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A Versatile Set of Aminooxy Amino Acids for the Synthesis of Neoglycopeptides

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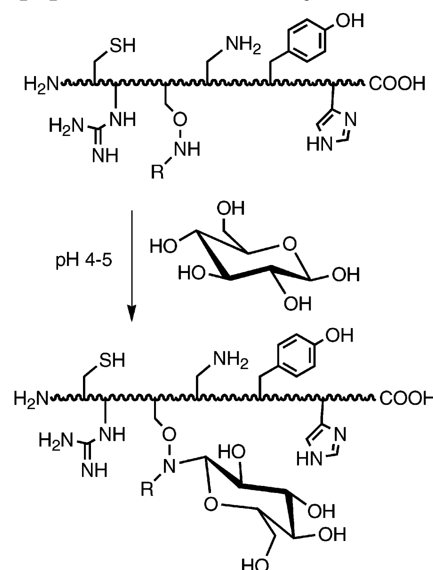
Four *N*-alkylaminooxy amino acids have been synthesized in 22–56% overall yield from readily available amino acid precursors. Each amino acid can be efficiently incorporated into peptides using Boc-chemistry-based solid-phase peptide synthesis, and in three of the four cases the resulting peptides can be chemoselectively glycosylated at the aminooxy side chains to generate neoglycopeptides. The range of *N*-alkylaminooxy amino acids prepared allows attachment of sugars at two-, three-, or four-atom distances from the peptide backbone, and each ensures that attached sugars adopt cyclic conformations. These derivatives provide convenient access to arrays of biologically relevant neoglycopeptides that may be used to probe the influence of attached sugars on the structure and function of peptides and proteins.

Introduction

Glycosylation of peptides and proteins induces a variety of structural and functional changes. Reported effects include enforcing extended peptide conformations, conferring resistance to proteolysis, increasing thermostability, and enabling intracellular transport.[1] Unfortunately, controlled studies of how attached sugars change the properties of peptides have been hampered by a lack of methods for the rapid, efficient synthesis of large numbers of glycopeptides. Although many elegant glycopeptide syntheses have been reported, they generally involve complicated techniques and use expensive, sensitive, glycosylated amino acid derivatives. To facilitate the synthesis of glycosylated peptides, researchers have increasingly turned to the synthesis of neoglycopeptides—peptides to which a sugar is attached via a nonbiological linkage. Although these substrates do not precisely duplicate natural glycopeptides, they can be produced quickly in large quantities, and some examples have been shown to retain native activity.[2]

An attractive strategy for neoglycopeptide synthesis relies on the chemoselective reaction of completely unprotected aminooxy-derivatized peptides and native reducing sugars.[3–5] The peptides can be made by well-established solid-phase peptide synthesis (SPPS) procedures, and the subsequent reaction of the aminooxy side chains with the aldehydes of reducing sugars proceeds selectively under mild aqueous conditions (Scheme 1). A key advantage of this approach is that a single peptide may be reacted with a variety of sugars, and the synthesis of combinatorial neoglycopeptide arrays is greatly facilitated.

SCHEME 1. General Synthesis of Neoglycopeptides (R = H or Alkyl)



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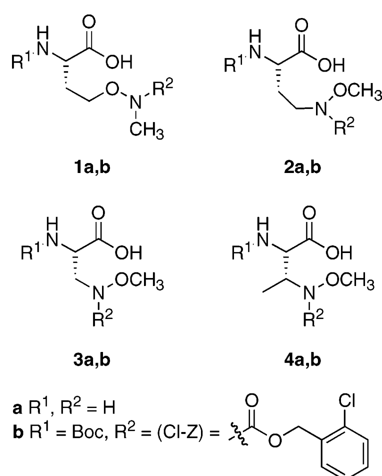
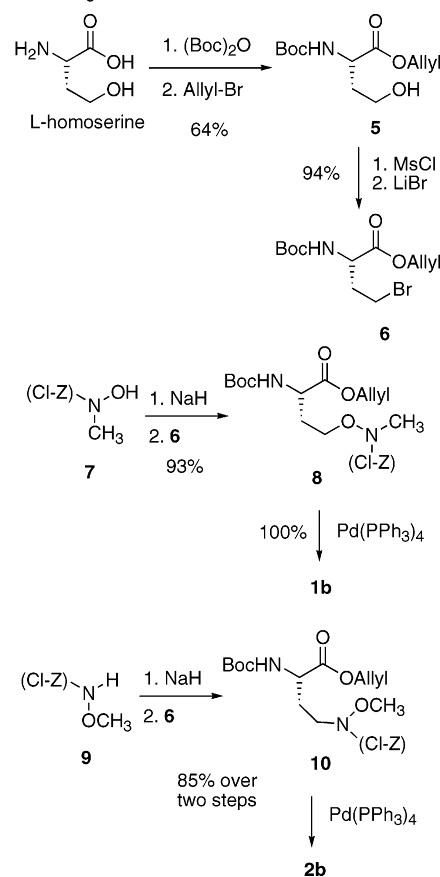
Early examples demonstrating this strategy unfortunately suffered from one of two problems that limited the biological relevance of the neoglycopeptides. Either the aminooxy side chain placed the sugar far from the peptide backbone or the sugar adopted a linear rather than cyclic conformation. In a preliminary communication,[4a] we introduced an aminooxy amino acid, **1a**, that solved both problems: it produces neoglycopeptides where the sugar is attached to the peptide via a short, four-atom link, and the *N*-methyl substitution of the aminooxy nitrogen forces attached sugars to adopt cyclic conformations. Nonetheless, aminooxy amino acid derivatives with shorter side chains are necessary both to increase the structural diversity available for neoglycopeptides and to better mimic natural glycopeptides.



Natural glycopeptides fall into two broad categories: O-linked, where the carbohydrate is attached via the oxygen of a serine or threonine side chain (a two-atom connection), and N-linked, where the carbohydrate is attached via the amide nitrogen of an asparagine side chain (a three-atom connection). Aminooxy amino acid derivatives that attach sugars at these side chain lengths would greatly expand the range of potential neoglycopeptides. Recently, two groups independently used Fmoc-chemistry-based SPPS to synthesize neoglycopeptides containing the aminooxy amino acid **2a**, onto which the sugar may be attached to mimic an asparagine-linked sugar (three-atom connection).[5]

To extend this series of aminooxy amino acids, we report the syntheses of aminooxy amino acids **1**−**4** from homoserine, serine, and threonine; their use in Boc-chemistry-based SPPS; and their application in conjugation reactions with reducing sugars to prepare neoglycopeptides. Overall, a set of aminooxy amino acids has been created for the synthesis of biologically relevant neoglycopeptides possessing sugar moieties at two-, three-, or four-atom distances from the peptide backbone. Because of their reactivity and because the *N*-alkylaminooxy side chain induces the sugar to adopt a cyclic conformation, aminooxy amino acids **1**−**4** should allow the practical synthesis of neoglycopeptide derivatives capable of mimicry of the structure and function of natural peptides and proteins.

SCHEME 2. Synthesis of 1b and 2b



Results and Discussion

Synthesis of Protected Aminooxy Amino Acids. Aminooxy amino acid **1b** was prepared in four steps and 56% overall yield from L-homoserine by a route featuring displacement of bromide **6** with the oxygen anion of hydroxylamine **7** (Scheme 2).[4a] To prepare **2b**, we envisioned simply reacting **6** with the isomeric methoxylamine derivative **9**. Bromide **6**, which is easily prepared on a large scale, would then serve as a common intermediate for both **1b** and **2b**.

Although ultimately successful, the reaction of **9** with **6** required several important modifications. Foremost among these was the use of increased temperature and reaction time. Whereas the reaction of **7** with **6** proceeded smoothly in 1 h at 0 °C, efficient reaction between **9** and **6** required 3−4 h and warming to rt. Because the anion of **9** appears be a more basic nucleophile than the anion of **7** (vide infra), we attributed this reduced reactivity to steric rather than electronic factors. Further evidence for problems with steric crowding came from attempts at Mitsunobu coupling[6] of **9** with alcohol **5**. Unlike successful reported cases with virtually identical amides but longer side-chain amino acid alcohols,[7] numerous attempts with our substrates led predominantly to recovered starting materials and only small amounts of the desired product **10**.

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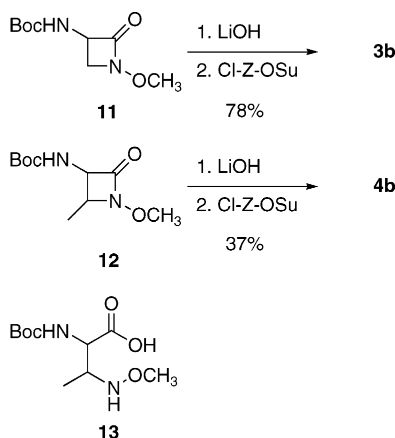
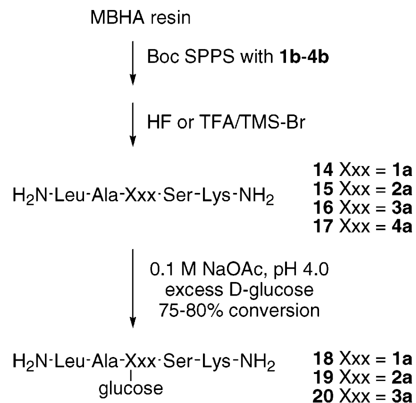
SCHEME 3. Synthesis of 3b and 4b



SCHEME 4. Synthesis of Model Peptides and Neoglycopeptides



The purification of **10** also proved problematic. In the purification of **8**, excess hydroxylamine **7** could be removed by extraction with aqueous NaOH. However, **9** proved to be less acidic than **7** and could not be removed by basic extraction during the purification of **10**. Because **9** and **10** also coelute on silica gel chromatography, we were forced to take the mixture of the two directly into the allyl ester deprotection. Treatment of the mixture of **9** and **10** with $Pd(PPh_3)_4$ and pyrrolidine cleanly converted the ester to the acid **2b**, which was now easily separated from **9** by chromatography. The overall yield of **2b** from **6** was 85%; **2b** was thus made in three steps and 51% overall yield from L-homoserine.

In designing the synthesis of **3b** and **4b**, we envisioned introducing the methoxyamino side chain intramolecularly via the *N*-methoxyamide of either Boc-L-Ser or Boc-L-allo-Thr. A search of the literature revealed that Miller et al.[8] and Floyd et al.[9] had already used this approach to synthesize the azetidinones **11** and **12**. From these known derivatives, basic ring opening followed by 2-chlorobenzyloxycarbonyl protection of the nitrogen was expected to afford **3b** and **4b** (Scheme 3).

In the event, treatment of **11** with LiOH cleanly hydrolyzed the amide, and the resulting *N*-methoxyamine could be protected in situ with *N*-(2-chlorobenzyloxycarbonyloxy)succinimide (Cl-Z-OSu) to yield 78% of **3b** after purification. Unfortunately, the analogous reaction with **12** was not as successful. Although TLC and NMR analysis showed that the hydrolysis of **12** to **13** was complete within 15 min, it proved difficult to protect the resulting methoxyamine. The original in situ protection conditions used to make **3b** led to <15% of **4b** after extensive purification, and attempts where the ring-opened product **13** was isolated and protected in various nonaqueous conditions yielded no desired product whatsoever. In the end, modifying the original conditions by using CH_3CN instead of THF allowed us to recover **4b** in 37% yield. Perhaps not unexpectedly, the additional methyl group appears to severely restrict the nucleophilicity of the nitrogen. Because we feared that a similar lack of reactivity of this hindered methoxyamine would limit glycosylation of peptides containing **4a**, the synthesis of **4b** was not optimized. Instead, its reactivity in peptide synthesis was explored.

Synthesis and Glycosylation of Aminooxy Peptides. All four aminooxy amino acids, **1b**–**4b**, were easily incorporated into peptides. For this study, we synthesized the four model peptides of sequence H_2N-Leu-Ala-Xxx-Ser-Lys-NH_2 where Xxx is one of **1a**–**4a** (Scheme 4). These peptides, **14**–**17**, were made on 4-methylbenzhydrylamine (MBHA) resin using in situ neutralization procedures for Boc-chemistry-based SPPS.[10] To ensure fast and efficient coupling, the in situ protocols generally rely on the use of large excesses of the activated amino acid being introduced (typically 1000 mol % of activated amino acid relative to the resin loading). When coupling **1b**–**4b**, we wanted to maintain rapid and complete reaction, but we could not afford to use such large excesses. For these amino acids, we reduced the amount used to 200 mol % but concomitantly reduced the volume of DMF to keep the concentration of the activated amino acid at the level found in the standard protocols. With this modification, single couplings of 25 min proved sufficient to maximize Xxx incorporation, and only small amounts (<3%) of any Xxx-deletion peptides were subsequently observed. After SPPS, the peptides were deprotected and cleaved from the solid resins with either liquid HF or a mixture of TFA, bromotrimethylsilane, and thioanisole.[11] The free peptides were purified by RP-HPLC and characterized by ESI-MS.

Glycosylation of each of these peptides was attempted with a large excess of D-glucose at 40 °C in a pH 4.0, 0.1 M NaOAc buffer. We previously found these conditions to be effective for a variety of peptides containing **1a**, and we considered them to be good for testing the glycosylation of the three new aminooxy side chains. These reactions were monitored by HPLC, and the products were identified by ESI-MS. Because of our previous work, **14** served as a control peptide, and as expected glycosylation of **14** proceeded cleanly to yield >75% of the D-glucose adduct after 5 h. Similar results were found with **15** and **16**, showing that the methoxyamino side chains of **2a** and **3a** undergo efficient chemoselective ligation with D-glucose. Data for the glycosylation of **16**

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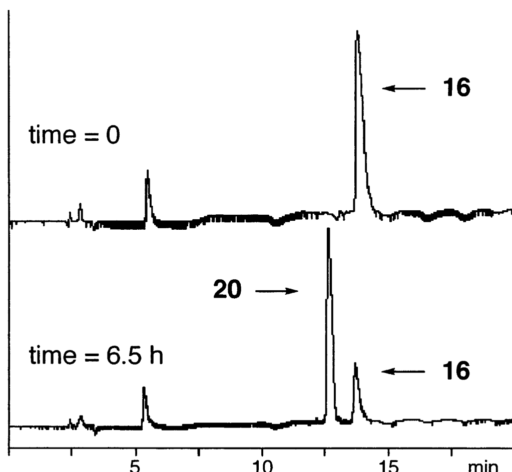

FIGURE 1. HPLC traces of 0 h (top) and 6.5 h (bottom) time points of the glycosylation of **16** with an excess of D-glucose in a 0.1 M NaOAc, pH 4.0 buffer. At 6.5 h, 75% of **16** has undergone glycosylation to form **20**.

is shown in Figure 1. In this case, after 6.5 h the chromatogram and ESI-MS analysis of the fractions corresponding to the peaks indicated 75% conversion to the monoglycosylated derivative **20**.

In contrast, glycosylation of **17** was not successful. After subjecting **17** to our standard conditions for 5 h, no evidence of any glycosylation was seen. Increasing the temperature to 60 °C and reacting for 24 h similarly resulted in no observable glycosylation. As in previous cases with peptides containing **1a**, more forcing conditions, such as evaporating the solvent under heating, led to nonspecific glycosylation and multiply glycosylated products. The steric hindrance that reduced the reactivity of the methoxyamino group in the protection of **13** appears also to prevent efficient glycosylation of the side chain of **4a**.

Conclusion

Three novel protected *N*-alkylaminooxy amino acids, **1b**–**3b**, were synthesized in few steps and high overall yield from readily available amino acid precursors. Each can be efficiently incorporated into peptides by standard SPPS procedures, and the resulting *N*-alkylaminooxy side chains of these peptides can be chemoselectively glycosylated under mild conditions. This set allows sugars adopting the cyclic conformation to be attached at two-, three-, and four-atom distances from the peptide backbone. These attributes and the potential to attach a variety of reducing sugars to *N*-alkylaminooxy peptides offer entry to neoglycopeptide libraries for studying the effects of glycosylation on peptide structure and activity.

Experimental Section

Allyl 2-(*N*-*tert*-Butoxycarbonyl)amino-4-hydroxybutanoate (5). A flask was charged with L-homoserine (1.00 g, 8.40 mmol), NaOH (336 mg, 8.40 mmol), H_2O (10 mL), and CH_3CN (10 mL). *tert*-Butyl dicarbonate (2.02 g, 9.24 mmol) was added, and the resulting solution was stirred for 14 h. The solvents were removed, and the flask contents were dried briefly under vacuum. The residue was triturated with Et_2O, dried under vacuum, and dissolved in DMF (25 mL). Allyl bromide (798 μL, 9.24 mmol) was added, and the reaction mixture was stirred for 19 h. The solvent volume was reduced, and the concentrated solution was dissolved in EtOAc (200 mL) and poured into a separatory funnel. The organic layer was washed with saturated $NaHCO_3$ (3 × 50 mL), H_2O (50 mL), 0.1 M $KHSO_4$ (2 × 50 mL), and saturated NaCl (50 mL) and then dried. Filtration of the drying agent, removal of the solvents, and drying under vacuum yielded 1.40 g (5.41 mmol, 64%) of **5** as a viscous oil that was used without further purification. ^1H NMR (400 MHz, $CDCl_3$): δ 5.91 (m, 1H), 5.40 (bd, 1H, J = 7.3 Hz), 5.35 (m, 1H), 5.27 (m, 1H), 4.66 (d, 2H, J = 5.8 Hz), 4.52 (m, 1H), 3.68 (m, 2H), 3.20 (bs, 1H), 2.18 (m, 1H), 1.64 (m 1H), 1.45 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.8, 156.7, 131.6, 119.2, 80.7, 66.3, 58.5, 50.8, 36.5, 28.5. An analytical sample was purified by column chromatography (EtOAc/hexanes, 30:70 to 40:60 to 50:50). FAB-HRMS: calcd for $C_{12}H_{21}NO_5Li$ $(M + Li)^+$ 266.1580, found 266.1578.

Allyl 2-(*N*-*tert*-Butoxycarbonyl)amino-4-bromobutanoate (6). A flask was charged with **5** (2.00 g, 7.72 mmol) and CH_2Cl_2 (25 mL). Methanesulfonyl chloride (717 μL, 9.26 mmol) and triethylamine (1.39 mL, 10.0 mmol) were added, and the resulting solution was stirred for 95 min. Lithium bromide (6.71 g, 77.2 mmol) and acetone (25 mL) were then added, and the mixture was stirred for 14 h. The solvents were removed, and the residue was dissolved in EtOAc (200 mL) and poured into a separatory funnel. The organic layer was washed with H_2O (3 × 80 mL), saturated $NaHCO_3$ (80 mL), and saturated NaCl (80 mL) and then dried. The solvent was removed, and column chromatography of the residue (EtOAc/hexanes, 20:80 to 30:70) afforded 2.34 g (7.25 mmol, 94%) of **6** as a white solid. Mp: 50–51 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 5.91 (m, 1H), 5.35 (dd, 1H, J = 1.1, 17.2 Hz), 5.27 (dd, 1H, J = 1.1, 10.3 Hz), 5.13 (bs, 1H), 4.65 (d, 2H, J = 5.9 Hz), 4.44 (m, 1H), 3.44 (d, 2H, J = 7.0 Hz), 2.42 (m, 1H), 2.22 (m, 1H), 1.45 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.7, 155.5, 131.5, 119.3, 80.5, 66.4, 52.7, 36.1, 28.5. FAB-HRMS: calcd for $C_{12}H_{21}BrNO_4$ $(M + H)^+$ 322.0654, found 322.0648. Anal. Calcd for $C_{12}H_{20}BrNO_4$: C, 44.73; H, 6.26; N, 4.35. Found: C, 44.49; H, 6.52; N, 4.19.

***N*-Methyl-*N*-(2-chlorobenzyloxycarbonyl)hydroxylamine (7).** A flask was charged with KOH (2.09 g, 37.2 mmol) and MeOH (20 mL). *N*-Methylhydroxylamine hydrochloride (3.10 g, 37.2 mmol) and more MeOH (20 mL) were added, and the resulting solution was stirred for 15 min. The precipitated KCl was removed by filtration through cotton, and the solids were rinsed with MeOH (10 mL). To the clear solution was added *N*-(2-chlorobenzyloxycarbonyloxy)succinimide (5.27 g, 18.6 mmol), and the mixture was stirred for 6 h. The solvent was removed, and the residue was dissolved in EtOAc (200 mL) and poured into a separatory funnel. The organic layer was washed with 0.1 M $KHSO_4$ (3 × 50 mL) and saturated NaCl (50 mL) and then dried. Removal of the solvent followed by drying under vacuum afforded 3.87 g (17.9 mmol, 96%) of **7** as a white solid, which was used without further purification. Mp: 66–69 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 7.41 (m, 2H), 7.28 (m, 2H), 6.60 (bs, 1H), 5.29 (s, 2H), 3.25 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.9, 133.8, 133.7, 129.9, 129.81, 129.78, 127.1, 65.6, 38.2. Anal. Calcd for $C_9H_{10}ClNO_3$: C, 50.13; H, 4.67; N, 6.50. Found: C, 50.22; H, 4.74; N, 6.33.

Allyl 2-(*N*-*tert*-Butoxycarbonyl)amino-4-[*O*-[*N*-(2-chlorobenzyloxycarbonyl)-*N*-methyl]amino]hydroxybutanoate (8). A flask was charged with **7** (837 mg, 3.88 mmol) and anhydrous DMF (5 mL). NaH (60% dispersion in mineral oil, 149 mg, 3.73 mmol) was added, and the mixture was stirred for 10 min and then cooled to 0 °C. Compound **6** (1.00 g, 3.10 mmol) was added, the sides of the flask were rinsed with additional DMF (2 mL), and the resulting solution was stirred for 90 min at 0 °C. The reaction mixture was poured into a separatory funnel and diluted with EtOAc (200 mL). The organic layer was washed with 0.1 M NaOH (5 × 50 mL), 0.1 M $KHSO_4$ (2 × 50 mL), and saturated NaCl (50 mL) and then dried. The solvent was removed, and column chromatography of the residue (EtOAc/hexanes, 30:70) and drying

under vacuum at 45 °C for 24 h afforded 1.32 g (2.89 mmol, 93%) of **8** as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 (m, 1H), 7.38 (m, 1H) 7.28 (m, 2H), 5.89 (m, 1H), 5.57 (bs, 1H), 5.22−5.34 (m, 4H), 4.62 (m, 2H), 4.46 (m, 1H), 3.99 (m, 2H), 3.18 (s, 3H), 2.18 (m, 1H), 2.04 (m, 1H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.0, 157.2, 155.6, 133.8, 133.6, 131.7, 129.8, 129.62, 129.60, 127.0, 118.8, 79.9, 70.6, 66.0, 65.2, 51.3, 36.6, 30.6, 28.4. FAB-HRMS: calcd for $C_{21}H_{30}ClN_2O_7$ $(M + H)^+$ 457.1742, found 457.1739. Anal. Calcd for $C_{21}H_{29}ClN_2O_7$: C, 55.20; H, 6.40; N, 6.13. Found: C, 55.17; H, 6.38; N, 6.07.

***N*-(2-Chlorobenzyloxycarbonyl)methoxylamine (9).** A flask was charged with KOH (1.12 g, 20.0 mmol) and MeOH (10 mL). Methoxylamine hydrochloride (1.67 g, 20.0 mmol) and more MeOH (5 mL) were added, and the resulting solution was stirred for 15 min. The precipitated KCl was removed by filtration through cotton, and the solids were rinsed with MeOH (5 mL). To the clear solution was added *N*-(2-chlorobenzyloxycarbonyloxy)succinimide (2.84 g, 10.0 mmol), and the mixture was stirred for 17 h. The solvent was removed, and the residue was dissolved in EtOAc (100 mL) and poured into a separatory funnel. The organic layer was washed with 0.1 M $KHSO_4$ (3 × 30 mL) and saturated NaCl (30 mL) and then dried. Removal of the solvent followed by drying under vacuum afforded 2.11 g (9.79 mmol, 98%) of **9** as a waxy solid, which was used without further purification. Mp: 35−38 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (s, 1H), 7.42 (m, 1H), 7.36 (m, 1H), 7.26 (m, 2H), 5.28 (s, 2H), 3.73 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.4, 133.7, 133.5, 130.0, 129.8, 129.7, 127.0, 64.82, 64.77. Anal. Calcd for $C_9H_{10}ClNO_3$: C, 50.13; H, 4.67; N, 6.50. Found: C, 50.11; H, 4.86; N, 6.26.

2-(*N*-*tert*-Butoxycarbonyl)amino-4-[*O*-[*N*-(2-chlorobenzyloxycarbonyl)-*N*-methyl]amino]hydroxybutanoic Acid (1b). A flask was charged with **8** (1.28 g, 2.80 mmol) and CH_2Cl_2 (10 mL). Triphenylphosphine (38 mg, 0.14 mmol) and pyrrolidine (246 μL, 2.94 mmol) were added, and the flask was flushed with N_2. Tetrakis(triphenylphosphine)palladium(0) (81 mg, 0.070 mmol) was then added, and the mixture was stirred for 40 min. The solvents were removed by rotary evaporation, and the residue was dissolved in EtOAc (125 mL) and poured into a separatory funnel. The organic layer was washed with 0.1 M $KHSO_4$ (3 × 50 mL) and saturated NaCl (50 mL), and then dried. The solvent was removed, and column chromatography of the residue (EtOAc/hexanes/AcOH, 40:60:0.5 to 50:50:0.5) afforded an oil that was further purified by size-exclusion chromatography (LH-20, $CHCl_3$). The resulting residue was dried under vacuum overnight at 55 °C to yield 1.16 g (2.79 mmol, 100%) of **1b** as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$, 55 °C): δ 8.60 (bs, 1H), 7.39 (m, 2H), 7.26 (m, 2H), 5.63 (bs, 1H), 5.29 (s, 2H), 4.43 (bs, 1H), 4.01 (m, 2H), 3.18 (s, 3H), 2.17 (m, 1H), 2.05 (m, 1H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$, 55 °C): δ 175.7, 157.6, 156.2, 133.93, 133.90, 130.1, 129.82, 129.80, 127.2, 80.6, 71.0, 65.5, 51.8, 36.7, 30.7, 28.5. FAB-HRMS: calcd for $C_{18}H_{26}ClN_2O_7$ $(M + H)^+$ 417.1429, found 417.1421. Anal. Calcd for $C_{18}H_{25}ClN_2O_7$: C, 51.86; H, 6.05; N, 6.72. Found: C, 52.03; H, 6.16; N, 6.67.

2-(*N*-*tert*-Butoxycarbonyl)-4-[*N*-[2-chlorobenzyloxycarbonyl]-*N*-methoxy]diaminobutanoic Acid (2b). A flask was charged with **9** (856 mg, 3.97 mmol) and anhydrous DMF (5 mL). Sodium hydride (60% dispersion in mineral oil, 152 mg, 3.81 mmol) was added, and the mixture was stirred for 30 min and then cooled to 0 °C. Compound **6** (1.02 g, 3.18 mmol) was added, the sides of the flask were rinsed with additional DMF (5 mL), and the resulting solution was stirred for 30 min at 0 °C. The ice bath was removed, and the reaction was allowed to warm to rt and stirred for an additional 3.5 h. The reaction mixture was poured into a separatory funnel and diluted with EtOAc (125 mL). The organic layer was washed with 0.1 M $KHSO_4$ (3 × 50 mL) and saturated NaCl (50 mL) and then dried. The solvent was removed, and column chromatography of the residue (EtOAc/hexanes, 20:80 to 25:75 to 30:70) afforded 1.48 g of a residue identified by ^{1}H NMR as an approximately 3:1 mixture of **9** and **10**. This residue was dissolved in CH_2Cl_2 (15 mL). Triphenylphosphine (42 mg, 0.16 mmol) and pyrrolidine (266 μL, 3.18 mmol) were added, and the flask was flushed with Ar. Tetrakis(triphenylphosphine)palladium(0) (92 mg, 0.080 mmol) was then added, and the mixture was stirred for 90 min. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and poured into a separatory funnel. The organic layer was washed with 0.1 M $KHSO_4$ (3 × 50 mL) and saturated NaCl (50 mL) and then dried. The solvent was removed, and column chromatography of the residue (EtOAc/hexanes/AcOH, 40:60:1 to 50:50:1) afforded an oil that was further purified by size-exclusion chromatography (LH-20, MeOH:CH_2Cl_2, 10:90). The resulting residue was dried under vacuum overnight at 50 °C to yield 1.121 g (2.69 mmol, 85%) of **2b** as a viscous oil. ^{1}H NMR (400 MHz, CD_3CN, 55 °C): δ 8.6 (bs, 1H), 7.48 (m, 1H) 7.42 (m, 1H), 7.33 (m, 2H), 5.67 (d, 1H, J = 8.1 Hz), 5.24 (s, 2H), 4.17 (bs, 1H), 3.67 (s, 3H), 3.61 (m, 2H), 2.15 (m, 1H), 1.93 (m, 1H), 1.40 (s, 9H). ^{13}C NMR (100 MHz, CD_3CN, 55 °C): δ 173.2, 156.4, 155.8, 134.0, 133.1, 130.0, 129.9, 129.5, 127.3, 79.4, 64.8, 61.9, 51.4, 45.6, 29.1, 27.7. FAB-HRMS: calcd for $C_{18}H_{26}ClN_2O_7$ $(M + H)^+$ 417.1429, found 417.1425. Anal. Calcd for $C_{18}H_{25}ClN_2O_7$: C, 51.86; H, 6.05; N, 6.72. Found: C, 51.92; H, 6.20; N, 6.58.

2-(*N*-*tert*-Butoxycarbonyl)-3-(*N*-2-chlorobenzyloxycarbonyl-*N*-methoxy)diaminopropanoic Acid (3b). A flask was charged with **11**[11] (544 mg, 2.52 mmol), LiOH·H_2O (211 mg, 5.02 mmol), H_2O (5 mL), and THF (10 mL), and the mixture was stirred for 20 h. $NaHCO_3$ (250 mg) and *N*-(2-chlorobenzyloxycarbonyloxy)succinimide (1.43 g, 5.04 mmol) were added, and the contents were stirred for an additional 21 h. The THF was removed, and the resulting aqueous mixture was diluted with brine (25 mL) and acidified to pH 4−5 with 0.1 M $KHSO_4$. The aqueous layer was extracted with EtOAc (4 × 40 mL), and the combined organic layers were then washed with 0.1 M $KHSO_4$ (2 × 50 mL) and brine (50 mL) and then dried. Removal of the solvent gave a residue that was purified by column chromatography (acetone/CH_2Cl_2/AcOH, 10:90:0.5 to 20:80:0.5) and then size-exclusion chromatography (LH-20, MeOH/CH_2Cl_2, 10:90) to yield 790 mg of **3b** (1.96 mmol, 78%). ^{1}H NMR (400 MHz, CD_3CN, 55 °C): δ 8.6 (bs, 1H), 7.48 (m, 1H), 7.43 (m, 1H), 7.34 (m, 2H), 5.63 (d, 1H, J = 8.0 Hz), 5.25 (s, 2H), 4.42 (bs, 1H), 3.97 (dd, 1H, J = 4.9, 14.8 Hz), 3.89 (dd, 1H, J = 7.2, 14.8 Hz), 3.66 (s, 3H), 1.40 (s, 9H). ^{13}C NMR (100 MHz, CD_3CN, 55 °C): δ 171.5, 156.4, 155.5, 133.8, 133.1, 130.0, 129.9, 129.5, 127.3, 79.6, 65.0, 61.8, 51.8, 49.5, 27.6. Anal. Calcd for $C_{18}H_{25}ClN_2O_7$: C, 50.69; H, 5.75; N, 6.95. Found: C, 50.62; H, 5.87; N, 6.59.

2-(*N*-*tert*-Butoxycarbonyl)-3-(*N*-2-chlorobenzyloxycarbonyl-*N*-methoxy)diaminobutanoic Acid (4b). A flask was charged with **12**[11] (435 mg, 1.89 mmol), LiOH·H_2O (159 mg, 3.78 mmol), H_2O (4 mL), and CH_3CN (8 mL), and the mixture was stirred for 90 min. $NaHCO_3$ (200 mg) and *N*-(2-chlorobenzyloxycarbonyloxy)succinimide (2.68 g, 9.45 mmol) were added, and the contents were stirred for an additional 68 h. The mixture was diluted with 0.1 M $KHSO_4$ (40 mL) and extracted with EtOAc (4 × 35 mL). The combined organic layers were washed with brine (25 mL) and then dried. Removal of the solvent gave a residue that was purified by column chromatography (acetone/CH_2Cl_2/AcOH, 5:95:0.5 to 10:90:0.5), additional chromatography (EtOAc/hexanes/AcOH, 40:60:0.5 to 50:50:0.5), and then size-exclusion chromatography (LH-20, MeOH:CH_2Cl_2, 10:90) to yield 288 mg of **4b** (0.691 mmol, 37%). ^{1}H NMR (400 MHz, CD_3CN, 55 °C): δ 7.50−7.56 (m, 2H), 7.41 (m, 2H), 5.74 (bs, 1H), 5.35 (d, 1H, J = 12.8 Hz), 5.30 (d, 1H, J = 13.0 Hz), 4.50 (m, 1H), 4.38 (m, 1H), 3.77 (s, 3H), 1.47 (s, 9H), 1.35 (d, 3H, J = 6.7 Hz). ^{13}C NMR (100 MHz, CD_3CN): δ 171.6, 157.0, 155.6, 133.6, 133.1, 130.1, 130.0, 129.4, 127.3, 79.4, 65.0, 63.4, 56.6, 56.2, 27.5, 14.1. FAB-HRMS: calcd for $C_{18}H_{26}ClN_2O_7$ $(M + H)^+$ 417.1429, found 417.1421. Anal. Calcd for $C_{18}H_{25}ClN_2O_7$: C, 51.86; H, 6.05; N, 6.72. Found: C, 51.91; H, 6.22; N, 6.60.

Representative Glycosylation Reaction. Lyophilized powder of **14** (0.65 mg) was dissolved in 0.1 M NaOAc, pH 4.0

buffer (500 μL). D-Glucose (21 mg, 0.12 mmol) was added, and the mixture was heated at 39 °C for 7 h. Semipreparative HPLC (Microsorb C18; 0−20% buffer B (CH_3CN + 0.08% TFA) in buffer A (H_2O + 0.10% TFA) over 25 min; 3 mL/min) of the mixture and lyophilization of the product fractions yielded the monoglycosylated product **18** (0.6 mg).

Data for **14**: t_R = 6.2 min (Kromasil C18 5 μm 100 Å, 5−20% B over 20 min, 1 mL/min); ESI-MS calcd for $C_{23}H_{47}N_8O_7$ $(M + H)^+$ 547.4, found 547.1.

Data for **15**: t_R = 7.7 min (Kromasil C18 5 μm 100 Å, 5−20% B over 20 min, 1 mL/min); ESI-MS calcd for $C_{23}H_{47}N_8O_7$ $(M + H)^+$ 547.4, found 547.1.

Data for **16**: t_R = 13.7 min (Microsorb C18 5 μm 100 Å, 0−20% B over 20 min, 1 mL/min); ESI-MS calcd for $C_{21}H_{45}N_8O_7$ $(M + H)^+$ 533.3, found 534.0.

Data for **17**: t_R = 13.2 min (Microsorb C18 5 μm 100 Å, 0−20% B over 20 min, 1 mL/min); ESI-MS calcd for $C_{23}H_{47}N_8O_7$ $(M + H)^+$ 547.4, found 548.0.

Data for **18**: t_R = 8.7 min (Kromasil C18 5 μm 100 Å, 5−20% B over 20 min, 1 mL/min); ESI-MS calcd for $C_{29}H_{57}N_8O_{12}$ $(M + H)^+$ 709.4, found 709.2.

Data for **19**: t_R = 7.1 min (Kromasil C18 5 μm 100 Å, 5−20% B over 20 min, 1 mL/min); ESI-MS calcd for $C_{29}H_{57}N_8O_{12}$ $(M + H)^+$ 709.4, found 709.2.

Data for **20**: t_R = 8.5 min (Kromasil C18 5 μm 100 Å, 5−20% B over 20 min, 1 mL/min); ESI-MS calcd for $C_{28}H_{55}N_8O_{12}$ $(M + H)^+$ 695.4, found 695.2.

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Supporting Information Available: General experimental procedures and ^1H NMR and ^{13}C NMR spectra for **1b**−**4b** and **5**−**9**; HPLC chromatograms of **14**−**20**. This material is available free of charge via the Internet at http://pubs.acs.org.

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