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N^{α}-Dmc protected amino acid aglycones: versatile hydrogenolysis stable acceptors for *O*-glycosylation

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Abstract—N^{α}-(4,4-Dimethyl-2,6-dioxocyclohexylidenemethylene) (Dmc) protected L-serine, L-threonine and L-homoserine have been prepared as *tert*-butyl esters in excellent yields. These hydrogenolysis stable acceptors underwent efficient α-*O*-glycosylation with an L-fucopyranosyl bromide donor and also allowed convenient protecting group manipulations to ultimately deliver novel glycoamino acid building blocks suitable for Fmoc based solid-phase glycopeptide synthesis.

The majority of proteins observed in Nature bear diverse *O*- and *N*-linked oligosaccharide structures,[1] and it has become increasingly clear that glycosylation modulates both the physicochemical properties and biological functions of these glycoproteins. For example, glycosylation influences uptake, distribution, excretion and proteolytic stability, yet is also implicated in communication between cells and in attachment of bacteria and viruses to host cells.[2] One such carbohydrate entity, sialyl Lewis X (sLe^X) is located on the cell-surface of many important celltypes,[3] and plays a key role in both inflammatory responses and metastasis of cancer cells.[4] In addition, other key *O*-linked fucosylated sequences have been identified in human factor IX, insect neuropeptides and epidermal growth factor (EGF) domains of assorted coagulation and fibrinolytic proteins.[5] Our interest in the synthesis of novel fucopeptide based inhibitors of sLe^X binding, as potential anti-inflammatory and anti-metastatic agents, revealed a dearth of established routes for the synthesis of appropriately protected α-linked fuco-amino acid building blocks suitable for Fmoc/^{t}Bu solid-phase peptide synthesis (SPPS). Consequently, to investigate their biological function in detail, efficient access to appropriate glycoamino acid building blocks is required for the construction of key fucose-bearing glycopeptides.

Only a handful of synthetic routes have been described to date. For example, boron trifluoride etherate promoted glycosylation of Fmoc-Thr-OH and Fmoc-Ser-OH with peracetylated L-fucose afforded the thermodynamic α-fucosides in 35% and 44% yields, respectively.[6] Clearly, this provided a rapid access to the required building blocks, however, the resulting mixtures required HPLC purification, thereby opposing scale-up of the one-step protocol. Traditionally, α-fucosyl amino acids are obtained by the in situ anomerisation reaction using nonparticipatory *O*-benzyl ether protected fucosyl donors. Since the *O*-glycosidic linkage of *O*-benzyl protected fucose is highly sensitive to acid (e.g., TFA), a protecting group exchange of *O*-benzyl for *O*-acetyl is paramount if the building blocks are to be utilised in an Fmoc-based SPPS strategy. Unfortunately, the Fmoc group itself is not hydrogenolysis stable[7] and to circumvent this problem, Kunz and co-workers α-fucosylated Fmoc-Thr-O^{t}Bu with a 2,3,4-tri-*O*-*p*-methoxybenzyl thiofucoside.[8] These sugar protecting groups could then be removed with ceric ammonium nitrate (CAN) and acetylated prior to the acidolytic deprotection of the *tert*-butyl ester (38% over three steps). Using a similar strategy, glycosylation of Z-Ser-OBn using 2,3,4-tri-*O*-benzylfucosyl bromide afforded the desired glycoamino acid.[9] From this intermediate, global deprotection using catalytic hydrogenolysis was followed by Fmoc incorporation, allyl esterification, sugar hydroxyl acetylation and allyl ester deprotection. It was observed that direct incorporation of the sugar acetates was not possible with the free acid, and thus dictated the excessive protecting group manipulations outlined. Therefore, in an

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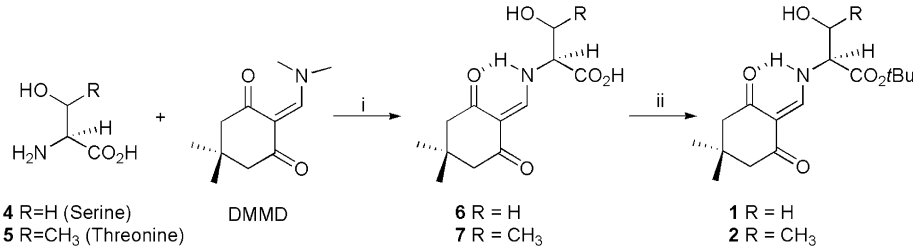
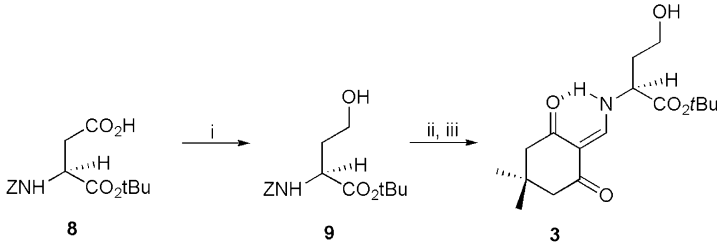
attempt to overcome some of the problems associated with the construction of these building blocks, this letter describes the synthesis and application of piperidine labile N^{α}-(4,4-dimethyl-2,6-dioxocyclohexylidenemethylene) (Dmc)[10] protected amino acids **1**–**3** as robust hydrogenolysis stable glycosyl acceptors for α-fucosylation.

Originally, the use of Dmc as an orthogonal amino protecting group for SPPS became redundant as it was superseded by its methyl derivative Dde[11] due to the latter's improved stability towards the Fmoc deprotection conditions. We reasoned however, that the former's stability to hydrogenolysis and lability towards piperidine would be an asset when used as a protecting group suitable for the preparation of α-fucosylated amino acids for SPPS. To this end, serine **4** and threonine **5** were protected as their corresponding N^{α}-Dmc derivatives **6** and **7** using 5,5-dimethyl-2-(dimethylaminomethylene)cyclohexane-1,3-dione (DMMD) and subsequently converted to the corresponding *tert*-butyl esters **1** and **2**[12] using the methodology described by Thierry et al.[13] (Scheme 1).

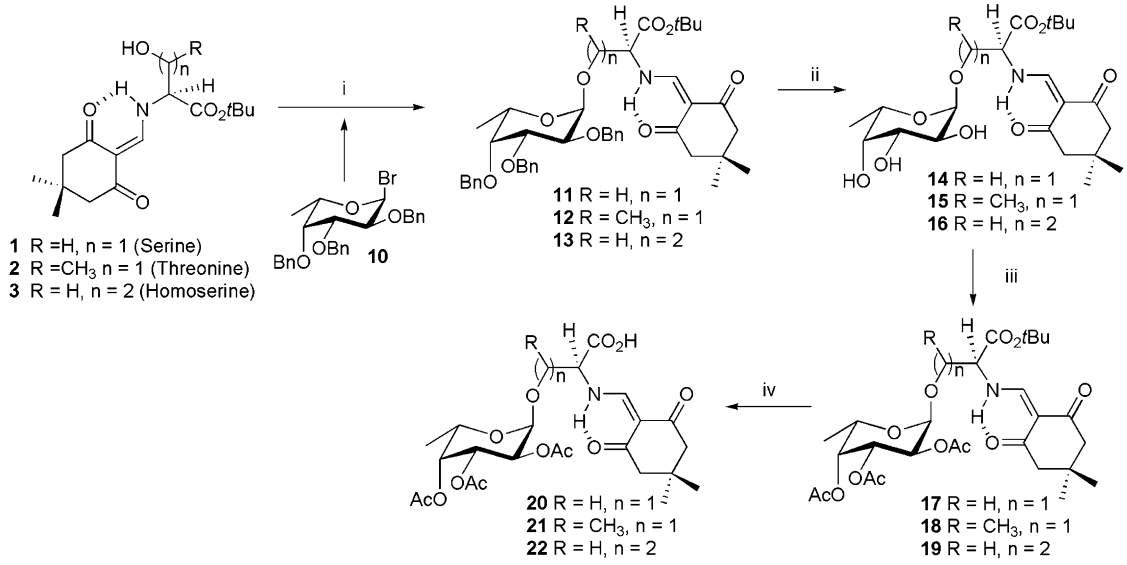
In addition, an efficient synthesis of N^{α}-Dmc-homoserine *tert*-butyl ester **3**[14] was accomplished in three steps from the commercially available Z-Asp-O^{t}Bu **8** (Scheme



Scheme 1. Reagents and conditions: (i) EtOH, (**6**; 83%, **7**; 86%); (ii) *tert*-butyl trichloroacetimidate, EtOAc, (**1**; 42%, **2**; 47%).



Scheme 2. Reagents and conditions: (i) *i*-BuOCOCl/Et_3N, THF; then $NaBH_4/H_2O$, 81%; (ii) H_2/Pd–C, EtOH; (iii) DMMD, EtOH, rt, 87%.



Scheme 3. Reagents and conditions (i) Et_4NBr, DCM, 4 Å MS (**11**; 63%, **12**; 73%, **13**; 40%); (ii) H_2, Pd/C, EtOH (**14**; 83%, **15**; 85%, **16**; 71%); (iii) Ac_2O, Py (**17**; 76%, **18**; 78%, **19**; 61%); (iv) TFA:DCM (1:1) 1.5 h (**20**; 92%, **21**; 91%, **22**; 92%).

2).[15] The β-carboxylic acid in **8** was first activated with isobutyl chloroformate and then reduced with $NaBH_4$ to yield Z-protected homoserine **9**. Replacement of Z with Dmc was achieved in one pot via hydrogenolysis in EtOH followed by derivatisation with DMMD to obtain **3** in high yield.

With the Dmc protected amino acid aglycones in hand, glycosylation employing 2,3,4-tri-*O*-benzylfucopyranosyl bromide **10**[16] as the donor, alongside tetrabutylammonium bromide promoted in situ anomerisation, afforded the desired α-glycosides **11–13** in extremely rewarding yields (Scheme 3). Compared to the equivalent procedures for Fmoc or Z-protected amino acids, this high yielding glycosylation may be rationalised in a manner akin to that described for glycosylation of O'Donnell Schiff bases of serine and threonine.[17] It could be reasonably argued that the nucleophilicity of the aglycones **1–3** may be enhanced due to the absence of unfavourable intramolecular hydrogen bonding between the α-NH and side chain-OH groups commonly observed with acyl or carbamoyl protected equivalents. In both cases, confirmation of the desired α-linkage was provided by the observed small coupling constants (δ, $J_{H\text{-}1',H\text{-}2'}$ = 3.3 Hz) in the ^{1}H NMR spectra. Catalytic hydrogenolysis of the glycosides gave the hydroxy sugars **14–16** which were subsequently acetylated to afford the fully protected adducts **17–19**. Final acidolytic cleavage of the *tert*-butyl ester relinquished the desired building blocks **20–22**[18] suitable for SPPS.

In conclusion we have described the synthesis of N^{α}-Dmc protected serine, threonine and homoserine 1-*O*-α-L-fucoside linked building blocks suitable for Fmoc/^{t}Bu SPPS. The stability of Dmc to both hydrogenolysis and acidolysis has been established and the employment of these building blocks in the construction of fucopeptide libraries is now ongoing and will be reported in due course.

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11. Bycroft, B. W.; Chan, W. C.; Chhabra, S. R.; Hone, N. D. *J. Chem. Soc., Chem. Commun.* **1993**, 778–779.
12. (a) N^{α}-Dmc-L-serine *tert*-butyl ester **1**: mp 135–136 °C; *m/z* (+ES) 312 (M+1, 100%); ν_{max} (KBr)/cm^{-1}: 3426 (O–H, N–H), 1735 (ester C=O), 1667 (amide C=O), 1606 (C=C); $[\alpha]_D^{25}$ +41.4 (*c* 0.1, MeOH); ^{1}H NMR δ ($CDCl_3$): 0.85 (6H, s, Dmc $C(CH_3)_2$), 1.34 (9H, s, $C(CH_3)_3$), 2.11, 2.13 (4H, 2 × s, Dmc 2 × CH_2), 3.87 (2H, m, β-CH_2), 4.06 (1H, m, α-CH), 4.96 (1H, t, *J* 5.9, OH), 7.98 (1H, d, *J* 14.3, Dmc C=CH), 11.19 (1H, dd, *J* 14.1, 8.2, NH); ^{13}C NMR δ ($CDCl_3$): 28.0 ($C(C_3)_3$), 28.2, 28.8 (Dmc $C(CH_3)_2$), 31.1 ($C(CH_3)_2$), 51.0, 51.3 (Dmc 2 × CH_2), 63.4 (β-CH_2), 64.8 (α-CH), 83.9 ($C(CH_3)_3$), 108.9 (Dmc C=CH), 158.0 (Dmc C=CH), 167.1 (CO *tert*-butyl ester), 196.6, 199.3 (CO dione); Found: C, 61.12; H, 8.09; N, 4.30. Calcd for $C_{16}H_{25}NO_5{\cdot}0.2H_2O$: C, 61.03; H, 8.14; N, 4.45; (b) N^{α}-Dmc-L-threonine *tert*-butyl ester **2**: mp 137–138 °C; *m/z* (+ES) 326 (M+1, 100%); ν_{max} (KBr)/cm^{-1}: 3423 (O–H, N–H), 1736 (ester C=O), 1667 (amide C=O), 1605 (C=C); $[\alpha]_D^{25}$ +21.8 (*c* 1.0, MeOH); ^{1}H NMR δ ($CDCl_3$): 1.04 (6H, s, Dmc $C(CH_3)_2$), 1.28 (3H, d, *J* 6.4, CH_3 Thr), 1.50 (9H, s, $C(CH_3)_3$), 2.32, 2.35 (4H, 2 × s, Dmc 2 × CH_2), 3.85 (1H, dd, *J* 4.5, 9.3, α-CH), 4.28 (1H, m, β-CH), 8.01 (1H, d, *J* 14.1, Dmc C=CH), 11.29 (1H, dd, *J* 9.8, 13.8, NH); ^{13}C NMR δ ($CDCl_3$): 19.9 (CH_3 Thr), 27.9 ($C(CH_3)_3$), 28.3, 28.7 (Dmc $C(CH_3)_2$), 31.1 ($C(CH_3)_2$), 51.0, 51.3 (Dmc 2 × CH_2), 67.9 (β-CH), 69.2 (α-CH), 83.6 ($C(CH_3)_3$), 108.0 (Dmc C=CH), 158.6 (Dmc C=CH), 167.7 (CO *tert* butyl ester), 196.6, 199.2 (CO dione); Found: C, 62.35; H, 8.36; N, 4.26. Calcd for $C_{17}H_{27}NO_5$: C, 62.75; H, 8.36; N, 4.30.
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14. N^{α}-Dmc-L-homoserine *tert*-butyl ester **3**: mp 150–152 °C ; R_f (acetone/pet.ether bp 40–60 °C, 1:1) 0.41; *m/z* (+ES) 326.2 (M+H); ν_{max} (KBr)/cm^{-1} 3331 (OH), 1718 (ester C=O); $[\alpha]_D^{25}$ + 3.02 (*c* 0.03, MeOH); ^{1}H NMR δ ($CDCl_3$): 1.05 (6H, s, Dmc $C(CH_3)_2$), 1.49 (9H, s, $C(CH_3)_3$), 1.97 (1H, ddd, *J* 14.0, 9.3, 4.5, β-CHa), 2.22 (1H, ddd, *J* 14.4, 9.7, 4.9, β-CH), 2.34, 2.38 (each 2H, s, Dmc 2 × CH_2), 3.19 (1H, m, CH_2OH), 3.68 (1H, m, γ-CHa), 3.79 (1H, m, γ-CHb), 4.29 (1H, m, *J* 8.9, 4.4, α-CH), 8.15 (1H, d, *J* 14.0, Dmc C=CH), 11.34 (1H, dd, *J* 12.4, NH). δ_C (62.9 MHz) 27.94 (*h*Ser-$C(CH_3)_3$), 28.50, 28.61 (Dmc $(CH_3)_2$), 31.12 (Dmc $C(CH_3)_2$), 35.33 (*h*Ser β-CH_2), 51.06, 51.42 (Dmc 2 × CH_2), 57.52 (*h*Ser γ-CH_2), 60.11 (*h*Ser α-CH), 83.36 (*h*Ser $C(CH_3)_3$), 107.94 (Dmc C=CH), 157.99 (Dmc C=CH), 167.30 (CO *tert* butyl ester), 196.80, 199.18 (CO dione); Found: C, 62.46; H, 8.40; N, 4.34; $C_{17}H_{27}NO_5$ requires C, 62.75; H, 8.36; N, 4.30.
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18. (a) N^{α}-Dmc-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-L-serine **20**: mp 121–122 °C; ν_{max} (KBr)/cm^{-1}: 1745 (ester C=O), 1668 (amide C=O), 1601 (C=C); δ_H (250 MHz, $CDCl_3$): 1.05 (6H, s, Dmc $(CH_3)_2$), 1.11 (3H, d, *J* 6.3, Fuc

CH_3), 1.97, 2.11, 2.15 (9H, 3 × s, 3 × $COCH_3$), 2.37 (4H, s, Dmc 2 × CH_2), 3.77 (1H, m, Ser β-CHa), 4.08 (1H, q, *J* 6.3, Fuc H5), 4.25 (1H, dd, *J* 10.0, 4.0, Ser β-CHb), 4.42 (1H, m, Ser α-CH), 5.09–5.25 (4H, m, Fuc H1, H2, H3 and H4), 8.23 (1H, d, *J* 14.0, Dmc C═CH), 11.45 (1H, dd, *J* 14.0, 8.0, Dmc NH); δ_C (62.9 MHz, $CDCl_3$): 16.3 (Fuc CH_3), 20.20, 21.00, 21.20 (3 × acetyl CH_3), 28.3, 28.6 (Dmc C(CH_3)$_2$), 31.07 (Dmc-*C* (CH_3)$_2$), 50.8, 51.2 (Dmc 2 × CH_2), 62.44 (Fuc C5), 64.8 (Ser α-CH), 67.0 (Ser β-CH_2), 68.0 (Fuc C2), 68.2 (Fuc C4), 71.1 (Fuc C3), 96.2 (Fuc C1), 108.00 (Dmc *C*═CH), 158.9 (Dmc C═*C*H), 170.0,170.5, 171.4 (3 × acetyl C═O), 171.9 (COOH), 195.6, 198.8 (Dmc dione); Found: C, 54.60; H, 6.52; N, 2.43; Calcd for $C_{24}H_{33}NO_{12}$: C, 54.64; H, 6.31; N, 2.66; (b) N^{α}-Dmc-O-(2,3,4-tri-O-acetyl-α- L-fucopyranosyl)- L-threonine **21**: mp 129–130 °C; ν_{max} (KBr)/cm^{-1}: 1745 (ester C═O), 1668 (amide C═O), 1601 (C═C); δ_H (250 MHz, $CDCl_3$): 1.06 (6H, s, Dmc (CH_3)$_2$), 1.08 (3H, d, *J* 6.3, Fuc CH_3), 1.19 (3H, d, *J* 6.1, Thr CH_3), 1.98, 2.12, 2.14 (9H, 3 × s, 3 × $COCH_3$), 2.39 (4H, s, Dmc 2 × CH_2), 4.14 (2H, m, Thr α-CH and Fuc H5), 4.49 (1H, m, Thr β-CH), 5.05 (1H, dd, *J* 10.5, 3.5, Fuc H3), 5.24 (3H, m, Fuc H1, H2, and H4), 8.19 (1H, d, *J* 14.3, Dmc C═CH), 11.33 (1H, dd, *J* 12.4, 8.8, Dmc NH); δ_C (62.9 MHz, $CDCl_3$): 15.3 (Fuc CH_3), 15.8 (Thr CH_3), 20.30, 20.60, 20.8 (3 × acetyl CH_3), 28.2, 28.4 (Dmc C(*C*H_3)$_2$), 31.2 (Dmc *C*(CH_3)$_2$), 51.3, 51.7 (Dmc 2 × *C*H_2), 65.2 (Fuc C5), 65.9 (Thr α-CH), 68.0 (Fuc C2), 68.4 (Fuc C4), 69.8 (Thr β-C), 70.8 (Fuc C3), 93.2 (Fuc C1), 108.3 (Dmc *C*═CH), 158.9 (Dmc C═*C* H), 169.7, 170.1, 170.7 (3 × acetyl C═O), 170.9 (COOH), 196.3, 199.6 (Dmc dione); Found: C, 54.45; H, 6.59; N, 2.39; Calcd for $C_{25}H_{35}NO_{12}$: C, 54.45; H, 6.51; N, 2.59; (c) N^{α}-Dmc-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-L-homoserine **22**: Oil, ν_{max} (KBr)/cm^{-1}: 1744 (ester C═O), 1662 (amide C═O), 1601 (C═C); δ_H (400 MHz, $CDCl_3$): 1.07 (6H, s, Dmc (CH_3)$_2$), 1.12 (3H, d, *J* 6.5, Fuc CH_3), 2.00, 2.08, 2.18 (9H, 3 × s, 3 × $COCH_3$), 2.26 (1H, m, *h*Ser β-CHa), 2.42 (4H, s, Dmc 2 × CH_2), 2.46 (1H, m, *h*Ser β-CHb), 3.55 (1H, m, *h*Ser γ-CHa), 3.86 (1H, m, *h*Ser γ-CHb), 4.10 (1H, q, *J* 6.8, Fuc H5), 4.39 (1H, m, *h*Ser α-CH), 5.05 (1H, d, *J* 3.5, Fuc H1), 5.13 (1H, dd, *J* 10.8, 3.6, Fuc H3), 5.27 (1H, d, *J* 2.8, Fuc H4), 5.35 (1H, dd, *J* 10.8, 3.8, Fuc H2), 8.26 (1H, d, *J* 14.2, Dmc C═CH), 11.46 (1H, dd, *J* 13.8, 8.5, Dmc NH); δ_C (100.6 MHz, $CDCl_3$): 15.82 (Fuc CH_3), 20.68, 20.72, 20.81 (3 × acetyl CH_3), 28.39, 28.52 (Dmc C(*C*H_3)$_2$), 31.33 (Dmc *C* (CH_3)$_2$), 32.18 (*h*Ser β-CH), 51.25, 50.90 (Dmc 2 × *C*H_2), 59.21 (*h*Ser α-CH), 62.58 (*h*Ser γ-CH_2), 65.14 (Fuc C4), 68.01 (Fuc C3), 68.10 (Fuc C2), 71.17 (Fuc C5), 96.34 (Fuc C1), 107.81 (Dmc *C*═CH), 158.80 (Dmc C═*C*H), 170.72, 170.91, 170.97 (3 × acetyl C═O), 171.64 (COOH), 198.43, 200.01 (Dmc dione); *m/z* HRMS (TOFES-) 540.2056 (M−H). Calcd for $C_{25}H_{34}NO_{12}$ (M−H) 540.2080.