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Solid-Phase Synthesis of Oxathiocoraline by a Key Intermolecular Disulfide Dimer

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Triostin,[1] Echinomycin,[2] Thiocoraline,[3] and BE-22179[4] (Figure 1) belong to a family of potent antitumoral bicyclic peptide antibiotics, all of marine origin but proceeding from distinct actinomycetes. These octadepsipeptides share a common general structure with two key heterocyclic units which act as bisintercalators to DNA.[5] The central disulfide or thioacetal bridge is flanked by two ester or thioester moieties which give shape to the symmetrical bicycle. The unusually high number of nonproteinogenic amino acids on such a small structure (NMe and D-aa) adds further difficulty to the synthesis of these depsipeptides. In the search for new compounds with anticancer activity, the preparation of new quinoxaline antibiotics was undertaken. Here we report the first and unique solid-phase synthesis of a member of this family, the oxathiocoraline **1**,[6] which exhibits high synthetic complexity [*N*Me amino acids, ester bonds, Cys(Me)].[7]

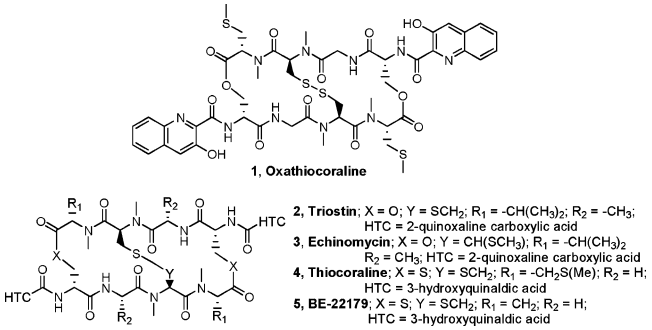
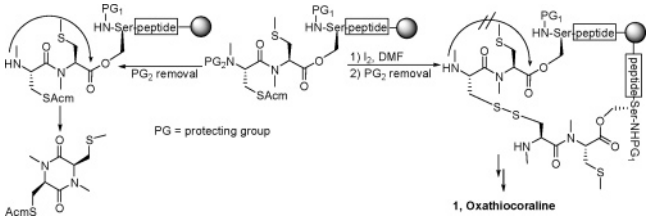


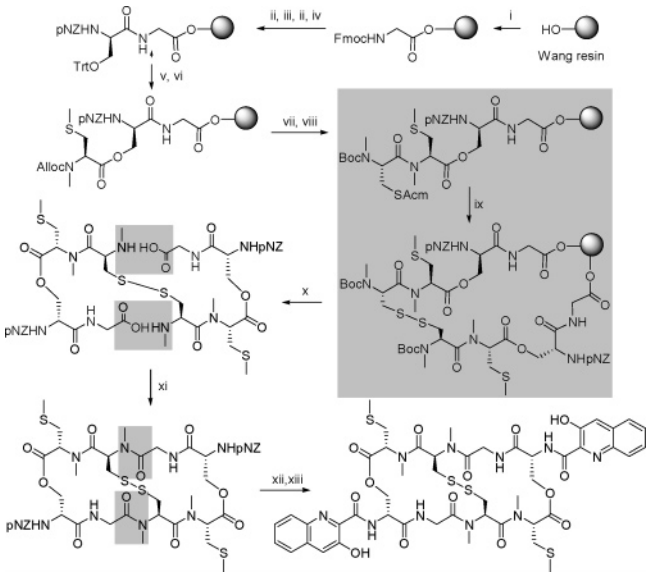
Figure 1.

From a synthetic point of view, the ester moiety imposes several additional drawbacks to the regular amide bond,[8] the most important being the risk of diketopiperazine (DKP) formation both at the resin level and within the chain. Furthermore, cysteines are prone to racemization when forming part of an ester. Moreover, *N*Me-Cys(Me) is prone to β-elimination to give didehydroalanine under basic conditions and to be easily oxidized. Finally, the presence of both a *N*Me-Cys(Me) residue and consecutive *N*Me amino acids calls for a delicate balance in the coupling conditions; while *N*Me-Cys(Me) would require no basic conditions throughout the elongation of the peptide, the coupling of *N*Me amino acids usually needs harsh (highly basic) conditions.[9] Initially, several attempts which covered a broad number of conventional strategies (stepwise synthesis, 4 + 4 fragment-coupling approach, choice of different starting points) were examined. Because of the strong propensity of the two consecutive *N*Me-Cys to form DKP and owing to the lability of the ester bond, the linear octadepsipeptide was never obtained. We reasoned that the presence of the disulfide in an earlier stage of the synthesis may restrict the flexibility of the chain, thereby minimizing DKP formation (Scheme 1). With this in mind, efforts were directed at obtaining a linear protected tetradepsipeptide of good purity, to achieve the intermolecular disulfide dimer prior to removal of the key Cys-protecting group.

Scheme 1. Formation of the Key Intermolecular Disulfide Dimer



Scheme 2. Synthesis of Oxathiocoraline[a]



[a] (i) Fmoc-Gly-OH, DIPCDI, DMAP, CH_2Cl_2−DMF (9:1); (ii) piperidine−DMF (1:4); (iii) Fmoc-D-Ser(Trt)-OH, HATU, HOAt, DIEA, DMF; (iv) *p*NZ-Cl, DIEA, DMF; (v) TFA−TIS−CH_2Cl_2 (2:2.5:95.5); (vi) Alloc-*N*Me-Cys(Me)-OH, DIPCDI, DMAP, CH_2Cl_2−DMF; (vii) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2; (viii) Boc-*N*Me-Cys(Acm)-OH, HATU, HOAt, DIEA, DMF; (ix) I_2 in DMF; (x) TFA−H_2O−CH_2Cl_2 (3:1:7); (xi) PyBOP, HOAt, DIEA, DMF; (xii) $Na_2S_2O_4$, ACN−EtOH−H_2O; (xiii) 3-hydroxyquinaldic acid, EDC·HCl, HOSu, CH_2Cl_2.

For the construction of the tetradepsipeptide (Scheme 2), Gly was chosen as the starting point since in this case the occurrence of DKP formation is minimized along the chain. As a solid support, Wang resin, was used.[10]

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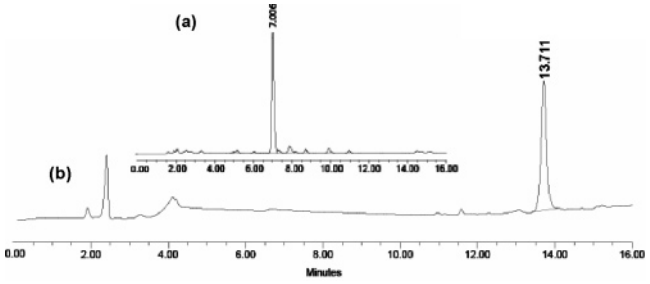

Figure 2. HPLC of (a) tetradepsipeptide; (b) oxathiocoraline.

As we started the synthesis of the tetradepsipeptide, we became aware that apart from the choice of the starting point, the choice of protecting groups was also crucial. A range of different protecting groups were required because of the fragility of the ester bond during chain elongation:[11] (a) Fmoc for the residues preceding the ester functionality;[11a] (b) *p*-nitrobenzyloxycarbonyl (*p*NZ) in the place of the heterocylic moiety;[11b] (c) trityl (Trt) for the protection of the side chain of Ser;[11c] (d) allyloxycarbonyl (Alloc) for the residue forming part of the ester;[11d] and (e) Boc for the *N*-terminal Cys.[11e] Other choices of protecting groups gave the tetradepsipeptide in poor yields. The optimal conditions for the synthesis of the tetradepsipeptide were thus as follows: Fmoc-Gly-OH was loaded onto Wang resin via symmetrical anhydride/DMAP; after removal of the Fmoc group, Fmoc-D-Ser(Trt)-OH was introduced using HATU/HOAt/DIEA in DMF. Next, the Fmoc group was removed before construction of the ester bond, and the free amine was reprotected with *p*NZ-Cl/DIEA. The *p*NZ group in this position was compatible with the ester bond, and thus could be removed at the tetradepsipeptide level on solid-phase or at a later stage in solution.

Next, the trityl group was cleaved by treatment with TFA−TIS−CH_2Cl_2 and elongation was continued through the side chain of Ser. To make the ester bond, Alloc-*N*Me-Cys(Me)-OH was introduced with DIPCDI/DMAP overnight.[12] Next, the Alloc group was removed under neutral conditions by using $Pd(PPh_3)_4/PhSiH_3$ in CH_2Cl_2. The introduction of Boc-*N*Me-Cys(Acm)-OH was accomplished with two treatments of HATU/ HOAt/DIEA.[13] In these conditions the tetradepsipeptide was obtained in 89% purity[14] (Figure 2a).

At this point the key intermolecular disulfide dimer was formed using I_2 in DMF.[15] Finally, to avoid sulfoxide formation, cleavage of the dimer from the resin was performed in the presence of H_2O with two treatments of TFA−H_2O−CH_2Cl_2 (3:1:6), with concomitant cleavage of the Boc group. After evaporation of the solvent, the dimer was lyophilized. Next, the crude dimer was dissolved in CH_2Cl_2−DMF (19:1) and preactivated with HOAt. After adjusting the pH to slightly basic conditions with DIEA, solid PyBOP was added to obtain, after 6 h, the *p*NZ-protected bicycle, with no signs of internal DKP formation.[16] The *p*NZ group was removed with $Na_2S_2O_4$ in ACN−EtOH−H_2O (4:1:1), and the 3-hydroxyquinaldic acid[17] was introduced with the rather mild coupling method, EDC·HCl and HOSu in CH_2Cl_2, to avoid over incorporation of carboxylic acid. The crude oxathiocoraline was obtained in 7% overall yield, after purification by semipreparative HPLC (Figure 2b).

In vitro activity of oxathiocoraline was evaluated in three tumor cell lines (MDA-MB-231, A549, HT29) and showed growth inhibitory effect, similar to other depsipeptides presently in clinical trials (see SI).

In conclusion, here we describe an optimal synthetic strategy for the solid-phase preparation of oxathiocoraline. The key step is the solid-phase formation of the disulfide dimer peptide, which prevents the formation of internal DKPs. Other drawbacks have been overcome by the use of a myriad of protecting groups such as Fmoc, *p*NZ, Alloc, and Boc for N-protection, Trt for the OH, and Acm for the SH, in combination with a number of coupling reagents: DIPCDI/DMAP for the ester formation, HATU /HOAT/ DIEA for the sequential incorporation of the amino acids, PyBOP/ HOAt/DIEA for the bis lactamization, and EDC·HCl/HOSu for the final incorporation of the cromophore. We presume that this strategy will be of general application for the preparation of other important bicyclic depsipeptides.

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Supporting Information Available: Experimental procedures, characterization material, and abbreviations. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Wang resin could be used in this case since the incorporation of the third residue was through the side chain of Ser, which avoided the formation of diketopiperazines at the resin level.
(11) The use of alternative protection was accompanied by dramatic side reactions. For instance (a) cleavage of the ester bond was observed with the use of piperidine. (b) The *p*NZ in this position was compatible with the ester bond. In other positions of the chain (third and fourth residue), the removal conditions (6 M $SnCl_2$, 1.6 mM HCl) effected cleavage of the ester functionality. (c) TBDMS could also be used at this point but the amino acid was not commercial. (d) Both the use of Fmoc and *p*NZ groups resulted in cleavage of the ester bond. (e) The use of other acid-labile groups (such as Trt) were also investigated but abandoned because of the difficulty in obtaining the protected *N*Me amino acid.
(12) A certain degree of racemization (2%) was observed; shorter times or reagents that minimize racemization (e.g., MSNT) gave lower conversions or lower purity products (MSNT requires large excess of base).
(13) Less equivalents of base were used to avoid side reactions.
(14) Alternatively, the heterocycle moiety can be introduced on solid-phase with DIPCDI/HOAt in DMF (see SI).
(15) This intermolecular disulfide dimer formation was showed to be also possible on another kind of resins, showing the robustness of this strategy.
(16) To exclude a 13-membered ring cyclization in this strategy, reduction of the disulfide bridge has been carried out showing the mass corresponding to the reduced peptide and not to the half structure (see SI).
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