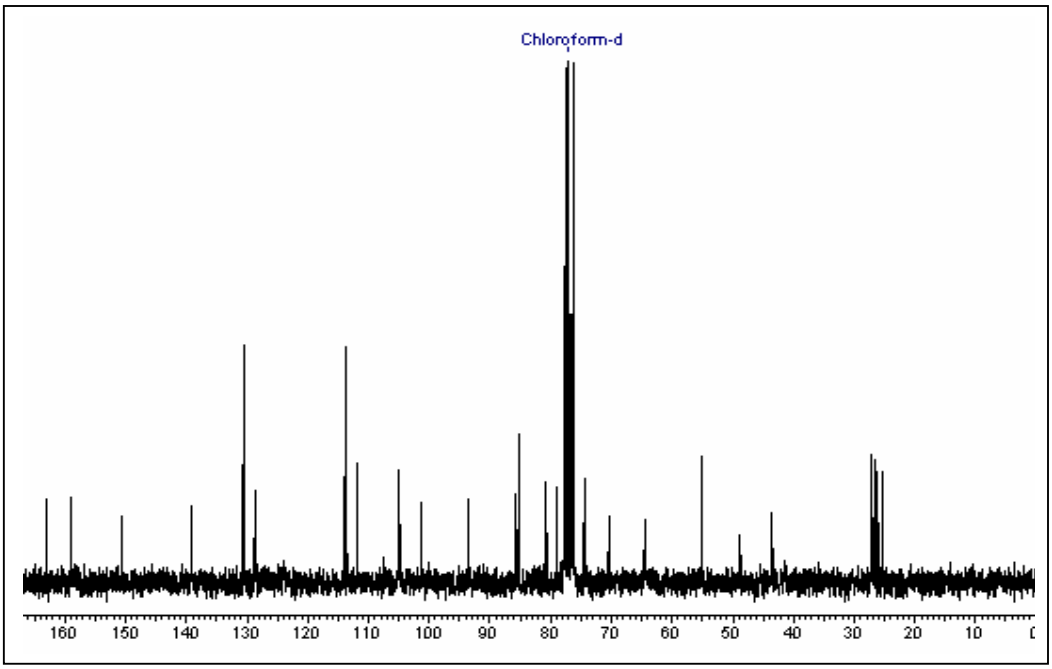
Found: C, 63.27; H, 4.90; N, 9.59; S, 2.69.

MS: m/z = 1179.91 ($M+Na^+$).

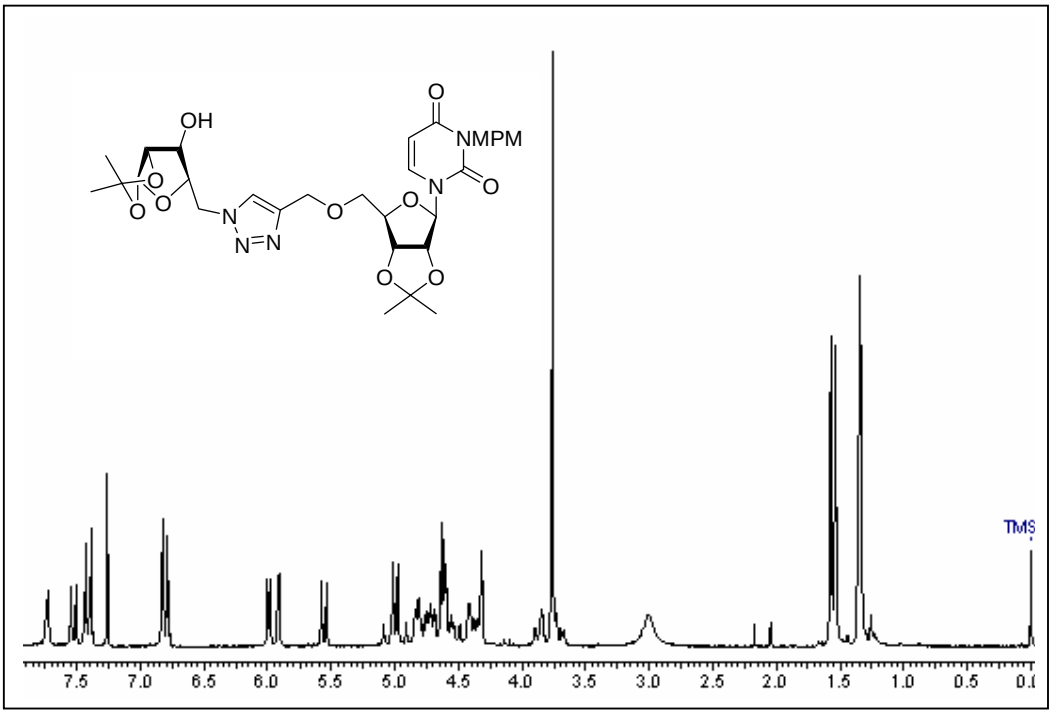
Chapter 3: Spectra



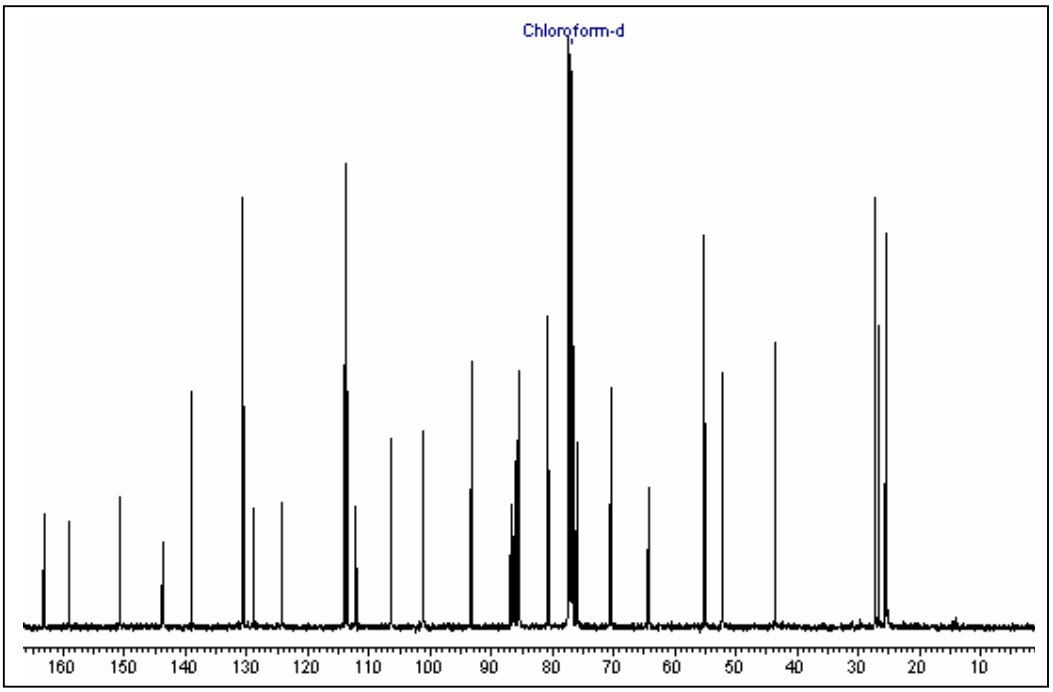
^{1}H NMR spectrum of Compound **203** (Table 1) in $CDCl_3$



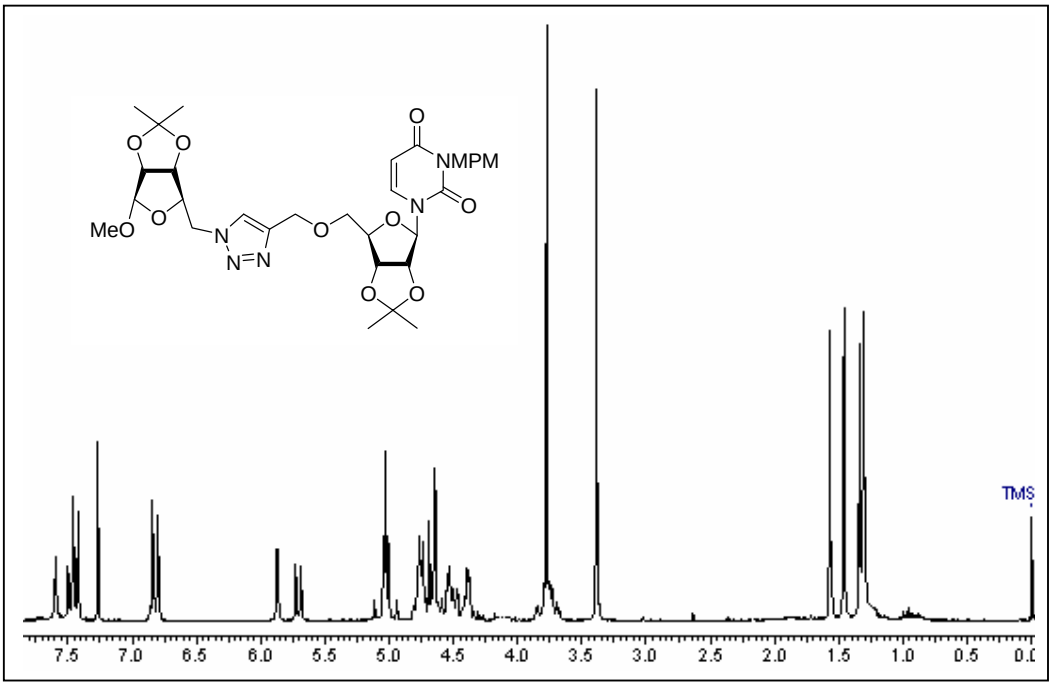
^{13}C NMR spectrum of Compound **203** (Table 1) in $CDCl_3$



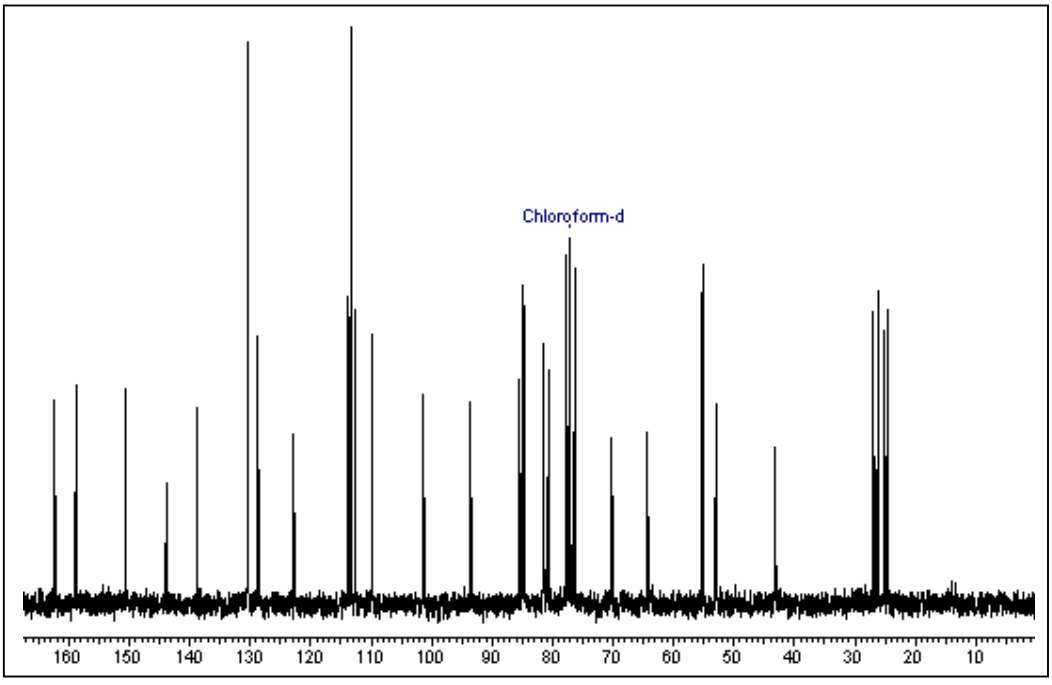
^{1}H NMR spectrum of Compound **204** (Table 1) in $CDCl_3$



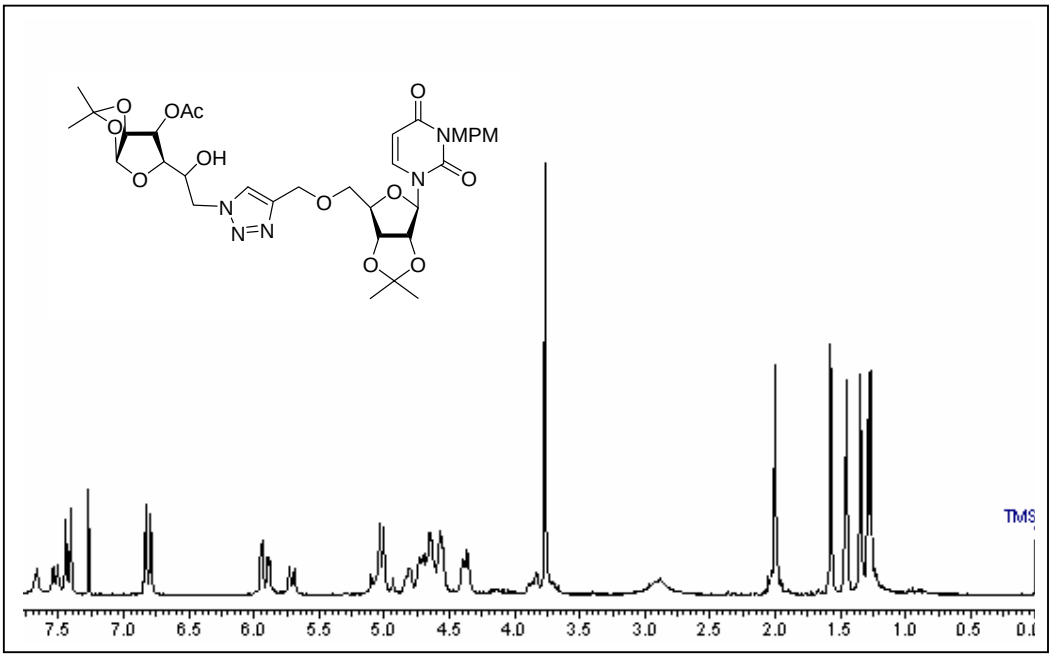
^{13}C NMR spectrum of Compound **204** (Table 1) in $CDCl_3$



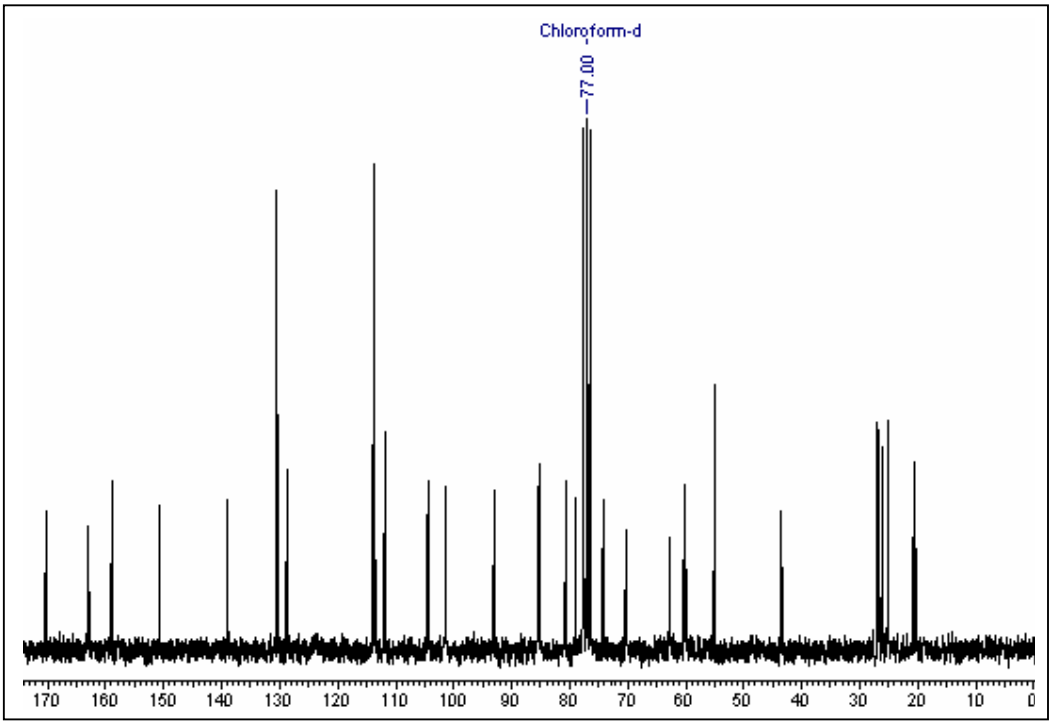
^{1}H NMR spectrum of Compound **205** (Table 1) in $CDCl_3$



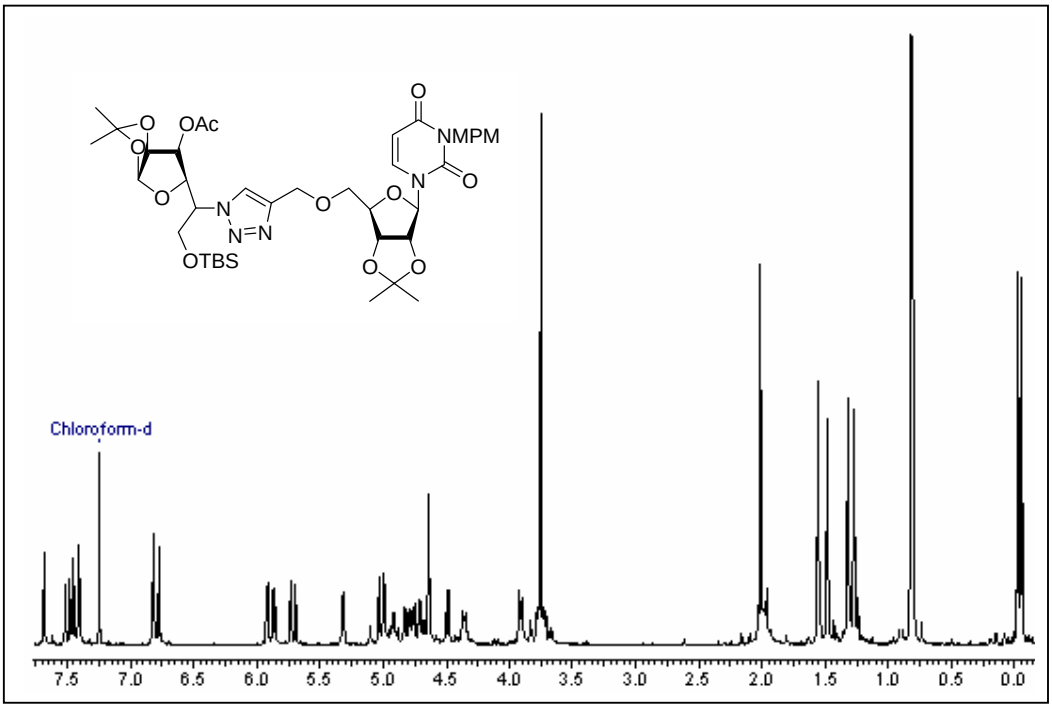
^{13}C NMR spectrum of Compound **205** (Table 1) in $CDCl_3$



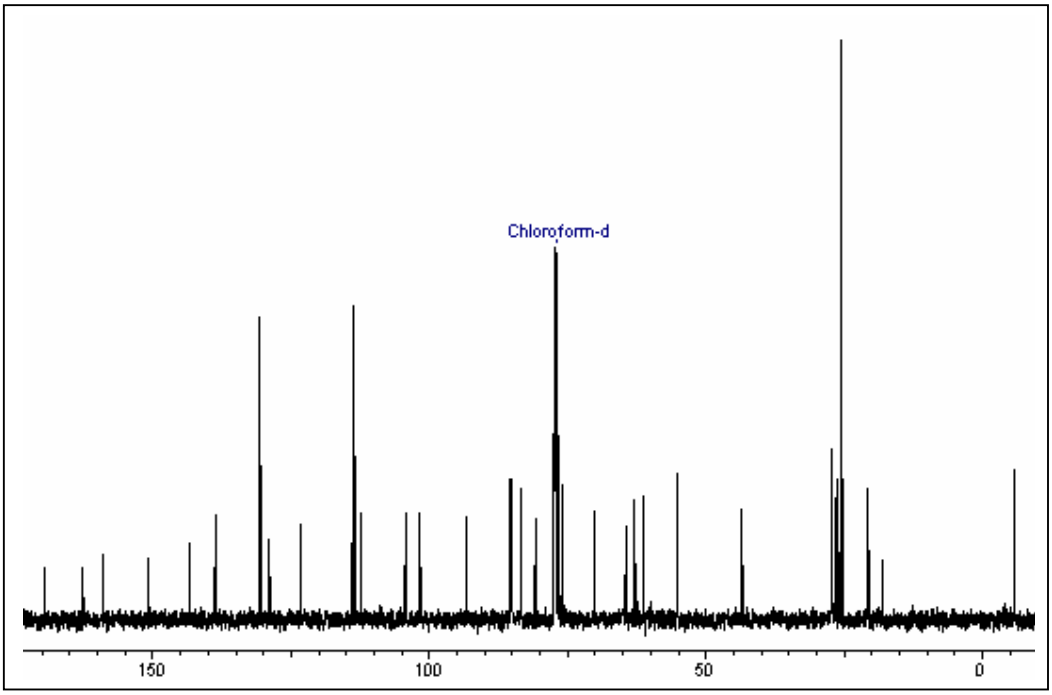
^{1}H NMR spectrum of Compound **206** (Table 1) in $CDCl_3$



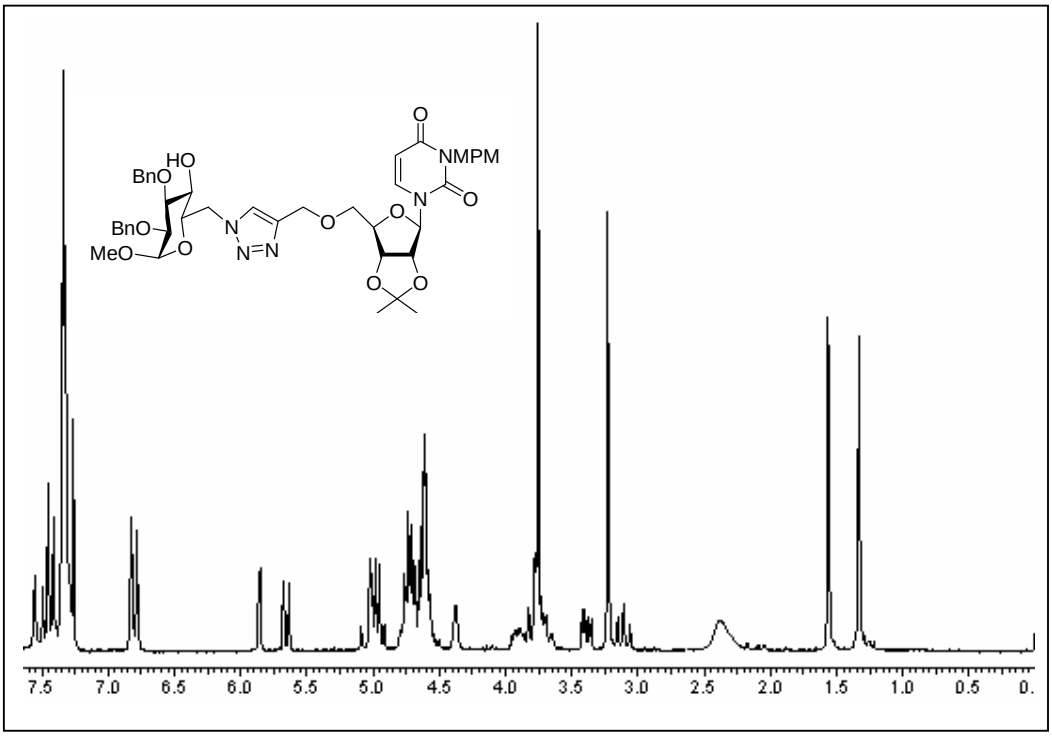
^{13}C NMR spectrum of Compound **206** (Table 1) in $CDCl_3$



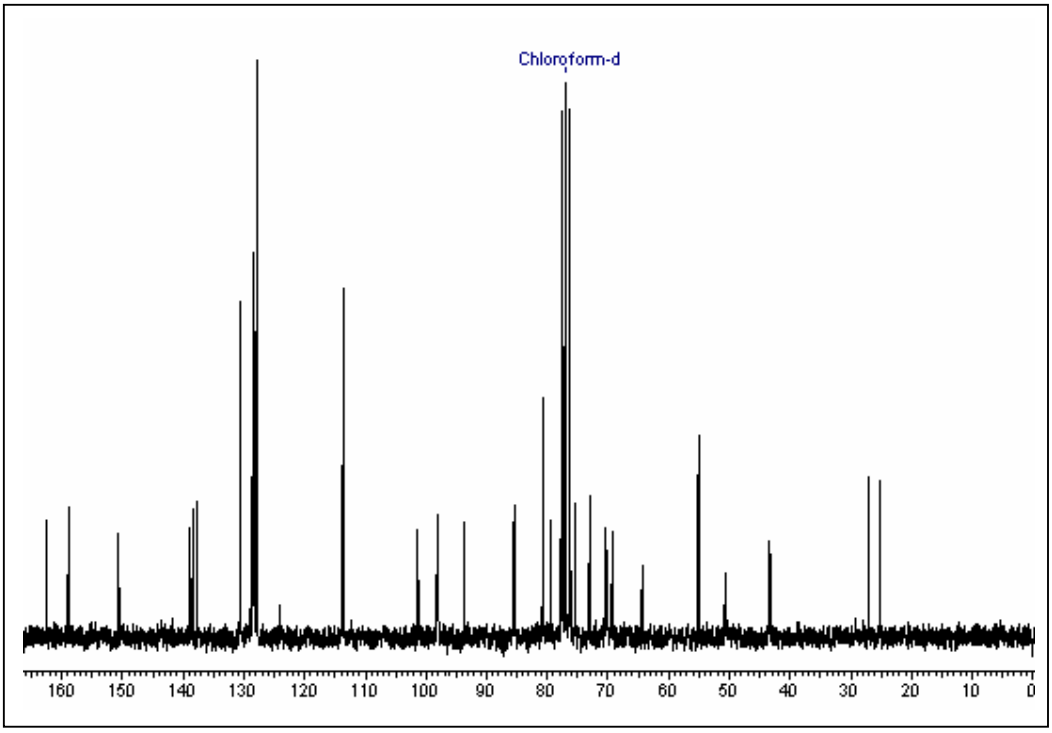
^{13}H NMR spectrum of Compound **207** (Table 1) in $CDCl_3$



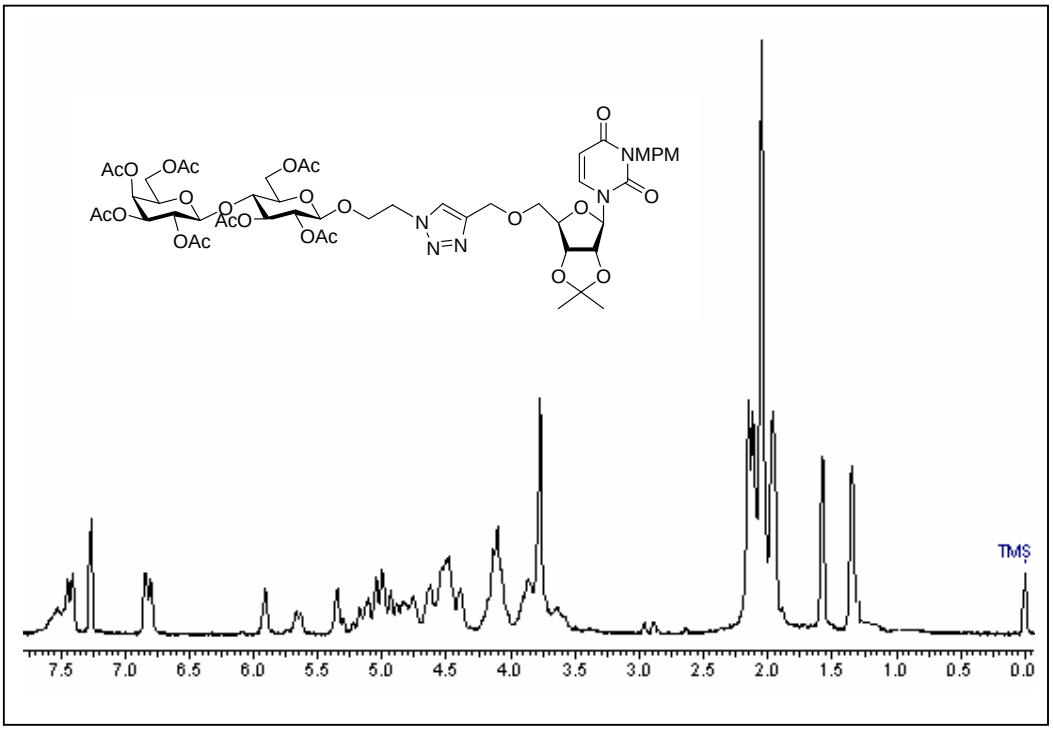
^{13}C NMR spectrum of Compound **207** (Table 1) in $CDCl_3$



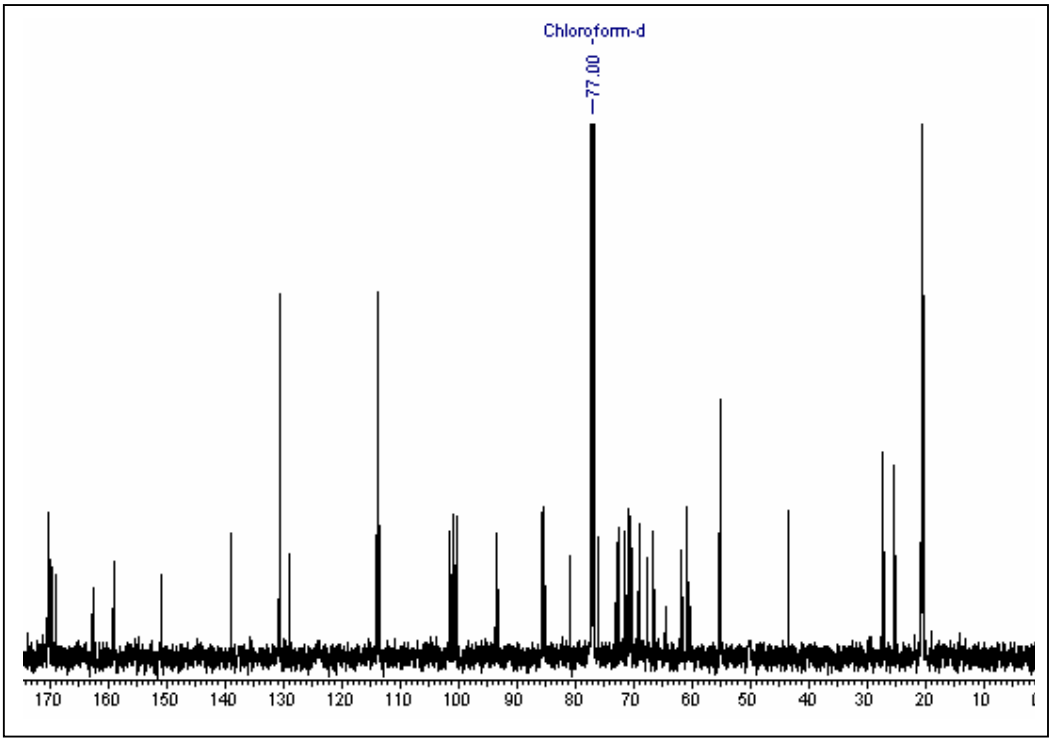
^{1}H NMR spectrum of Compound **208** (Table 1) in $CDCl_3$



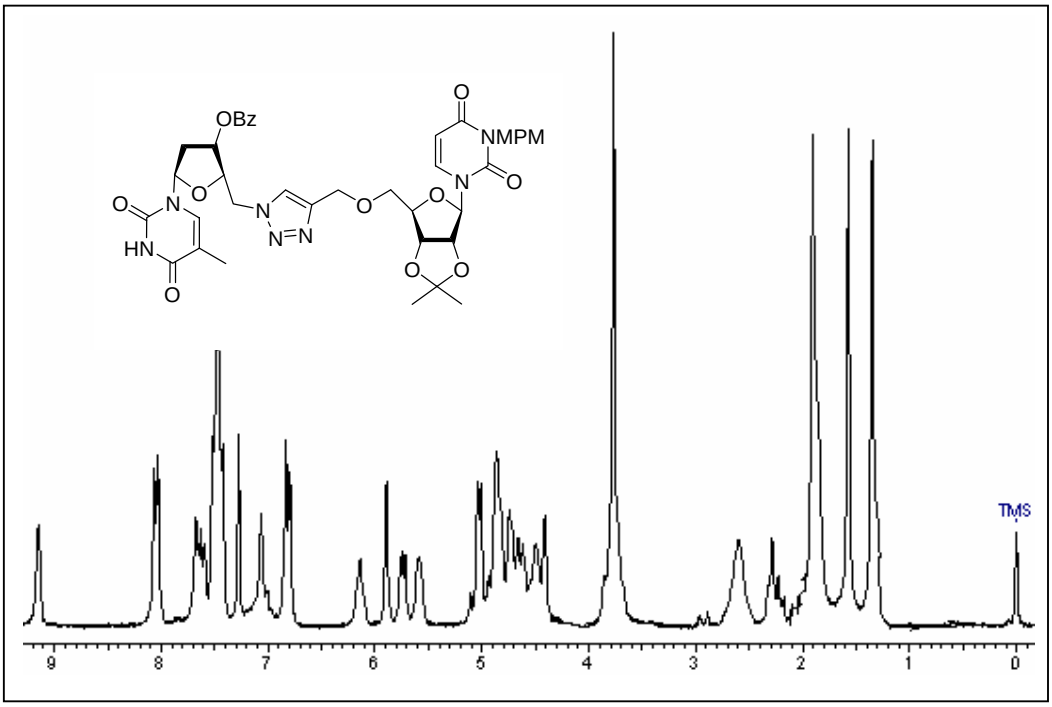
^{13}C NMR spectrum of Compound **208** (Table 1) in $CDCl_3$



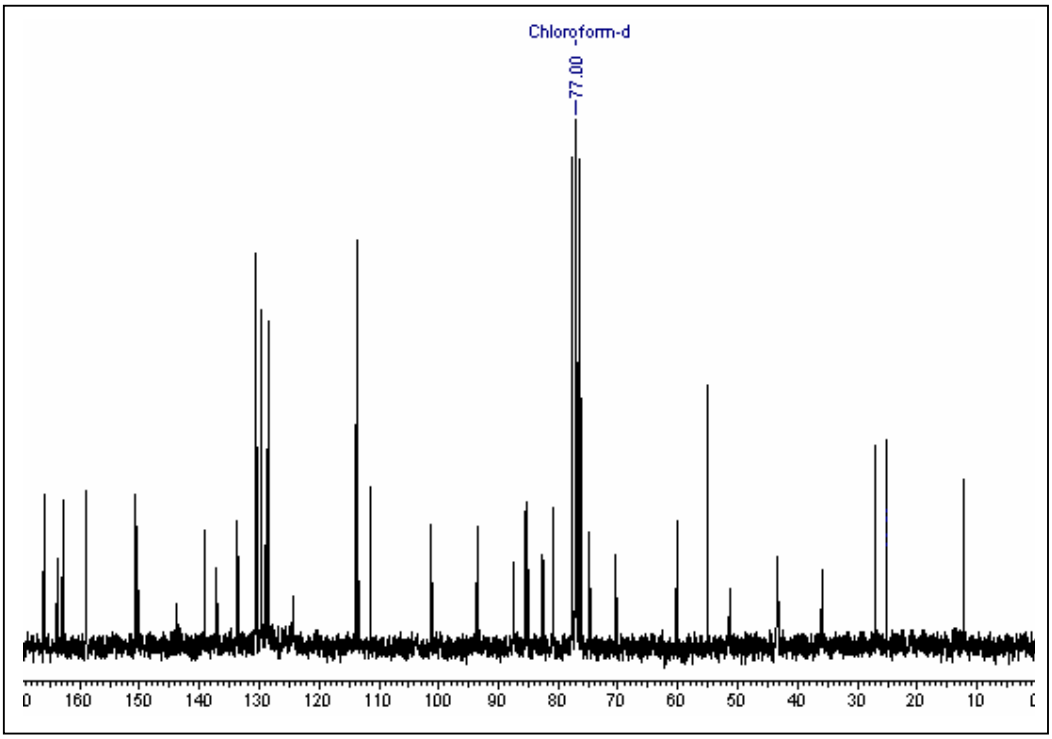
^{1}H NMR spectrum of Compound **209** (Table 1) in $CDCl_3$



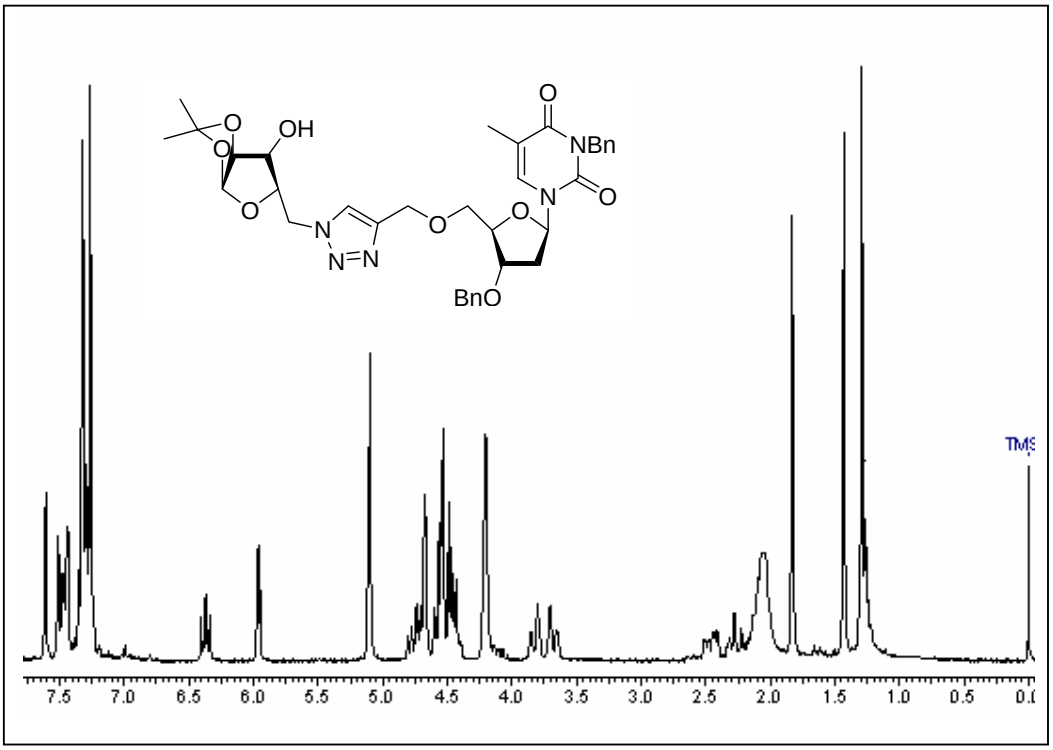
^{13}C NMR spectrum of Compound **209** (Table 1) in $CDCl_3$



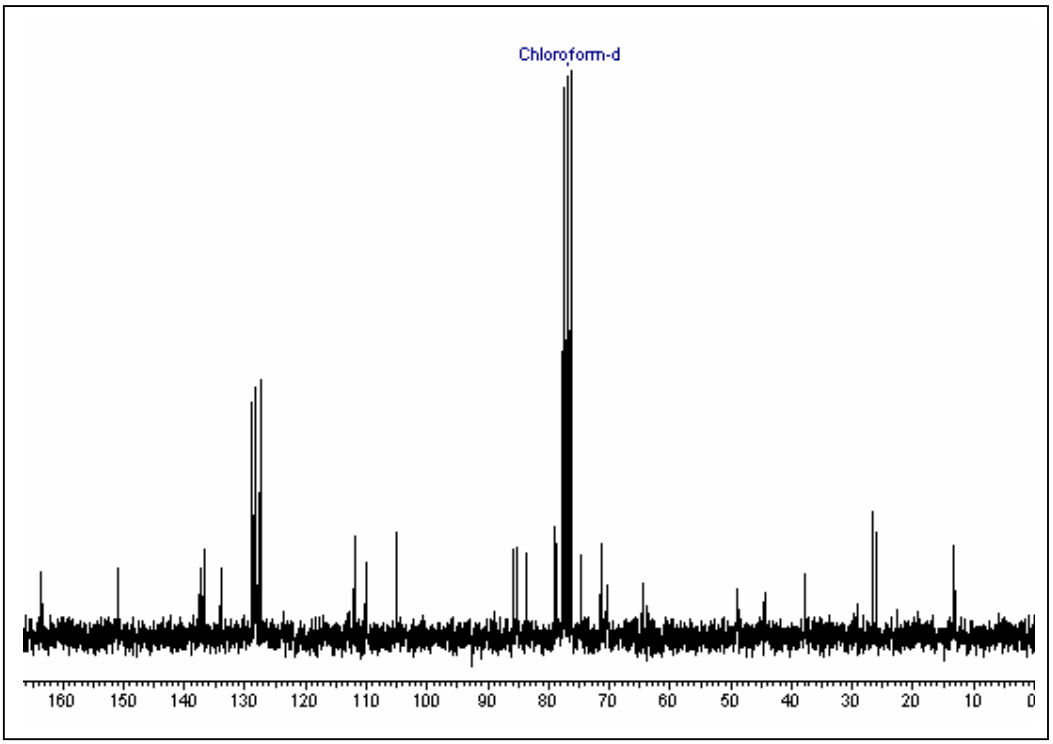
^{1}H NMR spectrum of Compound **210** (Table 1) in $CDCl_3$



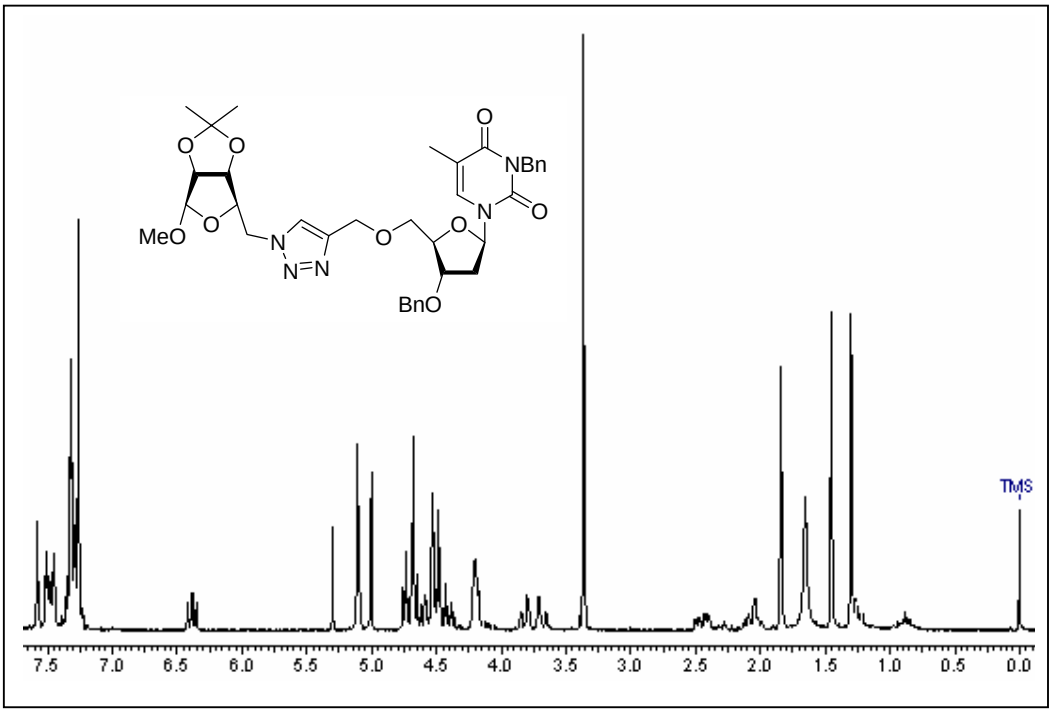
^{13}C NMR spectrum of Compound **210** (Table 1) in $CDCl_3$



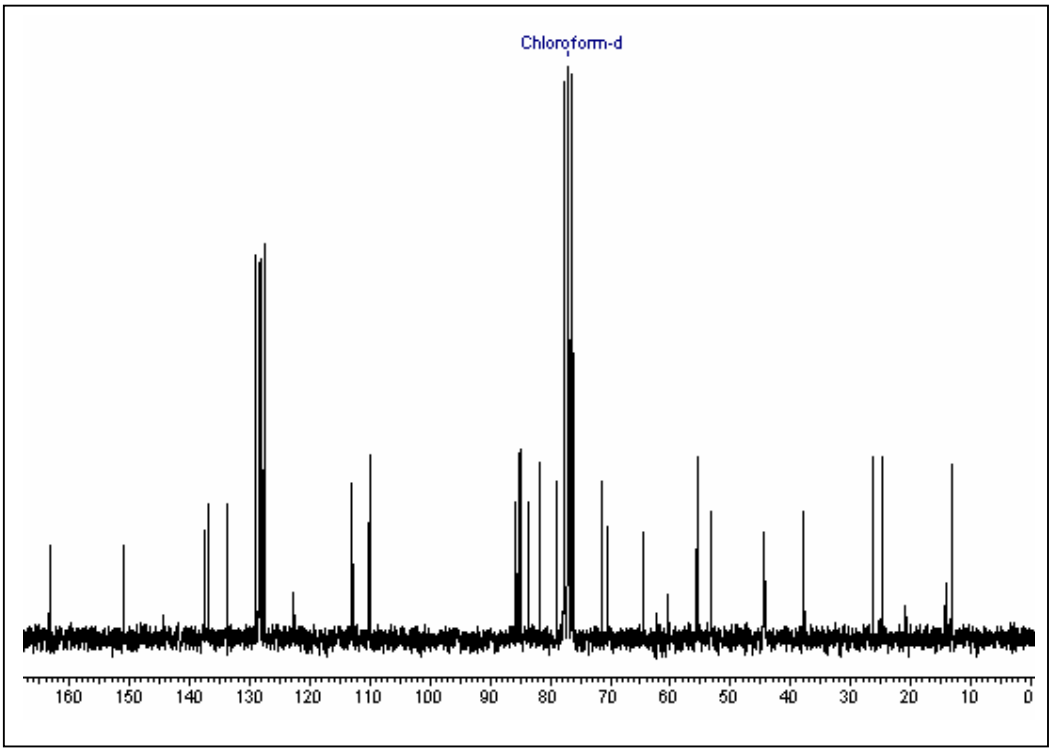
^{1}H NMR spectrum of Compound **213** (Table 2) in $CDCl_3$



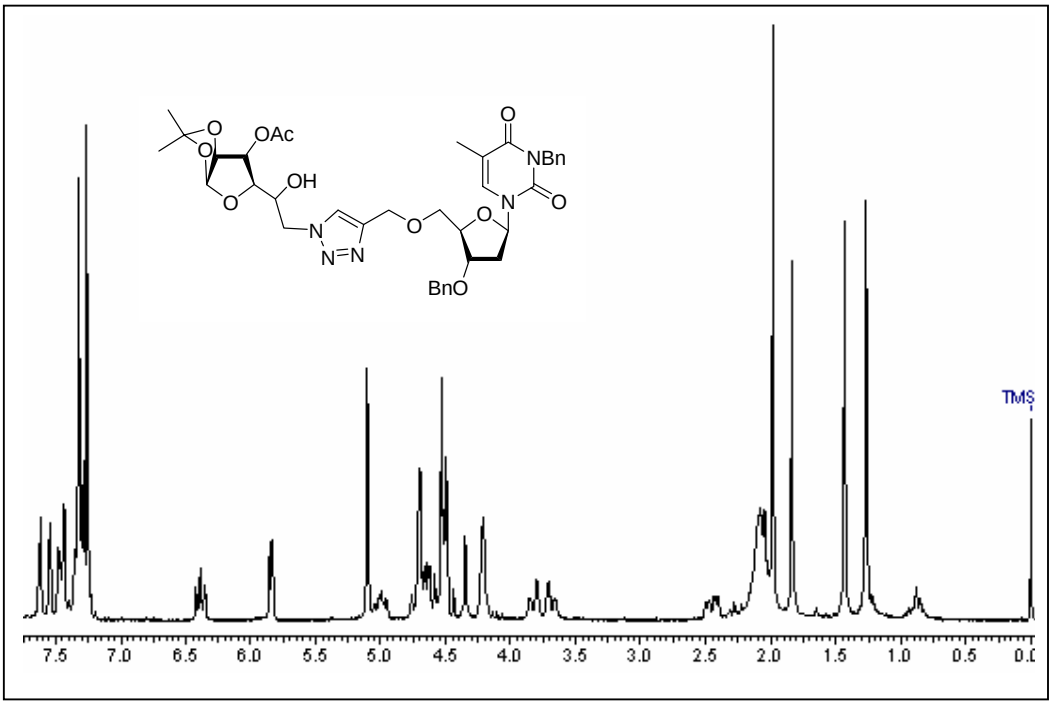
^{13}C NMR spectrum of Compound **213** (Table 2) in $CDCl_3$



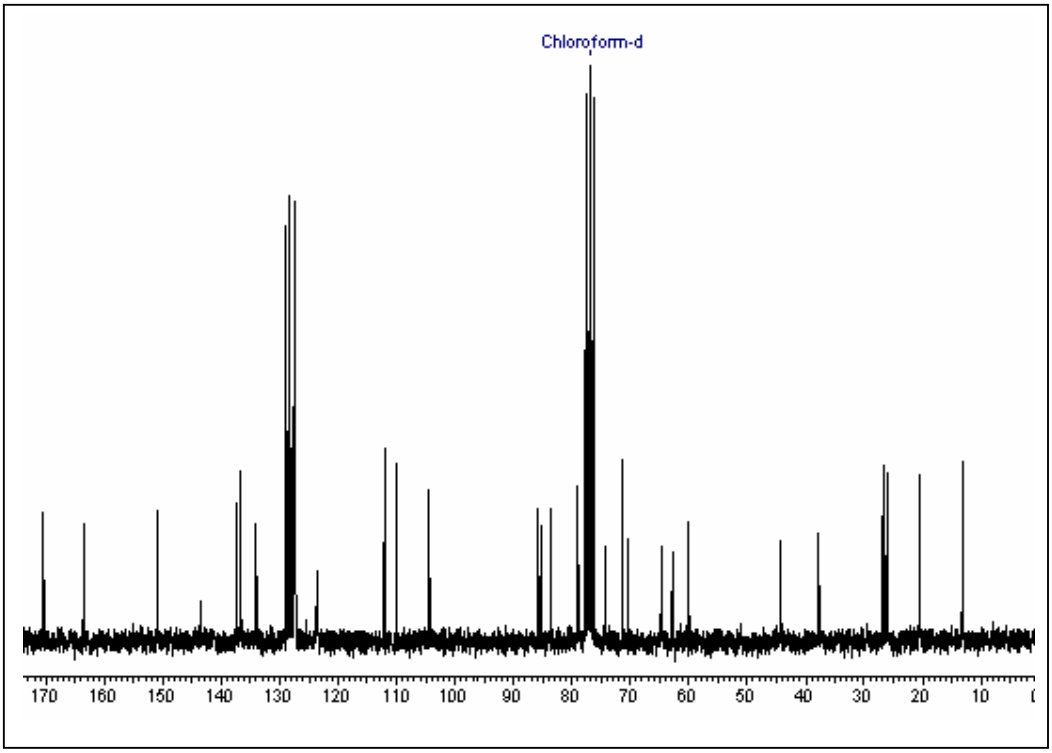
^{1}H NMR spectrum of Compound **214** (Table 2) in $CDCl_3$



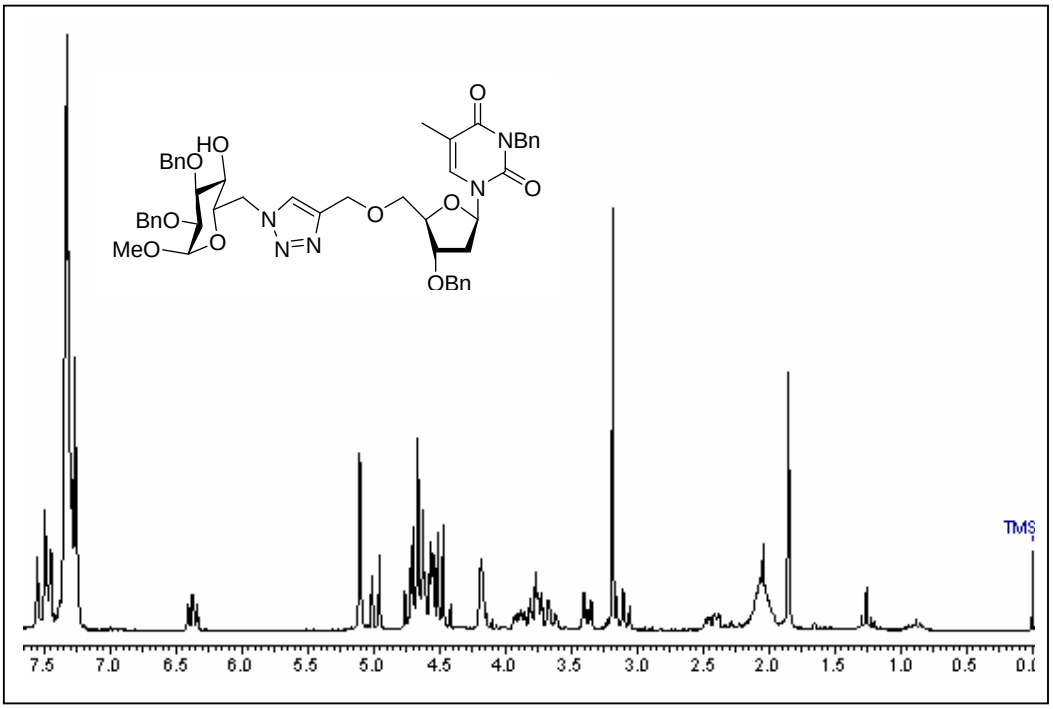
^{13}C NMR spectrum of Compound **214** (Table 2) in $CDCl_3$



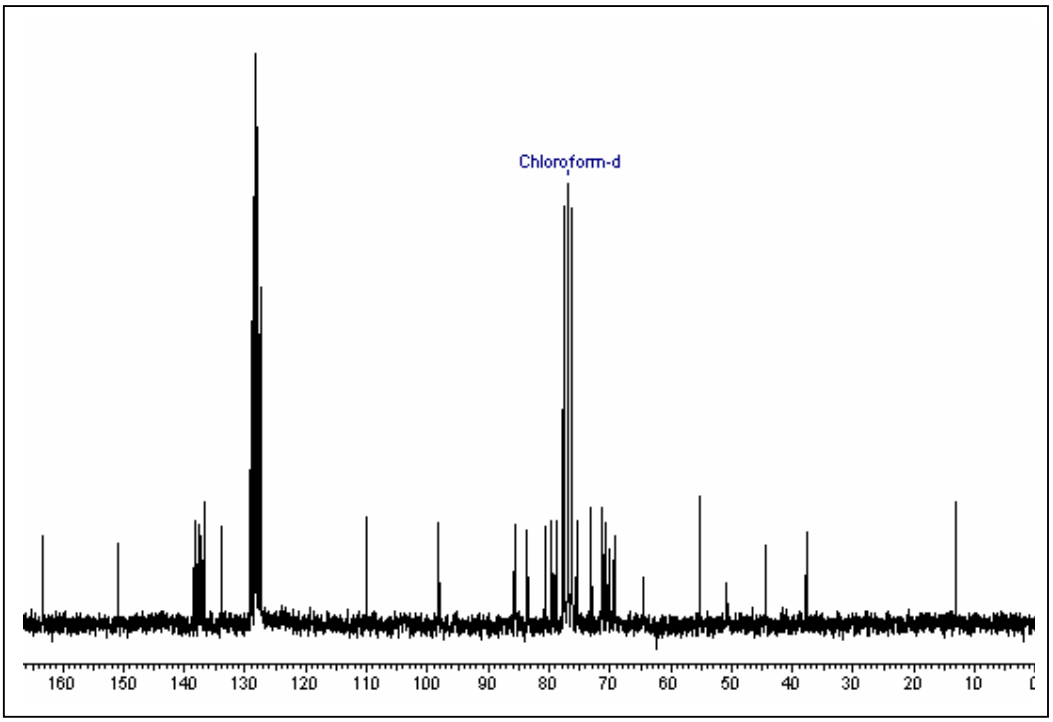
^{1}H NMR spectrum of Compound **215** (Table 2) in $CDCl_3$



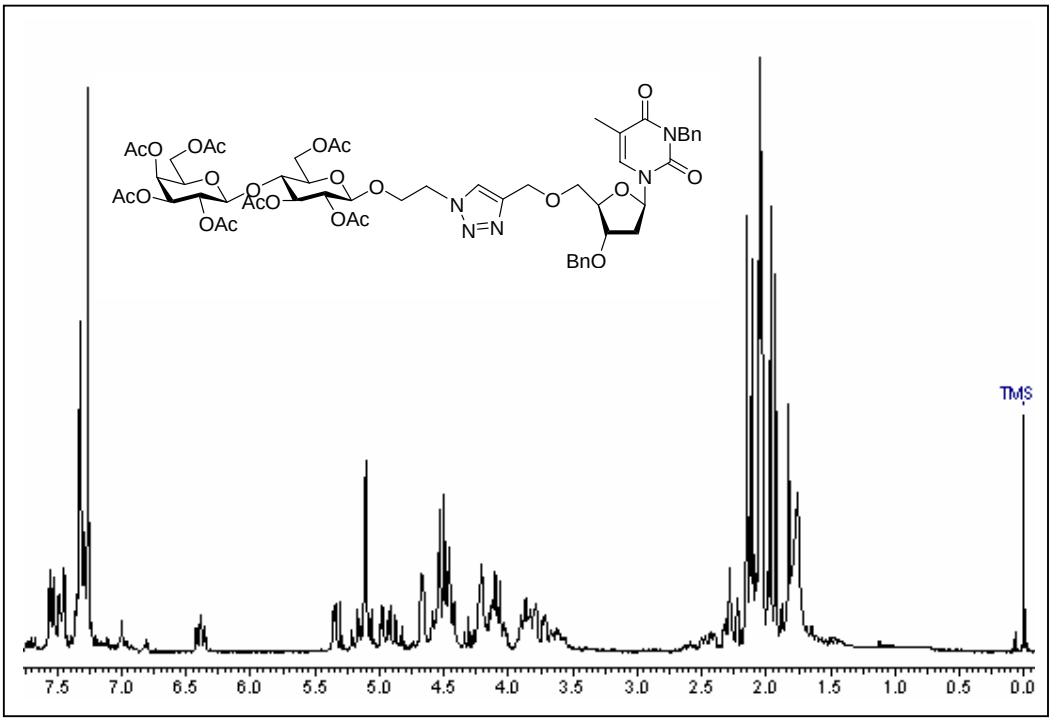
^{13}C NMR spectrum of Compound **215** (Table 2) in $CDCl_3$



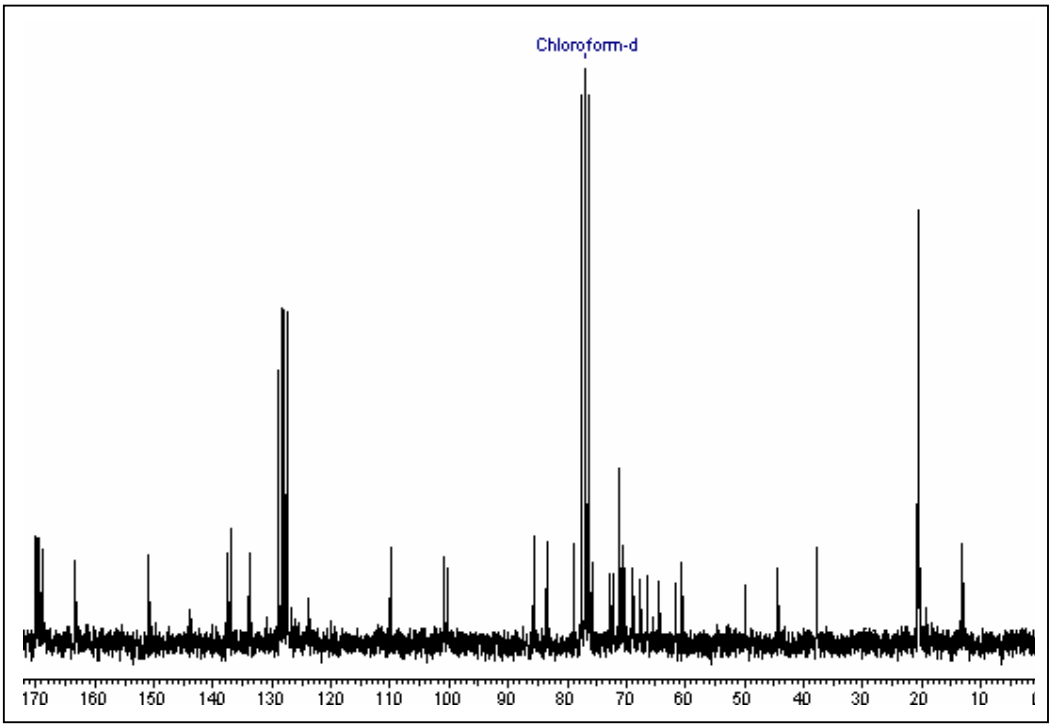
^{1}H NMR spectrum of Compound **216** (Table 2) in $CDCl_3$



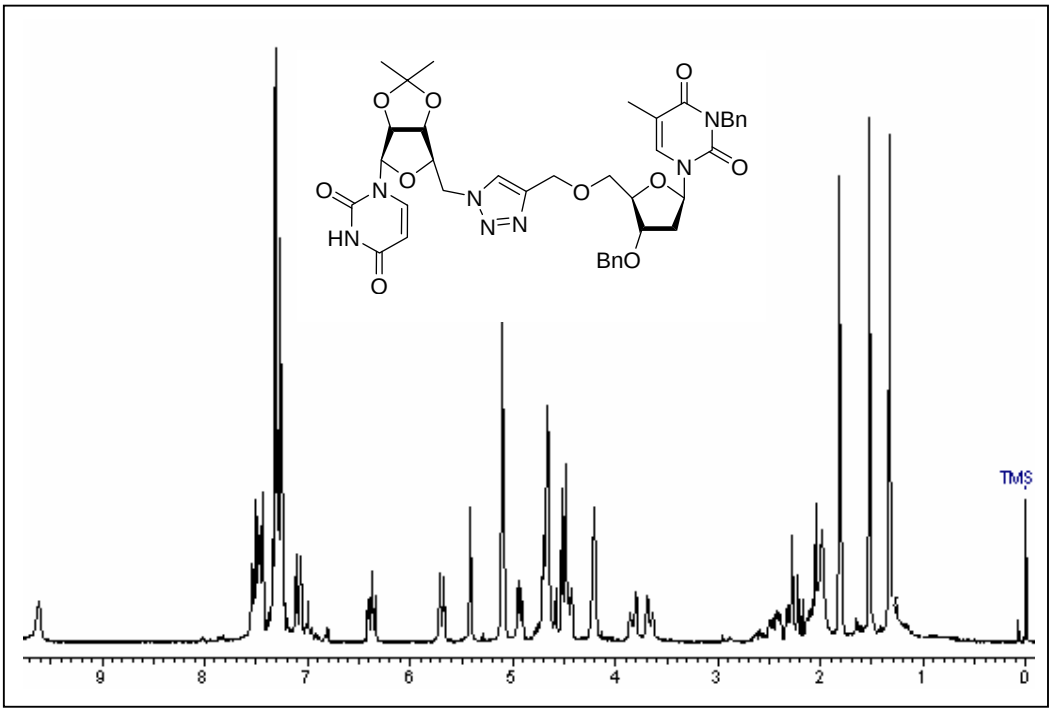
^{13}C NMR spectrum of Compound **216** (Table 2) in $CDCl_3$



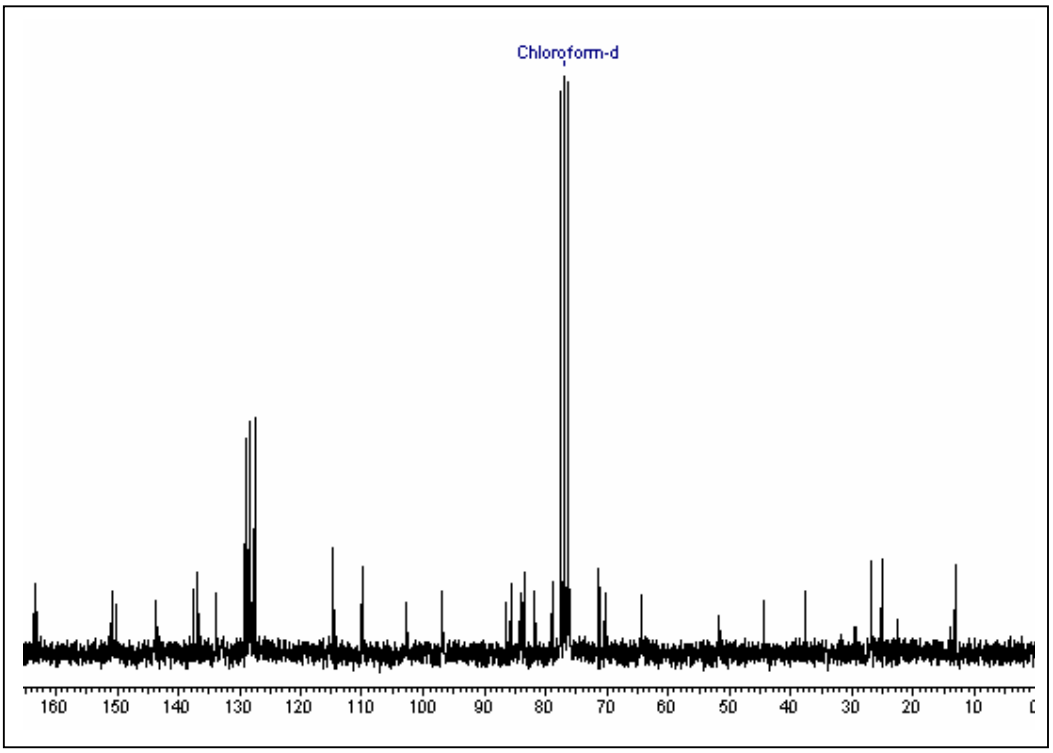
^{1}H NMR spectrum of Compound **217** (Table 2) in $CDCl_3$



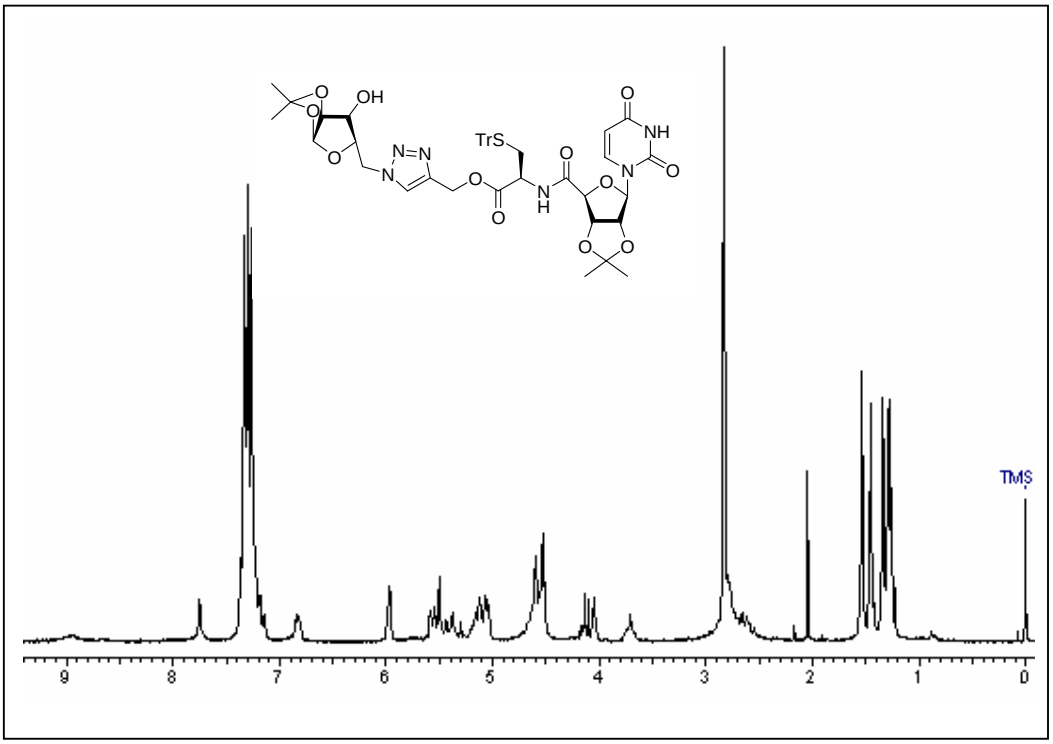
^{1}H NMR spectrum of Compound **217** (Table 2) in $CDCl_3$



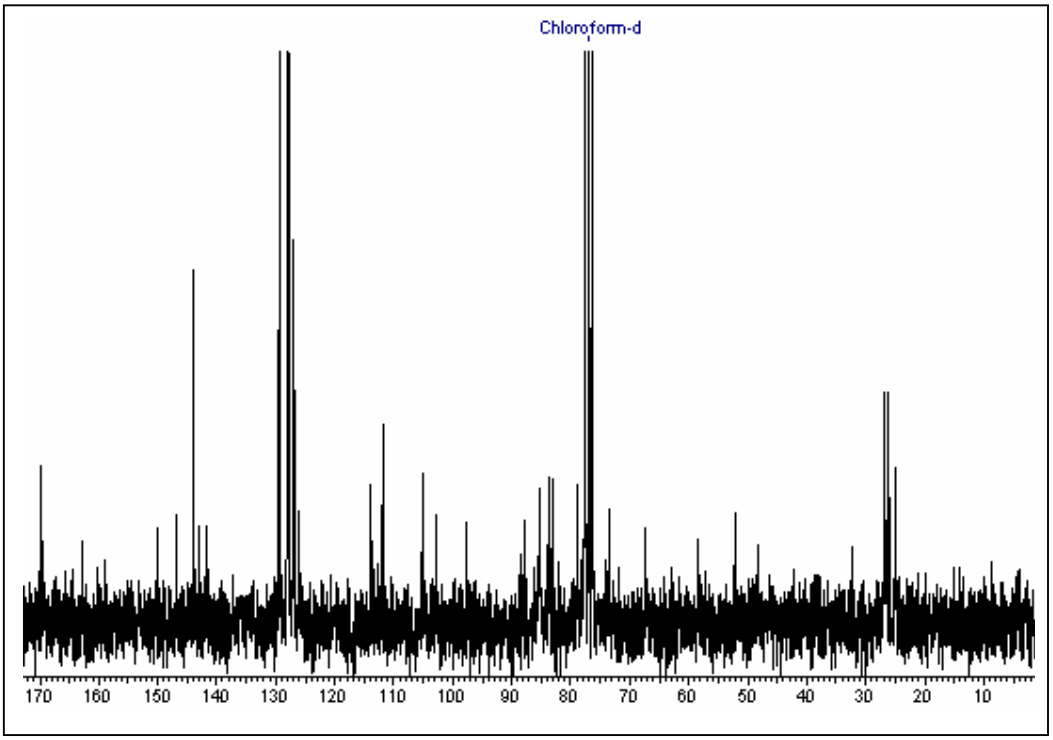
^{1}H NMR spectrum of Compound **218** (Table 2) in $CDCl_3$



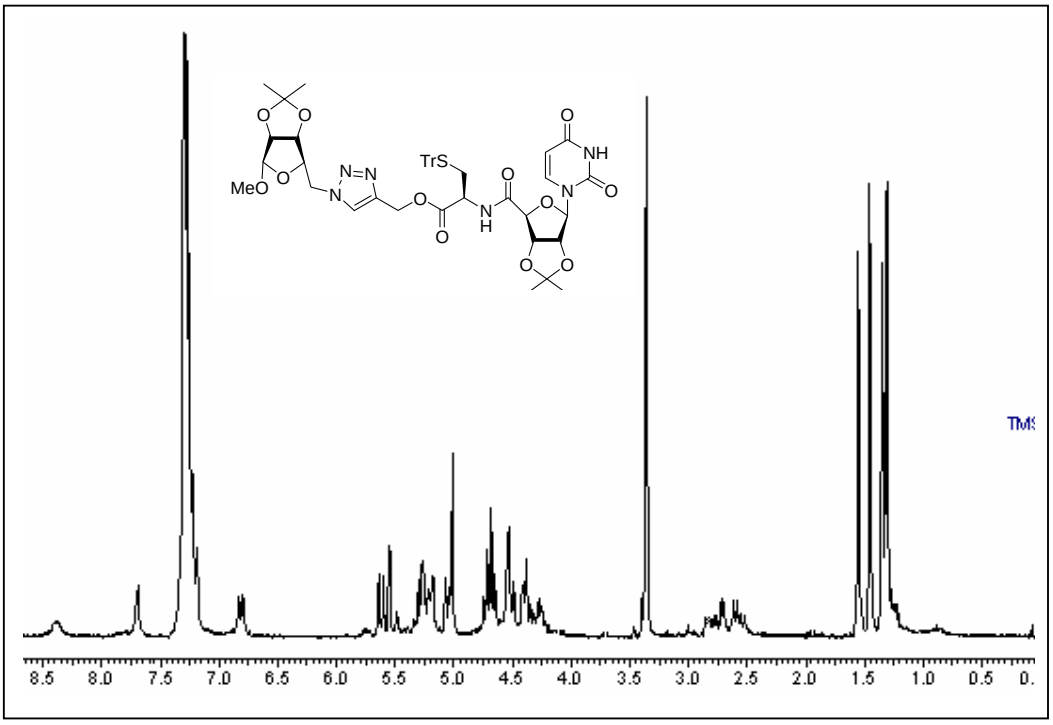
^{1}H NMR spectrum of Compound **218** (Table 2) in $CDCl_3$



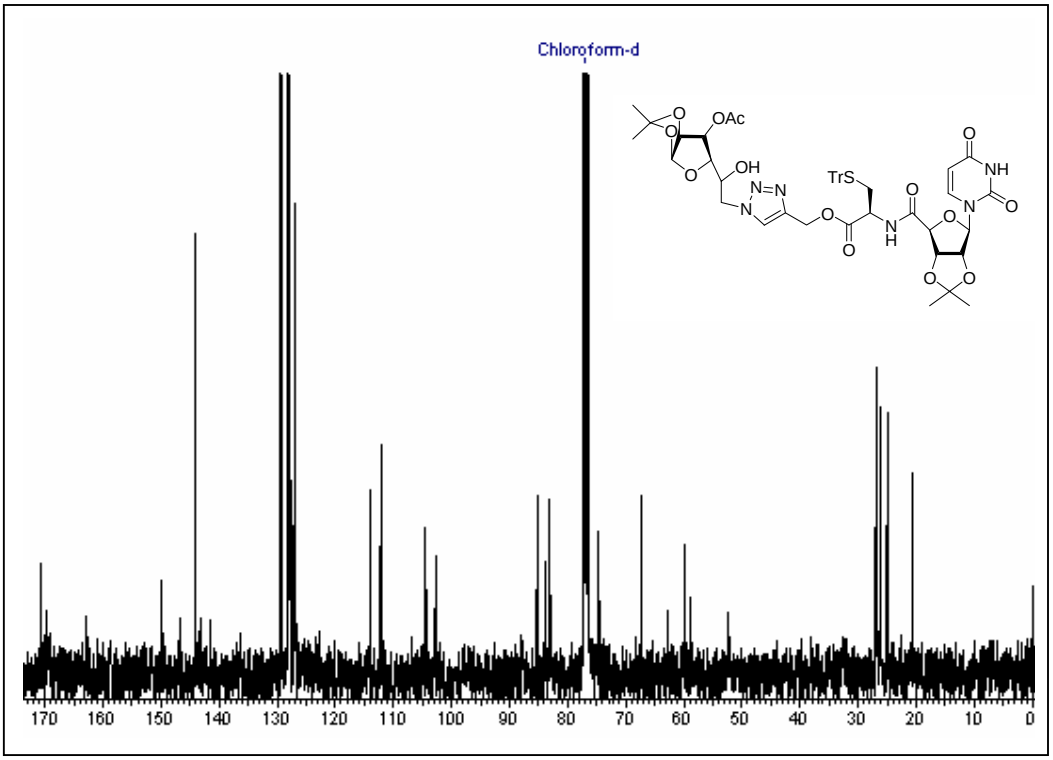
^{1}H NMR spectrum of Compound **224** (Table 3) in $CDCl_3$



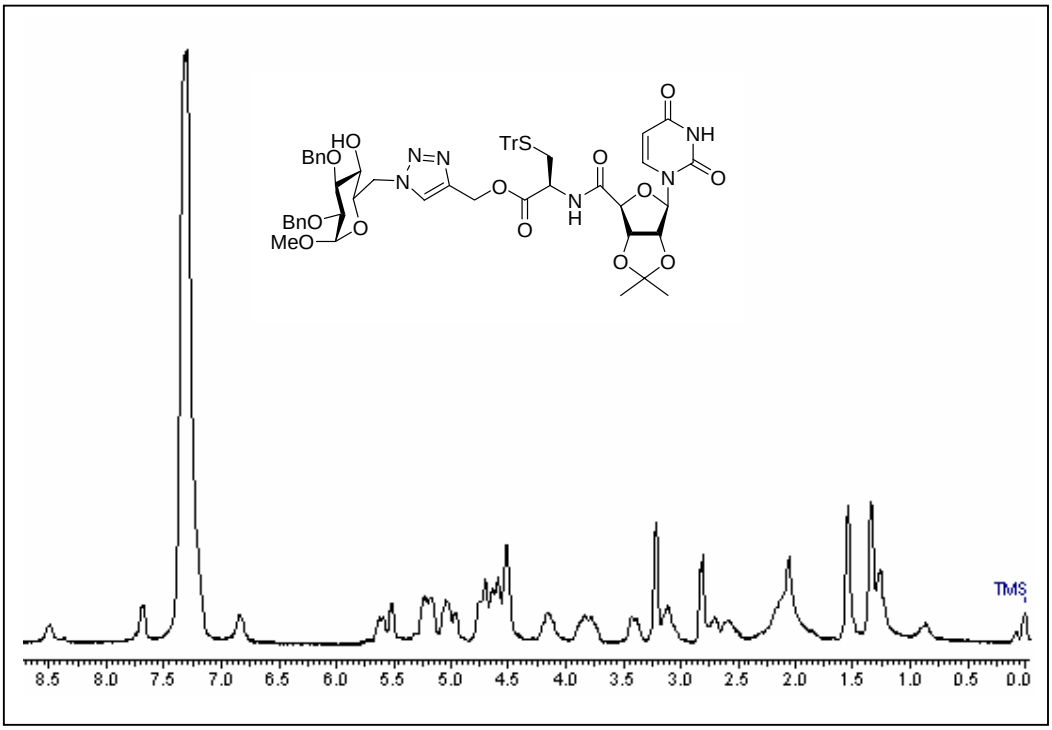
^{13}C NMR spectrum of Compound **224** (Table 3) in $CDCl_3$



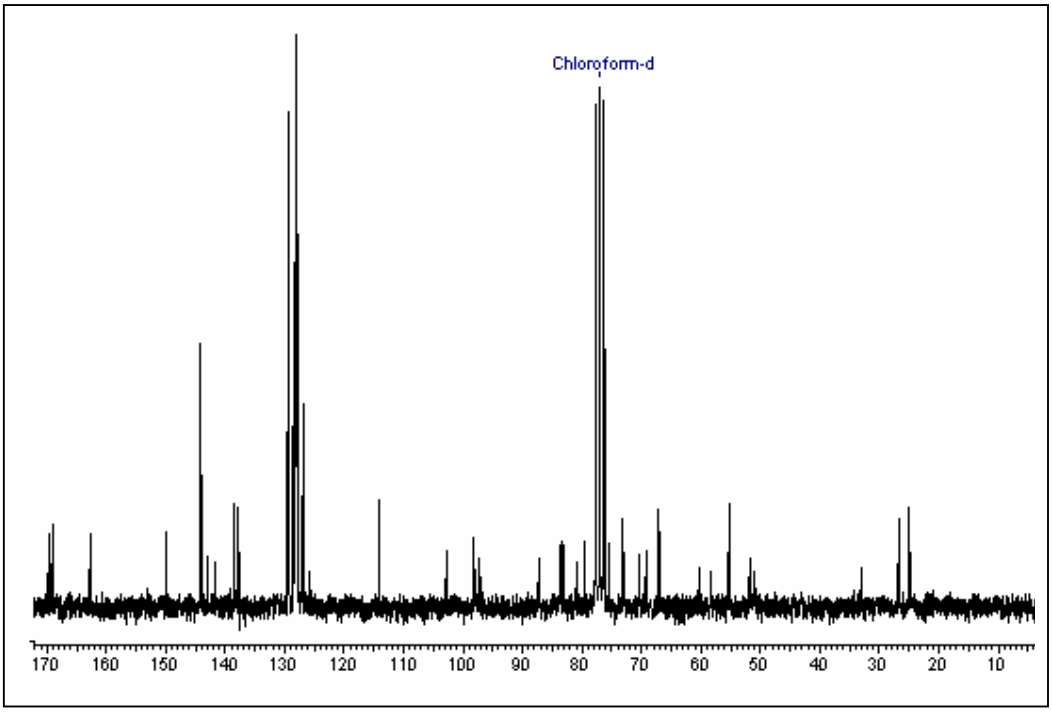
^{1}H NMR spectrum of Compound **225** (Table 3) in $CDCl_3$



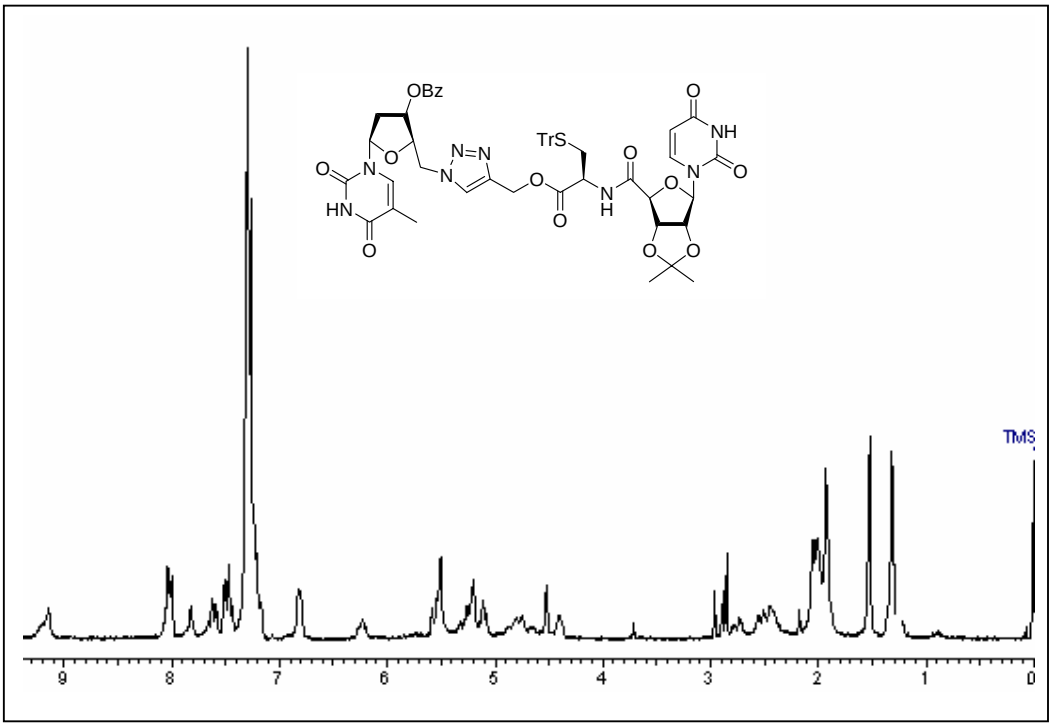
^{13}C NMR spectrum of Compound **226** (Table 3) in $CDCl_3$



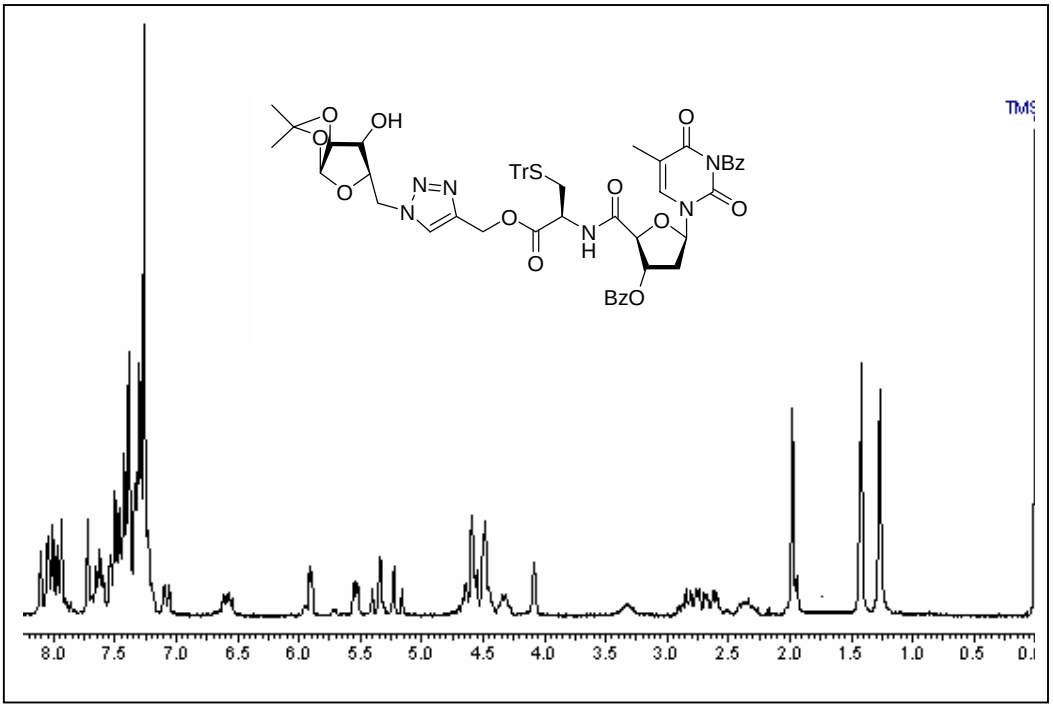
^{1}H NMR spectrum of Compound **227** (Table 3) in $CDCl_3$



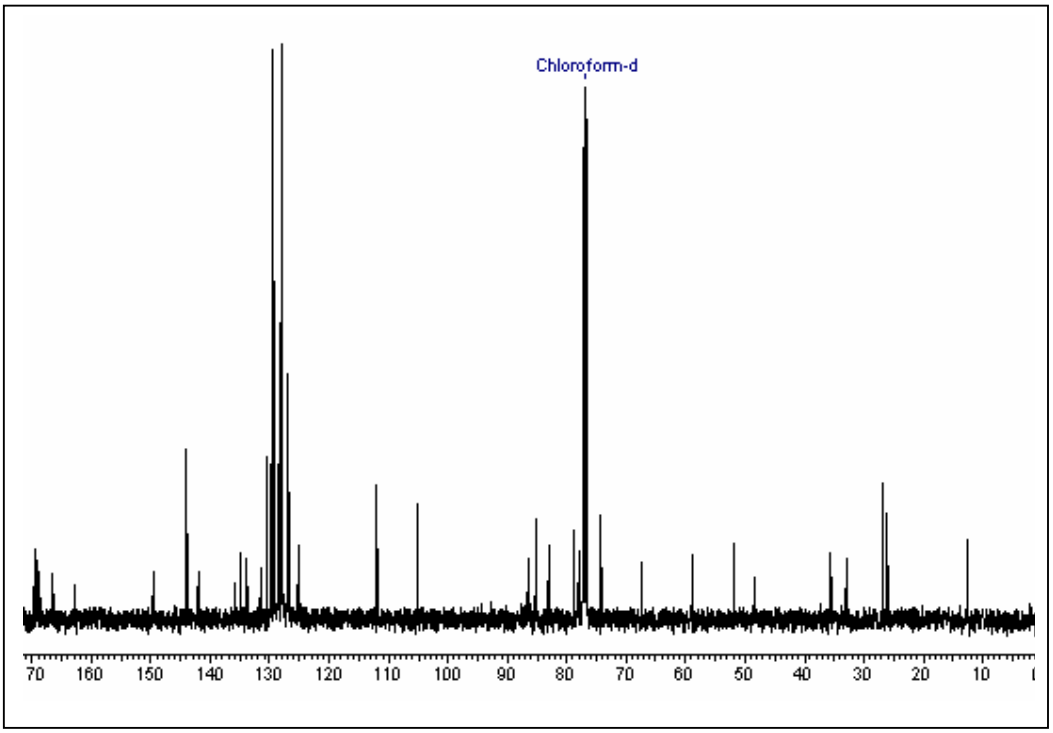
^{13}C NMR spectrum of Compound **227** (Table 3) in $CDCl_3$



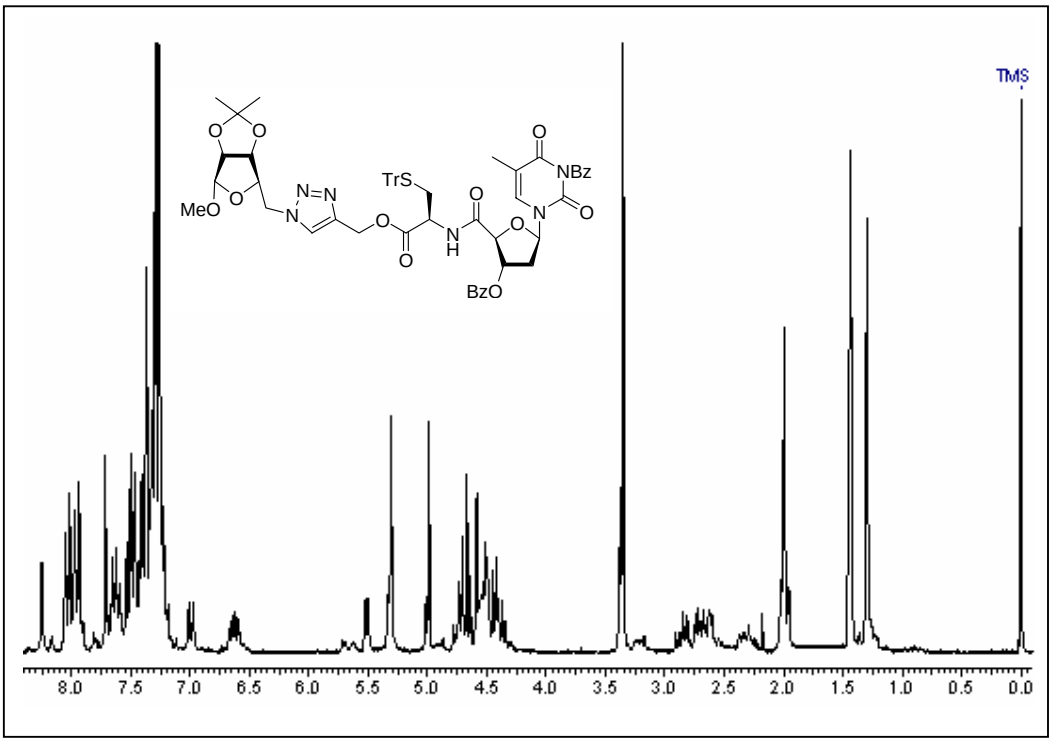
^{1}H NMR spectrum of Compound **228** (Table 3) in $CDCl_3$



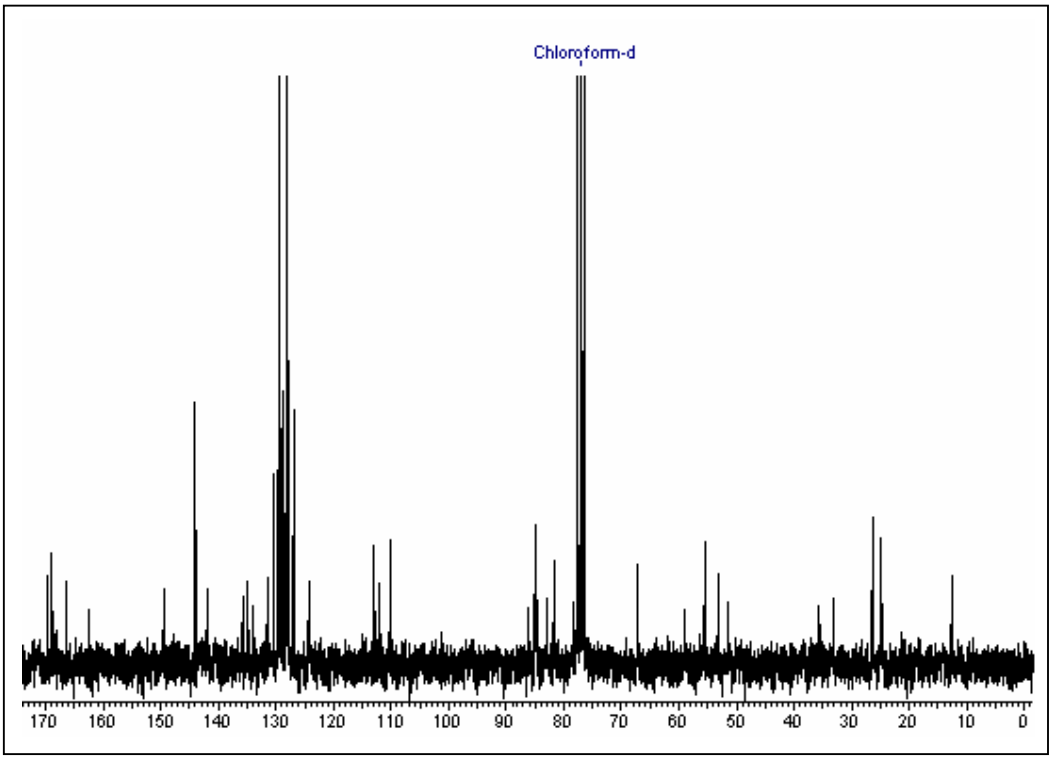
^{1}H NMR spectrum of Compound **232** (Table 4) in $CDCl_3$



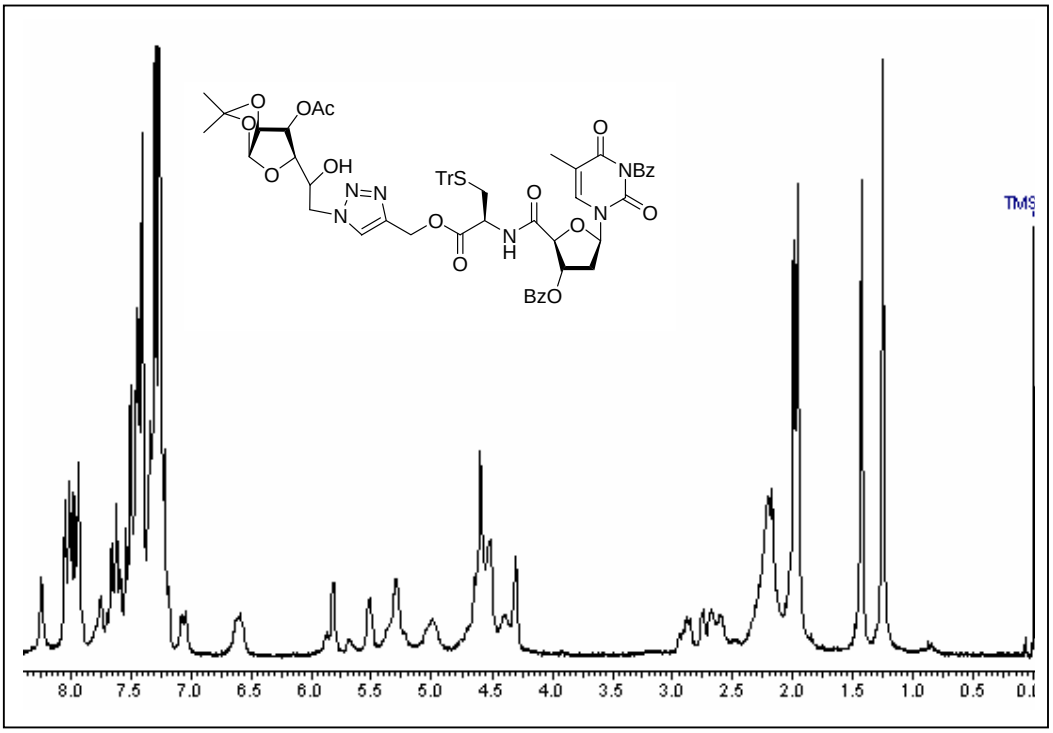
^{13}C NMR spectrum of Compound **232** (Table 4) in $CDCl_3$



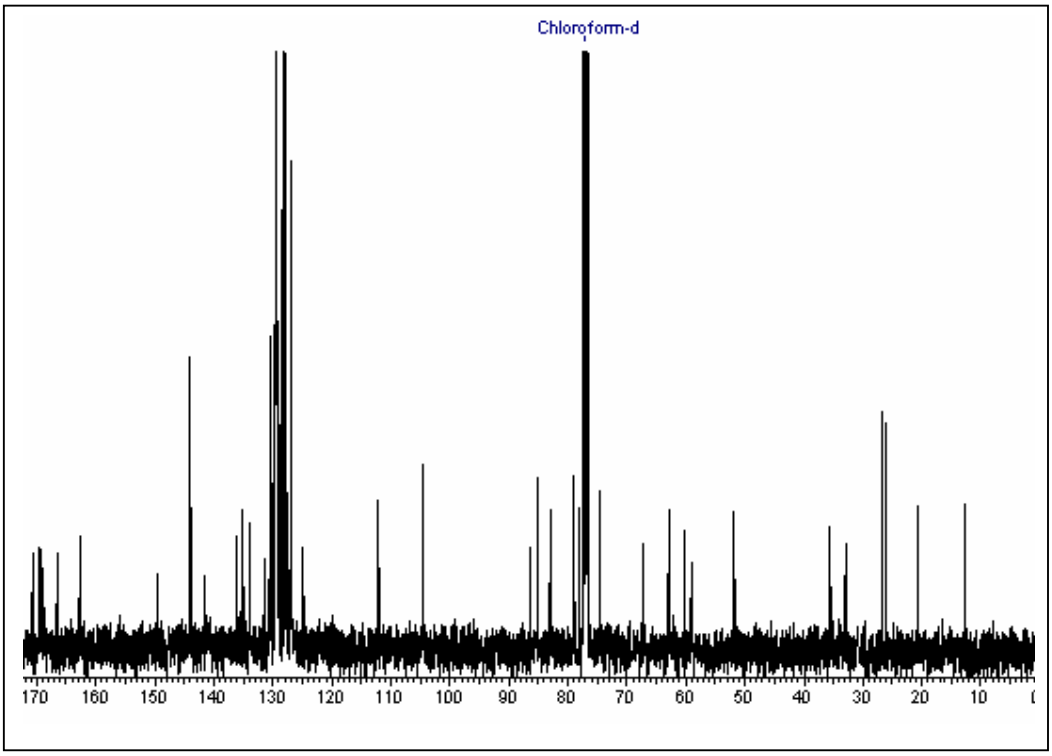
^{1}H NMR spectrum of Compound **233** (Table 4) in $CDCl_3$



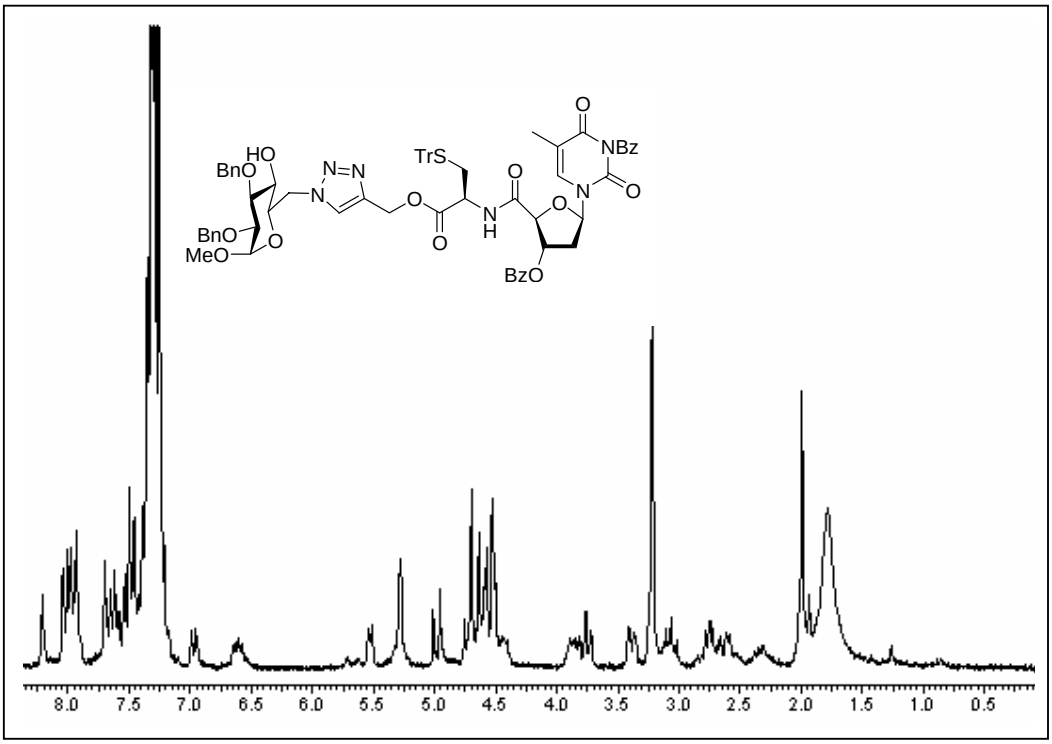
^{13}C NMR spectrum of Compound **233** (Table 4) in $CDCl_3$



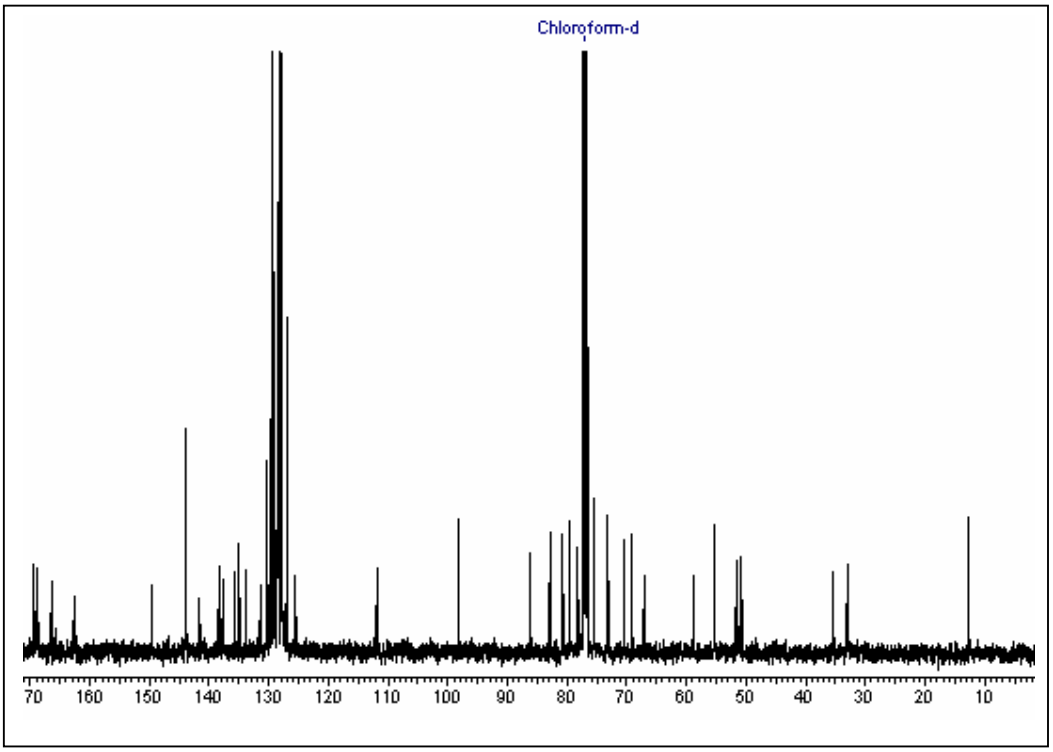
^{1}HNMR spectrum of Compound **234** (Table 4) in $CDCl_3$



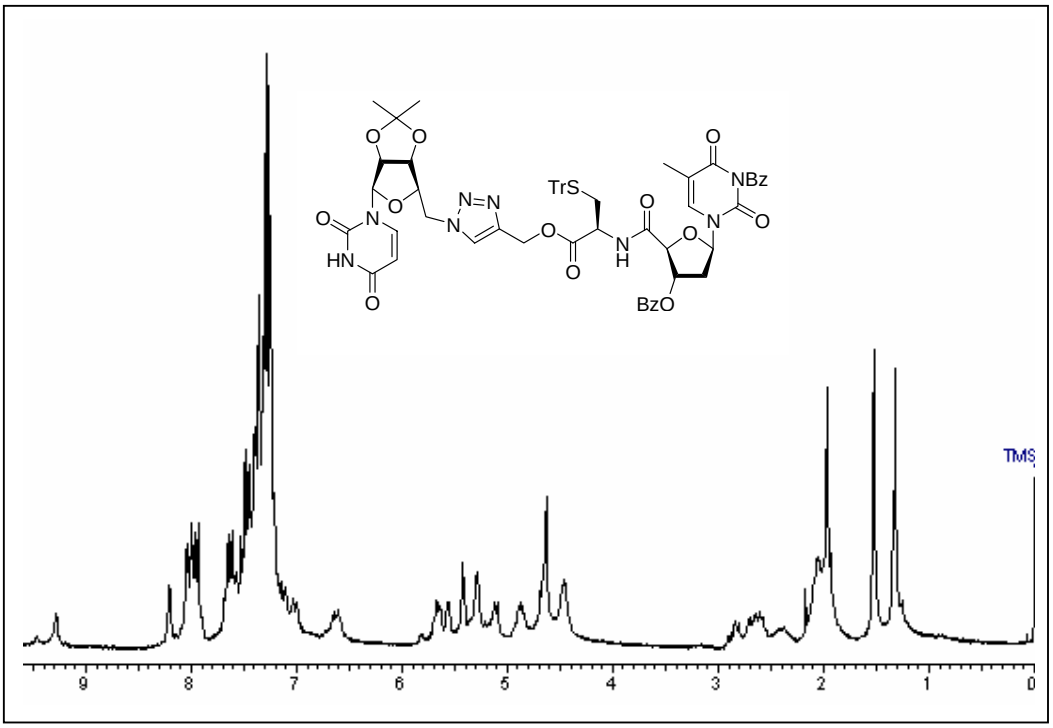
^{13}C NMR spectrum of Compound **234** (Table 4) in $CDCl_3$



^{1}H NMR spectrum of Compound **235** (Table 4) in $CDCl_3$



^{13}C NMR spectrum of Compound **235** (Table 4) in $CDCl_3$



^{1}H NMR spectrum of Compound **236** (Table 4) in $CDCl_3$

Chapter 3: References

3.9. References

1. Seeberger, P. H. *Chem. Comm.,* **2003,** 1115.
2. *Essentials of Glycobiology,* ed. Varki, A.; Cummins, R.; Esko, J.; Freez, H.; Hart, G.; Marth, J. Cold spring Harbor, NY, **1999**.
3. (a) Doores, K. J.; Gamblin, D. P.; Davis, B. G. *Chem. Eur. J.,* **2006,** *12,* 656. (b) Schroder, P. N.; Giannis, A. *Angew. Che. Int. Ed.,* **1999,** *38,* 1379.
4. Wilkinson, B. L.; Bornaghi, L. F.; Poulsena, S. A.; Houston, T. A. *Tetrahedron,* **2006,** *62,* 8115.
5. Takaya, K.; Nagahori, N.; Kurogochi, M.; Furuike, T.; Miura, N.; Monde, K.; Lee, Y. C.; Nishimura, S. I. *J. Med. Chem.,* **2005,** *48,* 6054.
6. Yarema, K.J.; Bertozzi, C.R. *Curr. Opin. Chem. Biol.,* **1998,** *2,* 49.
7. Chittaboina, S.; Xie, F.; Wang, Q. *Tetrahedron Lett.,* **2005,** *46*, 2331.
8. Ahmadibeni, Y.; Parang, K. *J. Org. Chem.,* **2006,** *71,* 6693.
9. Lee, L. V.; Mitchel, M.L.; Huang, S. J.; Fokin, V. V.; Sharpless, K. B.; Wong, C. H. *J. Am. Chem. Soc.,* **2003,** *125,* 9588.
10. Behr, J. B.; Gourlain, T.; Helimi, A.; Guillerm, G. *Bioorg. Med. Chem. Lett.,* **2003,** *13,* 1713.
11. Dondoni, A.; Giovannini, P. P.; Massi, A. *Org. Lett.,* **2004,** *17,* 2929.
12. Yeager, A. R.; Finney, N. S. *J. Org. Chem.,* **2004,** *69,* 613.
13. Stolz, F.; Reiner, M.; Blume, A.; Reutter, W.; Schmidt, R. R. *J. Org. Chem.,* **2004**, *69*, 665.
14. Chevalier, A. L.; Pierre, R.; Kanso, R.; Chambert, S.; Doutheau, A.; Queneau, Y. *Tetrahedron Lett.,* **2006,** *47*, 2431.
15. Wilkinson, B. L.; Bornaghi, L.F.; Poulsena, S.A.; Houston, T.A. *Tetrahedron,* **2006,** *62,* 8115.
16. Hotha, S.; Kashyap, S.; *J. Org. Chem.,* **2006**, *71*, 364.
17. Somu, R. V.; Boshoff, H.; Qiao, C.; Bennett, E. M.; Barry III, C. E. Aldrich, C C. *J. Med. Chem.,* **2006,** *49,* 31.
18. Zhang, D.; Miller, M. J.; *Current Pharmaceutical Design,* **1999,** *5,* 73.
19. Prepared by the method given in Danishefsky, S. J.; DeNinno, S. L.; Chen, S. H.; Boisvert, L.; Barbachyn, M. *J. Am. Chem. Soc.,* **1989,** *111,* 5810.
20. Rostovtsev, V. V.; Green, L. G,; Fokin, V. V.; Sharpless, K. B. *.Angew. Chem. Int. Ed*., **2002**, *41,* 2596.

21. Brier, S.; Lemaire, D.; DeBonis, S.; Forest, E.; Kozielski. *Biochemistry,* **2004,** *43,* 13072.
22. Epp, J. B.; Widlanski, T. S. *J. Org. Chem.,* **1999.** *64,* 293.
23. Prepared by the method given in Gurjar, M. K.; Kunwar, A. C.; Reddy, D. V.; Islam, A.; Lalitha, S. V. S.; Jagannadh, B.; Rama Rao, A. V. *Tetrahedron,* **1993,** *49,* 4373.
24. Prepared by the method given in Barrett, A. G. M.; Lebold, S. A. *J. Org. Chem.,* **1990.** *55,* 3853.

****

Chapter 4: Evaluation Of Antifungal Activity Of The New Molecules

4.1. Introduction and Present work

In order to evaluate the antifungal activity of new molecules, they are divided into four groups according to their structural scaffolds and these groups are as follows (Table 1).

Table 1

Sl. No.	Test compounds
Group 1	1,2,3-Triazole and 1,2,3,4-tetrazole fused tetracyclic compounds (**118, 123, 127, 132, 136, 140, 144, 148** and **150**)
Group 2	Glycosides derived from D-xylofuraose derived 1,2,3-triazole and 1,2,3,4-tetrazole fused tetracyclic compounds (**151α, 151β, 152α, 152β, 153α, 153β, 154α, 154β, 155α, 155β** and **156(α,β mix)**)
Group 3	Nucleosides derived from D-xylofuranose derived 1,2,3-triazole and 1,2,3,4-tetrazole fused tetracyclic compounds (**159, 161, 162, 163, 165** and **166**)
Group 4	Glycoconjugates of uridine and azido sugars containing 1,2,3-triazole (**203, 204, 205, 206, 207, 208** and **209**)

Following methods were employed to evaluate the biological activity.

1. Whole cell based plate assay.

2. Target-specific whole cell based assay: Haploinsufficiency assay.

3. Target based assay: Chitin synthase Non radioactive assay.

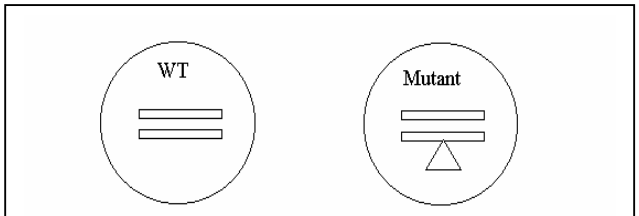
1. Whole cell based plate assay:

The whole cell based plate assays are carried out for the primary screening of antifungal compounds. The method involves the simple procedure that a disk of Whatmann paper impregnated with known amount of compound under test is placed on agar plate spread with fungal cell/spore suspension. After the incubation at specified temperature and time, the inhibition of fungal growth is measured. The

minimum concentration of test compound that gives the zone of inhibition is determined as minimum inhibitory concentration (MIC) for that fungal culture. The whole cell based plate assays provides a simple, easy and cost effective means for screening of antifungal compounds. However the fungal growth inhibition observed is not specific to particular target, hence these assays do not provide any clue regarding mode of action of antifungal compounds.

2. Target-specific whole cell based assay: Haploinsufficiency assay.

The target-specific whole cell based assays are based on hypersensitivity of yeast cells to target specific inhibitors, if diploid cells are mutated in one of the two copies of the target gene. When only one functional copy of a gene is present in a cell, then the amount of target protein made is usually half than that of the cell with two copies i.e. wild type. In the presence of a specific inhibitor, the cell with half the amount of the target protein (haploinsufficiency) will be more sensitive to the inhibitor, compared to the cell with normal amount (wild type) (Figure 1). Thus, for each validated target, hypersensitive test strains will be constructed by mutating one copy of the target gene in yeast, *Sacharomyces cerevisiae* (Table 2).



Strain	No. of copies of target gene	Level of target protein	Sensitivity to inhibitors of target protein	Sensitivity to inhibitors of other proteins
WT	Two	Normal	Normal	Normal
Mutant	One	Half	Hyper-sensitive	Normal

Figure 1: Haploinsufficiency

As the readout is relative cell density in the presence or absence of the inhibitor, the target-specific whole cell based screen can be setup only for those targets which are essential for growth or viability of the fungi. By comparing the relative growth of test strains to normal strains, it is possible to identify target-specific inhibitors from high throughput screens. Further, whole cell based assays are advantageous over enzymatic assays that these only identify lead compounds that can penetrate the fungal cells. Such screens have been successfully used for identifying antifungal lead compounds[1].

Table 2: *S. cerevisiae* mutant strains

Strains	Targets	Function
1536	Wild	
1500	ERG 11	Ergosterol synthesis
1485	TRL 1	tRNA splicing (tRNA ligase activity)
1487	YPD 1	Osmo-sensory signaling pathway via two-component system
1488	RSC 9	Chromatin remodeling complex
1492	CHS 2	Chitin synthase
1519	ARL 1	Magnesium, di-, tri- valent ion transport
1831	FAS 2	Fatty acid synthetase activity
1834	ILV 5	Acetohydroxy acid reductoisomerase activity
1845	FBA 1	Gluconeogenesis and glycolysis, Fructose-1,6-bisphosphate aldolase activity

In the present study, the assay was performed in *S. cerevisiae* using diploids that have both wild alleles as well as heterozygous diploids that have only one

functional allele as listed in table 1, using 96-well plate format medium throughput screening.

3. Target based assay: Chitin synthase Non radioactive assay.

Chitin, a β-1,4 linked *N*-acetyl-D-glucosamine polymer is a major structural component of the fungal cell wall and is absent in mammal and plant cells. Chitin synthase is the enzyme that catalyses the biosynthesis of chitin. This enzyme is the unique target for the development of antifungal compounds, as it is absent in mammals and plants (specific and less toxic), present in all fungi (broad spectrum) and it is essential for the survival of fungi (fungicidal). Thus Chitin synthase serves as an ideal target for the antifungal activity screening, both *in vivo* and *in vitro*.

Traditionally, chitin synthase activity is measured by radioactive assay using [^{14}C] UDP-*N*-acetyl-D-glucosamine as a substrate followed by quantitative determination of ^{14}C labelled chitin after acid precipitation.[2] Recently Lucero and coworkers developed a methodology for non-radioactive *in vitro* assay for estimation of chitin synthase activity.[3]

The method involves binding of synthesized chitin to a wheat germ agglutinin (WGA) coated surface followed by detection of the polymer with horseradish peroxidase (HRP) activity. A total membrane fraction from yeast cells, as a source of chitin synthase is incubated with the appropriate reagents for the production of chitin in microtitre plate wells coated with WGA (Step I, Figure 2). Then WGA conjugated to HRP (WGA-HRP) is added to bind to the immobilized chitin (Step II, Figure 2). HRP activity is monitored by the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) by hydrogen peroxide (Step III, Figure 2). This reaction gives a mixture of blue and yellow compounds. TMB (colorless), transfer complex (blue color) and oxidized TMB (yellow diimine) reach to an equilibrium resulting in a visible blue-green color.

Acidification of the reaction mixture to pH 1.0 arrests the peroxidase reaction, which produces a yellow color with a maximal absorption at 450 nm and an increase in molar absorbance.

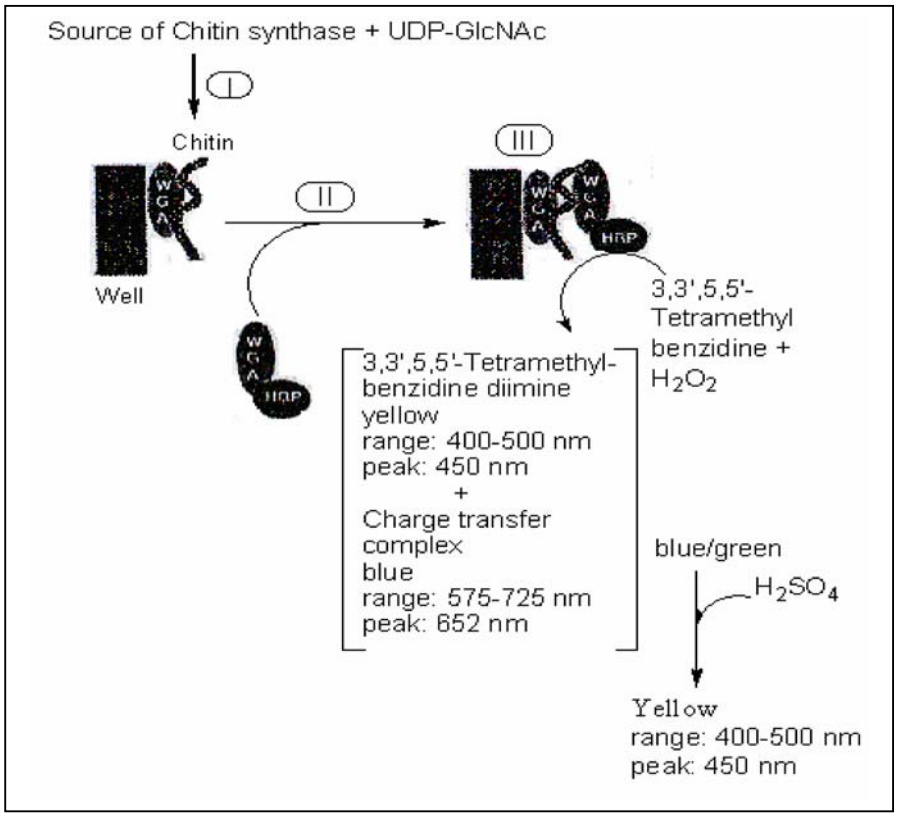


Figure 2: Steps of the chitin binding assay are (I) chitin synthesis and binding, (II) binding of WGA-HRP to immobilized chitin, and (III) colorimetric assay of HRP activity.

The method is highly sensitive, low dispersion, can be easily adopted to high throughput screening for chitin synthase activity in cell free extract and the assay can be performed without using any radioactive material. Thus the method is more advantageous over conventional radioactive assay method. Lucero and coworkers suggested that this assay may be employed for the screening of antifungal compounds for chitin synthase inhibitory activity in HTS mode.[3]

Chapter 4: Materials and Methods

1. Whole cell based Plate assay:

Test compounds **Group 1 - Group 4** (Table 1, page No. 234) were screened for the antifungal activity against *Candida albicans* (human pathogen) and *Fusarium oxysporum* (plant pathogen) using whole cell based plate assay. *C. albicans* and *F. oxysporum* were maintained on yeast extract peptone glucose (YPG) agar and potato dextrose agar slants, respectively. For the whole cell based assay both the fungi were grown in YPG broth under shaking conditions (180 rpm) for 24 h at 28 ^{o}C. The liquid culture of *C. albicans*/*F. oxysporum* (100 µl) was spread on YPG plate. The test compounds (**Group 1 - Group 4**) and amphotericin B were dissolved in DMSO (50% v/v). Whatmann filter paper (No. 4) discs were placed on YPG plates spread with fungal cultures. The test compounds (40, 60 and 100 µg/ml) and antifungal drug amphotericin B (15 µg/ml) were added on discs. The plates were then incubated for 24 h at 28 ^{o}C and then the zone of inhibition if any, was measured. The experiment was carried out in triplicate.

2. Haploinsufficiency assay:

Test compounds **Group 1 - Group 4** (Table 1, page No. 234) were screened for antifungal activity against *Sacharomyces cerevisae*; wild type and nine target specific mutant strains (as listed in table 2, page No. 236). Strains of *S. cerevisae* were maintained on YPD (10 gm/l yeast extract, 20 gm/l peptone, 20 gm/l dextrose and 20 gm/l agar) slants. The wild type and mutant strains of *S. cerevisae* were grown in 5 ml of YAD+S (Yeast nitrogen base w/o amino acids or ammonium sulfate: 1.7 gm/l, Ammonium sulfate: 5 gm/l, Dextrose monohydrate: 5 gm/l, Histidine HCl monohydrate: 20 mg/l, Uracil: 20 mg/l and Leucine: 100 mg/l) broth under shaking

condition (180 rpm) for 24 h at 28 ^{o}C. The liquid cultures were diluted suitably with YAD+S broth to get 0.1 absorbance at 600 nm. These liquid cultures were added to microtitre plate (80 µl/well). 20 µl of test compounds (**Group 1 – Group 4**) and fluconazole and nikkomycin Z as reference antifungal agents were added to the wells. After gentle mixing the plate was incubated for 24 h at 28 ^{o}C. Then using plate reader, absorbance (**A**) at 600 nm was measured.

The % inhibition of test culture was then calculated as follows.

$$\% \text{ inhibition} = \frac{A \text{ (Control)} - A \text{ (Test)}}{A \text{ (Control)}} \times 100$$

Control = DMSO (50%) + Culture

3. Chitin Synthase Non radioactive assay:

The mixed membrane fraction was extracted from hyphal cells of *Benjaminiella poitrasii* as described earlier by Deshpande *et al.*[4] This mixed membrane fraction was used as a source of chitin synthase activity. Chitin synthase activity was estimated using UDP-*N*-acetyl-D-glucosamine as a substrate.[3] The effect of test compounds (**Group 1 - Group 4**) (Table 1, page No. 234) on chitin synthase activity was studied. The microtitre plates were coated with wheat germ agglutinin (WGA). The assay mixture (100 µl) with and without test compounds (50 µg/ml) containing 32.5 µl of enzyme preparation and Tris –HCl (50 mM, pH 7.5), $MgCl_2$ (10 mM), GlcNAc (25 mM) and UDP- GlcNAc was added to wells. The plate was then shaken slowly on vortex shaker for 30 sec and then incubated at room temperature for 90 min. Finally, 100 µl of 1 µg/ml WGA-HRP in blocking buffer was added and after being gently shaken, plates were incubated for 15 min at room temperature. Plates were then emptied by vigorous shaking of their contents and washed with distilled

water. Then 100 µl of peroxidase substrate reagent was added. The reaction was stopped by the addition of 100 µl of 1 N H_2SO_4 which produces a stable yellow color, the absorbance (**A**) of which was measured spectrophotometrically at 430 nm.

The % inhibition was determined as follows.

$$\% \text{ Inhibition} = \frac{y - x}{y} \times 100$$

x = A (Test) – A (Substrate blank)

y = A [Control (Std)] - A (Substrate blank)

Chapter 4: Results and Discussions

1. Whole cell based Plate assay:

Plate assay was carried out for test compounds **Group1** to **Group 4** as listed in table 1 (page No. 234) using concentrations 40, 60 and 100 µg/ml and amphotericin B (15 µg/ml) was used as standard drug. Tables 3-6 show the inhibitions of vegetative growth of *Candida albicans* and *Fusarium oxysporum* in the presence of test compounds group 1, group 2, group 3 and group 4 respectively.

Effect of amphotericin B on the vegetative growth of *C. albicans* and *F. oxysporum*:

Concentration (15 µg/ml) – *C. albicans* (*C.A*) - 6-7 mm

F. oxysporum (*F.O*) - 4-5 mm.

A. Effect of Group 1 test compounds (table 1, page No. 234) on the vegetative growth of *C. albicans* and *F. oxysporum*:

Table 3 : Summary of the results of Group 1: Plate assay

Test Compounds	Zone of inhibition (mm)					
	40 µg/ml		60 µg/ml		100 µg/ml	
	C.A	*F.O*	*C.A*	*F.O*	*C.A*	*F.O*
118	10	7	12	9	18	12
123	8	12	12	14	16	16
127	8	8	10	12	16	14
132	10	9	10	10	16	12
136	10	8	13	10	16	12
140	9	7	11	10	13	12
144	10	6	12	8	15	10
148	8	7	11	9	16	12
150	10	ND	14	ND	16	ND

After analysing the results, we found that 1,2,3-traizole fused tetracyclic compounds (**Group 1**) were found to have inhibitory activity against vegetative growth of both *C. albicans* and *F. oxysporum* (Table 3). These test compounds

showed the zone of inhibition in the range of 8-10 mm and 6-12 mm against *C. albicans* and *F. oxysporum* respectively, at concentration 40 µg/ml. Amongst these compounds 1,2,3-traizole fused tetracyclic compounds (**118**), (**132**), (**136**) and (**144**) shown maximum inhibition zone of about 10 mm against *C. albicans* and 1,2,3-traizole (**123**) and (**132**) showed a maximum inhibition zone of about 12 mm and 9 mm respectively, against *F. oxysporum*. However, 1,2,3,4-tetrazole fused tetracyclic compound (**150**) shown inhibition only against *C. albicans* with 10 mm zone of inhibition at 40 µg/ml . We also observed the concentration dependant inhibition of vegetative growth; the inhibition was increased with the increase in concentration. 13-18 mm and 10-16 mm inhibition zone against *C. albicans* and *F. oxysporum* respectively, was observed at a maximal concentration of 100 µg/ml.

B. Effect of Group 2 test compounds (table 1, page No. 234) on the vegetative growth of *C. albicans* and *F. oxysporum*:

Table 4 : Summary of the results of Group 2: Plate assay

Test Compounds	Zone of inhibition (mm)					
	40 µg/ml		60 µg/ml		100 µg/ml	
	C.A	*F.O*	*C.A*	*F.O*	*C.A*	*F.O*
151 α	8	ND	10	ND	16	ND
151 β	7	ND	11	ND	14	ND
152 α	8	8	12	10	14	12
152 β	8	6	10	8	14	10
153 α	7	6	9	8	14	10
153 β	8	6	12	8	18	10
154 α	8	ND	11	ND	17	ND
154 β	9	ND	12	ND	16	ND
155 α	10	ND	12	ND	17	ND
155 β	9	11	12	13	18	15
156 (αβ)	9	9	12	11	16	12

The glycoside derivatives, (**Group 2**) were also found to have vegetative growth inhibitory activity (Table 4). The observed zone of inhibition was in the range of 7-10 mm at concentration 40 µg/ml against *C. albicans*. (**155α),** a α-allyl glycoside of 1,2,3,4-tetrazole was the one which showed maximum inhibition of about 10 mm and (**154β), (155β**) and (**156),** glycoside of 1,2,3,4-tetrazole showed 9 mm inhibition against *C. albicans* (40 µg/ml).

However, glycosides (**152α, 152β, 153α, 153β, 155β** and **156**) exhibited inhibition against *F. oxysporum* in the range of 6-11 mm at concentration 40 µg/ml. Amongst these glycosides, (**155β**), β-ally glycosides of 1,2,3,4-tetrazole had a maximum inhibition of about 11 mm and (**156**), homopropargyl glycoside of 1,2,3,4-tetrazole had 9 mm (in mixture) against *F. oxysporum*. All the glycosides exhibited a concentration dependant inhibition. A maximum of about 14-18 mm of inhibition zone was observed against *C. albicans* at concentration 100 µg/ml and similar effect was also observed against *F. oxysporum* (10-15 mm).

C. Effect of Group 3 test compounds (table 1, page No. 234) on the vegetative growth of *C. albicans* and *F. oxysporum*:

Table 5: Summary of the results of Group 3: Plate assay

Test Compounds	Zone of inhibition (mm)					
	40 µg/ml		60 µg/ml		100 µg/ml	
	C.A	*F.O*	*C.A*	*F.O*	*C.A*	*F.O*
159	11	6	13	8	16	10
161	10	8	12	10	18	12
162	8	8	10	10	18	12
163	9	ND	12	ND	16	ND
165	8	11	10	15	18	18
166	8	10	12	10	18	14

The plate assay of nucleoside derivatives (**Group 3**) also showed inhibitory activity against vegetative growth of both *C. albicans* and *F. oxysporum* at concentration 40 µg/ml, except (**163**) which was found active only against *C. albicans* (Table 5). The observed zone of inhibition was in the range of 8-11 mm and 6-11 mm against *C. albicans* and *F. oxysporum* respectively, at 40 µg/ml. Maximum inhibition was observed for uracil nucleoside (**159**) and adenine nucleoside (**161**) derived from 1,2,3-traizole (**118**), with 11 and 10 mm respectively, against *C. albicans*. Whereas, adenine nucleoside (**165**) and 6-chloroguanine nucleoside (**166**), which were derived from 1,2,3,4-tetrazole (**150**) showed maximum inhibition about 10 and 11 mm respectively, against *F. oxysporum* at concentration 40 µg/ml.

The increase in concentration of nucleosides increased the zone of inhibition that clearly shows the concentration dependant activity. The range of zone of inhibition for these compounds increased to 16-18 mm and 12-18 mm against *C. albicans* and *F. oxysporum* respectively, at concentration 100 µg/ml.

D. Effect of Group 4 test compounds (table 1, page No. 234) on the vegetative growth of *C. albicans* and *F. oxysporum*:

Table 6: Summary of the results of Group 4: Plate assay

Test Compounds	Zone of inhibition (mm)					
	40 µg/ml		60 µg/ml		100 µg/ml	
	C.A	*F.O*	*C.A*	*F.O*	*C.A*	*F.O*
203	8	6	11	8	18	10
204	9	7	10	9	16	12
205	9	8	12	10	18	12
206	10	9	12	11	14	13
207	10	6	12	8	16	10
208	8	9	10	11	16	14
209	8	9	10	11	16	13

In the case of entirely different molecular scaffolds, i.e. conjugates of uridine and azido sugars containing a metabolically stable motif, i.e. 1,2,3-triazole nucleus (**Group 4**), these compounds were also found to have inhibitory activity against vegetative growth of both *C. albicans* and *F. oxysporum* at concentration 40 µg/ml (Table 6). We found 8-10 mm and 6-9 mm zone of inhibition against *C. albicans* and *F. oxysporum* respectively (40 µg/ml). Glycoconjugates (**206**) and (**207**) and glycoconjugates (**204**) and (**205**) showed 10 mm and 9 mm respectively, against C. *albicans*, whereas glycoconjugates (**206**), (**208**) and (**209**) showed 9 mm against *F. oxysporum*, these were the compounds for which maximum inhibition was observed. Glycoconjugates also exhibited a concentration dependant inhibition.

Table 7: **Summary of the results of Group 1 to Group 4: Plate assay**

Test Compounds	Max. Zone of inhibition (mm) at 40 µg/ml		Test Compounds	Max. Zone of inhibition (mm) at 40 µg/ml	
	C. A	*F. O*		*C. A*	*F. O*
118	10	-	**159**	11	-
123	-	12	**161**	10	-
132	10	9	**165**	-	11
136	10	-	**166**	-	10
144	10	-	**204**	9	-
150	10	-	**205**	9	-
154β	9	-	**206**	10	9
155α	10	-	**207**	10	-
155β	9	11	**208**	-	9
156 (αβ)	9	9	**209**	-	9

In conclusion, the primary bioassay showed that, most of the newly synthesized compounds were found to have *in vivo* antifungal activity. Most active amongst the screened compounds are listed in table 7. But further screening was necessary to find out the activity against a particular target present in fungi and determination of detailed structure activity relationship for getting the lead molecule. In order to determine activity against specific targets, all the newly synthesized molecules were subjected to haploinsufficiency assay (*in vivo*) and Chitin synthase inhibitory assay (*in vitro*).

2. Haploinsufficiency assay (*in vivo*):

Haploinsufficiency (*in vivo*) assay was carried out for test compounds **Group 1** to **Group 4** (table 1, page No. 234) using concentration 100 µg/ml and tables 8-11 depict the observed % inhibitions against *S. cerevisiae*, wild type and selected mutant strains. Fluconazole and Nikkomycin Z were used as standard drugs.

Effect of Fluconazole and Nikkomycin Z on growth of *S. cerevisiae* wild type and mutant strains:

Fluconazole: (16 µg/ml) 70% inhibition was observed.

Nikkomycin Z: (100 µg/ml) 73% inhibition was observed.

A. Effect of Group 1 test compounds (table 1, page No. 234) on growth of *S. cerevisiae* wild type and mutant strains (conc. 100 µg/ml) (% inhibition):

Although most of the compounds have shown inhibitory activity in case of primary assay i.e. plate assay, when target specific assay was performed, following results were observed (Table 8). Triazole derived from β-D-arabinose (**127)** showed inhibition against targets YPD 1 (63%, strain 1487), RSC 9 (64%, strain 1488) and ILV 5 (57%, strain 1834) at concentration 100 µg/ml against wild strain (16%,

strain1536). Compound (**150),** a 1,2,3,4-tetrazole derived from α-D-xylofuranose is specifically active against target ILV 5 (60%, strain 1834) at concentration 100 µg/ml against wild strain (12%, strain1536).

Table 8: Summary of the results of Group 1: Haploinsufficiency

Yeast	**Test Compounds**								
	118	**123**	**127**	**132**	**136**	**140**	**144**	**148**	**150**
1536 (Wild)	56	11	16	ND	57	05	04	ND	12
1500	10	ND	07	32	9.9	04	ND	ND	22
1485	62	10	30	04	21	11	ND	16	17
1487	66	40	**63**	ND	ND	ND	ND	ND	02
1488	72	24	**64**	04	33	06	03	02	14
1492	ND	03	25	ND	04	ND	ND	ND	08
1519	10	09	13	17	13	10	21	06	18
1831	69	14	25	13	11	ND	ND	ND	10
1834	68	29	**57**	07	ND	ND	ND	ND	**60**
1845	11	03	12	ND	02	ND	ND	ND	03

B. Effect of Group 2 test compounds (table 1, page No. 234) on growth of *S. cerevisiae* wild type and mutant strains (conc. 100 µg/ml) (% inhibition):

Group 2 test compounds are the glycoside derivatives of tetracyclic compounds (**118**) and (**150**). Glycoside derivatives (**152β**) and (**156**) were found to be effective (Table 9). Compound (**152β**) a β-allyl glycoside derived from D-xylofuranose derived 1,2,3-traizole fused tetracyclic compound (**118**) has shown inhibitory activity against YPD 1 (66%, strain 1487) at 100 µg/ml against wild strain (10%, strain 1536). Compound (**156αβ**), homopropargyl glycoside derived from 1,2,3,4-tetrazole fused tetracyclic compound (**150**), was active specifically against

RSC 9 (75%, strain 1488) at 100 µg/ml concentration against wild strain (10%, strain1536).

Table 9: Summary of the results of Group 2: Haploinsufficiency

Yeast	**Test Compounds**										
	151 α	**151 β**	**152 α**	**152 β**	**153 α**	**153 β**	**154 α**	**154 β**	**155 α**	**155 β**	**156 (αβ)**
1536 (Wild)	05	04	50	10	04	06	06	02	ND	55	10
1500	ND	ND	03	ND	ND	48	ND	ND	ND	09	07
1485	12.3	08	53	27	05	12	ND	46	06	15	15
1487	ND	ND	60	**66**	ND	ND	19	ND	ND	25	08
1488	06	06	70	45	08	18	12	08	06	75	**75**
1492	ND	ND	06	06	06	ND	02	03	ND	ND	03
1519	ND	ND	10	13	06	10	ND	ND	ND	ND	ND
1831	08	ND	09	02	02	03	02	06	04	62	12
1834	ND	ND	ND	ND	ND	ND	ND	ND	ND	02	ND
1845	ND	ND	63	45	27	ND	ND	ND	ND	4.1	ND

C. Effect of Group 3 test compounds (table 1, page No. 234) on growth of *S. cerevisiae* wild type and mutant strains (conc. 100 µg/ml) (% inhibition):

In case of **group 3** test compounds, nucleoside derivative (**161),** a N-Bz adenine nucleoside derived from 1,2,3-traizole fused tetracyclic compound (**118**) was found specifically active against target YPD 1 (65%, strain 1487) against wild strain (16%, strain1536) (Table 10), while nucleoside (**166**), a 6-chloroguanine nucleoside derived from 1,2,3,4-tetrazole fused tetracyclic compound (**150**) showed inhibition against target RSC 9 (60%, strain 1488) at concentration 100 µg /ml against wild strain (04%, strain1536).

Table 10: Summary of the results of Group 3: Haploinsufficiency

Yeast	Test Compounds					
	159	**161**	**162**	**163**	**165**	**166**
1536 (Wild)	47	16	05	33	07	04
1500	05	ND	03	ND	05	05
1485	51	16	16	08	14	12
1487	65	**65**	10	ND	ND	ND
1488	65	38	11	08	18	**60**
1492	04	07	ND	14	06	06
1519	18	13	06	ND	09	ND
1831	50	14	14	03	02	09
1834	51	23	ND	19	ND	ND
1845	ND	ND	ND	ND	14	ND

D. Effect of Group 4 test compounds (table 1, page No. 234) on growth of *S. cerevisiae* wild type and mutant strains (conc. 100 µg/ml) (% inhibition):

Table 11: Summary of the results of Group 4: Haploinsufficiency

Yeast	Test Compounds						
	203	**204**	**205**	**206**	**207**	**208**	**209**
1536 (Wild)	ND	35	07	ND	ND	ND	47
1500	02	ND	08	ND	ND	ND	03
1485	12	12	17	11	ND	ND	17
1487	03	07	05	15	ND	ND	20
1488	13	10	27	08	1.0	**85**	70
1492	02	ND	10	04	ND	ND	05
1519	ND	ND	ND	ND	ND	ND	ND
1831	13	ND	14	02	ND	ND	28
1834	21	ND	02	02	ND	ND	ND
1845	ND	ND	06	ND	ND	ND	09

While in **group 4** test compounds, which are conjugates of uridine and azidosugars containing 1,2,3-triazole heterocyclic nucleus (**203-209**), glycoconjugate (**208**) was the only compound that found effective against RSC 9 (strain 1488) with 85% inhibition, at 100 μg/ml against wild strain (ND, strain1536) (Table 11).

Table 12: Summary of the results of Group 1 to Group 4: Haploinsufficiency

Test Compounds	Targets	% inhibition (100 μg/ml)
127	YPD 1	63
	RSC 9	64
	ILV 5	57
150	ILV 5	60
152β	YPD 1	66
156	RSC 9	75
161	YPD 1	65
166	RSC 9	60
208	RSC 9	85

In conclusion, the results from haploinsufficiency assay indicated that some of the test compounds (table 12) were found to be effective against different targets. Surprisingly, none of the test compounds (**Group 1** to **Group 4**) shown inhibitory activity against chitin synthase (CHS 2, strain 1492) in vivo (Nikkomycin Z, 73% inhibition, 100 μg/ml). Another observation was that the test compounds (**Group 1** to **Group 4**) also did not show any effect on lanosterol 14α-demethylase (ERG 11, 1500) for ergosterol synthesis (Fluconazole, 70% inhibition, 16 μg/ml).The inefficacy of these test compounds may be attributed to their poor cell wall penetration or due to the inadequate effective concentration into the fungal cell.

3. Chitin Synthase Non radioactive assay (*in vitro*):

All the newly synthesized compounds (**Group1- Group 4**) (Table 1, page No. 234) were also screened for *in vitro* inhibitory activity against Chitin synthase. The assay was carried out using concentration 50 µg/ml and table's 13-16 shows the observed % inhibitions of Chitin synthase activity. Nikkomycin Z was used as standard inhibitor.

Effect of Nikkomycin Z on Chitin synthase activity (% inhibition):

Concentration: 4 µg/ml, more than 90 % inhibition was observed.

A. Effect of Group 1 test compounds (table 1, page No. 234) on Chitin synthase activity (conc. 50 µg/ml) (% inhibition):

Table 13: Summary of the results of Group 1: Chitin synthase assay

Test compounds	% Inhibition	Test compounds	% Inhibition
118	62	**140**	50
123	47	**144**	18
127	53	**148**	34
132	62	**150**	52
136	73		

Group 1 test compounds, when screened for *in vitro* chitin synthase inhibitory activity using non radioactive assay exhibited promising results (Table 13). Tetracyclic compounds showed inhibition of Chitin synthase activity in the range of about 18 – 73%. Compound (**136**), a tetracyclic 1,2,3-triazole derived from α-D-glucofuranose showed the maximum inhibition (73%) at 50 µg/ml. Tetracyclic 1,2,3-triazoles (**118**) and (**132**) have shown 62% of inhibition, while 50-53% of inhibition was observed for tetracyclic 1,2,3-triazoles (**127**), (**140**) and tetracyclic 1,2,3,4-tetrazole (**150**) at concentration 50 µg/ml.

B. Effect of Group 2 test compounds (table 1, page No. 234) on Chitin synthase activity (conc. 50 µg/ml) (% inhibition):

Table 14: Summary of the results of Group 2: Chitin synthase assay

Test compounds	% Inhibition	Test compounds	% Inhibition
151α	47	**154α**	59
151β	39	**154β**	49
152α	20	**155α**	47
152β	61	**155β**	59
153α	48	**156 (αβ)**	54
153β	57		

Group 2 compounds also found effective against Chitin synthase activity *in vitro* as 20-61% of inhibition was observed (Table 14). The glycoside (**152β**) was one showed 61% as maximum inhibition, while glycosides (**153β**), (**154α**), (**155β**) and (**156**) were the other compounds having more than 50% of inhibition at concentration of 50 µg/ml.

C. Effect of Group 3 test compounds (table 1, page No. 234) on Chitin synthase activity (conc. 50 µg/ml) (% inhibition):

Table 15: Summary of the results of Group 31: Chitin synthase assay

Test compounds	% Inhibition
159	25
161	62
162	15
163	71
165	56
166	40

Group 3 compounds were also found effective against Chitin synthase activity *in vitro* and the observed inhibition was in the range of 25-71%. Nucleoside (**163**) showed maximum inhibition of 71% at concentration 50 µg/ml. Nucleoside (**161**) and (**165**) showed 62% and 56% inhibition respectively, at 50 µg/ml.

D. Effect of Group 4 test compounds (table 1, page No. 234) on Chitin synthase activity (conc. 50 µg/ml) (% inhibition):

Similarly as observed in group 1, 2 and 3, **group 4** test compounds also exhibited 47-90% inhibition *in vitro* (Table 16). Glycoconjugates (**204)** and (**205)** have shown 90% inhibition at 50 µg/ml. Whereas glycoconjugates (**203**), (**208**) and (**209**) were the other compounds which have shown more than 70% inhibition, while (**208**) showed 56% *in vitro* inhibition against chitin synthase activity at 50 µg/ml.

Table 16: Summary of the results of Group 4: Chitin synthase assay

Test compounds	% Inhibition
203	73
204	90
205	90
206	47
207	56
208	73
209	71

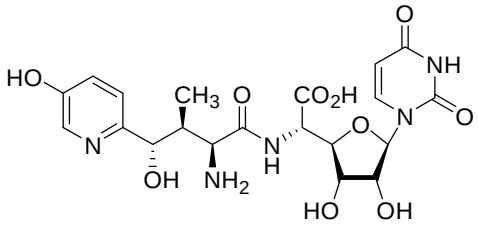
In conclusion, **Group 1** – **Group 4** compounds showed appreciable inhibition of Chitin synthase *in vitro*. Highest activity was seen in glycoconjugates (**204**) and (**205**) i.e. 90% inhibition of Chitin synthase activity at 50 µg/ml concentration against nikkomycin Z (4 µg/ml) where 90 % inhibition was observed. The inhibition exhibited by other compounds against this target *in vitro* cannot be neglected.

Table 17: Summary of the results of Group 1 to Group 4: Chitin synthase assay

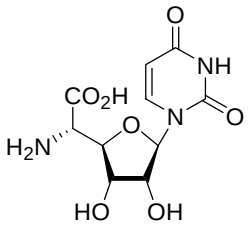
% Chs inhibition	Test Compounds
50 – 70%	118, 127, 132, 140, 150, 152β, 153β, 154α, 155β, 156, 161, 165 and 207
70 – 90%	136, 163, 203, 204, 205, 208 and 209

Discussions:

We had selected polyoxins and nikkomycins as the models for antifungal development. Polyoxins and nikkomycins are the naturally occurring peptidyl nucleosides and are the competitive inhibitors of Chitin synthase, an enzyme essential for chitin biosynthesis in the cell wall of fungi.



Nikkomycin Z (**1**)



Uracil polyoxin C (**2**)

These antibiotics are selectively toxic to pathogenic fungi, while being non toxic to bacteria, plant and animals and this property was driven our attention to select peptidyl nucleosides as model antifungals. Polyoxins and nikkomycins exhibit similar activity against chitin synthases from a variety of fungi, however are more potent against filamentous fungi than *C. albicans*. This behaviour may be due to the poor penetration of these antibiotics into the fungal cell due to the difference of cell membrane permeability. Nikkomycin Z (**1**), most potent of this class, exhibited activity against *C. albicans,* with MIC of 14 μg/ml.[5]

Although, peptidyl nucleosides are potent inhibitors of Chitin synthase, they suffer from limitations such as, they are prone to hydrolytic cleavage by dipeptidases and poor cell wall penetration due to lack of hydrophobicity. Thus peptidyl nucleosides are ineffective *in vivo*. Several modifications were carried out to increase the hydrolytic stability and cell wall penetration, remained unsuccessful. The synthetic modifications of peptidyl nucleosides in detail are discussed in Chapter 1.

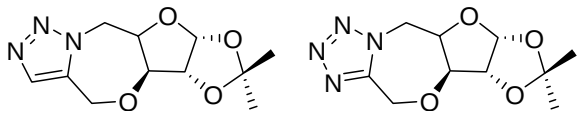
We have reported the synthesis of hitherto unknown molecules with anticipated antifungal activity in chapter 2 and chapter 3.

The new molecules are divided into four groups according to their structural components (Table 1, page No. 234). These compounds are derived from carbohydrate templates such as pentofuranoses and hexofuranoses, and nucleoside such as uridine. All the compounds have a 1,2,3-triazole or 1,2,3,4-tetrazole heterocycle either as fused ring such as in polycyclic compounds with or without nucleobase or as a metabolically stable linkage in nucleoside-sugar conjugates.

In our attempt to develop novel antifungal compounds possessing increased hydrolytic stability and improved cell wall penetration, so that, the compounds will exhibit antifungal activity. We have attempted to generate a library of novel molecules containing furanose nucleus as fused ring system and glycoconjugates derived from nucleoside uridine or thymidine.

The vital feature we have incorporated in newly synthesized molecules is either 1,2,3-triazole or 1,2,3,4-tetrazole heterocycle and a sugar moeity. These heterocycles are known for their marked biological activity including antimicrobial activity. The detailed account of properties and compounds containing these heterocycles is discussed in chapter 2 and 3.

Group 1 Compounds:



Group 1: Representative examples (**118**) and (**150**)

Group 1 compounds are the tetracyclic compounds containing furanose ring with diverse stereochemistry and was fused with either 1,2,3-triazole (e.g. **118**) or 1,2,3,4-tetrazole (e.g. **150**) instead of a side chain and does not contain nucleobase. In general, the preliminary screening for antifungal activity i.e. by plate assay against *C. albicans* (8-10 mm) and *F. oxisporum* (7-12 mm), although all the compounds exhibited growth inhibition at concentration 40 μg/ml, these compounds are less effective than amphotericin B (*C. albicans,* 6-7 mm and *F. oxisporum*, 4-5 mm at 15 μg/ml). Amongst these compounds the following trends are seen.

In case of *C. albicans*, following are the observations;

1. Xylofuranose configuration (**118, 132, 136** and **150**) is preferred over ribofuranose.
2. Introduction of tetrazole (**150**) in place of triazole, replacement of 7 membered ring (**136**) by 8 membered ring (**132**) or arabinose sugar (**127**) does not have any marked effect on the activity.

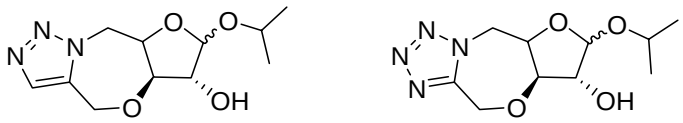
While in the case of *F. oxisporum*, the picture is drastically changed. Here tetrazole did not show any activity, whereas only one compound (**123**), α-D-ribofuranose derived tetracyclic triazole showed an average activity.

While evaluating the results of haploinsufficiency assay, it must be born in mind that we are looking for activity against a specific mutant, wherein it should be less sensitive to the wild strain. Here general activity figure are less significant. Some of the compounds found active against different targets, such as triazole (**127**) against YPD 1 (63%), RSC 9 (64%) and ILV 5 (57%), against wild 16% (strain 1536) and

tetrazole (**150**) against only ILV 5 (60%), against wild 12% (strain 1536). Thus the compounds (**127**) and (**150**) may further be studied at low concentrations to optimize the results. Surprisingly these results portray the lack of *in vivo* activity against Chs target as well as activity against 14α-demethylase (ERG 11) for ergosterol synthesis.

However, when these compounds were screened for *in vitro* activity against Chs using non-radioactive assay, showed some interesting results. The compounds showed 18 – 73% inhibition at 50 μg/ml, in comparison to reference inhibitor nikkomycin Z (4 μg/ml, >90%). Triazole (**136**), a α-D-glucofuranose derivative showed maximum of 73% inhibition, followed by triazoles (**118**) and (**132**) respectively derived from α-D-xylofuranose and α-D-glucofuranose which showed 62% inhibition. However no specific structural features can be associated with this activity. These results indicate that the compounds have poor cell wall penetration in haploinsufficiency assay due to insufficient hydrophobicity, although the compounds have affinity to the Chs enzyme target *in vitro* as indicated.

Group 2 Compounds:



Group 2: Representative examples (**151 α**, **151 β**) and (**154 α**, **154 β**)

In **group 2**, the compounds are the tricyclic **α** and **β** glycosides derived from opening of the 1,2-*O*-isopropylidene ring in tetracyclic 1,2,3-triazole (**118**) e.g. compounds (**151 α**, **151 β**) and 1,2,3,4-tetrazole (**150**) e.g. compounds (**154 α**, **154 β**). The compounds were designed to study the effect of *O*-alkyl chains and their configuration at anomeric carbon of furanose ring of tetracyclic compounds, instead of a nucleobase as in peptidyl nucleosides.

Amongst these compounds, glycosides **154β, 155α, 155β** and **156(αβ)** derived from tetracyclic 1,2,3,4-tetrazole compound (**150**) were found to be better inhibitors than those derived from tetracyclic 1,2,3-triazole compound (**118**) in case of plate assay. However, the incorporation of *O*-alkyl group with either **α**-configuration or **β**-configuration at anomeric carbon of tetracyclic 1,2,3-triazole (**118**) and 1,2,3,4-tetrazole (**150**) did not result in any increment in activity against vegetative growth of *C. albicans* and *F. oxisporum* than their parent compounds and amphotericin B (*C. albicans,* 6-7mm and *F. oxisporum*, 4-5 mm at 15 μg/ml).

Further these compounds were subjected to *in vivo* assay (haploinsufficiency) in *S. cerevisiae* mutant strians. The glycosidic modification of the tetracyclic 1,2,3-triazole (**118**) and 1,2,3,4-tetrazole (**150**) did not result in any *in vivo* inhibitory activity against Chs target (strain, CHS 2, 1492) and also against lanosterol 14α-demethylase (ERG 11) for ergosterol synthesis. The lack of *in vivo* activity shows that the glycosidic modification did not improve the activity in this case also.

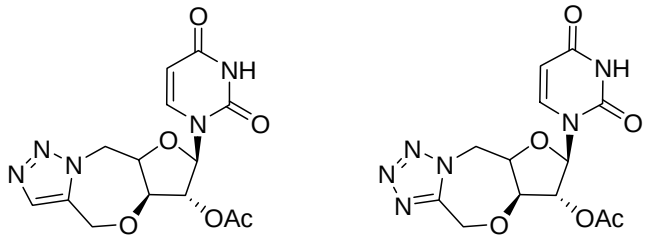
However, activity against different targets was observed e.g. glycoside (**152β**), β-allyl glycoside derivative of tetracyclic 1,2,3-triazole (**118**) showed activity against YPD 1 (66%) at 100 μg/ml against wild type (10%, strain 1536). Glycoside (**156**), homopropargyl derivative of tetracyclic 1,2,3,4-tetrazole (**150**) showed inhibition against RSC 9 (75%, 100 μg/ml) against wild type (10%, strain 1536), while the parent compound was ineffective.

While in case of *in vitro* Chs inhibitory activity of glycosides, we found 47-61% inhibition at 50 μg/ml in reference to nikkomycin Z (4 μg/ml, >90%), glycoside (**152β**) was the most effective (61%, 50 μg/ml) which was same as its parent compound triazoles (**118**) (62%, μg/ml). These results show that the glycosidic

modification of tetracyclic compounds retained the *in vitro* activity against Chs but did not enhance it.

Group 3 Compounds:

In another effort to modify the tetracyclic 1,2,3-triazole (**118**) and 1,2,3,4-tetrazole (**150**), these compounds were synthesized. **Group 3** compounds are the β-nucleoside derivatives, synthesized from diacetate of tetracyclic 1,2,3-triazole and 1,2,3,4-tetrazole by glycosidation of different nucleobases. These compounds contain uracil as a nucleobase, so as to mimic the structure of nikkomycin Z for ex. (**159**) and (**163**). In addition to uracil, other bases such as thymine, *N*-Bz adenine and 6-chloroguanine were also used, so as to study the effect of change in base on the biological activity. Compounds (**159-166**) were the nucleosides screened for antifungal activity.



Group 3: Representative examples (**159**) and (**163**) **β**-nucleosides

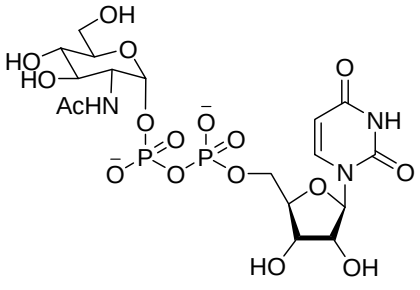
Compounds (**159**) and (**161**), the uracil and *N*-Bz adenine nucleosides of tetracyclic 1,2,3-triazole (**118**) showed (11 and 10 mm, 40 µg/ml) maximum growth inhibition against *C. albicans*, whereas compounds (**165**) and (**166**), the *N*-Bz adenine and 6-chloroguanine nucleosides of tetracyclic 1,2,3,4-tetrazole (**150**) showed (11 and 10 mm, 40 µg/ml) against *F. oxisporum* (amphotericin B, *C. albicans,* 6-7mm and *F. oxisporum*, 4-5 mm at 15 µg/ml). All other nucleoside derivatives showed less activity. There was no increment in inhibition compared to the inhibition shown by their parent compounds, even after incorporation of nucleobase.

Nucleoside derivatives also failed to show any *in vivo* activity (haploinsufficiency) against Chs target (strain, CHS 2, 1492) and lanosterol 14α-demethylase (ERG 11) in *S. cerevisiae* mutant strains. But nucleoside (**161**) and (**166**) showed activity against YPD 1(65%, 100 μg/ml) (16%, wild strain) and RSC 9 (60%, 100 μg/ml) (4%, wild strain). Thus introduction of base in tetracyclic compounds did not increase in their *in vivo* activity.

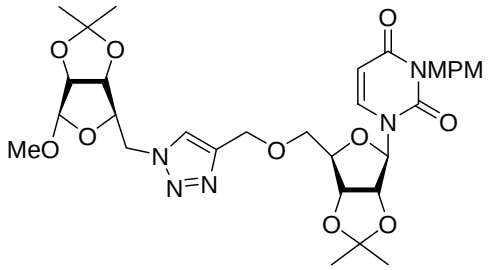
In case of *in vitro* Chs inhibitory activity nucleoside (**163**) was the most effective, which showed 71% inhibition at (50 μg/ml) (nikkomycin Z 4 μg/ml, >90%). These compounds also retained *in vitro* inhibitory activity against Chs. One can assume that these nucleosides also failed to cross the fungal cell wall, so as to achieve the sufficient concentration into the cell to affect the inhibition.

Group 4 Compounds:

We have also designed and synthesized **group 4** molecules in view of obtaining analogs of UDP-GlcNAc (**13**). These are the conjugates of nucleosides and sugars (**205**) linked by metabolically stable 1,2,3-triazole nucleus in the form of 1,4-disubstituted adducts to mimic the structure of UDP-GlcNAc (**13**), a natural substrate for Chitin synthase enzyme in the biosynthesis of fungal cell wall component, chitin.



UDP-GlcNAc (**13**)



Glycoconjugates (**205**)

Glycoconjugates (**group 4**) were also screened for *in vivo* and *in vitro* antifungal activity. The preliminary plate assay (**203-209**) showed the inhibition of

vegetative growth of both *C. albicans* (8-10 mm) and *F. oxisporum* (6-9 mm) at 40 μg/ml (amphotericin B, *C. albicans,* 6-7mm and *F. oxisporum*, 4-5 mm at 15 μg/ml). Glycoconjugates (**206**) and (**207**) showed a maximum growth inhibition (10 mm) against C. *albicans* at 40 μg/ml, whereas glycoconjugates (**206**), (**208**) and (**209**) showed maximum growth inhibition (9 mm) against *F. oxisporum*. The activities of glycoconjugates were also less than amphotericin B.

When haploinsufficiency assay against wild and mutant strains of *S. cerevisiae* was performed, the glycoconjugates (100 μg/ml) failed to exhibit activity against strain for Chitin synthase activity and lanosterol 14α-demethylase (ERG 11) for ergosterol synthesis *in vivo*. But compound (**208**) showed activity against RSC 9 (85%) against wild (ND). However, compounds (**204**) and (**205**) have shown a maximum *in vitro* inhibitory activity against Chitin synthase with 90% inhibition at 50 μg/ml in comparison to nikkomycin Z (4 μg/ml, >90%).

The observations from all these groups suggest that further modification of these compounds is necessary to enhance their bioactivity, solubility and hydrophobicity so that they exhibit better activity as compared to present antifungals. Another important observation from haploinsufficiency assay was that, none of the compounds from **group 1** to **group 4** showed inhibitory activity against *S. cerevisiae* mutant strain CHS 2 (strain 1492) and ERG 11 (strain 1500). Whereas the Chs assay showed good inhibition *in vitro*, this could be ascribed to poor cell wall penetration.

In short, further structural modifications are essential for desired antifungal activity at lower concentration and the present results have provided some guidelines for incorporation of chemical units in the future molecules.

4.4. Conclusion

In conclusion, test compounds **Group 1- Group 4** (Table 1, page No. 234) were screened for antifungal activity and following methods were employed; 1) Whole cell based plate assay, 2) Haploinsufficiency assay and 3) Chitin synthase non radioactive assay. The overall results are summarized in the following table (18).

Table 18

Test Compounds	**Plate assay**	**Haploinsufficiency (*in vivo*)**	**Chitin synthase assay (*in vitro*)**
Group 1 **Group 2** **Group 3** **Group 4**	All 33 compounds 7-11 mm (*C. albicans*) 6-12 mm (*F.oxysporum*)	127, 150, 152β, 156, 161, 166 and 208. YPD 1, RSC 9, ILV 5 No CHS 2 (1492) No ERG 11 (1500)	>50% inhibition, 118, 127, 132, 136, 140, 150, 152β, 153β, 154α, 155β, 156αβ, 161, 163, 165, 203, 204, 205, 207, 208 and 209.

Plate assay of the test compounds shows that the newly synthesized compounds possess *in vivo* activity against *C. albicans* as well as *F. oxysporum*, where as polyoxins and nikkomycins are not much effective against *C. albicans*. When we analyze the haploinsufficiency assay (*in vivo*) against *S. cerevisiae* wild and mutant strains, it was found that the compounds lack *in vivo* activity against chitin synthase as well as lanosterol 14α-demethylase of *S. cerevisiae*. The lack of *in vivo* activity against *S. cerevisiae* can be attributed to the poor cell wall penetration, although the compounds were having *in vivo* activity against *C. albicans* and *F. oxysporum*. This difference in *in vivo* activity may be due to the difference in permeability of cell membranes.

However, most of the compounds (Table 18) posses potent *in vitro* activities against chitin synthase that was witnessed by non-radioactive chitin synthase assay.

Table 19 gives the test compounds which are potential chitin synthase inhibitors which affect Chs *in* vitro and activity against *C. albicans* and *F. oxysporum in vivo* (plate assay).

Table 19

Test Compounds	**Plate assay (9-10 mm)**	**Chitin synthase assay (*in vitro*)**
Group 1	118, 132, 136 and 150	118, 132, 136 and 150
Group 2	155β	155β
Group 3	161 and 163	161 and 163
Group 4	204, 205 and 207	204, 205 and 207

We are successful in designing analogues of polyoxins and nikkomycins such as compounds **118, 132, 136, 150, 155β, 161, 163, 204, 205** and **207,** which are potential chitin synthase inhibitors. However, further studies like toxicity studies are required. Finally, any modifications leading to improvement in the cell penetration of these newly synthesized scaffolds would definitely result in promising lead structure with an *in vivo* activity for antifungal development. Conjugation of these compounds with lipophylic chain (fatty chains) or with transport proteins, as the structure of newly synthesized molecules provides facile sites for this kind of modifications by which the cell wall penetration can be increased and may result in molecules with improved antifungal activity.

Chapter 4: References

4.5. References

1. (a) Marr, K. A.; Rustad, T. R.; Rex, J. H.; White, T. C. Antimicrob. Agents Chemother., **1999,** *43,* 1303. (b) Rex, J. H.; Pfaller, M. A.; Walsh, T. J.; Chaturvedi, V.; Espinel-Ingroff, A.; Ghannoum, M. A.; Gosey, L. L.; Odds,F. C.; Rinaldi, M. G.; Sheehan, D. J.; Warnock, D. W. *Clin. Microbiol. Rev.,* **2001,** *14,* 643. (c) DeBacker, M. D.; Nellissen, B.; Logghe, M.; Viaene, J.; Loonen, I.; Vandoninck, S.; de Hoogt, R.; Dewaele, S.; Simons, F. A.; Verhasselt, P.; Vanhoof, G.; Contreras, R.; Luyten, W. H. *Nat. Biotechnol.,* **2001,** *19,* 235.
2. (a) Keller, F. A.; Cabib, E. *J. Biol. Chem.* **1971,** *246,* 160. (b) Choi, W. J.; Cabib, E. *Anal. Biochem.,* **1994,** *219,* 368.
3. Lucero, H. A.; Kuranda, M. J.; Bulik, D. A. *Anal. Biochem.,* **2002,** *305,* 97.
4. Deshpande, M. V.; O' Donnel, R.; Goody, G. W. *FEMS Microbiol. Lett.,* **1997,** *152,* 327.
5. Zang, D.; Miller, M. J. *Currrent Pharmaceutical design,* **1999,** *5,* 73.

List of Research Publications

List of Research Publications

1. Expedient synthesis of 1,2,3-triazole-fused tetracyclic compounds by intramolecular Huisgen ('click') reactions on carbohydrate-derived azido-alkynes

Srinivas Hotha, **Ramakrishna I. Anegundi** and Arvind A. Natu.

Tetrahedron Lett., **2005,** *46,* 4585-4588.

2. Diversity oriented synthesis of 1,2,3-triazole and 1,2,3,4-tetrazole-fused glycosides and nucleosides by intramolecular 1,3-dipolar cycloaddition reaction.

Ramakrishna I. Anegundi, Arvind A. Natu, Vedavati G. Puranik and Srinivas Hotha.

(Communicated to *J. Combi. Chem*.).

3. Diversity oriented synthesis of glycoconjugates containing 1,2,3-traizole, a metabolically stable linker.

Ramakrishna I. Anegundi, Arvind A. Natu and Srinivas Hotha.

(Manuscript under preparation).