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Dedicated to Professor Ionel Haiduc on the occasion of his 80th anniversary

SYNTHESIS AND ESI(+)-MS COMPLEXATION STUDIES OF NEW 24- AND 36-MEMBERED CYCLIC PEPTIDES

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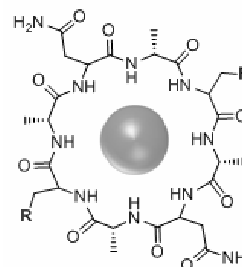
We present herein the synthesis and characterization of new 24- and 36 membered cyclic peptides composed of alternating D- and L-α-amino acids. The macrocyclic peptides were synthesized directly on the solid support by '*head-to-tail*' cyclization using Fmoc chemistry. All peptide products were purified by reversed-phase HPLC and characterized by mass spectrometry (ESI+-MS). Preliminary results concerning their ability to bind carboxylic acids small molecules are also presented.



INTRODUCTION

Cyclic peptides represent a class of natural and synthetic compounds that found important applications in various fields among which the most important are: pharmaceutics,[1] supramolecular chemistry[2] and, grace to their self-assembly ability, materials sciences.[3] Numerous examples of cyclic peptides-based drugs are reported in the literature.[1] This is mainly due to their increased stability towards the proteases cleavage as compared to linear peptides.[4]

In the field of the supramolecular chemistry, cyclic peptides or peptoids were used both as host molecules[5] and for construction of self-assembled structures.[3,6] Moreover, self-assembled cyclic peptides were reported to form channels[7] large enough to allow crossing of various small molecules. For example, Ghadiri et al. showed that cyclic peptides that contain alternate D/L amino acids in the sequence are able to associate by hydrogen bonding and to form nanopores permeable for glucose[7a] and glutamic acid.[7b] The use of cyclic peptides as host molecules has the great advantage of smooth modulation of the binding properties and cavity sizes, by modifying the nature and the number of the amino acids as well as the orientation of their side chains towards the interior[8] or the exterior[9] of the macrocycle cavity. Thus, these compounds are very appealing hosts and were used for complexation of both neutral and ionic molecules.[5,7]

The great applicability of this class of compounds together with the development of the solid phase synthesis methodologies led to an important progress of their synthetic methods. In

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most cases, the linear precursor is obtained by solid phase peptide synthesis while the key macrocyclization step can be preformed both in solution and on solid support. Usually, a pre-organization of the linear precursor is required (*i.e.* this can be achieved by introduction in the peptide sequence of proline or α-*N*-methylated residues[10] or D-amino acids[11]). On resin cyclization benefits from a reduced amount of the linear oligomers byproducts through a pseudodillution effect. The cyclization reaction can be realized head-to-tail, side chain-to-side chain, head-to-side chain or side chain-to-tail resulting in a large variety of compounds that can be prepared.[12]

In this context, we were interested in the synthesis of new cyclic peptides of various sizes containing alternating D and L amino acids as well as investigation of their structural and binding properties for neutral molecules. The D,L-alternation of the amino acids was proved to ensure the orientation of the amino-acids' side chains pointing out to the exterior of the cycle.[9] Thus, the complexation takes advantage of the amides groups that define the cavity while the reactive groups of the amino acids side chains can act as anchors for further functionalization.

RESULTS AND DISCUSSION

The peptides studied in this work (**I**: cyclo-NAHA2AHAHA2A; **II**: cyclo-NAKANAKA; **III**: cyclo-NAEANAEA and **IV**: cyclo-NA2ANA2A, Figure 1) were designed to contain both commercial and homemade amino acids and to have all the amino acids side-chains pointing out towards the exterior of the cavity. This geometry can be achieved by ensuring a D, L alternation of amino acids configuration in the peptides' sequences.[9] In addition, this alternation ensures a quasi-planarity (i.e. the amino acids belonging to the cycle define a plane) of the molecules. All Ala residues from the peptide sequence have a D configuration while all the other amino acids residues are L.

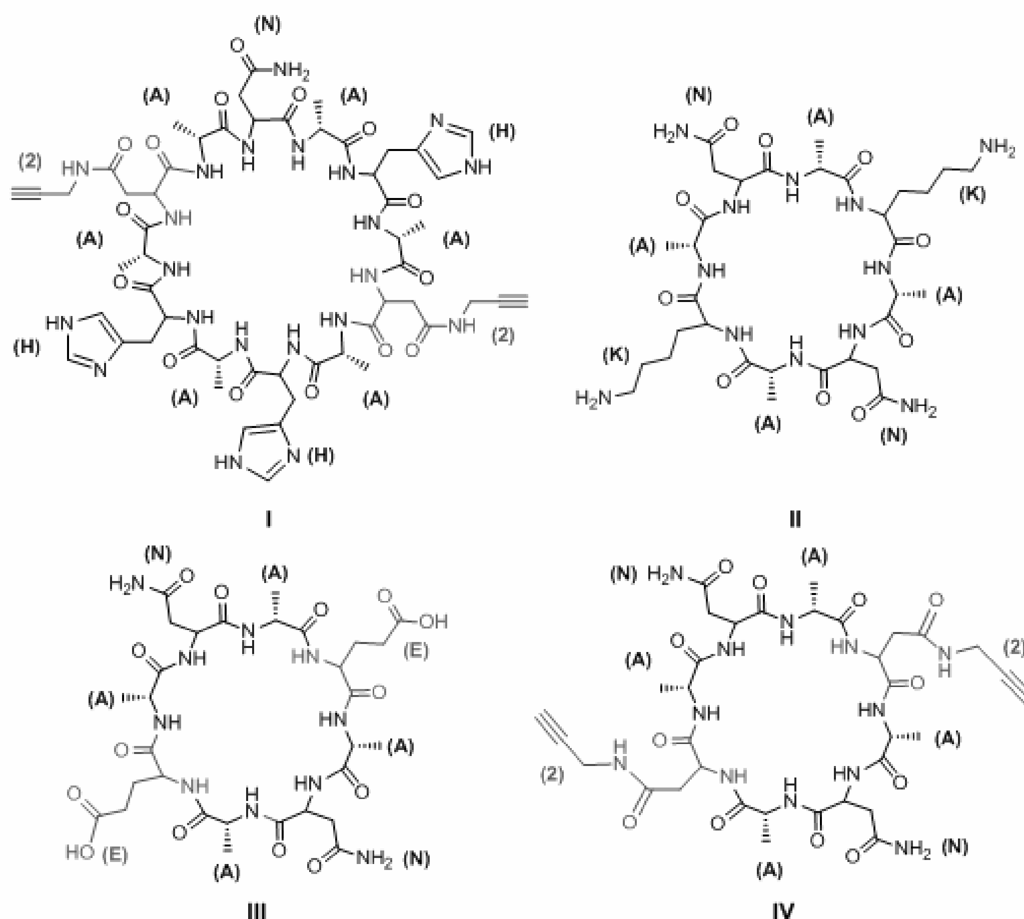
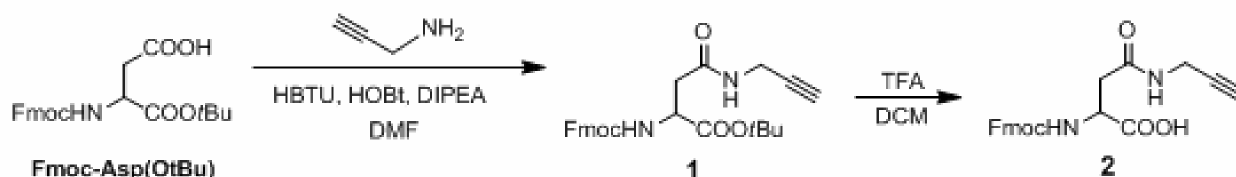


Fig. 1 – Structure of the synthesized cyclic peptides **I**: cyclo-NAHA2AHAHA2A; **II**: cyclo-NAKANAKA; **III**: cyclo-NAEANAEA; **IV**: cyclo-NA2ANA2A.



Scheme 1 – Synthesis of compounds **1** and **2**.

Introduction in the peptide sequence of a triple bond decorated amino acid would help convenient further functionalization of the cyclic peptides through orthogonal reactions such as click chemistry. Consequently, we firstly obtained compound **2** following the synthetic path presented in Scheme 1, using a procedure we previously developed for triple bond functionalization of Fmoc-Glu(OtBu).[13] HBTU/HOBt mediated amide coupling between Fmoc-Asp(OtBu) and propargyl amine provided fully protected amino acid **1**. This was submitted to TFA deprotection of the *t*-butyl group to afford compound **2** that can be directly used for solid phase synthesis of the cyclic peptides.

The synthesis of **2** and its incorporation in peptide sequences was also recently reported by Perrin *et al.* using a different synthetic strategy, namely EDC·HCl (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) / HOBt-mediated coupling of Fmoc-Asp(OtBu) with propargyl amine, TFA mediated deprotection *t*-butyl group and activation of carboxyl group as NHS (*N*-hydroxysuccinimide) ester.[14]

The cyclic peptides were next obtained using solid phase peptide synthesis, Fmoc chemistry,[15] and on resin *head-to-tail* macrocyclization. First step consisted in the attachment of Fmoc-Asp-ODmab (Dmab: 4-{*N*-[1-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-3-methylbutyl]-amino}benzyl) amino acid through its side chain carboxylic group to the solid support (MBHA Rink Amide resin). ODmab protecting group is orthogonal with peptide Fmoc synthesis and can be selectively deprotected before the macrocyclization.[16] Peptide elongation was performed by successive coupling / NH_2-deprotection reactions of the amino acids in the desired sequence. *N*-Fmoc protected amino acid couplings were carried-out using HBTU (*N,N,N',N'*-tetramethyl-O-(1H-benzotrialol-1-yl)uronium hexafluorophosphate) as coupling agent, HOBt (hydroxybenzotriazole) to reduce the racemisation and DIPEA (*N,N*-diisopropylethylamine) as base, in DMF as solvent. Deprotection of the amino groups of the growing peptide was achieved by treatment with a solution of piperidine/DMF (1/4, v/v). The efficiency of the coupling and deprotection reactions was checked by performing the ninhydrin-based Kaiser test. The resulted linear peptides were further treated with 2% hydrazine hydrate in DMF in order to complete deprotection of the α-carboxyl group of the first amino acid. Subsequent cyclization was achieved by reacting the α-carboxyl group of the first amino acid with the amino group of the last one, using HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b] pyridinium-3-oxide hexafluoro-phosphate) as coupling reagent.[17]

The final step of the synthesis was the cleavage from the resin concomitant with full deprotection of the amino acids side-chain protecting groups. After isolation, all peptides were analyzed by analytical reversed phase HPLC and mass spectrometry. Crude peptides were purified by semi-preparative reversed-phase HPLC. The purity of the peptides was checked by RP-HPLC (UV detection at 230 nm) and the pure products were characterized by MS. The HPLC chromatogram of peptide **I** is presented in Figure 2.

ESI(+)-MS spectra showed in all cases peaks corresponding to mono-, double and triple charged species as well as peaks corresponding to peptide – trifluoroacetic acid adducts. For example, the ESI(+)-MS spectrum of peptide I (Figure 3) displays peaks corresponding to the monocharged species at $m/z = 1257.63$ $[M+H^+]$ for the protonated molecular ion and at $m/z = 1372.66$ $[M+CF3COOH+H^+]$ for the peptide-TFA adduct, double charged species at $m/z = 686.83$ $[M+CF3COOH+2H^+]$ and $m/z = 629.32$ $[M+2H^+]$ as well as peaks corresponding to the triple charged species at $m/z = 458.23$ $[M+CF3COOH+3H^+]$ and $m/z = 419.88$ $[M+3H^+]$.

Next, we set to study the ability of the cyclic peptides to form complexes with small molecules. To this end, the binding affinity of peptides **II** and **III** towards a set of di- and tricarboxylic acids (Figure 4) was determined by ESI(+)MS and MS/MS experiments. MS-ESI(+) spectra of the complexes between peptides **II** or **III** and compounds **3**–**8** displayed characteristic peaks $m/z = 490.77$, $m/z = 525.81$, $m/z = 469.22$ corresponding to the double charged species [**II** + **7** + $2H^+$], [**II** + **5** + $2H^+$] and [**III** + **3** + $2H^+$], respectively, as well as to monocharged species at m/z=980.52 and m/z=1050.51 for [**II** + **6** + H^+] and [**III** + **5** + H^+], respectively.

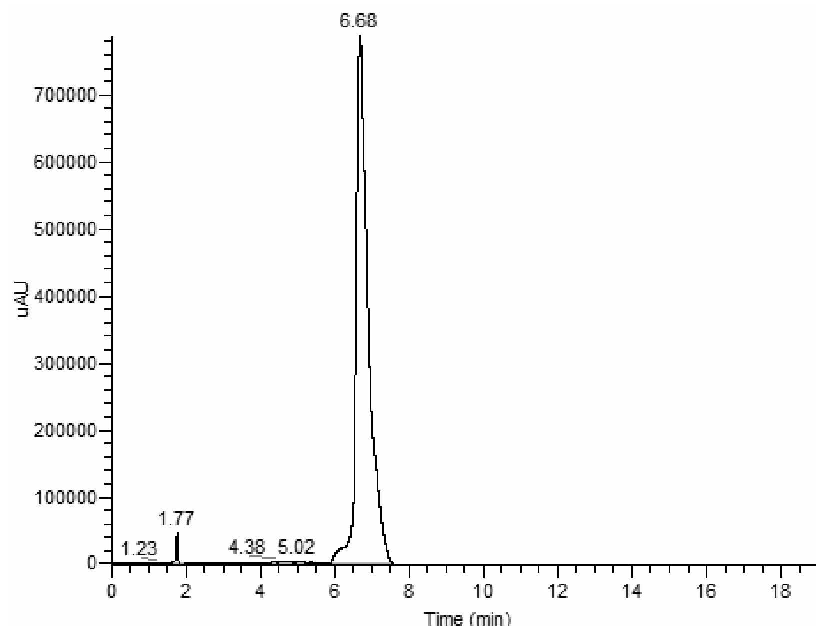


Fig. 2 – HPLC chromatogram (UV detection; 230 nm) of peptide **I**.

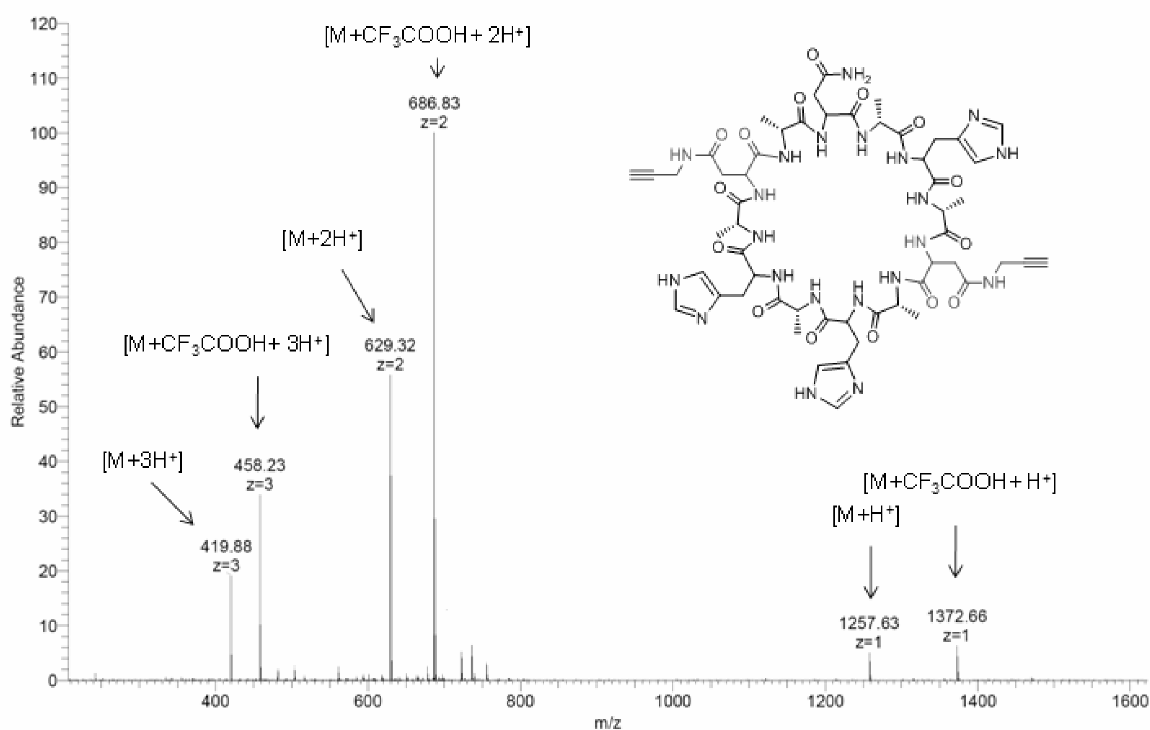


Fig. 3 – ESI(+)-HRMS spectrum of peptide **I.**

Figure 5 (left) shows the ESI(+)MS spectrum of the complex between **II** and **7** that presents peaks corresponding to peptide **II** at *m/z* = 296.18 [**II** + $3H^+$] and *m/z* = 385.76 [**II** + $2H^+$], peptide **II**–TFA (trifluoroacetic acid) adduct at *m/z* = 443.77 [**II** + TFA+ $2H^+$], peptide **II**–**7** complex at *m/z* = 490.77 [**II** + **7** + $2H^+$] and peptide **II**–**7**–TFA adduct at *m/z* = 548.28 [**II** +**7**+THA+$2H^+$], as well as the MS^2 experiment run for the complex between peptide **II** and **5** (Figure, right). Isolation of the peak corresponding to the complex **II** + **5** at *m/z* = 524.81, followed by its fragmentation by collision induced dissociation technique (CID, voltages of 5 eV) led to the formation of the protonated molecular ions corresponding to peptide **II** and guest **5** at *m/z* = 770.5 and *m/z* = 279.12, respectively.

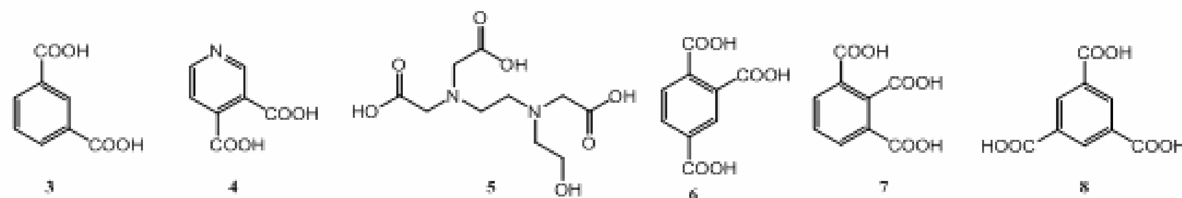


Fig. 4 – Structure of the di- and tri-carboxylic acids 3–8 used as guests in complexation studies.

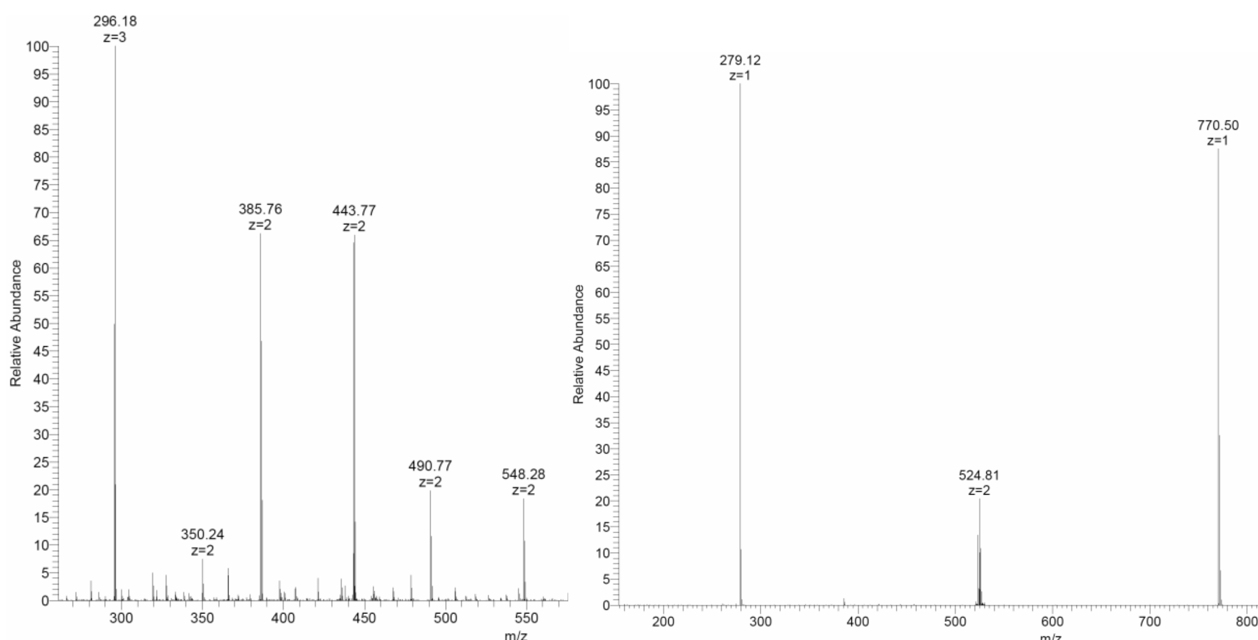


Fig. 5 – ESI(+)-MS spectrum of the complex between peptide **II**–acid carboxylic **7** (left) and ESI(+)-MS/MS (CID, collision energy 5 eV, peak isolation with 10 ppm) of peptide **II**–carboxylic acid **5 (**right).

As inferred from ESI(+)MS and MS/MS experiments, peptide **II** has the ability to form 1:1 complexes with compounds **5**, **6** and **7**, while peptide **III** forms 1:1 complexes with compounds **3** and **5**. Any of the other examined guest molecules formed no complexes with **II** or **III**.

EXPERIMENTAL

General experimental data: Chemicals and solvents of commercial grade were used for the synthesis and purification of the functionalized amino acids. DCM was dried by distillation over calcium hydride. DMF for peptide synthesis was used for the preparation of the cyclic peptides. Manual synthesis of the cyclic peptides was performed using a Stuart Flask Shaker. The NMR spectra were recorded at room temperature, in $CDCl_3$ or methanol-D_4 as solvents, on a Bruker Advance 400 (^{1}H at 400 MHz, ^{13}C at 100 MHz) spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) using residual solvent peak as internal reference (^{1}H, ^{13}C). Electrospray (ESI) and Atmospheric Pressure Chemical Ionisation (APCI) were recorded on a LTQ XL ORBITRAP (ThermoScientific) spectrometer, in positive ions mode, using external mass calibration. Thin layer chromatography (TLC) was carried on silica gel coated aluminium F_{254} plates. Preparative column chromatography was performed on silica gel (0.040-0.063 mm). Analytical HPLC analyses for all peptide derivatives were performed on a Agilent Technologies 1200 Series HPLC system, equipped with UV detection, using an analytical C_{18} VYDAC (300 Å, 4.6 mm×150 mm, 5 µm). The eluent was a linear gradient of water (0.1% TFA) and acetonitrile. The retention time (Rt). Semi-preparative RP-HPLC was carried out on a Dionex Ultimate 3000 Series (ThermoScientific) using a semi-preparative C_{18} VYDAC (300 Å, 20 mm × 250 mm, 10 µm) column and elution with the same gradient of solvents. A Christ Alpha 1-4 LSC freeze dryer was used for lyophilisation of the peptides.

Synthesis of the functionalized amino acids ***1*** *and* ***2*** ***tert-Butyl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-oxo-4-(prop-2-yn-1-ylamino) butanoate*** **(1)**: To a solution of propargyl amine (0.38 ml, 5.937 mmol) in dry DCM (18 ml), Fmoc-Asp-OtBu (2.036 g, 4.947 mmol), HBTU (1.86 g, 4.947 mmol), HOBt (0.668 g, 4.947 mmol) and DIPEA (1.635 ml, 9.895 mmol) were added and the solution was stirred at room temperature for 2h. The solution was washed with water (3x30 ml), the organic phase was dried on $MgSO_4$ and the solvent was removed under reduced pressure. The obtained residue was dissolved in DCM (0.5 mL), cold acetonitrile (5 mL) was added and the resulting precipitate was filtered to yield compound **1** (1.86 g, 84%) as a white solid. **m.p.:** 165–166 °C

^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.48 (s, 9H), 2.19 (s, 1H), 2.74 (dd, ^{2}J=15.6 Hz, ^{3}J=4.4 Hz, 1H), 2.89 (dd, ^{2}J=15.6 Hz, ^{3}J=4.4 Hz, 1H), 4.03 (s, 2H), 4.22 (t, ^{3}J=7.0 Hz, 1H), 4.32–4.42 (m, 2H), 4.47–4.49 (m, 1H), 5.95 (br. s, 1H), 6.01 (br. d, 1H) 7.31 (t, ^{3}J=7.2 Hz, 2H), 7.40 (t, ^{3}J=7.2 Hz, 2H), 7.60 (d, ^{3}J=7.2, 2H), 7.76 (d, ^{3}J=7.2 Hz, 2H). **^{13}C RMN-APT** (100 MHz, $CDCl_3$) δ ppm: 28.0, 29.3, 38.1, 47.3, 51.5, 67.3, 72.0, 79.3, 82.7, 120.1, 125.3, 127.2, 127.8, 141.4, 144.0, 156.3, 169.6, 169.9.
APCI(+)HRMS: calcd. for $C_{26}H_{29}N_2O_5$ 449.2071 found: 449.2075 $[M+H^+]$; calcd. for $C_{22}H_{21}N_2O_5$ 393.1445, found: 393.1449 $[M\text{-}tBu + H^+]$

2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-4-oxo-4-(prop-2-yn-1-ylamino)butanoic acid **(2)**: To a solution of **1** (1.843 g, 4.109 mmol) in 34.5 ml DCM, trifluoroacetic acid (11.5 ml, solution TFA in DCM 33%) and water (0.1 ml) were added and the solution was stirred at room temperature for 1h. The solvent was removed under reduced pressure, cold diethyl ether was added and the precipitate was filtered to provide compound **2** (1.487, 92%) as a white solid. **m.p.:** 184–185°C
^{1}H RMN (400 MHz, Methanol-D_4) δ ppm: 2.55 (t, ^{3}J=2.4 Hz, 1H), 2.70 (dd, ^{2}J=15.6 Hz, ^{3}J=7.2 Hz, 1H), 2.77 (dd, ^{2}J=15.6 Hz, ^{3}J=7.2 Hz, 1H), 3.94 (d, ^{3}J=2.4 Hz, 2H), 4.21 (t, ^{3}J=7.2 Hz, 1H), 4.28–4.34 (m, 2H), 4.55 (dd, ^{3}J=4.8 Hz, ^{4}J=7.2 Hz, 1H), 7.29 (t, ^{3}J=7.2 Hz, 2H), 7.36 (t, ^{3}J=7.2 Hz, 2H), 7.64 (dd, ^{3}J=7.2 Hz, ^{4}J=3.6 Hz, 2H), 7.77 (t, ^{3}J=7.2 Hz, 2H). **^{13}C RMN-APT** (100 MHz, Methanol-d_4) δ ppm: 29.5, 38.3, 48.3, 50.1, 68.1, 72.3, 80.4, 120.9, 126.3, 128.2, 128.8, 142.5, 145.2, 158.3, 171.9, 174.5. **APCI(+) HRMS** calcd. for $C_{22}H_{21}N_2O_5$ 393.1445 found:393.1446 $[M+H^+]$

General synthetic method for peptide synthesis: The synthesis of the cyclic peptides was carried out using MBHA Rink Amide resin (loading 0.56 mmol/g) as solid support. The resin was prepared by swelling (1h shaking in 2 ml DMF) and Fmoc-deprotection (2x15 min shaking with 2 ml piperidine/DMF 2/8 solution). The elongation of the peptide chain was achieved through sequential coupling and deprotection reactions for each amino acid. The amide coupling reactions were done using the appropriate reaction mixture containing the reagents in the following ratios: resin/amino acid/HBTU/HOBt/DIPEA 1/2/2/2/6 (for introduction of compound **2**); 1/3/3/3/9 (Fmoc-Asp-ODmab - first amino acid of the sequences); 1/4/4/4/12 (Fmoc-D-Ala-OH, Fmoc-His(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(OtBu)-OH) and resin/amino peptide/HATU/DIPEA 1/4/4/12 (cyclization). Deprotection of the Fmoc-protecting group for each amino acid was achieved by 2x10 min shaking with 2 ml piperidine/DMF 2/8 solution. Selective deprotection of the -ODmab group on the side-chain of the first amino acid was done by 2x3 min shaking with hydrazine hydrate 2% in DMF solution. The completion of the coupling after each step was checked using the Kaiser Test. The cleavage of the cyclic peptides from the solid support was done by gentle shaking for 2 h with 2 ml cleavage solution: TFA (trifluoroacetic acid) 95%, TIS (triisopropylsilane) 2.5% and water 2.5%. The peptides were precipitated in cold diethyl ether and separated by centrifugation. Cyclic peptides **I-IV** were purified by semi-preparative RP-HPLC using a linear gradient of water (0.1% TFA) - acetonitrile

Cyclic peptide I**:** Amino acid sequence: cyclo-**NAHA2AHAHA2A**. Cyclic peptide **I** was purified by semi-preparative RP-HPLC using a linear gradient of water (0.1% TFA) 95–65 %, acetonitrile: 5–35% (min. 2–16). R.t: 9.1 min. **ESI(+)-MS**: calcd. for $C_{54}H_{73}N_{21}O_{15}$ 1256.56, found: 1372.66 $[M + CF_3COOH + H^+]$, 1257.63 $[M + H^+]$, 686.83 $[M + CF_3COOH + 2H^+]$, 629.32 $[M + 2H^+]$, 458.22 $[M + CF_3COOH + 2H^+]$, 419.88 $[M + 3H^+]$.

Cyclic peptide II**:** Amino acid sequence: cyclo-**NAKANAKA**. Cyclic peptide **II** was purified by semi-preparative RP-HPLC using a linear gradient of water (0.1% TFA) 100–65 %- acetonitrile: 0–35% (min. 2-16). R.t: 8.8 min. **ESI(+)-MS**: calcd. for $C_{32}H_{56}N_{12}O_{10}$ 769.43 found: 885.50 $[M + CF_3COOH + H^+]$, 770.50 $[M + H^+]$, 385.75 $[M + CF_3COOH + 2H^+]$, 458.23 $[M + CF_3COOH + 2H^+]$, 443.25 $[M + 2H^+]$, 257.50 $[M + 3H^+]$.

Cyclic peptide III**:** Amino acid sequence: cyclo-**NAEANAEA**. Cyclic peptide **III** was purified by semi-preparative RP-HPLC using a linear gradient of water (0.1% TFA): 95–65% - acetonitrile: 5–35% (min. 2-16). R.t: 10.6 min. **ESI(+)-MS**: calcd. for $C_{30}H_{46}N_{10}O_{14}$ 771.33, found: 772.40 $[M+H^+]$, 887.43 $[M+CF_3COOH+H^+]$.

Cyclic peptide IV**:** cyclo-**NA2**ANA**2A**. Cyclic peptide **IV** was purified by semi-preparative RP-HPLC using a linear gradient of water (0.1% TFA): 90–65% - acetonitrile: 10–35% (min. 2–16). R.t: 7.8 min. **ESI(+)-MS**: Calcd for $C_{34}H_{48}N_{12}O_{12}$ 817.36, found: 955.44 $[M + CF_3COOH + Na^+]$, 933.46 $[M + CF_3COOH + H^+]$, 818.43 $[M + H^+]$.

CONCLUSIONS

In conclusion, we described here the successful preparation of four cyclic peptides containing commercial and in-house synthesized amino acids that display a D/L alternation of the amino acids within the sequence. The cyclic peptides were obtained by solid phase peptide synthesis, using on resin "*head to tail*" macrocyclization. The 24 and 36 membered cyclic peptides are decorated with functional groups (*i.e.* ethynyl, amino, carboxyl), oriented towards the exterior of their cavity. The ESI(+)-MS and MS/MS investigations showed the ability of some of these peptides to form 1:1 complexes with di- and tri- carboxylic acids. Further studies are underway to develop new applications based on the presence of the functional groups that were designed to allow attachment of various self-assembling units such as urea derivatives or hydrophobic chains in order to obtain nanoporous materials as well as to construct cyclic peptides-based cryptands with superior binding properties.

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REFERENCES

1. (a) K. Fosgerau and T. Hoffmann, *Drug Discov. Today,* **2015**, *20*, 122–128; (b) A. Tapeinou, M.-T. Matsoukas, C. Simal and T. Tselios, *Pept. Sci.,* **2015**, *104*, 453–461; (c) P. Thapa, M. J. Espiritu, C. Cabalteja and J.-P. Bingham, *Int. J. Pept. Res. Ther.*, **2014**, *20*, 545–551; (d) A. Tapeinou, M. T. Matsoukas, C. Simal and T. Tselios, *Biopolymers*, **2015**, *104*, 453–461; (e) S. H. Joo, *Biomol. Ther.*, **2012**, *20*, 19–26.
2. (a) R. J. Brea, C. Reiriz and J. R. Granja, *Chem. Soc. Rev.*, **2010**, *39*, 1448–1456. (b) S. Kubik "Synthetic peptide-based receptors" in: "Supramolecular Chemistry: From Molecules to Nanomaterials", J. Wiley & Sons, Chichester, 2012, p. 1179–1203; (c) S. Kubik "Cyclopeptide derived synthetic receptor" in: "Artificial Receptors for Chemical Sensors", Wiley-VCH, Weinheim, 2010, p. 135–167.
3. (a) C. Alemán, A. Bianco and M. Venanzi "Peptide Materials: From Nanostructures to Applications" J. Wiley & Sons, Chichester, 2013, p. 1–435; (b) S. Guha and A. Banerjee, *Macromol. Chem. Phys.*, **2009**, *210*, 1422–1432; (c) D. J. Rubin, S. Amini, F. Zhou, H. Su, A. Miserez and N. S. Joshi, *ACS Nano*, **2015**, *9*, 3360–3368; (d) A. Handelman, A. Natan and G. Rosenman, *Pept. Sci.*, **2014**, *20*, 487–493.
4. Á. Roxin and G. Zheng, *Future Med. Chem.*, **2012**, *4*, 1601–1618.
5. (a) A. Robert, B. P. Elmes and K. A. Jolliffe, *Chem. Commun.*, **2015**, *51*, 4951–4968; (b) A. N. Chermahini, M. Rezapour and A. Teimouri, *J. Incl. Phenom. Macrocycl. Chem.*, **2014**, *79*, 205–214; (c) N. Rodríguez-Vázquez, R. García-Fandiño, M. Amorín and J. R. Granja, *Chem. Sci.*, **2016**, *7*, 183–187; (d) J. R. Cochrane, A. Schmitt, U. Wille and C. A. Hutton, *Chem. Commun.*, **2013**, *49*, 8504–8506
6. E. Gazit, *Chem. Soc. Rev.*, **2007**, *36*, 1263–1269.
7. (a) J. R. Granja and M. R. Ghadiri, *J. Am. Chem. Soc.*, **1994**, *116*, 10785−10786; (b) J. Sanchez-Quesada, H. S. Kim and M. R. Ghadiri, *Angew. Chem., Int. Ed.*, **2001**, *40*, 2503−2506.
8. M. Favre, K. Moehle, L. Jiang, B. Pfeiffer and J. A. Robinson, *J. Am. Chem. Soc*., **1999**, *121*, 2679–2685.
9. D. Wang, L. Guo, J. Zhang, L. R. Jones, Z. Chen, C. Pritchard and R. W. Roeske, *J. Pept. Res.*, **2001**, *57*, 301–306.
10. J. Sanchez-Quesada, M. R. Ghadiri, H. Bayley and O. Braha, *J. Am. Chem. Soc.*, **2000**, *122*, 11758–11766.
11. S. F. Brady, S. L. Varga, R. M. Freidinger, D. A. Schwenk, M. Mendlowski, F. W. Holly and D. F. Veber, *J. Org. Chem.*, **1979**, *44*, 3101–3105.
12. C. J. White and A. K. Yudin, *Nat. Chem.*, **2011**, *3*, 509–524.
13. N. Candelon, N. D. Hădade, M. Matache, J.-L. Canet, F. Cisnetti, D. P. Funeriu, L. Nauton and A. Gautier, *Chem. Commun*., **2013**, *49*, 9206–9208.
14. L. Zhao, J. P. May, A. Blanc, D. J. Dietrich, A. Loonchanta, K. Matinkhoo, A. Pryyma and D. M. Perrin *Chem. Bio. Chem.*, **2015**, *16*, 1420–1425.
15. W. C. Chan and P. D. White, "Fmoc solid phase peptide synthesis: a practical approach", Oxford University Press, 2000, p. 1–325.
16. W. C. Chan, B. W. Bycroft, D. J. Evans and P. D. White, *J. Chem. Soc. Chem. Commun.*, **1995**, *21,* 2209–2210.
17. C. F. McCusker, P. J. Kocienski, F. T. Boyle and A. G. Schatzlein, *Bioorg. Med. Chem. Lett.* **2002**, *4*, 547–549.