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A novel method for the formation of *N*-glycosides using hydroxamate

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Abstract—Direct formation of Asn-linked carbohydrate by *N*-glycosylation has been difficult, because of the lack of nucleophilicity of carboxamide nitrogen. We report here the novel *N*-glycosylation using Asn hydroxamate as a glycosyl acceptor. Reaction with glycosyl fluoride or glycosyl trichloroacetimidate afforded *N*-glycoside and subsequent reduction with SmI_2 gave Asn-linked glucose. Carbamate derived hydroxamates proved to have even enhanced reactivity to give *N*-glycosides in high yields. © 2003 Published by Elsevier Science Ltd.

N-Glycosylation is an important post-translational modification of proteins. It is mediated by the multisubunit enzyme oligosaccharyltransferase (OST) that transfers tetradecasaccharide ($Glc_3Man_9GlcNAc_2$) from dolichol diphosphate (Dol-PP) to the asparagine (Asn) side chain of the nascent protein (Scheme 1, Eq. (1)).[1] Besides the size of the oligosaccharide (M.W. 2370) transferred, the remarkable feature of this transformation is that generally unreactive carboxamide nitrogen reacts as a nucleophile. In fact, direct formation of the *N*-glycoside linkage with Asn by chemical glycosylation is yet to be realized.[2] Conventionally, synthesis of Asn-linked oligosaccharide has been achieved by the coupling of glycosylamine derivatives, obtainable by the treatment of reducing sugar with ammonium bicarbonate[3a] or reduction of glycosyl azide,[3b] with activated aspartic acid (Eq. (2)). We report here the *N*-glycosylation using hydroxamate, which was used successfully for the formation of Asn-carbohydrate linkage.

Biosynthetic incorporation of *N*-linked oligosaccharide requires Asn-X-Ser/Thr (X: any amino acid except Pro) as the consensus sequence.[4] To reconcile this requirement with the exceptional activity of the Asn side chain, intramolecular hydrogen bonding between hydroxy group and carboxamide has been proposed.[5,6] In order to chemically activate the carboxamide group of Asn, our attention was turned to the use of hydroxamate as the nucleophile. It has been known that the acidity of hydroxamate is substantially higher than that of caboxamide.[7] In addition, the nucleophilicity of nitrogen should be enhanced by the α-effect of oxygen. Contemplating these, we surmised that the nitrogen of **1** may have enough nucleophilicity to serve as a glycosyl acceptor (Scheme 1). After coupling, removal of the benzyloxy group should be possible under reducing conditions (Eq. (3)).

In order to test this hypothesis, hydroxamate **1a** was prepared from Fmoc-Asp-O*t*Bu as depicted in Scheme 2. Our initial attempt was directed to the Mitsunobu-type reaction[8] between **1a** and tetra-*O*-benzyl glucose **2a**. Although the coupling reaction proceeded cleanly under Tsunoda's conditions[9] (TMAD, Bu_3P/toluene), the product proved to be the *O*-glycoside **3** (Table 1, entry 1). On the other hand, glycosyl fluoride **2b** gave *N*-glycoside **4** when activated by Ag(I)–Cp_2MCl_2[10] (entries 2–8). As silver salts, AgOTf and $AgSbF_6$ gave comparable results (entries 2, 5, 7), while $AgBF_4$ (entry 3) and $AgPF_6$ (entry 4) were not suitable for this purpose. Cp_2HfCl_2 (entry 6) was somewhat less effective than Cp_2ZrCl_2. Reaction with the trichloroacetimidate **2c** proceeded in a similar efficiency (entry 9). Subsequent removal of the benzyloxy group was achieved cleanly by SmI_2 mediated reduction (THF, rt, 10 min)[11] in the presence of MeOH (25 equiv.) to afford glycosyl asparagine **5** in 88% yield. Although the product obtained here is uncommon αGlc1→Asn,[12] this

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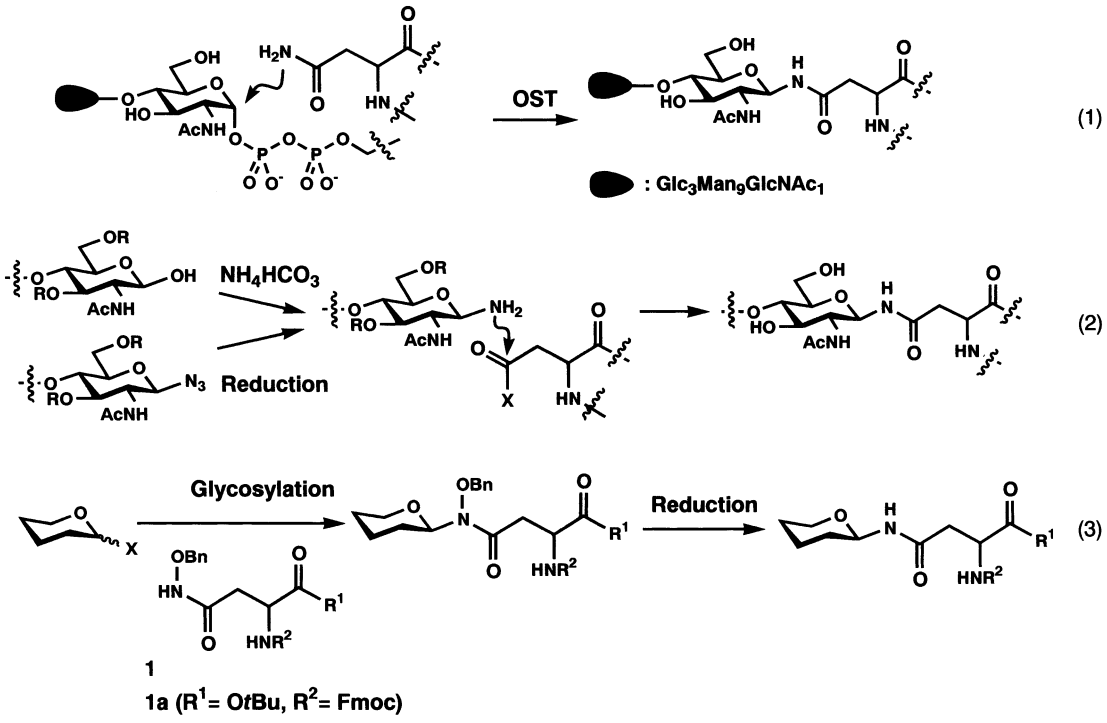
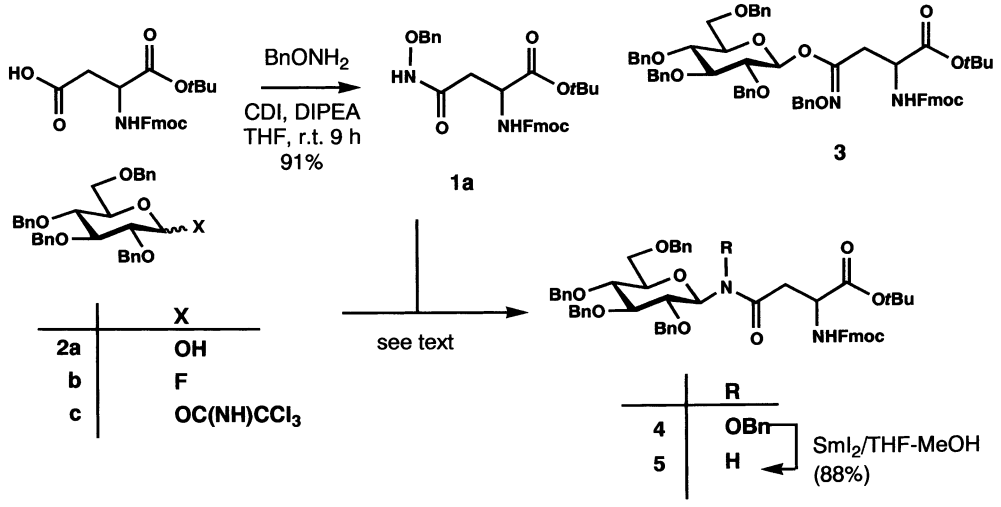


Scheme 1. Enzymatic and chemical formation of Asn-linked saccharide.



	X
2a	OH
b	F
c	OC(NH)CCl3

	R
4	OBn
5	H

Scheme 2. Formation of *N*-glycoside from hydroxamate.

result provides the first example of the formation of the Asn-linked carbohydrate by direct glycosylation.

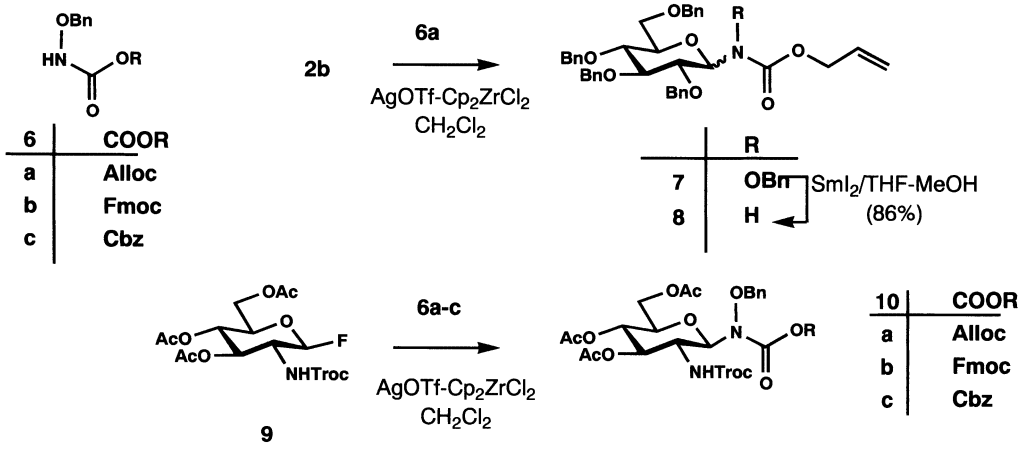
Carbamate-derived hydroxamate proved to have an even higher reactivity toward glycosyl donor (Scheme 3). Thus, **6a** was prepared from benzyloxyamine and allyl chloroformate and subjected to glycosylation with **2b**. This reaction proceeded smoothly at subzero temperature to afford the product in high yield (Table 1, entry 10). It was smoothly reduced to **8** under conditions identical to the preparation of **5**. More gratifyingly, the reaction of **6a**–**c** with trichloroethoxycarbonyl (Troc)-protected[13] glucosamine donor **9** was highly successful (entries 10–13). It selectively provided the β-linked *N*-glycosides **10a**–**c** in high yield. By contrast, reaction of **9** with **1a** did not proceed at all. Since the facile conversion of allyloxycarbonyl masked glycosylamine to *N*-glycosylated asparagine was reported previously,[14] this reaction might well provide a novel access to Asn-linked glycans.

In summary, a novel method for the formation of *N*-glycoside was developed, that was applied to the direct glycosylation of Asn side chain.

Table 1. Results of glycosylation reactions

Entry[a]	Donor	Hydroxamate	Reagents[d]	Temp./Time (h)	Product	Yield (%)	α:β
1	**2a**	**1a**	A	rt/3	**3**	95	β
2[b]	**2b**	**1a**	B	rt/3	**4**	53	5.5:1
3[c]	**2b**	**1a**	C	rt/24	**4**	18	1.7:1
4[c]	**2b**	**1a**	D	rt/24	**4**	Trace	ND
5[c]	**2b**	**1a**	E	rt/24	**4**	53	2:1
6[c]	**2b**	**1a**	F	rt/24	**4**	39	2.1:1
7[c]	**2b**	**1a**	E	rt/24	**4**	55	4:1
8[c]	**2b**	**1a**	G	−20°C–rt/10	**4**	48	1.8:1
9[b]	**2c**	**1a**	H	−78°C–rt/16	**4**	43	3.3:1
10[b]	**2b**	**6a**	B	−20°C–rt/5.5	**7**	90	3.5:1
11[b]	**9**	**6a**	B	−20°C/1	**10a**	83	β
12[b]	**9**	**6b**	B	−20°C/1	**10b**	84	β
13[b]	**9**	**6c**	B	−20°C/1	**10c**	87	β

[a] All reactions were performed in CH_2Cl_2, except entry 1 (toluene) and entry 7 (toluene–CH_2Cl_2), 9:1).
[b] Performed in the presence of molecular sieves 4A.
[c] Performed in the presence of molecular sieves AW-300.
[d] A: TMAD (3 equiv.)–Bu_3P (3 equiv.), B–F: AgX (1.0 equiv.)–Cp_2MCl_2 (0.5 equiv.) (B: X = OTf, M = Zr; C: X = BF_4, M = Zr; D: X = PF_6, M = Zr; E: X = SbF_6, M = Zr; F: X = SbF_6, M = Hf), G: $BF_3{\cdot}OEt_2$ (1.0 equiv.), H: TMSOTf (1.0 equiv.).



Scheme 3. Reaction with carbamate-derived hydroxamate.

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