Communication

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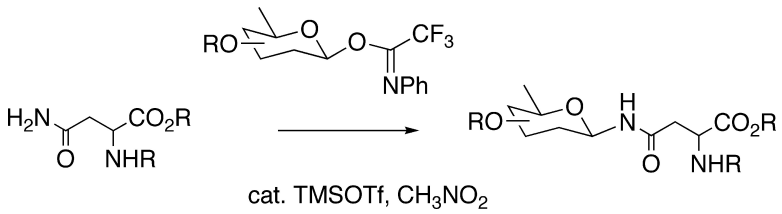


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# Efficient Stereoselective Synthesis of γ-*N*-Glycosyl Asparagines by N-Glycosylation of Primary Amide Groups

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Post-translation modification of proteins involves phosphorylation and glycosylation, which is an essential biological process to mature their functions. *N*-glycosides attached to asparagines at the γ-position through a β-glycosidic bond are found in various membrane proteins and play significant roles in biological processes on the cell surface.[1] To elucidate the biological role of these glycoproteins, their partial fragments, such as *N*-glycopeptides, have served as effective biochemical probes and are attractive synthetic targets.[2] Therefore, an effective methodology for the synthesis of the various *N*-glycosyl peptides is required.

Most of the established methodologies for linking asparagines and saccharides through an N-glycosidic bond to provide *N*-glycopeptides **1** involve amidation of the asparaginic acid **5** with the glycosylamines **4** or its equivalent (Scheme 1, path B).[3] However, the β-glycosylamines **4** are sufficiently unstable not only to epimerize at the anomeric position but also to hydrolyze to the lactol during the reaction. Furthermore, the asparaginic acid **5** in peptides easily undergoes cyclization to afford the corresponding succinimide by activation of the carbonyl group[4] (path C). To minimize succinimide formation, careful control of the reaction conditions is required. On the other hand, biological synthetic processes of *N*-glycoproteins involve N-glycosylation of the primary amides **2** with donor **3** (path A). The biosynthetic pathway suggests an efficient and alternative approach for the chemical synthesis of various *N*-glycosyl amides **1** from the corresponding stable amides **2**.[5] However, it is worth noting that the nitrogen of the amide group showed very poor nucleophilicity toward glycosylation. Additionally, O-glycosylation of the amide could also lead to a considerable side reaction. In 1989, Kahne et al. reported that the coupling of a *N*-silyl acetamide with a perbenzyl galactosyl sulfoxide provided α-*N*-glycosyl acetamide as a major product.[7,8] The *N*-trimethylsilyl group enhances the nucleophilicity of the amide nitrogen. However, the preparation of *N*-silyl asparagines is a difficult task because of their instability.

In this communication, we have demonstrated the stereoselective synthesis of glycosyl amino acids and peptides by N-glycosylation of primary amides without any amide activating groups. As illustrated in Table 1, treatment of acetamide **10** with 1.5 equiv of the glycosyl β-*N*-phenyltrifluoroacetoimidate **6a**[9] in the presence of a catalytic amount of TMSOTf at room temperature in nitromethane[10] provided the glycosyl acetamide **11** in excellent yield with complete β-selectivity (entry 1). Dichloromethane did not work well in the N-glycosylation as a solvent (entry 4). Both electron-withdrawing and -donating groups on the leaving group reduced the yield of **11**[11] (entries 5 and 6). Use of α-trichloroacetoimidate **9a**[12] as a donor provided the glycosyl acetamide **11** in dramatically reduced yield (42%) along with a significant amount of the glycosyl trichloroacetamide **12** (entry 7). The yield of **12** was 80% based on donor **9a**. N-substitution of the trifluoroimidate prevented the released trifluoroacetamide from being glycosylated with the donor

***Scheme 1***

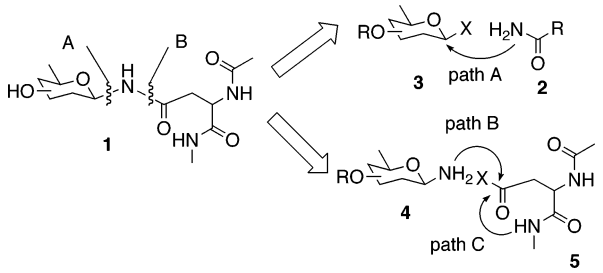
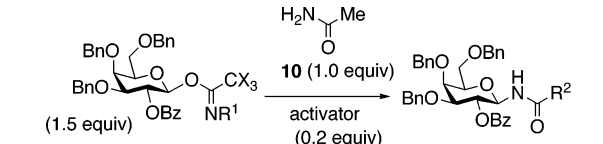



***Table 1.*** N-Glycosylation of Acetamide **10** with the Galactosyl Imidates **6a**−**9a**




**6a** : X = F, R = Ph
**7a** : X = F, R = p-MeOC$_6$H$_4$
**8a** : X = F, R = p-F C$_6$H$_4$
**9a** : X = Cl, R = H

**11** : R$^2$ = Me
**12** : R$^2$ = CCl$_3$

| entry | donor | activator | solvent | yield of **11** (%) |
|---|---|---|---|---|
| 1 | **6a** | TMSOTf | $CH_3NO_2$ | 98 |
| 2 | **6a** | TMSOTf | $CH_3CN$ | 70 |
| 3 | **6a** | TMSOTf | EtCN | 85 |
| 4 | **6a** | TMSOTf | $CH_2Cl_2$ | 46 |
| 5 | **7a** | TMSOTf | $CH_3NO_2$ | 66 |
| 6 | **8a** | TMSOTf | $CH_3NO_2$ | 75 |
| 7 | **9ac** | TMSOTf | $CH_3NO_2$ | 42 |
| 8 | **6a** | TESOTf | $CH_3NO_2$ | 53 |
| 9 | **6a** | TBSOTf | $CH_3NO_2$ | 87 |
| 10[a] | **6a** | TMSOTf | $CH_3NO_2$ | 71[b] |

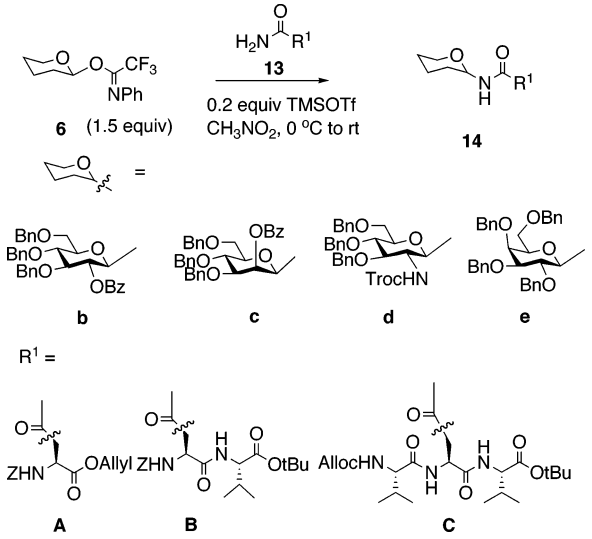
[a] With 1.5 equiv of acceptor used as base on donor **6a**. [b] The yield was estimated based on donor **6a**.

due to its steric hindrance. Both TESOTf and TBSOTf were found not to be effective for the N-glycosylation as a promoter in comparison with TMSOTf (entries 8 and 9). Use of excess acceptor **10** provided glycosyl amide in good yield based on the donor **6a** (entry 10).

We next investigated N-glycosylation of the protected asparagine **13A** with β-glycosyl imidates **6a**−**e**[12] (Table 2). The galactosyl, glucosyl, and mannosyl imidates **6a**−**c** attached with an acyl protecting group at the C2 position and underwent N-glycosylation to form the 1,2-trans glycosidic bond, providing the corresponding β-*N*-glucosyl, β-*N*-galactosyl, and α-*N*-mannosyl[13] asparagines **14aA**−**14cA** in excellent yields (91−98%) (entries 1−3). The *N*-Troc glucosamine **6d**[14] was stereoselectively converted to the corresponding *N*-glycosyl asparagines **14dA** in moderate yields (68%) with complete β-selectivity (entry 4). On the other hand, glycosidation of the perbenzyl-protected galactoside **6e** provided


10.1021/ja0450298 CCC: $30.25 

***Table 2.*** N-Glycosylation of Asparagin **13A** and Peptides **13B** and **13C** with Glycosyl Donors **6a**−**e**




| entry | donor | acceptor | product | yield (%) | α:β[a] |
|---|---|---|---|---|---|
| 1 | **6a** | **13A** | **14aA** | 98 | β only |
| 2 | **6b** | **13A** | **14bA** | 99 | β only |
| 3 | **6c** | **13A** | **14cA** | 91 | α only |
| 4 | **6d** | **13A** | **14dA** | 68 | β only |
| 5 | **6e** | **13A** | **14eA** | 68 | 77:23[a] |
| 6 | **6a** | **13B** | **14aB** | 94 | β only |
| 7 | **6b** | **13B** | **14bB** | 78 | β only |
| 8 | **6c** | **13B** | **14cB** | 88 | α only |
| 9 | **6d** | **13B** | **14dB** | 31 | β only |
| 10[b] | **6a** | **13C** | **14aC** | 39 | β only |
| 11[b] | **6b** | **13C** | **14bC** | 10 | β only |
| 12[b] | **6c** | **13C** | **14cC** | 19 | α only |
| 13[b] | **6d** | **13C** | **14dC** | 0 | |

[a] Ratio was estimated by HPLC analysis based on refractive index detection. [b] Two equivalents of donors **6** was used.

the α-linked *N*-glycosyl amide **14eA** in 68% yield with moderate selectivity (entry 5). To demonstrate the feasibility of N-glycosylation, the synthesis of glycopeptides **14B** and **14C** by N-glycosylation of peptides **13B** and **13C** with **6a**−**d** was investigated (entries 6−13). The glycosyl dipeptides, **14aB**-, **14bB**-, and **14cB**-modified glucose, galactose, and mannose, were prepared in good yields with excellent selectivity from dipeptide **13B**. The *N*-Troc glucosamine **6d** was converted to the corresponding glycosyl amide **14dB** in moderate yield. However, glycosylation of tripeptides resulted in the reduced yields of the corresponding glycopeptides **14aC**, **14bC**, and **14cC**. Unfortunately, the glucosaminyl tripeptide **14dC** was not obtained under these reaction conditions. The low efficiency of the reaction might have resulted from the low solubility of the tripeptides in nitromethane. To our knowledge, this is the first example of the synthesis of *N*-glycosyl peptides by N-glycosylation of nonactivated primary amides.

In conclusion, we have demonstrated the efficient and elegant synthesis of *N*-glycosides by N-glycosylation of asparagine-containing peptides with glycosyl *N*-phenyltrifluoroimidates utilizing a catalytic amount of TMSOTf in nitromethane. This coupling method allows for the synthesis of the various *N*-glycosyl amides from the primary amide derivatives, which are effective biochemical probes for elucidation of the role of glycopeptides.

**Supporting Information Available:** Experimental procedures for the N-glycosylation and full characterization for compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) $^1$H NMR signals of the anomeric position are broad at room temperature due to the syn−anti isomerization around the carbon−nitorgen double bond. We determined the anomeric configuration of glycosyl imidates **6a**−**e** by low-temperature NMR experiments to be β. In the case of glucose, galactose, and glucosamine derivatives, the H−H coupling constants at the anomeric position are 8.2 and 8.7 Hz. In the case of mannosyl imidate **6c**, NOE was observed between H1 and H3, and H1 and H5. Additionally, the $^1J_{C-H}$C−H coupling constant of C1 was 164 Hz: Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293−297.

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