SYNTHESES OF CELLOBIOSYL, MALTOSYL, AND LACTOSYL DERIVATIVES OF ASPARAGINE

D. DUNSTAN AND L. HOUGH

Department of Chemistry, Queen Elizabeth College, Campden Hill Road, London W8 7AH (Great Britain)

(Received August 11th, 1971; accepted for publication, September 29th, 1971)

ABSTRACT

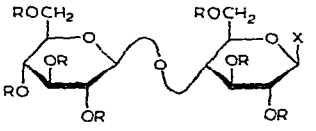
Three *N*-glycosylasparagines have been synthesised, namely, 1-*N*-(4-L-aspartyl)-4-*O*-β-D-glucopyranosyl-β-D-glucopyranosylamine, 1-*N*-(4-L-aspartyl)-4-*O*-α-D-glucopyranosyl-β-D-glucopyranosylamine, and 1-*N*-(4-L-aspartyl)-4-*O*-β-D-galactopyranosyl-β-D-glucopyranosylamine. They were obtained from the polyacetates of cellobiose, maltose, and lactose, *via* the 1-bromides, 1-azides, and then the 1-amines, followed by condensation with 1-benzyl *N*-benzyloxycarbonyl-L-aspartate. Two coupling reagents, dicyclohexylcarbodiimide and 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline are compared. The latter has the distinct advantage of ease of purification of the products by facilitating the removal of by-products. The mass spectra of the fully protected *N*-glycosylasparagines are discussed.

INTRODUCTION

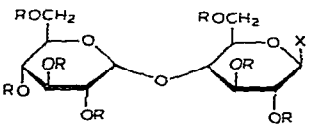
Asparagine derivatives linked to oligosaccharides are required as model compounds for the studies of glycoproteins, and maltose, lactose, and cellobiose were selected as readily available disaccharides for combination with asparagine. The use of dicyclohexylcarbodiimide (DCC) as a coupling reagent for synthesis has usually entailed conventional column chromatography[1] to remove last traces of *N,N'*-dicyclohexylurea. We have therefore investigated the use of the "dry-column technique" and another coupling reagent, namely 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline[2] (EEDQ).

DISCUSSION

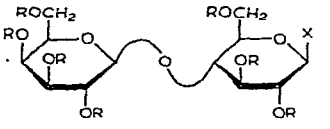
2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl azide (**1**), 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-β-D-glucopyranosyl azide (**2**), and 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl azide[3] (**3**) were obtained crystalline in >60% yield from the corresponding α-bromides by treatment with sodium azide in *N,N*-dimethylformamide rather than formamide[7]. Hydrogenation of these azides gave the corresponding β-D-glycosylamines **4**–**6**.



1 R = Ac, X = N_3
4 R = Ac, X = NH_2
7 R = Ac, X = $NHCOCH_2CH(CO_2CH_2Ph)NHCOOCH_2Ph$
10 R = H, X = $NHCOCH_2CH(CO_2H)NH_2$



2 R = Ac, X = N_3
5 R = Ac, X = NH_2
8 R = Ac, X = $NHCOCH_2CH(CO_2CH_2Ph)NHCOOCH_2Ph$
11 R = H, X = $NHCOCH_2CH(CO_2H)NH_2$



3 R = Ac, X = N_3
6 R = Ac, X = NH_2
9 R = Ac, X = $NHCOCH_2CH(CO_2CH_2Ph)NHCOOCH_2Ph$
12 R = H, X = $NHCOCH_2CH(CO_2H)NH_2$

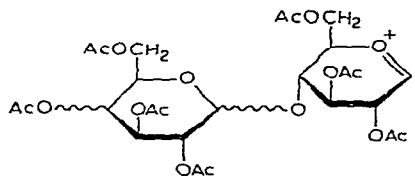
Compounds **4–6** were coupled to 1-benzyl *N*-benzyloxycarbonyl-L-aspartate by treatment with DCC in dichloromethane at room temperature for 6 h. Despite repeated recrystallization of the products, it was necessary to use column chromatography to remove the last traces of *N,N'*-dicyclohexylurea. The purification of the products by traditional methods of column chromatography on silica gel was difficult. However, pure materials were quickly and easily obtained by a "dry-column[9] technique". Recrystallization then gave analytically pure products **7–9** in ~60% yield.

In a parallel series of experiments, the glycosylamines **4–6** were coupled to the aspartate derivative by treatment with EEDQ[2] in a benzene–ethanol solution overnight at room temperature. As the time required for complete reaction was greater than that for the DCC reaction, slightly more decomposition of the amine occurred. However, the products **7**, **8**, and **9** were obtained pure, in ~65% yield, without resorting to chromatography.

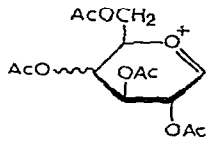
The *N*-glycosylasparagines **10–12** were obtained by hydrogenolysis of the protecting groups from **7–9** followed by *O*-deacetylation with ammonia in methanol. The maltosyl (**11**) and lactosyl (**12**) derivatives were very hygroscopic. The cellobiosyl derivative (**10**) was stable when kept at −15°, but **11** and **12** decomposed after about a month at this temperature.

The mass spectra of the fully protected *N*-glycosylasparagines **7**, **8**, and **9** were very similar; **7** and **8** gave a very small peak for the molecular ion at m/e 974; and correspondingly small peaks in these spectra at *m/e* 866 and 839 were due to the thermal elimination of benzyl alcohol and cleavage of the benzyl ester group from the molecule[4]. All three spectra had a peak at *m/e* 619 which was assigned to the disaccharide oxonium ion[5] **13**.

The peak at *m/e* 331 (assigned to **14**) was present in all of the spectra, and typical fragmentation patterns for polyacetates, involving the loss of acetic acid and ketene from this ion, gave peaks at *m/e* 289, 271, 247, 229, 211, 187, 169, 127, and 109.



13 (*m/e* 619)



14 (*m/e* 331)

The presence of the acetoxonium, diacetoxonium, and triacetoxonium ions characteristic of acetate spectra was confirmed by peaks at *m/e* 43, 103, and 145.

The use of n.m.r. spectrometry for determination of the configuration of the linkage of the carbohydrate moiety to L-asparagine was precluded by the complexity of the spectra; this confirms the observation of Jeanloz and Spinola[3] with the lactosyl derivative.

EXPERIMENTAL

General. — Melting points (uncorrected) were determined on a Reichert hot-stage. Kieselgel Merck 7734 (70–325 mesh) was used for dry columns. T.l.c. was carried out on plates (5 × 20 cm) coated with Merck Kieselgel G. Optical rotations were measured at 20° on a Perkin–Elmer 141 polarimeter. The mass spectra were determined by P.C.M.U., Harwell, with an A.E.I. MS-9 instrument at 70 eV. The temperature of the ion source was 240° for **7**, 180° for **8**, and 210° for **9**. All evaporations were carried out under reduced pressure.

N-Benzyloxycarbonyl-L-aspartic acid[6] was converted, *via* *N*-benzyloxycarbonyl-L-aspartic anhydride, into the 1-benzyl ester by the method of Cowley, Hough, and Peach[7].

Synthesis of the β-D-glycosyl azides **1**, **2**, *and* **3**. — The disaccharide octaacetate (10 g) was dissolved in the minimal volume of dichloromethane and the solution was cooled to 0°. Hydrogen bromide in glacial acetic acid (30 ml, 45% w/v) was added, and the reaction mixture was left to stand in an ice bath for 30 min. The glycosyl bromide was extracted into ice-cold dichloromethane, and the combined extracts were washed with ice–water, cold saturated aqueous sodium hydrogen carbonate, and ice–water again, and then dried and evaporated to a small volume.

This solution of the bromide was added to a solution of sodium azide (20 g) in *N*,*N*-dimethylformamide. The mixture was stirred at room temperature for 6 h and then poured into water, the organic layer was separated, and the aqueous layer extracted with dichloromethane. The combined extracts were washed with water, dried, and evaporated to yield crude azide. The following compounds were thus obtained. 2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl azide (**1**, 65%) was recrystallized from chloroform–ether and had m.p. 182–183°, $[\alpha]_D$ −31.2° (*c* 1.0, chloroform); lit.[8] m.p. 182–182.5°, $[\alpha]_D$ −30.9° (*c* 1.0,chloroform). 2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranos-

yl)-β-D-glucopyranosyl azide (**2**, 60%), eluted from silica gel with chloroform–ether (7:3), was recrystallized from methanol and had m.p. 90–91°, $[\alpha]_D$ +52.4° (*c* 1.0, chloroform); lit.[8] m.p. 91°, $[\alpha]_D$ +53.0° (*c* 1.0, chloroform). 2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl azide[3] (**3**, 60%), eluted from silica gel with ether–light petroleum (4:1), was recrystallized from ether–light petroleum and had m.p. 72–74°, $[\alpha]_D$ −22.0° (*c* 0.6, chloroform); **3** was homogeneous on t.l.c. (chloroform–acetone 7:3) (Found: C, 47.3; H, 5.3; N, 6.2. $C_{26}H_{35}N_3O_{17}$ calc.: C, 47.3; H, 5.3; N, 6.4%).

Synthesis of the N-*glycosylasparagines* **7**, **8**, *and* **9**. — The appropriate β-D-glycosyl azide (2 g) dissolved in methanol was hydrogenated over Adams' catalyst (0.2 g) at 45 lb/sq.in. for 3 h. The solvent was removed at room temperature (<25°), to yield the crude amine. This was sufficiently pure for use in the coupling procedures, and any recrystallization or further purification only increased decomposition of the product.

(*a*) *Coupling of the β-D-glycosylamines with DCC.* The amine (1.9 g) and 1-benzyl *N*-benzyloxycarbonyl-L-aspartate (1.1 g) were dissolved in dichloromethane, DCC (0.7 g) was added, and the reaction mixture was stirred at room temperature for 6 h. Glacial acetic acid (1 ml) was added and, after 30 min, the precipitated *N,N'*-dicyclohexylurea was filtered off and washed with dichloromethane. The filtrate and washings were combined, washed with water, dried, and evaporated at room temperature. The residue was eluted from a dry column[9] of silica gel with the solvents given below to give first *N,N'*-dicyclohexylurea and then the required product.

(*b*) *Coupling of the β-D-glycosylamines with EEDQ.* The amine (1.9 g) and 1-benzyl *N*-benzyloxycarbonyl-L-aspartate (1.1 g) were dissolved in a 1:1 mixture of benzene–ethanol, and EEDQ (0.8 g) was added. The mixture was stirred at room temperature overnight. The solvent was removed by evaporation, and the residue was washed with warm absolute ether. Pure material was then obtained by careful crystallization from the solvents given below.

The following compounds were thus obtained. 2,3,6-Tri-*O*-acetyl-1-*N*-(1-benzyl *N*-benzyloxycarbonyl-4-L-aspartyl)-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosylamine (**7**). Method (*a*) yielded 60% of **7** by elution with chloroform–methanol (15:1) and recrystallization from chloroform–ether; m.p. 187–190°, $[\alpha]_D$ +1.6° (*c* 0.7, chloroform), homogeneous on t.l.c. (chloroform–methanol, 15:1) (Found: C, 55.2; H, 5.3; N, 2.9. $C_{45}H_{54}N_2O_{22}$ calc.: C, 55.4; H, 5.6; N, 2.9%). Method (*b*) yielded 65% of **7** which, when recrystallized three times from chloroform–ether, had m.p. 188–190°, $[\alpha]_D$ +1.8° (*c* 0.7, chloroform), and was homogeneous on t.l.c. (chloroform–methanol, 15:1).

2,3,-6Tri-*O*-acetyl-1-*N*-[1-benzyl *N*-benzyloxycarbonyl-4-L-aspartyl]-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-β-D-glucopyranosylamine (**8**). Method (*a*) yielded 63% of **8** by elution with chloroform–ether (6:4) and recrystallization from ethanol–ether; m.p. 95–98°, $[\alpha]_D$ +68.0° (*c* 0.6, chloroform), homogeneous on t.l.c. (chloroform–ether, 6:4) (Found: C, 55.1; H, 5.2; N, 2.7. $C_{45}H_{54}N_2O_{22}$ calc.: C, 55.4; H, 5.6; N, 2.9%). Method (*b*) yielded 68% of **8** which, when recrystallized three times

from ethanol–ether, had m.p. 94–97°, $[\alpha]_D$ +68.0° (*c* 0.6, chloroform), homogeneous on t.l.c. (chloroform–ether, 6:4).

2,3,6-Tri-*O*-acetyl-1-*N*-[1-benzyl *N*-benzyloxycarbonyl-4-L-aspartyl]-4-*O*-(2,3,4, 6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosylamine[3] (**9**). Method (*a*) yielded 61% of **9** by elution with ethyl acetate–cyclohexane (8:3) and recrystallization from ethanol–ether; m.p. 90–92°, $[\alpha]_D$ +8.6° (*c* 0.75, chloroform), homogeneous on t.l.c. (ethyl acetate–cyclohexane, 8:2); lit.[3] m.p. 91–92°, $[\alpha]_D^{22}$ +9.5° (*c* 1.0, chloroform) (Found: C, 55.2; H, 5.6; N, 2.8. $C_{45}H_{54}N_2O_{22}$ calc.: C, 55.4; H, 5.6; N, 2.9%). Method (*b*) gave 65% of **9** which, when recrystallized three times from ethanol–ether, had m.p. 91–92°, $[\alpha]_D$ +8.5° (*c* 0.8, chloroform), and was homogeneous on t.l.c. (ethyl acetate–cyclohexane, 8:2).

Preparation of the N-*glycosylasparagines* **10**, **11**, *and* **12**. — The protected derivative **7**, **8**, or **9** (0.5 g) was dissolved in ethanol (100 ml) and acetic acid (10 ml) and hydrogenated over 10% palladium-on-charcoal (0.1 g) at a pressure of 45 lb/sq.in. for 3 h. The solution was then filtered, the solvent was removed at room temperature, and cyclohexane was distilled from the residue to remove traces of acetic acid. The acetylated product, without further purification, was then dissolved in dry methanol and ammonia was passed through the cooled solution to saturation. After storage at ~0° overnight, the solvent was removed at room temperature and the residue was washed with acetone to remove acetamide. The following compounds were thus obtained.

1-*N*-(4-L-aspartyl)-4-*O*-β-D-glucopyranosyl-β-D-glucopyranosylamine (**10**, 60%), crystallized from water–methanol–ether, had m.p. 250–252° (dec.), $[\alpha]_D$ −14° (*c* 0.45, water) (Found: C, 42.0; H, 6.1; N, 5.8. $C_{16}H_{28}N_2O_{13}$ calc.: C, 42.1; H, 6.1; N, 6.1%). 1-*N*-(4-L-aspartyl)-4-*O*-α-D-glucopyranosyl-β-D-glucopyranosylamine (**11**, 55%), crystallized from water–methanol, had m.p. 239–240° (dec.), $[\alpha]_D$ +7.0° (*c* 0.4, water) (Found: C, 42.5; H, 6.0; N, 5.8%). 1-*N*-(4-L-aspartyl)-4-*O*-β-D-galactopyranosyl-β-D-glucopyranosylamine (**12**, 60%), crystallized from water–methanol had m.p. 235° (dec.), $[\alpha]_D$ +1.0° (*c* 0.1, water) (Found C, 41.9; H, 6.5; N, 6.0%).

Compounds **11** and **12** were very hygroscopic and could only be handled for a few minutes in a moist atmosphere. They were stored in a vacuum desiccator over phosphoric oxide in a refrigerator, but after 4–6 weeks they had decomposed.

ACKNOWLEDGMENT

We thank the Medical Research Council for their financial support.

REFERENCES

1 C. H. BOLTON, L. HOUGH, AND M. Y. KHAN, *Biochem. J.*, 101 (1966) 194.
2 B. BELLEAU AND G. MALEK, *J. Amer. Chem. Soc.*, 90 (1968) 1652.
3 M. SPINOLA AND R. W. JEANLOZ, *Carbohyd. Res.*, 15 (1970) 361.
4 L. MESTER, A. SCHIMPL, AND M. SENN, *Tetrahedron Lett.*, (1967) 1697.
5 G. JOHNSON, W. RULIFFSON, AND R. GRAHAM COOKS, *Carbohyd. Res.*, 18 (1971) 243.
6 M. BERGMANN AND L. ZERVAS, *Ber.*, 65 (1932) 1192.
7 D. COWLEY, L. HOUGH, AND C. PEACH, *Carbohyd. Res.*, 19 (1971) 231.
8 A. BERTHO, *Ann.*, 562 (1949) 229.
9 B. LOEV AND K. M. SNADER, *Chem. Ind. (London)*, (1965) 15.