

Synthesis of a 2-Acetamido-2-deoxy- β -D-mannuronic Acid-Containing Artificial Glycolipid Corresponding to the Repeating Unit of a Teichuronic Acid from *Micrococcus luteus*

Yumiko OSA, Eisuke KAJI,* Keiko TAKAHASHI, Motoko HIROOKA, Shonosuke ZEN, and
Frieder W. LICHTENTHALER⁺

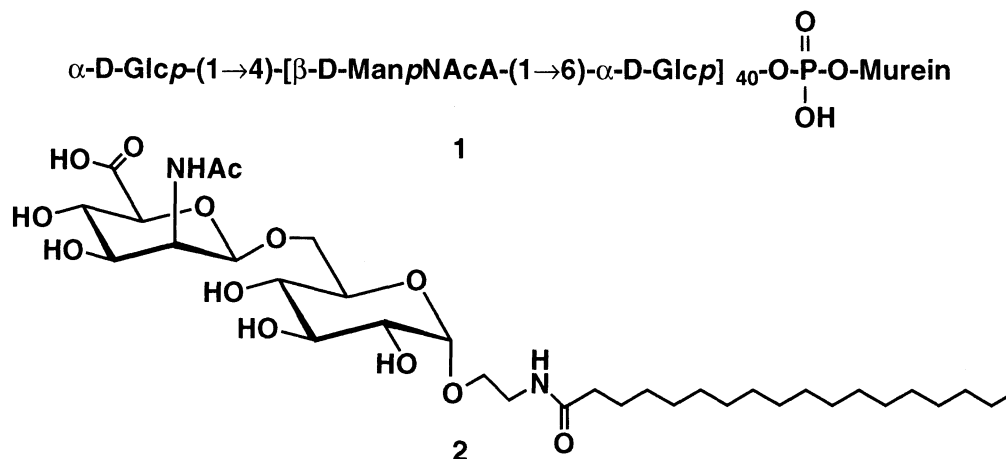
School of Pharmaceutical Sciences, Kitasato University, Shirokane, Minato-ku, Tokyo 108

⁺Institut für Organische Chemie, Technische Hochschule Darmstadt, D-64287 Darmstadt, Germany

A new type of artificial glycolipid constituted with 2-acetamido-2-deoxy- β -D-mannuronic acid was synthesized utilizing a readily accessible building block, methyl 2-(benzoyloxy)iminoglycosuronate as the glycosyl donor. Stereocontrolled β -glycosidation of the donor was as smoothly effected as the subsequent stereospecific reduction of (benzoyloxy)imino function. After anomeric activation, attachment of the spacer (2-aminoethanol)-linked stearic acid and final deblocking gave the designed glycolipid in good overall yield.

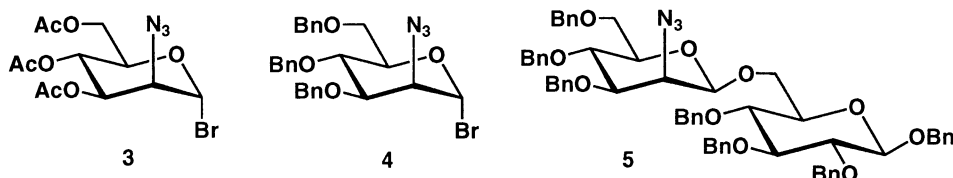
Teichuronic acid constitutes a cell wall component of some Gram-positive bacteria carrying an antigenic determinant, which conceivably is an immunologically active element against living organisms. In *Micrococcus luteus*, for example, the teichuronic acid is an acidic polysaccharide¹⁻³⁾ composed of the disaccharide repeating unit, 2-acetamido-2-deoxy- β -D-mannuronic acid-(1 $\rightarrow$ 6)- α -D-glucopyranose, which binds peptidoglycan (murein) through a phosphoric ester linkage as depicted in the formula (1).

Due to the recent progress in glycotechnology, which showed a variety of carbohydrates to be highly useful recognition markers for targeting drug delivery systems (DDS),⁴⁻⁶⁾ we have designed a novel artificial glycolipid 2, in which the above disaccharide is linked to stearic acid through a 2-aminoethanol spacer.

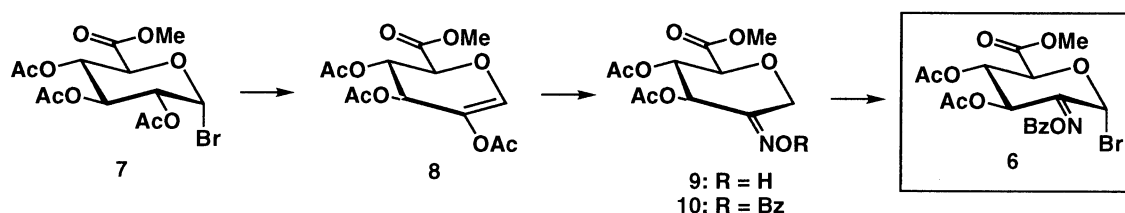


The disaccharide β -D-ManNAcA-(1 $\rightarrow$ 6)- α -D-Glc has previously been synthesized⁷⁾ by elaboration of the mannuronic acid portion from the 2-azido sugar 3, the acquisition of which requires 6 steps from D-glucose,⁸⁾

and, deplorably, shows no stereoselectivity in glycosidations ($\alpha : \beta = 1 : 1.1$).⁷⁾ Even though the more laboriously accessible donor **4** (11 steps from D-glucose)⁷⁾ allows stereospecific β -glycosidation with benzyl 2,3,4-tri-*O*-benzyl- β -D-glucopyranoside, the resulting disaccharide **5** was found unsuited for conversion into the uronic acid. Hence, new glycosyl donors are needed for the construction of β -D-ManNAcA-containing oligosaccharides.



We have designed a versatile glycosyl donor, namely 2-oximinoglycuronyl bromide **6** for the synthesis of the disaccharide repeating unit of **1**. On the basis of the methodology we have developed for the synthesis of β -D-mannosamine-containing oligosaccharides,⁹⁾ **6** should be readily accessible on a preparative scale by exposing the 2-hydroxyglucuronal ester **8** to the 3 step-sequence comprising hydroxylaminolysis, *O*-benzoylation, and photobromination — a concept that could be readily realized: the glucuronyl bromide **7**,¹⁰⁾ prepared in 2 steps from D-glucuronolactone in 75% yield, was subjected to reaction with diethylamine-tetrabutylammonium bromide (1.5 : 1.0 eq.) in DMF to give the 2-hydroxyglucuronal ester **8** in 54% yield.¹¹⁾ Oximation of **8** with excess hydroxylamine hydrochloride in pyridine afforded the oxime **9** (56% yield, not yet optimized), subsequent benzoylation with benzoyl chloride-pyridine gave (90%) the *O*-benzoyl oxime of *E*-configuration,¹²⁾ and the concluding photobromination¹³⁾ proceeded smoothly to provide the desired oximinoglycuronyl bromide **6** in an isolated yield of 95%. The bromide **6** was proved to be stable, crystalline substance,¹⁴⁾ storable in a refrigerator for months without decomposition.

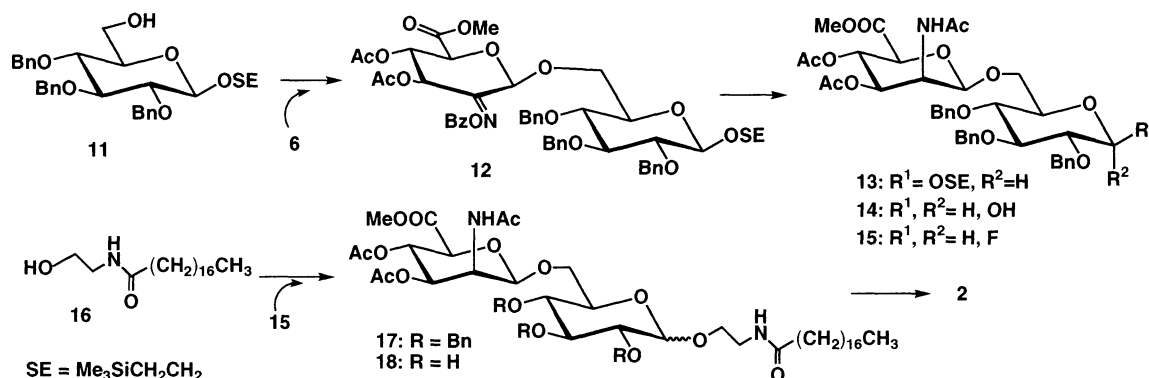


The utility of **6** as a β -selective glycosyl donor for the construction of β -D-ManNAcA-(1 $\rightarrow$ 6)- α -D-Glc was examined with partially blocked 2-(trimethylsilyl)ethyl β -D-glucopyranoside (**11**)¹⁵⁾ as the acceptor. Of the several procedures evaluated for β -glycosidation of **6** with **11**, the most effective one proved to be the use of silver aluminosilicate (a van Boeckel catalyst)¹⁷⁾ in dichloromethane (2 h at 25 °C): a β -selectivity of better than 20 : 1 (¹H NMR of the reaction mixture) was observed, allowing the isolation of anomerically pure **12** in a yield of 88%. The β -configuration of **12** unequivocally followed from ¹H NMR data.¹⁸⁾

Another key step for the construction of β -D-ManNAcA concerns stereocontrolled conversion of (benzoyl-oxo)imino function into the *manno*-configured acetamido group, which was successfully effected by hydroboration: Treatment of **12** with twelve molar excess of borane-THF complex in THF followed by *N*-acetylation led to the desired 2-acetamido-2-deoxy- β -D-mannuronate (**13**) in 77% yield,²¹⁾ of which the β -D-*manno* configuration was proved by the couplings $J_{1,2}$, $J_{2,3}$, and $J_{3,4}$ of 2.0, 3.1, and 9.0 Hz, respectively.

Assembly of the spacer-linked glycolipid (**2**) was achieved by activation of the reducing end of **13** (1-OSE $\rightarrow$ 1-OH $\rightarrow$ 1-F) and subsequent glycosidation with 2-(stearoylamino)ethanol (**16**).²²⁾ According to the

Magnusson's method,¹⁵⁾ 2-(trimethylsilyl)ethyl group was smoothly removed with TFA in CH₂Cl₂ (**13**→**14**, 83%), and the resulting 1-OH was fluorinated with DAST (**14**→**15**, 98%). The fluoride **15** was coupled with **16** in the presence of either SnCl₂-AgClO₄²³⁾ or Cp₂ZrCl₂-AgClO₄²⁴⁾ in CH₂Cl₂ to afford **17** in 53 or 64% yield, respectively. Both methods are of equal stereoselectivity, i.e., the α -glycoside is predominantly obtained in an α : β ratio of about 4 : 1. Subsequent de-*O*-benzylation was effected with Pd-C/H₂ (**17**→**18**, 97%), the concluding saponification with 1 M NaOH-MeOH (1 : 2), to smoothly provide the target glycolipid **2** in 86% yield.²⁵⁾ The biological evaluation of **2** will be discussed elsewhere.



In summation, a practical, straightforward reaction sequence has been developed for generating a highly useful β -D-ManNAcA donor from D-glycuronolactone (20% over 6 simple steps), i.e. the suitably blocked 2-(benzoyloxy)iminoglucuronate **6**. Its utility as an efficient glycosyl donor for the assembly of β -D-ManNAcA-containing oligosaccharides was amply demonstrated by the fact that both, β -glycosidation with **11** and reduction of the oximino group to 2-acetamido-2-deoxy- β -D-mannuronate (**13**), are proceeding in an essentially stereospecific manner. Thus, this approach has major advantages over previous methodology, and is presently being applied to the synthesis of a variety of other β -D-ManNAcA-containing oligosaccharides.

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References

- 1) H. R. Perkins, *Biochem. J.*, **86**, 475 (1963).
- 2) S. Hase and Y. Matsushita, *J. Biochem. (Tokyo)*, **68**, 723 (1970); **69**, 559 (1971); **72**, 1117 (1972).
- 3) Nasir-ud-Din and R. W. Jeanloz, *Carbohydr. Res.*, **47**, 245 (1976).
- 4) J. L. Bodmer and R. T. Dean, *Methods Enzymol.*, **112**, 298 (1985).
- 5) R. T. Lee and Y. C. Lee, "The Glycoconjugates," ed by M. L. Horowitz (1982), Vol. 4, Part B, P. 57.
- 6) Y. C. Lee, *Trends Glycosci. Glycotechnol.*, **4**, 251 (1992).
- 7) H. Paulsen, B. Helpap, and J. P. Lorentzen, *Liebigs Ann. Chem.*, **1987**, 431.
- 8) H. Paulsen, J. P. Lorentzen, and W. Kutschker, *Carbohydr. Res.*, **136**, 153 (1985).
- 9) E. Kaji and F. W. Lichtenthaler, *Trends Glycosci. Glycotechnol.*, **5**, 121 (1993).
- 10) G. N. Bollenback, J. W. Long, D. G. Benjamin, and J. A. Lindquist, *J. Am. Chem. Soc.*, **77**, 3310 (1955).
- 11) Treatment of **7** with DEA-NaI or DBU in various solvents resulted in less effective dehydrobrominations.

- 12) The benzoyloxime accumulated in an *E* : *Z* ratio of 40 : 1 as evidenced by ^1H NMR data: the quasi-equatorial H-1 of the *E*-isomer is deshielded by the oxime-benzoyl group to 4.93 ppm (versus 4.53 ppm for *Z*-isomer); H-3, in turn, shows substantial deshielding in the *Z*-isomer ($\rightarrow$ 6.35 ppm) versus a normal chemical shift (5.70 ppm) in the *E*-isomer. Additional evidence was secured by NOE experiments.
- 13) Photobromination was carried out by refluxing with 1 molar equiv. NBS in CCl_4 for 0.5 h under irradiation with 250 W tungsten lamp.
- 14) Compound **6**: mp 90-91 °C (Et_2O -pentane); $[\alpha]_{\text{D}}^{23} + 340.8^\circ$ (*c* 0.5, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ = 2.13, 2.21 (each 3H, s, 2 x COCH_3), 3.79 (3H, s, OCH_3), 4.67 (1H, d, H-5), 5.48 (1H, t, H-4), 6.24 (1H, d, H-3), 7.43 (1H, s, H-1), 7.51, 7.65, 8.04 (5H, aromatic H); $J_{3,4} = J_{4,5} = 9.5$ Hz; ^{13}C NMR (75 MHz, CDCl_3) δ = 20.45, 20.49 (2 x COCH_3), 53.32 (COOCH_3), 66.87 (C-3), 68.37 (C-4), 72.05 (C-1), 72.44 (C-5), 154.61 (C-2); MS (FAB) *m/z*: 472 $[\text{M}+1]^+$.
- 15) Compound **11** was readily prepared from 2-(trimethylsilyl)ethyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene- β -D-glucopyranoside¹⁶⁾ by reductive opening of the benzylidene-acetal with $\text{LiAlH}_4\text{-AlCl}_3$ in 76% yield: colorless syrup; $[\alpha]_{\text{D}}^{23} + 3^\circ$ (*c* 1, CHCl_3); MS (FAB) *m/z*: 551 $[\text{M}+1]^+$, 573 $[\text{M}+\text{Na}]^+$.
- 16) K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg, G. Magnusson, J. Dahmen, G. Noori, and K. Stenvall, *J. Org. Chem.*, **53**, 5629 (1988).
- 17) C. A. A. van Boeckel, T. Beetz, and S. F. van Aelst, *Tetrahedron*, **40**, 4097 (1984).
- 18) The β -D-anomers of 2-(benzoyloxy)iminoglycosides, have exceptionally small $J_{3,4}$ and $J_{4,5}$ coupling constants (4.5-6.5 Hz) originating from the steric congestion between 2-(acyloxy)imino group and aglycon, which distorts the pyranoid ring; the corresponding α -anomers exhibit normal J values (9-10 Hz), the ring adapting the $^4\text{C}_1$ -conformation.^{19,20)}
- 19) F. W. Lichtenthaler, E. Kaji, and S. Weprek, *J. Org. Chem.*, **50**, 3505 (1985).
- 20) E. Kaji, F. W. Lichtenthaler, T. Nishino, A. Yamane, and S. Zen, *Bull. Chem. Soc. Jpn.*, **61**, 1291 (1988).
- 21) In this process, the conditions are to be such that the substrate **12** can react in fairly diluted THF solution (e.g. 14 mM), otherwise the 6-carboxylic ester function is reduced to the primary alcohol along with the desired reduction of 2-oxyimino group.
- 22) N. S. Chandrakumar, V. L. Boyd, and J. Hajdu, *Biochim. Biophys. Acta*, **711**, 357 (1982).
- 23) T. Mukaiyama, Y. Murai, and S. Shoda, *Chem. Lett.*, **1981**, 431; T. Mukaiyama, Y. Hashimoto, and S. Shoda, *ibid.*, **1983**, 935.
- 24) K. Suzuki, H. Maeta, and T. Matsumoto, *Tetrahedron Lett.*, **30**, 4853 (1989); K. Suzuki, H. Maeta, T. Suzuki, and T. Matsumoto, *ibid.*, **30**, 6879 (1989).
- 25) Obtained as an anomeric mixture of α : β = ca. 4 : 1; $[\alpha]_{\text{D}}^{25} - 5.3^\circ$ (*c* 1, MeOH); MS (FAB) *m/z*: 751 $[\text{M} + 2\text{Na}]^+$; Selected ^1H NMR (300 MHz, CD_3OD) for the α -anomer δ = 2.02 (3H, s, NHAc), 3.20 (1H, dd, H-4), 3.39 (1H, dd, H-2), 3.57 (1H, d, H-5'), 3.58 (1H, dd, H-4'), 3.59 (1H, dd, H-3), 3.64 (1H, m, H-3'), 3.66 (1H, td, H-5), 3.68 (1H, dd, H-6a), 4.12 (1H, dd, H-6b), 4.48 (1H, dd, H-2'), 4.69 (1H, d, H-1'), 4.75 (1H, d, H-1); $J_{1,2}=4.0$, $J_{2,3}=10.0$, $J_{3,4}=8.0$, $J_{4,5}=10.0$, $J_{1',2'}=2.0$, $J_{2',3'}=4.0$, $J_{3',4'}=J_{4',5'}=9.0\text{Hz}$; ^{13}C NMR (75 MHz, CD_3OD) for the α -anomer δ = 54.80 (C-2'), 70.82 (C-6), 71.42 (C-5'), 72.34 (C-4), 73.14 (C-5), 73.78 (C-2), 74.47 (C-3'), 75.41 (C-4'), 78.62 (C-3), 100.31 (C-1), 101.67 (C-1'), 174.95 (C-6').

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