Indian Journal of Chemistry
Vol. 48B, March 2009, pp. 397-407

Synthesis of urea tethered glycosylated amino acids and glycopeptides mediated by DPPA employing N^{α}-Fmoc-Asp/Glu-5-oxazolidinones

G Nagendra, H P Hemantha & Vommina V Sureshbabu*

Peptide Research Laboratory, Department of Studies in Chemistry, Central College Campus, Bangalore University, Dr B. R. Ambedkar Veedhi, Bangalore 560 001, India

E-mail: hariccb@rediffmail.com

Received 24 April 2008; accepted (revised) 3 December 2008

The utility of diphenyl phosphoryl azide (DPPA) as azido transfer reagent for the insertion of urea moiety between β/γ carboxyl group of N^{α}-Fmoc-Asp/Glu-5-oxazolidinones and glycosyl amine has been demonstrated. Utility of this protocol for the synthesis of urea-linked neoglycopeptides has also been explored. The compounds are characterised by ^{1}H NMR, ^{13}C NMR and mass spectroscopy.

Keywords: DPPA, oxazolidinones, glycosylated urea, glycopeptidomimetics

Glycopeptides are a rapidly growing family of biologically important molecules. They contain covalently bound peptide and carbohydrate segments. Attachment of the carbohydrate often modifies the structure and function of the peptide/protein. Carbohydrates, which are exposed on the surface of protein, serve as domains for cell-molecule and cell-cell attachment. Interactions between the membrane bound glycoconjugate glycans and the carbohydrate binding proteins are important in mediating cellular processes such as cell growth regulation, cell-cell recognition, adhesion, cancer cell metastasis and parasitical infections[1,2].

Recent years have witnessed tremendous development in glycopeptide synthesis as these molecules are not easily available through gene technology since they are post-translational products resulting from the activity of glycosyl hydrolases and transferases[3-5]. Consequently, the availability of glycopeptide samples for several studies on structural[3, 6-8] as well as function aspectes[9,10] is largely met with chemical synthesis[11-15]. In glycoproteins, carbohydrates are attached through the oxygen in the side chain of serine/threonine in *O*-linked glycolproteins or thorough the carboxamide nitrogen of aspargine in case of *N*-linked glycoproteins[15,16]. Synthetic glycopeptides that contain a non-native linkage between the saccharide and peptide moieties are called as neoglycopeptides[17]. These glycopeptidomimetics are potentially useful because they emulate the function of natural compounds and possess enhanced *in vivo* stability and bioavailability.

There is a growing interest in the changes in the glycopeptide properties brought about by the introduction of non-native linkages like retro amide subunits[18], carbamate and ureido bonds[19]. It has been shown that the glycopeptidyl ureas are more water soluble than the natural glycopeptides and hence they acquire wide utility in various biological functions. Urea moiety acts as a structural element in enzyme inhibitors and in developing potent analogues of a peptide based drugs.

Reported procedures for the synthesis of the urea tethered glycosylated amino acids/peptides employ the use of sugar isocyanates obtained from sugar-1-acid or sugar-1-amine as building blocks. Ichikawa *et al.*[20], have used a multi-step protocol to prepare sugar-1-isocyanate starting from sugar amine and have coupled the former with various α,β-diamino acid derivatives. The disadvantages in this method include a) too many synthetic steps and b) difficult separation of anomeric isomers of sugar isocyanates. Alternatively, Ikegami *et al.*[21], reported an one pot conversion of sugar-1-acid into the corresponding isocyanate employing diphenyl phosphoryl azide (DPPA) and have coupled the isocyanates with amino acid ester to obtain the desired glycpeptidyl ureas. Again, the preparation of sugar-1-acid is a multi-step route by itself. Therefore, an alternate route of generating the isocyanate from the side chain carboxyl group of protected aspartic acid/glutamic acid is envisaged and coupled it with a sugar amine. This circumvents the cumbersome preparation of

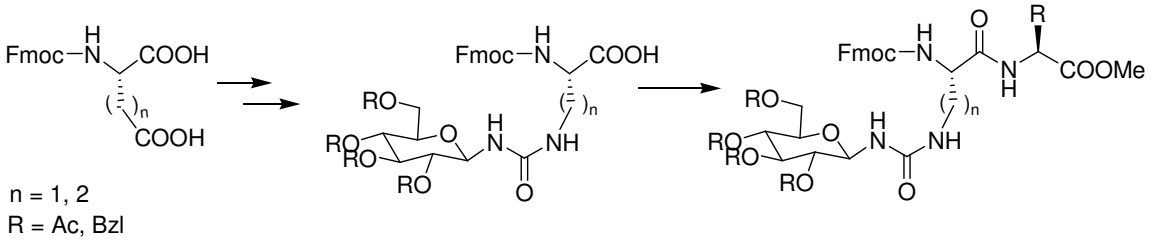
sugar isocyanate and allows for easily accessible carbohydrate and amino acid derivatives as the components of coupling reaction. Further, 5-oxazolidinones derived from Fmoc-Asp/Glu[22] was chosen as the bidentately and internally protected intermediates (with α-COOH protected) for the selective conversion of side chain -COOH group into isocyanates because these oxazolidinones can be prepared in a single high yielding step, are stable and do not cyclize into internal ureas upon conversion of the ω-COOH into isocyanate.

In organic synthetic reactions, diphenyl phosphoryl azide (DPPA) is used as an efficient azido transfer reagent for the preparation of peptidyl ureas. Recently DPPA mediated single-pot synthesis of α-peptidyl ureas *via* the modified Curtius rearrangement[23] is reported. In this report an useful application of DPPA for the synthesis of the urea linked glycosylated amino acids is reported. The use of DPPA is advantageous over earlier reports as it does not require preactivation of the carboxy group before reaction with azide donors and also avoids a sequence of multi-step syntheses *viz*., the formation of acid azide, its rearrangement into isocyante and coupling with a nucelophile can be operated in a single pot. The overall strategy for the reaction is given below (**Scheme I**).

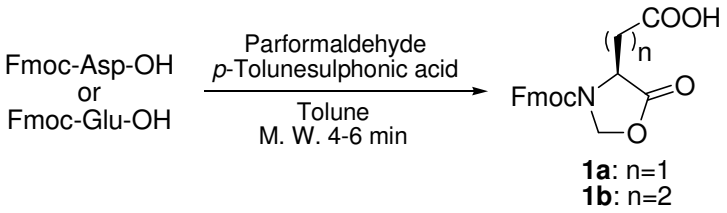
Results and Discussion

N^{α}-Fmoc protected Asp/Glu was converted to N^{α}-Fmoc-Asp/Glu-5-oxazolidinone (**Scheme II**) by subjecting the mixture of N^{α}-Fmoc-Asp/Glu with paraformaldehyde and catalytic amount of *p*-toluene sulfonic acid in toluene to microwave irradiation for 4-6 min. The desired 5-oxazolidinones were obtained in 95% yield after purified through column chromatography (silica gel 100-200 mesh using chloroform:methanol v/v).

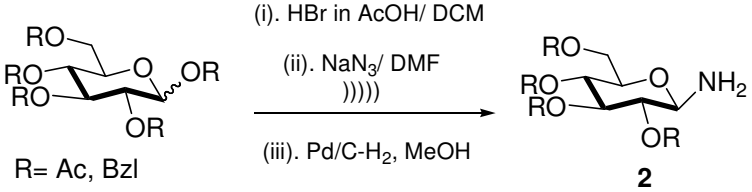
The required *o*-protected glycosyl amine (**Scheme III**) component was prepared by the known literature procedures. The hydroxyl groups were protected as their acetates or benzoates. The α-anomeric acetate or benzoate was converted to the bromo intermediate using HBr/AcOH in dry CH_2Cl_2 at RT which was then treated with NaN_3 in DMF under sonication in 30 min to obtain the 1-α-azido tetraacetyl/benzoyl sugar in quantitative yield. Catalytic hydrogenation of the azide using Pd-C/H_2 in MeOH yielded 1-β-amino-



Scheme I



Scheme II — Synthesis of N^{α}-Fmoc-Asp/Glu-5-oxazolidinone: i. Paraformaldehyde, PTSA, Toluene, MW (4-6 min)

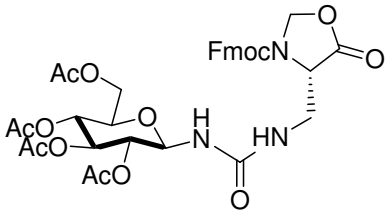
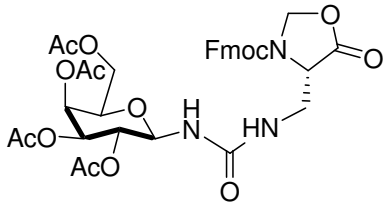
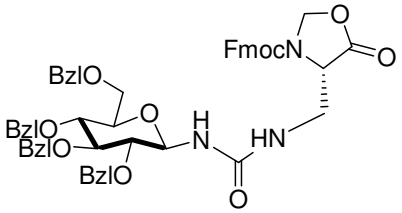
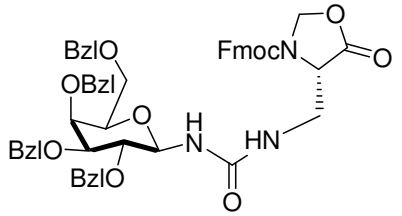
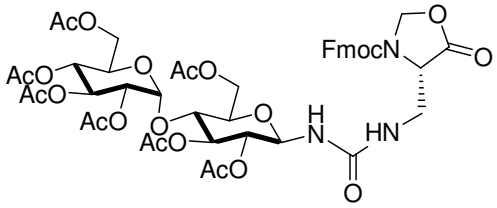


Scheme III — Synthesis of Glycosyl amine component

tetra acetyl/benzoyl sugar which was used directly without purification.

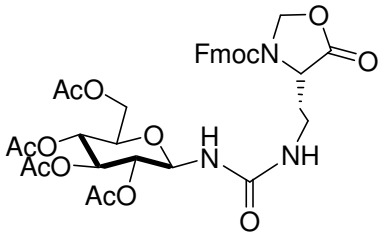
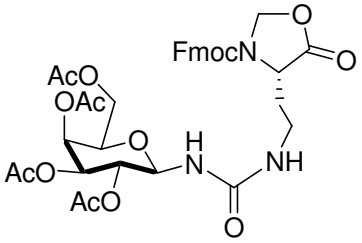
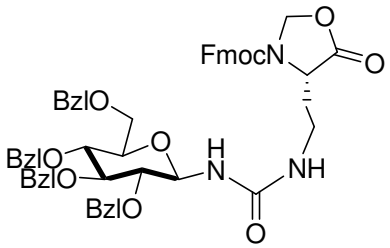
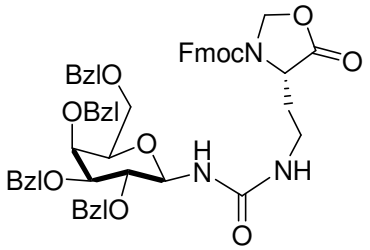
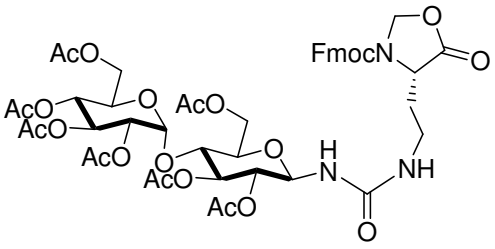
N^{α}- Fmoc-Asp/Glu-5-oxazolidinone **1**, diisopropyl ethylamine and DPPA were stirred in THF at 0º C and the reaction was monitored through TLC as well as IR. Upon complete formation of the acid azide that takes about 20 min, glycosyl amine **2** in THF was added and the reaction-mixture was subjected to sonication for 30 min. During the reaction, DPPA acts as carbonyl activating and azido transfer reagent, which brings about conversion of the acid to the acid azide. Rearrangement of the latter into the isocyanate with concomitant coupling of the glycosyl amine produces the ureido linked glycosyl oxazolidinone. The glycosylated oxazolidinones **3** were isolated in >80% yield and were characterized through mass and NMR spectroscopy. The reaction was also performed with the amine derived from *o*-protected disaccharide, and the corresponding ureido oxazolidinone was obtained in >70% yield (**Table I**). The cleavage of the oxazolidinone ring to regenerate the 1-acid group was demonstrated with all the 10 examples. Treatment of glycosylated oxazolidinone with 1*N* LiOH in THF for 20 min gave the desired glycosylated amino acid in

Table I — Synthesis of urea linked glycosylated oxazolidinones

Entry	Product	m.p. (°C)	Yield (%)	Mass [M+Na] (Calcd.)
3a		118-20	78	734.6944[a] (734.6692)
3b		148-50	87	734.2[b] (734.66)
3c		176-78	92	982.4[b] (982.27)
3d		140-42	90	982.8[b] (982.27)
3e		134-36	89	1034.9[b] (1034.33)

--Contd

Table I — Synthesis of urea linked glycosylated oxazolidinones--*Contd*

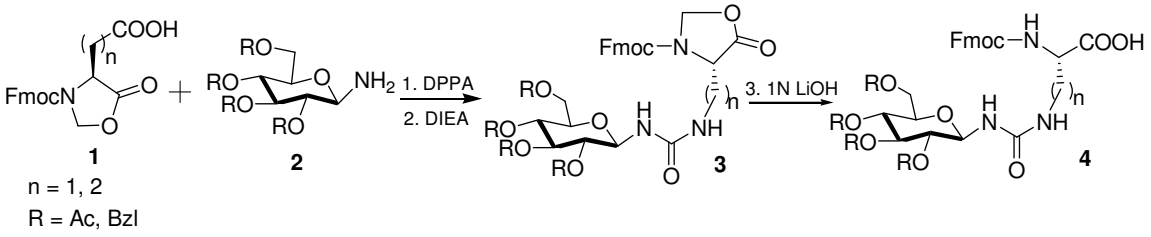
Entry	Product	m.p. (°C)	Yield (%)	Mass [M+Na] (Calcd.)
3f		110-12	85	748.6984[a] (748.6958)
3g		130-32	88	748.6904[a] (748.6958)
3h		112-14	85	996.4[b] (996.96)
3i		166-68	85	996.1[b] (996.96)
3j		84-86	78	1048.4[b] (1048.35)

[a] HRMS, [b] ES-MS

good yield (**Scheme IV**, **Table II**). The resulting glycosylated amino acids were coupled to amino acid esters employing DCC-HOBt as coupling agent. All the glycopeptides could be obtained in quantitative yields (**Scheme V**, **Table III**).

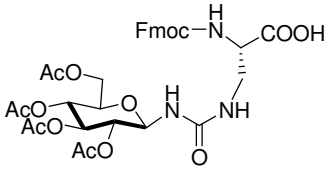
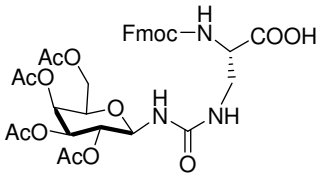
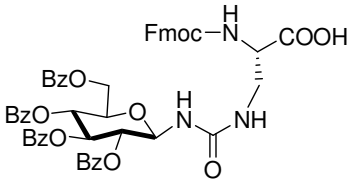
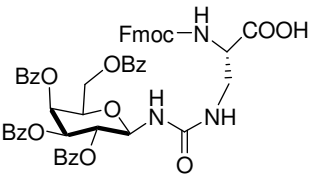
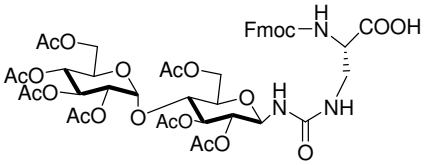
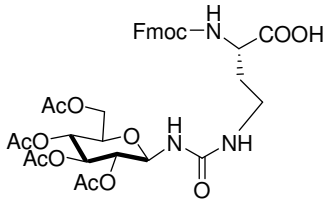
Conclusion

The synthesis of urea linked glycosylated amino acids mediated by DPPA has several advantages over the Curtius or Schmidt protocols. In this protocol, the handling and work-up procedure of the hazardous



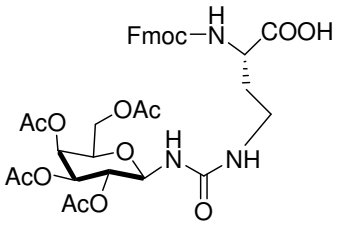
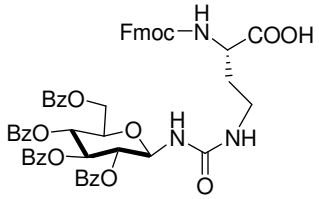
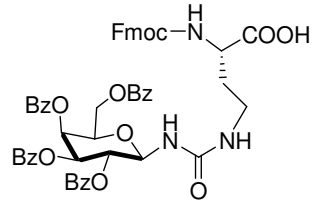
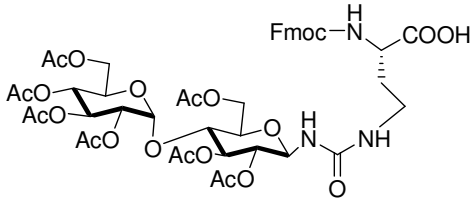
Scheme IV — Synthesis of ureido linked glycosylated amino acids: 1. DPPA (1.0 mmole), DIEA (1.0 mmole), THF, 2. 1*N* LiOH (1 equiv), THF

Table II — Synthesis of urea linked glycosylated amino acid

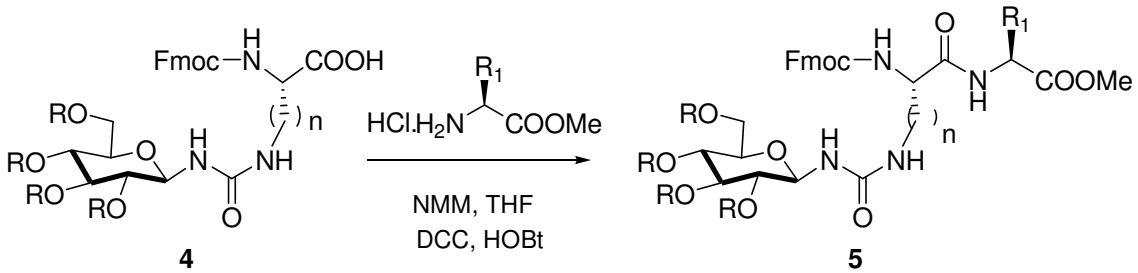
Entry	Product	m.p. (°C)	Yield (%)	Mass [M+Na] (Calcd.)
4a		123	82	722.2173[a] (722.2120)
4b		115	83	722.2173[a] (722.2132)
4c		127	86	914.4[b] (914.1)
4d		113	90	914.4[b] (914.2)
4e		105	71	1175.3[b] (1175.8)
4f		99	79	736.2336[a] (736.2308)

--Contd

Table II — Synthesis of urea linked glycosylated amino acid

Entry	Product	m.p. (°C)	Yield (%)	Mass [M+Na] (Calcd.)
4g		135	84	736.2336[a] (713.2310)
4h		102	87	928.4[b] (928.1)
4i		129	89	928.4[b] (928.2)
4j		107	72	1189.3[b] (1189.1)

[a] HRMS, [b] ES-MS



5a: n = 1 R_1= $CH(CH_3)_2$; 5b: n = 1, R_1 = CH_3; 5c: n = 1, R_1 = CH_3; 5d: n = 2, R_1= $CH(CH_3)_2$; 5e: n = 2 R_1= CH_3; 5f: n = 2, R_1 = CH_3: R = Ac or Bz.

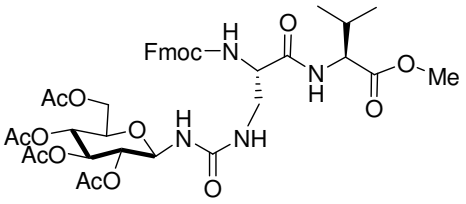
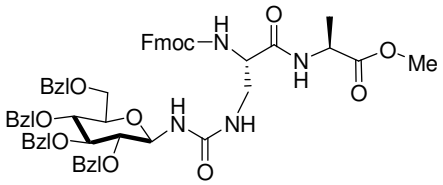
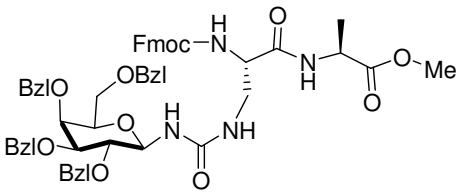
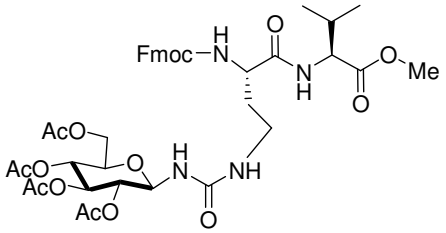
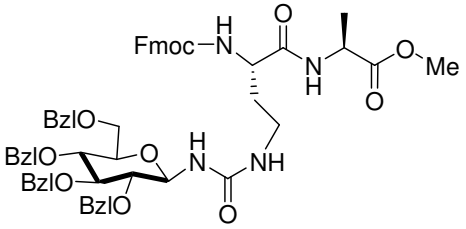
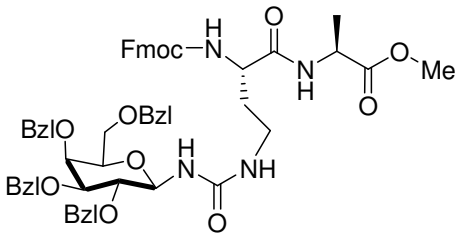
Scheme V — Synthesis of neoglycopeptides using ureido linked glycosylated amino acids: (i) reagent 4, DCC, HOBt, amino acid ester, *N*-methylmorpholine, DCM

azide and isocyanate is circumvented. All the products were obtained as pure crystalline solids which were characterised by IR, ^{1}H NMR, ^{13}C NMR and mass spectrometric procedures.

Experimental Section

All solvents were freshly distilled before use. Amino acids were used as received from Sigma-Aldrich Company, USA. Melting points were determined by open capillary method and are uncorrected. IR spectra were recorded on a Nicolet model impact 400D FT-IR spectrometer (KBr pellets, 3 cm^{-1} resolution). ^{1}H NMR spectra were recorded on a Bruker AMX 400 MHz spectrometer. Mass spectra were recorded on MALDI-TOF (KRATOS) mass

Table III — Synthesis of glycopeptides

Entry	Product	m.p. (°C)	Yield (%)	Mass[M+Na] (Calcd.)
5a		126-28	82	835.7024[a] (835.8162)
5b		126-28	82	1055.5[b] (1055.3)
5c		122-24	82	1055.8[b] (1055.3)
5d		152-54	85	849.4[b] (849.84)
5e		110-12	85	1069.7[b] (1069.3)
5f		164-66	85	1069.3[b] (1069.3)

[a] HRMS, [b] ES-MS

spectrometer. Unless or otherwise mentioned, all amino acids used, have L-configuration. TLC analysis was carried out using the precoated silica gel GF_{254} plates.

General procedure for the synthesis of N^{α}-Fmoc-Asp/Glu-oxazolidinones, 1

N^{α}-Fmoc-Asp/Glu-oxazolidinones were prepared by exposing the slurry of the reactants *p*-toluene

sulphonic acid (100 mg), paraformaldehyde (2 g) and N^{α}-Fmoc-Asp/Glu-OH (10 mmole) in toluene (20 mL) to microwave irradiation in an unmodified domestic microwave oven operated at 2450 MHz frequency at 80% power. 5-Oxazolidinone formation, as monitored by TLC (chloroform:methanol:acetic acid, 40:2:1 v/v), was found to be complete in 3 min.

General procedure for the synthesis of urea linked glycosylated Fmoc-Asp/Glu-oxazolidinone derivatives, 3

To a solution of N^{α}-Fmoc-Asp/Glu-oxazolidinone (10 mmole) in dry THF (20 mL), DPPA (10 mmole) and DIEA (10 mmole) were added consecutively at 0ºC and the reaction-mixture was stirred at the same temperature for 20 min. To this was added glycosyl amine (1.1 mmole) in THF and the reaction-mixture was subjected to sonication for 30 min/ till the completion as monitored by TLC. The precipitated crude ureido analogues were isolated by filtration and recrystallization using DMSO:water (8:2 v/v).

General procedure for the synthesis of ureido-linked amino acids, 4

1*N* Solution of LiOH (1 mmole) was added in one portion to a solution of side chain ureido linked glycopeptidyl N^{α}-Fmoc-Asp/Glu-oxazolidinone (1 mmole) in THF (10 mL) and stirred for an hr. The resulting solution was acidified with 6% HCl (10 mL) and extracted with EtOAc (2 × 10 mL). The combined organic extract was washed with brine and dried over anhyd. sodium sulphate. The solvent was removed *in vacuo* to leave the crude product as a colourless solid which was column purified ($CHCl_3$:MeOH:AcOH; 40:2:1 v/v).

General procedure for the synthesis of ureido linked glycopeptides, 5

To an ice-cold solution of ureido linked glycosylated amino acid (10 mmole) in THF (10 mL), NMM (11 mmole), amino free amino acid ester (11 mmole), HOBt (12 mmole) were added successively followed by DCC (12 mmole) and the reaction-mixture was allowed to stir at RT for 5-6 hr until completion (as moniterd by TLC). The DCU was filtered and the solvent was removed in *vacuo*. The reaction-mixture was taken into ethyl acetate. The organic layer was washed with 10% HCl (10 mL × 2), 5% Na_2CO_3 (10 mL × 2), brine solution (10 mL × 1) and dried over anhyd. sodium sulphate. Finally the solvent was removed under reduced pressure to afford the crude product, which was purified using column chromatography (EtOAc: hexane).

Fmoc-Asp-β-[NH-CO-NH-(2,3,4,6-tetra-O-acetyl-*β*-D-glucopyranosyl)]-oxazolidinone, 3a: IR (thin film): 1245, 1700, 1800 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.01-2.16 (4 s, 12 H), 2.71 (br s, 1 H), 3.3 (br s, 1 H), 4.12 (m, 1H), 4. 19 (t, J = 5.7 Hz, 1H), 4.48 (q, 1H), 4.61 (q, 1H), 5.01 (d, J = 6.7, 1H), 5.1 (d, J = 6.7 Hz , 2H), 5.51 (t, J = 5.9 Hz, 1H), 5.71 (t, J = 5.9 Hz, 1H), 5.93 (t, J = 5.1 Hz, 1H), 7.33-7.77 (m, 8H).

Fmoc-Asp-β-[NH-CO-NH-(2,3,4,6-tetra-O-acetyl-*β*-D-galactopyranosyl)]-oxazolidinone, 3b: IR (thin film): 1212, 1700, 1735, 1801 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.01-2.16 (4 s, 12 H), 2.71 (br s, 1H), 3.3 (br s, 1H), 4.12 (m, 1H), 4.19 (t, J = 6.2 Hz, 1H), 4.48(q, 1H), 4.61 (q, 1H), 5.01 (d, J = 5.6 Hz, 1H), 5.1 (d, J = 5.9 Hz, 2H), 5.51 (t, J = 5.9 Hz, 1H), 5.71 (t, J = 5.5 Hz, 1H), 5.93 (t, J = 5.8 Hz, 1H), 7.33-7.77 (m, 8H).

Fmoc-Asp-β-[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl-*β*-D-glucopyranosyl)]-oxazolidinone, 3c: IR (thin film): 1214, 1711, 1738, 1800 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.7 (br s, 1H), 3.29 (br s, 1H), 3.45 (m, 1H), 4.12 (m, 1H), 4.12 (m, 1H), 4.2 (t, J = 5.8 Hz, 1H), 4.4 (d, J = 5.9 Hz, 2H), 4.48 (dd, 1H), 4.61 (dd, 1H), 5.0 (d, J = 6.1 Hz, 1H), 5.1 (s, 2H), 5.51 (t, J = 6.2 Hz, 1H), 5.71 (t, J = 5.9 Hz, 1H), 5.93 (t, J = 5.9 Hz, 1H), 7.23-7.95 (m, 28H).

Fmoc-Asp-β-[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl-*β*-D-galactopyranosyl)]-oxazolidinone, 3d: IR (thin film): 1257, 1705, 1735, 1810 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.7 (br s, 1H), 3.29 (br s, 1H), 3.45 (m, 1H), 4.12 (m, 1H), 4.12 (m, 1H), 4.2 (t, J = 5.7 Hz, 1H), 4.4 (d, J = 5.9 Hz, 2H), 4.48 (dd, 1H), 4.61 (dd, 1H), 5.0 (d, J = 6.1 Hz, 1H), 5.1 (s, 2H), 5.51 (t, J = 6.4 Hz, 1H), 5.71 (t, J = 6.0 Hz, 1H), 5.93 (t, J = 6.2 Hz, 1H), 7.23-7.95 (m, 28H).

Fmoc-Asp-β-[NH-CO-NH-(2,2',3,3',4',6,6'-hepta-O-acetyl-*β*-D-maltosyl)]-oxazolidinone, 3e: IR (thin film): 1257, 1715, 1735, 1805 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.0-2.16 (7s, 21H), 2.72 (br s, 1H), 3.29 (br s, 1H), 3.88 (m, 1H), 3.94-4.05 (m, 3H), 4.19 (t, J = 5.5 Hz, 1H), 4.41 (d, J = 5.7 Hz, 2H), 4.23-4.26 (dd, 2H), 4.51 (q, 1H), 4.71 (d, J = 6.1 Hz, 1H), 4.78 (t, J = 5.9 Hz, 1H), 4.85 (q, 1H), 5.06 (t, J = 5.8 Hz, 1H), 5.12 (s, 2H), 5, 27 (t, J = 5.7 Hz, 1H), 5.41 (t, J = 5.9 Hz, 1H), 5.57 (d, J = 5.8 Hz, 1H), 7.33-7.77(m, 8H).

Fmoc-Glu-γ-[NH-CO-NH-(2,3,4,6-tetra-O-acetyl-*β*-D-glucopyranosyl)]-oxazolidinone, 3f: IR (thin

film): 1222, 1698, 1749, 1800 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.9-2.01 (br d, 2H), 2.95-3.12 (br d, 2H), 2.0-2.07 (4 s, 12H), 4.12 (m, 1H), 4.20 (t, *J* = 6.1 Hz, 1H), 4.43 (m, 2H), 4.45 (q, 1H), 4.62 (q, 1H), 4.99 (d, *J* = 6.3 Hz, 1H), 5.09 (s, 2H), 5.5 (t, *J* = 5.6 Hz, 1H), 5.71 (t, *J* = 5.9 Hz, 1H), 7.33-7.77 (m, 8H).

Fmoc-Glu-γ-[NH-CO-NH-(2,3,4,6-tetra-O-acetyl-*β*-D-galactopyranosyl)]-oxazolidinone, 3g: IR (thin film): 1222, 1698, 1749, 1800 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.9-2.01 (br d, 2H), 2.95-3.12 (br d, 2H), 2.0-2.07 (4 s, 12H), 4.12 (m, 1H), 4.20 (t, *J* = 5.9 Hz, 1H), 4.43 (m, 2H), 4.45 (q, 1H), 4.62 (q, 1H), 4.99 (d, *J* = 6.0 Hz, 1H), 5.09 (s, 2H), 5.5 (t, *J* = 5.8 Hz, 1H), 5.71 (t, *J* = 5.5 Hz, 1H), 7.33-7.77 (m, 8H).

Fmoc-Glu-γ-[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl-*β*-D-glucopyranosyl)]-OH, 3h: IR (thin film): 1222, 1702, 1749, 1800 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.61 (d, *J* = 5.7 Hz, 1H), 3.30 (d, *J* = 5.9 Hz, 1H), 4.13 (t, *J* = 5.8 Hz, 1H), 4.24 (d, *J* = 5.9 Hz, 2H), 4.28 (m, 1H), 4.48 (dd, 1H), 4.63 (dd, 1H), 4.66 (d, *J* = 5.5 Hz, 1H), 5.18 (d, *J* = 6.1 Hz, 2H), 5.5 (t, *J* = 6.1 Hz, 1H), 5.71 (t, *J* = 5.8 Hz, 1H), 5.92 (t, *J* = 5.8 Hz, 1H), 7.33-8.00 (m, 28H).

Fmoc-Glu-γ-[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl-*β*-D-galactopyranosyl)]-oxazolidinone, 3i: IR (thin film): 1222, 1698, 1749, 1800 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.91-2.01 (br d, 2H), 2.95-3.12 (br s, 2H), 4.12 (m, 1H), 4.20 (t, *J* = 5.5 Hz, 1H), 4.43 (d, *J* = 6.0 Hz, 2H), 4.48 (q, 1H), 4.62 (t, *J* = 5.8 Hz, 1H), 4.99 (d, *J* = 5.9 Hz, 1H), 5.09 (s, 2H), 5.50 (t, *J* = 5.4 Hz, 1H), 5.71 (t, *J* = 5.7 Hz, 1H), 5.93 (t, *J* = 6.1 Hz, 1H), 7.23-8.08 (m, 28H).

Fmoc-Glu-γ-[NH-CO-NH-(2,2',3,3',4',6,6'-hepta-O-acetyl-*β*-D-maltosyl)]-oxazolidinone, 3j: IR (thin film): 1251, 1700, 1739, 1802 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.00 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.05 (s, 3H), 2.10 (s, 1H), 2.16 (s, 1H), 2.61 (d, *J* = 5.8 Hz, 1H), 3.3 (d, 5.8, 1H), 3.8 (m, 1H), 3.39-4.06 (m, 3H), 4.13 (t, *J* = 5.6 Hz, 1H), 4.24 (m, 5H), 4.51 (dd, 1H), 4.71 (d, *J* = 5.6 Hz, 1H), 4.78 (t, *J* = 6.2 Hz, 1H), 4.85 (dd, 1H), 5.05 (t, *J* = 6.2 Hz, 1H), 5.16 (d, *J* = 5.9 Hz, 2H), 5.26 (t, *J* = 6.0 Hz, 1H), 5.41 (t, *J* = 5.5 Hz, 1H), 5.57 (d, *J* = 5.8 Hz, 1H), 7.33 (t, *J* = 6.3 Hz, 2H), 7.41 (t, *J* = 6.1 Hz, 2H), 7.52 (d, *J* = 5.8 Hz, 2H), 7.77 (d, *J* = 5.9 Hz, 2H).

Fmoc-Asp-β-[NH-CO-NH-(2,3,4,6-tetra-O-acetyl-*β*-D-glucopyranosyl)]-OH, 4a: IR (KBr): 1251, 1707, 1740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.99, 2.01, 2.03, 2.05 (4s, 12H), 2.10 (s, 1H), 2.16 (s, 1H), 2.61 (d, *J* = 2.9 Hz, 1H), 3.30 (d, *J* = 9.2 Hz, 1H), 3.80 (m, 1H), 3.39-4.06 (m, 3H), 4.13 (t, *J* = 7.1 Hz, 1H), 4.24 (m, 5H), 4.51 (dd, *J* = 6.9 Hz, 1H), 4.71 (d, *J* = 7.1 Hz, 1H), 4.78 (t, *J* = 9.0 Hz, 1H), 4.85 (dd, *J* = 6.9 Hz, 1H), 5.05 (t, *J* = 9.6 Hz, 1H), 5.26 (t, *J* = 9.8 Hz, 1H), 5.41 (t, *J* = 10.6 Hz, 1H), 5.57 (d, *J* = 6.2 Hz, 1H), 7.33 (t, *J* = 9.6 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.52 (d, *J* = 7.2 Hz, 2H), 7.77 (d, *J* = 4.7 Hz, 2H).

Fmoc-Asp-β-[NH-CO-NH-(2,3,4,6-tetra-O-acetyl-*β*-D-galactopyranosyl)]-OH, 4b: IR (KBr): 1250, 1707, 1740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.00 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.05 (s, 3H), 2.10 (s, 1H), 2.16 (s, 1H), 2.61 (d, *J* = 3 Hz, 1H), 3.30 (d, *J* = 9.0 Hz, 1H), 3.80 (m, 1H), 3.39-4.06 (m, 3H), 4.19 (t, *J* = 6.7 Hz, 1H), 4.24 (m, 5H), 4.51 (dd, *J* = 6.9 Hz, 1H), 4.71 (d, *J* = 7.1 Hz, 1H), 4.78 (t, *J* = 9.0 Hz, 1H), 4.85 (dd, *J* = 6.9 Hz, 1H), 5.05 (t, *J* = 9.6 Hz, 1H), 5.26 (t, *J* = 9.8 Hz, 1H), 5.41 (t, *J* = 10.6 Hz, 1H), 5.57 (d, *J* = 6.2 Hz, 1H), 7.33 (t, *J* = 9.6 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.52 (d, *J* = 7.2 Hz, 2H), 7.77 (d, *J* = 4.7 Hz, 2H).

Fmoc-Asp-β-[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl-*β*-D-glucopyranosyl)]-OH, 4c: IR (KBr): 1251, 1703, 1740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.00 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.05 (s, 3H), 2.10 (s, 1H), 2.16 (s, 1H), 2.61 (d, *J* = 3 Hz, 1H), 3.30 (d, *J* = 9.0 Hz, 1H), 3.80 (m, 1H), 3.39-4.06 (m, 3H), 4.20 (t, *J* = 7.2 Hz, 1H), 4.24-4.32 (m, 5H), 4.51 (dd, *J* = 6.9 Hz, 1H), 4.71 (d, *J* = 7.1 Hz, 1H), 4.78 (t, *J* = 9.0 Hz, 1H), 4.85 (dd, *J* = 7.1 Hz, 1H), 5.05 (t, *J* = 9.5 Hz, 1H), 5.26 (t, *J* = 9.8 Hz, 1H), 5.41 (t, *J* = 10.6 Hz, 1H), 5.57 (d, *J* = 9.6 Hz, 1H), 7. 33 (t, *J* = 6.20 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.52 (d, *J* = 7.2 Hz, 2H), 7.77 (d, *J* = 4.7 Hz, 2H).

Fmoc-Asp-β-[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl-*β*-D-galactopyranosyl)]-OH, 4d: IR (KBr): 1251, 1702, 1745 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.00 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.05 (s, 3H), 2.10 (s, 1H), 2.16 (s, 1H), 2.61 (d, *J* = 3 Hz, 1H), 3.30 (d, *J* = 9.0 Hz, 1H), 3.80 (m, 1H), 3.39-4.06 (m, 3H), 4.19 (t, *J* = 6.7 Hz, 1H), 4.34 (m, 5H), 4.51 (dd, *J* = 7.1 Hz, 1H), 4.71 (d, *J* = 7.1 Hz, 1H), 4.78 (t, *J* = 9.0 Hz, 1H), 4.85 (dd, *J* = 6.9 Hz, 1H), 5.05 (t, *J* = 9.5 Hz, 1H), 5.26 (t, *J* = 9.8 Hz, 1H), 5.41 (t, *J* = 10.6 Hz, 1H), 5.57 (d, *J* = 9.6 Hz, 1H), 7.33 (t, *J* = 6.2 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.52 (d, *J* = 7.2 Hz, 2H), 7.77 (d, *J* = 4.7 Hz, 2H).

Fmoc-Asp-β-[NH-CO-NH-(2,2',3,3',4',6,6'-hepta-O-acetyl-*β*-D-maltosyl)]-OH, 4e: IR (KBr): 1257, 1715, 1735 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.03-2.13 (s, 12H), 2.61 (d, *J* = 3 Hz, 1H), 3.30 (d, *J* = 9.0 Hz, 1H), 4.13 (t, *J* = 7.1 Hz, 1H), 4.24 (d, *J* = 7.0 Hz,

2H), 4.28 (m, 1H), 4.48 (dd, *J* = 7.1 Hz, 1H), 4.63 (dd, *J* = 6.9 Hz, 1H), 4.66 (d, *J* = 7.0 Hz, 1H), 5.50 (t, *J* = 9.6 Hz, 1H), 5.71 (t, *J* = 9 Hz, 1H), 5.92 (t, *J* = 7.1 Hz, 1H), 7.33 (t, *J* = 6.2 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.52 (d, *J* = 7.2 Hz, 2H), 7.77 (d, *J* = 4.7 Hz, 2H).

Fmoc-Glu-γ-[NH-CO-NH-(2,3,4,6-tetra-O-acetyl-*β*-D-glucopyranosyl)]-OH, 4f: IR (KBr): 1222, 1698, 1749 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.9-2.01 (br d, 2H), 2.95-3.12 (br d, 2H), 2.00-2.07 (4 s, 12H), 4.12 (m, 1H), 4.20 (t, *J* = 7.0 Hz, 1H), 4.43 (m, 2H), 4.45 (q, *J* = 7.1 Hz, 1H), 4.62 (q, *J* = 7.0 Hz, 1H), 4.99 (d, *J* = 7.1 Hz, 1H), 5.50 (t, *J* = 9.6 Hz, 1H), 5.71 (t, *J* = 9.0 Hz 1H), 5.93 (t, *J* = 7.1 Hz, 1H), 7.33-7.77 (m, 8H).

Fmoc-Glu-γ-[NH-CO-NH-(2,3,4,6-tetra-O-acetyl-*β*-D-galactopyranosyl)]-OH, 4g: IR (KBr): 1222, 1701, 1750 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.9-2.01 (br d, 2H), 2.95-3.12 (br d, 2H), 2.00-2.07 (4 s, 12H), 4.12 (m, 1H), 4.20 (t, *J* = 7 Hz, 1H), 4.43 (m, 2H), 4.45 (q, *J* = 7.1 Hz, 1H), 4.62 (q, *J* = 7.0 Hz, 1H), 4.99 (d, *J* = 7.1 Hz, 1H), 5.50 (t, *J* = 9.6 Hz, 1H), 5.71 (t, *J* = 9.0 Hz, 1H), 5.93 (t, *J* = 7.1 Hz, 1H), 7.33-7.77 (m, 8H).

Fmoc-Glu-γ-[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl-*β*-D-glucopyranosyl)]-OH, 4h: IR (KBr): 1222, 1698, 1749, 1800 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.9-2.01 (br d, 2H), 2.95-3.12 (br d, 2H), 4.20 (t, *J* = 7.0 Hz, 1H), 4.40 (d, *J* = 7.1 Hz, 2H), 4.28 (m, 1H), 4.48 (m, 1H), 4.63 (m, 1H), 4.66 (d, *J* = 7.0 Hz, 1H), 5.50 (t, *J* = 9.6 Hz, 1H), 5.71 (t, *J* = 7.1 Hz, 1H), 5.92 (t, *J* = 6.9 Hz, 1H), 7.33-8.06 (m, 28H).

Fmoc-Glu-γ-[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl-*β*-D-galactopyranosyl)]-OH, 4i: IR (KBr): 1222, 1700, 1745 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.91-2.01 (br d, 2H), 2.95-3.12 (br d, 2H), 4.20 (t, *J* = 7.1 Hz, 1H), 4.39 (m, 4H), 4.28 (m, 1H), 4.48 (m, 1H), 4.63 (m, 1H), 4.66 (d, *J* = 7.0 Hz, 1H), 5.50 (t, *J* = 9.6 Hz, 1H), 5.71 (t, *J* = 7.1 Hz, 1H), 5.92 (t, *J* = 6.9 Hz, 1H), 7.33-8.06 (m, 28H).

Fmoc-Glu-γ-[NH-CO-NH-(2,2',3,3',4',6,6'-hepta-O-acetyl-*β*-D-maltosyl)]-OH, 4j: IR (KBr): 1254, 1698, 1742 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.00 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.05 (s, 3H), 2.10 (s, 1H), 2.16 (s, 1H), 2.61 (d, *J* = 3 Hz, 1H), 3.32 (d, *J* = 9 Hz, 1H), 3.82 (m, 1H), 3.39-4.06 (m, 3H), 4.13 (t, *J* = 7.1 Hz, 1H), 4.24 (m, 5H), 4.51 (m, 1H), 4.71 (d, *J* = 7.0 Hz, 1H), 4.78 (t, *J* = 6.9 Hz, 1H), 4.85 (m, 1H), 5.05 (t, *J* =6.9 Hz, 1H), 5.26 (t, *J* =7.0 Hz, 1H), 5. 41 (t, *J* =7.1 Hz, 1H), 5.57 (d, *J* = 9.6 Hz, 1H), 7.33-7.77 (m, 8H).

Fmoc-Asp-β-[NH-CO-NH-(2,3,4,6-tetra-O-acetyl-*β*-D-glucopyranosyl)]-Val-OMe, 5a: IR (KBr): 1700, 1740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.93 (d, *J* = 6.6 Hz, 6H), 1.84 (m, 1H), 2.00-2.06 (s, 12H), 2.70 (br s, 1H), 3.29 (br s, 1H), 3.70 (s, 3H), 4.11 (m, 1H), 4.20 (t, *J* = 6.7 Hz, 1H), 4.40 (d, *J* = 7.0 Hz, 2H), 4.49 (dd, *J* = 6.9 Hz, 1H), 4.61 (dd, *J* = 7.1 Hz, 1H), 5.01 (d, *J* = 9.5 Hz, 1H), 5.51 (t, *J* = 9.6 Hz, 1H), 5.71 (t, *J* = 9.0 Hz, 1H), 5.93 (t, *J* = 4.3 Hz, 1H), 7.26-7.95 (m, 8H).

Fmoc-Asp-β-[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl-*β*-D-glucopyranosyl)]-Ala-OMe, 5b: IR (KBr): 1251, 1702, 1745 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.16 (d, *J* = 7.9 Hz, 3H), 2.70 (br s, 1H), 3.70 (s, 3H), 4.12 (m, 1H), 4.20 (t, *J* = 7.1 Hz, 1H), 4.40 (d, *J* = 7.0 Hz, 2H), 4.50 (d, *J* = 6.9 Hz, 1H), 4.61 (d, *J* = 7.1 Hz, 1H), 5.00 (d, *J* = 9.0 Hz, 1H), 5.50 (t, *J* = 9.6 Hz, 1H), 5.70 (t, *J* = 9.0 Hz, 1H), 5.93 (t, *J* = 4.3 Hz, 1H), 7.23-8.07 (m, 28H).

Fmoc-Asp-β-[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl-*β*-D-galactopyranosyl)]-Ala-OMe, 5c: IR (KBr): 1251, 1700, 1740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.16 (d, *J* = 7.9 Hz, 3H), 2.70 (br s, 1H), 3.70 (s, 3H), 4.12 (m, 1H), 4.20 (t, *J* = 6.7 Hz, 1H), 4.40 (d, *J* = 7.0 Hz, 2H), 4.50 (d, *J* = 6.9 Hz, 1H), 4.61 (d, *J* = 7.1 Hz, 1H), 5.00 (d, *J* = 9.0 Hz, 1H), 5.50 (t, *J* = 9.6 Hz, 1H), 5.70 (t, *J* = 9.0 Hz, 1H), 5.93 (t, *J* = 4.3 Hz, 1H), 7.23-8.07 (m, 28H).

Fmoc-Glu-γ-[NH-CO-NH-(2,3,4,6-tetra-O-acetyl-*β*-D-glucopyranosyl)]–Val-OMe, 5d: IR (KBr): 1222, 1700, 1749 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.93 (t, *J* = 6.6 Hz, 6H), 1.84 (m, 1H), 2.00-2.06 (s, 12H), 1.90-2.01 (br d, 2H), 2.95-3.12 (br d, 2H), 3.70 (s, 3H), 4.12 (m, 1H), 4.48 (m, 1H), 4.62 (m, 1H), 4.99 (d, *J* = 9.0 Hz, 1H), 5.50 (t, *J* = 9.6 Hz, 1H), 5.71 (t, *J* = 9.0 Hz, 1H), 5.93 (t, *J* = 4.3 Hz, 1H), 7.28-7.77 (m, 8H).

Fmoc-Glu-γ-[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl-*β*-D-glucopyransyl)]–Ala-OMe, 5e: IR (KBr): 1222, 1698, 1750 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.17 (d, *J* = 7.9 Hz, 3H), 1.90-2.01 (br d, 2H), 2.95-3.12 (br d, 2H), 3.69 (s, 3H), 4.11 (m, 1H), 4.47 (m, 1H), 4.62 (m, 1H), 5.00 (d, *J* = 9.0 Hz, 1H), 5.50 (t, *J* = 9.6 Hz, 1H), 5.71 (t, *J* = 9.0 Hz, 1H), 5.93 (t, *J* = 4.3 Hz, 1H), 7.23-8.07 (m, 28H).

Fmoc-Glu-γ-[NH-CO-NH-(2,3,4,6-tetra-O-benzoyl-*β*-D-galactopyranosyl)]–Ala-OMe, 5f: IR (KBr): 1222, 1700, 1750 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.17 (d, *J* = 7.9 Hz, 3H), 1.90-2.01 (br d, 2H), 2.95-3.12 (br d, 2H), 3.69 (s, 3H), 4.11 (m, 1H), 4.47 (m, 1H), 4.62 (m, 1H), 5.00 (d, *J* = 9.0 Hz, 1H), 5.50 (t, *J*

= 9.5 Hz, 1H), 5.71 (t, *J* = 9.0 Hz, 1H), 5.93 (t, *J* = 4.3 Hz, 1H), 7.23-8.07 (m, 28H).

Acknowledgement

Authors thank the DST (grant No. SR/S1/OC-26/2008), Govt. of India, New Delhi for financial support.

References

1 Dwek R A, *Chem Rev*, 96, **1996**, 683.
2 Sears P & Wong C H, *Angew Chem Int Edn,* 38, **1999**, 230.
3 Montreuil J, *Adv Carbohydr Chem & Biochem,* 37, **1980**, 157.
4 Varki A, *Glycobiology,* 3, **1993**, 97.
5 Kornfeld R & Kornfeld S, *Annu Rev Biochem*, 54, **1985**, 631.
6 Paulsen H, *Angew Chem Int Edn Engl,* 29, **1990**, 823.
7 Tanner W & Lehle L, *Biochim Biophys Acta,* 906, **1987**, 81.
8 Wieland F, *Biochemie,* 70, **1988**, 1493.
9 Lis H & Sharon N, *Eur J Biochem,* 218, **1993**, 1.
10 Marshall R D & Neuberger A, *Adv Carbohydrate Chem & Biochem,* 25, **1970**, 407.
11 Schwartz R T & Datema R, *Adv Carbohydrate Chem & Biochem,* 40, **1982**, 287.
12 Garg H G & Jeanloz R W, *Adv Carbohydr Chem & Biochem,* 43, **1985**, 135.
13 Meldal M, In *Neoglycoconjugates: Preparation and Applications*, Lee Y C & Lee R T, Eds Academic Press, San Diego, **1994**, 145.
14 Garg H G, Van dem Brunch K & Kunz H, *Adv Carbohydr Chem & Biochem,* 50, **1994**, 277.
15 Kunz H, *Angew Chem Int Ed Engl,* 26, **1987**, 294.
16 Marcaurelle L A & Bertozzi C R, *Chem –Eur J,* 5, **1999**, 1384.
17 Magnusson G, Chernak A Y, Khilberg J & Kononov L O, In *Neoglycoconjugates: Preparation and applications,* Lee Y C & Lee R T, Eds (Academic press, San Diego), **1994**, 53.
18 Hoffmann M, Burkh art F, Hessler G & Kessler H, *Helv Chim Acta,* 79, **1996**, 1591.
19 Ichikawa Y, Nishiyama T & Isobe M A, *Synlett,* **2000**, 1253.
20 Ichikawa Y, Ohara F, Kotsuki H & Nakano K, *Org Lett, 8*, **2006**, 5009.
21 Ikegami S, Sawada D, Sasayama S & Takahashi H, *Tett Lett*, 47, **2006**, 7219.
22 Sureshbabu V V, Tantry S J & Kantharaju, *Tett Lett*, 43, **2002**, 9461.
23 Sureshbabu V V, Chennakrishnareddy G & Narendra N, *Tett Lett*, 49, **2008**, 1408.