A Convergent Approach to the Chemical Synthesis of Asparagine-Linked Glycopeptides

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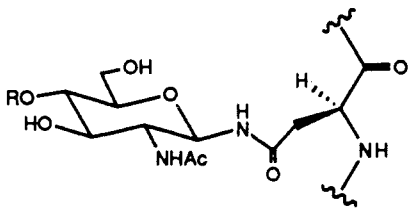
Summary: Reaction conditions for the direct coupling of glycosylamines to aspartic acid containing peptides are described.

A cotranslational process which is common to the biosynthetic pathways of many cell-surface and secreted eukaryotic proteins involves transfer of $(Glc)_3(Man)_9(GlcNAc)_2$ to an asparagine amide nucleophile to produce an asparagine-linked glycoprotein (1).[1] Because it occurs



1 R = $(Glc)_3(Man)_9(GlcNAc)$

cotranslocationally, glycosylation may play a role in the folding of glycoproteins.[2] In order to model the interactions between the nascent polypeptide chain and the attached oligosaccharide, we have undertaken the chemical synthesis of a series of glycopeptides. The existing methods of glycopeptide synthesis involve coupling of a glycosylamine to a suitably protected aspartic acid to give a protected asparagine–carbohydrate conjugate, followed by selective deprotection and elaboration of the glycoamino acid in a stepwise manner.[3-10] The O-glycosidic linkage present in complex oligosaccharides is not stable to the acidolytic deprotection conditions normally used in peptide synthesis;[3,6] therefore, application of this strategy to complex targets requires the use of specialized amino acid derivatives[3,10] or the elaboration of the oligosaccharide portion of the glycopeptide by chemical or enzymatic[8] methods. As a practical alternative, we are seeking to develop a convergent approach based on the coupling of an oligosaccharide β-glycosylamine to an aspartic acid containing peptide.[11] Large peptides could be made and purified by standard techniques, followed by the introduction of acid-sensitive and synthetically precious oligosaccharide in a late step. Realization of this strategy requires a high-yield coupling reaction for the formation of the glycopeptide amide bond and a protection/deprotection scheme which allows selective deprotection of the desired aspartic acid and, subsequently, mild deprotection of the product glycopeptide.[3] This paper focuses on the coupling reaction and reports the synthesis of four glycopeptide amides.

While the coupling of protected glycosyl amines (e.g. **2**, Chart I) to α-esters of aspartic acid (Asp) proceeds in good yield,[3,6,7,12] the coupling to Asp-containing peptides may be complicated by competing intramolecular succinimide formation.[13-15] In order to minimize succinimide formation and achieve a high-yield coupling, we have found that several factors must be carefully controlled. The activation of the peptide–aspartate carboxyl group, the minimization of base in the reaction medium, and the choice of protection for the carbohydrate hydroxyls all play critical roles (see Table I). Peptide **4**[15] was chosen for model studies because the valine residue adjacent to Asp was expected to hinder succinimide formation.[13] Activation of peptide **4** with diisopropylcarbodiimide (DIC) did not effect coupling, whereas coupling of the 1-hydroxybenzotriazole

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(4) Otvos, L., Jr.; Wroblewski, K.; Kollat, E.; Perczel, A.; Hollosi, M.; Fasman, G. D.; Ertl, H. C. L.; Thurin, J. *Pep. Res.* **1989**, *2*, 362.

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(7) (a) Garg, H. G.; Jeanloz, R. W. *Advances in Carbohydrate Chemistry and Biochemistry*; Academic Press, Inc.: New York, 1985; Vol. 43, pp 135–189. (b) Nakabayashi, S.; Warren, C. D.; Jeanloz, R. W. *Carbohydr. Res.* **1988**, *174*, 279.

(8) Thiem, J.; Wiemann, T. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 80.

(9) An example of the glycosylation of a simple amide has been reported; however, the α-anomer was the predominant product. Kahne, D.; Walker, S.; Cheng, Y.; Van Engen, D. *J. Am. Chem. Soc.* **1989**, *111*, 6881.

(10) Waldmann, H.; Marz, J.; Kunz, H. *Carbohydr. Res.* **1990**, *196*, 75.

(11) To our knowledge one attempt to implement this approach has been reported. The coupling of **2** to several di- and tripeptides was reported, albeit in low (ca. 20%) yield. However, the products were not deprotected. (a) Ishii, H.; Inoue, Y.; Chûjô, R. *Int. J. Pep. Prot. Res.* **1984**, *24*, 421. (b) Ishii, H.; Inoue, Y.; Chûjô, R. *Polymer J.* **1985**, *17*, 693.

(12) A solution of Boc-Asp(α-Bn) (3.0 equiv) and DIEA (5.3 equiv) in DMF was added to crude **2**.[23] (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP, 3.5 equiv)[20] was added, and the solution was stirred at 23 °C for 17 h. Silica gel chromatography afforded the glycosylated amino acid in 69% yield (none of the α-anomer was detected by ^{1}H NMR).

(13) Bodanszky, M.; Martinez, J. *J. Org. Chem.* **1978**, *43*, 3071. The rate of succinimide formation is dependent on the identity of the amino acid C-terminal to the aspartate.

(14) The attempted coupling of the peptide H_2N-WDAS-$CONH_2$[15] with **2** (3 equiv of BOP, 16 equiv of DIEA, DMF, 23 °C) afforded the succinimide as the major product by HPLC (30% isolated yield); none of the desired glycopeptide was detected. Additional reactions were not attempted due to the difficulty of the synthesis and purification of this peptide.

(15) Peptides **4**, **7**, **10**, and **12** were synthesized on the Kaiser oxime resin[16,17] using BOP or HBTU couplings, cleaved from the resin with ammonium acetate,[18] and deprotected using trifluoroacetic acid (TFA; **4** and **10**; Asp(tBu) was used) or hydrogenation (**7** and **12**; H_2/Pd on C/DMF/35–40 psi; Asp(Bn), Tyr(2,6-dichloroBn), Thr(Bn), and Ser(Bn) were used). Ac-WDAS-NH_2[14] was synthesized on the RapidAmide resin using the DuPont RaMPS system, and cleaved and deprotected with TFA/water/ethanedithiol/thioanisole (Asp(tBu) and Ser(tBu) were used). In the cases of peptides **7** and **12**, the corresponding succinimides were formed to varying extents during the synthesis and deprotection. In the most extreme case (**7**), the crude cleavage product contained ~ 55% succinimide **8** (under certain cleavage conditions, less than 5% of the desired peptide **7** was produced). The structure of **7** was supported by the FABMS fragmentation pattern (NIH MS facility). Succinimide **5** was formed as a byproduct of acetylation of H-DVF-NH_2 (peptide **4** was the major product). Each peptide, peptide succinimide, and glycopeptide was purified to homogeneity by reverse-phase HPLC (C4) and characterized by ^{1}H NMR and FAB mass spectrometry. The β stereochemistry at the anomeric position of each glycopeptide was confirmed by the magnitude of the coupling constant ($J_{1,2}$ = ca. 9 Hz).

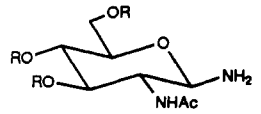
(16) (a) Kaiser, E. T. *Acc. Chem. Res.* **1989**, *22*, 47. (b) Kaiser, E. T.; et al. *Science* **1989**, *243*, 187.

(17) Jarrett, J. T.; Lansbury, P. T., Jr. *Tetrahedron Lett.* **1990**, *31*, 4561.

(18) Jarrett, J. T., unpublished results.

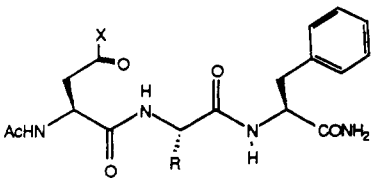
0022-3263/90/1955-5560$02.50/0

Chart I



2 R = Ac

3 R = H

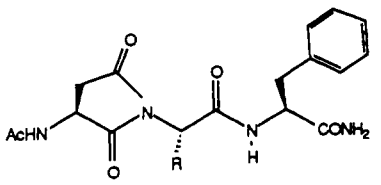


4 R = $CH(CH_3)_2$, X = OH

6 R = $CH(CH_3)_2$, X = NH(GlcNAc)

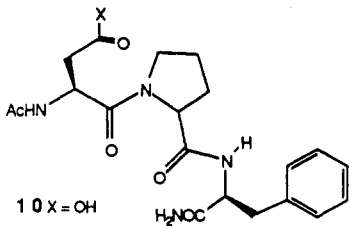
7 R = H, X = OH

9 R = H, X = NH(GlcNAc)



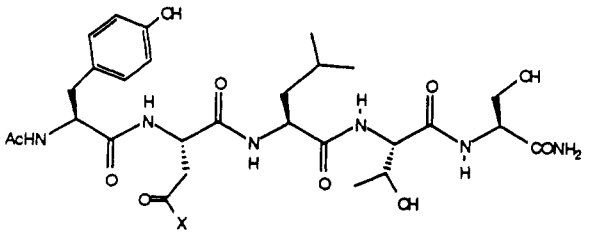
5 R = $CH(CH_3)_2$

8 R = H



10 X = OH

11 X = NH(GlcNAc)



12 X = OH

14 X = NH(GlcNAc)

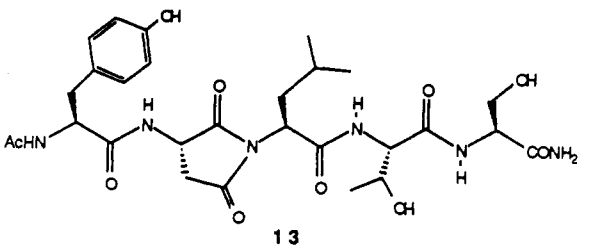


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Table I

entry	peptide[a]	glycosylamine	coupling reagent[b]	DIEA, equiv	product distribution (HPLC),[c] % glycopeptide	starting material	succinimide
1	4 (DVF)	3	HBTU/HOBt	0	95 (6)	0 (4)	5 (5)
2	4 (DVF)	2[d]	HBTU/HOBt	0	≤10	≥85 (4)	5 (5)
3	4 (DVF)	3	DIC/HOBt[e]	0	20 (6)	75 (4)	5 (5)
4	4 (DVF)	3	BOP/HOBt	0	>90 (6)	5 (4)	<5 (5)
5	4 (DVF)	3	BOP/HOBt	5	90 (6)	0 (4)	10 (5)
6	10 (DPF)	3	BOP[f]	1.5	95 (11, 58%)	5 (10)	NA
7	7 (DGF)	3	HBTU/HOBt	0	80 (9, 53%)	15 (7)	5 (8)
8	12 (YDLTS)	3	HBTU/HOBt	0	80 (14, 61%)	15 (12, 12%)	5 (13)

[a] The one-letter code is used for clarity: D, Asp; V, Val; F, Phe; P, Pro; G, Gly; Y, Tyr; L, Leu; T, Thr; S, Ser; W, Trp; A, Ala. [b] All reactions utilized 2 equiv of glycosylamine and 3 equiv of coupling reagent (1 equiv of HOBt) at room temperature, except where otherwise indicated. [c] ±5%, by absorbance (225 nm); purified yields in parentheses. [d] See footnote 23. In this case, 6 equiv (relative to peptide) glycosyl azide was reduced and coupled in situ. [e] The peptide was premixed with DIC (3 equiv) and HOBt (3 equiv) for 20 min before addition of glycosylamine. [f] 1.5 equiv of glycosylamine, 1.5 equiv of BOP.

(HOBt) ester, which was formed via DIC activation, proceeded slowly (see the table, entry 3). Optimal conditions[19] involved activation with (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP)[20] or 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (entries 1, 4, 6–8).[21] We have also observed that succinimide formation is directly related to the amount of base present in the coupling reaction (entry 4 vs entry 5); we recommend using the minimum required (2 equiv of glycosylamine or 1 equiv of glycosylamine with 1 equiv of diisopropylethylamine (DIEA)).[22] The choice of the amine component for the

(19) To a solution of Ac-YDLTS-NH_2 (**12**, 50 μmol, 31.9 mg) in DMF (500 μL) was added: GlcNAcNH_2 (Sigma, 100 μmol, 22.0 mg) in DMSO/DMF (850 μL DMSO/500 μL DMF); HBTU (150 μmol, 56.9 mg) in DMF (1 mL); and HOBt (50 μmol, 6.8 mg) in DMF (500 μL). The reaction mixture was stirred at 23 °C, and the reaction was monitored by reverse-phase HPLC (C4 analytical column; 95:5 → 0:100 over 20 min (H_2O (0.1% AcOH)–CH_3CN (0.1% AcOH))). When HPLC indicated no further conversion (3.5 h), the crude mixture was purified by HPLC (isocratic conditions: 88:12 H_2O (0.1% AcOH)–CH_3CN (0.1% AcOH)) to give Ac-YN(GlcNAc)LTS-NH_2 (**14**, 25.6 mg, 61% yield) and unreacted Ac-YDLTS-NH_2 (3.8 mg, 12%). Glycopeptide **14** was characterized by FABMS (MW(calc) = 840.4; $(M + H)^+$ = 841.5) and ^1H NMR (300 MHz, DMSO-d_6: Asn δCONHR, δ 8.2, d, J = 9 Hz; anomeric H1, δ 4.8, dd, J = 9, 9 Hz, confirms β stereochemistry).

(20) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219.

(21) Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927.

(22) Although the model reactions reported in the table involve the use of glycosylamine **3** as nucleophile and as base (2 equiv), we have recently shown that the reaction works equally well (and is more economical) with 1 equiv of **3** and 1 equiv of DIEA as base (other conditions identical to ref 19).

coupling reaction is also critical. Using our optimized coupling conditions,[19] peptide **4** was coupled to **3** to afford the glycopeptide **6** in good yield (entry 1). However, using the O-acetylated nucleophile **2**,[23] no significant coupling was observed (entry 2). This result, which is consistent with our earlier experience[14] and the low yields obtained in the past using **2** as the amine component,[11] may be due to the decreased nucleophilicity of **2** relative to **3**.[25]

In contrast to peptide **4**, peptide **7** should be optimally disposed for cyclization;[13] however, using our conditions,[19] a 53% yield of glycopeptide **9** was isolated with minimal succinimide formation (entry 7). Glycosylamine **3** has also been coupled to peptides **10** and **12**[26] to provide the glycopeptides **11** (58% purified yield) and **14** (61%), respectively (entries 6 and 8).[15,19] Our current focus is to test the limits of this reaction regarding the size of each component and to adapt this coupling procedure to a solid-phase methodology which allows for selective deprotection of a single carboxyl group at the desired aspartic acid. This procedure[19] has been used to successfully glycosylate resin-bound aspartic acid.[27] Preliminary ^{1}H NMR experiments of glycopeptides **6** and **11** indicate that the attached carbohydrate may influence the conformation of the peptide chain, possibly via the formation of a hydrogen bond.[28] The availability of a wide variety of synthetic glycopeptides will enable us to elucidate these important interactions.

Acknowledgment. We thank Beth Berger for synthesizing and purifying H_2N-WDAS-$CONH_2$[14] and Dr. Chris Warren for his helpful advice. We are grateful to Dr. Ioannis Papyannopoulos of the MIT MS facility (NIH Grant no. RR00317) for FABMS analyses. This work was supported by a grant from the Office of Naval Research (Molecular Recognition Program), the donors of the Petroleum Research Fund, administered by the American Chemical Society, and funds from the Camille and Henry Dreyfus Foundation (New Faculty Award) and Merck & Co. (Faculty Development Award).

(23) A method for the conversion of peracetylated oligosaccharides with GlcNAc at the reducing terminus to the β-glycosylamine and subsequent coupling to an amino-protected aspartic acid ester has been reported.[7] We have modified that procedure to minimize handling of the unstable glycosyl amine as follows: the β-glycosylazide[7b,8] was treated with 1,3-propanedithiol[24] (5 equiv) and diisopropylethylamine (3 equiv) in dimethylformamide (DMF) for 1.5 h at 23 °C to afford the β-glycosylamine **2**. Solvent was removed in vacuo, and the crude product was coupled directly.[12]

(24) Bayley, H.; Standring, D. N.; Knowles, J. R. *Tetrahedron Lett.* **1978**, 3633.

(25) A referee suggests that the observed difference in yield may simply be due to the lability of **2** under the reaction conditions. Although the rearrangement (see ref 7b) and dimerization (Paul, B.; Korytnyk, W. *Carbohydr. Res.* **1978**, *67*, 457) of **2** are precedented, we feel that this explanation is unlikely in light of the successful coupling of **2** to Boc-Asp(α-Bn) (see ref 12).

(26) Peptide **12** is derived from the glycosylation site of ovalbumin. Glabe, C. G.; Hanover, J. A.; Lennarz, W. J. *J. Biol. Chem.* **1980**, *255*, 9236.

(27) Fluorenylmethoxycarbonyl (Fmoc) protected aspartic acid bound to the polystyrene-based methylphenacyl resin[29] was treated with **3** (2 equiv), HBTU (3 equiv), and HOBt (1 equiv) in DMF/DMSO. After shaking for 25 h, the resin was photolyzed (350 nm, DMF/2 equiv of H_2O, 23 h, 23 °C) to provide the product Fmoc-Asn(GlcNAc), as well as some unreacted Fmoc-Asp (~4:1 glycoamino acid to starting material, by HPLC).

(28) Glycopeptides **11** and **6** were analyzed (^{1}H NMR, 300 MHz, DMSO) over the temperature range 20–50 °C. For **11**, the chemical shifts of two amide protons were relatively insensitive to temperature ($\Delta\delta/\Delta T \leq 3.5$ ppb/deg), indicating the participation of these protons in hydrogen bonds.[11] For **6**, one amide proton appears to be involved in hydrogen bonding. Details of these and other NMR experiments will be published elsewhere.

(29) Wang, S. S. *J. Org. Chem.* **1976**, *41*, 3258.

Photochemical Intramolecular Cyclization Reactions of Acylgermanes[1]

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Summary: The first photochemical intramolecular cyclization reaction of acyltriphenylgermanes to give 5- and 6-membered cyclic ketones bearing α-(triphenylgermyl)-methyl group is described.

There has been rapid development of a large number of remarkable organic reactions with functionalized silicon reagents. In contrast, development of germanium chemistry has been quite restricted. The utilization of organogermanium compounds in organic synthesis has, however, begun to generate considerable interest.[2] For example, acylgermanes under UV irradiation have recently been shown to mainly undergo a Norrish type-I reaction to generate a germyl and acyl radical pair, via the acylgermyl S_1 state.[3] On the other hand, photochemical reactions of acylsilanes,[4-6] lead to a nucleophilic siloxycarbene formed from the acylsilane T_1 state, which undergoes intermolecular reaction with a variety of reagents and/or proceeds to a Norrish type-II reaction involving γ-H abstraction and fragmentation. Consequently, the photochemical reactions characteristic of acylgermanes can be of interest as a novel means of generating acyl radicals.[7] The purpose of this paper is to demonstrate that acylgermanes are useful photoprecursors to acyl radicals and

(1) Presented in part at the 1989 International Chemical Congress of Pacific Basin Societies, Honolulu, December 17–22, 1989 (Abstracts of Papers, ORGN 439).

(2) (a) Oda, H.; Morizawa, Y.; Oshima, K.; Nozaki, H. *Tetrahedron Lett.* **1984**, *25*, 3217. Oda, H.; Oshima, K.; Nozaki, H. *Chem. Lett.* **1985**, 53. (b) Yamamoto, Y.; Hatsuya, S.; Yamada, J.-I. *J. Org. Chem.* **1990**, *55*, 3118. (c) Inoue, S.; Sato, Y. *Organometallics* **1987**, *6*, 2568. Inoue, S.; Sato, Y.; Suzuki, T. *Ibid.* **1988**, *7*, 739. (d) Kitching, W.; Olszowy, H. A.; Harvey, K. *J. Org. Chem.* **1982**, *47*, 1893. Wickham, G.; Young, D.; Kitching, W. *Organometallics* **1988**, *7*, 1187. (e) Sano, H.; Miyazaki, Y.; Okawara, M.; Ueno, Y. *Synthesis* **1986**, 776. (f) Kauffnann, T.; Ilchmann, G.; Koenig, R.; Wensing, M. *Chem. Ber.* **1985**, *118*, 391. (g) Livantsova, L. I.; Perelygina, O. P.; Zaitseva, G. S.; Baukov, Yu. I. *Zh. Obshch. Khim.* **1984**, *54*, 1925. (h) Stanczyk, W. *J. Organomet. Chem.* **1986**, *299*, 15. (i) Soderquist, J. A.; Negron, A. *J. Org. Chem.* **1989**, *54*, 2462. (j) Chatani, N.; Horiuchi, N.; Hanafusa, T. *J. Org. Chem.* **1990**, *55*, 3393. (k) Kiyooka, S.; Nakata, M. *Chem. Lett.* **1988**, 721. Kiyooka, S.; Shiota, F.; Shibuya, T. *Ibid.* **1987**, 495. Kiyooka, S.; Miyauchi, A. *Ibid.* **1985**, 1829.

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