ORGANIC LETTERS

2004
Vol. 6, No. 17
2929−2932

Assembling Heterocycle-Tethered *C*-Glycosyl and α-Amino Acid Residues via 1,3-Dipolar Cycloaddition Reactions

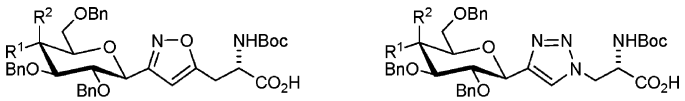
Alessandro Dondoni,* Pier Paolo Giovannini, and Alessandro Massi

Laboratorio di Chimica Organica, Dipartimento di Chimica, Università di Ferrara, Via L. Borsari 46, I-44100 Ferrara, Italy

adn@dns.unife.it

Received June 4, 2004

ABSTRACT



(R^1 = H, R^2 = OBn)
(R^1 = OBn, R^2 = H)

The 1,3-dipolar cycloadditions of *C*-glycosyl nitrile oxides and acetylenes to an alkyne and an azide, respectively, bearing a masked glycinyl moiety furnished disubstituted isoxazoles and triazoles. Unveiling the glycinyl group in these cycloadducts afforded *C*-glycosyl α-amino acids in which the two bioactive entities were tethered through rigid five-membered heterocycles. Optimized entries to the same compounds involved the use of unmasked but protected alkyne- and azide-containing amino acids as the partners of 1,3-dipolar cycloadditions.

In respect to native *O*- and *N*-glycosides, anomeric carbon-linked glycoconjugates are impervious to chemical and enzymatic degradation and do not undergo hydrogen bonding at the former anomeric position. Therefore there has been over the past 2 decades a great deal of effort by organic chemists to develop expedient syntheses of various *C*-glycosides.[1] In the realm of this rapidly growing field of carbohydrate chemistry, *C*-glycosyl amino acids such as isosteres of *O*-glycosyl serines and *N*-glycosyl asparagines have been attracting intense interest[2] because of their potential service as probes in studies of glycopeptide biological activity[3] and as leads for the development of new drugs against carbohydrate-based metabolic disorders.[4] A special family of *C*-glycosyl α-amino acid are the *C*-glycosylacetylene-phenylalanine residues reported by Meldal and Vasella and their co-workers.[5] These compounds were designed as rigidified systems by virtue of their acetylene bridges, which were expected to confer new chemical, structural, and biological characteristics on glycopeptides in which they were eventually incorporated. As a further contribution to this area, we thought it interesting to prepare another class of rigidified *C*-glycosyl α-amino acids in which the tether holding the sugar and amino acid residue was constituted of a heteroaromatic ring. Needless to say, such a structural motif is present in a natural product, i.e., the *C*-mannopyranosyltryptophan, the only native *C*-glycosyl amino acid so far discovered in which the heterocycle is represented by the rigid indole ring.[6] The method of executing our plan became readily apparent after considering the increasing attention that is being paid to the development of chemoselective ligation methodologies of biologically active entities via 1,3-dipolar cycloaddition reactions (1,3-DCRs).[7] Therefore we will present below efficient approaches to *C*-glycosylated isoxazole and triazole alanines

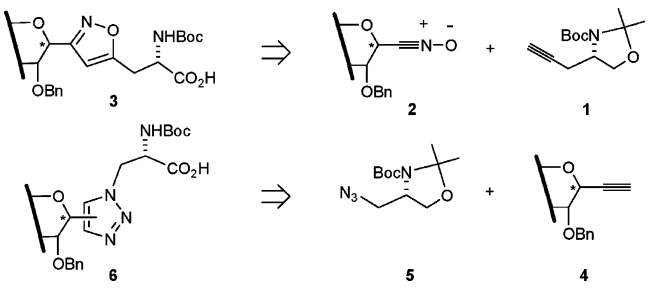
(1) Postema, M. H. D. *C-Glycoside Synthesis*; CRC Press: Boca Raton, 1995.
(2) Dondoni, A.; Marra, A. *Chem. Rev.* **2000**, *100*, 4395−4421.
(3) (a) Varki, A. *Glycobiology* **1993**, *3*, 97−130. (b) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683−720. (c) Varki A, Cummings, R.; Esko, J.; Freeze, H.; Hart, G.; Marth, J. *Essentials of Glycobiology*; Cold Spring Harbor Laboratory Press: New York, 1999.
(4) Wong, C.-H. *Carbohydrate-Based Drug Discovery*; Wiley-VCH: Weinheim, 2003.
(5) Lowary, T.; Meldal, M.; Helmboldt, A.; Vasella, A.; Bock, K. *J. Org. Chem.* **1998**, *63*, 9657−9668.
(6) (a) Beer, T.; Vliegenthart, J. F. G.; Löffler, A.; Hofsteenge, J. *Biochemistry* **1995**, *34*, 11785−11789. (b) Löffler, A.; Doucey, M.-A.; Jansson, A. M.; Müller, D. R.; Beer, T.; Hess, D.; Meldal, M.; Richter, W. J.; Vliegenthart, J. F. G.; Hofsteenge, J. *Biochemistry* **1996**, *35*, 12005−12014. (c) Doucey, M.-A.; Hess, D.; Blommers, M. J. J.; Hofsteenge, J. *Glycobiology* **1999**, *9*, 435−441.

10.1021/ol048963g CCC: $27.50 © 2004 American Chemical Society
Published on Web 07/30/2004

(see graphical abstract) via the venerable Huisgen 1,3-DCRs of nitrile oxides and azides to acetylenes.[8] This work stems from our recent studies on *C*-glycosyl amino acid[2] and heterocycle amino acid[9] synthesis as the basic operation toward the construction of designed bioactive molecules, peptides in primis, with new biological properties.

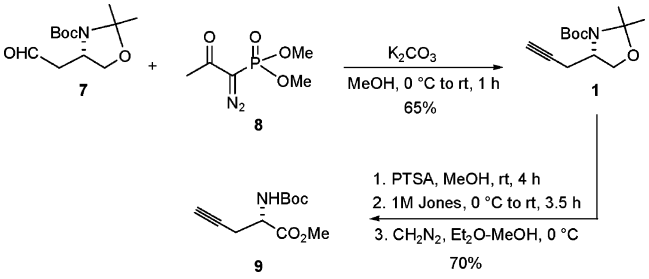
Toward such a target as the sugar and amino acid functionalized isoxazole **3** and triazole **6** systems via 1,3-DCRs, a retrosynthetic blueprint (Scheme 1) was carried out

Scheme 1. Synthetic Strategy



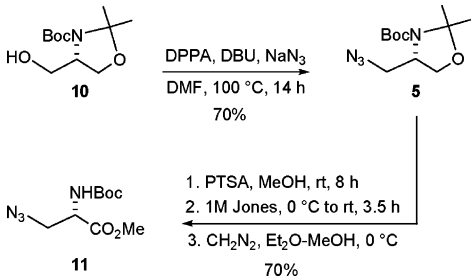
by taking into account the ready access to anomeric sugar nitrile oxides **2** and acetylenes **4** from recent chemistry developed in our and other laboratories.[10,11] Appropriate partners for the planned cycloadditions were the acetylene **1** and the azide **5**, both carrying the *N*-Boc oxazolidine ring as a valuable masked glycinyl moiety.[9a,12] The preparation of these new reagents was straightforward starting from the protected homoserinal[9] **7** and the phosphonate[13] **8** for the synthesis of **1** (Scheme 2), and the protected serinol[14] **10**

Scheme 2. Synthesis of Masked and Protected Ethynyl Functionalized Glycines **1** and **9**



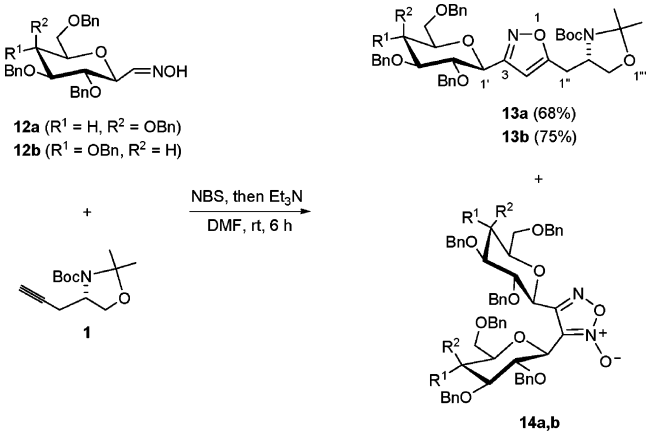
and commercially available reagents for the synthesis of **5** (Scheme 3).

Scheme 3. Synthesis of Masked and Protected Azido Functionalized Glycines **5** and **11**



Taking advantage of our earlier experience in nitrile oxide generation from *C*-glycosyl aldoximes,[10b] we first treated a DMF solution of the *C*-galactosyl oxime **12a** and alkyne **1** (10.0 equiv) with *N*-bromosuccinimide (NBS) and then added Et_3N dropwise (Scheme 4). Chromatography of the reaction

Scheme 4. Initial Approach toward Synthesis of *C*-Glycosyl Isoxazole Alanines



mixture furnished the 3,5-disubstituted isoxazole cycloadduct **13a** (4-H δ 6.23 ppm, DMSO-d_6, 120 °C) in good yield (68%) as the sole regioisomer,[15] alongside the furoxan side product **14a**.[10b] Noteworthy is that the unreacted dipolarophile **1** was recovered almost completely in very pure form. Under the same conditions, the *C*-glucosyl oxime **12b** and

(7) (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004−2021. (b) Fazio, F.; Bryan, M. C.; Blixt, O.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **2002**, *124*, 14397−14402. (c) Perez-Balderas, F.; Ortega-Munoz, M.; Morales-Sanfrutos, J.; Hernandez-Mateo, F.; Calvo-Flores, F. G.; Calvo-Asin, J. A.; Isac-Garcia, J.; Santoyo-Gonzalez, F. *Org. Lett.* **2003**, *5*, 1951−1954. (d) Lee, L. V.; Mitchell, M. L.; Huang, S.-J.; Fokin, V. V.; Sharpless, K. B.; Wong, C.-H. *J. Am. Chem. Soc.* **2003**, *125*, 9588−9589.

(8) Huisgen, R. *Angew. Chem., Int. Ed. Engl.* **1963**, *2*, 565−598; 633−645. For reviews, see: (a) Padwa, A. *1,3-Dipolar Cycloaddition Chemistry*; Wiley: New York, 1984. (b) Padwa, A.; Pearson, W. H. *Synthetic Applications of 1,3-Dipolar Cycloaddition Chemistry Toward Heterocycles and Natural Products*; Wiley: New York, 2003.

(9) (a) Dondoni, A.; Massi, A.; Minghini, E.; Sabbatini, S.; Bertolasi, V. *J. Org. Chem.* **2003**, *68*, 6172−6183. (b) Dondoni, A.; Massi, A.; Minghini, E.; Bertolasi, V. *Tetrahedron* **2004**, *60*, 2311−2326.

(10) *C*-Glycosyl nitrile oxides: (a) Baker, K. W. J.; March, A. R.; Parsons, S.; Paton, M.; Stewart, G. W. *Tetrahedron* **2002**, *58*, 8505−8513. (b) Dondoni, A.; Giovannini, P. P. *Synthesis* **2002**, 1701−1706.

(11) Ethynyl *C*-glycosides: (a) Nishika, T.; Ishikawa, M.; Isobe, M. *Synlett* **1999**, 123−125. (b) Dondoni, A.; Mariotti, G.; Marra, A. *J. Org. Chem.* **2002**, *67*, 4475−4486.

(12) (a) Garner, P.; Yoo, J. U.; Sarubu, R.; Kennedy, V. O.; Youngs, W. J. *Tetrahedron* **1998**, *54*, 9303−9316. (b) Dondoni, A.; Marra, A.; Massi, A. *J. Org. Chem.* **1999**, *64*, 933−944.

(13) Callant, P.; D'Haenens, L.; Vandewall, M. *Synth. Commun.* **1984**, *14*, 155−161.

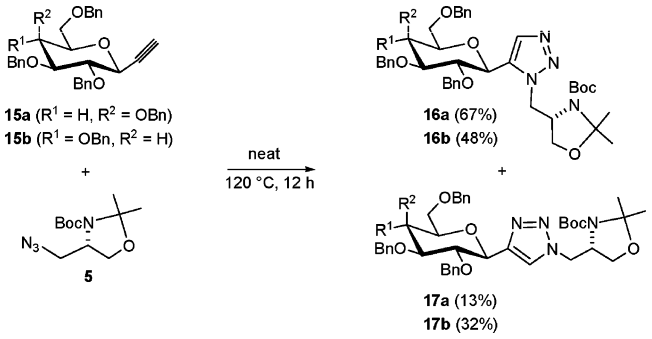
(14) Dondoni, A.; Perrone, D. *Org. Synth.* **1999**, *77*, 64−77.

(15) Grünanger, P.; Vita-Finzi, P. *The Chemistry of Heterocyclic Compounds. Isoxazoles*; Wiley: New York, 1991; Vol. 49, Part. 1, pp 24−55 and 183−186.

the dipolarophile **1** afforded the isoxazole **13b** (4-H δ 6.42 ppm, DMSO-d_6, 100 °C) in yield comparable to that for **13a** (75%).

Next, we examined the triazole synthesis by coupling the azide **5** with the ethynyl *C*-galactoside **15a**[11b] in 1:1 molar ratio at 120 °C under solvent-free conditions (Scheme 5).

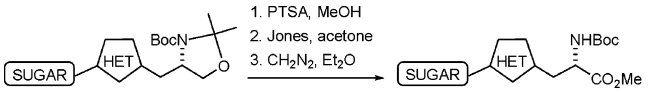
Scheme 5. Initial Approach toward Synthesis of *C*-Glycosyl Triazole Alanines

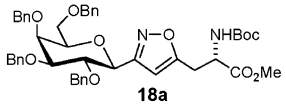
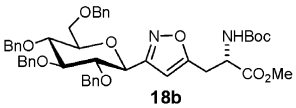
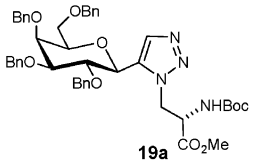
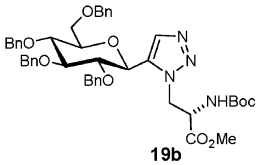
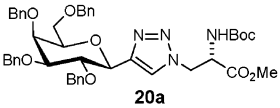
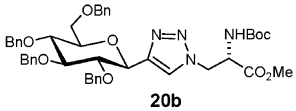


This fusion process furnished a mixture of 1,5- and 1,4-disubstituted triazoles **16a** and **17a** in 5:1 ratio (as determined by ^{1}H NMR analysis) and 80% overall yield after chromatographic purification. Under the same conditions the ethynyl *C*-glucoside **15b**[11b] and the azide **5** afforded the corresponding triazole regioisomers **16b** and **17b** in 1.5:1 ratio and 80% yield. The above product ratios were established after a careful characterization of each isomer. ^{1}H NMR spectra (DMSO-d_6, 100 °C) of 1,4-regioisomers **17a,b** exhibited the triazole proton (δ ~8.0 ppm) sensibly shifted downfield compared to the corresponding 1,5-isomers **16a,b** (δ ~7.6 ppm). This finding is in agreement with earlier assignments of 1,4- and 1,5-disubstitued triazole derivatives based on ^{1}H NMR spectroscopy.[16]

The final conversion of isoxazole and triazole cycloadducts **13a,b**, **16a,b**, and **17a,b** into the target α-amino acids was then achieved by acetonide removal and subsequent oxidation (see Supporting Information).[17] All carboxylic acids thus prepared were conveniently characterized as their methyl esters **18a,b**, **19a,b**, and **20a,b** shown in Table 1. It is important to point out that the yields of isolated **19a,b** and **20a,b** were substantially altered by the loss of material upon chromatography on silica gel of the crude reaction mixtures. It is also worth noting that **18a** can also be regarded as a

Table 1. Unveiling of Glycinyl Moiety in Cycloadducts **13a,b**, **16a,b**, and **17a,b**



entry	cycloadduct	product	yield (%)[a]
1	**13a**	 **18a**	75
2	**13b**	 **18b**	78
3	**16a**	 **19a**	48
4	**16b**	 **19b**	50
5	**17a**	 **20a**	45
6	**17b**	 **20b**	48

[a] Isolated yields.

conformationally constrained analogue of a *C*-galactosyl hydroxynorvaline methylene isostere (β-D-Gal-CH$_2$-Hnv). This amino acid has been recently synthesized[18,19] and used in immunological studies, such as for the induction of tolerance in autoimmune rheumatoid arthritis.

Of relevance to the elaboration of these amino acid esters into appropriate building blocks for peptide synthesis, was the demonstration that their *O*-benzyl protecting groups could be reductively removed without affecting the heterocyclic rings. Addressing this issue was especially important in the case of the isoxazole derivatives because the isoxazole ring can be easily cleaved by reducing agents.[20] Hence, the debenzylation of compounds **18a** and **19b** was carried out by Pd(OH)$_2$-catalyzed hydrogenation under mild conditions to afford the corresponding compounds with free hydroxy groups in very high yields (see Supporting Information).[21]

Aiming at simplifying access to the heterocycle amino esters of Table 1, we sought a more direct approach to these

(16) (a) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057−3064. (b) Wang, Z.-X.; Qin, H.-L. *Chem. Commun.* **2003**, 2450−2451 and references therein. Unfortunately, unambiguous structural assignment of cycloadducts **16a,b** and **17a,b** or their derivatives by NOE was unsuccessful. Nevertheless, the structures of all compounds derived from **16a,b** and **17a,b** were consistent with their respective ^{1}H and also ^{13}C NMR spectra (4-C, δ ~132 ppm; 5-C, δ ~135 ppm in 1,5-regioisomers. 4-C, δ ~144 ppm; 5-C δ ~124 ppm in 1,4-regioisomers). For ^{13}C NMR spectral data of 1,4- and 1,5-disubstituted triazoles, see: Crandall. J. K.; Crawley, L. C.; Komin, J. B. *J. Org. Chem.* **1975**, *40*, 2045−2047. Katritzky, A. R.; Lagowski, J. M. In *Comprehensive Heterocyclic Chemistry*; Potts, K. T., Ed.; Pergamon Press: Oxford, 1984; pp 15−16.

(17) Attempts to carry out the one-pot oxidative cleavage of the oxazolidine ring with the Jones reagent as described (see ref 12b) turned out to be fruitless.

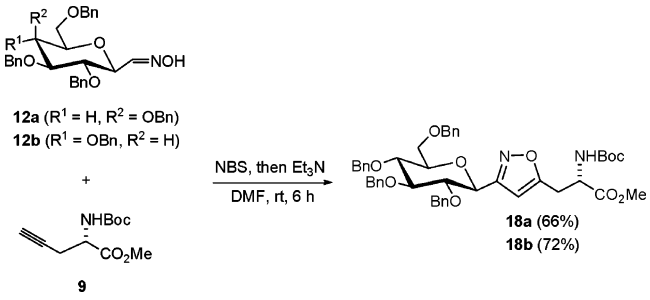
(18) Dondoni, A.; Giovannini, P. P.; Marra, A. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2380−2388.

(19) Wellner, E.; Gustafsson, T.; Bäcklund, J.; Holmdahl, R.; Kihlberg, J. *ChemBioChem* **2000**, *1*, 272−280.

(20) Grünanger, P.; Vita-Finzi, P. *The Chemistry of Heterocyclic Compounds. Isoxazoles*; Wiley: New York, 1991; Vol. 49, Part 1, p 278.

compounds using the readily available ethynyl and azido functionalized amino esters **9** and **11** (Schemes 2 and 3) as partners of the 1,3-DCRs. A matter of concern, however, was the stereochemical integrity of the unmasked glycinate moiety under the conditions of the 1,3-DCRs. Pressing forward nonetheless, nitrile oxide generation from the sugar oximes **12a** and **12b** in the presence of excess of the alkyne **9** at ambient temperature (Scheme 6) furnished the 3,5-

Scheme 6. Optimized Synthesis of *C*-Glycosyl Isoxazole Alanines



disubstituted isoxazoles **18a** and **18b** in comparable overall yields (~50% from **1**).

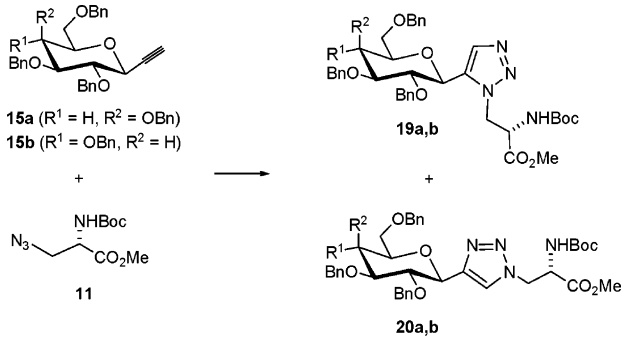
Next, we examined the synthesis of the triazoles **19** and **20**. These were achieved by heating the sugar acetylenes **15a** and **15b** with the azide **11** at 120 °C without solvent for 2 h (Table 2).[22] The corresponding 1,5- and 1,4-disubstituted triazoles **19a**/**20a** and **19b**/**20b** were obtained in almost identical ratios but in better overall yields (~60% vs ~40% from **5**) in respect to the previous route. This improved procedure reduced, in fact, the loss of material caused by the chromatographic purification of intermediates.

Looking at the above results from the viewpoint of a preparative efficiency, a disadvantage lies in the formation of mixtures of 1,4- and 1,5-disubstituted triazoles.[8] A simple solution to this longstanding problem has relied upon adopting the latest advance in this chemistry, that is,

(21) Crucial for the selective hydrogenolysis of benzyl groups in **18a** was the presence of acetic acid in the reaction medium. For a wider discussion on the effect of pH in the hydrogenation of isoxazole derivatives, see: Stork, G.; Danishefsky, S.; Ohashi, M. *J. Am. Chem. Soc.* **1967**, *89*, 5459−5460.

(22) A prolonged reaction time caused partial epimerization at the α-carbon of the target *C*-glycosyl α-amino acids.

Table 2. Cycloadditions of Sugar Alkynes **15a** and **15b** with the Azide **11** (1.0 equiv)



alkyne	thermal[a]	catalyzed[b]
15a	**19a** (68)[c] + **20a** (13)[c]	**20a** (80)[c]
15b	**19b** (47)[c] + **20b** (31)[c]	**20b** (82)[c]

[a] Neat, 120 °C, 2 h. [b] Toluene, CuI (0.1 equiv), DIPEA (1.0 equiv), rt, 15 h. [c] Isolated yields.

performing nonconcerted Cu(I)-catalyzed reactions. This produced only 1,4-disubstituted regioisomers.[16a,23] Thus, treatment of the sugar alkynes **15a** and **15b** with 1.0 equiv of the azide **11** and CuI (0.1 equiv) in toluene and addition of diisopropylethylamine (DIPEA)[7b] afforded high yields (Table 2) of the expected 1,4-disubstitutd triazoles **20a** and **20b** as single products.

In summary, we have developed synthetic routes based on effective 1,3-DCRs that provide a rapid entry to a new class of unnatural *C*-glycosyl amino acids featuring a five-membered heterocyclic moiety (isoxazole or triazole) between the sugar and amino acid entities. A primary service of these amino acids can be foreseen as building blocks for new glycopeptides.

Acknowledgment. We gratefully acknowledge MIUR (COFIN 2002) for financial support.

Supporting Information Available: Experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

OL048963G

(23) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V. Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596−2599.