

184. Total Synthesis of Cyclothialidine

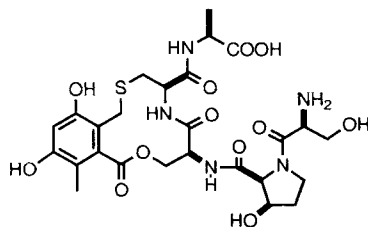
by Erwin Götschi*, Christian-Johannes Jenny, Peter Reindl, and Fabienne Ricklin

Pharma Division, Preclinical Research, F. Hoffmann-La Roche Ltd., CH-4002 Basel

(2. IX.96)

A total synthesis of cyclothialidine (**1**), a new DNA gyrase inhibitor isolated from *Streptomyces filipinensis*, is described. The synthetic concept was tested by preparing the lactone **13** (Scheme 2) which contains the characteristic bicyclic core entity of **1**. Key features of the synthesis of **1** are: preparation of 3,5-dihydroxy-2,6-dimethylbenzoic acid (**23**) from 3,5-dihydroxybenzoic acid (**19**) by two consecutive *Mannich* aminomethylation/hydrogenation sequences; benzylic *N*-bromosuccinimide bromination of an ester derivative **25** thereof and its subsequent coupling with Boc-Ser-Cys-OMe (**11**); cyclization of the ω -hydroxy acid **29** to the 12-membered lactone **30** using preferably *Mitsunobu* conditions; completion of the peptidic side chains of **1** using Boc strategy (Scheme 4). Optically pure *cis*-*N*-(*tert*-butoxycarbonyl)-3-hydroxy-L-proline ((–)-**14**) was obtained by resolution of the racemate *via* an efficient reaction sequence containing a lipase-catalyzed enantiospecific acetate hydrolysis (Scheme 3). The structure of natural **1** was confirmed by comparison with the synthetic material. The synthetic route described provides also easy access to analogues of **1**, *e.g.*, *via* the intermediate **30**.

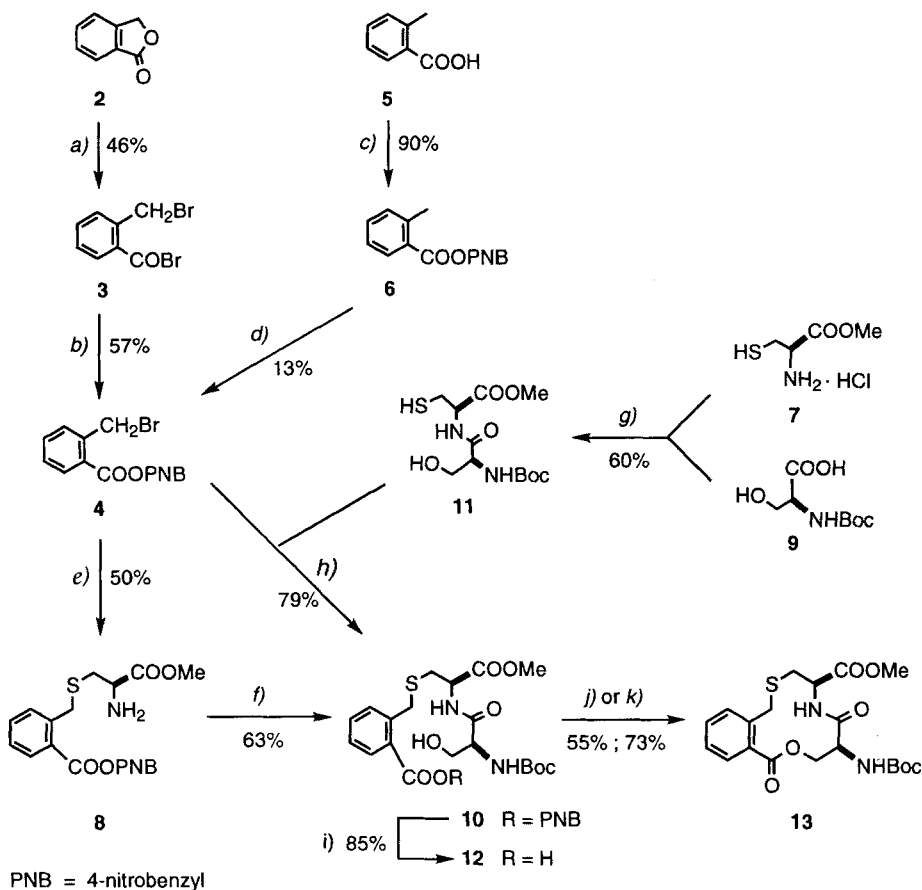
1. Introduction. – Cyclothialidine (**1**), a potent DNA gyrase inhibitor, was isolated from *Streptomyces filipinensis* NR 0484 [1] as a first representative of a new class of natural products [2]. Its unique structure was elucidated mainly by a combination of spectroscopical methods and amino-acid analysis [3] and features a 12-membered lactone ring that is fused to a highly substituted benzene ring, and partly integrated into a pentapeptide chain containing the rare amino acid *cis*-3-hydroxy-L-proline. The *meta*-position of the two OH groups on the phenyl ring differs from the typical *ortho/para*-substitution pattern of the orsellinic acid type macrolides produced by the polyketide biosynthetic pathway [4].



Cyclothialidine **1**

It is noteworthy that **1** was discovered in a DNA-supercoiling-assay-guided screening program aiming at new DNA gyrase inhibitors as potential antibacterials [5]. Its mode of action was shown to be inhibition of the ATPase activity conferred by the B subunit of DNA gyrase [6], the target of the coumarin antibiotics novobiocin and coumermycin [7].

yielded lactone **13** in 55% yield. The ease of the cyclization was indicated by the fact that **13** was already partly formed during the activation period at 0°. A better lactonization yield was achieved by subjecting **12** to Mitsunobu conditions [11] affording **13** in 73% yield.

Scheme 2. Synthesis of Lactone **13**

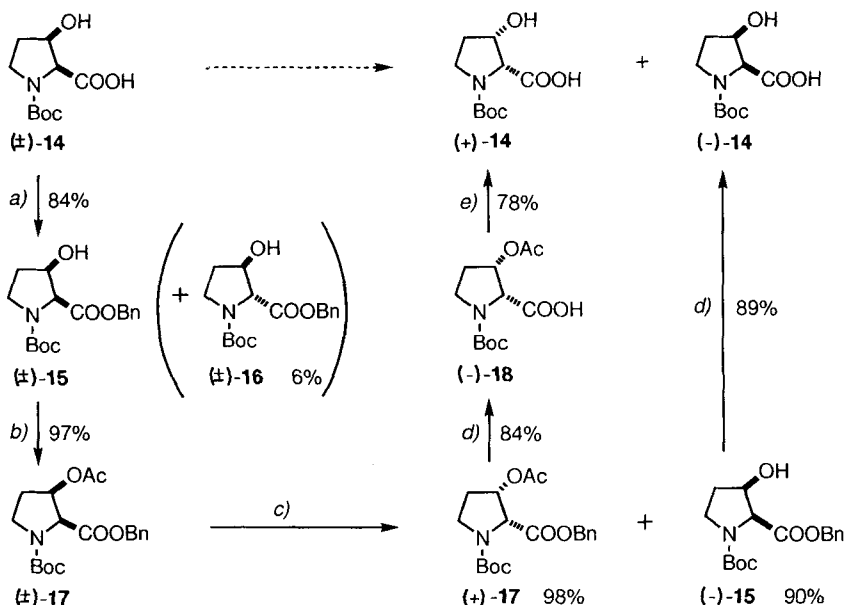
a) $\text{Ph}_3\text{P} \cdot \text{Br}_2$, 170°, 3 h. b) PNB alcohol, $(i\text{-Pr})_2\text{EtN}$, CH_2Cl_2 , 0° to r.t., 3 h. c) PNB bromide, K_2CO_3 , acetone, reflux, 4 h. d) NBS, CCl_4 , $h\nu$, reflux. e) **7**, Et_3N , CH_2Cl_2 , 0° to r.t., 3 h. f) **9**, EDC, MeCN, 0°, 4 h. g) DCC, MeCN, 4-methylmorpholine, 0°, 4 h. h) Et_3N , CH_2Cl_2 , 0° to r.t., 3 h. i) H_2 , Pd/C, AcOEt, r.t., 15 h. j) 2,2'-Bisthiobispyridine, Ph_3P , toluene, 0°, then 110°. k) DEAD, Ph_3P , toluene, 0° to r.t., 4 h.

For the lactone H-atoms, the ^1H -NMR spectrum of **13** in $(\text{D}_6)\text{DMSO}$ showed the typical features reported for **1** [3]. For the geminal methylene protons at C(1) and C(8), large differences of the chemical shifts with $\Delta\delta = 0.8$ and 0.45 ppm, respectively, were observed. The coupling constants $J(3,4)$ and $J(3',4)$ (12 and 5 Hz, resp.) and $J(7,8)$ and $J(7,8')$ (2 and 2 Hz, resp.) indicate the preference of an antiperiplanar/synclinal and a synclinal/synclinal arrangement of the cysteine (2 H—C(3), H—C(4)) and the serine (H—C(7), 2 H—C(8)) H-atoms, respectively, as it had been found for **1** in D_2O and in $(\text{D}_6)\text{DMSO}$.

Lactone **13** was used as starting material for further structural modifications. However, it was found eventually that the biological activity of **1** was linked to the presence of at least one phenolic OH group [8], and consequently, neither **13** nor any of its derivatives were found to exert DNA gyrase inhibitory activity.

2.2. *Preparation of cis-N-(tert-butoxycarbonyl)-3-hydroxy-L-proline ((-)-14)*. For the preparation of (-)-**14**, we started from the racemate ($\pm$)-**14** which we had prepared from L-proline in close analogy to the procedure of Häusler [12]. For the resolution of ($\pm$)-**14**, we initially examined various diastereoisomeric salts and succeeded to obtain the desired enantiomer by repeated recrystallization of its (+)-dehydroabietylamine salt, the procedure, however, being very tedious. Therefore, we applied in an analogous manner the lipase-catalyzed chemo- and enantioselective kinetic resolution method, used by Bhide *et al.* [13] for cleavage of the acetoxy group in the (3*R*)-enantiomer of methyl *cis*-3-acetoxy-*N*-(*tert*-butoxycarbonyl)-DL-prolinate, to the corresponding benzyl ester ($\pm$)-**17** (Scheme 3). The racemic acetate ($\pm$)-**17**, obtained in 81% yield by consecutive benzylation and acetylation of ($\pm$)-**14**, was vigorously stirred with *Candida cylindracea* in pH 7 phosphate buffer at room temperature for 48 h, causing predominant cleavage of the acetoxy function of the L-proline derivative. The hydroxy ester (-)-**15** and the unreacted acetoxy ester (+)-**17** were easily separated by chromatography and isolated in 90 and 98% yield, respectively. Hydrogenolysis of the benzyl ester (-)-**15** afforded the L-proline derivative (-)-**14** in 89% yield and with an enantiomeric purity of > 99% ee, as determined by gas chromatography for its methyl-ester derivative. The specific rotation of (-)-**14** was found to be significantly higher than that reported by Cooper *et al.* [14] for the enantiomeric (+)-**14**, indicating their product to have only *ca.* 80% ee. Upon cleavage of the Boc group in (-)-**14** with CF₃COOH, pure *cis*-3-hydroxy-L-proline was isolated, confirming the optical purity of (-)-**14**. As a curiosity, we repeatedly isolated (-)-**14** with a m.p. of 127–128° compared to 101–103° reported by Cooper *et al.* for the enantiomer, before we eventually obtained a sample of (-)-**14** with m.p. 106–107°, identical to the higher-melting material in NMR spectrum and optical rotation, and thus representing a different crystal modification. The efficacy of the lipase-catalyzed resolution method was further demonstrated by the conversion of (+)-**17** in 65% yield to the D-proline derivative (+)-**14** via hydrogenolysis of the benzyl ester and saponification of the acetoxy function of the resulting (-)-**18**. The (2*R*,3*S*)-enantiomer (+)-**14** was obtained in the lower-melting crystal modification, *i.e.*, with m.p. 106–107°, and showed exactly the opposite specific rotation of (-)-**14**. Both (-)-**14** and (+)-**14** exceeded considerably the optical rotations so far reported [14].

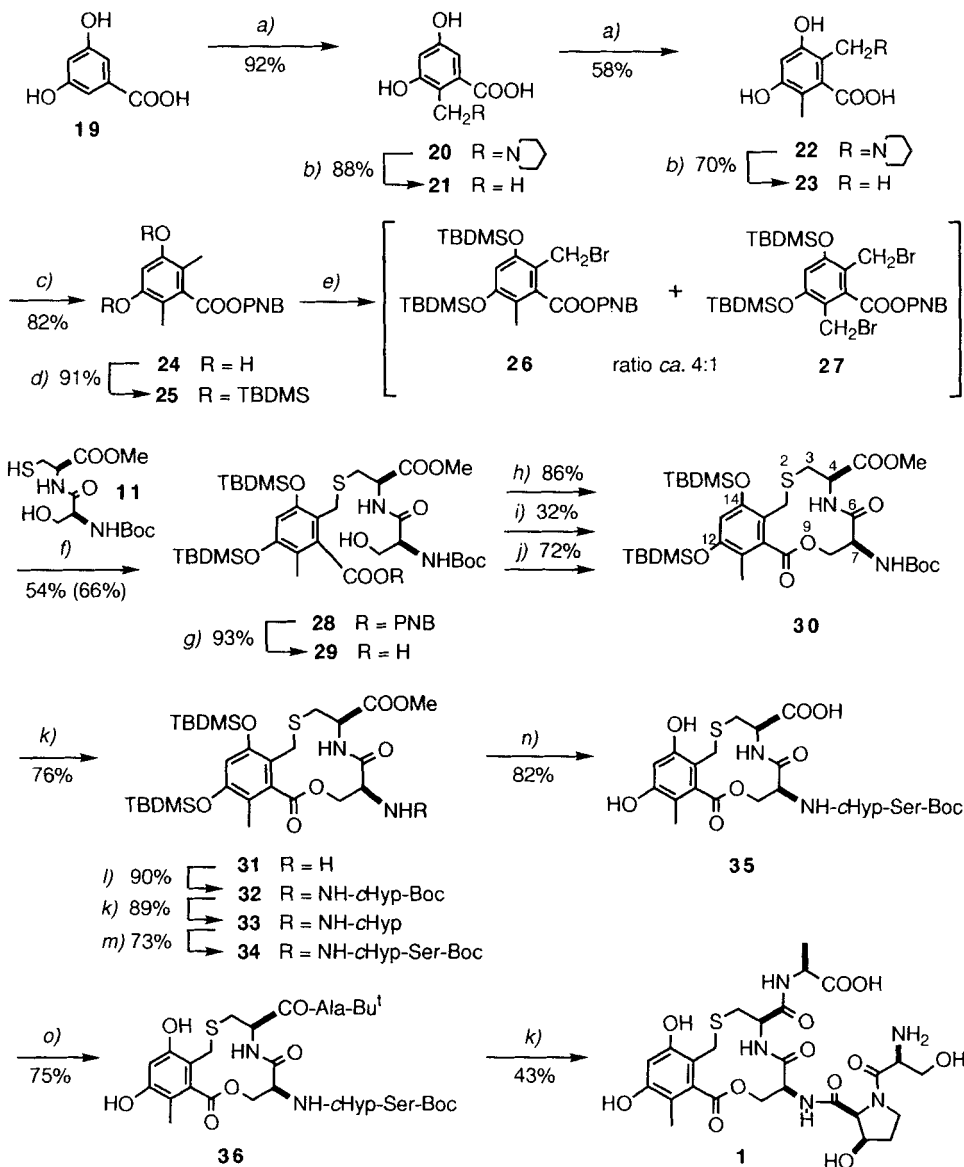
2.3. *Synthesis of Cyclothialidine (1)*. For the synthesis of **1** according to the proposed scheme, we needed the symmetrical 3,5-dihydroxy-2,6-dimethylbenzoic acid **23** (Scheme 4). The strategy used by Borchard and Sinhababu [15], *i.e.*, protecting the 2- and 6-position of 3,5-dihydrobenzoic acid (**19**) by bromination in order to achieve aminoalkylation in the 4-position, prompted us to examine the Mannich reaction for the direct *ortho*-methylation of **19**. Treatment of **19** with the Mannich reagent, obtained from piperidine and aqueous CH₂O in AcOH, produced in a highly regioselective manner the piperidin-1-ylmethyl derivative **20** which was hydrogenated in the presence of Pd/C and 1 equiv. of piperidine to afford in 81% overall yield 3,5-dihydroxy-2-methylbenzoic acid (**21**), a compound so far not easily accessible on a large scale [16]. By applying the same reaction

Scheme 3. Resolution of *cis*-3-Hydroxy-DL-proline

a) BnBr, K_2CO_3 , DMF, r.t. b) Ac_2O , pyridine, r.t. c) *Candida cylindraceae* lipase, Na-phosphate buffer, pH 7, r.t. d) H_2 , Pd/C, AcOEt. e) KOH, MeOH, r.t., then H_3O^+ .

sequence to **21**, we obtained **23** in 40% yield, the hydrogenolysis of the *Mannich* product **22** being performed in the presence of 1 equiv. of NaOH. Acid **23** was esterified by alkylation of its *N,N,N',N'*-tetramethylguanidinium salt with 4-nitrobenzyl bromide in DMF. The use of the guanidine as base proved to be superior to K_2CO_3 , the latter furnishing more of unwanted phenol ethers. The phenol groups of ester **24** were protected as *(t*-Bu) Me_2Si ethers, and the symmetrical 2,6-dimethylbenzoate **25** was then brominated using NBS in refluxing CCl_4 under irradiation with light. In contrast to the bromination of **6**, the reaction went smoothly, providing reliably a 4:1:1 mixture of the desired monobromide **26**, symmetrical dibromide **27**, and starting material **25**. This mixture was treated with thiol **11** and Et_3N in CH_2Cl_2 , and the resulting sulfide **28** was easily isolated in 54% (66% rel. to used **25**) yield by chromatography. The hydrogenolytic cleavage of **28** provided the ω -hydroxy acid **29** in 93% yield, the reaction again being rather slow, as found in the preparation of **12**. For the lactonization of the sterically demanding **29**, the thioester activation based on pyridine-2-thiol gave only a modest yield (32%) of **30**. Using the more reactive thioester derived from 4-(*tert*-butyl)-1-isopropyl-1*H*-imidazol-2-thiol [17], **30** was isolated in 72% yield. The *Mitsunobu* reaction, however, again provided the best cyclization result (86%). The 1H -NMR spectrum (D_6)DMSO of **30** exhibits the characteristic features discussed above for **13**. The lactone **30** represents a key intermediate which we have used for the preparation of analogues of **1** [8]. The methyl ester can be saponified, or the amino function can be unmasked, by choice.

Scheme 4. Synthesis of Cyclothialidine



a) CH₂O, piperidine, pH 5. b) H₂, Pd/C, aq. MeOH, base. c) 1. *N,N,N',N'*-Tetramethylguanidine, DMF, 2. PNB bromide. d) TBDMSCl, Et₃N, DMF. e) NBS, CCl₄, hv. 90°. f) Et₃N, CH₂Cl₂. g) H₂, Pd/C, AcOEt. h) DEAD, Ph₃P, toluene. i) 2,2'-Dithiobispyridine, Ph₃P, toluene, 0°, then 110°. j) 2,2'-Dithiobis[(4-*tert*-butyl)-1-isopropyl-1*H*-imidazole], Ph₃P, toluene, 0°, then 110°. k) CF₃COOH, 0°. l) Boc- α Hyp-OH, EDC, MeCN. m) Boc-L-Ser-OH, EDC, MeCN. n) 1. NH₄F, aq. THF, 2. NaOH, aq. THF. o) L-Ala-O-(*t*-Bu), EDC, MeCN.

For the synthesis of **1**, the missing amino acids were attached stepwise by a sequence of deprotection and coupling reactions. The Boc group in **30** was removed in CF_3COOH , and the resulting amine **31** was coupled to *cis*-*N*-(*tert*-butoxycarbonyl)-3-hydroxy-L-proline (Boc-*c*Hyp; (–)-**14**) using EDC in MeCN to afford **32**. In an analogous manner, **32** was converted to amine **33** which was acylated with Boc-L-Ser-OH to give **34**. After removal of the silyl protecting groups of **34** using NH_4F in aqueous THF, the methyl ester was saponified *in situ* with NaOH to provide carboxylic acid **35**. Direct EDC coupling of **35** with L-Ala-O-(*t*-Bu) gave only a poor result. However, upon previous formation of the hydroxy-succinimide ester of **35**, the reaction with the alanine ester was improved, and **36** was isolated in 75 % yield. Finally, both *tert*-butyl protecting groups were simultaneously removed in CF_3COOH , and the resulting amino acid was purified by reversed-phase chromatography and freeze-dried to yield **1** as white powder in 43 % yield.

When compared with an authentic sample, the synthetic material **1** could not be distinguished in various TLC and HPLC systems. The specific rotations were in good agreement, and the spectroscopic properties with regard to IR, UV, and MS were very similar, although we measured a higher specific absorption in the UV. The NMR spectra displayed some discrepancies with regard to the chemical shifts of the N-terminal serine protons (*Fig.*). It is known [3], however, that the position of these signals depends strongly on the degree of protonation of the amino function, and we concluded that the natural product had been isolated rather as a salt, whereas the synthetic product was obtained as the neutral inner salt. To unambiguously prove the identity of synthetic and natural material, we measured the spectrum of their mixture and indeed obtained only one set of serine proton signals. In addition, both natural and synthetic **1** exhibited the same inhibitory activity in a supercoiling assay using *E. coli* DNA gyrase [18].

3. Conclusions. – The DNA gyrase inhibitor cyclothialidine (**1**) can be considered as progenitor of a new class of antibacterial agents [8] [19]. We have described a synthetic route which provides access not only to the natural product but also to analogues of **1** that might have a potential as antibacterials. Key features of this route represent the synthesis of 3,5-dihydroxy-2,6-dimethylbenzoic acid using the *Mannich* reaction, the preferential monobromination of a derivative thereof, and the lactonization to the so far not described bicyclic ring system of **1**. In addition, an efficient kinetic resolution procedure was used for the preparation of *cis*-*N*-(*tert*-butoxycarbonyl)-3-hydroxy-L-proline. The structure of cyclothialidine (**1**) was confirmed by comparison of the synthetic material with the natural product.

The authors wish to thank their colleagues from Pharma Research New Technologies, *F. Hoffmann-La Roche Ltd.*, for NMR spectra (Dr. *W. Arnold*), mass spectra (Mr. *W. Meister*, Drs. *G. Oesterheldt*, *W. Vetter*), IR and UV spectra and specific rotations (Mrs. *J. Kohler*, Mr. *A. Bubendorf*), GC (Dr. *W. Walther*), and elemental analysis (Dr. *S. Müller*).

Experimental Part

General. Solvents and chemicals used for reactions were purchased from commercial suppliers and used without further purification. Reactions were carried out under N_2 or Ar. In the usual workup, aq. layers were back-extracted with the org. solvent used. Org. solns. were dried (Na_2SO_4), and evaporation of solvents was performed *in vacuo* at 20–40°. Prep. chromatography: Silica gel 60 (230–400 mesh, *Merck*). TLC: silica gel glass

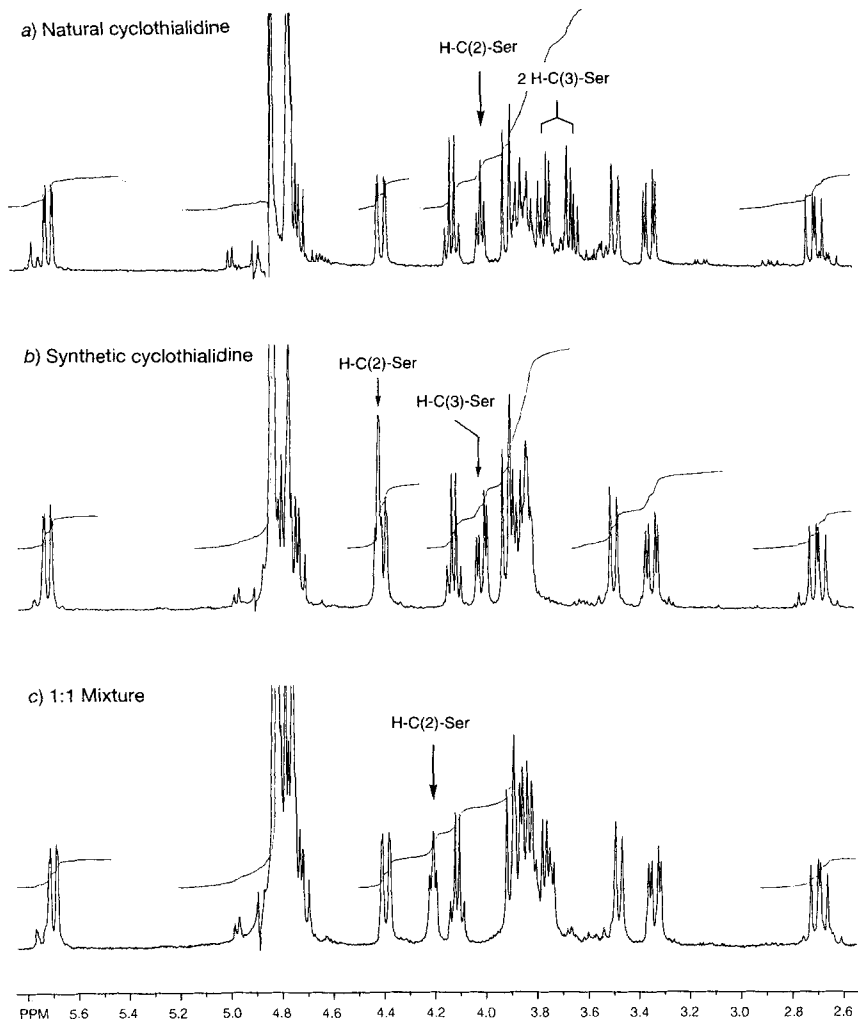


Figure. 400-MHz ^1H -NMR Spectra (D_2O) of a) natural cyclothialidine, b) synthetic cyclothialidine, c) a 1:1 mixture of natural and synthetic cyclothialidine. Ser = N-terminal serine moiety.

plates ('Kieselgel' 60 F_{254} , Merck); detection by UV and visualization by spraying with 1% aq. KMnO_4 soln. or 0.4% aq. $\text{K}_2\text{Pt}(\text{II})\text{I}_4$ soln. [20]. M.p.: Büchi SMP-20K; uncorrected. IR Spectra: Nicolet-20SXB spectrophotometer; data in cm^{-1} . UV Spectra: Uvikon 810; λ_{max} (log ϵ) in nm. Optical rotation: Perkin-Elmer-241 polarimeter; 10-cm cell, at 20° . ^1H -NMR: Bruker-AC-250 or Bruker-ARX-400; chemical shifts δ in ppm rel. to SiMe_4 ($=0$ ppm), coupling constants J in Hz. MS: electron ionization (EI), Finnigan-MAT SSQ 700; ion-spray (positive) ionization (ISP), PE-Sciex API III; high resolution (HR), Finnigan-MAT MAT95; m/z (rel. int.).

1. Synthesis of 13. 1.1. 2-(Bromomethyl)benzoyl Bromide (3). For 3 h, 1H-isobenzofuran-1-one (2; 31.5 g, 0.235 mol) and $\text{Ph}_3\text{P} \cdot \text{Br}_2$ (107.3 g, 0.247 mol) were heated with stirring to 170° for 3 h. The dark-red mixture was cooled and then distilled under reduced pressure to afford 3 (30.2 g, 46%). Colorless oil which solidifies on standing. B.p. $158\text{--}165^\circ/21$ mbar ([9]: $170\text{--}171^\circ/32$ mbar). Material of this quality was used in the next step.

1.2. *4-Nitrobenzyl 2-(Bromomethyl)benzoate (4)*. At 0°, (i-Pr)₂EtN (1.88 ml, 11 mmol) was added to a soln. of **3** (3.06 g, 11 mmol) and 4-nitrobenzyl alcohol (1.68 g, 11 mmol) in CH₂Cl₂ (25 ml). The mixture was stirred for 15 min at 0° and for 3 h at r.t., then diluted with CH₂Cl₂ (25 ml), washed with 1N H₂SO₄ (10 ml) and H₂O (2 × 10 ml), dried, and evaporated. The residual oil was crystallized twice from AcOEt/hexane: **4** (2.19 g, 57%). White crystals. M.p. 109–110°. ¹H-NMR (250 MHz, CDCl₃): 4.95 (s, CH₂Br); 5.48 (s, COOCH₂); 7.36–7.60 (m, 3 arom. H); 7.64 (d, *J* = 8.5, 2 arom. H (PNB)); 8.03 (dd, *J* = 7, 1.5, 1 arom. H); 8.26 (d, *J* = 8.5, 2 arom. H (PNB)). EI-MS: 270 ([*M* – Br]⁺). Anal. calc. for C₁₅H₁₂BrNO₄ (350.17): C 51.45, H 3.45, Br 22.82, N 4.00; found: C 51.58, H 3.47, Br 22.59, N 3.83.

1.3. *Alternative Route to 4. 4-Nitrobenzyl 2-Methylbenzoate (6)*. A stirred mixture of 2-methylbenzoic acid (**5**; 13.62 g, 0.1 mol), 4-nitrobenzyl bromide (25.92 g, 0.12 mol), and K₂CO₃ (41.5 g, 0.30 mol) in acetone (100 ml) was heated under reflux for 4 h. The mixture was cooled to 0° and then slowly added to an ice-cold, stirred mixture of 1N HCl (0.5 l) and AcOEt (1 l). The org. layer was separated, washed with brine (0.5 l), dried, and evaporated. The residual oil was crystallized from AcOEt/hexane to give **6** (24.5 g, 90%). White crystals. M.p. 89–90°. ¹H-NMR (250 MHz, CDCl₃): 2.62 (s, Me); 5.44 (s, CH₂); 7.22–7.32 (m, 1 arom. H); 7.40–7.50 (m, 1 arom. H); 7.61 (d, *J* = 8.5, 2 arom. H (PNB)); 7.98 (dd, *J* = 7, 1.5, 1 arom. H); 8.25 (d, *J* = 8.5, 2 arom. H (PNB)).

4-Nitrobenzyl 2-(Bromomethyl) Benzoate (4). To a soln. of **6** (271 mg, 1.0 mmol) in CCl₄ (5 ml) was added NBS (178 mg, 1.0 mmol). The suspension was heated under reflux with irradiation of light for 25 min, whereupon all the insoluble material was floating at the surface. The mixture was cooled in an ice-bath for 15 min, and insoluble material was filtered off. The soln. was evaporated, the residue chromatographed (hexane/AcOEt 10:1), and the purified product crystallized from CH₂Cl₂/hexane: **4** (45 mg, 13%). White crystals. M.p. 104°. TLC (hexane/AcOEt 2:1): *R*_f 0.31. ¹H-NMR (CDCl₃): identical to that given above (see 1.2).

1.4. *Methyl S-[2-[(4-Nitrobenzyloxy)carbonyl]benzyl]-L-cysteinate (8)*. To an ice-cold soln. of **4** (1.75 g, 5.0 mmol) and L-Cys-OMe·HCl (**7**; 0.86 g, 5.0 mmol) in CH₂Cl₂ (25 ml) was added Et₃N (1.39 ml, 10.0 mmol). The mixture was stirred for 15 min at 0° and for 3 h at r.t., then diluted with CH₂Cl₂ (25 ml), washed with 5% NaHCO₃ soln. (25 ml) and brine (2 × 25 ml), dried, and evaporated. The residual oil was chromatographed (AcOEt) to furnish a colorless oil (1.20 g) which solidified on standing. Trituration with hexane gave **8** (1.01 g, 50%). White powder. M.p. 45–51°. TLC (AcOEt): *R*_f 0.28. ¹H-NMR (250 MHz, CDCl₃): 1.65 (br. s, NH₂); 2.66 (dd, *J* = 13.5, 7.6, 1 H–C(3)(Cys)); 2.85 (dd, *J* = 13.5, 4.6, 1 H–C(3)(Cys)); 3.61 (dd, *J* = 7.6, 4.6, 1 H–C(2)(Cys)); 3.71 (s, COOMe); 4.11, 4.19 (2d, *AB*, *J*_{AB} = 13, ArCH₂S); 5.45 (s, COOCH₂); 7.32–7.40 (m, 2 arom. H); 7.45–7.53 (m, 1 arom. H); 7.62 (d, *J* = 8.5, 2 arom. H (PNB)); 7.98 (dd, *J* = 6.7, 0.5, 1 arom. H); 8.26 (d, *J* = 8.5, 2 arom. H (PNB)). ISP-MS: 405.4 ([*M* + H]⁺). Anal. calc. for C₁₉H₂₀N₂O₆S (404.44): C 56.43, H 4.98, N 6.93, S 7.93; found: C 56.39, H 4.98, N 6.85, S 7.99.

1.5. *Methyl N-[N-(tert-Butoxycarbonyl)-L-seryl]-S-[2-[(4-nitrobenzyloxy)carbonyl]benzyl]-L-cysteinate (10)*. To an ice-cold mixture of **8** (0.81 g, 2.0 mmol) and Boc-L-Ser-OH (**9**; 0.41 g, 2.0 mmol) in MeCN (6 ml) was added *N*-[3-(dimethylamino)propyl]-*N*-ethylcarbodiimide hydrochloride (EDC; 0.44 g, 2.3 mmol). The mixture was stirred at 0° for 4 h, then diluted with AcOEt (30 ml), and washed with 1N HCl (20 ml), 5% NaHCO₃ soln. (20 ml), and brine (2 × 20 ml). The org. layer was dried and evaporated and the crude product crystallized from AcOEt to give **10** (0.75 g, 63%). White crystals. M.p. > 78° (dec.). TLC (AcOEt): *R*_f 0.48. ¹H-NMR (250 MHz, CDCl₃): 1.45 (s, *t*-Bu); 2.87–3.00 (m, 2 H–C(3)(Cys)); 3.61–3.72 (m, 1 H); 3.73 (s, COOMe); 4.03–4.16 (m, 1 H); 4.07, 4.26 (2d, *AB*, *J*_{AB} = 12.4, ArCH₂S); 4.25–4.36 (m, 1 H); 4.77–4.87 (m, 1 H); 5.49 (s, COOCH₂); 5.57 (d, *J* = 8, NH(Ser)); 7.30–7.42 (m, 2 arom. H, NH(Cys)); 7.48–7.56 (m, 1 arom. H); 7.64 (d, *J* = 8.4, 2 arom. H (PNB)); 8.00 (dd, *J* = 6.8, 0.5, 1 arom. H); 8.26 (d, *J* = 8.5, 2 arom. H (PNB)). ISP-MS: 592.4 ([*M* + H]⁺), 536.4 ([*M* + H – C₄H₈]⁺), 492.4 ([*M* + H – C₄H₈ – CO₂]⁺). Anal. calc. for C₂₇H₃₃N₃O₁₀S (591.63): C 54.81, H 5.62, N 7.10, S 5.42; found: C 54.76, H 5.53, N 6.89, S 5.65.

1.6. *Alternative Route to 10. Methyl N-[N-(tert-Butoxycarbonyl)-L-seryl]-L-cysteinate (11)*. To a mixture of L-Cys-OMe·HCl (**7**; 171.6 g, 1 mol) and Boc-L-Ser-OH (**9**; 205.2 g, 1 mol) in MeCN (2.5 l) was added at 5° *N*-methylmorpholine (110 ml, 1 mol). To the stirred soln. was added over 30 min at 5–10° a soln. of *N,N'*-dicyclohexylcarbodiimide (206.3 g, 1 mol) in MeCN (2 l), and stirred was continued for 4.5 h at 5°. The precipitate formed was removed by filtration and the soln. evaporated. The residual oil was dissolved in AcOEt (1 l) and the soln. washed with 0.5N HCl (0.5 l), 5% NaHCO₃ soln. (0.5 l), and brine (0.5 l), dried, and evaporated. The residual oil was chromatographed (1.5 kg of silica gel, AcOEt/hexane 1:1) and the purified product crystallized from Et₂O/hexane: **11** (192.8 g, 60%). White crystals. M.p. 74–76°. TLC (AcOEt): *R*_f 0.38. [*α*]_D = +3.3 (*c* = 1, AcOEt). ¹H-NMR (250 MHz, CDCl₃): 1.46 (t, *J* = 9, SH), superimposed by 1.47 (s, *t*-Bu); 2.85–2.96 (m, OH); 3.01 (dd, *J* = 9, 4, 2 H–C(3)(Cys)); 3.63–3.77 (m, 1 H–C(3)(Ser)); 3.80 (s, COOMe); 4.06–4.17 (m, 1 H–C(3)(Ser)); 4.17–4.27 (m, H–C(2)(Ser)); 4.83 (dt, *J* = 8, 4, H–C(2)(Cys)); 5.54 (br. d, *J* = 8, NH(Ser)); 7.36 (br. d, *J* = 8, NH(Cys)). EI-MS: 266 ([*M* – C₄H₈]⁺). Anal. calc. for C₁₂H₂₂N₂O₆S (322.38): C 44.71, H 6.88, N 8.69, S 9.94; found: C 44.74, H 6.84, N 8.74, S 9.81.

Alcohol 10. Et₃N (2.78 ml, 20 mmol) was added to an ice-cold soln. of **4** (7.0 g, 20 mmol) and **11** (6.45 g, 20 mmol) in CH₂Cl₂ (100 ml). The soln. was stirred for 15 min at 0° followed by 3 h at r.t. and then washed with 5% NaHCO₃ soln. (100 ml) and brine (2 × 100 ml), dried, and evaporated. The residual oil was chromatographed (AcOEt/CH₂Cl₂ 1:1) and the purified product crystallized from Et₂O/hexane: **10** (9.31 g, 79%). White powder. ¹H-NMR (CDCl₃) and MS: identical to those of **10** prepared from **8** (see 1.5).

1.7. Methyl N-[N-(tert-Butoxycarbonyl)-L-seryl]-S-(2-carboxybenzyl)-L-cysteinate (12). A mixture of **10** (5.91 g, 10 mmol) and 5% Pd/C (1.18 g) in AcOEt (200 ml) was stirred under H₂ at r.t. for 15 h. The catalyst was removed by filtration and the soln. evaporated. The residue was stirred with MeOH (100 ml) at r.t. for 2 h and the precipitate filtered off. The clear soln. was evaporated, the residue taken up in AcOEt (150 ml), and the soln. washed with 1N HCl (100 ml) and brine (2 × 50 ml), dried, and evaporated. The residual oil was crystallized from Et₂O/hexane to give **12** (3.88 g, 85%). White powder. ¹H-NMR (250 MHz, (D₆)DMSO): 1.38 (s, *t*-Bu); 2.66 (dd, *J* = 14, 8, 1 H-C(3)(Cys)); 2.78 (dd, *J* = 14, 6, 1H-C(3)(Cys)); 3.43–3.67 (m, 2 H-C(2)(Ser)); 3.61 (s, COOMe); 3.98–4.11 (m, 1 H); 4.11 (s, ArCH₂S); 4.44–4.56 (m, 1 H); 4.81 (br., OH); 6.70 (d, *J* = 8, NH(Ser)); 7.33–7.42 (m, 2 arom. H); 7.45–7.54 (m, 1 arom. H); 7.84 (dd, *J* = 8, 0.5, 1 arom. H); 8.29 (d, *J* = 7.5, NH(Cys)); 12.98 (br., COOH). Anal. calc. for C₂₀H₂₈N₂O₈·0.15 H₂O (459.21): C 52.31, H 6.21, N 6.10, H₂O 0.59; found: C 52.16, H 6.30, N 6.10, H₂O (Karl Fischer titration) 0.56.

1.8. Methyl (4R,7S)-7-[1-(tert-Butoxycarbonyl)amino]-1,3,4,5,6,7,8,10-octahydro-6,10-dioxo-9,2,5-benzoxathiazacyclododecine-4-carboxylate (13). *Procedure A.* To a stirred suspension of finely powdered **12** (1.37 g, 3.0 mmol) in toluene (30 ml) were added at 0° Ph₃P (1.02 g, 3.9 mmol) and 2,2'-dithiobispyridine (0.86 g, 3.9 mmol). The mixture was stirred at 0° for 20 min and then diluted with toluene (120 ml). The yellow soln. was added dropwise over 1 h boiling toluene (60 ml). The mixture was heated under reflux for another hour, then cooled, and evaporated. The residual oil was chromatographed (AcOEt/CH₂Cl₂/hexane 1:1:1) and the purified product crystallized from CH₂Cl₂/hexane **13** (0.724 g, 55%). White powder. M.p. 206° (dec.). TLC (AcOEt/hexane 1:1): R_f 0.25. [α]_D = +71.2 (c = 1, MeOH). IR (KBr): 3414m, 3296m, 2978m, 1721vs, 1670s, 1655s, 1600m, 1517s. EI-MS: 382 ([M – CH=C(Me)₂]⁺). ¹H-NMR (250 MHz, (D₆)DMSO): 1.43 (s, *t*-Bu); 3.00 (dd, *J* = 14, 12, 1 H-C(3)); 3.11 (dd, *J* = 14, 5, 1 H-C(3)); 3.65 (s, COOMe); 3.77 (d, *J* = 11, 1 H-C(1)); 4.31 (br. dd, *J* = 12, 2, 1 H-C(8)); 4.40 (m, H-C(7)); 4.59 (d, *J* = 11, 1 H-C(1)); 4.68 (m, H-C(4)); 4.75 (br. dd, *J* = 12, 2, 1 H-C(8)); 7.36–7.57 (m, 4 arom. H); 7.84 (d, *J* = 7, NH); 8.32 (d, *J* = 8, NH). Anal. calc. for C₂₀H₂₆N₂O₇S (438.50): C 54.78, H 5.98, N 6.39, S 7.31; found: C 54.87, H 5.95, N 6.55, S 7.46.

Procedure B. To a stirred suspension of finely powdered **12** (1.37 g, 3.0 mmol) in toluene (75 ml) were added at 0° Ph₃P (1.02 g, 3.9 mmol) and 95% diethyl azodicarboxylate (0.64 ml, 3.9 mmol). Stirring was continued at 0° for 15 min and then at r.t. for 4 h. The mixture was evaporated and the residual oil dissolved in CH₂Cl₂ and submitted to chromatographic purification (*t*-BuOMe/hexane/CH₂Cl₂ 2:2:1 (→diethyl 1,2-hydrazinedicarboxylate), then 1:1:1). Crystallization from CH₂Cl₂/hexane gave **13** (0.97 g, 73%). ¹H-NMR ((D₆)DMSO): identical to that given above.

2. Resolution of (±)-14. **2.1. Benzyl cis-N-(tert-Butoxycarbonyl)-3-hydroxy-DL-prolinate ((±)-15) and Benzyl trans-N-(tert-Butoxycarbonyl)-3-hydroxy-DL-prolinate ((±)-16).** K₂CO₃ (1.38 g, 10 mmol) was added to a soln. of *cis*-N-(tert-butoxycarbonyl)-3-hydroxy-DL-prolinate ((±)-**14**; 2.31 g, 10.0 mmol) [12] and benzyl bromide (1.20 ml, 10.0 mmol) in DMF (20 ml). The mixture was stirred at r.t. for 8 h. The resulting suspension was diluted with AcOEt (150 ml) and washed with H₂O (4 × 50 ml). The org. layer was dried and evaporated and the residue chromatographed (AcOEt/hexane 1:1): (±)-**16** (0.18 g, 6%) and (±)-**15** (2.70 g, 84%; after crystallization from AcOEt/hexane).

Data of (±)-15: White crystals. M.p. 76–77°. TLC (AcOEt/hexane 1:1): R_f 0.28. ¹H-NMR (250 MHz, CDCl₃; rotamer 1/rotamer 2 ca. 2:1): 1.34, 1.46 (2s, *t*-Bu, rot. 1/rot. 2); 1.92–2.18 (m, 2 H-C(4), OH, rot. 1/rot. 2); 3.40–3.72 (m, 2 H-C(5), rot. 1/rot. 2); 4.31, 4.48 (2 d, *J* = 6, H-C(2), rot. 1/rot. 2); 4.55–4.69 (m, H-C(3), rot. 1/rot. 2); 5.15, 5.34 (2d, AB, *J*_{AB} = 14, COOCH₂, rot. 2); 5.21 (s, COOCH₂, rot. 1); 7.27–7.44 (m, 4 arom. H, rot. 1/rot. 2). Anal. calc. for C₁₇H₂₃NO₅ (321.37): C 63.54, H 7.21, N 4.36; found: C 63.52, H 7.28, N 4.16.

Data of (±)-16: Colorless oil. TLC (AcOEt/hexane 1:1): R_f 0.32. ¹H-NMR (250 MHz, CDCl₃; rotamer 1/rotamer 2 ca. 2:1): 1.34, 1.46 (2s, *t*-Bu, rot. 1/rot. 2); 1.52–1.62 (m, OH, rot. 1/rot. 2); 1.81–2.19 (m, 2 H-C(4), rot. 1/rot. 2); 3.52–3.73 (m, 2 H-C(5), rot. 1/rot. 2); 4.21, 4.33 (2d, *J* ≈ 1, H-C(2), rot. 1/rot. 2); 4.39–4.48 (m, H-C(3), rot. 1/rot. 2); 5.07–5.29 (m, COOCH₂, rot. 1/rot. 2); 7.30–7.40 (m, 4 arom. H, rot. 1/rot. 2).

2.2. Benzyl cis-3-Acetoxy-N-(tert-butoxycarbonyl)-DL-prolinate ((±)-17). A soln. of (±)-**15** (0.70 g, 2.18 mmol) and pyridine (0.02 ml, 0.25 mmol) in Ac₂O (3 ml) was stirred at r.t. for 6 h. The mixture was evaporated and the residual oil purified by chromatography (AcOEt/hexane 1:1): (±)-**17** (0.77 g, 97%). Colorless oil. TLC (AcOEt/hexane 1:2): R_f 0.54. ¹H-NMR (250 MHz, CDCl₃; rotamer 1/rotamer 2 ca. 2:1): 1.35, 1.47 (2s, *t*-Bu, rot. 1/rot. 2); 1.70, 1.79 (2s, MeCO, rot. 1/rot. 2); 2.02–2.24 (m, 2 H-C(4), rot. 1/rot. 2); 3.40–3.74 (m, 2 H-C(5),

rot. 1/rot. 2); 4.55, 4.63 (2d, $J = 7$, H-C(2), rot. 1/rot. 2); 5.05, 5.34 (2d, AB, $J_{AB} = 12$, COOCH₂, rot. 2); 5.17 (s, COOCH₂, rot. 1); 5.43 (ddd, $3 \times J = 7$, H-C(3), rot. 1/rot. 2); 7.29–7.43 (m, 4 arom. H, rot. 1/rot. 2). Anal. calc. for C₁₉H₂₅NO₆ (363.41): C 62.80, H 6.93, N 3.85; found: C 62.52, H 7.16, N 3.86.

2.3. *Benzyl cis-3-Acetoxycarbonyl-N-(tert-butoxycarbonyl)-D-prolinate* ((+)-**17**) and *Benzyl cis-N-(tert-butoxycarbonyl)-3-hydroxy-L-prolinate* ((-)-**15**). A mixture of (±)-**17** (1.09 g, 3 mmol) and *Candida cyclindracea* lipase (54 mg; *Fluka*, activity 36 U/mg) in 0.1M sodium phosphate buffer (pH 7, 18 ml) was vigorously stirred at r.t. for 48 h. The emulsion was extracted with AcOEt (70 ml) and the org. layer washed with brine (40 ml), dried, and evaporated. Chromatography (AcOEt/hexane 1:2) of the residual oil gave (+)-**17** (0.533 g, 98%) and (-)-**15** (0.436 g, 90%).

Data of (+)-17: Colorless oil. TLC (AcOEt/hexane 1:2): R_f 0.54. $[\alpha]_D = +0.7$ ($c = 1.0$, CH₂Cl₂). ¹H-NMR (CDCl₃): identical to that of (±)-**17** (see 2.2).

Data of (-)-15: Colorless oil. TLC (AcOEt/hexane 1:2): R_f 0.20. $[\alpha]_D = -24.1$ ($c = 1.5$, CH₂Cl₂). ¹H-NMR (CDCl₃): identical to that of (±)-**15** (see 2.1). Anal. calc. for C₁₇H₂₃NO₅ (321.37): C 63.54, H 7.21, N 4.36; found: C 63.25, H 7.41, N 4.34.

2.4. *cis-N-(tert-butoxycarbonyl)-3-hydroxy-L-proline* ((-)-**14**). A soln. of (-)-**15** (321 mg, 1 mmol) in AcOEt (10 ml) was hydrogenated for 30 min at r.t. in the presence of 5% Pd/C (64 mg). After a H₂ uptake of 25 ml, the catalyst was removed by filtration, the soln. evaporated, and the residual oil crystallized from hexane: (-)-**14** (206 mg, 89%). White crystals. M.p. 126–127°. $[\alpha]_D = -100.8$ ($c = 1.4$, CH₂Cl₂)¹; -13.5 ($c = 1.0$, AcOEt). ¹H-NMR (250 MHz, CDCl₃)²: 1.50 (s, *t*-Bu); 1.95–2.13 (m, 2 H-C(4)); 3.50–3.75 (m, 2 H-C(5)); 4.37 (d, $J = 5.5$, H-C(2)); 4.56–4.70 (m, H-C(3)). Anal. calc. for C₁₀H₁₇NO₅ (231.25): C 51.94, H 7.41, N 6.06; found: C 51.84, H 7.43, N 5.92.

2.5. *cis-3-Acetoxycarbonyl-N-(tert-butoxycarbonyl)-D-proline* ((-)-**18**). Hydrogenolysis of (+)-**17** (363 mg, 1 mmol) in an analogous manner as described for the preparation of (-)-**14** (see 2.4) gave (-)-**18** (230 mg, 84%). White crystals. M.p. 127–128° (from AcOEt/hexane)³. $[\alpha]_D = -12.1$ ($c = 0.8$, CH₂Cl₂)³. ¹H-NMR (250 MHz, CDCl₃): 1.44 (s, *t*-Bu); 2.06 (s, COMe); 2.01–2.32 (m, 2 H-C(4)); 3.43–3.57 (m, 1 H-C(5)); 3.57–3.74 (m, 1 H-C(5)); 4.51–4.66 (br. d, H-C(2)); 5.49 (ddd, $3 \times J = 7$, H-C(3)). Anal. calc. for C₁₂H₁₉NO₆ (273.29): C 52.74, H 7.01, N 5.13; found: C 52.81, H 6.91, N 4.98.

2.6. *cis-N-(tert-butoxycarbonyl)-3-hydroxy-D-proline* ((+)-**14**). A soln. of (-)-**18** (2.48 g, 9.1 mmol) in 0.5M methanolic KOH (24 ml) was stirred at r.t. for 2 h and then evaporated. The residual oil was dissolved in AcOEt (50 ml) and the soln. washed at 0° with 1N HCl (30 ml) and brine (2 × 20 ml), dried, and evaporated. The residual oil was crystallized twice from AcOEt/hexane: (+)-**14** (1.64 g, 78%). White crystals. M.p. 106–107°. $[\alpha]_D = +100.3$ ($c = 1.4$, CH₂Cl₂)¹; +12.9 ($c = 1.0$, AcOEt). ¹H-NMR (CDCl₃): identical to that of (-)-**14** (see 2.4). Anal. calc. for C₁₀H₁₇NO₅ (231.25): C 51.94, H 7.41, N 6.06; found: C 51.80, H 7.44, N 6.02.

3. *Synthesis of 1*. 3.1. *3,5-Dihydroxy-2-[(piperidin-1-yl)methyl]benzoic Acid* (**20**). Piperidine (228.5 ml, 2.32 mol) was added dropwise to a stirred soln. of 37% aq. CH₂O soln. (163 ml, 2.18 mol) in AcOH (135 ml), the temp. being kept at 18–25° by occasional cooling with an ice-bath. Stirring was continued for 0.5 h, whereupon this *Mannich* soln. was transferred into a dropping funnel. A soln. obtained by heating 3,5-dihydroxybenzoic acid (**19**; 300 g, 1.95 mmol) in 38% aq. EtOH soln. (0.7 l) was cooled to 25°, causing some **19** to crystallize again. To this

¹) *Cooper et al.* [14] reported (+)-**14** with a m.p. of 101–103° and $[\alpha]_D = +55.5$ ($c = 1.39$, CH₂Cl₂). We obtained (-)-**14** repeatedly with m.p. 126–127° before we encountered a sample of m.p. 106–107° with identical NMR spectrum and specific rotation, thus being a lower-melting modification. On standing for 1 year, the m.p. of the latter material was again at 125–126°. The optical purity of our material was checked by 2 methods: a) Samples of (-)-**14** and of (±)-**14** were treated with CH₂N₂ and the resulting methyl esters analyzed by GLC using a chiral column (*OV-61*, β-cyclodextrin, permethylated): (-)-**14** was found to contain < 0.5% of the other enantiomer. b) A sample of (-)-**14** (116 mg) was treated with CF₃COOH (30 min, 0°) followed by ion-exchange purification (*Dowex 50W X4*, acid form, elution with H₂O and 1.3M aq. NH₃) and crystallization (H₂O/EtOH) of the resulting product: *cis*-3-hydroxy-L-proline (48 mg, 73%). White crystals. M.p. 248–255° ([21]: 245–255°). $[\alpha]_D = -97$ ($c = 1$, H₂O) ([21]: $[\alpha]_D = -90.3$, -102.7 ($c = 1$, H₂O)). Based on these data, the material described by *Cooper et al.* [14] appears to have an enantiomeric purity (ee) of ca. 80%.

²) The NMR spectra of (-)-**14** and (+)-**14** (see 2.6) give rise to a broad *s* for the *t*-Bu group and a rather sharp *d* for H-C(2). However, we also observed that the exchange rate of atropisomeric forms was slowed down giving rise to 2 *s* for the *t*-Bu group and 2 *d* for H-C(2) in various ratios, and corresponding changes for the other signals.

³) The lower m.p. and specific rotation reported for (-)-**18** earlier [14] indicates a lower optical purity (ca. 80% ee) (see also *Footnote 1*).

mixture was added within 1 min 1/3 of the above *Mannich* soln. (0.18 l). The clear soln. obtained was seeded with product crystals⁴) and stirred at r.t. until a precipitate started to form. The residual 2/3 of the *Mannich* soln. (0.36 l) were added over 2.5 h, and stirring was continued for 15 h at r.t. The precipitate was collected by filtration, washed with AcOEt (1 l), and dried to afford **20** (449 g, 92%) as a pale-yellow solid which was used directly in the next step. M.p. > 300°. ¹H-NMR (250 MHz, D₂O): 1.40–1.70 (*m*, 6 H); 2.65–2.90 (*m*, 4 H); 3.84 (*s*, ArCH₂); 5.94, 5.97 (2*d*, *J* = 2, 2 arom. H). IR (KBr): 2669*m*, (br.), 1601*s*, 1499*m*, 1341*m*, 1138*m*, 1020*m*. EI-MS: 251 (16, *M*⁺), 208 (22), 166 (22), 137 (26), 84 (100, C₅H₁₀N⁺).

3.2. *3,5-Dihydroxy-2-methylbenzoic Acid (21)*. A well stirred suspension of **20** (200 g, 0.80 mol) in MeOH (2.0 l), H₂O (0.15 l), and piperidine (98.6 ml, 1.0 mol) was hydrogenated for 14 h at a H₂ pressure of 0.1 bar in the presence of 5% Pd/C (20 g). The pH of the mixture was set to 1 by the addition of 37% aq. HCl soln., the catalyst filtered off, and the soln. concentrated *in vacuo*. The residue was diluted with H₂O (1 l) and then extracted with AcOEt (3 × 1.5 l). The org. layer was washed successively with 2*N* HCl/15% NaCl soln. 4:1 (2 × 0.8 l) and with 15% NaCl soln. (0.8 l), dried, and evaporated to give **21** (118 g, 88%) as pale-yellow solid which was used directly in the next step. For analysis, a sample was crystallized from H₂O: White solid. M.p. 245° (dec.). ([16]: 245–246° (dec.)). ¹H-NMR (250 MHz, (D₆)DMSO): 2.16 (*s*, Me); 6.45, 6.62 (2*d*, *J* = 3.9, 2 arom. H); 9.20, 9.40 (2 br. *s*, 2 OH); 12.70 (br. *s*, COOH). EI-MS: 168 (100, *M*⁺), 150 (16), 122 (72, [*M* – CO₂ – H₂]⁺), 69 (26). Anal. calc. for C₈H₈O₄ (168.15): C 57.14, H 4.80; found: C 57.06, H 4.66.

3.3. *3,5-Dihydroxy-2-methyl-6-[(piperidin-1-yl)methyl]benzoic Acid (22)*. In an analogous manner, **21** (300.0 g, 1.78 mol) was subjected to the *Mannich* procedure described above (see 3.1). Before isolating the product, AcOEt (1 l) was added to the cooled mixture and stirring continued at 0° for 1 h. The precipitate was collected by filtration, washed with AcOEt (1 l), and dried to give **22** (274 g, 58%) as pale-yellow crystals which were used directly in the next step. ¹H-NMR (250 MHz, (D₆)DMSO): 1.58 (*m*, 6 H); 1.96 (*s*, Me); 2.81 (*m*, 4 H); 3.79 (*s*, ArCH₂); 6.32 (*s*, arom. H). IR (KBr): 3427*m*, 3108*m* (br.), 1602*s*, 1457*s*, 1491*s*, 1990*s*, 1960*s*, 1901*s*, 1118*s*. EI-MS: 265 (10, *M*⁺), 180 (30), 151 (28), 84 (100, C₅H₁₀N⁺).

3.4. *3,5-Dihydroxy-2,6-dimethylbenzoic Acid (23)*. To a suspension of **22** (520 g, 1.95 mol) in MeOH (4.5 l) was added with stirring 3*N* NaOH (650 ml, 1.95 mol). The clear soln. was hydrogenated for 14 h in the presence of 5% Pd/C (52 g) at a H₂ pressure of 0.1 bar. The pH of the mixture was set to 1 by the addition of 37% aq. HCl soln., the catalyst filtered off, and the soln. evaporated. The residue was diluted with H₂O (1 l) and extracted with AcOEt (3 × 2 l). The org. layer was washed with 2*N* HCl/15% NaCl soln. 4:1 (2 × 1 l) and with 15% NaCl soln. (1 l), dried, and evaporated. The solid residue was crystallized from dioxane/hexane: **23** (250.0 g, 70%). White crystals. M.p. 178–179°. ¹H-NMR (250 MHz, (D₆)DMSO): 1.92 (*s*, 2 Me); 6.38 (*s*, arom. H); 9.12 (*s*, 2 OH); 12.90 (br. *s*, COOH). EI-MS: 182 (92, *M*⁺), 164 (16), 136 (100, [*M* – CO₂ – H₂]⁺). Anal. calc. for C₉H₁₀O₄ (182.18): C 59.34, H 5.53; found: C 59.23, H 5.42.

3.5. *4-Nitrobenzyl 3,5-Dihydroxy-2,6-dimethylbenzoate (24)*. To a soln. of **23** (36.4 g, 0.20 mol) in DMF (0.2 l) was added at 0° *N,N,N',N'*-tetramethylguanidine (25.4 ml, 0.20 mol). After stirring for 15 min at r.t. (→ white precipitate), 4-nitrobenzyl bromide (43.2 g, 0.20 mol) was added and stirring continued for 6 h at r.t. The mixture was diluted with AcOEt (1 l) and washed with 1*N* HCl (2 × 0.2 l) and 15% NaCl soln. (3 × 0.4 l). The org. layer was dried and evaporated and the solid residue crystallized twice from AcOEt/hexane: **24** (52.0 g, 82%). Yellow crystals. M.p. 168–170°. ¹H-NMR (250 MHz, (D₆)DMSO): 1.89 (*s*, 2 Me); 5.46 (*s*, COOCH₂); 6.45 (*s*, 1 arom. H); 7.71, 8.27 (2*d*, *AB*, *J*_{*AB*} = 9, 4 arom. H (PNB)); 9.24 (*s*, 2 OH). Anal. calc. for C₁₆H₁₅NO₆ (317.30): C 60.57, H 4.77, N 4.41; found: C 60.57, H 4.83, N 4.28.

3.6. *4-Nitrobenzyl 3,5-Bis[(tert-butyl)dimethylsilyloxy]-2,6-dimethylbenzoate (25)*. To a stirred mixture of **24** (31.7 g, 0.1 mol) and (*t*-Bu)Me₂SiCl (33.2 g, 0.22 mol) in DMF (80 ml) was added at 0° Et₃N (33.4 ml, 0.24 mol), a precipitate being formed immediately. The mixture was stirred at 0° for 1.5 h, then diluted with AcOEt (0.5 l), and extracted with H₂O (5 × 0.2 l). The org. layer was dried and evaporated and the residual oil crystallized from hexane: **25** (49.8 g, 91%). White crystals. M.p. 121–122°. ¹H-NMR (250 MHz, CDCl₃): 0.20 (*s*, 4 MeSi); 1.00 (*s*, 2 *t*-Bu); 2.02 (*s*, 2 arom. Me); 5.43 (*s*, COOCH₂); 6.34 (*s*, 1 arom. H); 7.61, 8.24 (2*d*, *AB*, *J*_{*AB*} = 8.5, 4 arom. H (PNB)). Anal. calc. for C₂₈H₄₃NO₆Si₂ (545.83): C 61.61, H 7.94, N 2.57; found: C 61.63, H 7.85, N 2.63.

3.7. *4-Nitrobenzyl 2-(Bromomethyl)-3,5-bis[(tert-butyl)dimethylsilyloxy]-6-methylbenzoate (26) and 4-Nitrobenzyl 2,6-Bis(bromomethyl)-3,5-bis[(tert-butyl)dimethylsilyloxy]benzoate (27)*. To a soln. of **25** (27.30 g, 50 mmol) in CCl₄ (300 ml) was added NBS (8.90 g, 50 mmol). The stirred suspension was heated at reflux under irradiation with light (500 W) until all the insoluble material was floating at the surface of the soln. (50 min). The

⁴) The seeding of the soln. is not essential but seemed to cause the formation of a better filtrable precipitate. Seeding crystals were obtained in an analogous small-scale experiment.

mixture was cooled and stirred for 15 min at 0°. Insoluble material (succinimide) was removed by filtration and the soln. evaporated to furnish a pale yellow oil (33.7 g) containing (NMR analysis) starting material **25** (ca. 5.3 g, 19%), **26** (ca. 20.9 g, 67%), and **27** (ca. 5.0 g, 14%). ¹H-NMR (250 MHz, CDCl₃): **26**: 0.23, 0.28 (2s, 2 Me₂Si); 1.00, 1.04 (2s, 2 *t*-BuSi); 2.03 (s, arom. Me); 4.51 (s, CH₂Br); 5.48 (s, COOCH₂); 6.36 (s, 1 arom. H); 7.67, 8.25 (2d, *AB*, *J*_{AB} = 8, 4 arom. H (PNB)); **27**: 0.30 (s, 2 Me₂Si); 1.04 (s, 2 *t*-BuSi); 4.49 (s, CH₂Br); 5.53 (s, COOCH₂); 6.39 (s, 1 arom. H); 7.72, 8.25 (2d, *AB*, *J*_{AB} = 8, 4 arom. H (PNB)).

3.8. *Methyl N-[N-(tert-Butoxycarbonyl)-L-seryl]-S-[4,6-bis[(tert-butyl)dimethylsilyloxy]-3-methyl-2-[(4-nitrobenzyloxy)carbonyl]benzyl]-L-cysteinate (28)*. To a stirred soln. of **25/26/27** (33.7 g, containing ca. 20.9 g of **26**; see 3.7), and **11** (17.73 g, 55 mmol) in CH₂Cl₂ (0.25 l) was added at 0° Et₃N (7.67 ml, 55 mmol). Stirring was continued for 1 h at 0° and for 2 h at r.t. The soln. was washed with 5% aq. NaHCO₃ soln. (100 ml) and brine (2 × 100 ml), dried, and evaporated. The residual oil was chromatographed (AcOEt/hexane 1:2): **25** (4.75 g, 17.4%) and **28** (23.6 g, 54%; 66% rel. to converted **25**). Pale-yellow oil. ¹H-NMR (250 MHz, CDCl₃): 0.22, 0.24 (2s, 2 Me₂Si); 1.00, 1.01 (2s, 2 *t*-BuSi); 1.44 (s, Boc); 2.02 (s, arom. Me); 2.88 (dd, *J* = 14, 6, 1 H-C(3)(Cys)); 2.94 (dd, *J* = 14, 5, 1 H-C(3)(Cys)); 3.01 (m, OH); 3.68 (m, 1 H-C(3)(Ser)); 3.70 (s, COOMe); 3.74, 3.79 (2d, *AB*, *J*_{AB} = 12, ArCH₂S); 4.10 (m, 1 H-C(3)(Ser)); 4.28 (m, NH(Ser)); 4.73 (m, H-C(2)(Cys)); 5.50 (s, COOCH₂); 5.62 (d, *J* = 7, H-C(2)(Ser)); 6.36 (s, 1 arom. H); 7.11 (d, *J* = 7, NH(Cys)); 7.66, 8.25 (2d, *AB*, *J*_{AB} = 8, 4 arom. H (PNB)). ISP-MS: 866.5 ([*M* + H]⁺). Anal. calc. for C₄₀H₆₃N₃O₁₂SSi₂ (866.19): C 55.47, H 7.33, N 5.63, S 3.70; found: C 55.20, H 7.40, N 4.63, S 3.78.

3.9. *Methyl N-[N-(tert-butoxycarbonyl)-L-seryl]-S-[4,6-bis[(tert-butyl)dimethylsilyloxy]-2-carboxy-3-methylbenzyl]-L-cysteinate (29)*. A mixture of **28** (17.32 g, 0.02 mol) and 5% Pd/C (2.60 g) in AcOEt (0.2 l) was stirred under H₂ for 15 h at r.t. The mixture was filtered and the soln. evaporated. The residual oil was stirred with MeOH (0.2 l) for 2 h at r.t. and the fine precipitate formed removed by filtration. The solvent was evaporated, the residual oil dissolved in AcOEt (0.3 l), and the soln. washed with 1N HCl (0.1 l) and brine (3 × 0.05 l), dried, and evaporated. The residual oil was filtered through silica gel (200 g) using AcOEt: **29** (13.60 g, 93%). Pale-yellow foam. ¹H-NMR (250 MHz, (D₆)DMSO): 0.21, 0.23 (2s, 2 Me₂Si); 0.98 (s, 2 *t*-BuSi); 1.38 (s, Boc); 2.01 (s, arom. Me); 2.74–2.89 (m, 2 H-C(3)(Cys)); 3.47–3.63 (m, 2 H-C(3)(Ser)), superimposed by 3.60 (s, COOMe); 3.66 (s, ArCH₂S); 3.97–4.07 (m, H-C(2)(Ser)); 4.38–4.50 (m, H-C(2)(Cys)); 6.25 (s, 1 arom. H); 8.30 (br. s, NH(Cys)). Anal. calc. for C₃₃H₅₈N₂O₁₀SSi₂ (731.06): C 54.22, H 8.00, N 3.83; found: C 53.85, H 7.94, N 3.65.

3.10. *Methyl (4R,7S)-7-[(tert-Butoxycarbonyl)amino]-12,14-bis[(tert-butyl)dimethylsilyloxy]-1,3,4,5,6,7,8,10-octahydro-11-methyl-6,10-dioxo-9,2,5-benzoxathiazacyclododecine-4-carboxylate (30)*. By Lactonization of **29** under Mitsunobu Conditions. To a soln. of **29** (2.19 g, 3.0 mmol) in toluene (75 ml) were added at 0° Ph₃P (1.02 g, 3.9 mmol) and 95% diethyl azodicarboxylate (0.64 ml, 3.9 mmol). The mixture was stirred for 15 min at 0° followed by 4 h at r.t., and then evaporated. The residual oil was dissolved in CH₂Cl₂ and submitted to chromatographic purification (AcOEt/hexane 1:1): **30** (1.84 g, 86%). Amorphous solid. TLC (AcOEt): *R*_f 0.55. [*α*]_D = +58.3 (c = 0.7, AcOEt). ¹H-NMR (250 MHz, (D₆)DMSO): 0.21, 0.23 (2s, 2 Me₂Si); 0.98, 1.01 (2s, 2 *t*-BuSi); 1.42 (s, Boc); 1.97 (s, arom. Me); 2.83 (dd, *J* = 14, 10, 1 H-C(3)); 3.04 (dd, *J* = 14, 4, 1 H-C(3)); 3.50 (d, *J* = 11, 1 H-C(1)); 3.63 (s, COOMe); 3.86 (d, *J* = 11, 1 H-C(1)); 4.19 (dd, *J* = 11, 3, 1H-C(8)); 4.39 (m, H-C(7)); 4.58 (m, H-C(4)); 5.04 (dd, *J* = 11, 2, 1 H-C(8)); 6.32 (s, 1 arom. H); 7.16 (d, *J* = 8, *NH*Boc); 8.26 (d, *J* = 8, H-N(5)). ISP-MS: 730.5 ([*M* + NH₄]⁺), 713.5 ([*M* + H]⁺). Anal. calc. for C₃₃H₅₆N₂O₉SSi₂ (713.05): C 55.59, H 7.92, N 3.93, S 4.50; found: C 55.19, H 7.90, N 4.04, S 4.46.

By Lactonization of **29** with 2,2'-Dithiobispyridine. As described in Procedure A for **13** (see 1.8), **29** afforded **30** in 16% yield. When the activation period was extended from 20 min to 1 h at 0°, **30** was obtained in 32% yield.

By Lactonization of **29** with 2,2'-Dithiobis[4-(tert-butyl)-1-isopropyl-1H-imidazole] [17]. To a stirred soln. of **29** (2.19 g, 3.0 mmol) in toluene (30 ml) were added at 0° 2,2'-dithiobis[4-(tert-butyl)-1-isopropyl-1H-imidazole] (1.54 g, 3.9 mmol) and Ph₃P (1.02 g, 3.9 mmol). While stirring was continued at 0° for 1 h, a fine precipitate formed. The suspension was diluted with toluene (120 ml) and added over 1 h to boiling toluene (60 ml). After complete addition, the clear soln. was heated under reflux for 1 h, then cooled, and evaporated. The residual oil was stirred with hexane (40 ml) at 0° for 1 h. The crystals were filtered off, and the soln. was evaporated and the residue chromatographed (AcOEt/hexane 1:2): **30** (1.54 g, 72%). White foam. ¹H-NMR ((D₆)DMSO): identical to that given above.

3.11. *Methyl (4R,7S)-7-Amino-12,14-bis[(tert-butyl)dimethylsilyloxy]-1,3,4,5,6,7,8,10-octahydro-11-methyl-6,10-dioxo-9,2,5-benzoxathiazacyclododecine-4-carboxylate (31)*. A soln. of **30** (7.13 g, 10.0 mmol) in CF₃COOH (100 ml) was stirred at 0° for 30 min. The solvent was evaporated and the residue dissolved in AcOEt (200 ml). The soln. was washed with sat. NaHCO₃ soln. (2 × 60 ml) and brine (3 × 60 ml), dried, and evaporated. The residual oil was stirred with hexane (30 ml), and the crystals formed were recrystallized from AcOEt/hexane: **31** (4.66 g, 76%). White crystals. M.p. 154–155° (dec.) [*α*]_D = +41.4 (c = 1.0, AcOEt). ¹H-NMR (400 MHz, (D₆)DMSO): 0.21, 0.23

(2s, 2 Me₂Si); 0.97, 1.01 (2s, 2 *t*-BuSi); 1.95 (s, arom. Me); 2.25 (br. s, NH₂); 2.86 (dd, *J* = 14.3, 10.2, 1 H-C(3)); 3.06 (dd, *J* = 14.3, 4.7, 1 H-C(3)); 3.45 (d, *J* = 10.6, 1 H-C(1)); 3.65 (s, COOMe); 3.66 (m, H-C(7)); 3.84 (d, *J* = 10.6, 1 H-C(1)); 4.11 (dd, *J* = 10.8, 3.3, 1 H-C(8)); 4.68 (m, H-C(4)); 5.21 (dd, *J* = 10.8, 2.2, 1 H-C(8)); 6.32 (s, 1 arom. H); 8.45 (d, *J* = 8.5, H-N(5)). HR-MS (ESI): 613.2791 ([*M* + H]⁺, C₂₈H₄₉N₂O₇SSi₂⁺; calc. 613.2799).

3.12. *Methyl (4R,7S)-7-[(3R)-1-(tert-Butoxycarbonyl)-3-hydroxy-L-prolyl]amino]-12,14-bis[(tert-butyl)dimethylsilyloxy]-1,3,4,5,6,7,8,10-octahydro-11-methyl-6,10-dioxo-9,2,5-benzoxathiazacyclododecine-4-carboxylate (32)*. To an ice-cold mixture of **31** (3.06 g, 5.0 mmol) and (–)-**14** (1.27 g, 5.5 mmol) in MeCN (20 ml) was added EDC (1.05 g, 5.5 mmol). The mixture was stirred at 0° for 5 h, then diluted with AcOEt (200 ml), and washed with 1N HCl (50 ml), 5% NaHCO₃ soln. (50 ml), and brine (2 × 50 ml). The org. layer was dried and evaporated and the crude product crystallized from AcOEt/hexane: **32** (3.73 g, 90%). White crystals. M.p. 155–158° (dec.). TLC (AcOEt): R_f 0.35. [α]_D = +39.9 (*c* = 1.0, AcOEt). ¹H-NMR (400 MHz, (D₆)DMSO; rotamer 1/rotamer 2 ca. 1:1): 0.20, 0.21, 0.23, 0.24 (4s, 2 Me₂Si, rot. 1/rot. 2); 0.97, 1.02 (2s, *t*-BuSi); 1.30, 1.37 (2s, Boc, rot. 1/rot. 2); 1.82, 1.95 (2m, 2 H-C(4) (cHyp)); 1.97, 1.98 (2s, arom. Me, rot. 1/rot. 2); 2.55 (dd, *J* = 14, 12, 1 H-C(3)); 3.05, 3.06 (2dd, *J* = 14, 4, 1 H-C(3), rot. 1/rot. 2); 3.13, 3.22 (2m, 1 H-C(5) (cHyp), rot. 1/rot. 2); 3.45 (d, *J* = 10, 1 H-C(1)); 3.46 (m, 1 H-C(5) (cHyp)); 3.61 (s, COOMe); 3.85 (d, *J* = 10, 1 H-C(1)); 3.96, 4.04 (2dd, *J* = 10, 2.5, 1 H-C(8), rot. 1/rot. 2); 4.42 (m, H-C(2) (cHyp), H-C(3) (cHyp)); 4.56 (m, H-C(7)); 4.67 (m, H-C(4)); 5.57, 5.59 (2dd, *J* = 10, 2, 1 H-C(8), rot. 1/rot. 2); 6.03, 6.09 (2d, *J* = 3, OH(cHyp), rot. 1/rot. 2); 6.34 (s, 1 arom. H); 8.30, 8.34 (2d, *J* = 7, NH-C(7), rot. 1/rot. 2); 8.48, 8.51 (2d, *J* = 10, H-N(5), rot. 1/rot. 2). HR-MS (ESI): 848.3616 ([*M* + Na]⁺, C₃₈H₆₃N₃O₁₁SSi₂Na⁺; calc. 848.3620).

3.13. *Methyl (4R,7S)-12,14-Bis[(tert-butyl)dimethylsilyloxy]-1,3,4,5,6,7,8,10-octahydro-7-[(3R)-3-hydroxy-L-prolyl]amino]-11-methyl-6,10-dioxo-9,2,5-benzoxathiazacyclododecine-4-carboxylate (33)*. As described for **31** (see 3.11), with **32** (3.30 g, 4.0 mmol). The crude product was crystallized from Et₂O/hexane: **33** (2.58 g, 89%). White crystals. M.p. > 140° (dec.). TLC (acetone/hexane 1:1): R_f 0.10. [α]_D = +12.7 (*c* = 0.7, AcOEt). ¹H-NMR (400 MHz, (D₆)DMSO): major rotamer (> 80%): 0.21 (s, Me₂Si); 0.24, 0.25 (2s, 2 MeSi); 0.98, 1.02 (2s, 2 *t*-BuSi); 1.77 (m, 1 H-C(4) (cHyp)); 1.97 (s, arom. Me); 1.98 (m, 1 H-C(4) (cHyp)); 2.71 (dd, *J* = 14, 12, 1 H-C(3)); 2.97 (m, 1 H-C(5) (cHyp)); 3.09 (dd, *J* = 14.0, 4.8, 1 H-C(3)); 3.12 (m, 1 H-C(5) (cHyp)); 3.42 (d, *J* = 10.4, 1 H-C(1)); 3.63 (s, COOMe); 3.73 (d, *J* = 10.4, 1 H-C(1)); 3.90 (br. d, *J* ≈ 6, H-C(2) (cHyp)); 4.01 (dd, *J* = 10, 2.5, 1 H-C(8)); 4.48 (m, H-C(3) (cHyp)); 4.64 (m, H-C(4)); 4.74 (m, H-C(7)); 5.32 (d, OH (cHyp)); 5.50 (dd, *J* = 10, 2, 1 H-C(8)); 6.33 (s, 1 arom. H); 8.25 (d, *J* = 9, H-N(5)); 8.47 (d, *J* = 8, NH-C(7)). HR-MS (ESI): 726.3277 ([*M* + H]⁺, C₃₃H₅₆N₃O₉SSi₂⁺; calc. 726.3276).

3.14. *Methyl (4R,7S)-7-[(3R)-1-(N-(tert-Butoxycarbonyl)-L-seryl)-3-hydroxy-L-prolyl]amino]-12,14-bis[(tert-butyl)dimethylsilyloxy]-1,3,4,5,6,7,8,10-octahydro-11-methyl-6,10-dioxo-9,2,5-benzoxathiazacyclododecine-4-carboxylate (34)*. As described for **32** (see 3.12), **33** (1.45 g, 2.0 mmol) was coupled with *N*-Boc-L-serine (**9**; 0.41 g, 2.0 mmol). The product was purified by chromatography (acetone/hexane 1:1) and crystallized from CH₂Cl₂/hexane: **34** (1.33 g, 73%). White crystals. M.p. > 130° (dec.). TLC (acetone/hexane 1:1): R_f 0.17. [α]_D = +35.3 (*c* = 1.0, AcOEt). ¹H-NMR (400 MHz, (D₆)DMSO): major rotamer (ca. 80%): 0.20, 0.21, 0.23, 0.24 (4s, 4 MeSi); 0.97, 1.02 (2s, 2 *t*-BuSi); 1.36 (s, Boc); 1.91 (m, 1 H-C(4) (cHyp)); 1.97 (s, arom. Me); 2.05 (m, 1 H-C(4) (cHyp)); 2.58 (dd, *J* = 14, 12, 1 H-C(3)); 3.05 (dd, *J* = 14, 5, 1 H-C(3)); 3.40 (m, 1 H-C(3) (Ser)); 3.45 (d, *J* = 10, 1 H-C(1)); 3.57 (m, 1 H-C(3) (Ser), 1 H-C(5) (cHyp)); 3.62 (s, COOMe); 3.73 (m, 1 H-C(5) (cHyp)); 3.84 (d, *J* = 10, 1 H-C(1)); 4.01 (dd, *J* = 10, 2, 1 H-C(8)); 4.28 (m, H-C(2) (Ser)); 4.42 (m, H-C(3) (cHyp)); 4.54 (m, H-C(7)); 4.67 (d, *J* = 7, H-C(2) (cHyp)); 4.69 (m, H-C(4)); 4.74 (t, *J* = 5.5, OH(Ser)); 5.57 (dd, *J* = 10, 2.5, 1 H-C(8)); 6.08 (d, *J* = 3.6, OH (cHyp)); 6.33 (s, 1 arom. H); 6.79 (d, *J* = 8, NH(Ser)); 8.40 (d, *J* = 7.3, NH-C(7)); 8.47 (d, *J* = 9.4, H-N(5)). HR-MS (ESI): 935.3933 ([*M* + Na]⁺, C₄₁H₆₈N₄O₁₃SSi₂Na⁺; calc. 935.3940). Anal. calc. for C₄₁H₆₈N₄O₁₃SSi₂·0.35 H₂O (919.68): C 53.54, H 7.46, N 6.10, S 3.49, H₂O 0.70; found: C 53.31, H 7.60, N 6.05, S 3.51, H₂O (Karl Fischer titration) 0.70.

3.15. *(4R,7S)-7-[(3R)-1-(N-(tert-Butoxycarbonyl)-L-seryl)-3-hydroxy-L-prolyl]amino]-1,3,4,5,6,7,8,10-octahydro-12,14-dihydroxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiazacyclododecine-4-carboxylic Acid (35)*. To a soln. of **34** (0.91 g, 1.0 mmol) in 80% aq. THF (12.5 ml) was added NH₄F (0.20 g, 5.4 mmol). The mixture was stirred at r.t. for 40 min, and the pH of the resulting emulsion was then raised from 9.8 to 11.6 by the addition of 1N NaOH (3.5 ml). Subsequently, 1N NaOH (3.0 ml) was added dropwise (over 20 min), until the pH reached 12.0 (autotitration). The pH was lowered to 9.0 by the addition of 6N HCl (ca. 2 ml) and the mixture extracted with AcOEt/hexane 1:1 (2 × 20 ml). The org. layers were extracted with sat. Na₂CO₃ soln. (2 × 5 ml) and the combined aq. layers cooled to 0° and acidified to pH 2.5 by the addition of 6N HCl. Saturation of the clear soln. with NaCl afforded an oily precipitate which was filtered off and extracted with acetone (3 × 20 ml). Insoluble material was removed by filtration, the soln. evaporated, and the residue crystallized from acetone/hexane to give **35** (0.55 g,

82%). White crystals. M.p. > 205° (dec.). $[\alpha]_D = +21.3$ ($c = 1.0$, MeOH). $^1\text{H-NMR}$ (400 MHz, $(\text{D}_6)\text{DMSO}$): major rotamer (ca. 80%): 1.36 (*s*, Boc); 1.89 (*s*, arom. Me); 1.95, 2.00 (2*m*, 2 H–C(4) (*c*Hyp)); 2.57 (*dd*, $J = 14, 12$, 1 H–C(3)); 3.05 (*dd*, $J = 14, 4$, 1 H–C(3)); 3.38 (*m*, 1 H–C(3) (Ser)); 3.46 (*d*, $J = 10.3$, 1 H–C(1)); 3.56 (*m*, 1 H–C(5) (*c*Hyp)), H–C(2) (Ser)); 3.71 (*m*, 1 H–C(5) (*c*Hyp)); 3.84 (*d*, $J = 10.3$, 1 H–C(1)); 3.97 (*dd*, $J = 10, 2$, 1 H–C(8)); 4.28 (*m*, H–C(2) (Ser)); 4.42 (*m*, H–C(3) (*c*Hyp)); 4.50 (*m*, H–C(4), H–C(7)); 4.67 (*d*, $J = 7.4$, H–C(2) (*c*Hyp)); 4.76 (*t*, $J \approx 5$, OH (Ser)); 5.53 (*dd*, $J = 10, 2$, 1 H–C(8)); 6.05 (*br. s*, OH (*c*Hyp)); 6.45 (*s*, 1 arom. H); 6.82 (*d*, $J = 7.8$, NH (Ser)); 8.36 (*m*, H–N(5), NH–C(7)); 9.48, 9.50 (2*s*, 2 OH); 12.72 (*br. s*, COOH). CI-MS: 671.3 ($[M + H]^+$), 571.9 ($[M + H - \text{Boc}]^+$).

3.16. *tert*-Butyl N- $\{[(4R,7S)-7-\{[(3R)-1-(tert-Butoxycarbonyl)-L-seryl]-3-hydroxy-L-prolyl\}amino]-1,3,4,5,6,7,8,10\text{-octahydro-12,14-dihydroxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiazacyclododecin-4-ylcarbonyl}]\}$ -L-alaninate (**36**). To an ice-cold mixture of **35** (1.67 g, 2.5 mmol) and *N*-hydroxysuccinimide (0.43 g, 3.75 mmol) in MeCN (50 ml) was added EDC (0.72 g, 3.75 mmol), and the mixture was stirred at 0° for 3 h. *tert*-Butyl L-alaninate (0.68 g, 3.75 mmol) and *N*-methylmorpholine (0.41 ml, 3.75 mmol) were added and stirring was continued at r.t. for 2 h. The soln. was diluted with AcOEt (100 ml) and washed with 1*N* HCl (30 ml), H₂O (30 ml), sat. Na₂CO₃ soln. (30 ml), and H₂O (3 × 30 ml). The org. layer was dried and evaporated, and the crude product was crystallized from acetone/hexane to give **36** (1.51 g, 75%). M.p. > 180° (dec.). TLC (acetone): $R_f = 0.55$. $[\alpha]_D = -8.3$ ($c = 0.7$, MeOH). $^1\text{H-NMR}$ (400 MHz, $(\text{D}_6)\text{DMSO}$): major rotamer (ca. 80%): 1.26 (*d*, $J = 7.2$, Me(Ala)); 1.36, 1.39 (2*s*, 2 *t*-Bu); 1.89 (*s*, arom. Me); 1.98, 2.07 (2*m*, 2 H–C(4) (*c*Hyp)); 2.95 (*dd*, $J = 14, 12$, 1 H–C(3)); 3.06 (*dd*, $J = 14, 4$, 1 H–C(3)); 3.38, 3.55 (2*m*, 2 H–C(3) (Ser)); 3.57 (*d*, $J = 10$, 1 H–C(1)); 3.60, 3.69 (2*m*, 2 H–C(5) (*c*Hyp)); 3.85 (*d*, $J = 10$, 1 H–C(1)); 4.02 (*dd*, $J = 10, 2$, 1 H–C(8)); 4.08 (*q*, $J = 7$, H–C(2) (Ala)); 4.28 (*m*, H–C(2) (Ser)); 4.36 (*m*, H–C(7)); 4.47 (*m*, H–C(4), H–C(3) (*c*Hyp)); 4.65 (*d*, $J = 7.3$, H–C(2) (*c*Hyp)); 4.69 (*t*, $J = 5$, OH (Ser)); 5.55 (*dd*, $J = 10, 2.5$, 1 H–C(8)); 6.09 (*d*, $J = 2$, OH (*c*Hyp)); 6.46 (*s*, 1 arom. H); 6.81 (*d*, $J = 8$, NH (Ser)); 7.51 (*d*, $J = 7$, NH (Ala)); 8.18 (*d*, $J = 9$, H–N(5)); 8.77 (*d*, $J = 6$, NH–C(7)); 9.49, 9.50 (2*s*, 2 OH). HR-MS (ESI): 820.3018 ($[M + \text{Na}]^+$, C₃₅H₅₁N₅O₁₄SNa⁺; calc. 820.3051).

3.17. Cyclothialidine (= N- $\{[(4R,7S)-1,3,4,5,6,7,8,10\text{-Octahydro-12,14-dihydroxy-7-}\{[(3R)-3\text{-hydroxy-1-L-seryl-L-prolyl}]\}amino]-11\text{-methyl-6,10-dioxo-9,2,5-benzoxathiazacyclododecin-4-ylcarbonyl}]\}$ -L-alanine; **1**). A soln. of **36** (638 mg, 0.8 mmol) in CF₃COOH (8 ml) was stirred at 0° for 1.75 h. The solvent was evaporated and the residue dissolved in H₂O (3 ml). The pH was adjusted to 6 by the addition of 28% NaOH soln. and the soln. then subjected to column chromatography (MCI-Gel CHP20P (Mitsubishi Chemical Industries, Ltd.), H₂O (→salts), then 2% aq. MeCN). The product fraction was lyophilized: **1** (220 mg, 43%). White powder. $[\alpha]_D = -12.2$ ($c = 0.5$, H₂O) ($[\beta]$: -13.2 ($c = 1$, H₂O)). UV: 293 (3.57) ($[\beta]$: 293 (3.20)). IR (KBr): 3344*s* (*br.*), 1720*s*, 1655*vs*, 1598*s*, 1534*s*. $^1\text{H-NMR}$ (400 MHz, $(\text{D}_2\text{O})^5$): 1.36 (*d*, $J = 7.3$, Me(Ala)); 2.04 (*s*, arom. Me); 2.10, 2.25 (2*m*, 2 H–C(4) (*c*Hyp)); 2.68 (*dd*, $J = 15, 11$, 1 H–C(3)); 3.33 (*dd*, $J = 15, 4.8$, 1 H–C(3)); 3.48 (*d*, $J = 11$, 1 H–C(1)); 3.78–3.92 (*m*, 1 H–C(1), 1 H–C(3) (Ser), 2 H–C(5) (*c*Hyp)); 3.99 (*dd*, $J = 12.5, 4.5$, 1 H–C(3) (Ser)); 4.11 (*q*, $J = 7.3$, H–C(2) (Ala)); 4.38 (*dd*, $J = 12, 2.3$, 1 H–C(8)); 4.40 (*m*, H–C(2) (Ser)); 4.70–4.90 (*m*, H–C(4), H–C(7), H–C(2)/H–C(3) (*c*Hyp)); 5.70 (*dd*, $J = 12, 2.5$, 1 H–C(8)); 6.59 (*s*, 1 arom. H). HR-MS (ESI): 664.1886 ($[M + \text{Na}]^+$, C₂₆H₃₅N₅O₁₂SNa⁺; calc. 664.1901). Anal. calc. for C₂₆H₃₅N₅O₁₂S · 2.5 H₂O (686.69): C 45.48, H 5.87, N 10.20, S 4.67, H₂O 6.56; found: C 45.34, H 5.93, N 10.28, S 4.34, H₂O (Karl Fischer titration) 6.64.

REFERENCES

- [1] J. Watanabe, N. Nakada, S. Sawairi, H. Shimada, S. Ohshima, T. Kamiyama, M. Arisawa, *J. Antibiot.* **1994**, 47, 32.
- [2] R. J. Lewis, O. M. P. Singh, C. V. Smith, A. Maxwell, T. Skarzynski, A. J. Wonacott, D. B. Wigley, *J. Mol. Biol.* **1994**, 241, 128; K. Yamaji, M. Masubuchi, F. Kawahara, Y. Nakamura, A. Nishio, S. Matsukuma, M. Fujimori, N. Nakada, J. Watanabe, T. Kamiyama, submitted to *J. Antibiot.*
- [3] T. Kamiyama, N. Shimada, T. Ohtsuka, N. Nakayama, Y. Itezono, N. Nakada, J. Watanabe, K. Yokose, *J. Antibiot.* **1994**, 47, 37.
- [4] J. H. Richards, J. B. Hendrickson, 'Biosynthesis of Terpenes, Steroids, and Acetogenins', W. A. Benjamin, New York, 1964.
- [5] N. Nakada, H. Shimada, T. Hirata, Y. Aoki, T. Kamiyama, J. Watanabe, M. Arisawa, *Antimicrob. Agents Chemother.* **1993**, 37, 2656.

⁵⁾ For a comparison with natural cyclothialidine, see also the Figure.

- [6] N. Nakada, H. Gmünder, T. Hirata, M. Arisawa, *Antimicrob. Agents Chemother.* **1994**, *38*, 1966.
- [7] M. Gellert, M. H. O'Dea, T. Itoh, J. Tomizawa, *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 4474.
- [8] E. Goetschi, P. Angehrn, H. Gmuender, P. Hebeisen, H. Link, R. Masciadri, J. Nielsen, *Pharmacol. Ther.* **1993**, *60*, 367.
- [9] D. J. Burton, W. M. Koppes, *J. Org. Chem.* **1975**, *40*, 3026; W. Davies, W. H. Perkin, *J. Chem. Soc., Trans.* **1922**, *121*, 2202.
- [10] E. J. Corey, K. C. Nicolaou, *J. Am. Chem. Soc.* **1974**, *96*, 5614.
- [11] T. Kurihara, Y. Nakajima, O. Mitsunobu, *Tetrahedron Lett.* **1976**, *17*, 2455; O. Mitsunobu, *Synthesis* **1981**, *1*.
- [12] J. Häusler, *Anal. Chem.* **1983**, 982; J. Häusler, *ibid.* **1981**, 1073.
- [13] R. Bhide, R. Mortezaei, A. Scilimati, C. J. Sih, *Tetrahedron Lett.* **1990**, *31*, 4827.
- [14] J. Cooper, P. T. Gallagher, D. W. Knight, *J. Chem. Soc., Chem. Commun.* **1988**, 509; J. Cooper, P. T. Gallagher, D. W. Knight, *J. Chem. Soc., Perkin Trans. 1* **1993**, 1313.
- [15] R. T. Borchardt, A. K. Sinhababu, *J. Org. Chem.* **1981**, *46*, 5021.
- [16] O. Jacobsen, F. Wierss, *Chem. Ber.* **1883**, *16*, 1956; R. B. Woodward, W. A. Reed, *J. Am. Chem. Soc.* **1943**, *65*, 1569.
- [17] E. J. Corey, D. J. Brunelle, *Tetrahedron Lett.* **1976**, 3409.
- [18] H. Gmünder (Pharma Division, Preclinical Research, *F. Hoffmann-La Roche Ltd.*, CH-4002 Basel), personal communication.
- [19] E. Goetschi, P. Angehrn, H. Gmuender, P. Hebeisen, H. Link, R. Masciadri, P. Reindl, F. Ricklin, in 'Medicinal Chemistry: Today and Tomorrow' (Proceedings of AIMECS 95 – Tokyo, September 1995), Ed. M. Yamazaki, Blackwell Science Ltd., Oxford, 1996, pp. 263–270.
- [20] R. Munier, M. Machebœuf, *Bull. Soc. Chim. Biol.* **1949**, *31*, 1144.
- [21] J. C. Sheehan, J. G. Whitney, *J. Am. Chem. Soc.* **1963**, *85*, 3863; J. S. III Wolff, J. D. Ogle, M. A. Logan, *J. Biol. Chem.* **1966**, *241*, 1300.