ORGANIC LETTERS

2004
Vol. 6, No. 18
3123−3126

Expedient Synthesis of Triazole-Linked Glycosyl Amino Acids and Peptides

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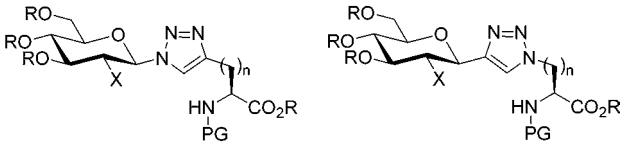
Received June 18, 2004

ABSTRACT

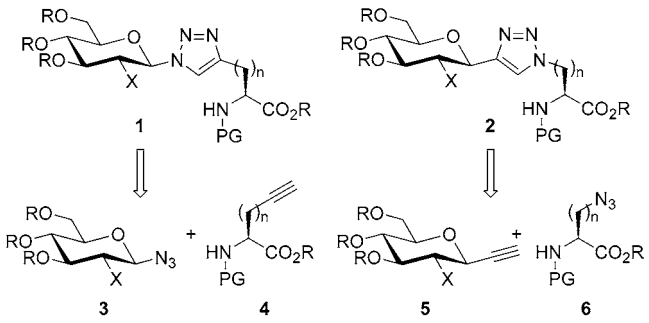


An expedient, high-yielding synthesis of two types of triazole-linked glycopeptides is described. These novel and stable glycopeptide mimics were prepared via Cu(I)-catalyzed [3 + 2] cycloaddition of either azide-functionalized glycosides and acetylenic amino acids or acetylenic glycosides and azide-containing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

Glycopeptides[1] 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 activity[3] (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 procedure for the [3 + 2] cycloaddition between organic azides and acetylenes ("click reactions"). As recently independently reported by the groups of Meldal[5] and Sharpless,[6] this reaction generally results in the corresponding 1,4-disubstituted 1,2,3-triazoles in high yields. Application of

Scheme 1. Retrosynthesis



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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.
(2) (a) Kunz, H. *Angew. Chem., Int. Ed.* **1987**, *26*, 294. (b) Spiro, R. G. *Glycobiology* **2002**, *12*, 43R. (c) Seitz, O. *ChemBioChem* **2000**, *1*, 214.
(3) (a)Wu, T. C.; Goekjian, P. G.; Kishi, Y. *J. Org. Chem.* **1987**, *52*, 4819. (b) Haneda, T.; Goekjian, P. G.; Kim, S. H.; Kishi, Y. *J. Org. Chem.* **1992**, *57*, 490.

10.1021/ol048841o CCC: $27.50
Published on Web 07/31/2004

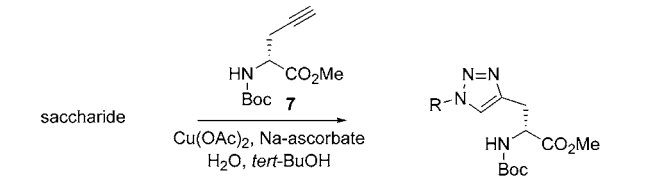
this reaction to either azidoglycosides **3** and acetylenic amino acids **4** or acetylenic glycosides **5** and azide-containing amino acids **6** would then result in the triazole-linked glycopeptides **1** and **2**, respectively, which constitute a novel compound class.[7] Besides the aim of constructing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

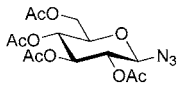
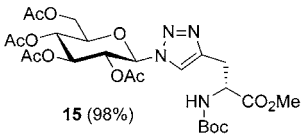
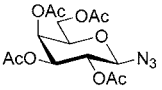
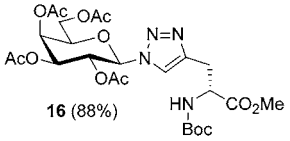
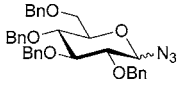
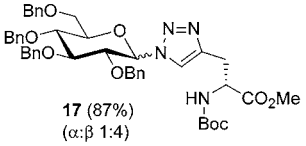
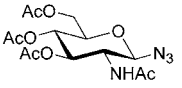
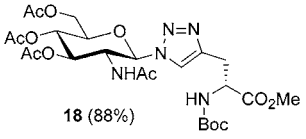
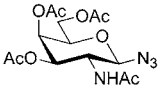
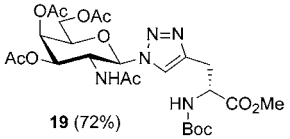
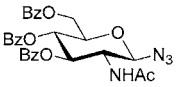
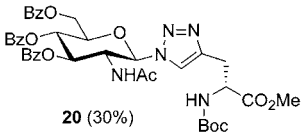
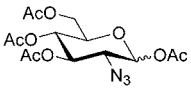
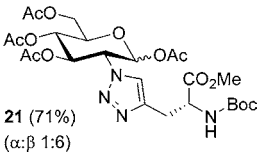
To probe the viability of our approach, various glycosides containing an anomeric azide functionality were prepared and reacted with (*R*)-*N*-Boc-propargylglycine methyl ester (**7**, Table 1).[9] The coupling was initially studied using azidoglucoside (**8**) and azidogalactoside (**9**)[10] in combination with different Cu(I) species (e.g., CuI, CuCl, and CuCN) and different bases (e.g., Et_3N 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 H_2O 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 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

Table 1. Variation of the Carbohydrate Moiety



entry	saccharide	glycopeptide[a,b]
1	 **8**	 **15** (98%)
2	 **9**	 **16** (88%)
3	 **10** (α:β 1:4)	 **17** (87%) (α:β 1:4)
4	 **11**	 **18** (88%)
5	 **12**	 **19** (72%)
6	 **13**	 **20** (30%)
7	 **14** (α:β 1:6)	 **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, H_2O/*tert*-BuOH 1:1 (v/v), rt, 16 h. [b] Yield of isolated product.

(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.; Schmidtmann, 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.

(5) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.

(6) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596.

(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.

(10) Shiozaki, M.; Arai, M.; Macindoe, W. M.; Mochizuki, T.; Kurakata, S.; Maeda, H.; Nishijima, M. *Chem.* Lett. **1996**, *9*, 735

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

(12) Prepared via tetra-*O*-acetyl-α-D-pyranosyl chloride from the corresponding glucosamine and galactosamine (ref 13) and subsequent treatment with NaN_3: 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 Me_3SiN_3, 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]

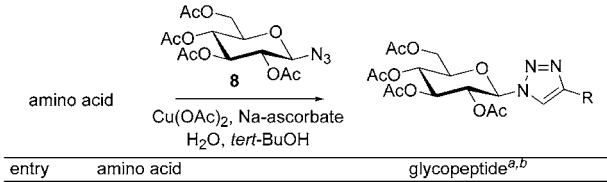
Table 2 shows the application of different acetylenic amino acids containing a variety of amino protecting groups in the cycloaddition with azidoglycoside **8**. From Table 1, the compatibility of the Boc group with these reaction conditions was already clear; entries 1 and 2 of Table 2 now show that Fmoc and Ts are also well-suited for this reaction. Increasing the length of the side chain of the amino acid[16] (entries 3 and 4) showed no significant change in reactivity, providing 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.

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.

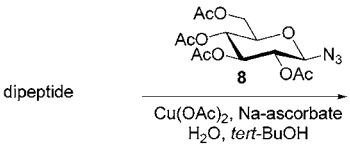
Table 2. Variation of the Amino Acid Moiety



entry	amino acid	glycopeptide[a,b]
1	**22**	**28** (70%)
2	**23**	**29** (64%)
3	**24**	**30** (70%)
4	**25**	**31** (76%)
5	**26**	**32** (85%, 1:1)
6	**27**	**33** (60%)

[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, H_2O/*tert*-BuOH 1:1 (v/v), rt, 16 h. [b] Yield of isolated product.

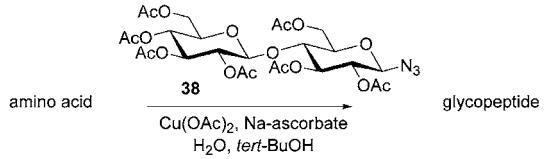
Table 3. Cycloaddition Reactions Involving a Dipeptide



entry	dipeptide	glycopeptide[a,b]
1	**34**	**36** (51%)
2	**35**	**37** (92%)

[a] Reagents and conditions: 1 equiv of azidoglycoside **8**, 1 equiv of dipeptide, 0.2 equiv of $Cu(OAc)_2$, 0.4 equiv of sodium ascorbate, H_2O/*tert*-BuOH 1:1 (v/v), rt, 16 h. [b] Yield of isolated product.

Table 4. Cycloaddition Reactions Involving a Disaccharide



entry	amino acid	glycopeptide[a,b]
1	**7**	**39** (71%)
2	**34**	**40** (81%)

[a] Reagents and conditions: 1 equiv of azidoglycoside **38**, 1 equiv of amino acid, 0.2 equiv of $Cu(OAc)_2$, 0.4 equiv of sodium ascorbate, H_2O/*tert*-BuOH 1:1 (v/v), rt, 16 h. [b] Yield of isolated product.

(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.

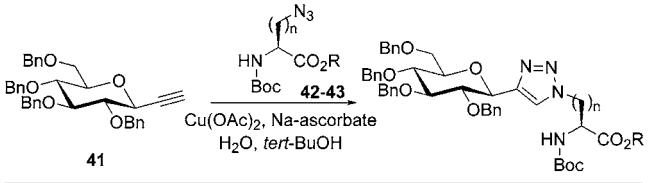
(17) Prepared from Boc-protected (*S*)-propargylglycine amide via dehydration: Cossu, S.; Giacomelli, G.; Conti, S.; Falorni, M. *Tetrahedron* **1994**, *50*, 5083.

(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 TfN_3.[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 containing a free carboxylic acid appeared to be somewhat harder to purify than their methyl ester counterparts, which 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.

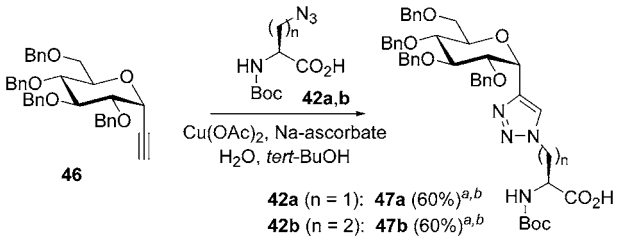
Table 5. Cycloaddition between a *β*-Acetylenic Glycoside and Azide-Containing Amino Acids



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, H_2O/*tert*-BuOH 1:1 (v/v), rt, 16 h. [b] Yield of isolated product.

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, H_2O/*tert*-BuOH 1:1 (v/v), rt, 16 h. [b]Yield of isolated product.

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.

Acknowledgment. S. A. M. W. van den Broek, B. C. J. van Esseveldt, B. Verheijen, and M. IJsselstijn are kindly acknowledged for the preparation of several compounds. Senter (Ministry of Economic Affairs, The Netherlands) is gratefully acknowledged for providing financial support.

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.

OL048841O

(19) Hepta-*O*-acetyl-*β*-lactosyl azide (**38**) was prepared from lactose by peracetylation followed by treatment with $SnCl_4$ and Me_3SiN_3.[8]

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