Synthesis of cyclic peptide mimetics via the successive ring expansion of lactams

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**Abstract:** A successive ring expansion protocol is reported that enables the controlled insertion of natural and non-natural amino acid fragments into lactams. Amino acids can be installed into macrocycles via an operationally simple and scalable iterative procedure, without the need for high dilution. This method is expected to be of broad utility, especially for the synthesis of medicinally important cyclic peptide mimetics.

Macrocycles have important roles across several scientific fields, including medicinal chemistry,[1] catalysis,[2] self-assembly,[3] molecular sensing[4] and supramolecular chemistry.[5] However, functionalised macrocycles are often difficult to make,[6] and in many cases, this can limit their utility. Macrocycles are typically prepared via the end-to-end cyclisation of linear precursors (**1** → **2**, Scheme 1A), which can be difficult and unpredictable processes;[7] the main problem is ensuring that the ‘ends’ of the linear molecule react whilst avoiding unwanted side reactions, especially intermolecular coupling. Macrocyclisation reactions are usually performed at high dilution to minimise such problems, but they are rarely avoided entirely, often resulting in low yielding, impractical processes, especially on large scale. While various strategies have emerged to address these problems,[8] high dilution methods remain the most commonly used, hence new versatile and scalable procedures for the synthesis of functionalised macrocycles will always be of value.

One approach is to assemble macrocycles via ring expansion (**5** → **2**, Scheme 1A).[9, 10] ‘Growing’ macrocycles in this way has a distinct advantage over most other approaches, in that rather than seeking to make the difficult macrocyclisation step easier,ring expansion enables it to be avoided altogether. One such method reported by our group is summarised in Scheme 1B; thus, cyclic β-keto esters (**6**)were shown to undergo a telescoped *C*-acylation/deprotection sequence, resulting in the overall insertion of β-amino acid derivatives (*e.g.* **7**) into ring enlarged products (**8**).[11] A key design feature of this method is that that the β-keto ester group (highlighted) needed to promote the rearrangement is regenerated upon ring expansion, meaning that controlled ‘Successive Ring Expansion’ (SuRE) can be achieved by repeating the same acylation/deprotection sequence on the product **8**. This proof-of-concept study highlighted the potential of SuRE-type methods, although its reliance on a relatively reactive β-keto ester motif is a drawback, as this limits the utility of the products; *e.g.* for medicinal applications, additional synthetic steps are needed to remove the metabolically labile β-keto ester.[12] Average yields of ≈50–60% and the fact that this method only works consistently well with β-amino acid derivatives were also identified as areas in need of improvement.

Herein we describe a new SuRE reaction mode, based on an operationally simple, versatile method for the ring expansion of lactams (**9** → **11**, Scheme 1C). As in our earlier work, successive ring expansion reactions can be performed, but in this case, a secondary lactam group (highlighted) is the repeating unit that enables subsequent iterations. Higher yields (typically ≈80–90%) and much improved substrate scope have been demonstrated, and furthermore, the only functional groups formed in the products are stable amides, auguring well for its use in applied fields that rely on the design and synthesis of macrocycles, especially the synthesis of cyclic peptide mimetics.[13]

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***Scheme 1*.** Macrocyclisation strategies and Successive Ring Expansion (SuRE).

The key ring expansion procedure was tested using 13-membered lactam **9a**, with the aim of performing an *N*-acylation to form an imide (**12**), which could then rearrange to form **11a** upon protecting group cleavage as shown in Scheme 2. Precedent for related transformations meant that these studies were undertaken with relatively high confidence;[14, 15] similar transamidation-type ring expansion processes have been reported by Hesse and coworkers[14a-b] and others,[14c-g] while recent research by Szostak et al. demonstrates the surprising ease with which *N-*acyl-Boc-carbamates undergo intermolecular transamidation.[15] Thus, the *N*-acylation of lactam **9a** was first attempted using Fmoc-protected amino acid chloride **10a**,andwas successfully achieved upon reaction with pyridine and DMAP at reflux in CH2Cl2. The resulting imide **12** was then treated with DBU in CH2Cl2 at RT, which promoted concomitant protecting group cleavage (**12** → **13**) and ring expansion (**13** → **14** → **11a**), furnishing 17-membered ring lactam **11a** in 91% yield over the telescoped two-step sequence. Next, having confirmed the viability of this ring expansion method, it was important to establish whether it could be applied successively; to test this, lactam **11a** was reacted under the same conditions, and with no additional optimisation, the 21-membered ring product **15a** was formed in 81% isolated yield. As the second ring expansion was performed in the same way as the first, the product also contains a secondary lactam group (highlighted), and hence is another potential starting substrate for further SuRE iterations (*vide infra*).



***Scheme 2*.** Successive ring expansion of lactam **9a**.

Having uncovered an efficient new ring expansion method, and proved that it can be applied successively, attention turned to exploring its scope (Scheme 3). First, other β-amino acid chlorides were tested in place of initial test substrate **10a** (Scheme 3A). *N­-*Methylated variant **11b** was prepared in high yield (from both Fmoc- and Cbz-protected amino acid chlorides), which is important given that *N*-methylation is of much interest as a tool to improve the druggability of cyclic peptides.[16] Branched β-amino acids are also tolerated (*e.g.* **11c**), as are protected primary amino acids, exemplified by the formation of lactam **11d** in high yield; of further note, this example was repeated on larger scale (10 mmol) with no significant drop in yield, and its structure was confirmed by X-ray crystallography.[17] Importantly, the method works consistently well across a range of ring sizes, with 6–12-membered lactams all undergoing ring expansion in high yield (78–90%) using acid chloride **10a** (Scheme 3B). In view of the well-known problems associated with making medium-sized rings,[9, 10] the formation of **11e** and **11f** using the standard method is particularly pleasing.

Cyclic peptides and their mimics are of significant medicinal interest,[13] hence we were keen to demonstrate that α-amino acid derivatives could also be used in this method. α-Amino acid derivatives proved to be much more challenging than their β-homologues in our published β-keto ester SuRE reactions (which only work consistently well with glycine derivatives),[11, 12]  but pleasingly, the current lactam ring expansions are far more robust. Thus, *N-*acylation and ring expansion can be achieved with a range of α-amino acid chlorides, including examples with hydrocarbon side chains, tryptophan, methionine and proline derivatives (**11l**–**u**,Scheme 3C).[18] Some variation in yield was observed across this series, but note that these reactions were all performed using the same conditions and are unoptimised. Cyclic peptoids are also of interest for their medicinal properties,[19] and we have demonstrated that 4 peptoid building blocks are well tolerated, furnishing expanded lactams **11v**–**y** in high yields (Scheme 3D).

Selected special cases (Scheme 3E) illustrate that the scope of this method is likely to extend beyond cyclic peptides. For example, the same rearrangement has been demonstrated on a linear amide (*N-*ethyl propanamide), to form diamide **11z**. 2-Azetidinone can also undergo *N-*acylation and ring expansion, with a Cbz-protection strategy favoured in this case, as this helped with the purification of the polar 8-membered ring product **11aa**.[20] Lactone groups can also be installed into ring-expanded products, by replacing the protected amine with a benzyl-protected alcohol; following ­*N-*acylation, the alcohol is revealed by hydrogenolysis to initiate ring expansion (**11ab**, **11ac**).[21] We anticipate that this will be useful for the synthesis of medicinally important lactones, *e.g.* macrolide-class natural products and their derivatives, which are well known for their anti-microbial properties.[22] It is also worth noting that every ring expansion reaction reported in this manuscript was performed using a 0.1 M reaction concentration, and hence should be easier to scale than typical high-dilution macrocycle syntheses.

One of the most important features of this research is the fact that all the products shown in Scheme 3 are potential starting materials for further ring expansion, as the secondary amide group (highlighted) produced in the reaction provides a synthetic handle for further iterations. To showcase this feature, 6 macrocycles formed via a second ring expansion reaction (**15a**–**f**) are illustrated in Scheme 4. These reactions, which were performed using the standard conditions, include macrocycles made with α- and β-amino acid derivatives and peptoid building blocks. Of course, these products represent a small fraction of those potentially accessible; by using the full array of linear fragments shown in Scheme 3, the synthesis of a much larger array of functionalised macrocycles should be possible. Finally, we have confirmed that ring expansion for a third time to form a tripeptide system is also possible (**16a**). Additional acid chloride **10a** was needed in this case to ensure that the *N*-acylation to proceeded to completion, but the isolated yield (77%) remained comparable with other examples.



**Scheme 3.** Lactam ring expansion scope. a Conditions A: i) To lactam **9** (1 equiv.), pyridine (6 equiv.) and DMAP (0.1 equiv.) in CH2Cl2 (7 mL/mmol of **12**) add **10** (1.5 equiv.) in CH2Cl2 (3 mL/mmol of **12**), 18 h at 50 °C; ii) DBU or piperidine (10 equiv.), CH2Cl2 (10 mL/mmol), RT, 18 h. b Conditions B: i) Acylation step as in conditions A; ii) H2, Pd/C, EtOAc (10 mL/mmol), RT, 18 h. c Also performed on 10 mmol scale (≈2.5 g, 94%).

In summary, a new SuRE protocol for the formation of cyclic peptide mimetics is described, based on the expansion of simple, readily available lactams. The method is operationally simple, scalable, and allows a wide range of peptide-containing macrocycles to be generated, typically in high yield. This is expected to be of use in the synthesis of macrocycles with medicinal applications, such as stapled peptides.[23] Cyclic peptide mimetics are also of much medicinal interest, and this method appears particularly well suited for the synthesis of this compound class; macrocycles based on short amino acid sequences and a non-peptidic portion are important in this area,[13] withexemplar macrocycle classes with proven therapeutic potential shown in Scheme 4 (**17** and **18**).[24] In this manuscript, we have mainly focused on the potential of SuRE to be an enabling technology in medicinal chemistry, but by increasing the freedom with which researchers can design and synthesise peptide-containing macrocycles, useful applications in other areas[2-5] are also anticipated to emerge in time.

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**Keywords:** Macrocycles • Medium-sized Rings • Ring Expansion • Cyclic peptides • Lactams



**Scheme 4.** Successive Ring Expansion reactions (SuRE). a Conditions A: i) To lactam **9** (1 equiv.), pyridine (6 equiv.) and DMAP (0.1 equiv.) in CH2Cl2 (7 mL/mmol of **12**) add **10** (1.5 equiv.) in CH2Cl2 (3 mL/mmol of **12**),18 h at 50 °C; ii) DBU (10 equiv.), CH2Cl2 (10 mL/mmol), RT, 18 h. b 4.5 equiv. of **10a** used.

[1] (a) E. M. Driggers, S. P. Hale, J. Lee, N. K. Terrett, *Nat. Rev. Drug Discov.* **2008**, *7*, 608; (b) E. Marsault, M. L. Peterson, *J. Med. Chem.* **2011**, *54*, 1961; (c) F. Giordanetto, J. Kihlberg *J. Med. Chem.* **2014**, *57*, 278; (d) A. K. Yudin, *Chem. Sci*. **2015**, *6*, 30.

[2] (a) S. Dawn, M. B. Dewald, D. Sobransingh, M. C. Paderes, A. C. Wibowo, M. D. Smith, J. A. Krause, P. J. Pellechia, L. S. Shimizu, *J. Am. Chem. Soc.* **2011**, *133*, 7025.

[3] J. Montenegro, M. R. Ghadiri, J. R. Granja, *Acc. Chem. Res*. **2013**, *46*, 2955.

[4] T. Ema, D. Tanida, T. Sakai, *J. Am. Chem. Soc.* **2007**, *129*, 10591.

[5] (a) K. E. Griffiths, J. F. Stoddart, *Pure Appl. Chem*. **2008**, *80*, 485; (b) N. H. Evans, P. D. Beer, *Chem. Soc. Rev*. **2014**, *43*, 4658; (c) M. Xue, Y. Yang, X. Chi, X. Yan, F. Huang, *Chem. Rev*. **2015**, *115*, 7398.

[6] (a) M. Hesse in *Ring Enlargement in Organic Chemistry,* Wiley-VCH, Weinheim, **1991**; (b) Practical Medicinal Chemistry with Macrocycles, E. Marsault, M. L. Peterson Eds, Wiley, **2017**.

[7] (a) J. Fastrez, *J. Phys. Chem*. **1989**, *93,* 2635; (b) J. C. Collins, K. James, *Med. Chem. Commun*. **2012**, *3*, 1489.

[8] For prominent macrocyclisation methods and review articles, see: (a) Z. J. Gartner, B. N. Tse, R. Grubina, J. B. Doyon, T. M. Snyder, D. R. Liu, *Science* **2004**, *305*, 1601; (b) A. Parenty, X. Moreau, and J.-M. Campagne, *Chem. Rev*., **2006**, *106*, 911; (c) R. Hill, V. Rai, A. K. Yudin, *J. Am. Chem. Soc.* **2010**, *132*, 2889; *130*, 15611; (d) C. J. White, A. K. Yudin, *Nat. Chem.* **2011**, *3*, 509; (e) A. Fürstner, Science **2013**, *341*, 1229713; (f) A. P. Treder, J. L. Hickey, M.-C. J. Tremblay, S. Zaretsky, C. C. G. Scully, J. Mancuso, A. Doucet, A. K. Yudin, E. Marsault, *Chem. Eur. J*. **2015**, *21*, 9249.

[9] (a) F. Kopp, C. F. Stratton, L. B. Akella, D. S. Tan, *Nat. Chem. Bio.* **2012**, *8*, 358; (b) R. A. Bauer, T. A. Wenderski, D. S. Tan, *Nat. Chem. Bio.* **2013**, *9*, 21. (c) J. E. Hall, J. V. Matlock, J. W. Ward, J. Clayden, *Angew. Chem. Int. Ed*. **2016**, *55*, 11153; (d) Z.-L. Li, X.-H. Li, N. Wang, N.-Y. Yang, X.-Y. Liu, *Angew. Chem. Int. Ed.* **2016** *55*, 15100.

[10] For a review, see; J. R. Donald, W. P. Unsworth, *Chem. Eur. J*. **2017**, *23*, 8780.

[11] C. Kitsiou, J. J. Hindes, P. I’Anson, P. Jackson, T. C. Wilson, E. K. Daly, H. R. Felstead, P. Hearnshaw, W. P. Unsworth, *Angew. Chem. Int. Ed*. **2015**, *54*, 15794

[12] L. G. Baud, M. A. Manning, H. L. Arkless, T. C. Stephens, W. P. Unsworth, *Chem. Eur. J*. **2017**, *9*, 2225.

[13] See references 1 and 3, and: (a) R. H. Kohli, C. T. Walsh, M. D. Burkart, *Nature* **2002**, *418*, 658; (b) J. Gavenonis, B. A. Sheneman, T. R. Siegert, M. R. Eshelman, J. A. Kritzer, *Nat. Chem. Biol.* **2014**, *10*, 716; (c) E. A. Villar, D. Beglov, S. Chennamadhavuni, J. A. Porco Jr, D. Kozakov, S. Vajda, A. Whitty, *Nat. Chem. Biol.* **2014**, *10*, 723; (d) W. Xu, Y. H. Lau, G. Fischer, Y. S. Tan, A. Chattopadhyay, M. de la Roche, M. Hyvönen, C. Verma, D. R. Spring, L. S. Itzhaki, *J. Am. Chem. Soc.* **2017**, *139*, 2245.

[14] See references 6a, 10 and: (a) R. Wälchli, A. Guggisberg, M. Hesse, *Tetrahedron Lett.* **1984**, *21*, 2205; (b) U. Kramer, A. Guggisberg, M. Hesse, H. Schmid, *Angew. Chem. Int. Ed.* **1978**, *17*, 200; (c) M. M. Shemyakin, V. K. Antonov, A. M. Shkrob, V. I. Shchelokov, Z. E. Agadzhanyan, *Tetrahedron***1965**, 21, 3537; (d) G. I. Glover, Robert B. Smith, H. Rapoport, *J. Am. Chem. Soc.* **1965**, *87*, 2003; (e) J. M. Muchowski, *Can. J. Chem.* **1970**, *48*, 1946, (f) A. N. Chulin, I. L. Rodionov, L. K. Baidakova, L. N. Rodionova, T. A. Balashova, V. T. Ivanov, *J. Peptide Sci.* **2005**, 11, 175; (g) V. N. Azev, A. N. Chulin, I. L. Rodionov **2014**, *50*, 145.

[15] Y. Liu, S. Shi, M. Achtenhagen, R. Liu, M. Szostak, *Org. Lett*. **2017**, *19* 1614.

[16] (a) J. Chatterjee, C. Gilon, A. Hoffman, H. Kessler, *Acc. Chem. Res*., **2008**, *41*, 1331; (b) J. Chatterjee, F. Rechenmacher, H. Kessler, *Angew. Chem. Int. Ed.* **2013**, *52*, 254; (c) D. P. Slough, H. Yu, S. M. McHugh, Y.-S. Lin, *Phys. Chem. Chem. Phys*. **2017**, *19*, 5377.

[17] CCDC 1559565 contains 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](http://www.ccdc.cam.ac.uk/data_request/cif).

[18] Products **11l–u**,generated from enantiopure amino acid derivatives, retained high negative [α]D values, suggesting that little/no epimerisation takes place during ring expansion (see Supporting Information).

[19] (a) S. B. Y. Shin, B. Yoo, L. J. Todaro, K. Kirshenbaum, *J. Am. Chem. Soc*. **2007**, *129*, 3218; (b) A. M. Webster, S. L. Cobb, *Tetrahedron Lett.* **2017**, *58*, 1010.

[20] For a related β-lactam expansion, see: A. Klapars, S. Parris, K. W. Anderson, S. L. Buchwald, *J. Am. Chem. Soc.* **2004**, *126*, 3529.

[21] For related ring expansion processes leading to lactone products, see; (a) E. J. Corey, D. J. Brunelle, K. C. Nicolaou, *J. Am. Chem. Soc.* **1977***, 99,* 7359; (b) J. E. Forsee, J. Aubé, *J. Org. Chem.* **1999**, *64*, 4381.

[22] I. B. Seiple, Z. Zhang, P. Jakubec, A. Langlois-Mercier, P. M. Wright, D. T. Hog, K. Yabu, S. R. Allu, T. Fukuzaki, P. N. Carlsen, Y. Kitamura, X. Zhou, M. L. Condakes, F. T. Szczypiński, W. D. Green, A. G. Myers, *Nature*, **2016**, *533*, 338.

[23] Y. H. Lau, P. de Andrade, Y. Wu, D. R. Spring, *Chem. Soc. Rev*. **2015**, *44*, 91.

[24] (a) H. R. Hoveyda, E. Marsault, R. Gagnon, A. P. Mathieu, M. Vézina, 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. Bhérer, D. F. Veber, M. L. Peterson, G. L. Fraser, *J. Med. Chem*. **2011**, *54*, 8305; (b) H. Karatas, Y. Li, L. Liu, J. Ji, S. Lee, Y. Chen, J. Yang, L. Huang, D. Bernard, J. Xu, E. C. Townsend, F. Cao, X. Ran, X. Li, B. Wen, D. Sun, J. A. Stuckey, M. Lei, Y. Dou, S. Wang, *J. Med. Chem*. **2017**, *60*, 4818.

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