Journal of Medicinal Chemistry

ARTICLE

pubs.acs.org/jmc

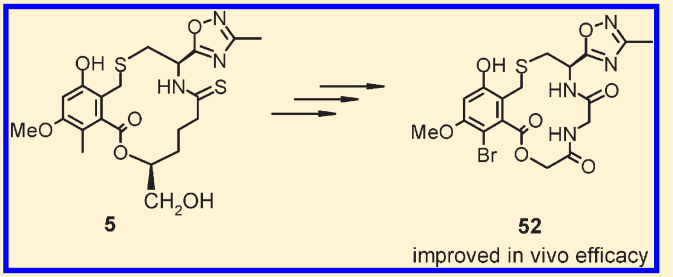
A New DNA Gyrase Inhibitor Subclass of the Cyclothialidine Family Based on a Bicyclic Dilactam−Lactone Scaffold. Synthesis and Antibacterial Properties†

Peter Angehrn, Erwin Goetschi,* Hans Gmuender,‡ Paul Hebeisen, Michael Hennig, Bernd Kuhn, Thomas Luebbers, Peter Reindl, Fabienne Ricklin, and Anne Schmitt-Hoffmann§

F. Hoffmann-La Roche Ltd., Discovery Chemistry, CH-4070 Basel, Switzerland

Supporting Information

ABSTRACT: The DNA gyrase inhibitor cyclothialidine had been shown to be a valuable lead structure for the discovery of new antibacterial classes able to overcome bacterial resistance to clinically used drugs. Bicyclic lactone derivatives containing in their 12−14-membered ring a thioamide functionality were reported previously to exhibit potent antibacterial activity against Gram-positive bacteria. Moderate in vivo efficacy, however, was demonstrated only for derivatives bearing hydrophilic substituents, which were found to have a favorable impact on pharmcokinetics, and to reduce metabolic degradation, in particular glucuronidation. The incorporation of an additional amide unit into the 14-membered monolactam-lactone scaffold of cyclothialidine analogues provided a new "dilactam" subclass of DNA gyrase inhibitors of inherently higher polarity. After adjusting their lipophilicity by methyl−halogen exchange at the benzene ring, compounds of this series did not require the thioamide functionality to exert a decent antibacterial potency and consequently exhibited improved pharmacokinetic properties resulting in a pronounced in vivo efficacy in a mouse septicaemia infection model.



INTRODUCTION

The emergence of resistance to clinically used drugs was recognized early on as a threat in the treatment of bacterial infections.[1] Bacterial resistance mechanisms caused by mutations in the binding site can be directed to a specific therapeutic agent, but cross-resistance to other members of the same family is quite common and can also affect different chemical classes. The use of a different mode of action, in particular the addressing of novel bacterial targets, is an obvious way out of this problem.[2]

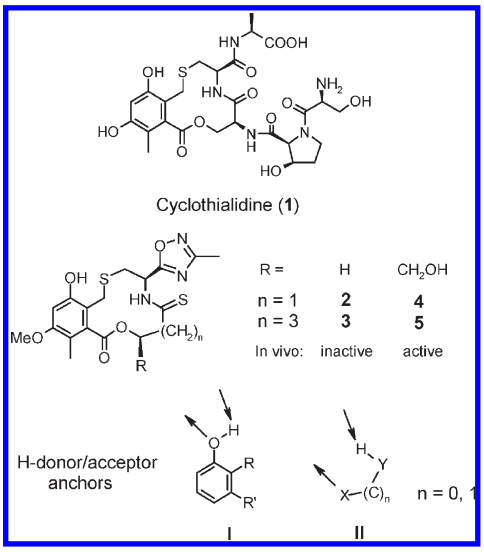
The success of the quinolones in the 1980s had reinforced the interest for DNA gyrase as a bacterial target.[3] By using target-based screening methods, cyclothialidine (**1**) and congeners of it were discovered independently by research groups at Roche[4] and at Glaxo[5] (GR122222X = cyclothialidine C[6]) and were shown to be potent inhibitors of the ATPase activity conferred by the B subunit of DNA gyrase (Chart 1).[7] This target had been already validated by the coumarin class of antibiotics,[8] e.g. novobiocin, which however, since the 1980s, was hardly used clinically. Therefore, new subunit B inhibitors of DNA gyrase were considered to have a potential for the development of drugs devoid of cross-resistance with established antibacterial agents.

Although lacking activity against intact bacterial cells, cyclothialidine was followed up by the Roche group in a medicinal chemistry program which provided analogues of potent antibacterial activity covering broadly the spectrum of Gram-positive bacteria.[9,10] This early, ligand-based work had established a broad knowledge of the structure−activity relationships (SAR) with regard to the DNA gyrase inhibitory activity and shown the importance of physicochemical properties for both antibacterial in vitro activity and in vivo efficacy. Furthermore, the ambivalent role of one of the phenol groups of **1** had been revealed: on one hand, the hydroxy group ortho to the thiomethyl side chain of the benzene ring was found to be essential for the binding to the enzyme,[11] and we have early on postulated its role as a hydrogen-bond donor and acceptor. On the other side, this main anchor group **I** of the cyclothialidine inhibitor family was also found to be its Achille's heel for being a target for glucuronidation, leading to rapid inactivation and clearance from the plasma of promising derivatives such as the bicyclic lactones **2** and **3**. Phenol conjugation as well as strong plasma protein binding were considered by us as major factors preventing the translation of the antibacterial potency into an adequate in vivo efficacy. Following our working hypothesis suggesting more hydrophilic derivatives to be less affected by these drawbacks, we eventually found analogues, e.g. the hydroxymethyl derivatives **4** and **5**, that displayed both improved pharmacokinetic properties and in vivo efficacy.[10]

The difficulties to achieve in vivo efficacy with cyclothialidine derivatives had raised interest in alternative subclasses. We had discovered that also *seco*-cyclothialidines can be good DNA

Received: October 28, 2010
Published: March 09, 2011

Chart 1



gyrase inhibitors, a finding that was also taken up by others,[9b,12] but for this as well as for other phenol subseries of the general structure **I**,[13] it so far has not been possible to combine a useful level of antibacterial activity with appropriate physicochemical properties. Other targeted screening programs have provided nonphenolic DNA gyrase subunit B inhibitors. Triazines[14] and alkyl-urea substituted *N*-heterocycles[15,16] were discovered by screening for ATPase inhibitors, pyrazoles by assaying the inhibition of chromosome partitioning in *Escherichia coli*,[17] and pyrrole-2-carboxamides by using a NMR guided screen.[18] Structural information on the binding mode of cyclothialidine,[19,20] novobiocin,[20] but also of the ATP analogue ADPNP,[21] to the ATP binding site has been used more and more to guide the optimization of lead structures, and in addition, it allowed to apply fragment-based in silico tools in the search for new classes of DNA gyrase inhibitors. Thus, a biased in silico "needle" screening for potential inhibitors featuring a generic hydrogen-bond donor/acceptor motif **II**, followed up by an extended hit validation process, provided several new nonphenolic DNA gyrase inhibitor classes such as indazoles and 2-hydroxymethyl-indoles.[22] Among the various nonphenolic series of subunit B inhibitors described in the past decade, many still represent only potential starting points for an optimization program. Others, however, such as ethyl-urea-benzimidazoles[15] or pyrrole-2-carboxamides[16] have been developed to potent antibacterials which show promising efficacy in vivo.

We have described for cyclothialidine analogues how well-balanced physicochemical properties are required to allow translation of potent enzyme inhibitory activity into significant efficacy in the therapy of infections.[10] Prototypical illustration of this concept is the conversion of the in vivo inactive, lipophilic lactones **2** and **3** into efficacious analogues **4** and **5**, respectively, the difference being made by an additional hydroxymethyl substituent. Herein we report on a new subclass of the cyclothialidine inhibitor family, which emerged from our continued efforts to improve on the conversion of potent enzyme inhibitory activity into significant in vivo efficacy within the 14-membered lactone series. To this end, we investigated not only new substitution patterns on the parent monolactam-lactones but also the incorporation of polar elements into the bicyclic scaffold.

CHEMISTRY

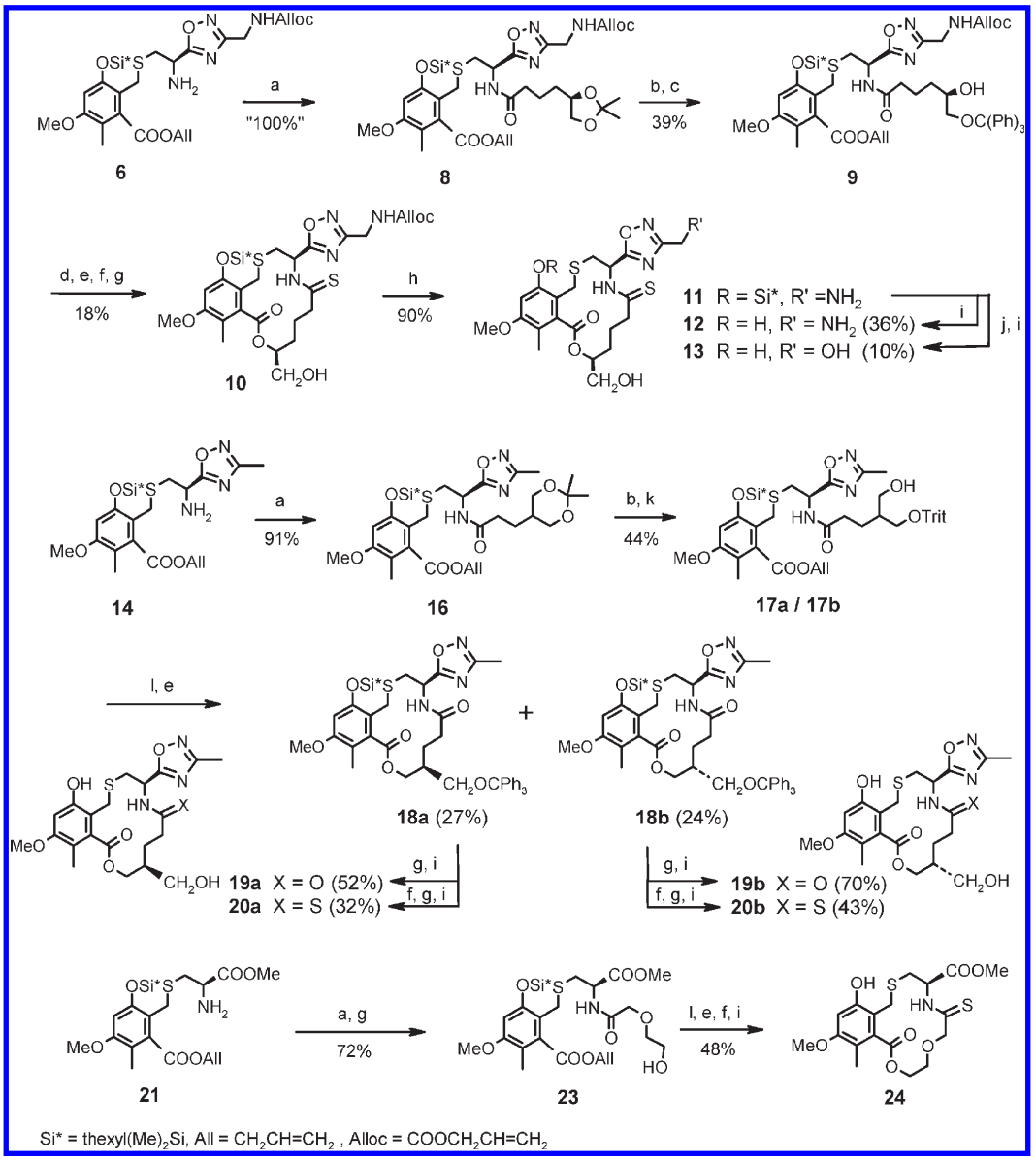
The preparation of new bicyclic lactones relied on the synthetic pathways described previously.[10] In general, a primary amine (**6**, **14**, **21**, **34b,c**, **35b,c**, **82**) was acylated with an appropriate linker unit (e.g., **7**, **15**, **22**, **44**). If required, the hydroxy function at the C-5 position of the linker moiety was unmasked and other functional groups were optionally protected, whereupon the allyl ester was cleaved and the resulting ω-hydroxy-carboxylic acid was lactonized. A reaction sequence consisting mainly in optional thiation of the lactam group, optional derivatization, and eventual cleavage of protecting groups provided the target compounds.

In the preparation of **12** and **13** (Scheme 1), ketal **8** was hydrolyzed and the primary alcohol was protected as trityl ether (**9**). Palladium-assisted cleavage of the allyl ester of **9** also caused partial cleavage of the allyl carbamate group, which had to be reestablished by subsequent treatment of the crude product with allyl chloroformate. After the lactonization, thiation, and trityl ether cleavage steps, the resulting alcohol **10** was converted to the target compounds. For the syntheses of the 13-hydroxymethyl derivatives **19a,b** and **20a,b**, amine **14**[10] was acylated with dioxolane **7**.[23] Upon hydrolysis of ketal **16** and protection of the primary alcohol as trityl ether, the mixture of diastereomeric allyl esters **17a**/**17b** was converted into the lactones **18a** and **18b**, which were separated by chromatography. The pure isomers were converted to the target compounds **19a,b** and **20a,b** in the usual manner. The stereochemical assignment for the 13-hydroxymethyl substituent was made possible by an X-ray crystal structure of lactone **19b**.[24] Analogously, the 9-oxa lactone **24** was assembled starting from amine **21**[10] and linker-acid **22**.[25]

For the synthesis of the 1-chloro- and 1-bromo-lactones **36**−**41** (Scheme 2), the required amino intermediates **34b,c** and **35b,c** were prepared starting from methyl ester **25**.[26] Treatment of **25** with sulfuryl chloride in dichloromethane provided a mixture of regioisomers from which the major component **26** could be isolated in pure form by crystallization. Saponification of the ester **26** afforded pseudoacid **27b**, which in its NMR spectrum (DMSO-d_6) also displayed signals of the corresponding *o*-formyl benzoic acid (ca. 20%). For the preparation of the corresponding bromo-derivative (**27c**), **25** was converted to pseudoacid **28**, which subsequently was brominated in dichloromethane with high regioselectivity. The resulting bromide **27c** exhibited the same pseudoacid/acid ratio in DMSO as **27b**. Treatment of **27b**, **c** with tetramethylguanidine and allyl bromide in DMF followed by silylation of the phenol group afforded aldehydes **30b,c**. As reductive thiolation[27] of **30b,c** with the cysteine derivatives **32** or **33**[10] was accompanied by extensive aldehyde to methyl reduction and partial debromination in the case of **30c**, the benzyl iodides **31b,c** were prepared and used for the alkylative coupling with thiols **32** and **33**.[28] Intermediates **34b,c** and **35b,c** were converted to the target compounds **36**−**41**, respectively, by subjecting them to the reaction sequence described previously for the preparation of **98**, **3**, and **100** (Table 1) from amines **14** and **6**, respectively.[10]

For the preparation of dilactams **47**−**50**, amine **14** was coupled with glycine derivative **44**, and the resulting product was converted to the bicyclic lactone **47** in 4 steps. Heating of dilactam **46a** with 0.8 equivalent of Lawesson's reagent in toluene provided three products which were separated by chromatography and thereafter desilylated by treatment with

Scheme 1[a]



[a] Reagents and conditions: (a) (*R*)-4-(2,2-dimethyl-[1,3]dioxolan-4-yl)-butyric acid (**7**), 3-(2,2-dimethyl-[1,3]dioxan-5-yl)-propionic acid (**15**), or (2-trityloxy-ethoxy)-acetic acid (**22**), for **8**, **16**, or **23**, respectively, EDC, MeCN, 0 °C; (b) 5 N HCl-EtOH, rt; (c) tritylpyridinium trifluoroborate, MeCN, rt; (d) (i) $Pd(PPh_3)_4$, 4-TMS-morpholine, AcOTMS, DCM, rt, (ii) $ClCOOCH_2CHCH_2$, NMM, DCM, 0 °C; (e) DEAD, PPh_3, toluene, 0−20 °C; (f) Lawesson's reagent, toluene, 80 °C; (g) pTsOH, MeOH, 20−60 °C; (h) $Pd(PPh_3)_4$, Me_2N-TMS, TFA-TMS, DCM, 0 °C; (i) NH_4F, MeOH, rt; (j) $NaNO_2$, 25% aq AcOH, 0 °C; (k) $ClC(Ph)_3$, pyridine, rt; (l) $Pd(PPh_3)_4$, morpholine,THF, 0 °C.

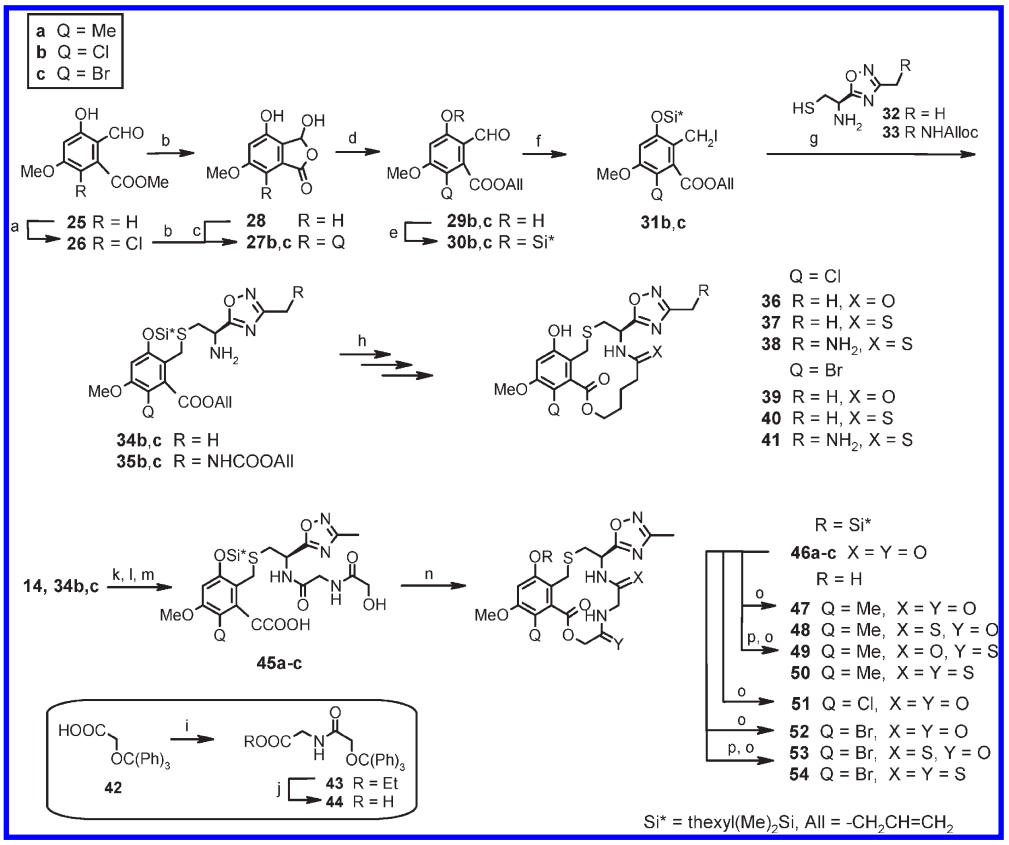
NH_4F in methanol, to give the two regioisomeric monothioxo derivatives **48** and **49** as well as the 8,11-dithioxo product **50**. The structural assignment for the monothioxo products is based on the marked low-field shift of the NH-proton of the thioamide functionality. Using the same reaction sequence, the chloro- and bromo-analogues **51**, and **52**-**54**, were prepared from the amines **34b** and **34c**, respectively.

Amine **34c** also served for the syntheses of lactones **58**−**59**, **63**−**66**, and **71**−**78** (Scheme 3). For the 9-methyl derivatives **58**−**59**, the linker was introduced stepwise, whereas for the other syntheses the readily available linker entities **60a**,[29] **60b**, and **67**[30] were attached in one step. The cyclic amines **71** and **78** were obtained from 9-Boc intermediate **69** by optional thiation and cleavage of protecting groups. *N*-acylation of amine **70** afforded derivatives **72**−**77**.

For the preparation of tertiary amine **81** (Scheme 4), intermediate **34c** was heated with lactone **79**, and the resulting acylation product **80** was subjected to the usual reaction sequence. To modify the substituent of the oxadiazole ring, carboxylic acid **85** was synthesized starting from benzyl iodide **31c** and L-cysteine methyl ester. Coupling of **85** with the amide-oximes **86a**,**b** followed by thermal cyclization provided analogues **87** and **88**. Further variations of the oxadiazole substitution were accomplished by modifying the aminomethyl substituent of lactones **91** and **92** using procedures described previously.[10]

dx.doi.org/10.1021/jm1014023 |*J. Med. Chem.* 2011, 54, 2207–2224

Scheme 2[a]



[a] Reagents and conditions: (a) SO_2Cl_2, DCM, rt; (b) 2 N NaOH, rt; (c) Br_2, DCM, 0−20 °C; (d) allyl bromide, 1,1,3,3-tetramethylguanidine, DMF, rt; (e) thexyldimethylchlorosilane, NEt_3, DMF, 0−20 °C; (f) tetramethyldisiloxane, TMSCl, NaI, MeCN, 0 °C; (g) **32** or **33**, respectively, NEt_3, DCM, rt; (h) prepared in analogous manner as **99**, **3**, and **100** starting from **34b,c** or **35b,c**, respectively, see Experimental Section; (i) Gly-OEt, EDC, NMM, MeCN, 0 °C; (j) KOH, MeOH, 60 °C; (k) **44**, EDC, MeCN, 0 °C; (l) pTsOH, MeOH, rt; (m) $Pd(PPh_3)_4$, morpholine,THF, 0 °C; (n) DEAD, PPh_3, toluene, 0−20 °C; (o) NH_4F, MeOH; (p) Lawesson's reagent, toluene, 80 °C.

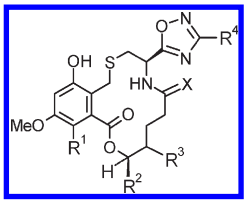
Conformational Aspects of the 14-Membered Lactones. During our previous work with 12−14-membered monolactams, e.g. **2** or **3**, the AX_2 spin system of the protons of the cysteamine entity was found to display in various solvents always a large and a small vicinal coupling constant, suggesting a high population of a conformation with a synclinal/antiperiplanar arrangement of these protons. This conformational feature was also found for **1** when bound to the ATPase site of DNA gyrase.[19,20] In the NMR spectrum of the excellent dilactam inhibitors, however, two small- to medium-sized vicinal coupling constants for the cysteamine protons were observed, indicating a synclinal/synclinal arrangement to be predominant. Interestingly, the X-ray analyses of the 14-memberd lactones **3** and **52** display ring conformations which do not correspond to the conformational features observed in solution (Figure 1). The crystal structure of monolactam **3** exhibits a synclinal/synclinal relation for the cysteamine hydrogens with the oxadiazole ring in a pseudoaxial position, whereas in that of dilactam **52**, the cysteamine hydrogens are synclinal/antiperiplanar and the oxadiazole is in a pseudoequatorial position. Both structures show an intramolecular hydrogen bond between the N(9/12) hydrogen and the lactone carbonyl. This hydrogen bond is more pronounced in the dilactam scaffold where it is part of a perfect β-turn motif. It is noteworthy that 3D structure generation for **3** and **52** proposes low-energy conformations, which are in agreement with the observed coupling constants in the solution NMR spectra. Whereas the calculation for **3** affords a conformation which can easily be superimposed with the "binding conformation" of **1**, the conformation of **52** in its bound state is presumably different from that proposed in Figure 1 (Figure 2).

BIOLOGICAL RESULTS AND DISCUSSION

As improving of in vivo efficacy within the class of the bicyclic 14-membered lactones represents a multidimensional problem, the impact of structural modifications of the lead compounds, **98** and **3** (Table 1), on their physicochemical and enzyme inhibitory properties, as well as on their antibacterial activity in vitro and in vivo, will be discussed in parallel. We used calculated log *P* (CLOGP) values for all compounds as a rough estimate of lipophilicity, which we consider (besides the intrinsic enzyme inhibitory activity) to be the major player in the optimization process. Lipophilicity/hydrophilicity properties control cell permeation, thus antibacterial activity, as well as solubility, protein binding, and susceptility for glucuronidation, hence pharmacokinetic behavior and in vivo efficacy. Regarding both DNA gyrase inhibitory activity, expressed as maximum noneffective concentration (MNEC) in a supercoiling assay[10] and antibacterial activity in vitro, assessed as minimum inhibitory concentration (MIC), parent lactam **98** was markedly surpassed by the

dx.doi.org/10.1021/jm1014023 |J. Med. Chem. 2011, 54, 2207–2224

Table 1. DNA Gyrase Inhibitory Activity, in Vitro and in Vivo Antibacterial Activity, and CLOGP of Monolactams. Comparison with Reference Compounds



compd	R^1	R^2	R^3	R^4	X	MNEC[a] (μg/mL)	MIC[b] (μg/mL)	ED_{50}[c] (mg/kg)	CLOGP[d]
98[f]	Me	H	H	Me	O	0.01	0.25	>25	1.70
3[f]	Me	H	H	Me	S	0.004	0.06	>25	2.37
5[f]	Me	CH_2OH	H	Me	S	0.005	0.5	12.5	1.41
99[f]	Me	H	H	CH_2OH	S	0.001	0.25	25	1.06
100[f,g]	Me	H	H	CH_2NH_2	S	0.002	0.25	8.5	1.05
12	Me	CH_2OH	H	CH_2NH_2	S	0.005	32	nd[e]	0.09
13	Me	CH_2OH	H	CH_2OH	S	0.01	10	nd	0.10
19a	Me	H	β-CH_2OH	Me	O	0.005	8	nd	0.23
19b	Me	H	α-CH_2OH	Me	O	0.1	64	nd	0.23
20a	Me	H	β-CH_2OH	Me	S	0.002	0.5	12.5	1.62
20b	Me	H	α-CH_2OH	Me	S	0.05	1	>25	1.62
36	Cl	H	H	Me	O	0.005	0.25	>25	1.76
37	Cl	H	H	Me	S	0.002	0.25	nd	2.58
38[g]	Cl	H	H	CH_2NH_2	S	0.002	0.12	6	1.27
39	Br	H	H	Me	O	0.005	0.12	25	1.92
40	Br	H	H	Me	S	0.005	0.12	>25	2.73
41[g]	Br	H	H	CH_2NH_2	S	0.05	0.5	12.5	1.42

[a] Supercoiling assay, *E. coli* gyrase, MNEC = maximum noneffective concentration. [b] Agar dilution (Mueller−Hinton agar); inoculum 10^4 CFU/spot, MIC = minimum inhibitory concentration, *Staphylococcus aureus* Smith. [c] In vivo efficacy, septicaemia in mice (*S. aureus* Smith), iv administration. [d] CLOGP = calculated log *P* (version 4.94, program BioByte Corp., Claremont, CA). [e] Not determined. [f] Monolactam reference compounds.[10] [g] Hydrochloride salt.

10-thioxo analogue **3**. As a matter of fact, from a large number of compounds prepared, only thiolactams seemed to have the potential for in vivo efficacy, which eventually was found for polar analogues such as the hydroxymethyl derivatives **4**, **5**, and **99**, or the amine **100**.[10]

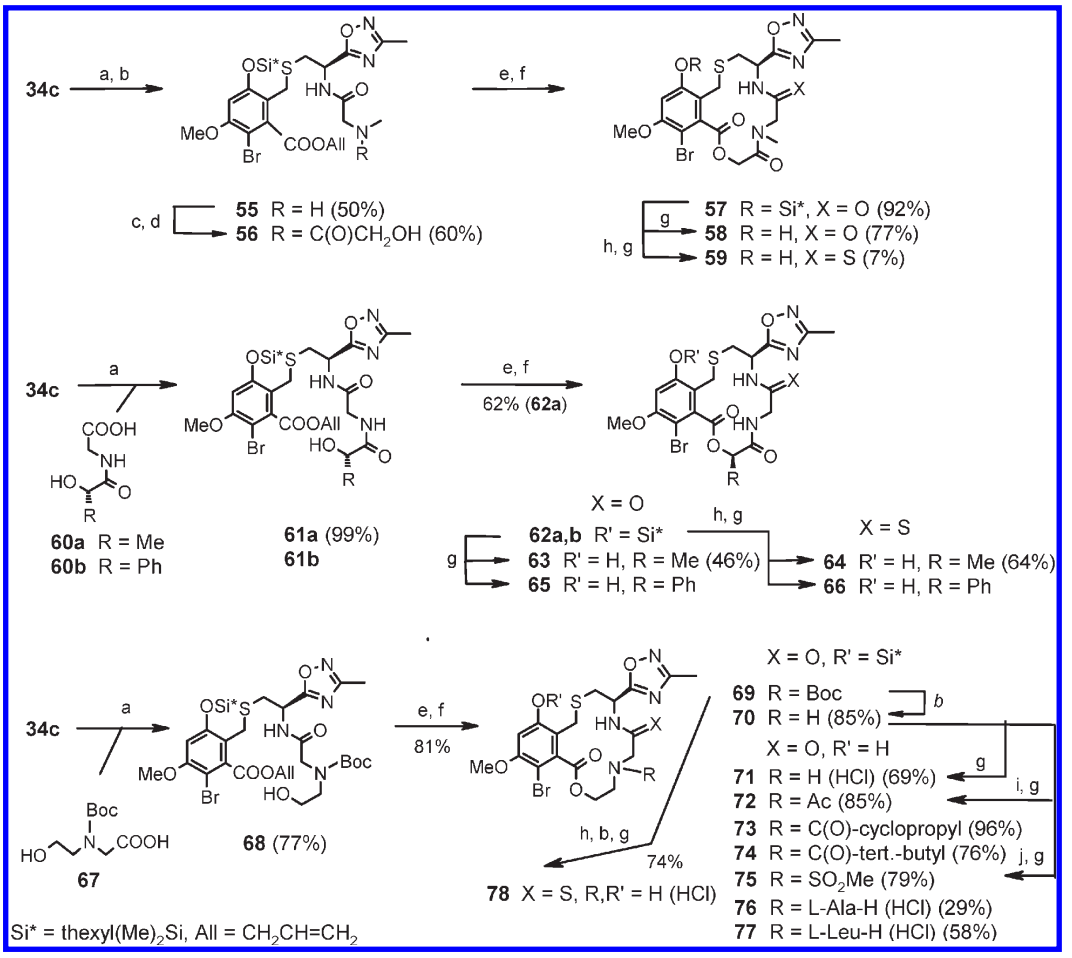
We started out by investigating further substitution patterns of the parent lactones **98** and **3**. Table 1 shows a compilation of biological data of novel derivatives of this series in comparison with that of key compounds (**98**, **3**, **5**, **99**, **100**) from our previous work in the 14-membered lactone series. The combination of two hydrophilic substituents in the same molecule provided the potent enzyme inhibitors **12** and **13**, which however displayed a strongly diminished antibacterial activity, presumably due to their pronounced hydrophilic character.

We probed systematically other positions of the core lactone for their suitability to carry polar substituents. Exemplary for this investigation are the two isomeric thiolactams **20a** and **20b** bearing a hydroxymethyl group in the 13β- and 13α-positions, respectively. We noticed a 25-fold stronger enzyme inhibiton for the β-hydroxymethyl derivative **20a** when compared to the α-substituted analogue **20b**. A similar differentiation had been observed already for the epimeric 10-oxo analogues **19a** and **19b**. Whereas, however, the superiority of the 13β-hydroxymethyl substitution was also reflected in a better antibacterial activity of **19a**, the MICs of **20a** and **20b** were similar. Nevertheless, only the 13β-substituted isomer **20a** was efficacious in vivo, although not surpassing the efficacy of our best compound. The fact that no lactam, i.e. no 10-oxo-analogue displaying in vivo efficacy, had been identified up to then was explained by the inherently higher polarity, giving rise to a weak antibacterial activity due to slow cell penetration. However, the failure of lactam **98** in the in vivo assay was not obvious. It displayed a good in vitro activity against *S. aureus* with only a small "plasma shift" when assessed in the presence of plasma proteins. We tried to moderately increase the lipophilicity by replacing the 1-methyl group by a chloro or a bromo atom. This modification had been shown previously to be beneficial for the in vitro potency of *seco*-cyclothialidines.[12a] We found that both halogen analogues of **98**, i.e. **36** and **39**, showed a slight improvement of both enzyme inhibitory and in vitro antibacterial activity, but most importantly, bromo analogue **39** displayed in vivo efficacy. The same modifications at thiolactam **3** led to a slightly decreased activity against *S. aureus*, and the 1-bromo analogue **40** was found inactive in vivo. For the more polar amine **100**, however, the combination of these lipophilic elements was helpful. In particular, the chloro derivative **38** was active in the mouse septicaemia model with an ED_{50} of 6 mg/kg. Obviously, and in accordance with our working hypothesis, it appears that halogenation is only helpful for polar compounds like **98** or **100** but not for lipophilic ones like **3**.

For tuning the lipophilicity in the 14-membered lactone series, we had used until that time four structural elements: the conservation of the amide functionality itself and polar substituents such as hydroxymethyl or aminomethyl for increasing hydrophilicity, and the thioamide group and halogenation of the benzene ring for amplifying hydrophobicity. An alternative way to enhance the hydrophilic character of target compounds

dx.doi.org/10.1021/jm1014023 |*J. Med. Chem.* 2011, 54, 2207–2224

Scheme 3[a]



[a] Reagents and conditions: (a) for **55**, **61a**, **61b**, or **68**, Boc-N(Me)CH_2COOH, **60a**, **60b**, or **67**, respectively, EDC, MeCN, 0 °C; (b) TFA, 0 °C; (c) (Ph_3COCH_2COOH, HOBT, EDC, MeCN, 0 °C; (d) pTsOH, MeOH, rt; (e) $Pd(PPh_3)_4$, morpholine, THF, 0 °C; (f) DEAD, PPh_3, toluene, 0−20 °C; (g) NH_4F, MeOH, rt; (h) Lawesson's reagent, toluene, 80 °C; (i) Ac_2O, pyridine, 60 °C; (j) cyclopropane-carbonyl chloride, 2,2-dimethyl-propionyl chloride, benzoyl chloride, or methanesulfonyl chloride, respectively, NEt_3, DCM, 0 °C, or L-Ala-OH or L-Leu-OH, respectively, EDC, MeCN, 0 °C.

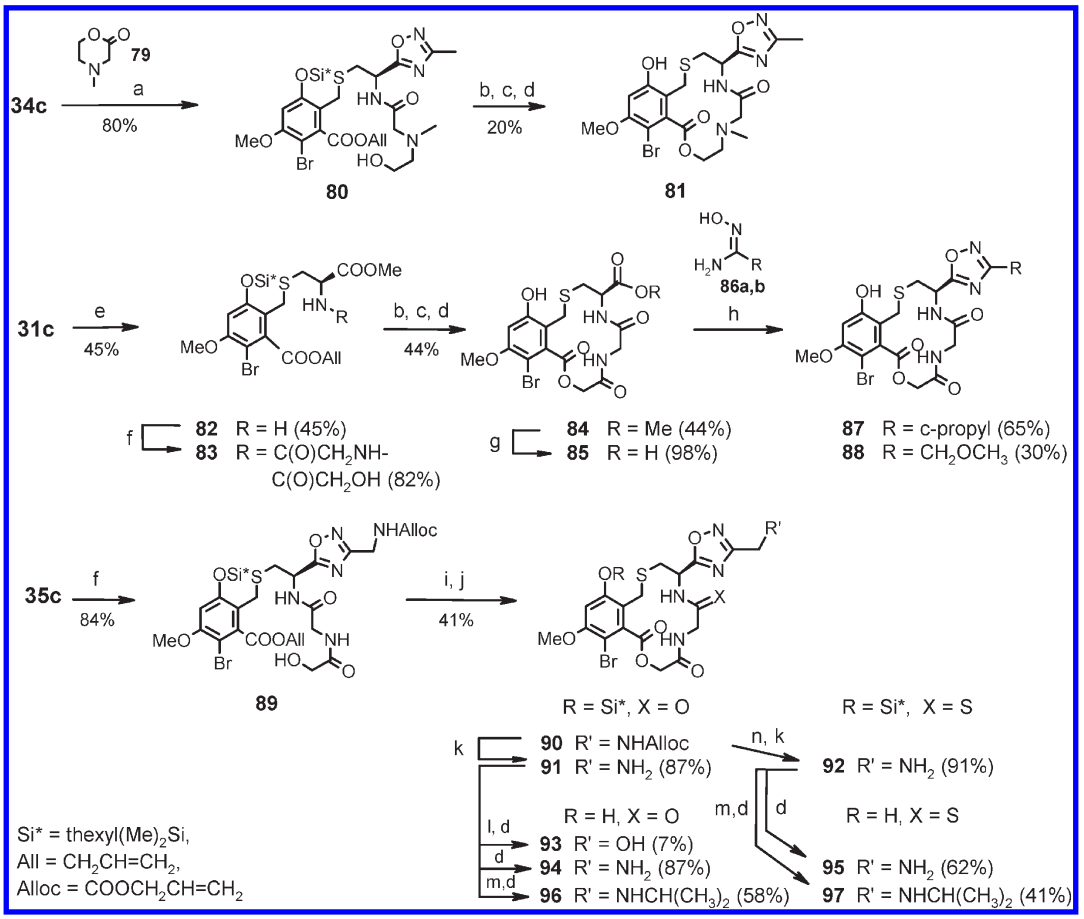
consists in using a more polar scaffold. In an early attempt to apply this strategy, it had been found that the cyclic ether **24**, the 12-oxa analogue of **101** (atom numbering of structure **101**),[10] displayed a significantly reduced antibacterial potency, which we had attributed to unfavorable binding properties as well as to the increased hydrophilicity when compared to the parent **101** (Figure 3). In contrast, dilactam **47**, containing a dipeptide moiety in the 14-membered lactone ring, was found to maintain the DNA gyrase inhibitory activity of the parent monolactam **98**. The antibacterial activity, however, was strongly affected by its polar character, making it also inefficacious in the therapeutic in vivo assay (Table 2). Therefore, we set out to increase the lipophilicity of **47** by either applying thiation of the amide groups or halogenation of the benzene ring. The 11-thioxo analogue **48** was a 10 times more potent DNA gyrase inhibitor, exhibited excellent antibacterial activity, and unlike its monolactam analogue **3**, was found to be efficacious in vivo. The 8-thioxo regioisomer **49** was inferior to **48**. The bis-thioxo product **50**, although equipotent to **48**, was not followed up.

The need to introduce a certain level of lipophilicity to warrant a decent antibacterial activity in vitro and in vivo had been satisfied up to then by keeping at least one thioamide functionality in the 12−14-membered bicyclic scaffolds, the noteworthy exception having been the bromo-lactam **39**. Taking into account the improved properties of halogenated monolactam-lactones, we also prepared the 4-chloro and the 4-bromo analogues of the new dilactams. In comparison to **47**, chloro-dilactam **51** displayed the same modest antibacterial activity but nonetheless was found to be active in vivo, like **39**. Bromo-dilactam **52**, however, being equipotent as enzyme inhibitor, was found to be 8 times more active in vitro against *S. aureus* Smith. To our surprise, this compound showed an excellent efficacy in vivo in the mouse septicaemia model with an ED_{50} of 3 mg/kg when compared to the ED_{50} values assessed for novobiocin (3 mg/kg) and vancomycin (1.5 mg/kg) in the same assay. The 11-thioxo analogue **53**, despite its superior antibacterial activity, was less efficacious in vivo than **52**, and the even more lipophilic bis-thioxo derivative **54** was found to exhibit both lower enzyme inhibitory and lower antibacterial activity.

The favorable biological properties of bromo-dilactam **52** let us consider it as a new lead structure. In particular, the fact that this compound was devoid of the potentially toxic and metabolically instable thioamide entity was regarded as an advantage.[32] We therefore explored further possibilities to optimize the dilactam scaffold of **52**, investigating at first the impact of substituents. It was found that *N*-methylation of the newly

dx.doi.org/10.1021/jm1014023 |*J. Med. Chem.* 2011, 54, 2207–2224

Scheme 4[a]



[a] Reagents and conditions: (a) **79**, 1-oxy-pyridin-2-ol, toluene, 80 °C; (b) $Pd(PPh_3)_4$, morpholine, THF, 0 °C; (c) DEAD, PPh_3, toluene, 0−20 °C; (d) NH_4F, MeOH, rt; (e) L-Cys-OMe, NEt_3, DCM, 0 °C; (f) (i) **44**, EDC, MeCN, 0 °C, (ii) pTsOH, MeOH, 20 °C; (g) 0.1 N NaOH, aq THF, rt; (h) (i) *N*-hydroxy-cyclopropanecarboxamidine (**86a**) or *N*-hydroxy-2-methoxy-acetamidine (**86b**), respectively, DCC, 1-oxy-pyridin-2-ol, DMF, 0 °C, (ii) toluene, 110 °C; (i) (i) $Pd(PPh_3)_4$, 4-TMS-morpholine, AcOTMS, DCM, rt, (ii) $ClCOOCH_2CHCH_2$, NMM, DCM, 0 °C; (j) DEAD, PPh_3, THF, 0−20 °C; (k) $Pd(PPh_3)_4$, $(Me)_2$N-TMS, TFA-TMS, DCM, rt; (l) $NaNO_2$, 25% aq AcOH, 0 °C; (m) Lawesson's reagent, toluene, 80 °C; (n) acetone, $NaBH_4$, aq AcOH, NaOAc, 0 °C.

introduced amide function (**58**) only slightly affected MNEC and MIC values, but that in vivo efficacy was lost. A similar effect was noted for the introduction of a 7β-methyl substituent (**63**), whereas a 7β-phenyl group (**65**) markedly reduced the enzyme inhibitory activity. To get a more complete SAR picture, the 11-thioxo analogues **59**, **64**, and **66**, were also prepared. Displaying in general a reduced enzyme inhibitory activity, they had slightly increased antibacterial potency, which, however, was not translated into in vivo efficacy.

The basic amine **71**, formally obtained by reduction of the 8-oxo carbonyl group of **52**, represents the 12-aza analogue of **98** (atom numbering of structure **98**). In contrast to the lowered enzyme inhibitory activity of the 9-oxa derivative **24**, when compared to its carba analogue **101**, amine **71** was found to be equally active as both monolactam **98** and dilactam **52**. Its antibacterial properties were less pronounced like those of dilactam **52**, but unlike **52** it showed only borderline in vivo efficacy. The corresponding thiolactam **78** was assayed inactive in vivo.

Attempts to improve the biological properties of amine **71** by further modifications at its amino function were unsuccessful (Table 3). *N*-Methylation (**82**) led to loss of in vivo efficacy, and *N*-acylation (**72**−**77**) mostly had a negative impact on the antibacterial activity, an exception being the pivaloyl derivative **74**, which however was inactive in mice.

Modification of the oxadiazole side chain of **52** along the line exercised earlier for the monolactams[10] provided similar results (Table 4). With the exception of cyclopropyl analogue **87**, being structurally closest to **52**, compounds **88**, **93**, **94**, and **96** lost their in vitro potency, probably due to their strong hydrophilic character. This is supported by the finding that the 11-thioxo analogues of **94** and **96**, the equipotent inhibitors **95** and **97**, regained antibacterial activity, and **97**, but not **95**, was also curative in the in vivo assay although four times less than dilactam **52**.

The impact of the three major structural modifications applied, i.e. polar substituents, methyl/halogen exchange, and monolactam/dilactam switch, can be summarized as follows:

DNA Gyrase Inhibiton. In the monolactam series, the attachment of 1 or 2 polar substituents was found to be tolerated in several positions, e.g. 14β, 13β, and the 3′-position of the oxadiazole, but in the 13α-position, it caused a strong reduction of activity. Both 1-chloro and 1-bromo substituents brought a 2-fold increase of activity for lactam **98**, but only 1-chloro

dx.doi.org/10.1021/jm1014023 |*J. Med. Chem.* 2011, 54, 2207–2224

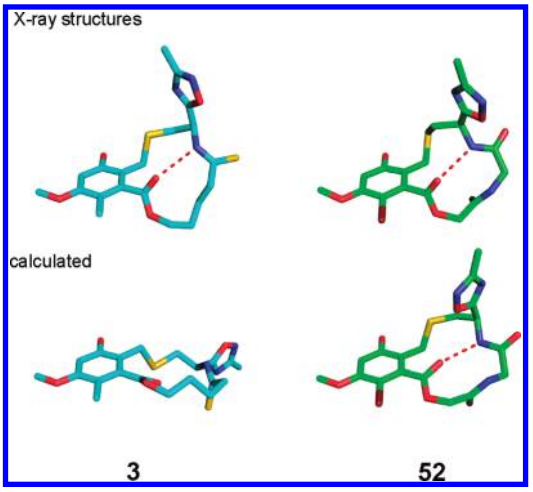


Figure 1. X-ray structures and calculated low-energy conformations of lactones **3** and **52**.[24] In the solid state, dilactam **52** adopts a β-turn-like conformation which is stabilized by a strong intramolecular hydrogen bond (d (O···HN) = 2.94 Å), whereas the corresponding hydrogen bond in thiolactam **3** is longer (d = 3.19 Å), indicating weaker stabilization of this inherently more flexible ring system. The oxadiazole substituent in the crystal structure occupies a pseudoaxial position in **3** but a pseudoequatorial positition in **52**. Interestingly, CORINA 3D structure generation[31a] proposes low-energy conformations where the heterocycle is pseudoequatorial in **3** and pseudoaxial for **52**, which is in agreement with the coupling constants found in the NMR spectra. The strong intramolecular hydrogen bridge is preserved in the calculated conformation of **52** (d = 2.83 Å).

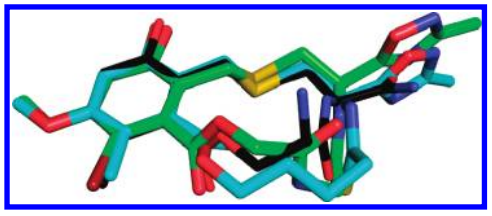
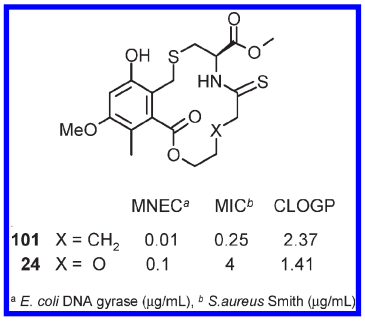


Figure 2. Overlay of **1** (black, structure truncated for clarity of view) bound to the ATP binding site of DNA gyrase[19] with low-energy conformations of **3** (cyan) and **52** (green). The conformation of **52** with the heterocycle in the pseudoequatorial position was adapted from the CORINA generated conformation of **3** (Figure 1) with subsequent energy minimization using Moloc.[31b] This conformation is within 1 kcal/mol of that of the relaxed X-ray structure of **52**.

improved thiolactam **3**. The 11-oxo dilactams showed a potent and robust inhibitory activity slightly better than that of cyclothialidine (MNEC 0.05 μg/mL), but they were 2−4 times weaker compared to **98**. Thiation of the 11-oxo group of dilactams had a favorable effect only for 4-methyl- but not for the 4-chloro- and the most interesting 4-bromo-derivatives.

Antibacterial Activity in Vitro. For the assessment of the antibacterial potency we have considered as indicator strain *S. aureus* Smith, which was also used in the mouse infection model. The antibacterial spectrum of the "dilactams" follows closely that of the "mono-lactams". It covers broadly the spectrum of Gram-positive bacteria, but only selected strains of Gram-negative bacteria, such as *Xanthomonas maltophilia*, *Haemophilus influencae*, *Neisseria meningitidis*, and *Moraxella catarrhalis* are susceptible.[10] Some general trends in the SAR can be seen from the MIC values of selected compounds compiled in Table 5. As



		MNEC[a]	MIC[b]	CLOGP
101	X = CH_2	0.01	0.25	2.37
24	X = O	0.1	4	1.41

[a] *E. coli* DNA gyrase (μg/mL), [b] *S.aureus* Smith (μg/mL)

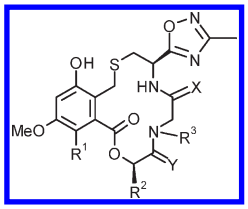
Figure 3. Comparison of biological properties and CLOGP of 12-carba- and 12-oxa-lactones **101** and **24** (atom numbering of structure **101**).

observed before, lipophilicity strongly influences the antibacterial potency and thiolactams were typically found to be 2−16 times more active than their oxo-analogues. As a general rule for mono- and dilactams, replacement of the methyl group at the phenyl ring by chloro or bromo was paid off well, the beneficial effect being, however, more pronounced for polar molecules, like **47** or **98**. The incorporation of a second amide entity into the lactam ring generally lowered the MIC by a factor of 8−32, with the exception of that against *Streptococcus pyogenes* (factor 2). Because of their higher lipophilicity, the original MIC values of thiolactams (**3**, **40**) were less affected by this added polarity than those of the corresponding 11-oxo compounds (**98**, **39**).

The antibacterial activity of the most interesting dilactam **52** was further characterized by testing it against a larger number of Gram-positive and Gram-negative isolates (Table 6). Against a set of highly resistant MRSA, **52** was found to be only slightly weaker than vancomycin and 2−4 times less active than linezolide. The lead compounds **3** and **5** were best in this comparison. Interestingly, the hydroxymethyl derivative **5** was equipotent to the parent thiolactam **3**, indicating that polar compounds might be less affected by the alterations presented by these resistant strains. MIC values of **52** against a set of vancomycin-resistant strains of *Enterococcus faecalis* were 2−8 times lower than those of linelozide and imipenem, as well as those of **3** and **5**, and a similar superiority of **52** was found for *Entercoccus faecium* strains resistant to vancomycin and imipenem. In line with this finding is the observation that for all enterococci tested, **5** was more active than the more lipophilic parent compound **3**. For penicillin-resistant *Streptococcus pneumonia*, *Haemophilus influencae*, and *Moraxella catarrhalis*, **52** was found to be 2−8 times more active than the thiolactams **3** and **5** and the reference compound linelozide. In the testing against the latter two species, MICs of **3** and **5** presumably were negatively affected by the presence of plasma proteins in the test medium ("plasma shift").

In Vivo Efficacy. The in vivo performance of the compounds displayed in Table 5 can be rationalized in the following way: Low intrinsic activity (MIC 8 ug/mL) is probably the main reason why the polar dilactam **47** was found inefficacious. Although very potent, the lipophilic thiolactams **3** and **40** were not active in vivo. In addition to unfavorable pharmacokinetic behavior, their MIC value is strongly affected by binding to plasma proteins as is evidenced by a "plasma shift" of 8. On the other side, the polar **98** is not efficacious despite a low MIC and a small "plasma shift" of 2, so that other parameters, e.g. high clearance by glucuronidation, have to be brought into play as explanation. The slightly more potent bromo analogue **39**,

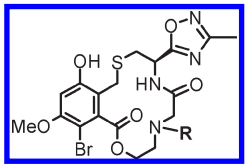
Table 2. DNA Gyrase Inhibitory Activity, in Vitro and in Vivo Antibacterial Activity, and CLOGP of Dilactams: Modifications at the Core



compd	R^1	R^2	R^3	X	Y	MNEC[a] (μg/mL)	MIC[b] (μg/mL)	ED_{50}[c] (mg/kg)	CLOGP[d]
47	Me	H	H	O	O	0.01	8	>25	0.57
48	Me	H	H	S	O	0.001	0.5	25	1.00
49	Me	H	H	O	S	0.005	2	>25	0.77
50	Me	H	H	S	S	0.002	0.5	nd[e]	1.20
51	Cl	H	H	O	O	0.02	8	25	0.63
52	Br	H	H	O	O	0.02	1	3	0.78
53	Br	H	H	S	O	0.05	0.12	12.5	1.21
54	Br	H	H	S	S	0.2	1	nd	1.41
58	Br	H	Me	O	O	0.05	2	>12.5	1.43
59	Br	H	Me	S	O	0.05	0.5	nd	1.86
63	Br	Me	H	O	O	0.05	2	>12.5	1.30
64	Br	Me	H	S	O	0.2	0.5	>12.5	1.73
65	Br	Ph	H	O	O	0.2	>64	nd	2.52
66	Br	Ph	H	S	O	1	0.5	>25	2.94
71[f]	Br	H	H	O	H,H	0.01	1	25	1.28
78[f]	Br	H	H	S	H,H	0.1	0.5	>25	1.71

[a] Supercoiling assay, *E. coli* gyrase, MNEC = maximum noneffective concentration. [b] Agar dilution (Mueller−Hinton agar); inoculum 10^4 CFU/spot, MIC = minimum inhibitory concentration, *Staphylococcus aureus* Smith. [c] In vivo efficacy, septicaemia in mice (*S. aureus* Smith), iv administration. [d] CLOGP = calculated log *P* (version 4.94, program BioByte Corp., Claremont, CA). [e] Not determined. [f] Hydrochloride salt.

Table 3. Biological Properties and CLOGP of 9-Aza-lactones



compd	R	MNEC[a] (μg/mL)	MIC[b] (μg/mL)	ED_{50}[c] (mg/kg)	CLOGP[d]
71[f]	H	0.01	1	25	1.28
81[f]	Me	0.05	1	>25	1.79
72	Ac	0.01	16	nd[e]	1.45
73	(CO)-cProp	0.10	8	nd	1.72
74	(CO)-tBu	0.05	0.5	>12	2.37
75	SO_2Me	0.10	64	nd	1.45
76[f]	Ala-H	0.10	>64	nd	1.20
77[f]	Leu-H	0.10	16	nd	2.66

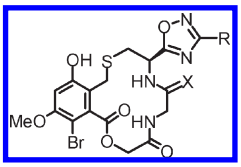
[a] Supercoiling assay, *E. coli* gyrase, MNEC = maximum noneffective concentration. [b] Agar dilution (Mueller−Hinton agar); inoculum 10^4 CFU/spot, MIC = minimum inhibitory concentration, *Staphylococcus aureus* Smith. [c] In vivo efficacy, septicaemia in mice (*S. aureus* Smith), iv administration. [d] CLOGP = calculated log *P* (version 4.94, program BioByte Corp., Claremont, CA). [e] Not determined. [f] Hydrochloride salt.

however, displaying only a modest "plasma shift" of 4, was assayed efficacious. The chloro analogue **38** was in vivo slightly better than amine **100** in accordance with its improved MIC value. Dilactam **97**, an *N*-isopropyl derivative of the amine **95**, was found active in vivo in contrast to the significantly more polar **95**. We have described earlier a reversed case where *N*-isopropylation of **100** led to an inefficacious compound.[10] Obviously, isopropylation of **95** adjusts lipophilicity appropriately, whereas in the case of **100**, it was increased too much.

The best compound **52** was the most polar one among those found active in vivo. Its modest antibacterial activity in vitro (MIC = 1 ug/mL against *S. aureus* Smith) was hardly affected by

dx.doi.org/10.1021/jm1014023 |*J. Med. Chem.* 2011, 54, 2207–2224

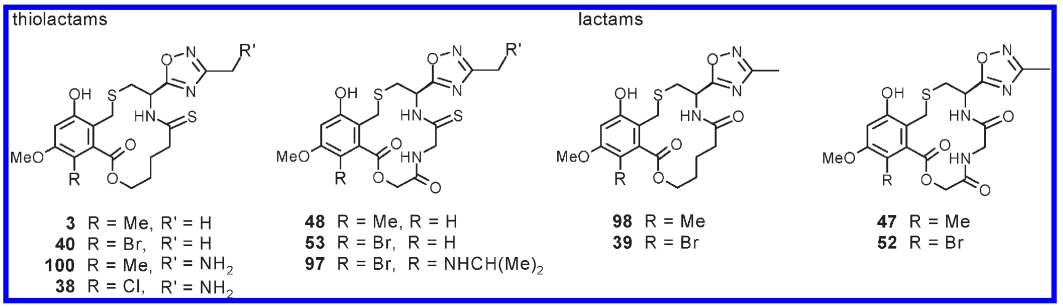
Table 4. Biological Properties and CLOGP of Dilactams: Modification of the Oxadiazole Substituent



compd	R	X	MNEC[a] (μg/mL)	MIC[b] (μg/mL)	ED_{50}[c] (mg/kg)	CLOGP[d]
52	Me	O	0.02	1	3	0.78
87	c-propyl	O	0.02	2	6	1.23
88	CH_2OMe	O	0.05	8	nd[e]	0.31
93	CH_2OH	O	0.02	32	nd	−0.53
94[f]	CH_2NH_2	O	0.10	32	nd	−0.54
95[f]	CH_2NH_2	S	0.05	1	>12	−0.01
96[f]	$CH_2NHCHMe_2$	O	0.02	16	nd	0.72
97[f]	$CH_2NHCHMe_2$	S	0.02	0.5	12	1.25

[a] Supercoiling assay, *E. coli* gyrase, MNEC = maximum noneffective concentration. [b] Agar dilution (Mueller−Hinton agar); inoculum 10^4 CFU/spot, MIC = minimum inhibitory concentration, *Staphylococcus aureus* Smith. [c] In vivo efficacy, septicaemia in mice (*S. aureus* Smith), iv administration. [d] CLOGP = calculated log *P* (version 4.94, program BioByte Corp., Claremont, CA). [e] Not determined. [f] Hydrochloride salt.

Table 5. DNA Gyrase Inhibitory Activity, in Vitro and in Vivo Antibacterial Activity, and Physicochemical Properties of Selected Mono- and Dilactams: Comparison with Reference Compounds



		MIC[b] (μg/mL)										
compd	MNEC[a] (μg/mL)	*E. c.* 25922	*X. m.* 1AC 739	*S. a.* 25923	*S. a.* Smith	*S. e.* 16/2	*E. f.* 6	*S. p.*b15	ED_{50}[c] (mg/kg)	CLOGP[d]	log *D*[e]	PS[f]
3[g]	0.004	>64	0.5	0.12	0.06	0.01	0.25	0.25	>25	2.52	3.09	8
40	0.005	>64	1	<0.12	<0.12	<0.12	0.12	0.12	>25	2.73	3.23	8
48	0.01	>64	4	1	0.5	<0.12	0.5	0.5	25	1.10	2.32	
53	0.05	>64	0.25	0.25	<0.12	<0.12	<0.12	0.12	12.5	1.31	2.61	
100[g,h]	0.002	64	4	0.5	0.25	<0.12	0.5	0.5	8.5	1.05	1.85	4
38[g]	0.002	64	4	0.5	0.12	<0.12	<0.12	0.25	6	1.27	1.89	
97[g]	0.02	>64	16	1	0.5	<0.12	0.25	0.25	12	1.25		
98[g]	0.01	>64	4	0.5	0.25	0.12	0.5	1	>25	1.70	1.99	2
39	0.005	64	2	<0.12	<0.12	<0.12	0.12	<0.12	25	1.91	2.18	4
47	0.01	>64	>64	32	8	1	8	2	>25	0.57	1.05	
52	0.02	>64	>64	4	1	<0.12	0.5	<0.12	3	0.78	1.40	2
novobiocin	0.1	>64	>64	0.25	0.12	0.25	4	1	3			
vancomycin		>64	>64	1	1	2	4	1	1.5	≤1		2

[a] Supercoiling assay, *E. coli* gyrase, MNEC = maximum noneffective concentration. [b] Agar dilution (Mueller−Hinton medium), inoculum 10^4 CFU/spot, MIC = minimum inhibitory concentration. Test organisms: *Escherichia coli* 25922, *Xanthomonas maltophilia* IAC 739, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* Smith, *Staphylococcus epidermidis* 16/2, *Enterococcus faecalis* 6, *Streptococcus pyogenes* b15. [c] In vivo efficacy, septicaemia in mice (*S. aureus* Smith), iv administration. [d] Calculated log *P*, see Table 1. [e] Octanol/water pH 7.4 shake-flask method. [f] "Plasma shift" = ratio MIC (BHI agar-mouse plasma (1:1), *S. a.* Smith)/MIC (BHI agar, *S. a.* Smith). [g] Reference compounds.[10] [h] Hydrochloride salt.

protein binding, resulting in a "plasma shift" of only 2. However, dilactam **52** obviously displays improved pharmacokinetic properties, which was demonstrated in a pharmacokinetic study in rats, where it achieved the highest plasma levels measured so

dx.doi.org/10.1021/jm1014023 |*J. Med. Chem.* 2011, 54, 2207–2224

Table 6. In Vitro Antibacterial Activity of 52 and of Reference Compounds against Multiresistant Gram-Positive and Gram-Negative Bacteria[a]

	S aureus (11 strains)	*E. faecalis* (16 strains)	*E. faecium* (23 strains)	*S. pneumoniae* (21 strains)	*H. influenzae* (21 strains)	*M. catarrhalis* (20 strains)
compd	$MIC_{50/90}$	$MIC_{50/90}$	$MIC_{50/90}$	$MIC_{50/90}$	$MIC_{50/90}$	$MIC_{50/90}$
52	4/4	0.25/0.5	0.5/0.5	0.5/2	1/4	1/2
3[b]	1/1	4/4	8/8	1/2	8/16	2/2
5[b]	1/1	1/1	1/2	2/4	8/8	2/2
penicillin[c,d]	256 />256 [c]			2/8 [d]	32/>32 [d]	8/32 [d]
linelozide	1/2	2/2	1/2	1/2	8/16	8/16
imipenem	32/64	2/2	64/>64			
vancomycin	2/4	2/256	256 />256			

[a] Agar dilution: Mueller−Hinton agar (inoculum 10^4 CFU/spot) for *S. aureus*, *E. faecium*, *E. faecalis*; Haemophilus test medium (inoculum 10^5 CFU/spot) for *H. influenzae*; Mueller−Hinton agar (inoculum 10^5 CFU/spot) and IsoSensitest (inoculum 10^4 CFU/spot) supplemented with sheep blood (5% v/v), for *M. catarrhalis* and *S. pneumoniae*, respectively. $MIC_{50/90}$ = minimum concentration (in μg/mL) that inhibits at least 50%, 90% of the strains tested. [b] Monolactam reference compounds.[10] [c] Data for methicillin. [d] Data for penicillin G.

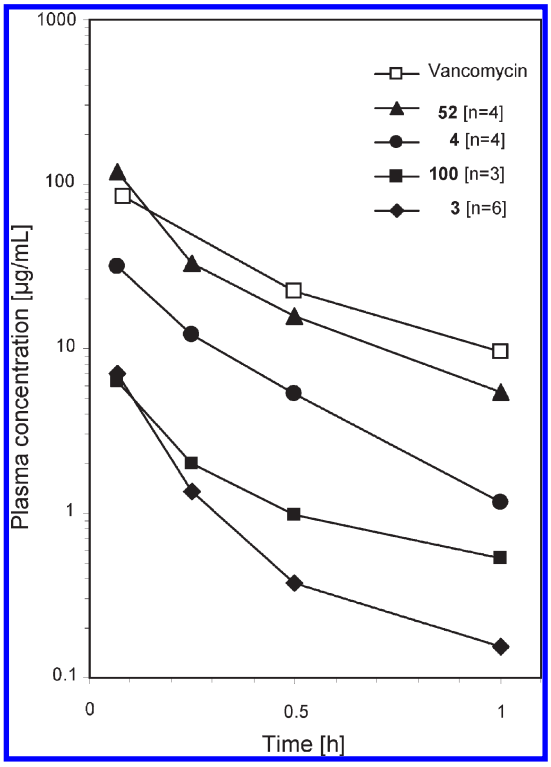


Figure 4. Total plasma concentration of **52** and of reference compounds[10] **4**, **100**, and **3** in rats ($n = 3-6$) after a single intravenous dose of 20 mg/kg (microsuspension in gelatin). Comparison with vancomycin data from ref 35.

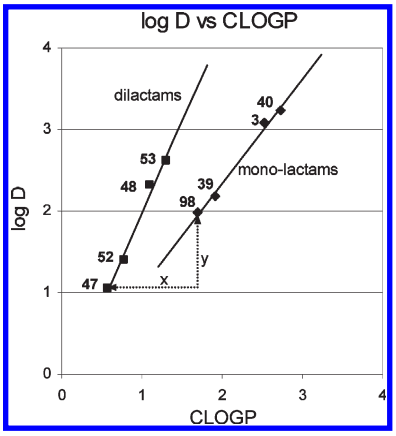


Figure 5. Correlation between calculated log *P* (CLOGP) and experimental log *D* values of two analogous series of monolactams and dilactams (see Table 5). The ratio *y*/*x* between derivatives differing only in the additional amide group is <1, indicating an overestimation of this hydrophilicity increment by the CLOGP value. The dilactam analogues are positioned along a steeper line (slope 2.2) as compared to the monolactams (line slope 1.3). This results from the overestimation in the CLOGP of the hydrophilic impact of the additional amide functionality in comparison to the lipophilicity contributions of methyl/bromine replacement (**52**), thiation (**48**), or both (**53**).

far for cyclothialidine analogues and approaching those measured for vancomycin (Figure 4).

The comparison of CLOGP values with experimental log *D* values reveals a charscteristic distinction between the monolactam and the dilactam series (Figure 5). The calculated log *P* value obviously overestimates the hydrophilicity impact of the additional amide functionality. This can be explained by a compensatory reduction of the hydrophilicity by the stronger intramolecular hydrogen bond of the dilactam scaffold (see Figure 1). As a consequence, the 11-thioxo-dilactams **48** and **53** have a higher log *D* but a smaller CLOGP value than the monolactams **98** and **39.** Nevertheless, dilactams **47** and **52** are significantly more polar than the monolactams **98** and **39**, which explains their reduced antibacterial effect. Being aware of the deficiencies of the CLOGP value, Figure 6 demonstrates anyway that a certain degree of lipophilicity is a prerequisite for a pronounced antibacterial potency. However, the general observation that high lipophilicity leeds to increased protein binding, assessed directly or deduced from the "plasma shift" of the antibacterial in vitro test, reinforced the view that only compounds of a certain hydrophilicity can exhibit efficacy in vivo. Figure 7 shows the ED_{50} values determined for all compounds of significant activity against *S. aureus* Smith plotted against their CLOGP value. In vivo efficacy ($ED_{50} \leq 25$ mg) was only found for derivatives with CLOGP between 0.78 and 1.92. Some inefficacious compounds within this lipophilicity window were possibly not tested high enough ($ED_{50} > 12$ mg/kg), but others (ED_{50} >25 mg/kg) must be hampered by other unfavorable properties such as metabolic degradation, e.g. glucuronidation.

We have focused on compounds occupying this favorable lipophilicity range, which seems to offer the best compromise for optimizing concomitantly cell permeation and pharmacokinetic

dx.doi.org/10.1021/jm1014023 |*J. Med. Chem.* 2011, 54, 2207–2224

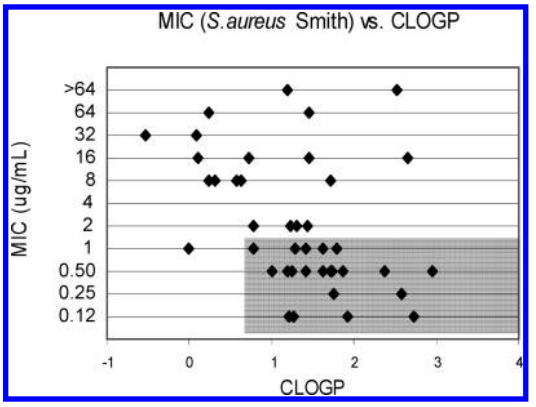


Figure 6. Antibacterial activity in vitro of all new compounds plotted against their CLOGP value. A certain degree of lipophilicity (CLOGP > 1) is required for a pronounced antibacterial potency.

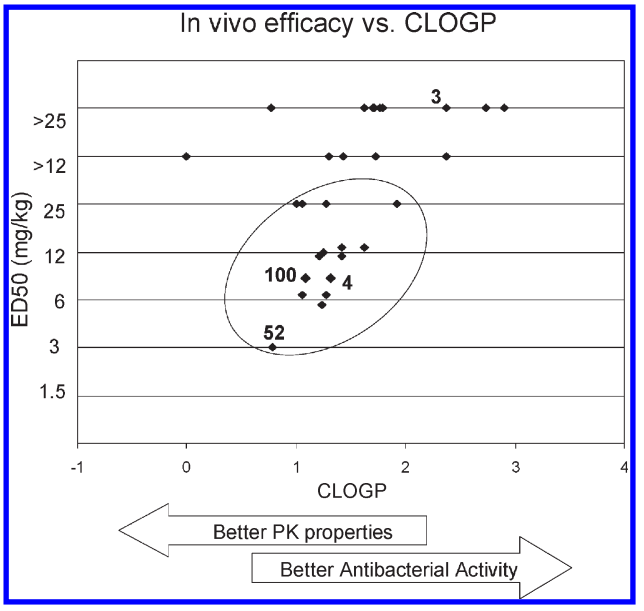


Figure 7. In vivo efficacy (septicaemia in mice, *S. aureus* Smith) plotted against CLOGP for all compounds with a MIC ≤ 2 μg/mL (*S. aureus* Smith) tested. For pharmacokinetic data of compounds **3**, **4**, **52**, and **100**, see Figure 4.

behavior. Figure 8 shows schematically how the stepwise application of structural lipophilicity modulaters on the parent lactone **98**, i.e. conversions A, B and C, influences hydrophilicity and biological properties, i.e. log *D*, ED_{50} and MIC values, of its derivatives.

CONCLUSIONS

Our continued effort to optimize bicyclic lactones derived from the natural product cyclothialidine was guided by the hypothesis that only a certain degree of hydrophilicity will allow the translation of a good antibacterial potency into in vivo efficacy. According to this hypothesis, and confirmed by the experience collected so far, efficacious compounds can only be found within a rather narrow lipophilicity window. The CLOGP value appeared to be a useful guidance tool for the modification program, although the conformational flexibility of the 14-memberd lactone rings together with the possibility to form an intramolecular hydrogen bond could provide specific properties

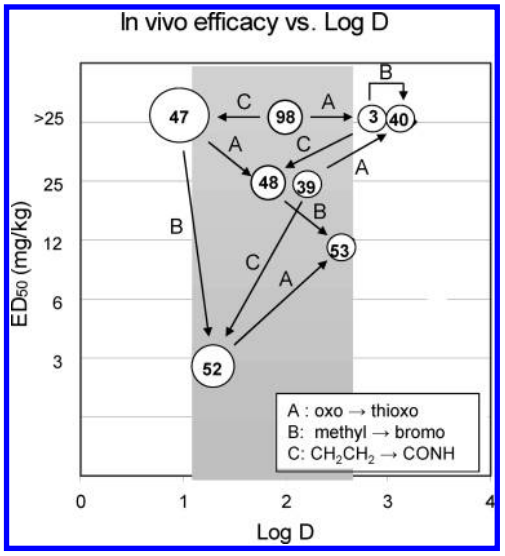


Figure 8. Impact on polarity (log *D*) and biological properties (in vivo efficacy, antibacterial activity) of chemical modifications on derivatives of parent monolactam **98**. The derivatives shown are interconnected by conversions A, B, and C. Small/large circle diameters indicate small/large MIC values.

to new series of interest, e.g. with regard to conformational adaption to different environments. Thus, it seemed obvious that new lactone subclasses of intrinsically improved biological properties had to be found empirically.

Further attempts to identify more favorable hydrophilic substitution patterns for the 14-membered monolactams provided only compounds of similar efficacies as those previously reported, but the use of a chloro- or bromo-substitute for the 1-methyl group of monolactams brought some progress with regard to the antibacterial activity both in vitro and in vivo.

A noteworthy step forward was accomplished by using a new and more polar scaffold obtained by incorporating an additional amide moiety into the lactone ring. Dilactam analogues were found to be still potent DNA gyrase inhibitors. Compared to the monolactams, they had a slightly reduced antibacterial potency due to their increased hydrophilic character, a drawback which, however, was compensated by more favorable physicochemical properties. The dilactam inhibitors cover the same antibacterial spectrum than the monolactams and were found to be particularly active against multiresistant strains of *Enterococcus faecalis*, *Entercoccus faecium* , and *Streptococcus pneumonia*. The 4-bromo-dilactam **52** displayed a significantly improved pharmacokinetic behavior, which resulted in a substantial in vivo efficacy with an ED_{50} of 3 mg/kg in a mouse septicaemia model. Regarding its antibacterial potency, polar character, as well as pharmacokinetic behavior, dilactam **52** resembles the properties of vancomycin, which, however, is addressing an extracellular target. The fact that the "dilactams" do not require a thioamide functionality to achieve in vivo efficacy makes them attractive.

Unfortunately, we did not succeed in further improving on the lead compound **52**, and only structurally close analogues displayed a similar in vivo effect. Nevertheless, the new dilactam subclass confirms that DNA gyrase inhibitors of the cyclothialidine family can indeed combine interesting antibacterial activity with satisfactory pharmacokinetic properties, and therefore, might earn further investigation.

EXPERIMENTAL SECTION

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. For standard workup procedures, organic solvents and aqueous (aq) solutions used are given in brackets, and if not stated otherwise, aq solutions of $NaHCO_3$ and Na_2CO_3 used were saturated, aq layers were back-extracted with the organic solvent used, organic solutions were dried with Na_2SO_4, and solvents were evaporated under reduced pressure 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). Normal phase silica gel (Silica Gel 60, 230−400 mesh, Merck) was used for preparative chromatography, and the eluents used are indicated in brackets. Melting points (mp) were determined on a Büchi SMP-20K and are uncorrected. ^{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 δ 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 ISP-MS and ISN-MS were recorded on a PE-Sciex API III. High-resolution mass spectra (HRMS) were determined on an Agilent LC/MS (series 1200, 6120 Q-TOF). Elemental analyses were carried out in our laboratory or by Solvias AG, Basel, and results are within ±0.4% of the theoretical values. Alternatively, target compounds without elemental analysis were analyzed by HRMS and their purity was assessed by HPLC (A: RP-18 (reverse phase), 250 mm × 4 mm, detector 278 nm, 0.01 M aq K,Na-phospate buffer (pH 7.0) containing tetrakis-(decyl)ammonium bromide (0.008 mol/L)/acetonitrile (38:62), 1 mL/min; B: C-18 (reverse phase), 2.1 mm × 30 mm, detector with various wavelengths 265 nm (70%), 225 (10%), 215, 220, 230, 250, 280 nm (4% each), eluent 0.01% aq HCOOH/5−95% MeCN−MeOH (4:1); 1.2 mL/min) and shown to be >95% if not stated otherwise.

Methyl 2-Chloro-6-formyl-5-hydroxy-3-methoxy-benzoate (26). To a solution of methyl 2-formyl-3-hydroxy-5-methoxybenzoate (**25**,[26] 25.4 g, 0.12 mol) in DCM (120 mL) was added over 15 min at 20 °C sulfuryl chloride (10.7 mL, 0.13 mol), causing a light evolution of gas. The mixture was stirred for 21 h at 20 °C and then evaporated. The residual material was crystallized from EtOAc to give a mixture of **26** and the 4-chloro regioisomer (23.6 g, 72:28 mixture of regioisomers). Repeated recrystallization from *t*-BuOMe provided pure **26** (8.33 g, 28%) as white crystals, mp 123−124 °C. ^{1}H NMR (DMSO-d_6) δ 3.82 and 3.93 (2 s, 2 × 3H), 6.72, 10.10, and 11.43 (3 s, 3 × 1H).

(*RS*)-7-Chloro-3,4-dihydroxy-6-methoxy-3*H*-isobenzofuran-1-one (27b). To **26** (0.65 g, 2.5 mmol) was added 2 N NaOH (2.5 mL, 5 mmol), and the mixture was stirred for 30 min. The solution was acidified with 3 N HCl at 0 °C, a white precipitate being formed. The mixture was extracted with EtOAc. The organic layer was washed with brine, dried, and evaporated to give **27b** (0.57 g, 99%) as white solid, mp >230 °C. ^{1}H NMR (DMSO-d_6) δ 3.87 (s, 3H), 6.48 (d, 1H, J = 8.7), 6.86 (s, 1H), 7.81 (d, 1H, J = 8.7), 10.62 (s, 1H). Signals of the corresponding acid (2-chloro-6-formyl-5-hydroxy-3-methoxy-benzoic acid, ca. 20%) δ 3.92 (s, 3H), 6.70 (s, 1H), 10.10 (s, 1H), 11.38 (s, 1H), 13.46 (br s, 1H). Anal. ($C_9H_7ClO_5$) C, H, Cl.

(*RS*)-3,4-Dihydroxy-6-methoxy-3*H*-isobenzofuran-1-one (28). To methyl 2-formyl-3-hydroxy-5-methoxybenzoate (**25,** 63.1 g, 0.30 mol) was added 2 N NaOH (375 mL, 0.75 mol), and the mixture was stirred at 20 °C for 0.5 h. Water (50 mL) was added, and the mixture was cooled in an ice-bath. Upon acidification to pH 2.5 by addition of 7 N HCl (ca. 70 mL), a precipitate was formed. The mixture was stirred for 0.5 h at 0 °C, and subsequently, the solid was collected by filtration and washed with cold H_2O (130 mL). The wet solid was dissolved in acetone (0.3 L)/EtOAc (0.6 L) and the solution was dried and evaporated. The resulting residue was recrystallized by dissolving it in hot acetone (0.11 L), followed by cooling and addition of hexane (0.4 L) to give pseudoacid **28** (51.6 g, 88%) as white solid, mp 153−153 °C. ^{1}H NMR (DMSO-d_6) δ 3.79 (s, 3H), 6.56 (d, 1H, J = 8), 6.66 (s, 1H), 6.76 (br s, 1H), 7.82 (d, 1H, J = 8), 10.42 (br s, 1H).

(*RS*)-7-Bromo-3,4-dihydroxy-6-methoxy-3*H*-isobenzofuran-1-one (27c). Bromine (12.8 mL, 0.25 mol) was added over 1 min to a stirred suspension of **28** (49.0 g, 0.25 mol) in DCM (0.5 L) precooled to 0 °C. The temperature rose to 12 °C and was allowed to rise to 20 °C over 10 min. Stirring was continued for 2 h at 20 °C, and thereafter, the yellow reaction mixture was evaporated completely. To a suspension of the solid residue in H_2O (0.5 L) was added at 0 °C 32% aq NaOH (100 mL). The mixture was stirred for 15 min to produce a dark-yellow solution of pH 12. By slowly adding 7 N HCl (ca. 100 mL) at 0 °C, the pH of the reaction mixture was lowered to 2.5. The precipitate produced was collected by filtration, washed with H_2O, and dried. The crude product was triturated with hot acetone (0.25 L), and after cooling to 0 °C and addition of hexane (0.25 L), **27c** (60.2 g, 88%) was isolated as white solid, mp >200 °C. ^{1}H NMR (DMSO-d_6) δ 3.88 (s, 3H), 6.46 (d, 1H, J = 8.5), 6.62 (s, 1H), 7.59 (d, 1H, J = 8.5), 10.64 (s, 1H). Signals of corresponding acid (2-bromo-6-formyl-5-hydroxy-3-methoxy-benzoic acid, ca. 20%) δ 3.93 (s, 3H), 6.67 (s, 1H), 10.05 (s, 1H), 11.42 (s, 1H), 13.45 (br s, 1H).

Allyl 2-Bromo-6-formyl-5-hydroxy-3-methoxy-benzoate (29c). *N,N,N′,N′*-Tetramethylguanidine (34.6 mL, 0.275 mol) was added at 20 °C to a solution of **27c** (68.8 g, 0.25 mol) in DMF (0.5 L). After stirring for 0.5 h, allyl bromide (27.5 mL, 0.325 Mol) was added to the reaction mixture and stirring was continued at 20 °C for 18 h. The mixture was evaporated, and Et_2O (0.4 L) was added to the residual oil. The mixture was extracted with 2% aq $NaHCO_3$ (0.4 L). The aqueous extract was set to pH 5 by addition of 7 N HCl and then extracted with EtOAc (1 L). The organic layer was washed with brine, dried, and evaporated. The remaining oil (57.2 g) was taken up in hot hexane (0.9 L), insoluble material was removed by filtration, and the solution was stirred at 0 °C to give **29c** (53.1 g, 67%) as white solid, mp 45 °C. ^{1}H NMR ($CDCl_3$) δ 3.96 (s, 3H), 4.91 (d, 2H, J = 6), 5.46 (d, 1H, J = 10), 5.47 (d, 1H, J = 17), 5.95−6.16 (m, 1H), 6.51 (s, 1H), 9.68 (s, 1H), 12.01 (s, 1H).

In addition, the byproduct allyl 3-allyloxy-6-bromo-2-formyl-5-methoxy-benzoate (8.0 g, 9%) was isolated from the Et_2O extract, and starting material **27c** (8.7 g, 13%) was recovered from the aqueous extract at pH 2.5 (EtOAc).

Allyl 2-Bromo-5-[dimethyl(thexyl)silyloxy]-6-formyl-3-methoxy-benzoate (30c). To a stirred mixture of **29c** (37.8 g, 0.12 mol) and (dimethyl)-(1,1,2-trimethyl-propyl)-chlorosilane (35.3 mL, 0.18 mol) in DMF (240 mL) was added at 0 °C NEt_3 (25.1 mL, 0.18 mol), a precipitate being formed immediately. The reaction mixture was stirred at 20 °C for 15 h, and then worked up (EtOAc, 1 N HCl, 5% aq NaCl). The resulting oil was crystallized from hexane to give **30c** (51.3 g, 93%) as white crystals, mp 88−89 °C. ^{1}H NMR ($CDCl_3$) δ 0.34 (s, 6H), 0.94 (d, 6H, J = 7), 0.98 (s, 6H), 1.68−1.84 (m, 1H), 3.92 (s, 3H), 4.91 (d, 2H, J = 6), 5.30 (d, 1H, J = 10), 5.42 (d, 1H, J = 16), 5.98−6.17 (m, 1H), 6.39 (s, 1H), 10.18 (s, 1H).

Allyl 2-Bromo-5-[dimethyl(thexyl)silyloxy]-6-iodomethyl-3-methoxy-benzoate (31c). Sodium iodide (10.34 g, 69.0 mmol) and trimethylchlorosilane (9.45 mL, 73.6 mmol) were added at 20 °C to a solution of **30c** (21.0 g, 46.0 mmol) in MeCN (23 mL), a brown precipitate being formed. The mixture was stirred for 5 min, subsequently cooled to 0 °C, and 1,1,3,3-tetramethyl-disiloxane (8.7 mL, 48.5 mmol) was added over 5 min. The mixture was stirred at 20 °C for 2 h, and then diluted with EtOAc, washed with H_2O, dried, and evaporated. On the basis of NMR analysis, the resulting brown oil (30 g) consisted of **31c** (ca.19.2 g, 73%), and 7-bromo-4-[dimethyl-(thexyl)silyloxy]-6-methoxy-3*H*-isobenzofuran-1-one (10.8 g) as the major byproduct. NMR ($CDCl_3$) of **31c**: δ 0.31 (s, 6H), 0.87 (d, 6H, J = 7), 0.99 (s, 6H), 1.64−1.82 (m, 1H), 3.79 (s, 3H), 4.34 (s, 2H), 4.86 (d, 2H, J = 6),

5.32 (d, 1H, J = 10), 5.48 (d, 1H, J = 16), 5.99−6.17 (m, 1H), 6.36 (s, 1H).

Allyl 2-[(*R*)-2-Amino-2-(3-methyl-[1,2,4]oxadiazol-5-yl)-ethylsulfanylmethyl]-6-bromo-3-[dimethyl(thexyl)silyloxy]-5-methoxy-benzoate (34c). NEt_3 (6.4 mL, 46 mmol) was added to a solution of crude **31c** (30 g containing ca. 19.2 g, 34 mmol, of **31c**) and (*R*)-2-mercapto-1-(3-methyl-1,2,4-oxadiazol-5-yl)-ethylcarbamic acid *tert*-butyl ester (**32**,[10] 11.9 g, 46 mmol) in DCM (90 mL). The reaction mixture was stirred at 20 °C for 2 h, and then worked up (EtOAc, 1 N HCl, brine). The resulting material was stirred with TFA (90 mL) at 0 °C for 0.5 h. The solution was evaporated, and the remaining oil was worked up (EtOAc, aq Na_2CO_3, brine). The crude product was purified by chromatography (EtOAc/hexane 1:3) to give **34c** (13,5 g, 66%) as a light-yellow foam. NMR ($CDCl_3$) δ 0.30 (s, 6H), 0.93 (d, 6H, J = 7), 0.99 (s, 6H), 1.67−1.85 (m, 1H), 1.84 (br s, 2H), 2.38 (s, 3H), 2.84 (dd, 1H, J = 13, 8), 3.06 (dd, 1H, J = 13, 5), 3.69 and 3.74 (2 d, 2 × 1H, J = 13), 3.84 (s, 3H), 4,12−4.20 (m, 1H), 4.86 (d, 2H, J = 6), 5.32 (d, 1H, J = 11), 5.47 (d, 1H, J = 16), 5.97−6.15 (m, 1H), 6.45 (s, 1H).

Allyl 2-Chloro-5-[dimethyl(thexyl)silyloxy]-6-iodomethyl-3-methoxy-benzoate (31b). Obtained by subjecting **27b** in analogous manner to the reaction sequence described above for the preparation of **31c** from **27c**. Brown oil, which, based on NMR analysis, consisted of **31b** (ca. 60%) and 7-chloro-4-[dimethyl-(thexyl)silyloxy]-6-methoxy-3*H*-isobenzofuran-1-one (ca. 32%) as the major byproduct. NMR ($CDCl_3$) of **31b**: δ 0.36 (s, 6H), 0.96 (d, 6H, J = 7), 1.08 (s, 6H), 1.65−1.83 (m, 1H), 3.84 (s, 3H), 4.39 (s, 2H), 4.90 (d, 2H, J = 6), 5.32 (d, 1H, J = 10), 5.48 (d, 1H, J = 16), 6.01−6.18 (m, 1H), 6.42 (s, 1H).

Allyl 2-[(*R*)-2-Amino-2-(3-methyl-[1,2,4]oxadiazol-5-yl)-ethylsulfanylmethyl]-6-chloro-3-[dimethyl(thexyl)silyloxy]-5-methoxy-benzoate (34b). Obtained by subjecting crude **31b** in analogous manner to the procedure described above for the preparation of **34c** from **31c**; light-yellow foam. NMR ($CDCl_3$) δ 0.31 (s, 6H), 0.93 (d, 6H, J = 7), 0.99 (s, 6H), 1.70−1.82 (m, 1H) superimposed by 1.79 (br s, 2H), 2.38 (s, 3H), 2.86 (dd, 1H, J = 14 and 8), 3.05 (dd, 1H, J = 14 and 5), 3.70 and 3.74 (2 d, 2 × 1H, J = 11), 3.85 (s, 3H), 4.15−4.21 (m, 1H), 4.86, 5.31, and 5.45 (3 d, 3 × 2H, J = 6, 10 and 18), 5.96−6.17 (m, 1H), 6.47 (s, 1H).

Allyl (*R*)-2-[2-Amino-2-(3-allyloxycarbonylaminomethyl-1,2,4-oxadiazol-5-yl)-ethylsulfanyl-methyl]-3-[dimethyl-(thexyl)silyloxy]-6-chloro-5-methoxybenzoate (35b). Obtained by subjecting **31b** in analogous manner to the procedure described above for the preparation of **34c** but replacing **31c** by **31b** and **32** by (*R*)-1-(3-allyloxycarbonylaminomethyl-1,2,4-oxadiazol-5-yl)-2-mercapto-ethylcarbamic acid *tert*-butyl ester (**33**);[10] light-yellow foam. NMR ($CDCl_3$) δ 0.31 (s, 6H), 0.93 (d, 6H, J = 7), 0.99 (s, 6H), 1.70−1.82 (m, 1H), 2.87 (dd, 1H, J = 13, 7), 3.05 (dd, 1H, J = 13, 4), 3.69 and 3.76 (2 d, 2 × 1H, J = 14), 3.85 (s, 3H), 4.15−4.24 (m, 1H), 4.52, 4.61, and 4.86 (3 d, 3 × 2H, J = 6), 5.20−5.50 (m, 4H), 5.84−6.15 (m, 2H), 6.47 (s, 1H).

Allyl (*R*)-2-[2-Amino-2-(3-allyloxycarbonylaminomethyl-1,2,4-oxadiazol-5-yl)-ethylsulfanyl-methyl]-6-bromo-3-[dimethyl(thexyl)silyloxy]-5-methoxybenzoate (35c). Obtained by subjecting **31c** in analogous manner to the procedure described above for the preparation of **34c** but replacing **32** by **33**; light-yellow foam. NMR ($CDCl_3$) δ 0.31 (s, 6H), 0.93 (d, 6H, J = 7), 0.99 (s, 6H), 1.76−1.80 (m, 1H) superimposed by 1.65−1.95 (br s, 2H), 2.87 (dd, 1H, J = 13, 7), 3.05 (dd, 1H, J = 13, 5), 3.67 and 3.72 (2 d, 2 × 1H, J = 13), 3.85 (s, 3H), 4.19 (dd, 1H, J = 7, 5), 4.52, 4.60, and 4,86 (3 d, 3 × 2H, J = 6), 5.18−5.55 (m, 5H), 5.84−6.15 (m, 2H), 6.45 (s, 2H).

(*R*)-8-(3-Aminomethyl-[1,2,4]oxadiazol-5-yl)-1-chloro-4-hydroxy-2-methoxy-10-thioxo-7,8,9,10,11,12,13,14-octahydro-5*H*-15-oxa-6-thia-9-aza-benzocyclotetradecen-16-one Hydrochloride (38). Obtained by subjecting amine **35b** in analogous manner to the reaction sequence described previously for the preparation of **100** from **6**;[10] white solid. ^{1}H NMR (DMSO-d_6) δ 1.60−2.10 (m, 4H), 2.34 (s, 3H), 2.58−2.74 and 2.86−3.00 (2 m, 2 × 1H), 3.08 (dd, 1H, J = 14, 11), 3.40 (dd, 1H, J = 14, 4), 3.69 (d, 1H, J = 11), 3.80 (s, 3H), 3.91 (d, 1H, J = 11), 4.06−4.20 (m, 1H), 4.31 (br s, 2H), 4.50−4.66 (m, 1H), 5.72 −5.88 (m, 1H), 6.72 (s, 1H), 8.67 (br s, 3H), 10.45 (s, 1H), 10.76 (d, 1H, J = 7). HRMS calcd for ($C_{19}H_{23}ClN_4O_5S_2$) 486.0798, found 486.0793.

By subjecting amine **34c** and 5-trityloxypentanoic acid in analogous manner to the reaction sequence described previously for the preparation of **98** and **3** from **14**,[10] compounds **39** and **40** were obtained:

(*R*)-1-Bromo-4-hydroxy-2-methoxy-8-(3-methyl-[1,2,4]-oxadiazol-5-yl)-8,9,11,12,13,14-hexahydro-5*H*,7*H*-15-oxa-6-thia-9-aza-benzocyclotetradecene-10,16-dione (39). White solid. ^{1}H NMR (DMSO-d_6) δ 1.60−2.10 (m, 5H), 2.34 (s, 3H), 2.33−2.48 (m, 1H), 2.88 (dd, 1H, J = 14, 11), 3.29 (dd, 1H, J = 14, 4), 3.68 (d, 1H, J = 11), 3.80 (s, 3H), 3.85 (d, 1H, J = 11), 4.04−4.16 (m, 1H), 4.54−4.65 (m, 1H), 5.13−5.25 (m, 1H); 6.64 (s, 1H), 8.66 (d, 1H, J = 8), 10.34 (s, 1H). MS (ISN) 498.0 [(M − 1)$^-$]. Anal. ($C_{19}H_{22}BrN_3O_6S \cdot 0.2EtOAc$) C, H, N.

(*R*)-1-Bromo-4-hydroxy-2-methoxy-8-(3-methyl-[1,2,4]-oxadiazol-5-yl)-10-thioxo-7,8,9,10,11,12,13,14-octahydro-5*H*-15-oxa-6-thia-9-aza-benzocyclotetradecen-16-one (40). White solid. ^{1}H NMR (DMSO-d_6) δ 1.60−2.10 (m, 4H), 2.34 (s, 3H), 2.58−2.73 and 2.85−2.99 (2 m, 2 × 1H), 3.04 (dd, 1H, J = 14, 11), 3.40 (dd, 1H, J = 14, 4), 3.66 (d, 1H, J = 11), 3.80 (s, 3H), 3.88 (d, 1H, J = 11), 4.06−4.19 and 4.51−4.64 (2 m, 2 × 1H), 5.69−5.81 (m, 1H), 6.64 (s, 1H), 10.37 (s, 1H), 10.63 (d, 1H, J = 8). MS (ISN) 514.0 [(M − 1)$^-$]. Anal. ($C_{19}H_{22}BrN_3O_5S_2$) C, H, N, Br.

Ethyl (2-Trityloxy-acetylamino)-acetate (43). To a mixture of 2-trityloxy-acetic acid (**42**,[10] 63.6 g, 0.20 mol), glycine ethyl ester hydrochloride (30.7 g, 0.22 mol), and 4-methyl-morpholine (24.2 mL, 0.22 mol) in MeCN (0.8 L) was added dropwise at 0−4 °C over 30 min a solution of DCC (45.4 g, 0.22 mol) in MeCN (0.2 L). The mixture was stirred at 0 °C for 3 h, a white precipitate being formed. The mixture was evaporated and the remaining material was stirred with EtOAc (0.5 L) at 0 °C for 15 min. Insoluble material was removed by filtration, and the clear solution was washed with 1 N HCl (0.5 L) and with brine. The organic layer was dried and evaporated to give **43** (82 g, "102%") as a white solid. An analytical sample was obtained by recrystallization (EtOAc/hexane) as white solid, mp 115−116 °C. ^{1}H NMR (DMSO-d_6) δ 1.21 (t, 3H, J = 7), 3.45 (s, 2H), 3.92 (d, 2H, J = 6), 4.12 (q, 2H, J = 7), 7.25−7.48 (m, 15H), 8.24 (t, 1H, J = 6). Anal. ($C_{25}H_{25}NO_4$) C, H, N.

(2-Trityloxy-acetylamino)-acetic Acid (44). A mixture of **43** (82.0 g, max. 0.2 mol) and KOH (14.6 g, 0.26 mol) in MeOH (100 mL) was stirred at 60 °C for 0.5 h. The mixture was cooled and evaporated, and the remaining oil was dissolved in H_2O (0.2 L). The solution was washed with *t*-BuOMe/hexane (2:1, 0.6 L) and then acidified to pH 3 at 0 °C by the addition of 5 N HCl, a white precipitate being formed. The mixture was extracted with EtOAc (0.4 L). Insoluble material in the organic layer (product) was isolated by filtration, and the filtrate was washed with brine, dried, and evaporated. The residue (25.3 g) and the isolated insoluble material were triturated together with EtOAc/hexane (1:1, 1 L) at 0 °C to give, after filtration and drying, **44** (60.0 g, 80%) as white solid, mp 183−184 °C. ^{1}H NMR (DMSO-d_6) δ 3.44 (s, 2H), 3.84 (d, 2H, J = 6), 7.25−7.50 (m, 15H), 8.14 (t, 1H, J = 6), 12.66 (s, 1H). Anal. ($C_{23}H_{21}NO_4$) C, H, N.

3-[Dimethyl(thexyl)silyloxy]-2-[(*R*)-2-[2-(2-hydroxy-acetyl-amino)-acetylamino]-2-(3-methyl-[1,2,4]oxadiazol-5-yl)-ethylsulfanylmethyl]-5-methoxy-6-methyl-benzoic Acid (45a). (i) A mixture of allyl 2-[(*R*)-2-amino-2-(3-methyl-[1,2,4]oxadiazol-5-yl)-ethylsulfanylmethyl]-3-[dimethyl(thexyl)silyloxy]-5-methoxy-6-methyl-benzoate (**14**,[10] 2.95 g, 5.5 mmol), **44** (2.44 g, 6.5 mmol), and EDC (1.53 g, 8.0 mmol) in MeCN (55 mL) was stirred at 0 °C for 5 h. Workup (EtOAc, 1 N HCl, aq $NaHCO_3$, brine) gave a viscous oil (5.38

g, ca. 100%). (ii) This oil (5.38 g) was stirred with pTsOH hydrate (1.0 g) in MeOH (30 mL) at 20 °C for 2 h. Workup (EtOAc, 5% aq $NaHCO_3$, brine) and chromatography (EtOAc/hexane 1:1) gave a light-yellow foam (3.1 g). (iii) To a solution of this material (3.1 g, 4.8 mmol) in THF (50 mL) were added at 0 °C morpholine (4.1 mL, 47 mmol) and $Pd(PPh_3)_4$ (0.115 g, 0.1 mmol). The mixture was stirred for 2 h at 0 °C, and then worked up (EtOAc, 1 N HCl, brine) to give **45a** (1.95 g, 58%) as light-yellow foam. ^{1}H NMR ($CDCl_3$) δ 0.28 (s, 6H), 0.94 (d, 6H, J = 7), 0.98 (s, 6H), 1.70−1.82 (m, 1H), 2.14 and 2.35 (2 s, 2 × 3H), 3.04 (dd, 1H, J = 12, 5), 3.17 (dd, 1H, J = 12, 7), 3.76 (s, 3H), 3.78 and 3.82 (2 d, 2 × 1H, J = 12), 4.12 (dd, 1H, J = 14.7, 4), 4.22 (s, 2H), 4.34 (dd, 1H, 14.7, 6), 5.36−5.48 (m, 1H), 6.37 (s, 1H), 7.32 (s, 1H), 7.55−7.64 and 7.78−7.90 (2 m, 2 × 1H).

(*R*)-1-[Dimethyl(thexyl)silyloxy]-3-methoxy-4-methyl-13-(3-methyl-[1,2,4]oxadiazol-5-yl)-9,10,13,14-tetrahydro-12*H*,16*H*-6-oxa-15-thia-9,12-diaza-benzocyclotetradecene-5,8,11-trione (46a). A solution of **45a** (1.43 g, 2.34 mmol) and PPh_3 (1.31 g, 5.0 mmol) in toluene (60 mL) was cooled to 0 °C. DEAD (0.78 mL, 5.0 mmol) was added, and the mixture was stirred for 1 h at 0 °C and for 15 h at 20 °C. The solution was evaporated and the residue was chromatographed (EtOAc/hexane 3:1) to give **46a** (0.58 g, 42%) as off-white foam. NMR ($CDCl_3$) δ 0.27 and 0.30 (2 s, 2 × 3H), 0.94 (d, 6H, J = 7), 0.99 (s, 6H), 1.70−1.84 (m, 1H), 2.04 and 2.29 (2 s, 2 × 3H), 2.94 (dd, 1H, J = 14, 4), 3.39 (dd, 1H, J = 14, 6), 3.63 (d, 1H, J = 11), 3.76 (s, 3H), 3.89 (d, 1H, J = 11), 3.97 (dd, 1H, J = 15, 5), 4.14 (dd, 1H, J = 15, 6), 4.54 and 5.00 (2 d, 2 × 1H, J = 12), 5.58−5.70 (m, 1H), 6.40 (s, 1H), 7.36 (d, 1H, J = 8), 8.38 (t, 1H, J = 6).

(*R*)-1-Hydroxy-3-methoxy-4-methyl-13-(3-methyl-[1,2,4]-oxadiazol-5-yl)-9,10,13,14-tetrahydro-12*H*,16*H*-6-oxa-15-thia-9,12-diaza-benzocyclotetradecene-5,8,11-trione (47). To a solution of **46a** (198 mg, 0.33 mmol) in MeOH (6 mL) was added NH_4F (20 mg, 0.1 mmol), and the mixture was stirred at 20 °C for 1 h. Workup (EtOAc, H_2O) and chromatography (EtOAc/hexane 1:1) followed by recrystallization (MeOH/EtOAc/hexane) of the crude product gave **47** (83 mg, 55%) as white solid. ^{1}H NMR (DMSO-d_6) δ 1.96 and 2.31 (2 s, 2 × 3H), 2.83 (dd, 1H, J = 15, 3), 3.28 (dd, 1H, J = 15, 7), 3.54 (d, 1H, J = 12), 3.74 (s, 3H), 3.80 (d, 1H, J = 12), 3.85−3.93 (m, 2H), 4.68 and 4.90 (2 d, 2 × 1H, J = 12), 5.52−5.63 (m, 1H), 6.55 (s, 1H), 7.51 (d, 1H, J = 8), 9.25 (t, 1H, J = 6), 9.85 (s, 1H). MS (ISN) 449.3 [$(M - 1)^-$]. Anal. ($C_{19}H_{22}N_4O_7S \cdot 0.2MeOH$) C, H, N, S.

(*R*)-1-Hydroxy-3-methoxy-4-methyl-13-(3-methyl-[1,2,4]-oxadiazol-5-yl)-11-thioxo-9,10,11,12,13,14-hexahydro-16*H*-6-oxa-15-thia-9,12-diaza-benzocyclotetradecene-5,8-dione (48), (*R*)-1-Hydroxy-3-methoxy-4-methyl-13-(3-methyl-[1,2,4]-oxadiazol-5-yl)-8-thioxo-7,8,9,10,13,14-hexahydro-12*H*,16*H*-6-oxa-15-thia-9,12-diaza-benzocyclotetra-decene-5,11-dione (49), and (*R*)-1-Hydroxy-3-methoxy-4-methyl-13-(3-methyl-[1,2,4]oxa-diazol-5-yl)-8,11-dithioxo-7,8,9,10,11,12,13,14-octahydro-16*H*-6-oxa-15-thia-9,12-diaza-benzocyclotetra-decen-5-one (50). A mixture of **46a** (295 mg, 0.50 mmol) and Lawesson's reagent (161 mg, 0.4 mmol) in toluene (5 mL) was heated to 80 °C for 0.5 h. The mixture was cooled and evaporated, and the residue was chromatographed (EtOAc/hexane 1:1) to give three products: F1 (Si*-**50**, 77 mg, 25%), F2 (Si*-**48**, 90 mg, 29%), and F3 (Si*-**49**, 51 mg, 16%), with R_f values (EtOAc/hexane 1:1) of 0.63, 0.45, and 0.40, respectively. The silylated intermediates were treated individually with NHF_4/MeOH as described in the preparation of **47** to give the following products:

48 (61 mg, 88%, from 90 mg F2), white solid. ^{1}H NMR (DMSO-d_6) δ 1.99 and 2.33 (2 s, 2 × 3H), 3.09 (dd, 1H, J = 15, 3), 3.31 (dd, 1H, J = 15, 7), 3.57 (d, 1H, J = 12), 3.74 (s, 3H), 3.81 (d, 1H, J = 12), 4.32 (d, 2H, J = 6), 4.76 and 4.91 (2 d, 2 × 1H, J = 13), 6.03−6.15 (m, 1H), 6.56 (s, 1H), 9.20 (t, 1H, J = 5), 9.30 (d, 1H, J = 8), 9.87 (s, 1H). HRMS calcd for ($C_{19}H_{22}N_4O_6S_2$) 466.0981, found 466.0978.

49 (33 mg, 85%, from 51 mg F3), white solid (see SI).

50 (6 mg, 10%, from 77 mg F1, after chromatography), white solid (see SI).

(*R*)-4-Chloro-1-hydroxy-3-methoxy-13-(3-methyl-[1,2,4]-oxadiazol-5-yl)-9,10,13,14-tetrahydro-12*H*,16*H*-6-oxa-15-thia-9,12-diaza-benzocyclotetradecene-5,8,11-trione (51). Obtained by subjecting amine **34b** in analogous manner to the reaction sequence described above for the preparation of **47** from **34a**; white solid. ^{1}H NMR (DMSO-d_6) δ 2.31 (s, 3H), 2.90 (dd, 1H, J = 14, 3), 3.24 (dd, 1H, J = 14, 7), 3.54 (d, 1H, J = 12), 3.81 (s, 3H), 3.86 (d, 1H, J = 12) superimposed by 3.80−3.96 (m, 2H), 4.71 and 4.90 (2 d, 2 × 1H, J = 13), 4.49−5.62 (m, 1H), 6.69 (s, 1H), 7.44 (d, 1H, J = 9), 9.30 (t, 1H, J = 6), 10.45 (s, 1H). HRMS calcd for ($C_{18}H_{19}ClN_4O_7S$) 470.0663, found 470.0659.

6-Bromo-3-[dimethyl(thexyl)silyloxy]-2-[(*R*)-2-[2-(2-hydroxy-acetylamino)-acetylamino]-2-(3-methyl-[1,2,4]oxadiazol-5-yl)-ethylsulfanylmethyl]-5-methoxy-benzoic Acid (45c). Amine **34c** (6.0 g, 10.0 mmol) was subjected in analogous manner to the reaction sequence described above (**45a** (i−iii)) to give crude **45c** (6.34, 94%) as slightly yellow foam. ^{1}H NMR ($CDCl_3$) δ 0.30 (s, 6H), 0.94 (d, 6H, J = 7), 0.99 (s, 6H), 1.70−1.82 (m, 1H), 2.36 (s, 3H), 3.04 (dd, 1H, J = 12, 5), 3.19 (dd, 1H, J = 12, 7), 3.72 and 3.79 (2 d, 2 × 1H, J = 12), 3.84 (s, 3H), 4.10 (dd, 1H, J = 14, 4), 4.22 (s, 2H), 4.36 (dd, 1H, 14, 6), 5.42−5.52 (m, 1H), 6.41 (s, 1H), 7.42−7.60 and 7.77−7.88 (2 m, 2 × 1H).

(*R*)-4-Bromo-1-[dimethyl(thexyl)silyloxy]-3-methoxy-13-(3-methyl-[1,2,4]oxadiazol-5-yl)-9,10,13,14-tetrahydro-12*H*,16*H*-6-oxa-15-thia-9,12-diaza-benzocyclotetradecene-5,8,-11-trione (46c). Subjecting **45c** (4.96 g, 7.4 mmol) to the lactonization procedure described in the preparation of **46a** afforded **46c** (2.39 g, 49%) as off-white foam. NMR ($CDCl_3$) δ 0.30 and 0.32 (2 s, 2 × 3H), 0.94 (d, 6H, J = 7), 0.99 (s, 6H), 1.70−1.85 (m, 1H), 2.32 (s, 3H), 2.96 (dd, 1H, J = 14, 4), 3.38 (dd, 1H, J = 14, 7), 3.62 (d, 1H, J = 11), 3.83 (s, 3H), 3.87 (d, 1H, J = 11), 3.98−4.19 (m, 2H), 4.58 and 4.99 (2 d, 2 × 1H, J = 12), 5.98−5.68 (m, 1H), 6.44 (s, 1H), 7.42 (d, 1H, J = 8), 7.76 (t, 1H, J = 6).

(*R*)-4-Bromo-1-hydroxy-3-methoxy-13-(3-methyl-[1,2,4]-oxadiazol-5-yl)-9,10,13,14-tetrahydro-12*H*,16*H*-6-oxa-15-thia-9,12-diaza-benzocyclotetradecene-5,8,11-trione (52). A mixture of **46c** (462 mg, 0.70 mmol) and NH_4F (50 mg, 1.35 mol) in MeOH (6 mL) was stirred at 20 °C for 1 h. The mixture was diluted with EtOAc (50 mL) and washed with H_2O. The organic layer was dried and evaporated, and the residue was purified by chromatography (acetone/hexane 1:1) and crystallized (MeOH/EtOAc/hexane) to give **52** (151 mg, 42%) as white solid. ^{1}H NMR (DMSO-d_6) δ 2.31 (s, 3H), 2.88 (dd, 1H, J = 14, 4), 3.22 (dd, 1H, J = 14, 7), 3.55 (d, 1H, J = 12), 3.80 (s, 3H), 3.86 (d, 1H, J = 12) superimposed by 3.78−3.89 (m, 2H), 4.74 and 4.90 (2 d, 2 × 1H, J = 12), 5.48−5.58 (m, 1H), 6.67 (s, 1H), 7.42 (d, 1H, J = 8), 9.31 (t, 1H, J = 6), 10.44 (s, 1H). HRMS calcd for ($C_{18}H_{19}BrN_4O_7S$) 514.0158, found 514.0154.

(*R*)-4-Bromo-1-hydroxy-3-methoxy-13-(3-methyl-[1,2,4]-oxadiazol-5-yl)-11-thioxo-9,10,11,12,13,14-hexahydro-16*H*-6-oxa-15-thia-9,12-diaza-benzocyclotetradecene-5,8-dione (53) and (*R*)-4-Bromo-1-hydroxy-3-methoxy-13-(3-methyl-[1,2,4]oxadiazol-5-yl)-8,11-dithioxo-7,8,9,10,11,12,13,14-octahydro-16*H*-6-oxa-15-thia-9,12-diaza-benzocyclotetra-decen-5-one (54). A mixture of **46c** (328 mg, 0.50 mmol) and Lawesson's reagent (161 mg, 0.4 mmol) in toluene (5 mL) was heated to 80 °C for 0.5 h. The mixture was cooled and evaporated, and the residue was chromatographed (EtOAc/hexane 1:1) to give two major products: F1 (Si*-**54**, 55 mg, 16%) and F2 (Si*-**53**, 75 mg, 22%), with R_f values (EtOAc) of 0.66 and 0.43, respectively. The silylated products were treated individually with NHF_4/MeOH as described in the preparation of **47** to give, after purification by chromatography (EtOAc/hexane) and crystallization (EtOAc/hexane), the following products:

53 (11 mg, 18%, from 75 mg F2), white solid. ^{1}H NMR (DMSO-d_6) δ 2.33 (s, 3H), 3.11 (dd, 1H, J = 15, 3), 3.32 (dd, 1H, J = 15, 8), 3.59 (d, 1H, J = 12), 3.81 (s, 3H), 3.82 (d, 1H, J = 12), 4.33 (d, 2H, J = 6), 4.78

and 4.92 (2 d, 2 × 1H, J = 13), 6.05−6.16 (m, 1H), 6.68 (s, 1H), 9.20−9.32 (m, 2H), 10.46 (s, 1H). HRMS calcd for ($C_{18}H_{19}BrN_4O_6S_2$) 529.9929, found 529.9927.

54 (5 mg, 11%, from 55 mg F1), white solid (see SI).

Allyl 2-{(*R*)-2-[3-(Allyloxycarbonylamino-methyl)-[1,2,4]-oxadiazol-5-yl]-2-[2-(2-hydroxy-acetylamino)-acetylamino]-ethylsulfanylmethyl}-6-bromo-3-[dimethyl(thexyl)silyloxy]-5-methoxy-benzoate (89). Reacting **35c** (7.0 g, 10.0 mmol) with **44** (4.5 g, 12.0 mmol) and treating the resulting product with pTsOH/MeOH, as described above in the preparation of **45a** (i−ii), gave **89** (6.81 g, 84%) as colorless foam. NMR ($CDCl_3$) δ 0.30 and 0.32 (2 × 3H), 0.93 (d, 6H, J = 7), 0.98 (s, 6H), 1.68−1.80 (m, 1H), 2.89−3.11 (m, 2H), 3.61 and 3.75 (2 d, 2 × 1H, J = 13), 3.85 (s, 3H), 3.97−4.16 (m, 4H), 4.48, 4.58, and 4,85 (3 d, 3 × 2H, J = 6), 5.15−5.37 (m, 4H), 5.47 (d, 1H, J = 17), 5.62 (t, 1H, J = 6), 5.80−6.14 (m, 2H), 6.46 (s, 1H), 7.31 (d, 1H, J = 8).

Allyl [5-((*R*)-4-Bromo-1-[dimethyl(thexyl)silyloxy]-3-methoxy-5,8,11-trioxo-7,8,9,10,11,12,13,14-octahydro-5*H*,16*H*-6-oxa-15-thia-9,12-diaza-benzocyclotetradecen-13-yl)-[1,2,4]-oxadiazol-3-ylmethyl]-carbamate (90). Allyl ester **89** (2.04 g, 2.50 mmol) was subjected successively to the ester cleavage and the lactonization procedures described above (**45a** (iii) and **46a**) to give **90** (0.77 g, 41%) as white foam. NMR ($CDCl_3$) δ 0.30 and 0.33 (2 s, 2 × 3H), 0.94 (d, 6H, J = 7), 1.00 (s, 6H), 1.68−1.84 (m, 1H), 2.97 (dd, 1H, J = 15, 3), 3.31 (dd, 1H, J = 15, 6), 3.60 (d, 1H, J = 11), 3.84 (s, 3H), 3.86 (d, 1H, J = 11), 4.15−4.22 (m, 2H), 4.47 and 4.62 (2 d, 2 × 2H, J = 6), 4.62 and 5.01 (2 d, 2 × 1H, J = 12), 5.20 (d, 1H, J = 10), 5.29 (d, 1H, J = 17), 5.44 (t, 1H, J = 6), 5.55−5.68 and 5.82−5.99 (2 m, 2 × 1H), 6.46 (s, 1H), 7.44 (t, 1H, J = 6), 7.48 (d, 1H, J = 8).

(*R*)-13-(3-Aminomethyl-[1,2,4]oxadiazol-5-yl)-4-bromo-1-[dimethyl(thexyl)silyloxy]-3-methoxy-11-thioxo-9,10,11,12,13,14-hexahydro-16*H*-6-oxa-15-thia-9,12-diaza-benzocyclotetradecene-5,8-dione (92). A mixture of **90** (0.36 g, 0.48 mmol) and Lawesson's reagent (0.70 g, 0.24 mmol) in toluene (5 mL) was heated to 80 °C for 0.5 h. The mixture was cooled and evaporated, and the residue was chromatographed (EtOAc/hexane 1:1) to give three products with R_f values (EtOAc/hexane 2:1) of 0.60, 0.46, and 0.29, respectively: To a solution of the product of the second fraction (R_f = 0.46, 77 mg) in DCM (1.4 mL) were added dimethylamino-trimethylsilane (0.10 mL, 0.6 mmol) and trimethylsilyl trifluoroacetate (0.10 mL, 0.6 mmol). The solution was stirred at 0 °C for 10 min, whereupon $Pd(PPh_3)_4$ (7 mg, 0.006 mmol) was added and stirring was continued for 2 h at 0 °C. The mixture was evaporated and the residual oil was worked up (EtOAc, 5% aq $NaHCO_3$, brine) to give, after chromatography (EtOAc) of the crude product, **92** (63 mg, 91%) as light-yellow foam. TLC: R_f = 0.11 (EtOAc).

(*R*)-4-Bromo-1-hydroxy-13-[3-(isopropylamino-methyl)-[1,2,4]oxadiazol-5-yl]-3-methoxy-11-thioxo-9,10,11,12,13,14-hexahydro-16*H*-6-oxa-15-thia-9,12-diaza-benzocyclotetradecene-5,8-dione Hydrochloride (97). (i) To a mixture of **92** (30 mg, 0.044 mmol), acetone (0.06 mL), NaOAc (10 mg), AcOH (0.07 mL), and H_2O (0.15 mL) in THF (0.3 mL) was added at 0 °C portionwise over 30 min $NaBH_4$ (10 mg, 0.26 mmol). Stirring was continued for 10 min, and the mixture was then worked up (EtOAc, aq $NaHCO_3$, brine). The residue was chromatographed (EtOAc) to yield a major product (17 mg). (ii) A solution of this material (17 mg) and NH_4F (10 mg) in MeOH (1 mL) was stirred at 20 °C for 0.5 h. After workup (EtOAc, H_2O), a solution of the resulting residue in EtOAc (1 mL) was treated with 3 N HCl−Et_2O (0.05 mL) and Et_2O (1 mL). The precipitate formed was isolated to give **97** (12 mg, 41%) as white solid. ^{1}H NMR (DMSO-d_6) δ 1.23 (d, 6H, J = 6), 3.19 (dd, 1H, J = 15, 3), 3.29 (dd, 1H, J = 15, 8), 3.64 (d, 1H, J = 11), 3.81 (s, 3H), 3.89 (d, J = 11), 4.22−4.50 (m, 3H), 4.82 and 4,88 (2 d, 2 × 1H, J = 12), 6.12−6.23 (m, 1H), 6.71 (s, 1H), 9.31 (t, 1H, J = 6), 9.35 (d, 1H, 8), 10.50 (s, 1H). HRMS calcd for ($C_{21}H_{26}BrN_5O_6S_2$) 587.0508, found 587.0509.

DNA Gyrase Inhibition. The MNEC (maximum noneffective concentration) was determined in a supercoiling assay as described previously.[10] 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 described previously.[10] Details are indicated in the table legends.

In Vivo Antibacterial Efficacy. The in vivo efficacy was determined in a septicaemia mice model as described previously.[10] *Staphylococcus aureus* Smith was used as test organism. In experiments with 0−1 survivals in a control group of 5−10 mice, the lowest dose affording 3−5 survivals out of a group of 5 infected mice was taken as the 50% effective dose (ED_{50}, in mg/kg).

■ ASSOCIATED CONTENT

Supporting Information. Additional synthetic procedures and characterization of new compounds; X-ray crystallographic information for compounds **3**, **19b**, and **52**. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

[†]X-ray structures of compounds **3**, **19b**, and **52**, are deposited with Cambridge Crystallographic Data Centre under CCDCs 797879, 797880, and 797881, respectively.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +41 61 711 7385. E-mail: goetschi@intergga.ch

Present Addresses

[‡]GeneData AG, PO Box, CH-4016 Basel, Switzerland.

[§]Basilea Pharmaceutica International Ltd., PO Box, CH-4005 Basel, Switzerland.

■ ACKNOWLEDGMENT

We thank Silvia Herzig, Serge Corpataux, and Corinne Lohri for chemical syntheses, Karin Kuratli, Veronique Schirmer, and Harry Straub for the biological evaluation, Christian Bartelmus and Wolf Arnold for spectroscopical analyses, and Björn Wagner for the determination of physicochemical parameters.

■ ABBREVIATIONS USED

ATP, 5′-adenosine triphosphate; ADPNP, 5′-adenylyl-β,γ-imidodiphosphate; MNEC, maximum noneffective concentration; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; CLOGP, calculated log P value.; SiO_2, silica gel; DCM, dichloromethane; DEAD, diethyl azodicarboxylate; DCC, N,N'-dicyclohexyl-carbodiimide; EDC, N-(dimethylamino-propyl)-N'-ethyl-carbodiimide hydrochloride; Lawesson's reagent, [2,4-bis-(4-methoxyphenyl)-2,4-dithioxo-1,3,2,-4-dithiaphosphetane]; MeCN, acetonitrile; TFA, trifluoroacetic acid; pTsOH, *p*-toluenesulfonic acid monohydrate; trityl, triphenylmethyl; thexyl, 1, 1, 2-trimethyl-propyl; Si *, dimethyl-(thexyl)silyl; Boc, *tert*-butoxycarbonyl; R_f, TLC retention time; SI, Supporting Information

■ ADDITIONAL NOTE

Systematic IUPAC (International Union of Pure and Applied Chemistry) chemical names were used for all new compounds.[11]

■ REFERENCES

(1) (a) Wood, M. J. Chemotherapy of Gram-positive nosocomial sepsis. *J. Chemother.* **1999**, *11*, 446–452. (b) Chopra, I. Antibiotic resistance in *Staphylococcus aureus*: concerns, causes and cures. *Expert Rev. Anti-Infect. Ther.* **2003**, *1*, 45–55. (c) Barrett, C. T.; Barrett, J. F. Antibacterials: are the new entries enough to deal with the emerging resistance problems?. *Curr. Opin. Biotechnol.* **2003**, *14*, 621–626.

(2) Singh, S. B.; Barrett, J. F. Empirical antibacterial drug discovery—foundation in natural products. *Biochem. Pharmacol.* **2006**, *71*, 1006–1015.

(3) Hooper, D. C.; Wolfson, J. S. Mode of action of new quinolones: new data. *Eur. J. Clin. Microbiol. Infect. Dis.* **1991**, *10*, 223–231.

(4) (a) Kamiyama, T.; Shimma, N.; Ohtsuka, T.; Nakayama, N.; Itezono, Y.; Nakada, N.; Watanabe, J.; Yokose, K. Cyclothialidine, a novel DNA gyrase inhibitor. II. Isolation, characterization and structure elucidation. *J. Antibiot.* **1994**, *47*, 37–45. (b) Nakada, N.; Shimada, H.; Hirata, T.; Aoki, Y.; Kamiyama, T.; Watanabe, J.; Arisawa, M. Biological characterization of cyclothialidine, a novel DNA gyrase inhibitor. *Antimicrob. Agents Chemother.* **1993**, *37*, 2656–2661.

(5) Lewis, J. R.; Singh, O. M. P.; Smith, C. V.; Skarzynsky, T.; Maxwell, A.; Wonacott, A. J.; Wigley, D. B. Crystallization of inhibitor complexes of an *N*-terminal 24 kDa fragment of the DNA gyrase protein. *J. Mol. Biol.* **1994**, *241*, 128–130.

(6) Yamaji, K.; Masubuchi, M.; Kawahara., F.; Nakamura, J.; Nishio, A.; Matsukuma, S.; Watanaba, J.; Fujimori, M.; Nakada, N.; Kamiyama, T. Cyclothialidine analogues, novel DNA gyrase inhibitors. *J. Antibiot.* **1997**, *50*, 402–411.

(7) Nakada, N.; Gmuender, H.; Hirata, T.; Arisawa, M. Mechanism of inhibition of DNA gyrase by cyclothialidine, a novel DNA gyrase inhibitor. *Antimicrob. Agents Chemother.* **1994**, *38*, 1966–1973.

(8) (a) Gellert, M.; O'Dea, M. H.; Itoh, C.; Tomizawa, J. Novobiocin and coumermycin inhibit DNA supercoiling catalysed by DNA gyrase. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 4474–4478. (b) Lambert, H. P.; O'Grady, F. W. Antibiotics and Chemotherapy. In *Coumarins*, 6th ed.; Lambert, H. P., O'Grady, F. W., Eds.; Churchill Livingstone: Edinburgh, 1992; pp 140−141.

(9) (a) Arisawa, M.; Goetschi, E.; Kamiyama, T.; Masciadri, R.; Shimada, H.; Watanabe, J.; Hebeisen, P.; Link, H. PCT Int. Appl. WO 9218490, 1992; *Chem. Abstr.* **1993**, *119*, 117285. (b) Geiwiz, J.; Goetschi, E.; Hebeisen, P.; Link, H.; Luebbers, T. European Patent Appl. EP 675122, 1995; *Chem. Abstr.* **1996**, *124*, 146213. (c) Goetschi, E.; Angehrn, P.; Gmuender, H.; Hebeisen, P.; Link, H.; Masciadri, R.; Nielsen, J. Cyclothialidine and its congeners: a new class of DNA gyrase inhibitors. *Pharmacol. Ther.* **1993**, *60*, 367–380. (d) Goetschi, E.; Angehrn, P.; Gmuender, H.; Hebeisen, P.; Link, H.; Masciadri, R.; Reindl, P.; Ricklin, F. The DNA gyrase inhibitor cyclothialidine: progenitor of a new class of antibacterial agents. In *Medicinal Chemistry: Today and Tomorrow*; Yamazaki, M., Ed.; Blackwell Science Ltd.: Oxford, 1996; pp 263−270. (e) Goetschi, E.; Angehrn, P.; Gmuender, H.; Hebeisen, P.; Kostrewa, D.; Link, H.; Luebbers, T.; Masciadri, R.; Reindl, P.; Ricklin, F.; Theil, F. P. From the DNA gyrase inhibitor cyclothialidine to a new class of antibacterial agents. In *Natural Product Chemistry at the Turn of the Century*; Atta-ur-Rahman, Choudhary, M. I., Khan, K. M., Eds.; Print Arts: Karachi, 2002; pp 489−497.

(10) Angehrn, P.; Buchmann, S.; Funk, C.; Goetschi, E.; Gmuender, H.; Hebeisen, P.; Kostrewa, D.; Link, H.; Luebbers, T.; Masciadri, R.; Nielsen, J.; Reindl, P.; Ricklin, F.; Schmitt-Hoffmann, A.; Theil, F.-P. New antibacterial agents derived from the DNA gyrase inhibitor cyclothialidine. *J. Med. Chem.* **2004**, *42*, 1487–1513.

(11) In contrast to our earlier papers, we used IUPAC nomenclature, which gives different numbering, e.g., 1- or 4-hydroxy for the essential phenol group, dependent on the core scaffold.

(12) (a) Hebeisen, P.; Angehrn, P.; Gmuender, H.; Goetschi, E.; Link, H.; Luebbers, T.; Schneider, F. New DNA gyrase inhibitors related to cyclothialidine: *seco*-analogues as potent antibacterial agents. Programme and Abstracts of the 20th International Congress of Chemotherapy, Sydney, Australia, June 29−July 3, 1997, Poster 042. (b) Rudolph, J.; Theis, H.; Hanke, R.; Endermann, R.; Johannsen, L.; Geschke, F.-U. *seco*-Cyclothialidines: new concise synthesis, inhibitory activity toward bacterial and human DNA topoisomerases, and antibacterial properties. *J. Med. Chem.* **2001**, *44*, 619–626.

(13) (a) Luebbers, T.; Angehrn, P.; Gmuender, H.; Herzig., S. Design, synthesis, and structure−activity relationship studies of new phenolic DNA gyrase inhibitors. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4708–4714. (b) Brvar, M.; Perdih, A.; Oblak, M.; Mašic, L. P.; Solmajer, T. In silico discovery of 2-amino-4-(2,4-dihydroxyphenyl)thiazoles as novel inhibitors of DNA gyrase B. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 958–962.

(14) Poyser, J. P.; Telford, B.; Timms, D.; Block, M. H.; Hales, N. J. Triazine derivatives and their use as antibacterials. WO Patent 99/01442, 1999.

(15) Charifson, P. S.; Grillot, A.-L.; Grossman, T. H.; Parsons, J. D.; Badia, M.; Bellon, S.; Deininger, D. D.; Drumm, J. E.; Gross, C. H.; LeTiran, A.; Liao, Y.; Mani, N.; Nicolau, D. P.; Perola, E.; Ronkin, S.; Shannon, D.; Swenson, L. L.; Tang, Q.; Tessier, P. R.; Tian, S.-K.; Trudeau, M.; Wang, T.; Wei, Y.; Zhang, H.; Stamos, D. Novel dual-targeting benzimidazole urea inhibitors of DNA gyrase and topoisomerase IV possessing potent antibacterial activity: intelligent design and evolution through the judicious use of structure-guided design and stucture−activity relationships. *J. Med. Chem.* **2008**, *51*, 5243–5263.

(16) (a) Basarab, G. S..; Gravestock, M. B.; Hauck, S. I. Pyrrole derivatives as dna gyrase and topoisomerase inhibitors. Patent WO2006/092599, 2006. (b) Basarab, G. S.; Bist, S.; Manchester, J. I. Patent WO2008/06470, 2008. (c) Bist, S.; Dangel, B.; Sherer, B. Heterocyclic urea derivatives and methods of use thereof. Patent WO2009/106885, 2009. (d) Basarab, G. S.; Bist, S.; Manchester, J. I.; Sherer, B. Alkyl urea substituted pyridines. U.S. Patent US 7,674,801, 2007.

(17) Tanitame, A.; Oyamada, Y.; Ofuji, K.; Suzuki, K.; Ito, H.; Kawasaki, M.; Wachi, M.; Yamagishi, J. Potent DNA gyrase inhibitors; novel 5-vinylpyrazole analogues with Gram-positive antibacterial activity. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2863–2866.

(18) (a) Basarab, G. S. Antibacterial pyrrolecarboxamides. WO2008/020227, 2008. (b) Gravestock, M. B.; Hull, K. G.; Fleming, P. R. Antibacterial pyrrolecarboxamides. WO2008/020229, 2008. (c) Illingworth, R. N.; Uria-Nickelsen, M. R.; Bryant, J.; Eakin, A. New inhibitors of bacterial topoisomerase GyrA/Par subunits. 48th Annual ICAAC Meeting, Washington, DC, October, 2008, Poster F1−2028. (d) Uria-Nickelsen, M. R.; Blodgett, A.; Eakin, A. 48th Annual ICAAC Meeting, Washington, DC, October, 2008Poster F1−2029.

(19) Kostrewa, D.; D'Arcy, A. Unpublished results.

(20) Lewis, J. R.; Singh, O. M. P.; Smith, C. V.; Skarzynsky, T.; Maxwell, A.; Wonacott, A. J.; Wigley, D. B. The nature of inhibition of DNA gyrase by the coumarins and the cyclothialidines revealed by X-ray crystallography. *EMBO J.* **1996**, *15*, 1412–1420.

(21) Wigley, D. B.; Davies, G. J.; Dodson, E. J.; Maxwell, A.; Dodson, G. Crystal structure of a *N*-terminal fragment of the DNA gyrase B protein. *Nature* **1991**, *351*, 624–629.

(22) Boehm, H.-J.; Boehringer, M.; Bur, D.; Gmuender, H.; Huber, W.; Klaus, W.; Kostrewa, D.; Kuehne, H.; Luebbers, T.; Meunier-Keller, N.; Mueller, F. Novel inhibitors of DNA gyrase: 3D structure based biased needle screening, hit validation by biophysical methods, and 3D optimization. A promising alternative to random screening. *J. Med. Chem.* **2000**, *43*, 2664–2674.

(23) Hori, K.; Hikage, N.; Inagaki, A.; Mori, S.; Nomura, K.; Yoshii, E. Total synthesis of tetronomycin. *J. Org. Chem.* **1992**, *57*, 2888–2902.

(24) X-ray structures of compounds **3**, **19b**, and **52**, are deposited with Cambridge Crystallographic Data Centre under CCDCs 797879, 797880, and 797881, respectively. The oxadiazole ring in compound **52** is disordered in the X-ray structure.

(25) Knutsen, L. J. S.; Andersen, K. E.; Lau, J.; Lundt, B. F.; Henry, R. F.; Morton, H. E.; Naerum, L.; Petersen, H.; Stephensen, H.; Suzdak, P. D.; Swedberg, M. D. B.; Thomsen, C.; Soerensen, P. O. Synthesis of novel GABA uptake inhibitors. 3. Diaryloxime and diarylvinyl ether derivatives of nipecotic acid and guvacine as anticonvulsant agents. *J. Med. Chem.* **1999**, *42*, 3447–3462.

(26) Broadhurst, M. J.; Hassall, C. D.; Thomas, G. J. Tetracycline studies. Part 5. New syntheses of anthracenes and anthraquinones through benzophenone carbanions. *J. Chem. Soc., Perkin Trans. 1* **1977**, 2502–2512.

(27) Geiwiz, J.; Goetschi, E.; Hebeisen, P. Concise synthesis of cyclothialidine analogues with ring sizes from 12 to 15: novel macrocyclization protocol involving reductive thiolation. *Synthesis* **2003** 1699–1704.

(28) Aizpurua, J. M.; Lecea, B.; Palomo, C. Reduction of carbonyl compounds promoted by silicon hydrides under the influence of trimethylsilyl-based reagents. *Can. J. Chem.* **1986**, *64*, 2342–2347.

(29) Joerres, V.; Keul, H.; Hoecker, H. Aminolysis of α-hydroxy acid esters with α-amino acid salts. First step in the synthesis of optically active 2,5-morpholinediones. *Macromol. Chem. Phys.* **1998**, *199*, 825–833.

(30) Lowe, G.; Vilaivan, T. Amino acids bearing nucleobases for the synthesis of novel peptide nucleic acids. *J. Chem. Soc., Perkin Trans. 1* **1997**, 539–546.

(31) (a) *CORINA*, version 3.46, 3D structure generator (www.molecular-networks.com); (b) *Moloc*, molecular modeling package, e.g. for generation of conformation libraries (www.moloc.ch).

(32) (a) Ruse, M. J.; Waring, R. H. The effect of methimazole on thioamide bioactivation and toxicity. *Toxicol. Lett.* **1991**, *58*, 37–41. (b) Ruse, M. J.; Waring, R. H. The metabolism of thionicotinamide in the rat. *Drug Metab. Drug Interact.* **1991**, *9*, 123–137.

(33) Merzouk, A.; Guibé, F.; Loffet, A. On the use of silylated nucleophiles in the palladium catalyzed deprotection of allylic carboxylates and carbamates. *Tetrahedron Lett.* **1992**, *33*, 477–480.

(34) Iwata, C.; Maezaki, N; Murakami, M.; Soejima, M.; Tanaka, T.; Imanishi, T. Asymmetric functionalization of prochiral 1,3-diols based on an efficient 1,6-chiral induction: the diastereoselective C−O bond fission in chiral β-arylsulfinyl acetal via two types of chelation control. *J. Chem. Soc., Chem. Commun.* **1992**, 516–518.

(35) Ngeleka, M.; Auclair, P.; Tardif, D.; Beauchamp, D.; Bergeron, M. G. Intrarenal distribution of vancomycin in endotoxemic rats. *Antimicrob. Agents Chemother.* **1989**, *33*, 1575–1579.