Carbohydrate Research, 96 (1981) 73–78
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

DERIVATIVES OF *S*-α- AND -β-D-HEXOPYRANOSYL THIOPHOSPHATES

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(Received April 20th, 1981; accepted for publication, May 12th, 1981)

ABSTRACT

Di-*tert*-butyl esters of the tetra-*O*-acetyl and tetra-*O*-benzyl derivatives of *S*-α- and -β-D-glucopyranosyl thiophosphates and *S*-α- and -β-D-galactopyranosyl thiophosphates were prepared by reaction of di-*tert*-butyl triethylammonium phosphorothioate with tetra-*O*-acetyl- or tetra-*O*-benzyl-hexopyranosyl halides.

INTRODUCTION

Phosphonate and thiophosphate analogs of naturally occurring phosphates are potentially valuable as regulators, activators, or inhibitors of metabolism[1]. Recently, a general approach to the synthesis of phosphonate analogs of α-D-hexopyranosyl phosphates was elaborated[2], and we now report methods for the synthesis of the thioester analogs of α-D-hexopyranosyl phosphates.

Thiophosphorylation of tetra-*O*-acetyl-α-D-glucopyranosyl bromide is a known reaction. Michalska *et al.*[3] reported that, using the triethylammonium salt of phosphorothioic acid blocked by formation of a 5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinyl ring, a product of *S*-glycosylation (the thioester) having the β-D configuration was obtained in high yield. However, from the biochemical point of view, analogs having the α-D configuration are far more interesting. Moreover, use of the 5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinyl ring-system as a protective group does not allow unblocking without cleavage of the S–P bond.

Di-*tert*-butyl triethylammonium phosphorothioate (**5**) appears to be the reagent of choice for the preparation of free *S*-alkylphosphorothioic acids[4]. The reagent is known to give *S*-alkylation with organic halides, and the *tert*-butyl groups may be readily removed by dealkylation with dry hydrogen chloride in chloroform without removal of other protecting groups[4]. We expected that the reaction of this reagent with glycosyl halides would produce derivatives of *S*-α-D-hexopyranosyl thiophosphates.

When treated with salt **5**, tetra-*O*-acetyl-α-D-glucopyranosyl or -galactopyranosyl bromide (**1** or **2**) gave only thioesters **6** and **7** having the β-D configuration, in

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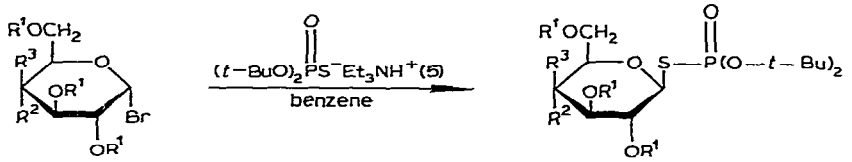
agreement with earlier observations[3,4]. The same result was obtained when tetra-*O*-benzyl-α-D-glucopyranosyl and -galactopyranosyl bromides (**3** and **4**) were used, even though the method used for preparation of the glycosyl halides should have given anomeric mixtures[5].

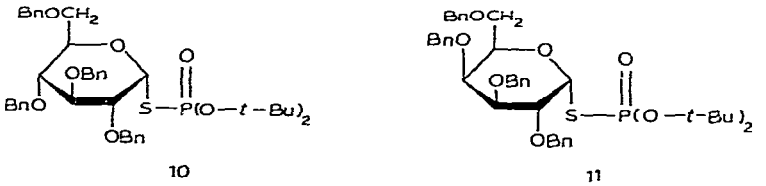


1	R^1 = Ac, R^2 = OAc, R^3 = H	6
2	R^1 = Ac, R^2 = H, R^3 = OAc	7
3	R^1 = Bn, R^2 = OBn, R^3 = H	8
4	R^1 = Bn, R^2 = H, R^3 = OBn	9

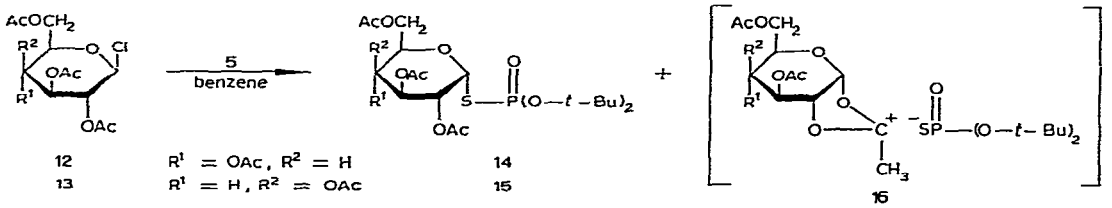
Bn = $PhCH_2$

Scheme 1

Thiophosphorylation of **3** and **4** in the presence of tetrabutylammonium bromide (added to anomerize the glycosyl halides[6]) afforded mixtures of α-D and β-D anomers. *S*-(Tetra-*O*-benzyl-α-D-galactopyranosyl) thiophosphate (**11**) could be separated from its β-D anomer (**9**) by chromatography, but the mixture of **8** and **10** could not be separated.

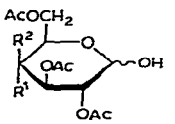


10 11



12	R^1 = OAc, R^2 = H	14
13	R^1 = H, R^2 = OAc	15

16



17
18

Scheme 2

Use of tetra-*O*-acetyl-β-D-glucopyranosyl and -galactopyranosyl chlorides[7] (**12** and **13**) offers a better route to the synthesis of phosphorothioic esters having the α-D configuration. The products of this reaction (**14** and **15**) are accompanied by only traces of the β-D anomers (**6** and **7**); however, considerable proportions of 2,3,4,6-tetra-*O*-acetyl-D-glucose (**17**) and -D-galactose (**18**) are formed This is probably due to participation of the 2-acetoxyl group, giving the unstable and non-detectable intermediate **16**, which is hydrolyzed on either the thin-layer plate or column of silica gel, to give **17** and **18** from **12** and **13**, respectively. Products **17** and **18** are contaminated with small proportions of the β-D anomers (**6** and **7**, respectively).

The *tert*-butyl groups in compounds **6–11**, **14**, and **15** may be readily removed without removing the benzyl or acetyl groups, by passing gaseous hydrogen chloride through a chloroform solution of the substrate. This reaction was, however, conducted on an analytical scale only.

The method for the synthesis of *S*-α-D-hexopyranosyl thiophosphates based on tetra-*O*-acetyl-α-D-hexopyranosyl chlorides (**12** and **13**) and di-*tert*-butyl triethylammonium phosphorothioate (**5**) seems to be the best, despite its low yield (which depends strongly on the temperature of, and the time required for, column chromatography, because of the instability of the *tert*-butyl esters **10**, **11**, **14**, and **15**)

EXPERIMENTAL

General. — Melting points were measured with a Thomas–Hoover Uni-melt melting-point apparatus and are uncorrected. ^{1}H-N m.r. spectra were recorded with a Perkin–Elmer R-32-90 spectrometer, with Me_4Si as the internal standard Optical rotations were measured with a Bendix ETL-NPL type 143A automatic polarimeter. Thin-layer chromatography (t.l.c.) was conducted on aluminum-backed plates of Silica Gel 60 (E. Merck). Column chromatography was performed on Silica Gel (230–400 mesh, E. Merck) according to the method described by Still *et al.*[8]. For elution, two solvents were used: solvent *A*, 7:3 (v/v) petroleum ether–ethyl acetate, and solvent *B*, 4:1 (v/v) petroleum ether–ethyl acetate. Gravity chromatography cannot be used for purification of di-*tert*-butyl phosphorothioic esters, because of their instability.

1-*O*-Acetyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (**19**) and -galactopyranose (**20**) were used for the synthesis of tetra-*O*-benzyl-D-glucopyranosyl and -galactopyranosyl bromides (**3** and **4**). Tetra-*O*-benzyl-D-glucopyranose[9,10] and -galactopyranose were obtained from methyl α-D-glucopyranoside and methyl α-D-galactopyranoside, respectively; acetylation to yield compounds **19** and **20** was achieved with acetic anhydride and pyridine, and was followed by evaporation of the acetylating reagents under vacuum, and purification of the products by chromatography. Tetra-*O*-acetyl-β-D-glucopyranosyl and -galactopyranosyl chlorides (**12** and **13**) were obtained according to the procedure of Farkas *et al.*[7]. Di-*tert*-butyl triethylammonium phosphorothioate (**5**) was prepared according to the procedure of Zwierzak and Gramze[4].

Thiophosphates **6–11**, **14**, and **15** are unstable at room temperature. T.l.c. of fractions from column chromatography always gave at the origin a spot of variable intensity, depending on the time and temperature of the column separations; yields, therefore, depended strongly on these two parameters. The best results were obtained by conducting the chromatography at 4°. Because of the instability of compounds **6–11**, **14**, and **15**, elemental analyses were not obtained.

Di-tert-*butyl* S-(*2,3,4,6-tetra*-O-*acetyl-β*-D-*glucopyranosyl*) *thiophosphate* (**6**). — A solution of tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**1**; 0.5 g) and **5** (0.4 g) in anhydrous benzene was heated at reflux temperature for 1 h. The precipitate of triethylamine hydrobromide was removed by filtration, and the solvent by evaporation. The crude, post-reaction mixture was purified by column chromatography using solvent *A* as the eluant; yield of **6**, 0.4 g; m.p. 98–100°, $[\alpha]_D^{25}$ +14.3° (*c* 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ 5.23 (t, 1 H, $J_{2,3} \simeq J_{3,4} \simeq 10.8$ Hz, H-3), 5.0–5.2 (m, 3 H, H-1,2,4), 4.22 (pd, 1 H, $J_{5,6}$ 4.8, $J_{6,6'}$ 12.7 Hz, H-6), 4.12 (pd, 1 H, $J_{5,6'}$ 2.0 Hz, H-6'), 3.57 (m, 1 H, $J_{4,5}$ 11.1 Hz, H-5), 2.08, 2.05, 2.03, 2.00 (4 s, 12 H, 4 Ac), and 1.55 and 1.54 (2 s, 18 H, 2 *t*-Bu).

Di-tert-*butyl* S-(*2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl*) *thiophosphate* (**7**). — Conversion of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**2**) into compound **7** was achieved according to the procedure described for **6**; m.p. of **7**, 90–94°, $[\alpha]_D^{25}$ +26.6° (*c* 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ 5.44 (bd, 1 H, $J_{3,4}$ 5.1 Hz, H-4), 5.27 (t, 1 H, $J_{1,2} = J_{2,3} = 10.1$ Hz, H-2), 5.07 (pd, 1 H, $J_{1,P}$ 5.1 Hz, H-1), 5.05 (pd, 1 H, H-3), 4.13 (m, 2 H, H-6,6'), 3.99 (m, 1 H, H-5), 2.16, 2.06, 2.03, 1.98 (4 s, 12 H, 4 Ac), and 1.55 and 1.54 (2 s, 18 H, 2 *t*-Bu).

Di-tert-*butyl* S-(*2,3,4,6-tetra*-O-*benzyl-β*-D-*glucopyranosyl*) *thiophosphate* (**8**). — A solution of 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (**19**; 2.0 g) in dichloromethane (10 mL) was cooled to 4° and treated with a saturated solution of HBr in dichloromethane (20 mL). After 20 min, the solvent was removed under vacuum. The crude, acetylated glycosyl bromide was extracted three times with 20 mL of anhydrous ether, and the extract was evaporated to dryness. The oily residue was dissolved in anhydrous benzene and treated with **5** (5.0 g). The solution was heated at reflux temperature for 1 h, filtered, evaporated, and purified by column chromatography using solvent *B*; yield of **8**, 1.3 g; colorless crystals, m.p. 71–74°, $[\alpha]_D^{25}$ +25.9° (*c* 1.0, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 1.52, 1.54 (2 s, 18 H, 2 *t*-Bu), 3.4–4.0 (m, 6 H, H-2,3,4,5,6,6'), 4.4–5.0 (m, 8 H, 4 benzyl), and 7.1–7.4 (m, 20 H, aromatic).

Di-tert-*butyl* S-(*2,3,4,6-tetra*-O-*benzyl-β*-D-*galactopyranosyl*) *thiophosphate* (**9**). — 1-*O*-Acetyl-2,3,4,6-tetra-*O*-benzyl-D-galactopyranose (**20**) was treated according to the procedure described for compound **19**, to give **9**; colorless syrup; $[\alpha]_D^{25}$ +16.0° (*c* 1.0, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 1.47, 1.49 (2 s, 18 H, 2 *t*-Bu), 3.3–3.7 (m, 4 H, H-3,5,6,6'), 3.85 (t, 1 H, $J_{1,2} = J_{2,3} = 9.5$ Hz, H-2), 3.98 (bd, 1 H, $J_{3,4}$ 3.4 Hz, H-4), 4.9 (pd, 1 H, $J_{1,P}$ 13.4 Hz, H-1), 4.3–5.0 (8 H, 4 benzyl), and 7.1–7.5 (m, 20 H, aromatic).

Di-tert-*butyl* S-(*2,3,4,6-tetra*-O-*benzyl-α- and -β*-D-*glucopyranosyl*) *thiophos-*

phate (**8** *plus* **10**). — Compound **19** (4.0 g) was transformed into the acetylated glycosyl bromide as described. The crude glycosyl bromide was dissolved in anhydrous benzene (20 mL) and treated with tetrabutylammonium bromide (2.9 g) The solution was stirred for 20 min; then **5** (7.0 g) was added, and the mixture was heated at reflux temperature for 1 h. The resulting mixture of anomers (**8** and **10**) was purified by column chromatography using solvent *B*; yield of mixture, 1.8 g; $[\alpha]_D^{25}$ +45.5° (*c* 1.0, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$) of **10** (obtained from a spectrum of the mixture of anomers): δ 4.08 (m, 1 H, H-2) and 6.14 (pd, 1 H, $J_{1,2}$ 4.5, $J_{1,P}$ 10.6 Hz, H-1).

Di-tert-*butyl* S-(*2,3,4,6-tetra*-O-*benzyl*-α- *and* -β-D-*galactopyranosyl*) *thiophosphate* (**9** *and* **11**). — Compound **20** (5.0 g) was transformed into a mixture of **9** and **11** according to the procedure described for **19**. Separation of the crude mixture of anomers was achieved by column chromatography using solvent *B*; yield of **11**, 0.8 g; colorless syrup; $[\alpha]_D^{25}$ +75.0° (*c* 1.0, $CHCl_3$); ^{1}H-n.m r. ($CDCl_3$): δ 1.49, 1.50 (2 s, 18 H, 2 *t*-Bu), 3.4–3.8 (m, 3 H, H-5,6,6'), 4.03 (m, 1 H, H-4), 4.21 (pd, 1 H, $J_{2,3}$ 9.1, $J_{3,4}$ 4.9 Hz, H-3), 4.32 (pd, 1 H, $J_{1,2}$ 4.5 Hz, H-2), 6.09 (pd, 1 H, $J_{1,P}$ 10.7 Hz, H-1), 4.0–5.0 (m, 8 H, 4 benzyl), and 7.3–7 5 (m, 20 H, aromatic)

Yield of **9**, 1.2 g.

Di-tert-*butyl* S-(*2,3,4,6-tetra*-O-*acetyl*-α-D-*glucopyranosyl*) *thiophosphate* (**14**). — A solution of compounds **12** (5.0 g) and **5** (4.0 g) in anhydrous benzene was heated at reflux temperature for 1 h. The precipitate was removed by filtration, and the solvent by evaporation. The crude syrup was separated by column chromatography using solvent *A*; yield of **14**, 0.6 g; colorless syrup: $[\alpha]_D^{25}$ +128° (*c* 1.0, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 1 55 (bs, 18 H, 2 *t*-Bu), 2 02, 2.03, 2 07, 2 09 (4 s, 12 H, 4 Ac), 4.12 (pd, 1 H, $J_{5,6'}$ 2.1, $J_{6,6'}$ 10.2 Hz, H-6'), 4.2–4.4 (m, 2 H, H-5,6), 5.09 (pd, 1 H, $J_{1,2}$ 5.6, $J_{2,3}$ 5.7 Hz, H-2), 5.12 (t, 1 H, $J_{4,5}$ 9.4, $J_{3,4}$ 9.7 Hz, H-4), 5.29 (t, 1 H, $J_{2,3}$ 9.7 Hz, H-3), and 6.03 (pd, 1 H, $J_{1,P}$ 11.9 Hz, H-1).

Yield of **17**, 1.5 g, contaminated with **6**.

Di-tert-*butyl* S-(*2,3,4,6-tetra*-O-*acetyl*-α-D-*galactopyranosyl*) *thiophosphate* (**15**). — Thiophosphorylation of **13** (4.0 g) was performed according to the procedure described for **12**; yield of **15**, 0.5 g; colorless crystals; m.p 99–102°, $[\alpha]_D^{25}$ +151° (*c* 1.0, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 1.55 (bs, 18 H, 2 *t*-Bu), 1.96, 2.00, 2.05, 2.12 (4 s, 12 H, 4 Ac), 4.04 (pd, 1 H, $J_{5,6'}$ 6.2, $J_{6,6'}$ 11.2 Hz, H-6'), 4.16 (pd, 1 H, $J_{5,6}$ 7.7 Hz, H-6), 4.46 (bt, 1 H, H-5), 5.10 (pd, 1 H, $J_{3,4}$ 3.3, $J_{2,3}$ 11.1 Hz, H-3), 5.31 (pd, 1 H, $J_{1,2}$ 5.2 Hz, H-2), 5.45 (pd, 1 H, $J_{4,5}$ 1 0 Hz, H-4), and 6.06 (pd, 1 H, $J_{1,P}$ 11.8 Hz, H-1).

Yield of **18**, 1.1 g.

ACKNOWLEDGMENT

This work was supported by a grant (GM26193) from the National Institutes of Health.

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