# New Antibacterial Agents Derived from the DNA Gyrase Inhibitor Cyclothialidine

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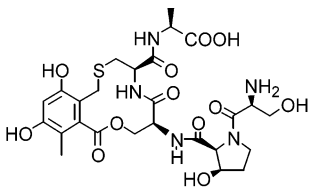
Cyclothialidine (**1**, Ro 09-1437) is a potent DNA gyrase inhibitor that was isolated from *Streptomyces filipinensis* NR0484 and is a member of a new family of natural products. It acts by competitively inhibiting the ATPase activity exerted by the B subunit of DNA gyrase but barely exhibits any growth inhibitory activity against intact bacterial cells, presumably due to insufficient permeation of the cytoplasmic membrane. To explore the antibacterial potential of **1**, we developed a flexible synthetic route allowing for the systematic modification of its structure. From a first set of analogues, structure−activity relationships (SAR) were established for different substitution patterns, and the 14-hydroxylated, bicyclic core (**X**) of **1** seemed to be the structural prerequisite for DNA gyrase inhibitory activity. The variation of the lactone ring size, however, revealed that activity can be found among 11- to 16-membered lactones, and even seco-analogues were shown to maintain some enzyme inhibitory properties, thereby reducing the minimal structural requirements to a rather simple, hydroxylated benzyl sulfide (**XI**). On the basis of these "minimal structures" a modification program afforded a number of inhibitors that showed in vitro activity against Gram-positive bacteria. The best activities were displayed by 14-membered lactones, and representatives of this subclass exhibit excellent and broad in vitro antibacterial activity against Gram-positive pathogens, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *and Enterococcus faecalis*, and overcome resistance against clinically used drugs. By improving the pharmacokinetic properties of the most active compounds (**94**, **97**), in particular by lowering their lipophilic properties, we were able to identify congeners of cyclothialidine (**1**) that showed efficacy in vivo.

## Introduction

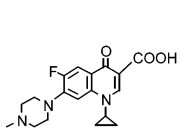
The emergence of bacterial resistance to most of the antibacterials used clinically represents a major challenge in today's antibiotic research, outweighing the task to develop drugs of higher potency, expanded spectrum of activity, and improved safety profile. In particular the spread of multiresistant Gram-positive bacteria during the last 2 decades is of major concern in the hospital environment, where the glycopeptides vancomycin and teicoplanin are considered as the last-resort drugs against methicillin-resistant *Staphylococcus aureus* (MRSA).[1] First reports on vancomycin-resistant *S. aureus*,[2] however, represent a serious threat to the therapy of nosocomial infections, which at present are mainly caused by MRSA and other highly resistant Gram-positive bacteria. Therefore, the search for new structural entities and novel targets of attack as a means to overcome bacterial resistance continues to be an important research area.

The identification of the bacterial type II topoisomerase DNA gyrase[3] as the biological target of the quinolones[4] and of the coumarin antibiotics[5] aroused a general interest in inhibitors of this enzyme as potential antibacterial drugs. DNA gyrase is an $A_2B_2$ holoenzyme that mediates various reactions essential for the bacterial cell, e.g. the introduction of negative superhelical turns into bacterias circular DNA.[6,7] The quinolones, e.g. ciprofloxacin (**2**), are DNA gyrase subunit A inhibitors that directly interfere with the processes between DNA and the gyrase enzyme.[4] This class of antibacterials has gained a strong position in the therapy of bacterial infections. Its use, however, might be hampered in the future by resistance development and undesired side

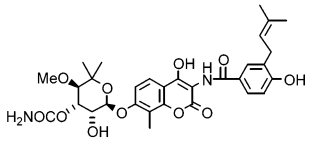
**Chart 1**



Cyclothialidine (**1**, RO 09-1437)



Ciprofloxacin (**2**)



Novobiocin (**3**)

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effects.[7] The coumarin antibiotics, such as novobiocin (**3**) and coumermycin, competitively inhibit the ATPase activity conferred by the DNA gyrase B subunit and thereby abolish the energy-dependent reactions catalyzed by DNA gyrase.[5] Rapid emergence of resistance as well as poor tolerability has restricted or even prevented their clinical use.[8] Therefore, the B subunit of DNA gyrase represents a presently unexploited target for antibacterial attack.

Nalidixic acid,[9] the progenitor of the quinolones, as well as the coumarin antibiotics,[10] had been discovered by the traditional search method relying on the growth inhibition of bacterial cultures. Target-based assays, e.g. enzyme inhibition assays, have been used less frequently for the identification of a new antibacterial principle but are playing an increasingly important role.[11] They have the advantage of detecting a potential antibacterial uncoupled from its ability to cross the bacterial membrane. The possibility to study DNA gyrase in vitro[12] provided a powerful tool in the search for new inhibitors of this enzyme, and the discovery of cyclothialidine (**1**, Ro 09-1437, Chart 1) represents an early example of this target-specific drug-finding technique.[13−15]

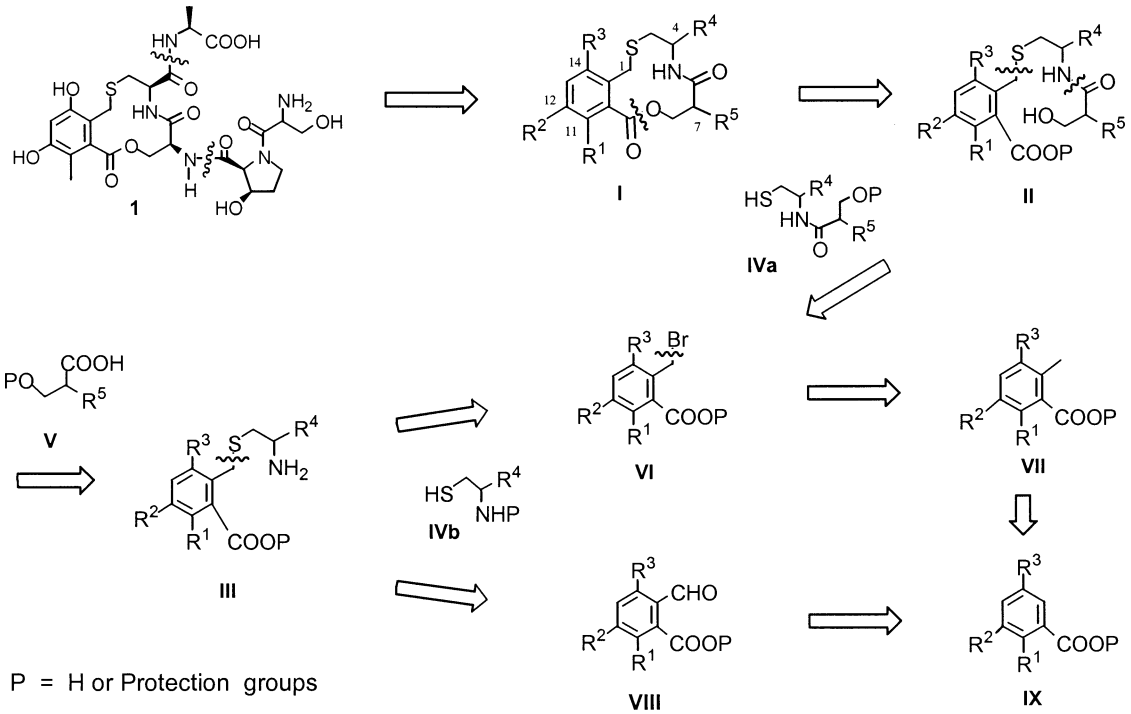
Cyclothialidine was found by screening microbial broths for the in vitro inhibition of the supercoiling activity of DNA gyrase and was eventually isolated in low yield from the fermentation broth of *Streptomyces filipinensis* NR0484.[13] The structure of **1** features a 12-membered lactone ring that is fused to a highly substituted benzene ring and partly incorporated in a pentapeptide chain.[14] Cyclothialidine was found to be a potent and selective inhibitor of DNA gyrase and its mode of action was shown to be inhibition of the ATPase activity exerted by the B subunit of DNA gyrase.[15] The fact that **1** barely exhibits any growth-inhibitory activity against intact bacterial cells was tentatively explained by poor penetration of the bacteria's cytoplasmic membrane[13]—a major drawback shared also by other natural congeners of **1** isolated subsequently.[16] Nevertheless, the compound was considered to be a promising lead structure whose modification might open up a route to a new class of antibacterials.

We have reported part of our approach in this task in preliminary form,[17−19] and herein, a more comprehensive account of this work is given. To explore and exploit the antibacterial potential of **1**, we applied a strategy addressing in a more or less consecutive manner the following tasks: (i) development of a flexible synthetic route allowing the preparation of many analogues of **1**, (ii) investigation of the structural requirements for DNA gyrase inhibitory activity, (iii) lead optimization toward in vitro antibacterial activity, and (iv) optimization for in vivo efficacy.

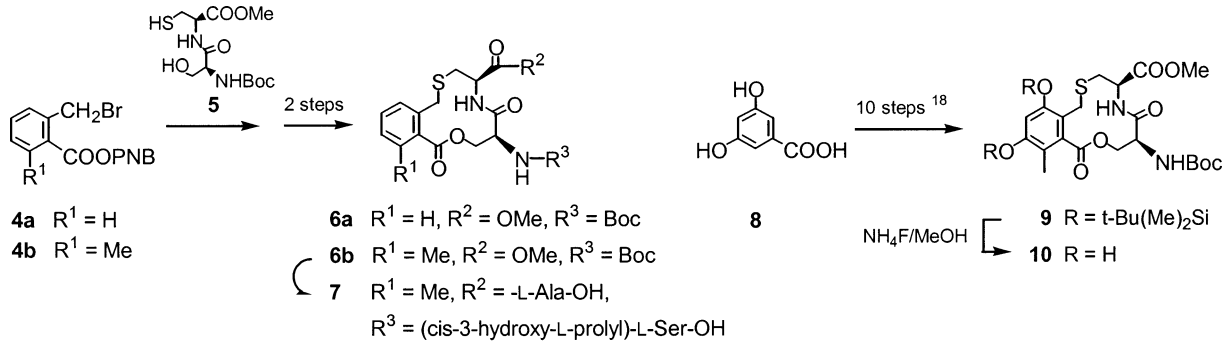
## Chemistry

As the limited amount of the natural product available did not allow for an efficient chemical modification program, we embarked on a total synthetic approach. To investigate the role of the substituents both on the lactone as well as on the benzene ring, preparation of a number of analogues **I** retaining the bicyclic core of **1** (Scheme 1) was envisioned. According to the retrosynthetic analysis of **1**, a classical lactonization of $\omega$-hydroxy acid **II** (P = H) is used for the formation of the macrocycle.[18] Disconnection of the peptidic side chain—stepwise or all at once—leads back to a benzylating agent, such as benzyl bromide **VI** or benzaldehyde **VIII**. Bromide **VI** prepared by benzylic bromination of *o*-toluate **VII** is employed to alkylate cysteine derivative **IVa** or **IVb** to afford thioether **II** or **III** (P = protecting group), respectively. The latter is converted to **II** by amidation with carboxylic acid **V**. Alternatively, thioether **III** is produced by reacting aldehyde **VIII** with thiol **IVb** in a reductive thiolation.[17b,20,21] Benzoic acid derivative **IX** serves as common starting material for

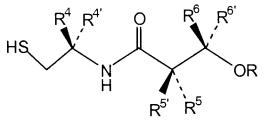
**Scheme 1**



**Scheme 2**




**Scheme 3.**[a] Preparation of Dipeptidic Building Blocks



| | | | | Cpnd | $R^4$ | $R^{4'}$ | $R^5$ | $R^{5'}$ | $R^6$ | $R^{6'}$ | R |
|---|---|---|---|---|---|---|---|---|---|---|---|
| L-Cys-OMe·HCl | + | Boc-L-Ser-OH | a → | **5** [18] | COOMe | H | NHBoc | H | H | H | H |
| $HSCH_2CH_2NH_2$·HCl | + | Boc-L-Ser-OH | a → | **11** | H | H | NHBoc | H | H | H | H |
| L-Cys-OMe·HCl | + | $Ph_3COCH_2CH_2COOH$ | b → | **12** | COOMe | H | H | H | H | H | $CPh_3$ |
| $HSCH_2CH_2NH_2$·HCl | + | $Ph_3COCH_2CH_2COOH$ | a → | **13** | COOMe | H | H | H | H | H | $CPh_3$ |
| D-Cys-OMe·HCl | + | Boc-D-Ser-OH | a → | **14** | H | COOMe | H | NHBoc | H | H | H |
| L-Cys-OMe·HCl | + | Boc-D-Ser-OH | a → | **15** | COOMe | H | H | NHBoc | H | H | H |
| D-Cys-OMe·HCl | + | Boc-L-Ser-OH | b → | **16** | H | COOMe | NHBoc | H | H | H | H |
| L-Cys-OMe·HCl | + | (*S*)-$CH_3CH(OH)CH_2COOH$ | a → | **17** | COOMe | H | H | H | H | Me | H |
| L-Cys-OMe·HCl | + | (*R*)-$CH_3CH(OH)CH_2COOH$ | b → | **18** | COOMe | H | H | H | Me | H | H |

[a] Reagents: (a) DCC, *N*-methylmorpholine, MeCN; (b) EDC, *N*-methylmorpholine, MeCN.

**VI** and **VIII**. Introduction of a methyl group affords **VII**,[18] whereas Vielsmeier formylation of **IX** gives aldehyde **VIII**. Furthermore, this synthetic scheme provides the option to modify the lactone core of **1** by variation of building block **V**. The reductive thiolation route proved to be particularly valuable for the regiospecific preparation of compounds having more complex aromatic substitution patterns.

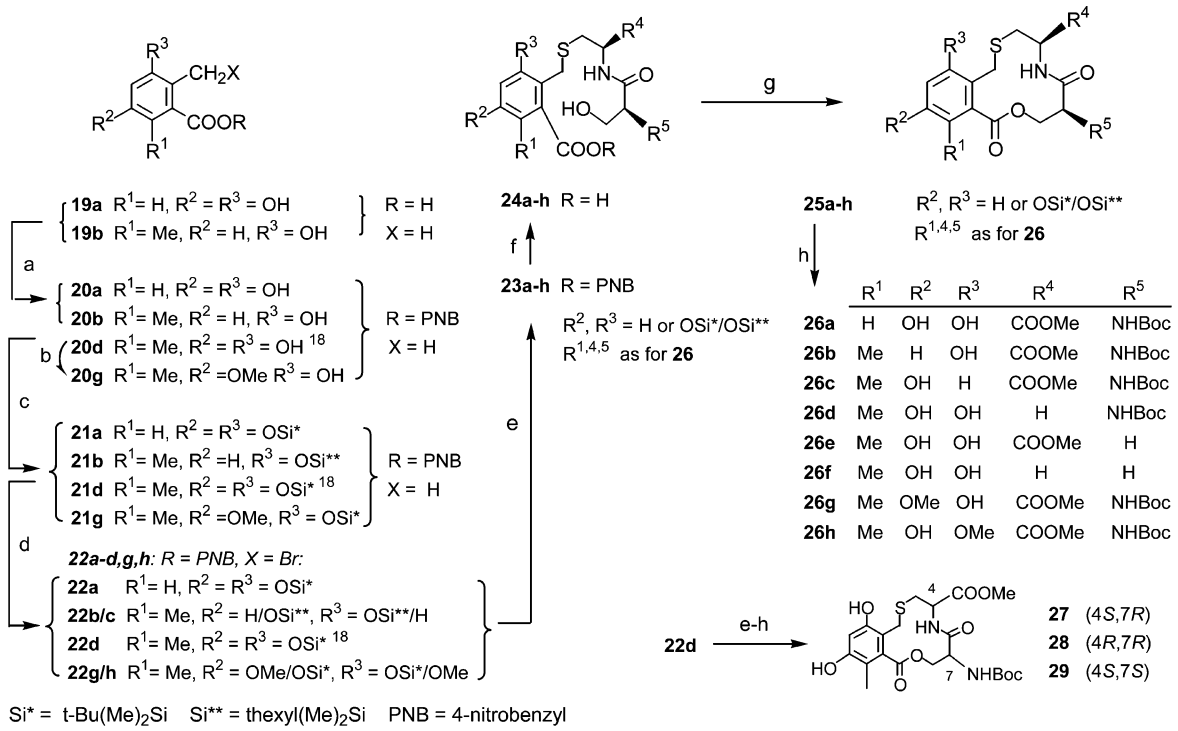
Our exploratory synthesis of **6a**[18] was adapted for the preparation of the 11-methyl analogue **6b** (Scheme 2). Starting from **6b**, the pentapeptide chain of the lead compound was completed by applying the methodology used later on for the total synthesis of **1**[18] to afford 12,14-deoxycyclothialidine (**7**). The first derivative bearing the original aromatic substitution pattern, **10**, was obtained by fluoride-catalyzed cleavage of the silyl protecting groups of the intermediate **9**, of which the synthesis from resorcilic acid (**8**) was previously described.[18]

The peptidic building blocks **11**−**18** were prepared, similarly to **5**,[18] by reaction of cysteine congeners with suitably substituted and protected 3-hydroxypropionic acids, using either DCC or EDC as coupling auxiliary (Scheme 3).

In Scheme 4 the syntheses of **26a**−**h** and **27**−**29**, analogues of **10** bearing different substitution patterns, are outlined. These compounds were prepared in a manner similar to the procedure applied for the synthesis of **10**. Thus, for 11-nor-analogue **26a**, 2-methylresorcilic acid (**19a**)[18] was converted to 4-nitrobenzyl (PNB) ester **20a**, and the phenol groups were protected as silyl ethers. The ester **21a** was subjected to bromination with *N*-bromosuccinimide (NBS). The resulting benzyl bromide **22a** was coupled with **5** in the presence of $Et_3N$, PNB ester **23a** was subsequently cleaved by hydrogenolysis, and the resulting hydroxy acid **24a** was cyclized to lactone **25a** using Mitsunobu conditions.[22] Cleavage of the silyl protecting groups afforded **26a**.

For the preparation of the monohydroxy derivatives **26b** and **26c**, 3-hydroxy-2,6-dimethylbenzoic acid[23] was converted to **21b**, which was subsequently brominated with NBS. The crude mixture of the two regioisomeric monobromides **22b/c** was reacted with thiol **5**, and thioethers **23b** and **23c** were separated by chromatography. Corroboration of the structures of the components of the mixture **22b/c** as well as that of thioethers **23b** and **23c** was based on NOE experiments. The isomers displaying a NOE between an aromatic hydrogen and the methyl singlet were assigned to structures **22b** and **23b**, whereas the isomers lacking this NOE were assigned to structures **22c** and **23c**, respectively. The thioethers **23b** and **23c** were processed further to **26b** and **26c**, respectively, in analogy to the synthesis of **26a**. Replacing in this scheme thiol **5** by thiols **11**−**13** afforded the target compounds **26d**−**f**, respectively. For the preparation of the regioisomeric monomethoxy derivatives **26g** and **26h**, **20d** was consecutively monomethylated and silylated, and the intermediate **21g** was subjected to NBS bromination. The crude bromination product (**22g/h**), containing approximately 32% and 36%

**Scheme 4**[a]




[a] Reagents and conditions: (a) 4-nitrobenzyl bromide, *N,N,N′,N′*-tetramethylguanidine, DMF, 20 °C; (b) MeI, $K_2CO_3$, acetone, 60 °C, 16 h; (c) t-Bu(Me)$_2$SiCl or thexyl(Me)$_2$SiCl, $Et_3N$, DMF; (d) NBS, $CCl_4$, 90 °C, *hν*; (e) (i) **5**, **11**−**16**, respectively, $Et_3N$, DCM, 20 °C, (ii) (for **25e**,**f**) pTsOH, MeOH, 60 °C, 1 h; (f) $H_2$, Pd−C, EtOAc; (g) DEAD, $PPh_3$, THF, 20 °C; (h) $NH_4F$, MeOH.

of the regioisomeric bromides **22g** and **22h**, respectively, was reacted with **5**. The mixture of the two resulting thioethers **23g** and **23h** was separated by chromatography, and the two methoxy series were individually processed further to afford the corresponding lactones **26g** and **26h**, respectively. The regiochemical assignment in this series was based on $^1H-^{13}C$ HMBC coupling experiments (**26g** and **26h**) and was eventually confirmed by an X-ray structure of **25g**.[24]

The stereoisomers **27**−**29** were prepared analogously to the parent compound **10** but with replacing intermediate **5** by thiols **14**−**16**, respectively.

The two isomeric 8-methyl derivatives **32** and **33** were obtained similarly by reacting the mixture **22g/h** with dipeptides **17** and **18**, respectively (Scheme 5). In these cases, the ring-closing step using Mitsunobu conditions proceeded with inversion of the configuration at C(8). The chiral integrity of the purified products was indicated by their NMR spectra lacking the signals of possible diastereoisomeric products.

Lactones **43** and **47** and the corresponding 7-(Boc-L-serylamino) derivatives **45** and **48** were prepared starting from hydroxy acid **34** and nitro acid **37**, respectively. In these syntheses, the carboxylic acid function was protected as a silyl ester during the benzylic bromination reaction.[25]

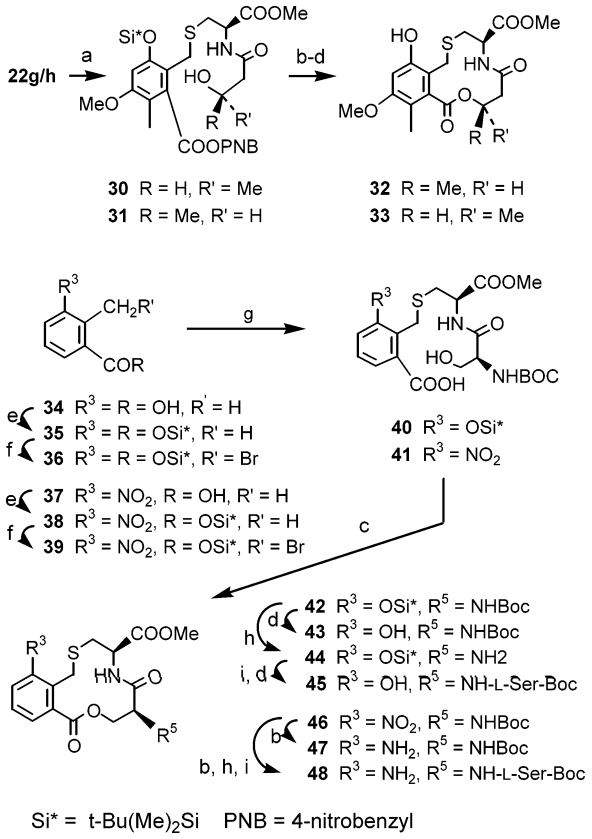
Derivatives **49**−**52** and **54**−**61** were prepared by side-chain modifications of compounds **25g**, **26g**, **50**, **10**, and **53**,[18] as outlined in Scheme 6.

To prepare derivatives of the most active 12-methoxy series in a more efficient way, we developed a regiospecific synthesis relying on benzaldehyde **67** as starting point for the elaboration of the sulfanyl side chain (Scheme 7). Thus, the synthesis of the key intermediates **76**, **77**, and **78** was achieved by benzylation of thiols L-Cys-OMe, **70**, or **75** in a reductive thiolation reaction of **67**, using triethylsilane in trifluoroacetic acid/dichloromethane.[17b,20,21] As this new route no longer required a NBS bromination step, it was possible to replace the PNB ester by an allyl ester, which later on could be cleaved more easily with Pd(0) catalysis.

For the preparation of the aldehyde **67**, 2-methylresorcilic acid derivative **62**[26] was subjected to Vielsmeyer formylation, and the 3-methoxy group in the resulting aldehyde **63** was regiospecifically cleaved due to the assistance of the neighboring formyl group.[27] Methyl ester **64** was converted to allyl ester **67** by consecutive saponification, alkylative esterification of the resulting pseudoacid **65** with allyl bromide, and silylation of the phenol group. For the preparation of the thiol **70**, L-Boc-cystine was coupled with 2 equiv of acetamide oxime, and the resulting condensation product was then thermally cyclized to the bis-3-methyloxadiazole **69**.[28] The disulfide function was finally reduced with tributylphosphine in 2,2,2-trifluoroethanol. For the preparation of **75**, 2-aminoacetonitrile (**71**) was converted to the amidoxime **73**, which thereafter was transformed to the oxadiazole **75** as described for **70**.

For the syntheses of the homologous lactones **86a**−**g** (Scheme 8), amine **76** was reacted in an EDC-mediated coupling reaction with carboxylic acids **82a**−**g**, which had been prepared by tritylation of methyl 2-hydroxyacetate followed by saponification (**82a**[29]) or by monotritylation of diols **79b**−**g** followed by two sequential oxidation reactions (**82b**−**g**). The trityl protecting group in the primary amidation products was cleaved off with pTsOH/MeOH. The resulting allyl esters **83a**−**g** were converted by Pd(0)-catalyzed ester cleavage to the corresponding hydroxy acids **84a**−**g**, which were subsequently cyclized to the lactones **85a**−**g** under Mit-

**Scheme 5**[a]




[a] Reagents and conditions: (a) **17** or **18**, respectively, $Et_3N$, DCM, 20 °C; (b) $H_2$, Pd−C, EtOAc; (c) DEAD, $PPh_3$, THF, 20 °C; (d) $NH_4F$, MeOH; (e) t-Bu(Me)$_2$SiCl, $Et_3N$ or imidazole, DMF; (f) NBS, $CCl_4$, 90 °C, *hν*; (g) **5**, $Et_3N$, DCM, 20 °C; (h) TFA; (i) Boc-L-Ser-OH, EDC, MeCN, 0 °C.

sunobu conditions. Cleaving of the silyl protecting group afforded the target lactones **86a**−**g.** The corresponding thioamides **87a**−**g** were obtained by heating **85a**−**g** with Lawesson's reagent in toluene and subsequent cleavage of the silyl protecting group.

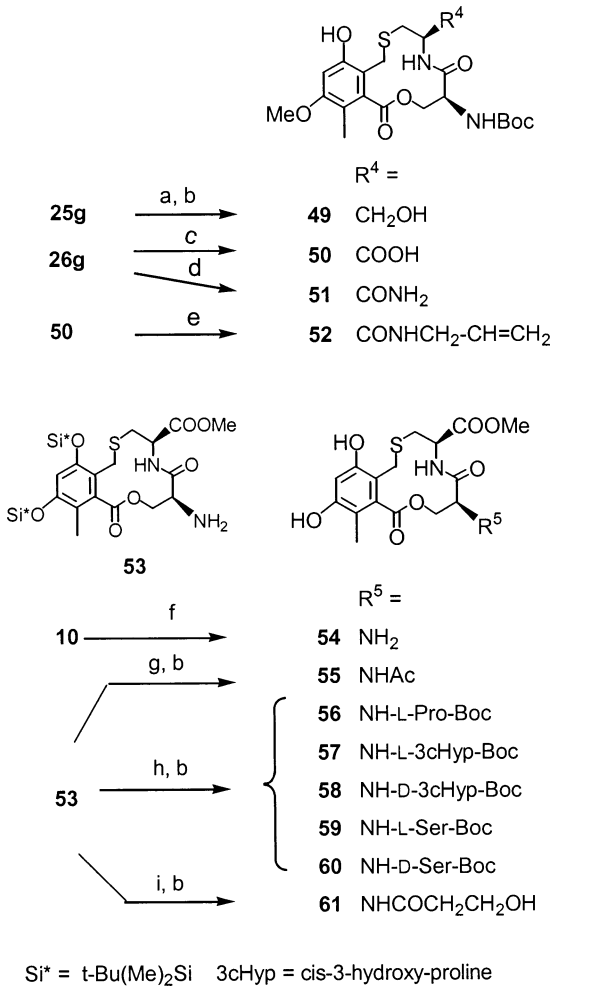
The seco-derivatives **90** and **91** were obtained by reductive thiolation of aldehyde **88** with *N*-Ac-L-Cys-OMe. Cleavage of the silyl group afforded the thioether **90**, whereas the preceding treatment of **89** with Lawesson's reagent followed by cleavage of the silyl ether gave **91**.

For the preparation of **93**, **94**, **96**, and **97**, the amine **77** was amidated with **82b** or **82d**, respectively. The trityl ethers were cleaved and the resulting products processed following the standard route used for the syntheses of **86b**,**d** and **87b**,**d**.

For the synthesis of **103** (Scheme 9), intermediate **77** was amidated with (*R*)-2-hydroxysuccinic acid-1-methyl ester.[30] After cleavage of the allyl ester, hydroxy acid **100** was cyclized under Mitsunobu conditions with concomitant inversion of the configuration at C(8). Treatment of the lactone **101** with Lawesson's reagent provided thioamide **102**. Reduction of the methyl ester function with $NaBH_4$ in MeOH followed by cleavage of the silyl protecting group afforded **103**.

For the syntheses of the homologous hydroxymethyl lactones **110** and **111**, the amine **77** was amidated with carboxylic acids **105a**, prepared by consecutive hydrogenation and saponification of **104**,[31] and with **105b**,[45]

**Scheme 6**[a]




[a] Reagents and conditions: (a) $NaBH_4$, MeOH, THF, 0 °C; (b) $NH_4F$, MeOH; (c) aq NaOH, THF; (d) $NH_3$, MeOH, 20 °C; (e) *N*-hydroxysuccinimide, EDC, MeCN, then allylamine; (f) TFA; (g) $Ac_2O$, 60 °C; (h) Boc-L-Pro-OH, Boc-L-3cHyp-OH, BOC-D-3cHyp-OH, BOC-L-Ser-OH, or BOC-D-Ser-OH, respectively, EDC, MeCN, 0 °C; (i) (i) $(Ph_3)_3COCH_2CH_2COOH$, EDC, MeCN, (ii) pTsOH, MeOH.

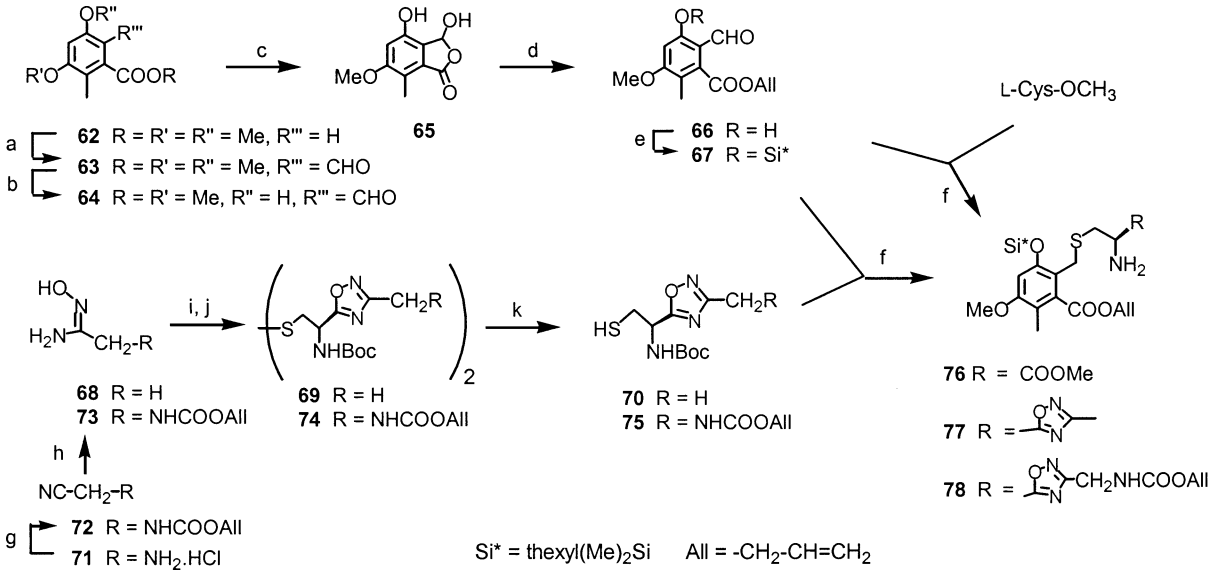
respectively. The acetonide group in the resulting products **106a**,**b** was hydrolyzed, and the primary alcohol of diols **107a**,**b** was protected as the trityl ether (**108a**,**b**). Cleavage of the allyl ester, lactonization, and cleavage of the protecting groups afforded the derivatives **110** and **111**, respectively.

For the preparation of compounds **114**−**118**, the amine **78** was converted to the protected thiolactam **112** in analogy to the synthesis of **97**. As cleavage of the allyl ester occurred under concomitant scission of the *N*-allyloxycarbonyl protecting group, we used $Pd(PPh_3)_4$ in the presence of trimethylsilylamine and trimethylsilyl trifluoroacetate[33] in order to prevent allylation of the unmasked aminomethyl group. The *N*-protecting group was subsequently reintroduced with allyl chloroformate under Schotten−Baumann conditions.

Subjecting **112** to the latter allyl cleavage protocol gave **113**, and subsequent removal of the silyl group afforded the aminomethyl derivative **114**, which was isolated as the hydrochloride salt.

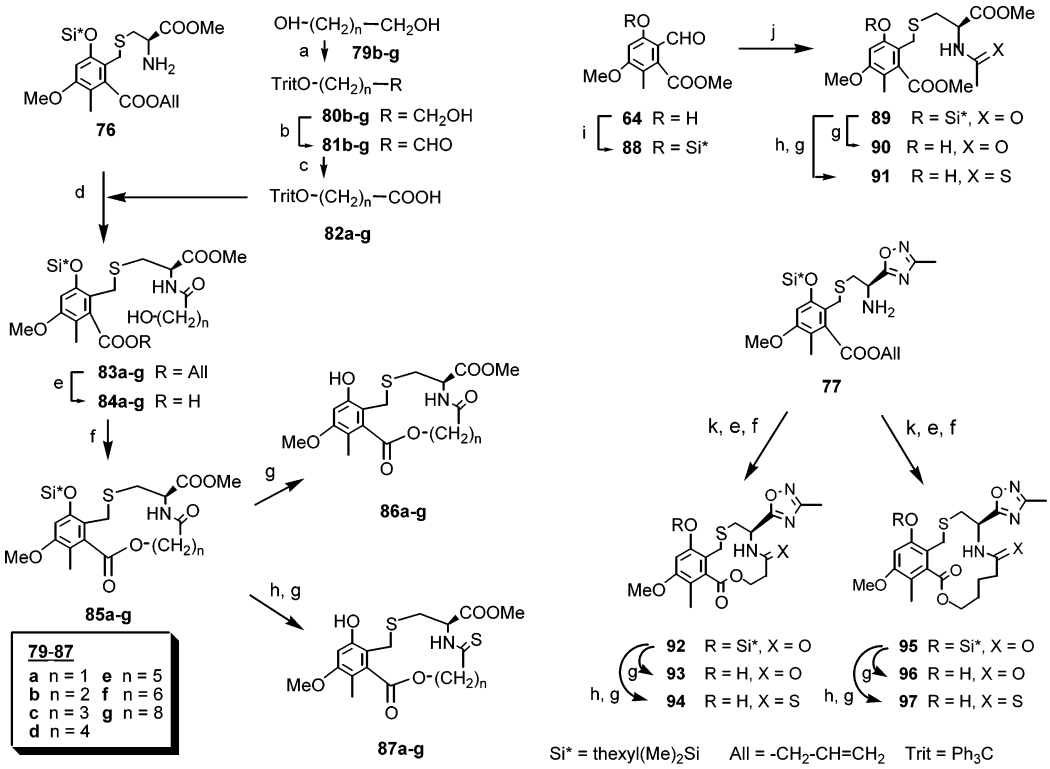
By acylating **113** with acetic anhydride or *N*-allyloxycarbonyl-L-Ala-OH[34] and subsequently cleaving of the

**Scheme 7**[a]




[a] Reagents and conditions: (a) DMF, $POCl_3$, DCM, 72 h, 40 °C; (b) $BCl_3$, DCM, 5 °C, 40 min; (c) 2.5 N KOH; (d) $CH_2{=}CHCH_2Br$, *N,N,N′,N′*-tetramethylguanidine, DMF; (e) thexyl(Me)$_2$SiCl, $Et_3N$, DMF; (f) $Et_3SiH$, TFA, DCM, 0 °C; (g) $ClCOOCH_2CH{=}CH_2$, $Et_3N$, MeCN; (h) $(NH_2OH)_2{\cdot}H_2SO_4$, NaOH, MeOH; (i) (L-Boc-Cys-)$_2$, 1-hydroxypyridin-2(1*H*)-one, DCC, DMF; (j) toluene (**69**) or dioxane (**74**), 100 °C, 7 h; (k) $PBu_3$, $CF_3CH_2OH$, $H_2O$, 0 °C.

**Scheme 8**[a]



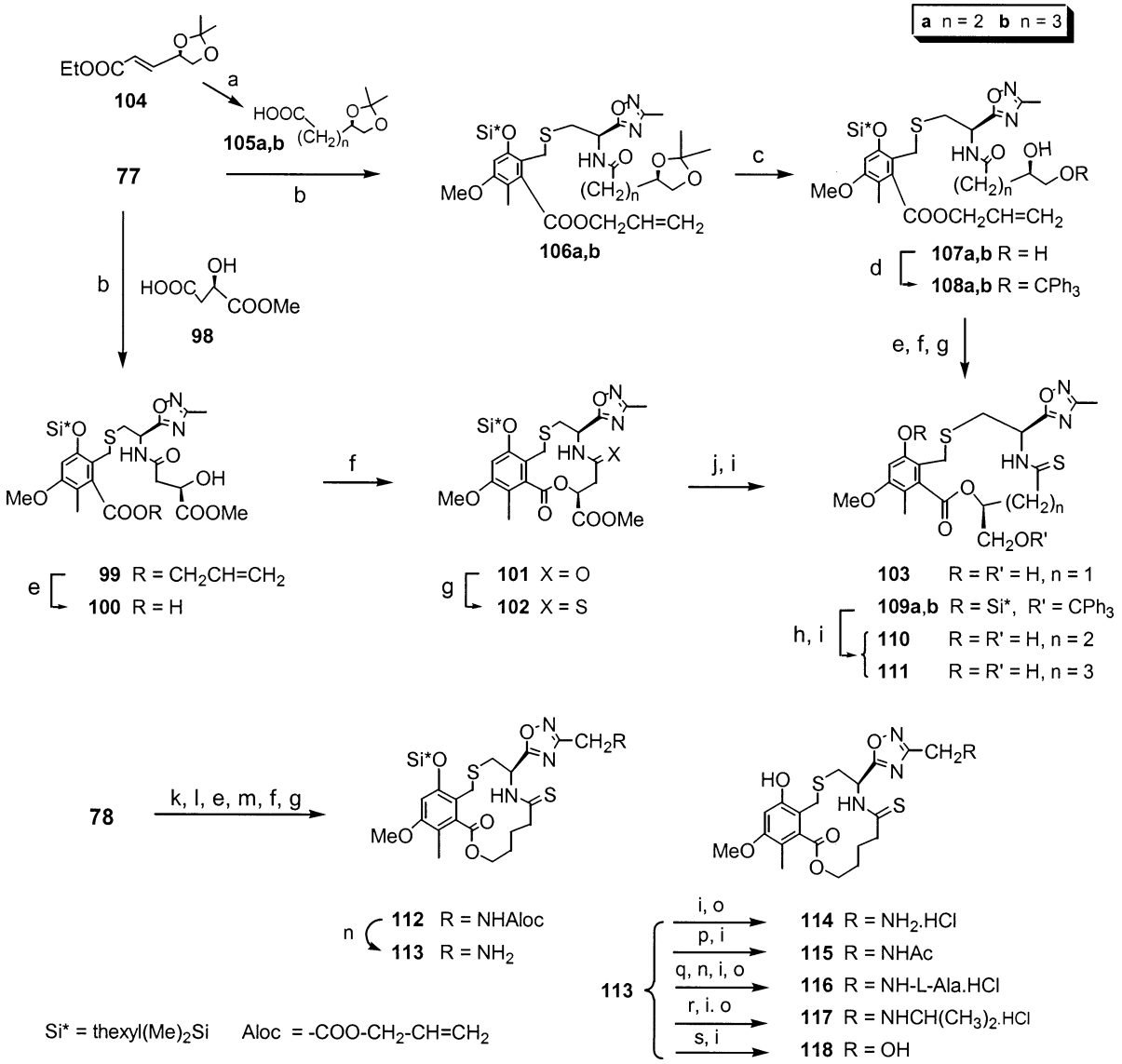

[a] Reagents and conditions: (a) $Ph_3CCl$, pyridine; (b) $(COCl)_2$, DMSO, DCM, −70 °C, then $Et_3N$; (c) $KMnO_4$, aq acetone; (d) (i) EDC, MeCN, 0 °C, (ii) pTsOH, MeOH, 20 °C; (e) $Pd(PPh_3)_4$, morpholine, THF, 0 °C; (f) DEAD, $PPh_3$, THF, 20 °C; (g) $NH_4F$, MeOH; (h) Lawesson's reagent, toluene, 80 °C; (i) thexyl(Me)$_2$SiCl, $Et_3N$, DMF; (j) Ac-L-Cys-OMe, $Et_3SiH$, TFA, DCM, 0 °C; (k) **82b** or **82d**, respectively, with conditions (d).

protecting groups, the derivatives **115** and **116**, respectively, were obtained. Reductive amination of **113** with acetone/$NaBH_4$ followed by deprotection of the phenol group afforded the isopropylamino derivative **117**. Nitrosation of **113** using $NaNO_2$ in 25% aqueous AcOH produced a product mixture, from which, after deprotection, the hydroxymethyl derivative **118** was isolated.

## Biological Results and Discussion

**DNA Gyrase Inhibition.** The DNA gyrase inhibitory activity of all target compounds was assessed in an in vitro supercoiling assay in which the introduction of superhelical turns into a relaxed plasmid was determined by gel electrophoresis.[12,35] For practical reasons, i.e., to allow more efficiency in the evaluation of new

**Scheme 9**[a]

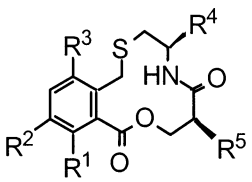



[a] Reagents and conditions: (a) (i) $H_2$, Pd−C, EtOAc, (ii) KOH, MeOH, 40 °C; (b) EDC, MeCN, 0 °C; (c) 80% AcOH; (d) $Ph_3CCl$, pyridine; (e) $Pd(PPh_3)_4$, morpholine (for **112**: TMS−morpholine, TMSOAc), THF, 0 °C; (f) DEAD, $PPh_3$, THF, 20 °C; (g) Lawesson's reagent, toluene, 80 °C; (h) pTsOH, MeOH, 60 °C; (i) $NH_4F$, MeOH; (j) $NaBH_4$, MeOH; (k) **82d**, EDC, $CH_3CN$, 0 °C; (l) pTsOH, MeOH, 20 °C; (m) $ClCOOCH_2CHCH_2$, *N*-methylmorpholine, DCM; (n) $Pd(PPh_3)_4$, TMS-DMA, TFA-TMS, DCM; (o) HCl; (p) $Ac_2O$, pyridine; (q) *N*-Aloc-L-Ala-OH, EDC, MeCN, 0 °C; (r) $NaBH_4$, acetone, aq AcOH, NaOAc, 0 °C; (s) $NaNO_2$, 25% aq AcOH.

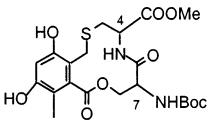
derivatives, a MNEC (maximum noneffective concentration) value was determined. In general, the MNEC value of a compound was 3−5 times lower than the $IC_{50}$, and 10−20 times lower than the concentration needed for complete inhibition of the supercoiling reaction.

According to our strategy, we focused our initial efforts on revealing the active principle of the lead structure, thereby depending on a total synthetic approach. Although the pentapeptide entity was initially considered also as a synthetic target, our main effort aimed at simpler derivatives **I** retaining the bicyclic lactone core and probing the importance of the various substituents of **1** (Table 1). Our first synthetic targets, lactones **6a** and **6b**, were found to be inactive in the supercoiling assay, indicating that either the phenolic hydroxy groups and/or the pentapeptide chain were important for activity. Starting from **6b**, we then completed the pentapeptide entity of **1**. The fact that 12,14-dideoxycyclothialidine (**7**) was devoid of DNA gyrase inhibitory activity was a clear-cut confirmation that the phenolic groups of **1** were essential, and thereafter we chose to remain with the original aromatic substitution pattern. With the derivative **10**, an analogue of **1** featuring truncated peptidic side chains, we obtained for the first time an active compound, demonstrating the crucial role of the phenolic hydroxy groups. The next investigation addressed the specific role of the different substituents of the bicyclic core. To this end, the five substituents of **10** were individually disconnected by preparing the five tetrasubstituted derivatives **26a**−**e**. It turned out that, with the exception of the 14-hydroxy group, all the other substituents of **10** could be omitted without significantly impairing the biological activity, i.e., only the 14-deoxyderivative **26c** was found to be inactive. A similar bias was also observed for the two regioisomeric methoxy derivatives **26g** and **26h**. Whereas **26g** retained the activity of **10**, the 14-methoxy isomer **26h** was found to be inactive. On the basis of

**Table 1.** DNA Gyrase Inhibition (MNEC) and in Vitro Antibacterial Activity (MIC) of Selected Compounds: Variation of $R^1$–$R^5$



**10, 27-29:**



| compd | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | configuration at C(4) | configuration at C(7) | MNEC[a] ($\mu$g/mL) | MIC[b] ($\mu$g/mL) *N. meningitidis* 69480 | MIC[b] ($\mu$g/mL) *M. luteus* ATCC8340 |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** (cyclothialidine) | | | | | | | | 0.05 | 128 | 128 |
| **6a** | H | H | H | COOMe | NHBoc | | | >1 | nd[c] | nd |
| **6b** | Me | H | H | COOMe | NHBoc | | | >1 | nd | nd |
| **7** (12,14-dideoxy-**1**) | | | | | | | | >100 | nd | nd |
| **10** | Me | OH | OH | COOMe | NHBoc | R | S | 0.2 | 16 | 16 |
| **26a** | H | OH | OH | COOMe | NHBoc | | | 0.5 | 128 | 128 |
| **26b** | Me | H | OH | COOMe | NHBoc | | | 1 | nd | 16 |
| **26c** | Me | OH | H | COOMe | NHBoc | | | >100 | nd | >128 |
| **26d** | Me | OH | OH | H | NHBoc | | | 1 | nd | 32 |
| **26e** | Me | OH | OH | COOMe | H | | | 0.25 | 32 | 16 |
| **26g** | Me | OMe | OH | COOMe | NHBoc | | | 0.2 | 16 | 16 |
| **26h** | Me | OH | OMe | COOMe | NHBoc | | | >1 | 128 | >128 |
| **26f** | Me | OH | OH | H | H | | | 20 | nd | >128 |
| **43** | H | H | OH | COOMe | NHBoc | | | >1 | nd | >128 |
| **45** | H | H | OH | COOMe | NH-L-Ser-Boc | | | 0.4 | 32 | 64 |
| **47** | H | H | NH2 | COOMe | NHBoc | | | >1 | nd | >128 |
| **48** | H | H | NH2 | COOMe | NH-L-Ser-Boc | | | >100 | >128 | >128 |
| **27** | Me | OH | OH | COOMe | NHBoc | S | R | 10 | 128 | 128 |
| **28** | Me | OH | OH | COOMe | NHBoc | R | R | 1 | 32 | 128 |
| **29** | Me | OH | OH | COOMe | NHBoc | S | S | 4 | 128 | 128 |

[a] Supercoiling assay, *E. coli* gyrase, MNEC = maximum noneffective concentration. [b] Agar dilution (BB2 medium supplemented with 1% Isovitalex, 7.5% sheep blood, and menadione). Inoculum $10^4$ CFU/spot, MIC = minimum inhibitory concentration. [c] Not determined.

these results, the 14-hydroxylated, bicyclic lactone **X** was identified as the partial structure common to all derivatives found to be active (Figure 1).

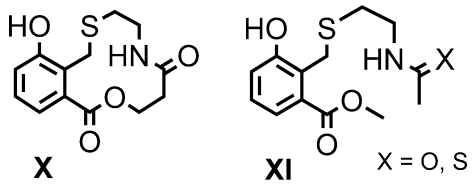



**Figure 1.** Minimal structural requirements for DNA gyrase inhibitory activity.

However, the other substituents contribute to the inhibitory activity. Thus, on removing both $R^4$ and $R^5$, the resulting compound **26f** showed only borderline activity. Removal of the 11-methyl ($R^1$) and of the 12-hydroxy group ($R^2$) led in **43** to a loss of activity, which remarkably was recovered in the 7-(Boc-L-seryl-amino) derivative **45**.

The observation that either of the two substituents at the macrocycle could be removed without significant loss of activity had raised the question whether the entire lactone ring was important for recognition by the enzyme. The answer was provided by the finding that **27**, the enantiomer of **10**, was merely inactive, emphasizing the importance of the three-dimensional shape of the lactone. The role of the stereochemistry at positions 4 and 7 was further probed by preparing the diastereoisomers **28** and **29**. Whereas at C(7) *S*- and *R*-configuration is tolerated, the data obtained indicates that in particular the *R*-configuration at C(4) is essential for optimal activity. This is also supported by the fact that the 7-unsubstituted **26e** exhibits higher activity than the 4-unsubstituted **26d** and by the virtually abolished activity of **26f**.

The replacement of the 14-hydroxy by a 14-amino group with **47** produced an inactive compound that could not be improved by the 7-(Boc-L-seryl-amino) substituent (**48**). The fact that the 14-hydroxy group could not be replaced by neither amino nor methoxy substituents led to the prediction that this group was acting both as hydrogen-bond donor and acceptor when binding to the DNA gyrase protein, a behavior not possible for amino or methoxy substituents. This assumption was eventually confirmed by X-ray structures of **1** bound to the gyrB protein.[36] These structures reveal the crucial role of the 14-hydroxy group, which maintains a hydrogen-bond network together with Asp75 and a tightly bound water molecule, as depicted in Figure

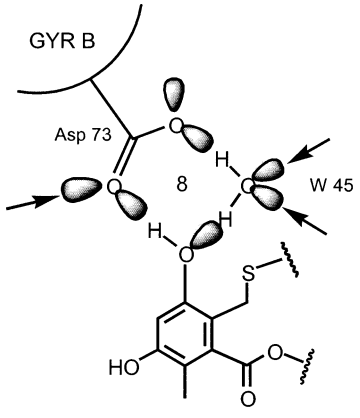
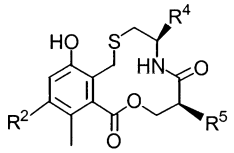
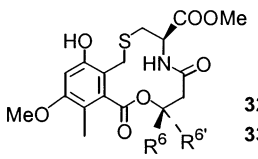



**Figure 2.** Binding mode of cyclothialidine at the DNA gyrase B protein.[36]

**Table 2.** DNA Gyrase Inhibition (MNEC) and in Vitro Antibacterial Activity (MIC) of Selected Compounds: Variation of $R^2$, $R^4$–$R^6$





**32** $R^6$ = Me, $R^{6'}$ = H
**33** $R^6$ = H, $R^{6'}$ = Me

| variation of | compd | $R^2$ | $R^4$ | $R^5$ | MNEC[a] ($\mu$g/mL) | MIC[b] ($\mu$g/mL) *N. meningitidis* 69480 | *M. luteus* ATCC8340 | *S. aureus* 887 | *S. pyogenes* b15 |
|---|---|---|---|---|---|---|---|---|---|
| $R^2$ | **1** | | | | 0.05 | 128 | 128 | >64 | 32 |
| | **10** | OH | COOMe | NHBoc | 0.2 | 16 | 16 | >64 | 32 |
| | **26**g | OMe | COOMe | NHBoc | 0.2 | 16 | 16 | 64 | 64 |
| $R^4$ | **49** | OMe | $CH_2OH$ | NHBoc | 2.5 | 64 | 64 | >64 | 64 |
| | **50** | OMe | COOH | NHBoc | 10 | nd[c] | >64 | >64 | >64 |
| | **51** | OMe | $CONH_2$ | NHBoc | 0.1 | 4 | 16 | >64 | 16 |
| | **52** | OMe | $CONHCH_2CHCH_2$ | NHBoc | 0.1 | 4 | 8 | 16 | 16 |
| $R^5$ | **86**b | OH | COOMe | H | 0.25 | 32 | 16 | >64 | 128 |
| | **54** | OH | COOMe | $NH_2$ | 1 | nd | >64 | >64 | nd |
| | **55** | OH | COOMe | NHAc | 0.4 | 32 | 128 | >64 | >64 |
| | **56** | OH | COOMe | NH-L-Pro-Boc | 2.5 | nd | 64 | >64 | >64 |
| | **57** | OH | COOMe | NH-L-3cHyp-Boc | 0.05 | 64 | 8 | >64 | 32 |
| | **58** | OH | COOMe | NH-D-3cHyp-Boc | 1 | nd | 128 | >64 | nd |
| | **59** | OH | COOMe | NH-L-Ser-Bo c | 0.1 | nd | 8 | >64 | nd |
| | **60** | OH | COOMe | NH-D-Ser-Boc | 1 | nd | >64 | >64 | nd |
| | **61** | OH | COOMe | $NHCOCH_2CH_2OH$ | 0.2 | nd | 16 | >64 | >64 |
| $R^6$ | **32** | | | | 0.4 | 8 | 16 | 16 | 16 |
| | **33** | | | | 4 | 64 | 64 | >64 | >64 |

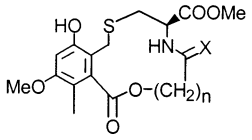
[a,b,c] See Table 1.

2. The information on the exact binding mode of **1** and of other ligands of the ATP binding site had been used by others for a biased needle screening aiming at new DNA gyrase inhibitors.[37]

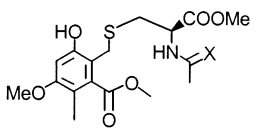
From our experience with a large number of analogues of **1**, the binding of the 14-hydroxy group seems to add a major contribution for their binding to the DNA gyrase protein. Modification of the substituents on cyclothialidine's core structure had revealed **X** as the minimal structural requirements for DNA gyrase inhibition. Nevertheless, other substituents add to the inhibitory potency by providing better binding and presumably by supplying a stabilizing influence with regard to the bioactive conformation of the lactone ring. This is supported by NMR data showing for active compounds a large and a small coupling constant between the protons at C(3) and C(4) as a characteristic feature. This suggests a synclinal/antiperiplanar arrangement of the hydrogens and implies a staggered (gauche) conformation about C$\alpha$ and C$\beta$ of the cysteine entity. This local conformation is also displayed by **1** when binding to the ATPase site of DNA gyrase.

In our search for compounds of improved antibacterial activity, we have extensively modified the substituents $R^1$, $R^2$, and $R^{4-6}$. For $R^1$, methyl was found to be better than hydrogen, whereas larger alkyl groups led to a decrease in affinity. Therefore, a number of 11-methyl derivatives are compiled in Table 2 to describe SAR found for the other substituents with regard to the enzyme inhibitory activity. For $R^2$, OH ~ OMe; for $R^4$, COOMe, $CONH_2$ > $CH_2OH$ ≫ COOH. An interesting feature of $R^5$ was the striking activity of 3-hydroxypropionylamino substituents, either free as in **61**, or as part of an L-amino acid (**57**, **59**), reminiscent of the 7-(3-hydroxy-L-prolinoylamino) entity of **1**. When embedded in a D-amino acid residue (**58**, **60**), this motif is less efficient. In addition, $R^5$ = H or NHAc allowed good inhibitory properties (**86b**, **55**).

**Table 3.** Variation of the Lactone Ring Size. MNEC and MIC Values of Selected Compounds



**86a-g, 87a-g**



**90, 91**

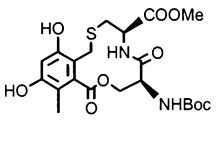
| compd | X | n | ring size | MNEC[a] ($\mu$g/mL) | MIC*b* ($\mu$g/mL) *S. a.* 1 | *S. a.* 2 |
|---|---|---|---|---|---|---|
| **86a** | O | 1 | 11 | >1 | >64 | >64 |
| **86b** | O | 2 | 12 | 0.5 | 64 | 64 |
| **86c** | O | 3 | 13 | 2 | 64 | 32 |
| **86d** | O | 4 | 14 | 0.05 | 4 | 2 |
| **86e** | O | 5 | 15 | 2 | >64 | 32 |
| **86f** | O | 6 | 16 | >1 | >64 | >64 |
| **86g** | O | 8 | 18 | >1 | nd[c] | nd |
| **87a** | S | 1 | 11 | 0.2 | >64 | >64 |
| **87b** | S | 2 | 12 | 0.05 | 2 | 2 |
| **87c** | S | 3 | 13 | 0.4 | 4 | 2 |
| **87d** | S | 4 | 14 | 0.01 | 0.5 | 0.25 |
| **87e** | S | 5 | 15 | 0.5 | 32 | 16 |
| **87f** | S | 6 | 16 | 0.5 | 16 | 16 |
| **87g** | S | 8 | 18 | >1 | nd | nd |
| **90** | O | – | – | 2 | >64 | >64 |
| **91** | S | – | – | 0.2 | 16 | 8 |

[a,c] See Table 1. [b] Agar dilution (Mueller–Hinton agar). Inoculum $10^4$ CFU/spot, MIC = minimum inhibitory concentration. *S. a.* 1: *S. aureus* ATCC25923; *S. a.* 2: *S. aureus* Smith.

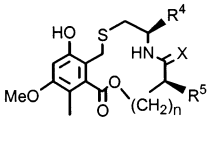
For $R^6$ at C(8), a clear preference for a $\beta$-methyl group (**32**) over a methyl group in the $\alpha$-position (**33**) was observed.

When examining the influence of the lactone ring size on the biological activity, we were surprised by the finding that DNA gyrase inhibitory activity was observed over a rather large structural scope, namely from 11- to 16-membered lactones (Table 3). Whereas in the 6-oxo series, activity was found from 12- to 15-membered lactones (**86a**–**e**), the 11- to 16-membered homo-

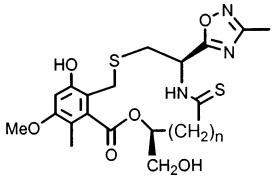
**Table 4.** DNA Gyrase Inhibition (MNEC), in Vitro and in Vivo Antibacterial Activity (MIC, $ED_{50}$) of Selected Compounds. Comparison with Reference Compounds



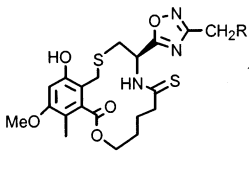
**10**



**26g, 86b, 87b, 86d, 87d, 93, 94, 96, 97**



**103, 110, 111**



**97** (R=H), **114-118**

| | | | | | | | MIC ($\mu$g/mL)[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| compd | $R^4$ | $R^5$ | R | X | n | MNEC[a] ($\mu$g/mL) | *E. c.* 25922 | *X. m.* AC739 | *S. a.* 25923 | *S. a.* Smith | *S. e.* 16/2 | *E. f.* 6 | *S. p.* b15 | $ED_{50}$[c] (mg/kg) |
| **10** | | | | | | 0.2 | >64 | >64 | >64 | 128 | 64 | 64 | 64 | nd[d] |
| **26g** | COOMe | NHBoc | | O | 1 | 0.2 | >64 | >64 | >64 | 128 | 16 | >64 | 128 | nd |
| **86b** | COOMe | H | | O | 1 | 0.5 | >64 | >64 | 64 | 64 | 8 | >64 | 32 | nd |
| **87b** | COOMe | H | | S | 1 | 0.05 | >64 | 8 | 2 | 2 | 0.5 | 4 | 4 | nd |
| **86d** | COOMe | O | | O | 3 | 0.05 | >64 | 16 | 4 | 2 | 1 | 4 | 4 | nd |
| **87d** | COOMe | S | | S | 3 | 0.01 | >64 | 8 | 0.5 | 0.25 | <0.12 | 1 | 1 | nd |
| **93** | 3-Me-ODA[e] | H | | O | 1 | 0.05 | >64 | 16 | 8 | 8 | 2 | 8 | 4 | >25 |
| **94** | 3-Me-ODA | H | | S | 1 | 0.02 | >64 | 4 | 0.5 | 0.25 | 0.12 | 1 | 1 | >25 |
| **96** | 3-Me-ODA | H | | O | 3 | 0.01 | >64 | 4 | 0.5 | 0.25 | 0.12 | 0.5 | 1 | >25 |
| **97** | 3-Me-ODA | H | (H) | S | 3 | 0.004 | >64 | 0.5 | 0.12 | 0.06 | 0.01 | 0.25 | 0.25 | >25 |
| **103** | | | | | 1 | 0.01 | >64 | 8 | 2 | 2 | 0.5 | 0.5 | 0.5 | 8.5 |
| **110** | | | | | 2 | 0.01 | >64 | 8 | 1 | 0.5 | <0.12 | 0.5 | 2 | 9.5 |
| **111** | | | | | 3 | 0.005 | >64 | 4 | 0.5 | 0.5 | <0.12 | 0.25 | 0.5 | 12.5 |
| **114** | | | $NH_2$·HCl | | | 0.002 | 64 | 4 | 0.5 | 0.25 | <0.12 | 0.5 | 0.5 | 8.5 |
| **115** | | | NHAc | | | 0.005 | >64 | 32 | 2 | 1 | <0.12 | 1 | 1 | 25 |
| **116** | | | NH-L-Ala·HCl | | | 0.005 | >64 | >64 | 8 | 2 | 1 | 8 | 2 | 25 |
| **117** | | | $NHCH(Me)_2$·HCl | | | 0.002 | 64 | 4 | 0.25 | 0.12 | <0.12 | 0.5 | 0.5 | >12.5 |
| **118** | | | OH | | | 0.001 | >64 | 8 | 0.5 | 0.25 | <0.12 | 1 | 0.5 | 25 |
| novobiocin | | | | | | 0.1 | >64 | >64 | 0.25 | 0.12 | 0.25 | 4 | 1 | 3 |
| vancomycin | | | | | | – | >64 | >64 | 1 | 1 | 2 | 4 | 1 | 1.5 |

[a] Supercoiling assay, *E.coli* gyrase, MNEC = maximum non effective concentration. [b] Agar dilution (Mueller–Hinton medium), inoculum $10^4$ CFU/spot, MIC = minimum inhibitory concentration. Test organisms: *E. coli* 25922, *X. maltophilia* IAC 739, *S. aureus* ATCC 25923, *S. aureus* Smith, *S. epidermidis* 16/2, *E. faecalis* 6, *S. pyogenes* b15. [c] In vivo efficacy, septicaemia in mice (*S. aureus* Smith), iv administration. [d] Not determined. [e] 3-Methyl-1,2,4-oxadiazol-5-yl.

logues **87a**–**f** of the 6-thioxo lactones displayed inhibitory properties. In both series, the 14-membered lactones (**86d**, **87d**) were the most active ones, followed by the 12-membered lactones and then by the others. The improved inhibitory activity was tentatively attributed to the increased lipophilicity and conformational flexibility of the larger rings, properties even more pronounced in the 6-thioxo series, and obviously optimally rewarded in the 14-membered lactones.

Considering the high degree of structural variability tolerated by the enzyme, it was not unexpected that we eventually identified "seco-compounds"—analogues lacking the lactone entity—that also inhibited DNA gyrase. Compounds **90** and **91** are simple representatives of this subclass.[17b,38] They demonstrate that the lactone entity is not essential for the binding and that the substructure common to all congeners of this inhibitor family can now be reduced further to a hydroxylated benzyl sulfide entity **XI** (Figure 1). It can be assumed that in all compounds found active, this pharmacophore is able to adopt a similar conformation when binding to DNA gyrase.

To increase the metabolic stability to enable in vivo efficacy, the ester function at C(4) was replaced. From the various five-membered heterocycles examined, the 1,2,4-oxadiazole[28] (ODA) was found to be the best and brought a further improvement observed already at the enzyme level. Thus, the oxadiazoles **93**, **94**, **96**, and **97** were 2–10 times more active in the supercoiling assay than the corresponding methyl esters (Table 4). Polar side chains, such as the hydroxymethyl groups at the lactone core (**103**, **110**, **111**) or amino and hydroxy functions at the ODA ring (**114**–**118**), introduced to improve the pharmacokinetic properties of the compounds, were in general well-tolerated and did not affect the excellent enzyme inhibitory activities found in particular for the 14-membered lactones.

**Antibacterial Activity in Vitro.** On the basis of the minimal structure **X**, many potent DNA gyrase inhibitors with activities similar to that of the lead compound have been prepared. However, with regard to the antibacterial activity, progress was rather modest. Cyclothialidine itself had been shown to be virtually inactive, and therefore, first we performed antibacterial in vitro testing using a set of rather sensitive strains requiring a test media supplemented with sheep blood (Tables 1 and 2). The compounds compiled in Table 1 were selected to demonstrate the importance of the various substituents $R^1$–$R^5$ as well as the stereochemical prerequisites of the lactone moiety. Their enzyme inhibitory activity was matched in a qualitative manner by the minimum inhibitory concentrations (MICs) determined in particular against *Neisseria meningitides* 69480 and *Micrococcus luteus* ATCC8340, confirming that the antibacterial activity was indeed a consequence of the inhibition of DNA gyrase. More data is shown in Table 2. The first synthetic compound found to be active in the enzyme assay (**10**) was only slightly better than **1** with regard to antibacterial activity. Methylation of the 12-hydroxy group, as exemplified by **26g**, effected

**Table 5.** In Vitro Antibacterial Activities of **97** and Reference Compounds against Multiresistant Gram-Positive Bacteria[a]

| | *S. aureus* (30)[b] | | | *E. faecalis* (16) | | | *E. faecium* (5) |
|---|---|---|---|---|---|---|---|
| compound | $MIC_{50}$ | $MIC_{90}$ | range | $MIC_{50}$ | $MIC_{90}$ | range | range |
| methicillin | 32 | >64 | 4−>64 | | | | |
| imipenem | 4 | >16 | 0.06−>64 | | | | |
| ciprofloxacin | 0.5 | >16 | 0.25−>16 | | | | |
| erithromycin | >16 | >16 | 0.5−>16 | | | | |
| tetracyclin | >16 | >16 | 0.5−>16 | | | | |
| novobiocin | | | | 4 | 8 | 4−8 | 2−8 |
| vancomycin | 1 | 2 | 0.5−2 | 2 | 4 | 1−4 | 1−>16 |
| **97** | 0.06 | 0.12 | 0.06−0.12 | 0.12 | 0.12 | 0.06−0.25 | 0.25−0.5 |

[a] Agar dilution (Mueller−Hinton agar). Inoculum $10^4$ CFU/spot; $MIC_{50}$ ($MIC_{90}$) = Minimum concentration (in $\mu$g/mL) that inhibits at least 50% (90%) of the strains tested. [b] The number of strains is in parentheses.

as an important improvement a modest activity against *S. aureus*. Variation of $R^4$ in the 12-methoxy series brought about some further progress, e.g. with the allyl amide **52**. Regarding the variation of $R^5$, representatives of the 12-hydroxy series are compiled. These compounds demonstrate that despite of a good enzyme inhibitory activity, the effect on bacterial cells remained marginal, probably due to their highly hydrophilic character. It can be noted that these $R^5$ substituents did not perform better in the 12-methoxy series (data not shown). Some further progress was marked by the 8-$\beta$-methyl derivative **32**.

With this modest level of in vitro antibacterial activity achieved, we changed the battery of test strains to more typical pathogens which could be tested in the absence of blood plasma (Mueller−Hinton media). In Table 3 the antibacterial performance of the homologues lactones **86a**−**g** and of their corresponding 6-thioxo analogues **87a**−**g** is displayed. Most importantly, the improved enzyme inhibitory property of the 14-membered lactones was also matched by a corresponding antibacterial activity, as can be seen by comparison of **86b** with **86d** and of **87b** with **87d**. The thioacetyl derivative **91**, representing the seco-subclass, exhibited an antibacterial activity corresponding to its activity at the enzyme level.

Modification work was continued, and key compounds featuring the major structural changes that led to an improved antibacterial potency are compiled in Table 4. Starting from **10**, the replacement of OH by OMe and the removal of the NHBoc group afforded **86b**, a derivative displaying a modest activity against *S. aureus*. It was then found that the introduction of a thioamide function not only improved the DNA gyrase inhibitory activity but also simultaneously caused a marked increase of the activity against Gram-positive strains. Although the MIC values of **87b** looked promising, the methyl ester function due to its metabolical liability was not suited for in vivo testing. We found an ODA ring to be an appropriate replacement for the ester group (**93**, **94**).[28] The 3-Me-ODA in position 4 indeed not only retained but improved both the enzyme−inhibitory and also the antibacterial activity in vitro when compared to the methyl ester group.

Lactone ring enlargement, e.g. going from **86b** to **86d** or from **87b** to **87d**, clearly brought about a further improvement. The outstanding enzyme inhibitory properties of the 14-membered lactones were also accompanied by a substantial increase in the antibacterial activity. Combining the most favorable structural elements, lactone **97** is the most potent compound in this series and displays broad-spectrum activity against Gram-positive bacteria, similar to or better than that of our reference compounds novobiocin and vancomycin. Nevertheless, typical Gram-negative bacteria, such as wild-type strains of *Escherichia coli, Klebsiella pneumoniae*, or *Pseudomonas aeruginosa*, were not susceptible, although activity was found against selected strains of *Xanthomonas maltophilia, Haemophilus influenzae, N. meningitides, and Moraxella catarrhalis.* To ensure that the desired target profile can be met by cyclothialidine congeners, we have tested **97** as a prototype of this new antibacterial class against a larger number of Gram-positive isolates (Table 5). The MICs of **97** against *S. aureus, Enterococcus faecalis*, and *Enterococcus faecium* were considerably lower than those of vancomycin. Even more important, **97** was equally inhibitory against antibiotic-susceptible and multiresistant strains and did not show any cross-resistance with other structural classes. As a consequence, the $MIC_{90}$ values against multiple-resistant staphylococci and enterococci were much lower than for established antibacterial classes, such as $\beta$-lactams, quinolones, macrolides, glycopeptides (vancomycin, teicoplanin), or rifamycins. Remarkably, clinical isolates resistant to novobiocin, a drug targeting the same binding site as **1**, were fully susceptible to **97**.[39]

The attachment of hydrophilic side chains to the best compounds (e.g. **94**, **97**) in order to lower their lipophilic character, and concomitantly increase their water solubility, led to a slight decrease of the antibacterial activity. Thus, the hydroxymethyl substituent of **103** or **111** lowered the in vitro activity to some extent when compared to that of the parent compounds **94** and **97**. A similar, nondetrimental effect was found for basic and polar substituents at the methyl group of the oxadiazole ring, as demonstrated for compounds **114**−**118**. Thus, tuning of the physicochemical properties of the most potent compounds appeared to be possible.

The excellent in vitro antibacterial activities of lipophilic compounds were significantly reduced by the presence of plasma proteins (Table 6). For the more hydrophilic compounds, this effect was less pronounced. This plasma-shift of the MIC values, due to protein binding of the compounds[40] and possibly also to a changed susceptibility of the bacteria, was assumed to play an important role with regard to the in vivo efficacy.

**Antibacterial Efficacy in Vivo.** The optimal translation of the in vitro antibacterial activity into an adequate in vivo efficacy turned out to be a difficult task. After compounds exhibiting an MIC value for *S. aureus*

**Table 6.** CLOGP, Protein Binding, Effect of Plasma Proteins on the in Vitro Antibacterial Activity, in Vitro Glucuronidation, and Cytotoxicity of Selected Compounds

| | | | in vitro antibacterial activity against *S. aureus* Smith | | | | |
|---|---|---|---|---|---|---|---|
| | | | MIC | | | | |
| compound | CLOGP[a] | % bound to protein[b] | BHI agar[c] (B) | 50% plasma[d] (P) | ratio P/B | % of total substrate glucuronidated[e] | cytotoxicity,[f] $IC_{50}$ (mg/mL) |
| **1** | −2.09 | | | | | | >800 |
| **86d** | 3.00 | | | | | | >50 |
| **97** | 3.34 | 96 | 0.03 | 0.25 | 8 | | 24 |
| **103** | 1.31 | 88 | | | | 25 | >50 |
| **110** | 1.80 | 94 | | | | 12 | >50 |
| **114** | 1.51 | 91 | 0.25 | 1 | 4 | 57 | |
| vancomycin | <1 | 25[g] | 1 | 2 | 2 | | |

[a] Calculated distribution coefficient, KOW_ClogP. See: Meylan, W. M.; Howard, P. H. *J. Pharm. Sci.* **1995**, *84*, 83−92. [b] Binding to mice plasma protein in percent. [c] MIC (μg/mL) determined in BHI (brain heart infusion) agar. [d] MIC (μg/mL) determined in BHI agar/mouse plasma (1:1). [e] In vitro glucuronidation estimated under standardized conditions (700 mg of rat liver microsomal protein, 1 mM UDPGA, and 40 μM substrate. Products formed after 1 h incubation time were analyzed by HPLC and expressed relative to the substrate added; see Fischer, M. B.; et al. *Drug Metab. Rev.* **2001**, *33*, 273−297). [f] Assessed with HeLa cells using the following method: Ohara H.; Terasima T. *GANN* **1972**, *63*, 317−327. [g] Data are from the following: Knudsen, J. D. et al., *Antimicrob. Agents Chemother.* **1997**, *41*, 1910−1915.

of <8 μg/mL had been identified, their efficacy in a septicaemia model in mice was tested. Unfortunately, out of a number of promising derivatives, among them **94** and **97**, none was able to cure the infected mice, and this was rather disappointing. Examination of plasma and urine samples revealed that our best compounds displayed unfavorable pharmacokinetics, i.e., they showed rapidly decreasing plasma concentrations. A major metabolite, which turned out to be the glucuronide of the phenolic parent compound, was discovered.[41] In contrast, inactive analogues of rather hydrophilic character, such as the carboxylic acid **50**, displayed a much longer plasma half-life. It was hypothesized that the reduction of the lipophilic properties of the best compounds might improve their pharmacokinetic behavior, and this concept indeed led to vivo efficacy. The hydroxymethyl group in the 8β-, 9β-, or 10β position, of the 12−14 membered lactones **103**, **110**, and **111**, respectively, did reduce the antibacterial potency by a factor 8 in comparison to their parent compounds (e.g. **94** and **97**). Nevertheless, these compounds were found to be active in the septicaemia model with an $ED_{50}$ of 8−12 mg/kg (iv administration). A similar performance was also found for the aminomethyl derivative **114**. The in vivo efficacy of the more hydrophilic derivatives **103**, **110**, and **114** can be attributed to lower protein binding and improved pharmacokinetics, both parameters comparing favorably with those of **97**, but remaining inferior to those of vancomycin (Table 6, Figure 3). The relatively high plasma levels achieved with the hydroxymethyl derivatives **103** and **110** is probably due to a slower glucuronidation rate, as was demonstrated in an in vitro experiment in comparison with **114** (Table 6). These compounds, while exhibiting a moderate in vitro activity comparable to that of vancomycin, seem to be closer to this reference compound than **97** and **114** in terms of hydrophilicity (CLOGP), susceptibility for glucuronidation, and protein binding. The amino derivative **114**, although showing a rapid decline of plasma concentrations due to glucuronidation, still remains efficacious due to its higher intrinsic antibacterial activity. The assumption that protein binding (plasma shift of MIC) and glucuronidation are particularly important for the more lipophilic derivatives helps to rationalize the in vivo performance of these derivatives.[40,42] The examples given do not yet match the efficacy of vancomycin or novobiocin, but they indicated means for further optimization by modifying the physicochemical properties.

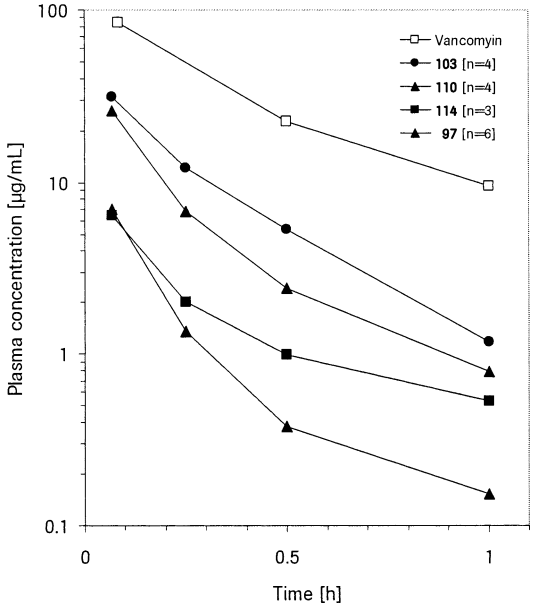



**Figure 3.** Total plasma concentrations of **97**, **103**, **110**, and **114** in rats ($n$ = 3−6) after a single intravenous dose of 20 mg/kg (microsuspension in gelatin). Comparison with vancomycin data from Ngeleka et al. (*Antimicrob. Agents Chemother.* **1989**, *33*, 1575−1579).

However, from many more compounds we had to learn that the window of optimal lipophilicity was rather small and that the glucuronidation rate was also depending on structural features of the substrates. This can be illustrated by compounds **115**−**118**, which showed only borderline activity in vivo. Whereas the weaker efficacy of the acylamino derivatives **115** and **116** can be attributed to their reduced antibacterial potency, the low efficacy of the rather lipophilic isopropylaminomethyl derivative **117** and of the rather hydrophilic hydroxymethyl compound **118** has to be tentatively explained by unfavorable pharmacokinetics, possibly due to either high protein binding, large tissue distribution, and/or rapid elimination, e.g. by glucuronidation.

**Cytotoxicity in Vitro.** It had been stated that cyclothialidine did not show any cytotoxic effect against HeLa cells up to 800 $\mu$g/mL, and this was in agreement with the finding that **1** was not inhibiting mammalian topoisomerases, e.g. $IC_{50}$ against calf thymus topoisomerase II >1000 $\mu$g/mL.[21] In view of the more lipophilic character of our best derivatives, which undoubtedly allowed them to exert their antibacterial activity, we wanted to ensure that the increased ability to penetrate cell membranes did not result in an unwanted toxic effect against mammalian cells. Thus, the cytotoxic potential of selected compounds was assessed with HeLa cells (Table 6). The absence of a significant toxicity confirmed that the affinity of cyclothialidine congeners is very specific for the bacterial type II topoisomerase.

## Conclusions

The screening technique applied in the discovery of cyclothialidine provided a lead compound whose outstanding inhibitory activity against the isolated bacterial enzyme was not translated into an adequate antibacterial activity. A flexible synthetic route has been developed that allowed us to prepare a variety of analogues, and the partial structure **XI** was identified as the pharmacophoric core of **1**. On the basis of this "minimal structure" many potent inhibitors of DNA gyrase, belonging to several subclasses, have been prepared, i.e., bicyclic lactones of different ring sizes as well as seco-derivatives lacking the lactone ring. Most importantly, congeners of **1** were found that indeed displayed considerable in vitro activity against Gram-positive pathogens. When analyzing the structural features that brought about the most potent activity against bacterial cells, it is evident that an increase of the lipophilic character of the compounds had a positive impact. At the enzyme level, these structural elements also contributed to a better affinity via improved hydrophobic interactions, whereas with regard to the antibacterial effect, faster permeation of the bacterial membrane in a presumably passive uptake was beneficial. It became obvious in our investigation that overly lipophilic compounds showing the most potent in vitro antibacterial activity did not fulfill the pharmacokinetic requirements for in vivo efficacy, a general problem for antibacterials targeting enzymes located in the cytoplasm. The optimization for in vivo activity by balancing physicochemical and antibacterial properties turned out to be very difficult, and this task was clearly aggravated by the fact that part of the active compounds was eliminated by glucuronidation of their essential phenol group. Nevertheless, by reducing the lipophilic character of the best compounds through selective introduction of hydrophilic substituents, derivatives displaying in vivo efficacy were identified.

Thus, we have demonstrated that the DNA gyrase inhibitory principle contained in cyclothialidine can be considered as the basis for a new class of antibacterial agents. The lack of cytotoxicity against mammalian cells, shown for optimized derivatives, supports the view that this class has the potential to provide clinically useful drugs.

## Experimental Section

**Chemistry. 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. If not stated otherwise, aqueous (aq) solutions of $NaHCO_3$, $Na_2CO_3$, and $NH_4Cl$ used in the standard workup were saturated, aqueous layers were back-extracted with the organic solvent used, and organic solutions were dried with $Na_2SO_4$. Evaporation of solvents and concentration of reaction mixtures were performed in vacuo at 20−40 °C on a Büchi rotary evaporator. Thin-layer chromatography (TLC) was carried out on silica gel glass plates (Kieselgel 60 F254, Merck) with detection by UV and visualization by spraying with 1% aq $KMnO_4$ solution or 0.4% aq $I_4K_2Pt$(II) solution (Meunier, R.; et al. *Bull. Soc. Chim. Biol.* **1949**, *31*, 1144). Normal-phase silica gel (Silica gel 60, 230−400 mesh, Merck) was used for preparative chromatography. Melting points (mp) were determined on a Büchi SMP-20K and are uncorrected. Optical rotation ($[\alpha]_D$) was measured on a Perkin-Elmer-241 polarimeter, 10 cm, at 20 °C. $^1$H NMR spectra were recorded on a Bruker-AC-250 or on a Bruker-ARX-400. If not stated otherwise, spectra were recorded at 250 mHz. Chemical shifts are reported as $\delta$ values (ppm) downfield from internal TMS in the indicated solvent. Coupling constants ($J$) are given in Hz; s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Low-resolution EI-mass spectra were recorded with a Finnigan-MAT SSQ 700 mass spectrometer; ISP-MS and ISN-MS were recorded on a PE-Sciex API III. High-resolution mass spectra (HRMS) were determined on a Finnigan-MAT MAT95. Elemental analyses were carried out in our laboratory or by Solvias AG (Basel, Switzerland) and results are within ±0.4% of the theoretical values. Target compounds that did not give satisfactory elemental analyses were analyzed by HRMS and their purity was assessed by HPLC with two different experimental systems and was shown to be >95% if not stated otherwise.

**Nomenclature.** The nomenclature system was used previously, and the numbering indicated in formula **I** (Scheme 1) was applied for target compounds.[27,28,31]

**Abbreviations:** $SiO_2$, silica gel; DCM, dichloromethane; DEAD, diethyl azodicarboxylate; DCC, *N*,*N*′-dicyclohexylcarbodiimide; EDC, *N*-(dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; NBS, *N*-bromosuccinimide; TFA, trifluoroacetic acid; L(D)-3cHyp, *cis*-3-hydroxy-L(D)-proline; trityl, triphenylmethyl; thexyl, 1,1,2-trimethylpropyl; $R_f$, chromatographic retention time.

**Methyl(4*R*,7*S*)-7-*tert*-butoxycarbonylamino-1,3,4,5,6,7,8,-10-octahydro-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (6b)** was prepared in five steps from 2,6-dimethylbenzoic acid using the methodology described for the synthesis of **6a**:[18] white powder, mp 150−151 °C; $[\alpha]_D = +31.7°$ ($c$ = 1, MeOH); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.41 (s, 9H, t-Bu), 2.27 (s, 3H, H−C(11)), 2.85 (dd, 1H, $J$ = 14, 9, H−C(3)), 3.11 (dd, 1H, $J$ = 14, 4, H−C(3)), 3.59 (d, 1H, $J$ = 11, H−C(1)), 3.63 (s, 3H, COOMe), 3.96 (d, 1H, $J$ = 11, H−C(1)), 4.23 (dd, 1H, $J$ = 11, 4, H−C(8)), 4.42−4.52 (m, 1H, H−C(7)), 4.62−4.74 (m, 1H, H−C(4)), 4.86 (dd, 1H, $J$ = 11, 2, H−C(8)), 7.15−7.22 (m, 2H, arom H), 7.27 (d, 1H, $J$ = 8, NH), 7.31 (t, 1H, $J$ = 7, arom H), 8.28 (d, 1H, $J$ = 8, NH); MS (ISP) 453.4 $(M + H)^+$, 470.2 $(M + NH_4)^+$. Anal. ($C_{21}H_{28}N_2O_7S$) C, H, N, S.

**12,14-Dideoxycyclothialidine (7)** was prepared from **6b** in seven steps using the methodology described for the synthesis of **1**:[18] white powder; $^1$H NMR (400 MHz, $D_2O$) $\delta$ 1.36 (d, 3H, $J$ = 7.3, Me(Ala)), 2.05−2.16, 2.20−2.31 (2 m, 2H, H−C(4) (cHyp), 2.33 (s, 3H, Me-C(11)), 2.69 (dd, 1H, $J$ = 15, 11, H−C(3)), 3.33 (dd, 1H, $J$ = 15, 4.5, H−C(3)), 3.60 (d, 1H, $J$ = 11, H−C(1)), 3.78−3.86 (m, 2H, H−C(5) (cHyp)), 3.86−3.95 (m, 1H), 3.98−4.07 (m, 2H), 4.11 (q, 1H, $J$ = 7.3, C(2) (Ala), 4.40 (dd, 1H, $J$ = 12, 2, H−C(8)), 4.46 (m, 1H, H−C(2) (Ser)), 4.70−4.90 (4H, H−C(4), H−C(7), H−C(2)/H−C(3) (cHyp)), 5.64 (dd, $J$ = 12, 2.5, H−C(8)), 7.26−7.34 (m, 2H, H−C(12), H−C(14)), 7.42 (t, 1H, $J$ = 7.6, H−C(13)); HRMS calcd for ($C_{26}H_{35}N_5O_{10}SH^+$) 610.2183, found 610.2181.

**General Preparation of Compounds I from *O*-Trialkylsilyl-Protected Precursors. Method A-1. Methyl (4*R*,7*S*)-7-*tert*-Butoxycarbonylamino-1,3,4,5,6,7,8,10-octahydro-12,14-dihydroxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (10).** A solution of methyl (4*R*,7*S*)-7-*tert*-butoxycarbonylamino-12,14-bis[(*tert*-butyl)dimethylsilyloxy]-1,3,4,5,6,7,8,10-octahydro-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate[18] (**9**, 2.54 g, 3.6 mmol) and $NH_4F$ (0.67 g, 18.0 mmol) in MeOH (72 mL) was stirred at 20 °C for 0.5 h. The solution was diluted with EtOAc (150 mL), washed with 5% aq NaCl (3 × 20 mL), dried, and evaporated. The remaining solid was crystallized from EtOAc/hexane to give **10** (1.47 g, 84%): white solid, mp 122−128 °C (dec); $[\alpha]_D = +54.3°$ ($c = 1$, EtOAc); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.42 (s, 9H), 1.89 (s, 3H), 2.84 (dd, 1H, $J$ = 14, 9), 3.02 (dd, 1H, $J$ = 14, 4), 3.42 (d, 1H, $J$ = 11), 3.63 (s, 3H), 3.81 (d, 1H, $J$ = 11), 4.19 (dd, 1H, $J$ = 11, 4), 4.31−4.41 (m, 1H), 4.46−4.57 (m, 1H), 4.88 (dd, 1H, $J$ = 11, 2), 6.44 (s, 1H), 7.22 (d, 1H, $J$ = 8), 8.18 (d, 1H, $J$ = 8), 9.49 (s, 1H), 9.51 (s, 1H); MS (ISP) 485.5 $(M + H)^+$. Anal. ($C_{21}H_{28}N_2O_9S$) C, H, N, S.

**General Procedures for the Preparation of Dipeptides 11−18. Method B-1. DCC as Coupling Auxiliary. *N*-(*N*-*tert*-Butoxycarbonyl-D-seryl)cysteamine (11).** To a mixture of cysteamine hydrochloride (5.68 g, 0.05 mol) and Boc-L-Ser-OH (10.26 g, 0.05 mol) in MeCN (0.15 L) was added at 0 °C *N*-methylmorpholine (5.5 mL, 0.05 mol). To the stirred solution was added over 30 min at −10 to −5 °C a solution of DCC (10.3 g, 0.05 mol) in MeCN (0.15 L), and stirring was continued for 3 h at 5 °C. The precipitate formed was removed by filtration and the filtrate was evaporated. The residual oil was dissolved in EtOAc (0.15 L), and the solution was washed with 0.5 N aq HCl, 5% aq $NaHCO_3$, and brine, dried, and evaporated. The residual oil was chromatographed ($SiO_2$; EtOAc/hexane 1:1) and the purified product was crystallized from EtOAc/hexane to give **11** (3.42 g, 26%): white crystals, mp 95−96 °C; $[\alpha]_D = -18.0°$ ($c = 0.4$, EtOH); $^1$H NMR ($CDCl_3$) $\delta$ 1.43 (t, 1H, $J$ = 9) superimposed by 1.46 (s, 9H), 2.62−2.73 (m, 2H), 3.14 (br s, 1H), 3.38−3.55 (m, 2H), 3.58−3.73 (m, 1H), 4.07−4.19 (m, 2H), 5.57 (br d, 1H, $J$ = 8), 7.07 (br s, 1H); MS (EI) 208 $(M - C_4H_8)^+$. Anal. ($C_{10}H_{20}N_2O_2S$) C, H, N, S.

Using method B-1, compounds **12**, **14**, **15**, and **18** were prepared similarly.

**Methyl (*R*)-2-(3-trityloxypropionylamino)-3-mercaptopropionate (12)** was prepared from l-Cys-OMe·HCl (8.58 g, 0.05 mol) and 3-trityloxypropionic acid (**82b**, 16.62 g, 0.05 mol): white crystals (from DCM/hexane) (19.32 g, 86%), mp 78−80 °C; $^1$H NMR ($CDCl_3$) $\delta$ 1.29 (t, 1H, $J$ = 9), 2.52 (t, 2H, $J$ = 5.7), 2.97 (dd, 2H, $J$ = 9, 4), 3.42 (t, 2H, $J$ = 5.7), 3.71 (s, 3H), 4.68−4.95 (m, 1H), 7.14 (d, 1H, 7.4), 7.19−7.34 (m, 9H), 7.42−7.48 (m, 6H).

**Methyl *N*-(*N*-*tert*-butoxycarbonyl-D-seryl)-D-cysteinate (14)** was prepared from d-Cys-OMe·HCl (1.72 g, 10.0 mmol) and Boc-D-Ser-OH (2.05 g, 10.0 mmol): white crystals (from $Et_2O$/hexane) (1.82 g, 56%), mp 73−75 °C; $[\alpha]_D = -4.3°$ ($c = 1$, EtOAc); $^1$H NMR ($CDCl_3$) "identical" with that of methyl *N*-(*N*-*tert*-butoxycarbonyl-L-seryl)-L-cysteinate[18] (**5**).

**Methyl *N*-(*N*-*tert*-butoxycarbonyl-D-seryl)-L-cysteinate (15)** was prepared from L-Cys-OMe·HCl (18.9 g, 0.11 mol) and Boc-D-Ser-OH (22.6 g, 0.11 mol): white solid (from $Et_2O$/hexane) (12.2 g, 34%), mp 104−106 °C; $^1$H NMR ($CDCl_3$) $\delta$ 1.47 (s, 9H) superimposed by 1.49 (t, 1H, $J$ = 9), 2.90−3.05 (br s, 1H), 3.02 (dd, 2H, $J$ = 8, 4), 3.77 (dd, 1H, $J$ = 12, 4), 3.79 (s, 3H), 4.11 (dd, 1H), 4.19−4.29 (m, 1H), 4.84 (dt, 1H, $J$ = 8, 4), 5.60 (br d, 1H, $J$ = 8), 7.43 (br d, $J$ = 8).

**Methyl (*R*)-2-((*R*)-3-hydroxybutyrylamino)-3-mercaptopropionate (18)** was prepared from l-Cys-OMe·HCl (8.2 g, 48.0 mmol) and (*R*)-3-hydroxybutyric acid (5.0 g, 48.0 mmol): white crystals (from EtOAc/hexane) (3.41 g, 32%), mp 64−66 °C; $[\alpha]_D = -1.3°$ ($c = 0.8$, EtOAc); $^1$H NMR ($CDCl_3$) $\delta$ 1.26 (d, 3H, $J$ = 6.2), 1.42 (t, 1H, $J$ = 9), 2.37 (dd, 1H, $J$ = 15, 8.4), 2.48 (dd, 1H, $J$ = 15, 3.3), 3.02 (dd, 2H, $J$ = 9, 4.3), 3.44 (d, 1H, $J$ = 3.6), 3.80 (s, 3H), 4.14−4.30 (m, 1H), 4.87−4.95 (m, 1H), 6.84 (d, 1H, $J$ = 8).

**Method B-2. EDC as Coupling Auxiliary. *N*-(3-Trityloxypropionyl)cysteamine (13).** To a mixture of cysteamine·HCl (0.45 g, 4.0 mmol) and 3-trityloxypropionic acid (**82b**, 1.33 g, 4.0 mmol) in MeCN (10 mL) were added at 0 °C *N*-methylmorpholine (0.44 mL, 4.0 mmol) and EDC (0.77 g, 4.0 mmol), and stirring was continued for 3 h at 0 °C. The mixture was diluted with EtOAc, washed with 1 N aq HCl, 5% aq $NaHCO_3$, and brine, dried, and evaporated. The residual oil was chromatographed ($SiO_2$; EtOAc/hexane 1:2) and the purified product was crystallized from EtOAc/hexane to give **13** (0.68 g, 43%): white solid, mp 151−153 °C; $^1$H NMR ($CDCl_3$) $\delta$ 1.29 (t, 1H, $J$ = 9), 2.48 (t, 2H, $J$ = 6), 2.56−2.67 (m, 2H), 3.32−3.47 (m, 4H), 6.64 (br t, 1H), 7.19−7.36 (m, 9H), 7.38−7.49 (m, 6H).

Using method B-2, compounds **16** and **17** were prepared similarly.

**Methyl *N*-(*N*-*tert*-butoxycarbonyl-L-seryl)-D-cysteinate (16)** was prepared from d-Cys-OMe·HCl (3.43 g, 20.0 mmol) and Boc-L-Ser-OH (4.1 g, 20.0 mmol): white solid (from $Et_2O$/hexane) (1.23 g, 20%), mp 104−105 °C; $^1$H NMR ($CDCl_3$) "identical" with that of **15**.

**Methyl (*R*)-2-((*S*)-3-hydroxybutyrylamino)-3-mercaptopropionate (17)** was prepared from l-Cys-OMe·HCl (3.43 g, 20.0 mmol) and (*S*)-3-hydroxy-butyric acid (2.1 g, 20.0 mmol): white crystals (from DCM/hexane) (1.86 g, 42%), mp 84−86 °C; $[\alpha]_D = +37.7°$ ($c = 0.9$, EtOAc); $^1$H NMR ($CDCl_3$) $\delta$ 1.26 (d, 3H, $J$ = 6.3), 1.37 (t, 1H, $J$ = 9), 2.37 (dd, 1H, $J$ = 15, 8.4), 2.47 (dd, 1H, $J$ = 15, 3), 2.94−3.14 (m, 2H), 3.48 (br s, 1H), 3.81 (s, 3H), 4.15−4.32 (m, 1H), 4.87−4.96 (m, 1H), 6.74 (d, 1H, $J$ = 7).

**General Procedure for the Preparation of Compounds I from Substituted Benzoic Acids VII. (a) Method C. Preparation of 4-Nitrobenzyl Esters. 4-Nitrobenzyl 3,5-Dihydroxy-2-methylbenzoate (20a).** To a solution of 3,5-dihydroxy-2-methylbenzoate[18] (**19a**, 33.6 g, 0.20 mol) in DMF (0.15 L) was added at 10 °C *N*,*N*,*N*′,*N*′-tetramethylguanidine (25.2 mL, 0.20 mol). After stirring for 30 min at 20 °C, 4-nitrobenzyl bromide (43.2 g, 0.20 mol) was added, and stirring was continued for 72 h at 20 °C. The mixture was concentrated, diluted with EtOAc (0.2 L), and washed with 2 N aq HCl (2 × 0.15 L), aq $NaHCO_3$ (2 × 0.15 L), and brine (0.15 L). The organic layer was dried and evaporated. The solid residue was recrystallized from EtOAc/hexane to give **20a** (47.1 g, 78%): yellow crystals, mp 156−158 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.18 (s, 3H), 5.42 (s, 2H), 6.51/6.72 (2 d, 2 × 1H, $J$ = 2.5), 7.71/8.27 (2 d, 2 × 2H, $J$ = 9), 9.37/9.58 (2 s, 2 × 1H).

**(b) Method D. Trialkylsilylation of Phenolic OH Groups. 4-Nitrobenzyl 3,5-Bis[(*tert*-butyl)dimethylsilyloxy]-2-methylbenzoate (21a).** To a stirred mixture of **20a** (3.03 g, 10.0 mmol) and (t-Bu)$Me_2$SiCl (4.52 g, 30.0 mol) in DMF (10 mL) was added at 0 °C $Et_3N$ (4.2 mL, 30.0 mmol), a precipitate being formed immediately. The reaction mixture was stirred at 0 °C for 6 h and then diluted with EtOAc (100 mL), extracted with 1 N aq HCl (50 mL) and $H_2O$ (4 × 50 mL), dried, and evaporated. The residual oil was crystallized from hexane to give **13** (3.20 g, 60%): white crystals, mp 72−73 °C; $^1$H NMR ($CDCl_3$) $\delta$ 0.20 (s, 6H), 0.22 (s, 6H), 0.98 (s, 9H), 1.02 (s, 9H), 2.33 (s, 3H), 5.41 (s, 2H), 6.50/7.00 (2 d, 2 × 1H, $J$ = 2.5), 7.59/8.25 (2 d, 2 × 2H, $J$ = 8.5).

**(c) Method E. Benzylic Bromination of 2-Methylbenzoates. 4-Nitrobenzyl 2-(Bromomethyl)-3,5-bis[(*tert*-butyl)dimethylsilyloxy]-6-methylbenzoate (22a).** To a solution of **21a** (10.64 g, 20 mmol) in $CCl_4$ (100 mL) was added NBS (3.56 g, 20 mmol). The stirred suspension was heated at reflux and irradiated with light (250 W) until all the insoluble material (succinimide) was floating at the surface of the solution (40 min). The mixture was cooled to 0 °C. Insoluble material was removed by filtration, the solution was evaporated, and the residual material was crystallized from hexane to give **22a** (11.54 g, 94%): white crystals, mp 101−103 °C; $^1$H NMR ($CDCl_3$) $\delta$ 0.22 (s, 6H), 0.30 (s, 6H), 0.98 (s, 9H), 1.06 (s, 9H), 4.96 (s, 2H), 5.46 (s, 2H), 6.51/7.06 (2 d, 2 × 1H, $J$ = 2.5), 7.62/8.25 (2 d, 2 × 2H, $J$ = 8.5).

**(d) Method F. Alkylative Thiolation of 2-Bromomethylbenzoates. Methyl *N*-[*N*-*tert*-Butoxycarbonyl-L-seryl]-*S*-[2,4-bis[(*tert*-butyl)dimethylsilyloxy]-6-(4-nitrobenzyloxycarbonyl)-benzyl]-L-cysteinate (23a).** A stirred solution of **22a** (6.1 g, 10.0 mmol) and **5**[18] (3.22 g, 10 mmol) in DCM (80 mL) was cooled to 0 °C and treated with $Et_3N$ (1.4 mL, 10 mmol). Stirring was continued for 1 h at 0 °C and for 1 h at 20 °C. The solution was washed with 5% aq $NaHCO_3$ and brine, dried, and evaporated. The residual oil (9.68 g) was chromatographed ($SiO_2$; EtOAc/hexane 1:2) to give **23a** (5.66 g, 66%), colorless foam: $^1$H NMR ($CDCl_3$) $\delta$ 0.19/0.26 (2 s, 2 × 6H), 0.97/1.03 (2 s, 2 × 9H), 1.43 (s, 9H); 2.86−3.10 (m, 3H), 3.65−3.75 (m, 1H) superimposed by 3.70 (s, 3H), 4.00−4.18 (m, 2H), 4.38 (m, 1H), 4.81 (m, 1H), 5.49 (s, 2H), 5.59 (d, 1H), 6.52/7.04 (2 d, 2 × 1H, $J$ = 2.5), 7.40 (br d, 1H, $J$ = 8), 7.63/8.25, (2 d, 2 × 2H, $J$ = 9).

**(e) Method G. Hydrogenative Cleavage of 4-Nitrobenzyl Esters. Methyl *N*-(*N*-*tert*-Butoxycarbonyl-L-seryl)-*S*-[2,4-bis[(*tert*-butyl)dimethylsilyloxy]-6-carboxylbenzyl]-L-cysteinate (24a).** A mixture of **23a** (5.66 g, 6.64 mmol) and 5% Pd−C (1.13 g) in EtOAc (70 mL) was stirred under $H_2$ at 20 °C for 18 h. The reaction mixture was filtered and the solution was evaporated. The residual oil was stirred with MeOH (60 mL) for 1 h at 20 °C. The mixture was evaporated and the residual oil was dissolved in EtOAc (100 mL). Insoluble material was removed by filtration, and the solution was washed with 1 N aq HCl (30 mL) and brine (2 × 20 mL), dried, and evaporated to give **24a** (4.30 g, 90%): white foam; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 0.20 (s, 6H), 0.24/0.25 (2 s, 2 × 3H), 0.95/0.99 (2 s, 2 × 9H), 1.38 (s, 9H), 2.68−2.87 (m, 2H), 3.40−3.60 (m, 2H) superimposed by 3.58 (s, 3H), 4.04/4,13 (2 d, 2 × 1H, $J$ = 12) superimposed by 3.95−4.07 (m, 1H), 4.28−4.46 (m, 1H), 4.72−4.80 (m, 1H), 6.44 (d, 1H, $J$ = 2.7), 6.58 (d, 1H, $J$ = 8), 6.89 (d, 1H, $J$ = 2.7), 8.15 (d, 1H, $J$ = 8), 13.05 (br s, 1H); MS (ISN) 715.5 $(M - H)^-$.

**(f) Method H-1. Cyclization of $\omega$-Hydroxy Carboxylic Acids. Methyl (4*R*,7*S*)-7-*tert*-Butoxycarbonylamino-12,-14-bis[(*tert*-butyl)dimethylsilyloxy]-1,3,4,5,6,7,8,10-octahydro-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (25a).** To a solution of **24a** (4.3 g, 6.0 mmol) in toluene (150 mL) were added at 0 °C TPP (2.04 g, 7.8 mmol) and 95% DEAD (1.28 mL, 7.8 mmol). The reaction mixture was stirred for 15 min at 0 °C and for 12 h at 20 °C and then evaporated. The residual oil was dissolved in DCM and submitted to chromatographic purification ($SiO_2$; EtOAc/hexane 1:1) to give **25a** (3.36 g, 80%): amorphous solid; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.20 (s, 6H), 0.24/0.27 (2 s, 2 × 3H), 0.98/1.04 (2 s, 2 × 9H), 1.49 (s, 9H), 2.95−3.08 (m, 1H), 3.20 (dd, 1H, $J$ = 14, 5), 3.76 (s, 3H), 3.86/4.13 (2 d, 2 × 1H, $J$ = 10.5), 4.48 (dd, 1H, $J$ = 11.4, 2); 4.54−4.64 (m, 1H), 4.81−4.93 (m, 1H), 5.03 (dd, 1H, $J$ = 11.4, 3), 6.46/6.90 (2 d, 2 × 1H, $J$ = 2.4), 7.21 (d, 1H, $J$ = 8); MS (ISP) 699.5 $(M + H)^+$.

**Methyl(4*R*,7*S*)-7-*tert*-butoxycarbonylamino-1,3,4,5,6,7,8,-10-octahydro-12,14-dihydroxy-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (26a)** was prepared from **25a** (1.40 g, 2.0 mmol) using method A-1 (**10**): white solid (from EtOAc/hexane) (0.80 g, 85%), mp 146−150 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 1.42 (s, 9H), 2.88 (dd, 1H, $J$ = 14, 11), 3.04 (dd, 1H, $J$ = 14, 4), 3.64 (s, 3H), 3.84/4.22 (2 d, 2 × 1H, $J$ = 10), 4.29 (dd, 1H, $J$ = 10, 2), 4.30−4.44 (m, 1H), 4.57−4.72 (m, 1H), 4.75 (dd, 1H, $J$ = 10, 3), 6.50/6.71 (2 d, 2 × 1H, $J$ = 2.4), 7.39 (d, 1H, $J$ = 8), 8.25 (d, 1H, $J$ = 8), 9.57/9.77 (2 s, 2 × 1H); MS (ISP) 471.3 $(M + H)^+$. Anal. ($C_{20}H_{26}N_2O_9S\cdot 0.3H_2O$) C, H, N, S.

**Synthesis of Methyl (4*R*,7*S*)-7-*tert*-butoxycarbonylamino-1,3,4,5,6,7,8,10-octahydro-14-hydroxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (26b). 4-Nitrobenzyl 3-hydroxy-2,6-dimethylbenzoate (20b)** was prepared from 3-hydroxy-2,6-dimethylbenzoic acid[26] (**19b**, 0.67 g, 4.0 mol), using method C (**20a**): pale-yellow crystals (from EtOAc/hexane) (0.92 g, 76%), mp 124−126 °C; $^1$H NMR ($CDCl_3$) $\delta$ 2.15 (s, 3 H), 2.19 (s, 3H), 4.80 (s, 1H), 5.45 (s, 2H), 6.74/6.92 (2 d, 2 × 1H, $J$ = 8), 7.62 (d, 2H, $J$ = 9), 7.62/8.25 (2 d, 2 × 2H, $J$ = 9).

**4-Nitrobenzyl 3-[Dimethyl(thexyl)silyloxy]-2,6-dimethylbenzoate (21b).** Using method D (**21a**), **20b** (0.91 g, 3.0 mmol) was silylated with (thexyl)$Me_2SiCl$ (0.65 mL, 3.3 mmol) to give **21b** (0.99 g, 74%): white crystals (from hexane), mp 77−78 °C; $^1$H NMR $CDCl_3$) $\delta$ 0.23 (s, 6H), 0.94 (d, 6H, $J$ = 6.8), 0.96 (s, 6H), 1.76 (m, 1H), 2.11/2.19 (2 s, 2 × 3H), 5.44 (s, 1H), 6.73/6.88 (2 d, 2 × 1H, $J$ = 8), 7.59/8.23 (2 d, 2 × 2H, $J$ = 9).

**4-Nitrobenzyl 2/6-(Bromomethyl)-3-[dimethyl(thexyl)-silyloxy]-6/2-methylbenzoate (22b/c).** Using method E (**22a**), **21b** (0.89 g, 2.0 mmol) was brominated with NBS (0.36, 2.0 mmol) to give a light-yellow oil (1.02 g) consisting (NMR analysis) of **21b** (0.32 g), **22b** (0.26 g, 25%), **22c** (0.36 g, 34%), and 4-nitrobenzyl 2,6-bis(bromomethyl)-3-[dimethyl(thexyl)-silyloxy)]benzoate (0.08 g). This mixture was directly used in the next step. **22b**: $^1$H NMR ($CDCl_3$) (partial) $\delta$ 0.26 (s, 6H), 2.14 (s, 3H), 4.49 (s, 2H), 5.49 (s, 2H), 6.79/7.12 (2 d, 2 × 1H, $J$ = 8.8), 7.66/8.24 (2 d, 2 × 2H, $J$ = 9). **22c**: $^1$H NMR ($CDCl_3$) (partial) $\delta$ 0.32 (s, 6H), 2.22 (s, 3H), 4.56 (s, 2H), 5.49 (s, 2H), 6.80/7.04 (2 d, 2 × 1H, $J$ = 8.8), 7.67/8.24 (2 d, 2 × 2H, $J$ = 9). Dibromo side product: $^1$H NMR ($CDCl_3$) (partial) $\delta$ 0.33 (s, 6H), 4.50/4.59 (2 s, 2 × 2H), 5.54 (s, 2H). (A NOE was observed between the aromatic methyl group and one of the phenyl hydrogens for the compound that was assigned structure **22b**, whereas for **22c** no NOE was found.)

**Methyl *N*-(*N*-*tert*-Butoxycarbonyl-L-seryl)-*S*-[6- and 4-(dimethyl(thexyl)silyloxy)-2-((4-nitrobenzyloxycarbonyl)-3-methylbenzyl)-L-cysteinate (23b and 23c).** Using method F (**23a**), the crude bromination mixture **22b/c** [1.0 g, containing **22b** (0.25 g, 0.48 mmol) and **22c** (0.35 g, 0.67 mmol)] was reacted with **5** (0.64 g, 2.0 mmol) to give, after chromatographic separation ($SiO_2$; EtOAc/hexane 1:2), **23b** (0.26 g, 71%) and **23c** (0.39 g, 76%). **23b**: colorless foam; TLC $R_f$ = 0.31 (EtOAc/hexane 1:1); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.26/0.28 (2 s, 2 × 3H), 0.94 (d, 6H, $J$ = 6.8), 0.98 (s, 6H), 1.45 (s, 9H), 1.75 (m, 1H), 2.22 (s, 3H), 2.92 (d, 2H, $J$ = 5), 3.01 (br t, 1H), 3.65 (m, 1H), 3.70 (s, 3H), 3.81/3.89 (2 d, 2 × 1H, $J$ = 13), 4.06 (m, 1H), 4.22 (m, 1H), 4.70 (m, 1H), 5.49 (s, 1H), 5.60 (br d, 1H), 6.79/6.98 (2 d, 2 × 1H, $J$ = 8.4), 7.03 (br d, 1H), 7.67/8.25 (2 d, 2 × 2H, $J$ = 8.8) (NOE between s at 2.22 and d at 6.98). **23c**: colorless foam; TLC: $R_f$ = 0.23 (EtOAc/hexane 1:1); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.25 (s, 6H), 0.94 (d, 6H, $J$ = 6.8), 0.97 (s, 6H), 1.45 (s, 9H), 1.75 (m, 1H), 2.13 (s, 3H), 2.77 (dd, 1H, $J$ = 14, 7), 2.90 (dd, 1H, $J$ = 14, 5), 2.95 (m, 1H), 3.71 (m, 1H) superimposed by 3.70 (d, 1H, $J$ = 9.6) and 3.72 (s, 3H), 3.76 (d, 1H, $J$ = 9.6), 4.11/4.27/4.45 (3 m, 3 × 1H), 5.47 (s, 1H), 5.59 (br d, 1H), 6.77/7.01 (2 d, 2 × 1H, $J$ = 8.4), 7.18 (br d, 1H), 7.64/8.25 (2 d, 2 × 2H, $J$ = 8.8) (No NOE between s at 2.13 and d at 6.77 or 7.01).

**Methyl(4*R*,7*S*)-7-*tert*-butoxycarbonylamino-1,3,4,5,6,7,8,-10-octahydro-14-hydroxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (26b)** was prepared from **23b** (0.25 g, 0.33 mmol) by consecutively applying methods G (**24a**), H-1 (**25a**), and A-1 (**10**): white crystals (from EtOAc/hexane) (46 mg); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.41 (s, 9H), 2.12 (s, 3H), 2.89 (dd, 1H, $J$ = 14, 9), 3.08 (dd, 1H, $J$ = 14, 4), 3.52 (d, 1H, $J$ = 11), 3.63 (s, 3H), 3.93 (d, 1H, $J$ = 11), 4.17 (dd, 1H, $J$ = 11, 3), 4.31−4.43 (m, 1H), 4.50−4.64 (m, 1H), 4.93 (dd, 1H, $J$ = 11, 2), 6.80/6.97 (2 d, 2 × 1H, $J$ = 8), 7.28 (d, 1H, $J$ = 8), 8.09 (d, 1H, $J$ = 8), 9.69 (s, 1H); MS (ISP) 469.1 $(M + H)^+$. Anal. ($C_{21}H_{28}N_2O_8S$) C, H, N, S.

**Methyl(4*R*,7*S*)-7-*tert*-butoxycarbonylamino-1,3,4,5,6,7,8,-10-octahydro-12-hydroxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (26c)** was prepared from **23c** (0.38 g, 0.50 mmol), using the reaction sequence of the preparation of **26b** from **23b**: white solid (79 mg); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.41 (s, 9H), 2.03 (s, 3H), 2.80 (dd, 1H, $J$ = 14, 9), 3.07 (dd, 1H, $J$ = 14, 4), 3.48 (d, 1H, $J$ = 11), 3.63 (s, 3H), 3.81 (d, 1H, $J$ = 11), 4.22 (dd, 1H, $J$ = 11, 3), 4.28−4.40 (m, 1H), 4.47−4.60 (m, 1H), 4.83 (dd, 1H, $J$ = 11, 2), 6.82/6.98 (2 d, 2 × 1H, $J$ = 8), 7.23 (d, 1H, $J$ = 8), 8.25 (d, 1H, $J$ = 8), 9.70 (s, 1H); MS (ISP) 469.4 $(M + H)^+$. Anal. ($C_{21}H_{28}N_2O_8S$) C, H, N, S.

***tert*-Butyl (*S*)-1,3,4,5,6,7,8,10-octahydro-12,14-dihydroxy-11-methyl-6,10-dioxo-9,2,5-ben zoxathiaazacyclododecine-7-carbamate (26d)** was prepared from **11** (1.23 g, 5.0 mmol) and crude 4-nitrobenzyl 2-(bromomethyl)-3,5-bis[(*tert*-butyl)-dimethylsilyloxy]-6-methylbenzoate (**22d**)[18] (3.37 g containing ca. 2.1 g, 3.35 mmol, of **22d**) by consecutively applying methods F (**23a**), G (**24a**), H-1 (**25a**), and A-1 (**10**): white solid (from EtOAc/hexane) (110 mg), mp 215 °C (dec); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.39 (s, 9H), 1.89 (s, 3H), 2.42−2.59 (m, lH), 2.65−2.75 (m, lH), 2.98−3.15 (m, lH), 3.50−3.70 (m, 1H) superimposed by 3.58/3.66 (2 d, 2 × 1H, $J$ = 11), 4.26−4.55 (m, 3H), 4.26−4.55 (m, 3H), 6.44 (s, lH), 7.03 (d, 1H, $J$ = 7), 8.13−8.24 (m, lH), 9.45/9.49 (2 s, 2 x lH); MS (ISP) 427 (M + H)$^+$. Anal. ($C_{19}H_{26}N_2O_7S$) C, H, N, S.

**Methyl (*R*)-1,3,4,5,6,7,8,10-Octahydro-12,14-dihydroxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (26e).** Using method F (**23a**), **12** (1.35 g, 3.0 mmol) and crude **22d**[18] (2.0 g containing ca. 1.25 g, 2.0 mmol, of **22d**) were coupled and the product was purified by chromatography ($SiO_2$, EtOAc/hexane 1:2) to give a white foam (0.72 g) [TLC $R_f$ = 0.87 (EtOAc/hexane 1:1)]. To cleave off the trityl group, a solution of this material and pTsOH·$H_2O$ (20 mg) in MeOH (10 mL) was heated to 60 °C for 1 h. The solution was evaporated and the residue was chromatographed ($SiO_2$; EtOAc/hexane 1:1) to give **23e** (0.38 g): white foam; TLC $R_f$ = 0.10 (EtOAc/hexane 1:1); $^1$H NMR ($CDCl_3$) $\delta$ 0.21/0.22/0.24/0.25 (4 s, 4 × 3H), 1.00/1.01 (2 s, 2 × 9H), 2.03 (s, 3H), 2.32−2.50 (m, 2H), 2.84 (dd, 1H, $J$ = 14, 4), 3.01 (dd, 1H, $J$ = 14, 5), 3.69 (s, 3H), 3.72 (d, 1H, $J$ = 12), 3.77−3.88 (m, 3H), 4.70−4.80 (m, 1H), 5.47 (s, 2H), 6.36 (s, 1H), 6.60 (d, 1H, $J$ = 8), 7.66/8.26 (2 d, 2 × 2H, $J$ = 9). Subjecting **23e** in a consecutive manner to methods G (**24a**), H-1 (**25a**), and A-1 (**10**) afforded **26e**: white solid (48 mg), mp 184−188 °C (dec); $^1$H NMR (400 mHz, DMSO-$d_6$) $\delta$ 1.86 (s, 3H), 2.40−2.47 (m, 1H), 2.58 (dd, 1H, $J$ = 15, 11), 2.84−2.93 (m, 1H), 3.06 (dd, 1H, $J$ = 15, 4), 3.65 (s, 3H), 3.66−3.69 (m, 2H), 4.47−4.53 (m, 1H), 4.59−4.69 (m, 2H), 6.42 (s, 1H), 8.55 (d, 1H, $J$ = 9), 9.45 (s, 1H), 9.49 (s, 1H). Anal. ($C_{16}H_{19}NO_7S$) C, H, N, S.

**1,3,4,5,6,7,8,10-Octahydro-12,14-dihydroxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine (26f).** Using the procedure of the preparation of **26e**, but replacing the thiol **12** by **13**, the lactone **26f** was prepared (110 mg): white solid (from EtOAc/hexane), mp 215−220 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.87 (s, 3H), 2.53−2.66 (m, 4H), 3.26−3.34 (m, 2H), 3.63 (s, 2H), 4.49−4.59 (m, 2H), 6.41 (s, 1H), 7.65 (t, 1H, $J$ = 6), 9.41 (s, 1H), 9.47 (s, 1H). Anal. ($C_{14}H_{17}NO_5S$·0.25$H_2O$) C, H, N, S.

**4-Nitrobenzyl 3-Hydroxy-5-methoxy-2,6-dimethylbenzoate (20g).** A mixture of 4-nitrobenzyl 3,5-dihydroxy-2,6-dimethylbenzoate[18] (**20d**, 95.1 g, 0.30 mol), methyl iodide (22.5 mL, 0.36 mol), and $K_2CO_3$ (49.8 g, 0.36 mol) in acetone (0.6 L) was heated at reflux for 16 h. Insoluble material was removed by filtration, and the filtrate was diluted with EtOAc (0.5 L), washed with 1 N aq HCl and brine, dried, and evaporated. Repeated crystallizations of the crude product (from EtOAc/DCM, DCM, and DCM/hexane) afforded **20g** (32.5 g, 32.7%): white crystals, mp 157−158 °C; $^1$H NMR ($CDCl_3$) $\delta$ 2.03/2.06 (2 × 3H), 3.77 (s, 3H), 4.71 (s, 1H), 5.44 (s, 2H), 6.42 (s, 2H), 7.61/8.24 (2 d, 2 × 2H, $J$ = 8.8). In addition, 4-nitrobenzyl 3,5-dimethoxy-2,6-dimethylbenzoate (35.4 g, 34%; white solid, mp 165−167 °C) and starting material (**20d**, 12.0 g, 12.6%) were isolated.

**4-Nitrobenzyl 3-[(*tert*-Butyl)dimethylsilyloxy]-5-methoxy-2,6-dimethylbenzoate (21g).** Working similarly as described in method D (**21a**), **20g** (28.8 g, 87 mmol) was reacted with (t-Bu)$Me_2$SiCl (15.7 g, 104 mmol) to give **21g** (36.8 g, 95%): white crystals (from hexane), mp 81−82 °C; $^1$NMR ($CDCl_3$) $\delta$ 0.21 (s, 6H), 1.01 (s, 9H), 2.02/2.04 (2 s, 2 × 3H), 3.76 (s, 3H), 5.43 (s, 2H), 6.38 (s, 1H), 7.61/8.24 (2 d, 2 × 2H, $J$ = 8.8).

**4-Nitrobenzyl 3-[(*tert*-Butyl)dimethylsilyloxy]-5-methoxy-2/6-(bromomethyl)-6/2-methylbenzoate (22g/22h).** Using method E (**22a**), **21g** (13.36 g, 30 mmol) was brominated with NBS (5.34 g, 30 mmol) in $CCl_4$ (150 mL) to give an oil (15.8 g) consisting (NMR analysis) of **21g** (ca. 2.5 g), **22g** (5.06 g, 32%), **22h** (5.69 g, 36%), and 4-nitrobenzyl 2,6-bis(bromomethyl)-3-[(*tert*-butyl)dimethylsilyloxy]-5-methoxybenzoate (2.5 g). **22g**: $^1$H NMR ($CDCl_3$) $\delta$ 0.24 (s, 6H), 1.01 (s, 9H), 2.05 (s, 3H), 3.84 (s, 3H), 4.54 (s, 2H), 5.48 (s, 2H), 6.41 (s, 1H), 7.67/8.24 (2 d, 2 × 2H, $J$ = 8). **22h**: $^1$H NMR ($CDCl_3$) $\delta$ 0.30 (s, 6H), 1.05 (s, 9H), 2.04 (s, 3H), 3.78 (s, 3H), 4.53 (s, 2H), 5.48 (s, 2H), 6.39 (s, 1H), 7.67/8.24 (2 d, 2 × 2H, $J$ = 8). 2,6-Bis-(bromomethyl) side product: $^1$H NMR ($CDCl_3$) $\delta$ 0.32 (s, 6H), 1.04 (s, 9H), 3.87 (s, 3H), 4.51 (s, 2H), 5.54 (s, 2H), 6.44 (s, 1H), 7.71/8.24 (2 d, 2 × 2H, $J$ = 8).

**Methyl *N*-[*N*-*tert*-Butoxycarbonyl-L-seryl]-*S*-[6/4-[(*tert*-butyl)dimethylsilyloxy]-4/6-methoxy-3-methyl-2-(4-nitrobenzyloxycarbonyl)benzyl]-L-cysteinate (23g and 23h).** Using method F (**23a**), the crude mixture **22g/h** (15.0 g, containing ca. 4.8 g, 9.2 mmol, of **22g**, and 5.4 g, 10.3 mmol, of **22h**) was reacted with **5** (9.63 g, 30 mmol), and the product mixture was separated by chromatography ($SiO_2$; EtOAc/hexane 2:3) to give **23g** (5.15 g, 27%) and **23h** (4.90 g, 26%). **23g**: white foam; TLC $R_f$ = 0.27 (EtOAc/hexane 1:1); $^1$H NMR ($CDCl_3$) $\delta$ 0.25/0.26 (2 s, 2 × 3H), 1.02 (s, 9H), 1.45 (s, 9H), 2.05 (s, 3H), 2.91 (d, 2H, $J$ = 5.4), 3.00 (br t, 1H), 3.62−3.72 (m, 1H), 3.70 (s, 3H), 3.74 (d, 1H, $J$ = 12), 3.77 (s, 3H), 3.81 (d, 1H, $J$ = 12), 4.02−4.12 (m, 1H), 4.16−4.28 (m, 1H), 4.66−4.76 (m, 1H), 5.49 (s, 2H), 5.59 (d, 1H, $J$ = 8), 6.40 (s, 1H), 7.06 (d, 1H, $J$ = 8), 7.66/8.28 (2 d, 2 × 2H, $J$ = 8.8). **23h**: white foam; TLC $R_f$ = 0.20 (EtOAc/hexane 1:1); $^1$H NMR ($CDCl_3$) $\delta$ 0.23 (s, 6H), 1.01 (s, 9H), 1.45 (s, 9H), 2.03 (s, 3H), 2.80 (dd, 1H, $J$ = 14, 6), 2.96 (dd, 1H, $J$ = 14, 4), 3.08 (br t, 1H), 3.62−3.76 (m, 1H), 3.72 (s, 3H), 3.74 (d, 1H, $J$ = 12), 3.80 (s, 3H), 3.81 (d, 1H, $J$ = 12), 4.06−4.17 (m, 1H), 4.22−4.32 (m, 1H), 4.75−4.87 (m, 1H), 5.47 (s, 2H), 5.59 (d, 1H, $J$ = 8), 6.42 (s, 1H), 7.17 (d, 1H, $J$ = 8), 7.65/8.25 (2 d, 2 × 2H, $J$ = 9).

**Methyl(*R*)-14-[(*tert*-Butyl)dimethylsilyloxy]-1,3,4,5,6,7,8,-10-octahydro-12-methoxy-11 -methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (25g).** Using method G (**24a**), **23g** (5.15 g, 6.72 mmol) was hydrogenated. The crude hydroxy acid (24g, 3.57 g, 84%, pale-yellow foam) was cyclized using method H-1 (**25a**) to give **25g** (1.83 g, 53%): white crystals (from EtOAc/hexane), mp 156−158 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.25/0.26 (2 s, 2 × 3H), 1.02 (s, 9H), 1.41 (s, 9H), 1.96 (s, 3H), 2.81 (dd, 1H, $J$ = 14, 10), 3.06 (dd, 1H, $J$ = 14, 4), 3.51 (d, 1H, $J$ = 11), 3.62 (s, 3H), 3.77 (s, 3H), 3.85 (d, 1H, $J$ = 11), 4.17 (dd, 1H, $J$ = 11, 4), 4.33−4.41/4.49−4.63 (2 m, 2 × 1H), 4.99 (dd, 1H, $J$ = 11, 2), 6.43 (s, 1H), 7.14 (d, 1H, $J$ = 8), 8.23 (d, 1H, $J$ = 8). The structure of **25g** was confirmed by X-ray analysis.[24]

**Methyl (*R*)-1,3,4,5,6,7,8,10-octahydro-14-hydroxy-12-methoxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (26g)** was prepared from **25g** by applying method A-1 (**10**): white crystals (from EtOAc/hexane), mp 138−142 °C (dec); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.41 (s, 9H), 1.94 (s, 3H), 2.90 (dd, 1H, $J$ = 14, 10), 3.04 (dd, 1H, $J$ = 14, 4), 3.40 (d, 1H, $J$ = 11), 3.63 (s, 3H), 3.71 (s, 3H), 3.81 (d, 1H, $J$ = 11), 4.24 (dd, 1H, $J$ = 11, 4), 4.30−4.38/4.48−4.58 (2 m, 2 × 1H), 4,87 (dd, 1H, $J$ = 11, 3), 6.53 (s, 1H), 7.27 (d, 1H, $J$ = 8), 8.15 (d, 1H, $J$ = 8), 9.73 (s, 1H); MS (ISP) 499.3 (M + H)$^+$. Anal. ($C_{22}H_{30}N_2O_9S$·0.05 $H_2O$) C, H, N, S. The structure assignment of **26g** is in agreement with a $^1$H−$^{13}$C HMBC NMR experiment which showed a correlation between C(15) and the phenolic hydrogen.

**Methyl (*R*)-1,3,4,5,6,7,8,10-Octahydro-12-hydroxy-14-methoxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (26h).** By applying the reaction sequence used to prepare **26g** from **23g**, **23h** was converted to **26h**: white solid (from EtOAc/hexane), mp 125−130 °C (dec); $^1$H NMR (400 mHz, DMSO-$d_6$) $\delta$ 1.41 (s, 9H), 1.99 (s, 3H), 2.90 (dd, 1H, $J$ = 14, 9), 3.04 (dd, 1H, $J$ = 14, 4), 3.40 (d, 1H, $J$ = 11), 3.63 (s, 3H), 3.71 (s, 3H), 3.81 (d, 1H, $J$ = 11), 4.22 (dd, 1H, $J$ = 11, 4), 4.32−4.40/4.50−4.58 (2 m, 2 × 1H), 4.87 (dd, 1H, $J$ = 11, 3), 6.53 (s, 1H), 7.27 (d, 1H, $J$ = 8), 8.14 (d, 1H, $J$ = 8), 9.72 (s, 1H); MS (ISP) 499.3 (M + H)$^+$. Anal. ($C_{22}H_{30}N_2O_9S$) C, H, N, S. The structure assignment of **26h** is

in agreement with a $^1H-^{13}C$ HMBC NMR experiment which showed a correlation between C(11) and the phenolic hydrogen.

**Methyl(4*S*,7*R*)-7-*tert*-butoxycarbonylamino-1,3,4,5,6,7,8,-10-octahydro-12,14-dihydroxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (27)** was prepared from **14** (1.61 g, 5.0 mmol) and crude **22d**[18] (4.0 g containing ca. 2.50 g, 4.0 mmol, of **22d**) using sequentially methods F (**23a**), G (**24a**), H-1 (**25a**), and A-1 (**10**): white solid (from EtOAc/hexane) (85 mg), mp 125−130 °C; $[\alpha]_D = -53.8°$ ($c = 0.8$, EtOAc); $^1H$ NMR "identical" to that of **10**; MS (ISP) 485 $(M + H)^+$. Anal. ($C_{21}H_{28}N_2O_9S$) C, H, N, S.

Compounds **28** and **29** were prepared similarly.

**Methyl(4*R*,7*R*)-7-*tert*-butoxycarbonylamino-1,3,4,5,6,7,8,-10-octahydro-12,14-dihydroxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (28)** was prepared from **15** (1.61 g, 5.0 mmol) and crude **22d**[18] (3.37 g, containing ca. 2.1 g, 3.35 mmol, of **22d**): white solid (from EtOAc/hexane) (150 mg), mp 198−200 °C; $[\alpha]_D = -62.5°$ ($c = 0.7$, EtOAc); $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.40 (s, 9H), 1.88 (s, 3H), 2.58 (dd, 1H, $J = 14, 11$), 3.04 (dd, 1H, $J = 14, 4$), 3.61 (d, 1H, $J = 11$), 3.66 (s, 3H), 3.77 (d, 1H, $J = 11$), 4.30−4.49 (m, 2H), 4.53−4.73 (m, 2H), 6.44 (s, 1H), 7.05 (d, 1H, $J = 8$), 8.82 (d, 1H, $J = 8$), 9.46 (s, 1H), 9.49 (s, 1H); MS (ISP) 485 $(M + H)^+$. Anal. ($C_{21}H_{28}N_2O_9S$) C, H, N, S.

**Methyl(4*S*,7*S*)-7-*tert*-butoxycarbonylamino-1,3,4,5,6,7,8,-10-octahydro-12,14-dihydrox y-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (29)** was prepared from **16** (0.97 g, 3.0 mmol) and crude **22d**[18] (2.0 g containing ca. 1.25 g, 2.0 mmol, of **22d**): white solid (from EtOAc/hexane) (96 mg), mp 195−198 °C; $[\alpha]_D = +60.5°$ ($c = 0.7$, EtOAc); $^1H$ NMR "identical" to that obtained for **28**; MS (ISP) 485 $(M + H)^+$. Anal. ($C_{21}H_{28}N_2O_9S$) C, H, N, S.

**4-Nitrobenzyl 3-[Dimethyl(thexyl)silyloxy]-2-[(*R*)-[2-((*S*)-3-hydroxybutyrylamino)-2-methoxycarbonylethyl]-sulfanylmethyl]-5-methoxy-6-methylbenzoate (30).** Using method F (**23a**), the crude mixture **22g/h** (1.57 g, containing ca. 0.50 g, 0.96 mmol, of **22g**) was reacted with **17** (0.66 g, 3.0 mmol). The less polar product was isolated by chromatography ($SiO_2$; EtOAc/hexane 1:2) to give **30** (0.47 g, 24%): white foam; TLC $R_f = 0.20$ (EtOAc/hexane 1:1); $^1H$ NMR ($CDCl_3$) $\delta$ 0.24/0.27 (2 s, 2 × 3H), 1.02 (s, 9H), 1.19 (d, 3H, $J = 6.3$), 2.05 (s, 3H), 2.14−2.28 (m, 2H), 2.79 (dd, 1H, $J = 14, 4.4$), 3.04 (dd, 1H, $J = 14, 5.2$), 3.69 (s, 3H), 3.70 (d, 1H, $J = 12.8$), 3.78 (s, 3H), 3.88 (d, 1H, $J = 12.8$), 4.00−4.14 (m, 1H), 4.68−4.77 (m, 1H), 5.43/5.50 (2 d, 2 × 1H, $J = 14$), 6.39 (s, 1H), 6.53 (d, 1H, $J = 8$), 7.66/8.28 (2 d, 2 × 2H, $J = 8.8$).

**Methyl (4*R*,8*R*)-1,3,4,5,6,7,8,10-Octahydro-14-hydroxy-12-methoxy-8,11-dimethyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (32).** Using consecutively methods G (**24a**), H-1 (**25a**), and A-1 (**10**), **30** (0.46 g, 0.7 mmol) was converted to **32** (0.07 g): white solid (from EtOAc/hexane), mp 217−222 °C; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.32 (d, 3H, $J = 6.4$), 1.91 (s, 3H), 2.48 (dd, 1H, $J = 14, 2$), 2.62 (dd, 1H, $J = 14, 10.5$), 2.69 (dd, 1H, $J = 14, 12$), 3.10 (dd, 1H, $J = 14, 4.6$), 3.34 (d, 1H, $J = 11$), 3.65 (s, 3H), 3.72 (s, 3H), 3.99 (d, 1H, $J = 11$), 4.65−4.73 (m, 1H), 5.65−5.75 (m, 1H), 6.50 (s, 1H), 8.45 (d, 1H, $J = 8.8$), 9.68 (s, 1H); MS (ISP) 398 $(M + H)^+$. Anal. ($C_{18}H_{23}NO_7S \cdot 0.2H_2O$) C, H, N.

**Methyl (4*R*,8*S*)-1,3,4,5,6,7,8,10-octahydro-14-hydroxy-12-methoxy-8,11-dimethyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (33)** was obtained from the crude mixture **22g/h** (3.15 g, containing ca. 1.0 g, 1.92 mmol, of **22g**) and **18** (1.33 g, 6.0 mmol) in analogy to the preparation of **32**: white solid (from EtOAc/hexane) (0.11 g), mp 123−125 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.41 (d, 3H, $J = 7$), 1.91 (s, 3H), 2.45 (dd, 1H, $J = 14, 9$), 2.75 (dd, 1H, $J = 14, 4$) superimposed by 2.77 (dd, 1H, $J = 14, 12$), 3.14 (dd, 1H, $J = 14, 4.5$), 3.31 (d, 1H, $J = 10$), 3.62 (s, 3H), 3.71 (s, 3H), 3.92 (d, 1H, $J = 10$), 4.28/5.46 (2 m, 2 × 1H), 6.46 (s, 1H), 8.56 (d, 1H, $J = 7$), 9.68 (s, 1H); MS (ISP) 398 $(M + H)^+$. Anal. ($C_{18}H_{23}NO_7S$) C, H, N, S.

**(*tert*-Butyl)dimethylsilyl 3-[(*tert*-Butyl)dimethylsilyloxy]-2-methylbenzoate (35).** A mixture of 3-hydroxy-2-methylbenzoic acid (**34**) (19.0 g, 0.125 mol), (t-Bu)$Me_2$SiCl (39.5 g, 0.263 mol), and imidazole (34 g, 0.5 mol) in DMF (100 mL) was heated to 70 °C for 6 h. The cold mixture was poured onto ice and then extracted with hexane (2 × 200 mL). The organic layer was washed with $H_2O$, 5% aq $NaHCO_3$, $H_2O$, and brine, dried, and evaporated, and the remaining oil was crystallized from hexane to give **35** (41.9 g, 88%): white crystals, mp 89−90 °C; $^1$NMR ($CDCl_3$) $\delta$ 0.21 (s, 6H), 0.37 (s, 6H), 1.01 (s, 9H), 1.02 (s, 9H), 2.46 (s, 3H), 6.93 (dd, 1H, $J = 8, 1$), 7.09 (t, 1H, $J = 8$), 7.51 (dd, 1H, $J = 8, 1$).

**(*tert*-Butyl)dimethylsilyl 3-[(*tert*-Butyl)dimethylsilyloxy]-2-bromomethylbenzoate (36).** Using method E (**22a**), **35** (39.8 g, 104 mmol) was brominated with NBS (18.6 g, 104 mmol) for 3 h to give **36** (40.2 g, 84%): white crystals (from hexane), mp 73−74 °C; $^1$NMR ($CDCl_3$) $\delta$ 0.30/0.40 (2 s, 2 × 6H), 1.02/1.07 (2 s, 2 × 9H), 5.08 (s, 2H), 6.99 (dd, 1H, $J = 8, 1$), 7.22 (t, 1H, $J = 8$), 7.56 (dd, 1H, $J = 8, 1$).

**Methyl *N*-(*N*-*tert*-Butoxycarbonyl-L-seryl)-*S*-[6-[(*tert*-butyl)dimethylsilyloxy]-2-carboxylbenzyl]-L-cysteinate (40).** Using method F (**23a**), **36** (2.5 g, 5.44 mmol) was reacted with **5** (1.77 g, 5.5 mmol). The reaction solution was washed with NaOAc/AcOH buffer (pH 4) and with brine, dried, and evaporated. The residue was chromatographed ($SiO_2$; EtOAc, then EtOAc−EtOH 5:1) to give **40** (1.0 g, 31%): white crystals (from DCM/hexane), mp 130−135 °C (dec); $^1H$ NMR (DMSO-$d_6$) $\delta$ 0.24/0.26 (2 s, 2 × 3H), 1.01 (s, 9H), 1.40 (s, 9H), 2.74−2.95 (m, 2H), 3.50−3.68 (m, 2H) superimposed by 3.60 (s, 3H), 4.03−4.17 (m, 2H), 4.40 (d, 1H, $J = 11$), 4.48−4,62 (m, 1H), 6.80 (d, 1H, $J = 8$), 7.09 (t, 1H, $J = 8$), 7.17 (d, 1H, $J = 8$), 7.27 (d, 1H, $J = 8$), 8.56 (d, 1H, $J = 8$).

**Methyl(4*R*,7*S*)-7-*tert*-Butoxycarbonylamino-1,3,4,5,6,7,8,-10-octahydro-14-hydroxy-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (43).** Using methods H-1 (**25a**) and A-1 (**10**), **40** (0.73 g, 1.25 mmol) was cyclized and the silyl group of the purified product **42** was subsequently cleaved off to give **43** (63 mg, 11%): white foam; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.42 (s, 9H), 2.92 (dd, 1H, $J = 14, 10$), 3.08 (dd, 1H, $J = 14, 4$), 3.64 (s, 3H), 3.93 (d, 1H, $J = 10$), 4.23 (dd, 1H, $J = 10, 2$), 4.32−4.44 (m, 2H), 4.57−4.83 (m, 2H), 7.04 (d, 1H, $J = 8$), 7.18 (t, 1H, $J = 8$), 7.27 (d, 1H, $J = 8$), 7.45 (d, 2H, $J = 8$), 8.31 (d, 2H, $J = 8$), 9.98 (s, 1H); HRMS calcd for ($C_{20}H_{26}N_2O_8SNa^+$) 477.1308, found 477.1306.

**Methyl (4*R*,7*S*)-7-Amino-14-[(*tert*-butyl)dimethylsilyloxy]-1,3,4,5,6,7,8,10-octahydro-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (44).** A solution of **42** (0.4 g, 0.7 mmol) in TFA (4 mL) was stirred at 0 °C for 0.5 h. The solvent was evaporated. The residue was dissolved in DCM and the solution was washed with aq $NaHCO_3$, dried, and evaporated. The residual material was chromatographed (EtOAc) to give **44** (165 mg, 51%): white foam; $^1H$ NMR (DMSO-$d_6$) $\delta$ 0.25/0.26 (2 s, 2 × 3H), 1.03 (s, 9H), 2.31 (br s, 2H), 2.91 (dd, 1H, $J = 14, 11$), 3.06 (dd, 1H, $J = 14, 4$), 3.60−3.72 (m, 1H), 3.66 (s, 3H), 3.91 (d, 1H, $J = 10$), 4.19 (dd, 1H, $J = 11, 3$), 4.68 (d, 1H, $J = 10$), 4.69−4.82 (m, 1H), 4.89 (dd, 1H, $J = 11, 2$), 7.08 (dd, 1H, $J = 8, 1$), 7.28 (t, 1H, $J = 8$), 7.40 (dd, 1H, $J = 8, 1$), 8.62 (d, 1H, $J = 8$).

**Methyl (4*R*,7*S*)-7-[[*N*-(*tert*-Butoxycarbonyl)-L-seryl]-amino]-14-hydroxy-1,3,4,5,6,7,8,10-octahydro-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (45).** A mixture of **44** (147 mg, 0.31 mmol), Boc-L-Ser-OH (64 mg, 0.31 mmol), and EDC (60 mg, 0.31 mmol) in MeCN (3 mL) was stirred at 0 °C for 2 h. The solution was diluted with EtOAc, washed with 5% aq $NaHCO_3$, 0.1 N aq HCl, and brine, dried, and evaporated. The residue was stirred with $NH_4F$ (46 mg, 1.25 mmol) in MeOH (3 mL) for 0.5 h at 20 °C. The solution was diluted with EtOAc, washed with 5% aq NaCl, dried, and evaporated. The residue was chromatographed ($SiO_2$; EtOAc) to give **45** (118 mg, 70%): white crystals (from DCM/$Et_2O$), mp 120−130 °C (dec); $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.36 (s, 9H), 2.69 (dd, 1H, $J = 14, 11$), 3.12 (dd, 1H, $J = 14, 4$), 3.44−3.72 (m, 2H), 3.63 (s, 3H), 3.95 (d, 1H, $J = 10$), 4.16−4.28 (m, 1H), 4.34 (dd, 1H, $J = 11, 2$), 4.44 (d, 1H, $J = 10$), 4.54−4.70 (m, 2H), 4.85 (dd, 1H, $J = 11, 3$), 5.53 (t, 1H, $J = 5$), 6.84 (d, 1H, $J = 7$), 7.08 (d, 1H, $J = 7$), 7.20 (t, 1H, $J = 7$), 7.36 (d, 1H, $J = 7$),

8.55/8.60 (2 d, 2 × 1H, $J$ = 8), 10.02 (s, 1H); HRMS calcd for ($C_{23}H_{31}N_3O_{10}SNa^+$) 564.1628, found 564.1632.

**(*tert*-Butyl)dimethylsilyl 3-Nitro-2-methylbenzoate (38).** To 3-nitro-2-methylbenzoic acid (**37**, 1.81 g, 10.0 mmol) and (t-Bu)$Me_2$SiCl (1.66 g, 11 mmol) in DMF (8 mL) was added $Et_3N$ (1.53 mL), and the mixture was stirred at 20 °C for 24 h. Hexane (80 mL) was added and the mixture was washed with 5% aq $NaHCO_3$ and with brine. The organic layer was dried and evaporated, and the remaining oil was crystallized from hexane to give **38** (1.62 g, 55%): white crystals, mp 60−62 °C; $^1$H NMR ($CDCl_3$) $\delta$ 0.40 (s, 6H), 1.01 (s, 9H), 2.65 (s, 3H), 7.38 (t, 1H, $J$ = 8), 7.83/8.04 (2 d, 2 × 1H, $J$ = 8).

**(*tert*-Butyl)dimethylsilyl 2-(Bromomethyl)-3-nitrobenzoate (39).** Using method E (**22a**), **38** (1.48 g, 5.0 mmol) was brominated with NBS (0.89 g, 5.0 mmol) to give **39** (1.39 g, 74%): white crystals (from hexane), mp 98−100 °C; $^1$H NMR ($CDCl_3$) $\delta$ 0.43 (s, 6H), 1.02 (s, 9H), 5.20 (s, 2H), 7.53 (t, 1H, $J$ = 8), 7.94/8.11 (2 d, 2 × 1H, $J$ = 8).

**Methyl *N*-(*N*-*tert*-Butoxycarbonyl-L-seryl)-*S*-[6-nitro-2-carboxylbenzyl]-L-cysteinate (41).** Using method F (**23a**), **39** (1.12 g, 3.0 mmol) was reacted with **5** (0.97 g, 3.0 mmol). The solvents were evaporated, and the residue was stirred with $NH_4F$ (0.3 g, 8.1 mmol) in MeOH (40 mL) for 0.5 h at 20 °C. The solvent was evaporated and the residue was taken up in $Et_2O$ (50 mL). The solution was extracted with aq $NaHCO_3$ (45 mL), and the basic extracts were acidified (pH 2) by the addition of 3 N aq HCl. The precipitated product was extracted with EtOAc, and the organic layer was washed with brine, dried, and evaporated to give **41** (0.69 g, 46%): white foam; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.38 (s, 9H), 2.72−2.92 (m, 2H), 3.40−3.60 (m, 2H) superimposed by 3.59 (s, 3H), 3.94−4.06 (m, 1H), 4.23/4.33 (2 d, 2 × 1H, $J$ = 12), 4.38−4.46 (m, 1H), 4.80 (br s, 1H), 6.62 (d, 1H, $J$ = 8), 7.61 (t, 1H, $J$ = 8), 8.04 (d, 2H, $J$ = 8), 8.19 (d, 1H, $J$ = 8), 13.75 (br s, 1H).

**Methyl (4*R*,7*S*)-14-Amino-7-*tert*-butoxycarbonylamino-1,3,4,5,6,7,8,10-octahydro-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (47).** Using method H-1 (**25a**), **41** (0.6 g, 1.2 mmol) was cyclized and the purified product (**46**, 0.25 g, 43%) was hydrogenated in EtOAc (12 mL) for 18 h at 20 °C in the presence of 5% Pd−C (0.06 g). The catalyst was filtered off and the solution was evaporated to give **47** (0.17 g, 31% from 41): white foam; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.43 (s, 9H), 2.97 (dd, 1H, $J$ = 14, 10), 3.13 (dd, 1H, $J$ = 14, 4), 3.63 (d, 1H, $J$ = 11), 3.64 (s, 3H), 4.20−4.50 (m, 3H), 4.60−4.80 (m, 2H), 5.30 (s, 2H), 6.86 (d, 1H, $J$ = 8), 6.95−7.10 (m, 2H), 7.48 (d, 1H, $J$ = 8), 8.14 (d, 1H, $J$ = 8); HRMS calcd for ($C_{20}H_{27}N_3O_7SNa^+$) 476.1468, found 476.1470.

**Methyl (4*R*,7*S*)-14-Amino-7-[[*N*-(*tert*-butoxycarbonyl)-L-seryl]amino]-1,3,4,5,6,7,8,10-octahydro-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (48).** In analogy to the preparation of **45**, **47** (91 mg, 0.2 mmol) was converted to **48** (68 mg, 63%): white foam; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.36 (s, 9H), 2.72 (dd, 1H, $J$ = 14, 11), 3.19 (dd, 1H, $J$ = 14, 4), 3.45−3.75 (m, 4H) superimposed by 3.63 (s, 3H), 4.15−4.27 (m, 1H), 4.33−4.45 (m, 2H), 4.50−4.70 (m, 2H), 4.76 (dd, 1H, $J$ = 11, 2), 5.30 (s, 2H), 5.43 (t, 1H, $J$ = 5), 6.80 (d, 1H, $J$ = 8), 6.87 (t, 1H, $J$ = 6), 7.06−7.13 (m, 2H), 8.44 (d, 1H, $J$ = 8), 8.57 (d, 1H, $J$ = 8); HRMS calcd for ($C_{23}H_{32}N_4O_9SNa^+$) 563.1788, found 563.1792.

***tert*-Butyl (4*R*,7*S*)-1,3,4,5,6,7,8,10-Octahydro-14-hydroxy-4-(hydroxymethyl)-12-methoxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-7-carbamate (49).** To a stirred solution of **25g** (123 mg, 0.20 mmol) in MeOH−THF (1:1, 1.4 mL) was added at 0 °C over 5 min $NaBH_4$ (78 mg, 2.0 mmol), and stirring was continued for 40 min. The mixture was diluted with EtOAc and washed with 1 N aq HCl and brine. The organic layer was dried and evaporated. The residue was treated with $NH_4F$/MeOH (method A-1) to give **49** (56 mg, 60%): white crystals (from EtOAc/hexane), mp 135−140 °C (dec); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.41 (s, 9H), 1.92 (s, 3H), 2.51 (dd, 1H, $J$ = 14, 11), 2.83 (dd, 1H, $J$ = 14, 4), 3.26−3.42 (m, 2H), 3.45 (d, 1H, $J$ = 11), 3.72 (s, 3H), 3.85 (d, 1H, $J$ = 11), 4.15 (dd, 1H, $J$ = 11, 2.5), 4.22−4.34 (m, 1H), 4.79 (t, 1H, $J$ = 5), 4.95 (dd, 1H, $J$ = 11, 2), 6.51 (s, 1H), 7.13 (d, 1H, $J$ = 8), 7.59 (d, 1H, $J$ = 8), 9.68 (s, 1H); MS (ISP) 471 (M + H)$^+$. Anal. ($C_{21}H_{30}N_2O_8S$·0.4$H_2O$) C, H, N.

**(4*R*,7*S*)-7-(*tert*-Butoxycarbonylamino)-1,3,4,5,6,7,8,10-octahydro-14-hydroxy-12-methoxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylic Acid (50).** A solution of **26g** (0.25 g, 0.5 mmol) in 80% aq THF (6.5 mL) was adjusted to pH 12 by the addition of 1 N NaOH (ca. 0.4 mL), and 0.1 N NaOH (6 mL) was then added at 20 °C at such a rate that the pH did not exceed 12 (autotitration; 20 min). The pH was lowered to 9 by the addition of 1 N aq HCl, and the solution was washed with EtOAc. The aqueous phase was set to pH 2.5 and subsequently extracted with EtOAc. The organic layer was washed with $H_2O$, dried, and evaporated to give **50** (0.21 g, 88%): white crystals (from acetone/hexane), mp 172−175 °C (dec); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.41 (s, 9H), 1.94 (s, 3H), 2.91 (dd, 1H, $J$ = 14, 8), 3.05 (dd, 1H, $J$ = 14, 4), 3.32 (d, 1H, $J$ = 11), 3.72 (s, 3H), 3.92 (d, 1H, $J$ = 11), 4.18 (dd, 1H, $J$ = 11, 2.5), 4.26−4.36/4,40−4.52 (2 m, 2 × 1H), 4.92 (dd, 1H, $J$ = 11, 2), 6.51 (s, 1H), 7.38 (d, 1H, $J$ = 7), 7.89 (d, 1H, $J$ = 7), 9.72 (s, 1H), 13.10 (br s, 1H); MS (ISN) 483.4 (M − H)$^-$. Anal. ($C_{21}H_{28}N_2O_9S$) C, H, N, S.

***tert*-Butyl (4*R*,7*S*)-4-Carbamoyl-1,3,4,5,6,7,8,10-octahydro-14-hydroxy-12-methoxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-7-carbamate (51).** A solution of **26g** (0.2 g, 0.4 mmol) in 5.5 N $NH_3$−MeOH (12 mL) was kept at 20 °C for 16 h. The solvent was evaporated and the residue crystallized from MeOH/$Et_2O$ to give **51** (0.13 g, 70%): white solid; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.41 (s, 9H), 1.93 (s, 3H), 2.76 (dd, 1H, $J$ = 14, 9), 3.02 (dd, 1H, $J$ = 14, 4), 3.36 (d, 1H, $J$ = 11), 3.72 (s, 3H), 3.93 (d, 1H, $J$ = 11), 4.17 (dd, 1H, $J$ = 12, 3), 4.19−4.30/4.36−4.48 (2 m, 2 × 1H), 4.96 (dd, 1H, $J$ = 12, 2), 6.50 (s, 1H), 7.27/7.40 (2 s, 2 × 1H), 7.53 (d, 1H, $J$ = 7), 7.88 (d, 1H, $J$ = 8), 9.71 (s, 1H); MS (ISP) 484.4 (M + H)$^+$. Anal. ($C_{21}H_{29}N_3O_8S$) C, H, N, S.

**(4*R*,7*S*)-7-*tert*-Butoxycarbonylamino-14-hydroxy-12-methoxy-11-methyl-6,10-dioxo-1,3,4,5,6,7,8,10-octahydro-9,2,5-benzoxathiaazacyclododecine-4-carboxylic Acid Allylamide (52).** A mixture of **50** (97 mg, 0.2 mmol), *N*-hydroxysuccinimide (35 mg, 0.3 mmol), and EDC (58 mg, 0.3 mmol) in MeCN (6 mL) was stirred at 0 °C for 3 h. A solution of allylamine (0.03 mL, 0.4 mmol) in MeCN (1 mL) was added, and stirring was continued for 1.5 h at 20 °C. The mixture was diluted with EtOAc, washed with 0.1 N aq HCl, 5% aq $NaHCO_3$, and brine, dried, and evaporated. The residue was chromatographed ($SiO_2$; EtOAc−hexane 1:2), and the silyl protecting groups in the purified product were cleaved off using method A-1 (**10**) to give **52** (30 mg, 29%): white solid (from EtOAc/hexane); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.41 (s, 9H), 1.93 (s, 3H), 2.72 (dd, 1H, $J$ = 14, 10), 3.03 (dd, 1H, $J$ = 14, 4), 3.42 (d, 1H, $J$ = 11), 3.62−3.74 (m, 2H) superimposed by 3.72 (s, 3H), 3.92 (d, 1H, $J$ = 11), 4.18 (dd, 1H, $J$ = 11, 3), 4.23−4.34/4.44−4.58 (2 m, 2 × 1H), 4.96 (dd, 1H, $J$ = 11, 2), 5.01 (d, 1H, $J$ = 10), 5.08 (d, 1H, $J$ = 18), 5.68−5.86 (m, 1H), 6.51 (s, 1H), 7.48 (d, 1H, $J$ = 7), 7.96 (d, 1H, $J$ = 8), 8.06 (t, 1H, $J$ = 5), 9.71 (s, 1H); MS (ISP) 524.5 (M + H)$^+$. Anal. ($C_{24}H_{33}N_3O_8S$) C, H, N, S.

**Methyl (4*R*,7*S*)-7-Amino-1,3,4,5,6,7,8,10-octahydro-12,-14-dihydroxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (54).** A solution of **10** (14 mg, 0.03 mmol) in TFA (0.3 mL) was stirred at 0 °C for 0.5 h. The solution was diluted with $H_2O$ (3 mL), and the pH was set to 4 by the addition of 2 N NaOH. The solution was subjected to reverse phase column chromatography (MCI-Gel CHP20P, Mitsubishi Ltd.) using 0.1% aq AcOH/0−15% MeCN as eluent. The product fraction was lyophilized to give **54** (3 mg, 27%): white powder; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.87 (s, 3H) superimposed by 1.88 (br s, 2H), 2.85 (dd, 1H, $J$ = 15, 10), 3.06 (dd, 1H, $J$ = 15, 4), 3.39 (d, 1H, $J$ = 11), 3.66 (s, 3H), 3.82 (d, 1H, $J$ = 11), 4.09 (dd, 1H, $J$ = 11, 3), 4.58−4.66 (m, 1H), 5.11 (dd, 1H, $J$ = 11, 2), 6.46 (s, 1H), 8.46 (d, 1H, $J$ = 7), 9.55 (br s, 2H); HRMS calcd for ($C_{16}H_{20}N_2O_7SH^+$) 385.1069, found 385.1065.

**Methyl (4*R*,7*S*)-7-Acetamido-1,3,4,5,6,7,8,10-octahydro-12,14-dihydroxy-11-methyl-6,10-dioxo-9,2,5-benzoxathia-**

**azacyclododecine-4-carboxylate (55).** A solution of **53**[18] (0.73 g, 1.2 mmol) in $Ac_2O$ (20 mL) and pyridine (0.1 mL) was heated to 60 °C for 1 h. The solvents were evaporated, and the remaining oil was subjected to method A-1 (**10**) to give **55** (0.39 g, 76%): white crystals (from MeOH/$Et_2O$), mp 140−145 °C (dec); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.90 (s, 3H), 1.94 (s, 3H), 2.88 (dd, 1H, $J$ = 14, 11), 3.08 (dd, 1H, $J$ = 14, 4) 3.50 (d, 1H, $J$ = 11), 3.63 (s, 3H), 3.76 (d, 1H, $J$ = 11), 4.08 (dd, 1H, $J$ = 12, 4), 4.37−4.49/4.61−4.70 (2 m, 2 × 1H), 5.07 (dd, 1H, $J$ = 12, 2), 6.45 (s, 1H), 8.25 (d, 1H, $J$ = 8), 8.29 (d, 1H, $J$ = 8), 9.49/9.51 (2 s, 2 × 1H); MS (ISP) 427 $(M + H)^+$. Anal. ($C_{18}H_{22}N_2O_8S$) C, H, N, S.

**Methyl (4*R*,7*S*)-7-[(2*S*,3*R*)-1-(*tert*-Butoxycarbonyl)-3-hydroxy-2-pyrrolidinecarboxamido]-1,3,4,5,6,7,8,10-octahydro-12,14-dihydroxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (57).** The acylation of the amine **53** with Boc-L-3cHyp-OH (EDC/MECN/0 °C) was previously described.[18] The silyl protecting groups in the purified coupling product (83 mg, 0.1 mmol) were cleaved off using method A-1 (**10**) to give **57** (37 mg, 56% from **53**): white solid (from MeOH/EtOAc/hexane), mp 180−183 °C (dec); $^1$H NMR (DMSO-$d_6$) two rotamers (ca. 5:4), data of major rotamer, $\delta$ 1.30 (s, 9H), 1.74−2.00 (m, 2H) superimposed by 1.89 (s, 3H), 2.58 (dd, 1H, $J$ = 14, 12), 3.04 (dd, 1H, $J$ = 14, 4), 3.06−3.23 (m, 1H), 3.40−3.58 (m, 1H) superimposed by 3.48 (d, 1H, $J$ = 11), 3.60 (s, 3H), 3.87 (d, 1H, $J$ = 11), 3.97 (dd, 1H, $J$ = 11, 3), 4.32−4.70 (m, 4H), 5.52 (dd, 1H, $J$ = 11, 2), 6.04 (d, 1H, $J$ = 3), 6.46 (s, 1H), 8.38 (d, 1H, $J$ = 8), 8.49 (d, 1H, $J$ = 7), 9.52 (s, 2H); HRMS calcd for ($C_{26}H_{35}N_3O_{11}SNa^+$) 620.1890, found 620.1890.

Starting from **53** (0.1 mmol), but replacing in the above procedure Boc-L-3cHyp-OH by either Boc-L-Pro-OH, Boc-D-3cHyp-OH,[18] Boc-L-Ser-OH, Boc-D-Ser-OH, or $Ph_3COCH_2CH_2$-COOH (**82b**), respectively, the derivatives **56**, **58**, **59**, **60**, and, after cleavage of the trityl protecting group as described in the synthesis of **23e**, **61** were prepared, respectively.

**Methyl (4*R*,7*S*)-7-[(*S*)-1-(*tert*-butoxycarbonyl)-2-pyrrolidinecarboxamido]-1,3,4,5,6,7,8,10-octahydro-12,14-dihydroxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (56):** white solid (from MeOH/EtOAc/hexane, 11 mg, 19%), mp 208−210 °C; $^1$H NMR (DMSO-$d_6$) two rotamers (3:2), data of major rotamer, $\delta$ 1.72−2.22 (m, 4H) superimposed by 1.91 (s, 3H), 2.89 (dd, 1H, $J$ = 14, 9), 3.09 (dd, 1H, $J$ = 14, 4), 3.24−3.41 (m, 2H), 3.48 (d, 1H, $J$ = 11), 3.62 (s, 3H), 3.76 (d, 1H, $J$ = 11), 4.14 (dd, 1H, $J$ = 11, 4), 4.22−4.38 (m, 2H), 4.64−4.75 (m, 1H), 4.93 (dd, 1H, $J$ = 11, 2.5), 6.45 (s, 1H), 8.28 (d, 1H, $J$ = 7), 8.40 (d, 1H, $J$ = 8), 9.52/9.55 (2 s, 2 × 1H); HRMS calcd for ($C_{26}H_{35}N_3O_{10}SNa^+$) 604.1941, found 604.1944.

**Methyl (4*R*,7*S*)-7-[(2*R*,3*S*)-1-(*tert*-butoxycarbonyl)-3-hydroxy-2-pyrrolidinecarboxami do]-1,3,4,5,6,7,8,10-octahydro-12,14-dihydroxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (58)**: white solid (from MeOH/EtOAc/hexane, 32 mg, 54%), mp 193−195 °C (dec); $^1$H NMR (DMSO-$d_6$) two rotamers (5:4), data of major rotamer, $\delta$ 1.30 (s, 9H), 1.74−1.96 (m, 2H) superimposed by 1.85 (s, 3H), 2.63 (dd, 1H, $J$ = 14, 12), 3.30−3.52 (m, 4H), 3.61 (d, 1H, $J$ = 11), 3.63 (s, 3H), 4.00−4.17 (m, 1H), 4.17 (d, 1H, $J$ = 5), 4.39−4.53 (m, 1H), 4.75−4.87 (m, 1H), 5.19 (dd, 1H, $J$ = 12, 2), 5.59−5.72 (m, 1H), 6.45 (s, 1H), 7.56 (d, 1H, $J$ = 8), 8.38 (d, 1H, $J$ = 8), 9.53/9.55 (2 s, 2 × 1H); HRMS calcd for ($C_{26}H_{35}N_3O_{11}SNa^+$) 620.1890, found 620.1893.

**Methyl (4*R*,7*S*)-7-[(*S*)-2-(*tert*-butoxycarbonylamino)-3-hydroxypropionamido]-1,3,4,5,6,7,8,10-octahydro-12,14-dihydroxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (59):** white solid (from MeOH/$Et_2O$/hexane, 22 mg, 38%); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.36 (s, 9H), 1.90 (s, 3H), 2.72 (dd, 1H, $J$ = 14, 12), 3.08 (dd, 1H, $J$ = 14, 4), 3.46−3.72 (m, 2H) superimposed by 3.49 (d, 1H, $J$ = 11) and 3.61 (s, 3H), 3.80 (d, 1H, $J$ = 11), 4.10 (dd, 1H, $J$ = 12, 3), 4.14−4.28 (m, 1H), 4.39−4.57 (m, 1H), 4.59−4.69 (m, 1H), 5.21−5.31 (m, 2H), 6.46 (s, 1H), 6.83 (d, 1H, $J$ = 9), 8.31 (d, 1H, $J$ = 7), 8.37 (d, 1H, $J$ = 9), 9.51/9.53 (2 s, 2 × 1H); HRMS calcd for ($C_{24}H_{33}N_3O_{11}SNa^+$) 594.1733, found 594.1733.

**Methyl (4*R*,7*S*)-7-[(*R*)-2-(*tert*-butoxycarbonylamino)-3-hydroxypropionamido]-1,3,4,5,6,7,8,10-octahydro-12,14-dihydroxy-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (60):** white solid (from $Et_2O$/hexane, 13 mg, 23%); $^1$H NMR (DMSO-$d_6$) two rotamers (5:1), data of major rotamer, $\delta$ 1.34 (s, 9H), 1.89 (s, 3H), 2.71 (dd, 1H, $J$ = 14, 12), 3.11 (dd, 1H, $J$ = 14, 4), 3.48 (d, 1H, $J$ = 11), 3.50−3.65 (m, 2H) superimposed by 3.61 (s, 3H), 3.74 (d, 1H, $J$ = 11), 4.00−4.16 (m, 2H), 4.42−4.58 (m, 1H), 4.58−4.72 (m, 1H), 5.02 (t, 1H, $J$ = 6), 5.08−5.19 (m, 1H), 6.45 (s, 1H), 6.97 (d, 1H, $J$ = 8), 8.08 (d, 1H, $J$ = 9), 8.24 (d, 1H, $J$ = 9), 9.51/9.53 (2 s, 2 × 1H); MS (ISP) 572 $(M + H)^+$; HRMS calcd for ($C_{24}H_{33}N_3O_{11}SNa^+$) 594.1733, found 594.1733.

**Methyl (4*R*,7*S*)-1,3,4,5,6,7,8,10-octahydro-12,14-dihydroxy-7-(3-hydroxypropionamid o)-11-methyl-6,10-dioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (61):** white solid (from MeOH/$Et_2O$/hexane, 20 mg, 43%); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.90 (s, 3H), 2.28−48 (m, 2H), 2.81 (dd, 1H, $J$ = 14, 11), 3.08 (dd, 1H, $J$ = 14, 4), 3.49 (d, 1H, $J$ = 11), 3.56−3.70 (m, 2H) superimposed by 3.62 (s, 3H), 3.78 (d, 1H, $J$ = 11), 4.11 (dd, 1H, $J$ = 12, 3), 4.38−4.50/4.60−4.70 (2 m, 2 × 1H), 4.74 (t, 1H, $J$ = 5), 5.11 (dd, 1H, $J$ = 12, 2), 6.45 (s, 1H), 8.25 (d, 1H, $J$ = 8), 8.29 (d, 1H, $J$ = 8), 9.51/9.53 (2 s, 2 × 1H); HRMS calcd for ($C_{19}H_{24}N_2O_9SNa^+$) 457.1281, found 457.1281.

**Preparation of Building Blocks 76−78. Methyl 2-Formyl-3,5-dimethoxy-6-methylbenzoate (63).** To a solution of DMF (0.34 L, 4.41 mol) in DCM (1 L) was slowly added $POCl_3$ (0.404 L, 4.41 mol). The mixture was stirred for 1.5 h at 20 °C. A solution of methyl 3,5-dimethoxy-2-methylbenzoate [26] (**62**, 618 g, 2.95 mol) in DCM (0.2 L) was added over 10 min. After being heated for 72 h at reflux temperature, the reaction mixture was cooled and poured into ice−water (3 L). The mixture was extracted with DCM (3.6 L), and the organic layer was washed with aq $Na_2CO_3$ (2 L) and $H_2O$ (2 × 2 L), dried, and evaporated. The solid residue was recrystallized from EtOAc/hexane to give **63** (646 g, 92%): white solid, mp 164−165 °C; $^1$H NMR ($CDCl_3$) $\delta$ 2.04 (s, 3H), 3.93 (s, 3H), 3.95 (s, 3H), 6.43 (s, 1H), 10.28 (s, 1H).

**Methyl 2-Formyl-3-hydroxy-5-methoxy-6-methylbenzoate (64).** A 1 M $BCl_3$−DCM solution (0.8 L) was added over 40 min at 5−10 °C to a solution of **63** (95.3 g, 0.40 mol) in DCM (0.25 L). The mixture was warmed to 20 °C over 0.5 h, and stirring was continued for 4 h. The clear solution was poured into ice−water (1.5 L), and the layers were separated. The organic layer was washed with $H_2O$, dried, and evaporated, and the solid residue was recrystallized from EtOAc/hexane to give **64** (77.3 g, 86%): white crystals, mp 118−119 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.91 (s, 3H), 3.80 (s, 3H), 3.86 (s, 3H), 6.58 (s, 1H), 10.08 (s, 1H), 11.07 (s, 1H).

**(*RS*)-3,4-Dihydroxy-6-methoxy-7-methyl-1,3-dihydroisobenzofuran-1-one (65).** A stirred mixture of **64** (44.8 g, 0.20 mol) and 2.5 N aq KOH (0.2 L) was warmed to 75 °C over 0.5 h and subsequently cooled to 5 °C. Upon addition of 10 N aq HCl, a precipitate formed which was isolated by filtration, washed with $H_2O$, and dried. Trituration with EtOAc/hexane afforded pseudoacid **65** (40.1 g, 98%): white solid, mp >255 °C (dec); $^1$H NMR (DMSO-$d_6$) $\delta$ 2.30 (s, 3H), 3.79 (s, 3H), 6.45 (d, 1 H, $J$ = 8.5), 6.71 (s, 1H), 7.64 (d, 1 H, $J$ = 8.5), 10.08 (s, 1H).

**Allyl 2-Formyl-3-hydroxy-5-methoxy-6-methylbenzoate (66).** To a solution of **65** (42.0 g, 0.2 mol) in DMF (1 L) was added *N*,*N*,*N*′,*N*′-tetramethylguanidine (25.2 mL, 0.2 mol). After stirring for 0.5 h, allyl bromide (33.8 mL, 0.4 Mol) was added, and stirring was continued for 3 h. The mixture was evaporated and the residual oil was dissolved in EtOAc (0.7 L). The solution was washed with brine, dried, and evaporated. The solid residue was crystallized twice from *tert*-butyl methyl ether to give **66** (43.3 g, 86%): white solid, mp 74−75 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.92 (s, 3H), 3.86 (s, 3H), 4.77 (d, 2H, $J$ = 6), 5.26 (d, 1H, $J$ = 10), 5.37 (d, 1H, $J$ = 17), 5.90−6.10 (m, 1H), 6.59 (s, 1H), 10.09 (s, 1H), 11.07 (s, 1H).

**Allyl 2-Formyl-5-methoxy-6-methyl-3-(dimethyl(thexyl)silyloxy)benzoate (67).** Using method D (**21a**), **66** (5.0 g, 0.02 mol) was silylated with (thexyl)$Me_2SiCl$ (4.33 mL, 0.022

mol) to give **67** (7.1 g, 90%): white solid (from hexane); mp 81−82 °C; $^1$H NMR ($CDCl_3$) $\delta$ 0.32 (s, 6H), 0.94 (d, 6H, $J$ = 7), 0.98 (s, 6H), 1.70−1.83 (m, 1H), 2.06 (s, 3H), 3.85 (s, 3H), 4.88 (d, 2H, $J$ = 6), 5.29 (d, 1H, $J$ = 10), 5.40 (d, 1H, $J$ = 16), 6.00−6.16 (m, 1H), 6.31 (s, 1H), 10.24 (s, 1H).

**Bis[(*R*)-2-*tert*-butoxycarbonylamino-2-(3-methyl-1,2,4-oxadiazol-5-yl)-ethyl] Disulfide (69).** To a solution of Boc-L-cystine (248.0 g, 0.56 mol), acetamidoxime (**68**, 89.0 g, 1.20 mol), and 1-hydroxypyridin-2(1*H*)-one (2.9 g, 0.026 mol) in THF (1 L) was added at 0 °C over 30 min a solution of DCC (250 g, 1.21 mol) in THF (0.8 L). The mixture was stirred for 16 h while the temperature was allowed to warm to 20 °C. The mixture was cooled to 0 °C and the precipitate was removed by filtration. The filtrate was concentrated in vacuo to a volume of about 0.5 L and then diluted with EtOAc (0.8 L). Upon the addition of $H_2O$ (1 L), a precipitate formed which was isolated by filtration to give Boc-L-cystine bis(acetamidoxime) ester (246.6 g, 79%) as white crystals, mp 134−136 °C. This material was taken up in toluene (0.8 L) and the mixture was heated at reflux for 3 h, the water formed being removed by a Dean−Stark trap. Addition of hexane (3 L) to the cooled mixture led to crystallization of the product **69** (215.0 g, 74%) as white crystals, mp 130−131 °C.

**(*R*)-2-Mercapto-1-(3-methyl-1,2,4-oxadiazol-5-yl)-ethylcarbamic Acid *tert*-Butyl Ester (70).** To a stirred solution of **69** (30 g, 58 mmol) in trifluoroethanol (50 mL) and $H_2O$ (6 mL) was added over 30 min at 0−5 °C tributylphosphine (18.1 mL, 14.84 g, 73.3 mmol), and the mixture was stirred for 3 h at 0 °C. The solvent was evaporated and the residue was chromatographed ($SiO_2$; EtOAc/hexane 1:3) to give **70** (24.1 g, 80%): colorless oil; $^1$H NMR ($CDCl_3$) $\delta$ 1.47(s, 9H), 2.41(s, 3H), 2.96−3.24 (m, 2H), 5.24−5.34 (m, 1H), 5.45−5.55 (br d, 1H, $J$ = 8).

***N*-Allyloxycarbonylaminoacetonitrile (72).** To a stirred suspension of aminoacetonitrile hydrochloride (**71**, 110.0 g, 1.19 mol) in MeCN (1.2 L) was slowly added $Et_3N$ (167.3 mL, 1.2 mol) at 20 °C. Allyl chloroformate (127.6 mL, 1.2 mol) was then added at 20 °C with cooling, followed by another portion of $Et_3N$ (167.3 mL, 1.2 mol), and stirring was continued for 2 h. Solid material was filtered off, and the solvents were evaporated. The residue was dissolved in EtOAc, and the solution was washed with $H_2O$, aq $NaHSO_4$, aq $NaHCO_3$, and brine, dried, and evaporated, and the residual oil was distilled to give **72** (132.6 g, 64%) as a colorless oil, bp 100 °C/0.07 mbar.

***N*-(Hydroxycarbamimidoylmethyl)carbamic Acid Allyl Ester (73).** To a solution of **72** (126.0 g, 0.9 mol) in MeOH (0.9 L) was added over 15 min with cooling in an ice bath a solution of hydroxylamine sulfate (73.9 g, 0.9 mol) and NaOH (36.0 g, 0.9 mol) in $H_2O$ (0.18 L), and the mixture was stirred for 15 h at 20 °C. The pH of the suspension was lowered from 8.7 to 7.0 by addition of 37% aq HCl. Solid material was filtered off and the solution was evaporated. Crystallization of the residue from EtOAc afforded **73** (128.8 g, 83%) as white crystals, mp 92−93 °C.

**(*R*)-2-Mercapto-1-(3-allyloxycarbonylaminomethyl-1,2,4-oxadiazol-5-yl)ethylcarbamic Acid *tert*-Butyl Ester (75).** To a solution of Boc-L-cystine (194.1 g, 0.44 mol), **73** (167.9 g, 0.97 mol), and 1-hydroxypyridin-2(1*H*)-one (2.5 g, 0.023 mol) in DMF (2 L) was added at 0−20 °C over 15 min a solution of DCC (200 g, 0.97 mol) in DMF (0.3 L), and the mixture was stirred for 3 h at 20 °C. The precipitate that formed was removed by filtration. The filtrate was concentrated to a volume of 0.5 L, diluted with EtOAc (4 L), and then washed with 5% aq NaCl, a precipitate being formed. The aqueous phase was separated, and the precipitate was isolated by filtration, triturated with MeOH (4 l), and dried. The white solid obtained (285 g, mp 147.5−149 °C) was heated in dioxane (1 L) at reflux for 7 h. The solvent was evaporated and the residue was crystallized twice, from t-BuOMe and from EtOAc/t-BuOMe, to give bis-[(*R*)-2-*tert*-butoxycarbonylamino-2-(3-allyloxycarbonylaminomethyl-1,2,4-oxadiazol-5-yl)ethyl] disulfide (**74**, 178.2 g, 68%) as white crystals, mp 118−128 °C. To a stirred solution of this material (30 g, 0.042 mol) in trifluoroethanol (90 mL) and $H_2O$ (6.4 mL) was added at 0−5 °C tributylphosphine (15.4 mL, 62 mmol), and stirring was continued for 2 h at 0 °C. The solvent was evaporated and the residue was chromatographed ($SiO_2$; EtOAc/hexane 1:1) to give **75** (23.1 g, 79%): white crystals (from EtOAc/hexane), mp 55−58 °C; $^1$H NMR ($CDCl_3$) $\delta$ 0.95 (t, 1H, $J$ = 8), 1.46 (s, 9H), 2.97−3.23 (m, 1H), 4.50/4.61 (2 d, 2 × 2H, $J$ = 6), 5.23−5.38 (m, 1H) superimposed by 5.22/5.32 (2 d, 2 × 1H, $J$ = 10), 5.45 (br s, 1H), 5.59 (d, 1H, $J$ = 8), 5.84−6.00 (m, 1H).

**General Procedures for the Reductive Thiolation of Aldehyde 67. Method I-1. Allyl (*R*)-2-[2-Amino-2-(methoxycarbonyl)ethylsulfanylmethyl]-3-[dimethyl(thexyl)-silyloxy]-5-methoxy-6-methylbenzoate (76).** To a stirred solution of **67** (3.14 g, 8.0 mmol) in TFA (16 mL), cooled to 0 °C, was added L-Cys-OMe•HCl (2.34 g, 13.6 mmol). After 2 min, $Et_3SiH$ (2.54 mL, 16 mmol) was added, and stirring was continued at 0 °C for 0.5 h. The mixture was evaporated and the residual oil was taken up in EtOAc. The solution was successively washed with $H_2O$, aq $Na_2CO_3$, and brine, dried, and evaporated. Chromatography of the crude product ($SiO_2$; EtOAc/hexane 1:1) afforded **76** (2.7 g, 66%): colorless foam; TLC $R_f$ = 0.47 (EtOAc); $^1$H NMR ($CDCl_3$) $\delta$ 0.29 (s, 6H), 0.94 (d, 6H, $J$ = 7), 1.00 (s, 6H), 1.63 (br s, 2H), 1.77 (m, 1H), 2.09 (s, 3H), 2.65 (dd, 1H, $J$ = 13.5, 8), 2.90 (dd, 1H, $J$ = 13.5, 4.5), 3.50 (dd, 1H, $J$ = 8, 4.5), 3.70 (s, 3H), 3.77 (s, 3H), 3.79 (s, 2H), 4.83 (d, 2H, $J$ = 6), 5.30 (d, 1H, $J$ = 10), 5.43 (d, 1H, $J$ = 17), 5.97−6.15 (m, 1H), 6.38 (s, 1H).

**Method I-2. Allyl (*R*)-2-[2-Amino-2-(3-methyl-1,2,4-oxadiazol-5-yl)ethylsulfanylmethyl]-3-[dimethyl(thexyl)silyloxy]-5-methoxy-6-methylbenzoate (77).** To a solution of **67** (13.8 g, 35.0 mmol) in TFA (38 mL), cooled to 0 °C, was added over 15 min a solution of **70** (13.7 g, 53 mmol) and $HSiEt_3$ (8.4 mL, 53 mmol) in DCM (35 mL). The solution was kept at 0 °C for 18 h. The mixture was subjected to a workup as described for **76**. Chromatography of the crude product ($SiO_2$; EtOAc/hexane 1:3) afforded **77** (14.1 g, 69%): pale-yellow oil; TLC $R_f$ = 0.34 (EtOAc/hexane 2:1); $^1$H NMR ($CDCl_3$) $\delta$ 0.29 (s, 6H), 0.93 (d, 6H, $J$ = 7); 0.99 (s, 6H), 1.66−1.88 (m, 1H), 1.80 (br s, 2H), 2.09 (s, 3H), 2.38 (s, 3H), 2.80 (dd, 1H, $J$ = 13, 8), 2.96 (dd, 1H, $J$ = 13, 5), 3.77/3.83 (2 d, 2 × 1H, $J$ = 12), 3.79 (s, 3H), 4.10 (dd, 1H, $J$ = 7, 4.5), 4.82 (dd, 2H, $J$ = 6), 5.32 (d, 1H, $J$ = 10), 5.44 (d, 1H, $J$ = 16), 5.95−6.15 (m, 1H), 6.39 (s, 1H). (Addition of TAE shift reagent gave no indication of the presence of the enantiomer). Allyl 5-methoxy-2,6-dimethyl-3-(dimethyl(thexyl)silyloxy)benzoate (1.7 g) was isolated as the major side product: colorless oil; TLC $R_f$ = 0.67 (EtOAc/hexane 2:1); $^1$H NMR ($CDCl_3$) $\delta$ 0.24 (s, 6H), 0.94 (d, 6H, $J$ = 7), 0.97 (s, 6H), 1.68−1.82 (m, 1H), 2.07/2.08/3.75 (3 s, 3 × 3H), 4.81 (d, 2H, $J$ = 6), 5.29 (d, 1H, $J$ = 10), 5.41 (d, 1H, $J$ = 17), 5.86−6.14 (m, 1H), 6.36 (s, 1H).

**Allyl (*R*)-2-[2-Amino-2-(3-allyloxycarbonylaminomethyl-1,2,4-oxadiazol-5-yl)-ethylsulfanylmethyl]-3-[dimethyl-(thexyl)silyloxy]-5-methoxy-6-methylbenzoate (78).** Using method I-2 (**77**), but replacing thiol **70** by **75**, **67** (5.22 g, 13.3 mmol) was converted to **78** (5.4 g, 64%): pale-yellow oil; TLC $R_f$ = 0.39 (EtOAc/hexane 2:1); $^1$H NMR ($CDCl_3$) $\delta$ 0.29 (s, 6H), 0.93 (d, 6H, $J$ = 7), 0.99 (s, 6H), 1.67−1.88 (m, 1H), 1.79 (br s, 2H), 2.08 (s, 3H), 2.81 (dd, 1H, $J$ = 14, 8), 3.03 (dd, 1H, $J$ = 14, 5), 3.73 (d, 1H, $J$ = 12), 3.77 (s, 3H), 3.81 (d, 1H, $J$ = 12), 4.07−4.17 (m, 1H), 4.50/4.58/4.80 (3 d, 3 × 2H, $J$ = 6), 5.22/5.26 (2 d, 2 × 1H, $J$ = 10), 5.30−5.45 (m, 1H), 5.37/5.44 (2 d, 2 × 1H, $J$ = 18), 5.83−6.14 (m, 2H), 6.39 (s, 1H).

**General Procedure for the Synthesis of ω-Trityloxyalkanoic Acids. 5-Trityloxypentanoic Acid (82d). (a) 5-Trityloxypentanol (80d).** To a solution of 1,5-pentanediol (**79d**, 150 mL, 1.50 mol) in pyridine (0.75 L) was added at 0 °C $Ph_3CCl$ (334.2 g, 1.5 mol). The mixture was stirred for 0.5 h at 0 °C followed by 16 h at 20 °C. Solvents were evaporated, and the residue was partitioned between EtOAc (1.5 L) and $H_2O$ (0.6 L). The organic layer was washed successively with 1 N aq HCl, aq $NaHCO_3$, and brine, dried, and evaporated. The crude product was purified by chromatography. t-BuOMe/hexane (1:3) eluted the bis-tritylated side product (TLC: $R_f$ = 0.8 (EtOAc/hexane 1:1)) and EtOAc/hexane (1:1) eluted the product, which was crystallized from hexane to give **80d** (242.2

g, 47%): white crystals, mp 68−69 °C; TLC: $R_f = 0.4$ (EtOAc/hexane 1:1); $^1$H NMR ($CDCl_3$) $\delta$ 1.23 (br t, 1H), 1.38−1.72 (m, 6H), 3.07 (t, 2H, $J = 6.5$), 3.56−3.66 (m, 2H), 7.17−7.34 (m, 9H), 7.40−7.49 (m, 6H).

**(b) 5-Trityloxypentanal (81d).** A solution of DMSO (75 mL, 1.05 mol) in DCM (0.12 L) was added over 25 min at −65 to −70 °C to a solution of oxalyl chloride (41.5 mL, 0.48 mol) in DCM (0.75 L). Stirring was continued for 10 min, and then a solution of **80d** (103.8 g, 0.30 mol) in DCM (0.45 L) was added over 25 min at −65 to −70 °C. After stirring for 10 min, $Et_3N$ (168 mL, 1.20 mol) was added over 5 min at −70 °C. Stirring was continued for 20 min, and the mixture was then warmed to 10 °C over 1 h and washed with $H_2O$ (1.6 L), and the aqueous layer was extracted with DCM (0.8 L). The organic layer was dried and evaporated, and the residue was crystallized from hexane (0.25 L) to give 5-trityloxypentanal (**81d**, 77.5 g, 75%): white crystals, mp 41 °C; $^1$H NMR ($CDCl_3$) $\delta$ 1.60−1.80 (m, 2H), 2.39 (dt, 2H, $J = 2, 7$), 3.08 (t, 2H, $J = 6$), 7.17−7.34 (m, 9H), 7.40−7.49 (m, 6H), 9.73 (t, 1H, $J = 2$).

**(c) 5-Trityloxypentanoic Acid (82d).** To a mixture of **81d** (86.1 g, 0.25 mol) in acetone (0.63 L) and $H_2O$ (0.25 L) was added portionwise, over 25 min, $KMnO_4$ (39.5 g, 0.25 mol), the temperature being kept below 28 °C. Stirring was continued at 20 °C for 2 h. The pH of the mixture was set to 5 by the addition of 3 N aq HCl, and 40% aq $NaHSO_3$ (ca. 1.5 L) was added over 45 min until the mixture became colorless, the pH being kept at 5. The mixture was acidified to pH 2 and extracted with EtOAc (1.5 L). The organic layer was washed with $H_2O$, dried, and evaporated. The residue was crystallized from EtOAc/hexane to give **82d** (70.4 g, 78%): white crystals, mp 146−148 °C; $^1$H NMR ($CDCl_3$) $\delta$ 1.59−1.82 (m, 4H), 2.34 (t, 2H, $J = 7$), 3.08 (t, 2H, $J = 6$), 7.18−7.34 (m, 9H), 7.39−7.48 (m, 6H), 10.54 (br s, 1H).

The trityloxy acid **82a** was prepared as described previously.[29] The trityloxy acids **82b,c** and **82e−g** were prepared using the procedure described for the synthesis of **82d** from **79d**.

**3-Trityloxypropionic acid (82b)** (starting from 1,3-propanediol): white crystals (EtOAc/hexane), mp 163−165 °C (lit.[43] mp 164−166); $^1$H NMR ($CDCl_3$) $\delta$ 2.64 (t, 2H, $J = 6$), 3.24 (t, 2H, $J = 6$), 7.17−7.32 (m, 9H), 7.36−7.45 (m, 6H).

**4-Trityloxybutyric acid (82c)** (starting from 1,4-butanediol): white crystals (EtOAc/hexane), mp 138−140 °C; $^1$H NMR ($CDCl_3$) $\delta$ 1.88−2.02 (m, 2H), 2.49 (t, 2H, $J = 7$), 3.13 (t, 2H, $J = 6$), 7.18−7.33 (m, 9H), 7.38−7.47 (m, 6H), 11.20 (br s, 1H).

**6-Trityloxyhexanoic acid (82e)** (starting from 1,6-hexanediol): white crystals (from EtOAc/hexane), mp 117−119 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.38−1.52 (m, 2H), 1.57−1.73 (m, 4H), 2.34 (t, 2H, $J = 7$), 3.06 (t, 2H, $J = 6$), 7.16−7.32 (m, 9H), 7.40−7.48 (m, 6H), 10.60 (br s, 1H).

**7-Trityloxyheptanoic acid (82f)** (starting from 1,7-heptanediol): colorless oil; $^1$H NMR ($CDCl_3$) $\delta$ 1.20−1.45 (m, 4H), 1.52−1.68 (m, 4H), 2.32 (t, 2H, $J = 7$), 3.04 (t, 2H, $J = 6$), 7.18−7.34 (m, 9H), 7.38−7.48 (m, 6H).

**9-Trityloxynonanoic acid (82g)** (starting from 1,9-nonanediol): colorless oil; $^1$H NMR ($CDCl_3$) $\delta$ 1.15−1.45 (m, 8H), 1.52−1.68 (m, 4H), 2.35 (t, 2H, $J = 8$), 3.04 (t, 2H, $J = 7$), 7.16−7.33 (m, 9H), 7.38−7.48 (m, 6H).

**General Procedure for the Synthesis of Lactones 86a−g. (a) Method J. Acylation of Amines 76−78. Allyl (*R*)-3-[Dimethyl(thexyl)silyloxy]-2-[2-(5-hydroxypentanoylamino)-2-(methoxycarbonyl)ethylsulfanylmethyl]-5-methoxy-6-methylbenzoate (83d).** To a suspension of **76** (76.8 g, 0.15 mol) and **82d** (64.9 g, 0.18 mol) in MeCN (0.6 L), cooled to 0 °C, was added EDC (34.5 g, 0.18 mol). The mixture was stirred at 0 °C for 8 h. The solvent was evaporated and the remaining oil was dissolved in EtOAc (0.8 L). The solution was washed successively with 1 N aq HCl and brine, dried, and evaporated. The solution of the residual oil (146.6 g) and $pTsOH \cdot H_2O$ (5.7 g, 0.03 mmol) in MeOH (0.3 L) was stirred at 20 °C for 3 h. The precipitated crystals were filtered off ($Ph_3COMe$), and the filtrate was evaporated. The residue was chromatographed ($SiO_2$; EtOAc/hexane 1:1) to afford **83d** (79.2 g, 86%): foam; TLC $R_f = 0.13$ (EtOAc/hexane 1:1); $^1$H NMR ($CDCl_3$) $\delta$ 0.27/0.30 (2 s, 2 × 3H), 0.95 (d, 6H, $J = 7$), 0.99 (s, 6H), 1.50−1.82 (m, 6H), 2.09 (s, 3H), 2.10−2.28 (m, 2H), 2.77 (dd, 1H, $J = 14$, 4), 3.08 (dd, 1H, $J = 14$, 5), 3.61 (t, 2H, $J = 6$), 3.69 (d, 1H, $J = 13$), 3.70 (s, 3H), 3.77 (s, 3H), 3.88 (d, 1H, $J = 13$), 4.68−4.78 (m, 1H), 4.85 (d, 2H, $J = 6$), 5.33 (d, 1H, $J = 10$), 5.46 (d, 1H, $J = 18$), 5.98−6.15 (m, 1H), 6.37 (s, 1H), 6.44 (d, 1H, $J = 8$).

**(b) Method K. Cleavage of Allyl Esters. (*R*)-3-[Dimethyl(thexyl)silyloxy]-2-[2-(5-hydroxypropanoylamino)-2-(methoxycarbonyl)ethylsulfanylmethyl]-5-methoxy-6-methylbenzoic Acid (84d).** To a solution of **83d** (79.0 g, 0.129 mol) in THF (1 L) were added morpholine (56.3 mL, 0.645 mol) and $Pd(PPh_3)_4$ (0.75 g, 0.65 mmol). The mixture was stirred at 0 °C for 2 h, and thereafter most of the solvent was evaporated. EtOAc (0.5 L) was added and the solution was washed with 2 N aq HCl and brine, dried, and evaporated to give **84d** (73.9 g, "100%"): colorless oil; $^1$H NMR ($CHCl_3$) $\delta$ 0.28/0.29 (2 s, 2 × 3H), 0.95 (d, 6H, $J = 7$), 1.00 (s, 6H), 1.55−1.88 (m, 6H), 2.17 (s, 3H), 2.25−2.50 (m, 2H), 2.91 (dd, 1H, $J = 13$, 4), 3.05 (dd, 1H, $J = 13$, 5), 3.62−3.82 (m, 2H), 3.70 (d, 1H, $J = 11$), 3.73 (s, 3H), 3.77 (s, 3H), 3.88 (d, 1H, $J = 11$), 4.66−4.76 (m, 1H), 6.36 (s, 1H), 6.68 (d, 1H, $J = 8$).

**(c) Method H-2. Cyclization of $\omega$-Hydroxycarboxylic Acids. Methyl (*R*)-1,3,4,5,6,7,8,9,10,12-Decahydro-16-[dimethyl(thexyl)silyloxy]-14-methoxy-13-methyl-6,12-dioxo-11,2,5-benzoxathiaazacyclotetradecine-4-carboxylate (85d).** To a stirred solution of **84d** (73.9 g, ca. 0.129 mol) and $PPh_3$ (43.2 g, 0.168 mol) in THF (2.5 L) was added at 0 °C, over 10 min, DEAD (26.4 mL, 0.168 mol). Stirring was continued for 1 h at 0 °C and for 15 h at 20 °C. The solution was evaporated, and the residue was stirred with DCM (0.2 L)/hexane (0.3 L) for 1 h at 0 °C. The crystals (diethyl hydrazodicarboxlate, 19.2 g) were filtered off, the filtrate was evaporated, and the residue was chromatographed ($SiO_2$; EtOAc/hexane 1:1) to give **85d** (55.9 g, 78%): foam; $^1$H NMR ($CDCl_3$) $\delta$ 0.28/0.29 (2 s, 2 × 3H), 0.95 (d, 6H, $J = 7$), 0.99 (s, 6H), 1.71−1.98 (m, 4H), 2.07 (s, 3H), 2.24−2.55 (m, 2H), 2.87−3.04 (m, 2H), 3.67 (d, 1H, $J = 11$), 3.73 (s, 3H), 3.77 (s, 3H), 3.93 (d, 1H, $J = 11$), 4.45−4.58 (m, 2H), 4.60−4.70 (m, 1H), 6.36 (s, 1H), 6.46 (d, 1H, $J = 8$).

**Method A-2. Liberation of *O*-Trialkylsilyl-Protected Phenols. Methyl (*R*)-1,3,4,5,6,7,8,9,10,12-Decahydro-16-hydroxy-14-methoxy-13-methyl-6,12-dioxo-11,2,5-benzoxathiaazacyclotetradecine-4-carboxylate (86d).** To a solution of **85d** (55.4 g, 0.1 mol) in MeOH (0.6 L) was added $NH_4F$ (3,7 g, 0.1 mol) and the mixture was stirred at 20 °C for 40 min. The mixture was concentrated to a volume of ca. 0.2 L, diluted with EtOAc (0.6 L), and washed with 5% aq NaCl (3 × 0.1 L). The organic layer was dried and evaporated, and the solid residue was recrystallized from MeOH/$Et_2O$ to give **86d** (33.82 g, 82%): white crystals, mp 165−67 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.60−2.03 (m, 5H) superimposed by 1.90 (s, 3H), 2.34−2.48 (m, 1H), 2.68 (dd, 1H, $J = 13$, 10), 3.04 (dd, 1H, $J = 13$, 4), 3.64 (s, 3H), 3.65 (d, 1H, $J = 11$), 3.69 (s, 3H), 3.75 (d, 1H, $J = 11$), 4.00−4.12 (m, 1H), 4.33−4.58 (m, 2H), 6.51 (s, 1H), 8.33 (d, 1H, $J = 8$), 9.73 (s, 1H); MS (ISP) 412.2 $(M + H)^+$. Anal. ($C_{19}H_{25}NO_7S$) C, H, N, S.

Using the procedures of the reaction sequence **76** → **83d** → **84d** → **85d** → **86d**, but replacing **82d** by **82a−c** and **82e−g**, respectively, the following lactones were prepared.

**Methyl (4*R*)-3,4,5,6,7,9-hexahydro-1*H*-13-hydroxy-11-methoxy-10-methyl-6,9-dioxo-8,2,5-benzoxathiaazacycloundecine-4-carboxylate (86a)** was prepared starting from **76** and **82a**: white crystals (from t-BuOMe/hexane), mp 179−182 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.99 (s, 3H), 2.99 (dd, 1H, $J = 14$, 6), 3.02 (dd, 1H, $J = 14$, 6), 3.44 (d, 1H, $J = 11$), 3.63 (s, 3H), 3.74 (s, 3H), 4.11 (d, 1H, $J = 11$), 4.63−4.76 (m, 1H), 4.76/4.86 (2 d, 2 × 1H, $J = 13$), 6.55 (s, 1H), 7.83 (d, 1H, $J = 8$), 9.90 (s, 1H); MS (ISN) 368.3 $(M - H)^-$. Anal. ($C_{16}H_{19}NO_7S$) C, H, N, S.

**Methyl (*R*)-1,3,4,5,6,7,8,10-octahydro-14-hydroxy-12-methoxy-11-methyl-6,10-dioxo-9 ,2,5-benzoxathiaazacyclododecine-4-carboxylate (86b)** was prepared starting from **76** and **82b**: white crystals (from MeOH/$Et_2O$/hexane),

mp 212−214 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.90 (s, 3H), 2.38−2.50 (m, 1H), 2.59 (dd, 1H, $J$ = 14, 11), 2.83−3.00 (m, 1H), 3.07 (dd, 1H, $J$ = 14, 4), 2.63−3.77 (m, 2H) superimposed by 3.65/3.71 (2 s, 2 × 3H), 4.47−4.73 (m, 3H), 6.49 (s, 1H), 8.56 (d, 1H, $J$ = 8), 9.69 (s, 1H); MS (ISP) 384 (M + H)$^+$. Anal. ($C_{17}H_{21}NO_7S$) C, H, N, S.

**Methyl (*R*)-3,4,5,6,7,8,9,11-octahydro-1*H*-15-hydroxy-13-methoxy-12-methyl-6,11-dioxo-10,2,5-benzoxathiaazacyclotridecine-4-carboxylate (86c)** was prepared starting from **76** and **82c**: white crystals (from MeOH/$Et_2O$), mp 126−129 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.91 (s, 3H) superimposed by 1.86−2.12 (m, 2H), 2.16−2.44 (m, 2H), 2.53 (dd, 1H, $J$ = 14, 12), 2.90 (dd, 1H, $J$ = 14, 4), 3.62 (s, 3H), 3.73 (s, 3H), 3.74 (d, 1H, $J$ = 12), 3.88 (d, $J$ = 12), 4.14−4.38 (m, 2H), 4.38−4.49 (m, 1H), 6.52 (s, 1H), 8.32 (d, 1H, $J$ = 8.5), 9.70 (s, 1H); MS (ISN) 396.2 (M − H)$^-$. Anal. ($C_{18}H_{23}NO_7S$) C, H, N, S.

**Methyl (*R*)-3,4,5,6,7,8,9,10,11,13-decahydro-1*H*-17-hydroxy-15-methoxy-14-methyl-6,13-dioxo-12,2,5-benzoxathiaazacyclopentadecine-4-carboxylate (86e)** was prepared starting from **76** and **82e**: white crystals (from MeOH/$Et_2O$), mp 202−204 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.32−1.80 (m, 6H), 1.90 (s, 3H), 2.04−2.24 (m, 2H), 2.66 (dd, 1H, $J$ = 14, 11), 3.08 (dd, 1H, $J$ = 14, 4), 3.63 (s, 2H), 3.64 (s, 3H), 3.73 (s, 3H), 4.08−4.20/4.30−4.42 (2 m, 2 × 1H) 4.42−4.54 (m, 1H), 6.52 (s, 1H), 8.30 (d, 1H, $J$ = 8.5), 9.74 (s, 1H); MS (ISN) 424.3 (M − H)$^-$. Anal. ($C_{20}H_{27}NO_7S$) C, H, N, S.

**Methyl (*R*)-1,3,4,5,6,7,8,9,10,11,12,14-dodecahydro-18-hydroxy-16-methoxy-15-methyl-6,14-dioxo-13,2,5-benzoxathiaazacyclohexadecine-4-carboxylate (86f)** was prepared starting from **76** and **82f**: white solid (from EtOAc/hexane), mp 200−202 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.18−1.76 (m, 8H), 1.93 (s, 3H), 1.95−2.25 (m, 2H), 2.73 (dd, 1H, $J$ = 13, 11), 2.97 (dd, 1H, $J$ = 13, 4), 3.62 (s, 3H), 3.67 (d, 1H, $J$ = 11), 3.73 (s, 3H), 3.77 (d, 1H, $J$ = 11), 4.02−4.16/4.25−4.29 (2 m, 2 × 1H), 4.29−4.41 (m, 1H), 6.51 (s, 1H), 8.23 (d, 1H, $J$ = 8), 9.72 (s, 1H); MS (ISN) 438.2 (M − 1)$^-$. Anal. ($C_{21}H_{29}NO_7S$) C, H, N, S.

**Methyl (*R*)-1,3,4,5,6,7,8,9,10,11,12,13,14,16-tetradecahydro-20-hydroxy-18-methoxy-17-methyl-6,16-dioxo-15,2,5-benzoxathiaazacyclooctadecine-4-carboxylate (86g)** was prepared starting from **76** and **82g**: white solid (from $Et_2O$), mp 170−174 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.10−1.72 (m, 12H), 1.92 (s, 3H), 2.00−2.20 (m, 2H), 2.87 (dd, 1H, $J$ = 13, 11), 3.16 (dd, 1H, $J$ = 13, 4), 3.52 (d, 1H, $J$ = 13), 3.65 (s, 3H), 3.71 (d, 1H, $J$ = 13), 3.73 (s, 3H), 4.25 (t, 2H, $J$ = 5), 4.48−4.60 (m, 1H), 6.53 (s, 1H), 8.30 (d, 1H, $J$ = 8), 9.82 (s, 1H); MS (ISP) 468.5 (M + H)$^+$. Anal. ($C_{23}H_{33}NO_7S$) C, H, N, S.

**Method L. Synthesis of 6-Thioxo Lactones. Methyl (*R*)-1,3,4,5,6,7,8,9,10,12-Decahydro-16-hydroxy-14-methoxy-13-methyl-12-oxo-6-thioxo-11,2,5-benzoxathiaazacyclotetradecine-4-carboxylate (87d).** A mixture of **85d** (3.32 g, 6.0 mmol) and Lawesson's reagent [2,4-bis(4-methoxyphenyl)-2,4-dithioxo-1,3,2,4-dithiaphosphetane] (2.70 g, 6.6 mmol) in toluene (80 mL) was heated to 80 °C for 0.5 h. The mixture was cooled and evaporated, and the residue was chromatographed ($SiO_2$; DCM, then EtOAc/hexane 1:1). The collected product [silyl-protected-**87d**, 2.9 g; TLC $R_f$ = 0.72 (EtOAc/hexane 1:1)] was deprotected using method A-2 (**86d**) to give **87d** (2.07 g, 80%): white crystals (from MeOH/$Et_2O$/hexane), mp 141−143 °C; TLC $R_f$ = 0.26 (EtOAc/hexane 1:1); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.60−2.00 (m, 4H) superimposed by 1.90 (s, 3H), 2.55−2.68/2.85−2.98 (2 m, 2 × 1H), 2.88 (dd, 1H, $J$ = 14, 12), 3.16 (dd, 1H, $J$ = 14, 4), 3.62 (d, 1H, $J$ = 10), 3.66 (s, 3H), 3.73 (m, 1H), 3.81 (d, 1H, $J$ = 10), 4.02−4.12/4.44−4.54/4.85−4.96 (3 m, 3 × 1H), 6.51(s, 1H), 9.76 (s, 1H), 10.28 (d, 1H, $J$ = 7); MS (ISN) 426.5 (M − H)$^-$. Anal. ($C_{19}H_{25}NO_6S2$) C, H, N, S.

Using the procedures of the synthesis of **87d**, the following 6-thioxo lactones were prepared starting from the silyl-protected lactones **87a**−**c** and **87e**−**g**, respectively.

**Methyl (*R*)-3,4,5,6,7,9-hexahydro-1*H*-13-hydroxy-11-methoxy-10-methyl-9-oxo-6-thioxo-8,2,5-benzoxathiaazacycloundecine-4-carboxylate (87a):** white crystals (from t-BuOMe/hexane), mp 164−166 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.99 (s, 3H), 3.05 (dd, 1H, $J$ = 15, 6), 3.15 (d, 1H, $J$ = 11), 3.22 (dd, 1H, $J$ = 15, 4), 3.65 (s, 3H), 3.74 (s, 3H), 4.17 (d, 1H, $J$ = 11), 5.04 (d, 1H, $J$ = 14), 5.28−5.39 (m, 1H), 5.40 (d, 1H, $J$ = 14), 6.54 (s, 1H), 9.67 (d, 1H, $J$ = 8), 9.94 (s, 1H); MS (ISN) 384.3 (M−H)$^-$. Anal. ($C_{16}H_{19}NO_6S_2$) C, H, N, S.

**Methyl (*R*)-1,3,4,5,6,7,8,10-octahydro-14-hydroxy-12-methoxy-11-methyl-10-oxo-6-thioxo-9,2,5-benzoxathiaazacyclododecine-4-carboxylate (87b):** white crystals (from EtOAc/hexane), mp 162−164 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.90 (s, 3H), 2.79−2.93 (m, 1H) superimposed by 2.85 (dd, 1H, $J$ = 14, 11), 3.21 (dd, 1H, $J$ = 14, 4), 3.34−3.50 (m, 1H), 3.58 (d, 1H, $J$ = 11), 3.67/3.71 (2 s, 2 × 3H), 3.75 (d, 1H, $J$ = 11), 4.57−4.68/4.80−4.94/5.23−5.34 (3 m, 3 × 1H), 6.49 (s, 1H), 9.72 (s, 1H), 10.58 (d, 1H, $J$ = 8); MS (ISP) 399.9 (M + H)$^+$. Anal. ($C_{17}H_{21}NO_6S_2$) C, H, N, S.

**Methyl (*R*)-3,4,5,6,7,8,9,11-octahydro-1*H*-15-hydroxy-13-methoxy-12-methyl-11-oxo-6-thioxo-10,2,5-benzoxathiaazacyclotridecine-4-carboxylate (87c)**: white crystals (from EtOAc/hexane), mp 93−96 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.92 (s, 3H), 2.02−2.36 (m, 2H), 2.56−2.72 (m, 2H), 2.84−2.98 (m, 1H), 3.05 (dd, 1H, $J$ = 14, 4), 3.64 (s, 3H), 3.66 (d, 1H, $J$ = 12), 3.73 (s, 3H), 3.83 (d, 1H, $J$ = 12), 4.14−4.36 (m, 2H), 4.90−5.02 (m, 1H), 6.51 (s, 1H), 9.71 (s, 1H), 10.27 (d, 1H, $J$ = 8); MS (ISN) 412.3 (M − H)$^-$. Anal. ($C_{18}H_{23}NO_6S_2$) C, H, N, S.

**Methyl (*R*)-3,4,5,6,7,8,9,10,11,13-decahydro-1*H*-17-hydroxy-15-methoxy-14-methyl-13-oxo-6-thioxo-12,2,5-benzoxathiaazacyclopentadecine-4-carboxylate (87e):** white solid (from t-BuOMe/hexane), mp 185−188 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.28−1.84 (m, 6H), 1.89 (s, 3H), 2.52−2.73 (m, 2H), 2.85 (dd, 1H, $J$ = 14, 11), 3.20 (dd, 1H, $J$ = 14, 4), 3.59/3.67 (2 d, 2 × 1H, $J$ = 11), 3.68 (s, 3H), 3.73 (s, 3H), 4.10−4.21/4.23−4.35 (2 m, 2 × 1H), 5.18−5.30 (m, 1H), 6.52 (s, 1H), 9.76 (s, 1H), 10.31 (d, 1H, $J$ = 8); MS (ISP) 440.2 (M + H)$^+$. Anal. ($C_{20}H_{27}NO_6S_2$) C, H, N.

**Methyl (*R*)-1,3,4,5,6,7,8,9,10,11,12,14-dodecahydro-18-hydroxy-16-methoxy-15-methyl-14-oxo-6-thioxo-13,2,5-benzoxathiaazacyclohexadecine-4-carboxylate (87f):** white solid (from EtOAc/hexane), mp 100−103 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.14−1.42 (m, 4H), 1.46−1.76 (m, 4H), 1.93 (s, 3H), 2.43−2.59/2.63−2.78 (2 m, 2 × 1H), 2.90 (dd, 1H, $J$ = 14, 12), 3.10 (dd, 1H, $J$ = 14, 3), 3.66 (s, 3H), 3.67 (d, 1H, $J$ = 11), 3.73 (s, 3H), 3.82 (d, 1H, $J$ = 11), 4.00−4.13/4.30−4.43 (2 m, 2 × 1H), 5.04−5.16 (m, 1H), 6.52 (s, 1H), 9.75 (s, 1H), 10.18 (d, 1H, $J$ = 7); HRMS calcd for ($C_{21}H_{29}NO_6S_2Na^+$) 478.1334, found 478.1334.

**Methyl (*R*)-1,3,4,5,6,7,8,9,10,11,12,13,14,16-tetradecahydro-20-hydroxy-18-methoxy-17-methyl-16-oxo-6-thioxo-15,2,5-benzoxathiaazacyclooctadecine-4-carboxylate (87g):** white solid (from $Et_2O$/hexane), mp 151−53 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.05−1.88 (m, 12H), 1.92 (s, 3H), 2.45−2.75 (m, 2H), 3.07 (dd, 1H, $J$ = 14, 12), 3.35 (dd, 1H, $J$ = 14, 4), 3.55 (d, 1H, $J$ = 13), 3.68 (s, 3H), 3.73 (s, 3H), 3.75 (d, 1H, $J$ = 13), 4.15−4.32 (m, 2H), 5.12−5.24 (m, 1H), 6.53 (s, 1H), 9.89 (s, 1H), 10.27 (d, 1H, $J$ = 7); HRMS calcd for ($C_{23}H_{33}NO_6S_2Na^+$) 506.1647, found 506.1650.

**Methyl 2-Formyl-5-methoxy-6-methyl-3-[dimethyl(thexyl)silyloxy]benzoate (88).** Using method D **(21a)**, **64** (11.2 g, 50.0 mmol) was silylated with (thexyl)$Me_2$SiCl (10.8 mL, 55 mmol) to give **88** (16.5 g, 90%): white crystals (from hexane), mp 68−69 °C; $^1$H NMR ($CDCl_3$) $\delta$ 0.32 (s, 6H), 0.94 (d, 6H, $J$ = 7), 0.98 (s, 6H), 1.68−1.84 (m, 1H), 2.04 (s, 3H), 3.85 (s, 3H), 3.95 (s, 3H), 6.31 (s, 1H), 10.24 (s, 1H).

**Methyl (*R*)-2-[2-acetylamino-2-(methoxycarbonyl)ethylsulfanylmethyl]-3-[dimethyl(thexyl)silyloxy]-5-methoxy-6-methylbenzoate (89)** was obtained by using method I-1 (**76**), but replacing L-Cys-OMe·HCl by Ac-L-Cys-OMe (0.18 g, 1.0 mol), and aldehyde **67** by **88** (0.37 g, 1.0 mol): colorless foam (0.36 g, 69%); TLC $R_f$ = 0.22 (EtOAc/hexane 1:1); $^1$H NMR ($CDCl_3$) $\delta$ 0.27/0.30 (2 s, 2 × 3H), 0.94 (d, 6H, $J$ = 7), 0.99 (s, 6H), 1.68−1.84 (m, 1H), 1.94 (s, 3H), 2.08 (s, 3H), 2.74 (dd, 1H, $J$ = 14, 4), 3.08 (dd, 1H, $J$ = 14, 5), 3.70 (d, 1H, $J$ = 13), 3.71 (s, 3H), 3.77 (s, 3H), 3.89 (d, 1H, $J$ = 13), 3.94 (s, 3H), 4.70−4.80 (m, 1H), 6.37 (s, 1H), 6.40 (d, 1H, $J$ = 8). In addition, the side product methyl 2,6-dimethyl-3-[dimethyl(thexyl)silanyloxy]-5-methoxybenzoate (0.08 g, 23%) was isolated as colorless oil: TLC $R_f$ = 0.55 (EtOAc/hexane 1:1); $^1$H NMR ($CDCl_3$) $\delta$ 0.11 (s, 6H), 0.83 (d, 6H, $J$ = 7), 0.85 (s, 6H),

1.58−1.75 (m, 1H), 1.93/1.94 (2 s, 2 × 3H), 3.63 (s, 3H), 3.77 (s, 3H), 6.24 (s, 1H).

**Methyl (*R*)-2-[2-Acetylamino-2-(methoxycarbonyl)-ethylsulfanylmethyl]-3-hydroxy-5-methoxy-6-methylbenzoate (90).** Using method A-2 (**86d**), **89** (360 mg, 0.69 mol) was converted to **90** (255 mg, 96%): white foam; TLC $R_f$ = 0.41 (EtOAc); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.93/1.96 (2 s, 2 × 3H), 2.68 (dd, 1H, $J$ = 14, 8), 2.80 (dd, 1H, $J$ = 14, 6), 3.61 (d, 1H, $J$ = 13), 3.62 (s, 3H), 3.68 (d, 1H, $J$ = 13), 3.74/3.80 (2 s, 2 × 3H), 4.40−4.52 (m, 1H), 6.53 (s, 1H), 8.27 (d, 1H, $J$ = 8), 9.78 (s, 1H). Anal. ($C_{17}H_{23}NO_7S$) C, H, N, S.

**Methyl (*R*)-3-Hydroxy-5-methoxy-2-[2-(methoxycarbonyl)-2-thioacetylamino-ethylsulf anylmethyl]-6-methylbenzoate (91).** Reacting **89** (276 mg, 0.5 mol) according to methods L (**87d**) and A-2 (**86d**) afforded **91** (160 mg, 78%): light-yellow foam; TLC $R_f$ = 0.60 (EtOAc); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.93/2.50 (2 s, 2 × 3H), 2.84 (dd, 1H, $J$ = 14, 9), 2.94 (dd, 1H, $J$ = 14, 5), 3.62 (d, 1H, $J$ = 13), 3.64 (s, 3H), 3.71 (d, 1H, $J$ = 13), 3.73/3.80 (2 s, 2 × 3H), 5.04−5.16 (m, 1H), 6.53 (s, 1H), 9.81 (s, 1H), 10.28 (d, 1H, $J$ = 7); MS (ISP) 402.4 (M + H)$^+$. Anal. ($C_{17}H_{23}NO_6S_2$) C, H, N, S.

Using the procedures of the reaction sequence **76** → **83d** → **84d** → **85d** → **86d/87d**, but replacing **76** by **77**, and **82d** by **82b** or **82d**, the lactones **93**, **94**, and **96**, **97** were prepared, respectively.

**(*R*)-1,3,4,5,6,7,8,10-Octahydro-14-hydroxy-12-methoxy-11-methyl-4-(3-methyl-1,2,4-oxadiazol-5-yl)-9,2,5-benzoxathiaazacyclododecine-6,10-dione (93):** white crystals (from MeOH), mp 220−223 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.91 (s, 3H), 2.34 (s, 3H), 2.50 (m, 1H), 2.70 (dd, 1H, $J$ = 15, 12), 2.90 (m, 1H), 3.27 (dd, 1H, $J$ = 15, 4), 3.72 (s, 3H), 3.75/3.82 (2 d, 2 × 1H, $J$ = 11), 4.50−4.72 (m, 2H), 5.33−5.47 (m, 1H), 6.50 (s, 1H), 8.87 (d, 1H, $J$ = 9), 9.73 (s, 1H); MS (ISN) 406.3 (M − H)$^-$. Anal. ($C_{18}H_{21}N_3O_6S$) C, H, N, S.

**(*R*)-1,3,4,5,6,7,8,10-Octahydro-14-hydroxy-12-methoxy-11-methyl-4-(3-methyl-1,2,4-oxadiazol-5-yl)-6-thioxo-9,2,5-benzoxathiaazacyclododecine-10-one (94)**: white solid (from EtOAc/hexane), mp 191−194 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.91 (s, 3H), 2.36 (s, 3H), 2.84−2.96 (m, 1H), 3.03 (dd, 1H, $J$ = 14, 11), 3.40 (dd, 1H, $J$ = 14, 4), 3.38−3,50 (m, 1H), 3.72 (s, 3H), 3.72/3.79 (2 d, 2 × 1H, $J$ = 10.5), 4.61−4.73 (m, 1H), 4.79−4.93 (m, 1H), 6.80−6.92 (m, 1H), 6.50 (s, 1H), 9.75 (s, 1H), 10.87 (d, 1H, $J$ = 8.5); MS (ISN) 422.4 (M − H)$^-$. Anal. ($C_{18}H_{21}N_3O_5S_2$) C, H, N, S.

**(*R*)-1,3,4,5,6,7,8,9,10,12-Decahydro-16-hydroxy-14-methoxy-13-methyl-4-(3-methyl-1,2,4-oxadiazol-5-yl)-11,2,5-benzoxathiaazacyclotetradecine-6,12-dione (96):** white crystals (from MeOH/$Et_2O$), mp 191−192 °C; $[\alpha]_D$ = −15.0° ($c$ = 0.5, EtOAc); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.58−1.90 (m, 5H), 1.91 (s, 3H), 1.93−2.08/2.31−2.48 (2 m, 2 × 1H), 2.34 (s, 3H), 2.88 (dd, 1H, $J$ = 14, 11.5), 3.25 (dd, 1H, $J$ = 14, 4), 3.70 (d, 1H, $J$ = 11), 3.73 (s, 3H), 3.86 (d, 1H, $J$ = 11), 4.02−4.16/4.50−4.62/5.10−5.22 (3 m, 3 × 1H), 6.52 (s, 1H), 8.64 (d, 1H, $J$ = 8), 9.74 (s, 1H); MS (ISP) 436.4 (M + H)$^+$. Anal. ($C_{20}H_{25}N_3O_6S$) C, H, N, S.

**(*R*)-1,3,4,5,6,7,8,9,10,12-Decahydro-16-hydroxy-14-methoxy-13-methyl-4-(3-methyl-1,2,4-oxadiazol-5-yl)-6-thioxo-11,2,5-benzoxathiaazacyclotetradecine-12-one (97):** white crystals (from acetone/hexane), mp 196−197°C; $[\alpha]_D$ = −26.2° ($c$ = 0.5, EtOAc); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.60−2.05 (m, 4H) superimposed by 1.91 (s, 3H), 2.34 (s, 3H), 2.56−2.72/2.84−3.00 (2 m, 2 × 1H), 3.03 (dd, 1H, $J$ = 14, 11.5), 3.40 (dd, 1H, $J$ = 14, 4), 3.69 (d, 1H, $J$ = 11), 3.73 (s, 3H), 3.89 (d, 1H, $J$ = 11), 4.04−4.18/4.48−4.62/5.68−5.80 (3 m, 3 × 1H), 6.52 (s, 1H), 9.77 (s, 1H), 10.60 (d, 1H, $J$ = 8); MS (ISP) 452.3 (M + H)$^+$. Anal. ($C_{20}H_{25}N_3O_5S_2$) C, H, N, S.

**Method J-2. Acylation of Amine 77. Allyl 3-[Dimethyl-(thexyl)silyloxy]-2-[(*R*)-2-((*R*)-3-hydroxy-3-(methoxycarbonyl)-propionylamino)-2-(3-methyl-1,2,4-oxadiazol-5-yl)-ethylsulfanylmethyl]-5-methoxy-6-methylbenzoate (99).** To a solution of **77** (4.29 g, 8.0 mmol) and (*R*)-2-hydroxysuccinic acid 1-methyl ester (**98**)[30] (1.78 g, 12.0 mmol) in MeCN (60 mL) was added EDC (2.3 g, 12.0 mmol), and the mixture was stirred at 0 °C for 4 h. The mixture was diluted with EtOAc and washed successively with 0.5 N aq HCl, 5% aq $NaHCO_3$, and brine. The organic layer was dried and evaporated to give **99** (4.31 g, 81%), colorless oil: $^1$NMR ($CDCl_3$) $\delta$ 0.26/0.30 (2 s, 2 × 3H), 0.94 (d, 6H, $J$ = 7), 0.98 (s, 6H), 1.73 (m, 1H), 2.09 (s, 3H), 2.36 (s, 3H), 2.65 (m, 2H), 2.83 (dd, 1H, $J$ = 14, 5), 3.21 (dd, 1H, $J$ = 14, 6), 3.63 (d, 1H, $J$ = 12), 3.78 (s, 3H), 3.80 (s, 3H), 3.87 (d, 1H, $J$ = 12), 4.48 (t, 1H, $J$ = 5), 4.85 (d, 2H, $J$ = 6), 5.33 (d, 1H, $J$ = 6), 5.34−5.42 (m, 1H), 5.45 (d, 1H, $J$ = 10), 5.95−6.12 (m, 1H), 6.38 (s, 1H), 6.88 (d, 1H, $J$ = 8).

**Methyl (4*R*,8*S*)-1,3,4,5,6,7,8,10-Octahydro-14-[dimethyl(thexyl)silyloxy]-12-methoxy-11-methyl-4-(3-methyl-1,2,4-oxadiazol-5-yl)-6,10-dioxo-9,2,5-oxathiaazabenzocyclododecine-8-carboxylate (101).** The allyl ester **99** (4.0 g, 6.0 mmol) was cleaved using method K (**84d**) and the resulting hydroxy acid **100** (3.6 g, 5.7 mmol) was cyclized using method H-2 (**85d**), to give **101** (2.25 g, 64%): amorphous solid; $^1$H NMR ($CDCl_3$) $\delta$ 0.26/0.31 (2 s, 2 × 3H), 0.94 (d, 6H, $J$ = 7), 1.00 (s, 6H), 1.77 (m, 1H), 2.19 (s, 3H), 2.38 (s, 3H), 2.86 (dd, 1H, $J$ = 14, 10), 2.89 (dd, 1H, $J$ = 14, 12.6), 3.08 (dd, 1H, $J$ = 14, 3), 3.26 (d, 1H, $J$ = 11), 3.28 (dd, 1H, $J$ = 14, 4), 3.78 (s, 3H), 3.84 (s, 3H), 4.09 (d, 1H, $J$ = 11), 5.51−5.61 (m, 1H), 5.92 (dd, 1H, $J$ = 12.6, 3), 6.39 (s, 1H), 6.75 (d, 1H, $J$ = 8.5).

**Methyl (4*R*,8*S*)-1,3,4,5,6,7,8,10-Octahydro-14-[dimethyl(thexyl)silyloxy]-12-methoxy-11-methyl-4-(3-methyl-1,2,4-oxadiazol-5-yl)-10-oxo-6-thioxo-9,2,5-oxathiaazabenzocyclododecine-8-carboxylate (102).** Using method K (**87d**), **101** (2.08 g, 3.4 mmol) was reacted with Lawesson's reagent to give **102** (1.66 g, 78%): white solid (from EtOAc/hexane), mp 132−135 °C; $^1$H NMR ($CDCl_3$) $\delta$ 0.25/0.31 (2 s, 2 × 3H), 0.94 (d, 6H, $J$ = 7), 1.00 (s, 6H), 1.76 (m, 1H), 2.19 (s, 3H), 2.38 (s, 3H), 3.12 (d, 1H, $J$ = 11), 3.13 (dd, 1H, $J$ = 14, 6), 3.28 (dd, 1H, $J$ = 14, 12), 3.49 (dd, 1H, $J$ = 14, 5), 3.54 (dd, 1H, $J$ = 14, 2.5), 3.77 (s, 3H), 3.86 (s, 3H), 4.13 (d, 1H, $J$ = 11), 5.94 (dd, 1H, $J$ = 12, 2.5), 6.00 (dd, 1H, $J$ = 6, 5), 6.39 (s, 1H), 8.45 (d, 1H, $J$ = 8.5).

**(4*R*,8*S*)-1,3,4,5,6,7,8,10-Octahydro-14-hydroxy-8-hydroxymethyl-12-methoxy-11-methyl-4-(3-methyl-1,2,4-oxadiazol-5-yl)-6-thioxo-9,2,5-benzoxathiaazacyclododecin-10-one (103).** To a solution of **102** (0.63 g, 1.0 mmol) in MeOH/THF (1:1, 10 mL) was added at 0 °C portionwise, over 1 h, $NaBH_4$ (0.19 g, 5 mmol). The reaction mixture was diluted with ice-cold 1 N aq HCl and then extracted with EtOAc. The organic layer was washed with 5% $NaHCO_3$ and brine, dried, and evaporated. The residue was chromatographed ($SiO_2$; EtOAc−hexane 1:1), and the silyl group of the purified product was subsequently cleaved off using method A-2 (**86d**), to give **103** (0.32 g, 70%): white crystals (from MeOH/$Et_2O$), mp 160 °C (dec); $[\alpha]_D$ = +14.0° ($c$ = 0.8, EtOAc); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.94 (s, 3H), 2.35 (s, 3H), 2.93 (dd, 1H, $J$ = 14, 2), 3.00 (dd, 1H, $J$ = 14, 10), 3.20 (dd, 1H, $J$ = 14, 2), 3.37 (d, 1H, $J$ = 11), 3.42 (dd, 1H, $J$ = 14, 4), 3.50−3.71 (m, 2H), 3.72 (s, 3H), 4.09 (d, 1H, $J$ = 11), 5.16 (t, 1H, $J$ = 7), 5.83−5.95 (m, 1H), 6.00−6.12 (m, 1H), 6.50 (s, 1H), 9.74 (s, 1H), 10.85 (d, 1H, $J$ = 8); MS (ISN) 452.3 (M − H)$^-$. Anal. ($C_{19}H_{23}N_3O_6S_2$) C, H, N, S.

**(*R*)-3-(2,2-Dimethyl-1,3-dioxolan-4-yl)propionic Acid (105a).** A mixture of ethyl (*R*)-3-(2,2-dimethyl-1,3-dioxolan-4-yl)acrylate[31] (**104**, 4.0 g, 20 mmol) and 5% Pd−C (0.4 g) in EtOAc (50 mL) was hydrogenated for 1 h at atmospheric pressure. The catalyst was filtered off and the solvent was evaporated. The residual oil was subjected to bulb-to-bulb distillation to afford ethyl (*R*)-3-(2,2-dimethyl-1,3-dioxolan-4-yl)propionate (2.9 g, 72%): colorless oil, bp ∼130 °C/0.1 mbar; $^1$H NMR ($CDCl_3$) $\delta$ 1.26 (t, 3H, $J$ = 7), 1.35/1.41 (2 s, 2 × 3H), 1.78−1.98 (m, 2H), 2.32−2.54 (m, 2H), 3.55 (dd, 1H, $J$ = 8, 7), 4.02−4.20 (m, 4H). A mixture of this material (2.8 g, 14.0 mmol) and KOH (1.18 g, 21.0 mmol) in MeOH (14 mL) was heated to 40 °C for 1.5 h. The solution was concentrated and partitioned between EtOAc and $H_2O$. The aqueous layer was acidified to pH 3 by the addition of 3 N aq HCl and then extracted with EtOAc. The organic layer was dried and evaporated to yield **105a** (1.97 g, 82%): colorless oil; $^1$H NMR ($CDCl_3$) $\delta$ 1.35/1.41(2 s, 2 × 3H), 1.75−1.99 (m, 2H), 2.38−2.59 (m, 2H), 3.57 (dd, 1H, $J$ = 8, 6.5), 4.02−4.20 (m, 2H).

**Allyl 2-[(*R*)-2-[(*R*)-3-(2,2-Dimethyl-1,3-dioxolan-4-yl)-propionylamino]-2-(3-methyl-1,2,4-oxadiazol-5-yl)ethylsulfanylmethyl]-3-[dimethyl(thexyl)silyloxy]-5-methoxy-6-methylbenzoate (106a).** Using Method J-2 (**99**), **77** (37.8 g, 70.6 mmol) was reacted with **105a** (18.7 g, 106 mmol) to give, after chromatographic purification ($SiO_2$; EtOAc/hexane 1:1), **106a** (38.4 g, 79%): pale-yellow oil; $^1$H NMR ($CDCl_3$) $\delta$ 0.26/0.30 (2 s, 2 × 3H), 0.94 (d, 6H, $J$ = 7), 0.98 (s, 6H), 1.35/1.40 (2 s, 2 × 3H), 1.65−1.95 (m, 3H), 2.10 (s, 3H), 2.30 (t, 2H, $J$ = 7), 2.36 (s, 3H), 2.84 (dd, 1H, $J$ = 14, 4), 3.20 (dd, 1H, $J$ = 14, 5), 3.52 (t, 1H, $J$ = 6), 3.63 (d, 1H, $J$ = 12), 3.78 (s, 3H), 3.87 (d, 1H, $J$ = 12), 4.00−4.16 (m, 2H), 4.83 (d, 2H, $J$ = 6), 5.22 (d, 1H, $J$ = 10), 5.34−5.43 (m, 1H), 5.44 (d, 1H, $J$ = 18), 5.95−6.14 (m, 1H), 6.38 (s, 1H), 6.62 (d, 1H, $J$ = 8).

**Allyl 3-[Dimethyl(thexyl)silyloxy]-2-[(*R*)-2-((*R*)-4-hydroxy-5-trityloxy-pentanoylamino)-2-(3-methyl-1,2,4-oxadiazol-5-yl)-ethylsulfanylmethyl]-5-methoxy-6-methylbenzoate (108a).** A solution of **106a** (9.7 g, 14 mmol) in 80% aq AcOH (56 mL) was heated to 60 °C for 20 min. The solvents were evaporated, and the solution of the residual oil in EtOAc was washed with aq $NaHCO_3$ and brine, dried, and evaporated to give crude **107a** (9.9 g) as a pale-yellow oil. A solution of this material and $(Ph)_3CCl$ (4.3 g, 15.4 mmol) in pyridine (7 mL) was stirred at 20 °C for 21 h. The mixture was evaporated, the residue was dissolved in EtOAc, and the solution was washed with 1 N aq HCl and brine, dried, and evaporated. The crude product was chromatographed ($SiO_2$; EtOAc/hexane 1:3) to give **108a** (8.5 g, 68%): colorless oil; $^1$H NMR ($CDCl_3$) $\delta$ 0.25/0.29 (2 s, 2 × 3H), 0.94 (d, 6H, $J$ = 7), 0.98 (s, 6H), 1.60−1.88 (m, 3H), 2.08 (s, 3H), 2.17−2.46 (m, 2H) superimposed by 2.34 (s, 3H), 2.84 (dd, 1H, $J$ = 14, 5), 3.05−3.17 (m, 2H), 3.23 (dd, 1H, $J$ = 14, 6), 3.64 (d, 1H, $J$ = 12), 3.75 (s, 3H), 3.75−3.82 (m, 1H), 3.85 (d, 1H, $J$ = 12), 4.82 (d, 2H, $J$ = 6), 5.27−5.47 (m, 3H), 5.96−6.10 (m, 1H), 6.37 (s, 1H), 6.68 (d, 1H, $J$ = 8), 7.19−7.36 (m, 10H), 7.40−7.47 (m, 5H).

**(4*R*,9*S*)-3,4,5,6,7,8,9,11-Octahydro-1*H*-15-hydroxy-9-hydroxymethyl-13-methoxy-12-methyl-4-(3-methyl-1,2,4-oxadiazol-5-yl)-6-thioxo-10,2,5-benzoxathiaazacyclotridecin-11-one (110).** Subjecting **108a** (4.3 g, 5.0 mmol) consecutively to methods K (**84d**), H-2 (**85d**), and L (**87d**) afforded **109a** (0.62 g) as a foam. A solution of this material (0.61 g, 1.0 mmol) and pTsOH·$H_2O$ (0.21 g, 1.1 mmol) in MeOH (16 mL) was heated for 25 min to 60 °C. The cooled mixture was diluted with EtOAc, washed with 5% aq $NaHCO_3$ and brine, dried, and evaporated. The residue was subjected to method A-2 (**86d**) to give **110** (0.22 g, 47%): white crystals (from MeOH), mp 198−199 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.95 (s, 3H), 2.14−2.28 (m, 2H), 2.34 (s, 3H), 2.64−2.80 (m, 1H), 2.84−2.98 (m, 1H), 2.96 (dd, 1H, $J$ = 14, 11), 3.34 (dd, 1H, $J$ = 14, 4), 3.53−3.61 (m, 2H), 3.68 (d, 1H, $J$ = 11), 3.72 (s, 3H), 3.94 (d, 1H, $J$ = 11), 4.97 (t, 1H, $J$ = 5), 5.36−5.47 (m, 1H), 5.88−6.00 (m, 1H), 6.50 (s, 1H), 9.71 (s, 1H), 10.55 (d, 1H, $J$ = 8); MS (ISP) 468.1 $(M + H)^+$. Anal. ($C_{20}H_{25}N_3O_6S_2$) C, H, N, S.

**(4*R*,10*S*)-1,3,4,5,6,7,8,9,10,12-Decahydro-16-hydroxy-10-hydroxymethyl-14-methoxy-13-methyl-4-(3-methyl-1,2,4-oxadiazol-5-yl)-6-thioxo-11,2,5-benzoxathiaazacyclotetradecin-12-one (111)** was prepared from **77** in analogous manner as **110**, but replacing **105a** by (*R*)-4-(2,2-dimethyl-1,3-dioxolan-4-yl)butyric acid[32] (**105b**): white solid (from EtOAc/hexane), mp 180−83 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.68−2.00 (m, 4H) superimposed by 1.96 (s, 3H), 2.34 (s, 3H), 2.56−2.71/2.73−2.88 (2 m, 2 × 1H), 3.03 (dd, 1H, $J$ = 14, 10), 3.34 (dd, 1H, $J$ = 14, 4), 3.44−3.66 (m, 2H), 3.68 (d, 1H, $J$ = 11), 3.73 (s, 3H), 3.95 (d, 1H, $J$ = 11), 4.86 (t, 1H, $J$ = 5), 4.98−5.09 (m, 1H), 5.73−5.85 (m, 1H), 6.51 (s, 1H), 9.74 (s, 1H), 10.54 (d, 1H, $J$ = 8); MS (ISP) 482.4 $(M + H)^+$. Anal. ($C_{21}H_{27}N_3O_6S_2$·$0.2H_2O$) C, H, N.

**(*R*)-4-(3-Allyloxycarbonylaminomethyl-1,2,4-oxadiazol-5-yl)-1,3,4,5,6,7,8,9,10,12-decahydro-16-[dimethyl(thexyl)-silyloxy]-14-methoxy-13-methyl-6-thioxo-11,2,5-benzoxathiaazacyclotetradecin-12-one (112).** Using the procedures of the reaction sequence **76** → **83d** → **84d** → **85d**, but replacing **76** by **78** and subsequently treating the resulting product with Lawesson's reagent as described in method L (**87d**), the lactone **112** was obtained. For the cleavage of the allyl ester, the following procedure[33] was used, causing concomitant cleavage of the *N*-allyloxycarbonyl protecting group, which subsequently was reintroduced: To a solution of the allyl ester intermediate (3.7 g, 5.0 mol) in DCM (50 mL) were added at 20 °C 4-trimethylsilyl-morpholine (2.66 mL, 15 mmol) and trimethylsilyl acetate (2.26 mL, 15 mmol), and the mixture was stirred for 5 min. $Pd(PPh_3)_4$ (115 mg, 0.1 mmol) was added and stirring was continued for 2 h. Solvents were evaporated, the residual oil was dissolved in MeOH (50 mL), the solution was evaporated again after standing for 0.5 h at 20 °C, and the remaining oil was evaporated once more from toluene (50 mL). A mixture of the residual oil, allyl chloroformate (0.79 mL, 7.5 mmol), and *N*-methylmorpholine (1.65 mL, 15 mmol) in DCM (50 mL) was stirred at 0 °C for 2 h and then evaporated. The residue was dissolved in EtOAc and the solution was washed with 1 N aq HCl and brine, dried, and evaporated. The crude *N*-allyloxycarbonyl-$\omega$-hydroxycarboxylic acid intermediate (3.82 g) was further processed to afford **112**: pale-yellow foam; $^1$H NMR ($CDCl_3$) $\delta$ 0.27/0.28 (2 s, 2 × 3H), 0.93 (d, 6H, $J$ = 7), 0.98 (s, 6H), 1.65−2.00 (m, 5H), 2.08 (s, 3H), 2.85−3.13 (m, 2H) superimposed by 3.02 (dd, 1H, $J$ = 13, 4), 3.20 (dd, 1H, $J$ = 13, 5), 3.63 (d, 1H, $J$ = 11), 3.78 (s, 3H), 3.94 (d, 1H, $J$ = 11), 4.30−4.43 (m, 1H), 4.50 (d, 2H, $J$ = 6), 4.60 (d, 2H, $J$ = 6), 5.18−5.37 (m, 3H), 5.81−6.02 (m, 2H), 6.38 (s, 1H), 8.48 (d, 1H, $J$ = 7).

**(*R*)-4-(3-Aminomethyl-1,2,4-oxadiazol-5-yl)-16-[dimethyl(thexyl)silyloxy]-1,3,4,5,6,7,8,9,10,12-decahydro-14-methoxy-13-methyl-6-thioxo-11,2,5-benzoxathiaazacyclotetradecin-12-one (113).** To a solution of **112** (0.97 g, 1.4 mmol) in DCM (19 mL) were added dimethylaminotrimethylsilane (1.32 mL, 8.4 mol) and trimethylsilyl trifluoroacetate (1.45 mL, 8.4 mmol). The solution was stirred at 20 °C for 10 min, and then $Pd(PPh_3)_4$ (97 mg, 0.084 mmol) was added and stirring was continued for 2.5 h. The mixture was evaporated and the residual oil was dissolved in EtOAc (50 mL). The solution was washed with 10% aq $NaHCO_3$ and brine, dried, and evaporated. The residue was chromatographed ($SiO_2$; EtOAc/hexane 1:2) to give **113** (0.67 g, 78%): foam; TLC $R_f$ = 0.27 (EtOAc); $^1$H NMR ($CDCl_3$) $\delta$ 0.27/0.28 (2 s, 2 × 3H), 0.93 (d, 6H, $J$ = 7), 0.98 (s, 6H), 1.58 (s, 2H), 1.70−2.10 (m, 5H), 2.08 (s, 3H), 2.87−3.19 (m, 2H) superimposed by 3.04 (dd, 1H, $J$ = 13, 4), 3.24 (dd, 1H, $J$ = 13, 5), 3.62 (d, 1H, $J$ = 11), 3.78 (s, 3H), 3.94 (s, 2H), 3.95 (d, 1H, $J$ = 11), 4.32−4.43/4.76−4.89/5.96−6.05 (3 m, 3 × 1H), 6.38 (s, 1H), 8.48 (d, 1H, $J$ = 8).

**(*R*)-4-(3-Aminomethyl-1,2,4-oxadiazol-5-yl)-1,3,4,5,6,7,8,9,10,12-decahydro-16-hydroxy-14-methoxy-13-methyl-6-thioxo-11,2,5-benzoxathiaazacyclotetradecin-12-one Hydrochloride (114).** A mixture of **113** (0.3 g, 0.5 mmol) and $NH_4F$ (0.1 g) in MeOH (10 mL) was stirred for 0.5 h at 20 °C and then partitioned between EtOAc and 5% aq NaCl. The organic layer was dried and evaporated. The solution of the residue (0.3 g) in EtOAc (20 mL) was filtered and then treated at 0 °C with 3 N HCl−$Et_2O$ (0.17 mL). The precipitate that formed was collected by filtration, washed with $Et_2O$, and dried to give **114** (0.22 g, 94%): white solid; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.60−2.08 (m, 5H) superimposed by 1.91 (s, 3H), 2.58−2.74/2.85−3.00 (2 m, 2 × 1H), 3.08 (dd, 1H, $J$ = 14, 11), 3.38 (dd, 1H, $J$ = 14, 4), 3.71 (d, 1H, $J$ = 11), 3.73 (s, 3H), 3.91 (d, 1H, $J$ = 11), 4.06−4.20 (m, 1H), 4.30 (s, 2H), 4.50−4.62 (m, 1H), 5.68−5.80 (m, 1H), 6.56 (s, 1H), 8.64 (br s, 3H), 9.87 (s, 1H), 10.74 (d, 1H, $J$ = 8); HRMS calcd for ($C_{20}H_{26}N_4O_5S_2$-$Na^+$) 467.1423, found 467.1423.

**(*R*)-*N*-[5-(1,3,4,5,6,7,8,9,10,12-Decahydro-16-hydroxy-14-methoxy-13-methyl-12-oxo-6-thioxo-11,2,5-benzoxathiaazacyclotetradecin-4-yl)-1,2,4-oxadiazol-3-ylmethyl]acetamide (115).** A mixture of **113** (30 mg, 0.05 mmol), $Ac_2O$ (0.5 mL), and pyridine (0.01 mL) was heated to 60 °C for 1 h. The solvents were evaporated, and the residue was chromatographed ($SiO_2$; EtOAc/hexane 1:1 and EtOAc). The silyl protecting group of the purified product [29 mg, TLC $R_f$ = 0.40 (EtOAc)] was cleaved off according to method A-2 (**86d**) to give **115** (19 mg, 75%): white solid; TLC $R_f$ = 0.23 (EtOAc); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.60−2.08 (m, 4H) superimposed by 1.86/

1.91 (2 s, 2 × 3H), 2.58−2.72/2.84−2.98 (2 m, 2 × 1H), 3.04 (dd, 1H, $J$ = 14, 11), 3.38 (dd, 1H, $J$ = 14, 4), 3.71 (d, 1H, $J$ = 11), 3.73 (s, 3H), 3.89 (d, 1H, $J$ = 11), 4.04−4.17 (m, 1H), 4.37 (d, 2H, $J$ = 6), 4.48−4.60 (m, 1H), 5.70−5.82 (m, 1H), 6.52 (s, 1H), 8.50 (t, 1H, $J$ = 6), 9.77 (s, 1H), 10.60 (d, 1H, $J$ = 7); HRMS calcd for ($C_{22}H_{28}N_4O_6S_2Na^+$) 531.1348, found 531.1346.

**(*S*)-2-Amino-*N*-[5-[(*R*)-1,3,4,5,6,7,8,9,10,12-decahydro-16-hydroxy-14-methoxy-13-methyl-12-oxo-6-thioxo-11,2,5-benzoxathiaazacyclotetradecin-4-yl]-1,2,4-oxadiazol-3-ylmethyl]propionamide Hydrochloride (116).** Using method J-2 (**99**), **113** (0.1 g, 0.16 mmol) was coupled with *N*-allyloxycarbonyl-L-alanine[34] (30 mg, 0.18 mmol) to give *N*-allyloxycarbonyl-L-alaninyl-**116** (102 mg, TLC $R_f$ = 0.36 (EtOAc/hexane 2:1)) as a foam. For the removal of the protecting groups and the formation of the HCl salt, this material was subjected to the procedures described for the preparation of **114** from **112**. The solution of the resulting oily product in $H_2O$ (2 mL) was lyophilized to give **116** (24 mg, 26%): white solid; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.36 (d, 3H, $J$ = 7), 1.62−2.10 (m, 4H) superimposed by 1.91 (s, 3H), 2.58−2.74/2.85−3.00 (2 m, 2 × 1H), 3.08 (dd, 1H, $J$ = 14, 11.5), 3.38 (dd, 1H, $J$ = 14, 4), 3.72 (d, 1H, $J$ = 11), 3.73 (s, 3H), 3.83−3.96 (m, 1H), 3.90 (d, 1H, $J$ = 11), 4.04−4.17 (m, 1H), 4.41−4.64 (m, 3H), 5.67−5.79 (m, 1H), 6.55 (s, 1H), 8.20 (br s, 3H), 9.13 (t, 1H, $J$ = 6), 9.84 (s, 1H), 10.70 (d, 1H, $J$ = 7); HRMS calcd for ($C_{23}H_{31}N_5O_6S_2H^+$) 538.1794, found 538.1794.

**(*R*)-1,3,4,5,6,7,8,9,10,12-Decahydro-16-hydroxy-4-[3-(isopropylamino)-methyl-1,2,4-oxadiazol-5-yl]-14-methoxy-13-methyl-6-thioxo-11,2,5-benzoxathiaazacyclotetradecin-12-one Hydrochloride (117).** To a mixture of **113** (61 mg, 0.1 mmol), acetone (0.1 mL), NaOAc (16 mg), AcOH (0.08 mL), and $H_2O$ (0.25 mL) in THF (0.5 mL) was added at 0 °C portionwise, over 30 min, $NaBH_4$ (20 mg, 0.5 mmol). Stirring was continued for 10 min, and the mixture was then diluted with EtOAc and washed with aq $NaHCO_3$ and brine. The organic layer was dried and evaporated. The residue was chromatographed ($SiO_2$; EtOAc/hexane 1:2) to yield a minor reaction product [8 mg, TLC $R_f$ = 0.66 (EtOAc/hexane 1:2); diisopropyl derivative] and a major product [40 mg, TLC $R_f$ = 0.36 (EtOAc/hexane 1:2)]. For the removal of the protecting group and the formation of the HCl salt, the latter material was subjected to the procedures described for the preparation of **114** from **113**, and the solution of the resulting oily product in $H_2O$ (2 mL) was lyophilized to give **117** (33 mg, 61%): white solid; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.26 (d, 6H, $J$ = 6.5), 1.62−2.08 (m, 4H) superimposed by 1.91 (s, 3H), 2.60−2.74/2.86−3.00 (2 m, 2 × 1H), 3.08 (dd, 1H, $J$ = 14, 11), 3.38 (dd, 1H, $J$ = 14, 4), 3.72 (d, 1H, $J$ = 11), 3.73 (s, 3H), 3.92 (d, 1H, $J$ = 11), 4.04−4.18 (m, 1H), 4.44 (s, 2H), 4.44−4.60 (m, 1H), 5.66−5.78 (m, 1H), 6.55 (s, 1H), 9.31 (br s, 2H), 9.85 (s, 1H), 10.71 (d, 1H, $J$ = 7); HRMS calcd for ($C_{23}H_{32}N_4O_5S_2H^+$) 509.1892, found 509.1890.

**(*R*)-1,3,4,5,6,7,8,9,10,12-Decahydro-16-hydroxy-4-(3-hydroxymethyl-1,2,4-oxadiazol-5-yl)-14-methoxy-13-methyl-6-thioxo-11,2,5-benzoxathiaazacyclotetradecin-12-one (118).** To a solution of **113** (1.0 g, 1.64 mmol) in 25% aq AcOH (34 mL) was added at 0 °C $NaNO_2$ (0.67 g, 8.5 mmol). The mixture was stirred at 0 °C for 1 h. After setting the pH to 8 by the addition of 14% aq NaOH, the mixture was extracted with EtOAc. The organic layer was washed with aq $NaHCO_3$ and brine, dried, and evaporated, and the residue was chromatographed ($SiO_2$; EtOAc/hexane 1:1). The material isolated from the most polar fraction [20 mg; TLC $R_f$ = 0.24 (EtOAc/hexane 1:1)] was subjected to method A-2 (**86d**) to give **118** (79 mg, 10%): white solid (from EtOAc/hexane); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.60−2.06 (m, 4H) superimposed by 1.91 (s, 3H), 2.56−2.71/2.84−2.98 (2 m, 2 × 1H), 3.04 (dd, 1H, $J$ = 14, 11.5), 3.38 (dd, 1H, $J$ = 14, 4), 3.71 (d, 1H, $J$ = 11), 3.73 (s, 3H), 3.91 (d, 1H, $J$ = 11), 4.04−4.16 (m, 1H), 4.47−4.62 (m, 1H) superimposed by 4.54 (d, 2H, $J$ = 6), 5.68−5.84 (m, 1H) superimposed by 5.73 (t, 1H, $J$ = 6), 6.52 (s, 1H), 9.79 (s, 1H), 10.61 (d, 1H, $J$ = 7); HRMS calcd for ($C_{20}H_{25}N_3O_6S_2Na^+$) 490.1082, found 490.1081.

**DNA Gyrase Inhibition.** A DNA supercoiling assay[12] was used to assess the DNA gyrase inhibitory activity. DNA gyrase $A_2B_2$ complex was reconstituted by incubation of equimolar quantities of its subunits at 25 °C for 0.5 h.[44] As described previously, relaxed pUC18 DNA was incubated with DNA gyrase at 37 °C for 0.5 h with the compound to be tested present at concentrations between 0.001 and 100 $\mu$g/mL.[35] Samples were analyzed by 0.8% agarose gel electophoresis, and the inhibiting activity of the compound tested was assessed by determining the MNEC, i.e., the highest inhibitor concentration not yet affecting the complete supercoiling of the plasmid. The MNEC, determined visually, was found to be 2−5 times lower than the $IC_{50}$ of the supercoiling reaction.

**In Vitro Antibacterial Activity.** Minimum inhibitory concentrations (MICs) for the test compounds against the test strains were determined by an agar dilution method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (National Committee for Clinical Laboratory Standards. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*, 3rd ed.; National Committee for Clinical Laboratory Standards: Villanova, PA, 1993; Vol. 13, no. 25, M7-A3.). Test medium 1, for test strains *N. meningititis* 69480, *S. aureus* 887, *S. pyogenes* b15, and *M. luteus* ATCC8340 was BB2 + 1% Isovitalex + 7.5% sheep blood + menadione. Test medium 2 for test strains *E. coli* 25922, *Xanthomonas maltophilia* AC 739, *S. aureus* 25923, *S. aureus* Smith, *S. epidermidis* 16/2, *S. pyogenes* b15, *S. faecalis* 6 was Mueller−Hinton agar. The compounds were incorporated into a melted medium of 50 °C just prior to the pouring and use of the plates. The inoculum of approximately $10^4$ colony-forming units (CFU) was prepared from appropiately diluted overnight cultures and applied to the agar surface with a multipoint inoculating device (Denley A400 multipoint inoculator). The lowest concentration of the compound that prevented the macroscopic growth of a culture after 18 h of incubation at 35 °C was taken as the minimal inhibitory concentration (MIC).

**In Vivo Antibacterial Efficacy.** The in vivo efficacy was determined in a septicaemia mice model. Septicaemia was induced in outbred Swiss albino mice (Jbm MoRo, weight, 16−20 g). Mice were infected by intraperitoneal injection of a diluted overnight culture of the test organism, *S. aureus* Smith, which was injected as a suspension in 4% gastric mucin. Bacterial challenge doses were about 4 times the number of organisms required to kill 50% of infected but unmedicated animals within 48 h. The test compounds were formulated as microsuspensions in gelatine and administered intravenously 1 and 3 h after bacterial challenge. Control and treatment groups at each dose were composed of five mice each. The 50% effective dose ($ED_{50}$, in milligrams per kilogram of body weight) was calculated by probit analysis as described by Finney (Finney, D. J. *Statistical methods in biological assay*, 3rd ed.; Charles Griffin & Co., Ltd.: London, 1978.) from the survival rates on day 4 after infection).

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