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Preliminary communication

Synthesis of β-D-(1→6)-linked disaccharides of *N*-fatty acylated 2-amino-2-deoxy-D-glucose: an approach to the lipid A component of the bacterial lipopolysaccharide

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As already described[1], the fundamental, carbohydrate structure of lipid A from *Salmonella* and *Escherichia coli* usually consists of a β-D-(1→6)-linked disaccharide of 2-deoxy-2-(D-3-hydroxytetradecanoylamino)-D-glucopyranose. In order to reconstitute such a β-linked disaccharide of a 2-(acylamino)-2-deoxy-D-glucopyranose, the use of oxazoline derivatives[2,3] appeared most suitable. In this connection, we have recently synthesized[4] such new oxazolines as **9** and **10** by the procedure of Matta and Bahl[5]. As an approach to the lipid A component of the bacterial lipopolysaccharide (LPS), we now describe a facile preparation of β-D-(1→6)-linked disaccharides of *N*-fatty acylated 2-amino-2-deoxy-D-glucose by the oxazoline method.

As we recently reported[3,6,7], when the glycosylation of the protected-sugar acceptors by oxazolines was conducted in 1,2-dichloroethane containing 10–20mM *p*-toluenesulfonic acid, the desired [even the β-D-(1→4)-linked] disaccharides of 2-acetamido-2-deoxy-D-glucose could be obtained in satisfactory yields. In view of this fact, the novel oxazolines **9** and **10**, prepared[4] from 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(tetradecanoylamino)-β-D-glucopyranose and 2-(DL-3-acetoxytetradecanoylamino)-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose, respectively, were used as glycosyl donors in the present disaccharide synthesis. On the other hand, as acceptors, we employed benzyl 3,4-di-*O*-acetyl- and 3,4-di-*O*-(2-butenyl)-2-deoxy-2-(tetradecanoylamino)-β-D-glucopyranoside (**4** and **5**) for coupling with **9**, and benzyl 2-(DL-3-acetoxytetradecanoylamino)-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**8**) for coupling with **10**. These acceptors were prepared as follows: *O*-deacetylation of **1** and selective tritylation of the 6-OH group of the product, followed by reacetylation, gave **2** {m.p. 153–154°, $[\alpha]_D$ +1.58° (*c* 1, chloroform}, whereas, if followed by crotylation, it gave **3** {m.p. 155–157°, $[\alpha]_D$ +1.17° (*c* 1.175, chloroform)}. Compounds **2** and **3** were hydrolyzed with 70% acetic acid, to afford the desired acceptors: **4**, $[\alpha]_D$ −22.07° (*c* 0.906, chloroform); and **5**, $[\alpha]_D$ −2.08°

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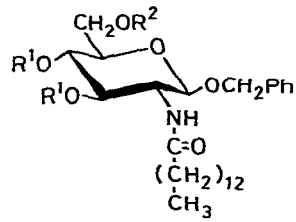
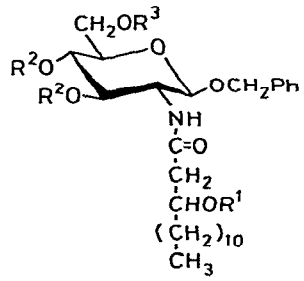




1 $R^1 = H$, $R^2 = Tr$
2 $R^1 = Ac$, $R^2 = Tr$
3 $R^1 = Cro$, $R^2 = Tr$
4 $R^1 = Ac$, $R^2 = H$
5 $R^1 = Cro$, $R^2 = H$

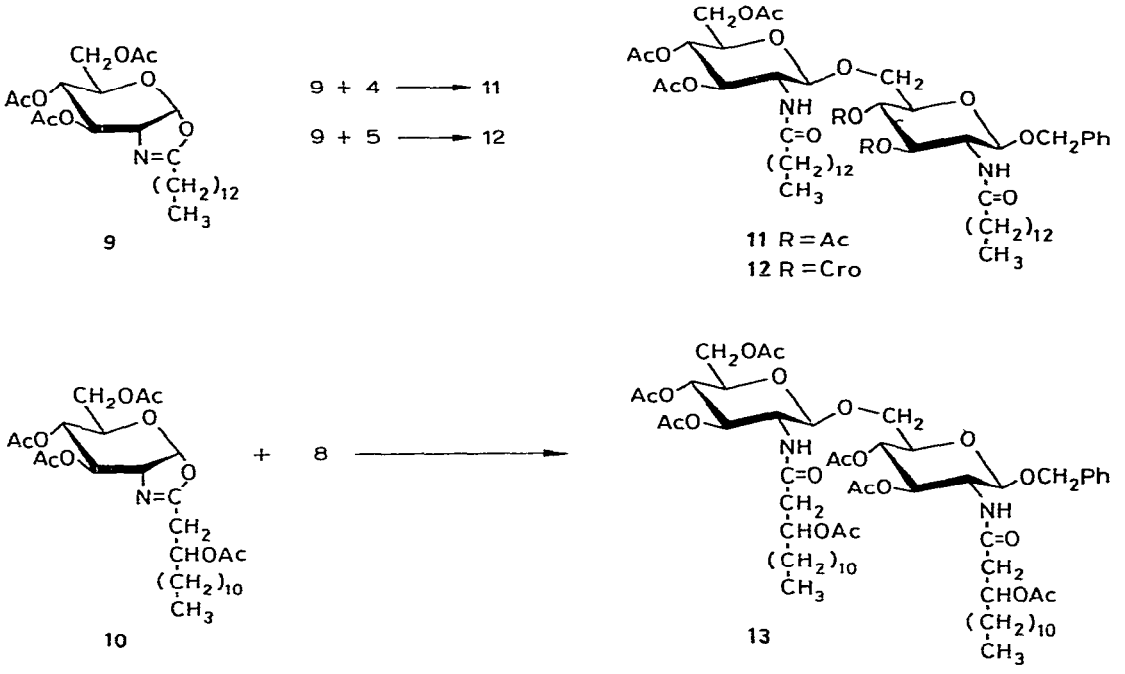
6 $R^1 = R^2 = R^3 = Ac$
7 $R^1 = R^2 = Ac$, $R^3 = Tr$
8 $R^1 = R^2 = Ac$, $R^3 = H$

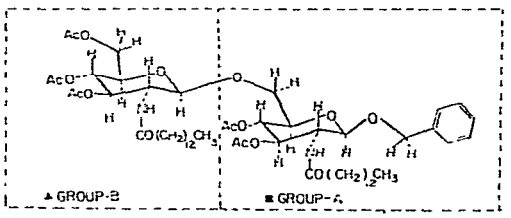
Cro = crotyl = 2-butenyl, Tr = Ph_3C

(*c* 2.205, chloroform), as amorphous materials. Similar treatment of benzyl 2-(DL-3-acetoxytetradecanoylamino)-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside[4] (**6**) gave **7**, and **7** gave **8**, $[\alpha]_D$ −17.87° (*c* 2.485, chloroform).

The glycosylation reaction was conducted by adding a solution of **9** or **10** (3 mol. equiv.) in 1,2-dichloroethane to a mixture of acceptor **4**, **5**, or **8** (1 mol. equiv.) and *p*-toluenesulfonic acid (0.33 mol. equiv.) in 1,2-dichloroethane; the final concentration of the acid was adjusted to ~20mM by adding 1,2-dichloroethane, and the mixture was stirred overnight at 60–70° in the case of **4** or **5**, and at 50° for **8**. When the oxazoline had almost completely disappeared, the mixture was extracted with chloroform as described earlier[6,7]. The resulting residue (containing disaccharide, a small amount of unreacted acceptor, and decomposition products derived from the oxazoline) was chromatographed on a column of silica gel. With 100:1 (v/v) chloroform–methanol as the eluant, the eluate gave the desired, β-D-(1→6)-linked disaccharides in 65–70% yield: **11**, $[\alpha]_D$ −22.95° (*c* 0.671, chloroform); **12**, $[\alpha]_D$ −9.67° (*c* 0.486, chloroform); and **13**, $[\alpha]_D$ −20.63° (*c* 0.538, chloroform), as amorphous materials. The evidence for the β-D-(1→6)-glucosidic linkage was given by the n.m.r. spectra. For example, the ^{1}H-n.m.r. spectrum (400 MHz) of **11** showed doublets for β-anomeric protons at δ 4.52 (*J* 8.7 Hz) and 4.62 (*J* 8.5 Hz), but no signals for α-anomeric protons were observed (see Fig. 1).

Finally, the disaccharides **11** and **13** were treated with methanolic sodium methoxide and the products hydrogenolyzed in the presence of 10% palladium–carbon catalyst, to yield 2-deoxy-6-*O*-[2-deoxy-2-(tetradecanoylamino)-β-D-glucopyranosyl]-2-(tetradecanoylamino)-D-glucose, m.p. 164–166°; and 2-deoxy-6-*O*-[2-deoxy-2-(DL-3-hydroxytetradecanoylamino)-β-D-glucopyranosyl]-2-(DL-3-hydroxytetradecanoylamino)-D glucose, m.p. 191–192°, respectively.





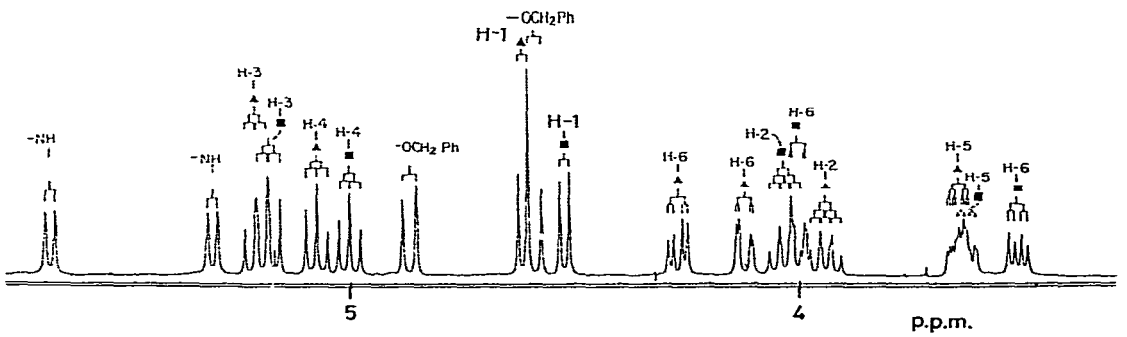
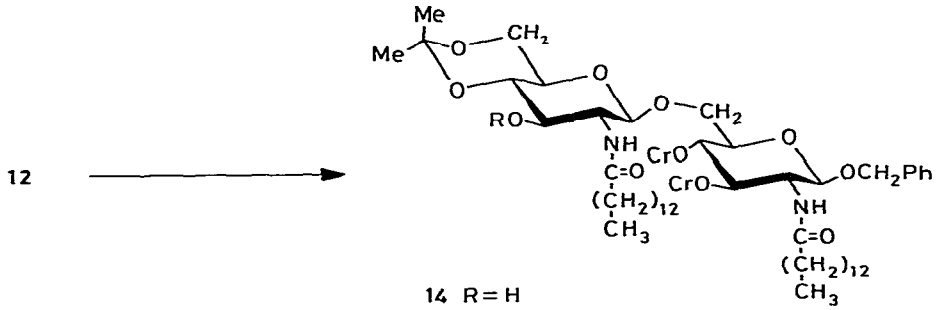


Fig. 1. ^{1}H-N.m.r. spectrum (400 MHz) of **11** in chloroform-*d*.

On the other hand, *O*-deacetylation of **12**, and treatment of the product with 2,2-dimethoxypropane in dry 1,4-dioxane containing a catalytic amount of *p*-toluenesulfonic acid[8], gave amorphous **14** in almost quantitative yield, $[\alpha]_D$ −34.01° (*c* 1.514, chloroform). The remaining, free hydroxyl group at C-3′ of **14** was temporarily protected with a (2-methoxyethoxy)methyl (Mem) group, in order to permit connection of it to 3-deoxy-D-*manno*-octulosonate (KDO) in the final, synthetic stage. The resulting, conveniently protected disaccharide **15**, $[\alpha]_D$ +1.33° (*c* 1.13, chloroform), may be useful for the preparation of some analogs of lipid A esterified with a variety of fatty acyl groups.



14 R = H

15 R = MeO(CH$_2$)$_2$OCH$_2$

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