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Synthesis of Urea-Tethered Neoglycoconjugates and Pseudooligosaccharides in Water

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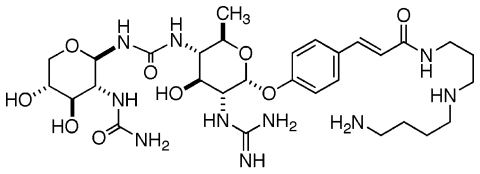
Abstract: A novel approach to the synthesis of urea glycosides in aqueous media has been explored. Steyermark's glucopyranosyl oxazolidinone was found to be a good synthon for anchoring glucosyl moieties onto amines and thiols. The present method was successfully applied to establish a new route for the synthesis of urea-tethered neoglycoconjugates and pseudooligosaccharides in water.

Introduction

Development of a new method for anchoring carbohydrate moieties onto biomolecules via nonnative glycosidic linkages, which is an alternative to glycosylation, has been an active area of numerous synthetic endeavors.[1] This trend motivated us to explore the synthesis of urea-tethered neoglycoconjugates and pseudooligosaccharides during our synthetic effort for the natural product, glycocinnasperimicin D (**1**).[2]

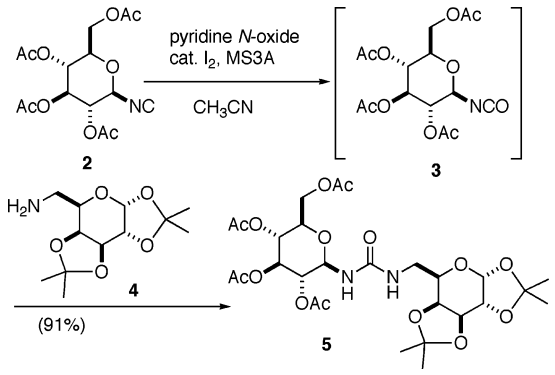


Glycocinnasperimicin D (**1**)

This aminosugar antibiotic possesses a unique structural feature, the urea-glycosyl linkage, which connects two highly functionalized aminosugars. The most challenging problem inherent in the total synthesis of **1** is the construction of this urea-glycosyl bond. To solve this problem, we developed a new method for the synthesis of urea glycoside, which involves the reaction of glycopyranosyl isocyanate with amine (Scheme 1).[3]

Oxidation of isonitrile **2** with pyridine *N*-oxide in the presence of a catalytic amount of iodine generated highly reactive isocyanate **3**, which was immediately treated with aminosugar **4** to afford the urea-linked disaccharide **5** in good yield. It should be noted that the reaction was carried out in organic solvent (CH_3CN) in the presence of water scavenger (MS3A) to avoid

Scheme 1



the hydrolysis of isocyanate **3**. In the course of this research work, we were fascinated with an approach to the urea glycoside synthesis *using unprotected sugars*, because such a protocol would lead to a new bioconjugate method *in water*.

In 1961, Steyermark reported the reaction of β-D-glucopyranosylamine **6** with phosgene, expecting to produce the known *N*,*N*′-di-β,β-D-glucopyranosyl urea **7**; however, the isolated product was actually the cyclic carbamate **8** in poor yield ranging from 6 to 30%.[4] The improved synthesis of oxazolidinone **8** was reported by Pinter and co-workers, who employed the reaction of glucopyranosyl phosphinimine with carbon dioxide and proposed the correct stereochemistry at the anomeric position in **8**.[5] The large coupling constant between H-1 and H-2 for **8** ($J_{1,2}$ = 9.2 Hz) unambiguously established the structure of diequatorial trans-annulation of the oxazolidinone to the pyranose ring. Since this Steyermark's glucopyranosyl oxazolidinone **8** has twisted structure and delocalization of the lone pair electrons on nitrogen into the carbonyl group was prevented,[6] the carbonyl group in oxazolidinone has increased kinetic reactivity toward nucleophilic attack with a decrease of the ring strain. In fact, we have disclosed that this unprotected glucopyranosyl derivative **8** behaves chemically as

† Kochi University.
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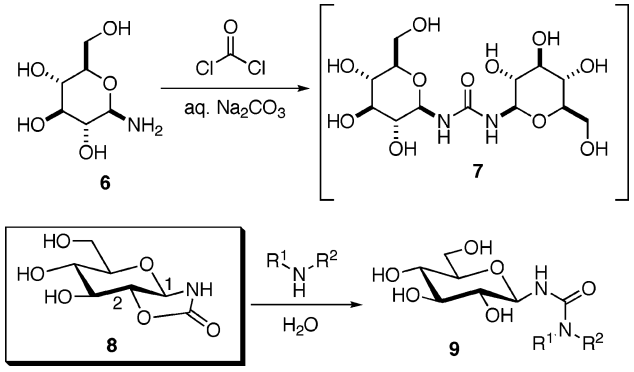
(1) (a) Taylor, C. M. *Tetrahedron* **1998**, 11317. (b) Davis, B. G. *Chem. Rev.* **2002**, *102*, 579.
(2) (a) Dobashi, K.; Nagaoka, K.; Watanabe, Y.; Nishida, M.; Hamada, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1985**, 1166. For the total synthesis of **1**, see: (b) Nishiyama, T.; Isobe, M.; Ichikawa, Y. *Angew. Chem., Int. Ed.* **2005**, *44*, 4372.
(3) (a) Ichikawa, Y.; Nishiyama, T.; Isobe, M. *Synlett* **2000**, 1253. (b) Ichikawa, Y.; Nishiyama, T.; Isobe, M. *J. Org. Chem.* **2001**, *66*, 4200.
(4) Steyermark, P. R. *J. Org. Chem.* **1962**, *27*, 1058.
(5) Kovacs, J.; Pinter, I.; Messmer, A. *Carbohydr. Res.* **1985**, *141*, 57.

10.1021/ja056253f CCC: $33.50

Scheme 2



an acylating agent in water: oxazolidinone **8** undergoes smooth ring-opening reaction with amines to give rise to the corresponding urea glucosides **9** (Scheme 2).[7]

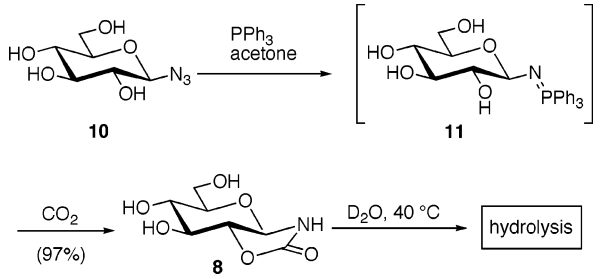
Herein, we wish to report the full details and further development of our approach for the synthesis of urea-tethered neoglycoconjugates and pseudooligosaccharides in aqueous media utilizing Steyermark's glucopyranosyl oxazolidinone.

Results and Discussions

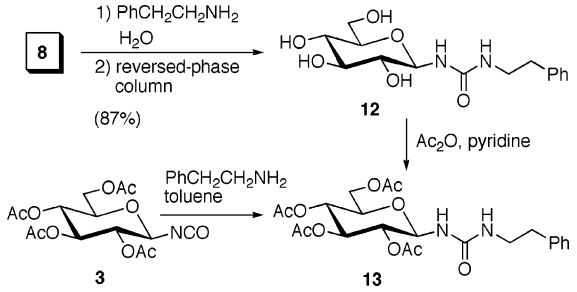
Stability of Oxazolidinone 8 in Water. Our initial effort focused on the hydrolysis of Steyermark's glucopyranosyl oxazolidinone **8**, which was prepared by the modified procedure of Pinter (Scheme 3). Treatment of glucopyranosyl azide **10** with triphenylphosphine in acetone gave phosphinimine **11**, which was successively treated with carbon dioxide in a one-pot process. The resulting white precipitate was collected and washed with acetone to furnish **8** in excellent yield. Surprisingly, hydrolysis of oxazolidinone in **8** occurred even in neutral media. In fact, a solution of **8** in D_2O at 40 °C was monitored by ^1H NMR, which showed that about 60% of **8** was hydrolyzed after 24 h.[8]

Reaction of 8 with Amines in Water. Satisfied with a high reactivity of **8**, we next turned our attention to examine the chemical behavior of **8** as an acylating reagent with amines in water. Gratifyingly, Steyermark's glucopyranosyl oxazolidinone **8** underwent smooth ring-opening reaction with 2-phenylethylamine in water; the reaction was completed within 1 h (Scheme 4). The resultant aqueous reaction mixture was directly loaded on reversed-phase column chromatography, and the product was eluted with stepwise gradient from water to 30% aqueous

Scheme 3



Scheme 4



methanol to furnish the urea glucoside **12** in 87% yield. The structure of **12** was confirmed by transforming into the corresponding acetate **13**, authentic sample of which was prepared by the reaction of Fischer's glucopyranosyl isocyanate **3**[9] with 2-phenylethylamine in organic solvent (anhydrous toluene).

Further studies focused on the reaction of **8** with a variety of amines to evaluate the generality and scope of this ring-opening reaction and to identify the steric factors of the alkyl groups in amine. The results are summarized in Table 1. Primary amines having no branches at the α-carbon, such as *n*-butylamine and benzylamine (entries 1 and 2), reacted with 1.2 equiv of **8** to give the urea glucosides **14a** and **14b** in good yields (95 and 88%). Alkyl substituents at the α-position of primary amine had considerable steric effects; in the case of (*S*)-(−)-α-methylbenzylamine and cyclohexylamine (entries 3 and 4), a slight excess of **8** (1.5 equiv), longer reaction time (2.0 h), and keeping the reaction mixture at 40 °C (entry 4) was necessary to obtain the urea glucosides **14c** and **14d** with satisfactory yields (80 and 84%). Secondary amines, diethylamine and pyrrolidine (entries 5 and 6), were less reactive than primary amines; however, use of 2 equiv of **8**, heating the reaction mixtures at 50−60 °C, and longer reaction times (3−6 h) resulted in sufficient yields (80 and 71% yield). The steric effect at the β-position of secondary amine considerably interfered with the reaction. For example, when diisobutylamine was employed (entry 7), hydrolysis of **8** predominated and only 8% of the product **14g** was isolated. In the case of more sterically congested secondary amine, diisopropylamine (entry 8), only hydrolysis of **8** was observed even after extended reaction times. Finally, aniline (entry 9) underwent urea glycosylation in water at 70 °C for 12 h to afford **14i** in 75% yield. It should be noted that heating a reaction mixture did not accelerate the hydrolysis of **8** due to the weaker basicity of aniline.

Reaction with Thiol. The importance of thiol-reactive reagents for the synthesis of glycoconjugates was well recognized in the field of neoglycoproteins and neoglycopeptides.

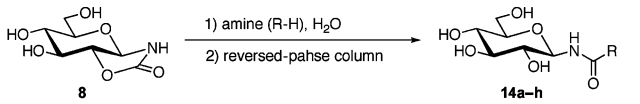
(6) The twisted structure of **8** was recognized in the highly reactive nature of nitrogen in oxazolidinone. Acetylation of **8** occurred on both the hydroxy groups and nitrogen in oxazolidinone under mild conditions (Ac_2O, pyridine, room temperature, 20 h) to furnish the tetraacetate **i**. See ref 4.

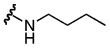
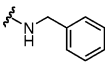
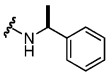
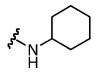
(7) (a) Ichikawa, Y.; Matsukawa, Y.; Isobe, M. *Synlett* **2004**, 1019. For the only reported example, to our knowledge, of the reaction of **8** with *N*-methylpiperazine in water, see: (b) Pinter, I.; Kovacs, J.; Toth, G. *Carbohydr. Res.* **1995**, *273*, 99. Analogous O-unprotected Zemplen's glucopyranosyl thiocarbamate and its reaction with amines was reported during our reserarch work. See: (c) Maya, I.; Lopez, O.; Fernandez-Bolanos, J. G.; Robina, I.; Fuentes, J. *Tetrahedron Lett.* **2001**, *42*, 5413. (d) Lopez, O.; Maya, I.; Fernandez-Bolanos, J. G. *Tetrahedron* **2004**, *60*, 61.

(8) ^1H NMR and TLC analysis indicated that the major hydrolysis products were α-, β-D-glucopyranose and *N*,*N*′-di-β,β-D-glucopyranosyl urea **7**, which was further confirmed by acetylation of the resultant hydrolysate and comparison with authentic samples. We thank Fumiyo Ohara (Kochi University) for these experiments.

(9) (a) Fischer, E. *Ber.* **1914**, *47*, 1377. (b) Johnson, T. B.; Bergmann, W. *J. Am. Chem. Soc.* **1932**, *54*, 3360. (c) Ichikawa, Y.; Matsukawa, Y.; Nishiyama, T.; Isobe, M. *Eur. J. Org. Chem.* **2004**, 586.

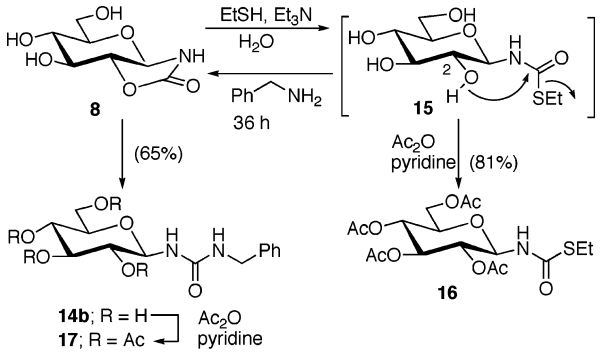
Table 1



entry	product (R =)		equiv. of **8**	*T* (°C)	reaction time (hr)	yield[a] (%)
1		**14a**	1.2	RT	1.0	95
2		**14b**	1.2	RT	1.0	88
3		**14c**	1.5	RT	2.0	80
4		**14d**	1.5	40	2.0	84
5		**14e**	2.0	60	3.0	71[b]
6		**14f**	2.0	50	6.0	80[c]
7		**14g**	2.0	50	12	8
8		**14h**	2.0	70	—	0
9		**14i**	1.5	70	12	75

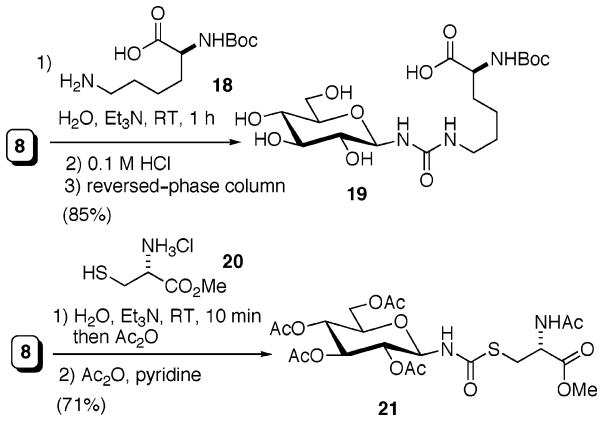
[a] Unless otherwise noted, yields were calculated after reversed-phase column purification. [b] The product was isolated after acetylation, because purification by reversed-phase column was difficult. [c] Since the product is hygroscopic, the yield was determined after acetylation.

Scheme 5



Several successful approaches to site-specific ligation of carbohydrate moieties at cysteine residues in proteins and/or peptides have been reported.[10] In this context, we were interested in the reactivity of **8** with sulfur nucleophiles, and we performed exploratory experiments testing the reaction of **8** with simple ethanethiol in water (Scheme 5). Although reaction of **8** with ethanethiol in water did not occur, addition of 1 equiv of triethylamine resulted in the smooth ring-opening reaction within 1 h, and the product **16** was isolated in 81% yield after acetylation.[11] During this investigation, we found that initially

Scheme 6



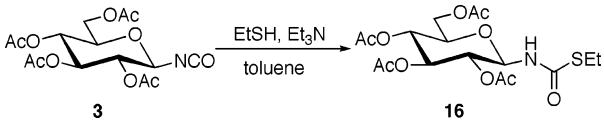
formed *N*-glucosyl thiolcarbamate **15** was unstable and appeared to go back to the starting material, which was confirmed by the following experiments: treatment of **8** with ethanethiol in water afforded an aqueous solution of **15** (checked by TLC analysis), which was subsequently subjected with benzylamine. Monitoring the reaction mixture showed that **15** gradually disappeared with concomitant formation of urea glucoside **14b**. After workup followed by acetylation provided the urea glucoside **17** in 65% yield. These experimental results confirmed that initial product **15** gradually regenerated **8** by intramolecular attack of C2 hydroxy group to the ethyl thiolcarbamate moiety,[12] and the resultant **8** was subsequently trapped with benzylamine to afford **14b**.

Synthesis of Glycosyl Amino Acid Conjugates. The reactivity of **8** toward amine and thiol appears to promise that Steyermark's glucopyranosyl oxazolidinoned **8** is a good synthon for anchoring carbohydrate moieties onto biomolecules in aqueous media. In this regard, we examined the reaction of **8** with amino acids in water (Scheme 6). Synthesis of urea-tethered glucosyl amino acid conjugate was carried out by the reaction of **8** with lysine ϵ-amino group in **18** in the presence of triethylamine. The reaction was completed at room temperature within 1 h, and the urea-tethered glucosyl lysine conjugate **19** was obtained in 85% yield. The reaction of **8** with L-cysteine methyl ester hydrochloride **20** in the presence of triethylamine was completed at room temperature for 10 min. The resulting reaction mixture was immediately treated with acetic anhydride to protect the remaining amino group in the cysteine moiety.[13] Concentration followed by acetylation furnished glucopyranosyl cysteine conjugate **21** in 71% yield.

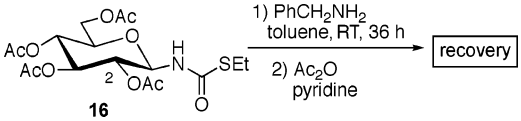
Synthesis of Urea-Tethered Pseudooligosaccharide in Aqueous Media. The chemistry of Steyermark's glucopyranosyl

(10) (a) Davis, N. J.; Flitsch, S. L. *Tetrahedron Lett.* **1991**, *32*, 6793. (b) Macindoe, W. M.; Oijen, A. H.; Boons, G.-J. *Chem. Commun.* **1998**, 847. (c) Davis, B. G.; Lloyd, R. C.; Jones, J. B. *J. Org. Chem.* **1998**, *63*, 9614. (d) Shin, I.; Jung, H.-J.; Lee, M.-R. *Tetrahedron Lett.* **2001**, *42*, 1325. (e) Cohen, S. B.; Halcomb, R. L. *J. Am. Chem. Soc.* **2002**, *124*, 2534. (f) Zhu, X.; Pachamuthu, K.; Schmidt, R. R. *J. Org. Chem.* **2003**, *68*, 5641.

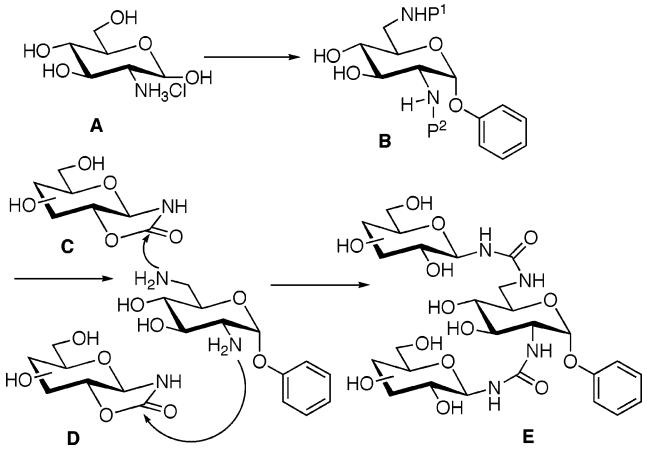
(11) Authentic sample of **16** was prepared by the reaction of Fischer's glucopyranosyl isocyanate **3** with ethanethiol and triethylamine in toluene.



(12) The existence of such an intramolecular attack of the C2 hydroxy group was also supported by the following experiments: treatment of **16** with benzylamine in toluene resulted in the near recovery of starting material.



Scheme 7

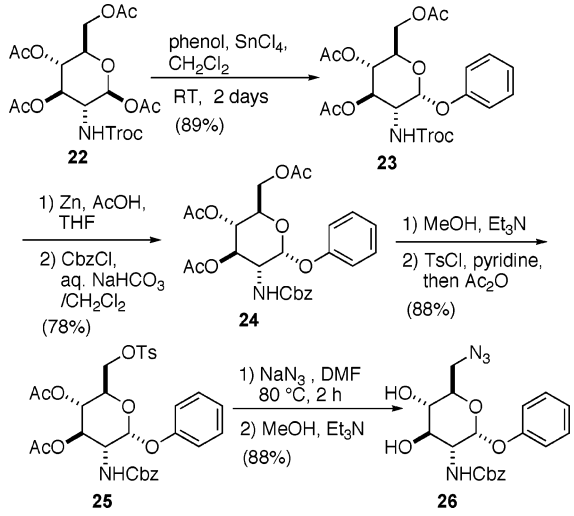


oxazolidinone **8** is much more precious than we initially expected, which finally led us to the synthesis of urea-linked pseudooligosaccharides in aqueous media. The synthetic plan is outlined in Scheme 7. Starting with commercially available D-glucosamine hydrochloride **A**, we set up to prepare phenyl 2,6-diaminoglycoside **B** as a core segment, which has two reactive sites for the urea glycosylation with Steyermark-type glycosyl oxazolidinones for the construction of β-(1 → 6) and β-(1 → 4) urea glycosyl bond. In addition, the phenyl group in **B** was envisioned to facilitate the final purification step by reversed-phase chromatography.

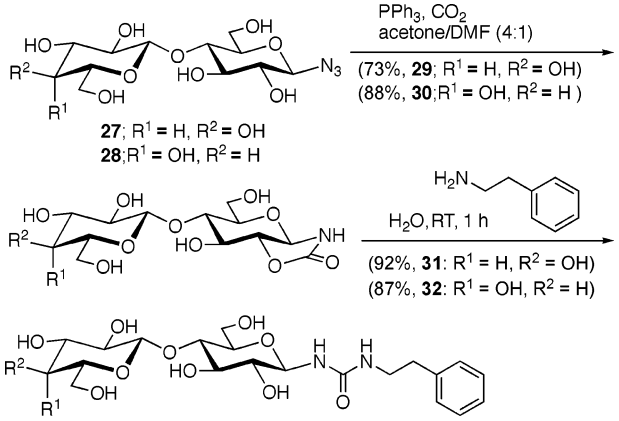
Synthesis of the phenyl 2,6-diaminoglycoside began with tin(IV) chloride-catalyzed glycosylation of the Troc-oxazolidinone **22** with phenol (Scheme 8). In this phenyl glycosylation, prolonged reaction time (2 days) resulted in a gradual decrease of the β-isomer proportion and concomitant increase in the formation of α-anomer to furnish α-phenyl glycoside **23** predominantly in 89% yield.[14] Treatment of **23** with zinc in a mixture of acetic acid and THF cleaved Troc-carbamate, and the resultant amine was immediately reprotected as its Cbz-derivative **24** in a 78% yield for two steps. Hydrolysis of acetyl groups in **24** (Et_3N, MeOH) gave triol, whose primary alcohol was selectively tosylated (TsCl, pyridine), and the remaining two hydroxy groups were acetylated (subsequent addition of Ac_2O) to afford **25** in an 88% yield. Displacement of the tosylate **25** with sodium azide in DMF and hydrolysis of the acetyl groups (Et_3N, MeOH) furnished the core segment **26** in an 88% yield.

Disaccharides **29** and **30**, containing the structural motif of Steyermark's glucopyranosyl oxazolidinone, were prepared from cellobiosyl and lactosyl azides **27** and **28** by using procedures similar to those described in Scheme 3. To check the reactivity of these disaccharide oxazolidinones **29** and **30**, reactions with

Scheme 8



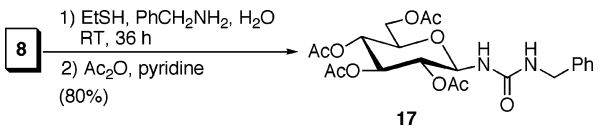
Scheme 9



2-phenylethylamine were carried out in water to result in the smooth formation of the corresponding urea glycosides **31** and **32** in 92 and 87% yield, respectively (Scheme 9).

The synthesis of urea-tethered pseudopentasaccharide began with the Staudinger reaction of azide **26** with triphenylphosphine in a mixture of water and *tert*-butyl alcohol (Scheme 10). Under this reaction condition, initially formed phosphinimine was hydrolyzed to afford the amine **33**, which was dissolved in aqueous media (H_2O/MeOH, 1:2) and then treated with 1.5 equiv of lactosyl oxazolidinone **30**. The formation of β-(1 → 6) urea intersaccharide bond was completed at room temperature within 24 h. The resultant mixture was treated with triethylamine to quench the excess **30** and then concentrated under reduced pressure to afford the residue, which was repeatedly washed with ethyl acetate to remove triphenylphosphine and triphenylphosphine oxide.[15] Hydrogenolysis of the Cbz group in **34** (H_2, 5% palladium on carbon, H_2O) gave the amine **35**, which was subsequently subjected to the second urea glycosylation with cellobiosyl oxazolidinone **29** (1.5 equiv) in water. The reaction mixture was stirred at room temperature for 24 h and then loaded on the reversed-phase column chromatography (eluted with H_2O followed by 9:1 H_2O/MeOH) to furnish urea-linked pseudopentasaccharide **36** in 73% overall yield starting from **26**. It should be noted that the whole procedure in this

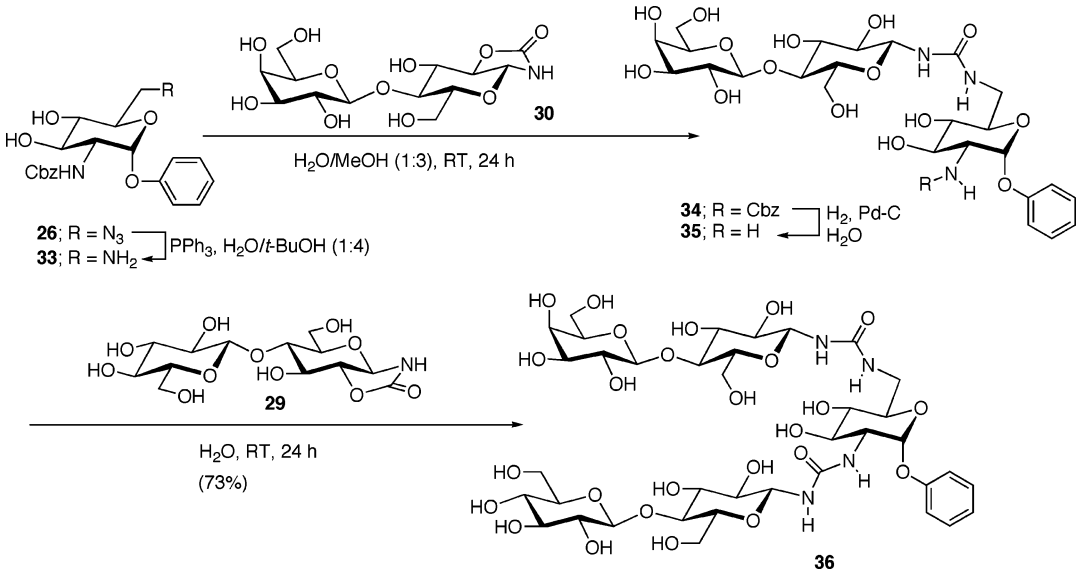
(13) Without protection of the amino group with acetic anhydride, the product gradually decomposed during isolation. These optimized conditions were established by the following experiments: competitive reaction of **8** with ethanethiol and benzylamine showed that initially formed major product was **15**. However, prolonged reaction time resulted in the disappearance of **15** and almost exclusive formation of **14b**. Upon concentration, the resultant product was treated with acetic anhydride and pyridine to afford the urea glucoside **17** in 80% yield.



(14) Nishiyama, T.; Ichikawa, Y.; Isobe, M. *Synlett* **2004**, 89.

(15) This operation was necessary for smooth hydrogenolysis of the Cbz group in **34**.

Scheme 10



reaction sequence (**26** → **33** → **34** → **35** → **36**) was carried out in a one-pot operation.

While most ^{1}H NMR signals of **36** are overlapped, ^{13}C NMR and FAB mass spectra confirmed the structure of this novel pseudopentasaccharide.[16] The ^{13}C NMR spectrum of **36** showed four aromatic carbons (δ 118.2, 124.0, 130.7, and 156.7), two urea carbonyl carbons (δ 159.8 and 160.2), and 30 carbons corresponding to the five hexopyranoses. Among ^{13}C NMR signals associated with the carbohydrate portions, the prominent chemical shifts assigned to two anomeric carbons linked to ureido nitrogen atom are δ 81.5 and 81.6, which are consistent with the β-stereochemistry.[17] The 103.2 and 103.6 ppm signals are assigned to the anomeric carbons of lactose and cellobiose moieties, and the upfield signal at 97.6 ppm to that of phenyl glycoside.

Conclusion

In this work exploring the chemitry of Steyermark's glucopyranosyl oxazolidinone **8**, we uncovered its utility for the synthesis of neoglycoconjugates in water. In fact, this strained cyclic carbamate **8** onto unprotected carbohydrate reacted with amine and thiol, which provides access to a variety of neoglycoconjugate and pseudooligosaccharide. Steyermark's glucopyranosyl oxazolidinone **8**, an old but renewed synthon for urea glycosylation, can be considered to be a water-soluble synthetic equivalent of Fischer's glucopyranosyl isocyanate **3**.

Experimental Section[18]

General Method for the Preparation of Urea Glucoside in Water. To a solution of *n*-butylamine (21 mg, 0.29 mmol) in water (4.0 mL) was added Steyermark's glucopyranosyl oxazolidinone **8** (71 mg, 0.35 mmol) in a single portion. After being stirred at room temperature for 1.0 h, the reaction mixture was directly passed through a reversed-phase column (H_2O followed by 10:1 H_2O/MeOH as eluent) to afford the urea glucoside **14a** as a viscous gum (76 mg, 95%).

Synthesis of Pseudopentasaccharide 36. To a solution of **26** (50 mg, 0.12 mmol) dissolved in a mixture of water (0.50 mL) and *tert*-butyl alcohol (2.0 mL) was added triphenylphosphine (35 mg, 0.13 mmol) in a single portion. After being stirred at 60 °C for 4 h, the reaction mixture was concentrated under reduced pressure to give **33**, which was dissolved in a mixture of water (1.0 mL) and MeOH (2.0 mL). Lactosyl oxazolidinone **30** (66 mg, 0.18 mmol) in methanol (1.0 mL) was added, and the resulting reaction mixture was stirred at room temperature for 24 h. Addition of triethylamine (0.10 mL) followed by stirring for about 6 h resulted in the disappearance of lactosyl oxazolidinone **30** (checked by TLC). The resultant reaction mixture was concentrated under reduced pressure to afford the residue, which was repeatedly washed with ethyl acetate to remove triphenylphosphine and triphenylphosphine oxide. The resulting **34** was dissolved in H_2O (5.0 mL), and palladium on carbon (5%, 20 mg) was added. The suspension was vigorously stirred under atmosphere of hydrogen at room temperature for 24 h. Palladium catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure. Finally, amine **35** was dissolved in H_2O (5.0 mL) and then treated with cellobiosyl oxazolidinone **29** (66 mg, 0.18 mmol). After being stirred at room temperature for 24 h, the resultant reaction mixture was loaded on the reversed-phase column chromatography (eluted with H_2O and 9:1 H_2O/MeOH) to furnish pseudopentasaccharide **36** (87 mg, 73% overall yield) as a white solid: mp 222−223 °C; $[\alpha]^{24}_D$ +43.9 (*c* 0.90, H_2O); IR (KBr) ν_{max} = 1654, 1560, 1279 cm^{-1}; ^{13}C NMR (D_2O, 100 MHz): δ 41.0, 54.8, 60.6, 60.8, 61.3, 61.7, 69.2, 70.1, 71.6, 71.9, 72.1, 72.2, 72.4, 72.5, 73.2, 73.8, 75.7, 75.9, 76.0, 76.1, 76.52, 76.56, 76.63, 78.8, 79.0, 81.5, 81.6, 97.6, 103.2, 103.6, 118.2, 124.0, 130.7, 156.7, 159.8, 160.2; HRMS (FAB) calcd for $C_{38}H_{61}N_4O_{26}$ $[M + H]^+$ 989.3574, found 989.3561.

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

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(16) For a recent example of related glycooligomers with thiourea linkage, see: Jimenez Blanco, J. L.; Bootello, P.; Ortiz Mellet, C.; Gutierrez Gallego, R.; Garcia Fernandez, J. M. *Chem. Commun*. **2004**, 92.
(17) The ^{13}C NMR of β-glucopyranosyl urea shows anomeric carbons in the region of 80−81 ppm, and the ^{13}C NMR chemical shift of α-ureido glycosidic carbon appears around 75−78 ppm. See ref 3b.
(18) For materials and methods, see the Supporting Information.