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Synthesis of the Mannopeptimycin Disaccharide and Its Conjugation with 4-Alkylidene-β-lactams

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The disaccharide moiety of the antibiotic mannopeptimycin has been synthesized as a (*N*-phenyl)trifluoroacetimidate donor, the reactivity of which was tested in conjugation with a 4-alkylidene-β-lactam acceptor. Key steps of the synthesis were $Bi(OTf)_3$-catalyzed glycosidations that proceeded in short times and with high yields and stereocontrol.

Introduction

Mannopeptimycins constitute a new class of glycopeptide antibiotics produced by *Streptomyces hygroscopicus* and they have proved active against some methicillin- and vancomycin-resistant bacteria.[1] The most active member of the family is represented by mannopeptimycin ε (Figure 1), the structure of which includes a cyclic hexapeptide with a *N*-linked mannose residue (common core for all mannopeptimycins) and a disaccharide moiety appended to a phenolic tyrosine residue. The disaccharide was found to be fundamental for the bioactivity and is made up of a 4-*O*-α-linked dimannosyl fragment bearing an isovaleryl group at the 4-OH of the outer terminus. The presence of this acyl substituent and its attachment site are both decisive for the antibacterial activity, other regioisomers being markedly less active.

Recently, we undertook a research program directed towards the synthesis of novel glycoconjugates of 4-alkylidene-β-lactam derivatives (such as the model compound **1**, Figure 2),[2] the glycoconjugation being a feasible option for attempting the achievement of improved biological performance.

Some synthetic members of this class of β-lactams[3] have been found to exhibit appreciable activity as antibiotics against resistant strains[4] and as inhibitors of human leukocyte elastase and matrix metalloproteases.[5] Besides the typical general advantages often associated with the attachment of sugars to bioactive molecules (improved solubility, stability, pharamacokinetic properties etc.),[6] a potential

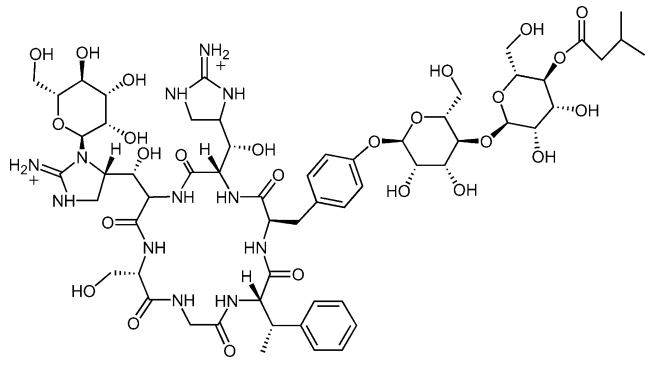

Figure 1. Structure of mannopeptimycin ε.

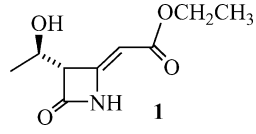

Figure 2. Structure of model 4-alkylidene-β-lactam **1**.

antibiotic may also benefit functionally from glycoconjugation, as evidenced by the presence of saccharidic moieties in several important active molecules such as vancomycin, teicoplanin, ramoplanin, moenomycin, mannopeptimycin itself etc.[7] Interestingly, it has been proposed that in some vancomycin analogues the saccharidic and non-saccharidic moieties may be involved at different stages in the inhibition of the peptidoglycan biosynthesis.[8] This view is strongly supported by the observation that in some cases the co-administration of the two unbound moieties results in equally efficient activity.[9] More generally, the functional duality of several antibiotics in inhibiting diverse stages of peptidoglycan biosynthesis is supported by a growing number of examples.[10] These considerations led us to evaluate the synthesis of alkylidene-β-lactams connected to sac-

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charidic moieties present in natural antibiotics, the feasible glycoconjugation of alkylidene-β-lactams having been demonstrated in a preliminary communication.[2] The mannose disaccharide of mannopeptimycin ε was considered an interesting target, since only a few reports concerning synthetic studies have appeared to date. Indeed, only one example of a synthesis of this disaccharide moiety has been published,[11] in a recent de novo approach based on a specifically prepared pyranone precursor[12] and requiring the en route construction of some stereogenic centres. On the other hand, several intermediates of this synthesis were usefully elaborated to provide structural analogues.[11]

Results and Discussion

Here we propose a straightforward alternative approach based on a single mannose precursor for the synthesis of both components of the target disaccharide. Both mannose residues are functionalized at 4-OH, so compound **2** (Figure 3) could itself serve both as an appropriate glycosyl acceptor at its 4-OH for the key glycosidation step and as a suitable precursor of the glycosyl donor **3**.

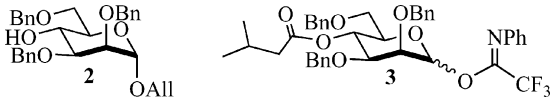


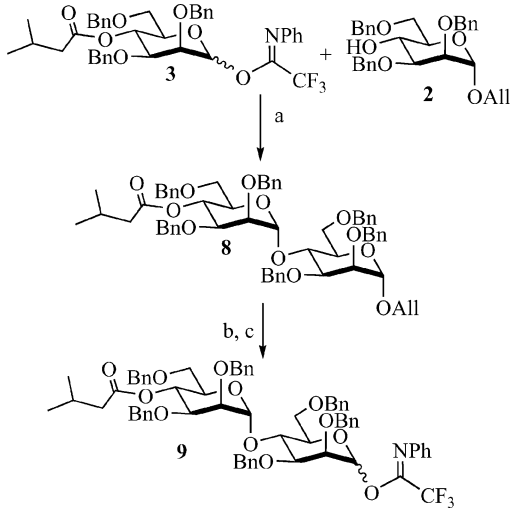
Figure 3. Precursors of the mannopeptimycine ε disaccharide.

The allyl system was chosen as the anomeric protecting group of **2**, to be selectively removable for the introduction of the (*N*-phenyl)trifluoroacetimidate leaving functionality.[13] Acceptor **2** was prepared by a known initial sequence (Fischer anomeric allylation and selective installation of a six-membered benzylidene) starting from D-mannose (Scheme 1).[14]

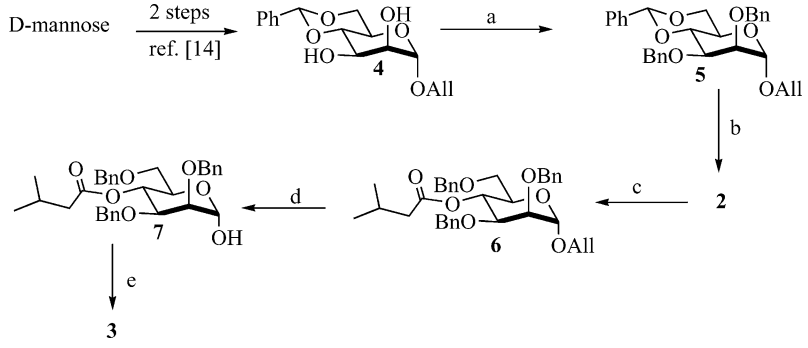
The resulting diol **4** was perbenzylated and then subjected to the regioselective reductive opening of the benzylidene ring. The latter step was not trivial, as several known methodologies such as Et_3SiH/trifluoroacetic acid,[15] Et_3SiH/copper(II) triflate[16] and $BH_3{\cdot}Et_3N/BF_3{\cdot}OEt_2$[17] afforded disappointing results. Acceptable yield (59%) and regiocontrol were eventually achieved with the combined $Et_3SiH/BF_3{\cdot}OEt_2$ system.[18]

An aliquot of **2** was then further elaborated to prepare donor **3**. The isovaleryl group was smoothly attached onto the 4-OH to furnish compound **6** in high yield. The downfield ^1H NMR shift of the 4-H (δ = 5.46 ppm, triplet, J = 10.0 Hz) confirmed the acylation at the desired site. Compound **6** was then deallylated with catalytic $PdCl_2$, and the crude compound **7**, after a simple filtration, was directly subjected to a final trifluoroacetimidoylation to yield donor **3** in 83% yield (from **6**).

The subsequent key coupling step was performed by taking advantage of $Bi(OTf)_3$-promoted activation of the trifluoroacetimidate donor, by a protocol we had recently reported (Scheme 2).[19] Consistently with that report, the reaction proceeded at low temperature to yield the target disaccharide **8** in high yield despite the sterically encum-



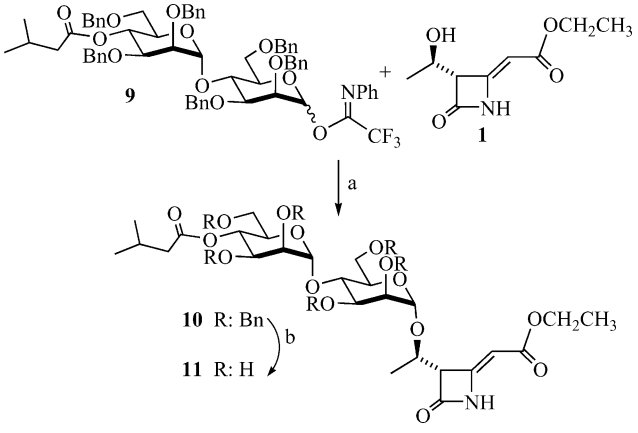
Scheme 2. Synthesis of the disaccharide donor of mannopeptimycin ε: Reagents and conditions: a) PhMe/1,2-DME (2:1), $Bi(OTf)_3$ (cat.) in dioxane, –60 °C to 0 °C, 73%; b) MeOH, $PdCl_2$ (cat.); c) DCM, $CF_3C(=NPh)Cl$, NaH, –10 °C to room temp., 75% (two steps).



Scheme 1. Synthesis of acceptor **2** and donor **3**. Reagents and conditions: a) DMF, NaH, BnBr, 0 °C to room temp., 88%; b) DCE, Et_3SiH, $BF_3{\cdot}OEt_2$, 0 °C to room temp., 59%; c) pyridine, isovaleryl chloride, 0 °C, 91%; d) MeOH, $PdCl_2$ (cat.); e) DCM, $CF_3C(=NPh)Cl$, NaH, –10 °C to room temp., 83% (two steps).

bered nucleophilic site of the acceptor. α-Selectivity was achieved, as expected, with a solvent mixture containing 1,2-dimethoxyehane[20] and without resorting to a donor bearing a 2-*O* participating acyl group, the installation of which would both have lengthened the overall synthetic sequence and have generated the nontrivial problem of its selective removal in the presence of the 4-*O*-isovaleryl group.

The desired disaccharide **8** was readily converted (Scheme 2) by the previously adopted sequence of anomeric deallylation and trifluoroacetimidoylation to afford donor **9** in a 75% overall yield after a single chromatographic purification. The reactivity of **9** was tested in its coupling with the β-lactam acceptor **1** to yield the desired conjugate **10** at very low temperature and in very short times (Scheme 3).



Scheme 3. Reagents and conditions: a) PhMe/1,2-DME (2:1), $Bi(OTf)_3$ (cat.) in dioxane, –70 °C to –55 °C, 76 –79%; b) MeOH/HCOOH (9:1), C/Pd, ultrasonication.

Indeed, the activation with catalytic $Bi(OTf)_3$ once more guaranteed a fast and efficient glycosidation with α-selectivity at low temperatures (less than one hour at –70 → –55 °C). It should be noted that the previously reported glycosidations of **1** with perbenzylated (*N*-phenyl)trifluoroacetimidate donors took several hours at room temperature under the agency of ytterbium triflate(III).[2] With both bismuth and ytterbium triflate the sensitive β-lactam ring proved stable, and the unreacted acceptor was recovered unaltered. Compound **10** was debenzylated by transfer hydrogenolysis under ultrasonic activation conditions[21] to yield the desired compound **11**. The outcome of this deprotection was critically determined by the amounts of palladium on charcoal employed. With high amounts of catalyst (more than 5 mg per mg of substrate) the deprotection was accompanied by reduction of the β-lactam exocyclic double bond and some migration of the isovaleryl group to the 6-OH. Debenzylation under hydrogen gave similar results. Transfer hydrogenolysis eventually succeeded through the use of a lower catalyst load (ca. 1 mg per mg of substrate) and by minimizing the exposure of the substrate to the reaction conditions.

Conclusions

We report a rapid synthesis of the disaccharide contained in the antibiotic mannopeptimycin ε in the form of a glycosyl donor. This compound was obtained by exploitation of a common synthetic route for both its residues and by the adoption of an efficient $Bi(OTf)_3$-promoted glycosidation as the key step. In addition, the practical activation of the disaccharide donor was applied for the glycoconjugation of the alkylidene β-lactam **1** to allow assessment of the antibiotic potential of chimeric structures presenting β-lactam scaffolds connected to saccharidic moieties contained in natural antibiotics of current use. Biological evaluation of glycosylated β-lactams is currently under investigation and will be reported in due course.

Experimental Section

General: ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ (internal standard $CHCl_3$ at δ = 7.26 ppm) or D_2O (internal standard HDO at δ = 4.80 ppm). Assignment of proton chemical shifts was based on decoupling experiments. Analytical thin-layer chromatography (TLC) was performed on aluminium plates precoated with 60 F_{254} silica gel as the adsorbent. Column chromatography was performed on Kieselgel 60 (63–200 mesh). Mass spectra were recorded in a reflection positive mode on a MALDI-TOF spectrometer.

Allyl 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (5): Sodium hydride (60% in oil, 97 mg, 2.4 mmol) was added at 0 °C to a solution of **4**[14] (251 mg, 0.81 mmol) and benzyl bromide (385 μL, 3.2 mmol) in DMF (4 mL). Once hydrogen evolution had ended, the mixture was allowed to warm to room temp. After 5 hours methanol (0.5 mL) was added, and after a further 20 minutes the mixture was diluted with dichloromethane. The organic phase was washed with water, dried with sodium sulfate and concentrated. The residue was purified by silica gel flash chromatography (eluent: petroleum ether/ethyl acetate from 95:5 to 8:2) to yield pure **5** (oil, 350 mg, yield 88%).

Compound 5: $[\alpha]_D^{25}$ = +39.4 (c = 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.70–7.20 (benzyl aromatic protons), 6.00–5.80 (m, 1 H, $-CH_2CH{=}CH_2$), 5.75 (s, 1 H, benzylidene nonaromatic CH), 5.31 (br. d, ^3J = 18.6 Hz, 1 H, $-CH_2CH{=}CH_{cis}H_{trans}$), 5.27 (br. d, ^3J = 10.2 Hz, 1 H, $-CH_2CH{=}CH_{cis}H_{trans}$), 4.96 (d, $J_{1,2}$ = 1.5 Hz, 1 H, 1-H), 4.95–4.74 (4 H, benzyl CH_2), 4.44–4.30 (2 H), 4.28–4.20 (br. dd, ^3J = 5.1, ^2J = 12.0 Hz, 1 H, $-CH_aH_bCH{=}CH_2$), 4.11 (dd, $J_{2,3}$ = 3.0, $J_{3,4}$ = 9.9 Hz, 1 H, 3-H), 4.05–3.90 (4 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.5, 137.9, 137.5, 133.3, 128.6–125.9, 117.4, 101.2, 98.3 (C-1), 79.0, 76.3, 76.2, 73.4, 72.9, 68.6, 67.7, 64.1 ppm.

Allyl 2,3,6-Tri-*O*-benzyl-α-D-mannopyranoside (2): Triethylsilane (0.85 mL, 5.4 mmol) and $BF_3{\cdot}OEt_2$ (170 μL, 1.3 mmol) were sequentially added at 0 °C to a solution of **5** (325 mg, 0.67 mmol) in anhydrous 1,2-dichloroethane (5.5 mL). The mixture was allowed to warm to room temp. After 3 hours the reaction was quenched with pyridine, and the solvent was removed under vacuum. Silica gel flash chromatography of the residue (eluent: petroleum ether/ethyl acetate, 85:15) yielded pure **2** (oil, 192 mg, yield 59%).

Compound 2: $[\alpha]_D^{25}$ = +9.0 (c = 1.4, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$): δ = 7.10–7.30 (benzyl aromatic protons), 6.00–5.80 (m, 1 H, $-CH_2CH{=}CH_2$), 5.26 (dq, ^3J = 15.6, ^2J = ^4J = 1.8 Hz, 1 H,

-$CH_2CH{=}CH_{cis}H_{trans}$), 5.19 (dq, ^3J = 10.2, $^2J = {}^4J$ = 1.8 Hz, 1 H, -$CH_2CH{=}CH_{cis}H_{trans}$), 4.96 (d, $J_{1,2}$ = 1.6 Hz, 1 H, 1-H), 4.75–4.59 (6 H, benzyl CH_2), 4.28–3.92 (m, 3 H, -$CH_2CH{=}CH_2$ and 5-H), 3.88–3.70 (5 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 138.1 (×3), 133.7, 128.3–127.5, 117.2, 97.1 (C-1), 79.6, 73.9, 73.4, 72.5, 71.8, 71.5, 70.3, 67.8 ppm. $C_{30}H_{34}O_6$ (490.59): calcd. C 73.45, H 6.99; found C 73.20, H 7.05.

Allyl 2,3,6-Tri-*O*-benzyl-4-*O*-isovaleryl-α-D-mannopyranoside (6): Isovaleryl chloride (45 μL, 0.35 mmol) was added at 0 °C to a solution of **2** (130 mg, 0.27 mmol) in anhydrous pyridine (3 mL). The mixture was stirred for 2 hour, and methanol was then added to quench the reaction. The mixture was concentrated, and the residue was purified by silica gel flash chromatography (eluent: petroleum ether/ethyl acetate, 9:1) to yield pure **6** as an oil (139 mg, yield 91%).

Compound 6 $[\alpha]_D^{25}$ = +23.0 (c = 1.0, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.10–7.30 (benzyl aromatic protons), 6.02–5.80 (m, 1 H, -$CH_2{=}CH$-CH_2), 5.46 (t, $J_{3,4} = J_{4,5}$ = 10.0 Hz, 1 H, 4-H), 5.29 (dq, ^3J = 18.0, $^2J = {}^4J$ = 1.6 Hz, 1 H, -$CH_2CH{=}CH_{cis}H_{trans}$), 5.19 (dq, ^3J = 9.8, $^2J = {}^4J$ = 1.6 Hz, 1 H, -$CH_2CH{=}CH_{cis}H_{trans}$), 4.97 (d, $J_{1,2}$ = 1.6 Hz, 1 H, 1-H), 4.82–4.45 (6 H, benzyl CH_2), 4.30–3.80 (5 H), 3.72–3.58 (m, 2 H, H_2-6), 2.18–2.00 (3 H), 0.96–0.86 (m, 6 H, isovaleryl CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 171.8, 138.1 (×3), 133.6, 128.2–127.4, 117.2, 97.3 (C-1), 77.3, 74.2, 73.3, 72.6, 71.7, 70.6, 69.9, 68.5, 67.9, 43.2, 25.3, 22.3 ppm. $C_{35}H_{42}O_7$ (574.71): calcd. C 73.15, H 7.37; found C 73.04, H 7.48.

2,3,6-Tri-*O*-benzyl-4-*O*-isovaleryl-α-D-mannopyranose (7): $PdCl_2$ (4 mg, 0.02 mmol) was added at room temperature to a solution of **6** (125 mg, 0.22 mmol) in methanol (1 mL). The mixture was kept whilst stirring for 5 hours and was then concentrated under vacuum. The residue was re-suspended in DCM/methanol (19:1), filtered through a short silica gel plug and concentrated under vacuum to yield **7** with a satisfactory purity (TLC, NMR) for direct subjection to the following step. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.10–7.30 (benzyl aromatic protons), 5.46 (t, $J_{3,4} = J_{4,5}$ = 9.8 Hz, 1 H, 4-H), 5.26 (br. s, 1 H, 1-H), 4.80–4.44 (6 H, benzyl CH_2), 4.14 (m, 1 H, 5-H), 3.91 (dd, $J_{2,3}$ = 2.8 Hz, 1 H, 3-H), 3.80 (dd, $J_{1,2}$ = 1.8 Hz, 1 H, 2-H), 3.69–3.44 (m, 2 H, 6-H_2), 2.16–1.96 (m, 3 H, isovaleryl CH_2 and CH), 0.89 and 0.87 (2×d, ^3J = 6.2 Hz, 6 H, isovaleryl methyl groups) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 172.0, 138.1 (×2), 137.4, 128.2–127.3, 92.6 (C-1), 76.7, 74.3, 73.2, 72.6, 71.6, 69.9, 68.6, 43.3, 25.3, 22.3 ppm.

2,3,6-Tri-*O*-benzyl-4-*O*-isovaleryl-D-mannopyranosyl (*N*-Phenyl)trifluoroacetimidate (3): (*N*-Phenyl)trifluoroacetimidoyl chloride (45 μL, 0.35 mmol) and sodium hydride (60% in oil, 12 mg, 0.29 mmol) were sequentially added at –10 °C to a solution of **7** in anhydrous dichloromethane (2 mL). The mixture was then allowed to warm to room temp., and after 4 hours the solvent was removed under vacuum. The residue was purified by column chromatography on neutral aluminium oxide (Brockman grade 2, eluent: petroleum ether/ethyl acetate, 9:1) to yield **3** (anomeric mixture) as an oil (129 mg, yield 83% over two steps).

Compound 3: β/α ca. 1.5:1. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.50–6.70 (aromatic protons), 6.28 (br. s, 1 H, α 1-H), 5.82 (br. s, 1 H, β 1-H), 5.51 (t, $J_{3,4} = J_{4,5}$ = 10.0 Hz, 1 H, β 4-H), 5.45 (t, $J_{3,4} = J_{4,5}$ = 10.0 Hz, 1 H, α 4-H), 4.98–4.44 (α/β benzyl CH_2), 4.16–4.00 (m, α/β 2-H and α 5-H), 3.92–3.56 (m, α/β 3-H and 6-H_2, and β 5-H), 2.20–1.92 (m, isovaleryl CH_2 and CH), 1.00–0.87 (m, isovaleryl methyl groups) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 171.8, 143.3, 137.6 (×2), 137.5, 129.2–127.3, 126.1, 120.6, 119.3, 95.3 (C-1), 78.6, 76.0, 75.4, 73.6, 73.4, 72.7, 72.2, 71.9, 69.6, 69.4, 68.0, 67.6, 43.2, 25.3, 22.3 ppm. $C_{40}H_{42}F_3NO_7$ (705.77): calcd. C 68.07, H 6.00; found C 67.96, H 5.94.

Allyl 2,3,6-Tri-*O*-benzyl-4-*O*-isovaleryl-α-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (8): Donor **3** (58 mg, 0.082 mmol) and acceptor **2** (32 mg, 0.065 mmol) were coevaporated three times in anhydrous toluene and dried under vacuum for one hour. The mixture was then dissolved under argon in toluene/DME (2:1, 1 mL) in the presence of freshly activated 4 Å AW molecular sieves and cooled to – 60 °C. After the system had been stirred for 15 minutes, a solution of $Bi(OTf)_3$ in dioxane (18 mg mL^{-1}, 115 μL, 0.003 mmol) was added. The mixture was warmed up to 0 °C over 50 minutes, and the reaction was then quenched with pyridine. The mixture was filtered through a short plug of silica gel, concentrated and purified by silica gel flash chromatography (eluent: petroleum ether/acetone, 9:1) to yield disaccharide **8** as an oil (47 mg, 73% yield).

Compound 8: $[\alpha]_D^{25}$ = +10.4 (c = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.20–6.95 (aromatic protons), 5.95–5.85 (m, 1 H, -$CH_2CH{=}CH_2$), 5.35 (t, $J_{3,4} = J_{4,5}$ = 10.0 Hz, 1 H, 4′-H), 5.31 (d, $J_{1,2}$ = 2.0 Hz, 1 H, 1′-H), 5.28 (dq, ^3J = 17.0, $^2J = {}^4J$ = 1.5 Hz, 1 H, -$CH_2CH{=}CH_{cis}H_{trans}$), 5.21 (dq, ^3J = 10.5, $^2J = {}^4J$ = 1.5 Hz, 1 H, -$CH_2CH{=}CH_{cis}H_{trans}$), 4.94 (d, $J_{1,2}$ = 2.0 Hz, 1 H, 1-H), 4.70–4.20 (benzyl CH_2), 4.24 (m, 1 H), 4.07 (t, $J_{3,4} = J_{4,5}$ = 10.0 Hz, 1 H, 4-H), 4.00 (m, 1 H), 3.91–3.84 (m, 2 H), 3.84–3.76 (m, 5 H), 3.69 (dd, $J_{2,3}$ = 2.5 Hz, 1 H, 2′-H), 3.49 (dd, $J_{5,6a}$ = 6.5, ^2J = 11.0 Hz, 1 H, 6_a′-H), 3.43 (dd, $J_{5,6b}$ = 3.0 Hz, 1 H, 6_b′-H), 2.20–1.92 (m, 3 H, isovaleryl CH_2 and CH), 0.87–0.83 (2×d, 6 H, isovaleryl methyl groups) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 171.8, 138.7, 138.5, 138.4, 138.2, 138.1, 138.0, 133.8, 128.4–127.2, 117.3, 100.1 (C-1′), 96.0 (C-1), 80.0, 75.2, 75.0, 73.6, 73.5, 73.1, 72.4, 72.1, 71.6, 71.5, 71.4, 71.0, 70.4, 70.0, 68.4, 68.0, 43.4, 25.4, 22.4 ppm. $C_{62}H_{70}O_{12}$ (1007.23): calcd. C 73.93, H 7.01; found C 73.82, H 6.98.

2,3,6-Tri-*O*-benzyl-4-*O*-isovaleryl-α-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranosyl (*N*-Phenyl)trifluoroacetimidate (9): Disaccharide donor **9** (anomeric mixture β/α ca. 8:1, 34 mg, 75% overall yield) was prepared from **8** (40 mg) by a deallylation/trifluoroacetimidoylation sequence analogous to that adopted in the synthesis of **3** from **6**.

Compound 9: β-Anomer. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.50–6.70 (aromatic protons), 5.74 (br. s, 1 H, 1-H), 5.36 (t, $J_{3,4} = J_{4,5}$ = 10.0 Hz, 1 H, 4′-H), 5.32 (d, $J_{1,2}$ = 2.0 Hz, 1 H, 1′-H), 4.96–4.20 (benzyl CH_2), 4.14–4.05 (m, 2 H, 2-H and 4-H), 3.98 (dd, $J_{5,6}$ = 1.6 Hz, ^2J = 11.0 Hz, 1 H, 6-H), 3.95–3.60 (m, 6 H), 3.58–3.40 (m, 2 H, 6′-H_2), 2.20–1.92 m, (3 H, isovaleryl CH_2 and CH), 1.00–0.87 (2×d, 6 H, isovaleryl methyl groups). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 171.8, 143.4, 138.4, 138.3 (×2), 138.2, 137.9, 137.4, 128.7–127.3, 124.3, 120.5, 119.3, 100.0 (C-1′), 95.7 (C-1), 81.7, 75.3, 74.0, 73.7, 73.5, 73.2, 72.2, 71.7, 71.6, 71.4, 70.2, 69.9, 68.4, 43.4, 25.4, 22.4 ppm. $C_{67}H_{70}F_3NO_{12}$ (1138.28): calcd. C 70.70, H 6.20; found C 70.56, H 6.24.

Ethyl ((2*Z*,3*S*)-3-{(1*R*)-1-[2,3,6-Tri-*O*-benzyl-4-*O*-isovaleryl-α-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranosyl-oxy]ethyl}-4-oxoazetidin-2-ylidene)acetate (10): Disaccharide donor **9** (20 mg, 17 μmol) and β-lactam acceptor **2** (6 mg, 30 μmol) were coevaporated three times in anhydrous toluene and dried under vacuum for one hour. The mixture was then dissolved under argon in toluene/DME (2:1, 0.7 mL) in the presence of freshly activated 4 Å AW molecular sieves and cooled to –70 °C. After the system had been stirred for 15 minutes a solution of $Bi(OTf)_3$ in dioxane (17 mg mL^{-1}, 27 μL, 0.7 μmol) was added. The mixture was allowed to warm gradually to –55 °C over 50 minutes, and the reaction was quenched with some drops of pyridine. The mixture was filtered

through a short plug of silica gel, concentrated and purified by silica gel flash chromatography (eluent: 4:1 petroleum ether/ethyl acetate) to yield the glycoconjugate **10** as a foam (16 mg, 79% yield).

Compound 10: $[\alpha]_D^{25}$ = +2.8 (c = 0.75, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 8.44 (br. s, 1 H, –NH), 7.55–6.05 (aromatic protons), 5.35 (t, $J_{3,4} = J_{4,5}$ = 10.0 Hz, 1 H, 4′-H), 5.29 (br. s, 1 H, 1′-H), 5.17 (br. s, 1 H, β-lactam vinyl CH), 5.02 (br. s, 1 H, 1-H), 4.65–4.30 (benzyl CH_2), 4.25–4.18 (m, 2 H, –OCH_2CH_3), 4.16 (m, 1 H, β-lactam -CHCH_3), 4.05–3.95 (2 H), 3.90–3.60 (8 H), 3.55–3.40 (m, 2 H, 6′-CH_2), 2.10–1.95 (m, 3 H, isovaleryl CH_2 and CH), 1.45 (d, ^3J = 6.4 Hz, 3 H, β-lactam -CHCH_3), 1.30 (t, ^3J = 6.4 Hz, 3 H, –OCH_2CH_3), 0.87 and 0.85 (2 × d, ^3J = 6.4 Hz, 6 H, isovaleryl methyl groups) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 171.8, 167.0, 165.4, 152.3, 138.6, 138.5 (×2), 138.4, 138.0 (×2), 128.5–127.3, 100.2 (C-1′), 99.0 (C-1), 90.7, 79.3, 75.2, 75.0, 74.0, 73.5, 73.2, 72.6, 72.4, 72.2, 71.6, 71.1, 70.6, 70.2, 68.6, 62.9, 60.3, 43.4, 25.4, 22.4, 19.9, 14.4 ppm. $C_{68}H_{77}O_{15}$ (1134.35): calcd. C 71.12, H 6.76; found C 70.98, H 6.80.

Ethyl ((2*Z*,3*S*)-3-{(1*R*)-1-[4-*O*-Isovaleryl-α-D-mannopyranosyl-(1→4)-α-D-mannopyranosyloxy]ethyl}-4-oxoazetidin-2-ylidene)-acetate (11): Compound **10** (7 mg) was dissolved with several portions of MeOH/formic acid (9:1, total volume ca. 2.5 mL) and added under argon to a vessel containing palladium on charcoal (10 mg), previously wetted with a few drops of the same solution. The vessel was ultrasonicated for 15 minutes at room temperature, and the mixture was then filtered through a plug of celite to yield compound **11** (3 mg) as a white solid.

Compound 11: ^1H NMR (400 MHz, D_2O): δ = 5.39 (br. s, 1 H, β-lactam vinyl CH), 5.30 (br. s, 1 H), 5.09 (t, $J_{3,4} = J_{4,5}$ = 10.0 Hz, 1 H, 4′-H), 5.00 (br. s, 1 H), 4.23 (dq, ^3J = 4.0 Hz, ^3J = 6.4 Hz, 1 H), 4.19 (q, ^3J = 7.2 Hz, 2 H, -OCH_2CH_3), 4.05–4.00 (2 H), 3.91 (dd, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 10.0 Hz, 1 H, H-3′), 3.90–3.75 (5 H), 3.70–3.55 (4 H), 2.29 (d, ^3J = 7.2 Hz, 2 H, isovaleryl CH_2), 2.04 (m, 1 H, isovaleryl CH), 1.36 (d, ^3J = 6.4 Hz, 3 H, β-lactam -CHCH_3), 1.25 (t, 3 H, ^3J = 7.2 Hz, -OCH_2CH_3), 0.91 (d, J = 6.8 Hz, 6 H, isovaleryl methyl groups) ppm.

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