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PAPER

Exploring the self-assembly of glycopeptides using a diphenylalanine scaffold†

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Diphenylalanine, a key building block for organic nanotechnology, forms discrete, rigid and hollow nanotubes that are assembled spontaneously upon their dilution from organic phase into aqueous solution. Here we report the efficient preparation of several *S*-linked glycosylated diphenylalanine analogues bearing different monosaccharide, di-saccharide and sialic acid residues. The self-assembly studies revealed that these glycopeptides adopted various structures and glycosylation could be a tool to manipulate the self-assembly process. Moreover, the solubility of these analogues was found to be much greater than diphenylalanine, which could open new applications based on these nanostructures.

Introduction

The formation of ordered nanostructures by simple organic building blocks is a new and promising direction in nanotechnology.[1] The diphenylalanine peptide **1** (Fig. 1), is considered as a key structural element as this small building block and its derivatives form spherical and tubular nanostructures that can be deposited using various techniques.[2] Moreover, it was also demonstrated that the addition of a hydrophobic group such as the 9-fluorenylmethoxycarbonyl (Fmoc), to the *N*-terminus of diphenylalanine produced more rigid and branched fibrils that are similar to the amyloid fibrils in longer polypeptides.[3] In addition, Fmoc-diphenylalanine creates material with the characteristic of a hydrogel with remarkable mechanical rigidity for the potential use in various biotechnological applications.[4] Nevertheless, the low solubility of diphenylalanine and its analogues in aqueous media has hampered several potential applications and studies aimed at understanding the mechanism of the self-assembly at the molecular level. Inspired by the role of glycosylation in the physiochemical and biological function of various biomacromolecules *e.g.* lipids, peptides and proteins,[5] we reasoned that glycosylation of diphenylalanine could have an impact on its solubility and self-assembly properties.

The effect of glycosylation on the self-assembly of peptides and proteins involved in health and diseases has been reported. For example, current data suggest that *O*-linked-β-*N*-acetylglucosamine (*O*-GlcNAc) has a significant role in normal function and the pathology of neurodegenerative disorders. In this regard, it has been reported that tau protein is extensively *O*-GlcNAcylated, in the healthy adult brain.[6] On the other hand, the phosophorylated structure of tau causes it to aggregate into paired helical filaments in the cytoplasm, which is one of the hallmarks of Alzheimer's disease.[6] In another example, it has been found that *O*-linked monosaccharide can affect the coil to β-structural transition of the prion peptide.[7] While the *O*-linked α-*N*-acetylgalactosamine, (α-GalNAc) at Ser 135, was found to suppress amyloid formation of the prion peptide, PrP(108-144), the same sugar at Ser132 shows opposite effect. Moreover, replacement of the α-GalNAc to β-GlcNAc did not lead to the same results, indicating that this effect is sugar specific.

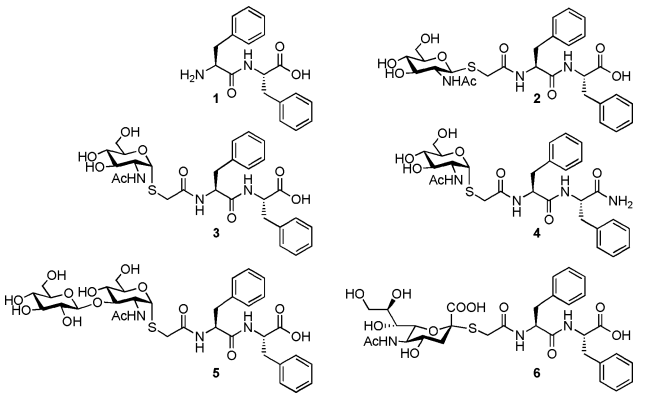

Fig. 1 The diphenylalanine scaffold and its glycosylated analogues used in this study.

The study of the self-assembly properties of amphiphilic systems based on lipid and peptides has also been investigated.[8] For example, it has been recently reported that glycosylation of fatty acids comprised of 18 carbons with a variety of monosaccharides

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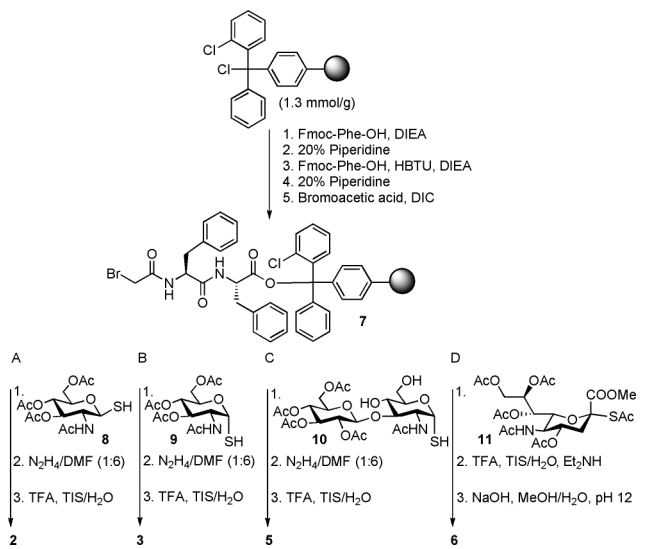
(β-D-glucose, β-D-galactose and β-D-mannose) promotes the formation of lipid nanotubes. Notably, only the β-D-glucose has triggered the self-assembly process.[9] In another interesting study, it was found that carbohydrate (β-D-galactose and α-D-mannose) conjugate Rod-Coil amphiphiles were found to self-assemble in different supramolecular structures depending on the molecular architecture.[10] In a related study, glycosylation was also reported to alter the aggregation kinetics of an amyloid-forming model peptide in a site-specific manner.[11] Together, these important studies support an important role for glycosylation in self-assembly processes. However, most of these studies were limited to probe the effect of monosaccharide on a particular scaffold and the use of diverse carbohydrate structures to examine their influence on the self-assembly and solubility properties has not been fully addressed. Herein, we report the design, synthesis and characterization of different *S*-linked glycosylated diphenylalanine analogues bearing carbohydrates of varying length and complexity (Fig. 1) and the influence of these saccharides on the self-assembly, morphology and the solubility of diphenylalanine peptides.

Results and discussion

The low solubility of the diphenylalanine and its analogues in aqueous media has hampered several potential applications and studies aiming at understanding the mechanism of the self-assembly at the molecular level. Thus, we wanted to examine whether we could enhance diphenylalanine solubility while retaining its self-assembly properties. Our endeavor to explore the effect of glycosylation on the diphenylalanine self-assembly and solubility started with the introduction of the monosaccharide, β-GlcNAc, to generate glycosylated diphenylalanine **2**. To achieve this goal we prepared diphenylalanine using Fmoc-solid phase peptide synthesis (Fmoc-SPPS) on 2-chlorotrityl resin (Scheme 1). To allow for a straightforward *N*-terminus glycosylation, the diphenylalanine was condensed with bromoacetic acid using DIC. The resultant bromo-dipeptide derivative was then subjected to nucleophilic substitution reaction with β-GlcNAc thiol[12] to provide corresponding *S*-linked glycopeptides in the protected form.[13] Subsequent treatment with hydrazine in DMF to remove the acetyl protecting groups followed by cleavage from resin using TFA yielded the *S*-β-GlcNAc diphenylalanine **2** (Scheme 1A). Similar procedure was then adopted, however using α-GlcNAc thiol[14] as a nucleophile to generate *S*-α-GlcNAc diphenylalanine 3 (Scheme 1B). Both glycopeptides were purified by using reverse phase HPLC to give, after lyophilization, the desired products in 27–30% isolated yield, which were further analyzed for structure integrity by mass spectrometry (Fig. 2) and NMR (ESI†).



Scheme 1 Synthesis of *S*-glycopeptides **2**–**6**. The synthesis of glycopeptide **4** was carried out in a similar manner to glycopeptide **3**, however using Rink amide resin.

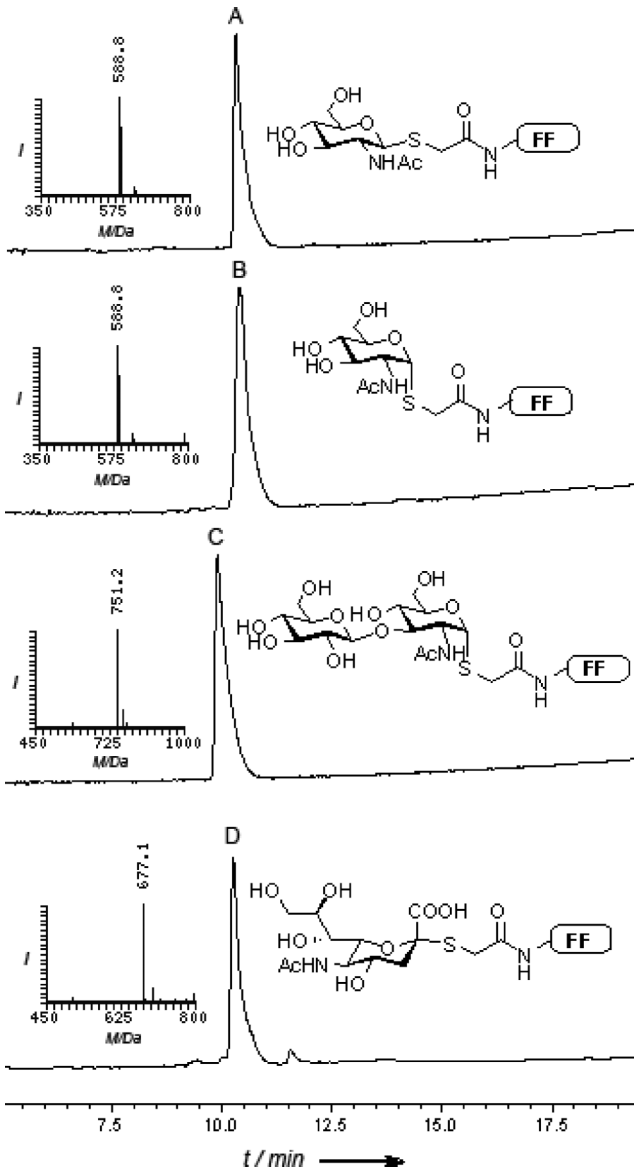


Fig. 2 Analytical HPLC traces and ESMS spectra of the pure *S*-glycopeptides (A) *S*-β-GlcNAc diphenylalanine (B) *S*-α-GlcNAc diphenylalanine (C) *S*-α-glucopyranosyl-(1→3)-D-glucosamine diphenylalanine (D) *S*-Neu5Ac diphenylalanine. FF stands for diphenylalanine.

Diphenylalanine often requires the addition of organic solvent or heating in order to dissolve it in aqueous media.[1,2] The fusion of β-GlcNAc to the diphenylalanine peptide to generate glycosylated diphenylalanine **2** enabled the glycopeptide to be

a slightly more water soluble than the parent diphenylalanine peptide, however this glycopeptide also produced insoluble self-assembled structures after a short period of incubation. Notably, the morphology of the nanostructures in a saturated glycosylated diphenylalanine **2** solution in water is different from the assemblies formed by the diphenylalanine peptide. While diphenylalanine **1** self-assembles into discrete tubular structures[2] (Fig. 3a–b), glycosylated diphenylalanine **2** forms bundles of fibers as was found by Transition electron microscopy (TEM) (Fig. 3c) as well as by Scanning electron microscopy (SEM) micrographs (Fig. 3d). Glycosylated diphenylalanine **2** was also dissolved in dimethyl sulfoxide (DMSO) then diluted in water, nevertheless, no ordered nanostructures were observed as analyzed by electron microscopy.

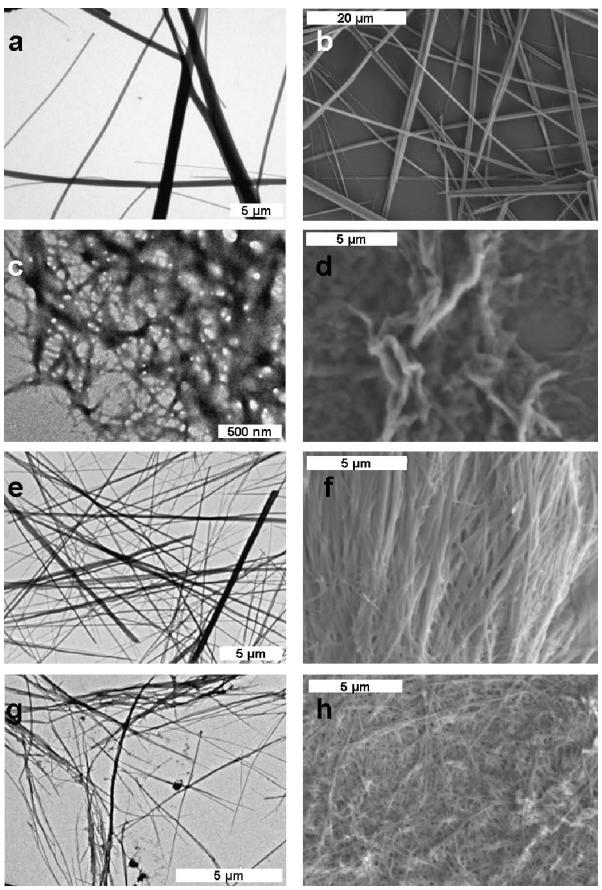
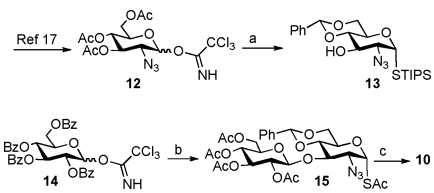


Fig. 3 Self assembled nanostructures of diphenylalanine and glycopeptide **2** and **3** after two hours of incubation at concentration of 6 mM. a–b) TEM and SEM micrograph respectively of diphenylalanine dissolved in HFIP/water; c–d) TEM and SEM micrograph respectively of glycosylated diphenylalanine **2** dissolved in water; e–f) TEM and SEM micrograph respectively of glycosylated diphenylalanine **3** dissolved in DMSO and water demonstrating the ordered nanostructures; g–h) TEM and SEM micrograph respectively of glycosylated diphenylalanine **3** dissolved in water demonstrating the ordered nanostructures.

Interestingly, the addition of α-GlcNAc thiol as a nucleophile to generate *S*-α-GlcNAc diphenylalanine **3** enabled a self assembly process and the formation of assemblies similar to those formed by diphenylalanine peptide, however with a similar solubility profile to glycopeptide **2**. Electron microscopy micrographs depict the elongated, discrete, rigid tubular structures (Fig. 3e–f). A saturated water solution of glycosylated diphenylalanine **3** enabled the formation of similar elongated discrete assemblies (Fig. 3g–h). The replacement of the *C*-terminal acid in amide derivative **4**, did not lead to changes in the morphology of the self-assembled structure.

One possible explanation of the differences of self-assembly structures between the *S*-α-GlcNAc diphenylalanine **3** and *S*-β-GlcNAc diphenylalanine **2** could be attributed to the position occupied by the sugar unit in the supramolecular structure. In glycopeptide **3**, it is possible that the sugar unit is pointing towards the channel that could be formed in a similar manner to the diphenylalanine case,[15] thus leaving the interaction between the aromatic residues intact and hence the ability to self assemble. On the other hand, the change in the configuration in glycopeptide **2** could place the sugar unit in a different position, which could interfere with the intramolecular aromatic stacking and disrupts self-assembly.

Our results on the self-assembly studies with *S*-β-GlcNAc glycosylated diphenylalanine **2** and *S*-α-GlcNAc glycosylated diphenylalanine **3** triggered us to prepare a glycosylated diphenylalanine analogue with a disaccharide unit bearing the α-GlcNAc, as we found that the α-configuration is preferential for the self-assembly process. This would give us the opportunity to examine how a more complex glycan would affect diphenylalanine self-assembly and solubility. For this goal, we synthesized a novel α-glucopyranosyl-(1→3)-D-glucosamine thiol **10** from corresponding acceptor **13** and donor **14**. The acceptor, 2-azo-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl triisopropylsilane **13** was synthesized from the known precursor **12**,[16] while the donor 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate **14** was prepared according to the reported synthesis from D-glucose.[17] The acceptor and the donor were subjected to Schmidt glycosylation[18] to yield the disaccharide that on global deprotection followed by peracetylation afforded the glycosyl azido-thioacetate **15**. Reaction of azido-thioacetate **15** with PPh_3 promoted thiazoline formation by an aza-Wittig reaction followed by acidic hydrolysis to furnish α-glucopyranosyl-(1→3)-D-glucosamine thiol **10** (Scheme 2). With **10** in hand, we then followed the reaction steps shown in Scheme 1C to afford the *S*-α-glucopyranosyl-(1→3)-D-glucosamine diphenylalanine **5** in 20% isolated yield.



Scheme 2 a) i. TIPS-SH, TMSOTf, CH_2Cl_2, Et_2O, –20 to 23 °C, 5 h, 74%; ii. NaOMe, MeOH, 23 °C, 45 min, 85%; iii. $PhCH(OMe)_2$, CSA, THF, 60 °C, 4 h, 67%; b) i. TMSOTf, 13, CH_2Cl_2, –78 °C to r.t., 4 h, 91%; ii. NaOH, MeOH, H_2O, 23 °C, 18 h; iii. Ac_2O, DMAP, pyr, 23 °C, 24 h, 57% over two steps; c) i. PPh_3, THF, 23 °C, 20 h; ii. TFA, MeOH, H_2O, 8 h, 0 °C, 66% over two steps.

We next examined the solubility and self-assembly properties of glycosylated diphenylalanine **5**. Notably, this glycopeptide did not require heating or the addition of any organic solvent and gave a very clear solution, even after 24 h of incubation, due to the high water-solubility of this glycopeptide. On the other hand, this

glycopeptide self-assembles to form elongated fibrils. However, the diameter of these nanostructures is approximately 10 nm, lower than those formed by glycosylated diphenylalanine **3**, as was found in the electron microscopy images (Fig. 4a).

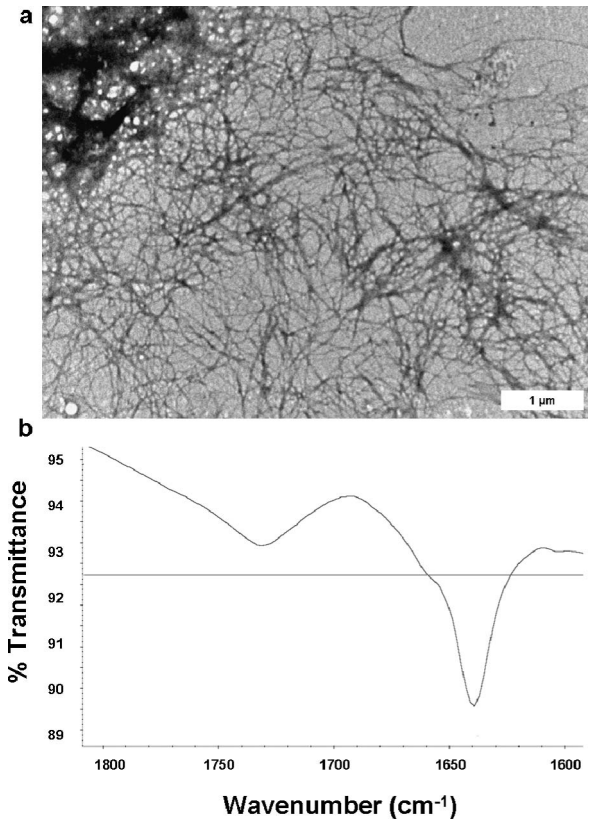


Fig. 4 Glycosylated diphenylalanine **5** self-assembles into ordered nano-structures after two hours of incubation at concentration of 6 mM. a. TEM micrograph of the assemblies. b. FTIR analysis of the assemblies indicating a β-sheet conformation with a major minimum peak at 1639 cm^{-1}.

Next we examined the nature of the secondary structures of the nano-assemblies using FTIR spectroscopy. The wavenumber amide I bands of diphenylalanine **5** were located with a major minimum peak at 1639 cm^{-1} (Fig. 4b). This vibrational peak is consistent with supramolecular organization of a peptide in a secondary structure of β-sheet conformation and consistent with the electron microscopy data described above.[19] These assemblies show ultrastructural similarity to β-sheet rich amyloidal fibrillar structures. The well-studied amyloidal assemblies indeed appear as thin entangled nano-fibrils with a diameter of 7–10 nm rich with β-sheet secondary structures as observed by IR spectroscopy.

Considering the biological importance of sialic acid, its unique chemical structure and its potential uses in drug development, we decided to study the effect of this sugar unit on the self-assembly and solubility of diphenylalanine. Sialic acid is a monosaccharide with nine-carbon backbone and negatively charged carboxylate group and typically is linked to the outermost unit of the glycoproteins. It plays vital roles in many physiological and pathological processes and has been used in the design of various carbohydrate-based antigens for vaccine development and various enzyme inhibitors.[20] In this regard, several templates were designed to present, multivalently, the sialic acid containing glycan.[21,22] Thus, the introduction of a new simpler scaffold that could be potentially used to present sialic acid containing glycan could be beneficial in various applications.

Recently, we have developed a *S*-alkylation strategy to synthesize *S*-linked α-(2→9)-octasialic acid with exclusive α-*S*-glycosidic bond formation.[23] By *in situ* selective deprotection of *S*-acetate in compound **11**[24] followed by *S*-alkylation, the epimerization of anomeric sulfur could be avoided. Thus, to achieve the synthesis of the diphenylalanine sialic acid derivative we used compound **11** for the *S*-alkylation step, in the presence of diethylamine (Scheme 1D). Subsequently, the fully protected glycopeptide was cleaved from resin using TFA followed by saponification to afforded *S*-Neu5Ac-diphenylalanine **6**. With glycopeptide **6** in hand, we then focused on the self-assembly analysis. Similar to **5**, glycosylated diphenylalanine **6** was also highly water-soluble. However, after its dissolution in water, glycosylated diphenylalanine **6** self assembled to form elongated and curved discrete nanostructures, the diameter of the fibrils is approximately 100 nm, as can be seen by TEM and SEM analysis (Fig. 5a–b). The nanostructure obtained in this case resembled the ones obtained by diphenylalanine **1** and the α-glycosylated diphenylalanine **3** in their discrete nature. Contrary to glycopeptide **1** and **3**, this glycopeptide was highly soluble and gave a clear solution even after 24 h.

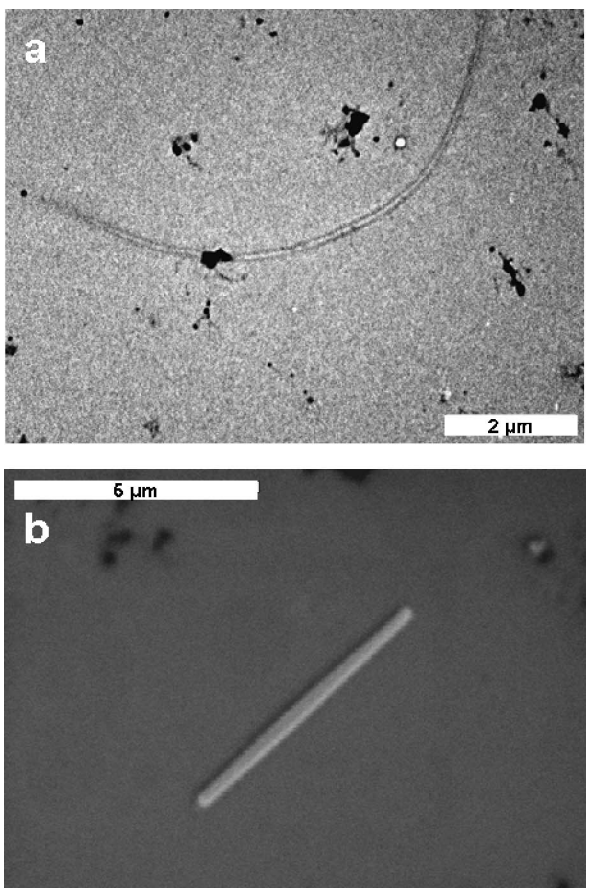


Fig. 5 Glycosylated diphenylalanine **6** self-assembles into ordered nano-structures after two hours of incubation at concentration of 6 mM. a–b. TEM and SEM micrograph respectively of the nanostructures.

Conclusions

We have shown that the introduction of different carbohydrate units into the diphenylalanine motif led to changes in the morphology of the self-assembled nanostructures. While α-glycosylated diphenylalanine **3** enabled the formation of assemblies similar to those formed by diphenylalanine **1**, the β-glycosylated diphenylalanine **2** gave no ordered nanostructures. On the other hand, the glycopeptide bearing disaccharide unit self-assembles to form elongated ordered nanostructure, however with different diameter than the diphenylalanine **1**, α-glycosylated peptide **3** and *S*-Neu5Ac-diphenylalanine **6**. We have also found that the addition of the carbohydrate moiety to the diphenylalanine building block, in particular the disaccharide and sialic acid units, have significantly increased the solubility of this peptide in water to initiate the self-assembly process. To best of our knowledge, this is the first example that explores variations of different carbohydrate units on the self-assembly of a peptidic scaffold. Taken together, our results could pave the way for new biotechnological applications based on the diphenylalanine motif and for better understanding of the self-assembly process at the molecular level. For example, these glycopeptide derivatives could be applied in those cases in which the use of organic solvents should be avoided such as drug delivery nanoassemblies, imaging agents encapsulating ferromagnetic elements, hydrogels for slow drug release and tissue engineering, antibacterial activity,[25] and as scaffold to present multivalently complex carbohydrates.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-500 MHz spectrometer. An internal standard, and *J* values are given in Hz. Mass of the materials was analyzed with LCQ Fleet Ion Trap (Thermo Scientific). Flash column chromatography was performed with silica gel (60–100 mesh). The reactions were carried out in oven-dried glassware under dry argon. Dichloromethane, diethyl ether, methanol and THF were dried before use. Fmoc-Phe-OH was purchased from Novabiochem and coupling reagents from Luxembourg Biotechnologies. Analytical thin-layer chromatography (TLC) was performed using thin layer chromatography on pre-coated plates (0.25 mm, silica gel 60 F254). Compound spots were visualized by UV light (254 nm) and were stained with phosphomolybdic acid.

SPPS

Solid-phase chemistry was carried out in syringes, equipped with teflon filters purchased from Torviq according to the Fmoc-strategy (Fmoc-SPPS) on 2-chlorotrityl resin (loading 1.3). The resin was pre-swollen in DCM followed by coupling Fmoc-Phe-OH to 2-chlorotrityl chloride resin using diisopropylethylamine (DIEA) in dichloromethane. The Fmoc group was removed with 20% piperidine in DMF. Peptide bond with second Fmoc-Phe-OH was formed using DIEA in the presence of 1-hydroxybenzotriazole (HOBt) and *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate (HBTU).

Bromoacetylation

A preactivated solution of bromoacetic acid (278 mg, 2 mmol) with DIC (0.37 mL, 2.4 mmol) in DMF (1.70 mL) for 15 min at 0 °C was added to the resin (77 mg, 0.1 mmol) with dipeptide and shaken well for 1 h. Resin was washed with DMF (×3).

General procedure for glycosylation of 8, 9, 10

The 1-thiosaccharide (5 equiv) was dissolved in DMF (4 mL), and was added to the resin (77 mg, 0.1 mmol) followed by the addition of TEA (133 mL, 9 mmol). The resin was shaken for 5 h and washed with DMF (×3).

Acetyl deprotection

The resin was treated with a solution of 4 mL of DMF/hydrazine (6 : 1) for 2.5 h, and washed with DMF (×3). The procedure was repeated twice.

Cleavage

A mixture of TFA, triisopropylsilane and water (95 : 2.5 : 2.5) was added to the dried peptide-resin and the reaction mixture was shaken for 1.5 h at r.t. The resin was removed by filtration and was washed with TFA (2 × 2 mL). TFA was evaporated and the residue was dissolved in 25% acetonitrile–water and lyophilized.

Peptide analysis and purification of 2, 3, 5

Analytical RP-HPLC was performed on a Thermo instrument (Spectra System p4000) using an analytical column (Jupiter 5 micron, C18, 300A 150 × 4.6 mm) and a flow rate of 1.2 mL min^{-1}. Preparative RP-HPLC was performed on an ECOM instrument using a preparative column (Jupiter 5 micron, C18, 300A, 250 × 10 mm) and a flow rate of 25 mL min^{-1}. Commercial peptide reagents were used without further purification. Buffer A: 99.9% water + 0.1% TFA, Buffer B: 99.9% ACN + 0.1% TFA.

S-β-GlcNAc diphenylalanine (2)

^{1}H NMR (500 MHz, DMSO-D6, 10% TFA-D) δ 8.36 (d, J = 7.8 Hz, 1H), 7.99 (d, J = 8.5 Hz, 1H), 7.71 (d, J = 9.2 Hz, 1H), 7.23–7.10 (m, 10 H), 4.50 (dd, J = 9.3, 4.6 Hz, 1H), 4.40 (dd, J = 8.7, 5.4 Hz, 1H), 4.31 (d, J = 10.3 Hz, 1H), 3.60 (dd, J = 11.9, 1.6 Hz, 1H), 3.48 (t, J = 10.3 Hz, 1H), 3.37 (dd, J = 11.9, 5.6 Hz, 1H), 3.24 (d, J = 14.4 Hz, 1H), 3.20–3.16 (m, 2H), 3.02 (m, 3H), 2.94 (dd, J = 13.9, 4.6 Hz, 1H), 2.88 (dd, J = 13.8, 8.7 Hz, 1H), 2.70 (dd, J = 13.8, 9.3 Hz, 1H), 1.75 (s, 3H); ^{13}C NMR (126 MHz, DMSO-D6, 10% TFA-D) δ 172.8, 171.3, 169.6, 168.9, 137.8, 137.6, 129.5, 129.4, 128.5, 128.3, 126.7, 126.5, 83.8, 81.4, 75.4, 70.6, 61.4, 54.6, 53.8, 53.7, 37.9, 36.9, 32.9, 23.0; ESI-MS: Calculated for $C_{28}H_{35}N_3O_9S$: 589.21 Da [M$^+$], Observed: 589.1 Da [M+H].

S-α-GlcNAc diphenylalanine (3)

^{1}H NMR (500 MHz, DMSO-D6, 10% TFA-D) δ 8.33 (d, J = 6.6 Hz, 1H), 8.02 (d, J = 8.6 Hz, 1H), 7.88 (d, J = 5.3 Hz, 1H), 7.24–7.11 (m, 10H), 5.36 (d, J = 5.1 Hz, 1H), 4.50 (dd, J = 9.0, 4.7 Hz, 1H), 4.40 (dd, J = 8.6, 5.4 Hz, 1H), 3.81 (dd, J = 10.6, 5.1 Hz, 1H), 3.67–3.63 (m, 1H), 3.62–3.59 (m, 1H), 3.46 (dd, J = 11.9, 5.6 Hz, 1H), 3.42–3.38 (m, 1H), 3.13 (t, J = 8.8 Hz, 1H), 3.07–3.01

(m, 3H), 2.93 (dd, J = 13.8, 4.6 Hz, 1H), 2.88 (dd, J = 13.9, 8.6 Hz, 1H), 2.67 (dd, J = 14.0, 9.0 Hz, 1H), 1.79 (s, 3H); ^{13}C NMR (126 MHz, DMSO-D6, 10% TFA-D) δ 173.0, 171.2, 170.2, 168.6, 137.9, 137.8, 129.7, 129.5, 128.6, 128.4, 126.8, 126.6, 83.1, 73.9, 71.1, 70.9, 61.0, 54.3, 54.0, 53.8, 38.1, 37.0, 32.3, 22.7; ESI-MS: Calculated for $C_{28}H_{35}N_3O_9S$: 589.21 Da [M$^+$], Observed: 589.1 Da [M+H].

S-α-glucopyranosyl-(1→3)-D-glucosamine diphenylalanine (5)

^{1}H NMR (500 MHz, DMSO-D6, 10% TFA-D) δ 8.35 (d, J = 7.8 Hz, 1H), 8.04 (d, J = 8.5 Hz, 1H), 7.63 (d, J = 6.9 Hz, 1H), 7.23–7.15 (m, 10H), 5.39 (d, J = 5.3 Hz, 1H), 4.49–4.45 (m, 1H), 4.39–4.33 (m, 2H), 3.91 (dt, J = 11.0, 5.6 Hz, 1H), 3.67–3.63 (m, 2H), 3.45 (dd, J = 11.9, 5.4 Hz, 1H), 3.33 (t, J = 9.5, 1H), 3.28–3.24 (m, 1H), 3.17–3.13 (m, 1H), 3.12–2.88 (m, 9 H), 2.85 (dd, J = 13.9, 8.9 Hz, 1H), 2.64 (dd, J = 13.8, 9.0 Hz, 1H), 1.75 (s, 3H); ^{13}C NMR (126 MHz, DMSO-D6) δ 171.6, 169.8, 168.8, 166.9, 136.5, 136.4, 128.2, 128.0, 127.1, 126.9, 125.4, 125.1, 101.5, 81.4, 79.2, 76.1, 75.4, 72.2, 68.9, 67.6, 60.0, 59.3, 52.6, 52.5, 51.3, 40.6, 36.6, 35.5, 30.6, 21.5; ESI-MS: Calculated for $C_{34}H_{45}N_3O_{14}S$: 751.80 Da [M$^+$], Observed: 751.2 Da [M+H].

Synthesis of glycopeptide 6

Procedure for glycosylation of 11. Protected Neu5Ac-thiol (309 mg, 0.56 mmol) was dissolved in DMF (1.17 mL) and was added to the resin (230 mg, 0.30 mmol). To this, freshly distilled diethylamine (350 mL, 3.37 mmol) was added, shaken for 5 h and washed with DMF (×3).

Acetyl and methylester hydrolysis. After the cleavage step the crude product was dissolved in methanol and the pH was adjusted to ~12 by the addition of 1 M NaOH (aq), stirred for 24 h at ambient temperature. After completion of the reaction on HPLC, 0.5 M HCl was added dropwise to adjust the pH to 4. The reaction mixture was concentrated, the crude product was dissolved in acetonitrile–water and HPLC purification as described above afforded the glycopeptide **6**.

^{1}H NMR (500 MHz, DMSO-D6, 10% TFA-D) δ 8.40 (d, J = 2.8 Hz, 1H), 8.39 (d, J = 3.5 Hz, 1H), 8.13 (d, J = 7.8 Hz, 1H), 7.35–7.22 (m, 10H), 4.55 (dd, J = 9.9, 4.4 Hz, 1H), 4.49 (dd, J = 8.7, 5.4 Hz, 1H), 3.72–3.59 (m, 4H), 3.53–3.47 (m, 2H), 3.41 (dd, J = 8.7, 1.7 Hz, 1H), 3.36–3.31 (m, 2H), 3.13 (dd, J = 13.9, 5.3 Hz, 1H), 3.03 (dd, J = 14.4, 4.8 Hz, 1H), 2.98 (dd, J = 14.0, 8.8 Hz, 1H), 2.78 (dd, J = 13.8, 9.8 Hz, 1H), 2.63 (dd, J = 12.5, 4.2 Hz, 1H),1.94 (d, J = 0.8 Hz, 3H), 1.67 (dd, J = 12.3, 10.9 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-D6, 10% TFA-D) δ 172.7, 171.9, 171.3, 171.1, 171.0, 167.0, 137.8, 137.5, 129.4, 129.3, 128.4, 128.2, 126.6, 126.4, 81.8, 76.1, 68.5, 62.9, 54.1, 53.9, 53.6, 53.5, 52.3, 52.2, 41.0, 37.5, 36.7, 32.7, 22.7; ESI-MS: Calculated for $C_{31}H_{39}N_3O_{12}S$: 677.72 Da [M$^+$], Observed: 678.1 Da [M+H].

Nanostructure sample preparation and imaging

Nanostructure preparation: the lyophilized powder of each of the synthesized glycopeptides, diphenylalanine **2**, **3**, **4**, **5**, **6**, were dissolved in pure water at a concentration of 6 mM, diphenylalanine **2** was incubated at 40 °C for 30 min. Glycosylated diphenylalanine **2**, **3**, were also dissolved in DMSO at a concentration of 60 mM then diluted in pure water at a concentration of 6 mM.

Transmission Electron Microscopy: After two hours and 24 h, 10 μL the nanostructures solutions were loaded on carbon coated copper grid, dried and stained with uranyl acetate, followed by imaging.

Scanning Electron Microscopy: After two hours and 24 h, 10 μL of the nanostructures solutions were deposited on slides, and then coated with gold. SEM measurements were performed on a JSM JEOL 6300 SEM operating at 5 kV.

Fourier Transform Infrared Spectroscopy: infrared spectra were recorded using Nicolet Nexus 470 FT-IR spectrometer with DTGS detector. Nanostructure solution sample were dried on polyethylene card to form thin film. The nanostructures deposits were resuspended with D_2O and dried. The resuspension procedure was repeated twice to ensure maximal hydrogen to deuterium exchange. The measurements were taken using a 4 cm^{-1} resolution and 1000 scans averaging. The transmittance minima values were determined by OMNIC analysis program (Nicolet).

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