Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

BENZYL 2,3,4-TRI-*O*-BENZYL-β-D-GLUCOPYRANOSIDURONIC ACID AND SOME RELATED COMPOUNDS

EMMANUEL ZISSIS AND HEWITT G. FLETCHER, JR.

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Maryland 20014 (U. S. A.)

(Received August 27th, 1969)

ABSTRACT

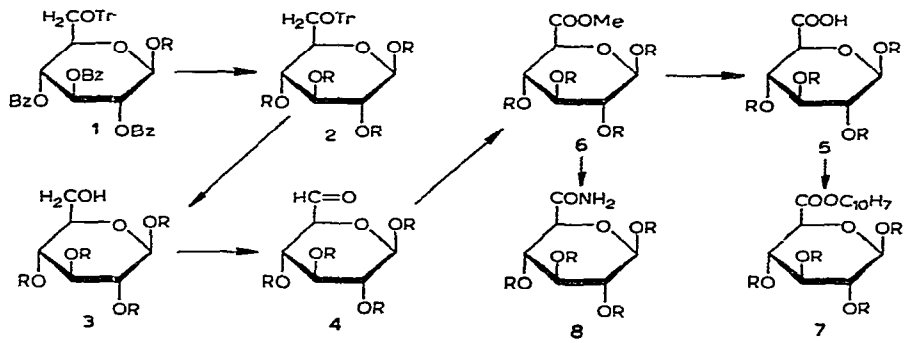
A D-glucuronic acid derivative fully protected with hydrogenolyzable groups except at C-6 has been synthesized. Successive tritylation and benzoylation of benzyl β-D-glucopyranoside gave benzyl 2,3,4-tri-*O*-benzoyl-6-*O*-trityl-β-D-glucopyranoside (**1**). The benzoyl groups in **1** were replaced with benzyl groups to give **2**, and the trityl group was then removed by hydrolysis, giving benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (**3**). Oxidation of **3** with the Pfitzner–Moffatt reagent afforded the corresponding aldehyde (**4**), which was further oxidized, with iodine and methanolic potassium hydroxide, to the methyl ester **6**. Alkaline hydrolysis of **6** gave the desired D-glucuronic acid derivative, benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosiduronic acid (**5**). The conversion of **3** into **5** was also achieved, in a one-step process, through the use of chromium trioxide and dilute sulfuric acid in acetone. The ester, **6**, was further characterized through the corresponding amide (**8**), and the steric accessibility of the carboxyl group in **5** was demonstrated through its conversion into the 2-naphthyl ester (**7**).

INTRODUCTION

The synthesis of alkali-labile derivatives of such polyfunctional molecules as the uronic acids presents unusual difficulties, inasmuch as the choice of protecting groups is, of necessity, restricted to those that may be removed under neutral or mildly alkaline conditions. For such syntheses, the benzyl ether group appears to be well suited and, indeed, Pravdić and Keglević[1] synthesized benzyl 2,3,4-tri-*O*-benzyl-D-glucopyranuronate, and it was later shown[2] that various 1-*O*-acyl derivatives of this compound could, through catalytic hydrogenolysis, serve as intermediates in the synthesis of 1-*O*-acyl-D-glucopyranuronic acids. We now report the synthesis of another type of partially protected D-glucuronic acid, namely, benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosiduronic acid (**5**), a compound of potential utility for the synthesis of D-glucuronic acid derivatives variously substituted at C-6. Some of these, such as the esters of D-glucuronic acid with alcohols, have been but little studied[3], and its esters with phenols have never, as far as we are aware, been prepared.

Benzyl β-D-glucopyranoside, a well-known and readily accessible substance[4], served as the starting point for the synthesis. The primary hydroxyl group in this

compound was masked through the action of trityl chloride but, as is frequently found in such "selective" tritylations, the immediate product was amorphous and required preparative, thin-layer chromatography for its purification. Direct benzoylation of the material in the tritylation mixture, however, afforded crystalline benzyl 2,3,4-tri-*O*-benzoyl-6-*O*-trityl-β-D-glucopyranoside (**1**) in an overall yield of 81%. The benzoyl groups in **1** were replaced by benzyl groups through conventional debenzoylation–benzylation with benzyl chloride and potassium hydroxide, and the product (**2**) was detritylated by heating with aqueous acetic acid. The partially substituted D-glucoside thus formed, namely, benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (**3**), was accompanied by a small proportion of a monoacetate which is, presumably, benzyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside. Acetylation by warm aqueous acetic acid has been observed by others[5].



Tr = Ph_3C , Bz = PhCO and R = $PhCH_2$

The conversion of the hydroxymethyl group on C-5 of **3** into a carboxyl group was initially attempted through the Heyns oxidation, a solution of the compound in aqueous *p*-dioxane being treated with oxygen in the presence of a platinum catalyst at temperatures approaching 90°. However, in our hands, this procedure was unsuccessful. It may be noted that variable results have been reported in the application of the Heyns oxidation to partially benzylated carbohydrate derivatives. Thus, whereas methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside[1] and methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside[6] were oxidized to the corresponding uronic acids, an attempt to oxidize the primary hydroxyl group in 1,2,3,5-tetra-*O*-benzyl-D-glucitol was unsuccessful[7].

As a second approach, we examined a two-step oxidation of **3**. The Pfitzner–Moffatt reagent[8] (methyl sulfoxide, pyridine, phosphoric acid, and *N*,*N*′-dicyclohexylcarbodiimide) readily converted **3** into the aldehyde, namely, benzyl 2,3,4-tri-*O*-benzyl-β-D-*gluco*-hexodialdo-1,5-pyranoside (**4**). Although this compound gave a crystalline (2,5-dichlorophenyl)hydrazone having the expected composition, purification of **4**, either through recrystallization or by chromatography, failed to give a product having an acceptable elemental analysis. Following a procedure devised by Inch, Ley, and Rich[9], a methanolic solution containing the aldehyde **4** and iodine

was treated with methanolic potassium hydroxide, and methyl (benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosid)uronate (**6**) was obtained in high yield. Both **6** and benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosiduronamide (**8**), which was readily prepared from **6**, were readily crystallizable compounds. Treatment of the methyl ester **6** with dilute alkali gave the desired benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosiduronic acid (**5**). Although **5** was crystalline and chromatographically homogeneous, and gave a satisfactory elemental analysis, its melting point was highly variable.

After the work just described had been completed, Matsui and coworkers[7] showed that 3-*O*-benzoyl-2,4,5,6-tetra-*O*-benzyl-L-gulitol could be oxidized to 3-*O*-benzoyl-2,4,5,6-tetra-*O*-benzyl-L-gulonic acid through the action of a solution of chromium trioxide and dilute sulfuric acid in acetone. Under these experimental conditions, we found **3** to be readily oxidized to **5**, the product being conveniently identified through conversion into its methyl ester (**6**). No attempt was made to maximize the yield of **5** (58%) in this one-step oxidation, but, as a preparative method, the procedure is unquestionably superior to the multi-step conversion developed earlier.

In order to demonstrate the steric accessibility of the carboxyl group in **5** for ester formation, the acid was condensed with 2-naphthol in the presence of *N,N'*-dicyclohexylcarbodiimide and pyridine. The product, namely, 2-naphthyl (benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosid)uronate (**7**), was obtained in 89% yield; it was crystalline, and gave a satisfactory elemental analysis.

EXPERIMENTAL

General methods. — Melting points are corrected values. Thin-layer chromatography was conducted on Silica Gel G (E. Merck AG, Darmstadt) by use of the solvent systems specified, the components being detected (unless otherwise noted) by spraying with 10% sulfuric acid and heating. Column chromatography was performed on No. 7734 Silica Gel (0.05–0.20 mm, E. Merck AG). N.m.r. spectra were recorded, for solutions in chloroform-*d*, with a Varian A-60 spectrometer and tetramethylsilane as the internal standard.

Benzyl 2,3,4-tri-O-benzoyl-6-O-trityl-β-D-glucopyranoside (**1**). — To a solution of benzyl β-D-glucopyranoside[4] (15.8 g) in pyridine (75 ml) was added trityl chloride (18.5 g), and the mixture was kept for 1 week at room temperature. Benzoyl chloride (27 ml) was then added and, after a further 18 h at room temperature, the excess of benzoyl chloride was decomposed by the addition of water (1 ml). The mixture was poured onto crushed ice, and the syrup that was precipitated was thoroughly washed with cold water; on storage under water overnight at +5°, the syrup crystallized; after decantation of the water, the solid was triturated with ethanol, washed with fresh ethanol, and dried; yield 50.5 g. Recrystallized from ethanol (25 ml/g), the product (**1**, 39.1 g, 81%) was obtained as elongated, flat prisms, m.p. 156.5–157.5°, $[\alpha]_D^{20}$ −12.6° (*c* 1.3, chloroform).

Anal. Calc. for $C_{53}H_{44}O_9$: C, 77.17; H, 5.38. Found: C, 77.00; H, 5.49.

*Benzyl 2,3,4-tri-*O*-benzyl-6-*O*-trityl-β-*D*-glucopyranoside* (**2**). — A mixture of **1** (68.8 g), *p*-dioxane (60 ml), and powdered potassium hydroxide (75 g) was gradually warmed to the boiling point, and stirred vigorously while benzyl chloride (60 ml) was added dropwise. After the addition was complete, the mixture was boiled and stirred for 2 h; it was then cooled and steam-distilled to remove the *p*-dioxane, benzyl chloride, and benzyl alcohol. The crude product was extracted with three 300-ml portions of ether, and the extracts were combined, washed with two 400-ml portions of water, dried (magnesium sulfate), and evaporated *in vacuo* to a mobile syrup which began to crystallize spontaneously after several days. Warm ethanol was added and, when crystallization was complete, the product (32.6 g, 50%) was removed by filtration. After recrystallization from chloroform–pentane and then from ethanol, compound **2** was obtained as large, rectangular prisms: m.p. 111–112°, $[\alpha]_D^{20}$ −3.6° (*c* 1, chloroform).

Anal. Calc. for $C_{53}H_{50}O_6$: C, 81.30; H, 6.44. Found: C, 81.27; H, 6.54.

*Benzyl 2,3,4-tri-*O*-benzyl-β-*D*-glucopyranoside* (**3**). — The trityl ether **2** (10 g) was dissolved in glacial acetic acid (80 ml), and the solution was warmed to 75–85°. Water (20 ml) was slowly added, care being taken to keep the mixture homogeneous. The temperature was held at 75–85° for 1 h and then, after storage overnight at room temperature, the precipitated triphenylmethanol was removed by filtration and washed with cold, 80% acetic acid. Concentration of the combined filtrate and washings yielded a second crop of triphenylmethanol which was removed by filtration. The filtrate was diluted with warm ethanol and then with a comparatively large volume of pentane; a stream of air was directed across the surface of the solution, and crystallization was allowed to progress until a solid mass of needle-shaped crystals was obtained; the cold mixture was filtered, the crystals (5.2 g) being washed with pentane. On being chilled, the filtrate yielded further fractions (1.8 g) which were dissolved in butyl alcohol–pentane and the contaminating triphenylmethanol was removed by filtration. On concentration and cooling, the filtrate yielded 1.0 g, to make a total yield of 6.2 g (90%) of **3**, still slightly contaminated with triphenylmethanol as shown by t.l.c. (6:1 benzene–ether). The compound may be crystallized from ethanol, ethanol–pentane, acetone–water, or cyclohexane, although chromatography on a column of silica gel with 6:1 benzene–ether is more effective in removing the last traces of triphenylmethanol as it gave pure **3** as needles (from ethanol), m.p. 105–106°, $[\alpha]_D^{20}$ −9.2° (*c* 1, chloroform).

Anal. Calc. for $C_{34}H_{36}O_6$: C, 75.53; H, 6.71. Found: C, 75.61; H, 6.69.

A repetition of the preparation, with use of 20.5 g of **2**, afforded 7.2 g of **3** as the first crop and then a second crop of crystals (3.4 g) which proved to be a mixture of **3**, triphenylmethanol, and a third product. Recrystallization of the second crop of crystals from ethanol removed most of the triphenylmethanol; the remainder (1.7 g) was chromatographed on a column of silica gel (35 g) with 6:1 benzene–ether. The third product moved the most rapidly of the three, and crystallized readily from ethanol to give 0.7 g (4.6%). Recrystallized from chloroform–pentane, benzyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside was obtained as needles, m.p.

116–117°, $[\alpha]_D^{20}$ −2.9° (*c* 1, chloroform); its n.m.r. spectrum included a three-proton singlet at τ 7.94 (OAc).

Anal. Calc. for $C_{36}H_{38}O_7$: C, 74.20; H, 6.57. Found: C, 74.33; H, 6.38.

*Benzyl 2,3,4-tri-*O*-benzyl-β-*D*-gluco-hexodialdo-1,5-pyranoside* (**4**). — The oxidation technique devised by Pfitzner and Moffatt[8] was used as described by Horton, Nakadate, and Tronchet[10]. To a solution of compound **3** (1 g) in methyl sulfoxide (4 ml) and benzene (1 ml) were added anhydrous pyridine (0.2 ml), 85% phosphoric acid (0.1 ml), and, finally, *N,N'*-dicyclohexylcarbodiimide (2 g). The mixture was stirred for 7 h at room temperature and kept overnight, the *N,N'*-dicyclohexylurea then being removed by filtration. A solution of oxalic acid monohydrate (2 g) in methanol (6 ml) was added to the filtrate, the mixture was stirred for 1 h, and filtered, and the filtrate was diluted with benzene and washed with aqueous sodium hydrogen carbonate solution (5%). The washings were combined, and extracted with ethyl acetate, and the extract was added to the benzene solution; the solution was dried (Drierite), and evaporated *in vacuo*. Benzene was added to the residue, the suspension was filtered, and the filtrate was evaporated *in vacuo* to yield a mobile syrup. This syrup was dissolved in a small volume of methanol, and the solution was kept for several days at +5°, to give a crystalline product that was removed by filtration at +5°, and washed with cold methanol. The crude **4** (0.8 g, 80% yield) thus obtained had m.p. ~65–75°, and could be recrystallized from methanol as prismatic needles, and from chloroform–pentane as radial clusters of needles, but these operations failed to yield chromatographically homogeneous material. Recrystallization from hot hexane caused partial conversion of the product into another substance, detected by t.l.c.; similarly, an attempt at preparative t.l.c. (5:1 benzene–ether) caused the product to become heterogeneous. With the fuchsin aldehyde reagent, the aldehyde gave a violet color, and, on t.l.c., spraying the compound with aniline hydrogen phthalate solution and then heating gave a brown spot.

A mixture of **4** (255 mg), (2,5-dichlorophenyl)hydrazine (100 mg), and methanol (5 ml) was heated on a steam bath until an almost dry residue remained. This was dissolved in a small volume of warm methanol; crystallization occurred spontaneously, to give the (2,5-dichlorophenyl)hydrazone of **4** (240 mg, 73%). The compound was successively recrystallized from methanol (once), cyclohexane (thrice), and methanol, to give needles, m.p. 138.5–139.5°, $[\alpha]_D^{20}$ −53.7° (*c* 1, chloroform). The (2,5-dichlorophenyl)hydrazone is somewhat unstable, both in solution and when exposed to the air on a t.l.c. plate.

Anal. Calc. for $C_{40}H_{38}Cl_2N_2O_5$: C, 68.86; H, 5.49; Cl, 10.16; N, 4.02. Found: C, 68.72; H, 5.73; Cl, 10.02; N, 3.97.

*Methyl (benzyl 2,3,4-tri-*O*-benzyl-β-*D*-glucopyranosid)uronate* (**6**). — An oxidation procedure similar to one used by Inch, Ley, and Rich[9] was employed. A stirred solution of **4** (2.3 g) and iodine (1.4 g) in methanol (23 ml) was heated to 55–60°, and was stirred while methanolic potassium hydroxide (4%, w/v) was added dropwise. After 45 min, the product began to crystallize from the hot solution; addition of the methanolic alkali was continued for another hour (until the color

of the iodine had disappeared; 20 ml of the alkaline solution was used). The mixture was allowed to cool to room temperature, and a few drops of water were added to incipient turbidity. Needle-like crystals of **6** were removed by filtration and washed with 50% aqueous methanol; wt. 2.1 g (86%). The product thus isolated contained an impurity that moved more slowly than the major part on t.l.c. with 20:1 benzene–ether, and this impurity was not removed by recrystallization from methanol, cyclohexane, acetone–water, or chloroform–pentane. Purification of a 200-mg sample was effected by preparative t.l.c. with 20:1 benzene–ether, and 175 mg of chromatographically homogeneous product was obtained; recrystallized twice from methanol, it had m.p. 109.5–110.5°, $[\alpha]_D^{20}$ −26.0° (*c* 1, chloroform).

Anal. Calc. for $C_{35}H_{36}O_7$: C, 73.92; H, 6.38. Found: C, 73.83; H, 6.33.

The largest fragment detected by mass spectroscopy was at 478, equivalent to *m/e* $PhCH_2$. The n.m.r. spectrum of the compound included a sharp, three-proton singlet at τ 6.25, very close to a similar signal at τ 6.28 assigned by Roy and Glaudemans[11] to the methyl group in 1,2,3,4-tetra-*O*-acetyl-6-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate)-β-D-glucopyranose.

*Benzyl 2,3,4-tri-*O*-benzyl-β-*D*-glucopyranosiduronamide* (**8**). — A solution of the methyl ester **6** (200 mg) in acetone (12 ml) was treated with ammonium hydroxide (6 ml) and kept at room temperature. After 1.5 h, an additional quantity (1 ml) of ammonium hydroxide could be added without causing precipitation of **6**. During the next 4 days, the mixture deposited a mass of silky needles. Water (1 ml) was added and, after 1 h, the crystals were removed by filtration and washed with aqueous acetone; wt. 103 mg. T.l.c. of the filtrate showed that a very small proportion of **6** was present; concentration of the solution afforded a second crop of crystals (84 mg), making a total yield of crude product of 187 mg (96%). Recrystallized from acetone and then from aqueous acetone, the amide had m.p. 193–194°, $[\alpha]_D^{20}$ −39.3° (*c* 1, chloroform).

Anal. Calc. for $C_{34}H_{35}NO_6$: C, 73.76; H, 6.37; N, 2.53. Found: C, 73.61; H, 6.11; N, 2.62.

*Benzyl 2,3,4-tri-*O*-benzyl-β-*D*-glucopyranosiduronic acid* (**5**). — (*A*) *From methyl (benzyl 2,3,4-tri-*O*-benzyl-β-*D*-glucopyranosid)uronate* (**6**). — A solution of pure **6** (164 mg) in acetone (15 ml) was cooled to 5° and treated dropwise with 0.1M potassium hydroxide (5.7 ml). After 2.5 h, the mixture was stirred with Amberlite IR-120 (H^+) ion-exchange resin, and the resin was removed by filtration and thoroughly washed with acetone. The filtrate and washings were combined, and evaporated to dryness, and the residue was crystallized, as needles, from warm cyclohexane, wt. 153 mg (96%), m.p. 127–131° (turbid melt). Recrystallized from acetone–cyclohexane, the product was chromatographically homogeneous (t.l.c. with 5:1 benzene–ether). Widely varying melting points were observed; an analytical sample, dried *in vacuo* at 100°, had m.p. 115–125°, $[\alpha]_D^{20}$ −34.1° (*c* 1, chloroform).

Anal. Calc. for $C_{34}H_{34}O_7$: C, 73.63; H, 6.18. Found: C, 73.33; H, 5.97.

(*B*) *From benzyl 2,3,4-tri-*O*-benzyl-β-*D*-glucopyranoside* (**3**). A procedure similar to that used by Matsui and coworkers[7] was employed. A solution of benzyl 2,3,4-tri-

O-benzyl-β-D-glucopyranoside (**3**, 0.5 g) in acetone (8 ml) was stirred while a solution of chromium trioxide (0.3 g) in 3.5M sulfuric acid (0.8 ml) was added dropwise. Stirring was continued for 1 h after the addition was complete, and the dark solution was then directly decanted from the chromium sesquioxide onto crushed ice. Acetone was used to wash the chromium sesquioxide, the washings being added to the ice. The crude product that was precipitated was removed by filtration and was washed with water; dried in the air, it weighed 0.3 g (58%).

Owing to the variability in the m.p. of the compound (described in *A*), identification was made here through conversion into the methyl ester **6**. A portion (0.1 g) of the material was treated with diazomethane in ether solution; on evaporation, a crystalline residue was obtained, and this was recrystallized from methanol to give clusters of long, prismatic needles (80 mg) which had m.p. 106–110°, alone and in admixture with compound **6** prepared from **4** as already described. The n.m.r. spectra of the two samples were identical.

*2-Naphthyl (benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosid)uronate* (**7**). — To a solution of **5** (0.65 g) and 2-naphthol (0.65 g) in a mixture of benzene (3 ml) and pyridine (0.3 ml) was added *N,N'*-dicyclohexylcarbodiimide (0.3 g); *N,N'*-dicyclohexylurea was promptly precipitated. After being kept overnight at room temperature, the mixture was diluted with benzene and filtered, and the residue was washed with cold benzene. The filtrate and washings were combined, concentrated, and cooled, to give 0.55 g of crystalline product. Preparative t.l.c. of the material in the filtrate, with 10:1 benzene–ether, afforded a second crop (0.16 g), and raised the yield to 89% of the theoretical. The product can be recrystallized from ether or from chloroform–pentane, and crystallizes as prismatic needles. When pure, it has m.p. 147–148° and $[\alpha]_D^{20}$ −19.4° (*c* 1, chloroform).

Anal. Calc. for $C_{44}H_{40}O_7$: C, 77.63; H, 5.92. Found: C, 77.87; H, 6.14.

ACKNOWLEDGMENTS

We thank Dr. T. D. Inch for drawing our attention to the oxidation procedure described in reference 9. We are also indebted to Mr. H. W. Diehl and Mr. E. W. Tracy for technical assistance. Elemental analyses, mass spectra, and n.m.r. spectra were provided by the staff of the Section on Microanalytical Services and Instrumentation of this Institute.

REFERENCES

1 N. PRAVDIĆ AND D. KEGLEVIĆ, *Tetrahedron*, 21 (1965) 1897.
2 D. KEGLEVIĆ, N. PRAVDIĆ, AND J. TOMASIĆ, *J. Chem. Soc. (C)*, (1968) 511.
3 C. A. MARSH, in G. J. DUTTON (Ed.), *Glucuronic Acid*, Academic Press, Inc., New York, N. Y., 1966, p. 51.
4 A. L. RAYMOND, R. S. TIPSON, AND P. A. LEVENE, *J. Biol. Chem.*, 130 (1939) 47.
5 V. PREY AND A. ASZALOS, *Monatsh. Chem.*, 91 (1960) 729; A. ASZALOS AND V. PREY, *Staerke*, 14 (1962) 50; D. H. BALL AND F. W. PARRISH, *J. Org. Chem.*, 27 (1962) 4120.
6 A. WACEK, F. LEITINGER, AND P. HOCHBAHN, *Monatsh. Chem.*, 90 (1959) 562.

7 M. MATSUI, M. SAITO, M. OKADA, AND M. ISHIDATE, *Chem. Pharm. Bull.* (Tokyo), 16 (1968) 1294.
8 K. E. PFITZNER AND J. G. MOFFATT, *J. Amer. Chem. Soc.*, 85 (1963) 3027; 87 (1965) 5661.
9 T. D. INCH, R. V. LEY, AND P. RICH, *J. Chem. Soc.* (C), (1968) 1693.
10 D. HORTON, M. NAKADATE, AND J. M. J. TRONCHET, *Carbohyd. Res.*, 7 (1968) 56.
11 N. ROY AND C. P. J. GLAUDEMANS, *J. Org. Chem.*, 33 (1968) 1559.