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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

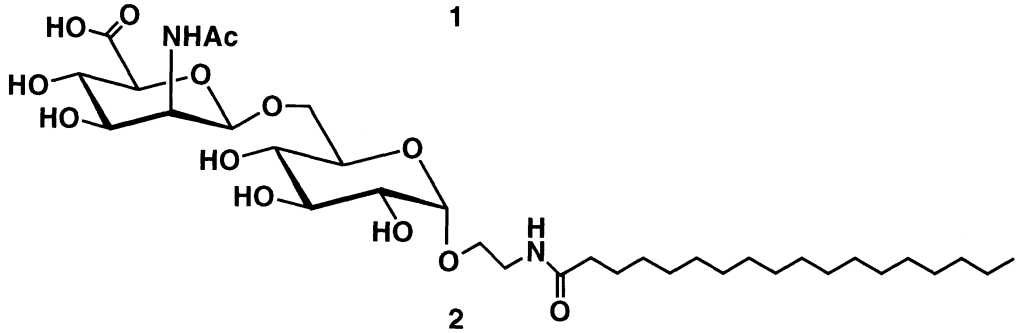
A new type of artificial glycolipid constituted with 2-acetamido-2-deoxy-β-D-mannuronic acid was synthesized utilizing a readily accessible building block, methyl 2-(benzoyloxy)iminoglycosuronate as the glycosyl donor. Stereocontrolled β-glycosidation of the donor was as smoothly effected as the subsequent 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 polysaccharide[1-3] composed of the disaccharide repeating unit, 2-acetamido-2-deoxy-β-D-mannuronic acid-(1→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.

α-D-Glc*p*-(1→4)-[β-D-Man*p*NAcA-(1→6)-α-D-Glc*p*]$_{40}$-O-P(=O)(OH)-O-Murein

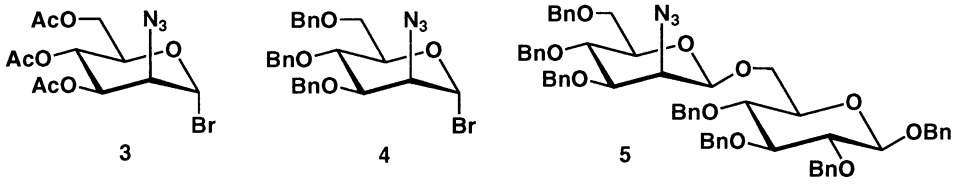
1



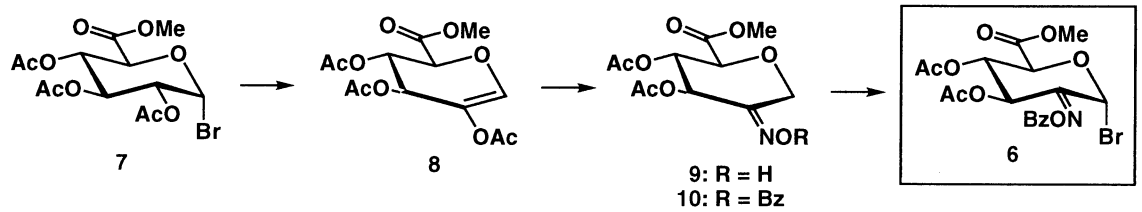
2

The disaccharide β-D-ManNAcA-(1→6)-α-D-Glc has previously been synthesized[7] 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 (α : β = 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-oxyiminoglycuronyl 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 photobromination[13] proceeded smoothly to provide the desired oximinoglucuronyl 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.

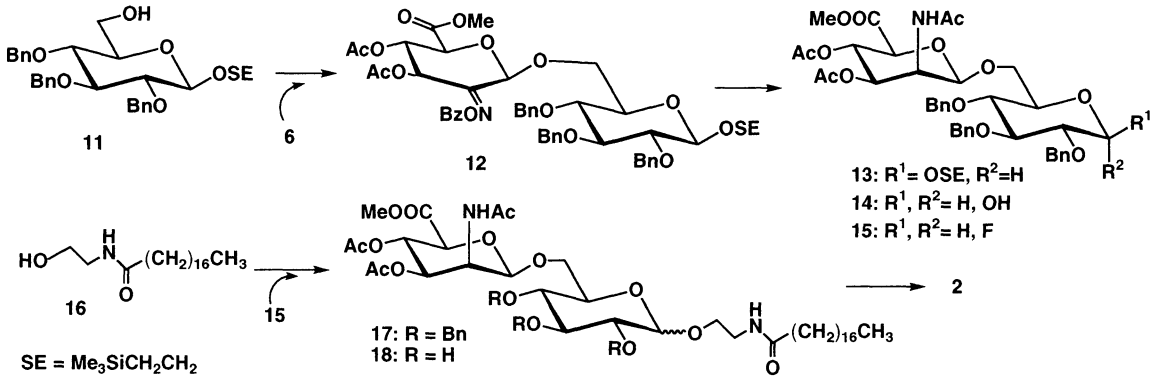


The utilily of **6** as a β-selective glycosyl donor for the construction of β-D-ManNAcA-(1→6)-α-D-Glc was examined with partially blocked 2-(trimethylsilyl)ethyl β-D-glucopyranoside (**11**)[15] as the acceptor. Of the several procedures evaluated for β-glycosidation of **6** with **11**, the most effective 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 (^{1}H 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 ^{1}H NMR data.[18]

Another key step for the construction of β-D-ManNAcA concerns stereocontrolled conversion of (benzoyloxy)imino function into the *manno*-configurated 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→1-OH→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 CH_2Cl_2 (**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 $SnCl_2$-$AgClO_4$[23] or Cp_2ZrCl_2-$AgClO_4$[24] in CH_2Cl_2 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/H_2 (**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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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 ^{1}H 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 (→ 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); $[\alpha]_D^{23}$ + 340.8° (*c* 0.5, $CHCl_3$); ^{1}H 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 $[M+1]^+$.

15) Compound **11** was readily prepared from 2-(trimethylsilyl)ethyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside[16] by reductive opening of the benzylidene-acetal with $LiAlH_4$-$AlCl_3$ in 76% yield: colorless syrup; $[\alpha]_D^{23}$ + 3° (*c* 1, $CHCl_3$); MS (FAB) m/z: 551 $[M+1]^+$, 573 $[M+Na]^+$.

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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 $^4C_1$-conformation.[19,20]

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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.

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25) Obtained as an anomeric mixture of α : β = ca. 4 : 1; $[\alpha]_D^{25}$ - 5.3° (*c* 1, MeOH); MS (FAB) m/z: 751 $[M + 2Na]^+$; Selected ^{1}H 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, $J_{3',4'}$= $J_{4',5'}$=9.0Hz; ^{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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