ChemPubSoc Europe

DOI: 10.1002/chem.201500068

CHEMISTRY A European Journal Full Paper

Macrocycles

Solid-Phase Parallel Synthesis of Functionalised Medium-to-Large Cyclic Peptidomimetics through Three-Component Coupling Driven by Aziridine Aldehyde Dimers

Adam P. Treder,[a] Jennifer L. Hickey,[b] Marie-Claude J. Tremblay,[a] Serge Zaretsky,[b] Conor C. G. Scully,[b] John Mancuso,[a] Annie Doucet,[a] Andrei K. Yudin,[b] and Eric Marsault*[a]

Abstract: The first solid-phase parallel synthesis of macrocyclic peptides using three-component coupling driven by aziridine aldehyde dimers is described. The method supports the synthesis of 9- to 18-membered aziridine-containing macrocycles, which are then functionalized by nucleophilic opening of the aziridine ring. This constitutes a robust approach for the rapid parallel synthesis of macrocyclic peptides.

Introduction

Macrocycles are cyclic molecules composed of 12 or more atoms. The synthesis of chemically and conformationally diverse macrocycles has been tackled by using several technologies, ranging from traditional combinatorial chemistry to methods allying chemistry and biology (for reviews, see reference [1]). Some of these approaches have become the platforms of emerging companies (for specific examples, see reference [2]; for reviews, see reference [1f,g]). One challenge inherent to the macrocyclisation reaction is the ability to bring the two termini of the linear precursor in close proximity to enable ring formation. Indeed, macrocyclisation is a unimolecular, largely entropy-driven reaction in which backbone flexibility and transannular interactions represent critical factors that can favour or disfavour the reaction.[3] As a result, macrocyclisation is often performed under high or pseudo-high dilution conditions to reduce the formation of undesired dimers and higher oligomers. The availability of a broad diversity of natural and unnatural amino acids has meant that the synthesis of macrocyclic peptides has received particular attention, and several methods have been optimised to synthesise these molecules incorporating variable degrees of nonpeptidic contents, using macrolactamisation, ring-closing metathesis, S_NAr or [3+2] cycloaddition to name a few (for reviews, see reference [1f,g]). Macrocycles have also been synthesised by using multicomponent reactions (for representative examples, see reference [4]).

In 2006, the Yudin group reported a novel class of amphoteric reagents in the form of aziridine aldehyde dimers,[5] and the group later demonstrated the capability of such compounds to disrupt the three-component Ugi reaction. When aziridine aldehyde dimers reacted with an isocyanide and a linear peptide possessing a free amino and carboxylate functions, aziridine amide-containing peptide macrocycles were isolated.[6] One of the distinguishing features of this macrocyclisation is the unusually high concentration at which it remains feasible (typically higher than 0.1 M), which constitutes a preparative advantage compared with other macrocyclisation reactions, which are usually performed at millimolar concentrations.[1g,h] The rationale behind this efficiency has been provisionally attributed to the maintenance, through each intermediate step, of a stabilising ion pair that facilitates chain folding and keeps the two ends in close proximity.[6d] The resulting endocyclic aziridine amide can be opened post-macrocyclisation with a variety of nucleophiles, which adds an extra point of diversity to introduce exocyclic substituents.[6a,f,7]

To further exploit the potential of this novel method, we report herein the development of a solid-phase approach to macrocyclisation with aziridine aldehydes. Given its optimisation for the synthesis of peptides, the potential of solid-phase synthesis (SPS) has been amply demonstrated in both high-throughput parallel synthesis and split-pool combinatorial chemistry.[8] SPS constitutes a tool of choice to build libraries that are suitable for hit identification and rapid analogue generation for hit-to-lead optimisation. Building on our recent application of the disrupted Ugi reaction with aziridine aldehyde dimers to the solid-phase synthesis of piperazinones,[9] we report herein the development of a solid-phase macrocyclisation protocol and aziridine ring-opening strategy.

[a] Dr. A. P. Treder, M.-C. J. Tremblay, Dr. J. Mancuso, A. Doucet, Prof. E. Marsault
Institut de Pharmacologie de Sherbrooke
Université de Sherbrooke, 3001, 12e av nord
Sherbrooke (QC) J1H 5N4 (Canada)
E-mail: eric.marsault@usherbrooke.ca

[b] Dr. J. L. Hickey, Dr. S. Zaretsky, Dr. C. C. G. Scully, Prof. A. K. Yudin
Davenport Research Laboratories
Department of Chemistry, University of Toronto
80 St. George Street, Toronto (ON) M5S 3H6 (Canada)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201500068.

Results and Discussion

We sought a process in which every synthetic step was implemented on solid support to maximise diversity generation with a minimal number of operations. Key steps of our design (Figure 1) included peptide elongation, pivotal three-compo-

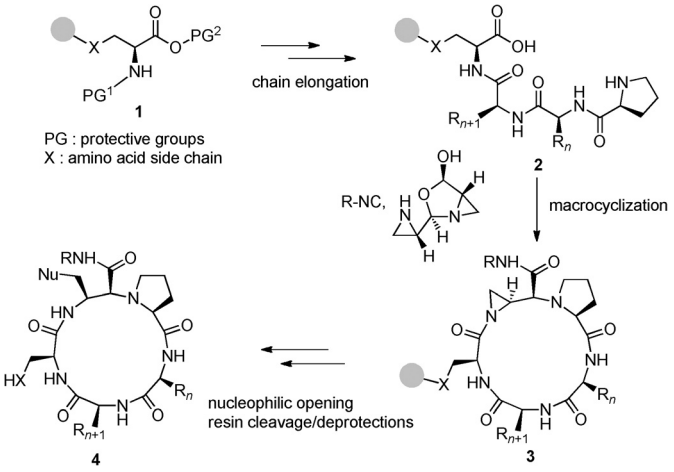
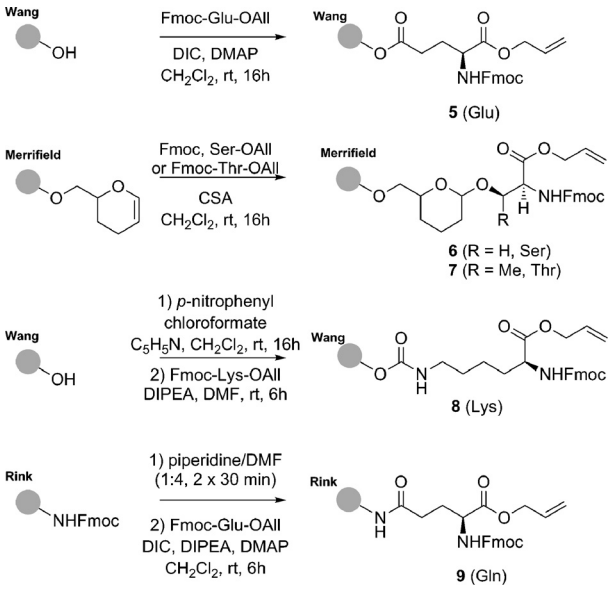


Figure 1. General synthetic approach (exemplified with all-L-amino acids and (*S*)-aziridine aldehyde dimer derived from L-Ser).

nent macrocyclisation, subsequent nucleophilic ring-opening of the newly formed aziridine, and final resin cleavage with simultaneous deprotection of side chains. To address these requirements, we decided to attach the precursor peptide to the resin through the side chain of a suitably protected C-terminal amino acid. Accordingly (Figure 1), we reasoned that a suitable general strategy might involve side chain attachment of the first amino acid, protected on the amino and the carboxylate positions, to deliver precursor **1**. Subsequent chain elongation, ideally by using standard Fmoc chemistry, followed by N- and C-terminal deprotections, would deliver linear precursor **2**. The latter would then undergo macrocyclisation in the presence of aziridine aldehyde dimer and isocyanide to produce macrocycle **3**. Finally, nucleophilic opening of the newly formed acyl aziridine **3** in situ followed by acid-mediated resin cleavage and concomitant side chain deprotection(s), would deliver the desired product **4**, which could be purified by reverse-phase HPLC. This synthetic approach offered a lot of flexibility to introduce diversity by varying ring size and stereochemistry as well as the substitution of amino aldehyde, nucleophile and amino acids side chains. Structurally, macrocycles resulting from this strategy are distinguished from macrocyclic peptides or depsipeptides by the presence of a 1,2-disubstituted diamine moiety bearing two controlled stereogenic centres (see below). Here, we report the successful implementation of this strategy and demonstrate its utility by synthesizing a representative set of 9- to 18-membered rings bearing diverse amino acids and nucleophiles.

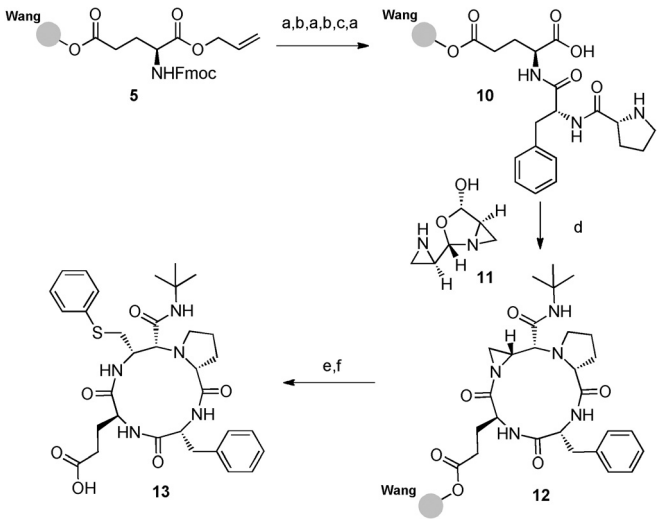
Several strategies were selected to attach different amino acids through their side chains to the solid support (Scheme 1). Fmoc-L-Glu-OAll was attached directly to Wang



Scheme 1. Strategies for side-chain attachment of the C-terminal amino acid.

resin through its side chain carboxylate in the presence of *N*,*N*′-diisopropyl carbodiimide (DIC) and 4-(*N*,*N*-dimethylamino)-pyridine (DMAP) in dichloromethane, to deliver precursor **5**.[10] For the attachment of Ser and Thr, Merrifield resin was first functionalised to generate Ellman resin bearing a tetrahydropyranyl (THP) linker by reaction with 3,4-dihydro-2*H*-pyran-2-methanol and sodium hydride.[11] Subsequent reaction with the hydroxyl group of Fmoc-L-Ser-OAll or Fmoc-L-Thr-OAll in the presence of camphorsulfonic acid in dichloromethane delivered precursors **6** and **7**, respectively.[11] Anchoring of Lys through its side chain started with reaction of Wang resin with *p*-nitrophenyl chloroformate in the presence of pyridine in dichloromethane, to generate the corresponding carbonate. Subsequent reaction with Fmoc-L-Lys-OAll in the presence of Hünig's base in *N*,*N*-dimethylformamide (DMF) delivered precursor **8**.[12] Finally, anchoring of Gln was prepared by amide bond formation between Fmoc-L-Glu-OAll and Rink amide resin in the presence of DIC, diisopropylethylamine (DIPEA), and DMAP in dichloromethane, to deliver **9**. Generally, resin loadings were determined after Fmoc cleavage and found to be approximately 0.40 mmol g^{-1} for THP-linked resins, or similar to suppliers' loadings (0.68 mmol of Fmoc-L-Glu-OAll/g, 0.66 mmol of Fmoc-L-Lys-OAll/g, 0.47 mmol of Fmoc-L-Gln-OAll/g). Loadings were confirmed by cleavage in trifluoroacetic acid (TFA)/dichloromethane (1:1). The purity of cleavage products was confirmed by LC-MS analysis and found to be generally excellent (> 95%, UV monitoring).

A typical synthesis is exemplified in Scheme 2. Starting from Fmoc-L-Glu-OAll (**5**) attached to Wang resin (Scheme 1), the Fmoc group was deprotected in the presence of 20% piperidine/DMF, followed by HATU-mediated couplings to assemble the precursor linear amino acid sequence. Deprotection of the allyl ester in the presence of palladium tetrakis(triphenylphosphane) and phenylsilane in dichloromethane, followed by Fmoc removal, delivered macrocyclisation precursor **10** anch-



Scheme 2. Detailed synthetic scheme exemplified for macrocycle **13**. Reagents and conditions: a) 20% piperidine/DMF, 30 min, rt (×2); b) Fmoc-AA-OH, HATU, DIPEA, DMF, 4 h, rt; c) $[Pd(PPh_3)_4]$, $PhSiH_3$, CH_2Cl_2, rt, 3 h; d) **11**, *t*Bu-NC, DCM:TFE (1:1), rt, 4 h; e) PhSH, DIPEA, DMF, rt, 16 h; f) TFA/CH_2Cl_2 (1:1), rt, 75 min.

ored on the resin through the Glu side chain.[13] Reactions were followed by LC-MS and driven to completion by using three equivalents of reagents. Typically, macrocyclisation precursors were more than 90% pure, as determined after mini-cleavage (2–5 mg resin in TFA/CH_2Cl_2, 1:1) by LC-MS analysis (UV monitoring).

The critical macrocyclisation step was implemented in the form of a disrupted three-component Ugi reaction in the presence of aziridine aldehyde dimer **11** (synthesised as reported in Refs. [14] and [9]) and *tert*-butyl isocyanide. Previous experience with the solution-phase macrocyclisation revealed that the reaction performs best in TFE as a solvent. However, this solvent did not give satisfactory resin swelling, which is critical for reaction completion on solid support.[8b] A preliminary screening of solvent conditions led us to choose CH_2Cl_2/TFE (1:1) as the best compromise between swelling and reactivity. Macrocyclisation was run in the presence of aziridine aldehyde dimer (1 equiv with respect to measured loading), *tert*-butyl isocyanide (2 equiv) in CH_2Cl_2/TFE (1:1) at ambient temperature, to deliver acyl aziridine-containing macrocycle **12** (Scheme 2). The acyl aziridine was ring-opened with thiophenol in situ in the presence of DIPEA in DMF. Finally, the macrocycle was cleaved from the resin by using TFA/CH_2Cl_2 (1:1). The crude material was collected by filtration and evaporation, and macrocycle **13** was isolated by preparative HPLC. This method was used to generate macrocycles **13**–**34** (Figure 2).

Several observations can be made regarding this sequence. First, the reaction performed well for macrocycles of various ring sizes, ranging from 9 atoms (**14**, **15** derived from dipeptides) to 18 atoms in the ring (**30**–**34** derived from pentapeptides). Notably, the yields for 9- and 12-membered rings were reasonable, considering that medium-sized homodetic peptides are notoriously difficult to cyclise.[15] Compared with homodetic cyclic tri- and tetrapeptides, which contain three or four amide bonds, respectively, the replacement of one amino acid by the substituted aminoethyl moiety originating from the three-component reaction, is expected to reduce ring strain through the removal of one amide bond. Second, macrocyclisation proved tolerant of all side chain functionalities, including nonpolar and polar side chains. Likewise, introduction of non-alpha-amino acids in the chain was well tolerated (e.g., **23**–**25**). Third, macrocyclisation was tolerant of variations in stereochemistry at every position, provided that the stereochemistry of the Pro residue on the N-terminus of the chain was matched to that of the aziridine aldehyde dimer (i.e., L-Pro reacts cooperatively with (*S*)-aziridine aldehyde dimer derived from L-Ser as in **14**–**26**, **28**–**33**, whereas D-Pro matches the (*R*)-aziridine aldehyde dimer derived from D-Ser as in **13**, **27**, **34**). Mismatched stereochemistry between the Pro residue and the aziridine aldehyde dimer typically gave intractable mixtures. This observation was rationalised by Zaretsky et al., who demonstrated in solution that a stereochemistry match between the aziridine aldehyde dimer and amino acid partners was paramount to obtaining good diastereoselectivity in the disrupted Ugi reaction with secondary amino acids.[16] For previous discussion on the importance of a terminal Pro residue, see references [5, 14, 16]; for the use of alternative isocyanides, see references [6f, 16].

The aziridine ring of intermediate **3** (Figure 1) was opened on resin with a variety of nucleophiles, exemplified here with different thiols (**13**, **18**, **21**, **22**, **28**, **29**) and thiobenzoic acid (**14**). It should be noted, however, that all attempts to isolate aziridine-containing macrocycle **3** by acid-mediated resin-cleavage failed, which reflects the relative instability of the aziridine moiety.[17]

As summarised in Figure 2, starting from 0.1 mmol resin (typically ca. 160 mg of a 0.6 mmol g^{-1} nominal loading resin), macrocycles were isolated after synthesis, preparative HPLC purification and lyophilisation, with isolated yields ranging from 7 to 32% (**18** and **23**, respectively) for the 7-, 9-, 11- or 13-step syntheses of ring systems built on di-, tri-, tetra- and pentapeptides, respectively.

Once the purity of the peptides was confirmed by LC-MS analysis, the macrocycles were analysed by using 1D ^1H and ^{13}C NMR, as well as by 2D ^1H-^1H and ^1H-^{13}C NMR spectroscopic techniques. Successful macrocyclisation was noted by the appearance of a new amide NH peak with 2D TOCSY cross-peaks to the adjacent linker region (Figure 3). The structure of compound **24** has the linker atoms both annotated and highlighted in red. We observed the corresponding cross-peaks from the new NH amide to the α-, β-, and γ-protons, as shown in the TOCSY spectrum. The nucleophilic aziridine ring-opening was confirmed to proceed with the same regioselectivity as observed in the solution-phase protocol, which was supported by NMR evidence of a downfield diastereotopic methylene at the β-position.

The next step was to compare the properties of this new family of macrocycles with those of reported drugs and clinical candidates. In terms of physicochemical and biological properties, macrocycles are considered an intermediate class between small organic molecules and large biomolecules.[1e] With molec-

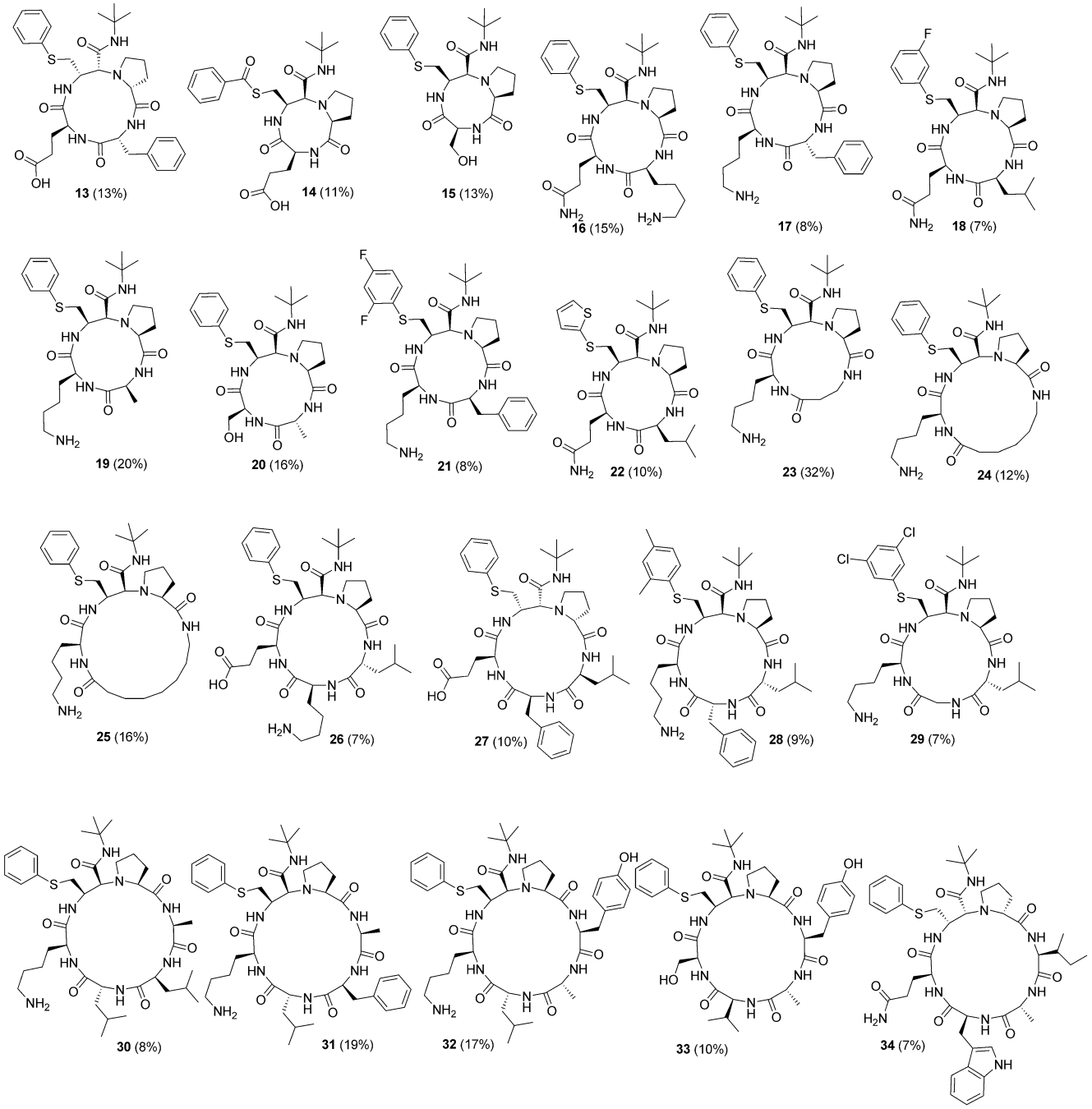


Figure 2. Macrocycles synthesised on solid phase by three-component macrocyclisation and subsequent aziridine ring-opening. Macrocycles were isolated as TFA (**13**–**29**) or formate (**30**–**34**) salts. Yields of **13**–**29** were calculated considering one TFA equivalent per net positive charge on the macrocycle, and **31**–**35** using formate as a counter-anion.

ular weights usually ranging between 500 and 2,000 Da, they combine large surface areas, usually associated with biomolecules such as antibodies, with the potential to reach suitable physicochemical properties conducive to oral bioavailability, usually associated with small molecules. These properties mean that macrocycles have attracted an increasing level of attention recently as an underexploited chemical space (for recent reviews, see references [1f–h, 18]). They have been used as drug candidates on most target classes, with currently around 70 macrocyclic drugs or clinical candidates issued mostly from natural products and peptides.[18a]

Accordingly, we sought to compare the chemical space covered by representative macrocyclic drugs and clinical candidates with the collection reported in this paper. As shown in Table 1, the newly reported macrocycles cover a broad range of molecular weights (449–860 g mol^{-1}), which is within the range of known macrocyclic peptidic and peptidomimetic drug candidates (Ulimorelin, Linopristin, Simeprevir, Vaniprevir,

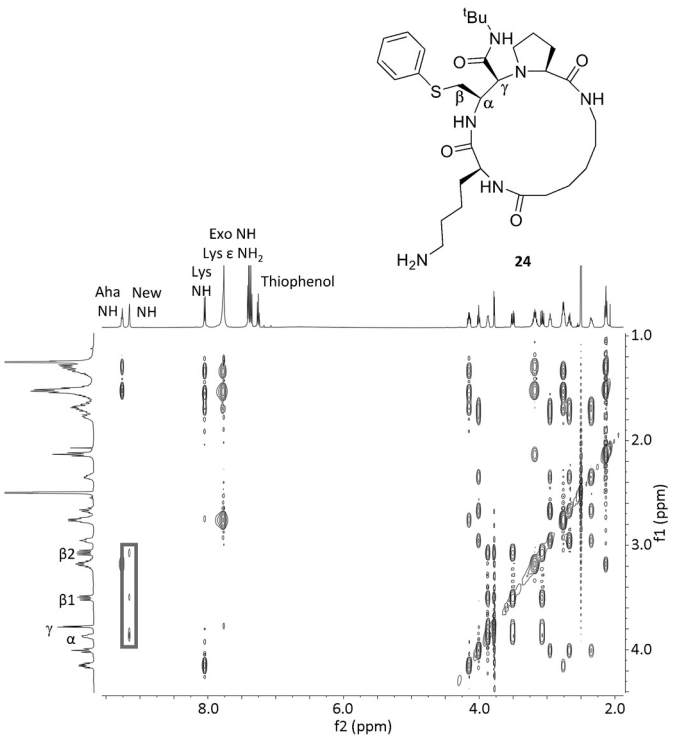


Figure 3. ^{1}H-^{1}H TOCSY NMR of compound **24**. The linker region is annotated and highlighted in red on the structure. Corresponding cross-peaks from the new NH to the adjacent linker atoms are highlighted on the TOCSY spectrum.

Table 1. Analysis of properties of newly reported macrocycles and comparison with macrocyclic drugs and development candidates.

Compound	Ring size	M_w [g mol^{-1}]	cLogP[a]	PSA [Å^2][a]
13	12	637.8	0.3	137.1
14	9	518.6	−1.2	139.9
15	9	448.6	0.5	108.0
16	12	617.8	−1.0	169.4
17	12	636.9	2.0	128.9
18	12	620.8	0.0	153.2
19	12	560.8	0.4	139.6
20	12	519.7	−0.1	137.2
21	12	672.8	2.5	119.9
22	12	608.8	−0.1	152.2
23	13	560.8	0.5	144.0
24	15	602.8	0.9	132.4
25	18	630.9	1.8	130.5
26	15	731.9	−1.7	179.5
27	15	751.0	−0.3	148.1
28	15	778.1	3.1	147.1
29	15	728.8	1.3	155.0
30	18	787.1	2.0	171.8
31	18	821.1	2.0	176.1
32	18	837.1	1.4	214.9
33	18	782.0	0.9	208.9
34	18	860.1	1.2	230.3
cyclosporin	11	1202.6	0.9	282.2
ulimorelin	18	538.7	2.8	97.8
linopristin	19	950.1	1.9	229.7
simeprivir	14	749.9	5.4	157.9
vaniprevir	20	755.9	4.9	193.4
octreotide	20	1019.2	−1.0	336.3

[a] Physicochemical properties were calculated by using QikProp.[19]

Octreotide, Cyclosporine).[18a,20] Likewise, their cLogP range from very hydrophilic (**26**, cLogP −1.7, close to Octreotide, cLogP −1.0) to reasonably lipophilic, taking as a reference Lipinski's parameters (**28**, cLogP 3.1, similar to Ulimorelin, cLogP 2.8, which possesses a bioavailability of 25%).[21] Their Polar Surface Areas (PSA) cover a broad range (**15**, 108 Å^2 to **34**, 230.4 Å^2), which overlaps with values predictive of an acceptable cellular permeability.[18a] These parameters are readily adjusted by varying ring size and substitution pattern. Finally, the analysis of principal moments of inertia (PMI, Figure 4)[22] reveals that this class of macrocycles covers a shape ensemble that overlaps with that of the aforementioned macrocyclic drugs and development candidates, independently of ring size. Overall, this class of macrocycles appears suitable to support drug discovery. The first biologically active molecules in this class were reported recently.[23]

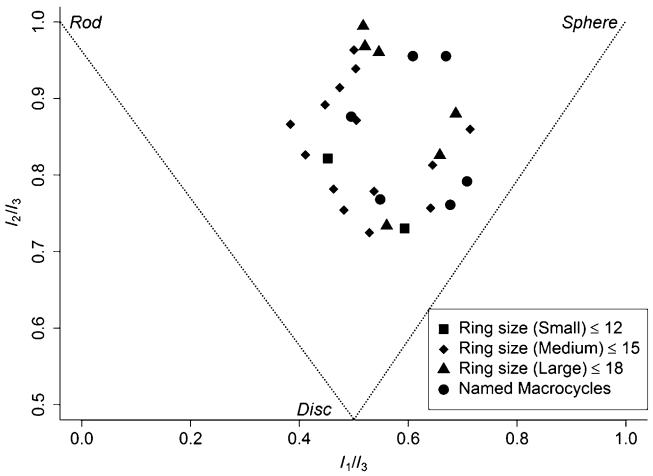


Figure 4. Distribution of Principal Moments of Inertia (PMI) of newly reported macrocycles, separated by ring size. Macrocyclic drugs and development candidates (purple) include cyclosporin, ulimorelin, linopristin, simeprevir, vaniprevir and octreotide.

Conclusion

The methodology reported herein provides a versatile tool for the solid-phase parallel synthesis of diversified libraries of macrocyclic peptidomimetics. The reaction sequence is completely implemented on solid-phase, which minimises transfers and user-intensive operational steps. The method was used to generate a library of several hundred macrocycles, 22 of which are reported herein. The synthesis was achieved by using 24-well Mettler–Toledo parallel synthesis blocks.[24] Typically, the synthesis of a subset of 48 macrocycles is achievable in two weeks by a single chemist (excluding purification and lyophilisation). The method is tolerant of a broad diversity in terms of ring size (9- to 18-membered rings) as well as the nature and stereochemistry of amino acids contained in the ring, providing molecules that also possess a nonpeptidic exocyclic element as an additional point of diversity or subsequent functionalisation. At a time when macrocycles are generating tremendous

interest in the drug discovery community, we anticipate that both the versatility of the method and the properties of the resulting macrocycles will make this technology broadly applicable.

Experimental Section

Representative experimental procedures: synthesis of macrocycle 13

Preparation of Fmoc-L-Glu-OAll anchored to Wang resin through the side chain: Wang resin (0.95 mmol g^{-1}, 9 g, 8.55 mmol, 1 equiv) was swollen in CH_2Cl_2 (150 mL) in a 250 mL glass solid-phase peptide synthesis reactor equipped with a fritted glass funnel. The resin was then further washed with CH_2Cl_2 (2×150 mL, 4 min cycle on an orbital shaker). Fmoc-L-Glu-OAll (8.75 g, 21.375 mmol, 2.5 equiv) and DMAP (157 mg, 1.28 mmol, 0.15 equiv) in CH_2Cl_2 (150 mL) was added to the resin, followed by DIC (3.31 mL, 21.375 mmol, 2.5 equiv). The reaction mixture was shaken overnight on an orbital shaker then drained and washed with CH_2Cl_2, MeOH, CH_2Cl_2, MeOH, CH_2Cl_2 (3×150 mL each, 4 min cycles). The resin was capped with acetic anhydride (3.2 mL, 34.2 mmol, 3 equiv) in the presence of pyridine (2.75 mL, 34.2 mmol, 3 equiv) in anhydrous CH_2Cl_2 (150 mL). Finally the resin was drained, washed (CH_2Cl_2, MeOH, CH_2Cl_2, MeOH, CH_2Cl_2; 3× 150 mL each, 4 min cycles) and dried in vacuo. Loading was confirmed by mini-cleavage (TFA/CH_2Cl_2 (1:1), 1.5 h) and was close to the manufacturer's specifications (0.68 mmol of Fmoc-L-Glu-OAll/g).

Fmoc deprotection: Resin (0.147 mg, 0.1 mmol) was transferred into a polypropylene disposable reactor (10 mL). Piperidine/DMF (20%, 8 mL) was added and the resin was shaken for 30 min. The resin was then drained and the above operation was repeated. Finally, the resin was drained and washed with DMF (5×8 mL).

Attachment of Fmoc-D-Phe-OH followed by Fmoc-D-Pro-OH: Fmoc-D-Phe-OH (116 mg, 0.3 mmol, 3 equiv) and HATU (97 mg, 0.255 mmol, 2.55 equiv) were dissolved in DMF (4 mL). DIPEA (89 μL, 0.51 mmol, 5.1 equiv) was added and the mixture was transferred to the resin. After 4 h agitation, the resin was drained and washed with DMF (2×), IPA, DMF, CH_2Cl_2 (3×), and diethyl ether (8 mL, 4 min cycles). The Fmoc group was deprotected by using 20% piperidine/DMF as described above, then Fmoc-D-Pro-OH was introduced similarly to Fmoc-D-Phe-OH.

Allyl ester deprotection: Resin (0.1 mmol, 1 equiv) was washed with anhydrous CH_2Cl_2 (8 mL). Phenylsilane (123 μL, 1 mmol, 10 equiv) in anhydrous CH_2Cl_2 (2 mL) was added, followed by $Pd(PPh_3)_4$ (35 mg, 0.03 mmol, 0.3 equiv) in anhydrous CH_2Cl_2 (2 mL). The resin was agitated for 3 h then the dark-coloured solution was drained. The resin was washed with 0.5% sodium diethyl dithiocarbamate/DMF (3×), isopropanol, DMF, CH_2Cl_2 (3×) and diethyl ether (8 mL, 3–4 min cycles), then dried in vacuo.

Fmoc deprotection, macrocyclisation and nucleophilic ring-opening of aziridine: The above resin (0.1 mmol) was swollen in DMF (30 min), drained and then piperidine/DMF (20%, 8 mL) was added. After 30 min agitation, the resin was drained and again 20% piperidine/DMF was added. The resin was agitated for 30 min, drained and washed with DMF (5×) then CH_2Cl_2 (5×8 mL, 4 min cycles) and finally with anhydrous CH_2Cl_2/TFE (1:1, 3×3 mL, 4 min cycles). Next, anhydrous CH_2Cl_2 (1 mL) was added, followed by a 0.1 M solution of (*R*)-aziridine aldehyde dimer **11** and *tert*-butyl isocyanide (22.6 μL, 0.2 mmol, 2 equiv) in TFE (1 mL). The reaction mixture was agitated for 4 h, then drained and washed with CH_2Cl_2 (4×8 mL, 4 min cycles). The resin was further washed with DMF (4×8 mL, 4 min cycles), then DMF (2.5 mL) was added, followed by PhSH (113 μL, 1.1 mmol, 11 equiv) and DIPEA (174 μL, 1.0 mmol, 10 equiv). The reaction mixture was agitated overnight then drained and washed with DMF, CH_2Cl_2, MeOH, CH_2Cl_2, MeOH, CH_2Cl_2 (3×8 mL each, 4 min cycles).

NB: The (*R*)-aziridine aldehyde dimer derived from D-Ser was used in the synthesis of macrocycles **13**, **27** and **34**, whereas the other macrocycles were obtained by reaction with (*S*)-aziridine aldehyde dimer derived from L-Ser.

Resin cleavage: The final macrocycle was cleaved with TFA/CH_2Cl_2 (1:1, 6 mL) for 75 min. The resin was then washed (4×) with TFA/CH_2Cl_2 (1:1). Combined filtrates were evaporated and purified by preparative HPLC (**13**–**29**) or MS-triggered preparative HPLC (**31**–**34**). Fractions containing the product were re-analysed by UPLC-MS, pooled and the product was isolated by lyophilisation.

Computational modelling and PMI analysis

Structures were built in Maestro 9.9 and energy minimised by using 10000 steps of the Polak–Ribier Conjugate Gradient method.[25] A Monte-Carlo molecular mechanics conformational search for each macrocycle was carried out by using the OPLS_2005 forcefield and a GB/SA implicit water model as implemented in Macromodel.[26] The electrostatic interaction cutoff distance was set to 20 Å, the van der Waals interaction cutoff distance to 8 Å, and the hydrogen-bonding interaction cutoff distance to 4 Å. Torsional sampling of amide bonds was performed and a 5 kcal mol^{-1} energy window was employed to discard high-energy structures. Mirror-image conformers were retained. Redundant conformers were discarded if they were found to be within an RMSD of 5 Å of existing conformers. All conformers were energy minimised by using 10000 steps of the Polak–Ribier Conjugate Gradient method.

The lowest energy conformation for each macrocycle was selected for PMI analysis. Principal axes of inertia (I_1, I_2, and I_3) were calculated by using a built-in script in Maestro.

Acknowledgements

This work was financially supported by the Consortium Québécois de Recherche sur le Médicament (CQDM). Dr. Andrew Roughton is gratefully acknowledged for helpful comments.

Keywords: drug discovery · macrocycles · peptides · small ring systems · solid-phase synthesis

[1] a) N. K. Bashiruddin, H. Suga, *Curr. Opin. Chem. Biol.* **2015**, *24C*, 131–138; b) S. E. Northfield, C. K. Wang, C. I. Schroeder, T. Durek, M. W. Kan, J. E. Swedberg, D. J. Craik, *Eur. J. Med. Chem.* **2014**, *77*, 248–257; c) J. R. Frost, J. M. Smith, R. Fasan, *Curr. Opin. Struct. Biol.* **2013**, *23*, 571–580; d) M. Gao, K. Cheng, H. Yin, *Biopolymers* **2015**, DOI: 10.1002/bip.22625; e) E. M. Driggers, S. P. Hale, J. Lee, N. K. Terrett, *Nat. Rev. Drug Discovery* **2008**, *7*, 608–624; f) E. Marsault, M. L. Peterson, *J. Med. Chem.* **2011**, *54*, 1961–2004; g) C. J. White, A. K. Yudin, *Nat. Chem.* **2011**, *3*, 509–524; h) A. K. Yudin, *Chem. Sci.* **2015**, *6*, 30–49.

[2] a) E. A. Jefferson, S. Arakawa, L. B. Blyn, A. Miyaji, S. A. Osgood, R. Ranken, L. M. Risen, E. E. Swayze, *J. Med. Chem.* **2002**, *45*, 3430–3439; b) E. Marsault, H. R. Hoveyda, R. Gagnon, M. L. Peterson, M. Vezina, C. Saint-Louis, A. Landry, J. F. Pinault, L. Ouellet, S. Beauchemin, S. Beaubien, A. Mathieu, K. Benakli, Z. Wang, M. Brassard, D. Lonergan, F. Bilodeau, M. Ramaseshan, N. Fortin, R. Lan, S. Li, F. Galaud, V. Plourde, M. Champagne, A. Doucet, P. Bherer, M. Gauthier, G. Olsen, G. Villeneuve, S. Bhat, L. Foucher, D. Fortin, X. Peng, S. Bernard, A. Drouin, R. Deziel, G.

Berthiaume, Y. L. Dory, G. L. Fraser, P. Deslongchamps, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4731 – 4735; c) B. N. Tse, T. M. Snyder, Y. Shen, D. R. Liu, *J. Am. Chem. Soc.* **2008**, *130*, 15611 – 15626.

[3] a) A. R. Bogdan, S. V. Jerome, K. N. Houk, K. James, *J. Am. Chem. Soc.* **2012**, *134*, 2127 – 2138; b) J. E. Delorbe, J. H. Clements, B. B. Whiddon, S. F. Martin, *ACS Med. Chem. Lett.* **2010**, *1*, 448 – 452; c) F. Enck, M. A. Marahiel, A. Geyer, *ChemBioChem* **2008**, *9*, 2597 – 2601.

[4] a) D. G. Rivera, A. V. Vasco, R. Echemendia, O. Concepcion, C. S. Perez, J. A. Gavin, L. A. Wessjohann, *Chem. Eur. J.* **2014**, *20*, 13150 – 13161; b) G. Koopmanschap, E. Ruijter, R. V. Orru, *Beilstein J. Org. Chem.* **2014**, *10*, 544 – 598; c) T. F. Niu, M. Sun, M. F. Lv, W. B. Yi, C. Cai, *Org. Biomol. Chem.* **2013**, *11*, 7232 – 7238.

[5] R. Hili, A. K. Yudin, *J. Am. Chem. Soc.* **2006**, *128*, 14772 – 14773.

[6] a) B. K. W. H. Chung, J. L. Hickey, C. C. G. Scully, S. Zaretsky, A. K. Yudin, *Med. Chem. Commun.* **2013**, *4*, 1124; b) R. Hili, V. Rai, A. K. Yudin, *J. Am. Chem. Soc.* **2010**, *132*, 2889 – 2891; c) X. Li, A. K. Yudin, *J. Am. Chem. Soc.* **2007**, *129*, 14152 – 14153; d) C. C. G. Scully, V. Rai, G. Poda, S. Zaretsky, D. C. Burns, R. S. Houliston, T. Lou, A. K. Yudin, *Chem. Eur. J.* **2012**, *18*, 15612 – 15617; e) A. K. Yudin, R. Hili, *Chem. Eur. J.* **2007**, *13*, 6538 – 6542; f) S. Zaretsky, C. C. G. Scully, A. J. Lough, A. K. Yudin, *Chem. Eur. J.* **2013**, *19*, 17668 – 17672.

[7] S. Zaretsky, J. Tan, J. L. Hickey, A. K. Yudin *Peptide Libraries in Methods in Molecular Biology* (Ed.: R. Derda), Springer Science Business Media, New York, **2015**, pp. 67 – 80.

[8] a) B. A. Bunin, J. A. Ellman, *J. Am. Chem. Soc.* **1992**, *114*, 10997; b) S. A. Kates, F. Albericio, *Solid Phase Synthesis a Practical Guide*, CRC Press, Boca Raton, **2000**.

[9] A. P. Treder, M. C. Tremblay, A. K. Yudin, E. Marsault, *Org. Lett.* **2014**, *16*, 4674.

[10] D. C. Kapeller, S. Bräse, *Synlett* **2011**, 161 – 164.

[11] T. F. Nishiguchi, S. Kuroda, M. Kajisaki, K. Saitoh, *J. Org. Chem.* **1998**, *63*, 8183 – 8187.

[12] G. Rossé, F. Ouertani, H. Schröder, *J. Comb. Chem.* **1999**, *1*, 397 – 401.

[13] a) T. Conroy, K. A. Jolliffe, R. J. Payne, *Org. Biomol. Chem.* **2009**, *7*, 2255 – 2258; b) D. T. Rijkers, J. A. den Hartog, R. M. Liskamp, *Bioorg. Med. Chem.* **2004**, *12*, 5099 – 5106.

[14] B. H. Rotstein, V. Rai, R. Hili, A. K. Yudin, *Nat. Protoc.* **2010**, *5*, 1813 – 1822.

[15] W. D. Meutermans, G. T. Bourne, S. W. Golding, D. A. Horton, M. R. Campitelli, D. Craik, M. Scanlon, M. L. Smythe, *Org. Lett.* **2003**, *5*, 2711 – 2714.

[16] S. Zaretsky, S. Adachi, B. Rotstein, J. L. Hickey, C. C. G. Scully, J. D. St Denis, R. Courtemanche, J. C. Y. Yu, B. K. W. Chung, A. K. Yudin, *J. Org. Chem.* **2014**, *79*, 9948 – 9956.

[17] Free aziridine-containing macrocycles have been observed to be stable to column chromatography, see for example references [6f,7] and, in certain cases, stability was also observed at high temperatures (65 °C in [D_6]DMSO) during variable-temperature NMR studies (Jennifer L. Hickey, Sai Chakka Kumar, Megan A. St. Denis, Monzur Morshed, Serge Zaretsky, Conor C. G. Scully, Andrew L. Roughton, and Andrei K. Yudin; unpublished results).

[18] a) F. Giordanetto, J. Kihlberg, *J. Med. Chem.* **2014**, *57*, 278 – 295; b) A. K. Oyelere, *Curr. Top. Med. Chem.* **2010**, *10*, 1359 – 1360; c) E. A. Villar, D. Beglov, S. Chennamadhavuni, J. A. Porco, Jr., D. Kozakov, S. Vajda, A. Whitty, *Nat. Chem. Biol.* **2014**, *10*, 723 – 731.

[19] N. QikProp v4.2; Schrödinger Inc.; New York, **2014**.

[20] H. R. Hoveyda, E. Marsault, R. Gagnon, A. P. Mathieu, M. Vezina, A. Landry, Z. Wang, K. Benakli, S. Beaubien, C. Saint-Louis, M. Brassard, J. F. Pinault, L. Ouellet, S. Bhat, M. Ramaseshan, X. Peng, L. Foucher, S. Beauchemin, P. Bherer, D. F. Veber, M. L. Peterson, G. L. Fraser, *J. Med. Chem.* **2011**, *54*, 8305 – 8320.

[21] a) C. A. Lipinski, *J. Pharmacol. Toxicol. Methods* **2000**, *44*, 235 – 249; b) D. F. Veber, S. R. Johnson, H. Y. Cheng, B. R. Smith, K. W. Ward, K. D. Kopple, *J. Med. Chem.* **2002**, *45*, 2615 – 2623.

[22] H. S. Beckmann, F. Nie, C. E. Hagerman, H. Johansson, Y. S. Tan, D. Wilcke, D. R. Spring, *Nat. Chem.* **2013**, *5*, 861 – 867.

[23] A. Roxin, J. Chen, C. C. Scully, B. H. Rotstein, A. K. Yudin, G. Zheng, *Bioconjugate Chem.* **2012**, *23*, 1387 – 1395.

[24] http://www.silicycle.com/ca/products/silicycle-miniblock-xt.

[25] S. I. Maestro v9.9, New York, NY, 2014.

[26] S. I. N. Y. Macromodel v10.3, NY, 2014.

Received: January 7, 2015
Published online on May 26, 2015