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Amino acid fluoride for glycopeptide synthesis†

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Abstract

The formation of *N*-glycosidic linkage between *N*-acetylglucosamine (GlcNAc) and asparagine (Asn) was effected using aspartic acid γ-fluoride in combination with either glycosyl azide or silyl carbamate, by the action of Lindlar catalyst or Bu_4NF. Further elongation of peptide chain was performed to give pentapeptide. This method was further applied into the synthesis of trisaccharidic asparagine, using *p*-methoxybenzyl assisted stereoselective β-mannosylation as the key transformation.

Keywords: azides; carbamates; fluorides; glycopeptides.

Amino acid fluorides are powerful and rapid reacting acylating species, which have proved effective both in solution phase and solid phase peptide synthesis.[1] Excellent reactivity of amino acid fluoride is well underscored in the synthesis of peptibols, which contain multiple numbers of sterically demanding aminoisobutylic acid residues.[2] The use of amino acid fluorides, especially in the field of glycopeptide synthesis,[3] seems promising, because strong activation of the carboxyl component is likely to be required in order to incorporate sterically demanding carbohydrate carrying components. We report here the first demonstration of the utility of acid fluorides for the formation of *N*-linked carbohydrate containing peptides.

Common to all types of *N*-linked glycoproteins is the presence of β-configurated *N*-glycosidic linkage between asparagine (Asn) and *N*-acetylglucosamine (GlcNAc) residues. Here, by using aspartic acid γ-fluoride in combination with either glycosyl azide or silyl carbamate as latent glycosyl amine (Scheme 1) the formation of this particular type of linkage was effected.

GlcNAc derived azide **1**[4] was reacted with properly protected fluorides **2a**–**c**,[5] in the presence of Lindlar catalyst and Me_3SiOMe (to trap HF) under H_2 (Method A). After chromatographic purification, corresponding glyco-amino acid products **3a**–**c** were isolated in 88–93% yield (Table 1). This procedure is compatible with Fmoc/*t*-Bu, Cbz/Bn, and Boc/*t*-Bu protected aspartic acid fluorides (entries 1–3). Besides Lindlar catalyst, 10% Pd–C deactivated by ethylenediamine [Pd(ene)][6] was also effective for this transformation (entry 4).

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† Dedicated to Prof. Pierre Sinaÿ, on the occasion of his 62nd birthday.

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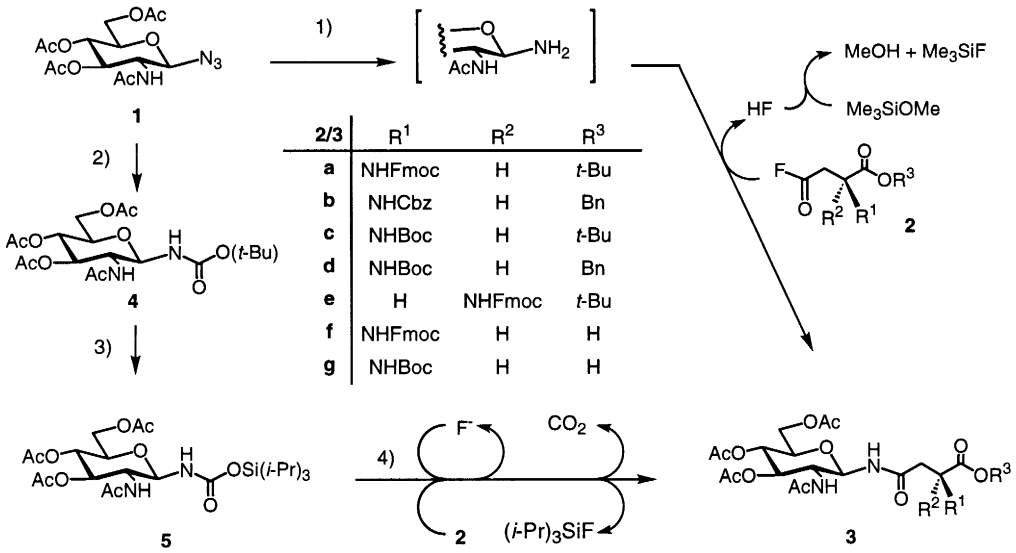


2/3	R^1	R^2	R^3
a	NHFmoc	H	*t*-Bu
b	NHCbz	H	Bn
c	NHBoc	H	*t*-Bu
d	NHBoc	H	Bn
e	H	NHFmoc	*t*-Bu
f	NHFmoc	H	H
g	NHBoc	H	H

Scheme 1. Synthesis of *N*-glycosylated asparagine. (1) Lindlar cat., Me_3SiOMe, H_2/THF or *i*-PrOH; (2) Lindlar cat., H_2, $(Boc)_2O$/EtOH, rt, 20 h, 87%; (3) $(i\text{-}Pr)_3SiOTf$ (1.5 equiv.), DBMP (1.7 equiv.)/CH_2Cl_2, rt, 40 min, 100%; (4) Bu_4NF (0.2 equiv.)/CH_2Cl_2

Table 1
Synthesis of glyco-amino acid **3** from **1** (Method A) or **5** (Method B) [a]Based on **1**. [b]Pd(ene) was used in place of Lindlar catalyst. [c]Calculated based on **5**.

entry	**2** (equiv.)	Method	solv.	time (h)	yield (%)
1	**a** (1.5)	**A**	THF	18	90[a]
2	**b** (1.5)	**A**	*i*-PrOH	19	93[a]
3	**c** (1.3)	**A**	THF	18	88[a]
4	**c** (1.3)	**A**	THF	18	92[a,b]
5	**a** (1.2)	**B**	CH_2Cl_2	20	82[c]
6	**b** (1.4)	**B**	$ClCH_2CH_2Cl$	18	87[c]
7	**c** (1.2)	**B**	CH_2Cl_2	40	78[c]
8	**d** (1.2)	**B**	CH_2Cl_2	20	84[c]

As an alternative precursor of glycosylamine, we envisaged Boc protected **4** assuming that it can be converted to the corresponding silyl carbamate **5** (Method B).[7] Coupling of the latter with acid fluoride was expected to proceed in the presence of a catalytic amount of Bu_4NF, with continuous regeneration of F^-. GlcNAc azide **1** was first converted to crystalline Boc derivative **4** (H_2, Lindlar cat., Boc_2O/EtOH, 33 h, 87%; m.p. 204–205°C) and then into silyl carbamate **5** under modified Ohfune's conditions[8] [$(i\text{-}Pr)_3SiOTf$, 2,6-di-*t*-butyl-4-methylpyridine (DBMP)/CH_2Cl_2, rt, 2 h, quantitative]. Subsequent reaction with fluorides **2a**–**d** was triggered by Bu_4NF (0.2 equiv.) to give **3a**–**d** in 78–87% yield (CH_2Cl_2, rt, 20 h, Table 1, entries 5–8).

Further elongation of peptide linkage was attempted as follows (Fig. 1). Free acids **3f** (1.2 equiv.) and **3g** (1.3 equiv.), prepared from **3a** (33%TFA/CH_2Cl_2, rt, 1.5 h) and **3d** (H_2, Pd/C, 1:1 AcOEt–EtOH, rt, 20 h), respectively, were coupled with tripeptide **6b** (prepared from Boc protected **6a** by treatment with 15% TFA/CH_2Cl_2, rt, 20 min), based on Carpino's in situ acid fluoride protocol [tetramethylfluoroformamidinium hexefluorophosphate (TFFH), $(i\text{-}Pr)_2NEt/CH_2Cl_2$, rt, 4 h][9] to afford tetrapeptides **7a** and **7b** in 67% and 91% yield, respectively. Compound **7b** was converted to silyl carbamate **7c** [$(i\text{-}Pr)_3SiOTf$, DBMP/CH_2Cl_2, rt, 2 h, 95%], which was then reacted with valine fluoride **8** (1.5 equiv.) in the presence of 0.2 equiv. of Bu_4NF in CH_2Cl_2 (rt, 20 h) to afford **9** in 92% yield.

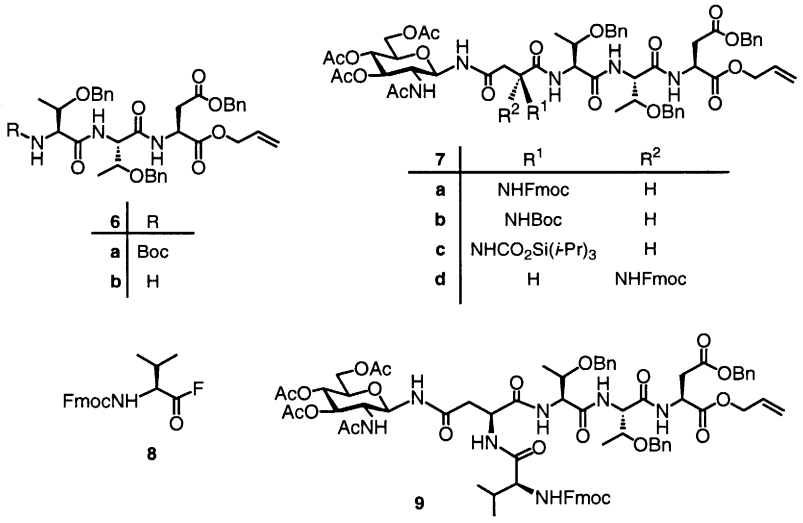


Fig. 1.

In order to ascertain the extent of epimerization during coupling reactions, enantiomeric fluoride **2e** was prepared from Fmoc-D-Asp-O(*t*-Bu) (Novabiochem) and converted to **3e** and **7d**, in a similar manner as described above. These glycopeptides are diastereomeric to **3a** and **7a**, with respect to the Asn residue. Stereochemical homogeneity of **3a**/**3e** and **7a**/**7d** was confirmed by 400 MHz ^{1}H NMR, and it was concluded that the extent of epimerization was negligible.

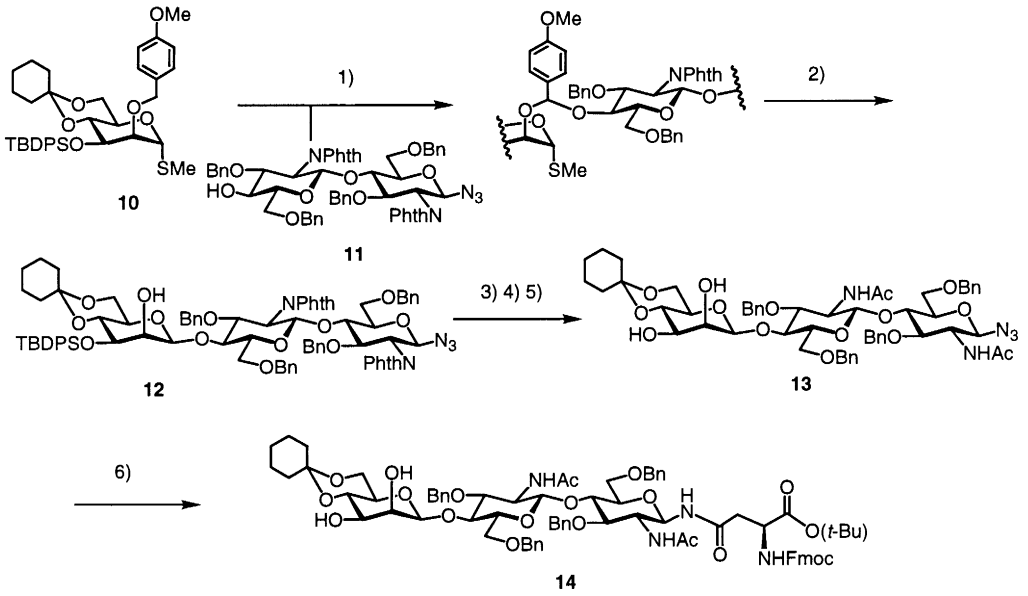
The applicability of the method to more functionalized systems was further explored using trisaccharide azide **13** synthesized by using *p*-methoxybenzyl-assisted intramolecular aglycon delivery.[10] β-Mannoside containing trisaccharide **12** [^{13}C NMR ($CDCl_3$) δ 99.8, 96.8 and 85.5 (anomeric carbons, $^1J_{C-H}$=162, 164 and 164 Hz, respectively)] was prepared from mannose donor **10**[10d] and disaccharide **11** and transformed into **13**. Coupling with fluoride **2a** gave **14** in 70% yield (Scheme 2).

In summary, acid fluorides were successfully used for the synthesis of *N*-glycosylated asparagine containing glycopeptides. Using either azide or silyl carbamate as a latent amino component, amide linkages can be formed under essentially neutral conditions (Lindlar cat. −Me_3SiOMe or cat. Bu_4NF).

1. Typical experimental procedure: Preparation of 3a

Method A: A mixture of compounds **1** (25.1 mg, 0.067 mmol) and **2a** (41.0 mg, 0.103 mmol), Me_3SiOMe (40 μl, 0.29 mmol) and Lindlar catalyst (10 mg) in THF (2 ml) was stirred under H_2 at room temperature for 18 h. The mixture was diluted with $CHCl_3$:MeOH (10:1, 10 ml), treated with aminopropyl silica gel (Sigma, 1.1 mmol/g, 0.10 g, rt, 0.5 h) and insoluble materials removed by filtration. The filtrate was evaporated in vacuo and the residue was chromatographed over silica gel (CH_2Cl_2:acetone 4:1∼1:1) to afford 44.7 mg (90%) of compound **3a**: δ_H ($CDCl_3$, 400 MHz) 6.09 (d, 1H, *J*=7.9 Hz, NH), 5.95 (d, 1H, *J*=8.4 Hz, NH), 5.2–5.0 (m, 3H), 4.6–4.0 (m, 7H), 3.74 (ddd, 1H, *J*=9.6, 4.0, 2.0 Hz, H–5), 2.85 (dd, 1H, *J*=16.5, 4.5 Hz, C*H*$_2$CONH), 2.70 (dd, 1H, *J*=16.5, 4.0 Hz, C*H*$_2$CONH), 2.07, 2.05, 2.04 and 1.96 (4s, 3H each, Ac), 1.44 (s, 9H, *t*-Bu); δ_C ($CDCl_3$, 100 MHz) 172.1, 171.8, 170.9, 170.5, 169.8, 169.1 and 155.9 (CO), 82.2 (C-1), 80.2 (Me_3C), 73.5, 72.9, 67.6, 67.1 (CH_2), 64.5, 61.7 (CH_2), 53.5, 51.0, 47.2, 38.0 (CH_2), 28.0, 23.3, 20.8, 20.7.

Method B: To a solution of compounds **5** (32.9 mg, 0.0625 mmol) and **2a** (30 mg, 0.075 mmol) in CH_2Cl_2 (1.5 ml), was added Bu_4NF (0.15 M, 60 μl, 0.009 mmol) and the mixture stirred at room



Scheme 2. Synthesis of trisaccharidic asparagine. (1) DDQ, MS4A/CH_2Cl_2, rt, 2.5 h; (2) MeOTf, DBMP, MS4A/$Cl(CH_2)_2Cl$, 40°C, 22 h, 78% over two steps; (3) $H_2N(CH_2)_2NH_2$/EtOH, reflux, 24 h; (4) Ac_2O/MeOH, rt, 2 h, 75% over two steps; (5) Bu_4NF/THF, rt, 45 min., 91%; (6) **2a** (1.9 equiv.), Me_3SiOMe (4.8 equiv.), Lindlar cat., H_2/THF, rt 26 h, 70%

temperature for 20 h. After processing as described for Method A, 37.7 mg (82%) of compound **3a** was obtained.

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