

Click glycoconjugation of per-azido- and alkynyl-functionalized β -peptides built from aspartic acid†

Marielle Barra,^{a,b} Olivier Roy,^{a,b} Mounir Traïkia^{a,b} and Claude Taillefumier^{*a,b}

Received 30th November 2009, Accepted 8th April 2010

First published as an Advance Article on the web 4th May 2010

DOI: 10.1039/b923275c

Azide- and alkynyl-containing homo- β^3 -peptides, of up to six residues in length, were synthesised in solution from aspartic acid. Their subsequent conjugation with monosaccharides bearing an azide or a terminal alkyne function was efficiently achieved by copper-mediated cycloadditions leading to two novel families of small glycoclusters. These compounds represent ideal tools to explore carbohydrate-mediated multivalent interactions.

Introduction

The synthesis of chemically well-defined multivalent glycoconjugates has aroused great interest over the past fifteen years.^{1,2} Obviously, they represent valuable tools for studying polyvalent interactions between carbohydrates and proteins which are key events in the communication between cells and their environment. Multivalent carbohydrate–protein interactions mediate many complex biological functions including immunity, cell-differentiation, inflammatory responses, microbial adhesion, cancer metastasis and signal transduction through cell membranes.³ Synthetic carbohydrate-based multivalent ligands with varied weights, shapes and valencies are, therefore, well-suited for the development of therapeutics that inhibit, for example, a particular lectin-mediated process² or in carbohydrate-based anticancer vaccine approaches.⁴ The binding studies conducted with such multivalent constructs also contribute to a better understanding of the so-called “cluster glycoside effect”.⁵ The Copper(I)-catalyzed Azide–Alkyne Cycloaddition (CuAAC)⁶ emerged a few years ago as a unique and powerful chemoselective ligation technique that was successfully applied to the preparation of macromolecular constructs such as glycodendrimers,⁷ and glycopolymers.⁸ A huge number of densely functionalized core molecules including calix[4]arene, cyclodextrins, cyclen-like molecules, benzene rings, monosaccharides, *etc.*, have been used for generating glycoclusters *via* the CuAAC click reactions.^{9,10} The clustering of carbohydrate ligands upon oligomeric chains like peptides¹¹ is attractive because the valency of the constructs is determined by the oligomer length. β -Peptides are undoubtedly the most thoroughly studied pseudopeptide chains since the pioneering reports from Gellman's and Seebach's groups in the mid-1990s.¹² The extraordinary interest in β -peptides is largely due to their high folding propensity from very short sequences;¹³ several new helical secondary structures have been discovered.¹⁴ This unique behaviour has led to numerous

applications of β -peptides as peptidomimetics.^{15–18} On the other side, the use of folded β -peptides as chemical platforms to control the spatial presentation of bioactive molecules has been scarcely studied to date.¹⁹

β -Peptides are of particular interest as peptide mimics since their amide bonds are resistant to a great variety of peptidases or proteases.²⁰ We therefore thought that β -peptides could also be used as enzymatically stable peptide-type structures for chemo-selective assembly of carbohydrate ligands. Little work has been done on the chemical ligation of β -peptides to give access to multivalent constructs.²¹ Due to our interest in the construction of glycosylated peptidomimetic chains,²² we report in this paper two complementary strategies for convenient glycoconjugation of short β^3 -homopeptides using the popular Copper(I)-catalyzed Azide–Alkyne Cycloaddition. One strategy involves an azide-functionalized β -peptide family (Fig. 1A) and the other an alkynyl-functionalized β -peptide family (Fig. 1B). Both β -peptide families contained up to six residues and were prepared in solution phase, by taking advantage of the β -amino acid character of aspartic acid. CuAAC reactions with monosaccharide-type partners are further reported.²³

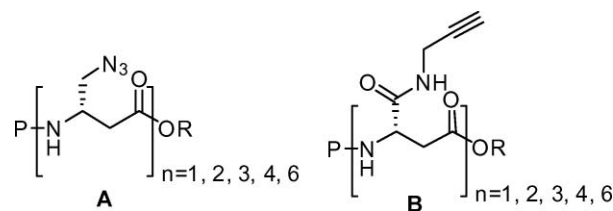


Fig. 1 Clickable β^3 -peptide oligomers from aspartic acid.

Results and discussions

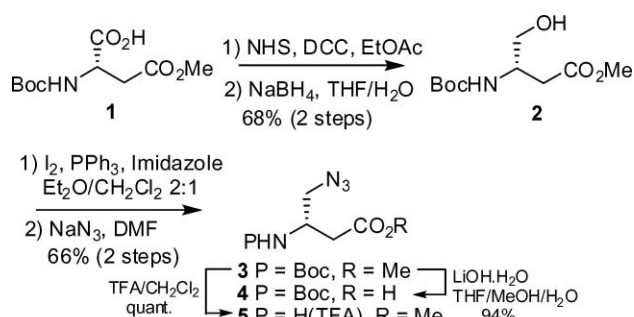
Synthesis of oligomers of family A

The β^3 -amino acid **3** bearing an azido methyl side-chain is available *via* a short synthetic sequence from *N*-(*tert*-butoxycarbonyl)-L-aspartic acid β -methyl ester **1** (Scheme 1). Reduction of the α -carboxylic was achieved by treatment of compound **1** with NHS and DCC to yield a *N*-hydroxysuccinimide ester, which was further exposed to sodium borohydride to afford the alcohol **2** in 68% yield

^aClermont Université, Université Blaise Pascal, Laboratoire SEESIB, BP 10448, F-63000, CLERMONT-FERRAND, France

^bCNRS, UMR 6504, Laboratoire SEESIB, F-63177, AUBIERE cedex, France. E-mail: claude.taillefumier@univ-bpclermont.fr; Fax: +33 (0)4 73 40 77 17; Tel: +33 (0)4 73 40 54 27

† Electronic supplementary information (ESI) available: ¹H and ¹³C NMR spectra of all new compounds, RP-HPLC chromatograms of compounds. See DOI: 10.1039/b923275c



Scheme 1 Synthesis of the azido-functionalized building blocks.

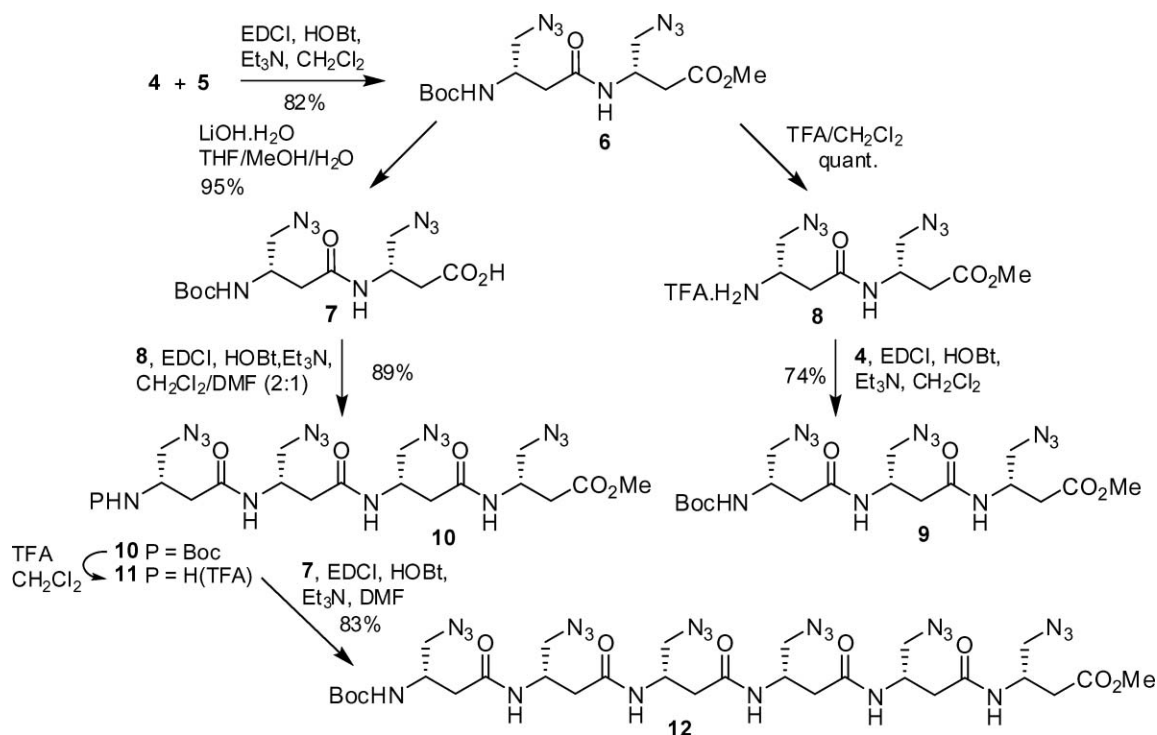
over two steps.^{24,25} The azide **3** was then prepared upon iodination of the alcohol using triphenylphosphine, iodine and imidazole,²⁶ followed by substitution with sodium azide. The methyl ester **3** was then converted to its respective acid **4** (LiOH·H₂O) and removal of the *N*-Boc protection (TFA–CH₂Cl₂) afforded the amine **5** quantitatively as a TFA salt.

Subsequent coupling of the two monomeric building blocks **4** and **5** under standard EDCI/HOBt (1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide/1-hydroxybenzotriazole) coupling conditions in CH₂Cl₂–Et₃N, yielded dimer **6** in 82% yield after flash chromatography. The same EDCI/HOBt acylating agents were used to prepare the longer oligomers **9**, **10** and **12**. Trimer **9** was prepared from the crude dimeric amine **8** (TFA–CH₂Cl₂) from **6** and monomer acid **4** in CH₂Cl₂, in 74% yield after silica gel chromatography (Scheme 2). For a convergent synthesis of the tetramer, amine **8** was reacted with acid **7** in a DMF–CH₂Cl₂ (1 : 2) mixture to give **10** in 89% yield. Similarly, hexamer **12** was synthesised in 83% yield by reacting the tetramer amine **11** with the dimer acid **7** in DMF as solvent. The yields for the coupling

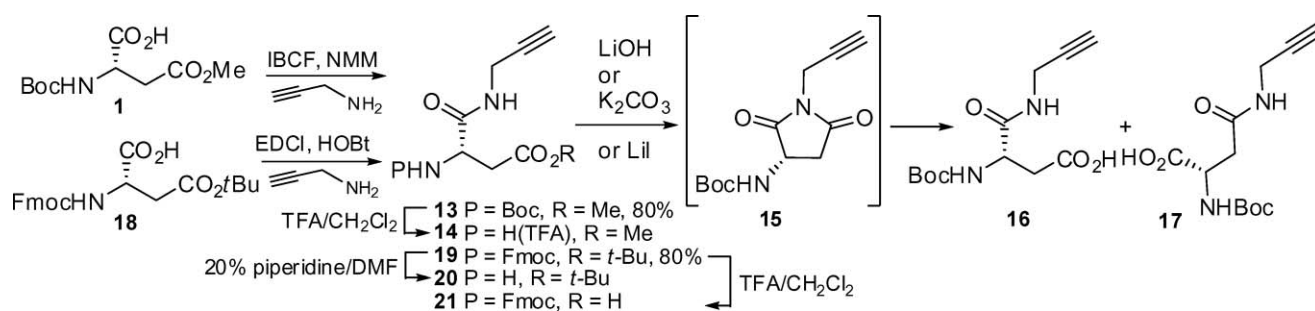
reactions did not decrease when approaching longer oligomers; however, the use of DMF was necessary to ensure homogeneous reaction mixtures during tetramer and hexamer synthesis. Moreover, their poor solubility in any organic solvent suitable for extraction techniques and SiO₂ chromatography precluded their purification in this way. Careful successive washing of the crude solids of **10** and **12** with 5% aq. citric acid solution, water, aq. saturated NaHCO₃ solution and water gave materials with good purity. Subsequent recrystallisation of hexamer **12** in THF–MeOH furnished a very pure analytical sample. The NMR spectra of the oligomers were recorded in CD₃OH as this solvent gave the best compromise for solubility and signal dispersion. Unfortunately, the poor solubility of the hexamer **12** in CD₃OH meant that only a weak ¹H NMR spectrum could be acquired. High-resolution mass spectra were obtained for all oligomers.

Synthesis of oligomers of family B

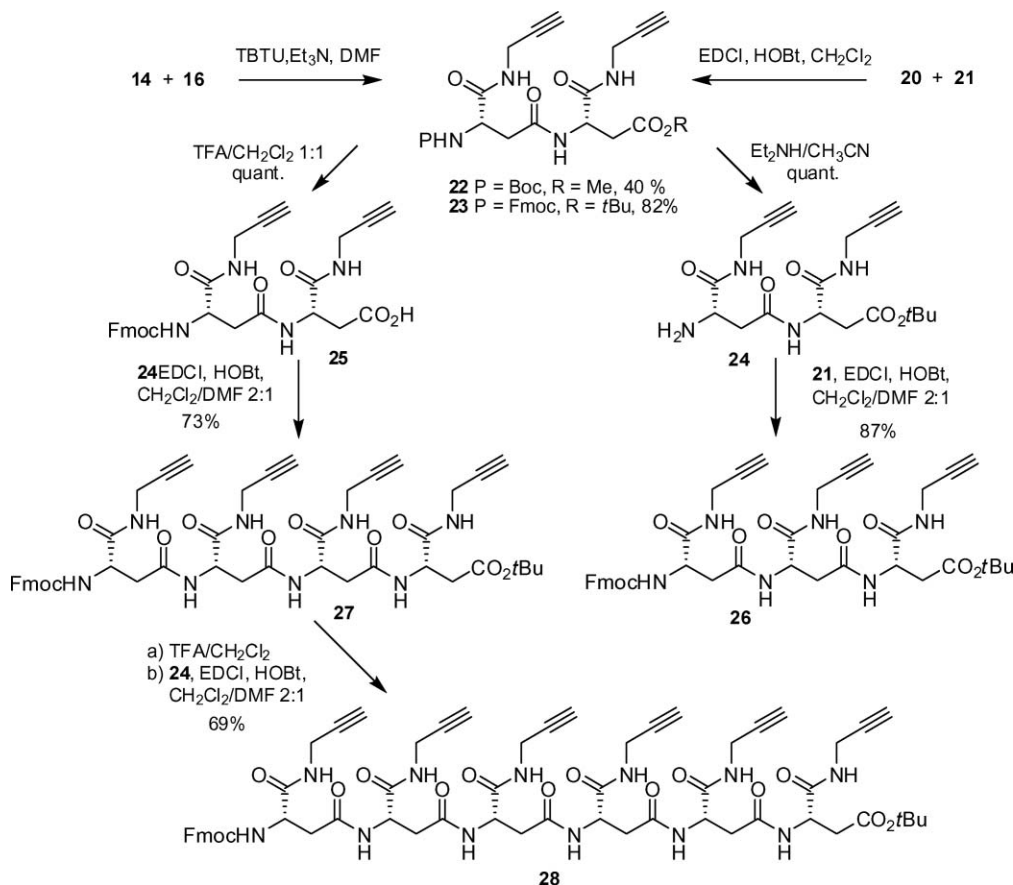
Homo-β³-peptides of family B are singular in the realm of β-peptides as they contain extra amide groups in the side-chains.²⁷ This series of β³-oligomers is functionalized with alkynyl groups for reaction with azide-containing compounds according to the principle of “click chemistry”. At first, we envisaged their synthesis from the Boc-Asp(OMe)-OH **1** used previously for family A synthesis. The latter was reacted with propargylamine using the isobutylchloroformate-based mixed anhydride approach to afford the amide **13** in 80% yield (Scheme 3).²⁸ Selective deprotection of *N*- or *C*-termini was attempted to prepare the required acid and amine for dimer synthesis. Removal of the *N*-Boc of **13** in TFA–CH₂Cl₂ furnished the corresponding amine **14** quantitatively as a TFA salt. Unfortunately, saponification of the methyl ester proved troublesome, the expected β-acid **16** was obtained together with



Scheme 2 Solution-phase synthesis of azido-functionalized β-peptide oligomers.



Scheme 3 Synthesis of the alkynyl-functionalized building blocks.

Scheme 4 Solution-phase synthesis of alkynyl-functionalized β -peptide oligomers.

the α -acid **17** in approximately the same proportion. The presence of the latter can be explained by base-mediated aspartimide formation and subsequent ring-opening of the aspartimide with water during the course of the reaction.²⁹ Similar issues occurred with LiOH and K_2CO_3 as bases and also under mild neutral conditions with LiI/EtOAc.³⁰ The two acids were isolable by flash chromatography. The acid **16** was coupled with its amine partner **14** using TBTU as acylating agent, to afford the dimer **22** in a modest yield of 40% (Scheme 4). The coupling reaction was accompanied by formation of aspartimide **15** which was isolable by chromatography, and which can partly explain the low yield of **22**. We then successfully employed a Fmoc/*t*Bu strategy which was believed to minimize aspartimide formation. Commercially available FmocAsp(*O**t*Bu)-OH **18** was converted into the propargylamide **19**³¹ under EDCI/HOBT conditions in

80% yield. The free carboxylic acid **21** was then obtained in quantitative yield by TFA- CH_2Cl_2 deprotection without formation of aspartimide by-product. Removal of the *N*-Fmoc protecting group, by treatment with 20% v/v piperidine in DMF, also resulted in a clean reaction. Despite basic cleavage conditions, the sterically hindered *O**t*Bu ester prevented aspartimide formation. The EDCI/HOBT coupling conditions were successfully used for synthesising all the members of family B (Scheme 4). Amine **20** was coupled with acid **21** in CH_2Cl_2 to give the dimer **23** in 82% yield. The longer oligomers were then synthesised as shown in Scheme 4. The yields of the coupling reactions were slightly affected by the increase of chain length and were, respectively, of 87% for the trimer, 73% for the tetramer and 69% for the hexamer. The coupling reactions were performed in a solvent mixture of DMF- CH_2Cl_2 (1:2) to ensure homogeneous reaction mixtures.

Table 1 Copper-Catalysed Azide-Alkyne cycloadditions of family A and deprotection of the sugar moieties

Reaction scheme details: Starting materials **3** ($n=1$), **6** ($n=2$), **9** ($n=3$), **10** ($n=4$), and **12** ($n=6$) are Boc-protected β -peptides. They react with 1 equiv. of propargyl- α -D-mannopyranoside (**29**) in DMF, catalyzed by $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.16 equiv.) and ascorbic acid (0.48 equiv.). This yields intermediates **30-34**. Subsequent treatment with Na/MeOH yields deacetylated products **35-39**.

Entry	Starting β^3 -peptide	Product	Yield (%) ^a	Deacetylated product ^b
1	3	30	79	35
2	6	31	88	36
3	9	32	86	37
4	10	33	82	38
5	12	34	76	39

^a Isolated yields. ^b Purity of compounds **35-39** was confirmed by a single, sharp peak on analytical RP-HPLC at 214 nm.

Given their poor solubility, the solids of **26-28** were purified by careful aqueous washing, following the same protocol used for purification of the tetramer and hexamer (**10** and **12**) in family A.

Click glycoconjugation

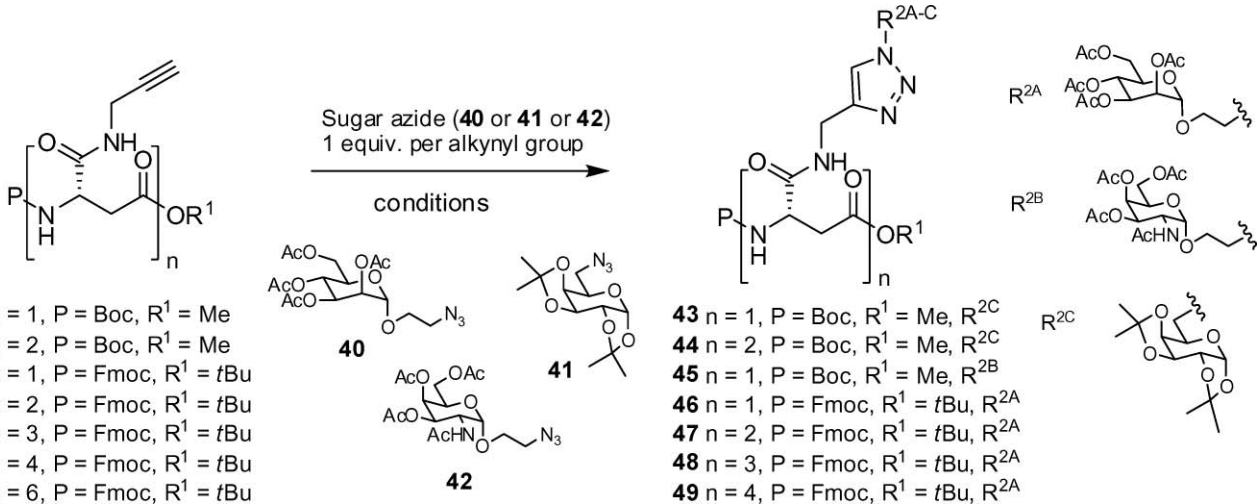
Glycoconjugation of the oligomers was investigated by Cu(I)-catalysed Huisgen azide-alkyne 1,3-dipolar cycloaddition reaction. The reaction can be performed with various commercial sources of copper(I) in numerous organic solvents or mixtures of water and miscible organic solvents. When using copper(I) salts, polytriazolyl-based ligands or tertiary amines are often added to increase the rate of CuAAC and to stabilise Cu(I) from disproportionation and/or reaction with oxygen. Copper(II) salts like CuSO_4 , in combination with a reducing agent such as ascorbic acid or sodium ascorbate, to produce Cu(I) *in situ*, are also common for CuAAC. The latter conditions were employed and the reactions conducted in DMF to ensure oligomers' solubility. Cycloaddition of the azido-functionalized β -peptides (**3**, **6**, **9**, **10** and **12**) with the propargyl- α -D-mannopyranoside **29**³² proceeded at room temperature in the presence of CuSO_4 (0.16 equiv.) and ascorbic acid (0.48 equiv.) with good yields ranging from 88 to 76% (see Table 1). Regardless of oligomer length, cycloadditions were carried out with one equivalent of sugar derivative **29** per azide function on the oligomer. The conjugates were isolated as solids after silica gel chromatography. Complete deprotections of the sugar moieties were then carried out by a standard Zemplén³³ deacetylation (Na/MeOH) followed by careful neutralization with cation exchange resin (Dowex 50W-X8 (H⁺)). Filtration provided the respective compounds **35-39** and reverse-phase HPLC confirmed purity (a single sharp peak for each).

Table 2 summarizes the cycloaddition reactions with the alkynyl-functionalized β -peptide oligomers (family B). Most of the reactions were performed in DMF, which was able to solubilize all the members of this family, except hexamer **28**.

CuSO_4 /ascorbic acid conditions were successfully employed for coupling the Boc/Me protected monomer **13** and dimer **22** with azido sugars **41** and **42**³⁴ (entries 1-3); however, with dimer **22**, increased amount of catalyst was necessary to ensure reaction completion. We also noticed a dramatic effect of the oligomer termini protecting groups Boc/Me vs. Fmoc/*t*-Bu.

In the Fmoc/*t*-Bu series, regardless of oligomer length, all of the reactions were carried out at room temperature (entries 4, 9, 11-12) with 1 equiv. of CuSO_4 and 3 equiv. of ascorbic acid.³⁵ For example, the number of alkynyl groups present on each oligomer meant that 1.0 and 0.25 equiv. of copper per alkynyl group were used, respectively, for the monomer **19** and tetramer **27**. Under these conditions, cycloadditions with the azido manno-derivative **40**³⁶ occurred with good yields, ranging from 70% (entry 11) to 82% (entry 4). The same conditions were employed for hexamer **28** but no reaction occurred (entry 13). This was attributed to the insolubility of the starting material (**28**). In the case of dimer **23**, cycloaddition with **40** was also performed with microwave irradiation, which allowed the reduction of the amount of copper and ascorbic acid by a third (entry 10). Finally, cycloaddition reactions with Cu(I) species were also attempted. Dimer **23** failed to react with azide **40** in the presence of CuI and DIPEA in DMF. However, the easy handling of $(\text{EtO})_3\text{P-CuI}$ complex in combination with DIPEA allowed efficient preparation (95% yield) of the monovalent conjugate **46** (entry 5) under microwave-assisted conditions, with toluene as solvent.¹⁰ Similar conditions were attempted to transform dimer **23** into its respective divalent conjugate **47** (entry 6); however, only a trace amount of **47** was detected. This was presumably due to the poor solubility of compound **23** in toluene, despite heating. Change of solvent from toluene to DMF and increased amount of catalyst (from 10 to 20 mol%), furnished the desired compound **47** but only in modest yield of 35% (entry 7). This last result gave supporting evidence of the particular behaviour of family B. Cu-trapping by the amide-rich compounds in family B could explain some of

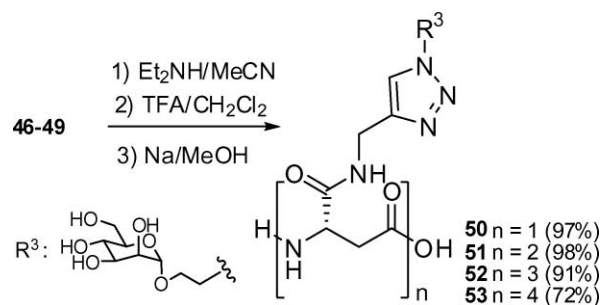
Table 2 Copper-Catalysed Azide-Alkyne cycloadditions of family B

						
<p>13 $n = 1$, $P = \text{Boc}$, $R^1 = \text{Me}$ 22 $n = 2$, $P = \text{Boc}$, $R^1 = \text{Me}$ 19 $n = 1$, $P = \text{Fmoc}$, $R^1 = t\text{Bu}$ 23 $n = 2$, $P = \text{Fmoc}$, $R^1 = t\text{Bu}$ 26 $n = 3$, $P = \text{Fmoc}$, $R^1 = t\text{Bu}$ 27 $n = 4$, $P = \text{Fmoc}$, $R^1 = t\text{Bu}$ 28 $n = 6$, $P = \text{Fmoc}$, $R^1 = t\text{Bu}$</p>						
Entry	Starting material	Sugar azide	Conditions ^{a,b}	Solvent	Product	Yield (%) ^d
1	13	41	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.08 equiv.), ascorbic acid (0.24 equiv.)	DMF	43	88
2	22	41	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.16 equiv.), ascorbic acid (0.48 equiv.)	DMF	44	65
3	22	42	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.16 equiv.), ascorbic acid (0.48 equiv.)	DMF	45	78
4	19	40	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1 equiv.), ascorbic acid (3 equiv.)	DMF	46	82
5	19	40	$\text{CuIP}(\text{OEt})_3$ (0.1 equiv.), DIPEA, MW ^c (30 min)	Toluene	46	95
6	23	40	$\text{CuIP}(\text{OEt})_3$ (0.1 equiv.), DIPEA, MW ^c (30 min)	Toluene	47 traces	—
7	23	40	$\text{CuIP}(\text{OEt})_3$ (0.2 equiv.), DIPEA, MW ^c (30 min)	DMF	47	35
8	23	40	CuI , DIPEA	DMF	none	—
9	23	40	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1 equiv.), ascorbic acid (3 equiv.)	DMF	47	74
10	23	40	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.32 equiv.), ascorbic acid (0.96 equiv.), MW ^c (85 min)	DMF	47	73
11	26	40	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1 equiv.), ascorbic acid (3 equiv.)	DMF	48	70
12	27	40	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1 equiv.), ascorbic acid (3 equiv.)	DMF	49	80
13	28	40	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1 equiv.), ascorbic acid (3 equiv.)	DMF	none	—

^a The reaction time was around 3–4 h (except for reactions under microwave irradiation). ^b All the reactions were conducted at room temperature except the reactions under microwave irradiation at -85°C . ^c A microwave oven was used. ^d Isolated yields.

our results. However, this phenomenon depends on the backbone termini protecting groups. For example, the difference in reactivity of dimers **22** (Boc/Me) and **23** (Fmoc/*t*-Bu) certainly arises from protecting group-dependent backbone conformations.

Removal of the *O*-acetyl groups to yield compounds suitable for biological assays was then undertaken. Treatment of **46** under Zemplén conditions (Na/MeOH) afforded a mixture of three compounds identified by LC-MS analysis. One of them was the expected deacetylated product; the second one, a compound which had lost both the acetyl and the Fmoc groups; and the third one resulted from intramolecular aspartimide formation between the amide nitrogen of the side-chain and the *t*-Bu ester. Despite careful control of the reaction conditions, partial cleavage of the Fmoc carbamate and aspartimide formation still occurred. Deacetylation with NaOMe was then attempted from the compound obtained by unmasking the amine of **46**. However, the desired deacetylated product was contaminated by a minor side-product resulting from intramolecular aspartimide formation. Finally, to circumvent the problems caused by the termini protecting-groups, it was decided to remove them prior to deacetylation. Compounds **46–49** were treated to remove the Fmoc carbamates (with Et_2NH in acetonitrile) and the resulting amines were purified by flash chromatography (Scheme 5). They were then exposed to TFA in CH_2Cl_2 to allow deprotection of

**Scheme 5** Sequential deprotection reactions of compounds **46–49**.

the *t*-Bu esters, and finally deacetylation with NaOMe in MeOH furnished the pure desired compounds **50–53** in good to excellent yields (72–98%).

Conclusion

Linear homo- β^3 -peptide scaffolds with pendant alkynyl or azido motifs on each residue were synthesised from monomer to hexamer length. They were further glycoconjugated through a 1,2,3-triazole, using azide–alkyne cycloadditions, to yield carbohydrate-linked β -peptides. These constructs feature an enzyme-stable pseudo-peptide chain, and a high carbohydrate density could

represent ideal tools for multivalent carbohydrate–protein binding studies. Work is currently in progress in our group to synthesise constructs for antitumoral immunotherapy.

Experimental section

General

Unless otherwise indicated, all reactions were carried out under a nitrogen atmosphere. Starting materials were obtained from commercial suppliers and used without further purification. A CEM Discover™ microwave oven was used for microwave-assisted reactions. Melting points were determined on a Reichert microscope apparatus and are uncorrected. Specific rotations were measured on a Jasco DIP-370 polarimeter using a 10 cm cell. IR spectra were recorded on Perkin-Elmer 881 or a Shimadzu FTIR-8400S spectrometers and wave numbers are expressed in cm^{-1} . NMR spectra were recorded on 400 or 500 MHz spectrometers. Chemical shifts are referenced to the residual solvent peak and J values are given in Hz. Where applicable, assignments were based on the combination of several 2D NMR techniques (COSY, TOCSY, HMBC, HSQC). TLC was performed on Merck TLC aluminium sheets, silica gel 60, F254. Flash chromatography was performed with Merck silica gel 60, 40–63 μm . HRMS were recorded on a Micromass Q-Tof Micro (3000 V) apparatus. HPLC analysis was performed on a Waters 590 instrument equipped with an Acclaim® 120 column (C18, 5 μm , 120 Å, 4.6 \times 250 mm) and a Waters 484 UV detector.

(S)-4-Azido-3-(*tert*-butoxycarbonylamino)butanoic acid (**4**)

To a solution of the protected amino acid **3**²⁵ (2.00 g, 7.74 mmol) in a mixture of THF–H₂O–MeOH (3 : 1 : 1, 230 mL) was added LiOH (0.974 g, 23.2 mmol) in one portion and the mixture was stirred at room temperature for 2 h. The solvents were removed under vacuum and the residue was dissolved in water and washed with EtOAc. The aqueous layer was acidified to pH = 1 with 1 N HCl, and extracted with EtOAc (3 \times 100 mL). The combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated, to give **4** (1.78 g, 94%) as a white solid. Mp 59–62 °C; $[\alpha]_{\text{D}}^{21}$ –31.9 (*c* 0.50 in MeOH); R_f 0.22 (CH₂Cl₂–MeOH, 9 : 1); ν_{max} (KBr)/ cm^{-1} 3414 and 3368 (NH), 2104 (N₃), 1722 (C=O, acid), 1699 and 1686 (C=O, Boc, amide); δ_{H} (400 MHz; Acetone-*d*₆) 1.96 (s, 9H, C(CH₃)₃), 2.60 (d, 2H, J 6.6 Hz, H₂), 3.46 (dd, 1H, J 5.1, 12.5 Hz, H₄), 3.51 (dd, 1H, J 6.3, 12.5 Hz, H_{4'}), 4.10 (m, 1H, H₃), 6.19 (d, 1H, J 6.9 Hz, NH); δ_{C} (100 MHz; Acetone-*d*₆) 29.5 (C(CH₃)₃), 37.9 (C₂), 49.7 (C₃), 55.5 (C₄), 80.2 (C(CH₃)₃), 156.9 (C=O, Boc), 173.5 (C₁); HRMS (ESI) m/z calcd. for C₁₀H₁₈N₄O₄ [M + Na]⁺: 267.1069, found: 267.1083.

(1-Azidomethyl-1-methoxycarbonylmethyl)methylammonium trifluoroacetate salt (**5**)

The protected amino acid **3** (0.50 g, 1.94 mmol) was dissolved in a 1 : 1 mixture of TFA–CH₂Cl₂ (6 mL) at 0 °C. After 1.5 h stirring, CH₂Cl₂ was added and the solvents were removed under vacuum. The residue was co-evaporated with CH₂Cl₂ (3 times) until complete disappearance of TFA to give **5** (0.528 g, 100%) as a colorless oil. $[\alpha]_{\text{D}}^{21}$ –5.7 (*c* 0.53 in CHCl₃; free amine); δ_{H} (400 MHz; CDCl₃) 2.74 (d, 1H, J 4.4, 17.0 Hz, H₂), 2.84 (dd,

1H, J 3.8, 17.1 Hz, H_{2'}), 3.41–3.92 (m, 6H, H₃, H₄, CO₂CH₃), 8.29 (bs, 3H, NH₃⁺); δ_{C} (100.1 MHz; CDCl₃) 33.6 (C₂), 47.7 (C₃), 51.4 (C₄), 52.4 (CO₂CH₃); 170.9 (C₁); HRMS (ESI) m/z calcd. for C₅H₁₀N₄O₂ [M + H]⁺: 159.0882, found: 159.0873.

General procedure of coupling reactions for azido-functionalized oligomers synthesis

The crude amine partner obtained as a TFA ammonium salt after TFA-mediated Boc carbamate deprotection was dissolved in CH₂Cl₂ (12 mL mmol^{–1}) under argon atmosphere and triethylamine (1.2 equiv.) was added at 0 °C. After 10 min stirring, the crude carboxylic acid partner, HOBt·H₂O (1.2 equiv.) and EDCI·HCl (1.2 equiv.) were added successively. The reaction mixture was allowed to warm to room temperature and stirred overnight. Work-up A or B was then applied to isolate the coupling product.

Work-up A. The reaction mixture was diluted with CH₂Cl₂, and washed successively with an aqueous citric acid (5%) solution, water, a saturated NaHCO₃ solution and brine. The organic layer was dried (MgSO₄), concentrated *in vacuo* and the residue was purified by flash chromatography.

Work-up B. The reaction mixture was concentrated *in vacuo* and the solid residue, placed in a fritted glass funnel (pore size 4), was washed successively with 5% aqueous citric acid (4 \times) water (4 \times), saturated NaHCO₃ (4 \times), and water (4 \times). The pure solid was then dried under vacuum.

Azide dimer (**6**)

The coupling between **4** (0.186 g, 0.762 mmol) and **5** (0.198 g, 0.727 mmol) was carried out according to the general coupling procedure (work-up A). The residue was purified by flash chromatography (EtOAc–cyclohexane 3 : 2) to yield the dimer **6** (0.24 g, 82%) as a white solid. Mp 107–113 °C; $[\alpha]_{\text{D}}^{20}$ –32.1 (*c* 0.19 in MeOH); R_f 0.30 (EtOAc–cyclohexane, 6 : 4); ν_{max} (KBr)/ cm^{-1} 3348 and 3292 (NH), 2105 (N₃), 1741 (C=O, ester), 1696 (C=O, Boc) and 1654 (C=O, amide); δ_{H} (400 MHz; CDCl₃) 1.41 (s, 9H, C(CH₃)₃), 2.43 (dd, 1H, J 5.8, 15.0 Hz, H_{2A}), 2.49 (dd, 1H, J 6.2, 15.0 Hz, H_{2'A}), 2.57 (dd, 1H, J 6.0, 16.3 Hz, H_{2B}), 2.62 (dd, 1H, J 6.0, 16.3 Hz, H_{2'B}), 3.38 (dd, 1H, J 6.2, 12.2 Hz, H_{4A}), 3.48–3.56 (m, 3H, H_{4'A}, 2 \times H_{4B}), 3.68 (s, 3H, CO₂CH₃), 4.01 (m, 1H, H_{3A}), 4.39 (m, 1H, H_{3B}), 5.50 (d, 1H, J 6.6 Hz, NH_A), 6.56 (d, 1H, J 8.4 Hz, NH_B); δ_{C} (100.1 MHz; CDCl₃) 28.2 (C(CH₃)₃), 35.5 (C_{2B}), 37.7 (C_{2A}), 45.7 (C_{3B}), 47.7 (C_{3A}), 52.0 (CO₂CH₃), 53.2 (C_{4B}), 53.4 (C_{4A}), 79.8 (C(CH₃)₃), 155.2 (C=O, Boc), 169.9 (C_{1A}), 171.3 (C_{1B}); HRMS (ESI) m/z calcd. for C₁₄H₂₄N₈O₅ [M + Na]⁺: 407.1767, found: 407.1762.

Azide trimer (**9**)

Removal of the Boc group of dimer **6** (0.500 g, 1.30 mmol) was carried out as described for the preparation of **5** to give the TFA ammonium salt **8** (0.518 g, 100%) as a colorless oil. The coupling reaction between **4** (0.123 g, 0.502 mmol) and **8** (0.200 g, 0.502 mmol) was carried out according to the general coupling procedure (work up A). The residue was purified by flash chromatography (CH₂Cl₂–MeOH 98 : 2 to 95 : 5) to yield the trimer **9** (0.190 g, 74%) as a white solid. Mp 154–157 °C; $[\alpha]_{\text{D}}^{25}$ –35.6 (*c* 0.18 in MeOH); R_f 0.31 (EtOAc–cyclohexane, 8 : 2); ν_{max}

(ATR)/cm⁻¹ 3352 and 3300 (NH), 2100 (N₃), 1730 (C=O, ester), 1680 (C=O, Boc), 1647 (C=O, amide); δ_{H} (500 MHz; CD₃OH) 1.39 (s, 9H, C(CH₃)₃), 2.26–2.37 (m, 2H, 2 × H_{2A}), 2.38–2.39 (m, 2H, 2 × H_{2B}), 2.51 (dd, 1H, *J* 8.0, 16.0 Hz, H_{2C}), 2.58 (dd, 1H, *J* 5.8, 16.0 Hz, H_{2C}), 3.35–3.41 (m, 2H, 2 × H_{4A}), 3.41–3.50 (m, 4H, 2 × H_{4B} and 2 × H_{4C}), 3.63 (s, 3H, CO₂CH₃), 4.01 (m, 1H, H_{3A}), 4.27 (m, 1H, H_{3B}), 4.31 (m, 1H, H_{3C}), 6.70 (d, 1H, *J* 8.3 Hz, NH_A), 8.04 (d, 1H, *J* 8.1 Hz, NH_B), 8.20 (d, 1H, *J* 8.0 Hz, NH_C); δ_{C} (100.1 MHz; CD₃OH) 28.7 (C(CH₃)₃), 36.9 (C_{2C}), 39.0 (C_{2B}), 39.5 (C_{2A}), 47.7 (C_{3C}), 48.2 (C_{3B}), 49.7 (C_{3A}), 52.3 (CO₂CH₃), 54.5 (C_{4C}), 54.6 (C_{4B}), 55.0 (C_{4A}), 80.4 (C(CH₃)₃), 157.5 (C=O, Boc), 172.1 (C_{1B}), 172.4 (C_{1A}), 172.6 (C_{1C}); HRMS (ESI) *m/z* calcd. for C₁₈H₃₀N₁₂O₆ [M + Na]⁺: 533.2309, found: 533.2303.

Azide tetramer (10)

Hydrolysis of the methyl ester of dimer **6** (0.500 g, 1.301 mmol) was carried out as described for the preparation of **4** to yield the crude carboxylic acid **7** (0.466 g, 97%).

The coupling of **7** (0.250 g, 0.675 mmol) and **8** (0.269 g, 0.675 mmol) was carried out according to the general coupling procedure (work up B) to yield the tetramer **10** (0.383, 89%) as a white solid. Mp 180–183 °C; $[\alpha]_{\text{D}}^{25}$ –30.7 (*c* 0.075 in MeOH); *R*_f 0.31 (EtOAc); ν_{max} (ATR)/cm⁻¹ 3298 and 3290 (NH), 2100 (N₃), 1738 (C=O, ester), 1686 (C=O, Boc); 1649 (C=O, amide); δ_{H} (400 MHz; CD₃OH) 1.40 (s, 9H, C(CH₃)₃), 2.34–2.50 (m, 6H, 2 × H_{2A}, 2 × H_{2B}, 2 × H_{2C}), 2.53 (dd, 1H, *J* 8.1, 16.2 Hz, H_{2D}), 2.59 (dd, 1H, *J* 5.8, 16.2 Hz, H_{2D}), 3.36–3.39 (m, 2H, 2 × H_{4A}), 3.39–3.49 (m, 6H, 2 × H_{4B}, 2 × H_{4C}, 2 × H_{4D}), 3.64 (s, 3H, CO₂CH₃), 4.04 (m, 1H, H_{3A}), 4.31–4.42 (m, 3H, H_{3C}, H_{3B}, H_{3D}), 6.75 (d, 1H, *J* 8.5 Hz, NH_A), 8.03 (d, 1H, *J* 8.1 Hz, NH_B), 8.09 (d, 1H, *J* 8.2 Hz, NH_C), 8.21 (d, 1H, *J* 8.0 Hz, NH_D); δ_{C} (100.1 MHz; CD₃OH) 28.7 (C(CH₃)₃), 37.0 (C_{2D}), 38.9 and 39.2 (C_{2C}, C_{2B}, C_{2C}), 39.5 (C_{2A}), 47.6 (C_{3C}, C₃), 48.1 (C_{3A}), 52.4 (CO₂CH₃), 54.7 and 55.1 (C_{4C}, C₄), 80.4 (C(CH₃)₃), 157.6 (C=O, Boc), 172.1 (C_{1B}), 172.2 (C_{1C}), 172.4 (C_{1A}), 172.8 (C_{1D}); HRMS (ESI) *m/z* calcd. for C₂₂H₃₆N₁₆O₇ [M + Na]⁺: 659.2851, found: 659.2858.

Azide hexamer (12)

Removal of the Boc group of the azide tetramer **10** (0.133 g, 0.209 mmol) was carried out as described for the preparation of **5** to yield the TFA ammonium salt **11** (0.135 g, 100%) as a white solid. The coupling of **7** (0.123 g, 0.502 mmol) and **11** (0.200 g, 0.502 mmol) was carried out according to the general coupling procedure (work up B) to yield the hexamer **12** (0.153 g, 83%) as a white solid. Mp 225–228 °C; $[\alpha]_{\text{D}}^{25}$ –10.0 (*c* 0.19 in DMF); *R*_f 0.31 (EtOAc); ν_{max} (ATR)/cm⁻¹ 3287 (NH), 2102 (N₃), 1730 (C=O, ester), 1682 (C=O, Boc), 1639 (C=O, amide); δ_{H} (400 MHz; CD₃OH) 1.45 (s, 9H, C(CH₃)₃), 2.27–2.76 (m, 12H, 12 × H₂); 3.15–3.54 (m, 12H, 12 × H₄); 3.76 (s, 3H, CO₂CH₃); 4.14–4.97 (m, 6H, 6 × H₃); 6.87 (m, 1H, NH); 7.71 (d, 1H, *J* 8.7 Hz, NH); 8.00 (m, 1H, NH); 8.12 (m, 2H, 2 × NH); HRMS (ESI) *m/z* calcd. for C₃₀H₄₈N₂₄O₉ [M + H]⁺: 889.4114, found: 889.4116.

(S)-Methyl 3-(tert-butoxycarbonylamino)-4-oxo-4-(prop-2-ynylamino)butanoate (13)

To a solution of **1** (0.512 g, 2.07 mmol) in THF (13 mL) were added *N*-methylmorpholine (0.252 mL, 2.488 mmol) and

isobutylchloroformate (0.339 mL, 2.488 mmol). The mixture was cooled at 0 °C and propargylamine (0.137 mL, 2.488 mmol) was added. After stirring at room temperature for 3 h, the mixture was filtered off and concentrated *in vacuo*. The residue was diluted with EtOAc, washed with water (3 times), dried (MgSO₄), filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (EtOAc–cyclohexane 1 : 1) to yield **13** (0.471 g, 80%) as a white solid. Mp 71–73 °C; $[\alpha]_{\text{D}}^{21}$ +18.9 (*c* 0.19 in CHCl₃); *R*_f 0.57 (EtOAc–cyclohexane, 7 : 3); ν_{max} (KBr)/cm⁻¹ 3330, 3385 and 3226 (NH, CspH), 1736 (C=O, ester), 1686 (C=O, Boc), 1655 (C=O, amide); δ_{H} (400 MHz; CDCl₃) 1.44 (s, 9H, C(CH₃)₃), 2.21 (t, 1H, *J* 2.8 Hz, C≡CH), 2.67 (dd, 1H, *J* 5.5, 16.9 Hz, H₂), 2.98 (dd, 1H, *J* 4.5, 16.9 Hz, H₂), 3.70 (s, 3H, CO₂CH₃), 3.94–4.06 (m, 2H, NHCHH), 4.50 (m, 1H, H₃), 5.7 (d, 1H, *J* 7.3 Hz, NH), 6.83 (m, 1H, *J* 2.8 Hz, NH); δ_{C} (100.1 MHz; CDCl₃) 28.2 (C(CH₃)₃), 29.2 (NHCH₂), 35.7 (C₂), 50.5 (CO₂CH₃), 52.0 (C₃), 71.6 (C≡CH), 79.1 (C(CH₃)₃), 80.6 (C≡CH), 155.5 (C=O, Boc), 170.4 (C₄), 172.2 (C₁); HRMS (ESI) *m/z* calcd. for C₁₃H₂₀N₂O₅ [M + Na]⁺: 307.1270, found: 307.1280.

Saponification of 13

Hydrolysis of the methyl ester of **13** (100 mg, 0.352 mmol) was carried out as described for the preparation of **4** to yield the acids **16** (38.0 mg, 40%) and **17** (36.1 mg, 38%).

(S)-3-(tert-Butoxycarbonylamino)-4-oxo-4-(prop-2-ynylamino)butanoic acid (16). 40%, yellow solid. Mp 124–127 °C; $[\alpha]_{\text{D}}^{21}$ +12.5 (*c* 1.12 in CHCl₃); *R*_f 0.61 (EtOAc–MeOH, 9 : 1); ν_{max} (KBr)/cm⁻¹ 3343 and 3255 (NH, Csp-H), 3102 (Csp²-H), 1719, 1685 and 1641 (C=O, acid, Boc, amide); δ_{H} (400 MHz; DMSO-*d*₆) 1.38 (s, 9H, C(CH₃)₃), 2.44 (dd, 1H, *J* 8.2, 16.3 Hz, H₂), 2.57 (dd, 1H, *J* 5.3, 16.3 Hz, H₂), 3.06 (t, 1H, *J* 2.4 Hz, C≡CH), 3.76–3.91 (m, 2H, NHCHH), 4.23 (m, 1H, H₃), 7.00 (d, 1H, *J* 8.2 Hz, NH), 8.23 (t, *J* 5.0 Hz, NH); δ_{C} (100 MHz; DMSO-*d*₆) 28.1 (C(CH₃)₃), 28.1 (NHCH₂), 36.5 (C₂), 50.9 (C₃), 72.8 (C≡CH), 78.2 (C(CH₃)₃), 81.0 (C≡CH), 155.1 (C=O, Boc), 170.8 (C₄), 172.0 (C₁); HRMS (ESI) *m/z* calcd. for C₁₂H₁₈N₂O₅ [M + Na]⁺: 293.1113, found: 293.1095.

(S)-2-(tert-Butoxycarbonylamino)-4-oxo-4-(prop-2-ynylamino)butanoic acid (17). White solid. $[\alpha]_{\text{D}}^{21}$ +47.7 (*c* 1.0 in CHCl₃); *R*_f 0.30 (EtOAc–MeOH, 9 : 1); δ_{H} (400 MHz; DMSO-*d*₆) 1.38 (s, 9H, C(CH₃)₃), 2.45 (dd, 1H, *J* 7.5, 15.4 Hz, H₂), 2.55 (dd, 1H, *J* 5.4, 15.4 Hz, H₂), 3.09 (t, 1H, *J* 2.3 Hz, C≡CH), 3.78–3.91 (m, 2H, NHCHH), 4.27 (m, 1H, H₃), 6.90 (d, 1H, *J* 8.4 Hz, NH), 8.31 (t, 1H, *J* 5.4 Hz, NH), 12.6 (se, 1H, H_{acid}); δ_{C} (100 MHz; DMSO-*d*₆) 27.9 (NHCH₂), 28.1 (C(CH₃)₃), 36.6 (C₂), 50.0 (C₃), 73.0 (C≡CH), 78.0 (C(CH₃)₃), 80.9 (C≡CH), 155.1 (C=O, Boc), 168.9 (C₁), 173.2 (C₄); HRMS (ESI) *m/z* calcd. for C₁₂H₁₈N₂O₅ [M + Na]⁺: 293.1113, found: 293.1108.

(S)-tert-Butyl 3-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4-oxo-4-(prop-2-ynylamino)butanoate (19)

To a solution of commercially available FmocAsp(OtBu)-OH (4.00 g, 9.72 mmol) in CH₂Cl₂ were added propargylamine (0.443 mL, 11.67 mmol) and HOBT·H₂O (1.57 g, 11.67 mmol) under an atmosphere of argon at 0 °C. The mixture was stirred for 10 min and EDCI·HCl (2.23 g, 11.67 mmol) was added in one portion. The reaction was stirred at rt for 12 h, then diluted with CH₂Cl₂ (400 mL) and washed successively with an aqueous

citric acid (5%) solution, water, a saturated NaHCO_3 solution and brine. The organic layer was dried (MgSO_4), concentrated *in vacuo* and the residue was purified by flash chromatography on silica gel (EtOAc–cyclohexane 3:7) to yield a white solid (3.48 g, 80%). Mp 104–106 °C; $[\alpha]_D^{25} +20.1$ (c 1 in CHCl_3 , lit. +16.5)³¹

(S)-tert-Butyl 3-amino-4-oxo-4-(prop-2-ynylamino) butanoate (20)

The amino acid **19** (2.00 g, 4.46 mmol) was dissolved in a 4:1 mixture of DMF–piperidine (100 mL) and the reaction mixture was stirred at room temperature for 1.5 h. The volatiles were removed under vacuum and the residue was purified by flash chromatography (CH_2Cl_2 –MeOH, 93:7) to yield the amine **20** (1.01 g, 100%) as a yellow oil. $[\alpha]_D^{25} -12.2$ (c 0.50 in CHCl_3); R_f 0.43 (CH_2Cl_2 –MeOH, 9:1); ν_{max} (ATR)/ cm^{-1} 3365 and 3298 (NH, Csp-H) 3066 and 3002 (Csp²-H), 2120 (C≡C), 1724 (C=O, ester), 1666 (C=O, amide); δ_{H} (400 MHz; CDCl_3) 1.40 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.75 (s, 2H, NH_2), 2.19 (t, 1H, J 2.3 Hz, C≡CH), 2.50 (dd, 1H, J 8.2, 16.6 Hz, H_2), 2.78 (dd, 1H, J 3.9, 16.6 Hz, H_2), 3.62 (dd, 1H, J 3.9, 8.2 Hz, H_3), 3.97 (m, 1H, $\text{CHHC}\equiv\text{CH}$), 4.03 (m, 1H, $\text{CHHC}\equiv\text{CH}$), 7.62 (m, 1H, NH); δ_{C} (100.1 MHz; CDCl_3) 28.0 ($\text{C}(\text{CH}_3)_3$), 28.9 ($\text{CH}_2\text{C}\equiv\text{CH}$), 40.4 (C_2), 51.9 (C_3), 71.3 (C≡CH), 79.5 (C≡CH), 81.2 ($\text{C}(\text{CH}_3)_3$), 171.0 (C_4), 173.2 (C_1); HRMS (ESI) m/z calcd. for $\text{C}_{11}\text{H}_{18}\text{N}_2\text{O}_3$ [$\text{M} + \text{H}$]⁺: 227.1396, found: 227.1409.

(S)-3-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-4-oxo-4-(prop-2-ynylamino) butanoic acid (21)

The amino acid **19** (2.00 g, 4.46 mmol) was dissolved in a 6:4 mixture of TFA– CH_2Cl_2 (10 mL) at 0 °C and stirred for 2 h. The reaction mixture was diluted with CH_2Cl_2 and evaporated *in vacuo*. The residue was co-evaporated with CH_2Cl_2 (3 times) until complete disappearance of TFA to yield **21** (1.75 g, 100%) as white solid. Mp 150–152 °C; $[\alpha]_D^{25} -16.2$ (c 0.32 in MeOH); R_f 0.38 (CH_2Cl_2 –MeOH, 9:1); ν_{max} (ATR)/ cm^{-1} 3368 and 3294 (NH, OH, Csp-H), 3066 and 3001 (Csp²-H), 1722 (C=O, acid), 1660 (C=O, amide); δ_{H} (400 MHz; Acetone- d_6) 2.63 (t, 1H, J 2.6 Hz, C≡CH), 2.77 (dd, 1H, J 7.1, 16.7 Hz, H_2), 2.90 (dd, 1H, J 5.8, 16.7 Hz, H_2), 4.00 (d, 2H, J 2.3 Hz, $\text{CH}_2\text{C}\equiv\text{CH}$), 4.24 (t, 1H, J 7.1 Hz, CH, Fmoc), 4.31 (dd, 1H, J 7.1, 10.3 Hz, CHH, Fmoc), 4.39 (dd, 1H, J 7.1, 10.3 Hz, CHH, Fmoc), 4.58 (t, 1H, J 6.0 Hz, H_3), 7.32 (m, 2H, H_{Ar}), 7.41 (t, 2H, J 7.5 Hz, H_{Ar}), 7.70 (d, 2H, J 7.5 Hz, H_{Ar}), 7.86 (d, 2H, J 7.5 Hz, H_{Ar}); δ_{C} (100.1 MHz; Acetone- d_6) 30.1 ($\text{CH}_2\text{C}\equiv\text{CH}$), 37.5 (C_2), 48.9 (CH, Fmoc), 53.3 (C_3), 68.4 (CH_2 , Fmoc), 73.1 (C≡CH), 80.0 (C≡CH), 121.8, 127.2, 128.9 and 129.5 (CH_{Ar}) 143.0 and 145.9 (C_{Ar}), 157.9 (C=O, Fmoc), 171.1 (C_4), 173.3 (C_1); HRMS (ESI) m/z calcd. for $\text{C}_{22}\text{H}_{20}\text{N}_2\text{O}_5$ [$\text{M} + \text{H}$]⁺: 393.1450, found: 393.1465.

Propargylamide dimer (22)

To a solution of the monomer amine (TFA ammonium salt) **14** (0.524 g, 1.759 mmol) and the acid **16** (0.455 g, 1.683 mmol) in DMF (21 mL) were added TBTU (0.675 g, 2.105 mmol) and Et_3N (0.469 mL, 3.367 mmol). The reaction mixture was stirred at room temperature under an atmosphere of argon for 4 h 30, then concentrated *in vacuo*, and the residue purified by flash chromatography on silica gel (CH_2Cl_2 –MeOH, 98:2) to yield the dimer **22** (0.272 g, 40%) and the aspartimide by-product **15** (17 mg, 10%).

Dimer 22. White solid. Mp 167–170 °C; $[\alpha]_D^{25} -96.0$ (c 0.025 in DMF); R_f 0.26 (EtOAc–cyclohexane, 7.5:2.5); ν_{max} (KBr)/ cm^{-1} 3321, 3304, 3268 and 3250 (NH, Csp-H), 1735 (C=O, ester), 1687 (C=O, Boc), 1638 (C=O, amide); δ_{H} (400 MHz; $\text{DMSO}-d_6$) 1.38 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.32 (dd, 1H, J 8.1, 14.5 Hz, H_{2A}), 2.54–2.57 (m, 2H, H_{2A} , H_{2B}), 2.71 (dd, 1H, J 6.5, 16.2 Hz, H_{2B}), 3.05–3.09 (m, 2H, $2 \times \text{C}\equiv\text{CH}$), 3.58 (s, 3H, CO_2CH_3), 3.80–3.87 (m, 4H, $\text{CHHC}\equiv\text{CH}$), 4.28 (m, 1H, H_{3A}), 4.59 (m, 1H, H_{3B}), 6.95 (d, 1H, J 8.4 Hz, NH_A), 8.24 (d, 1H, J 8.2 Hz, NH_B), 8.26 (t, 1H, J 5.5 Hz, NH'_B), 8.32 (t, 1H, J 5.5 Hz, NH'_A); δ_{C} (100.1 MHz; $\text{DMSO}-d_6$) 28.1 ($\text{C}(\text{CH}_3)_3$), 28.2 (2C , $\text{CH}_2\text{C}\equiv\text{CH}$), 35.9 (C_{2B}), 37.8 (C_{2A}), 49.0 (C_{3B}), 51.3 (C_{3A}), 51.4 (CO_2CH_3), 72.8 (C≡CH), 78.3 ($\text{C}(\text{CH}_3)_3$), 80.8, 81.0 (2C , C≡CH), 155.0 (C=O, Boc), 169.5 (C_{1A}), 169.9 (C_{4A}), 170.5 (C_{1B}), 171.1 (C_{4B}); HRMS (ESI) m/z calcd. for $\text{C}_{20}\text{H}_{28}\text{N}_4\text{O}_7$ [$\text{M} + \text{Na}$]⁺: 459.1856 found: 459.1837.

Aspartimide 15. White solid. Mp 113–115 °C; $[\alpha]_D^{25} +5.9$ (c 1.0 in CHCl_3); R_f 0.67 (EtOAc–MeOH, 3:1); ν_{max} (ATR)/ cm^{-1} 3400 and 3272 (NH, Csp-H), 1711 and 1695 (C=O, imide); δ_{H} (400 MHz; Acetone- d_6) 1.41 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.68 (m, 1H, C≡CH), 2.71 (dd, 1H, J 5.7, 17.8 Hz, H_2), 3.10 (dd, 1H, J 9.4, 17.8 Hz, H_2), 4.19 (dd, 1H, J 2.1, 17.1 Hz, NHCHH), 4.26 (dd, 1H, J 2.3, 17.1 Hz, NHCHH), 4.57 (m, 1H, H_3), 6.78 (d, 1H, J 6.7 Hz, NH); δ_{C} (100 MHz; Acetone- d_6) 29.0 (NHCH_2), 29.4 ($\text{C}(\text{CH}_3)_3$), 37.1 (C_2), 51.7 (C_3), 73.3 (C≡CH), 79.2 ($\text{C}(\text{CH}_3)_3$), 81.0 (C≡CH), 157.2 (C=O, Boc), 175.1 (C_1), 176.8 (C_4); HRMS (ESI) m/z calcd. for $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_4$ [$\text{M} + \text{Na}$]⁺: 275.1008, found: 275.1009.

General procedure of coupling reactions for alkynyl-functionalized oligomers synthesis

The crude carboxylic acid partner resulting from the *t*-Bu ester deprotection was dissolved in CH_2Cl_2 (5 mL mmol⁻¹) at 0 °C under an atmosphere of argon and the amine partner (1.0 equiv.) and HOBt· H_2O (1.4 equiv.) were added successively. The reaction mixture was stirred for 10 min and EDCI·HCl (1.5 equiv.) was added. The reaction mixture was allowed to warm to room temperature and stirred for 8 h. The mixture was concentrated *in vacuo* and the solid residue, placed in a fritted glass funnel (pore size 4), was washed successively with 5% aqueous citric acid (4 × 10 mL), water (4 × 10 mL), saturated NaHCO_3 solution (4 × 10 mL), water (4 × 10 mL) and methanol (3 × 10 mL, except for **23**). The pure solid was then dried under vacuum.

Propargylamide dimer (23)

The coupling between the amine **20** (0.105 g, 0.464 mmol) and the acid **21** (0.200 g, 0.510 mmol) was carried out according to the general procedure to yield the dimer **23** (0.228 g, 82%) as a white solid. Mp 153–155 °C; $[\alpha]_D^{25} -25.0$ (c 0.52 in DMF); R_f 0.20 (EtOAc–cyclohexane 6:4); ν_{max} (ATR)/ cm^{-1} 3289 (NH, Csp-H), 3066 (Csp²-H), 1730 (C=O, ester), 1687 (C=O, Fmoc), 1649 (C=O, amide); δ_{H} (400 MHz; Pyridine- d_5) 1.44 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.92 (dd, 1H, J 8.0, 15.7 Hz, H_{2B}), 2.99–3.13 (m, 3H, $2 \times \text{C}\equiv\text{CH}$, H_{2A}), 3.23–3.35 (m, 2H, J 6.3, 15.7 Hz, H_{2A} , H_{2B}), 4.18 (t, 1H, J 6.7 Hz, CH Fmoc), 4.22–4.47 (m, 5H, $2 \times \text{CH}_2\text{C}\equiv\text{CH}$, CHH Fmoc), 4.56 (m, 1H, CHH Fmoc), 5.48 (m, 1H, H_{3A}), 5.56 (m, 1H, H_{3B}), 7.25 (m, 2H, H_{Ar}), 7.41 (m, 2H, H_{Ar}), 7.60 (m, 2H, H_{Ar}), 7.84 (d, 2H, J 7.3 Hz, H_{Ar}), 9.21 (d, 1H, J 8.4 Hz, NH_A), 9.25 (m, 1H, NH'_A), 9.74 (m, 1H, NH'_B), 9.78 (d, 1H, J 8.5 Hz, NH_B);

δ_c (100.1 MHz; Pyridine- d_5) 28.3 (C(CH₃)₃), 29.6 and 29.7 (2C, CH₂C≡CH), 38.7 (C_{2B}), 39.6 (C_{2A}), 47.9 (CH Fmoc), 51.1 (C_{3B}), 53.4 (C_{3A}), 67.1 (CH₂ Fmoc), 72.5 and 72.6 (2C, C≡CH), 81.0 (C(CH₃)₃), 81.6, 81.7 (2C, C≡CH), 120.7, 125.9, 126.0, 127.8 and 128.4 (CH_{Ar}), 141.9 and 144.8 (C_{Ar}), 157.4 (C=O, Fmoc), 170.7 (C_{1B}), 171.2 (C_{1A}), 171.6 (C_{4A}), 172.7 (C_{4B}); HRMS (ESI) m/z calcd. for C₃₃H₃₆N₄O₇ [M + H]⁺: 601.2662, found: 601.2659.

Propargylamide trimer (26)

Removal of the Fmoc carbamate of the propargylamide dimer **23** (0.376 g, 0.626 mmol) was carried out as described for the preparation of **20** to yield the amine **24** (0.237 g, 100%) as a colorless oil. The coupling between the acid **21** (0.086 g, 0.219 mmol) and the dimer amine **24** (0.083 g, 0.219 mmol) was carried out according to the general procedure to yield the trimer **26** (0.143 g, 87%) as a white solid. Mp 207–212 °C; $[\alpha]_D^{21}$ –29.2 (*c* 0.25 in DMF); R_f 0.43 (CH₂Cl₂–MeOH, 9 : 1); ν_{\max} (ATR)/cm^{–1} 3283 (NH), 3075 and 3053 (Csp²-H), 1724 (C=O, ester), 1695 (C=O, Fmoc), 1641 (C=O, amide); δ_H (500 MHz; Pyridine- d_5) 1.42 (s, 9H, C(CH₃)₃), 2.90 (dd, 1H, *J* 8.1, 15.7 Hz, H_{2C}), 2.95–3.09 (m, 5H, H_{2A}, H_{2B}, 3 × C≡CH), 3.18 (dd, 1H, *J* 8.4, 14.2 Hz, H_{2A}), 3.22 (dd, 1H, *J* 8.3, 14.2 Hz, H_{2B}), 3.28 (dd, 1H, *J* 5.9, 15.7 Hz, H_{2C}), 4.17 (t, 1H, *J* 7.0 Hz, CH Fmoc), 4.20–4.44 (m, 7H, 3 × CH₂C≡CH, CHH Fmoc), 4.55 (dd, 1H, *J* 7.0, 10.4 Hz, CHH Fmoc), 5.43 (m, 1H, H_{3A}), 5.57 (m, 1H, H_{3C}), 5.77 (m, 1H, H_{3B}), 7.25, 7.40, 7.59 and 7.84 (8H, H_{Ar}), 9.18 (d, 1H, *J* 8.6 Hz, NH_A), 9.24 (m, 2H, NH_A NH_B), 9.77 (m, 1H, NH_C), 9.78 (d, 1H, *J* 8.6 Hz, NH_C), 9.84 (d, 1H, *J* 8.6 Hz, NH_B); δ_c (100.1 MHz; Pyridine- d_5) 28.4 (C(CH₃)₃), 29.7 and 29.8 (2C, CH₂C≡CH), 38.9 (C_{2C}), 39.4 (C_{2B}), 39.8 (C_{2A}), 48.0 (CH Fmoc), 51.3 (C_{3C}), 51.6 (C_{3B}), 53.6 (C_{3A}), 67.2 (CH₂ Fmoc), 72.6, 72.7 and 72.8 (3C, C≡CH), 81.4 (C(CH₃)₃), 81.7, 81.8 and 82.0 (3C, C≡CH), 120.9, 126.2, 127.9 and 128.5 (CH_{Ar}), 142.1 and 144.9 (C_{Ar}), 157.6 (C=O, Fmoc), 170.9 (C_{1C}), 171.4 (C_{1B}), 171.8 (2C, C_{1A}, C_{4A}), 172.7 (C_{4B}), 172.9 (C_{4C}); HRMS (ESI) m/z calcd. for C₄₀H₄₄N₆O₉ [M + H]⁺: 753.3248, found: 753.3257.

Propargylamide tetramer (27)

Deprotection of the *t*-Bu ester of dimer **23** (0.100 g, 0.166 mmol) was carried out as described for the preparation of **21** to yield the acid **25** (0.090 g, 100%) as a white solid which was used without purification.

The coupling between **24** (0.190 g, 0.502 mmol) and **25** (0.417 g, 0.766 mmol) was carried out according to the general procedure to yield the tetramer **27** (0.331 g, 73%) as a white solid. Mp 252–257 °C; $[\alpha]_D^{21}$ –35.2 (*c* 0.14 in DMF); R_f 0.88 (CH₂Cl₂–MeOH, 8 : 2); ν_{\max} (ATR)/cm^{–1} 3279 (NH, Csp²-H), 3059 (Csp²-H), 1721 (C=O, ester), 1694 (C=O, Fmoc), 1643 (C=O, amide); δ_H (500 MHz; Pyridine- d_5) 1.43 (s, 9H, C(CH₃)₃), 2.89 (dd, 1H, *J* 8.2, 15.7 Hz, H_{2D}), 2.94–3.07 (m, 7H, H_{2A}, H_{2B}, H_{2C}, 4 × C≡CH), 3.08–3.25 (m, 3H, H_{2A}, H_{2B}, H_{2C}), 3.28 (dd, 1H, *J* 5.8, 15.7 Hz, H_{2D}), 4.15–4.46 (m, 10H, 4 × CH₂C≡CH, CH Fmoc, CHH Fmoc), 4.55 (m, 1H, CHH Fmoc), 5.41 (m, 1H, H_{3A}), 5.55 (m, 1H, H_{3D}), 5.71 and 5.75 (2 m, 2H, H_{3B}, H_{3C}), 7.29, 7.39, 7.64 and 7.85 (H_{Ar}), 9.17 (d, 1H, *J* 8.4 Hz, NH_A), 9.20–9.31 (m, 3H, NH'), 9.69–9.88 (m, 4H, NH_B, NH_C, NH_D, NH'); δ_c (100.1 MHz; Pyridine- d_5) 28.5 (C(CH₃)₃), 29.8 and 29.9 (2C, CH₂C≡CH), 38.9 (C_{2D}), 39.4, 39.5 and 39.8

(3C, C₂), 48.1 (CH Fmoc), 51.3, 51.6 and 51.7 (3C, C₃), 53.7 (C_{3A}), 67.4 (CH₂ Fmoc), 72.7, 72.8 and 72.9 (4C, C≡CH), 81.3, 81.7 and 81.9 (3C, C≡CH), 102.9, 126.2, 126.2, 128.0, 128.2, 128.6 and 129.9 (CH_{Ar}), 136.5, 142.1 and 145.0 (C_{Ar}), 157.7 (C=O Fmoc), 171.0, 171.7, 171.9, 172.6, 172.7 and 172.9 (8C, 4 × C₁, 4 × C₄); HRMS (ESI) m/z calcd. for C₄₇H₅₃N₈O₁₁ [M + H]⁺: 905.3834, found: 905.3829.

Propargylamide hexamer (28)

Deprotection of the *t*-Bu ester of tetramer **27** (0.197 g, 0.218 mmol) was carried out as described for the preparation of **21**, the resulting acid was used in the next step without purification. Coupling of the latter (0.139 g, 0.164 mmol) with the dimer amine **24** (0.062 g, 0.164 mmol) was carried out according to the general procedure to yield the hexamer **28** (0.137 g, 69%) as a white solid. Mp > 300 °C; $[\alpha]_D^{21}$ too insoluble; R_f 0.38 (CH₂Cl₂–MeOH, 9 : 1); ν_{\max} (ATR)/cm^{–1} 3283 (NH); 1719 (C=O ester); 1637 (C=O, Fmoc, amide); δ_H (500 MHz; DMSO- d_6) 2.19–2.78 (m, 12H, 12 × H₂); 2.97–3.13 (m, 6H, 6 × C≡CH); 3.72–4.00 (m, 12H, 6 × CH₂C≡CH); 4.13–4.76 (m, 6H, 6 × H₃); 7.27–8.46 (m, 6H, 6 × NH); HRMS (ESI) m/z calcd. for C₆₁H₆₈N₁₂O₁₅ [M+H]⁺: 1209,5005, found 1209,5011.

Glycoconjugated monomer (30)

To a solution of the azide β-amino ester **3** (100 mg, 0.387 mmol) and sugar alkyne **29** (150 mg, 0.387 mmol) in DMF (3 ml) was added 0.1 M CuSO₄ solution (0.620 ml, 0.16 equiv.) and 0.1 M ascorbic acid solution (1.858 ml, 0.48 equiv.). The mixture was stirred for 4 h under an argon atmosphere. The solvent was evaporated and the residue taken up in CH₂Cl₂ was washed with water (4 × 75 ml) and brine. The organic layer was dried (MgSO₄), filtered, concentrated *in vacuo*, and the residue purified by flash chromatography on silica gel (CH₂Cl₂–MeOH 98 : 2–95 : 5) to yield the glycoconjugate **30** (197 mg, 0.306 mmol) in 79% as a white solid. Mp 52–54 °C; $[\alpha]_D^{21}$ +32.6 (*c* 0.28 in CHCl₃); R_f 0.44 (CH₂Cl₂–MeOH, 9.5 : 0.5); ν_{\max} (KBr)/cm^{–1} 3360 (NH), 3142 (Csp²-H), 1743 (C=O, ester), 1716 (C=O, Boc, amide); δ_H (400 MHz; CDCl₃) 1.28 (s, 9H, C(CH₃)₃), 1.86, 1.92, 2.00 and 2.03 (4 s, 12H, CH₃CO), 2.38–2.55 (m, 2H, H₂), 3.59 (s, 3H, CO₂CH₃), 3.97 (m, 1H, H_{5carb}), 4.0 (d, 1H, *J* 12.2 Hz, H_{6carb}), 4.18 (dd, 1H, *J* 4.7, 12.2 Hz, H_{6'carb}), 4.24 (m, 1H, H₃), 4.44–4.53 (m, 2H, H₄), 4.56 (d, 1H, *J* 12.4 Hz, OCHH), 4.73 (d, 1H, *J* 12.4 Hz, OCHH), 4.84 (s, 1H, H_{1carb}), 5.09 (s, 1H, H_{2carb}), 5.12–5.24 (m, 2H, H_{3carb}, H_{4carb}), 5.45 (d, 1H, *J* 7.9 Hz, NH), 7.60 (s, 1H, Csp²H); δ_c (100.1 MHz; CDCl₃) 20.3, 20.4, 20.5 and 20.6 (4 s, 4 × CH₃CO), 27.9 (C(CH₃)₃), 35.5 (C₂), 47.4 (C₃), 51.7 (C₄), 52.0 (CO₂CH₃), 60.4 (OCH₂), 62.1 (C_{6carb}), 65.8 (C_{4carb}), 68.4 (C_{5carb}), 68.7 (C_{3carb}), 69.2 (C_{2carb}), 79.7 (C(CH₃)₃), 96.4 (C_{1carb}), 124.0 (Csp²H), 143.0 (Csp²), 154.8 (C=O, Boc), 169.4, 169.5, 169.8 and 170.4 (4C, CH₃CO), 170.9 (C₁); HRMS (ESI) m/z calcd. for C₂₇H₄₀N₄O₁₄ [M + H]⁺: 645.2619 found: 645.2632.

Glycoconjugated dimer (31)

The cycloaddition between diazide **6** (75 mg, 0.194 mmol) and sugar alkyne **29** (150 mg, 0.388 mmol) was carried out as described for the preparation of **30** to give, after flash chromatography on silica gel (EtOAc–MeOH 98 : 2), **31** (197 mg, 88%), as a white solid. Mp 85–89 °C; $[\alpha]_D^{26}$ +32.7 (*c* 0.54 in CHCl₃); R_f 0.40 (EtOAc–MeOH, 98 : 2); ν_{\max} (KBr)/cm^{–1} 3342 and 3308 (NH), 3140 and

3074 (Csp²-H), 1745 (C=O, ester), 1664 (C=O, Boc, amide); δ_{H} (400 MHz; CDCl₃) 1.41 (s, 9H, C(CH₃)₃), 1.96, 2.02, 2.03, 2.10 and 2.12 (5 s, 24H, CH₃CO), 2.27 (dd, 1H, *J* 6.8, 15.5 Hz, H_{2A}), 2.32 (dd, 1H, *J* 5.1, 15.8 Hz, H_{2A}), 2.65 (d, 2H, *J* 5.2 Hz, H_{2B}), 3.69 (s, 3H, CO₂CH₃), 4.02–4.15 (m, 4H, 2 × H_{5carb}, 2 × H_{6carb}), 4.20–4.33 (m, 3H, H_{3A}, H_{6carb}), 4.49–4.59 (m, 4H, 4 × H₄), 4.64 (m, 1H, H_{3B}), 4.60–4.75 (m, 2H, OCH₂), 4.84 (t, 2H, *J* 10.2 Hz, OCH₂), 4.93, 4.95 (2 s, 2H, 2 × H_{1carb}), 5.13, 5.18 (2 s, 2H, 2 × H_{2carb}), 5.23–5.34 (m, 4H, 2 × H_{3carb}, 2 × H_{4carb}), 5.81 (d, 1H, *J* 7.8 Hz, NH_A), 6.87 (d, 1H, *J* 6.8 Hz, NH_B), 7.74 and 7.78 (2 s, 2H, 2 × Csp²H); δ_{C} (100.1 MHz; CDCl₃) 20.6, 20.7, 20.8 and 20.8 (8C, CH₃CO), 28.2 (C(CH₃)₃), 35.4 (C_{2B}), 36.7 (C_{2A}), 46.6 (C_{3B}), 47.9 (C_{3A}), 51.7 and 51.9 (2C, C₄), 52.1 (CO₂CH₃), 60.6 and 60.8 (2C, OCH₂), 62.3 (C_{6carb}), 65.9 (C_{4carb}), 68.6 (C_{5carb}), 68.9 and 69.0 (2C, C_{4carb}), 69.4 and 69.6 (2C, C_{2carb}), 79.9 (C(CH₃)₃), 96.3 and 96.7 (2C, C_{1carb}), 124.3 and 124.6 (2C, Csp²H), 143.1 and 143.6 (2C, Csp²), 155.1 (C=O Boc), 169.6 (CH₃CO), 169.9 (C_{1A}), 170.0, 170.0, 170.2 and 170.6 (CH₃CO), 170.9 (C_{1B}); HRMS (ESI) *m/z* calcd. for C₄₈H₆₈N₈O₂₅ [M + H]⁺: 1157.4374 found: 1157.4393.

Glycoconjugated trimer (32)

The cycloaddition between triazide **9** (100 mg, 0.196 mmol) and sugar alkyne **29** (227 mg, 0.598 mmol) was carried out as described for the preparation of **30** to give, after flash chromatography on silica gel (CH₂Cl₂–MeOH 9.5:0.5–9:1), **32** (281 mg, 86%), as a white solid. Mp 152–155 °C; $[\alpha]_{\text{D}}^{26} +52.2$ (*c* 0.52 in CHCl₃); *R*_f 0.46 (CH₂Cl₂–MeOH, 9:1); ν_{max} (KBr)/cm^{−1} 3302 (NH), 3068 (Csp²-H), 1748 (C=O, ester), 1701, 1670 and 1657 (C=O, Boc, amide); δ_{H} (400 MHz; CDCl₃) 1.42 (s, 9H, C(CH₃)₃), 1.94, 1.95, 2.01, 2.02, 2.10, 2.11 and 2.13 (7 s, 36H, CH₃CO), 2.30 (dd, 1H, *J* 4.1, 15.1 Hz, H_{2B}), 2.39 (dd, 1H, *J* 4.2, 15.1 Hz, H_{2B}), 2.46 (bs, 2H, H_{2A}), 2.79 (bs, 2H, H_{2C}), 3.71 (s, 3H, CO₂CH₃), 3.99–4.07 (m, 3H, 3 × H_{5carb}), 4.07–4.16 (m, 3H, 3 × H_{6carb}), 4.21 (m, 1H, H_{3A}), 4.24–4.38 (m, 5H, 3 × H_{6carb}, 2 × H₄), 4.41–4.57 (m, 2H, H₄), 4.56–4.75 (m, 7H, H_{3B}, H_{3C}, 2 × H₄, 3 × OCHH), 4.76–4.88 (m, 3H, OCHH), 4.89–5.03 (m, 3H, 3 × H_{1carb}), 5.12–5.21 (m, 3H, 3 × H_{2carb}), 5.21–5.34 (m, 6H, 3 × H_{3carb}, 3 × H_{4carb}), 6.40 (d, 1H, *J* 7.2 Hz, NH_A), 7.35 (d, 1H, *J* 8.5 Hz, NH_B), 7.42 (d, 1H, *J* 6.9 Hz, NH_C), 7.70, 7.83 and 7.96 (3 s, 3H, 3 × Csp²H); δ_{C} (100.1 MHz; CDCl₃) 20.6, 20.7 and 20.8 (12C, CH₃CO), 28.3 (C(CH₃)₃), 35.7 (C_{2A}), 35.9 (C_{2C}), 37.8 (C_{2B}), 46.6 (C_{3B}), 47.1 (C_{3C}), 48.1 (C_{3A}), 51.3 (C₄), 52.1 (CO₂CH₃), 52.2 and 52.7 (2C, C₄), 60.7, 60.6 and 60.8 (3C, OCH₂), 62.3 (3C, C_{6carb}), 66.0 (3C, C_{4carb}), 68.6 and 68.7 (3C, C_{5carb}), 68.9 and 69.1 (3C, C_{3carb}), 69.3 and 69.5 (3C, C_{2carb}), 79.9 (C(CH₃)₃), 96.5, 96.6 and 96.6 (3C, C_{1carb}), 124.6, 124.7 and 124.8 (3C, Csp²H), 143.0, 143.2 and 143.8 (3C, Csp²), 155.3 (C=O, Boc), 169.3 (C_{1B}), 169, 169.9 and 170.0 (CH₃CO), 170.1 (C_{1A}, CH₃CO), 170.6 and 170.7 (CH₃CO), 170.9 (C_{1C}); HRMS (ESI) *m/z* calcd. for C₆₉H₉₆N₁₂O₃₆ [M + 2H]²⁺: 835.3098 found: 835.3084.

Glycoconjugated tetramer (33)

The cycloaddition between tetraazide **10** (50 mg, 0.078 mmol) and sugar alkyne **29** (121 mg, 0.314 mmol) was carried out as described for the preparation of **30** to give, after flash chromatography on silica gel (EtOAc–MeOH 9:1), **33** (140 mg, 82%), as a white solid. Mp 183–185 °C; $[\alpha]_{\text{D}}^{21} +43.2$ (*c* 0.50 in CHCl₃); *R*_f 0.45 (CH₂Cl₂–MeOH, 9:1); ν_{max} (KBr)/cm^{−1} 3291 (NH), 3064 (Csp²-H), 1746

(C=O, ester), 1690 (C=O, Boc), 1653 (C=O, amide); δ_{H} (400 MHz; CDCl₃) 1.40 (s, 9H, C(CH₃)₃), 1.93, 1.94, 2.00, 2.01, 2.09, 2.10 and 2.11 (7 s, 48H, CH₃CO), 2.21–2.58 (m, 6H, 6 × H₂), 2.68 (d, 2H, *J* 5.9 Hz, 2 × H_{2D}), 3.69 (s, 3H, CO₂CH₃), 3.96–4.21 (m, 9H, 4 × H_{5carb}, 4 × H_{6carb}, H_{3A}), 4.22–4.34 (m, 4H, 4 × H_{6carb}), 4.46 (dd, 2H, *J* 5.8, 14.0 Hz, 2 × H₄), 4.49–4.74 (m, 13H, 3 × H₃, 2 × H₄, 8 × OCHH), 4.72–4.84 (m, 4H, 4 × H₄), 4.89–4.96 (m, 4H, 4 × H_{1carb}), 5.12–5.19 (m, 4H, 4 × H_{2carb}), 5.19–5.35 (m, 8H, 4 × H_{3carb}, 4 × H_{4carb}), 6.23 (d, 1H, *J* 7.5 Hz, NH_A), 7.03 (bs, 1H, NH), 7.69 (d, 1H, *J* 7.8 Hz, NH_B), 7.73, 7.79, 7.85 and 7.87 (4 s, 4H, 4 × Csp²H), 8.01 (d, 1H, *J* 7.6 Hz, NH); δ_{C} (100.1 MHz; CDCl₃) 20.6, 20.7 and 20.8 (16C, CH₃CO), 28.2 (C(CH₃)₃), 35.8 (C_{2D}), 36.0 (C₂), 36.8 (C_{2A}), 37.0 (C₂), 46.7 (2C, C_{3D}, C₃), 46.9 (C₃), 48.3 (C_{3A}), 52.0 (C₄), 52.1 (CO₂CH₃), 52.3, 52.4 and 52.6 (3C, C₄), 59.9, 60.2 and 60.5 (4C, OCH₂), 62.3 (4C, C_{6carb}), 65.8 and 65.9 (4C, C_{3carb}), 68.6, 68.6 and 68.7 (4C, C_{5carb}), 69.0 (4C, C_{4carb}), 69.2 and 69.3 (4C, C_{2carb}), 79.8 (C(CH₃)₃), 96.2, 96.3, 96.4 and 96.5 (4C, C_{1carb}), 124.6, 124.7 and 124.8 (4C, Csp²H), 142.9, 143.1 and 143.1 (4C, Csp²), 155.3 (C=O, Boc), 169.5, 169.6, 169.7, 169.9, 170.0, 170.0, 170.6, 170.7 (CH₃CO, C₁); HRMS (ESI) *m/z* calcd. for C₉₀H₁₂₄N₁₆O₄₇ [M + 2H]²⁺: 1091.8990 found: 1091.3982.

Glycoconjugated hexamer (34)

The cycloaddition between hexaazide **12** (40 mg, 0.045 mmol) and sugar alkyne **29** (104 mg, 0.27 mmol) was carried out as described for the preparation of **30** to give, after flash chromatography on silica gel (EtOAc–MeOH 9:1), **34** (110 mg, 76%), as a white solid. Mp 190–192 °C; $[\alpha]_{\text{D}}^{21} +53.7$ (*c* 0.49 in CHCl₃); *R*_f 0.25 (EtOAc–MeOH, 9:1); ν_{max} (KBr)/cm^{−1} 3363 and 3285 (NH), 3132 and 3084 (Csp²-H), 1748 (C=O, ester), 1655 (C=O, Boc, amide); δ_{H} (400 MHz; CDCl₃) 1.37 (s, 9H, C(CH₃)₃), 1.94, 1.96, 2.00, 2.01, 2.03, 2.08, 2.09 and 2.11 (8 s, 72H, CH₃CO), 2.17–2.60 (m, 10H, 10 × H₂), 2.69 (d, 2H, *J* 6.1 Hz, 2 × H_{2F}), 3.67 (s, 3H, CO₂CH₃), 3.97–4.18 (m, 12H, 6 × H_{5carb}, 6 × H_{6carb}), 4.19–4.37 (m, 7H, H_{3A}, 6 × H_{6carb}), 4.38–4.71 (m, 23H, 5 × H₃, 12 × H₄, 6 × OCHH), 4.72–4.86 (m, 6H, 6 × OCHH), 4.91–4.95 (m, 6H, 6 × H_{1carb}), 5.11–5.19 (m, 6H, 6 × H_{2carb}), 5.19–5.34 (m, 12H, 6 × H_{3carb}, 6 × H_{4carb}), 6.16 (d, 1H, *J* 6.2 Hz, NH_A), 6.97 (bs, 1H, NH), 7.47 (bs, 1H, NH), 7.54 (bs, 1H, NH), 7.61 (bs, 1H, NH), 7.83 (m, 1H, NH), 7.77, 7.83, 7.86, 7.88, 7.97 and 7.98 (6 s, 6H, 6 × Csp²H); δ_{C} (100.1 MHz; CDCl₃) 20.6, 20.7 and 20.8 (24C, CH₃CO), 28.2 (C(CH₃)₃), 35.9, 37.0, 37.1, 37.2, 37.4 and 37.5 (6C, C₂), 46.7, 47.0, 47.1, 47.4 and 48.1 (6C, C₃), 52.0 (C₄), 52.1 (CO₂CH₃), 52.4, 52.5, 52.6 and 53.0 (5C, C₄), 59.5, 60.2 and 60.5 (6C, OCH₂), 62.3 (6C, C_{6carb}), 65.9 (6C, C_{4carb}), 68.6, 68.8, 69.2 and 69.3 (18C, 6 × C_{2carb}, 6 × C_{3carb}, 6 × C_{5carb}), 79.7 (C(CH₃)₃), 95.7, 96.3, 96.5 and 96.6 (6C, C_{1carb}), 124.7 and 125.0 (6C, Csp²H), 142.8, 142.9 and 143.0 (6C, Csp²), 155.3 (C=O, Boc), 169.6, 169.7, 169.9, 170.0, 170.1, 170.2, 170.4, 170.6 and 171.0 (C₁, CH₃CO); HRMS (ESI) *m/z* calcd. for C₁₃₂H₁₈₀N₂₄O₆₉ [M + 2H]²⁺: 1603.5730 found: 1603.5726.

Deprotected glycoconjugated monomer (35)

To a solution of **30** (50 mg, 0.078 mmol) in dry MeOH (8 ml) was added a 0.1 M solution of NaOMe in MeOH (0.20 ml, freshly prepared from Na and MeOH before use). The reaction mixture was stirred for 5 h, then neutralized with Dowex 50W-X8 (H⁺ form, washed with H₂O and MeOH before use) and filtered through a

sintered glass funnel. The resin was washed with H₂O, MeOH was concentrated *in vacuo* and the remainder of the solution was lyophilised to yield **35** quantitatively (37 mg) as a white solid. Mp 74–77 °C; [α]_D²¹ +47.3 (*c* 0.27 in H₂O); *R*_f 0.45 (CH₂Cl₂–MeOH, 9:1); ν_{\max} (KBr)/cm^{−1} 3360 (NH, OH), 1724 (C=O, ester), 1693 (C=O, Boc, amide); δ_{H} (400 MHz; CD₃OD) 1.34 and 1.37 (2 s, 9H, C(CH₃)₃), 2.56 (dd, 1H, *J* 7.6, 16.0 Hz, H₂), 2.64 (dd, 1H, *J* 5.8, 16.0 Hz, H₂'), 3.53–3.90 (m, 9H, CO₂CH₃, 6 × H_{carb}), 4.36 (m, 1H, H₃), 4.48 (dd, 1H, *J* 8.2, 13.9 Hz, H₄), 4.59 (dd, 1H, *J* 4.7, 13.6 Hz, H₄'), 4.63 (d, 1H, *J* 12.4 Hz, OCHH), 4.80 (d, 1H, *J* 12.4 Hz, OCHH), 4.86 (s, 1H, H_{1carb}), 7.97 (s, 1H, Csp²H); δ_{C} (100.1 MHz; CD₃OD) 27.0 and 27.2 (C(CH₃)₃), 36.0 (C₂), 47.9 (C₃), 50.9 (CO₂CH₃), 52.5 (C₄), 59.2 (OCH₂), 61.5 (C_{6carb}), 67.2, 70.5, 71.0 and 73.5 (4C, CH_{carb}), 79.1 (C(CH₃)₃), 99.3 (C_{1carb}), 124.6 (Csp²H), 143.8 (Csp²), 155.9 (C=O, Boc), 171.1 (C₁); HRMS (ESI) *m/z* calcd. for C₁₉H₃₂N₄O₁₀ [M + H]⁺: 477.2197 found: 477.2198.

Deprotected glycoconjugated dimer (36)

Deacetylation of dimer **31** (50 mg, 0.043 mmol) was carried out as described for the preparation of **35** to give **36** (34 mg, 97%) as a white solid. Mp 130–132 °C; [α]_D²¹ +51.2 (*c* 0.40 in MeOH); *R*_f 0.45 (*n*-propanol–H₂O, 9:1); ν_{\max} (KBr)/cm^{−1} 3329 (NH, OH), 1724 (C=O, ester), 1685 and 1678 (C=O, Boc, amide); δ_{H} (400 MHz; D₂O) 1.20, 1.28 (2 s, 9H, C(CH₃)₃), 2.42 (m, 1H, H_{2A}), 2.48 (dd, 1H, *J* 5.5, 9.5 Hz, H_{2A}'), 2.65 (dd, 1H, *J* 8.7, 16.2 Hz, H_{2B}), 2.84 (dd, 1H, *J* 4.9, 16.2 Hz, H_{2B}'), 3.60–3.73 (m, 7H, CO₂CH₃, 4 × H_{carb}), 3.73–3.82 (m, 4H, H_{carb}), 3.83–3.98 (m, 4H, H_{carb}), 4.18–4.38 (m, 2H, H_{3A}, H₄), 4.49 (dd, 1H, *J* 2.7, 13.3 Hz, H₄), 4.53 (dd, 1H, *J* 8.6, 13.9 Hz, H₄'), 4.62–4.76 (m, 4H, H_{3B}, H₄, 2 × OCHH), 4.76–4.88 (m, 2H, OCHH), 4.93–5.01 (m, 2H, H_{1carb}), 8.07 and 8.11 (2 s, 2H, Csp²H); δ_{C} (100.1 MHz; D₂O) 27.2 and 27.5 (C(CH₃)₃), 36.1 (C_{2B}), 37.7 and 37.8 (2C, C₂), 46.8 (C_{3B}), 48.3 (C_{3A}), 52.6 (CO₂CH₃), 52.7, 53.4, 53.9, 59.5 (4C, 2 × C₄, 2 × OCH₂), 60.9 (2C, C_{6carb}), 66.7, 69.9, 70.5 and 72.9 (8C, 2 × C_{2carb}, 2 × C_{3carb}, 2 × C_{4carb}, 2 × C_{5carb}), 81.0 (C(CH₃)₃), 99.2 and 99.6 (2C, C_{1carb}), 125.9, 126.0 (2C, Csp²H), 143.4 and 143.6 (Csp²), 156.5 (C=O, Boc), 171.5 (C_{1A}), 172.6 (C_{1B}); HRMS (ESI) *m/z* calcd. for C₃₂H₅₂N₈O₁₇ [M + H]⁺: 821.3529 found: 821.3525.

Deprotected glycoconjugated trimer (37)

Deacetylation of trimer **32** (50 mg, 0.030 mmol) was carried out as described for the preparation of **35** to give **37** (34 mg, quantitative) as a white solid. Mp 174–177 °C; [α]_D²¹ +44.2 (*c* 0.20 in H₂O); *R*_f 0.65 (*n*-propanol–H₂O, 7:3); ν_{\max} (KBr)/cm^{−1} 3306 (NH, OH), 1736 (C=O, ester), 1687 (C=O, Boc), 1645 (C=O, amide); δ_{H} (400 MHz; D₂O) 1.17 and 1.26 (2 s, 9H, C(CH₃)₃), 2.39–2.49 (m, 3H, H₂), 2.53 (dd, 1H, *J* 6.1, 15.2 Hz, H₂), 2.64 (dd, 1H, *J* 8.8, 16.4 Hz, H_{2C}), 2.82 (dd, 1H, *J* 4.6, 16.4 Hz, H_{2C}'), 3.60–3.72 (m, 9H, CO₂CH₃, 6 × H_{carb}), 3.72–3.82 (m, 6H, 6 × H_{carb}), 3.83–3.97 (m, 6H, 6 × H_{carb}), 4.26 (m, 1H, H₃), 4.34–4.72 (m, 14H, 2 × H₃, 6 × H₄, 6 × OCHH), 4.94, 4.97 and 4.99 (3 s, 3H, H_{1carb}), 8.05, 8.09, 8.11 (3 s, 3H, Csp²H); δ_{C} (100.1 MHz; D₂O) 27.2 and 27.5 (C(CH₃)₃), 35.8, 37.5 and 37.8 (3C, C₂), 46.6, 47.1 and 48.1 (3C, C₃), 52.5 (CO₂CH₃), 52.7, 53.4 and 53.8 (3C, C₄), 59.5 and 59.7 (3C, C₇), 60.9 (3C, C_{6carb}), 66.7, 69.9, 70.5 and 73.0 (12C, 3 × C_{2carb}, 3 × C_{3carb}, 3 × C_{4carb}, 3 × C_{5carb}), 81.0 (C(CH₃)₃), 99.2 and 99.6 (3C, C_{1carb}), 125.7 and 125.8 (3C, Csp²H), 143.4 and 143.7 (3C, Csp²), 156.4

(C=O, Boc), 171.1, 171.3 and 172.6 (3C, C₁); HRMS (ESI) *m/z* calcd. for C₄₅H₇₂N₁₂O₂₄ [M + 2H]²⁺: 583.2464 found: 583.2451.

Deprotected glycoconjugated tetramer (38)

Deacetylation of tetramer **33** (40 mg, 0.018 mmol) was carried out as described for the preparation of **35** to give **38** (27 mg, quantitative) as a white solid. Mp 178–180 °C; [α]_D²² +33.1 (*c* 0.27 in H₂O); *R*_f 0.56 (*n*-propanol–H₂O, 7:3); ν_{\max} (KBr)/cm^{−1} 3296 (NH, OH), 3136 and 3099 (Csp²-H), 1736 (C=O, ester), 1683 (C=O, Boc), 1653 (C=O, amide); δ_{H} (400 MHz; D₂O) 1.15 and 1.23 (2 s, 9H, C(CH₃)₃), 2.36–2.58 (m, 6H, H₂), 2.64 (dd, 1H, *J* 8.8, 16.0 Hz, H_{2D}), 2.82 (d, 1H, *J* 16.0 Hz, H_{2D}'), 3.57–3.71 (m, 11H, CO₂CH₃, 8 × H_{carb}), 3.71–3.81 (m, 8H, H_{carb}), 3.82–3.98 (m, 8H, H_{carb}), 4.12–4.72 (m, 20H, 4 × H₃, 8 × H₄, 8 × OCHH), 4.94, 4.97 and 4.98 (3 s, 4H, H_{1carb}), 8.05, 8.07, 8.11 (3 s, 4H, Csp²H); δ_{C} (100.1 MHz; D₂O) 27.2 and 27.5 (C(CH₃)₃), 35.8, 37.2 and 37.4 (4C, C₂), 46.6, 47.0 and 48.1 (4C, C₃), 52.5 (CO₂CH₃), 52.6 and 53.4 (4C, C₄), 59.5 (4C, C₇), 60.9 (4C, C_{6carb}), 66.7, 69.9, 70.5 and 73.0 (16C, 4 × C_{2carb}, 4 × C_{3carb}, 4 × C_{4carb}, 4 × C_{5carb}), 80.9 (C(CH₃)₃), 99.2 (4 × C_{1carb}), 125.7 and 125.8 (4C, Csp²H), 143.6 and 143.7 (4C, Csp²), 156.4 (C=O, Boc), 170.9, 171.0, 171.3, 172.6 (4C, C₁); HRMS (ESI) *m/z* calcd. for C₅₈H₉₂N₁₆O₃₁ [M + 2H]²⁺: 755.3130 found: 755.3120.

Deprotected glycoconjugated hexamer (39)

Deacetylation of hexamer **34** (40 mg, 0.013 mmol) was carried out as described for the preparation of **35** to give **39** (28 mg, quantitative) as a white solid. Mp 189–192 °C; [α]_D²² +23.9 (*c* 0.23 in H₂O); *R*_f 0.40 (*n*-propanol–H₂O, 7:3); ν_{\max} (KBr)/cm^{−1} 3294 (NH, OH), 1732 (C=O, ester), 1678 and 1651 (C=O, Boc, amide); δ_{H} (400 MHz; D₂O) 1.14 and 1.22 (2 s, 9H, C(CH₃)₃), 2.33–2.61 (m, 10H, H₂), 2.67 (dd, 1H, *J* 9.4, 16.6 Hz, H_{2F}), 2.84 (d, 1H, *J* 5.1, 16.6 Hz, H_{2F}'), 3.57–3.71 (m, 15H, CO₂CH₃, 12 × H_{carb}), 3.71–3.81 (m, 12H, H_{carb}), 3.81–3.96 (m, 12H, H_{carb}), 4.17–4.87 (m, 30H, 6 × H₃, 6 × H₄, 6 × OCHH), 4.94, 4.97 and 4.98 (3 s, 6H, H_{1carb}), 8.05, 8.07 and 8.11 (3 s, 6H, Csp²H); δ_{C} (100.1 MHz; D₂O) 27.5 (C(CH₃)₃), 35.7, 37.8, 37.1, 37.2 and 37.3 (6C, C₂), 46.5, 46.6, 46.8, 46.9 and 47.0 (6C, C₃), 52.5 (CO₂CH₃), 52.7 (6C, C₄), 59.4 (6C, C₇), 60.9 (6C, C_{6carb}), 66.7, 69.9, 70.5 and 73.0 (24C, 6 × C_{2carb}, 6 × C_{3carb}, 6 × C_{4carb}, 6 × C_{5carb}), 80.9 (C(CH₃)₃), 99.2 (6C, C_{1carb}), 125.8 (6C, Csp²H), 143.6 (6C, Csp²), 156.4 (C=O, Boc), 170.5, 170.7, 170.8, 170.9, 171.2 and 172.6 (6C, C₁); HRMS (ESI) *m/z* calcd. for C₈₄H₁₃₂N₂₄O₄₅ [M + 2H]²⁺: 1099.4462 found: 1099.4504.

Glycoconjugated monomer (43)

The cycloaddition between alkyne **13** (99 mg, 0.351 mmol) and sugar azide **41** (100 mg, 0.351 mmol) was carried out as described for the preparation of **30**, except for CuSO₄ and acid ascorbic quantities, 0.1 M CuSO₄ solution (0.281 ml, 0.08 equiv.) and 0.1 M ascorbic acid solution (0.842 ml, 0.24 equiv.) were used, to give, after flash chromatography on silica gel (CH₂Cl₂–MeOH 8.5:1.5), **43** (175 mg, 88%), as a white solid. Mp 61–63 °C; [α]_D²³ −26.3 (*c* 0.32 in CHCl₃); *R*_f 0.59 (EtOAc); ν_{\max} (KBr)/cm^{−1} 3337 and 3320 (NH), 3140 (Csp²-H), 1736 (C=O, ester), 1714 and 1676 (C=O, Boc, amide); δ_{H} (400 MHz; CDCl₃) 1.27, 1.34, 1.38 and 1.46 (4 s, 12H, C(CH₃)₂), 1.41 (s, 9H, C(CH₃)₃), 2.68 (dd, 1H, *J* 5.2, 16.8 Hz, H₂), 2.96 (dd, 1H, *J* 3.8, 16.8 Hz, H₂'), 3.65 (s, 3H,

CO₂CH₃), 4.10–4.21 (m, 2H, H_{4carb}, H_{5carb}), 4.30 (dd, 1H, *J* 2.3, 4.7 Hz, H_{2carb}), 4.41 (dd, 1H, *J* 8.4, 14.0 Hz, H_{6carb}), 4.47–4.58 (m, 3H, H₃, NHCHH, H_{6carb}), 4.56 (dd, 1H, *J* 3.5 14.3 Hz, NHCHH), 4.61 (dd, 1H, *J* 1.9, 7.6 Hz, H_{3carb}), 5.48 (d, 1H, *J* 4.9 Hz, H_{1carb}), 5.65 (d, 1H, *J* 7.3 Hz, NH), 7.11 (bs, 1H, NH'), 7.66 (bs, 1H, Csp²H); δ_C (100.1 MHz; CDCl₃) 24.4, 24.9, 25.9 (3 × C(CH₃)₂), 28.3 (C(CH₃)₃), 35.2 (NCH₂), 35.9 (C₂), 50.6 (C_{6carb}), 50.7 (C₃), 52.0 (CO₂CH₃), 67.1 (C_{4carb}), 70.3 (C_{2carb}), 70.7 (C_{3carb}), 71.1 (C_{5carb}), 80.6 (C(CH₃)₃), 96.2 (C_{1carb}), 109.1 and 109.9 (2 × C(CH₃)₂), 155.5 (C=O, Boc), 170.7 (C₁), 172.2 (C₄); HRMS (ESI) *m/z* calcd. for C₂₅H₃₉N₅O₁₀ [M + H]⁺: 570.2775 found: 570.2755.

Glycoconjugated dimer (44)

The cycloaddition between dialkyne **22** (72 mg, 0.166 mmol) and sugar azide **41** (94 mg, 0.331 mmol) was carried out as described for the preparation of **30** to give, after flash chromatography on silica gel (AcOEt–MeOH 9.7:0.3), **44** (109 mg, 65%), as a white solid. Mp 124–127 °C; [α]_D²⁴ –40.5 (*c* 0.55 in CHCl₃); *R*_f 0.62 (EtOAc–MeOH, 9:1); ν_{max} (KBr)/cm^{–1} 3312 and 3277 (NH), 3144 and 3084 (Csp²–H), 1737 (C=O, ester), 1688 (C=O, Boc), 1658 and 1643 (C=O, amide); δ_H (400 MHz; Pyridine-*d*₅) 1.25, 1.26, 1.35, 1.36, 1.47, 1.54 and 1.56 (7 s, 24H, C(CH₃)₂), 1.38 (s, 9H, C(CH₃)₃), 2.96–3.08 (m, 2H, H_{2A}, H_{2B}), 3.16 (dd, 1H, *J* 7.9, 13.9 Hz, H_{2A}), 3.29 (dd, 1H, *J* 5.8, 16.0 Hz, H_{2B}), 3.54 (s, 3H, CO₂CH₃), 4.29 (dd, 1H, *J* 1.0, 7.9 Hz, H_{5carb}), 4.35 (d, 1H, *J* 7.6 Hz, H_{5carb}), 4.46–4.53 (m, 2H, 2 × H_{2carb}), 4.53–4.63 (m, 2H, 2 × H_{4carb}), 4.64–4.89 (m, 10H, 4 × NHCHH, 2 × H_{3carb}, 3 × H_{6carb}), 5.29 (m, 1H, H_{3A}), 5.55 (m, 1H, H_{3B}), 5.62–5.73 (m, 2H, 2 × H_{1carb}), 8.18 and 8.19 (2 s, 2H, 2 × Csp²H), 8.30 (d, 1H, *J* 8.3 Hz, NH_A), 9.46 (t, 1H, *J* 4.2 Hz, NH'), 9.67 (t, 1H, *J* 4.6 Hz, NH'), 9.75 (d, 1H, *J* 8.4 Hz, NH_B); δ_C (100.1 MHz; Pyridine-*d*₅) 24.7, 25.3, 26.4 and 26.5 (8C, C(CH₃)₂), 28.6 (C(CH₃)₃), 36.0 and 36.1 (2C, NHCH₂), 37.3 (C_{2B}), 39.6 (C_{2A}), 51.1 (3C, C_{3B}, 2 × C_{6carb}), 51.9 (CO₂CH₃), 53.2 (C_{3A}), 67.9 and 68.0 (2C, C_{4carb}), 71.2 (2C, C_{2carb}), 71.5 (2C, C_{3carb}), 71.9 (2C, C_{5carb}), 79.5 (C(CH₃)₃), 97.0 (2C, C_{1carb}), 109.3 and 110.0 (4C, C(CH₃)₂), 124.5 and 124.6 (2C, Csp²H), 146.3 and 146.5 (2C, Csp²), 156.9 (C=O, Boc), 171.5 and 171.7 (2C, C₁), 172.0 and 173.0 (2C, C₄); HRMS (ESI) *m/z* calcd. for C₄₄H₆₆N₁₀O₁₇ [M + H]⁺: 1007.4686 found: 1007.4679.

Glycoconjugated dimer (45)

The cycloaddition between dialkyne **22** (30 mg, 0.069 mmol) and sugar azide **42** (57 mg, 0.137 mmol) was carried out as described for the preparation of **30** to give, after flash chromatography on silica gel (AcOEt–MeOH 9.7:0.3), **45** (68 mg, 78%), as a white solid. Mp 119–122 °C; *R*_f 0.20 (CH₂Cl₂–MeOH, 93:7); δ_H (400 MHz; Pyridine-*d*₅) 1.40 (C(CH₃)₃), 2.00, 2.01, 2.01, 2.02, 2.03, 2.21 and 2.26 (7 s, 24H, CH₃COO, CH₃CON), 3.02–3.14 (m, 2H, H_{2A}, H_{2B}), 3.31 (dd, 1H, *J* 9.3, 13.6 Hz, H_{2A}), 3.29 (dd, 1H, *J* 5.1, 16.2 Hz, H_{2B}), 3.52 (s, 3H, CO₂CH₃), 3.94–4.08 (m, 2H, OCH₂CH₂), 4.14–4.55 (m, 8H, 2 × H_{5carb}, 4 × H_{6carb}, 2 × OCHHCH₂), 4.55–4.86 (m, 7H, 3 × NHCHH, 4 × OCH₂CHH), 5.00 (dd, 1H, *J* 6.3, 15.3 Hz, NHCHH), 5.06–5.17 (m, 2H, H_{2carb}), 5.35 (m, 1H, H_{3A}), 5.38 (d, 1H, *J* 3.3 Hz, H_{1carb}), 5.41 (m, 1H, H_{3B}), 5.44 (d, 1H, *J* 3.2 Hz, H_{1carb}), 5.59 (dd, 1H, *J* 3.1, 11.7 Hz, H_{3carb}), 5.76 (dd, 1H, *J* 2.1, 12.1 Hz, H_{3carb}), 5.80 (d, 1H, *J* 2.6 Hz, H_{4carb}), 5.88 (d, 1H, *J* 1.6 Hz, H_{4carb}), 8.06 (s, 1H, Csp²H), 8.19 (s, 1H, Csp²H), 8.39 (d,

1H, *J* 8.6 Hz, NH_A), 9.28 (m, 1H, NH'), 9.65 (m, 1H, NH'), 9.84 (d, 1H, *J* 7.6 Hz, NH_B); δ_C (100.1 MHz; Pyridine-*d*₅) 20.9, 21.0, 21.1 and 21.1 (6C, CH₃COO), 23.6 and 23.6 (2C, CH₃CON), 28.8 (C(CH₃)₃), 36.1 and 36.5 (2C, NHCH₂), 37.0 (C_{2B}), 39.9 (C_{2A}), 48.7 and 48.8 (2C, C_{2carb}), 50.1 and 50.3 (2C, OCH₂CH₂), 51.8 (C_{3B}), 52.1 (CO₂CH₃), 53.3 (C_{3A}), 62.8 and 63.0 (2C, C_{6carb}), 66.9 and 67.3 (2C, OCH₂CH₂), 67.9 (2C, C_{5carb}), 68.5 and 69.7 (2C, C_{4carb}), 69.2 and 69.3 (2C, C_{3carb}), 79.7 (C(CH₃)₃), 98.5 and 99.0 (2C, C_{1carb}), 123.8 (2C, Csp²H), 146.3 and 147.0 (2C, Csp²), 156.9 (C=O, Boc), 170.9, 171.0, 171.1, 171.3 and 171.4 (CH₃COO, CH₃CON), 171.9 (C₄), 172.1 and 172.3 (2C, C₁), 173.8 (C₄); HRMS (ESI) *m/z* calcd. for C₅₂H₇₆N₁₂O₂₅ [M + H]⁺: 1269.5137 found: 1269.5123.

Glycoconjugated monomer (46)

CuSO₄/ascorbic acid. The cycloaddition between alkyne **19** (100 mg, 0.223 mmol) and sugar azide **40** (93 mg, 0.223 mmol) was carried out as described for the preparation of **30** except for CuSO₄ and ascorbic acid quantities, 0.1 M CuSO₄ solution (2.23 ml, 1 equiv.) and 0.1 M ascorbic acid solution (6.69 ml, 3 equiv.) were used, to give, after flash chromatography on silica gel (CH₂Cl₂–MeOH 9.5:0.5), **46** (158 mg, 82%).

(EtO)₃PCuI. Alkyne **19** (134 mg, 0.30 mmol), sugar azide **40** (141 mg, 0.30 mmol), (EtO)₃PCuI (11 mg, 0.03 mmol) and diisopropyl ethyl amine (0.156 ml, 0.90 mmol) were dissolved in toluene (3 ml) and exposed to 30 W microwave irradiation at 80 °C for 30 min. After cooling to room temperature, the solution was concentrated *in vacuo*, and the residue was purified by flash chromatography on silica gel (AcOEt) to yield the glycoconjugate **46** (262 mg, 95%) as a white solid. Mp 77–80 °C; [α]_D²⁴ +25.8 (*c* 0.51 in CHCl₃); *R*_f 0.33 (CH₂Cl₂–MeOH, 95:5); ν_{max} (KBr)/cm^{–1} 3377 and 3348 (NH), 3142 (Csp²–H), 1746 (C=O, ester), 1678 (C=O, Fmoc, amide); δ_H (400 MHz; CDCl₃) 1.43 (s, 9H, C(CH₃)₃), 1.98, 2.03, 2.08 and 2.11 (4 s, 12H, CH₃CO), 2.69 (dd, 1H, *J* 5.7, 16.7 Hz, H₂), 2.84 (dd, 1H, *J* 5.5, 16.7 Hz, H₂'), 3.53 (m, 1H, H_{5carb}), 3.84 (m, 1H, OCHHCH₂), 4.03 (dd, 1H, *J* 2.1, 12.3 Hz, OCHHCH₂), 4.14–4.23 (m, 2H, 2H_{6carb}), 4.20 (t, 1H, *J* 7.3 Hz, CH Fmoc), 4.33–4.46 (m, 2H, CH₂ Fmoc), 4.47–4.67 (m, 5H, OCH₂CH₂, NHCH₂, H₃), 4.70 (bs, 1H, H_{1carb}), 5.13 (bs, 1H, H_{2carb}), 5.15–5.26 (m, 2H, H_{3carb}, H_{4carb}), 6.02 (d, 1H, *J* 8.7 Hz, NH), 7.18 (m, 1H, NH'), 7.30 (t, 2H, *J* 7.4 Hz, H_{Ar}), 7.39 (t, 2H, *J* 7.4 Hz, H_{Ar}), 7.52 (d, 2H, *J* 7.4 Hz, H_{Ar}), 7.62 (s, 1H, Csp²H), 7.75 (d, 2H, *J* 7.4 Hz, H_{Ar}); δ_C (100.1 MHz; CDCl₃) 20.6, 20.7 and 21.0 (4C, CH₃CO), 28.0 (C(CH₃)₃), 35.3 (NHCH₂), 37.4 (C₂), 47.1 (CH Fmoc), 49.6 (OCH₂CH₂), 51.3 (C₃), 62.1 (C_{6carb}), 65.7 (C_{4carb}), 66.1 (OCH₂CH₂), 67.1 (CH₂ Fmoc), 68.8 (C_{3carb}), 69.0 (C_{5carb}), 69.2 (C_{2carb}), 81.6 (C(CH₃)₃), 97.4 (C_{1carb}), 120.0 (CH_{Ar}), 123.3 (Csp²H), 125.0, 127.0 and 127.7 (CH_{Ar}), 141.2 and 143.6 (C_{Ar}), 145.0 (Csp²), 156.0 (C=O, Fmoc), 169.6 and 170.1 (2C, CH₃CO), 170.3 (C₁), 170.5 and 170.6 (2C, CH₃CO), 170.7 (C₄); HRMS (ESI) *m/z* calcd. for C₄₂H₅₁N₅O₁₅ [M + H]⁺: 866.3460 found: 866.3461.

Glycoconjugated dimer (47)

CuSO₄/ascorbic acid. The cycloaddition between alkyne **23** (100 mg, 0.166 mmol) and sugar azide **40** (140 mg, 0.333 mmol) was carried out as described for the preparation of **30**, except for CuSO₄ and ascorbic acid quantities, 0.1 M CuSO₄ solution (1.66 ml, 1 equiv.) and 0.1 M ascorbic acid solution (5.0 ml,

3 equiv.) were used, to give, after flash chromatography on silica gel (CH_2Cl_2 –MeOH 9.5:0.5 to 9:1), **47** (176 mg, 74%).

(EtO)₃PCuI. Alkyne **23** (75 mg, 0.125 mmol), sugar azide **40** (118 mg, 0.250 mmol), (EtO)₃PCuI (9 mg, 0.025 mmol) and diisopropyl ethyl amine (0.065 ml, 0.375 mmol) were dissolved in DMF (2 ml) and exposed to 15 W microwave irradiation at 80 °C for 30 min. After cooling to room temperature, the solution was concentrated in vacuo, and the residue was purified by flash chromatography on silica gel (AcOEt) to yield the glycoconjugate **46** (62 mg, 35%) as a white solid. Mp 116–119 °C; $[\alpha]_{\text{D}}^{24} +14.2$ (*c* 0.58 in CHCl_3); R_f 0.30 (CH_2Cl_2 –MeOH, 98:2); ν_{max} (KBr)/ cm^{-1} 3304 and 3295 (NH), 3082 (Csp²-H), 1748 (C=O, ester), 1659 (C=O, Fmoc, amide); δ_{H} (400 MHz; CDCl_3) 1.41 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.97, 1.99, 2.02, 2.03, 2.07, 2.08 and 2.09 (7 s, 24H, CH_3CO), 2.62 (dd, 1H, *J* 3.7, 14.5 Hz, H_{2A}), 2.72 (m, 1H, $\text{H}_{2'A}$), 2.74 (dd, 1H, *J* 5.4, 16.6 Hz, H_{2B}), 2.84 (dd, 1H, *J* 6.1, 16.6 Hz, $\text{H}_{2'B}$), 3.59 (m, 1H, $\text{H}_{5\text{carb}}$), 3.65 (m, 1H, $\text{H}_{5\text{carb}}$), 3.76–3.88 (m, 2H, 2 × OCHHCH_2), 3.98–4.10 (m, 4H, 2 × OCHHCH_2 , 2 × $\text{H}_{6\text{carb}}$), 4.13 (m, 1H, CH Fmoc), 4.19 (dd, 2H, *J* 5.1 12.5 Hz, 2 × $\text{H}_{6\text{carb}}$), 4.40 (m, 2H, CH_2 Fmoc), 4.35–4.46 (m, 3H, NHCHH), 4.52–4.56 (m, 4H, OCH_2CH_2), 4.64 (dd, 1H, *J* 6.3, 15.4 Hz, NHCHH), 4.69–4.74 (m, 2H, H_{3A} , $\text{H}_{1\text{carb}}$), 4.76 (s, 1H, $\text{H}_{1\text{carb}}$), 4.83 (m, 1H, H_{3B}), 5.16 (m, 1H, $\text{H}_{2\text{carb}}$), 5.17–5.30 (m, 5H, 2 × $\text{H}_{3\text{carb}}$, 2 × $\text{H}_{4\text{carb}}$, $\text{H}_{2\text{carb}}$), 6.29 (d, 1H, *J* 8.4 Hz, NH_A), 7.28 (t, 2H, *J* 7.3 Hz, H_{Ar}), 7.38 (t, 2H, *J* 7.2 Hz, H_{Ar}), 7.43 (m, 2H, NH_B , NH'), 7.55 (d, 1H, *J* 7.2 Hz, H_{Ar}), 7.56 (d, 1H, *J* 7.3 Hz, H_{Ar}), 7.68 and 7.69 (2 s, 2H, 2 × Csp²H), 7.74 (d, 2H, *J* 7.5 Hz, H_{Ar}), 7.85 (m, 1H, NH'); δ_{C} (100.1 MHz; CDCl_3) 20.6, 20.7 and 20.9 (8C, CH_3CO), 28.0 ($\text{C}(\text{CH}_3)_3$), 35.2 and 35.3 (2C, NHCH_2), 36.9 (C_{2B}), 38.1 (C_{2A}), 47.1 (CH Fmoc), 49.5 (2C, OCH_2CH_2), 49.6 (C_{3B}), 51.5 (C_{3A}), 62.2 (2C, $\text{C}_{6\text{carb}}$), 65.4 and 65.7 (2C, $\text{C}_{4\text{carb}}$), 66.1 and 66.2 (2C, OCH_2CH_2), 66.9 (CH2 Fmoc), 68.8, 68.9, 69.0, 69.1 and 69.3 (6C, 2 × $\text{C}_{2\text{carb}}$, 2 × $\text{C}_{3\text{carb}}$, 2 × $\text{C}_{5\text{carb}}$), 81.3 ($\text{C}(\text{CH}_3)_3$), 97.4 and 97.5 (2C, $\text{C}_{1\text{carb}}$), 120.0 (CH_{Ar}), 123.3 and 123.6 (2C, Csp²H), 124.9, 127.0 and 127.7 (CH_{Ar}), 141.2, and 143.6 (C_{Ar}), 144.8 and 145.6 (2C, Csp²), 156.1 (C=O, Fmoc), 169.6, 170.2, 170.4, 170.5, 170.6, 170.8, 171.3 and 171.4 (12C, C_1 , C_4 , CH_3CO); HRMS (ESI) *m/z* calcd. for $\text{C}_{65}\text{H}_{82}\text{N}_{10}\text{O}_{27}$ [*M* + *H*]⁺: 718.2748 found: 718.2753.

Glycoconjugated trimer (48)

The cycloaddition between alkyne **26** (38 mg, 0.05 mmol) and sugar azide **40** (63 mg, 0.151 mmol) was carried out as described for the preparation of **30**, except for CuSO_4 and ascorbic acid quantities, 0.1 M CuSO_4 solution (0.5 ml, 1 equiv.) and 0.1 M ascorbic acid solution (1.51 ml, 3 equiv.) were used, to give, after flash chromatography on silica gel (CH_2Cl_2 –MeOH 9.5:0.5), **48** (70 mg, 70%), as a white solid. Mp 132–135 °C; $[\alpha]_{\text{D}}^{25} +18.0$ (*c* 0.45 in CHCl_3); R_f 0.41 (CH_2Cl_2 –MeOH, 9:1); ν_{max} (KBr)/ cm^{-1} 3298 and 3285 (NH), 3130 and 3072 (Csp²-H), 1748 (C=O, ester), 1693 (C=O, Fmoc), 1643 (C=O, amide); δ_{H} (500 MHz; Pyridine-*d*₅) 1.42 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.99, 2.03, 2.08, 2.08 and 2.10 (5 s, 36H, CH_3CO), 2.92 (dd, 1H, *J* 8.1, 15.8 Hz, H_2), 2.95 (m, 1H, H_2), 2.97 (m, 1H, H_{2A}), 3.18 (m, 1H, $\text{H}_{2'A}$), 3.20 (m, 1H, H_2), 3.33 (m, 1H, H_2), 3.91–4.02 (m, 3H, $\text{H}_{5\text{carb}}$), 4.02–4.10 (m, 3H, OCHHCH_2), 4.17 (m, 1H, CH Fmoc), 4.20–4.36 (m, 4H, 3 × OCHHCH_2 , CHH Fmoc), 4.37–4.47 (m, 3H, $\text{H}_{6\text{carb}}$), 4.49–4.61 (m, 4H, 3 × $\text{H}_{6\text{carb}}$, CHH Fmoc), 4.68 (t, 2H, *J* 5.3 Hz, OCH_2CH_2), 4.71–4.89 (m, 10H, OCH_2CH_2 , NHCHH , NHCHH), 5.12, 5.14 and 5.17 (3 s,

3H, $\text{H}_{1\text{carb}}$), 5.43 (m, 1H, H_{3A}), 5.55 (m, 1H, H_3), 5.62–5.72 (m, 6H, 3 × $\text{H}_{2\text{carb}}$, 3 × $\text{H}_{3\text{carb}}$), 5.72–5.84 (m, 4H, H_3 , 3 × $\text{H}_{4\text{carb}}$), 7.26 (m, 2H, CH_{Ar}), 7.40 (t, 2H, *J* 6.9 Hz, CH_{Ar}), 7.60 (m, 2H, H_{Ar}), 7.84 (d, 2H, *J* 7.6 Hz, CH_{Ar}), 8.19, 8.32 and 8.34 (3 s, 3H, Csp²H), 9.07 (d, 1H, *J* 8.4 Hz, NH_A), 9.40 (m, 2H, 2 × NH'), 9.59 (d, 1H, *J* 8.5 Hz, NH), 9.72 (d, 1H, *J* 8.0 Hz, NH), 9.73 (m, 1H, NH'); δ_{C} (100.1 MHz; Pyridine-*d*₅) 20.9, 21.0 and 21.2 (12C, CH_3CO), 28.4 ($\text{C}(\text{CH}_3)_3$), 36.2 and 36.3 (2C, NHCH_2), 48.0 (CH Fmoc), 50.1 (3C, OCH_2CH_2), 51.5, 51.6 and 53.6 (3C, C_3), 63.1 (3C, $\text{C}_{6\text{carb}}$), 66.8 (3C, $\text{C}_{4\text{carb}}$), 67.1 and 67.2 (4C, 3 × OCH_2CH_2 , CH_2 Fmoc), 69.9 (3C, $\text{C}_{5\text{carb}}$), 70.29, 70.34 and 70.4 (6C, 3 × $\text{C}_{2\text{carb}}$, 3 × $\text{C}_{3\text{carb}}$), 81.1 ($\text{C}(\text{CH}_3)_3$), 98.4 (3C, $\text{C}_{1\text{carb}}$), 120.9 (CH_{Ar}), 126.1 and 126.2 (3C, Csp²H), 128.0 and 128.5 (CH_{Ar}), 142.1 (C_{Ar}), 145.0, 146.4, 146.5 and 147.0 (3 × C_6 , C_{Ar}), 155.8 (C=O, Fmoc), 170.5, 170.6, 170.7, 170.7, 171.1, 171.2, 171.5 and 173.1 (3 × C_1 , 3 × C_4 , CH_3CO); HRMS (ESI) *m/z* calcd. for $\text{C}_{88}\text{H}_{113}\text{N}_{15}\text{O}_{39}$ [*M* + *H*]⁺: 1002.8733 found: 1002.8732.

Glycoconjugated tetramer (49)

The cycloaddition between alkyne **27** (70 mg, 0.077 mmol) and sugar azide **40** (129 mg, 0.309 mmol) was carried out as described for the preparation of **30** excepted for CuSO_4 and ascorbic acid quantities, 0.1 M CuSO_4 solution (0.774 ml, 1 equiv.) and 0.1 M ascorbic acid solution (2.32 ml, 3 equiv.) were used, to give after flash chromatography on silica gel (CH_2Cl_2 –MeOH 9.5:0.5), **49** (157 mg, 80%), as a white solid. Mp 162–164 °C; $[\alpha]_{\text{D}}^{25} +20.7$ (*c* 0.57 in CHCl_3); R_f 0.88 (CH_2Cl_2 –MeOH, 8:2); ν_{max} (KBr)/ cm^{-1} 3300 (NH), 3144, 3078 and 3063 (Csp²-H), 1748 and 1647 (C=O, ester, Fmoc, amide); δ_{H} (500 MHz; Pyridine-*d*₅) 1.42 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.97, 1.99, 2.00, 2.03, 2.08, 2.08 and 2.10 (7 s, 48H, CH_3CO), 2.92 (dd, 1H, *J* 8.6, 15.6 Hz, H_{2D}), 2.93–3.02 (m, 3H, H_{2A} , H_{2B} , H_{2C}), 3.09 (dd, 1H, *J* 8.6, 13.9 Hz, H_2), 3.13–3.25 (m, 2H, $\text{H}_{2'A}$, H_2), 3.31 (dd, 1H, *J* 5.2, 15.6 Hz, $\text{H}_{2'D}$), 3.92–4.12 (m, 8H, 4 × $\text{H}_{5\text{carb}}$, 4 × OCHHCH_2), 4.13–4.37 (m, 6H, 4 × OCHHCH_2 , CH Fmoc, CHH Fmoc), 4.36–4.46 (m, 4H, $\text{H}_{6\text{carb}}$), 4.48–4.60 (m, 5H, 4 × $\text{H}_{6\text{carb}}$, CHH Fmoc), 4.77–4.94 (m, 16H, 8 × NHCHH , 8 × OCH_2CHH), 5.12, 5.13 and 5.18 (3 s, 4H, $\text{H}_{1\text{carb}}$), 5.41 (m, 1H, H_{3A}), 5.54 (m, 1H, H_{3D}), 5.67–5.76 (m, 14H, 2 × H_3 , 4 × $\text{H}_{2\text{carb}}$, 4 × $\text{H}_{3\text{carb}}$, 4 × $\text{H}_{4\text{carb}}$), 7.26 (m, 2H, H_{Ar}), 7.40 (t, 2H, *J* 7.5 Hz, H_{Ar}), 7.61 (m, 2H, H_{Ar}), 7.83 (d, 2H, *J* 7.5 Hz, H_{Ar}), 8.40 (d, 2H, *J* 5.6 Hz, H_{Ar}), 8.08, 8.20, 8.30 and 8.39 (4 s, 4H, Csp²H), 9.04 (d, 1H, *J* 8.5 Hz, NH_A), 9.35–9.49 (m, 3H, NH'), 9.56 (d, 1H, *J* 8.4 Hz, NH_D), 9.63 (m, 2H, NH_B , NH_C), 9.71 (t, 1H, *J* 5.1 Hz, NH'); δ_{C} (100.1 MHz; Pyridine-*d*₅) 20.9, 21.1 and 21.2 (16C, CH_3CO), 28.4 ($\text{C}(\text{CH}_3)_3$), 36.2 and 36.3 (4C, NHCH_2), 38.8 (C_{2D}), 39.5, 39.6 and 39.9 (3C, C_2), 48.1 (CH Fmoc), 50.1 (4C, OCH_2CH_2), 51.5 (C_{3D}), 51.6 and 51.6 (2C, C_3), 53.7 (C_{3A}), 63.1 (4C, $\text{C}_{6\text{carb}}$), 66.8 (4C, $\text{C}_{4\text{carb}}$), 67.2 (5C, 4 × OCH_2CH_2 , CH_2 Fmoc), 69.8, 70.3 and 70.4 (12C, 4 × $\text{C}_{2\text{carb}}$, 4 × $\text{C}_{3\text{carb}}$, 4 × $\text{C}_{5\text{carb}}$), 81.1 ($\text{C}(\text{CH}_3)_3$), 98.4 ($\text{C}_{1\text{carb}}$), 124.0, 125.1 (C_7), 120.9, 126.1, 128.0 and 128.6 (CH_{Ar}), 142.1, 145.0, 146.4, 146.5 and 146.9 (4 × Csp², C_{Ar}), 157.6 (C=O, Fmoc), 170.6, 170.7, 170.8, 171.1, 171.2, 171.5, 171.6, 172.1, 173.1 and 173.3 (4 × C_1 , 4 × C_4 , CH_3CO); HRMS (ESI) *m/z* calcd. for $\text{C}_{111}\text{H}_{144}\text{N}_{20}\text{O}_{50}$ [*M* + *H*]⁺: 1287.47 found: 1287.4717.

Deprotected glycoconjugated monomer (50)

The fully protected glycoconjugate **46** (100 mg, 0.116 mmol) was dissolved in a 2:1 CH_3CN –Et₃NH mixture (4.3 ml) at 0 °C. The

reaction mixture was then stirred for 0.5 h at 0 °C, 0.5 h at room temperature and concentrated *in vacuo*. The residue was co-evaporated with CH₂Cl₂ (3 × 10 ml) and purified by flash chromatography on silica gel (CH₂Cl₂–MeOH 9.5:0.5–9:1) to yield the unmasked amine coming from **50** in almost quantitative yield (72 mg). The next step was the deprotection of the *t*-Bu ester. A portion of the amine (32 mg, 0.050 mmol) was dissolved in a 1:1 CH₂Cl₂–TFA mixture (1 ml) and stirred at 0 °C for 2 h. The mixture was then concentrated and co-evaporated with CH₂Cl₂ (3 × 10 ml), the crude compound was then dissolved in dry MeOH and the pH was adjusted to 8 with a 0.04 M solution of NaOMe in MeOH (freshly prepared from Na and MeOH before use). The reaction mixture was stirred for 5 h, then neutralized with Dowex 50W-X8 (H⁺ form, washed with H₂O and MeOH before use) and filtered through a sintered glass funnel. The resin was washed with H₂O, MeOH, was concentrated *in vacuo* and the remainder of the solution was lyophilised to yield **50** (21 mg, 97%) as a white hygroscopic solid. [α]_D²⁵ +22.8 (*c* 0.27 in H₂O); *R*_f 0.54 (*n*-propanol–H₂O, 7:3); δ_{H} (400 MHz; CD₃OD) 2.84 (dd, 1H, *J* 8.4, 17.8 Hz, H₂), 2.96 (dd, 1H, *J* 4.6, 17.8 Hz, H₂'), 3.05 (m, 1H, H_{5carb}), 3.58 (m, 2H, H_{3carb}, H_{4carb}), 3.63 (dd, 1H, *J* 5.8, 11.9 Hz, H_{6carb}), 3.70–3.78 (m, 2H, H_{2carb}, H_{6carb}'), 3.83 (m, 1H, OCHHCH₂), 4.09 (m, 1H, OCHHCH₂), 4.17 (dd, 1H, *J* 4.6, 8.2 Hz, H₃), 4.51 (dd, 1H, *J* 4.6, 15.4 Hz, NHCHH), 4.54 (d, 1H, *J* 15.4 Hz, NHCHH), 4.61 (t, 2H, *J* 4.6 Hz, OCH₂CHH), 4.69 (s, 1H, H_{1carb}), 7.93 (s, 1H, Csp²H); δ_{C} (100.1 MHz; CD₃OD) 35.9 (2C, C₂, NHCH₂), 51.4 (C₃, OCH₂CH₂), 62.8 (C_{6carb}), 66.7 (OCH₂CH₂), 68.5 (C_{carb}), 71.9 (C_{2carb}), 72.4 (C_{carb}), 74.5 (C_{5carb}), 101.6 (C_{1carb}), 125.4 (Csp²H), 145.5 (Csp²), 169.4 and 173.4 (2C, C₁, C₄); HRMS (ESI) *m/z* calcd. for C₁₅H₂₅N₅O₉ [M + H]⁺: 420.1731 found: 420.1721.

Deprotected glycoconjugated dimer (51)

Deprotection of dimer **47** (100 mg, 0.069 mmol) was carried out as described for the preparation of **50** to give **51** (51 mg, 98%, three steps) as a white solid. Mp 142–145 °C; [α]_D²⁵ –19.1 (*c* 0.22 in H₂O); *R*_f 0.50 (*n*-propanol–H₂O, 7:3); δ_{H} (400 MHz; D₂O) 2.57 (dd, 1H, *J* 8.6, 16.0 Hz, H₂), 2.67 (dd, 1H, *J* 5.0, 16.0 Hz, H₂'), 2.75 (m, 1H, H₂), 2.79 (dd, 1H, *J* 6.1, 15.5 Hz, H₂'), 2.95 (m, 1H, H_{5carb}), 3.00 (m, 1H, H_{5carb}), 3.54–3.69 (m, 6H, H_{3carb}, H_{4carb}, H_{6carb}), 3.73 (dd, 1H, *J* 2.2, 12.2 Hz, H_{6carb}'), 3.74 (dd, 1H, *J* 2.2, 12.2 Hz, H_{6carb}'), 3.85 (m, 2H, H_{2carb}); 3.87–3.94 (m, 2H, OCHHCH₂), 3.96 (m, 1H, H₃), 4.01–4.12 (m, 2H, OCHHCH₂), 4.47 (d, 4H, *J* 4.1, NHCHH), 4.54–4.69 (m, 5H, H₃, OCH₂CHH), 4.79 (m, 2H, H_{1carb}), 7.92 and 7.96 (2 s, 2H, Csp²H); δ_{C} (100.1 MHz; D₂O) 34.5 and 34.6 (2C, NHCH₂), 38.7 (2C, C₂), 50.0 (2C, OCH₂CH₂), 51.2 and 51.8 (2C, C₃) 60.6 (2C, C_{6carb}), 65.2 and 65.4 (2C, OCH₂CH₂), 66.4 (2C, C_{carb}), 69.9 (2C, C_{2carb}), 70.4 (2C, C_{carb}), 72.7 (2C, C_{5carb}), 99.4 and 99.5 (2C, C_{1carb}), 124.4 and 124.5 (2C, Csp²H), 144.4 and 144.7 (2C, Csp²), 171.8, 173.5 and 177.5 (4C, 4 × C₁, 4 × C₄); HRMS (ESI) *m/z* calcd. for C₃₀H₄₈N₁₀O₁₇ [M + H]⁺: 821.3250 found: 821.3272.

Deprotected glycoconjugated trimer (52)

Deprotection of trimer **48** (50 mg, 0.025 mmol) was carried out as described for the preparation of **50** to give **52** (28 mg, 91%, three steps) as a white solid. Mp 139–141 °C; [α]_D²⁵ +18.9 (*c* 0.22 in H₂O); *R*_f 0.47 (*n*-propanol–H₂O, 7:3); δ_{H} (400 MHz; D₂O) 2.67 (dd, 1H, *J* 7.8, 15.4 Hz, H₂), 2.78–2.91 (m, 3H, H₂), 2.91–3.06 (m, 5H, 2 ×

H₂, 3 × H_{5carb}), 3.53–3.68 (m, 9H, 3 × H_{3carb}, 3 × H_{4carb}, 3 × H_{6carb}), 3.68–3.77 (m, 3H, H_{6carb}'), 3.81–3.86 (m, 3H, H_{2carb}), 3.89 (m, 3H, OCHHCH₂), 4.01–4.12 (m, 3H, OCHHCH₂), 4.35 (dd, 1H, *J* 5.7, 7.0 Hz, H₃), 4.43, 4.46 and 4.49 (3 s, 6H, NHCHH), 4.58–4.67 (m, 6H, OCH₂CHH), 4.71 (m, 2H, H₃), 4.77 (m, 3H, H_{1carb}), 7.91, 7.92 and 7.96 (3 s, 3H, Csp²H); δ_{C} (100.1 MHz; D₂O) 34.5 and 34.6 (3C, NHCH₂), 35.3, 35.5 and 36.8 (3C, C₂), 49.8 and 50.4 (3C, C₃), 50.1 (3C, OCH₂CH₂), 60.7 (3C, C_{6carb}), 65.4 (3C, OCH₂CH₂), 66.4 (3C, C_{carb}), 67.9 (3C, C_{2carb}), 70.4 (3C, C_{carb}), 72.7 (3C, C_{5carb}), 99.5 (3C, C_{1carb}), 124.5 and 124.6 (3C, Csp²H), 143.9, 144.5 and 144.5 (3C, Csp²), 168.4, 170.0, 171.7, 172.1, 172.3 and 174.0 (6C, 3 × C₁, 3 × C₄); HRMS (ESI) *m/z* calcd. for C₄₅H₇₁N₁₅O₂₅ [M + 2H]²⁺: 611.7450 found: 611.7446.

Deprotected glycoconjugated tetramer (53)

Deprotection of tetramer **49** (100 mg, 0.039 mmol) was carried out as described for the preparation of **50** to give **53** (45 mg, 72%, three steps) as a white solid. Mp 127–130 °C; [α]_D²⁵ –10.3 (*c* 0.31 in H₂O); *R*_f 0.10 (*n*-propanol–H₂O, 7:3); δ_{H} (400 MHz; D₂O) 2.63–2.76 (m, 2H, H₂), 2.78–2.91 (m, 2H, H₂'), 2.91–3.03 (m, 8H, 4 × H₂, 4 × H_{5carb}), 3.53–3.68 (m, 12H, 4 × H_{3carb}, 4 × H_{4carb}, 4 × H_{6carb}), 3.67–3.76 (m, 4H, 4 × H_{6carb}'), 3.85 (m, 4H, H_{2carb}), 3.89 (m, 4H, OCHHCH₂), 3.96–4.15 (m, 5H, 4 × OCHHCH₂, H₃), 4.35 (t, 1H, *J* 7.0 Hz, H₃), 4.42, 4.46, 4.49 (3 s, 8H, NHCHH), 4.55–4.67 (m, 8H, OCH₂CHH), 4.72 (t, 2H, *J* 6.7 Hz, H₃), 4.76 (m, 4H, H_{1carb}), 7.91, 7.92, 7.98 (4 s, 4H, Csp²H); δ_{C} (100.1 MHz; D₂O) 34.6 and 34.7 (4C, NHCH₂), 35.3 and 36.8 (4C, C₂), 49.8, 50.2, 50.4 and 50.5 (4C, C₃), 50.1 (4C, OCH₂CH₂), 60.7 (4C, C_{6carb}), 65.4 (4C, OCH₂CH₂), 66.4 (4C, C_{carb}), 69.9 (4C, C_{2carb}), 70.4 (4C, C_{carb}), 72.7 (4C, C_{5carb}), 99.5 (4C, C_{1carb}), 124.5, 124.5, 124.6 and 124.7 (4C, Csp²H), 144.7 (4C, Csp²), 168.4, 170.0, 171.6, 171.8, 172.1, 172.3, 173.9 and 174.0 (8C 4 × C₁, 4 × C₄); HRMS (ESI) *m/z* calcd. for C₆₀H₉₄N₂₀O₃₃ [M + 2H]²⁺: 833.3280 found: 833.3272.

Acknowledgements

This work was supported by funding from the Centre National de la Recherche Scientifique (CNRS) and Région Auvergne. We gratefully acknowledge Aurélie Job and Bertrand Légeret (Clermont-Université, Laboratoire SEESIB) for HPLC and mass spectrometry analysis. We thank Dr Alison A. Edwards for critical reading of the manuscript.

References

- 1 R. Roy, *Top. Curr. Chem.*, 1997, **187**, 241–274; Y. M. Chabre and R. Roy, *Curr. Top. Med. Chem.*, 2008, **8**, 1237–1285.
- 2 R. J. Pieters, *Org. Biomol. Chem.*, 2009, **7**, 2013–2025.
- 3 A. Varki, *Glycobiology*, 1993, **3**, 97–130; R. A. Dwek, *Chem. Rev.*, 1996, **96**, 683–720; C. R. Bertozzi and L. L. Kiessling, *Science*, 2001, **291**, 2357–2364; L. L. Kiessling, J. E. Gestwicki and L. E. Strong, *Angew. Chem.*, 2006, **118**, 2408–2429; L. L. Kiessling, J. E. Gestwicki and L. E. Strong, *Angew. Chem., Int. Ed.*, 2006, **45**, 2348–2368.
- 4 Z. Guo and Q. Wang, *Curr. Opin. Chem. Biol.*, 2009, **13**, 608–617; J. D. Warren, X. D. Geng and S. J. Danishefsky, *Top. Curr. Chem.*, 2007, **267**, 109–141; T. Freire, S. Bay, S. Vichier-Guerre, R. Lo-Man and C. Leclerc, *Mini-Rev. Med. Chem.*, 2006, **6**, 1357–1373; I. Bettahi, G. Dasgupta, O. Renaudet, A. A. Chentoufi, X. Zhang, D. Carpenter, S. Yoon, P. Dumy and L. BenMohamed, *Cancer Immunol. Immunother.*, 2009, **58**, 187–200.
- 5 Y. Lee and R. T. Lee, *Acc. Chem. Res.*, 1995, **28**, 321–327; M. Mammen, S.-K. Choi and G. M. Whitesides, *Angew. Chem.*, 1998,

- 110, 2908–2953; M. Mammen, S.-K. Choi and G. M. Whitesides, *Angew. Chem., Int. Ed.*, 1998, **37**, 2754–2794; R. T. Lee and Y. C. Lee, *Glycoconjugate J.*, 2000, **17**, 543–551; J. J. Lundquist and E. J. Toone, *Chem. Rev.*, 2002, **102**, 555–578; L. L. Kiessling, Pontrello, M. C. Schuster, Synthetic Multivalent Carbohydrate Ligands as Effectors or Inhibitors of Biological Processes, in *Carbohydrate-based Drug Discovery*, ed. C.-H. Wong, Wiley-VCH, Weinheim, Germany, 2003; Vol. 2, pp 575–608.
- 6 V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem.*, 2002, **114**, 2708–2711; V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596–2599; C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057–3064.
- 7 For an example of glycodendrimers prepared by CuAAC: Y. M. Chabre, C. Contino-Pépin, V. Placide, T. C. Shiao and R. Roy, *J. Org. Chem.*, 2008, **73**, 5602–5605.
- 8 For examples of glycopolymers prepared by CuAAC: L. Nurmi, J. Lindqvist, R. Randev, J. Syrett and D. M. Haddleton, *Chem. Commun.*, 2009, 2727–2729; M. Semsarilar, V. Ladmiral and Sébastien Perrier, *Macromolecules*, 2010, **43**, 1438–1443.
- 9 S. Cecioni, R. Lalor, B. Blanchard, J.-P. Praly, A. Imberty and S. E. M. S. Vidal, *Chem.-Eur. J.*, 2009, **15**, 13232–13240; C. Papin, G. Doisneau and J.-M. Beau, *Chem.-Eur. J.*, 2009, **15**, 53–57; S. Kamioka, T. Takahashi, S. Kawauchi, H. Adachi, Y. Mori, K. Fujii, H. Uekusa and T. Doi, *Org. Lett.*, 2009, **11**, 2289–2292; A. Marra, L. Moni, D. Pazzi, A. Corallini, D. Bridi and A. Dondoni, *Org. Biomol. Chem.*, 2008, **6**, 1396–1409; A. Vecchi, B. Melai, A. Marra, C. Chiappe and A. Dondoni, *J. Org. Chem.*, 2008, **73**, 6437–6440; A. Dondoni, *Chem.-Asian J.*, 2007, **2**, 700–708; A. Dondoni and A. Marra, *J. Org. Chem.*, 2006, **71**, 7546–7557.
- 10 F. Pérez-Balderas, M. Ortega-Muñoz, J. Morales Sanfrutos, F. Hernández-Mateo, F. G. Calvo-Flores, J. A. Calvo-Asín, J. Isac-García and F. Santoyo-González, *Org. Lett.*, 2003, **5**, 1951–54.
- 11 For examples of incorporation of sugar units onto preformed peptides, using CuAAC: N. Miller, G. M. Williams and M. A. Brimble, *Org. Lett.*, 2009, **11**, 2409–2412; D. J. Lee, K. Mandal, P. W. R. Harris, M. A. Brimble and S. B. H. Kent, *Org. Lett.*, 2009, **11**, 5270–5273; Y.-X. Chen, L. Zhao, Z.-P. Huang, Y.-F. Zhao and Y.-M. Li, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 3775–3778; J. Wang, H. Li, G. Zou and L.-X. Wang, *Org. Biomol. Chem.*, 2007, **5**, 1529–1540; S. I. van Kasteren, H. B. Kramer, H. H. Jensen, S. J. Campbell, J. Kirkpatrick, N. J. Oldham, D. C. Anthony and B. G. Davis, *Nature*, 2007, **446**, 1105–1109; S. Dedola, S. A. Nepogodiev and R. A. Field, *Org. Biomol. Chem.*, 2007, **5**, 1006–1017; H. Lin and C. T. Walsh, *J. Am. Chem. Soc.*, 2004, **126**, 13998–14003.
- 12 G. P. Dado and S. H. Gellman, *J. Am. Chem. Soc.*, 1994, **116**, 1054–62; D. Seebach, M. Overhand, F. N. M. Kühnle, B. Martinoni, L. Oberer, U. Hommel and H. Widmer, *Helv. Chim. Acta*, 1996, **79**, 913–941.
- 13 For reviews on β -peptides folding: D. Seebach and J. L. Matthews, *Chem. Commun.*, 1997, 2015–2022; W. F. DeGrado, J. P. Schneider and Y. Hamuro, *J. Pept. Res.*, 1999, **54**, 206–217; R. P. Cheng, S. H. Gellman and W. F. DeGrado, *Chem. Rev.*, 2001, **101**, 3219–3232; D. Seebach, J. V. Schreiber, P. I. Arvidsson and J. Frackenhohl, *Helv. Chim. Acta*, 2001, **84**, 271–279; T. A. Martinek and F. Fülöp, *Eur. J. Biochem.*, 2003, **270**, 3657–3666; D. Seebach, A. K. Beck and D. J. Bierbaum, *Chem. Biodiversity*, 2004, **1**, 1111–1239; G. Guichard, *Pseudo-Peptides in Drug Discovery*, ed. P. E. Nielsen, Wiley-VCH, Weinheim, 2004, 33–120.
- 14 U. Koert, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 1836–1837; D. Seebach, D. F. Hook and A. Glättli, *Biopolymers*, 2006, **84**, 23–37; Y. D. Wu, W. Han, D. P. Wang, Y. Gao and Y. L. Zhao, *Acc. Chem. Res.*, 2008, **41**, 1418–1427.
- 15 D. Seebach and J. Gardiner, *Acc. Chem. Res.*, 2008, **41**, 1366–1375; U. Arnold, M. P. Hinderaker, B. L. Nilsson, B. R. Huck, S. H. Gellman and R. T. Raines, *J. Am. Chem. Soc.*, 2002, **124**, 8522–8523; M. A. Gellman, S. Richter, H. Cao, N. Umezawa, S. H. Gellman and T. M. Rana, *Org. Lett.*, 2003, **5**, 3563–3565.
- 16 J. A. Kritzer, O. M. Stephens, D. A. Guarracino, S. K. Reznik and A. Schepartz, *Bioorg. Med. Chem.*, 2005, **13**, 11–16; O. M. Stephens, S. Kim, B. D. Welch, M. E. Hodsdon, M. S. Kay and A. Schepartz, *J. Am. Chem. Soc.*, 2005, **127**, 13126–13127; J. D. Sadowsky, W. D. Fairlie, E. B. Hadley, H. S. Lee, N. Umezawa, Z. Nikolovska-Coleska, S. Wang, D. C. S. Huang, Y. Tomita and S. H. Gellman, *J. Am. Chem. Soc.*, 2007, **129**, 139–154.
- 17 K. Gademann, M. Ernst, D. Hoyer and D. Seebach, *Angew. Chem., Int. Ed.*, 1999, **38**, 1223–1226; K. Gademann, M. Ernst, D. Seebach and D. Hoyer, *Helv. Chim. Acta*, 2000, **83**, 16–33; K. Gademann, T. Kimmerlin, D. Hoyer and D. Seebach, *J. Med. Chem.*, 2001, **44**, 2460–2468.
- 18 For examples of antibacterial properties: D. Liu and W. F. DeGrado, *J. Am. Chem. Soc.*, 2001, **123**, 7553–7559; P. I. Arvidsson, J. Frackenhohl, N. S. Ryder, B. Liechty, F. Petersen, H. Zimmermann, G. P. Camenisch, R. Woessner and D. Seebach, *ChemBioChem*, 2001, **2**, 771–773; E. A. Porter, B. Weisblum and S. H. Gellman, *J. Am. Chem. Soc.*, 2002, **124**, 7324–7330; R. F. Epand, T. L. Raguse, S. H. Gellman and R. M. Epand, *Biochemistry*, 2004, **43**, 9527–9535; M. A. Schmitt, B. Weisblum and S. H. Gellman, *J. Am. Chem. Soc.*, 2007, **129**, 417–428; B. P. Mowery, S. E. Lee, D. A. Kissounko, R. F. Epand, R. M. Epand, B. Weisblum, S. S. Stahl and S. H. Gellman, *J. Am. Chem. Soc.*, 2007, **129**, 15474–15476.
- 19 A. M. Brückner, P. Chakraborty, S. H. Gellman and U. Diederichsen, *Angew. Chem., Int. Ed.*, 2003, **42**, 4395–4399; A. S. Norgren and P. I. Arvidsson, *Org. Biomol. Chem.*, 2005, **3**, 1359–1361; G. L. Simpson, A. H. Gordon, D. M. Lindsay, N. Promsawan, M. P. Crump, K. Mulholland, B. R. Hayter and T. Gallagher, *J. Am. Chem. Soc.*, 2006, **128**, 10638–10639; A. S. Norgren and P. I. Arvidsson, *J. Org. Chem.*, 2008, **73**, 5272–5278.
- 20 D. Seebach, S. Abele, T. Sifferlen, M. Hänggi, S. Gruner and P. Seiler, *Helv. Chim. Acta*, 1998, **81**, 2218–2243; J. Frackenhohl, P. I. Arvidsson, J. V. Schreiber and D. Seebach, *ChemBioChem*, 2001, **2**, 445–455; D. F. Hook, F. Gessier, C. Noti, P. Kast and D. Seebach, *ChemBioChem*, 2004, **5**, 691–706; D. F. Hook, P. Bindschäedler, Y. R. Mahajan, R. Sebesta, P. Kast and D. Seebach, *Chem. Biodiversity*, 2005, **2**, 591–632.
- 21 P. Virta, M. Karskela and H. Lönnberg, *J. Org. Chem.*, 2006, **71**, 1989–1999.
- 22 O. Roy, S. Faure, V. Thery, C. Didierjean and C. Taillefumier, *Org. Lett.*, 2008, **10**, 921–924.
- 23 The spot synthesis of a glycotetrapeptide library from Fmoc-asparaginic acid propargylamide was reported whilst our work was in progress, the synthesis involved the preparation of glycoconjugated β -amino acid building blocks prior to their peptide coupling: K. Günther, C. Schips and T. Ziegler, *J. Carbohydr. Chem.*, 2008, **27**, 446–463.
- 24 C. S. Dexter and R. F. W. Jackson, *J. Org. Chem.*, 1999, **64**, 7579–7585.
- 25 Use of a mixed anhydride resulted in a lower yield: T. Markidis and G. Kokotos, *J. Org. Chem.*, 2001, **66**, 1919–1923.
- 26 The best yield was obtained by use of a mixture of solvents ($\text{Et}_2\text{O}-\text{CH}_2\text{Cl}_2$ 2 : 1): T. Hjelmggaard and D. Tanner, *Org. Biomol. Chem.*, 2006, **4**, 1796–1805.
- 27 S. Ahmed, R. Beleid, T. Sprules and K. Kaur, *Org. Lett.*, 2007, **9**, 25–28; K. Kaur, T. Sprules, W. Soliman, R. Beleid and S. Ahmed, *Biochim. Biophys. Acta, Proteins Proteomics*, 2008, **1784**, 658–665.
- 28 F. Sanda, K. Terada and T. Masuda, *Macromolecules*, 2005, **38**, 8149–8154.
- 29 Tlc monitoring showed a fast moving spot which had disappeared at the end of the reaction and that could correspond to an aspartimide by-product.
- 30 C. Mayato, R. L. Dorta and J. T. Vázquez, *Tetrahedron Lett.*, 2008, **49**, 1396–1398 and references cited therein.
- 31 The synthesis of this building block has been reported whilst our work was in progress: N. Pietrzik, C. Schips and T. Ziegler, *Synthesis*, 2008, 519–526.
- 32 T. Hasegawa, M. Numata, S. Okumura, T. Kimura, K. Sakurai and S. Shinkai, *Org. Biomol. Chem.*, 2007, **5**, 2404–2412.
- 33 G. Zemplén and E. Pascu, *Chem. Ber.*, 1929, **62B**, 1613.
- 34 R. Roy and J. M. Kim, *Angew. Chem., Int. Ed.*, 1999, **38**, 369–372.
- 35 For examples of papers in which use of a stoichiometric amount of $\text{CuSO}_4 \cdot x\text{H}_2\text{O}$ was required for azide-alkyne $[3 + 2]$ cycloaddition: C. Ornelas, J. R. Aranzaes, L. Salmon and D. Astruc, *Chem.-Eur. J.*, 2008, **14**, 50–64; M. Mourer, F. Hapiot, E. Monflier and S. Manuel, *Tetrahedron*, 2008, **64**, 7159–7163.
- 36 L. Liu, C. P. Li, S. Cochran and V. Ferro, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 2221–2226.