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A pincer auxiliary to force difficult lactamisations †

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A new method to cyclise peptides is reported based on insertion of a salicylaldehyde derived pincer auxiliary in the linear precursor sequence. H-βAla-Phe-OH and H-Phe-βAla-OH were chosen as representative model peptides.

Cyclic peptides constitute a versatile compound class.[1] Especially small cyclic peptides are promising in drug,[2] materials[3] and catalysis applications.[4] However, in general head-to-tail ring-closure of small cyclopeptides (strategy **A**, Scheme 1) has proven to be difficult.[5] In addition and in vast contrast to the cyclodipeptides (diketopiperazines), the majority of the (7-membered) monocyclic homodiketopiperazines, made up of an α- and a linear β-amino acid, are difficult to cyclise. Consequently, only a limited number of cyclotetrapeptides[6] and homodiketopiperazines[7] have been described. These rings have been closed successfully only due to the presence of turn-inducing residues and the application of high-dilution conditions. Also, the correct choice of the coupling site has shown to be crucial. To say it concisely, to date, no generally applicable and thus sequence independent methods exist to effect small peptide cyclisations. The main reason for the cyclisation failure is the predominant *trans* arrangement of the amide bond(s) in the linear precursor, preventing a correct spatial positioning of the terminal amine and the activated carboxylic group for cyclisation.[5,8] Unexpectedly, the inclusion of secondary amino acids (*e.g.* proline or sarcosine) in the linear precursor sequence, resulting in a conformationally unbiased tertiary amide bond, does not necessarily lead to efficient cyclisations.[9]

Recently, we have developed a novel auxiliary mediated combined tethered–templated strategy to cyclise medium-sized lactams.[10] In our search for new sequence independent methods for small peptide cyclisations we report an improved auxiliary, giving access to lactams containing multiple endocyclic amide bonds. Our approach is based on the use of a pincer auxiliary that facilitates *two* subsequent lactamisations by playing a dual tethering and templating role (strategy **B**, Scheme 1).

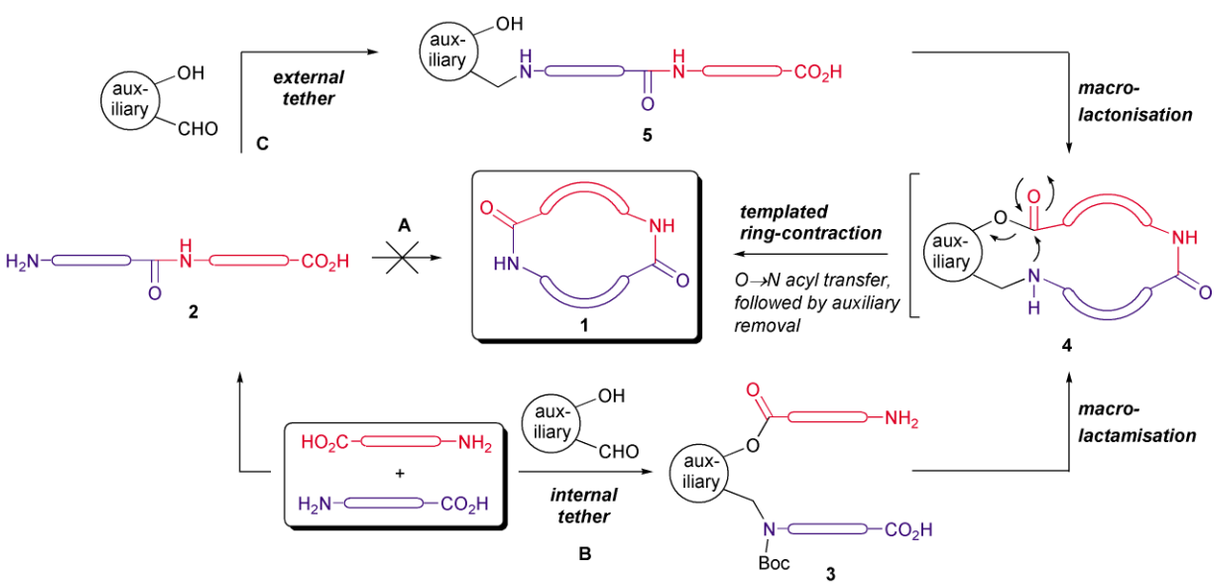
In strategy **B**, in the first macrolactamisation step (*i.e.* **3**⟶**4**) the auxiliary serves as an internal tether (or hinge) allowing an unconstrained approach of the mutually reactive amino and activated ester groups, followed by the final templated lactamisation by a transannular ring-contracting O⟶N acyl transfer reaction (*i.e.* **4**⟶**1**).

Another strategy (strategy **C**, Scheme 1), which has been applied successfully in the cyclisation of pentapeptides in a pioneering study by Meutermans and coworkers,[11] relies on functionalisation of the *N*-terminus of the peptide with a similar auxiliary now serving as an external tether. Activation of the carboxylic acid leads to a macrolactonisation reaction (*i.e.* **5**⟶**4**), still hampered by the predominant *trans* amide bonds in the linear precursor, followed by the same templated transannular ring-contraction to give the final lactam.

In the present communication the power of our novel internal auxiliary based method (strategy **B**, Scheme 1) will be demonstrated as compared to the other strategies (strategies **A** and **C**, Scheme 1). In our study the monocyclic homodiketopiperazine 3-benzyl[1,4]diazepane-2,5-dione (or cyclo(-Phe-βAla-)) **1a**, composed of phenylalanine and β-alanine, was used as a difficult to lactamise model cyclopeptide (Scheme 2).

Strategy A: That indeed ring-closure to give cyclo(-Phe-βAla-) **1a** by direct head-to-tail lactamisation of H-βAla-Phe-OH **2a** or H-Phe-βAla-OH **2b** (Scheme 3) is difficult is underscored by the finding that no cyclisation could be

† Electronic supplementary information (ESI) available: experimental details. See http://www.rsc.org/suppdata/ob/b3/b303836j/



Scheme 1 Cyclisation strategies towards bislactams.

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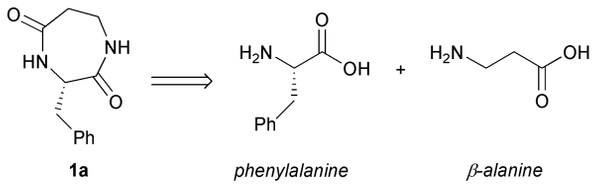
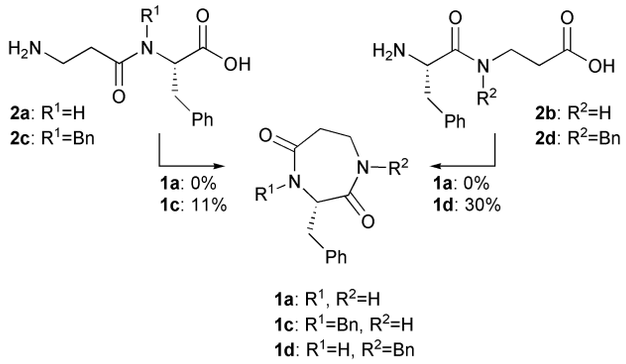


Scheme 2 Target homodiketopiperazine **1a**.



Scheme 3 Strategy A; direct cyclisation. *Reagents and conditions:* EDC (5 equiv), HOBt (4 equiv), DMF–CH_2Cl_2 (1:4), RT.

accomplished using the most powerful coupling reagents (*i.e.* EDC–HOBt, BOP, TBTU and PfPyU) at high dilution. Lactamisation of the amide *N*-benzylated precursors H-βAla-BnPhe-OH **2c** and H-Phe-BnβAla-OH **2d**, employing a tertiary amide bond under high-dilution conditions, indeed gave the corresponding homodiketopiperazines cyclo(-βAla-BnPhe-) **1c** and cyclo(-Phe-BnβAla-) **1d**, albeit in yields of only 11% and 30%, respectively.

Strategy B: The recent results published by Meutermans and coworkers[11] and our preliminary study,[10] prompted us to use salicylaldehyde-derived auxiliaries for the combined tethered–templated approaches towards cyclo(-Phe-βAla-) **1a**. Salicylaldehyde derived auxiliaries combine the high reactivity of an aryl ester towards aminolysis (enthalpic activation) with a correct spatial positioning of the secondary amine and carbonyl functional groups for the transannular ring-contraction reaction (entropic activation).

Synthesis of the linear cyclisation precursors **3a** and **3b** to test our internal tethered strategy was accomplished by a reductive amination reaction of H-βAla-OBn and H-Phe-OBn with 3-*tert*-butoxy-2-hydroxybenzaldehyde **6**,[12] followed by Boc-protection of the resulting amine and esterification with Cbz-Phe-OH or Cbz-βAla-OH, respectively (Scheme 4). After liberation of the carboxylic acid and amine functional groups by catalytic hydrogenolysis under neutral conditions, the tethered lactamisation precursors **3a** and **3b** were isolated as stable compounds.

The introduction of the sterically demanding *tert*-butoxy group at the *ortho* position with respect to the ester group has shown to be crucial because preliminary experiments, starting from salicylaldehyde, suffered from extensive side-reactions due to premature intermolecular aminolysis.[13]

Activation of the carboxylic acids of **3a** and **3b** by treatment with EDC–HOBt effected the tethered cyclisation reactions to give the macrocyclic lactams **4a** and **4b** in yields of 84% and 71%, respectively. Removal of the Boc and *tert*-butyl protective groups by stirring in TFA–dichloromethane was followed by the addition of solid $NaHCO_3$ to induce the final templated transannular O⟶N acyl transfer reaction furnishing the final homodiketopiperazines **1g** and **1h**, both in yields of 79%.

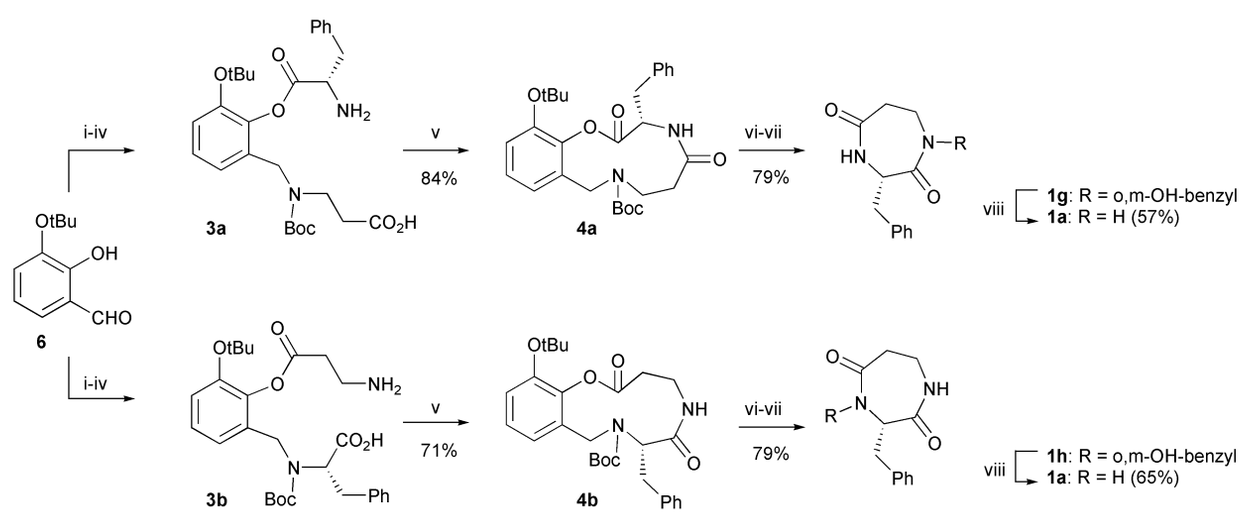
These results show that our approach, featuring an auxiliary which is inserted in the peptide bond, and serves as a tethering hinge or molecular pincer facilitating the mutually reactive terminal functional groups to approach one and another, is sequence independent. Also, due to the connection of the auxiliary onto the C-terminal amino acid as a tertiary amide (*i.e.* compounds **3**), this residue is shielded from racemisation, which often occurs with difficult peptide cyclisations.[5]

Removal of the remaining 1-(2,3-dihydroxybenzyl) group was accomplished by reduction with sodium in liquid ammonia after methylation of the phenolic-OH groups to give cyclo-(-Phe-βAla-) **1a** in overall yields of 57% (from **1g**) and 65% (from **1h**) (Scheme 4).

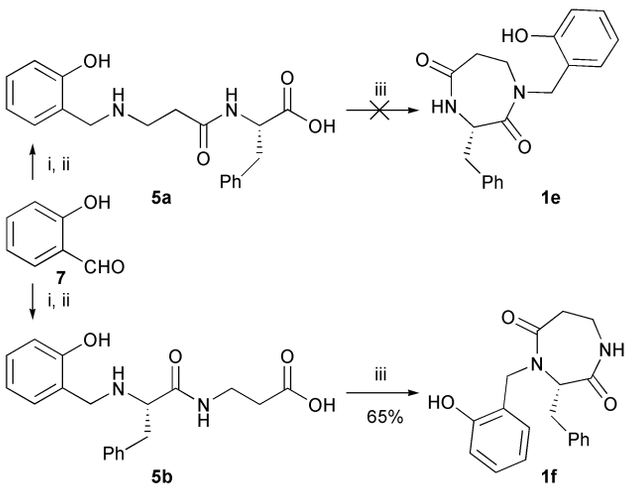
Strategy C: For the synthesis of **1a** *via* strategy C, the N-termini of H-βAla-Phe-OBn and H-Phe-βAla-OBn were reductively alkylated with salicylaldehyde **7**, followed by liberation of the carboxyl group by catalytic hydrogenolysis to furnish **5a** and **5b**, respectively.

The consecutive macrolactonisation and ring-contractive lactamisation reactions were conducted using the optimised conditions as developed by Meutermans and coworkers.[11] Activation of **5a** with BOP (1.1 equiv) using DiPEA (2 equiv) as the base in DMF under high dilution (1 mM) only gave complex reaction mixtures. On the other hand, **5b** cyclised smoothly to give homodiketopiperazine **1f** in a yield of 65% (Scheme 5). Although the reason for the cyclisation failure of **5a** is unclear to us,[14] it is obvious that strategy B, featuring an external tethering auxiliary, is strongly sequence dependent.

In conclusion, we have developed a new and powerful auxiliary mediated lactamisation strategy towards small cyclopeptides. This was demonstrated by the successful



Scheme 4 Strategy B; internal tether–template. *Reagents and conditions:* i, H-βAla-OBn or H-Phe-OBn, $Na(OAc)_3BH$, THF; ii, $(Boc)_2O$, CH_2Cl_2; iii, Cbz-Phe-OH or Cbz-βAla-OH, DCC, DMAP, CH_2Cl_2; iv, H_2, Pd/C, EtOAc–iPrOH = 4 : 1; v, EDC–HOBt (5 equiv); vi, TFA–CH_2Cl_2 = 1 : 1; vii, $NaHCO_3$, EtOAc; viii, MeI, K_2CO_3, followed by Na, NH_3(l).



Scheme 5 Strategy C; external tether–template. *Reagents and conditions:* i, H-βAla-Phe-OBn or H-Phe-βAla-OBn, $Na(OAc)_3BH$, CH_2Cl_2; ii, H_2, Pd/C, EtOAc–iPrOH = 4 : 1; iii, BOP (1.1 equiv), DiPEA (2 equiv), DMF (1 mM).

sequence independent ring-closure to a homodiketopiperazine, which could not be prepared efficiently using the currently known lactamisation methods. Currently, our novel internal auxiliary mediated combined tethered–templated approach is expanded towards the synthesis, of to date, inaccessible homodetic and heterodetic cyclotetrapeptides.

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12 The synthesis of the novel auxiliary **6** was easily accomplished in one step from commercially available 2,3-dihydroxybenzaldehyde (see electronic supplementary material).†

13 The shielding effect of *ortho*-alkoxy groups for aminolysis reactions of aryl esters has been described before. After removal of the *tert*-butyl group, the reactivity of the phenolic ester is enhanced due to the formation of a hydrogen bond between the *ortho*-phenolic OH and the ester carbonyl group. See: (*a*) G. T. Bourne, S. W. Golding, R. P. McGeary, W. D. F. Meutermans, A. Jones, G. R. Marshall, P. F. Alewood and M. L. Smythe, *J. Org. Chem.*, 2001, **66**, 7706–7713; (*b*) C. L. Beech, J. F. Coope, G. Fairley, P. S. Gilbert, B. G. Main and K. Plé, *J. Org. Chem.*, 2001, **66**, 2240–2245.

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