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## Nucleosides, Nucleotides and Nucleic Acids

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### Preparation of Potential Cell-Permeant Nucleoside-2',3'-Cyclic Phosphate Precursors

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## PREPARATION OF POTENTIAL CELL-PERMEANT NUCLEOSIDE-2',3'-CYCLIC PHOSPHATE PRECURSORS

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□ *Uridine-3'-phosphorothiolate triesters bearing lipophilic moieties were prepared via Michaelis-Arbuzov chemistry. Subsequent deprotection of the S-cholesteryl phosphorothiolate triester afforded the corresponding diester which underwent spontaneous Cyclization to cleanly afford uridine 2',3'-cyclic phosphate. This transesterification reaction could be expedited by treatment with iodine under mild, neutral conditions.*

**Keywords** Michaelis-Arbuzov; cell-permeant; phosphorothiolate; cyclic phosphate; precursor

### INTRODUCTION

Recent interest in 2',3'-cyclic nucleotide 3'-phosphodiesterase (EC 3.1.4.37, CNP) results from its intracellular templating role in microtubule formation<sup>[1]</sup> and its maintenance of extracellular myelination by oligodendrocytes.<sup>[2]</sup> CNP represents 4% of total myelin proteins and its malfunction in oligodendrocytes is a key feature of several disease states including Multiple Sclerosis, lead poisoning and schizophrenia.<sup>[3,4]</sup>

The two splice isoforms—CNPI (46 kD) and CNPII (48 kD)—are highly modified posttranslationally and are heterogenous in size and charge.<sup>[5]</sup> Such diversity results in differential subcellular localisation<sup>[6]</sup> and also can interfere with immunohistochemical staining of the appropriate epitopes.<sup>[3,7]</sup> Although CNP mediated hydrolysis of 2',3'-cyclic nucleotides into the corresponding 2'-monoesters has been investigated chemically,<sup>[8]</sup> the in vivo relevance of this activity is still uncertain as such substrates have not been detected in the brain.

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The development of membrane-permeant nucleotide substrate analogues of CNP would enable more definitive intracellular spatial visualisation of CNP, and could potentially form the basis of therapeutic intervention in diseases involving CNP malfunction. However, intracellular delivery of such analogues has not, to the authors' knowledge, been reported. This can be attributed to the polar nature of the anion and also the reactivity of five-membered cyclic phosphates.<sup>[9]</sup>

The simplest and most well-established strategy for delivering anionic nucleotides across cell membranes is to append lipophilic moieties to nonessential functionalities of bioactive compounds such as nucleoside 3',5'-cyclic phosphate second messengers, for example, *N*<sup>6</sup>,*O*<sup>2'</sup>-dibutyl guanosine 3',5'-cyclic phosphate.<sup>[10]</sup> More elegant and effective prodrug strategies for delivering charge-neutralized mononucleotides use "programmable linkers," which only release the drug upon internalization by target cells and exposure to cytoplasmic esterases or low endosomal pH.<sup>[11]</sup>

Phosphorothiolates represent one potential programmable linker as the P-S linkage is sensitive to the endosomal pH. In addition, enhanced intramolecular trans-esterification rates of the phosphorothiolate-linked dinucleoside UpsU compared with its natural congener UpU have been documented previously.<sup>[12]</sup> We report here our results concerning the preparation of lipophilic 3'-O-phosphorothiolate derivatives of uridine and subsequent transformation of the 3'-*O,S*-cholesteryl diester to the corresponding 2',3'-cyclic phosphate under mild neutral conditions.

## RESULTS AND DISCUSSION

First reported in 1974 by Hata and Sekine,<sup>[13]</sup> the synthesis of nucleoside S-phenyl phosphorothiolate esters via directed Michaelis-Arbuzov (M-A) chemistry has yielded intermediates in the preparation of nucleoside phosphoramidates, pyrophosphates, and phosphate diesters.<sup>[14]</sup> Subsequent development of this chemistry has principally focused on the permanent installation of inter- and intranucleoside phosphorothiolate diesters.<sup>[15]</sup> Activated disulfides bearing lipophilic moieties (**1a**, **b**; Figure 1) were prepared for this study.

Disulfide **1a** was prepared according to the procedure of Eckstein and coworkers.<sup>[16]</sup> The previously unreported **1b** was synthesized by DCC-mediated coupling of 11-bromoundecanoic acid with 1-dodecylamine and transformation of the resultant 11-bromoundecamide into the corresponding thiol *via* the thiuronium salt.<sup>[17]</sup> High yields from this scheme were critically dependent on exclusion of oxygen during final alkaline hydrolysis to liberate the thiol and during its subsequent isolation and transformation to **1b**.

We previously have described the utility of *o*-methylbenzyl phosphite intermediates for the synthesis of deoxyribonucleoside phosphorothiolate

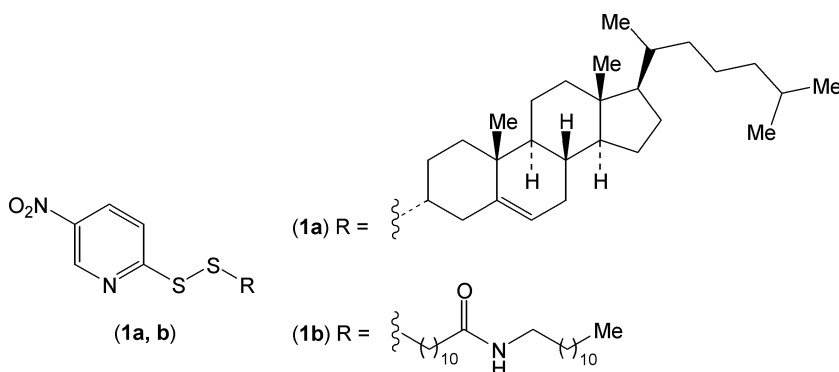
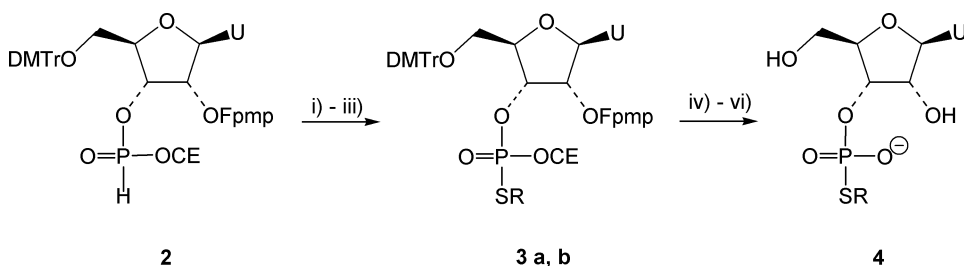


FIGURE 1 Activated disulfides used in Michaelis-Arbuzov reactions.

triesters.<sup>[18]</sup> The acid sensitivity of such intermediates precluded the application of this strategy for the preparation of lipophilic appended *ribonucleoside*-phosphorothiolates. We, thus, sought to apply a strategy starting from the activated *H*-phosphonate (**2**; Scheme 1).<sup>[19]</sup> The sensitivity of 5'-*S*-ribonucleoside phosphorothiolates towards cleavage in the presence of fluoride or base<sup>[12,20]</sup> led us to utilize the acid-sensitive 2'-*O*-Fmp-protecting group for the chemistry.

Following activation of the *H*-phosphonate **2** with *N,O*-bis(trimethylsilyl)acetamide, the trimethylsilylphosphite triester derivative of uridine was successfully treated in situ under ambient conditions with **1a** or **1b** to afford the corresponding *S*-alkyl uridine-3'-phosphorothioate triesters **3a** (96% pure by <sup>31</sup>P NMR) and **3b** (94%) after 24 hours or 12 hours, respectively. The only side-product observed was the phosphorothiolate



#### Key

DMTr: 4,4'-dimethoxytrityl

Fmp: 2-fluorophenyl-4'-methoxypiperidin-4-yl

CE: 2-cyanoethyl

R: cholesteryl (**3a,4**); *N*-decyl-11-undecanamidyl (**3b**)

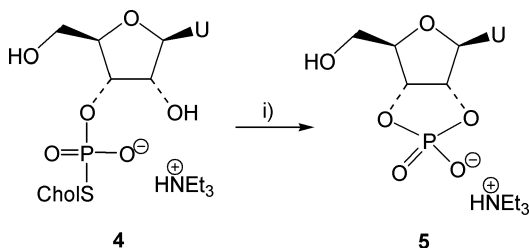
**SCHEME 1** Preparation of phosphorothiolate-derivatized nucleoside. Reagents and conditions: i) *N,O*-Bis(trimethylsilyl) acetamide (10 eq), CDCl<sub>3</sub>, 25°C, 2.5 hours; ii) **1a** (20 hours) or **1b** (12 hours) (1.5 eq), CDCl<sub>3</sub>, 25°C; iii) TCA (5 eq), pyrrole (20 eq), DCM, 25°C, 1 minute; v) sat. NH<sub>3</sub> in MeOH, 25°C, 26 hours; vi) HCl (aq), pH 3, MeOH/CHCl<sub>3</sub> (4:4:1), 25°C, 2 hours.

resulting from removal of the *S*-alkyl function. **3a** was subsequently isolated in 85% yield following chromatography. In contrast, purification of **3b** resulted in highly attenuated yields (typically 40%) principally attributable to its low solubility.

The 2'-acetal moiety retained its integrity during acid-mediated detritylation of **3a** and **3b** under anhydrous conditions and, thus, afforded the corresponding 5'-hydroxy nucleotides in 84% and 90% yields by  $^{31}\text{P}$  NMR. However spontaneous intramolecular cyclization of the phosphorothiolate triesters to the corresponding 3',5'-cyclic phosphate ( $t_{1/2}$  15 hours) was observed and only moderate yields (45% from **3a**; 22% from **3b**) were recovered following purification by silica gel column chromatography. Immediate removal of the 2-cyanoethyl group under mild conditions to yield the corresponding phosphorothiolate diesters, thus, was performed in order to inhibit the transesterification reaction. This proceeded cleanly for both products by  $^{31}\text{P}$  NMR, although the *N*-dodecyl-*S*-undecanamide diester (from **3b**) proved intractable. The 2'-protected *S*-cholesteryl diester (from **3a**) was isolated in 88% yield and the phosphorothiolate linkage was robust during work-up and handling. The 2'-ketal was hydrolysed cleanly at pH 3 to give **4** in 62% yield and 100% purity. The fully deprotected nucleotide was stored frozen at neutral pH (6.8). This significantly reduced (although did not eliminate) transesterification to the 2',3'-cyclic phosphate.

Cyclization was further expedited using iodine at neutral pH (Scheme 2);<sup>[20]</sup> competing hydrolysis to the phosphate monoester is not observed due to the high effective molarity (ca.  $10^7$  M) of the vicinal hydroxyl.<sup>[21]</sup> Exclusive formation of uridine 2',3'-cyclic phosphate in 95% yield by  $^{31}\text{P}$  NMR after 2 hours was observed (Figure 2).

We currently are seeking to employ lipophilic nucleoside and nucleotide phosphorothiolates, in particular cholesteryl derivatives,<sup>[22]</sup> as cyclic phosphate precursors for the investigation of the role of CNP in demyelination and also to the enigmatic function of biochemically related enzymes/domains in yeast and virus.<sup>[22]</sup>



**SCHEME 2** Cyclization of *S*-cholesteryl phosphorothiolate. Reagents and conditions: i)  $\text{I}_2$  (1eq), aqueous 0.1M TEAA (aq) /  $\text{CD}_3\text{OD}/\text{CDCl}_3$  (34:38:28 v/v/v), pH 6.8,  $25^\circ\text{C}$ , 2 hours.

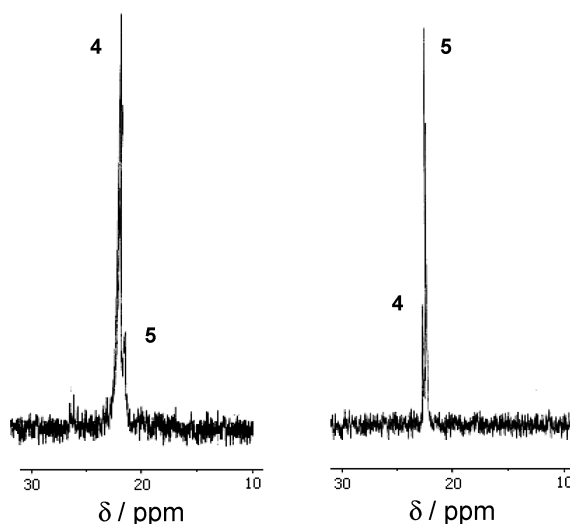


FIGURE 2  $^{31}\text{P}$  NMRs showing cyclization of **4** before (a) and after (b) addition of  $\text{I}_2$ .

## EXPERIMENTAL

### General Methods

NMRs were recorded on General Electric QE 300, Bruker DPX 300 or Bruker DRX 500 spectrometers. Electrospray (MS-ES+) mass spectroscopies were performed on a VG Auto Spec Mass Spectrometer operating at 8 kV and using 3-nitrobenzylalcohol as the matrix.

Columns were packed with dry silica (Merk 7736 Kieselgel 60 H). TLCs were carried out on Merk Kieselgel 60<sub>254</sub> and the spots visualized at 254 nm.

Chloroform and DCM were refluxed and distilled from phosphorus pentoxide (Aldrich, UK) and passed through activated basic alumina (Aldrich, UK) before use.  $\text{CDCl}_3$  (Aldrich, UK) was passed through activated basic alumina before use. EtOH and MeOH (GPR, Fisher, UK) were refluxed with the corresponding magnesium alkoxide and fractionally distilled onto 3 Å molecular sieves (Aldrich, UK). All other reagents were used as supplied from Fluka/RdH (UK).

***N*-dodecyl-11-(5-nitropyridyldisulfanyl)undecanamide (1b).** To a vigorously stirred suspension of 11-bromoundecanoic acid (500 mg, 1.88 mmol) and *N*-hydroxysuccinimide (230 mg, 2.06 mmol) in DCM (10 ml) was added a solution of *N,N'*-dicyclohexylcarbodiimide (770 mg, 3.77 mmol) in DCM (5 ml) under anhydrous conditions at 25°C. A clear solution was obtained after 5 minutes and stirring was maintained for an additional 2 hours during which precipitation of *N,N'*-dicyclohexylurea was observed. The reaction mixture was then filtered under an inert atmosphere and the filtrate added to a stirred solution of dodecylamine (690 mg, 3.76 mmol) and DMAP (10 mg, 0.09 mmol) in anhydrous DCM (10 ml). The reaction mixture was

heated at 35°C for 2 hours. Dichloromethane (40 ml) was added and the diluted solution successively washed with water (3 × 150 ml) and brine (60 ml), dried over sodium sulfate and filtered. Removal of the solvent under vacuum afforded a white solid residue which was further purified by triturating with hexane (200 ml). 11-Bromo-(*N*-dodecyl)undecanamide was isolated as a white solid (772 mg, 95%). A solution of the amide (25 mg, 0.58 mmol) and thiourea (48 mg, 0.63 mmol) in ethanol (15 ml) was refluxed for 6 hours in the absence of oxygen. The reaction mixture was then allowed to cool to 20°C and a 0.2 M solution of sodium hydroxide in argon-saturated ethanol (3 ml) added. The solution was heated under reflux for 2 hours, cooled to room temperature and acidified to pH 3 by addition of dilute HCl (aq). The solution was reduced in vacuo to give a solid residue, which was dissolved in DCM (70 ml) and washed with water (6 × 70 ml) followed by brine (100 ml). The organic phase was then dried over sodium sulfate, filtered, and concentrated under reduced pressure to yield the 11-mercapto-(*N*-dodecyl)undecanamide as a white solid (205 mg, 92% yield). The mercaptan (820 mg, 2.13 mmol) was dissolved in argon saturated DCM/methanol (40 ml, 85:15 v/v). A solution of bis(2-thio-5-nitropyridine)disulfide (1 g, 3.22 mmol) in argon-saturated DCM/methanol (60 ml, 50:50 v/v), was added dropwise over 10 minutes. Acetic acid (2.5 ml) was then added and the resulting orange solution stirred at 45°C for 48 hours. The solvent was removed in vacuo, the residue dissolved in chloroform (150 ml) and successively washed with 2 M aqueous sodium carbonate (20 ml), water (2 × 250 ml) and brine (40 ml). The organic phase was dried over sodium sulfate, filtered, and concentrated under vacuum. The solid obtained was further purified by triturating with acetonitrile (15 ml × 3), recovering each time the insoluble portion after centrifugation (15,000 rpm, 30 minutes). The desired product was isolated as a white powder after removal of the solvent in vacuo (795 mg, 64% yield).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta_H$ : 0.78–0.82 (3H, m, CH<sub>3</sub>(CH<sub>2</sub>)<sub>11</sub>), 1.67–1.85 (36H, m, (CH<sub>2</sub>)<sub>18</sub>), 2.07 (2H, t, *J* = 7.5 Hz, CH<sub>2</sub>C(O)NH), 2.76 (2H, t, *J* = 7.3 Hz, CH<sub>2</sub>SS), 3.16 (2 H, dt, *J*<sub>1</sub> = 6.7 Hz, *J*<sub>2</sub> = 6.4 Hz, CH<sub>2</sub>NHCO), 5.30 (1H, broad s, C(O)NH), 7.85 (1 H, d, *J* = 8.8 Hz, H3 Py-NO<sub>2</sub>), 8.33 (1 H, dd, *J*<sub>1</sub> = 8.8 Hz, *J*<sub>2</sub> = 2.5 Hz, H4 Py-NO<sub>2</sub>), 9.20 (1H, d, *J* = 2.5 Hz, H6 Py-NO<sub>2</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta_C$ : 13.10, 21.67, 24.77, 25.91, 27.42, 27.95, 28.06 (2C), 28.87 (2C), 28.32 (2C), 28.54 (2C), 28.62 (2 C), 28.68 (2 C), 30.89, 35.90, 38.03, 38.49, 76.19, 103.00, 118.09, 130.53, 144.08, 179.50, 171.94. Calc. MS-ES+, 5.60 × 10<sup>8</sup> eV: 541 [M+H], 563 [M+Na].

**S-Thiocholesteryl-2-cyanoethyl-5'-O-(4,4'-dimethoxytrityl)-2'-O-(1-fluorophenyl-4'-methoxypiperidyl-4'-yl)uridine 3'-phosphorothiolate (3a).** To a solution of 5'-O-(4,4'-dimethoxytrityl)-2-cyanoethyl-2'-O-(1-fluorophenyl-4'-methoxypiperidyl-4'-yl) uridine 3'-(hydrogen *H*-phosphonate)<sup>[20]</sup> (2; 136 mg, 0.16 mmol) in CDCl<sub>3</sub> (1.2 ml), was added *N,O*-bis(trimethylsilyl)

acetamide (0.39 ml, 1.6 mmol) at 25°C. The reaction was maintained under these conditions for 1 hour after which time complete reaction to the putative phosphite triester intermediate was inferred by  $^{31}\text{P}$  NMR analysis ( $\delta$  130.0 and 129.2 ppm). Solid disulfide **1a** (130 mg, 0.24 mmol) was added in situ with vigorous mixing and the reaction mixture maintained under ambient conditions for 20 hours. The reaction was subsequently diluted with ethyl acetate (40 ml) and washed with a 2 M  $\text{NaHCO}_3$  (aq) ( $2 \times 70$  ml), water ( $2 \times 70$  ml) and brine (20 ml). The organic phase was dried over sodium sulfate, filtered, and reduced in vacuo. The residue was purified by silica gel chromatography eluting with a gradient of methanol (0–3% v/v) in DCM. **3a** was isolated as a cream foam (173 mg, 85% yield).

$^{31}\text{P}$  NMR (121 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{P}}$ : 29.95 and 29.38.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{H}}$ : 0.55–2.07 (43H, m, aliphatic chol.), 3.09–3.11 (3H, 2s,  $\text{OCH}_3$ -Fpmp), 2.33–3.18 (11H, m, PSCH,  $(\text{CH}_2\text{CH}_2)_2\text{N} + \text{OCH}_2\text{CH}_2\text{CN}$ ), 3.31–3.49 (2H, m,  $\text{H}5' + \text{H}5''$ ), 3.70 (6H, s,  $\text{OCH}_3$ -DMTr), 4.30–4.11 (2H, m,  $\text{OCH}_2\text{CH}_2\text{CN}$ ), 4.35–4.39 (1H, m,  $\text{H}4'$ ), 4.91–4.82 (1H, m,  $\text{H}3'$ ), 4.96–5.08 (1H, m,  $\text{H}2'$ ), 5.14–5.16 (1H, m,  $\text{H}5$ ), 5.16–5.22 (1H, m, vinylic chol.), 6.10–6.22 (1H, m,  $\text{H}1'$ ), 6.74–7.24 (17H, m, ArH-DMTr + ArH-Fpmp), 8.60 (1H, broad s,  $\text{N}3\text{H}$ ), 8.66–8.70 (1H, m,  $\text{H}6$ ). MS-ES+,  $9.31 \times 10^6$  eV: 1273 [M+H] 1295 [M+Na].

**S-(N-dodecyl-11-undecanamidyl)-2-cyanoethyl-5'-O-(4,4'-dimethoxytrityl)-2'-O-(1-fluorophenyl-4'-methoxypiperidyl-4'-yl)uridine 3'-phosphorothiolate (3b).** Prepared as above except that the M-A reaction was complete within 12 hours (86 mg, 44% yield).

$R_{\text{f}}$  (dichloromethane/methanol 9:1) = 0.56.  $^{31}\text{P}$  NMR (121 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{P}}$ : 31.05 and 30.29.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{H}}$ : 0.87 (3H, t,  $J = 6.4$  Hz,  $(\text{CH}_2)_{11}\text{CH}_3$ ), 1.25–2.10 (36H, m,  $(\text{CH}_2)_8\text{CH}_2\text{C}(\text{O})\text{NH} + (\text{CH}_2)_{10}\text{CH}_2\text{NHC}(\text{O})$ ), 2.14 (2H, t,  $J = 7.6$  Hz,  $\text{CH}_2\text{C}(\text{O})\text{NH}$ ), 2.75 (2H, t,  $J = 6.3$  Hz,  $\text{OCH}_2\text{CH}_2\text{CN}$ ), 2.62–3.18 (10H, m,  $(\text{CH}_2\text{CH}_2)_2\text{N} + \text{PSCH}_2$ ), 3.05–3.28 (5H, m,  $\text{C}(\text{O})\text{NHCH}_2 + \text{OCH}_3$ -Fpmp), 3.40–3.65 (2H, m,  $\text{H}5' + \text{H}5''$ ), 3.80 (6H, s,  $\text{OCH}_3$ -DMTr), 4.18–4.36 (2H, m,  $\text{OCH}_2\text{CH}_2\text{CN}$ ), 4.31–4.44 (1H, m,  $\text{H}4'$ ), 4.90–5.01 (1H, m,  $\text{H}3'$ ), 5.09–5.18 (1H, m,  $\text{H}2'$ ), 5.27–5.30 (1H, m,  $\text{H}5$ ), 5.42–5.51 (1H, broad s,  $\text{C}(\text{O})\text{NH}$ ), 6.27–6.30 (1H, m,  $\text{H}1'$ ), 6.84–7.34 (17H m, ArH-DMTr + ArH-Fpmp), 7.65–7.80 (1H, m,  $\text{H}6$ ), 8.98–9.04 (1H, 2 broad s,  $\text{N}3\text{H}$ ). MS-ES+,  $2.20 \times 10^6$  eV: 1256 [M+H], 952 [M-DMTr+H].

**Ammonium salt of S-Cholesteryl-uridine-3'-O-phosphorothiolate (4).** To a stirred solution of **3a** (163 mg, 0.2 mmol) in DCM (2 ml) was added a 1 M solution of trichloroacetic acid in anhydrous DCM (1 ml) under an inert atmosphere at room temperature. The resulting deep orange solution was maintained under these conditions for 3 minutes and pyrrole (185  $\mu\text{l}$ , 4 mmol) added. After 20 minutes, the reaction mixture had decolorized and was immediately diluted with ethyl acetate (150 ml), washed with 2 M  $\text{NaHCO}_3$  (aq) ( $2 \times 300$  ml), water ( $3 \times 200$  ml) and brine (40 ml).



The organics were then separated, dried over sodium sulfate, filtered under reduce pressure, and concentrated in vacuo. The crude detritylated intermediate was purified by silica gel column chromatography eluting with 0–3% methanol in DCM to yield a white solid (87 mg, 45%) and used immediately in the subsequent step. To a solution of this detritylated nucleotide (61 mg, 0.06 mmol) was added methanolic ammonia (saturated at  $-15^{\circ}\text{C}$ ) in methanol (1 ml), and the reaction mixture maintained at ambient temperature for 28 hours. Evaporation of the volatiles under reduced pressure afforded the desired 2'-Fpmp-protected phosphorothiolate diester (51 mg, 88%). The phosphorothiolate diester derivative (30 mg, 0.03 mmol) was dissolved in 4.4:1 (v/v) methanol: $\text{CHCl}_3$  (5.4 ml)  $\text{HCl}$  (aq) added until the pH reached 3 (*ca.* 1 ml) and stirred under ambient conditions for 6 hours. Solvent was removed in vacuo and following coevaporation with acetonitrile. Hydrolysed Fpmp ketal was removed by trituration with diethyl ether (8 ml) to yield the product (**4**) (15 mg, 62% yield) which was stored frozen in 100 mM triethylammonium acetate (pH 6.8).  $^{31}\text{P}$  NMR (121 MHz,  $\text{CDCl}_3/\text{CD}_3\text{OD}$  6:4)  $\delta_P$ : 25.24;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3/\text{CD}_3\text{OD}$  6:4)  $\delta_H$ : 0.64–2.47 (43H, m, aliphatic chol.), 2.96–3.07 (1H, m, PSCH), 3.75–3.86 (2H, m,  $\text{H}5' + \text{H}5''$ ), 4.26–4.37 (2H, m,  $\text{H}3' + \text{H}4'$ ), 4.70–4.75 (1H, m,  $\text{H}2'$ ), 5.29–5.32. (1H, m, vinylic chol.), 5.69 (1H, d,  $J = 8.1$  Hz,  $\text{H}5$ ), 5.91 (1H, d,  $J = 5.3$  Hz,  $\text{H}1'$ ), 7.92 (1H, d,  $J = 8.1$  Hz,  $\text{H}6$ ). MS-ES,  $9.31 \times 10^6 \text{ eV}$ : 914 [M-].

**Triethylammonium salt of uridine-2',3'-O-cyclic phosphate (5).** A solution of the triethylammonium salt of phosphorothiolate monoester derivative **4** (15 mg, 0.02 mmol) in water, buffered at pH 6.8 in triethylammonium acetate 0.1 M, deuterated methanol and deuterated chloroform (1 ml, 34:38:28 v/v/v), was treated with elemental iodine (5 mg, 0.02 mmol) at  $25^{\circ}\text{C}$ . The reaction was monitored by TLC (dichloromethane and methanol (6:4 v/v + 0.2% of triethylamine)). After 2 hours the reaction was 95% complete and the product characterized by co-injection on C18 RP-HPLC with an authentic sample purchased from Aldrich.

$^{31}\text{P}$  NMR (202 MHz, 0.1M TEAA, methanol and dichloromethane 34:38:28:4)  $\delta_P$ : 22.21.

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