Synthesis of urea oligomers and their antibacterial activity†

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Facially amphiphilic urea oligomers were successfully prepared in a one-pot reaction by carbonyl diimidazole (CDI) coupling and showed greater antibacterial activity against both Gram-negative *Escherichia coli* and Gram-positive *Bacillus subtilis* than MSI-78.

We have been working on host defense peptide mimics by designing simple oligomers with cationic and non-polar groups that segregate onto opposite sides of the structure.[1] Both these unnatural oligomers and natural peptides show activity against Gram-negative and Gram-positive bacteria. Here we describe a novel series of aromatic ureas with internal NH···S hydrogen bonding and their antibacterial activity.

Facially amphiphilic structures in which polar and non-polar groups segregate to opposite sides of the overall conformation are ubiquitous in Nature. Examples span from α-helical peptides to steroids.[2] Of these structures, perhaps the most widely studied is the amphiphilic helix.[3] One example includes the Magainins which are a class of cationic peptides that position polar and non-polar residues on opposite sides of the helical cylinder.[3,4] Magainins represent one category of host defense peptides (HDPs), which are a large class of cationic, amphiphilic molecules implicated in the innate immune systems of various organisms from moths and frogs to humans.[2a,3d,4] Recently, much effort has focused on the design and synthesis of unnatural backbones which mimic the helical and antimicrobial nature of HDPs.[5] We have focused on facially amphiphilic structures which adopt extended as opposed to helical conformations and as a result represent very simple architectures which are readily produced in one-pot reactions.[1a,c,f]

This report describes a novel series of oligomers based on aromatic ureas. These oligomers have internal hydrogen bonding to limit their conformational flexibility and were determined to have potent antimicrobial activity against both *E. coli* and *B. subtilis*. The evaluation of oligomer length indicated an optimum for overall activity and selectivity of these aromatic ureas at $n = 3$.

Fig. 1 shows the chemical structure of two molecules, one is the previously reported arylamide[6] and the other is based on the new ureas reported here. In the arylamides, there are internal hydrogen bonds only between the thioether and amide NH which confines rotation about the ArC–NH single bond but not the ArC–C single bond as shown by the arrows. In contrast, the newly designed ureas have NH···S interactions on every ring. In addition to these positive interactions, the urea carbonyl would prefer to point away from the thioether to reduce electrostatic repulsion.[1d] As a result, these oligomers have elements of both positive and negative design. We wanted to determine the influence of these structural changes on antibacterial activity and selectivity.

We speculated that the direct oligomerization of **3** in the presence of CDI would yield a series of oligomers that could be separated by chromatography in one step as opposed to a lengthy step-wise methodology using protection/deprotection schemes. As shown in Fig. 2, when **3** is reacted in excess, this approach successfully yielded dimer, trimer, and tetramer as well as a fraction containing pentamer through octamer which was not further separated or considered for antibacterial studies.

Minimization using AM1 gave the expected overall conformation of the dimer, **4**, as the all-*trans* structure shown in Fig. 3. A limited AM1 search found that both the trimer and tetramer also adopted the all-*trans* conformation. For **4**, the energy difference between the all-*trans* conformation and one in which the carbonyl points toward a thioether, formed by rotating 180° around an ArC–N bond, is 16 kcal mol^{-1}. This agrees well with a value of 15 kcal mol^{-1} obtained from higher level methods for the corresponding energy difference between conformations of methylthioacetanilide,[1d] and is to be expected because of steric repulsion and loss of one internal NH···S bond. However, these ureas have an alternative conformation which differs from

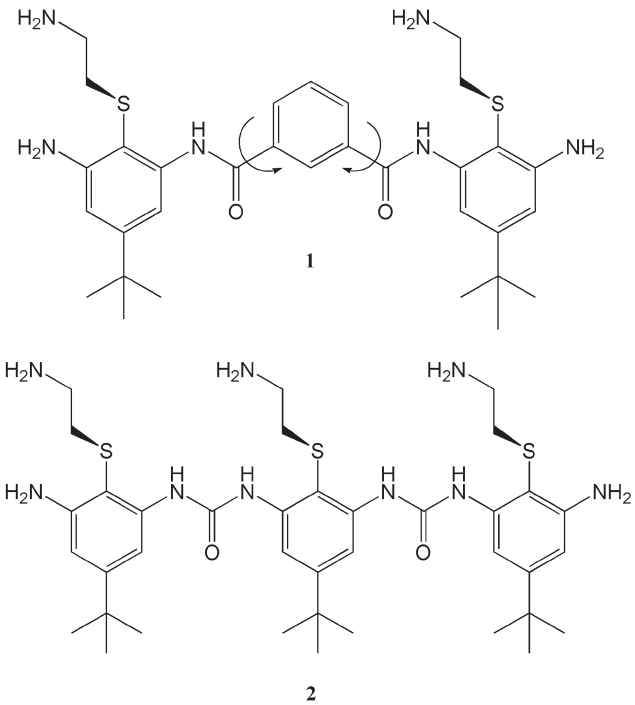

Fig. 1 The structure of arylamide **1** and urea trimer **2**.

† Electronic Supplementary Information (ESI) available: synthetic and computational procedures, antibacterial and hemolysis results are included. See http://www.rsc.org/suppdata/cc/b4/b413679a/

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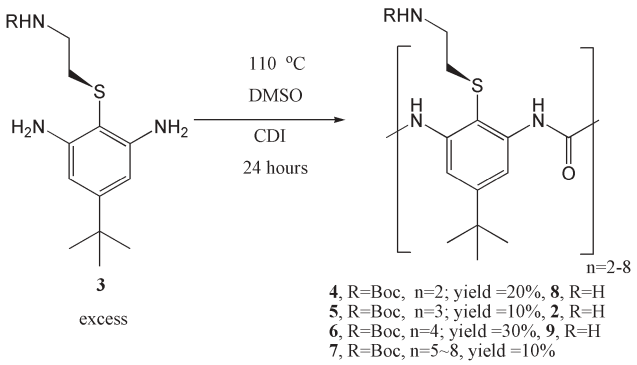

Fig. 2 Synthesis of urea oligomers.

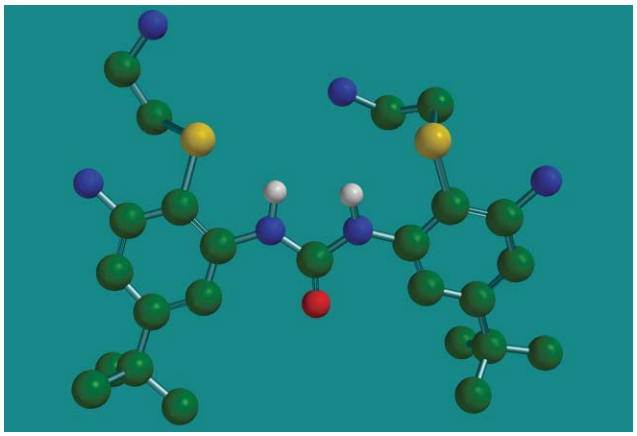

Fig. 3 The most stable conformation of the urea dimer from AM1 minimization (C green, H white, N blue, O red, S yellow).

the all-*trans* by rotating ~150° around one amide–carbonyl bond. This conformation still contains both NH···S hydrogen bonds but has the carbonyl on the same side as one thioether. It is only 3 kcal mol^{-1} higher in energy (with AM1) than the all-*trans* one. Density-functional theory (DFT) calculations using the HCTH functional and plane-wave basis set were performed on the urea backbone of **4**. Fig. 4 shows three key structures from the torsional curve for constrained geometry optimizations with fixed CN–CN dihedral angles. The lowest energy conformation is all-*trans* but the energy difference compared to a conformation with CN–CN ≈ 30° (or 150° from all-*trans*), is only 0.5 kcal mol^{-1} at this level. The torsional curve suggests that over the range from 150–210°, the energy differs by only 1 kcal mol^{-1}. The barrier height is large, 8 kcal mol^{-1}, but is only half the barrier height of a normal amide bond, such as found in **1**, as calculated for benzanilide with the same method. The CC–NC torsional barrier is ~3.5 kcal mol^{-1} with the same method. Though further study is warranted with still

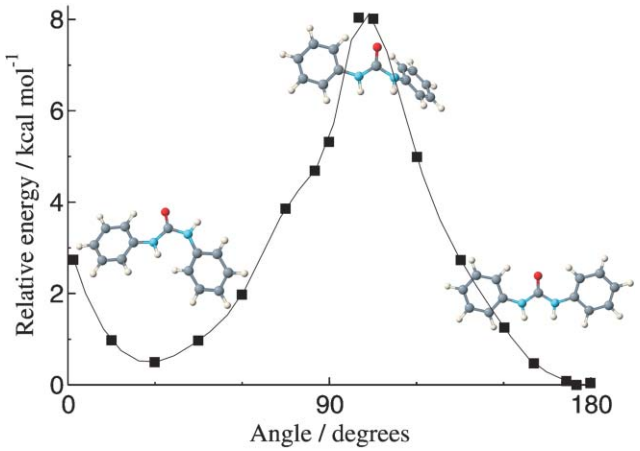

Fig. 4 DFT torsional potential for rotation around one amide–carbonyl bond. The conformations for CN–CN = 30°, 100°, and 180° are shown (C gray, H white, O red, N blue).

more accurate methods and larger model compounds, the computational results suggest that **4–6** favor the all-*trans* conformation but have greater conformational flexibility than the corresponding amides. Solvent titration studies using $CDCl_3$ and DMSO-d_6, confirm the presence of internal hydrogen bonding of the urea NHs. The ppm chemical shift change was determined to be only 0.47 for the urea NHs *versus* 1.65 for the Boc NHs of **5**.

Following deprotection of the Boc groups, antibacterial and hemolysis activity was determined. Table 1 shows the antibacterial results reported as minimal inhibitory concentration (MIC) against Gram-negative *E. coli* and Gram-positive *B. subtilis*, along with hemolysis (HC_{50}) data. The dimer, **8**, has a MIC of 4.5 μg ml^{-1} against both bacteria and its HC_{50} is 14 μg ml^{-1} giving a selectivity of 3.1. The trimer, **2**, and the tetramer, **9**, are more potent compounds with MIC values at 0.7 μg ml^{-1}, but are more hemolytic. However, because **2** is quite active it is more selective than either the dimer or tetramer. As a result, **2** represents a maximum in potency and selectivity. It is more active and selective than the corresponding arylamide **1**,[1,6] more potent than MSI-78, a magainin derivative, but not as selective (see Table 1), and has activity against *E. coli* comparable with Polymyxin B (MIC = 0.5 μg ml^{-1}).

Urea oligomers were successfully prepared in a one-pot reaction. Although chromatography is necessary to separate these oligomers, this route is rapid and has advantages over the multi-step method using protection groups. The trimer and tetramer are the most active oligomers; however, the hemolysis increases with increasing molecular weight in this series, making **2** the most potent and selective compound. The selectivity is lower than other arylamides with side chain modifications which may result from the increased hydrophobicity due to the number of *tert*-butyl

Table 1 Antibacterial and hemolysis activity of oligomers (μg ml^{-1})

Compound	MIC $_{E.\ coli}$	MIC $_{B.\ subtilis}$	HC_{50}	Selectivity $_{E.\ coli}$	Selectivity $_{B.\ subtilis}$
1[6]	12.5	—	12	0.96	—
8	4.5	4.5	14	3.1	3.1
2	0.7	0.7	3.5	5.0	5.0
9	0.7	0.7	1.75	2.5	2.5
MSI-78	12.5	—	120	9.6	—

groups. Decreasing the hydrophobicity by removing the *tert*-butyl groups is expected to generate more selective oligomers. These urea oligomers are also conformationally more flexible than similar arylamide structures, a property which could be utilized for other purposes including ion complexation.[7]

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Notes and references

1 (*a*) G. N. Tew, D. H. Liu, B. Chen, R. J. Doerksen, J. Kaplan, P. J. Carroll, M. L. Klein and W. F. DeGrado, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 5110–5114; (*b*) L. Arnt and G. N. Tew, *Langmuir*, 2003, **19**, 2404–2408; (*c*) L. Arnt, K. Nusslein and G. N. Tew, *J. Polym. Sci., Part A: Polym. Chem.*, 2004, **42**, 3860–3864; (*d*) R. J. Doerksen, B. Chen, D. Liu, G. Tew, W. F. DeGrado and M. L. Klein, *Chem. Eur. J.*, 2004, **10**, 5008–5016; (*e*) R. J. Doerksen, B. Chen and M. L. Klein, *Chem. Phys. Lett.*, 2003, **380**, 150–157; (*f*) L. Arnt and G. N. Tew, *J. Am. Chem. Soc.*, 2002, **124**, 7664–7665.

2 (*a*) A. Tossi, L. Sandri and A. Giangaspero, *Biopolymers*, 2000, **55**, 4–30; (*b*) P. B. Savage, C. H. Li, U. Taotafa, B. W. Ding and Q. Y. Guan, *FEMS Microbiol. Lett.*, 2002, **217**, 1–7.

3 (*a*) Y. R. Vandenburg, B. D. Smith, E. Biron and N. Voyer, *Chem. Commun.*, 2002, **16**, 1694–1695; (*b*) T. Wieprecht, O. Apostolov, M. Beyermann and J. Seelig, *Biochemistry*, 2000, **39**, 442–452; (*c*) M. H. Wu, E. Maier, R. Benz and R. E. W. Hancock, *Biochemistry*, 1999, **38**, 7235–7242; (*d*) Y. Shai, *Biopolymers*, 2002, **66**, 236–248; (*e*) R. I. Lehrer, A. K. Lichtenstein and T. Ganz, *Ann. Rev. Immunol.*, 1993, **11**, 105–128.

4 (*a*) K. Matsuzaki, Y. Mitani, K. Akada, O. Murase, S. Yoneyama, M. Zasloff and K. Miyajima, *Biochemistry*, 1998, **37**, 15144–15153; (*b*) K. Matsuzaki, O. Murase, N. Fujii and K. Miyajima, *Biochemistry*, 1996, **35**, 11361–11368.

5 (*a*) Y. Hamuro, J. P. Schneider and W. F. DeGrado, *J. Am. Chem. Soc.*, 1999, **121**, 12200–12201; (*b*) D. Liu and W. F. DeGrado, *J. Am. Chem. Soc.*, 2001, **123**, 7553–7559; (*c*) E. A. Porter, X. Wang, H. S. Lee, B. Weisblum and S. H. Gellman, *Nature*, 2000, **404**, 565; (*d*) E. A. Porter, B. Weisblum and S. H. Gellman, *J. Am. Chem. Soc.*, 2002, **124**, 7324–7330; (*e*) T. L. Raguse, E. A. Porter, B. Weisblum and S. H. Gellman, *J. Am. Chem. Soc.*, 2002, **124**, 12774–12785; (*f*) J. A. Patch and A. E. Barron, *J. Am. Chem. Soc.*, 2003, **125**, 12092–12093.

6 D. H. Liu, S. Choi, B. Chen, R. J. Doerksen, D. J. Clements, J. D. Winkler, M. L. Klein and W. F. DeGrado, *Angew. Chem. Int. Ed.*, 2004, **43**, 1158–1162.

7 S. Yagi, M. Ezoe, I. Yonekura, T. Takagishi and H. Nakazumi, *J. Am. Chem. Soc.*, 2003, **125**, 4068–4069.