ORGANIC LETTERS

2006
Vol. 8, No. 22
5009−5012

A New Approach to the Neoglycopeptides: Synthesis of Urea- and Carbamate-Tethered *N*-Acetyl-D-glucosamine Amino Acid Conjugates

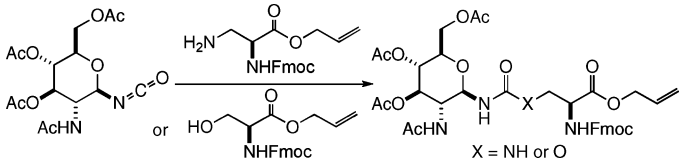
Yoshiyasu Ichikawa,* Fumiyo Ohara, Hiyoshizo Kotsuki, and Keiji Nakano

Faculty of Science, Kochi University, Akebono-cho, Kochi 780-8520, Japan

ichikawa@kochi-u.ac.jp

Received July 8, 2006

ABSTRACT



A novel approach to the synthesis of Fmoc-protected neoglycopeptide building blocks is described. Oxidation of *N*-acetyl-D-glucosamine isonitrile afforded the corresponding highly reactive glycopyranosyl isocyanate, which reacted with amino acid derivatives to furnish the corresponding urea- and carbamate-tethered Fmoc-protected *N*-acetyl-D-glucosamine amino acid conjugates in good yields.

Carbohydrates linked to the peptide backbone of proteins have become the focus of bioorganic and/or medicinal research work due to their involvement in diverse biochemical processes such as cellular recognition and adhesion.[1] Carbohydrates in natural glycoproteins are attached to the peptide backbones through the oxygen in the side chain of serine or threonine in *O*-linked glycoproteins or through the carboxamide nitrogen of asparagine in *N*-linked glycoproteins.[2] Although extensive research effort has been devoted to exploring the synthesis of accurately sequenced glycopeptides for biological and structural studies, total synthesis of the native glycopeptides still remains a challenging and time-consuming endeavor due to the difficulty of glycosyl-coupling reactions. In parallel, development of glycopeptide mimetics continues to attract much attention in order to supply homogeneous, stable, and readily accessible glycopeptide analogues for biological studies and therapeutic applications.[3] Accordingly, the covalent attachment of carbohydrates to peptides replaced with non-native linkages is of particular interest, with special attention having been paid to mimetics where the *O*-glycosidic linkage is replaced by carbon−carbon,[4] carbon−sulfur,[5] and carbon−aminooxy units.[6] For glycopeptide mimetics of natural glycopeptides with *N*-glycosidic linkages, the amide group in *N*-glycosides has been replaced by a retroamide subunit[7] and a urea glycosyl bond.[8]

During the course of developing a synthesis of various glycopeptide mimetics, we planned to pursue the solid-phase

(1) (a) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683. (b) Varki. A. *Glycobiology* **1993**, *3*, 97.

(2) (a) Marcaurelle. L. A.; Bertozzi, C. R. *Chem.—Eur. J.* **1999**, *5*, 1384. (b) H. Kunz. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 294.

(3) (a) Taylor, C. M. *Tetrahedron* **1998**, *54*, 11317. (b) Davis, B. G. *Chem. Rev.* **2002**, *102*, 579.

(4) (a) Burkhart, F.; Hoffmann, H.; Kessler, H. *Angew. Chem., Int. Ed.* **1997**, *36*, 1191. (b) Dondoni, A.; Mara, A. *Chem. Rev.* **2000**, *100*, 4395. (c) McGravey, G. J.; Benedum, T. E.; Schmidimann, F. W. *Org. Lett.* **2002**, *4*, 3591.

(5) (a) Davis, N. J.; Flitsch, S. L. *Tetrahedron Lett.* **1991**, *32*, 6793. (b) Jobron, L.; Hummel, G. *Org. Lett.* **2000**, *2*, 2265. (c) Knapp, S.; Myers, D. S. *J. Org. Chem.* **2001**, *66*, 3636. (d) Cohen, S. B.; Halcomb, R. L. *Org. Lett.* **2001**, *3*, 405. (e) Zhu, X.; Pachamuthu, K.; Schmidt, R. R. *J. Org. Chem.* **2003**, *68*, 5641 and references therein.

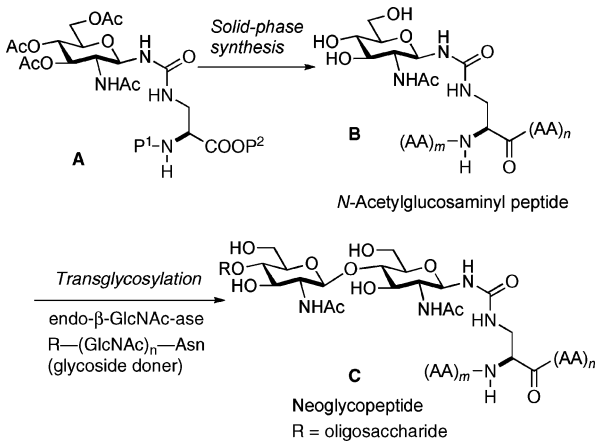
(6) Rodriguez, E. C.; Marcaurelle, L. A.; Bertozzi, C. R. J. *J. Org. Chem.* **1998**, *63*, 7134.

(7) Hoffmann, M.; Burkhart, F.; Hessler, G.; Kessler. H. *Helv. Chim. Acta* **1996**, *79*, 1519.

10.1021/ol0616788 CCC: $33.50
Published on Web 09/29/2006

synthesis of urea-tethered neoglycopeptides, the strategy for which is outlined in Scheme 1. Starting with urea-linked

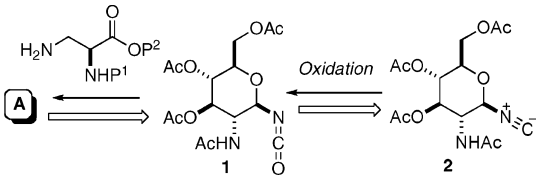
Scheme 1. Synthetic Strategy of Neoglycopeptides



N-acetyl-D-glucosamine amino acid conjugate **A**, solid-phase synthesis would give the *N*-acetyl-D-glucosaminyl peptide **B**. Transglycosylation of **B** using endo-β-GLcNAc-ase would provide the urea-tethered neoglycopeptide **C**.[9] In this paper, we report on the synthesis of the urea-tethered glycosyl amino acid (**A**, P^1 = Fmoc) and its use as a building block for solid-phase synthesis based on the Fmoc-strategy.

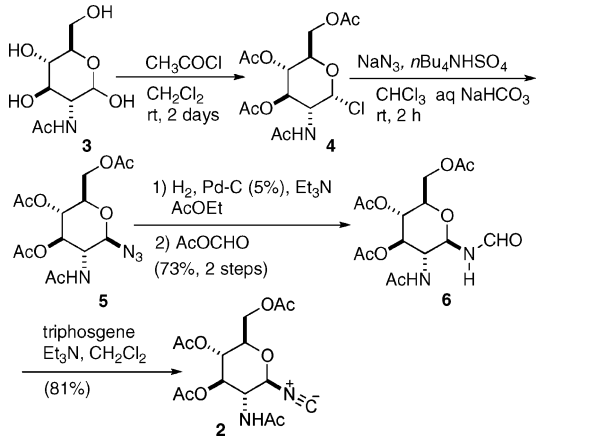
Our retrosynthetic analysis of **A** is shown in Scheme 2. A key feature of our plan was to construct the urea−glycosyl bond through a coupling reaction between isocyanate **1** and various α,β-diamino acid derivatives. Since such a highly functionalized isocyanate **1** was only postulated to be a transient intermediate by Pinter,[10] the crucial step in our approach was the synthesis of the reactive isocyanate **1**. To solve this problem, we planned to employ the oxidation of isonitrile **2** for the generation of **1** under mild reaction conditions.[11]

Scheme 2. Retrosynthetic Analysis of **A**



Starting with commercially available *N*-acetyl-D-glucosamine **3** (Scheme 3), Horton's protocol afforded α-chloro-*N*-acetyl-D-glucosamine acetate **4**,[12] which was further transformed into glycosyl azide **5** by the displacement reaction with sodium azide under phase-transfer conditions.[13] Catalytic hydrogenation of azide **5** followed by treatment of the resulting glycopyranosylamine with acetic formic anhydride furnished the formamide **6** in 73% yield over two steps. Dehydration of **6** with triphosgene/triethylamine gave the *N*-acetyl-D-glucosamine isonitrile **2** in good yield (81%).

Scheme 3. Preparation of *N*-Acetyl-D-glucosamine Isonitrile **2**



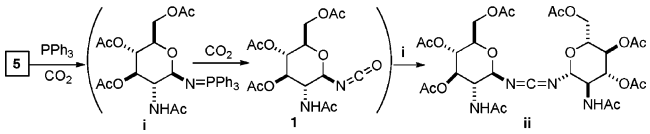
With the synthesis of isonitrile **2** established, we initially examined the synthesis of urea glycosides by the reaction of **1** with six different amines as summarized in Table 1. In a typical case (entry A), oxidation of **2** was carried out with pyridine *N*-oxide (3 equiv) and a catalytic amount of iodine (7 mol %) in acetonitrile in the presence of water scavenger (MS 3 Å).

The resulting solution, containing highly reactive isocyanate **1**, was immediately treated with phenethylamine (2.0 equiv).[14] To our delight, the urea glycoside **7a** was isolated in 92% yield. Primary amines having alkyl branches at the α-carbon (entry B and C) as well as secondary amine (entry D) smoothly reacted with **1** to afford the corresponding urea glycosides **7b**−**d** in good yields (>90%). Even in the case of a sterically hindered secondary amine, such as diisopropylamine (entry E), the corresponding urea glycoside **7e** was obtained in 91% yield. An application of **1** for the synthesis of urea-tethered disaccharide is represented in entry F, where aminosugar reacted with **1** to yield the urea-tethered pseudodisaccharide **7f** in 92% yield.

(8) Ichikawa, Y.; Nishiyama, T.; Isobe, M. *Synlett* **2000**, 1253.

(9) (a) Seitz, O.; Wong, C.-H. *J. Am. Chem. Soc.* **1997**, *119*, 8766. (b) Wang, L.-X.; Tang, M.; Suzuki, T.; Kitajima. K.; Inoue, Y.; Inoue, S.; Fan, J.-Q.; Lee, Y. *J. Am. Chem. Soc.* **1997**, *119*, 11137. (c) Mizuno, M.; Haneda, K.; Iguchi, R.; Muramoto, I.; Kawakami, T.; Aimoto, S.; Yamamoto, K.; Inazu, T. *J. Am. Chem. Soc.* **1999**, *121*, 284.

(10) Pinter reported reaction of azide **5** with triphenylphosphine and CO_2 and isolated the symmetrical carbodiimide **ii**. Although the intermediacy of isocyanate **1**, formed by reaction of iminophosphorane **i** with CO_2, was proposed, isolation or even detection of **1** has never been successful due to the rapid reaction of **i** with reactive isocyanate **1**. See: Kovacs, J.; Pinter, I.; Messmer, A. *Carbohydr. Res.* **1987**, *166*, 101.



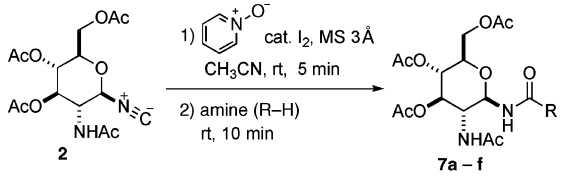
(11) Ichikawa, Y.; Nishiyama, T.; Isobe, M. *J. Org. Chem.* **2001**, *66*, 4200.

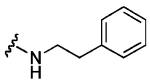
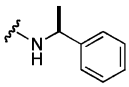
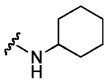
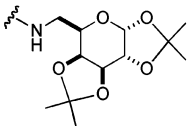
(12) Horton, D., Johnson, A. L.; McKusick, B. C. *Organic Syntheses*; Wiley: New York, 1973; Collect. Vol. V, p 1.

(13) Tropper, F. D.; Anderson, F. O.; Braun, S.; Roy, R. *Synthesis* **1992**, 619.

(14) Although silica gel TLC analysis of the reaction mixture showed the consumption of **2**, isocyanate **1** could not be observed by TLC. Accordingly, we employed 2 equiv of amines to optimize the yields.

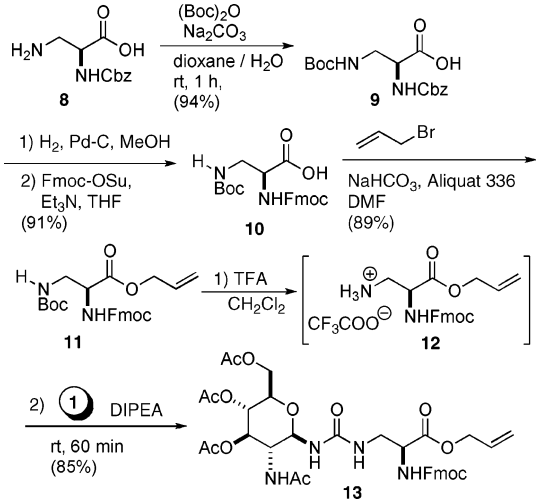
Table 1. Synthesis of Urea Glycosides from Isonitrile **2**



entry	product (R=)		yield (%)
A		**7a**	92
B		**7b**	92
C		**7c**	93
D		**7d**	91
E		**7e**	91
F		**7f**	92

Encouraged by the ready formation of urea glycosides by the reaction of **1** with amines, we next explored the synthesis of an Fmoc-protected *N*-acetyl-D-glucosamine amino acid conjugate **13** (Scheme 4). Boc-protection of α,*β*-diamino acid

Scheme 4. Synthesis of Urea-Linked *N*-Acetyl-D-glucosamine Amino Acid Conjugate **13**

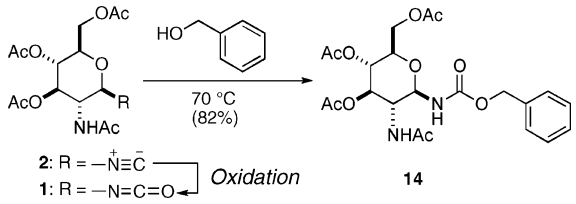


derivative **8**[15] followed by a deprotection−protection sequence gave the Fmoc-protected amino acid **10**.

Esterification of **10** furnished the fully protected α,*β*-diamino acid **11**. Removal of the *N*-Boc protecting group in **11** was carried out by treatment with TFA in CH_2Cl_2, and the resultant TFA salt **12** was treated with isocyanate **1** (2.0 equiv) in the presence of diisopropylethylamine at room temperature for 60 min. Fmoc-protected *N*-acetyl-D-glucosamine amino acid conjugate **13** was isolated in 85% yield after chromatographic purification.

During the course of developing our urea-tethered glycosyl amino acid conjugate, it was envisoned that the highly reactive isocyanate **1** would react with serine derivatives to afford a new type of carbamate-tethered *N*-acetyl-D-glucosamine amino acid conjugates. Accordingly, the reaction of **1** with benzyl alcohol was quickly investigated to check the reactivity of **1** toward alcohols (Scheme 5). To our

Scheme 5. Reaction of Isocyanate **1** with Benzyl Alcohol

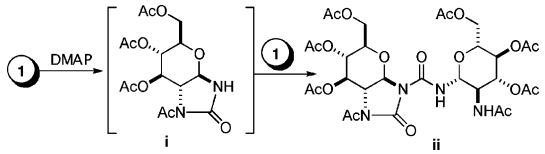


delight, treatment of **1** with benzyl alcohol (1.0 equiv) at room temperature for 5 h gave rise to the carbamate **14**, albeit in low yield (20%). However, use of excess benzyl alcohol (3.0 equiv) and heating the reaction mixture at 70 °C improved the yields up to 82%.

Our attention then turned toward the synthesis of carbamate-tethered *N*-acetyl-D-glucosamine amino acid conjugate **16** (Table 2). Treatment of isocyanate **1** with serine derivative **15** using reaction conditions similar to those employed in Scheme 5 (entries A−C) furnished the coupling product **16** in moderate yields (36−48%). Although a large excess of **15** (9 molar excess with respect to **1**) improved the yield up to 80% and excess **15** was recovered by chromatography (entry D), we attempted to improve the reaction by activating the isocyanate group in **1**. Although activation of **1** with 4-(*N*,*N*-dimethylamino)pyridine (DMAP) gave the dimeric product predominantly,[16] use of 20 mol % dibutyltin dilaurate successfully improved the coupling process in good yield (81%, entry F).[17] It should be noted that the reaction conditions in entry F are mild and neutral and that *β*-elimination and deprotection of acetyl/Fmoc groups was not observed.

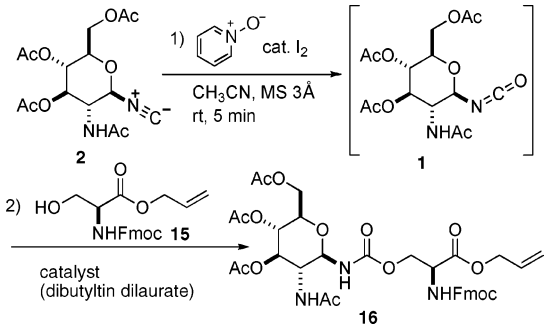
(15) Waki, M.; Kitajima, Y.; Izumiya, N. *Synthesis* **1981**, 266.

(16) Initial attempts to activate isocyanate **1** by DMAP resulted in the intramolecular attack of nitrogen in the *N*-acetyl group to the neighboring isocyanate (**1** → **i**) followed by dimerization to afford **ii**.



(17) (a) Abbate, F. W.; Ulrich, H. *J. Appl. Polym. Sci.* **1969**, *13*, 1929. (b) Greenwald, R. B.; Pendri, A.; Bolikal, D. *J. Org. Chem.* **1995**, *60*, 331.

Table 2. Optimization for the Synthesis of **16**



entry	isonitrile **2** (equiv)	serine **15** (equiv)	*T* (°C)	time (h)	catalyst (mol %)	yield (%)
A	1.0	3.0	rt	5	none	40
B	1.0	3.0	70	5	none	48
C	3.0	1.0	rt	5	none	36
D	1.0	10.0	70	15	none	80
E	1.0	3.0	70	12	20	64
F	3.0	1.0	rt	7	20	81

We have demonstrated that *N*-acetyl-D-glucopyranosyl isocyanate **1** is a valuable synthon for the preparation of urea- and carbamate-tethered amino acid conjugates. The highly reactive nature of isocyanate **1**, as demonstrated in the coupling reaction with the hydroxy group of the serine derivative **15** under mild conditions, is noteworthy when compared with the less reactive glycosyl isothiocyanate counterpart.[18] Further studies to the synthesis of neoglycopeptides using **13** and **16** is now in progress.[19]

Acknowledgment. We are grateful for financial support from a Grant-in-Aid for Scientific Research on Priority Areas (18032055 and 18037053) from MEXT. This work was supported in part by a Special Research Grant for Green Science from Kochi University.

Supporting Information Available: Full experimental procedures and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0616788

(18) For the reactivity of glycosyl isothiocyanates, see: Witczak, Z. J. *Adv. Carbohydr. Chem. Biochem.* **1986**, *44*, 91. For recent representative examples of thiourea conjugation strategy, see: (a) Lindhorst, T. K.; Kieburg, C. *Angew. Chem., Int. Ed.* **1996**, *35*, 1953. (b) Benito, J. M.; Gomez-Garcia, M.; Ortiz Mellet, C.; Baussanne, I.; Defaye, J.; Garcia Fernandez, J. M. *J. Am. Chem. Soc.* **2004**, *126*, 10355.

(19) In the synthesis of *O*-linked type glycopeptides, removal of the Fmoc group became a serious problem due to the base-catalyzed β-elimination (Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 294). To check this point, Fmoc-deprotection of **13** and **16** were carried out by treatment with 20% piperidine in DMF at room temperature for 10 min. In these reactions, β-elimination has never been observed, and the products and yields were confirmed after acetylation, which shows the compatibility of both **13** and **16** with standard solid-phase peptide synthesis.