Total Synthesis of NodRm^{IV} (S): a Sulfated Lipotetrasaccharide Symbiotic Signal from *Rhizobium meliloti*

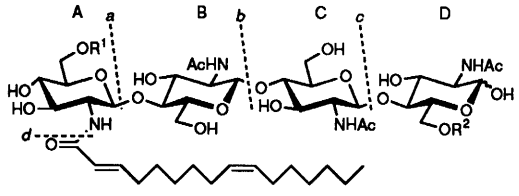
Denis Tailler, Jean-Claude Jacquinet and Jean-Marie Beau*

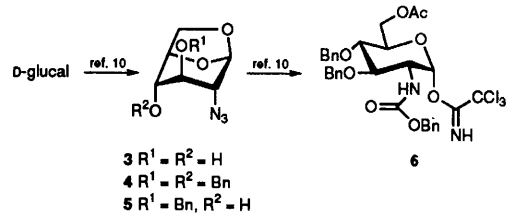
Université d'Orléans, Laboratoire de Biochimie Structurale, BP 6759, F-45067 Orléans Cedex 2, France

The stereocontrolled synthesis of the sodium salt of NodRm^{IV} (S), the bacterial chemical signal involved in the *Rhizobium meliloti*-alfalfa symbiosis, has been achieved in an overall yield of 8%.

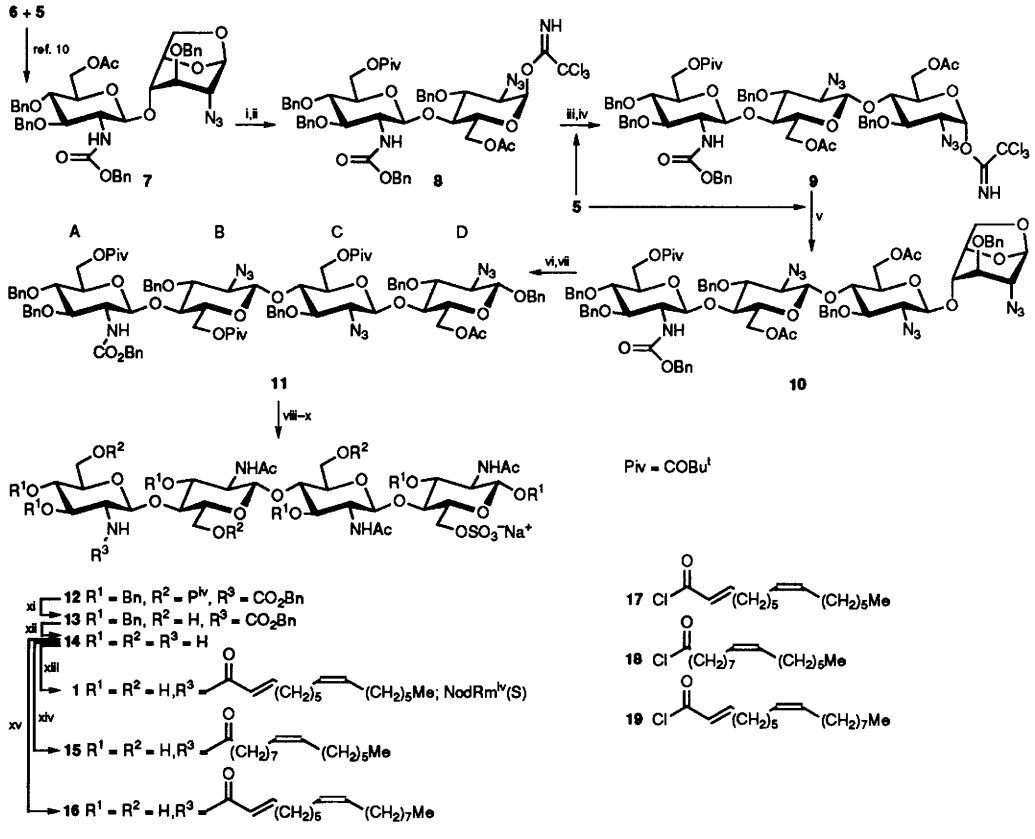
The initial stages of the symbiotic relationship between *Rhizobium* bacteria and leguminous plants are established by an exchange of chemical signals.[1] Flavonoids produced by the host plant induce, in the bacterium, the production of specific signal molecules (Nod factors) that elicit the morphogenesis of plant root nodules in which atmospheric nitrogen is reduced to ammonia. These molecules, now identified in several *Rhizobium*-legume symbiosis[1–7] were first characterized by Lerouge *et al.*[2] from an overproducing strain of *Rhizobium meliloti*. The substance, now called NodRm^{IV} (S), **1**, is a sulfated lipotetrasaccharide of *N*-acetyl-D-glucosamine specific for alfalfa. Another nodulation factor was later identified[2,3] as NodRm^{IV} (Ac, S) **2**. It is significant that the 6-*O*-sulfate group at the reducing unit determines the host specificity of the chemical signal which, upon removal, loses its ability to nodulate alfalfa but becomes capable of nodulating vetch, a nonhost plant.

These compounds, crucial for plant cell biology studies and available in only scarce amounts from rhizobial cultures, are ideal candidates for chemical synthesis. Here we report the





Scheme 1



Scheme 2 *Reagents and conditions*: i, MeONa, MeOH, room temperature then Me_3CCOCl, DMAP, pyridine, quant.; ii, Ac_2O : CF_3CO_2H (9 : 1, v/v), room temperature then $PhCH_2NH_2$ (10 equiv.) Et_2O, room temp. then CCl_3CN, DBU, CH_2Cl_2, room temperature, 1 h, 73%; iii, **5** (2 equiv.), $BF_3{\cdot}Et_2O$ (0.1 equiv.), CH_2Cl_2, −78 to −20 °C, 85%; iv, see ii, 73%; v, see iii, 91%; vi, see i, 81%; vii, see ii, then $PhCH_2OH$, $BF_3{\cdot}Et_2O$ (0.28 equiv.), $PhCH_3$, −20 °C, 1 h, 79%; viii, $NaBH_4$, $NiCl_2$, DME–EtOH, room temperature then Ac_2O, pyridine, room temperature.; ix, 2 mmol dm^{-3} MeONa, MeOH, room temperature, 52% for **11**; x, $SO_3{\cdot}Me_3N$, DMF, 50 °C, 16 h then Sephadex SP-C25(Na^+), 98%; xi, MeONa, MeOH, room temperature, 88%; xii, H_2, 10% Pd/C, AcOEt : MeOH : water (1 : 1 : 1), room temperature, 16 h, 97%; xiii, **17** (6 equiv.), $NaHCO_3$, AcOEt : MeOH : water (1 : 1 : 1), room temperature, 48 h, 60% plus 30% recovered starting amine; xiv, see xiii with **18** (6 equiv.), 60%; xv, see xiii with **19** (6 equiv.), 56%

total synthesis of NodRm^{IV} (S), compound **1** and simple lipidic analogues.

The synthesis of **1** programmed in the *a*, *b*, *c*, *d* sequence of coupling reactions (Scheme 1) is representative of a nonconventional construction of a linear oligosaccharide starting from the nonreducing end of the oligomer. It relies on (*i*) the iterative use of 1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose **3**, a useful building block[8] now easily available in high yields from D-glucal by 1,6-iodocyclization[9] and sodium azide treatment[10] and (*ii*) the α-trichloroacetimidate anomeric activation.[11] Two other syntheses have appeared[12,13] based on the use of adapted *N*-phthaloyl derivatives[14] of D-glucosamine.

The oligomerization started with disaccharide **7**, available from **5** and **6** in 91% yield.[10] Exchange of the acetate to a pivalate group, opening of the 1,6-anhydro ring followed by a selective de-*O*-acetylation at the anomeric centre[15] and trichloroacetimidate formation provided the α-imidate disaccharide **8**†‡ in 73% overall yield from **7** (Scheme 2). Coupling of the disaccharide donor **8** with alcohol **5** in the presence of 0.1 equiv. of $BF_3{\cdot}Et_2O$ smoothly led to a trisaccharide which was transformed to α-imidate **9**‡ in the three-step procedure described above (63% overall yield). Introduction of the final D-glucosaminyl moiety using the same strategy with α-imidate **9** and alcohol **5** provided the tetramer **10** in 91% yield.

Tetrasaccharide **11** in which the primary position on unit D was differentiated from the other primary positions was obtained by a standard sequence of transformations including exchange of the protecting groups at O_6 of units B and C, acetolysis, imidate formation and glycosylation with benzyl alcohol (64% overall yield from **10**). The intermediate imidate of this sequence also represents a useful tetramer donor for other biological objectives. Replacement of the three azido groups§ by *N*-acetyl groups in **11** followed by a selective removal of the *O*-acetate with 2 mmol dm^{-3} sodium methoxide in methanol provided the free primary alcohol of unit D. Sulfation by the trimethylamine–sulfur trioxide complex in DMF and exchange of the counterion readily provided the fully protected sodium sulfate **12** in 98% yield. Sequential deprotection of **12** with sodium methoxide gave the triol **13** (88%) and hydrogenolysis led to the free amine **14** (97%). Finally, selective *N*-acylation by the di-unsaturated acid chloride **17**¶ provided NodRm^{IV} (S) **1**‡ $[\alpha]_D$ −1.5 (*c* 1.23 water) in 60% yield (86% based on the recovered starting amine **14**). Similarly, the mono-unsaturated C16 : 1 side-chain analogue **15** and the di-unsaturated C18 : 2 analogue **16** were obtained in 60 and 56% yield, respectively using acid chlorides **18** and **19**.¶ The final lipotetrasaccharides **1**, **15** and **16** were purified by silica gel chromatography (ethyl acetate : methanol : water, 5 : 2 : 1 as eluent) and gel filtration on a Sephadex G-25 column. The ability of these synthetic compounds and other variants on the acyl chain to elicit nodulation events on the host plant will be reported in due course.[18]

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Footnotes

† All new compounds gave satisfactory spectral and analytical data.

‡ *Selected ^1H NMR data* ($CDCl_3$, 300 MHz; identification of the sugar units is that of NodRm^{IV} shown in Scheme 1): **8** δ 3.52 (dd, 1 H, $J_{1,2}$ 3.8, $J_{2,3}$ 9.5 Hz, B unit H_2), 6.34 (d, 1 H, B unit H_1), 8.72 (s, 1 H, C=NH). **9**: δ 3.62 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 9.5 Hz, C unit H_2), 4.28 (d, 1 H, $J_{1,2}$ 8 Hz, B unit H_1), 6.37 (d, 1 H, C unit H_1), 8.74 (s, 1 H, C=NH). **1**: δ (CD_3OD) 0.90 (m, 3 H, Me), 1.25–1.50 (m, CH_2 in the chain), 1.94, 2.00 and 2.03 (3 s, NAc of the major α isomer), 2.00–2.05 (m, 4 H, allylic $H_{8,11}$ in the chain), 2.18–2.25 (m, 2 H, allylic H_4 in the chain), 4.00 (m, 1 H, unit D H_5), 4.08 (dd, $J_{5,6}$ 2.0, $J_{6,6'}$ 10.8 Hz, unit D H_6 of the α isomer), 4.23 (dd, $J_{5,6'}$ 3.5 Hz, unit D $H_{6'}$ of the α isomer), 4.54–4.63 (5 d, *J* 8.0–8.5 Hz, 5 $H_1\beta$), 5.06 (d, $J_{1,2}$ 3.3 Hz, D unit $H_1\alpha$), 5.35 (m, 2 H, $H_{9,10}$ in the chain), 5.99 (dt, 1 H, $J_{2,4}$ 1.5, $J_{2,3}$ 15.5 Hz, H_2 in the chain), 6.83 (dt, 1 H, $J_{3,4}$ 7 Hz, H_3 in the chain).

§ This reduction of the azido groups to amino groups by the $NiCl_2$–$NaBH_4$ system is the only step giving a moderate yield in the synthetic sequence. Although not tested on **11**, the reduction of tri-azides (structurally very close to **11**) to the corresponding tri-amines can now be performed with a much higher efficiency using the tin(II) complexes method.[16]

¶ The di-unsaturated fatty acid chloride **17** was prepared from ethyl palmitoleate by syn-elimination of the corresponding α-phenyl selenoxide[17] (diphenyl diselenide, Pr^i_2NLi, THF, −78 °C then $NaIO_4$, $NaHCO_3$, EtOH–water, 62% of only the α,β-unsaturated *E* isomer), saponification (2.5 mol dm^{-3} KOH, EtOH–water, 80 °C, 9 h then 1 mol dm^{-3} aq. HCl, 88%) and acid chloride formation (oxalyl chloride, pyridine, CH_2Cl_2). The acid chloride was used immediately after its preparation. Acid chloride **19** was synthesized in similar yields by the same route starting from ethyl oleate.

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18 Part of this work was presented at the *XVIth International Carbohydrate Symposium*, Paris, July, 1992, Abst. A298 and at the *VIIth European Carbohydrate Symposium*, Cracow, Poland, August, 1993, Abstract A141.