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SHORT COMMUNICATION

Synthesis of lipooligosaccharide nodulation signals NodBj-V(RCO, MeFuc) and NodBj-IV(RCO, MeFuc) of *Bradyrhizobium japonicum*[1]

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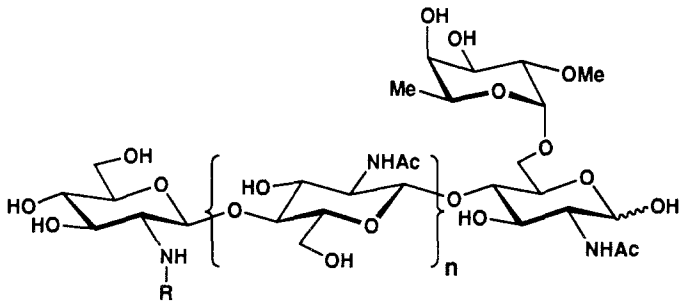
First total syntheses of Nod factors of *Bradyrhizobium japonicum* were described in a stereo- and regio-controlled manner.

Keywords: Nod factors, lipooligosaccharides, *Bradyrhizobium japonicum*, synthesis

Bradyrhizobium japonicum is a soil bacterium that forms nitrogen-fixing nodules specifically on the roots of the agronomically significant legume soybean. In 1992, Sanjuan *et al.* [2] reported the isolation and chemical characterization of a metabolite that was produced by *B. japonicum* strain USDA110 (Type I strain) and that is responsible for an early event of the nodulation process on the host legume.

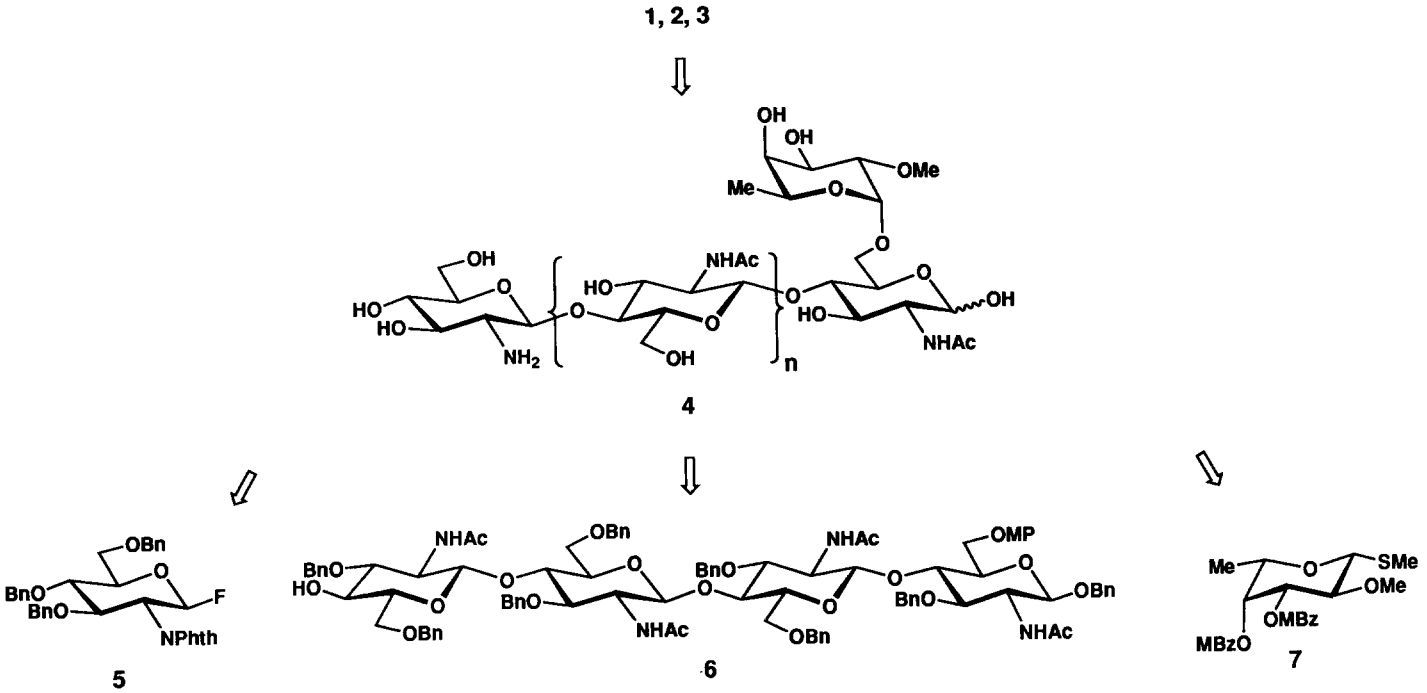


	R	n	
1	$COC_{17}H_{33}$ (9Z)	3	NodBj-V (C18:1,MeFuc)
2	$COC_{17}H_{33}$ (11Z)	3	NodBj-V (C18:1,MeFuc)
3	$COC_{17}H_{33}$ (11Z)	2	NodBj-IV (C18:1,MeFuc)

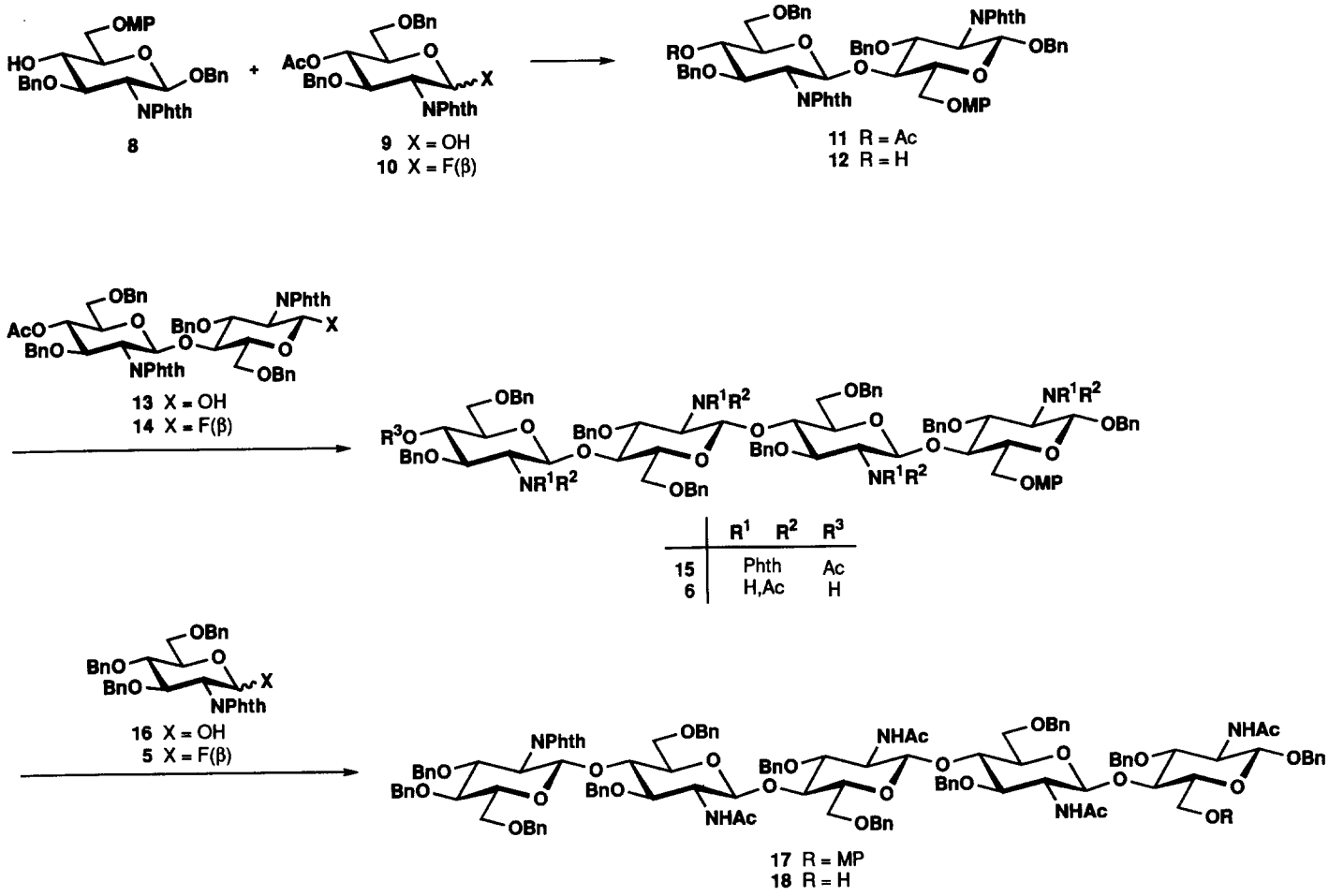
Structure 1,2,3

The structure was proposed to be a lipohexasaccharide **1**, or NodBj-V ($C_{18:1}$, MeFuc) [3]. The structure of the isolated fatty acid was proposed [2] as oleic acid by oxidation to a diol and EIMS analysis of its derivatives. In 1993, Carlson *et al.* [4] reported the structures and the biological activities of lipooligosaccharide nodulation signals (**2**, **3** and others) produced by *B. japonicum* USDA135 (Type I strain) and *B. japonicum* USDA61 (Type II strain). In order to clarify the structural requirements for the lipooligosaccharide to induce necessary morphological differentiation on the root of the host plants, synthetic analogues of these lipooligosaccharides are required. As part of our synthetic projects [5] on plant-physiologically active glycoconjugates, we describe here synthetic approaches to *B. japonicum* nodulation signals **1**, **2** and **3**. A versatile synthetic strategy was designed by use of a completely deprotected oligosaccharide **4** with a free amino function as a key precursor for the target molecules. The precursor then should be acylated with an activated fatty acid at the final step [6], so that final target molecules may carry any kind of fatty acid of our choice. Further bond disconnection of compound **4** led us to design two glycosyl donors **5** and **7** and a glycotetraosyl glycosyl acceptor **6**.

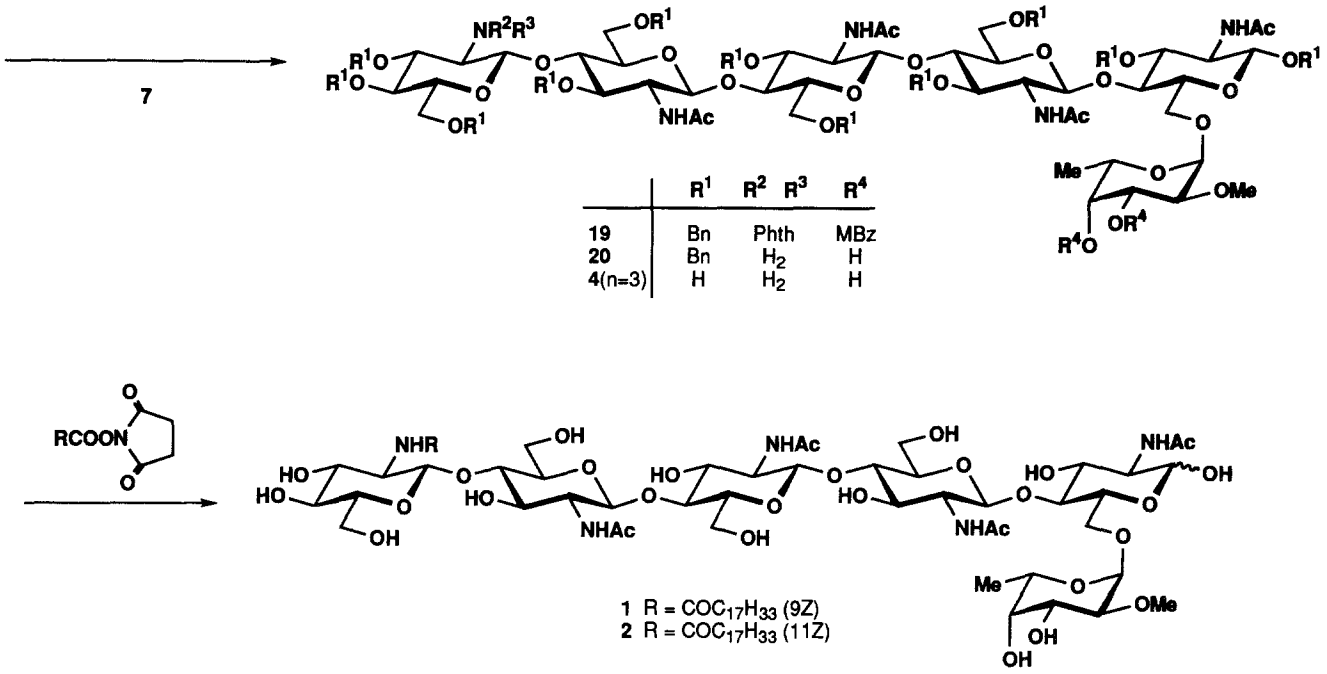
Glycosylation of compound [6] was carried out in the presence of $Cp_2Hf(OTf)_2$ [7, 8] and powdered molecular sieves 4A (MS4A) in $(ClCH_2)_2$ at $-23°$ with 1.5 equivalents of fluoride **10** readily obtainable from compound **9** [6] by treatment with DAST [8] to give 86% of **11**, which was saponified with NaOMe in 1:1 MeOH-THF to give 84% of a glycosyl acceptor **12**. Glycosyl donor **14** was prepared in 88% from already reported [9] chitobiosyl derivative **13** by treatment with DAST in $(ClCH_2)_2$. Glycosylation of **12** with 1.5 equivalents of **14** was performed according to Suzuki procedure as described above to give 99% of tetrasaccharide **15** that was, in turn, converted into the designed key intermediate **6** in two steps in 75% overall; 1, $NH_2NH_2 \cdot H_2O$ in EtOH, reflux; 2, Ac_2O in MeOH.



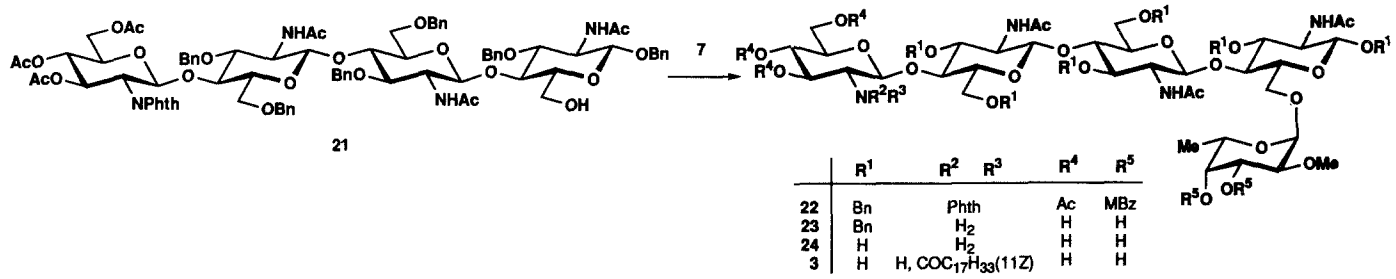
Scheme 1. (MP = 4-MeO-C_6H_5, MBz = 4-Me-C_6H_4CO).



Scheme 2



Scheme 2 (*cont.*)



Scheme 3

Further chain elongation was carried out by use of a glycosyl donor **5** that was prepared in a quantitative yield from reported compound **16** [10] by treatment with DAST. Glycosylation of **6** with **5** as described above gave 80% of **17** that was, in turn, treated with four equivalents of $(NH_4)_2Ce(NO_3)_6$ (CAN) [11] in 3:1 CH_3CN-H_2O to afford 79% of **18**.

Having prepared a pentasaccharide back-bone **18**, crucial stereoselective introduction of α-L-fucosyl residue at O-6^1 should be exploited. Fucosyl donor **7** was prepared from methylthio β-L-fucopyranoside [12] in four steps in 59% overall; 1, $Me_2C(OMe)_2$, $TsOH \cdot H_2O$ in Me_2CO; 2, MeI, NaH in DMF; 3, 90% TFA in $(CH_2Cl)_2$; 4, p-Me-C_6H_4COCl in Py. Coupling of **7** with **19** was achieved in a stereocontrolled manner in the presence of $CuBr_2$-Bu_4NBr-MS4A [13] in CH_3NO_2 to give 65% of **19** and no β-isomer could be detected by tlc. Compound **19** was efficiently converted into the designed precursor **4** via **20** in two steps in 90% overall; 1, $NH_2NH_2 \cdot H_2O$ in EtOH, reflux; 2, $Pd(OH)_2$-C, H_2 in 80% aq.MeOH. Treatment of **4** with 9*Z* and 11*Z*-octadecenoyl *N*-hydroxysuccinimide and subsequent purification by Bond Elute C_{18} [14] afforded the target molecules **1** (37%) and **2** (33%), respectively. ^{1}H-NMR data were in good agreement with those of natural **1** [2] and related compounds [4], thus providing synthetic evidence for the proposed structures.

Another Nod factor NodBj-IV (C18:1, MeFuc) **3** containing one less *N*-acetyl-D-glucosamine residue could readily be synthesized by employing already reported [6] tetrasaccharide glycosyl acceptor **21** and fucosyl donor **7**. Thus coupling between **21** and **7** was achieved stereoselectively under the conditions as described above to give 68% of **22** which was further converted into **3** via **23** and **24** in three steps in 41% overall as described for **2**.

In summary, lipooligosaccharide nodulation signals **1**, **2**, and **3** produced by type 1 and type 2 strains of *Bradyrhizobium japonicum* were synthesized for the first time in a regio- and stereo-control manner employing either glycotetraosyl **21** or glycopentaosyl intermediate **18** as a key glycosyl acceptor.

Table 1. Physical data for novel compounds.

Compounds	R_F	$[\alpha]_D$[a]	^{1}H-NMR[a] (δ_H)
1	0.46 in 2:1:1 ^{n}BuOH-EtOH-H_2O		in DMSO-d_6: 5.32 (m, C*H*=C*H*), 4.93 (*d*, 3.4 *Hz*, 1^{Fuc}), 4.84 (bs, 1^1), 4.30–4.40 (m, $1^{2,3,4,5}$), 1.80 (s, 4 × Ac), 1.05 (d, 6.4 Hz, 6^{Fuc}), 0.84 (t, 5.9 Hz, CH_2CH_3).
2	0.46 in 2:1:1 ^{n}BuOH-EtOH-H_2O		in DMSO-d_6: 5.31 (m, C*H*=C*H*), 4.93 (d, 3.4 Hz, 1^{Fuc}), 4.84 (bs, 1^1), 4.35–4.40 (m, $1^{2,3,4,5}$), 3.56 (s, OMe), 1.05 (d, 6.4 Hz, 6^{Fuc}), 0.84 (t, 5.8 Hz, CH_2CH_3).
3	0.58 in 2:1:1 ^{n}BuOH-EtOH-H_2O		in DMSO-d_6: 5.32 (m, C*H*=C*H*), 4.93 (d, 3.9 Hz, 1^{Fuc}), 4.84 (bs, 1^1), 4.30–4.40 (m, $1^{2,3,4}$), 1.82, 1.80, and 1.80 (3s, 3 × Ac), 1.05 (d, 6.9 Hz, 6^{Fuc}), 0.84 (t, 6.3 Hz, CH_2CH_3).
4 ($n = 3$)	0.04 in 2:1:1 ^{n}BuOH-EtOH-H_2O	−6.3° (4:1 MeOH-H_2O, c 0.3)	in D_2O: 5.16 (d, 3.3 Hz, 1^{Fuc}), 3.503 (s, OMe), 2.058, 2.053, 2.054, and 2.028 (4s, 4 × Ac), 1.20 (d, 6.3 Hz, 6^{Fuc}).
5	0.40 in 3:2 hexane-EtOAc	+74.5° (c 0.5)	5.87 (dd, 7.9 and 53.5 Hz, H-1)
6	0.40 in EtOAc	−43.1° (c 0.2)	6.45, 5.84, and 5.19 (3d, 9.5, 9.5, and 9.2 Hz, respectively, 3 × N*H*Ac), 3.75 (s, OMe), 1.98, 1.76, 1.74, and 1.74 (4s, 4 × Ac).
7	0.42 in 2:1 hexane-EtOAc	−171.5° (c 0.7)	5.61 (d, 3.6 Hz, H-4), 5.31 (dd, 3.6 and 9.9 Hz, H-3), 4.47 (d, 9.6 Hz, H-1), 3.52 (s, OMe), 2.44, 2.36, and 2.34 (3s, 3 × Me), 1.28 (d, 6.6 Hz, H-6).
10	0.38 in 3:2 hexane-EtOAc	+85.5° (c 0.6)	5.87 (dd, 8.6 and 53.0 Hz, H-1), 5.21 (t, 8.6 Hz, H-4), 1.96 (s, Ac).
11	0.37 in 1:1 hexane-EtOAc	+33.7° (c 1.1)	5.29 (d, 8.3 Hz, 1^2), 5.12 (t, 8.8 Hz, 4^2), 4.99 (d, 8.3 Hz, 1^1), 3.79 (s, OMe), 1.91 (s, Ac).
12	0.33 in 1:1 hexane-EtOAc	+15.4° (c 1.2)	5.28 (d, 7.8 Hz, 1^2), 4.99 (d, 7.8 Hz, 1^1), 3.79 (s, OMe).
14	0.40 in 1:1 hexane-EtOAc	+43.1° (c 0.3)	5.69 (dd, 7.8 and 53.7 Hz, 1^1), 5.33 (d, 8.3 Hz, 1^2), 5.16 (t, 9.3 Hz, 4^2), 1.93 (s, Ac).
15	0.37 in 4:1 PhMe-EtOAc	+35.4° (c 0.4)	5.28 (d, 8.2 Hz, 1^4), 5.12 (t, 9.2 Hz, 4^4), 5.08, 5.06, and 4.91 (3d, 7.0, 8.2, and 8.2 Hz, respectively, for $1^{2,3,4}$), 3.74 (s, OMe), 1.88 (s, Ac).
17	0.32 in 1:1 EtOAc-hexane	−29.3° (c 0.5)	6.45, 5.91, and 5.24 (3d, 9.2, 9.8, and 9.2 Hz, 3 × N*H*Ac), 5.20 (d, 8.2 Hz, 1^5), 3.74 (s, OMe), 1.96, 1.73, 1.72, and 1.69 (4s, 4 × Ac).
18	0.32 in 1:2 hexane-Me_2CO	−37.0° (c 0.6)	6.47, 5.66, and 5.50 (3d, 10.7, 7.9, and 7.6 Hz, respectively, 3 × N*H*Ac), 5.22 (d, 8.5 Hz, 1^5), 1.90, 1.85, 1.81 and 1.66 (4s, 4 × Ac).
19	0.44 in 2:3 hexane-EtOAc	−52.8° (c 0.5)	5.56 (dd, 3.5 and 10.3 Hz, 3^{Fuc}), 5.51 (d, 3.5 Hz, 4^{Fuc}), 5.18 (d, 8.3 Hz, 1^5), 5.03 (d, 3.4 Hz, 1^{Fuc}), 3.26 (s, OMe), 2.45 and 2.34 (2s, *Me*Bz), 1.98, 1.91, 1.75, and 1.71 (4s, 4 × Ac), 0.89 (d, 6.8 Hz, 6^{Fuc}).
20	0.56 in 8:1 $CHCl_3$-MeOH	−38.8° (MeOH, c 0.5)	3.52 (s, OMe), 1.873, 1.824, 1.816, and 1.816 (4s, 4 × Ac), 1.13 (d, 6.4 Hz, 6^{Fuc}).
22	0.37 in 2:3 hexane-Me_2CO	−15.0° (c 0.1)	5.04 (d, 3.9 Hz, 1^{Fuc}), 3.31 (s, OMe), 2.46 (s, 2 × *Me*Bz), 2.01, 1.99, 1.92, 1.89, 1.86, and 1.80 (6s, 6 × Ac), 0.93 (d, 6.8 Hz, 6^{Fuc}).
23	0.67 in 8:1 $CHCl_3$-MeOH	−46.5° (MeOH, c 0.5)	in CD_3OD: 4.99 (d, 3.9 Hz, 1^{Fuc}), 3.52 (s, OMe), 1.93, 1.88, and 1.82 (3s, 3 × Ac), 1.13 (d, 6.9 Hz, 6^{Fuc}).
24	0.07 in 2:1:1 ^{n}BuOH-EtOH-H_2O	−29.2° (4:1 MeOH-H_2O, c 0.6)	in D_2O: 4.94 (d, 2.9 Hz, 1^{Fuc}), 1.84, 1.83, and 1.81 (3s, 3 × Ac).

[a] Values of $[\alpha]_D$ and δ_H were recorded at 25° ± 3° for solutions in $CHCl_3$ and $CDCl_3$, respectively, unless otherwise indicated. Signal assignment for ^{1}H-NMR such as H-3^2 stands for a proton at C-3 of sugar residue 2.

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