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Total Synthesis of the Sulfated Lipooligosaccharide Signal Involved in Rhizobium Meliloti-Alfalfa Symbiosis[1]

Lai-Xi Wang, Chuan Li, Qin-Wei Wang, and Yong-Zheng Hui*

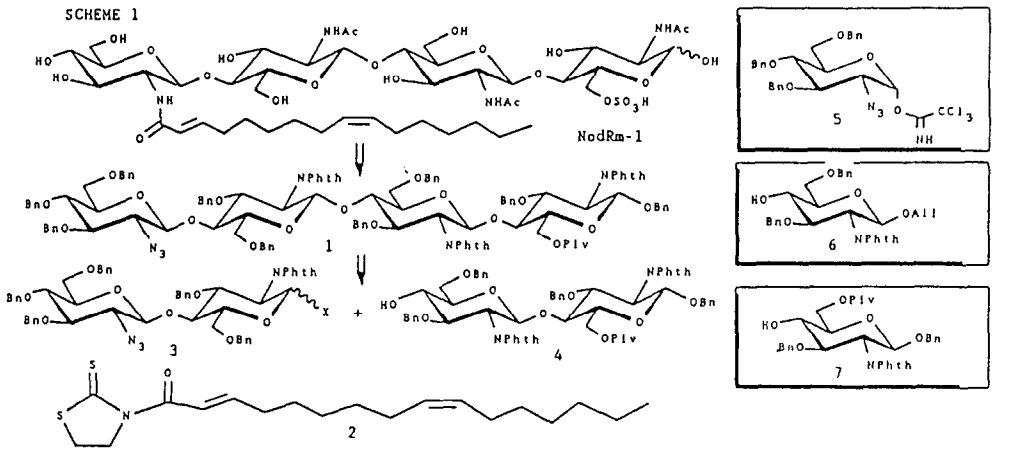
State Key Lab of Bioorganic and Natural Products Chemistry
Shanghai Institute of Organic Chemistry, Academia Sinica.
345 Lingling Lu, Shanghai 200032, China

Abstract: A total synthesis of a sulfated lipooligosaccharide, the nodulation signal involved in the symbiosis of rhizobium meliloti-alfalfa, was achieved in a stereocontrolled manner.

Recently, Lerouge et al first reported the characterization of the nodulation signal involved in R. meliloti-alfalfa symbiosis. The signal, called NodRm-1, was shown to be a sulfated lipotetrasaccharide of D-glucosamine which can elicit root hair deformation on alfalfa at 10^{-9} M but not on vetch (a heterogenous host).[2-4] Interestingly, the desulfated compound can elicit the same organogenesis and root morphology on vetch but not on alfalfa.[5-7] These findings, together with the natural scarcity and fancinating structure of NodRm-1, have aroused great interests in carrying out its chemical synthesis.[1,8] Herein we describe an unambiguous, total synthesis of NodRm-1.

The synthetic challenge comes from the regio- and stereochemistry and the variety of functional groups (Sulfur, Nitrogen, and unsatuation) in NodRm-1. As shown in Scheme 1, a suitably protected tetrasaccharide **1** and the activated 3-acylthiazoline-2-thione **2**[9] were designed as the key intermediates. Disconnection of **1** led to the disaccharides **3** and **4** which could, in turn, be synthesized from monosaccharides **5**, **6**, and **7**.

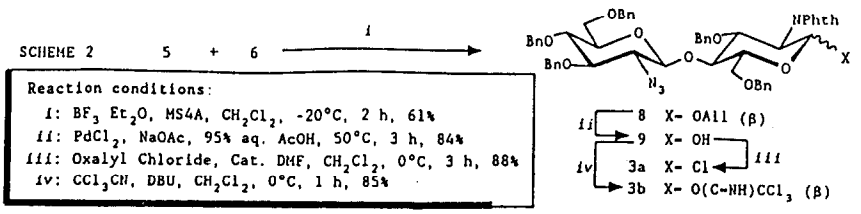
SCHEME 1



Ac = Acetyl; Bn = Benzyl; Phth = Phthaloyl; Piv = Trimethylacetyl

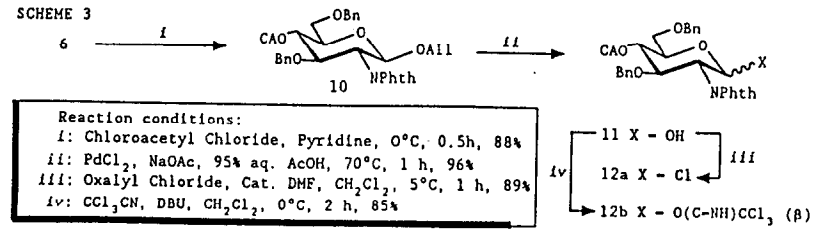
BF_3 Et_2O-promoted glycosylation of imidate **5**[10] with **6**[11] led to a 61% yield of the desired **8**, the ^{1}H-NMR data of which for H-1′ (δ 4.39, $J_{1',2'}$ 8.1 Hz) clearly indicated the β-D glycosidic linkage. De-O-allylation of

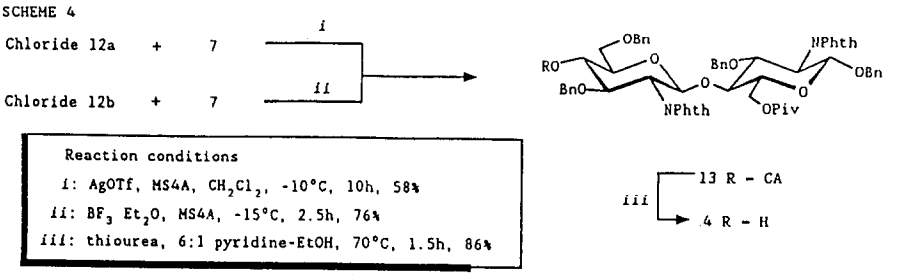
8 with $PdCl_2$-NaOAc[12] in 95% aq. AcOH gave hemiacetal **9** (84%) which was then converted into a glycosyl donor **3a** or **3b** as follows. Reaction of **9** with oxalyl chloride-DMF provided the chloride **3a** (88%), and treatment of **9** with DBU and CCl_3CN gave the desired β-imidate **3b** (85%) (Scheme 2).



To synthesize compound **4**, the 4-hydroxyl group in **6** was tentatively protected with chloroacetyl group to provide **10** (87%). Then **10** was changed into the hemiacetal **11**, which was finally converted into the chloride **12a** (89%) and imidate **12b** (85%) as shown in Scheme 3.

Glycosylation of **12a** and **7**[13,14] in the presence of (AgOTf)[15] gave **13** (58%) with a β-glycosidic linkage as expected from the β-directing effect of the N-phth group. Whereas BF_3 Et_2O-promoted coupling of **12b** with **7** led to a 76% yield of **13**. Then selective liberation of the 4'-OH was achieved by reaction of **13** with thiourea[16] to give compound **4** (86%) (Scheme 4).



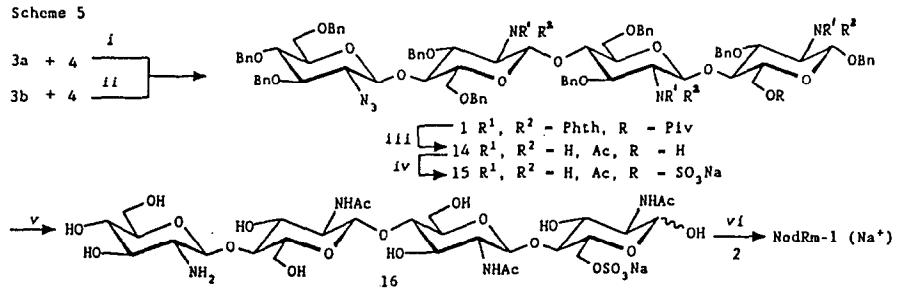


With suitable disaccharide donor and acceptor at hand, next the crucial couplings between them were examined. AgOTf-Promoted reaction of **3a** with **4** did afford the desired tetrasaccharide **1** (50%). Whereas the coupling of imidate **3b** with **4** in the presence of BF_3 Et_2O was shown to be more efficient, giving a 72% yield of compound **1** (Scheme 5). Again, the stereochemistry of compound **1** was confirmed by its ^{1}H-NMR spectrum.

The N-phth groups in **1**, which have once exerted activating and β-directing functions in glycosylations, were then replaced by N-acetyl groups to give **14** having 6-OH free in 3 steps (66% from **1**) (Scheme 5). Sulfation of **14** with SO_3-Py complex[17] in DMF proceeded smoothly to yield **15** (81%). Hydrogenolysis of **15** for both de-O-protection and simultaneous reduction of the azido function was performed with 10% Pd-C, giving free amino sugar **16** (83%) as a white foam after lyophilization. Finally, the selective N-acylation was achieved by reaction of **16** with activated fatty

acid 2[18] to provide the target NodRm-1 as its sodium salt, the ^{1}H-NMR data of which were in reasonable agreement with those reported.[3,8]

Scheme 5



Reaction conditions:

i: AgOTf, MS4A, CH_2Cl_2, -20°C, 6h, 50%. *ii*: BF_3 Et_2O, MS4A, -15°C, 3h, 72%.

iii: (a) NH_2NH_2 H_2O, EtOH, 95°C, 12 h; (b) Ac_2O, pyridine, 20°C, 6 h;
(c) KOH, 1:1 MeOH-THF, 20°C, 5 h, 66% in 3 steps. *iv*: (a) SO_3-Py complex, DMF, 50°C, 6 h;
(b) Sephadex LH-20 eluted with MeOH; (c) Dowex 50-x8 (Na^+) eluted with MeOH, 81%.
v: H_2, 10% Pd/C, 1:1:0.5 EtOH-THF-H_2O, 25°C, 24h; then Bio-gel P-2 and Dowex 50-x8 (Na^+), 83%
vi: 95% EtOH, Et_3N, 45°C, 3 days, 45%.

In summary, an unambiguous, total synthesis of the sulfated lipooligosaccharide nodulation signal (NodRm-1) was achieved in a regio- and stereo-controlled manner.

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13. Compound **7** was prepared by selective pivaloylation of benzyl 3-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (Ref. 14).
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18. **2** was synthesized by reaction of 2-trans-9-cis-hexadecadienoic acid (prepared from 7-cis-tetradecenal) with thiazolidine-2-thione, DCC, DME, 20°C, 6h, 93%.
19. New compounds gave satisfactory elemental analysis. Selected physical data for some key intermediates are given below. Values of $[\alpha]_D$ and $\delta_{H,C}$ (600 MHz) were measured at 25°C for solutions in $CHCl_3$ and $CDCl_3$, respectively, unless otherwise noted. **NodRm-1 (Na^+)**: $[\alpha]_D$ -1.2° (c 0.1, H_2O); δ_H (D_2O) 6.81 (dt, 1 H, J 7.1, 15.3 Hz, H-3 in chain), 5.98 (d, 1 H, J 15.3 Hz, H-2 in chain), 5.36 (m, 2 H, H-9,10 in chain), 5.09 (d, 0.7 H, J 3.3 Hz, H-1α), 4.61-4.50 (m, 3.3 H, β-anomeric H), 4.20 (dd, 1 H, J 7.5, 10.5 Hz, H-6a), 4.08 (dd, 1 H, J 2.0, 10.5 Hz, H-6b), 2.23-2.19 (m, 2 H, H-4 in chain), 2.04-2.00 (m, 4 H, H-8,11 in chain), 2.01, 2.00, and 1.98 (s each, 3 H each, NAc), 1.48-1.25 (m, 14 H, CH_2 in chain), 0.88 (m, 3 H, CH_3 in chain). **1**: $[\alpha]_D$ -9.6° (c 0.4); δ_H 4.88 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 5.25 (d, 1 H, $J_{1',2'}$ 8.5 Hz, H-1′), 5.08 (d, 1 H, $J_{1'',2''}$ 8.1 Hz, H-1′′), 4.40 (d, 1 H, $J_{1''',2'''}$ 8.0 Hz, H-1′′′), 1.22 (s, 9 H, $COCMe_3$). **2**: δ_H 6.82 (dt, 1 H, J 7.0, 14.6 Hz, H-3), 5.72 (d, 1 H, J 15.6 Hz, H-2), 5.31 (m, 2 H, cis-CH=CH), 4.58 (t, 2 H, J 7.1 Hz, CH_2N), 3.48 (t, 2 H, J 7.1 Hz, CH_2S), 2.24-2.02 (m, 6 H, H-4,8,11), 1.49-1.24 (m, 14 H, CH_2 in chain), 0.87 (t, 3 H, J 7.0 Hz, CH_3). **3a**: υ_{max}(film) 2080 cm^{-1} (N_3); δ_H 6.26 (d, 0.3 H, $J_{1,2}$ 3.8 Hz, H-1α), 6.01 (d, 0.7 H, $J_{1,2}$ 8.8 Hz, H-1β), 4.4 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1′). **3b**: $[\alpha]_D$ +12.2° (c 0.5); δ_H 8.53 (s, 1 H, NH), 6.46 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.43 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1′). **4**: $[\alpha]_D$ -7.1° (c 1.0); δ_H 5.26 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1′), 4.93 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 1.17 (s, 9 H, $COCMe_3$). **8**: $[\alpha]_D$ +8.7° (c 0.5); υ_{max} (film) 2080 cm^{-1} (N_3); δ_H 5.67 (m, 1 H, C<u>H</u>=CH_2), 5.15 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 5.08 and 5.00 (d each, 1 H each, J 10.2 and 17.4 Hz, CH=C<u>H</u>$_2$), 4.39 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1′). **9**: υ_{max}(film) 3450 (OH) and 2090 cm^{-1} (N_3); δ_H 7.68-7.60 (m, 4 H, phthaloyl), 5.38 (d, 0.65 H, $J_{1,2}$ 8.5 Hz, H-1β), 5.30 (d, 0.35 H, $J_{1,2}$ 3.6 Hz, H-1α), 4.42 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1′). **13**: $[\alpha]_D$ +9.8° (c 0.8); δ_H 5.25 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1′), 5.18 (t, 1 H, J 9.2 Hz, H-4′), 4.94 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 3.48 (s, 2 H, CH_2Cl), 1.16 (s, 9 H, $COCMe_3$). **14**: $[\alpha]_D$ -38.5° (c 0.4); υ_{max}(film) 3500 and3350 (OH and NH), 2080 (N_3), and 1670 cm^{-1} (NHAc); δ_H 6.52 (d, 1 H, J 7.5 Hz, NHAc), 5.75 (d, 1 H, J 7.8 Hz, NHAc), 4.66, 4.45, 4.35, and 3.96 (d each, 1 H each, J 6.7-8.1 Hz, 4 β-anomeric H), 1.92, 1.86, and 1.70 (s each, 3 H each, 3 NAc); δ_C 171.26, 170.60, and 170.09 (3 <u>C</u>OCH_3), 101.83, 100.97, 99.66, and 99.46 (4 β-anomeric C), 54.38, 54.31, and 52.63 (C-2,2′,2′′), 58.48 (C-6), 61.44 (C-2′′′), 23.51, 23.38, and 23.15 (3 CO<u>C</u>H_3). **15**: $[\alpha]_D$ -27.7° (c 0.3); δ_H (CD_3OD) (broad signals) 7.13-7.31 (m, 45 H, aromatic H), 1.90, 1.82, and 1.75 (s, 3 H each, 3 OAc). **16**: $[\alpha]_D$ +3.8° (c 0.1, H_2O); δ_H (D_2O) 5.13 (d, 0.8 H, $J_{1,2}$ 3.3 Hz, H-1α), 4.85 and 4.68 (d each, 1 H each, J 7.5 and 8.4 Hz, β-anomeric H), 4.20 (dd, 1 H, J 4.6, 11 Hz, H-6a), 4.08 (dd, 1 H, J 2.3, 11 Hz, H-6b), 2.00, 2.03, and 2.05 (s, 3 H each, 3 NAc).

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