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Synthesis of a Galactosylated 4-Hydroxylysine Building Block and Its Incorporation into a Collagen Immunodominant Glycopeptide

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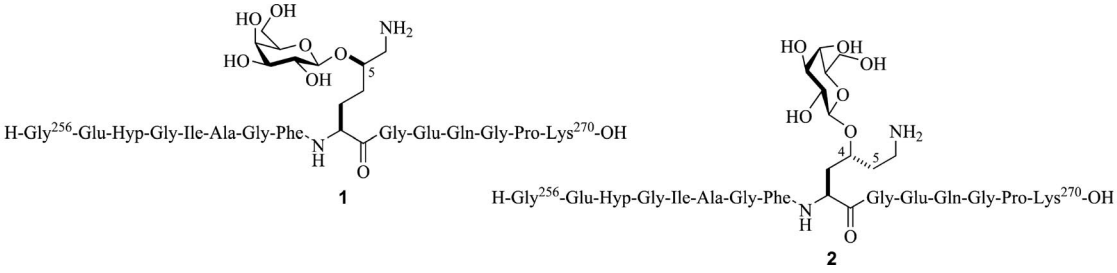
An analogue of the immunodominant glycopeptide from type II collagen encompassing residues 256–270 has been prepared by substituting β-D-galactopyranosyl-(2*S*,4*R*)-4-hydroxy-L-lysyl (Gal-4-Hyl) for β-D-galactopyranosyl-(2*S*,5*R*)-5-hydroxy-L-lysyl (Gal-5-Hyl) at position 264. The synthesis of the 4-hydroxylysine aglycon started from the known (2*S*,4*S*)-4-hydroxy-6-oxo-1,2-piperidinedicarboxylate (**3**) and involved selective ring-opening of **3**, lactone formation, and *N*-acylation of the lactone with glycyl esters. The resulting *N*-Fmoc-protected 4-hydroxylysyl-glycine dipeptide derivative was galactosylated in high yield to give a building block suitable for solid-phase peptide synthesis.

Introduction

In collagen-induced arthritis (CIA), a mouse model for rheumatoid arthritis (RA), autoreactive T cells of transgenic mice expressing MHC class II A^q molecule (or human DR4 and DR1 haplotypes) recognize a peptide encompassing residues 256–270 when it is post-translationally modified at position 264 (hydroxylation and glycosylation of the Lys side-chain).[1,2] The resulting β-D-galactopyranosyl-(2*S*,5*R*)-5-hydroxy-L-lysyl (Gal-5-Hyl) side-chain at position 264 serves as a critical T-cell receptor (TCR) contact point. The development of efficient routes to Gal-5-Hyl building blocks and synthetic CII-derived glycopeptides[3–5] has been instrumental in studies aimed at understanding the role of lysine hydroxylation and glycosylation in CIA. Interestingly, synthetic CII peptides with Gal-5-Hyl at position 264 {[Gal-5-Hyl264]CII(256–270) (**1**)} have been found to exert a protective effect against the development of CIA in neonatal treatment experiments.[6] Furthermore, [Gal-5-Hyl264]CII(259–273) complexed to A^q molecules was reported to prevent the development of CIA in mice while the nonglycosylated analogue/A^q complex did not.[7] Taken together, these findings are relevant to the development of new treatment against autoimmune arthritis based on CII glycopeptides. In particular, CII glycopeptide mimetics may prove useful as altered peptide ligands (APL)[8] to interfere with the T-cell response in CIA and RA.

We recently described the design and synthesis of several analogues of [Gal-5-Hyl264]CII(256–270) carrying diverse modifications at the critical hydroxylysine side-chain and their use as probes to explore the fine specificity of CII-reactive T cells involved in the initiation and/or regulation of CIA.[9] To further investigate the basis of epitope recognition by CII-reactive T cells, we became interested in synthesizing and evaluating the [Gal-5-Hyl264]CII(256–270) variant **2** obtained by substituting a galactosylated 4-hydroxylysyl residue for the galactosylated 5-hydroxylysyl residue at position 264. We have shown previously that



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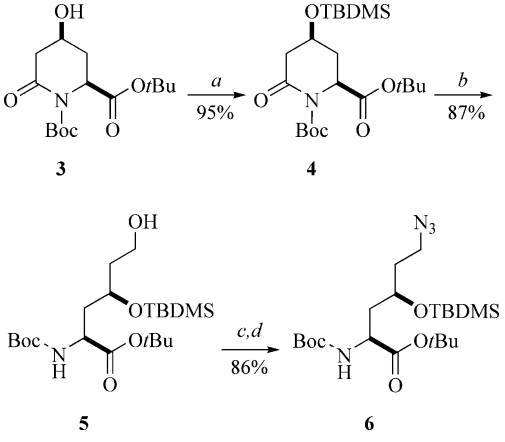
enantiopure 5-hydroxy- and 4-hydroxy-6-oxo-1,2-piperidinedicarboxylates are versatile building blocks for the synthesis of 5- and 4-hydroxylysine derivatives, respectively.[5,10] In continuation of this work, we now report the preparation of a *N*-Fmoc-protected galactosylated (2*S*,4*R*)-4-hydroxylysine derivative and its incorporation at position 264 of the CII(256–270) peptide.

Results and Discussion

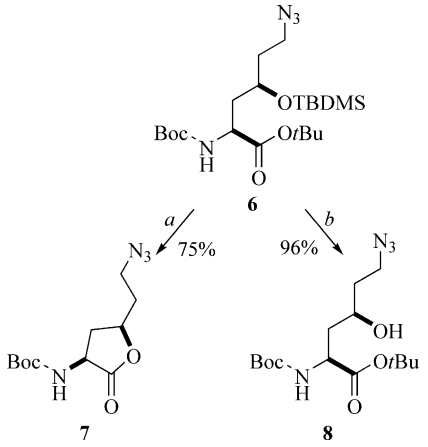
Our general approach to 4-hydroxylysine derivatives suitably protected for subsequent glycosylation and solid-phase synthesis using Fmoc chemistry started with di-*tert*-butyl (2*S*,4*S*)-4-hydroxy-6-oxo-1,2-piperidinedicarboxylate (**3**) (Scheme 1).



Scheme 1. Reagents and conditions: (a) TBDMSCl, imidazole, CH_2Cl_2; (b) $NaBH_4$, EtOH; (c) MsCl, DIPEA, CH_2Cl_2; (d) NaN_3, DMF.

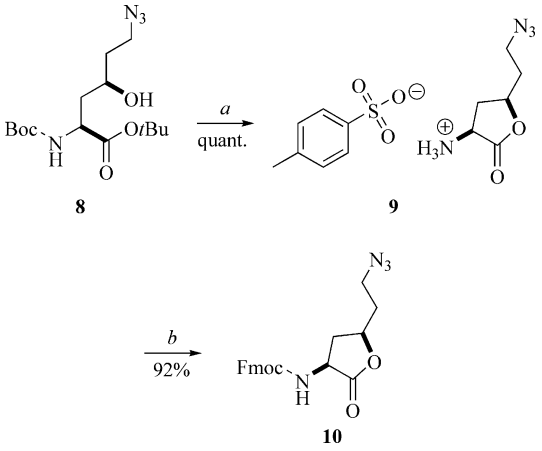
Diastereomerically pure **3** was prepared in three steps from Boc-Asp-O*t*Bu in an overall yield of 63%, as described previously.[10] Protection of the secondary alcohol prior to ring-opening with $NaBH_4$ in EtOH was mandatory for a successful and high-yielding conversion into the corresponding 1,3-diol. This is in contrast to (2*S*,5*S*)-5-hydroxy-6-oxo-1,2-piperidinedicarboxylate (5-hydroxylysine precursor) which was readily and selectively reduced to the corresponding 1,2-diol under the same conditions.[5] The primary alcohol **5** was then converted into azide **6** in 86% yield. The azide moiety, which can be selectively reduced to the corresponding primary amine on a solid-phase support prior to cleavage of the peptide from the resin, is used as a permanent protection for the ε-amino group. Our first attempt to remove the TBDMS protection by treatment with TBAF[11] resulted in spontaneous and quantitative formation of γ-lactone **7** (Scheme 2).

This side-reaction was completely reversed to the benefit of the desired 4-hydroxylysine derivative **8** by simply performing the reaction in a mixture of acetic acid and THF as solvent.[12] However, attempts to selectively remove the *N*-Boc protecting group of **8** in the presence of the *tert*-butyl ester under conditions previously developed for the



Scheme 2. Reagents and conditions: (a) TBAF, THF; (b) TBAF, AcOH, THF.

preparation of 5-hydroxylysine derivatives [i.e., *p*-toluenesulfonic acid (PTSA) in acetonitrile] resulted in quantitative lactonization to give PTSA salt **9** (Scheme 3).

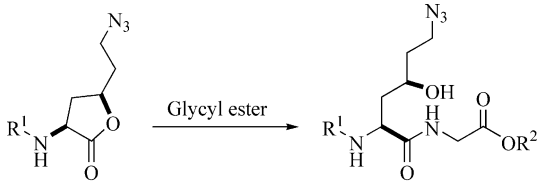


Scheme 3. Reagents and conditions: (a) PTSA, CH_3CN; (b) FmocOSu, K_2CO_3, acetone/H_2O.

Similarly, treatment of the *O*-protected derivative **6** under the same conditions also gave **9** quantitatively. The *N*-Fmoc-protected lactone **10** was easily obtained in 92% yield by reaction of **9** with FmocOSu in the presence of aqueous K_2CO_3 as base. We then envisioned that *N*-protected γ-lactones **7** and **10**, subject to selective ring-opening with a glycyl ester derivative {Gly is the amino acid residue immediately following Gal-5-Hyl in the sequence of the [Gal-5-Hyl264]CII(256–270) glycopeptide}, could be of practical value to generate 4-hydroxylysylglycyl dipeptide aglycons.[13] The *N*-acylation of various glycyl esters by lactones **7** and **10** has been examined and the results are reported in Table 1.

The Boc-protected γ-lactone **7** reacted readily with H-Gly-O*t*Bu and H-Gly-OAll in Et_2O to give the corresponding *N*-Boc-protected (2*S*,4*R*)-4-hydroxylysylglycyl esters **11** and **12** in 65 and 63% yields, respectively.[13,14] Alternatively, THF but not MeCN could be employed. Ring-opening was accompanied by some epimerization (18–25%

Table 1. *N*-Acylation of glycyl esters by lactones **7** and **10**.

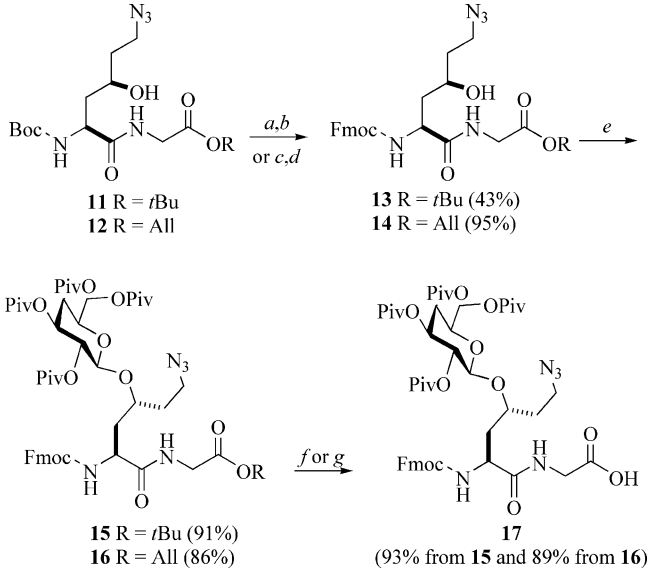


7 or **10** (2*S*,4*R*)-**11**-**13**

Entry	Lactone	R^1	Reagents	R^2	Solvent	Time [d]	Product	Epimer ratio[a]	% Yield[b]
1	**7**	Boc	HCl·H-Gly-O*t*Bu, Et_3N	*t*Bu	THF	3	**11**	70:30	34 (43)[c,d]
2	**7**	Boc	H-Gly-O*t*Bu	*t*Bu	THF	7	**11**	75:25	63
3	**7**	Boc	H-Gly-O*t*Bu	*t*Bu	Et_2O	7	**11**	78:22	65
4	**7**	Boc	H-Gly-O*t*Bu	*t*Bu	CH_3CN	7	**11**	–	0 (100)[c]
5	**7**	Boc	H-Gly-OAll	All	Et_2O	7	**12**	80:20	63
6	**7**	Boc	H-Gly-OAll	All	THF	7	**12**	82:18	48
7	**10**	Fmoc	H-Gly-O*t*Bu	*t*Bu	THF	4	**13**	–[e]	23
8	**10**	Fmoc	H-Gly-O*t*Bu, $AlMe_3$	*t*Bu	THF	2	**13**	–	0[f]

[a] Epimer ratio determined by analytical C_{18} RP-HPLC of the crude product. [b] Refers to the major (2*S*,4*R*) diastereomer after purification. [c] Starting material recovered is given as a percentage in parentheses. [d] The minor isomer was isolated in 12% yield. [e] Only one product was detected and characterized. [f] The starting material decomposed.

based on HPLC analysis of the crude product), probably at the α-carbon of the 4-Hyl residue. The epimers were easily separated from (2*S*,4*R*)-**11** and (2*S*,4*R*)-**12** by flash column chromatography on silica gel. The *N*-Fmoc-protected lactone **10** was a poorer substrate and the linear dipeptide **13** was only recovered in 23% yield. An attempt to increase the yield by conducting the reaction with $AlMe_3$ was not successful. Selective removal of the Boc protecting group of **11** and **12** followed by Fmoc reprotection gave protected dipeptide aglycons **13** and **14** in 43 and 95% yields, respectively (Scheme 4).



11 R = *t*Bu
12 R = All
13 R = *t*Bu (43%)
14 R = All (95%)
15 R = *t*Bu (91%)
16 R = All (86%)
17 (93% from **15** and 89% from **16**)

Scheme 4. Reagents and conditions: (a) PTSA, CH_3CN; (b) FmocOSu, K_2CO_3, acetone/H_2O; (c) TFA, CH_2Cl_2; (d) FmocOSu, $NaHCO_3$, THF/H_2O; (e) β-D-Gal(Piv)$_4$Br, silver silicate, 4-Å mol. sieves, CH_2Cl_2; (f) TFA, CH_2Cl_2; (g) $Pd(PPh_3)_4$, morpholine, THF.

Glycosylation of **13** and **14** with tetrapivaloylated galactosyl bromide under conditions optimized previously for the galactosylation of 5-Hyl mimetics afforded **15** and **16** in 91 and 86% yields, respectively. Removal of the *tert*-butyl ester in **15** gave galactosylated *N*-Fmoc-protected 4-hydroxylysylglycine dipeptide **17** in 93% yield. Similarly, deallylation of **16** by treatment with $Pd(PPh_3)_4$ gave **17** in 89% yield. With an overall yield of 31%, the route involving ring-opening of lactone **7** with H-Gly-OAll is clearly more efficient.[15] The *N*-Fmoc-protected Gal-4-Hyl-Gly building block **17** was next used for the solid-phase synthesis of glycopeptide **2**. Peptide assembly was performed on a polystyrene Wang resin[16] (20 mmol scale) by using a home-made peptide synthesizer.[17] Glycosylated dipeptide **17** was coupled with BOP in the presence of HOBt and DIEA. After elongation of the peptide, the azido function was reduced on the resin by treatment with triphenylphosphane (PPh_3) in THF/H_2O for 72 h. Cleavage from the resin was performed with TFA containing water and scavengers. At this stage of the synthesis, the purity of the precursor of **2** bearing a protected galactosyl moiety (**18**, see formula in Supporting Information) was 92.7% based on analytical C_{18} RP-HPLC (see the electronic supporting information). An additional step was necessary for the removal of the pivaloyl ester protecting groups. To avoid epimerization of the α-stereogenic centers of the amino acids along the peptide chain, a diluted solution of NaOMe in MeOH (40 mM) was used. Monitoring the reaction by C_{18} RP-HPLC indicated complete cleavage of all four pivaloyl groups after 16 h without significant formation of degradation byproducts. The purity of crude glycopeptide **2** was 75%. Finally, CII-derived glycopeptide **2** was purified by RP-HPLC and recovered in 63% yield based on the resin loading. The homogeneity of glycopeptide **2** was assessed by C_{18} RP-HPLC (>99%) and its structure was confirmed by electrospray-

Table 2. ^{1}H NMR chemical shifts of glycopeptide **2** in [D$_6$]DMSO after RP-HPLC purification.[a,b]

Residue	δ [ppm]				
	NH	$^{\alpha}$CH	$^{\beta}$CH	$^{\gamma}$CH	Others
Gly 256	n.d.	3.57, 3.51	–	–	–
Glu 257	8.52	4.64	1.92, 1.69	2.31	–
Hyp 258		4.36	2.02, 1.88	4.36	$^{\delta}$CH$_2$ 3.72, 3.55
Gly 259	8.25	3.77, 3.66	–	–	–
Ile 260	7.65 (7.84)	4.19 (4.19)	1.72 (1.69)	1.07, 1.38	$^{\delta}$CH$_3$ 0.77; $^{\gamma}$CH$_3$ 0.79
Ala 261	8.12 (8.22)	4.25 (4.26)	1.21 (1.21)	–	–
Gly 262	8.00	3.73	–	–	–
Phe 263	8.13 (8.07)	4.45 (4.49)	2.98, 2.87 (3.06, 2.92)	–	arom. 7.27, 6.75
4-Hyl 264	8.39	4.31	2.01	3.35	$^{\varepsilon}$NH 6.95; $^{\varepsilon}$CH$_2$ 2.95; $^{\delta}$CH$_2$ 1.75, 1.66
Gly 265	7.96	3.69	–	–	–
Glu 266	7.86 (7.88)	4.27 (4.27)	1.91, 1.80 (1.92, 1.82)	2.26 (2.24)	–
Gln 267	8.13	4.30	1.92, 1.75	2.14	–
Gly268	7.90 (7.96)	3.91, 3.61 (4.00, 3.82)	–	–	–
Pro 269		4.37 (4.46)	2.02, 1.88 (2.20, 1.96)	1.88 (1.78)	$^{\delta}$CH$_2$ 3.50, 3.55 (3.40, 3.47)
Lys 270	7.97 (8.17)	4.11 (4.17)	1.72, 1.58 (1.75, 1.62)	1.35	$^{\varepsilon}$NH n.d.; $^{\varepsilon}$CH$_2$ 2.78; $^{\delta}$CH$_2$ 1.53

[a] Signals for a minor isomer are indicated in parentheses. [b] Chemical shifts for the galactosyl moiety at position 4-Hyl 264 in **2** are: δ = 3.99 (1-H), 3.62 (3-H), 3.60, 3.56, 3.38, (6-H, 5-H, 4-H), 3.29 (2-H) ppm.

ionization time-of-flight (ESI-TOF) mass spectrometry (see the electronic supporting information) and ^{1}H NMR analysis (see Table 2).

In previous work we identified CII-reactive T cells [three hybridomas (A8E2, A2G10, and A9E5) and a recurrent pathogenic CD4$^+$ T cell clone (A9.2)] in DBA/1 (H-2q) mice immunized with bovine CII (bCII) that participate in CIA course and regulation.[18,19] We also showed previously that the hybridomas and the T-cell clone expressed closely related T-cell receptors (TCR) and recognized glycosylated peptides comprising the immunodominant bCII (256–270) epitope. The capacity of these cells to recognize glycopeptide **2** containing 4-Hyl instead of 5-Hyl substitution at position 264 will be reported in a forthcoming study.

Experimental Section

General Methods: Amino acid derivatives were purchased from NeoMPS (Strasbourg, France). THF was distilled from Na/benzophenone. CH_2Cl_2 and cyclohexane were distilled from CaH_2. Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} (Merck) with detection by UV light and charring with 1% (w/w) ninhydrin in ethanol followed by heating. Flash column chromatography was carried out on silica gel (0.063–0.200 nm). HPLC analysis was performed on a Nucleosil C_{18} column (5 μm, 3.9 × 150 mm) by using a linear gradient of A (0.1% TFA in H_2O) and B (0.08% TFA in CH_3CN) at a flow rate of 1.2 mL min^{-1} with UV detection at 214 nm. Optical rotations were recorded with a Perkin–Elmer polarimeter. ^{1}H and ^{13}C NMR spectra were recorded by using Bruker Advance DPX-300 and DPX-400 instruments. Mass spectra were recorded by using an ESI-TOF spectrometer (Bruker microTOF). Tetrapivaloylated galactosyl bromide was prepared by a two-step procedure starting from commercial D-galactose.[20] Silver silicate was prepared according to the reported procedure[21] and dried at 110 °C under high-vacuum for 6 h before use.

Di-*tert*-butyl (2*S*,4*S*)-4-{[*tert*-Butyl(dimethyl)silyl]oxy}-6-oxo-1,2-piperidinedicarboxylate (4): Imidazole (430 mg, 6.32 mmol) and TBDMSCl (476 mg, 3.16 mmol) were added to a solution of **3** (500 mg, 1.58 mmol) in CH_2Cl_2 (5.0 mL) at 0 °C. The mixture was allowed to reach room temperature and stirred overnight. After evaporation of the solvent, the residue was dissolved in AcOEt. The solution was washed with 1 N $KHSO_4$, brine, and water, dried with Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by filtration through a plug of silica gel (AcOEt/Hex, 2:8) to yield **4** (650 mg, 95%). HPLC: t_R = 18.16 min (linear gradient, 30–100% B, 20 min); colorless oil. $[\alpha]_D$ = –29.4 (c = 1.1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.48 (dd, J = 7.6, 6.7 Hz, 1 H, NCH), 4.12–4.03 (m, 1 H, CHO), 2.74 (ddd, J = 16.6, 5.3, 1.8 Hz, 1 H, CH_2CO), 2.49 (dd, J = 16.6, 8.5 Hz, 1 H, CH_2CO), 2.37–2.28 (m, 1 H, NCHCH_2), 2.08–1.96 (m, 1 H, NCHCH_2), 1.50 [s, 9 H, C(CH$_3$)$_3$], 1.46 [s, 9 H, C(CH$_3$)$_3$], 0.88 [s, 9 H, SiC(CH$_3$)$_3$], 0.07 (s, 3 H, SiCH$_3$), 0.05 (s, 3 H, SiCH$_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.0 (C), 168.9 (C), 152.0 (C), 83.5 (C), 82.2 (C), 64.4 (CH), 56.6 (CH), 44.4 (CH_2), 34.9 (CH_2), 27.9 (3 CH_3), 27.9 (3 CH_3), 25.8 (3 CH_3), 18.1 (C), –4.7 (CH_3), –4.8 (CH_3) ppm. $C_{21}H_{39}NO_6Si$ (429.25): calcd. C 58.71, H 9.15, N 3.26; found C 59.01, H 9.14, N 3.46.

***tert*-Butyl (2*S*,4*S*)-2-[(*tert*-Butoxycarbonyl)amino]-4-{[*tert*-butyl(dimethyl)silyl]oxy}-6-hydroxyhexanoate (5):** Compound **4** (500 mg, 1.16 mmol) was dissolved in EtOH (10.0 mL) and cooled to 0 °C. After portionwise addition of $NaBH_4$ (220 mg, 5.82 mmol), the mixture was allowed to reach room temperature and stirred overnight. After being quenched by water, the mixture was stirred for a further 10 min. Evaporation of the solvent gave an oil which was dissolved in AcOEt. The solution was washed with water and dried with Na_2SO_4. Evaporation of the filtrate afforded an oil which was filtered through a silica pad (AcOEt/Hex, 1:1). Pure **5** was recovered (440 mg, 87%). HPLC: t_R = 16.12 min (linear gradient, 30–100% B, 20 min); colorless oil. $[\alpha]_D$ = –2.9 (c = 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.26 (br. d, J = 7.3 Hz, 1 H, NH), 4.16–4.08 (m, 1 H, CHO), 4.08–4.00 (m, 1 H, NHCH), 3.82–3.68 (m, 2 H, CH_2OH), 2.22 (br. s, 1 H, OH), 1.98–1.63 [m, 4 H, CH_2CH(OH)CH_2] 1.45 [s, 9 H, C(CH$_3$)$_3$], 1.42 [s, 9 H, C(CH$_3$)$_3$], 0.90 [s, 9 H, SiC(CH$_3$)$_3$], 0.09 (s, 6 H, SiCH$_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.0 (C), 155.3 (C), 81.6 (C), 79.5 (C), 68.5 (CH), 59.5 (CH_2), 52.0 (CH), 38.8 (CH_2), 38.4 (CH_2), 28.3 (3 CH_3), 27.9 (3 CH_3), 25.8 (3 CH_3), 17.9 (C), –4.5 (CH_3), –4.8 (CH_3) ppm. $C_{21}H_{43}NO_6Si$ (433.29): calcd. C 58.16, H 9.99, N 3.23; found C 57.89, H 10.35, N 3.23.

***tert*-Butyl (2*S*,4*R*)-6-Azido-2-[(*tert*-butoxycarbonyl)amino]-4-{[*tert*-butyl(dimethyl)silyl]oxy}hexanoate (6):** *N*,*N*-Diisopropylethylamine

(DIPEA) (194 μL, 1.14 mmol) was added to a solution of **5** (330 mg, 0.76 mmol) in CH_2Cl_2 (5.0 mL) at ambient temperature. The solution was cooled to 0 °C and MsCl (88 μL, 1.14 mmol) was added through a hypodermic syringe. The mixture was allowed to reach room temperature and stirred for 3 h. The reaction was quenched by water, CH_2Cl_2 was evaporated and replaced by AcOEt. The organic phase was washed with 1 N $KHSO_4$, brine, saturated $NaHCO_3$, and water, dried with Na_2SO_4, filtered, and concentrated in vacuo. The residue was dissolved in DMF (10.0 mL). NaN_3 (148 mg, 2.28 mmol) was added to the solution which was heated at 80 °C for 8 h. After the solution had cooled to room temperature, water was added, and the solution was extracted twice with AcOEt. The combined organic layers were washed with water, dried with Na_2SO_4, filtered, and the solvents evaporated in vacuo. The crude product was purified by flash column chromatography (AcOEt/Hex, 1:9) to yield pure **6** (298 mg, 86% for two steps). HPLC: t_R 16.80 (linear gradient, 50–100% B, 20 min); colorless oil. $[a]_D$ = +1.0 (*c* = 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 5.22 (br. d, *J* = 7.1 Hz, 1 H, NH), 4.18–4.12 (m, 1 H, NHC*H*), 3.97–3.89 (m, 1 H, CHO), 3.36 (m, 2 H, CH_2N_3), 1.94–1.83 (m, 1 H, NHCHCH_2), 1.81–1.68 (m, 3 H, NHCHCH_2, CH_2CH$_2$N$_3$); 1.46 [s, 9 H, C(CH$_3$)$_3$], 1.43 [s, 9 H, C(CH$_3$)$_3$], 0.90 [s, 9 H, SiC(CH$_3$)$_3$], 0.09 (s, 3 H, SiCH$_3$), 0.07 (s, 3 H, SiCH$_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.8 (C), 155.3 (C), 81.7 (C), 79.5 (C), 67.0 (CH), 51.9 (CH), 47.5 (CH_2), 39.1 (CH_2), 35.8 (CH_2), 28.3 (3 CH_3), 27.9 (3 CH_3), 25.8 (3 CH_3), 17.9 (C), –4.5 (CH_3), –4.8 (CH_3) ppm. $C_{21}H_{42}N_4O_5Si$ (458.29): calcd. C 54.99, H 9.23, N 12.22; found C 55.23, H 9.30, N 12.44.

***tert*-Butyl *N*-[(3*S*,5*R*)-5-(Azidoethyl)-2-oxotetrahydro-3-furanyl]-carbamate (7):** Tetrabutylammonium fluoride (543 mg, 2.08 mmol) was added to a solution of **6** (250 mg, 0.429 mmol) in THF (20.0 mL) at 0 °C. The mixture was allowed to reach room temp. and stirred for 2 h. The reaction was quenched by adding a saturated solution of NH_4Cl. THF was evaporated and replaced by AcOEt. The organic layer was washed with 1 N $KHSO_4$, brine, and saturated $NaHCO_3$, dried with Na_2SO_4, filtered, and concentrated in vacuo. Crude **7** was filtered through a short plug of silica gel (AcOEt/Hex, 1:1) to afford pure **7** (281 mg, 75%). HPLC: t_R = 7.56 min (linear gradient, 30–100% B, 20 min); colorless oil. $[a]_D$ = +57.9 (*c* = 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 5.12 (br. s, 1 H, NH), 4.56–4.46 (m, 1 H, CHO), 4.42–4.35 (m, 1 H, NHC*H*), 3.53–3.48 (m, 2 H, CH_2N_3), 2.89–2.79 (m, 1 H, NHCHCH_2), 2.02–1.81 (m, 3 H, NHCHCH_2, CH_2CH$_2$N$_3$), 1.44 [s, 9 H, C(CH$_3$)$_3$] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.3 (C), 155.3 (C), 80.6 (C), 74.8 (CH), 51.3 (CH), 47.4 (CH_2), 36.5 (CH_2), 34.6 (CH_2), 28.2 (3 CH_3) ppm. $C_{11}H_{18}N_4O_4$ (270.13): calcd. C 48.88, H 6.71, N 20.73; found C 49.15, H 6.72, N 20.66.

***tert*-Butyl (2*S*,4*R*)-6-Azido-2-[(*tert*-butoxycarbonyl)amino]-4-hydroxyhexanoate (8):** Acetic acid (58 μL, 1.00 mmol) was added to a solution of **6** (92 mg, 0.200 mmol) in THF (2.0 mL) at 0 °C. TBAF (262 mg, 1.00 mmol) was added and the solution was stirred for 96 h at room temp. The reaction was quenched by adding a saturated solution of NH_4Cl. THF was evaporated and replaced by AcOEt. The organic layer was washed with 1 N $KHSO_4$, brine, and saturated $NaHCO_3$, dried with Na_2SO_4, filtered, and concentrated in vacuo. Purification of the crude product by flash column chromatography (AcOEt/Hex, 2:8) gave pure **8** (66 mg, 96%). HPLC: t_R = 10.93 min (linear gradient, 30–100% B, 20 min); colorless oil. $[a]_D$ = +15.1 (*c* = 1.1, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 5.39 (br. d, *J* = 7.7 Hz, 1 H, NH), 4.41–4.33 (m, 2 H, NHC*H*, O*H*), 3.70 (br. t, *J* = 9.7 Hz, 1 H, C*H*OH), 3.50–3.37 (m, 2 H, CH_2N_3), 1.90–1.80 (m, 1 H, NHCHCH_2), 1.76–1.57 (m, 3 H, CH$_2$CH$_2$N$_3$), 1.44 [s, 9 H, C(CH$_3$)$_3$], 1.43 [s, 9 H, C(CH$_3$)$_3$] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.7 (C), 157.0 (C), 82.5 (C), 80.6 (C), 64.1 (CH), 51.0 (CH), 48.3 (CH_2), 42.2 (CH_2), 35.6 (CH_2), 28.2 (3 CH_3), 27.9 (3 CH_3) ppm. $C_{15}H_{28}N_4O_5$ (344.21): calcd. C 52.31, H 8.19, N 16.27; found C 52.59, H 8.14, N 16.28.

(3*S*,5*R*)-5-(Azidoethyl)-2-oxotetrahydro-3-furanaminium 4-Methyl-1-benzenesulfonate (9): *p*-Toluenesulfonic acid (55 mg, 0.289 mmol) was added to a solution of **8** (50 mg, 0.145 mmol) in CH_3CN (2.0 mL) at 0 °C. The mixture was stirred for 6 h (until complete consumption of **8**). A large volume of cold Et_2O was added and the suspension was filtered. The precipitate was washed with CH_2Cl_2/Et_2O and dried under high vacuum to afford pure **9** (50 mg, 100%): TLC: R_f = 0.45 (AcOEt/pyridine/acetic acid/water, 8:2:0.5:1); white solid. $[a]_D$ = +25.4 (*c* = 1.0, MeOH); m.p. 234–240 °C (decomposition). ^1H NMR (300 MHz, CD_3OD): δ = 7.70 (d, *J* = 8.0 Hz, 2 H, arom. H), 7.23 (d, *J* = 7.9 Hz, 2 H, arom. H), 4.72–4.63 (m, 1 H, CHO), 4.40 (dd, *J* = 8.7, 12.0 Hz, 1 H, C*H*CO), 3.55–3.45 (m, 1 H, CH_2N_3), 2.87–2.78 (m, 1 H, CHCH_2CH), 2.37 (s, 3 H, CH_3Ph), 2.03–1.91 (m, 3 H, CHCH_2CH, CH_2CH$_2$N$_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.0 (C), 138.6 (C), 126.8 (2 CH), 124.0 (2 CH), 74.4 (CH), 47.6 (CH), 32.2 (CH_2), 31.4 (CH_2), 18.3 (CH_3) ppm. $C_{13}H_{18}N_4O_5S$ (342.10): calcd. C 45.61, H 5.30, N 16.36; found C 45.76, H 5.31, N 16.19.

[(9*H*-Fluoren-9-yl)methyl] *N*-[(3*S*,5*R*)-5-(Azidoethyl)-2-oxotetrahydro-3-furanyl]carbamate (10): Compound **9** (75 mg, 0.219 mmol) was dissolved in H_2O (1.0 mL). Solid K_2CO_3 (90 mg, 0.651 mmol) and FmocOSu (110 mg, 0.326 mmol) dissolved in acetone (1.0 mL) were added to the solution stirred at ambient temperature. After 3 h, acetone was evaporated and replaced by AcOEt. The solution was washed with 1 N $KHSO_4$, dried with Na_2SO_4, and concentrated in vacuo. Purification of the crude product by flash column chromatography (AcOEt/Hex, 1:1) gave pure **10** (79 mg, 92%). HPLC: t_R = 12.47 min (linear gradient, 30–100% B, 20 min); white solid. $[a]_D$ = +48.0 (*c* = 1.0, $CHCl_3$); m.p. 132–134 °C. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.76 (d, *J* = 7.4 Hz, 2 H, arom. H), 7.58 (d, *J* = 7.4 Hz, 2 H, arom. H), 7.40 (t, *J* = 7.4 Hz, 2 H, arom. H), 7.31 (t, *J* = 7.4 Hz, 2 H, arom. H), 5.48 (br. d, *J* = 4.8 Hz, 1 H, NH), 4.51–4.43 (m, 2 H, NHC*H*, C*H*O), 4.42 (d, *J* = 6.6 Hz, 2 H, CH_2OCO), 4.21 (t, *J* = 6.6 Hz, 1 H, $CHCH_2OCO$), 3.51–3.47 (m, 2 H, CH_2N_3), 2.84 (m, 1 H, NHCHCH_2), 2.04–1.83 (m, 1 H, NHCHCH_2), 1.80–1.59 (m, 2 H, CH_2CH$_2$N$_3$)^2 ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.0 (C), 155.9 (C), 143.6 (2 C), 141.3 (2 C), 127.8 (2 CH), 127.1 (2 CH), 125.0 (2 CH), 120.0 (2 CH), 74.9 (CH), 67.3 (CH_2), 51.6 (CH), 47.4 (CH_2), 47.0 (CH), 36.1 (CH_2), 34.5 (CH_2) ppm. HRMS (ESI): calcd. for $C_{21}H_{21}N_4O_4$ $[M + H]^+$ 393.1557; found: 393.1549.

***tert*-Butyl 2-({(2*S*,4*R*)-6-Azido-2-[(*tert*-butoxycarbonyl)amino]-4-hydroxyhexanoyl}amino)acetate [(2*S*,4*R*)-11]:** Lactone **7** (245 mg, 0.906 mmol) was placed in a 50 mL round-bottomed flask and dissolved in Et_2O (1.0 mL). HCl·H-Gly-O*t*Bu (761 mg, 4.54 mmol) was washed with a 1 N NH_4OH solution (50.0 mL) and extracted twice with CH_2Cl_2 (25.0 mL). The resulting residue was dissolved in Et_2O (3.0 mL) and added to the previous solution through a hypodermic syringe. The solution was stirred at room temperature and the reaction was monitored by TLC (AcOEt/Hex, 1:1). After 1 week, **7** had completely disappeared. The remaining solvent was evaporated and replaced by AcOEt. The resulting solution was washed with 1 N $KHSO_4$, brine, and water and the solvents evaporated in vacuo to give crude **11**. The crude product was purified by flash column chromatography (AcOEt/Hex, 1:1) to yield (2*S*,4*R*)-**11** (237 mg, 65%). HPLC: t_R = 8.77 min (linear gradient, 30–100% B, 20 min); colorless oil. $[a]_D$ = +14.5 (*c* = 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 6.88 (m, 1 H, N*H*CH$_2$), 5.70 (d, *J* = 7.9 Hz,

1 H, NHCHCO), 4.46–4.41 (m, 1 H, NHC*H*CO), 3.92 (d, *J* = 5.1 Hz, 2 H, NHCH_2CO), 3.81 (m, 1 H, C*H*OH), 3.51–3.37 (m, 2 H, CH_2N_3), 1.87–1.64 (m, 4 H, NHCHCH_2, CH_2CH$_2$N$_3$), 1.45 [s, 9 H, C(CH_3)$_3$], 1.43 [s, 9 H, C(CH_3)$_3$] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.9 (C), 168.9 (C), 156.7 (C), 82.5 (C), 80.5 (C), 65.1 (CH), 51.5 (CH), 48.4 (CH_2), 42.0 (CH_2), 41.9 (CH_2), 35.9 (CH_2), 28.2 (3 CH_3), 28.0 (3 CH_3) ppm. HRMS (ESI): calcd. for $C_{17}H_{32}N_5O_6$ [M + H]$^+$ 402.2347; found 402.2338.

***tert*-Butyl 2-({(2*R*,4*R*)-6-Azido-2-[(*tert*-butoxycarbonyl)amino]-4-hydroxyhexanoyl}amino)acetate (*epi*-11):** The diastereomer *epi*-**11** was obtained from crude **11** after purification by flash column chromatography (yield: 12%). HPLC: t_R = 8.37 min (linear gradient, 30–100% B, 20 min); colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.99 (m, 1 H, N*H*CH$_2$), 5.44 (d, *J* = 7.7 Hz, 1 H, Boc-N*H*), 4.38 (m, 1 H, NHC*H*CO), 4.01 (dd, *J* = 5.7, 18.1 Hz, 1 H, NHCH_2CO), 3.90–3.87 (m, 1 H, C*H*OH), 3.86 (dd, *J* = 4.9, 18.1 Hz, 1 H, NHCH_2CO), 3.46 (t, *J* = 6.4 Hz, 2 H, CH_2N_3), 1. 92–1.85 (m, 2 H, NHCHCH_2); 1.75–1.67 (m, 2 H, CH_2CH$_2$N$_3$), 1.45 [s, 9 H, C(CH_3)$_3$], 1.43 [s, 9 H, C(CH_3)$_3$] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.4 (C), 168.85 (C), 155.6 (C), 82.5 (C), 80.4 (C), 66.2 (CH), 52.0 (CH), 48.4 (CH_2), 41.9 (CH_2), 39.87 (CH_2), 36.3 (CH_2), 28.3 (3 CH_3), 28.0 (3 CH_3) ppm. HRMS (ESI): calcd. for $C_{17}H_{32}N_5O_6$ [M + H]$^+$ 402.2347; found 402.2347.

Allyl 2-({(2*S*,4*R*)-6-Azido-2-[(*tert*-butoxycarbonyl)amino]-4-hydroxyhexanoyl}amino)acetate [(2*S*,4*R*)-12]: Lactone **7** (187 mg, 0.692 mmol) was placed in a 50 mL round-bottomed flask and dissolved in THF (1.0 mL). TFA·H-Gly-OAll (793 mg, 3.46 mmol) was washed with a 1 N NH_4OH solution (50.0 mL) and extracted twice with CH_2Cl_2 (25.0 mL). After evaporation to dryness, the resulting residue was dissolved in THF (2.0 mL) and added to the previous solution through a hypodermic syringe. The white suspension was stirred at room temperature and the reaction was monitored by TLC (AcOEt/Hex, 1:1). After 1 week, **7** had completely disappeared. THF was evaporated and replaced by AcOEt. The solution was washed with 1 N $KHSO_4$, brine, and water and the solvents evaporated in vacuo. The crude product was purified by flash column chromatography (AcOEt/Hex, 1:1) to yield (2*S*,4*R*)-**12** (128 mg, 48%). HPLC: t_R = 7.57 min (linear gradient, 30–100% B, 20 min); colorless oil. $[\alpha]_D$ = +11.7 (*c* = 1.1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.23 (br. t, *J* = 5.3 Hz, 1 H, N*H*CH$_2$), 5.96–5.83 (m, 1 H, C*H*=CH$_2$), 5.71 (br. d, *J* = 7.7 Hz, 1 H, N*H*CH), 5.36–5.24 (m, 2 H, CH=CH_2), 4.64 (dt, *J* = 5.8, 1.3 Hz, 2 H, CH_2CH=CH$_2$), 4.48–4.43 (m, 1 H, NHC*H*CO), 4.13–3.95 (m, 2 H, NHCH_2CO), 3.80 (m, 1 H, C*H*OH), 3.50–3.35 (m, 2 H, CH_2N_3), 1.88–1.79 (m, 1 H, NHCHCH_2), 1.76–1.64 (m, 3 H, CH_2CH$_2$N$_3$), 1.43 [s, 9 H, C(CH_3)$_3$] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.2 (C), 169.5 (C), 156.8 (C), 131.3 (CH), 119.1 (CH_2), 80.7 (C), 66.2 (CH_2), 65.2 (CH), 51.5 (CH), 48.3 (CH_2), 41.9 (CH_2), 41.2 (CH_2), 35.9 (CH_2), 29.7 (CH_2), 28.2 (3 CH_3) ppm. HRMS (ESI): calcd. for $C_{16}H_{28}N_5O_6$ [M + H]$^+$ 386.2034; found 386.2027.

Allyl 2-({(2*R*,4*R*)-6-Azido-2-[(*tert*-butoxycarbonyl)amino]-4-hydroxyhexanoyl}amino)acetate (*epi*-12): The diastereomer *epi*-**12** was obtained from crude **12** after purification by flash column chromatography. *epi*-**12** could not be isolated in pure form and was contaminated by ca. 15% (2*S*,4*R*)-**12**. HPLC: t_R = 7.11 min (linear gradient, 30–100% B, 20 min); colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.36 (m, 1 H, N*H*CH$_2$), 5.95–5.82 (m, 1 H, C*H*=CH$_2$), 5.58 (m, 1 H, N*H*CH), 5.35–5.22 (m, 2 H, CH=CH_2), 4.62 (dt, *J* = 5.8, 1.4 Hz, 2 H, CH_2CH=CH$_2$), 4.44 (m, 1 H, NHC*H*CO), 4.17–3.93 (m, 2 H, NHCH_2CO), 3.94–3.84 (m, 1 H, C*H*OH), 3.44 (t, *J* = 6.7 Hz, 2 H, CH_2N_3), 1.96–1.78 (m, 2 H, NHCHCH_2), 1.74–1.62 (m, 2 H, CH_2CH$_2$N$_3$), 1.41 [s, 9 H, C(CH_3)$_3$] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.6 (C), 169.5 (C), 155.7 (C), 131.4 (CH), 119.0 (CH_2), 80.6 (C), 66.1 (CH_2), 65.3 (CH), 51.5 (CH), 48.3 (CH_2), 41.9 (CH_2), 41.2 (CH_2), 36.4 (CH_2), 29.7 (CH_2), 28.3 (3 CH_3) ppm. HRMS (ESI): calcd. for $C_{16}H_{28}N_5O_6$ [M + H]$^+$ 386.2034; found 386.2034.

***tert*-Butyl 2-{[(2*S*,4*R*)-6-Azido-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-4-hydroxyhexanoyl]amino}acetate (13):** *p*-Toluenesulfonic acid (95 mg, 0.499 mmol) was added to a solution of (2*S*,4*R*)-**11** (100 mg, 0.249 mmol) in CH_3CN (2.0 mL) at 0 °C. The mixture was stirred for 7 h [until complete consumption of (2*S*,4*R*)-**11**]. The reaction was quenched by the addition of aqueous 1 N NH_4OH (20.0 mL). The solution was extracted with CH_2Cl_2 (20.0 mL, 2 × 10.0 mL) and the combined organic layers were dried with Na_2SO_4, filtered, and concentrated in vacuo. The residue was dissolved in acetone (3.0 mL) and the same volume of water was added to the solution. Solid K_2CO_3 (103 mg, 0.745 mmol) and a solution of FmocOSu (126 mg, 0.373 mmol) in acetone (1.0 mL) were added to the solution which was stirred at room temperature for 3 h. Acetone was evaporated and replaced by AcOEt. The solution was washed with a saturated $NaHCO_3$ solution, brine, and 1 N $KHSO_4$, dried with Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash column chromatography (AcOEt/Hex, 6:4) to yield pure **13** (56 mg, 43% for two steps). HPLC: t_R = 12.71 min (linear gradient, 30–100% B, 20 min); colorless oil. $[\alpha]_D$ = +6.8 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.76 (d, *J* = 7.5 Hz, 2 H, arom. H), 7.58 (m, 2 H, arom. H), 7.39 (t, *J* = 7.5 Hz, 2 H, arom. H), 7.30 (t, *J* = 7.5 Hz, 2 H, arom. H), 6.95 (br. t, *J* = 5.0 Hz, 1 H, N*H*CH$_2$CO), 6.08 (br. d, *J* = 7.9 Hz, 1 H, N*H*CHCO), 4.56–4.48 (m, 1 H, NHC*H*CO), 4.46–4.35 (m, 2 H, CH_2OCO), 4.20 (t, *J* = 6.9 Hz, 1 H, C*H*CH$_2$OCO), 3.93 (d, *J* = 4.9 Hz, 2 H, NHCH_2CO), 3.86–3.80 (m, 1 H, C*H*OH), 3.46–3.41 (m, 2 H, CH_2N_3), 1.86–1.79 (m, 2 H, NHCHCH_2), 1.74–1.63 (m, 2 H, CH_2CH$_2$N$_3$), 1.45 [s, 9 H, C(CH_3)$_3$] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.7 (C), 168.9 (C), 157.2 (C), 143.7 (C), 143.6 (C), 141.3 (2 C), 82.6 (C), 67.4 (CH_2), 65.5 (CH), 52.1 (CH), 48.3 (CH_2), 47.1 (CH), 42.0 (CH_2), 41.7 (CH_2), 36.0 (CH_2), 28.0 (3 CH_3) ppm.

Allyl 2-{[(2*S*,4*R*)-6-Azido-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-4-hydroxyhexanoyl]amino}acetate (14): The dipeptide (2*S*,4*R*)-**12** was dissolved in CH_2Cl_2 (1.0 mL) and TFA (2.0 mL) was added to the resulting solution. The mixture was stirred at room temperature for 2 h. The solvents were evaporated in vacuo and coevaporated with hexane and the residue was dried under high vacuum. The residue was dissolved in THF (1.0 mL) and the same volume of water was added to the solution. Solid $NaHCO_3$ (39 mg, 0.464 mmol) and a solution of FmocOSu (79 mg, 0.234 mmol) in THF (1.0 mL) was added to the solution which was stirred at room temperature for 3 h. THF was evaporated and replaced by AcOEt. The solution was washed with a saturated $NaHCO_3$ solution, brine, and 1 N $KHSO_4$, dried with Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash column chromatography (AcOEt/Hex, 6:4) to yield pure **14** (75 mg, 95% for two steps). HPLC: t_R = 12.13 min (linear gradient, 30–100% B, 20 min); colorless oil. $[\alpha]_D$ = +4.9 (*c* = 0.9, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.75 (d, *J* = 7.5 Hz, 2 H, arom. H), 7.57 (m, 2 H, arom. H), 7.40 (t, *J* = 7.5 Hz, 2 H, arom. H), 7.36 (tt, *J* = 7.5, 1.0 Hz, 2 H, arom. H), 7.12 (m, 1 H, N*H*CH$_2$CO), 6.08 (br. d, *J* = 7.7 Hz, 1 H, N*H*CHCO), 5.95–5.82 (m, 1 H, C*H*=CH$_2$), 5.35–5.22 (m, 2 H, CH=CH_2), 4.62 (d, *J* = 5.8 Hz, 2 H, CH_2CH=CH$_2$), 4.58–4.51 (m, 1 H, NHC*H*CO), 4.46–4.38 (m, 2 H, CH_2OCO), 4.20 (t, *J* = 6.9 Hz, 1 H, C*H*CH$_2$OCO), 4.07–4.03 (m, 2 H, NHCH_2CO), 3.88–3.78 (m, 1 H, C*H*OH), 3.46–3.41 (m, 2 H, CH_2N_3), 1.91–1.79 (m, 2 H, NHCHCH_2), 1.74–1.63 (m, 2 H,

$CH_2CH_2N_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.9 (C), 169.4 (C), 157.2 (C), 143.7 (C), 143.5 (C), 141.3 (2 C), 131.3 (CH), 127.8 (2 CH), 127.1 (2 CH), 125.0 (2 CH), 120.0 (2 CH), 119.2 (CH_2), 67.4 (CH_2), 66.2 (CH_2), 65.7 (CH), 52.2 (CH), 48.4 (CH_2), 47.1 (CH), 41.6 (CH_2), 41.3 (CH_2), 35.9 (CH_2), 29.7 (CH_2) ppm. HRMS (ESI): calcd. for $C_{26}H_{30}N_5O_6$ $[M + H]^+$ 508.2191; found 508.2186.

***tert*-Butyl 2-{[(2*S*,4*R*)-6-Azido-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-4-*O*-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl)-hexanoyl]amino}acetate [Fmoc-(GalPiv$_4$)(4*R*)Hyl-Gly-O*t*Bu (15)]:** The *N*-Fmoc-protected dipeptide **13** (56 mg, 0.107 mmol) and tetrapivaloylated galactosyl bromide (93 mg, 0.160 mmol) were placed in an argon-filled round-bottomed flask and dissolved in CH_2Cl_2 (3.0 mL). Powdered 4-Å mol. sieves were added and the suspension was stirred for 30 min. The mixture was protected from light and silver silicate (200 mg) was added. The reaction was stirred for 8 h at room temperature. The dark-brown suspension was filtered through a plug of Celite® and the solvent was evaporated to give a brownish residue which was purified by flash column chromatography (Et_2O/Pent, 7:3) to give pure **15** (100 mg, 91%). HPLC: t_R = 13.29 min (linear gradient, 60–100% B, 20 min); colorless oil. $[\alpha]_D$ = –8.4 (c = 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.76 (d, J = 7.5 Hz, 2 H), 7.62 (m, 2 H), 7.39 (t, J = 7.5 Hz, 2 H), 7.32 (m, 2 H), 6.98 (m, 1 H), 6.29 (br. d, J = 7.1 Hz, 1 H), 5.42 (d, J = 2.9 Hz, 1 H), 5.29–5.23 (m, 1 H), 5.15 (dd, J = 10.4, 2.9 Hz, 1 H), 4.66 (d, J = 7.7 Hz, 1 H), 4.52 (m, 1 H), 4.44–4.25 (m, 3 H), 4.19–4.07 (m, 3 H), 4.04–4.00 (m, 1 H), 3.97–3.95 (m, 2 H), 3.36 (m, 2 H), 2.17–1.99 (m, 2 H), 1.91–1.80 (m, 1 H), 1.77–1.65 (m, 1 H), 1.44 (s, 9 H), 1.18 (s, 9 H), 1.17 (s, 9 H), 1.15 (s, 9 H), 1.11 (s, 9 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.8 (C), 177.4 (C), 176.9 (C), 176.8 (C), 172.8 (C), 168.3 (C), 156.5 (C), 143.8 (C), 143.7 (C), 141.2 (2 C), 127.7 (2 CH), 127.1 (2 CH), 125.3 (2 CH), 119.9 (2 CH), 99.8 (CH), 82.5 (C), 74.4 (CH), 71.3 (CH), 70.8 (CH), 69.0 (CH), 67.6 (CH_2), 66.4 (CH), 60.6 (CH_2), 52.5 (CH), 47.1 (CH_2), 47.0 (CH), 42.1 (CH_2), 39.0 (C), 38.8 (2 C), 38.7 (C), 33.7 (CH_2), 30.3 (CH_2), 28.0 (3 CH_3), 27.2 (3 CH_3), 27.1 (3 CH_3), 27.0 (6 CH_3) ppm. HRMS (ESI): calcd. for $C_{53}H_{76}N_5O_{15}$ $[M + H]^+$ 1022.5332; found 1022.5358.

Allyl 2-{[(2*S*,4*R*)-6-Azido-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]-amino}-4-*O*-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl)-hexanoyl]amino}acetate [Fmoc-(GalPiv$_4$)(4*R*)Hyl-Gly-OAll (16)]: The *N*-Fmoc-protected dipeptide **14** (54 mg, 0.106 mmol) and tetrapivaloylated galactosyl bromide (92 mg, 0.159 mmol) were placed in an argon-filled round-bottomed flask and dissolved in CH_2Cl_2 (3.0 mL). Powdered 4-Å mol. sieves were added and the suspension was stirred for 30 min. The mixture was protected from light and silver silicate (150 mg) was added. The reaction was stirred for 8 h at room temperature. The dark-brown suspension was filtered through a plug of Celite® and the solvent was evaporated to give a brownish residue which was purified by flash column chromatography (Et_2O/Pent, 8:2) to give pure **16** (92 mg, 86%). HPLC: t_R = 11.78 min (linear gradient, 60–100% B, 20 min); colorless oil. $[\alpha]_D$ = –12.0 (c = 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.76 (d, J = 7.4 Hz, 2 H), 7.61–7.64 (m, 2 H), 7.39 (t, J = 7.4 Hz, 2 H), 7.34–7.28 (m, 2 H), 7.06 (m, 1 H), 6.17 (br. d, J = 7.7 Hz, 1 H), 5.97–5.84 (m, 1 H), 5.41 (d, J = 3.1 Hz, 1 H), 5.32 (dq, J = 17.2, 1.4 Hz, 1 H), 5.26–5.22 (m, 2 H), 5.16 (dd, J = 10.4, 3.1 Hz, 1 H), 4.67 (d, J = 8.0 Hz, 1 H), 4.63 (d, J = 5.7 Hz, 2 H), 4.51–4.25 (m, 4 H), 4.22–4.16 (m, 1 H), 4.14–4.06 (m, 2 H), 4.06–3.98 (m, 2 H), 3.36 (t, J = 6.8 Hz, 2 H), 2.15–2.04 (m, 2 H), 1.92–1.78 (m, 1 H), 1.77–1.68 (m, 1 H), 1.18 (s, 9 H), 1.16 (s, 9 H), 1.14 (s, 9 H), 1.11 (s, 9 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.7 (C), 177.2 (C), 176.8 (C), 176.7 (C), 172.1 (C), 169.3 (C), 157.2 (C), 143.9 (2 C), 141.3 (2 C), 131.6 (CH), 127.7 (2 CH), 127.2 (2 CH), 125.3 (2 CH), 120.0 (2 CH), 118.9 (CH_2), 99.7 (CH), 74.9 (CH), 71.3 (CH), 70.8 (CH), 68.9 (CH), 67.3 (CH_2), 66.5 (CH_2), 65.9 (CH), 60.6 (CH_2), 52.2 (CH), 48.4 (CH_2), 47.1 (CH), 41.6 (CH_2), 41.3 (CH_2), 39.0 (C), 38.9 (C), 38.8 (C), 38.7 (C), 33.8 (CH_2), 30.3 (CH_2), 27.2 (3 CH_3), 27.1 (9 CH_3) ppm. HRMS (ESI): calcd. for $C_{52}H_{72}N_5O_{15}$ $[M + H]^+$ 1006.5019; found 1006.5011.

2-{[(2*S*,4*R*)-6-Azido-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-4-*O*-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl)hexanoyl]-amino}acetic Acid [Fmoc-(GalPiv$_4$)(4*R*)Hyl-Gly-OH (17)]: From the galactosylated dipeptide **15**: **15** (80 mg, 0.078 mmol) was dissolved in CH_2Cl_2 (1.0 mL) and TFA (2.0 mL) was added to the solution. The mixture was stirred at room temperature for 2 h. The solvents were evaporated in vacuo and co-evaporated with hexane. Crude **17** was purified by filtration through a short plug of silica gel (AcOEt/Hex/AcOH, 5:5:0.1) to give pure **17** (70 mg, 93%).

From the galactosylated dipeptide **16**: **16** (50 mg, 0.050 mmol) was dissolved in THF (1.0 mL) and morpholine (22 μL, 0.250 mmol) was added to the solution. $Pd(PPh_3)_4$ (5.8 mg, 0.005 mmol) was added to the mixture which was stirred at room temperature for 2 h. THF was evaporated and replaced by AcOEt. The solution was washed with 1 N $KHSO_4$ and brine, dried with Na_2SO_4, and concentrated in vacuo. Crude **17** was purified by flash column chromatography on silica gel (AcOEt/Hex/AcOH, 5:5:0.1) to give pure **17** (52 mg, 89%); colorless oil. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.75 (d, J = 7.4 Hz, 2 H), 7.62 (m, 2 H), 7.38 (t, J = 7.4 Hz, 2 H), 7.35–7.27 (m, 2 H), 7.11 (m, 1 H), 6.22 (br. d, J = 6.9 Hz, 1 H), 5.41 (d, J = 3.1 Hz, 1 H), 5.25 (dd, J = 10.4, 7.6 Hz, 1 H), 5.15 (dd, J = 10.4, 2.8 Hz, 1 H), 4.64 (d, J = 7.5 Hz, 1 H), 4.52–4.46 (m, 1 H), 4.40–4.34 (m, 2 H), 4.30–4.23 (m, 1 H), 4.17–4.06 (m, 4 H), 4.02–3.98 (m, 2 H), 3.34 (m, 2 H), 2.07–1.79 (m, 3 H), 1.77–1.48 (m, 1 H), 1.77–1.65 (m, 1 H), 1.17 (s, 9 H), 1.16 (s, 9 H), 1.15 (s, 9 H), 1.11 (s, 9 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.8 (C), 177.4 (C), 176.9 (C), 176.8 (C), 172.8 (C), 168.3 (C), 156.5 (C), 143.8 (C), 143.7 (C), 141.2 (2 C), 127.7 (2 CH), 127.1 (2 CH), 125.3 (2 CH), 119.9 (2 CH), 99.8 (CH), 82.5 (C), 74.4 (CH), 71.3 (CH), 70.8 (CH), 69.0 (CH), 67.6 (CH_2), 66.4 (CH), 60.6 (CH_2), 52.5 (CH), 47.1 (CH_2), 47.0 (CH), 42.1 (CH_2), 39.0 (C), 38.8 (2 C), 38.7 (C), 33.7 (CH_2), 30.3 (CH_2), 28.0 (3 CH_3), 27.2 (3 CH_3), 27.1 (3 CH_3), 27.0 (6 CH_3) ppm. HRMS (ESI): calcd. for $C_{49}H_{68}N_5O_{15}$ $[M + H]^+$ 966.4706; found 966.4688.

Gly^{256}-Glu-Hyp-Gly-Ile-Ala-Gly-Phe-(Gal)(4*R*)Hyl-Gly-Glu-Gln-Gly-Pro-Lys^{270} (2): The glycopeptide **2** was synthesized on Wang resin by using a homemade semiautomatic peptide synthesizer on a 20 μmol scale. For each coupling step, the reactants were introduced manually as a solution in DMF (2.0 mL). N^{α}-Fmoc amino acids (5.0 equiv.) with standard side-chain protecting groups were coupled twice by using BOP (5.0 equiv.), HOBt (5.0 equiv.), and DIEA (10.0 equiv.) in DMF for 20 min. The galactosylated building block **17** (1.5 equiv.) was coupled twice by using BOP (1.5 equiv.), HOBt (1.5 equiv.), and DIEA (3.0 equiv.) in DMF for 60 (1st coupling) and 20 min (2nd coupling). The washing of the resin and Fmoc deprotection (by using a freshly prepared solution of 20% piperidine in DMF) were performed automatically. The coupling and deprotection steps were monitored by the Kaiser test.[22] At the end of the elongation of the peptide chain, the resin was washed with CH_2Cl_2 and dried with Et_2O. The resin was placed in a syringe equipped with a frit and allowed to swell by addition of THF (1.0 mL). A solution of PPh_3 (10 equiv.) in a 3:1 mixture of THF/H_2O (4.0 mL) was added and the mixture was gently shaken for 72 h. The resin was washed with THF and CH_2Cl_2 and dried with Et_2O. A mixture of TFA/H_2O/TIPS/DTT

(8.8:0.5:0.2:0.5, 10.0 mL) was added to the resin. The mixture was gently shaken for 2.5 h and the resulting solution was flushed through a frit with cold Et_2O. The precipitate was recovered by centrifugation, dissolved in a mixture of AcOH and H_2O, and freeze-dried. The resulting protected glycopeptide **18** (purity of crude **18** estimated by RP-HPLC: 92.7%) was placed in a 50.0 mL round-bottomed flask and dissolved in a freshly prepared 40 mM solution of NaOMe in MeOH (25.0 mL). The deprotection was monitored by C_{18} RP-HPLC. After 16 h the solution was neutralized by the dropwise addition of AcOH and MeOH was evaporated in vacuo (purity of crude 2: 75%). Purification by semipreparative C_{18} RP-HPLC gave the glycopeptide **2** (27.5 mg, 63%, >99% purity). HPLC: t_R = 7.59 min (linear gradient, 5–65% B, 20 min). ^{1}H NMR (300 MHz, [D_6]DMSO): see Table 2. (ESI-TOF): calcd. for $C_{71}H_{113}N_{18}O_{28}$ $[M + H]^+$ 1665.7966; found 1665.8012.

Supporting Information (see also the footnote on the first page of this article): Formula of compound **18**, C_{18} RP-HPLC profiles for crude **18**, crude **2**, and purified **2**; the high-resolution ESI-MS spectrum of pure **2**, and ^{1}H NMR spectra of compounds **7**, **11**, **12** and **17**.

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