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Protecting Group Free Synthesis of Urea-linked Glycoconjugates: Efficient Synthesis of β-Urea glycosides in Aqueous Solution

Yoshiyasu Ichikawa,*a Takahiro Minami, Shohei Kusaba, Nobuyoshi Saeki, Yuta Tonegawa, Yumiko Tomita, Keiji Nakano,a Hiyoshizo Kotsuki,a and Toshiya Masuda,b

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A method for the protecting group free synthesis of β-urea-linked glycoconjugates has been developed. The one step process, involving reaction between urea and D-glucose, N-acetyl-D-glucosamine or D-xylose in acidic aqueous solution, furnishes the corresponding β-urea glycoside in modest yield. This simple and efficient procedure is applicable to the synthesis of β-urea tethered amino acid/carbohydrate conjugates.

Introduction

The urea glycosyl linkage has been known in Nature as a unique and important structural motif found in members of the glycosinolyl-spermidine amino-sugar antibiotic family. In these natural products, two amino sugars are connected via a urea glycosyl bond. Moreover, the synthesis of neoglycoconjugates, in which native and enzymatically labile glycosidic bonds are replaced by robust non-native linkers, have received considerable recent attention due to the increasing need to develop a new type of molecular tools in glyobiology and potential therapeutic agents.

Replacement of naturally occurring O- and N-glycosyl linkages with urea-glycosyl bonds is one strategy used to design new neoglycoconjugates. Although a number of new synthetic methods to access urea glycosides have been devised by us and other groups, all methods require the use of protected carbohydrates as intermediates. As a result, the reported synthetic routes to urea glycosides are often lengthy as a consequence of the need for protection/deprotection steps. The shortcomings of routes to urea glycosides that rely on the use of protected carbohydrates have directed our attention to a classical method involving acid-catalyzed condensation reaction of glucose with urea in water. Although well documented, the applications of this process to reactions of N-substituted urea derivatives are both rare and questionable.

In 1926, Helferich, a former student of Emile Fischer, reported the reaction of glucose with methyl-harnstoff (N-methylurea) in aqueous 6.5 M HCl to obtain “d-glucose-monomethylureid” (eq. 1). In 1953, Erickson investigated the reaction of long-chain octadeylurea with D-glucose. The observations made in these two precedents suggest that direct coupling of N-substituted ureas with unprotected carbohydrates could serve as a general method for the preparation of urea glycosides. However, the reliability of the two reports was questionable owing to the fact that both Helferich and Erickson characterized the reaction products only by using melting point, elemental analysis and optical rotation data. Furthermore, the yields in the reported reactions were exceedingly low and the stereochemistry at the anomeric position of the products was not determined. As a result of these issues, we have carried out an investigation of the one-step, acid promoted reactions of N-substituted ureas with carbohydrates. This effort has led to the development of a unique and efficient protecting group free method for the synthesis of urea-linked glycoconjugates.

Results and discussion

In the initial phase of this study, we aimed at characterization of the product (d-glucose-monomethylureid) formed in the reaction between glucose and N-methylurea reported by Helferich. For this purpose, we prepared the anomeric pair of 1-methyl-3-glucosylurea by employing our previously established “isocyanide method” (Scheme 1). Starting with commercially available pentaacetyl-β-D-glucose (1), β-glucosyl isocyanide 4 was prepared in a three step sequence involving (i) azide glucosylation of 1, (ii) catalytic hydrogenation of azide 2 followed by formylation of the produced glucosyl amine, and (iii)
dehydration of glucosyl formamide 3 with triphosgene and triethylamine. Oxidation of β-glucosyl isocyanide 4 with pyridine N-oxide in the presence of a catalytic amount of iodine and MS 3A (anhydrous conditions) generated the highly reactive glucosyl isocyanate 5, which, without isolation, was treated with methyamine. This process formed β-1-methyl-3-glucosylurea 6a in 90% yield. A similar set of transformations starting with α-glucosyl isocyanide 10, prepared from 1 in four steps, afforded α-1-methyl-3-glucosylurea 6b in 90% yield (Scheme 2).

Scheme 1 Synthesis of β-1-methyl-3-glucosylureas from β-glucosyl isocyanide

Scheme 2 Synthesis of α-1-methyl-3-glucosylurea from α-glucosyl isocyanide

With tetraacetyl derivatives of the two anemic 1-methyl-3-glucosylureas in hand, we next explored the acid-catalyzed condensation reaction of D-glucose (12) with N-methyurea in water as described by Helferich (Scheme 3). In initial experiments using the reported conditions (2.4 equivalents of N-methyurea, aqueous 5.5 M HCl at 50 °C for 16 d) the product gave only trace amounts of products. After some experiments varying acid catalysts (HCl, H₂SO₄, acidic resins), amount of N-methyurea, a range of temperature and time, we found that this reaction, using 6 M HCl, ten equivalent excess N-methyurea, room temperature and a three-day time period, resulted in higher product yields. Specifically, neutralization of the crude reaction mixture with sodium bicarbonate followed by concentration in vacuo afforded a solid residue, which when treated with Ac₂O and pyridine followed by chromatography produced a mixture of tetraacetyl β- and α-1-methyl-3-glucosylurea (6a and 6b) in a 94:6 ratio and a 68% yield. The products of this process were found to be identical to the independently synthesized glucosyl ureas (Scheme 1 and 2).

In order to demonstrate that our approach is truly ‘protecting free’, we further examined the work-up procedure to obtain a non-acylated N-methyurea glucoside. After some experiments, it was found that simply treating the reaction mixture with methanol and ether led to crystallization of the product. As a result, non-acylated N-methyurea glucoside 6c was isolated as crystals in 70% yield.

The high β-selectivity in this process is presumably the consequence of the fact that the reaction most likely proceeds under thermodynamically controlled conditions and that the urea group displays only a small anomeric effect. The product distribution dominating the formation of β-anomer 6a over α-isomer 6b seems to reflect the sterically driven preference for the bulky urea substituents at the pyranose anemic position to occupy the equatorial position.
reactions with pyrrolidineurea and N,N/dimethylurea took place considerably (Method A; 10 equivalents of urea, 6 M HCl, room temperature and three-day reaction time). The results show that reactions employing n-butyl and β-phenethyl urea generated the corresponding urea glucosides 13 and 14 in reasonable yields (entries A and B, 67% and 56%, respectively) and high β-selectivities (13a/13b = 93:7 and 14a/14b = 93:7). To our disappointment, cyclohexylurea and (R)-α-methylbenzylurea, both of which possess α-alkyl branching, reduced the yield considerably (Method A, entry C and D, 26% and 24%). Also, reactions with pyrrolidineurea and N,N-dimethylurea took place in low yields (Method A, entry E and F, 10% and 6%, respectively). In addition to the low yields, we sometimes encountered problems in purification steps to remove excess amount of urea.

In order to increase the yield and to reduce the amount of loading urea, we further investigated the conditions, which led to the observation that employing 2.4 M HCl, co-solvents such as ethyl acetate or acetonitrile, two equivalents of each urea, and a shorter reaction time (ca. 24 h) brought about much more efficient glucosyl urea formation (Method B). In the case of n-butylurea and phenetylurea (Method B, entries A and B), two equivalents of urea were enough to produce the products in comparable yields with those of Method A. Glucosylation of cyclohexylurea and (R)-α-methylbenzylurea employing Method B raised the yields considerably (entries C and D; 68 and 72%). Although yields in the case of ureas derived from secondary amines were still low even using Method B (entries E and F, 27 and 30%), increases in the amounts of the ureas (10 equiv) cause a significant improvement in the yields (Method B, entries E and F, 40 and 51%). It should be noted that all reactions using Method B generated products with high degree of β-selectivity (>90:10). Moreover, due to the high crystalline nature of β-urea glucosides, the minor α-anomers were easily removed by recrystallization.

The potential generality of the protecting group free synthesis of urea glucosides was explored by using N-acetyl-D-glucosamine (19) as a substrate and Method B conditions (Table 2). Preliminary experiments, which revealed that acetonitrile is a poor co-solvent to solubilize N-acetyl-d-glucosamine (19), suggested that ethyl acetate be used as the co-solvent. In addition, five equivalents of urea were necessary to obtain reasonable yields. By using modified Method B, we obtained the corresponding urea glucosamides 20-23 (entries A to D) in comparable yields to those observed for reactions of D-glucose (Table 1, entries A to D). Unfortunately, in the case of pyrrolidine urea (entry E), a low yield (5%) of the urea glucosaminde 24 was obtained. In each case, the β-anomer was

### Table 1: Protecting group free synthesis of N-Substituted urea glucosides

<table>
<thead>
<tr>
<th>Entry</th>
<th>Urea glucoside</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6 M HCl</td>
<td>67% (93:7)</td>
<td>2.4 M HCl/CH$_3$CN (1:4)</td>
</tr>
<tr>
<td>B</td>
<td>6 M HCl</td>
<td>56% (93:7)</td>
<td>2.4 M HCl/CH$_3$CN (1:4)</td>
</tr>
<tr>
<td>C</td>
<td>6 M HCl</td>
<td>26% (92:8)</td>
<td>2.4 M HCl/CH$_3$CN (1:6)</td>
</tr>
<tr>
<td>D</td>
<td>6 M HCl</td>
<td>24% (98:2)</td>
<td>2.4 M HCl/CH$_3$CN (1:6)</td>
</tr>
<tr>
<td>E</td>
<td>6 M HCl</td>
<td>10% (97:3)</td>
<td>2.4 M HCl/CH$_3$CN (1:6)</td>
</tr>
<tr>
<td>F</td>
<td>6 M HCl</td>
<td>6% (97:3)</td>
<td>2.4 M HCl/CH$_3$CN (1:6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.4 M HCl/CH$_3$CN (1:6)</td>
</tr>
</tbody>
</table>

*The reaction was carried out on 300-mg scale of D-glucose (12).

*Yields obtained employing 10 equiv of urea.

*The ratio was determined by 1H NMR analysis of the crude products after acetylation.

![Scheme 3](image-url)  
Scheme 3: Synthesis of 1-methyl-3-glucosylurea.
formed exclusively. The structures of 21, 22 and 24 were unambiguously confirmed by comparison with previously reported samples prepared from 19 in 6 steps using the isocyanide method (eq. 2). Protecting group free synthesis of urea glucosamides shows that this method is a convenient short step syntheses of β-urea glucosamides in which urea moieties are derived from primary amines.

Table 2 Synthesis of β-urea glucosamides starting from N-acetyl-D-glucosamine (19)

<table>
<thead>
<tr>
<th>entry</th>
<th>urea glucoside</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20</td>
<td>49</td>
</tr>
<tr>
<td>B</td>
<td>21</td>
<td>45</td>
</tr>
<tr>
<td>C</td>
<td>22</td>
<td>57</td>
</tr>
<tr>
<td>D</td>
<td>23</td>
<td>48</td>
</tr>
<tr>
<td>E</td>
<td>24</td>
<td>5</td>
</tr>
</tbody>
</table>

*The reaction was carried out on 300-mg scale of N-acetyl-D-glucosamine (19).

Further studies aimed at broadening the substrate scope of the process led us to explore the urea forming reaction of D-xylose (26) using Method B (Table 3). We are delighted to find that D-xylose (26) is a better substrate than hexoses, giving good yields of urea xylidoses 27–30 (entries A to D, 71–89%) with high degree of β-selectivity (≥ 98:2). Even in the reaction with pyrrolidineurea (entry E), the corresponding urea xyloside 31 was obtained in modest (41%) yield. The structures and β/α-selectivity of the products (27–31) were unambiguously determined by comparison with authentic samples synthesized by using the isocyanide method (Scheme 4).

Table 3 Protecting group free glycosylation of d-xylose (26)

<table>
<thead>
<tr>
<th>entry</th>
<th>urea xyloside</th>
<th>yield (%)</th>
<th>β/α (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>27</td>
<td>73</td>
<td>98:2</td>
</tr>
<tr>
<td>B</td>
<td>28</td>
<td>71</td>
<td>&gt;98:2</td>
</tr>
<tr>
<td>C</td>
<td>29</td>
<td>89</td>
<td>&gt;98:2</td>
</tr>
<tr>
<td>D</td>
<td>30</td>
<td>78</td>
<td>&gt;98:2</td>
</tr>
<tr>
<td>E</td>
<td>31</td>
<td>41</td>
<td>&gt;98:2</td>
</tr>
</tbody>
</table>

* The reaction was carried out on 300-mg scale of D-xylose (26).

** The ratio was determined by 1H NMR analysis of the crude products after acetylation.
Having developed an efficient method for the synthesis of \( \beta \)-urea glycosides starting with unprotected carbohydrates, our attention next focused on its application to the synthesis of urea-tethered amino acid-carbohydrate conjugates. For this purpose, we examined to install a urea group on a lysine derivative 35 (Scheme 5). Amide formation of 35 with dimethylamine using EDC in the presence of HOBt and deprotection of N/Boc group in 36 with TFA produce the amine 37. Transcarbamoylation of phenyl carbamate with 37 in the presence of the catalyst dibutyltin maleate furnished urea 38 in 80% yield.

Reactions of urea 38 (2 equiv) with D-glucose 12 in 2.4 M HCl and co-solvents were examined (Table 4). Although we could obtain the desired amino acid-glucose conjugate 39 with high \( \beta \)-selectivity, the yields were low in each co/solvent, acetonitrile (entry A, 9%, \( \beta/\alpha = 91:9 \)) and ethyl acetate (entry B, 19%, \( \beta/\alpha = 96:4 \)). Although raising the stoichiometry of urea 38 to 5 equivalents and use of acetonitrile as a co/solvent gave the product in only 20% yield (entry C), employing ethyl acetate as a co-solvent improved the yield to an acceptable level (entry D, 51%, \( \beta/\alpha = 95:5 \)). The presence of the \( \alpha \)-isomer 39b and the determination of the \( \beta/\alpha \)-selectivities of the reactions were made possible by the availability of authentic samples of 39a and 39b, prepared by using the isocyanide method starting with isocyanides 4 and 10 (Scheme 6).
Conclusion

An investigation of the reaction of glucose with N-substituted urea is revisited over 100 years later since the report by Helfferich, which led to a protecting group free method for the synthesis of urea glycosides. The established process is a good and simple method for the preparation of β-urea glycosides in which urea moieties contain primary amines. While the yields are only moderate, the reactions are both scalable and highly β-selective. This protecting group free method is complementary to one developed earlier based on reactions of glycosyl isocyanide intermediates.

Experimental

Synthesis of N'-methyl-N-2,3,4,6-tetra-O-acetyl-β-d-glucopyranosyl urea (6a) employing Method A

A solution of D-glucose 12 (500 mg, 2.78 mmol) and 1-methylurea (2.10 g, 27.87 mmol) in 6 N HCl (2.0 ml) was stirred at room temperature for 3 days. The reaction mixture was neutralized with solid NaHCO₃ and washed with CH₂Cl₂ to remove excess 1-methylurea. The aqueous layer was concentrated under reduced pressure to give crude urea glucoside as solids (2.68 g), which was dissolved in a mixture of pyridine (12 ml) and Ac₂O (6.0 ml). The solution was stirred at 50 °C for 3 hours, and the resulting reaction mixture was treated with saturated aqueous NaHCO₃. The separated aqueous layer was extracted with Et₂O, and the combined organic layers were washed with brine, dried (Na₂SO₄) and then concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (2:1 AcOEt/hexane) to afford a mixture of 1-methyl-3-glycosylurea 6 (764 mg, 68%).

Synthesis and isolation of N'-methyl-N-β-d-glucopyranosyl urea (6c).

To a solution of D-glucose 12 (1.0 g, 5.74 mmol) and N-methyl urea (2.0 g 24.4 mmol) in water (1.0 ml) was added conc. HCl (1.0 ml). After being stirred at room temperature for 3 days, MeOH (20 ml) and Et₂O (35 ml) were added. After standing the mixture at 0 °C for 2 days, the crystals formed are collected, washed with MeOH (5.0 ml) and Et₂O (5.0 ml), and air-dried to furnish 1/methyl/3/glucosylurea 6c (764 mg, 68%).

General method for the synthesis of N-Substituted urea glycosides using Method B

N'-butyl-1-N,2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl urea (13a). A solution of p-glucose 12 (300 mg, 1.67 mmol) and n-butylurea (387 mg, 3.33 mmol) dissolved in a mixture of CH₂CN (1.0 ml) and 2.4 N HCl (0.25 ml) was stirred at 50 °C for 1 day, and then was neutralized with solid NaHCO₃. The resulting reaction mixture was diluted with H₂O (ca. 2.0 ml) and washed with CH₂Cl₂. The separated aqueous layer was extracted with n-BuOH, and the combined organic extracts were concentrated under reduced pressure to afford the solids.

The resulting crude product was dissolved in a mixture of pyridine (10 ml) and Ac₂O (5.0 ml). The solution was stirred at 50 °C for 3 hours, and diluted with saturated aqueous NaHCO₃. The separated aqueous layer was extracted with Et₂O. The combined organic layers were washed with brine, dried (Na₂SO₄) and then concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (2:1 AcOEt/hexane) to afford n-butylurea glucoside 13a as a white solid (485 mg, 65%, β:α = 92:8). IR (KBr): νmax 3323, 2939, 2355, 1755, 1739 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 2.72 (s, 3H), 3.35 (brt, J = 9.5 Hz, 1H), 3.39 (t, J = 9.5 Hz, 1H), 3.48–3.57 (m, 1H), 3.54 (t, J = 9.5 Hz, 1H), 3.71 (dd, J = 12.0, 5.5 Hz, 1H), 3.88 (dd, J = 12.0, 5.5 Hz, 1H), 4.84 (brd, J = 9.5 Hz, 1H); ¹³C NMR (D₂O, 100 MHz) δ 26.8, 61.3, 67.2, 70.0, 72.5, 77.2, 77.6, 81.7, 160.8. HRMS(ESI): m/z calcld for C₁₀H₁₃N₂O₆ [M+H⁺] 237.1087, found 237.1081; m/z calcld for C₁₃H₁₉N₂O₆Na [M+Na⁺] 259.0906, found 259.0917.

Acknowledgments

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Notes and references


10. This work has been communicated in a preliminary form: Y. Ichikawa, S. Kusaba, T. Minami, Y. Tomita, K. Nakano and H. Kotsuki, Synlett, 2011, 1462-1466.
