Studies toward Lipid A: Synthesis of Differentially Protected Disaccharide Fragments†

Sian L. Griffiths,‡ Robert Madsen,‡ and Bert Fraser-Reid*,‡

Paul M. Gross Chemical Laboratory, Department of Chemistry, Duke University, Durham, North Carolina 27708, and Natural Products and Glycotechnology Research Institute, Inc. (NPG), Durham, North Carolina 27707

Received August 29, 1996 (Revised Manuscript Received March 6, 1997⊗)

Disaccharides containing two glucosamine units are readily synthesized where the two amine functionalities are orthogonally protected as the tetrachlorophthaloyl and pent-4-enoyl moieties. The 1→6 linked disaccharide has the potential to be developed toward a series of biologically interesting lipid A analogs.

Background

We have recently highlighted the use of both pent-4-enoyl and tetrachlorophthaloyl (TCP) as protecting groups for amine functionalities.[1] In particular when used on 2-deoxy-2-amino glucosamine derivatives, the TCP group has several attractive features as compared to those of other commonly used protecting groups, notably the relatively mild conditions that can be used for its removal.[1a,c,d,2] In this paper we report the further exploration of pent-4-enoyl-protected glucosamine derivatives and the subsequent generation of disaccharides containing two glucosamine units where the amine functionalities are orthogonally protected as the pent-4-enoyl and TCP moieties.

Introduction

Endotoxins are complex lipopolysaccharides (LPS) situated in the outer membrane of the cell wall of Gram-negative bacteria.[3] They are essential for cell survival, but during an infection they can be liberated from the cell wall and through a pathway involving reaction with the lipopolysaccharide binding protein can cause the release of agents such as the interleukins. High levels of LPS therefore cause a huge immune response leading to such pathological effects as fever, shock, and in extreme cases organ failure and death.[4] Indeed, the mortality rate of bacterial sepsis (systemic inflammatory response syndrome) is as high as 60%. The terminal disaccharide phospholipid of the LPS, known as lipid A (**1** from *Escherichia coli*) has been shown to be responsible for its biological activity.[3,5] Furthermore it has been shown that the number and the structure of the fatty acid chains present on the molecule may strongly influence its endotoxic activity.[6]

Recently a lipid A molecule was isolated from *Rhodbacter sphaeroides* which was nontoxic but was a potent antagonist of LPS-induced responses.[7] Christ and coworkers[8,9] synthesized the proposed structure of this lipid A molecule and thereby disproved the suggested structure; however, the synthetic material, also a potent antagonist, was used as the basis for their recently described lipid A analog E5531 (**2**).[10] To confer stability onto the molecule the 3 and 3′ acyl linkages were replaced with ether linkages and a methyl group was added to the free primary alcohol. E5531 was shown to inhibit LPS-induced processes *in vitro.* Additionally, *in vivo* studies showed that mice could be protected from a lethal injection of *E. coli* when E5531 was administered in conjunction with a β-lactam antibiotic, and now E5531 is in phase II clinical trials as a potential drug candidate.

The general structure of the lipid A molecule, which consists of two glucosamine units, requires that the amino groups be differentiated, and here was seen a potential application of our amine protecting group methodology. It was envisaged that an intermediate such as **A** could be generated that would allow for each of the four sites (R^1−R^4) occupied by a fatty acid chain to be independently manipulated, thereby giving access to a series of lipid A analogs from a common intermediate (Figure 1).

Results and Discussion

Early attempts to synthesize these disaccharides using *N*-pent-4-enoylated glycosyl chlorides as donor molecules were unsuccesful, in keeping with literature precedent that these 2-*N*-acyl-protected compounds are unreactive due to the formation of the intermediate oxazolines.[11] Our

† Supported by a grant from PHS AI 31862 (to B.F.-R.).

‡ Present addresses: S.L.G., Pharmacopeia Inc., Princeton, NJ 08540. R.M., Department of Organic Chemistry, Technical University of Denmark, Lyngby, Denmark. B.F.-R., Natural Products and Glycotechnology (NPG) Research Institute Inc., 4118 Swarthmore Road, Durham, NC 27707.

⊗ Abstract published in *Advance ACS Abstracts,* April 15, 1997.

(1) (a) Debenham, J. S.; Madsen, R.; Roberts, C.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 3302. (b) Madsen, R.; Roberts, C.; Fraser-Reid, B. *J. Org. Chem.* **1995**, *60*, 7920. (c) Debenham, J. S.; Fraser-Reid, B. *J. Org. Chem.* **1996**, *61*, 432. (d) Debenham, J. S.; Debenham, S. D.; Fraser-Reid, B. *Bioorg. Med. Chem.*, in press.

(2) Stangier, P.; Hindsgaul, O. *Synlett* **1996**, 179. Castro-Palomino, J. C.; Schmidt, R. R. *Tetrahedron Lett.* **1995**, *36*, 5343. See also *N,N*-diacetyl protection which requires more basic conditions (NaOMe/MeOH) for removal: Castro-Palomino, J. C.; Schmidt, R. R. *Tetrahedron Lett.* **1995**, *36*, 6871.

(3) Raetz, C. H. R. *Annu. Rev. Biochem.* **1990**, *59*, 129. Raetz, C. H. R. *J. Bacteriol.* **1993**, *175*, 5745.

(4) Parillo, J. E. *N. Engl. J. Med.* **1993**, *328*, 1471.

(5) Rietschel, E. T.; Brade, H. *Sci. Am.* **1992**, *267*, 4904.

(6) Zähringer, U.; Lindner, B.; Rietschel, E. T. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 211.

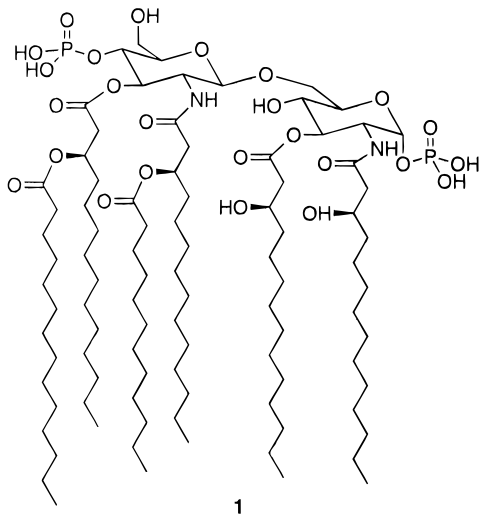
(7) (a) Qureshi, N.; Honovich, J. P.; Hara, H.; Cotter, R. J.; Takayama, K. *J. Biol. Chem.* **1988**, 263. (b) Qureshi, N.; Takayama, K.; Kurtz, R. *Infect. Immun.* **1991**, *59*, 441.

(8) Christ, W. J.; McGuinness, P. D.; Asano, O.; Wang, Y.; Mullarkey, M. A.; Perez, M.; Hawkins, L. D.; Blythe, T. A.; Dubuc, G. R.; Robidoux, A. L. *J. Am. Chem. Soc.* **1994**, *116*, 3637.

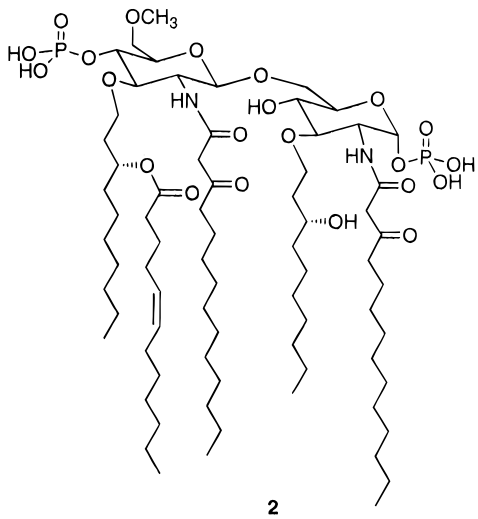
(9) For other synthetic work on lipid A molecules see: Kusumoto, S. In *Bacterial Endotoxic Lipopolysaccharides*; Morrison, D. C., Ryan, J. L., Eds.; CRC: Boca Raton, FL, 1992; p 81.

(10) Christ, W. J.; Asano, O.; Robidoux, A. L. C.; Perez, M.; Wang, Y.; Dubuc, G. R.; Gavin, W. E.; Hawkins, L. D.; McGuinness, P. D.; Mullarkey, M. A.; Lewis, M. D.; Kishi, Y.; Kawata, T.; Bristol, J. R.; Rose, J. R.; Rossignol, D. P.; Kobayashi, S.; Hishinuma, I.; Kimura, A.; Asakawa, N.; Katayama, K.; Yamatsu, I. *Science* **1995**, *268*, 80.

S0022-3263(96)01668-4 CCC: $14.00



1



2

Figure 1.

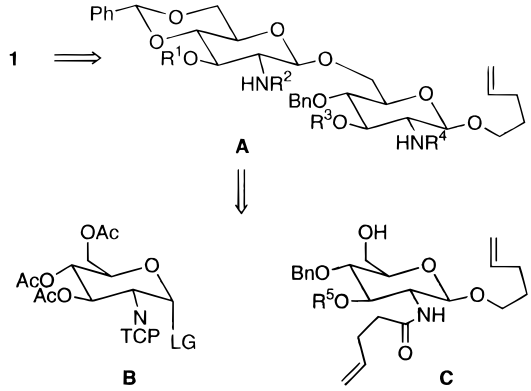
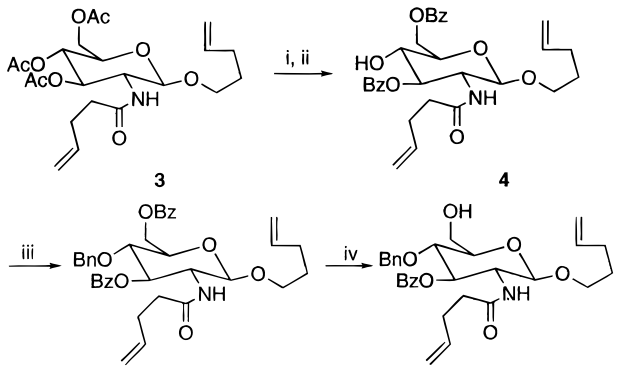


Figure 2.

retrosynthesis therefore led to coupling partners **B** and **C**, where the TCP group was situated on the donor molecule **B**, since we have previously shown that TCP-protected derivatives acted as excellent donor molecules.[1a,c,d] The use of a pentenyl coupling procedure involving **B** is incompatible with the *N*-pent-4-enoyl group on **C**, so instead a Koenigs−Knorr coupling reaction would be used to form the key glycosidic bond (Figure 2). The acceptor molecule **6** was synthesized in three steps from the previously reported pentenyl glycoside **3**.[1b] Hydrolysis of the acetate groups and subsequent treatment with benzoyl chloride at low temperature[12] gave the selectively protected 3,6-derivative **4** which could be crystallized in 81% yield. Benzylation of this material under acidic conditions using benzyl trichloroacetimidate following literature procedures[12a,13] was attempted, but the reaction was sluggish and only produced a poor yield of 4-benzyl ether **5**. Instead **4** was benzylated directly to afford **5** in 80% yield employing sodium hydride and excess benzyl bromide. Selective removal of the C-6 benzoate was accomplished following Shiba's procedure[12a] to afford coupling partner **6** in 75% yield (Scheme 1).

Scheme 1[a]



[a] Reagents: (i) NaOMe/MeOH; (ii) BzCl, py, −40 °C, 81% from **3**; (iii) BnBr, NaH, *n*−Bu_4NI DMF, 80%; (iv) 0.04 M NaOMe, 0 °C, 7 h, 75%.

The TCP-protected tetraacetate derivate **7** was synthesized as previously reported.[1d] Treatment with HBr in acetic acid and acetic anhydride overnight afforded the glycosyl bromide **8** which was used directly in the coupling reaction. The most efficient promoter for the glycosidic bond formation was found to be silver triflate,[14] which when used in conjunction with activated powdered 4 Å molecular sieves in dichloromethane afforded a pleasing 76% yield of the disaccharide **9** (Scheme 2). The *trans* linkage was ensured by the neighboring group participation of the TCP group during the coupling reaction.

With the desired disaccharide **9** in hand attention then turned to chemoselective deprotection of the the two amide protecting groups. The *N*-pent-4-enoyl group was easily removed from **9** by treatment with iodine (3 equiv) in a tetrahydrofuran/water mixture[1a,b] in approximately 8 min, affording the free amine **10** in 71% yield. Longer exposure to the reaction conditions led to the isolation of products where the iodine had additionally attacked the *O*-pentenyl double bond. The use of other solvent mixtures for the deprotection such as acetonitrile/water, which had been shown to give similar yields but with increased reaction times,[1b] led to mixtures of products.

The selective removal of the TCP group from **9** proved to be somewhat more challenging, not however due to

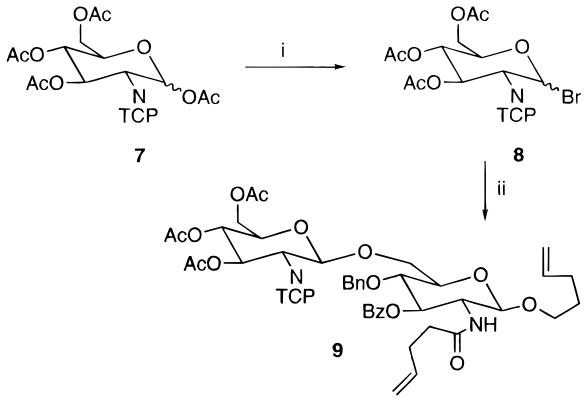
(11) For a review on the synthesis of oligosaccharides of 2-deoxy-2-amino sugars see: Banoub, J.; Boullanger, P.; Lafont, D. *Chem. Rev.* **1992**, *92*, 1167.

(12) (a) Imoto, M.; Yoshimura, H.; Yamamoto, M.; Shimamoto, T.; Sakaguchi, N.; Kusumoto, S.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2197. (b) El-Sokkary, R. I.; Silwanis, B. A.; Nashed, M. A.; Paulsen, H. *Carbohydr. Res.* **1990**, *203*, 319.

(13) Wessel, H-P.; Iversen, T.; Bundle, D. R. *J. Chem Soc., Perkin Trans. 1* **1985**, 2247.

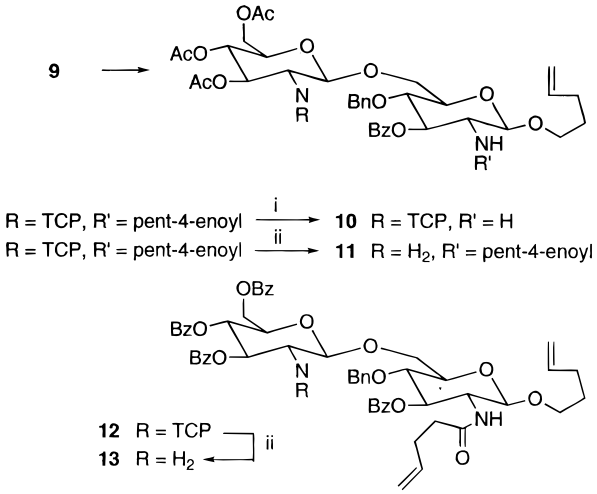
(14) Hanessian, S.; Banoub, J. *Carbohydr. Res.* **1977**, *53*, C13.

Scheme 2[a]



[a] Reagents: (i) HBr/Ac_2O; (ii) **6**, CH_2Cl_2, AgOTf, −20 °C to rt, 4 Å molecular sieves, 76%.

Scheme 3[a]



[a] Reagents: (i) I_2, THF/H_2O, 8 min; (ii) MeCN/THF/EtOH (2:1:1), ethylenediamine, 60 °C.

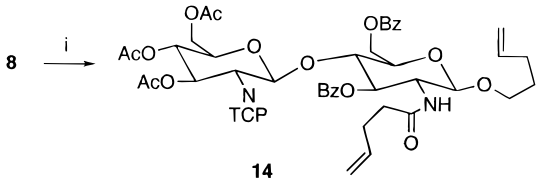
removal of the pent-4-enoyl group but because of concomitant removal of the C-6 acetate group. Under a variety of conditions the best yield that could be obtained for the free amine **11** was 43% along with about 30% of the disaccharide where both the TCP and the C-6 acetate group had been removed. However when the corresponding tetrabenzoate derivative **12**[15] was exposed to the deprotection conditions, a much more acceptable yield (64%) of the free amine **13** was obtained (Scheme 3).

Finally we briefly explored the synthesis of the corresponding 1→4 linked disaccharide where the previously synthesized **4** would be used as our acceptor molecule. Although **4** is both sterically and electronically unreactive, under the same conditions that were used before, the 1→4 linked ketobioside **14** was obtained in an acceptable 58% yield (Scheme 4).

In conclusion, we have reported the synthesis of disaccharides containing two glucosamine units where, given suitable protection of the hydroxy groups, the two amine functionalities are orthogonally protected. The 1→6 linked derivative **9** has the potential to be developed toward a key intermediate for the synthesis of a large number of biologically interesting lipid A analogs, in which each of the four fatty acid containing positions in the naturally occuring molecules would be individually

(15) Prepared from disaccharide **9** in two steps.

Scheme 4[a]



[a] Reagents: (i) **4**, CH_2Cl_2, AgOTf, −20 °C to rt, 4 Å molecular sieves, 58%.

accessible. Work toward this objective is currently in progress and will be reported in due course.

Experimental Section

General Procedures. All reactions were conducted under a dry argon atmosphere. THF was distilled from sodium benzophenone ketyl. Dichloromethane and acetonitrile were distilled from calcium hydride. Absolute ethanol was stored over 4 Å molecular sieves. Solutions of compounds in organic solvents were dried over sodium sulfate. TLC plates were Kieselgel 60 F254 (Merck Art. 5554). Flash column chromatography was done with silica gel (230−400 mesh, Merck). FAB mass spectra were recorded using a *m*-nitrobenzyl alcohol matrix with xenon as the fast atom. Accurate mass measurements were made using FAB at 10k resolution. Elemental analyses were conducted by Atlantic Microlab, Inc., P.O. Box 2288, Norcross, GA 30091.

Pent-4-enyl 3,6-Di-*O*-benzoyl-2-deoxy-2-(pent-4-enoylamino)-*β*-D-glucopyranoside (4). To a solution of pent-4-enyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(pent-4-enoylamino)-*β*-D-glucopyranoside (**3**) (4.5 g, 9.88 mmol) in CH_2Cl_2 (4 mL) was added 0.04 M NaOMe in MeOH (50 mL), and the mixture was stirred at rt for 1 h. The reaction was quenched with Amberlite IR-120(H^+), and the solution was filtered through Celite. The filter cake was further washed with MeOH (15 mL), and the combined filtrate was concentrated to give a crystalline residue which was dried under high vacuum overnight. To this residue dissolved in pyridine (40 mL) at −40 °C was added benzoyl chloride (2.5 mL, 21.5 mmol), and the reaction mixture was stirred at this temperature for 2 h. The mixture was diluted with $CHCl_3$ (150 mL) and washed successively with water (150 mL), 5% aqueous HCl (2 × 150 mL), and saturated aqueous $NaHCO_3$ (150 mL) solutions. The organic solutions were dried (Na_2SO_4) and concentrated to give a foam which was crystallized from Et_2O to afford **4** (4.28 g, 81%): mp 139−140 °C; $[\alpha]^{20}_D$ +9.7 (*c* 1, $CHCl_3$); ^1H NMR δ 8.10−8.04 (m, 4H, Ph), 7.62−7.55 (m, 2H, Ph), 7.48−7.40 (m, 4H, Ph), 5.85 (d, J = 9.2 Hz, NH), 5.77 (m, −CH═), 5.62 (m, −CH═), 5.46 (dd, J = 8.6, 10.5 Hz, 1H), 5.02−4.84 (m, 3H), 4.77−4.61 (m, 4H), 4.11 (m, 1H), 3.93−3.78 (m, 3H), 3.52 (m, 1H), 3.34 (d, J = 4.6 Hz, OH), 2.25−2.05 (m, 6H), 1.74−1.65 (m, 2H); ^{13}C NMR 172.6, 167.5, 167.1, 138.0, 136.7, 133.6, 133.3, 130.0, 129.8, 129.7, 129.2, 128.5, 128.4, 115.4, 114.9, 101.0, 76.2, 74.1, 69.7, 69.0, 64.0, 54.3, 36.0, 30.0, 29.4, 28.7. Anal. Calcd for $C_{30}H_{35}NO_8$: C, 67.02; H, 6.55; N, 2.61. Found: C, 67.18; H, 6.60; N, 2.57.

Pent-4-enyl 3,5-Di-*O*-benzoyl-4-*O*-benzyl-2-deoxy-2-(pent-4-enylamino)-*β*-D-glucopyranoside (5). To an ice-cooled solution of **4** (2.5 g, 4.65 mmol), Bu_4NI (0.3 g, 0.81 mmol), and BnBr (4 mL, 33.6 mmol, 7 equiv) in DMF (20 mL) was added NaH (0.25 g of a 60% oil dispersion, 6.25 mmol). The ice bath was removed, and the mixture was stirred at rt for 20 min. The reaction was quenched by the addition of AcOH, and the mixture was diluted with CH_2Cl_2 (70 mL) and washed with water (70 mL). The organic solution was dried, concentrated, then taken up in Et_2O, and filtered to remove crystalline impurities. The filtrate was concentrated and purified by column chromatography (95:5 CH_2Cl_2/EtOAc) to afford **5** (2.33 g, 80%): R_f = 0.52; mp 148−149 °C; $[\alpha]^{20}_D$ +4.9 (*c* 1, $CHCl_3$); ^1H NMR δ 8.10−8.04 (m, 4H, Ph), 7.64−7.57 (m, 2H, Ph), 7.52−7.44 (m, 4H, Ph), 7.16−7.06 (m, 5H, Ph), 5.84−5.55 (m, 3H), 5.48 (dd, J = 8.7, 10.5 Hz, 1H), 5.01−4.85 (m, 3H), 4.75 (d, J = 10.2 Hz, 1H), 4.63−4.50 (m, 5H), 4.28 (m,

1H), 3.94−3.76 (m, 3H), 3.47 (m, 1H), 2.24−2.03 (m, 6H), 1.71−1.60 (m, 2H); ^{13}C NMR 172.3, 167.0, 166.3, 138.1, 136.9, 133.7, 133.2, 130.0, 129.8, 129.8, 129.3, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 115.4, 114.9, 101.5, 75.9, 75.0, 73.1, 68.9, 63.3, 53.8, 36.0, 30.1, 29.4, 28.7. Anal. Calcd for $C_{37}H_{41}NO_8$: C, 70.80; H, 6.58; N, 2.23. Found: C, 70.93; H, 6.65; N, 2.27.

Pent-4-enyl 3-*O*-Benzoyl-4-*O*-benzyl-2-deoxy-2-(pent-4-enoylamino)-*β*-D-glucopyranoside (6). A solution of **5** (1.1 g, 1.75 mmol) in CH_2Cl_2 (2 mL) was treated with 0.04 M methanolic NaOMe (20 mL) at 0 °C for 7 h, and then the reaction was quenched with Amberlite IR-120(H^+). The reaction mixture was filtered through Celite, and the filter cake was rinsed with a further amount of MeOH (20 mL). The combined organic solution was concentrated to give a crystalline residue which was taken up in hot petroleum ether, cooled, and then filtered to afford **6** (0.69 g, 75%): mp 150−151 °C; $[\alpha]^{20}_D$ −39.4 (*c* 1, $CHCl_3$); ^{1}H NMR δ 8.05−8.00 (m, 2H, Ph), 7.63−7.56 (m, 1H, Ph), 7.48−7.42 (m, 2H, Ph), 7.19−7.10 (m, 5H, Ph), 5.85−5.71 (m, 2H), 5.61 (m, 1H), 5.46 (dd, *J* = 9.2, 10.7 Hz, 1H), 5.04−4.83 (m, 3H), 4.73 (d, *J* = 10.1 Hz, 1H), 4.61 (s, 2H), 4.51 (d, *J* = 8.3 Hz, 1H), 4.21 (m, 1H), 3.93−3.73 (m, 4H), 3.55−3.42 (m, 2H), 2.23−1.99 (m, 7H), 1.73−1.62 (m, 2H); ^{13}C NMR 172.4, 167.1, 138.0, 137.4, 136.8, 133.6, 129.9, 129.4, 128.6, 128.4, 128.3, 128.0, 115.1, 115.0, 101.5, 76.1, 75.7, 75.0, 74.9, 69.2, 61.6, 53.9, 35.9, 30.0, 29.3, 28.7. Anal. Calcd for $C_{30}H_{37}NO_7$: C, 68.81; H, 7.12; N, 2.67. Found: C, 68.91; H, 7.17; N, 2.62.

Pent-4-enyl (3,4,6-Tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-*β*-D-glucopyranosyl)-(1→6)-3-*O*-benzoyl-4-*O*-benzyl-2-deoxy-2-(pent-4-enoylamino)-*β*-D-glucopyranoside (9). To **7** (1 g, 1.63 mmol) in acetic anhydride (0.4 mL, 4.24 mmol) was added HBr (45% w/v solution in acetic acid, 1.5 mL, 8.44 mmol). The reaction mixture was stirred at rt for 24 h while being protected from light. After being diluted with $CHCl_3$ (20 mL), the reaction mixture was poured onto ice/water (20 mL). The organic layer was separated, washed with H_2O (2 × 30 mL) and a saturated aqueous $NaHCO_3$ solution (30 mL), dried, and concentrated to afford anomeric bromide **8** as a syrup. To this material, **6** (0.573 g, 1.08 mmol) (dried by azeotroping both together from toluene), and powdered 4 Å molecular sieves (0.5 g) in CH_2Cl_2 (5 mL) at −25 °C was added AgOTf (0.515 g, 2.00 mmol). The reaction mixture was removed from the cold bath, protected from light, and stirred at rt for 24 h. After the reaction was quenched with a saturated aqueous $NaHCO_3$ solution and brine (5 mL total), the mixture was filtered through Celite. The filter cake was washed with additional CH_2Cl_2 (20 mL), and the combined filtrate was washed with a saturated aqueous $NaHCO_3$ solution (30 mL), dried, and concentrated. Purification by column chromatography (10:1 CH_2Cl_2/EtOAc) afforded **9** (0.883 g, 76%): R_f = 0.56 (6:1 CH_2Cl_2/EtOAc); mp 120−123 °C; $[\alpha]^{20}_D$ = −3.6 (*c* 1, $CHCl_3$); ^{1}H NMR δ 7.98 (d, *J* = 8.2 Hz, 2H,), 7.59 (t, *J* = 7.0 Hz, 1H), 7.40 (t, *J* = 7.7 Hz, 2H), 7.12−7.05 (m, 3H), 6.86 (d, *J* = 7.5 Hz, 2H), 5.78−5.52 (4H, m), 5.44 (d, *J* = 8.4 Hz, 1H), 5.35 (dd, *J* = 8.8, 10.4 Hz, 1H), 5.21 (t, *J* = 9.5 Hz, 1H), 5.01−4.82 (m, 3H), 4.72 (d, *J* = 10.2 Hz, 1H), 4.41−4.30 (m, 5H), 4.20−3.99 (m, 3H), 3.81−3.72 (m, 3H), 3.63 (t, *J* = 8.8 Hz, 1H), 3.53 (m, 1H), 3.40 (m, 1H), 2.16−2.06 (m, 6H), 2.10 (s, 3H), 2.03 (s, 3H), 1.89 (s, 3H), 1.65−1.58 (m, 2H); ^{13}C NMR 172.1, 170.6, 170.5, 169.3, 166.7, 163.4, 162.3, 140.5, 138.0, 136.7, 136.7, 133.5, 129.8, 129.7, 129.2, 128.5, 128.1, 128.0, 127.5, 126.6, 101.1, 97.3, 76.5, 75.7, 74.6, 73.5, 71.8, 70.7, 68.6, 67.6, 61.9, 55.2, 53.7, 35.8, 29.9, 29.2, 28.6, 20.7, 20.6, 20.4. Anal. Calcd for $C_{50}H_{52}Cl_4N_2O_{16}$: C, 55.67; H, 4.86; N, 2.60. Found: C, 55.76; H, 4.86; N, 2.54.

Pent-4-enyl (3,4,6-Tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-*β*-D-glucopyranosyl)-(1→6)-3-*O*-benzoyl-4-*O*-benzyl-2-deoxy-2-amino-*β*-D-glucopyranoside (10). To a solution of **9** (0.05 g, 0.046 mmol) in THF (0.5 mL) was added an equal volume of H_2O (0.5 mL). Additional THF (0.5 mL) was then added until the turbid solution became clear. Iodine (0.035 g, 0.139 mmol) was then added in one portion at rt, and the resulting brown solution was stirred for 8 min. The reaction was quenched by the addition of solid $(NH_4)_2S_2O_3$ until the brown color disappeared. The mixture was concentrated, and the residue was taken up in $CHCl_3$ and washed with brine (10 mL). The organic layer was dried and concentrated, and purification by column chromatography (99:1 CH_2Cl_2/MeOH) afforded **10** (0.0328 g, 71%) as a white foam: R_f = 0.46 (97:3 CH_2Cl_2/MeOH); $[\alpha]^{20}_D$ = +1.0 (*c* 1.19, $CHCl_3$); ^{1}H NMR δ 8.02 (d, *J* = 7.4 Hz, 2H), 7.58 (t, *J* = 7.4 Hz, 1H), 7.47 (approximate t, *J* = 7.4, 7.8 Hz, 2H), 7.04 (m, 3H), 6.78 (2 overlapping doublets, *J* = 7.9, 7.3, 2H), 5.80−5.68 (m, 2H), 4.42−4.05 (m, 7H), 3.87−3.78 (m, 3H), 3.58−3.49 (m, 3H), 2.92 (m, 1H), 2.14−2.00 (m, 2H), 2.09 (s, 3H), 2.03 (s, 3H), 1.88 (s, 3H), 1.67 (m, 2H); ^{13}C NMR 170.7, 170.6, 169.3, 166.0, 140.8, 137.9, 136.6, 133.2, 129.7, 129.5, 128.6, 128.1, 127.9, 127.5, 127.5, 126.7, 115.0, 103.9, 97.4, 77.4, 76.6, 74.6, 74.3, 71.8, 70.8, 69.3, 68.5, 68.1, 56.5, 55.2, 30.2, 28.7, 20.7, 20.6, 20.5; HRMS (FAB) calcd for $C_{45}H_{47}Cl_4N_2O_{15}$ [$(MH)^+$] 995.1731, found 995.1712.

Pent-4-enyl (3,4,6-Tri-*O*-acetyl-2-deoxy-2-amino-*β*-D-glucopyranosyl)-(1→6)-3-*O*-benzoyl-4-*O*-benzyl-2-deoxy-2-(pent-4-enoylamino)-*β*-D-glucopyranoside (11). To a solution of **9** (0.05 g, 0.0463 mmol) in 2:1:1 MeCN/EtOH/THF (0.5 mL) was added ethylenediamine (6 μL, 0.09 mmol), and the solution was heated at 60 °C for 2 h. The reaction mixture was allowed to cool and then concentrated. The residue was purified by column chromatography (98:2 CH_2Cl_2/MeOH) to afford **11** (0.016 g, 43%) as a clear film: R_f = 0.41 (95:5 CH_2Cl_2/MeOH); $[\alpha]^{20}_D$ = −15.9 (*c* 1, $CHCl_3$); ^{1}H NMR δ 8.02 (dd, *J* = 1.0, 7.4 Hz, 2H), 7.58 (t, *J* = 7.4 Hz, 1H), 7.47 (t, *J* = 7.6 Hz, 1H), 7.19 (m, 3H), 7.12 (m, 2H), 5.78 (m, 1H), 5.62 (m, 2H), 5.44 (m, 1H), 5.04−4.84 (m, 5H), 4.74 (d, *J* = 9.8 Hz, 1H), 4.58 (ABq, 2H), 4.50 (d, *J* = 8.1 Hz, 1H), 4.28 (m, 2H), 4.20−4.07 (m, 3H), 3.87 (m, 1H), 3.75−3.70 (m, 3H), 3.63 (m, 1H), 3.46 (m, 1H), 2.96 (dd, *J* = 8.4, 9.3 Hz, 1H), 2.19−2.10 (m, 6H), 2.08 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 1.69−1.58 (m, 2H); ^{13}C NMR 172.1, 170.8, 170.7, 169.8, 166.6, 138.0, 137.1, 136.7, 133.5, 129.8, 129.2, 128.5, 128.4, 128.0, 128.0, 115.3, 114.9, 101.3, 77.1, 75.4, 74.7, 74.5, 71.9, 69.0, 69.0, 68.7, 62.0, 55.6, 53.9, 35.8, 30.0, 29.2, 28.6, 20.9, 20.8, 20.7; HRMS (FAB) calcd for $C_{42}H_{55}N_2O_{14}$ [$(MH)^+$] 811.3653, found 811.3661.

Pent-4-enyl (3,4,6-Tri-*O*-benzoyl-2-deoxy-2-amino-*β*-D-glucopyranosyl)-(1→6)-3-*O*-benzoyl-4-*O*-benzyl-2-deoxy-2-(pent-4-enoylamino)-*β*-D-glucopyranoside (13). To pent-4-enyl (3,4,6-tri-*O*-benzoyl-2-deoxy-2-tetrachlorophthalimido-*β*-D-glucopyranosyl)-(1→6)-3-*O*-benzoyl-4-*O*-benzyl-2-deoxy-2-(pent-4-enoylamino)-*β*-D-glucopyranoside (**12**)[15] (24.6 mg, 0.019 mmol) in 2:1:1 MeCN/EtOH/THF (0.4 mL) was added ethylenediamine (5.9 μL, 0.088 mmol), and the solution was heated at 60 °C for 2 h. The reaction mixture was allowed to cool and then concentrated. The residue was purified by column chromatography (99:1 CH_2Cl_2/MeOH) to afford **13** (12.8 mg, 66%) as a clear film: R_f = 0.41 (95:5 CH_2Cl_2/MeOH); $[\alpha]^{20}_D$ = −20.6 (*c* 1.28, $CHCl_3$); ^{1}H NMR δ 8.03−7.86 (m, 8H), 7.61−7.28 (m, 12H), 7.21−7.10 (m, 5H), 5.76 (m, 1H), 5.66−5.42 (m, 4H), 4.97−4.84 (m, 3H), 4.74 (d, *J* = 10.2 Hz, 1H), 4.62−4.42 (m, 6H), 4.19 (m, 2H), 4.01 (m, 1H), 3.92−3.82 (3H, m), 3.82−3.72 (m, 2H), 3.75 (m, 2H), 3.45 (m, 1H), 3.21 (m, 1H), 2.19−2.01 (m, 6H), 1.70−1.61 (m, 2H); ^{13}C NMR 172.1, 166.6, 166.3, 166.1, 165.4, 138.1, 137.1, 136.7, 133.5, 133.4, 133.1, 129.8, 129.7, 129.2, 129.0, 128.8, 128.5, 128.4, 128.4, 128.4, 128.1, 128.0, 115.3, 114.5, 101.3, 77.2, 76.8, 75.4, 74.7, 74.3, 72.1, 69.7, 69.1, 69.1, 63.2, 56.2, 53.8, 35.8, 30.0, 29.2, 28.7; HRMS (FAB) calcd for $C_{57}H_{61}N_2O_{14}$ [$(MH)^+$] 997.4123, found 997.4119.

Pent-4-enyl (3,4,6-Tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-*β*-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzoyl-2-deoxy-2-(pent-4-enoylamino)-*β*-D-glucopyranoside (14). Glycosyl bromide **8** was prepared in the same way as described for the synthesis of **9** from **7** (57.2 mg, 0.093 mmol). To this material, **4** (25 mg, 0.0465 mmol) (dried by azeotroping both together from toluene), and powdered 4 Å molecular sieves (100 mg) in CH_2Cl_2 (2 mL) at −25 °C was added AgOTf (26.3 mg, 0.1023 mmol). The reaction mixture was worked up in the same manner as described for **9**. Purification of the resulting residue by column chromatography (85:15 CH_2Cl_2/EtOAc) afforded **14** (29.5 mg, 58%) as a film: R_f = 0.29 (7:1 CH_2Cl_2/EtOAc); $[\alpha]^{20}_D$ = +52.7 (*c* 1.31, $CHCl_3$); ^{1}H NMR δ 8.15−8.02 (m, 2H), 7.69−7.64 (m, 2H), 7.60−7.52 (m, 3H), 7.49−7.38 (m, 3H), 5.76−5.46 (m, 5H), 5.04 (t, *J* = 9.7 Hz, 1H), 4.95−4.84 (m, 2H), 4.75 (d, *J* = 10.4 Hz),

4.56 (d, J = 13.4 Hz, 1H), 4.47 (d, J = 8.2 Hz, 1H), 4.24−4.10 (m, 4H), 3.88−3.70 (m, 3H), 3.52 (d, J = 10.7 Hz, 1H), 3.41 (m, 1H), 3.12 (d, J = 10.0 Hz, 1H), 2.22−2.02 (m, 4H), 2.02 (s, 3H), 1.88 (s, 3H), 1.78 (s, 3H), 1.67−1.50 (m, 4H); ^{13}C NMR 172.3, 170.5, 170.5, 169.0, 166.0, 165.4, 140.4, 137.8, 136.7, 136.6, 129.9, 129.8, 129.7, 129.1, 128.8, 128.6, 128.3, 115.4, 114.8, 101.1, 97.5, 75.7, 74.4, 72.1, 71.6, 70.4, 68.7, 67.2, 62.6, 60.5, 55.9, 54.0, 35.7, 29.8, 29.2, 28.5, 20.7, 20.5, 20.4. Anal. Calcd for $C_{50}H_{50}Cl_4N_2O_{17}$: C, 54.96; H, 4.61; N, 2.56. Found: C, 55.09; H, 4.58; N, 2.50.

JO961668S