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Note

Synthesis of a globotetraose trimer

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Abstract

The synthesis is described of a globotetraose trimer in 74% yield by the reaction of tris(2-aminoethyl)amine with the hydrophobic squaric decyl ester glycoside of globotetraose. The synthesis was readily monitored and purified using reversed phase HPLC. Unreacted squaric decyl ester globotetraoside was recovered rendering the method highly economical.

Keywords: P-Antigen; Globotetraose; Trimer; Multivalency; Hydrophobic; Squaric decyl ester

Multivalency has been suggested to be of importance in many carbohydrate–protein interaction events,[1] such as in the adhesion of various pathogenic bacteria to host tissue. A prototype example is the adhesion of P-fimbriated uropathogenic *Escherishia coli* to urinary tract epithelial cells, which is believed to involve a multivalent interaction between fimbrial papG adhesin[2] and the globoside glycolipid (P-antigen) present on the epithelial cell surface.[3] Furthermore, human parvovirus have been proposed to bind human erythroid progenitor cells via a unique and intriguing mode involving the enclosure of three globotetraose tetrasaccharides in one single binding site.[4]

In this note, we describe the synthesis of the trimeric globotetraose derivative **4** amenable for the study of multivalency effects in *E. coli* papG adhesin binding to uroepithelial cells, as well as for probing the possibility of a parvovirus B19 capsid binding site accommodating three globotetraose tetrasaccharide moieties. We have recently demonstrated the versatility of squaric decyl ester glycosides in neoglycoconjugate synthesis;[5] they react in high yields with amino-group carrying entities, such as microtiter plates, BSA, and Sepharose EAH 4B, and unreacted excesses of squaric decyl ester glycosides were easily recovered by means of C18 SPE. Hence, we envisioned that the squaric decyl ester glycoside **1**[5] (Scheme 1) would be an ideally suited globotetraose derivative for conjugation to a tris-amino functional carrier molecule to give the trimeric globotetraose **4**.

Thus, the squaric decyl ester glycoside **1** was dissolved in DMF and a solution of tris(2-aminoethyl)amine in DMF was added (Scheme 1). The reaction progress was monitored by drawing small aliquots from the reaction solution at regular intervals and analyzing the aliquots by reversed-phase HPLC. The squaramide butendione ring is an excellent UV-absorbent,[6] which facilitated the reaction progress monitoring (Fig. 1).

Formation of the monomer **2** was rapid, while formation of the dimer **3** and of the desired trimer **4** was slower. After 12 days, the reaction was stopped and purified by reversed-phase HPLC affording the trimer **4** (74%) together with some impure dimer **3**–trimer **4** mixture and recovered an excess of squaric decyl ester globotetraoside **1**. The products were analyzed and their structures confirmed with MALDI-TOF MS and ^{1}H NMR.

1. Experimental

General.—NMR experiments were recorded with Bruker DRX 400 MHz or Bruker DRX 500 MHz spectrometers at ambient temperature. ^{1}H NMR assignments were derived from COSY experiments. The

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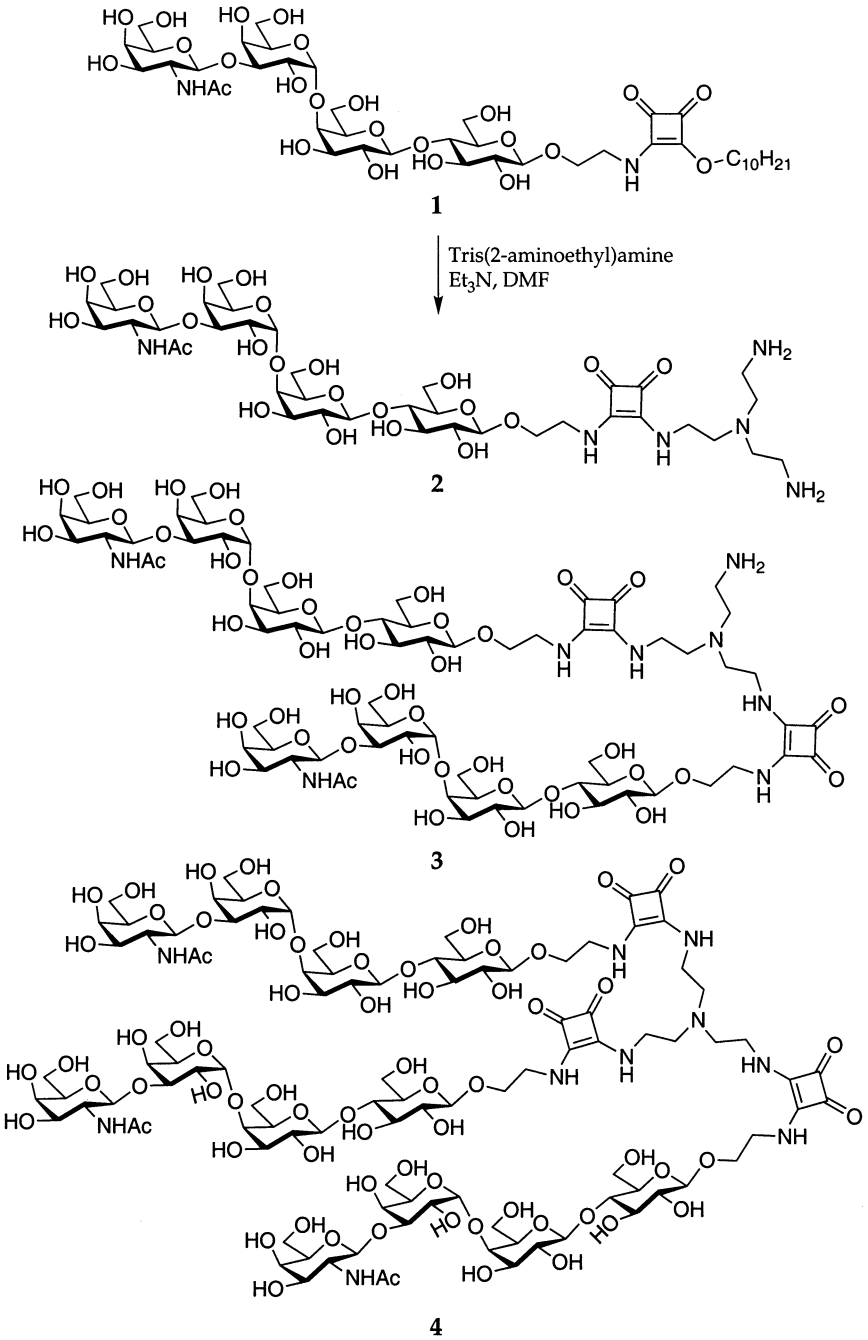
optical rotations were measured with a Perkin–Elmer 241 polarimeter. MALDI-TOF MS experiments were recorded with a Bruker Biflex III instrument (run in positive mode) using gentisic acid (2,5-dihydroxy benzoic acid) as matrix. Monitoring and purification of the conjugation reaction was performed on a Beckman System Gold HPLC instrument (C18 reversed phase).

Globotetraose trimer (**4**).—To a solution of **1** (10.1 mg, 10.2 μmol) in dry DMF (0.5 mL) was added tris-(2-aminoethyl)amine (1.8 μmol; 180 μL of a 10 mM solution in DMF) at ambient temperature. Et_3N was added after 24 h (1.4 μL, 10.2 μmol) and after 48 h (1.4 μL, 10.2 μmol). After 12 days, the solvent was evaporated under vacuum, and the residue was dissolved (sonication) in water (75 μL) and separated on HPLC affording pure unreacted **1** (4.5 mg, 94% yield based on calculated excess **1**), an dimer–trimer mixture (**3** + **4** + unidentified impurity, 1.6 mg), and pure trimer **4** (3.5 mg, 74%). ^{1}H NMR (500 MHz, D_2O): δ 5.04 (d, 1 H, J 4.2 Hz, H-1″), 4.76 (d, 1 H, J 8.4 Hz, H-1‴), 4.65 (d, 1 H, J 5.9 Hz, H-1′), 4.64 (d, 1 H, J 7.7 Hz, H-1), 4.50 (brt, 1 H, J 6.4 Hz, H-5″), 4.37 (brd, 1 H, J 2.3 Hz,



1 → **2** → **3** → **4**

Scheme 1.

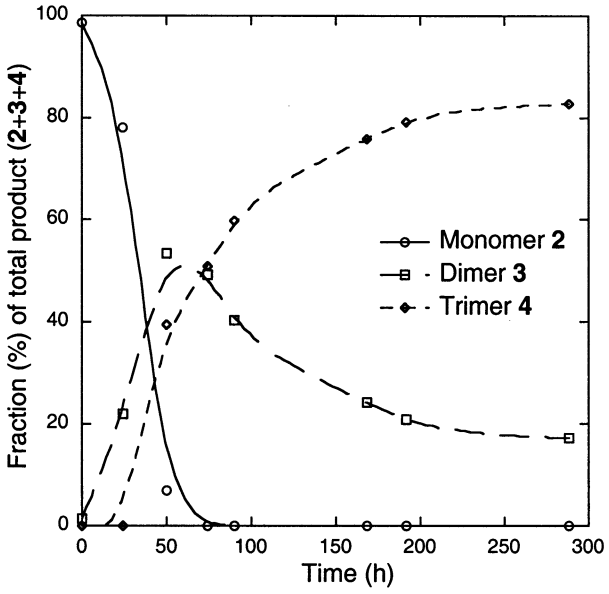


Fig. 1. Reaction monitoring with HPLC.

H-4″), 3.44 (brt, 1 H, *J* 8.0 Hz, H-2), 2.16 (s, 3 H, Ac). MALDI-TOF MS Calcd for $C_{102}H_{162}N_{10}NaO_{69}$ [M + Na]: 2653.9; Found: 2653.8.

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