184. Total Synthesis of Cyclothialidine

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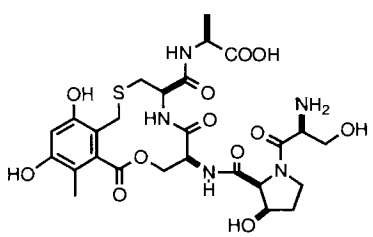
(2.IX.96)

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].



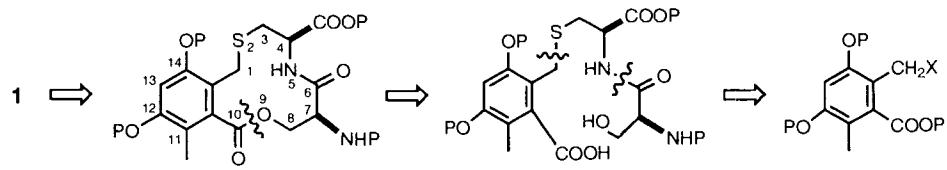
Cyclothialidine **1**

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].

Although hardly exhibiting any antibacterial activity against intact bacterial cells, probably due to insufficient penetration of the cytoplasmic membrane, **1** was considered to be a promising lead compound which by chemical modification of its structure might open a route to a new class of antibacterials. To explore and exploit the potential of **1** in this respect, we developed an efficient and flexible synthetic route allowing the preparation of a great variety of analogues [8].

The retrosynthetic analysis (*Scheme 1*) of **1** suggested the bicyclic lactone **I** to be a versatile key intermediate. For the formation of the macrocycle, a classical lactonization of an ω-hydroxy acid **II** seemed to be the method of choice. Disconnection of the peptide chain from the seco-acid **II** – either stepwise or all at once – would then lead back to a benzylating agent, such as a bromide **III** which could be derived from a 3,5-dihydroxy-2,6-dimethylbenzoate **IV**. Based on this analysis, we have realized a synthetic scheme which has proven its flexibility in many different syntheses. Herein we report in more detail on our synthesis of **1** [8].

Scheme 1. *Retrosynthetic Analysis*



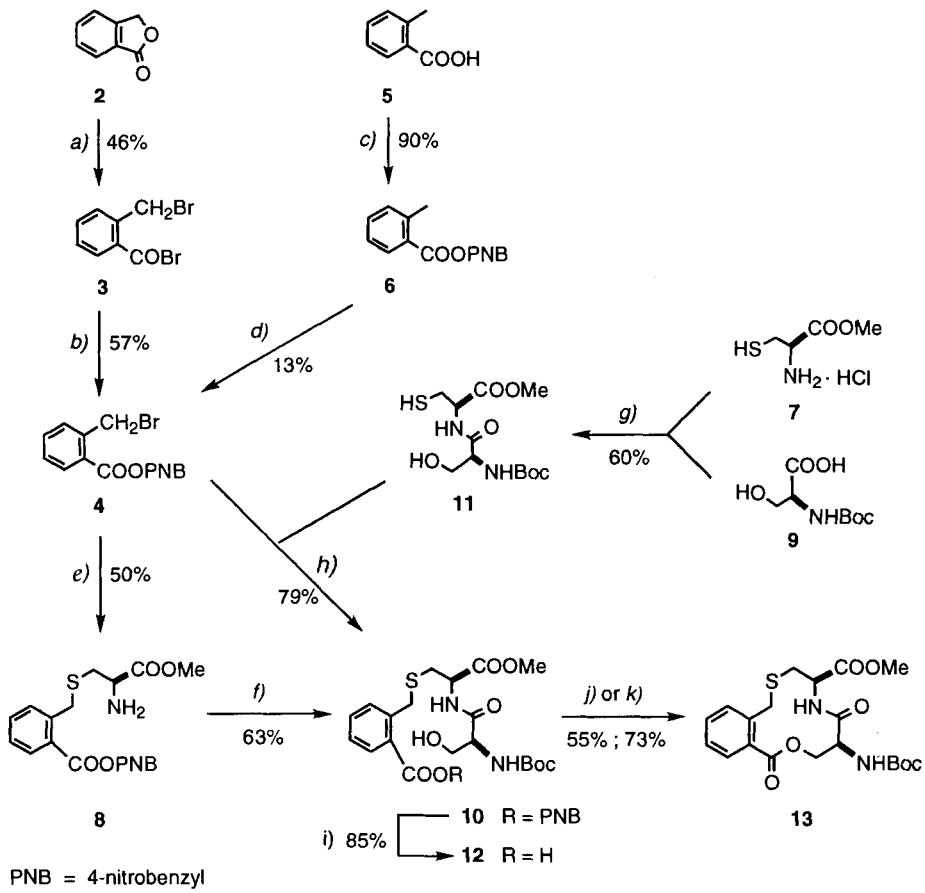
I II III X = Br IV X = H

P = Protecting groups

2. Results and Discussion. – 2.1. *Model Studies.* To examine the feasibility of our strategy, we first investigated the synthesis of the lactone **13** lacking the characteristic phenyl substitution pattern of **1** (*Scheme 2*). Phthalide (**2**) was heated with $Ph_3P \cdot Br_2$ according to *Burton* and *Koppes* [9], and the resulting benzoyl bromide **3** was treated in CH_2Cl_2 with 4-nitrobenzyl (PNB) alcohol in the presence of *Hünig*'s base to yield ester **4**. Looking for a more general access to *o*-(bromomethyl)benzoates **III**, we examined the *N*-bromosuccinimid (NBS) bromination of ester **6** which was prepared by alkylation of 2-methylbenzoic acid **5** with 4-nitrobenzyl bromide. This reaction, however, gave only a poor yield (13%) of **4** due to bromination of the 4-nitrobenzyl group. Bromide **4** was substituted by L-Cys-OMe (**7**) in CH_2Cl_2 in the presence of Et_3N, and the resulting amine **8** was acylated with Boc-L-Ser-OH (**9**) in MeCN using *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride (EDC) as the coupling reagent. A more direct route to **10** consisted in treating bromide **4** in CH_2Cl_2 in the presence of Et_3N with dipeptide **11** that was obtained in 60% yield from the DCC coupling of L-Cys-OMe (**7**) with Boc-L-Ser-OH (**9**) in MeCN. Probably due to the presence of a sulfide functionality, the hydrogenolytic cleavage of 4-nitrobenzyl ester **10** in AcOEt was rather slow and required a large amount of Pd catalyst (20% by weight of 5% Pd/C). Nevertheless, the reaction went to completion, and ω-hydroxy acid **12** was isolated in 85% yield. For the subsequent lactonization step, we first used the thioester-activation method of *Corey* and *Nicolaou* [10]. Thus, treatment of **12** with 2,2'-dithiobispyridine and Ph_3P in toluene

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) $Ph_3P \cdot Br_2$, 170°, 3 h. *b*) PNB alcohol, $(i\text{-Pr})_2EtN$, CH_2Cl_2, 0° to r.t., 3 h. *c*) PNB bromide, K_2CO_3, acetone, reflux, 4 h. *d*) NBS, CCl_4, *hv*, 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.

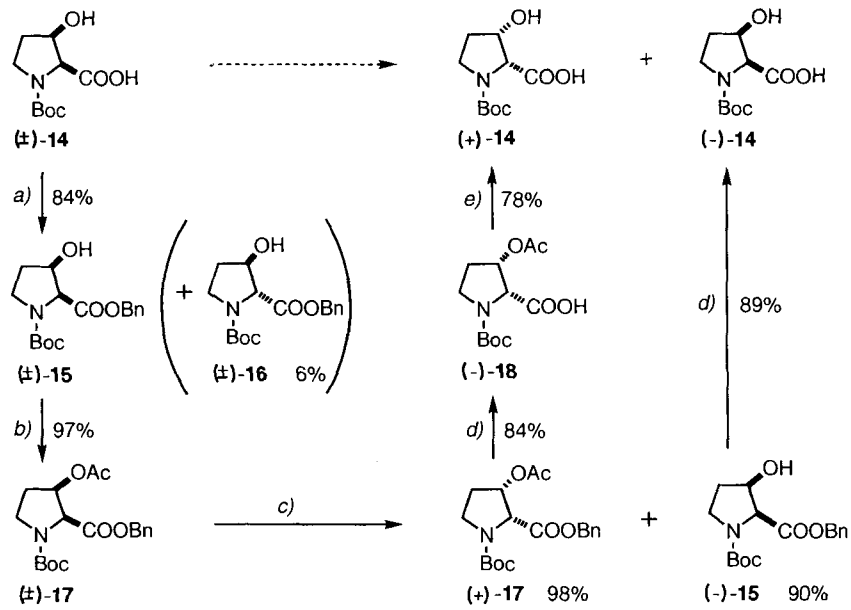
For the lactone H-atoms, the ^{1}H-NMR spectrum of **13** in (D_6)DMSO showed the typical features reported for **1** [3]. For the geminal methylene protons at C(1) and C(8), large differences of the chemical shifts with $\Delta\delta = 0.8$ and 0.45 ppm, respectively, were observed. The coupling constants $J(3,4)$ 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 (D_6)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 (±)-**14** which we had prepared from L-proline in close analogy to the procedure of *Häusler* [12]. For the resolution of (±)-**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 (±)-**17** (*Scheme 3*). The racemic acetate (±)-**17**, obtained in 81% yield by consecutive benzylation and acetylation of (±)-**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 CF_3COOH, 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 CH_2O 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

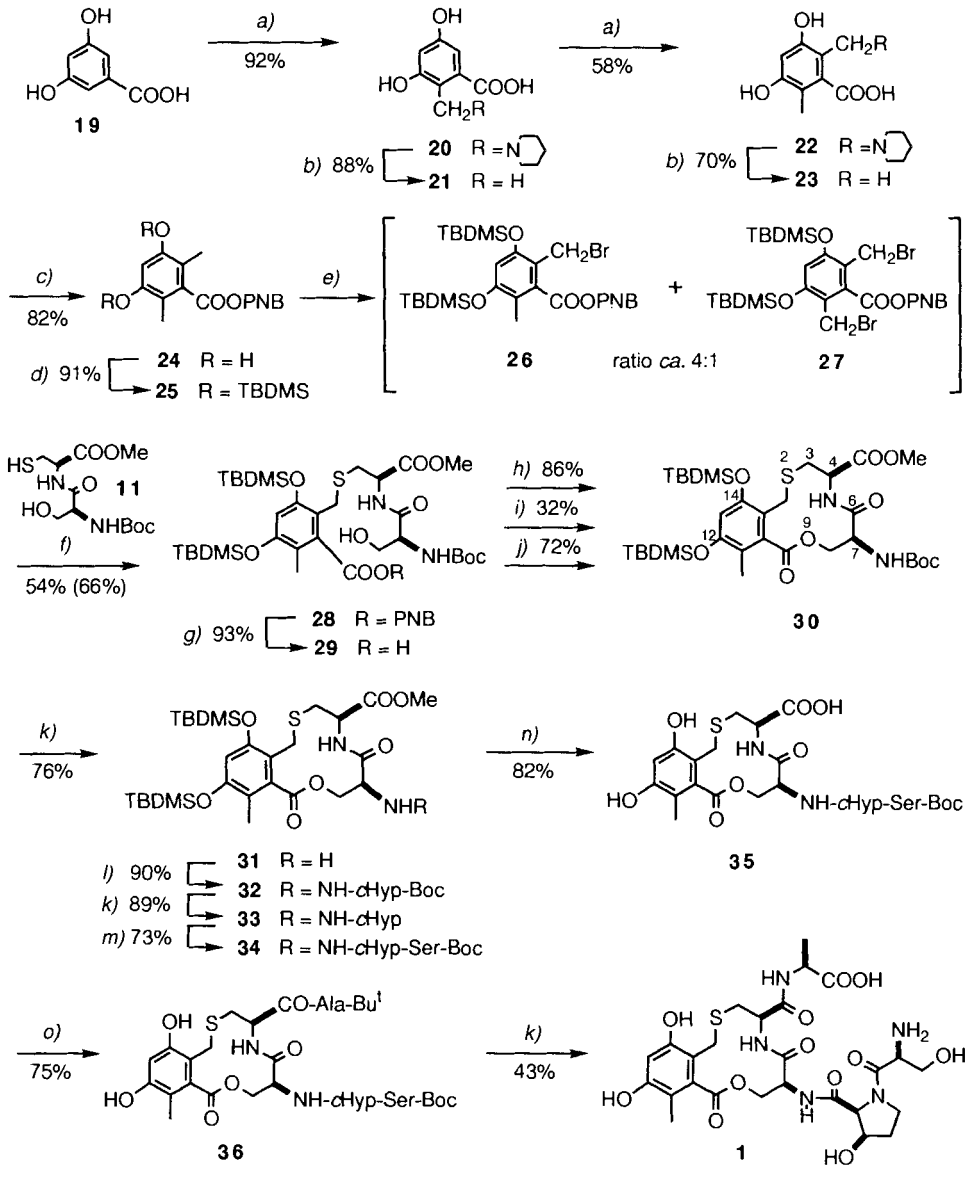
Scheme 3. *Resolution of* cis-*3-Hydroxy-*DL-*proline*



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 ^{1}H-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) CH_2O, piperidine, pH 5. *b*) H_2, Pd/C, aq. MeOH, base. *c*) 1. *N,N,N′,N′*-Tetramethylguanidine, DMF, 2. PNB bromide. *d*) TBDMSCl, Et_3N, DMF. *e*) NBS, CCl_4, *hv*. 90°. *f*) Et_3N, CH_2Cl_2. *g*) H_2, Pd/C, AcOEt. *h*) DEAD, Ph_3P, toluene. *i*) 2,2′-Dithiobispyridine, Ph_3P, toluene, 0°, then 110°. *j*) 2,2′-Dithiobis[(4-(*tert*-butyl)-1-isopropyl-1*H*-imidazole], Ph_3P, toluene, 0°, then 110°. *k*) CF_3COOH, 0°. *l*) Boc-*c*Hyp-OH, EDC, MeCN. *m*) Boc-L-Ser-OH, EDC, MeCN. *n*) 1. NH_4F, 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 cyclohialidine (**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

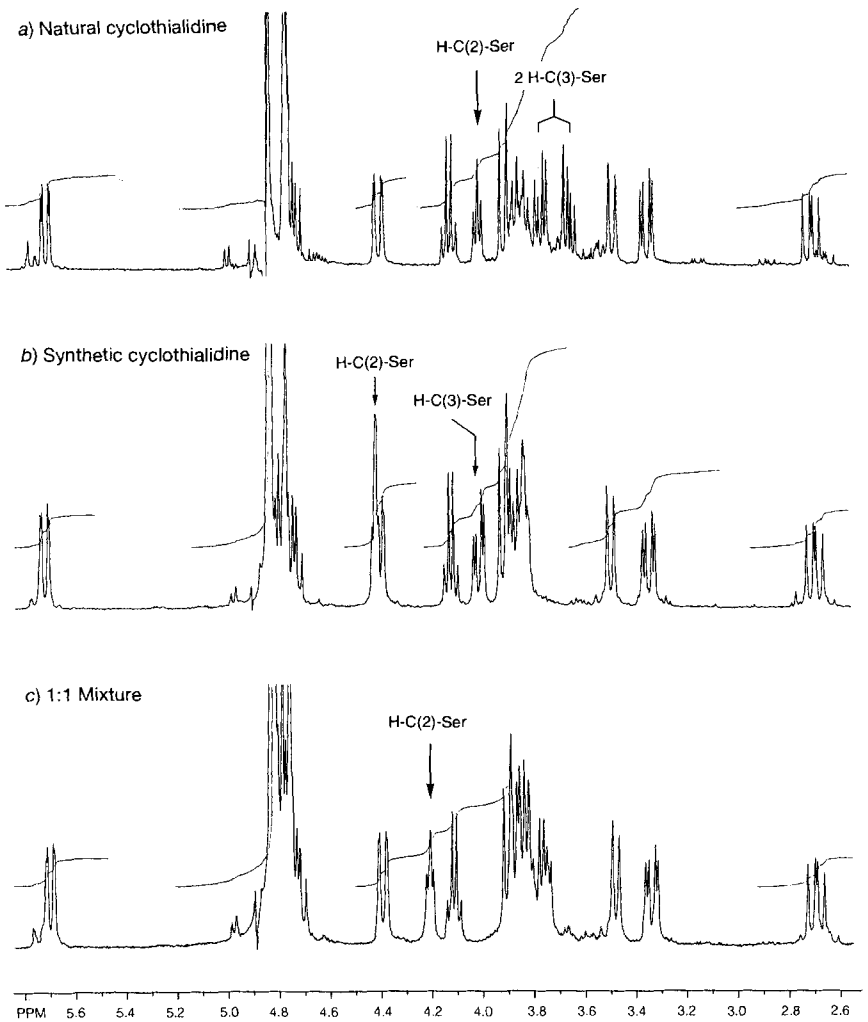


Figure. *400-MHz ^{1}H-NMR Spectra* (D_2O) *of* a) *natural cyclothialidine*, b) *synthetic cyclothialidine*, c) *a 1:1 mixture of natural and synthetic cyclothialidine*. Ser = N-terminal serine moiety.

plates ('Kieselgel' *60 F_{254}, Merck*); detection by UV and visualization by spraying with 1% aq. $KMnO_4$ soln. or 0.4% aq. $K_2Pt(II)I_4$ 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°. ^{1}H-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, 1*H*-isobenzofuran-1-one (**2**; 31.5 g, 0.235 mol) and $Ph_3P \cdot Br_2$ (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. 158–165°/21 mbar ([9]: 170–171°/32 mbar). Material of this quality was used in the next step.

1.2. *4-Nitrobenzyl 2-(Bromomethyl)benzoate* (**4**). At 0°, $(i\text{-}Pr)_2EtN$ (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 CH_2Cl_2 (25 ml). The mixture was stirred for 15 min at 0° and for 3 h at r.t., then diluted with CH_2Cl_2 (25 ml), washed with 1N $KHSO_4$ (10 ml) and H_2O (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°. ^{1}H-NMR (250 MHz, $CDCl_3$): 4.95 (*s*, CH_2Br); 5.48 (*s*, $COOCH_2$); 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 $C_{15}H_{12}BrNO_4$ (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 K_2CO_3 (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°. ^{1}H-NMR (250 MHz, $CDCl_3$): 2.62 (*s*, Me); 5.44 (*s*, CH_2); 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 CCl_4 (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 unsoluble material was floating at the surface. The mixture was cooled in an ice-bath for 15 min, and unsoluble material was filtered off. The soln. was evaporated, the residue chromatographed (hexane/AcOEt 10:1), and the purified product crystallized from CH_2Cl_2/hexane: **4** (45 mg, 13%). White crystals. M.p. 104°. TLC (hexane/AcOEt 2:1): R_f 0.31. ^{1}H-NMR ($CDCl_3$): 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 CH_2Cl_2 (25 ml) was added Et_3N (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 CH_2Cl_2 (25 ml), washed with 5% $NaHCO_3$ 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. ^{1}H-NMR (250 MHz, $CDCl_3$): 1.65 (br. *s*, NH_2); 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 (2*d*, *AB*, J_{AB} = 13, $ArCH_2S$); 5.45 (*s*, $COOCH_2$); 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 $C_{19}H_{20}N_2O_6S$ (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% $NaHCO_3$ 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. ^{1}H-NMR (250 MHz, $CDCl_3$): 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 (2*d*, *AB*, J_{AB} = 12.4, $ArCH_2S$); 4.25–4.36 (*m*, 1 H); 4.77–4.87 (*m*, 1 H); 5.49 (*s*, $COOCH_2$); 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 ($[M + H - C_4H_8]^+$), 492.4 ($[M + H - C_4H_8 - CO_2]^+$). Anal. calc. for $C_{27}H_{33}N_3O_{10}S$ (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% $NaHCO_3$ 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 Et_2O/hexane: **11** (192.8 g, 60%). White crystals. M.p. 74–76°. TLC (AcOEt): R_f 0.38. $[\alpha]_D$ = +3.3 (*c* = 1, AcOEt). ^{1}H-NMR (250 MHz, $CDCl_3$): 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 ($[M - C_4H_8]^+$). Anal. calc. for $C_{12}H_{22}N_2O_6S$ (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**. Et_3N (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 CH_2Cl_2 (100 ml). The soln. was stirred for 15 min at 0° followed by 3 h at r.t. and then washed with 5% $NaHCO_3$ soln. (100 ml) and brine (2 × 100 ml), dried, and evaporated. The residual oil was chromatographed (AcOEt/CH_2Cl_2 1:1) and the purified product crystallized from Et_2O/hexane: **10** (9.31 g, 79%). White powder. ^{1}H-NMR ($CDCl_3$) 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 H_2 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 Et_2O/hexane to give **12** (3.88 g, 85%). White powder. ^{1}H-NMR (250 MHz, (D_6)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*, ArCH_2S); 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 $C_{20}H_{28}N_2O_8S \cdot 0.15\ H_2O$ (459.21): C 52.31, H 6.21, N 6.10, H_2O 0.59; found: C 52.16, H 6.30, N 6.10, H_2O (*Karl Fischer* titration) 0.56.

1.8. *Methyl (4*R*,7*S*)-7-[(* 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° Ph_3P (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/CH_2Cl_2/hexane 1:1:1) and the purified product crystallized from CH_2Cl_2/hexane **13** (0.724 g, 55%). White powder. M.p. 206° (dec.). TLC (AcOEt/hexane 1:1): R_f 0.25. $[\alpha]_D = +71.2$ (*c* = 1, MeOH). IR (KBr): 3414*m*, 3296*m*, 2978*m*, 1721*vs*, 1670*s*, 1655*s*, 1600*m*, 1517*s*. EI-MS: 382 ([*M* – CH=C(Me)$_2$]$^+$). ^{1}H-NMR (250 MHz, (D_6)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 $C_{20}H_{26}N_2O_7S$ (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° Ph_3P (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 CH_2Cl_2 and submitted to chromatographic purification (*t*-BuOMe/hexane/CH_2Cl_2 2:2:1 (→diethyl 1,2-hydrazinedicarboxylate), then 1:1:1). Crystallization from CH_2Cl_2/hexane gave **13** (0.97 g, 73%). ^{1}H-NMR ((D_6)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**). K_2CO_3 (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 H_2O (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. ^{1}H-NMR (250 MHz, $CDCl_3$; *rotamer 1/rotamer 2 ca.* 2:1): 1.34, 1.46 (2*s*, *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 (2*d*, *AB*, J_{AB} = 14, $COOCH_2$, *rot. 2*); 5.21 (*s*, $COOCH_2$, *rot. 1*); 7.27–7.44 (*m*, 4 arom. H, *rot. 1/rot. 2*). Anal. calc. for $C_{17}H_{23}NO_5$ (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. ^{1}H-NMR (250 MHz, $CDCl_3$; *rotamer 1/rotamer 2 ca.* 2:1): 1.34, 1.46 (2*s*, *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 (2*d*, *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*, $COOCH_2$, *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 Ac_2O (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. ^{1}H-NMR (250 MHz, $CDCl_3$; *rotamer 1/rotamer 2 ca.* 2:1): 1.35, 1.47 (2*s*, *t*-Bu, *rot. 1/rot. 2*); 1.70, 1.79 (2*s*, 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 (2*d*, J = 7, H–C(2), *rot. 1/rot. 2*); 5.05, 5.34 (2*d*, *AB*, J_{AB} = 12, $COOCH_2$, *rot. 2*); 5.17 (*s*, $COOCH_2$, *rot. 1*); 5.43 (*ddd*, 3 × J = 7, H–C(3), *rot. 1/rot. 2*); 7.29–7.43 (*m*, 4 arom. H, *rot. 1/rot. 2*). Anal. calc. for $C_{19}H_{25}NO_6$ (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-Acetoxy*-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, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$): 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, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$): identical to that of (±)-**15** (see *2.1*). Anal. calc. for $C_{17}H_{23}NO_5$ (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 in at r.t. in the presence of 5% Pd/C (64 mg). After a H_2 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° [1]. $[\alpha]_D$ = −100.8 (c = 1.4, CH_2Cl_2)[1]; −13.5 (c = 1.0, AcOEt). ^{1}H-NMR (250 MHz, $CDCl_3$)[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 $C_{10}H_{17}NO_5$ (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-Acetoxy*-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, CH_2Cl_2)[3]. ^{1}H-NMR (250 MHz, $CDCl_3$): 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 × J = 7, H–C(3)). Anal. calc. for $C_{12}H_{19}NO_6$ (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° [1]. $[\alpha]_D$ = +100.3 (c = 1.4, CH_2Cl_2)[1]; +12.9 (c = 1.0, AcOEt). ^{1}H-NMR ($CDCl_3$): identical to that of (−)-**14** (see *2.4*). Anal. calc. for $C_{10}H_{17}NO_5$ (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. CH_2O 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

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[1]) *Cooper et al.* [14] reported (+)-**14** with a m.p. of 101–103° and $[\alpha]_D$ = +55.5 (c = 1.39, CH_2Cl_2). 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 CH_2N_2 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 CF_3COOH (30 min, 0°) followed by ion-exchange purification (*Dowex 50W X4*, acid form, elution with H_2O and 1.3M aq. NH_3) and crystallization (H_2O/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, H_2O) ([21]: $[\alpha]_D$ = −90.3, −102.7 (c = 1, H_2O)). 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 crystals[4]) 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°. ^{1}H-NMR (250 MHz, D_2O): 1.40–1.70 (*m*, 6 H); 2.65–2.90 (*m*, 4 H); 3.84 (*s*, ArCH_2); 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, $C_5H_{10}N^+$).

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), H_2O (0.15 l), and piperidine (98.6 ml, 1.0 mol) was hydrogenated for 14 h at a H_2 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 H_2O (1 l) and then extracted with AcOEt (3 × 1.5 l). The org. layer was washed successively with 2N 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 H_2O: White solid. M.p. 245° (dec.). ([16]: 245–246° (dec.)). ^{1}H-NMR (250 MHz, (D_6)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, $[M - CO_2 - H_2]^+$), 69 (26). Anal. calc. for $C_8H_8O_4$ (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. ^{1}H-NMR (250 MHz, (D_6)DMSO): 1.58 (*m*, 6 H); 1.96 (*s*, Me); 2.81 (*m*, 4 H); 3.79 (*s*, ArCH_2); 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, $C_5H_{10}N^+$).

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 3N 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 H_2 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 H_2O (1 l) and extracted with AcOEt (3 × 2 l). The org. layer was washed with 2N 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°. ^{1}H-NMR (250 MHz, (D_6)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, $[M - CO_2 - H_2]^+$). Anal. calc. for $C_9H_{10}O_4$ (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 1N 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°. ^{1}H-NMR (250 MHz, (D_6)DMSO): 1.89 (*s*, 2 Me); 5.46 (*s*, $COOCH_2$); 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 $C_{16}H_{15}NO_6$ (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)Me_2SiCl (33.2 g, 0.22 mol) in DMF (80 ml) was added at 0° Et_3N (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 H_2O (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°. ^{1}H-NMR (250 MHz, $CDCl_3$): 0.20 (*s*, 4 MeSi); 1.00 (*s*, 2 *t*-Bu); 2.02 (*s*, 2 arom. Me); 5.43 (*s*, $COOCH_2$); 6.34 (*s*, 1 arom. H); 7.61, 8.24 (2*d*, *AB*, J_{AB} = 8.5, 4 arom. H (PNB)). Anal. calc. for $C_{28}H_{43}NO_6Si_2$ (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 CCl_4 (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 unsoluble material was floating at the surface of the soln. (50 min). The

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[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°. Unsoluble 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%). ^{1}H-NMR (250 MHz, $CDCl_3$): **26**: 0.23, 0.28 (2*s*, 2 Me_2Si); 1.00, 1.04 (2*s*, 2 *t*-BuSi); 2.03 (*s*, arom. Me); 4.51 (*s*, CH_2Br); 5.48 (*s*, $COOCH_2$); 6.36 (*s*, 1 arom. H); 7.67, 8.25 (2*d*, *AB*, J_{AB} = 8, 4 arom. H (PNB)); **27**: 0.30 (*s*, 2 Me_2Si); 1.04 (*s*, 2 *t*-BuSi); 4.49 (*s*, CH_2Br); 5.53 (*s*, $COOCH_2$); 6.39 (*s*, 1 arom. H); 7.72, 8.25 (2*d*, *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 CH_2Cl_2 (0.25 l) was added at 0° Et_3N (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. $NaHCO_3$ 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. ^{1}H-NMR (250 MHz, $CDCl_3$): 0.22, 0.24 (2*s*, 2 Me_2Si); 1.00, 1.01 (2*s*, 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 (2*d*, *AB*, J_{AB} = 12, ArCH_2S); 4.10 (*m*, 1 H–C(3)(Ser)); 4.28 (*m*, NH(Ser)); 4.73 (*m*, H–C(2)(Cys)); 5.50 (*s*, $COOCH_2$); 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 (2*d*, *AB*, J_{AB} = 8, 4 arom. H (PNB)). ISP-MS: 866.5 ($[M + H]^+$). Anal. calc. for $C_{40}H_{63}N_3O_{12}SSi_2$ (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 H_2 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. ^{1}H-NMR (250 MHz, (D_6)DMSO): 0.21, 0.23 (2*s*, 2 Me_2Si); 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*, ArCH_2S); 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 $C_{33}H_{58}N_2O_{10}SSi_2$ (731.06): C 54.22, H 8.00, N 3.83; found: C 53.85, H 7.94, N 3.65.

3.10. *Methyl (4*R,7S*)-7-[(* tert-*Butoxycarbonyl)amino]-12,14-bis[(* tert-*butyl)dimethylsilyoxy]-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° Ph_3P (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 CH_2Cl_2 and submitted to chromatographic purification (AcOEt/hexane 1:1): **30** (1.84 g, 86%). Amorphous solid. TLC (AcOEt): R_f 0.55. $[\alpha]_D$ = +58.3 (*c* = 0.7, AcOEt). ^{1}H-NMR (250 MHz, (D_6)DMSO): 0.21, 0.23 (2*s*, 2 Me_2Si); 0.98, 1.01 (2*s*, 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, N*H*Boc); 8.26 (*d*, *J* = 8, H–N(5)). ISP-MS: 730.5 ($[M + NH_4]^+$), 713.5 ($[M + H]^+$). Anal. calc. for $C_{33}H_{56}N_2O_9SSi_2$ (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-1*H*-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-1*H*-imidazole] (1.54 g, 3.9 mmol) and Ph_3P (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. ^{1}H-NMR ((D_6)DMSO): identical to that given above.

3.11. *Methyl (4*R,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 CF_3COOH (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. $NaHCO_3$ 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.) $[\alpha]_D$ = +41.4 (*c* = 1.0, AcOEt). ^{1}H-NMR (400 MHz, (D_6)DMSO): 0.21, 0.23

(2*s*, 2 Me_2Si); 0.97, 1.01 (2*s*, 2 *t*-BuSi); 1.95 (*s*, arom. Me); 2.25 (br. *s*, NH_2); 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 ($[M + H]^+$, $C_{28}H_{49}N_2O_7SSi_2^+$; calc. 613.2799).

3.12. *Methyl (4*R,7S*)-7-{[(3*R*)-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% $NaHCO_3$ 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. $[\alpha]_D$ = + 39.9 (*c* = 1.0, AcOEt). ^{1}H-NMR (400 MHz, (D_6)DMSO; *rotamer 1*/*rotamer 2 ca.* 1:1): 0.20, 0.21, 0.23, 0.24 (4*s*, 2 Me_2Si, *rot. 1*/*rot. 2*); 0.97, 1.02 (2*s*, *t*-BuSi); 1.30, 1.37 (2*s*, Boc, *rot. 1*/*rot. 2*); 1.82, 1.95 (2*m*, 2 H–C(4) (*c*Hyp)); 1.97, 1.98 (2*s*, arom. Me, *rot. 1*/*rot. 2*); 2.55 (*dd*, *J* = 14, 12, 1 H–C(3)); 3.05, 3.06 (2*dd*, *J* = 14, 4, 1 H–C(3), *rot. 1*/*rot. 2*); 3.13, 3.22 (2*m*, 1 H–C(5) (*c*Hyp), *rot. 1*/*rot. 2*); 3.45 (*d*, *J* = 10, 1 H–C(1)); 3.46 (*m*, 1 H–C(5) (*c*Hyp)); 3.61 (*s*, COOMe); 3.85 (*d*, *J* = 10, 1 H–C(1)); 3.96, 4.04 (2*dd*, *J* = 10, 2.5, 1 H–C(8), *rot. 1*/*rot. 2*); 4.42 (*m*, H–C(2) (*c*Hyp), H–C(3) (*c*Hyp)); 4.56 (*m*, H–C(7)); 4.67 (*m*, H–C(4)); 5.57, 5.59 (2*dd*, *J* = 10, 2, 1 H–C(8), *rot. 1*/*rot. 2*); 6.03, 6.09 (2*d*, *J* = 3, OH(*c*Hyp), *rot. 1*/*rot. 2*); 6.34 (*s*, 1 arom. H); 8.30, 8.34 (2*d*, *J* = 7, NH–C(7), *rot. 1*/*rot. 2*); 8.48, 8.51 (2*d*, *J* = 10, H–N(5), *rot. 1*/*rot. 2*). HR-MS (ESI): 848.3616 ($[M + Na]^+$, $C_{38}H_{63}N_3O_{11}SSi_2Na^+$; calc. 848.3620).

3.13. *Methyl (4*R,7S*)-12,14-Bis[(tert-butyl)dimethylsilyloxy]-1,3,4,5,6,7,8,10-octahydro-7-{[(3*R*)-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 Et_2O/hexane: **33** (2.58 g, 89%). White crystals. M.p. > 140° (dec.), TLC (acetone/hexane 1:1): R_f 0.10. $[\alpha]_D$ = + 12.7 (*c* = 0.7, AcOEt). ^{1}H-NMR (400 MHz, (D_6)DMSO): major rotamer (> 80%): 0.21 (*s*, Me_2Si); 0.24, 0.25 (2*s*, 2 MeSi); 0.98, 1.02 (2*s*, 2 *t*-BuSi); 1.77 (*m*, 1 H–C(4) (*c*Hyp); 1.97 (*s*, arom. Me); 1.98 (*m*, 1 H–C(4) (*c*Hyp)); 2.71 (*dd*, *J* = 14, 12, 1 H–C(3)); 2.97 (*m*, 1 H–C(5) (*c*Hyp)); 3.09 (*dd*, *J* = 14.0, 4.8, 1 H–C(3)); 3.12 (*m*, 1 H–C(5) (*c*Hyp)); 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) (*c*Hyp)); 4.01 (*dd*, *J* = 10, 2.5, 1 H–C(8)); 4.48 (*m*, H–C(3) (*c*Hyp)); 4.64 (*m*, H–C(4)); 4.74 (*m*, H–C(7)); 5.32 (*d*, OH (*c*Hyp)); 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 ($[M + H]^+$, $C_{33}H_{56}N_3O_9SSi_2^+$; calc. 726.3276).

3.14. *Methyl (4*R,7S*)-7-{{(3*R*)-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 CH_2Cl_2/hexane: **34** (1.33 g, 73%). White chrystals. M.p. > 130° (dec.). TLC (acetone/hexane 1:1): R_f 0.17. $[\alpha]_D$ = + 35.3 (*c* = 1.0, AcOEt). ^{1}H-NMR (400 MHz, (D_6)DMSO): major rotamer (*ca.* 80%): 0.20, 0.21, 0.23, 0.24 (4*s*, 4 MeSi); 0.97, 1.02 (2*s*, 2 *t*-BuSi); 1.36 (*s*, Boc); 1.91 (*m*, 1 H–C(4) (*c*Hyp)); 1.97 (*s*, arom. Me); 2.05 (*m*, 1 H–C(4) (*c*Hyp)); 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) (*c*Hyp)); 3.62 (*s*, COOMe); 3.73 (*m*, 1 H–C(5) (*c*Hyp)); 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) (*c*Hyp)); 4.54 (*m*, H–C(7)); 4.67 (*d*, *J* = 7, H–C(2) (*c*Hyp)); 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 (*c*Hyp)); 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 ($[M + Na]^+$, $C_{41}H_{68}N_4O_{13}SSi_2Na^+$; calc. 935.3940). Anal. calc. for $C_{41}H_{68}N_4O_{13}SSi_2 \cdot 0.35\ H_2O$ (919.68): C 53.54, H 7.46, N 6.10, S 3.49, H_2O 0.70; found: C 53.31, H 7.60, N 6.05, S 3.51, H_2O (*Karl Fischer* titration) 0.70.

3.15. *(4*R,7S*)-7-{{(3*R*)-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 NH_4F (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. Na_2CO_3 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). Unsoluble 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}H-NMR (400 MHz, (D_6)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 ($[M + H - Boc]^+$).

3.16. tert-*Butyl* N-{*(4*R,*7*S*)-7-*{{*(3*R*)-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-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 1N HCl (30 ml), H_2O (30 ml), sat. Na_2CO_3 soln. (30 ml), and H_2O (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}H-NMR (400 MHz, (D_6)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 ($[M + Na]^+$, $C_{35}H_{51}N_5O_{14}SNa^+$; calc. 820.3051).

3.17. *Cyclothialidine* (= N-{*(4*R,*7*S*)-1,3,4,5,6,7,8,10-Octahydro-12,14-dihydroxy-7-*{[(3*R*)-3-hydroxy-1-*L*-*seryl*-*L*-*prolyl]amino*}-*11-methyl-6,10-dioxo-9,2,5-benzoxathiazacyclododecin-4-ylcarbonyl*}-*L*-*alanine*; **1**). A soln. of **36** (638 mg, 0.8 mmol) in CF_3COOH (8 ml) was stirred at 0° for 1.75 h. The solvent was evaporated and the residue dissolved in H_2O (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.*), H_2O (→salts), then 2% aq. MeCN). The product fraction was lyophilized: **1** (220 mg, 43%). White powder. $[\alpha]_D = -12.2$ ($c = 0.5$, H_2O) ([3]: −13.2 ($c = 1$, H_2O)). UV: 293 (3.57) ([3]: 293 (3.20). IR (KBr): 3344*s* (br.), 1720*s*, 1655*vs*, 1598*s*, 1534*s*. ^{1}H-NMR (400 MHz, D_2O)[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 ($[M + Na]^+$, $C_{26}H_{35}N_5O_{12}SNa^+$; calc. 664.1901). Anal. calc. for $C_{26}H_{35}N_5O_{12}S \cdot 2.5\ H_2O$ (686.69): C 45.48, H 5.87, N 10.20, S 4.67, H_2O 6.56; found: C 45.34, H 5.93, N 10.28, S 4.34, H_2O (*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. Shimma, 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.

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[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. Macheboeuf, *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.