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Greener peptoid synthesis in additive-free water-based media†

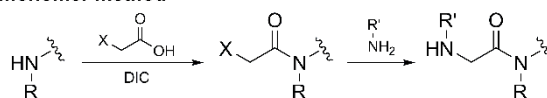
Nassirine Soumanou,^a Dorthe Lybye,^b Thomas Hjelmgaard,^{*b} and Sophie Faure^{*a}

Highly efficient procedures were developed for greener synthesis of sequence-defined peptoid oligomers. The optimised chain elongation procedure is carried out in water-based media in a unique *N* to *C* direction, and comprises a convenient one-pot two-step deprotection/coupling sequence for the installation of each new peptoid residue. The process is so efficient that the only work-up procedure required after each intermediate coupling step is aqueous extraction. In stark contrast to the traditional procedures, the only solvents apart from water used during the synthesis and workup processes are EtOH, MeOH or EtOAc. Furthermore, there is no requirement for specialised water-soluble protecting groups or inclusion of additives apart from the reagents in the reaction media.

Introduction

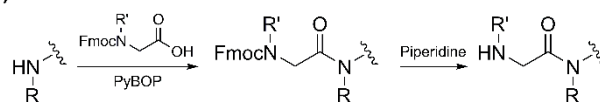
Originally developed in the late 1980's,^{1,2} peptoids (oligoamides of *N*-substituted glycines) possess a skeleton similar to that of peptides but differ in that the side-chains are appended to the nitrogen of the amides rather than to the α carbon (Scheme 1).³ Tremendous efforts have been made to understand and improve the folding properties of this class of peptidomimetics that lacks backbone chirality and hydrogen bond donors.^{4,5} Accordingly, structurally well-defined peptoids which mimic biological structures and functions⁶ and act as biomimetic nanomaterials have been developed.⁷ Peptoids are now amongst the most promising peptidomimetics to reach the drug market:⁸ they are far less susceptible to proteases and more cell-permeable than peptides,^{9,10} and they are highly suitable for combinatorial chemistry and high throughput screenings.¹¹ One of the many advantages of peptoids is indeed the facile synthesis. Thus, highly efficient submonomer and monomer methods for sequence-defined synthesis both in solution and on solid-phase using readily available starting materials have been developed (Scheme 1, (a) and (b)).^{12,13} However, no particular attention has been directed towards greener peptoid synthesis, and solvents such as DMF, NMP, THF and CH₂Cl₂ are routinely used during the synthesis and workup steps. In an effort to mitigate these sustainability issues, the first development of peptoid synthesis in water-based, additive-free media is presented herein (Scheme 1, (c)).

(a) Submonomer method



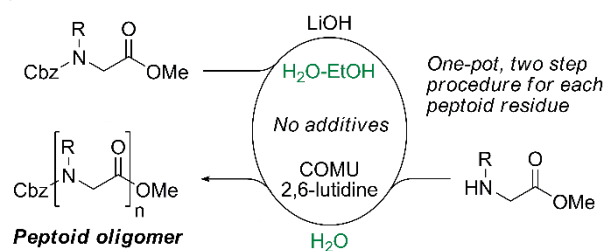
Solid-phase: DMF, DCM or NMP DMF, NMP or DMSO
Solution: DCM THF or EtOAc

(b) Monomer method



Solid-phase: DMF DMF or NMP
Solution: DCM DCM

(c) This work: monomer method in water-based solvents



Scheme 1 Classical methods to access peptoid oligomers: (a) submonomer and (b) monomer protocols with solvents traditionally employed in solid- or solution-phase synthesis, and (c) strategy developed in this work.

Various strategies have been explored for performing synthesis of the parent peptides in aqueous media, including the use of water-soluble protecting groups, nanoparticles and microwave-assistance to overcome solubility issues.¹⁴ However, the arguably most successful approach is the use of micellar aqueous media.¹⁵⁻¹⁹ In a recent example, efficient amide/peptide bond formation in an aqueous micellar medium

^a Université Clermont Auvergne, CNRS, ICCF, F-63000 Clermont-Ferrand, France. sophie.faure@uca.fr

^b Rockwool A/S, Hovedgaden 584, 2640 Hedehusene, Denmark. thomas.hjelmgaard@rockwool.com

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comprising 2 wt % TPGS-750-M (DL- α -tocopherol methoxypolyethylene glycol succinate)¹⁶ in combination with the uronium salt COMU ((1-cyano-2-ethoxy-2-oxoethylideneaminoxy) dimethylamino-morpholino-carbenium hexafluorophosphate) as coupling reagent was demonstrated.¹⁷ This methodology was further extended to the synthesis of various peptides of up to ten amino acid residues using a tandem Cbz deprotection/coupling sequence.^{18,19} Adaptation of these methodologies to peptoid synthesis was used as a starting point for the development presented herein.

Model study of peptoid synthesis in the C to N direction in water-based media

Synthesis of peptoids is commonly performed in the C to N direction. As a representative model system, it was chosen to initiate the study with the 1+1 coupling of the peptoid acid **1a** with the peptoid amines **2a** or **2b** (Table 1). The various peptoid acids **1** and peptoid amines **2** used herein were either commercially available or could readily be synthesised from commercially available reagents (see ESI). The 1+1 coupling reaction of peptoid acid **1a** with peptoid amine **2a** leading to the model dipeptoid **3** with a C-terminal *tert*-butyl ester was first studied in aqueous micellar media consisting of 2 wt % TPGS-750-M¹⁶ or SPGS-550-M (β -sitosterol methoxypolyethyleneglycol succinate)²⁰ in the presence of COMU and 2,6-lutidine at room temperature (Table 1, entries 1 and 2). Formation of the *N,N*-disubstituted amide in peptoids is more difficult than formation of the secondary amide in peptides, but the desired dipeptoid **3** was nonetheless obtained in excellent yields (89% and 94%, respectively), as well as in high crude purities (91% and 85%, respectively). It was then surprisingly found that the coupling reaction still proceeded in excellent crude purity (90%) and only slightly lower crude yield (85%) when using pure water as the reaction medium (entry 3). The inherent lipophilicity of the peptoid backbone compared to a peptide skeleton may explain this unexpected difference in reactivity in water. Increasing the reaction temperature to 30 °C only resulted in minor improvements in crude yield and purity (86% and 92%, respectively, entry 4). For comparison, performing the coupling reaction in a conventional solvent (CH₂Cl₂, entry 5) likewise furnished dipeptoid **3** in high yield (86%). However, the crude purity was moderate (61%), preventing the use of the crude material directly in further peptoid chain elongation.

Synthesis of the analogous model dipeptoid **4** with a methyl ester at the C-terminus was likewise first studied in 2 wt % TPGS-750-M at room temperature (entry 6). The desired product was obtained in high yield (89%) and excellent crude purity (98%). When performed at 30 °C (entry 7), the crude yield increased (95% vs. 89%) while the crude purity decreased (92% vs. 98%). Performing the reaction in pure water at room temperature provided the dipeptoid **4** in an excellent purity (98%), but the crude yield initially decreased to 68% (entry 8). However, in this case, increasing the reaction temperature to 30 °C had a

significant impact, providing dipeptoid **4** in excellent crude yield and purity (92% and 94%, respectively, entry 9).

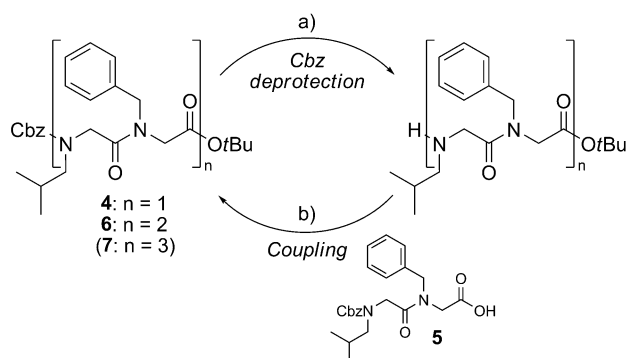
Importantly, these 1+1 coupling reactions were amenable to gram scale synthesis. It was, however, necessary to dilute the reaction medium to double the volume (amine concentration of 0.25 M instead of 0.50 M) to prevent the reaction mixture from turning into a paste when using micellar reaction media.

Table 1 Model dipeptoid synthesis in the C to N direction in water-based media

En.	Dipeptoid	Reaction medium	Temp. (°C)	Yield (%)	Purity (%) ^c
1	3	2% TPGS-750-M/H ₂ O	rt	89 ^b	91
2	3	2% SPGS-550-M/H ₂ O	rt	94 ^a	85
3	3	H ₂ O	rt	85 ^a	90
4	3	H ₂ O	30	86 ^a	92
5	3	CH ₂ Cl ₂	rt	86 ^b	61
6	4	2% TPGS-750-M/H ₂ O	rt	89 ^a	98
7	4	2% TPGS-750-M/H ₂ O	30	95 ^a	92
8	4	H ₂ O	rt	68 ^a	98
9	4	H ₂ O	30	92 ^a	94

Key: (a) Peptoid amine **2a** or **2b** (1.0 equiv., 0.5 M), peptoid acid **1a** (1.1 equiv.), COMU (1.1 equiv.), 2,6-lutidine (3.1 equiv.), overnight, further conditions in Table; (a) Crude yield; (b) Isolated yield; (c) Crude HPLC purity (214 nm).

The next step was to investigate the access to tetrapeptoids by block-coupling of the model dipeptoids after selective *N*- and *C*-terminal deprotection, respectively (Table 2). Unfortunately, *N*-terminal deprotection of methyl ester dipeptoid **4** resulted in diketopiperazine formation. This was not observed for *N*-terminal deprotection of dipeptoid **3** carrying the sterically hindered *tert*-butyl ester. In this case, however, the *C*-terminal deprotection to access the derived dipeptoid acid **5** required treatment with TFA in CH₂Cl₂. In spite of these undesirable deprotection conditions, dipeptoid acid **5** was still useful for studying the adaptation of the above-mentioned tandem Cbz deprotection/coupling sequence.^{18,19} Direct adaptation of the reaction sequence to synthesis of the model tetrapeptoid **6** by Cbz-deprotection of dipeptoid **4** in 2 wt % TPGS-750-M followed by coupling with dipeptoid acid **5** in the presence of COMU and 2,6-lutidine at room temperature proved unsuccessful (Table 2, entry 1). The major issue appeared to be the deprotection step since the desired tetrapeptoid **6** could be obtained in modest yield (36%) when the Cbz-deprotection was performed under conventional conditions (MeOH as solvent, entry 2). It was, however, possible to obtain a modest yield of tetrapeptoid **6** when using 2 wt % TPGS-750-M or 2 wt % SPGS-550-M as the reaction medium by increasing the reaction temperature to 30 °C and concomitantly decreasing the concentration to 0.25 M (41% and 17%, respectively, entries 3 and 4).

Table 2 Model oligomer synthesis in the C to N direction in water-based media

Entry	Oligomer	Reaction medium for deprotection step (a)	Temp. (°C)	Yield (%)
1	6	2 wt % TPGS-750-M/H ₂ O, HCl	Rt	-
2	6	MeOH	Rt	36
3	6	2 wt % TPGS-750-M/H ₂ O, HCl	30	41
4	6	2 wt % SPGS-550-M/H ₂ O, HCl	30	17
5	7	2 wt % TPGS-750-M/H ₂ O, HCl	30	-

Key: (a) Pd/C 10%, H₂, further conditions in Table; (b) Peptoid amine intermediate (1.0 equiv., 0.25-0.50 M), peptoid acid **5** (1.1 equiv.), COMU (1.05 equiv.), 2,6-lutidine (3.1 equiv.), 24 h, further conditions in Table.

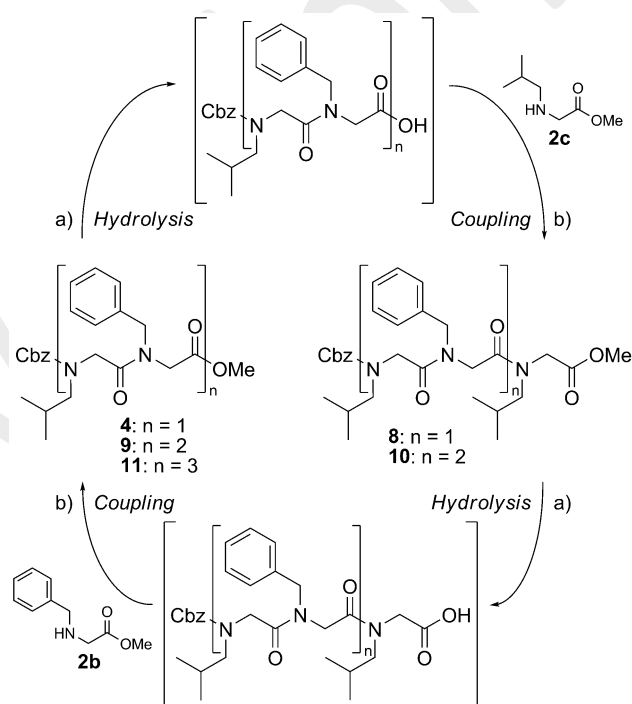
Attempts to improve the yield by inclusion of Oxyma as an additive together with COMU and 2,6-lutidine or DIPEA as base were unsuccessful. It was furthermore found that application of the most promising conditions (Table 2, entry 3) to a 4+2 block-coupling of tetrapeptoid **6** with dipeptoid acid **5** only furnished traces of the desired hexapeptoid **7** (entry 5).

This model study in the C to N direction represents the first demonstration of peptoid synthesis in aqueous reaction media and constitutes a first step towards more sustainable peptoid synthesis. Dipeptides were obtained in high yields and purities by 1+1 couplings. However, undesirable deprotection conditions were required for chain elongation beyond the dipeptides, only modest yields were obtained for the ensuing 2+2 block-couplings, and extension to 4+2 block-coupling proved unsuccessful. The development of an alternative strategy was therefore required.

Model study of peptoid synthesis in the N to C direction in water-based media

Contrary to peptides, the elongation of peptoids in the N to C direction is a feasible strategy since there are no issues with racemisation of backbone stereogenic C α centers (Table 3). One notable advantage is that only the first monomer requires protection at the N-terminal position and that this protecting group remains installed throughout the synthesis. The result is an improved atom economy, which is indeed found when calculating the atom economies for the formation of a model dipeptoid in the N to C versus in the C to N direction (39% vs. 33%, respectively, see ESI for details). Moreover, the ester

hydrolysis at the C-terminus may be performed under mildly basic aqueous conditions. The investigation of the N to C strategy started from the model dipeptoid **4** obtained above, extending the backbone with alternating peptoid amines **2c** and **2b** up to the model hexapeptoid **11** (Table 3, entries 1-4). The peptoid intermediates with free acids at the C-terminus were obtained by treatment with lithium hydroxide (1.1 equiv.) in MeOH-H₂O at 30 °C, acidification to pH 4, extraction with EtOAc and then evaporation. The crude products were used directly in the ensuing coupling steps in the presence of COMU and 2,6-lutidine at room temperature, both in 2 wt % TPGS-750-M (Method A) and in pure water (Method B).

Table 3 Model oligomer synthesis in the N to C direction in water-based media

Entry	Oligomer	Method A		Method B	
		Yield (%)	Purity (%)	Yield (%)	Purity (%)
1	Tripeptoid 8	62 ^a	88 ^a	58 ^a	85 ^a
2	Tetrapeptoid 9	77 ^a	81 ^a	71 ^a	87 ^a
3	Pentapeptoid 10	75 ^a	70 ^a	80 ^a	69 ^a
4	Hexapeptoid 11	43 ^b	84 ^b	53 ^b	85 ^b

Key: (a) LiOH-H₂O (1.1 equiv.), MeOH/H₂O 5:1 (0.2 M), 30 °C, 1 h; then HCl (1 M) to pH = 4; (b) peptoid acid intermediate (1.0 equiv., 0.35 M), peptoid amine **2c** or **2b** (1.3 to 1.5 equiv.), COMU (1.1 to 1.5 equiv.), 2,6-lutidine (3.1 equiv.), 2 wt % TPGS-750-M/H₂O (method A) or H₂O (method B), overnight at rt; ^a Crude yield and HPLC purity (214 nm); ^b Yield and HPLC purity (214 nm) after purification by silica gel chromatography.

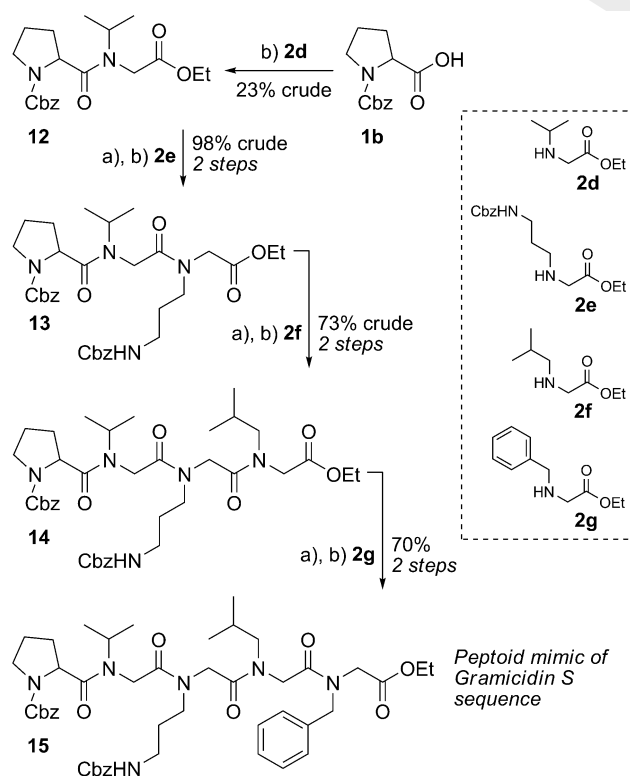
The reactions generally proceeded in good to high yields and purities (entries 1-4), and the inclusion of the micelles in the coupling steps did not appear to have any significant effect on the efficiency of the reactions (Method A vs. Method B). Peptoid hexamer **11** was accordingly obtained in similar overall yields (15% vs. 17%) and purities (84% vs. 85%) from dipeptoid **4** when performing the couplings in 2 wt % TPGS-750-M and in pure

water, respectively. Although the use of the aqueous micellar media already represents a major improvement compared to the traditional solvents, the unique possibility of performing the chain elongation in additive-free, environmentally benign solvents only is arguably even more intriguing from a sustainability point of view.

Application to the synthesis of a peptoid mimic of the Gramicidin S sequence

With efficient procedures for peptoid synthesis in additive-free, environmentally benign solvents in hand, the methods were applied to the synthesis of the protected linear pentapeptoid **15** (Scheme 2). This peptoid is a mimic of the peptidic sequence found in the antimicrobial Gramicidin S cyclodimer: *cyclo*(Pro-Val-Orn-Leu-D-Phe)₂.²¹ The first coupling between the protected proline **1b** and peptoid amine **2d** was particularly challenging due to the high steric hindrance of both reactants. Accordingly, the desired dipeptoid **12** was initially obtained in 17% yield. The yield could be increased slightly to 23% with the inclusion of 10% EtOAc as co-solvent and increasing the reaction temperature to 45°C.

Scheme 2 Synthesis of pentapeptoid mimic of the Gramicidin S sequence



Key: (a) LiOH·H₂O (1.2 equiv.), EtOH/H₂O 5:1 (0.2 M), 30 °C, 1 h; then HCl (1 M) to pH = 4; (b) peptoid acid intermediate (1.0 equiv., 0.35 M), peptoid amine **2d**, **2e**, **2f** or **2g** (1.2 equiv.), COMU (1.2 equiv.), 2,6-lutidine (3.1 equiv.), H₂O (10% EtOAc as co-solvent for synthesis of **12**), overnight at rt (45 °C for synthesis of **12**). Yields given for **12**, **13** and **14** are of crude products, while the yield of **15** is after purification by silica gel chromatography (96% HPLC purity (214 nm)).

By contrast, the ensuing stepwise elongation steps leading to the pentapeptoid **15** proceeded in good to excellent crude yields. The final pentapeptoid was purified by column chromatography, furnishing more than 300 mg product in excellent purity (96%) and 12% overall yield for the 7 steps from protected proline **1b**.

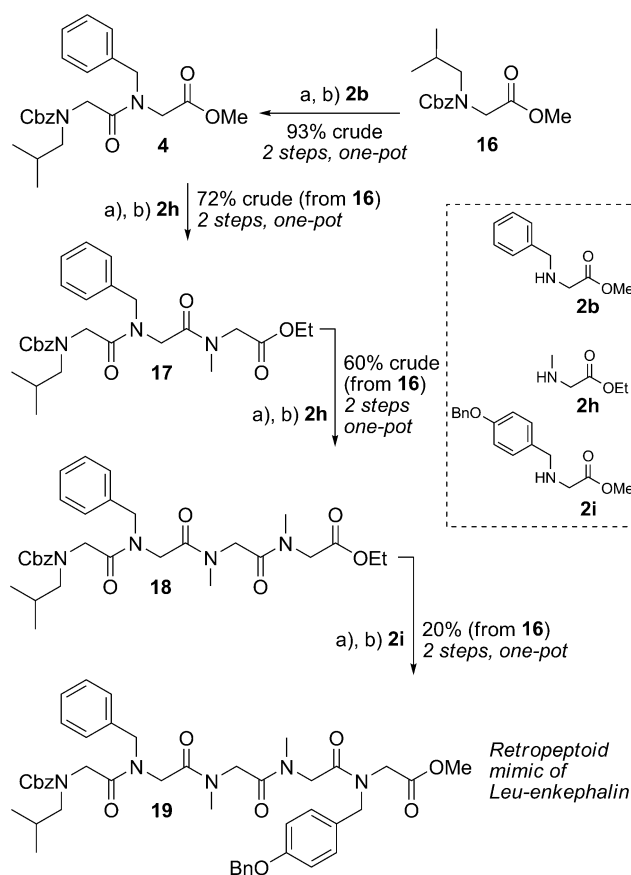
For comparison, the synthesis was also carried out with the inclusion of 2 wt% TPGS-750-M in the coupling steps, which provided pentapeptoid **15** in comparable purity and yield after purification (98% purity and 10% overall yield from protected proline **1b**).

One-pot two-step procedure and synthesis of a retropeptoid mimic of Leu-enkephalin

As a further improvement and simplification of the above method, it was then studied if the work-up by extraction after C-terminal deprotection could be omitted by developing a one-pot two-step procedure for incorporation of each peptoid residue. The preparation of a retropeptoid mimic of the endogenous opioid peptide neurotransmitter Leu-enkephalin²² (Tyr-Gly-Gly-Phe-Leu) was used to investigate this simplified protocol (Scheme 3).

Thus, after completion of each hydrolysis step, the reaction mixtures were acidified to pH 4 using 1M HCl. The EtOH was then evaporated, and the ensuing coupling steps were performed directly using the optimized conditions described above. This convenient one-pot two-step procedure proved highly efficient, and the desired Leu-enkephalin retropeptoid mimic **19** was obtained in excellent purity (97%) and 20% overall yield for the 8 steps from peptoid methyl ester **16**. The crude purities of the peptoid intermediates **4**, **17** and **18** were all excellent (≥94%) and only extraction with EtOAc was required after each coupling step. This highlights the efficiency of the developed method and the excellent crude purities are essential if the crude intermediate products are to be used directly in chain elongation without further purification.

Scheme 3 One-pot two-step procedure and synthesis of retropeptoid mimic of Leu-enkephalin



Key: (a) LiOH-H₂O (1.0 equiv.), EtOH/H₂O 2:3 (0.2 M), 30 °C, 1 h; then HCl (1 M) to pH = 4; then (b) peptoid acid intermediate (1.0 equiv., 0.35 M), peptoid amine **2b**, **2h** or **2i** (1.1 equiv.), COMU (1.1 equiv.), 2,6-lutidine (3.1 equiv.), H₂O, 4 h or overnight at 30 °C. Yields given for **4**, **17** and **18** are of crude products overall from **16**, while the yield of **19** is overall from **16** after purification by silica gel chromatography (97% HPLC purity (214 nm)).

To evaluate the sustainability of the optimised protocol in terms of waste produced, the E-factor²⁴ was calculated for the whole sequence leading to the retropeptoid mimic of Leu-enkephalin (see ESI for details). The E-factor for a previously published synthesis²⁵ of a retropeptoid mimic of Leu-enkephalin using a conventional solid-phase monomer method was calculated for comparison. The E-factor for the optimised protocol developed herein was found to be more than four times lower than the E-factor obtained for the conventional solid-phase monomer method when considering all solvents in the calculation, and 17 times lower when excluding water from the calculation (see ESI for details). In addition, the majority of the wastes produced from the use of the conventional methods is composed of hazardous solvents. The methods developed herein thus also represent a major improvement in terms of E-factor when compared to methods that are traditionally employed for the synthesis of peptoids.

Conclusions

Solvents such as DMF, NMP, THF and DCM are routinely used during the various processes required for the synthesis and work up of peptoids. The development work presented herein is the first that focuses on addressing these sustainability issues. Thus, the outcome of the initial model study in the classical C to N direction represents the first demonstration of peptoid synthesis in aqueous reaction media. Using this approach, dipeptoids were obtained in high yields and purities by 1+1 couplings of peptoid monomers using methods that were amenable to gram scale synthesis. However, undesirable deprotection conditions were required for chain elongation beyond the dipeptoids, only modest yields were obtained for the ensuing 2+2 block-couplings, and extension to 4+2 block-coupling proved unsuccessful. As an alternative approach, novel methods were therefore developed for synthesis in the opposite N to C direction. Chain elongation in this direction is one of the unique features of peptoids compared to peptides since there is no risk of backbone racemisation. The optimised chain elongation procedure in the N to C direction comprises a highly convenient and efficient one-pot two-step deprotection/coupling sequence for installing each new peptoid residue. In the first step of the process, the C-terminal ethyl or methyl ester of the peptoid chain is hydrolysed by reaction with LiOH in aqueous EtOH. After acidification with 1M HCl to pH 4 and evaporation of the EtOH, the ensuing coupling reaction with a peptoid amine is performed in water in the presence of COMU and 2,6-lutidine. This procedure was used in the synthesis of a retropeptoid mimic of Leu-enkephalin in 20% overall yield for the 8 steps from the starting monomer to the final pentapeptoid mimic. The process is so efficient that the only work-up procedure required after each intermediate coupling step is aqueous extraction. Importantly, the only solvents apart from water used during synthesis and workup processes were EtOH, MeOH or EtOAc. Compared to a classic method employed in the synthesis of peptoids, the procedures developed herein represent a major improvement in the E-factor (4 times lower when considering all solvents and 17 times lower when excluding water from the calculation). Furthermore, contrary to peptide synthesis in aqueous media, there was no requirement for specialised water-soluble protecting groups, and no need for inclusion of additives such as micelles in the reaction media. A central advantage in terms of atom economy of synthesis in the N to C direction is that only the first monomer requires protection at the N-terminal position and that this protecting group remains installed throughout the synthesis. The repeated installation and removal of large protecting groups such as Fmoc and Cbz is thus avoided. This improvement in atom economy was indeed exemplified by calculations performed for the formation of a representative model dipeptoid in the N to C and in the C to N direction, respectively. Moreover, the methyl or ethyl ester hydrolysis at the C-terminus may be performed under mildly basic conditions in aqueous EtOH. Compared to the conventional procedures, the novel and highly efficient method

developed herein thus represents a first and major step towards sustainable production of sequence-defined peptoid oligomers.

Conflicts of interest

There are no conflicts to declare.

Experimental

General experimental information

All solvents and chemicals obtained from commercial sources were used as received. 2 wt. % TPGS-750-M in H₂O, 2 wt. % SPGS-550-M in H₂O and 97% COMU were purchased from Sigma-Aldrich. The various peptoid monomers used herein were either commercially available or could readily be synthesised from commercially available reagents (see ESI). NMR spectra were recorded on a 400 MHz Bruker AC 400 spectrometer. Chemical shifts are referenced to the residual solvent peak and *J* values are given in Hz. The following multiplicity abbreviations are used: (s) singlet, (d) doublet, (m) multiplet and (br) broad. Where applicable, assignments were based on COSY, HMBC, HSQC and *J*-mod-experiments. Due to *cis/trans*-isomerism of the *N,N*-disubstituted amides in the peptoid backbones, multiple conformers (up to 2ⁿ rotameric forms for *n*-mers) may be visible in the NMR spectra, often rendering these spectra very complex.²⁶ Thin layer chromatography (TLC) was carried out on silica gel Kieselgel plates 60 F254 on alumina (Merck). Progression of reactions was, when applicable, followed by HPLC. Analytical HPLC was performed on a Agilent 1100 series coupled to DAD detector equipped with a Nucleodur 100-3 (C18, 125 mm, Ø 4.6 mm) with a flow of 1 mL/min using as solvent A: H₂O (0.1% TFA) and solvent B: MeCN; Gradient for analytical HPLC (17 min runs): 5% B (0-2 min), 5%→95% B (2-9 min), 95% B (9-12 min), 95%→5% B (12-14 min), 5% B (14-17 min). High-resolution mass spectra (HRMS) were recorded using electrospray ionization in positive mode (ESI+) on or a Q Exactive Quadrupole-Orbitrap Mass Spectrometer. Liquid chromatography mass spectroscopy (LC-MS) were recorded on a Q Exactive Quadrupole-Orbitrap Mass Spectrometer coupled to a UPLC Ultimate 3000 (Kinetex EVO C18; 1.7 µm; 100 mm x 2.1 mm column with a flow rate of 0.45 mL/min with the following gradient: a linear gradient of solvent B from 5% to 95% over 7.5 min (solvent A = H₂O + 0.1% formic acid, solvent B = MeCN + 0.1% formic acid) equipped with a DAD UV/VIS 3000 RS detector) or on a Qtof-micro Waters (3000V) with electrospray ionization coupled to an HPLC Alliance Waters system with a diode array detector (DAD) using a reverse-phase C18 Nucleosil column (100 mm x 2.1 mm, 5 µm pore size) with an H₂O/MeCN gradient and a flow rate of 0.2 mL/min.

General procedure A: Dipeptoid synthesis in the C to N direction by 1+1 coupling in water

To the peptoid acid (1.1 equiv.) in H₂O, 2 wt % TPGS-750-M/H₂O⁹ or 2 wt % SPGS-550-M/H₂O⁹ was added 2,6-lutidine (3.1 equiv.). The mixture was stirred for 5 min at room temperature whereupon the peptoid amine (1.0 equiv., 0.5 M) and COMU

(1.1 equiv.) were added. The mixture was stirred overnight at room temperature or 30 °C and was then extracted with EtOAc (2×). The combined organic layers were washed with 1 M HCl (3×), satd. aq. Na₂CO₃ (4×) and brine (1×). The organic layer was dried over Na₂SO₄, filtered and evaporated under reduced pressure to furnish the crude dipeptoid.

Dipeptoid 3. Dipeptoid **3** was synthesised by 1+1 coupling of peptoid acid **1a** (0.50 mmol) with peptoid amine **2a** (0.100 g, 0.45 mmol) according to General procedure A in 2 wt % TPGS-750-M/H₂O at room temperature. Dipeptoid **3** was obtained as a colourless oil (0.186 g, 0.40 mmol, 89%) after flash chromatography on silica gel using cyclohexane/EtOAc 70:30 as eluent. *R_f* (cyclohexane/EtOAc 90:10) = 0.34; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 0.77, 0.80, 0.82 and 0.85 (6H, 4×d, *J* = 6.7 Hz), 1.31, 1.33, 1.37 and 1.40 (9H, 4×s), 1.70-1.89 (1H, m), 3.08-3.21 (2H, m), 3.63, 3.81, 3.88 and 3.90 (2H, 4×s), 3.94, 4.01, 4.04 and 4.15 (2H, 4×s), 4.41, 4.52, 4.53 and 4.57 (2H, 4×s), 5.03, 5.06 and 5.09 (2H, 3×s), 6.91-7.33 (10H, m); ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 20.0, 20.1, 20.2 (2CH₃), 27.4, 27.5 (1CH), 27.9, 28.1, 28.2 (3CH₃), 47.9, 48.6, 48.7, 48.9, 49.0, 49.1 (2CH₂), 50.4, 50.6, 50.7, 50.9 (CH₂), 56.4, 56.3, 55.5, 55.4 (CH₂), 67.5, 67.4, 67.3 (1CH₂), 82.8, 82.7, 82.0, 81.5 (1C), 128.1, 128.7, 128.6, 128.5, 128.2, 128.0, 127.9, 127.8, 129.7, 127.4 (10CH), 136.8, 136.7, 136.4, 136.3, 135.6, 135.4 (2C), 157.1, 157.0, 156.6 (1C), 168.4, 168.3, 168.1 (1C), 169.4, 169.2, 169.1 (1C); HRMS (*m/z*): calcd for [M + H]⁺: 469.2697; found: 469.2676; LCMS: *t_r* = 6.70 min.

Dipeptoid 4. Dipeptoid **4** was synthesised by 1+1 coupling of peptoid acid **1a** (0.50 mmol) with peptoid amine **2b** (0.081 g, 0.45 mmol) according to General procedure A in H₂O at 30 °C. Dipeptoid **4** was obtained as a colourless oil (0.177 g, 92%) which was used without purification in the next step. *R_f* (cyclohexane/EtOAc 70:30) = 0.50; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 0.84 and 0.89 (6H, 2×d, *J* = 6.7 Hz), 1.82-1.89 (1H, m), 3.18-3.25 (2H, m), 3.60-3.70 (3H, m), 3.79-4.23 (4H, m), 4.49 and 4.65 (2H, 2×s), 5.11-5.15 (2H, s), 7.00-7.40 (10H, m); ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 19.9, 20.0 (2CH₃), 27.3, 27.4 (1CH), 47.0, 47.2, 47.4, 47.7, 48.4, 48.7, 48.8, 48.9, 49.0, 49.4, 49.5, 49.6 (2CH₂), 50.1, 50.2, 51.0, 51.3 (1CH₂), 52.1, 52.4 (1CH₃), 55.2, 55.4, 55.6 56.1, 56.2, 56.4 (1CH₂), 67.2, 67.3, 67.4 (1CH₂), 126.4, 126.8, 127.6, 127.8, 127.9, 128.0, 128.1, 128.4, 128.6, 128.9, 129.0 (10CH), 135.1, 135.3 (1C), 136.0, 136.1, 136.6, 136.7 (1C), 156.3, 156.5, 156.9, 157.0 (1C), 169.0, 169.2, 169.3, 169.5, 169.6, 169.7 (2C); HRMS (*m/z*): calcd for [M + H]⁺: 427.2227; found: 427.2226; LCMS: *t_r* = 5.70 min.

Tetrapeptoid 6. Peptoid acid **5** was obtained from dipeptoid **4** (0.130 g, 0.277 mmol, 1.1 equiv.) by deprotection of the *tert*-butyl ester (see ESI for general procedure). Tetrapeptoid **6** was then synthesized by Cbz deprotection of dipeptoid **4** (0.118 g, 0.252 mmol, 1.0 equiv.) followed by coupling with the above peptoid acid **5**. The procedure was carried out by adaptation of

the tandem Cbz deprotection/coupling sequence for peptide synthesis described in the literature,^{19,23} performing the reactions at 30 °C instead of at room temperature. Tetrapeptoid **6** was obtained as a colourless oil (0.076 g, 0.104 mmol, 41%) after flash chromatography on silica gel using cyclohexane/EtOAc 70:30 as eluent. R_f (cyclohexane/EtOAc 60:40) = 0.17; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 0.83-0.90 (12H, m), 1.40-1.50 (9H, s), 1.81-1.89 (2H, m), 3.21-3.28 (2H, m), 3.74-4.14 (8H, m), 4.53-4.72 (4H, m), 5.08-5.15 (4H, m), 7.00-7.37 (15H, m); HRMS (m/z): calcd for [M + H]⁺: 729.4222; found: 729.4229; LCMS: t_r = 6.49 min.

General procedure B: Peptoid acid by deprotection of methyl or ethyl ester for peptoid synthesis in the N to C direction

To a suspension of the peptoid (0.2 M) in MeOH/H₂O 5:1 (for synthesis of peptoids **8**, **9**, **10** and **11**) or EtOH/H₂O 5:1 (for synthesis of peptoids **13**, **14** and **15**) was added LiOH·H₂O (1.0-1.2 equiv.). The mixture was then stirred for 1 h at 30 °C. After completion of the reaction, the MeOH was evaporated. H₂O was added and the mixture was acidified to pH 4 with 1M HCl. The resulting mixture was extracted with EtOAc and the organic layer was dried over Na₂SO₄, filtered and evaporated under reduced pressure to furnish the crude peptoid acid which was used without further purification in the ensuing coupling step.

General procedure C: Peptoid coupling for peptoid synthesis in the N to C direction in aqueous media

To the peptoid acid (1.0 equiv., 0.35 M) in H₂O or 2 wt % TPGS-750-M/H₂O was added 2,6-lutidine (3.1 equiv.). The mixture was stirred for 5 min at room temperature and the peptoid amine (1.2-1.5 equiv.) and COMU (1.1-1.5 equiv.) were then added. After stirring overnight at room temperature, the mixture was extracted with EtOAc (3×). The combined organic layers were washed with 1M HCl (3×), satd. aq. Na₂CO₃ (4×) and brine (1×). The organic layer was dried over Na₂SO₄, filtered and evaporated under reduced pressure to furnish the crude peptoid.

Tripeptoid 8. Tripeptoid **8** was synthesised by methyl ester deprotection of dipeptoid **4** (0.197 g, 0.46 mmol, 1.0 equiv.) according to General procedure B followed by coupling with peptoid amine **2c** (0.087 g, 0.60 mmol, 1.3 equiv.) in the presence of COMU (0.257 g, 0.60 mmol, 1.3 equiv.) according to General procedure C in H₂O. Tripeptoid **8** was obtained as a colourless oil (0.145 g, 0.27 mmol, 58%) which was used in the ensuing reaction without further purification. R_f (cyclohexane/EtOAc 60:40) = 0.43; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 0.81-0.90 (12H, m), 1.81-1.83 (2H, m), 2.80-3.26 (4H, m), 3.66-3.71 (3H, m), 3.80-4.24 (6H, m), 4.52-4.67 (2H, m), 5.09-5.14 (2H, m), 7.03-7.37 (10H, m); ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 19.8, 19.9, 20.0, 20.1 (4CH₃), 27.01, 27.2, 27.3, 27.5, 27.7, 27.8 (2CH), 46.1, 46.2, 46.3, 46.9, 47.1, 47.4, 48.3, 48.4, 48.7, 48.9, 49.2 (3CH₂), 49.6, 50.2, 50.5, 50.8, 51.0 (1CH₂), 52.1, 52.2, 52.5, 52.7 (1CH₃), 55.3, 55.4, 55.6, 55.8, 56.0, 56.4, 56.5 (2CH₂), 67.3 (1CH₂), 126.6, 127.1, 127.2, 127.6, 127.7, 127.8, 127.9, 127.9, 128.0, 128.4, 128.7, 128.6, 128.7, 128.8, 129.0,

129.1 (10CH), 135.6, 135.7 (1C), 136.7, 136.8 (1C), 156.6, 157.1 (1C), 168.7, 168.8, 169.0, 169.2, 169.6, 170.1 (3C); HRMS (m/z): calcd for [M + H]⁺: 540.3068; found: 540.3065; LCMS: t_r = 5.70 min.

Tetrapeptoid 9. Tetrapeptoid **9** was synthesised by methyl ester deprotection of tripeptoid **8** (0.118 g, 0.218 mmol, 1.0 equiv.) according to General procedure B followed by coupling with peptoid amine **2b** (0.056 g, 0.31 mmol, 1.4 equiv.) in the presence of COMU (0.140 g, 0.31 mmol, 1.4 equiv.) according to General procedure C in H₂O. Tetrapeptoid **9** was obtained as a colourless oil (0.106 g, 0.154 mmol, 71%) and was used in the ensuing reaction without further purification. R_f (cyclohexane/EtOAc 60:40) = 0.43; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 0.81-0.91 (12H, m), 1.82-1.86 (2H, m), 2.88-3.26 (4H, m), 3.63-3.71 (3H, m), 3.79-4.28 (8H, m), 4.52-4.63 (4H, m), 5.09-5.16 (2H, m), 7.04-7.32 (15H, m); HRMS (m/z): calcd for [M + H]⁺: 687.3752; found: 687.3757; LCMS: t_r = 6.38 min.

Pentapeptoid 10. Pentapeptoid **10** was synthesised by methyl ester deprotection of tetrapeptoid **9** (0.082 g, 0.12 mmol, 1.0 equiv.) according to General procedure B followed by coupling with peptoid amine **2c** (0.026 g, 0.18 mmol, 1.5 equiv.) in the presence of COMU (0.077 g, 0.18 mmol, 1.5 equiv.) according to General procedure C in H₂O. Pentapeptoid **10** was obtained as a colourless oil (0.076 g, 0.095 mmol, 80%) and was used in the ensuing reaction without further purification. R_f (cyclohexane/EtOAc 30:70) = 0.44; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 0.81-0.91 (18H, m), 1.81-1.86 (3H, m), 2.91-3.26 (6H, m), 3.64-3.72 (3H, m), 3.79-4.28 (10H, m), 4.53-4.67 (4H, m), 5.11-5.13 (2H, m), 7.00-7.35 (15H, m); HRMS (m/z): calcd for [M + H]⁺: 800.4592; found: 800.4593; LCMS: t_r = 6.64 min.

Hexapeptoid 11. Hexapeptoid **11** was synthesised by methyl ester deprotection of pentapeptoid **10** (0.059 g, 0.074 mmol, 1.0 equiv.) according to General procedure B followed by coupling with peptoid amine **2b** (0.020 g, 0.114 mmol, 1.5 equiv.) in the presence of COMU (0.047 g, 0.114 mmol, 1.5 equiv.) according to General procedure C in H₂O. Hexapeptoid **11** was obtained as a colourless oil (0.037 g, 0.039 mmol, 53%) after purification by flash chromatography on silica gel using cyclohexane/EtOAc 20:80 as eluent. R_f (cyclohexane/EtOAc 20:80) = 0.47; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 0.80-0.89 (18H, m), 1.87-1.98 (3H, m), 3.00-3.23 (6H, m), 3.62-3.74 (3H, m), 3.83-4.26 (12H, m), 4.51-4.26 (6H, m), 5.10-5.13 (2H, m), 7.05-7.38 (20H, m); ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 20.0, 20.1, 20.2, 20.3 (6CH₃), 27.1, 27.2, 27.3, 27.5 (3CH), 46.4, 46.5, 46.8, 47.0, 47.2, 47.5, 47.7, 48.0, 48.5, 48.8, 48.9, 49.0, 49.1, 49.5 (3CH₂), 50.3, 50.4, 50.6, 50.8, 51.3, 51.5, 51.7 (6CH₂), 52.3, 52.4, 52.6, 52.7 (1CH₃), 55.6, 55.7, 56.3, 56.4 (3CH₂), 67.3 (1CH₂), 126.2, 126.3, 126.5, 126.6, 126.8, 127.0, 127.1, 127.2, 127.3, 127.4, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.5, 128.6, 128.7, 128.8, 128.9, 129.0, 129.1, 129.2, 129.3, 129.4, 129.5 (20CH), 135.3, 135.4, 135.8, 136.0 (2C), 136.8, 136.9, 137.1 (2C), 156.7, 157.1 (1C), 168.2, 168.5, 168.7, 169.2, 169.7, 169.8, 170.2 (6C); HRMS (m/z): calcd for [M + H]⁺: 947.5277; found: 947.5281; LCMS: t_r = 6.90 min.

Dipeptoid 12. Dipeptoid **12** was synthesised by coupling of protected proline **1b** (1.00 g, 4 mmol, 1.0 equiv.) with peptoid amine **2d** (0.70 g, 4.8 mmol, 1.2 equiv.) in the presence of COMU (3.44 g, 4.8 mmol, 1.2 equiv.) according to General procedure C in H₂O with 10% EtOAc as co-solvent at 45 °C. Dipeptoid **12** was obtained as a colourless oil (0.346 g, 0.92 mmol, 23%) and was used in the ensuing reaction without further purification. R_f (cyclohexane/EtOAc 40:60) = 0.34; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 0.83, 0.88, 0.96, 1.04, 1.10, 1.14, 1.22, 1.26 (6H, 8 \times d, J = 6.7 Hz), 1.21-1.30 (3H, m), 1.74-2.23 (4H, m), 3.40-3.60 (2H, m), 3.61-3.70 (1H, m), 3.96-4.36 (4H, m), 4.66-4.84 (1H, m), 5.00-5.19 (2H, m), 7.25-7.34 (5H, m); HRMS (m/z): calcd for [M + H]⁺: 377.2071; found: 377.2068; LCMS: t_r = 4.76 min.

Tripeptoid 13. Tripeptoid **13** was synthesised by ethyl ester deprotection of dipeptoid **12** (0.305 g, 0.81 mmol, 1.0 equiv.) according to General procedure B followed by coupling with peptoid amine **2e** (0.286 g, 0.97 mmol, 1.2 equiv.) in the presence of COMU (0.417 g, 0.97 mmol, 1.2 equiv.) according to General procedure C in H₂O. Tripeptoid **13** was obtained as a colourless oil (0.497 g, 0.976 mmol, 98%) and was used in the ensuing reaction without further purification. R_f (cyclohexane/EtOAc 90:10) = 0.36; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 0.82-1.18 (6H, m), 1.14-1.36 (3H, m), 1.61-2.16 (6H, m), 2.66-3.01 (2H, m), 3.19-3.94 (5H, m), 3.86-4.45 (6H, m), 4.64-4.84 (1H, m), 4.95-5.19 (4H, m), 5.36-5.89 (1H, m), 7.26-7.34 (10H, m); HRMS (m/z): calcd for [M + H]⁺: 625.3231; found: 625.3226; LCMS: t_r = 5.34 min.

Tetrapeptoid 14. Tetrapeptoid **14** was synthesised by ethyl ester deprotection of tripeptoid **13** (0.460 g, 0.73 mmol, 1.0 equiv.) according to General procedure B followed by coupling with peptoid amine **2f** (0.140 g, 0.89 mmol, 1.2 equiv.) in the presence of COMU (0.389 g, 0.89 mmol, 1.2 equiv.) according to General procedure C in H₂O. Tetrapeptoid **14** was obtained as a colourless oil (0.392 g, 0.53 mmol, 73%) and was used in the ensuing reaction without further purification. R_f (EtOAc) = 0.32; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 0.80-1.42 (15H, m), 1.48-2.16 (7H, m), 2.57-3.16 (4H, m), 3.19-3.94 (5H, m), 3.73-4.31 (7H, m), 4.31-4.86 (2H, m), 4.94-5.22 (4H, m), 5.58-5.97 (1H, m), 7.20-7.39 (10H, m); HRMS (m/z): calcd for [M + H]⁺: 738.4072; found: 738.4063; LCMS: t_r = 5.76 min.

Pentapeptoid 15 (peptoid mimic of Gramicidin S sequence). Pentapeptoid **15** was synthesised by ethyl ester deprotection of tripeptoid **14** (0.362 g, 0.491 mmol, 1.0 equiv.) according to General procedure B followed by coupling with peptoid amine **2g** (0.120 g, 0.589 mmol, 1.2 equiv.) in the presence of COMU (0.250 g, 0.589 mmol, 1.2 equiv.) according to General procedure C in H₂O. Pentapeptoid **15** was obtained as a colourless oil (0.302 g, 0.341 mmol, 70%) after flash chromatography on silica gel using EtOAc/MeOH 95:5 as eluent. R_f (EtOAc/MeOH 95:5) = 0.41; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 0.76-1.31 (15H, m), 1.54-2.22 (7H, m), 2.96-3.37 (4H, m), 3.38-3.70 (4H, m), 3.73-4.88 (14H, m), 4.95-5.20 (4H, m), 5.55-6.04 (1H, m), 7.28-7.33 (15H, m); ¹³C NMR (100 MHz, CDCl₃) δ

(ppm): 13.8, 13.9 (1CH₃), 18.9, 19.1, 19.3, 19.4, 19.5, 19.7, 19.8, 19.9, 20.0, 20.1, 20.8, 20.9, 21.0, 21.1, 21.2, 21.3 (4CH₃), 22.9, 23.1, 23.3, 23.4, 23.5, 23.6, 23.8, 23.9, 24.3, 24.4 (2CH₂), 26.8, 26.9, 27.3, 27.4 (1CH), 27.1, 29.5, 30.3 (2CH₂), 37.1, 37.2, 37.4, 37.5, 38.1, 38.3 (1CH₂), 41.6 (CH₂), 42.9, 43.3, 43.7, 44.9, 45.2, 45.4, 46.4, 46.7, 46.8, 47.0, 47.2, 47.4, 47.6, 47.7 (2CH₂), 47.8, 47.9 (1CH), 48.5, 48.7, 48.9, 49.0 (CH₂), 50.2, 50.6, 50.9, 51.1, 51.3, 51.4, 51.5, 51.7 (2CH₂), 55.3, 55.5, 55.6 (CH₂), 56.0, 56.2, 56.6, 56.8, 56.9, 57.1, 57.2 (1CH), 61.0, 61.2, 61.3, 61.3, 61.5, 61.6, 61.8 (CH₂), 65.8, 65.9, 66.0, 66.5, 66.6, 66.7, 67.2 (2CH₂), 126.2, 126.3, 126.6, 126.7, 127.2, 127.3, 127.4, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.3, 128.4, 128.5, 128.7, 128.8, 129.0 (15CH), 134.9, 135.0, 135.1, 135.4, 135.5, 135.7, 136.3, 136.4, 136.5, 136.7, 136.8 (3C), 153.9, 154.1, 154.2, 154.6, 154.7, 154.8 (C), 156.4, 156.6 (C), 167.8, 167.9, 168.0, 168.1, 168.2, 168.3, 168.4, 168.5, 168.6, 168.7, 168.8, 168.9, 169.0, 169.3, 169.4, 169.5, 169.6, 170.2, 170.3, 170.4, 170.6, 170.8, 171.0, 171.2, 171.4, 171.6, 171.7, 171.8 (4C), 173.4, 173.5, 173.6, 173.7 (1C); HRMS (m/z): calcd for [M + H]⁺: 885.4756; found: 885.4747; LCMS: t_r = 6.11 min.

General procedure D: Peptoid synthesis in the N to C direction using a one-pot two-step deprotection/coupling sequence in water-EtOH and water

To a suspension of the peptoid (1.0 equiv., 0.25 mmol) in EtOH/H₂O 2:3 (1.25 mL) was added LiOH·H₂O (1.0 equiv.). The mixture was stirred for 1 h at 30 °C. After full conversion (monitored by HPLC), the EtOH was evaporated, and the mixture was acidified to pH 4 with 1M HCl. 2,6-lutidine (3.1 equiv.), the peptoid amine (1.1 equiv.) and COMU (1.1 equiv.) were then added. The reaction mixture was stirred 4 h to overnight at 30 °C (while monitoring the reaction by HPLC). The aqueous mixture was extracted with EtOAc (2 \times 3 mL) and the organic layer was washed with 1M HCl (3 \times), satd. aq. Na₂CO₃ (4 \times) and brine (1 \times). The organic layer was dried over MgSO₄, filtered and evaporated under reduced pressure to furnish the crude peptoid which was used without further purification in the ensuing steps.

Pentapeptoid 19 (retropeptoid mimic of Leu-enkephalin).

Pentapeptoid **19** was obtained starting from peptoid methyl ester **16** (0.070 g, 0.25 mmol, 1.0 equiv.) via the intermediate dipeptoid **4**, tripeptoid **17** and tetrapeptoid **18** by repeating the one-pot two-step deprotection/coupling sequence according to General procedure D four times, using peptoid amine **2b** (0.050 g, 0.275 mmol, 1.1 equiv.), peptoid amine **2h**-HCl (0.042 g, 0.275 mmol, 1.1 equiv.), peptoid amine **2h**-HCl (0.042 g, 0.275 mmol, 1.1 equiv.), and then peptoid amine **2i** (0.078 g, 0.275 mmol, 1.1 equiv.) in the coupling steps, respectively. The crude final product was purified by flash chromatography on silica gel. Data for crude dipeptoid **4**: Colorless oil (100 mg, 0.234 mmol, 93% from **16**, 97% HPLC purity). NMR data as detailed above. Data for crude tripeptoid **17**: Colorless oil (92 mg, 0.18 mmol, 72% overall from **16**, 95% HPLC purity). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 0.86-0.75 (6H, m), 1.29-1.12 (3H, m), 1.92-1.68 (1H, m), 2.99-2.68 (3H, m), 3.26-3.10 (2H, m), 4.22-3.56 (8H, m),

4.59, 4.57, 4.50 and 4.48 (2H, 4×s), 5.07 and 5.05 (2H, 2×br s), 7.23-6.97 (10H, m).

Data for crude tetrapeptoid **18**: Colorless oil (87 mg, 0.15 mmol, 60% overall from **16**, 94% HPLC purity). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 0.78-0.94 (6H, m), 1.19-1.33 (3H, m), 1.75-1.99 (1H, m), 2.78-3.09 (6H, m), 3.10-3.29 (2H, m), 3.64-4.29 (10H, m), 4.51-4.65 (2H, m), 5.08-5.16 (2H, m), 7.01-7.42 (10H, m).

Data for pentapeptoid **19**: Colorless oil (42 mg, 0.051 mmol, 20% overall from **16**, 97% HPLC purity). *R_f* = 0.5 (AcOEt/MeOH 95:5); ¹H NMR (400 MHz, CDCl₃) δ (ppm): 0.74-0.94 (6H, m), 1.74-1.99 (1H, m), 2.73-3.11 (6H, m), 3.13-3.29 (2H, m), 3.62-3.73 (3H, m), 3.79-4.36 (10H, m), 4.43-4.68 (4H, m), 5.00-5.17 (4H, m), 6.87-6.99 (2H, m), 7.00-7.18 (2H, m), 7.19-7.45 (15H, m); ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 20.1, 20.2 (2CH₃), 27.2, 27.3, 27.6 (1CH), 35.6, 35.7, 35.8, 35.9, 36.0 (2CH₃), 46.4, 46.5, 46.7, 47.1, 47.4, 47.7, 48.9, 49.4, 49.5, 49.7, 49.8, 50.9, 51.0 (7CH₂), 52.3, 52.6 (CH₃), 55.3, 56.4 (CH₂), 67.3 (CH₂), 70.0, 70.1 (CH₂), 115.1, 115.4, 115.6, 115.7 (2CH), 126.5, 126.7, 126.8, 126.9, 127.0 (2CH), 127.4 (1C), 127.5, 127.6, 127.7, 127.9, 128.0, 128.1, 128.2, 128.3, 128.5, 128.7, 128.8, 129.0, 129.1, 130.2, 130.3 (15CH), 135.7, 135.8, 135.9 (C), 136.7, 136.8, 136.9, 137.0 (2C), 156.7, 157.1 (1C), 158.5, 158.6, 158.8 (1C), 167.9, 168.1, 168.2, 168.4, 168.5, 168.6, 168.7, 169.0, 169.1, 169.3, 169.4, 169.6, 170.1 (5C); HRMS (m/z): calcd for [M + H]⁺: 822.4072; found: 822.4056; LCMS: *t_r* = 6.18 min.

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