Chemoenzymatic Synthesis of Triazole-Linked Glycopeptides

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Abstract: Triazole-linked glycopeptides are prepared by C-terminal elongation of glycoamino acids with proteinogenic amino acids following a chemical or enzymatic coupling protocol. Two orthogonal routes for a chemoenzymatic strategy were explored, involving a click-reaction before amide bond formation or in reverse order. It was found that enzymatic peptide coupling under the influence of *alcalase* proceeds cleanly and in high yields, while the resulting dipeptides can be efficiently clicked to acetylene- or azide-containing sugars.

Key words: glycopeptides, azides, alkynes, click chemistry, enzymatic coupling

Since the development of the Cu(I)-catalyzed variant of the Huisgen [3+2] cycloaddition,[1] there has been a tremendous explosion of interest in the preparation of [1,2,3]-triazoles from acetylenes and organic azides.[2,3] The mild conditions for cycloaddition (*inter alia* aqueous solvents), the generally high yields and the wide functional group compatibility have led to broad application of the so-called 'click-reaction' to organic substrates including (unprotected) biopolymers such as proteins[4] and carbohydrates.[5,6] Of particular interest in this respect is the potential of a triazole to function as an amide isostere, a presumption that was put forward at an early occasion and that appears to be corroborated by several reports in recent literature.[7] As part of a program to obtain chemically and metabolically stable isosteres of *N*-glycopeptides, we have also applied Cu(I)-catalyzed Huisgen addition as recently described in the straightforward assembly of an array of triazolyl glycoamino acids from a wide range of carbohydrate and amino acid building blocks.[5] In particular, it was rewarding to find that the combination of *azido*sugars with *acetylenic* amino acids proceeded equally well as the inverse situation employing *acetylenic* sugars and *azido*amino acids.[5,8]

We have now extended our research into triazole-linked glycopeptides through elongation with proteinogenic amino acids via both conventional solution-phase chemical peptide synthesis and chemoenzymatic procedures. Chemoenzymatic peptide synthesis has received increasing interest over the last decade,[9,10] and is characterized by a number of specific advantages over chemical peptide synthesis: (a) enzymatic peptide coupling reactions are free from racemization, (b) side chain protection can mostly be omitted, (c) the coupling reagent (enzyme) can be recycled efficiently, (d) the process is environmentally friendly, and (e) the reaction proceeds under mild conditions. The greatest challenge in chemoenzymatic peptide synthesis is posed by incorporation of proline and/or non-proteinogenic amino acids such as D-, β- and unnatural amino acids.

We wish to report here that under the influence of *alcalase* as the biocatalyst, N-protected acetylenic and azidoamino acid methyl esters act as suitable acyl donors for condensation with a proteinogenic amino acid amide. *Alcalase*-catalyzed condensation was also applied to our triazole-linked glycoamino acids and compared with a traditional chemical peptide synthesis protocol. The inverse strategy, i.e. enzymatic coupling before click-reaction was found to be optimal, giving the desired triazole-linked glycodipeptides in high yield and purity.

In a typical enzymatic peptide synthesis protocol,[9] an N-protected amino acid (bearing a free carboxy group) is condensed with a C-protected amino acid. Since the reaction is equilibrium-controlled, the *thermodynamically* more stable starting materials (or hydrolysis products) are usually favored unless the desired condensation product precipitates from the solution. An alternative *kinetically* controlled approach involves C-activation of the N-protected amino acid. This is potentially more favorable since the yield is now predominantly determined by enzymatic properties such as substrate specificity and the ratio of synthesis versus hydrolysis.[9] The most suitable enzymes for enzymatic peptide synthesis include the majority of the commercially available serine, metallo-, and aspartate endo- and exoproteases.[11] Our synthetic efforts have focused on the condensation of a neutral and bulky N-protected unnatural amino acid [e.g. azidoornitine methyl ester (**1a**) and propargylglycine methyl ester (**1b**), Table 1] with a proteinogenic C-protected amino acid. To attain such a goal, subtilisine or carboxypeptidase Y seemed suitable candidates.[10] Based on the work of Chen et al.,[11] we focused our attention on the cheap and industrially available *alcalase*, a proteolytic enzyme mixture produced by *Bacillus licheniformis* (containing subtilisine Carlsberg as the major enzyme component), with high activity and stability in alcoholic solvents.

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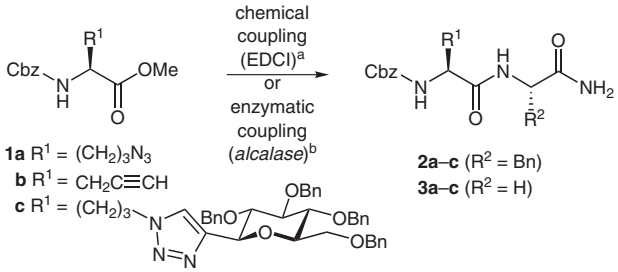
Table 1 Chemical and Enzymatic Synthesis of Dipeptides



Entry	Substrate		Chemical yield (%)[a]	Enzymatic yield (%)[b]	Dipeptide
1	**1a**	Phe-NH_2	69	77	**2a**
2	**1b**	Phe-NH_2	55	79	**2b**
3	**1c**	Phe-NH_2	86	40[c]	**2c**
4	**1a**	Gly-NH_2	80	93	**3a**
5	**1b**	Gly-NH_2	60	80	**3b**
6	**1c**	Gly-NH_2	82	10	**3c**

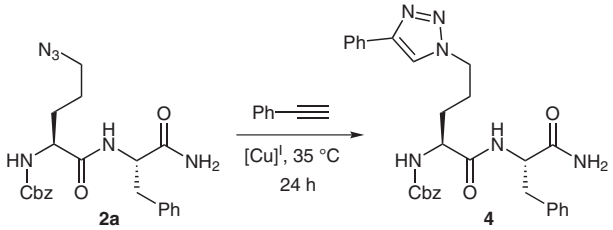
[a] Reaction conditions: 1. NaOH, THF; 2. Phe-NH_2 or Gly-NH_2, EDCI, HOAt, DIPEA, DMF.
[b] Reaction conditions: Phe-NH_2 or Gly-NH_2, *alcalase*, *t*-BuOH, DMF.
[c] Conversion of acyl donor as determined by HPLC.

In order to determine whether **1a–c** are suitable substrates for *alcalase*, first hydrolysis was investigated in a 1:1 mixture of *tert*-butyl alcohol and 1 M aqueous $KHCO_3$. It was observed that both **1a** and **1b** were completely hydrolyzed after five minutes, but the hydrolysis of the triazole-linked glycoamino acid methyl ester **1c** was much slower (complete conversion only after 24 h with a five-fold excess of *alcalase*). Possibly, the bulky glycosidic side-chain of **1c** is not well accommodated in the active site of the enzyme.

In the next step, *alcalase*-promoted condensation of **1a** and **1b** as the acyl donor with phenylalanine amide as acceptor was explored under anhydrous conditions (Table 1) and we were delighted to find that dipeptides **2a** and **2b** were formed in high yields (Table 1, entries 1 and 2). Similarly, dipeptides **3a** and **3b** were formed equally successfully by *alcalase*-mediated coupling of **1a** and **1b** to glycine amide (Table 1, entries 4 and 5) respectively. It was found that not only the isolated yields compared favorably to chemical coupling with EDCI, but the resulting dipeptides were formed with exclusive diastereoselectivity, i.e. with no detectable racemization. Not unexpectedly, under similar conditions the condensation of phenylalanine or glycine amide with our glycotriazolylamino acid methyl ester **1c** proceeded extremely slow (40% conversion of **1c** after 8 days) and did not lead to a satisfactory yield of the desired dipeptides.

Based on this observation, we were stimulated to investigate whether the inverse mode of actions, i.e. initial peptide coupling followed by Huisgen condensation with the carbohydrate moiety, would be more successful. Having the dipeptides **2a** and **3a** in hand, we first determined whether the C-terminal amide functionality would be disturbing in a Cu(I)-catalyzed [3+2] cycloaddition. To this end, a range of conditions was applied to a model system involving a mixture of **2a** and phenylacetylene (Table 2). Unfortunately, the reaction with standard $Cu(OAc)_2$/Na-ascorbate combination in a mixture of *tert*-butyl alcohol and water, conditions that served our goal so well earlier, failed in this case (Table 2, entry 1). The poor solubility of the substrates in *t*-BuOH–H_2O led us to switch to a THF–H_2O mixture, but also in this case no reaction took place (Table 2, entry 3). Presumably in these cases complexation of copper(I), added in substoichiometric amount, to the free amide functionality occurred, thereby catalyzing oxidation to Cu(II), as suggested by an observed color change from yellow to blue. Unfortunately, also with an excess of a copper(I) species, in the presence of a variety of tertiary amines in acetonitrile or THF, resulted in only minute formation of the desired product (Table 2, entries 5, 7 and 9). A more successful strategy was found by addition of tris(benzyltriazolylmethyl)amine (TBTA), a ligand reported by Fokin et al.[12] to protect copper from oxidation and disproportionation, while enhancing its catalytic activity. Indeed, addition of TBTA to any of the previously applied conditions led to a dramatic improvement in yield as well as a significant decrease in reaction time. In particular, the use of a CuI/TBTA combination in the presence of Et_3N in acetonitrile resulted in a conversion of more than 90% of **2a** into the desired triazolylpeptide **4** (Table 2, entry 8).

Table 2 Optimization of the [3+2] Cycloaddition of Dipeptide **2a** with Phenylacetylene



Entry	Cu(I) source	Base	Ligand	Solvent	Conversion[a] (%)
1	$Cu(OAc)_2$ Na-ascorbate[b]	–	–	*t*-BuOH–H_2O (1:1)	0
2	$Cu(OAc)_2$ Na-ascorbate[b]	–	TBTA[c]	*t*-BuOH–H_2O (1:1)	~50
3	$Cu(OAc)_2$ Na-ascorbate[b]	–	–	THF–H_2O (1:1)	0
4	$Cu(OAc)_2$ Na-ascorbate[b]	–	TBTA[c]	THF–H_2O (1:1)	~40
5	CuI[d]	DIPEA[d] 2,6-lutidine[d]	–	MeCN	~30
6	CuI[d]	DIPEA[d] 2,6-lutidine[d]	TBTA[d]	MeCN	~70
7	CuI[d]	Et_3N[d]	–	MeCN	0
8	CuI[d]	Et_3N[d]	TBTA[d]	MeCN	>90
9	CuBr[e]	–	PMDETA[e]	THF	0
10	CuBr[e]	–	TBTA[e]	THF	~70

[a] Conversion determined by TLC and LC-MS based on consumption of **2a**.
[b] 0.2 equiv of $Cu(OAc)_2$ and 0.4 equiv of Na-ascorbate.
[c] 0.2 equiv.
[d] 2 equiv.
[e] 1 equiv.

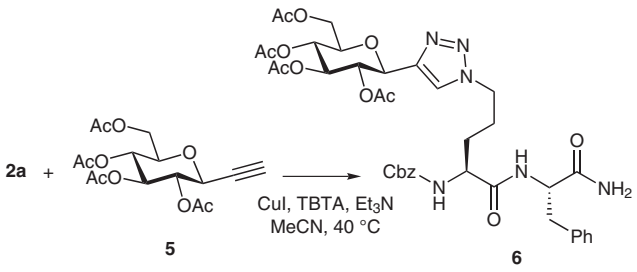
Having established suitable conditions for the cycloaddition of dipeptide **2a** with phenylacetylene, the optimization of the catalyst loading was investigated by condensation of **2a** with glucose-derived acetylene **5**[13a] (Table 3). It was found that reduction of the amount of catalyst from two equivalent to one gave a 100% conversion within 16 hours (Table 3, entry 1). With 50 mol% of copper, complete conversion was also attained within 24 hours (Table 3, entry 2) which was also the case with 20% copper (Table 3, entry 3). Further reduction of catalyst to 10 mol% failed to give complete conversion even after 48 hours (Table 3, entry 4), whereas under the action of 1 mol% catalyst no conversion was observed at all (Table 3, entry 5).

Since the reduction of the copper(I) catalyst loading below 20 mol% was found to be unsatisfactory for complete conversion to the product, all subsequent couplings were performed under identical conditions involving 20 mol% CuI, TBTA and Et_3N in acetonitrile. As summarized in Table 4, glycodipeptides **2c**, **3c**, **6**, and **8** were all obtained in excellent yields upon Huisgen condensation of *N*-Cbz-protected azidopropyl-containing dipeptides **2a** and **3a** with acetylenic *C*-glycosides **5** and **7**,[13b] irrespective of the carbohydrate O-protective groups (acetyl in **5** and benzyl in **7**). Deprotection of the acetyl protective groups of **6** using K_2CO_3/MeOH, proceeded smoothly to give the unprotected triazole-linked glycodipeptide **9** (Table 4).

Finally, the inverse mode of addition, i.e. condensation of *azido*sugar **10**[13c] with the *acetylenic* dipeptides **2b** or **3b** was also investigated. Much to our satisfaction, the desired glycodipeptides were formed uneventfully (Scheme 1) and in excellent yield.

In conclusion, a chemoenzymatic coupling strategy was successfully applied to the preparation of triazole-linked glycodipeptides. In particular, *alcalase*-induced condensation of an acetylenic or azidoamino acid methyl ester with an amino acid amide, followed by Cu(I)-catalyzed conversion to a triazole-linked glycopeptide proceeded with high conversion and good yield. Although the inverse approach, i.e. applying triazole-linked glycoamino

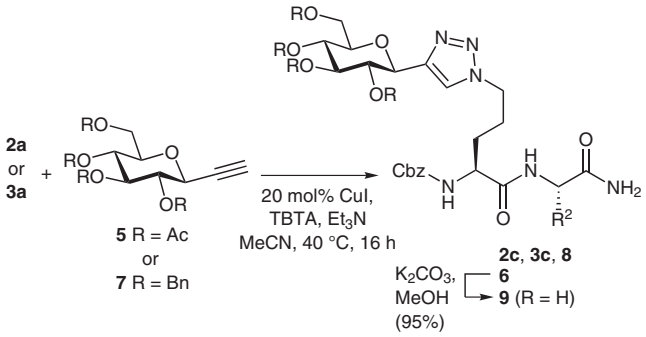
Table 3 Optimization of Catalyst Loading



Entry	Loading[a]	Concentration[b]	Time	Conversion[c]
1	100 mol%	1.1 mM	16 h	100%
2	50 mol%	1.8 mM	24 h	100%
3	20 mol%	12 mM	24 h	100%
4	10 mol%	3.7 mM	48 h	50%
5	1 mol%	5 mM	48 h	0%

[a] Loading of CuI, TBTA, and Et_3N.
[b] Concentration of substrates.
[c] Conversion determined by HPLC.

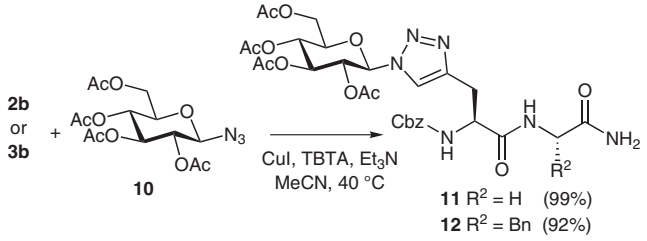
Table 4 Click Reaction of Dipeptides **2a** and **3a** with Glucosyl Acetylenes **5** and **7** and Deprotection of **6**



Entry	Dipeptide	Carbohydrate	Product	Yield (%)
1	**2a**[a]	**5** (R = Ac)	**6**	88
2	**2a**[a]	**7** (R = Bn)	**2c**	87
3	**3a**[b]	**5** (R = Ac)	**8**	62[c]
4	**3a**[b]	**7** (R = Bn)	**3c**	96

[a] **2a**: R^2 = Bn.
[b] **3a**: R^2 = H.
[c] Conversion ca. 70%.

acid donors was less successful, we are currently exploring alternative methods for this approach, for example, the use of no or less bulky carbohydrate protective groups, by using a more activated ester, or by the identification of a more active biocatalyst.



Scheme 1 Click-reaction of dipeptides **2b** and **3b** with glucosyl azide **10**.

^{1}H NMR spectra were recorded on a Varian Inova 400 (400 MHz) spectrometer and ^{13}C NMR spectra on a Bruker DMX300 (75 MHz) or Bruker DPX200 (50 MHz) spectrometer. Chemical shifts (δ) are given in ppm, downfield from tetramethylsilane. IR spectra were recorded on an ATI Mattson, Genesis series, or a Bruker Tensor 27 FTIR spectrometer. High-resolution mass spectra were recorded on a JEOL AccuTOF (ESI), or a MAT900 (EI, CI, and ESI). All reactions were performed under N_2 unless otherwise stated.

Before use in the coupling reactions, L-Phe-NH_2 was dried by co-evaporation with DMF (2 × 5 mL per mmol) and Gly-NH_2·HCl was first neutralized by treatment with an excess of *t*-BuNH_2, subsequently isolated as solid (Gly-NH_2) by repeated co-evaporation of the volatiles with EtOH and further dried by co-evaporation with DMF (2 × 5 mL per mmol). *t*-BuOH was dried by distillation under reduced pressure and kept under N_2. *Alcalase* was obtained from Novo Industrial (Denmark) as a brown aqueous liquid. For the hydrolysis experiments this enzyme solution was used as such. For the (anhyd) peptide coupling reactions the water in the *alcalase* solution was removed using the method of Chen et al.[11] As a matter of fact, the aqueous *alcalase* (0.5 mL) and absolute EtOH (1.0 mL) were mixed in an Eppendorf cup and the resulting suspension was agitated on a Vortex mixer for 5 min and centrifuged (3000 rpm) for 10 min to spin down the enzyme. The supernatant was decanted and the enzyme resuspended in absolute EtOH (1.0 mL), agitated for 5 min on a Vortex mixer and spun down by centrifugation. This procedure was repeated once with absolute EtOH (1.0 mL) and finally once with anhyd *t*-BuOH (1.0 mL). The resulting enzyme was resuspended in *t*-BuOH (1.0 mL) for use in the enzymatic peptide coupling reactions. All other commercially available reagents were used as received. HPLC analyses were performed with an Inertsil RP-18 column (25 × 0.46 cm) (RP-18, 5 μm). TfN_3 was prepared as described by Wong et al.[14]

Chemical Peptide Coupling; General Procedure

To a solution of **1** (2.5 mmol) in THF (12.5 mL) at 0 °C, was added aq 0.25 N NaOH solution (20.0 mL, 5.0 mmol). After 1 h at this temperature, the mixture was warmed to r.t. and stirring was continued. The extent of conversion was monitored by TLC analysis in EtOAc–heptane (1:1). When the methyl ester of **1** was completely hydrolyzed, the mixture was acidified with 1.0 N aq HCl until pH 2–3. The mixture was extracted with EtOAc (3 × 25 mL). The organic extracts were combined, dried (Na_2SO_4) and concentrated in vacuo, giving a yellow oil, corresponding to the free carboxylic acid (2.5 mmol). Part (1.0 mmol) of this amino acid was dried by co-evaporation with DMF (2 × 5 mL) and dissolved in DMF (2.5 mL). To the clear cooled (0–5 °C) solution, was added DIPEA (0.35 mL, 2.1 mmol), followed by EDCI (211 mg, 1.1 mmol) and HOAt (1-hydroxy-7-azabenzotriazole; 150 mg, 1.1 mmol) and the mixture was stirred for at least 0.5 h at 0–5 °C. Subsequently, a solution of Phe-NH_2 or Gly-NH_2 (1.1 mmol) in DMF (2.5 mL) was added and the mixture was stirred for 1 h at 0–5 °C. The mixture was warmed to r.t. and stirred overnight. TLC analysis (EtOAc) showed com-

plete conversion of the carboxylic acid starting material. The mixture was partitioned between EtOAc (75 mL) and H_2O (15 mL) to which a few drops of 1 N aq HCl were added. The aqueous layer was extracted with EtOAc (40 mL) and the combined organic phases were washed with 0.5 N aq HCl (40 mL), 1 M aq $KHCO_3$ (40 mL) and brine (40 mL), and subsequently dried (Na_2SO_4) and concentrated in vacuo.

Enzymatic Peptide Coupling; General Procedure

The *N*-Cbz-protected amino acid methyl ester **1** (1.0 mmol in the case of **1a** and **1b**, 0.5 mmol in the case of **1c**) was dried by co-evaporation with DMF (2 × 5 mL) and dissolved in *t*-BuOH (4.0 mL). After adding a solution of Phe-NH_2 or Gly-NH_2 (1.5 mmol) in DMF (2.0 mL), the mixture was stirred at 35 °C. Subsequently, the *alcalase* suspension in *t*-BuOH (1 mL) was added and the mixture was stirred at 35 °C. Samples were taken at regular time intervals and analyzed by HPLC. After almost complete conversion of **1**, the mixture was concentrated in vacuo to remove most of the volatiles. The residue was taken up in EtOAc (50 mL) and H_2O (20 mL), to which a few drops of 1 N aq HCl were added. The aqueous layer was extracted with EtOAc (30 mL) and the combined organic phases were washed with 1 M aq $KHCO_3$ (40 mL), 1 N aq HCl (40 mL) and brine (40 mL), dried (Na_2SO_4) and concentrated in vacuo. Analytically pure samples were obtained by recrystallization.

Optimized Click-Reaction; General Procedure

To a solution of the dipeptide (25 μmol) and the carbohydrate (25 μmol)[13] in MeCN (0.5 mL) were added solutions of Et_3N in MeCN (10 mM, 0.50 mL, 0.20 equiv) and TBTA in MeCN (10 mM, 0.50 mL, 0.20 equiv). N_2 was bubbled through the solution for 5 min. A solution of CuI in MeCN (10 mM, 0.50 mL, 0.20 equiv) was added and the mixture was stirred at 40 °C for 16 h. Brine (2 mL) was added and the mixture was extracted with EtOAc (2 × 4 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The product was purified by gradient flash chromatography (MeOH–CH_2Cl_2).

Cbz-L-Orn(N_3)-OMe (1a)

To a solution Cbz-L-Orn-OH (15 g, 56 mmol), K_2CO_3 (16.5 g, 84.5 mmol), and $CuSO_4 \cdot 5H_2O$ (143 mg, 0.570 mmol) in H_2O (160 mL) and MeOH (320 mL), was added freshly prepared TfN_3 in CH_2Cl_2 (± 0.41 M, 270 mL) and the reaction was stirred overnight at r.t. The mixture was concentrated in vacuo, acidified to pH 2 with 2 M aq HCl, and extracted with EtOAc (2 × 200 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo to yield crude Cbz-Orn(N_3)-OH. The crude intermediate was subsequently dissolved in MeOH (150 mL) and $SOCl_2$ (4.1 mL, 56 mmol) was added. After 4 h of reflux, and evaporation of the MeOH in vacuo, the residue was taken up in EtOAc (300 mL), and the EtOAc layer was washed with H_2O (2 × 150 mL) and brine (100 mL), dried ($MgSO_4$) and concentrated in vacuo to yield **1a** (15.6 g, 91%) as a colorless oil; R_f 0.53 (1:1 EtOAc–heptane).

IR (film): 3317, 3070, 3032, 2963, 2872, 2090, 1365, 1528 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 7.37–7.32 (m, 5 H), 5.32 (br d, *J* = 7.4 Hz, 1 H), 5.11 (s, 2 H), 4.42–4.38 (m, 1 H), 3.76 (s, 3 H), 3.31 (br s, 2 H), 1.98–1.58 (m, 4 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 172.4, 155.8, 135.8, 128.2, 127.9, 127.7, 66.8, 53.3, 53.1, 52.2, 50.4, 29.3, 24.5.

HRMS (CI): *m*/*z* calcd for $C_{14}H_{19}N_4O_4$ (M + H): 307.1406; found: 307.1407.

Cbz-L-Propargylglycine-OMe (1b)

To a suspension of L-propargylglycine[15] (80 mg, 0.71 mmol) in MeOH (1.5 mL) was added slowly $SOCl_2$ (114 μL, 1.56 mmol) at 0 °C. The mixture was refluxed for 2 h, cooled to r.t. and concentrated in vacuo. The product was suspended in dioxane (1 mL) and 2 N aq NaOH (0.35 mL) was added slowly at 0 °C. To the resulting solution was added $NaHCO_3$ (80.2 mg, 0.720 mmol) and subsequently Cbz-OSu (181 mg, 0.720 mmol) and the mixture was stirred for 18 h at r.t. The mixture was taken up in H_2O (10 mL) and EtOAc (10 mL) and the layers were separated. The aqueous phase was extracted with EtOAc (2 × 10 mL) and the combined organic layers were washed with aq sat. $NaHCO_3$ (15 mL), aq sat. NH_4Cl and brine, dried (Na_2SO_4) and concentrated in vacuo. Purification by column chromatography (heptane–EtOAc, 3:1) yielded **1b** (162 mg, 88%) as a colorless oil, which solidified upon storage at –20 °C; R_f 0.60 (1:1 EtOAc–heptane).

IR (film): 3369, 3295, 3032, 2958, 1714, 1511 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 7.35–7.28 (m, 5 H), 5.58 (br d, 1 H), 5.11 (s, 2 H), 4.55–4.50 (m, 1 H), 3.77 (s, 3 H), 2.80–2.70 (m, 2 H), 2.01 (t, *J* = 2.6 Hz, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 170.6, 155.4, 135.9, 128.3, 128.0, 127.9, 78.1, 71.7, 66.9, 52.6, 52.2, 22.5.

HRMS (CI): *m*/*z* calcd for $C_{14}H_{16}NO_4$ (M + H): 262.1079; found: 262.1092.

Cbz-L-Orn{4-(2,3,4,6-tetra-*O*-benzyl-β-D-glucosyl)[1,2,3]triazol-1-yl}-OMe (1c)

To a solution of Bn_4Glc-acetylene[13b] (7.50 g, 13.7 mmol) and Cbz-L-Orn(N_3)-OMe (4.20 g, 13.7 mmol) in *t*-BuOH (100 mL) was added a mixture of $Cu(OAc)_2$ (0.550 g, 2.75 mmol) and sodium ascorbate (1.1 g, 5.5 mmol) in H_2O (100 mL), and the reaction was stirred overnight at r.t.. After the addition of H_2O (150 mL), the mixture was extracted with CH_2Cl_2 (2 × 150 mL). The combined organic layers were washed with H_2O (100 mL), dried (Na_2SO_4) and concentrated in vacuo. Purification by gradient flash chromatography (EtOAc–heptane) yielded the clicked product **1c** (10.2 g, 87%) as a white solid; R_f 0.22 (1:1 EtOAc–heptane).

IR (film): 3321, 3028, 2950, 2863, 2249, 1951, 1722, 1515 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 7.41 (s, 1 H), 7.35–6.98 (m, 25 H), 5.30 (br d, *J* = 8.0 Hz, 1 H), 5.13–5.07 (m, 2 H), 4.94 (d, *J* = 10.9 Hz, 1 H), 4.90 (d, *J* = 10.9 Hz, 1 H), 4.85 (d, *J* = 10.5 Hz, 1 H), 4.64–4.49 (m, 5 H), 4.44–4.37 (m, 1 H), 4.35–4.27 (m, 3 H), 3.92 (t, *J* = 9.3 Hz, 1 H), 3.81 (t, *J* = 8.9 Hz, 1 H), 3.74–3.60 (m, 4 H), 3.69 (s, 3 H), 1.92–1.62 (m, 4 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 172.0, 155.7, 145.4, 138.3, 137.8, 137.7, 135.9, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.7, 127.6, 127.5, 127.4, 122.6, 86.6, 81.5, 79.1, 77.9, 75.3, 74.8, 74.5, 73.8, 73.1, 68.9, 66.8, 52.9, 52.3, 49.2, 29.2, 25.9.

HRMS (ESI): *m*/*z* calcd for $C_{50}H_{54}N_4O_9$ + Na (M + Na): 877.3788; found: 877.3805.

Cbz-L-Orn(N_3)-L-Phe-NH_2 (2a)

Chemical coupling; yield: 302 mg (69%). Enzymatic coupling; yield: 339 mg (77%); R_f 0.29 (2:1 EtOAc–heptane).

IR (film): 3304, 2470, 2098, 1679, 1640 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$/MeOD): δ = 7.38–7.17 (m, 10 H), 5.10 (d, *J* = 12.3 Hz, 1 H), 5.06 (d, *J* = 12.3 Hz, 1 H), 4.63 (dd, *J* = 5.9, 8.4 Hz, 1 H), 4.04 (dd, *J* = 5.7, 8.0 Hz, 1 H), 3.25–3.16 (m, 3 H), 2.93 (dd, *J* = 5.5, 8.6 Hz, 1 H), 1.74–1.44 (m, 4 H).

HRMS (ESI): *m*/*z* calcd for $C_{22}H_{26}N_6O_4$ + Na (M + Na): 461.19132; found: 461.19216.

Cbz-L-propargylglycine-L-Phe-NH_2 (2b)

Chemical coupling; yield: 536 mg (55%). Enzymatic coupling; yield: 312 mg (79%); R_f 0.25 (2:1 EtOAc–heptane).

IR (film): 3351, 2474, 2220, 2064, 1671, 1632 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$/MeOD): δ = 7.39–7.20 (m, 10 H), 5.10 (s, 2 H), 4.65–4.62 (m, 1 H), 4.28–4.25 (m, 1 H), 3.20–3.15 (m, 1 H), 3.00–2.95 (m, 1 H), 2.65–2.54 (m, 2 H), 2.12 (br s, 1 H).

HRMS (ESI): *m*/*z* calcd for $C_{22}H_{23}N_3O_4$ + Na (M + Na): 416.15863; found: 416.15939.

Cbz-L-Orn{4-(2,3,4,6-tetra-*O*-benzyl-β-D-glucosyl)[1,2,3]triazol-1-yl}-L-Phe-NH_2 (2c)

Chemical coupling; yield: 418 mg (86%). Click-reaction dipeptide **2c**; yield: 21 mg (87%); R_f 0.20 (2:1 EtOAc–heptane).

IR (film): 3369, 3282, 2945, 2828, 1696, 1645 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$/MeOD): δ = 7.71 (s, 1 H), 7.33–6.93 (m, 30 H), 5.06 (d, *J* = 12.2 Hz, 1 H), 5.03 (d, *J* = 12.2 Hz, 1 H), 4.89 (s, 2 H), 4.83 (d, *J* = 10.9 Hz, 1 H), 4.62–4.48 (m, 5 H), 4.44 (d, *J* = 11.9 Hz, 1 H), 4.25 (t, *J* = 7.0 Hz, 2 H), 4.24 (d, *J* = 10.9 Hz), 4.03 (dd, *J* = 5.8, 8.0 Hz, 1 H), 3.85–3.76 (m, 2 H), 3.73–3.66 (m, 3 H), 3.62–3.58 (m, 1 H), 3.16 (dd, *J* = 5.8, 14.0 Hz, 1 H), 2.90 (dd, *J* = 8.4, 14.0 Hz, 1 H), 1.78–1.48 (m, 4 H).

^{13}C NMR (75 MHz, $CDCl_3$/MeOD): δ = 174.4, 172.4, 157.3, 146.1, 138.8, 138.3, 138.2, 138.1, 137.0, 136.5, 129.5, 128.8, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.1, 128.0, 127.2, 123.8, 87.2, 82.2, 79.6, 78.4, 76.0, 75.4, 75.1, 74.3, 73.8, 69.3, 67.4, 54.8, 54.3, 50.0, 37.9, 29.2, 26.3.

HRMS (ESI): *m*/*z* calcd for $C_{58}H_{62}N_6O_9$ + Na (M + Na): 1009.44760; found: 1009.44631.

Cbz-L-Orn(N_3)-Gly-NH_2 (3a)

Chemical coupling; yield: 277 mg (80%). Enzymatic coupling; yield: 325 mg (93%); R_f 0.39 (EtOAc).

IR (film): 3343, 2483, 2241, 2072, 1662 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$/MeOD): δ = 7.36–7.30 (m, 5 H), 5.13 (d, 12.3 Hz, 1 H), 5.08 (d, *J* = 12.3 Hz, 1 H), 4.13–4.10 (m, 1 H), 3.93 (d, *J* = 17.1 Hz, 1 H), 3.81 (d, *J* = 17.1 Hz, 1 H), 3.34–3.31 (m, 2 H), 1.94–1.62 (m, 4 H).

HRMS (ESI): *m*/*z* calcd for $C_{15}H_{20}N_6O_4$ + Na (M + Na): 371.14437; found: 371.14351.

Cbz-L-propagylglycine-Gly-NH_2 (3b)

Chemical coupling; yield: 181 mg (60%). Enzymatic coupling; yield: 244 mg (80%); R_f 0.39 (EtOAc).

IR (film): 3291, 3066, 3036, 2941, 2409, 1658, 1532 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$/MeOD): δ = 7.34–7.27 (m, 5 H), 5.12 (d, *J* = 12.3 Hz, 1 H), 5.08 (d, *J* = 12.3 Hz, 1 H), 4.28 (t, *J* = 6.5 Hz, 1 H), 3.91 (d, *J* = 17.1 Hz, 1 H), 3.81 (d, *J* = 17.1 Hz, 1 H), 2.71 (ddd, *J* = 2.6, 6.2, 17.0 Hz, 1 H), 2.65 (ddd, *J* = 2.6, 6.8, 17.0 Hz, 1 H), 2.19 (t, *J* = 2.6 Hz, 1 H).

HRMS (ESI): *m*/*z* calcd for $C_{15}H_{17}N_3O_4$ + Na (M + Na): 326.11168; found: 326.11042.

Cbz-L-Orn{4-(2,3,4,6-tetra-*O*-benzyl-β-D-glucosyl)[1,2,3]triazol-1-yl}-Gly-NH_2 (3c)

Chemical coupling; yield: 361 mg (82%). Enzymatic coupling (using the standard procedure, but with 0.5 mmol of compound **1c**); yield: 43 mg (10%). Click-reaction; yield: 22 mg (96%); R_f 0.17 (2:1 EtOAc–heptane).

IR (film): 3343, 2945, 2824, 2483, 2232, 2072 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$/MeOD): δ = 7.73 (s, 1 H), 7.33–6.95 (m, 25 H), 5.10 (d, *J* = 12.3 Hz, 1 H), 5.09 (d, *J* = 12.3 Hz, 1 H), 4.91 (s, 2 H), 4.85 (d, *J* = 10.9 Hz, 1 H), 4.61–4.50 (m, 4 H), 4.47 (d, *J* = 12.0 Hz, 1 H), 4.34 (d, *J* = 7.0 Hz, 2 H), 4.23 (d, *J* = 11.0 Hz, 1 H), 4.14 (dd, *J* = 5.4, 8.4 Hz, 1 H), 3.91–3.70 (m, 7 H), 3.63–3.60 (m, 1 H), 2.02–1.60 (m, 4 H).

^{13}C NMR (50 MHz, $CDCl_3$/MeOD): δ = 173.0, 172.5, 157.1, 145.6, 138.3, 137.8, 137.7, 137.6, 136.2, 128.3, 128.3, 128.3, 128.2, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 123.6, 86.7, 81.7, 79.1, 78.0, 75.5, 75.0, 74.6, 73.8, 73.3, 68.8, 66.9, 54.5, 42.0, 28.5, 26.3.

HRMS (ESI): *m*/*z* calcd for $C_{51}H_{56}N_6O_9$ + Na (M + Na): 919.40065; found: 919.39922.

Cbz-L-Orn{4-(2,3,4,6-tetra-*O*-acetyl-β-D-glucosyl)[1,2,3]triazol-1-yl}-L-Phe-NH_2 (6)

Yield: 18 mg (88%); R_f 0.42 (EtOAc).

IR (KBr): 3307, 2955, 1753, 1675, 1533 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$/MeOD): δ = 7.71 (s, 1 H), 7.36–7.16 (m, 10 H), 5.37 (t, *J* = 9.4 Hz, 1 H), 5.23 (t, *J* = 9.7 Hz, 1 H), 5.19 (t, *J* = 9.7 Hz, 1 H), 5.09 (d, *J* = 12.1 Hz, 1 H), 5.05 (d, *J* = 12.1 Hz, 1 H), 4.75 (d, *J* = 10.0 Hz, 1 H), 4.63 (dd, *J* = 8.1, 14.3 Hz, 1 H), 4.37–4.21 (m, 3 H), 4.14 (dd, *J* = 2.0, 12.5 Hz, 1 H), 4.12–4.05 (m, 1 H), 3.91 (ddd, *J* = 2.0, 4.8, 12.4 Hz, 1 H), 3.17 (dd, *J* = 6.3 14.4 Hz, 1 H), 2.94 (dd, *J* = 8.2, 13.8 Hz, 1 H), 2.07 (s, 3 H), 2.06 (s, 3 H), 2.02 (s, 3 H), 1.88 (s, 3 H), 1.84–1.74 (m, 2 H), 1.68–1.59 (m, 1 H), 1.54–1.43 (m, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$/MeOD): δ = 173.5, 171.5, 170.9, 170.2, 169.9, 169.7, 143.9, 136.3, 135.7, 128.9, 128.2, 128.2, 128.0, 127.7, 126.6, 122.6, 75.8, 73.6, 72.8, 71.4, 68.1, 66.9, 61.9, 54.0, 53.5, 49.5, 37.2, 25.7, 20.3, 20.2, 20.0.

HRMS (ESI): *m*/*z* calcd for $C_{38}H_{46}N_6O_{13}$ + Na (M + Na): 817.30205; found: 817.29744.

Cbz-L-Orn{4-(2,3,4,6-tetra-*O*-acetyl-β-D-glucosyl)[1,2,3]triazol-1-yl}-Gly-NH_2 (8)

Yield: 11 mg (62%); R_f 0.11 (EtOAc).

IR (KBr): 3351, 2953, 1754, 1675, 1525 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$/MeOD): δ = 7.71 (s, 1 H), 7.33–7.28 (m, 5 H), 5.33 (t, *J* = 9.4 Hz, 1 H), 5.20 (t, *J* = 9.6 Hz, 1 H), 5.15 (t, *J* = 9.6 Hz, 1 H), 5.09 (d, *J* = 12.2 Hz, 1 H), 5.05 (d, *J* = 12.3 Hz, 1 H), 4.72 (d, *J* = 10.0 Hz, 1 H), 4.44–4.29 (m, 1 H), 4.26 (dd, *J* = 12.4, 4.8 Hz, 1 H), 4.17–4.08 (m, 1 H), 4.11 (dd, *J* = 12.5, 1.9 Hz, 1 H), 3.94–3.75 (m, 2 H), 3.88 (ddd, *J* = 9.6, 4.7, 2.0 Hz, 1 H), 2.05 (s, 3 H), 2.04 (s, 3 H), 1.99 (s, 3 H), 1.98–1.90 (m, 2 H), 1.85 (s, 3 H), 1.82–1.71 (m, 1 H), 1.63–1.51 (m, 1 H).

^{13}C NMR (75 MHz, $CDCl_3$/MeOD): δ = 172.3, 171.9, 170.8, 170.2, 169.9, 169.7, 156.7, 144.2, 135.8, 128.2, 128.0, 127.7, 122.7, 75.9, 73.6, 72.8, 71.4, 68.2, 66.9, 66.7, 62.0, 54.1, 42.0, 30.5, 28.5, 26.0, 20.3, 20.2, 20.1.

HRMS (ESI): *m*/*z* calcd for $C_{31}H_{40}N_6O_{13}$ + Na (M + Na): 727.25510; found: 727.25131.

Cbz-L-Orn{4-(β-D-glucosyl)[1,2,3]triazol-1-yl}-L-Phe-NH_2 (9)

To a solution of glucodipeptide **6** (12 mg, 15 μmol) in MeOH (2 mL) was added K_2CO_3 (0.29 mg, 1.5 μmol). The mixture was stirred overnight at r.t. It was neutralized with Amberlite IR-120 (5 mg) and stirring was continued for another 10 min. Product **9** was isolated after filtration in pure form as a white solid (8.9 mg, 95%).

^{1}H NMR ($CDCl_3$/MeOD, 400 MHz): δ = 7.86 (s, 1 H), 7.35–7.30 (m, 5 H), 7.26–7.16 (m, 5 H), 5.10 (d, *J* = 12.7 Hz, 1 H), 5.06 (d, *J* = 12.0 Hz, 1 H), 4.62 (dd, *J* = 8.6, 5.8 Hz, 1 H), 4.41 (d, *J* = 8.6 Hz, 2 H), 4.33 (t, *J* = 6.8 Hz, 2 H), 4.06 (dd, *J* = 7.9, 5.8 Hz, 1 H), 3.88 (d, *J* = 11.3 Hz, 1 H), 3.72 (dd, *J* = 12.1, 4.7 Hz, 1 H), 3.60–3.43 (m, 4 H), 3.18 (dd, *J* = 14.0, 5.8 Hz, 1 H), 2.92 (dd, *J* = 14.0, 8.8 Hz, 1 H), 1.86–1.75 (m, 2 H), 1.68–1.49 (m, 2 H).

^{13}C NMR ($CDCl_3$/MeOD, 75 MHz): δ = 173.7, 171.7, 156.4, 136.1, 135.6, 128.4, 127.6, 127.5, 127.3, 127.0, 125.9, 122.4, 79.9, 76.5, 73.5, 72.9, 69.4, 66.1, 61.0, 53.8, 53.3, 48.9, 36.7, 27.9, 25.2.

HRMS (ESI): *m*/*z* calcd for $C_{30}H_{38}N_6O_9$ + Na (M + Na): 649.25980; found: 649.26012.

Cbz-L-Ala{1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucosyl)[1,2,3]triazol-4-yl}-Gly-NH_2 (11)

Yield: 18 mg (99%); R_f 0.52 (EtOAc).

IR (KBr): 3292, 2948, 1753, 1687, 1535 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$/MeOD): δ = 7.93 (s, 1 H), 7.42–7.25 (m, 5 H), 5.99–5.90 (m, 1 H), 5.53 (s, 1 H), 5.50–5.45 (m, 2 H), 5.34–5.23 (m, 1 H), 5.11 (d, *J* = 12.4 Hz, 1 H), 5.08 (d, *J* = 12.4 Hz, 1 H), 4.49 (t, *J* = 6.2, 6.2 Hz, 1 H), 4.32 (dd, *J* = 12.6, 4.8 Hz, 1 H), 4.18 (dd, *J* = 12.6, 2.1 Hz, 1 H), 4.16–4.10 (m, 1 H), 3.92 (d, *J* = 17.1 Hz, 1 H), 3.84 (d, *J* = 17.0 Hz, 1 H), 3.29–3.18 (m, 2 H), 2.09 (s, 1 H), 2.08 (s, 1 H), 2.04 (s, 1 H), 1.83 (s, 1 H).

^{13}C NMR (75 MHz, $CDCl_3$/MeOD): δ = 172.1, 171.5, 170.5, 169.7, 169.3, 168.9, 156.3, 135.6, 127.9, 127.6, 127.4, 121.6, 85.0, 74.2, 72.1, 70.2, 67.3, 66.6, 61.1, 54.1, 41.8, 27.1, 19.7, 19.6, 19.1.

HRMS (ESI): *m*/*z* calcd for $C_{29}H_{36}N_6O_{13}$ + Na (M + Na): 699.22380; found: 699.22129.

Cbz-L-Ala{1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucosyl)[1,2,3]triazol-4-yl}-L-Phe-NH_2 (12)

Yield: 18 mg (92%); R_f 0.60 (EtOAc).

IR (KBr): 3351, 2942, 1748, 1693, 1666, 1524 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$/MeOD): δ = 7.74 (s, 1 H), 7.41–7.16 (m, 10 H), 5.96–5.87 (m, 1 H), 5.53 (s, 1 H), 5.49–5.41 (m, 2 H), 5.06 (s, 2 H), 4.63 (dd, *J* = 8.1, 5.9 Hz, 1 H), 4.42 (t, *J* = 6.4, 6.4 Hz, 1 H), 4.31 (dd, *J* = 12.6, 4.9 Hz, 1 H), 4.16 (dd, *J* = 12.6, 2.1 Hz, 1 H), 4.11 (ddd, *J* = 10.2, 4.8, 2.1 Hz, 1 H), 3.20–3.15 (m, 2 H), 3.05 (dd, *J* = 15.16, 6.86 Hz, 1 H), 2.96 (dd, *J* = 13.90, 8.27 Hz, 1 H), 2.09 (s, 3 H), 2.06 (s, 3 H), 2.04 (s, 3 H), 1.82 (s, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$/MeOD): δ = 173.6, 170.7, 170.5, 169.8, 169.4, 168.9, 156.1, 143.1, 136.2, 135.6, 128.6, 128.5, 128.0, 127.7, 127.4, 126.3, 121.4, 85.0, 74.3, 72.2, 70.1, 67.3, 66.6, 61.2, 53.9, 53.6, 36.8, 27.2, 19.8, 19.7, 19.2.

HRMS (ESI): *m*/*z* calcd for $C_{36}H_{42}N_6O_{13}$ + Na (M + Na): 789.27075; found: 789.26751.

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