An Improved Procedure for the Synthesis of Enaminones – Dimer Building Blocks in β-Strand Mimetics

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Abstract: @-Tides have been shown to have the same characteristics as a peptide in the β-strand conformation and to have the ability to self-associate into dimeric β-sheets. Aza-cyclohexaenaminones, obtained by condensation of a protected azacyclohexa-3,5-dione and amino acid esters, are the key building-blocks in the synthesis of @-tides. An improved three-step synthetic sequence to these enaminones has been developed that takes advantage of microwave-assisted chemistry in two of the steps to enhance the reaction rates. It was also found that the enaminone building blocks can be obtained by direct condensation of the aza-cyclohexa-3,5-dione with amino acid esters, without prior activation of the diketone. Multivariate design was used to optimize this microwave-assisted condensation, resulting in a short reaction time (300 s) and high yields (67–94%).

Key words: enaminones, β-strand mimetics, microwave-assisted synthesis, multivariate design, optimization

β-Strands and β-sheets are secondary structural elements involved in many protein–protein and protein–DNA interactions of importance in biological systems, and in the processes associated with disease as well as normal function.1 One such process is the molecular machinery of pilus assembly in uropathogenic Escherichia coli (UPEC), where β-sheet formation is crucial both during chaperone-mediated folding and in the transport of pilus subunits in the periplasm, as well as in the formation of mature pili on the bacterial cell-surface.2 In an effort to develop inhibitors of pilus assembly we have shown that peptides corresponding to the β-strand motifs from periplasmic chaperones and pilus subunits can inhibit key protein–protein interactions required for pilus assembly. Furthermore, a library of designed peptides was constructed and used to elucidate the structure–activity relationships for this interaction.3 Peptides have poor bioavailability as a consequence of low absorption and a high propensity to undergo degradation by peptidases. Therefore, we wanted to construct β-strand mimetics, which would ideally both improve bioavailability and biological affect, as compared to the pilus-derived peptides. In our design we thought about using alternating aza-cyclohexenones in between ordinary amino acid residues, in an attempt to rigidify the mimetic in an elongated β-strand conformation and to eradicate all peptide bonds (Figure 1). To our delight, Bartlett et al. have prepared such pentamer peptido-mimetics, so called @-tides, consisting of amino acids with 1,2-dihydro-3(6H)-pyridones at alternate positions.4a These β-strand mimetics were shown to have the same characteristics as a peptide in a β-strand conformation and to have the ability to self-associate into dimeric β-sheets. Here we present an improved microwave-assisted synthetic sequence to the desired @-tide building blocks, which also enable diversification desirable for library synthesis.

The previous synthesis of @-tide building blocks I started from commercially available 3,5-dichloropyridine 2, which consisted of some time-consuming steps that proceeded in modest yields (Scheme 1).4 The method also employed expensive and toxic reagents [e.g. Yb(OTf)3],

Figure 1 A scaffold with alternating aza-cyclohexenones is anticipated to adopt an extended β-strand conformation and preserve some of the hydrogen bonding interactions required for β-sheet formation. Enaminones 1 are key building blocks in the synthesis of such β-strand mimetics.

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Sn(OTf)$_2$] for the conjugate-addition–elimination step (Scheme 1, step f), which is inappropriate for pharmaceutical applications. The published method does therefore not appear to be completely satisfactory when making larger libraries of compounds, or in industrial applications. In an effort to develop an efficient and fast route to building blocks 6–14, which have diverse structures, our attention was first directed to the use of microwave-assisted synthesis to enhance the rate of the methoxylation of 3,5-dichloropyridine (2). In addition, it appeared that the synthetic sequence could be shortened by one step through direct condensation of alloc-azacyclohexadione with an amino acid to give the corresponding enaminone without prior activation of diketone 4 (cf. 5, Scheme 1).

It was found that the methoxylation of 3,5-dichloropyridine (2) progressed smoothly in DMF and that all starting material was consumed at a microwave temperature of 230 °C within a reaction time of 20 minutes. The isolated yield was comparable with that from the published procedure (60% vs. 68%), which was conducted at 80 °C for 38 hours with 8 equivalents of sodium methoxide. Thereafter, alloc-protected diketone 4 was prepared from 3,5-dimethoxypyridine 3 as described earlier starting by reduction of the pyridine ring and in situ alloc-protection of the resulting secondary amine. Subsequent hydrolysis of the enol ethers resulted in alloc-protected diketone 4. Diketone 4 is known to be somewhat labile and decomposes over time, but we found it to be stable for extensive periods of time when it was frozen in a benzene matrix (36.5 mg/mL).

Benzene is often used as a solvent in the synthesis of enamines, however, the low polarizability of benzene made us consider combining benzene with a solvent having a higher polarizability in order to assist microwave heating. Moreover, such a co-solvent would also increase the solubility of the reactants and hence enable synthesis of more diverse enaminones. Consequently, a small investigation of suitable co-solvents was performed and enaminone 6 was synthesized using a microwave temperature of 110 °C, a reaction time of six minutes in benzene containing either 4% DMSO, DMF, or N-methylpyrrolidine (NMP). The amount of enaminone 6 formed in the presence of the different co-solvents was compared using LC-MS and showed that up to twice as much product was formed when NMP was employed as the co-solvent compared to DMSO or DMF.

Initial attempts towards microwave-assisted synthesis of enaminone 6 directly from diketone 4 resulted in low yields, which indicated the decomposition of enaminone 6 and/or diketone 4 at these elevated temperatures and prolonged reaction times. Therefore, different reaction variables were optimized using multivariate experimental design in an effort to increase the yield. Multivariate experimental design is a tool that can maximize the knowledge of the reaction conditions with as few experiments as possible. In addition, a properly performed design also gives information about interaction effects between the different variables, which can not be obtained by changing the variables one by one. Thus, a full factorial design with three different variables, temperature, reaction time, and the amount of diketone 4 was set up. Three central points were also included so that the design would allow detection of curvature in the resulting model, giving a total of eleven experiments (Figure 2 and Table 1).
The yield for each experiment was calculated using LC-MS and a calibration curve was constructed using different concentrations of pure enaminone 6 corresponding to yields ranging from 10–90%. Calculated yields from the eleven experiments were then correlated with the different variables using multiple linear regression (MLR). This gave a model with five coefficients which explained 93% of the variation in the response ($R^2 = 0.93$) and had good predictive properties according to cross-validation with a $Q^2$ of 0.60. The regression coefficients (Figure 3, a) showed that the temperature was positively correlated with the yield while the reaction time and the amount of diketone did not significantly affect the yield.

Moreover, a negatively correlated cross-term between the temperature and time was in good agreement with the observation that the diketone and/or the product decomposed when the reaction times were too long or elevated temperatures were used. Furthermore, a negatively correlated square term of the temperature indicated that the optimal temperature actually is within the investigated interval. A plot of temperature against time, holding the diketone variable constant at the lowest setting and having the yield as response, predicted that optimal conditions for the reaction could be achieved with a temperature of 140 °C and a reaction time of five minutes (Figure 3, b). Thus, a diverse set of eight amino acids were selected and the corresponding enaminones were synthesized (Table 2, entries 1–8) in order to test the optimal conditions predicted by the model.

**Figure 2** Experimental design for optimization of enaminone synthesis. A full factorial design using three variables – temperature, reaction time, and amount of diketone 4 – resulting in a total of eleven experiments including three central points.

**Figure 3** Multiple linear regression (MLR) model of the reaction conditions used for synthesis of enaminone 6. a) Regression coefficients showing the investigated variables and their correlation to the yield of 6. b) Contour plot of the predicted yield (cf. boxes) as a function of temperature and reaction time using 1.05 equivalents of diketone 4 and the methyl ester of leucine.

**Table 1** Experimental Design for the Synthesis of 6

<table>
<thead>
<tr>
<th>Entry</th>
<th>Temperature (°C)</th>
<th>Time (s)</th>
<th>Diketone (equiv)</th>
<th>Yield (%)</th>
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<td>100</td>
<td>300</td>
<td>1.05</td>
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<tr>
<td>2</td>
<td>140</td>
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<td>1.05</td>
<td>100</td>
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<td>3</td>
<td>100</td>
<td>900</td>
<td>1.05</td>
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<td>4</td>
<td>140</td>
<td>900</td>
<td>1.05</td>
<td>69</td>
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<tr>
<td>5</td>
<td>100</td>
<td>300</td>
<td>1.75</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>140</td>
<td>300</td>
<td>1.75</td>
<td>66</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>900</td>
<td>1.75</td>
<td>48</td>
</tr>
<tr>
<td>8</td>
<td>140</td>
<td>900</td>
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<td>65</td>
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<tr>
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<td>120</td>
<td>600</td>
<td>1.4</td>
<td>81</td>
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<tr>
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<td>600</td>
<td>1.4</td>
<td>92</td>
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<tr>
<td>11</td>
<td>120</td>
<td>600</td>
<td>1.4</td>
<td>76</td>
</tr>
</tbody>
</table>

**Table 2** Experimental Design for the Synthesis of 6
Use of the optimized reaction conditions gave isolated yields in the range 67–94% of @-tide building blocks for six of the amino acids used (Table 2, entries 1–6). Furthermore, the reaction could be forced to completion by the addition of more diketone 4 (0.5 equiv) and repeating the reaction once more (cf. enamino 11, Table 2, entries 6 and 10). The synthesis of enamino 10 from proline shows that the method can be extended to secondary amines. However, proline has limited use as a building block in \( \beta \)-strand mimetics due to its tendency to initiate turn formation in peptides. In addition, enamino 10 was formed in a 1:1 cis/trans ratio (the amide bond), according to NMR spectroscopy, which may be a complicating factor in some peptidomimetics.

If the side chains of the amino acids are too polar, e.g. serine and threonine, the amount of NMP has to be raised to 10% in order for the reaction to proceed. Although the reaction appeared to proceed well according to LC-MS, the isolated yields were low after purification. This could be explained by the unfortunate fact that the resulting enamines 12 and 13 have similar chromatographic properties to NMP, which prevents the use of simple flash chromatography for purification. In order to circumvent this problem 12 and 13 were re-synthesized, using water-extractable DMF as a cosolvent instead of NMP, but only moderate yields (48% and 52%, respectively) were obtained (Table 2, entries 7,8). Fortunately, this setback can be avoided by using protecting groups such as \( t \)-Bu or Boc on amino acids with polar side-chains (Table 2, entries 2,3). Use of \( t \)-Bu-protected threonine for the synthesis of enamino 14 also raised the isolated yield to 73% compared to 52% when the side chain of threonine was left unprotected (Table 2, entries 8, 9).

Most commercially available polar amino acids carry acid labile protecting groups, such as Boc and \( t \)-Bu, on their side-chains. To allow orthogonal deprotection, the base labile methyl group was used for protection of the \( \alpha \)-carboxylic acid in the amino acids used to prepare most of the compounds 6–14. However, for hydrolyzing methyl esters the method has to be mild enough so as not to cause epimerization of the \( \alpha \)-carbon of the amino acid. Use of a 0.1 M aqueous solution of LiOH in THF is known to sup-

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**Table 2 Enaminone Synthesis Using Optimized Conditions**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>( R^1 )</th>
<th>( R^2 )</th>
<th>Cosolvent</th>
<th>Diketone (equiv)</th>
<th>Yield (%)</th>
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<tbody>
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<td>6</td>
<td>Me</td>
<td>Me</td>
<td>NMP</td>
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<tr>
<td>2</td>
<td>7</td>
<td>Me</td>
<td>Me</td>
<td>NMP</td>
<td>1.05</td>
<td>69</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>Me</td>
<td>Me</td>
<td>NMP</td>
<td>1.05</td>
<td>90</td>
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<tr>
<td>4</td>
<td>9</td>
<td>( t )-Bu</td>
<td>NMP</td>
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<td>94</td>
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<tr>
<td>5</td>
<td>10</td>
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<td>( t )-Bu</td>
<td>NMP</td>
<td>1.05</td>
<td>68</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>Me</td>
<td>Me</td>
<td>NMP</td>
<td>1.05</td>
<td>67</td>
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<tr>
<td>7</td>
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<td>Me</td>
<td>Me</td>
<td>DMF</td>
<td>1.05</td>
<td>48</td>
</tr>
<tr>
<td>8</td>
<td>13</td>
<td>( t )-Bu</td>
<td>Me</td>
<td>DMF</td>
<td>1.05</td>
<td>52</td>
</tr>
<tr>
<td>9</td>
<td>14</td>
<td>Me</td>
<td>Me</td>
<td>NMP</td>
<td>1.05</td>
<td>73</td>
</tr>
<tr>
<td>10</td>
<td>11</td>
<td>Me</td>
<td>Me</td>
<td>NMP</td>
<td>1.05 + 0.5( a )</td>
<td>100</td>
</tr>
</tbody>
</table>

\( a \) An additional portion of diketone 4 was added and the microwave irradiation procedure was repeated.
press epimerization. Enaminone 6 was hydrolyzed to enaminone 15 (Scheme 1) in quantitative yield using these conditions. To investigate if any racemization had occurred, the reaction mixture was analyzed by chiral HPLC [S,S] Whelk-O1). A single peak was obtained, indicating that hydrolysis proceeded without racemization. The synthesis was then repeated starting from racemic leucine. In this case the two enantiomers were detected with baseline separation confirming that synthesis of 15 had been achieved without racemization.

A new three-step synthetic sequence to @-tide building blocks has been developed. Microwave-assisted chemistry was used in two of the steps to enhance the reaction rates. Multivariate design was used to optimize the key step, i.e. synthesis of enaminones without prior activation of the intermediateaza-cyclohexa-3,5-dione, which is condensed with amino acid esters. Overall, this gave a fast and efficient synthetic method that allows the use of structurally diverse amino acids, for instance in the synthesis of @-tide libraries. Work is now in progress to synthesize @-tides that mimic the G1 β-strand of the type 1 pilus chaperone FimC. The ability of such @-tides to interfere with protein–protein interactions required for pilus assembly in uropathogenic E. coli will be investigated.

All reactions were carried out under an inert atmosphere with anhydrous solvents under anhydrous conditions, unless otherwise stated. TLC was performed on Silica Gel 60 F254 (Merck) with detection CHCl3. IR spectra were recorded on a PERKIN-ELMER 343 polarimeter at 20 °C. IR spectra were recorded on a SmithCreator microwave device using intended septum-sealed reaction vials. The 1H and 13C NMR spectra were recorded on a JEOL JMS-SX 102 spectrometer. Mass spectrometry was performed on a Waters micromass ZQ. High-resolution mass spectra [FAB+] were recorded on a JEOL JMS-SX 102 spectrometer.

The experiments were planned using full-factorial design and were done in randomized order. Sample analysis was carried out by diluting the reaction mixture (20 μL) with H2O–MeCN (1.98 mL, 95:5) before injection onto a LC-MS column. Integrated peak areas were quantified and the yield was determined using a calibration curve. The results were analyzed by MLR using MODDE 6.0.

Enaminones 6–14; General Procedure

Amino acid esters (0.2 mmol) and Et3N (28 μL, 0.2 mmol) were added to the alloc-protected diketone 4 (41.4 mg, 0.21 mmol) in NMP–benzene (10:90, 1.6 mL) and the solution was heated by microwave irradiation in a monomode instrument at 140 °C for 300 s. A sat. soln of NH4Cl (1.5 mL) was added and the mixture was extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with brine (5 mL), dried over MgSO4, and concentrated. Purification by flash column chromatography gave enaminones 6–14 as yellow oils.

Enaminone 6 (Scheme 1) in quantitative yield using these conditions. To investigate if any racemization had occurred, the reaction mixture was analyzed by chiral HPLC [S,S] Whelk-O1). A single peak was obtained, indicating that hydrolysis proceeded without racemization. The synthesis was then repeated starting from racemic leucine. In this case the two enantiomers were detected with baseline separation confirming that synthesis of 15 had been achieved without racemization.

A new three-step synthetic sequence to @-tide building blocks has been developed. Microwave-assisted chemistry was used in two of the steps to enhance the reaction rates. Multivariate design was used to optimize the key step, i.e. synthesis of enaminones without prior activation of the intermediate aza-cyclohexa-3,5-dione, which is condensed with amino acid esters. Overall, this gave a fast and efficient synthetic method that allows the use of structurally diverse amino acids, for instance in the synthesis of @-tide libraries. Work is now in progress to synthesize @-tides that mimic the G1 β-strand of the type 1 pilus chaperone FimC. The ability of such @-tides to interfere with protein–protein interactions required for pilus assembly in uropathogenic E. coli will be investigated.
1H NMR (400 MHz, CDCl3): δ = 8.17 (br s, 1 H), 7.50 (d, J = 7.6 Hz, 1 H), 7.36 (d, J = 8.1 Hz, 1 H), 7.20 (t, J = 14.1, 7.0 Hz, 1 H), 7.12 (t, J = 14.7, 7.1 Hz, 1 H), 7.00 (d, J = 3.0 Hz, 1 H), 5.85–5.96 (m, 1 H), 5.48 (br s, 1 H), 5.16–5.33 (m, 3 H), 4.60 (d, J = 5.7 Hz, 2 H), 4.26–4.33 (m, 1 H), 4.08–4.16 (m, 2 H), 4.05 (s, 2 H), 3.38 (dd, J = 14.8, 5.0 Hz, 1 H), 3.25–3.47 (m, 2 H), 2.14–2.29 (m, 2 H), 1.92–2.14 (m, 3 H), 1.46 (d, J = 7.1 Hz, 1 H), 1.35 (t, J = 7.2 Hz, 1 H), 0.90 (d, J = 6.6 Hz, 3 H).

IR: 2940, 2901, 1688, 1503, 1299, 922 cm–1.

HRMS (FAB): m/z calcd for C18H29N2O6 [M + H +], 369.2029; found, 369.2026.

Alloc-Ach-Thr-O-t-Bu (13)
The solvent used for the reaction was DMF–benzene (10:90). Purification by flash column chromatography (EtOAc–MeOH, 95:5) gave 13 as a yellow oil.

Yield: 36.9 mg (52%); [α]D –96 (c 1.0, CHCl3).

IR: 3299, 2977, 1659, 1544, 1239, 1108 cm–1.

1H NMR (400 MHz, CDCl3): δ = 6.15 (d, J = 7.4 Hz, 1 H), 5.83–5.96 (m, 1 H), 5.18–5.33 (m, 3 H), 4.60 (d, J = 5.1 Hz, 2 H), 4.24–4.43 (m, 3 H), 3.97–4.13 (m, 2 H), 3.87 (dd, J = 8.2, 3.0 Hz, 1 H), 1.46 (s, 9 H), 1.24 (d, J = 6.5 Hz, 3 H).

13C NMR (100 MHz, CDCl3): δ = 191.6, 169.2, 161.8, 154.9, 132.2, 118.1, 95.6, 83.4, 76.7, 66.8, 61.0, 50.5, 44.2, 27.9, 20.8, 20.0.

HRMS (FAB): m/z calcd for C18H29N2O6 [M + H +], 355.1869; found, 355.1860.

Alloc-Ach-Thr(OMe)-t-Bu(14)
Purification by flash column chromatography (EtOAc–MeOH, 80:20) gave pure 14 as a yellow oil.

Yield: 53.8 mg (73%); [α]D –12 (c 1.0, CHCl3).

IR: 3257, 3020, 2929, 1700, 1597, 1549, 1216 cm–1.

1H NMR (400 MHz, CDCl3): δ = 5.83–6.01 (m, 1 H), 5.43 (d, J = 7.4 Hz, 1 H), 5.05–5.36 (m, 3 H), 4.62 (d, J = 5.1 Hz, 2 H), 4.33 (s, 2 H), 4.23 (q, J = 5.7 Hz, 1 H), 3.98–4.13 (m, 2 H), 3.83 (dd, J = 8.2, 3.0 Hz, 1 H), 3.71 (s, 1 H), 1.22 (d, J = 6.5 Hz, 3 H), 1.11 (s, 9 H).

13C NMR (100 MHz, CDCl3): δ = 191.2, 170.2, 161.0, 154.8, 132.3, 118.0, 95.9, 74.7, 67.2, 66.7, 60.6, 52.5, 50.5, 44.4, 28.2, 21.6.

HRMS (FAB): m/z calcd for C18H29N2O6 [M + H +], 369.2026; found, 369.2029.

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(8) Modde 6.0, Umetrics AB Box 7960, 907 19 Umeå, Sweden.