Chemical Synthesis of NodRm-1: the Nodulation Factor Involved in *Rhizobium meliloti-legume* Symbiosis[1]

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A total synthesis of the sulfated lipotetrasaccharide (NodRm-1) is described. First, the disaccharide glycosyl donor—*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl chloride **3a** or the corresponding trichloroacetimidate **3b** — and the disaccharide glycosyl acceptor — benzyl *O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-benzyl-2-deoxy-2-phthalimido-6-*O*-pivaloyl-β-D-glucopyranoside **4** have been synthesized from monosaccharide moieties through a series of well established reactions. Then the crucial coupling between compound **4** and chloride **3a** or imidate **3b** gave the corresponding β-linked tetrasaccharide **2**. Transformation of the protecting group and subsequent 6-O-sulfation converted tetrasaccharide **2** into the sulfated, N-acetylated compound **25**. Finally, hydrogenolysis of compound **25**, followed by selective *N*-acylation with 3-acylthiazolidine-2-thione **29**, afforded NodRm-1 as its sodium salt.

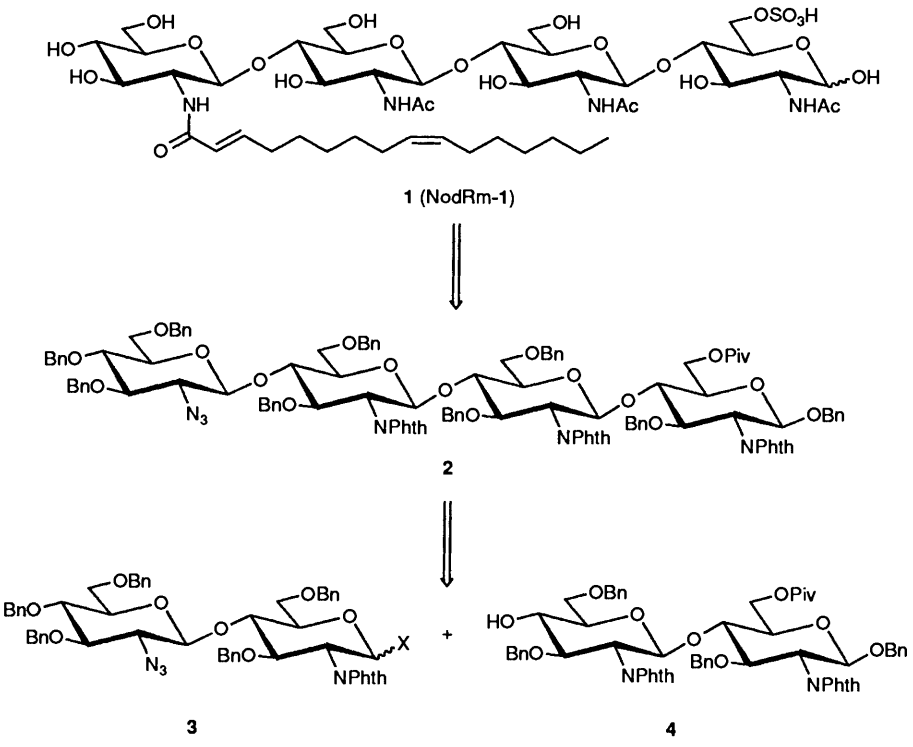
Rhizobia are symbiotic bacteria that elicit the formation of root nodules on their host leguminous plants, in which they fix nitrogen.[2] Recent studies[2,3] showed that the initial nodulation steps involved a reciprocal chemical communication between the plant and the rhizobium. On the one hand, the host legume releases flavonoid-type compounds that stimulate the coordinate expression of bacterial genes (nod genes) required for nodulation. On the other hand, these nod genes control the production of extracellular lipo-oligosaccharide nod factors that determine the host specificity of the bacterium and cause morphological changes in the plant root. In the 1990, Lerouge *et al.*[4] first purified and identified the nodulation factor (called NodRm-1) involved in *R. meliloti-alfalfa* symbiosis. This signal compound was shown to be a sulfated β-1,4-linked tetrasaccharide of D-glucosamine in which three amino groups were acetylated and one was acylated with a C_{16} diunsaturated fatty acid. Purified NodRm-1 can elicit root-hair deformation specifically on alfalfa (the homologous host) but not on vetch (a heterologous host). Interestingly, the compound without the sulfate group at C-6 (named NodRm-2) was able to elicit the same organogenesis and root morphology on vetch but not on alfalfa.[5-7] A study[8] also showed that slight chemical modifications of NodRm-1 such as reduction of the hemiacetal nature and hydrogenation of the unsaturation, as well as the desulfation, all resulted in considerable or even complete loss of its biological activity on alfalfa. These findings, together with the natural scarcity and novel structure of NodRm-1, have aroused great interest in its chemical synthesis.[1,9] Herein we report the details of an unambiguous, total synthesis of NodRm-1.

The synthetic challenges come from the regio- and stereochemistry and the variety of functional groups (sulfur, nitrogen, and unsaturation) in NodRm-1 **1**. Scheme 1 shows the retrosynthetic analysis. A tetrasaccharide precursor **2** was designed as the key intermediate that can allow the selective liberation of the 6-OH group for sulfation and has a 2-azido function at the non-reducing terminal residue for the selective *N*-acylation. Disconnection of intermediate **2** at the centre led to two key building blocks, the glycosyl disaccharide donor **3** and acceptor **4**. These disaccharides could, in turn, be prepared from suitable monosaccharide synthons through stereo controlled glycosidations.

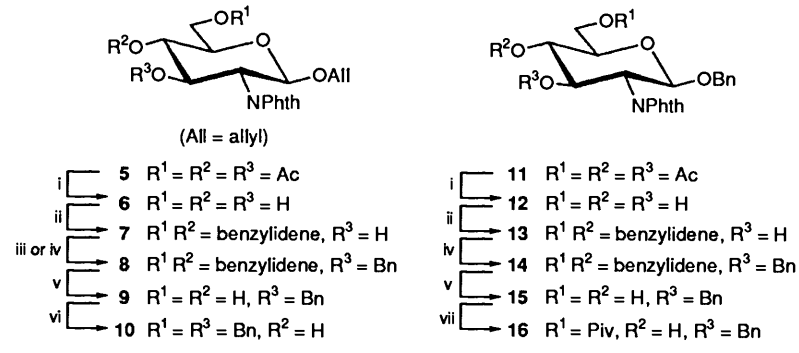
Results and Discussion

We chose the trichloroacetimidate method[10] for the stereoselective construction of the 2-azido-2-deoxy-β-D-glucosidic linkage, and the bulky 2-*N*-phthaloyl group for protecting the 2-amino functions because of its activating and β-directing effects during glycosidation. First of all, a series of monosaccharide synthons required for disaccharide synthesis were prepared as shown in Scheme 2. Starting from 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose,[11] allyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **5**[12] was synthesized in 81% yield by direct treatment with allyl alcohol in the presence of trimethylsilyl triflate (TMSOTf) in CH_2Cl_2. Acidic de-*O*-acetylation of compound **5**, followed by benzylidenation with α,α-dimethoxytoluene and catalytic toluene-*p*-sulfonic acid (TsOH) in *N*,*N*-dimethylformamide (DMF) gave the benzylidene compound **7**[13] in 85% yield in two steps *via* compound **6**.[13] Compound **7** was converted into 3-benzyl ether **8** in two ways. The first attempted benzylation of the 3-hydroxy group in compound **7** with benzyl trichloroacetimidate[14] in the presence of trifluoromethanesulfonic acid ('acidic' conditions) afforded the desired product (48%), whereas benzylation with benzyl bromide and sodium hydride in DMF ('basic' conditions) gave an 85% yield of compound **8**. Removal of the benzylidene acetal in compound **8** gave the diol **9**.[15] Selective benzylation at the primary hydroxy group of diol **9** was achieved by a modified procedure of Ogawa.[16] Thus, compound **9** was treated with bis(tributyltin) oxide in toluene with continuous azeotropic removal of water for 4 h, then was treated with benzyl bromide in the presence of tetrabutylammonium iodide (TBAI) instead of the bromide at 110 °C for 5 h, to give the 3,6-bis(benzyl ether) **10** in 75% yield. It should be noted that the modified procedure yielded the desired 6-*O*-benzylated product in an acceptable yield within a much shorter reaction period.

In a way similar to the preparation of diol **9**, the diol **15**[16] was synthesized starting from 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose *via* compounds **11**–**14**,[16] as shown in Scheme 2. The overall yield in 5 steps was 51%. Selective protection of the primary hydroxy group in diol **15** was efficiently achieved by reaction with 1.2 mol equiv. of pivaloyl chloride in pyridine to give the desired compound **16** (84%).



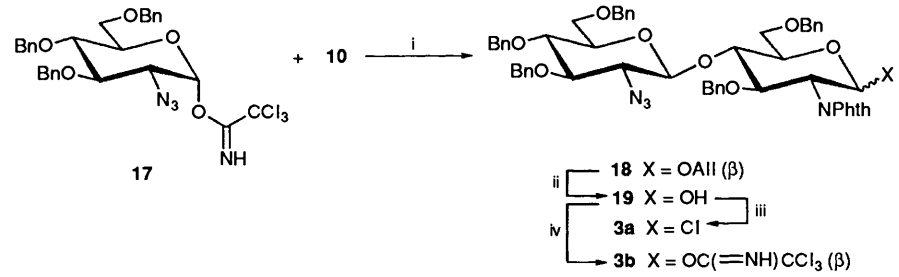
Scheme 1 Retrosynthetic analysis for compound **1**. Ac = acetyl; Bn = benzyl; Phth = phthaloyl; Piv = pivaloyl.



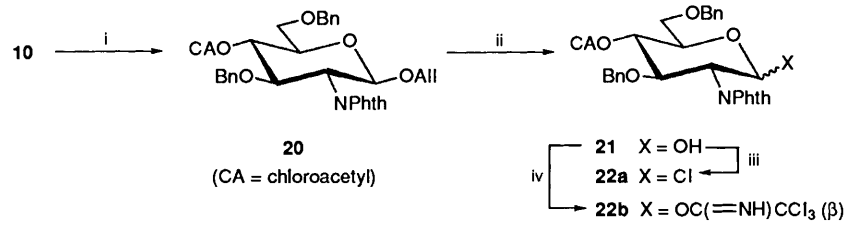
Scheme 2 *Reagents and conditions* (the data in parentheses are yields for benzyl glycoside series): i, Conc. HCl, acetone, 70 °C, 90% (92%); ii, α,α-dimethoxytoluene, TsOH (cat.), DMF, 50 °C, 95% (82%); iii, benzyl trichloroacetimidate, CF_3SO_3H, CH_2Cl_2, 0 °C, 48%; iv, BnBr, NaH, DMF, 0 °C, 85% (88%); v, 80% aq. AcOH, 70 °C, 93% (90%); vi, $(Bu_3Sn)_2O$, toluene, reflux with azeotropic removal of water for 4 h; then BnBr, Bu_4NI, toluene, 110 °C, 5 h, 75%; vii, PivCl (1.2 mol equiv.), pyridine, 0 °C, 4 h, 86%

Boron trifluoride–diethyl ether-promoted glycosidation of compound **10** with 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl trichloroacetimidate[17] **17** in CH_2Cl_2 in the presence of molecular sieves 4 Å (MS4A) proceeded smoothly to give the desired disaccharide **18** (61%) (see Scheme 3). The ^1H NMR spectrum of compound **18**, which showed a doublet at δ 4.39 with $J_{1',2'}$ 8.1 Hz for 1'-H, clearly indicated that the newly formed glycosidic linkage had the β-D configuration. Deallylation of compound **18** with palladium(II) chloride[18] and sodium acetate in 95% aq. AcOH afforded hemiacetal **19** (84%), which was then converted into suitable glycosyl donors (**3a** and **3b**) as follows. Reaction of compound **19** with oxalyl dichloride and a catalytic amount of DMF in CH_2Cl_2 provided the chloride **3a** in 88% yield. On the other hand, treatment of compound **19** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and trichloroacetonitrile afforded the imidate **3b** in good yield (Scheme 3).

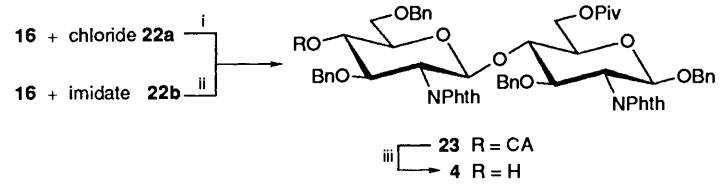
For the synthesis of monosaccharide glycosyl donors **22a** and **22b** that were required for the preparation of disaccharide **4**, the 4-hydroxy function in compound **10** was tentatively protected as the chloroacetyl (CA) ester which can be selectively removed later with thiourea without influencing other ester functions.[19] Thus, treatment of compound **10** with chloroacetyl chloride in pyridine afforded compound **20** (88%). Removal of the allyl group by reaction with $PdCl_2$–NaOAc in 95% aq. AcOH produced the hemiacetal **21** (96%). Then compound **21** was converted into chloride **22a** (89%) by reaction with oxalyl dichloride and a catalytic amount of DMF in CH_2Cl_2.[20] The desired imidate **22b** was easily obtained by treatment of compound **21** with CCl_3CN and DBU in CH_2Cl_2 (Scheme 4). Silver triflate (AgOTf)[11]-promoted glycosidation of compound **16** with chloride **22a** in CH_2Cl_2 in the presence of MS4A led to disaccharide **23** in 58% yield after column chromatography. The configuration of the newly generated anomeric centre was expected to be β-D due to the β-directing effect of the *N*-phthalimido group at the C-2 position. This was confirmed by its ^1H NMR data which showed a doublet at δ 5.25 with $J_{1',2'}$ 8.3 Hz for 1'-H. On the other hand, BF_3·Et_2O-promoted glycosidation of compound **16** with imidate **22b** gave a 76% yield of disaccharide **23** which was found to be identical with the



Scheme 3 *Reagents and conditions:* i, BF_3·Et_2O, MS4A, CH_2Cl_2, −20 °C, 2 h, 61%; ii, $PdCl_2$, NaOAc, 95% aq. AcOH, 50 °C, 3 h, 84%; iii, oxalyl dichloride, DMF (cat.), CH_2Cl_2, 0 °C, 3 h, 88%; iv, CCl_3CN, DBU, CH_2Cl_2, 20 °C, 1 h, 84%



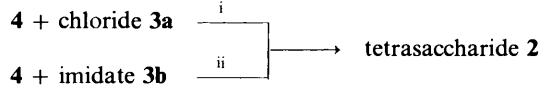
Scheme 4 *Reagents and conditions:* i, chloroacetyl chloride, pyridine, 0 °C, 0.5 h, 88%; ii, $PdCl_2$, NaOAc, 95% aq. AcOH, 70 °C, 1 h, 96%; iii, oxalyl dichloride, DMF (cat.), CH_2Cl_2, 5 °C, 1 h, 89%; iv, CCl_3CN, DBU, CH_2Cl_2, 20 °C, 2 h, 85%



Scheme 5 *Reagents and conditions:* i, AgOTf, MS4A, CH_2Cl_2, −10 °C, 10 h, 58%; ii, BF_3·Et_2O, MS4A, −15 °C, 2.5 h, 76%; iii, thiourea, (6:1) pyridine–EtOH, 70 °C, 1.5 h, 86%

product of the glycosyl chloride procedure. Selective liberation of the 4′-OH group in disaccharide **23** was achieved by reaction with thiourea[19] in 6:1 pyridine–EtOH at 70 °C, to give the glycosyl acceptor **4** in good yield (Scheme 5).

With a suitable disaccharide glycosyl donor and acceptor at hand, the crucial coupling reactions between them were examined. AgOTf-Promoted glycosidation of chloride **3a** with compound **4** provided the desired tetrasaccharide derivative **2**, but the yield (50%) was not satisfactory, whereas coupling of imidate **3b** with compound **4** in the presence of BF_3·Et_2O was shown to be more efficient, and afforded compound **2** in 72% yield (Scheme 6). Again, the stereochemistry of the newly formed glycosidic centre was confirmed by its ^1H–^1H 2D NMR (600 MHz) spectra. Four doublets at δ 4.88 (8.5 Hz), 5.25 (8.5 Hz), 5.08 (8.1 Hz), and 4.40 (8.0 Hz), which were assigned for the four anomeric protons 1-H, 1′-H, 1″-H and 1‴-H, respectively, clearly indicated that all four glycosidic linkages in tetrasaccharide **2** were in the β-D configuration.

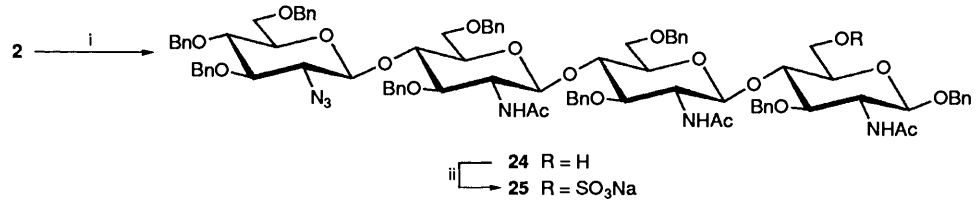


Scheme 6 *Reagents and conditions:* i, AgOTf, MS4A, CH_2Cl_2, −20 °C, 6 h, 50%; ii, BF_3·Et_2O, MS4A, −15 °C, 3 h, 72%

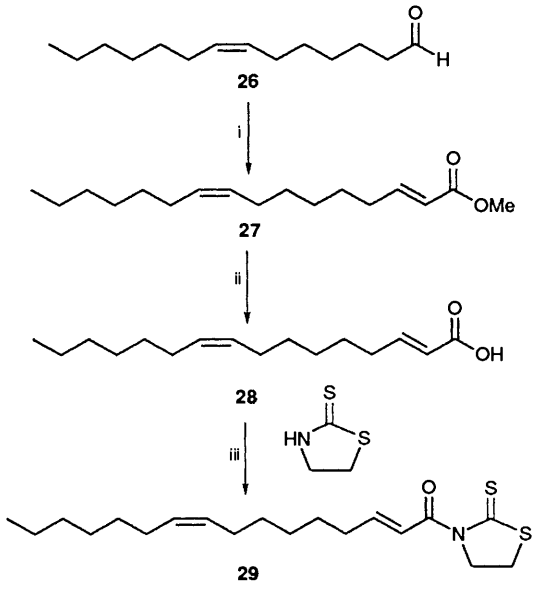
Having performed their activating and β-directing functions in the crucial glycosidation reactions, the phthaloyl protecting groups were then replaced by acetyl groups. Thus, treatment of compound **2** with hydrazine hydrate in ethanol under reflux, followed by *in situ* acetylation with acetic anhydride in pyridine, gave the *N*-acetylated product. However, high-performance TLC (HPTLC) [(20:1) $CHCl_3$–EtOH] examination revealed the existence of two components with R_f 0.59–0.61. This was probably due to partial de-*O*-pivaloylation by hydrazine and subsequent re-*O*-acetylation under the above conditions. Its ^1H NMR spectrum confirmed the presence of both *O*-pivaloyl and *O*-acetyl groups in the product. The product thus obtained was subjected to de-*O*-acylation with powdered potassium hydroxide in tetrahydrofuran (THF)-MeOH, to give a single compound, with R_F 0.38. This compound was determined to be the desired tetrasaccharide **24** in which the 6-hydroxy group was free and the three amino groups were *N*-acetylated (see Experimental section). The overall yield in 3 steps from compound **2** was 66%. Sulfation of compound **24** with sulfur trioxide–pyridine complex[21] in DMF at 50 °C proceeded smoothly to yield the key intermediate **25** (81%) as its sodium salt after ion exchange (Scheme 7).

The unsaturated fatty acid chain was synthesized starting from (*Z*)-tetradec-7-enal **26**. Wittig reaction of compound **26** with methyl (diethoxyphosphoryl)acetate gave methyl (2*E*,9*Z*)-hexadeca-2,9-dienoate **27** in 92% yield. Then compound **27** was saponified with 1.5 mol dm^{-3} KOH to yield (2*E*,9*Z*)-hexadeca-2,9-dienoic acid **28** (90%).* The chemical shifts and *J*-values, δ

* The attempted saponification of ester **27** with powdered potassium hydroxide in (1:1) THF–MeOH at 25 °C for 6 h didn't produce the desired α,β-unsaturated acid **28**, but instead gave (*Z*)-3-methoxyhexadec-9-enoic acid in 86% yield. This result clearly showed that methanol could easily undergo Michael addition to the α,β-unsaturated moiety under the above conditions. The structure of the product was confirmed by spectroscopy; R_f 0.12 in (5:1) hexane–diethyl ether; *m*/*z* (EI) 285 (M + 1, 8%); δ_H 10.5 (1 H, br s, OH), 5.35 (2 H, m, *z*-CH=CH), 3.64 (1 H, m, CHOMe), 3.38 (3 H, s, OMe), 2.56 (1 H, dd, *J* 7.3 and 15.4, 1/2 × CH_2CO), 2.49 (1 H, dd, *J* 5.1 and 15.4, 1/2 × CH_2CO), 2.01 (4 H, m, CH_2CH=$CHCH_2$), 1.20–1.60 (16 H, m, 8 × CH_2) and 0.9 (3 H, t, *J* 7.0, CH_2Me).



Scheme 7 *Reagents and conditions:* i, (*a*) NH_2NH_2-H_2O, EtOH, 95 °C, 16 h; then (*b*) Ac_2O, pyridine, 20 °C, 6 h; then (*c*) KOH, (1 : 1) MeOH–THF, 20 °C, 5 h, 66% in 3 steps. ii, (*a*) pyridine–sulfur trioxide complex, DMF, 50 °C. 6 h; then (*b*) Sephadex LH-20 eluted with MeOH; then (*c*) Dowex 50w-x8 (Na^+) eluted with MeOH, 81%.



Scheme 8 *Reagents and conditions:* i, Methyl (diethoxyphosphoryl)-acetate [$(EtO)_2P(=O)CH_2CO_2Me$], NaH, THF, 20 °C, 2.5 h, 92%; ii, THF, 1.5 mol dm^{-3} aq. KOH, 70 °C, 6 h; then 1 mol dm^{-3} aq. HCl, 90%; iii, DCC, ethylene glycol bismethyl ether (DME), 20 °C, 8 h, 85%

5.85 (d, *J* 15.4 Hz) and 7.10 (dt, *J* 7.0 and 14.6 Hz) for 2-H and 3-H, respectively, clearly indicated that the α,β-unsaturated bond had the desired *E* configuration. In order to conjugate it with an appropriate sugar moiety, the acid was activated in its 3-acylthiazolidine-2-thione form.[22] Thus, acid **28** was treated with thiazolidine-2-thione in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCC), to afford the activated compound **29** (85%) as a yellow syrup (Scheme 8).

Hydrogenolysis of compound **25** for both removal of benzyl ether protecting groups and simultaneous reduction of the azido function was performed under hydrogen in the presence of 10% palladium on carbon in THF–aq. EtOH and gave compound **30** (83%) as a white powder after lyophilization. Finally, selective *N*-acylation was achieved by the reaction of **30** with activated fatty acid **29** in 95% EtOH at 45 °C in the presence of a catalytic amount of triethylamine and gave the target molecule NodRm-1 **1** (45%) as its sodium salt after gel filtration purification (Scheme 9). The ^1H NMR spectrum (D_2O; 600 MHz) of the synthetic compound showed that the β-anomeric protons resonated at δ 4.61–4.50 as sets of doublets (*J* 8.5 Hz), and the α-anomeric hemiacetal proton of the terminal reducing sugar appeared at δ 5.09 as a doublet (*J* 3.3 Hz). Two downfield signals at δ 4.20 and 4.08 (each dd) were attributed to the two 6-H protons, clearly indicating the location of the sulfate group at C-6. The signals at δ 6.81 (dt, 1 H, *J* 7.1 and 15.3 Hz), 5.98 (d, 1 H, *J* 15.3 Hz) and 5.36 (m, 2 H) were assigned to the four olefinic protons at the *E*-conjugated double bond and the internal *Z*-double bond of the fatty acid chain, respectively. Other features of the ^1H NMR spectrum were in agreement with the structure reported.[4]

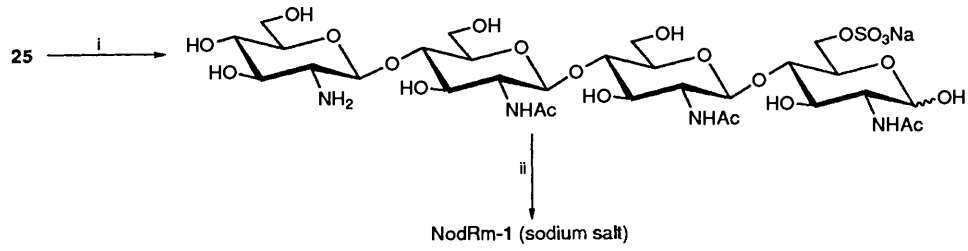
In summary, a total synthesis of the sulfated lipo-oligosaccharide nodulation factor (NodRm-1) was achieved in a regio- and stereo-controlled manner.

Experimental

Optical rotations, given in units of 10^{-1} deg cm^2 g^{-1}, were determined with a Perkin-Elmer Model 241 MC polarimeter at ambient temperature (20 °C). R_f Values were measured on HPTLC precoated plates (0.25 mm; E. Merck, Darmstadt, Germany) of Silica Gel $60F_{254}$. Reactions were monitored by TLC on precoated plates of Silica Gel $60F_{254}$ (0.25 mm; E. Merck, Darmstadt, Germany). Flash column chromatography was performed on Silica Gel 60 (200–300 mesh). Ratios of developing or eluting solvents in chromatography are specified in terms of volume. Extract and eluents were dried with anhydrous sodium sulfate, and concentrated under diminished pressure below 45 °C. IR spectra were recorded with a Shimadzu IR-27 spectrophotometer, using potassium bromide disks for solid samples, and film for liquid samples. Mass spectra were recorded with a VG QUATTRO MS instrument. ^1H NMR spectra were recorded at 600 MHz with a Bruker AMX-600 spectrometer, using tetramethylsilane as the internal standard and [^2H]chloroform as the solvent unless otherwise specified. *J* Values are given in Hz. ^{13}C NMR spectra were recorded at 100 MHz with a Bruker AMX-600 spectrometer. In most cases, only partial NMR data are reported; other spectral features were in accord with the proposed structures.

Allyl 3-O-*Benzyl*-4,6-O-*benzylidene*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranoside* **8**.—(a) *Benzylation with benzyl trichloroacetimidate.* Compound **7** (7.80 g, 17.8 mmol) and benzyl trichloroacetimidate (9.00 g, 36.0 mmol) were dissolved in $(CH_2Cl)_2$ (50 cm^3), then trifluoromethanesulfonic acid was added at 0 °C and the pH was maintained at ~ 3 (measured with universal indicator paper) for 2 h. The solution became dark brown as the reaction proceeded. After being neutralized with triethylamine, the reaction mixture was poured into ice–water, and extracted with CH_2Cl_2. The organic layer was washed successively with brine and water, dried, and concentrated. The residue was subjected to column chromatography with (7 : 1) light petroleum (30–60 °C)–EtOAc as the eluent to afford *title compound* **8** (4.60 g, 48%) as a syrup (Found: C, 70.4; H, 5.6; N, 2.4. $C_{31}H_{29}NO_7$ requires C, 70.6; H, 5.5; N, 2.65%); R_f 0.86 [(2 : 1) toluene–EtOAc]; $[\alpha]_D$ −12.8 (*c* 1.2, $CHCl_3$); δ_H 7.73–7.65 (4 H, br s, phthaloyl), 7.55–6.88 (10 H, m, 2 × Ph), 5.67 (1 H, m, CH_2=CH), 5.62 (1 H, s, benzylidene), 5.15 (1 H, d, $J_{1,2}$ 8.2, 1-H), 5.10 and 5.01 (1 H each, d each, *J* 17.2 and 10.3, CH_2=CH), 4.82 and 4.58 (2 H, ABq, J_{gem} 12.4, CH_2Ph), 4.40–4.18 (2 H, m, 6-H_2 and 4.15–3.75 (6 H, m, 2-, 3-, 4- and 5-H and OCH_2CH=CH_2).

(b) *Benzylation with benzyl bromide–sodium hydride.* To a solution of compound **7** (6.56 g, 15.0 mmol) in DMF (100 cm^3) was added sodium hydride (80%; 900 mg, 30.0 mmol) in portions, and the mixture was stirred for 30 min at room temperature. To the suspension was then added benzyl bromide (7.2 cm^3, 60.0 mmol) dropwise at 5 °C, and the resulting



Scheme 9 *Reagents and conditions:* i, H_2, 10% Pd/C, (1:1:0.5) EtOH–THF–water, 25 °C, 24 h; then Bio-gel P-2 and Dowex 50-x8 (Na^+), 83%; ii, Compound **29**, 95% EtOH, Et_3N (cat.), 45 °C, 3 days; then Bio-gel P-4 and Dowex 50w-x8 (Na^+), 45%

mixture was stirred at room temperature for 5 h. The mixture was poured into cold 5% aq. sodium hydrogen carbonate, and extracted with EtOAc. The organic layer was washed successively with brine and water, dried, and concentrated. Column chromatography of the residue, first with toluene containing 1% triethylamine as the eluent to remove benzyl bromide, then with (9:1) toluene–EtOAc containing 1% triethylamine as the eluent, gave compound **8** (6.73 g, 85%), which was identical with the product in procedure (a).

Allyl 3-O-*Benzyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranoside* **9**.—A solution of compound **8** (2.0 g, 1.89 mmol) in 80% aq. acetic acid (60 cm^3) was heated at 70 °C for 1.5 h, then was concentrated under reduced pressure, and the residue was co-evaporated with toluene to dryness. Column chromatography with (1:1) light petroleum (30–60 °C)–EtOAc as the eluent gave diol **9** (1.30 g, 93%) as an oil, R_f 0.53 [(10:1) $CHCl_3$–MeOH]; $[\alpha]_D$ +37.0 (*c* 0.5, $CHCl_3$) (lit.,[15] $[\alpha]_D$ +40.3); δ_H 7.78–7.68 (4 H, m, phthaloyl), 7.40–6.92 (5 H, m, Ph), 5.67 (1 H, m, CH_2=CH), 5.20 (1 H, d, $J_{1,2}$ 8.2, 1-H), 5.10–5.00 (1 H each, d each, *J* 17.5 and 10.4, CH=CH_2), 4.75 and 4.56 (2 H, ABq, J_{gem} 12.5, CH_2Ph), 4.32–3.52 (8 H, m, 2-, 3-, 4- and 5-H, 6-H_2 and OCH_2CH=).

Allyl 3,6-*Di*-O-*benzyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranoside* **10**.—A mixture of compound **9** (1.20 g, 2.73 mmol) and bis(tributyltin) oxide (1.22 g, 2.05 mmol) in toluene (60 cm^3) was stirred under reflux for 4 h with continuous azeotropic removal of water, then was concentrated to 30 cm^3. To the solution were added benzyl bromide (1.3 cm^3, 11.0 mmol) and TBAI (1.01 g, 2.73 mmol), and the resulting mixture was stirred at 110 °C for 5 h; TLC [(1:1) toluene–EtOAc] showed the complete reaction of substrate **9**, and a single spot more mobile than compound **9**. After being cooled to room temperature, the mixture was diluted with EtOAc, washed successively with aq. sodium thiosulfate, brine, and water, dried, and concentrated. Column chromatography of the residue with (5:1) toluene–EtOAc as the eluent afforded title compound **10** (1.08 g, 75%) as a syrup, R_f 0.61 [(2:1) toluene–EtOAc]; $[\alpha]_D$ +30.5 (*c* 0.5, $CHCl_3$) {lit.,[23] $[\alpha]_D$ + 31.4 (*c* 1, $CHCl_3$)}. Its ^1H NMR spectrum was in good agreement with the structure.

Benzyl 3-O-*Benzyl*-2-*deoxy*-2-*phthalimido*-6-O-*pivaloyl*-β-D-*glucopyranoside* **16**.—To a solution of diol **15**[16] (1.56 g, 3.18 mmol) in pyridine (20 cm^3) was added pivaloyl chloride (458 mg, 3.81 mmol). The mixture was stirred at 0 °C under argon for 4 h, then was poured into ice–water and extracted with CH_2Cl_2 (3 × 25 cm^3). The extracts were combined, washed successively with 1 mol dm^{-3} HCl, brine, and water, dried, and concentrated. Column chromatography of the residue with (5:1) light petroleum (30–60 °C)–EtOAc as the eluent gave *title compound* **16** (1.58 g, 86%) as a solid (Found: C, 68.95; H, 6.4; N, 2.3. $C_{33}H_{35}NO_8$ requires C, 69.1; H, 6.15; N, 2.4%); R_f 0.55 [(2:1) toluene–EtOAc]; $[\alpha]_D$ −16.6 (*c* 0.6, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3400 (OH) and 1725 (OPiv); δ_H 7.85–7.60 (m, 4 H, phthaloyl), 7.35–7.02 (10 H, m, 2 × Ph), 5.13 (1 H, d, $J_{1,2}$ 8.3, 1-H), 4.55 (1 H, dd, $J_{5,6a}$ 4.2, $J_{6a,6b}$ 12, 6-H[a]), 4.35 (1 H, dd, $J_{5,6b}$ 2.0, 6-H[b]), 4.23 (1 H, dd, $J_{2,3}$ 10.6, $J_{3,4}$ 8.2, 3-H), 4.17 (1 H, dd, 2-H), 3.62 (1 H, m, 5-H), 3.56 (1 H, t, $J_{4,5}$ 9.2, 4-H) and 1.27 (9 H, s, $COCMe_3$).

Allyl 4-O-(2′-*Azido*-3′,4′,6′-*tri*-O-*benzyl*-2′-*deoxy*-β-D-*glucopyranosyl*)-3,6-*di*-O-*benzyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranoside* **18**.—A mixture of 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl trichloroacetimidate **17**[17] (840 mg, 1.35 mmol), compound **10** (550 mg, 1.04 mmol), and MS4A (1.5 g) in CH_2Cl_2 (25 cm^3) was stirred at room temperature for 30 min, then was cooled to −20 °C. To the cold, stirred suspension was added dropwise a solution of boron trifluoride–diethyl ether (50 mm^3) in CH_2Cl_2 (2 cm^3) under nitrogen, and the resulting mixture was stirred for a further 2 h at that temperature. After being neutralized with triethylamine, the reaction mixture was filtered through a Celite pad, which was then washed with CH_2Cl_2 (2 × 10 cm^3). The filtrate and washings were combined and concentrated to dryness. The residue was subjected to column chromatography with (7:1) toluene–EtOAc as the eluent to give *disaccharide* **18** (625 mg, 61% based on **10**) as a syrup (Found: C, 70.5; H, 6.0; N, 5.5. $C_{58}H_{58}N_4O_{11}$ requires C, 70.6; H, 5.9; N, 5.7%); R_f 0.59 [(5:1) toluene–EtOAc]; $[\alpha]_D$ +8.7; ν_{max}(film)/cm^{-1} 2080 (N_3); δ_H 7.65 (4 H, s, phthaloyl), 7.35–6.78 (25 H, m, 5 × Ph), 5.67 (1 H, m, OCH_2CH=CH_2), 5.15 (1 H, d, $J_{1,2}$ 8.4, 1-H), 5.08 and 5.00 (1 H each, d each, *J* 10.2 and 17.4, OCH_2CH=CH_2), 4.39 (1 H, d, $J_{1',2'}$ 8.1, 1′-H), 4.21 (1 H, dd, $J_{2,3}$ 9.5, 2-H), 3.38 (1 H, dd, $J_{2',3'}$ 9.5, 2′-H) and 3.29 (1 H, t, $J_{3',4'}$ 9.1, 3′-H).

4-O-(2′-*Azido*-3′,4′,6′-*tri*-O-*benzyl*-2′-*deoxy*-β-D-*glucopyranosyl*)-3,6-*di*-O-*benzyl*-2-*deoxy*-2-*phthalimido*-D-*glucopyranose* **19**.—A mixture of compound **18** (888 mg, 0.899 mmol), palladium(II) chloride (397 mg, 2.25 mmol), and sodium acetate (738 mg, 9.0 mmol) in 95% aq. acetic acid (20 cm^3) was stirred at 50 °C for 3 h; TLC [(3:1) toluene–EtOAc] then showed the complete reaction of compound **18**, with a less mobile spot on TLC. The reaction mixture was diluted with EtOAc (100 cm^3) and filtered through a Celite pad. The filtrate was washed successively with 5% aq. $NaHCO_3$, brine, and water, dried, and concentrated. Column chromatography of the residue with (5:1) toluene–EtOAc as the eluent gave *title compound* **19** (716 mg, 84%) as an anomeric mixture (Found: C, 69.5; H, 5.8; N, 5.7. $C_{55}H_{54}N_4O_{11}$ requires C, 69.75; H, 5.75; N, 5.9%); R_f 0.19 (α) and 0.15 (β) [(5:1) toluene–EtOAc]; ν_{max}(film)/cm^{-1} 3450 (OH) and 2090 (N_3); δ_H 7.68–7.60 (4 H, m, phthaloyl), 7.31–6.80 (25 H, m, 5 × Ph), 5.38 (0.65 H, d, $J_{1,2}$ 8.5, 1-Hβ-isomer), 5.30 (0.35 H, d, $J_{1,2}$ 3.6, 1-Hα-isomer) and 4.42 (1 H, d, $J_{1',2'}$ 8.2, 1′-H).

Allyl 3,6-*Di*-O-*benzyl*-4-O-*chloroacetyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranoside* **20**.—Compound **10** (1.60 g, 3.02 mmol) was dissolved in $(CH_2Cl)_2$ (15 cm^3) containing pyridine (0.72 cm^3), then a solution of chloroacetyl chloride (0.73 cm^3, 9.06 mmol) in $(CH_2Cl)_2$ (3 cm^3) was added, and the resulting

mixture was stirred at 10 °C for 30 min. The reaction mixture was poured into 5% aq. $NaHCO_3$ and extracted with $(CH_2Cl)_2$. The organic layer was washed successively with brine and water, dried, and concentrated. Column chromatography of the residue with (7:1) toluene–EtOAc as the eluent afforded *compound* **20** (1.61 g, 88%) as a foam (Found: C, 65.3; H, 5.5; N, 2.1; Cl, 5.7. $C_{33}H_{32}ClNO_8$ requires C, 65.4; H, 5.3; N, 2.3; Cl, 5.85%); R_f 0.63 [(5:1) toluene–EtOAc]; $[\alpha]_D$ +51.3 (*c* 0.9, $CHCl_3$); ν_{max}(film)/cm^{-1} 1735 (chloroacetyl); δ_H 7.70 (4 H, s, phthaloyl), 7.40–6.95 (10 H, m, 2 × Ph), 5.67 (1 H, m, CH_2=C*H*), 5.21 (1 H, t, $J_{3,4} = J_{4,5}$ = 9.3, 4-H), 5.19 (1 H, d, $J_{1,2}$ 8.5, 1-H), 5.10 and 5.02 (1 H each, d each, *J* 10.3 and 17.2, CH_2=CH), 4.48 (1 H, dd, $J_{2,3}$ 9.0, $J_{3,4}$ 10.7, 3-H), 4.28 (1 H, dd, $J_{1,2}$ 8.5, 2-H), 4.25 (1 H, dd, $J_{5,6a}$ 4.8, $J_{6a,6b}$ 13.0, 6-H[a]), 4.01 (1 H, dd, $J_{5,6b}$ 6.4, 6-H[b]), 3.75 (1 H, m, 5-H), 3.72 (2 H, m, OCH_2CH=) and 3.63 (2 H, s, CH_2Cl).

3,6-*Di*-O-*benzyl*-4-O-*chloroacetyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranose* **21**.—A mixture of glycoside **20** (1.56 g, 2.57 mmol), palladium(II) chloride (1.13 g, 6.43 mmol), and sodium acetate (1.26 g, 15.4 mmol) in 90% aq. acetic acid (25 cm^3) was stirred at 70 °C for 1 h, then was cooled, diluted with EtOAc (60 cm^3), and filtered through a Celite pad. The filtrate was washed successively with 5% aq. $NaHCO_3$, brine, and water, dried, and concentrated. The residue was subjected to column chromatography with (5:1) toluene–EtOAc as the eluent to give the *hemiacetal* **21** (1.40 g, 96%) which was shown to be an anomeric mixture (Found: C, 63.4; H, 5.75; N, 2.3; Cl, 6.1. $C_{30}H_{28}ClNO_8$ requires C, 63.7; H, 5.0; N, 2.5; Cl, 6.3%); R_f 0.35 (α) and 0.31 (β) [(3:1) toluene–EtOAc]; ν_{max}(film)/cm^{-1} 3450 (OH) and 1735 (chloroacetyl); δ_H 7.68 (4 H, m, phthaloyl), 7.35–6.87 (10 H, m, 2 × Ph), 5.45 (0.3 H, t, $J_{3,4} = J_{4,5}$ = 9.2, 4-Hα-isomer), 5.37 (0.7 H, d, $J_{1,2}$ 8.5, 1-Hβ-isomer), 5.30 (0.7 H, t, $J_{3,4} = J_{4,5}$ = 9.3, 4-Hβ-isomer) and 5.24 (0.3 H, d, $J_{1,2}$ 3.7, 1-Hα-isomer).

3,6-*Di*-O-*benzyl*-4-O-*chloroacetyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranosyl Trichloroacetimidate* **22b**.—To a stirred solution of compound **21** (566 mg, 1.0 mmol) in CH_2Cl_2 (15 cm^3) were successively added trichloroacetonitrile (1.30 cm^3, 13.0 mmol) and DBU (76.1 mg, 0.5 mmol) at 0 °C under nitrogen. The mixture was stirred at 20 °C for 2 h, then was concentrated to give an oil, which was directly subjected to column chromatography with (9:1) toluene–EtOAc as the eluent to afford *compound* **22b** (604 mg, 85%) as a syrup (Found: C, 53.8; H, 4.25; N, 3.7; Cl, 19.9. $C_{32}H_{28}Cl_4N_2O_8$ requires C, 54.1; H, 4.0; N, 3.9; Cl, 20.0%); R_f 0.56 [(5:) toluene–EtOAc]; $[\alpha]_D$ +68.5 (*c* 0.2, $CHCl_3$); δ_H 8.58 (1 H, s, NH), 7.75–7.62 (4 H, m, phthaloyl), 7.40–6.92 (10 H, m, 2 × Ph), 6.45 (1 H, d, $J_{1,2}$ 8.4, 1-H), 5.34 (1 H, t, $J_{3,4} = J_{4,5}$ = 9.5, 4-H), 4.61–4.45 (m, 2- and 3-H and 3/2 × CH_2Ph), 4.40 (1 H, d, J_{gem} 12.3, 1/2 × CH_2Ph), 3.71 (2 H, s, CH_2Cl) and 3.70–3.62 (3 H, m, 5-H and 6-H_2).

Benzyl 3-O-*Benzyl*-2-*deoxy*-4-O-(3′,6′-*di*-O-*benzyl*-4′-O-*chloroacetyl*-2′-*deoxy*-2′-*phthalimido*-β-D-*glucopyranosyl*)-2-*phthalimido*-6-O-*pivaloyl*-β-D-*glucopyranoside* **23**.—(a) *Preparation from chloride* **22a**. A solution of oxalyl dichloride (2.13 cm^3, 24.7 mmol) in CH_2Cl_2 (2 cm^3) was added dropwise to a cold solution of hemiacetal **21** (1.40 g, 2.47 mmol) and DMF (0.10 cm^3, 1.24 mmol) in CH_2Cl_2 (8 cm^3). After being stirred for 1 h at 5 °C, the mixture was diluted with CH_2Cl_2 (40 cm^3) and poured into ice–water. The organic layer was washed with cold water (3 × 20 cm^3), dried, and concentrated to give 3,6-di-*O*-benzyl-4-*O*-chloroacetyl-2-deoxy-2-phthalimido-D-glucopyranosyl chloride **22a** (1.29 g, 89%) as a pale yellow foam which was shown to be an anomeric mixture, R_f 0.64 [(5:1) toluene–EtOAc]; δ_H 6.22 (0.3 H, d, $J_{1,2}$ 3.7, 1-Hα-isomer), 5.98 (0.7 H, d, $J_{1,2}$ 8.9, 1-Hβ-isomer), 5.43 (0.3 H, t, $J_{3,4} = J_{4,5}$ = 9.1, 4-Hα-isomer) and 5.30 (0.7 H, t, $J_{3,4} = J_{4,5}$ =9.3, 4-Hβ-isomer). The crude chloride **22a** was used for the next glycosidation step without further purification.

A mixture of chloride **22a** (1.20 g, 2.05 mmol), compound **16** (1.06 g, 1.85 mmol), and MS4A (2 g) in CH_2Cl_2 (15 cm^3) was stirred for 30 min, then was cooled to −10 °C. To the cold suspension was added silver trifluoromethanesulfonate (633 mg, 2.46 mmol). The resulting mixture was stirred at −10 to 10 °C for 10 h, then was diluted with CH_2Cl_2 (45 cm^3), and filtered through a Celite pad. The filtrate was washed successively with 1% aq. $NaHCO_3$ and water, dried, and concentrated to dryness. The syrupy residue was subjected to column chromatography with toluene–EtOAc (10:1 ⟶ 5:1) as the eluent to afford *disaccharide* **23** (1.20 g, 58%) as a syrup (Found: C, 67.6; H, 5.6; N, 2.4; Cl, 12.4. $C_{63}H_{61}ClN_2O_{15}$ requires C, 67.5; H, 5.5; N, 2.5; Cl, 12.65%); R_f 0.50 [(5:1) toluene–EtOAc]; $[\alpha]_D$ +9.8 (*c* 0.8, $CHCl_3$); δ_H 7.75–7.60 (8 H, m, 2 × phthaloyl), 7.35–6.82 (20 H, m, 4 × Ph), 5.25 (1 H, d, $J_{1',2'}$ 8.3, 1′-H), 5.18 (1 H, t, $J_{3',4'} = J_{4',5'}$ = 9.2, 4′-H), 4.94 (1 H, d, $J_{1,2}$ 8.3, 1-H), 4.49 (1 H, t, $J_{2',3'}$ 9.3, 3′-H), 4.38–4.30 (2 H, m, 2′- and 6-H[a]), 4.15–4.05 (2 H, m, 2- and 3-H), 3.90 (1 H, t, $J_{3,4} = J_{4,5}$ = 9.5, 4-H), 3.75–3.65 (1 H, m, 5′-H), 3.61 (1 H, dd, $J_{5,6b}$ 4.8, $J_{6a,6b}$ 12.3, 6-H[b]), 3.49–3.56 (2 H, m, 6′-H_2), 3.48 (2 H, s, CH_2Cl), 3.44 (1 H, m, 5-H) and 1.16 (9 H, s, $COCMe_3$).

(b) *Preparation from trichloroacetimidate* **22b**. To a stirred mixture of the imidate **22b** (852 mg, 1.20 mmol), compound **16** (584 mg, 1.0 mmol), and MS4A (1.50 g) in CH_2Cl_2 (15 cm^3) was added dropwise a solution of boron trifluoride–diethyl ether (61 mm^3, 0.5 mmol) in CH_2Cl_2 (2.0 cm^3) at −15 °C under Ar. After being stirred at −15 °C for 2.5 h, the reaction mixture was neutralized with triethylamine, diluted with CH_2Cl_2 (50 cm^3), and filtered through a Celite pad. The filtrate was washed successively with 5% aq. $NaHCO_3$, brine, and water, dried, and concentrated to dryness. Column chromatography with (7:1) toluene–EtOAc as the eluent gave the disaccharide **23** (852 mg, 76%) which was identical with the product isolated in procedure (a).

Benzyl 3-O-*Benzyl*-2-*deoxy*-4-O-(3′-6′-*di*-O-*benzyl*-2′-*deoxy*-2′-*phthalimido*-β-D-*glucopyranosyl*)-2-*phthalimido*-6-O-*pivaloyl*-β-D-*glucopyranoside* **4**.—A mixture of chloroacetate **23** (300 mg, 0.267 mmol) and thiourea (500 mg) in pyridine–ethanol (6:1; 14 cm^3) was stirred at 70 °C for 1.5 h, then was poured into ice–water, and extracted with CH_2Cl_2 (2 × 30 cm^3). The extracts were combined, washed successively with dil. hydrochloric acid and water, dried, and concentrated. Column chromatography of the residue with (5:1) toluene–EtOAc as the eluent gave *compound* **4** (240 mg, 85.8%) as a syrup (Found: C, 69.9; H, 5.9; N, 2.6. $C_{61}H_{60}N_2O_{14}$ requires C, 70.1; H, 5.8; N, 2.7%); R_f 0.26 [(5:1) toluene–EtOAc]; $[\alpha]_D$ −7.1 (*c* 1.0, $CHCl_3$); δ_H 7.82–7.65 (8 H, m, 2 × phthaloyl), 7.32–6.79 (20 H, m, 4 × Ph), 5.26 (1 H, d, $J_{1',2'}$ 8.2, 1′-H), 4.93 (1 H, d, $J_{1,2}$ 8.1, 1-H), 4.25–4.33 (2 H, m, 3′-H and 6′-H[a]), 4.16 (1 H, dd, $J_{2',3'}$ 9.3, 2′-H), 4.12–4.08 (2 H, m, 2- and 3-H), 3.92 (1 H, t, $J_{3,4} = J_{4,5}$ = 9.3, 4-H), 3.83–3.75 (2 H, m, 4′-H and 6′-H[b]), 3.62–3.52 (3 H, m, 5′-H and 6-H_2), 3.38 (1 H, m, 5-H) and 1.17 (9 H, s, $COCMe_3$).

4-O-(2-*Azido*-3,4,6-*tri*-O-*benzyl*-2-*deoxy*-β-D-*glucopyranosyl*)-3,6-*di*-O-*benzyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranosyl Trichloroacetimidate* **3b**.—To a stirred solution of hemiacetal **19** (430 mg, 0.454 mmol) in CH_2Cl_2 (10 cm^3) were successively added, at 0 °C, trichloroacetonitrile (1.18 cm^3, 11.8 mmol) and DBU (32 mg, 0.227 mmol). After being stirred for 1 h at 20 °C, the mixture was evaporated and the residue was directly chromatographed using (10:1) toluene–EtOAc as the eluent to give the *trichloroacetimidate* **3b** (416 mg, 84%) as a syrup (Found: C, 62.6; H, 5.1; N, 6.3; Cl, 9.6. $C_{57}H_{54}Cl_3N_5O_{11}$ requires C, 62.7; H, 5.0; N, 6.4; Cl, 9.75%); R_f 0.64 [(5:1) toluene–EtOAc]; $[\alpha]_D$ +12.2 (*c* 0.5, $CHCl_3$); δ_H 8.53 (1 H, s,

NH) 7.68 (4 H, s, phthaloyl), 7.32–6.91 (25 H, m, 5 × Ph), 6.46 (1 H, d, $J_{1,2}$ 8.3, 1-H) and 4.43 (1 H, d, $J_{1',2'}$ 8.1, 1'-H).

Benzyl O-2‴-*Azido*-3‴,4‴,6‴-*tri*-O-*benzyl*-2‴-*deoxy*-β-D-*glucopyranosyl*)-(1→4)-O-(3″,6″-*di*-O-*benzyl*-2″-*deoxy*-2″-*phthalimido*-β-D-*glucopyranosyl*)-(1→4)-O-(3′,6′-*di*-O-*benzyl*-2′-*deoxy*-2′-*phthalimido*-β-D-*glucopyranosyl*)(1→4)-3-O-*benzyl*-2-*deoxy*-2-*phthalimido*-6-O-*pivaloyl*-β-D-*glucopyranoside* **2**.—(a) *Preparation from chloride* **3a**. To a cold solution of hemiacetal **19** (426 mg, 0.450 mmol) and DMF (70 mm^3, 0.90 mmol) in CH_2Cl_2 (5 cm^3) was added dropwise a solution of oxalyl dichloride (0.20 cm^3, 2.25 mmol) in CH_2Cl_2 (1 cm^3) at 0 °C. The mixture was stirred at that temperature for 3 h, then was poured into ice–water, and extracted with CH_2Cl_2 (3 × 20 cm^3). The extracts were combined, washed successively with brine and water, dried, and concentrated to give a yellow syrup, which was chromatographed with (9:1) hexane–EtOAc as the eluent to obtain 4-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-D-glucopyranosyl chloride **3a** (382 mg, 88%) as a pale yellow syrup which was shown to be a mixture of the α- and β-anomer; R_f 0.69 [(5:1) toluene–EtOAc]; ν_{max}(film)/cm^{-1} 2080 (N_3); δ_H 7.68–7.56 (4 H, m, phthaloyl), 7.31–6.83 (25 H, m, 5 × Ph), 6.26 (0.3 H, d, $J_{1,2}$ 3.8, 1-Hα-isomer), 6.01 (0.7 H, d, $J_{1,2}$ 8.8, 1-Hβ-isomer) and 4.4 (1 H, d, $J_{1',2'}$ 8.2, 1'-H). The chloride thus obtained was used directly for the next reaction without further purification.

A mixture of the crude chloride **3a** (500 mg, 0.518 mmol), compound **4** (460 mg, 0.440 mmol), and MS4A (2.50 g) in CH_2Cl_2 (15 cm^3) was stirred at room temperature for 30 min. Then the mixture was cooled to −20 °C and silver trifluoromethanesulfonate (133 mg, 0.570 mmol) was added under Ar. The resulting mixture was stirred at −20 °C for 6 h; TLC [(5:1) toluene–EtOAc)] showed the complete reaction of chloride **3a**. After being diluted with CH_2Cl_2 (50 cm^3), the suspension was filtered through a Celite pad. The filtrate was washed successively with brine and water, dried, and concentrated. Column chromatography of the residue with (9:1) toluene–EtOAc as the eluent gave *tetrasaccharide* **2** (434 mg, 50%) as a syrup (Found: C, 70.5; H, 5.8; N, 4.15. $C_{116}H_{112}N_6O_{24}$ requires C, 70.6; H, 5.7; N, 4.3%); R_f 0.47 [(5:1) toluene–EtOAc]; $[\alpha]_D$ −9.6 (*c* 0.4, $CHCl_3$); ν_{max}(film)/cm^{-1} 2090 (N_3) and 1735 (OPiv); δ_H 7.85–7.59 (12 H, m, 3 × phthaloyl), 7.35–6.65 (45 H, m, 9 × Ph), 4.88 (1 H, d, $J_{1,2}$ 8.5, 1-H), 5.25 (1 H, d, $J_{1',2'}$ 8.5, 1'-H), 5.08 (1 H, d, $J_{1'',2''}$ 8.1, 1″-H), 4.40 (1 H, d, $J_{1''',2'''}$ 8.0, 1‴-H) and 1.22 (9 H, s, $COCMe_3$).

(b) *Preparation from imidate* **3b**. To a stirred mixture of the imidate **3b** (382 mg, 0.35 mmol), compound **4** (329 mg, 0.315 mmol), and MS4A (0.8 g) in CH_2Cl_2 (10 cm^3) was added a solution of boron trifluoride–diethyl ether (17 mm^3, 0.14 mmol) in CH_2Cl_2 (1 cm^3) under Ar at −15 °C. The resulting mixture was stirred at that temperature for 3 h, then was diluted with CH_2Cl_2 (30 cm^3) and filtered through a Celite pad. The filtrate was washed successively with 5% aq. $NaHCO_3$, brine, and water, dried, and concentrated. Column chromatography of the residue with (9:1) toluene–EtOAc as the eluent gave the tetrasaccharide **2** (448 mg, 72%) which was identical with the product elucidated in procedure (a).

Benzyl O-(2‴-*Azido*-3‴,4‴,6‴-*tri*-O-*benzyl*-2‴-*deoxy*-β-D-*glucopyranosyl*)-(1→4)-O-(2″-*acetimido*-3″,6″-*di*-O-*benzyl*-2″-*deoxy*-β-D-*glucopyranosyl*)-(1→4)-O-(2′-*acetimido*-3′,6′-*di*-O-*benzyl*-2′-*deoxy*-β-D-*glucopyranosyl*)-(1→4)-2-*acetimido*-3-O-*benzyl*-2-*deoxy*-β-D-*glucopyranoside* **24**.—A solution of compound **2** (98.7 mg, 50 mm^3) in EtOH–NH_2NH_2·H_2O (10:1; 11 cm^3) was stirred for 16 h at 95 °C. At the end of that time, TLC [(50:15:1) toluene–EtOH–1,4-dioxane] examination revealed the formation of two new spots, with R_f 0.67 (major) and 0.61 (minor). Then the ethanol and excess of hydrazine were evaporated off under reduced pressure and the residue was co-evaporated twice with ethanol (2 × 10 cm^3). The resulting residue was dissolved in (1:1) pyridine–acetic anhydride (4 cm^3). After being stirred at 20 °C for 6 h, the reaction mixture was concentrated, and the residual volatiles were co-evaporated with toluene–ethanol. The residue was then purified by chromatography on a Bio-beads S-X3 column (15 × 150 mm) eluted with toluene to give a major component, R_f 0.59–0.61 in (20:1) $CHCl_3$–EtOH, which was de-*O*-acylated as follows: the product was dissolved in (1:1) MeOH–THF (3 cm^3), powdered potassium hydroxide (50 mg) was added, and the mixture was stirred at 20 °C for 5 h, then was poured into water, and extracted with CH_2Cl_2 (12 cm^3). The extract was washed with water, dried, and concentrated. Column chromatography of the residue with (25:1) $CHCl_3$–EtOH as the eluent afforded *tetrasaccharide* **24** (53.6 mg, 66% from **2**) as a powder (Found: C, 68.6; H, 6.5; N, 5.0. $C_{93}H_{104}N_6O_{20}$ requires C, 68.7; H, 6.45; N, 5.2%); R_f 0.38 in [(20:1) $CHCl_3$–EtOH]; $[\alpha]_D$ −38.5 (*c* 0.4, $CHCl_3$); ν_{max}(film)/cm^{-1} 3500 and 3350 (OH and NH), 2080 (N_3) and 1670 (NHAc); δ_H 6.52 (1 H, d, *J* 7.5, N*H*Ac), 5.75 (1 H, d, *J* 7.8, N*H*Ac), 4.66 (1 H, d, *J* 6.7, anomeric H), 4.45 (1 H, d, *J* 6.7, anomeric H), 4.35 (1 H, d, *J* 8.1, anomeric H), 3.96 (1 H, d, *J* 8.2, anomeric H) and 1.92, 1.86 and 1.70 (each 3 H, each s, 3 × Ac); δ_C 171.26, 170.60 and 170.09 (3 × COMe), 101.83, 100.97, 99.66 and 99.46 (anomeric C), 61.44 (C-2‴), 58.48, (C-6), 54.38, 54.31 and 52.63 (C-2, -2′, -2″), and 23.51, 23.38 and 23.15 (3 × CO*Me*).

Benzyl O-(2-*Azido*-3,4,6-*tri*-O-*benzyl*-2-*deoxy*-β-D-*glucopyranosyl*)-(1→4)-O-(2-*acetimido*-3,6-*di*-O-*benzyl*-2-*deoxy*-β-D-*glucopyranosyl*)-(1→4)-O-(2-*acetimido*-3,6-*di*-O-*benzyl*-2-*deoxy*-β-D-*glucopyranosyl*)-(1→4)-2-*acetimido*-3-O-*benzyl*-2-*deoxy*-6-O-*sulfo*-β-D-*glucopyranoside*, *Sodium Salt* **25**.—Compound **24** (23.6 mg, 14.5 μmol) was dissolved in dry (1:1) DMF–pyridine (1 cm^3) and a solution of sulfur trioxide–pyridine complex (18.5 mg, 116 μmol) in dry DMF (1.5 cm^3) was added dropwise. The resulting solution was stirred at 50 °C for 6 h, then was cooled to 0 °C, and excess of the reagent was destroyed by addition of methanol (0.5 cm^3). Solvent was evaporated off under reduced pressure. The residue was dissolved in $CHCl_3$, and placed on a column (1.5 × 20 cm) of Sephadex LH-20 pre-equilibrated with (1:1) $CHCl_3$–MeOH. The column was eluted with the same solvent, and the fractions containing the product were collected. Then the product was subjected to chromatography on a column (1.0 × 15 cm) of Dowex 50w-x8 (Na^+) resin, pre-equilibrated with methanol, by elution with methanol to give *compound* **25** (20.3 mg, 81%) as a powder (Found: C, 63.4; H, 6.3; N, 4.5; S, 1.55. $C_{93}H_{103}N_6NaO_{23}S$·$2H_2O$ requires C, 63.3; H, 6.1; N, 4.8; S, 1.8%); R_f 0.51 [(5:1) $CHCl_3$–EtOH]; $[\alpha]_D$ −27.7 (*c* 0.3, $CHCl_3$); δ_H(CD_3OD) (only broad signals) 7.13–7.31 (45 H, m, 9 × Ph) and 1.90, 1.82 and 1.75 (3 H each, s each, 3 × NAc).

Methyl (2E,9Z)-*Hexadeca*-2,9-*dienoate* **27**.—To a suspension of sodium hydride (80%; 132 mg, 4.4 mmol) in THF (40 cm^3) was added a solution of methyl (diethoxyphosphoryl)acetate (924.7 mg, 4.4 mmol) in THF (5 cm^3), and the mixture was stirred for 30 min at 20 °C, then a solution of (*Z*)-tetradec-7-enal **26** (840 mg, 4.0 mmol; Sigma) in THF (4 cm^3) was added dropwise. A syrup appeared during the reaction. After being stirred for 2.5 h at 20 °C, the reaction mixture was poured into ice–water (50 cm^3), and extracted with diethyl ether (2 × 50 cm^3). The extracts were combined, washed successively with brine and water, dried, and concentrated. The residue was subjected to column chromatography with (7:1) hexane–diethyl ether as the eluent, to give compound **27** (980 mg, 92%) as an oil, R_f 0.73 [(5:1) hexane–diethyl ether]; *m*/*z* (EI): 267

(M + 1); δ_H 6.95 (1 H, dt, *J* 7.0 and 14.8, CH=CHCO), 5.82 (1 H, d, *J* 15.3, CH=CHCO), 5.38 (1 H, m, CH_2CH=$CHCH_2$), 3.73 (3 H, s, OMe), 2.20 (2 H, m, CH_2CH=CHCO), 2.01 (4 H, m, CH_2CH=$CHCH_2$), 1.22–1.54 (14 H, m, 7 × CH_2) and 0.88 (3 H, t, *J* 7.2, CH_2*Me*).

(2E,9Z)-*Hexadeca*-2,9-*dienoic Acid* **28**.—A solution of ester **27** (826 mg, 3.10 mmol) in THF (1 cm^3) was added to 1.5 mol dm^{-3} potassium hydroxide (35 cm^3), and the resulting mixture was stirred at 70 °C for 6 h, then was neutralized with 1 mol dm^{-3} HCl and the pH-value of the solution was adjusted to 3. The solution was extracted with CH_2Cl_2 (3 × 15 cm^3). The extracts were combined, washed successively with brine and water, dried, and concentrated. Column chromatography of the residue with (2:1) hexane–diethyl ether as the eluent gave compound **28** (710 mg, 90%) as a syrup, R_f 0.15 [(5:1) hexane–diethyl ether]; *m/z* (EI) 252 (M^+, 5%); δ_H 10.3 (1 H, br s, OH), 7.10 (1 H, dt, *J* 7.0 and 14.6, CH=CHCO), 5.85 (1 H, d, *J* 15.4, CH=CHCO), 5.35 (2 H, m, *Z*-CH=CH), 2.25 (2 H, m, CH_2CH=CHCO), 2.02 (4 H, m, CH_2CH=$CHCH_2$), 1.25–1.50 (14 H, m, 7 × CH_2) and 0.90 (3 H, t, *J* 7.0, CH_2*Me*).

N-[(2E,9*Z*)-*Hexadeca*-2,9-*dienoyl*]*thiazolidine*-2-*thione* **29**.—To a solution of acid **28** (100 mg, 0.397 mmol) and thiazolidine-2-thione (56.7 mg, 0.476 mmol) in ethylene glycol bismethyl ether (DME) (2.5 cm^3) was added DCC (98.2 mg, 0.476 mmol) in portions at 0 °C under N_2. Then the mixture was stirred at 20 °C. A solid appeared and the solution gradually became yellow. After reaction for 8 h, the mixture was poured into ice–water (10 cm^3) and extracted with diethyl ether (3 × 15 cm^3). The extracts were combined, washed with water, dried, and concentrated. Column chromatography of the residue with (5:1) hexane–diethyl ether as the eluent gave compound **29** (85%) as a yellow oil, R_f 0.27 [(5:1) hexane–diethyl ether]; *m/z* (EI): the molecular ion didn't appear; δ_H 6.82 (1 H, dt, *J* 7.0 and 14.6, 3-H), 5.72 (1 H, d, *J* 15.6, 2-H), 5.31 (2 H, m, *Z*-CH=CH), 4.58 (2 H, t, *J* 7.1, CH_2N), 3.48 (2 H, t, *J* 7.1, CH_2S), 2.24–2.02 (6 H, m, 4-, 8- and 11-H_2), 1.49–1.24 (14 H, m, CH_2 in chain) and 0.87 (3 H, t, *J* 7.0, Me).

O-(2‴-*Amino*-2‴-*deoxy*-β-D-*glucopyranosyl*)-(1→4)-O-(2″-*acetamido*-2‴-*deoxy*-β-D-*glucopyranosyl*)-(1→4)-O-(2′-*acetamido*-2′-*deoxy*-β-D-*glucopy-ranosyl*)-(1→4)-2-*acetamido*-2-*deoxy*-6-O-*sulfo*-D-*glucopyranose*, *Sodium Salt* **30**.—Compound **25** (4.0 mg, 2.31 μmol) was dissolved in a mixed solvent of THF–EtOH–water (1:1:0.5; 2.5 cm^3) containing 10% palladium on carbon (20 mg), and the resulting mixture was stirred under hydrogen for 24 h at 25 °C. Catalyst was removed by filtration, and the filtrate was evaporated to dryness. The residue was dissolved in water and lyophilized to give compound **30** (2.1 mg, 83%) as a powder, R_f 0.12 [(6:4:1) $CHCl_3$–MeOH–water]; $[\alpha]_D$ +3.8 (*c* 0.1, water); δ_H(D_2O) 5.13 (0.8 H, d, $J_{1,2}$ 3.3, 1-Hα-isomer), 4.85 (1 H, d, $J_{1',2'}$ 7.5, 1′-H), 4.68 (1 H, d, $J_{1'',2''}$ 8.5, 1″-H), 4.21 (1 H, dd, *J* 4.6 and 11, 6-H^a), 4.08 (1 H, dd, *J* 2.3 and 11, 6-H^b) and 2.00, 2.03 and 2.05 (each 3 H, each s, 3 × NAc). No signals for aromatic protons were detected.

O-{2-*Deoxy*-2-[(2E,9Z)-*hexadeca*-2,9-*dienamido*]-β-D-*glucopyranosyl*}-(1→4)-O-(2-*acetamido*-2-*deoxy*-β-D-*glucopy-ranosyl*)-(1→4)-O-(2-*acetamido*-2-*deoxy*-β-D-*glucopyranosyl*)-(1→4)-2-*acetamido*-2-*deoxy*-6-O-*sulfo*-D-*glucopyranose*-(*NodRm*-1), *Sodium Salt* **1**.—Compound **30** (2.0 mg, 2.24 μmol) was dissolved in 95% ethanol (1 cm^3) containing a catalytic amount of triethylamine, and to the solution was added compound **29** (7.93 mg, 22.4 μmol). The mixture was stirred at 45 °C for 72 h. Then the suspension was concentrated, and the residue was passed through a column of Bio-gel P-4. The fractions containing title product were combined and passed through a column of Dowex 50-x8 (Na^+). Lyophilization of the fractions gave compound **1** (1.12 mg, 45%) as a pale brown-yellow powder, R_f 0.39 [(50:40:10) $CHCl_3$–MeOH–water]; $[\alpha]_D$ −1.2 (*c* 0.1, water); δ_H (D_2O) 6.81 (1 H, dt, *J* 7.1 and 15.3, 3-H in chain), 5.98 (1 H, d, *J* 15.3, 2-H in chain), 5.36 (2 H, m, 9- and 10-H in chain), 5.09 (0.7 H, d, *J* 3.3, 1-Hα-isomer), 4.61–4.50 (3.3 H, m, β-anomeric H), 4.20 (1 H, dd, *J* 7.5 and 10.5, 6-H^a), 4.08 (1 H, dd, *J* 2.0 and 10.5, 6-H^b), 2.23–2.19 (2 H, m, 4-H_2 in chain), 2.04–2.00 (4 H, m, 8- and 11-H_2 in chain), 2.01, 2.00 and 1.98 (3 H each, s each, NAc), 1.48–1.25 (14 H, m, CH_2 in chain) and 0.88 (3 H, m, Me in chain).

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