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SYNTHESIS OF GLYCOSYLAMIDES AND 4-*N*-GLYCOSYL-L-ASPARAGINE DERIVATIVES

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ABSTRACT

A new synthesis of acylated glycosylamides by use of the reaction of acylglycosyl isothiocyanates with carboxylic acids catalyzed by triethylamine is described. Condensation with 1-benzyl *N*-(benzyloxy)carbonyl-L-aspartate, as carboxyl-containing compound, gave high yields of 4-*N*-glycosyl-L-asparagine derivatives, namely, the β-D-galactopyranosyl, 2-acetamido-2-deoxy-β-D-glucopyranosyl, β-lactosyl, and di-*N*-acetyl-β-chitobiosyl derivatives. The scheme of formation of the main- and by-products of the reaction of glycosyl isothiocyanate with carboxylic acids is discussed.

INTRODUCTION

The *N*-glycosylamide linkage as well as the *O*-glycosyl linkage are the main types of bond between carbohydrate and polypeptide chains in glycoproteins. Various 1-aminoacyl derivatives of glycosylamines have been synthesized during the last years. Among them, the derivatives of 4-*N*-glycosyl-L-asparagine [*N*-(L-aspart-4-oyl)-D-glycosylamine] seem to be the most interesting as models of structural fragments of glycoproteins[1]. In all cases, the glycosylamides were obtained by the usual methods of peptide synthesis, namely acylation of glycosylamine derivatives by *N*-protected derivatives of amino acids with the carbodiimide method[2–5], acylation by anhydrides[6,7] or chloride[8] of *N*-protected amino acids, and the method of mixed anhydrides[9].

In preceding communications[10–12], we have proposed a new way of synthesizing *N*-glycosylamides, which consists in the reaction of per-*O*-acylglycosyl isothiocyanates with carboxylic acids. A similar reaction is known in the interaction of alkyl and aryl isocyanates and isothiocyanates with aliphatic and aromatic acids[13,14]. As far as we know, this is the first time that this reaction has been used for the synthesis of glycosylamides. The starting acetylglycosyl isothiocyanates were obtained in high yields by treatment of acylglycosyl halides with silver[15–17] or ammonium thiocyanate[18].

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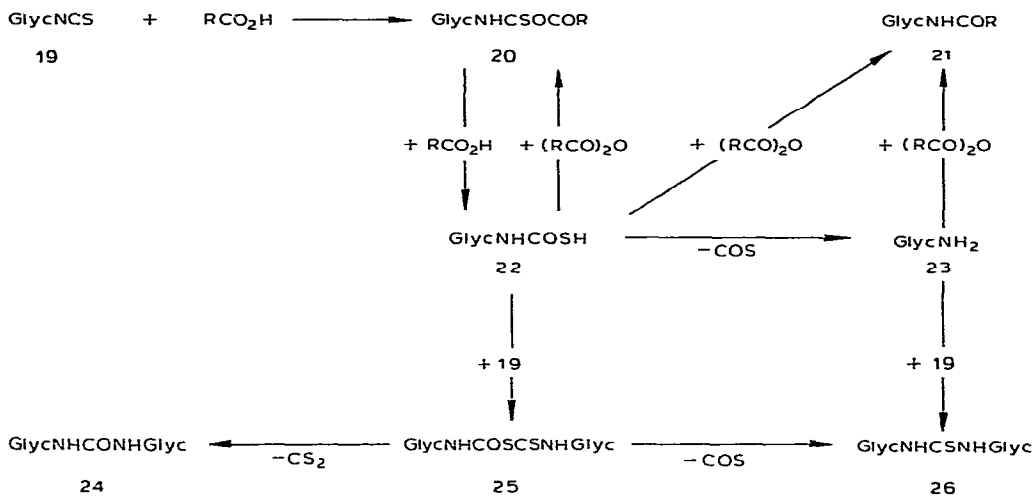
RESULTS AND DISCUSSION

We showed earlier[10,12] that the reaction of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl (**1**) and -D-galactopyranosyl isothiocyanate (**2**) with a slight excess of acetic or benzoic acid in the presence of 0.7–1.0 molar equivalent of triethylamine at room temperature produces the glycosylamides **6** and **7**, respectively, in 30–35% yield. The by-products were derivatives of *N*,*N'*-bis(glycosyl)urea (**9** and **10**) and *N*,*N'*-bis(glycosyl)thiourea (**11** and **12**), arising in a proportion similar to that of the desired compounds.

Synthesis of amides from isocyanates and carboxylic acids is usually considered to proceed *via* an intermediate mixed anhydride, which is further decarboxylated[14]. Apparently, the formation of the similar mixed anhydride **20** may follow the reaction of a glycosyl isothiocyanate **19** with a carboxylic acid, as shown in the hypothetical Scheme 1. The formation of disubstituted ureas in reactions with isocyanates is usually explained by the presence of moisture in the reaction mixture[14]. In



Glyc = acylated glycosyl residue

R = Me, Ph, or a *N*-protected amino acid residue

Scheme 1

addition, not only thioureas may arise from the reactions of isothiocyanates, but also ureas, when a derivative of thiocarbamic acid (such as **22**) is quite stable. However, the considerable proportion of urea derivatives cannot be explained by the possible presence of moisture in reagents and solvents, and the formation of **24** and **26** may depend on further rearrangements of the mixed anhydride **20**. By reacting with the excess of carboxylic acid, **20** may be converted into the thiocarbamic acid derivative **22**, which would give, with the starting isothiocyanate **19**, the anhydride **25**. After elimination of COS or CS_2, anhydride **25** may be converted into the thio-

TABLE I

YIELDS (%) OF **7**, **10**, AND **12** OBTAINED BY TREATMENT OF **2** WITH ACETIC ACID[a]

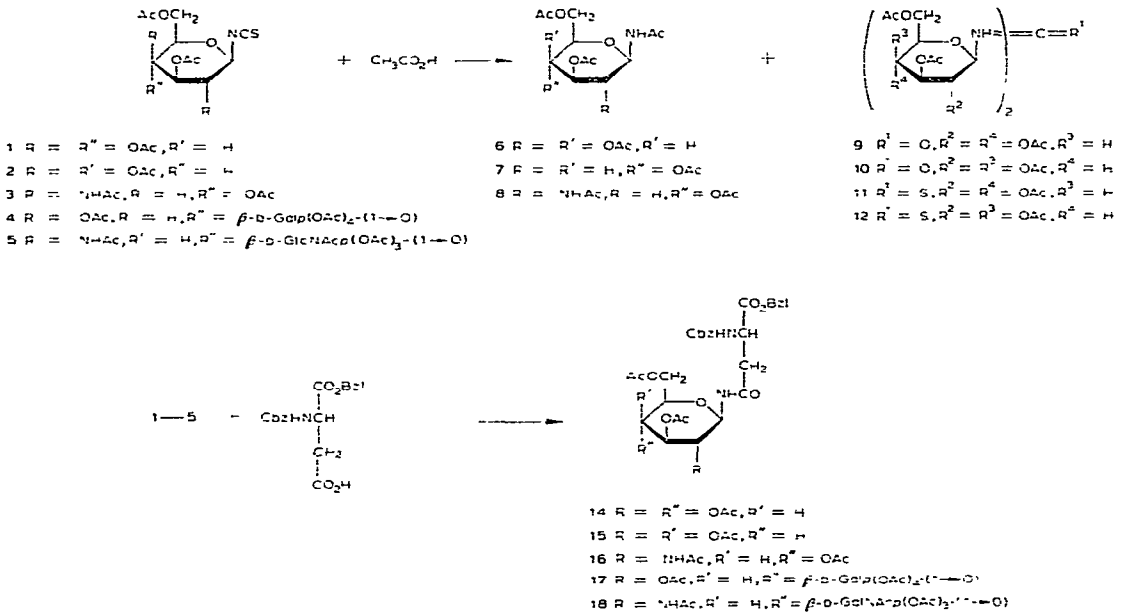
Triethylamine (Mol per mol of **2***)*	*Acetic acid*	*Yields of reaction products*[b]		
		7	**10**	**12**
0.1	1.4	68	7	25
0.3	1.4	70	12	18
0.5	1.4	61	22	17
0.7	1.4	52	34	14
1.1	1.4	54	38	8
1.5	1.4	51	41	8
0.3	2	71	6	23
0.3	3	68	7	25
0.3	5	68	5	27
1.4	3	76	4	20
1.4	10	71	4	25

[a]In toluene solution in the presence of triethylamine at 20°. [b]Estimated by densitometry of thin-layer chromatograms (see Experimental section).

urea **26** or urea **24** derivatives, respectively. Thus, the desired glycosylamide **21** may result from the action of a carboxylic acid anhydride on either **22**, or **23** arising from **22**.

These hypothetical transformations are not, apparently, exclusive of other possible reactions of glycosyl isothiocyanates with carboxylic acids in the presence of triethylamine, as autocatalysis at different stages of the process by the products formed (glycosylamine, urea derivatives) cannot be excluded. In the reaction of the isothiocyanate **2** with acetic acid, the reaction conditions were optimized to give the minimum by-products (urea and thiourea derivatives) by varying the molar ratios of catalyst to isothiocyanate, and of acid to isothiocyanate (see Table I). Increase of the molar proportion of acetic acid to isothiocyanate **2** from 1.4 to 3 (and higher), at approximately equal concentrations of **2** and catalyst, led to an increase in the yield of amide **7**. Excess of the acid leads, apparently, to the more complete conversion of isothiocyanate into the mixed anhydride **20**, and further into the thiocarbamic acid derivative **22**. The latter compound may produce urea and thiourea derivatives in the presence of the starting isothiocyanate in the system. It should be emphasized, as we showed earlier[12], that there is no direct formation of amide **21** from the mixed anhydride **20** *via* elimination of COS, as amide **7** was the main product of the reaction of isothiocyanate **2** with benzoic acid in the presence of an equivalent amount of acetic anhydride, and the corresponding 2,3,4,6-tetra-*O*-acetyl-1-*N*-benzoyl-β-D-galactopyranosylamine could not be found.

Despite of the fact that the excess of carboxylic acid led to an increase of the yield of amide **21**, we considered it inexpedient from a preparative point of view to use a considerable excess of the acid in the synthesis of 4-*N*-glycosylasparagine



derivatives. Variation of the proportion of triethylamine at a constant ratio (1.4:1) of acid to isothiocyanate showed that decrease of the catalyst concentration from about equimolar (used earlier[12]) to 0.1–0.3 mol per mol of **2** leads to an increase in the yield of amide **7** (up to 68–70%) at room temperature. This suggests that an increased amount of catalyst favors the side-reactions **22**→**25**→**24** + **26**. Treatment of **2** with acetic acid in toluene at 80° with the proportions of reagent and catalyst given in Table I led to some decrease of the yield (48–57%) of amide **7**, and resulted in approximately equal quantities of urea **10** and thiourea **12**.

Treatment of **2**, on a preparative scale, with acetic acid in toluene at room temperature in the presence of 0.1 molar equiv. of triethylamine gave amide **7** in a 75% yield. Under these optimal conditions, treatment of **1** and **2** with 1-benzyl *N*-(benzyloxy)carbonyl-L-aspartate[19] (**13**) resulted in a 70% yield of 2,3,4,6-tetra-*O*-acetyl-1-*N*-[1-benzyl *N*-(benzyloxy)carbonyl-L-aspart-4-oyl]-β-D-glucopyranosylamine[2] (**14**) and -galactopyranosylamine (**15**), respectively.

2,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-β-lactosyl isothiocyanate[18] (**4**) was condensed with aspartate **13** for the preparation of the known aspartylamide[3,5] (**17**) in 75% yield. Because the product obtained had a considerably higher melting point (163–164°) than that reported earlier[3,5] (91–92°), but a nearly identical optical rotation, its structure was further investigated. The i.r. spectrum of **17** indicates the presence of amide, aromatic, and ester groups. The ratio of aromatic to acetyl protons is ~1:2, which corresponds to the presence of two phenyl and seven acetyl groups (n.m.r. data). A molecular mass of 974 (MH^+, *m/e* 975) was estimated for **17** by field-desorption mass-spectrometry. In addition, the only peak detected in the range of *m/e* 500–1000 was that at *m/e* 867 ($MH^+ - PhCH_2OH$), of low intensity. Hydrolysis

of deacetylated **17** with 2.5M trifluoroacetic acid[20] yielded equimolar quantities of D-glucose and D-galactose, identified by g.l.c. in the form of trimethylsilyl ethers. Aspartic acid also was found to be a product of hydrolysis (t.l.c.). Finally, **17** was converted, by *O*-deacetylation, followed by catalytic hydrogenation, into 1-*N*-(L-aspart-4-oyl)-4-*O*-β-D-galactopyranosyl-β-D-glucopyranosylamine having physical constants in agreement with those previously described[5]. The differences in melting points for the amide **17** may be ascribed to polymorphism of the crystals. It should be noted that the synthesis of the same aspartylamides (**14**, **15**, and **17**) in the presence of increased proportions (up to one equivalent) of triethylamine resulted in yields of <40%.

The acylated isothiocyanates of 2-amino-2-deoxy sugars react in the same, smooth way with carboxylic acids to produce amides. Isothiocyanate **3** reacted with acetic acid in toluene at room temperature in the presence of one equivalent of triethylamine to give 2-acetamido-1-*N*-acetyl-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine[21,22] (**8**) in 70% yield. This high yield of amide, despite the increased concentration of catalyst, may be explained by the low solubility of the isothiocyanate **3** in toluene. This leads to a considerable excess of the acid over **3** in solution, thus favoring the desired direction of the reaction.

Isothiocyanate **3** reacted with aspartate **13** under the same conditions to produce the known[8,23] aspartylamide **16** in 70% yield. Per-*O*-acetylchitobiosyl isothiocyanate[18] (**5**) reacted with aspartate **13** in toluene only after prolonged heating, to give the known[24] derivative **18** of chitobiosylasparagine in 47% yield.

Thus, the present method of synthesis of glycosylamides by treatment of glycosyl isothiocyanates with carboxylic acids is more efficient than the previous methods based on the condensation of glycosylamines with acids and their derivatives, which only seldom produce yields of 60–65% of the desired compounds (only a single example of a more efficient reaction is known[7]). Moreover, the proposed method consists of only one stage, and appears to be simpler than the method that uses gycosyl azides.

EXPERIMENTAL

General. — Melting points were determined with a Boetius apparatus. Specific optical rotations were measured with a Perkin–Elmer Model 141 polarimeter. I.r. spectra were recorded with a UR-20 spectrophotometer for KBr discs. The field-ionization mass-spectrum was recorded with a Varian MAT CH-5-DF instrument, equipped with an EI-FI-FD combined ion-source. T.l.c. was performed on Silufol UV-254 plates (Chemapol, Czechoslovakia): the sugars were detected by u.v. light and by heating to 200–250°, and aspartic acid was detected with the ninhydrin reagent. Column chromatography was performed on Silica Gel L 40–100 μ (Chemapol, Czechoslovakia). Scanning of thin-layer chromatograms was performed with the aid of a narrow slit at 483 nm by use of an Opton Model PMQ-2 spectrophotometer. Solvents were evaporated *in vacuo* at 30–35°.

*Reaction of 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl isothiocyanate* (**2**) *with acetic acid in the presence of triethylamine at various ratios of amine to* **2** (*A*), *and acid to* **2** (*B*). — (*A*). A solution of **2** (0.1 mmol) and glacial acetic acid (0.14 mmol) in dry toluene (0.20 mL) containing triethylamine (0.01–0.15 mmol) was kept for 3 days at room temperature, or for 3.5 h at 80°, until complete disappearance of **2** (t.l.c.). The mixture was evaporated, and the residue dissolved in chloroform (0.5 mL), and analyzed by t.l.c. in 3:5 (v/v) benzene–2-butanone. A calibration mixture containing amide **7**, urea, and thiourea derivatives (**10** and **12**, respectively) in a molar ratio of 2:1:1 was used for quantitative estimation of the reaction products. The average yields of **7**, **10**, and **12** from three parallel experiments are presented in Table I; the relative error did not exceed 5%.

B. A solution of **2** (0.1 mmol) and glacial acetic acid (0.2–1.0 mmol) in dry toluene (0.20 mL) containing triethylamine (0.03 mmol) was kept for 3 days at room temperature, or for 3.5 h at 80°, after which it was analyzed as described under *A*. In the same way, **2** (0.1 mmol) was treated with acetic acid (0.3 and 1.0 mmol) in the presence of triethylamine (0.14 mmol) at room temperature. The results are given in Table I.

*1-*N*-Acetyl-2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosylamine* (**7**). — A mixture of **2** (1.17 g), glacial acetic acid (0.25 mL), and triethylamine (0.04 mL) in dry toluene (7 mL) was kept for 2 days at room temperature. After evaporation, the residue was chromatographed on a column (2.5 × 40 cm) with 97:97:3 to 17:17:6 (v/v) chloroform–ether–2-butanone. The sequence of eluted fractions was 880 mg (75%) of **7**, m.p. 172–173° (from 2-butanone–ether), $[\alpha]_D^{20}$ +34° (*c* 1, chloroform); 200 mg (18%) of thiourea **12**, m.p. 220–221° (from acetone–ether–hexane), $[\alpha]_D^{20}$ +8° (*c* 0.5, chloroform); and 70 mg (6%) of urea **10**, m.p. 233–234° (from 2-butanone–ether–hexane), $[\alpha]_D^{20}$ +11° (*c* 1, chloroform); the constants correspond to those presented earlier[12].

*2,3,4,6-Tetra-*O*-acetyl-1-*N*-[1-benzyl* N*-(benzyloxy)carbonyl-*L*-aspart-4-oyl]-β-*D*-glucopyranosylamine* (**14**). — A mixture of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate[15] (**1**, 195 mg), 1-benzyl *N*-(benzyloxy)carbonyl-L-aspartate[19] (**13**, 250 mg), and triethylamine (0.007 mL) in dry toluene (1.5 mL) was kept for 3 days at room temperature. After evaporation, the residue was dissolved in chloroform, and the solution was washed with a saturated solution of sodium hydrogencarbonate and evaporated. The residue crystallized from ethanol to give 240 mg (70%) of **14**, m.p. 143.5–144°, $[\alpha]_D^{20}$ +20° (*c* 1, chloroform); lit.[2] m.p. 142.5–143.5°, $[\alpha]_D^{22}$ +19° (*c* 2.01, chloroform).

*2,3,4,6-Tetra-*O*-acetyl-1-*N*-[1-benzyl* N*-(benzyloxy)carbonyl-*L*-aspart-4-oyl]-β-*D*-galactopyranosylamine* (**15**). — By use of the method described for the preparation of **14**, a mixture of **2** (195 mg) and **13** (250 mg) in toluene (1.5 mL) in the presence of triethylamine (7 μL) gave, after chromatography with 9:1 to 7:3 (v/v) tetrachloromethane–acetone, 239 mg (70%) of foamy **15**, $[\alpha]_D^{20}$ +29° (*c* 0.68, chloroform); ν_{max}^{KBr} 3360 (NH), 1755 (ester CO), 1530 (CONH), 770, 750, and 705 cm^{-1} (Ph).

Anal. Calc. for $C_{33}H_{38}N_2O_{14}$: C, 57.7; H, 5.6; N, 4.1. Found: C, 57.8; H, 5.6; N, 4.1.

Acid hydrolysis of **15** with M hydrochloric acid (24 h, 100°) resulted in the formation of D-galactose and L-aspartic acid (identified by t.l.c.).

2,3,6-Tri-O-acetyl-1-N-[1-benzyl N-(benzyloxy)carbonyl-L-aspart-4-oyl]-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-β-D-glucopyranosylamine (**17**). — Under the conditions described for the preparation of **14**, a mixture of 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl isothiocyanate[18] (**4**, 340 mg) and **13** (250 mg) in toluene (5 mL) in the presence of triethylamine (7 μL) gave, after crystallization from ethanol–ether, 340 mg (71%) of **17**, m.p. 163–164° (the same melting point was observed after crystallization from methanol and ethanol), $[\alpha]_D^{20}$ +10° (*c* 0.5, chloroform); lit.[3,5] m.p. 91–92° (from ethanol–ether), $[\alpha]_D^{20}$ +9.5° (*c* 1, chloroform) and +8.5° (*c* 0.8, chloroform), respectively; ν_{max}^{KBr} 3300 (NH), 1750 (ester CO), 1670 and 1550 (CONH), 770, 750, and 705 cm^{-1} (Ph); n.m.r. (dimethyl sulfoxide-d_6): δ 7.34 and 7.32 (s, 10 H, 2 Ph), 2.10, 2.04, 2.01, 2.00, 1.97, 1.90, and 1.85 (s, 21 H, 7 OAc); f.i.m.s.: *m/e* 975 (MH^+) and 867 ($MH^+ - PhCH_2OH$).

Anal. Calc. for $C_{45}H_{54}N_2O_{22}$: C, 55.4; H, 5.6; N, 2.9. Found: C, 55.3; H, 5.8; N, 2.9.

Compound **17** was *O*-deacetylated by the Zemplén method, and the product was hydrolyzed with 2.5M trifluoroacetic acid for 7 h at 100°. After evaporation, the mixture was dried by addition and distillation of toluene, per-*O*-trimethylsilylated, and analyzed by g.l.c.; this showed a ratio of D-glucose to D-galactose of 1:1. Aspartic acid was identified by t.l.c. (2:1:1, v/v, 1-butanol–acetic acid–water): R_F 0.30.

1-N-(L-Aspart-4-oyl)-4-O-β-D-galactopyranosyl-β-D-glucopyranosylamine. — *O*-Deacetylation of **17** (200 mg), followed by catalytic hydrogenation, was performed as previously described[5] to give 50 mg of instable title compound (yield 53%), m.p. 235° (dec., from aqueous methanol), $[\alpha]_D^{20}$ +1° (*c* 0.1, water); lit.[5] m.p. 235° (dec.), $[\alpha]_D$ +1.0° (*c* 0.1, water).

2-Acetamido-1-N-acetyl-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosylamine (**8**). — A mixture of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl isothiocyanate[17,18] (**3**, 0.39 g), glacial acetic acid (0.25 mL), and triethylamine (0.14 mL) in dry toluene (3 mL) was stirred at room temperature for 2 days. After evaporation, the residue was chromatographed on a column (2 × 30 cm) in 4:1 to 1:1 (v/v) chloroform–acetone to give 0.27 g (70%) of **8**, m.p. 240–241° (from methanol), $[\alpha]_D^{20}$ +22° (*c* 0.5, pyridine); lit.[21,22] m.p. 241° and 244–246°, $[\alpha]_D^{20}$ +22.8° (*c* 1.2, pyridine) and +41° (*c* 1, chloroform), respectively; ν_{max}^{KBr} 3320 (NH), 1745 (ester CO), 1665, and 1545 cm^{-1} (CONH).

2-Acetamido-3,4,6-tri-O-acetyl-1-N-[1-benzyl N-(benzyloxy)carbonyl-L-aspart-4-oyl]-2-deoxy-β-D-glucopyranosylamine (**16**). — By use of the procedure described for **8**, a mixture of isothiocyanate **3** (1.17 g), aspartate **13** (1.60 g), and triethylamine (0.63 mL) in toluene (25 mL) gave, after chromatography with 93:7 to 37:13 (v/v) chloroform–acetone, 1.45 g (70%) of **16**, m.p. 214–215° (from methanol), $[\alpha]_D^{20}$

+10° (*c* 0.5, chloroform); lit.[8,23] m.p. 214–217° (dec.) and 216–217°, $[\alpha]_D^{20}$ +28° (*c* 1.41, chloroform) and $[\alpha]_D^{21}$ +10.5° (*c* 0.353, chloroform), respectively.

*2-Acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-3,6-di-*O*-acetyl-1-*N*-[1-benzyl N-(benzyloxy)carbonyl-*L*-aspart-4-oyl]-2-deoxy-β-*D*-glucopyranosylamine* (**18**). — A suspension of hepta-*O*-acetyl-β-chitobiosyl isothiocyanate[18] (**5**, 1.40 g) and **13** (1.07 g) in toluene (25 mL) containing triethylamine (0.42 mL) was stirred for 50 h at 80°, and treated as described for **8**. Chromatography of the chloroform solution with 17:3 to 1:1 (v/v) chloroform–acetone yielded 0.83 g (43%) of **18**, m.p. 250–251° (from methanol), $[\alpha]_D^{20}$ −16° (*c* 0.2, chloroform); lit.[24] m.p. 250–253°, $[\alpha]_D$ −19° (*c* 0.26, chloroform); ν_{max}^{KBr} 3320 (NH), 1750 (ester CO), 1665 and 1550 (CONH), 725, and 675 cm^{-1} (Ph).

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