

A post-elongation strategy for the introduction of guanidinium units in the main chain of helical oligoureia foldamers

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ABSTRACT

The synthesis of hybrid urea-based foldamers containing isosteric guanidinium linkages at selected positions in the sequence is described. We used a post-elongation approach whereby the guanidinium moiety is introduced by direct transformation of a parent oligo(urea/thiourea) foldamer precursor. The method involves activation of the thiourea by treatment with methyl iodide and subsequent reaction with amines. To avoid undesired cyclization with the preceding urea moiety resulting in heterocyclic guanidinium formation in the main chain, the urea unit preceding the thiourea unit in the sequence was replaced by an isoatomic and isostructural γ -amino acid. The approach was extended to solid-phase techniques to accelerate the synthesis of longer and more functionalized sequences. Under optimized conditions an 8-mer hybrid oligomer incorporating a central guanidinium linkage was obtained in good overall yield and purity. This work also reports data related to the structural consequences of urea by guanidinium replacements in solution and reveals that helical folding is substantially reduced in oligomers containing a guanidinium group.

INTRODUCTION

Aliphatic N,N'-linked oligoureas¹ are a class of peptidomimetic oligomers which adopt a regular helical conformation stabilized by a network of H-bonds closing 12 and 14-membered pseudorings.² These non-peptide α -helicomimetic foldamers³ possess specific advantages for possible (bio)applications, such as high folding fidelity and stability to enzymatic proteolysis.⁴ Oligoureas do share high sequence modularity and synthetic accessibility with synthetic peptides.⁵ Variation of substitution patterns and the introduction of isosteric backbone modifications are two complementary approaches to generate different spacing and projection of side chains at the surface and fine tune the properties of the helix. We have previously reported heterogeneous urea-based backbones containing various proportion of urea bond surrogates including amide (A), carbamate (C),^{6,7} and thiourea (T)⁸ units and have shown that the folding propensity can be controlled by the ratio between urea (U) and isosteric units and by their relative distribution in the sequence (Figure 1a).

To further modulate the molecular recognition and biological properties of oligourea helices, we have now considered the replacement of the neutral urea linkage by a guanidinium (G) unit at selected positions in the sequence (Figure 1b). The guanidinium moiety is protonated over a wide range of pH and displays many useful features for molecular recognition in competitive solvents, serving as H-bond donor, charge pairing agent and being capable of cation- π -stacking interactions. It is a major functional group in proteins and natural products,⁹ a useful pharmacophore in medicinal chemistry and its physicochemical properties have been largely exploited in supramolecular chemistry for the design of anion receptors and sensors,¹⁰⁻¹³ and in organocatalysis.¹⁴ Guanidinium-rich molecules ranging from peptides to dendrimers and artificial oligomers efficiently enter living cells and have been widely explored as molecular transporters.¹⁵⁻²⁰

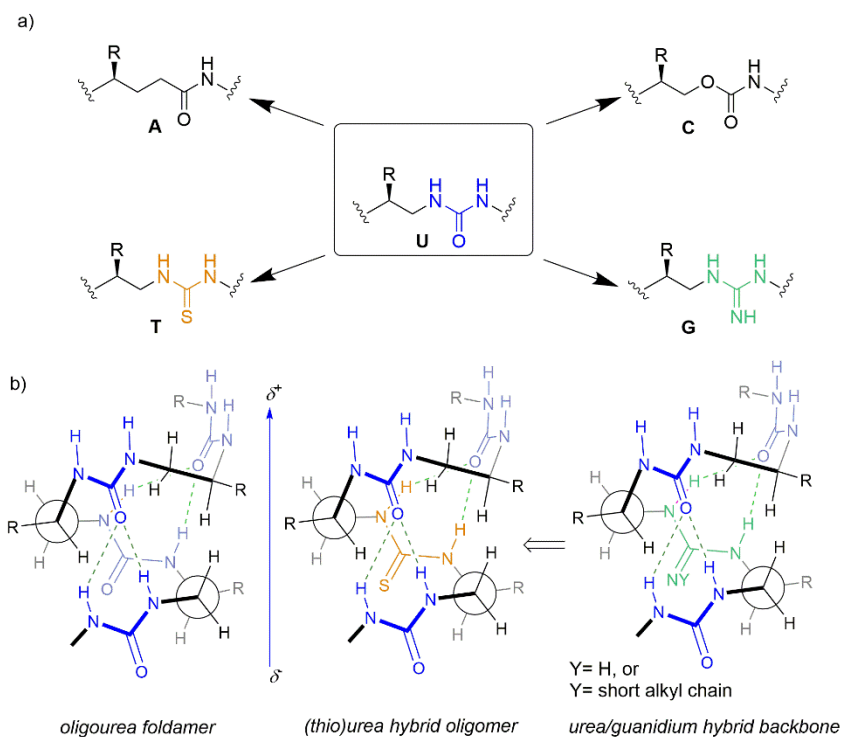


Figure 1. a) Urea-based unit in aliphatic N,N'-linked oligoureas and corresponding **A**, **C**, **G**, and **T** units as urea bond surrogates; b) Schematic representation of the polar helical conformation of oligo(thio)urea foldamers and illustration of the thiourea \rightarrow guanidinium replacement.

Oligomeric guanidines, *i.e.* backbones with N,N'-bridged guanidine units have also attracted interest as ligands of protein surfaces²¹ and as abiotic folded systems.²² For example, aromatic oligomeric N,N'-dimethyl-N,N'-diphenyl guanidines have been reported to fold into well-defined layered structures akin to that of corresponding aromatic oligoureas. Aliphatic oligomers with guanidinium backbones have also been developed as oligonucleotide²³⁻²⁶ and peptide^{27,28} mimics. Several solid-phase synthesis approaches to access aliphatic oligomeric guanidines have been reported and the most effective methods are based on the use of arylsulfonyl (e.g. Pbf: 2,2,4,6,7-Pentamethyldihydrobenzofuran-5-sulfonyl), and alkoxy carbonyl (e.g. Fmoc: 9-fluorenylmethoxycarbonyl, Cbz: carbobenzoxy and Troc: trichloroethoxycarbonyl) activated thiourea monomers.^{27,29-31} In these studies, the guanidylation reaction generally involves the

transformation of the thiourea moiety into a carbodiimide by treatment with EDC, Mukaiyama reagent or HgCl₂ in the presence of TEA and subsequent reaction with the terminal amine of the growing chain.³² This general approach and the use of N-((2-azidoalkyl)-Pbf-thioureas²⁷ as activated monomers are compatible with the solid-phase synthesis of aliphatic N,N'-linked oligourea foldamers on TFA-sensitive resins⁵ and well-suited for the preparation of cognate urea/guanidine hybrid oligomers. Yet, this approach requests the preparation of specific activated-thiourea building blocks and the preparation of highly labile arylsulfonyl isothiocyanate and we were more interested by the possibility to introduce the guanidinium moiety at a later stage in the synthesis by using urea/thiourea hybrid foldamers⁸ as advanced intermediates (Figure 1b). Following exploration of reaction conditions and optimization in solution using model oligomers, we have extended the approach to solid-phase synthesis and applied it to a longer oligomer carrying multiple functionalized side chains. In this work, we also report ¹H-NMR and CD (circular dichroism) data about the conformational preferences of the resulting urea/guanidinium (U/G) hybrids in direct comparison with the cognate urea/thiourea (U/T) and urea oligomers.

RESULTS AND DISCUSSION

Guanidinylation reaction of urea/thiourea hybrid oligomers in solution. Short-chain *N*-tert-butyloxycarbonyl protected dimer **1a** and tetramer **2a** were initially selected as model urea/thiourea hybrid oligomers (TU and UUTU sequences, respectively) to test the best conditions to transform the thiourea linkage into the corresponding guanidinium. The oligomers were synthesized in solution using activated building blocks **BB1** and **BB2** as described previously (Figure 2 and see general procedure for details).^{8,33}

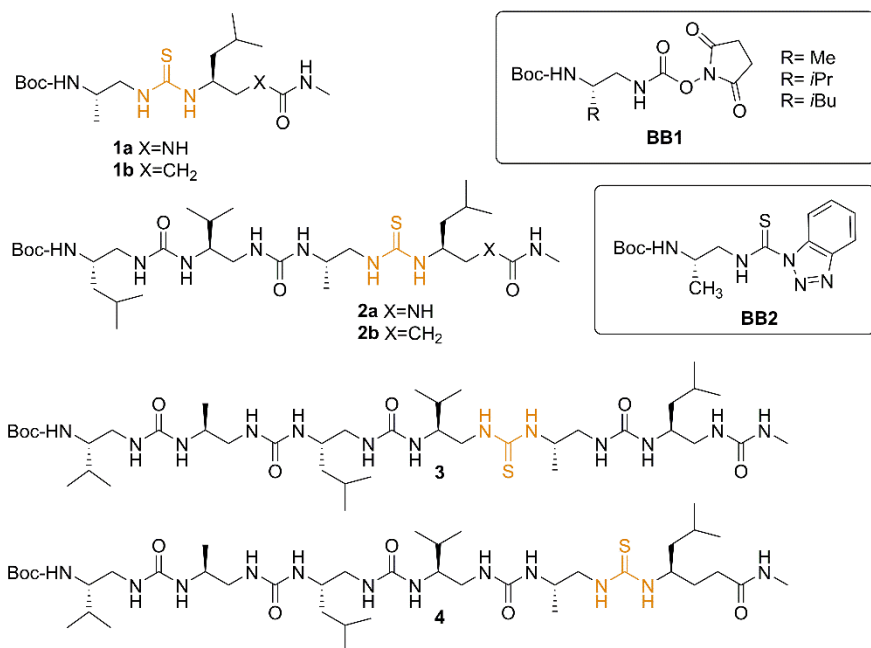
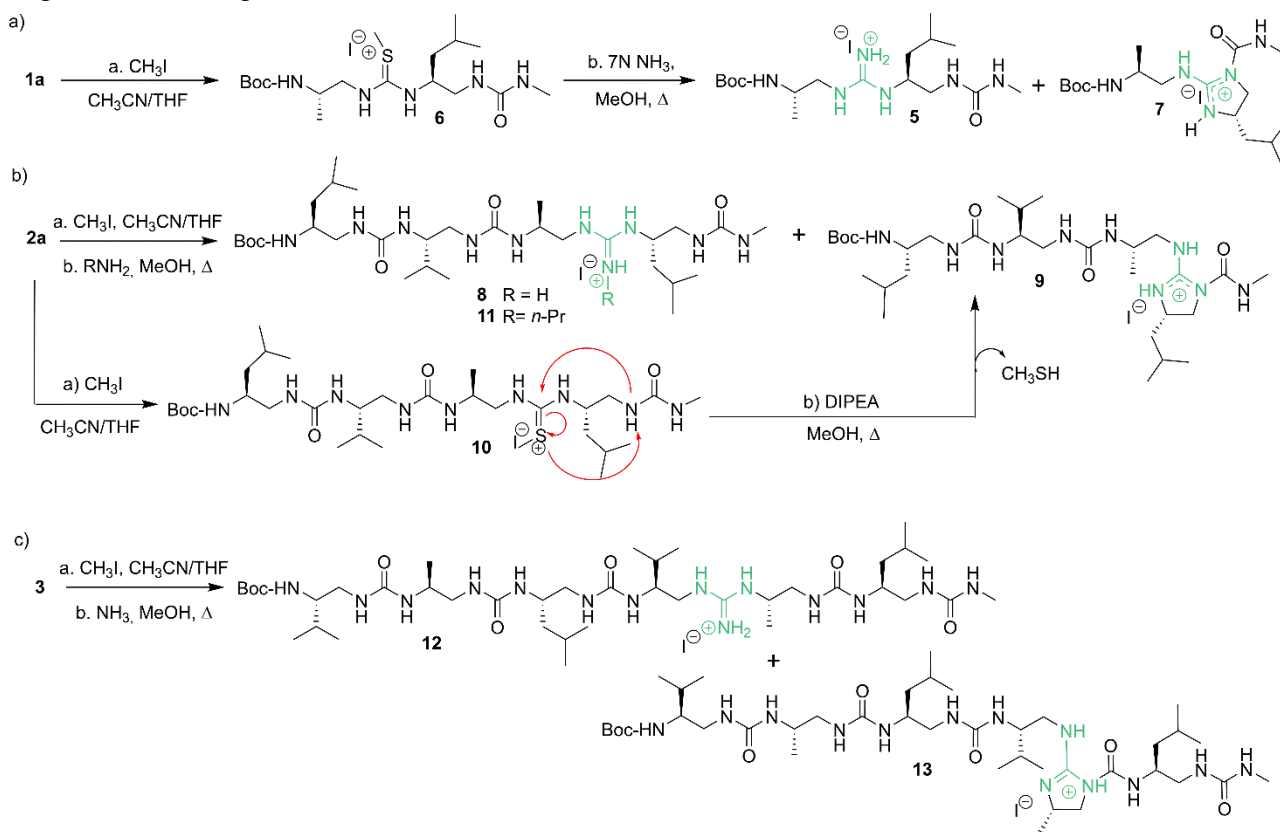


Figure 2. Sequences of target (thio)urea-based hybrid oligomers **1-4** for investigating guanidinylation reaction in solution. Formulae of activated building blocks **BB1** and **BB2** used to prepare oligomers **1**, **2** and **4**. Oligomer **3** was described previously.⁸

We performed the guanidinylation reaction by a two-step strategy (Rathke synthesis³⁴) involving the *S*-methylation of the thiourea linkage by reaction with iodomethane (CH₃I) to form the *S*-methylthiuronium iodide salt followed by a direct nucleophilic substitution in presence of the desired amine (Scheme 1).³⁵ Initial efforts were dedicated to the formation of *N,N'*-disubstituted guanidinium derivatives **5** (Scheme 1a). Dimer **1a** was thus first *S*-methylated with CH₃I to form intermediate **6** and subsequent substitution reaction was carried out in 7.0 N ammonia in CH₃OH as reaction medium with heating at 40-45 °C during the whole reaction process. The progress of the reaction was monitored by reversed phase HPLC (RP-HPLC) and showed complete disappearance of dimer **1a**. However, RP-HPLC analysis of the crude product after reaction workup revealed the presence of two main products in a ratio of 33:67 that were subsequently isolated by silica gel chromatography. After careful structural characterization, it appeared that under such conditions the major product was the cyclic *N,N',N''*-guanidine derivative **7** (Scheme 1a, Table 1 and see supporting information for detailed characterization). A similar ratio of both the expected guanidine

derivative **8** and the corresponding cyclic guanidine **9** as the main product was obtained when the reaction conditions were applied to tetramer **2a** (Scheme 1b, Table 1 and see supporting information). At this stage, we assumed that the heterocyclic guanidine products formed because the *N'* nitrogen of the neighbouring urea moiety was sufficiently nucleophilic in basic conditions to quickly react with the newly formed *S*-methylthiuronium intermediate. This hypothesis was further confirmed by reacting *S*-methylthiuronium **10** with DIPEA as base in pure MeOH (Scheme 1b). After 24 h, there was no more trace of **10** in the reaction mixture and cyclic guanidine **9** was observed as the main product (see supporting information for details).

Scheme 1. Synthesis of *N,N'*-disubstituted and *N,N',N''*-trisubstituted guanidinium oligomers from oligo(thio)urea oligomers **1a**, **2a** and **3**.



A similar outcome was observed when attempting to prepare the corresponding *N''*-substituted guanidine derivatives with *n*-propylamine as the nucleophile. The cyclic guanidine **9** was obtained

as the major product when starting from tetramer **2a** in MeOH (Table 1, entry 3). The influence of the nature of the solvent on this guanidinylation reaction was next evaluated and we found that the ratio between cyclic and linear products could be reversed in pure acetonitrile. Under these conditions, the desired linear *N,N',N''*-trisubstituted guanidinium compound **11** was formed as the main product (Scheme 1b, Table 1, entry 4). However, the reaction in acetonitrile was much slower than in methanol and required larger excess of the primary amine to convert the starting thiourea into the *N,N',N''*-trisubstituted guanidinium derivative. This guanidinylation reaction in acetonitrile was repeated with a 0.5M ammonia solution in 1,4-dioxane. The expected linear *N,N'*-disubstituted guanidinium compound **8** was obtained as the main compound (Table 1, entry 5), which confirms the strong influence of the solvent on the outcome of the reaction. The linear to cyclic product ratio was further improved by using THF as a co-solvent (Table 1, entry 6). These conditions were finally applied to known hexamer **3**⁸ (scheme 1c) and the corresponding linear guanidinium derivative **12** was obtained together with the cyclized product **13** with a 75:25 ratio (Table 1, entry 7).

Table 1. Outcome of the guanidinylation reaction on oligo(thio)ureas **1a**, **2a** and **3**

entry	substrate	nucleophile	equiv. of amine	(co)solvent ^a	time (h)	ratio linear:cyclic product ^b
1	1a	7N NH ₃	~ 150	CH ₃ OH	48	33:67 (5:7)
2	2a	7N NH ₃	~ 300	CH ₃ OH	24	26:74 (8:9)
3	2a	<i>n</i> -PrNH ₂	20	CH ₃ OH	24	15:85 (11:9)
4	2a	<i>n</i> -PrNH ₂	45 ^c	CH ₃ CN	120	73:27 (11:9)
5	2a	0.5M NH ₃ in 1,4-dioxane	45 ^c	CH ₃ CN	120	85:15 (8:9)
6	2a	0.5M NH ₃ in 1,4-dioxane	45 ^c	THF	38	97:3 (8:9)
7	3	0.5M NH ₃ in 1,4-dioxane	60 ^c	THF	150	75:25 (12:13)

^aThe reaction was carried out at 40-45°C; ^bDetermined by RP-HPLC analysis; ^cThe amine was added in several portions depending on the progress of the reaction.

Guanidinylation of γ -amino acid-containing oligo(thio)urea substrates. In order to avoid possible intramolecular cyclization leading to the incorporation of a heterocyclic guanidine in the foldamer backbone, (Scheme 1), we thought to substitute the urea residue (U unit) preceding the thiourea moiety by the corresponding γ^4 -amino acid derivative (A unit). Indeed, U and A units are isostructural and it has been shown previously that oligo(urea/ γ -amide) hybrids display similar helical folding propensities compared to homooligoureas.⁶ The corresponding dimer **1b** and tetramer **2b** equipped with thiourea and amide linkages (TA and UUTA sequences, respectively) were synthesized in solution according to previously reported procedures.^{6,8}

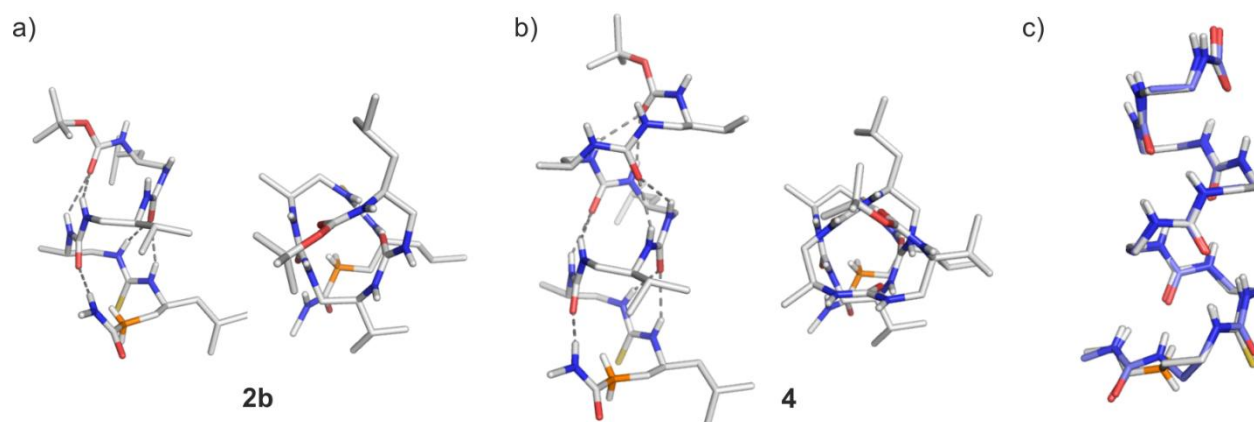


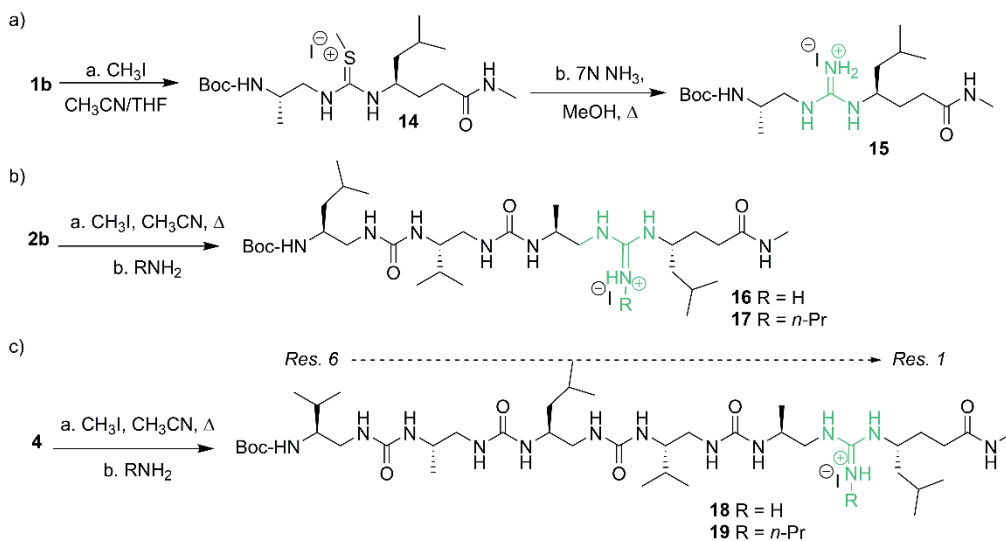
Figure 3. Crystal structures of (a) γ -residue-containing oligo(thio)urea **2b**, (b) hexamer **4**, and (c) structural alignment of the crystal structures of the hexamer **4** (light grey) with a cognate urea 6-mer (**U₆**)⁸ (slate blue), the side-chains have been omitted for clarity reasons, calculated RMSD = 0.150 Å. Only one of the two independent molecules present in the asymmetric unit of the crystal of **4** is represented.

X-ray diffraction analyses of single crystals of 4-mer hybrid **2b** provided detailed information on the structural consequences of the local U \rightarrow A replacement in **2**. As shown in Figure 3a, the structure of 4-mer **2b** is helical and compares well with the canonical 2.5-helical structure reported for the cognate U₄ homooligourea foldamer.⁷ Root-mean square deviations (RMSDs) calculated for the backbone asymmetric C atoms (CH(R)) of the two molecules highlights the correlation between

the two structures (RMSD for residues 1–4 = 0.129 Å). It is noteworthy that the intramolecular H-bond network is maintained with the thiocarbonyl group pointing towards the negative pole of the helix macrodipole similar to what was observed in longer helical oligo(thio)urea hybrids. These results provide further evidence that the combination of a γ -amino acid residue and a thiourea unit (TA sequence) at the first two positions of the sequence of oligoureases is tolerated and has limited influence on the overall helix geometry in the crystal.

The chemical transformation of the thiourea moiety into a guanidinium was next evaluated on dimer **1b** and tetramer **2b** (Scheme 2). Direct conversion of the S-methylthiuronium intermediate **14** derived from **1b** in 7N ammonia in CH₃OH gave the expected guanidinium-containing compound **15**. However, RP-HPLC analysis of the crude product indicated that the guanidinylation product **15** was contaminated by several by-products and after flash silica gel chromatography dimer **15** was recovered in only 20% yield (Table 2, entry 1).

Scheme 2. Two-step guanidinylation reaction of γ -residue-containing substrates **1-2b** and **4**.



Much better results were obtained when the guanidinylation reaction was conducted in a non protic solvent like CH₃CN. The reaction of S-methyluronium derivative of **2b** with either 0.5M ammonia

in 1,4-dioxane or *n*-propylamine as nucleophile in CH₃CN gave the expected guanidinium tetramers **16** and **17** in 55% or 46% yield respectively after purification by silica gel chromatography (Table 2, entries 3 and 4).

Table 2. Outcome of the guanidinylation reaction of model γ -amino acid-containing oligo(thio)urea substrates **1b**, **2b** and **4**.

entry	substrate	nucleophile	equiv. of amine	solvent ^a	time (h)	cmpd	yield (%)
1	1b	7N NH ₃	~ 150	MeOH	48	15	20
2	2b	0.5M NH ₃ /1,4-dioxane	45 ^b	CH ₃ CN	90	16	55
3	2b	0.5M NH ₃ /1,4-dioxane	40 ^b	THF	38	16	74
4	2b	<i>n</i> PrNH ₂	45 ^b	CH ₃ CN	90	17	46
5	4	0.5M NH ₃ /1,4-dioxane	20	CH ₃ CN	24	18	92
6	4	<i>n</i> PrNH ₂	20	CH ₃ CN	60	19	60

^aThe reaction was carried out at 40-45°C; ^bThe amine was added in several portions depending on the progress of the reaction.

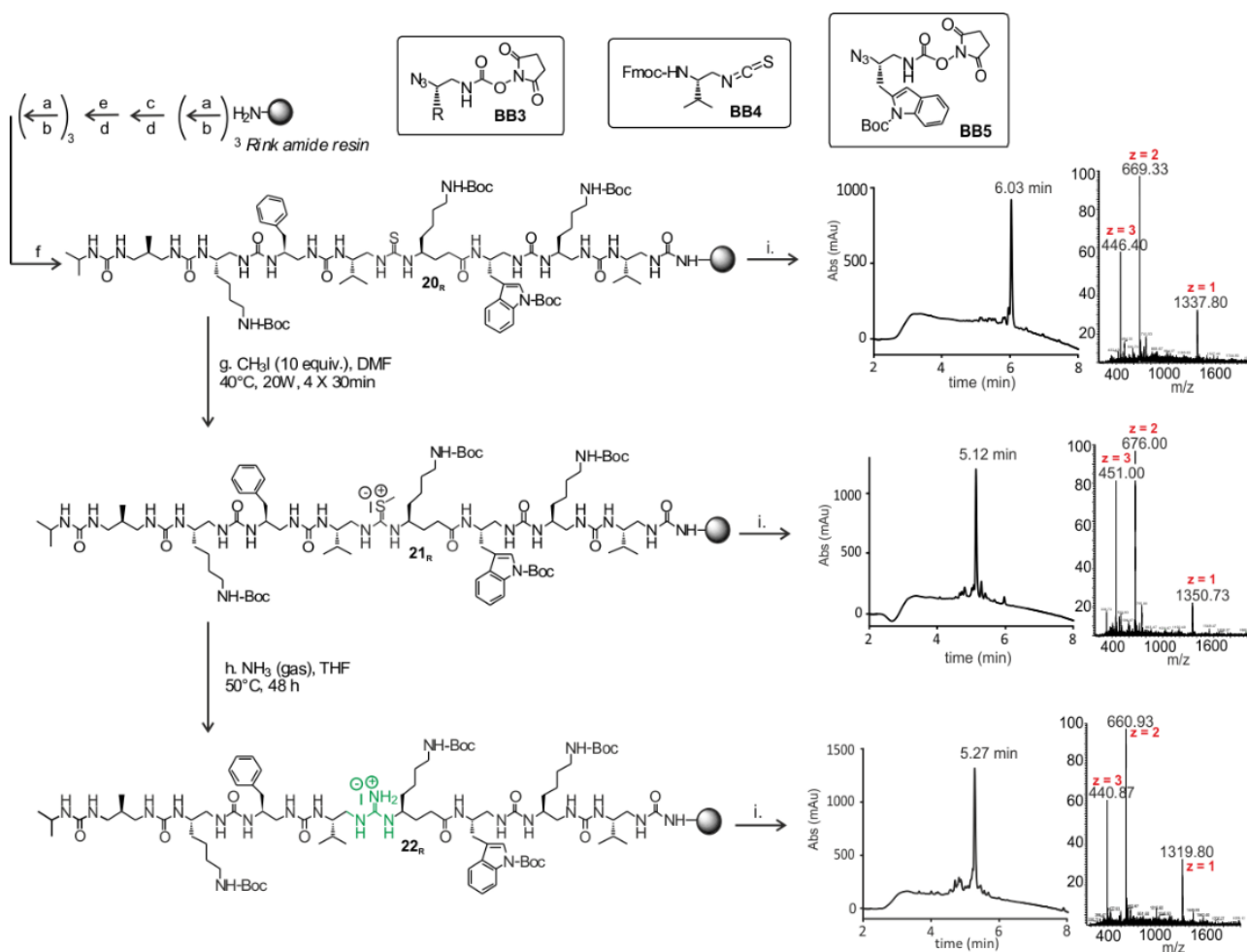
These results motivated us to test these optimized conditions on a longer and potentially more folded oligomer. The selected hexamer **4** equipped with a γ -Leu at first position (sequence UUUUTA, Figure 2) and composed of the two triads with Val, Ala, Leu side-chains was synthesized in solution (see general procedure for details). Single crystals suitable for X-ray diffraction studies were obtained and as shown in Figure 3b (see supporting information for details), oligomer **4** adopts a well-defined helical structure in the crystal. An overlay with the known structure of the corresponding 6-mer homooligourea U₆⁸ by fitting the six pairs of main chain asymmetric C atoms indicates a very close match (RMSD = 0.150 Å) between the two helices (Figure 3c).

Conversion of 6-mer **4** to the corresponding *N,N'*-di- or *N,N',N''*-trisubstituted guanidinium-containing oligomers **18** and **19** by treatment of its *S*-methylthiuronium salt with either 0.5 M ammonia solution in 1,4-dioxane or *n*-propylamine in CH₃CN was straightforward (Scheme 2c and Table 2 entries 4 & 5). The conversion to **19** required 60 hours to reach completion whereas conversion to **18** was nearly complete after only 24h as monitored by RP-HPLC analysis. Both oligo(guanidinium/amide/urea) hybrids **18** and **19** were recovered in 92% and 60% yield respectively after silica gel chromatography purification.

Guanidinylation reaction on solid support. We next applied this post-elongation guanidinylation method to the solid support with the aim to speed the access to more functionalized and biologically relevant sequences. We have recently reported an efficient solid phase methodology to prepare oligo(thio)ureas on TFA-labile resins.³⁶ This optimized SPS protocol was applied here to synthesize a resin-bound 8-mer oligo(thiourea/amide/urea) hybrid on Rink-amide resin (**20_R**) as a direct precursor for the guanidinylation steps (Scheme 3). This hybrid sequence derives from that of an antibacterial amphiphilic cationic oligourea sequence⁴ and contains (1) a thiourea bond between residues 4 and 5 and (2) a γ^4 -Lys residue at position 4 to prevent intramolecular guanidine formation. Activated azide succinimidyl carbamate monomers of type **BB3** with the side-chains of Ala, Val, Lys and Phe were used for urea formation in combination with *N*-Fmoc-aminoalkyl isothiocyanate **BB4** for thiourea insertion and commercially available *N*-Fmoc- γ^4 -Lys(Boc)-OH for amide bond formation. Because of the high reactivity of the indole side-chain towards methyl iodide, we employed a *N*-Boc protected version (**BB5**) of the Trp-derived azide building block during oligo(thio/amide/urea) hybrid SPS (Scheme 3 and see general procedure for details).³⁷ Successful elongation of the resin-bound oligomer **20_R** intermediate was confirmed by performing a

micro-TFA-cleavage of the resin. As depicted in scheme 3, the RP-HPLC profile of the crude material (**20**) showed a good purity, sufficient to continue with thiourea activation.

Scheme 3. Post elongation and on-resin approach to introduce guanidinium linkages in urea-based foldamers. (a-f) Synthesis of the resin bound 8-mer oligo(thiourea/amide/urea) hybrid **20_R**; (g) S-methylation of **20_R** and (h) guanidinylation reaction of **21_R** into **22_R**. The RP-HPLC chromatograms ($\lambda = 200$ nm) and ESI spectra correspond to the crude products isolated after each step (*i.e.* crude **20**, **21** and **22**) following micro-TFA-cleavage of few resin beads.



^aReaction conditions: (a) **BB3** or **BB5**, DIPEA, DMF, 70°C , 25 W (2×15 min); (b) PMe_3 , 1,4-dioxane/ H_2O , 70°C , 25 W (2×30 min); (c) Fmoc- γ -Lys(Boc)-OH, BOP, DIPEA, DMF, 70°C , 25 W (2×15 min); (d) DMF/piperidine; (e) **BB4**, DCM, 48h; (f) *i*PrNCO, DMF, 70°C , 25 W.

The S-methylation of the resin-bound thiourea oligomer **20_R** was performed by treatment with CH_3I in DMF under microwave irradiation at 40°C for 30 mins. The procedure was repeated 4 times to

ensure complete methylation. A micro-TFA-cleavage of the resin was performed to confirm the effective conversion of **20_R** into the resin-bound *S*-methylthiuronium iodide salt (**21_R**). In Scheme 3, the RP-HPLC profile essentially showed the presence of a single peak assigned to **21** by ESI-MS analysis.

The resin-bound methylthiourea **21_R** was initially treated with 0.5 N ammonia in 1,4-dioxane under the conditions optimized in solution to access to **22**. However, it appeared that the ammonia solution was not sufficiently concentrated to ensure conversion without an extended heating. Conversely, complete conversion to the resin-bound guanidinium **22_R** was obtained by treatment of **21_R** with a saturated solution of ammonia gas in THF for 48 hours at 50°C. The purity of the crude product after TFA cleavage was estimated to be $\approx 66\%$ by HPLC analysis. The expected guanidinium oligomer **22** was finally isolated in 17% overall yield (20 steps) after semi-preparative RP-HPLC.

Effects on the conformation of thiourea \rightarrow guanidinium replacements

The consequences on the helical conformation of oligo(thio)urea hybrids of discrete thiourea \rightarrow guanidinium replacements were investigated by NMR and ECD. We concentrated on oligomers **12**, **18**, **19**, and **22** which differ by the relative position of the backbone guanidinium in the sequence. The guanidinium unit is located close to the C-terminus in compounds **18** and **19**, whereas it occupies a more central position in **12** and **22**. NMR spectra of all compounds and their oligo(thio)urea precursors were recorded in the range 1-3 mM in CD₃CN (or CD₃OH) and proton resonances were assigned by using combination of homonuclear COSY, TOCSY, ROESY and heteronuclear ¹H-¹³C HSQC 2D experiments (see supporting information). As shown in Figure 4 for compounds **4**, **18** and **19**, thiourea and guanidinium NHs generally appear as broad signals and at higher frequencies compared to the urea NHs. Of note, the urea NH resonances of the two

guanidinium-containing 6-mers **18** and **19**, are not significantly affected by the T → G replacement and display qualitative features typical of a well-folded conformation : dispersion over a large range of chemical shifts (5.0-6.8 ppm); large vicinal coupling constants with β CH(R) protons (6-10 Hz) and a strong differentiation between vicinal coupling constants with diastereotopic α -methylene (α CH₂) protons of chiral diamine units.

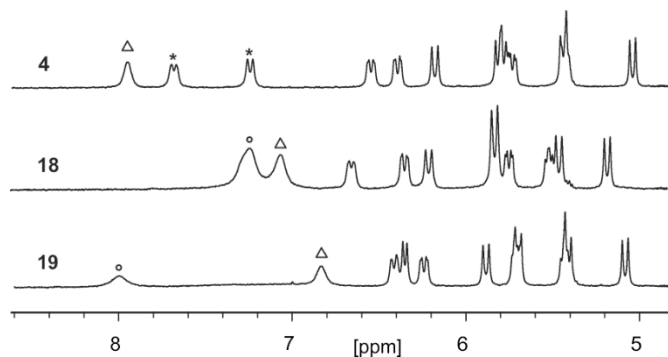


Figure 4. Part of ¹H NMR spectra (300 MHz) of oligomers **4**, **18-19** in CD₃CN (3mM) at 298 K, showing the NH region. The urea NHs signals are spread between 6.8 and 5.0 ppm. Thiourea NHs annotated with a star, guanidinium NHs annotated with a circle, amide NHMe annotated with a triangle.

The chemical shift variation ($\Delta\delta$) of α CH₂ of urea units was measured as a mean to further evaluate the effect of the guanidinium replacement on folding. It is now well-established^{2,33} that when placed in a helical environment, the α CH₂ protons of U units exhibit a high degree of anisochronicity (δ splitting) with $\Delta\delta$ reaching values generally above 1 ppm. The $\Delta\delta$ values for urea residues in **18** and **19** were found to be almost similar to those already reported for the cognate homo-urea 6-mer (U₆)⁸ and **4** suggesting that the helical structure running from residues 3-6 is not particularly affected by the introduction of the G and A units at positions 2 and 1.³⁸ (Figure 5, bottom). Further insight into the relative folding propensity of **4** and **18** at the residue level was gained by comparing hydrogen/deuterium (H/D) exchange data of backbone urea and amide protons (see Figures S96-99 and Tables S10 and S11 in supporting information). The first observation is that urea protons of central residues (NH and N'H of Val^{U3}, Leu^{U4} and N'H of Ala^{U5}) exchange at slow rates in both

molecules ($k_{\text{ex}} \leq 10 \times 10^{-3} \text{ min}^{-1}$) supporting the view that folding from residue 3-6 is not affected by the T \rightarrow G replacement. As expected, terminal urea protons (N'H of Val^{U6} and NH of Ala^{U5}) and Boc-NH proton which are exposed to the solvent exchange at much higher rate in both molecules. In contrast urea NH of the second residue was found to exchange at faster rate in **18** compared to **4** ($72 \times 10^{-3} \text{ min}^{-1}$ vs $11 \times 10^{-3} \text{ min}^{-1}$). The same holds true for the terminal amide proton which exchanges at a rate of $275 \times 10^{-3} \text{ min}^{-1}$ in **4**, but too fast for the rate to be determined in **18**. This is further supported by the observation that the amide NHMe proton resonance which appears ≈ 8 ppm in **4** is upfield shifted upon guanidinium replacement. These results confirm that folding is largely maintained over the first four urea-based residues but that the folding propensity of residues neighbouring the G units is reduced or eliminated. NMR evaluation of compounds **12** and **22** revealed a similar trend whereby the guanidinium moiety affects the helical character of the preceding residues in the sequence more significantly than those that follow. For example, a fall of anisochronicity at Leu^{U1} is observed in **12** compared to **3**, whereas the anisochronicity at Leu^{U4}, Ala^{U5} and Val^{U6} remains largely unchanged after the T \rightarrow G replacement (Figure 5, top). anisochronicities at U units in **22** were quite revealing, showing that residues in $i \pm 2$ relationship with the G unit (namely Trp^{U3} and Lys^{U7}) are comparatively more affected than other residues in the sequence (see supporting information). This drop of anisochronicity can be explained by a significantly reduced folding propensity of the guanidinium moiety and concomitant loss of canonical intramolecular H-bonds. In contrast, the marginally weaker anisochronicity values at residues 2, 6 and 8 suggests that the overall geometry of these residues is maintained on this face of the helix.

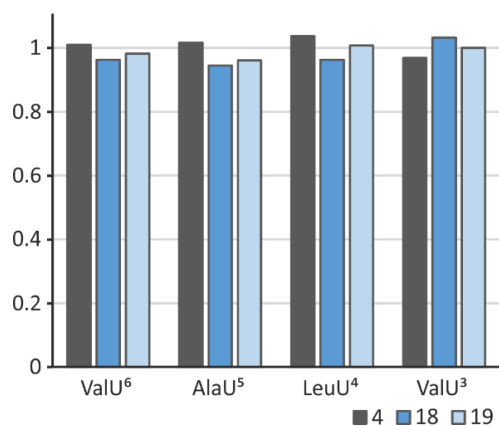
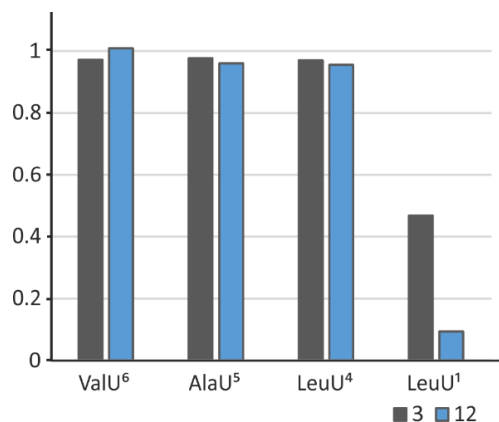


Figure 5. Anisochronicity at urea residues in oligo(thio/amide)urea **3** and **4** as well as related guanidinium hybrids **12**, **18**, and **19**. Ratios to the values measured for the cognate oligourea U_6^8 are reported.

Although ECD has been used extensively to characterize helix formation among homoligoureas,³³ its potential utility here, is complicated by the presence of multiple chromophores (ureas, thioureas and amides).^{6,8} Nevertheless, ECD analysis can provide qualitative information about the ability of modified oligomers to retain the typical signature (maximum of positive molar ellipticity $[\theta] \approx 203$ nm and a trough of weaker intensity at ≈ 188 nm) attributable to 2.5-helix formation. The ECD spectra of compounds **4**, **18** and **19** were measured at a concentration of 0.2 mM in 2,2,2-trifluoroethanol (TFE) and compared to that of the corresponding homooligourea U_6^8 (Figure 6). The CD signatures of **18** and **19** which contain a thiourea \rightarrow guanidinium replacement close to the C-terminus are reminiscent of that of the parent oligourea U_6 and have the hallmarks of a folded

structure in agreement with NMR analysis. A similar trend is observed when analysing the ECD spectrum of **22** (see Figure S95 in supporting information)

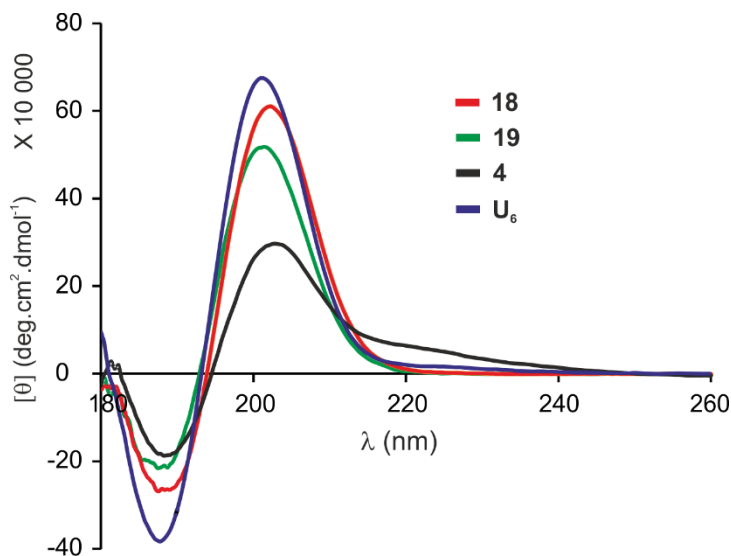


Figure 6. ECD spectra of oligomers **4**, **18** and **19** compared to that of the cognate homo-oligourea U_6^8 (all spectra were recorded in TFE at 0.2 mM).

CONCLUSIONS

The present work further expands the range of urea surrogates that can be introduced in the backbone of aliphatic oligourea foldamers to modulate their properties. While some of our earlier work focused on amide (A), carbamate (C) and thiourea (T) isosteric units, we have now exploited the reactivity of thioureas to introduce guanidinium linkages (G) at a late stage in the synthesis and possibly convert oligo(thio)ureas into the corresponding guanidinium/urea oligomers in a two-step procedure. One limitation of this approach was the competition with an intramolecular backbone cyclization that resulted in the formation of oligomers with heterocyclic guanidinium. Although this side reaction could be minimized by using moderately polar and non protic solvents, we found that the insertion of an isostructural γ -amino acid residue before the thiourea linkage was a simple alternative to cleanly install the guanidinium group in the sequence. By capitalizing on an efficient solid-phase synthesis of oligo(thio)ureas, we have adapted the guanidinylation procedure to solid

support enabling fast elaboration of urea-based oligomers containing backbone guanidiniums. Conformational studies in solution by NMR and ECD associated with XRD analyses confirm that the combination of a thiourea unit and a γ -amino acid residue and (T-A pair) in oligomeric precursors is compatible with helical folding with little deviation from the canonical 2.5-helical structure of oligoureas. Although spectroscopic characteristics of partially folded structures remain in oligoureas containing a guanidinium moiety, folding is significantly disrupted by the guanidinium replacement. A future goal of our research is to characterize the structures of oligo(urea/guanidinium) foldamers at atomic resolution using X-ray diffraction. It could also be of interest to evaluate how the nature of the counterion of the guanidinium linkage may affect the propensity for the oligomer to fold or unfold.³⁹⁻⁴⁴

Another development of this work facilitated by the compatibility with SPS techniques will be to evaluate the impact of such backbone modifications on the biological activities of foldamers in terms of recognition of biological surfaces and also cell penetration. Encouraging results have recently been obtained with derivatives of membrane-active antibacterial oligoureas containing various surrogate units including guanidinium.³⁷

EXPERIMENTAL SECTION

General procedures. All solvents and reagents were obtained from commercial sources. Rink amide MBHA resin (loading 0.52 mmol/g) was purchased from Merck-Millipore. Fmoc- γ -Lys(Boc)-OH and benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexa-fluorophosphate (BOP) reagent were purchased from PolyPeptide Laboratories France. Solid phase peptide synthesis grade organic solvents (DMF, DCM) were used for solid phase synthesis and were purchased from Carlo Erba. RP-HPLC-quality acetonitrile (CH₃CN) and MilliQ water were used for RP-HPLC

analyses and purification. Anhydrous solvents were dispensed from a solvent purification system (DCM, THF). Thin layer chromatography (TLC) was performed on silica gel 60 F254 (Merck) with detection by UV light and charring with 1% ninhydrin in ethanol followed by heating. Flash column chromatography was carried out on silica gel (40-63 μm , Merck). Chemical shifts are reported in parts per million (ppm, δ) relative to the ^1H or ^{13}C residual signal of the deuterated solvent used. ^1H NMR splitting patterns with observed first-order coupling are designated as singlet (s), broad singlet (bs), doublet (d), triplet (t), or quartet (q). Coupling constants (J) are reported in hertz.

Analytical RP-HPLC analyses were performed on a Dionex U3000SD using a Macherey-Nagel Nucleodur column (4.6 \times 100 mm, 3 μm) at a flow rate of 1 $\text{mL}\cdot\text{min}^{-1}$. The mobile phase was composed of 0.1% (v/v) TFA- H_2O (Solvent A) and 0.1% TFA- CH_3CN (Solvent B). The detection was performed at 200 nm and the column temperature in the oven was 25°C. Semi-preparative purifications of oligoureas were performed on a Dionex U3000SD system using a Macherey-Nagel Nucleodur column (100-6 C18ec, 10 \times 250 mm, 5 μm) at a flow rate of 4 $\text{mL}\cdot\text{min}^{-1}$. The mobile phase was similar as for the analytic system, unless otherwise notified.

Activated (S)-succinimidyl-2-[[[(tert-butoxy)carbonyl]amino]-2-X-ethyl]carbamate monomers **BB1** and **BB3** for the introduction of urea bonds and activated (S)-tert-butyl (1-(1H-benzo[d][1,2,3]triazole-1-carbothioamido)propan-2-yl)carbamate **BB2** used for the introduction of thiourea unit were prepared from corresponding *N*-Boc protected ethylene diamine derivatives using recently reported procedures.^{5,8,33} Compound **3** was synthesized as described previously.⁸ Solid-phase oligourea synthesis was performed manually under microwave irradiation (*vide infra*) on the Discover® System from CEM (CEM MWaves S.A.S., Orsay, France) using open reaction vessels and internal fiber optic temperature control.

Urea Formation in solution: General Procedure I. Boc-protected oligourea (1.0 equiv.) was dissolved in TFA (3 mL/g) cooled down in an ice/water bath and the reaction mixture was stirred at room temperature for 50 min under N₂ atmosphere. The reaction mixture was concentrated under reduced pressure, and the resulting residue was co-evaporated three times with cyclohexane. The crude product was then dissolved in CH₃CN (5 mL/1.5 mmol). DIPEA (3.0 equiv.) was added, and the mixture was cooled down to 0 °C prior to the dropwise addition of the following activated carbamate **BB1** (1.1 equiv.), dissolved in CH₃CN (5 mL). The pH of reaction must be basic, so if required additional portion of DIPEA was added. After completion of the reaction, the reaction mixture was evaporated under reduced pressure, dissolved in EtOAc, and washed with 1 M KHSO₄ solution, saturated solution of NaHCO₃ and brine. The organic layer was then dried over Na₂SO₄ and evaporated under reduced pressure. Flash column chromatography (CH₂Cl₂-MeOH (v/v), 98:2 to 95:5) over silica gel or trituration with Et₂O gave the expected compound.

Thiourea Formation: General Procedure II. Boc-protected oligourea (1 equiv.) was treated with TFA (3 mL/g) cooled down in an ice/water bath and the reaction mixture was stirred at room temperature for 50 min under N₂ atmosphere. The reaction mixture was then concentrated under reduced pressure, and the resulting residue was co-evaporated three times with cyclohexane. The crude product was dissolved in CH₃CN (5 mL/1.5 mmol). DIPEA (3.0 equiv.) was then added, and the mixture was cooled down to 0 °C prior to the dropwise addition of the thiocarbamoylbenzotriazole derivative **BB2** (1equiv.) dissolved in CH₃CN-CH₂Cl₂ (85:15, 7mL). The pH of reaction should be around 8, so if required additional amount of DIPEA was added. The reaction mixture was left to come back to RT and stirred overnight. CH₃CN was then evaporated under reduced pressure and the crude mixture was dissolved in EtOAc, washed twice with 1 M KHSO₄ solution, saturated solution of NaHCO₃, water and brine, dried over MgSO₄ and evaporated

under reduced pressure. Flash column chromatography (CH₂Cl₂-MeOH (v/v), 98:02 to 95:05) over silica gel gave the desired compound.

Heterodimer Boc-Ala^T-Leu^U-NHMe (1a). **1a** was prepared from Boc-Leu^U-NHMe (0.410 g, 1.5 mmol) and **BB2** (0.48 g, 1.5 mmol) as described in the general procedure **II** (0.52 g, 89%). ¹H NMR (DMSO-d₆, 300 MHz) δ 7.40 (bs, 2H), 6.80 (bs, 1H), 5.84 (bd, 2H), 4.33 (bs, 1H), 3.62 (bs, 1H), 3.50-3.22 (m, 3H), 3.17-2.98 (m, 2H), 2.56 (d, *J* = 4.6 Hz, 2H), 1.61 (m, *J* = 6.0 Hz, 1H), 1.41 (s+m, 10H), 1.29-1.19 (m, 1.5H), 1.02 (d, *J* = 6.6 Hz, 3H), 0.89 (d, *J* = 6.6 Hz, 3H), 0.86 (d, *J* = 6.6 Hz, 3H); ¹³C NMR (CD₃OD, 125 MHz) δ 182.9, 162.0, 158.3, 80.2, 54.1, 50.1, 47.6, 46.1, 42.6, 28.8, 27.1, 26.1, 23.7, 22.6, 18.7. HRMS (ESI-TOF) *m/z*: [M + Na]⁺ Calcd for C₁₇H₃₅N₅O₃NaS 412.2358; Found 412.2350. C₁₈ RP-HPLC (10–100% B in 10 min): *t_R* = 7.03 min.

Boc-Val^U-Ala^T-Leu^U-NHMe (24): **24** was prepared from **1a** following general procedure I White solid (0.49g, 71%), ¹H NMR (CD₃CN, 300 MHz) δ 6.96 (bs, 1H), 6.79 (bs, 1H), 5.93 (bs, 1H), 5.59 (bs, 1H), 5.39 (d, *J* = 10.5, 1H), 5.27 (t, *J* = 6.4, 1H), 4.98 (bs, 1H), 4.64 (bs, 1H), 4.15 (bs, 2H), 3.72 (bs, 1H), 3.55-3.45(m, 2H), 2.65 (d+bs, *J* = 4.7, 5H), 1.73-1.57 (m, 2H), 1.34-1.27 (m, 2H), 1.07 (d, *J* = 6.8, 3H), 0.97-0.87 (m, 12H); *t_R* = 7.60 min. (10–100% B in 10 min); ESI-MS (+): 517.3 [M+H]⁺, 1035.7 [2M+H]⁺.

Boc-Leu^U-Val^U-Ala^T-Leu^U-NHMe (2a). **2a** was prepared from **24** as described in the general procedure **I** and was isolated as a white solid (0.46g, 74%). ¹H NMR (DMSO-d₆, 300 MHz) δ 7.36 (bs, 2H), 6.67 (d, *J* = 6.8, 1H), 6.17-5.74 (m, 6H), 4.42 (bs, 1H), 3.89 (bs, 2H), 3.56-3.41 (m, 2H), 3.35-3.22 (m, 3H), 3.12-3.04 (m, 2H), 2.84 (bs, 2H), 2.56 (d, *J* = 4.7 Hz, 3H), 1.69-1.54 (m, 3H), 1.41 (s, 9H), 1.30-1.10 (m, 4H), 1.00 (d, *J* = 6.5 Hz, 3H), 0.9-0.81 (m, 18H); ¹³C NMR (CD₃OD, 125 MHz) δ 185.7, 162.2, 161.3, 161.2, 159.2, 80.2, 56.5, 53.6, 51.7, 50.1, 49.3, 46.6, 46.1, 45.8, 44.8, 43.4, 42.4, 31.9, 28.9, 26.9, 26.3, 26.1, 23.7, 23.5, 23.0, 22.7, 20.1, 19.0, 18.7. HRMS (ESI-

TOF) m/z: $[M + Na]^+$ Calcd for $C_{30}H_{61}N_9O_5NaS$ 682.4414; Found 682.4431. C_{18} RP-HPLC (10–100% B in 10 min): $t_R = 9.29$ min.

Boc- γ -Leu-NHCH₃ (25): Boc- γ -Leu-OH (1.4 g, 5.4 mmol) was suspended in CH₃CN (50 mL) and cooled in ice/water bath. After addition of DIPEA (3.76 mL, 21.6 mmol) all the suspension was dissolved. HBTU (2.46 g, 6.48 mmol) was added and the reaction mixture was stirred for 10 min. at 0°C. Methylamine hydrochloride (0.73 g, 10.8 mmol) was added in one portion. The reaction mixture became green and after 10 min. yellow. The reaction was left to reach RT and magnetic stirring was maintained overnight. CH₃CN was evaporated and the residue was dissolved in EtOAc (80 mL), washed with 1M KHSO₄ (5 × 35 mL), NaHCO₃ (3 × 35 mL, emulsion), brine (1 × 35 mL), dried over Na₂SO₄. The crude product was directly engaged to the next step without purification. Yellowish solid; (1.44g, 98% (crude, purity >95%)), ¹H NMR (CDCl₃, 300 MHz) δ 6.46 (bs, 1H), 4.30 (d, $J = 9.7$, 1H), 3.71-3.59 (m, 1H), 2.85 (d, $J = 4.8$, 3H), 2.25 (t, $J = 6.9$, 2H), 1.92-1.81 (m, 1H), 1.75-1.54 (m, 1H), 1.48 (s, 9H), 1.38 – 1.21 (m, 2H), 0.93 (d, $J = 6.7$, 3H), 0.92 (d, $J = 6.5$, 3H); $t_R = 6.93$ min. (10–100% B in 10 min); ESI-MS (+): 273.2 $[M+H]^+$, 545.4 $[2M+H]^+$.

Boc-Ala^T- γ Leu-NHMe (1b). **1b** was prepared according to procedure **II** starting from **25** (1.44 g, 5.3 mmol) and activated monomer **BB2** (1.73 g, 5.4 mmol) and was obtained as a white foam (1.5 g, 69%). ¹H NMR (CD₃CN, 300 MHz) δ 6.55+6.27 (s+bs, 2H), 5.58 (bs, 1H), 4.48 (bs, 1H), 3.75 (bs, 2H), 3.46 (bs, 2H), 2.70 (d, $J = 4.6$ Hz, 3H), 2.29-2.16 (m, 2H), 1.77-1.60 (m, 2H), 1.44 (s+m, 10H), 1.35-1.26 (m, 2H), 1.13 (d, $J = 6.7$ Hz, 3H), 0.95-0.92 (m, 6H); ¹³C NMR (CD₃OD, 125 MHz) δ 176.5, 158.4, 80.2, 53.4, 50.0, 47.8, 45.7, 33.7, 33.1, 28.8, 26.4, 26.1, 23.6, 23.0, 18.8. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{18}H_{36}N_4O_3NaS$ 411.2406; Found 411.2396. C_{18} RP-HPLC (10–100% B in 10 min): $t_R = 7.25$ min.

Boc-Val^U-Ala^T- γ Leu-NHMe (26): **26** was prepared from **1b** according to the general procedure I. White solid, (0.83g, 79%, flash chromatography CH₂Cl₂/CH₃OH, 98:2 to 95:5), ¹H NMR (CD₃CN, 300 MHz) δ 7.63 (bs, 1H), 6.93 (bs, 1H), 6.77 (bs, 1H), 5.37 (d, *J* = 9.8, 1H), 5.20 (t, *J* = 6.0, 1H), 4.99 (bs, 1H), 4.53 (bs, 1H), 4.28 (bs, 1H), 3.98 (bs, 1H), 3.58 (bs, 1H), 3.43 (bs, 1H), 2.36-2.23 (m, 2H), 2.67 (d+bs, *J* = 4.7, 4H), 1.71-1.60 (m, 2H), 1.47 (s, 9H), 1.38-1.24 (m, 3H), 1.08 (d, *J* = 6.8, 3H), 0.96-0.87 (m, 12H); *t*_R = 7.72 min. (10–100% B in 10 min); ESI-MS (+): 517.4 [M+H]⁺, 1033.7 [2M+H]⁺.

Boc-Leu^U-Val^U-Ala^T- γ Leu-NHMe (2b). **2b** was prepared from **26** as described in the general procedure I and was isolated as a white solid (1.6g, 88%). ¹H NMR (CD₃CN + 3% CDCl₃, 300 MHz) δ 7.81 (s, 1H), 7.45 (d, *J* = 6.0 Hz, 1H), 7.16 (d, *J* = 8.9 Hz, 1H), 5.51-5.44 (m, 3H), 5.36 (d, *J* = 10.0 Hz, 1H), 4.97 (d, *J* = 10.2 Hz, 1H), 4.58 (bm, 1H), 4.39 (bm, 1H), 4.13-4.03 (bm, 1H), 3.87 – 3.74 (m, 1H), 3.68-3.53 (m, 2H), 3.47 (ddd, *J* = 14.0 Hz, 7.7, 3.6, 1H), 2.68 (d, *J* = 4.7 Hz, 3H), 2.60-2.43 (m, 2H), 2.39-2.24 (m, 3H), 2.1-2.02 (m, 1H), 1.73-1.54 (m, 3H), 1.49 (s, 9H), 1.33-1.18 (m, 6H), 1.03 (d, *J* = 6.8, 3H), 0.97-0.85 (m, 18H); ¹³C NMR (CD₃OD, 125 MHz) δ 184.8, 176.6, 161.3, 161.1, 159.1, 80.2, 56.5, 52.5, 51.3, 50.0, 46.7, 46.4, 46.3, 44.8, 42.4, 33.6, 31.8, 31.8, 28.9, 26.5, 26.3, 26.2, 23.6, 23.6, 23.2, 22.7, 20.3, 19.3, 18.4. mp 206–207 °C. HRMS (ESI-TOF) *m/z*: [M + Na]⁺ Calcd for C₃₁H₆₂N₈O₅NaS 681.4462; Found 681.4470. C₁₈ RP-HPLC (10–100% B in 10 min): *t*_R = 9.23 min.

Boc-Ala^U-Leu^U-Val^U-Ala^T- γ Leu-NHMe (27): **27** was prepared from **2b** according to the general procedure I. White solid (0.71g, 79%), ¹H NMR (CD₃CN +3% CDCl₃, 300 MHz) δ 7.93 (bs, 1H), 7.61 (d, *J* = 6.8, 1H), 7.21 (d, *J* = 8.9, 1H), 6.37 (d, *J* = 8.8, 1H), 5.89-5.81 (m, 2H), 5.64 (d, *J* = 10.3, 1H), 5.51-5.48 (m, 2H), 4.96 (d, *J* = 10.0, 1H), 4.58 (bm, 1H), 4.44 – 4.34 (m, 1H), 4.15-4.05 (m, 1H), 3.98-3.85 (m, 1H), 3.80-3.45 (m, 6H), 2.68 (d, *J* = 4.6, 3H), 2.58 – 2.49 (m, 2H), 2.43-2.24

(m, 4H), 2.11-2.02 (m, 1H), 1.73-1.54 (m, 3H), 1.49 (s, 9H), 1.38-1.20 (m, 6H), 1.09 (d, $J = 6.9$, 3H), 1.03 (d, $J = 6.9$, 3H), 0.98-0.91 (m, 18H); $t_R = 9.98$ min. (10–100% B in 10 min then 100% B for 3 min); ESI-MS (+): 759.5 $[M+H]^+$, 1518.1 $[2M+H]^+$.

Boc-Val^U-Ala^U-Leu^U-Val^U-Ala^T- γ Leu-NHMe (4): **4** was prepared as described in the general procedure **I** and was isolated as a white solid (1.1g, 72% yield). ¹H NMR (CD₃CN, 300 MHz, see also Table S1) δ 7.94 (s, 1H), 7.68 (d, $J = 7.5$ Hz, 1H), 7.24 (d, $J = 9.3$ Hz, 1H), 6.55 (dd, $J = 9.7$ Hz, 2.6, 1H), 6.39 (dd, $J = 9.9$ Hz, 2.6 Hz, 1H), 6.18 (d, $J = 10.7$ Hz, 1H), 5.81 (d, $J = 10.2$ Hz, 1H), 5.78 (d, $J = 9.7$ Hz, 1H), 5.73 (dd, $J = 9.9$ Hz, 3.3 Hz, 1H), 5.46-5.41 (m, 2H), 5.04 (d, $J = 10.0$ Hz, 1H), 4.65-4.53 (m, 1H), 4.49-4.32 (m, 1H), 4.17-4.02 (m, 1H), 3.98-3.79 (m, 2H), 3.72-3.46 (m, 6H), 2.68 (d, $J = 4.7$ Hz, 3H), 2.61-2.49 (m, 2H), 2.43-2.24 (m, 5H), 2.1-2.02 (m, 1H), 1.77-1.55 (m, 4H), 1.50 (s, 9H), 1.38-1.28 (m, 3H), 1.24-1.17 (m, 2H), 1.08-0.81 (m, 30H); ¹³C NMR (CD₃OD, 75 MHz) δ 175.1, 160.5, 160.5, 160.3, 160.2, 160.2, 159.5, 158.3, 79.0, 56.4, 54.6, 51.2, 50.1, 46.1, 46.0, 45.5, 44.9, 43.3, 42.60, 41.5, 32.2, 30.7, 30.5, 27.5, 25.1, 25.0, 24.8, 22.3, 22.2, 21.9, 21.4, 18.9, 18.8, 17.9, 17.7, 16.9, 16.7. mp 208–209 °C. HRMS (ESI-TOF) m/z : $[M + Na]^+$ Calcd for C₄₁H₈₂N₁₂O₇NaS 909.6048; Found 909.6066. C₁₈ RP-HPLC (10–100% B in 10 min then 100% of B for 3 min): $t_R = 11.56$ min.

S-methylation reaction: General procedure III. To a solution of oligo(thio)urea (0.35 mmol in 20 mL) in a mixture of CH₃CN/THF (1:1, v/v) was added CH₃I (10 equiv.). The flask was tightly closed with a septum and the mixture was heated to 40-45°C. The monitoring of reaction was performed by RP-HPLC analysis. The reaction was completed after 2-5 hours. The solvent was evaporated and the crude material was directly engaged to the following step without any further purification.

Guanidinylation reaction with 7N NH₃ in CH₃OH: General procedure IV. The thiouronium iodide oligomer (0.14 mmol in 2 mL) was dissolved in a 7N ammonia solution in methanol, the flask was tightly closed with a septum and the reaction mixture was stirred at 40-45°C. The monitoring of the reaction was performed by RP-HPLC analysis. After 24-48 hours the reaction media was concentrated by evaporation under reduced pressure and the crude mixture was directly separated by silica gel column chromatography or semi-prep HPLC. In all cases, cyclic and/or linear substituted guanidiniums were formed.

Compounds 5 and 7. 5 and 7 were prepared from thiouronium iodide salt 6 according to the general procedure III and were isolated in a ratio of 33/67 after silica gel flash column chromatography (CH₂Cl₂-CH₃OH (v/v), from 98:2 to 95:5).

Compound 5 was recovered as a yellowish film in 11% yield (4 mg). ¹H NMR (CDCl₃, 300 MHz) δ 7.64 (bs, 1H), 7.42 (bs 2H), 6.13 (bs, 1H), 5.30 (bs, 1H), 4.82 (bs, 1H), 3.67 (bs, 1H), 3.56-3.41 (bm, 2H), 3.32-3.24 (m, 1H), 3.03 (dd, J = 14.0, 6.4 Hz, 1H), 2.78 (d, J = 4.8 Hz, 3H), 1.46 (s, 9H), 1.31 (d, J = 6.7 Hz, 3H), 0.97 (d, J = 6.6 Hz, 3H), 0.89 (d, J = 6.5 Hz, 2H). ¹³C (CDCl₃, 125MHz, cryoprobe) δ 160.5, 156.8, 156.7, 81.1, 51.3, 47.3, 47.0, 46.2, 29.7, 28.4, 28.3, 27.0, 24.9, 23.2, 21.5, 18.4. HRMS (ESI-TOF) m/z: [M]⁺ Calcd for C₁₇H₃₇N₆O₃ 373.2927; Found 373.2930. C₁₈ RP-HPLC (10-100% B in 10 min): t_R = 5.71 min.

Compound 7 was recovered as a yellowish film in 26% yield (12 mg); ¹H NMR (CDCl₃, 300 MHz) δ 9.64 (s, 1H), 9.48 (b sign, 0.5H), 7.47 (bs, 1H), 4.94 (d, J = 4.6 Hz, 1H), 4.68 (t, J = 8.8 Hz, 1H), 4.30-4.20 (m, 1H), 4.14-4.09 (m, 1H), 3.69-3.57 (m, 2H), 3.39 (m, 1H), 2.88 (d, J = 4.5 Hz, 3H), 2.02-1.89 (m, 1H), 1.85-1.79 (m, 1H), 1.58-1.54 (m, 1H), 1.47 (s, 9H), 1.34 (d, J = 6.6 Hz, 3H), 1.00 (t, J = 6.6 Hz, 6H); ¹³C NMR (HSQC, CDCl₃, 75MHz) 52.8, 52.2, 49.0, 46.5, 43.8, 29.5, 28.0,

24.2, 22.5, 22.5, 18.1. HRMS (ESI-TOF) m/z : $[M]^+$ Calcd for $C_{17}H_{34}N_5O_3$ 356.2662; Found 356.2668. C_{18} RP-HPLC (10–100% B in 10 min): $t_R = 6.04$ min.

Compounds 8 and 9. **8** and **9** were prepared from **2a** on a small scale (0.023 mmol) according to the general procedure **III** and **IV** and were formed in a ratio of 26/74. They were isolated after purification by semi-preparative RP-HPLC (25-65% B in 20 min).

Compound 8. 1H NMR (CD_3CN , 300 MHz) δ 7.50 (bs, 1H), 7.36 (bs, 1H), 7.13 (bs, 1H), 6.17 (bs, 1H), 6.06 (bs, 1H), 5.70-5.62 (m, 3H), 5.49 (bs, 1H), 5.42 (d, $J = 9.9$ Hz, 1H), 3.98 (bs, 1H), 3.79-3.68 (m, 1H), 3.64-3.43 (m, 3H), 3.40-3.21 (m, 3H), 3.05 (bs, 1H), 2.84 (bs, 1H), 2.69 (d, $J = 4.5$ Hz, 3H), 2.66-2.56 (m, 1H), 2.50-2.43 (m, 1H), 1.76-1.57 (m, 3H), 1.45 (s, 9H), 1.43-1.36 (m, 2H), 1.27-1.15 (m, 2H), 1.09 (d, $J = 6.7$ Hz, 3H), 0.95-0.89 (m, 18H); ^{13}C NMR (CD_3OD , 125 MHz, cryoprobe) δ 162.0, 161.5, 161.1, 159.2, 158.1, 80.3, 56.8, 56.4, 56.4, 52.4, 50.0, 46.6, 46.1, 45.6, 44.7, 42.7, 42.4, 31.9, 28.9, 26.2, 26.1, 23.5, 23.5, 22.6, 22.2, 20.2, 18.7, 18.6. HRMS (ESI-TOF) m/z : $[M]^+$ Calcd for $C_{30}H_{63}N_{10}O_5$ 643.4983; Found 643.4974. C_{18} RP-HPLC (10–100% B in 10 min): $t_R = 7.45$ min.

Compound 9: 1H NMR ($DMSO-d_6$, 300 MHz) δ 9.63 (s, 1H), 9.40 (t, $J = 6.2$ Hz, 1H), 7.63 (d, $J = 4.2$ Hz, 1H), 6.57 (d, $J = 8.7$ Hz, 1H), 6.29-6.20 (m, 1H), 5.97-5.92 (m, 2H), 5.76 (bs, 2H), 4.10 (bd, $J = 4.3$ Hz, 2H), 3.78-3.43 (m, 5H), 3.25-2.80 (m, 7H), 2.69 (d, $J = 4.1$ Hz, 3H), 1.75-1.44 (m, 6H), 1.38 (s, 9H), 1.29-1.21 (m, 4H), 1.07 (d, $J = 6.7$ Hz, 3H), 0.94-0.80 (m, 18H); ^{13}C NMR (HSQC, CD_3CN , 125 MHz) δ 54.1, 51.4, 48.5, 48.4, 45.1, 44.1, 43.1, 40.6, 29.6, 27.1, 24.8, 24.0, 23.5, 21.7, 21.1, 20.6, 18.3, 17.1. HRMS (ESI-TOF) m/z : $[M]^+$ Calcd for $C_{30}H_{60}N_9O_5$ 626.4717; Found 626.4697. C_{18} RP-HPLC (10–100% B in 10 min): $t_R = 7.26$ min.

Compound 9 (reaction in the presence of DIPEA). S-methylated tetramer **10** (0.017 mmol) was dissolved in MeOH (0.5 mL) and DIPEA was added (5 equiv., 15 μ L). The flask was closed with septum and the mixture was heated to 40-45°C. Cyclic product **9** was formed after 24 hours of stirring. The product was purified by semi-preparative RP-HPLC (30-100% B in 20 min) and ESI-MS analysis confirmed the formation of **9**.

Compound 12. Compound **12** was obtained from **3** (scale 0,035 mmol) according to the general procedures III and VI (THF as co-solvent) with a conversion of 56% for the guanidylation step using 60 equiv. of 0.5 M NH₃ in 1,4-dioxane (150h). The ratio of linear product **12** versus cyclic product **13** was 75/25. Compound **12** was isolated after semi-preparative RP-HPLC purification (40-60% B in 20 min). White solid. ¹H NMR (CD₃OH, 700 MHz, see also Table S4) δ 7.30 (m, NH), 6.65 (d, NH), 6.58 (m, NH), 6.40-6.37 (d, NH), 6.05-6.00 (m, NH), 5.95-5.87 (m, NH), 3.94 (m,1H), 3.87 (m,1H), 3.77-3.72 (m,2H), 3.69 (m,1H), 3.65-3.58 (m,2H), 3.55-3.48 (m, 2H), 3.46 (m,1H), 3.27 (m,1H), 3.16 (m,1H), 3.08-3.03 (m,2H), 2.87 (m,1H), 2.69 (d,3H), 2.56 (m,1H), 2.38-2.30 (m,2H), 1.75 (m,1H), 1.72-1.65 (m,2H),1.63 (m,1H),1.47 (s,9H),1.30 (m,2H),1.23-1.13 (m,5H), 1.03 (d,3H), 0.98-0.94 (dd,6H), 0.93-0.88 (m,18H). ¹³C NMR (CD₃OH, 700 MHz) δ 56.5,53.5, 48.9, 48.2,47.8, 47.2, 45.9, 45.1, 44.9, 44.4, 42.7, 41.6,44.4, 35.1, 30.6, 30.4,29.3, 27.4 (3C), 26.7, 25.9, 24.8, 22.3, 20.9 (2C), 18.7 (2C), 17.6, 16.9, 16.1,13.03. HRMS (ESI-TOF) m/z: [M]⁺ Calcd for C₄₀H₈₃N₁₄O₇ 871.6564; Found 871.6545. RP-HPLC (10–100% B in 10 min then 100% B for 2 min): t_R = 8.22 min.

Compound 15. **15** was prepared from **1b** according to general procedure **III** and **IV** in CH₃OH and was recovered in 20% yield (10mg). ¹H NMR (CD₃CN, 300 MHz) δ 7.53 (bs, 1H), 7.15 (bs, 2H), 6.83 (s, 1H), 5.72 (s, 1H), 3.61 (bs, 2H), 3.34-3.26 (bm, 1H), 3.14-3.04 (bm, 1H), 2.72 (d, J = 4.8 Hz, 3H), 2.35 – 2.27 (m, 2H), 1.88-1.60 (m, 4H), 1.46 (s, 9H), 1.42-1.30 (m, 1H), 1.21 (d, J = 6.7

Hz, 3H), 0.95 (d, $J = 6.6$ Hz, 3H), 0.88 (d, $J = 6.6$ Hz, 3H); ^{13}C NMR (HSQC, CD_3CN , 75MHz) δ 50.2, 46.6, 46.2, 42.3, 31.2, 30.1, 27.1, 25.3, 24.6, 22.3, 20.8, 16.5. HRMS (ESI-TOF) m/z : $[\text{M}]^+$ Calcd for $\text{C}_{18}\text{H}_{38}\text{N}_5\text{O}_3$ 372.2969; Found 372.2984. C_{18} RP-HPLC (10–100% B in 10 min): $t_{\text{R}} = 5.88$ min.

Guanidinylation reaction with *n*-propylamine: General procedure V. S-methylated oligomer intermediate was dissolved in CH_3CN or CH_3OH (0.034 mmol/mL) and *n*-propylamine (10 equiv.) was added. The flask was closed tightly with septum and the reaction mixture was heated to 40–45°C. The monitoring of the reaction was performed by RP-HPLC analysis. Additional amounts of *n*-propylamine were added regularly. Reaction was stopped when no further progress was observed by RP-HPLC analysis.

Compounds 9 and 11: **9** and **11** were prepared on small scale (0.034 mmol) in CH_3CN from **2a** according to the general procedures **III** and **V** in the presence of *n*-propylamine (45 equiv.) for 120 hours. The ratio of linear tetramer **11** versus cyclic product **9** was 73/27 and both compounds were isolated after semi-preparative RP-HPLC purification (30–100% B in 20 min). Yields after purification for compound **9** was 16% and for compound **11** was 26%.

When the reaction was performed in CH_3OH in presence of *n*-propylamine (20 equiv.) after 24 hours compound **11** and **9** were obtained in a ratio of 15/85 and were isolated after semi-RP-HPLC purification (30–100% B in 20 min).

Compound 11. ^1H NMR (CD_3CN , 300 MHz, see also Table S3) δ 6.02 (bs, 1H), 5.39 (d, $J = 6.9$, 1H), 5.08 (bs, 1H), 3.83 (bm, 1H), 3.71 (bm, 1H), 3.59 (bm, 3H), 3.33 (dd, $J = 14.0$ Hz, 3.4 Hz, 2H), 3.21–3.15 (m, 3H), 3.08–3.00 (m, 1H), 2.94 (dd, $J = 14.2$ Hz, 6.7 Hz, 1H), 2.68 (s, 3H), 2.66–2.52 (m, 2H), 1.76–1.62 (m, 5H), 1.46 (s, 9H), 1.42–1.21 (m, 3H), 1.18 (d, $J = 6.8$ Hz, 3H), 1.02–0.88 (m, 21H); ^{13}C NMR (HSQC, CD_3CN , 75MHz) δ 54.7, 51.2, 49.0, 48.6, 45.6, 45.1, 44.8, 43.0,

42.7, 40.9, 40.4, 30.0, 27.7, 25.5, 24.4, 22.1, 21.4, 20.6, 18.7, 17.4, 17.2, 10.4. HRMS (ESI-TOF) m/z : $[M]^+$ Calcd for $C_{33}H_{69}N_{10}O_5$ 685.5452; Found 685.5467. C_{18} RP-HPLC (10–100% B in 10 min): $t_R = 7.81$ min.

Compound 17. 17 was obtained from **2b** after 90 hours according to the general procedures **III** and **V** (conversion 82% for the guanidylation step, 45 equiv. *n*-propylamine, CH_3CN) as a yellowish foam in 46% yield after silica gel flash column chromatography (CH_2Cl_2 – CH_3OH (v/v), from 98:2 to 92:8). 1H NMR (CD_3CN , 300 MHz, see also Table S5) δ 8.02 (bs, 1H), 6.96 (bs, 1H), 6.00 (d, $J = 6.0$ Hz, 1H), 5.74 (dd, $J = 8.0$ Hz, 3.4, 1H), 5.49 (pseudo-t, $J = 6.0$ Hz, 1H), 5.42 (d, $J = 9.7$ Hz, 1H), 5.17 (d, $J = 9.6$ Hz, 1H), 3.88 – 3.72 (m, 1H), 3.70-3.51 (m, 4H), 3.35-3.27 (m, 2H), 3.21 (q, $J = 9.6$ Hz, 2H), 3.10 – 2.98 (m, 1H), 2.71 (d, $J = 4.7$ Hz, 3H), 2.68-2.58 (m, 2H), 2.31 (t, $J = 6.2$ Hz, 2H), 1.76-1.62 (m, 6H), 1.46 (s, 9H), 1.38-1.22 (m, 3H), 1.19 (d, $J = 6.7$ Hz, 3H), 1.03-0.87 (m, 21H); ^{13}C NMR (CD_3CN , 75MHz) δ 54.7, 50.0, 48.6, 46.1, 45.0, 42.9, 42.4, 41.0, 30.9, 30.1, 27.9, 25.0, 24.7, 21.8, 21.3, 20.6, 18.5 17.4, 10.4. HRMS (ESI-TOF) m/z : $[M]^+$ Calcd for $C_{34}H_{70}N_9O_5$ 684.5500; Found 684.5491. C_{18} RP-HPLC (10–100% B in 10 min): $t_R = 6.04$ min.

Compound 19. 19 was obtained from **4** after 60 hours according to the general procedures **III** and **V** (conversion 96% for the guanidylation step, 20 equiv. of *n*-propylamine) as a yellowish foam in 60% yield after silica gel flash column chromatography (CH_2Cl_2 – CH_3OH (v/v), from 98:2 to 92:8). 1H NMR (CD_3CN , 400 MHz, see Table S7) δ 8.02 (bs, 1H), 6.88 (bs, 1H), 6.43 (d, $J = 8.4$ Hz 1H), 6.37 (d, $J = 7.0$ Hz, 1H), 6.26 (dd, $J = 9.7$ Hz, 1.8, 1H), 5.92 (d, $J = 10.2$ Hz, 1H), 5.73 (d, $J = 10.3$ Hz, 1H), 5.53 (dd, $J = 6.9$ Hz, 4.8 Hz, 1H), 5.44 (d, $J = 10.3$, 1H), 5.22 (d, $J = 10.0$ Hz, 1H), 3.99-3.92 (m, 1H), 3.89-3.82 (m, 1H), 3.75-3.47 (m, 8H), 3.32 (dt, $J = 10.3$ Hz, 4.9, 1H), 3.20 (dd, $J = 12.4$ Hz, 6.8 Hz, 2H), 3.08 – 3.01 (m, 1H), 2.71 (d, $J = 4.7$ Hz, 3H), 2.62 – 2.50 (m, 1H), 2.45-2.25 (m, 5H), 1.87-1.80 (m, 1H), 1.73-1.65 (m, 7H), 1.63-1.56 (m, 1H), 1.49 (s, 9H), 1.42-1.31 (m, 1H),

1.25-1.13 (m+d, d, $J = 6.7$ Hz, 5H), 1.03-0.86 (m, 30H); ^{13}C NMR (75 MHz, CD_3CN) δ 174.4, 160.3, 160.2, 159.5, 159.3, 158.0, 155.0, 79.3, 56.4, 53.7, 50.3, 49.0, 47.7, 47.1, 46.0, 46.0, 45.8, 43.6, 43.1, 42.8, 42.6, 41.6, 31.6, 30.9, 30.6, 27.9, 25.3, 25.1, 24.8, 22.6, 22.6, 21.8, 20.9, 19.1, 19.0, 18.0, 17.9, 17.5, 17.4, 10.7. HRMS (ESI-TOF) m/z : $[\text{M}]^+$ Calcd for $\text{C}_{44}\text{H}_{90}\text{N}_{13}\text{O}_7$ 912.7086; Found 912.7078. C_{18} RP-HPLC (10–100% B in 10 min then 100% B for 3 min): $t_{\text{R}} = 9.23$ min.

Guanidinylation reaction with 0.5M NH_3 in 1,4-dioxane: General procedure VI. S-methylated oligomer intermediate (0.34 mmol) was dissolved in CH_3CN (10 mL). The mixture was heated to 40–45°C and the solution of 0.5M ammonia in 1,4-dioxane (20 equiv., 13.6 mL) was added. The flask was closed tightly with septum. The monitoring of the reaction was followed by RP-HPLC. The reaction was stopped when no further progress was observed.

Compound 16. 16 was obtained from **2b** after 90 hours according to the general procedures **III** and **VI** (conversion 70% for the guanidylation step, 45 equiv. of 0.5 M NH_3 in 1,4-dioxane) as a creamy solid with 55% yield after silica gel flash column chromatography (CH_2Cl_2 – CH_3OH (v/v), from 97:3 to 92:8). ^1H NMR (CD_3CN , 300 MHz) δ 7.30 (bs, 2H), 7.08 (bs, 2H), 6.96 (bs, 1H), 5.65 (bs, 1H), 5.53 (bd, $J = 10.3$ Hz, 1H), 5.46 (bd, $J = 9.3$ Hz, 1H), 5.39 (d, $J = 9.8$ Hz, 1H), 5.20 (d, $J = 10.2$ Hz, 1H), 4.06 – 3.96 (m, 1H), 3.81-3.71 (m, 1H), 3.64-3.50 (m, 3H), 3.42-3.31 (m, 2H), 2.72+2.83-2.65 (d + bm, $J = 4.7$ Hz, 3H+1H), 2.59-2.22 (m, 4H), 1.76-1.57 (m, 5H), 1.47 (s, 9H), 1.41-1.36 (m, 2H), 1.28-1.18 (m, 2H), 1.07 (d, $J = 6.7$ Hz, 3H), 0.94-0.87 (m, 18H); ^{13}C NMR (CD_3OD , 125 MHz with cryoprobe) δ 176.1, 161.4, 161.0, 159.2, 158.0, 80.3, 56.9, 56.4, 51.3, 49.9, 46.6, 45.9, 45.1, 44.7, 42.5, 33.3, 32.1, 31.8, 28.9, 26.4, 26.2, 26.2, 23.5, 23.5, 22.6, 22.2, 20.2, 18.8, 18.6. HRMS (ESI-TOF) m/z : $[\text{M}]^+$ Calcd for $\text{C}_{31}\text{H}_{64}\text{N}_9\text{O}_5$ 642.5030; Found 642.5049. C_{18} RP-HPLC (45–70% B in 25 min): $t_{\text{R}} = 13.71$ min.

Compound 18. **18** was obtained from **4** after 24h according to the procedures **III** and **VI** (conversion 98% for the guanidylation step, 20 equiv. of 0.5 M NH₃ in 1,4-dioxane). The crude material was purified by silica gel flash column chromatography (CH₂Cl₂–CH₃OH (v/v), from 95:5 to 94:6), to give **18** a yellowish foam in 92% yield. ¹H NMR (CD₃CN, 400 MHz) δ 7.22 (b, 2H), 7.06 (bs, 1H), 6.62 (d, *J* = 6.7 Hz, 1H), 6.31 (dd, *J* = 9.6 Hz, 2.1, 1H), 6.18 (d, *J* = 10.7 Hz, 1H), 5.82-5.78 (2xd, 2H), 5.71 (dd, *J* = 9.4 Hz, 3.0, 1H), 5.54 (dd, *J* = 6.8 Hz, 4.9 Hz, 1H), 5.43 (d, *J* = 10.3 Hz, 1H), 5.24 (d, *J* = 10.0 Hz, 1H), 4.05 (bp, 1H), 3.86-3.75 (m, 2H), 3.65-3.38 (m, 9H), 2.69-2.63 (m+d, *J* = 4.7 Hz, 4H), 2.54 (ddd, *J* = 14.2 Hz, 11.2 Hz, 4.7, 1H), 2.47-2.42 (bp, 1H), 2.37-2.23 (m, 4H), 1.76-1.66 (m, 6H), 1.46 (s, 9H), 1.36 (t, *J* = 6.5 Hz, 2H), 1.17 (t, *J* = 7.2 Hz, 2H), 1.03 (t, *J* = 7.1 Hz, 7H), 0.93-0.82 (m, 24H); ¹³C NMR (75 MHz, CD₃CN) δ 174.2, 160.7, 160.1, 159.4, 159.3, 158.0, 157.0, 79.4, 56.5, 54.8, 50.0, 48.0, 47.7, 46.9, 46.0, 45.9, 43.9, 43.6, 43.2, 42.6, 41.2, 32.3, 30.7, 30.6, 30.4, 27.9, 25.4, 25.0, 24.8, 22.4, 22.3, 21.6, 21.1, 19.2, 19.1, 18.0, 17.9, 17.4. HRMS (ESI-TOF) *m/z*: [M]⁺ Calcd for C₄₁H₈₄N₁₃O₇ 870.6617; Found 870.6591. C₁₈ RP-HPLC (10–100% B in 10 min then 100% B for 3 min): *t*_R = 9.11 min.

Solid phase synthesis of resin-bound oligo(thio)urea 20_R. **20_R** was synthesized with **BB3** building blocks according to the recently reported solid phase oligo(thio)urea synthesis procedure by locally using *N*-Fmoc isothiocyanate building block **BB4** and azide Trp-type activated monomer **BB5** under microwave irradiation with the azide strategy on a 50 μmol scale, starting from NovaPeg Rink Amide resin (111 mg, 0.45 mmol/g).³⁷ Coupling of *N*-Fmoc-γ-Lys-OH (1.5 equiv. relative to the resin loading, 1.5 mmol, 45 mg) was performed in DMF (3 mL) in presence of DIPEA (3.0 equiv.) and BOP (3.0 equiv.) under microwave irradiation (50 W, 50 °C, ramp time 5 min, hold time 30 min). This coupling step was repeated once. Fmoc removal was performed with a solution of piperidine in DMF (2:8, v/v, 1 mL), three times 20 min.

Compound 20. **20** was obtained from resin-bound **20_R** (16 μmol) by performing cleavage of the resin in TFA/TIS/H₂O solvent mixture (95:2.5:2.5, v/v/v, 2.5 mL). After cold Et₂O precipitation, the crude material was purified by semi-preparative RP-HPLC (10-45% of B in 20 min at 4 mL·min⁻¹) to furnish 3.9 mg of pure **20** in 17 % yield. ¹H NMR (CD₃OH, 400 MHz, see Table S8); HRMS (ESI-TOF) m/z: [M + H]⁺ Calcd for C₆₄H₁₁₃N₂₁O₈S 1336.8880; Found 1336.8904. RP-HPLC (10–100% B in 10 min): $t_{\text{R}} = 6.04$ min.

Solid phase synthesis of resin-bound 21_R. Resin bound oligo(thio)urea **20_R** (26 μmol) was engaged in methylation activation with CH₃I (10.0 equiv. relative to resin loading) in DMF (0.5 mL) under microwave irradiation (40°C, 20 W, 30 min). The resin was successively washed with DMF (3 \times 3 mL) and DCM (3 \times 3 mL). This alkylation step was repeated three times with the same washings between each activation.

Solid phase synthesis of resin-bound 22_R. The S-methylated resin-bound oligomer **21_R** was washed with anhydrous THF (3 \times 3 mL), then transferred in a sealed reactor. Anhydrous THF (5 mL) was added and ammonia gas was bubbled into the reactor for 30 min. The reactor was then sealed and stirred for 48 hours at 50 °C.

Compound 22. **22** was obtained from resin-bound **22_R** by performing cleavage of the resin in TFA/TIS/H₂O solvent mixture (95:2.5:2.5, v/v/v, 2.5 mL). After cold Et₂O precipitation, the crude material was purified by semi-preparative RP-HPLC (15-41% of B in 20 min at 4 mL·min⁻¹) to furnish 6 mg of pure **22** in 17% yield. ¹H NMR (CD₃OH, 400 MHz, see Table S9); HRMS (ESI-TOF) m/z: [M]⁺ Calcd for C₆₄H₁₁₅N₂₂O₈ 1319.9268; Found 1319.9262. RP-HPLC (10–100% B in 10 min): $t_{\text{R}} = 5.35$ min.

Circular dichroism

Circular dichroism (CD) experiments were performed on a Jasco J-815 spectrometer. Data are expressed in terms of the total molar ellipticity [$\text{deg}\cdot\text{cm}^2\cdot\text{dmol}^{-1}$]. CD spectra of the oligomers (0.2 mM) were acquired in spectrograde trifluoroethanol between 180 and 260 nm by using a rectangular quartz cell with a path length of 1 mm. To reduce the signal-to-noise ratio all spectra were recorded on an average of four consecutive scans.

Crystallographic data

See the supporting information for details. CCDC-1563646 and CCDC-1563645 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

ASSOCIATED CONTENT

***Supporting Information**

Additional experimental procedures and characterization data of all compounds, supplemental Figures S1–S79 and Tables S1–S8 (PDF)

Crystallographic data for **2b** (CIF)

Crystallographic data for **4** (CIF)

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