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COMMUNICATIONS

Published on Web 03/19/2003

Stereoselective Synthesis of α- and β-Glycosylamide Derivatives from Glycopyranosyl Azides via Isoxazoline Intermediates

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Recently, interest in the synthesis of glycosyl amides has grown because of the recognition of the importance of glycoproteins in diverse biochemical processes including intercellular communication, cell recognition, and cell growth.[1] In addition, glycopeptides can improve the absorption of poorly bioavailable drugs and peptides by enhancing membrane transport.[2] Typical naturally occurring glycoproteins have a β-*N*-linkage to an *N*-acetylglucosamine (Figure 1).

Recently, *N*-glucopyranosyl- and *N*-galactopyranosyl glycoprotein derivatives have been found in nature.[3,4] Nephritogenoside (Figure 2), isolated from the glomerular basement membrane of rats, is a glycopeptide with activity for the induction of glomerulnephritis.[4] This is the first example of a compound incorporating an α-*N*-glycosidic bond between glucose and asparagine.[4]

Several syntheses of nephritogenoside have been reported.[5] The stereoselective preparation of the α-*N*-glycopeptide linkage has proven to be a difficult task in these approaches. The key α-*N*-glucosylamide linkage in these syntheses has been made most frequently using Jeanloz's methodology in which a glycosyl-α-azide is reduced to anomeric amine and coupled with an aspartyl containing dipeptide.[5a−c] While this approach gave the desired glycodipeptide in good yield (65−80%), the stereoselectivity at the anomeric center was poor: this method resulted in α/β mixtures of glucosylamide product with ratios ranging from 1:5 to 5:1. The extent of anomerization of the intermediate α-glucosylamine depends on reducing conditions and protecting groups on the glycosyl moiety.[6]

Anomerization of 1-amino glycopyranosyl derivatives is so problematic that a number of alternative approaches to the synthesis of α-glycopeptide linkages have been developed which avoid intermediate formation of the free amine. For example, Fraser-Reid[5d,e] has shown that condensation of *n*-pentenyl- or thioglycosides[5f] with aspartic acid in the presence of NBS (or NIS) and acetonitrile affords α-*N*-linked glycopeptides. Alternatively, reaction of α-glycosyl azide with a carboxylic acid in the presence of tertiary phosphine, the Staudinger reaction,[7a] also affords α-*N*-linked glycopeptides, although anomerization remains as a significant problem.[7b−d]

The goal of this research was to develop a methodology for the stereoselective synthesis of an α-*N*-glycopeptide linkage from readily available 1-azidoglucopyranosyl derivatives **1** and **2**. In this approach, the stereochemistry of the intermediate isoxazoline **3** would control the stereochemistry at the newly formed anomeric center.

Our lab has recently reported the stereospecific synthesis of α- and β-glucopyranosyl (and glycosyl) azides from α- or β-glycopyranosyl chlorides, respectively.[8] Treatment of either β-azide **1** or α-azide **2** with Ph_3P in refluxing 1,2-dichloroethane in the presence of 4 Å molecular sieves for 15 h gave isoxazoline **3** (Scheme 1).

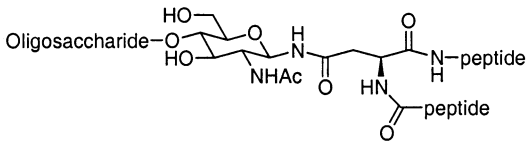
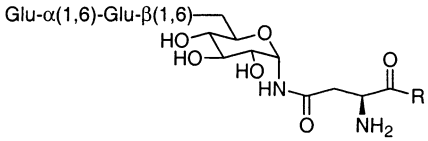


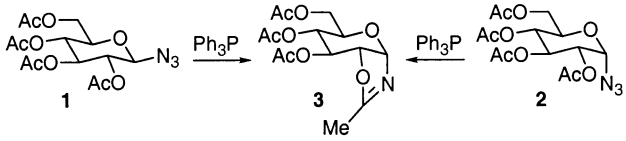
Figure 1. β-*N*-linkage to an *N*-acetylglucosamine in naturally occurring glycoproteins.



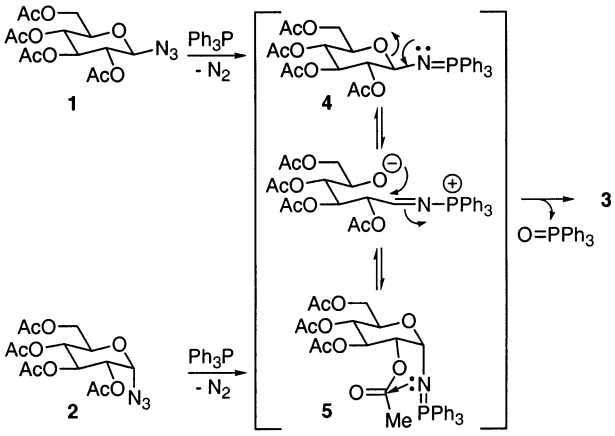
R = -NH-P-L-F-G-I-A-G-E-D-G-P-T-G-P-S-G-I-V-G-Q-OH

Figure 2. Structure of nephritogenoside.

Scheme 1. Synthesis of Glucopyranosyl Isoxazoline (**3**)



Scheme 2. Mechanism for the Formation of Isoxazoline **3**

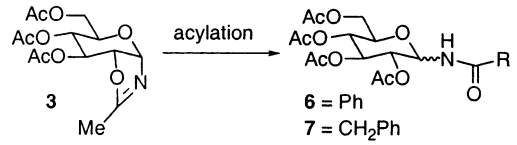


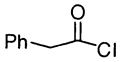
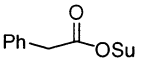
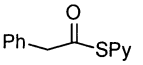
Formation of isoxazoline **3** from either azide can be explained by the mechanism shown in Scheme 2 involving α/β anomerization of the intermediate phosphorimines **4** and **5**.[7c,d] Isoxazoline formation from **4** cannot occur due to strain in the resulting product. Accordingly, epimerization followed by cyclization gave exclusively α-isoxazoline **3**.

Monitoring the reaction mixture by ^1H NMR indicated that isoxazoline **3** was the only glucosyl derivative observed in the NMR spectrum following the disappearance of starting material. Because the isoxazoline was relatively moisture sensitive, preliminary coupling studies with activated acid derivatives were undertaken

10.1021/ja028694u CCC: $25.00

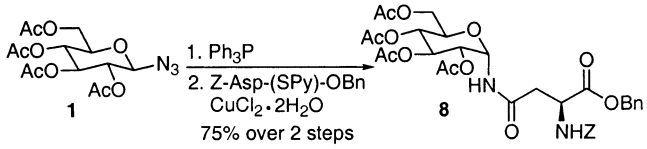
Table 1. Coupling Reactions of Glucopyranosyl Isoxazoline **3**[a]



entry	reagent[b]	equiv	additive[b]	temp (°C)[c]	yield (%)[d]	α/β[e]
1		4	-	83	**7** (51)	>19/1
2		4	-	83	**7** (25)	1/4
3		1.3	-	83	**7** (67)	5/1
4	"	1.3	$CuCl_2 \cdot 2H_2O$	30	**7** (80)	98/2

[a] All reactions were performed in 1,2-dichloroethane in the presence of 4 Å molecular sieves. All acylation reactions were run for 24 h. A listing of all acylating agents and reaction conditions investigated is summarized in the table in the Supporting Information. [b] Used in acylation step. Additives were added after reagents. [c] For acylation step. [d] Isolated yield (>95%). [e] Determined by ^{1}H NMR spectroscopy or HPLC analysis.

Scheme 3. Synthesis of α-*N*-Aspartyl Glucosylamine **8**



to determine the optimum acylating reagents for the reaction. The results are summarized in Table 1.

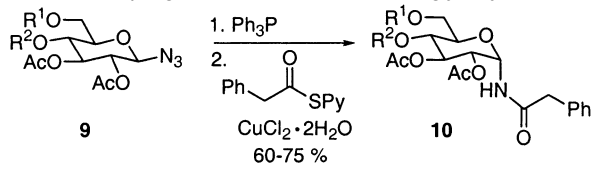
Acylation of isoxazoline **3** (formed in situ) with the reactive acid chloride gave the α-adduct in a highly stereoselective process (entry 1). However, acid chlorides were inappropriate reagents for general glycopeptide synthesis, and alternative derivatives were investigated.[9] Attempts to utilize *N*-hydroxysuccinimidyl (entry 2) or pentafluorophenyl esters in the acylation protocol met with mixed success because the yields and the α/*β* selectivity were poor. The thiopyridyl ester (entry 3), on the other hand, gave the α-adduct with high selectivity. By adding metal salts[9] to coordinate the pyridyl moiety, and presumably increasing the electrophilicity of the reagent, we accomplished the coupling reaction *at room temperature* to give *exclusively* the α-glucopyranosyl adduct in excellent yield (entry 4).

The generality of the thiopyridyl coupling methodology was demonstrated by the synthesis of α-glucopyranosyl asparagine derivative **8**. In situ isoxazoline formation by treatment of azide **1** was followed by acylation with the thiopyridyl ester of *N*-Z-protected aspartic benzyl ester in the presence of $CuCl_2 \cdot 2H_2O$ to give exclusively the α-asparagine adduct (Scheme 3).

The thiopyridyl coupling reactions were extended using di- and triglycosyl azides (**9a** and **9b**) to demonstrate the generality of the methodology (Scheme 4).

In conclusion, a stereoselective synthesis of α-*N*-glycopyranosyl amides has been developed that employs the readily available

Scheme 4. Coupling Reactions with Di- and Triglycosyl Azides



glycosyl azides as a starting material. Studies to extend this methodology to the synthesis of nephritogenoside, 2-*N*-acetamido-2-deoxy-*β*-glucosylamides, and to solid-phase synthesis are underway and will be reported in due course.

Acknowledgment. We thank the National Cancer Institute (CA-82169) for generous financial support. We also thank Dr. Yiu-Fai Lam (NMR) and Mr. Noel Whittaker (mass spectrometry) for their assistance in obtaining analytical data. This manuscript is dedicated to our colleague Robert Dorfman on the occasion of his 60th birthday.

Supporting Information Available: Extended version of table, experimental procedures, and spectral data of all new compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Varki, A. *Glycobiology* **1993**, *3*, 97−130. (b) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683−720. (c) Kobata, A. *Glycoconjugate J.* **2000**, *17*, 443−464. (d) *Glycoproteins and Disease*; Montreuil, J., Vliegenthart, J. F. G., Schachter, H., Eds.; Elsevier: Amsterdam, 1996. (e) Herzner, H.; Reipen, T.; Schultz, M.; Kunz, H. *Chem. Rev.* **2000**, *100*, 4495−4537.

(2) Polt, R.; Porreca, F.; Szabo, L. Z.; Bilsky, E. J.; Davis, P.; Abbruscato, T. J.; Davis, T. P.; Horvath, R.; Yamamura, H. I.; Hruby, V. J. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7114−7118.

(3) (a) Wieland, F.; Heitzer, R.; Schaefer, W. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 5470−5474. (b) Paul, G.; Lottspeich, F.; Wieland, F. *J. Biol. Chem.* **1986**, *261*, 1020−1024.

(4) (a) Shibata, S.; Miura, K. *J. Biochem.* **1987**, *89*, 1737−1749. (b) Shibata, S.; Takeda, T.; Natori, Y. *J. Biol. Chem.* **1988**, *263*, 12483−12485. (c) Takeda, T.; Sawaki, M.; Ogihara, Y.; Shibata, S. *Chem. Pharm. Bull.* **1989**, *37*, 54−56.

(5) (a) Teshima, T.; Nakajima, K.; Takahashi, M.; Shiba, T. *Tetrahedron Lett.* **1992**, *33*, 363−366. (b) Takeda, T.; Kojima, K.; Ogihara, Y. *Carbohydr. Res.* **1993**, *243*, 79−89. (c) Zhang, H.; Wang, Y.; Thürmer, R.; Al-qawasmeh, R. A.; Voelter, W. *Pol. J. Chem.* **1999**, *73*, 101−115 and references therein. (d) Ratcliffe, A. J.; Konradsson, P.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1990**, *112*, 5665−5667. (e) Nair, L. G.; Fraser-Reid, B.; Szardenings, A. K. *Org. Lett.* **2001**, *3*, 317−319 and references therein. (f) Sasaki, M.; Tachibana, K. *Tetrahedron Lett.* **1991**, *32*, 6873−6876.

(6) (a) Takeda, T.; Utsuno, A.; Okamoto, N.; Ogihara, Y. *Carbohydr. Res.* **1990**, *207*, 71−19. (b) Zhang, H.; Wang, Y.; Thürmer, R.; Parvez, K.; Atta-ur-Rahman, I. C.; Voelter, W. *Z. Naturforsch* **1999**, *54b*, 692−698.

(7) (a) For a review on Staudinger reaction, see: Gololobov, Y. G.; Kasukhin, L. F. *Tetrahedron* **1992**, *48*, 1353−1406. (b) Inazu, T.; Kobayashi, K. *Synlett* **1993**, 869−870. (c) Boullanger, P.; Maunier, V.; Lafont, D. *Carbohydr. Res.* **2000**, *324*, 97−106. (d) Kovács, L.; Ösz, E.; Domokos, V.; Holzer, W.; Györgydeák, Z. *Tetrahedron* **2001**, *57*, 4609−4621 and references therein.

(8) Soli, E. D.; Manoso, A. S.; Patterson, M. C.; DeShong, P.; Favor, D. A.; Hirschmann, R.; Smith, A. B. *J. Org. Chem.* **1999**, *64*, 3171−3177.

(9) A listing of all acylating agents and reaction conditions investigated is summarized in the table in the Supporting Information.

JA028694U