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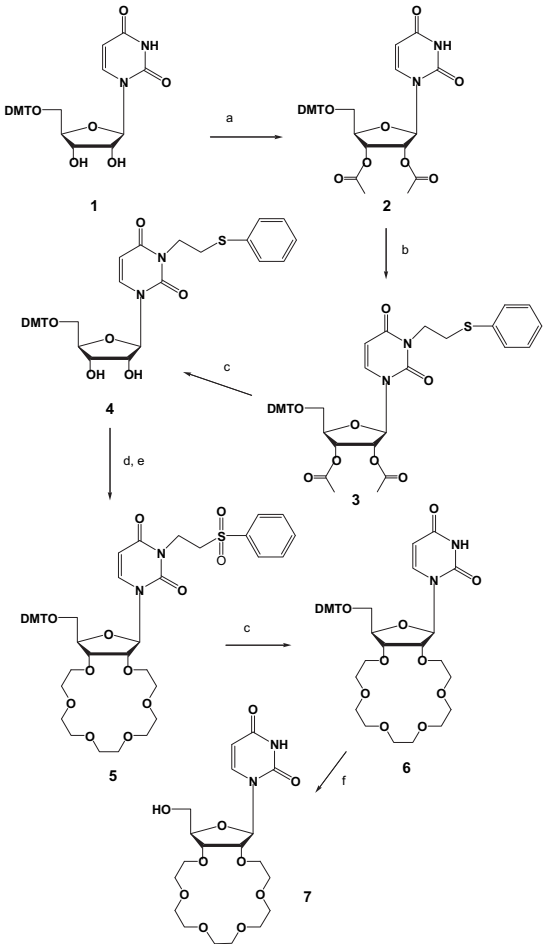
expected to confer partial positive charging to the modified nucleosides: these can show interesting biological properties per se or, if attached to an oligonucleotide chain, enhance their cell-membrane permeability. Potential ionophoric activity may also be envisaged in a design where a central nucleosidic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