THE SYNTHESIS OF *O*-α-D-MANNOPYRANOSYL-(1 → 6)-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-(1 → 4)-2-ACETAMIDO-2-DEOXY-D-GLUCOSE*

MOHAMMED SHABAN† AND ROGER W. JEANLOZ‡

Laboratory for Carbohydrate Research, Departments of Biological Chemistry and Medicine, Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts 02114 (U. S. A.)

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ABSTRACT

2-Acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-4-*O*-(2-acetamido-3-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-β-D-glucopyranose was obtained from di-*N*-acetylchitobiose by formation of the 4,6-benzylidene acetal, acetylation, and removal of the benzylidene group. Condensation with tetra-*O*-acetyl-α-D-mannopyranosyl bromide gave crystalline *O*-(tetra-*O*-acetyl-α-D-mannopyranosyl)-(1 → 6)-*O*-(2-acetamido-3-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucose which was *O*-deacetylated to give the crystalline, title compound. This trisaccharide was further characterized by an undecaacetate. The trisaccharide is useful as a reference compound for the structure determination of glycopeptides as well as a starting material for their synthesis.

INTRODUCTION

The carbohydrate moiety of numerous glycoproteins isolated from blood or secretions is composed of oligosaccharides linked to the protein part through an alkali-resistant glycosylamine linkage between asparagine and 2-acetamido-2-deoxy-D-glucose. The oligosaccharides are composed of 2-acetamido-2-deoxy-β-D-glucopyranosyl and α-D-mannopyranosyl residues forming a core located near the protein–carbohydrate link. To this core may be attached branches composed of D-galactose, 2-acetamido-2-deoxy-D-glucose, L-fucose, and sialic acid residues[1]. In a study of the structure of the carbohydrate moiety of beef-pancreatic ribonuclease B, a trisaccharide derivative composed of one molecule of D-mannose and two molecules of 2-acetamido-2-deoxy-D-glucose linked to asparagine was isolated, and the structure of *O*-α-D-

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†On leave of absence from the Faculty of Science, Alexandria University, Alexandria, Egypt.

‡To whom inquiries should be sent.

mannopyranosyl-(1 → 3)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)-2-acetamido-1-*N*-(aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine was proposed on the basis of periodate oxidation studies[2]. Since other structures similar to, but not identical with, this structure have been proposed for other glycoproteins[3], the synthesis as reference compounds of various trisaccharides containing a di-*N*-acetylchitobiose and an α-D-mannose residue has been undertaken as a part of a program to synthesize fragments of carbohydrate chains of glycoproteins and glycolipids. Interest in the synthesis of carbohydrate structures containing both the 2-acetamido-2-deoxy-β-D-glucopyranosyl and the α-D-mannopyranosyl residues has been greatly increased by the work of Burger and of Sachs and their respective associates, who have established that both wheat-germ agglutinin and concanavalin A (an agglutinin isolated from jack bean meal) aggregate cancer and virus-transformed cells. The action of the wheat-germ agglutinin and concanavalin A on the cells is strongly inhibited by 2-acetamido-2-deoxy-D-glucose and di-*N*-acetylchitobiose[4], and by methyl α-D-mannopyranoside[5], respectively, thus suggesting the presence of 2-acetamido-2-deoxy-β-D-glucopyranosyl and α-D-mannopyranosyl residues at the surface of these cells.

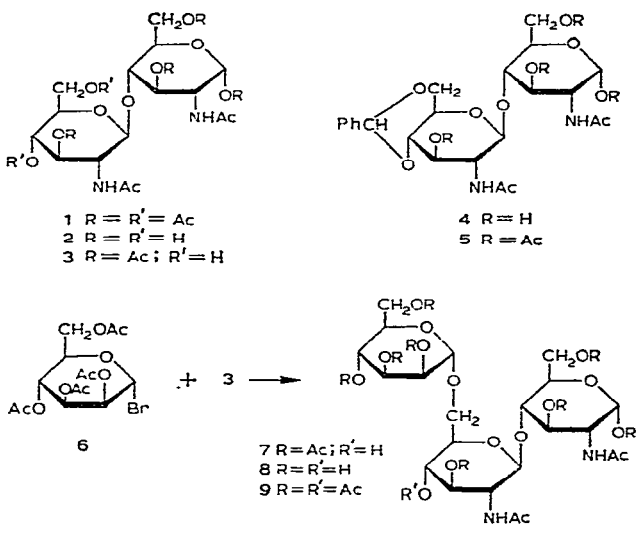
In the present paper we report the synthesis and characterization of *O*-α-D-mannopyranosyl-(1 → 6)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-D-glucose [*O*-α-D-mannopyranosyl-(1 → 6)-di-*N*-acetylchitobiose]. This trisaccharide may be prepared by a Koenigs–Knorr reaction of either a tetra-*O*-acetyl-α-D-mannopyranosyl halide with a suitably protected di-*N*-acetylchitobiose derivative or of a *O*-α-D-mannopyranosyl-(1 → 6)-2-acetamido-2-deoxy-α-D-glucopyranosyl halide with a suitably blocked 2-amino-2-deoxy-D-glucose derivative. The first alternative was elected because it avoids the use of the relatively unstable 2-acetamido-2-deoxy-D-glucopyranosyl halides[6], and uses the stable and reactive tetra-*O*-acetyl-α-D-mannopyranosyl halide — which gives practically only the α-anomer[7–10] — and the relatively accessible di-*N*-acetylchitobiose.

DISCUSSION

Octaacetyl-chitobiose (**1**) was prepared from chitin by a modification of the Bergmann acetolysis procedure[11,12]. In the latter method, incomplete acetolysis of the chitin was observed after the stated reaction time (36 h at room temperature and 10 h at 55°). When the reaction was performed for a longer time, the yield of oligosaccharides was enormously decreased, with a concomitant increase in the 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-glucose produced. Since the physical state of the chitin seemingly was responsible for the incomplete dissolution, the product was first dissolved in 85% phosphoric acid at room temperature for 4 h and then reprecipitated by dilution[13] to give a fine powder, probably partially degraded, that was readily soluble in the acetolysis mixture. A shorter reaction time (12 h at room temperature and 8 h at 50°) gave an increased yield of the oligosaccharides. The reaction product, after separation of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-glucose, was fractionated by adsorption chromatography on silica gel to give octaacetyl-chitobiose in 6% yield.

Di-*N*-acetylchitobiose (**2**), obtained by *O*-deacetylation of the octaacetyl derivative **1**, was treated with benzaldehyde in the presence of fused zinc chloride to give 2-acetamido-2-deoxy-4-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-α-D-glucopyranose (**4**) in 56% yield. Acetylation of **4** with acetic anhydride and pyridine gave crystalline 2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-4-*O*-(2-acetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-α-D-glucopyranose (**5**). Compound **5** was also obtained without isolation of the intermediate **4**, which was acetylated directly after the removal of benzaldehyde. The latter procedure gave a better overall yield, probably because hydrolysis of the 4,6-benzylidene group in the aqueous zinc chloride solution was minimized during the isolation. *O*-Deacetylation of **5** gave **4** in good yield. The benzylidene group of **5** was removed with hot, dilute acetic acid to give 2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-4-*O*-(2-acetamido-3-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-α-D-glucopyranose (**3**). The configuration at C-1 of the reducing amino sugar moiety of the intermediates **3** and **5** could not be established with certainty by a comparison of the molar rotations with those of the respective components (Table I). Since the reactions used for the transformation of **4** into **5** and of **5** to **3** are not expected to cause changes of the anomeric configuration, the more-likely α-configuration attributed to **5** was also attributed to **4** and **3**. It is also of interest that all of the other known derivatives of di-*N*-acetylchitobiose, where a mixture of anomers may exist, crystallize in the α configuration.

Condensation of **3** with tetra-*O*-acetyl-α-D-mannopyranosyl bromide[24] (**6**) in the presence of mercuric cyanide gave crystalline *O*-(tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-*O*-(2-acetamido-3-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose (**7**) in 66% yield. As in similar, previously described condensations[9,25], no products resulting from other possible condensation modes, such as reaction to give the β-anomer or condensation with the free hydroxyl group at C-4, were observed. The α-D configuration of the



O-mannopyranosyl-(1 → 6) linkage is indicated by comparison of the molar rotations of **7** with the sum of the molar rotations of **6** and methyl tetra-*O*-acetyl-α- and β-D-mannopyranosides (Table I), as described in earlier publications[8–10]. It is evident that the anomeric acetoxyl group of **7** retained the same configuration as that of the parent compound **3**, since it was not subjected during condensation to conditions that might have caused inversion.

TABLE I

MOLECULAR ROTATIONS OF PREPARED COMPOUNDS COMPARED WITH THE SUM OF THEIR CONSTITUENTS

Compound	$[M]_D$ (*degrees*) × 10^{-2}
Methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside[a] (**10**) (Ref. 14) +2-acetamido-2-deoxy-α-D-glucose[b] (Ref. 15)	−108
Compound **10**+2-acetamido-2-deoxy-β-D-glucose[b] (Ref. 16)	−297
Compound **4**[c]	−123
Methyl 2-acetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside[a] (**11**) (Ref. 17) +2-acetamido-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose[a] (**12**) (Ref. 18)	+296
Compound **11**+2-acetamido-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose[a] (**13**) (Ref. 19)	−42
Compound **5**[d]	+76
Methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside[a] (**14**) (Ref. 20) +Compound **12**	+260
Compound **14**+Compound **13**	−78
Compound **3**[e]	+113
Methyl tetra-*O*-acetyl-α-D-mannopyranoside[a] (**15**) (Ref. 21)+Compound **3**	+284
Methyl tetra-*O*-acetyl-β-D-mannopyranoside[a] (**16**) (Ref. 21)+Compound **3**	−64
Compound **7**[a]	+268
Methyl α-D-mannopyranoside[b] (Ref. 22)+di-*N*-acetyl-chitobiose (**17**) (Ref. 11) at equilibrium[b]	+243
Methyl β-mannopyranoside isopropyl alcoholate[b] (Ref. 23)+Compound **17**	−62
Compound **8** at equilibrium[c]	+199
Compound **15**+α-chitobiose octaacetate[a] (**18**) (Ref. 12)	+509
Compound **16**+Compound **18**	+211
Compound **9**[a]	+357

[a]Optical rotation determined in chloroform, [b]in water, [c]in 1:1 water–methanol, [d]in 1:1 chloroform–methanol, and [e]in methanol.

Saponification of the *O*-acetyl groups of **7** gave the crystalline trisaccharide **8**, whereas acetylation gave the undecaacetyl derivative **9**.

EXPERIMENTAL

General. — Melting points below 300° were determined with a Mettler FP-2 apparatus and correspond to "corrected melting points"; above 300° with a Büchi

melting point determination apparatus and were not corrected. Optical rotations were determined, in semimicro tubes, with a Perkin–Elmer Model 141 polarimeter. The chloroform used was analytical-reagent grade and contained about 0.75% of ethanol. Infrared spectra were recorded, for potassium bromide discs, with a Perkin–Elmer Model 237 spectrophotometer. Column chromatography was performed on Silica Gel Merck (70–325 mesh; E. Merck, Darmstadt, Germany), used without pretreatment. The ratio of weight of substance to weight of absorbent was 1:80 to 1:120. The volume of the fractions eluted was 3–4 ml per gram of the substance to be chromatographed. The ratio of the diameter of the column to its length was 1:12. Thin-layer chromatography was performed on precoated Silica Gel G plates (layer thickness 0.25 mm; E. Merck, Darmstadt, Germany); all compounds showed only one spot; the spots were revealed by spraying with 1:1:18 (v/v) anisaldehyde–conc. sulfuric acid–ethanol and heating for a few min at 100–110°. Evaporations were conducted *in vacuo* with a bath temperature below 45°. Solutions of less than 5 ml were evaporated under a stream of nitrogen. Microanalyses were performed by Dr. W. Manser, Zürich, Switzerland.

2-Acetamido-1,3,6-tri-O-*acetyl-2-deoxy-4*-O-(*2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl*)-α-D-*glucopyranose* (*octaacetyl-chitobiose*) (**1**). — Chitin (215 g, Pfanstiehl Laboratories, Inc., Waukegan, Illinois) was added gradually to 85% phosphoric acid (4 liters) at room temperature with stirring. After 4 h, the viscous syrup was filtered through a cheesecloth, and the filtrate was added in a thin stream to an ice–water mixture (6 liters) with vigorous stirring. After being kept overnight at room temperature, the product was filtered off on a sintered-glass funnel and successively washed with water, acetone, and ether, and dried to a finely powdered, white solid (200 g). This pretreated chitin (80 g) was gradually added with stirring to a cold mixture of acetic anhydride (400 ml) and conc. sulfuric acid (55 ml). Stirring was continued for 12 h at room temperature and a further 8 h at 50° to ensure complete dissolution of chitin. The solution was poured into ice (2 liters), and then treated with solid sodium acetate (450 g). After 12 h at room temperature, the solution was neutralized with solid potassium hydrogen carbonate and extracted with chloroform. The extract was washed with water, dried (sodium sulfate), and evaporated. The gummy product was triturated with methanol, and the insoluble residue (12 g) was filtered off and washed with methanol. This solid was chromatographed on a column of silica gel (300 g) with 9:1 chloroform–ethanol. Fractions having R_F 0.45 on t.l.c. with the same solvent were collected and evaporated to give 8 g of **1**. Crystallization from methanol gave needles, m.p. 308–309° (dec.); lit.[11]: m.p. 308–309°.

2-Acetamido-2-deoxy-4-O-(*2-acetamido-2-deoxy-α*-D-*glucopyranosyl*)-β-D-*glucopyranose* (**2**). — A solution of **1** (1 g) in methanol (40 ml) was treated with 0.1M sodium methanolate in methanol (5 ml) for 16 h at 4°. The solution was deionized by passage through Dowex-50 (H^+) resin, and then evaporated. Crystallization of the residue from methanol gave 600 mg (95%) of needles, m.p. 258° (dec.); lit.[12]: m.p. 260–262° (dec.).

2-Acetamido-2-deoxy-4-O-(*2-acetamido-4,6*-O-*benzylidene-2-deoxy-β*-D-*gluco-*

pyranosyl)-α-D-glucopyranose (**4**). — *From* **2**. Dry compound **2** (340 mg) was added to a solution of freshly fused zinc chloride (600 mg) in benzaldehyde (15 ml), and the mixture was shaken for 24 h at room temperature in an atmosphere of nitrogen. The mixture was poured into a cold mixture of water (100 ml) and hexane (200 ml), and the aqueous layer was rapidly separated, treated with a few drops of dilute ammonia, and passed through Rexyn-300 resin (H^+, OH^-). The solution was lyophilized, and the residue containing traces of inorganic material was crystallized from a minimum amount of water. Three recrystallizations from 50% methanol gave 230 mg (56%) of fine needles, m.p. 304–306° (dec.); $[\alpha]_D^{20}$ −24° (no change after 24 h, *c* 0.8, 50% methanol); i.r. data ν_{max}^{KBr} 1650 (CONH); 3300, 3390, and 3490cm^{-1} (NH and OH).

Anal. Calc. for $C_{23}H_{32}N_2O_{11}$: C, 53.92; H, 6.30; N, 5.46; O, 34.34. Found: C, 53.80; H, 6.28; N, 5.43; O, 34.20.

From **5**. A solution of **5** (100 mg) in methanol (70 ml) was treated with 0.1M sodium methanolate in methanol (2 ml) for 16 h at 4°. The cold mixture was rapidly passed through Dowex-50 (H^+) and evaporated. Crystallization of the residue as just described gave 70 mg (94%), m.p. and mixed m.p. 304–305° (dec.), having the same i.r. spectrum as that of the product obtained from **2**.

2-Acetamido-1,3,6-tri-O-acetyl-2-deoxy-4-O-(2-acetamido-3-O-acetyl-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-α-D-glucopyranose (**5**). — *From* **2**. Dry **2** (250 mg) was added to a solution of freshly fused zinc chloride (200 mg) in benzaldehyde (10 ml) and the mixture was shaken for 24 h at room temperature under nitrogen. Hexane (150 ml) was added to the mixture and the benzaldehyde–hexane solution was decanted from the separated, gummy residue. The residue was washed three more times with hexane and then treated with pyridine (20 ml) and acetic anhydride (10 ml) for 16 h at room temperature. The mixture was diluted with chloroform (250 ml) and successively washed with cold water, cold saturated potassium hydrogen carbonate solution, and water, and evaporated without being dried. Pyridine was removed by repeated addition and distillation of toluene, and the residue, contaminated with traces of benzaldehyde, was triturated with hexane and filtered off. Crystallization from 1:1 chloroform–methanol gave 350 mg (87%) of fine needles, decomposing without melting at 340°, $[\alpha]_D^{20}$ +11.3° (*c* 0.5, 1:1 chloroform–methanol); i.r. data: ν_{max}^{KBr} 1655 (CONH), 1740 (OAc), and 330 cm^{-1} (NH); t.l.c. in 9:1 chloroform–ethanol: R_F 0.45, in 3:2 benzene–methanol: R_F 0.66.

Anal. Calc. for $C_{31}H_{40}N_2O_{15}$: C, 54.71; H, 5.92; N, 4.12; O, 35.26. Found: C, 54.61; H, 5.94; N, 4.12; O, 35.38.

From **4**. A solution of **4** (30 mg) in pyridine (1 ml) was treated with acetic anhydride (1.5 ml) for 16 h at room temperature. Evaporation of the mixture and crystallization of the residue from 1:1 methanol–chloroform gave 32 mg (84%), decomposing without melting at 340° and having the same i.r. spectrum as the material obtained from **2**.

2-Acetamido-1,3,6-tri-O-acetyl-2-deoxy-4-O-(2-acetamido-3-O-acetyl-2-deoxy-β-D-glucopyranosyl)-α-D-glucopyranose (**3**). — A mixture of compound **5** (680 mg) in 50% acetic acid (15 ml) was heated for 30 min at 100°. The solution was evaporated,

and the residue was dried by repeated addition and distillation of methanol and toluene. Crystallization of the residue from methanol–ether gave 455 mg (77%) of needles, m.p. 302–304° (dec.); $[\alpha]_D^{20}$ +19° (*c* 1.3, methanol); i.r. data: ν_{max}^{KBr} 1655 (CONH); 1740 (OAc), 3400 cm^{-1} (broad, OH); t.l.c. in 3:2 benzene–methanol: R_F 0.43.

Anal. Calc. for $C_{24}H_{36}N_2O_{15}$: C, 48.66; H, 6.13; N, 4.73; O, 40.51. Found: C, 48.47; H, 6.12; N, 4.74; O, 40.31.

O-(*Tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-(*1*→*6*)-O-(*2-acetamido-3*-O-*acetyl-2-deoxy*-β-D-*glucopyranosyl*)-(*1*→*4*)-*2-acetamido-1,3,6-tri*-O-*acetyl-2-deoxy*-α-D-*glucopyranose* (7). — A solution of **3** (530 mg) and mercuric cyanide (500 mg) in 1:1 benzene–nitromethane (140 ml) was concentrated at atmospheric pressure to 120 ml. The mixture, after being cooled to room temperature, was treated with a solution of tetra-*O*-acetyl-α-D-mannopyranosyl bromide[24] (**6**, 1 g) in benzene (5 ml), and the mixture was stirred for 2 days at room temperature. Additional amounts of the bromide (0.5 g) and mercuric cyanide (0.5 g) were added, and the mixture was stirred for a further 2 days. The mixture was filtered, and the filtrate was diluted with 1,2-dichloroethane (100 ml), and successively washed with water, cold saturated potassium hydrogen carbonate solution, and water, and dried (sodium sulfate). The residue obtained after evaporation was chromatographed on a column of silica gel with elution at first with 1:1 ethyl acetate–ether and then with 9:1 chloroform–ethanol. Fractions obtained with the latter eluent gave, on evaporation, the chromatographically pure **7** which crystallized from chloroform–pentane to give 545 mg (66%) of microcrystals, m.p. 136–138°; $[\alpha]_D^{20}$ +29° (*c* 1.1, chloroform); i.r. data: ν_{max}^{KBr} 1670 (CONH), 1740 (OAc), 3360 cm^{-1} (NH and OH); t.l.c. in 7:3 benzene–methanol: R_F 0.52, in 9:1 chloroform–ethanol: R_F 0.33.

Anal. Calc. for $C_{38}H_{54}N_2O_{24}$: C, 49.46; H, 5.90; N, 3.04; O, 41.61. Found: C, 49.50; H, 5.89; N, 2.99; O, 41.44.

O-α-D-*Mannopyranosyl*-(*1*→*6*)-O-(*2-acetamido-2-deoxy*-β-D-*glucopyranosyl*)-(*1*→*4*)-*2-acetamido-2-deoxy*-α-D-*glucopyranose* (**8**). — A solution of **7** (100 mg) in methanol (10 ml) was treated with 0.1M sodium methanolate in methanol (2 ml) for 16 h at 4°. The solution was passed through a bed of Dowex 50 (H^+) and then evaporated. Crystallization of the residue from methanol–acetone gave 60 mg (94%) of microcrystals, m.p. 186–188°; $[\alpha]_D^{20}$ +36° → +34° (after 24 h, *c* 1.4, 50% methanol); i.r. data: ν_{max}^{KBr} 1650 (CONH), 3360 cm^{-1} (NH and OH).

Anal. Calc. for $C_{22}H_{38}N_2O_{16}$: C, 45.06; H, 6.53; N, 4.78; O, 43.64. Found: C, 45.00; H, 6.55; N, 4.26; O, 43.51.

O-(*Tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-(*1*→*6*)-O-(*2-acetamido-3,4-di*-O-*acetyl-2-deoxy*-β-D-*glucopyranosyl*)-(*1*→*4*)-*2-acetamido-1,3,6-tri*-O-*acetyl-2-deoxy*-α-D-*glucopyranose* (**9**). — A solution of **8** (150 mg) in pyridine (2 ml) was treated with acetic anhydride (2 ml) for 16 h at room temperature. The mixture was evaporated, and the residue was dried by repeated addition and distillation of toluene. Crystallization of the residue from chloroform–pentane gave 137 mg (88%) of microcrystals, m.p. 134–135°; $[\alpha]_D^{20}$ +37° (*c* 0.6, chloroform); i.r. data: ν_{max}^{KBr} 1650 (CONH), 1735

(OAc), 3360 cm^{-1} (NH); t.l.c. in 9:1 chloroform–ethanol, R_F 0.45.

Anal. Calc. for $C_{40}H_{56}N_2O_{25}$: C, 49.79; H, 5.84; N, 2.90; O, 41.46. Found: C, 49.71; H, 5.86; N, 2.82; O, 41.49.

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