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Synthesis and Folding propensity of Aliphatic Oligoureas containing repeats of proline-type Units

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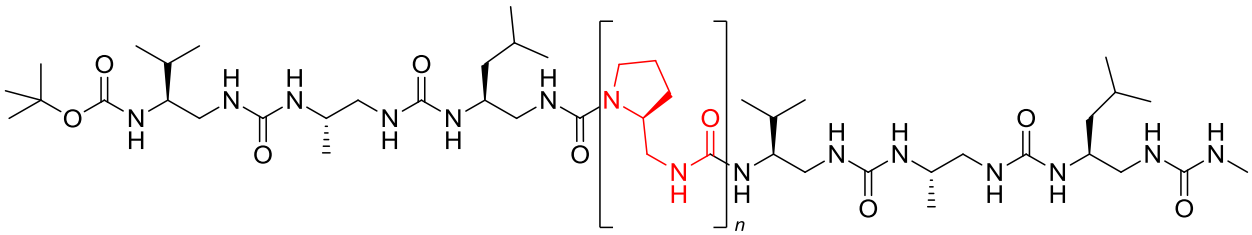


n = 1-7

ABSTRACT: The synthesis and conformational analysis of aliphatic oligoureas containing multiple adjacent *N*-alkylated units derived from proline (i.e., Pro[u]) is reported. The insertion of tri-substituted ureas in the main chain of *N*,*N*'-linked oligourea foldamers locally impairs the characteristic three centred-hydrogen bonding pattern associated with the formation of 2.5-helical structures. Three series of oligomers have been studied : one series in which the Pro[u] repeat is flanked on both sides by canonical urea residues (e.g. oligomers **2-6**), one series with canonical residues on either side of the Pro[u] repeat (oligomers **12** and **23**), and one series consisting exclusively of Pro[u] residues (oligomers **25** and **26**). Spectroscopic (NMR and electronic circular dichroism) and X-ray diffraction studies reveal that the 2.5-helix formed by oligomers of *N*,*N*'-

disubstituted ureas is robust enough to accommodate short oligopyrrolidine segments $(Pro^{u})_n$ (n < 7) which alone display no intrinsic folding propensity.

INTRODUCTION

The introduction of tertiary amides in the backbone of α-peptides, by locally decreasing the hydrogen bond donor capability and populating both cis- and trans-amide bond conformers, generally has dramatic consequences on the structure and dynamics of the peptide main chain. N-methylation for exemple, is a well appreciated strategy to modulate the conformation but also the potency and the membrane permeability of bioactive peptides.[1-3] In proline containing peptides, these effects are coupled with an increased conformational constraint imposed by the pyrrolidine ring. With a φ angle in the range -60° ± 20°, proline is the most conformationally restricted of the proteinogenic amino acids. As a result, proline tends to be excluded from α-helical and β-sheet structures, though it is frequently found at their ends and also in transmembrane helices.[4] A remarkable feature is the propensity of proline repeats to adopt helical secondary structures to the exclusion of hydrogen bonding such as the left-handed polyproline II (PPII) helix (with all amides in trans conformation) which predominates in polar solvents and plays crucial role in biological processes (e.g. protein-protein recognition, assembly of collagen triple helices[5,6]). Electronic effects (ie $n \rightarrow \pi^*$ interaction) have been shown to play a substantial role in the stabilization of the PPII helical conformation.[7,8] Synthetic proline-rich sequences that can adopt PPII helical conformation have been developed as cell penetrating agents[9] and as molecular ruler (by enabling controlled inter-ligand distances).[10,11,12]

Significant efforts have also been directed towards the elaboration of non natural peptidomimetic tertiary amide oligomers that can form defined non-hydrogen bonded structures. These include homooligomers of diversely substituted proline derivatives and analogues,[13-16] homologated proline residues,[17-22] and N-alkyl glycines.[23-26] Non canonical folded structures stabilized by H-bonds have also been described for hybrid oligoamides made of alternating secondary and tertiary

amides.[27-29]

We have previously investigated[30] the consequences of inserting *N*-(pyrrolidin-2-ylmethylamine) units[31] as proline analogues at discrete positions in the backbone of aliphatic *N*,*N*'-disubstituted urea oligomers, a class of peptidomimetic helical foldamers.[32-36] We found that despite the loss of one H-bond donor site, the geometry of the canonical 2.5 helix of oligoureas is not significantly impaired by the presence of multiple and non-adjacent pyrrolidine units. The tri-substituted urea which is created at the pyrrolidine insertion site still has the ability to form intramolecular H-bonds. In addition, the CO−N−Cβ−Cα angle (φ) values measured for pyrrolidine units (≈ −96°) in the crystal structures of corresponding oligomers match the ϕ values in the structure of the canonical 2.5-helix of oligoureas (i.e., φ ≈ −101°). However, our results also indicate that the pyrrolidine unit is endowed with a lower 2.5-helix propensity compared to canonical units thus suggesting that by changing the ratio of proline-type residues to canonical units, it may be possible to tune the stability of the 2.5 helical structure. In the present work, we have explored whether the helix geometry of aliphatic oligoureas may accommodate multiple adjacent pyrrolidine residues. Oligomers containing 2 to 7 consecutive pyrrolidine units flanked by canonical residues have been synthesized and characterised in solution and in solid state to determine the extent to which these oligopyrrolidine segments can be enforced in the 2.5 helical conformation. Furthermore, we have investigated new oligoureas consisting exclusively of pyrrolidine units and trisubstituted ureas to evaluate the possibility for oligopyrrolidine-ureas to adopt non canonical folding patterns.

RESULTS AND DISCUSSION

To evaluate the influence of multiple adjacent pyrrolidine residues on the 2.5-helix geometry of oligoureas, we have prepared a series of oligomers derived from the previously described heptamer **1** with one central pyrrolidine unit. The resulting oligomers **2**-**6** contain two to seven consecutive pyrrolidine residues flanked by three canonical units with aliphatic side chains of Ala, Val and Leu amino acid residues (See Figure 1).

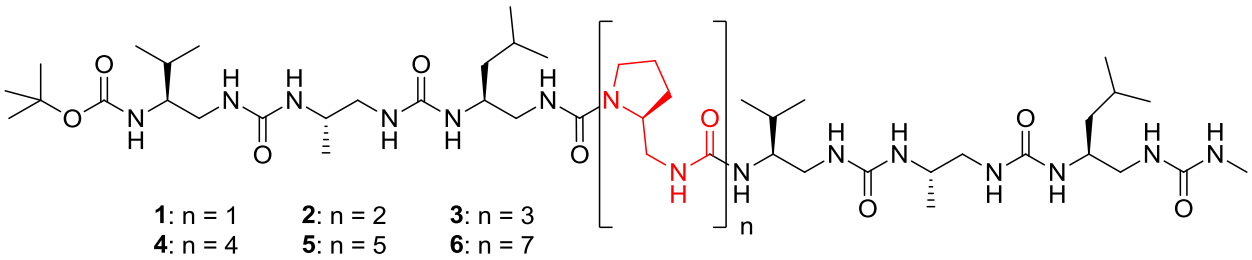
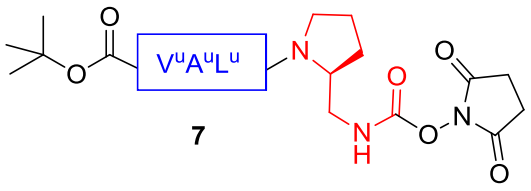
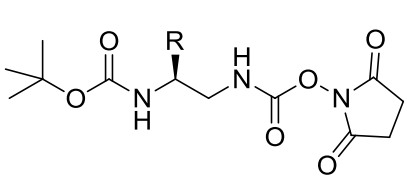


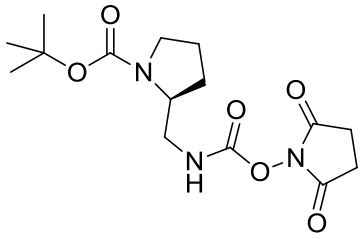
Figure 1. Sequences of oligoureas **1**-**6** containing one to seven adjacent pyrrolidine residues (shown in red)

Oligomers **2**-**6** ranging from 8-13 residues were synthesized by condensation of two (**2**-**5**) to three (**6**) segments.[30] Our approach involves the preparation of a first activated segment (**7**) containing a terminal pyrrolidine residue terminated by a succinimidyl carbamate. The presence of an *N*-alkylated residue (e.g. pyrrolidine) at the segment junction position is recommended to prevent a possible intramolecular cyclization of the activated oligourea that would lead to the formation of a heterocyclic biuret derivative.[30,37]





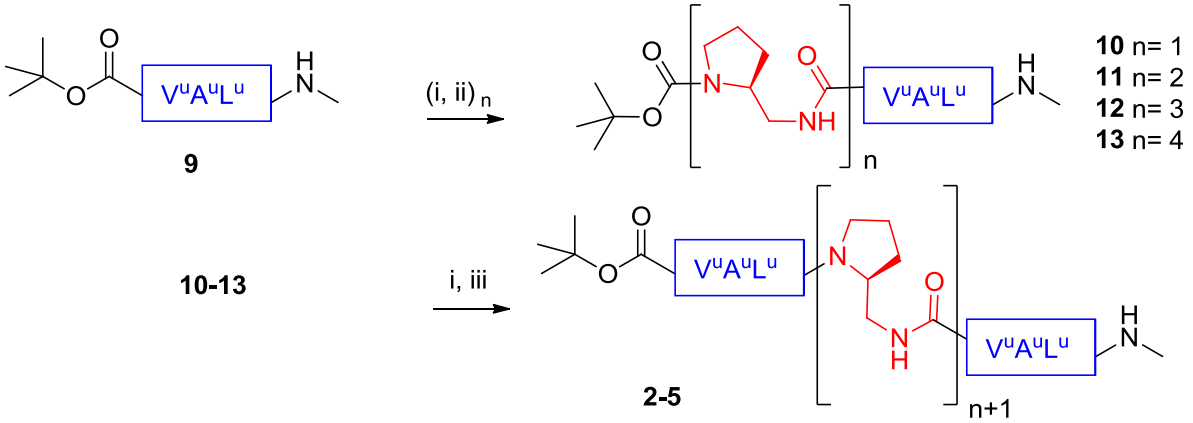
8 **a** R = iBu **b** R = Me **c** R = iPr



8d

The different segments were all synthesized stepwise from activated building blocks **8a-d**.[30,38,39] The segment condensation between activated oligourea **7** and oligomers **10-13** leading to oligoureas **2-5** proceeded in good yields ranging from 56 to 80 % (Scheme 1).

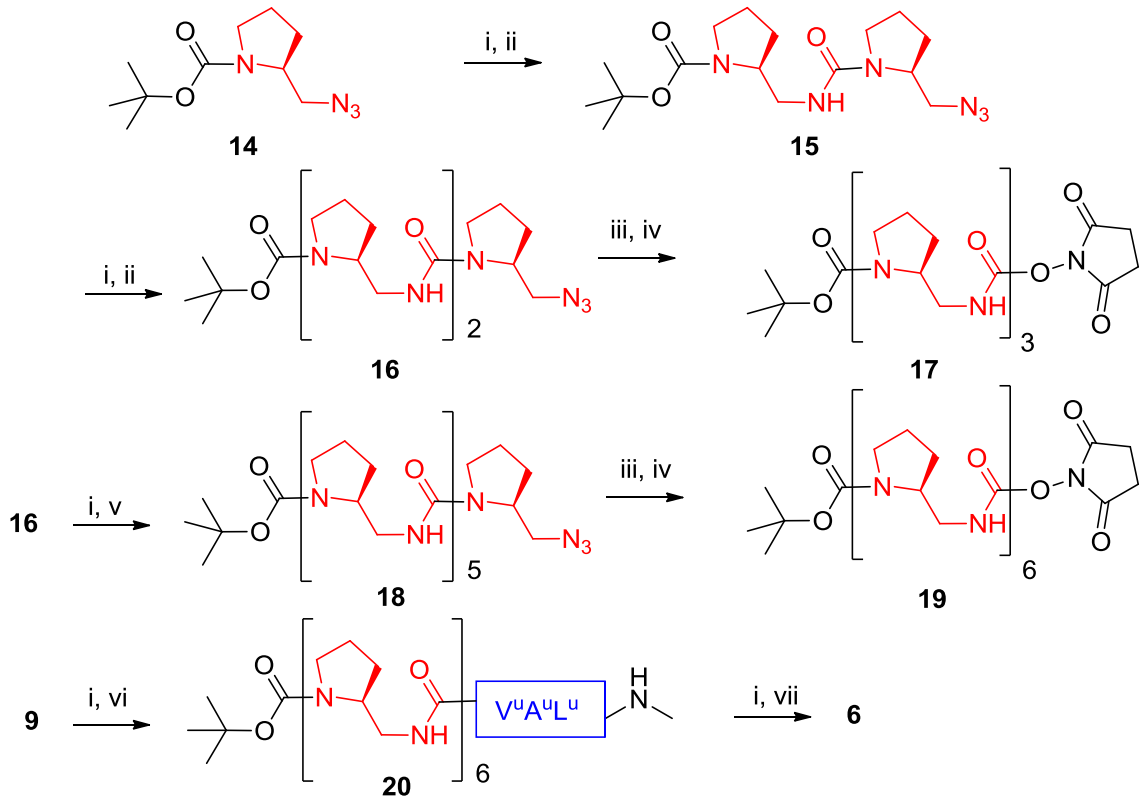
Scheme 1. Synthesis of oligoureas **2-5**[a]



[a]Reaction conditions : (*i*) TFA, 45 min; (*ii*) **8d**, DIEA, CH_3CN; (*iii*) **7**, DIEA, CH_3CN

For the preparation of the longest oligomer **6**, another activated segment of six pyrrolidine units **19** was prepared and coupled to the TFA salt derived from **9** prior to the final condensation with **7** (Scheme 2). The 12-mer **6** was thus readily obtained in good yields over the two segment condensation steps (55 and 60% respectively)

Scheme 2. Synthesis of oligourea 6[a]



[a]Reaction conditions : (*i*) TFA, 45 min; (ii) **8d**, DIEA, CH_3CN; (*iii*) H_2, Pd/C, EtOH; (*iv*) DSC, CH_2Cl_2; (*v*) **17**, DIEA, CH_3CN; (*vi*) **19** , DIEA, CH_3CN; (*vii*) **7**, DIEA, CH_3CN;

We have next compared the folding propensity of oligomers **1**-**6** in solution, first using electronic circular dichroism (ECD) in 2,2,2-trifluoroethanol (TFE). All spectra display a characteristic ECD signature indicative of 2.5-helical folding with a positive maximum at 203 nm, zero crossing at 193 nm and a weaker negative maximum at 188 nm (Figure 2). However, the per residue molar ellipticity (PRME) value at 203 nm for the molecule **2** (2.3×10^4 deg cm^2 dmol^{-1}) with two consecutive pyrrolidine units is significantly lower than in **1** (3.9×10^4 deg cm^2 dmol^{-1}) which is indicative of a destabilization of the canonical helical structure. The absolute PRME values further decrease gradually with the insertion of additional pyrrolidine residues (**3**-**6**), the

signal of **6** becoming poorly defined below 193 nm. Overall these results point to diminished helical folding caused by additional adjacent proline type residues.

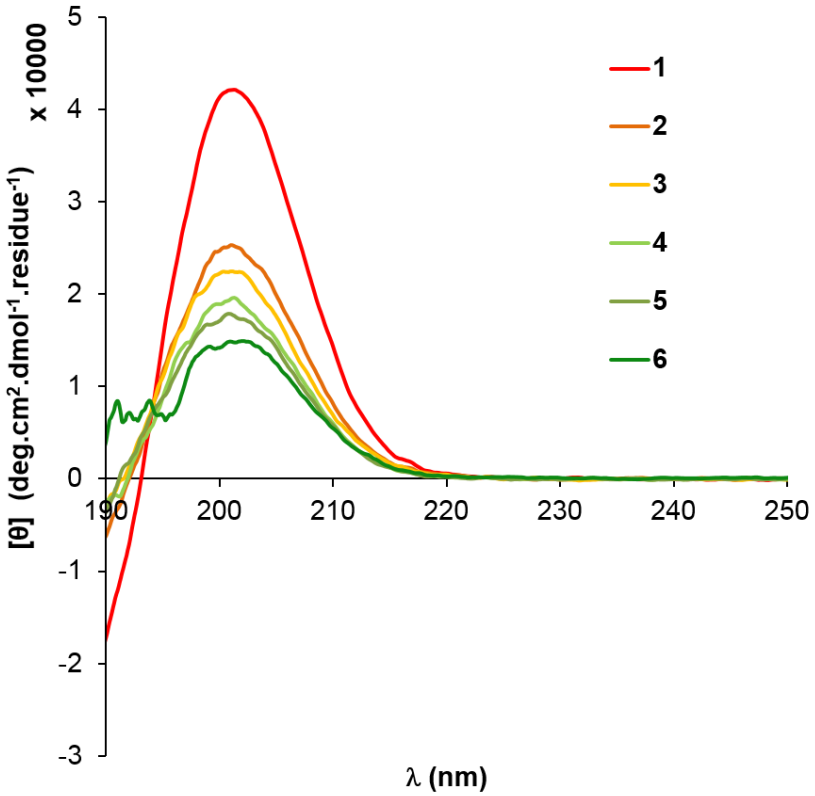


Figure 2. ECD spectra of molecules **1**-**6** (TFE, 0.2 mM)

These observations are supported by ^{1}H NMR spectroscopy experiments. ^{1}H NMR spectra of molecules **2**-**6** were recorded in CD_3OH at 3 mM. A lower solubility of oligomers is noticed upon increasing the number of pyrrolidine units in the chain with **6** being hardly soluble in CD_3OH. The frequencies of ^{1}H atoms were assigned using homonuclear DQF-COSY, TOCSY and ROESY experiments (see Tables S1-S3 in supporting information). However, spin systems of Pro^{u} residues in **3** and **5** could not be unequivocally resolved because of resonance overlaps and peak broadening. The NMR spectra of **2** and **3** are very similar to that of **1** and display features

typical of oligourea helices such as significant dispersion of urea NH signals (See Figs S3, S6 and S11 for illustration), high diastereotopicity of main chain CH_2 protons (*vide infra*), and large vicinal coupling constants between NHs and $^{\beta}$CH protons of canonical residues.[32,35,40] However, these characteristics progressively disappear in oligomers with more than three consecutive pyrrolidine units. The signals broaden and the dispersion of NH signals decreases significantly in oligomer **4** and **5**. The ^{1}H NMR spectrum of **4** was poorly resolved precluding unambiguous sequence assignment.

The anisochronicity values ($\Delta\delta$) of main chain CH_2 protons of acyclic/canonical residues at both ends of the sequence were extracted from spectra of oligomers **2**, **3** and **5** and compared to that of **1** (Figure 3). These values are sensitive to the number of pyrrolidine residues (n) in the sequence and decreased rapidly when $n > 2$ to reach values below that generally observed for 2.5-helical oligoureas in the case of **5**. Only the $\Delta\delta$ values for the first two residues (Leu^{u}1 and Ala^{u}2) are moderately affected among all oligomers. Overall these data are in line with ECD results and confirm that the introduction of multiple adjacent pyrrolidine residues in the central part of an oligourea sequence significantly alter the 2.5-helical geometry in solution.

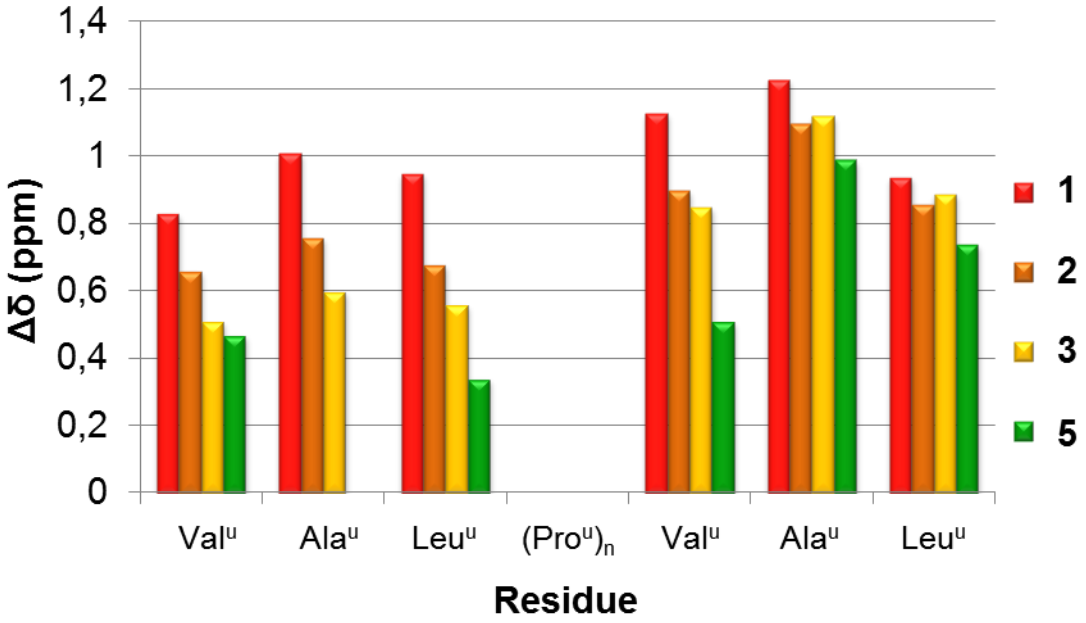
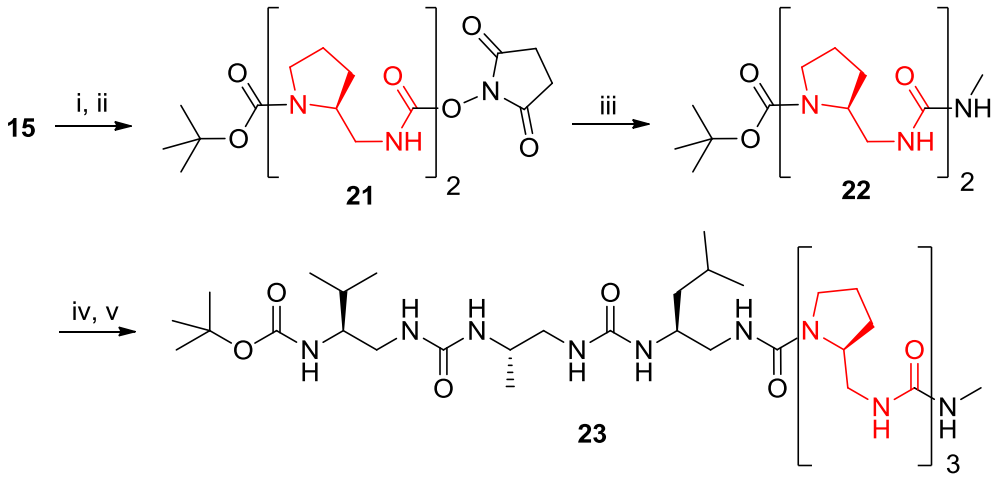


Figure 3. Chemical shift differences $\Delta\delta$ (ppm) of main chain CH_2 protons of canonical residues in the sequence of oligomers **1**, **2**, **3** and **5**

To determine which canonical segment in the sequence is the most efficient to induce helicity of the Pro^u repeat, we have compared **3** with urea hexamers **12** and **23** (see scheme 3 for the synthesis of **23**) consisting of three contiguous pyrrolidine units linked to a canonical segment at either end of the sequence.

Scheme 3. Synthesis of oligourea **23**[a]



[a]Reaction conditions : (*i*) H_2, Pd/C, EtOH; (*ii*) DSC, CH_2Cl_2; (*iii*) CH_3NH_2·HCl, DIEA, CH_3CN; (*iv*) TFA, 45 min; (*v*) **7**, DIEA, CH_3CN.

ECD experiments reveal that the PRME value measured for **12** at 203 nm is about the same as that observed for **3** (Figure 4). This result suggests that three canonical residues at the start of the sequence (residues 1-3) in 6-mer **12** are sufficient to promote the same level of helicity as observed in 9-mer **3**. The carbonyl groups of pyrrolidine units in **12** (residues 3-6) are believed to be engaged in 12- and 14-H-bonded rings typical of 2.5-helices with urea NHs of canonical residues. An additional 12-membered H-bonded ring may be formed between the first trisubstituted urea (at the junction of residues 4 and 5) and the Boc carbonyl oxygen. In contrast, the ECD spectrum recorded for 6-mer **23** gives a poor signature that can hardly be assigned to a 2.5 helical structure (Figure 4), thus indicating that a pyrrolidine unit repeat at the first three positions (residues 1-3) is not compatible with a canonical helical arrangement .

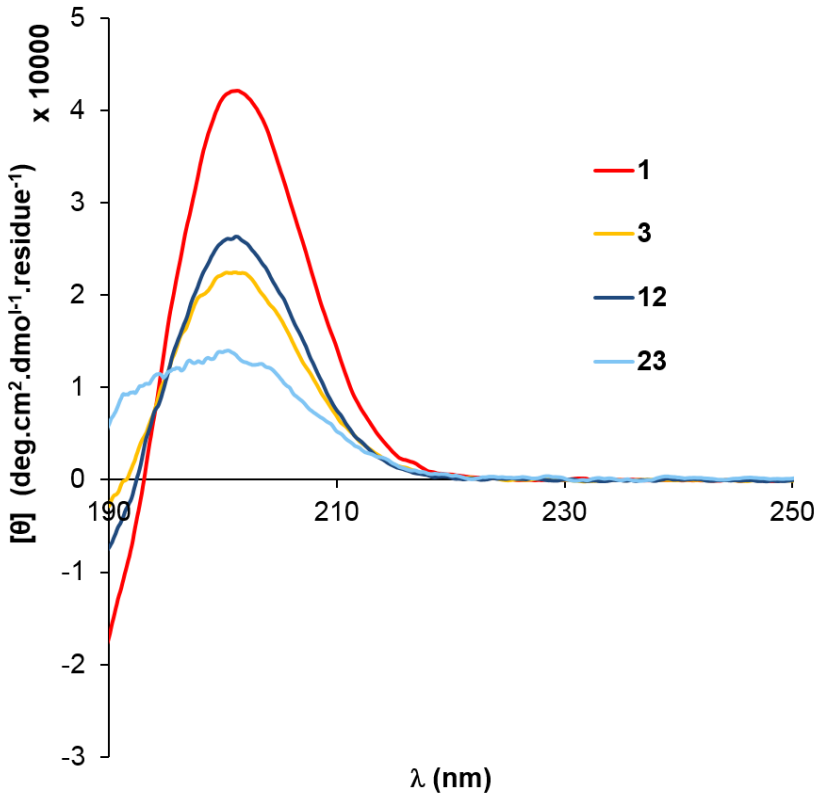


Figure 4. ECD spectra of **1**, **3**, **12** and **23** recorded in TFE at 0.2 mM

We obtained single crystal of **2** and **3** suitable for X-ray diffraction (XRD) analysis and solved their structure in the $P2_1$ and C2 space groups respectively (The crystal structure of **2** contains two independent molecules in the asymmetric unit) (Table 1). XRD analysis revealed that both molecules adopt a helical structure in the crystal with an overall geometry close to the canonical helix of oligoureas (Figure 5). Though the centre part of **3** tends to be slightly bent, the mean backbone torsion angles for each pyrrolidine residues are similar to those measured for **1** (CCDC 836810) and match well values generally found in 2.5-helical structures of oligoureas (

Table).[30,33,34]

Table 1. X-Ray cristallographic data of oligoureas **2** and **3**

Compound	**2**	**3**
CCDC code	-	-
Formula	C55.5 H101 N18 O11	C58 H109 N19 O11
M	1196.54	1248.64
Crystal system	monoclinic	Monoclinic
Space group	$P2_1$	C2
***a*/Å**	10.379(2)	18.609(4)
***b*/Å**	33.918(7)	20.000(4)
***c*/Å**	19.627(4)	21.577(4)
α/°	90	90
β/°	99.06(3)	107.06(3)
γ/°	90	90
***V*/Å^3**	6823(2)	7677(3)
T /K	293(2)	213(2)
Z	4	4
ρ/g cm^{-1}	1.165	1.080
size (mm)	-	0.1x0.01x0.01
λ/ Å	1.54178	1.54178
μ/mm^{-1}	0.675	0.621
Independent reflections	15187	5332
measured reflections	15187	-
parameters/restraints	1558/2	794/1
***R*1, *wR*2**	0.0514/0.1337	0.0832/0.2188
goodness of fit	1.036	1.053

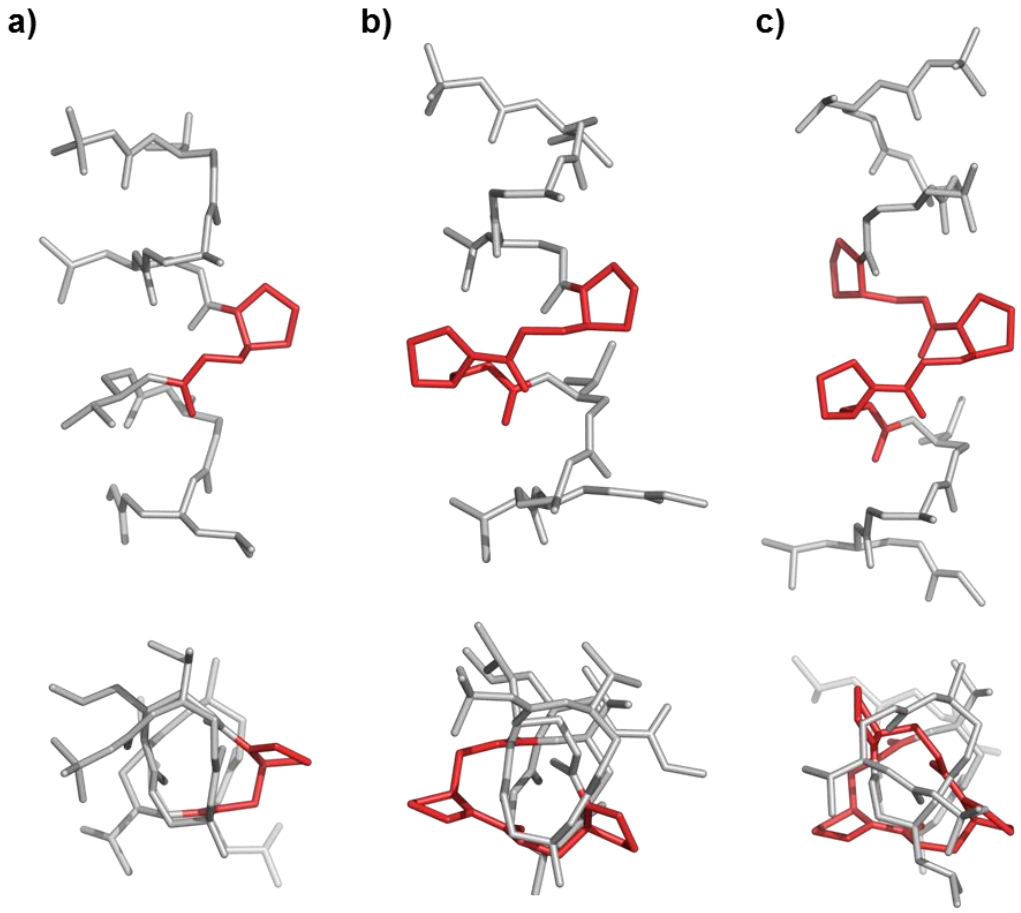
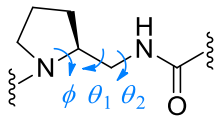


Figure 5. Comparison of X-Ray diffraction structures (side- and top views) of oligoureas (a) **1**[30] (CCDC 836810), (b) **2** and (c) **3**. Pyrrolidine units are coloured in red.

Table 2. Dihedral angles measured for each pyrrolidine type residues in **1**, **2** and **3**. (The P1 position corresponds to the terminal residue (Leu^{u}) coupled to methyl amine).



Cmpd	Res.	φ	θ_1	θ_2
1	P4	-104,3°	58,7°	91,2°
2	P4	-97,1°	45,9°	86,3°
	P5	-100,3°	66,6°	74,1°
3	P4	-95,1°	50,3°	93,2°
	P5	-98,6°	54,2°	73,3°
	P6	-90,2°	68,9°	87,9°

All possible intramolecular 12- and 14-membered H-bonded rings involving disubstituted urea NHs are formed in the three crystal structures. However, differences are observed for **3** in the H-bond network at the trisubstituted urea positions. At these positions involving pyrrolidine units, only 12-membered pseudorings between the remaining secondary amide (N'(*i*)) and C=O at position i+2 (O(*i*+2)) can be formed. In the structures of **1** and **2**, the N'(*i*) positions of tribustituted ureas (N'5 in **1** and both N'5 and N'6 in **2**) are all within a hydrogen-bonded distance to the corresponding carbonyl oxygens at i+2 positions (D(N···O) = 2.9-3.1 Å) (Figure 6a). Though two of these interactions closing 12-membered H-bonded rings are conserved in the structure of **3** (D(N'7···O9) = 3.0 Å and D(N'6···O8) = 2.9 Å), the distance between O7 and amide N'5 is much higher (D(N···O) = 4.3 Å) (Figure 6b). This reorganisation may be due to a steric repulsion between the two pyrrolidine rings of residues at P4 and P6. This observation further supports the conclusion drawn from solution studies indicating that the destabilization of the helical structure is largely amplified in **3** compared to **1** and **2**.

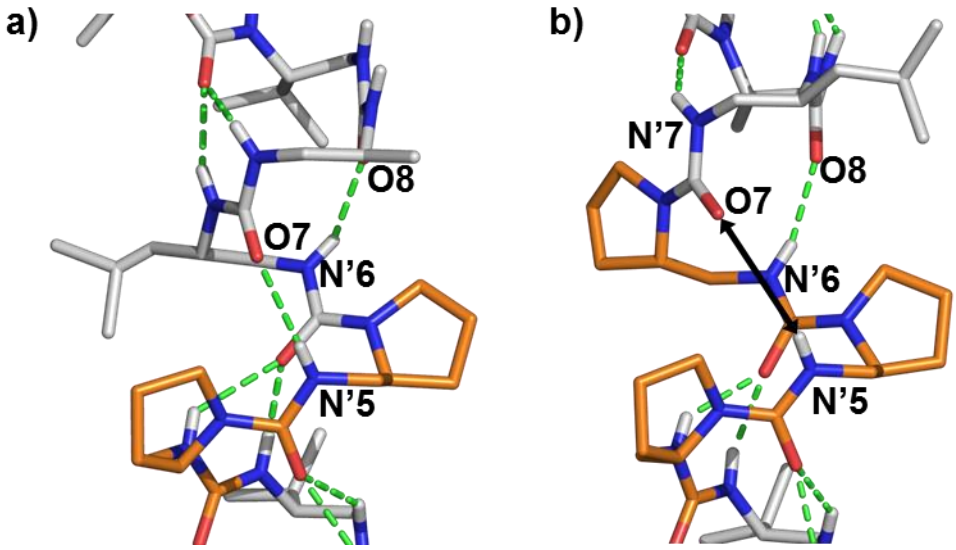
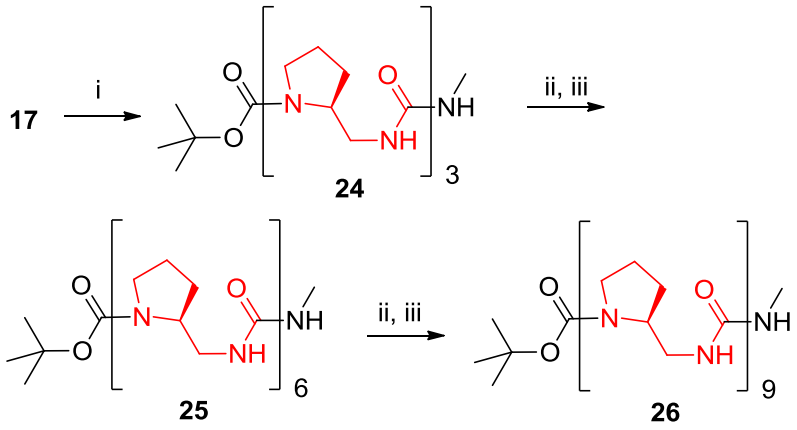


Figure 6 : Hydrogen bond details at the pyrrolidine positions in a) molecule **2** and b) molecule **3**. Main chain N'5 and O7 atoms are within a H-bond distance in **2 (**D(N···O) = 3.14 Å) but not in **3 (**D(N···O) > 4 Å).

To investigate whether oligomers built exclusively from *N*-(pyrrolidin-2-ylmethyl)ureido units can adopt a specific secondary structure, we have prepared two oligoureas containing six and nine pyrrolidine units (Scheme 4). Successive condensation steps using the activated segment **17** containing three pyrrolidine residues on a trimeric segment **24** afforded **25** and **26** in 40 and 48 % yield respectively.

Scheme 4. Synthesis of oligoureas **25** and **26** containing 6 and 9 pyrrolidine residues.



[a]Reaction conditions : (*i*) CH_3NH_2·HCl, DIEA, CH_3CN; (*ii*) TFA, 45 min; (*iii*) **17**, DIEA, DMF.

Oligoureas **25** and **26** exhibited poor ECD spectra in TFE, which reflects the absence of a well-defined folded conformation (Figure 7). These observations are confirmed by ^{1}H NMR analysis in CD_3OH at 3 mM. The NHs signals are broad and poorly dispersed and $^{\alpha}CH_2$ protons of the main chain are all overlapped between 3.0 and 3.5 ppm. These features indicate a lack of conformational homogeneity that may result from the presence of rapidly interconverting populations with various ratios of urea *cis-trans* isomers[41] and / or from interchain aggregation even at the low concentrations used for ECD measurements. It remains to be seen whether longer oligomers of pyrrolidine units could eventually adopt preferential conformations.

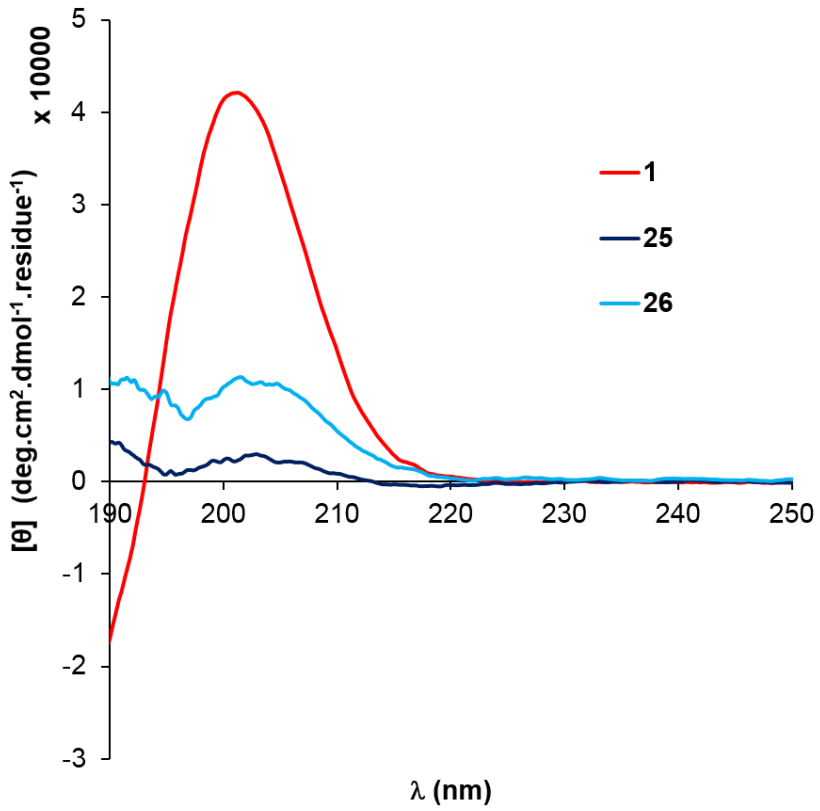


Figure 7. ECD spectra of **1**, **25** and **26** recorded in TFE at 0.2 mM

CONCLUSION

We have shown previously that the helix conformation of aliphatic *N*,*N*'-linked oligoureas tolerates the insertion of one or multiple non adjacent trisubstituted ureas such as those formed by insertion of pyrrolidine (Pro^u) units. This approach has been exploited to prepare long helical segments by fragment condensation.[30] We have now extended this work to oligoureas containing Pro^u repeats. ECD and NMR studies suggest that in solution the helix propensity of oligoureas decreases with the length of the repeat. Helices with $(Pro^u)_2$ and $(Pro^u)_3$ have been characterized by X-ray diffraction and reveal little deviation compared to canonical 2.5-helices. Oligomers consisting exclusively of pyrrolidine units, i.e. $(Pro^u)_6$ and $(Pro^u)_9$ have also been studied but do not show evidence of a dominant folded structure. The absence of defined secondary structure

elements could be due to several factors such as insufficient chain length to stabilize folded populations, uncontrolled urea *cis-trans* isomerization and/ or aggregation. The introduction of hydroxypyrrolidine derivatives (Hyp^u) or more constrained pyrrolidine type units with 3-aminopyrrolidine skeleton for example could be considered in future studies to modulate *cis-trans* isomerisation and possibly promote main chain organization and folding of *N*-alkyl-*N*,*N*'-linked oligoureas into well-defined secondary structures.

EXPERIMENTAL SECTION

Activated monomers **8** (**a** R = *i*Bu; **b** R = Me; **c** R = *i*Pr)[38] and **8d**[30], oligomers **1**[30], **7**[30] and **9**[34] were prepared using previously described procedures.

Urea formation: General procedure

Boc-protected oligourea (1.0 eq) was dissolved in TFA (3 ml / g) and stirred for 45 min. The reaction mixture was then concentrated under reduced pressure and the resulting residue was coevaporated three times with cyclohexane. The crude product was then dissolved in CH_3CN (5 ml / g). DIEA (3.0 eq) was then added and the mixture was cooled to 0°C prior to the dropwise addition of the following carbamate, **11a-d**, dissolved in CH_3CN. After completion of the reaction, the reaction mixture was evaporated, dissolved in EtOAc and treated with saturated $NaHCO_3$ aqueous solution, 1M $KHSO_4$ aqueous solution and brine. The organic layer was then dried over Na_2SO_4 and evaporated. Flash column chromatography (CH_2Cl_2-MeOH (v/v), 90:10) over silica gel gave the desired compound as a white product.

Boc-Pro^u-Val^u-Ala^u-Leu^u-NHMe (10). 10 was prepared from **8d** (0.064 g, 0.189 mmol) and **9** (0.100 g, 0.199 mmol) as described in the general procedure (0.090 g, 72 %). ^1H NMR: (300MHz, CD_3OH) δ = 6.43-6.29 (m, 2H, NH), 6.23-5.98 (m, 4H, NH), 5.92 (s, 1H, NH), 5.80

(d, J = 9.7 Hz, 1H, NH), 4.09-3.94 (m, 1H, CHN), 3.94-3.74 (m, 2H, CHN), 3.72-3.31 (m, 5H, CHN-CH_2N), 3.20-2.82 (m, 2H, CH_2N), 2.73 (d, J = 4.5 Hz, 3H, CH_3N), 2.64-2.30 (m, 4H, CH_2N), 2.03-1.81 (m, 4H, CH_2), 1.82-1.60 (m, 2H,CH), 1.50 (s, 9H, CH_3), 1.34-1.20 (m, 2H, CH_2), 1.04 (d, J = 6.8 Hz, 3H, CH_3), 0.98-0.89 (m, 12H, CH_3). ESI-MS (MW 627.82): m/z 628.3 $[M + H]^+$, 650.4 $[M + Na]^+$, 1277.1 $[2M + Na]^+$, C_{18} RP-HPLC (A : 0.1% TFA in H_2O, B: 0.1% TFA in MeOH, 50-100% B, 1 ml/min, 5 min): t_R= 5.62 min.

Boc-Val^u-Ala^u-Leu^u-Pro^u-Pro^u-Val^u-Ala^u-Leu^u-NHMe (2). 2 was prepared from **7** (0.021 g, 0.030 mmol) and **10** (0.020 g, 0.032 mmol) as described in the general procedure. (0.020 g, 56 %).^{1}H NMR : (300MHz, CD_3OH) δ = 6.73 (3, 1H, NH), 6.57 (m, 1H, NH), 6.52 (d, J = 9.8 Hz, 1H, NH), 6.43 (m, 2H, NH), 6.35 (m, 2H, NH), 6.23 (m, 1H, NH), 6.13-5.96 (m, 4H, NH), 5.92-5.85 (m, 2H, NH), 5.82 (m, 1H, NH), 4.34-4.12 (m, 1H, CHN), 4.09-3.83 (m, 4H, CHN), 3.70-3.39 (m, 15H, CHN-CH_2N), 3.05-2.95 (m, 1H, CH_2N), 2.88-2.74 (m, 3H, CH_2N), 2.73 (d, J = 4.6 Hz, 3H, CH_3N), 2.65-2.33 (m, 4H, CH_2N), 2.09-1.83 (m, 6H, CH_2), 1.81-1.56 (m, 6H, CH-CH_2), 1.48 (s, 9H, Boc), 1.34-1.22 (m, 4H, CH_2), 1.13-1.02 (m, 6H, CH_3), 0.99-0.85 (m, 24H, CH_3). ESI-MS (Mw 1124.47) : m/z 585.0 $[M+2Na]^{2+}$, 1146.8 $[M+Na]^+$, C_{18} RP-HPLC (A : 0.1% TFA in H_2O, B: 0.1% TFA in MeOH, 50-100% B, 1 ml/min, 5 min): t_R= 6.46 min.

Boc-Pro^u-Pro^u-Val^u-Ala^u-Leu^u-NHMe (11). 11 was prepared from **8d** (0.217 g, 0.030 mmol) and **10** (0.280 g, 0.032 mmol) as described in the general procedure. (0.320 g, 70 %).^{1}H NMR : (300MHz, CD_3CN) δ = 6.85 (m, 1H, NH), 6.26 (m, 1H, NH), 6.14 (d, J = 8.6 Hz, 1H, NH), 6.11 (d, J = 9.8 Hz, 1H, NH), 6.04 (m, 1H, NH), 5.74 (d, J = 9.7 Hz, 1H, NH), 5.67 (d, J = 10.8 Hz, 1H, NH), 5.62 (m, 1H, NH), 5.22 (d, J = 10.3 Hz, 1H, NH), 4.29-4.15 (m, 2H, CHN), 4.08-3.92 (m, 1H, CHN), 3.91-3.77 (m, 1H, CHN), 6.68-3.48 (m, 5H, CHN, CH_2N), 3.46-3.13 (m, 5H, CH_2N), 2.77-2.68 (m, 1H, CH_2N), 2.65 (d, J = 4.7 Hz, 3H, CH_3N), 2.55-2.30 (m, 3H, CH_2N), 2.12-2.25 (m, 1H, CH_2N), 2.07-1.50 (m, 10H, CH, CH_2), 1.47 (s, 9H, Boc), 1.21-1.12 (m, 2H,

CH_2), 0.98 (d, J = 6.9 Hz, 3H, CH_3), 0.92 (d, J = 6.5 Hz, 3H, CH_3), 0.90 (d, J = 6.7 Hz, 3H, CH_3), 0.85 (d, J = 6.7 Hz, 3H, CH_3), 0.81 (d, J = 6.8 Hz, 3H, CH_3). ESI-MS (MW 753.98) : m/z 776.5 $[M+Na]^+$, C_{18} RP-HPLC (A : 0.1% TFA in H_2O, B: 0.1% TFA in MeOH, 50-100% B, 1 ml/min, 5 min): t_R= 5.86 min.

Boc-Val^u-Ala^u-Leu^u-Pro^u-Pro^u-Pro^u-Val^u-Ala^u-Leu^u-NHMe (3). 3 was prepared from **7** (0.027 g, 0.038 mmol) and **11** (0.030 g, 0.040 mmol) as described in the general procedure. (0.030 g, 60 %).^{1}H NMR: (300MHz, CD_3OH) δ = 6.68 (m, 2H, NH), 6.51 (m, 1H, NH), 6.45 (d, J = 9.9 Hz, 1H, NH), 6.42-6.14 (m, 5H, NH), 6.08 (d, J = 9.2 Hz, 1H, NH), 6.01-5.81 (m, 6H, NH), 4.21-4.10 (m, 1H, CHN), 4.08-3.97 (m, 2H, CHN), 3.95-3.81 (m, 3H, CHN), 3.68-3.17 (m, 19H, CHN-CH_2N), 3.17-3.02 (m, 2H, CH_2N), 3.01-2.79 (m, 3H, CH_2N), 2.73 (d, J = 4.6 Hz, 3H, CH_3N), 2.70 (m, 1H, CH_2N), 2.65-2.49 (m, 1H, CH_2N), 2.49-2.34 (m, 1H, CH_2N), 2.11-1.90 (m, 12H, CH_2) 1.79-1.60 (m, 4H, CH), 1.47 (s, 9H, Boc), 1.33-1.22 (m, 4H, CH_2), 1.13-1.02 (m, 6H, CH_3), 0.99-0.84 (m ; 24H, CH_3). ESI-MS (MW 1250.62): m/z 1250.6 $[M+H]^+$, 1276.9 $[M+Na]^+$, C_{18} RP-HPLC (A : 0.1% TFA in H_2O, B: 0.1% TFA in MeOH, 50-100% B, 1 ml/min, 5 min): t_R= 6.27 min.

Boc-Pro^u-Pro^u-Pro^u-Val^u-Ala^u-Leu^u-NHMe (12). 12 was prepared from **8d** (0.135 g, 0.397 mmol) and **11** (0.300 g, 0.397 mmol) as described in the general procedure. (0.260 g, 75 %).^{1}H NMR : (300MHz, CD_3OH) δ = 6.69 (m, 1H, NH), 6.56 (m, 1H, NH), 6.46-6.15 (m, 5H, NH), 6.10 (d, J = 8.8 Hz, 1H, NH), 6.05-5.88 (m, 2H, NH), 4.29-3.81 (m, 5H, CHN), 3.68-3.50 (m, 5H, CHN-CH_2N), 3.44-3.23 (m, 8H, CH_2N), 3.22-3.03 (m, 3H, CH_2N), 2.81-2.74 (m, 1H, CH_2N), 2.73 (d, J = 4.7 Hz, 3H, CH3), 2.66-2.51 (m, 1H, CH_2N), 2.51-2.38 (m, 1H, CH_2N), 2.12-1.81 (m, 12H, CH_2), 1.79-1.59 (m, 2H, CH), 1.50 (s, 9H, Boc), 1.34-1.21 (m, 2H, CH_2), 1.06 (d, J = 6.8 Hz, 3H, CH_3), 0.97-0.86 (m, 12H, CH_3). ESI-MS (MW 880.13): m/z 902.6 $[M+Na]^+$, C_{18} RP-

HPLC (A : 0.1% TFA in H_2O, B: 0.1% TFA in MeOH, 50-100% B, 1 ml/min, 5 min): t_R= 5.94 min.

Boc-Val^u-Ala^u-Leu^u-Pro^u-Pro^u-Pro^u-Pro^u-Val^u-Ala^u-Leu^u-NHMe (4). 4 was prepared from **7** (0.102 g, 0.143 mmol) and **12** (0.120 g, 0.136 mmol) as described in the general procedure. (0.140 g, 82 %).^{1}H NMR : (300MHz, DMSO) δ = 6.88 (m, 1H, NH), 6.79 (m, 1H, NH), 6.57 (d, J = 9.1 Hz, 1H, NH), 6.42 (m, 1H, NH), 6.20 (m, 1H, NH), 6.10-5.71 (m, 12H, NH), 3.96-3.46 (m, 10H, CHN), 3.26-2.55 (m, 28H, CH_2N), 2.55 (d, 3H, CH_3N), 1.93-1.72 (m, 16H, CH_2), 1.70-1.52 (m, 4H, CH), 1.39 (s, 9H, Boc), 1.18-1.07 (m, 4H, CH_2), 0.99-0.91 (m, 6H, CH_3), 0.90-0.75 (m, 24H, CH_3). ESI-MS (MW 1376.78): m/z 689.0 $[M+2H]^{2+}$, 1398.8 $[M+Na]^+$, C_{18} RP-HPLC (A : 0.1% TFA in H_2O, B: 0.1% TFA in MeOH, 50-100% B, 1 ml/min, 5 min): t_R= 6.29 min.

Boc-Pro^u-Pro^u-Pro^u-Pro^u-Val^u-Ala^u-Leu^u-NHMe (13). 13 was prepared from **8d** (0.049 g, 0.143 mmol) and **12** (0.120 g, 0.136 mmol) as described in the general procedure. (0.104 g, 76 %).^{1}H NMR : (300MHz, CD_3CN) δ = 6.87 (m, 1H, NH), 6.53-6.40 (m, 2H, NH), 6.32 (m, 1H, NH), 6.27-6.14 (m, 2H, NH), 6.08 (m, 1H, NH), 5.99 (m, 1H, NH), 5.89-5.69 (m, 3H, NH), 4.46-4.32 (m, 1H, CHN), 4.30-4.12 (m, 2H, CHN), 4.10-3.93 (m, 2H, CHN), 3.90-3.77 (m, 2H, CHN), 3.67-3.49 (m, 5H, CH_2N), 3.47-3.12 (m, 11H, CH_2N), 3.11-2.81 (m, 2H, CH_2N), 2.78-2.70 (m, 1H, CH_2N), 2.66 (d, J = 4.7 Hz, 3H, CH_3N), 2.60-2.30 (m, 3H, CH_2N), 2.13-1.77 (m, 13H, CH_2), 1.75-1.50 (m, 5H, CH-CH_2), 1.48 (s, 9H, Boc), 1.25-1.15 (m, 2H, CH_2), 1.00 (d, J = 6.8 Hz, 3H, CH_3), 0.93 (d, J = 6.5 Hz, 3H, CH_3), 0.92 (d, J = 6.7 Hz, 3H, CH_3), 0.87 (d, J = 6.7 Hz, 3H, CH_3), 0.82 (d, J = 6.7 Hz, 3H, CH_3). ESI-MS (MW 1006.31): m/z 503.8 $[M+2H]^{2+}$, 1006.4 $[M+H]^+$, C_{18} RP-HPLC (A : 0.1% TFA in H_2O, B: 0.1% TFA in MeOH, 50-100% B, 1 ml/min, 5 min): t_R= 6.01 min.

Boc-Val^u-Ala^u-Leu^u-Pro^u-Pro^u-Pro^u-Pro^u-Pro^u-Val^u-Ala^u-Leu^u-NHMe (5). 5 was prepared from **7** (0.074 g, 0.104 mmol) and **13** (0.100 g, 0.099 mmol) as described in the general

procedure. (0.090 g, 60 %).^{1}H NMR : (300MHz, DMSO) δ = 7.0-6.75 (m, 2H, NH), 6.56 (d, J = 8.9 Hz, 1H, NH), 6.41 (m, 1H, NH), 6.20 (m, 1H, NH), 6.09-5.72 (m, 13H, NH), 4.18-3.72 (m, 6H, CHN), 3.63-3.30 (m, 31H, CHN-CH_2N), 3.30-2.75 (m, 6H, CH_2N), 2.72 (d, 3H, CH_3N), 2.11-1.71 (m, 20H, CH_2), 1.69-1.50 (m , 4H, CH), 1.39 (s, 9H, Boc), 1.29-1.16 (m, 4H, CH_2), 0.99-0.90 (m, 6H, CH_3), 0.90-0.74 (m, 24H, CH_3). ESI-MS (MW 1502.94) : m/z 771.8 $[M+K]^{2+}$, 1524.8 $[M+Na]^{+}$, C_{18} RP-HPLC (A : 0.1% TFA in H_2O, B: 0.1% TFA in MeOH, 50-100% B, 1 ml/min, 5 min): t_R= 6.34 min.

Boc-Pro^{u}-2-Azidomethyl-pyrrolidine (15). 15 was prepared from **8d** (0.729 g, 2.137 mmol) and **14** (0.509 g, 2.25 mmol) as described in the general procedure. (0.710 g, 89 %).^{1}H NMR: (300MHz, $CDCl_3$) δ = 6.38 (s, 1H, NH), 4.16-4.01 (m, 2H, CHN), 3.56-3.11 (m, 8H, CH_2N), 2.11-1.78 (m, 8H, CH_2), 1.49 (s, 9H, Boc). ESI-MS (MW 352.43) : m/z 353.0 $[M+H]^{+}$, 726.9 $[2M+Na]^{+}$, C_{18} RP-HPLC (A : 0.1% TFA in H_2O, B: 0.1% TFA in MeOH, 50-100% B, 1 ml/min, 5 min): t_R= 5.40 min.

Boc-Pro^{u}-Pro^{u}-2-Azidomethyl-pyrrolidine (16). 16 was prepared from **8d** (0.609 g, 1.78 mmol) and **15** (0.662 g, 1.87 mmol) as described in the general procedure. (0.665 g, 74 %).^{1}H NMR : (300MHz, $CDCl_3$) δ = 6.91 (s, 1H, NH), 6.54 (s, 1H, NH), 4.28-4.15 (m, 1H, CHN), 4.16-4.03 (m, 2H, CHN), 3.54-3.08 (m, 12H, CH_2N), 2.10-1.83 (m, 10H, CH_2), 1.83-1.64 (m, 2H, CH_2), 1.49 (s, 9H, Boc). ESI-MS (MW 478.59): m/z 501.3 $[M+Na]^{+}$, 979.0 $[2M+Na]^{+}$, C_{18} RP-HPLC (A : 0.1% TFA in H_2O, B: 0.1% TFA in MeOH, 50-100% B, 1 ml/min, 5 min): t_R= 5.62 min.

Boc-Pro^{u}-Pro^{u}-Pro^{u}-OSu (17). 16 (0.640 g, 0.418 mmol) was dissolved in ethanol. Pd/C 10% (0.060 g) was introduced under Ar atmosphere. The reaction mixture was put under vacuum then flushed with H_2 (three times) and the mixture was finally stirred at room temperature under H_2 for 12h. Pd/C was then removed by microfiltration, washed with EtOH and the solvent was then completely evaporated under vacuum. Disuccinimidyl carbonate (0.410 g, 1.62 mmol) was

suspended in dry CH_2Cl_2 (10 mL) and the amine (0.610 g, 1.35 mmol) was added portionwise. The reaction mixture was stirred at room temperature under N_2 atmosphere for 4h. After 4h stirring a white precipitate was formed (HOSu). CH_2Cl_2 was added and the precipitate was filtered and washed with CH_2Cl_2. Organic phase was washed with a 1M KHSO4 aqueous solution and water, dried over Na_2SO_4, and concentrated under reduced pressure. 1mL of CH_2Cl_2 was added to the residue and the product **17** was precipitated as a white solid by adding Et_2O (0.770 g, 96%). ^{1}H NMR : (300MHz, $CDCl_3$) δ = 8.23 (s, 1H, NH), 7.08 (s, 1H, NH), 6.63 (s, 1H, NH), 4.33-4.19 (m, 1H, CHN), 4.18-4.02 (m, 2H, CHN), 3.55-3.03 (m, 12H, CH_2N), 2.82 (s, 4H, CH_2), 2.10-1.80 (m, 10H, CH_2), 1.79-1.65 (m, 2H, CH_2), 1.49 (s, 9H, Boc). ESI-MS (MW 593.67) : m/z 594.1 $[M+H]^+$.

Boc-Pro^u-Pro^u-Pro^u-Pro^u-Pro^u-2-Azidomethyl-pyrrolidine (18). 18 was prepared from **17** (0.053 g, 0.089 mmol) and III.**16** (0.045 g, 0.094 mmol) as described in the general procedure. (0.060 g, 75 %).^{1}H NMR : (300MHz, $CDCl_3$) δ = 7.29-7.06 (m, 4H, NH), 6.63 (s, 1H, NH), 4.32-4.01 (m, 6H, CHN), 3.61-3.02 (m, 24H, CH_2N), 2.15-1.83 (m, 20H, CH_2), 1.80-1.67 (m, 4H, CH_2), 1.50 (s, 9H, Boc). ESI-MS (MW 857.06) : m/z 857.2 $[M+H]^+$, 879.3 $[M+Na]^+$, C_{18} RP-HPLC (A : 0.1% TFA in H_2O, B: 0.1% TFA in MeOH, 50-100% B, 1 ml/min, 5 min): t_R= 6.07 min.

Boc-Pro^u-Pro^u-Pro^u-Pro^u-Pro^u-Pro^u-OSu (19). 18 (0.630 g, 0.740 mmol) was dissolved in ethanol. Pd/C 10% (0.060 g) was introduced under Ar atmosphere. The reaction mixture was put under vacuum then flushed with H_2 (three times) and the mixture was finally stirred at room temperature under H_2 for 12h. Pd/C was then removed by microfiltration, washed with EtOH and the solvent was then completely evaporated under vacuum. Disuccinimidyl carbonate (0.225 g, 0.880 mmol) was suspended in dry CH_2Cl_2 (10 mL) and the amine (0.610 g, 0.734 mmol) was added portionwise. The reaction mixture was stirred at room temperature under N_2 atmosphere

for 4h. After 4h stirring a white precipitate was formed (HOSu). CH_2Cl_2 was added and the precipitate was filtered and washed with CH_2Cl_2. Organic phase was washed with a 1M $KHSO_4$ aqueous solution and water, dried over Na_2SO_4, and concentrated under reduced pressure. 1mL of CH_2Cl_2 was added to the residue and the product **19** was precipitated as a white solid after addition of Et_2O (0.690 g, 97%). ^{1}H NMR : (300MHz, $CDCl_3$) δ = 8.31 (s, 1H, NH), 7.31-7.22 (m, 3H, NH), 7.18 (s, 1H, NH), 6.63 (s, 1H, NH), 4.28-4.03 (m, 6H, CHN), 3.53-3.05 (m, 24H, CH_2N), 2.83 (s, 4H, CH_2), 2.11-1.84 (m, 20H, CH_2), 1.81-1.66 (m, 4H, CH_2), 1.48 (s, 9H, Boc). ESI-MS (MW 972.14) (in MeOH the methlyl ester is formed Mw 888): m/z 889.2 $[M+H]^+$, 911.4 $[M+Na]^+$, C_{18} RP-HPLC (A : 0.1% TFA in H_2O, B: 0.1% TFA in MeOH, 50-100% B, 1 ml/min, 5 min): t_R= 5.68 min.

Boc-Pro^u-Pro^u-Pro^u-Pro^u-Pro^u-Pro^u-Val^u-Ala^u-Leu^u-NHMe (20). 20 was prepared from **19** (0.020 g, 0.020 mmol) and **9** (0.011 g, 0.020 mmol) as described in the general procedure. (0.015 g, 55 %). ^{1}H NMR : (300MHz, CD_3CN) δ = 6.87 (m, 1H, NH), 6.65 (m, 1H, NH), 6.43 (m, 1H, NH), 6.52-6.38 (m, 2H, NH), 6.35-6.14 (m, 4H, NH), 6.12-5.99 (m, 1H, NH), 5.90-5.79 (m, 2H, NH), 5.76 (m, 1H, NH), 4.43-4.31 (m, 1H, CHN), 4.28-4.18 (m, 1H, CHN), 4.14-3.71 (m, 6H, CHN), 3.70-3.49 (m, 4H, CHN-CH_2N), 3.74-3.07 (m, 20H, CH_2N), 3.05-2.82 (m, 3H, CH_2N), 2.66 (d, J = 4.7 Hz, 3H, CH_3N), 2.57-2.27 (m, 4H, CH_2N), 1.97-1.73 (m, 24H, CH_2), 1.71-1.54 (m, 2H, CH), 1.47 (s, 9H, Boc), 1.27-1.09 (m, 2H, CH_2), 1.00 (d, J = 6.8 Hz, 3H, CH_3), 0.94-0.77 (m, 12H, CH_3). ESI-MS (MW 1258.60) : m/z 652.1 $[M+2Na]^{2+}$, 1280.8 $[M+Na]^+$, C_{18} RP-HPLC (A : 0.1% TFA in H_2O, B: 0.1% TFA in MeOH, 50-100% B, 1 ml/min, 5 min): t_R= 6.13 min.

Boc-Val^u-Ala^u-Leu^u-Pro^u-Pro^u-Pro^u-Pro^u-Pro^u-Pro^u-Pro^u-Val^u-Ala^u-Leu^u-NHMe (6). 6 was prepared from **7** (0.008 g, 0.011 mmol) and **20** (0.015 g, 0.011 mmol) as described in the general procedure. (0.013 g, 60 %).^{1}H NMR : (300MHz, CD_3CN) δ = 6.84 (m, 1H, NH), 6.41 (m, 1H, NH), 6.30 (m, 2H, NH), 6.23-6.13 (m, 2H, NH), 6.11-5.95 (m, 3H, NH), 5.89-5.79 (m, 4H, NH),

5.77-5.48 (m, 8H, NH), 4.41-4.31 (m, 1H, CHN), 4.27-4.13 (m, 1H, CHN), 4.10-3.75 (m, 6H, CHN), 3.70-3.13 (m, 20H, CHN-CH_2N), 3.10-2.73 (m, 10H, CH_2N), 2.67 (d, J = 4.6 Hz, 3H, CH_3N), 2.62-2.08 (m, 15H, CH_2N), 1.94-1.54 (m, 32H, CH-CH_2), 1.44 (s, 9H, Boc), 1.33-1.09 (m, 4H, CH_2), 1.08-0.99 (m, 6H, CH_3), 0.93-0.78 (m, 24H, CH_3). ESI-MS (MW 1798.27) : m/z 900.0 $[M+2H]^{2+}$, C_{18} RP-HPLC (A : 0.1% TFA in H_2O, B: 0.1% TFA in MeOH, 50-100% B, 1 ml/min, 5 min): t_R= 6.43 min.

Boc-Pro^u-Pro^u-OSu (21). 15 (0.120 g, 0.306 mmol) was solubilized in ethanol. Pd/C 10% was introduced under Ar. The reaction mixture was put under vacuum then flushed with H_2 (three times) and the mixture was finally stirred at room temperature under H_2 atmosphere for 12h. Pd/C was then removed by microfiltration, washed with EtOH and the solvent was then completely evaporated under vacuum. Disuccinimidyl carbonate (0.094 g, 0.367 mmol) was suspended in dry CH_2Cl_2 (8mL) and the amine (0.100 g, 0.306 mmol) was added portionwise. The reaction mixture was stirred at room temperature under N_2 for 4h. After 4h stirring a white precipitate was formed (HOSu). CH_2Cl_2 was added and the precipitate was filtered off and washed with CH_2Cl_2. The organic layers were combined, washed with a 1M $KHSO_4$ aqueous solution and water, dried over Na_2SO_4, and concentrated under reduced pressure. 1mL of CH_2Cl_2 was added to the residue and the product **24** was precipitated as a white solid after addition of Et_2O (0.130 g, 90%). ^{1}H NMR : (300MHz, $CDCl_3$) δ = 8.14 (s, 1H, NH), 6.53 (s, 1H, NH), 4.27-4.06 (m, 2H, CHN), 3.48-3.30 (m, 5H, CH_2N), 3.29-3.11 (m, 3H, CH_2N), 2.83 (s, 4H, CH_2), 2.10-1.69 (m, 8H, CH_2), 1.47 (s, 9H, Boc). ESI-MS (MW 476.52) : m/z 490.1 $[M+Na]^+$, 956.8 $[2M+Na]^+$

Boc-Pro^u-Pro^u-NHMe (22). Methylamine hydrochloride (0.188 g, 0.280 mmol) was dissolved in CH_3CN. DIPEA (0.14 ml, 0.830 mmol) was then added and the mixture was cooled to 0°C prior to the dropwise addition of **21** (0.130 g, 0.280 mmol), dissolved in CH_3CN. After completion, the

reaction mixture was evaporated, dissolved in EtOAc and treated with saturated $NaHCO_3$ aqueous solution, 1M $KHSO_4$ aqueous solution and brine. The organic layer was then dried over Na_2SO_4 and evaporated. Flash column chromatography (CH_2Cl_2-MeOH (v/v), 95:5) over silica gel gave **22** (0.090 g, 84%). ^{1}H NMR : (300MHz, CD_3OH) δ = 6.64 (m, 1H, NH), 6.33 (m, 1H, NH), 6.04 (m, 1H, NH), 4.07-3.81 (m, 2H, CHN), 3.49-3.04 (m, 8H, CH_2N), 2.73 (d, J = 4.6 Hz, 3H, CH_3N), 2.08-1.72 (m, 8H, CH_2), 1.49 (m, 9H, Boc). ESI-MS (MW 383.49) : m/z 384.0 $[M+H]^+$, 406.1 $[M+Na]^+$, 788.8 $[2M+Na]^+$, C_{18} RP-HPLC (A : 0.1% TFA in H_2O, B: 0.1% TFA in MeOH, 50-100% B, 1 ml/min, 5 min): t_R= 4.20 min.

Boc-Val^u-Ala^u-Leu^u-Pro^u-Pro^u-Pro^u-NHMe (23). 23 was prepared from **7** (0.053 g, 0.074 mmol) and **22** (0.030 g, 0.078 mmol) as described in the general procedure. (0.035 g, 51 %).^{1}H NMR : (300MHz, CD_3OH) δ = 7.15 (m, 1H, NH), 6.96 (m, 1H, NH), 6.76 (m, 1H, NH), 6.62-6.50 (m, 2H, NH), 6.23-5.93 (m, 5H, NH), 4.15-3.79 (m, 5H, CHN), 3.76-3.62 (m, 1H, CHN), 3.61-3.06 (m, 16H, CH2N), 2.94-2.78 (m, 2H, CH_2N), 2.75 (d, J = 4.6 Hz, 3H, CH_3N), 2.09-1.80 (m, 12H, CH_2), 1.78-1.62 (m, 2H, CH), 1.47 (s, 9H, Boc), 1.37-1.26 (m, 2H, CH_2), 1.08 (d, J = 6.6 Hz, 3H, CH_3), 0.96-0.92 (m, 12H, CH_3). ESI-MS (MW 880.13) : m/z 463.0 $[M+2Na]^{2+}$, 902.6 $[M+Na]^+$, C_{18} RP-HPLC (A : 0.1% TFA in H_2O, B: 0.1% TFA in MeOH, 50-100% B, 1 ml/min, 5 min): t_R= 5.64 min

Boc-Pro^u-Pro^u-Pro^u-NHMe (24). Methylamine hydrochloride (0.044 g, 0.65 mmol) was dissolved in CH_3CN. DIPEA (0.3 ml, 0.151 mmol) was then added and the mixture was cooled down to 0°C prior to the dropwise addition of **17** (0.350 g, 0.590 mmol), dissolved in CH_3CN. After completion, the reaction mixture was evaporated, dissolved in EtOAc and treated with saturated $NaHCO_3$ aqueous solution, 1M $KHSO_4$ aqueous solution and brine. The organic layer was then dried over Na_2SO_4 and evaporated. Flash column chromatography (CH_2Cl_2-MeOH (v/v), 95:5) over silica gel gave **21** (0.268 g, 90%). ^{1}H NMR : (300MHz, CD_3CN) δ = 6.61 (m,

1H, NH), 6.33 (m, 1H, NH), 2.74 (m, 1H, NH), 5.37 (m, 1H, NH), 4.02-3.74 (m, 3H, CHN), 3.41-3.11 (m, 10H, CH_2N), 3.08-2.82 (m, 2H, CH_2N), 2.67 (d, J = 4.7 Hz, 3H, CH_3N), 2.00-1.71 (m, 12H, CH_2), 1.47 (s, 9H, Boc). ESI-MS (MW 509.64): m/z 510.3 $[M+H]^+$, 532.3 $[M+Na]^+$, C_{18} RP-HPLC (A : 0.1% TFA in H_2O, B: 0.1% TFA in MeOH, 50-100% B, 1 ml/min, 5 min): t_R= 4.78 min.

Boc-Pro^u-Pro^u-Pro^u-Pro^u-Pro^u-Pro^u-NHMe, (25). 25 was prepared from **17** (0.110 g, 0.186 mmol) and **22** (0.100 g, 0.196 mmol) as described in the general procedure. (0.070 g, 40 %).^{1}H NMR : (300MHz, CD_3OH) δ = 7.06-6.89 (m, 4H, NH), 6.75 (s, 1H, NH), 6.33 (s, 1H, NH), 6.05 (s, 1H, NH), 4.06-3.79 (m, 6H, CHN), 3.49-3.18 (m, 18H, CH_2N), 3.13-2.97 (m, 6H, CH_2N), 2.73 (d, J = 4.0 Hz, 3H, CH_3N), 2.10-1.77 (m, 24H, CH_2), 1.48 (s, 9H, Boc). ESI-MS (MW 888.11): m/z 910.6 $[M+Na]^+$, C_{18} RP-HPLC (A : 0.1% TFA in H_2O, B: 0.1% TFA in MeOH, 50-100% B, 1 ml/min, 5 min): t_R= 6.47min.

Boc-Pro^u-Pro^u-Pro^u-Pro^u-Pro^u-Pro^u-Pro^u-Pro^u-Pro^u-NHMe, (26). 26 was prepared from **17** (0.040 g, 0.067 mmol) and **25** (0.060 g, 0.067 mmol) as described in the general procedure. (0.041 g, 48 %).^{1}H NMR : (300MHz, CD_3OH) δ = 7.11-6.84 (m, 7H, NH), 6.76 (s, 1H, NH), 6.30 (s, 1H, NH), 6.06 (s, 1H, NH), 4.08-3.79 (m, 9H, CHN), 3.53-3.18 (m, 27H, CH_2N), 3.17-2.97 (m, 9H, CH_2N), 2.73 (d, J = 4.1 Hz, 3H, CH_3N), 2.10-1.75 (m, 36H, CH_2), 1.48 (s, 9H, Boc). ESI-MS (MW 1266.58): m/z 1266.5 $[M+H]^+$, C_{18} RP-HPLC (A : 0.1% TFA in H_2O, B: 0.1% TFA in MeOH, 50-100% B, 1 ml/min, 5 min): t_R= 6.98 min.

ASSOCIATED CONTENT

*S Supporting Information

General methods, copies of ^{1}H NMR spectra, HPLC profiles and ESI-MS spectra for compounds **2-6**, **10-13**, and **15-26**, tables of ^{1}H chemical shifts of compounds **2**,**3** and **5** and ORTEP drawings of structures of **2** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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