

## Solid-Phase Synthesis of Conformationally Constrained Peptidomimetics Based on a 3,6-Disubstituted-1,4-diazepan-2,5-dione Core

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**Abstract:** Starting from a Cl-tryptyl linked hydroxylamine, a hydroxamic dipeptide having serine in the second position was prepared by using DMTMM as the coupling agent. Mitsunobu cyclization carried out under microwave heating gave very good yields of a 3,6-disubstituted-perhydro-diazepin-2,5-dione. This heterocycle can be used as a new platform for combinatorial chemistry or as a constraint to rigidify a small peptide.

In the search for new and more effective peptidomimetics, the synthesis of peptides containing a ring system that acts as a conformationally restricted core has been extensively employed to prepare new leads for the drug discovery process.<sup>1</sup> When inserted into peptide sequences, such constraint can enhance the biological properties as, for example, the binding with the active site or the stability of the modified peptide to endopeptidases. Different approaches have been pursued to introduce a (macro)cyclic structure inside a peptide chain. Head-to-tail,<sup>2</sup> residue-to-residue,<sup>3</sup> and residue-to-backbone cyclization<sup>4</sup> are the most common strategies employing natural  $\alpha$ -amino acids that also can be carried out on solid phase.

To get an effective constraint, the introduction of relatively small or medium size rings is preferred. Whereas the six-member diketopiperazine ring has been largely employed for this goal,<sup>5</sup> there are few literature reports

concerning the seven-member diazepane ring.<sup>6</sup> Although this heterocycle offers an attractive structure for the development of new biologically active compounds,<sup>7</sup> all the syntheses reported until now describe the preparation of 1,4-diazepan-2,5-diones. The syntheses proceeded through the lactonization of the terminal NH<sub>2</sub> and the  $\beta$ -COOH of a dipeptide containing aspartic acid<sup>6</sup> or the head-to-tail cyclization of a dipeptide containing phenylalanine.<sup>8</sup>

Looking for a new general and versatile synthesis of this class of heterocycles, we decided to follow an alternative route based on the construction of a serine-containing dipeptide linked to a hydroxylamine resin followed by cyclization of the hydroxyhydroxamate under Mitsunobu conditions.<sup>9</sup> This approach provides the new 3,6-disubstituted-diazepan-2,5-dione skeleton having an amino group in position 6 that can be employed for the elongation of the peptide chain or to increase the molecular diversity. Moreover, if the substituent in position 3 carries a protected carboxylic group, it is possible to have two sites for peptide elongation.

To optimize the reaction conditions for each step we decided to initially explore the cyclization of a simple model compound in the homogeneous phase. Thus, *O*-benzylhydroxylamine was reacted with *N*-Boc-PheOH in the presence of DMTMM as the coupling agent.<sup>10</sup> The hydroxamate **1** was deprotected (TFA/CH<sub>2</sub>Cl<sub>2</sub>/Et<sub>3</sub>SiH 1/1/0.1) and further coupled with Boc-Ser-OH. Product **2**, isolated in 72% yield after a simple aqueous workup, was ready to undergo the cyclization.

The first attempt was made with use of classical Mitsunobu conditions, DEAD/PPH<sub>3</sub> in THF at room temperature.<sup>11</sup> Cyclization occurred after an overnight reaction but compound **3** was isolated, after column chromatography, in 31% yield. The other compounds recovered in the reaction mixture were unreacted starting material and the alkylated hydrazine dicarboxylate **4** typical of a tricky Mitsunobu reaction.<sup>12</sup> Attempts to change the

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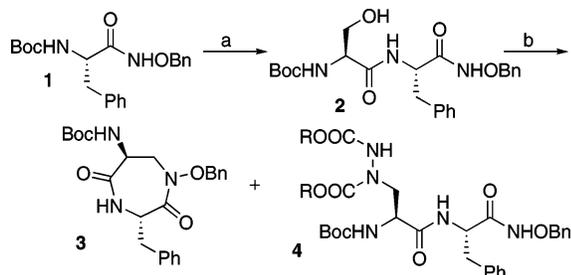
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TABLE 1. Cyclization Conditions

reagents	conditions	yields <sup>a</sup> 3, 4
DEAD/PPh <sub>3</sub> 1.1.	THF, rt, 12 h	31%, 40%
DIAD/PPh <sub>3</sub> 1:1	THF, rt, 12 h	46%, 36%
DTAD/PPh <sub>3</sub> 1:1	THF, rt, 12 h	42%, 26%
DTAD/PPh <sub>3</sub> 3:1	THF, rt, 12 h	47%, 28%
DIAD/PPh <sub>3</sub> 1.1	toluene, reflux, 12 h	63%, 26%
DIAD/PPh <sub>3</sub> 1.1	DMF, rt, 12 h	46%, 22%
DIAD/PPh <sub>3</sub> 1.1	DMF, MW, 210 °C, 10 min	75%, 4%

<sup>a</sup> Yield after separation by column chromatography. Starting material is also present.

### SCHEME 1. Synthesis of 1,4-Diazepan-2,5-dione Core<sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) TFA:CH<sub>2</sub>Cl<sub>2</sub>:Et<sub>3</sub>SiH 1:1:0.1, 6 h, rt, DMTMM, N-BocSerOH, NMM, THF, 6 h, rt. (b) See Table 1.

nature of the azodicarboxylate and to increase the dilution did not improve the yield of **3** (see Table 1).

The presence of product **4** obtained even with hindered di-*tert*-butyl azodicarboxylate (DTAD), although in a smaller amount than with DEAD or DIAD, suggested that dipeptide **2** was reluctant to cyclize probably because of the preferential transoid conformation assumed by the peptide bond. Indeed, the <sup>1</sup>H NMR spectrum of **2**, registered in *d*<sub>6</sub>-dms<sub>o</sub> at room temperature, showed two signals for the amide NH at δ 7.70 and 7.93 integrating for 0.8 and 0.2 H, respectively. The signals collapsed to a singlet in a range from 60 to 90 °C. Thus we considered that heating the reaction mixture would accelerate the trans-cis equilibration allowing a rapid replacement of the cis isomer consumed by the cyclization and consequently decreasing the amount of byproduct **4**.<sup>13</sup> When the cyclization was carried out in boiling toluene product **3** was obtained in 65% yield with the formation of a considerably smaller amount of **4**. When heating was more efficiently carried out under microwave irradiation<sup>14</sup> (DIAD/PPh<sub>3</sub> in DMF at 100 W for 10 min in a sealed tube)<sup>15</sup> product **3** was obtained in 75% yield and **4** was formed in a negligible amount.<sup>16</sup>

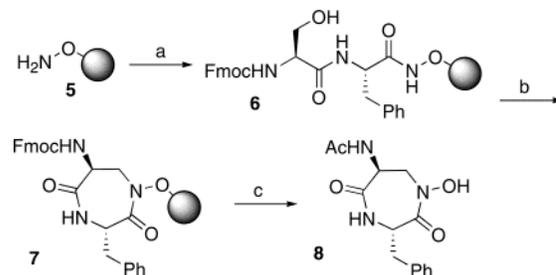
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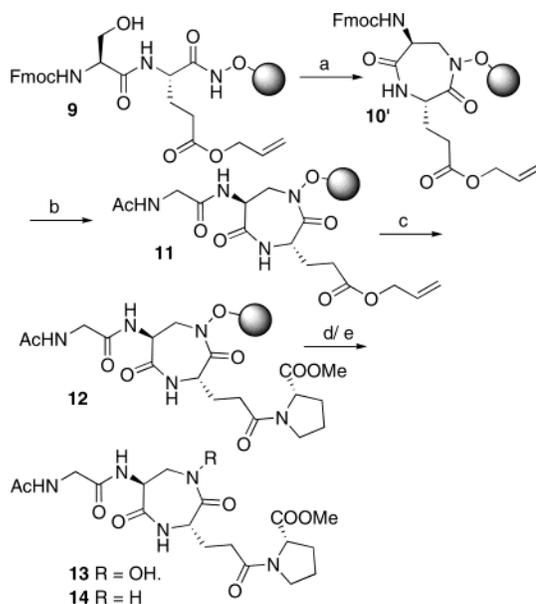
(16) To the best of our knowledge, in the literature there is only one example involving a microwave-promoted Mitsunobu reaction. Steinreiber, A.; Stadler, A.; Mayer, S. F.; Faber, K.; Kappe, C. O. *Tetrahedron Lett.* **2001**, *42*, 6283.

### SCHEME 2. Solid-Phase Synthesis of 1,4-Diazepan-2,5-dione<sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) N-FmocPheOH, DMTMM, DIPEA, NMP, 4 h, rt and piperidine 25% in DMF. (b) DIAD, PPh<sub>3</sub>, DMF, MW, 60 W, 210 °C. (c) Piperidine, 25% DMF followed by As<sub>2</sub>O, CH<sub>2</sub>Cl<sub>2</sub>, DIPEA, rt 1 h, TFA:CH<sub>2</sub>Cl<sub>2</sub>:TFE 10:40:40, rt, 6 h.

### SCHEME 3. Synthesis of Peptidomimetic Scaffold<sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) DIAD, PPh<sub>3</sub>, DMF, MW, 60 W, 210 °C. (b) 25% piperidine in DMF followed by N-AcGlyOH, DMTMM, DIPEA, NMP, rt, 4 h. (c) Pd(PPh<sub>3</sub>)<sub>4</sub> (3 equiv), CHCl<sub>3</sub>:AcOH:NMM 37:2:1, HProOMe, DMTMM, DIPEA, NMP, rt, 6 h. (d) TFA:CH<sub>2</sub>Cl<sub>2</sub>:TFE, 10:40:40, rt, 6 h. (e) SmI<sub>2</sub> 0.1 M in THF, 8 h, rt and workup as in ref 23.

For the solid-phase approach we chose hydroxylamine linked to a PS-DVB 2-chlorotrytyl resin.<sup>17</sup> Coupling with FmocPheOH gave compounds **6** (Scheme 2). Cyclization was carried out with DMF as the solvent, in the presence of 2 equiv of DIAD and 4 equiv of PPh<sub>3</sub> in a sealed tube under microwave irradiation (60 W, 210 °C). After 6 min, the beads were recovered by filtration, washed several times with DMF and CH<sub>2</sub>Cl<sub>2</sub>, and submitted to a second round of reaction under the same conditions.

After 3 cycles, the color test for free OH<sup>18</sup> carried out on the beads was negative. The Fmoc protecting group was removed, the NH<sub>2</sub> was acetylated to simplify the NMR spectra, and product **8** was removed from the resin with TFA/CH<sub>2</sub>Cl<sub>2</sub> 1/1 and isolated in 66% yield.

(17) We used the commercially available resin from Novabiochem.

(18) Attardi, M. E.; Taddei, M. *Tetrahedron Lett.* **2001**, *42*, 2927.

The versatility of this synthetic approach is outlined in Scheme 3. FmocGlu(OAll)OH<sup>19</sup> was linked to the hydroxylamine resin, deprotected, and coupled with N-FmocSerOH.<sup>20</sup> Cyclization of compound **9** was carried out under standard conditions under microwave activation to give product **10** (free OH test negative). Deprotection of the Fmoc group and coupling with N-AcGlyOH gave product **11**. Deprotection of the allyloxy group (Pd(PPh)<sub>4</sub> CHCl<sub>3</sub>, AcOH, NMM 37/2/1)<sup>21</sup> was followed by coupling with H-ProOMe until free carboxylic acid disappeared.<sup>22</sup>

Cleavage from the resin with TFA/CH<sub>2</sub>Cl<sub>2</sub> 1/1 gave the *N*-hydroxy-derivative **13** in 75% yield, whereas with SmI<sub>2</sub> in THF solution<sup>23</sup> **14** was isolated in 51% yield after aqueous workup and a short path silica gel column.

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In conclusion, with the crucial assistance of microwave irradiation, we have expanded the use of the Miller cyclization of hydroxy hydroxamates to the synthesis of new 3,6-disubstituted-1,4-diazepan-2,5-diones that can be employed as new scaffolds for combinatorial chemistry and as conformational constraints for short peptides. The extension of this approach to macrocyclization is actually underway in our laboratory and will be reported in due course.

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**Supporting Information Available:** Experimental procedures and characterization data for compounds **3**, **8**, **13**, and **14**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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