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ORIGINAL ARTICLE

Microwave-assisted reaction of glycosylamine with aspartic acid

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Abstract The synthesis of *N*-protected glycosyl amino acids from amines has been investigated and it was found that, under microwave conditions, glycosylamines could be hydrolyzed leading to new products containing a glycosyl ester linkage. The efficiency of the microwave-induced glycosylation of aspartic acid was studied comparing the microwave activity between amide and ester bond formation. Different sugar moieties have been employed to demonstrate the simple and reproducible coupling methodology. New glycosyl ester compounds were further characterized by NMR spectroscopy.

Keywords Glycosylamino acids · Triazine-based coupling reagent · Microwave-assisted synthesis

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Abbreviations

DCM	Dichloromethane
FCC	Flash column chromatography
Fmoc	9*H*-Fluoren-9-ylmethoxycarbonyl
H2BC	Heteronuclear two bond correlation
HMBC	Heteronuclear multiple bond correlation
HSQC	Heteronuclear single quantum coherence
MS	Mass spectrometry
MW	Microwave
NMM	*N*-Methylmorpholine
NMR	Nuclear magnetic resonance
RP-HPLC	Reverse phase-high performance liquid chromatography
SPGPS	Solid phase glycopeptide synthesis
TBCR	Triazine-based coupling reagent
t-Bu	*tert*-Butyl
TFA	Trifluoroacetic acid
TLC	Thin layer chromatography

Introduction

Glycoproteins, which can be co- or post-translationally modified proteins by a carbohydrate moiety, are involved in different biological processes, such as signalling, cell adhesion and cell growth regulation (Varki 1999; Rademacher 1988). Because of the heterogeneity of the potential specific sites of glycosylation present on each protein and of the diversity of enzymes involved in protein glycosylation (glycosyl transferases and glycosidases), the role of the sugars in proteins and peptides is an important and highly relevant field of study.

Two main forms of protein glycosylation are generally found: *O*- and *N*-protein glycosylation. In general, natural *O*-linked glycoproteins are synthesized by addition of the

sugar moieties to the hydroxyl side chain of serine, threonine, tyrosine, or hydroxylysine residues in polypeptides in the Golgi apparatus. Naturally occurring *N*-glycoproteins and glycopeptides include an asparagine modified by a carbohydrate moiety with an *N*-acetyl glucosamine. This modification is within a consensus sequence Asn-Xaa-Ser/Thr (where Xaa is any amino acid except Pro) and the glycosidic bond links the Asn side chain to the glycan portion.

In spite of the great progress made towards the difficult syntheses of glycopeptides and glycoproteins there are still unmet challenges. Great attention was given to the formation of the glycosidic bond and its maintenance during the repetitive coupling and deprotection steps involved in the synthesis of glycopeptides. N^{α}-protected amino acids that present distinct glycosylated side chains are important building blocks (Meldal 1994a; Meldal 1994b; Meldal and Bock 1994) in the solid phase glycopeptide synthesis (SPGPS).

A variety of procedures leading to *N*-, *O*-, and ester-linked amino acid building blocks is reported in the literature (Christiansen-Brams et al. 1993; Van Ameijde et al. 2002; Toshuki 1994; Ying et al. 2005; Meldal and Bock 1994; Keglevic et al. 1973, 1976; Keglevic and Valentekovic 1976) including an optimized large-scale microwave-based synthesis of *N*-β-linked glycans to side chains of Asn and Gln (Paolini et al. 2007). In fact, microwave (MW) technology is widely and advantageously used in different fields of organic synthesis, because reactions are usually clean, efficient and require short times (Kappe 2004; Kappe and Dallinger 2006). The optimization of different processes has to be systematically investigated, so the advantages and benefits of this new technology could be fully exploited.

Herein, we examine the effects of microwave irradiation on the coupling reaction of β-D-glucopyranosylamine with the side chain carboxylic group of aspartic acid previously reported (Paolini et al. 2007), discussing how the thermal conditions can favor the formation of a new product containing a glycosyl ester linkage.

Materials and methods

Thin layer chromatographies (TLCs) were carried out on SiO_2 (Merck; 60 Å F254) and spots located with: UV light (254, 366 nm), Fluram® (Fluka; fluorescamine, 4-phenylspyro [furan-2(3*H*),1′-isobenzofuran]-3,3′-dione) in acetone, and vanilline (absolute ethanol/H_2SO_4 10:1).

Microwave reactions were carried out in a CEM Explorer 48® microwave instrument, equipped with 35 and 10 mL vessels, respectively. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, on Varian Inova or Mercuryplus spectrometers in deuterated chloroform solution and are reported in parts per million (ppm), with the residual solvent peaks used as reference. Analytical RP-HPLC were performed on a Alliance, model 2695 instrument (equipped with a diode array detector, Waters Corporation, Milford, Massachusets) using a Jupiter C18 (5 µm, 250 × 4.6 mm) column (Phenomenex) at 1 mL/min or on an Acquity UPLC instrument (Waters) coupled to a single quadrupole ZQ ESI-mass spectrometer (Micromass) using a 2.1 × 50 mm, 1.7 µm Acquity BEH C18 column at 30°C, with a flow rate of 0.45 mL min^{-1}. The solvent system used was A (0.1% TFA in H_2O) and B (0.1% TFA in CH_3CN). High-resolution mass spectrometry was performed in a Waters Q-TOF micromass. The products were lyophilized with an Edwards Modulyo apparatus.

Protected amino acid Fmoc-L-Asp-O*t*-Bu was purchased from Novabiochem AG (Laufelfingen, Switzerland) and HPLC grade acetonitrile from Carlo Erba (Italy).

Glycosylamines were prepared according to standard methods. Conversion to amine GlcAc4-NH_2 was accomplished in excellent yield via the corresponding glycosylazide, GlcAc4-N_3 analogously to the reported method (Soli et al. 1999). The triazine-based coupling reagent DMTMM BF_4 [4-(4,6-dimethoxy-1,3,5-triazin-2-yl-)-4-methylmorpholinium tetrafluoroborate] was obtained as described (Kaminski et al. 2005a, b).

General procedure for the coupling reaction

Glycosylamines (GlcAc4-NH_2 **1**, GalAc4-NH_2 **2**, ManAc4-NH_2 **3**, 1 mmol), Fmoc-L-Asp-O*t*-Bu (1 mmol), NMM (1 mmol), and DMTMM BF_4 (1 mmol) were dissolved in CH_3CN (5 mL). The microwave tube was charged with the mixture, sealed and placed into the microwave synthesizer at reaction conditions described in Table 1. The solvent

Table 1 Comparative study of different reaction conditions between L-glucosylamine and aspartic acid side chain to obtain ester and/or amide derivatives **5** and **8**, respectively

Entry	Temp (°C)	Time (min)	Power (W)	Yield[a] (%)	Amide **5**/ester **8**[b]
1	70	5	100	80	94/6
2	100	3	100	48	55/45
3	130	1	100	52	28/72
4	130	1	200	58	15/85
5	150	1	100	69	13/87
6	150	1	200	76	8/92

All reactions were performed in a sealed vessel specific for the monomode microwave synthesizer CEM Explorer 48® in acetonitrile using N^{α}-Fmoc-L-Asp-O*t*-Bu, NMM, and the coupling reagent DMTMM BF_4

[a] For the mixture of compounds **5** and **8**

[b] Calculated by RP-HPLC or/and ^{1}H NMR analysis

was then evaporated under reduced pressure, the residue was dissolved in $CHCl_3$ and washed successively with water, 0.5 M aqueous $KHSO_4$, water, 0.5 M aqueous $NaHCO_3$, and water again. Organic layer was dried with Na_2SO_4, filtered and concentrated to dryness. The compounds were separated by flash column chromatography (FCC) (AcOEt/hexane 1:1). The solvents were evaporated under reduced pressure and the residues dried in a vacuum desiccator with P_2O_5 and KOH to constant weight affording compounds **5**–**10**.

Fmoc-L-Asn(GlcAc4)-O*t*-Bu (**5**). R_f [AcOEt/hexane 1:1, UV, fluorescamine and vanilline] = 0.25.
Fmoc-L-Asn(GalAc4)-O*t*-Bu (**6**). R_f [AcOEt/hexane 1:1, UV, fluorescamine and vanilline] = 0.25.
Fmoc-L-Asn(ManAc4)-O*t*-Bu (**7**) R_f [AcOEt/hexane 1:1, UV, fluorescamine and vanilline] = 0.27.

Characterization of the products is in accordance with the literature (Van Ameijde et al. 2002; Ying et al. 2005).

***N*$^{\alpha}$-Fmoc-L-Asp(GlcAc4)-O*t*-Bu (8)** R_f [AcOEt/hexane 1:1, UV, fluorescamine and vanilline] = 0.52; QTOF MS: *m*/*z* calcd for $[C_{37}H_{43}O_{15}N]$ 741.2633, found $(M + H)^+$ 742.2519. ^{13}C and ^1H NMR data are reported in Tables 2 and 3, respectively.

***N*$^{\alpha}$-Fmoc-L-Asp(GalAc4)-O*t*-Bu (9)** R_f [AcOEt/hexane 1:1, UV, fluorescamine and vanilline] = 0.53; QTOF MS: *m*/*z* calcd for $[C_{37}H_{43}O_{15}N]$ 741.2633, found $(M + Na)^+$ 764.2687. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.47 (s, 9H, *t*-Bu), 1.99, 2.02, 2.03, 2.14 (4 s, 12H, 4 × $COCH_3$), 2.85–3.12 (m, 2H, Asp β-CH_2), 4.00–4.18 (m, 3H, 6-CH_2, H-5), 4.22 (pt, J = 7.0 Hz, 1H, Fmoc-CH), 4.30–4.48 (m, 2H, Fmoc-CH_2), 4.54 (ddd, J = 8.2, 4.1 Hz, 1H, Asp α-CH), 5.09 (dd, J = 10.3, 3.3 Hz, 1H, H-3), 5.34 (dd, J = 10.3, 8.2 Hz, 1H, H-2), 5.42 (dd, J = 3.3, 0.8 Hz, 1H, H-4), 5.72 (d, J = 8.2 Hz, 1H, H-1), 5.81 (d, J = 8.2 Hz, 1H, NH), 7.31 (pt, J = 7.4 Hz, 2H, Fmoc-Ar), 7.40 (pt, J = 7.4 Hz, 2H, Fmoc-Ar), 7.62 (m, 2H, Fmoc-Ar), 7.76 (d, J = 7.7 Hz, 2H, Fmoc-Ar); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.6, 20.5 (CO*C*H_3), 27.8 (C(CH_3)), 36.8 (Asp β-CH_2), 47.1 (Fmoc-CH), 50.6 (Asp α-CH), 60.9 (C-6), 66.7 (C-4), 67.2 (Fmoc-CH_2), 67.7 (C-2), 70.7 (C-3), 71.7 (C-5), 82.9 (C(CH_3)), 92.4 (C-1), 120.0, 125.1, 127.1, 127.7, 141.3, 143.7 (Fmoc-Ar), 156.0 (Asp α-NH-CO), 169.1 (Asp α-CO, Asp β-CO), 169.6, 169.9, 170.0, 170.3(*C*OCH_3).

***N*$^{\alpha}$-Fmoc-L-Asp(ManAc4)-O*t*-Bu (10, α anomer)** R_f [AcOEt/hexane 1:1, UV, fluorescamine and vanilline] = 0.55; QTOF MS: *m*/*z* calcd for $C_{37}H_{43}O_{15}N$ = 741.2633, found $(M + Na)^+$=764.3274. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.46 (s, 9H, *t*-Bu), 2.00, 2.02, 2.08, 2.20 (4 s, 12H, 4× $COCH_3$), 2.90-3.10 (m, 2H, Asp β-CH_2), 4.00–4.12 (m, 2H, 6-CHCH, H-5), 4.23 (pt, J = 6.7 Hz, 1H, Fmoc-CH), 4.28–4.46 (m, 3H, Fmoc-CH_2, 6-C*H*CH), 4.57 (ddd, J = 8.1, 4.2 Hz, 1H, Asp α-CH), 5.24–5.40 (m, 3H, H-2, H-3, H-4), 5.81 (d, J = 8.0 Hz, 1H, N–H), 6.12 (s, 1H, H-1), 7.31 (pt, J = 7.4 Hz, 2H, Fmoc-Ar), 7.39 (pt, J = 7.4 Hz, 2H, Fmoc-Ar), 7.61 (m, 2H, Fmoc-Ar), 7.76 (d, J = 7.6 Hz, 2H, Fmoc-Ar); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.6, 20.7 (CO*C*H_3), 27.8 (C(*C*H_3)), 36.8 (Asp-β-CH_2), 47.1 (Fmoc-CH), 50.9 (Asp-α-CH), 62.0 (C-6), 65.5 (C-4), 67.4 (Fmoc-CH_2), 68.2 (C-2), 68.6 (C-3), 70.9 (C-5), 83.2 (*C*(CH_3)), 91.1 (C-1), 119.9, 125.1, 127.1, 127.7, 141.3, 143.8 (Fmoc-Ar), 156.1 (Asp α-NH-CO), 169.6 (Asp α-CO, Asp β-CO), 169.7, 169.9, 170.0, 170.7 (*C*OCH_3).

Table 2 ^{13}C NMR ($CDCl_3$, 100 MHz) chemical shifts (δ, ppm) of compounds **5** and **8**

Atom	Compounds	
	5	**8**
C-1	78.1	92.0
C-2	70.6	70.1
C-3	72.6	72.6
C-4	68.0	67.6
C-5	73.7	72.8
6-CH_2	61.5	61.4
Asn α-CH or Asp α-CH	51.0	50.5
Asn β-CH_2 or Asp β-CH_2	38.0	36.7
Fmoc-CH	47.1	47.1
Fmoc-CH_2	67.2	67.3
Fmoc-Ar	125.1, 127.0, 127.7, 120.0, 141.3, 143.8	125.1, 127.1, 127.8, 120.0, 141.3, 143.7
C(CH_3)$_3$	82.4	83.0
C(*C*H_3)$_3$	27.9	28.8
COCH_3	171.1, 170.5, 169.9, 169.5	170.7, 170.1, 169.4, 169.4
CO*C*H_3	20.6, 20.6, 20.5	20.5, 20.5, 20.6
Asn β-CO or Asp β-CO	170.7	169.1
Asn α-CO or Asp α-CO	169.7	169.1
Asn α-NH-CO or Asp α-NH-CO	156.1	156.0

***N*$^{\alpha}$-Fmoc-L-Asp(ManAc4)-O*t*-Bu (10, β anomer)** R_f [AcOEt/hexane 1:1, UV, fluorescamine and vanilline] = 0.5; QTOF MS: *m*/*z* calcd for $C_{37}H_{43}O_{15}N$ = 741.2633, found $(M + Na)^+$ = 764.3279. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.46 (s, 9H, *t*-Bu), 2.01, 2.05, 2.06, 2.25 (4 s, 12H, 4 × $COCH_3$), 2.90–3.10 (m, 2H, Asp β-CH_2), 3.80 (ddd, J = 9.6, 5.2, 2.4 Hz, H-5), 4.13 (dd, J = 12.5, 2.4 Hz, 1H, 6-C*H*CH), 4.22 (pt, J = 6.6 Hz, 1H, Fmoc-CH), 4.28–4.40 (m, 3H, Fmoc-CH_2, 6-CHC*H*), 4.51 (ddd, J = 8.6,

Table 3 ^{1}H NMR ($CDCl_3$, 400 MHz) chemical shifts (δ, ppm) of compounds **5** and **8**

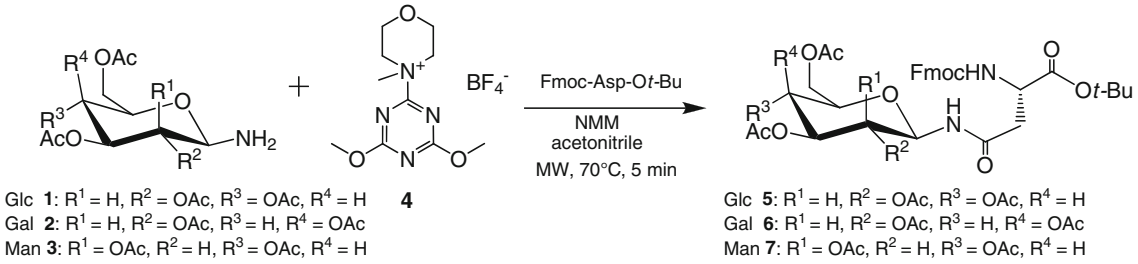
Atom	Compounds	
	5	**8**
H-1	5.23, dd (pt), $J = 9.3$ Hz	5.73, d, $J = 8.4$ Hz
H-2	4.91, dd (pt), $J = 9.5$ Hz	5.14, m
H-3	5.30, dd (pt), $J = 9.5$ Hz	5.26, dd (pt), 9.4 Hz
H-4	5.05, dd (pt), $J = 9.9$ Hz	5.12, m
H-5	3.78, ddd, $J = 9.9$, 4.2, 2.0 Hz	3.83, ddd, $J = 10.0$, 4.4, 2.1 Hz
6-CH_2	4.28, m	4.28, dd, $J = 12.5$, 4.3 Hz
	4.03, d, $J = 12.2$ Hz	4.09, dd, $J = 12.6$, 2.0 Hz
Asn α-CH or Asp α-CH	4.49, ddd, $J = 8.2$, 4.4, Hz	4.53, ddd, $J = 8.2$, 4.2, Hz
Asn β-CH_2 or Asp β-CH_2	2.65–2.90, m	2.85–3.09, m
Fmoc-CH	4.21, dd (pt), $J = 7.1$ Hz	4.22, dd (pt), $J = 7.3$ Hz
Fmoc-CH_2	4.42, dd, $J = 10.5$, 7.3 Hz	4.32–4.46, m
	4.25–4.36, m	
Fmoc-Ar	7.75, d, $J = 7.5$ Hz	7.76, d, $J = 7.5$ Hz
	7.59, d, $J = 7.5$ Hz	7.60, m
	7.39 (pt), $J = 7.4$ Hz	7.39 (pt), $J = 7.6$ Hz
	7.31 (pt), $J = 7.4$ Hz	7.31 (pt), $J = 7.1$ Hz
$C(CH_3)_3$	1.44, s	1.46, s
$COCH_3$	2.06, 2.02, 2.01	2.06, 2.03, 2.02, 2.015
Asn α-NH or Asp α-NH	5.92, d, $J = 8.5$ Hz	5.76, d, $J = 8.2$ Hz
1-NH	6.41, d, $J = 9.2$ Hz	-

4.6 Hz, 1H, Asp α-CH), 5.14 (dd, $J = 10.0$, 3.0 Hz, 1H, H-3), 5.30 (pt, $J = 9.0$ Hz, 1H, H-4), 5.50 (d, $J = 3.0$ Hz, 1H, H-2), 5.80 (d, $J = 7.4$ Hz, 1H, N–H), 5.87 (s, 1H, H-1), 7.31 (pt, $J = 7.4$ Hz, 2H, Fmoc-Ar), 7.39 (pt, $J = 7.4$ Hz, 2H, Fmoc-Ar), 7.61 (m, 2H, Fmoc-Ar), 7.76 (d, $J = 7.6$ Hz, 2H, Fmoc-Ar); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.5, 20.8 ($CO\mathit{C}H_3$), 27.8 ($C(\mathit{C}H_3)$), 36.8 (Asp β-CH_2), 47.1 (Fmoc-CH), 50.5 (Asp α-CH), 62.0 (C-6), 65.4 (C-4), 67.4 (Fmoc-CH_2), 68.0 (C-2), 70.6 (C-3), 73.3 (C-5), 83.2 ($\mathit{C}(CH_3)$), 90.8 (C-1), 119.9, 125.1, 127.1, 127.7, 141.3, 143.8 (Fmoc-Ar), 156.1 (Asp α-NH-CO), 169.6 (Asp α-CO, Asp β-CO), 169.9, 170.0, 170.1, 170.7 ($\mathit{C}OCH_3$).

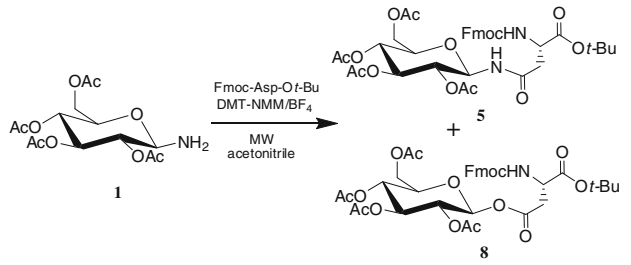
tert-Butyl-deprotection of Fmoc-protected amino acid

The pure *t*-Bu-protected compound **8** was dissolved in a mixture of TFA and DCM (1:1). The resulting mixture was stirred for 3 h at room temperature. The solvents were evaporated off. Fmoc-protected glycosyl amino acid **11** was obtained by suspension in water and lyophilization.

N^{α}-Fmoc-L-Asp(GlcAc4)-OH (11) R_f [AcOEt/hexane 1:1, UV] = 0.65; QTOF MS: *m/z* calcd for [$C_{33}H_{35}O_{15}N$] 685.2007, found 686.2192. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.02, 2.03, 2.04, 2.09 (4 s, 12H, 4 × $COCH_3$), 2.89–3.14 (m, 2H, Asp β-CH_2), 3.82 (m, 1H, H-5), 4.21–4.24 (m, 3H, 6-CH_2, Fmoc-CH) 4.38–4.41 (m, 2H, Fmoc-CH_2) 4.63 (pt, $J = 7.0$ Hz, 1H, Asp α-CH), 5.09–5.18 (m, 2H, H-3, H-2), 5.24 (pt, $J = 9.9$ Hz, 1H, H-4), 5.74 (d, $J = 8.2$ Hz, 1H, H-1), 5.90 (d, $J = 8.2$ Hz, 1H, NH), 7.30 (pt, $J = 7.4$ Hz, 2H, Fmoc-Ar), 7.40 (pt, $J = 7.4$ Hz, 2H, Fmoc-Ar), 7.62 (m, 2H, Fmoc-Ar), 7.76 (d, $J = 7.7$ Hz, 2H, Fmoc-Ar); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.3 ($CO\mathit{C}H_3$), 36.4 (Asp β-CH_2), 46.7 (Fmoc-CH), 50.6 (Asp α-CH), 61.0 (C-6), 67.3 (C-4), 67.4 (Fmoc-CH_2), 67.7 (C-2), 69.8 (C-3), 72.1



Scheme 1 Microwave-assisted synthesis of *N*-glycosylamino acids utilizing a triazine-based coupling reagent



Scheme 2 General coupling reaction between β-D-glucopyranosylamine (**1**) and Fmoc-Asp-O*t*-Bu

(C-5), 91.7 (C-1), 120.0, 125.1, 127.1, 127.7, 141.3, 143.7 (Fmoc-Ar), 156.0 (Asp α-NH-CO), 169.1 (Asp α-CO, Asp β-CO), 169.6, 169.9, 170.0, 170.3 ($COCH_3$).

Results and discussion

We previously reported (Paolini et al. 2007) an efficient MW-assisted synthesis of compounds **5** and **6** by coupling *O*-peracetylated glycosylamines **1** and **2** to aspartic acid side chain in the presence of NMM and the new triazine-based coupling reagent (TBCR) DMTMM BF_4 (**4**) (Kaminski et al. 2005a, b; Kaminski 1994). Shifting the equilibrium of the coupling reaction favours the formation of the fully protected Asn(SugAc4)-derivatives **5** and **6** (Scheme 1, Sug = Glc, Gal, Man). The reaction has been now extended also to the mannosylamine **3** giving compound **7**.

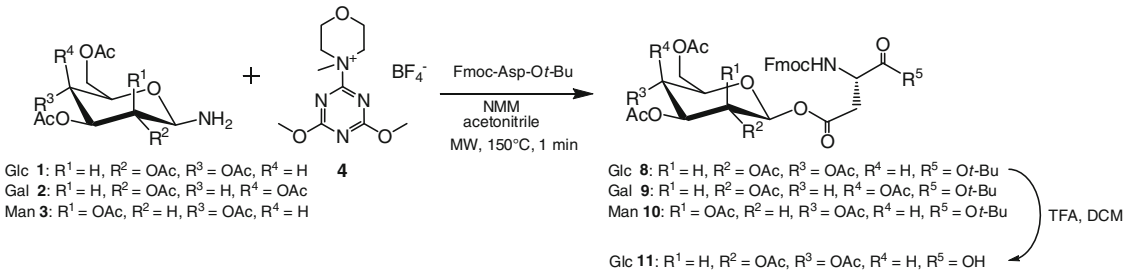
Moreover, in these conditions, we observed the formation of a new product in very low yield (6%) further characterized as the glucosyl ester N^{α}-Fmoc-L-Asp(GlcAc4)-O*t*-Bu (**8**). In the light of the observed formation of this compound during the MW-assisted coupling reaction, we undertook a thorough study of the latter by varying the reaction conditions (i.e., different MW power, temperature, and reaction times) in an attempt to optimize the generation of the orthogonally protected ester **8** (Scheme 2, Table 1).

Shortening the reaction time with concomitant increase in MW power and reaction temperature led to a progressive increase of the ester bond formation. Monitoring the coupling reaction by RP-HPLC and NMR revealed that extended reaction time at lower energy and temperature generated almost exclusively the *N*-glucosyl amide derivative **5** (Table 1, entry 1). Purification of the product obtained under these conditions yielded 80% of pure **5**. Short reaction times at higher energy and temperature led to a gradual increase of the ester/amide ratio (Table 1, entries 2, 3, and 5) with a maximum of 87/13 at 150°C and 100 W (Table 1, entry 5). Moreover, when MW power was increased from 100 to 200 W (Table 1, entry 6) the **5/8** ratio reached 8/92. Noteworthy, by decreasing coupling reaction time to 1 min we were able to avoid the thermal decomposition of the N^{α}-Fmoc protecting group with the advantage of a significant increase in the overall yields.

The glucosyl asparagine derivatives **5** and **8** were successfully purified independently from the reaction mixtures by FCC and characterized by spectroscopic methods.

Further experiments to obtain compound **8** were then performed. The coupling reaction of 2,3,4,6-tetra-*O*-acetyl-D-glycopyranose (1 mmol) with aspartic acid (1 mmol) in the presence of DMTMM (1 mmol), and NMM (1.5 mmol) under MW-assisted reaction conditions afforded compound **8**. By using the traditional ester-formation strategy, i.e., DIPEA (4 mmol) was added to a solution of α-D-glucopyranosyl bromide tetraacetate (2 mmol) and Fmoc-Asp-O*t*-Bu (2 mmol) in DCM, giving compound **8** after 2 days stirring of the solution at room temperature.

Structural determination of the ester **8** (Scheme 2) was achieved mainly on the basis of NMR spectroscopy. In fact, the ^{1}H NMR spectrum shows the signal of the easily attributable anomeric proton as a simple doublet at higher chemical shift with respect to the doublet of doublets (pt) observed for the same proton in compound **5**. Moreover, in the fully decoupled carbon-13 spectrum the signal of the corresponding anomeric carbon, easily recognized by HSQC, HMBC, and H2BC 2D-NMR experiments, appears now at δ 92.0 ppm as expected for this kind of derivative (δ 78.1 in compound **5**, see Tables 2, 3).



Scheme 3 General coupling reaction between β-D-glycopyranosylamine (**1–3**) and Fmoc-Asp-O*t*-Bu leading to Fmoc-Asp($SugAc_4$)-O*t*-Bu **8–10**

In the absence of MW irradiation under traditional thermal conditions, the reaction reported in Scheme 2 yielded exclusively the amide **5**. The reaction with no MW irradiation has been also performed using a pressure-resistant vessel at longer reaction times. After 24 h at 100–130°C no product **8** was observed and product **5** was obtained in very low yield (18% evaluated by HPLC).

It is well-known that glycosylamine, heated in a suitable solvent for more than 2 h, is prone to dimerization giving the corresponding diglycosylamine (Klein 1958; Vetter and Gallop 1995). Moreover, several reactions have been performed under heavy MW conditions with amines and no hydroxylation has been observed (Seijas et al. 2007; Gelens et al. 2005; Liautard et al. 2008). In this context, under MW irradiation, the glucosylamine **1** displayed the unexpected hydrolysis at C-1 by loss of ammonia, as detected by MS, reacting with the activated carboxylic group of the aspartic acid. As a consequence of the hydrolysis, we may conclude that microwaves irradiation is essential to the ester-type bond formation.

The general application of the MW coupling methodology leading to Fmoc-Asp(GlcAc$_4$)-O*t*-Bu (**8**) (150°C) was demonstrated by reaction of β-D-galactosylamine and β-D-mannosylamine with Fmoc-Asp-O*t*-Bu (Scheme 3). In the case of N^{α}-Fmoc-Asp(ManAc4)-O*t*-Bu a mixture of α/β anomers (ratio 2:1) was obtained.

Compound N^{α}-Fmoc-L-Asp(GlcAc4)-O*t*-Bu (**8**) was deprotected selectively at the α-carboxyl function by a TFA solution in DCM, obtaining the anomerically pure building block N^{α}-Fmoc-L-Asp(GlcAc$_4$)-OH (**11**), compatible for Fmoc/*t*-Bu-based SPGPS.

In summary, this report not only demonstrates the high efficiency of the microwave-assisted glycosylation of aspartic acid, but also compares the microwave activity between amide and ester bond formation. Hydrolysis of glycosylamines by extreme MW irradiation is observed, leading to new products containing a glycosyl ester linkage. The successful optimization of the synthesis of these building blocks fulfills an unmet need and suggests an additional application for MW-assisted synthesis otherwise difficult to achieve in a fast way by other methodologies.

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