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# Synthesis and evaluation of glucuronic acid derivatives as alkylating agents for the reversible masking of internucleoside groups of antisense oligonucleotides

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**Abstract**

2-Iodoethyl (methyl α-D-glucopyranosid)uronate and 2-iodoethyl (methyl β-D-glucopyranosid)thiouronate were prepared in five steps by an efficient synthetic route starting from D-glucuronic acid. Both compounds were used to alkylate dithymidine phosphorothioate and phosphorodithioate diesters, leading to the corresponding phosphotriesters **12** to **15**. Hydrolytic stability of **12–15** was studied in different biological media. The enzymatic hydrolysis of **12–15** was accompanied by another reaction resulting in formation of the dithymidine phosphodiesters. Several possible mechanisms for these reactions are proposed. © 1997 Elsevier Science Ltd.

*Keywords:* Glucuronic acid; Oligodeoxynucleoside phosphorothioate and phosphorodithioate; Antisense; Prodrug

## 1. Introduction

Oligonucleotides are a promising class of compounds for gene therapy. Their in vitro and in vivo efficiency was demonstrated as antioncogene as well as antiviral agents [1]. Two main obstacles, which are oligonucleotides cellular uptake and enzymatic stability, are still limiting the use of these compounds as antisense agents.

The anionic nature of oligonucleotides and high molecular weight complicate their uptake by cells. Non ionic oligonucleotides, obtained by chemical modification of the sugar-phosphate backbone such as methylphosphonodiesters, have been reported to enter cells more efficiently than their polyanionic counterparts through a different mechanism [2]. But this type of oligomers offers a lower binding affinity to RNA targets. Additionally, the methylphosphonates are unable to elicit RNase H activity. This intracellular enzyme plays a key role in the antisense

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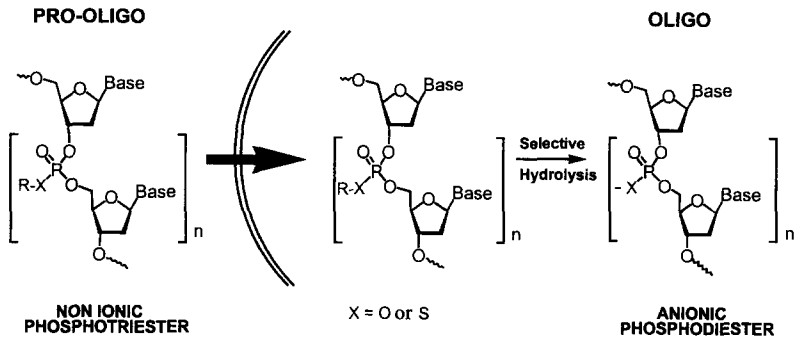



Scheme 1. Principle of the 'Pro-oligo approach'.

activity of oligonucleotides because it digests the target RNA strand of the formed RNA/DNA duplex [3].

The resistance of oligonucleotides to nuclease degradation has been improved by various chemical modifications of the sugar moiety [4–6] or of the phosphate backbone [7,8]. However, at this time, only phosphorothioate and phosphorodithioate derivatives are known to induce intracellular RNase H activity [9,10].

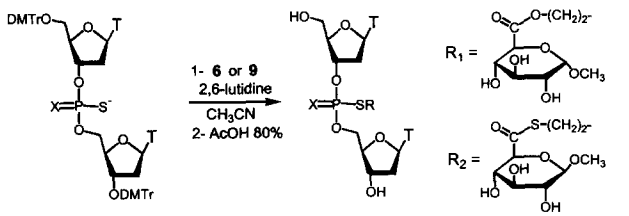
In this context, we considered the 'pro-oligo approach' [11], which is based on the transient masking of the phosphates negative charges with biocleavable substituents which could be selectively removed by intracellular enzymes yielding the oligonucleotide phosphodiester (Scheme 1).

The transient protective group must be stable enough in biological fluids but must be selectively and rapidly removed upon intracellular enzymatic activation. In a previous work, experiments were carried out with dithymidine phosphorothioate and phosphorodithioate models [11]. Thus, the esterases removable PivaloylOxyMethyl (POM) or *S*-AcylThioEthyl (SATE) protective groups were recently successfully introduced into the chimeric oligonucleotides [12,13]. Even if the pro-oligonucleotide concept seems of interest, one can anticipate that a fully protected oligonucleotide containing approximately 20 phosphate groups and bearing the lipophilic protecting moieties will have a poor solubility in aqueous solution. This may limit its application in antisense therapy.

In this article, the synthesis of a novel potentially enzymatically labile substituent derived from D-glucuronic acid is described. The high hydrophilicity of this compound can increase the solubility of the protected oligonucleotide in aqueous media. In addition, it is known that cell membranes are covered by glycosidic receptors that may play a role in receptor-mediated endocytosis [14]. In the present case, glucuronic residues located on the oligonucleotide chain may improve the oligonucleotides uptake via specific receptor-mediated transport.

To evaluate the potential of this new substituent, dithymidine phosphorothioate and phosphorodithioate triesters models were used. Masking of the phosphorothioate charge was achieved by a post-synthesis alkylation (Scheme 2).

We now describe the syntheses of glucuronic acid derivatives **6**, **9** as alkylating agents and preliminary data on the stability of the dithymidine phosphotriesters bearing such residues in culture medium and in cells' extract.

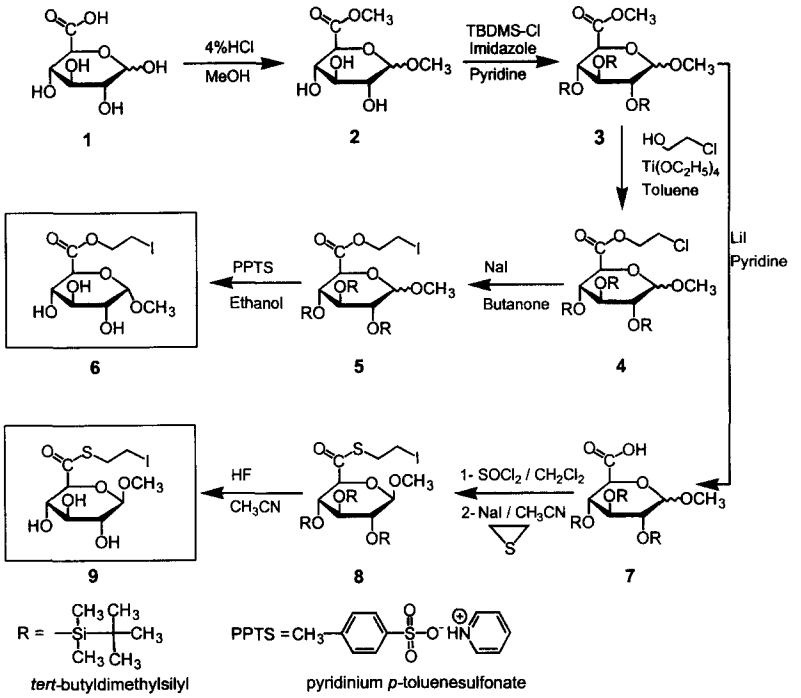



Scheme 2. Alkylation of phosphorothioate and phosphorodithioate diesters.

## 2. Results and discussion

The synthesis of compounds **6** and **9** has been achieved in five steps by two different approaches. Formation of ester **6** proceeds through a transesterification step of **3** and formation of the thioester **9** via an hydrolysis of the methyl ester **3** to generate the acid **7** (Scheme 3).

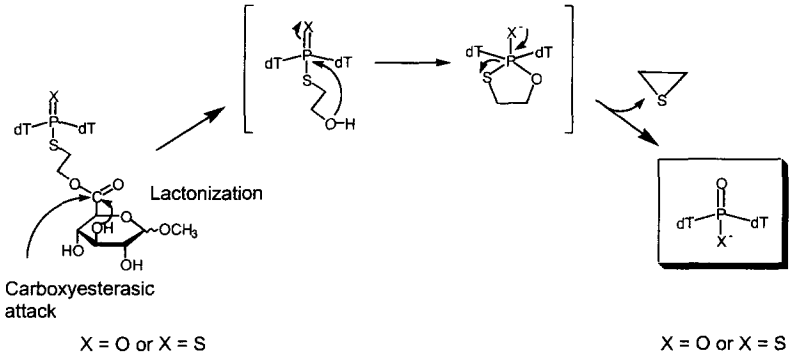
The common precursor **3** was obtained in two steps. The first step involved simultaneous protection of the anomeric position of the **1** and esterification of




Scheme 3. Synthesis of alkylating reagents: 2-iodoethyl (methyl β-D-glucopyranosid)uronate (**6**) and 2-iodoethyl (methyl β-D-glucopyranosid) thiouronate (**9**).

the carboxylate in anhydrous methanol containing acetyl chloride [15,16]. During this step, an equilibrium between methyl (methyl α,β-D-glucopyranosid)uronate **2** and methyl α,β-D-glucofuranosidurono-6,3-lactone [15] was observed, that was shifted towards the ester **2** by heating of the reaction mixture. To avoid lactonization, it was necessary to protect the hydroxyl groups of **2** by a suitable protecting group which had to be stable during the transesterification and the nucleophilic substitution steps (Steps 3 and 4 for **6**, Scheme 3) and the esterification (Step 4 for **9**). Then, this group had to be cleaved under neutral conditions to avoid degradation of the alkylating derivatives **6** and **9**. The *tert*-butyldimethylsilyl group was chosen, resulting in the precursor **3** [17]. The 2-chloroethyl (methyl 2,3,4-tri-*O*-*tert*-butyldimethylsilyl-α,β-D-glucopyranosid)uronate **4** has been obtained by a transesterification in presence of titanium tetraethoxide and 2-chloroethanol [18]. Compound **6** was then obtained by nucleophilic substitution of the chlorine atom with sodium iodide in butanone [19], followed by removal of the silyl protecting groups in **5** by reaction with pyridinium *p*-toluenesulfonate. Further purification resulted in the α-anomer of **6** [20]. Synthesis of the thioester **9** needed another strategy involving hydrolysis of the methyl ester functionality of **3** using lithium iodide [21], followed by an esterification of the free acid **7**. The yield of **7** was low, however alternative deprotection experiments involving potassium *tert*-butoxide or sodium hydroxide failed, because one *tert*-butyldimethylsilyl group was removed during the reaction. The following step allowed the direct transformation of the acid **7** in **8** via the generation in situ of an acyl chloride intermediate [22]. Finally, cleavage of the silyl protecting groups was performed with 40% aqueous hydrofluoric acid in acetonitrile [23]. Dinucleoside phosphorothioate and phosphorodithioate diesters **10** and **11** were synthesized using the hydrogenophosphonate method described by Stawinski et al. [24,25]. Alkylation of these diester dimers was carried out with iodo-derivatives **6** and **9** in the presence of 2,6-lutidine, as shown in Scheme 2. Alkylation of the phosphorodithioate **11** was faster than that of **10**, 3 and 7 days respectively, presumably due to the lower delocalization of the negative charge on the phosphorus atom.

After removal of the 4,4′-dimethoxytrityl group, the dinucleoside phosphotriesters **12–15** were purified by HPLC. Structures of phosphotriesters **12–15** were confirmed by $^{1}H$, $^{31}P$ NMR spectroscopy, mass spectrometry and UV spectroscopy. Unfortunately, a rapid decomposition of phosphotriesters **14** and **15** (40% of degradation within 2 h of incubation in $H_2O$ at room temperature) was observed, which made their purification difficult. The main product of decompo-




Scheme 4. Proposed competitive mechanisms of hydrolysis of dimer phosphotriesters.

sition of the phosphorothioate triester **14** was identified as the phosphorothioate diester **10**. Similarly, decomposition of the phosphorodithioate triester **15** led to the corresponding phosphorodithioate diester **11**. These observations suggest that an intramolecular reaction *via* a lactonization rather than a nucleophilic attack at the phosphorus atom could have occurred, which would lead to a phosphodiester and a phosphorothioate diester dimers respectively (Scheme 4). This intramolecular hydrolysis in **14** and **15** could be explained by a better leaving group ability of a thiolate compared to an alkoxide, with the consequence that a thioester functionality is more sensitive to hydrolysis than an ester function. The lack of stability of **14** and **15** made their use uncertain in further experiments and convinced us to carry out stability studies in biological media exclusively with the dimer triesters **12** and **13**.

In order to evaluate these compounds as substrates for esterases, a first set of experiments was conducted, which consisted in incubating phosphotriesters **12** and **13** with pig liver esterase (mixture of 7 isoenzymes) in phosphate buffer (Table 1). The half-life, $t_{1/2}$, of **12** was 2.2 h in this mixture and 3 h in the phosphate buffer alone. It seemed that this enzyme hydrolysed very slowly compound **12**. This fact can be explained by the spatial hindrance of the sugar moiety or its high hydrophilicity, since several authors reported that esterases have, in general, a higher activity with lipophilic esters [26,27].

The hydrolysis of **12** and **13** in cell extracts (CE) and in culture medium (CM), containing RPMI 1640 and 10% heat inactivated foetal calf serum was further investigated. As shown in Table 1, half-lives of dimers **12** and **13** in cell extracts were longer as compared to culture medium, which was opposite to our previous observation obtained with phosphotriesters bearing other biocleavable protecting group [28]. This may indicate that **12** and **13** are not efficient substrates for the esterases which are present in the CM and in the CE. Moreover, because the half-lives of **12** and **13** are slightly higher in RPMI than in CM (RPMI + 10% FCS), one can suggest that the observed decomposition is mainly due to a non-enzymatic process. We think that the decomposition of **12** and **13** is a result of lactonization process under the used experimental conditions (Scheme 4). The lactonization primarily occurs in the aqueous media, although a complementary esterases-mediated hydrolysis may take place.

Consequently, the glucuronic acid transient phosphate protecting groups cannot be used in a pro-oligonucleotide approach. The corresponding phosphotriesters are neither stable enough in aqueous solutions, nor are efficient substrates for intracellular

Table 1
Half-lives (h) of phosphotriesters **12** and **13** and resulting phosphodiesters

| | RPMI 1640 | Culture medium (CM) | Cell extract (CE) | Pig liver esterase (PLE) |
|---|---|---|---|---|
| **12** | 2.30 ± 0.10 | 2.05 ± 0.05 | 4.70 ± 0.30 | 2.20 ± 0.10 |
| | | | $POO^-$ formed | |
| **13** | 4.10 | 3.00 | 5.00 | 1.00 |
| | | | $POS^-$ formed | |

The hydrolysis mixtures contained $5.10^{-5}$ M dimer **12** or **13** in: RPMI 1640, Culture Medium (CM) constituted of RPMI 1640 supplemented with 10% heat desactivated foetal calf serum, CEM-SS cell supernatant (CE) or 8u/mL PLE [EC(3.1.1.1); Aldrich] in Phosphate buffer (pH 7,4; 20mM), and incubated at 37 °C. The nature of dimer phosphodiesters had been confirmed by co-injection with authentical samples.

esterases. It would be of interest to study derivatives bearing another sugar component, like the D-allopyranosiduronic acid, in order to avoid the lactonization process and to increase phosphotriesters stability in aqueous media. These compounds would still be soluble in aqueous media, and should become better substrates for esterases because of their lower hydrophilicity. Evaluation of new transient biocleavable moieties of the internucleoside groups is in progress.

## 3. Experimental

*General procedures.*—The $^{1}H$, $^{13}C$ and $^{31}P$ spectra were recorded with a Bruker AC 250 (250 MHz) spectrometer. $^{1}H$ chemical shifts were measured relative to $CDCl_3$ fixed at 7.24 ppm, $CD_3COCD_3$ fixed at 2.04 ppm or $CD_3CN$ set at 1.96 ppm. Standard proton decoupling techniques were used where required for characterisation. The presence of two different anomers has made difficult the exact assignment of the resonance signals. $^{13}C$ spectra were measured relative to $CDCl_3$ fixed at 77.01 ppm or $C_5D_5N$ fixed at 149.8, 135.5, 123.6 ppm and taken as internal reference. Conventional full characterization including elemental analysis of final products **6** and **9** could not be made because of the small amounts obtained in pure form that were totally used during alkylation reaction, but chromatographic homogeneity in several systems, detailed high field NMR data and FAB high resolution mass analyses indicate that the described products were pure. $^{31}P$ NMR chemical shifts were reported relative to $H_3PO_4$ taken as external standard. FAB mass spectra were recorded on a JEOL DX300 mass spectrometer operating with a JMA-DA 5000 mass data system in positive or negative ion mode using a mixture (1:1, v/v) of glycerol and thioglycerol (G–T) or 3-nitrobenzylalcohol (NBA) as a matrix. FAB high resolution mass spectra for **6** and **9** were obtained with a VG micromass ZAB 2-SEQ spectrometer in a positive mode detection, on glycerol matrix. Thin layer chromatography (TLC) was carried out on Kieselgel $60F_{254}$ plates (E. Merck), with detection by UV light and sulfuric acid spray. Silica Gel 60 (E. Merck) was used for column chromatography. Stability of the phosphotriesters **12** and **13** was determined by reverse-phase HPLC using on-line cleaning method [29] on a Waters Assoc. unit equipped with a 600E model multisolvent delivery system and controller, a 486 tunable absorbance detector, a 715 model autosampler and a base-line 810 data workstation. A six-port 4010 rheodyne valve allowed switching between the precolumn (Guard-Pak, $C_{18}$) and the analytical column (Hypersil, $C_{18}$ $100 \times 4.6$ mm, 3 $\mu$m). Products were eluted using a linear gradient of 0.05 M ammonium acetate buffer (pH 7) to 40% acetonitrile over a 35 min period with a flow rate of 1 mL/min and detection at 260 nm. For each time-point, a 100 $\mu$L aliquot containing dimer phosphotriester at concentration $5 \times 10^{-5}$ M was incubated at 37 °C before injection.

*Methyl (methyl α,β-D-glucopyranosid)uronate* (**2**). —Acetyl chloride (50 mL, 0.7 mol, 4% HCl) was added dropwise to a stirred suspension of D-glucuronic acid (10 g, 51 mmol) and dry MeOH (800 mL) at 0 °C. When the reaction mixture became clear at room temperature, it was heated at 65 °C for 6 h. The reaction mixture was neutralised with 30% $NH_4OH$ and concd under reduced pressure. The ester **2** and the methyl α,β-D-glucofuranosidurono-6,3-lactone formed were separated by silica gel column chromatography (100:0 → 9:1 $CH_2Cl_2$–MeOH gradient) to give the product **2** (7.9 g, 70%); $^{1}H$ NMR ($CD_3COCD_3$): δ 4.70 (d, 0.6 H, $J_{1,2}$ 3.5 Hz, H-1α), 4.25 (d, 0.4 H, $J_{1,2}$ 7.7 Hz, H-1β), 4.00 (d, 0.6 H, $J_{4,5}$ 9.2 Hz, H-5α), 3.85 (d, 0.4 H, $J_{4,5}$ 9.5 Hz, H-5β), 3.70 (s, 3 H, $COOCH_3$); 3.65–3.40 (m, 2 H, H-3, H-4), 3.43 (s, 1.2 H, $OCH_3$), 3.38 (m, 0.6 H, H-2α), 3.37 (s, 1.8 H, $OCH_3$), 3.20 (q, 0.4 H, H-2β); FABMS: *m/z* 445, $[2\ M + H]^+$; 223, $[M + H]^+$.

*Methyl (methyl 2,3,4-tri-*O*-tert-butyldimethylsilyl-α, β-D-glucopyranosid)uronate* (**3**).—To a soln of **2** (9.32 g, 4.2 mmol) and imidazole (2.85 g, 42 mmol) in dry pyridine (10 mL), *tert*-butylchlorodimethylsilane (3.7 g, 25.1 mmol) was added. After stirring for 60 h at room temperature, the reaction was quenched with $H_2O$ (3 mL). The mixture was concd and then diluted with $CH_2Cl_2$. The organic phase was washed with satd aq $NaHCO_3$, dried and concd under reduced pressure to a yellow oil. Product **3** (14.2 g, 60%) was obtained after purification on a silica gel column (100:0 → 92:8 cyclohexane–EtOAc gradient); $^{1}H$ NMR ($CDCl_3$): δ 4.62 (d, 0.3 H, $J_{1,2}$ 6.9 Hz, H-1β), 4.58 (d, 0.7 H, $J_{4,5}$ 3.6 Hz, H-1α), 4.12 (d, 1 H, $J_{4,5}$ 9.3 Hz, H-5), 3.98–3.68 (m, 5 H, $COOCH_3$, H-3, H-4), 3.68–3.60 (dd, 1 H, H-2), 3.55 (s, 0.9 H, $OCH_3$), 3.42 (s, 2.1 H, $OCH_3$), 1.00–0.80 (m, 27 H, 3 *t*BuSi), 0.21–0.05 (m, 18 H, 3 $CH_3Si$); $^{13}C$ NMR ($CDCl_3$): δ 170.7, 170.2 (COO −), 100.3, 96.3 (C-1), 74.3–70.3 (C-2, C-3, C-4, C-5), 56.2, 55.7 ($OCH_3$), 52.2, 51.7 ($COOCH_3$), 26.1–25.6 ($C(CH_3)_3$), 18.5–17.8 ($C(CH_3)_3$), −5.3–4.1 ($CH_3Si$); FABMS: *m/z* 565, $[M + H]^+$.

*2-Chloroethyl (methyl 2,3,4-tri-*O*-tert-butyldimethylsilyl-α,β-*D*-glucopyranosid)uronate* (**4**).—A soln of **3** (1 g, 1.7 mmol), chloroethanol (3 mL, 0.38 mol), and titanium (IV) ethoxide (370 μL, 17.8 mmol) in dry toluene (10 mL) was stirred at 110 °C during 20 h. Half the toluene was removed under reduced pressure and water (300 mL) was added. The aq soln was extracted with ether yielding, after evaporation, **4** (750 mg, 73%); $^1$H NMR ($CDCl_3$): δ 4.60 (d, 0.25 H, $J_{1,2}$ 6.8 Hz, H-1β), 4.54 (d, 0.75 H, $J_{1,2}$ 3.4 Hz, H-1α), 4.48–4.42 (m, 1 H, $COOCH_2$), 4.28–4.00 (m, 2 H, $COOCH_2$, H-5), 3.75–3.55 (m, 4 H, H-4, H-3, $CH_2Cl$), 3.51–3.44 (m, 1 H, H-2), 3.46 (s, 0.75 H, $OCH_3$), 3.31 (s, 2.25 H, $OCH_3$), 1.00–0.80 (m, 27 H, 3 *t*BuSi), 0.21–0.05 (m, 18 H, $3CH_3Si$); $^{13}$C NMR ($CDCl_3$): δ 170.3, 169.6 (COO −), 100.5, 96.3 (C-1), 74.4–70.5 (C-2, C-3, C-4, C-5), 64.9, 64.6 ($CH_2O$), 56.4, 55.8 ($OCH_3$), 41.4, 40.9 ($CH_2Cl$), 26.2–25.8 ($C(CH_3)_3$), 18.7–17.9 ($C(CH_3)_3$), −5.3–4.1 ($CH_3Si$); FABMS: *m/z* 635, $[M + H]^+$.

*2-Iodoethyl (methyl 2,3,4-tri-*O*-tert-butyldimethylsilyl-α,β-*D*-glucopyranosid)uronate* (**5**).—Compound **4** (245 mg, 0.4 mmol) and sodium iodide (96 mg, 0.64 mmol) were dissolved in dry butanone (3 mL). The reaction mixture was stirred at 80 °C for 24 h, then sodium chloride was filtered off and the soln was concd under reduced pressure. The oil was dissolved in EtOAc and successively washed with $Na_2S_2O_3$, $NaHCO_3$ and water. The residual material after concentration was purified on a silica gel column with a gradient of EtOAc (0 → 5%) in cyclohexane to give **5** (146 mg, 52%); $^1$H NMR ($CDCl_3$): δ 4.60 (d, 0.25 H, $J_{1,2}$ 6.9 Hz, H-1β) 4.55 (d, 0.75 H, $J_{1,2}$ 3.5 Hz, H-1α), 4.53–4.20 (m, 2 H, $COOCH_2$), 4.10 (d, 1 H, $J_{4,5}$ 9.1 Hz, H-5), 3.87–3.68 (m, 2 H, H-3, H-4), 3.63–3.55 (m, 1.75 H, H-2, $OCH_3$), 3.42 (s, 2.25 H, $OCH_3$) 3.36–3.26 (m, 2 H, $CH_2I$), 0.95–0.80 (m, 27 H, 3 *t*BuSi), 0.15–0.00 (m, 18 H, 3 MeSi); $^{13}$C NMR ($CDCl_3$): δ 169.8, 169.2 (COO −), 100.4, 96.2 (C-1), 74.3–70.4 (C-2, C-3, C-4, C-5), 65.9, 65.0 ($CH_2O$), 56.3, 55.7 ($OCH_3$), 26.1–25.7 ($C(CH_3)_3$), 18.5–17.8 ($C(CH_3)_3$), −0.5–0.9 ($CH_2I$), −5.3–4.1 ($CH_3Si$).

*2-Iodoethyl (methyl α-*D*-glucopyranosid)uronate* (**6**).—The silylated derivative **5** (200 mg, 0.28 mmol) was dissolved in absolute EtOH (3 mL) and pyridinium *p*-toluenesulfonate (175 mg, 0.7 mmol) was added. The reaction mixture was stirred at 55 °C for 65 h. The solvent was removed under reduced pressure and the product purified on a silica gel column (100:0 → 90:10 $CH_2Cl_2$–MeOH gradient) to give **6** (41 mg, 41%); $^1$H NMR ($CDCl_3$): δ 4.65 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.47 (t, 2 H, *J* 6.9 Hz, $COOCH_2$), 4,12 (d, 1 H, $J_{4,5}$ 9.2 Hz, H-5), 3.79–3.80 (m, 2 H, H-4, H-3), 3.60–3.58 (m, 1 H, H-2), 3.48 (s, 3 H, $OCH_3$), 3.35 (t, 2 H, *J* 6.9 Hz, $CH_2I$); $^{13}$C NMR ($CDCl_3$): δ 169.2 (COO −), 99.8 (C-1), 73.4–70.6 (C-2, C-3, C-4, C-5), 65.4 ($OCH_2$), 55.7 ($OCH_3$), −0.6 ($CH_2I$); FABMS: *m/z* 385, $[M + Na]^+$; 363, $[M + H]^+$; FABHRMS: *m/z* 362.9958 (Calcd. for $[M + H]^+$ 362.9941).

*Methyl 2,3,4-tri-*O*-tert-butyldimethylsilyl α,β-*D*-glucopyranuronic acid* (**7**).—Compound **3** (8.46 g, 15 mmol) was added to lithium iodide (8 g, 60 mmol) in dry pyridine (300 mL). After stirring for 5 h at 115 °C, a mixture of $H_2O$ (200mL) and 6 N HCl (150 mL) was added to reach pH 2 of the reaction mixture, then the mixture was extracted with $CH_2Cl_2$. A silica gel column chromatography (100:0 → 80:20 $CH_2Cl_2$–MeOH gradient) allowed to separate 15% of **7** (1.2 g, 2.2 mmol), 45% of starting ester **3** (re-used to increase the yield); $^1$H NMR ($CDCl_3$): δ 4.78 (d, 0.2 H, $J_{1,2}$ 6.8 Hz, H-1β), 4.50 (d, 0.8 H, $J_{1,2}$ 3.6 Hz, H-1α), 4.15 (d, 1 H, $J_{4,5}$ 9.2 Hz, H-5), 3.85–3.75 (m, 3 H, H-2, H-3, H-4), 3.50 (s, 0.6 H, $OCH_3$), 3.45 (s, 2.4 H, $OCH_3$), 0.95–0.83 (m, 27 H, 3 *t*BuSi), 0.15–0.05 (m, 18 H, 3 MeSi); FAB$^-$MS: *m/z* 549, $[M - H]^-$.

*2-Iodoethyl (methyl 2,3,4-tri-*O*-tert-butyldimethylsilyl β-*D*-glucopyranosid)thiouronate* (**8**).—Compound **7** (600 mg, 1 mmol) was dissolved in dry $CH_2Cl_2$ (1 mL) and thionyl chloride (255 μL, 3.5 mmol) was added dropwise under argon. The reaction was stirred for 5 h at room temperature, then the excess of thionyl chloride was removed under reduced pressure. The acid chloride was diluted with dry MeCN (1.5 mL) in the presence of sodium iodide (235 mg, 1.5 mmol). The soln was cooled to −20 °C and cold ethylene sulfide (135 μL, 2.3 mmol) was added under argon. The mixture was stirred for 2 h at room temperature, then poured onto an ice-water mixture and extracted with $CH_2Cl_2$. The organic phase was neutralised with satd aq $NaHCO_3$, washed with water, dried with $Na_2SO_4$ and concd to give an oil which was purified by chromatography on a silica gel column (100:0 → 7:3 $CH_2Cl_2$–cyclohexane) to furnish **8** (490 mg, 68%); $^1$H NMR ($CDCl_3$): δ 4.65 (d, 1 H, $J_{1,2}$ 6.8 Hz, H-1), 4.30 (s, 1 H, H-5), 4.10 (d, 1 H, $J_{4,3}$ 3.4 Hz, H-4), 3.65 (d, 1 H, $J_{3,4}$ 3.4 Hz, H-3), 3.50 (d, 1 H, $J_{2,1}$ 6.8 Hz, H-2), 3.45 (s, 3 H, $OCH_3$), 3.40–2.90 (m, 4 H, $SCH_2CH_2I$), 0.85–0.70 (m, 27 H, *t*BuSi), 0.05–0.09 (m, 18 H, 3 MeSi); $^{13}$C NMR ($CDCl_3$): δ 199.1 (COS −), 102.8 (C-1), 85.6–72.7 (C-2, C-3, C-4, C-5), 56.7 ($OCH_3$), 30.7 ($SCH_2$), 25.7–25.4 ($C(CH_3)_3$), 17.8–17.6 ($C(CH_3)_3$), 1.3

($CH_2I$), −5,4–4,4 ($SiCH_3$); FABMS: *m/z* 743, $[M + Na]^+$.

*2-Iodoethyl (methyl β-D-glucopyranosid)thiouronate* (**9**).—In a polypropylene vessel, HF (40% aq, 0.6 mL) was added to a soln of **8** (490 mg, 0.68 mmol) in $CH_3CN$ (12 mL). The reaction was stirred for 24 h at room temperature and the reaction mixture was purified by chromatography on a silica gel column (100:0 → 85:15 $CH_2Cl_2$–MeOH gradient) to give **9** (162 mg, 63%); $^1H$ NMR ($CD_3CN$): δ 4.25 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 3.90 (d, 1 H, $J_{4,5}$ 9.2 Hz, H-5), 3.48 (s, 3 H, $OCH_3$), 3.40–3.20 (m, 5 H, H-4, H-3, H-2, $SCH_2$), 3.15 (t, 2 H, *J* 6.7 Hz, $CH_2I$); $^{13}C$ NMR ($CD_5N$): δ 197.0 (COS −), 105.8 (C-1), 81.7–73.3 (C-2, C-3, C-4, C-5), 57.0 ($OCH_3$), 29.4 ($SCH_2$), 0 ($CH_2I$); FABMS: *m/z* 757, $[2 M + H]^+$; 379, $[M + H]^+$; FABHRMS: *m/z* 378.9743 (Calc. for $[M + H]^+$ 378.9712).

*Dithymidine [ethyl (methyl α-D-glucopyranosid)uronate]phosphorothiolate* (**12**).—Phosphorothioate diester **10** (215 mg, 0.17 mmol) was dissolved in MeCN (5 mL). Then 2,6-lutidine (30 μL, 0.25 mmol) and **6** (246 mg, 0.68 mmol) were added. The reaction mixture was stirred for 7 d at 50 °C. Tritylated phosphorothioate triester (190 mg, 0.14 mmol) was obtained after a purification by a silica gel column chromatography (100:0 → 98:2 $CH_2Cl_2$–MeOH gradient). The same alkylation method was applied to the synthesis of phosphotriester **13–15**. The dimethoxytrityl groups were cleaved by treatment with 8:2 AcOH–$H_2O$ (1 mL). The soln was stirred for 3.5 h at room temperature and detritylated **12** was chromatographed on a silica gel column (92:8 → 88:12 $CH_2Cl_2$–MeOH). HPLC (100:0 → 60:40 TEAOAc 0.05 M–$CH_3CN$), $t_r$ (min): **12**: 22.8, 23.8; **13**: 28.1, 29.0; **14**: 26.7, 27.1; **15**: 31.7, 32.5. $^{31}P$ NMR ($CD_3OD$): δ (**12**): 29.19, 29.13; δ (**13**): 97.54, 97.23; δ (**14**): 28.99, 28.68; δ (**15**): 97.47, 97.21; FABMS: for **12**, *m/z* 819, $[M + Na]^+$; for **13**, *m/z* 813, $[M + H]^+$, for **14**, *m/z* 813, $[M + H]^+$, for **15**, *m/z* 829, $[M + H]^+$.

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