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Skeletal diversity by sequential one-pot and stepwise routes using morpholine ester scaffolds

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ABSTRACT

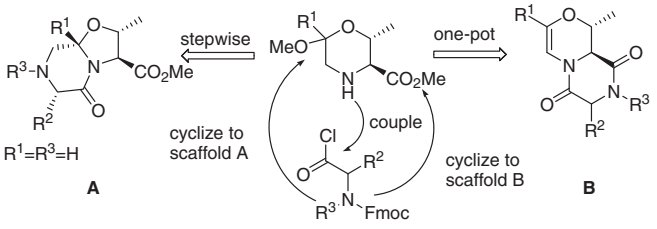
A skeletal diversity approach starting from morpholine acetals has been achieved by tuning a three-step process from stepwise to sequential one-pot to provide diverse scaffolds containing either a 2-oxopiperazine or a diketopiperazine skeleton.

Diversity-Oriented Synthesis (DOS)[1] is a powerful concept for developing large collections of structurally diverse small molecules, with the aim to explore larger portions of the chemical space in drug discovery issues and in the investigation of biological pathways. The principles of DOS span from the concept of generating structurally diverse compounds from a divergent approach consisting in a complexity-generating reaction followed by cyclization steps and appendage diversity, to the development of different cyclic structures through the build/couple/pair approach.[2] Also, among the synthetic strategies to produce new chemical entities, cascade, multicomponent, and multistep one-pot reactions have gained wide acceptance because they increase synthetic efficiency by decreasing the number of laboratory operations required and the quantities of chemicals and solvents used.[3] Thus, these reactions can facilitate ecologically and economically favorable syntheses. Recently, we turned our attention to the chemistry of morpholines,[4] as this heterocycle represents a common motif in medicinal chemistry, being found in several bioactive molecules, such as TACE, MMP, and TNF inhibitors.[5] Among the privileged heterocycles, diketopiperazines (DKPs) have been found to possess important biological properties, such as antitumor, antimicrobial, and antiviral activities.[6] Also, piperazines and 2-oxopiperazines have been widely recognized as 'privileged scaffolds' in medicinal chemistry, due to their presence as structural elements in a vast array of natural products and biologically active compounds.[7]

We envisioned the possibility of using morpholine-based heterocycles to achieve a skeletal diversity through the manipulation of the couple/pair approach. Specifically, the strategic approach was conceived to access different scaffolds by the combination of morpholine acetals with some α-amino acids via a one-pot process or a stepwise route (Scheme 1).

The modulation of the synthetic process using identical coupling partners proved to give two different bicyclic structures as a consequence of different mechanisms involved in the three-steps routes.

Morpholine derivative **2** was achieved from O-protected threonine and dimethoxyacetaldehyde as a separable mixture of diastereomers, as reported.[4b] Interestingly, the reaction of unprotected threonine methyl ester with phenacyl bromide in *N*-methylpyrrolidone (NMP), followed by cyclization in acidic methanol gave access to phenyl-substituted morpholine compound **4** as a single stereoisomer in 36% overall yield (Scheme 2). Diagnostic NOE correlation between the methoxy group at C-6 and H-2 allowed the



Scheme 1. Skeletal diversity through a couple-cyclize approach.

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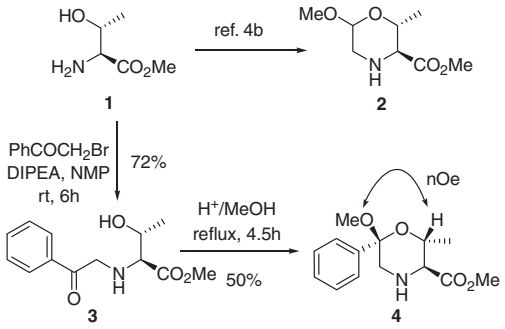


Scheme 2. Synthesis of morpholine ester scaffolds.

assignment of the C-6 absolute configuration, as shown in Scheme 2 and Figure 1.

Bicyclic compounds containing both the 1,4-dihydrooxazine and the diketopiperazine nuclei were obtained through a sequential one-pot route embracing the three steps (Fig. 2). Specifically, the process consisted of coupling of Fmoc-protected α-amino acyl chlorides with morpholine derivatives **2** or **4**, followed by acid-mediated double bond formation to give the 1,4-dihydro-oxazine nucleus and final Fmoc-deprotection with concomitant cyclization to give the diketopiperazine cycle. This process was conceived by applying a sequence of base-, acid-, and again base-mediated reactions, all carried out in one-pot. Thus, the coupling of the Fmoc-amino acid residue to the morpholine acetal was achieved by using the corresponding Fmoc-amino acid chloride and 2 equiv of 2,6-lutidine as base, as described by Carpino et al.[8] The use of standard COOH activators proved to be less efficient than the acyl chlorides in the coupling reaction. After completion of the reaction, as judged by TLC, 5 equiv of *p*-toluenesulfonic acid were added, and the mixture was diluted with toluene to allow for the generation of the intermediate 1,4-dihydroxazine ring by the azeotropical elimination of methanol. Such an amount of *p*TSA was assessed as the lowest to achieve complete conversion to the oxazine intermediate. Final treatment with an excess of diethylamine gave the final

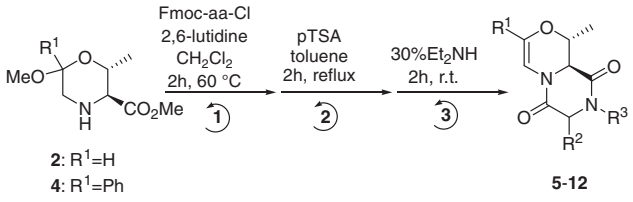


Figure 2. Sequential one-pot process to bicyclic diketopiperazines **5**–**12**.

bicyclic diketopiperazine by virtue of Fmoc-deprotection and concomitant intramolecular cyclization of the free amine to the ester carbonyl group. This process proved to be effective for a number of amino acids (Table 1),[9] though phenylalanine and leucine showed reduced yields, possibly due to the lowered stability of the corresponding precursors in the process. Particularly effective was the reaction with proline derivatives, giving the highest yields in the overall three-step process using both morpholines **2** and **4**.

The stepwise process[10] consisted of an acid–base workup after the coupling step, followed by treatment of the resulting adduct in refluxing toluene (0.2 M) and in the presence of 1 equiv of *p*TSA (Fig. 3). This protocol produced a different reaction outcome as a consequence of a different mechanism taking part during the second step of the process, giving bicyclic 2-oxopiperazines with

Table 1
Sequential one-pot process to bicyclic diketopiperazines **5**–**12**

Compd	Amino acid	R_1	R_2	R_3	Yield (%)
5	L-Val	Ph	$CH(CH_3)_2$	H	32
6	L-Pro	Ph	$(CH_2)_3$		92
7	L-Pro	H	$(CH_2)_3$		76
8	D-Pro	H	$(CH_2)_3$		69
9	L-(Bn)Ser	H	CH_2OBn	H	48
10	D-Val	H	$CH(CH_3)_2$	H	77
11	L-Phe	H	CH_2Ph	H	34
12	L-Leu	H	$CH_2CH(CH_3)_2$	H	15

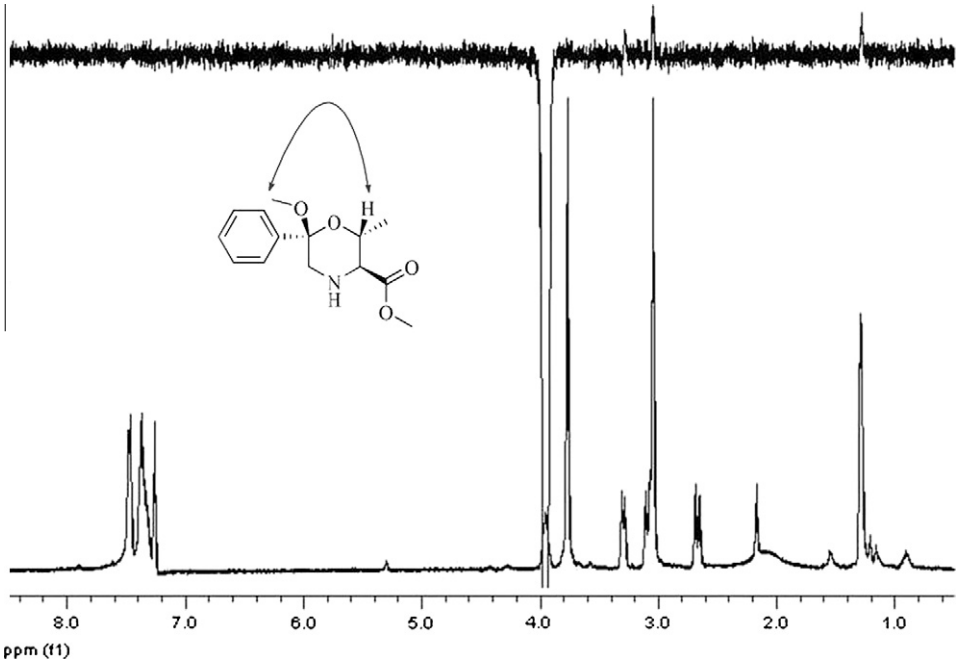


Figure 1. Diagnostic NOE peaks between the irradiated proton H-2 and the methoxy group at C-6 of compound **4**.

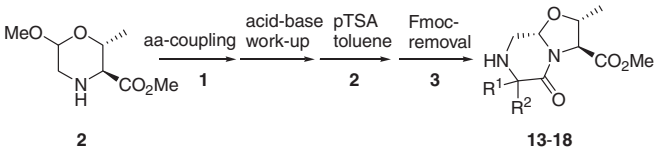
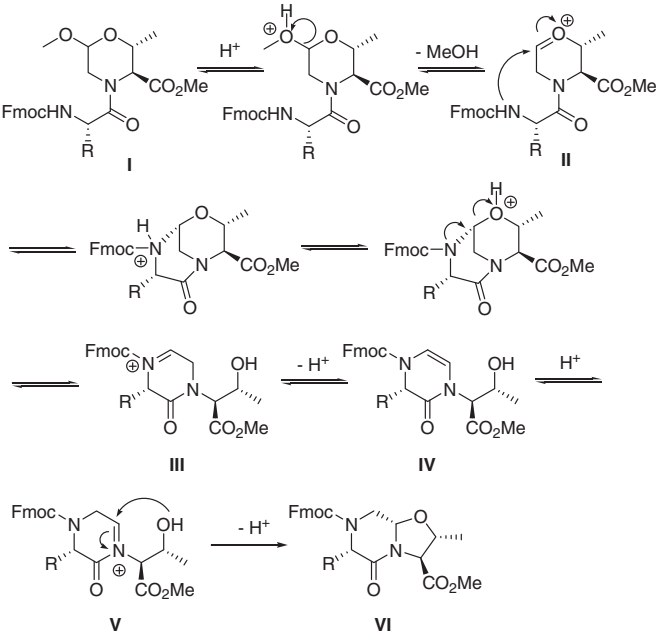


Figure 3. Stepwise process to bicyclic 2-oxopiperazines **13**–**18**.

complete diastereoselectivity. This result was achieved by the removal of 2,6-lutidine and its salts from the reaction mixture and by the use of fixed amounts of *p*TSA and toluene. Adduct **I** (Scheme 3), after protonation, is thought to undergo an intramolecular attack by the protected nitrogen atom to the oxonium moiety (structure **II**), followed by ring-opening of the morpholine ring to the iminium **III**. This species isomerizes by a deprotonation–protonation process to the shifted iminium intermediate **V**, following final cyclization to give the Fmoc-protected bicyclic 2-oxopiperazine **VI**.

According to this mechanism, the reaction of **2** with Fmoc-Pro-Cl under these conditions did not proceed to the bicyclic 2-oxopiperazine scaffold but gave the corresponding bicyclic diketopiperazine after Fmoc-deprotection (Table 2, compd **7**).

Moreover, morpholine derivative **4**, bearing a phenyl group at position 6 did not give the corresponding bicyclic 2-oxopiperazine, due to the altered reactivity at the benzylic position which did not allow for the rearrangement as described in Scheme 3.



Scheme 3. Hypothesized mechanism for the formation of the 2-oxopiperazine ring.

Table 2
Stepwise process to 2-oxopiperazinones **13**–**18**

Compd	L-Amino acid	R_1	R_2	Yield (%)
13	Val	H	$CH(CH_3)_2$	76
14	Ala	H	CH_3	31
15	Leu	H	$CH_2CH(CH_3)_2$	41
16	Phe	H	CH_2Ph	67
17	Phg	H	Ph	41
18	α-Me-Ala	CH_3	CH_3	55
7	Pro	H	$-(CH_2)_3-$	—[a]

[a] The corresponding bicyclic diketopiperazine was obtained after Fmoc-removal.

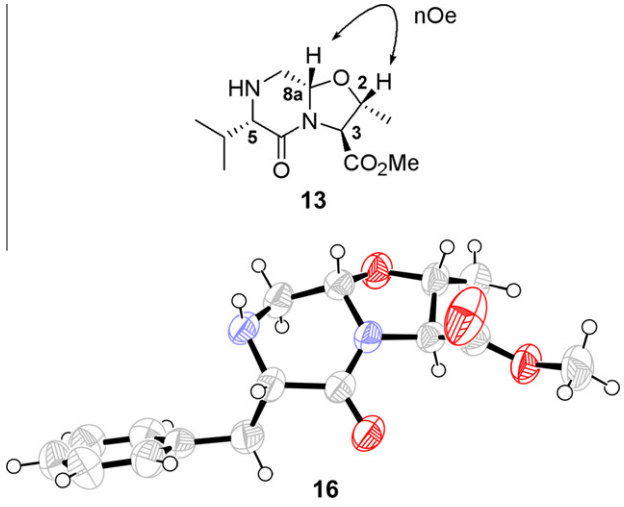


Figure 4. NOE correlation for compound **13** and X-ray structure of **16**.

NOE experiments on compound **13** were carried out to establish the correct stereochemistry of the bicyclic 2-oxopiperazine scaffold, as outlined in Figure 4. In particular, key NOE correlations between H-8a and H-2 of **13** unequivocably assessed the methylenic group to be oriented in *trans* with respect to the carbomethoxy group. This was in agreement with crystallographic data obtained for compound **16**,[11] showing the predicted stereochemical assignment of the newly-formed stereocenter and showing the six-membered ring in a boat-like conformation.

The herein reported new bicyclic scaffolds are particularly suited for peptidomimetic chemistry as dipeptide isosteres, and appendage diversity may be achieved by using the chemistry at the double bond for scaffold B of Figure 1, as already reported,[4b] or applying scaffold A in peptide chemistry by virtue of the presence of carboxy and amino groups.

In conclusion, the process involving morpholine acetals and suitable Fmoc-amino acid derivatives leads to two different bicyclic scaffolds depending on the reaction mode. Specifically, by carrying out the three-step process in one-pot with increasing amounts of alternated base–acid–base reactants, the bicyclic diketopiperazine is formed preferentially, whereas the stepwise route, by removing 2,6-lutidine and using the same reagents, leads to the formation of a different bicyclic skeleton containing the 2-oxopiperazine ring, as a consequence of ring opening of the morpholine nucleus. The concept of obtaining skeletal diversity by using same reagents in a different manner may provide new efficient ways to expand the access to chemical diversity by modulating both the reagents and the process. Application of these scaffolds in the generation of compound libraries through appendage diversity will be reported in due course.

Acknowledgments

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9. *General 'one-pot' procedure B*: morpholine acetal **2** or **4** (1 equiv) was dissolved in toluene (10 mL/mmol), then 2,6-lutidine (2 equiv) and Fmoc-aa-Cl (1 equiv) were sequentially added. The reaction mixture was brought to 60 °C and stirred for 2 h, then allowed to return at rt. Additional toluene (5 mL/mmol) and *p*-toluenesulfonic acid monohydrate (5 equiv) were added, then the mixture was placed in a single-necked round-bottomed flask equipped with a reflux condenser and a dropping funnel containing 4 Å molecular sieves and it was refluxed for 2 h. The reaction mixture was brought back at rt and 30% diethylamine solution in CH_3CN (5 mL/mmol) was added. The reaction mixture was stirred for 2 h at rt, then diluted with EtOAc (40 mL/mmol), washed with 5% $NaHCO_3$ and brine. The organic layers were dried over anhydrous Na_2SO_4, filtered and evaporated. A dark brown oil was obtained and purified by flash chromatography (EtOAc–petroleum ether 2:1), giving the title 2,5-diketopiperazine compounds as orange-yellow oils (for R = H) or pale yellow solids (for R = Ph).
10. *General 'stepwise' procedure A*: morpholine acetal **2** (1 equiv) was dissolved in anhydrous CH_2Cl_2 (4 mL/mmol), then 2,6-lutidine (2 equiv) and Fmoc-aa-Cl (1 equiv) were sequentially added. The reaction mixture was brought to 60 °C and stirred for 2 h under a nitrogen atmosphere. The reaction mixture was then diluted with CH_2Cl_2 and sequentially washed with 5% HCl, 5% $NaHCO_3$ and brine. The organic layers were dried over Na_2SO_4, filtered and evaporated to give a brownish foam. The crude product was then dissolved in toluene (5 mL/mmol) and *p*-toluenesulfonic acid monohydrate (1 equiv) was added. The reaction mixture was placed in a single-necked round-bottomed flask equipped with a reflux condenser and a dropping funnel containing 4 Å molecular sieves and refluxed for 2 h, then it was allowed to return to rt. EtOAc was added, and the organic solution was washed with 5% $NaHCO_3$ and brine. The organic layers were dried over anhydrous Na_2SO_4, filtered and evaporated to give a dark brown oil. The crude product was dissolved in 30% diethylamine in CH_3CN (5 mL/mmol) and allowed to react for 2 h at rt. The reaction mixture was then evaporated to obtain a pale brown solid, which was purified by flash chromatography (EtOAc–petroleum ether 3:1), to give the title bicyclic 2-oxopiperazine compounds as dark orange oils.
11. CCDC 769053.