SYNTHESIS AND ANTI-HIV ACTIVITY OF [d4U]-SPACER-[HI-236] BIFUNCTIONAL HIV-1 REVERSE TRANSCRIPTASE INHIBITORS

BY

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Declaration

I declare that "Synthesis and anti-HIV Activity of [d4U]-spacer-[HI-236] Bifunctional HIV-1 Reverse Transcriptase Inhibitors" is my own work and that all sources that I have used or quoted have been indicated and acknowledged by means of complete references.

Signed by candidate

Clare Imbosa Muhanji

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Contents

Abbreviations

ABC	(1*S*,4*R*)-4-[2-Amino-6-(cyclopropyl-amino)-9*H*-purin-9-yl]-2-cyclopentene-1-methanol succinate
AcBr	Acetyl Bromide
AcOH	Acetic Acid
AIDS	Acquired Immunodeficiency Syndrome
Amino Acids	A, Alanine; C, Cystein; D, Aspartate; E, Glutamate; F, Phenylalanine; G, Glycine; H, Histidine; I, Isoleucine; K, Lysine; L, Leucine; M, Methionine; N, Asparagine; P, Proline; Q, Glutamine; R, Arginine; S, Serine; T, Threonine; V, Valine; W, Tryptophan; Y, Tyrosine
α-APA	α-(2,6-Dichlorophenyl)-α-(2-acetyl-5-methylanilino)acetamide
APTS	8-Aminopyrene-1,3,6-trisulfonate
AZT	3'-Azido-2',3'-dideoxythymidine
B:	Generic base
BnBr	Benzyl bromide
Boc	*tert*-Butyl carbonate
BOP	Benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluoro phosphate
br	Broad
brs	Broad singlet
brt	Broad triplet
Bu_3SnH	Tributyltin hydride
$BuNH_2$	Butylamine
t-BuOK	Potassium *tert*-butoxide
BVDU	Bromovinyldeoxyuridine
CAN	Ceric ammonium nitrate
cat.	Catalytic
cDNA	Complementary deoxynucleic acid
CF_3COOH	Trifluoroacetic acid
CH_2Cl_2	Methylene chloride
CH_3CN	Acetonitrile
CS_2	Carbondisulfide

δ	Chemical shift in ppm
d	Doublet
dd	Doublet of doublets
d_2U	2',3'-Dideoxyuridine
d_4T	2',3'-Didehydro-2',3'-dideoxythymidine
d_4U	2',3'-Didehydro-2',3'-dideoxyuridine
DABO	Dihydroalkoxybenzyloxopyrimidine
DAPY	Diarylpyrimidine
DATA	Diaryltriazine
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	1,3-Dicyclohexylcarbodiimide
DCE	Dichloroethane
dCTP	Deoxycytidine triphosphate
ddC	2',3'-Dideoxycytidine
ddI	2',3'-Dideoxyinosine
Delavirdine	1-(5-Methanesulfonamido-1*H*-indol-2-yl-carbonyl)-4-[3-(1-methylethyl-amino)pyridinyl]piperazine monomethane sulfonate
DIA	(Diisopropylamino)phosphine
DIAD	Diisopropylazodicarboxylate
DIEA	Diisopropylethylamine
DMA	*N*,*N*-Dimethylacetamide
DMAP	*N*,*N*-Dimethylaminopyridine
DME	1,2-Dimethoxyethane
DMF	Dimethylformamide
DMP	2,2-Dimethoxypropane
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
dsDNA	Double-stranded deoxyribonucleic acid
dTTP	Deoxythymidine triphosphate
EDC	Ethylene dichloride
Efavirenz	(-)-6-Chloro-4-cyclopropylethynyl-4-trifluoromethyl-1,4-dihydro-2*H*-3,1-benzoxazin-2-one
E1	Unimolecular elimination

E2	Bimolecular elimination
EDTA	Ethylenediaminetetraacetate monosodium
EI	Electron impact
Enfuvirtide	36-amino acid peptide
Et_3N	Triethylamine
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
EtOH	Ethanol
eq.	Equivalent
FAB	Fast atom bombardment
FDU	5-Fluoro-2'-deoxyuridine
(-)-FTC	(-)-β-L-3'-thia-2',3'-dideoxy-5-fluorocytidine
gp	Glycoprotein
GR	Glutathione Reductase
HAART	Highly active anti-retroviral therapy
HBY 097	(*S*)-4-Isopropoxycarbonyl-6-methoxy-3-(methylthiomethyl)-3,4-dihydroquinoxaline-2(1*H*)-thione
HCl	Hydrochloric acid
HCMV	Cytomegalovirus
HEPT	1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)thymine
HI-236	*N'*-(5-bromo-2-pyridyl)-*N*-[2-(2,5-dimethoxyphenyl)ethyl]thiourea
HIV-1	Human Immunodeficiency Virus type 1
HOBT	1-Hydroxybenzotriazole
HRMS	High-resolution mass spectrometry
Hz	Hertz
IDU	5-Iodo-2'-deoxyuridine (Idoxuridine)
IR	Infrared spectometry
ITU	Imidoylthiourea
J	Coupling constant
LAH	Lithium aluminium hydride
Lit.	Literature
LTR	Long Terminal Repeat
m	Multiplet
M^+	Molecular ion

MA	HIV-1 matrix protein
MeOH	Methanol
MHz	Mega hertz
MKC-442	6-Benzyl-1-(ethoxymethyl)-5-isopropyluracil
Mp	Melting point
mRNA	Messenger ribonucleic acid
MsCl	Methanesulfonyl chloride
m/z	Mass to charge ratio
NBS	*N*-Bromosuccinamide
NEM	Nucleotide excision mechanism
Nevirapine	11-Cyclopropyl-5,11-dihydro-4-methyl-6*H*-dipyrido(3,2-b:2',3'-f)(1,4)diazepin-6-one
NMM	*N*-Methylmorpholine
NMR	Nuclear Magnetic resonance
NNIBP	Non-nucleoside inhibitor binding pocket
NNIBS	Non-nucleoside inhibitor binding site
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
PG	Protecting group
$P(OEt)_3$	Triethyl phosphite
Pd/C	Palladium on carbon
Pet ether	Petroleum ether
PETT	Phenylethylthiazolylthiourea
PFA	Phosphonoformate
Phospho	Phosphonate
PLM (II)	Plasmepsin (II)
PMBCl	*p*-Methoxybenzyl chloride
PMPA	(*R*)-9-(2-Phosphonylmethoxypropyl)adenine
PPh_3	Triphenylphosphine
$(PPh_3)_4Pd$	Tetrakis(triphenylphosphine)palladium
PQ	Primaquine
Ribavirin	1-β-D-Ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide
RNA	Ribonucleic acid
RNase H	Ribonuclease H

RT	Reverse transcriptase enzyme
rt	Room temperature
s	Singlet
SATE	*S*-Acyl-2-thioethyl
S_N1	Unimolecular nucleophilic substitution
S_N2	Bimolecular nucleophilic substitution
ssRNA	Single-stranded ribonucleic acid
t	Triplet
TBDMSCl	*tert*-Butyldimethylsilyl chloride
TBDPS	*tert*-Butyldiphenylsilyl
3TC	(-)-β-L-3'-Thia-2',3'-dideoxycytidine
TFA	Trifluoroacetic acid
TFT	5-Trifluoromethyl-2'-deoxyuridine (Trifluorothymidine or Trifluridine)
THF	Tetrahydrofuran
THP	Tetrahydropyran
TIBO	(+)-(*S*)-4,5,6,7-Tetrahydro-8-chloro-5-methyl-6-(3-methyl-2-butenyl)imidazo[4,5,1jk][1,4]benzodiazepine-2(1*H*)-thione
TIPS	Triisopropylsilyl
TLC	Thin layer chromatography
TMSCl	Trimethylsilyl chloride
TSAO	2',5'-bis-*O*-(*tert*-Butyldimethylsilyl)-3'-spiro-5'-(4"-amino-1",2"-oxathiole-2",2"-dioxide)pyrimidine)
p-TsOH	*para*-Toluenesulfonic acid monohydrate
UV	Ultra violet
q	Quartet

Abstract

This thesis describes the design and synthesis of bifunctional drugs combining a nucleoside (d4U) and non-nucleoside (HI-236) reverse transcriptase inhibitor linked via different spacers between C-5 of the NRTI and O-1 of the NNRTI.

Three targets were successfully synthesized in a divergent manner from uridine in 13 steps for the butyne target and 19 steps for targets bearing PEG-propyne units using Sonogashira coupling as a key step. The most challenging step of the synthesis involved Boc deprotection and thiourea condensation in the final step, which suffered from anomeric cleavage with loss of the sugar moiety. As a result, the target with a three-carbon propynyl spacer could not be accessed.

Progress towards the synthesis of a bifunctional system bearing a saturated and more flexible tether is highlighted in Chapter 4. The key reactions included Sonogashira coupling of iodo nucleosides, 2',3'-dideoxygenation of the vicinol diol, phenolic alkylation and condensation of amine with thiourea reagent. The synthesis surmounted several challenges, with chemoselective distinction of unsaturation via late introduction of the d4U double bond using Corey-Winter methodology as the highlight.

Preliminary biological results indicate that the d4U-butyne-HI-236 bifunctional to have the smallest EC_{50} anti-HIV activity of any NRTI-spacer-NNRTI bifunctional inhibitor synthesized to date. The work presented in this thesis has established a synthetic platform for the production of a library of active bifunctional compounds used to probe the two drug target sites of HIV-1 reverse transcriptase synergistically.

CHAPTER 1

Introduction

1.1 Human Immunodeficiency Virus (HIV)

HIV, the causative agent of AIDS, is a single-stranded RNA that belongs to the family of Retroviridae. Its genetic information is not encoded as DNA, but as RNA and therefore is reverse-transcribed into DNA. The HIV virus comprises two distinct viruses as HIV-1 and HIV-2, which differ in origin and gene sequence. HIV-1 carries a *vpu* gene whereas HIV-2 carries the *vpx* gene. These strains of HIV share similar molecular structures in that they contain a single-stranded RNA genome and both viruses cause AIDS with a similar spectrum of symptoms. Like all viruses, HIV-1 is an intracellular parasite and cannot multiply without the host cell.[1]

1.2 Epidemiology of HIV/AIDS

About 40.3 million people are living currently (2005) with HIV/AIDS and the great majority are in the developing world. With a population of about 600 million, sub-Saharan Africa accounts for over two-thirds of the world's HIV-infected people, including 80% of the world's HIV-infected women and children. In 2005, approximately 3.1 million AIDS deaths occurred globally, at least 2.4 million of them in sub-Saharan Africa. In South Africa, AIDS has orphaned an estimated 660,000 children,[2] with the epidemic being concentrated in the eastern and southern parts of the continent.[3] The epidemic's toll continues to mount even in countries already experiencing high HIV prevalence rates. Six countries, all in Southern Africa, have prevalence rates higher than 20%: Swaziland (38.8%), Botswana (37.3%), Lesotho (28.9%), Zimbabwe (24.6%), South Africa (21.5%) and Namibia (21.3%).[2]

1.3 Structure of HIV-1

The HIV-1 structure involves outer and inner cores. The outer core consists of a lipid bilayer acquired from the host cell, while the inner core contains two proteins, p24

and p17, as matrix proteins surrounding the nucleocapsid containing the genetic material. A single virion has an icosahedral shape with a knobby-looking envelope. The knobs are comprised of the envelope glycoproteins (gp) 120 and 41.[4a] Beneath the envelope is the viral matrix (p17), which, aside from structural maintenance, enables the DNA copy of the viral genome to be transmitted to the host nucleus. Inside this is the p24-capsid. It encapsulates nine genes: *gag*, *pol*, *env*, *tat*, *rev*, *nef*, *vif*, *vpu* and *vpr*, which carry all the information needed to make new viruses (Fig. 1.1).[5]

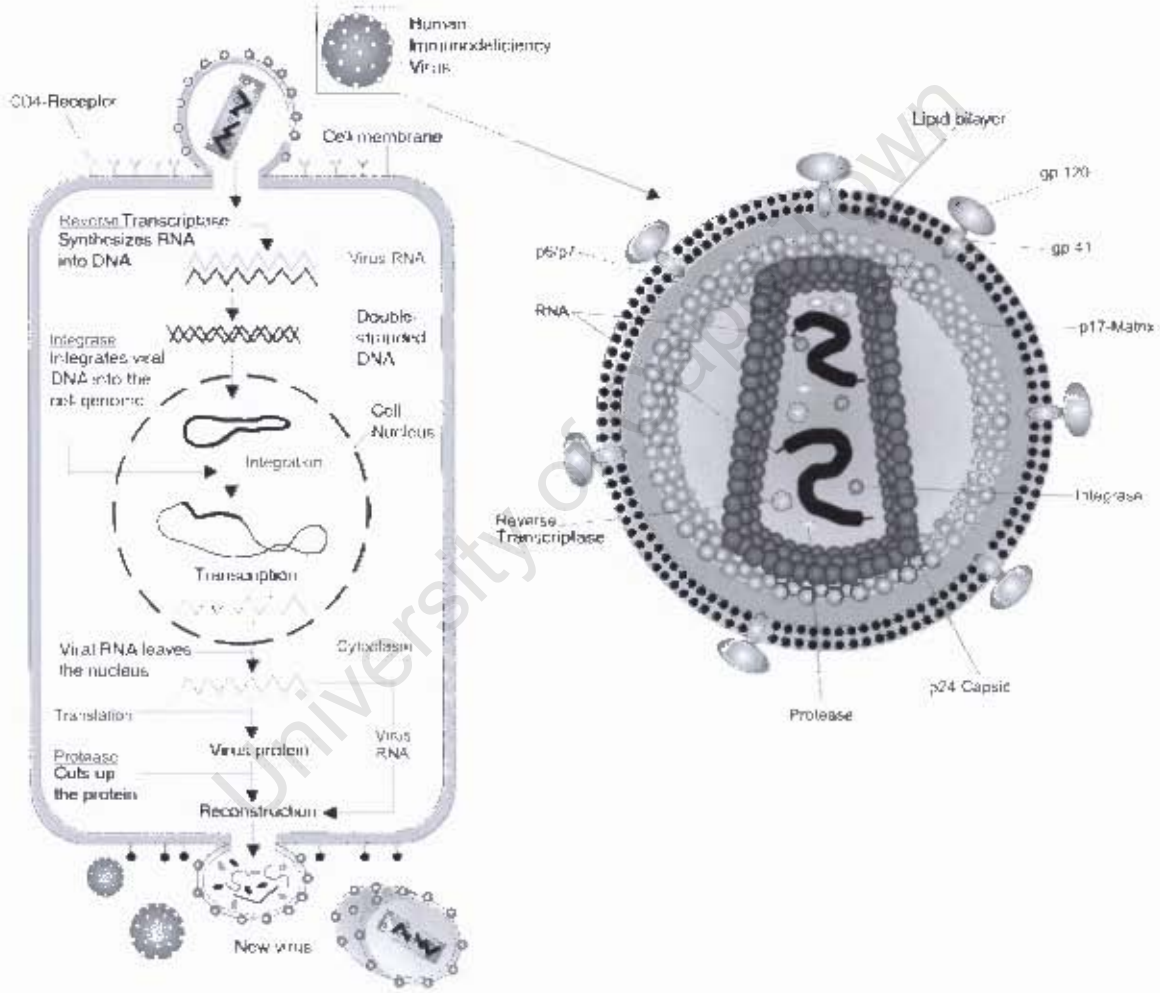


Fig 1.1: Structure and life cycle of HIV-1 virus

1.4 Life-Cycle of HIV

HIV begins its infection of a susceptible host cell by binding to the CD4 receptor on the host cell (Fig. 1.2). CD4 is present on the surface of many lymphocytes, which are a critical part of the body's immune system.[6] Two chemokine receptors: CCR5

and CXCR4 are coreceptors needed for HIV to enter the cell.[7] The HIV glycoprotein 120 interacts with the CD4 molecule on the surface of the target cell. Following CD4 binding, a central material change in the HIV gp120/gp41 complex is induced by interaction of gp120 with chemokine receptors CCR5 or CXCR4. The chemokine receptors, CCR5 and CXR4 are co-receptors for macrophage-tropic HIV-1 (R5-HIV-1) and T-cell line-tropic HIV-1 (X4-HIV-1), respectively. The change in conformation exposes gp41 allowing it to initiate fusion of the membranes.[4a] This is a complex process that has been investigated exhaustively leading to development of the fusion inhibitor Fuzeon™ (Enfuvirtide, T-20).[4b]

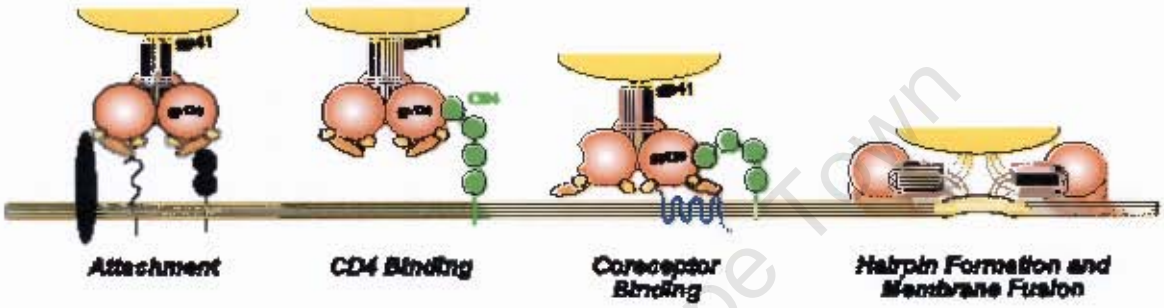


Fig. 1.2: The three steps of HIV cell entry.

Following fusion of the virus with the host cell, HIV enters the cell. Partial uncoating of the viral core occurs and the RNA-genetic material of the virus, is released into the host cell cytoplasm where it undergoes reverse transcription into DNA. An enzyme in HIV called reverse transcriptase (RT) is necessary to catalyze this conversion of viral RNA into DNA. Initially the viral enzyme (RT) copies the viral RNA genome into a single minus strand of DNA[5] via polymerisation of deoxynucleoside triphosphates into a complementary DNA (cDNA) strand using an RNA template. Like other DNA polymerases, this enzyme can only add nucleotides to the 3' end of a pre-existing primer, base-paired to the template. Once a cDNA copy of mRNA is synthesized, the mRNA is removed by ribonuclease (RNase H). The single-stranded DNA (ssDNA) is then converted by RT to double-stranded DNA (dsDNA). The entire process yields a dsDNA molecule that is longer than the template RNA and which has a long terminal repeat (LTR) at each end. Once the genetic material of HIV has been changed into DNA, this viral DNA enters the host cell nucleus (a process facilitated by the HIV proteins *vpr* and MA) where it can be integrated into the genetic material of the cell. The enzyme integrase catalyzes this process.[6]

Activation of the host cells using RNA polymerase II results in the transcription of viral DNA into messenger RNA (mRNA), which is then translated into viral proteins. The proteins pass through the Golgi apparatus where they are glycosylated into gp120 and gp41 HIV envelope proteins. The *gag* and *gag-pol* polyproteins associate with the inner surface of the plasma membrane and interact with gp41 present in the plasma membrane. As p55 and p160 polyproteins accumulate on the inner surface of the plasma membrane, they aggregate and commence assembly to form the virion. As assembly continues, the structure extrudes from the cell. The virus buds from the cell and acquires a lipid coat, carrying the gp120 and gp41 proteins. During (or soon after) the budding of the new HIV particle from the host cell membrane, the viral proteinase in p160 becomes active, resulting in the cleavage of p160 and p56 into the various subunits and generating the mature form of HIV.[5]

1.5 HIV Pathogenesis

AIDS results from selective depletion of CD4-positive helper T-lymphocytes.[8] The function of CD4 cells is to help CD8-positive cytotoxic T-lymphocytes (CTL or killer cells) to destroy other cells expressing foreign antigens and also to enhance antibody production by B-lymphocytes. Thus, CD4 cells represent a key component of the immune system. In the healthy individual, about 1200 CD4 cells circulate per μL blood; when CD4 counts drop below 400/μL, opportunistic infections start to occur.[1] HIV eventually kills the helper T-cells that are vital for the immune system, and the decline in CD4+ T-cells eventually has a great effect on humoral response functions, specifically the functioning of the B-cells. The helper T-cells activate both the cytotoxic T-cells and the B-lymphocytes. Helper T-cell depletion inhibits the B-cells from differentiating into plasma cells and memory cells, thus imparing the immune system's ability to fight against foreign antigens that have entered the body.[9] A person with a CD4 level below 200/μL is considered to be in the AIDS phase of the disease.

1.6 The Reverse Transcriptase Enzyme (RT)

HIV-1 RT is a dimer of two related chains; a 66-kD subunit (p66) and a 51-kD subunit (p51), which is derived from p66 by proteolytic cleavage. The p66 subunit contains

the polymerase and RNase H active sites as well as the NNRTI binding pocket (Fig. 1.3). The p51 polypeptide corresponds to the polymerase subdomain of p66, and comprises the first 440 amino acids of p66.[11] The subdomains within the polymerase domain of each subunit have been named fingers, palm, thumb and connection. The additional 120 residues at the carboxyl terminus of p66 comprise the RNase H domain. The arrangement of the subdomains in the two subunits is dramatically different. In p66, the subdomains are arranged to form a cleft in which the template-primer binding cleft and active site residues are buried. There is, therefore, only one functional polymerase active site per p66/p51 heterodimer.[12]

The RT enzyme is an essential viral enzyme, which is responsible for converting the genomic single-stranded RNA of HIV into double-stranded DNA (dsDNA), which subsequently becomes integrated into the host genome. HIV-1 RT is a multifunctional enzyme that exhibits two distinct enzymatic activities: (i) a DNA polymerase activity that can use either RNA or DNA as a template and (ii) an endonucleolytic ribonuclease H (RNase H) activity that specifically degrades the RNA strand of a RNA-DNA hybrid.[13] Reverse transcription is carried out using the following catalytic activities:

(i) RNA-dependent DNA polymerisation to form an RNA-DNA hybrid
(ii) RNase H degradation of the RNA strand from the RNA-DNA hybrid
(iii) DNA-dependent DNA polymerisation to form a dsDNA.

As a result of its crucial role in the life cycle of HIV, it has been considered to be a good drug target.[14]

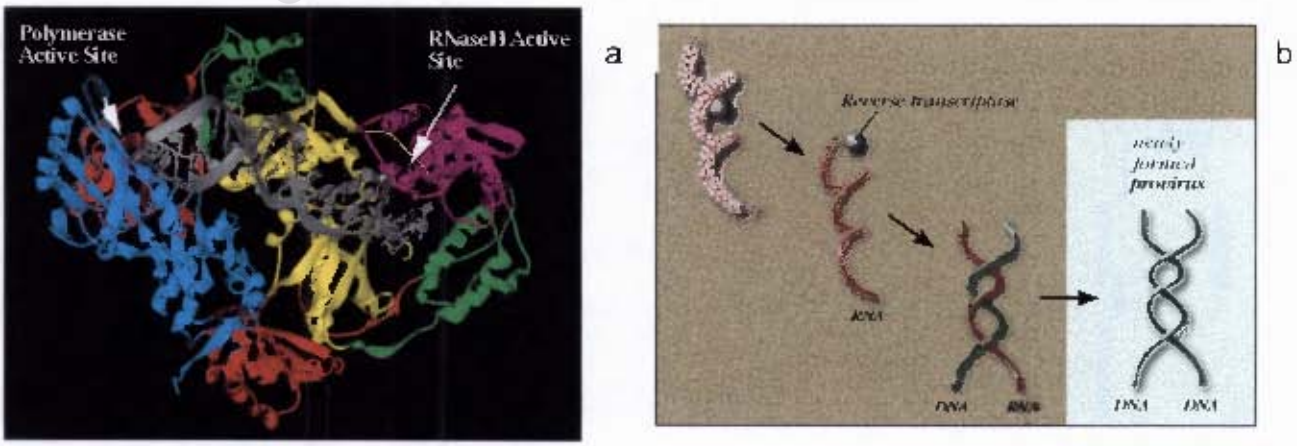


Fig. 1.3. (a) Structure of the HIV-1 reverse transcriptase enzyme adapted from Huang *et al.*[10] (b) RNA-directed synthesis followed by DNA-directed synthesis.

1.7 HIV-1 Reverse Transcriptase Inhibitors

Drugs that inhibit the functions of the Reverse Transcriptase enzyme are referred to as reverse transcriptase inhibitors. They are classified into two classes as:

a) Nucleoside and nucleotide analogues (NRTI)
b) Non-nucleoside reverse transcriptase inhibitors (NNRTI)

1.7.1 Nucleoside Reverse Transcriptase Inhibitors

Currently, seven drugs belonging to the 2',3'-dideoxynucleosides (ddNs) have been approved by the Food and Drug Administration for the treatment of HIV and are commercially available. They include, Zidovudine (AZT, Retrovir),[15] Stavudine (d4T, Zerit),[16] Zalcitabine (ddC, Hivid),[17] didanosine (ddI, Videx),[18] Lamivudine (3TC, Epivir),[19] Abacavir (ABC, 1592489, Ziagen),[20] and Emtricitabine ((-) FTC, Emtriva™).[21] One nucleotide analogue, Tenofovir disoproxil fumarate (PMPA, Viread™) has also been approved by the FDA and is currently in clinical use[22] (Fig. 1.4).

2',3'-Dideoxynucleosides are the most important class of compounds active against HIV. They act as DNA-chain terminators and competitive inhibitors of viral reverse transcriptase (RT).[23] Elongation is blocked because the chain terminators lack the 3'-OH functional group essential for incorporation of additional nucleotides. NRTIs are not highly specific and can inhibit normal cellular polymerases, causing serious side effects. They must first be phosphorylated intracellularly to their 5'-triphosphate form by cellular kinases before they act as chain terminators in the RNA-directed DNA polymerisation reaction catalysed by HIV-1 RT. In their 5'-triphosphate form, these dideoxynucleosides compete with the natural substrate (dTTP, dCTP, etc) of RT.[24] d4T shows selective anti-HIV activity comparable to that of AZT in vitro. However, d4T is less toxic and less inhibitory to mitochondrial DNA replication than AZT.[25]

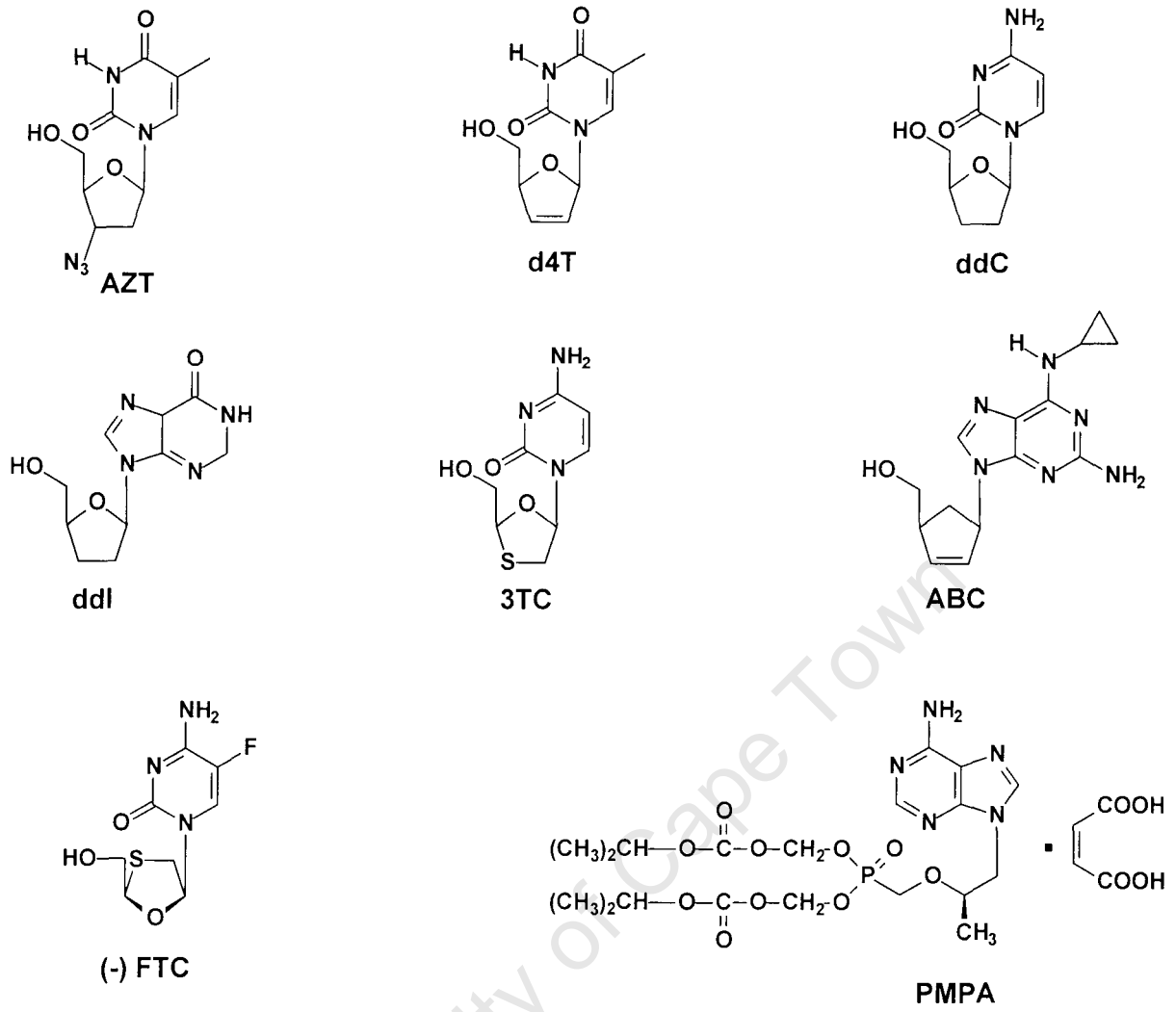


Fig. 1.4: Nucleoside analogues currently used to treat HIV/AIDS

1.7.2 The Structure of HIV-1 RT in Complex with Nucleoside Inhibitors

Nucleotide analogues bind at the dNTP-binding site (Fig. 1.5), adjacent to the 3' terminus of the primer strand that is located in the palm subdomain of the p66 subunit.[12] The catalytic triad of aspartic acids, (Asp110, Asp185 and Asp186), which are conserved in most polymerases are also located at this site. The nucleotide analogue binding site is composed of both protein and nucleic acid. The nucleic acid part of the binding site is made up of the 3'-primer terminus, which possesses the 3'-OH group that is the site for the covalent attachment of the incoming substrate. The base of the 3'-primer terminal nucleotide also helps to bind incoming substrate *via* base-stacking interactions. The first base in the template overhang contributes base-specific H-bond donor and acceptor groups that guide dNTP selection based on Watson-Crick base pairing rules. Based on modelling experiments, residues Asp185

and Asp186 of the conserved Tyr-Met-Asp-Asp motif and Asp110 are believed to bind the triphosphate moiety of the incoming dNTP *via* one or two chelated Mg^{2+} ions.[12]

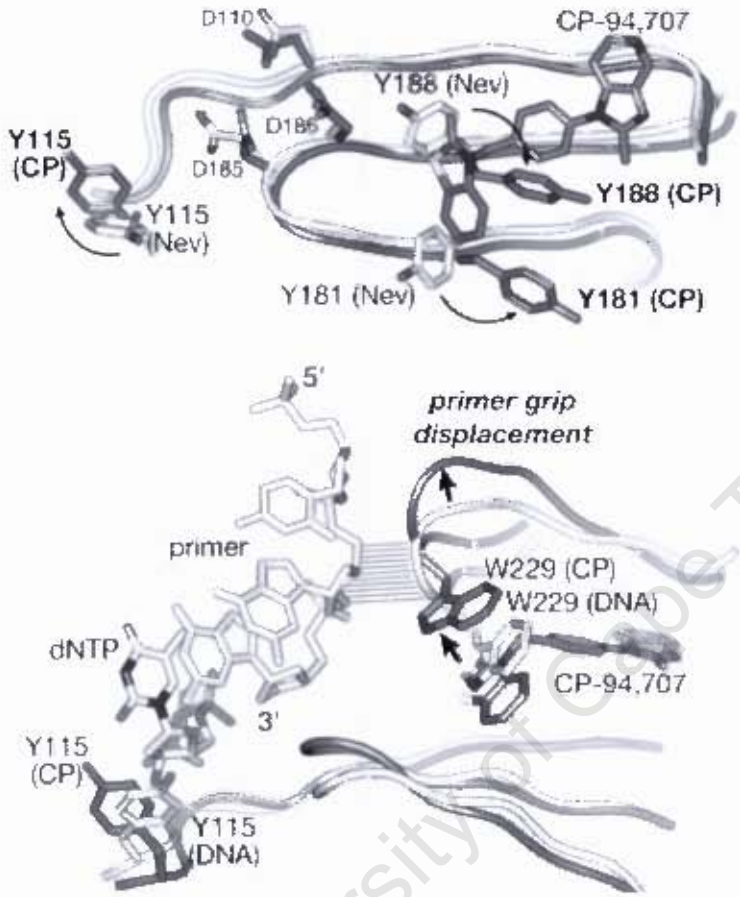


Fig. 1.5: Superposition of HIV-1 RT in complex with an inhibitor and in ternary complex with primer template DNA and incoming dNTP. Displacement of TRP-229 and concomitant displacement of the primer grip are indicated with arrows. Nonspecific contacts between the primer grip and the primer strand DNA are indicated with parallel lines.[26]

1.7.3 Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI)

In 1987, when the 1st NRTI (AZT) was approved for the treatment of HIV infection, the understanding of the replication cycle of the HIV virus was limited and the molecular targets that are known today to be central to HIV replication had not been identified.[27] A broad screening of the Janssen compound library in the early nineties led to the discovery of TIBO[28] and α-APA[29] as anti-HIV agents. This was followed by systematic lead optimization, which yielded the first-generation NNRTIs Tivirapine, a TIBO derivative, and Loviride, an α-APA derivative.[14,30] These compounds were effective against wild-type HIV-1 but had lower potency when tested against common

NNRTI-resistant mutants. Chemical modifications were introduced in these TIBO and α-APA derivatives, resulting in a systematic structure-based molecular modelling study which played a key role in understanding the three dimensional structure-activity relationships in these two chemically distinct series.[14]

The disclosure of TIBO compounds inspired the search for more potent and selective RT inhibitors.[27] To date, more than 30 structurally diverse NNRTIs have been identified.[31] They include DABO derivatives,[32] HEPT derivatives,[33] TSAO derivatives,[34] Thiocarboxanilide derivatives,[35] Quinoxaline derivatives[36] and PETT derivatives[37] (Fig. 1.6). The number of compounds within the NNRTIs is rapidly increasing and by the end of 1998, the following three compounds had been approved for clinical use by the food and drug administration: Nevirapine (Viramune®),[38] Delavirdine (Rescriptor®)[39] and Efavirenz (Sustiva™)[40] (Fig. 1.7).

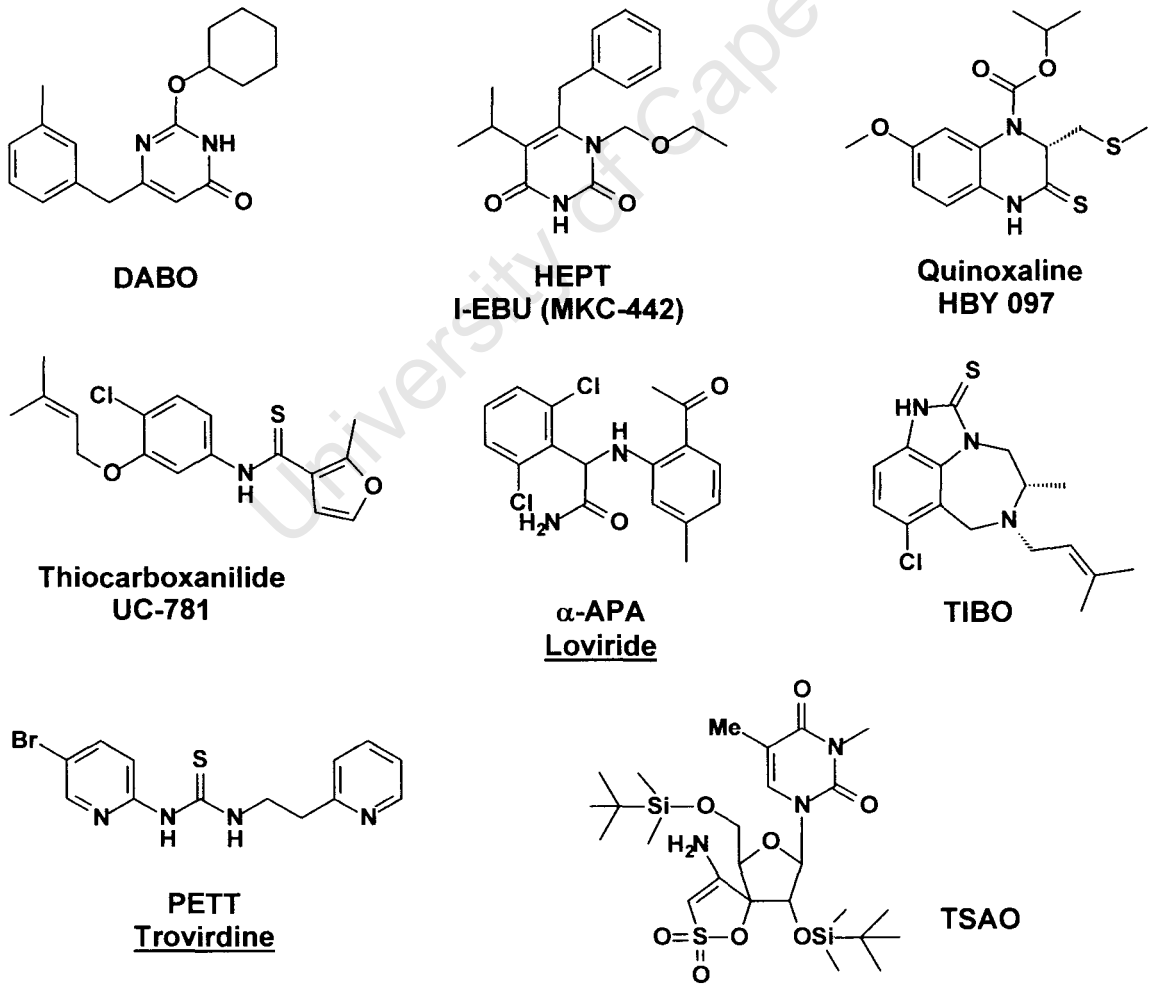
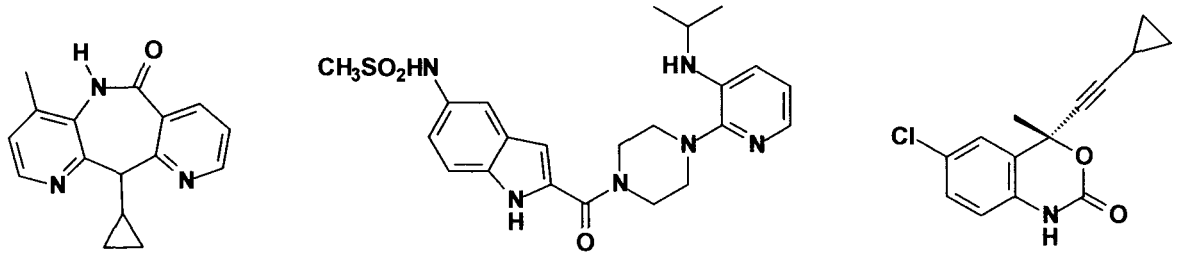


Fig. 1.6: Chemical structures of some selected NNRTI inhibitors

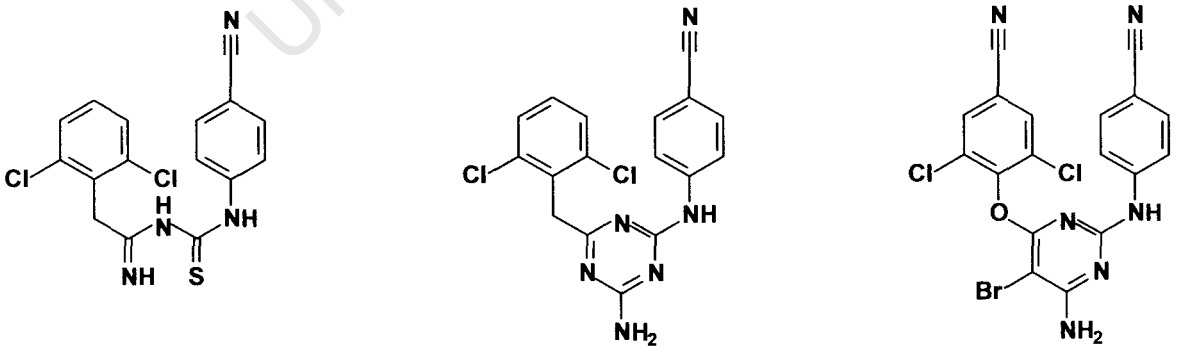


Nevirapine **Delavirdine** **Efavirenz**

Fig. 1.7: NNRTIs approved for the treatment of HIV/AIDS

Soon after the NNRTIs were being used in clinical settings, it became apparent that this class of inhibitors was vulnerable to HIV's high mutation rate, which resulted in a rapid selection of resistant strains. This tempered the initial enthusiasm and even led some groups to abandon NNRTI research altogether.[30]

By early 2000, structural activity studies around the α-APAs had produced imidazoylthiourea (ITU) derivatives. An effort to improve the metabolic stability of the ITUs led to the serendipitous discovery of DATA compounds. These compounds have a triazine ring that replaced the unstable thiourea moiety of ITU (Fig. 1.8). Molecular modelling of DATA compounds suggested that replacing the central triazine ring with a pyrimidine ring would improve the activity further. This structural modification led to the discovery of etravirine, a diarylpyrimidine (DAPY) derivative, that is highly potent and effective against wild-type and drug-resistant HIV-1 variants.[27]



ITU R100943 **DATA R106168** **DAPY Etravirine**

Fig. 1.8: Chemical structures of second-generation NNRTIs.

1.7.4 The NNRTI Binding Pocket

NNRTIs bind at a largely non-conserved site. Detailed structural information of the RT enzyme in complex with the inhibitor is useful for effective drug design. The NNRTI binding site ('pocket') is located in the p66 domain at about 10Å distance from the substrate-binding site. The ligands in this pocket are of mainly hydrophobic nature with substantial aromatic character with the most prominent ones being Y181, Y188, F227, L100, L234, V179 and K103. The pocket region also contains a few hydrophilic residues (Lys101, Lys103, Ser105, Asp192, Glu224 and Glu1138) and backbone atoms that may form hydrogen bonds to non-nucleoside compounds, which all include hydrogen-bond donor and acceptor groups.[12]

1.7.5 Interaction of NNRTIs with their Pocket Site at the HIV-1 RT

The NNRTIs block the HIV-1 reaction through interaction with an allosterically located, non-substrate binding site.[41] The NNRTI binding in this pocket causes a repositioning of the three-stranded beta-sheet in the p66 subunit, which contains the catalytic aspartic acid residues 100, 185 and 186. This inhibits HIV-1 RT by locking the active catalytic site into an inactive conformation, reminiscent of the conformation observed in the inactive p51 subunit.[42] Steady state kinetic studies carried out by Anderson *et al.* have revealed that NNRTI binding to HIV-1 RT does not prevent the binding of nucleotide triphosphate substrates to the enzyme, but instead blocks the chemical step of NRTI incorporation.[43]

When bound into their pocket of HIV-1 RT, the first-generation NNRTIs maintain a very similar conformational 'butterfly-like' shape. They roughly overlay each other in the binding pocket and appear to function as π-electron donors to aromatic side-chain residues lining the pocket.[44]

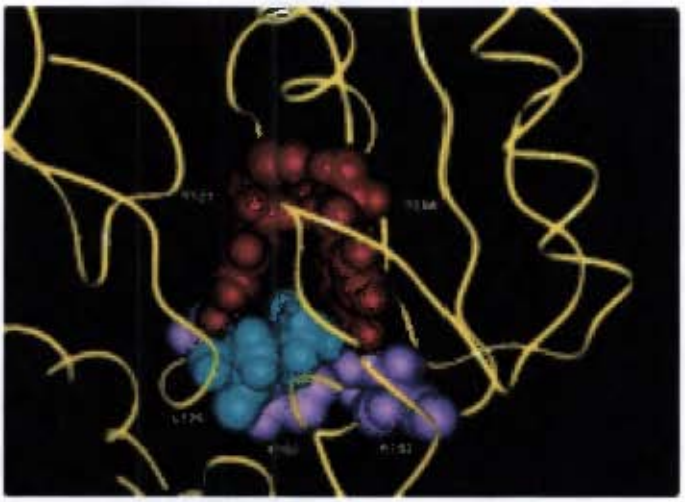


Fig. 1.9: Close-up of the future binding pocket area in unliganded RT. Amino acid residue 100 (shown in blue) makes contact with residues Y181 and Y188 (shown in red) which undergo major structural rearrangement prior to non-nucleoside inhibitor binding. L100 also makes contact with K101 and K103 (purple) in the uncomplexed state.[45]

In crystal structures of unliganded HIV-1 RT, the non-nucleoside inhibitor-binding site (NNIBS) does not exist (Fig. 1.9). During the process of inhibitor binding, significant conformational changes occur in the orientation of the side chains of Tyr181 and Tyr188 leading to the formation of the hydrophobic pocket accommodating the inhibitor. It is evident from a comparison of the various RT structures that the NNIBS has a very flexible structure, and that this flexibility apparently allows the enzyme to accommodate structurally diverse inhibitors having different shapes and sizes. In fact, side-chain residues adapt themselves to each bound inhibitor in a highly specific manner, closing about the surface of the drug to make tight van der Waals contacts.[46]

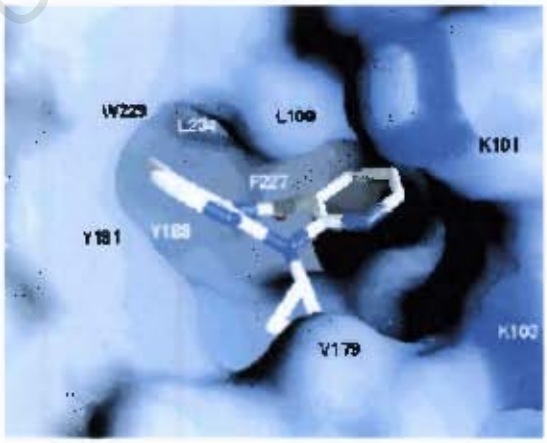


Fig. 1.10: Structure of Nevirapine in the NNRTI binding pocket.[27]

A three-dimensional structure of HIV-1 RT complexed with nevirapine (Fig. 1.10) demonstrates that it binds to a hydrophobic pocket on the enzyme near to but distinct from the *pol* active site. Nevirapine was shown to interact with two tyrosine residues at positions 181 and 188, which had been shown previously, by affinity labelling and point mutant studies, to be involved in binding.[47] The thiocarboxanilides UC-781 bind to their pocket in a similar fashion to other NNRTIs, i.e. through hydrogen bonding with the main-chain oxygen of Lys101 together with hydrophobic interactions with Leu100, Val106, Val179, Tyr188, Phe227, Leu234 and His235. The thiocarboxanilide UC-781 also makes important hydrophobic interactions with Try229.[44] The dimethylally group of TIBO (Wing 1 of butterfly) interacts with amino acid residues Pro95, Tyr181, Tyr188, Gly190 and Trp229, while the benzodiazepinone group (Wing II) interacts with Lys101, Lys103, His235 and Tyr318.[48]

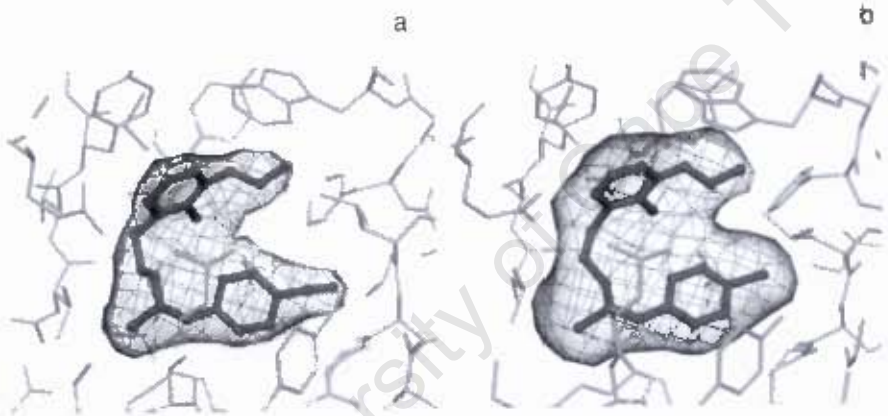


Fig. 1.11: Simulated annealing omit electron density maps showing the bound inhibitors at the NNRTI pocket of HIV-1 RT. *a*, PETT-1; *b*, PETT-2.[50]

Comparison of PETT inhibitors with nevirapine and TIBO reveals that these inhibitors adopt conformations that occupy similar volumes of space within the drug pocket despite their diverse structures (Fig. 1.11). When trovirdine and other PETT molecules were docked into the composite binding site, the composite binding pocket showed space around the phenyl ring in the Wing 2 (ring B) region of the binding site. The 5-bromopyridyl group binds tightly in this region,[49] with an intramolecular hydrogen bond between the ring B pyridyl nitrogen atoms and one of the nitrogen atoms of the thiourea group. Ring A is positioned at the "top" of the NNRTI pocket, and thus forms ring stacking interactions with the side chains of both Tyr188 and Tyr 181. The sulphur atom makes a number of van der Waals contacts with the

backbone of Lys101. There is a single hydrogen bond to the main chain from one of the thiourea nitrogens to the carbonyl of Lys101 (Fig. 1.12).[50]

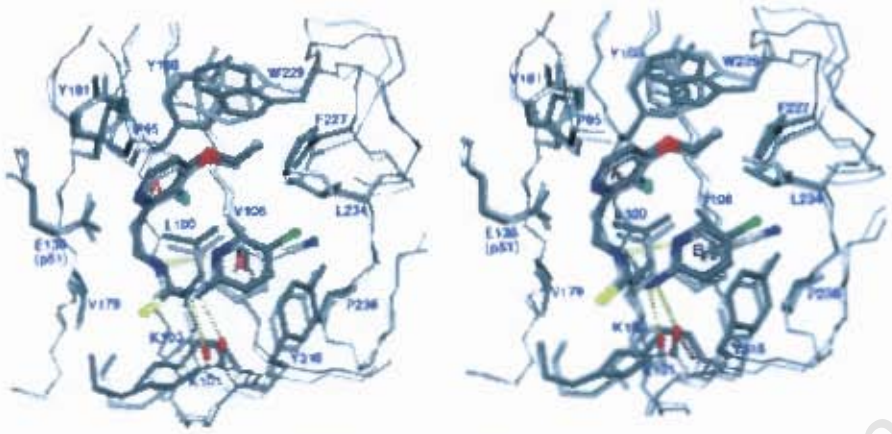


Fig. 1.12: Stereo view showing PETT-1 and PETT-2 positioned in the NNRTI binding pocket of HIV-1 RT. RT side chains that form the NNRTI binding site are marked. The inhibitors are in atom colours with PETT-2 shown in a darker hue. The protein for the PETT-1 complex is shown in *light gray*, whereas that for the PETT-2 complex is shown in *dark gray*. The positions of hydrogen bonds are marked in *broken yellow lines*.[50]

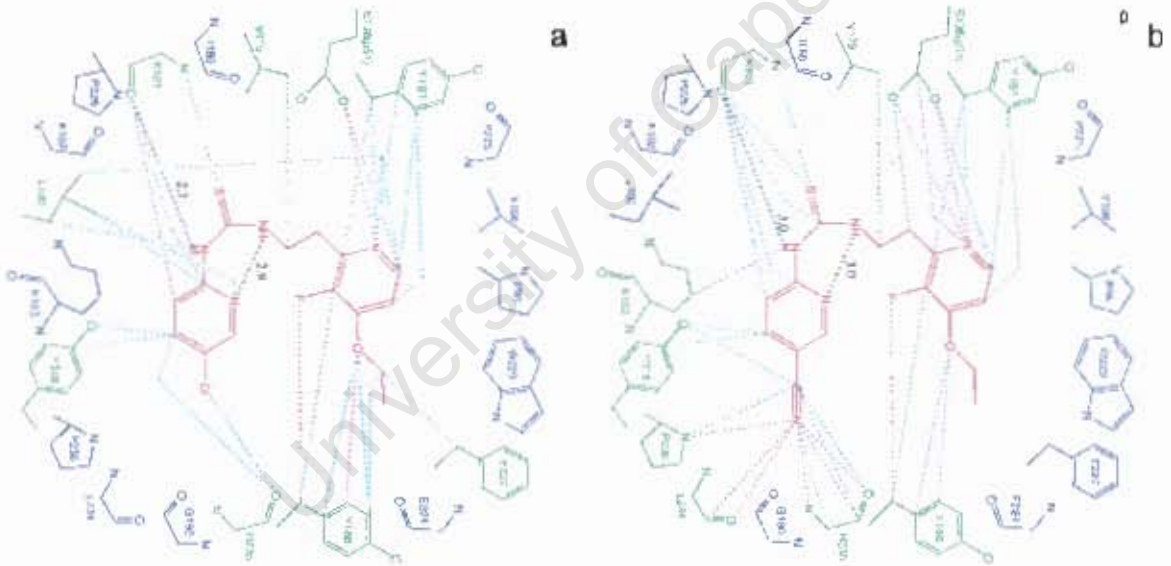
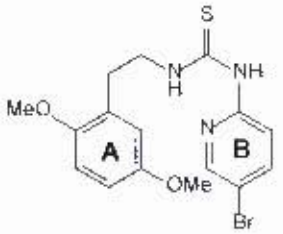


Fig. 1.13: **Schematic diagrams showing the intermolecular interactions between PETT inhibitors (in *red*) and the surrounding residues of HIV-1 RT for (*a*) PETT-1 and (*b*) PETT-2.** Residues that contact the NNRTI with a minimum inter-atomic distance of 3.6 Å are shown in *green*, whereas other residues lining the binding pocket are shown in *blue*. The individual distances between the NNRTI and the protein atoms are shown as *dashed lines* (distances 3.3 Å in *pink* and 3.6 Å in *light blue*). Hydrogen bonds together with their distances are shown in *black*.[50]

Structural studies around PETT led to the discovery of *N*-[2-(2,5-dimethoxyphenylethyl)]-*N'*-[2(5-bromopyridyl)]-thiourea (HI-236) (Fig. 1.14), which was designed to optimize occupancy of the binding pocket.[51] Docking experiments of HI-236 revealed that the 2-methoxy group was situated beneath the ethyl linker and

fits favourably into a cavity of the binding pocket, providing additional contact with the protein residues.

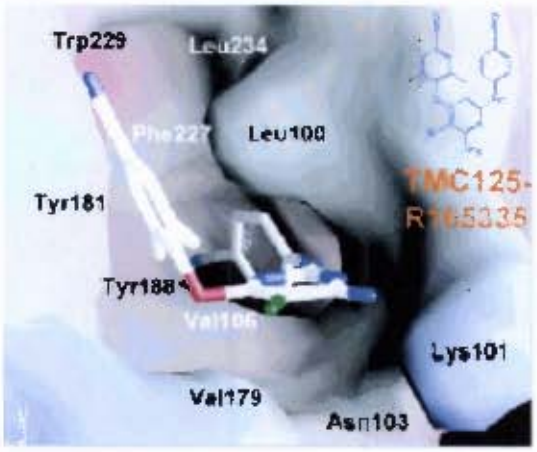


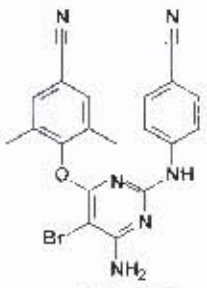
HI-236

Fig. 1.14. Structure of HI-236

Structure-activity studies of Ring A revealed that the methoxy substitution is more favourable at the *meta* (to the alkyl side chain) position, compared to the *para* position. Fluorine substitution is favourable at the *ortho* and *meta* positions, whereas chlorine was only favourable at the *ortho* position. Similarly, a hydrophobic group is more desirable than a polar group or hydrophilic group at the *para* position.[52]

The crystal structure of HIV-1 RT in complex with ITU (R100943), DATA and DAPY derivatives showed that these compounds bind in a unique "horseshoe" or "U" mode (Fig. 1.15) compared to the butterfly-like α-APA.[14] DAPY derivatives can adapt to changes in the NNRTI binding pocket. The torsional flexibility of the DAPY structure permits access to numerous conformational variants, and their compact structure permits repositioning and reorientation when mutations in the binding pocket are present.[27] The ability of etravirine (a DAPY derivative) to bind the RT enzyme in more than one conformationally distinct mode explains the exceptional spectrum of activity observed for this compound.[27]





TMC 125
R165335

Fig. 1.15: "Horseshoe" conformation of the second-generation NNRTI (TMC125).

1.8 Mutations and Drug Resistance

The HIV-1 reverse transcriptase enzyme makes numerous errors and unlike mammalian polymerase enzyme, it does not have 3'-exonuclease proofreading activity and cannot, therefore, proofread and correct the mistakes made during DNA synthesis. This results in rapid emergence of drug-resistant mutants.

1.8.1 NRTI Resistance

There are two currently known biochemical mechanisms of NRTI drug resistance. The first mechanism is mediated by mutations that allow the RT enzyme to discriminate against NRTIs during DNA synthesis, thereby preventing their addition to the growing DNA chain. The second mechanism is mediated by nucleotide excision mechanism (NEM) that increase the rate of hydrolytic removal of the chain-terminating NRTI and enable continued DNA synthesis.[53,54] In the second mechanism, the oxygen anion of a nucleoside diphosphate or triphosphate is used as a pyrophosphate nucleophile to attack and cleave the 3'/5'-phosphate bond of the primer, producing an unblocked primer and a dinucleoside tri- or tetraphosphate containing the dideoxynucleoside monophosphate from the primer terminus linked through its phosphate group to the distal phosphate of the free nucleoside di- or triphosphate (Fig. 1.16).[54]

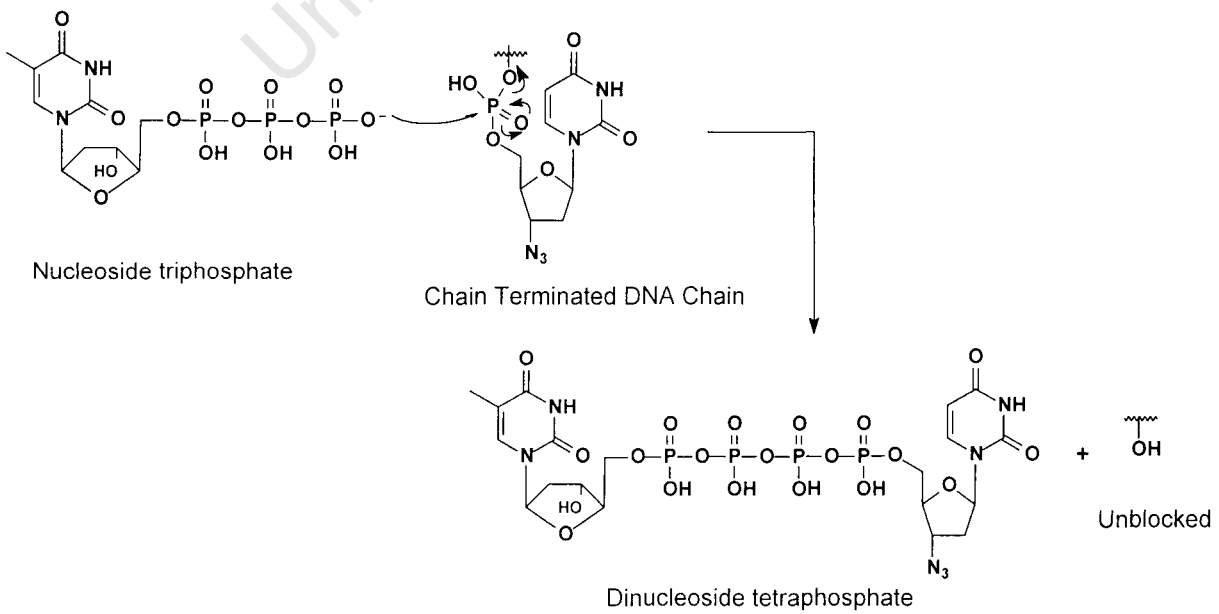


Fig. 1.16: Removal of NRTI from the primer terminus through dinucleoside polyphosphate synthesis

Biochemical and structural modelling studies suggest that the bulky azido group of AZT interferes with the formation of a dead-end catalytic complex by sterically preventing the addition of the next dNTP (Fig. 1.17). This explains why NEMs cause the highest levels of phenotypic resistance to AZT.

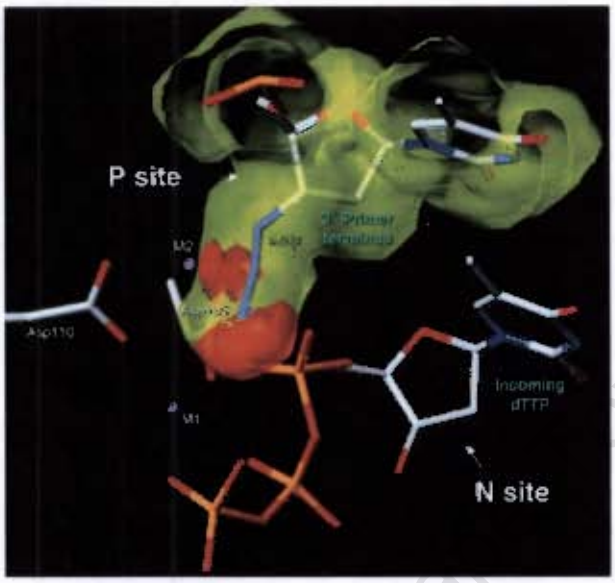


Fig. 1.17 Steric hindrance when an AZT-terminated primer is bound to RT at the P site. The figure, based on the structure of the ternary RT-DNA-dNTP complex, shows that the distance between the azido of AZT and D185 would cause steric conflict; the distance between D185 and the first and second azido nitrogens is less than the sum of the van der Waals radii.

The M184V mutation causes the high-level lamivudine resistance observed and emerges rapidly in patients receiving lamivudine monotherapy. This mutation (M184V) and other NRTI resistance mutations interfere with the effects of the NEMs. The mutational antagonism between the NEMs and several of the mutations that act by allowing RT to discriminate against NRTIs explains the clinical synergism observed with dual NRTI combinations such as Zidovudine/Lamivudine, Stavudine/Lamivudine, Zidovudine/didanosine and Stavudine/didanosine.[53]

1.8.2 NNRTI Resistance

NNRTIs have been notorious for rapidly triggering the emergence of drug-resistant HIV variants. Resistance usually emerges when an NNRTI is administered as a monotherapy. NNRTI resistance mutations arise rapidly and most often directly in contact with the NNRTI molecule. They are associated with changes in the binding of NNRTI to RT. The most common mutations are Lys103Asn (K103N) and Tyr181Cys

(Y181C).[38] The Y181C mutation causes a loss of aromaticity, by reducing favourable π-π interaction, whereas the K103N mutation indirectly affects NNRTI potency by stabilizing the closed form of the non-nucleoside inhibitor binding pocket (NNIBP) through the formation of a hydrogen bond between the Asn103 side-chain amide and the Tyr188 phenoxy oxygen, reducing the rate of inhibitor entry.[38] The Leu100Ile mutation causes steric interference between the β-branched isoleucine and a bound NNRTI. The Gly190Ala mutation causes resistance through steric conflict of the methyl side chain and the bound inhibitor.[44]

Mutations associated with resistance to nevirapine involve residues Y181, K103, V106, V108, Y188, L100 and G190, which have van der Waals contact with the inhibitor. Mutations of these residues lead to the weakening of the inhibitor binding to RT.[55] The most common resistance mutation observed for nevirapine in vivo is Y181C, which is prevented from emerging by co-administration of AZT. The mutually antagonistic effects of different resistance mutations (ie Y181C or L100I versus T215Y) and the hypersensitivity that is seen under some conditions (i.e. with P236L mutation towards some NNRTIs), argues in favour of the combined use of NNRTIs with NRTIs and different NNRTIs with one another.[56]

1.9 Combination Therapy

Combination therapy is a treatment regime that combines two or more drugs, in which each drug can work on the same or different drug-target, with either independent mechanisms of inhibition or patterns of resistance. The drug entities are either co-formulated in the same tablet or capsule (e.g. Combivir (Zidovudine/Lamivudine) in HIV-1 treatment) or co-administered in separate tablets or capsules (e.g. AZT and Nevirapine). Combination therapy has been used to exploit the synergistic and additive activities of individual drugs and is commonly used in the treatment of tuberculosis, cancer, leprosy, malaria and HIV. In the case of HIV, combination therapy has been coined the term "highly active anti-retroviral therapy" (HAART), which is a treatment regime that commonly combines the use of two NRTIs and one NNRTI or protease inhibitor.[57] Combinations of NRTI and NNRTIs have been found to decrease viral load, increase CD4 count, decrease mortality and delay disease progression to AIDS. While achieving synergism in their

anti-HIV action, a combination of different drugs reduces the risk of HIV drug-resistance development and diminishes toxic side-effects through reduction of the individual doses.[44]

1.9.1 The Double-Drug Strategy

The double-drug strategy involves the combination of two different classes of inhibitors into a single molecular entity. The aim is to enhance activity of the individual drugs, and thus prevent resistance as well as improve physicochemical characteristics. Such compounds may be divided into two types as:

(a) Compounds containing cleavable linkers that can unleash two different drugs intracellularly and thus act as prodrugs. These compounds can target two active sites in the same substrate or different steps of the virus/parasite life cycle. The design of these drugs requires that:
    (i) They contain a linker that is stable outside the target cell
    (ii) Once in the cytoplasm, they should regenerate the parent compounds.

(b) Compounds containing non-cleavable linkers that target two active sites that are located in close proximity.

1.9.1.1 Double-Drugs in Cancer and Malaria

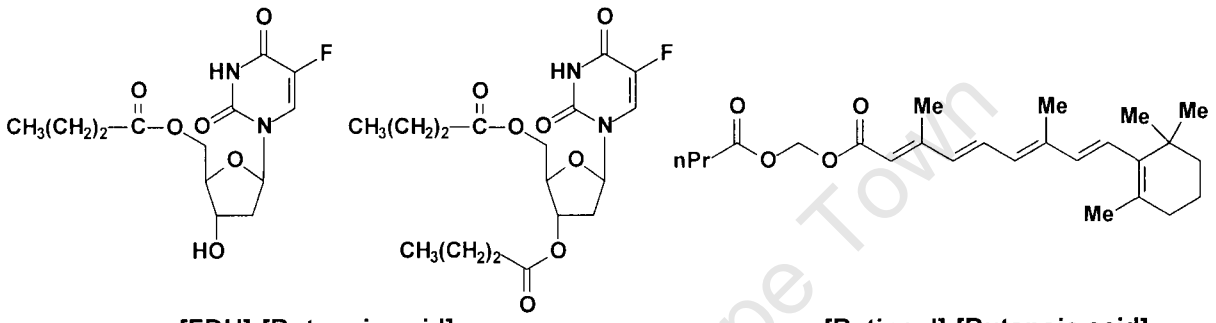
5'-*O*-Butanoyl and 3'-*O*-retinoyl esters of 5-fluoro-2'-deoxyuridine (FDU) (Fig. 1.18) have been synthesized to act as anticancer double prodrugs that would serve as a depot to release two active drugs that act through different mechanisms. The nucleotide derivative of FDU could act as a competitive inhibitor for thymidylate synthase whereas retinoic acid and butyric acid were expected to induce cell differentiation. The ester derivatives exhibited comparable activity to FDU.[58]

Retinoids have been reported to induce differentiation and arrest proliferation in a wide spectrum of cancer cells and are currently used for treatment of promyelocytic leukaemia. Butyric acid is an effective inhibitor of cell proliferation and inducer of cytodifferentiation. Retinoyloxymethyl butyrate, a mutual prodrug combining butyric

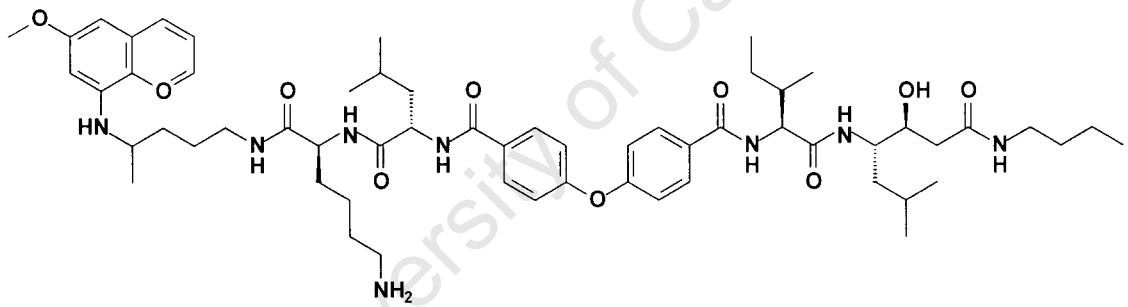
acid and *all-trans*-retinoic acid was evaluated for anticancer activity and was found to be more potent than the parent drugs.[59] Furthermore, the differentiation activity elicited by the double-drug was greater than that of the combined parent acids. The large increase in activity was attributed to two factors:

(i) The *all-trans*-retinoic acid fragment imparted lipophilicity and facilitated the penetration of butyric acid to the cellular target site.

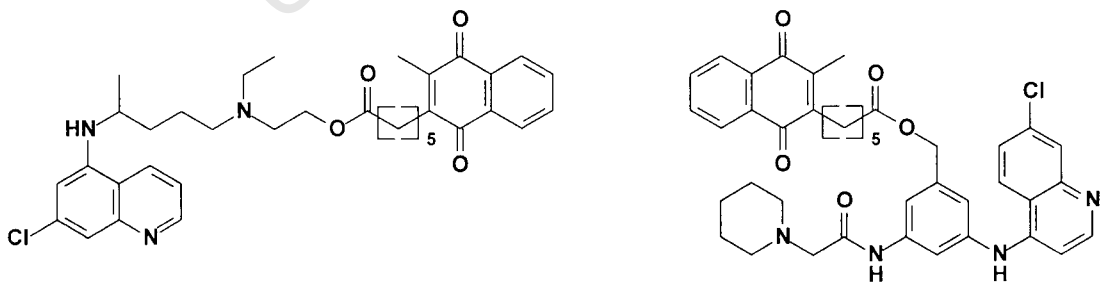
(ii) The intracellularly released *all-trans*-retinoic acid and butyric acid affected the cells synergistically.



[FDU]-[Butanoic acid] **[Retinoyl]-[Butanoic acid]**



[Primaquine]-4,4'-oxy-bis(benzoic acid)-[PLM II]



[4-aminoquinoline]-[GR Inhibitor]

Fig. 1.18: Cancer and Malaria double-drugs

The statine-based inhibitor of Plasmepsin (II) (PLM II) was linked to the antimalarial drug Primaquine (PQ) using dicarboxylic acid linkers (Fig. 1.18). PLM II is one of the aspartic proteases involved in the degradation of haemoglobin during the

intraerythrocytic cycle of *Plasmodium falciparum*. Primaquine is highly active against all malaria species infecting humans. Its toxicity levels can be minimised and the activity increased by converting it to a peptide prodrug. The PQ-Statine double-drugs showed remarkable improvement in the inhibition of both PLM II activity and *P. falciparum* growth in vitro. The double drugs kill the parasites mainly by inhibiting PLM II and consequently the digestion of haemoglobin that is essential for the survival of the parasite.[60]

Biot *et al.*[61,62] synthesized double-headed antimalarial prodrugs that target two essential functions of the malarial parasite, namely glutathione regeneration and heme detoxification, with the aim of exploring their synergistic or additive effects. The double drugs combined a glutathione reductase (GR) inhibitor to a 4-aminoquinoline moiety with a bioreversible linker. However, these double-drugs exhibited poor inhibition activity.

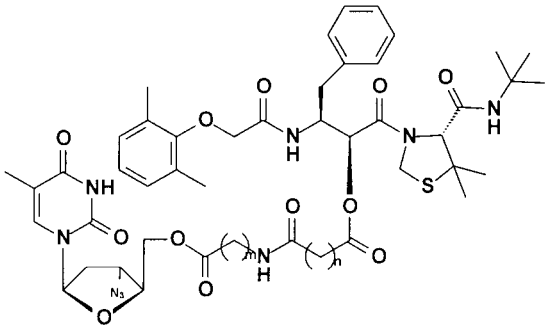
1.9.1.2 Double-drugs in HIV

(a) NRTI/Protease/Chemokine Receptor Double-Drugs

A combination of HIV protease inhibitors and AZT into a single molecule has also been successfully used to improve poor membrane permeation. The protease inhibitors (e.g., KNI-727), unable to penetrate the cell membrane were linked to AZT by a cleavable linker and this resulted in a considerable boost in potency in the cell assay[63] (Fig. 1.19).

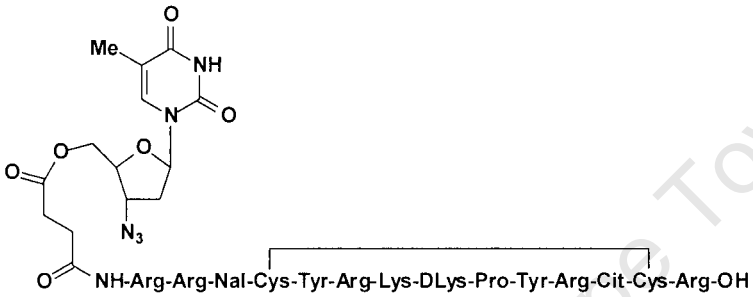
Tamamura *et al.*[64] employed the double-drug strategy to synthesize a bifunctional drug that incorporated a chemokine receptor, CXCR4 inhibitor (T140) and an NRTI (AZT) linked by succinate. T140 is a 14-amino acid residue peptide which inhibits infection of target cells by T cell-line-tropic strains of HIV through specific binding to its chemokine receptor, CXCR4. An equimolar mixture of AZT and T140 caused a remarkable increase in anti-HIV activity compared to the single compounds. This result led to the synthesis of conjugated compounds which combined each entity into a single molecule. The conjugate drugs exhibited a synergistic effect for anti-HIV in

vitro. The mechanism of action is based on the hydrolysis of the enzymatically labile ester 5'-*O*-bond between the NRTI and the spacer.

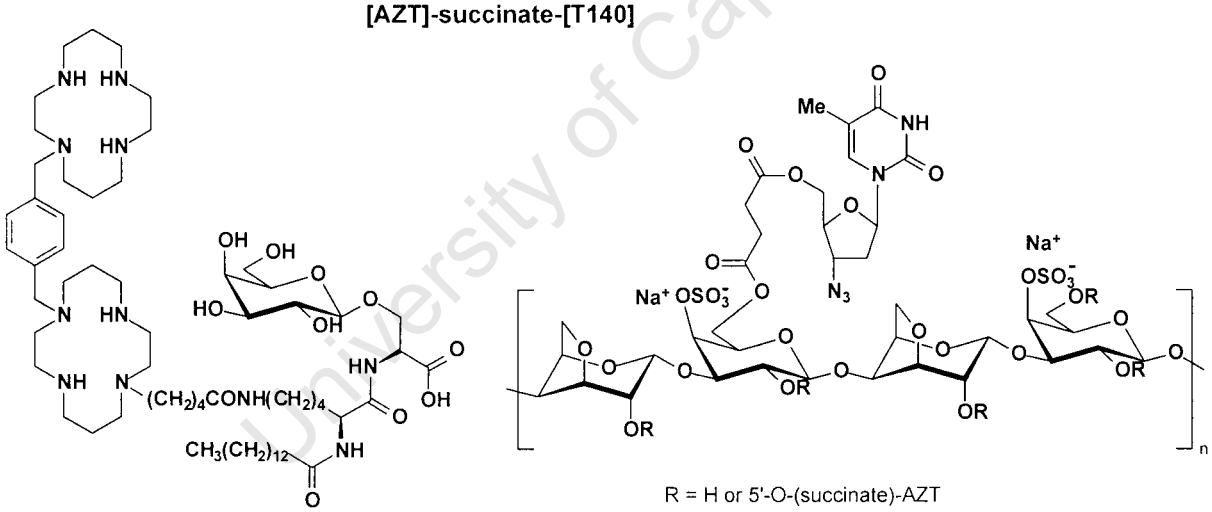


m=1, n=2

[AZT]-succinic-[KNI-727] conjugate



[AZT]-succinate-[T140]



Gal-AMD3100 Conjugates

[AZT]-succinate-[κ-carrageenan]

Fig. 1.19: Structure of NRTI (AZT), Protease (KNI-727), Chemokine Receptor (T140, Gal, AMD3100) and κ-Carrangeenan Conjugates.

Daoudi *et al.*[65] synthesized bifunctional compounds combining bicyclam AMD3100 and a galactosylceramide (GalCer) analogue in a single molecule with the aim of inhibiting several steps of the complex virus/cell cascade interactions (virus/cell adsorption/fusion processes). The double-drug [Gal]-[AMD3100] conjugate (Fig. 1.19) exhibited lower potencies than that of AMD3100. AMD3100 is a CXCR4

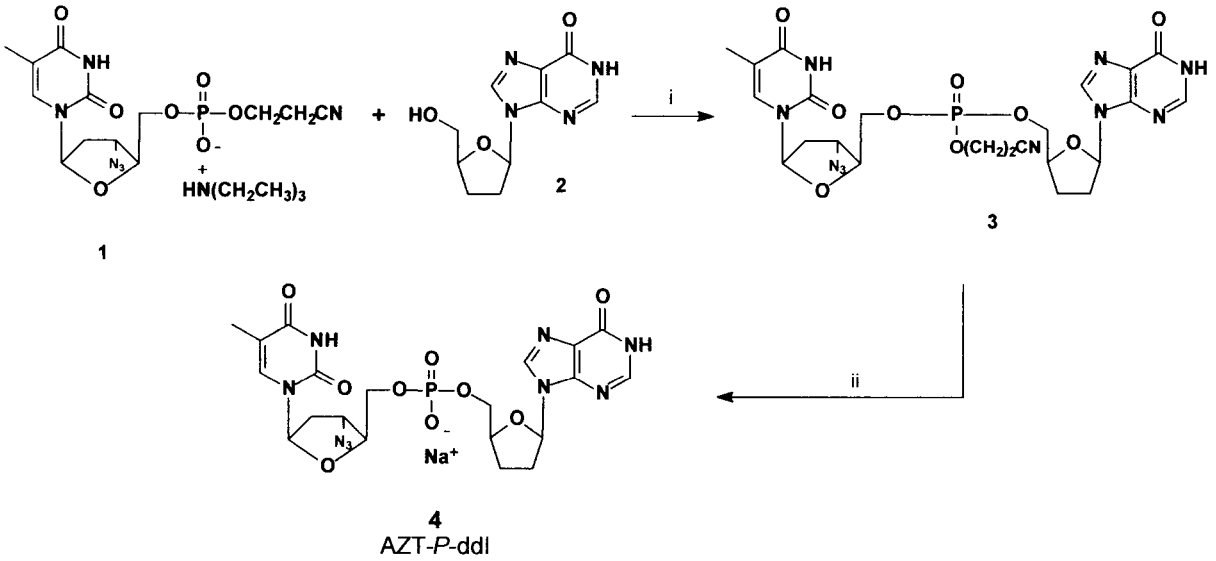
antagonist and interferes with the viral binding to the cellular co-receptors, whereas GalCer provides an attachment platform for the virus through its gp120 and/or gp41 onto the cell. When compared to the activity of GalSer, the bipharmacophore conjugate moderately increased its antiviral activity.

Similarly, AZT coupled onto κ-carrangeenan using a succinate diester spacer was synthesized with the aim of enhancing AZT intracellular uptake and testing synergism of the prodrug.[66] Carrageenans are natural sulphated polysaccharides extracted from different species of red seaweed that have a common structural backbone of D-galactose residues. The κ-form is characterised by a repeating unit of 4-sulfate-β-D-galactopyranose linked 1→3 and 3,6-anhydro-α-D-galactopyranose linked 1→4. The κ-carrangeenan was expected to act not only as a drug-delivery carrier for AZT, but also as an anti-HIV agent which would act synergistically with AZT. Carrangeenans possess anti-HIV activity and inhibit the binding of the virions to the cell (gp120 adsorption inhibitors) as well as the cell-to-cell fusion. The [κ-carrangeenan]-succinate diester-[AZT] conjugate inhibited the binding of the virions to the MT-4 cells and concomitantly delivered AZT to these cells to further inhibit the RT.

(b) Reverse Transcriptase Double-Drugs

(i) NRTI homo/heterodimers

Several homo- and heterodimers targeting the HIV reverse transcriptase enzyme have been synthesized by attaching the linker at the 5', 5 or N-3 positions of the nucleosides. Ijichi *et al.*[67] have reported the synthesis of nucleotide heterodimers of AZT, ddI and Ribavirin. The dimers were formulated as a mixed phosphate diester via the 5'-hydroxyl groups of the nucleoside, with the aim of releasing two nucleosides at the active site. Thus, 5'-*O*-phosphorylation of AZT gave AZT cyanoethyl phosphate **1**, which condensed with ddI **2** in the presence of *p*-toluenesulfonyl chloride to afford heterodimer [AZT]-cyanoethylphosphate-[ddI] **3**. Deprotection of the cyanoethyl group with 1N NaOH led to the heterodimer **4** (Scheme 1.1). Similarly, [ddI-phospho-Ribavirin] **5** (EC_{50} > 17.5 μM) and [AZT-phospho-Ribavirin] **6** (EC_{50} = 0.004 μM) heterodimers (Fig. 1.20) were synthesized using the same procedure. The heterodimers **4** and **6** showed enhanced anti-HIV activity relative to their monomers. Furthermore, AZT-P-ddI **4** (EC_{50} = 0.002 μM) was ten times less toxic than AZT to human granulocytes macrophage progenitor cells.



Scheme 1.1. *Reagents and Conditions*: (i) *p*-TsCl, CH_2Cl_2 (ii) NaOH.

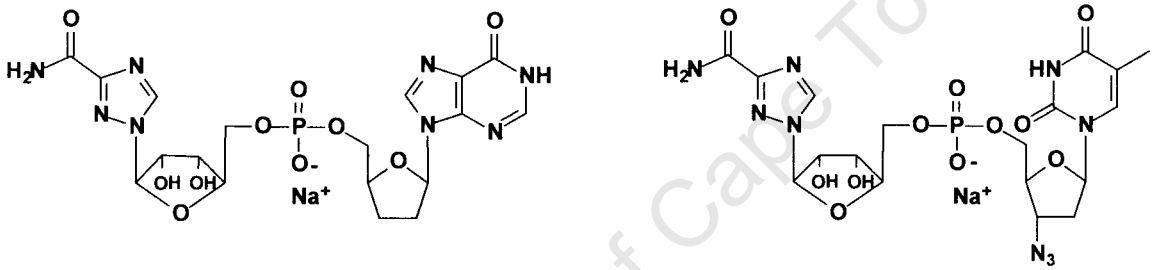
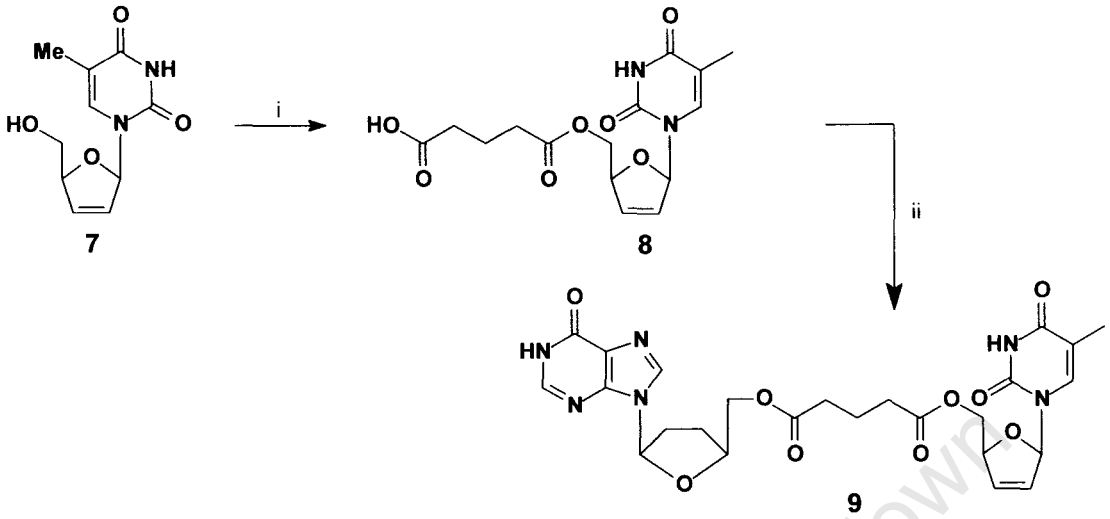


Fig. 1.20: Structures of AZT, ddI, Ribavirin and HEPU heterodimers

In a similar fashion, homo- and heterodimers of ddI, AZT and d4T have been synthesized by Mohamed *et al.*[68] with the aim of enhancing the antiviral activity of their components (Scheme 1.2). They used an ester linkage to link to a glutaric acid spacer. The synthetic strategy involved converting d4T **7** into half ester **8** by treatment with glutaric anhydride in methylene chloride, followed by an EDC-promoted esterification of the glutarate with ddI to form the heterodimer **9** in good yield (Scheme 1.2). AZT-ddI and ddI-ddI heterodimers bearing an ester linkage were also synthesized using this methodology. Other spacers used in this class of compounds were the carbonates and carbamates to form AZT and d4T homo- and heterodimer carbonates **10** and carbamates **11**, respectively (Fig. 1.21). Following intracellular hydrolysis of the carbonate or carbamate, the two nucleosides would be regenerated in the cytoplasm. The carbonates displayed anti-HIV activity comparable to AZT, while the carbamates displayed low anti-HIV activities. No synergistic effects

on the inhibition of HIV replication was detected for the carbonates **10a-c** and carbamates **11a-c**.



Scheme 1.2. *Reagents and Conditions*: (i) Glutaric anhydride, Et_3N, CH_2Cl_2; 85%. (ii) ddI/DMAP/EDC, HCl, CH_2Cl_2/DMF; 80%.

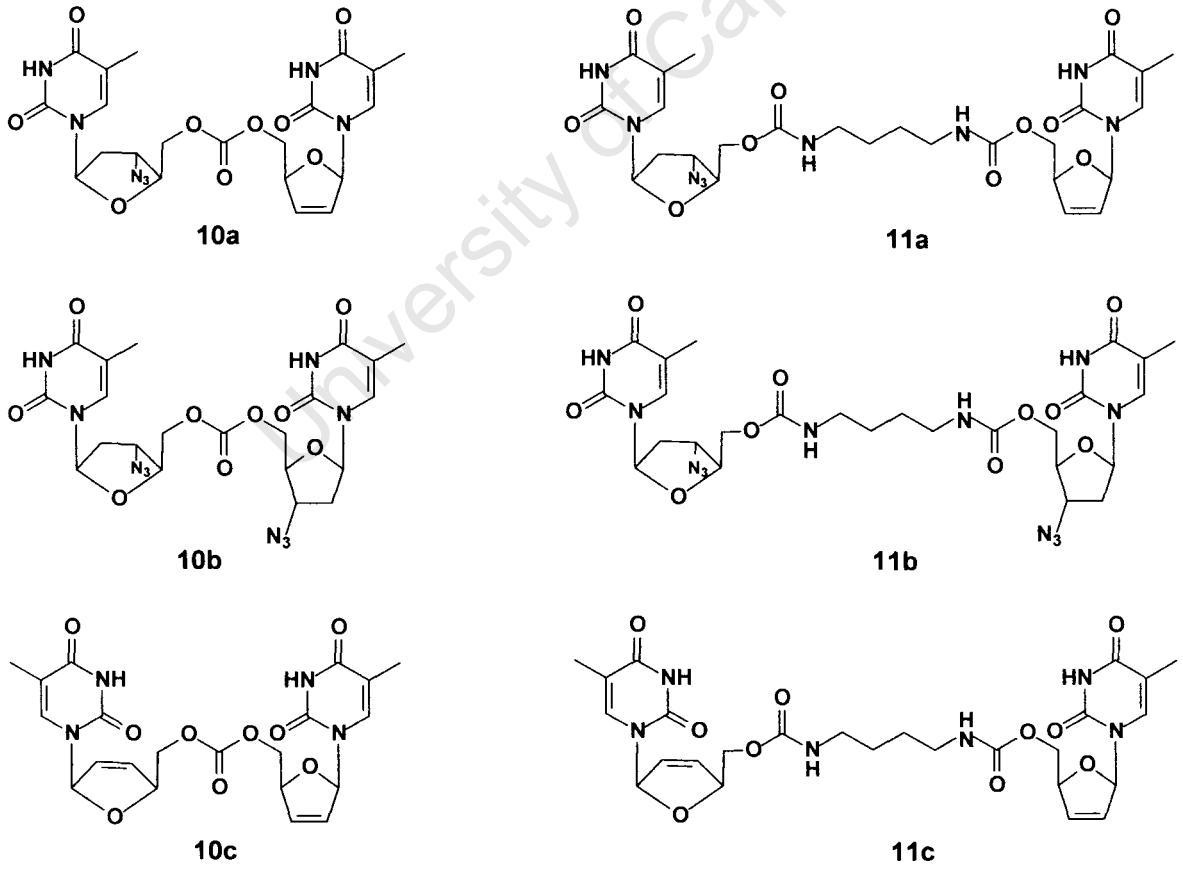
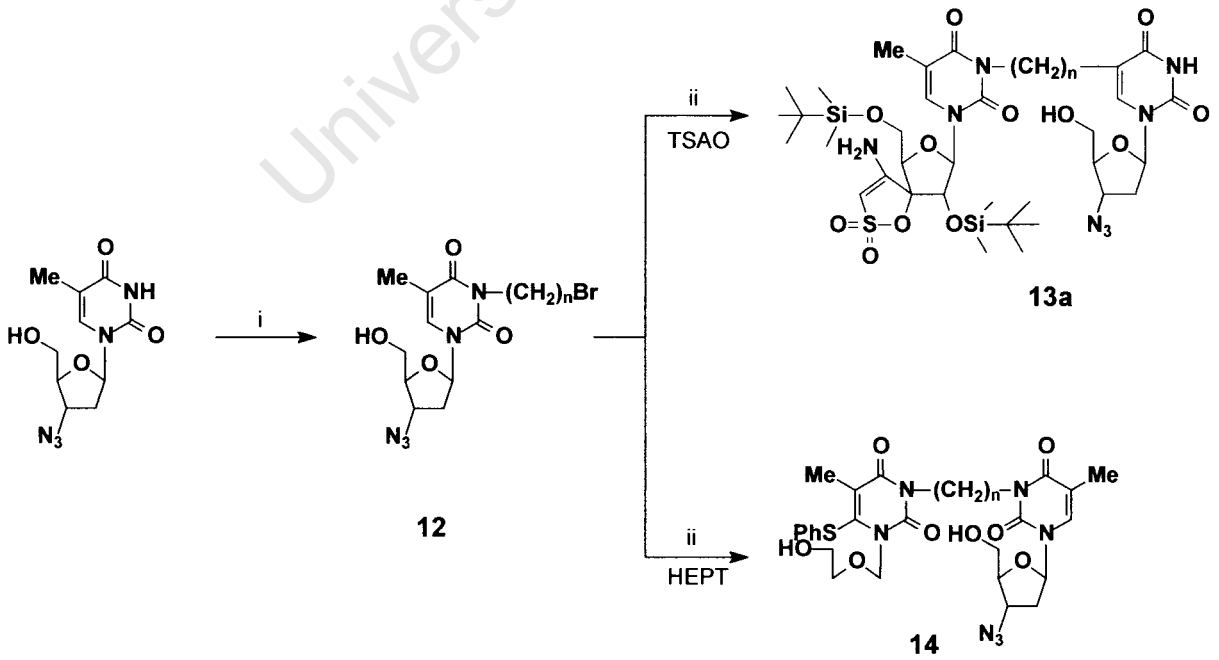


Fig. 1.21: Structure of AZT/d4T homo- and heterodimer linked by a carbonate or carbamate spacer

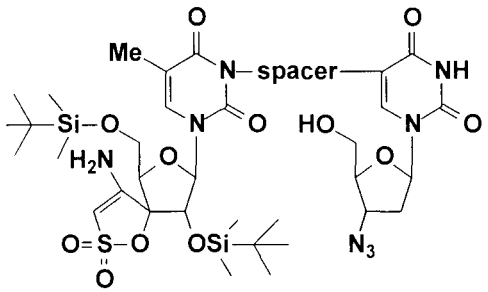
(ii) NRTI/NNRTI heterodimers

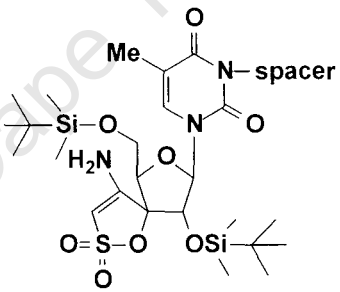
The combination of a nucleoside and a non-nucleoside RTI into a single molecular entity was advocated by Nanni *et al* in 1993.[69] This was supported by structural[11, 69] and biochemical[43,70] studies which indicated that linking the compounds with an appropriate spacer resulted in improved inhibitory capacity. The first heterodimers incorporating a NRTI with an NNRTI were synthesized by the Velázquez group in 1995.[71] They reported the synthesis of a family of anti-HIV heterodimers based on combining AZT with either TSAO-T or HEPT via a polymethylene spacer between the N-3 of the thymine base of both compounds (Scheme 1.3). TSAO derivatives were first synthesized in 1992 and represent a unique structural class of NNRTI as they specifically interact at the interface between the p51 and p66 subunits of HIV-1 RT. The prototype compound of this family is the thymine derivative designated TSAO-T and the most selective compound is its 3-*N*-methyl substituted derivative TSAO-m^{3}T. The synthetic strategy for formation of **13** and **14** involved selective N-3 alkylation of AZT with a dibromoalkyl reagent followed by reaction of the N-3-bromoalkyl AZT intermediate with TSAO-T or HEPT. The polymethylene spacer was varied from n = 3-6. Thus, treatment of AZT with 2 equiv of 1,3-dibromopropane in dry acetone:DMF (1:1) and in the presence of K_2CO_3 gave the N-3 substituted derivative **12**. Subsequent reaction of **12** with TSAO-T or HEPT under basic conditions gave heterodimers **13** and **14** in 50-60% yield.



Scheme 1.3. *Reagents and Conditions*: (i) $(CH_2)_nBr_2$ (n = 3-9), K_2CO_3, acetone, DMF (1:1). (ii) K_2CO_3, acetone, DMF (1:1).

The most active compound in the series was [TSAO-T]-$(CH_2)_3$-[AZT] **13a** (EC_{50} = 0.10 μM) incorporating a short spacer. Heterodimers bearing polymethylene linkers [-$(CH_2)_n$-] with n = 4-6 showed good antiviral activity, while longer spacers with n > 7 showed diminished activity. The precursors to the dimers were also tested. Specifically, AZT derivatives such as **12** proved inactive irrespective of the chain length of the methylene spacer. The activity of the TSAO-T-spacer derivatives **13d** decreased with increasing length of the spacer. Replacement of AZT with d4T resulted in improved anti-HIV activity. No marked differences in activity were observed when the spacer consisted of 1-butynyl, 1-butenyl or ethoxyethyl moieties. The attachment of a propynyl spacer to the C-5 position of the pyrimidine base of AZT showed comparable antiviral activity to that of the AZT heterodimer **13a** bearing a propylene spacer between two N-3 positions, whereas the corresponding d4T analogue of **13b** led to a 5-fold decrease in anti-HIV potency.





	spacer
13b	$-CH_2-C\equiv C-$
13c	$-(CH_2)_3-$

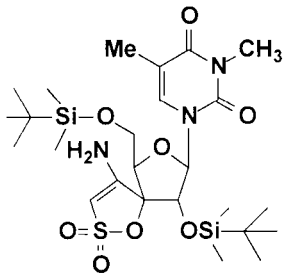
13d

Fig. 1.22

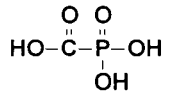
Spacer rigidity in the C-5 series did not markedly influence the antiviral potency since the heterodimer **13c** bearing a flexible propyl group as spacer was endowed with anti-HIV-1 activity comparable to that of the corresponding propynyl analogue **13b** (Fig. 1.22). Converting AZT, d4T and thymidine heterodimers to phenoxyphosphoramidate[72] heterodimers had no improved activity over the corresponding non-phosphorylated analogues. Overall, the d4T heterodimers had better inhibitory efficacy than AZT.

Combinations of TSAO-m^3T and foscarnet (PFA) (Fig. 1.23) as individual entities at a variety of concentrations revealed that these compounds displayed additive antiviral

activity.[73] These results inspired the synthesis of a single molecule combining a TSAO derivative with foscarnet through a labile phosphate ester bond. PFA is an effective antiviral agent approved for intravenous treatment of Human Cytomegalovirus (HCMV) Retinitis in patients with AIDS.[74] It is also effective against HIV replication and inhibits HIV-1 RT by blocking the pyrophosphate binding site.[75]

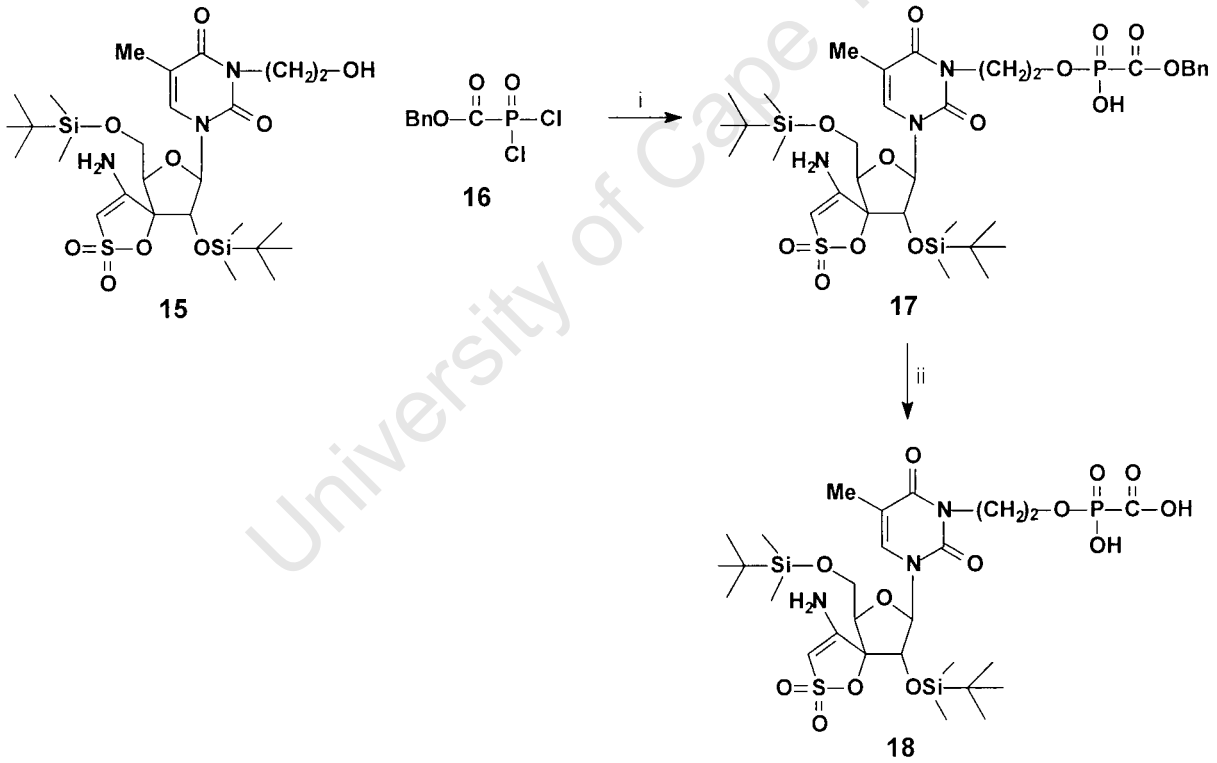


TSAO-m^{3}T



Phosphonoformate (PFA)

Fig. 1.23: Structure of TSAO-m^{3}T and Foscarnet



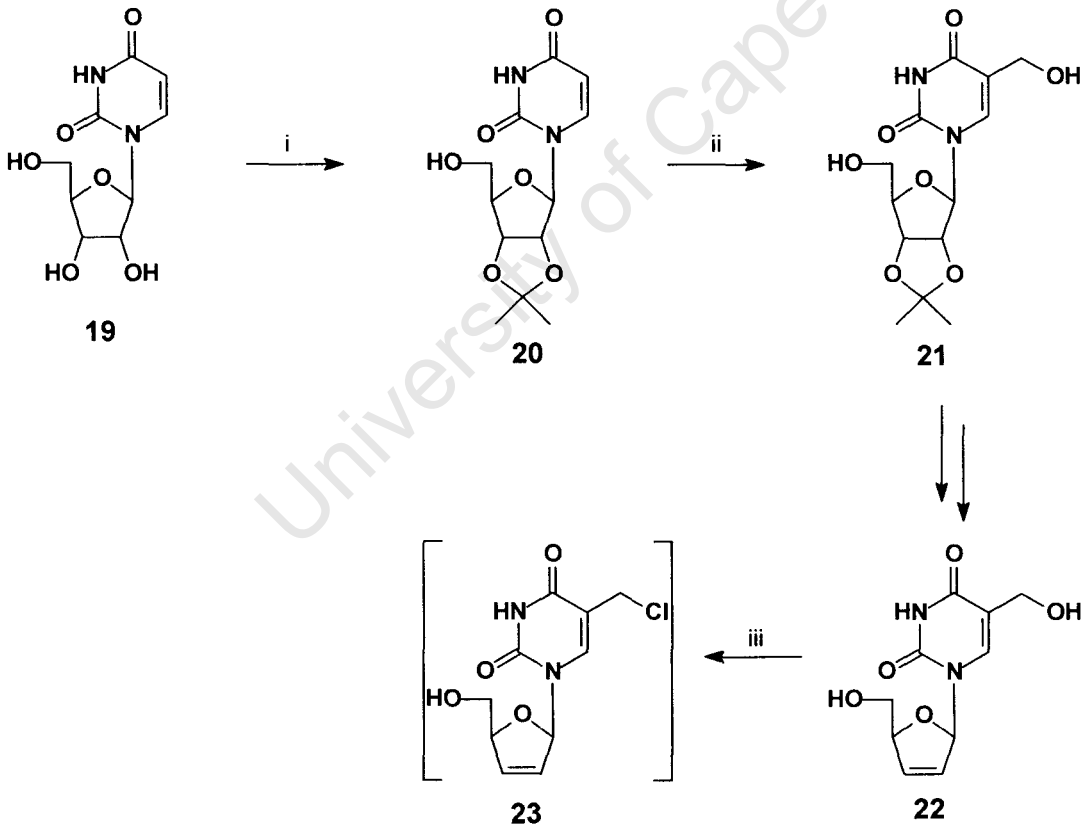
15 **16** **17** **18**

Scheme 1.4. *Reagents and Conditions*: (i) (a) **16**, Et_3N, CH_2Cl_2, -20 °C (b) H_2O; 54%. (ii) H_2, Pd/C, MeOH; 60%.

Thus, treatment of TSAO derivative **15** with the foscarnet-based phosphorylating reagent **16** in the presence of triethylamine led to the formation of the PFA diester **17** in moderate yield. Catalytic hydrogenolysis of **17** yielded the [TSAO-T]-[PFA] conjugate **18** in 60% yield (Scheme 1.4). Unfortunately, conjugate **18** was less active

than the parent compound **15**, displaying no additive or synergistic anti-HIV activity and thus indicating the activity to be due to the TSAO part of the molecule and not the PFA part.

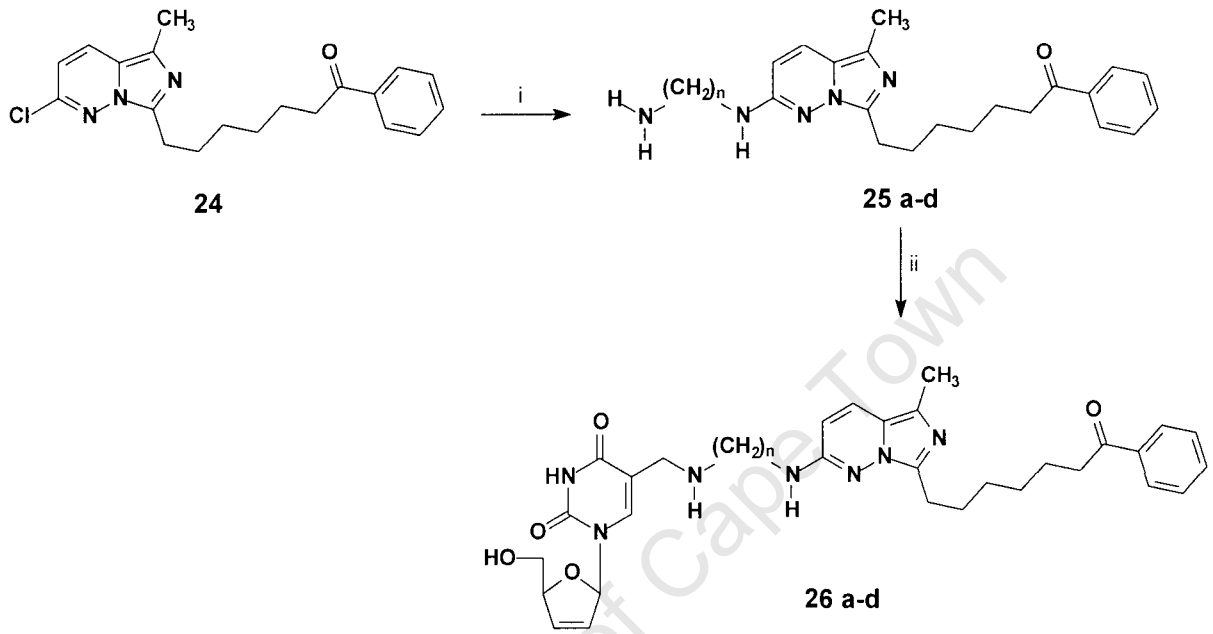
The design, synthesis and anti-HIV-1 evaluation of several non-cleavable heterodimers of the general formula [d4T]-NH$(CH_2)_n$–NH-[imidazo[1,5-b]pyridazine] (n = 6-12) involving a C-5 linkage to d4T was reported by Ladurée *et al.*[76] in 1998. Imidazo[1,5-*b*]pyridazine was chosen as the NNRTI due to its exceptional potency. The synthetic strategy involved synthesis of the NNRTI linked to a 1,n-diaminoalkyl spacer followed by substitution onto d4T via a C-5 chloromethyl group. The synthesis of the d4T coupling derivative (Scheme 1.5) involved C-5 hydroxymethylation of protected uridine **20** to give **21** in good yield followed by C-2'/3' double-bond introduction to afford **22** and selective chlorination of the hydroxymethyl group in the presence of the C-5' hydroxyl group of **22** to give **23**.



Scheme 1.5. *Reagents and Conditions*: (i) 2,2-DMP, APTS, MeCOMe. (ii) CH_2O, Et_3N, 60 °C; 93%. (iii) TMSCl, 1,4-dioxane, 60 °C.

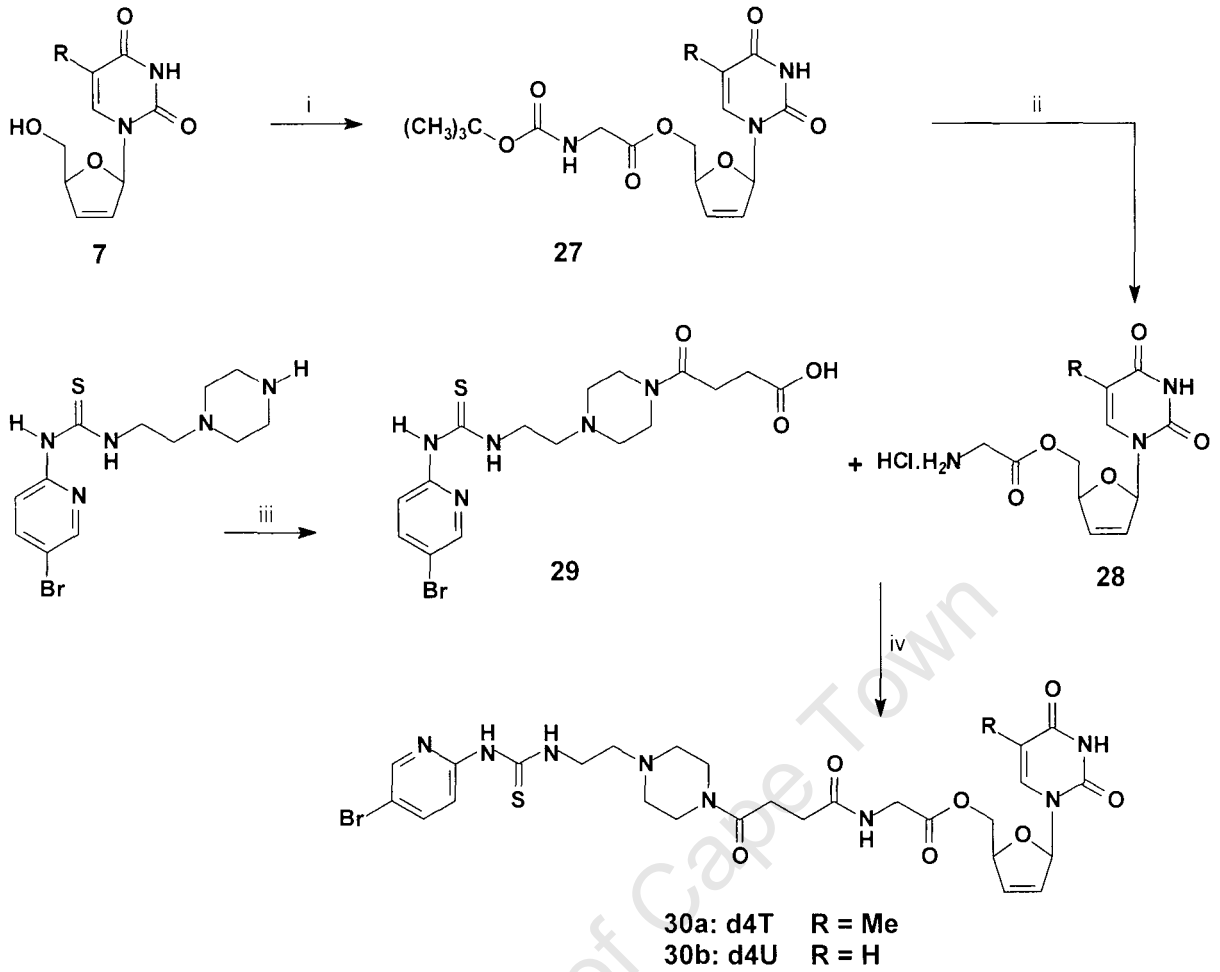
Imidazo[1,5-*b*]pyridazine **24** was synthesized in seven steps from alanine, and was heated with a 1,n-diaminoalkane at 120 °C to give monomers **25 a-d** via aromatic

nucleophilic substitution. Condensation of **25 a-d** with **23** in DMF gave heterodimers **26 a-d** in 72-85% yield (Scheme 1.6). The anti-HIV activity of the NNRTI linked to its spacer **25 a-d** was evaluated independently and the results revealed a decrease in activity compared to the unsubstituted NNRTI. The activity of the heterodimers **26a-d** was comparable to that of d4T and thus mainly due to the nucleoside part of the molecule.



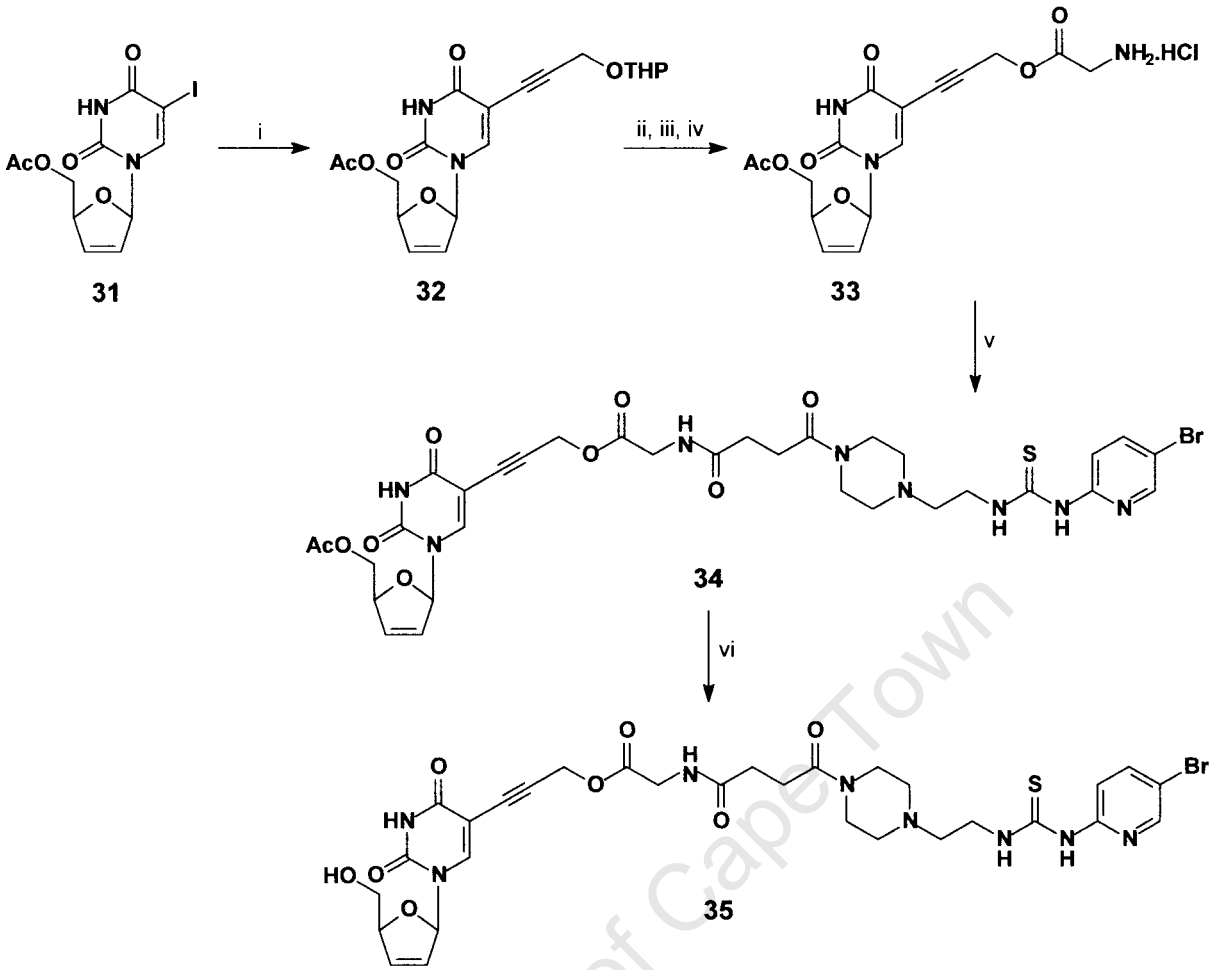
Scheme 1.6. *Reagents and Conditions*: (i) $H_2N–(CH_2)_n–NH_2$; a: n=6, b: n=8, c: n=10, d: n=12. (ii) d_4ClMUrd **23**, DIEA, DMF.

Similarly, the same group reported the synthesis of a NRTI/NNRTI heterodimer of the formula [NRTI]-Glycyl-Succinyl-[Trovirdine].[77] Nucleosides d4U and d4T were used as the NRTI and spacer linking was achieved via the C-5' hydroxyl group of NRTI with the *N*-piperazine of trovirdine. A succinyl-glycine moiety was chosen as a spontaneously cleavable linker, and introduced via coupling of the NRTIs with Boc-gly-OH using DCC in the presence of DMAP in DMF to give the 5'-esters **27**. Deprotection of Boc using 4M HCl/dioxane afforded the amine hydrochlorides **28** which were condensed with trovirdine analogue derivatives **29** using BOP/HOBt in the presence of triethylamine in DMF to afford the heterodimers **30 a-c** in 35-45% yield. The heterodimers were unfortunately devoid of antiviral activity at non-toxic concentrations (Scheme 1.7).



Scheme 1.7. *Reagents and Conditions*: (i) HOOC–CH_2–NHBoc, DCC, DMAP, DMF; 78%. (ii) 4M HCl/Dioxane; 87%. (iii) succinic anhydride, CH_2Cl_2; 64%. (iv) Et_3N, HOBt, BOP, DMF; 35-45%.

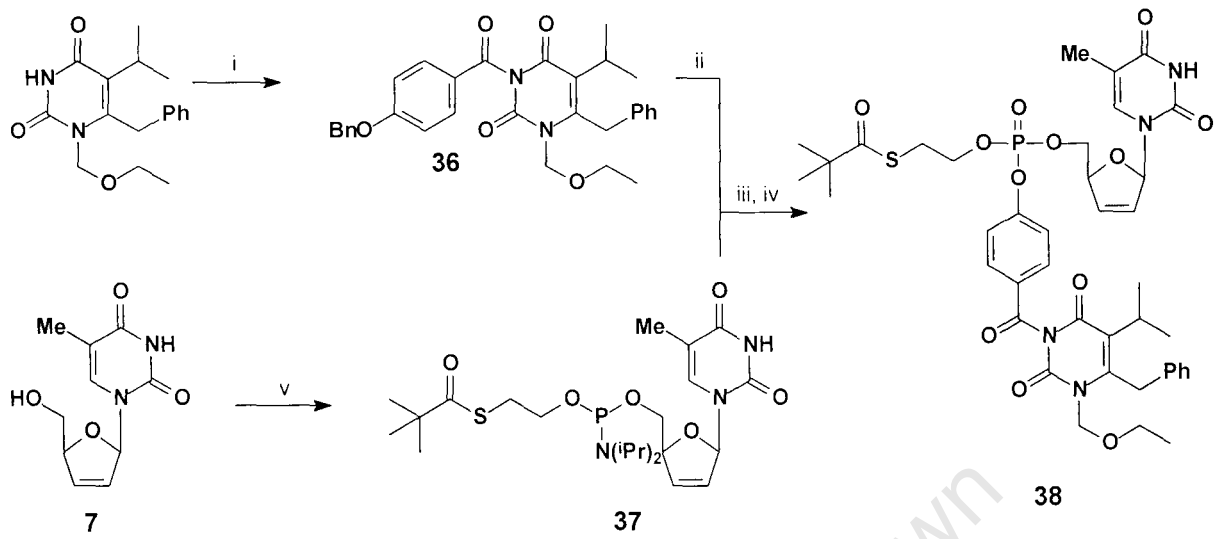
The synthesis by of an analogous system using C-5/NRTI as the attachment point[78,79] (Scheme 1.8) was accomplished employing a Sonogashira reaction. Pd(0) mediated Sonogashira coupling at C-5 of **31** with a C-3 propynyloxy spacer protected as its THP ether produced **32**. Deprotection of THP using CF_3COOH in CH_2Cl_2 followed by condensation of the resultant alcohol with Boc-Gly-OH using DCC in the presence of DMAP and subsequent deprotection of the Boc group using 4M HCl in dioxane afforded the amine hydrochloride **33**. Condensation of amine **33** with trovirdine derivative **29** followed by deprotection of the acetate group with cyanide ion gave the heterodimer **35** in 35-45% yield for the last step. The heterodimers displayed inferior anti-HIV activity (compound **35** displayed an IC_{50} > 20 μM) compared to the parent compounds. The lack of activity was attributed to the wrong positioning of the linker to either NRTI or NNRTI with respect to their active sites in the enzyme.



Scheme 1.8: *Reagents and Conditions*: (i) alkyne, $(PPh_3)_4Pd$, CuI, Et_3N, DMF; 67%. (ii) CF_3COOH, CH_2Cl_2, CH_3OH (iii) $HOOC–CH_2–NHBoc$, DCC, DMAP, DMF (iv) 4M HCl/Dioxane (v) **29**, Et_3N, HOBt, BOP, DMF; 48%. (vi) NaCN, MeOH; 38%.

Pedersen *et al.*[80] has recently reported the synthesis and antiviral activities of double-prodrugs against HIV based on the mixed *S*-acyl-2-thioethyl (SATE) prodrug approach. The SATE prodrugs were first introduced in 1993 by Imbach *et al* as a carboxyesterase-labile protecting group for the ddU nucleotide.[81] The double pro-drug in question incorporated d4T as a 5'-phosphate and as a SATE ester linking through the phosphate to N-3 of MKC-442 (a HEPT derivative) via a cleavable *p*-hydroxybenzoyl linker. The synthesis involved protecting the N-3 position of MKC-442 with a *p*-benzyloxybenzoyl group to give **36** in 86% yield. The amidite **37** was successfully reacted with the debenzylated analogue of **36** using DIEA as a base and tetrazole as an activating agent, which was followed by oxidation with *tert*-butylhydroperoxide to give **38** as a mixture of two diastereomers in 38% overall yield for the 3 steps (Scheme 1.9). The double-prodrug **38** had good activities against HIV-

1 (EC_{50} = 0.03 μM) and Y181C mutant (EC_{50} = 2.7 μM). The authors conclude that compound **38** acts as a prodrug and that the active drug contains the d4T moiety.



Scheme 1.9. *Reagents and Conditions*: *p*-Benzyloxybenzoic acid chloride, DIEA, pyridine; 86%. (ii) H_2, Pd/C (iii) **37**, 1*H*-tetrazole, CH_3CN (iv) *tert*-butylhydroperoxide; 38% (3 steps). (v) (*S*-pivaloyl-2-thioethyl)-*N,N*-bis(diisopropylamino)phosphine, DIA, 1*H*-tetrazole, CH_3CN; 94%.

In conclusion, most approaches have pursued the easier synthetic option of connecting the tether via the C-5' OH or N-3 of the nucleoside base. For the C-5 heterodimers, linker attachment has been achieved via a Pd(0) Sonogashira coupling (compound **35**) or hydroxymethylation of uridine (compound **26a-d**). Most of the heterodimers were designed as prodrugs linked with a cleavable tether, with the aim of regenerating the parent drugs once in the cell cytoplasm.

1.10 Objective of the Study

The use of combinations of different drugs is designed to prevent resistant HIV strains by effectively suppressing viral replication, thus denying HIV the opportunity to produce new mutations. This principle holds provided there is minimised cross-resistance between the drugs. NNRTIs act by slowing down the chemical step catalyzed by RT, and this retardation allows the two-step binding of NRTI to come to equilibrium leading to tighter binding of the nucleotide. The research in this thesis aimed to:

(i) Synthesize and evaluate the anti-HIV activity of heterodimers of the general formula [d4T]-spacer-[HI-236] involving a biologically non-

cleavable spacer, in the search for evidence of synergism between the two drugs and hence sites.

(ii) Evaluate a structure-activity relationship by using different types of linkers.

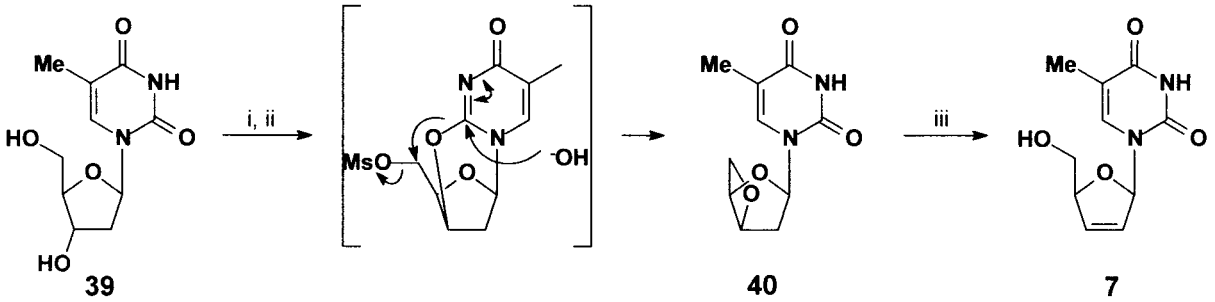
(iii) Synthesize and evaluate the anti-HIV activity of HI-236 linked to a spacer.

HI-236 was selected due to its potency against wild-type and NNRTI resistant HIV-1 strains (carrying the NNRTI resistant mutations K103N, V106A and Y181C). Furthermore, the relative simplicity of its structure, ease of its synthesis and options for spacer attachment also made HI-236 an attractive NNRTI choice. D4T is currently used to treat HIV, and was chosen due to its higher inhibitory effect and lower toxicity compared to AZT.

1.11 Strategies for the Synthesis of d4T

1.11.1 Synthesis of d4T from 2'-deoxythymidine

Horwitz *et al.*[82] reported the first synthesis of d4T **7** in 1964 as a potentially novel anti-cancer drug. The strategy involved conversion of 3',5'-anhydrothymidine **40** to d4T by a base-mediated elimination reaction. Thus, 2'-deoxythymidine **39** was readily converted into 1-(3',5'-anhydro-2-deoxy-β-D-threo-pentofuranosyl)-thymine **40** by converting it first into its 3',5'-di-*O*-mesyl derivative, and then by heating the crude product under reflux with an excess of aq. sodium hydroxide. Oxetane formation from the dimesylate of **39** proceeded via intermediacy of the pyrimidine base with the ring closing step as shown. Elimination of oxetane **40** was achieved by treatment with *t*-BuOK in DMSO (Scheme 1.10).

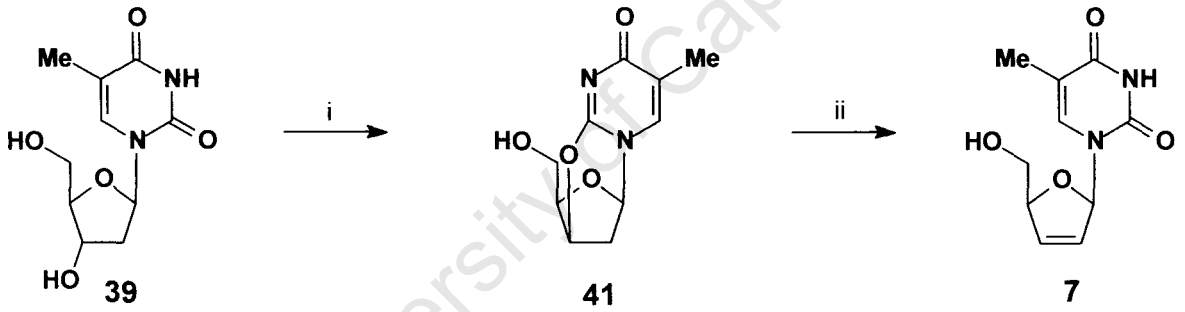


Scheme 1.10. *Reagents and Conditions*: (i) $MeSO_2Cl$, C_6H_5N (ii) aq. NaOH, reflux (iii) *t*-BuOK, DMSO; 57%.

Prolonged exposure of d4T to strong base at high temperature led to decomposition to give thymine as an undesired product thereby decreasing the overall yield.

In an attempt to overcome the decomposition of d4T just mentioned, Mansuri *et al.* [83] precipitated the 5'-alkoxide potassium salt of d4T by adding solvents such as toluene and acetone during work-up. Skonezny *et al*,[84] in a US patent, disclosed an improved process for producing d4T from the oxetane intermediate **40** by eliminating with potassium hydroxide in isopropanol.

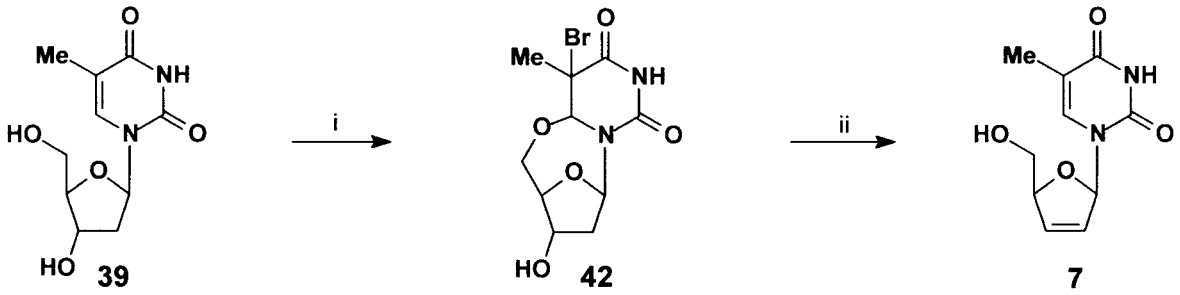
In a modified procedure of Horwitz, Reese *et al.* (Scheme 1.11) converted 2'-deoxythymidine **39** directly in one step into anhydro derivative **41** in 68% yield via a 3'-sulfite ester leaving group. The key elimination reaction to form d4T in 76% yield was achieved by reacting anhydro compound **41** with NaH in *N,N*-dimethylacetamide at 100 °C.[85]



Scheme 1.11. *Reagents and Conditions*: (i) $(PhO)_2SO$, 1-methylimidazole, DMA; 65%. (ii) NaH, DMA, 100 °C; 81%.

Lipshutz *et al.* [86] developed a method based on transient cyclonucleoside formation across the C-5'-oxygen of the sugar and C-6 of the pyrimidine base (Scheme 1.12) as a protection of the C-5' hydroxyl group, thereby permitting introduction of unsaturation into the sugar moiety prior to unravelling via *β*-elimination to the target nucleoside. The cyclonucleoside **42** was prepared by an electrophilic addition reaction of deoxythymidine with *N*-bromosuccinimide and catalytic trifluoroacetic acid (TFA) in DMF. In a 'one pot' procedure, the alcohol was then treated with trifluoromethanesulfonic anhydride in pyridine/THF followed by DBU to introduce the 2',3'-double bond via elimination of the triflate, followed by reductive elimination of the

bromine and alkoxy groups in the base with zinc dust in acetic acid to furnish d4T in good overall yield.



Scheme 1.12. *Reagents and Conditions*: (i) NBS, DMF, cat. TFA; 62%. (ii) (a) Tf_2O/pyr, THF (b) DBU (c) Zn/HOAc; 82% (3 steps).

1.11.2 Synthesis of d4T from 5-methyluridine

Luzzio *et al.*[87] demonstrated an efficient synthetic route to nucleoside olefins in the uridine and thymidine series, utilizing a Garegg-Samuelsson[88] iodine/triphenylphophine/imidazole-promoted deoxygenation of the 2',3'-hydroxyl groups as the key step (Scheme 1.13). The mechanism of the reaction involves reaction of imidazole with triphenylphosphine diiodide to form an imidazolephosphonium salt **I**. Complex **I** then reacts with the vicinol diol to form bis-oxyphosphonium salt **II**. Nucleophilic substitution of one of the oxyphosphonium groups of **II** to form **III** by iodide followed by iodide-mediated elimination furnishes alkene **IV** (Fig. 1.24).

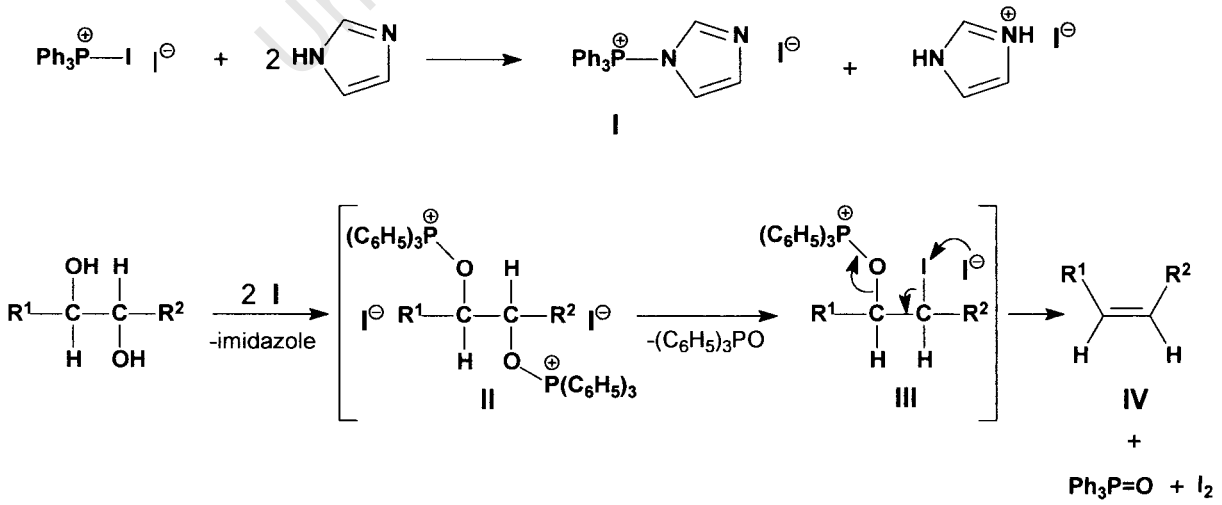
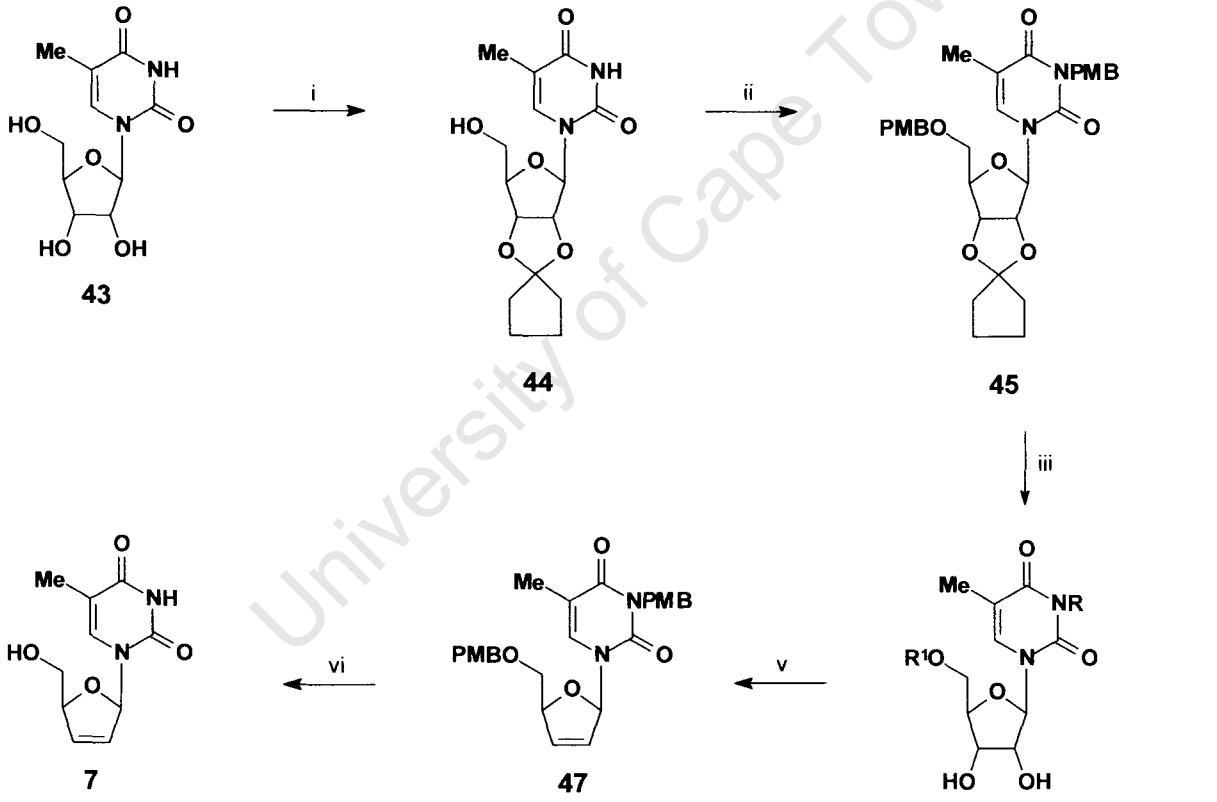


Fig. 1.24: Mechanism of Garegg-Samuelsson elimination

In order to avoid anhydronucleoside formation during olefination, the synthesis of **46** required a selective C-5' hydroxyl protection. Thus, protection of the 2',3'-hydroxyl of 5-methyluridine **43** with 1,1-dimethoxycyclopentane and *p*-toluenesulfonic acid provided the cyclopentylidene ketal **44** in excellent yield. Protection of the C-5' hydroxyl group of **44** as its N^3-5'-*O*-dibenzyl or *p*-methoxybenzyl ether **45** was accomplished in 93% yield by treatment with NaH/BnBr (or PMBCl) in 1,2-dimethoxyethane. Deprotection of the cyclopentylidene group was accomplished by employing 30% aq. TFA to furnish dibenzyl diol **46a**, which was subjected to the olefination conditions to give the desired olefin **47** in good yield. However, the procedure is sensitive to the C-5' hydroxyl protecting group. Since olefination of the 5'-silylated derivative **46b**, obtained directly from 5-methyluridine without the need to protect/deprotect the C-2' and C-3' hydroxyl groups, was unsuccessful.



Scheme 1.13. *Reagents and Conditions*: (i) *p*-toluenesulfonic acid, 1,1-dimethoxycyclopentane; 95%. (ii) 4-methoxybenzyl bromide, NaH, DME; 75%. (iii) aq. dichloroacetic acid (66%); 96%. (iv) TBDMSCl, imidazole, DMF (v) I_2, PPh_3, imidazole, toluene/CH_3CN (2:1); 82%. (vi) CAN, CH_3CN/H_2O; 68%.

By comparison, Mansuri *et al.*[89] explored three other methods for the conversion of the vicinol diol functionality of nucleosides to olefinic analogues. The methods

investigated included the Corey-Winter[90] reaction, olefin formation from 2',3'-*O*-methoxymethylidene cyclic ortho ester **49** and reductive elimination of 2',3'-haloacetates **52**. In the Corey-Winter procedure (Scheme 1.14), 5-methyluridine **43** was protected as its 5'-*O*-trityl ether and then converted to 1,3-dioxolane-2-thione (cyclic thionocarbonate) **48**, which fragmented with triethyl phosphite to furnish the protected olefin. However, the overall yield of protected olefin from uridine was only 30%. Deprotection of the trityl group under acidic conditions resulted in cleavage of the glycosidic bond, leading to an overall yield of 12% of the desired olefin **7**. The mechanism of the Corey-Winter step involves attack by the thiophilic phosphorus at sulphur, followed by a chelotropic elimination of CO_2 with expulsion of triphenylphosphine sulphide (Fig. 1.25).

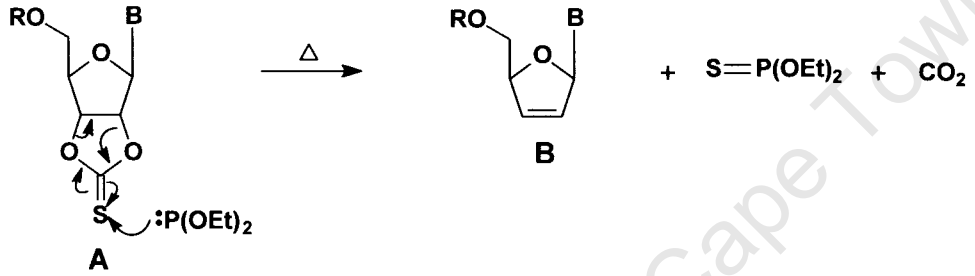
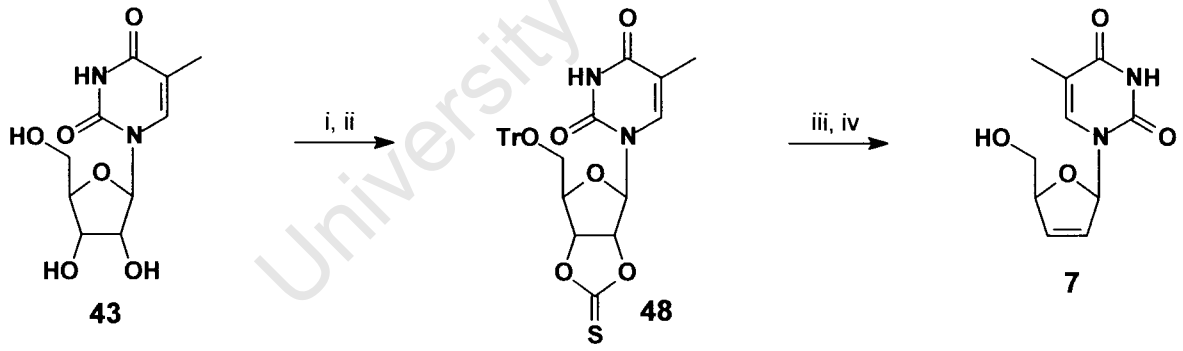


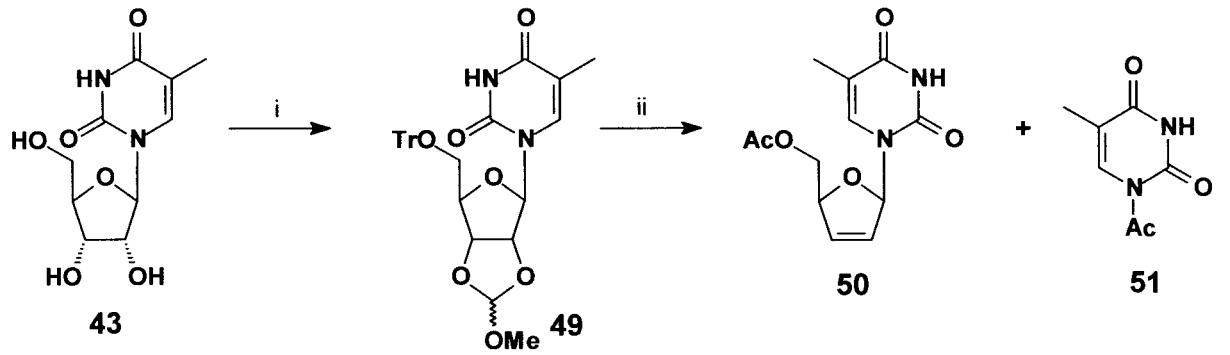
Fig. 1.25: The mechanism of Corey-Winter deoxygenation



Scheme 1.14. *Reagents and Conditions*: (i) Ph_3CCl, pyridine (ii) 1,1'-thiocarbonyldiimidazole, THF; 67% (2 steps). (iii) triethyl phosphite, 160 °C; 45%. (iv) cat. *p*-TsOH, $CHCl_3$/MeOH (5:1); 40%.

In the second approach (Scheme 1.15), 2-methoxy-1,3-dioxolane **49** was subjected to an acid-catalysed fragmentation to give the olefin **50** in 2 steps and 22% overall yield. In this case, anomeric cleavage of the olefin also occurred to form N^1-acetyl uracil **51**. Addition of a base (triethylamine or $NaHCO_3$) to neutralise the acid did not improve the yield. The mechanism for this elimination involves protonation of the

methoxy group to form **I** followed by expulsion of methoxyl group under anchimeric assistance to form **II**, and fragmentation with loss of CO_2 to form the olefin (Fig. 1.26).



Scheme 1.15. *Reagents and Conditions*: (i) trimethyl orthoformate, pyridinium *p*-toluenesulfonate, THF, rt; 68%. (ii) *p*-TsOH, acetic anhydride, 140 °C; 40%.

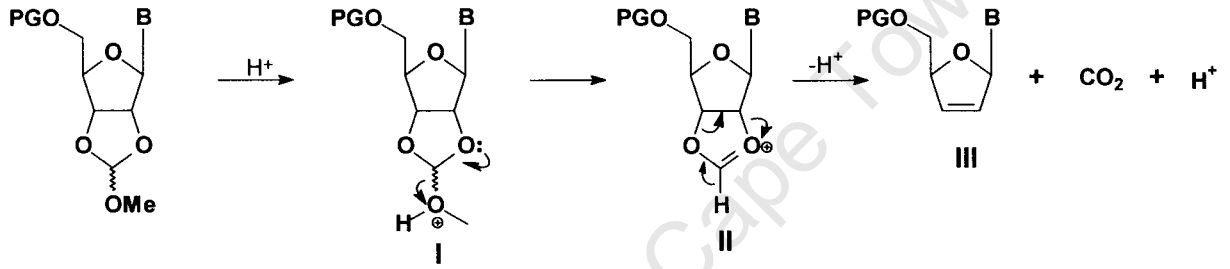
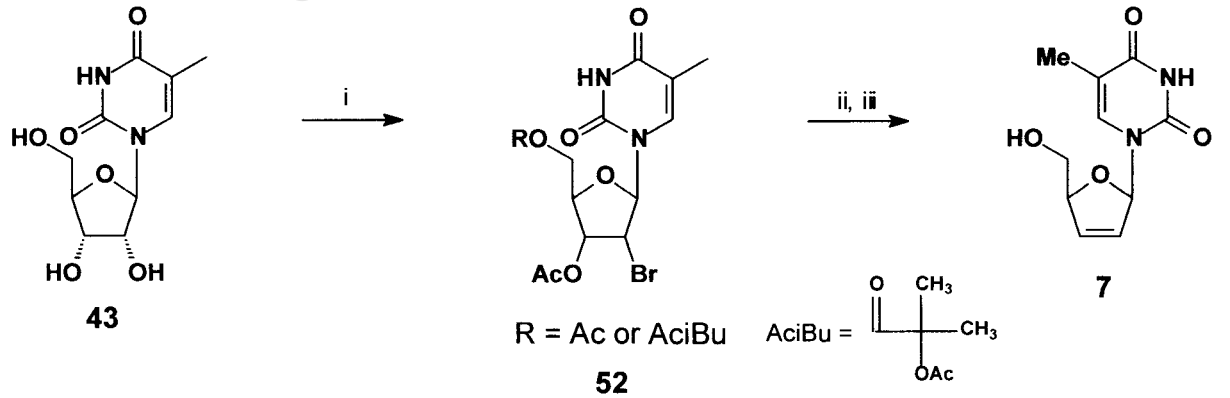


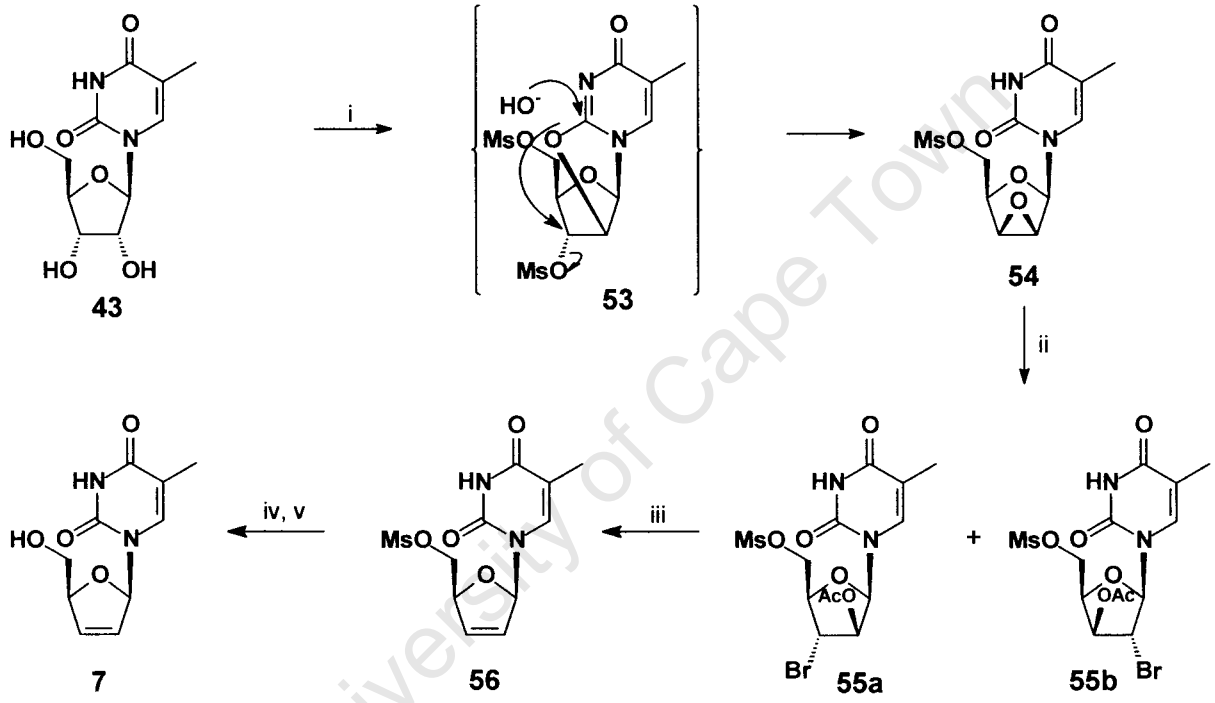
Fig. 1.26: The mechanism of reductive elimination of orthoesters

The third method (Scheme 1.16) involved reacting uridine or thymine with Mattock's bromide (2-acetoxyisobutyryl bromide)[91] to give a mixture of acetates **52**, which were reduced with activated zinc-copper couple to give the desired olefinic product in 38% overall yield. A major limitation of this method was the formation of large amounts of thymine by-product during the reductive syn-elimination of bromoacetate.



Scheme 1.16. *Reagents and Conditions*: (i) 2-acetoxyisobutyryl bromide, CH_3CN; 67%. (ii) Zn/Cu, DMF, rt (iii) NH_3, MeOH; 55%.

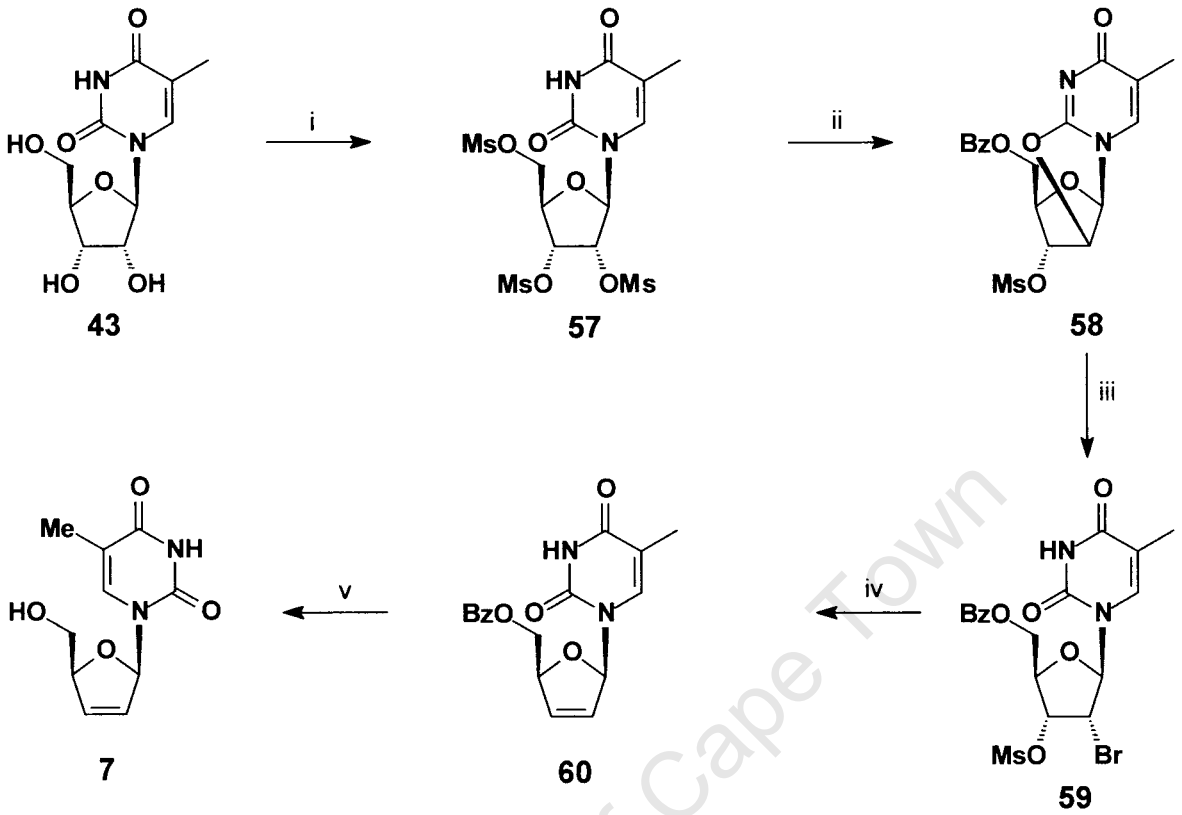
Chen *et al.* (Scheme 1.17) from the Bristol-Myers Squibb group, minimized extensive nucleoside bond cleavage by inverting the stereochemistry to proceed via a *trans* haloacetate. This was achieved by base-mediated conversion of the trimesylate of **43** into epoxide **54** via intermediate **53,** which opened with acetyl bromide to afford a regioisomeric mixture of *trans* bromoacetates **55a-b**. β-Elimination with Zn/Cu couple as before afforded 5'-mesyl d4T **56** under mild reductive elimination conditions, without significant nucleoside bond cleavage[92] as a result of superior *trans* stereoelectronic alignment in the elimination step.



Scheme 1.17. *Reagents and Conditions*: (i) (1) MsCl, NMM, acetone (2) 1N $NaOH/H_2O$; 82%. (ii) (1) AcBr, MeOH (2) AcBr, CH_2Cl_2; 92%. (iii) Zn/Cu, MeOH; 88%. (iv) $PhCO_2Na$, DMF; 91%. (v) $BuNH_2$; 90%.

Alternatively, the use of a sulfonyl group at the 3'-position of 5-methyluridine instead of an acetyl group in the syn bromomesylate **59** completely avoided the undesired cleavage of the base (Scheme 1.18). D4T was prepared employing this methodology in 75% overall yield starting from the readily available ribonucleoside 5-methyluridine.[93] The key step in the synthesis was the zinc-induced reductive elimination of bromomesylate **59**, which afforded d4T without nucleoside bond cleavage. Sodium benzoate acted as a base to form anhydro ring **58** as well as a

nucleophile to displace the 5'-mesyl group, preparing the way for the final facile deprotection with butylamine.



Scheme 1.18. *Reagents and Conditions*: (i) MsCl, NMM, acetone; 97%. (ii) $PhCO_2Na$, CH_3CONH_2; 90%. (iii) AcBr, MeOH, EtOAc; 98%. (iv) Zn, EtOAc/MeOH, cat. AcOH; 97%. (v) $BuNH_2$, 70 °C; 90%.

D4T has also been synthesized by ring-closing metathesis[94] (Scheme 1.19, Fig. 1.27). This methodology necessitated the simultaneous creation of two vinyl groups to afford **62**, which in turn led to a new route to 2',3'-didehydro-2',3'-dideoxynucleosides. Treatment of 5-methyluracil derivative **62** with Grubbs' reagent in DCM resulted in efficient ring closure to form the protected d4T **63**.[95]

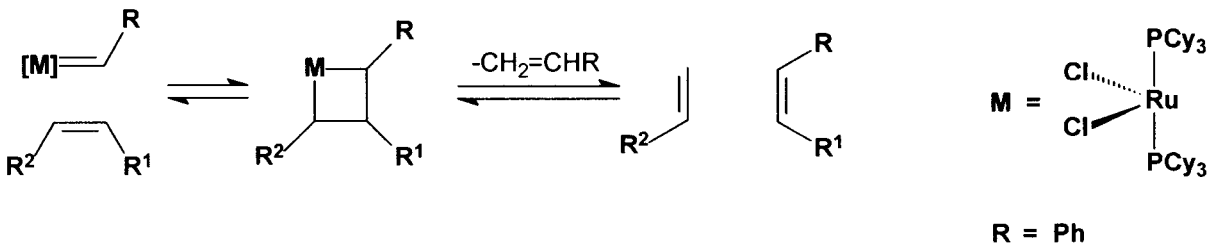
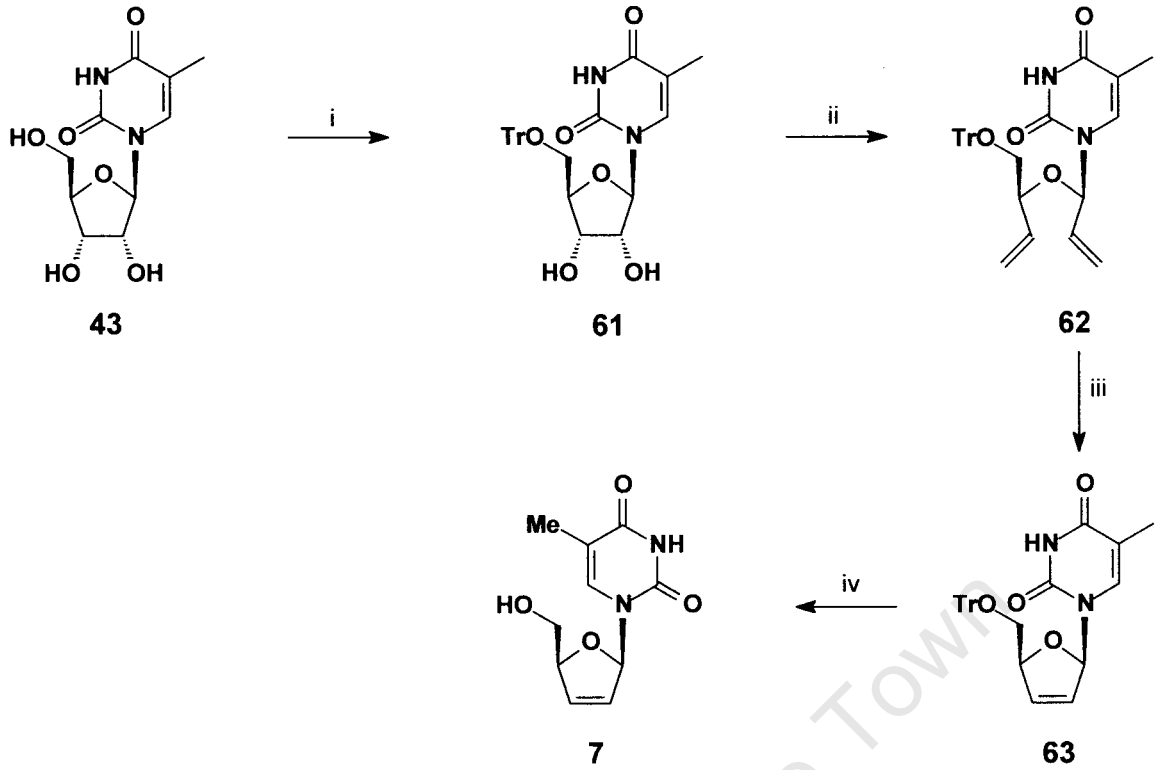


Fig. 1.27: Mechanism of olefin metathesis

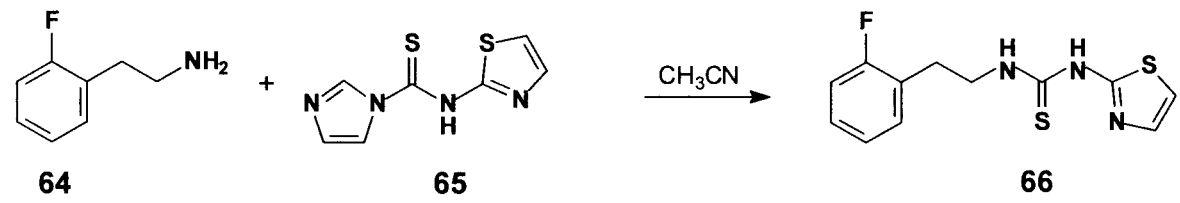


Scheme 1.19. *Reagents and Conditions*: (i) TrCl, pyridine (ii) (a) $NaIO_4$, $EtOH/H_2O$ (b) Ph_3PCH_3Br, *t*-BuOK, toluene (iii) AcOH; 80% (iv) Grubbs' reagents, CH_2Cl_2.

Apart from the Bristol–Myers-Squibb synthesis, most of the syntheses are only amenable to producing research quantities of d4T and not for production purposes, because of the involvement of protecting groups.

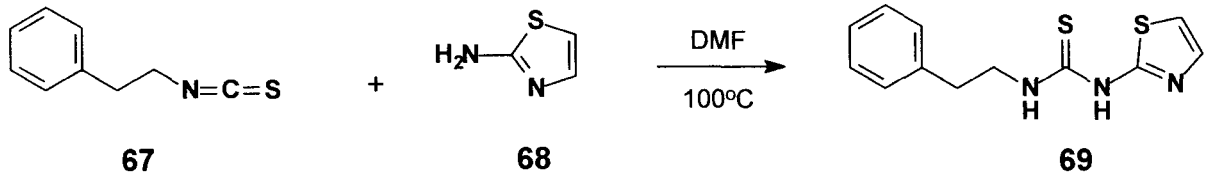
1.12 Synthesis of PETT

Phenethylthiazolylthiourea (PETT) compounds were first synthesized in 1995 by Bell *et al.*[96] as potent inhibitors of HIV-1, resulting in a structure-activity relationship profile amongst various substituents in their structure. The PETT derivatives were synthesized according to two general methods. In the first route, thiourea analogue **66** was prepared by condensation of the amine **64** with the thiocarbonyl reagent **65** derived from 2-aminothiazole and thiocarbonyldiimidazole (Scheme 1.20).



Scheme 1.20: Synthesis of PETT compounds from thiocarbonylimidazole thiazole.

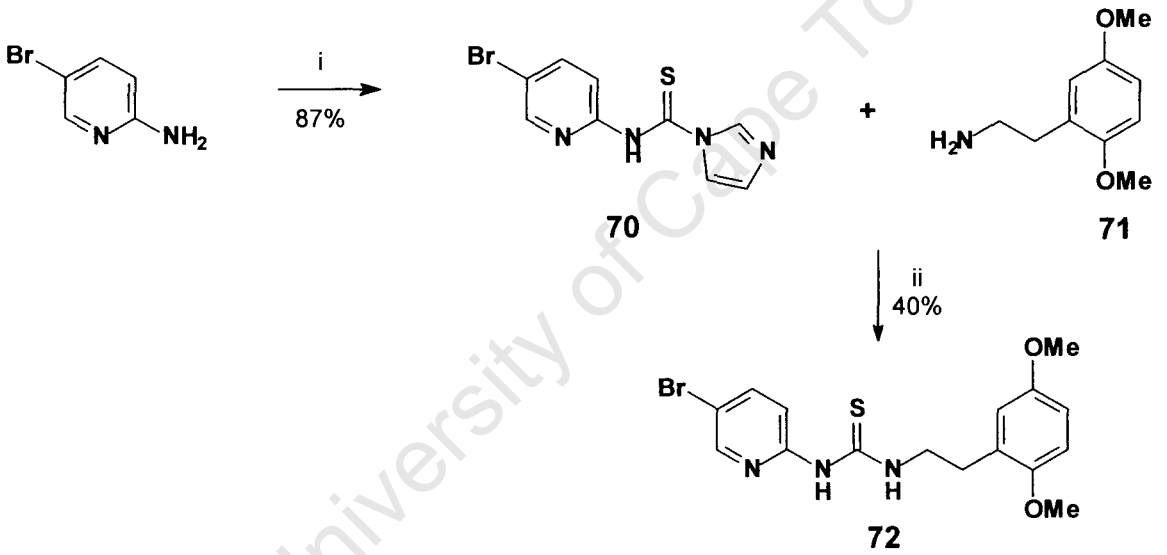
The second method (Scheme 1.21) to the thiourea analogue **69** involved reacting phenethyl isothiocyanate **67** with a heterocyclic amine (2-aminothiazole) **68**.



67 **68** **69**

Scheme 1.21: Synthesis of PETT compounds from phenethyl isothiocyanate.

HI-236 **72** was synthesized according to the first method by condensation of amine **71** with thiocarbonyl reagent **70** derived from 1,1'-thiocarbonyldiimidazole and 2-amino-5-bromo pyridine (Scheme 1.22).[97]



70 **71** **72**

Scheme 1.22. *Reagents and Conditions*: (i) 1,1'-thiocarbonyldiimidazole, acetonitrile, rt, 12-15h. (ii) DMF, 100 °C, 16h.

CHAPTER 2

Synthesis of Thiourea Derivatives for this Thesis

2.1 Synthesis of Thiourea derivatives

Various thiourea derivatives (Fig. 2.1) were prepared either to probe the origin of HIV activity in the bifunctional compounds or as models and/or intermediates in the synthesis. Thus, two NNRTIs linked to a spacer (**77** and **78**) were synthesized and evaluated for possible anti-HIV activity. Thiourea derivatives **74-76** were model compounds synthesized to optimize conditions for the final condensation step. Compound **73** was synthesized as a substrate for alkylation in the synthesis of target compound **95** (Chapter 4). Compound **79** was an intermediate in the synthesis of **73**. The NNRTIs synthesized are shown in Fig. 2.1. This Chapter describes their synthesis.

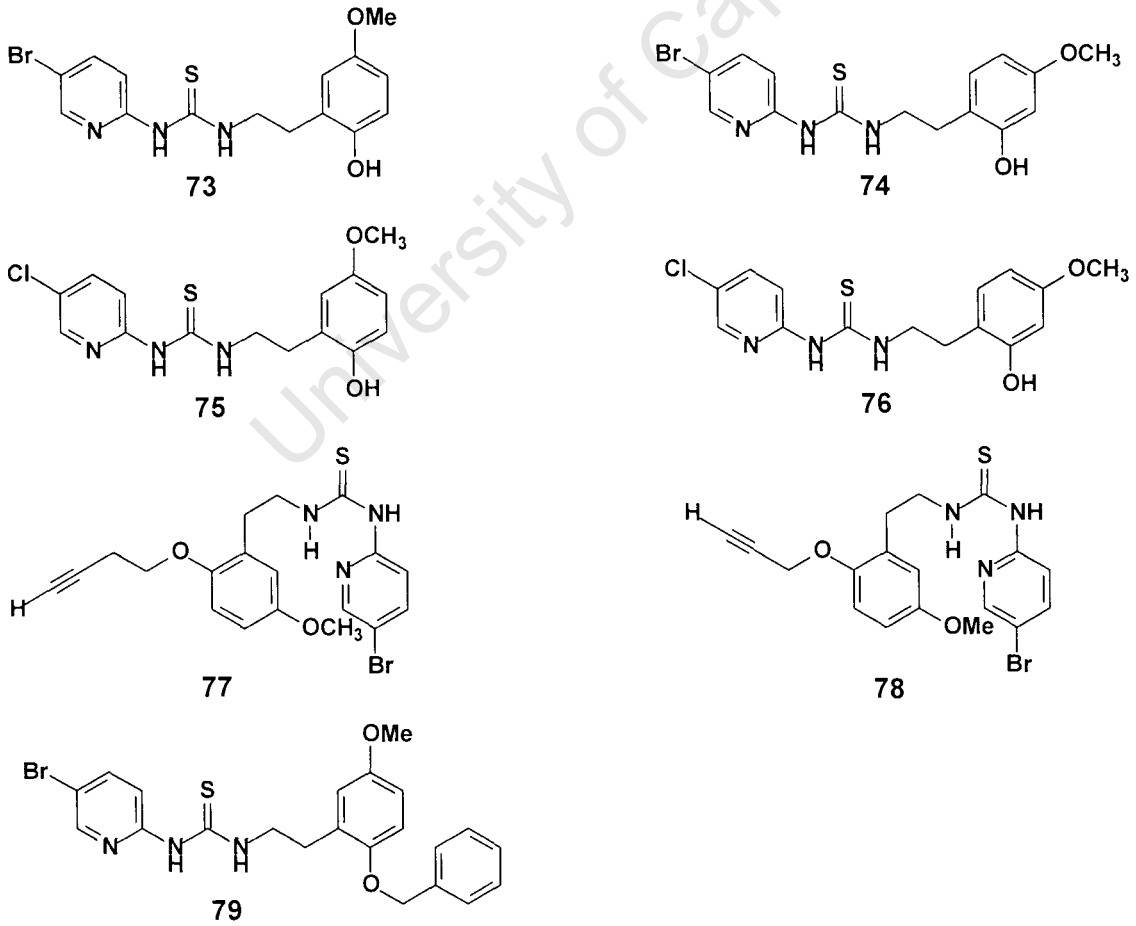


Fig. 2.1: Structure of NNRTI and NNRTI linked to a spacer

The retrosynthetic analysis of **73** (Fig. 2.2) revealed amine **80** derived from reduction of Henry derivative **83** to be a key intermediate. Converting **80** to **73** could be envisaged to proceed via two routes depending on the timing of introduction of the thiourea moiety. The first route (**a**) involved condensation of amine **80** with thiourea reagent **89** to give **79**. The second route (**b**) involved extra steps as a result of protection of amine **80** with a Boc group. The resultant Boc-protected amine was considered to be an attractive intermediate to purify. Catalytic hydrogenolysis of **82** would furnish **81** and this substrate was considered to be a key intermediate in the synthesis of the target molecules via alkylation.

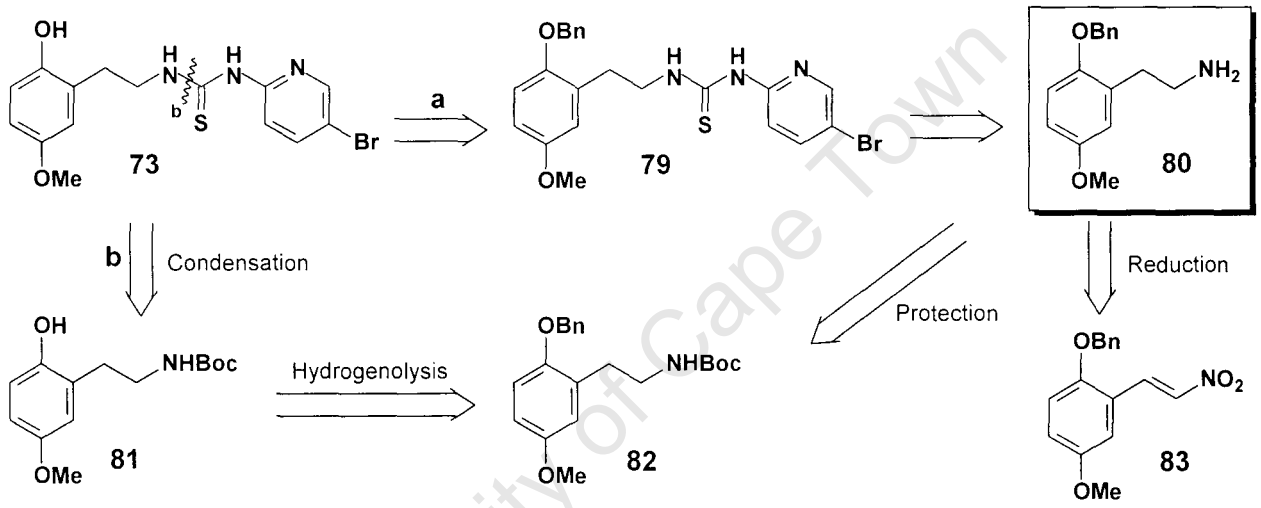


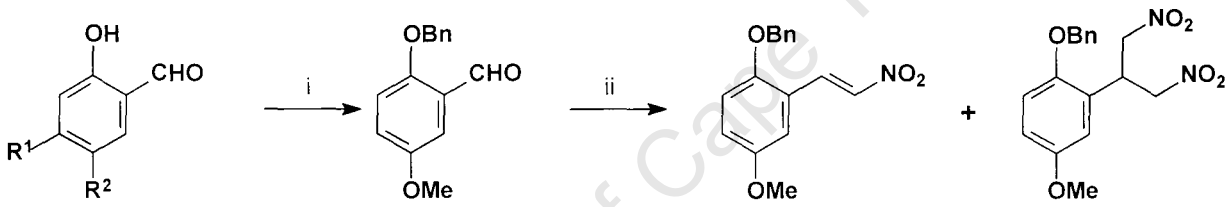
Fig. 2.2: Retrosynthetic analysis of thiourea derivatives

The starting amine **80** required in the synthesis of phenylethylbromopyridylthiourea (PETT) derivative **73** is not commercially available and was thus synthesized in 6 steps from aldehyde **84a** which is expensive. Thus, for optimisation purposes in the synthesis of **80**, the regioisomer and less expensive aldehyde **84b** was used (Scheme 2.1). However, for clarity, this series won't be described, since it served its purpose as a model sequence with no complications on transposing to the desired series.

Thus, for the actual synthesis, the phenolic hydroxyl group of aldehyde **84a** was first protected as its benzyl ether to give **85** in good yield. Its ^{1}H NMR spectrum displayed diagnostic peaks at δ_H 7.41 (5H, m) for the aromatic protons and a singlet at δ_H 5.15 for the benzylic methylene group, while the ^{13}C NMR spectrum displayed

corresponding signals at δ_C 136.3, 128.7, 128.2 and 127.3 for the aromatic carbons and δ_C 71.4 for the benzylic carbon. The melting point (44-45 °C) of a recrystallised sample was in agreement with the literature value[98] 47-48 °C, and this sample returned an acceptable combustion microanalysis.

In the next step, the benzylated aldehyde **85** was condensed with nitromethane in a Henry reaction[99] at 70 °C using ammonium acetate as promoter to give nitrostyrene derivative **83** in 75% yield and as a bright yellow solid after recrystallization from EtOH. Condensation of the aldehyde with nitromethane at reflux resulted in the desired product together with by-product **86** which was formed as a result of subsequent Michael addition onto **83**. Thus, the temperature of the reaction was carefully monitored.



84a: R^1 = H, R^2 = OMe
84b: R^1 = OMe, R^2 = H

85 **83** **86**

Scheme 2.1. *Reagents and Conditions*: (i) BnBr, K_2CO_3, EtOH, reflux, 16 h; 95%. (ii) CH_3NO_2, NH_4OAc, 70 °C, 14 h; 75%.

Mechanistically, the ammonium acetate provides both a proton source to activate the carbonyl oxygen as well as a base to abstract the acidic protons on nitromethane. The nitromethane anion formed then attacks the electrophilic carbonyl carbon generating intermediate **88**. An *E2* elimination of water subsequently results in the formation of *β*-nitrostyrene **83** (Fig. 2.3). At higher temperatures, the nitrostyrene **83** acts as a Michael acceptor and undergoes a conjugate addition with a second nitromethane anion to form by-product **86** which was isolated when the reaction was conducted over a prolonged period at reflux (≈ 78 °C).

The spectroscopic and analytical data for **83** was in agreement with the assigned structure. The ^{1}H NMR spectrum of **83** displayed two doublets at δ_H 8.12 and δ_H 7.79 for the nitroethenyl group with a large coupling constant $J_{1,2}$ of 13.4 Hz, while its ^{13}C

NMR spectrum displayed diagnostic signals at δ_C 136.1 for C-1 and δ_C 135.4 for C-2. The Mp of this compound was in agreement with the literature value.[99] The absence of aldehydic signals for both the ^1H (at δ_H 10.51) and ^{13}C (at δ_C 189.4) NMR spectra of **83** confirmed the assigned structure. The large coupling constant between H-1 and H-2 indicated that these protons are *trans* to each other and thus an *E*-stereochemistry. If the protons were *cis*, the coupling constant J_{cis} would have been between 10-12 Hz. The *E*-geometry is consistent with an *E*2 elimination.

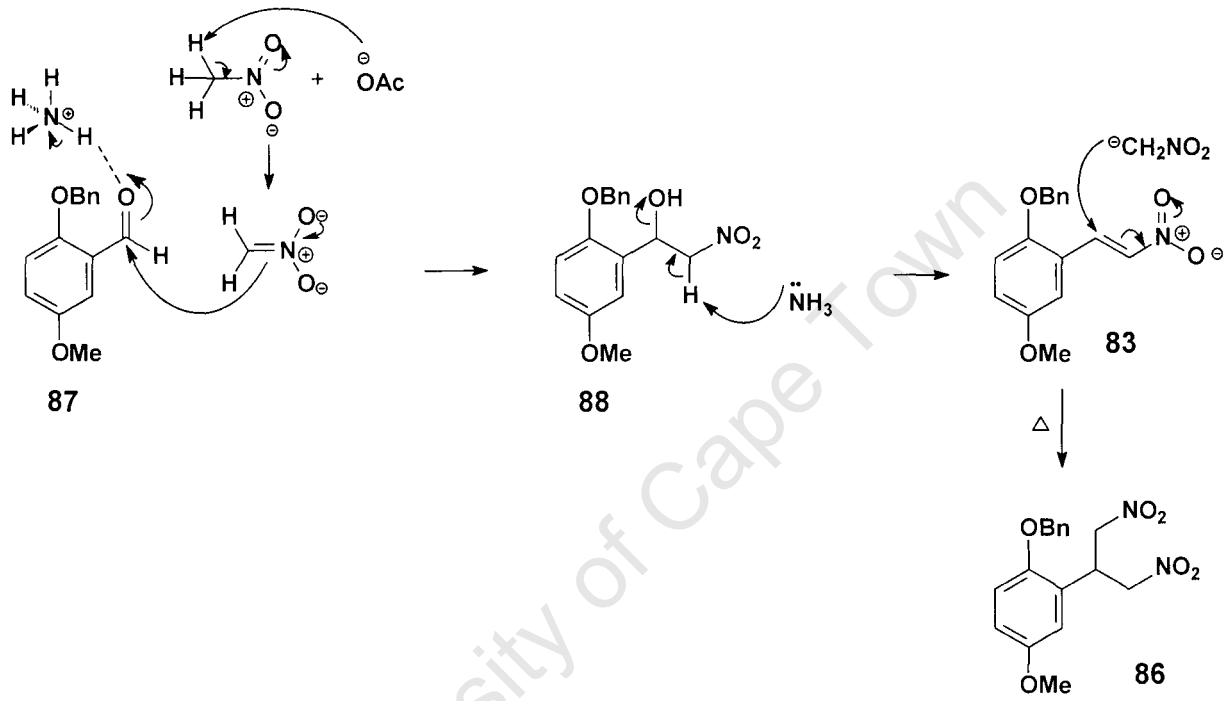
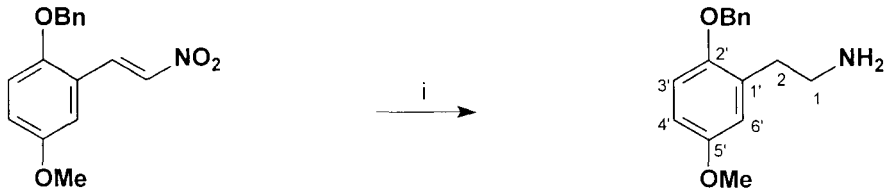


Fig. 2.3: Mechanism of formation of nitrostyrene

Reduction of nitrostyrene **83** by lithium aluminium hydride (LAH) in THF at reflux yielded the amine **80** in good yield (Scheme 2.2). The reaction could be followed by tlc towards the formation of the polar amine. Conventional extractive work-up could not be pursued in view of **80**'s water solubility. Thus, work-up involved adding saturated aq. Na_2CO_3 solution dropwise at 0 °C. The precipitate formed was filtered and the filter-cake washed with large volumes of methanol. Following complete evaporation of all solvent, the ^1H NMR spectrum of the crude amine **80** revealed the presence of a broad singlet resonating at δ_H 3.25 for the amine protons and two triplets at δ_H 2.73 and δ_H 2.63 representing the two sets of methylene protons. The ^{13}C NMR spectrum revealed the presence of ethyl carbons at δ_C 42.1 for C-1, and δ_C 34.6 for C-2 consistent with formation of a β-phenethylamine. As expected, the amine

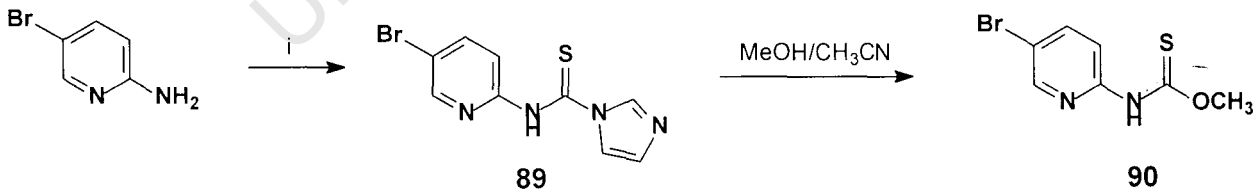
was very polar towards tlc, running with an Rf = 0.3 with an EtOAc/MeOH (9:1) system. Thus it wasn't purified but used in the next step. However, tlc indicated it to be predominantly a single compound, which was corroborated by the ^{1}H NMR spectrum.



83 **80**

Scheme 2.2. *Reagents and Conditions*: (i) $LiAlH_4$, THF, reflux, 4 h; 80%.

With amine **80** in hand, attention focused on exploring the two possible routes to **73**. The thiourea reagent **89** required for condensation with the amine was synthesized by condensation of 2-amino-5-bromopyridine with 1,1'-thiocarbonyldiimidazole in CH_3CN at room temperature for 12h (Scheme 2.3). Filtration of the precipitate that formed gave **89** in good yield, which was recrystallized from methanol/CH_3CN. This treatment resulted in substitution of the imidazole group by methoxy to form **90**, as indicated by the ^{1}H NMR spectrum which revealed the presence of a methoxy singlet resonating at δ_H 3.99 and a broad singlet at δ_H 11.56 for the NH proton. The presence of a thiocarbonyl was confirmed by the ^{13}C NMR spectrum, which revealed a diagnostic signal at δ_C 188.7.

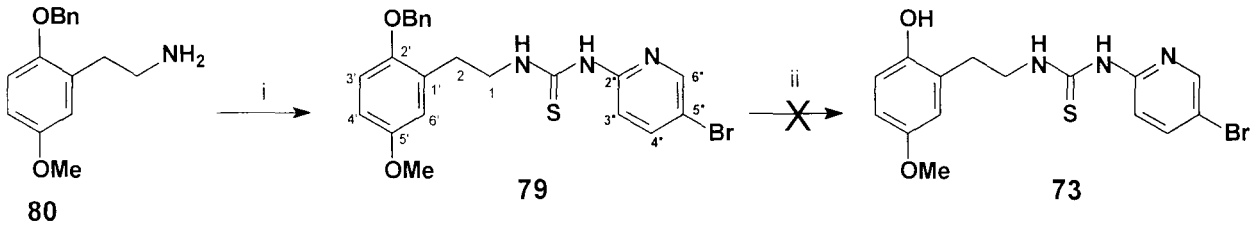


89 **90**

Scheme 2.3. *Reagents and Conditions*: (i) 1,1'-Thiocarbonyldiimidazole, CH_3CN, rt, 12 h; 87%.

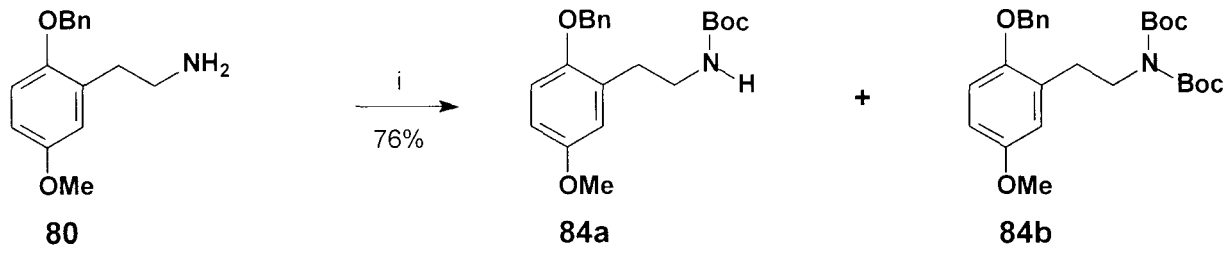
In view of the lability of **89** towards nucleophilic substitution, it was decided to use the precipitate of **89** without further processing. Thus, amine **80** was condensed with precipitated thiourea derivative **89** in DMF at 100 °C to give the protected derivative **79** in 37% yield over two steps after column chromatography (Scheme 2.4). The ^{1}H NMR spectrum of **79** revealed the presence of two broad singlets resonating at δ_H

11.18 and δ_H 8.80 for the two NH protons, while its ^{13}C NMR spectrum displayed a diagnostic signal at δ_C 179.0 for the thiocarbonyl carbon.



Scheme 2.4. *Reagents and Conditions*: (i) **89**, DMF, 100 °C, 16 h; 37%. (ii) H_2, Pd/C, rt.

An attempt to deprotect the benzyl group of **79** was unsuccessful and did not result in the desired product **73**, but instead led to recovered starting material. In this case, the thiourea scaffold in the molecule probably coordinates with the palladium catalyst, thus inhibiting the deprotection as anticipated in the planning. Hydrogenolysis of amine **80** using palladium-on-carbon catalyst did not yield the desired product either, presumably due to poisoning of the catalyst by the amine group. It was therefore envisaged that protection of the amine as a Boc would provide a better route to the desired thioureas **73**-**76**. It was felt that this protecting group would serve appropriately in subsequent steps including the Sonogashira coupling reaction. The Boc group is also relatively stable under basic conditions since the carbonyl group is relatively hindered and its electrophilicity diminished due to mesomeric stabilisation of oxygen and nitrogen. The strong resistance of the Boc group under basic conditions would allow chemoselective deprotection of the benzoyl group later in the synthesis, without disturbance of the double and triple bond functional groups. To this end, amine **80** was protected as its Boc-carbamate, by reacting with *di-tert*-butyldicarbonate (1.2 equiv.) and Et_3N in acetonitrile at room temperature to give **84a** in 76% yield (2 steps with LAH reduction) after purification by column chromatography (Scheme 2.5). The use of a catalytic amount of DMAP led to incomplete reaction thus necessitating the addition of more *di-tert*-butyldicarbonate up to 1.5 equiv. This resulted in formation of the desired carbamate in 36% overall yield for the two steps, together with di-boc by-product **84b** in 28% yield. In the absence of DMAP, the nucleophilic amine attacks *di-tert*-butyldicarbonate directly to form the Boc-protected amine **84a** (Fig. 2.4), which is not nucleophilic enough to undergo further substitution.



Scheme 2.5. *Reagents and Conditions*: (i) $(Boc)_2O$, Et_3N, DMAP, CH_3CN, rt, overnight.

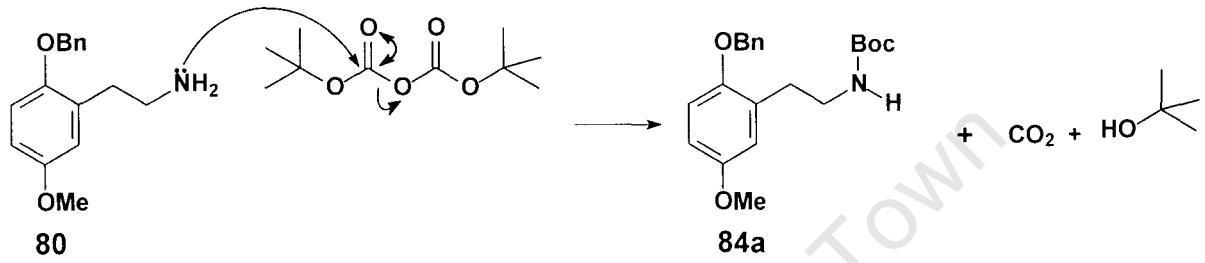


Fig. 2.4: Mechanism of formation of **84b** in the absence of DMAP

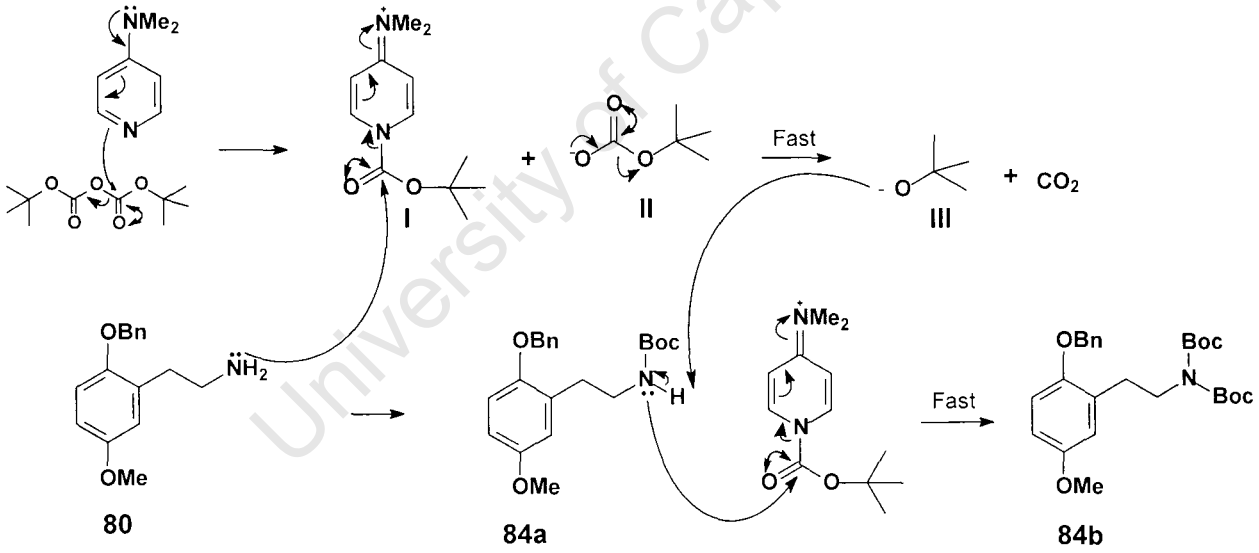
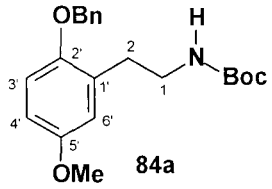


Fig. 2.5: Mechanism of formation of **84a** and **84b** in the presence of DMAP

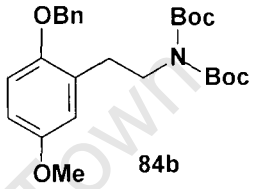
In the presence of DMAP, the latter reacts with *di-tert*-butyldicarbonate to form intermediate **I** which acts as a Boc transfer agent (Fig. 2.5). The butoxide anion **III** formed acts as a base. Since the carbamate NH hydrogen of mono-Boc derivative **84a** is relatively acidic, deprotonation occurs resulting in a fast second substitution to furnish **84b**. Thus in this case, it was better to leave out the DMAP.

The ^{1}H NMR spectrum of **84a** revealed the presence of a methyl singlet at δ_H 1.42, and a downfield shift in the methylene protons adjacent to nitrogen to δ_H 3.37. By comparison the ^{1}H NMR spectrum for compound **84b** had these protons resonating downfield at δ_H 3.89 consistent with the electron-withdrawing effect of the second Boc group. The ^{13}C NMR spectrum of **84a** revealed the presence of a carbamate carbonyl carbon at δ_C 155.9 and a methyl carbon at δ_C 27.8. The carbonyl carbon for compound **84b** resonated at δ_C 153.7.

Table 2.1:



84a



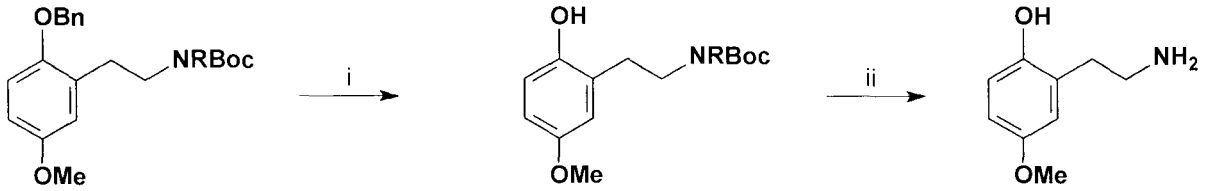
84b

Nucleus	Signal ($CDCl_3$, ppm)	
	84a	**84b**
H-1	3.37 (2H, q, *J* 6.4 Hz)	3.86 (2H, t, *J* 7.4 Hz)
H-2	2.83 (2H, t, *J* 6.4 Hz)	2.95 (2H, t, *J* 7.4 Hz)
C=O	155.9	153.7
$OC(CH_3)_3$	77.0	81.8
C-1	40.8	46.4

The other significant difference between these two compounds was the difference in the quarternary carbon resonating at δ_C 77.0 and δ_C 81.8 for **84a** and **84b**, respectively. Some of the important signals are shown in Table 2.1. The presence of a carbonyl group was also confirmed by the IR spectrum of these carbamates. Compound **84a** displayed a strong carbonyl signal at 1707 cm^{-1}, while **84b** displayed two strong peaks at 1776 and 1736 cm^{-1}, for the C=O asymmetric and symmetric stretches respectively.

Hydrogenolytic debenzylation of **84a-b** in the presence of 10% palladium-on-carbon in ethanol at room temperature led to phenols **81a-b** in good yield. The ^{1}H NMR spectrum of these compounds revealed the absence of a benzylic methylene singlet at δ_H 5.03 and δ_H 5.04 for δ_C **81a** and δ_C **81b** respectively, as well as aromatic protons at δ_H 7.45-7.29. The debenzylation was also confirmed by its ^{13}C NMR

spectrum, which showed only six aromatic carbons and no benzylic methylene carbons at δ_C 70.9 and δ_C 70.8 for **81a** and **81b**, respectively. The IR spectrum of **81a-b** displayed a diagnostic broad peak between 3398-3295 cm^{-1} for the hydroxyl stretch.



84a R = H
84b R = Boc

81a R = H
81b R = Boc

91

Scheme 2.6. *Reagents and Conditions*: (i) H_2, Pd/C, EtOH, rt, 5 h; 66%. (ii) CF_3COOH/CH_2Cl_2 (1:2), 0 °C, 30 min; 31%.

Deprotection of the Boc group on **81a** or **81b** was accomplished with trifluoroacetic acid in methylene chloride (1:2) at 0 °C to provide the desired amine **91** (Scheme 2.6). The mechanism involves protonation of the carbonyl oxygen to form **I** (Fig. 2.6). Elimination of a stable *tert*-butyl cation **III** via S_N1 gives carbamic acid **II** which undergoes decarboxylation to give the desired amine. The reaction is driven by the relative stability of the *tert*-butyl cation **III**.

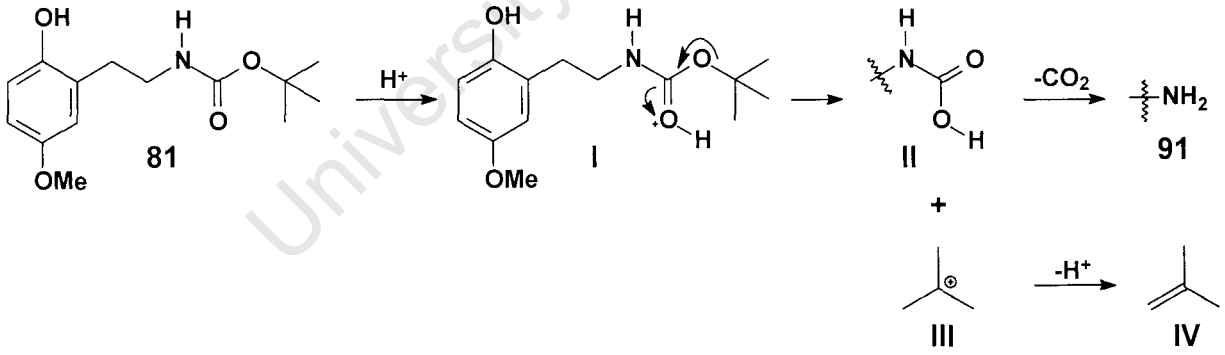
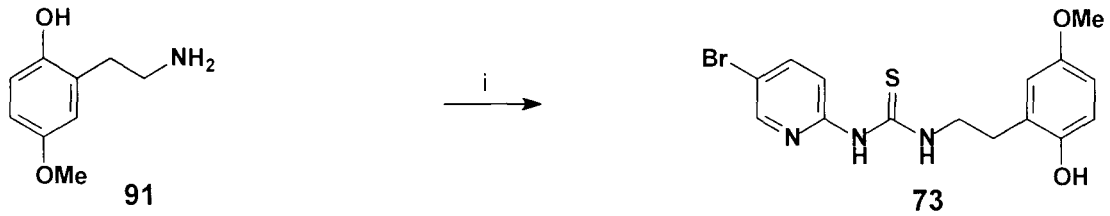


Fig. 2.6: Mechanism of deprotection of Boc

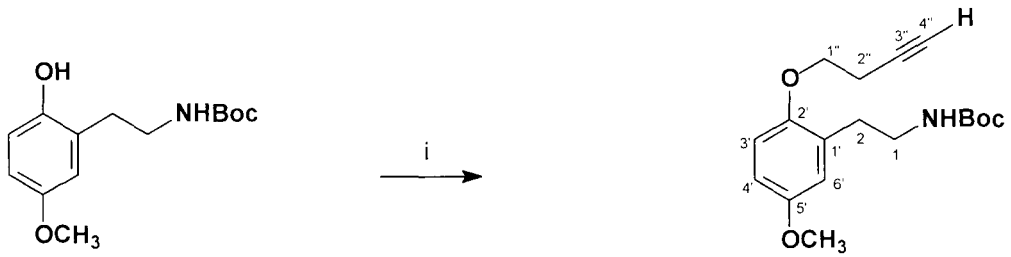
For elaboration into the HI-236 end of the molecule, amine **91** was condensed with thiourea reagent **89** to give **73** in 44% yield (Scheme 2.7) after column chromatography. The ^1H NMR spectrum of **73** displayed resonances for the thiourea NH protons at δ_H 11.12 and δ_H 10.59 whereas, its ^{13}C NMR spectrum showed the presence of a thiocarbonyl carbon at δ_C 179.0 which was downfield to that of a urea.

Compounds **74-76** were synthesized in a similar manner described for **73** and the structures were assigned on the basis of spectroscopic analysis.



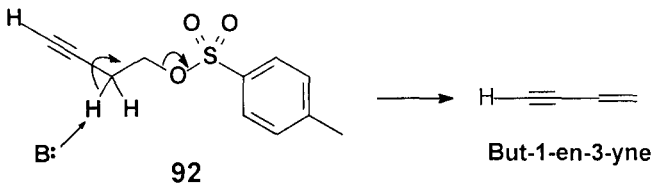
Scheme 2.7. Reagents and Conditions: (i) **89**, DMF, 100 °C, 1 h; 44%.

A further advantage of producing Boc-derivative **81** was that it could be used to access key intermediate **93** required in Sonogashira coupling as well as models **77** and **78** via alkylation of phenolic carbamate **81a** with 3-butyn-1-ol activated as its tosylate **92**. The alkylation reaction turned out to be unexpectedly troublesome. Heating carbamate **81a** with 3.0 equiv. K_2CO_3 and 3.0 equiv. butynyl tosylate in acetonitrile at reflux for 5 days gave the desired product **93** in only 31% yield, along with a 60% recovery of starting material (Scheme 2.8). The use of a large excess of K_2CO_3 and tosylate did not improve the yield, although the reaction didn't produce any other by-products according to tlc, and variation of solvent and base brought no significant improvement to the result either. The use of NaH as a base in either THF or DME led to decomposition of starting material. The poor yield and the incompleteness of the reaction prompted an investigation of the factors influencing this reaction. After a number of reactions using different equivalents of K_2CO_3, it was discovered that the rate of the alkylation reaction was dependent on the amount of potassium carbonate used. The slow conversion of the substrate to product was attributed to the fast rate of elimination of the tosylate to form but-1-en-3-yne (Fig. 2.7) in view of the acidity of the allylic hydrogens. This rationale was confirmed by minimising the amount of base, to 1.5 equiv. base and using a large excess of tosylate (6 equiv.). Thus, alkylation of **81a** under these conditions (1.5 equiv K_2CO_3 and 6 equiv. tosylate) yielded the alkylated carbamate **93** after 5 days in 70% yield. Alkylation of **81b** with butynyl tosylate **92** did not yield any product, probably due to steric hindrance as a result of the two bulky tert-butyl groups on nitrogen.



81a **93**

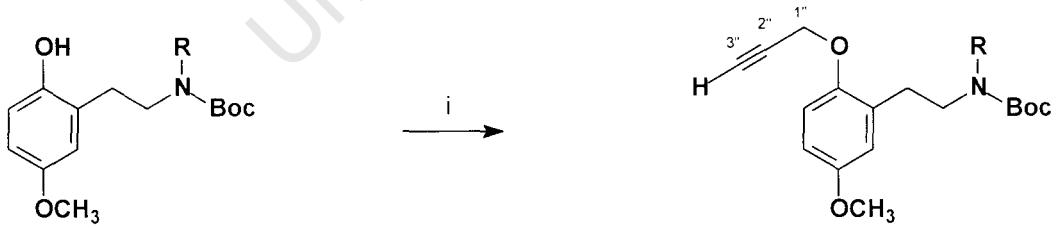
Scheme 2.8. *Reagents and Conditions:* (i) **92**, K_2CO_3, CH_3CN, reflux, 5 d; 70%.



92

Fig. 2.7: Mechanism of elimination of the tosylate

The ^1H NMR spectrum for compound **93** displayed a signal for the alkyne proton (H-4") at δ_H 2.03 which appeared as a triplet with a small coupling constant of *J* 2.7 Hz, due to allylic coupling with the H-2" signals. Additional signals in the spectrum at δ_H 2.66 for H-2" and a triplet at δ_H 4.05 for H-1" confirmed that alkylation had taken place. The ^{13}C NMR spectrum displayed diagnostic signals at δ_C 80.7 and δ_C 69.8 for the alkyne carbons. The presence of an alkyne was further confirmed by the IR spectrum, which displayed a weak signal at 3309 cm^{-1} for the **≡C-H** stretch as well as a weak signal at 2413 cm^{-1} for the alkyne **C≡C** stretch.



81a R = H
81b R = Boc

94a R = H
94b R = Boc

Scheme 2.9. *Reagents and Conditions*: (i) Propargyl bromide, K_2CO_3, CH_3CN, reflux.

For introduction of the shorter C-3 propynyl tether, **81a** was treated with equimolar quantities of K_2CO_3 and propargyl bromide resulting in low yields (31%) of the alkylated derivative **94a** and recovered starting material. The optimised conditions from before, in which an excess of propargyl bromide (6 equiv.) and 1.5 equiv. K_2CO_3

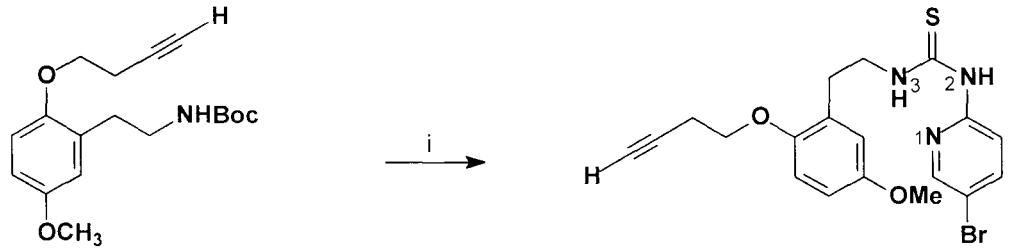
led to the desired carbamate **94a** in 75% yield after 20 h. In contrast to the incomplete reaction observed in the alkylation of **81a** using equimolar concentration of propargyl bromide and K_2CO_3, the use of *bis*-Boc **81b** significantly enhanced the conversion to the target alkyne **94b** in 70% yield (Scheme 2.9) in 1 h (Table 2.2).

Table 2.2. Optimization of alkylation of **81**

	Propargyl bromide	K_2CO_3	Time	Yields
81a	6 equiv	1.5 equiv	5 days	71% (**94a**)
81a	3 equiv	3 equiv	20 h	31% (**94a**)
81b	3 equiv	3 equiv	1 h	70% (**94b**)

The spectroscopic data of **94a-b** was consistent with the assigned structures and the presence of the alkyne group was evident in both the ^1H and ^{13}C NMR spectra. Thus, the ^1H NMR spectrum of **94b** displayed a diagnostic signal at δ_H 2.47 and δ_H 4.65 for the alkyne proton and H-1'', respectively. The ^{13}C NMR spectrum provided further confirmation with new signals at δ_C 79.0 and δ_C 75.2 for the alkyne carbons and δ_C 56.7 for C-1''.

Compound **77** was then produced in the following way. Deprotection of the Boc group in **93** using trifluoroacetic acid in dichloromethane (1:10), liberation of the amine with diisopropylethylamine, total evaporation of all the solvent followed by condensation of the resultant oil with thiocarbonyl derivative **89** in DMF at 100 °C afforded the coupled product **77** in unoptimised 25% yield (for the two steps) following column chromatography (Scheme 2.10). The ^1H NMR spectrum of compound **77** displayed a combination of aromatic ring hydrogens corresponding to the two rings as well as two broad singlets at δ_H 11.13 and δ_H 8.44 for NH-3 and NH-2, respectively. In addition, the ^{13}C NMR spectrum displayed a diagnostic signal at δ_C 179.2 corresponding to the thiocarbonyl carbon. The presence of the thiocarbonyl group was further confirmed by the IR spectrum, which displayed a weak signal at 1137 cm^{-1} for a C=S stretch.

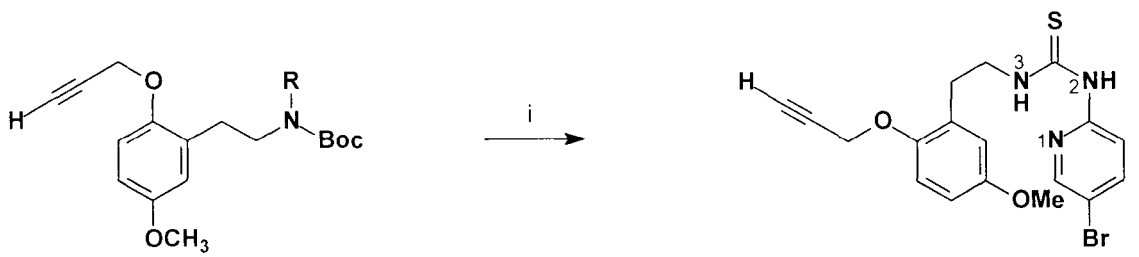


93 **77**

Scheme 2.10. *Reagents and Conditions:* (i) CF_3COOH, CH_2Cl_2, 0 °C, 1 h and then DIEA, rt, 10 min followed by **89**, DMF, 100 °C, 1 h; 25%.

The synthesis of **78** bearing a propyne spacer was performed in an identical manner to **77**. Deprotection of the Boc groups of **94a-b** under the same conditions as used for **93** followed by total evaporation of solvent and reaction with thiourea reagent **89** afforded **78** in low yield (17%) along with a complex mixture of products, presumably as a result of decomposition of the alkyne under acidic conditions (Scheme 2.11). The triflate salt **I** as well as the diisopropylethylamine salt **III** are fairly acidic and probably promoted dealkylation as depicted in Scheme 2.12.

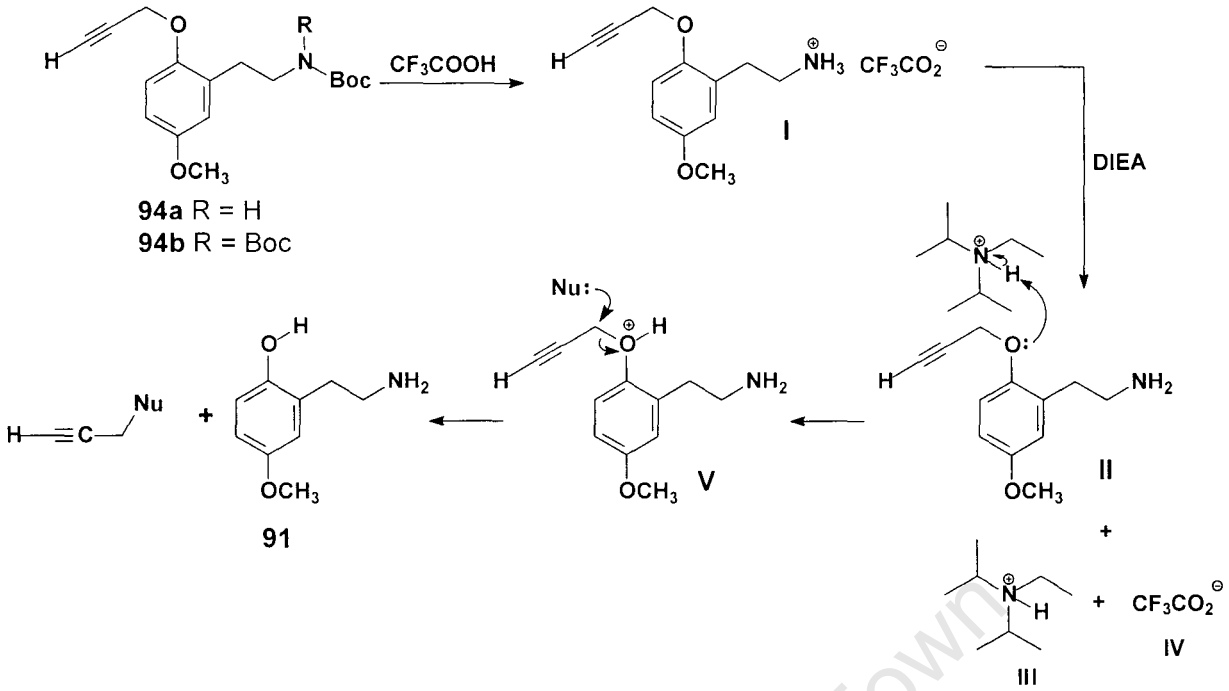
The structure of **78** was confirmed by both ^1H and ^{13}C NMR spectroscopy, which revealed a similar set of resonances to **77**. Thus the expected downfield shift for N-3 to δ_H 11.13 was observed in the ^1H NMR spectrum as a result of intramolecular hydrogen bonding of these protons with N-1 of the pyridine ring. The ^{13}C NMR spectrum of this compound displayed a diagnostic thiocarbonyl signal at δ_C 179.1. The presence of a thiocarbonyl group was further confirmed by the IR spectrum which displayed a weak signal at 1137 cm^{-1}.



94a R = H
94b R = Boc

78

Scheme 2.11. *Reagents and Conditions*: (i) CF_3COOH/CH_2Cl_2 (1:10), 0 °C, 1 h and then **89**, DMF, 100 °C, 16 h; 17%.



Scheme 2.12

Thioureas bearing a tether were synthesized according to the procedure described for the synthesis of HI-236 and were obtained in low yields. Optimisation of conditions for their synthesis needs to be done. These compounds are currently being tested for anti-HIV activity.

CHAPTER 3

Synthesis of d4U-Alkyne-HI-236

3.1 Strategy for the Synthesis of [d4U]-Spacer-[HI-236]

The bifunctional molecules **95-99** targeted in this thesis are shown in Fig. 3.1. The C-5 position on the pyrimidine ring of the base was selected as the attachment point of the tether due to its anticipated low interference with base-pairing in DNA.[100] In fact, tethering at the C-5 position with a flexible chain of about 10Å has been reported[101] to permit the triphosphate to be generated and thus be incorporated into nucleic acids. It was decided to lengthen the spacer using ethylene glycol units in view of their anticipated water solubility.

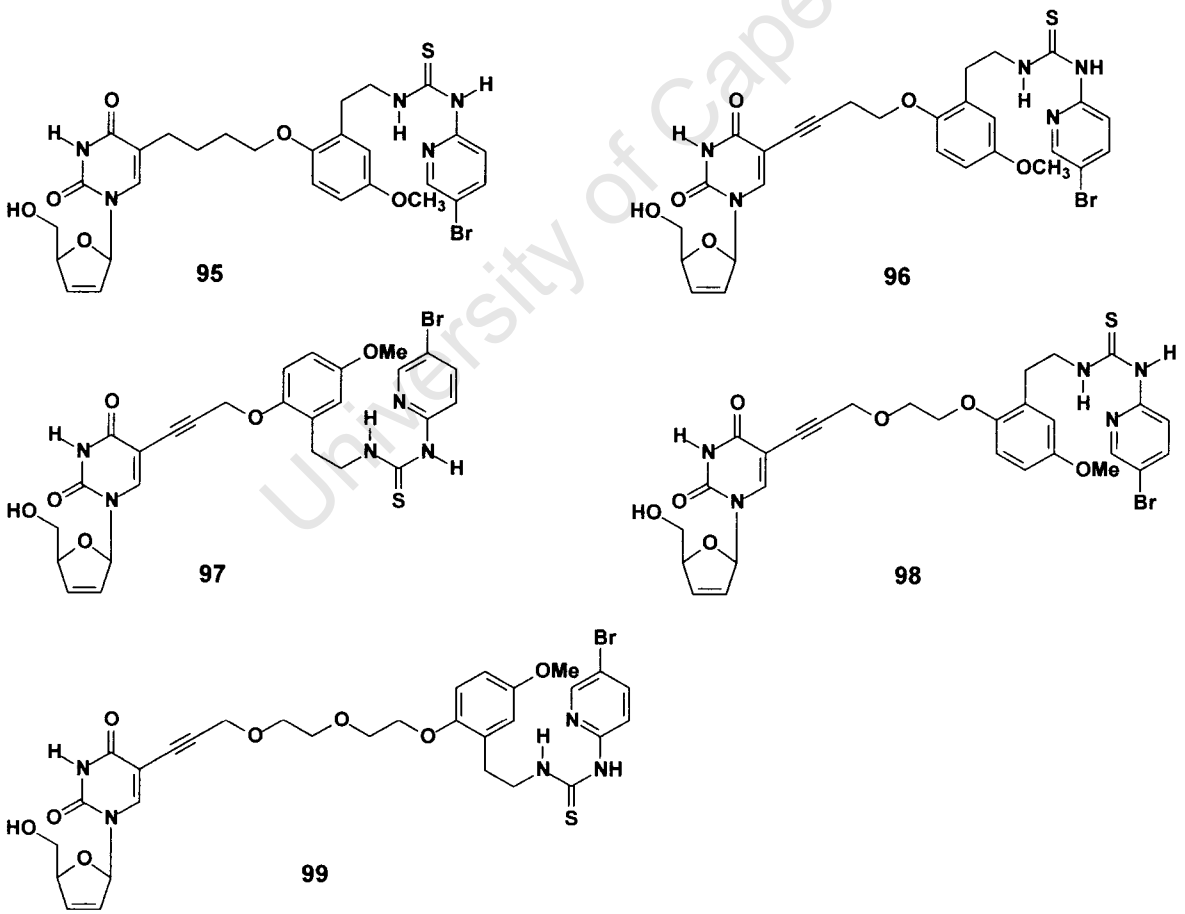


Fig. 3.1: Structure of bifunctional target molecules

Structural studies[49,51,52] around HI-236 by Uckun and co-workers in the original work on the drug revealed that the C-1 oxygen substituent of HI-236 is located at the entrance of the NNRTI pocket. This position was, therefore, chosen as the attachment point to the linker (tether). Attachment of the linker to the C-5 position of the nucleoside was thought to be accessible via a palladium cross-coupling Sonogashira[102] reaction of a terminal alkyne with a 5-iodo nucleoside. C-5 tethered heterodimers have also been developed extensively by the Ladurée[78,79] group in which a Sonogashira coupling strategy has also been demonstrated. Full awareness of their work only occurred once the work in this thesis was in progress. Furthermore, C-5 substituted pyrimidine nucleosides constitute a class of biologically significant molecules and the C-5 alkenyl and alkynyl substituted ones have shown various antiviral activities. For example, (*E*)-5(2-bromovinyl)-2'-deoxyuridine (BVDU) shown in Fig 3.2 is a highly potent and selective anti-Herpes agent, which inhibits Herpes Simplex Virus type 1 (HSV-1) and is currently in clinical use.[103] Other C-5 substituted nucleosides in clinical use include 5-iodo-2'-deoxyuridine (IDU) and 5-trifluoromethyl-2'-deoxyuridine (TFT) which are both administered as eye drops or ophthalmic cream in the treatment of herpes virus.[104]

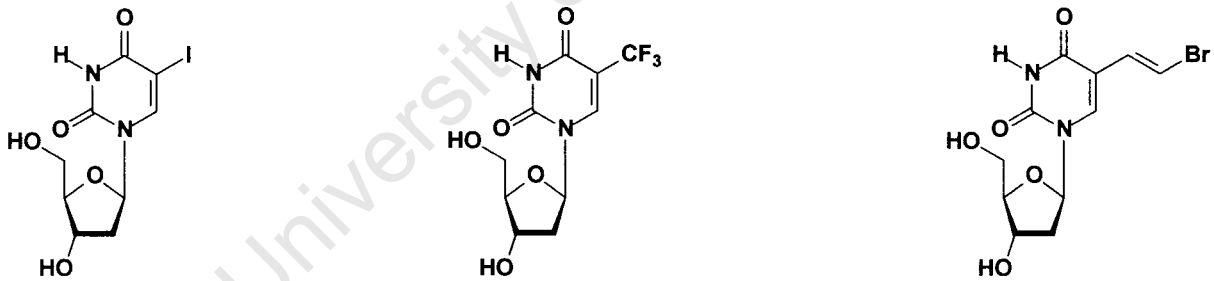


Fig. 3.2: C-5 substituted nucleosides in clinical use

3.1.1 Retrosynthesis of [d4T]-alkyne-[HI-236]

Two possible strategies were envisioned for the synthesis of the alkynyl target molecules **96-99**, with appropriate retrosynthetic analysis depicted in Fig. 3.3.

1. The first strategy (**a**) would involve a divergent approach via palladium cross-coupling reaction of an alkyne of type **A** with iodo-d4U **100** followed by

deprotection of the benzoyl and Boc groups and then condensation of the resultant amine with thiourea derivative **89** to furnish the target compounds **96-99**. It was anticipated that the use of sodium methoxide as a nucleophile for deprotection of the benzoyl group, as well as trifluoroacetic acid for deprotection of the Boc group might present a potential problem regarding nucleoside bond cleavage. Attention would therefore focus on optimising conditions for these two key reactions.

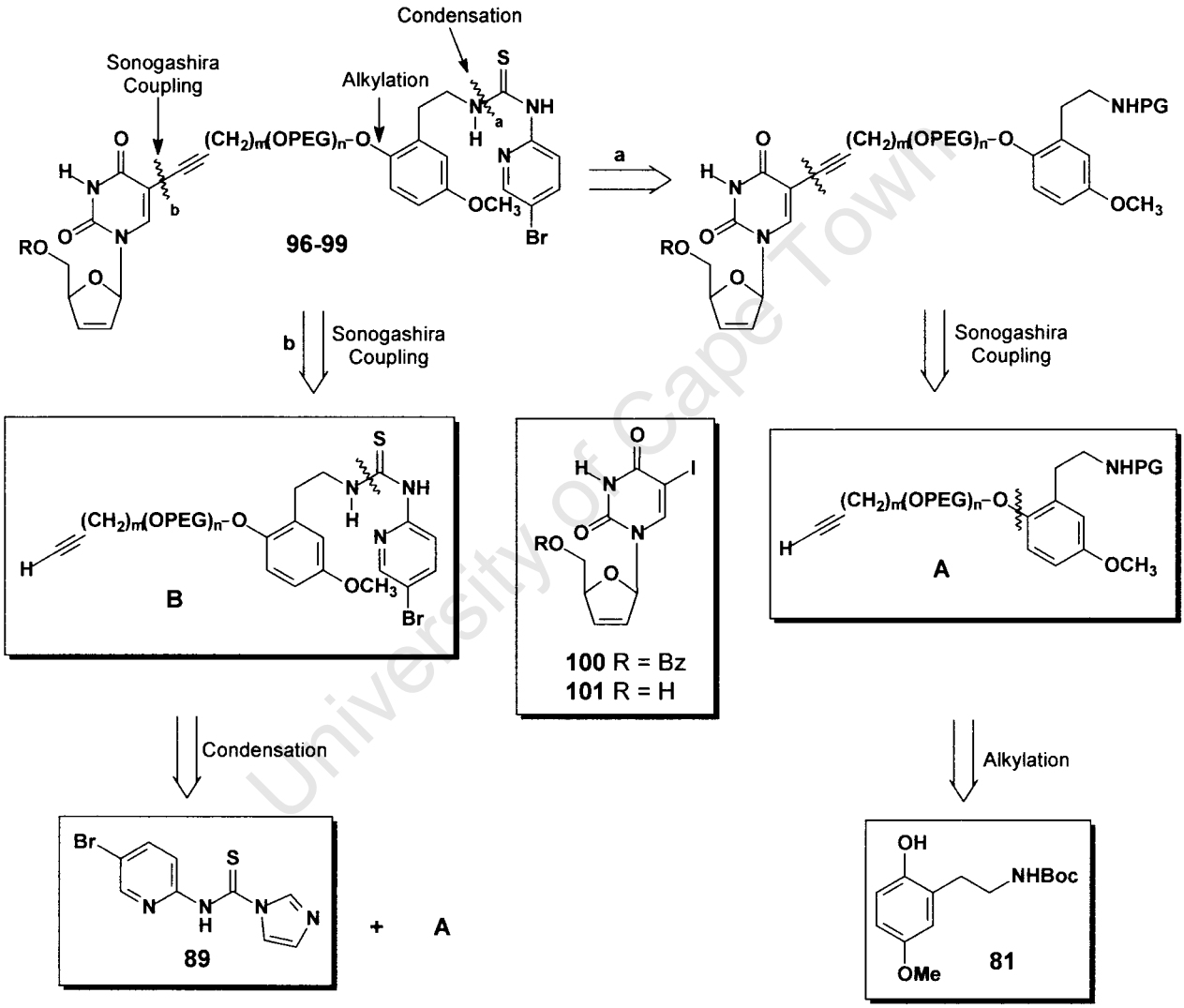


Fig. 3.3: Retrosynthetic analysis of heterodimers bearing an alkyne spacer

2. The second strategy (**b**) was more convergent and involved Sonogashira coupling of an alkyne of type **B** with iodo-d4U **100** followed by deprotection of the benzoyl group to furnish the target compounds directly. Attention would focus on the reactivity and potential interference of the thiocarbonyl group

under Sonogashira coupling conditions. It was anticipated that the presence of a bromopyridine ring might compete with the iodopyrimidine in the cross-coupling step, although literature precedence suggested that it is possible to regioselectively couple an iodonucleoside in the presence of an aryl bromide.[105] The use of CuI as a co-catalyst has been found to inhibit coupling of aryl bromides but enhance the coupling of aryl iodides.[106]

The key 5-iodo nucleoside **100** would be synthesized according to the Bristol-Myers-Squibb[93] procedure outlined in Scheme 1.17.

3.1.2 Literature Overview on Sonogashira Coupling

Sonogashira coupling of 5-iodouridine with terminal alkynes has been extensively studied.[107] The mechanism of this reaction starts with oxidative insertion of Pd(0) into the aryl-halide bond. CuI activates the alkyne by forming a copper acetylide, which undergoes transmetallation with the palladium complex to form the alkynyl-Pd-R intermediate. Reductive elimination of Pd(0) leads to the coupled product (Fig. 3.4).

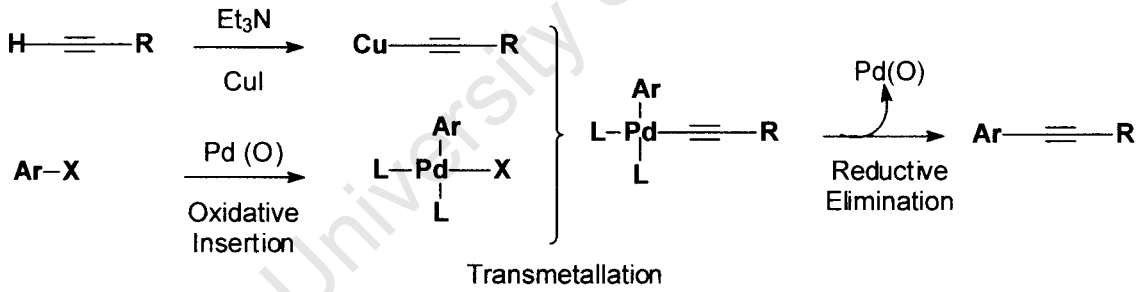
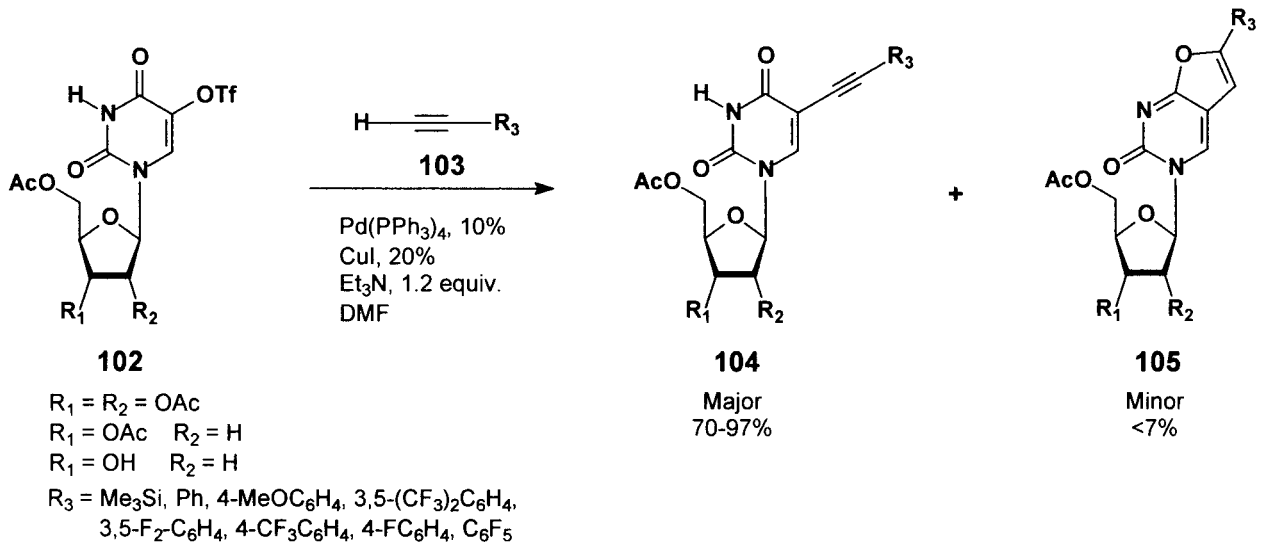


Fig. 3.4: Mechanism of Sonogashira coupling

Regarding literature precedent for our approach, Crisp and Flynn have demonstrated that the C-5 alkynyl derivatives **104** could be obtained in high yield (70-90%) by cross-coupling of acetylated uridine triflate **102** with a range of terminal alkynes **103** (Scheme 3.1) using tetrakis(triphenylphosphine)palladium(0) as a catalyst. Slight elevation of reaction temperature increased the rate of coupling. In this case, furanopyrimidine by-products **105** were isolated in low yield (7%) or in trace amounts.[108]



102

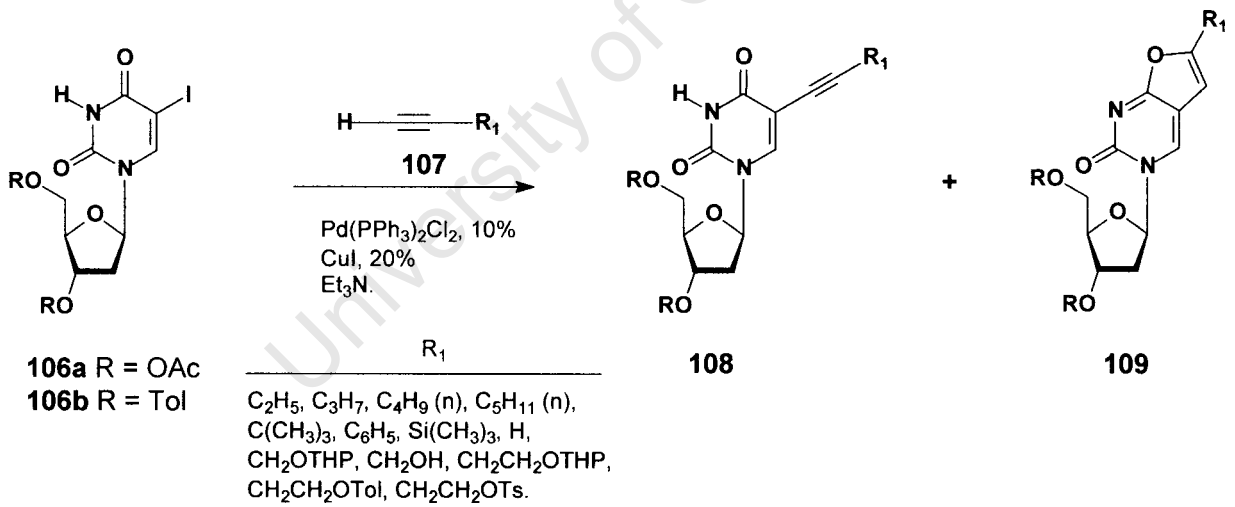
$R_1 = R_2 = OAc$
$R_1 = OAc \quad R_2 = H$
$R_1 = OH \quad R_2 = H$
$R_3 = Me_3Si$, Ph, 4-$MeOC_6H_4$, 3,5-$(CF_3)_2C_6H_4$, 3,5-F_2-C_6H_4, 4-$CF_3C_6H_4$, 4-FC_6H_4, C_6F_5

104 Major 70-97%

105 Minor <7%

Scheme 3.1

Robins *et al* applied the Sonogashira reaction to couple protected 2'-deoxythymidine derivatives **106** with several alkynes **107** in triethylamine at 50 °C in the presence of bis(triphenylphosphine)palladium (II) chloride and copper (I) iodide (Scheme 3.2).



106a R = OAc
106b R = Tol

R_1: C_2H_5, C_3H_7, C_4H_9 (n), C_5H_{11} (n), $C(CH_3)_3$, C_6H_5, $Si(CH_3)_3$, H, CH_2OTHP, CH_2OH, CH_2CH_2OTHP, CH_2CH_2OTol, CH_2CH_2OTs.

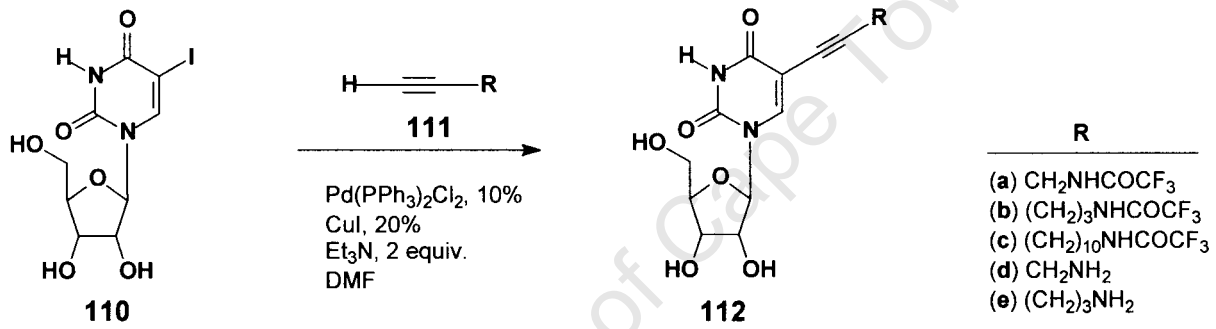
108 **109**

Scheme 3.2

In this case, the extent of cyclization was dependent on the reactants. Thus, coupling of **106a** with (trimethylsilyl)acetylene gave the target product **108** in 80% yield, whereas the use of 1-hexyne, 4-(*p*-toluyloxy)butyne, 4-tetrahydropyranyloxy)butyne or 4-(trityloxy)butyne under the same conditions gave the cyclized furanopyrimidin-2-one compound **109** as the major product. Conversely, coupling reactions with the same range of alkynes but using the nucleoside protected as its *p*-toluyl ester

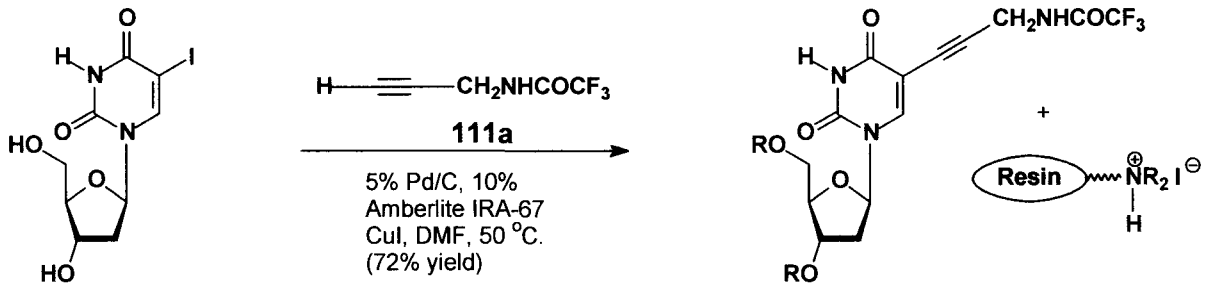
proceeded smoothly with minimal formation of deiodinated and furanoprimidin-2-one by-products.[109]

Hobbs[110] (Scheme 3.3) attempted to use the conditions optimised by Robins *et al* to couple propargylamine **111** with unprotected 5-iodouridine **110** but failed owing to the nucleoside's insolubility in triethylamine. Successful coupling was achieved to form the coupled products **112** in good yields (70-90%) by using DMF as a solvent and tetrakis(triphenylphosphine)palladium as a catalyst instead of the Pd(II) used by Robins. He concluded that the successful coupling was a result of using Pd(0) instead of Pd(II). A review of these conditions by Robins revealed that the use of DMF significantly improved the rate of coupling and not the Pd(0).[111]



Scheme 3.3

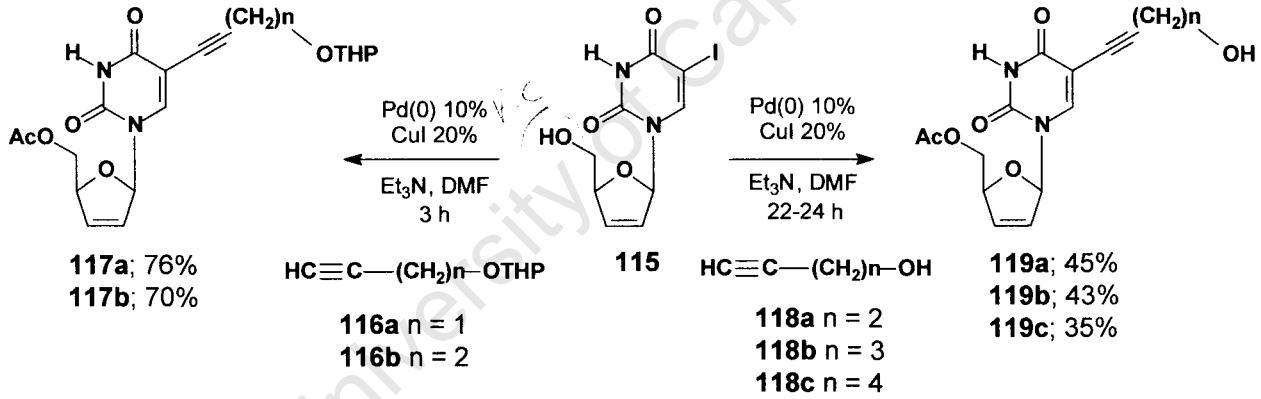
Sonogashira coupling of protected propargylamine **111a** with 2'-deoxynucleoside **113** by the Robins group in the presence of triethylamine as a base furnished the target nucleoside **114** together with triethylammonium salts that were difficult to separate. To improve the purification of these nucleosides, Garg *et al.*[112] developed a heterogeneous protocol that employed a palladium-on-carbon catalyst and a resin-bound tertiary amine (Amberlite IRA-67) as a base. These conditions furnished the coupled product **114** in good yield (Scheme 3.4). The study involved a cross-coupling reaction of nucleoside **113** in the presence of Amberlite IRA-67 and Pd(0) (10 mol%) which resulted in the coupled compound **114** in 79% yield after filtration of the reaction media and purification by column chromatography. The authors then substituted Pd(0) with a heterogeneous transition-metal catalyst (Pd/C) to further simplify the purification process.



113 **114**

Scheme 3.4

C-5 alkynylated d4T analogues **117** and **119** have been synthesized via the smooth and efficient coupling of alkynes **116** and **118** with 5'-*O*-acetyl-5-iodo d4U **115** in DMF under co-catalysis of Pd and CuI (Scheme 3.5). Reaction with unprotected alkynes **118** gave the coupled compounds **119** after 22-24 h in low yields (35-45%), whereas the protected alkynes **116** gave the target compounds in good yields (70-76%).[113]



117a; 76%
117b; 70%

HC≡C—(CH$_2$)n—OTHP

116a n = 1
116b n = 2

115

HC≡C—(CH$_2$)n—OH

118a n = 2
118b n = 3
118c n = 4

119a; 45%
119b; 43%
119c; 35%

Scheme 3.5

In summary, the use of CuI as a co-catalyst has been shown to give better results. A mole ratio of 2:1 copper to palladium has been shown to offer the best coupling conditions for alkynes, as the production of side products (cyclic furanopyrimidine) is minimized. It is noteworthy that performing the reaction in the presence of CuI has also been reported to increase the conversion rate to by-products. Solvent has been reported to be an important determinant for successful coupling of terminal alkynes with iodonucleosides.[111] The use of DMF as a solvent has been shown to reduce the percentage of cyclic by-product formed. A considerable percentage of this cyclized by-product was isolated when longer reaction times were employed or when an electron-withdrawing group on the nucleoside was present. Optimal conditions have

been found to be 2.0-2.5 equiv of terminal alkyne, 10% $Pd(PPh_3)_4$, 20% CuI and 1.2 equiv Et_3N in DMF. Pd (0) has been reported to give better results than Pd (II).[107]

3.2 Strategy (a): Synthesis of Target Compounds 96-99

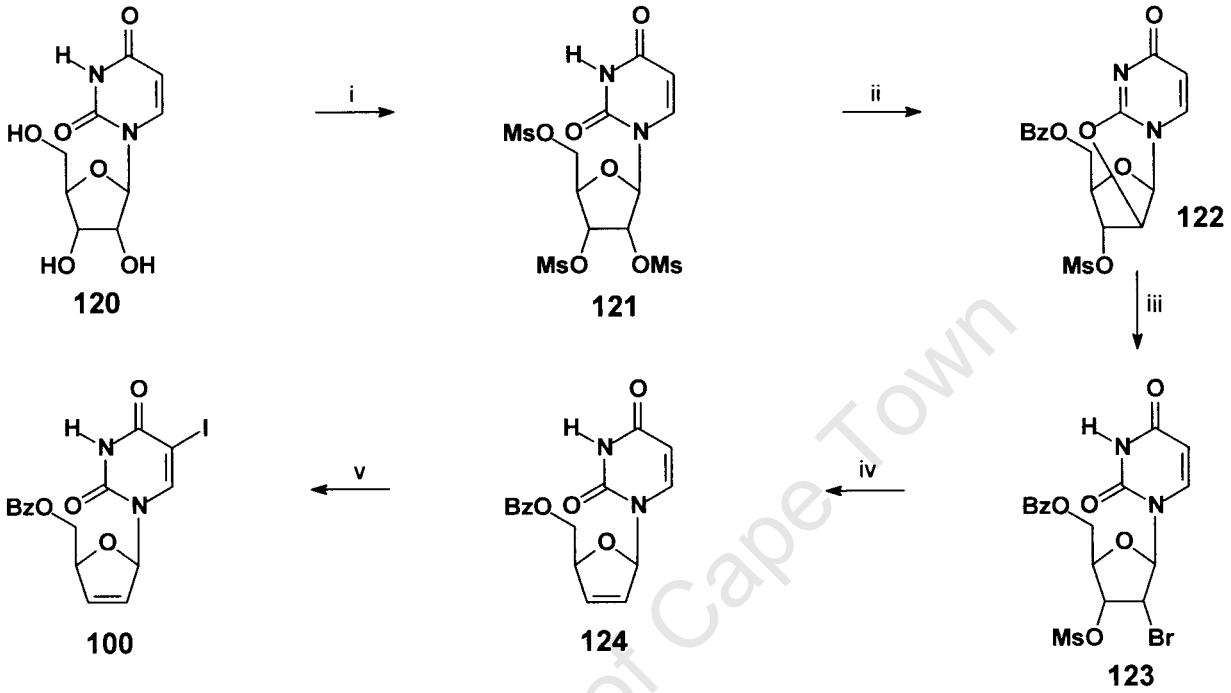
3.2.1 Synthesis of [d4U]-butyne-[HI-236]

3.2.1.1 Synthesis of iodo-d4U

Having successfully synthesized alkynes **93** and **94** (Schemes 2.8 and 2.9, Chapter 2), attention focused on the synthesis of iodo-d4U derivative **100** (Scheme 3.6). The 5'-*O*-benzoyl d4U derivative **124** was synthesized in four steps according to the Bristol-Myers Squibb procedure outlined in Scheme 1.18 in Chapter 1.[93] Reaction of uridine with methanesulfonyl chloride (3 eq.) in pyridine furnished trimesylate **121** in good yield. The product was isolated by adding the reaction mixture into ice-cold water, filtration of the precipitate formed and washing the filter cake with water. Following extensive drying on the pump, the ^1H NMR spectrum of **121** revealed the presence of three mesylate methyl singlets resonating at δ_H 3.22, δ_H 3.33 and δ_H 3.35 while its ^{13}C NMR spectrum displayed resonances at δ_C 37.9, 37.9 and 36.9 for the methyl groups.

The next step involved chemoselective transformation of the C-5' centre as well as preparation of C-2' for bromide substitution. Thus, the 2,2'-anhydrouridine **122** was synthesized by treatment of **121** with sodium benzoate in acetamide at 115 °C. After 1 h, the product was poured into ice-cold water, stirred at 0 °C for 15 min and the solid filtered to give the target compound in 75% yield. The ^1H NMR spectrum of **122** displayed the absence of an NH singlet at δ_H 11.51 in **121** as well as the presence of additional resonances in the downfield region for the aromatic protons of the benzoyl group. Its ^{13}C NMR spectrum displayed the appearance of a carbonyl carbon at δ_C 170.6 and aromatic carbons, confirming the presence of a benzoyl group, which could be assigned to the C-5' position in view of the relative deshielding of the diastereotopic H-5' protons. There was also a significant downfield shift in the C-2' carbon from δ_C 76.0 in **121** to δ_C 86.0, thus confirming that displacement of the

mesylate and formation of the anhydro ring had taken place at this carbon. C-2' over C-3' attack by the pyrimidine base is observed in view of the 5-*exo-tet* cyclization being faster than the 6-*exo-tet* process as well as the C-3' substitution forming a bridged compound.



Scheme 3.6. *Reagents and Conditions*: (i) MsCl, pyridine, 0 °C, 5 h; 71%. (ii) NaOBz, CH_3CONH_2, 115 °C, 1 h; 75%. (iii) CH_3COBr, EtOAc/MeOH (10:1), reflux, 1 h; 97%. (iv) Zn, EtOAc/MeOH, rt; 89%. (v) I_2, CAN, CH_3CN, 60 °C, 1 h; 80%.

Nucleophilic attack by bromide ion at the 2'-position using acetyl bromide in MeOH/EtOAc (1:10) gave *cis*-bromomesylate **123** in high yield (97%). Introduction of the bromine atom was evident in the ^1H NMR spectrum which displayed an upfield shift in the H-2' protons from δ_H 5.69 in **122** to δ_H 4.67 in **123**. Similarly, the ^{13}C NMR spectrum of **123** displayed an upfield shift of the C-2' resonance from δ_C 86.0 in **122** to δ_C 47.5 consistent with substitution by bromine. The mechanism of this reaction involves S_N2 displacement by bromide at C-2' with a protonated pyrimidine ring as a leaving group. The net result is inversion of configuration to afford the *syn* stereochemistry in **123**.

Reductive elimination of bromomesylate **123** employing zinc metal in ethyl acetate and methanol containing catalytic acetic acid afforded the 5'-*O*-benzoate ester of d4U

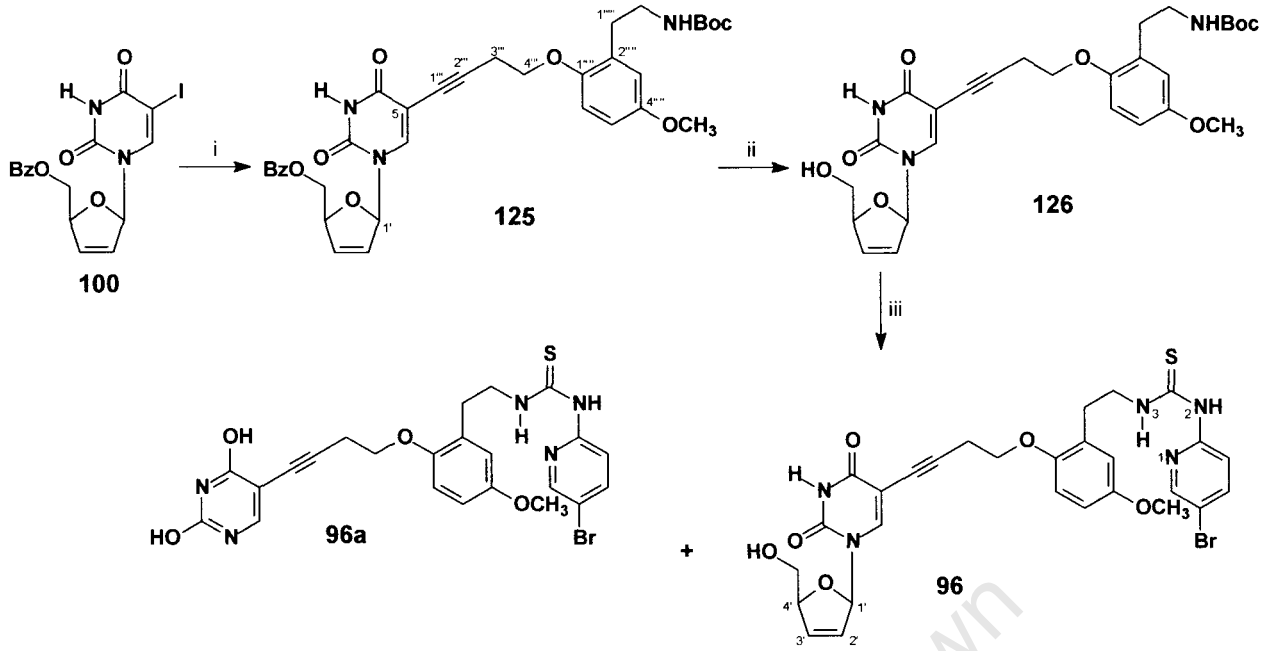
124 in 89% yield with no detectable cleavage of the nucleoside. The downfield shift of the C-2' and C-3' carbons in the ^{13}C NMR spectrum of **124** from δ_C 75.6 and δ_C 47.5 in **123** to δ_C 133.6 and δ_C 127.2 respectively, confirmed the introduction of the double bond via elimination of bromine and mesylate groups. Elimination of the mesylate was further confirmed by the absence of the mesylate methyl carbon at δ_C 38.8. Although *syn* elimination is energetically more demanding than *anti*, undoubtedly the potent leaving ability of the sulfonyl group assisted the elimination.

Halogen-substituted nucleosides have been shown to exhibit interesting chemotherapeutic, biochemical and biophysical properties.[103] They have also been utilized as intermediates for coupling with terminal alkynes to give 5-alkynyl nucleosides with antiviral activity.[107,114] The first 5-iodination of uridine was described by Prusoff *et al* using an iodine/nitric acid system.[115] Dale *et al*[116] subsequently reported iodination of 5-mercuriuridine derivatives using elemental iodine in aqueous alcohol. *N*-Iodosuccinimide as well as iodine monochloride have also been utilized for iodination of pyrimidine nucleosides. The CAN-mediated C-5 halogenation of uracil derivatives was first reported by Asakura, *et al* in 1990, in which molecular iodine as well as NaI and LiI were used as the iodine source.[114] Ultimately in this work, iodination of **124** to its 5-iodo d4U **100** was effected using elemental iodine and CAN at 60 °C in 80% yield. This reaction was found to be dependent on time and temperature. Thus, higher temperatures and long reaction times led to isolation of a by-product as a result of migration of the benzoyl group from the C-5'-hydroxyl to the pyrimidine nitrogen. Iodination was confirmed by the ^1H NMR spectrum which revealed the absence of a doublet at δ_H 5.33 for H-5 and a downfield shift in H-6 from δ_H 7.34 in **124** to δ_H 7.86. H-6 also appeared as a singlet in the ^1H NMR spectrum of **100**. The ^{13}C NMR spectrum of **100** revealed that C-5 had shifted upfield from δ_C 102.7 in **124** to δ_C 68.9, indicating iodination at C-5.

3.2.1.2 Sonogashira Coupling, Deprotection and final Condensation

Sonogashira coupling of 5-iodo-5'-*O*-benzoyl d4U **100** with alkyne **93** yielded the desired coupled product **125** in 94% yield within 3 h at room temperature (Scheme 3.7). The ^{1}H NMR spectrum data for **125** revealed signals for both the alkynyl and d4U moieties in the ratio of 1:1. A successful coupling was further confirmed by the absence of an alkyne proton at around δ_H 2.04. The ^{13}C NMR spectrum of **125** displayed diagnostic resonances at δ_C 141.6 (C-6), 150.5 (C-1''''), 100.7 (C-2'''), 91.3 (C-1'''), 90.7 (C-1'), 72.4 (C-5) and 40.6 (C-2'''') thus confirming the presence of both the nucleoside and alkyne. The structure was further confirmed by 2D NMR. The HMBC spectrum revealed a correlation between the methylene protons at δ_H 2.68 (H-3''') with the carbons at δ_C 100.7 (C-2'''), 91.3 (C-1'''), 72.3 (C-5) and 66.6 (C-4''').

With **125** in hand, only benzoyl deprotection, Boc removal and thiourea elaboration remained for the synthesis of the target. It was feared that the basic conditions of deprotecting the benzoyl group would cleave the nucleoside. Gratifyingly, the benzoyl group on **125** was deprotected using sodium methoxide (cat.) in methanol at 0 °C to furnish **126** in 70% isolated yield. The reaction was monitored by tlc and after all the starting material was converted to product, the base was diluted by adding water and the aqueous phase extracted with large volumes of ethyl acetate. The crude product was purified by column chromatography using 80% ethyl acetate in hexane. Debenzoylation was confirmed by the upfield shift in the diasteretopic protons at H-5' from δ_H 4.64 and δ_H 4.50 in **125** to δ_H 3.88 (dd, *J* 2.7, 12.4 Hz) and δ_H 3.78 (dd, *J* 3.0, 12.4 Hz), respectively. The presence of a broad hydroxyl singlet resonating at δ_H 1.96, as well as the absence of aromatic protons for the benzoyl group also confirmed the loss of the benzoyl group. Similarly, its ^{13}C NMR spectrum displayed an upfield shift in the C-5' carbon from δ_C 65.1 in **125** to δ_C 62.9. The absence of a carbonyl carbon resonating at δ_C 166.2 as well as carbons for the aromatic group further confirmed debenzoylation had taken place. Other signals were intact indicating that no other transformation had taken place.



Scheme 3.7. *Reagents and Conditions*: (i) Alkyne **93**, $(PPh_3)_4Pd$, CuI, Et_3N, DMF, THF, rt, 4 h; 94%. (ii) NaOMe, MeOH, 0 °C, 20 min; 50%. (iii) CF_3COOH, CH_2Cl_2, 0 °C, 30 min then **89**, DMF, 100 °C, 1 h; 41%.

The key condensation reaction required extensive optimization and the isolation conditions proved critical to ensure a reproducible result. Thus, deprotection of the Boc group on **126** using trifluoroacetic acid in methylene chloride at 0 °C furnished the crude amine as a triflate salt. The reaction could be monitored by tlc to form a very polar primary amine spot (Rf = 0.4 in 10% MeOH in CH_2Cl_2). Since the product couldn't be extracted from water into an organic solvent, work-up involved adding Hünig's base to liberate the amine, and condensing directly with thiocarbonyl derivative **89** in DMF at 100 °C. This resulted in the formation of product **96** but with accompanying nucleoside bond cleavage to generate compound **96a**. TLC analysis of the amine intermediate employing different solvent systems (10% MeOH in EtOAc, 5% MeOH in CH_2Cl_2, 10% MeOH in CH_2Cl_2, 100% THF, 50% hexane in THF, 2% MeOH in THF and 10% MeOH in THF) revealed one product, thus showing that the degradation of the product did not take place during Boc deprotection. The cleavage appeared to take place during the final condensation reaction, presumably promoted by the ammonium salt from the Hünig's base treatment ($(^iPr)_2EtN^+H\ CF_3CO_2^-$). Addition of K_2CO_3 and MeOH to neutralise the acid (CF_3COOH) prior to heating in the condensation with **89** resulted in nucleoside bond cleavage, presumably as a result of attack by the hydroxide ion generated during neutralisation. To circumvent this problem, the work-up was changed to first adding diisopropylethylamine to

liberate the amine, evaporation of the solvent on the rotary evaporator without extensive heating to remove excess trifluoroacetic acid followed by addition of K_2CO_3 and MeOH to make the reaction mixture basic. Filtration of the potassium-triflate and excess carbonate followed by removal of all volatiles furnished a crude amine presentable for condensation with thiocarbonyl derivative **89** in DMF at 100 °C for 1 h to furnish the target compound **96** in 41% overall yield. Despite efforts to make the reaction mixture basic, impurities as a result of the nucleoside cleaving were isolated. Some breakdown also seemed to occur on the acidic silica-gel. The impurity co-eluted with the target compound and was not easy to remove by column chromatography. However, repetitive preparative thin layer chromatography followed by recrystallization from methylene chloride/hexane furnished a pure sample of **96** which was subjected to anti-HIV activity testing. The ^{1}H NMR spectrum of a mixture of **96** and **96a** is shown in Fig. 3.5. It is clear from the spectrum that certain signals double up indicating common fragments. Furthermore, the sugar resonances are diminished and only gave single signals in both ^{1}H and ^{13}C spectra indicating that this grouping was only present in one of the compounds (**96**). Some signals appeared together such as the low-field thiourea protons NH-1 and NH-2 for **96** and **96a**, given that these protons are far away from the cleavage point to the sugar. Resonances for positions closer to the latter did, however, appear as two. Notably, the pyrimidine NH of 96 appeared as a singlet, with the pyrimidine ring of **96a** probably being enolised. A very good marker for the two compounds was the aromatic methoxy group appearing as two singlets as shown in Fig. 3.5a, resonating at δ_H 3.62 and δ_H 3.61 for **96** and **96a**, respectively. Resonances for the aglycon part integrated for one proton each, whereas those of the phenyl ABC splitting pattern as well as the pyridine ABX splitting pattern integrated for two protons each. Similarly, in the ^{13}C NMR spectrum, the presence of **96a** was confirmed by the presence of additional signals for the uracil, linker and the HI-236 part. Specifically, the spectrum displayed two sets of resonances for the following carbons: C-4 (δ_C 162.7, 161.7), C-5 (δ_C 73.9, 73.7), C-2" (δ_C 98.4, 97.3), C-1" (89.8, 89.6) and one set of resonances for the sugar part. Important resonances in the ^{13}C spectrum are shown in Fig. 3.5b and Table 3.1 (Page 83).

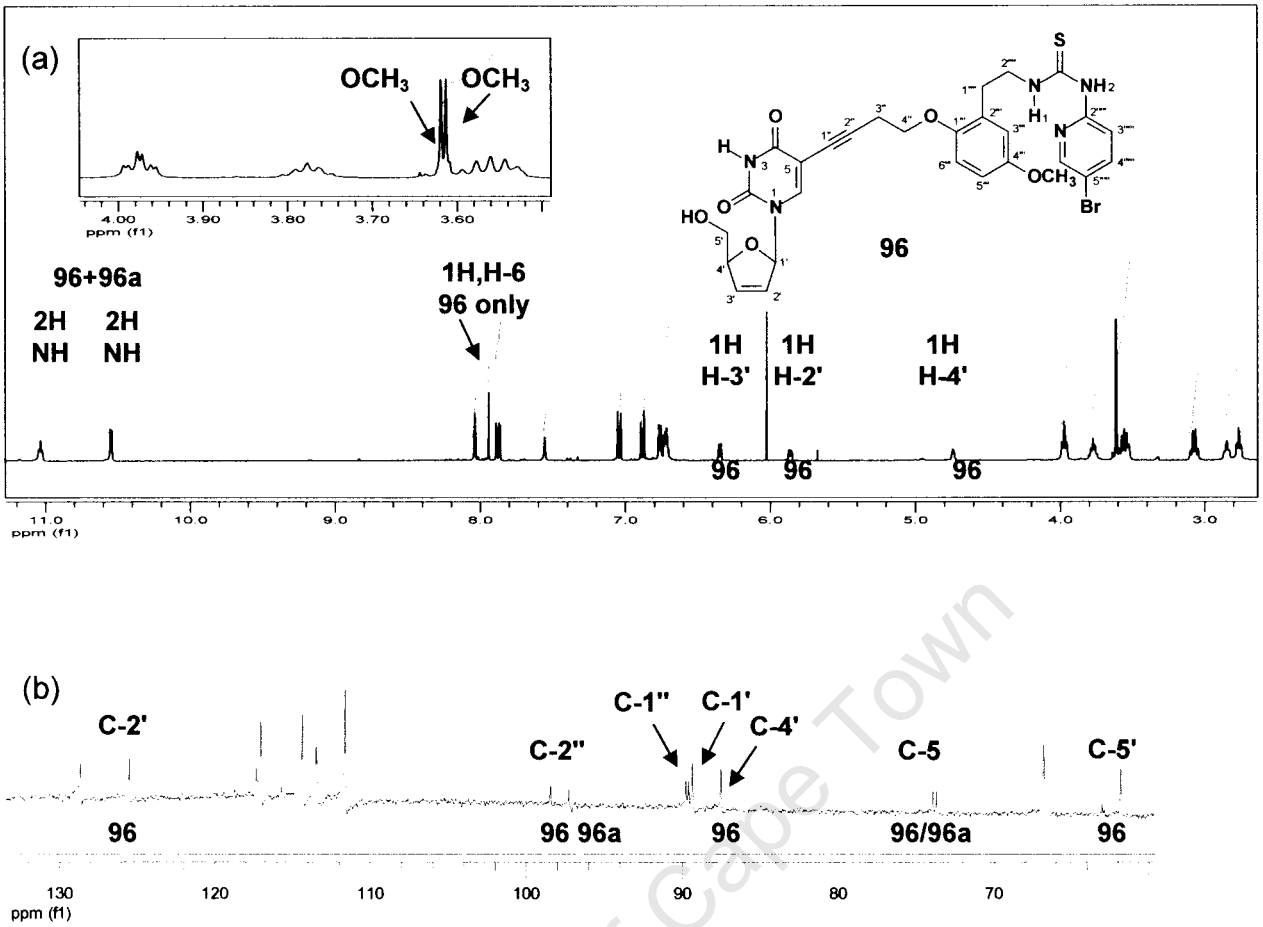
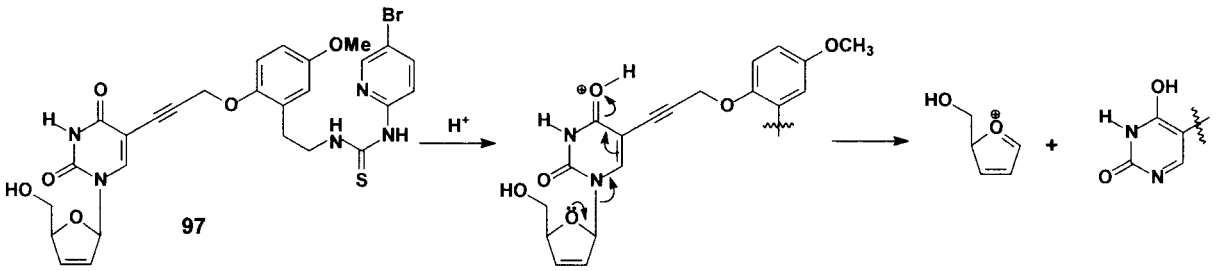


Fig. 3.5: (a) ^{1}H and (b) a section of ^{13}C NMR spectra showing a mixture of **96** and **96a**.

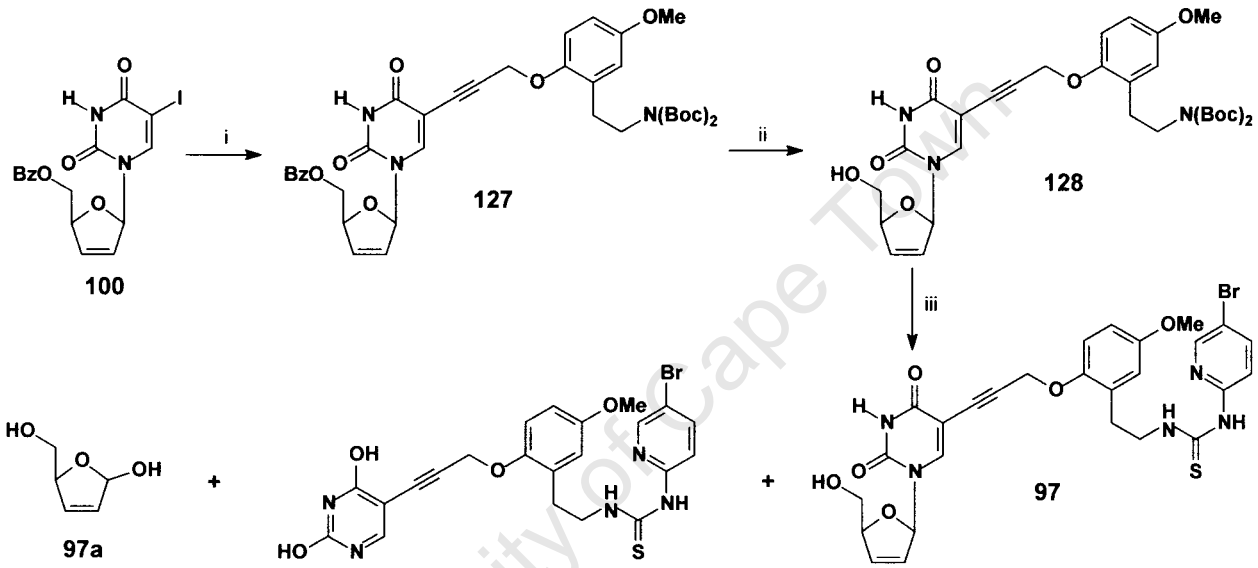
A plausible explanation for the cleavage relates to the leaving ability of the pyrimidine base being favoured by both allylic and resonance (oxygen) stabilising effects of the resultant oxocarbenium ion as shown in Scheme 3.8. The cleavage could well have been favoured by acidic residues in the medium or even on the silica-gel chromatography. The latter perception is supported by the observation that multiple chromatography resulted in extremely low yields of product with by-product formation.



Scheme. 3.8: Plausible mechanism of degradation of **97**

3.2.2 Synthesis of [d4U]-propyne-[HI-236]

Similarly, synthesis of **97** bearing a propyne spacer was pursued according to the procedure described above (Scheme 3.7) for the synthesis of **96**, the only difference being that the diboc-derivative **94b** was used. The structures of **127** and **128** were confirmed by spectroscopic analysis and high resolution mass spectroscopy. Compound **127** displayed resonances in its ^{1}H and ^{13}C NMR spectra for molecular entities of both **100** and **94**.



Scheme 3.9. *Reagents and Conditions*: (i) Alkyne **94b**, $(PPh_3)_4Pd$, CuI, Et_3N, DMF, THF, rt, 4 h; 94%. (ii) NaOMe, MeOH, 0 °C, 20 min; 74%. (iii) CF_3COOH, CH_2Cl_2, 0 °C, 30 min then **89**, DMF, 100 °C, 1 h; 40%.

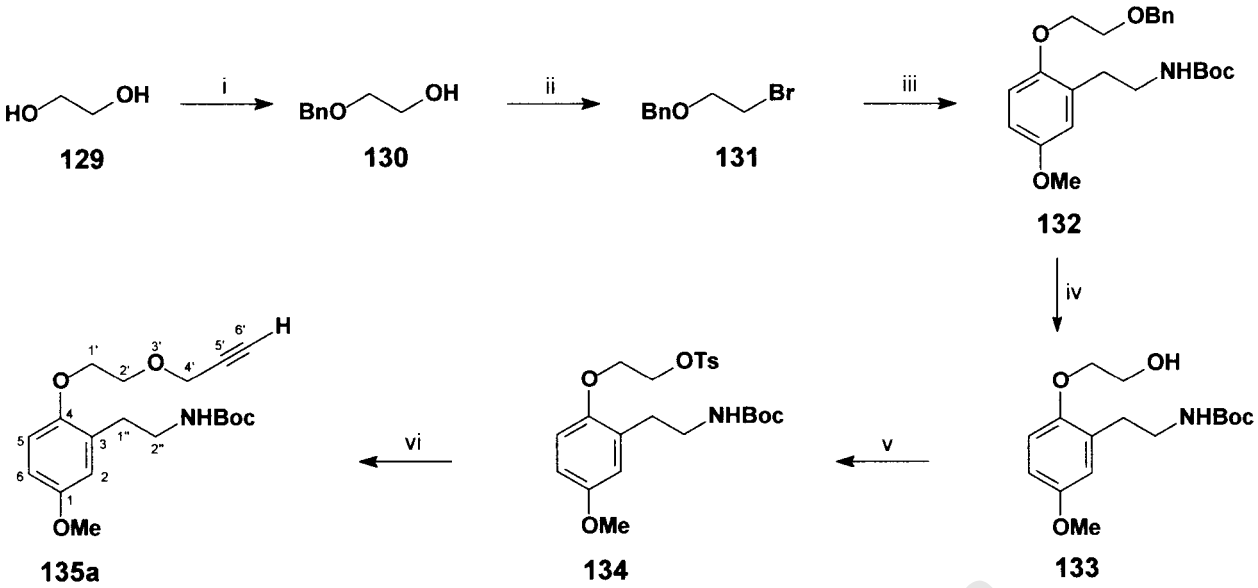
Deprotection of the benzoyl group of **127** furnished **128** in good yield after column chromatography purification, with the Boc groups intact and the absence of the benzoyl group in both the ^{1}H and ^{13}C spectra. Deprotection of the Boc groups of **128**, according to the procedure described for **126**, followed by condensation of the resultant amine with thiourea derivative **89** in DMF at 100 °C furnished a product which unfortunately decomposed to mainly the cleaved product following multiple chromatographic purification. A similar mechanism of degradation of **97** is presumed to operate as outlined in Scheme 3.8, involving acid-catalysed elimination. Isolation of the aglycon derivative **97a** provided evidence for its elimination. In view of this complication, pursuit of this target was discontinued. Future work might involve

hydrogenation of the triple and double bond before condensation in order to minimise the cleavage problem described.

3.2.3 Synthesis of [d4U]-monoPEG-propyne-[HI-236]

With a plausible yet low-yielding route developed of the prototype, attention was turned towards synthesizing heterodimers with longer spacers for structure-activity purposes. A polyethylene (PEG) unit was chosen as the repeating unit in view of its synthetic accessibility as well as its promotion of water solubility. It was envisaged that an equivalent NBoc-derivative with a modified spacer would be prepared for Sonogashira coupling as in strategy (**a**) described previously. The PEG linkers were incorporated via two nucleophilic substitution reactions, and were targeted as mono and di. The overall synthetic scheme for the initial phase of the synthesis is shown in Scheme 3.10. Following the method described by Marshall *et al.*,[117] 1,2-ethanediol **129** was mono-protected as its monobenzyl ether **130** in 53% yield with BnBr and NaH in THF. The product was easily isolated by distillation. Although only a moderate yield, the mono-benzylation could be carried out on a large scale. The presence of a benzyl group in the product was confirmed by the ^{1}H NMR spectrum, which revealed aromatic protons integrating for 5 protons resonating at δ_H 7.34 as well as methylene protons resonating at δ_H 4.56. The ^{13}C NMR spectrum displayed a methylene carbon at δ_C 73.3.

Treatment of alcohol **130** with carbon tetrabromide in methylene chloride smoothly yielded the bromide **131** in 95% yield. Work-up involved evaporating the solvent and purifying the crude product directly by column chromatography. Mechanistically, reaction of triphenylphosphine with carbon tetrabromide generates a bromophosphonium ion *in situ* which then reacts with the alcohol to give an alkoxyphosphonium ion (Fig. 3.6). The phosphonium ion intermediate then undergoes nucleophilic attack by bromide ion, displacing triphenylphosphine oxide. The driving force for the cleavage of the C-O bond is the formation of the strong phosphine oxide bond (P=O).



Scheme 3.10. *Reagents and Conditions*: (i) BnBr, NaH, THF, reflux, 20 h; 53%. (ii) PPh_3, CBr_4, CH_2Cl_2, 0 °C, 30 min; 95%. (iii) **81a**, K_2CO_3, CH_3CN, reflux, 20 h; 87%. (iv) H_2, Pd/C, EtOH, rt, 18 h; 87%. (v) TsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C-rt, 20 h; 76%. (vi) Propargyl bromide, K_2CO_3, reflux, 20 h; 74%.

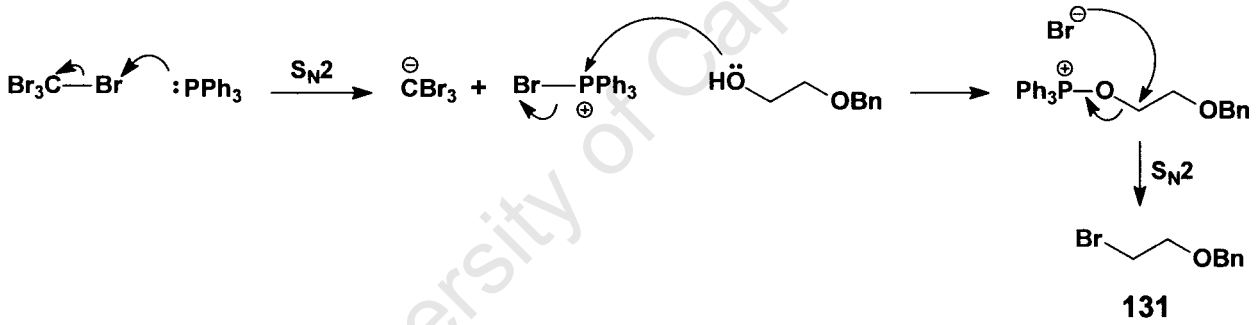


Fig. 3.6: Mechanism of formation of bromide **131**.

The ^1H NMR spectrum of compound **131** revealed the absence of the hydroxyl proton at δ_H 2.52, while its ^{13}C NMR spectrum revealed an upfield shift of the carbon at C-1 from δ_C 61.8 in alcohol **130** to δ_C 30.4, thus confirming substitution by bromine had taken place.

The next step in the reaction sequence was the nucleophilic substitution of the bromide by **81a** to give **132**. In contrast to the difficulty encountered in alkylating phenol **81a** with tosylate **92**, alkylation of phenol **81a** with bromide **131** in the presence of K_2CO_3 as a base in acetonitrile afforded the desired compound **132** smoothly in 87% isolated yield. The ^1H NMR spectrum of alkylated carbamate

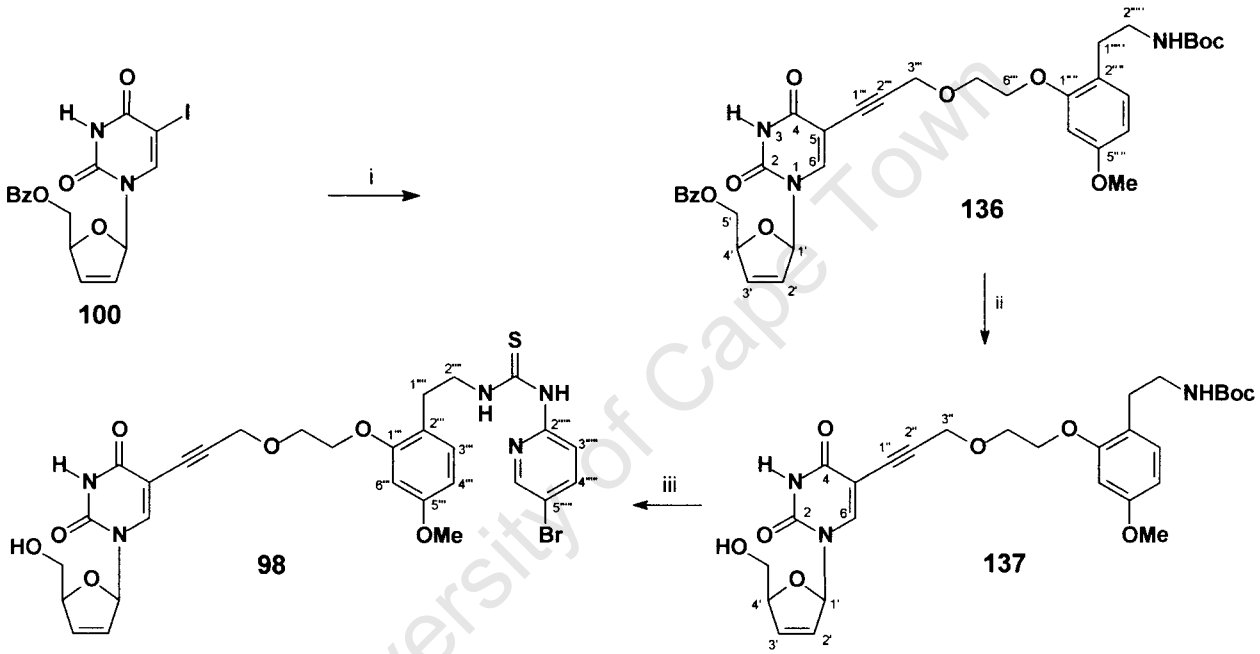
revealed a downfield shift of the protons α to bromine at δ_H 3.50 for the bromide **131** to δ_H 4.11 as well as the presence of signals for the phenolic moiety, thus confirming that the alkylation had taken place.

Catalytic hydrogenolysis of **132** using palladium-on-carbon catalyst in ethanol furnished **133** in 87% yield. The appearance of a hydroxyl proton as a broad singlet resonating at δ_H 1.25 and the absence of the characteristic benzylic methylene singlet at δ_H 4.63 for **132** provided evidence that debenzylation had taken place.

Three possible reactions were considered for the conversion of alcohol **133** to alkyne **135a**. The first involved direct alkylation with propargyl bromide to furnish alkyne **135a** in a single step. The second strategy relied on Mitsunobu coupling of propargyl alcohol with **133** and the last strategy was conversion of the hydroxyl group to a good leaving group, in this case a tosylate, followed by substitution with propargyloxide anion. The second method was attempted first and resulted in the desired target in only 41% yield. Of the other two methods, it turned out to be more efficient to substitute with propargyloxy anion, rather than alkylate with propargyl bromide, probably due to the softness of propargyloxide anion. Thus, to this end, the hydroxyl group on **133** was converted to the tosylate **134** in 76% yield by reacting it with *p*-toluenesulfonyl chloride in the presence of triethylamine and a catalytic amount of DMAP in methylene chloride. The ^{1}H NMR spectrum of **134** displayed aromatic protons with an AB coupling (J_{AB} 8.2 Hz) resonating at δ_H 7.75 and δ_H 7.28 as well as a methyl singlet at δ_H 2.39. The presence of additional aromatic signals in the ^{13}C NMR spectrum further confirmed the presence of a tosylate group.

Alkylation of tosylate **134** was accomplished in the presence of NaH and a large excess of propargyl alcohol in THF at reflux. Aqueous work-up followed by purification by column chromatography furnished the alkyne **135a** in 74% yield. An upfield shift of the H-2' signal in the ^{1}H NMR spectrum from δ_H 4.30 in tosylate **134** to δ_H 3.88 in **135a** confirmed the displacement of the tosylate. The ^{13}C NMR spectrum of compound **135a** displayed signals for the alkyne carbons at δ_C 74.7 for C-5' and δ_C 79.6 for C-6'.

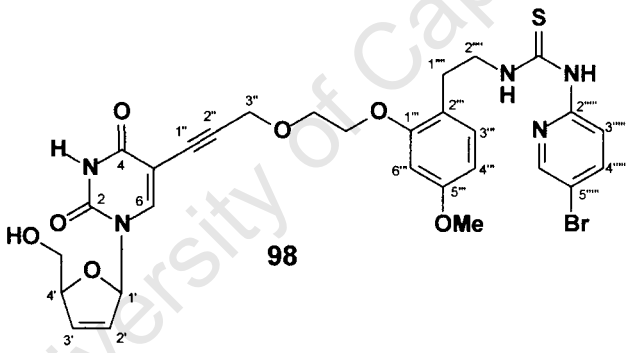
Sonogashira coupling of iodo-d4U **100** with alkyne **135a** yielded the coupled product **136** in 92% yield (Scheme 3.11). The ^{1}H NMR spectrum of **136** displayed the absence of the alkyne proton at δ_H 2.45 in **135a**. Some of the resonances for **136** derived from **100** and **135a** included a broad singlet at δ_H 8.21 (1H, H-6), a multiplet with allylic coupling at δ_H 6.97 (1H, H-1'), a singlet at δ_H 4.40 (2H, H-3'''), a singlet at δ_H 3.73 (3H, OCH_3) and a multiplet at δ_H 3.34 (2H, H-2''''). The ^{13}C NMR spectrum displayed diagnostic signals for the alkyne carbons at δ_C 99.8 for C-1''' and 89.9 for C-2''', and the anomeric carbon of the sugar (C-1') at δ_C 90.7.



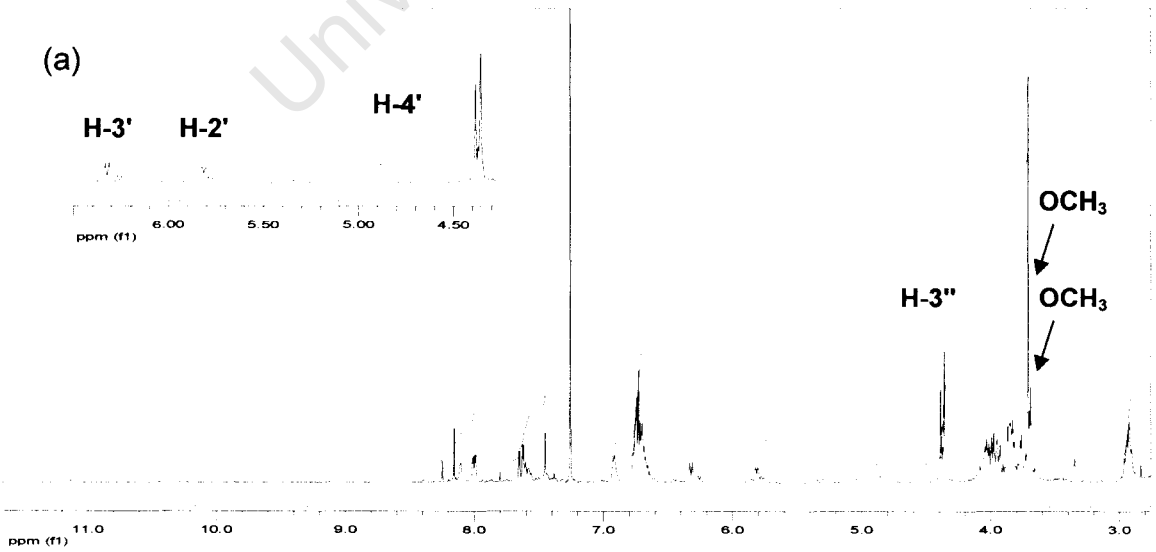
Scheme 3.11. *Reagents and Conditions*: (i) Alkyne **135a**, $(PPh_3)_4Pd$, CuI, Et_3N, DMF, THF, rt, 4 h; 92%. (ii) NaOMe, MeOH, 0 °C, 30 min; 69%. (iii) CF_3COOH, CH_2Cl_2, 0 °C, 30 min then **89**, DMF, 100 °C, 1 h; 40%.

Deprotection of the benzoyl group proceeded well with sodium methoxide in methanol to give the deprotected compound **137** in 69% yield. The ^{1}H NMR resonance for **137** exhibited an expected upfield shift for the diastereotopic H-5' protons to δ_H 3.90 and δ_H 3.79 as a result of a loss in the deshielding effect of the carbonyl group. Similarly, the ^{13}C NMR spectrum displayed the absence of a carbonyl carbon at δ_C 166.2 and an upfield shift of C-5' from δ_C 65.0 in **136** to δ_C 62.7 in **137**. The deprotection was further confirmed by the presence of a broad band in the IR spectrum at 3535 cm^{-1} for the hydroxyl group.

Using the optimised one-pot procedure (Pages 69-70), the Boc group on **137** was deprotected employing trifluoroacetic acid in methylene chloride, and the resultant amine after processing condensed with thiourea derivative **89** in DMF at 100 °C to furnish a mixture of products containing **98** in 40% combined yield after chromatography. The ^{1}H NMR spectrum of the mixture, shown in Fig. 3.7a, revealed two sets of signals for the sugar vinyl protons (H-2', H-3'), the two allylic methylene singlets (H-3") at δ_H 4.39 and δ_H 4.36 and two methoxy singlets at δ_H 3.71 and δ_H 3.69. Further purification by column chromatography and preparative tlc led to separation of the two compounds. The ^{1}H NMR spectra of the mixture of **98** with the unidentified by-product is shown in Fig. 3.7a. Importantly, though Fig. 3.7b shows a pure sample of **98**, with the vinyl signals of d4U integrating correctly as one proton relative to the downfield protons of the thiourea. Also, the ^{13}C NMR spectrum of **98** rendered one set of resonances with every carbon assignable. It is not clear what the structure of the second compound is. Work is currently underway to resolve its structure, and **98** is currently being tested for possible anti-HIV activity.



98



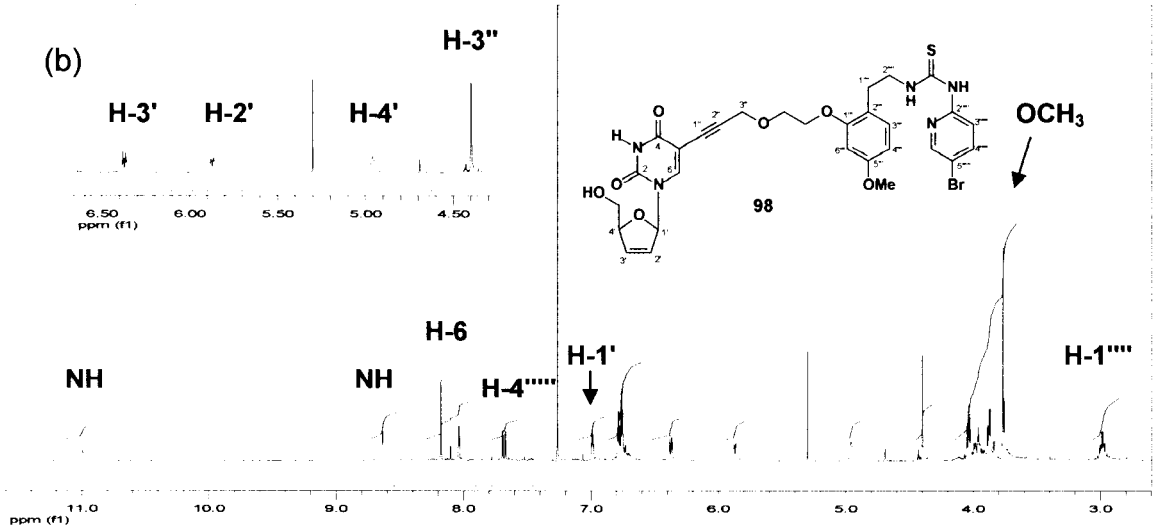
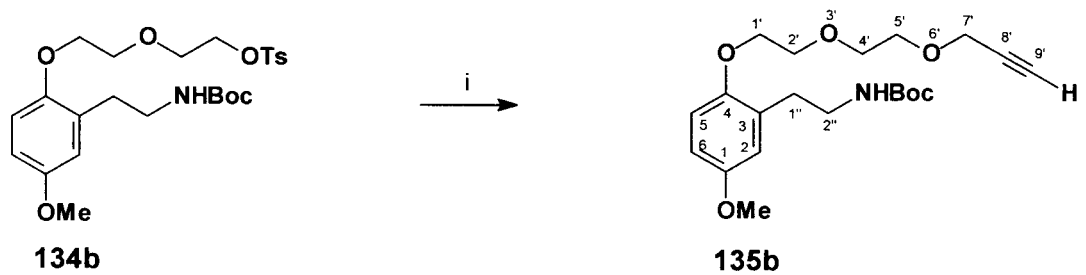


Fig. 3.7: ^{1}H NMR Spectrum of **98**. (a) shows a mixture of the two compounds while (b) represent the purified compound **98**.

3.2.4 Synthesis of [d4U]-diPEG-propyne-[HI-236]

The synthesis of di-PEG alkyne **135b** was similarly accomplished according to the procedure described for **135a**. Thus, diethylene glycol was selectively benzylated using benzyl bromide and NaH in THF. Treatment of the protected glycol with carbon tetrabromide, followed by alkylation of the resultant bromide with phenol **81a** in the presence of NaH in DME gave the corresponding alkylated compound. Catalytic hydrogenolysis gave the corresponding alcohol, which was converted to its tosylate in good yield. Alkylation of this tosylate with propargyloxy anion yielded **135b** in high yield (91%) (Scheme 3.12). The structures of these compounds were unequivocally confirmed by both ^{1}H and ^{13}C NMR spectra, IR, elemental analysis and/or HRMS.



134b **135b**

Scheme 3.12. *Reagents and Conditions*: (i) Propargyl bromide, NaH, THF, reflux, 20 h; 91%.

The synthesis of **99** (Scheme 3.13) was accomplished using the procedure described for **98**. Specifically, palladium cross-coupling of alkyne **135b** with **100** afforded the

coupled compound **138** in 82% yield after column chromatography. The ^{1}H and ^{13}C NMR spectra of **138**, shown in Fig 3.8, revealed resonances derived from both **138b** and **100**. Some important resonances in the ^{1}H NMR spectrum include 6.88 (H-1'), 7.64 (H-6), 4.21 (H-3'''), 3.71 (OCH_3) and 2.76 (H-1''''). Similarly, its ^{13}C NMR spectrum displayed corresponding resonances at 142.3 (C-6), 90.7 (C-1'), 58.9 (C-3'''), 55.6 (OCH_3) and 31.0 (C-1'''').

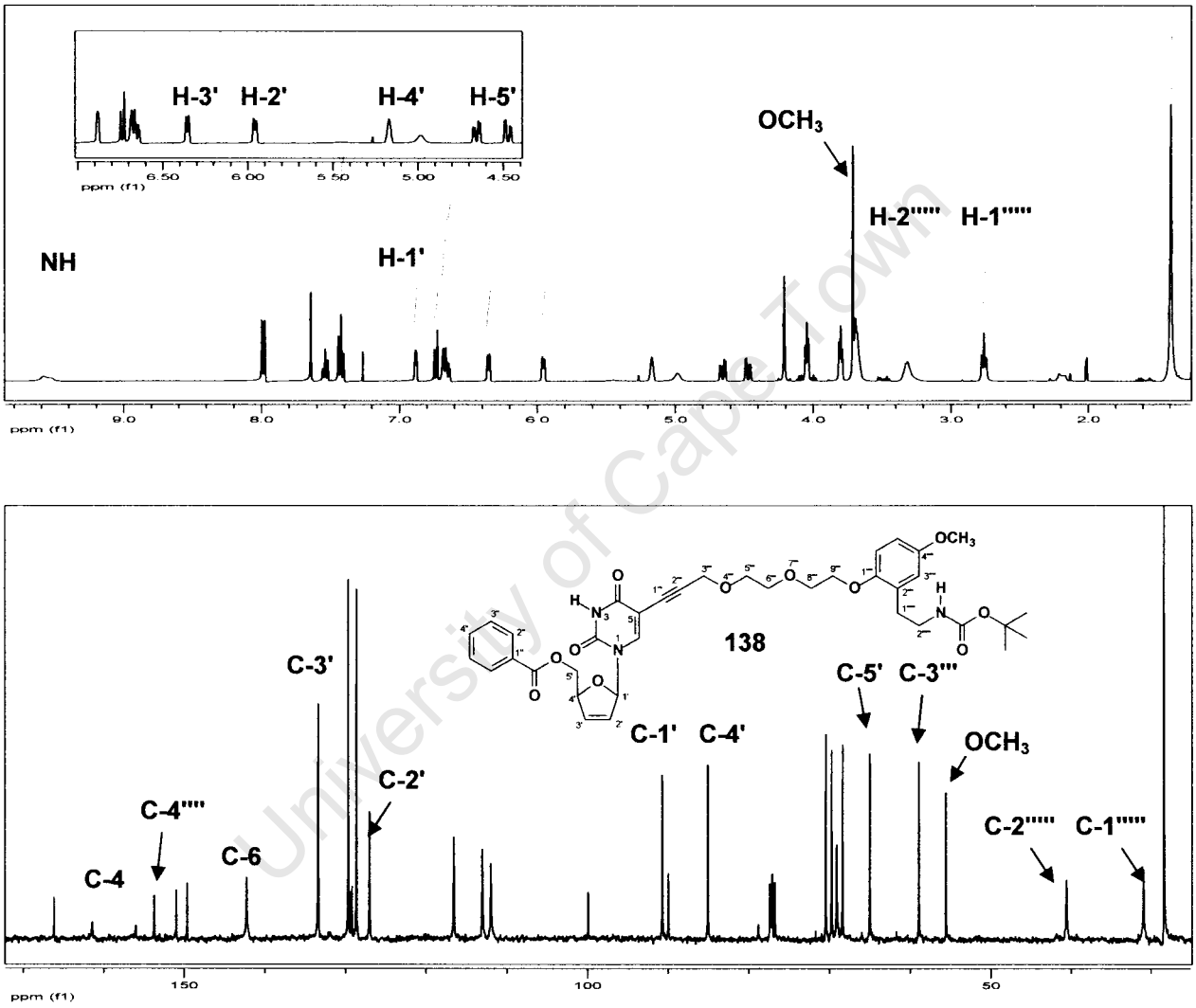
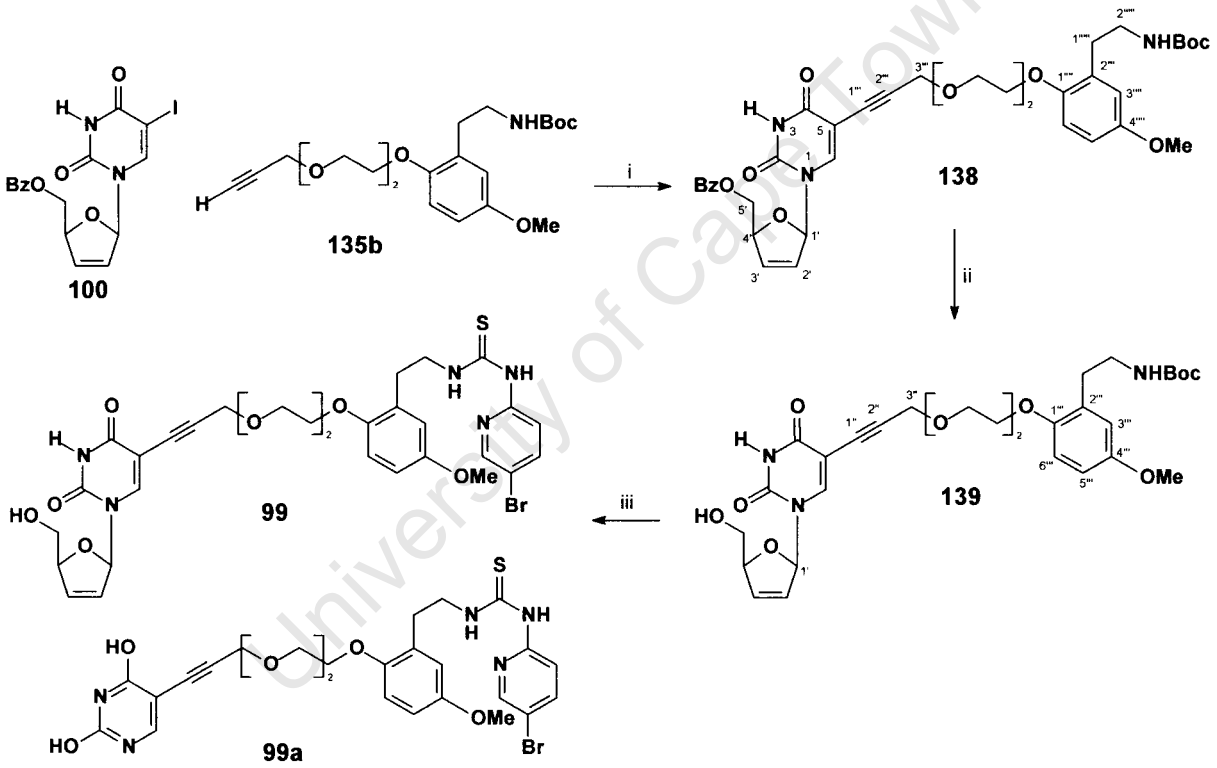


Fig. 3.8: ^{1}H and ^{13}C NMR spectra of **138**.

The benzoyl group on **138** was deprotected using the standard established conditions of NaOMe in MeOH. After aqueous work-up, **139** was isolated in 86% yield after purification by column chromatography. The structure of compound **139** was unequivocally assigned on the basis of its spectroscopic data, using a combination of both 1D and 2D ^{1}H and ^{13}C NMR as well as IR techniques and high-

resolution mass spectrometry. The ^{1}H and ^{13}C NMR spectra of **139** revealed the absence of resonances for the benzoyl group. The ^{1}H NMR spectrum revealed that H-5' protons had shifted upfield from δ_H 4.65 and δ_H 4.47 in **138** to δ_H 3.87 and δ_H 3.66, indicating debenzoylation. The appearance of a broad singlet at δ_H 2.41, corresponding to the hydroxyl group, supported the absence of a benzoate ester. Further evidence was given in the ^{13}C NMR spectrum which displayed an upfield shift in the C-5' resonance from δ_C 65.0 in **138** to δ_C 62.8 as well as the absence of a carbonyl carbon at δ_C 166.2. Furthermore, the IR spectrum of **139** revealed a broad band at 3400-3200 cm^{-1} characteristic of an alcohol, and no carbonyl stretching frequency.



Scheme 3.13. *Reagents and Conditions*: (i) Alkyne **135b**, $(PPh_3)_4Pd$, CuI, Et_3N, DMF, THF, rt, 4 h; 82%. (ii) NaOMe, MeOH, 0 °C, 30 min; 86%. (iii) CF_3COOH, CH_2Cl_2, 0 °C, 30 min then **89**, DMF, 100 °C, 1 h; 40%.

The focus then shifted to the troublesome deprotection of the Boc group and subsequent coupling of the resultant amine to furnish the target compound **99**. Once again, **139** was deprotected with trifluoroacetic acid in methylene chloride and processed according to the optimised conditions described for compound **126**. This resulted in isolation of a mixture of closely-running compounds (on tlc) in an

estimated combined yield of about 60%, after column chromatography. Compound **99** was isolated as a mixture of two closely-running compounds which were separated by further chromatography and recrystallization. Pure **99** is currently being tested for its anti-HIV activity. The ^{1}H NMR spectra shown in Fig. 3.9 depicts the various stages of the purification. Once again, as with **96**, a number of NMR markers for distinguishing the two components in the mixture were identified. These included thiourea NH at around δ_H 11.10 and δ_H 8.86, the H-3" allylic methylene at δ_H 4.33 and the aromatic methoxy at δ_H 3.75. The impurity **99a** is likely to be the cleavage product.

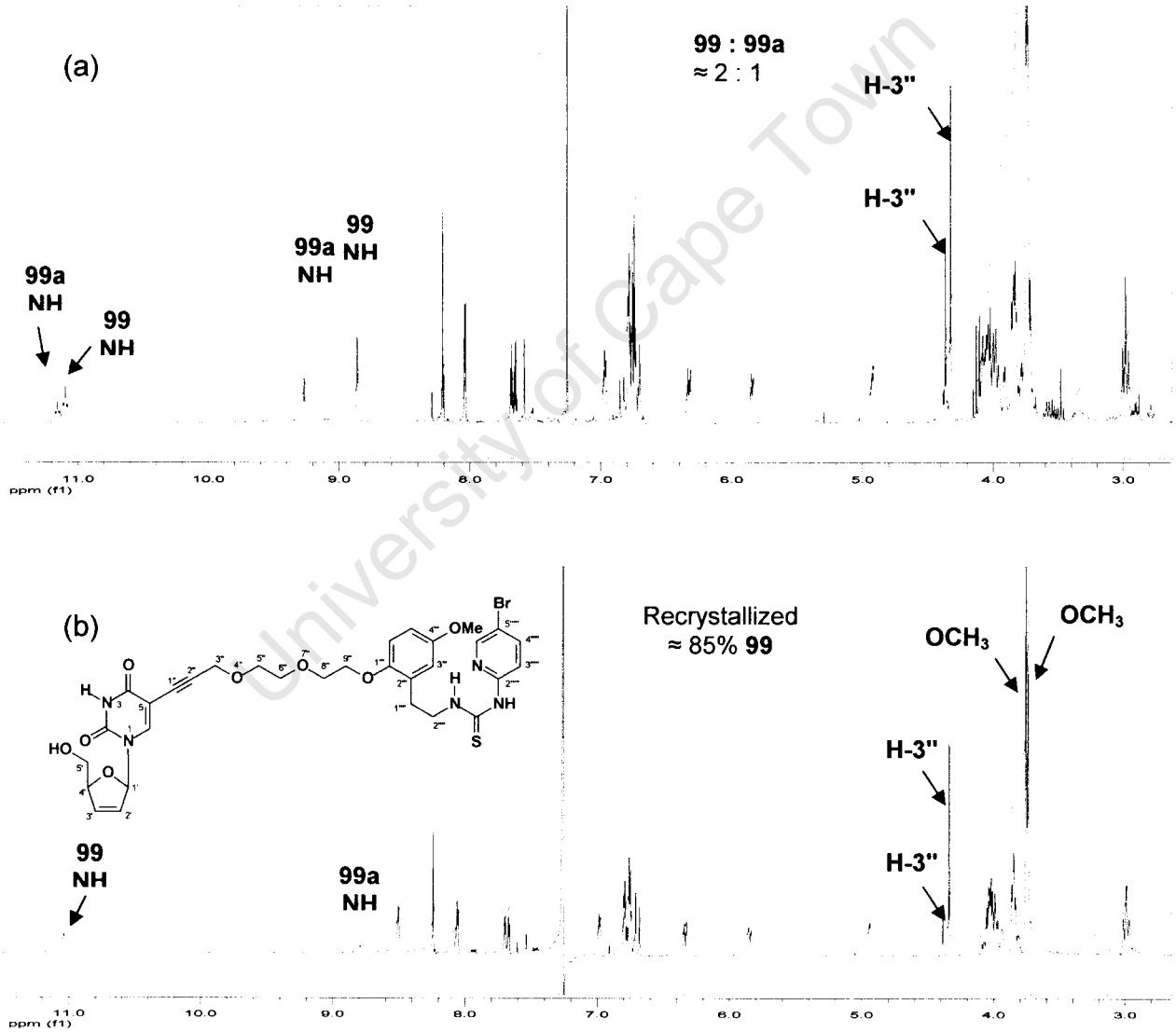


Fig. 3.9: ^{1}H NMR spectrum showing **99** and **99a** (a) mixture of **99** and **99a** (b) **99** with trace impurities of **99a**.

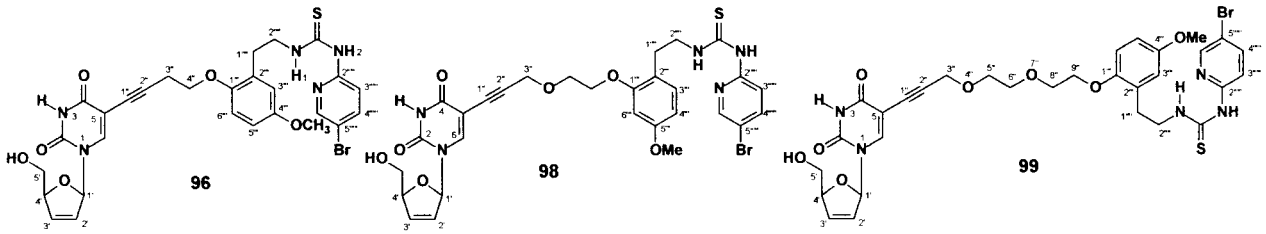


Table 3.1. Some ^{13}C NMR chemical shifts for **96**, **98** and **99**.

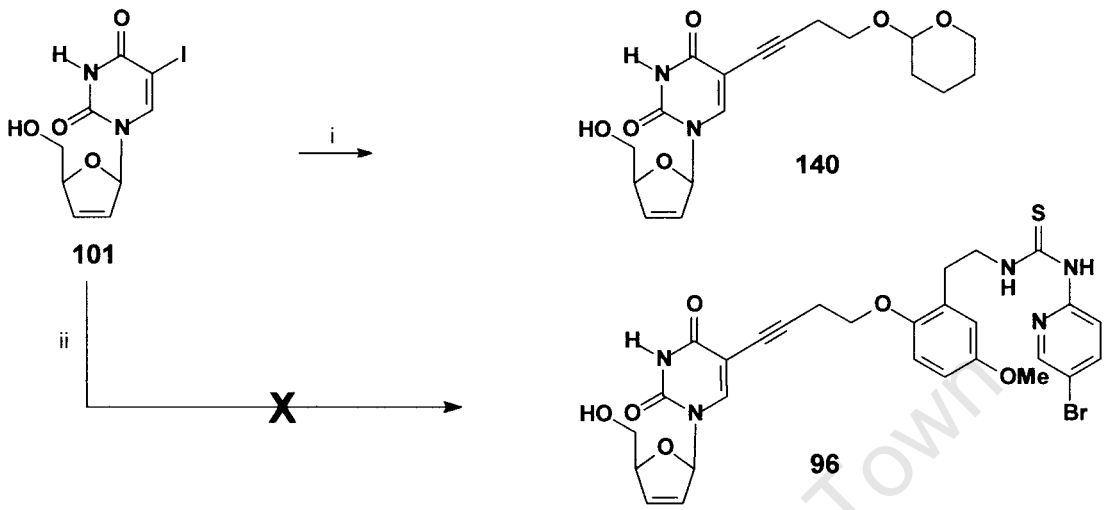
Position	96	98	99
4	162.7	161.9	161.4
6	144.8	144.6	144.9
1'	89.4	90.3	90.3
5'	61.8	62.8	62.8
1"	89.8	95.5	89.4
3"	20.1	59.3	59.2
1'''	150.3	151.2	151.8
6'''	113.4	112.9	111.7
1''''	29.1	30.0	30.1
2''''	44.6	46.0	45.8
4'''''	141.2	141.1	141.1
5'''''	113.4	113.5	112.7
6'''''	145.7	146.7	146.6
C=S	179.1	179.1	179.1

Three bifunctional compounds were synthesized employing this strategy. The final condensation step proved troublesome and more work needs to be done to optimise conditions for this step. Decomposition of these compounds seemed to also occur during purification by chromatography.

3.3 Strategy (b): Condensation followed by Sonogashira

Given the problems of contamination with impurities that were difficult to remove in the final condensation step just described, it was decided to synthesize the target compounds using strategy (**b**). It was hoped that this would result in a much cleaner reaction with fewer polar impurities that could be easily removed by column chromatography. Initially, it was decided to pursue a convergent Sonogashira coupling with unprotected nucleoside **101** in order to gain direct access to the final target. To this end, the coupling strategy began with some preliminary studies on

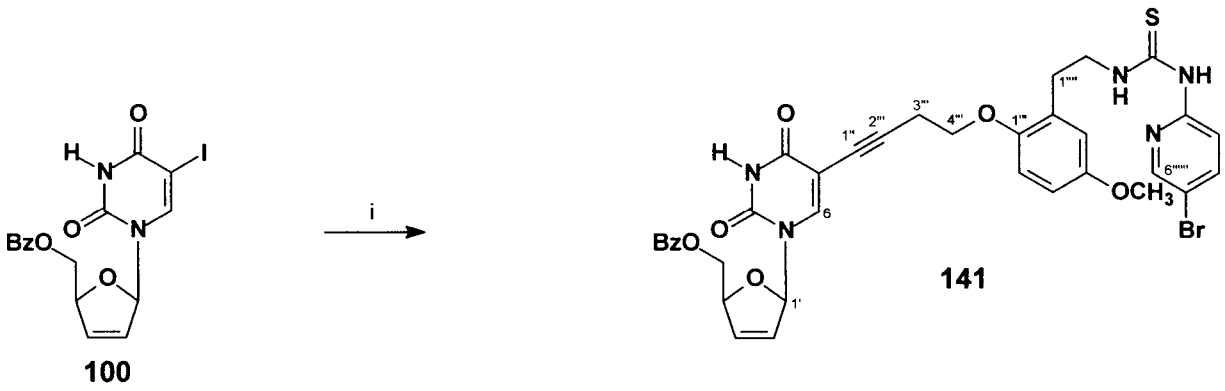
nucleoside **101** and but-3-yn-1-ol protected as its THP ether. Attempted coupling of these two produced the desired coupled product **140** in only 38% yield after column chromatography (Scheme 3.14).



Scheme 3.14. *Reagents and Conditions*: (i) $HC{\equiv}CCH_2CH_2OTHP$ (2 equiv.), $(PPh_3)_4Pd$ (10%), CuI (50%), Et_3N (2 equiv.), DMF, THF, rt. (ii) alkyne **77** or **78** (0.5 equiv.), $(PPh_3)_4Pd$ (10%), CuI (50%), Et_3N (2 equiv.), DMF, THF, rt.

Moreover, an attempt to couple alkynes **77** or **78** (Chapter 2) with **101** was not successful. The most plausible explanation for these discouraging results was that homo-coupling of the alkyne seemed to compete with cross-coupling as judged by tlc which revealed a non-polar spot whose ^1H NMR spectrum revealed the absence of the alkyne proton. Attention was therefore directed to using 5'-*O*-benzoyl protected nucleoside **100**. Gratifyingly, Sonogashira coupling of iodo d4U **100** with alkyne **77** yielded the coupled product **141** in 54% yield (Scheme 3.15) based on **77**. The lower yield was attributed to the use of an excess amount of **100** instead of using an excess of the alkyne **77**. Owing to selectivity observed during this coupling, it is likely that it would be possible to obtain higher yields employing the optimised conditions (2 equiv. alkyne) for this coupling. Owing to limited amount of compound **141** obtained, it was not possible to get a good ^{13}C NMR spectrum. However, its ^1H NMR spectrum revealed resonances for both the nucleoside **100** and alkyne **77**, thus confirming that the coupling had taken place. Specifically, resonances were observed at δ_H 7.67 (1H, s, H-6), δ_H 6.92 (1H, m, H-1'), δ_H 3.77 (3H, s, OCH_3), δ_H 2.68 (2H, t, *J* 6.2 Hz, H-1'''') and of importance was the doublet at δ_H 8.11 with a small coupling constant (*J* 3.2 Hz) assigned to H-6'''' on the pyridine ring. Thus, in view of time constraints and a

lack of material, the debenzoylation reaction was not optimised. However, this convergent route appears to hold great promise for future work.



Scheme 3.15. *Reagents and Conditions*: (i) Alkyne **77** (0.5 equiv.), $(PPh_3)_4Pd$ (10%), CuI (50%), Et_3N (2 equiv.), DMF, THF, rt, 4 h; 54%.

3.4 Biological Results and Discussion

The inhibitory activity of the test compounds was explored against HIV-1 (IIIB) replication in MT-2 cell cultures. The prototype bifunctional [d4U]-butyne-[HI-236] **96** was evaluated by comparison to HI-236 and d4T for inhibition of HIV-1 multiplication in cells of lymphocytic lineage (MT-2). Preliminary studies of the target compound revealed that it inhibited the cells with an EC_{50} value of 200 nM. This is the lowest value of any bifunctional NNRTI-spacer-NRTI compound reported to date and indicated our prototype to be the most promising candidate for this type of inhibitor. The EC_{50} value lies between that of d4T ($\approx$ 2 μM) and that of HI-236 ($\approx$ 5 nM), holding promise that we are on an interesting track of pursuit. The notion of synergism hasn't been proven, but importantly, nor has it been excluded. This is in contrast to an earlier study involving linkage of the spacer to trovirdine by Ladurée and co-workers[77] which led to dimers which did not display any anti-HIV activity, and which could thus exclude synergism. Importantly, the work strongly suggests that the connection point to HI-236 is a good choice.

3.5 Future Work

The synthetic work accomplished in this thesis has established a healthy platform for future study. Understandably, many questions now present themselves as:

1) The synthesis of the alkynyl spacer compounds still needs to be optimized regarding the final step. Options include changing the Boc protecting group and/or further investigation of the reaction conditions. The choice of alkynyl spacer is also a key issue.
2) A small library of compounds needs to be prepared. With the EC50 result obtained, emphasis shifts towards focusing on incremental changes to the spacer length, i.e from 3 carbons to five carbons.
3) The question of much longer spacers and their influence on the entropy of activation needs to be addressed.
4) Computational studies (eg QSAR studies) need to be implemented to develop a better understanding of the compounds interacting with HIV-1 RT and to thus be able to develop a predictive model to work with.

Nucleoside analogues have to be phosphorylated intracellularly to their 5'-triphosphate form using nucleoside kinases before the chemical step of polymerization. Some of them (eg d4T) are known to be poor substrates for these kinases and have thus prompted the development of prodrugs that bypass initial kinase dependency by intracellular delivery of the monophosphorylated analogue. AZT and d4T prodrugs exhibit higher potency against HIV compared to the parent nucleoside in vitro. Future work would involve converting [d4T]-spacer-[HI-236] bifunctional molecules into phosphoramidate prodrugs and compare their activity to the non-phosphorylated parent drugs.

CHAPTER 4

Towards the Synthesis of [d4U]-butane-[HI-236]

4.1 Retrosynthetic Analysis of [d4U]-butane-[HI-236]

The question of flexibility in the linker and its effect on activity focused on converting the directional triple bond to a less rigid polymethylene grouping. It was understood that a chemoselective route needed to be devised in order to preserve the double bond of the nucleoside based on d4T. This precluded the convergent strategy (**b**) described previously in which both double and triple bonds are generated in the same product.

The synthetic plan for the synthesis of double-drug **95** containing a C_4-alkyl tether is shown in Fig. 3.1 and is centered on the construction of the key intermediate **A** which was perceived to be available by dideoxygenation of a uridine derivative. It was thus clear that the synthesis of **A** would require a good reductive elimination strategy to generate the double bond at the 2',3'-position. Iodouridine **E** was considered to be a versatile intermediate as it would allow the introduction of the tether at the C-5 position by making use of palladium cross-coupling chemistry to form intermediate **D**. Two possible synthetic strategies, both using **A** but differing in the level of convergency, were envisaged:

(i) The first strategy would involve a convergent route (**a**) in which synthons **A** and **73** would be coupled via a phenolic *O*-alkylation reaction involving Mitsunobu conditions or via the sulfonate ester of **A**. Of concern was the competing nucleophilicity of the thiourea towards alkylation.

(ii) The second strategy also involved **A** but via a more "linear" route (**b**), involving alkylation of phenolic carbamate **81a**, followed by condensation with thiocarbonyl derivative **89**. It was noted that this strategy would require deprotection of the Boc group followed by condensation of the resultant amine, a step that had already proved to be problematic.

In spite of the latter, of the two strategies, (ii) was considered to be the better option because of the anticipated interference from the thiourea sulphur in the alkylation

step in strategy (i). Thus, the initial strategy for the synthesis of key intermediate **A** was to investigate a good protecting-group strategy for the following key reactions in order as: (i) Sonogashira coupling of **E** with a protected alkyne, (ii) hydrogenation of the triple bond on **D** and (iii) 2',3'-dideoxygenation of vicinol diol to form the olefin. The primary hydroxyl group on uridine could be protected selectively as a silyl ether, while the vicinol diol would be protected as a ketal.

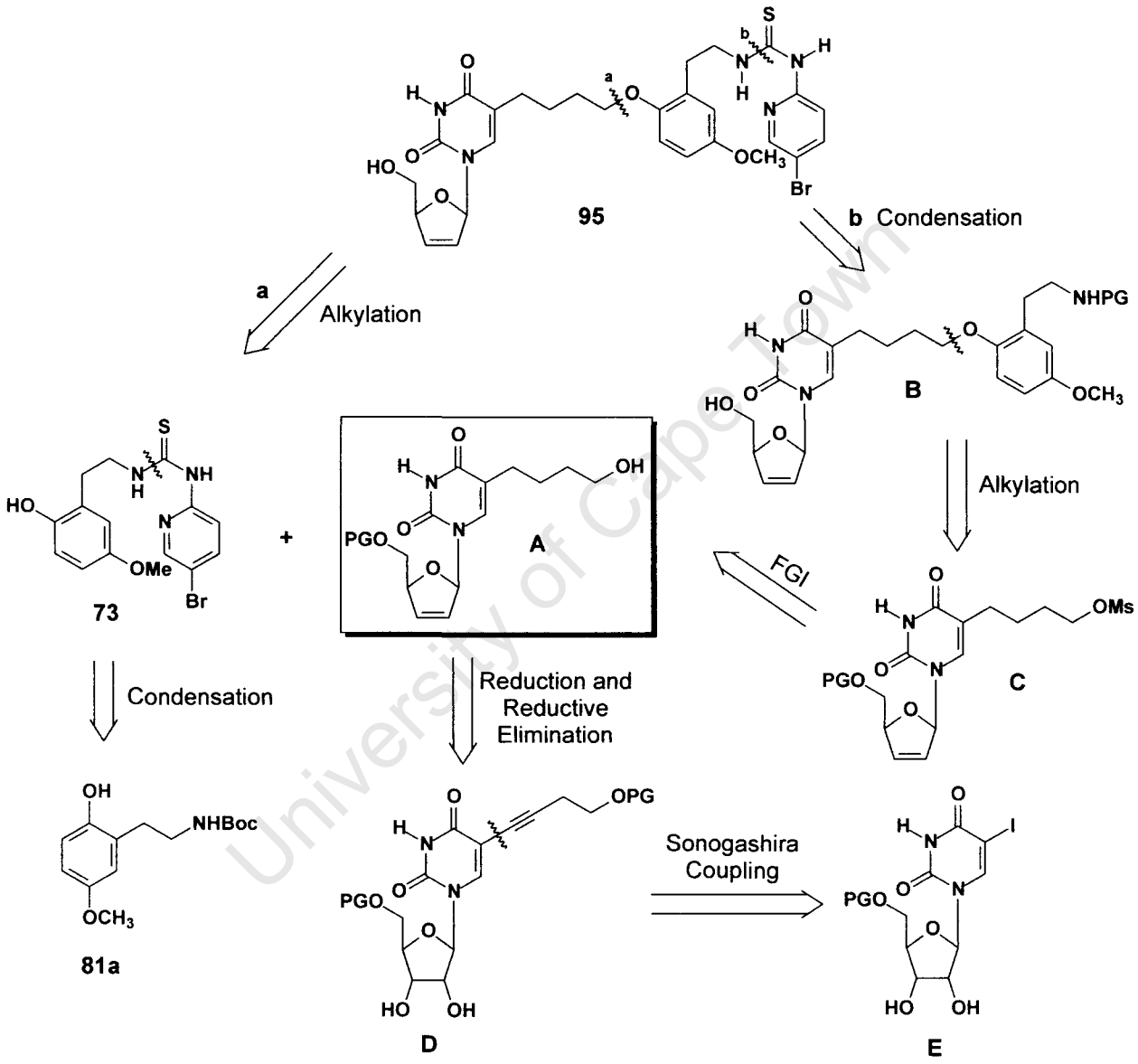


Fig. 4.1: Retrosynthetic analysis of [d4U]-butane-[HI-236]

4.2 Synthesis of key intermediate A

Preparation for the Sonogashira coupling began with iodination of uridine using the procedure of Wah[118] using molecular iodine and $AgNO_3$ at 0 °C in methanol to give

110 in 83% isolated yield reproducibly (Scheme 4.1). The main advantage of using silver salts is that following heterolytic cleavage of iodine, precipitation of AgI removes the residual iodide ions from the reaction rapidly. In this reaction, $AgNO_3$ acts as a Lewis-acid catalyst and coordinates with iodine generating an electrophilic iodo species, which is attacked by the alkene **I** to give intermediate **II** (Fig. 4.2). Isolation of the product was facile, involving AgI filtration, and crystallization of the residue following methanol removal.

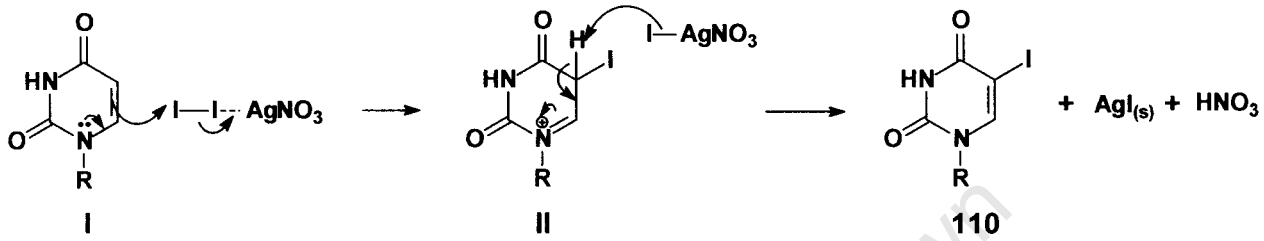
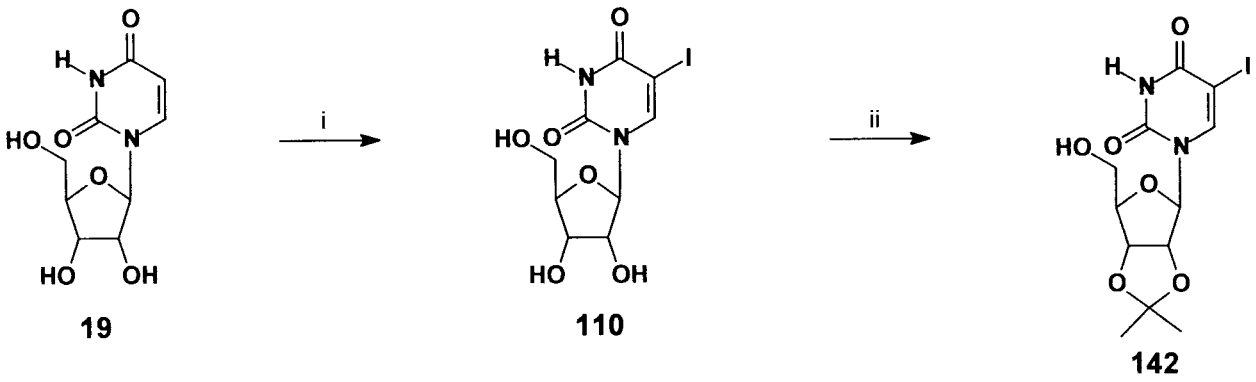


Fig. 4.2: Mechanism of iodination of uridine

Formation of **110** was corroborated by its ^1H NMR spectrum which revealed the collapse of H-6 at δ_H 8.45 from a doublet to a singlet. Similarly, the ^{13}C NMR spectrum revealed an upfield shift in C-5 to δ_C 69.9, thus confirming that C-5 substitution by iodine had taken place.

5-Iodouridine **110** was then chemoselectively protected at the 2' and 3' positions as an acetonide **142** in high yield using the standard method introduced by Levene and Stiller[119] in the 1930's of acetone and conc. H_2SO_4 with $CuSO_4$ as an added dehydrating agent. The method takes advantage of the *cis* disposition of the two hydroxyl groups resulting in desired differentiation of the various hydroxyl groups. Following reaction, the acidic medium was quenched with KOH in MeOH, the salts filtered, the solvent evaporated and the residue recrystallized from methanol to give **142** in high yield. The ^1H NMR spectrum of **142** displayed two diastereotopic methyl singlets at δ_H 1.50 and δ_H 1.36, while the ^{13}C NMR spectrum confirmed the formation of the ketal by displaying a quaternary ketal carbon resonance at δ_C 114.5 as well as two methyl carbons at δ_C 27.2 and δ_C 25.3.

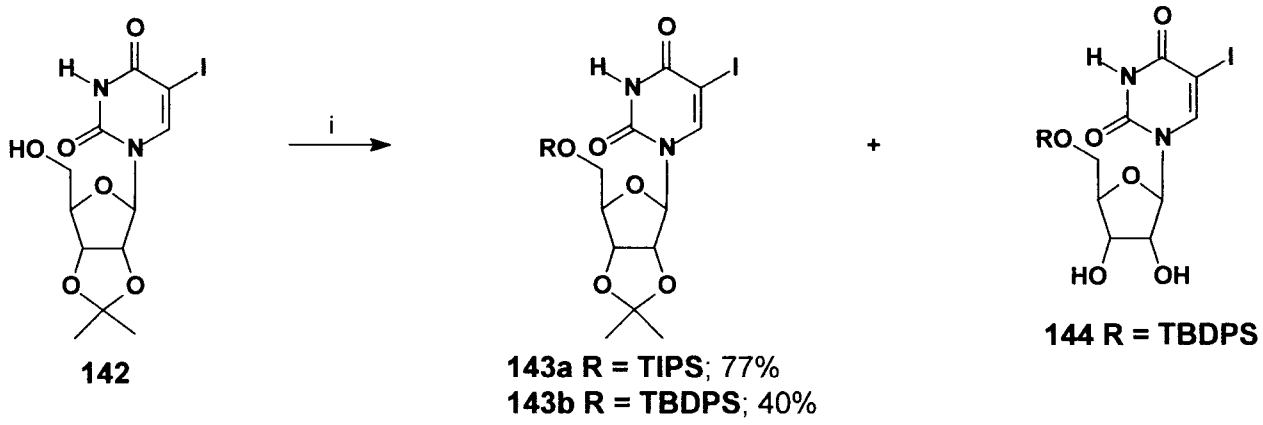


19 **110** **142**

Scheme 4.1. *Reagents and Conditions*; (i) I_2, $AgNO_3$, MeOH, 0 °C, 30 min; 83%. (ii) Acetone, $CuSO_4$, H_2SO_4, 0°C-rt, 22 h; 95%.

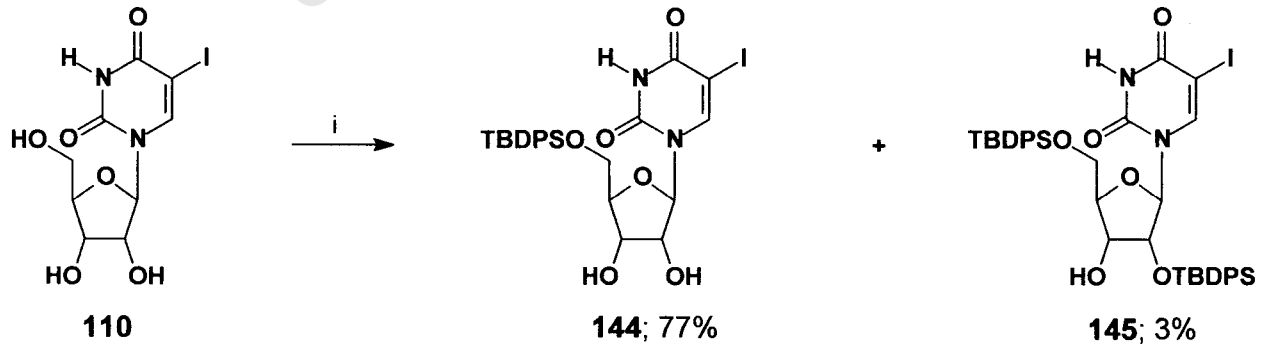
In preparation for the pivotal Sonogashira coupling, initially it was considered necessary to protect the 5'-hydroxyl group of **142**, which was carried out using triisopropylsilyl chloride and imidazole as a base in DMF at room temperature to give triisopropylsilyl ether **143a** in 77% yield (Scheme 4.2). By comparison, protection of the 5'-hydroxyl group as its *tert*-butyldiphenylsilyl ether **143b** was only achieved in 40% yield. Interestingly, the by-product of the latter reaction, obtained in 60% yield, was the deprotected ketal **144**. The deprotection reaction presumably used the imidazole hydrochloride as acid catalyst, and allowed release of steric strain between the bulky silyl group and pyrimidine base by rupturing the 5,5-bicyclic ring system that brings them into closer proximity compared to the sugar ring by itself.

The ^1H NMR spectrum for compound **143a** showed two doublets for the diastereotopic isopropyl methyl groups integrating for 18 protons at δ_H 1.09, as well as a methine multiplet at δ_H 1.18. Similarly, the ^{13}C NMR spectrum displayed corresponding carbon resonance at δ_C 18.0 and δ_C 12.0. Spectroscopic data for **143b** showed aromatic protons at δ_H 7.41-7.71 integrating for 10 protons as well as a *t*-butyl methyl singlet resonating at δ_H 1.12 integrating for 9 protons. Its ^{13}C NMR spectrum displayed additional signals in the aromatic region, a *t*-butyl methyl signal at δ_C 27.1 and a quaternary carbon signal at δ_C 19.3 for the *t*-butyl group.



Scheme 4.2. *Reagents and Conditions*: (i) TIPSCl, imidazole, DMF, rt, 12 h.

The deprotection of the ketal group during the silylation reaction to form **143b** prompted a reappraisal of the chemoselective protection of the hydroxyl groups of **110**. Treatment of **110** with TBDPSCl (1.5 equiv.) in the presence of imidazole (1.5 equiv.) yielded the desired nucleoside **144** in 49% yield, together with a by-product **145** (30% yield), which had two silyl ether groups. To minimise formation of the by-product, the amount of silyl chloride used was minimised to 1.0 equiv, however this resulted in incomplete reaction. The starting nucleoside **110** is soluble in water, and TLC was carried out on the aqueous phase revealing the presence of unreacted starting material. Treatment with 1.2 equivalents of silyl chloride significantly improved the yield of mono-TBDPS **144** to 77% with the di-TBDPS being formed in only 3% yield (Scheme 4.3). The ^{1}H and ^{13}C NMR spectroscopic data of **144** indicated the anticipated additional signals in the aromatic and highfield regions, confirming the presence of a silyl group.

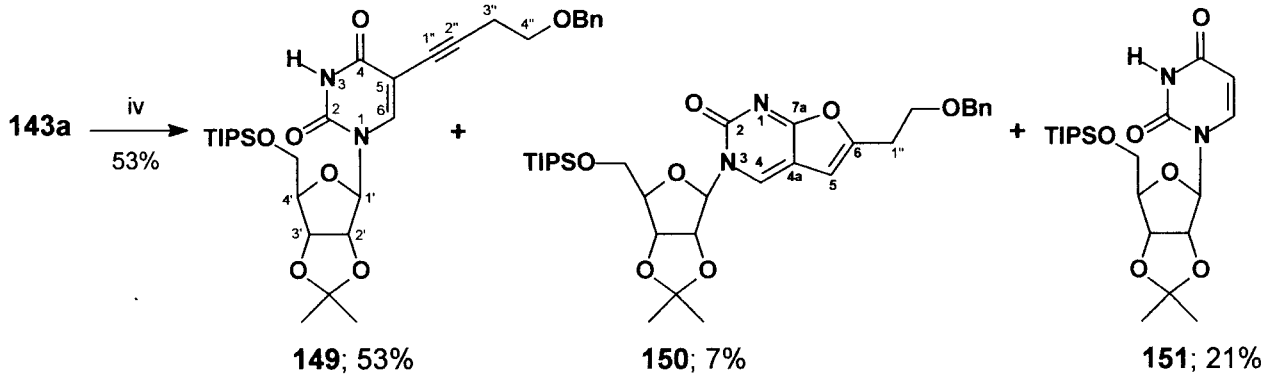


Scheme 4.3. *Reagents and Conditions*: (i) TBDPSCl (1.2 equiv.), imidazole, DMF, rt, 12 h.

With 5-iodouridine derivatives (**143a**, **143b** and **144**) in hand, Sonogashira coupling with protected alkynol was attempted. The benzyl (Bn), *p*-methoxybenzyl (PMB) and tetrahydropyran (THP) protecting groups were selected to protect the hydroxyl group on 3-butyn-1-ol and to provide a range of comparisons in the Sonogashira coupling. The benzyl and PMB groups were selected in anticipation of their stability towards acid hydrolysis of the acetonide needed to introduce the 2'/3' double-bond. The tetrahydropyranyl ether was selected in view of its anticipated stability towards hydrogenation post-coupling. The hydroxyl group protections were achieved following standard literature procedures, and the structures of the protected 3-butyn-1-ol derivatives **146**, **147** and **148** were confirmed by comparison of their ^{1}H and ^{13}C NMR spectra to those reported in the literature.[120,121,122]

Sonogashira coupling of **143a** with 3-butyn-1-ol protected as its benzyl ether **146** gave **149**, in 53% yield after column chromatography (Scheme 4.4). The by-products identified included cyclized furanopyrimidine **150** (7%) as well as deiodinated product **151** (21%).

The spectroscopic data for compound **149** was consistent with the assigned structure and the presence of the alkyne group was evident in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum for **149** revealed two triplets at δ_H 2.68 (H-3") and δ_H 3.62 (H-4"), a singlet at δ_H 4.53 for the benzylic methylene protons and a multiplet at δ_H 7.30 for the aromatic protons. Similarly, the ^{13}C NMR spectrum displayed additional signals at δ_C 100.5 (C-1"), 91.4 (C-2"), 68.1 (C-4") and 20.9 (C-3") compared to **143a**. Also present were signals for the aromatic carbons as well as signals for the benzylic methylene carbon at δ_C 72.9. A downfield shift of C-5 from δ_C 68.4 in **143a** to δ_C 72.2 further supported that the coupling had occurred. The structure was further confirmed by 2D NMR spectroscopy. The HMBC spectrum revealed a ^{3}J correlation between the singlet at δ_H 7.68 (H-6) and the alkynyl carbon at δ_C 100.5 (C-1").



Scheme 4.4. *Reagents and Conditions*: (i) $(PPh_3)_4Pd$, CuI, alkyne **146**, Et_3N, DMF, THF, rt, 16 h.

By-product **150**, although only obtained in low yield, proved to have an interesting furanopyrimidine structure shown in Scheme 4.4, via a CuI-catalyzed 5-*endo-dig* cyclization between the C-4 pyrimidine oxygen and acetylenic bond (Fig 4.3). In this case, CuI activates the triple bond by forming a complex **II**. This is followed by intramolecular addition of oxygen across the activated triple bond with subsequent proton transfer and release of CuI to give the furanopyrimidine product **150**.

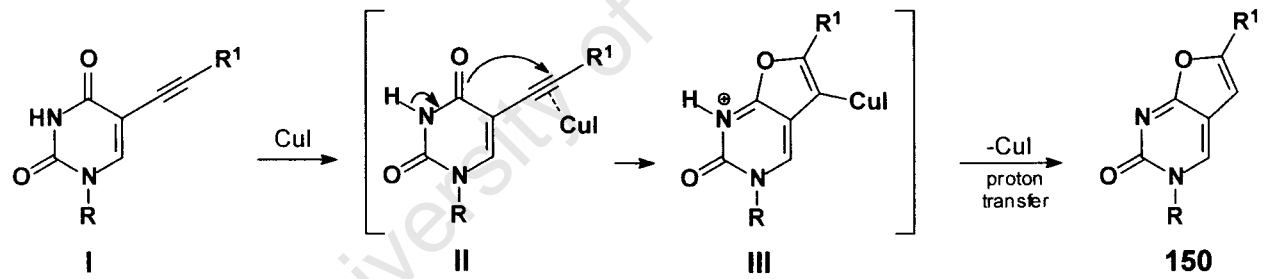


Fig 4.3: Plausible CuI-catalyzed mechanism for cyclization of furanopyrimidine

Furanopyrimidine derivatives are interestingly fluorescent and have been reported to display important potency and exclusive selectivity against varicella zoster virus (VZR).[123] Support for structure **150** was provided by the appearance of an additional triplet with an allylic coupling (*J* 1.8 Hz) at δ_H 6.13 for H-5 (see numbering in Scheme 4.4). The ^{13}C NMR spectrum of **150** displayed a downfield signal at δ_C 171.9, which was assigned to C-7a. Furthermore, ^3J HMBC correlations were identified between the proton at δ_H 6.13 (H-5) and the carbons at δ_C 171.9 (C-7a), 156.7 (C-6), 135.6 (C-4), 107.3 (C-4a) and δ_C 29.2 (C-1") confirming that cyclization had occurred. Another

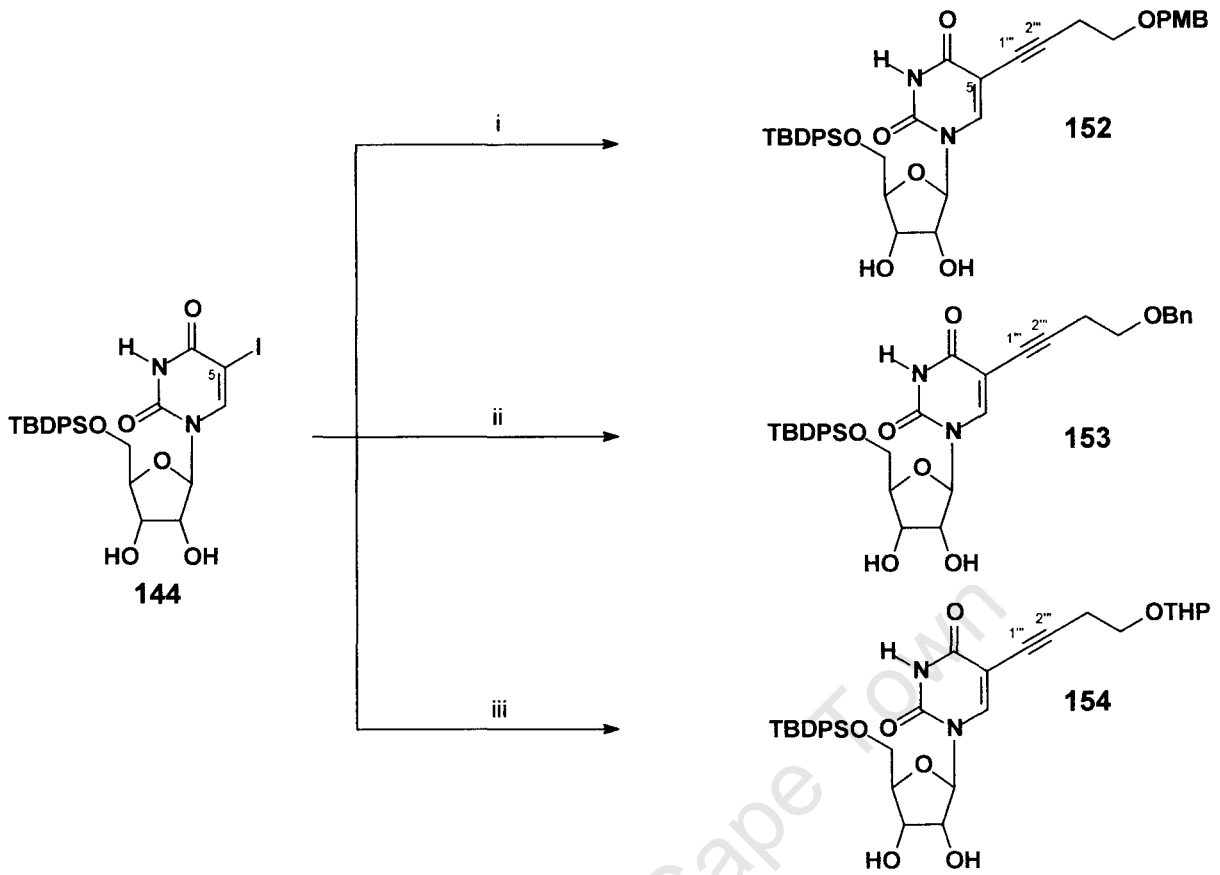
important correlation was between the proton at δ_H 8.34 (H-4) and the carbon at δ_C 100.1 (C-5).

Literature precedence suggested that formation of the furanopyrimidine was dependent on the protecting groups on the nucleoside.[108,109] In this case, the isolation of **151** suggested that the presence of the ketal pushes the bulky phenyl rings of the silyl ether into close proximity with the nucleoside base, thus leading to steric hindrance which inhibits the transmetallation step of the reaction. This suggested that a nucleoside unprotected at the 2',3'-position would be a better substrate for this coupling.

To this end, a series of Sonogashira reactions were performed on **144** using a number of different protected 3-butyn-1-ols (Scheme 4.5). Thus, compound **144** was subjected to a cross-coupling reaction with 4-methoxybenzylbut-3-yne to form **152** in 48% yield following column chromatography. The ^{1}H NMR spectrum of **152** was similar to that of **149** except for the absence of ketal signals, and its ^{13}C NMR spectrum revealed signals at δ_C 100.3 and δ_C 91.9 for C-1'' and C-2'', respectively. Another significant signal was the downfield shift in C-5 to δ_C 72.5.

Similarly, the use of a 3-butyn-1-ol protected as its benzyl ether gave the coupled product **153** in 76% yield. No cyclic furanopyrimidine by-product was formed in these reactions. The absence of furanopyrimidine supports the steric-strain rationale discussed before suggesting that in ketal **149**, the C-5' silyloxy and C-1' base groups are relatively close together compared to **152**. Thus for **149**, furanopyrimidine formation of **150** reduces the steric bulk of the C-5 substituted base. Spectroscopic data for compound **153** confirmed that the coupling had taken place.

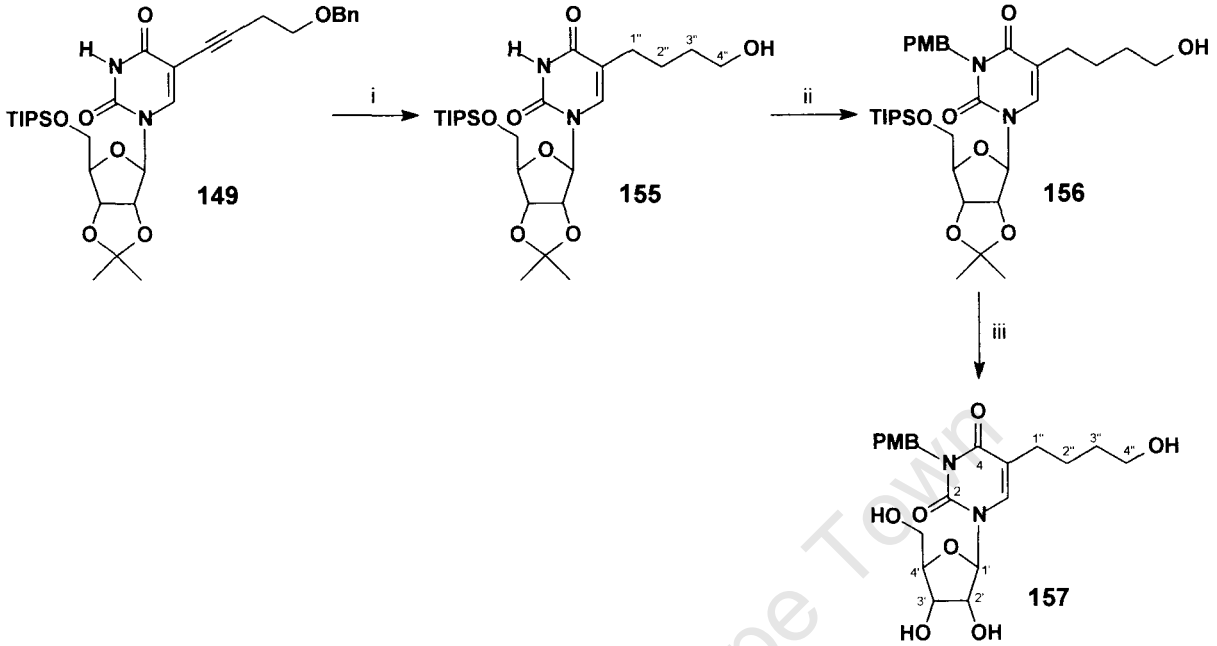
Sonogashira coupling of the 3-butyn-1-ol protected as a tetrahydropyran ether with 5-iodouridine **144** proved to give the most pleasing result furnishing the coupled product **154** in 97% yield. Generally, alkynols protected as THP ethers gave excellent yields during palladium cross-coupling reactions. The ^{13}C NMR spectrum of **154** displayed diagnostic signals at δ_C 99.4 and δ_C 90.5 for the alkynyl carbons at C-1''' and C-2''', respectively.



Scheme 4.5. *Reagents and Conditions*: (i) $HC{\equiv}CCH_2CH_2OPMB$ **147** (1.5 equiv.), $(PPh_3)_4Pd$ (10%), CuI (50%), Et_3N (2 equiv.), DMF, THF, rt; 48%. (ii) $HC{\equiv}CCH_2CH_2OBn$ **146** (1.5 equiv.), $(PPh_3)_4Pd$ (10%), CuI (50%), Et_3N (2 equiv.), DMF, THF, rt; 76%. (iii) $HC{\equiv}CCH_2CH_2OTHP$ **148** (3 equiv.), $(PPh_3)_4Pd$ (10%), CuI (50%), Et_3N (2 equiv.), DMF, THF, rt; 97%.

Having optimised conditions for Sonogashira coupling, the next step in the reaction sequence was hydrogenation of the alkyne functionality which was carried out using a palladium-on-carbon catalyst. Catalytic hydrogenation/hydrogenolysis of **149, 152** and **153** would be followed by reprotection of the primary hydroxyl group with a PMB group to prepare for introduction of the C-2'/3' double bond. The PMB group was chosen in view of its selective removal with CAN. The coupled product **149** was thus reduced with hydrogen in the presence of 10% palladium-on-carbon to give alcohol **155**. Filtration of the catalyst followed by purification of the crude product by column chromatography afforded **155** in 71% yield (Scheme 4.6). The ^1H NMR spectrum of **155** revealed additional methylene signals at δ_H 2.30 for H-1" and 1.05 for H-2", while its ^{13}C NMR spectrum displayed an upfield shift of the signals at C-1" and C-2" from δ_C 100.5 and δ_C 91.4 in **149** to δ_C 32.1 and δ_C 26.7, respectively. There was also a

significant downfield shift in the C-5 carbon to δ_C 115.2, which further confirmed a loss in the shielding effect caused by the alkyne.



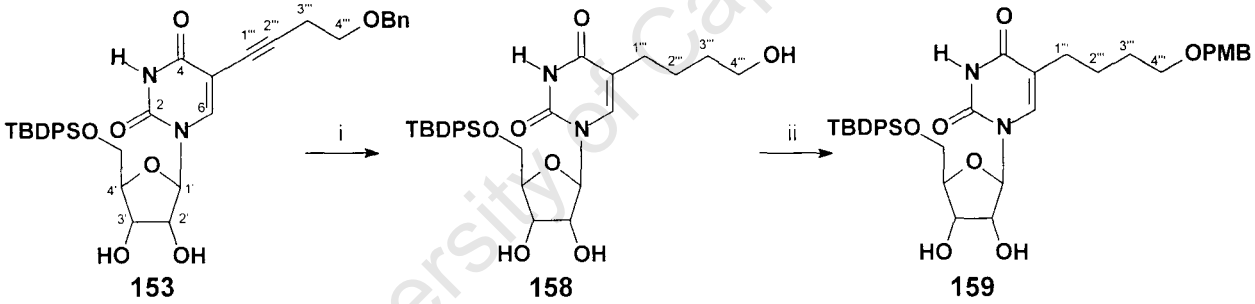
Scheme 4.6. *Reagents and Conditions*: (i) H_2, Pd/C, EtOH, rt, 2 d; 71%. (ii) PMBCl, NaH, DMSO, THF, rt, 21 h; 75%. (iii) 2M HCl, MeOH, rt, 24 h; 87%.

Protection of the primary hydroxyl group of **155** via its alkoxide ion as its *p*-methoxybenzyl ether resulted in imide **156** in good yield due to N-alkylation. This was concluded on the basis that resonance in the C-4" hydroxyl proton could be seen at δ_H 2.28 indicating that C-4" alkylation had not taken place. Furthermore the NH imide proton had disappeared. Compound **156** also showed two sets of aromatic protons (4H) of an AB system resonating at δ_H 7.41 (2H, d, *J* 8.6 Hz) and δ_H 6.78 (2H, d, *J* 8.6 Hz), diastereotopic benzylic methylene protons with a large geminal coupling at δ_H 5.04 (d, *J* 13.5 Hz) and δ_H 4.96 (d, *J* 13.5 Hz), and a methoxy singlet at δ_H 3.73 for the *p*-methoxybenzyl group. Similarly, the ^{13}C NMR spectrum had signals showing the presence of an aromatic ring, a methoxy signal at δ_C 55.0 and a methylene carbon at δ_C 43.8.

Acid hydrolysis of acetonide **156** using 2M HCl led to concomitant cleavage of the TIPS group and subsequent formation of triol **157**. Reducing the strength of the acid such as using acetic acid in water (7:3) failed to generate the expected diol. This negative result was attributed to relief of steric strain imposed by the bulky

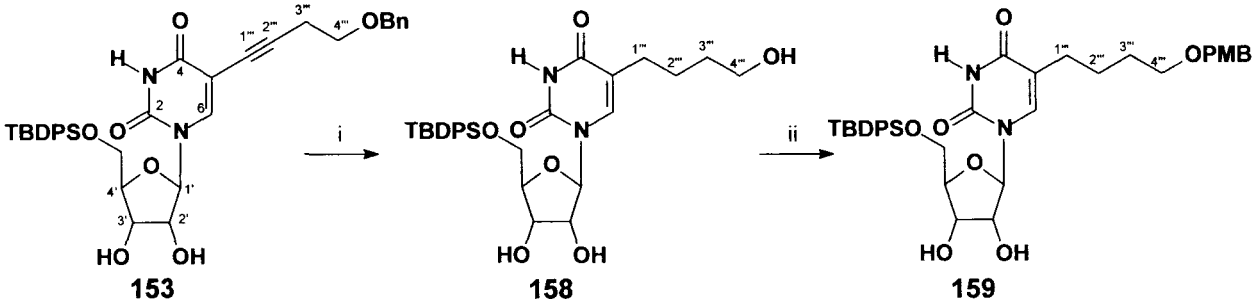
triisopropyl group. The ^{1}H NMR spectrum of **157*** revealed the absence of the triisopropylsilyl ether methyl signals at δ_H 1.04 and the appearance of four broad singlets resonating at δ_H 4.20, δ_H 3.46, 3.28 and δ_H 2.09 for the hydroxyl groups. The choice of TIPS as a protecting group for the 5'-OH group was based on the fact that it was easier to cleave at the end of the synthesis. However, its instability under acidic conditions prompted us to shift to using TBDPS as a protecting group.

In view of positive results in the 2',3'-unprotected series, it was decided to focus on the **152-154** series containing the C-5' TBDPS group and with free C-2'/3'-hydroxyl groups. Thus, hydrogenation of the triple bond in **152** and **153** led to hydrogenolysis of the PMB and benzyl protecting groups, respectively to form **158** (Scheme 4.7). The ^{1}H NMR spectrum of **158** showed additional signals in the upfield region for the methylene protons, while its ^{13}C NMR spectrum revealed the absence of alkyne signals at δ_C 99.4 and δ_C 90.5, thus confirming that reduction had taken place.



Scheme 4.7. *Reagents and Conditions*: (i) H_2, Pd/C, EtOH, rt, 1 day; 83%. (ii) PMBCl, NaH, THF, rt, 16 h; 27%.

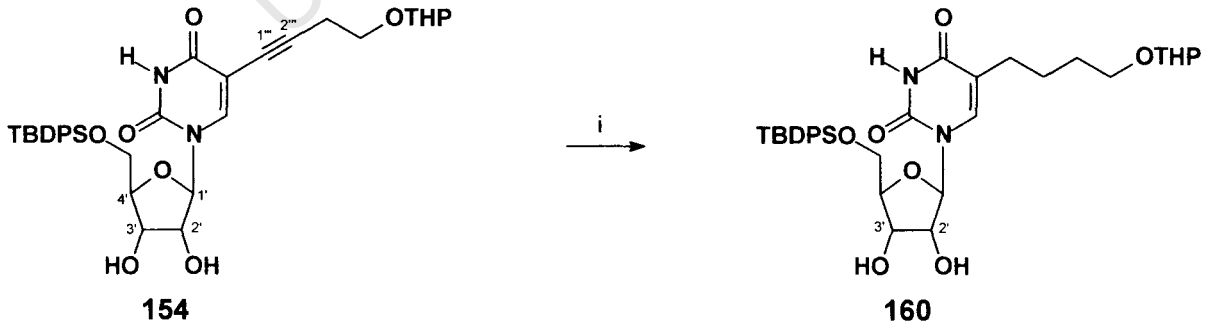
* This compound was not fully characterised and its ^{1}H NMR data is as follows: δ_H (300 MHz, $CDCl_3$); 7.53 (1H, s, H-6), 7.38 (2H, d, *J* = 8.7 Hz, H-2''', H-6'''), 6.79 (2H, d, *J* = 8.7 Hz, H-3''', H-5'''), 5.73 (1H, d, *J* = 3.9 Hz, H-1'), 5.04 (1H, d, *J* = 13.7 Hz, ArCH_2O), 4.98 (1H, d, *J* = 13.7 Hz, ArCH_2O), 4.31 (2H, m, H-2', H-3'), 4.20 (1H, brs, OH), 4.09 (3H, m, H-4', H-4''), 3.94 (1H, brd, *J* = 11.3 Hz, H-5'), 3.79 (1H, brd, *J* = 11.3 Hz, H-5'), 3.75 (3H, s, OCH_3), 3.46 (1H, brs, OH), 3.28 (1H, brs, OH), 2.33 (2H, t, J = 7.1 Hz, H-1''), 2.09 (1H, brs, OH), 1.58 (4H, m, H-2'', H-3'').



Scheme 4.7. *Reagents and Conditions*: (i) H_2, Pd/C, EtOH, rt, 1 day; 83%. (ii) PMBCl, NaH, THF, rt, 16 h; 27%.

An attempt to reprotect the primary hydroxyl group of **158** with *p*-methoxybenzyl (PMB) chloride under basic conditions via the alkoxide led to the desired compound in only 27% yield. The low yield was attributed to the cleavage of the TBDPS group which was isolated as a by-product (TBDPSOH) of this reaction. Silyl ethers are known to undergo base-promoted migration reactions from one functional group to the other and the cleavage could have arisen in an attempt to shift to the less hindered side-chain primary hydroxyl group.

The focus then shifted to hydrogenation of the triple bond of coupled THP ether **154** (Scheme 4.8) to give diol **160**. Filtration of the catalyst followed by column chromatography gave **160** in 83% yield. In this case, a catalytic amount (0.1 eq.) of triethylamine was added to the reaction mixture to avoid deprotection of the THP group.



Scheme 4.8. *Reagents and Conditions*: (i) H_2, Pd/C, EtOH, 2 days; 95%.

It is noteworthy that the rate of hydrogenation was dependent on the amount of triethylamine used. Excluding triethylamine led to deprotection of THP ether while the use of 1 mol equivalent of Et_3N slowed the reaction up to 48 h. The ^{13}C NMR

spectrum of **160** showed an upfield shift of the carbons resonating at δ_C 99.4 (C-1''') and δ_C 90.5 (C-2''') to δ_C 25.7 and δ_C 25.4 for the methylene carbons, but importantly, retention of signals for both the THP and TBDPS groups.

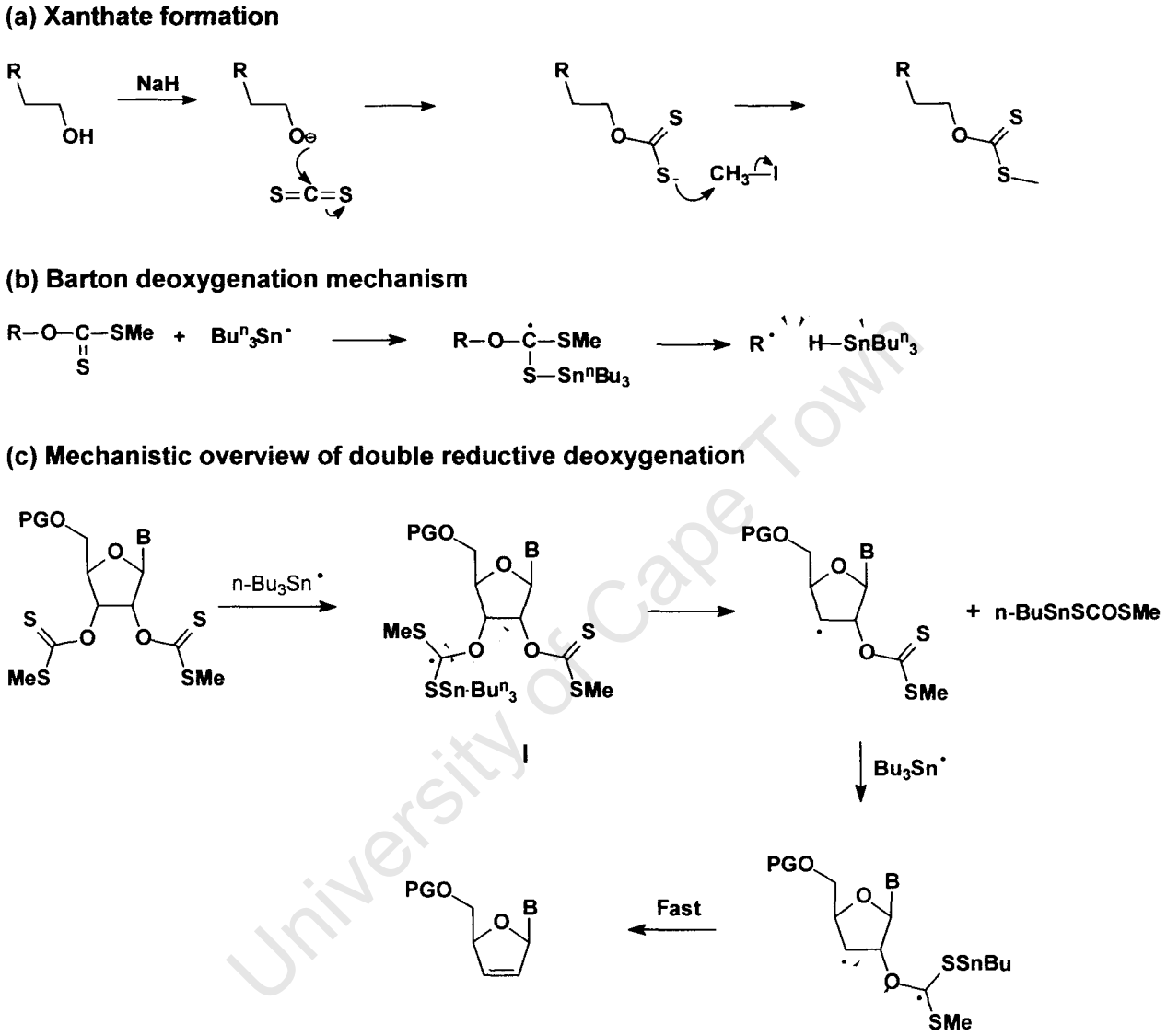
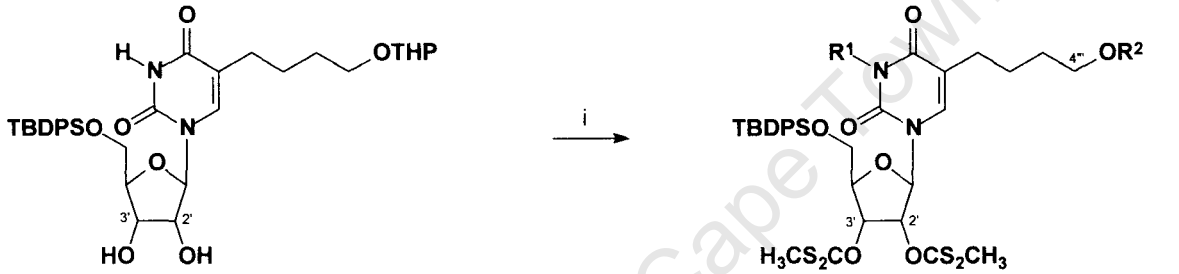


Fig. 4.4: Radical deoxygenation of *bis*-xanthate

With intermediate **160** in hand, the scene was now set for the crucial introduction of the C2'/3' double-bond. Several sets of conditions have been reported in the literature including: (i) the Barton-McCombie deoxygenation[124], (ii) reductive elimination of bromoacetates[91,92] and bromomesylates[93], (iii) the Garreg-Samuelsson I_2/PPh_3/imidazole promoted deoxygenation[88], (iv) olefin metathesis[95] and (v) the Corey-Winter procedure.[90] The Barton procedure involves deoxygenation of a

xanthate ester under tin hydride radical conditions, the various aspects of which are depicted in Fig. 4.4.

In the Barton procedure, a methyl xanthate is prepared by treatment of the relevant alcohol with NaOH or NaH and CS_2 to give a xanthate anion RO-CS-SNa, followed by treatment of this with methyl iodide (Fig. 4.4). In our case, reaction of diol **160** with carbon disulphide and sodium hydride followed by iodomethane gave five products as seen on TLC. Separation of the compounds by column chromatography allowed isolation of *N*-methyl-*tris*-xanthate **161a,** *tris*-xanthate **161b,** *N*-methyl-*bis*-xanthate **161c** and *bis*-xanthate **161d** in 22%, 7%, 29% and 15% yield, respectively (Scheme 4.9).



161a R^1 = Me, R^2 = OCS_2CH_3; 22 %
161b R^1 = H, R^2 = OCS_2CH_3; 7%
161c R^1 = Me R^2 = H; 29%
161d R^1 = H R^2 = H; 15%

Scheme 4.9. *Reagents and Conditions*: (ii) CS_2, NaH, Et_3N, THF, MeI, rt, 24 h.

The occurrence of *N*-methylation in **161a** and **161c**, as well as deprotection of the THP ether and subsequent formation of *O*-xanthate was evident in both the ^1H and ^{13}C NMR spectra of these compounds. The deprotection of the THP group was evident in both the ^1H and ^{13}C NMR spectrum, which revealed the absence of signals for the tetrahydropyran ring. Similarly, the presence of three thiocarbonyl carbons in the ^{13}C NMR spectrum of **161a** and **161b** corroborated that THP deprotection had occurred, which was followed by formation of the *O*-xanthate. The ^1H NMR spectrum of **161a** revealed the presence of three *S*-methyl singlets at δ_H 2.60, 2.58 and 2.54. There was also a significant shift in the H-2', H-3' and H-4''' protons from δ_H 4.31, 4.49 and 3.80 in **160** to δ_H 6.25, 6.47 and 4.43, respectively. Similarly, its ^{13}C NMR spectrum, assigned with the aid of an HSQC and HMBC correlation, exhibited diagnostic signals at δ_C 215.8, 214.7 and 214.6 which were assigned to the thiocarbonyl carbons. C-2', C-3' and C-4''' displayed a downfield shift from δ_C 70.4,

74.2 and 62.2 in **160** to δ_C 78.8, 78.0 and 73.6, respectively. A similar trend was observed in the ^1H and ^{13}C NMR spectra of compounds **161b-d**. Compounds **161a** and **161c** displayed an *N*-methyl singlet in the ^1H NMR at δ_H 3.30 and 3.31, respectively. Similarly, the ^{13}C NMR displayed corresponding signals at δ_C 28.0. The most important signals for the compounds are shown in Table 4.1. The presence of a hydroxyl group in **161c-d** was also confirmed by their IR spectra, which displayed broad stretches between ν 3300-3500 cm^{-1}.

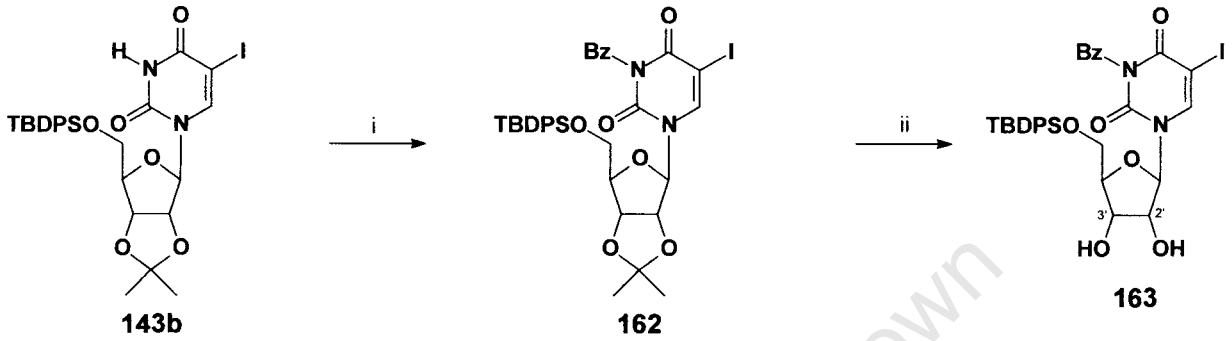
Table 4.1: Some Important ^1H NMR chemical shifts for **160** and **161a-d**

	160	**161a**	**161b**	**161c**	**161d**
H-2'	4.31	6.25	6.26	6.24	6.23
H-3'	4.49	6.47	6.47	6.46	6.46
H-4'''	3.80	4.43	4.44	3.49	3.49
	3.19				
C-2'	70.4	78.8	78.9	79.0	78.8
C-3'	74.2	78.0	77.9	77.9	78.0
C-4'''	62.2	73.6	73.6	62.2	62.2

THP ethers are labile under acidic conditions, but it is unusual to have deprotection of THP taking place under basic conditions. The mechanism for this transformation is unknown.

The formation of various side-products prompted the redesign of the synthesis to include protection of the NH group with a benzoyl group. Thus the synthesis returned to intermediate **143b** and several sets of conditions were investigated for its *N*-benzoylation (Scheme 4.10). Eventually, *N*-benzoyl nucleoside **162** was synthesized in 90% yield by treatment of isopropylidene derivative **143b** with sodium hydride in freshly distilled 1,2-dimethoxyethane (DME) followed by addition of freshly distilled benzoyl chloride. The results from the optimization reaction of *N*-benzoylation of **143b** are shown in Table 3.2. Treatment of **143b** with *n*-butyllithium in THF at -78 °C followed by benzoyl chloride, and then NH_4Cl afforded no detectable amount of product and only resulted in decomposition of the starting material as judged by TLC.

Reaction of **143b** and sodium hydride (NaH) in tetrahydrofuran (THF) followed by addition of BzCl at 0 °C led to a 15% conversion whereas warming the solution in a DMF/THF (1/3) solvent mixture to room temperature led to a 50% conversion. The best method involved stirring **143b** and sodium hydride in DME at room temperature for 30 min to form the anion, cooling the mixture to -78 °C and thereafter adding the benzoyl chloride dropwise.



Scheme 4.10. *Reagents and Conditions*: (i) (a) Base / solvent; (b) BzCl. (ii) HCl/EtOAc, rt, 45 min; 73%.

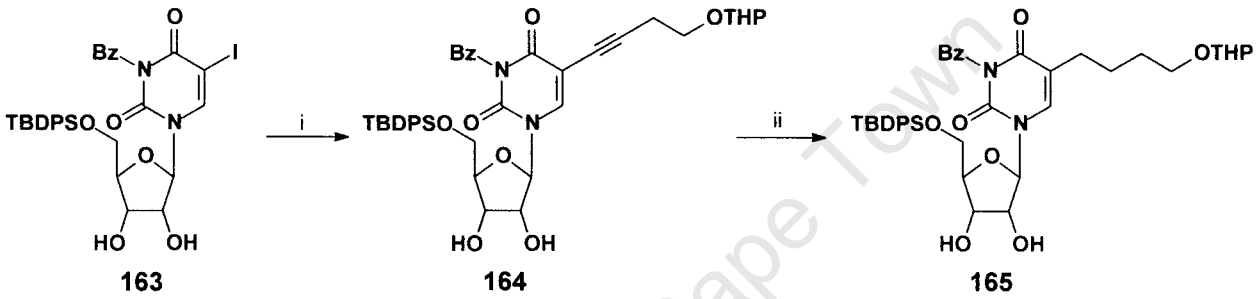
Table 4.2. Optimization of the *N*-benzoylation Reaction

Base	***Solvents***	[a]***T, °C***	***time***	[d]***Yield, %***
n-Butyllithium	THF	-78	1h	decomp
(^{i}Pr)$_2$NEt/ DMAP	CH_2Cl_2	[b]24	1d	40
(^{i}Pr)$_2$NEt/ DMAP	CH_2Cl_2	[b]24	2d	58
NaH	THF	0		15
NaH	DMF/THF	[b]24	12h	50
NaH	DMF/THF	[b]80	12h	60
NaH	DME	[b]24	24h	54
NaH	DME	[c]rt→ -78→rt	2h	90

[a] Refers to reaction temp. [b] BzCl was added dropwise to the anion at 0 °C before warming the reaction mixture to the indicated temperature. [c] BzCl added at -78 °C. [d] Column chromatography yields

The ^{1}H NMR spectrum for the desired benzoate **162** displayed signals at δ_H 7.88, 7.62 and δ_H 7.43 for the new aromatic ring protons. The ^{13}C NMR spectrum displayed an additional diagnostic peak at δ_C 167.4 for the new carbonyl carbon as well as additional signals in the aromatic region.

Acid hydrolysis of the isopropylidene derivative **162** was found to be a difficult task due to the sensitivity of the TBDPS group and several sets of conditions were explored. Initial attempts with 2M HCl in MeOH, 2M HCl in THF and 2M HCl in water all led to the cleavage of the TBDPS group. Varying reaction conditions (reaction time, temperature and concentration) during hydrolysis of the nucleoside with 80% acetic acid in water was also unsuccessful. Ultimately, treatment of the acetonide with saturated HCl acid in ethyl acetate (≈ 7.5 N), a method introduced in 1993 by Gardner *et al*[125], afforded the target diol **163** as a significantly more polar compound in 73% yield after column chromatography. The absence of methyl resonances was noted in both the ^{1}H (δ_H 1.53 and δ_H 1.30) and ^{13}C (δ_C 27.1 and δ_C 25.2) spectra.

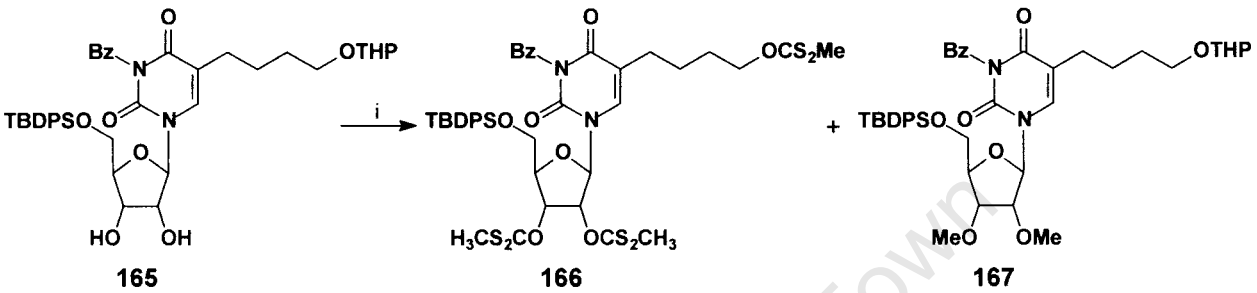


163 **164** **165**

Scheme 4.11. *Reagents and Conditions*: (i) $(PPh_3)_4Pd$ (10 %), CuI (50%), alkyne (2 equiv.), Et_3N (2 equiv.), DMF, THF, 60 °C, 4 h; 93%. (ii) H_2, Pd/C, EtOH, rt, 2 days; 84%.

Sonogashira coupling of nucleoside **163** with 4-(2-tetrahydropyranyloxy)-1-butyne as before gave the coupled product **164** in 93% yield after column chromatography (Scheme 4.11). The ^{13}C NMR spectrum of **164** displayed resonances at δ_C 101.2 and 93.0 for the alkyne carbon. Reduction of the triple bond using 10% Pd/C in a solution of ethanol in THF led to diol **165** (84% yield). Reaction of **165** with carbon disulfide and sodium hydride (4 equiv.) in the presence of a catalytic amount of Et_3N followed by subsequent quenching of the reaction with methyl iodide led to xanthate **166** in low yield as well as a by-product **167** (15%) (Scheme 4.12), which had the C-2', C-3' hydroxyl groups methylated as methyl ethers. This product arose out of incomplete xanthate formation before methylation with methyl iodide. The use of long reaction times and higher temperatures failed to provide an adequate solution to this problem and thus this approach was abandoned. The mechanism for the deprotection of the THP group was again unknown.

The ^{1}H NMR spectrum of the product revealed the presence of two compounds in a ratio of 1:1, which were not possible to separate by column chromatography. However, detailed ^{1}H and ^{13}C NMR spectral analysis revealed the mixture to be a combination of **166** and **167**. The ^{1}H NMR spectrum showed two methoxy singlets at δ_H 3.35 and three *S*-methyl singlets at δ_H 2.59, 2.57 and 2.49, while its ^{13}C NMR spectrum displayed corresponding carbons at δ_C 19.7, 19.5 and 19.5. The presence of a thiocarbonyl was also confirmed by the carbons at δ_C 214.9, 214.8 and 214.6.



165 **166** **167**

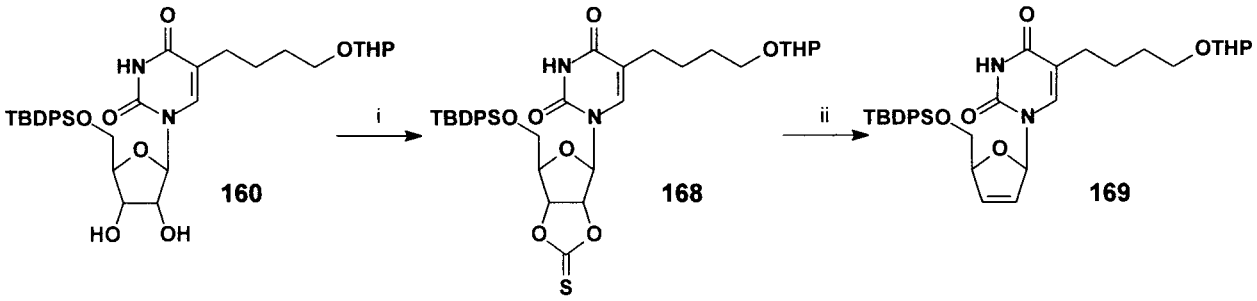
Scheme 4.12. *Reagents and Conditions*: (i) CS_2, NaH, Et_3N, THF, MeI, rt, 22 h; 15%.

Other deoxygenation methodologies were considered and eventually it was decided to explore the Corey-Winter[90] methodology involving fragmentation of a cyclic thionocarbonate by triethyl phosphite to form the olefin. It was anticipated that the conditions of the thionocarbonylation reaction would leave the two protecting groups intact and thus provide the selectivity required to complete the synthesis. The mechanism is outlined in Fig 1.21 (Section 1.12.2, Chapter 1).

To this end, diol **160** was first converted to its 2',3'-thionocarbonate **168** employing 1,1'-thiocarbonyldiimidazole in refluxing 1,2-dichloroethane. Evaporation of the solvent followed by purification of the crude product using column chromatography afforded **168** in 70% yield (Scheme 4.13). It was necessary to closely monitor the progress of this reaction, since prolonged reaction times led to erratic isolated yields, through formation of an un-identified by-product.

Compound **168** revealed a downfield shift in its ^{1}H NMR spectrum for the protons at δ_H 4.31 (H-2') and δ_H 4.49 (H-3') in **160** to δ_H 5.62 and 5.48, respectively, which supported the formation of the thionocarbonate. This was further confirmed by the ^{13}C NMR spectrum which displayed a diagnostic signal at δ_C 189.4 for the thiocarbonyl carbon. The deshielding effect of the thiocarbonyl resulted in a downfield

shift of the C-2' and C-3' carbons to δ_C 88.0 and δ_C 85.0, respectively from δ_C 70.4 and δ_C 74.2 in **160**.



Scheme 4.13. *Reagents and Conditions*: (i) 1,1'-Thiocarbonyldiimidazole, DCE, reflux, 1 h; 70%. (ii) $(EtO)_3P$, 160 °C, 4 h; 40%.

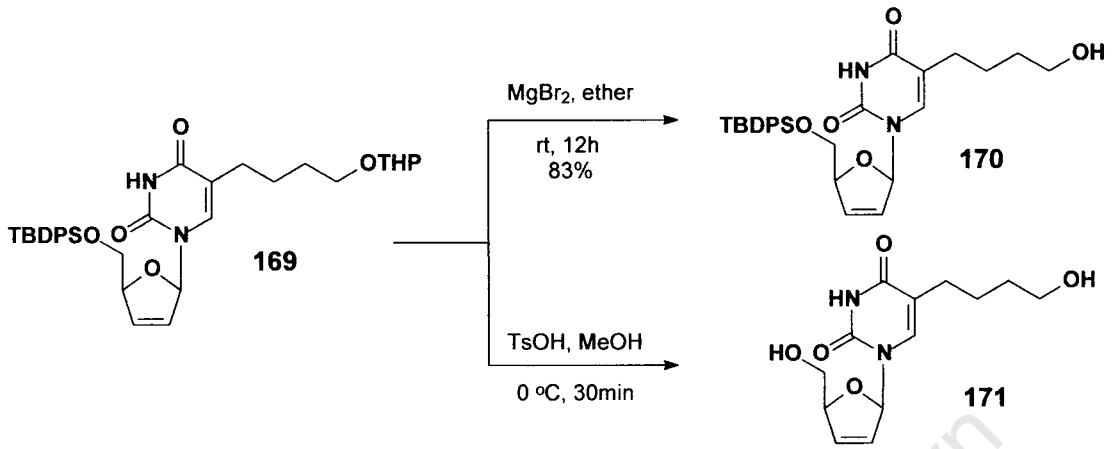
Thermolysis of thionocarbonate **168** employing triethyl phosphite at 150 °C led to formation of **169** in 40% isolated yield. In this case, starting material was recovered and an unidentified by-product was isolated. Subjecting the thionocarbonate to 170 °C for 4 h led to decomposition of the starting material. Several sets of conditions were explored and the best involved heating **168** at 160 °C for 4 h to form **169** in 40% yield as just mentioned.

The ^{1}H NMR spectrum of **169** revealed a shift in H-1', H-2' and H-3' from δ_H 5.56, 5.62 and 5.48 in **168** to δ_H 7.04, 5.85 and δ_H 6.37, respectively, while its ^{13}C NMR spectrum displayed a shift in C-2' and C-3' from δ_C 88.0 and δ_C 85.5 in **168** to δ_C 126.3 and δ_C 134.8, respectively, all of which was consistent with formation of the alkene.

The THP group on **169** was chemoselectively deprotected employing magnesium bromide in ether to furnish **170** in 83% yield (Scheme 4.14) with the mechanism of the reaction shown in Fig. 4.5. The use of *p*-toluenesulfonic acid in methanol resulted in undesired deprotection of both the THP and TBDPS groups to give **171**.

The ^{1}H NMR spectrum for compound **170** revealed the absence of the THP signals in the upfield region of the spectrum. This was further confirmed by its ^{13}C NMR spectrum which displayed the correct number of resonances for the carbons in **170**,

revealing the loss of the THP carbons. With key intermediate **170** in hand, attention focused on the two possible strategies for the end-game.



Scheme 4.14: Selective deprotection of THP in the presence of a silyl ether

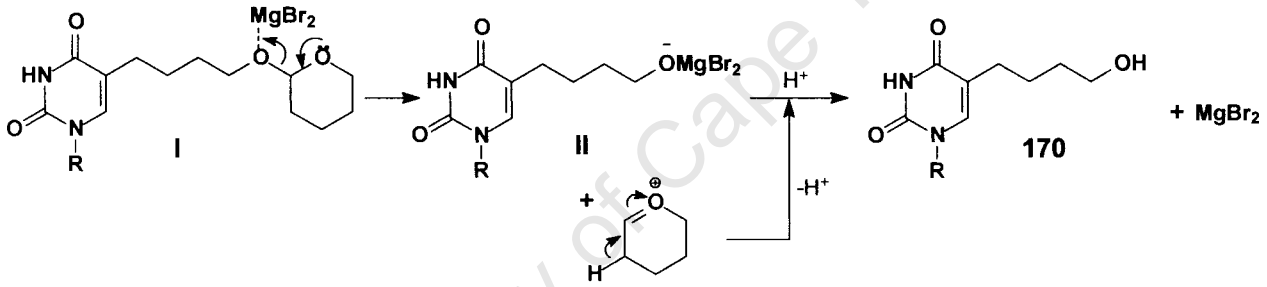
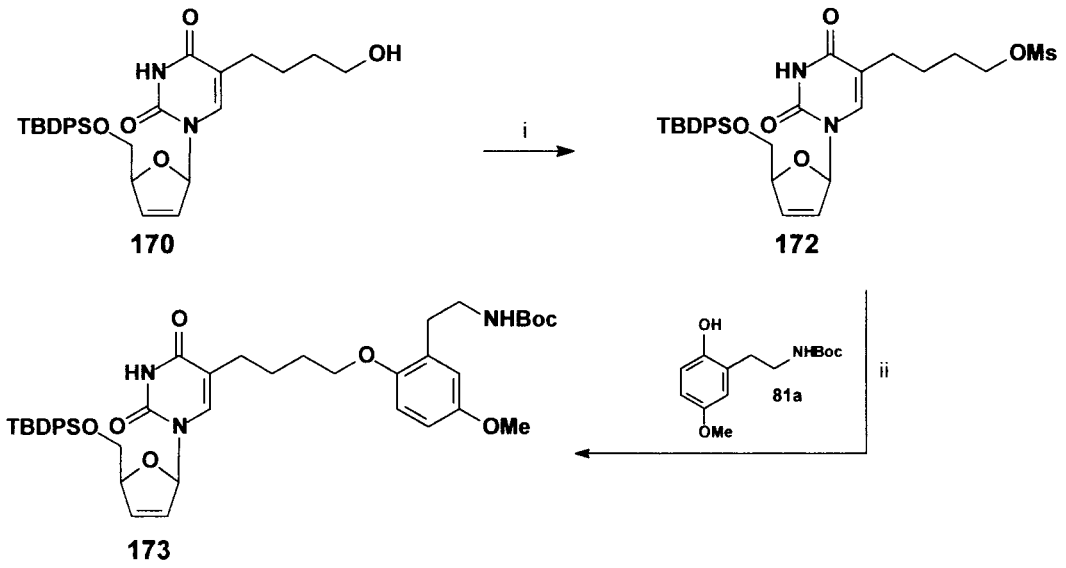


Fig. 4.5: Possible mechanism of THP deprotection

4.3 End-game via Strategy (a)

As previously discussed, strategy (**a**) was chosen for completing the synthesis in view of concern in the convergent strategy around thiocarbonyl alkylation. The major challenge anticipated from previous work was the introduction of the thiourea unit in the final step. Thus, the primary hydroxyl group on **170** was transformed to its mesylate **172** in 47% yield, by reacting it with methanesulfonyl chloride and triethylamine and a catalytic amount of DMAP in methylene chloride at 0 °C to room temperature (Scheme 4.15). Nucleophilic reaction of phenol **81a** with mesylate **172** in THF using NaH as a base resulted in formation of **173** in an unoptimised 38% yield.



Scheme 4.15. *Reagents and Conditions*: (i) MsCl, Et_3N, DMAP, CH_2Cl_2, rt, 1 h; 47%. (ii) **81a**, NaH, THF, rt, 12h; 38%.

Owing to insufficient material obtained for compound **173** (17 mg) coupled with the problems encountered during deprotection of the Boc group followed by condensation of the resultant amine in the synthesis of target compounds **96-99** the material was not committed to the last step. However, this sequence has laid the foundation for a scale-up followed by deprotection and condensation of **174** to the target.

In conclusion, Sonogashira coupling of 5'-O-protected iodouridine was performed in high yields employing the alkyne protected as a THP ether. Dideoxygenation of the 2',3'-diol was accomplished using Corey-Winter methodology. Converting the diol to a bis-xanthate led to deprotection of THP in an unusual mechanism.

The most challenging aspect of the d4U-butane-HI-236 synthesis was the dideoxygenation of the 2',3'-diol to form the alkene. The key to the success of this synthesis lies in the selection of protecting groups that would withstand several key reactions. Protection of the 5'-hydroxyl group with a benzoyl group was not attempted and this could probably have provided the selectivity required.

CHAPTER 5
Conclusion

Three bifunctional compounds with the general formula [d4U]-spacer-[HI-236] were synthesized via a linear strategy and evaluated for their biological activity. The bifunctional compounds were linked at the C-5 position of the nucleoside base via a Sonogashira reaction. Conditions for this reaction were optimized and high yields were obtained for this step. The last key step led to the cleavage of the target molecules and future work is required to optimize conditions for this step without the nucleoside cleavage taking place. A convergent strategy involving Sonogashira coupling of a tethered HI-236 fragment directly with iodo-d4U has afforded promising preliminary results and is worth following up.

Preliminary biological results on compound **96** showed that it displayed anti-HIV activity (EC_{50} = 200 nM). This proves that linking the two compounds by a spacer does enhance the activity. The point of attachment of the NRTI as well as the NNRTI is correct allowing the two compounds to interact with their target site. Future work would involve molecular modeling studies to provide insight into the mode of interaction of these substrates with their respective active sites.

Thiourea derivatives bearing a linker at the 2-position were synthesized with the aim of evaluating their anti-HIV activity. These compounds are currently being tested and will provide an insight into the synergism or additive activity of the bifunctional drugs.

Studies towards the synthesis of a bifunctional target with a flexible linker resulted in the synthesis of the key intermediate **170**. Optimum conditions were obtained for one of the key reactions, the Sonogashira coupling reaction. Future work would involve scaling-up the synthesis of **170** and completing the synthesis.

CHAPTER 6

Experimental Section

General Procedures

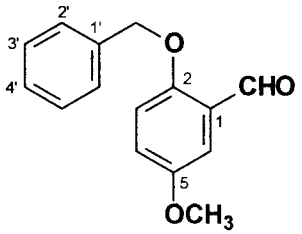
All reactions were performed in oven-dried glassware under an inert atmosphere of dry nitrogen. All starting materials were purchased from Sigma-Aldrich, South Africa except the solvents, acids and common salts. The following reaction solvents were distilled from the indicated drying agents: methylene chloride (P_2O_5), 1,2-dichloroethane (P_2O_5), *N,N*-dimethylaniline (CaH_2), diethyl ether (P_2O_5), tetrahydrofuran (sodium, benzophenone), toluene (sodium), methanol (Mg), acetonitrile (CaH_2), triethylamine (CaH_2), dimethoxyethane (Na, benzophenone).

^{1}H NMR spectra were recorded on a Varian Mercury Spectrometer at 300 MHz or on a Varian Unity Spectrometer at 400 MHz. ^{13}C NMR spectra were recorded at 75 MHz on a Varian Mercury Spectrometer or at 100 MHz on a Varian Unity Spectrometer. The spectra were recorded in $CDCl_3$, Acetone-d_6, MeOD or DMSO-d_6 with tetramethylsilane as the internal standard. Chemical shifts are reported in ppm (δ); multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). Coupling constants, *J*, are reported in Hertz. High resolution mass spectra were recorded on a VG70 SEQ micromass spectrometer, Mass Spectrometry Service, School of Chemistry, University of Witwatersrand. Infrared (IR) absorptions were measured on a Perkin Elmer Spectrum One FT-IR spectrometer. Peaks are reported in cm^{-1} with indicated relative intensities: s (strong); m (medium); w (weak). Melting points were determined using a Reichert-Jung Thermovar hot-stage microscope and are uncorrected. Elemental analysis for C, H and N were carried out using a Fisons EA 110 CHN Elemental Analyser. Optical rotations were obtained using a Perkin Elmer 343 polarimeter at 20 °C. The concentration *c* refers to g/100 mL.

The reaction progress was monitored by TLC using Merck silica-gel 60 F_{254}. Visualisation was accomplished with UV light or spraying with anisaldehyde and/or

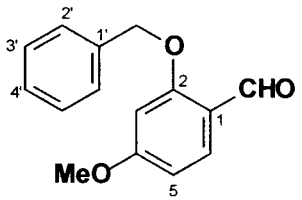
cerium ammonium sulphate spray reagents and then heating at 150 °C. Column chromatography was performed with Merck silica-gel 60 (70-230 mesh).

2-Benzyloxy-5-methoxybenzaldehyde (85a)



A solution of 2-hydroxy-5-methoxybenzaldehyde **84a** (4.35 mL, 34.8 mmol), benzyl bromide (6.22 mL, 52.2 mmol) and anhydrous K_2CO_3 (14.4 g, 104.4 mmol) in EtOH (35 mL) was stirred at reflux for 16 h. The K_2CO_3 was filtered through a pad of Celite, washed with large volumes of EtOAc and the solvent removed under reduced pressure. The residual mass was dissolved in ether (100 mL). The Et_2O solution was washed twice with 50 mL portions of saturated NaCl solution, aqueous sodium hydroxide (5%) and water. The Et_2O solution was dried over $MgSO_4$, evaporated under reduced pressure and the crude product recrystallised from EtOH to give colourless crystals (8.04 g, 95%). mp: 44-45 °C, [lit.[98] 47-48 °C]; IR ($CHCl_3$): ν_{max} 3692 (w), 3017 (m), 2872 (m), 2838 (w), 1683 (s), 1493 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 10.51 (1H, s, CHO), 7.41 (5H, m, ArH), 7.34 (1H, d, J = 3.3 Hz, H-6), 7.10 (1H, dd, J = 3.3, 9.1 Hz, H-4), 7.01 (1H, d, J = 9.1 Hz, H-3), 5.15 (2H, s, CH_2O), 3.80 (3H, s, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 189.4 (CHO), 155.8 (C-2), 153.9 (C-5), 136.3 (C-1'), 128.7 (C-3', C-5'), 128.2 (C-4'), 127.3 (C-2', C-6'), 125.6 (C-1), 123.4 (C-6), 115.2 (C-3), 110.4 (C-4), 71.4 ($ArCH_2O$), 55.8 (OCH_3); EI HRMS: *m/z* found 242.09699 (M^+). $C_{15}H_{14}O_3$ (M^+) requires 242.09429; Found C, 74.35; H, 5.81. $C_{15}H_{14}O_3$ requires C, 74.36; H, 5.82.

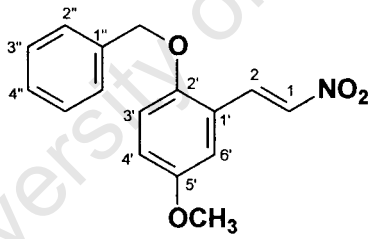
2-Benzyloxy-4-methoxybenzaldehyde (85b)



Benzyl bromide (7.04 mL, 59.2 mmol) was added dropwise to a suspension of 2-hydroxy-4-methoxybenzaldehyde **84b** (6.00 g, 39.4 mmol) and anhydrous K_2CO_3

(16.4 g, 118.3 mmol) in EtOH (35 mL). The mixture was stirred at reflux for 16 h. The K_2CO_3 was removed by filtration and the filtrate concentrated under reduced pressure. The crude product was dissolved in Et_2O (100 mL). The Et_2O solution was washed twice with 50 mL portions of saturated NaCl solution, aqueous sodium hydroxide (5%) and water. The Et_2O solution was dried over $MgSO_4$, evaporated under reduced pressure and the crude product recrystallised from EtOH to give colourless crystals (6.09 g, 64%). mp: 55–56 °C [lit.[98] 65-66 °C]; IR ($CHCl_3$): ν_{max} 3691 (w), 2865 (w), 2843 (w), 2772 (w), 1676 (s), 1603 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 10.39 (1H, d, J = 0.8 Hz, CHO), 7.84 (1H, d, J = 9.0 Hz, H-6), 7.37 (5H, m, ArH), 6.56 (1H, ddd, J = 0.8, 2.8, 9.0 Hz, H-5), 6.52 (1H, d, J = 2.8 Hz, H-3), 5.16 (2H, s, ArCH_2O), 3.84 (3H, s, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.2 (CHO), 166.1 (C-2), 162.8 (C-4), 136.0 (C-1'), 130.5 (C-6), 128.7 (C-2', C-6'), 128.3 (C-4'), 127.3 (C-3', C-5'), 119.4 (C-1), 106.2 (C-5), 99.3 (C-3), 70.5 (ArCH_2O), 55.6 (OCH_3); EI HRMS found *m/z* 242.09578 (M^+). $C_{15}H_{14}O_3$ requires (M^+) 242.09429; Found C, 74.23; H, 5.81. $C_{15}H_{14}O_3$ requires C, 74.36; H, 5.82.

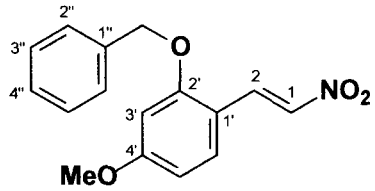
2-(2-Benzyloxy-5-methoxyphenyl)-1-nitroethene (83a)



A solution of 2-benzyloxy-5-methoxybenzaldehyde **85a** (5.00 g, 20.6 mmol), nitromethane (130 mL) and ammonium acetate (1.59 g, 20.6 mmol) were heated at 70 °C for 14 h. The cooled solution was diluted with CH_2Cl_2 (70 mL) and washed with saturated NaCl solution (2 × 250 mL) and water (250 mL), dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The crude product was recrystallised from absolute EtOH to afford yellow needles (4.38 g, 75%). mp: 112-114 °C [lit.[99] 114-116 °C]; IR ($CHCl_3$): ν_{max} 3692 (w), 3607 (w), 1632 (m) (C=C), 1515 (m), 1498 (s), 1343 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.16 (1H, d, J = 14.0 Hz, H-2) 7.81 (1H, d, J = 14.0 Hz, H-1), 7.41 (5H, m, ArH), 6.97 (3H, m, H-3', H-4', H-6'), 5.16 (2H, s, ArCH_2O), 3.79 (3H, s, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.8 (C-2'), 152.9 (C-5'), 138.6 (C-1), 136.1 (C-1''), 135.1 (C-2), 128.8 (C-2'', C-6''), 128.4 (C-4''), 127.4 (C-3'', C-5''), 120.1 (C-1'), 119.1 (C-6'), 116.0 (C-4'), 114.3 (C-3'), 71.3 (ArCH_2O), 55.8

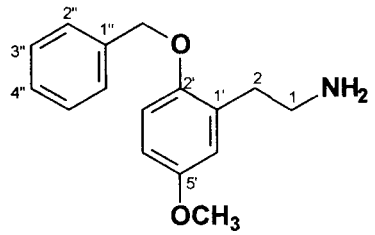
(OCH_3); EI HRMS: *m/z* found 285.09932. $C_{16}H_{15}NO_4$ requires (M^+) 285.10011; Found C, 67.37; H, 5.27; N, 5.00. $C_{16}H_{15}NO_4$ requires C, 67.36; H, 5.30; N 4.91.

2-(2-Benzyloxy-4-methoxyphenyl)-1-nitroethene (83b)



2-Benzyloxy-4-methoxybenzaldehyde **85b** (4.28 g, 17.7 mmol) was treated with nitromethane (120 mL) and ammonium acetate (1.36 g, 17.7 mmol). The mixture was heated at 70 °C for 14 h. The solution was diluted with CH_2Cl_2 (80 mL) and washed with saturated NaCl solution (2 × 100 mL) and water (100 mL). The organic layer was dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The crude product was recrystallised from EtOH to afford yellow needles (4.19 g, 83%). mp: 109-112 °C [lit.[126] 115-116 °C]; IR ($CHCl_3$): ν_{max} 3691 (w), 3607 (w), 1606 (s) (C=C), 1510 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.12 (1H, d, *J* = 13.4 Hz, H-2), 7.79 (1H, d, *J* = 13.4 Hz, H-1), 7.41 (6H, m, ArH, H-6'), 6.56 (2H, m, H-3', H-5'), 5.18 (2H, s, CH_2O), 3.83 (3H, s, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.2 (C-2'), 160.1 (C-4'), 136.1 (C-1), 135.5 (C-1''), 135.4 (C-2), 134.1 (C-6'), 128.8 (C-2'', C-6''), 128.5 (C-4''), 127.4 (C-3'', C-5''), 112.6 (C-1'), 106.3 (C-5'), 100.0 (C-3'), 70.8 (OCH_2), 55.5 (OCH_3); EI HRMS: *m/z* found 285.09965. $C_{16}H_{15}NO_4$ (M^+) requires 285.10011; Found C , H, 5.25; N, 4.95. $C_{16}H_{15}NO_4$ requires C, 67.36; H, 5.30; N, 4.91.

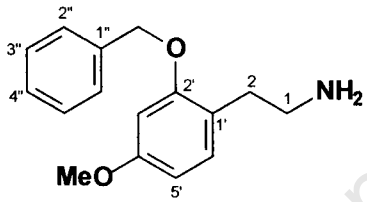
2-(2-Benzyloxy-5-methoxyphenyl)-ethylamine (80a)



A solution of nitroethene **83a** (1.35 g, 4.7 mmol) in THF (20 mL) was added dropwise over a period of 1 h to a stirred and refluxing suspension of lithium aluminium hydride (0.72 g, 18.9 mmol) in THF (10 mL). The mixture was refluxed for 4 h, cooled to 0°C and saturated aq. Na_2SO_4 added cautiously. Stirring was continued for an additional 30 min, the precipitate formed was filtered through a pad of Celite® and washed with

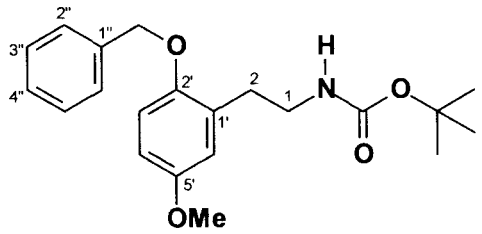
large volumes of MeOH. The filtrate was evaporated under reduced pressure and the crude product (1.04 g, 85%) used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.42 (2H, dd, *J* = 1.6, 6.9 Hz, H-2", H-6"), 7.37 (2H, dd, H-3", H-5"), 7.30 (1H, tt, *J* = 1.6, 6.9 Hz, H-4"), 6.91 (1H, d, *J* = 8.8, H-3'), 6.72 (1H, d, *J* = 3.0 Hz, H-6'), 6.69 (1H, dd, *J* = 3.0, 8.8 Hz, H-4'), 3.66 (3H, s, OCH_3), 3.25 (br s, NH_2), 2.73 (2H, t, *J* = 7.1 Hz, H-2), 2.63 (2H, t, *J* = 7.1 Hz, H-1); ^{13}C NMR (75 MHz, DMSO-d_6): δ 153.1 (C-5'), 150.2 (C-2'), 137.6 (C-1"), 129.9 (C-1'), 128.3 (C-3", C-5"), 127.5 (C-4"), 127.2 (C-2", C-6"), 116.3 (C-6'), 113.2 (C-3'), 111.1 (C-4'), 69.8 (ArCH_2O), 55.2 (OCH_3), 42.1 (C-1), 34.6 (C-2).

2-(2-Benzyloxy-4-methoxyphenyl)-ethylamine (80b)



A solution of nitroethene **83b** (1.55 g, 5.5 mmol) in THF (25 mL) was added dropwise to a stirred and refluxing suspension of LAH (0.83 g, 21.8 mmol) in THF (20 mL). The mixture was refluxed for 4 h, cooled to 0 °C, saturated aq. Na_2SO_4 added cautiously, filtered and the filtrate evaporated. The crude product (1.12 g, 80%) was used in the next step without further purification. ^{1}H NMR (300 MHz, CD_3OD): δ 7.44 (2H, m, H-2", H-6"), 7.37 (3H, m, H-3", H-4", H-5"), 7.05 (1H, d, *J* = 8.2 Hz, H-6'), 6.59 (1H, d, *J* = 2.2 Hz, H-3'), 6.46 (1H, dd, *J* = 2.2, 8.2 Hz, H-5'), 5.07 (2H, s, ArCH_2O), 4.80 (3H, s, OCH_3), 2.85 (2H, t, *J* = 7.3 Hz, H-1), 2.77 (2H, t, *J* = 7.3 Hz, H-2); ^{13}C NMR (75 MHz, CD_3OD): δ 161.0 (C-2'), 158.9 (C-4'), 138.8 (C-1"), 131.9 (C-6'), 129.6 (C-3", C-5"), 128.9 (C-4"), 128.5 (C-2", C-6"), 121.6 (C-1'), 105.9 (C-5'), 100.9 (C-3'), 71.1 (ArCH_2O), 55.8 (OCH_3), 42.9 (C-1), 34.5 (C-2).

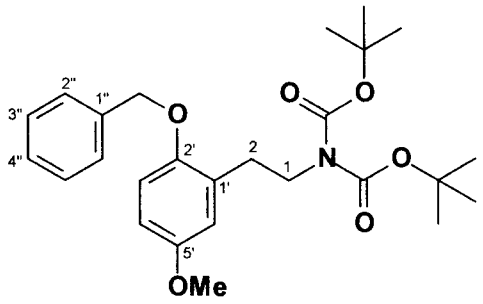
N-[2-(2-Benzyloxy-5-methoxyphenyl)ethyl]-*tert*-butylcarbamate (82a)



Amine **80a** (1.20 g, 4.7 mmol) in acetonitrile (10 mL), triethylamine (1.32 mL, 9.5 mmol) and DMAP (0.02 g) were treated with di-*tert*-butyldicarbonate (3.11 g, 14.2 mmol) in acetonitrile (5 mL) at room temperature for 20 h. The solvent was evaporated *in vacuo* and the residual mass subjected to column chromatography employing EtOAc / petroleum ether (15 / 85) to give carbamate **82a** as colourless crystals (0.73 g, 58%), along with dicarbamate by-product **82c** as a colourless oil (0.62 g, 28%).

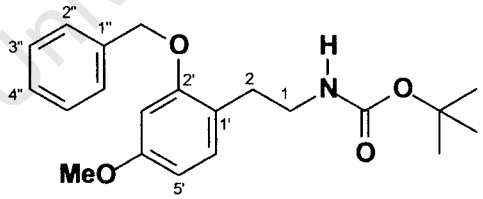
82a mp: 103-104 °C; IR ($CHCl_3$): ν_{max} 3691 (w), 3453 (w) (NH), 1707 (s) (CO), 1503 (s) (C=C) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.41 (5H, m, ArH), 6.84 (1H, d, J = 8.1 Hz, H-3'), 6.73 (1H, dd, J = 3.2, 8.1 Hz, H-4'), 6.69 (1H, d, J = 3.2 Hz, H-6'), 5.03 (2H, s, ArCH_2O), 4.70 (1H, brs, NH), 3.76 (3H, s, OCH_3), 3.37 (2H, q, J = 6.4 Hz, H-1), 2.83 (2H, t, J = 6.4 Hz, H-2), 1.42 (9H, s, OC(CH_3)$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.9 (C=O), 153.9 (C-5'), 151.0 (C-2'), 137.4 (C-1''), 128.6 (C-3'', C-5''), 128.0 (C-1'), 127.9 (C-4''), 127.3 (C-2'', C-6''), 116.8 (C-6'), 113.0 (C-4'), 112.0 (C-3'), 79.0 (OC(CH_3)$_3$), 70.9 (ArCH_2O), 55.7 (OCH_3), 40.8 (C-1), 30.9 (C-2), 27.8 (OC(CH_3)$_3$); EI HRMS: *m/z* found 301.13383 [(M^+ - *t*-butyl) + H]. $C_{21}H_{27}NO_4$ requires 301.13409 [(M^+ - *t*-butyl) + H]; Found C, 70.50; H, 7.62; N, 3.86. $C_{21}H_{27}NO_4$ requires C, 70.56; H, 7.61; N 3.92.

N-[2-(2-Benzyloxy-5-methoxyphenyl)ethyl]-di-(*tert*-butylcarbamate) (82c)



82c: IR ($CHCl_3$): ν_{max} 3692 (w), 3610 (w), 2973 (m), 2936 (m), 1776 (s) (CO), 1736 (s) (CO), 1691 (s) (C=C), 1594 (m), 1501 (s), 1455 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.45 (2H, d, *J* = 7.2 Hz, H-2", H-6"), 7.36 (2H, t, *J* = 7.2 Hz, H-3", H-5"), 7.29 (1H, t, *J* = 7.2 Hz, H-4"), 6.80 (1H, d, *J* = 8.8 Hz, H-3'), 6.73 (1H, d, *J* = 3.2 Hz, H-6'), 6.68 (1H, dd, *J* = 3.2, 8.8 Hz, H-4'), 5.04 (2H, s, ArCH_2O), 3.86 (2H, t, *J* = 7.4 Hz, H-1), 3.74 (3H, s, OCH_3), 2.95 (2H, t, *J* = 7.4 Hz, H-2), 1.44 (18H, s, 2 × OC(CH_3)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.7 (C=O), 152.4 (C-5'), 151.1 (C-2'), 137.7 (C-1"), 129.0 (C-1'), 128.4 (C-3", C-5"), 127.6 (C-4"), 127.2 (C-2"), 116.9 (C-6'), 113.0 (C-3'), 112.0 (C-4'), 81.8 (2 × O*C*(CH_3)$_3$), 70.8 (ArCH_2O), 55.6 (OCH_3), 46.4 (C-1), 30.1 (C-2), 28.0 (2 × OC(CH_3)$_3$); EI HRMS: *m/z* found 457.24465 (M^+). $C_{26}H_{35}NO_6$ requires (M^+) 457.24644.

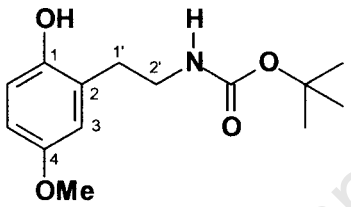
N-[2-(2-Benzyloxy-4-methoxyphenyl)ethyl]-*tert*-butylcarbamate (82b)



Amine **80b** (1.12 g, 4.3 mmol) and triethylamine (1.21 mL, 8.7 mmol) in CH_3CN (10 mL) were treated with di-*tert*-butyldicarbonate (1.2 mL, 5.2 mmol) in CH_3CN (5 mL) at rt for 20 h. After the reaction was complete, the solvent was evaporated *in vacuo* and the residual mass subjected to column chromatography employing EtOAc / petroleum ether (15 / 85) to give colourless crystals (1.13 g, 73%). mp: 84-85 °C (from hexane/EtOAc); IR ($CHCl_3$): ν_{max} 3692 (w), 3452 (m) (NH), 2981 (m), 2936 (m), 1707 (s) (CO), 1614 (s) (C=C), 1589 (m), 1507 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.44 (2H, m, H-2", H-6"), 7.39 (2H, t, *J* = 7.2 Hz, H-3", H-5"), 7.34 (1H, m, H-4"),

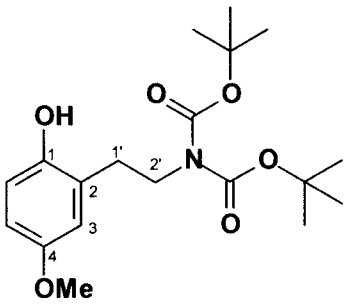
7.06 (1H, d, *J* = 8.4 Hz, H-6'), 6.53 (1H, d, *J* = 2.0 Hz, H-3'), 6.46 (1H, dd, *J* = 2.0, 8.4 Hz, H-5'), 5.05 (2H, s, ArCH_2O), 4.76 (1H, brs, NH), 3.77 (3H, s, OCH_3), 3.36 (2H, d, *J* = 6.0 Hz, H-1), 2.80 (2H, t, *J* = 6.0 Hz, H-2), 1.44 (9H, s, OC(CH_3)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.4 (C-4'), 157.4 (C-2'), 155.8 (C=O), 136.8 (C-1''), 130.8 (C-6'), 128.4 (C-3'', C-5''), 127.8 (C-4''), 127.1 (C-2'', 6''), 120.0 (C-1'), 104.4 (C-5'), 99.7 (C-3'), 78.6 (O*C*(CH$_3$)$_3$), 69.9 (Ar*C*H$_2$O), 55.1 (OCH_3), 40.8 (C-1), 29.9 (C-2), 28.3 (OC(*C*H$_3$)$_3$); EI HRMS: *m/z* found 357.19290 (M^+). $C_{21}H_{27}NO_4$ (M^+) requires 357.19401; Found C, 70.57; H, 7.62; N, 3.95. $C_{21}H_{27}NO_4$ requires C, 70.56; H, 7.61; N, 3.92.

N-2-(2-*t*-Butyloxycarbonylaminoethyl)-4-methoxyphenol (81a)



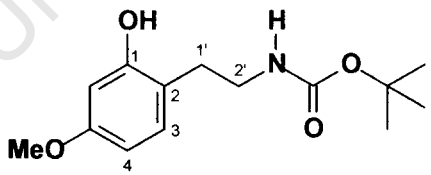
Hydrogen gas was introduced at atmospheric pressure into a flask containing a suspension of the carbamate **82a** (1.09 g, 3.1 mmol), 10% palladium-on-carbon (10 mol %) and ethanol (20 mL). The mixture was stirred at room temperature for 5 h. The catalyst was filtered through a pad of Celite®, washed with EtOAc (3 × 10 mL) and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography using EtOAc / petroleum ether (3 / 7) to give colourless crystals (0.57 g, 69%). mp: 116-117 °C; IR ($CHCl_3$): ν_{max} 3602 (w), 3463 (m) (NH), 3330 (br) (OH), 3016 (m), 2937 (w), 1690 (s) (CO), 1505 (s) (C=C) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.77 (1H, d, *J* = 8.8 Hz, H-6), 6.69 (1H, brs, OH), 6.67 (1H, dd, *J* = 3.0, 8.8 Hz, H-5), 6.63 (1H, d, *J* = 3.0 Hz, H-3), 4.92 (1H, brs, NH), 3.74 (3H, s, OCH_3), 3.30 (2H, q, *J* = 7.2 Hz, H-2'), 2.80 (2H, t, *J* = 7.2 Hz, H-1'), 1.45 (9H, s, OC(CH_3)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.9 (C=O), 153.2 (C-4), 148.7 (C-1), 117.6 (C-2), 116.6 (C-6), 116.1 (C-3), 112.8 (C-5), 80.0 (O*C*(CH$_3$)$_3$), 55.7 (OCH_3), 41.0 (C-2'), 31.4 (C-1'), 28.4 (OC(*C*H$_3$)$_3$); EI HRMS: *m/z* found 267.14329 (M^+). $C_{14}H_{21}NO_4$ (M^+) requires 267.14706; Found C, 62.82; H, 7.92; N, 5.16. $C_{14}H_{21}NO_4$ requires C, 62.94; H, 7.92; N, 5.24.

N-2-(2-*bis*{*t*-Butyloxycarbonyl}aminoethyl)-4-methoxyphenol (81c)



The carbamate **82c** (0.62 g, 1.4 mmol) was subjected to hydrogenolysis over a palladium-on-carbon catalyst (20 mol %) for 22 h. The catalyst was removed by filtration through a pad of Celite and washed with large volumes of EtOAc. The crude product was purified by column chromatography employing ethyl acetate / pet ether (3 / 7) to give the title compound as a colourless oil (0.39 g, 79%). IR ($CHCl_3$): ν_{max} 3691 (w), 3607 (w), 3393 (br) (OH), 1776 (s) (CO), 1721 (s) (CO), 1677 (s) (C=C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.78 (1H, d, J = 8.6 Hz, H-6), 6.67 (1H, dd, J = 3.1, 8.6 Hz, H-5), 6.63 (1H, d, J = 3.1 Hz, H-3), 6.56 (1H, brs, OH), 3.72 (3H, s, OCH_3), 3.72 (2H, t, J = 7.8 Hz, H-2'), 2.85 (3H, t, J = 7.8 Hz, H-1'), 1.50 (18H, s, 2 × $OC(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.2 (2 × C=O), 153.0 (C-4), 150.0 (C-1), 124.9 (C-2), 116.7 (C-6), 116.0 (C-3), 113.3 (C-5), 83.0 ($OC(CH_3)_3$), 55.7 (OCH_3), 46.2 (C-2'), 30.7 (C-1'), 28.0 (2 × $OC(CH_3)_3$); EI HRMS: *m/z* found 367.19968 (M^+). $C_{19}H_{29}NO_6$ (M^+) requires 367.19949.

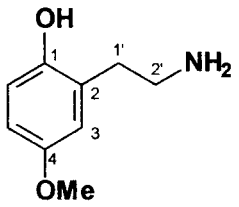
N-2-(2-*t*-Butyloxycarbonylaminoethyl)-5-methoxyphenol (81b)



Hydrogen gas was introduced at atmospheric pressure into a flask containing a suspension of the carbamate **82b** (1.11 g, 3.1 mmol), 10% palladium-on-carbon (20 mol %) and ethanol (20 mL). The mixture was stirred at room temperature for 20 h. The catalyst was filtered through a pad of Celite and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography using EtOAc / petroleum ether (3 / 7) to give colourless crystals (0.54 g, 65%). mp 100-101 °C (from EtOAc); IR ($CHCl_3$): ν_{max} 3691 (w), 3600 (w), 3463 (m) (NH), 3295 (br) (OH), 1686 (s) (CO), 1622 (s) (C=C), 1505 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.68 (1H,

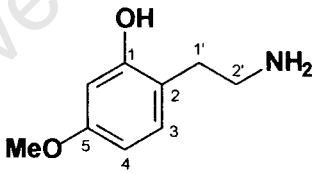
brs, OH), 6.93 (1H, d, J = 8.1 Hz, H-3), 6.46 (1H, d, J = 2.7 Hz, H-6), 6.38 (1H, dd, J = 2.7, 8.1 Hz, H-4), 4.98 (1H, brs, NH), 3.74 (3H, s, OCH_3), 3.27 (2H, q, J = 6.6 Hz, H-2'), 2.76 (2H, t, J = 6.6 Hz, H-1'), 1.45 (9H, s, $OC(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.7 (C=O), 157.1 (C-1), 155.9 (C-5), 130.8 (C-3), 117.3 (C-2), 105.6 (C-4), 102.0 (C-6), 80.1 ($OC(CH_3)_3$), 55.2 (OCH_3), 40.9 (C-2'), 30.5 (C-1') 28.4 ($OC(CH_3)_3$); EI HRMS: *m/z* found 267.14729 (M^+). $C_{14}H_{21}NO_4$ (M^+) requires 267.14706; Found C, 63.03; H, 7.97; N, 5.25. $C_{14}H_{21}NO_4$ requires C, 62.94; H, 7.92; N, 5.24.

2-(2-Aminoethyl)-4-methoxyphenol (91a)



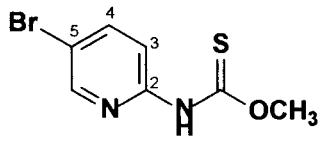
To a solution of the carbamate **81a** (0.20 g, 0.8 mmol) in CH_2Cl_2 (1.0 mL) was added trifluoroacetic acid (1.0 mL) in CH_2Cl_2 (1.0 mL) at 0°C. The mixture was stirred at 0°C for 30 min. Diisopropylethylamine (2.0 mL) was added and the solvent evaporated. The resulting amine was used in the next step without further purification.

2-(2-Aminoethyl)-5-methoxyphenol (91b)



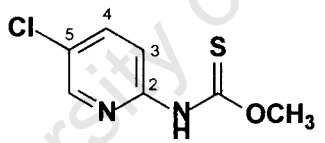
Trifluoroacetic acid (1.0 mL) in CH_2Cl_2 (1.0 mL) was added dropwise to a solution of the carbamate **81b** (0.20 g, 0.8 mmol) in CH_2Cl_2 (1.0 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min. Diisopropylethylamine (2.0 mL) was added and the solvent evaporated. The resultant amine was used in the next step without further purification. ^1H NMR (300 MHz, CD_3OD): δ 6.93 (1H, d, J = 8.3 Hz, H-3), 6.37 (1H, d, J = 2.7 Hz, H-6), 6.32 (1H, dd, J = 2.7, 8.3 Hz, H-4), 3.71 (3H, s, OCH_3), 2.93 (2H, m, H-2'), 2.74 (2H, t, J = 6.9 Hz, H-1').

N-(5-Bromo-2-pyridyl)]-methoxy-thiocarbamate (90a)



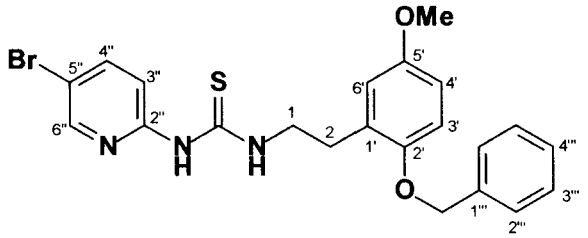
1,1'-Thiocarbonyldiimidazole (1.84 g, 10.3 mmol) and 2-amino-5-bromopyridine (1.78 g, 10.3 mmol) were added to dry acetonitrile (10 mL) at room temperature. The reaction mixture was stirred for 12 h and the precipitate filtered, washed with cold acetonitrile and recrystallized from acetonitrile/methanol to afford **90a** as colourless needles (2.91 g, 87%). mp: 150-151 °C; IR (KBr): ν_{max} 3468 (w) (NH), 1691 (m) C=C), 1134 (w) (C=S) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 11.56 (1H, brs, NH), 8.48 (1H, d, *J* = 2.4 Hz, H-6), 8.03 (2H, m, H-3, H-4), 3.99 (OCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 188.7 (C=S), 150.1 (C-2), 148.6 (C-6), 140.2 (C-4), 117.9 (C-3), 114.9 (C-5), 57.1 (OCH_3); Found C, 34.18; H, 2.82; N, 11.25; S, 12.86. $C_7H_7BrN_2OS$ requires C, 34.02; H, 2.86; N, 11.34; S, 12.97.

N-(5-chloro-2-pyridyl)-methoxy-thiocarbamate (90b)



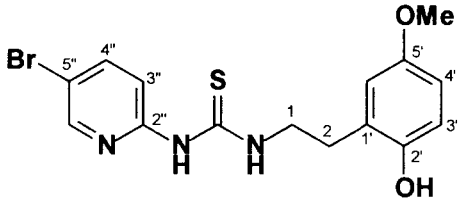
1,1'-Thiocarbonyldiimidazole (3.68 g, 20.6 mmol) was added to a solution of 2-amino-5-chloropyridine (2.65 g, 20.6 mmol) in acetonitrile at room temperature. The mixture was stirred for 12 h and the precipitate filtered, washed with cold acetonitrile. The crude product was recrystallised from acetonitrile/methanol to give **90b** as colourless needles (2.85 g, 58%). mp: 154-156°C; IR (KBr): ν_{max} 3468 (w) (NH), 1691 (m) C=C), 1134 (w) (C=S) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 11.53 (1H, br s, NH), 8.36 (1H, d, *J* = 2.8 Hz, H-6), 7.88 (2H, dd, *J* = 2.8, 8.8 Hz, H-3, H-4), 3.94 (3H, s, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 188.8 (C=S), 149.9 (C-2), 146.4 (C-6), 137.5 (C-4), 126.5 (C-5), 117.6 (C-3), 57.2 (OCH_3); Found C, 41.69; H, 3.43; N, 12.96; S, 15.14. $C_7H_7ClN_2OS$ requires C, 41.49; H, 3.48; N, 13.82; S, 15.82.

N-[2-(2-Benzyloxy-5-methoxyphenyl)ethyl]-*N'*-[2-(5-bromopyridyl)]-thiourea (79)



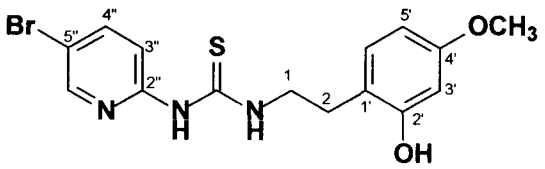
Amine **80a** (0.18 g, 0.7 mmol) was added to a suspension of thiocarbonyl derivative **89** (0.22 g, 0.8 mmol) in DMF (5 mL). The reaction mixture was heated to 100 °C for 16 h. The mixture was poured into ice-cold water (5 mL) and stirred for 30 min. The precipitate formed was filtered and washed with cold water (2 × 5 mL). The crude product was purified by column chromatography (30% EtOAc in pet ether) to give a yellow solid (0.12 g, 37%). mp: 141-142 °C ($CHCl_3$/MeOH); IR ($CHCl_3$): ν_{max} 3691 (w), 3607 (w), 3416 (w) (NH), 1602 (w), 1505 (w), 1138 (w) (C=S) cm^{-1}; ^1H NMR (300 MHz, CD_3Cl): δ 11.18 (1H, brs, NH), 8.80 (1H, brs, NH), 8.03 (1H, d, *J* = 2.6, H-6''), 7.65 (1H, dd, *J* = 2.6, 8.7 Hz, H-4''), 7.37 (5H, m, ArH), 6.86 (1H, d, *J* = 9.0 Hz, H-3'), 6.84 (1H, d, *J* = 3.2 Hz, H-6'), 6.74 (1H, dd, *J* = 3.2, 9.0 Hz, H-4'), 6.67 (1H, d, *J* = 8.7 Hz, H-3''), 5.02 (2H, s, ArCH_2O), 4.03 (2H, dd, *J* = 6.6 Hz, H-1), 3.75 (3H, s, OCH_3), 3.03 (2H, t, *J* = 6.6 Hz, H-2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 179.0 (C=S), 153.6 (C-5'), 151.6 (C-2''), 151.1 (C-2'), 146.7 (C-6''), 141.1 (C-4''), 137.3 (C-1'''), 128.8 (C-1'), 128.5 (C-3''', C-5'''), 127.8 (C-4'''), 127.2 (C-2''', C-6'''), 117.7 (C-6'), 113.2 (C-3''), 112.9 (C-3'), 112.6 (C-5''), 111.5 (C-4'), 70.7 (ArCH_2O), 55.6 (OCH_3), 45.7 (C-1), 30.0 (C-2); EI HRMS: *m/z* found 471.05937 (M^+). $C_{22}H_{22}N_3O_2BrS$ (M^+) requires 471.06161; Found C, 55.48; H, 4.68; N, 8.59; S, 6.17. $C_{22}H_{22}N_3O_2BrS$ requires C, 55.94; H, 4.69; N, 8.90; S, 6.79.

N-[2-(2-Hydroxy-5-methoxyphenyl)ethyl]-*N'*-[2-(5-bromopyridyl)]-thiourea (73)



To a solution of amine **91a** (0.18 g, 0.7 mmol) in DMF (20 mL), was added thiocarbonyl derivative **89** (0.22 g, 0.8 mmol). The reaction mixture was heated to 100 °C and stirred for 15 h. The reaction mixture was poured into ice-cold water (2 mL) and the suspension was stirred for 30 min. The product was filtered, washed with water (5 mL), dried, and further purified by column chromatography using EtOAc / petroleum ether (2 / 8) to furnish the titled compound as a pale yellow solid (0.14 g, 44%). mp: 201-203 °C (from EtOAc); IR (KBr): ν_{max} 3468 (w) (NH), 3300-3200 (br) (OH), 2959 (w), 1594 (s) (C=C), 1179 (m), 1115 (w) (C=S) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 11.12 (1H, brs, NH), 10.59 (1H, brs, NH), 8.90 (1H, brs, OH), 8.15 (1H, d, J = 2.4 Hz, H-6"), 7.92 (1H, dd, J = 2.4, 9.0 Hz, H-4"), 7.09 (1H, d, J = 9.0 Hz, H-3"), 6.73 (1H, d, J = 2.9 Hz, H-6'), 6.72 (1H, d, J = 8.7 Hz, H-3'), 6.63 (1H, dd, J = 2.9, 8.7 Hz, H-6'), 3.78 (2H, q, J = 6.8 Hz, H-1), 3.62 (3H, s, OCH_3), 2.84 (2H, t, J = 6.8 Hz, H-2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 179.0 (C=S), 152.2 (C-5'), 151.9 (C-2"), 149.1 (C-2'), 145.7 (C-6"), 141.1 (C-4"), 125.8 (C-1'), 116.4 (C-6'), 115.3 (C-3'), 114.3 (C-3"), 112.1 (C-4'), 111.6 (C-5"), 55.2 (OCH_3), 44.9 (C-1), 28.8 (C-2); EI HRMS: *m/z* found 381.01454 (M^+). $C_{15}H_{16}N_3O_2BrS$ (M^+) requires 381.01466; Found C, 47.09; H, 4.38; N, 10.17. $C_{15}H_{16}N_3O_2BrS$ requires C, 47.13; H, 4.22; N, 10.99.

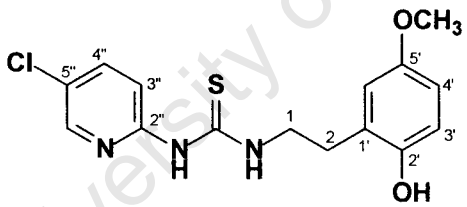
N-[2-(2-Hydroxy-4-methoxyphenyl)ethyl]-*N'*-[2-(5-bromopyridyl)]-thiourea (74)



Thiocarbonyl derivative **89** (0.25 g, 0.9 mmol) was reacted with amine **91b** (0.13 g, 0.8 mmol) in DMF (10 mL) at 100 °C for 16 h. The reaction mixture was poured into ice-cold water (5 mL) and the suspension was stirred for 30 min. The product was

filtered, washed with water (5 mL), dried, and further purified by column chromatography using ethyl acetate / petroleum ether (3 / 7) to furnish the title compound as a pale yellow crystals (0.12 g, 41%). mp: 192-194 °C (ethyl acetate); IR (KBr): ν_{max} 3468 (w) (NH), 3300-3200 (br) (OH), 2959 (w), 1594 (s) (C=C), 1179 (m), 1115 (w) (C=S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.11 (1H, brs, NH), 10.59 (1H, brs, NH), 9.40 (1H, brs, OH), 8.18 (1H, d, *J* = 2.4 Hz, H-6''), 7.93 (1H, dd, *J* = 2.4, 9.0 Hz, H-4''), 7.10 (1H, d, *J* = 9.0 Hz, H-3''), 7.03 (1H, d, *J* = 8.1 Hz, H-6'), 6.40 (1H, d, *J* = 2.4 Hz, H-3'), 6.35 (1H, dd, *J* = 2.4, 8.1 Hz, H-5'), 3.74 (2H, q, *J* = 6.7 Hz, H-1), 3.67 (3H, s, OCH_3), 2.80 (2H, t, *J* = 6.7 Hz, H-2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 179.0 (C=S), 158.9 (C-4'), 156.1 (C-2'), 152.3 (C-2''), 145.8 (C-6''), 141.2 (C-4''), 130.8 (C-6'), 117.2 (C-1'), 114.3 (C-3''), 111.5 (C-5''), 104.1 (C-5'), 101.2 (C-3'), 54.8 (OCH_3), 45.2 (C-1), 27.9 (C-2); EI HRMS: *m/z* found 381.01454 (M+). $C_{15}H_{16}N_3O_2BrS$ (M^+) requires 381.01466; Found C, 47.39; H, 4.23; N, 10.76. $C_{15}H_{16}N_3O_2BrS$ requires C, 47.13; H, 4.22; N, 10.99.

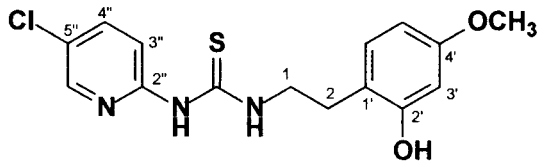
N-[2-(2-Hydroxy-5-methoxyphenyl)ethyl]-*N'*-[2-(5-chloropyridyl)]-thiourea (75)



Thiocarbonyl derivative **90b** (0.23 g, 1.0 mmol) was added to a solution of amine **91a** (0.13 g, 0.7 mmol) in DMF (1.0 mL). The reaction mixture was heated at 100 °C for 16 h. The mixture was poured into ice-cold water and the suspension stirred for 30 min. The product was filtered, washed with cold water, dried and the crude product purified by column chromatography using ethyl acetate / pet ether (3 / 7) to furnish **75** as colourless crystals (0.11 g, 43%). mp: 184-186 °C (ethyl acetate); IR (KBr): 3412 (w) (NH), 3400-3300 (br) OH), 2940 (w), 1602 (s) (C=C), 1161 (m) (C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.10 (1H, brt, *J* = 4.8 Hz, NH), 10.58 (1H, brs, NH), 8.88 (1H, brs, OH), 8.04 (1H, d, *J* = 2.8 Hz, H-6''), 7.79 (1H, dd, *J* = 2.8, 8.8 Hz, H-4''), 7.10 (1H, d, *J* = 8.8 Hz, H-3''), 6.70 (1H, d, *J* = 3.2 Hz, H-6'), 6.68 (1H, d, *J* = 8.8 Hz, H-3'), 6.59 (1H, dd, *J* = 3.2, 8.8 Hz, H-4'), 3.74 (2H, q, *J* = 6.8 Hz, H-1), 3.58 (3H, s, OCH_3), 2.80 (2H, t, *J* = 6.8 Hz, H-2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 179.0 (C=S),

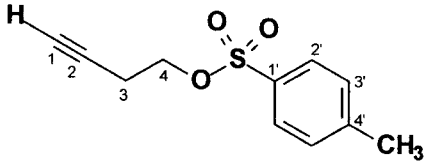
152.0 (C-5'), 151.9 (C-2''), 149.1 (C-2'), 143.5 (C-6''), 138.6 (C-4''), 125.8 (C-1'), 123.5 (C-5''), 116.3 (C-6'), 115.3 (C-3'), 113.8 (C-3''), 112.1 (C-4'), 55.2 (OCH_3), 44.9 (C-1), 28.8 (C-2); Found C, 53.12; H, 4.77; N, 12.34. $C_{15}H_{16}O_2N_3ClS$ requires C, 53.33; H, 4.77; N, 12.44.

N-[2-(2-Hydroxy-4-methoxyphenyl)ethyl]-*N'*-[2-(5-chloropyridyl)]-thiourea (76)



Thiocarbonyl derivative **90b** (0.31 g, 1.3 mmol) was reacted with crude amine **91b** in DMF (1 mL) at 100 °C for 16 h. The reaction mixture was poured into ice-cold water (5 mL) and the suspension was stirred for 30 min. The product was filtered, washed with water, dried, and further purified by column chromatography using ethyl acetate / petroleum ether (3 / 7) to furnish the title compound as pale yellow solid (0.20 g, 70%). mp: 208-209 °C (from ethyl acetate); IR (KBr): 3464 (w) (NH), 3400-3300 (br) OH), 1685 (s) (C=C), 1209 (s), 1191 (m), 1135 (m) (C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.07 (1H, brt, *J* = 4.6 Hz, NH), 10.56 (1H, brs, NH), 9.38 (1H, brs, OH), 8.06 (1H, d, *J* = 2.8 Hz, H-6''), 7.78 (1H, dd, *J* = 2.8, 9.0 Hz, H-4''), 7.10 (1H, d, *J* = 9.0 Hz, H-3''), 6.98 (1H, d, *J* = 8.3 Hz, H-6'), 6.35 (1H, d, *J* = 2.7 Hz, H-3'), 6.30 (1H, dd, *J* = 2.7, 8.3 Hz, H-5'), 3.69 (2H, q, *J* = 6.8 Hz, H-1), 3.62 (3H, s, OCH_3), 2.75 (2H, t, *J* = 6.8 Hz, H-2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 179.0 (C=S), 158.9 (C-4'), 156.2 (C-2'), 152.0 (C-2''), 143.6 (C-6''), 138.6 (C-4''), 130.8 (C-6'), 123.5 (C-5''), 117.2 (C-1'), 113.9 (C-3''), 104.2 (C-5'), 101.2 (C-3'), 54.8 (OCH_3), 45.2 (C-1), 28.0 (C-2).

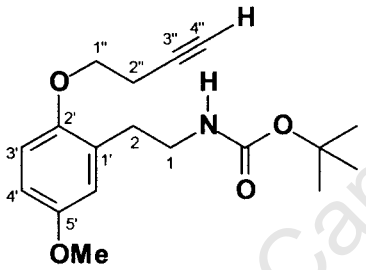
4-Tosyloxybut-1-yne (92)



p-Toluenesulfonyl chloride (6.14 g, 29.7 mmol) in CH_2Cl_2 (5 mL) was added dropwise to an ice-cold solution of but-3-yn-1-ol (1.5 mL, 19.8 mmol) containing triethylamine

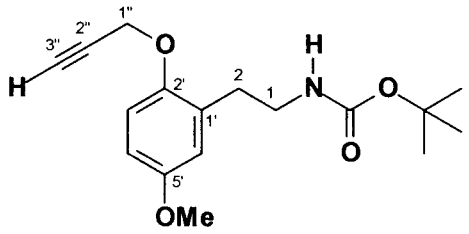
(4.12 mL, 29.7 mmol) and DMAP (0.02 g) in CH_2Cl_2 (10 mL). The mixture was stirred at 0 °C for 1 h. The organic layer was washed with $NaHCO_3$ (10 mL), water (2 × 10 mL) and then dried ($MgSO_4$), and the solvent evaporated under reduced pressure. The crude product was purified by column chromatography using ethyl acetate / petroleum ether (15 / 85) to give a colourless oil (4.44 g, 100%).[127] ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.75 (2H, d, *J* = 7.4 Hz, H-2', H-6'), 7.31 (2H, d, *J* = 7.4 Hz, H-3', H-5'), 4.06 (2H, t, *J* = 7.0 Hz, H-4), 2.50 (2H, td, *J* = 2.7, 7.0 Hz, H-3), 2.40 (3H, s, CH_3), 1.95 (1H, t, *J* = 2.7 Hz, H-1); ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.9 (C-1'), 132.6 (C-4'), 129.7 (C-2'), 127.7 (C-3'), 78.3 (C-2), 70.6 (C-1), 67.3 (C-4), 21.4 (C-3), 19.2 (CH_3).

[2-(2-But-3-ynyloxy-5-methoxyphenyl)ethyl]-*tert*-butylcarbamate (93)



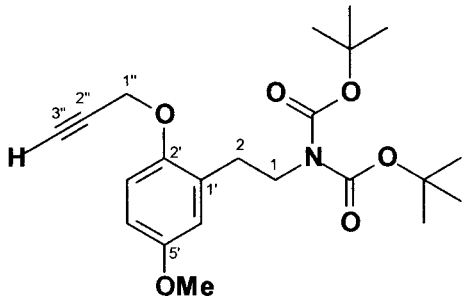
The tosylate **92** (1.69 g, 8.0 mmol) was added dropwise over 1 h (1.5 equiv. each day for 5 d) to a refluxing and stirring mixture of the phenol **81a** (0.34 g, 1.3 mmol) and anhydrous potassium carbonate (1.11 g, 8.0 mmol) in dry acetonitrile (50 mL). The mixture was stirred at reflux for 5 d. The solid was removed by filtration and the filtrate concentrated *in vacuo*. Purification by silica-gel column chromatography (15% ethyl acetate in petroleum ether) afforded a colourless solid (0.10 g, 61% yield based on recovered starting material (0.20 g)). mp: 76-77 °C (EtOAc/Pet ether); IR ($CHCl_3$): ν_{max} 3455 (w) (NH), 3309 (w) (≡C-H), 2413 (w) (C≡C), 1707 (s) (CO), 1602 (s, C=C) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.75 (3H, m, H-3', H-4', H-6'), 4.70 (1H, brs, NH), 4.05 (2H, t, *J* = 6.8 Hz, H-1"), 3.75 (3H, s, OCH_3), 3.36 (2H, q, *J* = 6.6 Hz, H-1), 2.79 (2H, t, *J* = 6.6 Hz, H-2), 2.66 (2H, dt, *J* = 2.7, 6.8 Hz, H-2"), 2.03 (1H, t, *J* = 2.7 Hz, H-4"), 1.42 (9H, s, $OC(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.9 (C=O), 153.9 (C-5'), 150.6 (C-2'), 129.3 (C-1'), 116.8 (C-6'), 112.8 (C-3'), 112.0 (C-4'), 80.7 (C-3"), 78.9 ($OC(CH_3)_3$), 69.8 (C-4"), 66.8 (C-1"), 55.6 (OCH_3), 40.7 (C-1), 30.9 (C-2), 28.4 ($OC(CH_3)_3$), 19.7 (C-2"); EI HRMS: *m/z* found 319.17756 (M^+). $C_{18}H_{25}NO_4$ (M^+) requires 319.17836. Found: C, 67.10; H, 7.66; N, 3.67. $C_{18}H_{25}NO_4$ requires C, 67.89; H, 7.89; N, 4.39.

[2-(2-Prop-2-ynyloxy-5-methoxyphenyl)ethyl]-*tert*-butylcarbamate (94a)



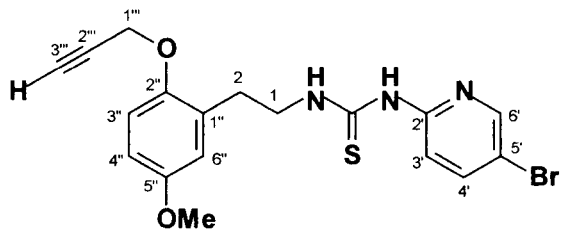
Propargyl bromide (2.00 mL, 22.4 mmol) in CH_3CN (2 mL) was added dropwise to a stirring and refluxing suspension of the phenol **81a** (1.00 g, 3.7 mmol) and potassium carbonate (0.78 g, 5.6 mmol) in CH_3CN (20 mL). The mixture was refluxed for 48 h, K_2CO_3 filtered through a pad of Celite and the filtrate evaporated under reduced pressure. Column chromatography of the residue employing ethyl acetate / petroleum ether (1 / 4) afforded the title compound as colourless crystals (0.86 g, 75%). mp: 49-50°C (EtOAc/pet ether); IR ($CHCl_3$): ν_{max} 3691 (w), 3454 (w) (NH), 3308 (w) (≡C-H), 3022 (m), 2124 (w) (C≡C), 1707 (s) (CO), 1501 (s, C=C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 6.90 (1H, d, *J* = 9.3 Hz, H-3'), 6.72 (2H, m, H-4', H-6'), 4.65 (3H, d, *J* = 2.4 Hz, H-1", NH), 3.75 (3H, s, OCH_3), 3.35 (2H, q, *J* = 6.8 Hz, H-1), 2.79 (2H, t, *J* = 6.8 Hz, H-2), 2.47 (1H, t, *J* = 2.4 Hz, H-3"), 1.42 (9H, s, $OC(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.9 (C=O), 154.3 (C-5'), 149.8 (C-2'), 129.6 (C-1'), 116.7 (C-6'), 113.6 (C-3'), 112.0 (C-4'), 79.0 (C-3", $OC(CH_3)_3$), 75.2 (C-2"), 56.7 (C-1"), 55.6 (OCH_3), 40.6 (C-1), 30.9 (C-2), 28.4 ($OC(CH_3)_3$); EI HRMS: *m/z* found 305.16244 (M^+). $C_{17}H_{23}NO_4$ (M^+) requires 305.16271; Found: C, 66.90; H, 7.54; N, 4.54. $C_{17}H_{23}NO_4$ requires C, 66.86; H, 7.59; N, 4.59.

N-tert-Butyloxycarbonyl-[2-(2-prop-2-ynyloxy-5-methoxyphenyl) ethyl]-*tert*-butylcarbamate (94b)



Propargyl bromide (80% in toluene, 0.3 mL, 3.2 mmol) in CH_3CN (1 mL) was added dropwise to a stirring and refluxing suspension of phenol **81b** (0.39 g, 1.1 mmol) and potassium carbonate (0.44 g, 3.2 mmol) in CH_3CN (10 mL). The mixture was refluxed for 1 h, filtered and the filtrate evaporated under reduced pressure. Column chromatography employing EtOAc / petroleum ether (1 / 5) afforded **94b** as a colourless oil (0.32 g, 74%). IR ($CHCl_3$): ν_{max} 3691 (w), 3308 (w) (≡C-H), 3016 (m), 3022 (s), 3024 (s), 1776 (w), 1737 (m) (CO), 1708 (m) (CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 6.91 (1H, d, *J* = 9.9 Hz, H-3'), 6.71 (2H, m, H-4', H-6'), 4.65 (2H, d, *J* = 2.4 Hz, H-1''), 3.79 (2H, t, *J* = 7.3 Hz, H-1), 3.75 (3H, s, OCH_3), 2.89 (2H, t, *J* = 7.3 Hz, H-2), 2.45 (1H, t, *J* = 2.4 Hz, H-3''), 1.47 (18H, s, 2 × $OC(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.3 (C-5'), 152.3 (2 × C=O), 150.1 (C-2'), 129.6 (C-1'), 116.8 (C-6'), 113.8 (C-3'), 112.0 (C-4'), 81.9 ($C(CH_3)_3$), 79.2 (C-2''), 75.1 (C-3''), 56.9 (C-1''), 55.6 (OCH_3), 46.5 (C-1), 30.1 (C-2), 28.0 ($C(CH_3)_3$); EI HRMS: *m/z* found 405.21491 (M^+). $C_{22}H_{31}NO_6$ (M^+) requires 405.21514.

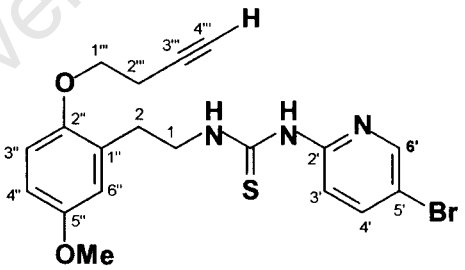
N-[2-(2-Propyn-2-yloxy-5-methoxyphenyl)ethyl]-*N'*-[2-(5-bromo pyridyl)]-thiourea (78)



Trifluoroacetic acid (0.1 mL) was added dropwise to an ice-cold solution of the carbamate **94** (0.35 g, 1.1 mmol) in CH_2Cl_2 (1.0 mL). The mixture was stirred at 0 °C for 2 h. Diisopropylethylamine (0.3 mL) was added and the mixture stirred for an

additional 10 min. The solvent was evaporated and the crude amine dried under vacuum for 1 h. Thiocarbonyl reagent **89** (0.39 g, 1.4 mmol) was added to the crude amine in DMF (1.0 mL) and the mixture stirred at 100 °C for 1 h. DMF was removed by co-evaporation with toluene and the residue subjected to column chromatography employing 15% ethyl acetate in pet ether to give the title compound as colourless needles (0.08 g, 17%). mp: 121-122 °C (from pet ether/EtOAc); IR ($CHCl_3$): ν_{max} 3691 (w), 3416 (w) (NH), 3307 (w) (≡C-H), 3174 (br) (NH), 1602 (m) (C=C), 1137 (w) (C=S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 11.13 (1H, brs, NH), 8.29 (1H, brs, NH), 8.12 (1H, d, *J* = 2.4 Hz, H-6'), 7.69 (1H, dd, *J* = 2.4, 8.8 Hz, H-4'), 6.95 (1H, d, *J* = 8.8 Hz, H-3'), 6.82 (1H, d, *J* = 3.1 Hz, H-6''), 6.76 (1H, dd, *J* = 3.1, 8.8 Hz, H-4''), 6.59 (1H, d, *J* = 8.8 Hz, H-3''), 4.67 (2H, d, *J* = 2.2 Hz, H-1'''), 4.01 (2H, q, *J* = 6.7 Hz, H-1), 3.76 (3H, s, OCH_3), 3.01 (2H, t, *J* = 6.7 Hz, H-2), 2.47 (1H, t, *J* = 2.2 Hz, H-3'''); ^{13}C NMR (75 MHz, $CDCl_3$): δ 179.1 (C=S), 154.2 (C-5''), 151.7 (C-2'), 150.0 (C-2''), 146.7 (C-6'), 141.1 (C-4'), 129.2 (C-1''), 117.6 (C-6''), 113.5 (C-3''), 113.3 (C-3'), 112.6 (C-5'), 111.5 (C-4''), 79.0 (C-2'''), 75.3 (C-3'''), 56.8 (C-1'''), 55.5 (OCH_3), 45.7 (C-1), 29.8 (C-2); Found: C, 51.23; H, 4.50; N, 8.62. $C_{18}H_{18}BrN_3O_2S$ requires C, 51.44; H, 4.32; N, 10.00.

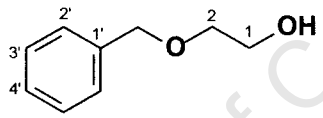
N-[2-(2-Butyn-3-yloxy-5-methoxyphenyl)ethyl]-*N'*-[2-(5-bromopyridyl)]-thiourea (77)



Trifluoroacetic acid (0.1 mL) was added dropwise to an ice-cold solution of the carbamate **93** (0.098 g, 0.37 mmol) in CH_2Cl_2 (1.0 mL). The mixture was stirred at 0 °C for 30 min. Diisopropylethylamine (0.3 mL) was added and the mixture stirred for an additional 10 min. The solvent was evaporated and the crude amine dried under vacuum for 1 h. Thiocarbonyl reagent **89** (0.125 g, 0.44 mmol) was added to the crude amine in DMF (1.0 mL) and the mixture stirred at 100 °C for 1 h. DMF was removed by co-evaporation with toluene and the residue subjected to column chromatography employing 15% ethyl acetate in pet ether to give the title compound

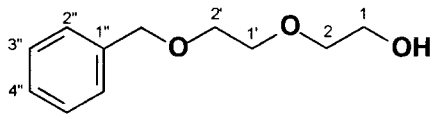
77 as colourless needles (0.033 g, 25%). mp: 155-156 °C (from pet ether / EtOAc); IR ($CHCl_3$): ν_{max} 3691 (w), 3415 (w) (NH), 3308 (w) (≡C-H), 3176 (w), 3048 (w), 2360 (w) (C≡C), 1591 (m), 1578 (m), 1559 (m), 1511 (s), 1163 (w) (C=S), 1138 (m) (C=S), 1097 (m) (C=S), 775 (w) (C-S) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 11.13 (1H, brs, NH), 8.44 (1H, brs, NH), 8.10 (1H, d, *J* = 2.4 Hz, H-6'), 7.68 (1H, dd, *J* = 2.4, 8.7 Hz, H-4'), 6.76 (3H, m, H-3', H-4'',H-6''), 6.61 (1H, d, *J* = 8.7 Hz, H-3''), 4.03 (4H, m, H-1, H-1'''), 3.75 (3H, s, OCH_3), 3.00 (2H, t, *J* = 6.6 Hz, H-2), 2.68 (2H, td, *J* = 2.6, 6.9 Hz, H-2'''), 2.04 (1H, t, *J* = 2.6 Hz, H-4'''); ^{13}C NMR (75 MHz, $CDCl_3$): δ 179.2 (C=S), 153.8 (C-5''), 151.6 (C-2''), 150.9 (C-2'), 146.9 (C-6'), 141.2 (C-4'), 128.9 (C-1''), 117.8 (C-6''), 113.1 (C-3'), 112.8 (C-3''), 112.7 (C-5'), 111.6 (C-4''), 80.8 (C-4''') 69.9 (C-3'''), 66.9 (C-1'''), 55.6 (OCH_3), 45.8 (C-1), 30.0 (C-2), 19.8 (C-2'''); Found: C, 52.01; H, 4.46; N, 9.00. $C_{19}H_{20}BrN_3O_2S$ requires C, 52.54; H, 4.64; N, 9.67.

2-Benzyloxyethanol (130)



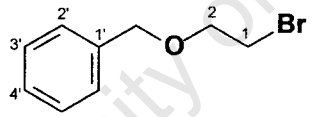
Benzyl bromide (7.60 mL, 64.4 mmol) was added dropwise to a suspension of NaH (60% in mineral oil, 1.55 g, 64.4 mmol) and 1, 2-ethanediol (10.80 mL, 193.4 mmol) in THF (40 mL). The mixture was refluxed for 20 h. The mixture was dissolved in EtOAc (60 mL) and washed with NH_4Cl (40 mL) and H_2O (2 × 40 mL). The EtOAc solution was dried over $MgSO_4$, the solvent evaporated and the crude product purified by distillation (bp: 102 °C/0.7 mm Hg [lit.[117] 90-95 °C/0.7 mm Hg]) to give **130** (5.19 g, 53%) as a colourless oil. ^1H NMR (300 MHz, $CDCl_3$): δ 7.34 (5H, m, ArH), 4.56 (2H, s, ArCH_2O), 3.74 (2H, t, *J* = 4.6 Hz, H-2), 3.58 (2H, t, *J* = 4.6 Hz, H-1), 2.52 (1H, s, OH); ^{13}C NMR (75 MHz. $CDCl_3$): δ 138.0 (C-1'), 128.7, 128.4, 127.8, (ArCH), 73.3 ($ArCH_2O$), 71.5 (C-2), 61.8 (C-1).

2-(2-Benzyloxyethoxy)ethanol (130b)



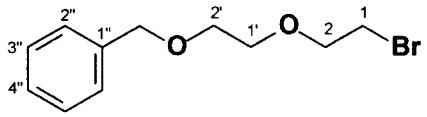
Benzyl bromide (2.24 mL, 18.9 mmol) was added dropwise to a suspension of NaH (60% in mineral oil, 0.76 g, 31.4 mmol) and diethylene glycol (6.00 g, 56.5 mmol) in THF (40 mL). The mixture was refluxed for 20 h. The mixture was dissolved in EtOAc (60 mL) and washed with NH_4Cl (40 mL) and H_2O (2 × 40 mL). The EtOAc solution was dried over $MgSO_4$, the solvent evaporated and the crude product purified by distillation (bp: 180 °C/0.7 mm Hg [lit.[128] 171-173 °C) to give **130b** (2.22 g, 60% yield) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33 (5H, m, ArH), 4.56 (2H, s, ArC*H*$_2$O), 3.64 (8H, m, 4 × CH_2O), 2.79 (1H, brs, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.8 (C-1"), 128.1, 127.5, 127.4 (Ar*C*H), 73.0 (Ar*C*H$_2$O), 72.3 (C-2'), 70.1 (OCH_2), 69.2 (OCH_2), 61.4 (C-1).

2-Benzyloxy-1-bromoethane (131)



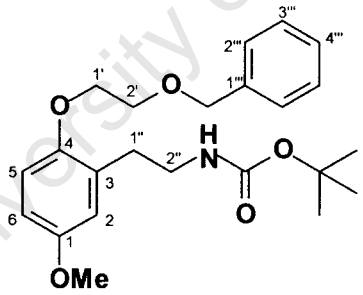
To a solution of the alcohol **130** (2.00 g, 13.1 mmol) and PPh_3 (4.14 g, 15.8 mmol) in CH_2Cl_2 (20 mL) was added CBr_4 (5.23 g, 15.8 mmol) at 0°C. The reaction mixture was stirred at 0°C for 30 min and the solvent evaporated. The crude product was purified by column chromatography using CH_2Cl_2 / pet ether (2 / 8) to give a colourless oil (2.68 g, 95%).[129] ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36 (5H, m, ArH), 4.60 (2H, s, ArC*H*$_2$O), 3.80 (2H, t, *J* = 6.2 Hz, H-2), 3.50 (2H, t, *J* = 6.2 Hz, H-1); ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.8 (C-1'), 128.5, 127.8, 127.7 (Ar*C*H), 73.1 (Ar*C*H$_2$O), 70.0 (C-2), 30.4 (C-1).

2-(2-Benzyloxy)ethoxy-1-bromoethane (131b)



To a solution of the alcohol **130b** (1.96 g, 1.0 mmol) and PPh_3 (3.14 g, 12.0 mmol) in CH_2Cl_2 (20 mL) was added CBr_4 (3.98 g, 12.0 mmol) at 0°C. The reaction mixture was stirred at 0°C for 30 min and the solvent evaporated. The crude product was purified by column chromatography using CH_2Cl_2 / pet ether (3 / 7) to give a colourless oil (2.36 g, 91%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36 (5H, m, ArH), 4.59 (2H, s, ArCH_2O), 3.81 (2H, t, *J* = 6.3 Hz, H-2), 3.67 (4H, m, 2 × CH_2O), 3.47 (2H, t, *J* = 6.3 Hz, H-1); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.0 (C-1"), 128.1 (C-2", C-6"), 127.4 (C-3", C-5"), 127.3 (C-4"), 73.0 (ArCH_2O), 70.9 (C-2'), 70.3 (CH_2O), 69.2 (CH_2O), 30.1 (C-1); EI HRMS: *m/z* found 258.02407 (M^+). $C_{11}H_{15}BrO_2$ (M^+) requires 258.02554.

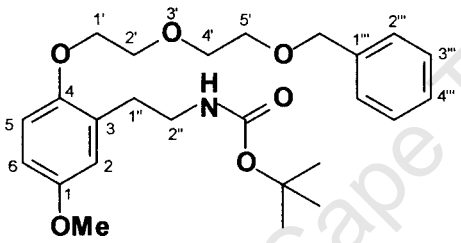
3-(2-*t*-Butyloxycarbonylaminoethyl)-4-(2-benzyloxyethoxy)anisole (132)



Bromide **131** (0.73 g, 3.4 mmol) in CH_3CN (1 mL) was added dropwise to a stirring and refluxing suspension of K_2CO_3 (0.47 g, 3.4 mmol) in a solution of phenol **81a** (0.30 g, 1.1 mmol) dissolved in CH_3CN (10 mL). The mixture was refluxed for 20 h, the K_2CO_3 filtered through a pad of Celite, the latter washed with ethyl acetate (3 × 30 mL) and the solvent reduced *in vacuo*. The crude product was purified by column chromatography using ethyl acetate / pet ether (2 / 8) to afford **132** as colourless crystals (0.39 g, 87%). mp: 56–58 °C (from EtOAc/pet ether); IR ($CHCl_3$): ν_{max} 3692 (w), 3452 (w) (NH), 1706 (s) (CO), 1502 (s) (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36 (5H, m, ArH), 6.78 (1H, d, *J* = 8.8 Hz, H-5), 6.72 (1H, d, *J* = 3.0 Hz, H-2), 6.70 (1H, dd, *J* = 3.0, 8.8 Hz, H-6), 4.73 (1H, brs, NH), 4.63 (2H, s, ArCH_2O), 4.11 (2H, t, *J*

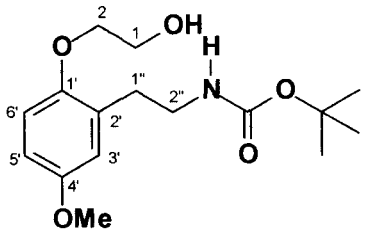
= 4.8 Hz, H-1'), 3.83 (2H, t, *J* = 4.8 Hz, H-2'), 3.76 (3H, s, OCH_3), 3.35 (2H, d, *J* = 6.4 Hz, H-2''), 2.80 (2H, t, *J* = 6.4 Hz, H-1''), 1.42 (9H, s, $OC(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.0 (C=O), 153.8 (C-1), 151.0 (C-4), 138.1 (C-1'''), 129.3 (C-3), 128.4 (C-2''', C-6'''), 127.7 (C-4'''), 127.6 (C-3''', C-5'''), 116.7 (C-2), 113.0 (C-5), 112.0 (C-6), 78.8 ($OC(CH_3)_3$), 73.3 ($ArCH_2O$), 68.8 (CH_2O), 68.4 (OCH_2), 55.7 (OCH_3), 40.7 (C-2''), 31.0 (C-1''), 28.4 ($OC(CH_3)_3$); EI HRMS: *m/z* Found 401.22044 (M^+). $C_{23}H_{31}NO_5$ (M^+) requires 401.22022; Found C, 68.90; H, 7.80; N, 3.37. $C_{23}H_{31}NO_5$ requires C, 68.80; H, 7.78; N, 3.49.

3-(2-*tert*-Butyloxycarbonylaminoethyl)-4-(5-benzyloxy-3-oxapentyl oxy)anisole (132b)



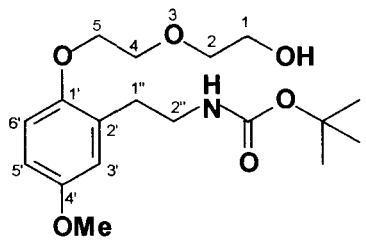
Bromide **131b** (0.52 g, 2.0 mmol) in DME (4 mL) was added dropwise to a stirring and refluxing suspension of NaH (0.06 g, 2.5 mmol) in a solution of phenol **81a** (0.27 g, 1.0 mmol) dissolved in DME (16 mL). The mixture was refluxed for 20 h, the crude product dissolved in EtOAc (20 mL) and washed with NH_4Cl (10 mL) and H_2O (2 × 10 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent reduced *in vacuo*. The crude product was purified by column chromatography using ethyl acetate / pet ether (3 / 7) to afford **132b** as a colourless oil (0.35 g, 78%). IR ($CHCl_3$): ν_{max} 3691 (w), 3684 (w), 3453 (w) (NH), 3011 (m), 2933 (m), 1706 (s) (CO), 1502 (s) (C=C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.30 (5H, m, ArH), 6.78 (1H, d, *J* = 8.7 Hz, H-5), 6.71 (1H, d, *J* = 2.8 Hz, H-2), 6.69 (1H, dd, *J* = 2.8, 8.7 Hz, H-6), 4.88 (1H, brs, NH), 4.58 (2H, s, $ArCH_2O$), 4.09 (2H, t, *J* = 4.9 Hz, CH_2O), 3.84 (2H, t, *J* = 4.9 Hz, CH_2O), 3.74 (5H, m, CH_2O, OCH_3), 3.65 (2H, m, CH_2O), 3.34 (2H, m, *J* = 6.4 Hz, H-2''), 2.79 (2H, t, *J* = 6.4 Hz, H-1''), 1.41 (9H, s, $OC(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.0 (C=O), 153.8 (C-1), 151.0 (C-4), 138.2 (C-1'''), 129.4 (C-3), 128.3 (ArCH), 127.7 (ArCH), 127.6 (ArCH), 116.6 (C-2), 113.1 (C-5), 112.0 (C-6), 78.8 ($OC(CH_3)_3$), 73.2 (CH_2O), 70.8 (CH_2O), 69.9 (CH_2O), 69.5 (CH_2O), 68.5 (CH_2O), 55.6 (OCH_3), 40.7 (C-2''), 31.0 (C-1''), 28.4 ($OC(CH_3)_3$); EI HRMS: *m/z* Found 445.24814 (M^+). $C_{25}H_{35}NO_6$ (M^+) requires 445.24644.

2-[2-(2-*tert*-Butyloxycarbonylaminoethyl)-4-methoxy]phenoxy-1-ethanol (133)



Hydrogen gas was introduced at atmospheric pressure to the carbamate **132** (0.37 g, 0.9 mmol) and a suspension of 10% palladium-on-carbon (10 mol%) in ethanol (5 mL). The mixture was stirred at room temperature for 18 h. The catalyst was filtered through a pad of Celite, washed with EtOAc (3 × 10 mL) and the filtrate evaporated *in vacuo*. The crude product was purified by column chromatography using EtOAc / pet ether (4 / 6) to give **133** as colourless crystals (0.25 g, 87%). mp: 84-85 °C (from EtOAc/pet ether); IR ($CHCl_3$): ν_{max} 3691 (w), 3458 (m, br) (NH, OH), 2934 (m), 1704 (s) (CO), 1502 (s) (C=C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.70 (3H, m, H-3', H-5', H-6'), 4.87 (1H, brs, NH), 4.03 (2H, t, J = 4.1 Hz, H-2), 3.95 (2H, t, J = 4.1 Hz, H-1), 3.75 (3H, s, OCH_3), 3.31 (2H, m, J = 6.7 Hz, H-2''), 2.78 (2H, t, J = 6.7 Hz, H-1''), 1.42 (9H, s, $OC(CH_3)_3$), 1.25 (1H, brs, OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.5 (C=O), 153.9 (C-4'), 151.4 (C-1'), 128.9 (C-2'), 117.1 (C-3'), 112.6 (C-6'), 112.0 (C-5'), 79.7 ($OC(CH_3)_3$), 70.6 (C-2), 61.6 (C-1), 55.9 (OCH_3), 41.0 (C-2''), 32.6 (C-1''), 28.6 ($OC(CH_3)_3$); EI HRMS: *m/z* found 311.17278 (M^+). $C_{16}H_{25}NO_5$ (M^+) requires 311.17327. Found: C, 61.70; H, 8.03; N, 4.14. $C_{16}H_{25}NO_5$ requires C, 61.72; H, 8.09; N, 4.50.

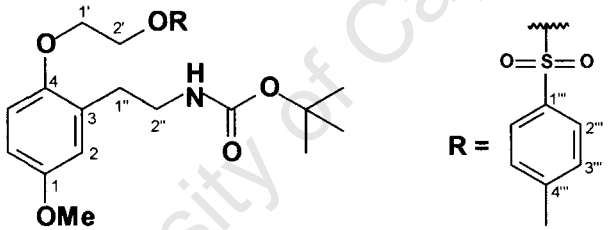
5-[2-(2-*tert*-Butyloxycarbonylaminoethyl)-4-methoxy]phenoxy-3-oxa-1-pentanol (133b)



Hydrogen gas was introduced at atmospheric pressure to the carbamate **132b** (0.22 g, 0.5 mmol) and a suspension of 10% palladium-on-carbon (10 mol%) in ethanol (15 mL). The mixture was stirred at room temperature for 18 h. The catalyst was filtered

through a pad of Celite, washed with EtOAc (3 × 10 mL) and the filtrate evaporated *in vacuo*. The crude product was purified by column chromatography using EtOAc / pet ether (1 / 1) to give **133b** as a colourless oil (0.16 g, 92%). IR ($CHCl_3$): ν_{max} 3692 (w), 3604 (w), 3452 (w) (NH), 3392 (br) (OH), 3011 (m), 2980 (m), 2934 (m), 1705 (s) (CO), 1503 (s) (C=C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.78 (1H, d, *J* = 9.3 Hz, H-6'), 6.70 (2H, m, H-5', H-3'), 4.89 (1H, brs, NH), 4.10 (2H, t, *J* = 4.6 Hz, CH_2O), 3.85 (2H, t, *J* = 4.6 Hz, CH_2O), 3.75 (5H, m, OCH_3, CH_2O), 3.67 (2H, m, CH_2O), 3.36 (2H, m, *J* = 6.2 Hz, H-2''), 2.79 (2H, t, *J* = 6.2 Hz, H-1''), 1.63 (1H, brs, OH), 1.40 (9H, s, $OC(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.0 (C=O), 153.9 (C-4'), 151.0 (C-1'), 129.3 (C-2'), 116.7 (C-3'), 113.0 (C-6'), 112.0 (C-5'), 72.6 (C-5), 69.8 (CH_2O), 68.4 (CH_2O), 61.8 (C-1), 55.6 (OCH_3), 40.8 (C-2''), 31.1 (C-1''), 28.4 ($OC(CH_3)_3$); EI HRMS: *m/z* found 355.19828 (M^+). $C_{18}H_{29}O_6N$ (M^+) requires 355.19949.

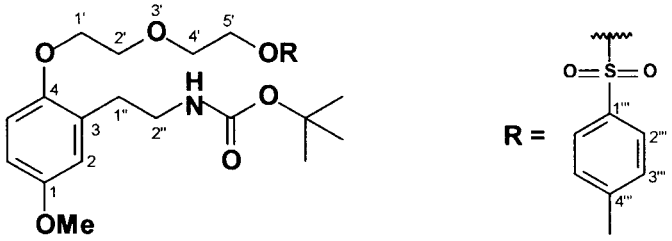
3-(2-*tert*-Butyloxycarbonylaminoethyl)-4-(2-*p*-toluenesulphonyloxy ethoxy)anisole (134a)



p-Toluenesulfonyl chloride (0.29 g, 1.5 mmol) was added portionwise to a stirring solution of the alcohol **133** (0.24 g, 0.8 mmol) containing triethylamine (0.2 mL, 1.5 mmol) and a catalytic amount of DMAP (0.02 g) in CH_2Cl_2 (10 mL) at 0°C. The reaction mixture was stirred at room temperature for 20 h. The solvent was evaporated under reduced pressure and the crude product subjected to column chromatography employing EtOAc / pet ether (3 / 7) to afford the title compound as a colourless oil (0.27 g, 76%). IR ($CHCl_3$): ν_{max} 3690 (w), 3451 (w) (NH), 1706 (s) (CO), 1367 (s) ($S(=O)_2$), 1164 (s) ($S(=O)_2$) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (2H, d, *J* = 8.2 Hz, H-2''', H-6'''), 7.28 (2H, d, *J* = 8.2 Hz, H-3''', H-5'''), 6.62 (3H, m, H-2, H-5, H-6), 4.77 (1H, brs, NH), 4.30 (2H, t, *J* = 4.7 Hz, H-2'), 4.05 (2H, t, *J* = 4.7 Hz, H-1'), 3.68 (3H, s, OCH_3), 3.22 (2H, q, *J* = 6.8 Hz, H-2''), 2.65 (2H, t, *J* = 6.8 Hz, H-1''), 2.38 (3H, s, CH_3), 1.38 (9H, s, $OC(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.9 (C=O), 154.1 (C-1), 150.2 (C-4), 145.0 (C-1'''), 132.9 (C-4'''), 129.9 (C-3''', C-5'''), 129.3 (C-3), 127.8 (C-2''', C-6'''), 116.7 (C-2), 112.0 (C-5), 111.9 (C-6), 78.8 ($OC(CH_3)_3$), 68.4

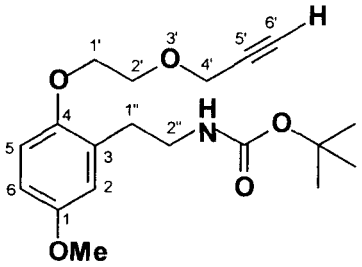
(C-1'), 66.4 (C-2'), 55.5 (OCH_3), 40.5 (C-2''), 30.9 (C-1''), 28.4 ($OC(CH_3)_3$), 21.5 (CH_3); EI HRMS: *m/z* found 465.18130 (M^+). $C_{23}H_{31}NO_7S$ (M^+) requires 465.18212.

3-(2-*tert*-Butyloxycarbonylaminoethyl)-4-(5-*p*-toluenesulphonyloxy-3-oxa-pentyloxy)-anisole (134b)



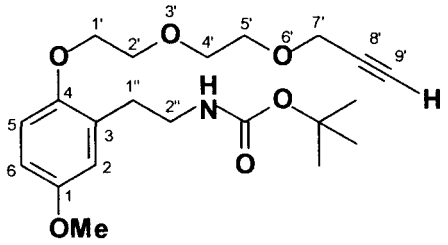
p-Toluenesulfonyl chloride (0.18 g, 1.0 mmol) was added portionwise to a stirring solution of the alcohol **133b** (0.17 g, 0.5 mmol) containing triethylamine (0.07 mL, 0.5 mmol) and DMAP (cat.) in CH_2Cl_2 (15 mL) at 0°C. The reaction mixture was stirred at room temperature for 20 h. The solvent was evaporated under reduced pressure and the crude product subjected to column chromatography employing EtOAc / pet ether (4 / 6) to afford the title compound **134b** as a colourless oil (0.16 g, 83%). IR ($CHCl_3$): ν_{max} 3693 (w), 3453 (w) (NH), 3023 (m), 3007 (m), 2980 (m), 2934 (m), 1706 (s) (CO), 1502 (s) (C=C), 1367 (s) ($S(=O)_2$), 1168 (s) ($S(=O)_2$), 1064-1021 (w) (S=O); ^1H NMR (400 MHz, $CDCl_3$): δ 7.76 (2H, d, *J* = 7.9 Hz, H-2''', H-6'''), 7.28 (2H, d, *J* = 7.9 Hz, H-3''', H-5'''), 6.73 (1H, d, *J* = 8.8 Hz, H-5), 6.68 (2H, m, H-2, H-6), 4.80 (1H, brs, NH), 4.17 (2H, t, *J* = 4.7 Hz, H-5'), 3.98 (2H, t, *J* = 4.7 Hz, H-4'), 3.75 (4H, m, 2 × CH_2O), 3.73 (3H, s, OCH_3), 3.29 (2H, q, *J* = 6.7 Hz, H-2''), 2.74 (2H, t, *J* = 6.7 Hz, H-1''), 2.39 (3H, s, CH_3), 1.39 (9H, s, $OC(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.9 (C=O), 153.9 (C-1), 150.9 (C-4), 144.8 (C-1'''), 133.1 (C-4'''), 129.8 (C-2'''), 129.3 (C-3), 127.9 (C-3'''), 116.6 (C-2), 113.0 (C-5), 112.0 (C-6), 78.8 ($O_2C(CH_3)_3$), 70.0 (CH_2O), 69.3 (CH_2O), 68.8 (CH_2O), 68.3 (CH_2O), 55.6 (OCH_3), 40.6 (H-2''), 31.0 (H-1''), 28.4 ($OC(CH_3)_3$), 21.5 (CH_3); EI HRMS: *m/z* found 509.20675 (M^+). $C_{25}H_{35}NO_8S$ (M^+) requires 509.20834.

3-(2-*tert*-Butyloxycarbonylaminoethyl)-4-(3-oxahex-5-ynyloxy) anisole (135a)



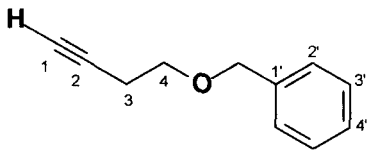
Propargyl alcohol (0.20 mL, 3.52 mmol) was added dropwise to a suspension of NaH (60% in mineral oil, 0.056 g, 2.35 mmol) in THF (10 mL) at 0 °C. The mixture was stirred for 1 h and the tosylate **134a** (0.27 g, 0.59 mmol) added. The mixture was heated at reflux for 20 h. The crude product was dissolved into EtOAc (15 mL), washed with NH_4Cl (10 mL), water (2 × 10 mL), dried ($MgSO_4$), and the solvent evaporated under reduced pressure. The crude product was purified by column chromatography employing EtOAc / pet ether (3 / 7) to furnish the title compound **135a** as a colourless oil (0.15 g, 74%). IR ($CHCl_3$): ν_{max} 3674 (w), 3452 (m) (NH), 3307 (m) (≡C-H), 2121 (w) (C≡C), 1703 (s) (CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 6.78 (1H, d, *J* = 7.8 Hz, H-5), 6.69 (2H, m, H-2, H-6), 4.79 (1H, brs, NH), 4.26 (2H, d, *J* = 2.3 Hz, H-4'), 4.10 (2H, m, H-1'), 3.88 (2H, m, H-2'), 3.75 (3H, s, OCH_3), 3.35 (2H, q, *J* = 6.7 Hz, H-2"), 2.80 (2H, t, *J* = 6.7 Hz, H-1"), 2.45 (1H, t, *J* = 2.3 Hz, H-6'), 1.42 (9H, s, $OC(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.0 (C=O), 153.9 (C-1), 150.9 (C-4), 129.4 (C-3), 116.7 (C-2), 113.0 (C-5), 112.0 (C-6), 79.6 (C-5'), 78.9 ($OC(CH_3)_3$), 74.7 (C-6'), 68.4 (CH_2O), 68.3 (CH_2O), 58.5 (C-4'), 55.7 (OCH_3), 40.8 (C-2"), 31.0 (C-1"), 28.4 ($OC(CH_3)_3$); EI HRMS: *m/z* found 349.18937 (M^+). $C_{19}H_{27}NO_5$ (M^+) requires 349.18892.

3-(2-*tert*-Butyloxycarbonylaminoethyl)-4-(3,6-dioxanon-8-ynyloxy) anisole (135b)



Propargyl alcohol (0.24 mL, 4.0 mmol) was added dropwise to a suspension of NaH (60% mineral oil, 0.05 g, 2.2 mmol) in THF (10 mL) at 0 °C. The mixture was stirred for 1 h and the tosylate **134b** (0.17 g, 0.3 mmol) added. The mixture was heated at reflux for 20 h. The crude product was dissolved into EtOAc (15 mL), washed with NH_4Cl (10 mL), water (2 × 10 mL), dried ($MgSO_4$), and the solvent evaporated under reduced pressure. The crude product was purified by column chromatography employing EtOAc / pet ether (4 / 6) to furnish the title compound **135b** as a colourless oil (0.12 g, 91 %). IR ($CHCl_3$): ν_{max} 3693 (w), 3607 (w), 3453 (w), 3308 (m), 3012 (m), 2980 (m), 2934 (m), 2120 (w) (C≡C), 1706 (s) (CO), 1502 (s) (C=C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.74 (1H, d, *J* = 8.6 Hz, H-5), 6.66 (2H, m, H-2, H-6), 4.90 (1H, brs, NH), 4.17 (2H, d, *J* = 2.4 Hz, H-7'), 4.05 (2H, t, *J* = 4.9 Hz, H-1'), 3.80 (2H, t, *J* = 4.9 Hz, H-2'), 3.71 (3H, s, OCH_3), 3.70 (4H, m, 2 × CH_2O), 3.31 (2H, q, *J* = 6.7 Hz, H-2''), 2.76 (2H, t, *J* = 6.7 Hz, H-1''), 2.42 (1H, t, *J* = 2.4 Hz, H-9'), 1.39 (9H, s, $OC(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.0 (C=O), 153.8 (C-1), 151.0 (C-4), 129.4 (C-3), 116.6 (C-2), 113.1 (C-5), 112.0 (C-6), 79.6 (C-8'), 78.7 ($OC(CH_3)_3$), 74.6 (C-9'), 70.5 (C-1'), 69.8 (OCH_2), 69.1 (OCH_2), 68.5 (OCH_2), 58.3 (C-7'), 55.6 (OCH_3), 40.7 (C-2''), 31.0 (C-1''), 28.4 ($OC(CH_3)_3$); EI HRMS: *m/z* found 393.21448 (M^+). $C_{21}H_{31}NO_6$ (M^+) requires 393.21514.

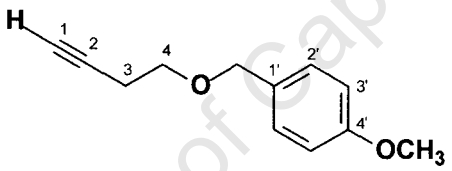
4-Benzyloxybut-1-yne (146)



3-Butyn-1-ol (2.50 mL, 33.4 mmol) was added dropwise to a suspension of NaH (60% mineral oil, 1.60 g, 40.1 mmol) in THF (20 mL) and the mixture was stirred for

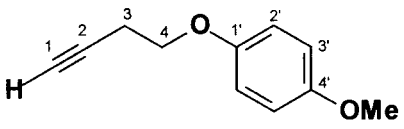
30 minutes at 0°C. Benzyl bromide (4.80 mL, 40.1 mmol) was added dropwise followed by tetrabutylammonium iodide (1.24 g, 3.4 mmol). This was stirred for 12 h at room temperature. The reaction mixture was quenched using MeOH (10 mL). The methanol was evaporated under reduced pressure. Water was added to the mixture and the product extracted using EtOAc (3 × 30 mL), dried over $MgSO_4$ and the solvent removed *in vacuo*. The crude product was purified by column chromatography using increasing amounts of ethyl acetate in petroleum ether to afford **146** as a colourless oil (3.45 g, 65%).[120] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37 (5H, m, ArH), 4.58 (2H, s, ArCH_2O), 3.63 (2H, t, *J* = 7.0, H-4), 2.52 (2H, td, *J* = 2.6, 7.0, H-3), 2.02 (1H, t, *J* = 2.6, H-1); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.1 (C-1'), 128.4 (C-2', C-6'), 127.6 (C-3', C-5'), 127.6 (C-4'), 81.3 (C-2), 72.9 (C-1), 69.4 (ArCH_2O), 68.2 (C-4), 19.9 (C-3).

4-(4-Methoxybenzyloxy)but-1-yne (147a)



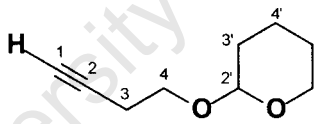
To a stirred suspension of NaH (0.51 g, 21.4 mmol) in DMSO (2 mL) was added dropwise a THF solution (30 mL) of 3-butyn-1-ol (1.08 mL, 14.3 mmol) under nitrogen. After *ca.* 30 min at 0 °C, a solution of *p*-methoxybenzyl chloride (2.90 mL, 21.4 mmol) in THF (30 mL) was added and stirring continued for 12 h. The mixture was poured into saturated NH_4Cl (50 mL) and then extracted with ether (3 × 50 mL). The organic extracts were washed with sat. NaCl (2 × 100 mL), dried over $MgSO_4$ and the solvent evaporated under reduced pressure. The product was purified by column chromatography to afford a colourless oil (2.46 g, 91%).[121] ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.27 (2H, d, *J* = 8.6 Hz, H-2', H-6'), 6.88 (2H, d, *J* = 8.6 Hz, H-3', H-5'), 4.49 (2H, s, ArCH_2O), 3.80 (3H, s, OCH_3), 3.58 (2H, t, *J* = 7.0 Hz, H-4), 2.49 (2H, td, *J* = 2.6, 7.0 Hz, H-3), 1.99 (1H, t, *J* = 2.6 Hz, H-1); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.3 (C-4'), 130.1 (C-1'), 129.3 (C-2', C-6'), 113.8 (C-3', C-5'), 81.3 (C-2), 72.6 (ArCH_2O), 69.2 (C-1), 67.8 (C-4), 55.2 (OCH_3), 19.8 (C-3).

4-(4-Methoxyphenoxy)but-1-yne (147b)



A mixture of 3-butyn-1-ol (1.00 mL, 13.4 mmol), *p*-methoxyphenol (4.98 g, 40.1 mmol), triphenylphosphine (4.56 g, 17.4 mmol) and diisopropylazodicarboxylate (3.40 mL, 17.4 mmol) in CH_2Cl_2 (15 mL) were heated at reflux for 20 h. The solvent was removed *in vacuo* and the crude product purified by column chromatography employing ethyl acetate / pet ether (1 / 9) to give **147b** as colourless crystals (0.50 g, 51%). mp: 44-45 °C (from pet ether/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.87 (2H, d, *J* = 9.2 Hz, ArH), 6.82 (2H, d, *J* = 9.2 Hz, ArH), 4.05 (2H, t, *J* = 7.1 Hz, H-4), 3.77 (3H, s, OCH_3), 2.64 (2H, td, *J* = 2.8, 7.1 Hz, H-3), 2.00 (1H, t, *J* = 2.8 Hz, H-1); ^{13}C NMR (75 MHz, $CDCl_3$): δ 154.2 (C-4'), 152.6 (C-1'), 115.9 (C-2', C-6'), 114.7 (C-3', C-5'), 80.6 (C-2), 69.8 (C-1), 66.9 (C-4), 55.7 (OCH_3), 19.6 (C-3); Found C, 74.65; H, 6.95. $C_{11}H_{12}O_2$ requires C, 74.98; H, 6.86.

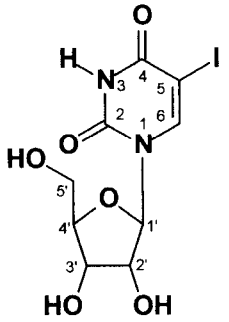
4-(2-Tetrahydropyranyloxy) but-1-yne (148)



To a stirred ice-cold solution of 3-butyn-1-ol (5.00 mL, 66.8 mmol) and 3,4-dihydro-2*H*-pyran (6.70 mL, 73.5 mmol) in dry dichloromethane (80 mL) was added *p*-toluenesulphonic acid (0.13 g, 0.7 mmol). The mixture was stirred for 10 min, the ice-bath removed and the solution stirred at rt for 1 h. The mixture was partitioned between ether (150 mL) and a solution made up of saturated brine (40 mL), saturated sodium bicarbonate (40 mL) and of water (50 mL). The organic phase was washed twice with saturated brine (100 mL), dried ($MgSO_4$) and the solvent evaporated. The product was purified by distillation at 51 °C / 0.7 mm Hg to give a colourless liquid (9.76 g, 95%).[122] ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.62 (1H, t, *J* = 3.2 Hz, H-2'), 3.86 (1H, m, H-6'), 3.80 (1H, m, H-4), 3.55 (1H, m, H-4), 3.49 (1H, m, H-6'), 2.46 (2H, td, *J* = 2.6, 7.0 Hz, H-3), 1.95 (1H, t, *J* = 2.6 Hz, H-1), 1.81 (1H, m, H-5'), 1.69 (1H, tt, H-3'), 1.58 (1H, m, H-3') 1.56 (2H, m, H-4'), 1.51 (1H, m, H-5'); ^{13}C NMR

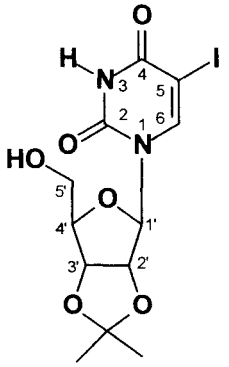
(100 MHz, $CDCl_3$): δ 98.7 (C-2'), 81.3 (C-2), 69.0 (C-1), 65.4 (C-4), 62.1 (C-6'), 30.4 (C-3'), 25.3 (C-4'), 19.8 (C-3), 19.3 (C-5').

5-Iodouridine (110)



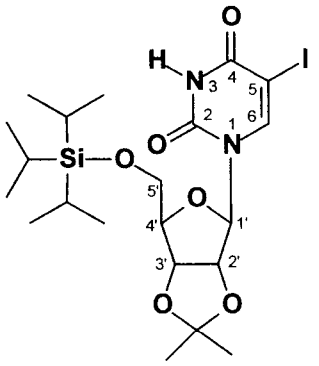
A suspension of uridine (10.00 g, 41.0 mmol) in methanol (200 mL) was sequentially treated with silver nitrate (6.97 g, 41.0 mmol) and iodine (10.41 g, 41.0 mmol) at 0°C, under nitrogen. The mixture was stirred for 30 minutes. A pale yellow precipitate formed which was filtered through a pad of Celite and washed with large volumes of methanol. The filtrate was concentrated *in vacuo* and methylene chloride added to induce crystallization. The product was recrystallized from methanol to afford 5-iodouridine as colourless crystals (12.63 g, 83%). $[\alpha]_D^{20}$ -28.5° (*c* 1.00, MeOH); mp: 205-206 °C (dec) (lit.[114,115] 207-209 °C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.63 (1H, s, NH), 8.45 (1H, s, H-6), 5.71 (1H, d, *J* = 5.0 Hz, H-1'), 5.35 (1H, d, *J* = 5.0, 2'-OH, exchanges with D_2O), 5.20 (1H, t, *J* = 4.4 Hz, 5'-OH, exchanges with D_2O), 5.01 (1H, d, *J* = 5.0 Hz, 3'-OH, exchanges with D_2O), 4.02 (1H, dd, *J* = 5.0, 9.4 Hz, H-2'), 3.97 (1H, dd, *J* = 5.0, 9.4 Hz, H-3'), 3.85 (1H, m, H-4'), 3.67 (1H, ddd, *J* = 2.8, 4.4, 12.0 Hz, H-5'), 3.56 (1H, ddd, *J* = 2.8, 4.4, 12.0 Hz, H-5'); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.1 (C-4), 151.0 (C-2), 145.8 (C-6), 89.0 (C-1'), 85.4 (C-4'), 74.6 (C-2'), 70.1 (C-3'), 69.9 (C-5), 60.9 (C-5'); Found: C, 29.92; H, 3.05; N, 8.00. $C_9H_{11}IN_2O_6$ requires C, 29.21; H, 3.00; N, 7.57.

2',3'-*O*-isopropylidene-5-iodouridine (142)



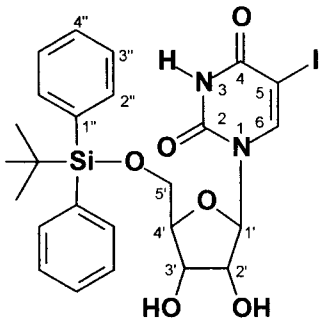
A mixture of 5-iodouridine **110** (1.00 g, 2.7 mmol) in acetone (40 mL), anhydrous copper sulphate (0.43 g, 2.7 mmol) and sulphuric acid (0.1 mL, 2.7 mmol) was stirred between 0-25 °C for 4 h. The reaction mixture was quenched with potassium hydroxide (0.30 g, 5.4 mmol). The precipitate formed was filtered through a pad of Celite and then washed with large volumes of methanol. The solvent was evaporated under reduced pressure to give a crude product (1.05 g, 95%) which was recrystallised from methanol to give white fluffy needles. $[\alpha]_D^{20}$ -45.7° (*c* 1.00, MeOH); mp: 216-218 °C (lit.[130] 225-227 °C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.71 (1H, brs, NH), 8.32 (1H, s, H-6), 5.82 (1H, d, *J* = 2.5 Hz, H-1'), 5.15 (1H, t, *J* = 5.1 Hz, 5'-OH), 4.91 (1H, dd, *J* = 2.5, 6.5 Hz, H-2'), 4.75 (1H, dd, *J* = 3.8, 6.5, H-3'), 4.09 (1H, q, *J* = 3.8 Hz, H-4'), 3.58 (2H, m, H-5'), 1.48 (3H, s, CH_3), 1.28 (3H, s, CH_3); ^{13}C NMR (75 MHz, DMSO): δ 160.5 (C-4), 150.0 (C-2), 146.0 (C-6), 112.8 ($O_2C(CH_3)_2$), 91.3 (C-1'), 86.8 (C-4'), 83.8 (C-2'), 80.2 (C-3'), 69.4 (C-5), 61.1 (C-5'), 26.9 ($O_2C(CH_3)_2$), 25.1 ($O_2C(CH_3)_2$); Found: C, 35.52; H, 3.54; N, 6.87. $C_{12}H_{15}IN_2O_6$ requires C, 35.14; H, 3.69; N, 6.83.

2',3'-*O*-(Isopropylidene)-5'-*O*-triisopropylsilyl-5-iodouridine (143a)



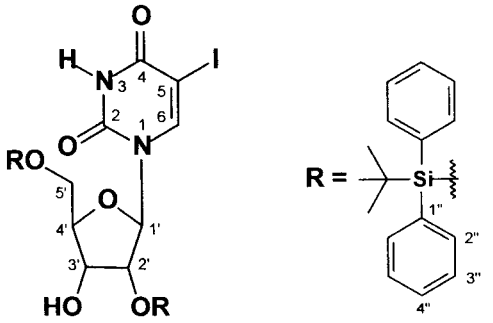
Nucleoside **142** (0.50 g, 1.2 mmol) was dissolved in DMF (5 mL) and imidazole (0.17 g, 2.4 mmol) was added with stirring. Triisopropylsilyl chloride (0.4 mL, 1.8 mmol) was added dropwise and the mixture stirred at room temperature for 24 h. The work-up involved adding EtOAc (20 mL) to the contents of the flask and washing with water (3 × 10 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The residue was purified by column chromatography using 20% EtOAc in petroleum ether to afford the title compound **143a** as a colourless oil (0.53 g, 77%). $[\alpha]_D^{20}$ -44.4° (*c* 1.10, $CHCl_3$); IR ($CHCl_3$): ν_{max} 3380 (w) (NH), 1718 (s) (CO), 1693 (s) (CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.51 (1H, brs, NH), 7.89 (1H, s, H-6), 5.90 (1H, d, *J* = 3.2, H-1'), 4.85 (1H, dd, *J* = 2.8, 6.4 Hz, H-3'), 4.70 (1H, dd, *J* = 3.2, 6.4 Hz, H-2'), 4.30 (1H, m, H-4'), 4.01 (1H, dd, *J* = 3.2, 11.6 Hz, H-5'), 3.89 (1H, dd, *J* = 2.6, 11.6 Hz, H-5'), 1.59 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.18 (3H, m, 3 × CH), 1.09 (18H, d, *J* = 6.0 Hz, 6 × CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.6 (C-4), 149.6 (C-2), 144.6 (C-6), 114.5 ($O_2C(CH_3)_2$), 92.1 (C-1') 86.5 (C-4'), 85.0 (C-2'), 80.1 (C-3'), 68.4 (C-5), 63.3 (C-5'), 27.2 ($O_2C(CH_3)_2$), 25.3 ($O_2C(CH_3)_2$), 18.0 (3 × $CH(CH_3)_2$), 12.0 (3 × $CH(CH_3)_2$).

5'-*O*-(*tert*-Butyldiphenylsilyl)-5-iodouridine (144)



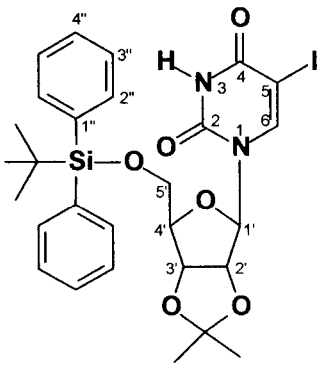
5-iodouridine **110** (2.21 g, 6.0 mmol) was dissolved in dry DMF (20 mL) and imidazole (0.81 g, 12.0 mmol) added. *tert*-Butyldiphenylsilyl chloride (2.3 mL, 9.0 mmol) was added dropwise under nitrogen and the mixture stirred at room temperature for 12 h. The mixture was diluted with $NaHCO_3$ and extracted with EtOAc (3 × 50 mL). The organic layer was washed with H_2O (3 × 100 mL), dried over $MgSO_4$ and the solvent evaporated under reduced pressure. Purification by column chromatography employing petroleum ether / ethyl acetate (1 / 1) yielded **144** as a white solid (1.79 g, 54%). $[\alpha]_D^{20}$ -8.6° (*c* 1.00, MeOH); mp 194-195 °C (from MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, CD_3OD): δ 8.03 (1H, s, H-6), 7.71 (4H, m, ArH), 7.43 (2H, m, ArH), 7.41 (4H, m, ArH), 5.93 (1H, d, *J* = 4.8 Hz, H-1'), 4.23 (2H, m, H-2', H-3'), 4.05 (1H, m, H-4'), 3.98 (1H, dd, *J* = 2.1, 11.7 Hz, H-5'), 3.83 (1H, dd, *J* = 3.3, 11.7 Hz, H-5'), 1.12 (9H, s, $C(CH_3)_3$); ^{13}C NMR (100 MHz, CD_3OD): δ 161.4 (C-4), 150.4 (C-2), 144.8 (C-6), 135.7 (C-2", C-6"), 135.5 (C-2", C-6"), 133.0 (C-1"), 132.7 (C-1"), 130.0 (C-4"), 129.9 (C-4"), 127.9 (C-3", C-5"), 127.8 (C-3", C-5"), 88.9 (C-1'), 85.5 (C-4'), 74.5 (C-2'), 70.4 (C-3'), 68.3 (C-5), 63.9 (C-5'), 26.6 ($C(CH_3)_3$), 19.1 ($C(CH_3)_3$); FABHRMS: *m/z* found 609.09286 (M^+ + H). $C_{25}H_{29}IN_2O_6Si$ (M^+ + H) requires 609.09178.

2',5'-*O*-*Bis*-(*tert*-Butyldiphenylsilyl)-5-iodouridine (145)



Obtained from the **144** procedure as a white solid (1.51 g, 30%). $[\alpha]_D^{20}$ +25.6° (*c* 1.98, $CHCl_3$); mp: 162-164 °C (from $MeOH/CH_2Cl_2$); IR ($CHCl_3$): ν_{max} 3383 (w) (NH), 1722 (s) (CO), 1694 (s) (CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.21 (1H, brs, NH), 7.60 - 7.26 (20H, m, ArH), 7.37 (1H, s, H-6), 6.12 (1H, d, *J* = 6.9 Hz, H-1'), 4.39 (2H, m, H-2', H-3'), 4.18 (1H, brs, H-4'), 3.86 (1H, dd, *J* = 1.8, 11.7 Hz, H-5'), 3.72 (1H, dd, *J* = 2.4, 11.7 Hz, H-5'), 3.15 (1H, d, *J* = 1.2 Hz, OH), 1.12 (9H, s, $C(CH_3)_3$), 0.94 (9H, s, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.2 (C-4), 149.6 (C-2), 143.2 (C-6), 135.6, 135.6, 135.4, 135.3 (C-2", C-6"), 132.3, 132.0, 131.9, 131.5 (C-1"), 130.7, 130.5, 130.2, 130.0 (C-4"), 128.3, 128.1, 128.0, 128.0 (C-3", C-5"), 86.9 (C-1'), 85.4 (C-4'), 75.2 (C-2'), 71.7 (C-3'), 69.8 (C-5), 64.3 (C-5'), 27.2 ($C(CH_3)_3$), 26.9 ($C(CH_3)_3$), 19.2 ($C(CH_3)_3$), 19.0 ($C(CH_3)_3$); FABHRMS: *m/z* found 847.20800 (M^+ + H). $C_{41}H_{47}IN_2O_6Si_2$ (M^+ + H) requires 847.20955.

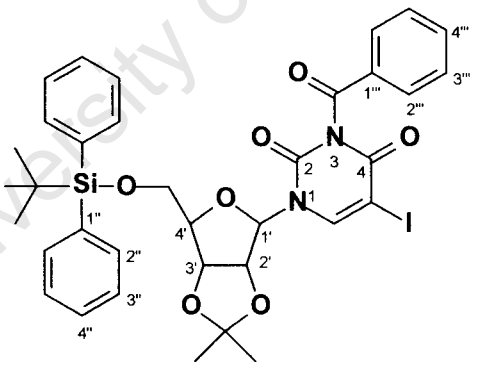
2',3'-Isopropylidene-5'-O-(*tert*-butyldiphenylsilyl)-5-iodouridine (143b)



To a solution of the nucleoside **144** (3.53 g, 5.8 mmol) in dry CH_2Cl_2 (40 mL) was added 2,2'-dimethoxypropane (20 mL) and a catalytic amount of *p*-toluenesulphonic acid monohydrate. The mixture was stirred at room temperature for 24 h. The solvent was concentrated *in vacuo* and the compound subjected to column chromatography

using ethyl acetate / petroleum ether (4 / 6) to give a colourless solid (2.84 g, 76%). $[\alpha]_D^{20}$ -11.2° (*c* 1.00, $CHCl_3$); mp: 217-218 °C (from MeOH/$CHCl_3$); IR ($CHCl_3$): ν_{max} 3380 (w) (NH), 1718 (s) (CO), 1694 (s) (CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.80 (1H, brs, NH), 7.89 (1H, s, H-6), 7.65 (4H, m, 2 × H-2", 2 × H-6"), 7.43 (4H, m, 2 × H-3", 2 × H-5"), 7.38 (2H, m, 2 × H-4"), 5.82 (1H, d, *J* = 3.3 Hz, H-1'), 4.77 (1H, dd, *J* = 3.3, 6.3 Hz, H-2'), 4.70 (1H, dd, *J* = 3.0, 6.3 Hz, H-3'), 4.29 (1H, m, H-4'), 3.98 (1H, dd, *J* = 3.0, 11.7 Hz, H-5'), 3.82 (1H, dd, *J* = 4.2, 11.7 Hz, H-5'), 1.57 (3H, s, $O_2C(CH_3)_2$), 1.33 (3H, s, $O_2C(CH_3)_2$), 1.09 (9H, s, $C(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.8 (C-4), 149.6 (C-2), 144.9 (C-6), 135.6 (C-2", C-6"), 135.5 (C-2", C-6"), 132.7 (C-1"), 132.6 (C-1"), 130.1 (C-4"), 130.0 (C-4"), 127.9 (C-3", C-5"), 127.9 (C-3", C-5"), 114.4 ($O_2C(CH_3)_2$), 92.8 (C-1'), 86.5 (C-4'), 84.7 (C-2'), 80.4 (C-3'), 68.5 (C-5), 64.0 (C-5'), 27.2 ($O_2C(CH_3)_2$), 27.1 ($C(CH_3)_3$), 25.3 ($O_2C(CH_3)_2$), 19.3 ($C(CH_3)_3$); Found C, 52.10; H, 5.15; N, 4.34. $C_{28}H_{33}IN_2O_6Si$ requires C, 51.85; H, 5.13; N, 4.32.

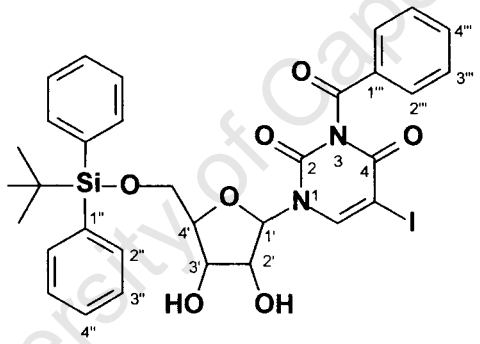
N-Benzoyl-2',3'-isopropylidene-5'-*O*-(*tert*-butyldiphenylsilyl)-5-iodouridine (162)



NaH (60% in mineral oil, 0.15 g, 6.1 mmol) was added to a solution of **143b** (1.97 g, 3.0 mmol) dissolved in freshly distilled 1,2-dimethoxyethane (DME) (30 mL) at 0 °C. The mixture was stirred at room temperature for 30 min and cooled to -78 °C for another 30 min. Benzoyl chloride (0.4 mL, 3.0 mmol) was added dropwise and the mixture allowed to warm to room temperature over 1 h. The solvent was removed under reduced pressure and the residual mass dissolved in ethyl acetate (50 mL). The organic layer was washed with aq. NH_4Cl (40 mL), water (2 x 40 mL), dried with $MgSO_4$ and filtered. After removal of the solvent under reduced pressure, the residue was purified by column chromatography using petroleum ether / EtOAc (7 / 3) to

afford the title compound **162** as a colourless solid (1.50 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (1H, s, H-6), 7.88 (2H, d, *J* = 7.6 Hz, H-2''', H-6'''), 7.68 (4H, m, 2 × H-2'', 2 × H-6''), 7.62 (1H, t, *J* = 7.6 Hz, H-4'''), 7.43 (8H, m, 2 × H-3'', 2 × H-4'', 2 × H-5'', H-3''', H-5'''), 5.83 (1H, d, *J* = 2.8 Hz, H-1'), 4.86 (1H, d, *J* = 2.8, 6.3 Hz, H-2'), 4.68 (1H, d, *J* = 3.3, 6.3 Hz, H-3'), 4.33 (1H, m, H-4'), 3.98 (1H, d, *J* = 3.0, 11.7 Hz, H-5'), 3.82 (1H, d, *J* = 4.4, 11.7 Hz, H-5'), 1.53 (3H, s, $O_2C(CH_3)_2$), 1.30 (3H, s, $O_2C(CH_3)_2$), 1.12 (9H, s, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.4 (ArCO), 158.8 (C-4), 148.9 (C-2), 144.9 (C-6), 135.6 (C-2'', C-6''), 135.5 (C-2'', C-6''), 135.1 (C-4''') 132.6 (C-4''), 132.6 (C-4''), 130.5 (C-2''', 6'''), 130.1 (C-1''), 130.1 (C-1''), 129.1 (C-1'''), 127.9 (C-3'', C-5''), 127.9 (C-3'', C-5''), 127.9 (C-3''', C-5'''), 114.2 ($O_2C(CH_3)_2$), 93.8 (C-1'), 87.0 (C-4'), 84.9 (C-3'), 80.7 (C-2'), 68.1 (C-5), 64.1 (C-5'), 27.1 ($O_2C(CH_3)_2$), 27.1 ($C(CH_3)_3$), 25.2 ($O_2C(CH_3)_2$), 19.2 ($C(CH_3)_3$).

N-Benzoyl-5'-*O*-(*tert*-butyldiphenylsilyl)-5-iodouridine (163)



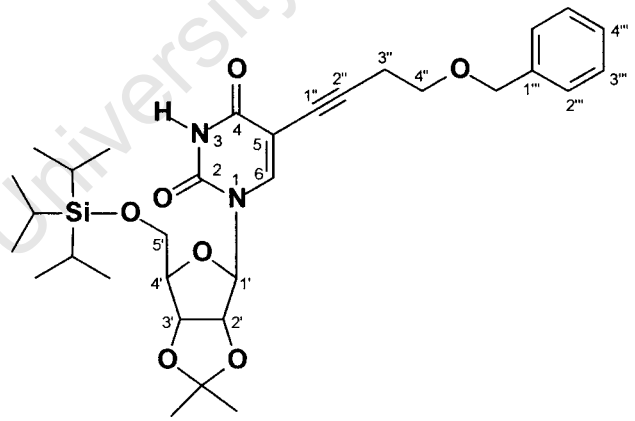
A saturated solution of HCl in ethyl acetate (40 mL) was added to the nucleoside **162** (5.01 g, 6.7 mmol). The mixture was stirred for 30 min and the reaction quenched with saturated Na_2CO_3. The organic layer was washed with water (3 x 20 mL), dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. Purification by column chromatography yielded a colourless solid (3.09 g, 82%). $[\alpha]_D^{20}$ +2.2° (*c* 0.94, $CHCl_3$); mp: 79-82 °C; IR ($CHCl_3$): ν_{max} 3534 (OH), 1754 (m) (CO), 1701 (m) (CO), 1663 (s) (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 (1H, s, H-6), 7.90 (2H, d, *J* = 7.5 Hz, H-2''', H-6'''), 7.65 (5H, m, 2 × H-2'', 2 × H-6'', H-4'''), 7.46 (8H, m, 2 × H-3'', 2 × H-4'', 2 × H-5'', H-3''', H-5'''), 5.84 (1H, d, *J* = 5.3 Hz, H-1'), 4.34 (1H, t, *J* = 5.3 Hz, H-2'), 4.28 (1H, m, H-3'), 4.19 (1H, d, *J* = 2.4 Hz, H-4'), 3.97 (1H, dd, *J* = 2.0, 12.0 Hz, H-5'), 3.79 (1H, dd, *J* = 2.8, 12.0 Hz, H-5'), 1.12 (9H, s, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.5 (C_6H_5OCN), 158.8 (C-4), 150.2 (C-2), 143.9 (C-6), 135.7 (C-2'', C-6''), 135.6 (C-2'', C-6''), 135.5 (C-4'''), 133.7 (C-4''), 133.7 (C-4''), 132.5 (C-1''), 132.3

(C-1''), 130.9 (C-1'''), 130.2 (C-2''', C-6'''), 128.5 (C-3''', C-5'''), 128.1 (C-3'', C-5''), 128.1 (C-3'', C-5''), 91.3 (C-1'), 86.6 (C-4'), 76.1 (C-3'), 71.3 (C-2'), 68.5 (C-5), 63.7 (C-5'), 27.2 ($C(CH_3)_3$), 19.4 ($C(CH_3)_3$); Found C, 53.90; H, 4.76; N, 3.79. $C_{32}H_{33}IN_2O_7Si$ requires C, 53.94; H, 4.67; N, 3.93.

General Procedure for Sonogashira Coupling (GP1)

Distilled triethylamine (2 equiv.), protected butyne (2 equiv.) and THF (5 mL) were added to a stirring solution of 5-iodouridine (1 equiv.) in dry DMF (2.5 mL). The mixture was thoroughly degassed with nitrogen for 1 h. CuI (0.5 equiv.) and $(PPh_3)_4$ Pd (0.1 equiv.) were added to the degassed solution under a nitrogen atmosphere. The mixture was left stirring at room temperature for 4 h. The solvent was removed under reduced pressure and the residue dissolved in $CHCl_3$ (50 mL), washed twice successively with 25 mL portions of 5% aq disodium EDTA, water and then dried over $MgSO_4$. Following removal of solvent, the residue was purified by column chromatography using EtOAc in petroleum ether.

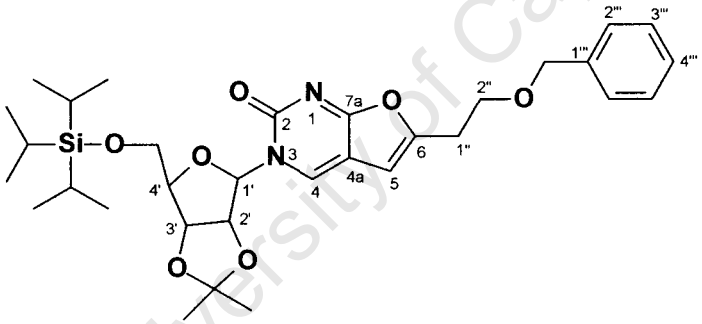
5-(4-Benzyloxybutyn-1-yl)-2',3'-*O*-(isopropylidene)-5'-*O*-triisopropyl silyluridine (149)



According to GP1: Nucleoside **143** (0.68 g, 1.2 mmol), 4-benzyloxybut-1-yne **146** (0.29 g, 1.8 mmol), CuI (0.05 g, 0.2 mmol), $(PPh_3)_4$ Pd (0.14 g, 0.12 mmol), 24 h. Purification by column chromatography using 20% EtOAc in petroleum ether gave **149** as a yellow oil (0.20 g, 36%) and a cyclic by-product **150** as a colourless oil (0.077 g, 14%).

149: IR ($CHCl_3$): ν_{max} 3691 (w), 3384 (w) (NH), 3029 (w), 2946 (w), 2867 (w), 1718 (s) (CO), 1602 (m) (C=C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.68 (1H, s, H-6), 7.30 (5H, m, ArH), 5.92 (1H, d, *J* = 3.2 Hz, H-1'), 4.82 (1H, dd, *J* = 3.3, 6.2 Hz, H-3'), 4.68 (1H, dd, *J* = 3.2, 6.2 Hz, H-2'), 4.53 (2H, s, $ArCH_2O$), 4.27 (1H, m, H-4'), 3.97 (1H, dd, *J* = 2.4, 11.4 Hz, H-5'), 3.87 (1H, dd, *J* = 3.0, 11.4 Hz, H-5'), 3.62 (2H, t, *J* = 7.4 Hz, H-4''), 2.68 (2H, t, *J* = 7.4 Hz, H-3''), 1.57 (3H, s, $O_2C(CH_3)_2$), 1.34 (3H, s, $O_2C(CH_3)_2$), 1.10 (3H, m, 3 × $CH(CH_3)_2$), 1.05 (18H, d, *J* = 6.4 Hz, 3 × $CH(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.8 (C-4), 149.3 (C-2), 142.1 (C-6), 138.0 (C-1'''), 128.3 (C-2''', 6'''), 127.7 (C-3''', 5'''), 127.6 (C-4'''), 114.3 ($OC(CH_3)_2$), 100.5 (C-2''), 91.8 (C-1'), 91.4 (C-1''), 86.4 (C-4'), 84.9 (C-2'), 80.1 (C-3'), 72.9 ($ArCH_2O$), 72.2 (C-5), 68.1 (C-4''), 63.3 (C-5'), 27.2 ($O_2C(CH_3)_2$), 25.3 ($O_2C(CH_3)_2$), 20.9 (C-3''), 17.9 ($CH(CH_3)_2$), 11.8 ($CH(CH_3)_2$).

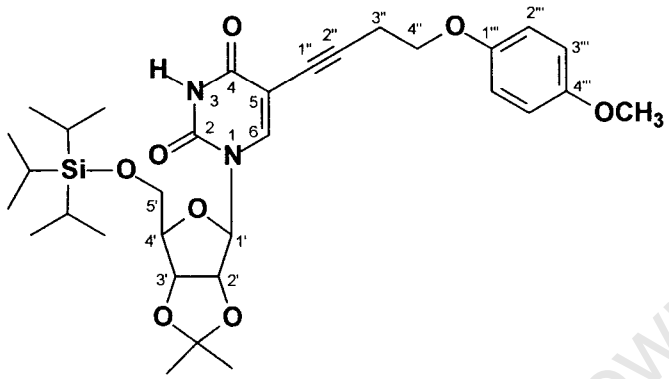
6-(2-Benzyloxylethyl)-3-(2',3'-isopropylidene-5'-*O*-triisopropylsilyl tetrahydrofuran-2-yl)-3*H*-furo [2,3-*d*] pyrimidin-2-one (150)



150: $[\alpha]_D^{20}$ -0.6 ° (*c* 2.8, $CHCl_3$); IR ($CHCl_3$): ν_{max} 3691 (w), 3384 (w) (NH), 2945 (m), 2868 (m), 1716 (s) (CO), 1679 (s) (C=C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.34 (1H, s, H-4), 7.30 (5H, m, ArH), 6.13 (1H, d, *J* = 0.9 Hz, H-5), 6.10 (1H, d, *J* = 1.8 Hz, H-1'), 4.82 (1H, dd, *J* = 3.6, 6.2 Hz, H-3'), 4.76 (1H, dd, *J* = 1.8, 6.2 Hz, H-2'), 4.52 (2H, s, OCH_2Ar), 4.39 (1H, m, H-4'), 4.08 (1H, dd, *J* = 2.4, 11.7 Hz, H-5'), 3.90 (1H, dd, *J* = 3.6, 11.7 Hz, H-5'), 3.77 (2H, t, *J* = 6.5 Hz, H-2''), 2.94 (2H, td, *J* = 0.9, 6.5 Hz, H-1''), 1.59 (3H, s, $O_2C(CH_3)_2$), 1.35 (3H, s, $O_2C(CH_3)_2$), 1.08 (3H, m, $CH(CH_3)_2$), 1.05 (9H, d, *J* = 5.4 Hz, $CH(CH_3)_2$), 1.04 (9H, d, *J* = 6.0 Hz, $CH(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.9 (C-7a), 156.7 (C-6), 154.5 (C-2), 137.8 (C-1'''), 135.6 (C-4), 128.3 (C-2''', C-6'''), 127.7 (C-3''', C-5'''), 127.6 (C-4'''), 113.9 ($O_2C(CH_3)_2$), 107.3 (C-4a), 100.1 (C-5), 94.2 (C-1'), 88.1 (C-4'), 86.4 (C-2'), 79.3 (C-3'), 73.1 (OCH_2Ar), 66.7 (C-2''), 63.2 (C-5'), 29.2 (C-1''), 27.2 ($O_2C(CH_3)_2$), 25.3 ($O_2C(CH_3)_2$), 17.9

($CH(CH_3)_2$), 11.8 ($CH(CH_3)_2$); FABHRMS: *m/z* found 599.31533 (M^+ + Na). $C_{32}H_{46}N_2O_7Si$ (M^+ + Na) requires 599.31524.

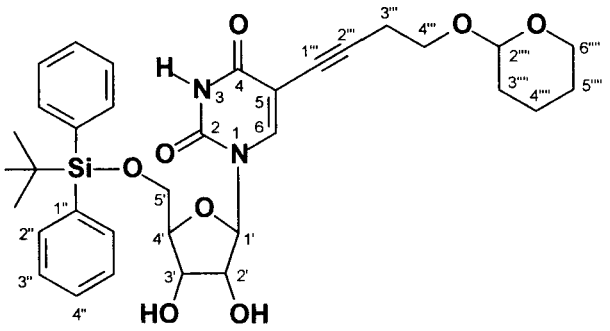
2',3'-Isopropylidene-5'-*O*-(triisopropylsilyl)-5-[4-(4-methoxy phenoxy)-butyn-1-yl]uridine (149b)



According to GP1: Nucleoside **143a** (0.26 g, 0.5 mmol), 4-(4-methoxyphenoxy)but-1-yne **147b** (0.02 g, 1.4 mmol), $(Ph_3P)_4Pd$ (0.054 g, 0.05 mmol), CuI (0.026 g, 0.14 mmol), Et_3N (0.13 mL, 0.9 mmol), DMF (1.3 mL), THF (2.0 mL), rt, 22 h. Purification by column chromatography employing petroleum ether / EtOAc (2 / 3) gave a pale yellow oil (0.14 g, 50%).

IR ($CHCl_3$): ν_{max} ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.16 (1H, brs, NH), 7.73 (1H, s, H-6), 6.84 (2H, d, *J* = 9.4 Hz, ArH), 6.82 (2H, d, *J* = 9.4 Hz, ArH), 5.94 (1H, d, *J* = 3.2 Hz, H-1'), 4.84 (1H, dd, *J* = 2.8, 6.4 Hz, H-3'), 4.70 (1H, dd, *J* = 3.2, 6.4 Hz, H-2'), 4.29 (1H, m, H-4'), 4.08 (2H, t, *J* = 7.3 Hz, H-4''), 4.00 (1H, dd, *J* = 2.2, 11.4 Hz, H-5'), 3.89 (1H, dd, *J* = 3.0, 11.4 Hz, H-5'), 3.75 (3H, s, OCH_3), 2.83 (2H, t, *J* = 7.3 Hz, H-3''), 1.58 (3H, s, $O_2C(CH_3)_2$), 1.35 (3H, s, $O_2C(CH_3)_2$), 1.16 (3H, m, 3 × $CH(CH_3)_2$), 1.07 (18H, d, *J* = 6.4 Hz, 3 × $CH(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.6 (C-4), 154.1 (C-4'''), 152.5 (C-1'''), 149.1 (C-2), 142.3 (C-6), 115.8 (C-3''', C-5'''), 114.6 (C-2''', C-6'''), 114.3 ($O_2C(CH_3)_2$), 100.3 (C-2''), 91.9 (C-1''), 90.8 (C-1'), 86.5 (C-4'), 85.0 (C-2'), 80.0 (C-3'), 72.5 (C-5), 66.6 (C-4''), 63.3 (C-5'), 55.7 (OCH_3), 27.2 ($O_2C(CH_3)_2$), 25.3 ($O_2C(CH_3)_2$), 20.6 (C-3''), 17.9 ($CH(CH_3)_2$), 11.8 ($CH(CH_3)_2$).

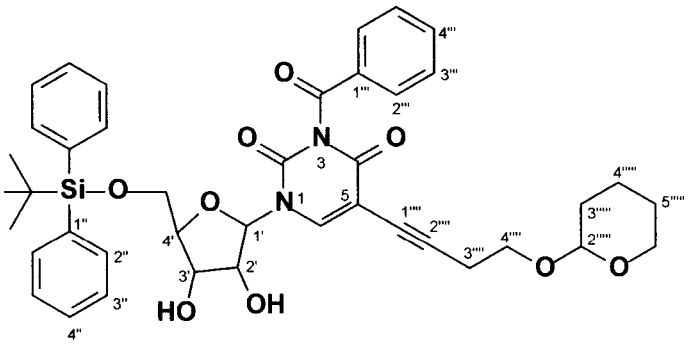
5-[4-(2-Tetrahydropyranyloxy)-but-1-ynyl]-5'-*O*-(*tert*-butyldiphenyl silyl)uridine (154)



According to GP1: Triethylamine (0.8 mL, 5.6 mmol), nucleoside **144** (1.70 g, 2.8 mmol), dry THF (30 mL), DMF (15 mL), 4-(2-tetrahydropyranyloxy)but-1-yne **148** (1.29 g, 8.4 mmol), $(Ph_3P)_4Pd$ (0.33 g, 0.3 mmol) and CuI (0.27 g, 1.4 mmol). The mixture was stirred for 3 hours and the product purified by column chromatography using petroleum ether / ethyl acetate (1 / 8) to give **154** as a yellow solid (1.73 g, 98%).

$[\alpha]_D^{20}$ +31.0° (*c* 1.09, $CHCl_3$); mp 165–167 °C (from $CHCl_3$); IR ($CHCl_3$): ν_{max} 3690 (w), 3386 (w) (NH), 3500 (br) (OH), 2947 (m), 2933 (m), 2860 (m), 2241 (w) (C≡C), 1694 (s) (CO), 1620 (w) (C=C) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 11.59 (1H, s, NH), 7.79 (1H, s, H-6), 7.62 (4H, m, ArH), 7.44 (6H, m, ArH), 5.80 (1H, d, *J* = 5.9 Hz, H-1'), 5.41 (1H, d, *J* = 5.1 Hz, 2'-OH), 5.10 (1H, d, *J* = 4.2 Hz, 3'-OH), 4.52 (1H, dd, *J* = 3.3, 3.6 Hz, H-2''''), 4.11 (1H, t, *J* = 5.1, 5.9 Hz, H-2'), 4.04 (1H, d, *J* = 4.2 Hz, H-3'), 3.92 (1H, m, H-4'), 3.84 (1H, dd, *J* = 2.9, 11.6 Hz, H-5'), 3.71 (2H, m, H-5', H-6''''), 3.52 (1H, m, H-4'''), 3.35 (1H, m, H-6''''), 3.32 (1H, m, H-4'''), 2.41 (2H, t, *J* = 6.9 Hz, H-3'''), 1.65 (2H, m, H-4''''), 1.56 (2H, m, H-3''''), 1.41 (2H, m, H-5''''), 1.02 (9H, s, $C(CH_3)_3$); ^{13}C NMR (75 MHz, DMSO-d_6): δ 161.4 (C-4), 149.6 (C-2), 142.1 (C-6), 135.1 (C-2'', C-6''), 134.9 (C-2'', C-6''), 132.4 (C-1''),132.2 (C-1''), 129.9 (C-4''), 129.8 (C-4'') 127.9 (C-3'', C-5''), 127.9 (C-3'', C-5''), 99.4 (C-2''''), 97.8 (C-2''''), 90.5 (C-1'''), 87.7 (C-1'), 84.4 (C-4'), 73.3 (C-2'), 72.9 (C-5), 69.7 (C-3'), 64.6 (C-4'''), 63.8 (C-5'), 61.3 (C-6''''), 30.0 (CH_2), 26.6 ($C(CH_3)_3$), 24.9 (CH_2), 20.2 (C-3'''), 18.9 ($C(CH_3)_3$), 18.7 (CH_2); Found C, 64.05; H, 6.04; N, 4.21. $C_{34}H_{42}N_2O_8Si$ requires C, 64.33; H, 6.67; N, 4.41.

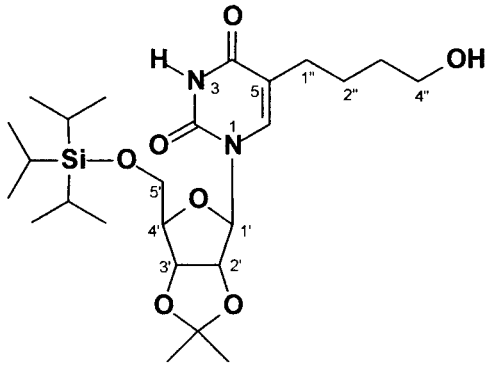
N-Benzoyl-5'-*O*-(*tert*-butyldiphenylsilyl)-5-[4-(2-tetrahydropyranyl oxy)butynyl]uridine (164)



According to GP1; Nucleoside **163** (1.12 g, 1.6 mmol), Et_3N (0.43 mL, 3.1 mmol), 4-(2-tetrahydropyranyloxy)but-1-yne **148** (0.48 g, 3.1 mmol), $(Ph_3P)_4Pd$ (0.18 g, 0.16 mmol) and CuI (0.15 g, 0.8 mmol), DMF (2 mL), THF (20 mL), rt, 2 h. Column chromatography employing EtOAc / pet ether (1 / 1) gave a colourless oil (0.89 g, 77%).

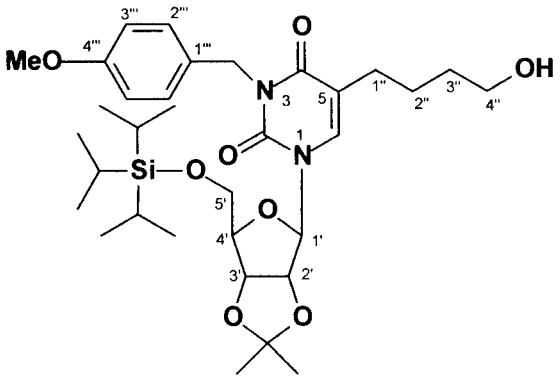
IR ($CHCl_3$): ν_{max} 3693 (w), 3531 (br) (OH), 3026 (w), 3011 (w), 2947 (m), 2860 (w), 2241 (w) (C≡C), 1755 (s) (CO), 1708 (s) (CO), 1669 (s) (CO); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02 (1H, s, H-6), 7.89 (2H, m, H-2'''/6'''), 7.65 (5H, m, 2 × H-2"/6", H-4'''), 7.44 (8H, m, 2 × H-3"/5", 2 × H-4", 2 × H-3'''/5'''), 5.82 (1H, d, *J* = 5.2 Hz, H-1'), 4.56 (1H, m, H-2''''), 4.30 (1H, t, *J* = 5.2 Hz, H-2'), 4.25 (1H, t, *J* = 5.2 Hz, H-3'), 4.15 (1H, m, H-4'), 4.03 (1H, brs, OH), 3.95 (1H, dd, *J* = 2.4, 11.6 Hz, H-5'), 3.81 (1H, m, H-6''''), 3.73 (2H, m, H-5', H-4''''), 3.45 (2H, m, H-4'''', H-6''''), 3.17 (1H, brs, OH), 2.52 (2H, t, H-3''''), 1.78 (1H, m, CH_2), 1.67 (1H, m, CH_2), 1.51 (4H, m, CH_2), 1.09 (9H, s, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.0 (C_6H_5OCN), 160.9 (C-4), 149.6 (C-2), 141.3 (C-6), 135.8 (C-2", C-6"), 135.7 (C-2", C-6"), 135.5 (C-4'''), 132.6 (C-1"), 132.5 (C-1"), 131.4 (C-1'''), 130.8 (C-2''', C-6'''), 130.4 (C-4"), 130.3 (C-4"), 129.4 (C-3''', C-5'''), 128.2 (C-3", C-5"), 128.2 (C-3", C-5"), 101.2 (C-2''''), 99.1 (C-2''''), 93.0 (C-1''''), 91.4 (C-1'), 86.6 (C-4'), 76.3 (C-2'), 71.7 (C-5), 71.4 (C-3'), 65.5 (C-6''''), 63.8 (C-5'), 62.5 (C-4''''), 30.7 (CH_2), 27.2 ($C(CH_3)_3$), 25.6 (CH_2), 21.2 (C-3''''), 19.7 (CH_2), 19.4 ($C(CH_3)_3$); FABHRMS: *m/z* found 761.28827 (M^+ + Na). $C_{41}H_{46}N_2O_9Si$ (M^+ + Na) requires 761.28701.

5-(4-Hydroxybutyl)-2',3'-(isopropylidene)-5'-*O*-(triisopropylsilyl) uridine (155)



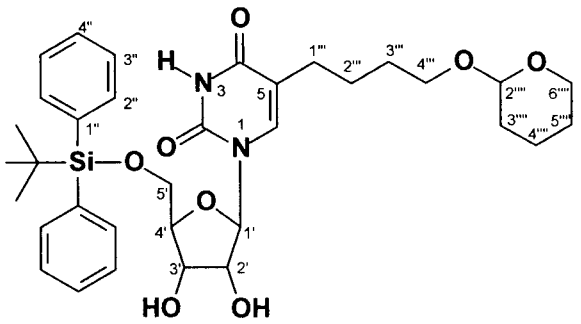
A solution of the nucleoside (0.20 g, 0.3 mmol) in ethanol was hydrogenated over a palladium-on-carbon catalyst (10 mol%) at atmospheric pressure and temperature for 2 days. After removal of the catalyst by filtration, the solvent was evaporated under reduced pressure and the crude product purified by column chromatography employing EtOAc / pet ether (1 / 1) to give a colourless oil (0.12 g, 71%). $[\alpha]_D^{20}$ -15.3 ° (*c* 1.30, $CHCl_3$); IR ($CHCl_3$): ν_{max} 3690 (w), 3390 (m) (NH), 3192 (br) (OH), 2945 (s) (CO), 1688 (s) (CO), 1603 (w) (C=C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 9.51 (1H, brs, NH), 7.18 (1H, s, H-6), 5.84 (1H, d, *J* = 2.8 Hz, H-1'), 4.76 (1H, dd, *J* = 2.8, 6.3 Hz, H-2'), 4.81 (1H, dd, *J* = 3.4, 6.3 Hz, H-3'), 4.18 (1H, m, H-4'), 3.93 (1H, dd, *J* = 2.8, 11.1 Hz, H-5'), 3.86 (1H, dd, *J* = 4.2, 11.1 Hz, H-5'), 3.62 (2H, s, H-4''), 2.30 (2H, s, H-1''), 2.28 (2H, s, H-3''), 1.55 (3H, s, $O_2C(CH_3)_2$), 1.33 (3H, s, $O_2C(CH_3)_2$), 1.10 (3H, m, 3 × $CH(CH_3)_2$), 1.05 (20H, m, 3 × $CH(CH_3)_2$, H-2''); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.8 (C-4), 150.2 (C-2), 136.9 (C-6), 115.2 (C-5), 114.5 ($O_2C(CH_3)_2$), 91.9 (C-1'), 86.3 (C-4'), 84.2 (C-2'), 80.1 (C-3'), 63.4 (C-5'), 62.2 (C-4''), 32.1 (C-1''), 27.2 ($O_2C(CH_3)_2$), 26.7 (C-2''), 25.4 ($O_2C(CH_3)_2$), 25.2 (C-3''), 17.9 ($CH(CH_3)_2$), 11.9 ($CH(CH_3)_2$); FABHRMS: *m/z* found 513.30041 (M^+ + H). $C_{25}H_{44}N_2O_7Si$ (M^+ + H) requires 513.29959.

5-[4-(4-Methoxybenzyl)-butyl]-2',3'-(isopropylidene)-5'-*O*-(tri isopropylsilyl)uridine (156)



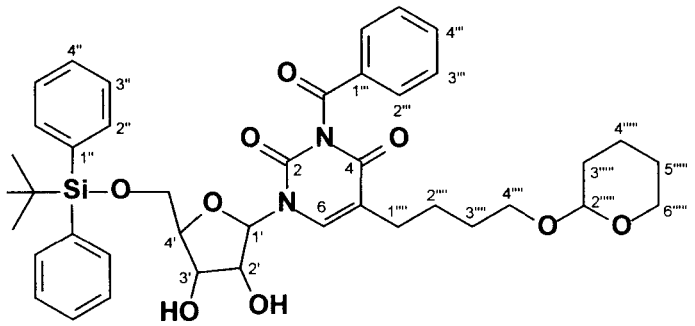
To a stirred suspension of NaH (60% in mineral oil, 0.03 g, 1.0 mmol) in DMSO (1.0 mL) was added dropwise a THF solution (4 mL) of the nucleoside **155** (0.35 g, 0.7 mmol) under N_2. After 45 min at room temperature, *p*-methoxybenzyl chloride (0.16 g, 1.0 mmol) was added and stirring continued for 20 hours at rt. The mixture was poured into saturated NH_4Cl (10 mL) (ice-mixture) and extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with water (2 × 10 mL), dried over $MgSO_4$ and the solvent evaporated under reduced pressure. The product was purified by column chromatography using pet ether / ethyl acetate (1 / 5) to give the title compound **156** as a colourless oil (0.32 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41 (2H, d, *J* = 8.6 Hz, H-2''', H-6'''), 7.18 (1H, s, H-6), 6.78 (2H, d, *J* = 8.6 Hz, H-3''', H-5'''), 5.82 (1H, d, *J* = 2.8 Hz, H-1'), 5.04 (1H, d, *J* = 13.5 Hz, ArCH_2O), 4.96 (1H, d, *J* = 13.5 Hz, ArCH_2O), 4.82 (1H, dd, *J* = 3.4, 6.3 Hz, H-3'), 4.75 (1H, dd, *J* = 2.8, 6.3 Hz, H-2'), 4.20 (1H, m, H-4'), 3.93 (1H, dd, *J* = 2.8, 11.1 Hz, H-5'), 3.84 (1H, dd, *J* = 4.2, 11.1 Hz, H-5'), 3.73 (3H, s, OCH_3), 3.61 (2H, t, *J* = 6.2 Hz, H-4''), 2.28 (3H, m, H-1'', OH), 1.55 (7H, m, H-2'', H-3'', $O_2C(CH_3)_2$), 1.33 (3H, s, $O_2C(CH_3)_2$), 1.04 (21H, m, 3 × C*H*$(CH_3)_2$, 3 × CH(CH_3)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.0 (C-4), 158.9 (C-4'''), 150.6 (C-2), 134.8 (C-6), 130.7 (C-2''', C-6'''), 128.9 (C-1'''), 114.2 ($O_2C(CH_3)_2$), 114.2 (C-5), 113.5 (C-3''', C-5'''), 93.0 (C-1'), 86.2 (C-4'), 84.6 (C-2'), 80.2 (C-3'), 63.3 (C-5'), 62.1 (C-4''), 55.0 (OCH_3), 43.8 (ArCH_2O), 32.1 (C-3''), 27.4 (C-1''), 27.1 ($O_2C(CH_3)_2$), 25.3 ($O_2C(CH_3)_2$), 25.1 (C-2''), 17.8 (3 × CH$(CH_3)_2$), 17.8 (3 × CH$(CH_3)_2$), 11.8 (3 × C*H*$(CH_3)_2$).

5-[4-(2-Tetrahydropyranyloxy)-butyl]-5'-*O*-(*tert*-butyldiphenylsilyl) uridine (160)



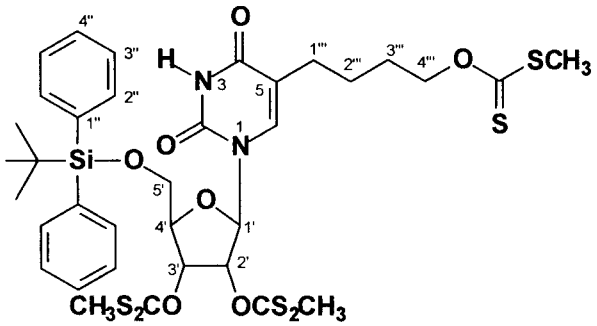
Hydrogen gas was added at atmospheric pressure to a suspension of the nucleoside **154** (0.58 g, 0.9 mmol), 10% palladium-on-carbon (10 mol %) and a catalytic amount of Et_3N (0.5 mL) in ethanol (5 mL). The reaction mixture was stirred at room temperature for 20 h. The residue was filtered over a pad of Celite, washed with large volumes of MeOH and the filtrate evaporated *in vacuo*. The crude product was purified by column chromatography employing 80% EtOAc in petroleum ether to give **160** as a colourless oil (0.49 g, 84%). $[\alpha]_D^{20}$ +18° (*c* 1.00, $CHCl_3$); IR ($CHCl_3$): ν_{max} 3690 (w), 3391 (m) (NH), 3210-3500 (br) (OH), 2932 (s), 2860 (m), 1683 (s) (CO), 1465 (m) cm^{-1}; ^1H NMR (300 MHz, CD_3OD): δ 7.70 (4H, m, 2 × H-2", 2 × H-6"), 7.42 (6H, m, 2 × H-3", 2 × H-4", 2 × H-5"), 7.39 (1H, s, H-6), 6.00 (1H, d, *J* = 5.4 Hz, H-1'), 4.49 (1H, t, *J* = 3.3 Hz, H-2""), 4.31 (2H, m, H-2', H-3'), 4.06 (2H, m, H-4', H-5'), 3.91 (1H, dd, *J* = 2.5, 11.2 Hz, H-5'), 3.80 (1H, m, H-4'"), 3.53 (1H, m, H-6""), 3.46 (1H, m, H-6""), 3.19 (1H, m, H-4'"), 1.92 (1H, m, H-1'"), 1.64 (1H, m, H-1'"), 1.81 (2H, m, CH_2), 1.50 (4H, m, 2 × CH_2), 1.32 (4H, m, 2 × CH_2), 1.10 (9H, s, $C(CH_3)_3$); ^{13}C NMR (100 MHz, CD_3OD): δ 164.5 (C-4), 151.4 (C-2), 136.3 (C-6), 135.5 (C-2", C-6"), 135.3 (C-2", C-6"), 133.6 (C-1"), 132.7 (C-1"), 130.1 (C-4"), 130.0 (C-4"), 127.9 (C-3", C-5"), 127.9 (C-3", C-5"), 115.1 (C-5), 99.0 (C-2""), 88.4 (C-1'), 85.1 (C-4'), 74.2 (C-3'), 70.4 (C-2'), 67.2 (C-6""), 64.1 (C-5'), 62.2 (C-4'"), 30.7 (C-3'"), 29.3 (C-5""), 26.5 ($C(CH_3)_3$), 25.7 (C-1'"), 25.4 (C-2'"), 19.5 (CH_2), 19.2 ($C(CH_3)_3$); FABHRMS: *m/z* found 661.29314 (M^+ + Na). $C_{34}H_{46}N_2O_8Si$ (M^+ + Na) requires 661.29212.

N-Benzoyl-5'-*O*-(*tert*-butyldiphenylsilyl)-5-[4-(2-tetrahydropyranyl oxy)butyl]uridine (165)



Nucleoside **164** (0.81 g, 1.1 mmol) was hydrogenated over palladium-on-carbon catalyst (0.06 g, 0.6 mmol) in EtOH (5 mL) to give **165** as a colourless oil (0.67 g, 83%). IR ($CHCl_3$): ν_{max} 3694 (w), 3594 (w), 3028 (w), 3010 (w), 2945 (m), 2861 (w), 1752 (s) (CO), 1706 (CO), 1666 (s) (CO); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (2H, dd, *J* = 1.6, 8.4 Hz, H-2'''), 7.65 (5H, m, ArH), 7.53 (1H, s, H-6), 7.42 (8H, m, ArH), 5.85 (1H, d, *J* = 6.0 Hz, H-1'), 4.50 (1H, t, *J* = 3.6, 5.2 Hz, H-2''''), 4.30 (2H, m, H-2', H-3'), 4.15 (1H, m, H-4'), 4.00 (1H, dd, *J* = 2.4, 11.7 Hz, H-5'), 3.83 (1H, dd, *J* = 3.0, 11.7 Hz, H-5'), 3.78 (1H, m, OCH_2), 3.62 (1H, m, OCH_2), 3.46 (1H, m, OCH_2), 3.26 (1H, m, OCH_2), 2.17 (1H, m, H-1''''), 2.04 (1H, m, H-1''''), 1.78 (1H, m, CH_2), 1.66 (1H, m, CH_2), 1.46 (8H, m, 4 × CH_2), 1.08 (9H, s, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.7 (C_6H_5OCN), 162.3 (C-4), 150.2 (C-2), 135.5 (C-2'', C-6''), 135.3 (C-2'', C-6''), 135.1 (C-4'''), 132.8 (C-1'''), 132.3 (C-1''), 131.5 (C-1''), 130.4 (C-4''), 130.1 (C-4''), 130.1 (C-2''', C-6'''), 129.9 (C-3''', C-5'''), 129.2 (C-6), 128.0 (C-3'', C-5''), 127.9 (C-3'', C-5''), 115.4 (C-5), 98.8 (C-2''''), 90.5 (C-1'), 85.8 (C-4'), 75.5 (C-3'), 70.9 (C-2'), 67.2 (C-6''''), 63.6 (C-5'), 62.3 (C-4''''), 30.7 (C-3''''), 29.4 (C-5''''), 27.1 (C-1''''), 27.0 ($C(CH_3)_3$), 25.4 (C-2''''), 22.7 (CH_2), 19.6 (CH_2), 19.3 ($C(CH_3)_3$).

5-[4-(*S*-Methyldithiocarbonyloxy)butyl]-5'-*O*-(*tert*-butyldiphenyl silyl)-2',3'-*bis*-(*S*-methyldithiocarbonyloxy)uridine (161b)

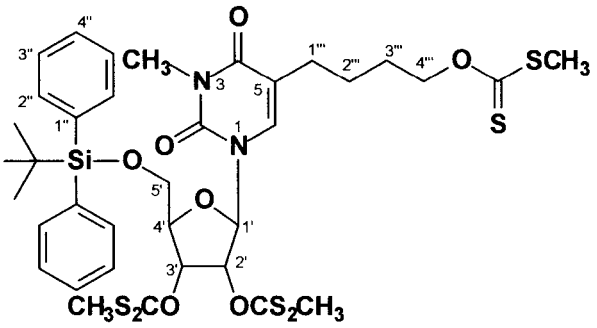


To a solution of the nucleoside **160** (0.92 g, 1.4 mmols) in dry THF under nitrogen were added a catalytic amount of imidazole (0.02 g) and NaH (60% in mineral oil, 0.13 g, 5.4 mmol) at 0 °C. After 10 minutes, CS_2 (0.6 mL, 10.1 mmols) was added dropwise. Stirring was continued for an additional 30 min and iodomethane (0.5 mL, 8.1 mmols) was added. The mixture was stirred at room temperature overnight. The suspension was hydrolysed with H_2O (40 mL) and extracted with EtOAc (3 × 40 mL). The combined organic extracts were washed with saturated aqueous NH_4Cl solution (100 mL) and brine (100 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The product was purified by column chromatography using pet ether / EtOAc (1 / 8) to give **161b** as a colourless oil (0.08 g, 7%), along with three by-products, also all as colourless oils.

161b: $[\alpha]_D^{20}$ -9.3° (*c* 1.00, $CHCl_3$); IR ($CHCl_3$): ν_{max} 3691 (w), 3388 (w) (NH), 2930 (m), 2859 (w), 1716 (s) (CO), 1692 (s) (CO), 1602 (w), 1463 (m), 1114 (s) (C=S), 1095 (s) (C=S), 1076 (s) (C=S), 621 (C-S) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.66 (1H, brs, NH), 7.69 (4H, m, 2 × H-2"/H-6"), 7.42 (6H, m, 2 × H-3"/H-5", 2 × H-4"), 7.37 (1H, s, H-6), 6.52 (1H, d, *J* = 7.6 Hz, H-1'), 6.47 (1H, dd, *J* = 2.0, 5.8 Hz, H-3'), 6.25 (1H, dd, *J* = 5.8, 7.6 Hz, H-2'), 4.43 (2H, t, *J* = 6.5 Hz, H-4'''), 4.41 (1H, m, H-4'), 4.13 (1H, d, *J* = 11.3 Hz, H-5'), 4.03 (1H, dd, *J* = 1.9, 11.3 Hz, H-5'), 2.60 (3H, s, SCH_3), 2.58 (3H, s, SCH_3), 2.54 (3H, s, SCH_3), 1.99 (1H, m, H-1'''), 1.84 (1H, m, H-1'''), 1.55 (2H, m, H-3'''), 1.39 (2H, m, H-2'''), 1.14 (9H, s, $C(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 215.8 (C=S), 214.7 (C=S), 214.6 (C=S), 162.7 (C-4), 150.3 (C-2), 135.6 (C-2", C-6"), 135.3 (C-2", C-6"), 132.8 (C-1"), 131.8 (C-1") 130.3 (C-4"), 130.1 (C-4"), 128.1 (C-3", C-5"), 128.0 (C-3", C-5"), 116.0 (C-5), 84.8 (C-1'), 78.0 (C-3'), 78.8 (C-2'), 73.6 (C-4'''), 83.7 (C-4'), 63.8 (C-5'), 27.9 (C-3'''), 27.1 ($C(CH_3)_3$), 26.7 (C-1'''), 25.3 (C-2'''), 19.4

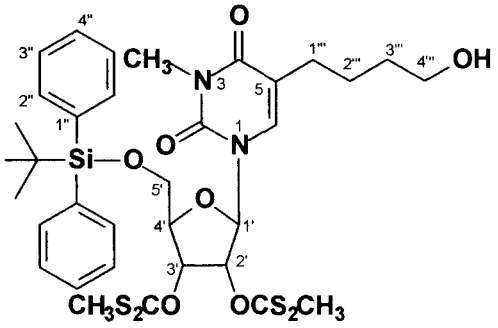
(SCH_3), 19.4 (SCH_3), 19.3 (SCH_3), 18.9 ($C(CH_3)_3$); FABHRMS: *m/z* found 847.11197 (M^+ + Na). $C_{35}H_{44}N_2O_7S_6Si$ (M^+ + Na) requires 847.11397.

3-*N*-Methyl-5-[4-(*S*-methylthiocarbonyloxy)-butyl]-5'-*O*-(*tert*-butyl diphenylsilyl)-2',3'-*bis*-(*S*-methyldithiocarbonyloxy)uridine (161a)



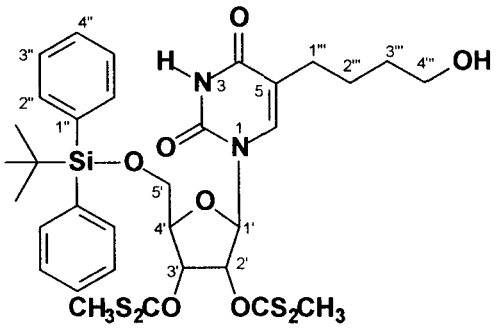
161a (253 mg, 22%): $[\alpha]_D^{20}$ -5.0° (*c* 1.00, $CHCl_3$); IR ($CHCl_3$): ν_{max} 3692 (w), 3606 (w), 2959 (m), 2932 (m), 2860 (w), 1710 (s) (CO), 1669 (s) (CO), 1645 (s) (C=C), 1465 (m), 1099 (s) (C=S), 1077 (s) (C=S) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.69 (4H, m, 2 × H-2"/6"), 7.40 (6H, m, 2 × H-3"/5", 2 × H-4"), 7.34 (1H, s, H-6), 6.55 (1H, d, *J* = 7.4 Hz, H-1'), 6.47 (1H, dd, *J* = 2.4, 6.0 Hz, H-3'), 6.26 (1H, t, *J* = 6.0, 7.4 Hz, H-2'), 4.44 (2H, t, *J* = 6.5 Hz, H-4'''), 4.39 (1H, d, *J* = 2.4 Hz, H-4'), 4.13 (1H, d, *J* = 12.2 Hz, H-5'), 4.02 (1H, d, *J* = 12.2 Hz, H-5'), 3.32 (3H, s, NCH_3), 2.60 (3H, s, SCH_3), 2.58 (3H, s, SCH_3), 2.58 (3H, s, SCH_3), 2.01 (1H, m, H-1'''), 1.90 (1H, m, H-1'''), 1.56 (2H, m, H-3'''), 1.38 (2H, m, H-2'''), 1.14 (9H, s, $C(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 215.7 (C=S), 214.6 (C=S), 214.5 (C=S), 162.8 (C-4), 151.1 (C-2), 135.5 (C-2", C-6"), 135.3 (C-2", C-6"), 133.3 (C-6), 132.8 (C-1"), 131.9 (C-1"), 130.2 (C-4"), 130.1 (C-4"), 128.1 (C-3", C-5"), 128.0 (C-3", C-5"), 114.9 (C-5), 85.9 (C-1'), 83.5 (C-4'), 78.9 (C-2'), 77.9 (C-3'), 73.6 (C-4'''), 63.7 (C-5'), 28.0 (C-3'''), 27.9 (C-1'''), 27.1 ($C(CH_3)_3$), 27.0 (NCH_3), 25.3 (C-2'''), 19.4 (SCH_3), 19.3 (SCH_3), 19.3 (SCH_3), 18.9 ($C(CH_3)_3$); FABHRMS: *m/z* found 861.13098 (M^+ + Na). $C_{36}H_{46}N_2O_7S_6Si$ (M^+ + Na) requires 861.12961.

3-*N*-Methyl-5-(4-hydroxybutyl)-5'-*O*-(*tert*-butyldiphenylsilyl)-2',3'-*bis*-(*S*-methyldithiocarbonyloxy)uridine (161c)



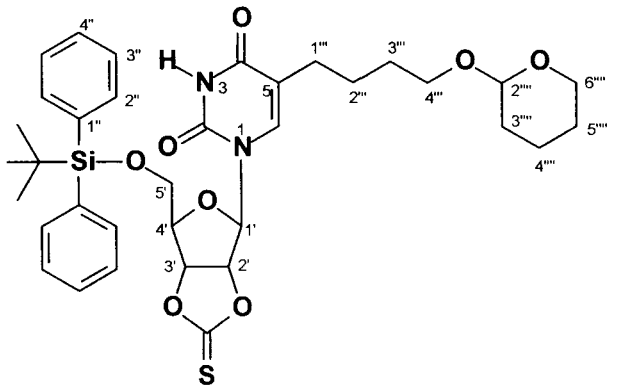
161c (288 mg, 29%): $[\alpha]_D^{20}$ -2.5° (*c* 1.00, $CHCl_3$); IR ($CHCl_3$): ν_{max} 3691 (w), 3612 (w), 3483 (br) (OH), 2932 (m), 2860 (m), 1709 (s) (CO), 1667 (s) (CO), 1644 (s) (C=C), 1466 (m), 1100 (s) (C=S), 1078 (s) (C=S), 720 (w) (C-S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.69 (4H, m, 2 × H-2"/6"), 7.42 (2H, m, 2 × H-4"), 7.37 (4H, m, 2 × H-3"/5"), 7.33 (1H, s, H-6), 6.52 (1H, d, *J* = 7.2 Hz, H-1'), 6.46 (1H, dd, *J* = 2.4, 5.9 Hz, H-3'), 6.24 (1H, dd, *J* = 5.9, 7.2 Hz, H-2'), 4.38 (1H, m, H-4'), 4.10 (1H, dd, *J* = 2.4, 11.4 Hz, H-5'), 4.00 (1H, dd, *J* = 2.0, 11.4 Hz, H-5'), 3.49 (2H, t, *J* = 6.2 Hz, H-4'''), 3.31 (3H, s, NCH_3), 2.59 (3H, s, SCH_3), 2.57 (3H, s, SCH_3), 2.02 (1H, m, H-1'''), 1.90 (1H, m, H-1'''), 1.35 (4H, m, H-2''', H-3'''), 1.13 (9H, s, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 214.6 (C=S), 214.5 (C=S), 163.0 (C-4), 151.0 (C-2), 135.6 (C-2", C-6"), 135.3 (C-2", C-6"), 133.3 (C-6), 132.9 (C-1"), 132.0 (C-1"), 130.2 (C-4"), 130.0 (C-4"), 128.1 (C-3", C-5"), 128.0 (C-3", C-5"), 115.4 (C-5), 86.1 (C-1'), 83.4 (C-4'), 79.0 (C-2'), 77.9 (C-3'), 63.7 (C-5'), 62.2 (C-4'''), 32.1 (C-3'''), 28.0 (NCH_3), 27.2 (C-1'''), 27.1 ($C(CH_3)_3$), 25.1 (C-2'''), 19.4 (SCH_3), 19.4 (SCH_3), 19.3 ($C(CH_3)_3$); FABHRMS: *m/z* found 771.17104 (M^+ + Na). $C_{34}H_{44}N_2O_7S_4Si$ (M^+ + Na) requires 771.16982.

5-(4-Hydroxybutyl)-5'-*O*-(*tert*-butyldiphenylsilyl)-2',3'-*bis*(*S*-methyldithiocarbonyloxy)uridine (161d)



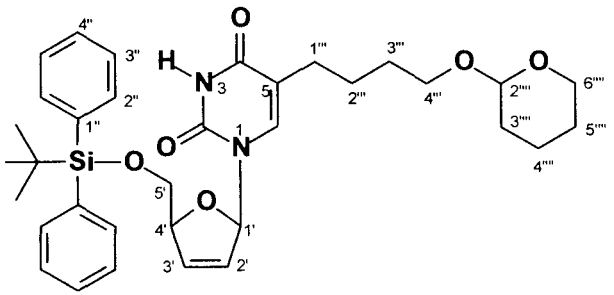
161d (147 mg, 15%): $[\alpha]_D^{20}$ -4.1° (*c* 1.00, $CHCl_3$); IR ($CHCl_3$): ν_{max} 3691 (w), 3602 (w), 3389 (m), (NH), 2931 (s), 2860 (m), 1715 (s) (CO), 1691 (s) (CO), 1602 (w), 1463 (m), 1114 (s) (C=S), 1095 (s) (C=S), 1077 (s) (C=S), 669 (C-S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 9.44 (1H, brs, NH), 7.69 (4H, m, 2 × H-2"/6"), 7.39 (2H, m, 2 × H-4"), 7.38 (4H, m, 2 × H-3"/5"), 7.35 (1H, s, H-6), 6.52 (1H, d, *J* = 7.2 Hz, H-1'), 6.46 (1H, dd, *J* = 2.2, 6.1 Hz, H-3'), 6.23 (1H, t, *J* = 6.1, 7.2 Hz, H-2'), 4.37 (1H, m, H-4'), 4.12 (1H, dd, *J* = 2.2, 11.6 Hz, H-5'), 4.01 (1H, dd, *J* = 1.4, 11.6 Hz, H-5'), 3.49 (2H, t, *J* = 6.0 Hz, H-4'''), 2.59 (3H, s, SCH_3), 2.57 (3H, s, SCH_3), 1.90 (1H, m, H-1'''), 1.86 (1H, m, H-1'''), 1.34 (4H, m, 2 × H-2''', 2 × H-3'''), 1.13 (9H, s, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 214.7 (C=S), 214.5 (C=S), 163.4 (C-4), 150.5 (C-2), 135.6 (C-2", C-6"), 135.3 (C-2", C-6"), 135.1 (C-6), 132.8 (C-1"), 131.9 (C-1"), 130.2 (C-4"), 130.1 (C-4"), 128.1 (C-3", C-5"), 128.0 (C-3", C-5"), 116.5 (C-5), 84.8 (C-1'), 83.6 (C-4'), 78.8 (C-2'), 78.0 (C-3'), 63.7 (C-5'), 62.2 (C-4'''), 32.1 (C-3'''), 27.1 ($C(CH_3)_3$), 26.5 (C-1'''), 25.1 (C-2'''), 19.4 (SCH_3), 19.4 (SCH_3), 19.3 ($C(CH_3)_3$); FABHRMS: *m/z* found 757.15491 (M^+ + Na). $C_{33}H_{42}N_2O_7S_4Si$ (M^+ + Na) requires 757.15417.

5-[4-(2-Tetrahydropyranyloxy)-butyl]-5'-*O*-(*tert*-butyldiphenylsilyl)-2', 3'-*O*-thiocarbonyluridine (168)



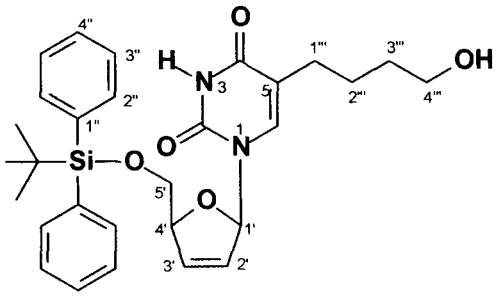
1,1'-Thiocarbonyldiimidazole (0.69 g, 3.9 mmols) was added to a solution of the nucleoside (0.62 g, 1.0 mmols) in 1, 2-dichloroethane (10 mL). The reaction mixture was refluxed for 1 h. The solvent was evaporated and the product purified by column chromatography using petroleum ether / ethyl acetate (1 / 1) to give **168** as a colourless oil (0.52 g, 79%). $[\alpha]_D^{20}$ + 9.6 ° (*c* 1.50, $CHCl_3$); IR ($CHCl_3$): ν_{max} 3693 (w), 3386 (w) (NH), 2946 (w), 2860 (w), 1810 (m), 1694 (s) (CO), 1134 (m) (C=S), 1090 (m) (C=S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 9.43 (1H, brs, NH), 7.61 (4H, t, *J* = 7.9 Hz, 2 × H-2"/6"), 7.39 (2H, m, 2 × H-4"), 7.32 (4H, m, 2 × H-3"/5"), 7.00 (1H, s, H-6), 5.62 (1H, d, *J* = 7.5 Hz, H-2'), 5.56 (1H, s, H-1'), 5.48 (1H, dd, *J* = 3.8, 7.5 Hz, H-3'), 4.51 (1H, t, *J* = 2.8, 4.4 Hz, H-2""), 4.42 (1H, m, H-4'), 3.86 (3H, m, 2 × H-5', H-4'"), 3.73 (1H, m, H-6""), 3.46 (1H, m, H-4'"), 3.38 (1H, m, H-6""), 2.31 (2H, m, H-1'"), 1.59 (2H, m, H-4""), 1.56 (4H, m, H-2'", H-5""), 1.52 (4H, m, H-3'", H-3""), 1.03 (9H, s, $C(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 189.4 (C=S), 163.3 (C-4), 149.9 (C-2), 138.6 (C-6), 135.4 (C-2", C-6"), 135.4 (C-2", C-6"), 132.7 (C-1"), 132.6 (C-1"), 129.9 (C-4"), 129.9 (C-4"), 127.8 (C-3", C-5"), 127.7 (C-3", C-5"), 116.0 (C-5), 99.0 (C-2""), 95.1 (C-1'), 88.0 (C-2'), 87.7 (C-4'), 85.5 (C-3'), 67.1 (C-6""), 63.3 (C-5'), 62.4 (C-4'"), 29.2 (C-5""), 26.7 ($C(CH_3)_3$), 26.3 (C-1'"), 25.4 (C-3'", C-3""), 24.9 (C-2'", C-4""), 19.6 ($C(CH_3)_3$); FABHRMS: *m/z* found 703.24944 $[M+Na]^+$. $C_{35}H_{44}N_2O_8SSi$ $[M+Na]^+$ requires 703.24852.

5-[4-(2-Tetrahydropyranyloxy)-butyl]-5'-*O*-(*tert*-butyldiphenylsilyl)-2',3'-didehydro-2',3'-dideoxyuridine (169)



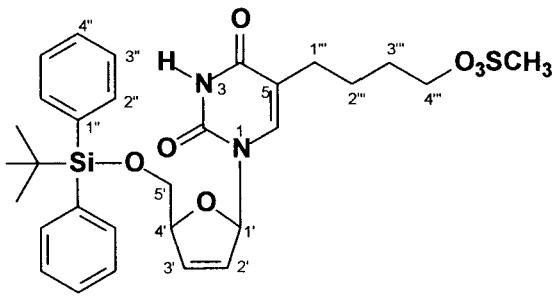
Thionocarbonate **168** (0.30 g, 0.5 mmol) was heated at 160 °C in triethylphosphite (2 mL) for 4 h. After conversion of the starting material had been indicated by TLC, the solvent was removed under reduced pressure and the crude product subjected directly to chromatographic purification using 40% ethyl acetate in petroleum ether to give **169** as a colourless oil (0.20 g, 75%). $[\alpha]_D^{20}$ +3.2 ° (*c* 1.50, $CHCl_3$); IR ($CHCl_3$): ν_{max} 3690 (w), 2943 (m), 2861 (w), 1700 (m) (CO), 1664 (s) (CO), 1640 (s) (CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.94 (4H, m, 2 × H-2", 2 × H-6"), 7.38 (6H, m, 2 × H-3", 2 × H-4", 2 × H-5"), 7.04 (1H, m, H-1'), 6.97 (1H, s, H-6), 6.37 (1H, dq, *J* = 1.6, 6.1 Hz, H-3'), 5.85 (1H, dq, *J* = 1.6, 2.0, 6.1 Hz, H-2'), 4.91 (1H, m, H-4'), 4.51 (1H, t, *J* = 3.3, 4.2 Hz, H-2""), 3.83 (3H, m, 2 × H-4"', H-5'), 3.63 (1H, m, H-6""), 3.47 (1H, m, H-5'), 3.26 (1H, m, H-6""), 2.06 (2H, m, H-1"'), 1.64 (1H, m, CH_2), 1.51 (5H, m, CH_2), 1.44 (2H, m, CH_2), 1.36 (2H, m, CH_2), 1.06 (9H, s, $C(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.7 (C-4), 151.2 (C-2), 135.5 (C-2", C-6"), 135.4 (C-2", C-6"), 134.8 (C-3'), 133.3 (C-6), 132.9 (C-1"), 132.9 (C-1"), 130.0 (C-4"), 129.9 (C-4"), 127.8 (C-3", C-5"), 127.8 (C-3", C-5"), 126.3 (C-2'), 114.6 (C-5), 98.9 (C-2""), 90.6 (C-1'), 86.7 (C-4'), 67.3 (C-4"'), 65.9 (C-6""), 62.3 (C-5'), 30.7 (CH_2), 29.4 (CH_2), 27.4 (C-1"'), 26.9 ($C(CH_3)_3$), 25.5 (CH_2), 25.3 (CH_2), 19.7 (CH_2), 19.3 ($C(CH_3)_3$).

5-(4-Hydroxybutyl)-5'-*O*-(*tert*-butyldiphenylsilyl)-2',3'-didehydro-2',3'-dideoxyuridine (170)



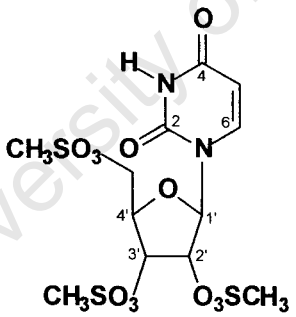
$MgBr_2$ (0.06 g, 0.5 mmol) was added to a solution of the nucleoside (0.11 g, 0.2 mmol) in ether (5 mL). The resulting suspension was stirred at room temperature for 12 h. Methanol (2 mL) was added dropwise and the solution stirred for 30 min. The solvent was removed under reduced pressure and the residue subjected to column chromatography employing EtOAc / petroleum ether (8 / 2) to give the title compound **170** as a colourless oil (0.08 g, 83%). IR ($CHCl_3$): ν_{max} 3691 (w), 3391 (w) (NH), 3178 (br) (OH), 2933 (w), 2860 (w), 1810 (w), 1688 (s) (CO) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.21 (1H, brs, NH), 7.64 (4H, m, 2 × H-2"/H-6"), 7.39 (6H, m, 2 × H-4", 2 × H-3"/H-5"), 6.99 (1H, s, H-6), 6.98 (1H, dd, *J* = 1.4, 3.4 Hz, H-1'), 6.37 (1H, dt, *J* = 1.6, 6.1 Hz, H-3'), 5.84 (1H, dq, *J* = 1.6, 6.1 Hz, H-2'), 4.92 (1H, m, H-4'), 3.83 (2H, t, *J* = 4.8 Hz, H-5'), 3.48 (2H, t, *J* = 6.2 Hz, H-4'''), 2.04 (1H, m, H-1'''), 1.94 (1H, m, H-1'''), 1.61 (1H, brs, OH), 1.33 (4H, m, H-2''', H-3'''), 1.08 (9H, s, $C(CH_3)_3$); ^{13}C NMR (75 MHz, CD_3OD): δ 165.8 (C-4), 152.7 (C-2), 137.5 (C-6), 136.6 (C-2", C-6"), 136.5 (C-2", C-6"), 135.9 (C-3'), 134.8 (C-1"), 134.3 (C-1"), 131.1 (C-4"), 131.0 (C-4"), 128.9 (C-3", C-5"), 128.8 (C-3", C-5"), 127.2 (C-2'), 116.1 (C-5), 91.3 (C-1'), 88.7 (C-4'), 67.3 (C-4'''), 62.6 (C-5'), 33.1 (C-3'''), 27.5 ($C(CH_3)_3$), 27.4 (C-1'''), 25.9 (C-2'''), 20.2 ($C(CH_3)_3$).

5-[4-(Methanesulfonyloxy)-butyl]-5'-*O*-(*tert*-butyldiphenylsilyloxy)-2',3'-didehydro-2',3'-dideoxyuridine (172)



Et_3N (0.03 mL, 0.2 mmol) and a catalytic amount of dimethyaminopyridine (0.01 g) were added to a solution of the nucleoside (0.05 g, 0.1 mmol) in CH_2Cl_2 (2 mL). The mixture was cooled to 0°C and MsCl (0.01 mL, 0.1 mmol) was added dropwise. The mixture was stirred at room temperature for 12 h. The residue was dissolved in CH_2Cl_2 (10 mL), washed with H_2O (3 × 5 mL), dried with $MgSO_4$, filtered and the solvent evaporated under reduced pressure. Column chromatography using petroleum ether / EtOAc (4 / 6) gave a colourless oil (25 mg, 46%).

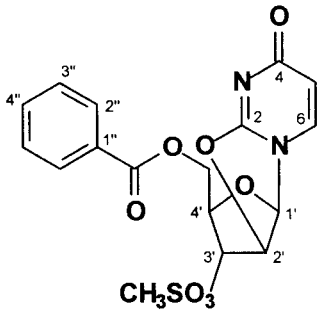
Uridine-2',3',5'-*tri*mesylate (121)



Methanesulfonyl chloride (8.7 mL, 113 mmol) was added to a cold and stirring solution of uridine **120** (6.1 g, 25 mmol) in pyridine (38 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 5 h and then poured into ice-water (250 mL) with stirring. The mixture was stirred for 5 min. The precipitate formed was collected by filtration and washed with water (3 × 100 mL) and dried to give **121** as a white solid (8.49 g, 71%). mp: 170-171 °C (lit.[130] 184-186 °C (dec)); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.51 (1H, d, *J* = 1.6 Hz, NH), 7.69 (1H, d, *J* = 8.0 Hz, H-6), 5.97 (1H, d, *J* = 4.4 Hz, H-1'), 5.69 (1H, dd, *J* = 1.6, 8.0 Hz, H-5), 5.60 (1H, t, *J* = 5.3 Hz, H-2'), 5.35 (1H, t, *J* = 5.3 Hz, H-3'), 4.53 (1H, m, H-4'), 4.46 (2H, m, H-5'), 3.35 (3H, s, O_3SCH_3), 3.33 (3H, s, O_3SCH_3), 3.22 (3H, s, O_3SCH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.9 (C-4),

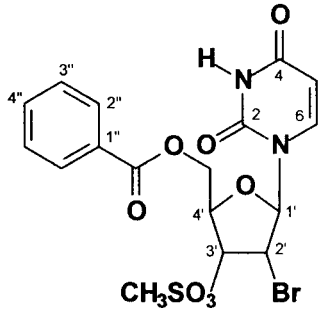
150.3 (C-2), 141.5 (C-6), 102.3 (C-5), 88.7 (C-1'), 78.5 (C-4'), 76.0 (C-2'), 74.0 (C-3'), 67.3 (C-5'), 37.9 (O_3SCH_3), 37.9 (O_3SCH_3), 36.9 (O_3SCH_3).

5'-*O*-Benzoyl-3'-*O*-methanesulfonyl-2'-anhydrouridine (122)



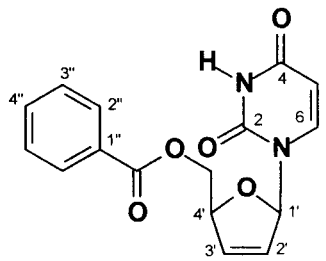
Trimesylate **121** (3.0 g, 6.3 mmol) was added to a stirred slurry of sodium benzoate (3.16 g, 21.9 mmol) in acetamide (25 g) at 115 °C. The reaction mixture was stirred at 115 °C for 65 min and then poured into ice-water (600 mL). The mixture was stirred at 0 °C for 15 min. The white solid was filtered, washed with water and dried to give **122** (1.93 g, 75%) as colourless needles. mp: 243-244 °C (lit.[130] 226-227 °C (dec)); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (3H, m, H-6, H-2", H-6"), 7.65 (1H, m, H-4"), 7.49 (2H, t, *J* = 7.8 Hz, H-3", H-5"), 6.45 (1H, d, *J* = 5.6 Hz, H-1'), 5.87 (1H, d, *J* = 7.6 Hz, H-5), 5.69 (1H, d, *J* = 5.6 Hz, H-2'), 5.62 (1H, d, *J* = 2.4 Hz, H-3'), 4.77 (1H, m, H-4'), 4.33 (1H, dd, *J* = 4.8, 12.1 Hz, H-5'), 4.22 (1H, dd, *J* = 7.4, 12.1 Hz, H-5'), 3.43 (3H, s, O_3SCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.6 (C=O), 165.1 (C-4), 159.2 (C-2), 136.5 (C-6), 133.5 (C-4"), 129.2 (C-2", C-6"), 128.9 (C-1"), 128.7 (C-3", C-5"), 109.0 (C-5), 89.7 (C-1'), 86.0 (C-2'), 82.1 (C-4'), 80.9 (C-3'), 62.5 (C-5'), 37.5 (O_3SCH_3).

5'-*O*-Benzoyl-2'-bromo-3'-*O*-methanesulfonyl-2'-deoxyuridine (123)



Acetyl bromide (2.5 mL, 33.9 mmol) was added dropwise to a stirring mixture of 5'-benzoyl-3'-*O*-methanesulfonyl-2'-anhydrouridine **122** (1.90 g, 4.8 mmol) in ethyl acetate (50 mL) and MeOH (5 mL). The mixture was refluxed for 1 h and then cooled. EtOAc (50 mL) was added and the organic layer washed with saturated aq. $NaHCO_3$ (50 mL) followed by brine (50 mL). The organic layer was dried over $MgSO_4$, the solvent removed *in vacuo* and the crude product recrystallized from EtOAc/pet ether to give **123** as a white solid (2.23 g, 97%). ^1H NMR (300 MHz, $CDCl_3$): δ 9.49 (1H, brs, NH), 8.01 (2H, m, H-2", H-6"), 7.60 (1H, m, H-4"), 7.46 (2H, m, H-3", H-5"), 7.31 (1H, d, *J* = 8.1 Hz, H-6), 6.12 (1H, d, *J* = 5.4 Hz, H-1'), 5.60 (1H, d, *J* = 8.1 Hz, H-5), 5.27 (1H, m, H-3'), 4.74 (1H, m, H-2'), 4.65 (3H, m, H-4', H-5'), 3.18 (3H, s, SCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.9 (C_6H_5OCO), 162.8 (C-4), 150.0 (C-2), 139.6 (C-6), 133.8 (C-4"), 129.6 (C-2", C-6"), 129.0 (C-1"), 128.7 (C-3", C-5"), 103.4 (C-5), 91.9 (C-1'), 80.7 (C-4'), 75.6 (C-3'), 62.2 (C-5'), 47.5 (C-2'), 38.8 (O_3SCH_3).

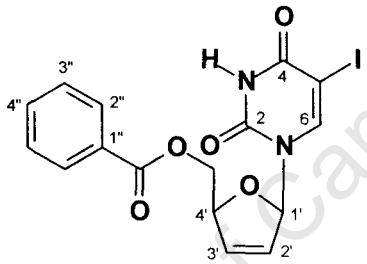
5'-Benzoyl-2',3'-didehydro-2',3'-dideoxyuridine (124)



Acetic acid (0.25 mL) and zinc dust (0.50 g, 7.69 mmol) were added to a suspension of **123** (2.19 g, 4.61 mmol) in a mixture of EtOAc (40 mL) and MeOH (13 mL) at 18 °C. After 3.5 h, the excess Zn was removed by filtration and the cake was washed with a 3:1 mixture of EtOAc/MeOH (2 × 20 mL). The solvent was removed *in vacuo* and more 3:1 mixture of EtOAc/MeOH (6 mL) added. To this solution was then added

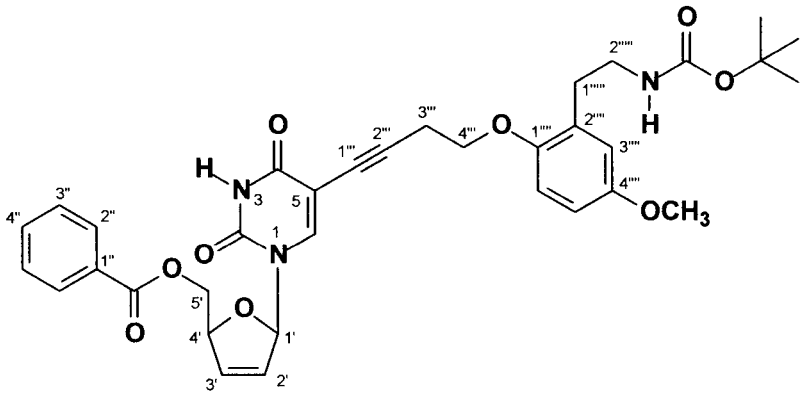
water (75 mL) and the solution stirred for 30 min. The resulting precipitate was filtered and the product washed with water and dried to give **124** as colourless crystals (1.14 g, 83%). mp: 141-142 °C (lit.[82c] 138.5-139 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.10 (1H, brs, NH), 7.99 (2H, m, H-2", H-6"), 7.60 (1H, m, H-4"), 7.46 (2H, m, H-3", H-5"), 7.34 (1H, d, *J* = 8.3 Hz, H-6), 7.00 (1H, m, H-1'), 6.39 (1H, dt, *J* = 1.7, 5.9 Hz, H-3'), 5.89 (1H. dq, *J* = 1.4, 5.9 Hz, H-2'), 5.33 (1H, d, *J* = 8.3 Hz, H-5), 5.16 (1H, m, H-4'), 4.70 (1H, dd, *J* = 3.6, 12.7 Hz, H-5'), 4.53 (1H, dd, *J* = 2.9, 12.7 Hz, H-5'); ^{1}H NMR (300 MHz, $CDCl_3$): δ 166.1 (C_6H_5OCO), 163.0 (C-4), 150.6 (C-2), 139.8 (C-6), 133.6 (C-4"), 135.6 (C-3'), 129.6 (C-2", C-6"), 128.6 (C-3", C-5"), 127.2 (C-2'), 102.7 (C-5), 89.8 (C-1'), 84.8 (C-4'), 64.6 (C-5').

5'-Benzoyl-2',3'-didehydro-2',3'-dideoxy-5-iodouridine (100)



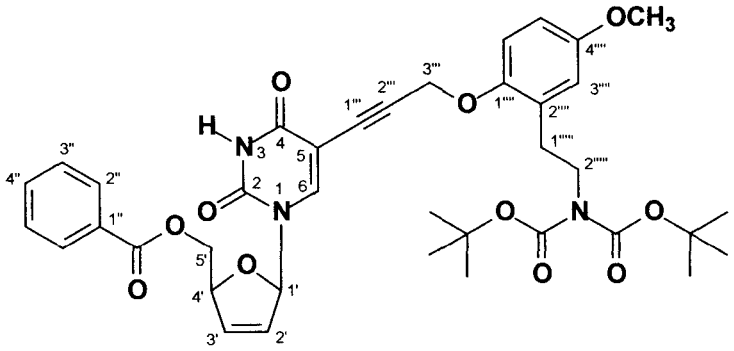
Cerium ammonium nitrate (IV) (0.77 g, 1.4 mmol) and iodine (0.43 g, 1.68 mmol) were added to a solution of 5'-benzoyl-2',3'-didehydro-2',3'-dideoxyuridine **124** (0.88 g, 2.8 mmol) in CH_3CN (40 mL). The mixture was stirred at 35 °C for 4 h. EtOAc (170 mL) was added to the residue and the solid product formed was removed by filtration. The filtrate was washed with a solution of $NaHSO_3$ (2 × 100 mL), water (2 × 100 mL), the organic layer dried over $MgSO_4$ and the solvent removed under reduced pressure. Recrystallization using CH_2Cl_2/pet ether gave **100** as white needles (1.05 g, 85%). mp: 167-168 °C; IR ($CHCl_3$): ν_{max} 3694 (w) (NH), 3606 (w), 3381 (w), 3020 (w), 1720 (s) (CO), 1694 (s) (CO), 1638 (m) cm^{-1}; ^{1}H NMR (300 MHz, Acetone-d_6): δ 10.37 (1H, brs, NH), 8.04 (2H, m, H-2", H-6"), 7.86 (1H, s, H-6), 7.64 (1H, m, H-4"), 7.53 (2H, m, H-3", H-5"), 6.88 (1H, m, H-1'), 6.60 (1H, dt, *J* = 1.7, 6.1 Hz, H-3'), 6.16 (1H, dq, *J* = 1.1, 6.1 Hz, H-2'), 5.25 (1H, m, H-4'), 4.63 (1H, dd, *J* = 4.4, 12.3 Hz, H-5'), 4.57 (1H, dd, *J* = 3.2, 12.3 Hz, H-5'); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.4 (C_6H_5OCO), 159.9 (C-4), 150.2 (C-2), 144.1 (C-6), 133.5 (C-4"), 133.5 (C-3'), 129.8 (C-2", C-6"), 129.3 (C-1"), 128.8 (C-3", C-5"), 127.1 (C-2'), 90.6 (C-1'), 85.1 (C-4'), 68.9 (C-5), 65.0 (C-5').

5-[4-{2-(2-*tert*-Butoxycarbonylaminoethyl)-4-methoxyphenoxy}but-1-ynyl]-5'-*O*-benzoyl-2',3'-didehydro-2',3'-dideoxyuridine (125)



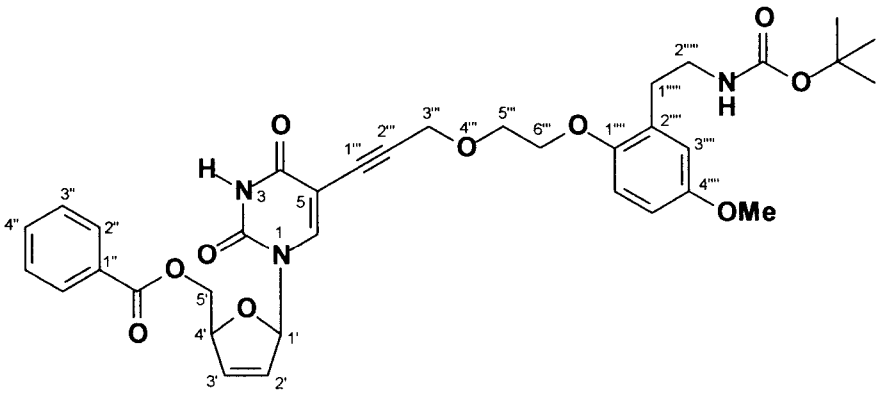
According to GP1: Nucleoside **100** (0.07 g, 0.2 mmol), alkyne **93** (0.10 g, 0.3 mmol), $(PPh_3)_4Pd$ (0.02 g, 0.02 mmol), CuI (0.02 g, 0.1 mmol), triethylamine (0.05 mL, 0.3 mmol), DMF (2.5 mL), THF (5.0 mL). The residue was purified by column chromatography using a gradient from petroleum ether / EtOAc (1 / 1) to EtOAc / MeOH (9 / 1) to give the coupled product as a yellow oil (0.095 g, 94%). $[\alpha]_D^{20}$ -14.4 ° (*c* 1.60, $CHCl_3$); IR ($CHCl_3$): ν_{max} 3692 (w), 3606 (w), 3451 (w) (NH), 3385 (w) (NH), 3011 (m), 2934 (m), 2243 (w) (C≡C), 1707 (s) (CO), 1502 (s), 1453 (m); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.79 (1H, brs, NH), 8.00 (2H, d, *J* = 8.6 Hz, H-2", H-6"), 7.61 (1H, s, H-6), 7.53 (1H, t, *J* = 7.7 Hz, H-4"), 7.42 (2H, t, *J* = 7.7, 8.6 Hz, H-3", H-5"), 6.91 (1H, m, H-1'), 6.70 (3H, m, H-3"", H-5"", H-6""), 6.39 (1H, dt, *J* = 1.7, 5.8 Hz, H-3'), 5.99 (1H, dq, *J* = 1.4, 5.8 Hz, H-2'), 5.20 (1H, m, H-4'), 4.83 (1H, brs, NH), 4.64 (1H, dd, *J* = 4.3, 12.5 Hz, H-5'), 4.50 (1H, dd, *J* = 3.0, 12.5 Hz, H-5'), 3.94 (2H, t, *J* = 6.8 Hz, H-4'"), 3.74 (3H, s, OCH_3), 3.32 (2H, m, H-2""'), 2.77 (2H, t, *J* = 6.9 Hz, H-1""'), 2.68 (2H, t, *J* = 6.8 Hz, H-3'"), 1.40 (9H, s, $OC(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.2 (C_6H_5OCO), 161.4 (C-4), 153.9 (C-4""), 153.9 (NHCO), 150.5 (C-1""), 149.4 (C-2), 141.6 (C-6), 133.5 (C-3'), 133.3 (C-4"), 132.0 (C-1"), 129.7 (C-2", C-6"), 129.2 (C-2""), 128.6 (C-3", C-5"), 127.0 (C-2'), 116.7 (C-3""), 112.9 (C-6""), 112.0 (C-5""), 100.7 (C-2'"), 91.3 (C-1'"), 90.7 (C-1'), 85.0 (C-4'), 78.8 ($O_2C(CH_3)_3$), 72.4 (C-5), 66.7 (C-4'"), 65.1 (C-5'), 55.6 (OCH_3), 40.6 (C-2""'), 31.0 (C-1""'), 28.4 ($OC(CH_3)_3$), 20.8 (C-3'"); FABHRMS: *m/z* found 654.24300 $[M+Na]^+$. $C_{34}H_{37}N_3O_9$ $[M+Na]^+$ requires 654.24274.

5-[3-{2-(2-*bis*(*tert*-butoxycarbonylaminoethyl)-4-methoxyphenoxy} prop-1-ynyl]-5'-*O*-benzoyl-2',3'-didehydro-2',3'-dideoxyuridine (127)



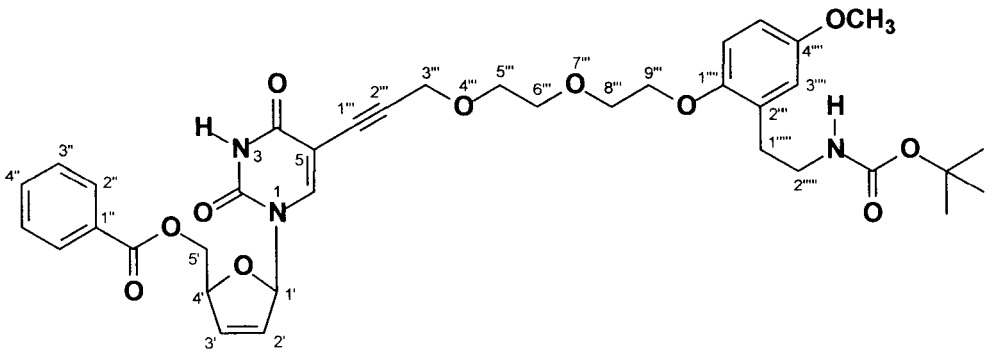
According to GP1: Nucleoside **100** (0.19 g, 0.4 mmol), alkyne **94b** (0.28 g, 0.7 mmol), $(PPh_3)_4Pd$ (0.05 g, 0.04 mmol), CuI (0.04 g, 0.1 mmol), triethylamine (0.1 mL, 0.8 mmol), DMF (2.5 mL), THF (5.0 mL). Purification by column chromatography employing ethyl acetate / petroleum ether (6 / 4) afforded a yellow oil (0.26 g, 84%). $[\alpha]_D^{20}$ -20° (*c* 0.30, $CHCl_3$); IR ($CHCl_3$): ν_{max} 3691 (w), 3606 (w), 3384 (w) (NH), 2930 (m), 2855 (w), 2243 (w) (C≡C), 1777 (m) (CO), 1721 (s) (CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.05 (1H, brs, NH), 7.98 (2H, m, H-2", H-6"), 7.67 (1H, s, H-6), 7.53 (1H, tt, *J* = 1.6, 7.5 Hz, H-4"), 7.42 (2H, t, *J* = 7.5 Hz, H-3", H-5"), 6.88 (1H, t, *J* = 1.5 Hz, H-1'), 6.87 (1H, d, *J* = 8.4 Hz, H-6""), 6.68 (1H, dd, *J* = 3.0, 8.4 Hz, H-5""), 6.65 (1H, d, *J* = 3.0 Hz, H-3""), 6.36 (1H, dt, *J* = 1.6, 6.0 Hz, H-3'), 5.96 (1H, dq, *J* = 1.2, 6.0 Hz, H-2'), 5.19 (1H, m, H-4'), 4.67 (3H, m, 1 × H-5', 2 × H-3'"), 4.47 (1H, dd, *J* = 2.8, 12.4 Hz, H-5'), 3.75 (2H, t, *J* = 7.3 Hz, H-2""), 3.71 (3H, s, OCH_3), 2.83 (2H, t, *J* = 7.3 Hz, H-1""), 1.44 (18H, s, 2 × $OC(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.1 (C_6H_5OCO), 161.1 (C-4), 154.1 (C-4""), 152.4 (NCO), 150.2 (C-1""), 149.5 (C-2), 142.7 (C-6), 133.4 (C-4"), 133.4 (C-3'), 129.7 (C-2", C-6"), 129.3 (C-1"), 129.1 (C-2""), 128.6 (C-3", C-5"), 127.0 (C-2'), 116.8 (C-3""), 113.9 (C-6""), 112.0 (C-5""), 99.8 (C-2'"), 90.9 (C-1'), 89.7 (C-1'"), 85.3 (C-4'), 81.9 ($OC(CH_3)_3$), 77.7 (C-5), 64.9 (C-5'), 57.6 (C-3'"), 55.6 (OCH_3), 46.4 (C-2""), 30.0 (C-1""), 28.0 (2 × $OC(CH_3)_3$); FABHRMS: *m/z* found 740.28021 $[M+Na]^+$. $C_{38}H_{43}N_3O_{11}$ $[M+Na]^+$ requires 740.27951.

5-[4-{2-(2-*tert*-Butoxycarbonylaminoethyl)-4-methoxyphenoxy}hexa-4-oxa-1-ynyl]-5'-*O*-benzoyl-2',3'-didehydro-2',3'-dideoxyuridine (136)



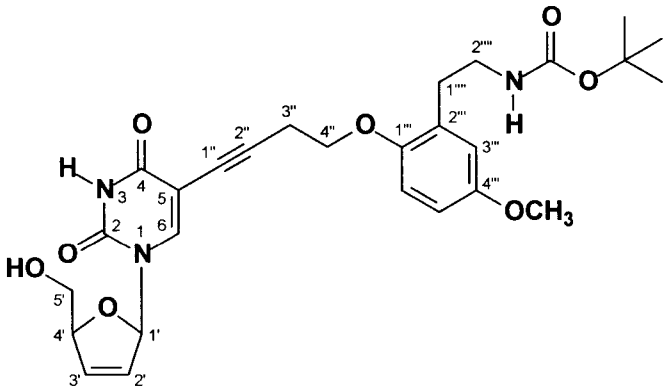
According to GP 1: Nucleoside **100** (0.27 g, 0.6 mmol), alkyne **135a** (0.32 g, 0.9 mmol), $(PPh_3)_4Pd$ (0.07 g, 0.06 mmol), CuI (0.06 g, 0.3 mmol), Et_3N (0.17 mL, 1.2 mmol), DMF (2.5 mL), THF (5.0 mL). The crude product was purified by column chromatography employing EtOAc / pet ether (6 / 4) to give the title compound **136** as a yellow oil (0.36 g, 92%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.36 (1H, brs, NH), 7.98 (2H, m, H-2", H-6"), 7.66 (1H, s, H-6), 7.52 (1H, m, H-4"), 7.43 (2H, m, H-3", H-5"), 6.90 (1H, m, H-1'), 6.74 (1H, d, *J* = 9.0 Hz, H-6""), 6.66 (2H, m, H-3"", H-5""), 6.37 (1H, dt, *J* = 1.6, 5.9 Hz, H-3'), 5.97 (1H, dq, *J* = 1.6, 5.9 Hz, H-2'), 5.18 (1H, m, H-4'), 4.94 (1H, brs, NH), 4.66 (1H, dd, *J* = 4.4, 12.5 Hz, H-5'), 4.48 (1H, dd, *J* = 2.9, 12.5 Hz, H-5'), 4.25 (2H, s, H-3'"), 4.05 (2H, m, CH_2O), 3.83 (2H, m, OCH_2), 3.72 (3H, s, OCH_3), 3.32 (2H, m, H-2""), 2.76 (2H, t, *J* = 6.2 Hz, H-1""), 1.39 (9H, s, $OC(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.1 (C_6H_5OCO), 161.3 (C-4), 156.0 (NHCO) 153.7 (C-4""), 150.9 (C-1""), 149.5 (C-2), 142.2 (C-6), 133.4 (C-4"), 133.3 (C-3'), 129.6 (C-2", C-6"), 129.3 (C-1"), 129.1 (C-2""), 128.6 (C-3", C-5"), 127.0 (C-2'), 116.5 (C-3""), 113.0 (C-6""), 111.9 (C-5""), 99.8 (C-2'"), 90.7 (C-1'), 89.9 (C-1'"), 85.0 (C-4'), 78.8 ($O_2C(CH_3)_3$), 77.2 (C-5), 68.3 (OCH_2), 68.1 (OCH_2), 65.0 (C-5'), 58.9 (C-3'"), 55.5 (OCH_3), 40.6 (C-2""), 30.8 (C-1""), 28.3 ($OC(CH_3)_3$).

5-[9-{2-(2-*tert*-Butyloxycarbonylaminoethyl)-4-methoxyphenoxy}-nona-4,7-dioxa-1-ynyl]-5'-*O*-benzoyl-2',3'-didehydro-2',3'-dideoxy uridine (138)



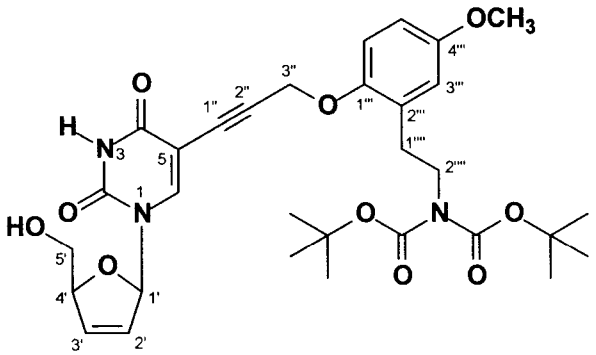
According to GP 1: Nucleoside **100** (0.110 g, 0.25 mmol), alkyne **135b** (0.119 g, 0.30 mmol), $(PPh_3)_4Pd$ (0.029 g, 0.025 mmol), CuI (0.024 g, 0.125 mmol), Et_3N (0.07 mL, 0.5 mmol), DMF (5 mL), THF (10 mL). Column chromatography employing EtOAc / pet ether (1 / 1) gave the title compound **138** as a yellow oil (0.154 g, 82%). $[\alpha]_D^{20}$ +30.6 ° (*c* 1.20, $CHCl_3$); IR ($CHCl_3$): ν_{max} 3693 (w), 3607 (w), 3452 (w) (NH), 3384 (w) (NH), 3011 (w), 2980 (w), 2935 (w), 1703 (s) (CO), 1502 (m) (C=C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.58 (1H, brs, NH), 7.98 (2H, m, H-2", H-6"), 7.64 (1H, s, H-6), 7.54 (1H, m, H-4"), 7.42 (2H, t, *J* = 7.8 Hz, H-3", H-5"), 6.88 (1H, m, H-1'), 6.73 (1H, d, *J* = 8.8 Hz, H-6""), 6.66 (2H, m, H-3"", H-5""), 6.35 (1H, d, *J* = 6.1 Hz, H-3'), 5.95 (1H, m, H-2'), 5.17 (1H, m, H-4'), 4.98 (1H, brs, NH), 4.65 (1H, dd, *J* = 4.3, 12.5 Hz, H-5'), 4.47 (1H, dd, *J* = 2.8, 12.5 Hz, H-5'), 4.21 (2H, s, H-3'"), 4.05 (2H, t, *J* = 4.9 Hz, CH_2O), 3.80 (2H, t, *J* = 4.9 Hz, CH_2O), 3.71 (3H, s, OCH_3), 3.69 (4H, m, 2 × CH_2O), 3.32 (2H, brs, H-2""), 2.76 (2H, t, *J* = 6.8 Hz, H-1""), 1.39 (9H, s, $OC(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.2 (C_6H_5OCO), 161.5 (C-4), 156.0 (NHCO), 153.7 (C-4""), 151.0 (C-1""), 149.6 (C-2), 142.3 (C-6), 133.4 (C-3'), 133.4 (C-4"), 129.7 (C-2", C-6"), 129.3 (C-1"), 129.2 (C-2""), 128.6 (C-3", C-5"), 127.0 (C-2'), 116.6 (C-3""), 113.1 (C-6""), 112.0 (C-5""), 99.9 (C-2'"), 90.7 (C-1'), 90.0 (C-1'"), 85.1 (C-4'), 78.8 ($O_2C(CH_3)_3$), 70.5 (2 × OCH_2), 69.8 (OCH_2), 69.1 (C-5), 68.4 (OCH_2), 65.0 (C-5'), 58.9 (C-3'"), 55.6 (OCH_3), 40.6 (C-2""), 31.0 (C-1""), 28.4 ($OC(CH_3)_3$); FABHRMS: *m/z* found 728.27796 $[M+Na]^+$. $C_{37}H_{43}N_3O_{11}$ $[M+Na]^+$ requires 728.27951.

5-[4-{2-(2-*tert*-Butoxycarbonylaminoethyl)-4-methoxyphenoxy}but-1-ynyl]-2',3'-didehydro-2',3'-dideoxyuridine (126)



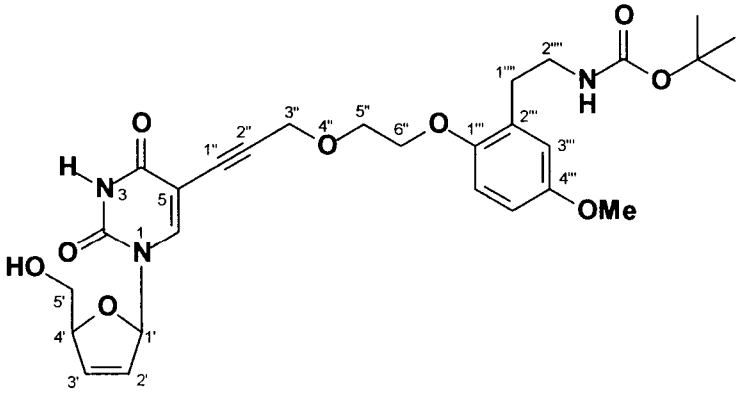
The nucleoside **125** (0.085 g, 0.1 mmol) was dissolved in MeOH (1 mL) and the residue cooled to 0 °C. Sodium methoxide in methanol (0.1 mL, 1M, 0.1 mmol) was added and the mixture stirred at 0 °C for 20 min. Water (5 mL) was added to the mixture and the residue extracted with ethyl acetate (3 × 15 mL). The organic extracts were combined, dried ($MgSO_4$), filtered and the solvent evaporated *in vacuo*. Column chromatography employing petroleum ether / ethyl acetate (2 / 8) gave **126** as a colourless oil (0.056 g, 79%). IR ($CHCl_3$): ν_{max} 3690 (w), 3521 (br) (OH), 3386 (w) (NH), 2411 (w) (C≡C), 1697 (s) (CO), 1602 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.98 (1H, brs, NH), 8.00 (1H, s, H-6), 6.97 (1H, m, H-1'), 6.75 (1H, d, *J* = 9.6 Hz, H-6'''), 6.69 (2H, m, H-3''', H-5'''), 6.36 (1H, dt, *J* = 1.7, 5.8 Hz, H-3'), 5.82 (1H, dq, *J* = 1.7, 5.8 Hz, H-2'), 4.90 (2H, brs, H-4', NH), 4.05 (2H, t, *J* = 6.1, Hz, H-4''), 3.88 (1H, dd, *J* = 2.7, 12.4 Hz, H-5'), 3.78 (1H, dd, *J* = 3.0, 12.4 Hz, H-5'), 3.74 (3H, s, OCH_3), 3.33 (2H, m, H-2''''), 2.82 (2H, t, *J* = 6.1 Hz, H-3''), 2.77 (2H, m, *J* = 6.3 Hz, H-1''''), 1.96 (1H, brs, OH), 1.40 (9H, s, $OC(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.9 (C-4), 156.4 (NHCO), 153.8 (C-4'''), 150.7 (C-1'''), 149.8 (C-2), 143.7 (C-6), 135.2 (C-3'), 129.3 (C-2'''), 125.9 (C-2'), 116.8 (C-3'''), 113.0 (C-6'''), 111.9 (C-5'''), 100.1 (C-2''), 91.3 (C-1''), 90.2 (C-1'), 87.6 (C-4'), 79.3 ($OC(CH_3)_3$), 72.4 (C-5), 66.8 (C-4''), 62.9 (C-5'), 55.6 (OCH_3), 40.5 (C-2''''), 31.1 (C-1''''), 28.4 ($OC(CH_3)_3$), 20.9 (C-3'').

5-[3-{2-(2-*bis*-(*tert*-Butoxycarbonylaminoethyl)-4-methoxyphenoxy}-prop-1-ynyl]-2',3'-didehydro-2',3'-dideoxyuridine (128)



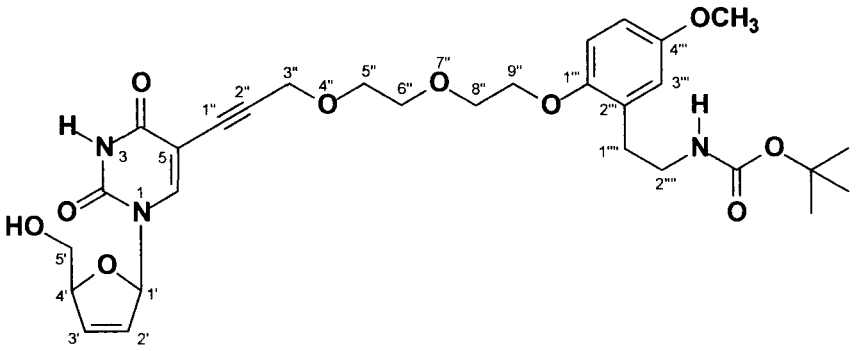
Sodium methoxide in methanol (0.1 mL, 1M, 0.1 mmol) was added to a solution of the nucleoside **127** (0.067 g, 0.09 mmol) in MeOH (1.0 mL) at 0 °C, and the mixture was stirred at 0 °C for 90 min. H_2O (5 mL) was added and the product extracted with ethyl acetate (3 × 10 mL). The organic extracts were combined, dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure. The product was purified by column chromatography employing a gradient of ethyl acetate / petroleum ether (8 / 2) to 100% ethyl acetate to give the title compound **128** as a colourless oil (0.042 g, 74%). $[\alpha]_D^{20}$ +22.4 ° (*c* 0.70, $CHCl_3$); IR ($CHCl_3$): ν_{max} 3693 (w), 3539 (br) (OH), 3385 (w) (NH), 2243 (w) (C≡C), 1777 (m) (CO), 1697 (s) (CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.72 (1H, brs, NH), 8.34 (1H, s, H-6), 6.95 (1H, m, H-1'), 6.93 (1H, d, *J* = 9.2 Hz, H-6'''), 6.70 (2H, m, H-3''', H-5'''), 6.33 (1H, dt, *J* = 1.8, 6.2 Hz, H-3'), 5.82 (1H, dt, *J* = 1.8, 6.2 Hz, H-2'), 4.92 (1H, m, H-4'), 4.78 (2H, s, H-3''), 3.92 (1H, brd, *J* = 11.2 Hz, H-5'), 3.79 (3H, m, 1 × H-5', 2 × H-2''''), 3.73 (3H, s, OCH_3), 3.18 (1H, brs, OH), 2.91 (1H, m, H-1''''), 2.84 (1H, m, H-1''''), 1.41 (18H, s, 2 × $OC(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.3 (C-4), 154.0 (C-4'''), 152.4 ($N(CO)_2$), 150.2 (C-1'''), 149.6 (C-2), 145.5 (C-6), 134.8 (C-3'), 129.5 (C-2'''), 126.1 (C-2'), 117.0 (C-3'''), 113 (C-6'''), 111.9 (C-5'''), 99.1 (C-2''), 90.3 (C-1'), 88.6 (C-1''), 87.7 (C-5), 82.1 (C-4'), 78.1 ($O_2C(CH_3)_3$), 62.7 (C-5'), 57.5 (C-3''), 55.6 (OCH_3), 46.9 (C-2''''), 29.9 (C-1''''), 27.9 (2 × $OC(CH_3)_3$).

5-[4-{2-(2-*tert*-Butoxycarbonylaminoethyl)-4-methoxyphenoxy}hexa-4-oxa-1-ynyl]-2',3'-didehydro-2',3'-dideoxyuridine (137)



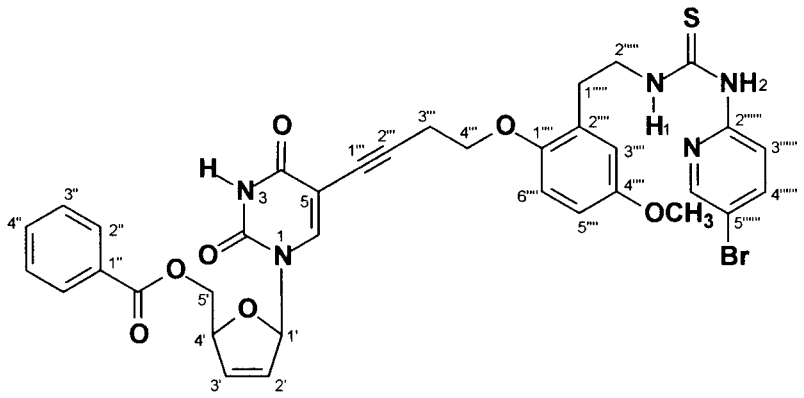
To an ice-cold solution of the nucleoside **136** (0.36 g, 0.6 mmol) in MeOH (2 mL) was added NaOMe in methanol (0.1 mL, 1 M, 0.1 mmol). The mixture was stirred at 0 °C for 30 min. The residue was diluted with H_2O (5 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The crude product was purified by column chromatography employing ethyl acetate / pet ether (6 / 4) to give **137** as a colourless oil (0.21 g, 69%). $[\alpha]_D^{20}$ +28.3 ° (*c* 1.10, $CHCl_3$); IR ($CHCl_3$): ν_{max} 3692 (w), 3535 (br) (OH), 3383 (w) (NH), 2344 (w) (C≡C), 1713 (s) (CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.03 (1H, brs, NH), 8.21 (1H, s, C-6), 6.97 (1H, m, H-1'), 6.76 (1H, d, *J* = 9.3 Hz, H-6'''), 6.67 (2H, m, H-3''', H-5'''), 6.34 (1H, dt, *J* = 1.7, 6.0 Hz, H-3'), 5.83 (1H, dq, *J* = 1.7, 6.0 Hz, H-2'), 4.96 (1H, brs, NH), 4.91 (1H, s, H-4'), 4.40 (2H, s, H-3''), 4.08 (2H, t, *J* = 4.6 Hz, CH_2O), 3.90 (1H, brd, *J* = 11.1 Hz, H-5'), 3.88 (2H, t, *J* = 4.6 Hz, CH_2O), 3.79 (1H, d, *J* = 11.1 Hz, H-5'), 3.73 (3H, s, OCH_3), 3.62 (1H, brs, OH), 3.34 (2H, m, H-2''''), 2.77 (2H, t, *J* = 6.1 Hz, H-1''''), 1.40 (9H, s, $OC(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.6 (C-4), 156.4 ($NHCO_2$), 153.8 (C-4'''), 150.9 (C-1'''), 149.7 (C-2), 144.7 (C-6), 135.1 (C-3'), 129.3 (C-2'''), 126.0 (C-2'), 116.8 (C-3'''), 113.2 (C-6'''), 111.8 (C-5'''), 99.2 (C-2''), 90.2 (C-1'), 89.2 (C-1''), 87.7 (C-4'), 79.2 ($O_2C(CH_3)_3$), 77.0 (C-5), 68.4 (CH_2O), 68.2 (CH_2O), 62.7 (C-5'), 59.1 (C-3''), 55.6 (OCH_3), 40.5 (C-2''''), 31.0 (C-1''''), 28.4 ($OC(CH_3)_3$).

5-[4-{2-(2-*tert*-Butoxycarbonylaminoethyl)-4-methoxyphenoxy}nona-4,7-dioxa-1-ynyl]-2',3'-didehydro-2',3'-dideoxyuridine (139)



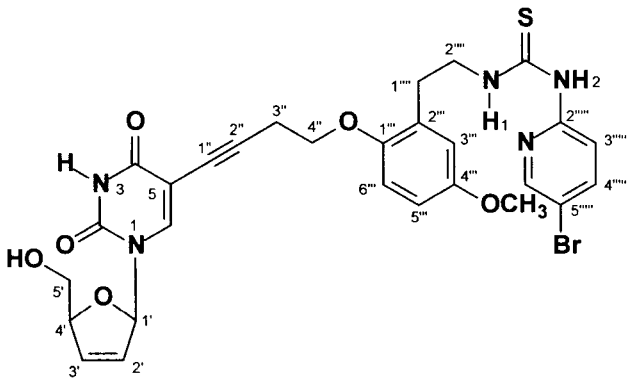
To a cold solution of the nucleoside **138** (0.095 g, 0.13 mmol) in MeOH (2 mL) was added NaOMe (0.1 mL, 1M, 0.1 mmol). The mixture was stirred at 0 °C for 30 min. The base was diluted with H_2O (5 mL) and extracted with ethyl acetate (3 x 20 mL). The combined organic extracts were dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The crude product was purified by column chromatography employing ethyl acetate / pet ether (6 / 4) to give **139** as a colourless oil (0.07 g, 86%). $[\alpha]_D^{20}$ -16.8 ° (*c* 0.50, $CHCl_3$); IR ($CHCl_3$): ν_{max} 3692 (w), 3611 (w), 3500-3200 (br) (OH), 3449 (w) (NH), 3387 (w), 3012 (m), 2933 (m), 2877 (m), 2240 (w) (C≡C), 1697 (s) (CO), 1502 (m) (C=C), 1457 (m) (C=C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.47 (1H, brs, NH), 8.18 (1H, s, C-6), 6.92 (1H, m, H-1'), 6.74 (1H, d, *J* = 9.0 Hz, H-6'''), 6.65 (2H, m, H-3''', H-5'''), 6.29 (1H, dt, *J* = 1.6, 6.1 Hz, H-3'), 5.79 (1H, dq, *J* = 1.6, 6.1 Hz, H-2'), 5.07 (1H, brs, NH), 4.87 (1H, m, H-4'), 4.32 (2H, s, H-3''), 4.04 (2H, m, CH_2O), 3.87 (1H, brd, *J* = 12.2 Hz, H-5'), 3.79 (2H, m, CH_2O), 3.71 (7H, m, 2 x CH_2O), OCH_3), 3.66 (1H, brd, *J* = 12.2 Hz, H-5'), 3.30 (2H, d, *J* = 6.3 Hz, H-2''''), 2.75 (2H, t, *J* = 6.3 Hz, H-1''''), 2.41 (1H, brs, OH), 1.38 (9H, s, $OC(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.8 (C-4), 156.3 ($NHCO_2$), 153.8 (C-4'''), 151.0 (C-1'''), 149.9 (C-2), 144.8 (C-6), 135.0 (C-3'), 129.4 (C-2'''), 126.0 (C-2'), 116.7 (C-3'''), 113.2 (C-5'''), 112.0 (C-6'''), 99.2 (C-2''), 90.2 (C-1'), 89.1 (C-1''), 87.7 (C-4'), 79.0 ($O_2C(CH_3)_3$), 77.7 (C-5), 70.5 (OCH_2), 69.8 (OCH_2), 69.1 (OCH_2), 68.5 (OCH_2), 62.8 (C-5'), 59.1 (C-3''), 55.6 (OCH_3), 40.6 (C-2''''), 30.9 (C-1''''), 28.4 ($OC(CH_3)_3$); FABHRMS: *m/z* found 624.25430 $[M+Na]^+$. $C_{30}H_{39}N_3O_{10}$ $[M+Na]^+$ requires 624.25332.

[Benzoyl-d4U]-butyne-[HI-236] (141)



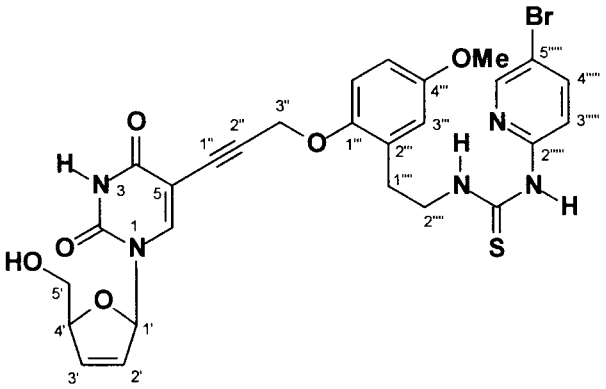
According to GP1: Alkyne **77** (0.020 g, 0.048 mmol), nucleoside **100** (0.025 g, 0.058 mmol), Pd (0) (0.006 g, 0.005 mmol), CuI (0.004 g, 0.02 mmol), Et_3N (0.01 mL, 0.010 mmol), DMF (0.5 mL), THF (1.0 mL) to give the title compound **141** as a white solid (0.019 g, 54%). mp: 95-97 °C (from pet ether/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.00 (1H, brs, NH-2), 8.83 (1H, brs, NH-1), 8.11 (1H, d, *J* = 3.2 Hz, H-6''''''), 8.02 (2H, m, H-3'''''', H-4''''''), 7.67 (1H, s, H-6), 7.53 (2H, m, H-2'', H-6''), 7.43 (2H, m, H-3'', H-5''), 7.30 (1H, m, H-4''), 7.20 (1H, brs, NH), 6.92 (1H, m, H-1'), 6.80 (1H, d, *J* = 2.5 Hz, H-3''''), 6.76 (1H, d, *J* = 8.9 Hz, H-6''''), 6.72 (1H, dd, *J* = 2.5, 8.9 Hz, H-5''''), 6.37 (1H, dt, *J* = 1.6, 6.0 Hz, H-3'), 6.06 (1H, m, H-2'), 5.21 (1H, m, H-4'), 4.67 (1H, dd, *J* = 4.0, 12.3 Hz, H-5'), 4.52 (1H, dd, *J* = 3.0, 12.3 Hz, H-5'), 3.98 (2H, m, H-4'''), 3.77 (3H, s, OCH_3), 3.55 (2H, m, H-2''''''), 2.95 (1H, m, H-3'''), 2.84 (1H, m, H-3'''), 2.68 (2H, t, *J* = 6.2 Hz, H-1''''''); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.7 (C-4), 155.2 (C_6H_5OCO), 153.9 (C-4''''), 152.0 (C-2''''''), 150.8 (C-1''''), 149.4 (C-2), 147.2 (C-6''''''), 141.8 (C-4''''''), 140.7 (C-6), 133.3 (C-4''), 133.1 (C-3'), 129.8 (C-2'', C-6''), 129.5 (C-2''''), 129.5 (C-1''), 128.6 (C-3'', C-5''), 127.5 (C-2'), 116.7 (C-3''''), 113.7 (C-6''''), 112.7 (C-5''''), 111.6 (C-3''''''), 111.6 (C-5''''''), 100.4 (C-2'''), 92.5 (C-1'''), 90.7 (C-1'), 85.0 (C-4'), 72.1 (C-5), 66.7 (C-4'''), 65.2 (C-5'), 55.6 (OCH_3), 40.0 (C-2''''''), 30.4 (C-1''''''), 29.7 (C-3''').

[d4U]-butyne-[HI-236] (96)



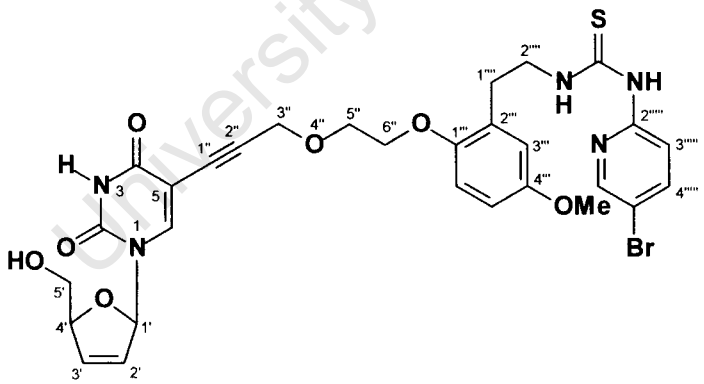
Trifluoroacetic acid (0.1 mL) was added to a cold solution of the nucleoside **126** (0.020 g, 0.04 mmol) in CH_2Cl_2 (1 mL). The mixture was stirred for 30 min at 0 °C and diisopropylethylamine (1.0 mL) added. The reaction mixture was stirred for 10 min and the solvent removed *in vacuo*. Methanol (1.0 mL) and K_2CO_3 (0.002 g) were added and the mixture stirred for an additional 10 min. Inorganic salts were removed by filtration through a pad of Celite and washed with large volumes of methanol. The solvent was removed *in vacuo* and the resultant amine dried under vacuum for 1 h. DMF (1.0 mL) and thiocarbonyl reagent **89** (0.013 g, 0.05 mmol) were added and the mixture was heated at 100 °C for 1 h. DMF was removed by co-evaporation with toluene and the product purified by column chromatography using petroleum ether / EtOAc (2 / 8) to give **96** as a pale yellow solid (0.012 g, 41%). mp: 200-202 °C (from $MeOH/CH_2Cl_2$); ^1H NMR (400 MHz, DMSO-d_6): δ 11.04 (1H, brt, *J* = 4.4 Hz, NH-1), 10.55 (1H, brs, NH-2), 8.04 (1H, d, *J* = 2.2 Hz, H-6''''), 7.94 (1H, s, H-6), 7.87 (1H, dd, *J* = 2.2, 8.6 Hz, H-4''''), 7.56 (1H, brs, NH), 7.04 (1H, d, *J* = 8.6 Hz, H-3''''), 6.88 (1H, d, *J* = 8.8 Hz, H-6'''), 6.75 (3H, m, H-1', H-3''', H-5'''), 6.35 (1H, dt, *J* = 1.6, 6.0 Hz, H-3'), 5.86 (1H, m, H-2'), 4.74 (1H, m, H-4'), 3.98 (2H, t, *J* = 6.4 Hz, H-4''), 3.78 (2H, m, H-2''''), 3.62 (3H, s, OCH_3), 3.58 (2H, m, H-5'), 2.85 (2H, t, *J* = 6.5 Hz, H-1''''), 2.76 (2H, t, *J* = 6.5 Hz, H-3''); ^{13}C NMR (100 MHz, DMSO-d_6): δ 179.0 (C=S), 162.7 (C-4), 153.2 (C-4'''), 152.2 (C-2''''), 150.3 (C-1'''), 149.8 (C-2), 145.6 (C-6''''), 141.2 (C-6), 141.2 (C-4''''), 135.4 (C-3'), 128.6 (C-2'''), 125.5 (C-2'), 117.0 (C-3'''), 114.4 (C-3''''), 113.4 (C-6'''), 111.6 (C-5'''),111.6 (C-5''''), 98.4 (C-2''), 89.8 (C-1''), 89.4 (C-1'), 87.5 (C-4'), 73.9 (C-5), 66.8 (C-4''), 61.8 (C-5'), 55.2 (OCH_3), 44.6 (C-2''''), 29.1 (C-1''''), 20.1 (C-3'').

[d4U]-propyne-[HI-236] (97)



Nucleoside (0.042 g, 0.07 mmol) in methylene chloride (1.0 mL) was treated with trifluoroacetic acid (0.1 mL) at 0 °C. The reaction was stirred for 30 min. K_2CO_3 and MeOH (1 mL) were added and the residue stirred for a further 10 min. The K_2CO_3 was filtered through a pad of Celite and washed with MeOH (3 × 10 mL). The solvent was removed *in vacuo* and the amine dried under vacuum. The crude amine formed was reacted with thiourea derivative **89** (0.029 g, 0.10 mmol) in DMF (1.0 mL) at 100 °C for 1h. The crude product was purified by column chromatography using ethyl acetate / hexane (8 / 2) to give a pale yellow solid (0.012 g, 49%).

[d4U]-monoPEGpropyne-[HI-236] (98)

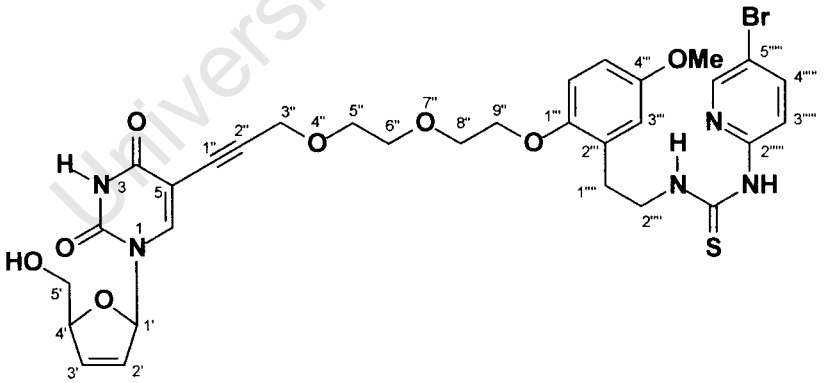


Nucleoside (0.147 g, 0.26 mmol) in methylene chloride (1.0 mL) was treated with trifluoroacetic acid (0.1 mL) at 0°C. The reaction was stirred for 30 min. Diisopropylethylamine (0.4 mL) was added and the solvent evaporated. The residue was dissolved in MeOH (1 mL), K_2CO_3 added and the mixture stirred for 10 min. The K_2CO_3 was filtered through a pad of Celite, washed with MeOH (3 × 10 mL) and the solvent evaporated *in vacuo*. The crude amine was dried under vacuum for 1 h and then reacted with thiourea derivative **89** (0.097 g, 0.34 mmol) in DMF (1.0 mL) at 100 °C for 1h. The crude product was purified by column chromatography using ethyl

acetate / hexane (8 / 2) to give a pale yellow solid (0.071 g, 40%) as a mixture of two compounds. Repetitive column chromatography using 4% MeOH in CH_2Cl_2 afforded **98** (7 mg) and **98a** (10 mg).

98. mp: 79-82 °C; $[\alpha]_D^{20}$ +53.3 ° (*c* 0.10, $CHCl_3$); IR ($CHCl_3$): ν_{max} 3691 (m) (NH), 3607 (w), 3385 (w), 3022 (m), 2928 (m), 2855 (w), 1719 (m) (CO), 1698 (m) (CO), 1602 (m) (C=C), 1221 (s) (C=S) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 11.00 (1H, brt, NH), 8.64 (2H, brs, 2 × NH), 8.18 (1H, s, H-6), 8.03 (1H, d, *J* = 2.4 Hz, H-6''''), 7.68 (1H, dd, *J* = 2.4, 8.8, H-4''''), 6.99 (1H, m, H-1'), 6.77 (4H, m, H-3'''', H-3''', H-5''', H-6'''), 6.37 (1H, dt, *J* = 1.6, 5.8 Hz, H-3'), 5.87 (1H, dt, *J* = 1.7, 5.8 Hz, H-2'), 4.96 (1H, m, H-4'), 4.40 (1H, s, H-3''), 4.05-3.77 (8H, m, H-5', H-2'''', 2 × CH_2O), 3.77 (3H, s, OCH_3), 2.99 (2H, t, *J* = 6.4 Hz, H-1''''); ^{13}C NMR (100 MHz, $CHCl_3$): δ 179.1 (C=S), 161.9 (C-4), 153.4 (C-4'''), 152.2 (C-2''''), 151.2 (C-1'''), 149.7 (C-2), 146.7 (C-6''''), 144.6 (C-6), 141.1 (C-4''''), 135.1 (C-3'), 129.2 (C-2'''), 126.1 (C-2'), 117.6 (C-3'''), 117.6 (C-5''''), 113.5 (C-3''''), 112.9 (C-6'''), 111.7 (C-5'''), 99.7 (C-2''), 95.5 (C-1''), 90.3 (C-1'), 87.7 (C-4'), 77.3 (C-5), 68.4 (OCH_2), 68.4 (OCH_2), 62.8 (C-5'), 59.3 (C-3''), 55.6 (OCH_3), 46.0 (C-2''''), 30.0 (C-1'''').

[d4U]-*di*PEGpropyne-[HI-236] (98)

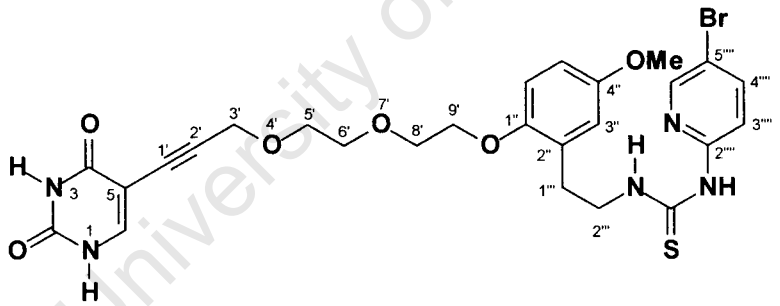


Nucleoside (0.047 g, 0.08 mmol) in methylene chloride (1.0 mL) was treated with trifluoroacetic acid (0.1 mL) at 0°C. The reaction was stirred for 30 min. Diisopropylethylamine (0.4 mL) was added and the solvent evaporated. The residue was dissolved in MeOH (1 mL), K_2CO_3 added and the mixture stirred for 10 min. The K_2CO_3 was filtered through a pad of Celite, washed with MeOH (3 × 10 mL) and the solvent evaporated *in vacuo*. The crude amine was reacted with thiourea derivative **89** (0.031 g, 0.1 mmol) in DMF (1.0 mL) at 100 °C for 1h. The crude product was

purified by column chromatography using ethyl acetate / hexane (8 / 2) to give a mixture of two products (0.034 g, 61%) as a white solid.

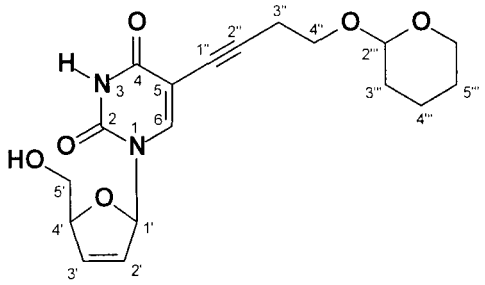
99: mp: 139-140 °C (from pet ether/CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.10 (1H, t, *J* = 5.1 Hz, NH), 8.86 (1H, brs, NH), 8.21 (1H, s, C-6), 8.03 (1H, d, *J* = 2.4 Hz, H-6''''), 7.69 (1H, dd, *J* = 2.4, 8.7 Hz, H-4''''), 6.97 (1H, m, H-1'), 6.84 (1H, d, *J* = 8.7 Hz, H-3''''), 6.74 (3H, m, H-3''', H-5''', H-6'''), 6.32 (1H, dt, *J* = 1.6, 5.9 Hz, H-3'), 5.84 (1H, dq, *J* = 1.6, 5.9 Hz, H-2'), 4.92 (1H, m, H-4'), 4.33 (2H, s, H-3''), 4.02 (6H, m, 3 × CH_2O), 3.84 (2H, m, CH_2O), 3.75 (5H, m, H-2'''', OCH_3), 2.98 (2H, t, *J* = 6.6 Hz, H-1''''); ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.1 (C=S), 161.6 (C-4), 153.8 (C-4'''), 151.8 (C-1'''), 151.3 (C-2''''), 149.8 (C-2), 146.6 (C-6''''), 144.9 (C-6), 141.1 (C-4''''), 135.0 (C-3'), 128.9 (C-2'''), 126.1 (C-2'), 117.6 (C-3'''), 113.7 (C-3''''), 112.9 (C-5''''), 112.7 (C-6'''), 111.7 (C-5'''), 99.4 (C-2''), 90.3 (C-1'), 89.4 (C-1''), 87.7 (C-4'), 76.7 (C-5), 70.7 (CH_2O), 70.0 (CH_2O), 69.2 (CH_2O), 68.6 (CH_2O), 62.8 (C-5'), 59.2 (C-3''), 55.6 (OCH_3), 45.8 (C-2''''), 30.0 (C-1'''').

[Uracil]-*di*PEGpropyne-[HI-236] (99a)



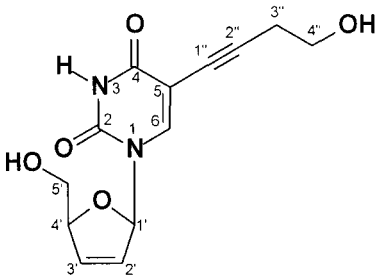
99a: mp: 119-123 °C (from pet ether/CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.18 (1H, t, *J* = 5.1 Hz, NH), 9.42 (1H, brs, NH), 8.02 (1H, d, *J* = 2.4 Hz, H-6''''), 7.65 (1H, dd, *J* = 2.4, 8.7 Hz, H-4''''), 7.59 (1H, s, H-6), 6.85 (1H, d, *J* = 8.7 Hz, H-3''''), 6.74 (3H, m, H-3'', H-5'', H-6''), 4.36 (2H, s, H-3'), 4.02 (6H, m, 3 × CH_2O), 3.85 (2H, m, CH_2O), 3.73 (5H, m, OCH_3, C-2'''), 2.98 (2H, t, *J* = 6.8 Hz, H-1'''); ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.0 (C=S), 163.0 (C-4), 153.7 (C-4''), 152.1 (C-2''''), 151.2 (C-1''), 149.4 (C-2), 146.5 (C-6), 144.9 (C-6''''), 141.1 (C-4''''), 128.9 (C-2''), 117.7 (C-3''), 113.9 (C-3''''), 112.9 (C-6''), 112.9 (C-5''''), 111.6 (C-5''), 90.4 (C-2'), 87.7 (C-1'), 77.4 (C-5), 70.7 (CH_2O), 69.9 (CH_2O), 69.2 (CH_2O), 68.5 (CH_2O), 59.2 (C-3'), 55.6 (OCH_3), 45.7 (C-2'''), 30.0 (C-1''').

5-[4-(2-Tetrahydropyranyloxy)-but-1-ynyl]-2',3'-didehydro-2',3'-dideoxyuridine (140)



According to GP1: Iodo-d4U **101** (0.100 g, 0.3 mmol), alkyne **148** (0.101 g, 0.66 mmol), Pd(0) (0.038 g, 0.03 mmol), CuI (0.031 g, 0.16 mmol), Et_3N (0.09 mL, 0.7 mmol), DMF (2.5 mL), THF (5 mL). Purification by column chromatography using EtOAc / hexane (3/2) furnished **140** as a colourless oil (0.041 g, 37%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.10 (1H, brs, NH), 8.02 (1H, s, H-6), 6.96 (1H, m, H-1'), 6.33 (1H, dt, *J* = 1.6, 5.9 Hz, H-3'), 5.83 (1H, dd, *J* = 1.6, 5.9 Hz, H-2'), 4.91 (1H, brs, H-4'), 4.64 (1H, m, H-2'''), 3.83 (4H, m, 1 × H-4'', 1 × H-5', 2 × H-6'''), 3.61 (1H, m, H-4''), 3.50 (1H, m, H-5'), 2.64 (2H, t, *J* = 7.0 Hz, H-3''), 2.10 (1H, brs, OH), 1.79 (1H, m, CH_2), 1.70 (1H, m, CH_2), 1.56 (4H, m, 2 × CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.8 (C-4), 149.8 (C-2), 143.7 (C-6), 134.9 (C-3'), 126.1 (C-2'), 100.3 (C-2''), 98.9 (C-2'''), 91.5 (C-1''), 90.2 (C-1'), 87.5 (C-4'), 72.2 (C-5), 65.7 (C-4''), 63.0 (C-6'''), 62.4 (C-5'), 30.5 (CH_2), 25.4 (CH_2), 21.1 (C-3''), 19.4 (CH_2).

5-(4-Hydroxybut-1-ynyl)-2',3'-didehydro-2',3'-dideoxyuridine (140a)



p-Toluenesulfonic acid (cat.) was added to a solution of **140** (0.041 g, 0.12 mmol) in MeOH (1 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min. The solvent was removed *in vacuo* and the crude product purified by column chromatography employing EtOAc / hexane (4/1) to give **140a** as a colourless oil (0.010 g, 27%). ^{1}H NMR (300 MHz, CD_3OD): δ 8.13 (1H, s, H-6), 6.93 (1H, m, H-1'), 6.42 (1H, dt, *J* = 1.6, 6.2 Hz, H-3'), 5.91 (1H, dq, *J* = 1.6, 6.2 Hz, H-2'), 4.88 (1H, m, H-4'), 3.76 (2H,

m, H-4''), 3.67 (2H, m, H-5'), 2.56 (2H, t, J = 6.6 Hz, H-3''); ^{13}C NMR (75 MHz, CD_3OD): δ 164.9 (C-4), 151.8 (C-2), 145.5 (C-6), 136.4 (C-3'), 127.0 (C-2'), 100.8 (C-2''), 92.1 (C-1''), 91.5 (C-1'), 89.3 (C-4'), 73.5 (C-5), 63.6 (C-4''), 61.5 (C-5'), 24.5 (C-3'').

Anti-HIV Evaluation

1×10^5 MT-2 cells per millilitre were infected with HIV-1 IIIB at a 0.1 multiplicity of infection (MOI). 100 μL of this solution was mixed with each serial dilution of inhibitor in triplicate on a 96-well plate. Mock infected cells were also mixed with inhibitor in a similar manner. After five days of incubation, a cell-permeable tetrazolium dye (MTT) was added. The MTT reaction was stopped after 5 h by adding acidified isopropanol. The plates were gently shaken overnight, quantitated on a plate reader and the absorbance measured at 595 nm. The absorbance values were then plotted versus inhibitor concentration to generate EC_{50} and CC_{50} values. The 50% effective concentration (EC_{50}) and 50% cytotoxic concentration (CC_{50}) of the test compounds were defined as the compound concentrations required to inhibit cell viability (MT-2) by 50% or to reduce by 50% the number of viable cells in mock-infected cell cultures, respectively.

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