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A General Approach to the Aza-Diketomorpholine Scaffold

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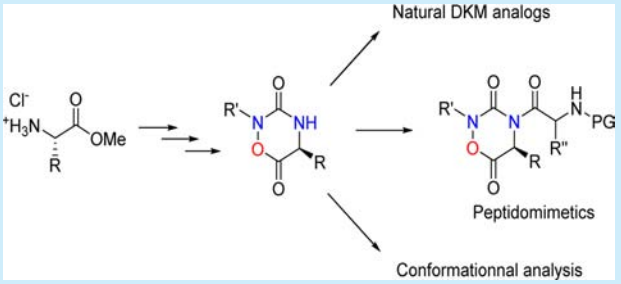
Supporting Information

ABSTRACT: A stereoconservative three-step synthesis to access to 1,2,4-oxadiazine-3,6-dione is presented. This underexplored platform could be considered as a constrained oxy-azapeptide or an aza-diketomorpholine, the methodology being then successfully applied to produce enantiopure aza-analogs of diketomorpholine natural products. Importantly, the first crystal structures were obtained and compared to diketomorpholine and diketopiperazine structures. Finally, a straightforward procedure concerning the coupling of this heterocyclic scaffold with various amino acids to afford original pseudodipeptide analogs was described.



Nitrogen- or oxygen-containing heterocycles involving a wide range of natural and bioactive molecules have attracted considerable attention due to their interesting potential in organic and medicinal chemistry.[1−3] Accordingly, recent curiosity was devoted to heterocycles bearing both nitrogen and oxygen as adjacent atoms.[4−6] Although such a cyclic platform was generally less studied, its importance for organic chemists and drug designers is well established.[7−9] Indeed, conformational restriction by cyclization is also a popular tactic for optimizing the hit-to-lead process (Figure 1).

By introducing rigidity in the original scaffold, a positive effect is often observed in structure-based drug design. Thus, the 2,5-diketopiperazine (DKP) motif, well exemplified among natural products,[10] represents the smallest cyclodipeptide structure and also clearly a valuable peptidomimetic pattern.[11,12] The closely related diketomorpholine (DKM) moiety, less described and studied,[13−15] could be then considered as a constrained cyclic depsipeptidomimetic structure. In this study, a procedure to access 1,2,4-oxadiazine-3,6-diones (1,2,4-Oxds) is proposed by incorporating a ring into the oxyazapeptide backbone to define aza-diketomorpholine (aza-DKM) as an original heterocycle (Figure 1).

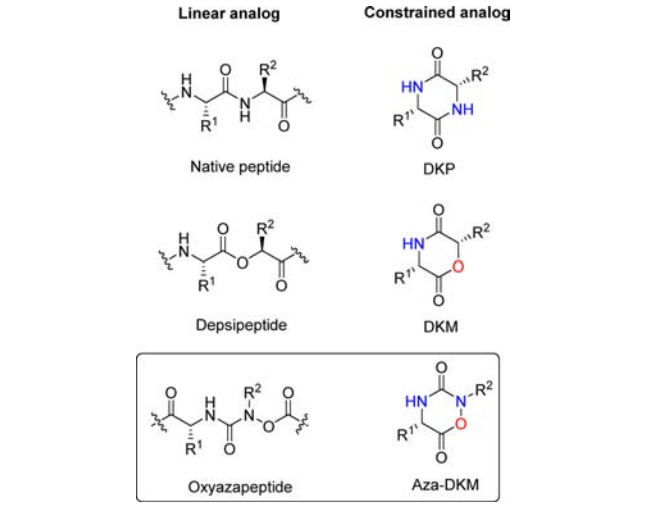
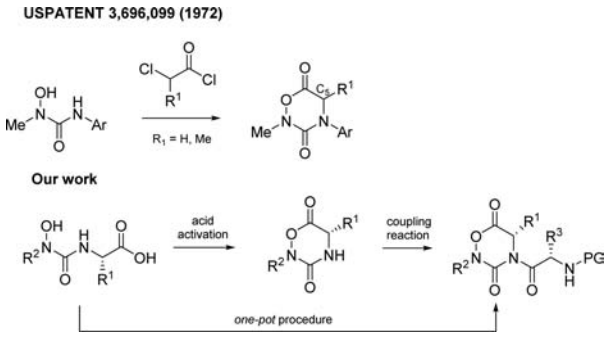


Figure 1. General structure of native peptides or peptidomimetics and their corresponding constrained analogs.

A review of the literature highlights that 1,2,4-Oxds were rarely reported. To the best of our knowledge, a unique and old patent exposes a synthetic pathway through condensation of hydroxyureas on chloroacetyl chlorides, affording 1,2-methyl-4-aryl-Oxd[Gly] or Oxd[Ala] with virtually no characterization or stereochemical details (Scheme 1).[16] Moreover the hydroxyurea formation needs experimental precaution such as handling the air sensitive reagent (isonitrile)[17] while the commercially availability of chiral chloroacetyl chlorides is uncommon.

Scheme 1. Synthetic Strategies To Build Aza-DKMs



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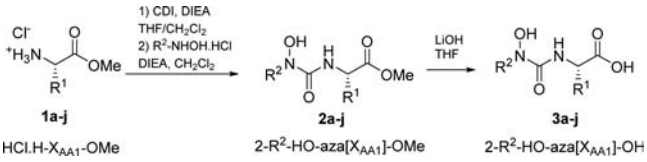
Performing synthetic assays by following the suggested procedure, in our hands, we constantly observed the linear chlorinated product formation. In this context, we propose an efficient and versatile three-step procedure that allowed a library of 2,5- or 2,4,5-functionalized aza-DKMs to be obtained (Scheme 1).

After construction of the starting building blocks from commercially available amino methyl ester with no special experimental precautions (no inert atmosphere or dry solvents), oxy-azapeptides were obtained and suitably deprotected to allow their cyclization after carboxylic acid activation. The feasibility of the coupling of cyclooxy-azapeptides with various N-protected amino acids was then studied, and a more efficient one-pot route has been developed. Aza-analogs of representative examples of natural DKMs were also synthetically produced as a last challenge.

The beginning of our chemical investigation required a synthetic access to oxyazapeptides. This peptidomimetics family in which the C^{α} group is replaced by an α-amino group and the NH function is substituted by an OH group was mostly studied by Katritzky et al.[18,19] Especially, the construction of the O–N^{α} bond, which involves a coupling reaction between substituted hydroxylamines and acyl imidazoles, is versatile to increase peptide stability.

Inspired by the work of Katritzky et al.,[19] a one-pot two-step reaction between various commercially available amino methyl esters, carbonyldiimidazole (CDI), and *N*-methyl-, *N*-benzyl-, and *N*-isopropyl hydroxylamine was proposed. The resulting esters **2a–j** were then submitted to mild saponification conditions to afford the original desired carboxylic acids **3a–j** in 85–92% yields (Table 1).

Table 1. Synthetic Access to Oxyazadipeptides 2a–j and 3a–j

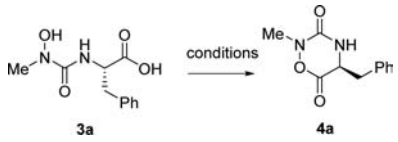


entry	X_{AA1}	R^2	2	yield (%)[a]	3	yield (%)[a]
1	Phe	Me	**2a**	95	**3a**	90
2	Ser(Bn)	Me	**2b**	90	**3b**	90
3	Tyr(Bn)	Me	**2c**	90	**3c**	90
4	Cys(Me)	Me	**2d**	81	**3d**	87
5	Ile	Me	**2e**	90	**3e**	85
6	Val	Bn	**2f**	97	**3f**	85
7	Trp	Bn	**2g**	92	**3g**	91
8	Phe	*i*Pr	**2h**	98	**3h**	87
9	(D)-Phe	*i*Pr	**2i**	98	**3i**	87
10	Leu	iPr	**2j**	96	**3j**	92

[a]Yield of isolated product.

The intramolecular cyclization to access the aza-DKM moiety required then a suitable carboxylic acid activation of the oxyazadipeptide previously formed. Several different experimental conditions were screened on the model compound **3a**. The best result was obtained by using HATU, the guanidinium derivative particularly useful in peptide synthesis for carboxylic acid activation (1.2 equiv), in the presence of DIEA (3.0 equiv), for 1 h at room temperature (Table 2, entries 7–8).[20] While a total conversion was observed

Table 2. Optimization of the Cyclization Step Using the Oxyazadipeptide 3a



entry	conditions	solvent	conversion (%)[a]	yield (%)[b]
1	BOP, TEA	DMF	0	0[c]
2	BOP, NMM	DMF	42	15
3	BOP, NMM	CH_3CN	45	23
4	DCC/DMAP	CH_2Cl_2	55	43
5	HBTU, DIEA	DMF	95	58
6	HBTU, DIEA	CH_3CN	95	65
7	HATU, DIEA	DMF	100	60
8	HATU, DIEA	CH_3CN	100	75

[a]Conversion was determined by HPLC and LCMS analysis. [b]Yield of isolated product. [c]No reaction.

in DMF and acetonitrile, cyclic compounds were revealed to be unstable to conventional aqueous workup, with a small amount of the linear starting material being recovered after ring opening of **4a**. Avoiding the aqueous workup step, the use of acetonitrile allowed direct purification of the mixture on silica gel (cyclohexane/EtOAc) leading to the desired 1,2,4-Oxd model **4a** in good yields.

To investigate the feasibility of cyclization, the initially prepared oxyazadipeptides **3b–j** bearing different side chains were then tested. According to the procedure previously established, a family of innovative heterocyclic compounds **4a–j** was prepared. The total conversion and the good yields that were obtained proved the reliability and the large scope of the cyclization reaction (Figure 2). Furthermore, starting with both

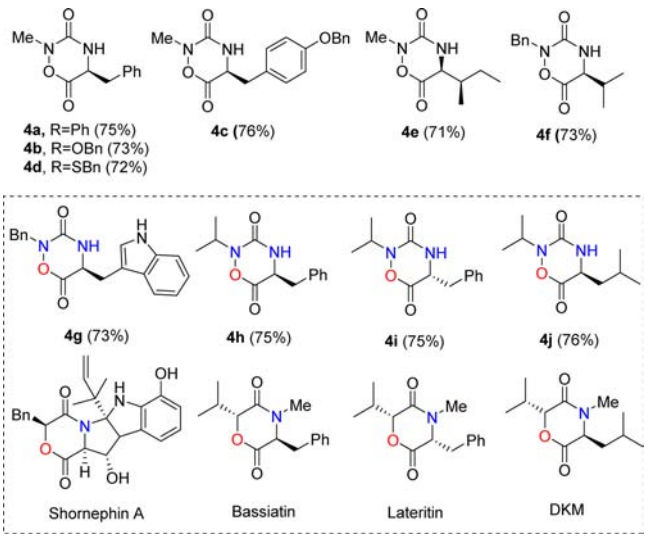


Figure 2. Access to novel functionalized 1,2,4-Oxd scaffolds.

the appropriate hydroxylamine and amino ester, we performed the synthesis of aza-analogs of the known natural product containing the DKM motif, anticipating that they could be of great interest for medicinal chemists.

We thus describe the synthesis of (*i*) a simplified aza-DKM analog **4g** of the core of the Shornephine A, an *Aspergillus sp.* metabolite highlighted as a useful inhibitor of P-glycoprotein

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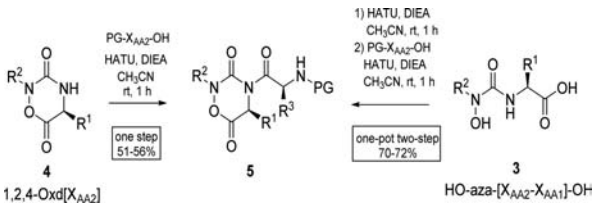
Org. Lett. 2017, 19, 492–495

potentially interesting in multidrug resistant cancers; (*ii*) an analog **4h** of Bassiatin, a platelet aggregation inhibitor isolated from *Beauveria bassiana*; (*iii*) an aza-DKM analog **4i** of Lateritin extracted from *Gibberella lateritium* described to inhibit the growth of various human cancer cell lines, Gram-positive bacteria, and *Candida albicans*;[21] and (*iv*) finally of the aza-analog **4j** of a natural cyclodepsipeptide isolated from *Fusarium sporotrichioides* (Figure 2).[22,23]

The retention of the enantiopurity was then confirmed with compounds **4h** and **4i** by chiral HPLC. While the enantiomeric mixture of **4h** and **4i** revealed the presence of two peaks of equal proportions, the analysis of each stereomer shows a single retention-time peak indicating that the initial stereogenic center is totally preserved during the cyclization.

The feasibility of a coupling reaction of aza-DKM moieties with various *N*-protected amino acids was then naturally explored. Despite the instability of 1,2,4-Oxd previously described and observed in an aqueous medium, not allowing conventional extraction workup, fruitful coupling conditions afforded **5a**−**c** derivatives in correct yields starting from 1,2,4-Oxd **4** (Table 3, entries 1−3). By limiting the purification steps,

Table 3. Peptide Coupling with the 1,2,4-Oxd[X_{AA1}-X_{AA2}] Platform



entry	S.M[a]	PG-X_{AA2}	5	overall yield (%)[b]
1	**4a**	Cbz-Ser(*t*-Bu)	**5a**	51
2	**4f**	Cbz-Phe	**5b**	56
3	**4g**	Boc-Ala	**5c**	52
4	**3a**	Cbz-Ser(*t*-Bu)	**5a**	70
5	**3f**	Cbz-Phe	**5b**	72
6	**3g**	Boc-Ala	**5c**	70

[a]Starting material. [b]Yield of isolated product.

the overall yield could be significantly improved to excellent overall yields, employing an original one-pot procedure starting from the linear oxyazapeptide **3** (Table 3, entries 4−6).

Importantly, the crystal structures of the compounds **4c** and **5a** as representative examples of this new family of constrained peptidomimetics were obtained (Figure 3). The asymmetric unit of **4c** comprises two independent molecules exhibiting

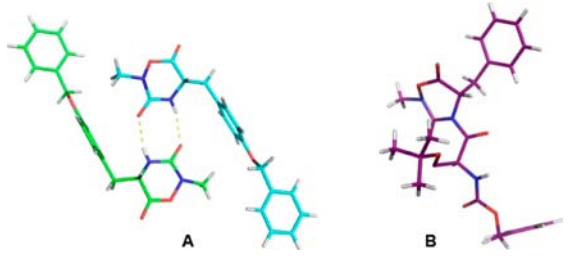
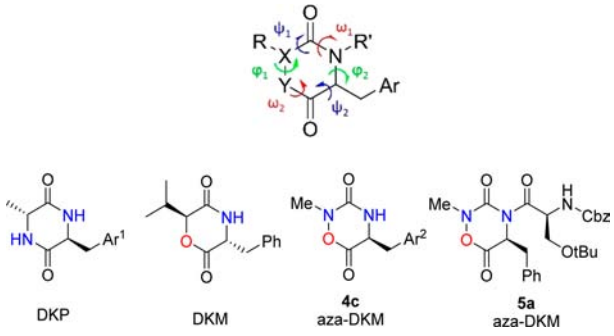


Figure 3. (A) X-ray crystal structure of the aza-DKM **4c**. The two independent molecules of **4c** are shown in green and cyan. The intermolecular hydrogen bonds are indicated as dashed lines. (B) X-ray crystal structure of the aza-DKM **5a** (in purple).

comparable structures which were connected by two hydrogen bonds (Figure 3A). The superimposition of the two structures show that the heterocyclic rings adopt similar pseudoboat conformations with a single rotation of ∼180° of the aryl moieties (Figure S1). It is noteworthy that the incorporation of a protected amino acid on the aza-DKM platform induces a reversal of its pseudoboat conformation via the pyramidal nitrogen inversion of the nitrogen bearing the methyl group (Figure 3B, Table 3).

Comparison of the conformation of the 1,2,4-Oxd **4c** to the DKM and DKP structures of the literature allowed us to evaluate the impact of the presence of carbon, nitrogen, or oxygen atoms in X and Y positions on the conformation of the heterocycle (Table 4, Figure 4).

Table 4. Dihedral Angle Measurement of DKP, DKM, and Aza-DKM Cycles



angles	DKP	DKM	4c[a]	5a
φ_1	65.4	−43.3	−45.6 ± 8.8	54.1
Ψ_1	−53.0	32.9	30.6 ± 9.4	−38.2
ω_1	−10.1	7.1	16.5 ± 0.4	−14.2
φ_2	27.8	−37.8	−39.0 ± 9.6	50.1
Ψ_2	−17.6	27.3	28.8 ± 9.7	−33.9
ω_2	−7.9	12.2	12.4 ± 1.4	−13.3

[a]Average values, Ar^1 = *p*-hydroxylphenyl, Ar^2 = *p*-benzyloxyphenyl.

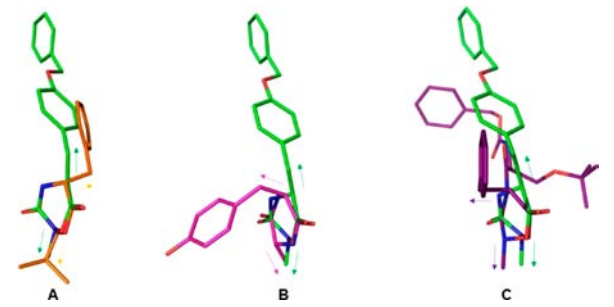


Figure 4. Superimposition of the crystal structures of aza-DKM (in green) with (A) DKM (in orange), (B) DKP (in magenta), and (C) aza-DKM derivative **5a**. Hydrogens atoms have been omitted for clarity.

First, we observed that 1,2,4-Oxd **4c** can adopt a pseudoboat conformation with similar torsion angles to the case of the DKM derivative (Figure 4A).[24] In this context, the isopropyl and methyl groups are projected toward similar directions while the aryl and the phenyl groups are on the opposite face of the ring since they are connected on a carbon of different stereochemistry. In contrast, we noted that the structural properties of the DKP ring were different than those of the DKM or aza-DKM derivatives and that the pseudoboat

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conformation of the DKP projects the substituents toward different directions (Table 3, Figure 4B).[25] Consequently, we showed that the single substitution of the carbon in X by a nitrogen did not change markedly the conformation of the heterocyclic ring (Table 3) while the additional replacement of the oxygen in position Y affects the overall structure of the heterocycle and thus the orientation of the substituents. As expected, the conformational inversion of the aza-DKM in **5a** also modulates the relative orientations of the methyl and aryl groups (Figure 4C).

Finally, intrigued by the instability of DKM reported by Capon et al.,[23] the exposure to water of **4g** and **5c** derivatives was evaluated. The LC/MS analysis revealed that both compounds exhibited a similar opening into the corresponding carboxylic acid derivative, 50% of the starting heterocycle being recovered after 13 h and 9 h, respectively. While **4g** was totally opened into the corresponding carboxylic acid after 76 h, **5c** reached a heterocycle/carboxylic acid 10:90 equilibrium after 26 h in water, the same ratio observed even after 7 days.

In conclusion, an efficient, simple, and unprecedented methodology to synthesize a new class of peptidomimetics has been developed by the original stereoconservative cyclization of oxy-azapeptides. A three-step sequence using commercially available starting materials, mild reaction conditions, and easy handling led to a 1,2,4-Oxd platform called aza-diketomorpholine, in good yields. Some examples illustrated the use of these heterocycles for the successful synthesis of Bassiatin and Shornephine A analogs. Concerned by the great pharmaceutical significance of peptidomimetics, aza-DKM represents a promising skeleton to elaborate novel constrained pseudodipeptide derivatives, provided their aqueous stability could be improved. The crystal structures of the aza-DKM allowed us to describe the conformation of the heterocycle and the orientation of the substituents which is mandatory to rationally design bioactive compounds. Further structural studies and theoretical calculations on derivatives are in progress to tentatively rationalize the conformational preference of the aza-DKM.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b03656.

Synthetic details; ^{1}H,^{13}C NMR; and X-ray data (unless otherwise specified, absolute configuration of amino acids used is "L") (PDF)

Crystallographic data for **4c** (CIF)

Crystallographic data for **5a** (CIF)

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Notes

The authors declare no competing financial interest.

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