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Synthesis and NMR characterization of a novel crown-ether ring-fused uridine analogue

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ABSTRACT

The chemical synthesis and ^{1}H NMR analysis of a novel bicyclic uridine derivative, with a 18-crown-6 ether moiety fused at the ribose 2- and 3-positions, as first example of a hitherto unknown class of ribose-modified nucleosides, are here described. NMR-based conformational analysis studies showed for the modified nucleoside a marked preference for an N-type sugar puckering and the nucleobase in the *anti* conformation, with the uracil favouring the coordination of a sodium ion hosted in the crown ether.

1. Introduction

Since Cram and Pedersen first proposed the synthesis of macrocyclic polyether compounds, commonly defined as 'crown ethers', a plethora of different crown ether derivatives have been described in the literature over the past three decades and their synthetic and analytical potential investigated.[1] The incorporation of crown ether moieties into chiral scaffolds is interesting, providing effective chiral receptors, potentially useful as chiral reagents and catalysts for enantioselective reactions, or chiral selectors in chromatographic techniques.[2] In this context, many carbohydrate-based crown ethers, with one or more crown ethers attached to pyranosidic or furanosidic skeletons, have been described, proving to be suitable chiral phase transfer catalysts and/or models for the study of chiral recognition in enzymatic processes,[3] or useful amphiphilic macrocycles with tunable complexation and self-aggregation properties.[4]

To the best of our knowledge, very few examples of hybrid molecules combining nucleoside scaffolds with these macrocycles have appeared in the literature. The first report concerned the synthesis and biological evaluation of crown ether-linked aryl-5′-phosphate diesters derivatives of AZT (3′-azido-thymidine) and DDU (2′,3′-dideoxyuridine), proposed as the first prototypes of a new class of nucleotide prodrugs.[5] The design and synthesis of another class of AZT prodrugs, 5′-conjugated to cyclam- and bicyclam residues through a flexible five carbon atom linker, have been described.[6] Remarkably, the conjugation of DNA fragments with amphiphilic polyaza crown ethers, attached at opposite ends of the oligonucleotide backbone, has been shown to significantly stabilize DNA duplexes.[7] More recently, the synthesis and physico-chemical characterization of an adenosine derivative bearing a benzo-15-crown-5 ether moiety at the N6 nitrogen atom have also been reported.[8,9]

In the search for effective, selective and non-toxic antiviral and/or antitumoral therapeutics, a variety of strategies have been devised to design novel nucleoside analogues, mostly modified at the level of the sugar moiety. Among the plethora of known ribose-modified nucleosides, considerable attention has been focused on four, five and six atom ring-fused bicyclic nucleosides as conformationally locked substrates, preferentially recognized by cellular kinases[10] or privileged building blocks for biologically active modified oligonucleotides.[11] Nucleoside ribose-fused crown ethers have not been described in the literature and the effect of the insertion of these macrocycles on the nucleoside sugar conformation cannot be easily a priori predicted. Further interest in combining nucleosides with crown ethers may be found in the excellent binding abilities of these macrocycles towards biologically relevant cations as Na^+ and K^+,

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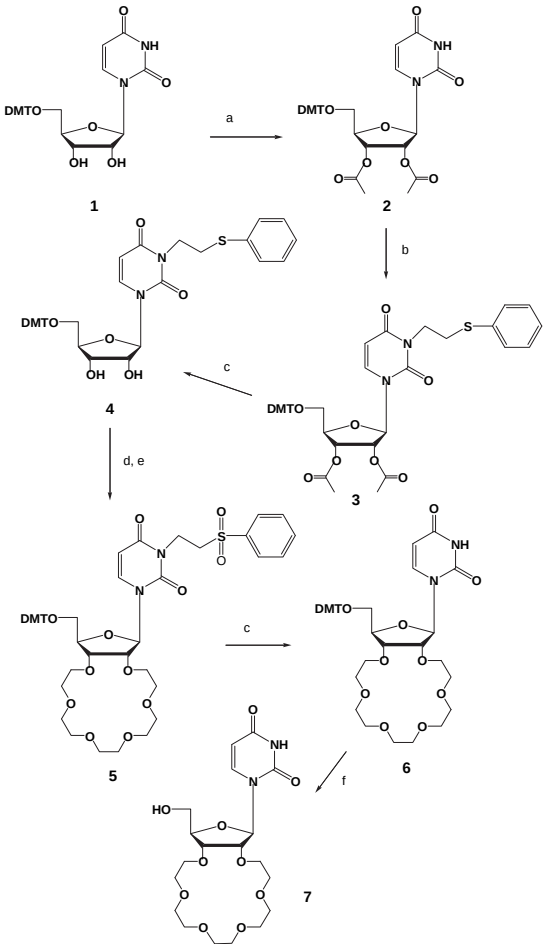
expected to confer partial positive charging to the modified nucleosides: these can show interesting biological properties per se or, if attached to an oligonucleotide chain, enhance their cell-membrane permeability. Potential ionophoric activity may also be envisaged in a design where a central nucleosidic core is decorated with crown ether moieties and amphiphilic appendages.

We have recently anticipated a general, straightforward synthetic scheme for the preparation of new bicyclic ribonucleoside derivatives, carrying a 18-crown-6 ether moiety fused at the *cis*-oriented 2- and 3-hydroxyls of the ribose.[12] To prove the feasibility of this strategy, we here describe novel uridine derivative **7** (Scheme 1), chosen as a model synthetic target, and analyze its peculiar conformational behaviour in solution as inferred from NMR data.



Scheme 1. a) Acetic anhydride, pyridine, rt, 12 h; (b) 2-(phenylthio)ethanol, ADDP, tri-*n*-butylphosphine, benzene, 10 min at 0 °C, then 18 h at rt; (c) 2 M NaOH in dioxane/H_2O, 1:1 (v/v), 50 °C, 8 h; (d) NaH, penta(ethylene glycol) di-*p*-toluenesulfonate, THF, reflux, 12 h; (e) *m*-CPBA, rt, 1 h; (f) 1% TFA in CH_2Cl_2 (v/v), rt, 1 h.

2. Synthesis

The overall synthetic procedure to obtain crown-ether ring-fused derivative **7** is described in Scheme 1. A crucial prerequisite for the selective alkylation of the 2′ and 3′-OH of a ribonucleoside is the choice of suitable protecting groups for the nucleobase, which have to be highly resistant to very basic media required for the crown ether formation step. Previously, some of us had showed the 2-(phenylthio)ethyl group as a very efficient protection to transiently mask the N3 position of thymidine, thus allowing easy manipulation of the ribose moieties.[13] 2-(Phenylthio)ethyl is a ‘safety catch’ protecting group, which can be removed in two steps: first, a mild oxidation promotes the conversion of the thioether to sulfone, followed by its effective removal through an aq basic treatment. It can be easily inserted into the uracil moiety through a high yielding Mitsunobu condensation of the sugar-protected ribonucleoside with 2-(phenylthio)ethanol and is very stable to a variety of conditions, including strongly basic solutions.

Starting from commercially available 5′-*O*-DMT-uridine **1**, addition of an excess of acetic anhydride in pyridine gave sugar-protected derivative **2**. This was then reacted with 2-(phenylthio) ethanol in the presence of tri-*n*-butylphosphine and ADDP in benzene, giving fully protected nucleoside **3** in 89% yields. Successive treatment of this substrate with a 2 M NaOH solution in dioxane/H_2O 1:1 (v/v) allowed the clean deprotection of the 2′,3′-hydroxyls, thus affording **4** in almost quantitative yields. Desired 18-crown-6-ether-fused derivative **5** was obtained by reacting **4** with a small excess of penta(ethylene glycol) ditosylate in the presence of NaH in THF. Appropriate dilution conditions were adopted to avoid undesired polymerization events; indeed, TLC monitoring of the reaction mixture showed the formation of the target compound in very satisfactory yields. Not unexpectedly, assuming, as the first event, the alkylation on one OH group, the close proximity of the vicinal OH associated with the templating effect of the metal cation (Na^+ in this case) strongly favoured the cyclization process over undesired dimerization, as deduced from the complete absence, in the reaction mixture, of side products. As the starting nucleoside disappeared, the resulting thioether was directly oxidized—by addition of *m*-chloroperbenzoic acid (*m*-CPBA), in a one pot procedure—to sulfone **5**, isolated after column chromatography in 70% for the two steps. Complete removal of the 2-(phenylsulfonyl)ethyl group was then achieved by treatment with 2 M NaOH solution in dioxane/H_2O 1:1 (v/v). Final deprotection involved the reaction of 5′-*O*-DMT-protected nucleoside **6** with a 1% TFA solution in CH_2Cl_2 for 1 h at rt. After repeated washings with organic solvents, the reaction mixture was simply purified by gel filtration chromatography on a G25 Sephadex column, eluted with H_2O/EtOH 4:1 (v/v), giving the pure target compound **7** in 80% yields. Following the described procedures, **7** was prepared in seven steps and 46% overall yield from commercially available 5′-*O*-DMT-uridine **1**.

All the synthesized compounds were purified by column chromatography, in all cases allowing the isolation of homogeneous compounds, as checked by HPLC analysis, and fully characterized by ^1H and ^{13}C NMR spectroscopy and MALDI and/or ESI-MS methods.

3. NMR analysis and structural studies

In order to elucidate the conformational features of synthesized compound **7**, a detailed NMR analysis was performed. The 1D ^1H NMR proton spectrum of **7** (700 MHz, D_2O, T=25 °C) showed the presence of signals at δ_H 7.90 (H6), 6.09 (H1′), 5.89 (H5), 4.32 (H2′), 4.23 (H4′), 4.12 (H3′), 3.97 and 3.83 ppm (H5′$_a$ and H5′$_b$, respectively). Furthermore, a severe overlapping of signals was observed at δ_H 3.70 ppm, attributed to the methylene groups of the crown ether moiety. Proton (700 MHz, T=25 °C) and carbon (175 MHz, T=25 °C) assignments were obtained through an in-depth analysis of two-dimensional COSY, HSQC and HMBC NMR experiments. 2D NOESY experiments were also acquired and studied in order to confirm the assignment and obtain structural information. The NOE pattern was consistent with the assignment we have done. Among others, we observed strong and diagnostic NOE effects between H6 and H1′, H2′, H3′, and between H5′$_a$/H5′$_b$ and H2′ that could be indicative of the conformation adopted by **7**.

Thus, these NOE contacts have been used as restraints in structural calculations. On this basis, an initial structure of **7** was generated and minimized to eliminate any possible conformational bias. The dynamics started at 1000 K using the consistent valence

force field, as implemented in the Discover software (Accelrys, San Diego, USA). Thereafter, the temperature was decreased stepwise to 250 K. The final step was again to energy-minimize in order to refine the structures thus obtained, successively using the steepest descent and the quasi-Newton–Raphson (VA09A) algorithms.

Out of 200 structures generated, 50 structures with the lowest energies were selected. As expected, the superimposition of these 50 structures (Fig. 1) (RMSD=0.03±0.03) revealed that the nucleosidic core of the molecule is well structured, while a conformational heterogeneity is observed for the crown ether moiety. The lack of violations and the low RMSD value suggest that the minimized conformation of **7** is consistent with the experimentally determined restraints. Interestingly, the nucleobase strongly prefers an *anti* glycosidic conformation. Furthermore, whereas the

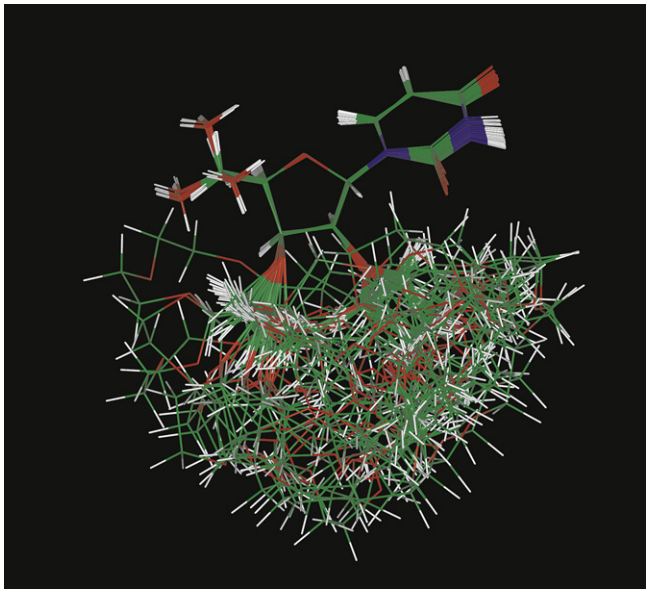


Figure 1. Superimposition of the best 50 structures of **7** in the absence of coordinated cations. The molecule is depicted in coloured 'stick' (carbons, green; oxygens, red; hydrogens, white).

unmodified uridine is characterized by a balanced distribution of the populations of ribose conformers between the North and South conformation,[14] it seems that the presence of the crown ether strongly selects the N-type conformation (C2′-*exo*) of the sugar. The net prevalence of the N-type conformation has been also confirmed by the coupling constants between H1′ and H2′, H2′ and H3′ and H3′ and H4′, being 3.0, 5.3, 6.0 Hz, respectively. Using the equation:[15]

$$\%N = J_{3'4'}/J_{1'2'} + J_{2'3'}$$

the population of the N-type conformation (%N) for **7** is found to be 72%, compared to 56% for uridine.[16] VT-^{1}H NMR experiments also clearly indicated a high rigidity for the ribose moiety in **7**, with no detectable variation in the *J* values in the temperature range 288–328 K.

Taking into consideration the high binding abilities of 18-crown-6 ethers towards Na^+ and K^+, a series of ^{1}H NMR-based titrations have been performed. In all cases, when alkali metal cations—i.e., sodium and potassium, both given in the form of chlorides or perchlorates—were progressively added to the host molecule solution up to a large excess, no detectable changes, either at the level of the chemical shifts or of *J* values, were observed in the ^{1}H NMR spectra. In analogy with the case of the carbohydrate-fused bis-crown ether we have recently studied,[17] the absence of effects in our system upon addition of sodium or potassium ions can only be explained assuming that this nucleoside is already coordinated with one sodium ion, presumably incorporated during the NaH-promoted cyclization step to give **5** and never lost in the successive synthetic steps.[18] On this basis, new sets of calculation were successively performed on **7** imposing the presence of one sodium ion. Particularly, the calculations were carried out by using the same constraints and procedures used for those performed in the absence of the cation. Furthermore, we have used additional distance restraints in the range of 2.4–5.0 Å between the oxygens of the crown ether and the sodium ion. The width of this range, if necessary, guarantees the Na^+ ion to freely move towards other functional groups of the molecule and, at the same time, to remain in the proximity of the crown ether. Also in this case, out of 200 structures generated, 50 structures with the lowest energies were selected. The superimposition of these 50 structures (Fig. 2) turned out to be excellent, having a root mean square deviation (RMSD) value of 0.03±0.02. The lack of violations and the low RMSD value suggest that the minimized conformation of **7** in the presence of sodium is also consistent with the experimentally determined restraints. This superimposition clearly shows that the sugar and the uracil base possess the same relative orientation in all the structures (Fig. 2, panel A), which is also perfectly

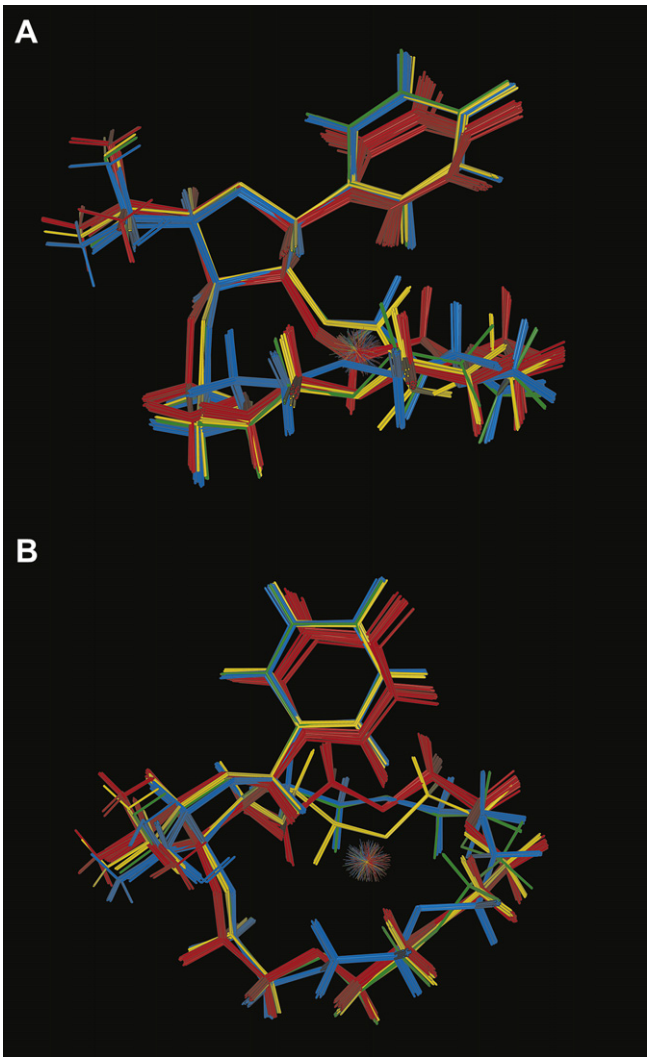


Figure 2. Two different orientations of the superimposition of the best 50 structures of **7** in the presence of Na^+. The orientation showed in panel A highlights the perfect superimposition of the base and sugar moieties. The structural heterogeneity of the crown ether is reported in panel B. The four main conformations computed for **7** are reported in red, yellow, blue and green.

superimposable to the structure obtained in the absence of Na^+ (Fig. 3). Nevertheless, in the first case, only four main conformations have been found for the crown ether moiety (Fig. 2, panel B), very similar to each other and with the sodium ion always fixed in the same position.

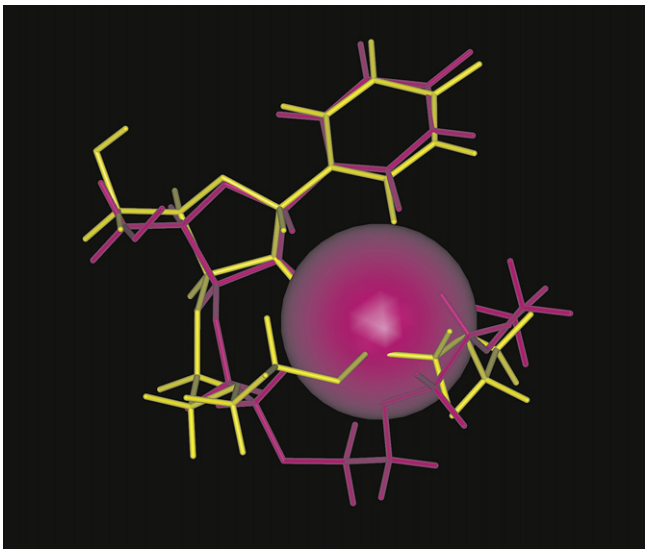


Figure 3. Superimposition of the structures obtained for **7** without (yellow) and with (magenta) the sodium ion.

Interestingly, the nucleobase is oriented in such a way that O2 points right towards the ion, providing an additional coordination site for sodium binding (Fig. 3), thus resulting into a highly functionalized lariat ether. The present results show that the 18-membered macrocycle 18-crown-6 ether, when *cis*-fused to the 2′ and 3′ positions of a nucleoside moiety, is indeed able to partially freeze the ribofuranose pseudorotational cycle, analogously to three, four or five-membered ring-fused bicyclic nucleosides[19] essentially locking the sugar in a C3′-*endo* (N-type) conformation.

4. Conclusions

The synthesis of a novel crown-ether ring-fused uridine derivative is described here, realized through a simple, high yielding procedure from 5′-*O*-DMT-uridine. Key step allowing the straightforward manipulation of the ribose moieties was the selective protection of the nucleobase with the two-stage protecting group 2-phenylthioethyl, inserted at N3 via a Mitsunobu reaction.

Interestingly, NMR-based conformational analysis studies carried out on **7** show that the crown ether-fused ribofuranose predominantly adopts an N-type conformation, with the nucleobase in an *anti* conformation about the *N*-glycosidic bond. Furthermore, the well known cation binding properties of crown ethers could be strongly enhanced in **7** because of the presence of uracil with the O2 protruding towards the crown ether. This, in a sort of lariat ether-structure, can provide an additional binding site for the coordination of one sodium or potassium ion, thereby contributing to a further stabilization of the cation–crown ether complex.

Preliminary biological evaluation on this modified nucleoside, studied at a maximum concentration of 10 μM, showed no relevant antiviral activity when tested against HIV-1 and a variety of other viruses.

Future work will address the complete biological evaluation of the synthesized compound, as well as its further elaboration into a variety of amphiphilic conjugates—through 5′-derivatization with different lipophilic moieties—for the development of potential artificial ionophores.

5. Experimental section

5.1. Materials and methods

TLC analyses were carried out on silica gel plates from Merck (60, F_{254}). Reaction products on TLC plates were visualized by UV light and then by treatment with a 10% $Ce(SO_4)_2/H_2SO_4$ aqueous solution. For column chromatography, silica gel from Merck (Kieselgel 40, 0.063–0.200 mm) was used. HPLC analyses were performed on a Beckman System Gold instrument equipped with a UV detector module 166 and a Shimadzu Chromatopac C-R6A integrator. By HPLC analysis on a Nucleosil 100-5 C18 Supelco analytical column (250×4.6 mm, 5 μm), eluted with a linear gradient from 0 to 100% in 30 min of CH_3CN in H_2O, flow=0.8 mL/min, detection at λ=264 nm, all the synthesised compounds resulted to be more than 98% pure. For the ESI-MS analyses a Waters Micromass ZQ instrument—equipped with an Electrospray source—was used in the positive and/or negative mode. MALDI TOF mass spectrometric analyses were performed on a PerSeptive Biosystems Voyager-De Pro MALDI mass spectrometer in the Linear mode using 2,5-dihydroxybenzoic acid as the matrix. NMR spectra were recorded on Bruker WM-400 or Varian INOVA 500 spectrometers. All the chemical shifts are expressed in parts per million with respect to the residual solvent signal; *J* values are in hertz. The following abbreviations were used to explain the multiplicities: s=singlet; d=doublet; t=triplet; q=quartet; m=multiplet; b=broad; dd=double doublet. Peak assignments have been carried out on the basis of standard $^1H-^1H$ COSY and HSQC experiments.

5.2. Nuclear magnetic resonance

NMR spectra for compound **7** were determined in D_2O (Aldrich, Milwaukee, USA). The NMR samples were prepared at a concentration range of 1.0–10.0 mM. Upon varying the concentration, no significant change was observed in the shape or distribution of the ^1H NMR resonances, thus allowing to exclude aggregation phenomena under these conditions. NMR spectra were recorded with a Varian^{Unity} INOVA 700 MHz spectrometer. Phase sensitive NOESY spectra[20] were recorded with mixing times in the range 250–500 ms (T=25 °C). TOCSY spectrum[21] with mixing time of 100 ms was recorded. ROESY and TOCSY were recorded using TPPI[22] procedure for quadrature detection. {$^1H-^{13}C$}-HSQC[23] and {$^1H-^{13}C$}-HMBC[24] were optimized for $^1J_{C-H}$=135 Hz and $^{2,3}J_{C-H}$=10 Hz, respectively. In all the 2D experiments, time domain data consisted of 2048 complex points in t_2 and 400–512 fids in t_1 dimension. The relaxation delay was kept at 1.2 s for all the experiments. All the experiments were acquired at T=25 °C, and the spectra were calibrated relative to HDO (4.75 ppm) as the internal standard. The NMR data were processed on a SGI Octane workstation using FELIX 98 software (Accelrys, San Diego, USA) and on a iMAC using the software iNMR (www.inmr.net).

5.3. Structure calculations

Cross peak volume integrations were performed with the program FELIX 98, using the NOESY experiment collected at mixing time of 250 ms and with a relaxation delay of 3 s. The NOE volumes were then converted to distance restraints after they were calibrated using known fixed distances of $H5'_a$ and $H5'_b$ of the ribose and H5 and H6 of the base. Then an NOE restraint file was generated with three distance classifications as follows: strong NOEs (1.5 Å$\leq r_{ij} \leq$3.5 Å), medium NOEs (3.0 Å$\leq r_{ij} \leq$4.5 Å) and weak NOEs (4.0 Å$\leq r_{ij} \leq$5.5 Å). A total of five NOEs derived distance restraints were used. Three-dimensional structures, which satisfy NOE were constructed by simulated annealing calculations. All the calculations used a distance dependant macroscopic dielectric constant of

80×*r* and an infinite cut-off for nonbonded interactions to partially compensate for the lack of the solvent.[25] The initial structure of **7** was built using a completely random array of atoms. Using the steepest descent followed by quasi-Newton–Raphson method (VA09A) the conformational energy was minimized. Restrained simulations were carried out in vacuo for 42 ns at 1000 K using the CVFF force field as implemented in Discover software (Accelrys, San Diego, USA). Then, the temperature was decreased stepwise until 250 K. The final step was again to energy-minimize so to refine the structures obtained, using successively the steepest descent and the quasi-Newton–Raphson (VA09A) algorithms. Both dynamic and mechanic calculations were carried out by using a 10 (kcal/mol)/Å^2 flatwell restraints. A total of 200 structures were generated for both calculations (with and without Na^+).

Illustrations of structures were generated using the INSIGHT II program, version '98 (Accelrys, San Diego, USA). All the calculations were performed on a PC running Linux RedHat Enterprise WS 4.0.

5.3.1. Synthesis of ***3****.* 5′-*O*-DMT-uridine **1** (500 mg, 0.92 mmol) was treated with anhydrous pyridine (3 mL) and acetic anhydride (1.5 mL). The reaction mixture was left under stirring at room temperature. After 12 h, the reaction mixture was concentrated under reduced pressure, diluted with CH_2Cl_2, transferred into a separatory funnel and washed twice with water. The organic phase, dried over anhydrous Na_2SO_4, was filtered, concentrated under reduced pressure and purified by column chromatography. Elution of the column with CH_2Cl_2 containing growing amounts of CH_3OH (from 0 to 5%), in the presence of a few drops of triethylamine, allowed desired compound **2** (576 mg, 0.92 mmol) in almost quantitative yields.

Compound **2** (576 mg, 0.92 mmol) was dissolved in anhydrous benzene (3 mL) at 0 °C and treated with 2-(phenylthio)ethanol (100 μL, 0.92 mmol) and *n*-tributylphosphine (270 μL, 1.1 mmol). After 10 min, the reaction mixture was taken to rt, treated with ADDP (280 mg, 1.1 mmol) and left, under stirring, at rt for 18 h. The solution was then taken to dryness, redissolved in ethyl acetate and washed twice with water. The organic layer was concentrated under reduced pressure and purified by silica gel chromatography. Eluting the column with *n*-hexane/ethyl acetate, 3:2 (v/v), in the presence of a few drops of triethylamine, furnished desired compound **3** in a pure form (639 mg, 0.82 mmol) in 89% yield.

Compound **3**: oil, R_f=0.3 [*n*-hexane/ethyl acetate, 7:3 (v/v)]. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.67 (1H, d, *J*=7.5 Hz, H-6); 7.44–6.83 (18H, overlapped signals, aromatic protons); 6.22 (1H, d, *J*=5.5 Hz, H-1′); 5.59–5.54 (2H, overlapped signals, H-2′ and H-3′); 5.37 (1H, d, *J*=7.5 Hz, H-5); 4.23 (1H, m, H-4′); 4.17–4.13 (2H, m, Ph–S–CH_2–CH_2–N–*Uridine*); 3.78 (6H, s, 2×(OCH_3 of *DMT* group)); 3.52–3.45 (2H, m, H_2-5′); 3.15 (2H, t, *J*=7.5 and 7.0 Hz, Ph–S–CH_2–CH_2–N–*Uridine*); 2.10 (6H, s, 2×(CH_3C=O)). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 169.5 and 169.4 (2×(CH_3C=O)); 162.0 (C-4); 150.8 (C-2); 143.7 (C-6); 158.6, 137.6, 135.4, 134.9, 134.8, 134.6, 130.0, 129.8, 128.8, 128.4, 127.9, 127.1, 126.3, 125.7 and 113.2 (aromatic carbons); 102.2 (C-5); 87.3 (quaternary carbon of *DMT* group); 86.4 (C-1′); 81.6 (C-4′); 73.0 (C-2′); 70.7 (C-3′); 62.2 (C-5′); 55.1 (2×(OCH_3 of *DMT* group)); 40.6 (Ph–S–CH_2–CH_2–N–*Uridine*); 29.5 (Ph–S–CH_2–CH_2–N–*Uridine*); 20.4 and 20.3 (2×(CH_3C=O)). MALDI-MS (positive ions): calcd for $C_{42}H_{42}N_2O_{10}S$, 766.2560; *m*/*z*, found 789.13 ([M+Na]$^+$, 100); 805.08 ([M+K]$^+$, 45); 486.26 ([(M−DMT)+Na]$^+$, 10); 502.45 ([(M−DMT)+K]$^+$, 5). HRMS (ESI-MS, positive ions): calcd for $C_{42}H_{42}N_2O_{10}SNa$, 789.2458; *m*/*z*, found 789.2439 [M+Na]$^+$.

5.3.2. Synthesis of ***4****.* Compound **3** (639 mg, 0.82 mmol) was treated with 5 mL of a 2 M solution of NaOH in dioxane/H_2O, 1:1 (v/v) and left for 8 h at 50 °C. Then the reaction mixture was concentrated under reduced pressure, redissolved in CH_2Cl_2 and washed twice with water. The organic phase, dried over anhydrous Na_2SO_4, was filtered, concentrated under reduced pressure and purified by column chromatography eluted with CH_2Cl_2 containing growing amounts of CH_3OH (from 0 to 5%), in the presence of a few drops of triethylamine. Desired compound **4** (550 mg, 0.80 mmol) was isolated in a pure form in 97% yields.

Compound **4**: oil, R_f=0.5 [CH_2Cl_2/CH_3OH, 97:3 (v/v)]. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.80 (1H, d, *J*=8.0 Hz, H-6); 7.65–6.81 (18H, overlapped signals, aromatic protons); 5.86 (1H, d, *J*=3.5 Hz, H-1′); 5.65 (2H, br s, exchangeable signal, 2×(OH)); 5.38 (1H, d, *J*=8.0 Hz, H-5); 4.37 (1H, t, *J*=5.0 and 5.0 Hz, H-3′); 4.24 (1H, apparent t, *J*=3.5 and 4.0 Hz, H-2′); 4.19–4.14 (3H, overlapped signals, (Ph–S–CH_2–CH_2–N–*Uridine*) and H-4′); 3.47–3.43 (2H, m, H_2-5′); 3.16 (2H, apparent t, *J*=8.0 and 7.5 Hz, Ph–S–CH_2–CH_2–N–*Uridine*). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 162.3 (C-4); 151.0 (C-2); 144.2 (C-6); 158.5, 138.1, 135.2, 129.9, 128.8, 128.4, 128.0, 127.8, 126.9, 125.8 and 113.1 (aromatic carbons); 101.3 (C-5); 91.0 (C-1′); 86.8 (quaternary carbon of *DMT* group); 83.9 (C-4′); 75.2 (C-2′); 69.7 (C-3′); 62.1 (C-5′); 55.0 (2×(OCH_3 of *DMT* group)); 40.4 (Ph–S–CH_2–CH_2–N–*Uridine*); 29.7 (Ph–S–CH_2–CH_2–N–*Uridine*). ESI-MS (positive ions): calcd for $C_{38}H_{38}N_2O_8S$, 682.2349; *m*/*z*, found 704.33 ([M+Na]$^+$, 100); 720.23 ([M+K]$^+$, 40). HRMS (ESI-MS, positive ions): calcd for $C_{38}H_{38}N_2O_8SNa$, 705.2247; *m*/*z*, found 705.2222 [M+Na]$^+$.

5.3.3. Synthesis of ***5****.* Compound **4** (550 mg, 0.80 mmol), dissolved in anhydrous THF (17 mL) was treated with NaH (60% dispersion in mineral oil, 96 mg, 2.40 mmol); after 10 min, pentaethylene glycol di-*p*-toluenesulfonate (655 mg, 1.2 mmol) was added and the reaction mixture was left under stirring at room temperature. After 20 min, anhydrous THF (12 mL) was added and the reaction mixture was left at reflux for 12 h. Upon consumption of the starting nucleoside, *m*-CPBA (414 mg, 2.4 mmol) was added to the mixture and the reaction left at rt, under stirring for 1 h. The reaction mixture was then concentrated under reduced pressure, redissolved in CH_2Cl_2 and washed twice with a satd. $NaHCO_3$ solution in water. The organic phase, dried over anhydrous Na_2SO_4, was filtered, concentrated under reduced pressure and purified by column chromatography. Eluting the column with CH_2Cl_2 containing growing amounts of CH_3OH (from 0 to 5%), in the presence of a few drops of triethylamine, gave pure compound **5** (512 mg, 0.56 mmol) in 70% yield for the two steps.

Compound **5**: oil, R_f=0.3 [CH_2Cl_2/CH_3OH, 97:3 (v/v)]. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.97 (1H, d, *J*=7.0 Hz, H-6); 7.68–6.82 (18H, overlapped signals, aromatic protons); 5.87 (1H, br s, H-1′); 5.73 (1H, d, *J*=8.0 Hz, H-5); 4.53 (1H, apparent t, *J*=4.5 and 4.0 Hz, H-2′); 4.37 (1H, t, *J*=5.0 and 5.0 Hz, H-3′); 4.26 (2H, t, *J*=7.0 and 7.0 Hz, Ph–SO_2–CH_2–CH_2–N–*Uridine*); 4.21 (1H, m, H-4′); 3.98–3.57 (22H, overlapped signals, 5×(O–CH_2–CH_2–O) and H_2-5′); 3.49 (2H, t, *J*=7.0 and 7.0 Hz, Ph–SO_2–CH_2–CH_2–N–*Uridine*). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 161.9 (C-4); 150.1 (C-2); 144.3 (C-6); 158.5, 138.9, 138.4, 135.3, 135.1, 133.7, 129.9, 129.6, 129.1, 128.0, 127.8, 126.9 and 113.1 (aromatic carbons); 100.9 (C-5); 89.0 (C-1′); 86.7 (C-4′ and quaternary carbon of *DMT* group); 80.9 (C-2′); 75.3 (C-3′); 70.6, 70.4, 70.3, 69.6 and 69.3 (5×(O–CH_2–CH_2–O)); 60.7 (C-5′); 55.0 (OCH_3 of *DMT* group); 52.4 (Ph–SO_2–CH_2–CH_2–N–*Uridine*); 34.6 (Ph–SO_2–CH_2–CH_2–N–*Uridine*). MALDI-MS (positive ions): calcd for $C_{48}H_{56}N_2O_{14}S$, 916.3452; *m*/*z*, found 938.94 ([M+Na]$^+$, 100); 954.93 ([M+K]$^+$, 30); 636.68 ([(M−DMT)+Na]$^+$, 10); 652.77 ([(M−DMT)+K]$^+$, 5). HRMS (ESI-MS, positive ions): calcd for $C_{38}H_{38}N_2O_8SNa$, 939.3350; *m*/*z*, found 939.3319 [M+Na]$^+$.

5.3.4. Synthesis of ***6****.* Compound **5** (512 mg, 0.56 mmol) was treated with 5 mL of a 2 M solution of NaOH in dioxane/H_2O, 1:1 (v/v) and left for 8 h at 50 °C. The reaction mixture was then concentrated under reduced pressure, redissolved in CH_2Cl_2 and washed twice with water. The organic phase, dried over anhydrous Na_2SO_4, was filtered, concentrated under reduced pressure and

purified by column chromatography. Eluting the column with CH_2Cl_2 containing growing amounts of CH_3OH (from 0 to 5%), in the presence of a few drops of triethylamine, gave pure desired compound **6** (400 mg, 0.53 mmol) in 95% yields.

Compound **6**: oil, R_f=0.3 [CH_2Cl_2/CH_3OH, 95:5 (v/v)]. ^1H NMR ($CDCl_3$, 500 MHz): δ 8.09 (1H, d, J=8.0 Hz, H-6); 7.92–6.83 (13H, overlapped signals, aromatic protons); 5.94 (1H, br s, H-1′); 5.29 (1H, d, J=8.0 Hz, H-5); 4.35 (1H, m, H-3′); 4.23–4.21 (2H, br s, H-2′ and H-4′); 3.96–3.59 (20H, overlapped signals, 5×(O–CH_2–CH_2–O)); 3.79 (6H, s, 2×(OCH_3)); 3.56–3.45 (2H, m, H_2-5′). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 162.7 (C-4); 149.6 (C-2); 144.3 (C-6); 158.5, 140.2, 135.2, 135.0, 130.0, 129.4, 128.0, 127.9, 127.0 and 113.1 (aromatic carbons); 101.7 (C-5); 88.3 (C-1′); 86.8 (quaternary carbon of *DMT* group); 81.0 (C-4′); 80.8 (C-2′); 75.4 (C-3′); 70.6, 70.4, 70.3, 69.7 and 69.4 (5×(O–CH_2–CH_2–O)); 60.7 (C-5′); 55.1 (OCH_3 of *DMT* group). MALDI-MS (positive ions): calcd for $C_{40}H_{48}N_2O_{12}$, 748.3207; m/z, found 468.49 ([(M–DMT)+Na]$^+$, 100); 484.45 ([(M–DMT)+K]$^+$, 30). HRMS (ESI-MS, positive ions): calcd for $C_{40}H_{48}N_2O_{12}Na$, 771.3105; m/z, found 771.3119 [M+Na]$^+$.

5.3.5. Synthesis of ***7***. Compound **6** (50 mg, 0.067 mmol) was treated with a 1% TFA solution in CH_2Cl_2 (v/v, 1 mL total volume) under stirring at room temperature for 1 h. The reaction mixture was then concentrated under reduced pressure, redissolved in diethyl ether and washed twice with distilled water. The aqueous phase was concentrated under reduced pressure and purified on a Sephadex G25 column eluted with H_2O/EtOH, 4:1 (v/v). From UV spectrophotometric measurements, fractions absorbing at λ=264 nm were collected and taken to dryness, yielding pure **7** (24 mg, 0.053 mmol) with 80% yields.

Compound **7**: colourless oil, R_f=0.3 [CH_2Cl_2/CH_3OH, 7:3 (v/v)]. ^1H NMR (D_2O, 700 MHz, 25 °C): δ 7.90 (1H, d, J=8.0 Hz, H-6); 6.09 (1H, d, J=3.0 Hz, H-1′); 5.89 (1H, d, J=8.0 Hz, H-5); 4.32 (1H, dd, J=5.3 and 3.0 Hz, H-2′); 4.23 (1H, m, H-4′); 4.12 (1H, dd, J=5.3 and 6.0 Hz, H-3′); 3.97 (1H, AB part of an ABX system, J=3.0 and 12.0 Hz, H-5_a′); 3.83 (1H, m, H-5_b′); 3.80–3.69 (20H, overlapped signals, 5×(O–CH_2–CH_2–O)). ^{13}C NMR (D_2O, 125 MHz): δ 161.7 (C-4); 152.0 (C-2); 143.4 (C-6); 104.8 (C-5); 90.5 (C-1′); 84.3 (C-4′); 82.4 (C-2′); 78.6 (C-3′); 72.4, 72.3, 72.1, 72.0, 71.8 and 71.7 (5×(O–CH_2–CH_2–O)); 63.0 (C-5′). MALDI-MS (positive ions): calcd for $C_{19}H_{30}N_2O_{10}$, 446.1900; m/z, found 468.90 ([M+Na]$^+$, 100); 484.85 ([M+K]$^+$, 25). HRMS (ESI-MS, positive ions): calcd for $C_{19}H_{30}N_2O_{10}Na$, 469.1798; m/z, found 469.1821 [M+Na]$^+$.

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