Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron 63 (2007) 10758–10763
Tetrahedron

Synthesis of water-soluble nucleotide-calixarene conjugates and preliminary investigation of their in vitro DNA replication inhibitory activity

Grazia M. L. Consoli,[a,*] Giuseppe Granata,[a] Eva Galante,[a] Isabella Di Silvestro,[b] Laura Salafia[b] and Corrada Geraci[a,*]

[a]*Istituto di Chimica Biomolecolare, C.N.R., Via del Santuario 110, I-95028 Valverde (CT), Italy*
[b]*Agroindustry Advanced Technologies, Centro Ricerche e Sviluppo, Blocco Palma I, Zona Industriale, I-95030 Catania, Italy*

Received 4 May 2007; revised 31 May 2007; accepted 5 June 2007
Available online 24 July 2007

Abstract—Calix[4]arenes bearing four thymine or adenine 2′-deoxynucleotide moieties have been synthesized and characterized by NMR and ESI-MS analysis. Due to their amphiphilic nature, the conjugates (**2a** and **2b**) obtained tend to self-assemble in aqueous medium by stacking interactions. Their good water solubility makes **2a** and **2b** promising candidates for bioorganic applications. A preliminary study has provided evidence of their inhibitory activity toward the replication of a *Penicillium digitatum* DNA fragment via PCR (polymerase chain reaction).

1. Introduction

The design of systems capable of molecular recognition in aqueous medium is desirable, especially for the development of compounds compatible with the biological environment and having applications in drug delivery, and binding and detection of biomolecules. For such purposes, nucleotide functionalities are very interesting because of their ability to establish multiple and cooperative non-covalent interactions.[1] This has stimulated interest in the development of nucleotide conjugates that exploit the self-complementarity of nucleotides in recognition phenomena.[2] Nucleobase, nucleoside or nucleotide moieties have also been introduced onto calix[4]arene macrocycles to afford interesting self-assembling systems.[3] In this paper, we report the synthesis of novel water-soluble nucleotide-calixarene conjugates and a preliminary investigation of their potential application in biological fields.

2. Results and discussion

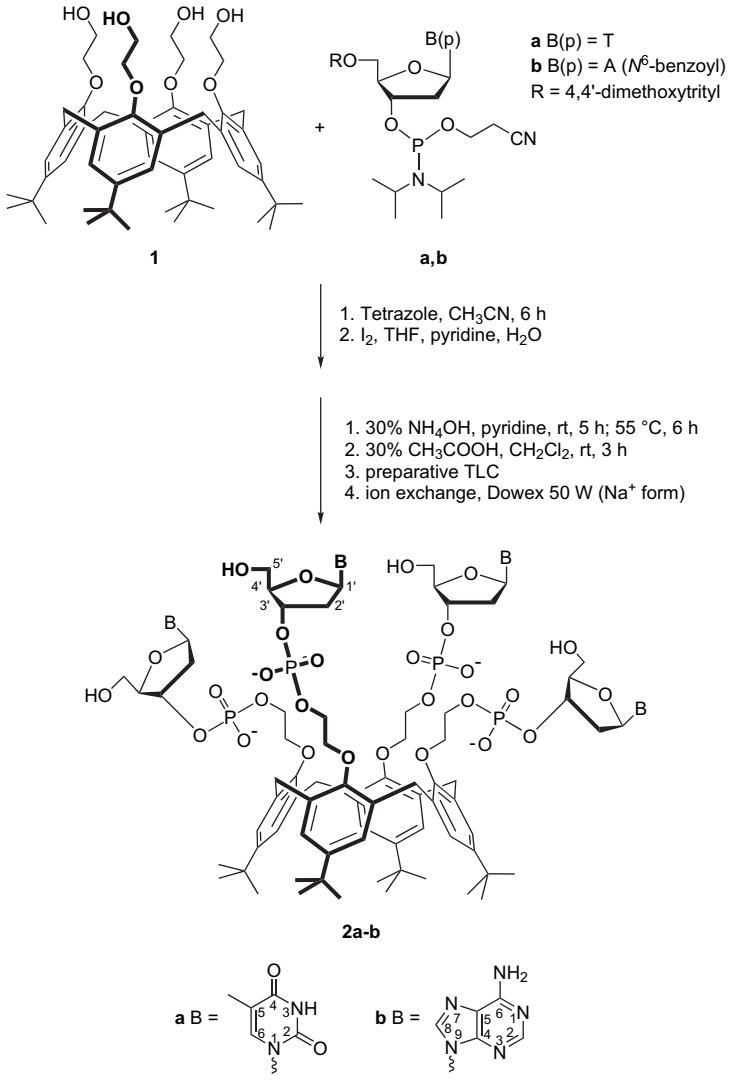
Starting from calix[4]arene **1** fixed in the *cone* conformation,[4] and adapting the procedure reported in a previous paper for the synthesis of partially functionalized nucleotide-calix[4]arene hybrids,[5] tetra-nucleotide-calix[4]arene derivatives **2a** and **2b** were obtained in 63–65% yield (Scheme 1). The increase of the hydrophilic moiety with respect to the previously reported nucleotide-calixarenes,[5] confers a good water solubility (1×10^{-2} M) on derivatives **2a** and **2b** and makes them more promising candidates for bioorganic applications.

Compounds **2a** and **2b** were characterized by NMR and ESI-MS analysis. The presence of one AX system for the calixarene Ar–CH_2–Ar groups, one resonance for the *tert*-butyl groups, and one pattern of signals for the nucleotide pendants in the ^{1}H NMR spectra of **2a** and **2b** in MeOD confirmed the exhaustive functionalization of the calixarene macrocycle. Well-resolved spectra, and no temperature and concentration dependence of the ^{1}H NMR measurements (concentration range 0.25–5 mM), indicated that compounds **2a** and **2b** exist mainly as monomeric species in MeOD solution.

Nevertheless, ESI-MS spectra of **2a** and **2b** showed in addition to the singly and doubly charged quasimolecular ion peaks (*m/z* 2086.2 $[M+2Na-H]^+$ and *m/z* 1043.5 $[M+2Na]^{2+}$ for **2a**; *m/z* 2122.0 $[M+2Na-H]^+$and *m/z* 1061.6 $[M+2Na]^{2+}$ for **2b**), peaks for dimeric (*m/z* 1383.9 for **2a** $[2M+3Na]^{3+}$, and *m/z* 1407.7 for **2b** $[2M+3Na]^{3+}$), trimeric (*m/z* 1561.0 for **2a** $[3M+2Na+2\ K]^{4+}$, and *m/z* 1591.8 for **2b** $[3M+6Na-2H]^{4+}$), and traces of tetrameric (*m/z*

Keywords: Calixarene; Nucleotide; Self-assembly; Molecular recognition; Polymerase chain reaction.
* Corresponding authors. Tel.: +39 095 7212136; fax: +39 095 7212141; e-mail: corrada.geraci@icb.cnr.it

0040–4020/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2007.06.123



Scheme 1. Synthesis of compounds **2a** and **2b**.

1641.1 for **2a** $[4M+K+4H]^{5+}$, and *m*/*z* 1697.8 for **2b** $[4M+8Na-3H]^{5+}$) self-assembled structures.[6]

Like other amphiphilic calixarene derivatives that self-organize in supramolecular structures such as solid lipid nanoparticles,[7] micelles[8] or vesicles,[9] compounds **2a** and **2b** tend to form aggregates under aqueous conditions. Initial indications of this were the broadening of their proton spectra passing from MeOD to D_2O as solvent (Figs. 1 and 2), and the frothing of their aqueous solutions when shaken.

Since aromatic protons undergo an upfield shift due to stacking interactions,[10] diamagnetic shielding effects observed by comparing the proton spectra of **2a** and **2b** with those of the corresponding free mononucleotides, 2′-deoxythymidine 5′-monophosphate (dTMP) and 2′-deoxyadenosine 5′-monophosphate (dAMP) disodium salts, showed that the nucleobases of **2a** and **2b** were involved in stacking interactions in D_2O solution.

In particular, upfield shifts of the thymine H-6 ($\Delta\delta$=0.12 ppm) and adenine H-8 ($\Delta\delta$=0.52 ppm) and H-2 ($\Delta\delta$=0.24 ppm) protons were observed when a 5 mM solution of **2a** or **2b** was compared with an equimolar solution of dTMP and dAMP, respectively. The same $\Delta\delta$ shifts were measured when a 5 mM solution of **2a** or **2b** was compared with 4-fold more concentrated solutions (20 mM) of dTMP or dAMP, thus excluding a possible concentration

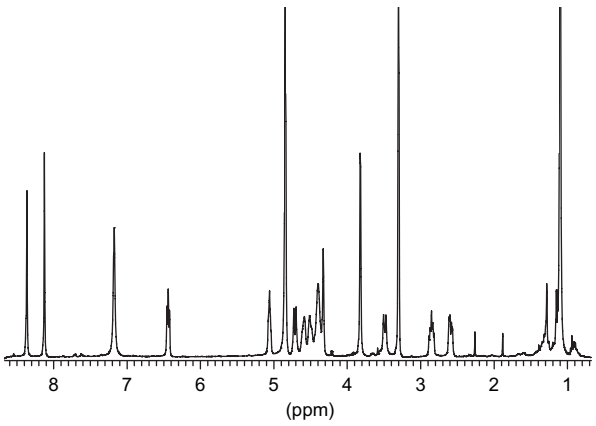


Figure 1. ^{1}H NMR spectrum of **2b** (400 MHz, 297 K, MeOD, 5 mM).

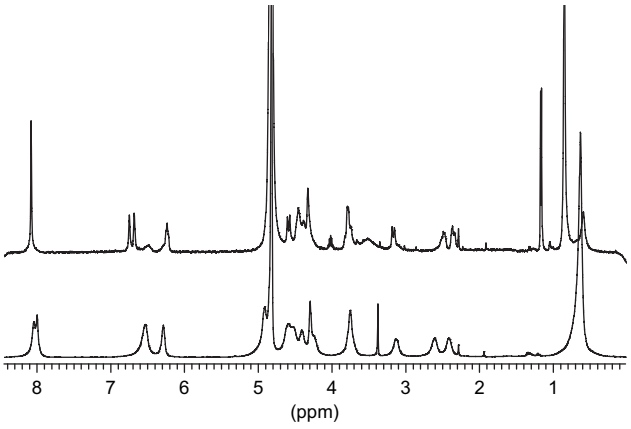


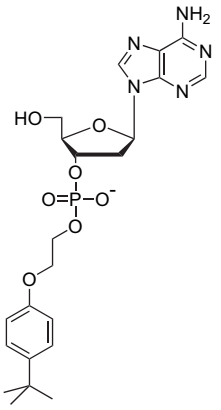
Figure 2. ^{1}H NMR spectrum of **2b** (400 MHz, 297 K, D_2O) at different concentrations: (top) 0.25 mM; (bottom) 5 mM.

effect due to the tetravalence of the nucleotide-calixarene hybrids.

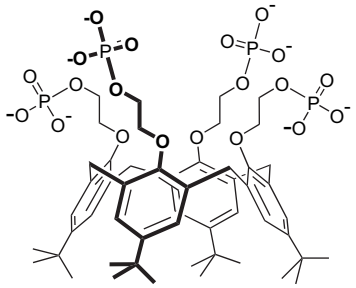
Considering the multivalent structure of **2a** and **2b,** the observed diamagnetic shielding could be due to both intramolecular and intermolecular stacking interactions. The concentration and temperature dependence of the aromatic and *tert*-butyl proton chemical shifts of **2a** and **2b**, corroborated the existence of intermolecular interactions. In particular, downfield shifts of the adenine H-2 ($\Delta\delta$=0.20 ppm) and H-8 ($\Delta\delta$=0.16 ppm), and calixarene *tert*-butyl ($\Delta\delta$=0.31 ppm) and ArH ($\Delta\delta$=0.17 and 0.24 ppm) protons, were observed when the 5 mM solution of **2b** was diluted to 0.25 mM concentration (Fig. 2). Similarly, downfield shifts of the resonances relative to the thymine H-6 ($\Delta\delta$=0.02 ppm) and calixarene *tert*-butyl ($\Delta\delta$=0.04 ppm) and ArH ($\Delta\delta$=0.09 ppm) protons resulted on diluting the 5 mM solution of **2a** to 0.5 mM.

Diamagnetic deshielding effects and sharpening of the ^{1}H NMR spectra observed by heating **2a** and **2b** from 294 to 330 K, were also consistent with the disassembly of aggregate species. Studies are in course to characterize the self-assembly (morphology and size) of **2a** and **2b** in aqueous medium.

The low toxicity and immunogenicity[11] shown by water-soluble calixarenes, combined with their anticoagulant, and antithrombotic,[12] antiangiogenic, and anticancer,[13] antiviral,[14] antimicrobial, and antifungal[15] activities, also open up prospects for biological applications of the nucleotide-calixarene conjugates. The ability of these compounds to establish DNA-like interactions[3d,5] and recognize amino acid-like compounds[5] has been reported. Due to the presence of the nucleotide moieties, compounds **2a** and **2b** can, in principle, interfere with DNA replication by recognition and binding of complementary nucleotides, nucleic acids, and/or proteins. This possibility was investigated by a polymerase chain reaction (PCR) assay, which is a fast, easy, and cheap way to identify inhibitors of the DNA replication.[16] Experiments were carried out according to the protocol drawn up by Pedersen et al.[17] When compound **2a** or **2b** were added to the PCR mixture, containing *Penicillium digitatum* DNA as template, a pair of primers, *Thermus aquaticus* Taq polymerase, and dNTPs, marked reduction or disappearance of the band corresponding to the amplified DNA fragment was detected by agarose gel electrophoresis (Fig. 3).



3



4

To evaluate the role of the calixarene platform in the PCR amplification inhibition, nucleotide derivative **3** (sodium salt), lacking the macrocyclic structure, was synthesized. When **3** was added to the PCR mixture, also at 4-fold higher concentration than **2b**, no inhibitory effect on DNA replication was observed (Fig. 3). This finding suggested that the multivalency effect, spatial preorganization of the nucleotide moieties, and/or steric hindrance are all factors influencing the inhibition.

Inhibitors of DNA amplification can act on different components of the PCR mixture.[18] Since the DNA polymerization arrested by **2a** and **2b** can be reinitiated through the addition of another aliquot of Taq DNA polymerase to the PCR medium during a second run, an inhibitory mechanism directly involving the Taq polymerase can be hypothesized. Probably the nucleotide-calixarene–DNA polymerase interaction disrupts the binding of the natural substrates to the enzyme sites, resulting in the inhibition of the polymerization process.

DNA polymerase from *T. aquaticus* belongs to the family of DNA polymerase I enzymes engaged in the repair of DNA lesions in prokaryotic organism.[19] It is known that the dNTP·Taq polymerase binding is primarily determined by

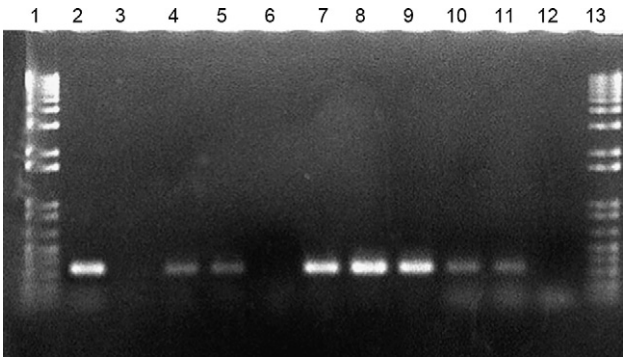


Figure 3. Agarose gel electrophoresis of PCR products: molecular marker (lane 1 and 13); positive control (lane 2), negative control (lane 12), in the presence of 20, 10, and 5 μM solution of **2a** (lanes 3–5) and **2b** (lanes 6–8), and in the presence of 30, 50, 80 μM solution of **3** (lanes 9–11).

recognition and binding of the phosphate moieties, which are anchored by three positively charged residues, Arg659, Lys663, and Arg587, and by two polar residues, His639 and Gln613.[20]

Similarly, the electrostatic potential distribution on the inhibitor and polymerase molecular surface could guide the initial binding of **2a** and **2b** to the enzyme. To verify this, PCR was carried out under the same experimental conditions but in the presence of tetraphosphorylated calixarene **4** (sodium salt, 20 μM) lacking the nucleoside moiety. The observed PCR inhibition indicated that electrostatic interactions between the negatively charged phosphate groups of the calixarene derivatives (**2a** and **2b**, **4**) and the positively charged amino acids of the Taq polymerase are involved in the molecular recognition. As in the natural DNA replication apparatus, additional non-covalent interactions established by the nucleoside moieties of **2a** and **2b**, could be important in determining the strength, specificity, and selectivity of the nucleotide-calixarene–polymerase binding. Further studies of this are in progress.

3. Conclusions

In summary, the present paper reports the synthesis of the first water-soluble calixarene derivatives bearing four nucleotide units at the narrow rim. Because of their amphiphilic nature, the synthesized hybrids show a remarkable propensity to self-assemble in polar solvents. We have begun a more detailed study to better characterize the nature of the self-assembly.

Noteworthy are the biological potentialities of the compounds reported here. A preliminary study has provided evidence of their capability to inhibit in vitro DNA replication during PCR amplification. This feature could open a new scenario for biomedical applications of the nucleotide-calixarene conjugates, such as the design of new antiviral, antibacterial, and anticancer agents.

4. Experimental

4.1. General

^{1}H NMR spectra were acquired on a Bruker Avance™ 400 spectrometer at 400.13 MHz (^{1}H). Chemical shifts (δ) are given in parts per million and referenced to: (a) TMS as internal standard for the experiments in CD_3OD; (b) the residual HOD signal (δ=4.82 ppm) for the experiment in D_2O. ESI-MS experiments were recorded in positive ion mode on a Perseptive Biosystems ESI Mariner™ mass spectrometer. Polymerase chain reaction was carried out in a GeneAmp PCR System (2400 Perkin–Elmer). Preparative TLC was performed using silica gel plates (Kieselgel 60 F_{254}, 1 mm Merck). All chemicals were reagent grade and were used without further purification.

4.2. Synthetic procedures and ^{1}H NMR data

4.2.1. Synthesis of compounds 2a and 2b. A solution of protected 2′-deoxynucleoside phosphoramidite **a** or **b** (0.6 mmol) in anhydrous CH_3CN (8 mL) and tetrazole 0.45 M in the same solvent (2.5 mL), was added dropwise to a stirring solution of compound **1** (50 mg, 0.06 mmol) dissolved in anhydrous CH_3CN (3 mL). *Nucleotide deprotection protocol*: The mixture was stirred at rt for 6 h and then treated with an excess of 0.1 M iodine solution in THF/H_2O/pyridine (9:1:0.1). After evaporation under vacuum, the residue was dissolved in CH_2Cl_2 (40 mL) and the solution was washed by 5% aqueous solutions of sodium metabisulfite and sodium sulfate. The organic layer was dried by anhydrous sodium sulfate and in vacuo. The residue was dissolved in pyridine (2 mL) and concd ammonia (40 mL) was added. The suspension was stirred at rt for 5 h and then (except for compound **2a**) at 55 °C for 6 h. After removal of the solvent under vacuum, the residue was taken up in CH_2Cl_2 (2 mL), 30% aqueous HOAc (25 mL) was added and the mixture was stirred at rt for 3 h. Removal of the solvent under vacuum left a residue that was washed with CH_2Cl_2 (10 mL) and CH_3CN (10 mL), and collected by filtration. Pure compounds **2a** and **2b** (ammonium salts) were obtained by silica gel preparative TLC (^{i}PrOH/H_2O/concd ammonia 75:20:5) in 65 and 63% yields, respectively.

4.2.1.1. Compound 2a. δ_H (CD_3OD, 297 K) 1.15 (s, 36H, 4×C(CH_3)$_3$), 1.88 (s, 12H, 4×thymine–CH_3), 2.26 (ddd, 4H, $J_{2',2''}$=−13.9 Hz, $J_{2',1'}$=7.8 Hz, $J_{2',3'}$=6.5 Hz, 4×H-2′), 2.44 (ddd, 4H, $J_{2'',2'}$=−13.9 Hz, $J_{2'',3'}$=2.4 Hz, $J_{2'',1'}$=6.0 Hz, 4×H-2″), 3.48 and 4.68 (AX system, 8H, J=12.8 Hz, 4×ArCH_2Ar), 3.77 (dd, 4H, $J_{5'',5'}$=−12.3 Hz, $J_{5'',4'}$=3.1 Hz, 4×H-5″), 3.83 (dd, 4H, $J_{5',5''}$=−12.3 Hz, $J_{5',4'}$=2.8 Hz, 4H, 4×H-5′), 4.18 (br s, 4H, 4×H-4′), 4.30 (br s, 8H, 4×OCH_2), 4.50 (br s, 8H, 4×OCH_2), the H-3′ resonance is obscured by the residual HOD signal, 6.31 (dd, 4H, $J_{1',2''}$=6.0 Hz, $J_{1',2'}$=7.8 Hz, 4×H-1′), 7.22 (s, 8H, 8×ArH), 7.85 (s, 4H, 4×H-6).

4.2.1.2. Compound 2b. δ_H (CD_3OD, 297 K) 1.09 (s, 36H, 4×C(CH_3)$_3$), 2.59 (dd, 4H, $J_{2'',2'}$=−13.4 Hz, $J_{2'',1'}$=6.1 Hz, 4×H-2″), 2.85 (ddd, 4H, $J_{2',2''}$=−13.5 Hz, $J_{2',1'}$=7.9 Hz, $J_{2',3'}$=6.0 Hz, 4×H-2′), 3.48 and 4.70 (AX system, 8H, J=12.6 Hz, 4×ArCH_2Ar), 3.82 (br s, 8H, 4×H-5′ and 4×H-5″), 4.33 (br s, 4H, 4×H-4′), 4.40 (br s, 8H, 4×OCH_2), 4.51 (d, 4H, J=11.2 Hz, 2×OCH_2), 4.58 (d, 4H, J=11.2 Hz, 2×OCH_2), 5.06 (br t, 4H, $J_{2',3'}$=6.0 Hz, 4×H-3′), 6.44 (dd, 4H, $J_{1',2''}$=6.1 Hz, $J_{1',2'}$=7.9 Hz, 4×H-1′), 7.17 (br s, 8H, 8×ArH), 8.12 (s, 4H, 4×H-2), 8.36 (s, 4H, 4×H-8).

4.3. Synthesis of compound 3

A solution of protected 2′-deoxyadenosine phosphoramidite **b** (0.13 mmol) in anhydrous CH_3CN (2 mL) and tetrazole 0.45 M in the same solvent (1 mL) was added dropwise to a stirring solution of 2-(4-(*tert*-butyl)-phenoxy)-ethanol (110 mg, 0.57 mmol) dissolved in anhydrous CH_3CN (0.3 mL). The same nucleotide deprotection protocol reported for compound **2b** was used. The final residue was taken up with ammonium bicarbonate aqueous solution and washed with AcOEt. The aqueous layer was dried under vacuum and the residue was purified by silica gel preparative TLC (^{i}PrOH/H_2O/concd ammonia 85:10:5) to give compound **3** (ammonium salt) in 90% yield. δ_H (CD_3OD,

297 K) 1.22 (s, 9H, $C(CH_3)_3$), 2.61 (dd, 1H, $J_{2'',2'}=-13.6$ Hz, $J_{2'',1'}=6.1$ Hz, H-2″), 2.85 (ddd, 1H, $J_{2',2''}=-13.6$ Hz, $J_{2',1'}=8.0$ Hz, $J_{2',3'}=6.1$ Hz, H-2′), 3.82 (br s, 2H, H-5′ and H-5″), 4.16 (br m, 1H, H-4′), 4.18–4.24 (overlapped signals, 2H×OCH_2, 1H×OCH_2), 4.33 (br m, 1H×OCH_2), 5.03 (br t, 1H, $J_{2',3'}=6.1$ Hz, H-3′), 6.39 (dd, 1H, $J_{1',2''}=6.1$ Hz, $J_{1',2'}=7.9$ Hz, H-1′), 6.79 (d, 2H, $J=8.5$ Hz, 2×ArH), 7.21 (d, 2H, $J=8.5$ Hz, 2×ArH), 8.16 (s, 1H, H-2), 8.26 (s, 1H, H-8).

4.4. Synthesis of compound 4

To a stirring solution of compound **1** (30 mg, 0.036 mmol) in anhydrous CH_2Cl_2 (0.5 mL) at 0 °C, anhydrous Et_3N (0.2 mL) was added followed by bis(2,2,2-trichloroethyl)-phosphorochloridate (138 mg, 0.36 mmol). The reaction mixture was stirred for 30 min at 0 °C and overnight at rt. Then it was quenched by addition of saturated aqueous sodium bicarbonate and extracted with CH_2Cl_2. The combined organic layers were washed with aqueous NaCl, dried by anhydrous sodium sulfate, and the solvent was removed. The residue dissolved in pyridine/AcOH (12 mL, v/v 5:1) was kept at 0 °C, then activated Zn (600 mg) was added. The reaction mixture was stirred overnight at rt. Solid was filtered off and after removal of the solvent, the residue was treated with 5 M NaOH and washed with CH_2Cl_2. The aqueous layer was treated with 2 N HCl to give a precipitate collected by filtration. After washing by 0.1 N HCl and water, pure compound **4** was obtained in 60% yield. δ_H (D_2O, 297 K) 1.17 (s, 36H, 4×$C(CH_3)_3$), 3.50 and 4.58 (AX system, 8H, $J=12.6$ Hz, 4×$ArCH_2Ar$), 4.29 (br s, 8H, 4×OCH_2), 4.44 (br m, 8H, 4×OCH_2), 7.30 (br s, 8H, 8×ArH).

Sodium salts of compounds **2a** and **2b**, **3** and **4** were obtained by passing through a column of Dowex®-50 W (H_2O/MeOH 1:1 v/v).

4.5. PCR assay

The standard reaction mixture for PCR (100 μL final volume) contained 10 μL of 10×PCR buffer, 1.5 mM $MgCl_2$, 0.5 μM primer 20-nucleotide-long ITS 549, 0.5 μM primer 24-nucleotide-long ITS 212d, 0.2 mM dNTPs, 2.5 units Taq DNA Polymerase Native (Invitrogen Life Technologies), 20 ng *P. digitatum* DNA template. To the reaction mixture variable amounts of inhibitor **2a**, **2b** (5, 10, or 20 μM), **3** (30, 50, or 80 μM) or **4** (20 μM) were added. PCR setting was 1 step at 94 °C for 30 s, followed by 40 cycles at 94 °C for 30 s, at 48 °C for 30 s, at 72 °C for 30 s, 1 step at 72 °C for 10 min, and 1 final step at 4 °C for 10 min. Ten microliters of each reaction mixture was applied to each lane in 1.5% agarose gel (containing 1 μg of ethidium bromide per mL of gel) in 1×TAE buffer (tris-acetate–EDTA), which was also the running buffer. Electrophoresis migration was performed at 70 V for 1.5 h.

Acknowledgements

This work is part of the project 'Design and synthesis of novel nucleotide derivatives as agents in biochemical recognition phenomena' (C.N.R.). Thanks are due to Dr. D. Garozzo (I.C.T.P., C.N.R., Catania) for ESI-MS measurements.

References and notes

1. (a) Sivakova, S.; Rowan, S. J. *Chem. Soc. Rev.* **2005**, *34*, 9; (b) Sessler, J. L.; Lawrence, C. M.; Jayawickramarajah, J. *Chem. Soc. Rev.* **2007**, *36*, 314.
2. (a) Iwaura, R.; Yoshida, K.; Masuda, M.; Yase, K.; Shimizu, T. *Chem. Mater.* **2002**, *14*, 3047; (b) Iwaura, R.; Yoshida, K.; Masuda, M.; Mayumi, O.-K.; Mitsuru, Y.; Shimizu, T. *Angew. Chem., Int. Ed.* **2003**, *42*, 1009; (c) Moreau, L.; Barthélémy, P.; Li, Y.; Luo, D.; Prata, C. H.; Grinstaff, M. W. *Mol. BioSyst.* **2005**, *1*, 260; (d) Moreau, L.; Ziarelli, F.; Grinstaff, M. W.; Barthélémy, P. *Chem. Commun.* **2006**, 1661.
3. (a) Sidorov, V.; Kotch, F. W.; El-Kouedi, M.; Davis, J. T. *Chem. Commun.* **2000**, 2369; (b) Kim, S. J.; Kim, B. H. *Tetrahedron Lett.* **2002**, *43*, 6367; (c) Kotch, F. W.; Sidorov, V.; Lam, Y.-F. ; Kayser, K. J.; Li, H.; Kaucher, M. S.; Davis, J. T. *J. Am. Chem. Soc.* **2003**, *125*, 15140; (d) Kim, S. J.; Kim, B. H. *Nucleic Acids Res.* **2003**, *31*, 2725.
4. Cobben, P. L. H. M.; Egberink, R. J. M.; Bomer, J. G.; Bergveld, P.; Verboom, W.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1992**, *114*, 10573.
5. Consoli, G. M. L.; Granata, G.; Galante, E.; Cunsolo, F.; Geraci, C. *Tetrahedron Lett.* **2006**, *47*, 3245.
6. In the ESI-MS spectra of **2a** and **b**, each monomeric, dimeric, trimeric, and tetrameric species presents a cluster of peaks. The values reported in the text are relative to the maximum intensity peak for each cluster distribution.
7. (a) Shahgaldian, P.; Da Silva, E.; Coleman, A. W.; Rather, B.; Zaworotko, M. J. *Int. J. Pharm.* **2003**, *253*, 23; (b) Dubes, A.; Moudrakovski, I. L.; Shahgaldian, P.; Coleman, A. W.; Ratcliffe, C. I.; Ripmeester, J. A. *J. Am. Chem. Soc.* **2004**, *126*, 6236.
8. (a) Shinkai, S.; Mori, S.; Koreishi, H.; Tsubaki, T.; Manabe, O. *J. Am. Chem. Soc.* **1986**, *108*, 2409; (b) Capuzzi, G.; Fratini, E.; Pini, F.; Baglioni, P.; Casnati, A.; Teixeira, J. *Langmuir* **2000**, *16*, 188; (c) Kellermann, M.; Bauer, W.; Hirsch, A.; Shade, B.; Ludwig, K.; Böttcher, C. *Angew. Chem., Int. Ed.* **2004**, *43*, 2959.
9. (a) Markowitz, M. A.; Bielski, R.; Regen, S. L. *Langmuir* **1989**, *5*, 276; (b) Tanaka, Y.; Myiachi, M.; Kobuke, Y. *Angew. Chem., Int. Ed* **1999**, *38*, 505; (c) Lee, M.; Lee, S.-J.; Jiang, L.-H. *J. Am. Chem. Soc.* **2004**, *126*, 12724; (d) Micali, N.; Villari, V.; Consoli, G. M. L.; Cunsolo, F.; Geraci, C. *Phys. Rev. E* **2006**, *73*, 051904; (e) Strobel, M.; Kita-Tokarczyk, K.; Taubert, A.; Vebert, C.; Heiney, P. A.; Chami, M.; Meier, W. *Adv. Funct. Mater.* **2006**, *16*, 252.
10. Waugh, J. S.; Fessenden, R. *J. Am. Chem. Soc.* **1957**, *79*, 846.
11. (a) Gansey, M. H. B. G.; De Haan, A. S.; Bos, A. S.; Verboom, W.; Reinhoudt, D. N. *Bioconjugate Chem.* **1999**, *10*, 613; (b) Da Silva, E.; Shahgaldian, P.; Coleman, A. W. *Int. J. Pharm.* **2004**, *273*, 57; (c) Da Silva, E.; Lazar, A. N.; Coleman, A. W. *J. Drug. Del. Sci. Technol.* **2004**, *14*, 3; (d) Paquet, V.; Zumbuehl, A.; Carreira, E. M. *Bioconjugate Chem.* **2006**, *17*, 1460.
12. (a) Hwang, K. M.; Qi, Y. M.; Liu, S. Y.; Lee, T. C.; Choy, W.; Chen, J. U.S. Patent 5,196,452, 1991; (b) Hwang, K. M.; Qi, Y. M.; Liu, S. Y.; Lee, T. C.; Choy, W.; Chen, J. U.S. Patent 5,409,959, 1995; *Chem. Abstr.* **1996**, *123*, 959c.

13. Blaskovich, M. A.; Ling, Q.; Delarue, F. L.; Sun, J.; Park, H. S.; Coppola, D.; Hamilton, A. D.; Sebti, S. M. *Nat. Biotechnol.* **2000**, *18*, 1065.
14. Hwang, K. M.; Qi, Y. M.; Liu, S. Y.; Lee, T. C.; Choy, W.; Chen, J. U.S. Patent 5,441,983, 1995; *Chem. Abstr.* **1996**, *123*, 275992d.
15. (a) Harris, M. Pat. WO 95/19974, 1995; *Chem. Abstr.* **1996**, *124*, 55584C; (b) Tanaka, M.; Kikuchi, A. Jap. Pat. 7,187,930, 1995; *Chem. Abstr.* **1996**, *123*, 220827y.
16. Mulli, K. B.; Ferré, F.; Gibbs, R. A. *The Polymerase Chain Reaction*; Birkhauser: Boston, 1994.
17. Pedersen, L. H.; Skouboe, P.; Boysen, M.; Soule, J.; Rossen, L. *Int. J. Food Microbiol.* **1997**, *35*, 169.
18. (a) Delarue, M.; Poch, O.; Tordo, N.; Moras, D.; Argos, P. *Protein Eng.* **1990**, *3*, 461; (b) Eom, S. H.; Wang, J.; Steitz, T. A. *Nature* **1996**, *382*, 278.
19. Li, Y.; Kong, Y.; Korolev, S.; Waksman, G. *Protein Sci.* **1998**, *7*, 1116.
20. (a) Sitohy, M.; Chobert, J.-M.; Gaudin, J.-C.; Haertlé, T. *Int. J. Biol. Macromol.* **2001**, *29*, 259; (b) Hyun, C.; Filippich, L. J.; Hughes, I. *J. Biochem. Biophys. Methods* **2005**, *62*, 63; (c) Gening, L. V.; Klinchea, S. A.; Reshetnjak, A.; Grollman, A. P.; Miller, H. *Nucleic Acids Res.* **2006**, *34*, 2579.