This is a repository copy of Solid-phase methodology for synthesis of O-alkylated aromatic oligoamide inhibitors of α-helix-mediated protein-protein interactions.

White Rose Research Online URL for this paper:
http://eprints.whiterose.ac.uk/83421/

Version: Published Version

**Article:**

https://doi.org/10.1002/chem.201204098

**Reuse**
Unless indicated otherwise, fulltext items are protected by copyright with all rights reserved. The copyright exception in section 29 of the Copyright, Designs and Patents Act 1988 allows the making of a single copy solely for the purpose of non-commercial research or private study within the limits of fair dealing. The publisher or other rights-holder may allow further reproduction and re-use of this version - refer to the White Rose Research Online record for this item. Where records identify the publisher as the copyright holder, users can verify any specific terms of use on the publisher’s website.

**Takedown**
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.
A major effort in modern bio-organic chemistry focuses on the design, synthesis and structural characterisation of foldamers, non-natural oligomers that adopt well-defined secondary, tertiary and quaternary structures. One ultimate objective of such studies is to recapitulate the functional behaviour of biomacromolecules. Particular emphasis has been placed on inhibitors of α-helix-mediated protein–protein interactions—an endeavour that in its own right represents a major challenge. The development of synthetic methodologies that allow access to small-to-medium sized libraries of foldamers incorporating diverse side chains, represents the cornerstone upon which such studies are pursued. In this regard, it is noteworthy that the most robust methodology exists for peptoids, β-peptides and more recently oligoureas; templates that have seen the most significant use in a biological context. and others have recently reported on the use of aromatic oligoamides as potential α-helix mimetics. Rather than topographical mimicry of the α-helix (as is the case for and other foldamers), these compounds mimic an α-helix by presenting key side chains in a rod-like template in a spatial orientation that matches that of the α-helix (Figure 1). Although solution methods for assembly of very large and long aromatic oligoamides have been described, a significant advance in this area would be the ready availability of solid-phase methods tolerant to a diverse array of side chains; this would facilitate library generation and ease of purification. Other than our own preliminary report on N-alkylated aromatic oligoamides, only a limited number of reports have been described on the synthesis of benzamidines and related aromatic oligoamides that meet the criteria outlined above. Herein, we describe such a method that can be used for synthesis of 3-O-alkylated aromatic oligobenzamides. Using microwave irradiation, trimers can be assembled on a solid support in 2.5 h in sufficient purity for screening purposes. The methodology is tolerant to a large and diverse collection of monomers and amenable to synthesis of significantly longer oligomers. The approach and our observations in developing it should have general applicability for synthesis of aromatic oligoamide foldamers.

In developing our approach we sought to avoid implementation of novel protecting group chemistries and constrained ourselves to the use of Fmoc (Fmoc = fluorenlymethoxycarbonyl) as a semi-permanent protecting group and permanent acid labile protecting groups on the side chains. Herein, we describe such a method that can be used for synthesis of 3-O-alkylated aromatic oligobenzamides. Using microwave irradiation, trimers can be assembled on a solid support in 2.5 h in sufficient purity for screening purposes. The methodology is tolerant to a large and diverse collection of monomers and amenable to synthesis of significantly longer oligomers. The approach and our observations in developing it should have general applicability for synthesis of aromatic oligoamide foldamers.
As is shown, a full array of peptide based side chains covering the entirety of functionality found in native peptide side chains is accessible (with the exception of cysteine, arginine and histidine), whilst several non-natural side chains and chiral side chains can also be incorporated. There are several noteworthy points as follows: 1) for benzylic side chains (e.g., 3e–k) it was necessary to use tin(II) chloride for nitro group reduction (3 to 4 in Scheme 1) as opposed to palladium on charcoal, so as to avoid cleavage of the side chain, 2) for the hydrolysis step (4 to 5 in Scheme 1), care was be required to avoid cleavage of the side chain, requiring use of lithium hydroxide and mild conditions (e.g., room temperature). Cleavage of the side chain by elimination of the phenol occurred under forcing conditions—a feature that prevented us from obtaining a monomer mimicking histidine. Additionally, for side chains possessing an electron-withdrawing group to the phenol, E1CB was promoted (not shown). Finally, for the tert-butyl ester side chain 1m we observed deprotection of the tert-butyl group using sodium hydroxide presumably via a ketene intermediate. We also synthesised an Fmoc-protected monomer mimicking glycine; our design positions the phenolic oxygen as the atom mimicking the α position of the amino acid within the helix and therefore this represents a poor mimic of glycine and would require protection during synthesis. We therefore protected commercially available 3-methyl-4-aminobenzoic acid with Fmoc to furnish the glycine mimic 1s.

A general outline of the solid-phase synthesis (SPS) method is illustrated in Scheme 2. In terms of developing this methodology, the amide bond forming reaction is challenging as the substrate is a deactivated aniline. We selected acid labile Wang resin for these studies and achieved resin loading using thionyl chloride or Ghosez/C29s reagent either directly to the resin or to glycine loaded resin. We attempted a series of screening experiments using the isopropyl monomer 1a, chloroform as solvent and microwave assistance (using a CEM peptide synthesiser) to identify suitable coupling regents. From our screening experiments, only activating agents that form acid chlorides proved successful (i.e., thionyl chloride and Ghosez’s reagent). This was not entire-
ly surprising given that our prior studies\cite{21,22,24} in the absence of microwave, indicated to us that strongly activated acids (e.g., acid chlorides) would be necessary to mediate amide bond formation. We then proceeded to develop an optimised method by attempting oligomer synthesis and broadening the monomer set. Unfortunately, the majority of monomers were found to be poorly soluble in chloroform, so we resorted to the use of DMF. Using in situ formation of the acid chloride from Ghosez's reagent and microwave irradiation, no amidine formation was observed. Similarly, with pre-activation or isolation of the acid chloride followed by microwave assistance, we were unable to effect the amidine formation. An explanation for these results was obtained from LC-MS analysis of the reaction mixture, which revealed capping of the immobilised aniline by both DMF and Ghosez’s reagent to give a stable amidine (Figure 2).

This capping reaction, which is observed even when the acid chloride is used directly, indicates that the solvent reversibly reacts with the acid chloride to generate the Vilsmeier intermediate, which can then cap the aniline. We did not observe this behaviour for synthesis of N-alkylated aromatic oligoamides—one explanation is that capping of an N-alkylated aniline results in an unstable intermediate, which cannot lose a proton to form the amidine (Figure 2). With these results in hand we performed a solvent screen to identify more polar solvents, which would not lead to such side reactions.

From our solvent screen we identified N-methylpyrrolidinone (NMP) as a suitable solvent with which to perform solid-phase coupling to give the aromatic benzamides. After further screening and optimisation we established that direct use of the acid chlorides obtained from thionyl chloride or pre-activation using Ghosez/C29s reagent prior to coupling in the microwave synthesiser could effect coupling in high yield (Scheme 2). The use of acid chlorides was preferable for the majority of alkyl/aryl Fmoc-protected monomers 1 as these could be precipitated and stored for at least one month with no decomposition. For the more highly functionalised monomers that tended not to precipitate upon reaction with thionyl chloride it was preferable to use the in situ method (these highly functionalised monomers tended to be less stable as acid chlorides). We found that for direct addition of acid chlorides, a single cycle of coupling at 50°C for 30 min in the absence of base was sufficient to achieve high conversion, however, for longer oligomers we used double couplings. For Fmoc removal no special optimisations were required and 20% piperidine in NMP was sufficient (Scheme 2). Care was required with the global deprotection reaction, which we performed off-line from the synthesiser; certain side chains (see below) were found to be susceptible to cleavage by elimination with the indole side chain a notable example, thus this stage of the procedure requires careful monitoring.

With these observations and optimisations established, we demonstrated the versatility of the method by synthesising a sufficient number of trimers 7–24 (Table 1) so as to demonstrate that each monomer in the set could couple and be coupled to. In addition we also illustrated that amino acids (other than glycine could be appended to the C-terminus 27 (through use of different amino acid loaded Wang resins) and to the N-terminus. The only problematic monomer was 1k with the resulting oligomer undergoing cleavage of the benzylidene under the standard deprotection conditions required to cleave the phenolic tert-butyl protecting group. In addition, whilst we observed reasonable coupling with

![Figure 2. Mechanism for capping of anilines during SPS via Vilsmeier intermediates.](image)

<p>| Table 1. Oligomers synthesised.[^{[a]}] |
|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Trimer</th>
<th>R(^1)</th>
<th>R(^2)</th>
<th>R(^3)</th>
<th>Final purity [%]</th>
<th>Yield [%]</th>
<th>Precipitate</th>
<th>HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>95</td>
<td>73</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>e</td>
<td>e</td>
<td>e</td>
<td>95</td>
<td>92</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>h</td>
<td>a</td>
<td>90</td>
<td>71</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>a</td>
<td>h</td>
<td>e</td>
<td>90</td>
<td>82</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>e</td>
<td>i</td>
<td>a</td>
<td>99</td>
<td>86</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>g</td>
<td>j</td>
<td>a</td>
<td>99</td>
<td>99</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>e</td>
<td>g</td>
<td>a</td>
<td>95</td>
<td>78</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>a</td>
<td>s</td>
<td>a</td>
<td>99</td>
<td>64</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>a</td>
<td>d[^{[b]}]</td>
<td>a</td>
<td>99</td>
<td>73</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>a</td>
<td>f[^{[c]}]</td>
<td>a</td>
<td>99</td>
<td>79</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>a</td>
<td>k</td>
<td>a</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>a</td>
<td>l</td>
<td>a</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>a</td>
<td>m[^{[d]}]</td>
<td>a</td>
<td>99</td>
<td>69</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>a</td>
<td>n[^{[d]}]</td>
<td>a</td>
<td>99</td>
<td>–</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>a</td>
<td>o[^{[d]}]</td>
<td>a</td>
<td>99</td>
<td>–</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>a</td>
<td>p</td>
<td>a</td>
<td>90</td>
<td>69</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>a</td>
<td>q[^{[e]}]</td>
<td>a</td>
<td>99</td>
<td>–</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>a</td>
<td>r[^{[e]}]</td>
<td>a</td>
<td>99</td>
<td>–</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

\[^{[a]}\] For an explanation of the R groups, see Scheme 1. \[^{[b]}\] By using in situ activation of monomer with thionyl chloride. \[^{[c]}\] By using in situ activation of monomer with Ghosez's reagent.
monomer 1l, we were unable to isolate and characterise the resulting trimer (18). Finally we also illustrated the versatility and power of the method through synthesis of longer oligomers 25, 26 and 28 (up to a hexamer). This foldamer was obtained in 10 h using double couplings and the NMR spectrum is given in Figure 3a for the product obtained directly from the resin. This spectrum is typical of the spectral data that is obtained immediately following resin cleavage and indicates that the oligomers are obtained in sufficient purity for preliminary screening. In several instances, this was not the case, however, cleaner material could be obtained by preparative HPLC.

To illustrate the potential for longer oligomers to act as mimics of extended helices, we performed molecular modelling on the hexamer 28 as is illustrated in Figure 3b (see the Supporting Information for more details). With all side chains located on one face, hexamer 28 can project side chains in such an orientation so as to mimic five consecutive side chains along an $\alpha$-helical surface, whilst rotation around the terminal Ar–CO bond permits the hexamer 28 to mimic a sixth chain. This demonstrates that such oligomers could find use in the inhibition of more extended $\alpha$-helix mediated protein–protein interactions. Finally we obtained several crystal structures of a representative trimer 29 (described previously)[21,24] comprising isopropyl monomers and with a C-terminal methyl ester and N-terminal nitro group (Figure 3c–e). These exemplify the salient points of our previously published analysis of the conformational preference of these oligomers,[21,23,40] that is, they adopt a rod-like conformation with free rotation around the Ar–CO axes and rotation around the Ar–NH axes restricted through S(5) intramolecular hydrogen bonding. Critically, side chains adopt both syn and anti orientations with respect to one another, whilst variations along the backbone permit the side chains to project in subtly different orientations.[23]

In conclusion, we have developed a method for synthesis of aromatic oligoamides containing a diverse array of natural and non-natural amino acid side chains using a microwave-assisted automated peptide synthesiser. A four-step monomer synthesis allows generation of Fmoc-protected building blocks for SPS with trimers accessible in 2.5 h in sufficient purity for screening. These foldamers represent good templates to act as mimetics of the $\alpha$-helix and hence as inhibitors of protein–protein interactions. Our method represents a useful tool with which to obtain protein–protein interaction inhibitors by sequence based design[9] and for library generation to screen against unknown targets. Our own future efforts in this area will describe detailed studies on aromatic oligoamide helix mimetics targeted against a broad range of protein–protein interactions.

**Experimental Section**

**General procedure for oligomer formation—single coupling.** Fmoc-protected pre-loaded Wang resin (127 mg, 0.1 mmol, 1 equiv) was loaded onto a CEM microwave peptide synthesiser after being swelled for a total of 30 min in NMP and CH$_2$Cl$_2$ solutions. A series of washes (3/C$_3$NMP), deprotection (2/C$_3$20% Piperidine/NMP, total of 3.5 min at 75°C) and further washes (5/C$_3$NMP) prepared the resin for coupling. Fmoc-protected acyl chloride of 1a–s (0.4 mmol, 4 equiv) obtained by pre-activation or prepared separately was dissolved in NMP (2.5 mL), delivered to the reaction vessel and submitted to microwave irradiation at 50°C for 30 min. A final series of filtered washes of the reaction vessel (3 × NMP) finishes a coupling cycle.

**Acknowledgements**

This work was supported by the European Research Council [ERC-StG-240324] and the Engineering and Physical Sciences Research Council
Keywords: amides · foldamers · helical structures · protein–protein interactions · solid-phase synthesis