Phototransamidation as a Method for the Synthesis of *N*-Glycosyl Asparagines

Kristóf Vízvárdi, Christian Kreutz, Alexander S. Davis, Vincent P. Lee, Benjamin J. Philmus, Ondrej Simo,† and Katja Michael*
Department of Chemistry, University of Hawaii, Honolulu 96822, U. S. A.
†*Department of Chemistry, University of the Pacific, Stockton 95211, U. S. A.*

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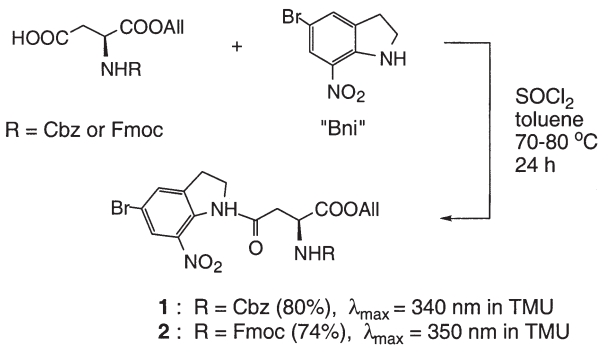
N-Glycosyl asparagines were synthesized by a mild photochemical coupling method in which a photoreactive amide of an aspartic acid's β-carboxyl group is condensed with an aminosaccharide. Upon excitation, the γ-carbon becomes susceptible to nucleophilic attack and the obtained *N*-glycosyl asparagines, which may be useful building blocks for the synthesis of *N*-glycopeptides and neoglycopeptides, are generated in good yields.

N-Glycopeptides are important tools for glycobiology because as partial structures of complex *N*-glycoproteins,[1] they can serve as model compounds in biochemical and biophysical studies. In *N*-glycopeptides, typically a β-*N*-acetylglucosamine is linked to an asparagine side chain via an amide bond. An elegant way to synthesize *N*-glycopeptides is by condensing a glycosylamine with an activated form of an aspartic acid containing peptide.[2] However, a drawback of this approach is that aspartimides are produced to some extent during the critical coupling step. The amount of aspartimide formed clearly correlates with the amount of base present in the reaction mixture.[3]

Our interest in glycopeptides led us to the idea to test a new concept for establishing the amide linkage between glycosyl moieties and the aspartic acid side chain. Based on the discovery that *N*-acyl-5-bromo-7-nitroindoline can undergo photocoupling with nucleophiles in the absence of tertiary amines,[4,5] the following initial questions were raised: Is 5-bromo-7-nitroindoline-derivatized aspartic acid reactive enough in order to undergo phototransamidation with the relatively weakly nucleophilic glycosylamines, and is this reaction efficient when the starting materials are reacted in equimolar amounts? In order to explore the applicability and scope of this photochemical reaction at the glycosyl amino acid level, several natural and unnatural aminosaccharides including the weaker nucleophilic glycosylamines, and photoreactive aspartic acid substrates were synthesized.

Amidation of the commercially available 5-bromo-7-nitroindoline (Bni) with Cbz-Asp-OAll and Fmoc-Asp-OAll in the presence of thionyl chloride furnished the photoreactive amino acids Cbz-Asp(Bni)OAll (**1**) and Fmoc-Asp(Bni)OAll (**2**) in 80% and 74% yield, respectively (Scheme 1). The two glycosylamines **3**[6,7] and **4**[7] derived from *N*-acetylglucosamine and chitobiose (Scheme 2), as well as the more nucleophilic and partially unprotected aminomethyl-*C*-glycosides **5**[8] and **6**[9] derived from glucose (Scheme 3) were synthesized for testing their performance as amine nucleophiles in the photolysis experiments. The equipment needed for the light induced coupling reaction is inexpensive and comprises an UV lamp and a Pyrex reaction container that allows for water cooling and inert gas bubbling.

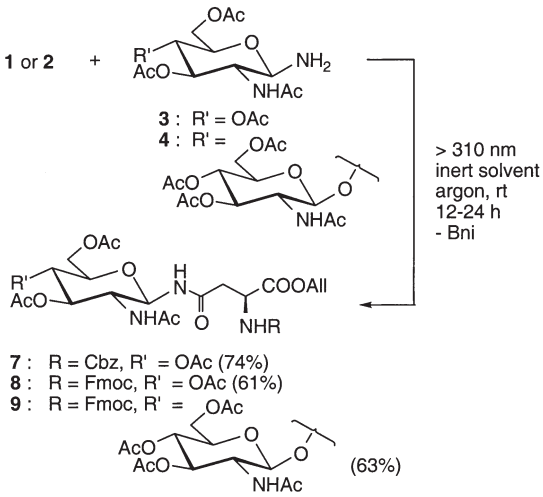
The amino acids **1** and **2** successfully underwent phototransamidation with glycosylamines **3** and **4** (Scheme 2) and with the aminomethyl-*C*-glycosides **5** and **6** (Scheme 3) in inert



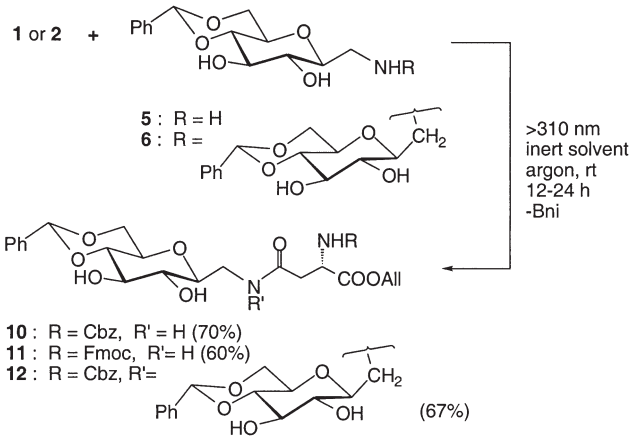
Scheme 1. Synthesis of photoreactive asparagine derivatives.

solvents such as tetrahydrofuran or *N*,*N*,*N*′,*N*′-tetramethylurea (TMU). The two starting materials were employed in nearly equimolar amounts with one of the two reactants in a slight excess between 1.1 and 1.3 molar equivalents. The *N*-glycosyl Cbz and Fmoc protected allyl asparaginates **7**-**12** were produced in 60-74% yield,[10] which is in a similar range when compared to the condensation of glycosylamines with acid chlorides of aspartic acid.[11] We have not observed any anomerization or racemization in the amino acid moiety. The orthogonally protected glycosyl amino acids **7**-**12** can be used as building blocks for the synthesis of glycopeptides and neoglycopeptides.

In the phototransamidation of Fmoc protected asparagine derivative **2** and aminomethyl-*C*-glycoside **5** we observed the presence of approximately 10% dibenzofulvene. We attribute this partial loss of the Fmoc group to the basicity of the primary amine



Scheme 2. Photocoupling of glycosylamines derived from glucosamine and chitobiose with photoreactive asparagine derivatives to orthogonally protected 1-*N*-(4-L-aspartyl)-β-D-glycosylamines.

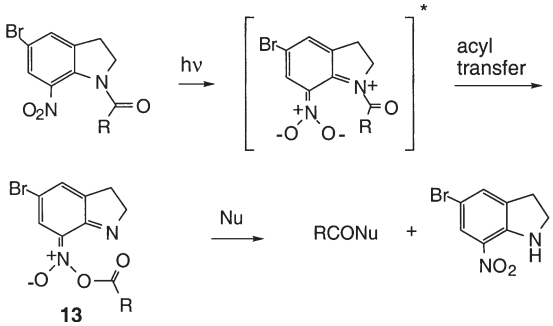


Scheme 3. Photocoupling of partially unprotected monomeric and dimeric amino-*C*-glycosides derived from glucose with photoreactive asparagine derivatives to unnatural *C*-glycosyl asparagine derivatives.

5. Fmoc cleavage was not apparent when the less basic glycosyl amines **3** and **4** were used.

It has been suggested that, upon photoexcitation, *N*-acyl-5-bromo-7-nitroindolines undergo intermolecular acyl transfer to an adjacent nitro group oxygen, leading to the activated intermediate **13** whose fate is solvent dependent. In organic solvents the prevailing reaction pathway is the acylation of a nucleophile and simultaneous liberation of Bni (Scheme 4).[12–14] Thus, *N*-acyl-5-bromo-7-nitroindolines such as **1** and **2** are latent activated esters, which become functional when exposed to light. Intermediate **13** is active enough to react with the relatively weakly nucleophilic glycosylamines **3** and **4**.

In conclusion, phototransamidation is a useful method for generating *N*-glycosyl asparagines in good yields, and the two reactants can be economically utilized in nearly equimolar amounts. The strength of this method lies in its mild activation under neutral conditions. Therefore, together with the ability of the *N*-acyl-5-bromo-7-nitroindoline group to function as a protecting group in the dark, this method has great potential to be applicable to the more demanding amide formation between glycosylamines and aspartic acid containing peptides. Research directed toward a light induced convergent *N*-glycopeptide synthesis is currently underway.



Scheme 4. Proposed photolysis mechanism in inert organic solvents.[12–14]

Gratitude is expressed to Prof. Carlo Unverzagt for insightful discussions.

References and Notes

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9 The synthesis of *C*-glycoside **6** will be published separately (Simo, O.; Gross, P.H.).
10 Exemplified photochemical coupling procedure: The asparagine derivative **1** (0.072 mmol) and glycosylamine **3** (0.086 mmol) were dissolved in 6 mL dry THF in a photoreactor (microscale photochemical reaction assembly, model 7880, pyrex, ACE Glass). After argon purging for 30 min the reaction mixture was irradiated with a mercury gaseous discharge lamp (Pen-Ray, 5.5 watt, low pressure, cold cathode) under a continuous argon flow at 23 °C. After 18 h TLC indicated consumption of **1**, while small amounts of **3** were still present. Normally, irradiation is stopped at this point, but in this case another 0.036 mmol of **1** was added and irradiation was continued for 7 h. After removal of the solvent the remainder was chromatographed on silica with ethyl acetate/hexanes 3:1. $R_f = 0.25$ (ethyl acetate/hexanes 3:1); yield of **7**: 61%. ^{1}H NMR (300 MHz, dmso-d_6, 293 K): δ 8.66 (m, 1H, NH-1); 7.89 (m, 1H, NH-2); 7.62 (m, 1H, NH-α), 7.40-7.23 (m, 5H, aromatic); 5.83 (m, 1H, allylic CH); 5.27 (m 1H, allylic terminal CH, $J_{trans} = 17.4$ Hz); 5.16 (m, 2H, allylic terminal CH); 5.14, (d, 1H, H-1, $J_{H\text{-}1/H\text{-}2} = 9.15$ Hz, coupling constant measured after homodecoupling); 5.06 (dd, 1H, H-3, $J_{H\text{-}2/H\text{-}3} = J_{H\text{-}3/H\text{-}4} = 10.5$ Hz); 5.00 (s, 2H, benzylic CH_2); 4.79 (dd, 1H, H-4, $J_{H\text{-}4/H\text{-}5} = 9.8$ Hz); 4.54 (m, 2H, allylic aliphatic CH_2); 4.44 (m, 1H, H-α); 4.16 (m, 1H, H-6); 3.89 (m, 1H, H-6′); 3.84 (m, 1H, H-2); 3.78 (m, 1H, H-5); 2.61 (m, 1H, H-β); 2.49 (m, 1H, H-β′); 1.97, 1.96 (2s, 3H, Ac); 1.94 (s, 3H, Ac); 1.88 (s, 3H, Ac); 1.71, 1.69 (2s, 3H, Ac); ^{13}C NMR (125 MHz, dmso-d_6, 293 K) δ 171.1, 170.0, 169.5, 169.3 (5 × C=O); 155.8 (C=O, urethane); 136.8 (aromatic); 132.3 (olefinic CH, allyl); 128,3, 127.9, 127.7 (aromatic); 117.5, 117.6 (2 × olefinic CH_2, allyl); 78.0 (C-1); 73.4 (C-3); 72.3 (C-5); 68.3 (C-4), 65.6 (CH_2, Cbz); 65.0 (aliphatic CH_2, allyl); 61.8 (C-6); 52.1 (C-2); 50.2, 50.3 (2 × C-α); 36.8 (C-β); 22.6 (CH_3, acetyl); 20.5 (CH_3, acetyl); 20.4 (2 × CH_3, acetyl). The signal duplication of two acetyl-CH_3 groups in the ^{1}H NMR spectrum as well as the signal duplication of C-α and the terminal allylic carbon in the ^{13}C NMR spectrum indicates two conformational isomers, most likely cis/trans isomers of the urethane. FAB MS: $m/z = 636$ ($M+H^+$); $m/z = 674$ ($M+K^+$).
11 For example, coupling of Fmoc-Asp(Cl)OPfp with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine affords the glycosyl amino acid in 72% yield: I. Christiansen-Brams, M. Meldal, and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, **1993**, 1461.
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