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Stereoselective synthesis of glycosyl amides by traceless Staudinger ligation of unprotected glycosyl azides

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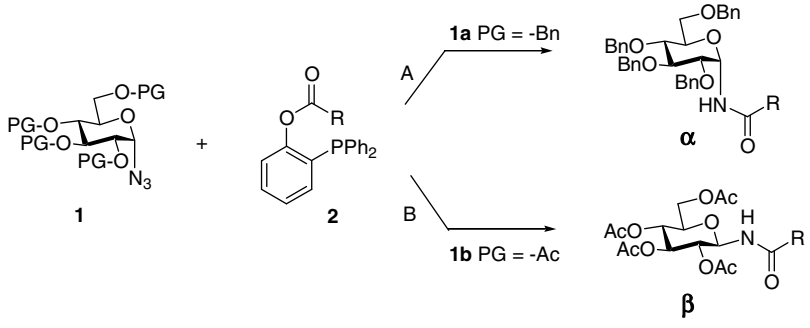
Abstract—The stereoconservative Staudinger ligation of unprotected α- and β-glucosyl azides with diphenylphosphanyl-phenyl esters to afford α- and β-glucosyl amides is described.

Keywords: Glycosyl amides; Glycosyl azides; Neoglycoconjugates; Stereoselective synthesis

1. Introduction

α-Glycosyl amides represent a little known class of non-hydrolyzable monosaccharide derivatives that may find useful application as sugar mimics and neoglycoconjugates. Their stereoselective synthesis has proven difficult because glycosyl amines are not configurationally stable and undergo easy α to β anomerization. Various methods have been proposed to get around this problem by reducing α-glycosyl azides in the presence of acylating agents, but only a few have been reported to afford α-glycosyl amides, most of which require two steps and/or have been described for a limited number of substrates.[1–6] Much work has been devoted to the Staudinger reduction–acylation process,[7–13] which consists of the reduction of glycosyl azides with phosphines in the presence of carboxylic acids or derivatives. An aza-ylide (iminophosphorane) is formed, which can be trapped by the acylating agent before the free amine is formed. However, anomerization remains a significant problem also for Staudinger reduction intermediates and, unless the acylation step is very fast, β-amides or mixtures of anomers are often obtained.[14]

We have recently reported that the traceless Staudinger ligation of *O*-benzyl-α-glycosyl azides **1a** with diphenylphosphanyl-phenyl esters **2** in polar aprotic solvents yields α-glycosyl amides with good yields and selectivity (Scheme 1, path A).[6] The phosphines employed, which



Scheme 1. Traceless Staudinger ligation of protected glycosyl azides.

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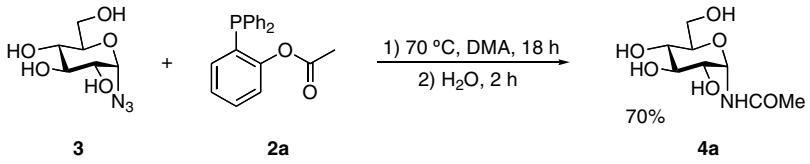


Scheme 2. Staudinger reduction–acetylation of the unprotected α-glucosylazide **3** with **2a**.

are stable to air, allow the fast intramolecular trapping of the reduction intermediates affording direct formation of the amide link and preventing anomerization. However, the process depends critically on the nature of the sugar protecting groups: the same phosphines react with tetra-*O*-acetyl-glycosyl azides **1b** in a non-stereoconservative fashion and afford β-glycosyl amides (Scheme 1, path B). The dependence of the ligation stereochemistry on the nature of the sugar protecting group appeared to be related to the electron-withdrawing effect of the acetates,[15,16] which reduces the rate of the acylation step and favors the anomerization. This effect enforces the use of benzyl ethers as protecting groups in the synthesis of α-glycosyl azides.

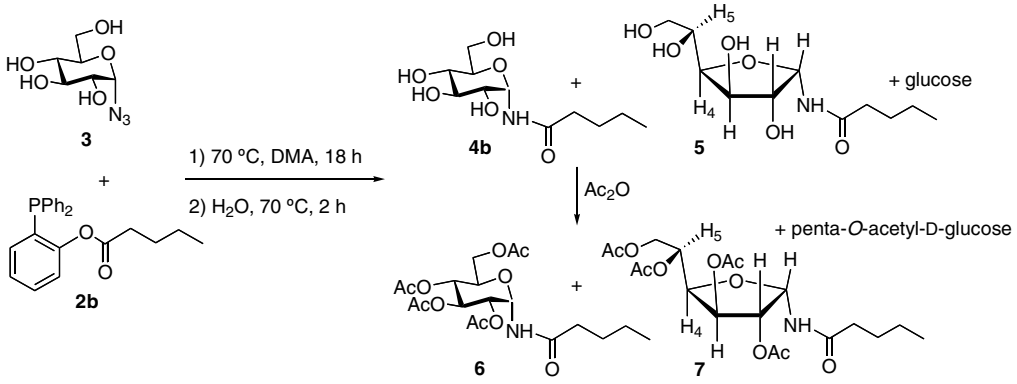
Here we report our initial results on the Staudinger ligation of unprotected α and β glucosyl azides with phosphines **2**. We were able to identify conditions that allowed a clean and stereoconservative reaction to occur in good to moderate yields, affording α or β glucosylamides depending on the configuration of the starting azide. Isolation of the resulting glucosylamides from the reaction by-products, notably triarylphosphine oxides, was remarkably simplified and could be performed by water extraction in all the cases examined.

2. Results and discussion

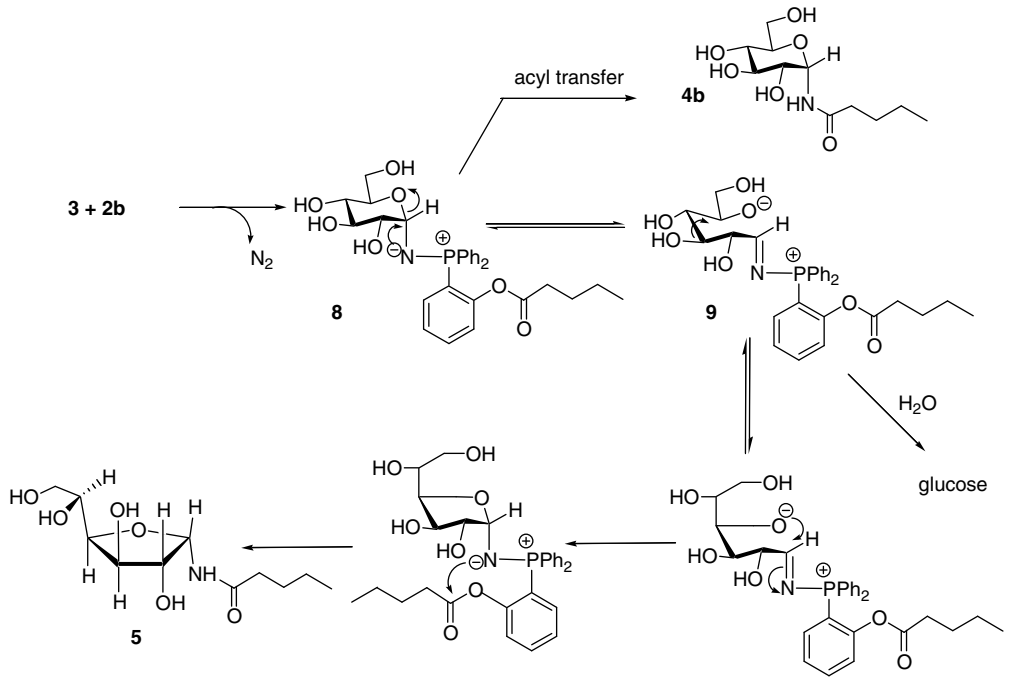
As a first step, the reaction of **2a** with the unprotected α-glucosyl azide **3** was examined under the same conditions optimized for the ligation of the benzyl derivative **1a**. After 18 h of reaction in dimethylacetamide (DMA) at 70 °C, the mixture was hydrolyzed by adding water and α-*N*-acetylglucosylamine **4a** was isolated in 70% yield by extracting the hydrophobic by-products in CH_2Cl_2 (Scheme 2). The process appeared to be totally stereoconservative. The anomeric configuration of **4a** was assigned on the basis of the vicinal coupling constant of the anomeric proton (at 5.45 ppm) $J_{1,2} = 5.4$ Hz.

Extension of this method to the pentanoyl phosphine **2b** (Scheme 3) yielded a complex mixture of compounds in the water fraction that were separated by reverse-phase HPLC and characterized as the expected pentanamide **4b** (50% yield), the α-*N*-pentanoyl-glucofuranosylamine isomer **5** (15%) and glucose (equilibrium mixture of anomers, 30%). The configuration of **4b** was assigned on the basis of the anomeric proton signal (5.45 ppm, $J_{1,2} = 5.4$ Hz). The structure of **5** was assigned on the basis of ESIMS and of the ^{1}H NMR (D_2O), which is consistent with the spectrum of the known α-D-glucofuranoside.[17] The anomeric configuration of **5** was further confirmed to be α by the NOESY spectrum (D_2O), which showed a strong crosspeak for the H-1 and H-2 protons and no crosspeak for the H-1 and H-3 protons (Scheme 3). The presence of glucose in the reaction crude was identified by ^{1}H NMR spectroscopy and confirmed upon acetylation of the crude, which allowed the isolation of the known *N*-pentanoyl-tetra-*O*-acetyl-α-D-glucopyranosylamine **6**[6] together with the tetra-*O*-acetyl *N*-furanosylamide **7**, and α and β penta-*O*-acetyl-glucose (Scheme 3).

Furanoside **5** must clearly derive from a ring-opening process occurring after the azide reduction step, presumably from the iminophosphorane **8** (Scheme 4) to afford the phosphinimine **9**, which can undergo ring-closure to yield **5**. Hydrolysis of the same intermediate **9** accounts for the formation of D-glucose in the reaction mixture. The alternative hypothesis that glucose could be formed



Scheme 3. Staudinger ligation of **3** with phosphine **2b** in DMA.

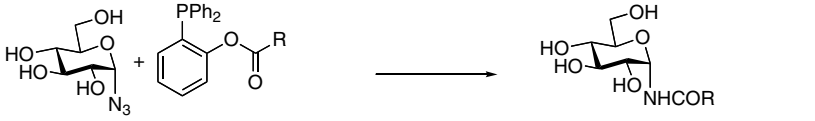


Scheme 4. Mechanism for the formation of furanosylamide **5** and glucose in the ligation of **3** with **2b**.

by direct hydrolysis of the starting azide was discarded because **3** was recovered unaltered after treatment with water in DMA at 70 °C for 24 h.

After this preliminary analysis, the reaction conditions were optimized using NMR as the analytical technique. This series of experiments allowed us to draw the following general conclusions. First, the formation of the α-furanosylamide **5** is favored by a temperature increase and can be reduced to ~5% by running the ligation at temperatures below 40 °C. Second, the azide conversion is favored by an increase in solvent polarity, which appears to accelerate the acyl transfer step. This increase is best realized by adding 1,3-dimethyltetrahydro-2(1*H*)pyrimidinone (DMPU, 1–2%) to the DMA solution.

Eventually, the best conditions identified consisted in using a 98:2 DMA–DMPU solvent mixture and running the reaction at 40 °C for 4 h, before proceeding to the hydrolysis step by adding 5% of H_2O and stirring at 40 °C for an additional 2 h. Under these conditions the α-glucopyranosylamide **4b** was isolated in 65% yield and in an excellent anomeric ratio (Scheme 5 and Table 1, entry 2). This product was exclusively contaminated by 4% of furanosylamide **5**, which could be separated by automated or open-air reverse-phase chromatography (see Section 4). When the same reaction conditions were extended to phosphines **2c–f** (Scheme 5, Table 1, entries 5, 7, 9, 11) the anomeric ratios were found to be consistently good; the yields were moderate. Variable amounts of furanosylamide were formed, depending on

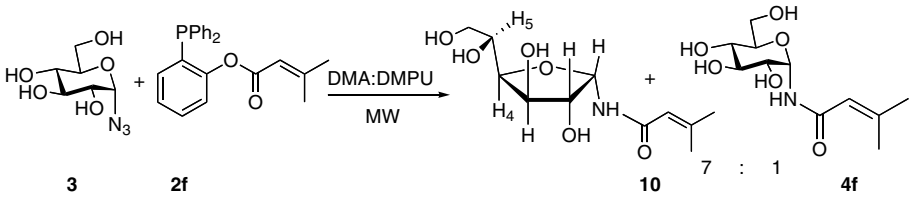


Scheme 5. Synthesis of α-glucosylamides **4** by ligation of **3** with **2** in DMA–DMPU.

Table 1. Synthesis of α-glucosylamides **4** by ligation of **3**

Entry	Phosph.	Product	Conditions	Yield[a] (%)	α:β[a]	% Furanose[a]
1	**2a**	**4a**	DMA, 70 °C, 18 h	70[b]	⩾99:1	—
2	**2b**	**4b**	98:2 DMA–DMPU 40 °C, 4 h	70(65[b])	98:2	4
3	**2b**	**4b**	98:2 DMA–DMPU MW, 120 °C, 10 min	65[b]	98:2	3
4	**2b**	**4b**	98:2 DMA–DMPU MW, 50 °C, 50 min	65[b]	98:2	2
5	**2c**	**4c**	98:2 DMA–DMPU 40 °C, 4 h	56	98:2	2
6	**2c**	**4c**	98:2 DMA–DMPU MW, 50 °C, 50 min	40[b]	98:2	2
7	**2d**	**4d**	98:2 DMA–DMPU 40 °C, 4 h	25	98:2	10
8	**2d**	**4d**	98:2 DMA–DMPU MW, 50 °C, 50 min	35[b]	98:2	4
9	**2e**	**4e**	98:2 DMA–DMPU 40 °C, 4 h	40	98:2	9
10	**2e**	**4e**	98:2 DMA–DMPU MW, 50 °C, 50 min	50[b]	98:2	2
11	**2f**	**4f**	98:2 DMA–DMPU 40 °C, 4 h	40	95:5	40
12	**2f**	**4f**	98:2 DMA–DMPU MW, 50 °C, 50 min	9[b]	98:2	52[b]

[a] Yields and diastereomeric ratios were estimated on the basis of the ^{1}H NMR spectrum of the crude water extract.
[b] Isolated yields.



Scheme 6. Reaction of **3** with **2f** under microwave irradiation.

the acyl chain transferred. Only 2% of furanose was formed together with glutaroylamide **4c** (Table 1, entry 5), ~10% with the aspartic and glutamic acid derivatives **4d** and **4e** (Table 1, entries 7 and 9), and up to 40% with the 3-methyl-butenoate derivative **4f** (Table 1, entry 11). Compared with the results previously obtained on 2,3,4,6-tetra-*O*-benzyl-α-D-glucosylazide (**1a**)[6] this approach affords a higher α/β selectivity and a much easier purification of the amide products, which can be isolated by water extraction from the crude reaction mixtures.

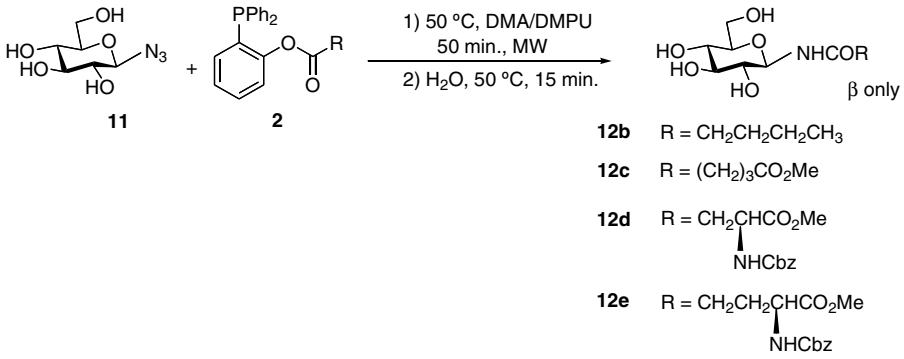
In an effort to improve the yields, the use of microwave (MW) heating was explored (Table 1, entries 3, 4, 6, 8, 10, and 12).[18] Initial experiments revealed that the reaction of **3** and **2b** could be completed in 10 min under MW irradiation at 120 °C (Table 1, entry 3) with yield and selectivity comparable to those achieved in 4 h at 40 °C without MW (compare with Table 1, entry 2). Furthermore, even at 50 °C under MW irradiation the reaction was complete in only 50 min (Table 1, entry 4) and the amount of furanoside was reduced to 2%. Similar results were obtained with phosphines **2c**–**e**: in all cases the amount of furanoside was consistently reduced to 2–4%, the α/β selectivity was unchanged and the yields were generally increased, especially for the most difficult transfer reactions (compare Table 1, entries 7 and 8, 9 and 10). Surprisingly, MW irradiation during the ligation of **3** with **2f** favored the formation of furanosylamide **10** (Scheme 6), which was formed in 7:1 ratio with the isomeric pyranosylamide **4f** and could be isolated in 52% yield by flash chromatography.

To probe the scope of the microwave-assisted process, we also examined the ligation of the unprotected β-glucosylazide **11** with representative phosphines (Scheme 5, Table 2). The process efficiently affords the β-glucosylamides **12**, characterized by a vicinal coupling constant of the anomeric proton $J_{1,2}$ of 9.2 Hz. Yields were generally good and the selectivity for the pyranose isomer was excellent. Also in this case, the main advantage of

Table 2. MW-assisted ligation of β-glucosylamine **11**[a]

Entry	Phosph.	Product	R	Y[b] (%)	% Furanoside[c]
1	**2b**	**12b**	$-(CH_2)_3CH_3$	70	2
2	**2c**	**12c**	$-(CH_2)_3CO_2Me$	75	2
3	**2d**	**12d**	$-CH_2-CH(NHCbz)-CO_2Me$	32	2
4	**2e**	**12e**	$-(CH_2)_2-CH(NHCbz)-CO_2Me$	38	2

[a] All reactions were run in 98:2 DMA–DMPU for 50 min at 50 °C under MW irradiation.
[b] Isolated yield.
[c] Estimated on the basis of the ^{1}H NMR spectra of the crude water extract.



Scheme 7. Synthesis of β-glucosylamides by MW-assisted ligation of the unprotected β-glucosyl azide **11**.

this procedure over the one described for the corresponding tetra-*O*-acetyl azide **1b**[6] lies in the easy purification of the resulting amides from the reaction mixtures (Scheme 7).

3. Conclusions

Elaboration of unprotected carbohydrates has been receiving much attention[19] and one method for the synthesis of β-D-2-deoxy-2-*N*-acetyl-glucopyranosyl-asparagine has been recently described.[20] The results reported here show that unprotected α or β glucosyl azides can be stereoselectively transformed into the corresponding amides by traceless Staudinger ligation using phosphines **2**. Crucial to yield and selectivity was an appropriate solvent selection; DMA–DMPU mixtures (98:2) were found to afford the best results. Microwave acceleration allowed shortening of the reaction time to 50 min and improvement in selectivity. Remarkably, reaction of **3** with the 3-methyl-2-butenoyl phosphine **2f** under MW irradiation led to the isolation of furanosylamide **10** in moderate yields. All ligation products could be isolated from the crude reaction mixture by simple water extraction, and were further purified by flash chromatography either on direct or reverse-phase silica gel. The process described here affords the best stereoselectivity achieved so far in the synthesis of some α-glycosylamides, such as the aminoacid derivatives **4d** and **4e**. Work is in progress to improve the yields of such difficult ligation reactions.

4. Experimental

4.1. General

Solvents were dried by standard procedures: dichloromethane, methanol, *N*,*N*-diisopropylethylamine, and triethylamine were dried over calcium hydride; *N*,*N*-dimethylacetamide (DMA), 1,3-dimethyltetrahydro-2(1*H*)pyrimidinone (DMPU), chloroform, and pyridine were dried over activated molecular sieves. Reactions requiring anhydrous conditions were performed under argon. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded at 400 MHz on a Bruker AVANCE-400 instrument. Chemical shifts (δ) for ^{1}H and ^{13}C spectra are expressed in ppm relative to internal Me_4Si as standard. Signals were abbreviated as s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra were obtained with a Bruker ion-trap Esquire 3000 apparatus (ESI ionization). HPLC separation of **4b** and **5** was performed with an Atlantis reverse-phase column (dc 19 × 100 mm, 5 μm, ESIMS detector, Waters) using a H_2O–CN_3CN gradient (from 100:0 to 70:30) over 6 min (**4b**: $t_R = 3.6$ min), **5** ($t_R = 4.1$ min). Thin layer chromatography (TLC) was carried out with precoated Merck F_{254} silica gel plates. Flash chromatography (FC) was carried out with Macherey-Nagel Silica Gel 60 (230–400 mesh); reverse-phase open-air flash chromatography was carried out with silica gel Cosmosil 75 C_{18}-OPN (75 mesh Nacalai tesque).[21] Reverse-phase automated chromatography was carried out with a Biotage System SP1 (25+M dc 25 × 150 mm column, C-18 silica 40–63 μm). Azides **3** and **11** were synthesized starting from the corresponding known peracetylated sugars[6] by sodium methoxide deprotection. Phosphines **2a**–**d** and **2f** have been previously described.[6]

4.2. Synthesis of *N*-benzyloxycarbonyl-L-glutamic acid 5-(2-diphenylphosphanyl-phenyl)ester 1-methyl ester 2e

A solution of the *o*-diphenylphosphinophenol[6] (200 mg, 0.715 mmol, 1 equiv), the commercially available *N*-carbobenzyloxy-L-glutamicacid 1-methyl ester (255 mg, 0.86 mmol, 1.2 equiv) and *N*,*N*-dimethylaminopyridine (8.8 mg, 0.072 mmol, 0.1 equiv) in dry CH_2Cl_2 (0.1 M) was added, at room temperature and under nitrogen, to a suspension of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (193.3 mg, 1 mmol, 1.4 equiv) and dry *N*,*N*-diisopropylethylamine (172.6 μL, 1 mmol, 1.4 equiv) in dry CH_2Cl_2. The mix-

ture was stirred at rt for 2 h, monitoring by TLC (1:1 hexane–AcOEt). The reaction mixture was diluted with CH_2Cl_2 and extracted with 10% aqueous HCl and water; the organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by flash chromatography using 7:3 hexane–AcOEt as the eluant to afford **2e** in 55% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42–7.29 (m, 11H), 7.17–7.08 (m, 2H), 6.86 (m, 1H), 5.40 (d, 1H, $J = 7.2$ Hz, N*H*), 5.12 (s, 1H, $J = 12.3$ Hz, CH_2-Ph), 4.35 (q, 1H, $J = 4.8$ Hz, C*H*), 2.35 (m, 2H, CH_2–COO), 2.07 (m, 1H, CH_2–CH, Ha), 1.86 (m, 1H, CH_2–CH, Hb).

4.3. General procedure for stereoselective ligation of 3 in DMA–DMPU (procedure A)

Phosphine (1.2 equiv) was added, at room temperature, to a 0.1 M solution of azide **3** (1 equiv) in 98:2 *N*,*N*-dimethylacetamide and DMPU. The solution was stirred for 4 h at 40 °C, then water was added and the mixture was stirred for an additional 2 h at the same temperature. The solvent was evaporated under reduced pressure, and the residue was diluted with water and extracted with CH_2Cl_2. The water layer was evaporated under reduced pressure and the crude was purified as indicated below for each compound.

4.4. General procedure for stereoselective ligation of 3 and 11 under microwave irradiation (procedure B)

Phosphine (1.2 equiv) was added, at room temperature, to a 0.1 M solution of the azide (1 equiv), in 98:2 *N*,*N*-dimethylacetamide and DMPU. The solution was heated to 50 °C in the microwave oven. After 50 min water (10%) was added and the mixture was stirred for an additional 10 min at the same temperature in the MW oven. The solvent was evaporated under reduced pressure, and the residue was diluted with CH_2Cl_2 and extracted with water. The water layer was evaporated under reduced pressure and the crude was purified by flash chromatography as indicated below.

4.5. *N*-Acetyl-α-D-glucopyranosylamide 4a

Crude purified by flash chromatography: 80:20:2 $CHCl_3$–MeOH–H_2O, $R_f = 0.1$, 70% yield. $[\alpha]_D$ +111.6 (*c* 0.2, MeOH); ^{1}H NMR (400 MHz, D_2O): δ 5.48 (d, 1H, $J_{1,2} = 5.5$ Hz, H-1), 3.71 (dd, 1H, $J_{1,2} = 5.5$ Hz, $J_{2,3} = 10.2$ Hz, H-2), 3.69–3.66 (m, 2H, H-6a and H-6b), 3.63 (dd, 1H, $J_{2,3} = J_{3,4} = 10.2$ Hz, H-3), 3.43 (m, 1H, H-5), 3.34 (dd, 1H, $J_{3,4} = J_{4,5} = 10.2$ Hz, H-4), 2.01 (s, 3H, –CH_3); ^{13}C NMR (100 MHz, D_2O): δ 176.01, 76.51 C-1, 73 C-2, 72.62 C-5, 69.34 C-3, 69.34 C-4, 60.48 C-6, 21.96 (–CH_3); ESIMS *m*/*z*: 244.1 $[M+Na]^+$.

4.6. *N*-Pentanoyl-α-D-glucopyranosylamide 4b

Crude purified by flash chromatography: 80:20:2 $CHCl_3$–MeOH–H_2O, or by open-air chromatography on Cosmosil 75 C_{18}-OPN: 95:5 H_2O–MeOH, or by automated chromatography (H_2O–CH_3CN gradient); yield 65%. ^{1}H NMR (400 MHz, D_2O): δ 5.60 (d, 1H, $J_{1,2} = 5.4$ Hz, H-1), 3.83 (dd, 1H, $J_{1,2} = 5.4$ Hz, $J_{2,3} = 9.4$ Hz, H-2), 3.86–3.71 (m, 3H, H-3, H-6a and H-6b), 3.53–3.49 (m, 1H, H-5), 3.46 (dd, 1H, $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4), 2.39 (m, 2H, –COCH_2–), 1.62 (m, 2H, –*C*H_2–CH_2–CH_3), 1.35 (m, 2H, –*C*H_2–CH_3), 0.92 (t, 3H, $J = 7.5$ Hz, –CH_3); ^{13}C NMR (100 MHz, D_2O): δ 179.37, 76.52 C-1, 73.03 C-2, 72.68 C-5, 69.35 C-3, 69.30 C-4, 60.51 C-6, 35.34 (–CO*C*H_2–), 27.51 (–*C*H_2–CH_2–CH_3), 21.54 (–*C*H_2–CH_3), 13.01 (–CH_3); ESIMS *m*/*z*: 286.29 $[M+Na]^+$.

4.7. *N*-Pentanoyl-α-D-glucofuranosylamide 5

Purified from the ligation in pure DMA by reverse-phase HPLC; 80:20:2 $CHCl_3$–MeOH–H_2O, $R_f = 0.3$. $[\alpha]_D$ +48.5 (*c* 0.4, MeOH); ^{1}H NMR (400 MHz, D_2O): δ 5.71 (d, 1H, $J_{1,2} = 4$ Hz, H-1), 4.23 (dd, 1H, $J_{2,3} = 0$ Hz, H-3), 4.08 (dd, 1H, $J_{3,4} = 2.4$ Hz, $J_{1,2} = 4$ Hz, H-2), 3.98 (dd, 1H, $J_{4,5} = 9.4$ Hz, $J_{3,4} = 2.4$, H-4), 3.79 (dd, 1H, $J_{4,5} = 9.4$ Hz, $J_{5,6} = 6$ Hz, H-5), 3.70 (dd, 1H, $J_{5,6} = 6$ Hz, $J_{6a,6b} = 9$ Hz, H-6a), 3.52 (dd, 1H, $J_{5,6} = 6$ Hz, $J_{6b,6a} = 9$ Hz, H-6b), 2.25 (m, 2H, –COCH_2–), 1.48 (m, 2H, –*C*H_2–CH_2–CH_3), 1.25 (m, 2H, –*C*H_2–CH_3), 0.79 (t, 3H, $J = 7.5$ Hz, –CH_3); ^{13}C NMR (100 MHz, D_2O): δ 178.12, 80.94 C-1, 78.68 C-4, 75.55 C-3, 74.91 C-2, 68.86 C-5, 63.34 C-6, 35.40 (–CO*C*H_2–), 27.22 (–*C*H_2–CH_2–CH_3), 21.52 (–*C*H_2–CH_3), 12.92 (–CH_3). ESIMS *m*/*z*: 286.29 $[M+Na]^+$.

4.8. *N*-Pentanedioic acid-α-D-glucopyranosylamide methyl ester 4c

Purification by flash chromatography (80:20:2 $CHCl_3$–MeOH–H_2O; $R_f = 0.2$) or Cosmosil 75 C_{18}-OPN (95:5 H_2O–MeOH); procedure B, yield 40%. $[\alpha]_D$ +75.4 (*c* 0.1, MeOH); ^{1}H NMR (400 MHz, D_2O): δ 5.52 (d, 1H, $J_{1,2} = 5.6$ Hz, H-1), 3.64 (m, 1H, $J_{2,3} = 9.6$ Hz, H-2), 3.63 (dd, 1H, $J_{6a,6b} = 10$ Hz, $J_{5,6} = 2.4$ Hz, H-6a), 3.58 (t, 1H, $J_{4,5} = 4$ Hz, H-4), 3.54 (s, 3H, OMe), 3.54 (m, 1H, H-6b), 3.34 (m, 1H, H-5), 3.27 (t, 1H, $J_{3,4} = 8.8$ Hz, H-3), 2.32–2.25 (m, 4H, *C*H_2–CO–N, *C*H_2–$COOCH_3$), 1.75 (m, 2H, CH_2–*C*H_2–CH_2); ^{13}C NMR (100 MHz, D_2O): δ 177.75 (CO–N), 176.44 (CO_2Me), 80.94 C-1, 78.68 C-4, 75.55 C-3, 74.91 C-2, 68.86 C-5, 63.34 C-6, 53 (OCH_3), 35.40 (–CO*C*H_2–), 34.71–32.72 (CH_2COO–, *C*H_2–CO–N), 20.40 (CH_2–CH_2–CH_2); ESIMS *m*/*z*: 330.3 $[M+Na]^+$.

4.9. *N*α-Benzyloxycarbonyl-*N*γ-α-D-glucopyranosyl-L-asparagine-*O*-methyl ester 4d

Purified by flash chromatography (80:20:2 $CHCl_3$–MeOH–H_2O; $R_f = 0.35$) or automated reverse-phase chromatography (H_2O–CH_3CN gradient); procedure B, yield 35%. $[\alpha]_D$ +57.2 (*c* 0.2, MeOH); ^1H NMR (400 MHz, D_2O): δ 7.42–7.28 (m, 5H, Ph), 5.48 (d, 1H, $J_{1,2} = 5.6$ Hz, H-1), 5.06 (br s, 2H, CH_2–O), 4.55 (t, 1H, $J = 6.4$ Hz, CH–N), 3.72 (dd, 1H, $J_{2,3} = 5.6$ Hz, H-2), 3.54–3.76 (m, 4H, H-3, H-4, H-5, H-6a), 3.68 (s, 3H, O–CH_3), 3.34 (m, 1H, H-6b), 2.88 (d, 2H, $J = 6.4$ Hz, CH_2–CO); ^{13}C NMR (100 MHz, D_2O): δ 179.37, 128.77, 128.40, 127.64, 76.64 C-1, 72.98 C-3, 72.61 C-5, 69.24 C-2, 69.13 C-4, 67.26 (CH_2–O–), 60.51 C-6, 53.14 (O–Me), 50.83 (–CH–N); ESIMS *m*/*z*: 465.18 $[M+Na]^+$.

4.10. *N*α-Benzyloxycarbonyl-*N*γ-α-D-glucopyranosyl-L-glutamine-*O*-methyl ester 4e

Purified by flash chromatography (80:20:2 $CHCl_3$–MeOH–H_2O; $R_f = 0.33$); procedure B, yield 50%. $[\alpha]_D$ +50.4 (*c* 0.3, MeOH) ^1H NMR (400 MHz, D_2O): δ 7.42–7.28 (m, 5H, Ph), 5.52 (d, 1H, $J_{1,2} = 5.5$ Hz, H-1), 5.10 (s, 2H, CH_2–O), 4.20 (q, 1H, $J = 5$ Hz, $J_{CH-CH_2} = 9$ Hz, CH–N), 3.72 (m, 4H, H-2, O–CH_3), 3.54–3.76 (m, 6H, H-3, H-4, H-5, H-6a, H-6b), 2.47 (t, 2H, $J = 7$ Hz, CH_2–CH_2–CH), 2.18 (m, 1H, Ha, CH_2–CH), 1.95 (m, 1H, Hb, CH_2–CH); ESIMS *m*/*z*: 457.3 $[M+H]^+$.

4.11. *N*-3-Methyl-2-butenoyl-α-D-glucopyranosylamide 4f

The compound was purified by flash chromatography (80:20:2 $CHCl_3$–MeOH–H_2O); procedure A, yield 38%. ^1H NMR (400 MHz, D_2O): δ 5.75 (m, 1H, CH=$C(CH_3)_2$), 5.56 (d, 1H, $J_{1,2} = 4$ Hz, H-1) 3.9–3.3 (m, 6H, H-6a, H-6b, H-5, H-4, H-3, H-2), 2.03 (d, 3H, $J = 1.6$ Hz, CH_{3a}), 1.88 (d, 3H, $J = 1.6$ Hz, CH_{3b}); ESIMS *m*/*z*: 284.1 $[M+Na]^+$.

4.12. *N*-3-Methyl-2-butenoyl-α-D-glucofuranosylamide 10

The compound was purified by flash chromatography (80:20:2 $CHCl_3$–MeOH–H_2O, $R_f = 0.29$); procedure B, yield 52%. $[\alpha]_D$ +71.8 (*c* 0.1, MeOH); ^1H NMR (400 MHz, D_2O): δ 5.81 (d, 1H, $J_{1,2} = 3.8$ Hz, H-1), 5.77 (m, 1H, CH=$C(CH_3)_2$), 4.28 (dd, 1H, $J_{2,3} = 1.4$ Hz, $J_{3,4} = 2.65$ Hz, H-3), 4.14 (dd, 1H, $J_{2,3} = 1.4$ Hz, $J_{1,2} = 3.8$ Hz, H-2), 4.03 (dd, 1H, $J_{4,5} = 8.8$ Hz, $J_{3,4} = 2.65$ Hz, H-4), 3.84 (dd, 1H, $J_{4,5} = 8.8$ Hz, $J_{5,6} = 6.2$ Hz, H-5), 3.75 (dd, 1H, $J_{5,6a} = 6.2$ Hz, $J_{6a,6b} = 12$ Hz, H-6a), 3.57 (dd, 1H, $J_{5,6b} = 6.2$ Hz, $J_{6a,6b} = 12$ Hz, H-6b), 2.02 (s, 3H, CH_{3a}), 1.83 (s, 3H, CH_{3b}); ^{13}C NMR (100 MHz, D_2O): δ 170.05 (CO), 155.38 (*C*=CH), 116.87 (*CH*=C), 81.05 C-1, 78.72 C-4, 75.69 C-3, 75.11 C-2, 69.02 C-5, 63.47 C-6, 26.39 (CH_{3a}), 19.56 (CH_{3b}); ESIMS *m*/*z*: 284.1 $[M+Na]^+$.

4.13. *N*-Pentanoyl-β-D-glucopyranosylamide 12b

The compound was purified by flash chromatography (80:20:2 $CHCl_3$–MeOH–H_2O); procedure B, yield 70%. ^1H NMR (400 MHz, D_2O): δ 4.88 (d, 1H, $J_{1,2} = 9.2$ Hz, H-1), 3.70 (dd, 1H, $J_{5,6a} = 2.4$ Hz, $J_{6a,6b} = 12.4$ Hz, H-6a), 3.64 (dd, 1H, $J_{5,6b} = 5.2$ Hz, $J_{6a,6b} = 12.4$ Hz, H-6b), 3.50–3.49 (m, 2H, H-5, H-4), 3.28–3.37 (m, 2H, H-3, H-2), 2.34 (m, 2H, –$COCH_2$–), 1.51 (m, 2H, –CH_2–CH_2–CH_3), 1.33 (m, 2H, –CH_2–CH_3), 0.82 (t, 3H, $J = 7.5$ Hz, CH_3); ^{13}C NMR (100 MHz, D_2O): δ 178.75, 78.74 C-1, 76.50 C-2, 77.85 C-5, 77.53 C-3, 71.73 C-4, 69.14 C-6, 35.52 (–COCH_2–), 27.24 (–CH_2–CH_2–CH_3), 21.55 (–CH_2–CH_3), 12.97 (CH_3); ESIMS *m*/*z*: 286.29 $[M+Na]^+$.

4.14. *N*-Pentanedioic acid-β-D-glucopyranosylamide methyl ester 12c

The compound was purified by flash chromatography (80:20:2 $CHCl_3$–MeOH–H_2O); procedure B, yield 75%. ^1H NMR (400 MHz, D_2O): δ 4.90 (d, 1H, $J_{1,2} = 9.2$ Hz, H-1), 3.82 (dd, 1H, $J_{5,6a} = 2.4$ Hz, H-6a), 3.68 (m, 1H, H-6b), 3.65 (s, 3H, OCH_3), 3.43–3.48 (m, 2H, H-4, H-5), 3.38–3.30 (m, 2H, H-2, H-3), 2.38 (m, 2H, CH_2COO–), 2.32 (m, 2H, CH_2CON–), 1.88 (m, 2H, CH_2–CH_2–CH_2); ESIMS *m*/*z*: 330.3 $[M+Na]^+$.

4.15. *N*α-Benzyloxycarbonyl-*N*γ-β-D-glucopyranosyl-L-asparagine-*O*-methyl ester 12d

The compound was purified by flash chromatography (80:20:2 $CHCl_3$–MeOH–H_2O); procedure B, yield 32%. ^1H NMR (400 MHz, D_2O): δ 7.45–7.35 (m, 5H, Ph), 5.10 (br s, 2H, CH_2–O), 4.88 (d, 1H, $J_{1,2} = 9.2$ Hz, H-1), 4.51 (t, 1H, $J = 6.4$ Hz, *CH*–N), 3.70 (s, 3H, O–CH_3), 3.85–3.23 (m, 6H, H-2, H-3, H-4, H-5, H-6a, H-6b), 2.98 (m, 2H, –CH_2); ESIMS *m*/*z*: 465.18 $[M+Na]^+$.

4.16. *N*α-Benzyloxycarbonyl-*N*γ-β-D-glucopyranosyl-L-glutamine-*O*-methyl ester 12e

The compound was purified by flash chromatography (80:20:2 $CHCl_3$–MeOH–H_2O); procedure B, yield 38%. ^1H NMR (400 MHz, D_2O): δ 7.45–7.32 (m, 5H,Ph), 5.10 (s, 2H, CH_2–O), 4.91 (d, 1H, $J_{1,2} = 9.2$ Hz, H-1), 4.22 (m, 1H, CH–N), 3.70 (s, 3H, OCH_3), 3.81 (dd, 1H, $J_{5,6a} = 2$ Hz, $J_{6a,6b} = 14.4$ Hz, H-6a), 3.67 (dd, 1H, $J_{5,6b} = 7.2$, H-6b), 3.52–3.48 (m, 2H, H-4, H-5), 3.29–3.40 (m, 2H, H-3, H-2), 2.40 (t, 2H, $J = 7$ Hz, CH_2-CO), 2.17 (m, 1H, Ha, CH_2–CH–N), 1.98 (m, 1H, Hb,

CH_2–CH–N); ^{13}C NMR (100 MHz, D_2O): δ 178.37, 176.26, 128.83, 128.46, 127.69, 79.27 C-1, 77.52 C-5, 76.82 C-2, 76.48 C-3, 69.26 C-4, 67.25 (CH_2–O–), 60.57 C-6, 53.83 (–CH–), 53.01 (O–Me), 31.76, (CH_2–CO), 26.21 (CH_2-CH); ESIMS m/z: 457.3 $[M+H]^+$.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.04.023.

References

1. Ratcliffe, A. J.; Fraser-Reid, B. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1805–1810.
2. Ratcliffe, A. J.; Konradsson, P.; Fraser-Reid, B. *J. Carbohydr. Res.* **1991**, *216*, 323–335.
3. Damkaci, F.; DeShong, P. *J. Am. Chem. Soc.* **2003**, *125*, 4408–4409.
4. Bianchi, A.; Bernardi, A. *Tetrahedron Lett.* **2004**, *45*, 2231–2234.
5. Bianchi, A.; Russo, A.; Bernardi, A. *Tetrahedron: Asymmetry* **2005**, *16*, 381–386.
6. Bianchi, A.; Bernardi, A. *J. Org. Chem.* **2006**, *71*, 4565–4577.
7. Staudinger, H.; Meyer, J. *Helv. Chim. Acta* **1919**, *2*, 635–646.
8. Gololobov, Y. G.; Kasukhin, L. F. *Tetrahedron* **1992**, *48*, 1353–1407.
9. Saxon, E.; Armstrong, J. I.; Bertozzi, C. R. *Org. Lett.* **2000**, *2*, 2141–2143.
10. Lin, F. L.; Hoyt, H. M.; Van Halbeek, H.; Bergman, R. G.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2005**, *127*, 2686–2695, and references cited therein.
11. Laughlin, S. T.; Bertozzi, C. R. *Nat. Protocols* **2007**, *2*, 2930–2944.
12. Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett.* **2000**, *2*, 1939–1941.
13. He, Y.; Hinklin, R. J.; Chang, J.; Kiessling, L. L. *Org. Lett.* **2004**, *6*, 4479–4482.
14. Kovács, L.; Ösz, E.; Domokos, V.; Holzer, W.; Györgydeák, Z. *Tetrahedron* **2001**, *57*, 4609–4621, and references cited therein.
15. Mootoo, D. R.; Konradsson, P.; Udodong, U.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1988**, *110*, 5583–5584.
16. Ottoson, H.; Udodong, U.; Wu, Z.; Fraser-Reid, B. *J. Org. Chem.* **1990**, *55*, 6068–6070.
17. Norrild, J. C.; Eggert, H. *J. Am. Chem. Soc.* **1995**, *117*, 1479–1484.
18. de la Hoz, A.; Diaz-Ortiz, A. *Adv. Org. Synth.* **2005**, *1*, 119–171.
19. Wang, C.-C.; Lee, J.-C.; Luo, S.-Y.; Kulkarni, S. S.; Huang, Y.-W.; Lee, C.-C.; Chang, K.-L.; Hung, S.-C. *Nature* **2007**, *446*, 896–899.
20. Doores, K. J.; Mimura, Y.; Dwek, R. A.; Rudd, P. M.; Elliott, T.; Davis, B. G. *Chem. Commun.* **2006**, 1401–1403.
21. www.nacalai.co.jp/en/cosmosil.