DOI: 10.1002/chem.200600815

Total Solid-Phase Synthesis of the Azathiocoraline Class of Symmetric Bicyclic Peptides

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Abstract: Thiocoraline is a potent antitumor agent isolated from the marine organism *Micromonospora sp.* This symmetric bicyclic depsipeptide binds the minor groove of DNA. Here we report two solid-phase strategies for the syntheses of azathiocoraline and its analogues. The thioester linkage was replaced by an amide bond to improve the compound's pharmacokinetic properties. The first strategy is based on a convergent (4+4) approach, whilst the second is a stepwise synthesis, cyclizations in both approaches occurring on the solid support. These two strategies were designed to overcome problems caused by the presence of consecutive noncommercial *N*-methyl amino acids, to avoid epimerization during cyclization and/or fragment condensation, and to form the disulfide bridge under solid-phase conditions. The heterocyclic moiety was added in the last step of the synthesis to assist the preparation of libraries of new compounds with potential therapeutic applications.

Keywords: bisintercalators · coupling reagents · cyclic peptides · natural products · on-resin cyclization · peptides

Introduction

Thiocoraline is a potent new antitumor agent that has been isolated from the marine organism *Micromonospora sp*.[1] It exhibits a complex depsipeptide structure that shows biological activity of interest, and the research efforts of many scientific groups and pharmaceutical companies have consequently focused on this peptide in order to improve knowledge about its mode of action and to study its potential application in cancer therapy. Thiocoraline shares several common motifs with a family of antitumor peptide antibiotics, which includes BE-22179, triostin A and echinomycin.[2] This group of peptides is characterized by: a) bicyclic structures, b) C_2 symmetry, c) DNA intercalation chromophore moieties, d) ester or thioester linkages at the terminal part of the peptide chains,[3] e) disulfide or analogous bridges in the middle of the peptide chain, f) the presence of several *N*-methyl amino acids, and g) non-natural amino acids of D configuration. The *N*-terminal amino function in thiocoraline is thus capped with 3-hydroxyquinaldic acid, which acts as an intercalating chromophore group, whilst the two peptide chains are bridged by thioester and disulfide linkages from Cys residues, these being the components that afford the disulfide *N*-methylated bridge and D configuration, as well as the two Cys(Me) residues. All these characteristics confer on this family of peptides the capacity to bind with DNA by bisintercalation and thus to alter the vital cell cycle. Thiocoraline inhibits DNA elongation by DNA polymerase α at a concentration that blocks cell cycle progression and clonogenicity.[4] Peptide synthesis now faces the challenge of obtaining this family of molecules: a task that is crucial if they are to be used for therapeutic applications.

Here we discuss two solid-phase syntheses of aza analogues of thiocoraline (Scheme 1), in which the thioester bridges have been replaced by amide ones with the aim of improving pharmacokinetics. Azathiocoraline should be resistant to enzyme degradation and more soluble than the natural peptide.

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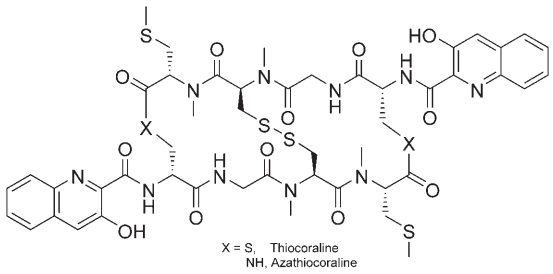


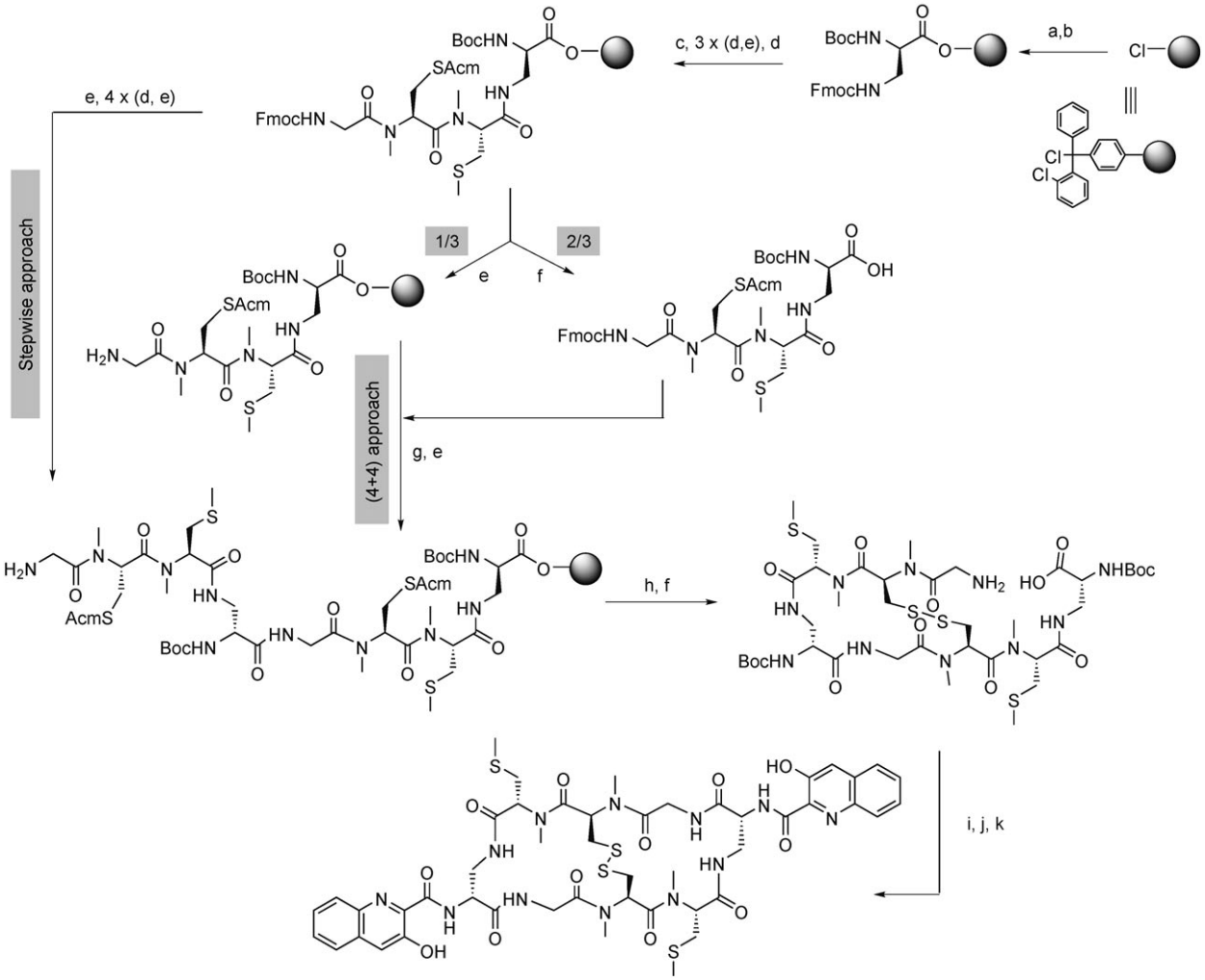
Scheme 1. Structures of thiocoraline and azathiocoraline.

The synthesis of these kinds of peptides represents a challenge because, in addition to being bicyclic (disulfide and lactam), they contain consecutive *N*-methyl amino acids and the quinaldic acid moiety. Consecutive *N*-methyl amino acids can undergo internal diketopiperazine (DKP) formation[5] and, furthermore, *N*Me-Cys(Me) easily undergoes an elimination side-reaction to produce didehydroalanine. Finally, the quinaldic acid moiety contains a hydroxy group, which may, depending on the coupling method used, require protection. To the best of our knowledge, this study is the first example of a solid-phase synthesis of this kind of molecule.[6,7,8] The establishment of a robust solid-phase synthetic strategy should facilitate the development of drug discovery programmes based on these natural products.

The strategy makes provision for the following issues: incorporation of a heterocyclic moiety in the last step to facilitate efficient synthesis of analogues with distinct chromophores, and a cyclization step with optimum amide bond formation, taking advantage of the symmetry to simplify the synthetic strategy and avoiding DKP formation favored by the presence of consecutive *N*-methyl amino acids.

Results and Discussion

Protection Scheme and synthesis of *N*-methyl cysteines: For the azathiocoraline analogues (Scheme 2), D-2,3-diaminopropionic acid (Dap) was used instead of D-Cys. The syn-



Scheme 2. Solid-phase strategy developed for the preparation of azathiocoraline by a (4+4) approach: a) Boc-D-Dap(Fmoc)-OH, DIEA, CH_2Cl_2; b) MeOH; c) piperidine/DMF (1:4), piperidine/DBU/toluene/DMF (1:1:4:14); d) Fmoc-AA-OH/HATU/DIEA, DMF; e) piperidine/DMF (1:4); f) TFA/CH_2Cl_2 (1:99); g) PyOAP/DIEA, CH_2Cl_2; h) I_2, DMF; i) EDC·HCl/HOAt/DIEA, CH_2Cl_2 (1 mM); j) TFA/H_2O (19:1); k) 3-Hydroxyquinaldic acid/EDC·HCl/HOSu/DIEA, CH_2Cl_2.

thetic approaches follow a fluorenylmethoxycarbonyl (Fmoc)/*tert*-butoxycarbonyl (Boc) strategy with use of a chlorotrityl chloride resin (CTC), which minimizes the formation of DKPs and allows cleavage of protected peptides under very mild acid conditions.[9] The acetamidomethyl (Acm) group, an orthogonal protecting group to the Fmoc and Boc groups, was chosen to mask the thiol group of the Cys during peptide elongation and to facilitate the direct formation of a disulfide bridge. H-*N*Me-Cys-OH, the precursor of the two Cys-based amino acids, was synthesized by the reduction of cyclic L-thiaproline with sodium and ammonium as previously described.[6,10] H-*N*Me-Cys-OH was *S*-methylated with MeI in a $NaHCO_3$ solution[11] and the Acm group was introduced by treatment with *N*-(hydroxymethyl)acetamide in a water solution with the addition of trifluoroacetic acid (TFA), catalyzed with trifluoromethanesulfonic acid.[10,12] The Fmoc group was introduced by treatment with the most reactive reagent, Fmoc-Cl.[12]

In our hands, direct methylation of Boc-Cys(Me/Acm)-OH did not work properly.[13] Furthermore, *N*-methylation under solid-phase conditions with use of the 2-nitrobenzenesulfonyl protecting group, as described by Miller and Scanlan, provoked β-elimination, mainly at the *N*Me-Cys(Me) residue,.[14]

The chromophore group, 3-hydroxyquinaldic acid, was synthesized by a new efficient strategy developed from commercially available 3-aminoquinoline.[15]

Convergent (4+4) approach: The first solid-phase strategy used for the preparation of azathiocoraline followed a convergent (4+4) approach (Scheme 2). Because of the symmetric character of the peptide, both fragments were prepared from only one synthesis. Although macrolactamization can be performed in any position, the most favorable option appeared to be between the α-amino function of Gly—as the Gly was not hindered—and the carboxylic acid function of D-Dap. The α-amino function of D-Dap, the carboxylic component, was thus protected in the form of a carbamate, thereby preventing the formation of oxazolone and minimizing epimerization.[16] In addition, the first amino acid (D-Dap) grows through the side chain, thereby avoiding DKP formation.

Fragment synthesis thus began with limited anchoring of Boc-D-Dap(Fmoc)-OH on the CTC resin (0.7 mmol of protected amino acid per g of resin, the functionalization being 1.5 mmol g^{-1}).[17] The stepwise synthesis was carried out through the β-amino function of D-Dap.[18] The first Fmoc group was removed with piperidine/DMF (1:4) and additional treatment with piperidine/DBU/DMF/toluene (5:5:70:20). These conditions facilitated the complete removal of this highly unreactive carbamate.[19] Couplings were performed with 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium hexafluorophosphate 3-oxide (HATU) and *N*,*N*-diisopropylethylamine (DIEA), which assured complete acylation of the *N*-methylamino acids. The coupling reaction was tested by the De Clercq test[20] and the removal of Fmoc groups was followed by UV measurements, which indicated a 95–97% yield per cycle. Once the tetrapeptide had been assembled, 2/3 of the protected peptide resin was cleaved with TFA/CH_2Cl_2 (1:99) and, without prior purification and with use of (7-azabenzotriazol-1-yloxy)-tris(pyrrolidino)phosphonium hexafluorophosphate (PyAOP) and DIEA as coupling reagents, it was coupled to the peptide resin, from which the Fmoc group had previously been removed.[21,22] After removal of the Fmoc group, the disulfide bridge was formed under solid-phase conditions with I_2 (5 equiv, 2.5 equiv × Acm) in DMF for 10 min (Figure 1a, b), by a method developed in our laboratory.[23] This on-resin oxidation greatly facilitates the removal of the excess of reagent and soluble side-products by simple filtration and extensive washing with $CHCl_3$ to remove I_2. The cyclic peptide was cleaved from the resin and, without further purification, the second cyclization was attempted at 1 mM concentration in CH_2Cl_2 (Figure 1c). Several coupling reagents, such as *N*,*N*′-diisopropylcarbodiimide (DIPCDI)/hydroxybenzotriazole (HOBt)/DIEA, DIPCDI/HOAt/DIEA, PyOAP/DIEA, 1-ethyl-3-(3′-dimethylamino-propyl)-carbodiimide hydrochloride (EDC·HCl)/7-aza-hydroxybenzotriazole (HOAt)/DIEA, were tested. The use of the water-soluble carbodiimide EDC·HCl and HOAt achieved a cleaner crude product and had the additional advantage that the reagents were removed in the aqueous workup treatment. DIEA was added to neutralize the trifluoroacetate salt and to avoid unwanted trifluoroacetylation, as in the synthesis of Kahalalide F.[24] After removal of the Boc group with TFA/H_2O (19:1), the 3-hydroxyquinaldic acid component was incorporated by use of EDC·HCl/hydroxysuccinimide (HOSu)/DIEA (Figure 2d). The use of more reactive additives such as HOAt or HOBt in place of HOSu resulted in more complex crude products because of over-acylation of the hydroxy function of the heterocyclic moiety.[25] The final product was purified (3.5% overall nonoptimized yield) by preparative TLC with CH_2Cl_2/MeOH (95:5) as eluents (Figure 1e). The product was characterized by HPLC and HRMS.[26]

Solid-phase bicyclization approach: The second strategy (Scheme 3), used to synthesize [*N*Me-Leu^4, *N*Me-Leu^8]azathiocoraline, an analogue of azathiocoraline in which *N*Me-Cys(Me) is substituted by *N*Me-Leu, started with the attachment of D-Dap onto the CTC resin through the β-amino group. This allowed the formation of the two cycles under solid-phase conditions, taking advantage of the pseudo-dilution phenomenon[27] associated with the solid-phase and allowing easy removal of the coupling reagents used for lactamization by simple filtration and washing. An additional advantage of this strategy is that the bicyclic peptide obtained after cleavage from the resin is unsymmetrical with respect to the α-amino function of D-Dap. The α-amino group that was anchored to the resin was therefore unprotected and hence ready to be acylated, while the other was protected by the Boc group, which can be removed either before acylation or after the first acylation has been performed. In the first case, both α-amino groups are acylated

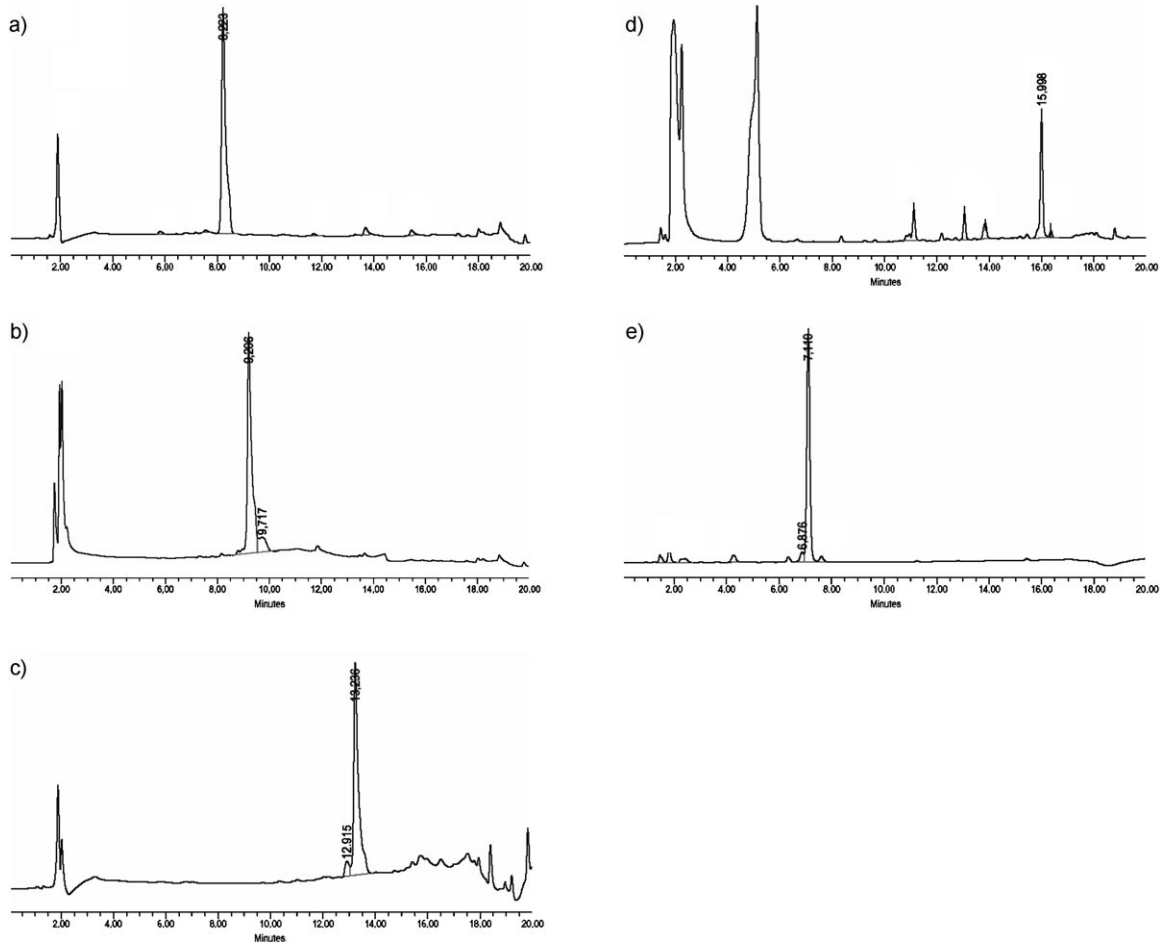


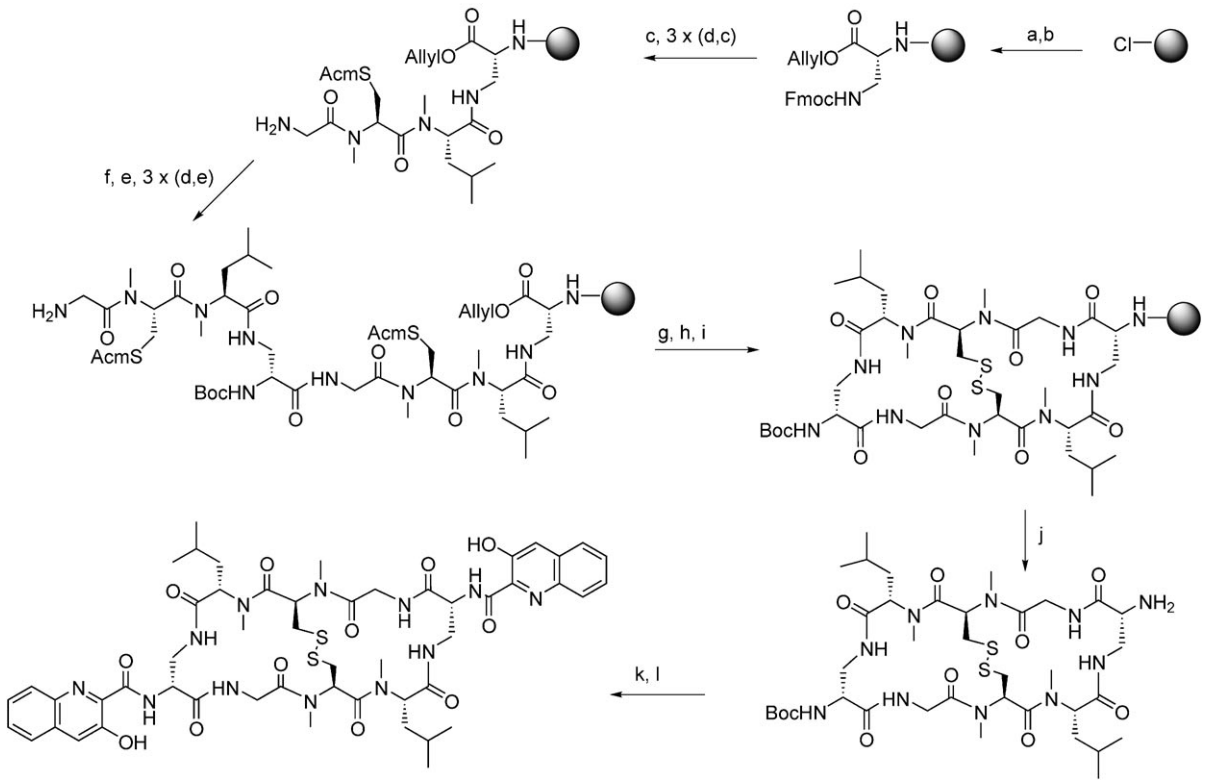
Figure 1. HPLC chromatograms of: a) linear octapeptide, b) after formation of disulfide bridge on solid support, c) after macrolactamization, d) crude azathiocoraline, e) purified azathiocoraline. Reversed-phase C_{18} columns were used for the analysis with elution by linear gradient (over 15 min) of 0.036% TFA in CH_3CN and 0.045% TFA in H_2O from 1:9 to 7:3 in a–d and from 5:5 to 9:1 in e.

with the same intercalating moiety, while in the second strategy two distinct moieties can be incorporated, thus introducing a new source of diversity.[28]

This scheme requires the orthogonal protection of the carboxylic function of D-Dap, which was achieved with the aid of an allyl ester. Limited attachment of H-D-Dap(Fmoc)-Oallyl,[29] to the CTC resin through the α-amino group was also performed, and a stepwise elongation of the peptide chain was carried out with HATU/DIEA (Figure 2a). Removal of the allyl ester was accomplished with $[Pd(PPh_3)_4]$ (0.1 equiv) in the presence of $PhSiH_3$ (10 equiv) as scavenger.[30] The formation of the disulfide bridge was achieved as indicated above. Cyclization on the solid support was incomplete after use of DIPCDI/HOBt, as shown by the ninhydrin test, but was efficiently accomplished with DIPCDI/HOAt. The partially protected bicyclic peptide was cleaved as in the case of the previous peptide (Figure 2b). Finally, the Boc group was removed, the 3-hydroxyquinaldic acid was incorporated, and the target was purified as above (Figure 2c, d). The product was characterized by HPLC and HRMS.[31]

Conclusion

Here we report two optimized solid-phase strategies for the preparation of azathiocoralines, both of which overcome the problems caused by the presence of consecutive noncommercial *N*-methyl amino acids. These two strategies were designed for addition of the heterocyclic moiety in the last step of the synthesis to favor the preparation of libraries, to avoid epimerization during cyclization and/or fragment condensation, and to form the disulfide bridge under solid-phase conditions, thereby facilitating the removal of the excess of I_2. Whilst the first strategy takes advantages of symmetry to provide a rapid synthesis, the second provides an unsymmetrical compound that may be of interest for exploration of the relationship between symmetry and activity while allowing most steps under solid-phase conditions. A cornerstone of the two approaches is the concourse of the distinct coupling reagents. Under solid-phase conditions, aminium/uronium and phosphonium salts are used, thereby facilitating the removal of excess and side-products by filtration and washing. Whilst HATU is used for the stepwise



Scheme 3. Solid-phase strategy developed for the preparation of azathiocoraline analogues by a stepwise approach. a) H-D-Dap(Fmoc)-OAllyl, DIEA, CH_2Cl_2; b) MeOH; c) piperidine/DMF (1:4), piperidine/DBU/toluene/DMF (1:1:4:14); d) Fmoc-AA-OH/HATU/DIEA, DMF; e) piperidine/DMF (1:4); f) Boc-D-Dap(Fmoc)-OH/HATU/DIEA, DMF; g) $[Pd(PPh_3)_4]$, $PhSiH_3$, CH_2Cl_2; h) I_2, DMF; i) DIPCDI/HOAt, DMF; j) TFA/CH_2Cl_2 (1:99); k) TFA/H_2O (19:1); l) 3-hydroxyquinaldic acid/EDC·HCl/HOSu/DIEA, CH_2Cl_2.

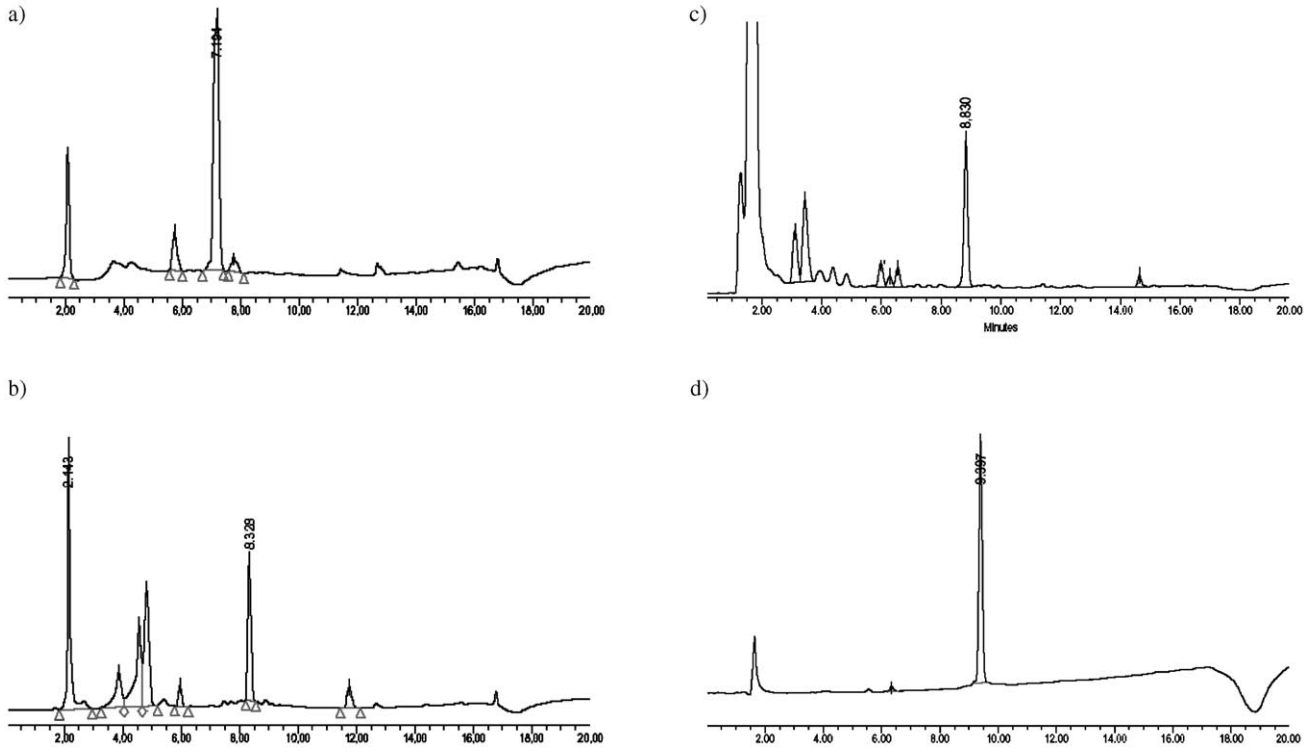


Figure 2. HPLC chromatograms of: a) linear octapeptide, b) after formation of the bicyclic peptide on solid support, c) after the coupling of the 3-hydroxyquinaldic acid, d) purified [*N*Me-Leu[4], *N*Me-Leu[8]]azathiocoraline. Reversed-phase C_{18} columns were used for the analysis with elution by linear gradient (over 15 min) of 0.036% TFA in CH_3CN and 0.045% TFA in H_2O from 0:1 to 1:0 in a and b, 5:5 to 10:0 in c, and from 5:5 to 9:1 in d.

elongation of the peptidic chain to assure complete acylation of *N*-methylamino acids, PyAOP is preferred for fragment condensation in the first (4+4) approach because it does not cause capping of the amino function in slow couplings. For solid-phase cyclization, HOAt gave better results than HOBt as an additive to DIPCDI. The cyclization and incorporation of 3-hydroxyquinaldic acid was performed in solution with EDC·HCl to allow easy removal of carbodiimide derivatives during the workup. While HOAt is the best additive for cyclization, the less reactive HOSu is preferable for the final acylation because it prevents over-incorporation of the carboxylic acid. These state-of-the-art strategies are valid for a broad range of thiocoraline analogues and should contribute to the discovery of new compounds with therapeutic applications.

Experimental Section

Materials and equipment: Protected amino acid derivatives, HOBt, PyAOP and Fmoc-Cl were purchased from Applied Biosystems (Framingham, MA), Bachem (Bubendorf, Switzerland), Albatross (Montreal, Canada) and NovaBiochem (Läufelfingen, Switzerland). CTC resin was a gift from Rohm & Haas (Philadelphia, PA). DIEA, DIPCDI, piperidine, TFA, ammonia, iodomethane, allyl bromide, 2-quinoxalinecarboxylic acid and quinaldic acid were from Aldrich (Milwaukee, WI), and EDC· HCl was a gift from Luxembourg Industries (Tel Aviv, Israel). DMF, CH_2Cl_2, acetonitrile (HPLC grade), methanol (HPLC grade), dioxane, Et_2O, TBME and EtOAc were from SDS (Peypin, France). (R)-(−)-Thiazolidine-4-carboxylic acid, trifluoromethanesulfonic acid, *N*-(hydroxymethyl)acetamide, and *N*-hydroxysuccinimide were obtained from Fluka (Buchs, Switzerland). All commercial reagents and solvents were used as received, with the exception of DMF and CH_2Cl_2, which were degassed with nitrogen to remove volatile contaminants (DMF) and stored over activated 4 Å molecular sieves (Merck, Darmstadt, Germany) (DMF) or $CaCl_2$ (CH_2Cl_2). Et_2O was stored over Na.

Solution reactions were performed in round-bottomed flasks. Organic solvent extracts were dried over anhydrous $MgSO_4$, followed by solvent removal under reduced pressure at temperatures below 40 °C.

Solid-phase syntheses were performed in polypropylene syringes (2.5 mL) fitted with polyethylene porous discs. Solvents and soluble reagents were removed by suction. The Fmoc group was removed with piperidine/DMF (1:4, 1×1 min, 3×5 min, 1×10 min). Washings between deprotection, coupling and final deprotection steps were performed with DMF (5×1 min) and CH_2Cl_2 (5×1 min) with use of 5 mL solvent per g resin for each wash. Peptide synthesis transformations and washes were performed at 25 °C.

HPLC columns (Symmetry C18 reversed-phase column, 5.0 μm× 4.6 mm×150 mm) were obtained from Waters (Ireland). Analytical HPLC was performed on a Waters instrument containing two solvent delivery pumps (Waters 1525), an automatic injector (Waters 717 autosampler), a dual wavelength detector (Waters 2487) and a system controller (Breeze V3.20), and on an Agilent 1100 instrument incorporating two solvent delivery pumps (G1311A), an automatic injector (G1329A) and a DAD (G1315B). UV detection was at 215 or 220 nm, and linear gradients of CH_3CN (+0.036% TFA) to H_2O (+0.045% TFA) were run at a flow rate of 1.0 mL min^{-1} for 15 min.

IR spectra were obtained by using a Nicolet 510 FT-IR spectrophotometer. MALDI-TOF and ES(+)-MS analyses of peptide samples were performed on an Applied Biosystems VoyagerDE RP, by using ACH matrix, in a Waters Micromass ZQ spectrometer, and in an Agilent Ion Trap 1100 Series LC/MSDTrap. ^{1}H NMR (400 Hz) and ^{13}C NMR (100 MHz) spectroscopy was performed on a Varian Mercury 400. Chemical shifts (δ) are expressed in parts per million downfield from tetramethylsilane. Coupling constants are expressed in Hertz.

HCl·H*N*Me-Cys-OH:[10] (R)-(−)-Thiazolidine-4-carboxylic acid (8 g, 60.2 mmol) and sodium were added sequentially in small portions to liquid ammonia (200 mL), thereby preserving the excess of sodium. Ammonium chloride was then added. The solvent was removed under reduced pressure, and the product was dissolved in the minimum possible amount of water and was acidified with hydrochloric acid until pH 2. The water was removed under reduced pressure and the product was extracted with methanol to afford the title compound (6.9 g, 40.1 mmol, 66%) as a white solid. ^{1}H NMR (D_2O, 400 MHz): δ = 3.93 (m, 1H; CH^{α}), 3.03 (m, 2H; CH_2^{β}), 2.65 ppm (s, 3H; *N*Me); ^{13}C NMR (D_2O, 100 MHz): δ = 169.9 (CO Cys), 60.7 (CH^{α}), 32.0 (CH_2^{β}), 22.9 ppm (CH_3, NMe); ES(+): *m*/*z*: calcd for $C_4H_9NO_2S$ 135.0; found: 136.0 $[M+H]^+$.

Fmoc-*N*Me-Cys(Me)-OH: HCl·H*N*Me-Cys-OH[10] (4.1 g, 23.8 mmol) was dissolved in H_2O/THF (1:1, 170 mL) and the mixture was cooled (4 °C). Iodomethane (2.1 mL, 33.3 mmol) was dissolved in THF (68 mL), and $NaHCO_3$ (5.3 g, 71.4 mmol) in H_2O (68 mL). Both solutions were sequentially added dropwise to the amino acid solution. After the addition was complete, the mixture was stirred for 4 h at 25 °C, the product was acidified to pH 5, and the solvent was removed under reduced pressure. The product was then dissolved in H_2O/dioxane (1:1, 80 mL) and the solution was cooled to 4 °C. Fmoc-Cl (10 g, 38.8 mmol) was dissolved in dioxane (8 mL) and the amino acid solution was added. The mixture was stirred for 2 h at 4 °C and for 3 days more at 25 °C, the pH being maintained at 9–10. Dioxane was removed and the aqueous solution was washed with TBME (3×50 mL), the aqueous layer was acidified with HCl until pH 2, and the product was extracted with EtOAc (3×50 mL). The solvent was removed under reduced pressure to give the product (1.23 g, 71% purity and 7% yield), which was purified by MPLC. Analytical HPLC (t_R = 12.8 min, conditions 3:7 to 7:3, 99% purity); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 9.1 (brs, 1H; COOH), 7.75 (m, 2H; 2×CH_{arom} Fmoc), 7.60 (m, 2H; 2×CH_{arom} Fmoc), 7.55 (m, 2H; 2×CH_{arom} Fmoc), 7.34 (m, 2H; 2×CH_{arom} Fmoc), 4.87 & 4.67 (1:2) (m, 1H; CH^{α}), 4.46 (m, 2H; CH_2 Fmoc), 4.29 & 4.22 (1:2) (m, 1H; CH Fmoc), 3.11 & 2.82 (1:2) (m, 2H; CH_2^{β}), 2.96 & 2.89 (1:2) (s, 3H; *N*Me), 2.12 & 1.88 ppm (1:2) (s, 3H; SMe); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 175.0 (CO Cys), 157.5 (CO Fmoc), 143.9 (2×C_{arom} Fmoc), 141.6 (CO_{arom} Fmoc), 128.0 (2×CH_{arom} Fmoc), 127.4 (2×CH_{arom} Fmoc), 125.3 (2×CH_{arom} Fmoc), 120.3 (2×CH_{arom} Fmoc), 68.0 (CH_2 Fmoc), 58.6(CH^{α}), 47.3 (CH Fmoc), 33.4 (CH_2^{β}), 32.1 (CH_3, NMe), 15.7 ppm (CH_3, SMe); MALDI-TOF MS (DHB): calcd for $C_{20}H_{21}NO_4S$: 371.1; found: 372.0 $[M+H]^+$, 393.9 $[M+Na]^+$, 409.9 $[M+K]^+$.

Fmoc-*N*Me-Cys(Acm)-OH: HCl·H*N*Me-Cys-OH[10] (4.1 g, 23.8 mmol) was dissolved in H_2O (12.3 mL) and the solution was purged with Ar. *N*-(Hydroxymethyl)acetamide (3.54 g, 39.7 mmol) was added, and the mixture was cooled to 4 °C under Ar. A solution of trifluoroacetic acid in trifluoromethanesulfonic acid (95:5, 41 mL) was added, the mixture was stirred for 16 h at 25 °C, the product was dissolved in H_2O (2% Na_2CO_3)/dioxane (1:1, 80 mL), and the solution was then cooled to 4 °C. Fmoc-Cl (10 g, 38.8 mmol) was dissolved in dioxane and added to the amino acid solution, and the mixture was stirred for 2 h at 4 °C and for 3 days more at 25 °C with the pH at 9–10. The dioxane was removed, the aqueous solution was washed with TBME (3×50 mL), the aqueous layer was acidified with HCl until pH 2, and the product was extracted with EtOAc (3×50 mL). The solvent was removed under pressure and coevaporated with Et_2O to give a white solid (3.0 g, 71% purity, 33% yield). The product was purified by MPLC. Analytical HPLC (t_R = 8.8 min, conditions 3:7 to 7:3, 99% purity); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.76 (m, 2H; 2×CH_{arom} Fmoc), 7.58 (m, 2H; 2×CH_{arom} Fmoc), 7.39 (m, 2H; 2×CH_{arom} Fmoc), 7.29 (m, 2H; 2×CH_{arom} Fmoc), 7.01 & 6.55 (1:3.7) (m, 1H; NH Acm), 4.95 & 4.73 (m, 1H; CHα), 4.55, 4.25 (m, 4H; CH_2 Fmoc, CH_2 Acm), 4.21 (m, 1H; CH Fmoc), 3.25 & 2.9 (m, 2H; CH_2β), 2.91 & 2.90 (s, 3H; *N*Me), 2.02 & 1.99 ppm (s, 3H, Acm); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 172.6 (CO Cys), 157.9 (CO Fmoc), 156.0 (CO Acm), 144.0 (2×C_{arom} Fmoc), 141.6 (CO_{arom} Fmoc), 128.0 (2×CH_{arom} Fmoc), 127.4 (2×CH_{arom} Fmoc), 125.3 (2×CH_{arom} Fmoc), 120.2 (2×CH_{arom} Fmoc), 68.5 (CH_2 Fmoc), 58.8 (CH^{α}), 47.4 (CH Fmoc), 42.1 (CH_2 Acm),

32.0 (CH_2^{β}), 31.3 (CH_3, *N*Me), 23.1 ppm (CH_3, Acm); MALDI-TOF MS (DHB): *m/z*: calcd for $C_{22}H_{24}N_2O_5S$: 428.1; found: 429.2 $[M+H]^+$, 451.2 $[M+Na]^+$, 467.2 $[M+K]^+$.

H-D-Dap(Fmoc)-Oallyl: DIEA (164 mL, 0.94 mmol) was added to a suspension of Boc-D-Dap(Fmoc)-OH (200 mg, 0.47 mmol) in EtOAc (5 mL). The mixture was stirred at 25 °C until dissolution was complete, and allyl bromide (1 mL, 11.6 mmol) was then added. The resulting mixture was heated at reflux for 1 h, cooled, filtered, diluted with EtOAc (5 mL) and finally washed with aqueous $NaHCO_3$ (5%, 3×5 mL), HCl (1 N, 3×5 mL) and NaCl (sat, 3×5 mL). The organic layer was then concentrated to give a crude oil, which was purified by crystallization from Et_2O and dioxane to afford Boc-D-Dap(Fmoc)-Oallyl (128 mg, 0.27 mmol, 58%) as a white, amorphous powder. The Boc group was removed by treatment with a solution of TFA/H_2O (95:5). The mixture was stirred for 1.5 h at 25 °C, the solvent was removed in vacuo, and the remaining acid was eliminated by co-evaporations with Et_2O to afford the title compound (97 mg, 0.26 mmol, 98%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.70 (m, 2H; 2×CH_{arom} Fmoc), 7.55 (m, 2H; 2×CH_{arom} Fmoc), 7.30 (m, 2H; 2×CH_{arom} Fmoc), 7.21 (m, 2H; 2×CH_{arom} Fmoc), 5.88 (m, 1H; CH allyl), 5.28 (dm, *J* = 17.2 Hz, 1H; =C*H*H′ allyl), 5.18 (dm, J = 10.6 Hz, 1H; =CH*H*′ allyl), 4.68 (m, 1H; CH^{α}), 4.59 (m, 1H; CH Fmoc), 4.32, 4.10 (m, 2H; CH_2 allyl or CH_2 Fmoc), 3.56 ppm (m, 2H; CH_2^{β}); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 167.6 (CO Dap), 157.6 (CO Fmoc), 143.9 (2×C_{arom} Fmoc), 141.4 (CO_{arom} Fmoc), 130.0 (CH allyl), 128.1 (2×CH_{arom} Fmoc), 127.4 (2×CH_{arom} Fmoc), 125.2 (2×CH_{arom} Fmoc), 120.1 (2×CH_{arom} Fmoc), 67.6 (OCH_2 allyl), 67.4 (CH_2 Fmoc), 53.2 (CH Fmoc), 47.0 (CH^{α}), 41.0 ppm (CH_2^{β} allyl); ES(+): *m/z*: calcd for $C_{21}H_{22}N_2O_4$: 366.2; found: 367.1 $[M+H]^+$, 389.1 $[M+Na]^+$.

{[Boc-D-Dap(&^{1})-Gly-MeCys(Acm)-MeCys(Me)&^{2}][H-Gly-MeCys-(Acm)-MeCys(Me)&^{1}][Boc-D-Dap(&^{2})-O-CTC resin]}[32]—(4+4) approach: CTC resin (150 mg, 1.6 mmol g^{-1}) was placed in a 10 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was then washed with DCM (5×1 min) and a solution of Boc-D-Dap(Fmoc)-OH (45 mg, 0.21 mmol) and DIEA (123 μL, 1.33 equiv) in CH_2Cl_2 (2.5 mL) was then added. After 5 min, more DIEA (60 μL, 0.66 equiv) was added and the mixture was stirred for 55 min at 25 °C. The reaction was terminated by addition of MeOH (120 μL) and the mixture was stirred for a further 10 min. The Boc-D-Dap(Fmoc)-O-CTC resin was subjected to the following washings/treatments: filtration, CH_2Cl_2 (5×30 s), DMF (5×30 s), piperidine/DMF (1:4, 1×1 min, 3×5 min, 1×10 min), piperidine/DBU/toluene/DMF (5:5:20:70, 2×5 min). Loading, calculated by measuring absorbance at 290 nm, was 0.61 mmol g^{-1}.

The elongation of the peptide was achieved by sequential addition of Fmoc-AA-OH with HATU and DIEA (1:1:2) as coupling reagents in DMF in pre-activation mode. The mixture was stirred for 35 min and after filtration the corresponding colorimetric test indicated the completion of the coupling. Next, the peptide resin was washed with DMF (5×30 s) and treated with a solution of piperidine/DMF (1:4) to remove the Fmoc group, and the coupling plus Fmoc removal was then measured, as shown in Table 1.

The peptide resin was split into fractions (1/3 and 2/3). The former was treated with a piperidine/DMF (1:4) solution as described above, whilst the latter was cleaved from the resin with a TFA/H_2O solution (1:99, 5×30 s), and filtrates were collected over H_2O (3 mL, 60 mL H_2O per gram resin). The combined filtrates were concentrated to dryness under reduced pressure to provide the protected tetrapeptide (34.4 mg, 43 μmol, 78% yield). Analytical HPLC (t_R = 8.7 min, conditions 3:7 to 10:0, 88% purity); MALDI-TOF MS (DHB): *m/z*: calcd for $C_{37}H_{50}N_6O_{10}S_2$: 802.3; found: 825.5 $[M+Na]^+$, 841.5 $[M+K]^+$.

Table 1. Report on the synthesis of the tetrapeptide resin.[a]

Fmoc-AA-OH	Amount [mg (equiv)]	Test	Yield [%]
Fmoc-*N*Me-Cys(Me)-OH	102, (3)	ninhydrin (−)	90
Fmoc-*N*Me-Cys(Acm)-OH	118, (3)	De Clercq (−)	96
Fmoc-Gly-OH	136, (5)	De Clercq (−)	

[a] In all cases the coupling reactions were performed in DMF for 35 min.

Nonpurified lyophilized product was added to the unprotected peptide resin fraction with PyOAP (22.4 mg, 43 μmol) and DIEA (22 μL, 129 μmol) in DMF. The mixture was stirred overnight at 25 °C. After a positive De Clercq test without filtration, we added more PyOAP (22 mg, 43 μmol) and DIEA (22 μL, 129 μmol) to the mixture for 4 h, followed by more PyOAP (11 mg, 22 μmol) and DIEA (11 μL, 65 μmol) for a further 2 h until the De Clercq test was negative. We removed the Fmoc group as described above and measured fragment coupling yields, which were 81%. An aliquot was cleaved from the resin for analysis by HPLC-MS. Analytical HPLC (t_R = 8.3 min, conditions 1:9 to 7:3, 91% purity); MALDI-TOF MS (DHB): *m/z*: calcd for $C_{44}H_{78}N_{12}O_{15}S_4$: 1142.5; found: 1143.4 $[M+H]^+$, 1165.4 $[M+Na]^+$.

{[Boc-D-Dap(&^{1})-Gly-MeCys(Acm)-MeCys(Me)&^{2}][H-Gly-MeCys-(Acm)-MeCys(Me)&^{1}][Boc-D-Dap(&^{2})-O-CTC resin]}—Stepwise Approach: CTC resin (300 mg, 1.6 mmol g^{-1}) was placed in a 10 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was then washed with DCM (5×1 min), and a solution of Boc-D-Dap(Fmoc)-OH (90 mg, 0.7 mmol) and DIEA (244 μL, 4.7 equiv) in CH_2Cl_2 (2.0 mL) was then added. After 5 min, more DIEA (122 μL, 2.3 equiv) was added and the mixture was stirred for 55 min at 25 °C. The reaction was terminated by the addition of MeOH (240 μL) and the mixture was stirred for a further 10 min. The Boc-D-Dap(Fmoc)-O-CTC resin was subjected to the following washings/treatments: filtration, CH_2Cl_2 (5×30 s), DMF (5×30 s), piperidine/DMF (1:4, 1×1 min, 3×5 min, 1×10 min), piperidine/DBU/toluene/DMF (5:5:20:70, 2×5 min). Loading, calculated by measuring absorbance at 290 nm, was 0.64 mmol g^{-1}.

The elongation of the peptide was achieved by sequential addition of Fmoc-AA-OH with HATU and DIEA (1:1:2) as coupling reagents in DMF in pre-activation mode. The mixture was stirred for 35 min and after filtration the corresponding colorimetric test indicated completion of the coupling. Next, the peptide resin was washed with DMF (5×30 s) and treated with a solution of piperidine/DMF (1:4) to remove the Fmoc group, and the coupling plus Fmoc removal was then measured, as shown in Table 2.

An aliquot was cleaved for analysis by HPLC-MS. This technique showed two close peaks with the same mass. The global linear synthesis yield was 78%. Analytical HPLC (t_R = 8.3, 8.4 min, conditions 1:9 to 7:3, 83% purity); ES(+): *m/z*: calcd for $C_{44}H_{78}N_{12}O_{15}S_4$: 1142.5; found: 1143.9 $[M+H]^+$.

{[Boc-D-Dap&^{1}-Gly-MeCys(&^{2})-MeCys(Me)&^{3}][H-Gly-MeCys(&^{2})-MeCys(Me)&^{1}][Boc-D-Dap(&^{3})-O-CTC resin]}—disulfide bond formation on solid support: A solution of I_2 (122 mg, 5 equiv, 2.5 equiv×Acm) in DMF was added to the peptide resin and the mixture was stirred for 10 min at 25 °C. After filtration, the resin was washed repeatedly with DMF (5×30 s), CH_2Cl_2 (10×30 s) and $CHCl_3$ (5×30 s). HPLC-MS analysis of an aliquot cleaved from the resin indicated that the disulfide bond formation had been completed. The peptide resin was cleaved with a solution of TFA/H_2O (1:99, 5×30 s), and filtrates were collected over H_2O (18 mL, 60 mL H_2O per gram resin). The combined filtrates were evapo-

Table 2. Report on the synthesis of the octapeptide resin synthesized by carboxylic acid anchoring.[a]

Fmoc-AA-OH	Amount [mg (equiv)]	Test	Yield [%]
Fmoc-*N*Me-Cys(Me)-OH	214, (3)	ninhydrin (−)	95
Fmoc-*N*Me-Cys(Acm)-OH	247, (3)	De Clercq (−)	97
Fmoc-Gly-OH	285, (5)	De Clercq (+)	97
	285, (5)	De Clercq (−)	
Boc-D-Dap(Fmoc)-OH	246, (3)	ninhydrin (−)	97
Fmoc-*N*Me-Cys(Me)-OH	214, (3)	ninhydrin (−)	98
Fmoc-*N*Me-Cys(Acm)-OH	247, (3)	De Clercq (−)	97
Fmoc-Gly-OH	285, (5)	De Clercq (−)	94

[a] In all cases the coupling reactions were performed in DMF for 35 min.

rated to dryness under reduced pressure to provide the cyclic peptide (86 mg, 57% yield). Analytical HPLC (t_R = 9.4 min, conditions 1:9 to 7:3, 82% purity); MALDI-TOF MS (DHB): *m/z*: calcd for $C_{38}H_{66}N_{10}O_{13}S_4$: 998.4; found: 999.3 $[M+H]^+$, 1021.3 $[M+Na]^+$, 1037.3 $[M+K]^+$.

{[Boc-D-Dap(&[1])-Gly-MeCys(&[2])-MeCys(Me)&[3]][Boc-D-Dap(&[3])-Gly-MeCys(&[2])-MeCys(Me)&[1]]}: Cyclic peptide (85 mg, 85 μmol) dissolved in CH_2Cl_2 (85 mL, 1 mM) was added to a solution of HOAt (53 mg, 340 μmol) in the minimum amount possible of DMF. DIEA (30 μL, 170 μmol) and later EDC·HCl (65 mg, 340 μmol) were added and the mixture was stirred for 35 min at 25 °C. The solvent was removed in vacuo, a solution of CH_2Cl_2/Et_2O (1:1, 6 mL) was added, and the organic layer was washed with aqueous $NaHCO_3$ (5%, 2×4 mL), HCl (1 N, 2× 4 mL) and NaCl (sat, 2×4 mL). The solvent was removed under reduced pressure. Analytical HPLC (t_R = 9.4 min, conditions 1:9 to 7:3, 88% purity); ES(+): *m/z*: calcd for $C_{38}H_{64}N_{10}O_{12}S_4$: 980.4; found: 982 $[M+H]^+$.

Azathiocoraline: The bicyclical peptide was dissolved in TFA/H_2O (19:1, 3 mL) and the mixture was vigorously stirred for 1.5 h at 25 °C. TFA was evaporated under reduced pressure and the residual acid was removed by co-evaporation with Et_2O. H_2O was added and the product was lyophilized. A white solid was obtained (16.7 mg). Unprotected bicycle peptide (16.7 mg, 21.4 μmol) was dissolved in CH_2Cl_2 (250 μL), and DIEA (11.1 μL) was then added. 3-Hydroxyquinoline-2-carboxylic acid (12.3 mg, 64.2 μmol) was pre-activated with EDC·HCl (12.4 mg, 64.2 μmol) and HOSu (7.4 mg, 11 μmol) in CH_2Cl_2 (1 mL), and after 15 min the solution was added to the peptide solution. The solution was stirred for 16 h at 25 °C and more coupling reagents were then added: EDC·HCl (12.4 mg, 64.2 μmol) and HOSu (7.4 mg, 11 μmol) for two more days. The crude product was purified by PTLC (SiO_2, CH_2Cl_2/MeOH 95:5 as eluent) to afford the final product (2.5 mg). Analytical HPLC (t_R = 7.1 min, conditions 5:5 to 9:1); HRMS: *m/z* calcd for $C_{48}H_{58}N_{12}O_{12}S_4$: 1122.3180; found: 1123.3253 $[M+H]^+$.

{[Boc-D-Dap(&[1])-Gly-MeCys(Acm)-MeLeu&[2]][H-Gly-MeCys(Acm)-MeLeu&[1]][CTC resin-D-Dap(&[2])-Oallyl]}: CTC resin (50 mg, 1.6 mmol g^{-1}) was placed in a 10 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was then washed with DCM (5× 1 min), and a solution of H-D-Dap(Fmoc)-Oallyl (12.8 mg, 35 μmol) and DIEA (40 μL, 6.67 equiv) in CH_2Cl_2 (0.5 mL) was then added. After 5 min, more DIEA (20 μL, 3.33 equiv) was added and the mixture was stirred for 55 min at 25 °C. The reaction was terminated by addition of MeOH (40 μL) and the mixture was stirred for a further 10 min. The CTC resin-*N*H-D-Dap(Fmoc)-Oallyl resin was subjected to the following washings/treatments: filtration, CH_2Cl_2 (5×30 s), DMF (5×30 s), piperidine/DMF (1:4, 1×1 min, 3×5 min, 1×10 min), piperidine/DBU/toluene/DMF (5:5:20:70, 2×5 min). Loading, calculated by measuring absorbance at 290 nm, was 0.43 mmol per gram. Elongation of the peptide was achieved by sequential addition of Fmoc-AA-OH with HATU, and DIEA as coupling reagent in DMF was added to the peptide resin. The mixture was stirred for 35 min and after filtration the corresponding colorimetric test indicated completion of the coupling. In this case, the resin was washed with DMF (5×30 s) and treated with a solution of piperidine/DMF (1:4, 1×1 min, 3×5 min, 1×10 min) to remove the Fmoc group. Absorbance was determined in each case to obtain the yield of the coupling as shown in Table 3.

An aliquot was cleaved from the resin and analyzed as described above. We achieved an 81% yield of linear peptide and 79% purity of the crude product. Analytical HPLC (t_R = 7.3 min, conditions 0:1 to 1:0, 79% purity); MALDI-TOF MS (DHB): *m/z*: calcd for $C_{46}H_{82}N_{12}O_{13}S_2$: 1074.6; found: 1075.7 $[M+H]^+$, 1097.7 $[M+Na]^+$, 1113.7 $[M+K]^+$.

{[Boc-D-Dap(&[1])-Gly-MeCys(&[2])-MeLeu&[3]][CTCresin-D-Dap(&[3])-Gly-MeCys(&[2])-MeLeu&[1]]}: Allyl ester was removed with three treatments with $[Pd(PPH_3)_4]$ (6 mg, 0.1 equiv) and $PhSiH_3$ (62 μL, 10 equiv) dissolved in CH_2Cl_2 for 15 min under Ar. The remaining Pd was removed with three treatments of diethyldithiocarbamate sodium in DMF (0.2 M) for 15 min. A solution of I_2 (81 mg, 5 equiv, 2.5 equiv × Acm) in DMF was added and the solution was stirred for 10 min. After filtration, the peptide resin was washed repeatedly with DMF (5×30 s), CH_2Cl_2 (10×30 s) and $CHCl_3$ (5×30 s). HPLC-MS analysis of the aliquot cleaved from the resin indicated completion of disulfide bond formation.

Table 3. Report on the synthesis of the octapeptide resin synthesized by amino anchoring.[a]

Fmoc-AA-OH	Amount [mg (equiv)]	Test	Yield [%]
Fmoc-*N*Me-Leu-OH	24, (3)	ninhydrin (−)	97
Fmoc-*N*Me-Cys(Acm)-OH	27, (3)	De Clercq (−)	95
Fmoc-Gly-OH	37, (5)	De Clercq (−)	99
Boc-D-Dap(Fmoc)-OH	26, (3)	ninhydrin (−)	95
Fmoc-*N*Me-Leu-OH	24, (3)	ninhydrin (−)	98
Fmoc-NMe-Cys(Acm)-OH	27, (3) 27, (3)	De Clercq (+) De Clercq (+)	99
Fmoc-Gly-OH	37, (5)	De Clercq (−)	97

[a] In all cases the coupling reactions were performed in DMF for 35 min.

In a first attempt, the cycling step was performed with DIPCDI (2 equiv) and HOBt (2 equiv) as coupling reagents in DMF for 40 min. Because the ninhydrin test was positive, a second coupling was performed with more reactive reagents DIPCDI (2 equiv)/HOAt (2 equiv) for 40 min. The ninhydrin test was clearer and a second coupling with same reagents was required to complete the cyclization. Analytical HPLC (t_R = 8.3 min, conditions 0:1 to 1:0, 31% purity); MALDI-TOF MS (DHB): *m/z*: calcd for $C_{37}H_{64}N_{10}O_{10}S_2$: 872.4; found: 873.7 $[M+H]^+$, 895.6 $[M+Na]^+$, 911.5 $[M+K]^+$.

[*N*Me-Leu[4], *N*Me-Leu[8]]azathiocoraline: The peptide was cleaved from the resin as described above. After removal of the solvent, a solution of TFA/H_2O (95:5) was added and the mixture was stirred for 1.5 h at 25 °C. The solvent was removed under reduced pressure, H_2O was then added, and the product was lyophilized. The 3-hydroxyquinoline-2-carboxylic acid was coupled as described in the synthesis of azathiocoraline. The product was characterized by HPLC-MS. Analytical HPLC (t_R = 9.4 min, conditions 5:5 to 9:1); HRMS: *m/z* calcd for $C_{52}H_{66}N_{12}O_{12}S_2$: 1114.4365; found: 1115.4437 $[M+H]^+$.

Acknowledgements

This work was partially supported by funds from CICYT (BIO2002–2301, BQU2003–00089, and PETRI 95–0658-OP), Generalitat de Catalunya (Grup Consolidat and Centre de Referència en Biotecnología) and PharmaMar. The help of Dr. Jan Spengler in the hexafluoroacetone protection (Ref. [13]) is gratefully acknowledged.

[1] F. Romero, F. Espliego, J. Perez Baz, T. Garcia de Quesada, D. Gravalos, F. De la Calle, J. L. Fernandez-Puentes, *J. Antibiot.* **1997**, *50*, 734–737.
[2] a) M. Waring, *J. Path. Biol.* **1992**, 1022–1034; b) H. Otsuka, J. Shoji, *Tetrahedron* **1967**, *23*, 1535–1542.
[3] This peptide can be considered to be made up of two antiparallel symmetric chains with the amino terminals capped by the chromophore groups.
[4] E. Erba, D. Bergamaschi, S. Ronzoni, M. Faretta, S. Taverna, M. Bonfanti, C. V. Catapano, G. Faircloth, J. Jimeno, M. D'Incalci, *British J. Cancer* **1999**, *80*, 971–980.
[5] M. Teixidó, F. Albericio, E. Giralt, *J. Pept. Res.* **2005**, *57*, 153–166.
[6] Some elegant solution syntheses of thiocoraline and related molecules have recently been published: a) D. L. Boger, S. Ichikawa, *J. Am. Chem. Soc.* **2000**, *122*, 2956–2957; b) D. L. Boger, J. K. Lee, *J. Org. Chem.* **2000**, *65*, 5996–6000; c) M. Shin, K. Inouye, N. Higuchi, Y. Kyogoku, *Bull. Chem. Soc. Jpn.* **1984**, *57*, 2211–2215; d) D. L. Boger, J. H. Chen, K. W. Saionz, *J. Am. Chem. Soc.* **1996**, *118*, 1629–1644; e) D. L. Boger, M. W. Ledeboer, M. Kume, M. Searcey, Q. Jin,

J. Am. Chem. Soc. **1999**, *121*, 11375–11383; f) D. L. Boger, M. W. Ledeboer, M. Kume, Q. Jin, *Angew. Chem.* **1999**, *111*, 2533–2536; *Angew. Chem. Int. Ed.* **1999**, *38*, 2424–2426; ; g) Y. B. Kim, H. K. Kim, J. Y. Park, S. K. Kim, *Bioorg. Med. Chem. Lett*, **2004**, *14*, 541–544; h) K. B. Lorenz, U. Diederichsen, *J. Org. Chem.* **2004**, *69*, 3917–3927.

[7] During the preparation of this manuscript the solid-phase synthesis of TANDEM, a cyclic depsipeptide with some features in common with thiocoraline, but lacking the *N*-methyl amino acids and the free hydroxy group of the heterocycle, has been published: J. P. Malkinson, M. K. Anim, M. Zloh, M. Searcey, A. J. Hampshire, K. R. Fox, *J. Org. Chem.* **2005**, *70*, 7654–7661.

[8] For a short report on the solid-phase synthesis of complex peptides, see: M. A. Ciufolini, in *Strategies and Tactics in Organic Synthesis*, Vol. 6 (Ed.: M. Harmata), Elsevier, Amsterdam, NL, **2005**, pp. 25–26.

[9] K. Barlos, D. Gatos, W. Schäfer, *Angew. Chem.* **1991**, *103*, 571–572; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 590–593.

[10] J-F. Liu, X-X. Tang, B. Jiang, *Synthesis* **2002**, 1499–1501.

[11] Use of MeBr resulted in a *S*-permethylation followed by a β-elimination reaction.

[12] F. Albericio, A. Grandas, A. Porta, E. Pedroso, E. Giralt, *Synthesis* **1987**, 271.

[13] Methylation with MeI under basic conditions was achieved only on a small scale (<100 mg), whilst on larger scales β-elimination of Cys(SMe) was observed, as well as *N*-methylation of the amide bond of the Acm protecting group. Alternatively, treatment of these derivatives with paraformaldehyde and *p*-toluenesulfonic acid or with CH_2I_2 under basic conditions did not result in the formation of the corresponding oxazolone, reduction of which could have afforded the target compounds. Finally, hexafluoroacetone was used to protect both the amino and the carboxyl group, but the subsequent *N*-methylation did not work.

[14] a) S. C. Miller, T. S. Scanlan, *J. Am. Chem. Soc.* **1998**, *120*, 2690–2691; b) S. C. Miller, T. S. Scanlan, *J. Am. Chem. Soc.* **1997**, *119*, 2301–2302.

[15] Commercial 3-aminoquinoline was transformed into 3-hydroxyquinoline by the Bucherer reaction (93%) and protected as a MOM ether (67%). Then, 1,2-addition of methyllithium to the quinoline ring (88%), followed by oxidation of the methyl group to a carboxylic acid in a two-step procedure afforded the target compound in an overall yield of 37%: E. Riego, N. Bayó, C. Cuevas, F. Albericio, M. Álvarez, *Tetrahedron* **2005**, *61*, 1407–1411. In our hands, this method has given clearly better results than the previous method described by D. L. Boger, J. Chen, *J. Org. Chem.* **1995**, *60*, 7369–7371.

[16] P. Lloyd-Williams, F. Albericio, E. Giralt, *Chemical Approaches to the Synthesis of Peptides and Proteins*, CRC, Boca Raton (FL, USA), **1997**.

[17] The use of Cl-Trt-Cl-resins of higher loadings (>0.5 mmol g^{-1}) for the preparation of hydrophobic peptides usually results in complex crude products: C. Chiva, M. Vilaseca, E. Giralt, F. Albericio, *J. Pept. Sci.* **1999**, *5*, 131—140. Unreacted reactive sites were capped with MeOH/DIEA.

[18] This strategy also prevents the formation of DKPs. This peptide is prone to undergoing this side-reaction because of the presence of *N*-methyl amino acids and Gly.

[19] S. A. Kates, N. A. Solé, M. Beyermann, G. Barany, F. Albericio, *Pept. Res.* **1996**, *9*, 106–113.

[20] In our hands, this test showed a superior performance to the ninhydrin and chloranil tests for the detection of primary and secondary amines. a) A. Madder, N. Farcy, N. G. C. Hosten, H. De Muynck, P. J. De Clercq, J. Barry, A. P. Davis, *Eur. J. Org. Chem.* **1999**, 2787–2791; b) J. Vázquez, G. Qushair, F. Albericio, Colorimetric Tests for Solid Phase Synthesis. In *Methods in Enzymology*, Vol. 369 (Ed.: G. Morales), Academic Press, San Diego, **2003**, 21–35.

[21] Phosphonium derivatives are more convenient than uronium reagents such as HATU for rather slow couplings, since the latter can terminate the peptide chain through a guanylidation reaction. F. Albericio, J. M. Bofill, A. El-Faham, S. A. Kates, *J. Org. Chem.* **1998**, *63*, 9678–9683.

[22] Alternatively, the elongation of the full sequence can be performed stepwise by the same synthetic protocol (Scheme 1). In this case, yields of both strategies were very similar.

[23] a) C. García-Echeverría, F. Albericio, M. Pons, G. Barany, E. Giralt, *Tetrahedron Lett.* **1989**, *30*, 2441–2444; b) D. Andreu, F. Albericio, N. A. Solé, M. C. Munson, M. Ferrer, G. Barany, "Formation of disulfide bonds in synthetic peptides and proteins", in *Methods in Molecular Biology*, *Vol. 35*: *Peptide Synthesis Protocols* (Eds.: M. W. Pennington, B. M. Dunn), Humana Press, Totowa, N. J., **1994**, 91–169.

[24] J. C. Jiménez, À. López-Macià, C. Gracia, S. Varon, M. Carrascal, C. Cuevas, M. Royo, E. Giralt, F. Albericio, unpublished results.

[25] During the coupling with EDC·HCl/HOAt, incorporation of three units of 3-hydroxyquinaldic acid was detected.

[26] Analytical HPLC (t_R = 7.1 min, 81% purity, conditions 50% ACN (0.036% TFA), 50% H_2O (0.045% TFA) to 90% ACN (0.036% TFA), 10% H_2O (0.045% TFA) in 15 min); HRMS: *m*/*z* calcd for $C_{48}H_{58}N_{12}O_{12}S_4$: 1122.3180; found: 1123.3253 $[M+H]^+$.

[27] F. Albericio, R. P. Hammer, C. Garcia-Echeverria, M. A. Molins, J. L. Chang, M. C. Munson, M. Pons, E. Giralt, G. Barany, *Int. J. Pept. Protein Res.* **1991**, *37*, 402–413.

[28] These thiocoraline analogues with two different bisintercalation chromophore groups should allow the study of the effects on biological activity both of broken symmetry and of the presence of two distinct chromophore groups.

[29] This was prepared from commercially available Boc-D-Dap(Fmoc)-OH.

[30] P. Gómez-Martínez, M. Dessolin, F. Guibé, F. Albericio, *J. Chem. Soc. Perkin Trans. 1* **1999**, 2871–2874.

[31] Analytical HPLC (t_R = 9.3 min; conditions 50% ACN (0.036% TFA), 50% H_2O (0.045% TFA) to 90% ACN (0.036% TFA), 10% H_2O (0.045% TFA) in 15 min; HRMS: *m*/*z* calcd for $C_{52}H_{66}N_{12}O_{12}S_2$: 1114.4365; found: 1115.4437 $[M+H]^+$.

[32] For the nomenclature used see: J. Spengler, J. C. Jiménez, E. Giralt, F. Albericio. Nomenclature for Cyclic and Branched Homo- and Heterodetic Peptides, *J. Peptide Res.* **2005**, *65*, 550–555.

Received: June 9, 2006
Published online: September 5, 2006

