Novel Type of Rigid C-Linked Glycosylacetylene−Phenylalanine Building Blocks for Combinatorial Synthesis of C-linked Glycopeptides

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C-linked 3- and 4-(glycosylacetylene)−Fmoc-phenylalanines were synthesized as rigid building blocks for synthesis of libraries of neoglycopeptide templates. Perbenzylated 1,5-galactonolactone, 1,5- gluconolactone, and 1,5-mannonolactone were reacted with cerium TMS−acetylide and the products reduced to the *C*-glycosylacetylene−TMS derivatives. The gluco and galacto configurations yielded exclusively the *β*-*C*-glycoside, whereas the mannonolactone gave a mixture of α-C- and *β*-C-anomer. The products were subjected to acetolysis and TMS cleavage. The resulting per-acetylated glycosylacetylenes were cross-coupled with the (*R*,*S*)-N^{α}-acetyl-3- and 4-iodophenylalanine *O*-methyl esters by Pd(0) catalysis in piperidine to give the eight possible C-linked glycosyl amino acid building blocks **33**−**40** as diastereomeric pairs. These were O-deacetylated. As an example of further conversion into neoglycopeptide templates the *N*-acetyl group of the 4-linked *galacto*-diastereomeric pair **43** was hydrolyzed selectively by acylase 1 and separated from the (*R*)-stereoisomer. The product was converted to 4-(*β*-*C*-galactosylacetylene)(N^{α}-Fmoc-)-L-phenylalanine (**52**) by reaction with Fmoc−OSu. Acid hydrolysis of galactosyl acetylene phenyl alanine **43** at elevated temperature afforded the conversion of the acetylene to the 1′-oxo derivative which was also N^{α}-Fmoc protected. The building blocks were used in the glycopeptide synthesis of three neoglycopeptide templates **54**, **55**, and **56** known to bind to murine MHC class II E^{k} and to present the glycan for interaction with the T-cell receptor. This template has previously been employed to elicit a carbohydrate specific T-cell response.

The structural diversity of N- and O-linked oligosaccharides correlates with their role in cellular communication and regulatory functions. This has recently been highlighted by the discovery of the ubiquitous family of the galactose binding galectins that are involved in such important processes as development, metastasis, and apoptosis.[1] Similarly, the mannose 6-phosphate receptor is involved in intracellular protein sorting and binds to glycopeptide templates with high affinities.[2] The key role played by the recognition of carbohydrate epitopes by E-, L-, and P-selectins in the process of cell−cell adhesion and inflammation is well recognized, and the identification of the SLe^{x} tetrasaccharide as the ligand for selectin binding has generated enormous interest in the design and synthesis of carbohydrate-based inhibitors of SLe^{x}-selectin binding as novel therapeutic agents. As a rule, the natural oligosaccharide ligands require a lengthy and costly synthesis and are not accessible in sufficiently large amounts. This has led to considerable efforts toward developing carbohydrate mimics. In a particularly attractive class of such mimics a large portion of the oligosaccharide is replaced by non-carbohydrate and particularly by peptide scaffolds.[3] As a wide range of amino acids are commercially available, this approach offers great potential for the preparation of glycopeptide libraries. It is also attractive, because several glycopeptides mimicking the oligosaccharide have displayed relatively higher affinities to their receptors,[2,4] while oligosaccharides often bind with relatively low affinities. However, while small glycopeptides are an attractive alternative to complex oligosaccharides, glycopeptides containing naturally occurring (O- or N-linked) carbohydrate−amino acid linkages could be anticipated to be poor therapeutics. They are metabolically unstable and possess a relatively short biological half-life. The delivery of such polar compounds into the cell across the lipid bilayer is generally difficult. Finally, while the flexibility associated with the linkage between sugar and peptide is not necessarily a negative attribute, decreased flexibility should improve the interaction with receptors or enzymes by reducing the entropy of binding. Indeed, considerable effort has been allocated to the synthesis of conformationally rigid oligosaccharide analogues for the investigation of carbohydrate−protein binding.[5]

We have envisaged the preparation of a new class of glycosyl−amino acid building blocks comprised of glycosylacetylenes C-linked to aromatic amino acids of the general type **1** (Schemes 1 and 2). Over the last years,

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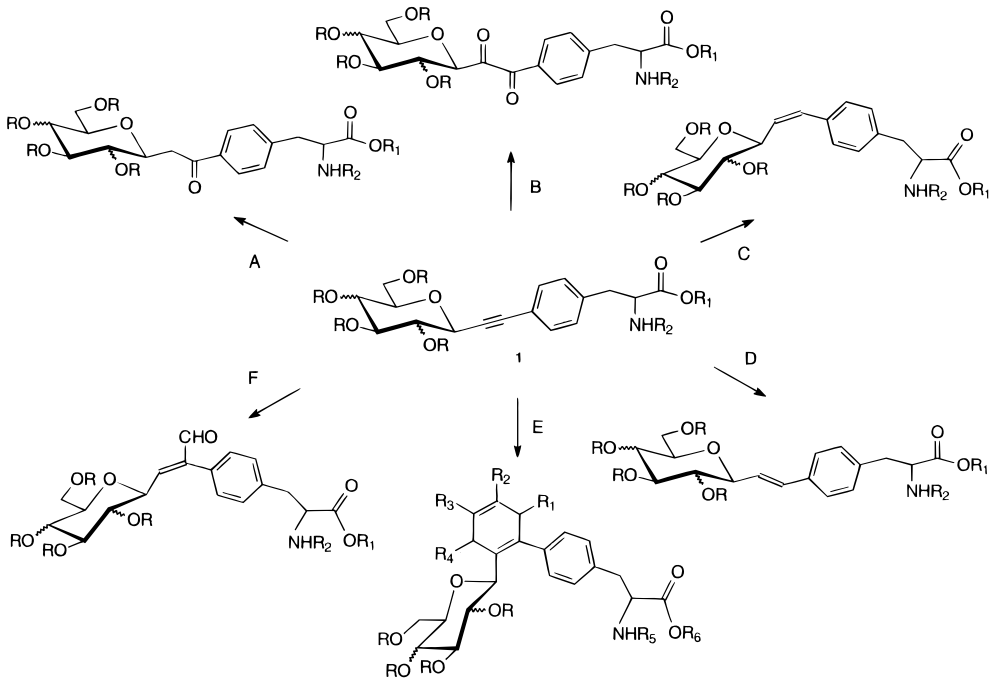
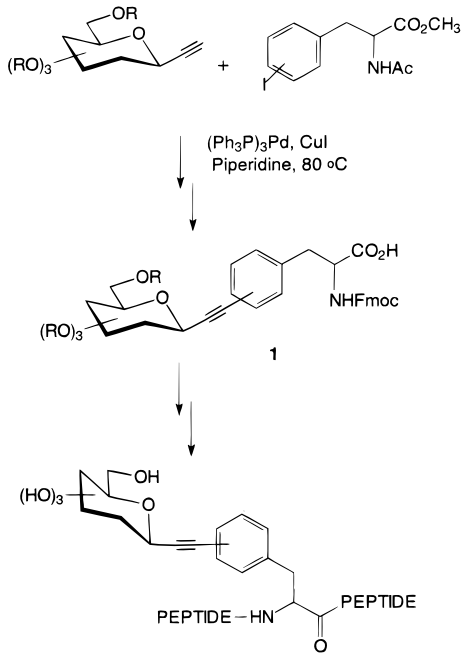
Scheme 1



Scheme 2



efficient methods for the synthesis of O- and N-linked glycopeptides have been reported.[6−11] In the most successful approaches glycopeptide synthesis is preceded by the preparation of the required glycosyl−amino acid building blocks that are incorporated into the peptide chain using standard peptide synthesis protocols. Recently, one of us has published several syntheses of oligomers of *C*-glycosylacetylenes.[12−20] The monomers possess a number of attractive features which may contribute to solve many of the above-mentioned problems associated with N- or O-linked glycopeptides. Similar to other *C*-glycosides, acetylenoglycopeptides should be tolerant of both the acidic and basic conditions normally used for glycopeptide synthesis, and their carbohydrate−protein linkages should be metabolically stable. The acetylene bridge between the sugar and amino acid is less polar than the normal ether or amide linkage of *O*- or *N*-glycopeptides and may confer improved membrane transport properties. This bridge is also rigid, so that acetylenoglycopeptides should present the saccharide moieties in a much less flexible fashion than naturally occurring glycopeptides.

However, the most intriguing feature of these molecules is linked to the chemical versatility of the acetylene functionality and to the many ways in which **1** can be converted into a number of other building blocks (Scheme 1). There is thus enormous potential for creating molecular diversity from these monomers. Acetylenes can be hydrated to ketones (A), oxidized into α,β diketones (B), [21] reduced to either (*E*)- or (*Z*)-alkenes (C and D),[22,23] converted to cyclohexadienes by Diels−Alder reaction with dienes (E),[24] and transformed into α,β unsaturated

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aldehydes by rhodium-catalyzed hydroformylation (F).[25] Many of these products could be further elaborated, e.g. by reductive amination of the ketones, α,β diketones, or α,β unsaturated aldehydes or by Michael addition to the α,β unsaturated aldehydes. As presented in Schemes 1 and 2 an indefinitely large number of glycopeptides can be envisaged resulting from a combination of the large number of conceivable glycosylacetylene−amino acid building blocks with the large number of subsequent synthetic manipulations. Related to this is a recent report[20] on a Bergman cyclization of 1,2-bis(2′-glucosylalkynyl)-benzenes to provide 2,3-diglucosylated naphthalenes.

Reported here is the preparation of building blocks **33**−**38** and **41**−**53** where glucosyl, galactosyl, and mannosyl residues are β-linked via the ethynediyl bridge to the *meta* or *para* position of phenylalanine (Figure 1 and Scheme 6). The preparation of the conjugates **39** and **40**, where phenylalanine is linked to an α-mannosylacetylene is also described. The preparation of the building blocks involves the reductive introduction of the "anomeric" ethynyl moiety, resolution of the racemic phenylalanines, alkynyl−aryl coupling,[26−28] and appropriate protection/deprotection of the glycosyl and phenylalanyl moieties. For the incorporation of these glycosyl−amino acid building blocks into glycopeptides, we planned to activate the carboxyl group in the presence of the free hydroxyl groups using TBTU.[29]

We also report the synthesis of C-linked glycosyl−acetylene-conjugated analogues of the mouse hemoglobin-derived decapeptide Hb(67−76), VITAFNEGLK substituted at position 72, binding well to the MHC Class II E^k molecule, and possibly eliciting a carbohydrate specific T-cell response. These conjugates were synthesized by multiple-column peptide synthesis (MCPS) employing the building blocks **52** and **53** (Scheme 6). Previous studies suggest that T-cells may recognize glycans that are in direct contact with the T-cell receptor when displayed on the Hb(67−76) epitope.

Synthesis of the Glycosylacetylenes. The glucosylacetylenes **2** and **3** were prepared as reported.[12] Similarly, we synthesized the galactosylacetylenes **4** and **5**, as illustrated in Scheme 3. The known lactone **6**[30] was converted in 91% yield to the ketose **7** by reaction with cerium TMS−acetylide. The hemiacetal was reduced by treatment with triethylsilane and boron trifluoride etherate to provide the β-linked C-glycoside **8** (71%). None of the corresponding α-anomer could be isolated; however, traces (4%, product characterized only by NMR) of the TES derivative **9** were formed by silyl exchange. The alkyne was desilylated with sodium hydroxide, yielding 99% of the benzylated alkyne **4**. However, unlike the glucose case,[12] where the benzyl groups could be removed within a few hours by acetolysis (acetic anhydride/*O*-(trimethylsilyl)trifluoromethanesulfonate), debenzylation

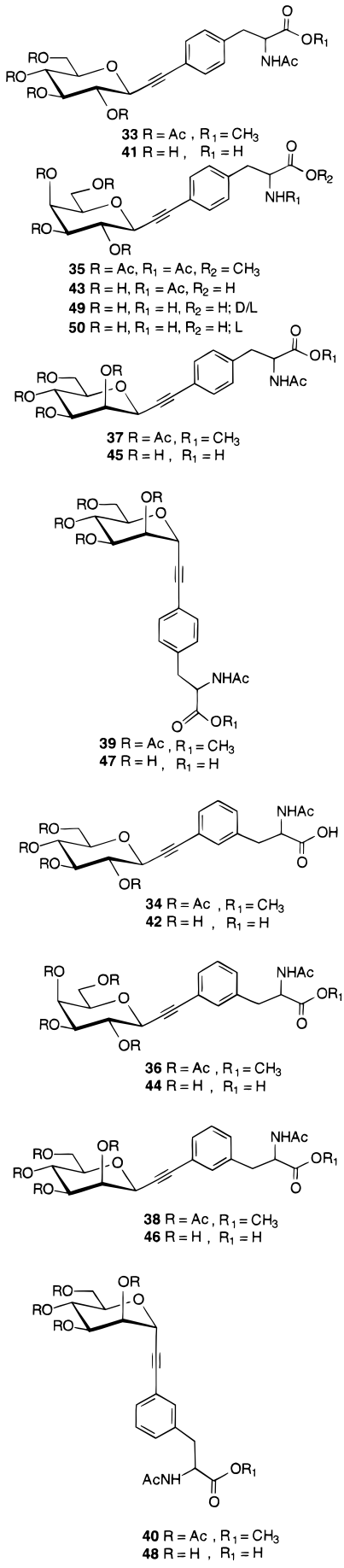

Figure 1.

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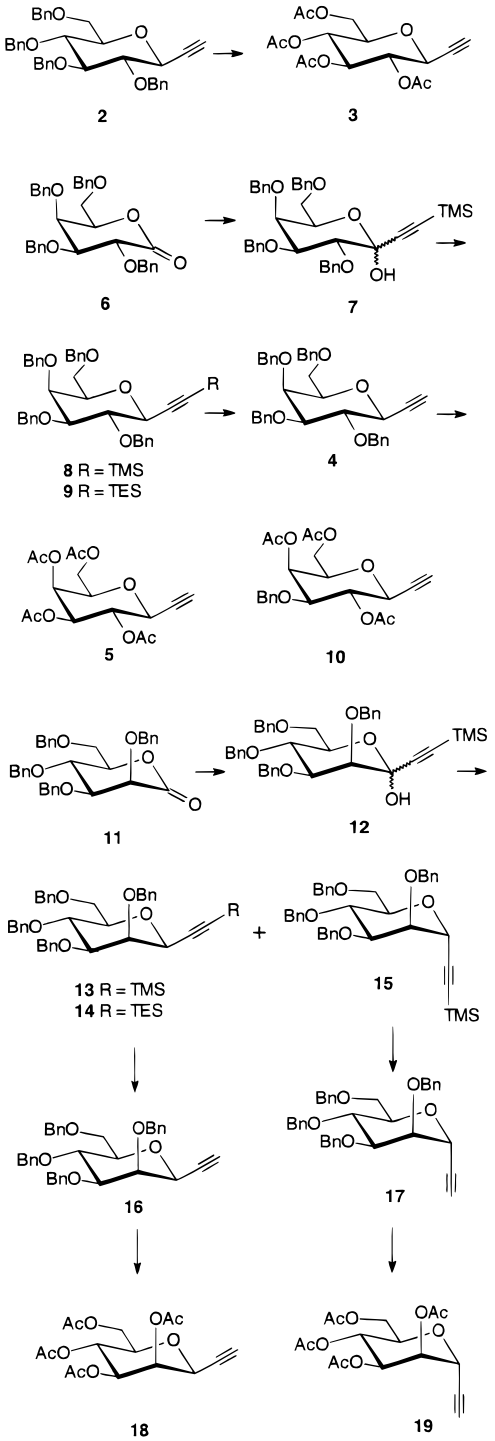
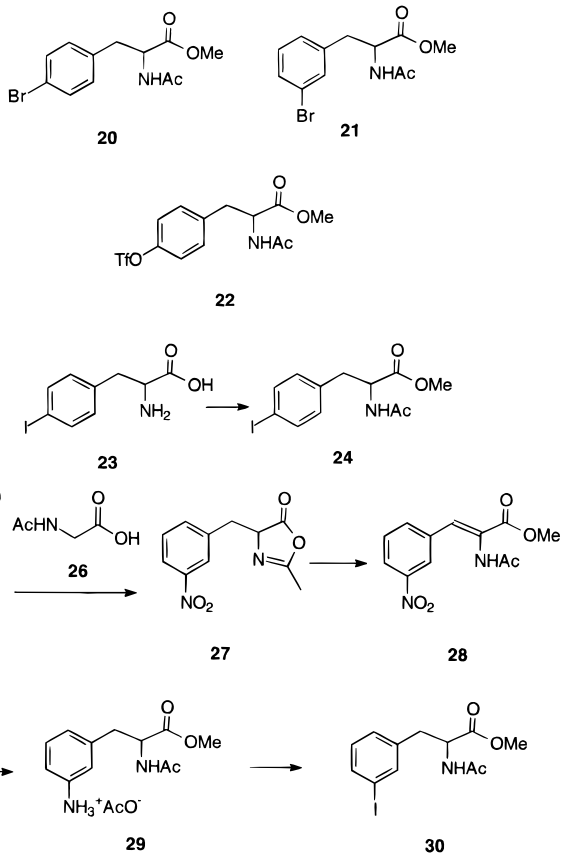
Scheme 3



of the *galacto*-isomer was very sluggish, and extended reaction times were required for complete conversion. Shorter reaction times resulted in large amounts of the 3-*O*-benzyl derivative **10**. Nevertheless, after 3 days the desired tetraacetate **5** was obtained in 81% yield. Attempts to shorten the reaction by heating resulted in decomposition, leading to large amounts of tarry byproducts that complicated the purification.

For the synthesis of the α- and *β*-mannosylacetylenes **16**−**29** (Scheme 3), we treated the mannopyranosyl lactone **11**[30] similarly to **6** to yield 93% of the ketose **12**. However, whereas the reduction of the glucose and galactose acetylenes with triethylsilane and boron trifluoride etherate gave only the *β*-anomers, reduction of **12**

Scheme 4



resulted in both the α- and *β*-C-mannosides **13** and **15**, respectively. Chromatography provided pure **13** in 55% yield**,** while **15** was contaminated by a small amount of the TES derivative **14**. Treatment of the pure *β*-anomer **13** with sodium hydroxide provided a 98% yield of **16.** Similar treatment of the mixture of **14** and **15** followed by chromatographic separation afforded pure **17** in 22% yield from ketose **12**. Acetolysis of the perbenzylated acetylenes **16** and **17** provided the tetraacetates **18** and **19** in 72% and 61% yields, respectively. Again, acetolysis was protracted; the byproduct being the 3-*O*-benzyl-2,4,6-triacetate. It appears that, at the least for glycosylacetylenes, acetolysis of the equatorial benzyloxy group of a pair of vicinal cis benzyloxy groups proceeds much more slowly.

Synthesis of Aromatic Amino Acid Derivatives. Racemic mixtures of the 3-bromo-*N*-acetylphenylalanine methyl ester **20**,[31] the 4-bromo-*N*-acetylphenylalanine methyl ester **21,**[31] and the 4-*O*-triflate of *N*-acetyl-L-tyrosine methyl ester **22**[32] were prepared as described previously. Commercially available racemic 4-iodophenylalanine **23** was acetylated and esterified with dimethoxypropane and hydrogen chloride to yield 97% of racemic **24** (Scheme 4). The 3-iodo-*N*-acetylphenylalanine methyl ester was prepared by condensation of 3-nitrobenzaldehyde (**25**) with *N*-acetylglycine (**26**) followed by treatment of the azalactone **27** with methanol to provide the crystalline enamide **28** in an overall yield of 27%. Hydrogenation of **28** in the presence of Pd/C yielded 96% of the 3-aminophenylalanine derivative **29** that was transformed into the iodo analogue **30** by diazotization and treatment with potassium iodide (48%).

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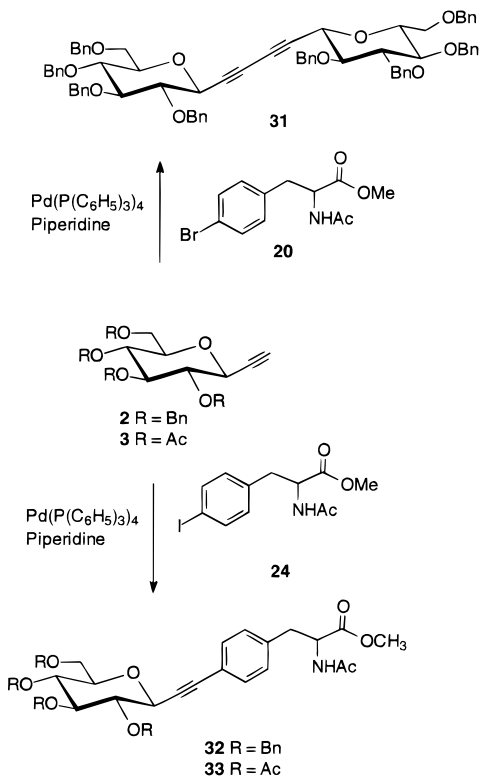
Scheme 5



Cross-Coupling. Initial attempts at cross-coupling involved the reaction of the bromo derivative **20** and the benzylated glucosylacetylene **2**. However, the use of standard conditions (triethylamine, copper iodide, $(Ph_3)_2PdCl_2$, room temperature) resulted in an 82% yield of the known butadiyne **31**[12] (Scheme 5) and none of the desired cross-coupled product **32**. Since yields in these cross-coupling reactions have been reported to be very sensitive to the solvent,[33] a number of organic bases (triethylamine, *N*-ethylmorpholine, piperidine, and *N,N,N′,N′*-tetramethylethylenediamine) were investigated as solvents, without positive result. Similarly, replacement of the catalyst with $(Ph_3)_4Pd$ gave none of the cross-coupled product, nor did increasing the reaction temperature (up to 120 °C in *N,N,N′,N′*-tetramethylethylenediamine). Similarly, attempted coupling of the glucosylacetylene **2** and the tyrosine triflate **22** under a variety of conditions led only to the butadiyne **31**.

In contrast, the reaction between **2** and the 4-iodophenylalanine **24** at room temperature in diethylamine with copper iodide and $(Ph_3)_4Pd$ gave a 20% yield of the desired cross-coupled products **32** (Scheme 5). The diastereomeric pair was most conveniently separated later by enzymatic differentiation as described below. Encouraged by this result, we optimized the reaction conditions. Best results were obtained by heating the iodide **24** and the acetylene **2** at 80 °C in the presence of copper iodide and $(Ph_3)_4Pd$ in piperidine. Under these conditions, we obtained **32** in a 81% yield. Piperidine has recently been reported[20] to be the solvent of choice for the cross-coupling of **2** with other iodobenzene derivatives. Unfortunately, debenzylation by treatment of **32** with acetic anhydride and *O*-(trimethylsilyl)trifluoromethanesulfonate led to decomposition. However, cross coupling of the acetylated alkyne **3** with **24** proceeded equally well, the only problem being that during the course of the reaction the acetoxy groups were partially cleaved. The crude product was thus acetylated prior to purification. The acetylated glycosylacetylenes **3, 5, 18,** and **19** were then coupled with the iodinated phenylalanine derivatives **24** and **30** leading to the products **33**−**40** (Figure 1) in yields between 69% and 91%. Deacetylation with sodium methoxide and methanol was followed by saponification of the methyl ester with sodium hydroxide to give **41**−**48**, *each as pairs of diastereoisomers,* in yields of over 95%.

N$^{\alpha}$-Deacetylation of the Glycosyl Acetylene Amino Acids. Two methods for the N$^{\alpha}$-deacetylation of the free acids **41**−**48** were tested. Nonenzymatic deacetylation requires forcing conditions,[34] but the glycosylacetylene amino acid conjugates possessing a carboxyl group may well be substrates for deacetylating enzymes that would ensure mild conditions. Also, enzymes should be selective for the configuration of the amino acid moiety and facilitate the isolation of one enantiomerically and diastereoisomerically pure amino acid.

Chemical Deacetylation. Deacetylation of the galactose derived acetamide **43** was optimized (Scheme 6). Initially, **43** was hydrolyzed in 1 N HCl at 90 °C. TLC and analytical RP-HPLC showed the slow formation of two major and one minor product, all with shorter retention times on RP-HPLC. After neutralization, the products were isolated by semiprepartaive RP-HPLC and characterized by NMR and ES-MS or MALDI-TOF-MS. The expected (racemic) amine **49** was obtained in 29% yield. The faster migrating **51** was obtained in 43% yield. It showed a higher mass (by 18 units) than **49**. For an unambiguous characterization, **51** was peracetylated under standard conditions. The NMR spectrum of the resulting tetraacetate showed the anomeric proton as a ddd at a low field, evidencing the formation of a ketone by regioselective hydration of the alkyne. A minor product eluting last must be the acetamido ketone corresponding to **51**, as evidenced by a peak in the MALDI-TOF-MS at 412.7 and a ddd for H-3 at 3.80 ppm. The hydration/N-deacetylation ratio was not influenced by the reaction temperature, as shown by further experiments. Extended reaction times lead to a quantitative transformation to **51.** This unexpected hydration is preparatively useful and illustrates the versatility of the acetylene functionality.

Enzymatic Deacetylation. The stereoisomerically pure phenylalanines were prepared in two steps by enzymatic resolution[35] of the mixture α-*R,S*-stereoisomeric acetamides followed by Fmoc protection. The enzymatic deacetylation of the acetamide **43** was performed with the acylase I from *Aspergillus melleus*, available from Sigma, at 30 °C in phosphate buffer at pH 7.5 instead of pH 8 as indicated by Sigma. This enzyme has been demonstrated to be highly selectively for the L-configuration of the α-carbon atom and hydrolyze only N-acetylated amino acids with the L-configuration. The deacetylation was conveniently followed by HPLC. After 24 h the intensity of the HPLC peak for product and starting material were equal and constant.

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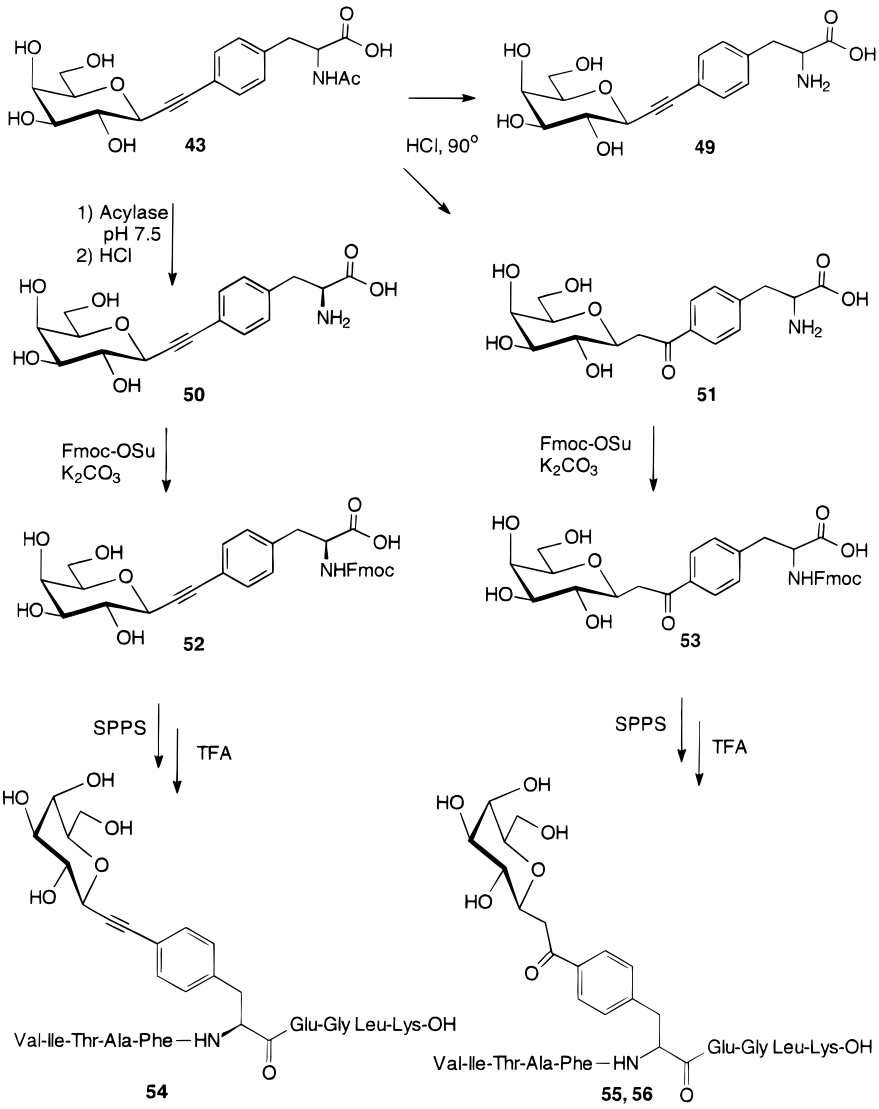
Scheme 6



Preparative HPLC and hydrolysis of the methyl ester provided 41% of **50**.

Protection of the Building Block with Fmoc. The mixtures of the diastereoisomeric amino acid conjugates **49** and **51** and the diastereomerically pure amino acid conjugate **50** were transformed into the Fmoc derivatives using Fmoc−OSu under standard conditions. The acids precipitated upon acidification of the reaction mixture. Product remaining in the supernatant was isolated by preparative RP-HPLC.

Peptide Synthesis. The peptide syntheses were performed on custom-made PEGA resin.[36,37] The resin was derivatized by a hydroxymethyl phenoxyacetic acid (HMPA) linker. The linker was coupled to the resin by activation with *O*-(benzotriazol-1-yl)-*N*,*N*,*N*,*N*-tetramethyluronium tetrafluoroborate (TBTU) and 4-ethylmorpholine (NEM). The first amino acid, lysine, was coupled as Fmoc-Lys(Boc)-COOH by activation with 1-(mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MSNT) and *N*-methylimidazol.[38] Fmoc cleavages were effected by treatment with 20% piperidine in DMF. The successive amino acids were coupled as Pfp esters. The glycosylacetylene amino acids were coupled for 3 h after activation with TBTU. The resin was washed thoroughly with DMF after Fmoc removal and after each acylation step. Acidolytic cleavage and deprotection of the peptide with 95% aqueous TFA gave the desired crude glycosylacetylene-containing peptide **54** which was then purified by semipreparative RP-HPLC and fully characterized by ES-MS and by 1D- and 2D-^{1}H NMR spectroscopy. The ketones **53** yielded the peptides **55** and **56** which could easily be separated by HPLC and were similarly characterized.

Conclusion

A range of eight glycosylacetylene amino acid building blocks containing C-linked glycan with a configuration corresponding to Glc, Gal, and Man have been synthesized. The building blocks were formed by the palladium(0)-catalyzed cross-coupling reactions of glycosylacetylenes with iodophenylalanines. In the Glc- and Gal-containing building blocks the C-glycosides were β-linked

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whereas the Man was α- and β-linked. It has been demonstrated that these building blocks can be obtained in the diastereomerically pure form by enzymatic resolution of the N-acetate followed by Fmoc protection. It was possible to perform chemical modification of the acetylene by hydrolysis to the ketone. The different building blocks were compatible with the conditions of Fmoc-based peptide synthesis and have been used in the synthesis of C-linked glycopeptide T-cell antigens. The building blocks can be employed in the synthesis of C-linked, spaced, and templated glycopeptide libraries.[3]

Experimental Section

General Methods. Optical rotations were measured at 22 ± 2 °C. Flash chromatography was performed on Silica Gel 60, 40−63 μM. Acylase I from Aspergillus melleus was purchased from Sigma as lyophilized powder. Suitable protected N^{α}-Fmoc amino acid−OPfp esters were purchased from Bachem (Switzerland) and NovaBiochem (Switzerland). PEGA resin was custom synthesized at Carlsberg. All commercial reagents were used as supplied and HPLC grade solvents (LabScan, Dublin) were used for chromatography. ^{1}H NMR chemical shifts were assigned on the basis of ^{1}H−^{1}H and ^{1}H−^{13}C correlation spectroscopy, and in cases of spectral overlap the shifts were derived from the center of the cross-peaks in the appropriate 2D experiment. For ^{1}H NMR spectra recorded in $CD_3COOD-H_2O$ the HOAc signal at 2.03 ppm was used as an internal reference. For the assignment of signals ^{1}H−^{1}H-COSY, ^{1}H−^{1}H double quantum filtered phase sensitive COSY and NOE in rotating frame (ROESY) spectroscopy experiments were used. MALDI-TOF MS was performed using a matrix of α-cyano-4-hydroxycinnamic acid. Unless otherwise stated, all reactions were carried out at room temperature. Organic solution were dried (Na_2SO_4) prior to concentration under vacuum at <40 °C (bath). Microanalyses were carried out by the microananlytical laboratory at the University of Copenhagen. Analytical, semipreparative, and preparative reverse phase HPLC separations were performed using analytical RCM (8 × 100 mm), Delta PAK (15 um, 300 Å, 25 × 200 mm), or 50 × 300 mm radial pressure C-18 columns with flow rates of 1, 10, and 20 cm^3 min^{-1}, respectively. Detection was at 215 and 280 nm. Solvent system: A, 0.1% TFA in water; B, 0.1% TFA in 90% acetonitrile−10% water.

4,5,6,8-Tetra-*O*-benzyl-1,1,2,2-tetradehydro-1,2-dideoxy-1-*C*-(trimethylsilyl)-D-galactooct-3-ulopyranose (7). $CeCl_3 \cdot 7H_2O$ (1.043 g, 2.80 mmol) was heated under vacuum at 120 °C for 1 h and then 140 °C for 1 h. The flask was cooled to 0 °C and flushed with argon, and then freshly distilled THF (10 mL) was added. The suspension was allowed to stir at room temperature for 2 h and then cooled to −78 °C. In a separate flask a solution of Li−TMS acetylene was prepared by dissolving TMS acetylene (0.5 mL, 3.54 mmol) in THF (4 mL) and then cooling the mixture to −78 °C before adding 1.6 M *n*-BuLi in hexane (2 mL). After being stirred for 45 min at −78 °C, the $CeCl_3$ suspension was treated with the freshly prepared acetylene solution (5 mL). The resulting yellow slurry was allowed to stir at −78 °C for 30 min before 2,3,4,6-tetra-*O*-benzylgalactopyranosyl lactone[1] **6** (758 mg, 1.40 mmol) in THF (10 mL) was added dropwise. After being stirred for 2 h while warming to room temperature the reaction was diluted with EtOAc and then washed with 0.1 HCl, water, and a saturated NaCl solution. The organic layer was then dried (Na_2SO_4) and evaporated to provide an oil that was chromatographed (6:1 petroleum ether/EtOAc) to give the product **7**, as an anomeric mixture (810 mg, 91%) of an oil, R_f 0.16 (6:1 petroleum ether/EtOAc). ^{1}H NMR (250 MHz, $CDCl_3$): δ 6.9−7.5 (m, 20 H, Ph), 0.015, −0.005 (combined 9H, Si(CH_3)$_3$). Anal. Calcd for $C_{39}H_{44}O_6Si \cdot {}^1/_2H_2O$ (645.87): C, 72.53; H, 7.02. Found: C, 72.52; H, 7.19.

3,7-Anhydro-4,5,6,8-tetra-*O*-benzyl-1,1,2,2-tetradehydro-1,2-dideoxy-1-*C*-(trimethylsilyl)-D-glycero-L-mannooctitol (8). Ketose **7** (860 mg, 1.35 mmol) was dissolved in 7:3 CH_3CN/CH_2Cl_2 (25 mL) and cooled to −10 °C. Separately, two stock solutions were prepared: solution A, Et_3SiH (1.4 mL) in 2:1 CH_3CN/CH_2Cl_2 (6 mL); solution B, BF_3OEt_2 (0.56 mL) in CH_3CN (4 mL). To the solution of the ketose was added first solution A (4.65 mL, 5.6 mmol Et_3SiH) followed by solution B (2.9 mL, 5.3 mmol BF_3OEt) and the reaction allowed to stir at −10 °C for 1 h, before being diluted with EtOAc and washed with water and a saturated NaCl solution. After drying (Na_2SO_4) and evaporation of the solvent the residue was chromatographed (9:1 petroleum ether/EtOAc) to give the product **8** as an oil: R_f 0.52 (6:1 petroleum ether/EtOAc); $[\alpha]_D$ −11.6° (*c* 0.9, $CHCl_3$); selected ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.10 (1 H), 3.61 (1 H), 3.57 (1 H), 4.04 (1 H), 4.10 (1 H), 3.68 (2 H); ^{13}C NMR δ 102.55, 90.50, 70.62, 77.21, 83.26, 73.71, 79.02, 68.37. Complete and assigned NMR data are presented in the Supporting Information Tables 1 and 2. Anal. Calcd for $C_{39}H_{44}O_5Si$ (620.86): C, 75.45; H, 7.14. Found: C, 75.20; H, 7.09.

3,7-Anhydro-4,5,6,8-tetra-*O*-benzyl-1,1,2,2-tetradehydro-1,2-D-glycero-L-mannooctitol (4). TMS derivative **8** (2.69 g, 4.33 mmol) was dissolved in 5:1 CH_3OH/CH_2Cl_2 (150 mL), 1 N NaOH (8 mL) was added, and the reaction was stirred for 1 h before being neutralized by the addition of 1 N HCl (8 mL). The solvent was then evaporated and the residue partitioned between EtOAc and water. The organic layer was washed with water and a saturated solution of NaCl, before being dried (Na_2SO_4) and evaporated to give the product **4** (2.35 g, 99%) as an oil that solidified upon standing: R_f 0.15 (9:1 petroleum ether/EtOAc): $[\alpha]_D$ +5.9° (*c* 0.8, $CHCl_3$); selected ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.68(1 H), 4.20 (1 H), 3.73 (1 H), 3.70 (1 H), 4.15 (1 H), 4.21(1 H), 3.77 (2 H); ^{13}C NMR δ 73.72, 79.02, 70.00, 77.32, 83.36, 73.68, 78.78, 68.55. Complete and assigned NMR data are presented in the Supporting Information Tables 1 and 2. Anal. Calcd for $C_{36}H_{36}O_5$ (548.68): C, 78.81; H, 6.61. Found: C, 78.44; H, 6.67.

3,7-Anhydro-4,5,6,8-tetra-*O*-acetyl-1,1,2,2-tetradehydro-1,2-D-glycero-L-mannooctitol (5). Benzylated derivative **4** (2.35 g, 4.28 mmol) was dissolved in Ac_2O (100 mL) and then cooled to 0 °C before TMSOTf (5.5 mL, 28.5 mmol) was added. The reaction was then warmed to room temperature and stirred for 72 h, before the dark brown solution was cooled to 0 °C and quenched by the cautious addition of a solution of $NaHCO_3$. Dilution of the reaction mixture with EtOAc was followed by washing of the organic layer with a solution of $NaHCO_3$, water, and a saturated solution of NaCl. After drying (Na_2SO_4), the organic layer was evaporated to provide a dark brown oil that was chromatographed (2:1 petroleum ether/EtOAc), yielding the product **5** (1.25 g, 81%) as light yellow solid: R_f 0.28 (2:1 petroleum ether/EtOAc); $[\alpha]_D$ +26.6° (*c* 1.1, $CHCl_3$); selected ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.51(1 H), 4.17 (1 H), 5.39 (1 H), 4.98 (1 H), 5.40 (1 H), 3.89 (1 H), 4.11 (2 H); ^{13}C NMR δ 77.82, 75.61, 68.79, 68.16, 71.34, 67.19, 74.51, 61.50. Complete and assigned NMR data are presented in the Supporting Information Tables 1 and 2. Anal. Calcd for $C_{16}H_{20}O_9$ (356.33): C, 53.93; H, 5.66. Found: C, 53.92; H, 5.48.

4,5,6,8-Tetra-*O*-benzyl-1,1,2,2-tetradehydro-1,2-dideoxy-1-*C*-(trimethylsilyl)-D-mannooct-3-ulopyranose (12). $CeCl_3 \cdot 7H_2O$ (1.10 g, 2.95 mmol) was heated under vacuum at 120 °C for 1 h and then 140 °C for 1 h. The flask was then cooled to 0 °C and flushed with argon, and then freshly distilled THF (10 mL) was added. The suspension was allowed to stir at room temperature for 2 h and then cooled to −78 °C. In a separate flask a solution of Li−TMS acetylene was prepared by dissolving TMS acetylene (0.5 mL, 3.54 mmol) in THF (4 mL) and then cooling the mixture to −78 °C before adding 1.6 M *n*-BuLi in hexane (2 mL). After being stirred for 45 min at −78 °C, the $CeCl_3$ suspension was treated with the freshly prepared acetylene solution (5.25 mL). The resulting yellow slurry was allowed to stir at −78 °C for 30 min before 2,3,4,6-tetra-*O*-benzylmannopyranosyl lactone[1] (**11**) (791 mg, 1.47 mmol) in THF (10 mL) was added dropwise. After being stirred for 2 h while warming to room temperature, the reaction was diluted with EtOAc and then washed with 0.1 HCl, water, and a saturated NaCl solution. The organic layer was then dried (Na_2SO_4) and evaporated to provide an oil that was chromato-

graphed (6:1 petroleum ether/EtOAc) to give, as an anomeric mixture, the product **12** (870 mg, 93%) as an oil: R_f 0.26 (6:1 petroleum ether/EtOAc); ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.9−7.5 (m, 20 H, Ph), 0.002, −0.01 (combined 9H, Si (Si(CH_3)$_3$). Anal. Calcd for $C_{39}H_{44}O_6Si$ (636.86): C, 73.55; H, 6.96. Found: C, 72.10; H, 7.04.

3,7-Anhydro-4,5,6,8-tetra-*O*-benzyl-1,1,2,2-tetradehydro-1,2-dideoxy-1-*C*-(trimethylsilyl)-D-glycero-D-galactooctitol (13) and 3,7-Anhydro-4,5,6,8-tetra-*O*-benzyl-1,1,2,2-tetradehydro-1,2-dideoxy-D-glycero-D-talooctitol (17). Ketose **12** (870 mg, 1.37 mmol) was dissolved in 7:3 CH_3CN/CH_2Cl_2 (25 mL) and cooled to −10 °C. Separately, two stock solutions were prepared: solution A, Et_3SiH (1.4 mL) in 2:1 CH_3CN/CH_2Cl_2 (6 mL); solution B, BF_3OEt (0.56 mL) in CH_3CN (4 mL). To the solution of the ketose was added first solution A (4.7 mL, 5.6 mmol Et_3SiH) followed by solution B (3.0 mL, 5.3 mmol BF_3OEt). The reaction was allowed to stir at −10 °C for 1 h, before being diluted with EtOAc and washed with water and a saturated NaCl solution. After drying (Na_2SO_4) and evaporation of the solvent the residue was chromatographed (9:1 petroleum ether/EtOAc) to provide two products. The more polar product, R_f 0.27 (6:1 petroleum ether/EtOAc), was the β-TMS acetylene **13** (465 mg, 55%) obtained as an oil that solidified upon standing: $[\alpha]_D$ −39.2° (*c* 0.4, $CHCl_3$); selected ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.01 (1 H), 3.86 (1 H), 3.38 (1 H), 3.76 (1 H), 3.30 (1 H), 3.63 (1 H), 3.65 (1 H); ^{13}C NMR δ 101.69, 90.87, 69.56, 75.79, 83.34, 74.49, 79.87, 69.34. Complete and assigned NMR data are presented in the Supporting Information Tables 1 and 2. Anal. Calcd for $C_{39}H_{44}O_5Si$ (620.86): C, 75.45; H, 7.14. Found: C, 74.98; H, 7.12.

The less polar product, R_f 0.35 (9:1 petroleum ether/EtOAc), was a mixture of the α-TMS acetylene **15** and the β-TES acetylene **14**. This impure product was then dissolved in 5:1 CH_3OH/CH_2Cl_2 (12 mL), and 1 N NaOH (0.6 mL) was added. After being stirred for 1 h the reaction was neutralized by the addition of 1 N HCl (0.6 mL), the solvent evaporated, and the residue partitioned between EtOAc and water. The organic layer was washed with water and a saturated solution of NaCl, before being dried (Na_2SO_4) and evaporated to give the partially deprotected product **17** (186 mg, 22%, 2 steps) as an oil: R_f 0.23 (6:1 petroleum ether/EtOAc); $[\alpha]_D$ +23.5° (*c* 0.9, $CHCl_3$); selected ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.41 (1 H), 4.73 (1 H), 3.75 (1 H), 3.94 (1 H), 3.89 (2 H), 3.71 (1 H), 3.64 (1 H); ^{13}C NMR δ 74.71, 80.68, 68.96, 75.82, 83.33, 74.58, 79.93, 69.36. Complete and assigned NMR data are presented in the Supporting Information Tables 1 and 2. Anal. Calcd for $C_{36}H_{36}O_5$ (548.68): C, 78.81; H, 6.61. Found: C, 78.39; H, 6.85.

3,7-Anhydro-4,5,6,8-tetra-*O*-benzyl-1,1,2,2-tetradehydro-1,2-D-glycero-L-galactooctitol (16). TMS derivative **13** (4.08 g, 6.57 mmol) was dissolved in 5:1 CH_3OH/CH_2Cl_2 (150 mL); then 1 N NaOH (10 mL) was added and the reaction stirred for 1 h before being neutralized by the addition of 1 N HCl (10 mL). The solvent was then evaporated and the residue partitioned between EtOAc and water. Washing of the organic layer with water and a saturated solution of NaCl was followed by drying (Na_2SO_4) and evaporation of the solvent to give the product **16** (3.54 g, 98%) as an oil that solidified upon standing: R_f 0.15 (6:1 petroleum ether/EtOAc); $[\alpha]_D$ −29.1° (*c* 0.5, $CHCl_3$); selected ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.38 (1 H), 4.05 (1 H), 3.88 (1 H), 3.46 (1 H), 3.84 (1 H), 3.68 (1 H), 3.63 (1 H). Complete and assigned ^{1}H NMR data are presented in the Supporting Information Tables 1 and 2. Anal. Calcd for $C_{36}H_{36}O_5$ (548.68): C, 78.81; H, 6.61. Found: C, 78.10; H, 6.81.

3,7-Anhydro-4,5,6,8-tetra-*O*-acetyl-1,1,2,2-tetradehydro-1,2-D-glycero-L-galactooctitol (18). Benzylated derivative **16** (3.54 g, 6.45 mmol) was dissolved in Ac_2O (100 mL) and then cooled to 0 °C before TMSOTf (8 mL, 41.4 mmol) was added. The reaction was then warmed to room temperature and stirred for 72 h, before the dark brown solution was cooled to 0 °C and quenched by the cautious addition of a solution of $NaHCO_3$. Dilution of the reaction mixture with EtOAc was followed by washing of the organic layer with a solution of $NaHCO_3$, water, and a saturated solution of NaCl. After drying (Na_2SO_4), the organic layer was evaporated to provide a dark brown oil that was chromatographed (2:1 petroleum ether/EtOAc), yielding the product **18** (1.65 g, 72%) as light yellow solid: R_f 0.25 (2:1 petroleum ether/EtOAc); $[\alpha]_D$ −41.5° (*c* 1.0, $CHCl_3$); selected ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.51 (1 H), 4.45 (1 H), 5.52 (1 H), 5.06 (1 H), 5.25 (1 H), 3.67 (1 H), 4.25 (1 H), 4.14 (1 H); ^{13}C NMR δ 75.42, 76.81, 68.02, 69.44, 71.41, 65.50, 76.49, 62.59. Complete and assigned NMR data are presented in the Supporting Information Tables 1 and 2. Anal. Calcd for $C_{16}H_{20}O_9$ (356.33): C, 53.93; H, 5.66. Found: C, 53.86; H, 5.45.

3,7-Anhydro-4,5,6,8-tetra-*O*-acetyl-1,1,2,2-tetradehydro-1,2-D-glycero-L-talooctitol (19). Benzylated derivative **17** (164 mg, 0.3 mmol) was dissolved in Ac_2O (10 mL) and cooled to 0 °C, and TMSOTf (0.4 mL, 2.07 mmol) was added. The reaction was warmed to room temperature, stirred for 30 h, and then worked up as described for **18**. Chromatography (2:1 petroleum ether/EtOAc) yielded the product **19** (65 mg, 61%) as light yellow solid: R_f 0.21 (2:1 petroleum ether/EtOAc); $[\alpha]_D$ +30.5° (*c* 0.8, $CHCl_3$); selected ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.76 (1 H), 4.76 (1 H), 5.32 (1 H), 5.46 (1 H), 5.26 (1 H), 4.17 (1 H), 4.29 (1 H), 4.11 (1 H); ^{13}C NMR δ 79.15, 75.98, 66.93, 70.91, 69.28, 65.91, 71.59, 62.30. Complete and assigned NMR data are presented in the Supporting Information Tables 1 and 2. Anal. Calcd for $C_{16}H_{20}O_9$ (356.33): C, 53.93; H, 5.66. Found: C, 53.75; H, 5.62.

***N*-Acetyl-4-iodo-(DL)-phenylalanine Methyl Ester (24).** 4-Iodo-(DL)-phenylalanine (**23**) (100 mg, 0.343 mmol) was suspended in CH_3OH (10 mL), and then Ac_2O (65 μL, 0.689 mmol) and $NaHCO_3$ (62 mg, 0.74 mmol) were added. After being stirred for 20 min the solution became clear, and after 45 min the reaction was complete. The solution was then evaporated to give a residue that was immediately resuspended in 2,2-dimethoxypropane (10 mL). Concentrated HCl (350 μL) was added, and after being stirred for 4 h the reaction was evaporated. Chromatography (9:1 CH_2Cl_2/CH_3OH) gave the product **24** (116 mg, 97%) as a white solid: R_f 0.74 (19:1 CH_2Cl_2/CH_3OH); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.641 (d, 2 H, ^3J 8.2 Hz, H-2, H-6), 6.897 (d, 2 H, ^3J 8.2 Hz, H-3, H-5), 5.936 (d, 1 H, $J_{CH,NH}$ 7.0 Hz, NH), 4.894 (ddd, 1 H, $J_{CH,NH}$ 7.0, $J_{\alpha,\beta}$ 5.9, $J_{\alpha,\beta'}$ 5.5 Hz, H-α), 3.759 (s, 3 H, OCH_3), 3.145 (dd, 1 H, $J_{\alpha,\beta}$ 5.9, $J_{\beta,\beta'}$ 13.9 Hz, H-β), 3.085 (dd, 1 H, $J_{\alpha,\beta}$ 5.5, $J_{\beta,\beta'}$ 13.9 Hz, H-β'), 2.021 (s, 3 H, NHCOCH_3); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 171.810, 169.555 (C=O), 137.615 (C-2, C-6), 135.511 (C-4), 131.204 (C-3, C-5), 92.603 (C-1), 52.892 (C-α), 52.413 (OCH_3), 37.355 (C-β), 23.116 (NHCOCH_3). Anal. Calcd for $C_{12}H_{14}INO_3$ (347.15): C, 41.52; H, 4.04; N, 4.03. Found: C, 41.45; H, 3.99; N, 4.02.

Methyl 3′-nitro-α-acetamidocinnamate (28). *N*-Acetylglycine **26** (5.24 g, 44.7 mmol), 3-nitrobenzaldehyde (**25**) (10 g, 66.2 mmol), and sodium acetate (2.71 g, 33.1 mmol) were suspended in Ac_2O (30 mL), and the reaction was heated at reflux for 1 h. During the course of the reaction the solution turned dark brown and, upon cooling to room temperature, solidified. The yellow-brown solid obtained was then suspended in ice water, filtered off, and washed extensively with cold water before being dissolved in CH_2Cl_2. After drying (Na_2SO_4), the organic solution was evaporated to yield **27** as a yellow-brown solid. This product was not purified further but was instead immediately dissolved in CH_3OH (200 mL), and the solution was heated at reflux for 9 h. Evaporation of the solvent provided a brown oil that was chromatographed (19:1 CH_2Cl_2/CH_3OH) to give a slightly colored product. Dissolution of this product in CH_3OH and then decolorization with activated carbon gave the product **28** (4.3 g, 27%) as a yellow-white solid, pure by ^{1}H NMR and TLC. The product could be recrystallized from EtOAc: R_f 0.22 (1:1 petroleum ether/EtOAc); ^{1}H NMR (DMSO-d_6, 250 MHz) δ 9.839 (s, 1 H, NH), 8.467 (s, 1 H, H-2), 8.220 (dd, 1 H, ^4J 1.9, ^3J 8.0 Hz, H-6), 8.046 (d, 1 H, ^3J 8.0 Hz, H-4), 7.726 (t, 1 H, ^3J 8.0, H-5), 7.315 (s, 1 H, H-β), 3.756 (s, 3 H, OCH_3), 2.031 (s, 3 H, NHCOCH_3); ^{13}C NMR (DMSO-d_6, 62.5 MHz) δ 170.210, 166.046 (C=O), 148.714 (C-1), 136.836 (C-4), 136.073 (C-3), 130.967 (C-5), 129.486 (C-β), 128.917 (C-α), 124.630 (C-2), 124.369 (C-4), 53.241 (OCH_3), 23.281 (NHCOCH_3). Anal. Cald. for $C_{12}H_{12}N_2O_5$ (264.24): C, 54.55; H, 4.58; N, 10.60. Found: C, 54.50; H, 4.58; N, 10.60.

***N*-Acetyl 3-amino-(DL)-phenylalanine Methyl Ester Acetate Salt (29).** Cinnamate **28** (4.2 g, 15.9 mmol) was dissolved in acetic acid (250 mL) and then 10% Pd/C (1.5 g) added. The reaction was allowed to stir for 20 h under H_2, before the catalyst was filtered away and the solvent evaporated to yield the product **25** (4.5 g, 96%) as a brown oil. ^{1}H NMR showed the product as pure: R_f 0.24 (19:1 CH_2Cl_2/CH_3OH); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.058 (t, 1 H, ^3J 7.7 Hz, H-5), 6.573 (m, 1 H, H-4), 6.481 (m, 1 H, H-6), 6.458 (m, 1 H, H-2), 6.110 (d, 1 H, $J_{CH,NH}$ 7.7 Hz, NH), 4.823 (ddd, 1 H, $J_{CH,NH}$ 7.7, $J_{\alpha,\beta}$ 5.8, $J_{\alpha,\beta'}$ 6.1 Hz, H-α), 3.713 (s, 3 H, OCH_3), 3.034 (dd, 1 H, $J_{\alpha,\beta}$ 5.8, $J_{\beta,\beta'}$ 13.8 Hz, H-β), 2.963 (dd, 1 H, $J_{\alpha,\beta}$ 6.1, $J_{\beta,\beta'}$ 13.8 Hz, H-β'), 1.957 (s, 3 H, NHCOC*H*$_3$); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 172.149, 169.728 (C=O), 146.141 (C-1), 136.982 (C-3), 129.426 (C-5), 119.506 (C-6), 115.969 (C-2), 114.080 (C-4), 52.992 (C-α), 52.206 (OCH_3), 37.667 (C-β), 23.000 (NHCO*C*H$_3$).

***N*-Acetyl-3-iodo-(DL)-phenylalanine Methyl Ester (30).** *N*-Acetyl-3-amino-(DL)-phenylalanine methyl ester (**29**) (15.2 g, 51.7 mmol) was dissolved in 1 N HCl (130 mL and the solution cooled to 0 °C. To the reaction was added, in 2 mL portions, $NaNO_2$ (4.71 g, 68.3 mmol) as a cold solution in water (150 mL). During the course of the addition the temperature of the mixture was kept below 5 °C by the addition of ice to the reation mixture. After the last aliquot of $NaNO_2$ solution was added, the reaction was allowed to stir for 30 min at 0−5 °C before KI (21.5 g, 129.5 mmol) was added, in 3 g portions. The reaction immediately turned purple, and gas evolution occurred. The flask was then removed from the ice bath and was heated at 80 °C for 1 h. After cooling of the flash to room temperature, ethyl acetate was added and then the aqueous layer was washed with ethyl acetate until no product was observed in the organic layer. The combined organic extracts were then dried (Na_2SO_4), and the solvent was evaporated. Chromatography (19:1 CH_2Cl_2/CH_3OH) gave the product as a yellow solid. Recrystallization from EtOAc/petroleum ether gave the product **30** (8.6 g, 48%) as white crystals: R_f 0.44 (19:1 CH_2Cl_2/CH_3OH); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.589 (m, 1 H, H-6), 7.667 (s, 1 H, H-2), 7.087 (m, 1 H, H-4), 7.026 (t, 1 H, ^3J 7.6 Hz, H-5), 6.025 (d, 1 H, $J_{CH,NH}$ 6.2 Hz, NH), 4.855 (ddd, 1 H, $J_{CH,NH}$ 6.2, $J_{\alpha,\beta}$ 5.8, $J_{\alpha,\beta'}$ 5.7 Hz, H-α), 3.740 (s, 3 H, OCH_3), 3.109 (dd, 1 H, $J_{\alpha,\beta}$ 5.8, $J_{\beta,\beta'}$ 13.9 Hz, H-β), 3.019 (dd, 1 H, $J_{\alpha,\beta}$ 5.7, $J_{\beta,\beta'}$ 13.9 Hz, H-β'), 2.010 (s, 3 H, NHCOC*H*$_3$); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 171.747, 169.619 (C=O), 138.338 (C-2), 136.147 (C-6), 136.145 (C.3), 130.205 (C-5), 128.408 (C-4), 94.375 (C-1), 53.038 (C-α), 52.415 (OCH_3), 37.352 (C-β), 23.079 (NHCO*C*H$_3$). Anal. Calcd for $C_{12}H_{14}INO_3$ (347.15): C, 41.52; H, 4.04; N, 4.03. Found: C, 42.02; H, 3.84; N, 4.06.

General Procedure for Pd-Coupling Reactions. The acetylene (55 mg, 0.15 mmol) and the iodide (45 mg, 0.13 mmol) were dissolved in piperidine (2 mL) and the flask purged with argon while heating to 80 °C. Following the simultaneous addition of $(Ph_3P)_4Pd$ (7.5 mg, 6.5 μmol) and CuI (2.5 mg, 13 μmol) the reaction was stirred at 80 °C for 1 h before being cooled and evaporated. The residue was then redissolved in 1:1 acetic anhydride/pyridine (5 mL) and stirred overnight, and the reaction was quenched by cooling the solution to 0 °C and adding methanol (3 mL). Evaporation of the solvent gave a brown oil that was chromatographed (EtOAc) to give the coupled product, R_f 0.45 (EtOAc).

***N*-Acetyl 4-*C*-(3,7-anhydro-4,5,6,8-tetra-*O*-acetyl-1,1,2,2-tetradehydro-1,2-D-glycero-D-gulooctityl)-DL-phenylalanine methyl ester (33):** yield 61 mg, 81%; selected ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.41 (1 H), 5.18 (1 H), 5.16 (1 H), 5.06 (1 H), 3.74 (1 H), 4.28 (1 H), 4.15 (1 H), 7.34 (2 H), 7.04 (2 H), 4.86 (1 H), 3.17 (1 H), 3.05 (1 H); ^{13}C NMR δ 86.79, 82.96, 69.35, 73.49, 71.21, 68.15, 75.91, 62.03, 120.33, 132.13 (2 C), 129.23 (2 C), 137.17, 52.93, 37.77. Complete and assigned NMR data are presented in the Supporting Information Tables 3 and 4. Anal. Calcd for $C_{28}H_{33}NO_{12}\cdot{}^1/_2H_2O$ (584.58): C, 57.53; H, 5.86; N, 2.40. Found: C, 57.29; H, 5.62; N, 2.44.

***N*-Acetyl 4-*C*-(3,7-anhydro-4,5,6,8-tetra-O-acetyl-1,1,2,2-tetradehydro-1,2-D-glycero-D-mannooctityl)-DL-phenylalanine methyl ester (35):** yield 68.5 mg, 91%; ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.41 (1 H), 5.18 (1 H), 5.16 (1 H), 5.06 (1 H), 3.74 (1 H), 4.28 (1 H), 4.15 (1 H), 7.34 (2 H), 7.04 (2 H), 4.86 (1 H), 3.17 (1 H), 3.05 (1 H); ^{13}C NMR δ 86.48, 82.21, 69.71, 68.53, 71.41, 67.34, 74.45, 61.57, 120.36, 132.11 (2 C), 129.20 (2 C), 137.13, 52.92, 37.75. Complete and assigned NMR data are presented in the Supporting Information Tables 3 and 4. Anal. Calcd for $C_{28}H_{33}NO_{12}\cdot{}^1/_2H_2O$ (584.58): C, 57.53; H, 5.86; N, 2.40. Found: C, 57.31; H, 5.52; N, 2.39.

***N*-Acetyl 4-*C*-(3,7-anhydro-4,5,6,8-tetra-*O*-acetyl-1,1,2,2-tetradehydro-1,2-D-glycero-D-galactooctityl)-DL-phenylalanine methyl ester (37):** yield 68.7 mg, 91%; ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.67 (1 H), 5.60 (1 H), 5.12 (1 H), 5.29 (1 H), 3.72 (1 H), 4.30 (1 H), 4.16 (1 H), 7.11 (2 H), 7.03 (2 H), 4.86 (1 H), 3.15 (1 H), 3.05 (1 H); ^{13}C NMR δ 86.53, 82.29, 69.04, 69.82, 71.52, 65.66, 76.40, 62.65, 120.34, 132.03 (2 C), 129.21 (2 C), 137.11, 52.90, 37.74. Complete and assigned NMR data are presented in the Supporting Information Tables 3 and 4. Anal. Calcd for $C_{28}H_{33}NO_{12}\cdot{}^1/_2H_2O$ (584.58): C, 57.53; H, 5.86; N, 2.40. Found: C, 57.35; H, 5.88; N, 2.42.

***N*-Acetyl 4-*C*-(3,7-anhydro-4,5,6,8-tetra-*O*-acetyl-1,1,2,2-tetradehydro-1,2-D-glycero-D-talooctityl)-DL-phenylalanine methyl ester (39):** yield 52.1 mg, 69%; ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.97 (1 H), 5.42 (1 H), 5.54 (1 H), 5.32 (1 H), 4.24 (1 H), 4.32 (1H), 4.15 (1 H), 7.41 (2 H), 7.08 (2 H), 4.87 (1 H), 3.19 (1 H), 3.09 (1 H); ^{13}C NMR δ 90.24, 81.17, 67.70, 71.20, 69.56, 65.99, 71.58, 62.39, 119.95, 132.11 (2 C), 129.33 (2 C), 137.49, 52.94, 37.74. Complete and assigned NMR data are presented in the Supporting Information Tables 3 and 4. Anal. Calcd for $C_{28}H_{33}NO_{12}\cdot{}^1/_2H_2O$ (584.58): C, 57.53; H, 5.86; N, 2.40. Found: C, 57.11; H, 5.56; N, 2.40.

***N*-Acetyl 3-*C*-(3,7-anhydro-4,5,6,8-tetra-*O*-acetyl-1,1,2,2-tetradehydro-1,2-D-glycero-D-gulooctityl)-DL-phenylalanine methyl ester (34):** yield 65.6 mg, 87%; ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.40 (1 H), 5.21 (1 H), 5.25 (1 H), 5.12 (1 H), 3.74 (1 H), 4.27 (1 H), 4.15 (1 H), 7.15 (1 H), 7.08 (1 H), 7.23, (1 H) 7.30 (1 H), 4.86 (1 H), 3.13 (1 H), 3.04 (1 H); ^{13}C NMR δ 86.84, 82.94, 69.34, 73.47, 71.19, 68.15, 75.94, 62.02, 121.78, 132.67, 136.23, 130.05, 128.56, 130.77, 52.96, 37.54. Anal. Calcd for $C_{28}H_{33}NO_{12}\cdot H_2O$ (593.58): C, 56.66; H, 5.94; N, 2.36. Found: C, 56.95; H, 5.59; N, 2.37.

***N*-Acetyl-3-*C*-(3,7-anhydro-4,5,6,8-tetra-*O*-acetyl-1,1,2,2-tetradehydro-1,2-D-glycero-D-mannooctityl)-DL-phenylalanine methyl ester (36):** yield 61 mg, 81%; ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.38 (1 H), 5.47 (1 H), 5.05 (1 H), 5.44 (1 H), 3.95 (1 H), 4.14 (1 H), 4.14 (1 H), 7.17 (1 H), 7.07 (1 H), 7.22, (1 H) 7.31 (1 H), 4.85 (1 H), 3.11 (1 H), 3.03 (1 H); ^{13}C NMR δ 86.49, 83.15, 69.67, 68.47, 71.36, 67.32, 74.45, 61.55, 121.80, 132.61, 136.19, 129.96, 128.50, 130.73, 52.92, 37.51. Anal. Calcd for $C_{28}H_{33}NO_{12}\cdot{}^1/_2H_2O$ (584.58): C, 57.53; H, 5.86; N, 2.40. Found: C, 57.51; H, 5.62; N, 2.38.

***N*-Acetyl-3-*C*-(3,7-anhydro-4,5,6,8-tetra-*O*-acetyl-1,1,2,2-tetradehydro-1,2-D-glycero-D-galactooctityl)-DL-phenylalanine methyl ester (38):** yield 52 mg, 69%; ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.66 (1 H), 5.60 (1 H), 5.11 (1 H), 5.29 (1 H), 3.72 (1 H), 4.29 (1 H), 4.16 (1 H), 7.12 (1 H), 7.07 (1 H), 7.22, (1 H) 7.28 (1 H), 4.46 (1 H), 3.14 (1 H), 3.03 (1 H); ^{13}C NMR δ 86.56, 82.27, 69.03, 69.80, 71.51, 65.65, 76.41, 62.63, 121.78, 132.55, 136.23, 129.95, 128.54, 130.67, 52.93, 37.53. Anal. Calcd for $C_{28}H_{33}NO_{12}\cdot{}^1/_2H_2O$ (584.58): C, 57.53; H, 5.86; N, 2.40. Found: C, 57.39; H, 5.64; N, 2.36.

***N*-Acetyl-3-*C*-(3,7-anhydro-4,5,6,8-tetra-*O*-acetyl-1,1,2,2-tetradehydro-1,2-D-glycero-D-talooctityl)-DL-phenylalanine methyl ester (40):** yield 53.5 mg, 71%; ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.97 (1 H), 5.42 (1 H), 5.53 (1 H), 5.32 (1 H), 4.23 (1 H), 4.33 (1 H), 4.15 (1 H), 7.20 (1 H), 7.12 (1 H), 7.27, (1 H) 7.38 (1 H), 4.86 (1 H), 3.15 (1 H), 3.05 (1 H); ^{13}C NMR δ 90.26, 81.15, 67.68, 71.18, 69.53, 65.98, 71.62, 62.40, 121.39, 132.65, 136.45, 130.23, 128.65, 130.74, 53.02, 37.52. Anal. Calcd for $C_{28}H_{33}NO_{12}$ (575.57): C, 58.43; H, 5.78; N, 2.43. Found: C, 58.08; H, 5.66; N, 2.43.

General Procedure for Deacylation Reactions. The coupled product (ca. 1 mmol) was dissolved in CH_3OH (20 mL), a small piece of sodium was added, and the reaction stirred for 2 h and evaporated. The residue was redissolved in water (20 mL), stirred for an additional 2 h, and then brought to pH

2−3 by the addition of IR 120 (H+) resin. After filtration of the resin the solution was evaporated to give the product, pure by NMR.

***N*-Acetyl 4-*C*-(3,7-anhydro-1,1,2,2-tetradehydro-1,2-D-glycero-D-gulooctityl)-DL-phenylalanine (41).** The conjugate **33** (641 mg, 1.11 mmol) yielded the product **41** (425 mg, 97%) as a foam: ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.34 (1 H), 3.51 (1 H), 3.57 (1 H), 3.56 (1 H), 3.53 (1 H), 3.94 (1 H), 3.74 (1 H), 7.50 (2 H), 7.28 (2 H), 4,63 (1 H), 3.24 (1 H), 3.01 (1 H), 1.95 (3 H); ^{13}C NMR δ 89.96, 85.21, 71.01, 69.91, 77.03, 73.77, 80.28, 61.23, 120.18, 132.35 (2 C), 129.76 (2 C), 138.65, 54.67, 37.17.

***N*-Acetyl-4-*C*-(3,7-anhydro-1,1,2,2-tetradehydro-1,2-D-glycero-D-mannooctityl)-DL-phenylalanine (43).** The conjugate **35** (730 mg, 1.27 mmol) yielded the product **43** (485 mg, 97%) as a foam: ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.27 (1 H), 3.81 (1 H), 3.68 (1 H), 4.02 (1 H), 3.43 (1 H), 3.58 (2 H), 7.52 (2 H), 7.29 (2 H), 4,64 (1 H), 3.25 (1 H), 3.02 (1 H), 1.95 (3 H); ^{13}C NMR δ 86.60, 85.45, 71.43, 71.08, 73.74, 69.24, 79.36, 61.51, 120.26, 132.36 (2 C), 129.76 (2 C), 138.51, 54.55, 37.10.

***N*-Acetyl 4-*C*-(3,7-anhydro-1,1,2,2-tetradehydro-1,2-D-glycero-D-galactooctityl)-DL-phenylalanine (45).** The conjugate **37** (640 mg, 1.11 mmol) yielded the product **45** (420 mg, 96%) as a foam: ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.51 (1 H), 4.10 (1 H), 3.59 (1 H), 3.66 (1 H), 3.30 (1 H), 3.94 (1 H), 3.76 (1 H), 7.50 (2 H), 7.29 (2 H), 4.52 (1 H), 3.24 (1 H), 3.02 (1 H), 1.95 (3 H); ^{13}C NMR δ 86.71, 84.71, 70.50, 71.72, 73.75, 66.96, 80.55, 61.48, 120.36, 132.31 (2 C), 129.74 (2 C), 138.35, 54.35, 37.00.

***N*-Acetyl 4-*C*-(3,7-anhydro-1,1,2,2-tetradehydro-1,2-D-glycero-D-talooctityl)-DL-phenylalanine (47).** The conjugate **39** (272 mg, 0.47 mmol) yielded the product **47** (183 mg, 98%) as a foam: ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.97 (1 H), 4.18 (1 H), 4.09 (1 H), 3.71 (1 H), 3.88 (1 H), 3.86 (1 H), 3.81 (1 H), 7.48 (2 H), 7.27 (2 H), 4,60 (1 H), 3.24 (1 H), 2.99 (1 H), 1.94 (3 H); ^{13}C NMR δ 89.75, 83.08, 70.11, 72.32, 71.54, 67.33, 76.02, 61.33, 120.05, 132.18 (2 C), 129.75 (2 C), 138.81, 54.97, 37.31.

***N*-Acetyl-3-*C*-(3,7-anhydro-1,1,2,2-tetradehydro-1,2-D-glycero-D-gulooctityl)-DL-phenylalanine (42).** The conjugate **34** (647 mg, 1.12 mmol) yielded the product **42** (435 mg, 98%) as a foam: ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.35 (1 H), 3.49 (1 H), 3.54 (1 H), 3.53 (1 H), 3.50 (1 H), 3.95 (1 H), 3.75 (1 H), 7.42 (1 H), 7.46 (1 H), 7.38, (1 H) 7.32 (1 H), 4.59 (1 H), 3.23 (1 H), 2.98 (1 H), 1.95 (3 H); ^{13}C NMR δ 86.96, 85.02, 71.00, 77.04, 73.76, 69.91, 80.30, 61.23, 121.68, 132.80, 137.88, 130.76, 129.19, 130.55, 55.09, 37.15.

***N*-Acetyl-3-*C*-(3,7-anhydro-1,1,2,2-tetradehydro-1,2-D-glycero-D-mannooctityl)-DL-phenylalanine (44).** The conjugate **36** (651 mg, 1.13 mmol) yielded the product **44** (435 mg, 98%) as a foam: ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.28 (1 H), 3.86 (1 H), 3.68 (1 H), 4.02 (1 H), 3.76 (1 H), 3.79 (2 H), 7.45 (1 H), 7.49 (1 H), 7.40, (1 H) 7.36 (1 H), 4.66 (1 H), 3.24 (1 H), 3.01 (1 H), 1.96 (3 H); ^{13}C NMR δ 86.54, 85.50, 71.40, 71.07, 73.75, 69.24, 79.38, 61.55, 121.87, 132.79, 137.46, 130.87, 129.23, 130.47, 54.34, 36.77.

***N*-Acetyl-3-*C*-(3,7-anhydro-1,1,2,2-tetradehydro-1,2-D-glycero-D-galactooctityl)-DL-phenylalanine (46).** The conjugate **38** (675 mg, 1.17 mmol) yielded the product **46** (447 mg, 97%) as a foam: ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.66 (1 H), 4.11 (1 H), 3.79 (1 H), 3.66 (1 H), 3.46 (1 H), 3.95 (1 H), 3.76 (1 H), 7.47 (1 H), 7.41 (1 H), 7.43, (1 H) 7.36 (1 H), 4.63 (1 H), 3.23 (1 H), 3.00 (1 H), 1.96 (3 H); ^{13}C NMR δ 86.70, 84.71, 70.49, 71.71, 73.75, 66.96 80.57, 61.49, 121.91, 132.72, 137.63, 130.77, 129.21, 130.44, 54.65, 36.92. ES-MS: found $(M + H)^+$, *m/e* 394.3; $C_{19}H_{23}NO_8$ requires M, 393.39.

***N*-Acetyl-3-*C*-(3,7-anhydro-1,1,2,2-tetradehydro-1,2-D-glycero-D-talooctityl)-DL-phenylalanine (48).** The conjugate **40** (272 mg, 0.47 mmol) yielded the product **48** (182 mg, 98%) as a foam: ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.97 (1 H), 4.19 (1 H), 4.09 (1 H), 3.71 (1 H), 3.97 (1 H), 3.96 (1 H), 3.81 (1 H), 7.42 (1 H), 7.45 (1 H), 7.38, (1 H) 7.32 (1 H), 4.61 (1 H), 3.22 (1 H), 2.97 (1 H), 1.95 (3 H); ^{13}C NMR δ 89.72, 83.16, 70.09, 72.31, 71.56, 67.32, 76.06, 61.35 121.68, 132.66, 137.68, 130.62, 129.22, 130.53, 54.68, 36.94.

4-*C*-(3,7-Anhydro-1,1,2,2-tetradehydro-1,2-D-glycero-D-galactooctityl)-DL-phenylalanine (49) and 4-*C*-(3,7-Anhydro-1-oxo-1,2-D-glycero-D-galactooctit-1-yl)-DL-phenylalanine (51). Method 1: Chemical Deacetylation. The *N*-acetyl derivative **43** (10 mg, 330 μmol) was dissolved in 30 mL of 1 N HCl and the solution stirred at 90 °C. The reaction was followed by analysis of 10 μL samples on analytical RP-HPLC using a linear gradient 0−50% B over 40 min. Two fast eluting products were formed. After 6 h the starting material had disappeared and the reaction mixture was cooled to room temperature and neutralized by addition of 5% NaOH solution. Preparative RP-HPLC using a linear gradient 0−30% B over 90 min gave the product **49** (33.7 mg, 29%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.24 (1 H), 3.77 (1 H), 3.64 (1 H), 3.98 (1 H), 3.73 (3 H), 7.54 (2 H), 7.31 (2 H), 4.28 (1 H), 3.34 (1 H), 3.02 (1 H); ^{13}C NMR δ 86.33, 85.88, 71.37, 71.08, 73.74, 69.24, 79.41, 61.55, 121.25, 132.88 (2 C) 129.97 (2 C), 135.78, 54.55, 35.99. ES-MS: $(M + H)^+$, *m/z* 352.2; $C_{17}H_{21}NO_7$ requires M, 351.355. Furthermore, the compound **51** (52.0 mg, 43%) was isolated: ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.48 (1 H), 3.23 (1 H), 3.80 (1 H), 3.51 (1 H), 3.58 (1 H), 3.90 (1 H), 3.57 (1 H), 3.59 (2 H), 7.92 (2 H), 7.40 (2 H), 4.26 (1 H), 3.35 (1 H), 3.22 (1 H); ^{13}C NMR δ 202.49, 171.99, 41.73, 76.52, 71.02, 74.31, 69.52, 78.96, 61.54, 141.01, 130.22 (2 C) 129.53 (2 C), 136.28, 54.49, 35.10. ES-MS: found for $(M + H)^+$, *m/e* 370.5; $C_{17}H_{23}NO_8$ requires M, 369.371.

3-*C*-(3,7-Anhydro-1,1,2,2-tetradehydro-1,2-D-glycero-D-galactooctityl)-L-phenylalanine (50). Method 2: Acylase-Catalyzed Reaction. The *N*-acetyl derivative **43** (102 mg, 259 μmol) was dissolved in 10 mL of PBS buffer (pH = 7.5). Acylase I (326 mg, 163 units) was added. The mixture was stirred for a total of 48 h, during which the pH was kept at 7.5 by addition of a saturated NaHCO3 solution. The reaction was followed by analytical RP-HPLC of 5 μL samples of the reaction mixture using a gradient buffer A → buffer B 100:0 → 50:50 over 40 min. When no evolution was observed anymore, the mixture was acidified by addition of 1 N HCl solution and filtered. Purification by preparative RP-HPLC yielded a fraction of impure starting material (72.4 mg) and 58.7 mg (62%) of pure product **50**: $[\alpha]_D$ +5.8° (*c* 2.0, H_2O, pH = 1); ES-MS $(M + H)^+$ *m/z* 352.1, $C_{17}H_{21}NO_7$ requires M, 351.355; ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.24 (1 H), 3.76 (1 H), 3.64 (1 H), 3.98 (1 H), 3.72 (3 H), 7.54 (2 H), 7.31 (2 H), 4.35 (1 H), 3.36 (1 H), 3.22 (1 H); ^{13}C NMR δ 86.28, 85.89, 71.35, 71.06, 73.73, 69.23, 79.40, 61.53, 121.32, 132.88 (2 C) 129.97 (2 C), 135.55, 54.18, 35.83.

N^{α}-(Fluroren-9-ylmethoxycarbonyl)-4-*C*-(3,7-anhydro-1,1,2,2-tetradehydro-1,2-D-glycero-D-galactooctityl)phenylalanine (52, DL Mixture). The glycosyl amino acid **49** (30 mg, 85 μmol) was dissolved in 5 mL of water, and the pH was adjusted to 10 by addition of a 1 M solution of K_2CO_3. Fmoc−OSu (32 mg, 94 μmol) was added as solution in 2 mL of acetone. After stirring of the reaction overnight TLC (15:5:2.5 DCM/MeOH/AcOH) and analytical C-18 HPLC showed complete conversion of the stating material. The reaction mixture was acidified to pH = 2 by addition of 1 N HCl thereby precipitating the Fmoc derivative. The solution was extracted 4 times with EtOAc, and the organic extracts were combined, dried over $MgSO_4$, and evaporated. Semipreparative C18-HPLC purification yielded 25.1 mg of **52** (DL form, 47%). ES-MS: found for $(M + Na)^+$, *m/e* 596.2; $C_{32}H_{31}NO_9$ requires M, 573.598.

N^{α}-(Fluroren-9-ylmethoxycarbonyl)-4-*C*-(3,7-anhydro-1,1,2,2-tetradehydro-1,2-D-glycero-D-galactooctityl)-L-phenylalanine (52). The amino acid derivative **50** (45 mg, 129 μmol) was dissolved in 10 mL of water, and the pH was adjusted to 10 by dropwise addition of a 1 M solution of K_2CO_3. A solution of Fmoc−OSu (48 mg, 142 μmol) in 2 mL of acetone was added. The reaction mixture was stirred at room temperature, and every few hours the pH was readjusted to 10 by addition of 1 M K_2CO_3. After 12 h TLC (15:5:2.5 or 15:5:2.5 DCM/MeOH/AcOH) showed that the reaction was finished. The reaction mixture was diluted to 20 mL and washed with 10 mL of diethyl ether. The water phase was transferred to two centrifuge tubes and under agitation acidified to pH 2 by addition of 1 N HCl. After centrifugation at 7000 r/min for 10 min the supernatant was decanted from the white precipitate. The precipitate was dispersed in water and collected by

centrifugation. This was repeated twice. The precipitate was lyophilized to yield 55.8 mg of white product. Product remaining in the supernatant and washings was recovered by running a preparative RP-HPLC of the combined concentrated solutions, and after lyophilization additional (7.71 mg) white power could be obtained (85% total yield of **52**). The two fractions were identical as analyzed by NMR spectroscopy and mass spectrometry: $[\alpha]_D$ +6.5° (*c* 0.8, DMF); ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.99 (1 H), 3.58 (1 H), 3.30 (1 H), 3.71 (1 H), 3.40 (1 H), 3.50 (2H), 7.2−7.6 (4 H), 4.20 (1 H), 3.11 (1 H), 2.90 (1 H); ^{13}C NMR δ 89.29, 84.72, 72.14, 71.42, 75.11, 69.45, 80.11, 61.59, 121−136 (6 C), 56.05, 37.12; ES-MS found for $(M + Na)^+$ 596.2, $C_{32}H_{31}NO_9$ requires M, 573.598.

***N*$^\alpha$-(Fluroren-9-ylmethoxycarbonyl)-3-*C*-(3,7-anhydro-1-oxo-1,2-D-glycero-D-galactooctityl)-DL-phenylalanine (53).** The amino acid derivative **51** (52 mg, 141 μmol) was dissolved in 10 mL of water, and the pH was adjusted to 10 by dropwise addition of a 1 M solution of K_2CO_3. A solution of Fmoc−OSu (53 mg, 157 μmol) in 2 mL of acetone was added. The reaction mixture was stirred at room temperature, and every few hours the pH was readjusted to 10 by addition of 1 M K_2CO_3. After 20 h analytical HPLC showed that the reaction was finished. The reaction was worked up as for compound **52**. Product remaining in the supernatant and washings was recovered by preparative HPLC. A total of 66.6 mg (77%) of white product **53** was obtained: ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.27 (1 H), 3.10 (1 H), 3.66 (1 H), 3.35 (1 H), 3.29 (1 H), 3.73 (1 H), 3.27 (1 H), 3.45 (1 H), 3.33 (1 H), 7.35−7.95 (4 H), 4.24 (1 H), 3.17 (1 H), 2.95 (1 H); ^{13}C NMR δ 198.81, 173.91, 42.22, 77.38, 71.37, 75.53, 69.43, 79.63, 61.16, 141.55, 129−130 (4 C), 136.29, 55.88, 37.13; ES-MS $(M + Na)^+$ *m*/*z* 596.2, $C_{32}H_{31}NO_9$ requires M, 573.598.

Preparation of the PEGA Resin. Syntheses of the peptides were performed in DMF using PEGA resin prepared in the laboratory.[36,37] The resin (5 g, loading 0.2 mmol/g) was packed into a 50 cm^3 disposable syringe fitted with a Teflon filter. The syringe was connected to a suction flask through a Teflon tube with a manual 2-way Teflon valve, and the resin was swelled in DMF, treated with 20% piperidine in DMF for 10 min, and washed 6 times with DMF. The resin was derivatized with the ((hydroxymethyl)phenoxy)acetic acid (HMPA linker). The HMPA linker (455 mg, 2.5 mmol), *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) (750 mg, 2.37 mmol), and 4-ethylmorpholine (315 μL, 2.5 mmol) were dissolved in DMF, and after 10 min the solution was added to the resin and left to react overnight. The resin was washed carefully with DMF and dichloromethane. *N*$^\alpha$-Fmoc-Lys(Boc)-COOH (1.75 mg, 3.75 mmol) and *N*-methylimidazole (231 mg, 223 μL, 2.81 mmol) were dissolved in dichloromethane (20 cm^3), and 1-(mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MSNT) (1.11 g, 3.71 mmol) was added. After 5 min the solution was added to the resin and allowed to react for 3 h. The resin was washed thoroughly with dichloromethane, DMF, and diethyl ether and dried in vacuo.

General Procedure for the SPPS of Peptides. Derivatized PEGA resin (0.2 mmol/g capacity, 0.66 equiv compared to the amount of glycosylated building block employed) was transferred into a syringe equipped with a Teflon filter. Apart from the glycosyl−amino acids all amino acids used were *N*$^\alpha$-Fmoc-amino acid OPfp (pentafluorophenyl) esters. *N*$^\alpha$-Fmoc deprotection was effected by successive 2 and 20 min treatments of the resin with 20% piperidine in DMF. The washing procedure (6 times with DMF) was repeated after each coupling/Fmoc deprotection step. Each *N*$^\alpha$-Fmoc-amino acid OPfp ester (3 equiv) and Dhbt-OH (3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine) (1 equiv) were dissolved in DMF. The solutions were added to the resins and then left for a minimum of 3 h. After each coupling step the resin was washed, the *N*$^\alpha$-Fmoc group removed, and the resin washed as described above. In the case of the glycosyl building blocks **52** and **53** only 1.5 equiv was employed. These were preactivated in DMF by adding 0.95 equiv of TBTU and 1.6 equiv of NEM for 20 min. These solutions were then transferred to the resins and allowed to react for 3 h. In the case of the synthesis using glycosyl−amino acid **53**, the resin was washed and the free amino groups in excess were capped by treatment of the resin with a 20% solution of Ac_2O in DMF for 20 min. The resin

Table 1. ^{1}H NMR Data (δ with Hz in Parentheses) the O-linked Glycopeptides 54−56 Measured in CD_3COOD/D_2O (1:1) at 600 MHz at 300 K

	54	55	56
		Val	
α-H	3.95	3.94	3.95
β-H	2.24	2.23	2.23
γ-H_3	1.01	1.00	1.01
γ-H_3	1.00	0.99	1.00
		Ile	
NH	8.38 (8.0)	8.38 (8.0)	8.38 (8.0)
α-H	4.34	4.32	4.40
β-H	1.84	1.84	1.85
γ-H_2	1.51/1.16	1.51/1.17	1.53/1.18
γ-H_3	0.90	0.87	0.88
δ-H_3	0.85	0.84	0.84
		Thr	
NH	8.12 (8.0)	8.11 (7.5)	8.27 (8.5)
α-H	4.40	4.40	4.30
β-H	4.22	4.21	4.30
γ-H_3	1.15	1.13	1.16
		Ala	
NH	8.00 (6.5)	8.00 (6.0)	8.12 (5.5)
α-H	4.32	4.31	4.30
β-H_3	1.28	1.22	1.24
		Phe	
NH	7.84 (7.0)	7.89 (7.0)	7.89 (7.0)
α-H	4.59	4.57	4.58
β-H_2	3.02/2.94	3.00/2.92	3.03/2.87
Phenyl	7.12 ortho	7.11 ortho	7.12 ortho
7.27 meta	7.26 meta	7.28 meta	
7.21 para	7.22 para	7.24 para	
		Phe (Conjugate)	
NH	7.97 (8.0)	8.02 (8.0)	8.06 (7.0)
α-H	4.61	4.66	4.63
β-Ha	3.12	3.22	3.10
β-Hb	3.04	3.09	3.07
		Glu	
NH	8.01 (7.5)	8.04 (7.5)	8.25 (7.5)
α-H	4.34	4.34	4.37
β-H_2	2.11/1.95	2.10/1.96	1.93/1.78
γ-H_2	2.41	2.39	2.03
		Gly	
NH	7.92 (6.0)	8.07 (6.0)	8.23 (6.0)
α-H_2	3.96	3.94	3.95
		Leu	
NH	7.84 (7.5)	7.85 (7.0)	7.90 (7.0)
α-H	4.43	4.41	4.42
β-H_2	1.61	1.60	1.60
γ-H	1.61	1.60	1.60
δ-H_3	0.90/0.85	0.90/0.85	0.91/0.85
		Lys	
NH	8.11 (8.0)	8.12 (7.5)	8.09 (8.0)
α-H	4.43	4.42	4.40
β-H	1.92/1.77	1.90/1.75	1.89/1.75
γ-H	1.45	1.43	1.43
δ-H	1.69	1.68	1.68
ϵ-H	3.02	3.02	3.01
NH_2	7.46	7.46	7.45
		Gal−Acetylene	
2-Ha		3.51 (1.0)	3.49 (2.0)
2-Hb		3.28 (17.0, 9.0)	3.31 (17.0, 9.0)
3-H	4.24 (9.5)	3.86	3.88
4-H	3.84 (9.5)	3.63	3.64
5-H	3.68 (3.5)	3.66	3.68 (3.0)
6-H	4.04	3.99	4.00
7-H	3.71	3.66	3.68
8-Ha	3.81 (7.5)	3.69	3.70
8-Hb	3.76 (12.0, 4.5)	3.64	3.61
2-H′, H-6′	7.43	7.95	7.97
3-H′, H-5′	7.21	7.38	7.36

was washed as usual, and the peptide synthesis was continued using OPfp esters. After cleavage of the final N^{α}-Fmoc group the resin was washed with DMF, CH_2Cl_2, and ether and dried. Cleavage of the glycopeptides was achieved by treatment with 95% TFA for 2 h. The resin was filtered off and washed 4 times with 95% TFA. The solution was concentrated and the glycopeptides precipitated by addition of ether. The crude glycopeptides were dissolved in water−TFA and analyzed by RP-HPLC using a linear gradient from 0 to 100% B over 50 min. Purification by semipreparative RP-HPLC using a linear gradient from 3 to 50% B over 70 min gave the pure glycopeptides. Using the building block **52** (L-epimer) the pure glycopeptide **54** (45.6 mg, 57%) (ES-MS: $(M + H)^+$, *m*/*z* 1310.9; $C_{63}H_{97}N_{11}O_{20}$ requires M, 1310.51) was obtained. Synthesis with the DL-mixture of **52** obtained from **49** gave the mixture of the two epimers of **54** in impure form after preparative RP-HPLC.

The building block **53** gave the two epimeric peptides **55** (29.6 mg, 34%) (ES-MS: $(M + H)^+$, *m*/*z* 1329.0; $C_{63}H_{97}N_{11}O_{20}$ requires M, 1328.52) and **56** (28.7 mg, 33%) (ES-MS: $(M + H)^+$, *m*/*z* 1328.9, $C_{63}H_{97}N_{11}O_{20}$ requires M, 1328.52), which could be easily separated by RP-HPLC. The capping of free amino functions had avoided the formation of the shorted sequence VITAFEGLK. The ^{1}H NMR spectra of **54**−**56** were all assigned by 2D-NMR spectroscopy, and the data are presented in Table 1.

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Supporting Information Available: Tables S1−S12 of the fully assigned ^{1}H and ^{13}C NMR data for compounds **4**, **5**, **8**, **13**, **16**−**19**, and **33**−**53** (12 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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