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Chemical synthesis of the pyruvic acetal-containing trisaccharide unit of the species-specific glycopeptidolipid from *Mycobacterium avium* serovariant 8

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ABSTRACT

The functionalized, pyruvic acetal-containing haptenic trisaccharide, *p*-trifluoroacetamidophenyl 6-deoxy-2-*O*-{3-*O*-[4,6-*O*-(*S*)-(1-methoxycarbonylethylidene)-3-*O*-methyl-β-D-glucopyranosyl]-α-L-rhamnopyranosyl}-α-L-talopyranoside (**19**), a component of the glycolipid from *Mycobacterium avium* serovar 8 was synthesized. For the preparation of the terminal pyruvic acetal-containing unit, benzyl 2-*O*-benzyl-3-*O*-methyl-β-D-glucopyranoside (**6**) was condensed with methyl 2,2-di(ethylthio)propionate (**1**) in the presence of SO_2Cl_2–CF_3SO_3H catalyst to yield benzyl 2-*O*-benzyl-4,6-*O*-(*S*)-(1-methoxycarbonylethylidene)-3-*O*-methyl-β-D-glucopyranoside (**7S**), which was then converted into the suitably substituted glycosyl donor 2-*O*-acetyl-4,6-*O*-(*S*)-(1-methoxycarbonylethylidene)-3-*O*-methyl-α-D-glucopyranosyl trichloroacetimidate (**11**). The disaccharide glycosyl acceptor *p*-nitrophenyl *endo*-3,4-*O*-benzylidene-6-deoxy-2-*O*-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-α-L-talopyranoside (**15**) was glycosylated with **11** in the presence of trimethyl trifluoromethanesulfonate to furnish the protected trisaccharide *p*-nitrophenyl 2-*O*-{3-*O*-[2-*O*-acetyl-4,6-*O*-(*S*)-(1-methoxycarbonylethylidene)-3-*O*-methyl-β-D-glucopyranosyl]-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl}-*endo*-3,4-*O*-benzylidene-6-deoxy-α-L-talopyranoside (**16**). After deprotection, this gave the spacer-armed unprotected haptenic trisaccharide **19**.

INTRODUCTION

Besides *Mycobacterium tuberculosis*[1] and *M. leprae*[2,3], the agents of human tuberculosis and leprosy, respectively, another "atypical" mycobacterium[4] may also cause infections in humans. The fact that up to 50% of patients with AIDS are infected with the *M. avium* complex[5–7] has evoked a growing interest in the epidemiology, immunochemistry, and structure elucidation of *Mycobacterium* antigens, and their partial or complete syntheses.

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Member serovariants of the *M. avium* complex are distinguished by a set of highly characteristic tetrapeptide-containing surface glycolipids (GPLs)[8–10], and the general structure of a GPL is shown in **A**. The antigenic specificity is due to the structural variability at the nonreducing terminus of the haptenic oligosaccharide.

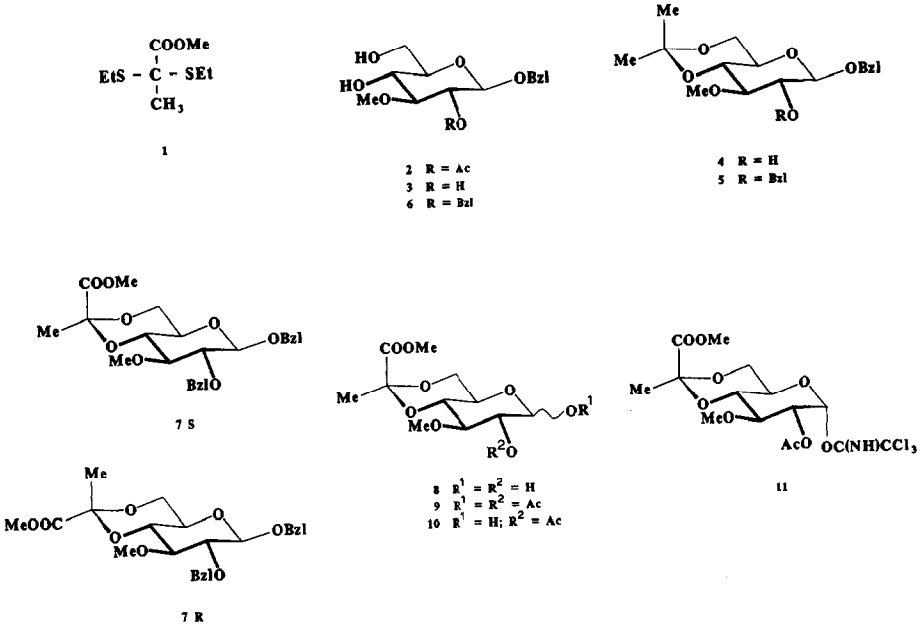
Fatty acyl-CO-NH-D-Phe-D-allo Thr-D-Ala-L-Alaninol-*O*-3,4-di-*O*Me-α-L-Rha*p*
|
O-Oligosaccharide
(A)

The structure of the haptenic oligosaccharide of the *M. avium* serovar. 8[11] is: 2-*O*-{3-*O*-[4,6-*O*-(*S*)-1-carboxyethylidene-3-*O*-methyl-β-D-glucopyranosyl]-α-L-rhamnopyranosyl}-6-deoxy-α-L-talopyranose. We now report the synthesis of this trisaccharide carrying a *p*-trifluoroacetamidophenyl spacer.

RESULTS AND DISCUSSION

There are numerous methods[12–21] for the synthesis of pyruvic acetals of carbohydrates, but the yield or the stereochemical outcome of these reactions is not satisfactory. We used a new reagent, methyl 2,2-di(ethylthio)propionate (**1**) for the synthesis of 4,6-*O*-(1-methoxycarbonylethylidene) acetals. This compound can be easily prepared from methyl pyruvate and ethanethiol in the presence of HCl, and then purified by distillation under reduced pressure. For the activation of **1**, a SO_2Cl_2–trifluoromethanesulfonic acid[22] mixture (1:1, 1 M solution in 7:3 toluene–diethyl ether) was applied.

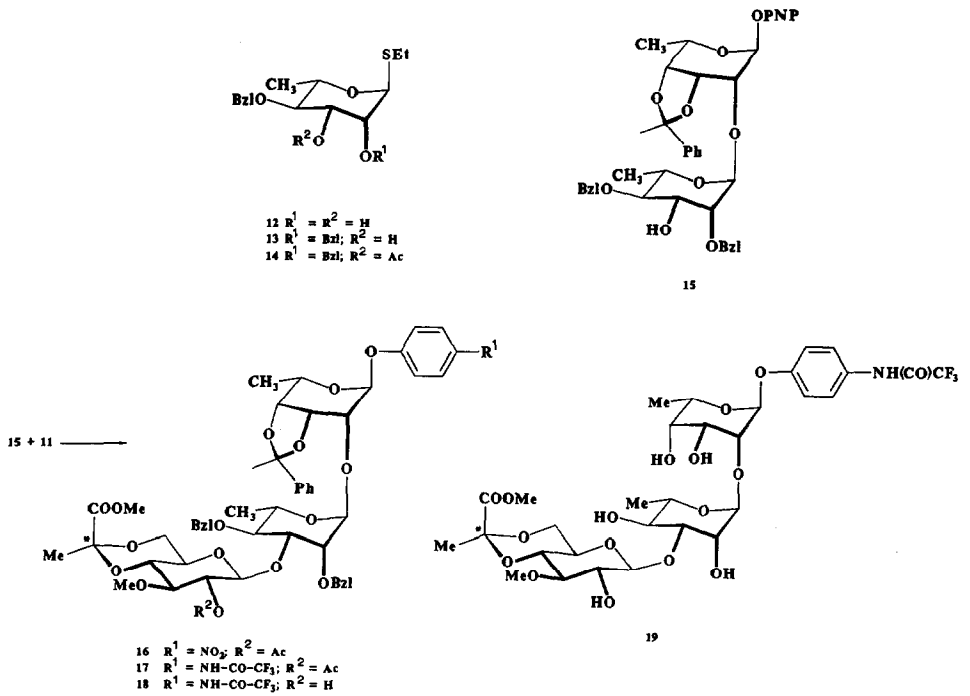
Benzyl 2-*O*-acetyl-3-*O*-methyl-β-D-glucopyranoside (**2**) was obtained from benzyl 2,4,6-tri-*O*-acetyl-3-*O*-methyl-β-D-glucopyranoside[24] by Zemplén transesterification (catalytic amount of NaOMe)[23]. Compound **2** was selected since the anomeric center could be selectively deprotected, and the participating group at position 2 ensured the β-selectivity of the glycosylation. However, the coupling reaction of **2** with **1** in the presence of SO_2Cl_2–trifluoromethanesulfonic acid gave a complex reaction mixture. This observation suggested the change of the *O*-acetyl function at position 2 to a temporary, easily removable, ether-type protecting group. Thus, benzyl 3-*O*-methyl-β-D-glucopyranoside[24] (**3**) was converted into its 4,6-*O*-isopropylidene derivative **4**, using 2,2-dimethoxypropane and *p*-toluenesulfonic acid catalyst[25]. Compound **4** was then benzylated, and subsequent acid hydrolysis of the isopropylidene group led to benzyl 2-*O*-benzyl-3-*O*-methyl-β-D-glucopyranoside (**6**). TLC analysis of the reaction of **6** with **1** in the presence of SO_2Cl_2–trifluoromethanesulfonic acid revealed the presence of only two products, which were separated and identified as benzyl 2-*O*-benzyl-4,6-*O*-(*S*)-(1-methoxycarbonylethylidene)-3-*O*-methyl-β-D-glucopyranoside (**7S**) and the corresponding diastereoisomer **7R**. The yields were acceptable (45 and 24%, respectively), and an advantage of the catalyst employed is that, because of its strong acid character, the formation of the thermodynamically stable product (**7S**) is favoured.



Catalytic hydrogenation of **7S** over 10% Pd–C and subsequent acetylation gave 1,2-di-*O*-acetyl-4,6-*O*-(*S*)-(1-methoxycarbonylethylidene)-3-*O*-methyl-D-glucopyranose (**9**), and removal of the anomeric acetyl group with hydrazine acetate[26] afforded 2-*O*-acetyl-4,6-*O*-(*S*)-(1-methoxycarbonylethylidene)-3-*O*-methyl-D-glucopyranose (**10**). This was then transformed into the trichloroacetimidate donor **11** using the procedure described by Schmidt[27].

The preparation of the suitably protected crystalline disaccharide glycosyl acceptor **15** and its use in the synthesis of complex oligosaccharides have been reported previously[28,29]. The synthesis of **15** was slightly modified in that a new rhamnosyl donor, ethyl 3-*O*-acetyl-2,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**14**) was applied. For the preparation of this new donor, we utilized our earlier observation that phase-transfer alkylation of thioglycosides at position 2 proceeds more easily and with better selectivity than the alkylation of *O*-glycosides. In the present case, ethyl 4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**12**) was benzylated under phase-transfer catalysis conditions to produce ethyl 2,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**13**) in a yield of 64%. Conventional acetylation of **13** then gave the rhamnosyl donor **14**. Glycosylation of *p*-nitrophenyl *endo*-3,4-*O*-benzylidene-α-L-talopyranoside[29] with the donor **14** and subsequent deacetylation of O-3 of the prepared disaccharide, using NaOMe in MeOH, then yielded the crystalline **15**.

Glycosylation of the "core" disaccharide acceptor **15** with **11** in the presence of trimethylsilyl trifluoromethanesulfonate promoter at −50°C furnished the trisac-



charide **16** (42%). The β configuration of the newly formed glycosidic linkage was proved by careful analysis of the ^1H and ^{13}C NMR spectra of **16**, which also revealed that, fortunately, no isomerization at the acetalic carbons occurred during the glycosylation.

The *p*-nitrophenyl aglycon of the trisaccharide **16** was converted into *p*-trifluoroacetamidophenyl (→ **17**) by means of hydrogenation and subsequent treatment with trifluoroacetic anhydride. In accordance with earlier observations[23], removal of the 2-OAc group from **17** required vigorous conditions. The benzyl and the benzylidene groups were removed conventionally (H_2/10% Pd–C) to yield the unprotected, spacer-armed trisaccharide **19**. The ^{13}C NMR spectrum of **19** was well resolved, but the complete assignment of the ^1H NMR spectrum required various 1D and 2D techniques (DQ-COSY, single and double $^1H,^1H$ RELAY, H, C COSY, and 1D NOE). The ^1H and ^{13}C NMR data are shown in Table I.

The preparation of neoglycoprotein-type artificial antigens from *p*-trifluoroacetamidophenyl glycosides is well documented[30].

EXPERIMENTAL

General methods.—Melting points were determined on a Kofler apparatus. Optical rotations were measured in $CHCl_3$ at 20°C with a Perkin–Elmer 241

TABLE I

^{13}C and ^{1}H NMR data (chemical shifts in ppm) for trisaccharide **19**

Atom	Chemical shift	
	^{13}C	^{1}H
1	99.34	5.60
2	78.56	4.00
3	73.60	3.63
4	67.35	4.08
5	69.24	3.76
6(CH_3)	16.74	1.22
1′	104.28	5.02
2′	71.58	4.15
2′	82.14	3.81
4′	72.48	3.58
5′	70.64	3.77
6′(CH_3)	18.00	1.25
1″	106.21	4.68
2″	75.12	3.30–
3″	83.61	
4″	78.38	
5″	66.93	–3.45
6″a,b	66.19	4.03–4.11

polarimeter. Reactions were monitored by TLC on Kieselgel 60 F_{254} (Merck) with *A*, 8:2 hexane–EtOAc; and *B*, 7:3 hexane–EtOAc; and with detection by UV light and/or by charring with aq 50% H_2SO_4. Column chromatography was performed on Kieselgel 60 (Merck, 70–230 mesh). All solvents were distilled from an appropriate drying agent. Evaporations were performed under reduced pressure at 40°C. The ^{1}H (200 MHz) and ^{13}C NMR (50.3 MHz) spectra were recorded with a Bruker WP-200 SY spectrometer for solutions in $CDCl_3$ (internal Me_4Si).

Methyl 2,2-di(ethylthio)propionate (**1**).—Ethanethiol (16 mL) was added dropwise to chilled methyl pyruvate (10 mL), then HCl was bubbled through the mixture for 1 h. The reaction was monitored by GLC. The mixture was poured onto ice, neutralized with satd aq $NaHCO_3$, and extracted with CH_2Cl_2 (3 × 150 mL). The organic layers were combined and washed with water (3 × 250 mL), dried, then concentrated in vacuo. The yellow crude product was purified by distillation under diminished pressure, under Ar, to give **1** (16 g, 89%) as a colourless liquid, bp 110°C/8 mmHg; ^{1}H NMR: δ 3.78 (s, 3 H, $COOCH_3$), 2.70 (dd, 4 H, SCH_2CH_3), 1.81 (s, 3 H, CH_3), 1.13 (t, 6 H, SCH_2CH_3). Anal. Calcd for $C_8H_{16}O_2S_2$: C, 46.13; H, 7.74; S, 30.76. Found: C, 46.09; H, 7.71; S, 30.80.

Benzyl 2-O-acetyl-3-O-methyl-β-D-glucopyranoside (**2**).—Benzyl 2,4,6-tri-*O*-acetyl-3-*O*-methyl-β-D-glucopyranoside[24] (2.0 g, 4.5 mmol) was dissolved in MeOH (50 mL), and NaOMe (20 mg) was added. The solution was stirred for 5 h at room temperature, then neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered off, the filtrate was concentrated in vacuo, and the crude syrup was

purified on a column of silica gel (9:1 CH_2Cl_2–acetone), to give **2** (670 mg, 42%); mp 90–92°C; $[\alpha]_D$ +158.7° (*c* 0.46, MeOH); ^{1}H NMR: δ 7.40–7.31 (m, 5 H, Ph), 5.02 (t, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 9 Hz, H-2), 4.56 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 3.62 (s, 3 H, OCH_3), 2.70 and 2.10 (2 bs, each 1 H, HO-4,6). Anal. Calcd for $C_{16}H_{22}O_7$: C, 58.88; H, 6.79. Found: C, 58.87; H, 6.79.

Benzyl 4,6-O-isopropylidene-3-O-methyl-β-D-glucopyranoside (**4**).—To a stirred suspension of benzyl 3-*O*-methyl-β-D-glucopyranoside (**3**; 4.5 g, 16 mmol) in 2,2-dimethoxypropane (20 mL) was added *p*-toluenesulfonic acid (100 mg). The reaction was monitored by TLC (*A*), and after 1 h the mixture was diluted with CH_2Cl_2, washed with aq $NaHCO_3$, dried, and concentrated in vacuo to yield **4** (5 g, 97%); R_f 0.4 (*A*); $[\alpha]_D$ −64.0° (*c* 0.4); ^{1}H NMR: δ 7.35 (s, 5 H, Ph), 4.92 and 4.63 (d, 2 H, PhCH_2), 4.44 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.95 (dd, 1 H, $J_{6a,6b}$ 10.5, $J_{6a,5}$ 5.5 Hz, H-6a), 3.80 (t, 1 H, $J_{3,4} = J_{4,5} = 10$ Hz, H-4), 3.67 (t, 1 H, $J_{6a,6b} = J_{6b,5} = 10.5$ Hz, H-6b), 3.60 (s, 3 H, OCH_3), 3.50 (dt, 1 H, $J_{1,2} = J_{2,3} = 8$, $J_{2,OH}$ 2 Hz, H-2), 3.25 (m, 2 H, H-5,3), 2.55 (bs, 1 H, OH), 1.51 and 1.42 [2 s, 6 H, C(CH_3)$_2$]. Anal. Calcd for $C_{17}H_{24}O_6$: C, 62.95; H, 7.46. Found: C, 63.08; H, 7.47.

Benzyl 2-O-benzyl-4,6-O-isopropylidene-3-O-methyl-β-D-glucopyranoside (**5**).—To a stirred suspension of **4** (966 mg, 3 mmol) and KOH (3 g) in *N*,*N*-dimethylformamide (10 mL) was added benzyl chloride (411 μL, 1.2 equiv). The mixture was stirred for 10 min at room temperature, then diluted with EtOAc, washed with water until neutral, and concentrated in vacuo to give **5** (1.17 g, 94%); R_f 0.62 (*A*); $[\alpha]_D$ −45.5° (*c* 0.59); ^{1}H NMR: δ 7.30 (m, 10 H, 2 Ph), 4.92, 4.87, 4.72 and 4.64 (2 d, each 1 H, PhCH_2), 4.55 (d, 1 H, $J_{1,2}$ 7 Hz, H-1), 3.95 (dd, 1 H, $J_{6a,6b}$ 11, $J_{6a,5}$ 5.5 Hz, H-6a), 3.79 (t, 1 H, $J_{6a,6b}$ 11, $J_{6b,5}$ 10.5 Hz, H-6b), 3.62 (t, 1 H, $J_{3,4} = J_{4,5} = 10$ Hz, H-4), 3.58 (s, 3 H, OCH_3), 3.35 (m, 2 H, H-2,3), 3.21 (dt, 1 H, $J_{4,5}$ 10, $J_{5,6b}$ 10.5, $J_{5,6a}$ 5.5 Hz, H-5), 1.5 and 1.41 [2 s, 6 H, C(CH_3)$_2$]. Anal. Calcd for $C_{24}H_{30}O_6$: C, 69.55; H, 7.30. Found: C, 69.02; H, 7.22.

Benzyl 2-O-benzyl-3-O-methyl-β-D-glucopyranoside (**6**).—A solution of **5** (1.0 g, 2.4 mmol) in aq AcOH (70%, 15 mL) was kept at 50°C for 2 h, then concentrated in vacuo. The crystalline crude product (0.9 g) was recrystallized from cyclohexane to yield **6** (780 mg, 87%); R_f 0.07 (*A*); mp 84–85°C; $[\alpha]_D$ −42.0° (*c* 0.71); ^{1}H NMR: δ 7.32 (m, 10 H, 2 Ph), 4.97, 4.90, 4.71 and 4.67 (2 d, each 1 H, PhCH_2), 4.57 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.92 (dd, 1 H, $J_{6a,6b}$ 11, $J_{6a,5}$ 3 Hz, H-6a), 3.78 (dd, 1 H, $J_{6a,6b}$ 11, $J_{6b,5}$ 5 Hz, H-6b), 3.62 (s, 3 H, OCH_3), 3.53 (t, 1 H, $J_{4,5} = J_{3,4} = 10$ Hz, H-4), 3.37 (m, 2 H, H-2,5), 3.20 (t, 1 H, $J_{2,3} = J_{3,4} = 10$ Hz, H-3), 2.7 (bs, 1 H, OH), 2.11 (bs, 1 H, OH). Anal. Calcd for $C_{21}H_{26}O_6$: C, 67.36; H, 7.0. Found: C, 67.30; H, 6.92.

Benzyl 2-O-benzyl-4,6-O-(1-methoxycarbonylethylidene)-3-O-methyl-β-D-glucopyranoside (**7**).—To a solution of **6** (500 mg, 1.4 mmol) in dry CH_2Cl_2 (7 mL) were added **1** (375 mg, 1.8 mmol) and activated 4A molecular sieves (500 mg), and the suspension was stirred under Ar for 40 min at room temperature. After cooling to −78°C, 1:1 CF_3SO_3H–SO_2Cl_2 (4 mL; 1 M in 7:3 toluene–diethyl ether) was added in one portion, and the mixture was allowed to warm up to room tempera-

ture. After 3 h, the mixture was neutralized by addition of satd aq $NaHCO_3$, then diluted with EtOAc (50 mL), and the organic layer was washed with water (3 × 20 mL), dried, and concentrated in vacuo. The resulting crude syrup was purified by column chromatography using 85:15 hexane–EtOAc as eluant. Yield: **7S**, 283 mg (45%); **7R**, 152 mg (24%).

7S: $[\alpha]_D$ −23.9° (*c* 0.88); ^{1}H NMR: δ 7.3 (bs, 10 H, 2 Ph), 4.9, 4.83, 4.72, and 4.62 (2 d, each 1 H, PhCH_2), 4.56 (d, 1 H, $J_{1,2}$ 7 Hz, H-1), 4.08 (dd, 1 H, $J_{6a,6b}$ 11, $J_{6a,5}$ 5 Hz, H-6a), 3.81 (s, 3 H, $COOCH_3$), 3.71 (t, 1 H, $J_{6a,6b} = J_{6b,5} = 10$ Hz, H-6b), 3.62 (s, 3 H, OCH_3), 3.4 (m, 4 H, H-2,3,4,5), 1.55 (s, 3 H, CH_3); ^{13}C NMR: δ 169.79 ($COOCH_3$), 102.47 (C-1), 98.70 (acetalic C), 25.15 (C-CH_3). Anal. Calcd for $C_{25}H_{30}O_8$: C, 65.49; H, 6.59. Found: C, 64.99; H, 6.46.

4,6-O-(S)-(1-Methoxycarbonylethylidene)-3-O-methyl-D-glucopyranose (**8**).—To a solution of **7** (1.0 g, 2.2 mmol) in EtOH (100 mL) was added Pd–C (10%, 100 mg), and the mixture was vigorously stirred under H_2 overnight at room temperature. The catalyst was filtered off, the cake was washed with EtOH (2 × 20 mL), and the washings were combined and concentrated in vacuo, to yield **8** (600 mg, 96%); R_f 0.3 (75:25 CH_2Cl_2–acetone); $[\alpha]_D$ +12.5° (*c* 0.65, MeOH); ^{1}H NMR: δ 5.21 (d, 0.5 H, $J_{1,2}$ 3 Hz, H-1α), 4.68 (d, 0.5 H, $J_{1,2}$ 8 Hz, H-1β), 4.99, 4.20, 3.18 and 2.20 (4 bs, each 0.5 H, 2 HO$\alpha + \beta$), 3.84 (s, 3 H, $COOCH_3$), 3.65 (s, 3 H, OCH_3), 1.54 (s, 3 H, CH_3). Anal. Calcd for $C_{11}H_{18}O_8$: C, 47.48; H, 6.52. Found: C, 47.11; H, 6.33.

1,2-Di-O-acetyl-4,6-O-(S)-(1-methoxycarbonylethylidene)-3-O-methyl-D-glucopyranose (**9**).—Compound **8** (600 mg, 2.1 mmol) was acetylated by using the standard pyridine (20 mL)–Ac_2O (2 mL) procedure, and the crude product was purified on a short column of silica gel (7:3 hexane–EtOAc) to yield **9** (750 mg, 96%); R_f 0.38 (*B*); mp 62–63°C; $[\alpha]_D$ +61.2° (*c* 0.74); ^{1}H NMR: δ 6.20 (d, 0.5 H, $J_{1,2}$ 4 Hz, H-1α), 5.66 (d, 0.5 H, $J_{1,2}$ 8 Hz, H-1β), 3.84 and 3.86 (s, 3 H, $COOCH_3$), 3.55 and 3.59 (s, 3 H, OCH_3), 2.18, 2.09, 2.07 and 2.06 (4 s, 6 H, OAc), 1.54 and 1.56 (2 s, 3 H, CH_3). Anal. Calcd for $C_{15}H_{22}O_{10}$: C, 49.72; H, 6.12. Found: C, 49.75; H, 6.10.

2-O-Acetyl-4,6-O-(S)-(1-methoxycarbonylethylidene)-3-O-methyl-D-glucopyranose (**10**).—A solution of **9** (600 mg, 1.7 mmol) in dry *N,N*-dimethylformamide (10 mL) was cooled to 0°C, then hydrazine acetate (200 mg) was added. The mixture was stirred for 6 h at 0°C, diluted with EtOAc (100 mL), washed with satd aq NaCl (2 × 20 mL), dried, and concentrated in vacuo, and the resulting syrup was chromatographed on silica gel, using eluant *B*, to yield **10** (360 mg, 67%) and **9** (100 mg).

10: R_f 0.17 (*B*); $[\alpha]_D$ +68.2° (*c* 1.2); ^{1}H NMR: δ 5.37 (t, 0.5 H, $J_{1,2} = J_{1,OH} = 3.5$ Hz, H-1α), 3.85 (s, 3 H, $COOCH_3$), 3.57 and 3.59 (s, 3 H, OCH_3), 3.05 (d, 0.5 H, $J_{1,OH}$ 3.5 Hz, OHα), 2.15 and 2.17 (s, 3 H, OAc), 1.55 and 1.56 (s, 3 H, CH_3). Anal. Calcd for $C_{13}H_{20}O_9$: C, 48.75; H, 6.29. Found: C, 48.70; H, 6.12.

2-O-acetyl-4,6-O-(S)-(1-methoxycarbonylethylidene)-3-O-methyl-α-D-glucopyranosyl trichloroacetimidate (**11**).—Compound **10** (200 mg, 0.62 mmol) was dissolved in dry *N,N*-dimethylformamide (7 mL), then trichloroacetonitrile (1.3

mL) and DBU (310 μL) were added to this solution. The mixture was stirred at room temperature for 10 min, then diluted with EtOAc (30 mL), washed with satd aq NaCl (3 × 20 mL), dried, and concentrated. The brown syrup was purified by column chromatography (*A*), to give **11** (190 mg, 66%) as a yellowish syrup, R_f 0.45 (*B*). The compound was immediately used for coupling.

Ethyl 2,4-di-O-benzyl-1-thio-α-L-rhamnopyranoside (**13**).—To a solution of ethyl 4-*O*-benzyl-1-thio-α-L-rhamnopyranoside[31] (**12**; 2.45 g, 5.2 mmol) in CH_2Cl_2 (15 mL) were added tetrabutylammonium bromide (662 mg), benzyl bromide (1.5 mL, 1.5 equiv), and aq 20% NaOH (15 mL), and the mixture was vigorously stirred at room temperature for 12 h. It was then diluted with CH_2Cl_2, and the organic layer was washed with water, dried, and concentrated. Column chromatography (eluant *A*) of the crude product yielded amorphous **13** (2.05 g, 64%); $[\alpha]_D$ −91.3° (*c* 0.64); ^{1}H NMR: δ 7.30 (m, 10 H, 2 Ph), 5.33 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.90–4.50 (m, 4 H, PhCH_2), 4.04 (dd, 1 H, $J_{4,5}$ 10, $J_{5,6a}$ 6 Hz, H-5), 3.91 (dt, 1 H, $J_{3,4} = J_{3,OH} = 10$, $J_{2,3}$ 4 Hz, H-3), 3.82 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 4 Hz, H-2), 3.36 (t, 1 H, $J_{3,4} = J_{4,5} = 10$ Hz, H-4), 2.55 (m, 2 H, CH_2), 2.40 (d, 1 H, $J_{3,OH}$ 10 Hz, OH), 1.3 (m, 6 H, 2 CH_3). Anal. Calcd for $C_{22}H_{28}O_4S$: C, 68.01; H, 7.26. Found: C, 67.56; H, 7.20.

Ethyl 3-O-acetyl-2,4-di-O-benzyl-1-thio-α-L-rhamnopyranoside (**14**).—Conventional acetylation of **13** (1.58 g, 4.07 mmol) with Ac_2O (3 mL) in pyridine (30 mL) and subsequent chromatographic purification (eluant *A*) gave **14** (1.70 g, 97%); $[\alpha]_D$ −56.7° (*c* 1.70); ^{1}H NMR: δ 7.30 (m, 10 H, 2 Ph), 5.25 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), 5.12 (dd, 1 H, $J_{2,3}$ 3, $J_{3,4}$ 10 Hz, H-3), 4.70–4.50 (m, 4 H, PhCH_2), 4.11 (dd, 1 H, $J_{4,5}$ 10, $J_{5,6}$ 6 Hz, H-5), 3.96 (dd, 1 H, $J_{1,2}$ 2, $J_{2,3}$ 3 Hz, H-2), 3.65 (t, 1 H, $J_{3,4} = J_{4,5} = 10$ Hz, H-4), 2.55 (m, 2 H, CH_2), 1.91 (s, 3 H, acetyl CH_3), 1.3–1.10 (m, 6 H, 2 CH_3). Anal. Calcd for $C_{24}H_{30}O_5S$: C, 66.95; H, 7.02. Found: C, 66.72; H, 6.93.

p-*Nitrophenyl* endo-*3,4-O-benzylidene-6-deoxy-2-O-(2,4-di-O-benzyl-α-L-rhamnopyranosyl)-α-L-talopyranoside* (**15**).—A solution of **14** (900 mg, 2.1 mmol) and *p*-nitrophenyl *endo*-3,4-*O*-benzylidene-6-deoxy-α-L-talopyranoside[29] (1.1 g, 2.6 mmol) in dry CH_2Cl_2 (20 mL) containing 4A molecular sieves (3 g) was stirred for 30 min under Ar, then methyl triflate (1.6 mL, 14.7 mmol) was added. After 1.5 h, pyridine was injected, and the mixture was filtered through Celite, concentrated, and co-concentrated with toluene (2 × 20 mL). Column chromatography (eluant *B*) of the crude product gave *p*-nitrophenyl 2-*O*-(3-*O*-acetyl-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-*endo*-3,4-*O*-benzylidene-6-deoxy-α-L-talopyranoside (1.16 g, 72%) which was *O*-deacetylated with NaOMe (100 mg) in MeOH (20 mL) to yield **15** (900 mg, 92%). For physical data, see ref 28.

p-*Nitrophenyl 2-O-{3-O-[2-O-acetyl-4,6-O-(S)-(1-methoxycarbonylethylidene)-3-O-methyl-β-D-glucopyranosyl]-2,4-di-O-benzyl-α-L-rhamnopyranosyl}*-endo-*3,4-O-benzylidene-6-deoxy-α-L-talopyranoside* (**16**).—A mixture of **11** (150 mg, 0.32 mmol), the disaccharide **15** (250 mg, 0.34 mmol), and 4A molecular sieves (500 mg) in dry CH_2Cl_2 (10 mL) was stirred under Ar at room temperature for 30 min. It was then cooled to −40°C, $Me_3SiOSO_2CF_3$ (30 μL in 2 mL of CH_2Cl_2) was added

dropwise, and, after 10 min, the reaction was quenched by the addition of 1 mL of dry pyridine. The mixture was diluted with CH_2Cl_2 (50 mL), filtered, washed with water (2 × 20 mL), dried, and evaporated. The crude product was purified on a silica gel column (75:25 hexane–EtOAc) to afford **16** as a colourless syrup (131 mg, 42%); R_f 0.54 (6:4 hexane–EtOAc); $[\alpha]_D$ −82.9° (*c* 0.38); ^{1}H NMR: δ 8.20 and 7.10 (2 bd, 4 H, *p*-nitrophenyl) 7.70–7.20 (m, 15 H, 3 Ph), 5.82 (s, 1 H, PhC*H endo*), 5.60 (d, 1 H, $J_{1,2}$ 6.5 Hz, H-1), 4.97 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 9 Hz, H-2″), 4.88 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1′), 4.80–4.50 (PhCH_2), 4.69 (d, 1 H, $J_{1,2}$ 8 Hz, H-1″), 3.81 (s, 3 H, $COOCH_3$), 3.48 (s, 3 H, OCH_3), 1.83 (s, 3 H, OAc), 1.50 (s, 3 H, pyruvyl CH_3), 1.25 (bd, 6 H, CH_3). Anal. Calcd for $C_{52}H_{59}NO_{19}$: C, 62.33; H, 5.93; N, 1.40. Found: C, 61.85; H, 5.86.

p-*Trifluoroacetamidophenyl 2-*O*-{3-*O*-[2-*O*-acetyl-4,6-*O*-(*S*)-(1-methoxycarbonylethylidene)-3-*O*-methyl-β-*D*-glucopyranosyl]-2,4-di-*O*-benzyl-α-*L*-rhamnopyranosyl}*-endo*-3,4-*O*-benzylidene-6-deoxy-α-*L*-talopyranoside* (**17**).—A solution of **16** (80 mg, 0.08 mmol) in EtOAc (10 mL) was vigorously stirred under H_2 and in the presence of Adam's catalyst (10 mg), at room temperature for 2 h. Pyridine (200 μL) and trifluoroacetic anhydride (150 μL) were added consecutively to the mixture, and stirring was continued for an additional 1 h. The catalyst was filtered off, the cake was washed with EtOAc (2 × 10 mL), and the filtrate was concentrated and then purified by column chromatography (99:1 CH_2Cl_2–EtOAc) to give **17** as a colourless syrup (80 mg, 94%); R_f 0.68 (92:8 CH_2Cl_2–EtOAc); $[\alpha]_D$ −76.31° (*c* 0.11); ^{1}H NMR: δ 7.30 and 7.10 (2 d, 4 H, *p*-trifluoroacetamidophenyl), 7.70–7.3 (m, 15 H, 3 Ph), 5.78 (s, 1 H, PhC*H endo*), 5.46 (d, 1 H, $J_{1,2}$ 6.5 Hz, H-1), 4.96 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 9 Hz, H-2″), 4.91 (d, 1 H, $J_{1,2}$ 2 Hz, H-1′), 4.73 (d, 1 H, $J_{1,2}$ 8 Hz, H-1″), 3.81 (s, 3 H, $COOCH_3$), 3.48 (s, 3 H, OCH_3), 1.86 (s, 3 H, OAc), 1.49 (s, 3 H, pyruvyl CH_3), 1.30 (bd, 6 H, CH_3). Anal. Calcd for $C_{54}H_{60}NF_3O_{18}$: C, 60.72; H, 5.66. Found: C, 60.11; H, 5.38.

p-*Trifluoroacetamidophenyl* endo-*3,4-*O*-benzylidene-6-deoxy-2-*O*-{2,4-di-*O*-benzyl-3-*O*-[4,6-*O*-(*S*)-(1-methoxycarbonylethylidene)-3-*O*-methyl-β-*D*-glucopyranosyl]-α-*L*-rhamnopyranosyl}-α-*L*-talopyranoside* (**18**).—Sodium methoxide (30 mg) was added to a solution of **17** (80 mg, 0.07 mmol) in MeOH (5 mL) and the mixture was stirred for 5 h. After neutralization (Amberlite IR-120; H^+) and concentration of the mixture, the crude syrup was purified on a short column of silica gel (8:2 CH_2Cl_2–EtOAc) to give **18** as a colourless syrup (42 mg, 58%). The product was applied for the next step without characterisation.

p-*Trifluoroacetamidophenyl 6-deoxy-2-*O*-{3-*O*-[4,6-*O*-(*S*)-(1-methoxycarbonylethylidene)-3-*O*-methyl-β-*D*-glucopyranosyl]-α-*L*-rhamnopyranosyl}-α-*L*-talopyranoside* (**19**).—To a solution of **18** (40 mg, 0.04 mmol) in MeOH (10 mL) was added Pd–C catalyst (10%, 20 mg), and the suspension was vigorously stirred under H_2 for 4 h. The catalyst was filtered off, the filtrate was concentrated in vacuo, and the crude material was purified on a short column of silica gel (99:1 CH_2Cl_2–MeOH) to furnish **19** as a colourless glass (25 mg, 83%); R_f 0.45 (92:8 CH_2Cl_2–MeOH);

$[\alpha]_D$ −77.6° (*c* 0.22, MeOH). Anal. Calcd for $C_{31}H_{42}NF_3O_{17}$: C, 49.14; H, 5.59; N, 1.86; F, 7.56. Found: C, 49.32; H, 5.23.

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