

Expedient Synthesis of Triazole-Linked Glycosyl Amino Acids and Peptides

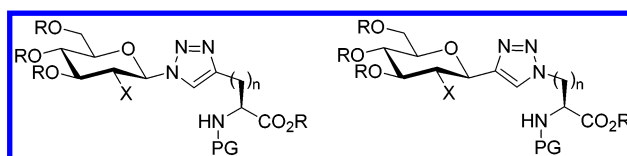
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ABSTRACT



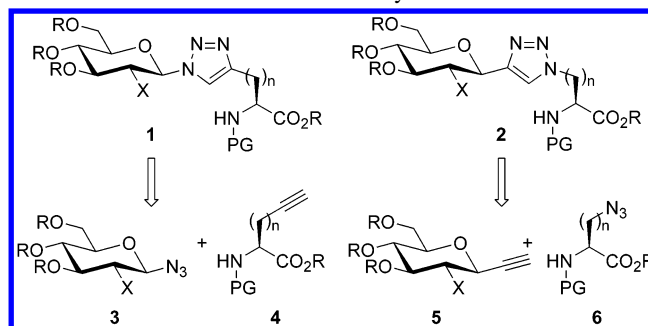
An expedient, high-yielding synthesis of two types of triazole-linked glycopeptides is described. These novel and stable glycopeptide mimics were prepared via Cu(I)-catalyzed [3 + 2] cycloaddition of either azide-functionalized glycosides and acetylenic amino acids or acetylenic glycosides and azide-containing amino acids.

Glycopeptides¹ constitute a class of natural compounds, involved in a number of important biological functions. By far the most commonly encountered members of this family are N- and O-linked glycopeptides.² Synthesis of such glycopeptides is complicated by the sensitivity of the glycosidic linkage between the (oligo)saccharide and the peptide toward chemical and enzymatic hydrolysis. Synthesis of (unnatural) amino acids, with the amino acid side chain connected to the sugar unit via an isosteric linkage, may lead to chemically and metabolically more stable analogues with potential biological activity³ (e.g., inhibitory activity toward glycosidases) or provide means to elucidate biochemical pathways.

Approaches to obtain stable glycopeptide analogues often involve the synthesis of C-linked glycopeptides. Most of

these syntheses, however, are rather complex and often feature low overall yields.⁴ Our research efforts aim at an efficient, high-yielding synthesis of triazole-linked glycopeptides such as **1** and **2** (Scheme 1), via a mild, Cu-catalyzed

Scheme 1. Retrosynthesis



procedure for the [3 + 2] cycloaddition between organic azides and alkynes ("click reactions"). As recently independently reported by the groups of Meldal⁵ and Sharpless,⁶ this reaction generally results in the corresponding 1,4-disubstituted 1,2,3-triazoles in high yields. Application of

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(1) *Glycopeptides and Related Compounds: Synthesis, Analysis and Applications*; Large, D. G., Warren, C. D., Eds.; Marcel Dekker: New York, 1997.

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this reaction to either azidoglycosides **3** and acetylenic amino acids **4** or acetylenic glycosides **5** and azide-containing amino acids **6** would then result in the triazole-linked glycopeptides **1** and **2**, respectively, which constitute a novel compound class.⁷ Besides the aim of constructing stable glycopeptide mimics, the resulting substituted triazoles may display relevant biological activity against various targets.⁸

To probe the viability of our approach, various glycosides containing an anomeric azide functionality were prepared and reacted with (*R*)-*N*-Boc-propargylglycine methyl ester (**7**, Table 1).⁹ The coupling was initially studied using azidoglucose (**8**) and azidogalactose (**9**)¹⁰ in combination with different Cu(I) species (e.g., CuI, CuCl, and CuCN) and different bases (e.g., Et₃N and DIPEA). Optimal results were obtained using modified Sharpless conditions,⁶ involving 0.2 equiv of Cu(OAc)₂ and 0.4 equiv of sodium ascorbate in a 1:1 (v/v) mixture of H₂O and *tert*-BuOH. This eventually provided the targeted glycoamino acids **15** and **16** in 98 and 88% yields (entries 1 and 2).

Under the same conditions, the benzylated glycosyl azide **10**¹¹ reacted smoothly to give the corresponding adduct **17** with retention of the anomeric configuration (entry 3).

Likewise, the glucosamine- and galactosamine-derived azides **11** and **12**,¹² respectively, reacted in an efficient manner with the acetylenic amino acid **7** to give the

(4) For entries into C-glyco amino acid synthesis, see for example: (a) Taylor, C. M. *Tetrahedron* **1998**, *54*, 11317. (b) Du, Y.; Lindhardt, R. J. *Tetrahedron* **1998**, *54*, 9913. (c) Dondoni, A.; Marra, A. *Chem. Rev.* **2000**, *100*, 4395. (d) Vincent, S. P.; Schleyer, A.; Wong, C.-H. *J. Org. Chem.* **2000**, *65*, 4440. (e) Xu, X.; Fakha, G.; Sinou, D. *Tetrahedron* **2002**, *58*, 7539. (f) Lane, J. W.; Halcomb, R. L. *J. Org. Chem.* **2003**, *68*, 1348. (g) Turner, J. J.; Leeuwenburgh, M. A.; Van der Marel, G. A.; Van Boom, J. H. *Tetrahedron* **2001**, *42*, 8713. (h) Dondoni, A.; Mariotti, G.; Marra, A.; Massi, A. *Synthesis* **2001**, *14*, 2129. (i) Westermann, B.; Walter, A.; Flörke, U.; Altenbach, H.-J. *Org. Lett.* **2001**, *3*, 1375. (j) McGarvey, G. J.; Benedum, T. E.; Schmidtman, F. W. *Org. Lett.* **2002**, *4*, 3591. (k) Dondoni, A.; Giovannini, P. P.; Marra, A. *J. Chem. Soc., Perkin Trans. 1* **2002**, 2380. (l) Nolen, E. G.; Kurish, A. J.; Wong, K. A.; Orlando, M. D. *Tetrahedron Lett.* **2003**, *44*, 2449. (m) Palomo, C.; Oiarbide, M.; Landa, A.; Concepción González-Rego, M.; García, J. M.; Gonzáles, A.; Odriozola, J. M.; Martín-Pastor, M.; Linden, A. *J. Am. Chem. Soc.* **2002**, *124*, 8637.

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(7) For earlier reports on [3 + 2] cycloadditions of azidoglycosides and acetylenes, see: (a) Bröder, W.; Kunz, H. *Carbohydr. Res.* **1993**, *249*, 221. (b) Al-Masoudi, N. A.; Al-Soud, Y. A. *Tetrahedron Lett.* **2002**, *43*, 4021. (c) Marco-Contelles, J.; Jiménez, C. A. *Tetrahedron* **1999**, *55*, 10511. (d) Peto, C.; Batta, G.; Gyorgydeak, Z.; Sztaricskai, F. *Carbohydr. Chem.* **1996**, *15*, 465 and references therein.

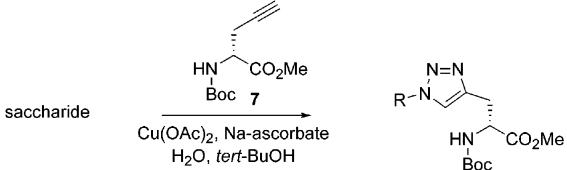
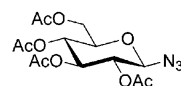
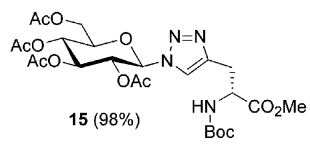
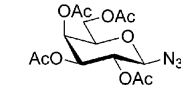
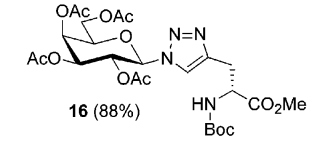
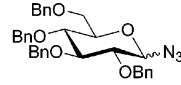
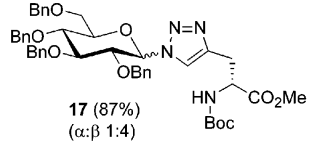
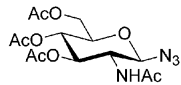
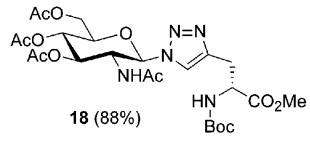
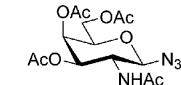
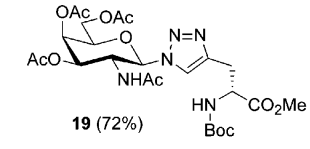
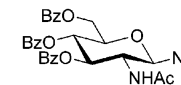
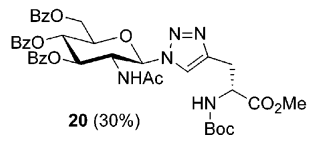
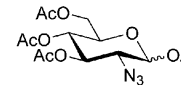
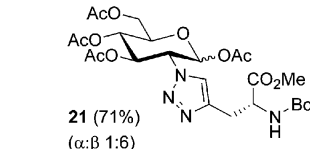
(8) For examples of biologically active triazoles, see: (a) Alvarez, R.; Velazquez, S.; San, F.; Aquaro, S.; De, C.; Perno, C. F.; Karlsson, A.; Balzarini, J.; Camarasa, M. J. *J. Med. Chem.* **1994**, *37*, 4185. (b) Velazquez, S.; Alvarez, R.; Perez, C.; Gago, F.; De, C.; Balzarini, J.; Camarasa, M. J. *Antivir. Chem. Chemother.* **1998**, *9*, 481. (c) Genin, M. J.; Allwine, D. A.; Anderson, D. J.; Barbachyn, M. R.; Emmert, D. E.; Garmon, S. A.; Graber, D. R.; Grega, K. C.; Hester, J. B.; Hutchinson, D. K.; Morris, J.; Reischer, R. J.; Ford, C. W.; Zurenko, G. E.; Hamel, J. C.; Schaadt, R. D.; Stapert, D.; Yagi, B. H. *J. Med. Chem.* **2000**, *43*, 953.

(9) Enantiopure propargylglycine is commercially available but in our hands was prepared via an enzymatic resolution process: (a) Wolf, L. B.; Sonke, T.; Tjen, K. C. M. F.; Kaptein, B.; Broxterman, Q. B.; Schoemaker, H. E.; Rutjes, F. P. J. T. *Adv. Synth. Catal.* **2001**, *343*, 662. (b) Sonke, T.; Kaptein, B.; Boesten, W. H. J.; Broxterman, Q. B.; Kamphuis, J.; Formaggio, F.; Toniolo, C.; Rutjes, F. P. J. T.; Schoemaker, H. E. In *Stereoselective Biocatalysis*; Patel, R. N., Ed.; Marcel Dekker: New York, 2000; p 23.

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(11) Prepared from 2,3,4,6-tetra-*O*-benzylglucose by treatment with DBU and DPPA: Mizuno, M.; Shioiri, T. *Chem. Commun.* **1997**, 2165.

Table 1. Variation of the Carbohydrate Moiety

		
entry	saccharide	glycopeptide ^{a,b}
1		 15 (98%)
2		 16 (88%)
3		 17 (87%) (α : β 1:4)
4		 18 (88%)
5		 19 (72%)
6		 20 (30%)
7		 21 (71%) (α : β 1:6)

^a Reagents and conditions: 1 equiv of azidoglycoside, 1 equiv of amino acid derivative, 0.2 equiv of Cu(OAc)₂, 0.4 equiv of sodium ascorbate, H₂O/*tert*-BuOH 1:1 (v/v), rt, 16 h. ^b Yield of isolated product.

corresponding products **18** and **19** in good yields (entries 4 and 5). The benzoyl-protected glucosamine **13**¹³ surprisingly gave a somewhat lower yield of the glycosylamino acid **20** (entry 6). Finally, the 2-azido functionalized glucose derivative **14**¹⁴ gave the desired coupling product in 77% yield (entry 7). Clearly, there were no notable differences in

(12) Prepared via tetra-*O*-acetyl- α -D-pyranosyl chloride from the corresponding glucosamine and galactosamine (ref 13) and subsequent treatment with NaN₃: Lehnhoff, S.; Goebel, M.; Karl, R. M.; Kloesel, R.; Ugi, I. *Angew. Chem., Int. Ed.* **1995**, *34*, 1104.

(13) Prepared by reaction of the benzoylated thioglycoside with ICl and Me₃SiN₃, respectively.

(14) Prepared from the corresponding glucosamine via azido transfer, followed by acetylation: Alper, P. B.; Hung, S.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 6029.

reactivity between the several monosaccharides or between the α - and β -isomers. Preliminary experiments to determine the stability of the triazole linkage in compound **15** revealed that this moiety is stable under a variety of acidic and basic conditions.¹⁵

Table 2 shows the application of different acetylenic amino acids containing a variety of amino protecting groups in the

Table 2. Variation of the Amino Acid Moiety

entry	amino acid	glycopeptide ^{a,b}
1		28 (70%)
2		29 (64%)
3		30 (70%)
4		31 (76%)
5		32 (85%, 1:1)
6		33 (60%)

^a Reagents and conditions: 1 equiv of azidoglycoside, 1 equiv of amino acid derivative, 0.2 equiv of $\text{Cu}(\text{OAc})_2$, 0.4 equiv of sodium ascorbate, $\text{H}_2\text{O}/\text{tert-BuOH}$ 1:1 (v/v), rt, 16 h. ^b Yield of isolated product.

cycloaddition with azidoglycoside **8**. From Table 1, the compatibility of the Boc group with these reaction conditions was already clear; entries 1 and 2 of Table 2 now show that Fmoc and Ts are also well-suited for this reaction. Increasing the length of the side chain of the amino acid¹⁶ (entries 3 and 4) showed no significant change in reactivity, providing

(15) Triazole linkage appeared to be stable using, for example: 2.5 M HCl in EtOAc, 2 h, rt; 1 M aqueous HCl, >5 days, rt; 1 M HCl in MeOH, 6 h, reflux; K_2CO_3 in MeOH, 4 h, rt; 1.25 M aqueous NaOH, 24 h, reflux.

(16) van Esseveldt, B. C. J.; van Delft, F. L.; de Gelder, R.; Rutjes, F. P. J. T. *Org. Lett.* **2003**, 5, 1717.

Table 3. Cycloaddition Reactions Involving a Dipeptide

entry	dipeptide	glycopeptide ^{a,b}
1		36 (51%)
2		37 (92%)

^a Reagents and conditions: 1 equiv of azidoglycoside **8**, 1 equiv of dipeptide, 0.2 equiv of $\text{Cu}(\text{OAc})_2$, 0.4 equiv of sodium ascorbate, $\text{H}_2\text{O}/\text{tert-BuOH}$ 1:1 (v/v), rt, 16 h. ^b Yield of isolated product.

products **30** and **31** in good yields. Similarly, α -methyl-substituted propargylglycine **26** (entry 5) afforded the glycoamino acid **32** in 85% yield.

In addition, subjection of the acetylenic aminonitrile **27**¹⁷ gave the desired adduct **33** in 60% yield.

Tables 3 and 4 show that the scope of the click reactions can be extended to dipeptides and disaccharides, respectively.

Table 4. Cycloaddition Reactions Involving a Disaccharide

entry	amino acid	glycopeptide ^{a,b}
1		39 (71%)
2		40 (81%)

^a Reagents and conditions: 1 equiv of azidoglycoside **38**, 1 equiv of amino acid, 0.2 equiv of $\text{Cu}(\text{OAc})_2$, 0.4 equiv of sodium ascorbate, $\text{H}_2\text{O}/\text{tert-BuOH}$ 1:1 (v/v), rt, 16 h. ^b Yield of isolated product.

For example, the two protected dipeptides **34** and **35**¹⁸ were successfully coupled to glycosyl azide **8**, affording the glycopeptides **36** and **37** in 51 and 92% yields, respectively.

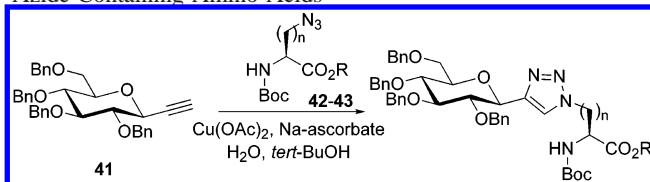
(17) Prepared from Boc-protected (*S*)-propargylglycine amide via dehydration: Cossu, S.; Giacomelli, G.; Conti, S.; Falorni, M. *Tetrahedron* **1994**, 50, 5083.

(18) Dipeptides **34** and **35** were synthesized using a standard coupling reaction involving PyBOP.

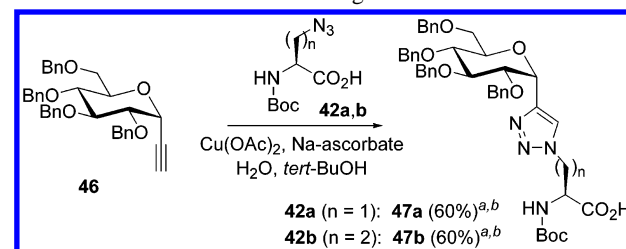
Inversely, the disaccharide azide **38**¹⁹ was coupled to amino acid **7** and dipeptide **34** in satisfactory yields (Table 4). Considering the fact that disaccharides and dipeptides couple very well under these conditions, we anticipate that also larger and more complex oligosaccharides and oligopeptides can be successfully ligated in this way.

Since all examples so far comprise the combination of azidoglycosides with acetylenic amino acids, we were intrigued whether ligation of constituents with inverted functionality, with the triazole-N1 position bound to the amino acid, would also be feasible. To this end, acetylenic glycosides and azide-containing amino acid derivatives were prepared: the α - and β -acetylenic glucose derivatives **41** and **46**, respectively, from 2,3,4,6-tetra-*O*-benzyl-D-glucose,²⁰ and the required amino acid azides (**42a–c** and **43**) from the corresponding amines via azido transfer using TfN₃.²¹ Evidently, these “reversed” triazole-linked glycopeptides were also readily obtained via the Cu-catalyzed [3 + 2] cycloaddition. Both the α - and β -glycopeptide products were formed uneventfully in yields ranging from 60 to 84% (Table 5 and Scheme 2). In general, amino acid derivatives

Table 5. Cycloaddition between a β -Acetylenic Glycoside and Azide-Containing Amino Acids



Scheme 2. Cycloaddition between an α -Acetylenic Glycoside and Azide-Containing Amino Acids



^a Reagents and conditions: 1 equiv of acetylenic glycoside **46**, 1 equiv of azide, 0.2 equiv of Cu(OAc)₂, 0.4 equiv of sodium ascorbate, H₂O/*tert*-BuOH 1:1 (v/v), rt, 16 h. ^b Yield of isolated product.

may explain the slightly lower yields. Furthermore, there is again virtually no change in reactivity upon elongation of the amino acid side chain as can be inferred from Table 5, entries 1–3, and Scheme 2.

In conclusion, we have developed a straightforward, versatile, and high-yielding method for the synthesis of a novel class of glycopeptides. Both α - and β -triazole-linked glycopeptides can be efficiently prepared using a variety of suitably functionalized (oligo)saccharides and (oligo)peptides. Currently, we aim at further expanding the scope of this application and more specifically at the synthesis of triazole-containing mimics of biologically active glycopeptides.

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Supporting Information Available: Experimental procedures and spectroscopic data of the triazole-linked glycopeptides. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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entry	amino acid	n	R	glycopeptide ^a	yield (%) ^b
1	42a	1	H	44a	70%
2	42b	2	h	44b	73%
3	42c	3	H	44c	71%
4	43	1	Me	45	84%

^a Reagents and conditions: 1 equiv of acetylenic glycoside **41**, 1 equiv of azide, 0.2 equiv of Cu(OAc)₂, 0.4 equiv of sodium ascorbate, H₂O/*tert*-BuOH 1:1 (v/v), rt, 16 h. ^b Yield of isolated product.

containing a free carboxylic acid appeared to be somewhat harder to purify than their methyl ester counterparts, which

(19) Hepta-*O*-acetyl- β -lactosyl azide (**38**) was prepared from lactose by peracetylation followed by treatment with SnCl₄ and Me₃SiN₃.⁸

(20) Dondoni, A.; Mariotti, G.; Marra, A. *J. Org. Chem.* **2002**, 67, 4475.

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