CHAPTER 2

Synthesis of Nα-(Purinyl/Pyrimidinyl acetyl)-4-Amino Proline with Potential Use in Chiral PNA Synthesis
2.1 Introduction

There is a constant quest to search for better drugs that are specific and have less side effects. In this aspect, Peptide Nucleic Acid (PNA) invented by Nielsen et al.,¹ have carved a place as one of the forerunners in antisense/antigene therapeutics. PNA has emerged as a novel nucleic acid mimic that binds DNA/RNA with high efficiency and maintains the sequence specificity. These properties, along with their stability towards nucleases and proteases and ease of synthesis in large scale has made PNAs very attractive candidates for antisense technology.²,³ Recently there is an influx of reports on modification of PNA to overcome some of the drawbacks observed in PNA as already discussed in the previous chapter. Attempts aimed at structural modifications to improve PNA properties for modulating DNA/RNA recognition and facilitating biological applications have centered around modification of PNA backbone⁴,⁵ and introduction of asymmetry into achiral PNA (Figure 1). Nielsen et al. have introduced a chiral amino acid,⁶ D/L-lysine, at the end of the PNA 1a and have shown that this induces chirality in the whole molecule to attain a helical structure, whose sense depends on the chirality of the amino acid. Koch et al.⁷ have prepared PNA-peptide chimera, 1b in which the peptide chain induces chirality into the molecule. Bergmann et al.⁸ have synthesized DNA-PNA chimeras⁹,¹⁰ 1c on CPG resin. In all these cases, the asymmetry is achieved by terminal modification. Garner,¹¹ Taddie¹² and Diederichsen¹³ have introduced the nucleobase through different spacers on α-C of glycine 2a-c, and peptides were prepared using these monomers alternately with other amino acids like glycine. Dueholm et al.¹⁴ and Kosynkina et al.¹⁵ have introduced chirality by incorporation of various amino acids into the PNA backbone itself as shown in structure 3a,b. Nielsen et al.¹⁶ have also prepared a flexible, positively charged analogue by attaching the nucleobase to N(2-aminoethyl)glycine unit through an ethyl side chain as in 4. While the present work was in progress,¹⁷ Lowe et al.¹⁸ reported introduction of the nucleobase at 4-position of 4-hydroxy-L-proline as in 5 and used this in combination with glycine to build PNA. Most of these modifications have shown interesting, but not substantial alterations, in the properties of the resulting peptides as compared to PNA.
2.1.1 Rationale for present work

In the present work it was envisioned to incorporate chirality and controlled
rigidity simultaneously into the PNA backbone to modulate the bio/physico-chemical
properties. This, in principle can be done by bridging the \( \alpha / \beta \)-C of ethylenediamine unit of
PNA with the \( \alpha'' \)-C of glycine unit (A and B, Figure 2) or the \( \alpha' / \beta' \)-C on sidechain to
which nucleobase is connected (C and D, Figure 2). These types of structural
modifications introduce conformational constrains into the backbone and create asymmetric centers. While A/B approach creates two asymmetric centers in the backbone, the approaches C/D generate only one chiral center in the backbone and one in the side chain. A common feature of all these modifications from a practical point is that, they can all be derived from the easily available trans-4-hydroxy-L-proline 6. For the sake of simplicity and straightforwardness, the approach A has been explored in this work.

![PNA monomer unit](image)

**Figure 2**

The objectives of this chapter are:

(i) Synthesis of N\textsuperscript{\alpha}-(thymin-1-yl)-4-aminopropyl diastereomers for use in PNA synthesis.


(iii) Synthesis of dipeptides using prolyl monomers and

2.2 Present work

2.2.1 Synthesis of \((2R/S, 4R/S)-4-N(Fmoc)proline methyl ester\) (10)

\(\text{trans-4-Hydroxy-L-proline}\) is a naturally occurring amino acid of collagen. It has a five-membered heterocyclic ring with two chiral centers at C2 and C4, apart from having three functional groups (N1, 2-COOH and 4-OH), which are amenable to easy modification. Further, the oxidation of 4-hydroxy to 4-keto enables functionalisation of C3 and C5 which are \(\alpha\) to the 4-keto moiety. The possibility of such derivatisation has made this compound versatile. This is reflected in several reports on synthesis of various chiral natural products and chiral molecules using this as starting material, well summarised in a recent review by Remuzon.\(^\text{19}\) In the present work, the synthesis of all four chiral building blocks for prolyl PNA was achieved by sequential and systematic inversion at C-2 and C-4 centers leading to all 4 diastereomers of 4-hydroxyproline.

2.2.2 \(2S, 4S\)-N(Fmoc)-N\(^\alpha\)(\(\text{-Boc}\))proline methyl ester

The synthesis of \((2S, 4S)-4-N(\text{Fluorenylmethoxycarbonyl})-N\(^\alpha\)(\text{thymin-1-yl})\) proline methyl ester (11) was achieved in 6 steps (Scheme 1) starting from \(\text{trans-4-hydroxy-L-proline}\) (6). The compound 6 was converted to its methyl ester by dissolving it in refluxing methanol in the presence of \(\text{SOCl}_2\). The resulting proline methyl ester was isolated as hydrochloride and then protected at the ring nitrogen using Boc-azide to obtain \((2S, 4R)-4\)-hydroxy N\(^\alpha\)(\(\text{-butoxycarbonyl}\))proline methyl ester (7). The compound 7 was converted to its 4-O-mesyl derivative 8 in very good yields by treatment with mesyl chloride in pyridine.
The appearance of a weak peak in IR at 2400 cm\(^{-1}\) and a downfield shift of \(\delta\) from 4.3 ppm to 5.2 ppm H4 in the \(^1\)H NMR indicated the formation of mesyl derivative 8. The 4R mesylate 8 was stirred with sodium azide in DMF to give the 4S-azido derivative 9 in almost quantitative yield (98%). This step is accompanied by an inversion of configuration at 4-position due to \(S_n2\) displacement reaction. The azide 9, shows a characteristic peak in IR at 2105 cm\(^{-1}\), along with the disappearance of H4 peak at 5.2 ppm and appearance of a new peak at 4.2 ppm in \(^1\)H NMR. The 2S,4S-azide 9 after hydrogenation gave the corresponding amine using 10% Pd/C as catalyst. The free amine thus obtained was protected using Fmoc-Cl by following the standard procedure to obtain N4 protected.
Figure 3: $^1$H NMR spectra of compounds a: 9(2S,4S) and b: 13(2S,4R) in CDCl$_3$. 
amino(N1-Boc)proline methyl ester 10 in moderate yield. This pathway with a single inversion step results in the overall transformation of 2S,4R proline (6) to 2S,4S proline derivative (10).

2.2.3 2S,4R-N(Fmoc)-Nα(t-Boc)proline methyl ester (14)

The synthetic methodology to prepare 14 from trans-4-hydroxy-L-proline (6) consists of two successive inversion steps, giving rise to overall retention of configuration at C4 position of the proline ring (Scheme 2). The (2S,4R)-4-hydroxy-Nα(t-butoxycarbonyl)proline methyl ester (7) was converted to 2S,4S-tosylate 12 under Mitsunobu conditions using DEAD, triphenylphosphine and methyl p-toluenesulfonate following the reported procedure. This step goes through inversion of configuration at C4. The diethylcarbodiimide formed during the reaction could not be completely removed by column chromatography and hence the impure tosylate was directly converted to azide which could be purified by successive silica gel column chromatographies.

![Chemical Structures](image-url)

**Scheme 2**
The 2S,4S tosylate 12 was treated with sodium azide in DMF to obtain the 2S,4R-azide 13 that was purified and characterized. This step is again associated with S_n2 inversion of the 4S-tosylate to 4R-azide leading to an overall retention of the configuration at C4 from compound 7. The hydrogenation of 2S,4R-azide 13 gave the free amine, which was treated with Fmoc-Cl as described earlier, to obtain the protected monomer 14 in moderate yield.

2.2.4 2R/4S-N(Fmoc)-N^t-Boc)proline methyl ester (20)

The previous procedures gave compounds with both (R, S) stereochemistries at 4-position while that at C2 was same as the starting material i.e., 2S. The synthesis of compounds with 2R configuration could be achieved by initial epimerization at C2.
Figure 4: $^{13}$C NMR spectra of compounds a: 19(2S,4R) and b: 23(2R,4R) in CDCl$_3$. 
Figure 5: a: $^{13}$C NMR and b: $^1$H NMR spectra of compound 15(2S,4R) in CDCl$_3$. 
This was done by treating 2S,4R-hydroxyproline with acetic acid and acetic anhydride followed by hydrolysis of the resulting lactone with 2N HCl to give 16 in good yield (Scheme 3). A similar set of reactions, as earlier described for the L isomer (2S,4R, 6) were used to obtain 4S,2R-derivatives 20; (i) esterification of carboxyl group in 16, (ii) N<sup>a</sup>-protection with Boc-azide gave the Boc methyl ester 17, (iii) conversion of 17 into 4R-mesyl derivative gave mesylate 18, (iv) treatment of this mesylate 18 with sodium azide/DMF (displacement with inversion) to give 4S-azido derivative 19 and (v) reduction of 19 to 4S-amino compound and subsequent protection with FmocCl to obtain fully protected 20.

2.2.5 2R/4R-N(Fmoc)-N<sup>a</sup>(t-Boc)proline methyl ester (24)

The last of the epimer 24 (4R,2R derivative) was obtained from 17 by following a path as in Scheme 4 identical to Scheme 2, which involved two successive inversion steps; (i) tosylation of 4R-hydroxy 17 function under Mitsunobu conditions yielded 4S-tosyl derivative 22, (ii) compound 22 in subsequent reaction with sodium azide underwent an inversion to yield the 4R-azido 23, with an overall retention of configuration at C4, (iii) the azide 23 was transformed to the fully protected 4R,2R aminoproline (24).

Thymine was treated with bromoethylacetate in presence of K<sub>2</sub>CO<sub>3</sub> to obtain thymin-1-yl ethylacetate, which was hydrolyzed with 2N NaOH to obtain thymin-1-yl acetic acid. The N<sup>a</sup> Boc group of 10 (scheme 1), 14 (scheme 2), 20 (scheme 3), 24 (scheme 4) were deprotected with 50% TFA/DCM to give the free secondary amine which was then coupled with thymin-1-yl acetic acid, following DCC-HOBT method, well established in literature for peptide coupling, to obtain the desired compounds 11, 15, 21 and 25 respectively in good yield (85%). Thus all possible stereoisomers of 4-amino-N<sup>a</sup>(thymin-1-yl acetyl) prolines which are the T-monomeric units for the designed 4-aminoprolyl nucleic acids were obtained starting from 6 with overall yields in the range of 28-30%.
2.3 Alternative method for monomer synthesis via a common precursor

The above strategy involving condensation of prolyl ring NH with N-carboxy-methylated nucleobase although successful for synthesis of T-monomers, presents problems for synthesis of monomers of other nucleobases, in particular, that of purines. In case of coupling of thymin-1-yl aceticacid, the yields are better with free amine of 10 and 14, than with the corresponding TFA-salts and Fmoc protection reaction yields were also low. This could be due to relative stereochemical disposition of substituents at 2 and 4 which may sterically hinder the coupling of ring NH with thymine acetic acid. An alternative route was explored towards a general synthesis of prolyl nucleic acid monomers which consisted of direct coupling of individual nucleobases (T, C, 6-Cl-2-aminopurine and A) with suitably protected N⁶-(bromoacetyl)proline. Such a strategy has been reported to be efficient in large scale synthesis of monomers of open chain peptide nucleic acids.²⁵
Figure 6: FAB-MS of compounds a: $21(2R,4S)$ and b: $25(2R,4R)$ in MNBA/LSIMS and NBA/LSIMS respectively.
Scheme 5

From a synthetic point of view, the combination of protecting groups as in the 4R-azido-N\textsuperscript{2}-(bromoacetyl)proline benzyl ester (28) is convenient, since in a subsequent
catalytic hydrogenation step, simultaneous transformation of 4-azido to 4-amino and deprotection of 2-benzyl ester to carboxylic acid can be achieved to get the free amino acid. It is also possible to selectively reduce 4-azido to 4-amino by catalytic transfer hydrogenation using ammonium formate/Pd-C without affecting the benzyl ester moiety. With this working rationale, 4R-hydroxy-L-proline 6 was converted into its benzyl ester by reaction with benzyl alcohol and p-toluenesulphonic acid (Scheme 5) followed by Boc protection of Nα using Boc-azole to get 26 which was transformed in two further steps to the 4R-azido derivative 27. The removal of Nα-Boc with TFA afforded the free amine, which upon treatment with bromoacetylchloride in presence of aq. Na₂CO₃, afforded the desired common intermediate 28. This reaction gave poor yields when triethylamine was employed as a base as per literature procedure²⁵ and the colored impurities from reaction mixture could not be easily removed by routine purification. The individual alkylation of the nucleobases thymine and 2-amino-6-chloropurine (precursor for guanine) with 28 was done by reaction of the two components in DMF in presence of K₂CO₃ as a base, followed by chromatographic purification to obtain 29 and 30 respectively, in excellent yields (95%). The alkylation of adenine and cytosine were achieved by reaction of the respective sodium salt (prepared by using NaH) with 28 to give corresponding azides 31 and 32 in moderate yields (Scheme 5). While the alkylation of thymine and cytosine is regiospecific, providing only N1-substitution products, that of purines is known²⁶ to be non-regiospecific leading to significant N7-substituted product along with the desired major product from N9-substitution. In the present work with adenine, N9-substituted product (31) was major. The N7-substituted product (<5%) was easily removed by chromatographic purification. For synthesis of G-monomer (34b), direct alkylation of guanine is not a facile reaction and hence 2-amino-6-chloropurine was alkylated with 28 to obtain exclusively the N9 regioisomer 30. The compound 29 was hydrogenated to the 4-amino-2-carboxylic acid derivative with 10% Pd-C and the amino acid derivative was protected with Boc-azole to obtain 33. Compounds 30-32 were then hydrogenated using ammonium formate and 10% Pd-C and protected with Boc-azole to get the protected monomers 34a-36 respectively. 34a was then hydrolyzed to the desired G-monomer 34b by 1N NaOH.
Figure 7: a: $^{13}$C NMR and b: $^1$H NMR spectra of compound 35(2S,4R) in DMSO-$d_6$ and CDCl$_3$ respectively.
Scheme 6
Figure 8: a: $^{13}$C NMR and b: $^1$H NMR spectra of compound 42 in CDCl$_3$.
This formally completed the synthesis of all the nucleobase 4\textit{R},2\textit{S} monomers required for building prolyl nucleic acids. This methodology coupled with that described in section 2.2 enables the synthesis of all possible 4-aminoproline stereoisomers, corresponding to N1 (T and C) and N9 (A and G) substitution with the four nucleobases.

2.3.1 Synthesis of T monomers for solid phase peptide synthesis

It was planned to synthesize homooligomers of these prolyl monomers and test their hybridizing capability with DNA oligomers. Solid phase peptide synthesis was adopted for this purpose (see Chapter 3) and the Boc-protected monomers were synthesized as follows. The azides 9 and 19 were deprotected at N\textsuperscript{o} with TFA as discussed earlier and the resulting amine was reacted with excess bromoacetylchloride in presence of aqueous Na\textsubscript{2}CO\textsubscript{3} in dioxane to obtain the bromo derivative 37 and 38 respectively in 68% yield (Scheme 6). Compounds 37 and 38 were stirred with thymine and K\textsubscript{2}CO\textsubscript{3} to obtain the 4-azido thyminyl prolines 39 and 40 respectively in good yield. These azides 39 and 40 were further hydrogenated to the free amine and protected with Boc-azide to obtain the 4-N(\textit{t}-butoxycarbonyl)-N\textsuperscript{o}(thymin-1-yl) proline methyl esters (41, 42) which were purified by column chromatography. The hydrolysis of the esters 41 and 42 with 2N NaOH to obtain the corresponding acids 43 and 44 gave a very low yield when neutralized with 4N HCl as reported earlier.\textsuperscript{22} Alternatively when Dowex-50 H\textsuperscript{+} was used for neutralization of the resulting Na salt of the acid, the yields were excellent. The methyl ester 41 and 42 were similarly hydrolyzed to obtain pure acids 43 and 44. The optical rotation of 44 obtained from NaOH hydrolysis and 33 obtained by hydrogenation gave equal but opposite rotation, indicating no observable racemization. These were used for solid phase peptide synthesis.

2.4 Synthesis and characterization of chiral PNA dimers 48 and 49

To examine the efficacy of synthesized monomers in polymer building reactions, the syntheses of dipeptides 48 and 49 were accomplished from 11\textit{a}, 11\textit{b} and 21. This was achieved by deprotection of 11\textit{a} and 21 with 2N aq. NaOH in methanol to give the corresponding acids 45 and 47. The neutralization of these acids was conveniently
achieved by adding Dowex-50 H⁺, to bring the reaction mixture to pH 7. This method of neutralization resulted in excellent yields (Scheme 7).

Scheme 7
The deprotection of C4 amino function in 11b with piperidine gave the common amino component 46. The acid component 45 was condensed with amine 46 in the presence of DCC-HOBT to give the dipeptide 49a (NH-4S,2S;4S,2S-OH) with all cis conformation. The acid component 47 was then condensed with the amine 46 in presence of DCC-HOBT to give the prolyl PNA dipeptide 48a (NH-4S,2R;4S,2S-OH) with one trans conformation (Scheme 7). The structural integrity of these two dipeptides 48a and 49a was established by $^1$H NMR and the diagnostic mass peaks (M+1) at 723 and 811 respectively in FAB-MS. The dipeptide 48a was deprotected by hydrogenation to obtain the free amine 48b. Similarly the dipeptide 49a upon deprotection with piperidine gave the free amine 49b which was characterized by $^1$H NMR.

The coupling yield of these dipeptides was observed to be very low. In this context Farese et al. have also reported an alternative method where they couple the aminoethylglycine unit on the PNA monomer and then couple the thymine acetic acid to the backbone secondary amine of the same. This method gave better coupling yield for solution phase synthesis of short peptides as the yields of direct coupling of monomers are observed to be low perhaps due to the bulky base group.

2.4.1 Spectral properties: $^1$H and $^{13}$C NMR

All compounds including those obtained as the intermediates in earlier pathways were unambiguously characterized for structural purity by $^1$H and $^{13}$C NMR. In general NMR spectra of N1-acyl substituted compounds (both t-Boc and carboxymethylated nucleobases) showed characteristic presence of syn and anti rotamer mixtures originating from the restricted rotation about the tertiary amide bond (>N-CO). The energy barriers for such rotation are high, leading to resolution of syn and anti rotamers at ambient temperature on NMR time scale, as shown by a doubling of relevant resonances. An equilibrium composition of approximately 80:20 was noticed in most of the compounds in CDCl$_3$, although the assignment of specific resonances to individual rotamers was not possible due to complex and overlapping spectral patterns for prolyl ring protons in 200 MHz spectra. Meltzer et al. have shown by NOE correlation experiments that both E and Z form of isomers exist due to the tertiary amide linkages (Figure 11), and the ratio of
Figure 9: $^1$H NMR (500 MHz) spectrum of compound 48b in D$_2$O.
Figure 10: FAB-MS of dipeptides a: 49a and b: 48a in MNBA.
the two depends on the solvent. In D$_2$O, E and Z enantiomers are in 1:1 ratio and in DMSO-$d_6$ the E isomer predominates (80:20). The enantiomeric pairs (9:23)/(13:19) and (11a:25)/(15:21) exhibited equal but opposite optical rotations, thus establishing the optical purity of compounds maintained in all these pathways.

![E isomer](image1) ![Z isomer](image2)

**Figure 11**

$^{13}$C NMR is valuable in identification of N7/N9 regioisomers in alkylation reactions of purines. In case of 7- and 9- methyladenines it has been established that the N9 isomer exhibits downfield shifts of 6 and 4 ppm for C5 and C6 respectively and the

<table>
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<th>Compound</th>
<th>C2</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
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<td>159.8</td>
<td>111.7</td>
<td>151.9</td>
<td>145.9</td>
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<tr>
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<td>149.9</td>
<td>118.7</td>
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<td>149.7</td>
<td>118.5</td>
<td>155.5</td>
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</tr>
<tr>
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<td>118.2</td>
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</tr>
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<td>118.3</td>
<td>155.4</td>
<td>141.2</td>
</tr>
</tbody>
</table>

upfield shifts of 10 and 4.5 ppm for C4 and C8 compared to the corresponding chemical shifts of N7 regioisomer. This pattern was also noticed with A-monomer of peptide-nucleic acids. The $^{13}$C NMR spectral data of compounds 31 and 35 prepared presently by alkylation of adenine was consistent with the data reported for N9-isomer (Table 1).
2.4.2 Circular dichroism of dipeptides 48b and 49b

For a preliminary examination of the structural effects induced by different relationships of the chiral centers in dipeptides 48b and 49b, the CD spectra of the prolyl PNA are shown in Figure 12. These show characteristically different Cotton effects in the region 240-300 nm, with 48b having only a negative CD band at 265 nm and 49b both negative and positive CD-bands at 288 and 255 nm respectively. These bands though weak, are completely absent in open chain PNA molecules\textsuperscript{27} and must arise in 48b and 49b due to asymmetry in nucleobase stacking induced by the chiral peptide backbone. Substantial differences are also noticed in relative CD patterns in the region 210-230 nm, originating from stereochemical differences in the peptide linkage. The two dipeptides differ in prolyl ring stereochemistry at only one center and it is remarkable that this single change affects the sign, amplitude and band position of the CD spectrum. A detailed discussion on CD data of PNA oligomers is presented in the next chapter.

\[ \text{Figure 12: CD spectra of PrNA dipeptides 48b and 49b.} \]
2.5 Synthesis of PNA monomers

PNA is known to show self-ordered helicity due to induced helical structure arising from terminal chiral center of the lysine amino acid. Hence, incorporation of the prolyl nucleic acid moiety on the terminal end of the PNA oligomer would be interesting. With this rationale, PNA monomers (53 - 56) were synthesized by a shorter route as shown in the Scheme 8. The advantage of this scheme is that purification was carried out at a later stage when 52 was obtained by column chromatography (60% from Boc azide).

Diaminoethane was converted to its mono-N-t-Boc derivative 50 by treating it with 0.1 equivalent of Boc-azide under high dilution conditions. The di-t-Boc derivative being insoluble in water can be easily removed. When compound 50 was alkylated efficiently with ethylbromoacetate in presence of one equivalent of K₂CO₃ as base under dilute condition, it was possible to get aminoethylglycine 51 in good yield. This was further treated with bromoacetyl chloride to yield the corresponding bromo derivative 52. In this case the method followed by Meltzer et al. using TEA as base gave poor yield and the resulting material was highly colored. Alternatively using aq. Na₂CO₃ in dioxane as the base and by adding excess bromoacetyl chloride the corresponding bromocompound was obtained. The colorless oil obtained after chromatographic purification was used in alkylation of the base. The intermediate 52 can be stored at 0 °C and used as required.

The alkylation of 52 with thymine and cytosine is established to be regiospecific. Thymine was reacted with 52 using K₂CO₃ as base to obtain 53a in high yield. In case of cytosine, the N4-amino group was protected as carboxybenzyl derivative, and used for alkylation employing NaH as the base to provide N1-substituted product 55a. Although adenine is known to form both N7 and N9-substitution, N7-alkylation product was not present when NaH was used as the base to form sodium-adenalide, which was then reacted with 52 to obtain 52a in moderate yield.
Scheme 8
Figure 13: a: $^1$H NMR and b: $^{13}$C NMR spectra of compound 57a in CDCl$_3$ and DMSO-$d_6$ respectively.
The alkylation of 2-amino-6-chloropurine with 52 was smooth with K$_2$CO$_3$ as the base to yield the corresponding 56 in excellent yields. All the compounds exhibited $^1$H & $^{13}$C NMR data which is consistent with the reported data.\textsuperscript{22} 2-Amino-6-chloropurine-aeg 52 was hydrogenated with 10% Pd-C to yield 2-amino purine derivative 57a which is a fluorescent monomer. The compounds 53a, 54a, 55a, and 57a were hydrolysed to the corresponding acids 53b, 54b 55b, and 57b, which were used for solid phase synthesis. The fluorescent 57b was further incorporated in PNA using solid phase synthesis to obtain a fluorescent PNA. Since the solid phase peptide synthesis was carried out manually and the completion of the reaction was estimated, the capping step was avoided. The 6-amino of 54b and 2-amino of 57b did not require protection as they were tested to be unreactive during peptide coupling conditions.

2.6 Conclusions

In this chapter, the design and preparation of stereochemically defined monomer building blocks, based on 4-aminoproline backbone, which are required for synthesis of conformationally rigid, polychiral PNA is demonstrated. The possible effects of stereostructural constrains on the backbone is preliminarily seen as induced chirality in base stacking in the model dipeptides which exhibit significant differences in their CD patterns upon change of stereochemistry even at a single site on backbone. The conformationally defined building blocks reported here with different nucleobases (A, T, C and G) when used in appropriate rational combinations, would lead to a library of chiral PNAs with diverse backbone geometry, that are useful for hybridization screening with appropriate DNA/RNA targets. It is also possible to design the homologues of these monomers (see Figure 2) from some of the intermediates, to access prolyl PNAs with controlled extension of backbones. The solid phase synthesis of homostereooligomers from these monomeric units to generate polychiral PNAs and evaluating their biophysical properties are discussed in the next chapter.
2.7 Experimental

All reagents were obtained from commercial sources and used without further purification. NaH was obtained from Aldrich as a 60% suspension in paraffin oil and the paraffin coating was washed off with pet-ether before use to remove the oil. All the solvents were dried according to literature procedures. Infrared spectra were recorded on a Perkin Elmer 599B instrument. $^1$H NMR (200 MHz), $^{13}$C NMR (50 MHz) spectra were recorded on Bruker ACF200 spectrometer fitted with an Aspect 3000 computer. All chemical shifts are with reference to TMS as an internal standard and are expressed in $\delta$ scale (ppm). The values given are directly from the computer printout. TLCs were carried out on (E.Merck 5554) precoated silicagel 60 F254 plates. TLCs were visualised with UV light and/or ninhydrin spray, followed by heating. Optical rotations were measured on JASCO DIP-181 polarimeter and CD spectra were obtained on a JASCO J600 instrument. All TLCs were run using DCM containing appropriate amounts of methanol. In NMR spectra that show splitting of peaks due to presence of rotameric mixtures, arising from the tertiary amide linkage, the major rotamer is designated as $\text{ma}$ and the minor rotamer as $\text{mi}$. The ratio of major:minor rotamers is 80:20 unless otherwise mentioned. In cases where minor isomer is $<10\%$ (not prominent), only the peaks of major rotamer are reported. Melting points of the compounds reported are uncorrected.

2$S,4R$-Hydroxy-$N^2$(t-butoxycarbonyl)proline methyl ester (7): $\text{trans-4}$-Hydroxy-L-proline 6 (5.2 g, 39.6 mmol) was suspended in dry methanol and cooled in an ice bath. Thionyl chloride (3.1 mL, 41.6 mmol) was added slowly with vigorous stirring and the resulting solution was refluxed for 6 hr. The solvent was removed under reduced pressure using KOH trap and the solid obtained was further dried under high vacuum to obtain the methyl ester hydrochloride as white crystalline solid (6.8 g, 95%). This methyl ester hydrochloride (6.5 g, 35 mmol) was dissolved in 50 mL of dioxane:water (1:1) and Boc-azide (5.8 g, 41 mmol) was added dropwise followed by 8.5 ml of TEA. The mixture was stirred at ambient temperature for 16 hr. The resulting solution was concentrated to half its volume, extracted with ether (50 mL x 4), the ethereal layer was washed with brine and the solvent evaporated to yield compound 7 (8.01 g, 90%).
IR (neat): 3400, 2980-2900, 1735 and 1670 cm⁻¹. ¹H NMR, (CDCl₃ +D₂O) δ: 4.48-4.40 (m, 2H, H₂ and H₄), 3.72 (s, 3H, COOCH₃), 3.60-3.44 (m, 2H, H₅A & H₅B), 2.4-2.32 (m, 1H, H₃A), 2.08-2.00 (m, 1H, H₃B) and 1.45-1.42 (d, 9H, 3 x CH₃).

2S,4R-O-Methysulphonate-N²(t-butoxycarbonyl)-proline methylester (8): Mesyl chloride (1.15 mL, 14.6 mmol) was added dropwise into a solution of compound 7 (3.0 g, 12.2 mmol) in dry pyridine (50 mL) at 0 °C with constant stirring. After the addition was complete, the ice-bath was removed and stirring continued at ambient temperature for 2 h. Pyridine was removed under reduced pressure and the residue was taken in water and extracted with DCM. The organic layer was washed with dil. aq KHSO₄ (2 x 50 mL), brine (50 mL) and dried over Na₂SO₄. The organic solvent was evaporated to yield a colorless oily substance which solidified at low temperature. The mesylate 8 (3.75 g, 96 %) obtained was used without further purification.

IR (nujol): 3019, 2400 (w, OMs), 1740, 1690, 1405, and 1215 cm⁻¹. ¹H NMR, (CDCl₃) δ: 5.32-5.22 (m, 1H, H₄), 4.52-4.35 (m, 1H, H₂), 3.85-3.73 (m, 2H, H₅A & H₅B), 3.75 (s, 3H, COOCH₃), 2.72-2.52 (br, 1H, H₃A), 2.35-2.17 (m, 1H, H₃B), 1.48 & 1.43 (s, 9H, 3 x CH₃).

2S,4S-Azido-N²(t-butoxycarbonyl)proline methyl ester (9): The mesylate 8 (3.2 g, 9.9 mmol) was taken in dry DMF (20 mL) to which NaN₃ (1.6 g, 24.7 mmol) was added and the mixture was stirred at 50 °C for 4.5 hr. The removal of DMF under vacuum, gave a residual oil which was suspended in water (100 mL) and the product was extracted with ether (60 mL x 3). The residue (2.45 g) obtained after removal of ether was purified by silica gel column chromatography using pet-ether/ethylacetate (0-50 %) eluant system to yield the azide 9 as a colourless oil (2.54 g, 94 %).

IR. (neat): 3010, 2105 (s, N₃), 1730, 1702 and 1399 cm⁻¹. ¹H NMR, (CDCl₃) δ: 4.43 (dq, J=6.0, 8.8, 5.3 Hz, H₂), 4.33 (br-m, 1H, H₄), 3.76 (s, 3H, OCH₃), 3.74 (m, 1H, H₅A), 3.48 (m, 1H, H₅B), 2.47 (m, 1H, H₃A), 2.17 (dt, J=4.3, 1.3 Hz, 1H, H₃B), 1.47 (s, 9H, C(CH₃)₃). ¹³C NMR, (CDCl₃) δ: 172.2 (ma) and 171.9 (mi) (C(OOMe), 154.0 (mi) and 153.4 (ma) (OCON<), 80.5 (CMe₃), 59.3 (mi) and 58.3 (ma) (C₄), 57.7 (ma) and 57.4
(mi) (C2), 52.3 (OCH₃), 51.3 (mi) and 50.8 (ma) (C5), 36.0 (ma) and 35.1 (mi) (C3), 28.3 (C(CH₃)₃). [α]D²⁰ = -31.3° (c = 0.4, MeOH).

2S,4S-N(Fluorenylmethoxy carbonyl)-N'-(t-butoxycarbonyl)proline methyl ester (10a): The azide 9 (2.0 g, 7.4 mmol) was dissolved in methanol (10 mL) and 10 % Pd-C (0.2 g) was added. The mixture was agitated in Parr hydrogenation apparatus for 4 h, under 40 psi H₂ pressure. Pd-C was filtered off over celite. Methanol was removed to give cis-4-amino-L-proline derivative in quantitative yield. This was taken in dioxane (7.5 mL) containing 10% aq. Na₂CO₃ (10 mL) and cooled in an ice bath. Fmoc chloride (2.1 g, 8.5 mmol) was slowly added in portions at 0 °C and stirring was continued at 0 °C for 4 h. The reaction mixture was further stirred at ambient temperature for 16 hr. The reaction mixture was then concentrated to half its volume and water (50 mL) was added before extracting with ether (50 mL x 3). The pale yellow residue after removal of ether was purified on a silica gel column using pet-ether/ethyl acetate gradient as eluant to obtain 10a (2.2 g, 63 %) as a white solid.

¹H NMR, (CDCl₃) δ: 7.78 (d, J=8 Hz, 2H, Ar), 7.60 (d, J=8 Hz, 2H, Ar), 7.45-7.3 (m, 4H, Ar), 5.85 (br, 1H, NH-Boc), 4.45-4.15 (m, 5H, H₄, H₂, CH & OCH₂ of Fmoc), 3.80 (2s, 3H, COOCH₃), 3.80-3.48 (m, 2H, H₅A & H₅B), 2.62-2.43 (m, 1H, H₃A), 2.10-1.95 (m, 1H, H₃B), 1.52 & 1.47 (s, 9H, 3 x CH₃).

2S,4S-N(Fluorenylmethoxy carbonyl)-N'-(thymin-1-yl)proline methyl ester (11a): Compound 10 (2.02 g, 4.3 mmol) was taken in 50 % TFA/DCM (5 mL), and stirred at ambient temperature for 1 hr. The solvent was removed under reduced pressure using KOH trap and the free amine was taken in water and neutralized with 10 % aq. NaHCO₃ followed by repeated extraction with ethyl acetate (50 mL x 4). The combined organic layer was dried over anhydrous Na₂SO₄ and the solvent removed to obtain the free amine. The residual oil was desiccated over KOH for 12 h. The free amine (4.3 mmol), HOBT (0.54 g, 4.3 mmol) and thymine acetic acid (0.866 g, 4.8 mmol) were dissolved in dry DMF (10 mL). The solution was cooled in an ice bath, DCC (0.9 g, 4.5 mmol) was added and the reaction mixture was stirred for 1 h in an ice bath and then for 3 h at RT. The reaction mixture was filtered to remove DCU and washed thoroughly with cold DCM.
The collective filtrate was washed successively with 10% aq. Na₂CO₃ (50 mL x 2), dil. KHSO₄ (50 mL x 2) and brine (40 mL). The organic layer was separated, dried over anhydrous Na₂SO₄ and the solvent was removed under vacuum. The oily residue was purified by silica gel column chromatography using DCM/MeOH (0-10 %) gradient as the eluant to obtain the title compound 11 in pure form (1.69 g, 74.9%).

TLC: 10% MeOH/CH₂Cl₂ , Rf 0.4. ¹H NMR, (CDCl₃) δ: 8.62 (s, 1H, T NH), 7.76-7.26 (m, 8H, Fmoc), 7.07 (mi) & 7.05 (ma) (s, 1H, T H6), 5.98 (ma) (d, J = 11 Hz, C4 NH) & 5.30 (mi, br, C4 NH), 4.73-4.16 (m, overlapping signals, 7H, H2, H4, T CH₂, Fmoc CH and OCH₃), 4.0-3.57 (br-m, 2H, H5), 3.78 (ma) & 3.76 (mi) (s, 3H, COOCH₃), 2.62-2.45 (br-m, 1H, H3B), 2.21-2.04 (m, 1H, H3A) & 1.93 (s, 3H, T CH₃). ¹³C NMR, (CDCl₃) δ: 172.7(COO), 166.1 (mi) & 165.4 (ma)(>NCO), 164.4 (NCO), 155.8 (T C2), 151.3 (T C4), 141.2 (T C6), 143.5, 127-124, 119 (C Ar), 110.4 (T C5), 66.5 (CH₂), 57.9 (OCH₃), 52.6 (C2), 51.9 (T CH₂), 50.8 (C4), 48.6 (C3), 46.85 (CH), 34.5 (C3), 12.2 (T CH₃).

[α]D²⁰ (c = 0.4, MeOH) = -38.8°; FAB-MS:[M+1] = 533.

2S,4S-N(Carboxybenzyl)-N"(thymin-1-y1)proline methyl ester (11a): The compound 10b was deprotected with TFA and coupled with thymine acetic acid using DCC/HOBT coupling method, following the procedure for 11a to obtain 11b in good yield.

¹H NMR, (CDCl₃) δ: 8.92 (s, 1H, T NH), 7.26 (m, 5H, Ar), 7.07 (mi) & 7.05 (ma) (s, 1H, T H6), 5.98 (ma) 4.98 (s, 2H, Ar), 4.62-4.16 (m, overlapping signals, 4H, H2, H4, T CH₂), 3.98-3.57 (br-m, 2H, H5), 3.73 (ma) & 3.69 (mi) (s, 3H, COOCH₃), 2.61-2.42 (br-m, 1H, H3B), 2.27-2.06 (m, 1H, H3A) & 1.93 (s, 3H, T CH₃).

2S,4S-O-p-Toluenesulfonyl-N"(t-butoxycarbony1)proline methyl ester (12): Proline methyl ester 7 (3.5 g, 14.3 mmol) was taken in dry THF (100 mL) along with PPh₃ (4.6 g, 17.5 mmol). To the stirred mixture DEAD (3.1g, 17.8 mmol) in 20 mL of THF was added slowly at 0 °C and stirred for 2 min., followed by dropwise addition of methyl p-toluenesulfonate (3.3 g, 17.1 mmol) in 20 mL of THF. The resulting solution was stirred at 0 °C for 20 min. and then at room temperature overnight. Solvent was removed under vacuum and the residue was directly purified by repeated silica gel column
chromatographies. The purification was repeated to remove diethylhydrazine dicarboxylate to obtain the pure tosylate 12.

IR: (neat), 3092, 1750, 1705, 1592 and 1402 cm⁻¹. ¹H NMR, (CDCl₃) δ: 7.78 (d, J=8.0 Hz, 2H, Ar), 7.38 (d, J=8.0 Hz, 2H, Ar), 5.05 (br, 1H, H₄), 4.63-4.30 (m, 1H, H₂), 3.78-3.65 (m, 2H, H₅A & H₅B), 3.70 (s, 3H, COOCH₃), 2.52-2.21 (m, 2H, H₃A & H₃B), 2.45 (s, 3H, ArCH₃), 1.52 & 1.40 (s, 9H, 3 x CH₃).

2S,4R-Azido N⁴(β-butoxycarbonyl)proline methyl ester (13): The pure tosylate 12 was taken in DMF and reacted with NaN₃ (1.5 g, 22 mmol) as reported for the preparation of compound 8 to obtain the corresponding azide 13 (1.3 g) in pure form after column chromatography.

IR. (neat): 3010, 2105 (s, N₃), 1730, 1702 and 1399 cm⁻¹. ¹H NMR, (CDCl₃) δ: 4.36 (dt, J = 6.9, 7.6 Hz, H₂), 4.19 (m, 1H, H₄), 3.74 (s, 3H, OCH₃), 3.64 (dd, J = 5.3, 12 Hz, H₅A), 3.47 (dt, J = 3.8, 12 Hz, H₅B), 2.32 (m, 1H, H₃A), 2.16 (m, 1H, H₃B), 1.46 (ma) and 1.41 (mi) [s, 9H, C(CH₃)₃]. ¹³C NMR, (CDCl₃) δ: 172.5 (ma) and 172.2 (mi) (CO), 153.6 (mi) and 152.9 (ma) (NCO), 80.0 (CCH₃), 59.0 (mi) and 58.5 (ma) (C₄), 51.7 (OCH₃), 51.1 (mi) and 50.9 (ma) (C₅), 35.9 (ma) and 35.0 (mi) (C₃), 28.8 (C(CH₃)₃).

[α]D²⁰ = -60.9° (c = 0.4, MeOH).

2S,4R-N(Fluorenylmethoxycarbonyl)-N⁴(β-butoxycarbonyl)proline methyl ester (14): The azide 13 (1 g, 3.7 mmol) was hydrogenated with 10 % Pd-C (0.1 g) in methanol and the amine obtained was further protected with Fmoc-Cl by following the procedure reported for 10 to obtain the required compound 14 (1.08 g, 63 %) after column chromatographic purification.

¹H NMR, (CDCl₃) δ: 7.78 (d, J=8 Hz, 2H, Ar), 7.60 (d, J=8 Hz, 2H, Ar), 7.45-7.30 (m, 4H, Ar), 5.85 (br, 1H, NH), 4.45-4.15 (m, 5H, H₄, H₂, CH & OCH₂ of Fmoc), 3.80 (2s, 3H, COOCH₃), 3.80-3.48 (m, 2H, H₅A & H₅B), 2.62-2.43 (m, 1H, H₃A), 2.10-1.95 (m, 1H, H₃B) 1.48 & 1.45 (s, 9H, C(CH₃)₃).

2S,4R-N(Fluorenylmethoxycarbonyl)-N⁴(thymin-1-yl)proline methyl ester (15): The above compound 14 (0.8 g, 1.7 mmol) was deprotected with 50 % TFA/DCM (5 mL) to obtain the corresponding free amine (0.5 g, 79 %, 1.36 mmol) which was coupled with
thymine acetic acid (0.26 g, 1.45 mmol) in the presence of HOBT (0.18 g, 1.38 mmol) and DCC (0.3 g, 1.4 mmol) in DMF (20 mL) to afford the required compound 15 (0.53 g, 79.8 %).

$^1$H NMR, (CDCl$_3$), $\delta$: 9.75 (s, 1H, $T$ NH), 7.76-7.25 (m, 8H, Fmoc), 7.02 (mi) & 6.96 (ma) (s, 1H, $T$ H6), 6.34 (ma) & 6.12 (mi) (d, 1H, J = 7.4 Hz, C-4 NH), 4.82-3.89 (m, 7H, H2, H4, $T$ CH$_2$, Fmoc CH$_3$, OCH$_2$), 3.72 (s, 3H, COOCH$_3$), 3.80-3.66 (m, 2H, H$_5$A,B), 2.44 (mi) & 2.17 (ma) (br-m, 2H, H$_3$A,B), 2.1 (s, 3H, $T$ CH$_3$). $^{13}$C NMR, (CDCl$_3$), $\delta$: 172.2 (COO), 166.3 (mi) & 165.5 (ma) ($\angle$C=CO), 164.3 (NCOO), 156.1 ($T$ C2), 151.6 ($T$ C4), 141.3 ($T$ C6), 143.2, 127.8-124.5, 119.8 (all C-Ar) 110.8 ($T$ C5), 66.3(CH$_2$), 57.9 (OCH$_3$), 52.6 (C2), 51.4 ($T$ CH$_2$), 50.7 (C4), 48.9 (C5), 46.2(CH), 35.0 (C3), 12.2 ($T$ CH$_3$). [ $\alpha$ ]$_D^{20}$ = -22.5 (c = 0.4, MeOH). FAB-MS: [M+1] = 533.

$4R$-allo-Hydroxy-D-proline hydrochloride (16): To a solution of acetic anhydride (28 mL) and glacial acetic acid (85 mL) at 50 °C was added 4-hydroxy proline 6 (7.2 g, 54.9 mmol) and the mixture was refluxed for 6 h. After 6 h the mixture was cooled and solvents removed under reduced pressure to get a thick oil. This oil was taken in 2N HCl (100 mL) and refluxed for 3.5 hr. The reaction mixture was treated with activated charcoal and filtered. The aqueous solution was concentrated and the $4R$-allo-hydroxy-D-proline hydrochloride 16 was crystallized from water as fine white needles. $^{13}$C NMR, (D$_2$O) $\delta$: 172.6, 69.7, 59.1, 54.3 and 37.6.

$2R,4R$-Hydroxy-$N^\alpha$(t-butoxycarbonyl)proline methyl ester (17): The ($2R,4R$)-4-hydroxyproline hydrochloride 16 (6.5 g, 35.9 mmol) was protected as the corresponding Boc-methyl ester as described for the L-isomer 7 to the Boc-proline methyl ester 17 (6.98 g, 80 %).

IR (nujol): 3450, 2950, 2860, 1730, and 1660 cm$^{-1}$. $^1$H NMR, (CDCl$_3$) $\delta$: 4.39-4.26 (m, 2H, H2, H4), 3.79 (d, 3H, COOCH$_3$), 3.66-3.49 (m, 2H, H5), 2.37-2.25 (m, 1H, H3A), 2.12-2.04 (m,1H, H3B) and 1.46 & 1.45 (s, 9H, 3 x CH$_3$). $^{13}$C NMR, (CDCl$_3$), $\delta$: 70.4, 69.4, 57.5, 55.1, 54.6, 52.3, 52.1, 38.5, 37.8, 28.2 & 28.1.
2R,4R-O-Methylsulfonyl N\textsuperscript{\textprime}-(\textit{t}-butoxycarbonyl)proline methyl ester (18): The Boc-methyl ester 17 (2.4 g, 10 mmol) was reacted with mesyl chloride (1.2 mL, 15 mmol) in pyridine as in case of 8 followed by usual work up to obtain the mesylate 18 as a thick oil (2.92 g, 92 %).

IR (nujol): 3019, 2400(w, OMbs), 1740, 1690, 1405, and 1215 cm\textsuperscript{-1}. \textsuperscript{1}H NMR, (CDCl\textsubscript{3}) \( \delta \): 5.32-5.22 (m, 1H, H\textsubscript{4}), 4.52-4.35 (m, 1H, H\textsubscript{2}), 3.85-3.73 (m, 2H, H\textsuperscript{5}A & H\textsuperscript{5}B), 3.75 (s, 3H, COOCH\textsubscript{3}), 3.05 (s, 3H, SO\textsubscript{2}CH\textsubscript{3}), 2.72-2.52 (br, 1H, H\textsuperscript{3}A), 2.35-2.17 (m, 1H, H\textsuperscript{3}B), 1.48 & 1.43 (s, 9H, C(CH\textsubscript{3})\textsubscript{3}).

2R,4S-Azido-N\textsuperscript{\textprime}-(\textit{t}-butoxycarbonyl)proline methyl ester (19): Mesylate 18 (2.42 g, 7.4 mmol) and NaN\textsubscript{3} (1.25 g, 18.7 mmol) were stirred in DMF followed by standard workup to obtain the azide 19 (1.82 g, 91 %) following the procedure for the preparation of compound 9.

\textsuperscript{1}H NMR, (CDCl\textsubscript{3}) \( \delta \): 4.36 (dt, J = 6.9, 7.6 Hz), 4.19 (m, 1H, H\textsubscript{4}), 3.74 (s, 3H, OCH\textsubscript{3}), 3.64 (dd, J = 5.3, 12 Hz, H\textsuperscript{5}A), 3.47 (dt, J = 3.8, 12 Hz, H\textsuperscript{5}B), 2.32 (m, 1H, H\textsuperscript{3}A), 2.16 (m, 1H, H\textsuperscript{3}B), 1.46 (ma) and 1.41 (mi) (s, 9H, C(CH\textsubscript{3})\textsubscript{3}). \textsuperscript{13}C NMR, (CDCl\textsubscript{3}) \( \delta \): 172.5 (ma) and 172.2 (mi) (CO), 153.6 (mi) and 152.9 (ma) (NCO), 80.0 (C(CH\textsubscript{3})\textsubscript{3}), 59.04 (mi) and 58.5 (ma) (C\textsubscript{4}), 51.7 (OCH\textsubscript{3}), 51.1 (mi) and 50.9 (ma), (C\textsubscript{5}), 35.9 (ma) and 35.0 (mi) (C\textsubscript{3}), 28.80(C(CH\textsubscript{3})\textsubscript{3}). [\alpha]D\textsuperscript{20} = +60.9 (c = 0.4, MeOH).

2R,4S-N(Fluorenylmethoxycarbonyl)-N\textsuperscript{\textprime}-(\textit{t}-butoxycarbonyl)proline methyl ester (20): The azide 19 (1.03 g, 3.8 mmol) was reduced using H\textsubscript{2}, 10 % Pd-C (0.1 g) and the resulting amine was protected with Fmoc-Cl (1.225 g, 4.7 mmol) by following the procedure used for the L-isomer 10 to get the protected 4-amino-D-proline derivative 20 (1.32 g, 74 %).

\textsuperscript{1}H NMR, (CDCl\textsubscript{3}) \( \delta \): 7.78 (d, J=8 Hz, 2H, Ar), 7.60 (d, J=8 Hz, 2H, Ar), 7.45-7.30 (m, 4H, Ar), 5.85 (br, 1H, NH), 4.45-4.15 (m, 5H, H\textsubscript{4}, H\textsubscript{5}, CH & OCH\textsubscript{2} of Fmoc), 3.80 (2s, 3H, COOCH\textsubscript{3}), 3.81-3.48 (m, 2H, H\textsuperscript{5}A & H\textsuperscript{5}B), 2.62-2.43 (m, 1H, H\textsuperscript{3}A), 2.10-1.95 (m, 1H, H\textsuperscript{3}B), 1.48 & 1.45 (s, 9H, C(CH\textsubscript{3})\textsubscript{3}).

2R,4S-N(Fluorenylmethoxycarbonyl)-N\textsuperscript{\textprime}(thymin-1-yl)proline methyl ester (21): Compound 20 (1.1 g, 2.3 mmol) after Boc-deprotection with TFA/DCM gave the
corresponding free amine (0.71 g, 1.9 mmol) which was then coupled with thymine acetic acid (0.368, 2.1 mmol), in the presence of HOBT (0.25 g, 1.9 mmol) and DCC (0.45 g, 2.2 mmol) to give the desired compound 21 (0.79g, 82%) as in case of compound 11.

$^1$H NMR, (CDCl$_3$) δ: 9.82 (s, 1H, $T\text{ NH}$), 7.76-7.25 (m, 8H, Fmoc), 7.02 (mi) & 6.94 (ma) (s, 1H, $T\text{ H6}$), 6.40 (ma) & 6.18 (mi) (d, J = 7.4 Hz, C4 NHz), 4.83-3.89 (m, 7H, H2, H4, $T\text{ CH}_2$, Fmoc CH, CH$_2$), 3.71 (ma) & 3.73 (mi) (s, 3H, OCH$_3$), 3.78-3.72 (m, 2H, H5A,B), 2.44 (mi) & 2.22 (ma) (brm, 2H, H3A,B), 2.11 (s, 3H, $T\text{ CH}_3$). $^{13}$C NMR, (CDCl$_3$), δ: 171.8 (COO), 165.2 (mi) & 164.6 (ma) (>NCO), 163.2 (NCOO), 156.4 ($T\text{ C2}$), 151.9 ($T\text{ C4}$), 141.5 ($T\text{ C6}$), 143.3, 127.8-124.5, 119.5 (all $T\text{ Ar}$), 111.3 ($T\text{ C5}$), 66.3 (CH$_2$), 58.2 (OCH$_3$), 52.9 (C2), 51.9 ($T\text{ CH}_2$), 51.0 (C4), 49.2 (C5), 46.8 (CH), 35.4 (C3), 12.6 ($T\text{ CH}_3$). [α]$_D^{20}$ = +22.8 ° (c = 0.4, MeOH). FAB-MS: [M+1] = 533.

2R,4S-O-p-Toluenesulfonate-N$^{$(t$)$-butoxycarbonyl}$proline methyl ester (22): The Boc-methyl ester 17 (2.41 g, 9.7 mmol) was reacted with PPh$_3$ (3.16 g, 14.3 mmol), DEAD (2.10 g, 12.2 mmol) and methyl p-toluenesulfonate (2.25 g, 12.5 mmol) as in case of 12 to obtain the tosylate 22.

IR(neat): 3092, 1750, 1705, 1592 and 1402 cm$^{-1}$. $^1$H NMR, (CDCl$_3$) δ: 7.78 (d, J=8.0 Hz, 2H, Ar), 7.38 (d, J=8.0 Hz, 2H, Ar), 5.05 (br, 1H, H4), 4.63-4.30 (m, 1H, H2), 3.78-3.65 (m, 2H, H5A & H5B), 3.70 (s, 3H, OCH$_3$), 2.52-2.20 (m, 2H, H3A & H3B), 2.45 (s, 3H, ArCH$_3$), 1.52 & 1.43 (s, 9H, 3 x CH$_3$).

(2R,4R)-4-Azido-N$^{$(t$)$-butoxycarbonyl}$proline methyl ester (23): The tosylate 22 was reacted with NaN$_3$ (1.05 g, 15 mmol) in DMF (20 mL) to obtain the azide 23 (1.36 g) following the procedure as reported for compound 9.

IR. (neat): 3010, 2105 (s, N$_3$), 1730, 1702 and 1399 cm$^{-1}$. $^1$H NMR, (CDCl$_3$) δ: 4.43 (dq, J=6.0, 8.8, 5.3 Hz, H2), 4.33 (brm, 1H, H4), 3.76 (s, 3H, COOCH$_3$), 3.74 (m, 1H, H5A), 3.48 (m, 1H, H5B), 2.47 (m, 1H, H3A), 2.17 (dt, J=4.3, 1.3 Hz, 1H, H3B), 1.49 & 1.43 (s, 9H, C(CH$_3$)$_3$). $^{13}$C NMR, (CDCl$_3$ δ: 172.2 (ma) and 171.9 (mi) (COO), 154.0 (mi) and 153.4 (ma) (OCON<$), 80.5 (C(CH$_3$)$_3$), 59.3 (mi) and 58.3 (ma) (C4), 57.7 (ma) and
57.4 (mi) (C2), 52.3 (OCH₃), 51.3 (mi) and 50.8 (ma) (C5), 36.0 (ma) and 35.1 (mi) (C3), 28.3 (C(CH₃)₃). [α]D³⁰ = +31.3 (c = 0.4, MeOH).

2R,4R-N(Fluorenlymethoxy carbonyl)-N⁶(t-butoxy carbonyl) proline methyl ester (24): The azide 23 (1.1 g, 4 mmol) was reduced using H₂-10% Pd-C (0.1 g) and the resulting amine was protected with Fmoc-Cl (1.2 g, 4.5 mmol) by following the procedure used for the L-isomer 10 to get the protected aminoproline 24 (1.28 g, 68%).

¹H NMR, (CDCl₃) δ: 7.78 (d, J=8 Hz, 2H, Ar), 7.60 (d, J=8 Hz, 2H, Ar), 7.45-7.30 (m, 4H, Ar), 5.85 (br, 1H, NH), 4.45-4.15 (m, 5H, H4, H2, CH & OCH₂ of Fmoc), 3.80 (mi) & 3.78 (ma) (s, 3H, COOCH₃), 3.80-3.48 (m, 2H, H5A & H5B), 2.62-2.43 (m, 1H, H3A), 2.10-1.95 (m, 1H, H3B), 1.52 & 1.47 (s, 9H, C(CH₃)₃).

2R,4R-N(Fluorenlymethoxy carbonyl)-N⁶(thymin-1-yl)proline methyl ester (25): The free amine (0.62 g, 80%, 1.6 mmol) obtained from compound 24 (0.98 g, 2.1 mmol) after Boc-deprotection with TFA/DCM, was coupled with thymine acetic acid (0.31 g, 1.75 mmol), HOBT (0.2 g, 1.6 mmol) and DCC (0.35 g, 1.7 mmol) to afford the required compound 23 (0.74 g, 85%) by following the procedure as in case of 11.

¹H NMR, (CDCl₃) δ: 7.76-7.26 (m, 8H, Fmoc), 7.08 (mi) & 7.01 (ma) (s, 1H, T H6), 6.05 (ma) & 5.40 (mi) (d, 2H, J = 7.4 Hz, C4 NH), 4.75-4.15 (m, 7H, H2, H4, T CH₂, Fmoc CH, CH₂), 3.98-3.56 (br-m, 2H, H5), 3.77 (ma) & 3.75 (mi) (s, 3H, OCH₃), 2.50 (br-m, 2H, H3A,B), 1.93 (s, 3H, T CH₃). ¹³C NMR, (CDCl₃) δ: 172.7 (COO), 166.2 (mi), & 165.5 (ma) (>NCO), 164 (NCOO), 156.0 (T C2), 151.6 (T C4), 141.4 (T C6), 143.5, 127.4-124.7, 119 (C-Ar), 110.6 (T C5), 66 (CH₂), 58.1 (OCH₃), 52.8 (C2), 52.0(T CH₂), 51.0 (C4), 48.9 (C5), 46.8 (CH), 34.8 (C3), 12.3 (T CH₃). [α]D³⁰ = +38.63 , (c = 0.4, MeOH), FAB-MS: [M+1]=533.

2R,4S-Hydroxy-N⁶(t-butoxy carbonyl) proline benzyl ester 26: To trans-4-hydroxy-L-proline 6 (3.35 g, 25.0 mmol) was added benzyl alcohol (25 mL), p-toluene sulfonic acid (6.1 g, mmol) and toluene (100 mL) and the mixture was refluxed with continuous removal of water using Dean-Stark apparatus. The mixture was cooled and dry diethyl ether was added, upon which, white crystals of p-toluene sulfonate salt of proline benzyl ester
separated. The solvent was filtered off and the crystalline salt was taken in DMSO (30 mL) triethylamine (9.5 mL) and Boc-azide (3.5 g, 0.25 mmol) was added. The mixture was stirred overnight at ambient temperature. The resultant mixture was taken in equal amount of water, extracted with ether (50 mL x 3), and the organic layer was washed sequentially with KHSO₄ (50 mL x 2), water (50 mL), 10% aq. Na₂CO₃ (50 mL x 2) and water (50 mL). Upon concentration of ether layer compound 26 was obtained.

¹H NMR, (CDCl₃) δ: 7.25 (s, 5H, ArH), 5.31 (m, 2H, ArCH₂), 4.45 (m, 1H, H2), 4.23 (m, 1H, H4), 3.65 (m, 2H, H5A,B), 2.30-2.55 (m, 2H, H3), 1.38 and 1.28 (s, 9H, C(CH₃)₃).

2S,4R-Azido-N⁶-(t-butoxycarbonyl)proline benzyl ester 27: Compound 26 (1.7 g, 7 mmol) was taken with p-toluenemethylsulfonate (1.65 g, 8 mmol), PPh₃ (2.3 g, 8.7 mmol) and DEAD (1.55 g, 8.9 mmol) in THF (60 mL) and stirred at RT for 6 hr. The resulting mixture was concentrated and purified by silicagel column chromatography and the fractions containing the tosylate were collected. The solvent was removed by evaporation and the product (1.8 g, 4.6 mmol) and stirred with NaN₃ (1.05 g, 15 mmol) in DMF to obtain compound 27 (1.62 g, 92%).

¹H NMR, (CDCl₃) δ: 7.35 (s, 5H, ArH), 5.15 (m, 2H, ArCH₂), 4.45 (m) and 4.34 (m) (2 x t, 1H, J = 7.5 Hz, H2), 4.13 (brm, 1H, H4), 3.66 (m, 1H, H5A) 3.42 (m, dd, J=3.5, 11.4 Hz, H5B), 2.30 (m, 1H, H3A), 2.13 (m, 1H, H3B) 1.48 & 1.42 (s, 9H, C(CH₃)₃).

2S,4R-Azido-N⁶-(bromoacetyl)proline benzyl ester 28: Compound 27 (3.5 g) was treated with 50% TFA in DCM for 1 h and the residue obtained after evaporation of solvent was dissolved in dioxane (15 mL) containing 10% aq. Na₂CO₃ (25 mL, pH 8.0). The mixture was cooled in ice bath and bromoacetyl chloride was added in two portions (2.5 eq. each). The pH was then adjusted to 8.0 by addition of aq. Na₂CO₃. The mixture was concentrated to half its volume and extracted with DCM (50 mL x 4). The dried organic layer upon concentration and column chromatographic purification gave 28 (3.1 g, 80%) as a colorless oil.

¹H NMR, (CDCl₃) δ: 7.37 (s, 5H, ArH), 5.16 (m, 2H, ArCH₂), 4.72 (m) and 4.63 (m) (dt, 1H, J = 7.0 Hz, H2), 4.30 (m, 1H, H4), 3.92 (m, 1H, H5A), 3.65 (m, 1H, H5B), 3.81 (s, 2H, CH₂Br), 2.36 (m, 1H, H3A), 2.18 (m, 1H, H3B). MS: (M+1) = 368
2S,4R-Azido-N\textsuperscript{N\textsuperscript{a}}-(thymin-1-yl acetyl)proline benzyl ester 29: A mixture of 28 (1.25 g, 0.3 mmol), thymine (0.6 g, 0.3 mmol) and solid K\textsubscript{2}CO\textsubscript{3} (0.7 g, 0.3 mmol) in dry DMF (20 mL) was stirred at 25 C for 3 h. The residue obtained after aqueous work up was purified by column chromatography using DCM-MeOH (0-10%) as eluant to obtain 29 (yield 90%), as a white solid.

\(^1\)H NMR, (CDCl\textsubscript{3}) \(\delta\): 9.45 (brs, 1H, NH), 7.38 (m) and 7.32 (ma) (s, 5H, Ar-H), 7.05 (ma) and 6.85 (mi) (s, 1H, 7-CH), 5.20 (m, 2H, ArCH\textsubscript{2}), 4.84 (mi, q, J = 5.8, 7.8 Hz, H-2), 4.65 (ma, q, J = 13.7, 2.0 Hz, H2), 4.60 (d, 1H, J=16.2, 7 CHA), 4.37 (d, 1H, J=16.2 Hz, 7 CHB), 4.35 (ma) and 4.20 (mi) (m, 1H, H4), 3.92 (dd, 1H, J = 11.6, 5.8 Hz, H5A), 3.70 (dt, J = 11, 4.0 Hz, 1H, H5B), 2.35 (m, 2H, H3A,B), 1.95 (s, 3H, 7 CH\textsubscript{3}). \(^{13}\)C NMR, (CDCl\textsubscript{3}) \(\delta\): 170.8 (COO), 166.2 (CONH), 156.1 (T-C2), 151.6 (T-C4), 141.3 (T-C6), 136.0, 128.8, 128.4, 128.0 (all Ar-C), 111.1 (T-C5), 67.2 (Ar-CH\textsubscript{2}), 58.2 (C4), 52.4 (T-CH\textsubscript{2}), 48.8 (C5), 34.6 (C3), 12.5 (7-CH\textsubscript{3}).

2S,4R-Azido-N\textsuperscript{N\textsuperscript{a}}-(2-amino-6-chloro-purin-9-yl acetyl)proline benzyl ester 30: A mixture of 2-amino-6-chloro-purine (0.73 g, 4.3 mmol), compound 28 (1.1g, 4.3 mmol) and K\textsubscript{2}CO\textsubscript{3} (0.46 g, 4.3 mmol) was taken in DMF (10 mL) and stirred at RT for 6 hr. The solution was filtered and the filtrate was evaporated to yield a foam which was purified by column chromatography to obtain 30 (1.28 g) as pure material in 96% yield.

M.P. = 93 C. \(^1\)H NMR, (CDCl\textsubscript{3}) \(\delta\): 8.00 (mi) and 7.80 (ma) (s, H8), 5.31 (br-s, 2H, NH\textsubscript{2}), 5.23 (mi, m, ArCH\textsubscript{2}), 5.11 (dd, J=12.2, ArCH\textsubscript{2}), 4.78-4.62 (m, 1H, H2), 4.60 (mi) and 4.61 (ma, t, J = 7.6 Hz, NCH\textsubscript{2}), 4.35 (ma) and 4.20 (mi, m, H4), 3.89 (ma) and 3.81 (mi, dd, 1H, J = 3.1, 12.9 Hz, H5A), 3.64 (dd, J = 3.3, 10.1 Hz, H5B), 2.40 (mi), and 2.37 (ma, dq, 1H, J = 14.0, 7.5 Hz, H3A), 2.17 (dq, 1H, J = 14, 7.5 Hz, H3B). \(^{13}\)C NMR (DMSO-d\textsubscript{6}) \(\delta\): 170.2 (COO), 164.4 (>NCO), 159.1 (Pu-C2), 153.5 (Pu-C4), 150.5 (Pu-C6), 142.8 (Pu-C8), 134.7, 128.2-127.4 (Ar-C), 123.4 (Pu-C5), 66.5 (Ar-CH\textsubscript{2}), 59.2 (C2), 57.5 (CH\textsubscript{2}), 50.9 (C4), 44.0 (C5), 33.7 (C3). \([\alpha]\)\textsubscript{D} = -42.5 (c = 0.4, MeOH).

2S,4R-Azido-N\textsuperscript{N\textsuperscript{a}}-(adenin-9-yl acetyl)proline benzyl ester 31: To NaH (0.04 g, 2.6 mmol) in dry DMF, adenine (0.34 g, 2.6 mmol) was added and stirred at 75 C for 15 min till the effervescence ceased. The flask was cooled, bromo compound 28 (0.65 g, 2.6
mmol) in DMF (5 mL) was added and the mixture stirred at 75 °C for 1 hr. DMF was removed under vacuum and the residue was taken in water, extracted with DCM and purified by column chromatography using MeOH/DCM mixture to obtain the pure compound 31 in 36% (0.25 g) yield.

¹H NMR, (CDCl₃) δ: 8.31 (ma) and 8.30 (mi) (s, 1H, A H8), 7.93 (ma) and 7.81 (mi) (s, 1H, A H2), 7.38 (mi) and 7.33 (ma) (s, 5H, ArH), 6.36 (ma) and 6.26 (mi) (brs, 2H, A NH₂), 5.25 (mi) and 5.15 (ma) (2 x dd, J = 12.5 Hz, 2H, ArCH₂), 4.95 (ma, s, A CH₂) and 4.92 (mi, t, J = 7.5 Hz, A CH₂), 4.65 (t, 1H, J = 9 Hz, H2), 4.35 (ma) and 4.18 (mi) (m, 1H, H4), 3.92 (q, 1H, J = 5.2, 10 Hz, H5A), 3.71 (q, 1H, J = 2.5, 10 Hz, H5B), 2.35 (m, 2H, H3A,B). ¹³C NMR, (CDCl₃) δ: 170.6 (COOBn), 165.2 (mi) and 164.7 (ma) (>NCO), 155.5 (A C-6 & NCOO), 152.7 (A C2), 149.7 (A C4), 141.1 (A C8), 134.9-128.9 (all Ar-C), 118.5 (A C5), 68.02 (mi) and 67.1 (ma) (ArCH₂), 59.3 (C2), 57.9 (ma) and 57.2 (mi) (C4), 51.3 (A CH₂), 44.3 (ma) and 43.8 (mi) (C5), 36.4 (mi) and 34.08 (ma) (C3).

[α]D²⁰ = -44.0 (c = 0.3, MeOH); [M+1] = 421.

2S,4R-azido-N"-(cytosin-1-yl acetyl)proline benzyl ester 32: Reaction of cytosine (0.75 g, 6 mmol) in DMF with NaH (0.09 g, 5.9 mmol) and compound 28 (1.47 g, 5.8 mmol) following the above procedure gave 32 in 50% (0.38 g) yield after column purification.

M.P. = 85 °C, ¹H NMR, (CDCl₃+D₂O) δ: 7.45-7.15 (m, 6H, ArH and C H6), 5.9(d, J = 8.0 Hz, C H5), 4.95-5.53 (m, 2H, ArCH₂), 4.82-4.45 (overlapping multiplets, 3H, N-CH₃, H2), 4.35 (brm, 1H, H4), 3.95 (brm, 1H, H5B), 3.70 (brm, 1H, H5A), 2.05-2.55 (overlapping multiplet, 2H, H3A,B). ¹³C NMR, (CDCl₃) δ: 171.3 (COO), 166.7 (NCO), 155.0 (C2), 147.7 (C-C6), 136.2 (C5), 129-128 (Ar-C's), 94.6 (C4), 66.6 (Ar-CH₂), 60.0 (C2), 58.1 (C4), 52.0 (CH₂), 50.1 (C5), 34.4 (C3). [α]D²⁰ = -51.3 (c = 0.3, MeOH).

2S,4R-N-(t-butoxycarbonyl)-N"-(thymin-1-yl acetyl) proline 33: Compound 29 (2 g, 5.4 mmol) in MeOH was hydrogenated under pressure (30 psi) using 10%Pd-C (200 mg) as catalyst for 15 h. The catalyst was filtered off and the free amino acid product was isolated by evaporation of solvent. This compound (1.4 g, 5 mmol) was dissolved in dioxane:water (1:1, 20 mL), treated with Boc-azole (0.8 mL) and stirred at 25 °C for 24
h. with pH maintained at 9.0 by addition of 4N NaOH. The reaction mixture was concentrated to 10 mL, neutralized with Dowex 50H\(^+\) and filtered. Removal of solvent from the filtrate afforded the required product which was purified by crystallization from MeOH.

\(^1\)H NMR, (D\(_2\)O), \(\delta\): 7.39 (d, \(J = 1.2\) Hz, \(\text{T H6}\)), 4.70 (t, \(J = 12.1\) Hz, N-CH\(_2\)), 4.52 (t, \(J = 9.2\) Hz, H2), 4.36 (m, 1H, H4), 4.20 (m) and 3.95 (m) (dd, H5B), 2.25-2.50 (bt, 2H, H3A,B), 1.90 (s, 3H, \(\text{T CH}_3\)), 1.46 (s, 9H, C(CH\(_3\))\(_3\)). \(^13\)C NMR, (DMSO-\(d_6\)) \(\delta\): 175.6 (COOMe), 167.3 (NCO), 157.8 (T-C5), 152.7 (T-C4), 143.8 (T-C6), 111.4 (T-C5), 59.1 (OCH\(_3\)), 52 (C2), 50.6 (T-CH\(_2\)), 50.3 (C4), 49.8 (C5), 35.0 (C3), 12.3 (T-CH\(_3\)). \([\alpha]_D\)\(^{20}\) - 14.7 (c = 0.3, MeOH).

\(2S,4R\)-N-(t-butoxycarbonyl)-N\(^a\)-(A/G/C-yl acetyl)proline benzyl ester 34a-36: The 4R-azido compounds 29-31 (1.0 g) were individually dissolved in methanol (50 mL) and reacted with ammonium formate (4 eq) and 10% Pd-C (0.1 g) for 24 h, after which the catalyst was removed by filtration over celite. The filtrate was evaporated under vacuum and the residue dissolved in water:dioxane (1:1, 15 mL). This was treated with Boc-azide (1.5 eq) and TEA (1.5 eq) at 50 °C for 15 h, after which the products were isolated by chromatographic purification (yields 60-70%).

\(2S,4R\)-N-(t-butoxycarbonyl)-N\(^a\)-(2-amino-6-chloropurin-9-yl acetyl)proline benzyl ester 34a: \(^1\)H NMR, (CDCl\(_3\)) \(\delta\): 7.85 (ma) and 7.70 (mi) (s, 1H, G H8), 7.30 (m, 5H, ArH), 4.97-5.45 (overlapping signals, 4H, NH\(_2\) and ArCH\(_2\)), 4.82 (dd, 2H, NCH\(_2\)), 4.65 (t, \(J = 4.2\) Hz, H2), 4.40 (brm, 1H, H4), 4.05 (brm, 1H, H5A), 3.65 (brm, 1H, H5B), 2.45 (ma, m) and 2.22 (ma, t) (2H, H3A,B), 1.47 (s, 9H, C(CH\(_3\))\(_3\)). \([\alpha]_D\)\(^{20}\) = -20.5 (c = 0.2, MeOH).

\(2S,4R\)-N-(t-butoxycarbonyl)-N\(^a\)-(guanin-9-yl acetyl)proline benzyl ester 34b: A mixture of 34a (0.5g, 1 mmol) and 1N aq. NaOH (2 mL) was stirred at RT for 2 h after which it was neutralized with ion-exchange resin, Dowex 50H\(^+\). MeOH (5 mL) was added, the resin was filtered off and the product that slowly precipitated was collected (yield 60%).
1H NMR, (DMSO-d_6:D_2O) δ: 7.70 (s, 1H, G H8), 4.92 (q, 2H, J = 19.5 Hz, N-CH_3), 4.28 (q, 1H, J = 8.1, 4.8 Hz, H2), 4.18 (t, 1H, J = 7.2 Hz, H4), 3.84 (q, 1H, J = 9.6, 5.8 Hz, H5A), 3.40 (q, 1H, J = 9.6, 7.2, H5B), 2.10 (dq, 1H, H3A,B), 1.40 (s, 9H, C(CH_3)_3).

2S,4R-N-(t-butoxycarbonyl)-N''-(Adenin-9-yl acetyl)proline benzyl ester 35: 1H NMR, (CDCl_3) δ: 8.29 (s, 1H, A H8), 7.82 (ma) and 7.72 (mi) (s, 1H, H2), 7.32-7.36 (m, 5H, ArH), 6.48 (bri, 2H, NH_2), 5.75 (brd, ma) and 5.52 (br, mi, 1H, NHBoc), 5.25 (mi) and 5.13 (ma) (dd, 2H, ArCH_2), 4.90 (dd, J = 15.6 Hz, 2H, A CH_3), 4.64 (t, 1H, J = 5.8 Hz, H2), 4.36 (br, 1H, H4), 3.96 (dd, 1H, H5A), 3.78 (brm, 1H, H5B), 2.50-2.05 (brm, 2H, H3A,B), 1.49 (s, 9H, C(CH_3)_3). 13C NMR, (CDCl_3) δ: 171.4 (ma) and 171.1 (mi) (COOR), 165.5 (CONH), 155.4 (C6 and OCON<), 152.4 (A C2), 149.3 (A C4), 141.6 (A C8), 135.1-128 (Ar), 118.2 (A C5), 79.8 (CMe_3), 67.9 (mi) and 67.1 (ma) (ArCH_2), 57.8 (C2), 51.4 (C4), 50.0 (A NCH_2), 44.7 (C5), 34.8 (C3), 28.3 (C(CH_3)_3), [α]_D^20 = -20.7 (c = 0.3, MeOH).

2S,4R-N-(t-butoxycarbonyl)-N''-(cytosin-1-yl acetyl)proline benzyl ester 36: 1H NMR, (CDCl_3+D_2O) δ: 7.25-7.40 (overlapping signals, ArH, C H6), 5.90 (d, J = 6.36 Hz, 1H, C H5), 5.10 (overlapping q, 2H, ArCH_2), 4.55-4.95 (brm, 2H, N-CH_2), 4.15-4.50 (brm, 2H, H3A,B), 1.45 (s, 9H, C(CH_3)_3). [α]_D^20 = -49.6 (c = 0.3, MeOH).

General Procedure for preparation of 4-azido-N''-(bromoacetyl)proline methyl ester 37, 38: The 4-azido Boc proline methyl esters (9, & 19) were deprotected with 50% TFA/DCM and the TFA was removed by repeated evaporation with ether under vacuum using KOH trap. The free amines were taken in 10% Na_2CO_3 in dioxane/water 1:1, and bromoacetyl chloride (2.5 eq, each) was added in two lots. The starting material was consumed in 5 min and the reaction mixtures were brought to pH 8.0 by adding 10% aq. Na_2CO_3, and the required bromo compounds were extracted using DCM. Further column purification yielded the required materials 37 and 38 respectively in good yield (68-75%).

2S,4S-azido-N''-(bromoacetyl)proline methyl ester 37: 1H NMR, (CDCl_3) δ: 4.72-4.45 (br-m, 2H, H2, H4), 3.80-3.55 (m, 2H, H5A,B), 3.82 (s, 3H, COOCH_3), 3.72 (s, 2H, COCH_2Br), 2.40-2.01 (m, 2H, H3A,B). 13C NMR (CDCl_3), δ : 172.3 (COO), 165.9
(CON), 58.3 (O-CH₃), 55.7 (ma), 55.2 (mi) (COCH₂Br), 52.44 (mi) 52.38 (ma) (C₂), 51.8 (C₄), 37.62 (C₅), 27.0 (C₃). MS: (m/e) = 290.

2R,4S-azido-N"-(bromoacetyl)proline methyl ester 38: ¹H NMR, (CDCl₃) δ: 4.75-4.6 (br-m, 1H, H₂), 4.45-4.35 (m, 1H, H₄), 4.05-3.98 (m 1H, 5HA), 3.82(s, 2H, COCH₂Br), 3.76 (s, 3H, COOCH₃), 3.71-3.60 (m, 1H, H₅B), 2.50-2.25 (m, 2H, H₃A,B). ¹³C NMR, (CDCl₃) δ : 172.2 (COO), 165.8 (CON), 58.4 (O-CH₃), 55.7 (ma), 55.2 (mi)(COCH₂Br), 52.4 (C₂), 51.8 (C₄), 37.7 (C₅), 27.2 (C₃). MS: (M+1) = 291

4-Azido-N"-(thymin-1-yl acetyl)proline methyl ester 39, 40: General procedure: A mixture of compound 37/38 (1.25 g, 0.3 mmol), thymine (0.6 g, 0.3 mmol) and solid K₂CO₃ (0.7 g, 0.3 mmol) in dry DMF (20 mL) was stirred at 25 °C for 3 h. The solvent was removed and the product 39/40 respectively was obtained after usual workup. trans Isomer 40 was purified easily by crystallizing from methanol (yield 90%). cis Isomer 39 was purified by column chromatography by elution with DCM-MeOH to obtain product as white solid.

2S,4S-Azido-N"-(thymin-1-yl acetyl)proline methyl ester 39: ¹H NMR, (CDCl₃) δ : 9.2 (b, 1H, NH), 7.01 (s, 1H, T-6H), 4.72-4.63 (m, 1H, H₂), 4.46 (d, 1H, J = 16 Hz, TCHA), 4.34-4.25 (m, 1H, H₄), 4.23 (d, 1H, J = 16 Hz, TCHB), 3.81-3.73 (m, 1H, H₅A), 3.72 (s, 3H, OCH₃), 3.68-3.54 (m, 1H, H₅B), 2.52-2.43 (m, 1H, H₃A), 2.13-2.02 (m, 1H, H₃B), 1.97 (s, 3H, T-CH₃), 1.45 (s, 9H, C(CH₃)₃).

2R,4S-Azido-N"-(thymin-1-yl acetyl)proline methyl ester 40 : IR (neat): 3400, 2952, 2923, 2105, 1735, 1700, 1665, 1642, 1462 and 1441 cm⁻¹. ¹H NMR, (CDCl₃) δ : 6.95 (s, 1H, 7H6), 4.48 (d, 1H, J=18, T CHA), 4.20 (d, 1H, J=18, T CHB), 4.76-4.65 (mi) & 4.48-4.38 (ma) (m, 1H, H₂), 4.30-4.20 (ma) & 4.15-4.06 (mi) (m, 1H, H₄), 3.85-3.75 (m, 1H, H₅A), 3.71 (mi) & 3.63 (ma) (s, 3H, COOCH₃), 3.65-3.53 (m, 1H, H₅B), 2.42-2.25 (mi) & 2.23-2.05 (ma) (m, 2H, H₃A,B), 1.88 (s, 3H, T-CH₃). ¹³C NMR, (CDCl₃) δ: 175.6 (COOMe), 167.3 (NCO), 157.8 (T-5C), 152.7 (T-4C), 143.8 (T-6C), 111.4 (T-5C), 59.1 (OCH₃), 52.0 (C₂), 50.6 (T-CH₂), 50.3 (C₄), 49.8 (C₅), 35.0 (C₃), 12.3 (T-CH₃).
N4-(Butoxycarbonyl)-N'^-(thymin-1-yl acetyl)proline methyl ester 41, 42: General procedure: The azido compounds 39, 40 were converted to the corresponding amino compounds by hydrogenation using 10% Pd-C in methanol. The free amine was protected with Boc-azide to get the Boc derivative 41 and 42, which were purified by column chromatography to obtain pure material.

2S,4S-N-(t-Butoxycarbonyl)-N'^-(thymin-1-yl acetyl)proline methyl ester 41: $^1$H NMR, (CDCl$_3$) $\delta$: 9.40 (br, 1H, NH), 7.06 (s, 1H, TH6), 5.60 (d, OCONH), 4.68 (d, 1H, J=16, T CHA), 4.33 (d, 1H, J=16, T CHB), 4.52-4.42 (m, 2H, H4, H2), 3.78 (mi) & 3.73 (ma) (s, 3H, COOCH$_3$), 3.98-3.80 (m, 1H, H5A), 3.60-3.51 (m, 1H, H5B), 2.53-2.42 (m, 1H, H3A), 2.17-2.0 (m, 1H, H3B), 1.97 (s, 3H, T-CH$_3$), 1.49 (s, 9H, 3 x CH$_3$).

2R,4S-N-(Butoxycarbonyl)-N'^-(thymin-1-yl acetyl)proline methyl ester 42: $^1$H NMR, (CDCl$_3$) $\delta$: 7.05 (s, 1H, TH6), 4.80 (d, 1H, J=18, T CHA), 4.10 (d, 1H, J=18, T CHB), 4.70-4.55 (m, 1H, H2), 4.50-4.42 (m, 1H, H4), 3.95-3.83 (m, 1H, H5A), 3.73 (s, 3H, COOCH$_3$), 3.65-3.55 (m, 1H, H5B), 2.30-2.05 (m, 2H, H3A,B), 1.88 (s, 3H, T-CH$_3$), 1.45 (s, 9H, 3 x CH$_3$). $^{13}$C NMR (CDCl$_3$) $\delta$: 175.6 (COOMe), 167.9 (>NCO), 157.8 (T-C5), 152.7 (T-C4, NCOO), 143.8 (T-C6), 111.4 (T-C5), 81.8 (C(CH$_3$)$_3$), 59.1 (OCH$_3$), 52.0 (C2), 50.6 (C3), 50.3 (C4), 49.8 (C5), 35.0 (C3), 28.6 (C(CH$_3$)$_3$), 12.3 (T-CH$_3$).

Hydrolysis of Boc-methyl esters of 43, 44 General procedure: The Boc-methyl esters of 41 and 42 were hydrolysed using cold 2N aq NaOH in methanol. Hydrolysis was complete within 5-15 min. The solution was neutralized using Dowex-50H$^+$ until the pH of the solution was 7.0 and filtered. The filtrate was concentrated and the resultant pale yellow solid was precipitated from methanol/pet-ether mixture.

2S,4S-N-(t-Butoxycarbonyl)-N'^-(thymin-1-yl acetyl)proline 43: $^1$H NMR, (D$_2$O) $\delta$: 7.37 (s, 1H, TH6), 4.50 (d, 1H, J=18, T CHA), 4.10 (d, 1H, J=18, T CHB), 4.35-4.20 (m, 1H, H2), 4.07-3.95 (m, 1H, H4), 3.80-3.65 (m, 1H, H5A), 3.60-3.45 (m, 1H, H5B), 2.88-2.75 (mi) & 2.72-2.50 (ma) (m, 2H, H3A), 2.35-2.15 (mi) & 2.15-2.0 (ma) (m, 2H, H3,B), 1.88 (s, 3H, T-CH$_3$), 1.45 (s, 9H, 3 x CH$_3$).
2R,4S-N-(β-Butoxycarbonyl)-N’-(thymin-1-yl acetyl)proline 44: \(^1\)H NMR, (D\(_2\)O) δ: 7.37 (s, 1H, T-H6), 4.50 (d, 1H, J=18, 7C-CH\(_A\)), 4.12 (d, 1H, J=18, 7C-CH\(_B\)), 4.72-4.55 (m, 1H, H\(_2\)), 4.51-4.42 (m, 1H, H\(_4\)), 3.95-3.83 (m, 1H, H\(_5\)A), 3.73 (s, 3H, COOCH\(_3\)), 3.65-3.55 (m, 1H, H\(_5\)B), 2.31-2.05 (m, 2H, H\(_3\)A,B), 1.88 (s, 3H, T-CH\(_3\)), 1.45 (s, 9H, (CH\(_3\))\(_3\)).

2S,4S-N(Carboxybenzyl)-N’(thymin-1-yl)proline 45: The methyl ester 11b (100 mg) was hydrolysed with 2N NaOH and neutralised with Dowex 50H\(^+\) to obtain the free acid 45 (72 mg).

\(^1\)H NMR, (DMSO-d\(_6\)) δ: 7.25 (s, 5H, Ar), 6.9 (s, 1H, T-H6), 5.01 (s, 2H, Ar), 4.50-4.07 (b-m, 4H, 7C-H\(_2\), H\(_2\), H\(_4\) overlapping multiplets), 3.80-3.65 (m, 1H, H\(_5\)A), 3.60-3.45 (m, 1H, H\(_5\)B), 2.72-2.50 (bm, 2H, H\(_3\)A), 2.16 (m, 2H, H\(_3\)B), 1.88 (s, 3H, T-CH\(_3\)). FAB MS(M+1) = 431.

2S,4S-Amino-N’(thymin-1-yl)proline methyl ester 46: The compound 11a (200 mg) was deprotected by stirring with 20% piperidine/DMF solution (2 mL). The solvent was removed and desiccated over KOH.

\(^1\)H NMR, (D\(_2\)O) δ: 7.25 (s, 1H, T-H6), 4.80 (d, 1H, J=18, T CH\(_A\)), 4.10 (d, 1H, J=18, T CH\(_B\)), 4.70-4.55 (m, 1H, H\(_2\)), 4.50-4.42 (m, 1H, H\(_4\)), 3.95-3.83 (m, 1H, H\(_5\)A), 3.73(s, 3H, COOCH\(_3\)), 3.65-3.55 (m, 1H, H\(_5\)B), 2.50 (br-m, 1H, H\(_3\)B), 2.04 (m, 1H, H\(_3\)A) 2.30-1.88 (s, 3H, T-CH\(_3\)). MS(M+1) = 311.

2S,4S-N(Fluorenylmethoxycarbonyl)-N’(thymin-1-yl)proline 47: The methyl ester 21 (120 mg) was hydrolysed with 2N NaOH and neutralised with Dowex 50H\(^+\) to obtain the free acid 45(89 mg).

\(^1\)H NMR, (D\(_2\)O) δ: 7.76-7.26 (m, 8H, Fmoc), 7.27 (mi) & 7.25 (ma) (s, 1H, T H6), 5.98 (ma) (d, J = 11 Hz, C4 NH) & 5.30 (mi, br, C4 NH), 4.73-4.16 (m, overlapping signals, 7H, H\(_2\), H\(_4\), T CH\(_2\), Fmoc CH and OCH\(_2\)), 4.0-3.57 (br-m, 2H, H\(_5\)), 2.05 (m, 2H, H\(_3\)A,B), 1.93 (s, 3H, T CH\(_3\)).

**Dipeptide Nucleic acids 48 and 49: General method:** The thymine monomers 45 and 47 (carboxyl component, 0.12 mmol) were condensed separately with the amino components
48 (30 mg, 0.1 mmol) in DMF (500 μL) in presence of DCC (25 mg, 0.12 mmol) and HOBT (15 mg, 0.1 mmol) at 25 °C for 4 h. The usual work up afforded the protected dipeptide products 48a and 49a respectively, which were quantitatively deprotected in one step using either H2/Pd-C or piperidine:DMF to yield the free dipeptides 48b and 49b respectively.

48b: $^1$H NMR, (D$_2$O), 500 MHz, δ: 7.38 (2H, T CH), 4.70-4.28 (8H, T CH$_2$ x 2, H4, H2 x 2), 4.16-3.67 (m, 4H, H5A,B x 2), 3.75 (s, 3H, OCH$_3$), 2.80-2.12 (m, 4H, H3A,B x 2), 1.88 (s, 6H, T CH$_3$ x 2). UV λ$_{max}$ = 269 nm.

49b: $^1$H NMR, (D$_2$O), 500 MHz, δ: 7.42,7.38 (2H, T CH), 4.80-4.40 (8H, T CH$_2$ x 2, H4, H2 x 2), 4.20-3.70 (m, 4H, H5A,B x 2), 3.77, 3.73 (s, 3H, OCH$_3$), 2.80-2.12 (m, 4H, H3A,B x 2), 1.89,1.87 (s, 6H, T CH$_3$ x 2). UV λ$_{max}$=280 nm.

48a: $^1$H NMR, (DMSO- d$_6$) δ: 11.30 (s, 2H, T NH x 2), 5.03 (s, 2H, CBz CH$_2$), 4.57-3.81 (m, 8H, H2, H4 x 2, T CH$_2$ x 2), 3.56 (s, 3H, OCH$_3$), 3.40-3.20 (br, 4H, H5A,B x 2 H), 2.30-2.55 (br, 4H, H3A,B x 2), 1.75 (s, 6H, T CH$_3$ x 2). FAB-MS: [M+1] = 723.

49a: $^1$H NMR, (DMSO- d$_6$) δ: 7.95-7.25 (m, 10H, Fmoc-Ar, T CH), 4.67-4.16 (m, 10H, H2, H4 x 2, Fmoc CH, CH$_2$, T CH$_2$ x 2), 3.91-3.54 (m, 4H, H5A,B x 2), 3.70 (s, 3H, OCH$_3$), 2.65-2.20 (br, 4H, H3A,B x 2), 1.82,1.80 (s, 6H, T CH$_3$ x 2). FAB-MS: [M+1] = 811.

N1-(t-Butoxycarbonyl)-1,2-diaminoethane 50:

1,2-Ethlenediamine (20 g, 0.33 mol) was taken in dioxane:water (500 mL, 1:1), and cooled in an ice bath. Boc azide (5g, 35 mmol) in dioxane (50 mL) was slowly added with stirring and the pH was maintained at 10.0 by continuous addition of 4N NaOH. The mixture was stirred for 8 hr. and the resulting solution was concentrated to 100 mL. The N1,N2-diboc derivative not being soluble in water, precipitated, and it was removed by filtration. The corresponding N1-monoBoc derivative 45 was obtained by repeated extraction from the filtrate with ethylacetate. Removal of solvents yielded the monoBoc ethylenediamine 50 (3.45 g, 63%).
$^1$H NMR, (CDCl$_3$) 90 MHz $\delta$: 5.21 (b, 1H, NH), 3.32 (t, 2H, J= 8 Hz), 2.54 (t, 2H, J=8 Hz), 1.42 (s, 9H).

**Ethyl N-(2-tert-Butoxycarbonyl aminoethyl)glycinate 51:** The N1-mono-Boc-1,2-diaminoethane 50 (3.2 g, 20 mmol) was treated with bromoethyl acetate (2.25 mL, 20 mmol) in acetonitrile (100 mL) in presence of K$_2$CO$_3$ (2.4 g, 20 mmol) and the mixture was stirred at ambient temperature for 5 h. The solid that separated was removed by filtration and filtrate was evaporated to obtain 51 (4.3 g, 83%) as a colorless oil.

$^1$H NMR, (CDCl$_3$) $\delta$: 5.02 (br, 1H), 4.22 (q, 2H, J=8 Hz), 3.35 (s, 2H), 3.20 (t, 2H, J=6 Hz), 2.76 (t, 2H, J=6 Hz), 1.46 (s, 9H), 1.28 (t, 3H, J=8,)

**Ethyl N-(tert-Butoxycarbonyl aminoethyl)-N-(bromoacetyl)glycinate 52:** Compound 51 (4.0 g, 14 mmol) was taken in 10% aq. Na$_2$CO$_3$ (75 mL) and dioxane (60 mL). Bromoacetyl chloride ( 6.5 mL, 0.75 mol) was added in two shots with rigorous stirring. The reaction was complete within 5 min. The reaction mixture was brought to pH 8.0 by adding more 10% aq. Na$_2$CO$_3$ and concentrated to remove dioxane. The product was extracted from the aqueous layer with DCM and was purified by column chromatography to obtain 52 as a colorless oil in good yield (4.2 g, 80%).

$^1$H NMR (CDCl$_3$) $\delta$: 5.45 (br, 1H), 3.28 (q, 2H, J = 8 Hz), 4.14 (s, 2H), 4.00 (s, 2H), 3.53 (t, 2H), 3.28 (q, 2H), 1.46 (s, 9H), 1.23 (t, 3H, J = 8 Hz). [M+1]= 380.

**N-( t-Butoxycarbonyl aeg)thyminth Ethyl Ester 53a:** Compound 52 (4.0 g, 11.6 mmol) was stirred with anhydrous K$_2$CO$_3$ (1.56 g 11.8 mmol) in DMF with thymine (1.4 g, 11.2 mmol) to obtain the desired compound in good yield. DMF was removed under reduced pressure and the oil obtained was purified by column chromatography.

$^1$H NMR, (CDCl$_3$) $\delta$: 9.00 (br, 1H, NH), 7.05 (mi) 6.98 (ma) (s, 1H, 7-CH), 5.65 (ma), 5.05 (mi) ( br, 1H, NH), 4.58 (ma), 4.44 (mi) (s, 1H, 7-CH$_2$), 4.20 (mi), 4.05 (ma) (s, 1H, 7CH$_3$), 4.25 (m, 2H, OCH$_3$), 3.55 (m, 2H), 3.36 (m, 2H), 1.95 (s, 3H, 7-CH$_3$), 1.48 (s, 9H), 1.289 (m, 3H). $^{13}$C NMR, (CDCl$_3$) $\delta$: 170.8, 169.3, 167.4, 164.3, 156.2, 151.2, 141.1, 110.2, 79.3, 61.8, 61.2, 48.5, 48.1, 47.7, 38.4, 28.1, 13.8, 12.2.
N-(t-Butoxycarbonyl aeg)-N4-(benzylxoycarbonyl)cytosine Ethyl Ester 54a: A mixture of NaH (0.25 g, 6.2 mmol) and N4-(bezyloxycarbonyl)cytosine (1.24 g, 6.2 mmol) was taken in DMF and stirred at 75 °C till the effervescence ceased. The mixture was cooled and 52 (2.0 g, 6.1 mmol) was added to obtain cytosine monomer 54a (1.62 g, 50%) in moderate yield.

\[ \text{H NMR, (CDCl}_3\text{): } 7.65 \text{ (d, 1H, J=8, H6), 7.35 (s, 5H, Ar), 7.25 (d, 1H, J=8, H5), 5.70 (br, 1H, NH), 5.20 (s, 2H, ArCH}_2\text{), 4.71 (ma), & 4.22 (br-s, 2H), 4.15 (q, 2H), 4.05 (s, 2H), 3.56 (m, 2H), 3.32 (m, 2H), 1.48 (s, 9H), 1.25(t, 3H).} \]

N-(t-Butoxycarbonyl aeg)Adenine Ethyl Ester 55a: NaH (0.246 g, 6.1 mmol) was taken in DMF (15 mL) and adenine (0.8 g, 6.1 mmol) was added. The mixture stirred at 75 °C till the effervescence ceased and the mixture was cooled before adding 52(2.0 g, 6.1 mmol). The reaction mixture was heated at 75 °C for 1 h and DMF was removed. The thick oil was taken in water and extracted with ethylacetate and purified by column chromatography to obtain the product 55a.

\[ \text{H NMR, (CDCl}_3\text{): } 8.32 \text{ (s, 1H), 7.95 (mi), 7.90 (ma) (s, 1H), 5.93 (ma) & 5.80 (mi), (br, 2H), 5.13 (ma) & 4.95 (mi), 4.22 (mi) & 4.05 (ma) (s, 2H), 4.20 (m, 2H), 3.65 (ma) & 3.55 (mi), (m, 2H), 3.40 (ma) & 3.50 (mi),(m, 2H), 1.42 (s, 9H), 1.25(m, 3H).} \]

N-(t-Butoxycarbonyl aeg)-2-amino-6-chloropurine Ethyl Ester 56: A mixture of 2-amino-6-chloropurine (1.14 g, 6.8 mmol), K$_2$CO$_3$ (0.932 g, 7 mmol) and 52 (2.4 g, 7 mmol) were taken in DMF (20 mL) and stirred at RT for 4 h, K$_2$CO$_3$ was removed by filtration and DMF was removed under reduced pressure. The resulting residue was purified by column chromatography to obtain 56 in excellent yield (2.65 g, 98%).

\[ \text{H NMR, (CDCl}_3\text{): } 7.89 (mi) & 7.85 (ma) (s, 1H), 7.30 (s, 1H), 5.80 (br, 1H), 5.18 (br, 2H), 5.02 (ma) & 4.85 (mi)(s, 2H), 4.18 (mi) & 4.05 (ma) (s, 2H), 3.65 (ma) & 3.16 (mi), (m, 2H), 3.42 (ma) & 3.28 (mi) (m, 2H), 1.50 (S, 9H), 1.26 (m, 3H).} \]

N-(t-Butoxycarbonyl aeg)-2-amino-purine Ethyl Ester 57a: Hydrogenation of 56 with 10% Pd-C, at 40 psi H$_2$ pressure for 6-8 h gave the required compound 57a in good yield after column purification (80%).
$^1$H NMR, (CDCl$_3$) δ: 8.65 (s, 1H), 7.93 (mi) & 7.90 (ma) (s, 1H), 5.80 (br, 1H), 5.40 (br 1H), 5.00 (ma) & 4.85 (mi) (s, 2H), 4.17 (mi) & 4.05 (ma) (s, 2H), 4.25 (m, 2H), 3.67 (ma) & 3.56 (mi) (m, 2H), 3.4 (ma), & 3.32 (mi) (m, 2H), 1.45 (s, 9H), 1.25 (m, 3H). $^{13}$C NMR (CDCl$_3$) δ: 169.8 (mi) & 169.6 (ma) (COO), 167.3 (ma) & 166.9 (mi) (CON<), 159.9 (4C2), 156.1 (OCON), 153.4 (4-C4), 148.6 (4-C6), 143.7 (4-C8), 126.8 (4-C5), 79.7 (ma) & 79.2(mi) (C(CH$_3$)$_3$), 62.13 (mi) & 61.4 (ma) (OCH$_3$), 48.47, 43.0, 38.65(all CH$_2$), 28.25[(CH$_3$)$_3$], 13.91 (OCH$_2$CH$_3$).

Hydrolysis of ethyl ester of PNA monomers 53a-55a, 57a: General method: The ethyl esters 48a-52a (2g) were hydrolysed using 2N aq NaOH (5 mL) in methanol (5 mL) and the resulting acid was neutralized with activated Dowex-50H$^+$ till the pH of the solution was 7.0. The resin was removed by filtration and the filtrate was concentrated to obtain the resulting Boc-protected acid in excellent yield (>85%).

N-(2-ter-Butoxycarbonyl-aminoethyl)-N-(thymin-1-yi)glycine 53b: $^1$H NMR, (D$_2$O) δ: 7.28 (s, 1H), 4.80 (merged with HDO) (ma) & 4.72 (mi), 4.28 (mi) & 4.15 (ma) (s, 2H), 4.20 (m, 2H), 3.55 (m, 2H), 3.34 (ma) & 3.20 (mi) (m, 2H), 1.90 (s, 3H), 1.45 (s, 9H). $^{13}$C NMR, (D$_2$O) δ: 167.5, 156, 151.3, 142.4, 108.7, 78.5, 48.05, 47.4, 41.0, 38.5, 28.5 & 12.2.

N-(2-ter-Butoxycarbonyl-aminoethyl)-N-(N-benzyloxy carbonyl cytosin-1-yi)glycine 54b: $^1$H NMR (D$_2$O) δ: 7.89 (ma) & 7.87 (mi) (d, 1H, J= 8), 7.41 (m, 5H, Ar), 7.18 (ma) & 7.14 (mi) (d, 1H, J=8), 5.19 (s, 2H, Ar), 4.78 (merged with HDO) (ma), 4.72(mi) & 4.28 (mi), 4.17 (ma) (s, 2H), 4.23 (m, 2H), 3.57 (m, 2H), 3.34 (ma), 3.20 (mi) (m, 2H), 1.45 (s, 9H). $^{13}$C NMR, (D$_2$O) δ: 163.1, 154.9, 153.2, 150.7, 136.0, 128.5, 128.1, 127.9, 93.9, 66.5, 48.7, 47.6, 41.2, 38.5, 28.5,

N-(2-ter-Butoxycarbonyl-aminoethyl)-N-(adenin-9-yl)glycine 55b: $^1$H NMR, (D$_2$O) δ: 8.32 (s, 1H), 8.23 (s, 1H), 5.38 (ma) & 5.2 (mi), 4.2 (ma) & 4.03 (mi)(s, 2H), 3.65 (ma) & 3.5 (mi) (t, J=7Hz, 2H), 3.41 (ma) & 3.20 (mi) (t J=7Hz, 2H), 1.40 (ma) & 1.28 (mi) (s, 9H). $^{13}$C NMR, (D$_2$O) δ: 175, 168.9, 158.4, 152.3, 149.6, 148.1, 145.2, 118.3, 81.6, 51.8, 50.2, 49.0, 48.6, 45.7, 45.5, 38.5 & 28.4.
N-(2-\textit{t}-Butoxycarbonyl-aminoethyl)-N-(2-amino purin-9-yl)glycine 57b: M.P. = 207
°C. \textsuperscript{1}H NMR, (D\textsubscript{2}O) \textdelta: 8.70 (s, 1H), 8.33 (s, 1H), 5.31 & 5.12 (s, 2H), 4.17 & 4.07 (s, 2H), 3.70 (t, 2H), 3.58 (t, 2H), 3.40 (t, 2H), 3.23 (t, 2H), 1.42 (d, 9H). \textsuperscript{13}C NMR, (DMSO-\textit{d}_6), \textdelta: 171.6 (m\textit{i}) & 171.07 (m\textit{a}) (COO), 167.3 (m\textit{a}) & 166.9 (m\textit{i})(CON<), 159.9 (A-C\textit{2}), 156.1 (OC\textit{ON}), 153.4(-A\textit{C}\textit{4}), 148.6(A-C\textit{6}), 143.7(A-C\textit{8}), 126.8(A-C\textit{5}), 78.7 (C(CH\textsubscript{3})\textsubscript{3}), 50.2, 48.9, 43.0, 38.65 (all NCH\textsubscript{2}), 28.25 (C(CH\textsubscript{3})\textsubscript{3}).
2.8 References


