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Synthesis of all four nucleoside-based β-amino acids as protected precursors for the synthesis of polyamide-DNA with alternating α-amino acid and nucleoside-β-amino acids

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ABSTRACT

A simple approach is described for the synthesis of all four orthogonally protected nucleoside-β-amino acids from commercially available starting materials. Synthesis of a model tetrameric DNA sequence in 5′–3′direction employing trityl strategy and glycine as α-amino acid alternating with nucleoside-β amino acids is described.

1. Introduction

Novel oligonucleotide (ON) analogues that can form stable duplexes or triplexes with target nucleic acids are highly desired synthetic objectives because of their use as therapeutic agents.[1] Various types of modified oligonucleotides[2] have been developed over the last two decades as potential antisense and antigene agents. The more recent developments, such as splice correcting and exon skipping[3] strategies require highly robust nucleic acid analogues that are stable under physiological conditions as single strands as well as in the form of duplexes with complementary RNA sequences. The study of unnatural dephosphono nucleic acid oligomers would be highly beneficial for siRNA applications as well.[4a] This approach would maintain the chirality and 3′–5′ directionality of the backbone, along with the enzymatic stability of the replaced phosphate linkage by unnatural linker group. The replacement of the internucleoside sugar–phosphate linkages by the robust amide,[4–8] carbamate,[9a–c] carbazoyl[9d] or guanidino-[10] linkages is studied in the literature. The exploration of an amide bond for this purpose may be an interesting choice because the well established solid phase peptide synthesis (SPPS) methodology can be directly extended and could be advantageous as in case of synthesis of peptide nucleic acids (PNA)[8a–c] and analogues containing chiral α-amino acids.[8d–i] Several four- and five-atom amide-linked (6/7 atom repeating units) deoxyribo/ribo[4–7] ON analogues are studied till date, but the chemistry development in most instances has been restricted to the synthesis of thymine (T–T dimers). The other dephosphono linkers also have not been extensively explored for the synthesis of mixed purine–pyrimidine sequences probably because of the difficulties encountered during the synthesis of building blocks corresponding to all four nucleobases required for the synthesis of any desired nucleobase sequence. The complete replacement of sugar phosphate backbone as in PNA has been extremely successful[8] and has found advantages in diagnostic applications[11] as well as in splice correction antisense applications.[3] The development of cyclic, chiral analogues of PNA containing 6/7-atom repeating units was an offshoot aimed at either getting better RNA selectivity as antisense agents, or directional selectivity for binding to specific RNA sequences.[8] The relevant examples are those of pyrrolidine-based NA analogues, such as pyrrolidinyl[12]/POM-PNA[13] and bepPNA[14] or those comprising prolyl-aminopyrrolidine-2-carboxylic acid[15] and prolyl-2-aminocyclopentanecarboxylic acid (prolyl-ACPC backbone) (Fig. 1).[16,17] The prolyl-ACPC backbone and its homologues[17b] are the alternating α/β amino acid backbone exhibited preferential binding with complementary DNA. Our initial work was based on the prolyl-ACPC backbone and in that case the alternating nucleoside-β-amino acids with natural α-amino acids (Fig. 1) gave rise to similar alternating α/β amino acid backbone scaffolds using well-established peptide chemistry.[18a] We studied thyminyl homooligomer sequences, in which thyminyl-β-amino acid was used in conjunction with natural α-L-amino acids and found that these sequences displayed RNA selective binding.

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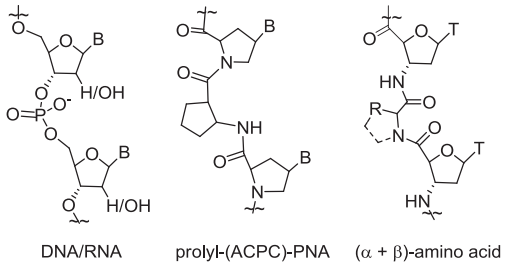


Fig. 1. DNA/RNA and backbone alterations.

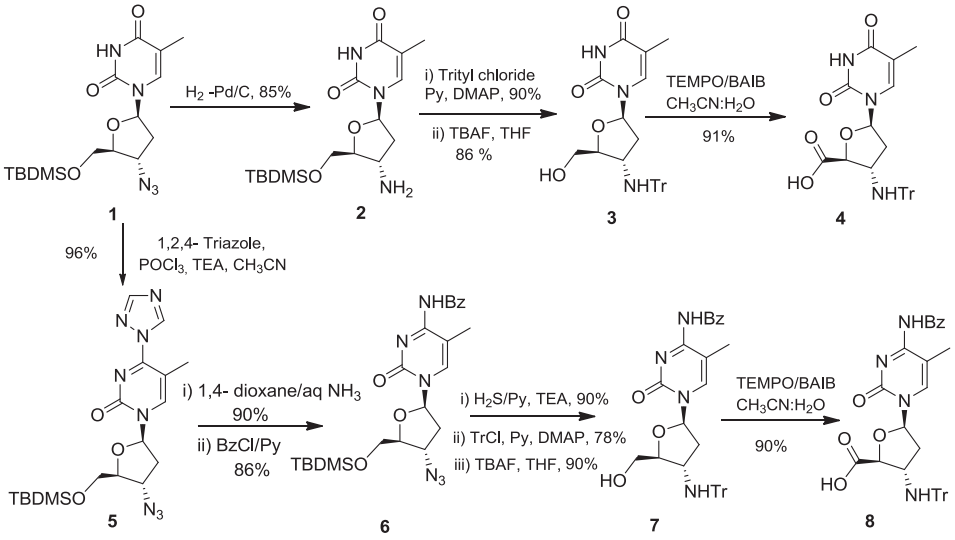
The thyminyl-β-amino acids were also synthesized by others for synthesizing phoshonamide internucleoside linkages[18b] and also for their use in sequences for studies in foldamer chemistry.[18c–f] For our interests in synthesizing (α+β) amino acid backbone, we further studied the effect of chirality of the α-amino acids by synthesizing the thymine dimers linked with L-proline, D-proline and prochiral glycine units on base stacking interactions.[19] To further exploit this interesting backbone, synthesis of all four natural nucleoside-based β-amino acids as building blocks is required. In this paper, we describe the first approach towards the synthesis of all four appropriately protected natural nucleoside-based β-amino acids and show their utility in the synthesis of a model (α-amino acid+nucleoside-β amino acid) tetrameric sequence of dephosphono polyamide-DNA.

2. Results and discussion

In our earlier strategy, we used Fmoc protected 3′-deoxy-3′-aminothymidine as nucleoside-β-amino acid monomer and rink amide resin as the solid support.[18a] Exocyclic amino protection is not required for thymine nucleobase during oligomer synthesis, and Fmoc strategy worked well. Also cleavage from the rink amide MBHA resin requires strongly acidic conditions (TFA:TFMSA/20% TFA in DCM), which may not be compatible for mixed base sequences containing purine nucleosides due to likely depurination in strongly acidic medium. We decided to use the trityl protection for 3′-sugar-amino function to be deprotected by trichloroacetic acid at each coupling step. This altered strategy would accommodate the orthogonal protection of nucleobases (benzoyl for adenine and cytosine and isobutyryl for guanine). We chose the succinate ester at 5′-end as a linker of the growing oligomer via succinamide linker to the support. The succinate ester can be cleaved concomitantly with the nucleobase deprotection, using aqueous/methanolic ammonia at the end of synthesis leaving a 5′-OH group. This strategy has been previously applied successfully for the synthesis of oligonucleotide phosphonamidates on Controlled Pore Glass (CPG) support.[20] The MBHA-succinate linker strategy has been previously used for the synthesis of glycopeptides[21] but has not been employed for the synthesis of oligopeptides.

The TEMPO–BAIB method[22] used earlier by us[18a,19] and others[18c–f] for oxidation of 5′-primary hydroxyl group in 3′-deoxy-3′-azidothymidine to get the 4′-carboxylic acid would be used as general procedure for protected tritylaminonucleosides. Thus, the 3′-deoxy-3′-tritylaminothymidine-4′-carboxylic acid **4** was obtained as follows (Scheme 1). The 5′-TBDMS-protected 3′-deoxy-3′-azidothymidine **1**[20a] was hydrogenated over Pd–C for reduction of azide to amine to get compound **2**. Reaction of **2** with trityl chloride, followed by deprotection of 5′-silyl ether gave compound **3** in good yield. The 3′-tritylamino-3′-deoxythymidine **3** was obtained in comparable yields and purity as reported earlier.[20] Compound **3** was easily converted to the corresponding acid **4** under TEMPO–BAIB oxidation conditions[18,19] (60% overall yield). The 3′-tritylamino-2′,3′-dideoxy-N^4-benzoyl-5-methylcytidine was synthesized from **1** as shown in Scheme 1. Compound **1** was converted to 1,2,4-triazolyl nucleoside derivative **5** by treatment with $POCl_3$ and 1,2,4-triazole in the presence of triethylamine.[20b] The cytidine derivative was obtained upon treatment of **5** with aq ammonia in dioxane. The free exocyclic amino group was further protected by treatment with BzCl in dry pyridine to get N^4-Bz protected cytidine derivative **6** in good yields. Compound **6** was further reduced to 3′-amino-5-methylcytidine derivative, which was subsequently protected as 3′-tritylamino group followed by the removal of silyl ether to get compound **7**.[20a] These altered reaction sequence as compared to the reported route[20b] avoided formation of 3′-NHTr-benzoyl derivative, which was formed during N^4-benzoylation. We used H_2S/pyridine for the reduction of azide to amine[7a] as reduction with Pd–C was found to be sluggish and less efficient. Oxidation to 4′-carboxylic acid derivative **8** was accomplished using TEMPO–BAIB oxidation conditions and the product was recovered by trituration with ether and acetone (42% overall yield).

For the synthesis of β-amino acid derivatives of adenine and guanine nucleosides, we used the commercially available 3′-amino-3′-deoxy purine nucleosides as starting materials. The synthesis of



Scheme 1. Synthesis of protected thymine/5-methylcytosine-β-amino acids.

the starting nucleoside derivatives is earlier reported from exocyclic amino protected 2′-deoxyxylo nucleosides.[20] Studies are also reported using commercial 3′-tritylamino-2′,3′-dideoxy purine nucleosides, when exocyclic amino protection involved either transient protection using TMS-chloride or peracylation/hydrolysis.[23] In either case, some undesired side products were obtained. The reactivity of nucleosides is quite sensitive to the substituent groups and we had to consider appropriate changes in the route to get the desired products in good yields. To get clean reaction products, we decided to use 5′-*O*-TBDMS protection as for the pyrimidine derivatives. The 3′-amino-2′,3′-dideoxyadenosine **9a** (Scheme 2) was converted to 3′-tritylamino derivative by treatment with trityl chloride in pyridine followed by silylation of 5′-OH with TBDMSCl to get the desired 3′-and 5′-protected derivative **10**. Subsequent protection of the exocyclic amino functionality with *N*-benzoyl tetrazole at higher temperature[24] afforded **11a** in good yields. The protection of exocyclic amino group proved to be challenging as extensive depurination was encountered even at −5 to −10 °C when benzoyl chloride was used as the acylating reagent. Benzoylation of 3′-NH-trityl group was not observed under these conditions. Compound **11a** was then desilylated to get the free hydroxyl function at 5′ position in **11b**, which was further subjected to oxidation using TEMPO–BAIB conditions to afford corresponding acid **14** (27% overall yield). The 3′-amino-2′,3′-dideoxyguanosine **9b** (Scheme 2) was converted to 3′-tritylamino derivative by treatment with trityl chloride followed by treatment with TBDMSCl to afford 3′-and 5′-protected derivative **12**. Exocyclic amino group was protected with isobutyryl chloride in pyridine to get **13a**. Removal of the 5′-silyl protecting group in **13a** gave compound **13b**, which on oxidation with TEMBO–BAIB afforded 4′-carboxylic acid derivative **15** (37% overall yield) (Scheme 2). All the purified compounds in Schemes 1 and 2 were characterized by ^{1}H, ^{13}C and mass spectrometry analysis and by comparing with the known compounds. The new compounds β-amino acid monomers **4**, **8**, **14** and **15** were further characterized by HRMS analysis.

These trityl protected nucleoside amino acids were stored in refrigerator with trace amounts of pyridine. Synthesis of a model tetrameric nucleoside sequence 5′-^{Me}C(gly)A(gly)^{Me}C(gly)T (**18**) was then planned using synthesized monomeric units and trityl glycine (**17**).[25] Choice of the resin for solid phase synthesis was influenced by the swelling properties of the resin and avoiding harsh acidic deprotection conditions considering sensitivity of the glycosidic bond towards acid. Amino functionalized resin has to be selected such that the final cleavage conditions are compatible towards stability of glycosidic bond. With this in mind, the first building block was modified with succinate ester linker at its 5′ position and was loaded on the resin through an amide bond. The resulting ester bond at 5′ position can be hydrolyzed under basic condition after oligomer synthesis. We used both CPG[20] and MBHA[21] resins for solid phase synthesis. The MBHA resin, which has better swelling properties gave much higher yield compared (coupling yield ~80% at each step) to the synthesis when CPG was used. The coupling efficiencies at each step could be monitored using Kaiser test[26] or by measurement of trityl cation. Thus, the succinate modified monomer[20] **16** was loaded on the MBHA resin using solid phase peptide coupling protocol (Scheme 3).[21] Tetrameric sequence was assembled using repetitive cycles, purified (21% isolated yield) and analyzed by mass spectrometry using ESI-MS technique.

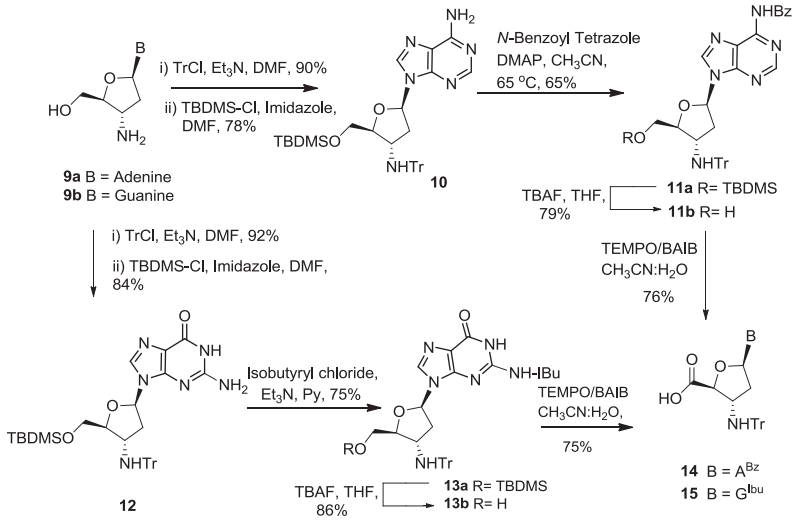
3. Conclusions

In conclusion, the synthesis of all four protected natural nucleoside based β-amino acids was accomplished using simple TEMPO/BAIB method and we further tested the synthesis of a model tetrameric nucleobase sequence 5′-^{Me}C(gly)A(gly)^{Me}C(gly)T **18** where ^{Me}C, A and T are nucleoside-β-amino acids corresponding to the 5-methyl-cytosine, adenine and thymine, and glycine is used as the alternating α-amino acid. Synthesis of longer PNA needs small changes in our protocols. Current studies are going on for optimizing condition for solid phase synthesis for improving coupling reactions and yield of the final oligomers so that the synthesis of longer oligomers can be achieved in greater purity.

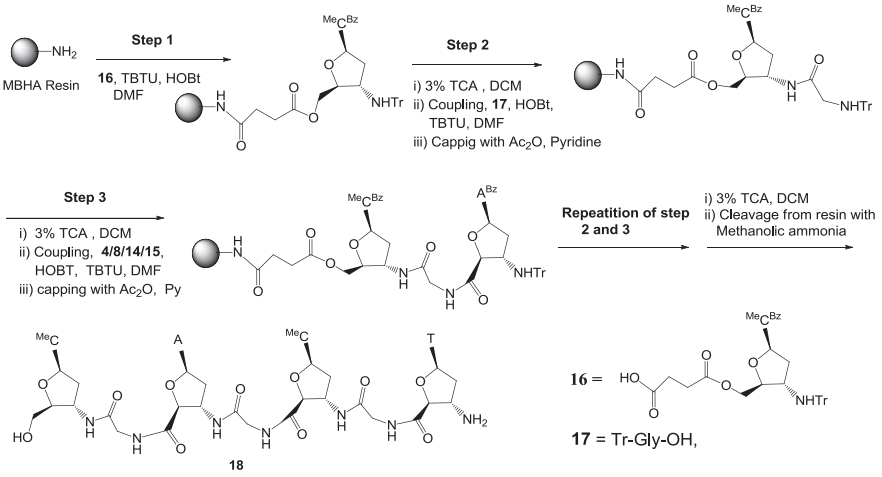
4. Experimental section

4.1. General

All the reagents were purchased from Sigma–Aldrich and used without purification. 3′-Amino purine nucleosides were purchased from Metkinen Chemistry, Finland. All solvents used were dried and distilled according to standard protocols. Analytical TLCs were performed on Merck 5554 silica 60 aluminium sheets. Column chromatography was performed for purification of compounds on silica gel (60–120 mesh, Merck). For acid-sensitive (trityl-containing) compounds, the column packed and equilibrated with 0.5% triethylamine. TLCs were performed using dichloromethane–methanol or petroleum ether–ethyl acetate solvent systems. Visualization



Scheme 2. Synthesis of protected adenine/guanine-β-amino acids.



Scheme 3. Schematic representation of solid phase synthesis of (α+β amino acid) tetramer.

was accomplished with UV light and/or by spraying with perchloric acid reagent and heating. The analytical data, such as melting point and specific rotation was obtained for the new compounds **4**, **8**, **14** and **15** and these were further characterized using IR, ^{1}H and ^{13}C NMR spectra and HRMS ^{1}H and ^{13}C NMR spectra were obtained using Bruker ACF 200 (200 MHz) or 400 (400 MHz) spectrometers and chemical shifts are reported on δ scale in parts per million (ppm) with the solvent indicated as the internal reference. The IR spectra were recorded on a Perkin Elmer Spectrum one-FT-IR spectrophotometer in chloroform.^{1}H NMR data are reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; br, broad; br s, broad singlet; m, multiplet and/or multiple resonance), number of protons. Mass spectra were recorded on Thermo Finnigan Surveyor MS-Q spectrometer (Mass spectra for tetramer was recorded on Aquity Ultra performance LC-MS, Waters), while HRMS was recorded by using a Micromass Q-Tof micro (YA-105) spectrometer and Thermo Scientific Q-exactive mass spectrometer. Specific rotation was recorded on Bellingham+Stanley Ltd, ADP 220 polarimeter and the concentration (c) is expressed in gram per 100 mL.

4.2. (1′*R*, 3′*S*, 4′*R*)-3′-Amino-5′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxythymidine (2)

A mixture of 3′-azido-5′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxythymidine **1** (3 g, 7.87 mmol) and 10% palladium on charcoal (0.3 g) in 30 mL methanol/ethyl acetate mixture (1:1, v/v) was stirred under hydrogen atmosphere (60 psi) at room temperature for 4 h. The reaction mixture was filtered through Celite and the solvent was evaporated in vacuo to give 3′-amino-5′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxythymidine **2** (2.37 g, 85%) as white foam.^{1}H NMR (200 MHz, $CDCl_3$): 7.51 (s, 1H), 6.26 (t, *J*=6.07 Hz, 1H), 3.90 (m, 2H), 3.72 (m, 1H), 3.64 (m, 1H), 2.19 (m, 2H), 1.91 (s, 3H), 0.93 (s, 9H), 0.11 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): 164.1, 150.5, 135.4, 110.5, 87.4, 84.3, 62.9, 51.4, 41.7, 25.8, 18.3, 12.5, −5.42, −5.47; MS (ESI) *m*/*z* 356 (M^{+}+1, 7), 378 (M^{+}+Na, 100).

4.3. (1′*R*, 3′*S*, 4′*R*)-3′-Tritylamino-3′-deoxythymidine (3)

Compound **2** (2 g, 5.63 mmol) was dissolved in dry pyridine (10 mL) and to it trityl chloride (2.04 g, 7.32 mmol) and catalytic amount of DMAP was added. Reaction mixture was allowed to stir at room temperature for 8 h. Solvent was removed under reduced pressure to afford crude 5′-*O*-(*tert*-butyldimethylsilyl)-3′-tritylamino-3′-deoxythymidine (3.03 g, 90%), which was then dissolved in 15 mL anhydrous THF and to it 1 N TBAF in THF (5.41 mL, 5.41 mmol) was added. Reaction was stirred at RT for 1 h. THF was removed in vacuo and residue dissolved in dichloromethane, washed with water. Organic layer was dried over Na_2SO_4 and concentrated. Compound was purified by column chromatography (30% EtOAc/petroleum ether) to afford 3′-tritylamino-3′-deoxythymidine **3** (1.49 g, 90%) as a white foam.^{1}H NMR (200 MHz, $CDCl_3$): 8.72 (br s, 1H), 7.54 (m, 6H), 7.14–7.33 (m, 10H), 6.02 (dd, *J*=6.57, 6.19 Hz, 1H), 3.60–3.87 (m, 3H), 3.32 (m, 1H), 2.26 (br s, 1H), 1.81 (s, 3H), 1.26–1.52 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): 163.8, 150.3, 146.0, 135.9, 128.5, 128.0, 126.7, 110.8, 86.6, 84.5, 71.1, 61.9, 53.2, 39.7, 12.4. MS (ESI) *m*/*z* 506 (M^{+}+Na, 100).

4.4. (1′*R*, 3′*S*, 4′*R*)-3′-Azido-5′-*O*-(*tert*-butyldimethylsilyl)-5-methyl-3′-deoxy-4-(1,2,4-triazol-1-yl)thymidine (5)

Triethylamine (10.95 mL, 78.94 mmol) was added drop wise over a portion of 10 min to a stirred mixture 1,2,4-triazole (5.97 g, 86.61 mmol) and phosphorus oxychloride (1.83 mL, 19.68 mmol) in 60 mL CH_3CN at 0 °C. The solution of compound **1** (3 g, 7.87 mmol) in 15 mL dry CH_3CN was then added drop wise and reaction mixture was allowed to stir at room temperature for 2.5 h. The solvent was evaporated in vacuo. The resulting brown solid was dissolved in ethyl acetate (100 mL). Organic layer washed with saturated $NaHCO_3$ solution followed by water, dried over Na_2SO_4 and concentrated to afford crude product **5** (3.26 g, 96%) as a white solid, which was used immediately for next reaction.

4.5. (1′*R*, 3′*S*, 4′*R*)-N^4-Benzoyl-3′-azido-5′-*O*-(*tert*-butyldimethylsilyl)-5-methyl-2′,3′-dideoxycytidine (6)

Compound 5 (3.26 g, 7.54 mmol) was dissolved in 1,4-dioxane (30 mL) and 15 mL concentrated aqueous ammonia was added. Reaction mixture was allowed to stir at rt for 2 h. After completion of the reaction, solvents were removed in vacuo. The residue was dissolved in dichloromethane and washed with water. Organic layer was dried over Na_2SO_4 and concentrated to afford a quantitative yield of 3′-azido-5′-*O*-(*tert*-butyldimethylsilyl)-5-methyl-2′,3′-dideoxycytidine (2.56 g, 6.73 mmol) as a colourless foam. This crude material was then azeotroped with pyridine and redissolved in pyridine (15 mL), and cooled externally at 0 °C. To this solution, benzoyl chloride (0.938 mL, 8.08 mmol) was added drop wise and the mixture was then allowed to attain rt and stir additionally for 14 h at rt. The reaction was quenched with 2 N NH_3 (5 mL) and was further stirred for 30 min. The solvent was evaporated in vacuo and residue was dissolved in ethyl acetate, washed with saturated

$NaHCO_3$ solution, dried over Na_2SO_4 and concentrated in vacuo. This crude was purified on column chromatography (25% EtOAc/petroleum ether) to afford 6 (2.79 g, 86%) as a colourless foam.[1]H NMR ($CDCl_3$, 200 MHz): 8.33 (m, 2H), 7.66 (s, 1H), 7.44–7.58 (m, 3H), 6.23 (t, J=6.31 Hz, 1H), 4.27 (m, 1H), 3.90–4.02 (m, 2H), 3.80–3.86 (m, 1H), 2.49–2.58 (m, 1H), 2.30 (m, 1H), 2.12 (s, 3H), 0.95 (s, 9H), 0.15 (s, 6H).

4.6. (1′*R*, 3′*S*, 4′R)-*N*^{4}-Benzoyl-5-methyl-3′-tritylamino-2′, 3′-dideoxycytidine (7)

Compound **6** (2.7 g, 5.57 mmol) was dissolved in 15% Et_3N in dry pyridine (10 mL) and H_2S gas was bubbled into the solution at 0 °C for 10 min. Reaction mixture was then stirred at room temperature for 30 min. Solvent was removed in vacuo and the residue was purified by column chromatography (5% MeOH/CH_2Cl_2) to afford in 90% yield of *N*^{4}-benzoyl-5-methyl-3′-amino-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-dideoxycytidine (2.29 g, 4.99 mmol). The following amounts were used in subsequent tritylation reaction; solvent, anhydrous pyridine, trityl chloride (1.58 g, 5.67 mmol) and catalytic amount of DMAP. Reaction mixture was allowed to stir at room temperature for 8 h. Solvent was removed in vacuo and the residue was purified by column chromatography (30% EtOAc/petroleum ether) to afford *N*^{4}-benzoyl-5-methyl-3′-tritylamino-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-dideoxycytidine (2.90 g, 78%), which was then subjected for desilylation. Following amounts were used in desilylation reaction; *N*^{4}-benzoyl-5-methyl-3′-tritylamino-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-dideoxycytidine (2.90 g, 4.14 mmol), 15 mL anhydrous THF as a solvent and TBAF 1 N in THF (6.21 mL, 6.21 mmol). Reaction was stirred at rt for 1 h. THF was removed in vacuo and residue dissolved in dichloromethane, washed with water. Organic layer was dried over Na_2SO_4 and concentrated. Compound was purified by column chromatography (35% ethyl acetate/petroleum ether) to give product **7** (2.18 g, yield 90%) as a white solid. [1]H NMR (200 MHz, $CDCl_3$): 8.31 (s, 1H), 8.28 (m, 1H), 7.39–7.55 (m, 10H), 7.18–7.39 (m, 10H), 6.03 (dd, J=6.70, 5.30 Hz, 1H), 3.66–3.93 (m, 3H), 3.32 (q, J=13.26, 6.94 Hz, 1H), 2.03 (s, 3H), 1.73 (br s, 1H), 1.25–1.42 (m, 2H); [13]C NMR (50 MHz, $CDCl_3$): 179.4, 159.6, 147.8, 146.0, 137.2, 137.0, 132.4, 129.8, 129.5, 128.1, 126.8, 111.6, 86.7, 85.4, 71.1, 61.8, 52.9, 40.3, 13.5; MS (ESI) m/z 587 (M^++1, 15), 609 (M^++Na, 100).

4.7. General procedure for synthesis of 3′-tritylamino-2′, 3′-dideoxy-purine nucleosides[23]

Et_3N (1.15 mL, 8.26 mmol) was added to a stirred solution of 3′-amino-2′,3′-dideoxy purine nucleoside **9a**/**9b** (7.51 mmol) in dry DMF (80 mL). The reaction mixture was stirred at 60 °C for 30 min. Trityl chloride (2.24 g, 8.26 mmol) was added to reaction mixture in two portions and allowed to stir for 3 h at room temperature. The reaction mixture poured on 120 mL cold water and the flask kept in freezer for 2 h. The obtained precipitate was filtered and washed thoroughly with cold water. White solid desiccated overnight to give crude 3′-tritylamino-2′,3′-dideoxyadenosine (3.32 g, 90%)/3′-tritylamino-2′,3′-dideoxyguanosine (4.13 g, 92%), which was used as such for next reaction.

4.8. (1′*R*, 3′*S*, 4′*R*)-5′-*O*-(*tert*-Butyldimethylsilyl)-3′-tritylamino-2′,3′-dideoxyadenosine (10)

3′-Tritylamino-2′,3′-dideoxyadenosine (3.32 g, 6.74 mmol), TBDMSCl (1.32 g, 8.77 mmol) and imidazole (1.37 g, 20.22 mmol) was suspended in 15 mL dry DMF and the reaction was allowed to stir overnight at rt. DMF was removed in vacuo and the residue was redissolved in ethyl acetate (150 mL). Organic layer was washed with water followed by brine, dried over Na_2SO_4 and concentrated in vacuo. Product was purified by column chromatography (3% MeOH/CH_2Cl_2) to afford 5′-*O*-(*tert*-butyldimethylsilyl)-3′-tritylamino-2′,3′-dideoxyadenosine **10** (3.19 g, 78%). [1]H NMR (200 MHz, $CDCl_3$): 8.30 (s, 1H), 7.91 (s, 1H), 7.51–7.56 (m, 6H), 7.17–7.32 (m, 9H), 6.29 (dd, J=6.19, 5.93 Hz, 1H), 5.71 (br s, 2H), 3.87 (m, 1H), 3.60–3.82 (m, 2H), 3.44 (m, 1H), 2.02 (br s, 1H), 1.71–1.79 (m, 2H), 0.82 (s, 9H), −0.03 (s, 3H), −0.04 (s, 3H); [13]C NMR (50 MHz, $CDCl_3$): 155.3, 152.8, 149.4, 146.1, 138.4, 128.6, 128.0, 126.6, 119.4, 87.1, 83.6, 71.2, 63.6, 54.7, 41.1, 25.8, 18.3, −5.4; MS (ESI) m/z 607 (M^++1, 10), 629 (M^++Na, 100); HRMS (ESI): calcd for $C_{35}H_{42}N_6O_2Si$: 607.3211, found: 607.3205.

4.9. (1′*R*, 3′*S*, 4′*R*)-*N*^{6}-Benzoyl-5′-*O*-(*tert*-butyldimethylsilyl)-3′-tritylamino-2′,3′-dideoxyadenosine (11a)

5′-*O*-(*tert*-Butyldimethylsilyl)-3′-tritylamino-2′,3′-dideoxyadenosine **10** (3.19 g, 5.26 mmol) dissolved in dry acetonitrile (40 mL). To it *N*-benzoyl tetrazole (1.82 g, 9.89 mmol) and 4-dimethylaminopyridine (0.604 g, 5.25 mmol) was added. The reaction mixture is allowed to stir at 65 °C for 90 min. After completion of reaction, solvent was removed in vacuo and residue dissolved in dichloromethane. Organic layer washed with saturated $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated to give crude product. This crude was purified by column chromatography (1% MeOH/CH_2Cl_2) to afford **11a** (2.42 g, 65%) as a white solid. [1]H NMR (200 MHz, $CDCl_3$): 9.15 (br s, 1H), 8.75 (s, 1H), 8.13 (s, 1H), 8.00 (d, J=6.70 Hz, 2H), 7.49–7.59 (m, 8H), 7.15–7.32 (m, 10H), 6.36 (dd, J=6.06, 5.69 Hz, 1H), 3.64–3.91 (m, 3H), 3.51 (m, 1H), 2.08 (br s, 1H), 1.64–1.88 (m, 2H), 0.81 (s, 9H), −0.02 (s, 3H), −0.03 (s, 3H); [13]C NMR (50 MHz, $CDCl_3$): 164.6, 152.4, 151.1, 149.2, 146.0, 140.9, 133.6, 132.6, 128.7, 128.5, 128.0, 127.8, 126.6, 122.8, 87.2, 83.9, 71.1, 63.4, 54.5, 41.0, 25.8, 18.3, −5.45, −5.53; MS (ESI) m/z 711 (M^++1, 100), 733 (M^++Na, 70).

4.10. (1′*R*, 3′*S*, 4′*R*)-*N*^{6}-Benzoyl-3′-tritylamino-2′,3′-dideoxyadenosine (11b)

Compound **11a** (2.4 g, 3.38 mmol) dissolved in anhydrous THF (15 mL) and 1 N TBAF in THF (5.07 mL, 5.07 mmol) was added. Reaction was stirred at rt for 1 h. THF was removed in vacuo and the residue was dissolved in dichloromethane, washed with water and then brine. Organic layer was dried over Na_2SO_4, concentrated in vacuo and purified by column chromatography (3% MeOH/CH_2Cl_2) to get product **11b** (1.59 g, 79%) as a white solid. [1]H NMR (200 MHz, $CDCl_3$): 9.26 (br s, 1H), 8.61 (s, 1H), 8.03 (m, 3H), 7.49–7.56 (m, 8H), 7.20–7.33 (m, 10H), 6.24 (dd, J=7.45, 6.31 Hz, 1H), 3.68–3.78 (m, 3H), 3.55 (d, J=11.32 Hz, 1H), 2.30 (m, 1H), 2.04 (br s, 1H), 1.76 (m, 1H); MS (ESI) m/z 597 (M^++1, 20), 619 (M^++Na, 100).

4.11. (1′*R*, 3′*S*, 4′*R*)-5′-*O*-(*tert*-Butyldimethylsilyl)-3′-tritylamino-2′,3′-dideoxyguanosine (12)

3′-Tritylamino-2′,3′-dideoxyguanosine (4.13 g, 8.12 mmol), TBDMSCl (1.59 g, 10.56 mmol) and imidazole (1.65 g, 24.36 mmol) was suspended in 15 mL dry DMF and the reaction was allowed to stir overnight at rt. DMF was removed in vacuo and the residue was dissolved in ethyl acetate (150 mL). Organic layer was washed with water, dried over Na_2SO_4 and concentrated in vacuo The crude product was purified by column chromatography (3% MeOH in DCM) to afford **12** (4.23 g, 84%) as a white solid. [1]H NMR (200 MHz, DMSO-d_6): 10.64 (br s, 1H), 7.46–7.50 (m, 7H), 7.14–7.32 (m, 9H), 6.45 (br s, 1H), 5.89–5.95 (t, J=6.19 Hz, 1H), 3.74–3.80 (m, 1H), 3.46–3.68 (m, 2H), 3.34 (m, 1H, merged with H_2O), 1.45–1.58 (m, 1H), 1.23–1.30 (m, 1H), 0.79 (s, 9H), −0.07 (s, 6H); [13]C NMR (50 MHz, $CDCl_3$): 159.1, 153.3, 151.3, 146.2, 135.4, 128.6, 128.0, 126.7, 117.0, 87.0, 83.0, 71.1, 63.5, 54.6, 40.3, 25.9, 18.4, −5.40, −5.52; MS

(ESI) *m*/*z* 623 (M^++1, 10), 645 (M^++Na, 100), 242 (30); HRMS (ESI): calcd for $C_{35}H_{42}N_6O_3Si$: 623.3160, found: 623.3145.

4.12. Synthesis of 5′-*O*-(*tert*-butyldimethylsilyl)-N^2-isobutyryl-3′-tritylamino-2′,3′-dideoxyguanosine (13a)

To solution of compound **12** (4.23 g, 6.80 mmol) in anhydrous pyridine (20 mL), triethylamine (0.95 mL, 6.80 mmol) was added and the mixture was stirred for 15 min. To this reaction mixture *iso*-butyryl chloride (1.43 mL, 13.6 mmol) was added dropwise and reaction was allowed to stir for 1 h. Pyridine was removed in vacuo and reaction mixture dissolved in ethyl acetate, washed with saturated $NaHCO_3$ solution, then water. Organic layer dried over Na_2SO_4 and concentrated to give crude product. The product was purified by column chromatography (1% MeOH/CH_2Cl_2) to afford **13a** (3.53 g, 75%) as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$): 7.58 (s, 1H), 7.52 (d, *J*=7.93 Hz, 6H), 7.28 (t, *J*=7.93 Hz, 6H), 7.20 (t, *J*=7.32 Hz, 3H), 5.97 (dd, *J*=6.72, 4.88 Hz, 1H), 3.83 (m, 1H), 3.67–3.77 (ABX, J_{AB}=11.30 Hz, 2H), 3.47 (m, 1H), 2.57–2.59 (septet, *J*=6.41 Hz, 1H), 1.68–1.71 (m, 1H), 1.54–1.57 (m, 1H), 1.21–1.25 (m, 6H) 0.80 (s, 9H), −0.05 (s, 3H), −0.06 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): 178.1, 155.4, 147.7, 147.2, 146.1, 136.5, 128.6, 128.1, 127.9, 126.7, 121.1, 87.1, 83.0, 71.1, 63.4, 54.6, 40.4, 36.5, 25.9, 18.9, 18.4, −5.45, −5.55; MS (ESI) *m*/*z* 693 (M^++1, 10), 715 (M^++Na, 100).

4.13. (1′*R*, 3′*S*, 4′*R*)-N^2-Isobutyryl-3′-tritylamino-2′,3′-dideoxyguanosine (13b)

This compound is obtained in 91% yield via desilylation of **13a** as described in the synthesis of **11b**. Compound was purified by column chromatography (using 3% methanol in DCM) to afford **13b** as white solid. ^{1}H NMR (200 MHz, $CDCl_3$): 7.60 (s, 1H), 7.52 (d, *J*=7.93 Hz, 6H), 7.18–7.38 (m, 9H), 5.98 (dd, *J*=6.44, 6.57 Hz, 1H), 3.85–3.96 (m, 2H), 3.53–3.64 (m, 2H), 2.68 (septet, *J*=6.95 Hz, 1H), 2.06 (br s, 1H), 1.83 (m, 1H), 1.52 (m, 1H), 1.20 (dd, *J*=6.82 and 3.28 Hz, 6H); MS (ESI) *m*/*z* 579 (M^++1, 10), 601 (M^++Na, 100).

4.14. General procedure for synthesis of nucleoside-β-amino acids

(Diacetoxyiodo)benzene (2.2 mmol) and TEMPO (0.25 mmol), were added to the solution of protected 2′,3′-deoxy-3′-tritylamino nucleosides **3**/**7**/**11b**/**13b** (1 mmol) in 5 mL CH_3CN/H_2O mixture (1:1). The reaction mixture was stirred at 25 °C for 3 h. Acetonitrile was removed completely in vacuo. The crude product was extracted with ethyl acetate. The organic phase was dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was triturated sequentially with diethyl ether and acetone, filtered and dried in vacuo.

4.15. (1′*R*, 3′*S*, 4′*S*)-3′-Deoxy-3′-tritylaminothymidine-4′-carboxylic acid (4)

This compound is obtained as a white solid (0.45 g, 91%) starting from compound **3** by general procedure described above. R_f (10% MeOH/CH_2Cl_2) 0.51; mp: 214–217 °C; $[\alpha]_D^{25}$ +30.9 (*c* 1.03, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6+drop of D_2O): 8.73 (s, 1H, H6), 7.42–7.44 (m, 6H, aromatic), 7.22–7.26 (m, 6H, aromatic), 7.12–7.16 (m, 3H, aromatic), 5.87 (dd, *J*=5.52,5.02 Hz, 1H, H1′), 4.08 (d, *J*=5.78 Hz, 1H, H4′), 2.89 (dd, *J*=11.54, 5.52 Hz, 1H, H3′), 1.64 (s, 3H, CH_3), 1.21 (m, 1H, H2″), 1.00 (m, 1H, H2′); ^{13}C NMR (100 MHz, DMSO-d_6+drop of D_2O): 173.9, 164.0, 150.1, 146.5, 138.5, 128.7, 127.6, 126.1, 107.5, 86.2, 85.6, 70.7, 56.6, 38.9, 12.3; IR (ν_{max}, cm^{-1}) ($CHCl_3$): 3620, 3399, 3018, 2975, 2896, 1690, 1215; HRMS (ESI): calcd for $C_{29}H_{27}N_3O_5$: 520.1848, found: 520.1846.

4.16. (1′*R*, 3′*S*, 4′*S*)-2′,3′-Dideoxy-3′-tritylamino-N^4-benzoyl-5-methylcytidin-4′-carboxylic acid (8)

This compound is obtained as a white solid (0.54 g, 90%) starting from compound **7** by general procedure described above. R_f (10% MeOH/CH_2Cl_2) 0.59; mp: 204–207 °C; $[\alpha]_D^{26}$ +115.9 (*c* 1.07, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6+drop of D_2O): 8.84 (br s, 1H, NH), 8.12 (s, 2H, aromatic), 7.57 (m, 1H, aromatic), 7.43–7.47 (m, 9H, aromatic), 7.23–7.27 (m, 6H, aromatic), 7.13–7.16 (m, 3H, aromatic), 5.96 (t, *J*=4.77 Hz, 1H, H1′), 4.22 (d, *J*=5.77 Hz, 1H, H4′), 3.05 (m, 1H, H3′), 1.89 (s, 3H, CH_3), 1.40 (m, 1H, H2″), 1.06 (m, 1H, H2′); ^{13}C NMR (100 MHz, DMSO-d_6+drop of D_2O): 174.4, 173.7, 160.4, 147.9, 146.6, 142.7, 136.6, 132.7, 129.4, 128.9, 128.0, 127.0, 126.4, 108.7, 87.3, 86.5, 70.8, 56.5, 39.1, 13.5; IR (ν_{max}, cm^{-1})($CHCl_3$): 3619, 3318, 3018, 2976, 2897, 1703, 1652, 1215; HRMS(ESI): calcd for $C_{36}H_{32}N_4O_5$: 601.2451, found: 601.2452.

4.17. (1′*R*, 3′*S*, 4′*S*)-2′,3′-Dideoxy-3′-tritylamino-N^6-benzoyl-adenin-4′-carboxylic acid (14)

This compound is obtained as a white solid (0.46 g, 76%) starting from compound **11b** by general procedure described above. R_f (10% MeOH/CH_2Cl_2) 0.52; mp: 262–270 °C; $[\alpha]_D^{26}$ +5.1 (*c* 0.79, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6+drop of D_2O): 8.63 (s, 1H, C2–H), 8.46 (s, 1H, C8–H), 7.97 (d, *J*=7.28 Hz, 2H, aromatic), 7.66 (m, 1H, aromatic), 7.51–7.55 (m, 2H, aromatic), 7.44–7.46 (m, 6H, aromatic), 7.24 (m, 6H, aromatic) 7.07–7.16 (m, 3H, aromatic), 6.35–6.38 (dd, *J*=6.27, 4.52 Hz, 1H, H1′), 4.30 (d, *J*=6.27 Hz, 1H, H4′), 3.47 (dd, *J*=13.05, 6.53 Hz, 1H, H3′), 1.62–1.69 (m, 1H, H2″), 1.31–1.34 (m, 1H, H2′); ^{13}C NMR (100 MHz, DMSO-d_6+drop of D_2O): 173.0, 165.5, 151.7, 151.5, 150.1, 146.2, 142.4, 133.3, 132.4, 128.58, 128.50, 128.4, 127.8, 126.3, 125.5, 84.6, 83.5, 70.7, 58.1, 37.3; IR (ν_{max}, cm^{-1}) ($CHCl_3$): 3619, 3399, 3018, 2976, 2896, 1707, 1216; HRMS(ESI): calcd for $C_{36}H_{30}N_6O_4$: 611.2407, found: 611.2404.

4.18. (1′*R*, 3′*S*, 4′*S*)-2′,3′-Dideoxy-3′-tritylamino-N^2-isobutyryl-guanosin-4′-carboxylic acid (15)

Analogous method was used for the synthesis of this compound starting from compound **13b**. In this case, after completion of the reaction mixture was directly diluted with ethyl acetate and extracted with water. The organic phase was dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was triturated sequentially with diethyl ether and acetone, filtered and dried in vacuo to get white solid (0.44 g, 75%). R_f (10% MeOH/CH_2Cl_2) 0.51; mp: 235–238 °C; $[\alpha]_D^{26}$ +33.0 (*c* 1.75, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6+drop of D_2O): 8.49 (s, 1H, C8–H), 7.40 (d, *J*=7.78 Hz, 6H, aromatic), 7.10–7.23 (m, 10H, aromatic), 5.96–5.99 (dd, *J*=5.52, 3.76 Hz, 1H, H1′), 4.17 (d, *J*=6.77 Hz, 1H, H4′), 3.11–3.16 (m, 1H, H3′), 2.72–2.75 (m, 1H, *i*Bu-CH), 1.50–1.53 (m, 1H, H2″), 1.08–1.12 (2d, 6H, *i*Bu-CH_3), 0.99 (m, 1H, H2′); ^{13}C NMR (100 MHz, DMSO-d_6+drop of D_2O): 180.4, 174.0, 155.2, 147.8, 146.6, 138.83, 128.9, 128.1, 127.9, 126.6, 120.3, 85.2, 84.8, 70.7, 57.0, 39.5, 35.0, 19.2, 19.1; IR (ν_{max}, cm^{-1}) ($CHCl_3$): 3393, 3016, 2975, 2895, 1685, 1609, 1215; HRMS (ESI): calcd for $C_{33}H_{32}N_6O_5$: 593.2512, found: 593.2510.

4.19. Pre-loading of MBHA resin

MBHA resin (60 mg, 1.75 mmol/g, 0.024 mmol) was washed and swelled in DCM for 1 h in solid phase flask. The resin was drained and washed with DMF. To a solution of N^4-benzoyl-5-methyl 3′-tritylamino-2′,3′-dideoxycytidine-5′-succinylate[20a] **16** (16.19 mg, 0.024 mmol) in 500 μL DMF, DIPEA (12.47 μL, 0.072 mmol) and TBTU (9.63 mg, 0.028 mmol) was added. To this solution HOBt (3.24 mg, 0.024 mmol) in 100 μL of DMF was added. This activated monomer solution was added to the resin and the suspension was allowed for gentle shaking for 12 h. Resin was drained and washed

with DMF (2×), DCM (2×) and pyridine (2×). Unreacted amino groups capped with 10% acetic anhydride in pyridine for 1 h. The nucleoside loading on solid support was determined by spectrophotometric determination of the concentration of trityl cation at 410 nm released after detritylation using 3% TCA in DCM. Calculated loading value for resin using molar extinction coefficient[27] 35,300 M^{-1} cm^{-1} at 410 nm for trityl cation was 35.2 μmol/g, which was good enough for next synthesis.

4.20. Solid phase synthesis of model tetramer 18

Synthesis of a model tetrameric nucleoside sequence 5′-C(gly)A(gly)C(gly)T was then undertaken using protected nucleoside β amino acid monomeric units and trityl glycine (**17**) on preloaded MBHA resin. The following synthetic condition were used,

(i) Deprotection of trityl group using 3% trichloroacetic acid (3 min×3) (ii) Neutralization using 5% DIPEA in DCM (3min×2) (iii) coupling using 3 equiv of monomer, 9 equiv DIPEA, 3 equiv TBTU and 1.5 equiv of HOBt as activator with respect to loading value of resin. All are premixed in DMF prior to addition to resin. This suspension was added to the resin. Reaction time 5 h. (iv) Capping of unreacted amino groups using 10% acetic anhydride in pyridine (10 min×2).

After the synthesis was complete, the oligomer **18** was cleaved from solid support using aqueous methanolic ammonia at 55 °C for 8 h. The terminal trityl group was deprotected before cleavage using 3% TCA in DCM or after cleavage using 80% aqueous acetic acid. Oligomer was extracted from resin in 20% methanol in water. Analytical purification was achieved on reverse phase C18 column using 5% acetonitrile in 0.1 M TEAA buffer as eluent system and monitored at 260 nm (retention time for tetramer 3.54 min) and further characterized by ESI-mass spectrometry. Mass calcd for $C_{46}H_{58}N_{20}O_{15}$ 1130.4391 ESI mass observed *m*/*z* for ($C_{46}H_{58}N_{20}O_{15}^{+1}$ 1129.7) ($C_{46}H_{58}N_{20}O_{15}^{+2}$, 565.22).

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Supplementary data

^{1}H, ^{13}C and mass spectra of selected compounds in Schemes 1 and 2. HRMS spectra of new compounds **4**, **8**, **10**, **12**, **14** and **15**. HPLC and ESI and MALDI-TOF-TOF mass spectra of oligomer **18**. Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.tet.2012.11.028.

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