Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron 61 (2005) 9506–9512
Tetrahedron

Synthesis and conformational studies of amide-linked cyclic homooligomers of a thymidine-based nucleoside amino acid☆

Tushar Kanti Chakraborty,* Dipankar Koley, Sripadi Prabhakar, Rapolu Ravi and Ajit Chand Kunwar *

Indian Institute of Chemical Technology, Hyderabad 500 007, India

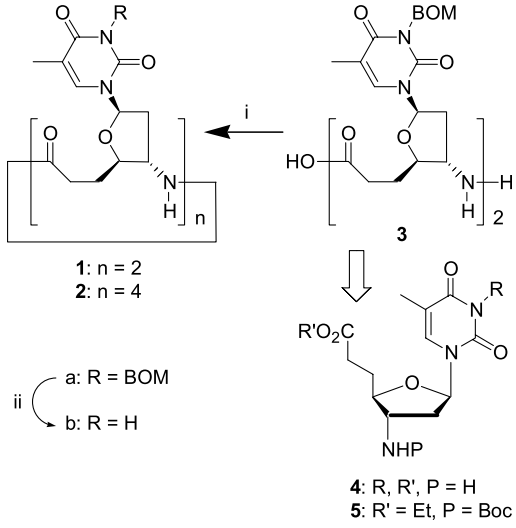
Received 30 May 2005; revised 11 July 2005; accepted 28 July 2005

Available online 18 August 2005

Abstract—Cyclic homooligomers of a thymidine-based nucleoside amino acid were synthesized from the linear dimer using BOP reagent in the presence of DIPEA under dilute conditions. Conformational analysis by NMR and constrained MD studies revealed that all the cyclic products had symmetrical structures. The NH and CO groups in these molecules point in opposite directions with near perpendicular orientation with respect to the plane of the macrocyclic ring having CO on the same side as the base.

1. Introduction

Cyclisation of linear biopolymers is widely used to constrain their conformational degrees of freedom and induce desirable structural biases permitting enhanced receptor selectivity and binding affinity with additional properties like decreased susceptibility to degradation in biological systems.[1] Cyclic DNAs and RNAs, for example, have been studied extensively for their unusual chemical and biological activities.[2] However, synthesis of such cyclic DNAs remains a challenging task,[3] thereby limiting exploratory studies, especially in discovering potential leads for drug discovery. It was envisaged that the replacement of the phosphodiester linkages with amide bonds would not only facilitate the assembly of such substrates using standard solid- or solution-phase peptide synthesis methods, but would also help to enhance their stability towards nucleases. Amide-linked oligonucleotides have been studied extensively for potential therapeutic applications involving antisense strategy.[4] However, their cyclic versions have remained largely unexplored. Herein, we report the synthesis and conformational studies of amide-linked cyclic homooligonucleotides **1** and **2**, which were prepared, as shown in Scheme 1, by cyclisation of the linear dimer **3** of the monomeric building block **4**,[4h] a thymidine-based nucleoside amino acid (Taa).



Scheme 1. Reagents and conditions: (i) BOP reagent, DIPEA, DMF, 0 °C to rt, 10 h; (ii) H_2, Pd-C (10%), THF–MeOH (1:1), rt, 0.5 h.

1.1. Synthesis of the cyclic homooligomers

The starting material for our synthesis was the fully protected monomer Taa **5a**.[5,6] While the *tert*-butoxycarbonyl (Boc) group was deprotected using TFA–CH_2Cl_2 (1:3), saponification of the ethyl ester was carried out with LiOH in dioxane–water (1:1). Reaction of Boc-Taa(BOM)-OH with H_2N-Taa(BOM)-OEt using the conventional solution phase method using *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate (TBTU) and 1-hydroxybenzotriazole (HOBt) as coupling agents in

☆ IICT Communication no. 050409.

Keywords: Nucleoside amino acids; Cyclic DNAs; Cyclic RNAs; Cyclic peptides; Conformation; NMR.
* Corresponding authors. Tel.: +91 40 27193154; fax: +91 40 27193108/27160387; e-mail: chakraborty@iict.res.in

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.07.089

presence of *N*-methylmorpholine (NMM) in CH_3CN gave the protected dimer, Boc-[Taa(BOM)]$_2$-OMe in 75% yield. Saponification of the protected dimer was followed by Boc-deprotection under the conditions mentioned above to furnish the intermediate **3**, which was directly subjected to cyclisation using benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP reagent) in the presence of *N*,*N*-diisopropylethylamine (DIPEA) in amine-free DMF as solvent to furnish a mixture of products, **1a** and **2a**, in 16 and 28% yields, respectively. They were separated by standard silica gel column chromatography and hydrogenated using 10% Pd-C in THF–MeOH (1:1) to furnish the BOM-deprotected products **1b** and **2b** in quantitative yields. The purified products were fully characterized by spectroscopic methods before using them in the conformational studies.

All the products were characterized by positive ion electrospray ionization (ESI) mass spectra.[7] The spectra showed the expected $[M+Na]^+$ ion to confirm the molecular weight of the product, and sometimes $[2M+Na]^+$ ion was also found. Interestingly, the spectra of some of the products showed $[M+2Na]^{2+}$. Although, the *m*/*z* value of $[M+2Na]^{2+}$ ion matches with that of $[M+Na]^+$ ion of lower homologue, these ions were identified by isotopic distribution patterns and also by the mass differences between the ^{12}C and ^{13}C isotopic peaks, that is, a difference of 1 Da in the case of $[M+Na]^+$ ion and 0.5 Da in the case of $[M+2Na]^{2+}$ ion. Further the $[2M+Na]^+$ and $[M+2Na]^{2+}$ ions were confirmed by MS/MS, which resulted in the corresponding $[M+Na]^+$ ion in addition to the other characteristic fragment ions.[8]

1.2. Conformational analysis. NMR studies

The conformational analysis of **1a** and **2a** were carried out by NMR spectroscopy at 500 MHz. The studies were undertaken in 5–10 mM solution at 30 °C in $CDCl_3$ and DMSO-d_6 for **1a**, whereas structures of **2a**, due to its inadequate solubility in $CDCl_3$, were investigated in DMSO-d_6. The presence of only one set of peaks in **1a** and **2a** is consistent with a two and four fold symmetry, respectively, in the NMR time frame.

While extensive decoupling experiments and simulations of the spectra were used to obtain the couplings, the

Table 1. ^{1}H NMR chemical shifts (δ, ppm) and coupling constants (*J*, Hz) of **1a** ($CDCl_3$, 500 MHz, 30 °C)

Ring protons		Base protons[a]	
H1′	6.15 (t, *J*=6.9 Hz)	C5-Me	2.01 (s)
H2′	2.27 (ddd, *J*=6.2, 10.2, 14.3 Hz)	H6	7.14 (s)
H2″	1.71 (ddd, *J*=6.6, 7.6, 14.3 Hz)	H7	5.68 (d, *J*=10.2 Hz)
H3′	4.32 (dddd, *J*=6.6, 8.2, 8.3, 10.2 Hz)	H7′	5.47 (d, *J*=10.2 Hz)
H4′	3.55 (ddd, *J*=3.2, 8.2, 11.0 Hz)	H9	4.72 (d, *J*=11.6 Hz)
H5′	2.37 (dddd, *J*=1.8, 11.0, 12.2, 13.5 Hz)	H9′	4.70 (d, *J*=11.6 Hz)
H5″	2.07 (dddd, *J*=2.0, 3.2, 7.1, 13.5 Hz)	NH	6.55 (d, *J*=8.3 Hz)
H6′	2.33 (ddd, *J*=1.8, 7.1, 14.3 Hz)	Ph	7.29–7.40 (m)
H6″	2.03 (ddd, *J*=2.0, 12.2, 14.3 Hz)		

[a] H7,7′; H9,9′ and Ph protons are from BOM groups.

Table 2. ^{1}H NMR Chemical shifts (δ, ppm) and coupling constants (*J*, Hz) of **1a** (DMSO-d_6, 500 MHz, 30 °C)

Ring protons		Base protons[a]	
H1′	6.04 (t, *J*=6.4 Hz)	C5-Me	1.86 (s)
H2′	2.30 (ddd, *J*=5.7, 9.5, 13.7 Hz)	H6	7.52 (s)
H2″	1.99 (m)	H7	5.34 (d, *J*=9.8 Hz)
H3′	4.10 (m)	H7′	5.32 (d, *J*=9.8 Hz)
H4′	3.71 (ddd, *J*=3.5, 7.9, 10.0 Hz)	H9	4.58 (br s)
H5′	2.12 (m)	H9′	4.58 (br s)
H5″	1.95 (m)	NH	7.80 (d, *J*=7.8 Hz)
H6′	2.24 (m)	Ph	7.25–7.35 (m)
H6″	2.12 (m)		

[a] H7,7′; H9,9′ and Ph protons are from BOM groups.

Table 3. ^{1}H NMR chemical shifts (δ, ppm) and coupling constants (*J*, Hz) of **2a** (DMSO-d_6, 500 MHz, 30 °C)

Ring protons		Base protons[a]	
H1′	6.15 (t, *J*=6.7 Hz)	C5-Me	1.86 (s)
H2′	2.30 (ddd, *J*=14.2, 9.1, 5.7 Hz)	H6	7.57 (s)
H2″	2.15 (m)	H7	5.33 (d, *J*=9.8 Hz)
H3′	4.25 (m)	H7′	5.31 (d, *J*=9.8 Hz)
H4′	3.60 (ddd, *J*=8.6, 7.0, 4.2 Hz)	H9	4.58 (S)
H5″	1.95 (m)	H9′	4.58 (S)
H5′	1.86 (m)	NH	8.12 (d, *J*=8.0 Hz)
H6′	2.26 (ddd, *J*=5.8, 9.1, 14.9 Hz)	Ph	7.24–7.34 (m)
H6″	2.16 (m)		

[a] H7,7′; H9,9′ and Ph protons are from BOM groups.

assignments were carried out with the help of DQFCOSY experiments,[9] and ROESY experiments[10] provided the information on the proximity of protons. The spectral parameters are given in Tables 1–3. Some of the important long-range NOEs seen in the ROESY spectra of **1a** and **2a** are shown in Figures 1 and 3, respectively.

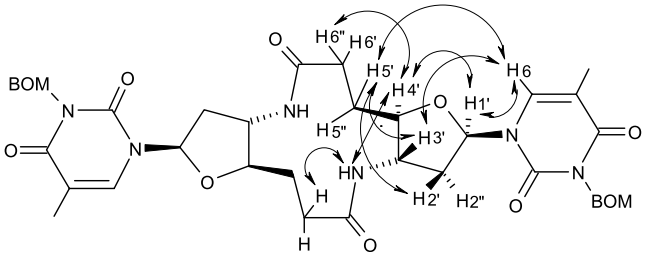


Figure 1. Schematic representation of some of the diagnostic long-range NOEs seen in the ROESY spectrum of **1a** in $CDCl_3$.

The conformational analysis of **1b** and **2b** could not be carried out due to line-broadening and overlapping signals both in $CDCl_3$ and DMSO-d_6, making it very difficult to derive the spectral parameters.

1.3. Conformational analysis of 1a

The spectral data of **1a** suggested that its 12-membered macrocyclic ring was very rigid. The vicinal couplings, $^3J_{H4'-H5'}=11.0$ Hz, $^3J_{H5'-H6''}=12.2$ Hz, $^3J_{H5''-H6''}=2.0$ Hz, $^3J_{H5'-H6'}=1.8$ Hz, $^3J_{H4'-H5''}=3.2$ Hz are consistent with values of about 60°and $-60°$ for C_4–C_5–C_6–CO and C_3–C_4–C_5–C_6, respectively. This is further supported by the NOE correlations H4′↔H6″ and H3′↔H5′ shown in Figure 1. The resulting structure had the NH and CO pointing approximately perpendicular to the plane of the macrocyclic ring with CO on the same side as the base. The information on the sugar pucker was derived with the help of PSUEROT programme,[11] which indicates that the sugar ring takes a single $^{O}_{4}T$ conformation[12] with $P=69.2$ and $\nu_{max}=39.6°$. In nucleosides $^{O}_{4}T$ pucker is in between the C2′ endo and C3′ endo sugar puckerings, which are the lowest energy conformations. The structure is consistent with the NOEs between H2′↔H5′, H1′↔H4′ and H3′↔H5′ (Fig. 1). The information on the orientation of the base was obtained from the distinct NOEs between the base proton, H6 and sugar protons. The presence of NOEs between H2′↔H6, H3′↔H6, and H5′↔H6 very clearly supported the presence of *anti* conformation of the base. Yet the NOE correlation H1′↔H6 implies a significant population of molecule with *syn* conformation. Such a situation is often encountered in nucleosides and nucleotides where both *syn* and *anti* conformations[12] are observed in solution.

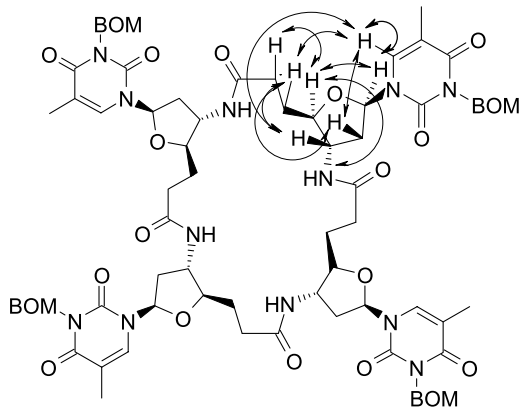


Figure 3. Schematic representation of some of the diagnostic long-range NOEs seen in the ROESY spectrum of **2a** in DMSO-d_6.

The cross-peak intensities in the ROESY spectra were used for obtaining the restraints in the molecular dynamics (MD) calculations[13] on **1a**. Molecular dynamics calculations were carried out using Sybyl 6.8 program on a Silicon Graphics O2 workstation. The Tripos force field, with default parameters, was used throughout the simulations. The detailed protocol of the MD calculations is provided in the Section 4. Figure 2 depicts the ensemble of the backbone-superimposed structures of the 20 samples, collected during 600 ps simulated annealing protocol, which clearly shows the proposed structure of the molecule. The average pair wise backbone RMSD for the structures is $0.24+0.15$ Å.[14]

1.4. Conformational analysis of 2a

For **2a**, though the chemical shift values were obtained from the DQFCOSY spectra, it was not possible to derive all the couplings due to spectral complexity and overlap. However, ROESY data showed NOEs (Fig. 3), which were similar to those for **1a**, suggesting a very similar structure for the tetramer.

Few of the couplings, which could be obtained, as well as weaker NOEs, point towards averaging of the spectral parameters, which may arise due to several other conformations contributing to the structure, due to the larger macrocyclic ring. The predominant structure, however, resembles that of **1a**. The NOEs between the base proton, H6 and the protons in the sugar ring, are consistent with the presence of both *syn* and *anti* conformation, with the latter being dominant.

Cyclic homooligomers of nucleoside amino acids constitute a new class of novel molecular entities that display interesting 3-D structures, reminiscent of the structures of peptide nanotubes. The NH and CO groups in these molecules point in opposite directions with near

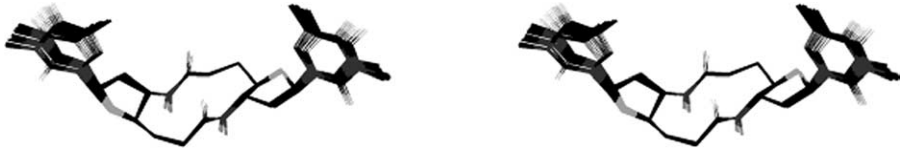


Figure 2. Stereo view of the 20 superimposed energy-minimized structures of **1a** sampled during 100 cycles of the 600 ps constrained MD simulations following the simulated annealing protocol. For clarity the protons (except amide protons) and the BOM groups are not shown.

perpendicular orientation with respect to the plane of the macrocyclic ring having CO on the same side as the base. This study will be useful in creating various de novo amide-linked cyclic homo- as well as heterooligomers using other nucleoside amino acids as well. The well-defined structures of these macrocyclic peptides will be useful to carry out investigations into many interesting molecular recognition processes, especially those involving base-pairing and may find many applications similar to those exhibited by cyclic DNAs and RNAs.

2. Experimental

2.1. General procedures

All reactions were carried out in oven or flame-dried glassware with magnetic stirring under nitrogen atmosphere using dry, freshly distilled solvents, unless otherwise noted. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm silica gel plates with UV light, I_2, 7% ethanolic phosphomolybdic acid-heat and 2.5% ethanolic anisaldehyde (with 1% AcOH and 3.3% concd H_2SO_4)-heat as developing agents. Silica gel finer than 200 mesh was used for flash column chromatography. Yields refer to chromatographically and spectroscopically homogeneous materials unless otherwise stated. Melting points are uncorrected. IR spectra were recorded as KBr pellets on FT-IR. Mass spectra were obtained under liquid secondary ion mass spectrometric (LSIMS) and electrospray ionisation (ESI) techniques. Optical rotations were measured with a digital polarimeter.

2.2. Details of NMR studies

NMR spectra were recorded using a 500 MHz spectrometer at 30 °C with 5–10 mM solutions in appropriate solvents using TMS as internal standard and the solvent signals as secondary standards and the chemical shifts (δ) are shown in ppm. Multiplicities of NMR signals are designated as s (singlet), d (doublet), t (triplet), q (quartet), br (broad), m (multiplet, for unresolved lines), etc. ^{13}C NMR spectra were recorded at 75 and 125 MHz with complete proton decoupling. The proton chemical shift assignments were carried out with the help of two-dimensional double quantum filtered correlation spectroscopy (DQFCOSY)[9] and nuclear Overhauser effect spectroscopy (NOESY)/rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments,[10] the later also provided the information on the proximity of protons. All the experiments were carried out in the phase sensitive mode.[15] The spectra were acquired with 2×256 or 2×192 free induction decays (FID) containing 8–16 transients with relaxation delays of 1.0–1.5 s. The ROESY experiments were performed with mixing time of 0.3 s and a spin-locking field of about 2.5 kHz was used. The two-dimensional data were processed with Gaussian apodization in both the dimensions.

2.3. Details of molecular dynamics studies

Molecular mechanics/dynamics calculations were carried out using Sybyl 6.8 program on a Silicon Graphics O2 workstation. The Tripos force field, with default parameters, was used throughout the simulations.

A dielectric constant of 47 Debye for DMSO solvent was used in all minimizations as well as in MD runs. Minimizations were done first with steepest decent, followed by conjugate gradient methods for a maximum of 2000 iterations each or RMS deviation of 0.005 kcal/mol, whichever was earlier. The energy-minimized structures were then subjected to MD studies. A number of inter atomic distance constraints (more than three bond away) were used in the MD studies that were derived from the rOe cross-peaks from the ROESY spectrum. Distance constraints have been obtained from 0.3 s mixing time ROESY experiments by using the volume integral and two-spin approximation. Force constant of 15 kcal/Å were applied in the form of flat bottom potential well with the lower and upper bounds obtained by subtracting and adding 10% to the distances obtained above. These constraints are given in Table 4.[13]

Table 4. NOE constraints used in MD simulation study of compound **1a**

S.no.	From	To	Upper bound	Lower bound
1	H2′	H6	2.713	2.220
2	H3′	H6	2.868	2.347
3	H5′	H6	3.425	2.802
4	H3′	H5′	2.860	2.340
5	H1′	H4′	2.496	2.042
6	NH	H4′	3.439	2.810

No H-bonding constraint was used. The energy-minimized structures were subjected to constrained MD simulations for duration of 600 ps using 100 cycles, each of 6 ps period, of the simulated annealing protocol. The atomic velocities were applied following Boltzmann distribution about the center of mass, to obtain a starting temperature of 700 K.[16]

After simulating for 1 ps at high temperature, the system temperature was reduced exponentially over a 5 ps period to reach a final temperature of 300 K. Structures were sampled after every five cycles, leading to an ensemble of total 20 structures. The sampled structures were energy-minimized without constraints, by using the above-mentioned protocol and the superimposed structures obtained by backbone alignment are shown in the paper, in Figure 2. To determine the backbone and the average pair-wise heavy atom RMSD, the structures were analyzed using the MOLMOL program.[14]

2.3.1. Synthesis of 5a. To a solution of Boc-Taa-OEt (**5b**, 1.21 g, 2.94 mmol) in CH_3CN (10 mL) at room temperature was added DBU (1.32 mL, 8.83 mmol) with stirring. After 5 min at the same temperature, BOM-Cl (0.61 mL, 4.41 mmol) was added to it and stirring continued for 1 h. The reaction was quenched with saturated aqueous NH_4Cl solution, extracted with EtOAc. The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated in vacuo. Purification by column chromatography (SiO_2, 28–30% EtOAc in petroleum ether eluant) gave the desired compound **5a** (1.32 g, 85%) as a white semisolid. Data for **5a**: $R_f=0.4$ (silica gel, 30% EtOAc in

petroleum ether); $[\alpha]_D^{26}$ +42.1 (c=5.5 in $CHCl_3$); IR (neat) ν_{max} 3373, 2927, 1710, 1660, 1523 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.32–7.28 (m, 5H, aromatic), 7.13 (s, 1H, H6), 6.16 (t, J=6.0 Hz, 1H, H1′), 5.48 (s, 2H, N–CH_2), 4.70 (m, 3H, BocNH and Ph–CH_2), 4.14 (q, J=7.15 Hz, 2H, ester CH_2), 3.98 (m, 1H), 3.72 (m, 1H), 2.50 (m, 2H), 2.25 (m, 2H), 2.15 (m, 2H), 1.95 (s, 3H, C5–CH_3) 1.45 (s, 9H, Boc), 1.26 (t, J=7.15 Hz, 3H, ester-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 163.1, 155.2, 150.7, 137.8, 133.6, 128.1, 127.4, 110.4, 84.3, 83.2, 79.9, 77.2, 72.1, 70.4, 60.4, 53.7, 38.1, 30.5, 28.5, 28.2, 14.0, 13.1; MS (LSIMS) m/z (%) 532 (16) $[M+H]^+$; HRMS (LSIMS) calcd for $C_{27}H_{37}N_3O_8Na$ 554.2478, found 554.2498.

2.3.2. Synthesis of 3. To a solution of **5a** (334 mg, 0.629 mmol) in dioxane–water (6 mL, 1:1) at 0 °C was added LiOH–H_2O (79.2 mg, 1.88 mmol) with stirring and the temperature was allowed to rise from 0 °C to room temperature over 3 h. Then the reaction mixture was neutralized by DOWEX 50×80–100 ion exchange acidic resin and filtered. The filtrate was concentrated in vacuo. The mixture was then diluted with CH_2Cl_2, washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo to obtain the acid Boc-[Taa(BOM)]-OH.

In another round bottom flask, a solution of **5a** (341 mg, 0.692 mmol) in CH_2Cl_2 (4 mL) was taken. To this solution was added trifluoroacetic acid (1 mL) with stirring at 0 °C. Stirring was continued for 2 h as the temperature was allowed to rise slowly from 0 °C to room temperature. The reaction mixture was then concentrated in vacuo to give TFA [Taa(BOM)]-OEt.

The crude acid Boc-[Taa(BOM)]-OH was dissolved in CH_3CN (4 mL) and NMM (0.07 mL, 0.619 mmol) was added. Then it was sequentially treated with HOBT·H_2O (42.5 mg, 0.314 mmol) and TBTU (222 mg, 0.619 mmol) at room temperature. After 30 min, compound TFA [Taa(BOM)]-OEt dissolved in CH_3CN (4 mL) containing NMM (0.1 mL, 0.963 mmol) was added to the reaction mixture at room temperature. After being stirred for 12 h at room temperature, the reaction mixture was quenched with saturated aqueous NaH_2PO_4 solution and the solvent was evaporated under reduced pressure. The water phase was extracted with CH_2Cl_2, washed with water, brine, dried (Na_2SO_4), concentrated in vacuo. Purification by column chromatography (SiO_2, 1.8% MeOH in $CHCl_3$ as eluant) afforded the protected linear dimer Boc-[Taa(BOM)]$_2$-OEt (432 mg, 75%). Data for Boc-[Taa(BOM)]$_2$-OEt: R_f=0.45 (silica gel, 3% MeOH in $CHCl_3$); $[\alpha]_D^{26}$ +36.2 (c=1.9 in $CHCl_3$); IR (KBr) ν_{max} 3348, 2975, 1711, 1665, 1531 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.3–7.17 (m, 10H, aromatic), 7.08 (s, 2H, H6), 6.93 (d, J=7.15 Hz, 1H, N–H), 6.08 (m, 2H, H1′), 5.51 (d, J=7.15 Hz, 1H, BocNH), 5.41 (s, 4H, N–CH_2), 4.64 (s, 4H, Ph–CH_2), 4.19 (m, 1H), 4.1 (m, 2H), 3.95 (m, 1H), 3.72 (m, 2H), 2.5–2.4 (m, 2H), 2.38–2.08 (m, 10H), 1.97 (s, 3H, one of the C5-methyls), 1.95 (s, 3H, other C5–CH_3) 1.42 (s, 9H, Boc), 1.26 (t, J=7.15 Hz, 3H, ester-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1, 172.3, 163.1, 155.4, 150.7, 137.8, 133.8, 133.6, 128.1, 127.5, 110.5, 84.7, 84.4, 83, 80.1, 77.1, 72.1, 70.5, 60.5, 53.8, 52.4, 37.7, 32.4, 30.3, 29.1, 28.5, 28.2, 14.0, 13.1; MS (LSIMS) m/z (%) 918 (20) $[M+H]^+$; HRMS (LSIMS) calcd for $C_{47}H_{60}N_6O_{13}Na$ 939.4116, found 939.4089.

To a solution of Boc-[Taa(BOM)]$_2$-OEt (295 mg, 0.321 mmol) in dioxane–water (4 mL, 1:1) at 0 °C was added LiOH–H_2O (40.5 mg, 0.965 mmol) with stirring. Stirring was continued for 3 h as the temperature was allowed to rise slowly from 0 °C to room temperature. Then the reaction mixture was neutralized by DOWEX 50×80–100 ion exchange acidic resin and filtered. The filtrate was concentrated in vacuo. The mixture was then diluted with CH_2Cl_2, washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo to obtain the acid Boc-[Taa(BOM)]$_2$-OH. The acid was dissolved in CH_2Cl_2 (3 mL) To this solution was added trifluoroacetic acid (0.75 mL) with stirring at 0 °C. Stirring was continued for 3 h as the temperature was allowed to rise slowly from 0 °C to room temperature. The reaction mixture was then concentrated in vacuum to give the TFA salt of the crude acid, **3**, which was used directly in the next step.

2.3.3. Synthesis of 1a and 2a. To the TFA salt of the crude acid **3** in amine free dry DMF (32.1 mL, 10^{-2} M) was added BOP reagent (156.5 mg, 0.353 mmol) at 0 °C and the reaction mixture was stirred for 15 min. This was followed by the slow addition of DIPEA (0.27 mL, 1.6 mmol) to the reaction mixture and stirring was continued for 10 h at room temperature. Evaporation of the DMF under reduced pressure gave a residue that was dissolved in CH_2Cl_2, washed with saturated aqueous NH_4Cl solution, saturated aqueous $NaHCO_3$ solution, brine, dried (Na_2SO_4) and concentrated in vacuum. The crude was purified by column chromatography (SiO_2, 3.8–4.0% MeOH in $CHCl_3$ as eluant) to afford the cyclic dimer **1a** (39 mg, 16%) and the cyclic tetramer **2a** (139 mg, 28%) as solids. Data for **1a**: R_f=0.24 (silica gel, 7% MeOH in $CHCl_3$); $[\alpha]_D^{26}$ +17.5 (c=0.95 in $CHCl_3$); IR (KBr) ν_{max} 3291, 2924, 2854, 1713, 1650, 1547 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) see Table 1; ^1H NMR (DMSO-d_6, 500 MHz) see Table 2; ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 163.1, 151.3, 137.1, 133.7, 128.6, 128.2, 111.7, 83.0, 82.4, 73.0, 71.0, 51.6, 39.0, 31.3, 29.6, 27.9, 13.2; MS (ESI) m/z (%) 793 (100) $[M+Na]^+$, 810 (20) $[M+K]^+$; HRMS (ESI) calcd for $C_{40}H_{46}N_6O_{10}Na$ 793.3173, found 793.3211. Data for **2a**: R_f=0.32 (silica gel, 7% MeOH in $CHCl_3$), $[\alpha]_D^{26}$ +30.6 (c=0.76 in $CHCl_3$), IR (KBr) ν_{max} 3296, 2924, 2854, 1713, 1650, 1547 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) see Table 3; ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.9, 162.1, 150, 137.5, 135.1, 127.6, 126.8, 126.7, 108.6, 83.4, 81.9, 70.4, 69.7, 51.3, 35.6, 31.5, 28.4, 12.0; MS (ESI) m/z (%) 1563 (100) $[M+Na]^+$; HRMS (ESI) calcd for $C_{80}H_{92}N_{12}O_{20}Na$ 1562.6370, found 1562.6345.

2.3.4. Synthesis of 1b. To a solution of **1a** (30 mg, 0.038 mmol) in THF–MeOH (2 mL, 1:1) was added 10% Pd on C (10 mg). The mixture was hydrogenated under atmospheric pressure using of a H_2-filled balloon for 30 min. The reaction mixture was filtered through a short pad of Celite, and the filter cake was washed with MeOH. The filtrate and the washings were combined and concentrated in vacuum to get a quantitative yield of **1b** (20 mg). Data for **1b**: R_f=0.18 (silica gel, 20% MeOH in $CHCl_3$), $[\alpha]_D^{26}$ −21.9 (c=0.47 in DMSO), IR (KBr) ν_{Max}

3421, 1664, 1562 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 11.06 (s, 1H, NH of thymine), 8.24 (br s, 1H, CONH), 7.46 (s, 1H, H6), 6.06 (d, $J=6.6$ Hz, H1′), 3.91 (m, 1H, H3′), 3.77 (m, 1H, H4′), 2.35–1.92 (m, 6H), 1.79 (s, 3H, 5-Me); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.9, 163.6, 150.2, 136.0, 109.6, 82.4, 79.9, 52.2, 37.2, 30.5, 28.5, 11.9; MS (ESI) *m/z* (%) 553 (100) $[M+Na]^+$; HRMS (ESI) calcd for $C_{24}H_{30}N_6O_8Na$ 553.2022, found 553.2043.

2.3.5. Synthesis of 2b. Compound **2b** was synthesized from **2a** in quantitative yield following the same procedure described above for the synthesis of **1b**. Data for **2b**: $R_f=$ 0.17 (silica gel, 20% MeOH in $CHCl_3$), $[\alpha]_D^{26}$ +57.1 ($c=$ 0.24 in DMSO); IR (KBr) ν_{Max} 3434, 2927, 1700, 1551 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 11.28 (s, 1H, NH of thymine), 8.19 (d, $J=9.1$ Hz, 1H, CONH), 7.50 (s, 1H, H6), 6.10 (d, $J=6.9$ Hz, 1H, H1′), 4.23 (m, 1H, H3′), 3.55 (m, 1H, H4′), 2.33–1.86 (m, 6H), 1.81 (s, 3H, 5-Me); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.4, 163.6, 150.3, 136.1, 109.8, 82.9, 82.3, 51.8, 36.1, 32.0, 29.0, 11.9; MS (ESI) *m/z* (%) 1083 (20) $[M+Na]^+$; HRMS (ESI) calcd for $C_{48}H_{60}N_{12}O_{16}Na$ 1083.4147, found 1083.4190.

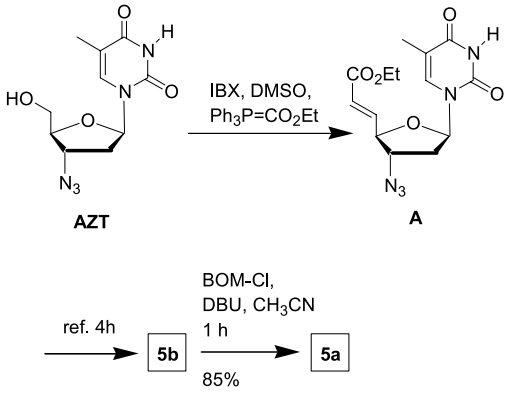
Acknowledgements

We thank CSIR, New Delhi for research fellowships (D.K. and R.R.) and DST, New Delhi for financial support.

References and notes

1. (a) Deber, C. M.; Madison, V.; Blout, E. R. *Acc. Chem. Res.* **1976**, *9*, 106–113. (b) Milner-White, E. J. *Trends Pharmacol. Sci.* **1989**, *10*, 70–74. (c) Fairlie, D. P.; Abbenante, G.; March, D. R. *Curr. Med. Chem.* **1995**, *2*, 654–686. (d) Cuniasse, P.; Raynal, I.; Yiotakis, A.; Dive, V. *J. Am. Chem. Soc.* **1997**, *119*, 5239–5248. (e) Cavallaro, V.; Thompson, P.; Hearn, M. *J. Pept. Sci.* **1998**, *4*, 335–343. (f) Benito, J. M.; Blanco, J. L. J.; Mellet, C. O.; Fernández, J. M. G. *Angew. Chem., Int. Ed.* **2002**, *41*, 3674–3676. (g) Fan, L.; Hindsgaul, O. *Org. Lett.* **2002**, *4*, 4503–4506. (h) Jaunzems, J.; Oelze, B.; Kirschning, A. *Org. Biomol. Chem.* **2004**, *2*, 3448–3456. (i) Kazmaier, U.; Hebach, C.; Watzke, A.; Maier, S.; Mues, H.; Volker Hu, V. *Org. Biomol. Chem.* **2005**, *3*, 136–145.
2. (a) Hsu, C.-Y.L.; Dennis, D. *Nucleic Acids Res.* **1982**, *10*, 5637–5647. (b) Ulanovsky, L.; Bodner, M.; Trifonov, E. N.; Choder, M. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 862–866. (c) Ross, P.; Weinhouse, H.; Aloni, Y.; Michaeli, D.; Weinberg-Ohana, P.; Mayer, R.; Braun, S.; de Vroom, E.; van der Marel, G. A.; van Boom, J. H.; Benziman, M. *Nature* **1987**, *325*, 279–290. (d) Rao, M. V.; Reese, C. B. *Nucleic Acids Res.* **1989**, *17*, 8221–8239. (e) Rumney, S., IV; Kool, E. T. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1617–1619. (f) Prakash, G.; Kool, E. T. *J. Am. Chem. Soc.* **1992**, *114*, 3523–3527. (g) Gamper, H. B.; Reed, M. W.; Cox, T.; Virosco, J. S.; Adams, A. D. *Nucleic Acids Res.* **1993**, *21*, 145–150. (h) Diener, T. O. *Trends Microbiol.* **1993**, *1*, 289–294. (i) Rubin, E.; Rumney, S.; Kool, E. T. *Nucleic Acids Res.* **1995**, *23*, 3547–3553. (j) Kool, E. T. *Annu. Rev. Biophys. Biomol. Struct.* **1996**, *25*, 1–28. (k) Mazumder, A.; Uchida, H.; Neamati, N.; Sunder, S.; Jaworska-Maslanka, M.; Wickstrom, E.; Zeng, F.; Jones, R. A.; Mandes, R. F.; Chenault, H. K.; Pommier, Y. *Mol. Pharmacol.* **1997**, *51*, 567–575. (l) Kool, E. T. *Acc. Chem. Res.* **1998**, *31*, 502–510. (m) Escaja, N.; Pedroso, E.; Rico, M.; Gonzalez, C. *J. Am. Chem. Soc.* **2000**, *122*, 12732–12742. (n) Li, T.; Liu, D.; Chen, J.; Lee, A. H. F.; Qi, J.; Chan, A. S. C. *J. Am. Chem. Soc.* **2001**, *123*, 12901–12902.
3. (a) Hsu, C.-Y. J.; Dennis, D.; Jones, R. A. *Nucleosides Nucleotides* **1985**, *4*, 377–389. (b) Barbato, S.; De Napoli, L.; Mayol, L. *Tetrahedron Lett.* **1987**, *28*, 5727–5728. (c) Capobianco, M. L.; Carcuro, A.; Tondelli, L.; Garbesi, A.; Bonara, G. M. *Nucleic Acids Res.* **1990**, *18*, 2661–2669. (d) Ross, P.; Mayer, R.; Weinhouse, H.; Amikam, D.; Huggirat, Y.; Benziman, M.; de Vroom, E.; Fidder, A.; de Paus, P.; Sliedregt, L. A. J.; van der Marel, G. A.; van Boom, J. H. *J. Biol. Chem.* **1990**, *265*, 18933–18943. (e) De Napoli, L.; Montesarchio, D.; Picciall, G.; Santacroce, C.; Mayol, L.; Galeone, A.; Messere, A. *Gazz. Chim. Ital.* **1991**, *121*, 505–508. (f) Battistini, C.; Fustinoni, S. *Tetrahedron* **1993**, *49*, 1115–1132. (g) Zeng, F.; Jones, R. A. *Nucleosides Nucleotides* **1996**, *15*, 1679–1686. (h) Alazzouzi, E.; Escaja, N.; Grandas, A.; Pedroso, E. *Angew. Chem., Int. Ed.* **1997**, *36*, 1506–1508. (i) Frieden, M.; Grandas, A.; Pedroso, E. *Chem. Commun.* **1999**, 1593–1594. (j) Smietana, M.; Kool, E. T. *Angew. Chem., Int. Ed.* **2002**, *41*, 3704–3707. (k) Smietana, M.; Johnson, R. B.; Wang, Q. M.; Kool, E. T. *Chem. Eur. J.* **2004**, *10*, 173–181.
4. (a) Lebreton, J.; Mesmaeker, A. D.; Waldner, A.; Fritsch, V.; Wolf, R. M.; Freiser, S. M. *Tetrahedron Lett.* **1993**, *34*, 6383–6386. (b) Lebreton, J.; Waldner, A.; Fritsch, V.; Wolf, R. M.; Mesmaeker, A. D. *Tetrahedron Lett.* **1994**, *35*, 5225–5228. (c) Mesmaeker, A. D.; Lebreton, J.; Waldner, A.; Fritsch, V.; Wolf, R. M. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 873–878. (d) Mesmaeker, A. D.; Waldner, A.; Lebreton, J.; Hoffmann, P.; Fritsch, V.; Wolf, R. M.; Freier, S. M. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 226–229. (e) Bergmann, F.; Bannwarth, W.; Tam, S. *Tetrahedron Lett.* **1995**, *36*, 6823–6826. (f) Waldner, A.; Mesmaeker, A. D.; Wendenborn, S. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2363–2366. (g) Mesmaeker, A. D.; Lesueur, C.; Bevierre, M.-O.; Fritsch, V.; Wolf, R. M. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2790–2794. (h) Fujii, M.; Yamamoto, K.; Hidaka, J. *Tetrahedron Lett.* **1997**, *38*, 417–420. (i) Mesmaeker, A. D.; Leberton, J.; Jouanno, C.; Fritsch, V.; Wolf, R. M.; Wendeborn, S. *Synlett* **1997**, 1287–1290. (j) Goodnow, R. A., Jr.; Richou, A.-R.; Tam, S. *Tetrahedron Lett.* **1997**, *38*, 3195–3198. (k) Goodnow, R. A., Jr.; Tam, S.; Pruess, D. L.; McComas, W. W. *Tetrahedron Lett.* **1997**, *38*, 3199–3202. (l) Robins, M.; Doboszewski, B.; Nilsson, B. L.; Peterson, M. A. *Nucleosides Nucleotides Nucleic Acids* **2000**, *19*, 69–86. (m) Wilds, C. J.; Minasov, G.; Natt, F.; von Matt, P.; Altmann, K.-H.; Egli, M. *Nucleosides, Nucleotides Nucleic Acids* **2001**, *20*, 991–994. (n) Li, X.; Zhan, Z.-Y.J.; Knipe, R.; Lynn, D. G. *J. Am. Chem. Soc.* **2002**, *124*, 746–747. (o) Li, X.; Lynn, D. G. *Angew. Chem., Int. Ed.* **2002**, *41*, 4567–4569.
5. Compound **5a** was synthesized from **5b**, which was prepared from 3′-Azido-3′-deoxythymidine (AZT) by a slight modification of the reported procedure [Ref. 4h], in which the oxidation of AZT and olefination of the resulting aldehyde was accomplished in an one-pot process using iodoxybenzoic acid (IBX) in the presence of stabilized ylide, $Ph_3P=CHCO_2Et$ (Ref. 6). The resulting product **A** was then transformed into **5b**

following the earlier reported steps (Ref. 4h). Treatment of **5b** with BOM-Cl in the presence of DBU gave **5a** in 85% yield



6. Lerner, C.; Siegrist, R.; Schweizer, E.; Diederich, F.; Gramlich, V.; Jakob-Roetne, R.; Zurcher, G.; Borroni, E. *Helv. Chim. Acta* **2003**, *86*, 1045–1062.
7. (a) Bruins, A. P.; Covey, T. R.; Henion, J. D. *Anal. Chem.* **1987**, *59*, 2642–2646. (b) Iribane, J. V.; Thomson, B. A. *J. Chem. Phys.* **1976**, *64*, 2287–2294.
8. Cole, R. B. *Electrospray Ionization Mass Spectrometry*; Wiley: New York, 1997.
9. (a) Cavanagh, J.; Fairbrother, W. J.; Palmer, A. G., III; Skelton, N. J. *Protein NMR Spectroscopy*; Academic: San Diego, 1996. (b) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986.
10. Hwang, T. L.; Shaka, A. J. *J. Am. Chem. Soc.* **1992**, *114*, 3157–3159.
11. de Leeuw, F. A. A. M.; Altona, C. Quant. Chem. Progr. Exch., No. 463, University of Indiana at Bloomington, 1983.
12. Saenger, W. *Principles of Nucleic Acid Structure*; Springer: New York, 1984.
13. Kessler, H.; Griesinger, C.; Lautz, J.; Müeller, A.; van Gunsteren, W. F.; Berendsen, H. J. C. *J. Am. Chem. Soc.* **1988**, *110*, 3393–3396.
14. Etezady-Esfarjani, T.; Hilty, C.; Wüthrich, K.; Rueping, M.; Schreiber, J.; Seebach, D. *Helv. Chim. Acta* **2002**, *85*, 1197–1209.
15. States, D. J.; Haberkorn, R. A.; Ruben, D. J. *J. Magn. Reson.* **1982**, *48*, 286–292.
16. For details see: (a) Sybyl Force Field Manual, version 6.6, 1999, pp 217—229 (http://www.tripos.com/custResources/applicationNotes/triad/index.html). (b) Evans, J. N. S. *Biomolecular NMR Spectroscopy*; Oxford University Press: Oxford, 1995.