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SYNTHESIS OF THE ROOT NODULE-INDUCING FACTOR NodRm-IV(C16:2,S) OF *RHIZOBIUM MELILOTI* AND RELATED COMPOUNDS[1]

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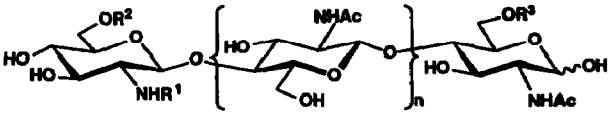
Abstract: Nod factors NodRm-IV(RCO,S) that carry natural as well as unnatural fatty acids were synthesized in a stereocontrolled manner.

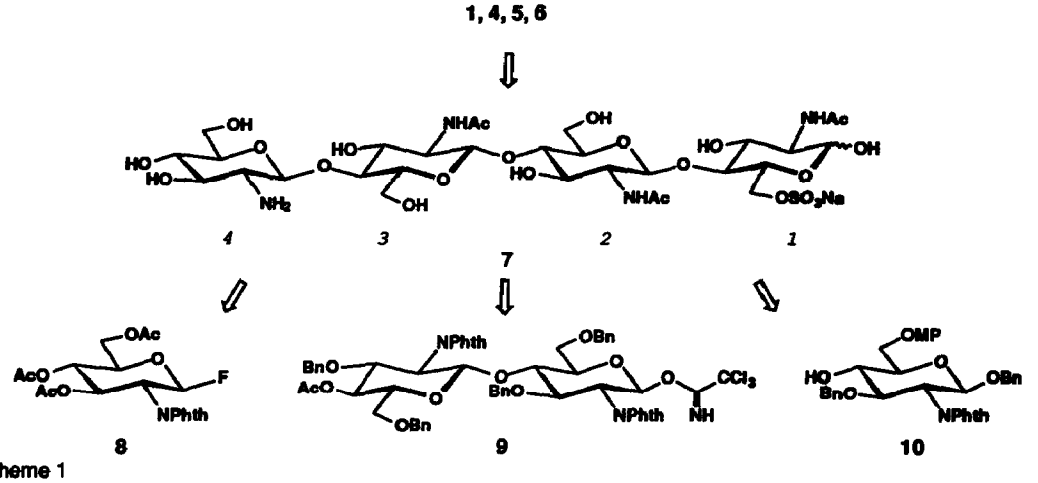
Rhizobiumn-legume symbiosis results in the formation of root nodules where nitrogen can be fixed, thus providing a natural alternative to the use of ammonium fertilizer. In 1990, NodRm-IV(C16:2,S)[2] was isolated as a major alfalfa-specific nodulation signal from the culture media of *Rhizobium meliloti* strains that overexpressed symbiotic extracellular Nod signals and the structure was reported to be a sulfated lipooligosaccharide **1** (n=2) by Lerouge et al[3]. Other Nod signals **2** and **3** of related structures that were produced by *R. meliloti* have also been reported[4,5,6]. It is to be noted that in 1992 Nicolaou and his co-workers[8] reported a first synthesis of **1** (n=2) and **2** (n=2). In addition, other synthetic approaches towards related Nod factors have also been reported[9] in the preliminary forms.

As part of our project on the synthesis of complex glycoconjugates that have plant hormon activity, we describe here a versatile approach to the synthesis of NodRm-IV (C16:2,S) **1** (n=2) and analogues unnatural compounds **4**, **5**, and **6**, aiming at the elucidation of the biological significance of fatty acyl groups linked to the non-reducing end $GlcNH_2$ residue[7].



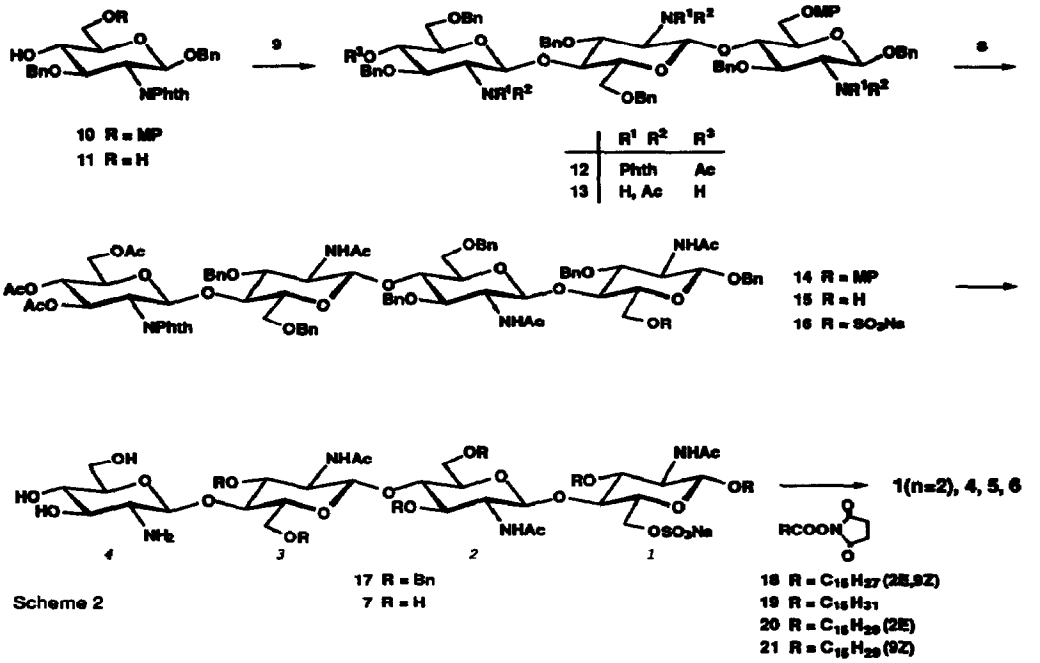
	R^1	R^2	R^3	n
1	$COC_{15}H_{27}$ (2E,9Z)	H	SO_3Na	2,3
2	$COC_{15}H_{27}$ (2E,9Z)	Ac	SO_3Na	2,3
3	$COC_{15}H_{25}$(2E,4E,9Z)	H	SO_3Na	1,2,3
4	$COC_{15}H_{31}$	H	SO_3Na	2
5	$COC_{15}H_{29}$(2E)	H	SO_3Na	2
6	$COC_{15}H_{29}$(9Z)	H	SO_3Na	2

Based on a retrosynthetic bond disconnections we designed a key intermediate **7** so that any acyl group can be introduced efficiently at the final step of our synthetic sequence. Compound **7** can be further disconnected into two glycosyl donors **8** and **9** and a glycosyl acceptor **10**.



Scheme 1

The glycosyl donor **9** was already reported[10] and two other synthons **8** and **10** were readily available as follows. 1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose was converted into **8**[11] in two steps, *1*) NH_2NH_2•AcOH in DMF[12], *2*) DAST in $(ClCH_2)_2$[13], 73% overall. Readily available compound **11**[14] was treated with p-MeOPhOH, Ph_3P and DEAD in CH_2Cl_2 to give 97% of **10**[11]. Having three designed synthons **8**, **9**, and **10** in hands, we carried out chain extention experiments directed toward a key intermediate **7**. Coupling of **9** with 1.1 equivalents of **10** in the presence of catalytic amount (0.2 equivalents) of BF_3•OEt_2 in CH_2Cl_2 at -78° afforded 69% of **12**[11], which was treated first with 1:11 NH_2NH_2-EtOH, then 1:2 Ac_2O-MeOH to afford 99% of **13**[11]. Glycosylation of **13** with 3 equivalents of **8** was promoted by 1:2 Cp_2HfCl_2-AgOTf[15] in $(ClCH_2)_2$ to give 79% of **14**. Introduction of sulfate at O-6*I* of the tetrasaccharide **14** was performed in 2 steps to give **16**[11] via **15**[11]; *1*) $(NH_4)_2Ce(NO_2)_6$ (CAN) in 3:1 CH_3CN-H_2O[16]; *2*) Et_3NSO_3 in DMF at 50°, then Dowex 50 (Na^+) in 8:1 MeOH-H_2O, 70% overall. Compound **16** was then converted into the designed intermediate **7** in 2 steps via **17**; *1*) NH_2NH_2•H_2O in EtOH; *2*) Pd-black, H_2 in 4:1 MeOH-H_2O, then Sephadex LH-20 in 4:1 MeOH-H_2O; 88% overall. Final N-acylation of **7** was carried out with O-acyl-N-hydroxysuccinimide **18**~**21** in 4:1 MeOH-H_2O. The reaction mixture was submitted to Sephadex LH-20 to give a mixture of recovered **7** and the N-acylated product from which the desired product was further purified by use[17] of short column of C_{18}Silica (Bond elute, Uniflex Co.). The column was first washed by 1:4 MeOH-H_2O to recover **7** and then eluted in 4:2:1 MeOH-H_2O-$CHCl_3$ to afford target compounds **1** (97%), **4** (50%), **5** (55%), and **6** (72%), respectively[18]. ^{1}H-NMR data of synthetic **1** were in complete agreement with those[3] of the natural sample.



Scheme 2

In summary, an efficient and practical synthetic route toward a variety of Nod factors NodRm-IV(RCO,S) has been developed by employing glycotetraose **7** as a key intermediate.

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References and Notes

1 Part 12 in the series "Plant and microbial glycoconjugate". For part 11, see N. Hong, Y. Nakahara, and T. Ogawa, *Proc. Japan Acad.*, **69(B)** 55-60 (1993). A part of this work was orally presented at the annual meeting of the Japan society of bioscience, biotechnology, and agricultural chemistry, April 1992.

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11 Physical data for novel compounds are presented below. Values of $[\alpha]_D$ and $\delta_{H,C}$ were recorded at 25°±3° for solutions in $CHCl_3$ and $CDCl_3$, respectively, unless otherwise indicated. Signal assignment such as H-3^2 stands for a proton at C-3 of sugar residue *2*. **1**: R_F 0.51 in 2:1:1 nBuOH-EtOH-H_2O; δ_H (CD_3OD) 6.73 (dt, 6.9 and 15.6 Hz, C*H*=), 5.87 (d, 15.7 Hz, C*H*=), 4.96 (d, 3.5 Hz, 1^1), 4.52, 4.42, and 4.42 (3d, 8.3 Hz, $1^{2,3,4}$), 4.15 (dd, 2.9 and 10.7 Hz, 6^1), 3.97 (dd, 1.9 and 10.7 Hz, 6^1), 1.96, 1.90 and 1.88 (3s, 3 x NAc). **4**: R_F 0.52 in 2:1:1 nBuOH-EtOH-H_2O; δ_H (4:2:1 CD_3OD-D_2O-$CDCl_3$) 5.14 (d, 3.4 Hz, 1^1), 4.62, 4.55, 4.54 (3d, 8.2 Hz, $1^{2,3,4}$), 4.21 (bd, 10.4 Hz, 6^1), 4.13 (bd, 10.4 Hz, 6^1), 2.08, 2.04, 2.02 (3s, 3 x NAc). **5**: R_F 0.50 in 2:1:1 nBuOH-EtOH-H_2O; δ_H (CD_3OD) 6.81 (dt, 7.3 and 15.1Hz, C*H*=), 5.94 (d, 15.1 Hz, C*H*=), 5.05 (d, 3.4 Hz, 1^1), 4.61, 4.50, 4.50 (3d, 8.3 Hz, $1^{2,3,4}$), 4.24 (dd, 2.9 and 10.7 Hz, 6^1), 4.05 (dd, 1.9 and 10.7 Hz, 6^1), 2.04, 1.98, 1.96 (3s, 3 x NAc). **6**: R_F 0.50 in 2:1:1 nBuOH-EtOH-H_2O; δ_H (CD_3OD) 5.33-5.36 (m, -C*H*=C*H*-), 5.06 (d, 3.4 Hz, 1^1), 4.62, 4.52, 4.47 (3d, 8.3 Hz, $1^{2,3,4}$), 4.25 (dd, 3.4 and 10.7 Hz, 6^1), 4.06 (dd, 2.0 and 10.7 Hz, 6^1), 2.05, 1.98, 1.96 (3s, 3 x NAc). **7**: $[\alpha]_D$ -3.1° (c 0.3, 4:1 MeOH-H_2O); R_F 0.16 in 2:2:1 nBuOH-EtOH-H_2O; δ_H (4:1 CD_3OD-D_2O) 5.20 (d, 3.7 Hz, 1^1), 5.05, 4.82, and 4.71 (3d, 8.2~8.8Hz, $1^{2,3,4}$), 4.30 (dd, 3.4 and 10.8 Hz, 6^1), 4.19 (dd, 1.6 and 10.4 Hz, 6^1), 2.16, 2.12, and 2.09 (3s, 3Ac). **8**: $[\alpha]_D$ +68° (c 1.1); R_F 0.50 in 10:1 CH_2Cl_2-EtOAc; δ_H 6.11 (dd, 7.8 and 52.3 Hz, H-1), 2.14, 2.05 and 1.88 (3s, 3Ac). **10**: $[\alpha]_D$ -22° (c 1.0); R_F 0.41 in 3:2 hexane-EtOAc; δ_H 5.18 (d, 8.2 Hz, H-1), 3.78 (s, OMe). **12**: $[\alpha]_D$ +41° (c 1.1); R_F 0.22 in 3:2 hexane-EtOAc; δ_H 5.30 (d, 8.5 Hz, 1^1), 5.12 and 4.93 (2d, 7.9 Hz, $1^{2,3}$), 3.76 (s, OMe), 1.89 (s, Ac). **13**: $[\alpha]_D$ -9.4° (c 1.0, MeOH); R_F 0.50 in EtOAc; δ_H 1.86, 1.82 and 1.77 (3s, 3Ac). **14**: $[\alpha]_D$ -44° (c 1.0); R_F 0.46 in 2:3 hexane-Me_2CO; δ_H 5.45 (d, 8.2 Hz, 1^1), 3.74 (s, OMe), 2.00, 1.97, 1.94, 1.85, 1.69, and 1.67 (6s, 6Ac). **15**: $[\alpha]_D$ -45° (c 0.7); R_F 0.39 in 10:1 EtOAc-EtOH; δ_H 5.44 (d, 8.5 Hz, 1^4), 2.01, 1.96, 1.89, 1.85, and 1.73 (6s, 6Ac). **16**: $[\alpha]_D$ -27° (c 0.5, MeOH); R_F 0.42 in 5:1 $CHCl_3$-MeOH; δ_H(CD_3OD) 5.47 (d, 8.5 Hz, 1^4), 4.68 and 4.45 (2d, 8.2 and 7.9 Hz, two of $1^{1,2,3}$), 2.01, 1.91, 1.90, 1.85, 1.80 and 1.80 (6s, 6Ac). **17**: $[\alpha]_D$ -7.6° (c 0.6, MeOH); R_F 0.52 in 2:1 $CHCl_3$-MeOH; δ_H (CD_3OD) 7.1~7.4 (m, 6Ph), 1.90, 1.90 and 1.76 (3s, 3Ac). **18**: Prepared from 7*Z*-tetradecenal in 3 steps, *1*) Ph_3PCHCO_2Me in PhH, *2*) 1:10 2.0M NaOH-dioxane, *3*) N-hydroxysuccimide-DCC in THF; 55% overall. R_F 0.39 in 10:1 PhMe-EtOAc; δ_H 7.28 (td, 6.9 and 15.8 Hz, H-3), 6.02 (td, 1.7 and 15.8 Hz, H-2), 0.88 (t, 6.9 Hz, Me).
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