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An Unsaturated Peptidomimetic Assembly Derived from a Carbohydrate‡

YING-KIT CHUNG,[a] TIMOTHY D. W. CLARIDGE,[a] GEORGE W. J. FLEET,[a] STEPHEN W. JOHNSON,[a] JOHN H. JONES,[a]* KEITH W. LUMBARD[b] and ANDREW V. STACHULSKI[b,c]

[a] Dyson Perrins Laboratory, University of Oxford, OX1 3QY, UK
[b] Ultrafine UFC Ltd, Synergy House, Guildhall Close, Manchester Science Park, Manchester M15 6SY, UK
[c] Present address: Robert Robinson Laboratories, University of Liverpool, L69 7ZD, UK

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Abstract: A strategy has been established for the synthesis of peptidomimetics derived from unsaturated carbohydrates, and exemplified by the use of methyl 2,6-anhydro-7-azido-3,7-deoxy-4,5-*O*-isopropylidene-D-*lyxo*-hept-2-enonate **9** as a dipeptide 'monomer' which can be elaborated from either end. Selective reduction of **9** gives a protected pseudodipeptide ester suitable for use as an amino component, and saponification gives an azido acid suitable for use as a carboxyl component. The 'dimer' product of coupling these two components with TBTU can be similarly elaborated at either end to give a 'trimer', and a further cycle of selective reduction and coupling gave a 'tetramer', **17**, a pseudo-octapeptide. Copyright © 2003 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: unsaturated peptidomimetics; carbohydrates; azido acids

INTRODUCTION

Peptidomimetics derived from carbohydrates are of particular current interest because of the potential for precise stereochemical design that carbohydrate building blocks offer. The examples reported so far all incorporate saturated carbohydrate amino-acid residues [e.g. 1–7], and thus have backbones that mimic peptides in which the principal backbone constraints on conformation are the peptide bonds. In peptides containing $\alpha\beta$-unsaturated amino-acid residues, which occur naturally and have been the subject of diverse studies, there are the further constraints associated with trigonal carbons at the α-positions. We now report, and outline in Schemes 1 and 2, an exploration of the feasibility of synthesizing peptidomimetics derived from unsaturated carbohydrate amino acids.

Our key intermediate was the protected azido $\alpha\beta$-unsaturated methyl ester **9**, which was obtained from penta-*O*-acetyl-β-D-galactopyranose **1**. In **9**, the azido function is a potential amino group 1,4-juxtaposed to a blocked carboxyl group, so that **9** is equivalent overall to a fully protected dipeptide. Intermediate or 'monomer' **9** is thus in principle open to elaboration from either end: selective reduction would give an amino component for coupling with a suitable carboxyl component; saponification would give a carboxyl component for coupling with a suitable amino component. Both strategies were successfully demonstrated, as shown in Schemes 1 and 2. No indications of side reactions involving intramolecular Michael addition or cycloaddition were detected at any stage. Of particular note is the repeated application of selective azide reduction before coupling, which was performed in three successive cycles to give the

*Correspondence to: Dr John H. Jones, Balliol College Oxford OX1 3BJ, UK; e-mail: John.Jones@balliol.ox.ac.uk
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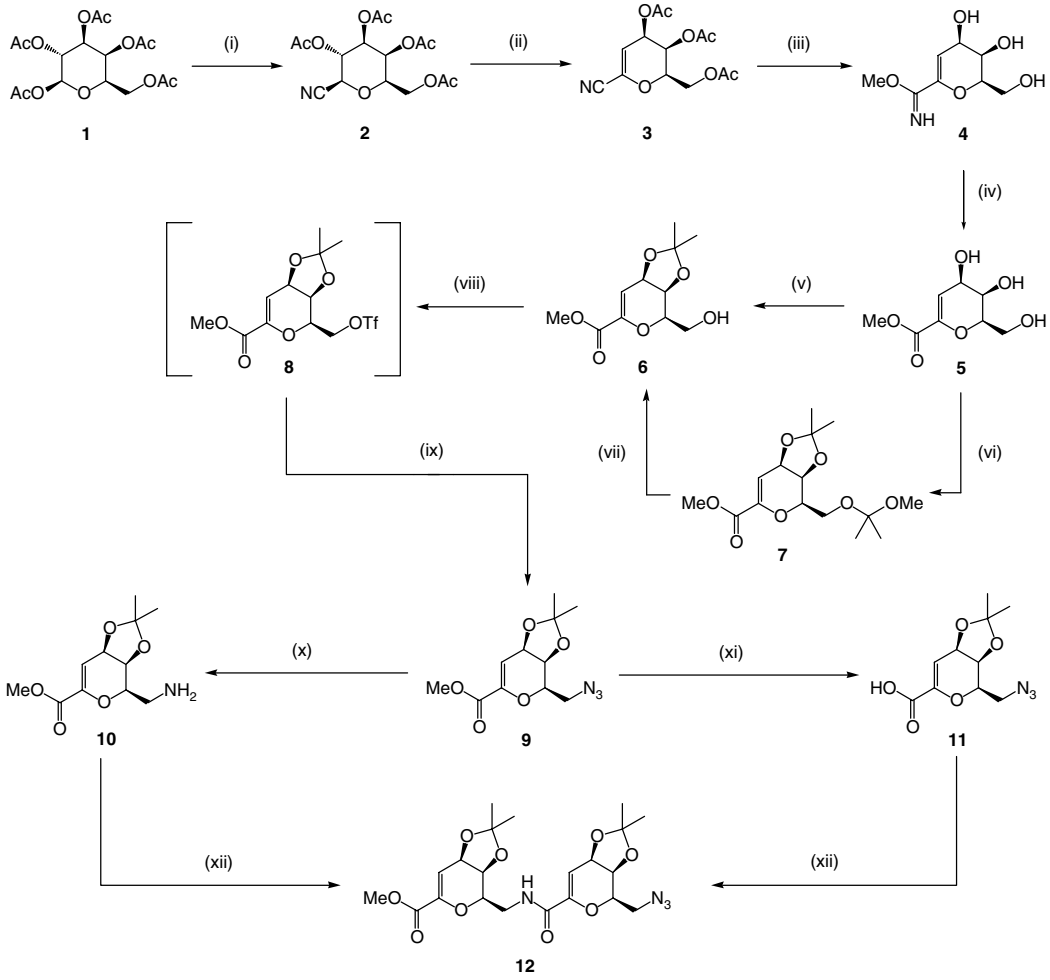
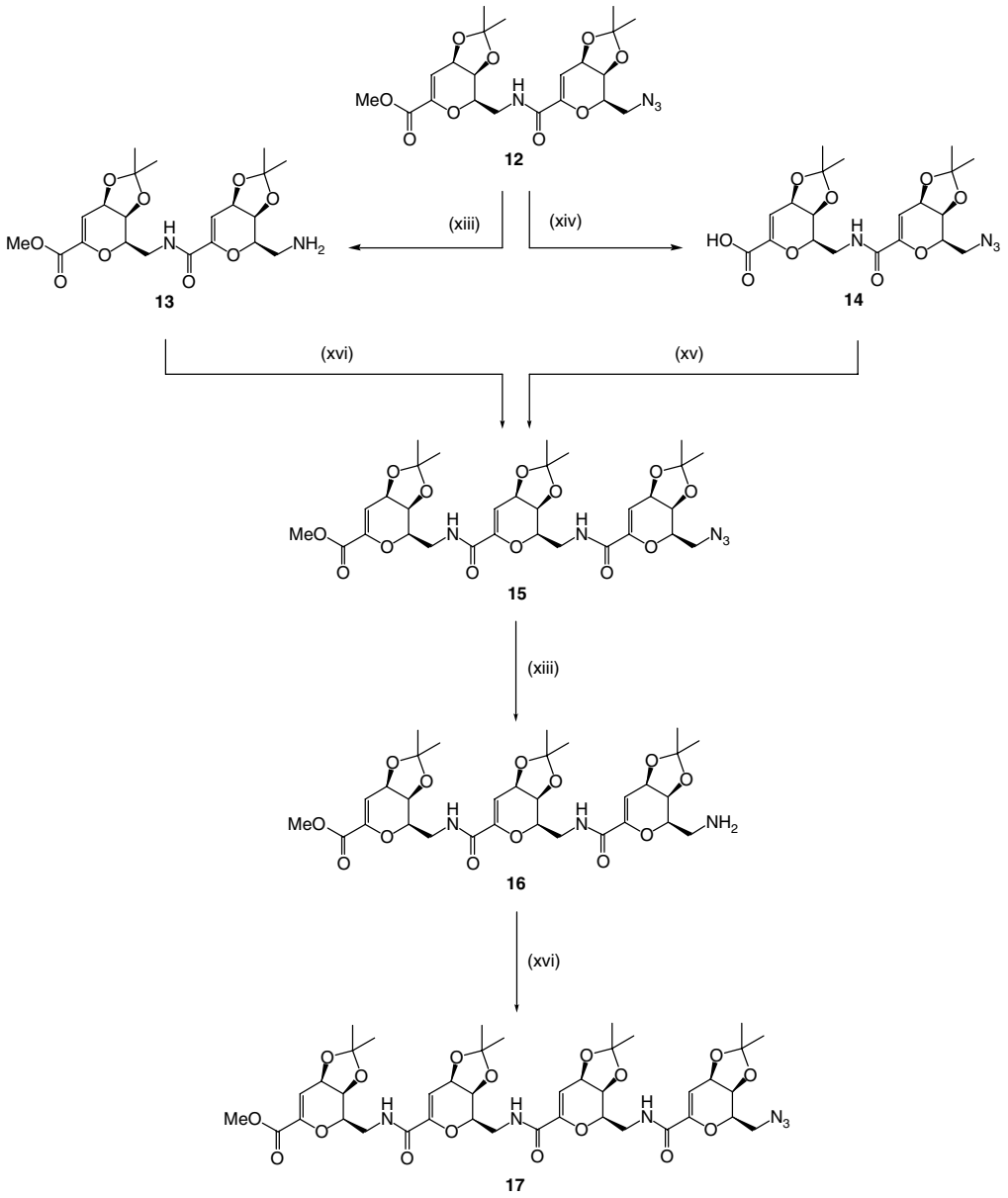


Scheme 1 Reagents and conditions; (i) TMSCN, $BF_3.Et_2O$, anhydrous CH_3NO_2; (ii) DBU, 3Å molecular sieves, DCM, 12 h; (iii) NaOMe, MeOH; (iv) Amberlyst 15 H^+ resin, H_2O; (v) acetone, TsOH; (vi) 2,2-dimethoxypropane, TsOH; (vii) MeOH, TsOH; (viii) Tf_2O, py, DCM; (ix) NaN_3, DMF; (x) Lindlar's catalyst, MeOH, H_2; (xi) NaOH (1M), MeOH, H_2O, Amberlite IR 120 H^+ resin (xii) TBTU, TEA, DMF.

'tetramer' or protected octapeptide analogue **17**: there are examples [8] of Lindlar's catalyst being used to reduce azides in the presence of alkene groups in the literature, but none of the complexity involved in the present exercise.

NMR and IR studies of tetramer **17** in chloroform showed that a degree of organization was present. The wealth of NMR data obtained led to a turn-like structure being proposed as illustrated in Scheme 3. Although this is consistent with all the information that has been collected, a more rigorous examination of the data *via* molecular modelling is required before this structure can be confirmed.

Synthetic assembly of the hexamer by the same (i.e. saponification of the tetramer **17**, followed by TBTU coupling with the dimer amino component **13**) methodology was attempted. In our brief preliminary report of this work [9], we reported that after gel filtration a chromatographically homogeneous hexamer was obtained which had five distinct and well separated NH signals in its NMR spectrum with other evidence of conformational organization. There was, however, an unassigned broad signal in the NMR spectrum at 3.1 ppm, which at that time was judged to be an unidentified low molecular weight artefact. Subsequent work found that at low temperature the broad signal resolves into four methyl resonances (Scheme 4), consistent with the restricted rotation expected in a side product [10,11] derived from reaction of the amino component **13** with the TBTU reagent. This suspicion was confirmed by deliberate reaction of **13** with TBTU



Scheme 2 Reagents and conditions; (xiii) Lindlar's catalyst, MeOH, H_2; (xiv) NaOH (1M), MeOH, H_2O, Amberlite IR 120 H^+ resin; (xv) **10**, TBTU, TEA, DMF; (xvi) **11**, TBTU, TEA, DMF.

and characterization of the product as **18**; comparison of the NMR spectrum of this with the supposed hexamer showed that the 'hexamer' was in fact an almost 1 : 1 mixture of the tetramer **17** and the by product **18**.

EXPERIMENTAL

Exhaustive spectroscopic and analytical data were collected for all the compounds described, confirming their formulation as pure single isomers of the structures indicated.

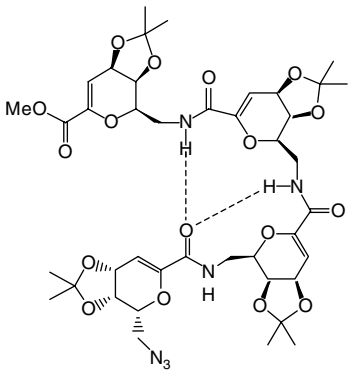
3,4,5,6-Tetra-*O*-acetyl-β-D-galactopyranosyl cyanide (2)

Trimethylsilyl cyanide (14 ml, 3 equiv) was added to a stirred solution of 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose **1** (14.0 g, 36.0 mmol) in anhydrous nitromethane (280 ml), followed by addition of $BF_3.Et_2O$ (0.7 ml), all at room temperature. Evaporation after 1 h gave a black oil, which was crystallized from MeOH, affording **2** as a yellow crystalline solid (9.59 g, 75%) of

m.p. 166°–168 °C, $[\alpha]_D^{26} = +37.8$ (*c* 1.0, $CHCl_3$). Lit [12] m.p. 168°–169 °C, $[\alpha]_D^{20} = +37.2$ (*c* 0.98, $CHCl_3$).

4,5,6-Tri-*O*-acetyl-2,5-anhydro-3-deoxy-D-*lyxo*-hept-2-enononitrile (3)

To a solution of **2** (2.0 g, 5.6 mmol) in anhydrous DCM (45 ml) were added 3 Å molecular sieves (2.0 g). The solution was stirred at room temperature for 6 h before being cooled in an ice-bath for 30 min. DBU (1.0 ml, 1.2 equiv) was then added dropwise and the reaction mixture was stirred at room temperature for a further 12 h. The mixture was filtered and the solvent was evaporated leaving a black oil, from which **3** (0.936 g, 56%) was obtained by flash column chromatography as a white crystalline solid of m.p. 115°–117 °C, $[\alpha]_D^{24} = -55.0$ (*c* 1.0, $CHCl_3$). Lit [13] m.p. 113°–115 °C, $[\alpha]_D^{20} = -52.0$ (*c* 1.321, $CHCl_3$). Some **2** was also recovered (0.446 g, 1.25 mmol, 22%).



Scheme 3 Proposed secondary structure of tetramer **17**.

Methyl 2,5-anhydro-3-deoxy-D-*lyxo*-hept-2-enonimidate (4)

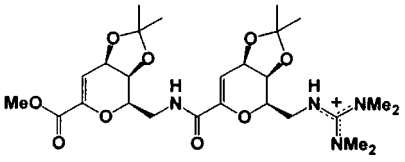
Unsaturated nitrile **3** (0.774 g, 2.61 mmol) was dissolved in MeOH (10 ml) at room temperature, and methanol NaOMe was added dropwise until the apparent pH was 9. The mixture was stirred overnight, during which a white precipitate formed. After keeping the mixture in the refrigerator for a further 12 h, the solids were filtered off, affording **4** (0.436 g, 82%), which was sufficiently pure for use: m.p. 187°–189 °C, $[\alpha]_D^{24} = -66.0$ (*c* 1.0, DMSO). Lit [14] m.p. 192°–193 °C, $[\alpha]_D = -64.0$ (*c* 1.05, DMSO).

Methyl 2,5-anhydro-3-deoxy-D-*lyxo*-hept-2-enonate (5)

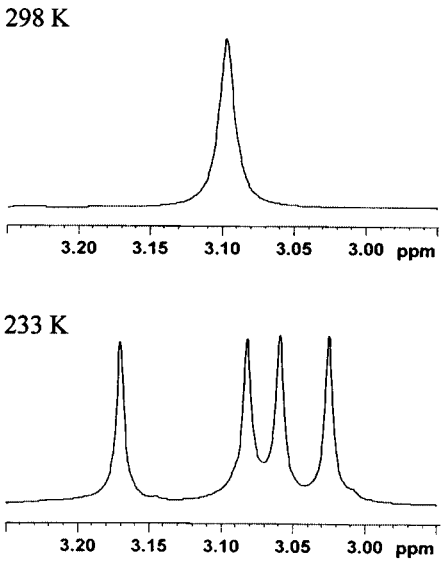
Imidic ester **4** (0.481 g, 2.37 mmol) was dissolved in water (25 ml), which was acidified with Amberlyst 15 (H^+ form) resin until the pH was 2 and then stirred for 2 h at room temperature. The resin was filtered off and the water was evaporated giving **5** (0.308 g, 64%) which was pure enough to be used: m.p. 142°–146 °C, $[\alpha]_D^{24} = -42.0$ (*c* 1.0, H_2O). Lit [15] m.p. 143°–144 °C, $[\alpha]_D = -42.0$ (*c* 1.03, H_2O).

Methyl 2,5-anhydro-3-deoxy-4,5-*O*-isopropylidene-D-*lyxo*-hept-2-enonate (6)

By reaction of 5 with acetone. 4-Toluenesulfonic acid (0.024 g, 0.12 mmol) was added to a stirred



18



Scheme 4 ^{1}H NMR spectra of side-product **18** at 298K and 233K.

solution of ester **5** (0.248 g, 1.22 mmol) in acetone (12 ml). The reaction mixture was stirred at room temperature for 24 h. DCM (30 ml) was added and the mixture was washed with saturated aqueous $NaHCO_3$ (3 × 30 ml), dried, and evaporated, forming a cloudy oil. Flash column chromatography gave **6** (0.248 g, 84%) as a colourless oil of $[\alpha]_D^{24} = +52.7$ (*c* 1.0, $CHCl_3$). MS: MNH_4^+ found 262.1291, $C_{11}H_{20}NO_6$ requires 262.1283.

By reaction of 5 with 2,2-dimethoxypropane. Compound **5** (0.226 g, 1.10 mmol) was dissolved in 2,2-dimethoxypropane (10 ml) followed by the addition of 4-toluenesulfonic acid (0.030 g, 0.15 mmol). The mixture was stirred under argon for 24 h. DCM (30 ml) was added and the mixture was washed with saturated aqueous $NaHCO_3$ (3 × 30 ml), dried, and evaporated. Flash column chromatography gave **6** as a colourless oil (0.136 g, 51%). Also isolated as a cloudy oil, was the by-product **7** (0.089 g, 25%): $[\alpha]_D^{24} = +39.2$ (*c* 1.0, $CHCl_3$). Found C, 56.78; H, 7.75; $C_{15}H_{24}O_7$ requires C, 56.95; H, 7.65%). Further **6** was obtained from the by-product **7** as follows. 4-Toluenesulphonic acid (0.022 g, 0.11 mmol) was added to a stirred solution of **7** (0.087 g, 0.28 mmol) in MeOH (10 ml). After 1 h, DCM (20 ml) was added and the mixture was washed with saturated aqueous NaHCO (3 × 15 ml), dried, and evaporated, giving **6** (0.049 g, 71% from **7**) which was identical to the material obtained directly.

Methyl 2,5-anhydro-6-azido-3,6-deoxy-4,5-*O*-isopropylidene-D-*lyxo*-hept-2-enonate, 'monomer' 9

Compound **6** (0.263 g, 1.07 mmol) was dissolved in DCM (12 ml) and cooled to −40 °C for 15 min. Pyridine (0.45 ml, 5.35 mmol, 5 equiv) was added dropwise, followed by trifluoromethanesulphonic anhydride (0.60 ml, 3.2 mmol, 3 equiv). DCM (30 ml) was added after 10 min and the mixture was washed with 1 M aqueous HCl (30 ml), followed by pH 7 buffer (85 g KH_2PO_4/14.5 g NaOH/950 ml H_2O: 30 ml), dried, and evaporated, giving an orange oil (crude trifluoromethanesulphonate **8**) which was immediately redissolved in DMF (12 ml) and treated with NaN_3 (0.218 g, 3.20 mmol, 3 equiv). Water (25 ml) was added to the mixture after 10 min. Extraction with DCM (6 × 50 ml), drying and flash column chromatography gave **9** as a colourless oil (0.189 g, 66%) of $[\alpha]_D^{24} = +30.1$(*c* 1.4, $CHCl_3$). Found C, 49.25; H, 5.76; N, 15.58; $C_{11}H_{15}N_3O_5$ requires C, 49.07; H, 5.62; N, 15.61%.

Amino ester 10

Compound **9** (0.125 g, 0.46 mmol) was dissolved in MeOH (3 ml) and Lindlar's catalyst (0.150 g) was added. The flask was flushed with argon and then with hydrogen and the solution was stirred at room temperature. After 1.5 h, the mixture was filtered through celite and the methanol was evaporated, giving **10** as an orange oil (0.106 g, 95%) of $[\alpha]_D^{24} = +49.6$(*c* 1.0, $CHCl_3$) MS: MH^+ found 244.1185, $C_{11}H_{18}NO_5$ requires 244.1184.

Azido acid 11

NaOH (1 M, 0.22 ml, 1 equiv) was added dropwise to a stirred solution of **9** (0.059 g, 0.22 mmol) in MeOH (0.5 ml). The reaction mixture was stirred at room temperature for 1 h. The MeOH was evaporated and the mixture was dissolved in H_2O (15 ml). Amberlite IR 120 H^+ resin was added to the solution until the pH was 1 and then removed by filtration. The filtrate was freeze-dried, forming **11** as an amorphous white solid (0.047 g, 84%) of m.p. 74°–77 °C, $[\alpha]_D^{23} = +51.4$ (*c* 1.0, $CHCl_3$). MS: M^- found 254.0777, $C_{10}H_{12}N_3O_5$ requires 254.0789.

Dimer 12

Azido acid **11** (0.137 g, 0.54 mmol) was dissolved in anhydrous DMF (1 ml) followed by treatment with TBTU (0.21 g, 0.65 mmol, 1.2 equiv) and TEA (0.11 ml, 0.76 mmol, 1.4 equiv). The mixture was stirred for 5 min before the addition of **10** (0.131 g, 0.54 mmol, 1.0 equiv pre-dissolved in 1 ml of DMF). After 3 days, the DMF was removed *in vacuo*, finally by co-evaporation with toluene. Flash column chromatography gave **12** as a pale yellow oil (0.155 g, 61%) of $[\alpha]_D^{24} = +58.0$ (*c* 1.0, $CHCl_3$). MS: MNa^+ found 503.1754, $C_{21}H_{28}N_4O_9Na$ requires 503.1759.

Selective reduction of dimer 12

Lindlar's catalyst (0.130 g) was added to a solution of **12** (0.136 g, 0.28 mmol) in MeOH (2 ml) at room temperature, which was then flushed with argon followed by hydrogen. After 1 h, the mixture was filtered through celite, and the MeOH was evaporated giving **13** (0.130 g, 100%) as an orange oil of $[\alpha]_D^{20} = +70.3$ (*c* 1.0, $CHCl_3$). MS: MH^+ found 455.2030, $C_{21}H_{31}N_2O_9$ requires 455.2015.

Saponification of dimer 12

NaOH (0.22 ml, 0.22 mmol, 1 M) was added to a stirred solution of **12** (0.103 g, 0.215 mmol) in MeOH (2.0 ml). After 1 h at room temperature, the MeOH was evaporated leaving an oily residue, which was redissolved in water (4 ml). Amberlite IR 120 resin was added until the pH was 2. The mixture was filtered and freeze dried affording **14** (0.062 g, 62%) as an amorphous white solid of $[\alpha]_D^{24} = +45.0$ (*c* 0.5, $CHCl_3$). MS: MH^+ found 467.1778, $C_{20}H_{27}N_4O_9$ requires 467.1773.

Trimer 15

By coupling 10 with 14. TBTU (0.100 g, 0.30 mmol, 1.2 equiv) was added to a solution of **14** (0.117 g, 0.25 mmol) in DMF (1 ml) at room temperature and the mixture was stirred for 5 min. Amine **10** (0.061 g, 0.25 mmol, pre-dissolved in 1 ml of DMF) was added to the mixture, followed by TEA (0.07 ml, 0.50 mmol, 2 equiv). After 3 days, the DMF was evaporated *in vacuo*, finally by co-evaporation with toluene, forming a brown oil. Flash chromatography gave **15** (0.050 g, 29%) as an amorphous white solid, identical with the product of the alternative procedure which follows.

By coupling 11 with 13. TBTU (0.167 g, 0.50 mmol, 1.2 equiv) was added to a stirred solution of **13** (0.190 g, 0.42 mmol) in DMF (1 ml) at room temperature. After 5 min, acid **11** (0.110 g, 0.42 mmol, predissolved in 1 ml of DMF) was added to the mixture, followed by TEA (0.11 ml, 0.84 mmol, 2 equiv). After 3 days, the DMF was evaporated *in vacuo,* finally by co-evaporation with toluene, forming a brown oil. Flash chromatography gave **15** (0.126 g, 43%) as an amorphous white solid of $[\alpha]_D^{21} = +49.1$(*c* 0.715, $CHCl_3$). MS: MH^+ found 692.2779, $C_{31}H_{42}N_5O_{13}$ requires 692.2767.

Tetramer 17

Lindlar's catalyst (0.170 g) was added to a solution of **15** (0.176 g, 0.25 mmol) in MeOH (5 ml) at room temperature. The system was flushed with argon followed by hydrogen. After 1 h, the mixture was filtered through celite. Evaporation gave **16** (0.169 g, 100%) as a pale orange oil. TBTU (0.100 g, 0.31 mmol, 1.2 equiv) was added to a stirred solution of this oil in DMF (1.5 ml) at room temperature. After 5 min, acid **11** (0.066 g, 0.26 mmol, pre-dissolved in 1.5 ml of DMF) was added to the mixture, followed by TEA (0.07 ml, 0.52 mmol, 2 equiv). After 3 days the DMF was evaporated *in vacuo*, finally by co-evaporation with toluene forming a brown oil. Gel filtration (Sephadex LH20, MeOH) followed by flash chromatography gave **17** (0.097 g, 42%) as an amorphous solid of $[\alpha]_D = +18.2$ (*c*, 4.06, $CHCl_3$). Found C, 54.71; H, 6.04; N, 9.34; $C_{41}H_{54}N_6O_{17}$ requires C, 54.54; H, 5.98; N, 9.31%.

By product 18

TBTU (0.017 g, 1 equiv) was added to a stirred solution of the dimer amine **13** (0.024 g, 0.054 mmol) in DMF (1 ml) under nitrogen. After 20 min, TEA (0.0075 ml, 1 equiv) was added. After 4 days, the solvent was removed and the residue was purified by repeated flash chromatography on neutral alumina to give **18** (0.019 g) as a clear oil of $[\alpha]_D^{25} + 16.2$ (*c*, 0.30, $CHCl_3$); MS: MH^+ found 553.2867, $C_{26}H_{41}N_4O_9$ requires 553.2874.

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