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Synthesis of the Fungal Lipo-Chitooligosaccharide Myc-IV (C16:0, S), Symbiotic Signal of Arbuscular Mycorrhiza

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A new synthesis of the fungal lipo-chitooligosaccharide Myc-IV (C16:0, S), which was recently reported to be a major symbiotic signalling molecule in arbuscular mycorrhiza, is described. Key steps include the oxidative cleavage of a 4,6-*O*-benzylidene acetal to prepare a disaccharidic glycosyl acceptor, and stereoselective glycosylations with 2-methyl-5-*tert*-butylphenyl thioglycosyl donors.

Introduction

The association of arbuscular mycorrhizal (AM) fungi (Glomeromycota group) with plant roots is the oldest and ecologically most important symbiotic relationship between higher plants and microorganisms.[1] It is more than 400 million years old; the rhizobia–legume endosymbiosis appeared only 60 million years ago.[2] Inside plant-root cells, these fungi transport rare or poorly soluble mineral nutrients such as phosphorus, copper, and zinc from the soil to the plant, which in turn supplies carbohydrates to the fungus. The plant benefits from its fungal partner by improved nutrition, water control, and disease resistance, all of which could be beneficial to low-input sustainable agriculture. The fungus depends on its host plant for carbon, and its development is strictly under the control of the plant. The AM fungi endosymbiosis starts with an exchange of chemical signals between the root and the microsymbiont, priming both partners for the subsequent association. Each partner produces chemical mediators. The signals produced by the host plant were identified recently.[3,4] They are strigolactones, carotenoid lactones secreted by plant roots, which rapidly stimulate the development of AM fungi at concentrations as low as 0.1 pM. Forty years ago, these molecules were characterized as seed-germination stimulants of witchweeds (*Striga* spp) and broomrapes (*Orobanche* and *Phelipanche* spp), parasitic plants from the *Orobanchaceae* family.[5] More recently, it has been shown that strigolactones act as a new class of hormones in regulating plant architecture.[6] It was recently revealed that strigolactones induce the overexpression of short-chain chitin oligomers by AM fungi.[7]

By analogy with the rhizobial Nod factors, which induce molecular responses in the host root, the Myc factors were proposed early as compounds released in the rhizosphere by AM fungi.[8–10] These are diffusible factors that are recognized by plant hosts and are necessary for the establishment of a successful mycorrhizal association. Myc factors, like Nod factors, activate calcium spiking.[11] This is consistent with the hypothesis of biologists that the "Myc" signalling pathways, which are older, could have been used by nitrogen-fixing bacteria for their recognition by plants (the "Nod" signalling pathways). Nod factors are recognized by receptor-like kinases that contain sugar-binding lysine-motif domains.[12] Analogous AM-specific receptors most probably also exist but need to be characterized. As the chitin backbone of the Nod factor molecule is more typical of fungi than bacteria, it was proposed earlier that the diffusible AM factors could be Nod-factor-like molecules or chitin oligomers.[9,13] Myc factors (11 compounds isolated to date, Figure 1) were finally structurally characterized in 2011, having been isolated from *Glomus intraradices* (Glomeromycota), and were found to have structures very similar to the Nod factors.[14] The Myc factors were purified from exudates from germinating spores of the AM fungus. The biologically active compounds characterized by mass spectrometry (MS) by Dénarié et al.[14] consist of a mixture of sulfated and non-sulfated lipo-chitooligosaccharides (LCOs) present at a concentration between 10^{-5}

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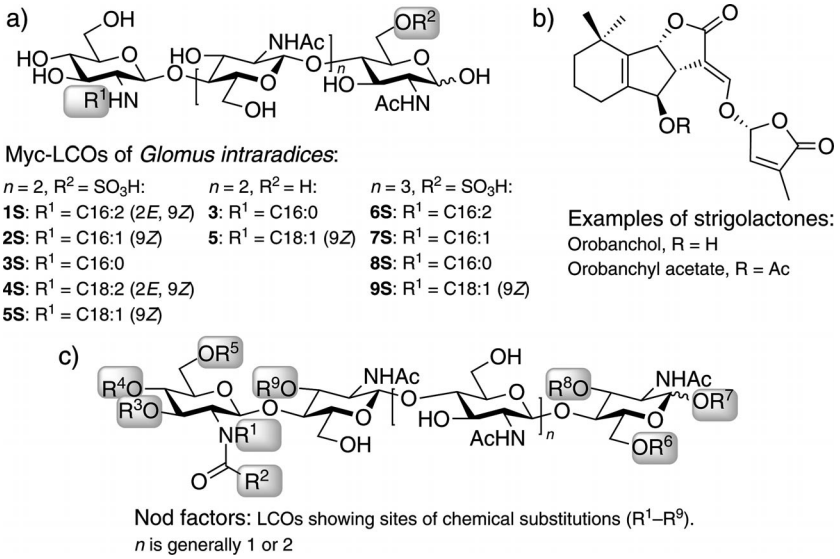


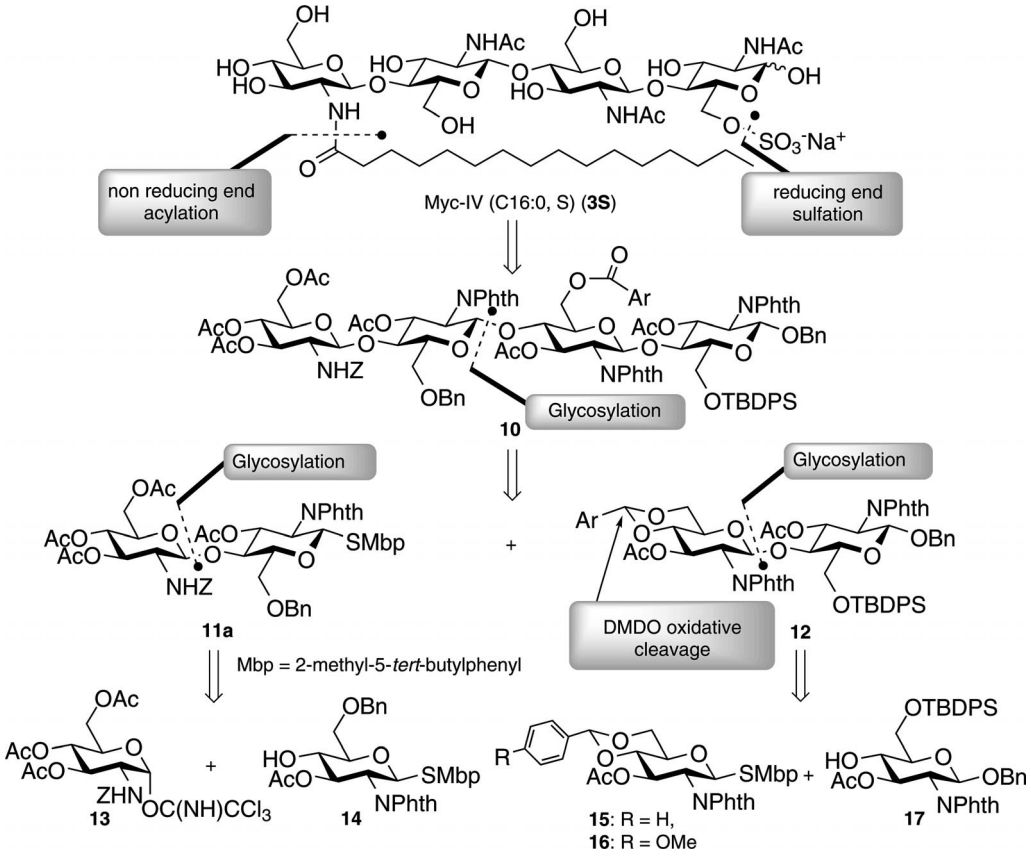
Figure 1. Molecular signalling molecules involved in the initiation of arbuscular mycorrhizal (AM) symbiosis. a) Myc factor structures isolated to date from *Glomus intraradices*. b) Two examples of strigolactone structures. c) Structures of most of the Nod factors.

and 10^{-11} M in the medium. As for Nod factors, they share a common backbone of four or five (β1–4)-linked *N*-acetylglucosamine units, *N*-acylated at the non-reducing end unit with a common fatty acid group, such as stearic (C18:0), oleic (C18:1), or palmitic (C16:0) acid. The only *O*-substitution is the *O*-sulfation at the C-6 position of the monosaccharide residue at the reducing end of the molecule. The synthesis of the major Myc factors (i.e., **3**, **3S**, **5**, and **5S**) has been performed to unambiguously prove the structures established by MS. This synthesis of LCOs **3**, **3S**, **5**, and **5S** was accomplished using recombinant *Escherichia coli* strains coexpressing the *nodBC* genes (encoding the chitooligosacchahe *nodH* gene (encoding the chito-oligosaccharide sulfotransferase) from *Sinorhizobium meliloti*, as described by Samain et al.[15,16] This straightforward approach produced a mixture of chito tetrasaccharides and pentasaccharides.[14a] The characterized Myc factors have simpler structures than the Nod factors. Some of them had already been isolated as Nod factors [compound **1S** is NodRm-IV (S), **2S** is produced by *Rhizobium meliloti*[17,18] and *Rhizobium* sp. GRH2[19] (as is **5** in minor amounts)], and had been synthesized by various groups in recent decades chemically[20–23] or by genetically engineered bacteria.[24] Another one of them (compound **3S**) had been synthesized as an "unnatural" Nod factor.[21] The crucial role of Nod factors in an agronomically and ecologically important symbiosis stimulated synthetic interest soon after their discovery in 1990. Because these molecules are produced by rhizobia or AM fungi as mixtures, and only in minute quantities, chemical synthesis greatly facilitates biochemical and physiological studies of the sensing mechanisms involved in Nod and Myc factor signalling.[25,14b]

Myc factors or their analogues could be used to stimulate mycorrhization for a broad range of applications in agriculture with higher specificity than the strigolactones (see above),[3,4,6] which are also involved as chemical mediators in the arbuscular mycorrhizal symbiosis. LCO Myc factors could also be used to stimulate root system development, which is essential to improve water and mineral uptake, especially in parts of the world where soil fertility and water availability are poor. These remarkable biological activities prompted us to develop a new chemical synthesis of Myc factors using new tools in oligosaccharide chemistry and learning from past syntheses[26] of Nod factors. Thus we considered: (i) selection of a suitable glycosylation procedure able to cleanly and efficiently produce the difficult (β1–4) glycosidic bonds between two 2-acetamido-2-deoxy-D-glucopyranosyl units; (ii) choice of an oligomerization strategy that would provide the oligosaccharide with the highest efficacy; (iii) selective differentiation of identical functional groups, e.g., primary or secondary hydroxy and amino groups present in the chitin fragment.

Results and Discussion

Our new synthesis of an LCO used a method developed in our group for the formation of 4-OH-acceptors. The oxidative cleavage of 4,6-*O*-benzylidene acetals of various glycopyranosides was carried out with dimethyldioxirane (DMDO),[27] and regioselectivity was ensured by placement of a suitable protecting group at the C-3 position. Similarly to the strategy of Hui et al.,[23] we chose a blockwise synthesis for the preparation of tetrasaccharide **10** from two preformed disaccharidic moieties **11a** and **12** (Scheme 1). As direct glycosylation with *N*-acetylglucosamine derivatives is difficult,[28] *N*-protected derivatives were used, and selective differentiation of the amino groups was achieved by using a benzyloxycarbonyl (Z) protecting group for the non-reducing terminal residue and orthogonal phthalimido (Phth) protecting groups for the other amino groups. The TBDPS (*tert*-butyldiphenylsilyl) protecting group at the C-6 posi-



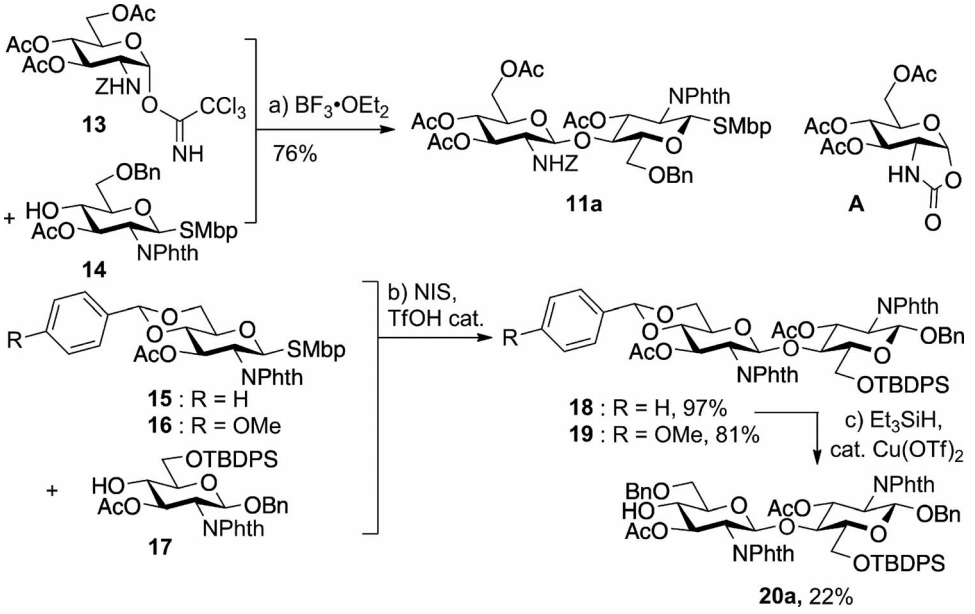
Scheme 1. Retrosynthetic analysis of sulfated glycolipid **3S** Myc-IV (C16:0, S).

tion of the reducing terminal unit was chosen to be stable over several steps of the synthesis. A glycosylation between α-imidate donor **13** and acceptor **14** would give disaccharide **11a** with the nitrogen atoms on the two sugar units differentiated. The other glycosylation reactions, i.e., the synthesis of disaccharide **12** and tetrasaccharide **10** would be carried out using thioglycoside donors.

The synthesis of Myc (C16:0, S) **3S** started with the formation of monomers **13**, **14**, **15**, **16**, and **17**,[29] which were efficiently obtained by known procedures from commercially available D-glucosamine hydrochloride. Glycosylation of α-imidate donor **13** with acceptor **14** in toluene at –78 °C resulted in an efficient (76%) 1,2-*trans* glycosylation, as already observed with an α-imidate donor bearing *O*-benzyl protecting groups in our total synthesis of NodRm-IV S in 1994.[22] Lower yields were observed when dichloromethane was used as solvent (23–38%), or when the reaction was carried out at 0 °C in toluene (68%). We observed the formation of the unreactive oxazolidinone **A**[30] under these glycosylation conditions, resulting from participation by the NHZ group. The reactivity of the non-malodorous 2-methyl-5-*tert*-butylphenylthio group[31,32] (SMbp) was sufficient to allow the coupling of **15** (**16**) with acceptor **17** using NIS-TfOH as promoters without affecting the TBDPS group, providing disaccharides **18** and **19** in high yields (97 and 81%, respectively). Reductive opening of the 4,6-*O*-arylidene acetal of disaccharide **18** proved to be inefficient with any of the classical methods attempted [Et_3SiH/TFA (trifluoroacetic acid), Et_3SiH/cat. $Cu(OTf)_2$, $NaBH_3CN$ (excess)/trimethylsilyl chloride (excess)].[33] At best, a poor yield (22%) of benzyl derivative **20a** was obtained, probably due to the incompatibility of the reducing reagents with the ester and/or silyl groups of substrate **18**[34] (Scheme 2).

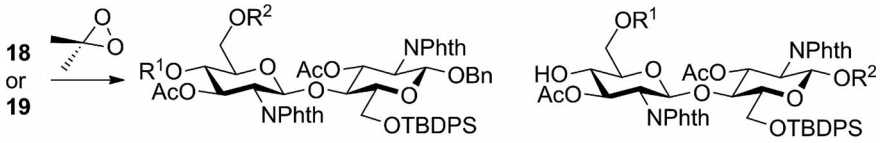
Oxidative ring opening of **18** and **19** was studied as an alternative route to an acceptor that could be used in the next glycosylation (Table 1). We have previously shown that the regioselective oxidative cleavage of the 4,6-*O*-benzylidene protection of monosaccharides is governed by the nature of the hydroxy protecting group at the C-3 position.[27] In this case, an acetate group provided a good selectivity (4:1) in favour of the 4-alcohol. When this method was applied to disaccharides **18** and **19**, the expected 4-OH compounds were formed in moderate yields (48–57%), along with traces of the 6-OH derivatives (**21** and **24**). For the reaction with **18** (Table 1, entry 1), we detected traces of diol **22**. We also observed, when **19** was used as starting material, the formation of 4-OH derivative **25** resulting from oxidation of the benzyl group at the anomeric position. However, the 4-methoxyaryl group favoured the oxidation by DMDO at the benzylic position, and so a better yield for the oxidative ring opening was obtained with compound **19** (57%) than with **18** (48%).

The crucial 2 + 2 coupling between disaccharide acceptor **20b** and donor **11a** was first performed using NIS-TfOH as promoter (Table 2, entry 1) to give tetrasaccharide **26** in low



Scheme 2. Formation of disaccharides **11a**, **18**, and **19**. a) **14** (1 equiv.), **13** (2 equiv.), BF_3·OEt_2 (0.5 equiv.), toluene, 7 h, –78 °C to room temp. b) **17** (1 equiv.), **15** or **16** (1.5–1.7 equiv.), NIS (2.5 equiv.), TfOH (0.15 equiv.), MS 4 Å, CH_2Cl_2, 1 h 30 min, –10 °C. c) Et_3SiH (2 equiv.), $Cu(OTf)_2$ (0.2 equiv.), CH_2Cl_2/CH_3CN, 3 h, room temp.

Table 1. Regioselective oxidative cleavage of the 4,6-*O*-benzylidene group of disaccharides by DMDO.



20b: R^1 = H; R^2 = Bz
21: R^1 = Bz; R^2 = H
23: R^1 = H; R^2 = MBz
24: R^1 = MBz; R^2 = H

22: R^1 = H, R^2 = Bn
25: R^1 = MBz, R^2 = H

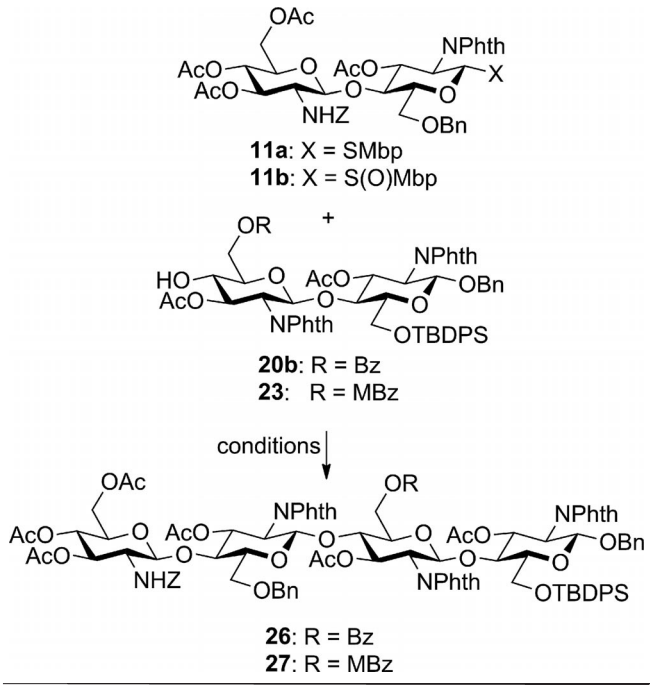
Entry	Starting material	Product[a] (yield [%])[b]	By-products[a] (yield [%])[b]
1	**18**	**20b** (48)	**21** (<5), **22** (<5)
2	**19**	**23** (57)	**24** (<5); **25** (10)

[a] Conditions: DMDO (ca. 0.1 M in acetone; 5 equiv.), 96 h, 5 °C. [b] Yield after silica gel chromatography. MBz = 4-methoxybenzoyl.

yield (36%). The stereochemistry of the product was proved from its ^1H NMR spectrum. Simply stirring acceptor **20b** (or **23**) and donor **11a** with molecular sieves (MS 4 Å) for >1 h instead of 10 min before the addition of the promoters increased the yield of tetrasaccharide **26** (or **27**) to 46% (or 48%) (Table 2, entries 2 and 5). When sulfoxide **11b**[35] was used as donor, together with the appropriate promoters, tetrasaccharide **26** was not obtained efficiently (Table 2, entry 4). Finally, as for the formation of disaccharide **11a**, changing the solvent of the glycosylation from CH_2Cl_2 to toluene resulted in an increase of the yield of tetrasaccharide **26** to 61% (Table 2, entry 3).[36]

The *N*-Phth groups in **26** were then selectively replaced by *N*-acetyl groups in the presence of the NHZ group using a two-step procedure [$(NH_2CH_2)_2$ then Ac_2O] to give compound **28** with three NHAc groups, which was difficult to separate from *N*,*N*′-diacetyl ethylenediamine (Scheme 3). Using the same sequence, the deprotection of **27** unfortunately led to a mixture of products due to the difficult aminolysis of the 4-methoxybenzoyl (MBz) group. Hence, the synthesis was continued with tetrasaccharide **28**. Deprotection of the TBDPS group using NH_4F in MeOH under reflux conditions smoothly gave the 6-OH tetrasaccharide, which was then easily sulfated by the SO_3·pyridine complex, using the standard procedure, to give compound **29**. Final methanolysis of the *O*-acetate groups, followed by hydrogenolysis of the Z-group, gave the free chito-oligosaccharide (CO) **30**.[23a] After chromatography on silica gel, this compound was subjected to selective *N*-acylation using an excess of palmitoyl chloride **31**[37] by heating (40 °C) in the presence of $NaHCO_3$. The target compound, Myc (C16:0, S) **3S**, was purified by chromatography on silica gel (ethyl acetate/methanol/water, 5:2:1 as eluent) and gel filtration through a Sephadex G-25 column. The regioselectivity of

Table 2. Final glycosylation for the synthesis of the tetrasaccharidic unit.



Entry	Donor	Acceptor	Donor/ acceptor	Conditions[a]	Product (yield [%])[e]
1	**11a**	**20b**	1.5:1	A[a]	**26** (36)
2	**11a**	**20b**	2:1	B[b]	**26** (46)
3	**11a**	**20b**	1:1.3	C[c]	**26** (61)
4	**11b**	**20b**	1:1.1	D[d]	–
5	**11a**	**23**	1:1.1	B[b]	**27** (48)

[a] A: CH_2Cl_2, MS 4 Å (10 min, room temp.), then NIS (2.5 equiv.), TfOH (0.15 equiv.), 1 h, –10 °C to room temp. [b] B: CH_2Cl_2, MS 4 Å (≥1 h, room temp.), then NIS (2.5 equiv.), TfOH (0.15 equiv.), 1.5 h, –10 °C to room temp. [c] C: toluene, MS 4 Å (≥1 h, room temp.), then NIS (1.2 equiv.), TfOH (0.2 equiv.), 3 h, –30 °C. [d] D: CH_2Cl_2, MS 4 Å (≥1 h, room temp.), then Tf_2O (1.2 equiv.), TTBP (2,4,6-tri-*tert*-butylpyrimidine; 2 equiv.), 1.5 h, –78 °C to –30 °C. [e] Yield after silica gel chromatography.

the final acylation was unambiguously proved by tandem mass spectrometry (MS/MS) data, as described for natural **3S**.[14a] No β-elimination of the fatty acyl chain was observed, as would be expected for *O*-acyl groups,[14a,38] which conclusively showed that the *N*-acyl derivative had been obtained. The fragmentation of the LCOs by MS/MS was studied extensively, and the results obtained for **3S** are consistent with the MS/MS data for LCOs (**32**,[22] **33**,[39] and **34**[40]) previously synthesized in our group (Figure 2 and Supporting Information). In all cases, we observed, by MS/MS from the parent molecular ion, the fragment ions: [M – H_2O], $^{0,2}A_4$, Y_3, [Y_3 – H_2O], [$^{0,2}A_4$ – (non reducing terminal residue)], and Y_2.[41]

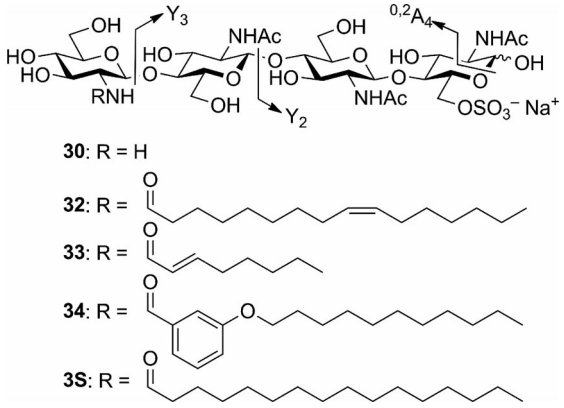
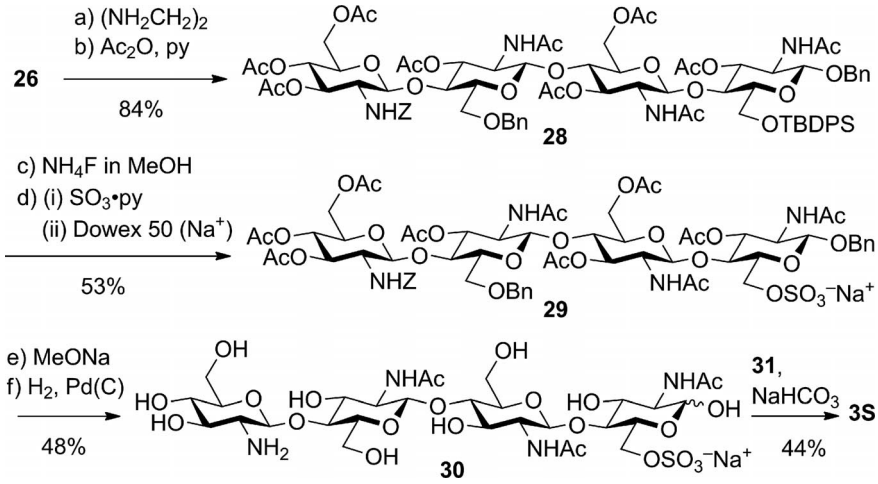


Figure 2. Diagnostic fragmentations by tandem mass spectrometry for sulfated CO (**30**) and LCOs (**32**–**34** and **3S**).

Conclusions

We have achieved the synthesis of lipo-chitooligosaccharide **3S** using robust glycosylation procedures (α-imidate and β-thioglycoside donors), combined with new protecting groups for this class of compound, without relying on azido



Scheme 3. Final deprotections and functionalizations. a) $(NH_2CH_2)_2$ (150 equiv.), EtOH, 12 h, 60 °C; b) Ac_2O (275 equiv.), pyridine, 48 h, room temp.; c) NH_4F (0.5 M in MeOH; 5 equiv.), 12 h, reflux; d) *(i)* SO_3·pyridine (3 equiv.), DMF, 48 h, r.t. *(ii)* Dowex® 50 (Na^+), MeOH. e) MeONa (1 M in MeOH; 2 equiv.), MeOH, 12 d, room temp.; f) H_2, Pd (10% on C), EtOAc/H_2O/EtOH (1:1:0.1), 16 h, room temp.; g) **31** (6 equiv.), $NaHCO_3$, DMF/THF/H_2O (5:3:2), 48 h.

chemistry to differentiate the amino groups. The disaccharide acceptor was prepared by a dimethyldioxirane-mediated oxidative ring opening of 4,6-*O*-benzylidene acetals, as recently reported by our group. New syntheses of LCO analogues are currently in progress in our laboratory, and these analogues could be valuable in improving mycorrhization or nodulation for plants of interest.

Experimental Section

General Remarks: See Supporting Information.

(2-Methyl-5-*tert*-butylphenyl) (3,4,6-Tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (11a): Acceptor **14**[29] (300 mg, 0.49 mmol, 1.0 equiv.) and donor **13**[29] (571 mg, 0.98 mmol, 2.0 equiv.) were cooled in toluene (3.6 mL) to –78 °C. $BF_3{\cdot}OEt_2$ (30 μL, 0.245 mmol, 0.5 equiv.) was added dropwise to this solution. The reaction mixture was stirred at this temperature for 2 h, then it was warmed slowly to room temperature over a period of 5 h, and then neutralized with triethylamine (200 μL). The volatiles were evaporated under reduced pressure, and the crude material was purified by chromatography on silica gel (heptane/EtOAc, 8:2 to 1:1) to give disaccharide **11a** (380 mg, 76%) as an amorphous white solid. $[\alpha]_D^{25}$ = +5.3 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, CD_3CN): δ = 7.94–7.82 (m, 4 H, Ar), 7.50 (d, *J* = 2.1 Hz, 1 H, Ar), 7.45–7.32 (m, 9 H, Ar), 7.29 (dd, *J* = 7.9, *J* = 2.1 Hz, 1 H, Ar), 7.25–7.18 (m, 1 H, Ar), 7.13 (d, *J* = 7.9 Hz, 1 H, Ar), 5.62 (m, 1 H, 3B-H), 5.56 (d, $J_{B\text{-}1,B\text{-}2}$ = 10.5 Hz, 1 H, 1B-H), 5.52–5.45 (m, 1 H, NH), 5.19–5.02 (m, 3 H, 3A-H, OCH_2Ph), 4.91 (t, $J_{A\text{-}4,A\text{-}3}$ = $J_{A\text{-}4,A\text{-}5}$ = 10.0 Hz, 1 H, 4A-H), 4.68–4.51 (m, 3 H, 1A-H, CH_2Ph), 4.36–4.18 (m, 2 H, 2B-H, 6A-H), 4.06–3.91 (m, 2 H, 4B-H, 6′A-H), 3.69–3.56 (m, 3 H, 6B-H, 6′B-H, 5A-H), 3.56–3.36 (m, 2 H, 5B-H, 2A-H), 2.18 (s, 3 H, Me), 2.00 (s, 3 H, Ac), 1.98 (s, 3 H, Ac), 1.92 (s, 3 H, Ac), 1.86 (s, 3 H, Ac), 1.26 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, CD_3CN): δ = 171.6 (2 C, COMe), 171.4 (C, COMe), 170.9 (C, COMe), 163.9 [2 C, $N(CO)_2$], 157.2 (C, NHCO), 139.9 (C, Ar), 138.5 (C, Ar), 138.4 (C, Ar), 136.3 (CH, Ar), 136.2 (CH, Ar), 132.8 (C, Ar), 132.5 (C, Ar), 131.7 (CH, Ar), 131.5 (CH, Ar), 129.9 (3 CH, Ar), 129.7 (C, Ar), 129.4 (3 CH, Ar), 129.1 (3 CH, Ar), 127.0 (2 CH, Ar), 125.0 (CH, Ar), 124.6 (CH, Ar), 122.3 (C, Ar), 101.7 (CH, C-1A), 85.4 (CH, C-1B), 79.9 (CH, C-5B), 76.3 (CH, C-4B), 74.1 (CH_2, CH_2Ph), 73.4 (CH, C-3A or 3B-H), 73.0 (CH, C-3A or C-3B), 72.7 (CH, C-5A), 70.0 (CH, C-4A), 69.6 (CH_2, C-6B), 67.6 (CH_2, OCH_2Ph), 63.1 (CH_2, C-6A), 57.5 (CH, C-2A), 55.5 (CH, C-2B), 35.4 (C, *t*Bu), 31.9 (3 CH_3, *t*Bu), 21.3 (3 CH_3, Ac), 21.2 (CH_3, Ac), 20.8 (CH_3, Me) ppm. IR (film): ṽ = 3030, 2960, 2874, 2355, 1778, 1715, 1610 cm^{-1}. MS (ESI): *m*/*z* (%) = 1047 (100) $[M + Na]^+$. HRMS (ESI): calcd. for $C_{54}H_{60}N_2NaO_{16}S$ $[M + Na]^+$ 1047.3687; found 1047.3645.

Benzyl (3-*O*-Acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (18): Acceptor **17**[29] (1.00 g, 1.47 mmol, 1.00 equiv.) and donor **15**[29] (1.30 g, 2.21 mmol, 1.50 equiv.) were stirred with molecular sieves (4 Å; 0.8 g) in CH_2Cl_2 (5 mL) at room temperature for 30 min. NIS (826 mg, 3.67 mmol, 2.50 equiv.) was then added, and the suspension was cooled to –10 °C. TfOH (19 μL, 0.22 mmol, 0.15 equiv.) was added, and then the reaction mixture was allowed to warm to room temperature and stirred for 1.5 h. The molecular sieves were removed by filtration, and the filtrate was poured into $NaHCO_3$ (saturated aq.). The mixture was extracted with CH_2Cl_2, and the organic phase was washed with $Na_2S_2O_3$ (saturated aq.), dried with Na_2SO_4, and concentrated under reduced pressure. The crude material was purified by chromatography on silica gel (toluene/acetone, 98:2 to 96:4) to give disaccharide **18** (1.57 g, 97%) as an amorphous white solid. $[\alpha]_D^{25}$ = –0.5 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.82–7.57 (m, 12 H, Ar), 7.46–7.29 (m, 11 H, Ar), 7.16 (d, *J* = 7.0 Hz, 1 H, Ar), 7.04 (t, *J* = 7.0 Hz, 2 H, Ar), 6.95 (d, *J* = 7.3 Hz, 2 H, Ar), 5.81 (t, $J_{A\text{-}3,A\text{-}2}$ = $J_{B\text{-}3,B\text{-}4}$ = 9.2 Hz, 1 H, 3A-H), 5.70–5.65 (m, 1 H, 3B-H), 5.66 (d, $J_{A\text{-}1,A\text{-}2}$ = 8.0 Hz, 1 H, 1A-H), 5.48 (s, 1 H, CHPh), 5.22 (d, $J_{B\text{-}1,B\text{-}2}$ = 8.2 Hz, 1 H, 1B-H), 4.63 (d, *J* = 12.0 Hz, 1 H, CH_2Ph), 4.37 (dd, $J_{A\text{-}6,A\text{-}6'}$ = 10.4, $J_{A\text{-}6,A\text{-}5}$ = 4.6 Hz, 1 H, 6A-H), 4.28 (d, *J* = 12.0 Hz, 1 H, CH_2Ph), 4.20 (dd, $J_{B\text{-}2,B\text{-}3}$ = 10.7, $J_{B\text{-}2,B\text{-}1}$ = 8.2 Hz, 1 H, 2B-H), 4.19–4.13 (m, 2 H, 2A-H, 4B-H), 3.79 (br. d, $J_{B\text{-}6,B\text{-}6'}$ = 11.5 Hz, 1 H, 6B-H), 3.75–3.65 (m, 2 H, 4A-H, 6′A-H), 3.63 (dd, $J_{A\text{-}5,A\text{-}4}$ = 9.5, $J_{A\text{-}5,A\text{-}6}$ = 4.6 Hz, 1 H, 5A-H), 3.58 (dd, $J_{B\text{-}6',B\text{-}6}$ = 11.5, $J_{B\text{-}6',B\text{-}5}$ = 4.0 Hz, 1 H, 6′B-H), 3.41 (br. dd, $J_{B\text{-}5,B\text{-}4}$ = 9.5, $J_{B\text{-}5,B\text{-}6'}$ = 4.0 Hz, 1 H, 5B-H), 1.94 (s, 3 H, Ac), 1.84 (s, 3 H, Ac), 1.06 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.2 (C, COMe), 169.9 (C, COMe), 167.9 [4 C, $2N(CO)_2$], 137.2 (C, Ar), 137.0 (C, Ar), 136.3 (2 CH, Ar), 136.1 (2 CH, Ar), 134.4 (4 CH, Ar), 133.8 (C, Ar), 133.4 (C, Ar), 131.8 (2 C, Ar), 131.4 (2 C, Ar), 129.9 (2 CH, Ar), 129.4 (CH, Ar), 128.5 (2 CH, Ar), 128.3 (CH, Ar), 127.9 (2 CH, Ar), 127.8 (CH, Ar), 127.7 (2 CH, Ar), 127.6 (2 CH, Ar), 126.5 (2 CH, Ar), 126.4 (CH, Ar), 123.8 (2 CH, Ar), 123.6 (2 CH, Ar), 101.9 (CH, CHPh), 97.5 (CH, C-1A), 96.7 (CH, C-1B), 79.5 (CH, C-4A), 75.3 (CH, C-5B), 74.2 (CH, C-4B), 71.8 (CH, C-3B), 70.5 (CH_2, CH_2Ph), 69.9 (CH, C-3A), 68.9 (CH_2, C-6A), 66.3 (CH, C-5A), 62.6 (CH_2, C-6B), 55.9 (CH, C-2A), 55.3 (CH, C-2B), 27.1 (3 CH_3, *t*Bu), 21.1 (CH_3, Ac), 20.7 (CH_3, Ac), 19.7 (C, *t*Bu) ppm. IR (film): ṽ = 2955, 2911, 2852, 1779, 1747, 1720, 1387 cm^{-1}. MS (ESI): *m*/*z* (%) = 1123 (35) $[M + Na]^+$. HRMS (ESI): calcd. for $C_{62}H_{60}N_2NaO_{15}Si$ $[M + Na]^+$ 1123.3661; found 1123.3715.

Benzyl (3-*O*-Acetyl-6-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (20b): Benzylidene acetal **18** (485 mg, 0.44 mmol) was added to a solution of freshly distilled dimethyldioxirane[42] in acetone (22 mL, ≈ 0.1 M), and the mixture was stirred at 5 °C for 96 h. The crude material was purified by chromatography on silica gel (heptane/EtOAc, 9:1 to 8:3) to give alcohol **20b** (240 mg, 48%) as an amorphous white solid. $[\alpha]_D^{25}$ = +4.9 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.06 (d, *J* = 6.0 Hz, 2 H, Ar), 7.84–7.61 (m, 13 H, Ar), 7.57 (t, *J* = 7.0 Hz, 1 H, Ar), 7.49–7.40 (m, 5 H, Ar), 7.39–7.27 (m, 3 H, Ar), 7.12–7.01 (m, 3 H, Ar), 6.96 (d, *J* = 7.0 Hz, 1 H, Ar), 5.71–5.62 (m, 2 H, 3A-H, 3B-H), 5.60 (d, $J_{A\text{-}1,A\text{-}2}$ = 8.2 Hz, 1 H, 1A-H), 5.20 (d, $J_{B\text{-}1,B\text{-}2}$ = 8.5 Hz, 1 H, 1B-H), 4.73 (dd, $J_{A\text{-}6,A\text{-}6'}$ = 12.0, $J_{A\text{-}6,A\text{-}5}$ = 3.4 Hz, 1 H, 6A-H), 4.67–4.57 (m, 2 H, 6′A-H, CH_2Ph), 4.32 (t, $J_{B\text{-}4,B\text{-}3}$ = $J_{B\text{-}4,B\text{-}5}$ = 9.5 Hz, 1 H, 4B-H), 4.27 (d, *J* = 12.0 Hz, 1 H, CH_2Ph), 4.23 (dd, $J_{B\text{-}2,B\text{-}3}$ = 10.7, $J_{B\text{-}2,B\text{-}1}$ = 8.5 Hz, 1 H, 2B-H), 4.13 (dd, $J_{A\text{-}2,A\text{-}3}$ = 11.0, $J_{A\text{-}2,A\text{-}1}$ = 8.2 Hz, 1 H, 2A-H), 3.81 (br. d, $J_{B\text{-}6,B\text{-}6'}$ = 11.0 Hz, 1 H, 6B-H), 3.73–3.63 (m, 3 H, 4A-H, 5A-H, 6′B-H), 3.36 (br. d, $J_{B\text{-}5,B\text{-}4}$ = 9.5 Hz, 1 H, 5B-H), 3.12 (d, $J_{OH,A\text{-}4}$ = 4.0 Hz, 1 H, OH), 1.88 (s, 3 H, Ac), 1.83 (s, 3 H, Ac), 1.06 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.3 (C, COMe), 170.3 (C, COMe), 168.1 [2 C, $N(CO)_2$], 167.8 (C, PhCO), 167.5 [2 C, $N(CO)_2$], 137.3 (2 C, Ar), 136.4 (2 CH, Ar), 136.1 (2 CH, Ar), 134.4 (2 CH, Ar), 133.7 (2 CH, Ar), 131.5 (3 C, Ar), 130.1 (2 CH, Ar), 129.9 (CH, Ar), 129.8 (CH, Ar), 129.6 (C, Ar), 128.8 (2 CH, Ar), 128.3 (2 CH, Ar), 127.9 (2 CH, Ar), 127.7 (4 CH, Ar), 127.6 (2 CH, Ar), 123.8 (2 CH, Ar), 123.7 (2 C, Ar), 123.6 (2 CH, Ar), 96.9 (CH, C-1A), 96.7 (CH, C-1B), 75.4 (CH, C-5B), 74.5 (CH, C-5A), 73.3 (2 CH, C-3A, C-4B), 71.1 (CH, C-3B), 70.5 (CH_2,

CH_2Ph), 70.0 (CH, C-4A), 63.7 (CH_2, C-6A), 62.4 (CH_2, C-6B), 55.4 (CH, C-2B), 55.1 (CH, C-2A), 27.1 (3 CH_3, *t*Bu), 20.9 (CH_3, Ac), 20.8 (CH_3, Ac), 19.7 (C, *t*Bu) ppm. IR (film): $\tilde{\nu}$ = 2935, 1777, 1745, 1715, 1385, 1274, 1226, 1070, 1040, 719, 701 cm^{-1}. MS (ESI): *m*/*z* (%) = 1139 (100) $[M + Na]^+$. HRMS (ESI): calcd. for $C_{62}H_{60}N_2NaO_{16}Si$ $[M + Na]^+$ 1139.3610; found 1139.3616.

Benzyl (3,4,6-Tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (26): Acceptor **20b** (120 mg, 0.107 mmol, 1.30 equiv.) and donor **11** (84 mg, 0.082 mmol, 1.00 equiv.) were stirred with molecular sieves (4 Å; 80 mg) in anhydrous toluene (1.6 mL) at room temperature for 1 h. NIS (23 mg, 0.102 mmol, 1.20 equiv.) was then added, and the suspension was cooled to –30 °C. TfOH (1.5 μL, 0.016 mmol, 0.20 equiv.) was added, and the reaction mixture was stirred at –30 °C for 3 h. Et_3N was added, and the molecular sieves were removed by filtration. The filtrate was washed with $Na_2S_2O_3$ (saturated aq.), dried with Na_2SO_4, and concentrated under reduced pressure. The crude material was purified by chromatography on silica gel (toluene/acetone, 95:5 to 88:12) to give tetrasaccharide **26** (98 mg, 61%) as an amorphous white solid. $[\alpha]_D^{25}$ = +3.7 (*c* = 0.3, $CHCl_3$). ^1H NMR (600 MHz, CD_3CN): δ = 7.92 (d, *J* = 7.7 Hz, 2 H, Ar), 7.88–7.63 (m, 16 H, Ar), 7.58 (t, *J* = 7.7 Hz, 1 H, Ar), 7.53–7.18 (m, 20 H, Ar), 7.09 (t, *J* = 7.3 Hz, 1 H, Ar), 7.01 (t, *J* = 7.3 Hz, 1 H, Ar), 6.93 (d, *J* = 7.7 Hz, 1 H, Ar), 5.65 (t, $J_{C\text{-}3,C\text{-}2}$ = $J_{C\text{-}3,C\text{-}4}$ = 10.3 Hz, 1 H, 3C-H) (see Figure 3), 5.58 (t, $J_{D\text{-}3,D\text{-}2}$ = $J_{D\text{-}3,D\text{-}4}$ = 10.0 Hz, 1 H, 3D-H), 5.51 (br. t, $J_{B\text{-}3,B\text{-}2}$ = $J_{B\text{-}3,B\text{-}4}$ = 10.0 Hz, 1 H, 3B-H), 5.47–5.42 (m, 2 H, 1C-H, NH), 5.33 (d, $J_{B\text{-}1,B\text{-}2}$ = 8.1 Hz, 1 H, 1B-H), 5.13–4.98 (m, 4 H, 3A-H, 1D-H, OCH_2Ph), 4.85 (t, $J_{A\text{-}4,A\text{-}3}$ = 9.5 Hz, 1 H, 4A-H), 4.62–4.49 (m, 5 H, 1A-H, 6C-H, CH_2Ph, CH_2Ph), 4.28 (d, *J* = 12.5 Hz, 1 H, CH_2Ph), 4.24 (dd, $J_{A\text{-}6,A\text{-}6'}$ = 12.5, $J_{A\text{-}6,A\text{-}5}$ = 4.4 Hz, 1 H, 6A-H), 4.19 (t, $J_{D\text{-}4,D\text{-}3}$ = $J_{D\text{-}4,D\text{-}5}$ = 10.0 Hz, 1 H, 4D-H), 4.11–3.87 (m, 7 H, 6′A-H, 2B-H, 4B-H, 2C-H, 4C-H, 6′C-H, 2D-H), 3.74–3.59 (m, 5 H, 6B-H, 6′B-H, 5C-H, 6D-H, 6′D-H), 3.56–3.45 (m, 2 H, 5A-H, 5B-H), 3.39–3.31 (m, 2 H, 2A-H, 5D-H), 1.95 (s, 3 H, Ac), 1.93 (s, 3 H, Ac), 1.86 (s, 3 H, Ac), 1.83 (s, 3 H, Ac), 1.80 (s, 3 H, Ac), 1.63 (s, 3 H, Ac), 0.91 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, CD_3CN): δ = 171.6 (C, COMe), 171.5 (3 C, COMe), 171.4 (C, COMe), 171.3 (C, COMe), 170.9 (C, COPh), 169.1 (C, NCO), 168.9 [2 C, $N(CO)_2$], 168.8 (C, NCO), 166.7 [2 C, $N(CO)_2$], 157.2 (C, NHCO), 139.8–124.5 (42CH, 12 C, Ar), 101.4 (CH, C-1A), 98.7 (CH, C-1B), 98.0 (2 CH, C-1C, C-1D), 76.9 (CH, C-4C), 76.3 (CH, C-4B), 76.0 (CH, C-5D), 75.7 (CH, C-5B), 74.6 (CH, C-4D), 74.1 (CH, C-5C), 73.9 (CH_2, CH_2Ph), 73.5 (CH, C-3A), 72.7 (CH, C-5A), 72.0 (CH, C-3B), 71.8 (CH, C-3C), 71.7 (CH_2, CH_2Ph), 71.5 (CH, C-3D), 69.9 (CH, C-4A), 69.3 (CH_2, C-6B), 67.6 (CH_2, OCH_2Ph), 64.2 (CH_2, C-6C), 63.5 (CH_2, C-6D), 63.0 (CH_2, C-6A), 57.6 (CH, C-2A), 56.5 (CH, C-2B), 56.3 (2 CH, C-2C, C-2D), 27.6 (3 CH_3, *t*Bu), 21.4 (CH_3, Ac), 21.3 (3 CH_3, Ac), 21.2 (2 CH_3, Ac), 20.4 (C, *t*Bu) ppm. IR (film): $\tilde{\nu}$ = 2947, 1777, 1744, 1713, 1384, 1223, 1025, 720, 698 cm^{-1}. MS (ESI): *m*/*z* (%) = 1983 (70) $[M + Na]^+$. HRMS (ESI): calcd. for $C_{105}H_{104}N_4NaO_{32}Si$ $[M + Na]^+$ 1983.6301; found 1983.6265.

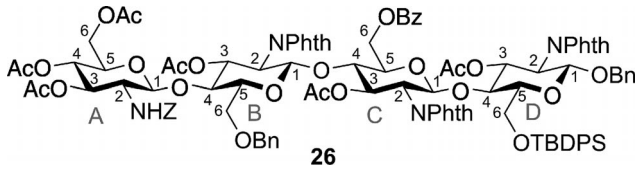


Figure 3. Numbering for compound **26** and the following tetramers.

Benzyl (3,4,6-Tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-β-D-glucopyranoside (28): Tetrasaccharide **26** (448 mg, 0.228 mmol, 1.0 equiv.) and ethylenediamine (2.29 mL, 34 mmol, 150 equiv.) were stirred in ethanol (39 mL) at 60 °C for 12 h. The volatiles were evaporated under reduced pressure, and the crude product was used in the next step without purification. The residue was stirred with acetic anhydride (5.9 mL, 63 mmol, 275 equiv.) in pyridine (71 mL) at room temperature for 2 d. The volatiles were evaporated under reduced pressure, and the crude product was purified by chromatography on silica gel (CH_2Cl_2/MeOH, 1:0 to 96:4) to give **28** (330 mg, 84%) as an amorphous white solid. $[\alpha]_D^{25}$ = –33.8 (*c* = 1.0, $CHCl_3$). ^1H NMR (500 MHz, CD_3CN): δ = 7.84–7.74 (m, 4 H, Ph), 7.52–7.26 (m, 21 H, Ph), 6.41–6.33 (m, 2 H, NH, NH), 5.92 (d, $J_{NH,D\text{-}2\ or\ C\text{-}2}$ = 9.5 Hz, 1 H, NH), 5.13–4.94 (m, 6 H, 3B-H, 3C-H, 3D-H, NH, OCH_2Ph), 4.88 (t, $J_{A\text{-}4,A\text{-}3}$ = $J_{A\text{-}4,A\text{-}5}$ = 9.8 Hz, 1 H, 4A-H), 4.77 (d, *J* = 12.0 Hz, 1 H, CH_2Ph), 4.67 (d, $J_{C\text{-}1,C\text{-}2\ or\ D\text{-}1,D\text{-}2}$ = 8.2 Hz, 1 H, 1C-H or 1D-H), 4.62–4.43 (m, 7 H, 1A-H, 3A-H, 1B-H, 1C-H or 1D-H, CH_2Ph, CH_2Ph), 4.35–4.25 (m, 2 H, 6A-H, 6C-H), 4.17 (dd, $J_{C\text{-}6',C\text{-}6}$ = 12.2, $J_{C\text{-}6,C\text{-}5}$ = 5.5 Hz, 1 H, 6′C-H), 4.04–3.93 (m, 3 H, 6′A-H, 4D-H, 6D-H), 3.92–3.81 (m, 3 H, 2B-H, 4B-H, 6′D-H), 3.73 (t, $J_{C\text{-}4,C\text{-}3}$ = $J_{C\text{-}4,C\text{-}5}$ = 9.2 Hz, 1 H, 4C-H), 3.69–3.55 (m, 4 H, 6B-H, 6′B-H, 2C-H, 2D-H), 3.55–3.41 (m, 5 H, 2A-H, 5A-H, 5B-H, 5C-H, 5D-H), 2.05 (s, 3 H, Ac), 2.01 (s, 6 H, Ac), 1.98 (s, 6 H, Ac), 1.89 (s, 6 H, Ac), 1.84 (s, 3 H, Ac), 1.82 (s, 3 H, Ac), 1.65 (s, 3 H, Ac), 1.11 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, CD_3CN): δ = 172.0 (C, COMe), 171.6 (3 C, COMe), 171.5 (C, COMe), 171.4 (C, COMe), 171.2 (2 C, COMe), 171.1 (C, COMe), 170.9 (C, COMe), 157.2 (C, NHCO), 139.8 (C, Ph), 139.2 (C, Ph), 138.5 (C, Ph), 137.2 (CH, Ph), 137.0 (CH, Ph), 135.2 (C, Ph), 134.4 (C, Ph), 132.6 (CH, Ph), 131.4 (CH, Ph), 131.3 (CH, Ph), 129.9 (3 CH, Ph), 129.8 (CH, Ph), 129.7 (2 CH, Ph), 129.4 (CH, Ph), 129.3 (2 CH, Ph), 129.1 (7 CH, Ph), 129.0 (CH, Ph), 128.9 (2 CH, Ph), 128.3 (CH, Ph), 102.1 (CH, C-1A), 101.5 (CH, C-1B), 101.2 (2 CH, C-1C, C-1D), 77.3 (CH, C-4C), 76.6 (CH, C-5D), 76.0 (CH, C-4B), 75.3 (2 CH, C-4D, C-5B or C-5C), 74.3, 74.1, 74.0, 73.9 (4 CH, C-3B, C-3C, C-3D, C-5B or C-5C), 73.8 (CH_2, CH_2Ph), 73.6 (CH, C-3A), 72.6 (CH, C-5A), 71.5 (CH_2, CH_2Ph), 69.9 (CH, C-4A), 69.4 (CH_2, C-6B), 67.5 (CH_2, OCH_2Ph), 63.9 (CH_2, C-6D), 63.7 (CH_2, C-6C), 63.0 (CH_2, C-6A), 57.5 (CH, C-2A), 55.7 (2 CH, C-2C, C-2D), 54.9 (CH, C-2B), 27.7 (3 CH_3, *t*Bu), 23.6 (2 CH_3, Ac), 23.5 (CH_3, Ac), 21.6 (3 CH_3, Ac), 21.5 (CH_3, Ac), 21.3 (2 CH_3, Ac), 21.2 (CH_3, Ac), 20.4 (C, *t*Bu) ppm. IR (film): $\tilde{\nu}$ = 3292, 2939, 1743, 1655, 1534, 1428, 1368, 1225, 1030, 740, 698 cm^{-1}. MS (MALDI): *m*/*z* (%) = 1657 (100) $[M + Na]^+$. HRMS (MALDI): calcd. for $C_{82}H_{102}N_4NaO_{29}Si$ $[M + Na]^+$ 1657.6297; found 1657.6324.

Benzyl (3,4,6-Tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-sodium Sulfonato-β-D-glucopyranoside (29): Compound **28** (310 mg, 0.189 mmol, 1.0 equiv.) and NH_4F (0.5 M in MeOH; 1.89 mL, 0.948 mmol, 5.0 equiv.) were stirred at 70 °C for 12 h. The volatiles were removed under reduced pressure. The crude material was purified by chromatography on silica gel (CH_2Cl_2/MeOH, 1:0 to 96:4) to give benzyl (3,4,6-tri-*O*-acetyl-2-benzyloxycarbonylamino-

2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-β-D-glucopyranoside (191 mg, 72%) as an amorphous white solid. ^{1}H NMR (500 MHz, CD_3CN): δ = 7.45–7.29 (m, 15 H), 6.52 (d, J = 9.5 Hz, 1 H), 6.38–6.31 (m, 2 H), 5.35 (d, J = 9.0 Hz, 1 H), 5.13–4.94 (m, 6 H), 4.88 (t, J = 9.8 Hz, 1 H), 4.84 (d, J = 12.1 Hz, 1 H), 4.64–4.44 (m, 7 H), 4.33 (dd, J = 12.0, J = 1.1 Hz, 1 H), 4.29 (dd, J = 12.4, J = 4.4 Hz, 1 H), 4.16 (dd, J = 12.0, J = 4.6 Hz, 1 H), 3.96 (dd, J = 12.4, J = 2.0 Hz, 1 H), 3.88–3.55 (m, 11 H), 3.54–3.48 (m, 1 H), 3.45–3.32 (m, 3 H), 2.97 (br. t, J = 5.2 Hz, 1 H), 2.08 (s, 3 H), 2.01 (s, 3 H), 1.98 (s, 3 H), 1.97 (s, 3 H), 1.96 (s, 6 H), 1.89 (s, 3 H), 1.85 (s, 3 H), 1.83 (s, 3 H), 1.82 (s, 3 H) ppm. MS (ESI): *m*/*z* (%) = 1419 (100) $[M + Na]^+$. HRMS (ESI): calcd. for $C_{66}H_{84}N_4NaO_{29}$ $[M + Na]^+$ 1419.5119; found 1419.5115.

This alcohol was stirred with SO_3·pyridine (65 mg, 0.408 mmol, 3.0 equiv.) in DMF (10 mL) at room temperature for 2 d. The reaction mixture was concentrated under reduced pressure. The pyridinium salt was exchanged to the sodium salt by eluting over Dowex® (Na^+ form) ion-exchange resin, and the volatiles were removed under reduced pressure. The crude product was purified by chromatography on silica gel (EtOAc/*i*PrOH/H_2O/Et_3N, 8.8:0.8:0.4:0.1 to 7.6:1.6:0.8:0.1) to give **29** (150 mg, 74%) as a white amorphous solid. $[\alpha]_D^{25}$ = –28.4 (c = 0.35, $CHCl_3$). ^{1}H NMR (500 MHz, CD_3CN): δ = 7.40–7.32 (m, 15 H), 6.61–6.51 (m, 2 H), 6.45 (dd, J = 11.0, J = 10.0 Hz, 1 H), 6.37 (dd, J = 7.5, J = 6.5 Hz, 1 H), 5.08–4.99 (m, 5 H), 4.87–4.79 (m, 3 H), 4.61–4.54 (m, 5 H), 4.32–4.26 (m, 3 H), 4.10–4.07 (m, 2 H), 3.97–3.92 (m, 2 H), 3.85–3.76 (m, 4 H), 3.61–3.57 (m, 3 H), 3.53–3.48 (m, 3 H), 3.46–3.42 (m, 2 H), 3.38–3.34 (m, 2 H), 2.06–1.85 (10s, 30 H) ppm. IR (film): $\tilde{\nu}$ = 3303, 2926, 1741, 1651, 1539, 1428, 1369, 1226, 1032, 741, 699 cm^{-1}. MS (ESI): *m*/*z* (%) = 1475 (100) $[M – Na]^-$. HRMS (ESI): calcd. for $C_{66}H_{83}N_4O_{32}S$ $[M – Na]^-$ 1475.4711; found 1475.4711.

(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-2-acetamido-6-*O*-sodium Sulfonato-2-deoxy-D-glucopyranose (30): Compound **29** (75 mg, 0.0500 mmol, 1.0 equiv.) was stirred with NaOMe (1 M in MeOH; 100 μL, 2.0 equiv.) in MeOH (1.4 mL) at room temperature for 12 d. The solvent was removed under reduced pressure. The residue was dissolved in a mixture of EtOAc/H_2O/EtOH (1:1:0.1), and Pd(10% on C; 75 mg) was added. The reaction mixture was stirred for 16 h under H_2. The catalyst was then removed by filtration, and rinsed with EtOAc, H_2O, and MeOH. The solvents were removed from the filtrate under reduced pressure, and the crude product was purified by chromatography on silica gel ($CH_3CN/H_2O/NH_{3\,(aq)}$, 7:3:0.1 to 7:3.5:0.1) to give chito-oligosaccharide **30**[23a] (20 mg, 48%) as a white amorphous solid. $[\alpha]_D^{25}$ = +182 (c = 1.8, H_2O) [ref.[23a] +3.8 (c = 0.1, H_2O)]. ^{1}H NMR (500 MHz, D_2O): δ = 4.97 (d, J = 3 Hz, 0.5 H, 1D-Hα), 4.65 (d, J = 8.5 Hz, 1 H, 1-Hβ), 4.51 (d, J = 8.5 Hz, 0.5 H, 1D-Hβ), 4.44–4.37 (m, 2 H, 1-Hβ), 4.08–3.85 (m, 3 H), 3.54–3.28 (m, 20 H), 2.90 (t, J = 9 Hz, 1 H, 2A-H), 1.87 (s, 3 H), 1.85 (s, 3 H), 1.82 (s, 3 H) ppm. MS (ESI): *m*/*z* (%) = 867 (100) $[M – Na]^-$. HRMS (ESI): calcd. for $C_{30}H_{51}N_4O_{23}S$ $[M – Na]^-$ 867.2683; found 867.2665.

Myc-IV (C16:0, S) (3S): Compound **30** (12 mg, 0.013 mmol, 1 equiv.) and $NaHCO_3$ (6.8 mg, 0.081 mmol, 6 equiv.) were stirred in a mixture of DMF (294 μL) and H_2O (84 μL) at 40 °C in an Eppendorf tube. A solution of palmitoyl chloride (13 μL, 0.021 mmol, 3 equiv.) in anhydrous THF (81 μL) was then added to the mixture. After 24 h, further $NaHCO_3$ (6.8 mg) and further palmitoyl chloride (13 μL, 3 equiv.) in THF (81 μL) were added. After 48 h, the volatiles were removed under reduced pressure, and the crude product was purified by chromatography on silica gel (EtOAc/MeOH/H_2O, 8:2:1 to 6/2:1) and then by Sephadex G-25 (H_2O). Myc-IV (C16:0, S) **3S** (6.8 mg, 44%) was obtained after lyophilisation as a white powder. $[\alpha]_D^{25}$ = –4.1 (c = 0.26, MeOH). ^{1}H NMR (600 MHz, CD_3OD) partial data: δ = 5.05 (d, J = 3.0 Hz, 0.6 H, 1D-Hα), 4.61 (d, J = 8.5 Hz, 1 H, 1-Hβ), 4.54 (d, J = 8.5 Hz, 0.4 H, 1D-Hβ), 4.50 (d, J = 8.5 Hz, 1 H, 1-Hβ), 4.46 (d, J = 8.5 Hz, 1 H, 1-Hβ), 4.06 (br. d, J = 9.5 Hz, 1 H, 6-H), 3.98 (br. d, J = 9.5 Hz, 1 H, 6-H), 1.95, 1.89, 1.87 (3 s, 9 H, 3 NHAc), 1.55–1.47 [m, 2 H, NH(CO)CH_2], 1.24–1.15 (m, 26 H, 13 CH_2), 0.80 (t, J = 7.0 Hz, CH_3) ppm. MS (ESI): *m*/*z* (%) = 1105 (30) $[M – Na]^-$. HRMS (ESI): calcd. for $C_{46}H_{81}N_4O_{24}S$ $[M – Na]^-$ 1105.4961; found 1105.5002. For the MS/MS data, see Supporting Information.

Supporting Information (see footnote on the first page of this article): General methods, experimental procedures, characterization data and copies of ^{1}H and ^{13}C NMR spectra of new compounds.

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