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Carbohydrate Research 340 (2005) 557–566
Carbohydrate RESEARCH

# Synthesis of glycosyl amino acids by light-induced coupling of photoreactive amino acids with glycosylamines and 1-*C*-aminomethyl glycosides

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Received 5 October 2004; accepted 31 December 2004
Available online 29 January 2005

**Abstract**—The glycosylamines of *O*-acetyl-protected GlcNAc and chitobiose, as well as two partially unprotected 1-*C*-aminomethyl glucosides, were photochemically coupled with orthogonally protected *N*-aspartyl-5-bromo-7-nitroindoline derivatives. The reactions proceeded under neutral conditions by irradiation with near-UV light. The glycosyl asparagines with *N*- or *C*-glycosyl linkages were afforded in 60–85% yield on a 10–70 mg scale. Moreover, the ability of a highly photoreactive *N*-glutamyl-4-methoxy-7-nitroindoline derivative to acylate amino saccharides was tested. Upon irradiation in the presence of a dimeric 1-*C*-aminomethyl glycoside, or a glycosylamine, the corresponding glycosyl glutamines were obtained in 50% and 30% yield, respectively. Preparations of the photoreactive aspartates and the 1-*C*-aminomethyl glycosides are also described.


*Keywords:* *C*-Glycosides; Glycosyl amino acids; Photochemical reaction; Transamidation

## 1. Introduction

In the synthesis of complex biopolymers, mild reaction conditions for the individual manipulation steps are not only desirable in order to extend the scope of an orthogonal protecting group strategy, but they may also be necessary to avoid or minimize irreversible side reactions. For example, in the convergent synthesis of *N*-glycopeptides, the aspartic acid to be coupled with a glycosylamine can suffer from substantial aspartimide formation, a competing intramolecular cyclization involving, as a nucleophile, the adjacent amide nitrogen of the amino acid located at the *C*-terminal side of Asp.[1–3] Since the extent of aspartimide formation depends partially on the amount of base present in the coupling mixture, neutral reaction conditions may help to reduce the amount of aspartimide formed in the convergent synthesis of *N*-glycopeptides.

Our interest in *N*-glycopeptides led us to the idea to explore a base-free photochemical coupling method, at first on the glycosyl amino acid level, in order to assess its usefulness for *N*-glycopeptide synthesis. *N*-Acyl-7-nitroindolines are attractive photoreactive species that are known to acylate nucleophiles upon irradiation with near-UV light in the absence of tertiary amines. Almost 30 years ago it was discovered that *N*-acyl-5-bromo-7-nitroindolines undergo photosolvolysis,[4] and this was followed by their application as light-sensitive protecting groups,[5] and their use in fragment condensation in peptide chemistry.[6] In recent times, an *N*-acyl-7-nitroindoline was incorporated into a photolabile linker for solid-phase synthesis,[7] and *N*-acyl-5,7-dinitroindolines were applied to acylate aliphatic amines, generating amides, and carbamates.[8,9] Our preliminary results using *N*-acyl-5-bromo-7-nitroindolines in glycosyl asparagine synthesis were recently reported in a

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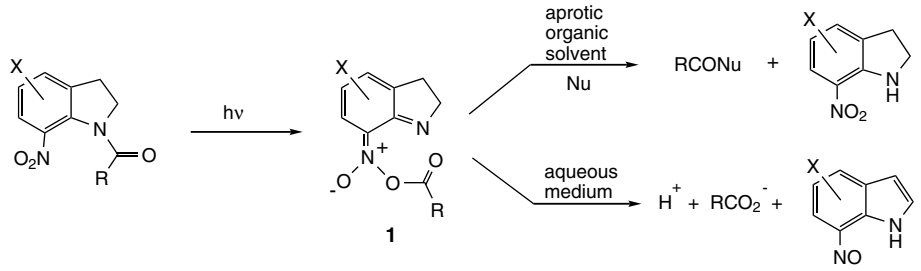
doi:10.1016/j.carres.2004.12.023




**Scheme 1.** Proposed photolysis mechanisms of *N*-acyl-nitroindolines in the presence of a nucleophile in an inert organic solvent, and in water.[13] (X = ring substituent, e.g., 4-methoxy, 5-bromo, etc.)

communication.[10] Furthermore, a number of *N*-acyl-7-nitroindoline derivatives with varying substituents have been developed as effective caged compounds, which undergo flash photolysis to release carboxylates in aqueous reaction media.[11–14]

The light-induced acylation of nucleophiles in aprotic organic solvents is believed to proceed via a nitronic anhydride **1** resulting from acyl transfer to the adjacent nitro group upon excitation.[13] In inert organic solvents, nucleophiles present in the reaction mixture become acylated by **1**, and recyclable 7-nitroindoline is released. Under aqueous conditions, however, intermediate **1** releases a carboxylic acid and a 7-nitrosoindole (Scheme 1).[11,13]

Since glycosyl amino acids and glycopeptides may be of value for medicinal chemistry, another important issue is their stability toward enzymatic degradation under physiological conditions. Stability toward enzymatic hydrolysis can be introduced by the incorporation of foreign structural elements, such as *C*-glycosides,[15–20] that may convert glycoconjugates into poor substrates for glycoamidases. For example, a *C*-glycopentapeptide with an insertion of a methylene group between the asparagine's side chain amide group and the anomeric center of GlcNAc showed resistance toward *N*-glycanase-catalyzed hydrolysis and was in fact a potent inhibitor of this enzyme.[21]

Here we report the photochemical acylation of two glycosylamines and two 1-*C*-aminomethyl glycosides with orthogonally protected *N*-aspartyl-5-bromo-7-nitroindoline derivatives under neutral reaction conditions, which produces glycosyl asparagines with natural *N*- or unnatural *C*-glycosyl linkages. We also describe the photoacylation of amino saccharides using a highly photoreactive glutamate derivative with an incorporated 4-methoxy-7-nitroindoline moiety.[12] The syntheses of the photoreactive asparagines, and the syntheses of the 1-*C*-aminomethyl β-D-glucosides used in this study are also described.

## 2. Results and discussion

### 2.1. General considerations

The *O*-acetylated GlcNAc and chitobiose derived glycosylamines **2**[22–24] and **3**[25–27] are common reactants in *N*-glycosyl amino acid synthesis and *N*-glycopeptide synthesis by conventional methods. Recently, we have subjected **2** and **3**, as well as the *C*-glycosidic amino saccharide **5** and its dimeric derivative **9** (Fig. 1) to light-induced glycosyl asparagine synthesis.[10] These glycosyl asparagines may be useful building blocks for the synthesis of *N*-glycopeptides and neoglycopeptides. This was recently demonstrated by means of the *N*-chitobiosyl asparagine **14**, which was deprotected and used as a building block in the synthesis of a glycododecapeptide partial structure of a human cell-adhesion protein.[28]

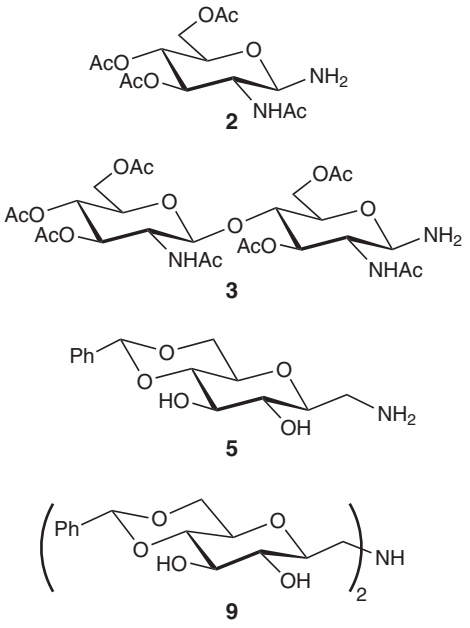



**Figure 1.** Amino saccharides used for light-induced glycosyl amino acid synthesis.

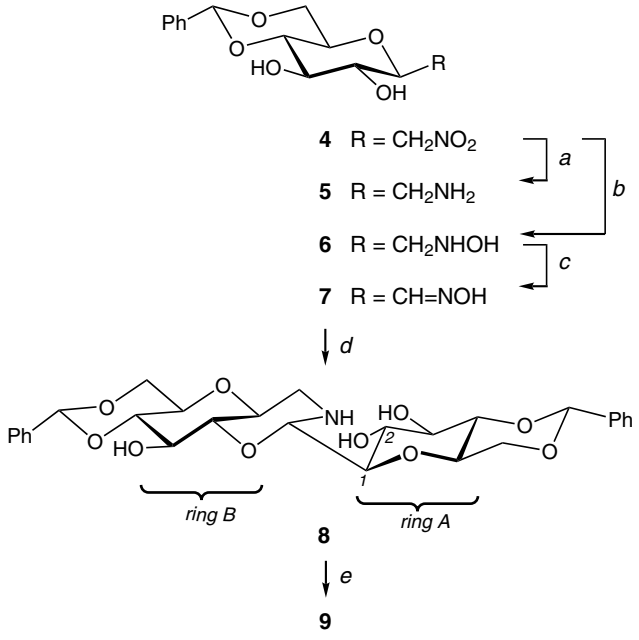
### 2.2. Generation of the 1-*C*-aminomethyl β-D-glucopyranosides

In an earlier report the reduction of the known 1-*C*-nitromethyl β-D-glucopyranoside **4** with elemental iron in aqueous tetrahydrofuran under a carbon dioxide atmosphere generated the 1-*C*-aminomethyl 4,6-*O*-benzylidene-β-D-glucopyranoside (**5**) in 78% yield.[29,30] We found that hydrogenation of **4** in the presence of Raney

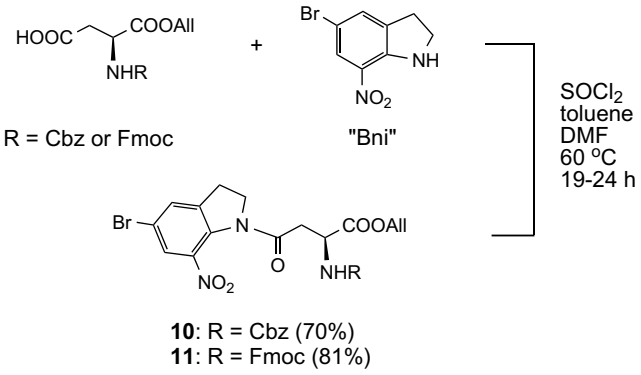
nickel furnishes **5** in 83% yield (Scheme 2). A sufficiently pure product that can be used for further synthetic manipulations without purification is obtained by a simpler work-up.

The dimeric *C*-glycoside **9** was generated by serendipity in a high-yielding reaction sequence (Scheme 2). In an attempt to reduce 1-*C*-nitromethyl β-D-glucopyranoside **4** by palladium-catalyzed hydrogenation to **5**, hydroxylamine **6** was obtained instead. Oxidation of **6** with air produced *cis*/*trans* oxime **7**. Hydrogenation of **7** in the presence of Raney nickel did not afford *C*-glycoside **5**, but unexpectedly, the dimeric aminal **8**.

The structure of **8** is supported by mass spectrometry, elemental analysis, and NMR spectroscopy. The aminal proton has a chemical shift of 4.29 ppm, and the HSQC spectrum reveals its direct connectivity to the aminal carbon at 84.9 ppm. In the HMBC spectrum, which produces heteronuclear cross peaks between protons and carbon atoms two and sometimes three bonds apart, H-1 of glucose ring B (Scheme 3) shows only two cross peaks, that is, one to C-2, and another one to the aminal carbon. The only other proton displaying an HMBC cross peak with the aminal carbon is a proton of the methylene group of the 1-oxa-3-azacyclohexane ring. We did not observe a homonuclear $^{3}J$ coupling between the aminal proton and H-1 in the one-dimensional $^{1}$H NMR spectrum, and in the HOHAHA spectrum only a weak cross peak between these two protons appears. The small $^{3}J_{1,\mathrm{aminal}}$ coupling of <1 Hz indicates that




**Scheme 2.** Synthesis of monomeric and dimeric *C*-glycosides **5** and **9** used for photoacylation: (a) $H_2$, Raney nickel, MeOH, rt, 15 h; (b) $H_2$, Lindlar catalyst, MeOH, rt, 8–10 h; (c) air, $NH_4OH$, MeOH, rt, 10–16 h; (d) $H_2$, Raney nickel, py, $H_2O$, rt, 2–2.5 d; (e) 1. $NaBH_4$, AcOH, py, MeOH; 2. $NH_4Cl$ aq, 3. NaOH, MeOH, $H_2O$ (pH 9).




**Scheme 3.** Synthesis of photoreactive and orthogonally protected asparagines.

the two glucose rings lie in approximately perpendicular planes under the conditions used for this NMR analysis.

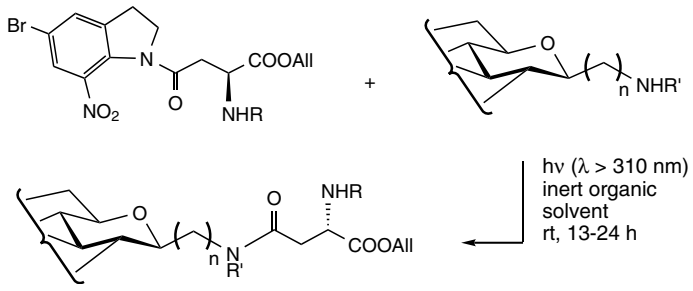
Reduction with sodium borohydride converted aminal **8** almost quantitatively into the secondary amine **9**. Even though initially our synthetic efforts were directed toward a different product, we felt that this dimeric 1-*C*-aminomethyl glucoside could be useful in syntheses of neoglycopeptides mimicking branched disaccharides.

### 2.3. Generation of the photoreactive asparagine derivatives

Following a procedure similar to the known amidation of 5-bromo-7-nitroindoline (Bni),[6] the commercially available Bni was condensed with Cbz-Asp-OAll or Fmoc-Asp-OAll in the presence of thionyl chloride and a small amount of DMF to give the photoreactive amino acids Cbz-Asp(Bni)OAll (**10**) and Fmoc-Asp(Bni)OAll (**11**) in 70% and 81% yield, respectively (Scheme 3). In organic solvents all *N*-acyl-5-bromo-7-nitroindolines have an absorption band in the near-UV region at ~350 nm, and they clearly show luminescence, a useful property that facilitates monitoring their formation or their consumption, as well as their purification by chromatography. This luminescence is characteristic for the *N*-acyl-7-nitroindolines with a 5-bromo substituent. We did not observe it in derivatives in which the 5-bromo substituent is replaced by a 5-cyano, 5-carboxymethyl, or a 4-methoxy group.

### 2.4. Generation of *N*- and *C*-glycosyl amino acids by phototransamidation

The ability of the two photoreactive asparagines **10** and **11** to acylate the weakly nucleophilic glycosylamines **2** and **3**, as well as the more nucleophilic and partially unprotected glucose-derived 1-*C*-aminomethyl glucosides **5** and **9** was tested. All photochemical experiments were conducted in THF and/or *N*,*N*,*N*′,*N*′-tetramethylurea (TMU) according to Scheme 4. Both solvents are stable



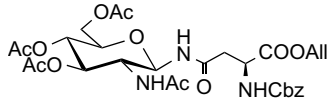
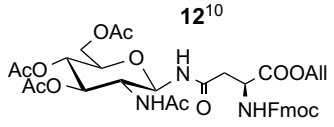
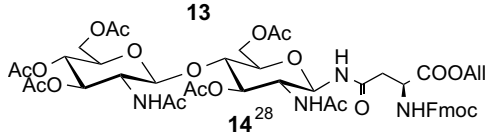
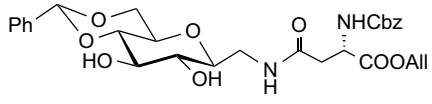
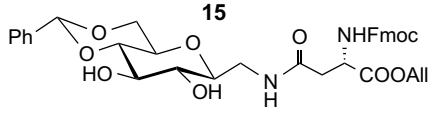
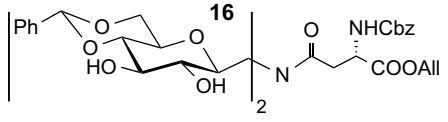

**Scheme 4.** General scheme for the synthesis of *N*- and *C*-glycosyl asparagines by phototransamidation. R = Cbz or Fmoc, R′ = H or another *C*-glycoside, *n* = 0 or 1.

under the photochemical reaction conditions applied, and TMU increases the solubility of the polar reactants.

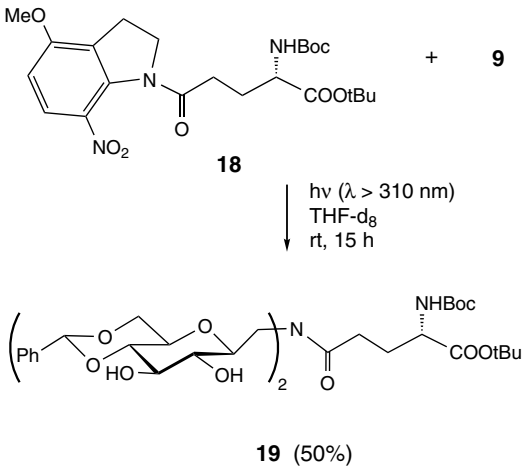
The inexpensive equipment needed comprises a mercury lamp and a Pyrex reaction vessel allowing for water cooling and inert gas purging. Small-scale reactions can also be performed by irradiating the reactants in a standard 5-mm NMR tube. It is important to filter out the high-energy wavelengths of mercury lamps in these phototransamidations, since otherwise the reactants undergo photodecomposition. Therefore, quartz glass reactors are to be avoided, but Pyrex is well suited for this purpose due to its natural cutoff with an onset of 310 nm.

Table 1 summarizes the phototransamidations performed. The amino acids **10** and **11** successfully undergo phototransamidation with glycosylamines **2** and **3**, and with the 1-*C*-aminomethyl β-D-glucosides **5** and **9**. The two starting materials were employed in nearly equimolar amounts with one of the two reactants, typically the amino saccharide component, in 10% excess. Similar to the condensation of glycosylamines with acid chlorides of aspartic acid,[31] the *N*-glycosyl Cbz- and Fmoc-protected allyl asparaginates **12**–**17** were produced in 60–85% yield. We did not observe any anomerization in the sugar or epimerization in the amino acid moiety.

**Table 1.** Glycosyl asparagines synthesized

| Photoreactive asparagine | Amino saccharide | Amino acid/sugar ratio | Solvent | Glycosyl asparagine | Yield[a] |
|---|---|---|---|---|---|
| **10** | **2** | 1:1.10 | THF |  **12**[10] | 74% |
| **11** | **2** | 1:1.10 | THF |  **13** | 61% |
| **11** | **3** | 1:1.14 | THF/TMU 5:1 |  **14**[28] | 63% |
| **10** | **5** | 1.11:1 | THF |  **15** | 70% |
| **11** | **5** | 1:1.11 | TMU |  **16** | 60% |
| **10** | **9** | 1:1.13 | THF |  **17** | 85% |

[a] All yields refer to chromatographically purified products.




**Scheme 5.** Light-induced coupling of Boc-Gln(4-methoxy-7-nitroindoline)-OtBu with the *C*-glycosidic secondary amine **9**.

Our results indicate that this photochemical method may be promising for the convergent synthesis of *N*-glycopeptides, that is, the light-induced coupling of nitroindoline derivatized peptides with aminosaccharides under neutral conditions.

In the phototransamidation of Fmoc-protected aspartate derivative **11** and *C*-glycoside **5**, we observed the presence of approximately 10% dibenzofulvene. Possibly, this partial loss of the Fmoc group is due to the basicity of the primary amino group in **5**. Fmoc cleavage was not apparent when the less basic glycosyl amines **2** and **3** were used.

The known *N*-glutamyl-4-methoxy-7-nitroindoline **18** is the protected precursor of a compound that can efficiently release glutamate upon flash photolysis in aqueous media.[11–13] Its high efficiency in flash photolysis experiments has been attributed to the electron-releasing effect of the 4-methoxy substituent *para* to the 7-nitro group, which increases the extinction coefficient and the quantum yield.[12] In order to look into the influence of the ring substituent of *N*-acyl-7-nitroindolines in phototransamidations, we were interested in studying glutamate derivative **18**, which has a 4-methoxy instead of a 5-bromo substituent in the indoline ring. The ability of this electron-rich photoreactive glutamate to acylate amino saccharides was tested by irradiation of **18** in the presence of glycosylamine **2**, and in the presence of 1-*C*-aminomethyl β-D-glucoside **5** in THF. Surprisingly, the photochemical experiment with glycosylamine **2** showed only 30% of *N*-glycosyl glutamine formation based on NMR analysis of the crude mixture. Due to the inefficient conversion to the *N*-glycosyl glutamine, we decided not to isolate any photoproducts from this reaction. Similarly, the photochemical reaction of **18** with the more nucleophilic amino saccharide **9** gave the *C*-glucopyranosyl glutamine **19** in only 50% yield after chromatography (Scheme 5). In both cases, practically all of the photoreactive starting material **18** had been consumed, which indicates that due to its high reactivity, one or more unproductive side reactions were competing with the desired phototransamidations.

## 3. Conclusions

The *N*-aspartyl-5-bromo-7-nitroindolines **10** and **11** are latent nitronic anhydrides, which become activated upon irradiation with near-UV light. In inert organic solvents (THF and/or TMU) amino saccharides, including the relatively weakly nucleophilic glycosylamines **2** and **3** and the partially unprotected 1-*C*-aminomethyl β-D-glucopyranosides **5** and **9**, become acylated. The overall reaction is a phototransamidation, which generates glycosyl asparagines in fair-to-good yields (60–85%). Carbohydrate derivative **9** is a novel dimeric *C*-glycoside, similar to the one produced in the L-fucose series.[32] It can be efficiently synthesized starting from the known 4,6-*O*-benzylidene-protected 1-*C*-nitromethyl β-D-glucopyranoside **4** via the novel aminal **8**.

In the glycosyl amino acid syntheses shown, the two reactants are economically utilized in nearly equimolar amounts, and the 5-bromo-7-nitroindoline liberated is recyclable. The major strength of this method lies in its mild activation by light under neutral reaction conditions. Therefore, together with the ability of the 5-bromo-7-nitroindoline group to function as a protecting group for carboxyl groups in the dark, the phototransamidations demonstrated here pave the way for the convergent synthesis of glycopeptides with *N*- and *C*-glycosyl linkages.

The known highly photoreactive 4-methoxy-7-nitroindoline derivative of glutamic acid (**18**) produces glycosyl glutamines in lower yield (30–50%), especially when the weakly nucleophilic glycosylamine **2** is utilized. The fact that **18** is completely consumed upon irradiation indicates that one or more unproductive side reactions also take place. We conclude that the 4-methoxy-7-nitroindoline derivatives are too reactive to efficiently perform phototransamidations under our reaction conditions. Apparently, the *N*-acyl-5-bromo-7-nitroindolines do not show these side reactions, at least not to a large extent, and the natural and unnatural glycosyl amino acids are generated much more efficiently. A detailed study of indoline ring substituent effects on phototransamidations is currently underway.

## 4. Experimental

### 4.1. General methods

$^{1}H$ and $^{13}C$ NMR spectra were taken at 300 MHz using a Varian Mercury spectrometer or at 500 MHz using a

Varian Unity Inova spectrometer. Protons of diastereotopic methylene groups were assigned with or without 'prime', that is, H-6 (downfield) and H-6′ (upfield). Thin-layer chromatography (TLC) was performed on aluminum or glass plates coated with Silica Gel 60 $F_{245}$. TLC plates were developed with a staining solution [$P_2O_5 \times 24MoO_3 \times xH_2O$ (10 g), $(NH_4)_2Ce(NO_3)_6$ (5 g), $H_2O$ (450 mL), and $H_2SO_4$ (50 mL)] and heat. Mass spectra were measured on a VG Analytical 70SE or a JEOL LCmate mass spectrometer. Elemental analyses were carried out at Desert Analytics, Tucson, AZ. Optical rotations were measured with an O.C. Rudolph & Sons polarimeter, model 956, or with a Jasco DIP-370 polarimeter. Toluene and THF were dried by standard methods and distilled. *N*,*N*,*N*′,*N*′-Tetramethylurea was freshly distilled. All other solvents were reagent grade. Cbz-Asp-OAll was synthesized from Cbz-Asp-anhydride and allyl alcohol as previously described for Cbz-Asp-OBn,[33] and Fmoc-Asp-OAll was either synthesized in the same way or purchased from Novabiochem. 5-Bromo-7-nitroindoline was purchased from Lancaster synthesis.

Phototransamidation reactions were performed at ambient temperature under argon either in a microscale photochemical reaction assembly, model 7880, ACE Glass, pyrex, (reaction scale 20–50 mg), or in an NMR tube (reaction scale ~10 mg). For irradiation up to four mercury gaseous discharge lamps (Pen-Ray, 5.5 W, low pressure, cold cathode) were used. In the photochemical reaction assembly, the reaction mixtures were purged with argon for 20 min prior to irradiation. For small-scale phototransamidations the NMR tubes containing the reaction mixtures were thoroughly flushed with argon and tightly capped. During irradiations the reaction mixtures were kept at ambient temperature by water cooling. Irradiations were continued until TLC or NMR spectroscopy indicated the consumption of the photoreactive amino acids. Upon completion, the solvents were evaporated under reduced pressure, and the crude mixtures were chromatographed on silica gel (230–400 mesh, Natland Int. Corporation).

### 4.2. Preparation of 1-*C*-aminomethyl 4,6-*O*-benzylidene-β-D-glucopyranoside (5)

Raney nickel (slurry, 3 g) was added to a suspension of nitro derivative **4** (3.11 g, 10 mmol) in MeOH (40 mL). The reaction mixture was degassed by evacuating the flask and filling with hydrogen three times and then stirred under $H_2$ using a rubber balloon until silica TLC (6:1 $CHCl_3$–EtOH) indicated that the reaction was complete (~15 h). The white product was dissolved in boiling MeOH, the catalyst was filtered off, and the methanolic filtrate was concentrated in vacuo to a small volume. The amine **5** was filtered off after cooling, washed with a minimal amount of MeOH, and dried (2.34 g, 83%). All analytical data concurred with the ones published earlier.[29]

### 4.3. Aminal 8

A suspension of **4** (9.34 g, 30 mmol) and Lindlar catalyst (700 mg) in MeOH (150 mL) was flushed with $H_2$ three times and stirred under $H_2$ using a rubber balloon at ambient temperature until silica TLC indicated the consumption of the fast-migrating compound **4** (8–10 h). The precipitated gray crude product **6**, 8.5 g, was filtered off and used without purification. Air was bubbled through a stirred suspension of crude **6** (6.0 g) in MeOH (240 mL) and $NH_4OH_{concd}$ (100 mL) until silica TLC indicated the consumption of **6** as the slow-migrating spot (10–16 h). The reaction mixture was filtered and concentrated to a small volume in vacuo. The white precipitate (oxime **7**, *cis*/*trans* mixture) was filtered off and was recrystallized from $H_2O$ to give pure **7** (4.4 g, 70% over two steps): mp 227–230 °C (dec), $R_f$ 0.34, 0.39 ($SiO_2$; 10:1 $CHCl_3$–EtOH). Raney nickel (0.7 g) was added to a suspension of **7** (2 g, 6.77 mmol) in pyridine (10 mL) and $H_2O$ (20 mL), and the reaction flask was evacuated and filled with $H_2$ three times. Hydrogenation using a rubber balloon at ambient temperature for 50–60 h produced a precipitate that was filtered off, and was digested with pyridine (35 mL). The pyridine solution was filtered from the catalyst and evaporated in vacuo to a syrup. MeOH precipitated aminal **8**, which was filtered off, washed with MeOH, and dried to give a white solid (1.43 g, 78%): mp 223–225 °C (dec), $[\alpha]_D^{22}$ −55 (pyridine), $R_f$ 0.41 ($SiO_2$; 10:1 $CHCl_3$–EtOH). Since the molecule is unsymmetrical, the sugar ring with the two free hydroxyl groups is designated 'A', the other one 'B' (Scheme 2) for NMR assignments. $^1$H NMR (500 MHz, $Me_2SO$-$d_6$, 298 K): $\delta$ 7.47–7.42 (m, 4H, arom.); 7.39–7.35 (m, 6H, arom.); 5.58 (s, 1H, CH–Ph); 5.57 (s, 1H, CH–Ph); 5.25 [d, 1H, $^3J_{3,OH\text{-}3}$ 4.7 Hz, OH-3 (A)]; 5.18 [d, 1H, $^3J_{3,OH\text{-}3}$ 4.6 Hz, OH-3 (B)]; 5.16 [d, 1H, $^3J_{2,OH\text{-}2}$ 5.5 Hz, OH-2 (A)]; 4.29 (d, 1H, $^3J_{CH,NH}$ 11.4 Hz, O–CH–N); 4.20–4.14 [m, 2H, H-6(A), H-6(B)]; 3.70 [dd, 1H, $^3J_{5,6} = {}^2J_{6,6'}$ 10.1 Hz, H-6′(A)]; 3.69–3.58 [m, 2H, H-6′(B), H-3(B)]; 3.53 [m, 1H, H-2(A)]; 3.48–3.40 [m, 3H, H-4(B), H-5(B), H-3(A)], 3.37 [dd, 1H, $^3J_{3,4} = {}^3J_{4,5}$ 9.1 Hz, H-4(A)]; 3.33 [m, 1H, H-5(A), overlapped with $H_2O$]; 3.28 [dd, 1H, $^3J_{1,2}$ 9.8 Hz; $^3J_{1,O\text{–}CH\text{–}N}$ 1.7 Hz, H-1(A)]; 3.20 [m, 1H, H-1(B)]; 3.16–3.10 [m, 2H, H-2(B), CH (C-1–$CH_2$–N)]; 2.64–2.53 [m, 2H, NH, CH′ (C-1–$CH_2$–N)]; $^{13}$C NMR: (125.8 MHz, $Me_2SO$-$d_6$, 298 K): $\delta$ 137.8, 137.7 (arom., quart.); 128.8, 128.7, 128.0, 126.4, 126.3 (arom.); 100.9, 100.5 (CH–Ph); 84.9 (O–CH–N); 82.0, 81.9 [C-2(B), C-4(B)]; 80.7 [C-4(A)]; 80.6 [C-1(A)]; 74.2 [C-3(A)]; 73.6 [C-1(B)]; 70.8 [C-3(B)]; 70.5 [C-5(A), C-5(B)]; 69.9 [C-2(A)]; 67.9 [C-6(A)]; 67.8 [C-6(B)]; 46.9 (C-1–*C*$H_2$–N). ESIMS: (*m*/*z*): $[M+H]^+$ calcd for

$C_{28}H_{34}NO_{10}$, 544.2; found, 544.2. Anal. Calcd for $C_{28}H_{33}NO_{10}$: C, 61.87; H, 6.12; N, 2.58. Found: C, 61.21; H, 6.31; N, 2.52.

### 4.4. *N*,*N*-[Bis-*C*-(4,6-*O*-benzylidene-β-D-glucopyranosyl methyl)]amine (9)

$NaBH_4$ (151.3 mg, 4 mmol) was added to a stirred suspension of aminal **8** (1.087 g, 2 mmol) in 2:1 pyridine–HOAc (10 mL) and MeOH (8 mL) at rt during 20 min. The resulting solution was stirred for 1 h. Solvents were removed under reduced pressure. Diethyl ether (40 mL) was added to the remaining gel, and the suspension was vigorously stirred for 2 h, filtered, and washed with ether. The crude product was dissolved in MeOH (15 mL) under reflux, and $NH_4Cl$ (428 mg, 8 mmol) in $H_2O$ (10 mL) was added. A white product precipitated after a few minutes. The suspension was refluxed for an additional 5 min, cooled, and filtered. The white hydrochloride was washed with water and dried (966 mg, 83%). NaOH (2 N, 0.52 mL, 1.04 mmol) was added to a stirred suspension of the hydrochloride (582 mg, 1 mmol) in MeOH (20 mL) and $H_2O$ (7 mL). The resulting solution (pH 9) was then stirred at rt for 20 min. MeOH was removed from the reaction mixture under reduced pressure, $H_2O$ (20 mL) was added, and the product was filtered off, washed with $H_2O$, and dried (490 mg, 90%): mp 233–234 °C, $[\alpha]_D^{22}$ −44 (DMF), $R_f$ 0.21 ($SiO_2$; 10:1 $CHCl_3$–EtOH). The assignment of the proton NMR signals were made based on the HOHAHA and 1D TOCSY spectra. $^1$H NMR (500 MHz, THF-$d_8$, 298 K): $\delta$ 7.47–7.46 (m, 4H, arom.); 7.31–7.26 (m, 6H, arom.); 5.50 (s, 2H, 2 × CH–Ph); 4.20 (dd, 2H, $^3J_{6,6'}$ 10.3 Hz, $^3J_{5,6}$ 4.3 Hz, 2 × H-6); 3.62 (2H, m, 2 × H-6′); 3.51 (2H, dd, $^3J_{2,3} = {}^3J_{3,4}$ 8.5 Hz, 2 × H-3); 3.40 (m, 2H, 2 × H-1); 3.37–3.30 (m, 6H, 2 × H-2, 2 × H-4, 2 × H-5); 2.96 [dd, 2H, $^2J_{CH-N,CH'-N}$ 12.3 Hz, $^3J_{1,CH-N}$ 3.7 Hz, 2 × CH (C-1–$CH_2$–N)]; 2.79 [dd, 2H, $^3J_{1,CH'-N}$ 6.2 Hz, 2 × CH′ (C-1–$CH_2$–N)] ppm. APC-IMS: (*m*/*z*): $[M+H]^+$ calcd for $C_{28}H_{36}NO_{10}$, 546.2; found, 546.3. Anal. Calcd for $C_{28}H_{35}NO_{10}$: C, 61.64; H, 6.47; N, 2.57. Found: C, 61.71; H, 6.81; N, 2.59.

### 4.5. *N*-α-Benzyloxycarbonyl-β-(5-bromo-7-nitroindolin-1-yl)-L-aspartamide-α-allyl ester (10)

Cbz-Asp-OAll (200 mg, 0.65 mmol) and 5-bromo-7-nitroindoline (302 mg, 1.25 mmol) were suspended in anhydrous toluene (6 mL) and DMF (0.01 mL, 0.13 mmol) and warmed to 60 °C under argon. Freshly distilled $SOCl_2$ (0.081 mL, 0.93 mmol) was injected, and the mixture was stirred at 60 °C. After 19 h TLC analysis indicated the consumption of Cbz-Asp-OAll. The reaction mixture was allowed to cool and was quenched by addition of $H_2O$ (20 mL). The mixture was extracted 3× with EtOAc, and the crude product was chromatographed on silica with 1:1 EtOAc–hexanes. The desired Cbz-Asp(Bni)-OAll (**10**) was obtained as a brown syrup (241 mg, 70%). TLC: $R_f$ 0.31 ($SiO_2$; 1:1 EtOAc–hexanes). The NMR spectrum showed absence of extraneous lines except for the $H_2O$ signal at 3.35 ppm and the $Me_2SO$-$d_6$ signal at 2.50 ppm. $^1$H NMR (300 MHz, $Me_2SO$-$d_6$, 298 K): $\delta$ 7.86, 7.84 (2 × m, 2H, H-4, H-6, indoline); 7.69 (d, 1H, $^3J_{NH,\alpha}$ 8.1 Hz, NH); 7.37–7.29 (m, 5H, arom., Cbz); 5.89 (m, 1H, –C*H*=$CH_2$); 5.31 (dtd, 1H, $^3J_{trans}$ 17.3 Hz, –CH=C$H_2$, *trans*); 5.20 (dtd, 1H, $^3J_{cis}$ 10.5 Hz, –CH=C$H_2$, *cis*); 5.04 (s, 2H, –C$H_2$–Ph); 4.59 (m, 2H, –O–C$H_2$–, allyl); 4.54 (m, 1H, H-α); 4.25 (t, 2H, $^3J_{2,3}$ 8.1 Hz, H-2, H-2′, indoline); 3.20 (m, 2H, H-3, H-3′, indoline); 3.07 (dd, 1H, $^2J_{\beta,\beta'}$ 16.8 Hz, $^3J_{\alpha,\beta}$ 5.4 Hz, H-β); 2.96 (dd, 1H, $^3J_{\alpha,\beta'}$ 7.8 Hz, H-β′); $^{13}$C NMR (126 MHz, $Me_2SO$-$d_6$, 298 K): $\delta$ 170.8, 168.1 [2 × C=O (amide, ester)]; 155.8 [C=O (Cbz)]; 143.7, 136.8, 133.2 (arom.); 132.2 [–*C*H=$CH_2$ (allyl)]; 132.0 (C-4, indoline); 128.3, 127.8, 127.6 (arom.); 124.3 (C-6, indoline); 117.7 (–CH=*C*$H_2$, allyl); 115.4 (arom.); 65.6 ($CH_2$, Cbz); 65.2 (O–$CH_2$–, allyl); 50.4 (C-α); 49.4 (C-2, indoline); 36.5 (C-β); 28.3 (C-3, indoline). FABMS: (*m*/*z*): $[M+H]^+$ calcd for $C_{23}H_{23}BrN_3O_7$ 532.07; found, 532.2.

### 4.6. *N*-α-(Fluoren-9-ylmethoxycarbonyl)-β-(5-bromo-7-nitroindolin-1-yl)-L-aspartamide-α-allyl ester (11)

Fmoc-Asp-OAll (246 mg, 0.62 mmol) and 5-bromo-7-nitroindoline (302 mg, 1.25 mmol) were suspended in anhydrous toluene (6 mL) and DMF (0.01 mL, 0.13 mmol) and warmed to 60 °C under argon. Freshly distilled $SOCl_2$ (0.081 mL, 0.93 mmol) was injected, and the mixture was stirred at 60 °C. After 15 h silica TLC indicated an incomplete consumption of Fmoc-Asp-OAll ($R_f$ 0.34, $SiO_2$; 9:1 $CHCl_3$–MeOH). More $SOCl_2$ (0.03 mL, 0.31 mmol) was injected, and the mixture was allowed to react for additional 8 h. The reaction mixture was allowed to cool and was quenched by addition of $H_2O$ (20 mL). The mixture was extracted 3× with EtOAc, and the crude product was chromatographed on silica with 3:4 EtOAc–hexanes. The desired Fmoc-Asp(Bni)-OAll (**11**) was obtained as a yellow foam (311 mg, 81%). TLC: $R_f$ 0.20 ($SiO_2$; 3:4 EtOAc–hexanes). The NMR assignments were made based on HOHAHA and HSQC spectra. $^1$H NMR (500 MHz, $Me_2SO$-$d_6$, 298 K): $\delta$ 7.87 (d, 2H, $^3J$ 7.6 Hz, Fmoc); 7.84, 7.82 (2 × s, 2H, H-4, H-6, indoline); 7.72 (d, 1H, $^3J_{NH,\alpha}$ 8.1 Hz, NH); 7.68 (d, 2H, $^3J$ 7.5 Hz, arom. Fmoc); 7.40 (t, 2H, $^3J$ 7.4 Hz, arom. Fmoc); 7.30 (m, 2H, arom. Fmoc); 5.87 (m, 1H, –C*H*=$CH_2$); 5.29 (dtd, 1H, $^3J_{trans}$ 17.2 Hz, –CH=C$H_2$, *trans*); 5.17 (dd, 1H, $^3J_{cis}$ 10.5 Hz, –CH=C$H_2$, *cis*); 4.57 (m, 2H, O–$CH_2$–, allyl); 4.52 (m, 1H, H-α); 4.33–4.28 (m, 2H, $CH_2$, Fmoc); 4.27–4.20 (m, 3H, H-2, H-2′, indoline, CH, Fmoc); 3.20 (m, 2H, H-3, H-3′, indoline); 3.05 (dd, 1H, $^2J_{\beta,\beta'}$

16.7 Hz, $^{3}J_{\alpha,\beta}$ 5.3 Hz, H-β); 2.96 (dd, 1H, $^{3}J_{\alpha,\beta'}$ 7.7 Hz, H-β′). $^{13}$C NMR (126 MHz, $Me_2SO$-$d_6$, 298 K): δ 170.8, 168.1 [2 × C=O (amide, ester)]; 155.8 [C=O (Fmoc)]; 143.7, 140.7, 140.3, 133.2 (4 × arom., no CH); 132.2 (–*C*H=$CH_2$, allyl); 132.0 (C-4, indoline); 127.6 (CH, arom. Fmoc); 127.08, 127.06 (CH, arom. Fmoc and arom., no CH); 125.2 (CH, arom. Fmoc); 124.3 (C-6, indoline); 120.1 (CH, arom. Fmoc); 117.7 (–CH=*C*$H_2$, allyl); 115.4 (arom.); 65.8 ($CH_2$, Fmoc); 65.2 (–O–$CH_2$–, allyl); 50.4 (C-α); 49.4 (C-2, indoline); 46.6 (CH, Fmoc); 36.5 (C-β); 28.4 (C-3, indoline). FABMS: (*m*/*z*): $[M+H]^+$ calcd for $C_{30}H_{27}BrN_3O_7$, 620.1; found, 620.1; $[M+Li]^+$ calcd for $C_{30}H_{26}BrLiN_3O_7$, 626.1; found, 626.1. Anal. Calcd for $C_{30}H_{26}BrN_3O_7$: C, 58.07; H, 4.22; N, 6.77; Br, 12.88; O, 18.05. Found: C, 57.65; H, 4.04; N, 6.66; Br, 13.25; O, 18.42.

### 4.7. Preparation of *N*-γ-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-α-benzyloxycarbonyl-L-asparagine allyl ester (12)

The synthesis and characterization of this glycosyl asparagine has been described.[10] $[\alpha]_D^{20}$ +2 (*c* 1, $CHCl_3$).

### 4.8. *N*-γ-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-α-fluoren-9-ylmethoxycarbonyl-L-asparagine allyl ester (13)

The photoreactive amino acid **11** (50 mg, 0.081 mmol) and glycosylamine **2** (30.7 mg, 0.089 mmol) were dissolved in 10 mL of dry THF. The mixture was irradiated for 14 h under a continuous argon flow. After evaporation of the solvent the crude product was chromatographed on $SiO_2$ with 97.5:2.5 $CH_2Cl_2$–MeOH. The purified glycosyl amino acid **14** was obtained as a white solid (35 mg, 61%). TLC: $R_f$ 0.61 ($SiO_2$; 9:1 $CHCl_3$–MeOH). The NMR spectrum showed absence of extraneous lines except for the $H_2O$ signal at 3.35 ppm and the $Me_2SO$-$d_6$ signal at 2.50 ppm. $^1$H NMR (300 MHz, $Me_2SO$-$d_6$, 298 K): 8.69 (2 × d, 1H, NH-1); 7.88 (2 × d, 1H, NH-1); 7.98–7.84 (m, 3H, NH-2, 2 arom.); 7.78–7.60 (m, 3H, NH-α, 2 arom.); 7.41 (dd, 2H, 2 arom.); 7.32 (dd, 2H, 2 arom.); 5.86 (m, 1H, –$CH_2$–C*H*=$CH_2$); 5.28 (d, 1H, $^{3}J_{trans}$ 17.1 Hz, terminal allyl, *trans*); 5.22–5.12 (m, 3H, terminal allyl, *cis*, H-1); 5.10 (ddd, 1H, $^{3}J_{3,4} = {}^{3}J_{4,5}$ 9.9 Hz, H-4); 4.82 (dd, 1H, $^{3}J_{2,3}$ 9.9 Hz, H-3); 4.63–4.42 (m, –C*H*$_2$–CH=$CH_2$, H-α); 4.38–4.10 (m, 4H, –C*H*$_2$–CH– of Fmoc, H-6, H-6′); 4.00–3.77 (m, 3H, –$CH_2$–C*H*– of Fmoc, H-2, H-5); 2.75–2.44 (m, 2H, H-β, H-β′); 1.99, 1.97, 1.96 (3 × s, 6H, 2 × Ac); 1.90 (s, 3H, Ac); 1.74, 1.72 (2 × s, 3H, Ac). FABMS: (*m*/*z*): $[M+H]^+$ calcd for $C_{36}H_{42}N_3O_{13}$, 724; found, 724; $[M+Li]^+$ calcd for $C_{36}H_{41}LiN_3O_{13}$, 730; found, 730; $[M+K]^+$ calcd for $C_{36}H_{41}KN_3O_{13}$, 762; found, 762.

### 4.9. Preparation of *N*-γ-[2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-β-D-glucopyranosyl]-*N*-α-fluoren-9-ylmethoxycarbonyl-L-asparagine allyl ester (14)[28]

The photoreactive amino acid **11** (51 mg, 0.082 mmol) and glycosylamine **3** (59 mg, 0.093 mmol) were dissolved in 5 mL of anhyd THF and 1 mL of TMU. The mixture was irradiated for 13 h under a continuous argon flow. During the course of the reaction the product partially precipitated out. After evaporation of the solvent the crude product was chromatographed on $SiO_2$ with 9:1 $CHCl_3$–MeOH. The purified glycosyl amino acid **14** was obtained as a white solid (52 mg, 63%): TLC: $R_f$ 0.30 ($SiO_2$; 9:1 $CHCl_3$–MeOH); FABMS: (*m*/*z*): $[M+H]^+$ calcd for $C_{48}H_{59}N_4O_{20}$, 1011; found, 1011; $[M+K]^+$ calcd for $C_{48}H_{58}KN_4O_{20}$, 1049; found, 1049. The $^1$H and $^{13}$C NMR spectra matched the ones previously reported for **14**.[28]

### 4.10. *N*-γ-(4,6-*O*-Benzylidene-β-D-glucopyranosylmethyl)-*N*-α-benzyloxycarbonyl-L-asparagine allyl ester (15)

The photoreactive amino acid **10** (71.2 mg, 0.134 mmol) and 1-*C*-aminomethyl glucoside **5** (33.8 mg, 0.120 mmol) were dissolved in 10.5 mL of anhyd THF, and the mixture was irradiated for 24 h under a continuous argon flow. After evaporation of the solvent, the crude product was chromatographed with 3:1 EtOAc–hexanes. The purified glycosyl amino acid **15** was obtained as a colorless film (48 mg, 70%): $[\alpha]_D^{20}$ +38 (*c* 1, $CHCl_3$); TLC: $R_f$ 0.16 ($SiO_2$; 3:1 EtOAc–hexanes); $R_f$ 0.40 ($SiO_2$; 9:1 $CHCl_3$–MeOH). The NMR spectrum showed absence of extraneous lines except for the $H_2O$ signal at 3.30 ppm and the $Me_2SO$-$d_6$ signal at 2.50 ppm. $^1$H NMR: (300 MHz, $Me_2SO$-$d_6$, 298 K) δ 7.98 (dd, 1H, $^{3}J_{\gamma,CHN} = {}^{3}J_{\gamma,CH'N}$ 4.8 Hz, NH-γ); 7.65 (m, 1H, H-α); 7.46–7.25 (m, 10H, arom.); 5.86 (m, 1H, –C*H*=$CH_2$, allyl); 5.54 (s, 1H, Ph–CH, benzylidene); 5.34–5.22 [m, 3H, OH-2, OH-2, –CH=C*H*$_2$ (allyl, *trans*)]; 5.18 (d, 1H, $^{3}J_{cis}$ 10.8 Hz, –CH=C*H*$_2$, allyl, *cis*); 5.02 (s, 2H, $CH_2$, Cbz); 4.55 (m, 2H, –O–$CH_2$, allyl); 4.47 (m, 1H, H-α); 4.17 (m, 1H, H-6); 3.70–3.54 [m, 2H, H-6′, CH (C-1–$CH_2$–N)]; 3.47–3.20 (m, 4H, H-3, H-4, H-5, H-1, overlapped with $H_2O$); 3.10–2.90 [m, H-2, CH′ (C-1–$CH_2$–N)]; 2.64, (dd, 1H, $^{2}J_{\beta,\beta'}$ 16.5 Hz, H-β); 2.55 (m, 1H, H-β′). FABMS: (*m*/*z*): $[M+H]^+$ calcd for $C_{29}H_{35}N_2O_{10}$, 571; found, 571; $[M+K]^+$ calcd for $C_{29}H_{34}KN_2O_{10}$, 609; found, 609.

### 4.11. *N*-γ-(4,6-*O*-Benzylidene-β-D-glucopyranosyl methyl)-*N*-α-fluoren-9-ylmethoxycarbonyl-L-asparagine allyl ester (16)

Photoreactive amino acid **11** (50 mg, 0.080 mmol) and *C*-glycoside **5** (25 mg, 0.089 mmol) were dissolved in

10 mL of freshly distilled TMU and irradiated for 24 h. After evaporation of the solvent under reduced pressure, the crude product was chromatographed on $SiO_2$ with 97:3 $CHCl_3$–MeOH. The purified *C*-glycosyl amino acid **16** was isolated as a white solid: 32 mg, 60% yield. TLC: $R_f$ 0.5 ($SiO_2$; $CHCl_3$–MeOH 9:1). Compound **16** showed two equally populated conformers in $Me_2SO$, most likely due to *cis*/*trans* isomerization of the carbamate. The NMR spectrum showed the absence of extraneous lines except for the $H_2O$ signal at 3.30 ppm and the $Me_2SO$-$d_6$ signal at 2.50 ppm. $^1H$ NMR (300 MHz, $Me_2SO$-$d_6$, 298 K) $\delta$ 7.99 (dd, 1H, $^3J_{NH\text{-}\gamma,CHN} = {}^3J_{NH\text{-}\gamma,CH'N}$ 5.4 Hz, NH-γ); 7.88 (d, 2H, $^3J$ 7.5 Hz, arom. Fmoc); 7.76–7.65 (m, 3H, NH-α, arom. Fmoc); 7.46–7.27 [m, 9H, arom. Fmoc, Ph]; 5.86 [m, 1H, –C*H*=$CH_2$ (allyl)]; 5.55, 5.53 (2 × s, 1H, benzylidene); 5.34–5.23 [m, 3H, CH=C$H_2$ (allyl, *trans*), OH-3, OH-2]; 5.17 [dd, 1H, $^3J_{cis}$ 10.5 Hz, CH=C$H_2$ (allyl, *cis*)]; 4.53 [d, 2H, –O–$CH_2$– (allyl)]; 4.47 (m, 1H, H-α); 4.33–4.11 [m, 4H, CH, $CH_2$ (Fmoc), H-6]; 3.71–3.58 [m, 2H, H-6′, CH (C-1–$CH_2$–N)]; 3.46–3.22 (m, 4H, H-3, H-4, H-5, H-1, overlapped with $H_2O$); 3.11–2.90 [m, 2H, H-2, CH′ (C-1–$CH_2$–N)]; 2.72–2.48 (m, 2H, H-β, H-β′). FABMS: (*m*/*z*): $[M+H]^+$ calcd for $C_{36}H_{39}N_2O_{10}$, 559.26; found, 559.3. $[M+Li]^+$ calcd for $C_{36}H_{38}LiN_2O_{10}$, 665.27; found, 665.3.

### 4.12. *N*-γ,*N*-γ-[Bis-(4,6-*O*-benzylidene-β-D-glucopyranosyl methyl)]-*N*-α-benzyloxycarbonyl-L-asparagine allyl ester (17)

The photoreactive amino acid **10** (9 mg, 0.017 mmol) and the dimeric *C*-glycoside **9** (10.4 mg, 0.019 mmol) were dissolved in 0.6 mL THF-$d_8$ in a 5-mm NMR tube under argon. The mixture was irradiated with four mercury lamps for 20 h. The solvent was evaporated, and the crude product was flash chromatographed on $SiO_2$ with 95:5 $CHCl_3$–MeOH. The *C*-glycosyl amino acid was obtained as a white film (12 mg, 85%). TLC: $R_f$ 0.20 ($SiO_2$; 95:5 $CHCl_3$–MeOH). The NMR spectrum showed the absence of extraneous lines except for the $H_2O$ signal at 3.35 ppm and the $Me_2SO$-$d_6$ signal at 2.50 ppm. The NMR spectra of **17** are quite complex due to two diastereotopic glucose rings, as well as the existence of two equally populated conformers. Due to partial signal overlap in the HOHAHA, HSQC, and HMBC spectra, the two conformers and the signals of the diastereotopic glucose rings were not separately assigned. $^1H$ NMR (500 MHz, $Me_2SO$-$d_6$, 298 K): $\delta$ 7.39–7.28 (m, 16H, NH, arom.); 5.88 [m, 1H, –C*H*=$CH_2$ (allyl)]; 5.56, 5.55 [2s, 2H, 2 × Ph–H (benzylidene)]; 5.44 (m, 1H, OH-2); 5.35 (d, 1H, $^3J_{OH\text{-}3,3}$ 4.9 Hz, OH-3); 5.30 [d, 1H, $^3J_{trans}$ 17.3 Hz, –CH=C$H_2$ (allyl, *trans*)]; 5.27 (1H, d, $^3J_{OH\text{-}3,3}$ 4.6 Hz, OH-3); 5.22–5.16 [m, 2H, –CH=C$H_2$ (allyl, *cis*), OH-2]; 5.09–5.00 [m, 2H, $CH_2$ (Cbz)]; 4.62–4.51 [m, 3H, –O–$CH_2$– (allyl), H-α]; 4.19–4.08 (m, 2H, 2 × H-6); 4.02, 3.98 [2 × d, 1H, CH (C-1–$CH_2$–N)]; 3.81, 3.74 [2 × d, 1H, CH (C-1–$CH_2$–N)]; 3.69–3.58 (m, 2H, 2 × H-6′); 3.58–3.33 [m, 7H, 2 × H-1, 2 × H-3, CH′ (C-1–$CH_2$–N), 2 × H-4, overlapped with $H_2O$]; 3.32–3.12 [m, 3H, 2 × H-5, CH′ (C-1–$CH_2$–N)]; 3.07–2.94 (m, 2H, 2 × H-2); 2.94–2.81 (m, 2H, H-β, H-β′); $^{13}C$ NMR (126 MHz, $Me_2SO$-$d_6$, 298 K): $\delta$ 171.4, 171.2 (C=O, α); 170.0, 169.8 (C=O, β); 155.80, 155.77 (C=O, Cbz); 137.81, 137.76, 136.9, 136.8 (arom.); 132.4 [–*C*H=$CH_2$ (allyl)]; 128.8, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 126.3 (arom.); 117.5, 117.4 (–CH=*C*$H_2$, allyl); 100.64, 100.61 [Ph–*C*H (benzylidene)]; 80.9, 80.8, 80.7 (C-4); 80.0, 79.9, 79.2, 79.1 (C-1); 74.0, 73.82, 73.77 (C-3); 73.2, 73.1, 72.5, 72.3 (C-2); 70.1, 70.0, 69.9 (C-5); 67.94, 67.87 (C-6); 65.6 [Ph–$CH_2$–O– (Cbz)]; 64.9 [–O–$CH_2$– (allyl)]; 50.8 (C-α); 50.27 (C-1–*C*$H_2$–N); 48.8 (C-1–*C*$H_2$–N); 34.8 (C-β). HRFABMS: (*m*/*z*): $[M+H]^+$ calcd for $C_{43}H_{51}N_2O_{15}$, 835.3289; found, 835.3336.

### 4.13. *N*-δ,*N*-δ-[Bis-*C*-(4,6-*O*-benzylidene-β-D-glucopyranosylmethyl)]-*N*-α-*tert*-butoxycarbonyl-L-glutamine *tert*-butyl ester (19)

The photoreactive glutamate **18** (11.6 mg, 0.024 mmol) and 1-*C*-aminomethyl glucoside **9** (19.8 mg, 0.036 mmol) were dissolved in THF-$d_8$ in an NMR tube under argon. The mixture was irradiated for 15 h with four mercury lamps. The solvent was evaporated under reduced pressure, and the crude product was chromatographed on $SiO_2$ with 95:5 $CHCl_3$–MeOH. The *C*-glycosyl glutamine **19** was obtained as a white solid (10 mg, 50%): TLC: $R_f$ 0.46 ($SiO_2$; 95:5 $CHCl_3$–MeOH). The NMR spectrum showed the absence of extraneous lines except for the THF signals at 3.58 and 1.73 ppm. The two diastereotopic glucose spin systems, designated a and b, were identified based on 1D TOCSY and HOHAHA spectra. $^1H$ NMR (500 MHz, THF-$d_8$ + $D_2O$, 298 K): $\delta$ 7.47–7.45 (m, 4H, arom.); 7.35–7.24 (m, 6H, arom.); 6.38 (d, NH, not fully deuterium exchanged); 5.51 (s, 1H, CH–Ph); 5.49 (s, 1H, CH–Ph); 4.24 [dd, 1H, $^3J_{6,6'}$ 10.4 Hz, $^3J_{5,6}$ 4.7 Hz, H-6 (a)]; 4.19 [dd, 1H, $^3J_{6,6'}$ 10.2 Hz, $^3J_{5,6}$ 4.8 Hz, H-6 (b)]; 3.97 (dd, 1H, $^3J_{\alpha,\beta}$ 9.3 Hz, $^3J_{\alpha,\beta'}$ 4.7 Hz, H-α); 3.90 [m, 1H, CH (C-1–$CH_2$–N) (a)]; 3.82 [d, 1H, $^2J_{CHN,CH'N}$ 13.8 Hz, CH (C-1–$CH_2$–N) (b)]; 3.69–3.61 [m, 3H, H-6′ (a), H-6′ (b), CH′ (C-1–$CH_2$–N) (b)]; 3.60–3.51 [m, 5H, CH′ (C-1–$CH_2$–N) (a), H-3 (a), H-3 (b), H-1 (a), H-1 (b)]; 3.41–3.27 [m, 4H, H-4 (a), H-4 (b), H-5 (a), H-5 (b)], 3.17 [m, 1H, H-2 (a)]; 3.12 [dd, 1H, $^3J_{1,2} = {}^3J_{2,3}$ 9.0 Hz, H-2 (b)]; 2.65–2.51 (m, 2H, H-γ, H-γ′); 2.03 (m, 1H, H-β); 1.85 (m, 1H, H-β′); 1.45 (s, 9H, *t*-Bu), 1.43 (s, 9H, *t*-Bu) ppm. FABMS: (*m*/*z*): $[M+H]^+$ calcd for $C_{42}H_{59}N_2O_{15}$, 831.3915; found, 831.3923.

## Acknowledgements

We are grateful to Wesley Yoshida for the recording of TOCSY, HMBC and HSQC NMR spectra, and to Mike Burger for mass spectrometric analysis. We also thank Dr. John Corrie for supplying the glutamine derivative **18** and for helpful discussions. Acknowledgment is made to the Donors of the American Chemical Society Petroleum Research Fund for support of this research given to K.M. (PRF Type G 36892-G4).

## Supplementary data

$^1$H NMR spectra for compounds **10**, **12**–**17**, and **19** are provided in the Supplemental data section, which is available in the electronic version of this paper. Supplemetary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2004.12.023.

## References

1. Bodanszky, M.; Natarajan, S. *J. Org. Chem.* **1975**, *40*, 2495–2499.
2. Bodanszky, M.; Kwei, J. Z. *Int. J. Pept. Protein Res.* **1978**, *12*, 69–74.
3. Tam, J. P.; Riemen, M. W.; Merrifield, R. B. *Pept. Res.* **1988**, *1*, 6–18.
4. Amit, B.; Ben-Efraim, D. A.; Patchornik, A. *J. Am. Chem. Soc.* **1976**, *98*, 843–844.
5. Goissis, G.; Erickson, B. W.; Merrifield, R. B. *Pept., Proc. Am. Pept. Symp., 5th* **1977**, 559–561.
6. Pass, S.; Amit, B.; Patchornik, A. *J. Am. Chem. Soc.* **1981**, *103*, 7674–7675.
7. Nicolaou, K. C.; Safina, B. S.; Winssinger, N. *Synlett* **2001**, *SI*, 900–903.
8. Helgen, C.; Bochet, C. G. *Synlett* **2001**, 1968–1970.
9. Helgen, C.; Bochet, C. G. *J. Org. Chem.* **2003**, *68*, 2483–2486.
10. Vizvardi, K.; Kreutz, C.; Davis, A. S.; Lee, V. P.; Philmus, B. J.; Simo, O.; Michael, K. *Chem. Lett.* **2003**, *32*, 348–349.
11. Papageorgiou, G.; Ogden, D. C.; Barth, A.; Corrie, E. E. T. *J. Am. Chem. Soc.* **1999**, *121*, 6503–6504.
12. Papageorgiou, G.; Corrie, J. E. T. *Tetrahedron* **2000**, *56*, 8197–8205.
13. Morrison, J.; Wan, P.; Corrie, J. E. T.; Papageorgiou, G. *Photochem. Photobiol. Sci.* **2002**, *1*, 960–969.
14. Papageorgiou, G.; Lukeman, M.; Wan, P.; Corrie, J. E. T. *Photochem. Photobiol. Sci.* **2004**, *3*, 366–373.
15. Levy, D. E.; Tang, C. *The Chemistry of C-Glycosides*, 1st ed.; Pergamon: Oxford, 1995.
16. Meo, P.; Osborn, H. M. I. The Synthesis of C-Linked Glycosides. In *Carbohydrates*; Osborn, H. M. I., Ed.; Elsevier Science: Oxford, 2003; pp 337–384.
17. Györgydeák, Z.; Pelyvás, I. F. *Glycoscience* **2001**, *1*, 691–747.
18. Gross, P. H.; Franz, A. H.; Samoshin, V. V. *Recent Res. Dev. Org. Chem.* **2004**, *8*, 255–280.
19. Gross, P. H. *Carbohydr. Polym.* **1998**, *37*, 215–217.
20. Gross, P. H.; Samoshin, V. V.; Franz, A.; Gallagher, J.; Gremyachinskij, D.; Lu, J.; Phiasivongsa, P.; Simo, O.; Wei, Y. Q. *Glycobiology* **2001**, *11*, 907–908.
21. Wang, L.-X.; Tang, M.; Suzuki, T.; Kitajima, K.; Inoue, Y.; Inoue, S.; Fan, J.-Q.; Lee, Y. C. *J. Am. Chem. Soc.* **1997**, *119*, 11137–11146.
22. Bolton, C. H.; Hough, L.; Khan, M. Y. *Biochem. J.* **1966**, *101*, 184–190.
23. Szilágyi, L.; Györgydeák, Z. *Carbohydr. Res.* **1985**, *143*, 21–41.
24. Waldmann, H.; Kunz, H. *Liebigs Ann. Chem.* **1983**, 1712–1725.
25. Spinola, M.; Jeanloz, R. W. *J. Biol. Chem.* **1970**, *245*, 4158–4162.
26. Kunz, H.; Waldmann, H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 883–885.
27. Kunz, H.; Waldmann, H.; März, J. *Liebigs Ann. Chem.* **1989**, 45–49.
28. Wagner, M.; Dziadek, S.; Kunz, H. *Chem. Eur. J.* **2003**, *9*, 6018–6030.
29. Drew, K. N.; Gross, P. H. *Tetrahedron* **1991**, *47*, 6113–6126.
30. Wang, X.; Gross, P. H. *J. Org. Chem.* **1995**, *60*, 1201–1206.
31. Christiansen-Brams, I.; Meldal, M.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1461–1471.
32. Phiasivongso, P.; Gallagher, J.; Chen, C.-N.; Jones, P. R.; Samoshin, V. V.; Gross, P. H. *Org. Lett.* **2002**, *4*, 4587–4590.
33. Bergmann, M.; Zervas, L.; Salzmann, L. *Ber. Dtsch. Chem. Ges.* **1933**, *66*, 1288–1290.