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Antiviral and Tumor Cell Antiproliferative SAR Studies on Tetracyclic Eudistomins—II

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Abstract—In a search for the minimum pharmacophore of the naturally occurring tetracyclic eudistomins, five structural analogues (**4–8**) were evaluated for their in vitro antiviral and tumor cell antiproliferative activities. For the synthesis of these derivatives both intra- and intermolecular Pictet–Spengler reactions have been used. Opening of the β-carboline annulated 7-membered D-ring in **6** and **7** resulted in a complete loss of activity. On the other hand, replacement of either the oxygen atom or the sulfur atom in the 7-membered ring by a methylene group in **5** and **8**, respectively, is allowed. These results combined with previous SAR data underline the crucial importance of the D-ring in eudistomins as a scaffold for the correct positioning of both basic nitrogen atoms. Also bioisosteric replacement of the bicyclic indole system with a dimethoxyphenyl group, to give the isoquinoline skeleton, is allowed. The tricyclic isoquinoline derivative **4** is, so far, the most promising antiviral analogue; it combines a high potency (MIC at 100 ng/mL (340 nM)) with high MCC/MIC ratios (ranging from 1000 to 5000 against HSV-1, HSV-2, vaccinia virus, and vesicular stomatitis virus). © 1997 Elsevier Science Ltd.

Introduction

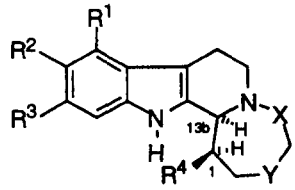
Treatment of viral infections by chemotherapy commenced about three decades ago. The first commercial antiviral drug (i.e., idoxuridine, which was used against herpes simplex keratitis virus) was registered in the early 1960s. Hitherto, most of the registered antiviral drugs are nucleoside analogues. The applications of these drugs are rather limited, emphasizing the demand for new antiviral compounds with unique structures. In the search for novel antiviral lead compounds marine species in particular proved to be a rich source.[1] In our laboratories we focused on the synthesis of tetracyclic eudistomins, which were first isolated from the colonial tunicate *Eudistoma olivaceum*. Of the five different classes of eudistomins that were isolated, the [1,6,2]-oxathiazepine 7-membered ring containing tetracyclic eudistomins (Chart 1) showed the most potent antiviral activities by far.[2] In addition, tumor cell antiproliferative activity has also been reported for eudistomins.[2d]

Although three total syntheses towards tetracyclic eudistomins were developed,[3] only limited structure–activity relationship (SAR) investigations have been published.[4] We already demonstrated that in debromo-eudistomin K (**1**) the natural 1*S*,13b*S* absolute configuration is essential for the biological activity.[4a] In this configuration the axially orientated C(1)-amino group is on the same side of the molecule as the lone pair at N(6). Deletion or substitution of the C(1) amino group by an hydroxy group as in **3** is not allowed, indicating an ionic interaction of the protonated C(1)-amino group with the receptor. Substitution of the indole-N proton for a methyl group only moderately lowered the biological potency. In the same study 10-methoxy debromoeudistomin K (**2**) showed a 10–30-fold increase in both antiviral and tumor cell antiproliferative activity up to the level of the most potent natural eudistomins C and E.

On the basis of these observations we anticipated the involvement of both the axially oriented C(1)-amino group and the bridgehead nitrogen atom together with the aromatic nucleus in the enantioselective binding process with the receptor. To gain further insight into the SAR of these highly potent compounds, eudistomin analogues with the oxathiazepine ring O and S atoms replaced by a methylene moiety were synthesized (**5** and **8**, respectively). In addition, the isoquinoline derivative **4** is described, as a bioisosteric analogue to the indole nucleus. To demonstrate the importance of the 7-membered ring as a scaffold for a correct spatial positioning of the aliphatic N atoms, also the two diastereomeric ring-opened β-carboline derivatives **6** and **7** have been prepared. Biologic activities of the known (**1**,**2**) and new (**4–8**) eudistomin derivatives were compared to standard antiviral and antitumor drugs.

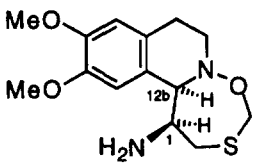
Chemistry

The key reaction for the construction of the tetrahydro-β-carboline skeleton is the Pictet–Spengler (PS) condensation. For the synthesis of the tetracyclic eudistomin-like β-carboline skeletons two strategies using the PS reaction are applicable (Scheme 1): (I) Intermolecular PS reaction followed by closure of the D-ring. (II)



4-8 have been obtained and tested as racemates

Eudistomin	R1	R2	R3	R4	X	Y
L	H	Br	H	NH_2	O	S
K	H	H	Br	NH_2	O	S
K(sulfoxide)	H	H	Br	NH_2	O	S=O
C	H	OH	Br	NH_2	O	S
E	Br	OH	H	NH_2	O	S
F	H	OH	Br	$NC_2H_4O_2$	O	S
K(debromo) 1	H	H	H	NH_2	O	S
2	H	OCH_3	H	NH_2	O	S
3	H	H	H	OH	O	S
5	H	H	H	NH_2	CH_2	S
8	H	H	H	NH_2	O	CH_2



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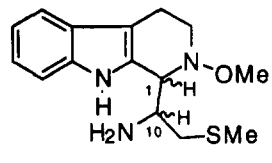


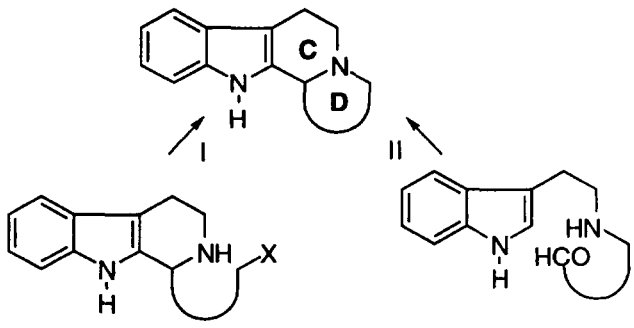
6 (threo), 7 (erythro)

Chart 1. Naturally tetracyclic eudistomin derivatives together with the discussed synthetically derived analogues.

Intramolecular PS reaction to give the tetracyclic skeleton in a simultaneous fashion.

Strategy I has been successfully applied by the groups of Nakagawa[3c,d] and Still.[3e,f] The PS reaction gave the tricyclic β-carboline skeleton in high yields in a *cis* diastereoselective fashion. However, closure of the 7-membered oxathiazepine ring by Pummerer-type reactions was difficult and proceeded in maximum yields of only 22%. Most likely these low yields may be attributed to the ambident nucleophilicity of the alkoxyamine moiety.

Kirkups and our group showed that simultaneous closure of the CD ring system can be efficiently carried out by application of strategy II to give the tetracyclic skeleton in yields up to 98%. Unfortunately, the *trans* diastereomer was always isolated as the major product. As we described earlier, the reason for the *trans* diastereoselectivity is hindered attack of the indole-β bond on the side of a cyclic iminium-ion intermediate where the (amino) substituent is present.[5] Consequently, the diastereomeric outcome of the reaction in the β-carboline series is determined in the beginning of the reaction sequence and is kinetically controlled.



Scheme 1. Synthetic strategies towards tetracyclic β-carbolines.

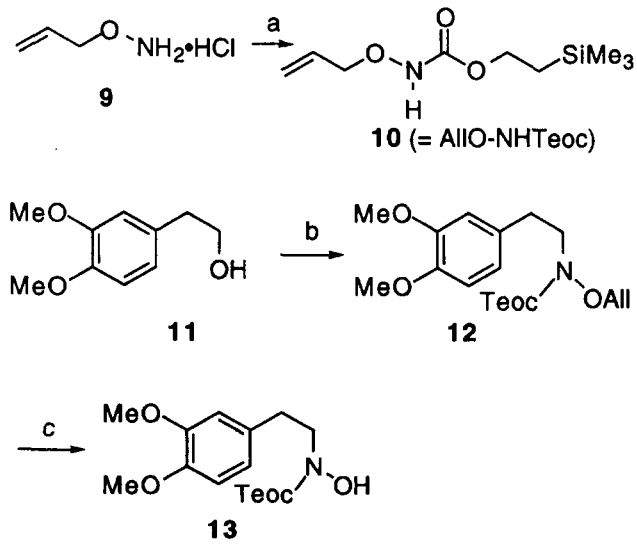
For the synthesis of the new eudistomin derivatives **4–8** described in this paper both strategies have been applied. Tetracyclic derivative **5**, lacking the hydroxyl amine moiety, and the tricyclic derivatives **6** and **7** have been synthesized using strategy I. Both derivatives **4** and **8** incorporating the 7-membered [1,2]-oxazepine ring have been prepared using strategy II.

Isoquinoline eudistomin analogue 4

It is known that the PS condensation is less effective in the isoquinoline series than in the β-carboline series.[6] The electron-rich pyrrole ring in indole is much more susceptible toward electrophilic species than an unsubstituted phenyl group. Therefore in our approach the electron-rich 2-(3,4-dimethoxyphenyl)-ethyl amine was used as a tryptamine isostere.

The N-protected *N*-hydroxy analogue **12** was synthesized by a Mitsunobu reaction of *O*-allyl-*N*-TEOC-hydroxylamine **10** and 2-(3,4-dimethoxyphenyl)-ethanol **11** (Scheme 2).

Miller and Maurer described the Mitsunobu reactions of N,O-diprotected hydroxylamines with primary alcohols using *O*-benzyl-*N*-benzyloxycarbonyl hydroxylamine.[7] Since deprotection by hydrogenolysis is accompanied by scission of the N–O bond, we decided



Scheme 2. Application of the Mitsunobu reaction for the synthesis of β-phenyl-*N*-hydroxy ethylamines. Reagents: (a) 2-(trimethylsilyl)ethoxycarbonyl chloride, DiPEA, THF; (b) **10**, DIAD, PPh_3, THF; (c) $Pd(OAc)_2$, PPh_3, $Et_3N{\cdot}HO_2CH$, $MeCN{:}H_2O$ (4:1), reflux.

to use protecting groups that can be removed by other methods. Hydroxamate **10** was envisaged carrying a [[2-(trimethylsilyl)ethyl]oxy]carbonyl (TEOC) N-protecting group combined with the hydroxyl group protected as its allyl ether.[8]

O-Allyl-*N*-TEOC-hydroxylamine **10** was synthesized from *O*-allylhydroxylamine hydrochloride hydrate **9** and TEOC-Cl in 98% yield (Scheme 2).

With hydroxamate **10** efficient Mitsunobu reactions could indeed be accomplished (Scheme 2). Triphenylphosphine/diisopropyl azodicarboxylate mediated alkylation of **10** with 2-(3,4-dimethoxyphenyl)ethanol **11** afforded **12** in a yield of 64%, after purification by column chromatography. Removal of the allyl group in **12** by treatment with palladium(II)acetate/triethylammonium formate gave **13** in a yield of 98%.

The nucleophilic coupling of the chloromethyl sulfide **14**[3b] with the sodium salt of the hydroxamate **13** gave (crude) **15** in a yield of 73% (Scheme 3).

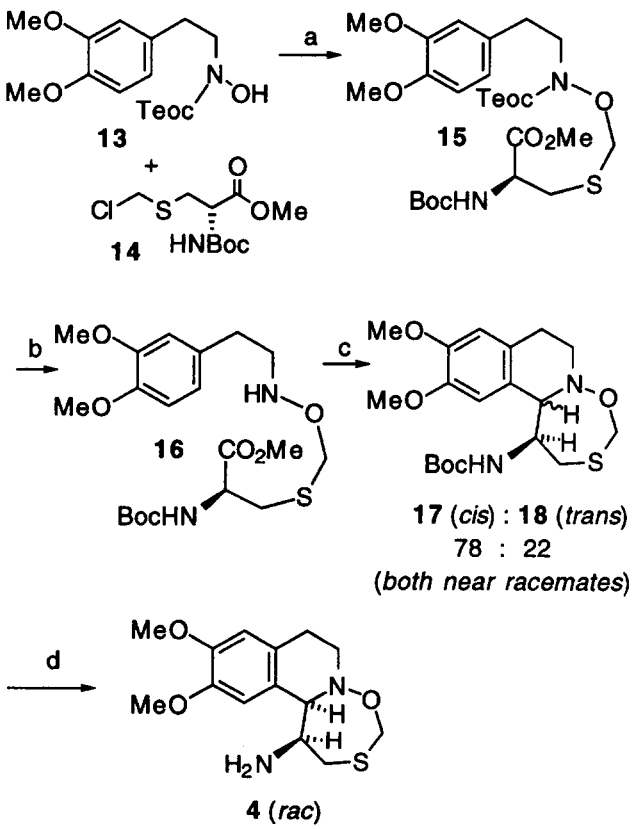
To avoid racemization the sodium alkoxide from **13** was added at such a rate that the pH of the reaction mixture remained nearly neutral as was previously described.[4] Removal of the TEOC protective group in **15** was accomplished by treatment with KF_2H_2O/*n*-Bu_4NCl/MeCN to give **16** in 98% yield. Although the optical integrity was not determined after the above described reaction steps, from previous work it may be assumed that no appreciable racemization had occurred.[3b]

The aldehyde precursor for the intramolecular PS reaction was generated in situ by DIBAL reduction of the methyl ester **16** at −75 ° C (Scheme 3). Addition of TFA at −75 °C induced the PS condensation. After warming to room temperature TLC analysis showed that starting aldehyde was still present in the reaction mixture. This observation is in contrast to the β-carboline series that gave smooth cyclizations at −75 °C, emphasizing the large difference in reactivity. After stirring for 30 min at room temperature complete consumption of the aldehyde was observed. Work up and purification by column chromatography gave the two diastereomeric *cis*:*trans* isoquinolines (**17** and **18**, respectively) in 60% yield in a ratio of 78:22, respectively.

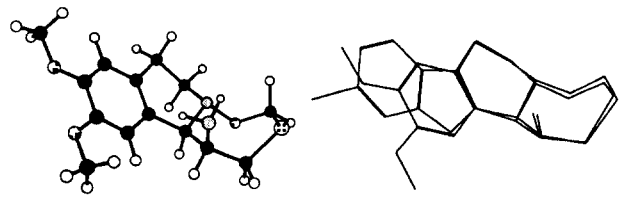
As a consequence of the slow cyclization reaction **17** and **18** were obtained as near racemates. It is likely that racemization can be avoided in future attempts by allowing the reaction to proceed at low temperature (−20 °C).

Comparison of the NMR and TLC data with the eudistomin type β-carboline series suggested that the desired *cis* diastereomer (lowest R_f at TLC) had been formed in excess. Also, for the *cis* diastereomer **17** a smaller geminal (^2J) coupling constant was observed for the thioacetal protons at C(4) (viz. ca. 9 Hz for the *cis* and ca. 11 Hz for the *trans* diastereomer). In addition, the δ-values of the (BOC) *t*-butyl singlets in the *cis*:*trans* diastereomers (namely at 1.24 and 1.47 ppm, respectively) corresponded with the δ-values found for the BOC protected *cis* and *trans* analogues of debromo eudistomin K **1** (1.17 and 1.52 ppm[3b]).

That these experimental data indeed pointed in the right direction was unambiguously proven by X-ray crystal structure analysis of **17** (Chart 2). Also an overlay presentation of the X-ray structures of the isoquinoline derivative **17** and N(1)-*p*-bromobenzoyl functionalized eudistomin K[1b] has been included, which shows that the conformation of the aliphatic bicyclic ring system in both N(1) functionalized derivatives is



Scheme 3. Synthesis of the eudistomin isoquinoline derivative **4**. Reagents: (a) NaH, NaI, DME; (b) $KF{\cdot}2H_2O$, *n*-Bu_4NCl, MeCN, 50 °C; (c) i. DIBAL, CH_2Cl_2, −75 °C, ii. TFA, −75 °C→rt; (d) TMSI, MeCN (**17** only).



X-ray structure of 17 ***Overlay of 17 with and the X-ray structure of eudistomin K***

Chart 2. Three-dimensional representations of the bioactive conformation. For clarity reasons the originally present N(1)-BOC protective group in **17** and the N(1)-*p*-bromobenzoyl group in the eudistomin K derivative have been replaced by a hydrogen atom.

essentially the same. An extensive NMR study revealed the same solid and solution state conformations for N(1)-*p*-bromobenzoyl functionalized eudistomin K.[1h] The study also showed the same solution state conformation of the aliphatic ring system for the naturally occurring N(1) unprotected derivatives.

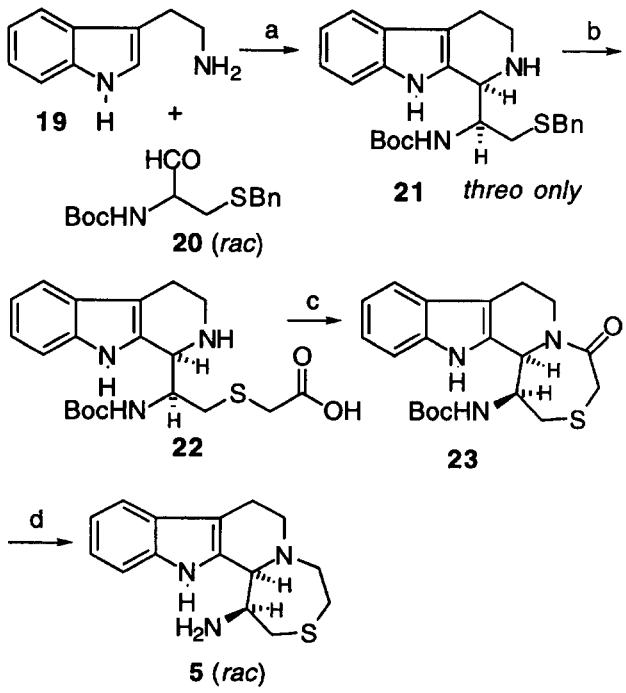
Because the electronic and steric nature of the dimethoxy phenyl group differs fundamentally from an indole moiety, the mechanistic conclusions drawn for the β-carboline series (vide supra) are not applicable for the isoquinoline series. To explain the reversal in diastereoselectivity in the isoquinoline series further investigations will be necessary.

Finally, the BOC-group was removed using the TMSCl/NaI method to generate TMSI in situ giving the eudistomin isoquinoline analogue **4** in 81% yield (Scheme 3).

Desoxa carba debromoeudistomin K (5)

As stated in the introduction of the chemistry section the intermolecular PS strategy was chosen for the synthesis of **5**. Therefore, predominant formation of the desired *cis* diastereomer was anticipated. Also, the deoxo carba derivative **5** lacks the ambident nucleophilic alkoxyamine moiety thus preventing problems with the closure of the D-ring.

The key step is the PS reaction of *rac*-*S*-benzyl-*N*-BOC-cysteinal **20** with tryptamine **19**. The aldehyde **20** was prepared by DIBAL reduction of the known corresponding methyl ester.[9] TFA catalyzed PS cyclization of aldehyde **20** with tryptamine in dichloromethane proceeded smoothly within 1 h at room temperature to give solely the anticipated *threo* diastereomer **21** in 54% yield (structure proof follows).



Scheme 4. Synthesis of the eudistomin deoxo carba derivative **5**. Reagents: (a) TFA, CH_2Cl_2; (b) (i) Na, NH_3(l), (ii) methyl chloroacetate, DBU, benzene, (iii) 2 N NaOH, dioxane; (c) TBTU, DiPEA, DMF; (d) TMSI, MeCN.

The benzyl protective group in **21** was removed by reduction with sodium in liquid ammonia. Alkylation of the thiol with methyl bromoacetate in benzene with DBU as a base, followed by saponification of the methyl ester with 2 N NaOH in dioxane gave **22** in an overall yield of 55% (three steps). Closure of the 7-membered ring, by activation of the carboxyl moiety as its *N,N,N',N'*-tetramethyluronium salt by treatment with TBTU in DMF with DIPEA as a base, proceeded smoothly to give the tetracyclic compound **23** in 82% yield.

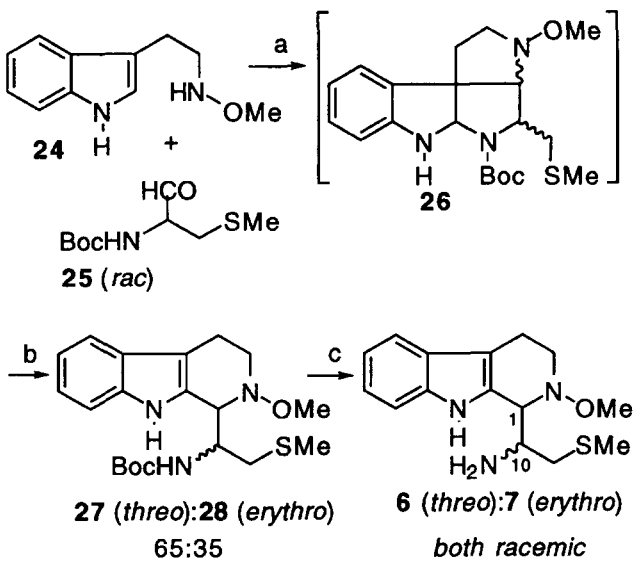
At this stage it was clear from 1H NMR data that indeed the anticipated *cis* diastereomer had been formed in the intermolecular PS reaction. Due to the high flexibility of the [1,6,2]-oxathiazepine ring in the natural eudistomin series reliable *cis/trans* assignments could only be made by comparison of the ^{1}H NMR data of the analogous diastereomers or by X-ray crystallography. In contrast the amide moiety in **23** gives a rigid 7-membered lactam. Thus, the small coupling constant (<1 Hz) found for H(1)–H(13b), which corresponds to a dihedral angle of 90°, unambiguously points to a *cis* relationship between these protons.

Reduction of the lactam with DIBAL at −5 °C, followed by removal of the BOC protective group by treatment with iodotrimethylsilane gave racemic **5** in an overall yield of 43%.

Noteworthy is the relatively efficient synthesis of **5**. With the natural tetracyclic [1,6,2]-*oxa*thiazepine ring containing analogues, which are at best synthesized using the intramolecular PS reaction (vide supra), high overall yields cannot be reached mainly due to diastereoselectivity towards the undesired *trans* diastereomer.

Tricyclic eudistomin analogues 6 and 7

TFA catalyzed Pictet–Spengler reaction of known N_b-methoxytryptamine **24**[10] and *rac*-*S*-methyl-*N*-BOC-cysteinal **25**[11] gave complete consumption of the starting materials in 15 min. ^{1}H NMR spectroscopy of a worked up aliquot of the reaction mixture after 1 h showed, besides both *cis* and *trans* β-carbolines **27** and **28**, the presence of the corresponding spiro intermediates **26**. The spiro compounds **26** were easily recognized in the ^{1}H NMR spectrum by the characteristic absorptions of H(6) (triplet at 6.6 ppm) and H(8) (doublet at δ 6,8 ppm), which are typical for indolenines. Prolonged treatment with TFA in chloroform for two days gave exclusive formation of both *cis/trans* β-carbolines **27** and **28** in an isolated yield of 60%. The formation of spiro



Scheme 5. Synthesis of the D-ring-opened eudistomin derivatives **6** and **7**. Reagents: (a) TFA, CH_2Cl_2; (b) two days; (c) TMSI, MeCN.

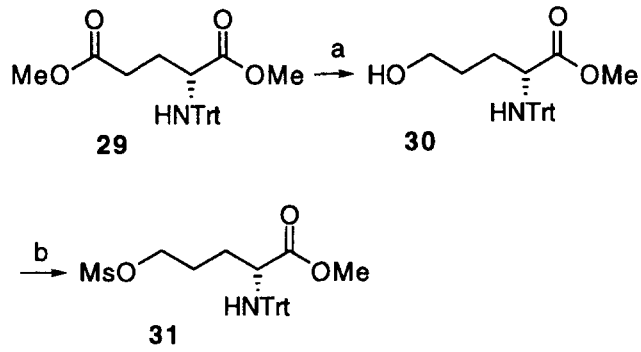
intermediates in the Pictet–Spengler reaction is well described in the literature.[12] The formation of the kinetic spiro intermediate is especially found with N_b-alkoxy tryptamines due to the high reactivity of the intermediate *N*-alkoxy iminium ion.[5]

NMR spectroscopy of the mixture of both diastereomers **6** and **7** showed a *threo*:*erythro* ratio of 65:35. Removal of the BOC protective group by treatment of the mixture of both diastereomers **27**:**28** with iodotrimethylsilane gave **6** (*threo*) and **7** (*erythro*) in 68% yield in a 56:44 ratio. These *threo* and *erythro* diastereomers are configurationally equal with the *cis* and *trans* tetracyclic eudistomin diastereomers, respectively.

Although the C(1)-amino group is not attached at a rigid ring system, *threo*/*erythro* assignment was still possible by NMR spectroscopy. The low field shift of the indole-NH protons of **6** and **7**, which absorbed at 9.84 ppm and 9.31 ppm, respectively, was consistent with donation of a hydrogen bond to the C-1 nitrogen atom. These hydrogen bonds result in a semi-rigid structure to give a well-defined dihedral angle for H-C(1)-C(10)-H.[13] The observed coupling constants $J_{H(1)-H(10)}$ were 3.5 Hz for **6** and 8.5 Hz for **7**, corresponding to dihedral angles of 60 (*threo*) and 180 (*erythro*), respectively. Molecular modeling of both diastereomers confirmed the presence of these hydrogen bonds. The calculated dihedral angles after PM3 minimization of the *threo* (**6**) and the *erythro* (**7**) diastereomers for H-C(1)-C(10)-H were 70° and 173°, respectively, which are in close agreement with the values derived from the measured coupling constants.

Desthia carba debromoeudistomin K (8)[14]

Application of the intramolecular PS condensation in the construction of the 7-membered 1,2-oxazine ring in



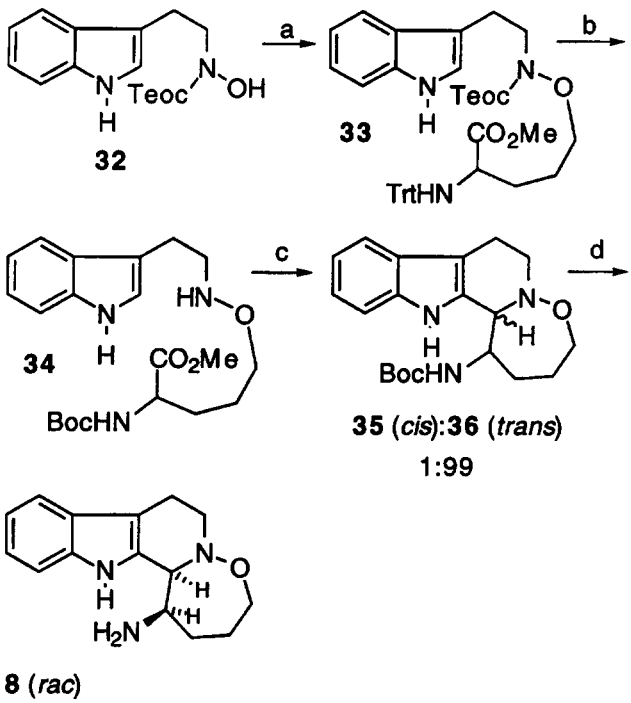
Scheme 6. Selective functionalization of the δ-carboxyl group of glutamic acid by shielding of the α-carboxylate with the bulky *N*-trityl protective group. Reagents: (a) $LiALH_4$, Et_2O, 0 °C; (b) MsCl, Et_3N, Et_2O, −20 °C.

the desthia carba derivative was already carried out by Kirkup and coworkers.[15] However, only the undesired *trans* derivative was obtained as a racemate. We undertook the synthesis of the *cis* desthia carba analogue using a modified intramolecular PS reaction strategy. In earlier approaches toward natural eudistomin derivatives with a [1,6,2]-oxathiazepine using the intramolecular PS reaction a BOC-protected C(1)-amino gave the best *cis*:*trans* ratios.[5] Thus, to facilitate formation of the anticipated *cis* isomer we replaced the C(1)-azide functionality, as used by Kirkup et al.,[15] by a BOC-protected amino group.

The synthesis of the intramolecular PS precursor *O*-alkylated N_b-oxotryptamine **34** was accomplished by nucleophilic coupling of N_b-TEOC protected N_b-hydroxy-tryptamine **32**[3b] with methyl (*R*)-5-methylsulfonyloxy-2-tritylamino-pentanoate **31** (Scheme 6). For this chiral α-amino ester fragment **31**, D-glutamic acid was used as the starting material. The carboxyl moiety of α-amino acids can be sterically shielded by trityl protection of the amino group. In the case of glutamic acid this gives the possibility for selective chemical manipulation of the γ-carboxyl group. Using this strategy, the terminal primary alcohol **30** could be obtained in one step by selective reduction of (D)-Trt-Glu(OMe)-OMe **29** (Scheme 6).[16] Addition of lithiumaluminum hydride, while carefully monitoring the progress of the reaction by TLC, to a stirred solution (at 0 °C) of the dimethyl ester **29** in ether gave the γ-mono alcohol **30** in 85% yield.

Treatment of the alcohol **30** at low temperature (−20 °C) with methanesulfonyl chloride and triethylamine in ether gave the mesylate **31** in high yield.[17] It should, however, be noted that the mesylate **31** was only stable below −10 °C. Nucleophilic coupling of **31** with the sodium alkoxide of N_b-TEOC-N_b-hydroxy tryptamine **32** in DME was initially carried out at 0 °C, but no conversion of the starting materials could be observed. After standing of the reaction mixture at room temperature overnight, all starting material had been consumed (Scheme 7) to give **33** in 67% yield.

As a consequence of the long exposure to the basic conditions used in the nucleophilic coupling, **33** was



Scheme 7. Synthesis of the eudistomin desulfo carba derivative **8**. Reagents: (a) (i) NaH, DME, (ii) **31**; (b) (i) 0.1 N HCl, F_3CCH_2OH, (ii) $(BOC)_2O$, Et_3N, CH_2Cl_2, (iii) $KF{\cdot}2H_2O$, *n*-Bu_4NCl, MeCN; (c) (i) DIBAL, CH_2Cl_2, −75 °C, (ii) TFA; (d) TMSI, MeCN (**35** only).

isolated as a (near) racemate. As the trityl protective group will not survive the acid catalyzed PS reaction this group was replaced by the BOC protective group. The trityl protective group was removed by titration with 0.1 M HCl in 2,2,2-trifluoroethanol of **33** giving the free amine in 70% yield. Subsequent treatment with di-*tert*-butyl dicarbonate and triethylamine in dichloromethane gave the BOC-protected product in 92% yield. Removal of the TEOC protective group with tetrabutyl ammonium chloride/potassium fluoride in acetonitrile gave the desired precursor for the intramolecular PS reaction **34** in 95% yield.

As shown in Scheme 7, reduction of the methyl ester with DIBAL at −75 °C followed by treatment of the obtained aldehyde with TFA resulted in cyclization in 72% yield. Initial inspection of the crude reaction mixture by both TLC and NMR spectroscopy indicated the presence of only the *trans* diastereomer **36**. Close examination of the isolated fractions obtained after purification of the reaction mixture by flash chromatography revealed, however, the presence of the desired *cis* diastereomer **35** in only 1.3% yield.

This >97% diastereomeric excess towards the undesired *trans* diastereomer is in sharp contrast to the 40% d.e. for the *trans* diastereomer found for the oxathiazepine containing analogues. Apparently, this is the result of a different conformation of the intermediate 7-membered ring incorporating the electrophilic iminium ion due to substitution of the sulfur atom by a methylene group.

Subsequent removal of the BOC protective group from the isolated *cis* diastereomer **35** by treatment with iodotrimethylsilane gave the desthia carba eudistomin analogue **8** in 64% yield.

The *cis/trans* assignments were made using 400 MHz ^{1}H NMR data of the C(1)-N BOC protected structures **35** and **36**. In the *natural* eudistomin series (i.e., with a [1,6,2]-oxathiazepine ring) it was found that *cis/trans* assignment was possible using the δ-values of the indole-NH protons of both diastereomers. X-ray crystal structure analysis of the C(1)-N BOC-protected *trans* diastereomer revealed that the C(1)-amino group occupies an equatorial position, which allows formation of an intramolecular hydrogen bond between the indole-N proton and the urethane carbonyl.[3b] This difference in spatial position of the C(1)-amino group and its ability to form a hydrogen bond with the indole-NH was clearly seen in the ^{1}H NMR spectra. The indole-NH of the *trans* diastereomer absorbed 1.4 ppm lower than in the *cis* isomer. X-ray structure analysis of the *cis* diastereomer showed the amino substituent occupies an axial position in which no such intramolecular hydrogen bond is possible.

Shankar and coworkers succeeded in obtaining an X-ray crystal structure from the *trans* desthia carba analogue with the NH_2 group functionalized with a 4-nitrobenzoyl group.[18] This X-ray analysis revealed that the functionalized C(1)-amino side chain occupied an equatorial position together with a hydrogen bond between the amide carbonyl and the indole-N proton. In the NMR spectrum of the C(1)-N BOC-protected *trans* carba analogue **36** also a downfield shift was observed for the indole NH proton (i.e., $\Delta\delta = 1.0$ ppm) compared to the *cis* diastereomer, suggesting the same spatial orientation of the C(1)-amino side groups in *cis/trans* desthia carba derivatives as found in both eudistomin diastereomers incorporating a [1,6,2]-oxathiazepine ring. It is also noteworthy that the (BOC) *t*-butyl singlets in the NMR spectra of the natural and desthia carba series were located at exact the same δ values (namely 1.17 ppm in the *cis* diastereomer and 1.52 ppm in the *trans* diastereomer). Due to broadening and overlap of the NMR signals no *cis/trans* assignments could be made on the basis of the vicinal coupling constants of H(1) and H(13b). The NMR spectrum of **8** was in good agreement with the spectrum as published by Kurihara and coworkers.[14]

Antiviral and antitumor structure–activity relationships

Antiviral activities

The derivatives **1**, **2** and **4–8** depicted in Chart 1 were evaluated for their inhibitory effects on the replication of a number of viruses:

DNA viruses: herpes simplex virus type-1 (HSV-1) (strain KOS), HSV-2 (strain G), vaccinia virus and

Table 1. Cytotoxicity and antiviral activity of the synthetically derived eudistomins in HeLa cell cultures

Compd code	Minimum cytotoxic conc. (mg/mL)[a]	Minimum inhibitory concentration (μg/mL)[b]		
		Vesicular stomatitis virus	Coxsackie virus B4	Polio virus-1
1	≥4	0.2	0.7	0.7
2	0.4	0.02	0.02	0.02
4	34	0.2	4	1
5	3100	40	20	20
6	40	>10	>10	>10
7	340	>10	>10	>10
8	340	7	7	7
BVDU	>400	>400	>400	>400
DHPA	>400	300	>400	>400
Ribavirin	>400	20	70	70
C-c^{3}Ado	>400	2	>400	>400

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50%. The data represent average values of two separate experiments. The assays were found to be reproducible with relative standard deviations of <20%.

thymidine kinase (TK) deficient (TK$^-$) HSV-1 (strain B2006).

(+)-RNA viruses: Coxsackie virus B4, polio virus-1, Sindbis virus, and Semliki forest virus.

(−)-RNA viruses: vesicular stomatitis virus, and para-influenza-3 virus.

(±)-RNA viruses: reovirus-1.

The antiviral tests were performed in HeLa (a human epithelial cell line derived from a cervix carcinoma) or Vero (a simian fibroblast cell line derived from African green monkey kidney) or PRK (primary rabbit kidney) or E_6SM (human embryonic skin–muscle fibroblast) cell lines. The cells used are non-dividing stationary cell monolayers. All antiviral tests were carried out following established procedures.[19] As reference compounds, *E*-5-(2-bromovinyl)-2′-deoxyuridine (BVDU), (*S*)-9-(2,3-dihydroxypropyl)adenine (DHPA), ribavirin, and carbocyclic 3-deazaadenosine (C-c^{3}Ado) were included in the antiviral tests. The observed antiviral data should always be considered in relation with the cytotoxic data. Cytotoxicity was evaluated by microscopic examination of cell monolayers not infected by the virus but exposed to the compounds, which then allows determination of the minimal cytotoxic concentration (MCC) in direct comparison with the or minimal (50%) (virus) inhibitory concentration (MIC_{50}). The antiviral activities were considered significant if the MCC:MIC ratios were equal or greater than 10.[20] Significant antiviral data are printed in italics in the Tables 1–3.

Table 1 shows that **1**, **2**, **4**, and the reference compounds ribavirin and C-c^{3}Ado were active against vesicular stomatitis virus (MCC:MIC = 20). Against the Coxsackie B4 and polio-1 viruses only **2** showed significant activity, although its high cytotoxity to the HeLa host cells lowered the MCC:MIC ratio. No significant activity was found for the desthia carba derivative **5**, the two diastereomeric ring opened analogues **6** and **7**, and for the desoxa carba derivative **8**.

Table 2 shows that in Vero host cells **1**, **2**, **4**, **5**, **8**, and C-c^{3}Ado all showed activity against Coxsackie B4 virus. The same compounds also showed activity against parainfluenza-3, reovirus-1, Sindbis, and Semliki forest virus (not **5** and C-c^{3}Ado). It is noteworthy that the MCC:MIC ratios were larger than 50 for the isoquinoline derivative **4** as well as for the desoxa and the desthia carba derivatives **5** and **8**, respectively. Compounds **6** and **7** showed no activity at all.

The same trend was found for the activity of **1**, **2**, **4**, **5**, **6**, **7** and **8** against HSV-1, HSV-2, vaccinia, and vesicular stomatitis virus, presented in Table 3. Especially the

Table 2. Cytotoxicity and antiviral activity of the synthetically derived eudistomins in Vero cell cultures

Compound code	Minimum cytotoxic concentration (μg/mL)[a]	Minimum inhibitory concentration (mg/mL)[b]				
		Para-influenza-3 virus	Reo-virus-1	Sindbis virus	Coxsackie virus B4	Semliki forest virus
1	≥4	*0.2*	*0.4*	*0.2*	*0.4*	*0.2*
2	≥1	0.7	*0.07*	*0.02*	*0.07*	*0.07*
4	40	*0.2*	*0.2*	*0.07*	*0.2*	*0.7*
5	≥100	*7*	*0.7*	*2*	*2*	20
6	≥100	>40	>40	>40	>40	>40
7	≥100	>40	>40	>40	>40	>40
8	>400	*4*	*2*	*7*	*7*	*7*
BVDU	>400	>400	>400	>400	>400	>400
DHPA	>400	70	70	150	70	>400
Ribavirin	>400	*20*	*70*	*40*	70	*40*
C-c^{3}Ado	>400	*2*	*2*	*20*	20	>400

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50%. The data represent average values of two separate experiments. The assays were found to be reproducible with relative standard deviations of <20%.

Table 3. Cytotoxic and antiviral activity of the synthetically derived eudistomins in primary rabbit kidney (PRK) or E_6SM cell cultures

Compound code	Minimum cytotoxic concentration (MCC) (μg/mL)[a]	Minimum inhibitory concentration (MIC) (μg/mL)[b]					
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinic virus	Vesicular stomatitis virus	Herpes simplex virus-1 (TK-)(B2006)	Herpes simplex virus-1 (TK^-/TK^+) VMW1837
1	≥10	*0.7*	*0.2*	*0.1*	*0.2*	*0.7*	
2	≥1	*0.045*	*0.02*	*0.02*	*0.02*	*0.04*	
4*c*	≥100	*0.1*	*0.1*	*0.07*	*0.1*	*0.07*	*0.02*
5*c*	100	*2*	*2*	*1*	*7*	*0.04*	*1*
6	200	>100	70	>100	>100	>100	100
7	>100	>40	>40	>40	>40	>40	>40
8	400	*2*	*2*	*2*	*1*	*2*	*2*
BVDU	≥400	*0.04*	*7*	*7*	>200	150	*10*
DHPA	≥400	>100	>100	*20*	*20*	>100	>400
Ribavirin	≥400	>200	>200	*20*	>400	>200	100
C-c^{3}Ado	≥400	>200	>200	*0.7*	*2*	150	150

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50%. The data represent average values of two separate experiments. The assays were found to be reproducible with relative standard deviations of <20%.
[c]Virus grown in E6SM cell cultures.

MCC:MIC ratios of 1000–5000 for **4** for these viruses emphasize its significant antiviral potency. It is noteworthy that, in contrast to HeLa host cells (see Table 1), in PRK host cells activity was also found for **5** and **8** against vesicular stomatitis virus. No specific anti-HIV 1 or anti-HIV 2 activities were found for any of the synthetically derived eudistomins (data not shown). Nonetheless, the broad-spectrum of potent antiviral activity of the eudistomins **1**, **2**, **4**, **5** and **8** in comparison to each of the reference drugs suggests their potential as a new class of antiviral compounds.

Antitumor activities

The inhibitory effects on the proliferation of murine leukemia cells (L1210), HTLV-1$^-$ infected human T-lymphoblast cells (Molt/4F), murine mammary carcinoma cells (FM3A), and human T-lymphocyte cells (CEM) were evaluated following established procedures.[21] For **1**, a marked inhibitory effect was observed, while **2** appeared to be a very potent cytostatic compound with IC_{50} values down to 5 ng/mL (16 nM) for the investigated tumor cells.[3b] These data of **1** and **2** in combination with the data of the new eudistomin analogues **4–8** are presented in Table 4.

Compound **4** was somewhat more potent than **1** in these antiproliferative assays, as in the antiviral tests. The desthia derivative **8** was only slightly less potent than **1**, in contrast to the antiviral test (10-fold decrease for **8** compared to **1**). Similarly for **2**, the antiproliferative activity was more pronounced than the antiviral activity. On the other hand, the desoxa derivative **5** was only weakly growth-inhibitory to the tumor cells. The ring opened derivatives **6** and **7** showed no significant tumor cell growth inhibition.

Compounds **1** and **4** were subjected to further in vitro antiproliferative activity determinations against breast cancer (MCF7 and EVSAT), colon cancer (WIDR), ovarian cancer (IGROV), melanoma (M19), renal cancer (A498), and non-small cell lung cancer (HOP92) cell lines, following established procedures (Table 5).[22]

Table 4. Inhibitory effects of synthetically derived eudistomins on the proliferation of murine leukemia cells (L1210), human T-lymphoblast cells (Molt/4F), FM3A, and CEM cells

Compound code	IC_{50}[a] (μg/mL)			
	L1210	Molt-4F	FM3A	CEM
1	0.11 ± 0.01[b]	0.12 ± 0.02		
2	0.005 ± 0.0005[b]	0.0062 ± 0.0006	0.0073 ± 0.0003	0.0055 ± 0.002
4	0.058 ± 0.013	0.079 ± 0.018	0.120 ± 0.01	0.076 ± 0.006
5	5.3 ± 1.29	5.13 ± 2.36	13.3 ± 6.6	4.57 ± 9.2
6	7.95 ± 5.94	18.3 ± 91	48.2 ± 1.8	7.31 ± 5.62
7	15 ± 4	16.5 ± 4	28 ± 4	15.5 ± 0.8
8	0.2 ± 0.06	0.25 ± 0.02	0.77 ± 0.06	0.3 ± 0.12

[a]Concentration required to inhibit tumor cell proliferation by 50%.
[b]Similar results were obtained with the P388 cell line by Dr P. Lelieveld from TNO-CIVO Institutes, Zeist, The Netherlands.

Table 5. Inhibitory effects of synthetically derived eudistomins on the proliferation of human breast cancer cells (MCF7, EVSAT), colon cancer (WIDR), ovarian cancer (IGROV), melanoma (M19), renal cancer (A498), and non-small cell lung cancer (HOP92)[a]

Compound code	IC_{50}[b] (μg/mL)						
	MCF7	EVSAT	WIDR	IGROV	M19	A498	HOP92
1	0.100	0.140	0.210	0.420	0.220	0.075	0.280
4	0.062	0.194	0.150	0.099	0.109	0.230	0.203
DOX	0.008	0.006	0.020	0.028	0.005	0.005	
5-FU	0.210	0.650	0.260	0.280	0.160	0.088	

[a]These results were obtained in the Laboratory of Experimental Chemotherapy and Pharmacology, Department of Medical Oncology, Rotterdam Cancer Institute (Dr Daniel den Hoed Kliniek), The Netherlands.
[b]Concentration required to inhibit tumor cell proliferation by 50%. The assays were found to be reproducible with relative standard deviations of <20%.

Doxorubicin (**DOX**) and 5-fluorouracil (**5-FU**) were used as reference compounds. The antiproliferative potencies of **1** and **4** fell roughly in between those of **DOX** and **5-FU**.

Discussion

The newly synthesized derivatives **4**, **5**, and **8**, as well as **1** and **2**, displayed a broad-spectrum antiviral activity against both DNA- and RNA-type viruses. These eudistomins compared favourably both with regard to in vitro potency and spectrum of activity to the reference drugs BVDU, DHPA, ribavirin, and C-c[3]Ado. No activity was found against the retrovirus HIV.

In the synthetic eudistomins presented in this paper, the general rank-order of antiviral potency was **2** > **4** > **1** > **5** and **8**. With respect to cytotoxicity (MCC:MIC ratio) the order was **4** > **5** and **8** > **1** and **2**. Thus, the tricyclic isoquinoline derivative **4** is, so far, the most promising antiviral candidate; it combines a high potency (MIC at ca. 100 ng/mL (340 nM)) with MCC:MIC ratios ranging from 1000–5000 (against HSV-1, HSV-2, vaccinia and vesicular stomatitis viruses). Since **4** was obtained and tested as a racemate (in contrast to **1** and **2**), an even higher potency might be expected from optically pure material with the correct 1*S*, 13b*R* configuration.

The tumor cell antiproliferative activity of these synthetic eudistomins followed roughly the same pattern: **2** ≫ **4** > **1** > **8** ≫ **5**. Interestingly, in the desthia derivative **8** and the 10-methoxy derivative **2** the antiproliferative activity was more pronounced than the antiviral activity. To the contrary, in the desoxy derivative **5** the antiviral activity was more pronounced. These results indicate that in the eudistomin class a further separation of antiviral and antitumor properties might be possible.

Combining all the SAR data of the natural and synthetic eudistomins a number of conclusions can now be formulated. (a) All active compounds possess a 7-membered ring with a *cis*-configuration at the two chiral centers and an amino group at C(1). NMR data of **1**, **2**, **4**, **5** and **8** revealed that the 7-membered ring in these derivatives had the same conformation as was found in the natural eudistomins. The amino group at C(1) is axially oriented as well as the lone pair of the bridgehead N(6). X-ray structures of eudistomin K and **17** (Chart 2) further support this observation. The absence of activity in the ring opened analogues **6** and **7** underline the function of the 7-membered ring as a scaffold for the correct spatial positioning of the two nitrogen atoms. (b) Both the oxygen and the sulfur atom in the 7-membered ring of eudistomins may be replaced by a methylene group, supporting that the thioacetal moiety itself is not essential in the intermolecular binding process with the biological target. (c) Substitution at the indole moiety is allowed. Introduction of a methyl group at the pyrrolo nitrogen does not significantly alter the activity. Potency can be increased by bromine, hydroxy and methoxy substituents at positions 9, 10 and 11. The most potent antiviral and antiproliferative eudistomin is the 10-methoxy derivative **2**. (d) Bioisosteric replacement of the indole nucleus by a dimethoxyphenyl group in **4** is allowed resulting in the most significant antiviral compound so far in the eudistomin class.

The presence of both tumor cell antiproliferative and broad-spectrum antiviral activity suggests that eudistomins presumably interact on biochemical processes that are essential for both tumor cell and virus growth. This has been observed more often for antiviral compounds.[23] The exact mechanisms of the biological actions of eudistomins are at present under investigation at the Rega Institute (Leuven, Belgium).

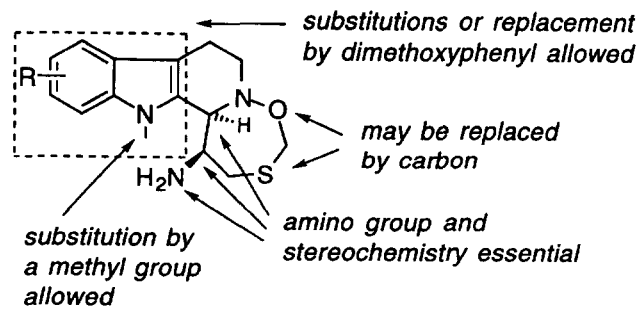


Chart 3. Schematic representation of the SAR conclusions.

Experimental

Magnetic resonance spectra were measured on a Bruker WH-90 or on a Bruker AM-400 spectrometer. Chemical shift values are reported as δ-values relative to tetramethylsilane as an internal standard; deuteriochloroform was used as a solvent. Mass spectra were obtained with a double focusing VG 7070E spectrometer. For the determination of optical rotations a Perkin–Elmer 241 polarimeter was used. All solvents were commercially obtained and used unpurified unless stated otherwise. Thin-layer chromatography (TLC) was carried out by using silca gel F_{254} plates (thickness 0.25 mm). Spots were visualized with a UV hand lamp, iodine vapor, ninhydrin solution containing 3 mL acetic acid and 0.3 g ninhydrin in 100 mL *n*-butanol, or Cl_2-TDM.[24] Column chromatography was carried out using silica 60H (Merck).

***O*-Allyl-*N*-(2-trimethylsilylethyloxycarbonyl)-hydroxylamine (10).** To *O*-allylhydroxylamine hydrochloride hydrate (1.9 g, 16.8 mmol) and TEOC-Cl (3.7 g, 20.5 mmol) dissolved in dry THF (15 mL) was added gradually diisopropylethylamine (6.5 mL, 4.8 g, 37.5 mmol). After stirring of the reaction mixture for 6 h, EtOAc (50 mL) was added and the mixture was subsequently washed with two portions 10% citric acid, satd $NaHCO_3$ and brine. After drying ($MgSO_4$) and evaporation of the volatiles in vacuo the residue was subjected to column chromatography (EtOAc:hexanes, 1:4) to give 3.6 g (98%) of **10** as a colorless oil; R_f 0.50 (EtOAc:hexanes, 1:4); EIMS (70 eV), *m/z* (relative intensity) 174 ([M-C_3H_7]$^+$, 12), 73 ([C_3H_9Si]$^+$, 100), 41 ([C_3H_5]$^+$, 38); ^{1}H NMR (90 MHz) δ 7.35 (br s, 1H, NH), 6.20–5.74 (m, 1H, H_2C=CH), 5.41–5.19 (m, 2H, H_2C=CH), 4.37–4.12 (m, 4H, 2*OCH_2), 1.08–0.89 (m, 2H, $SiCH_2$), 0.00 (s, 9H, $Si(CH_3)_3$).

***N*-[2-(trimethylsilyl)ethyloxycarbonyl]-*N*-(allyloxy)-2-(3,4-dimethoxyphenyl)ethylamine (12).** To 2-(3,4-dimethoxyphenyl)ethanol (1.0 g, 5.5 mmol), **10** (1.6 g, 7.1 mmol) and triphenylphosphine (1.87 g, 7.1 mmol) dissolved in dry THF (10 mL) was added gradually diisopropyl azodicarboxylate (1.40 mL, 1.44 g, 7.1 mmol) causing a slightly exothermic reaction. After 1 h the volatiles were evaporated in vacuo and the residue was subjected to column chromatography (EtOAc:hexanes, 1:6) to yield 0.86 g (64%) of **12** as a colorless oil; R_f 0.30 (EtOAc:hexanes, 1:4); EIMS (70 eV), *m/z* (relative intensity) 381 ([M]$^+$, 6), 338 ([M-C_3H_7]$^+$, 2), 151 ([$C_9H_{11}O_2$]$^+$, 22), 73 ([C_3H_9Si]$^+$, 100), 41 ([C_3H_5]$^+$, 6); ^{1}H NMR (90 MHz) δ 6.76 (s, 3H, PhH_3), 6.24–5.78 (m, 1H, H_2C=CH), 5.44–5.19 (m, 2H, H_2C=CH), 4.33 (d, 2H, J = 5.9 Hz, $NOCH_2$), 4.23–4.04 (m, 2H, OCH_2CH_2Si), 3.84 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.67 (dd, 2H, J = 8.0 Hz and J = 6.7 Hz, NCH_2), 2.85 (dd, 2H, J = 7.8 Hz and J = 6.5 Hz, $PhCH_2$), 1.04–0.86 (m, 2H, $SiCH_2$), 0.00 (s, 9H, $Si(CH_3)_3$) together with 0.48 g of an impure fraction, contaminated with **10**. Because the R_f values of the starting compound **10** and product **12** only differ slightly (i.e., 0.36 and 0.30) purification by flash chromatography was difficult and it is therefore recommended not to use an excess of **10**.

***N*-[2-(trimethylsilyl)ethyloxy)carbonyl]-*N*-hydroxy-2-(3,4-dimethoxyphenyl)ethylamine (13).** To **12** (1.3 g, 3.4 mmol) dissolved in acetonitrile:water (4:1, 25 mL) under an argon atmosphere was subsequently added: triethylammonium formate (4.4 g, 10 mmol), triphenylphosphine (40 mg, 0.15 mmol), and $Pd(OAc)_2$ (11 mg, 0.05 mmol). The reaction mixture was heated at reflux for 30 min. Work up was accomplished by the addition of EtOAc (50 mL) and subsequent washings with three portions of water and brine. After drying ($MgSO_4$), the volatiles were evaporated in vacuo and the residue was subjected to column chromatography (EtOAc:hexanes, 1:2) to give 1.14 g (98%) of **13** as a colorless oil; R_f 0.35 (EtOAc:hexanes, 1:1); EIMS (70 eV), *m/z* (relative intensity) 341 ([M]$^+$, 0.5), 151 ([$C_9H_{11}O_2$]$^+$, 31), 73 ([C_3H_9Si]$^+$, 100); ^{1}H NMR (90 MHz) δ 7.21 (br s, 1H, OH), 6.78–6.70 (m, 3H, PhH_3), 4.19–4.01 (m, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.71 (t, 2H, J = 6.8 Hz, NCH_2), 2.87 (t, 2H, J = 6.8 Hz, $PhCH_2$), 0.99–0.80 (m, 2H, $SiCH_2$), 0.00 (s, 9H, $Si(CH_3)_3$).

Methyl 2-(*tert*-butyloxycarbonylamino)-3-[*N*-(2-(3,4-dimethoxyphenyl)ethyl)aminoxymethyl-sulfanyl]-propionate (16). This experiment was carried out under an argon atmosphere employing flame-dried glass equipment. To **13** (0.99 g, 2.9 mmol), dissolved in freshly distilled 1,2-dimethoxyethane (10 mL), NaH (87 mg of a 80% suspension, 2.9 mmol) was added and the suspension was stirred until a clear solution appeared (hydrogen gas evolved). This solution was added gradually (in ca. 6 h) to a stirred solution of **14**,[3b] (1.4 g, 4.9 mmol) and NaI (0.7 g, 4.7 mmol) in freshly distilled 1,2-dimethoxyethane (50 mL). Work up was accomplished by the addition of satd NH_4Cl (2 mL) followed by concentration of the suspension in vacuo. The residue was dissolved in EtOAc and subsequently washed with water and brine. The organic layer was dried ($MgSO_4$) and the solvent was evaporated in vacuo to yield 1.24 g (73%) of crude **15** that was almost homogeneous by TLC. The crude **15** was dissolved in dry acetonitrile (25 mL). To this solution tetrabutylammonium chloride (1.76 g, 6.3 mmol) and potassium-fluoride dihydrate (0.79 g, 8.4 mmol) were added and the resulting suspension was stirred at 50 °C overnight. Work up was accomplished by evaporation of the volatiles in vacuo followed by purification of the residue by chromatography (EtOAc:hexanes, 1:2) affording 0.85 g (98%) of **16** as a colorless oil; R_f 0.40 (EtOAc:hexanes, 1:1); CIMS (70 eV), *m/z* (relative intensity) 445 ([M+1]$^+$, 6), 151 ([$C_9H_{11}O_2$]$^+$, 58), 148 (100), 57 ([C_4H_9]$^+$, 85); ^{1}H NMR (90 MHz) δ 6.78–6.72 (m, 3H, PhH_3), 5.95 (br d, 1H, J = 8.8 Hz, NH), 4.91–4.81 (AB, 2H, OCH_2S), 3.89 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.78 (s, 3H, CO_2CH_3), 3.22–3.74 (m, 6H, $PhCH_2CH_2$ and SCH_2CH), 1.44 (s, 9H, $C(CH_3)_3$).

***Rac-cis*-1-(*tert*-butyloxycarbonylamino)-10,11-dimethoxy-1,2,7,8,12b-pentahydro[1,6,2]-oxathiazepino-[2′,3′-*a*]-isoquinoline (17) and *Rac-trans* 1-(*tert*-butyloxycarbonyl-**

amino)-10,11-dimethoxy-1,2,7,8,12b-pentahydro-[1,6,2]-oxathiazepino-[2′,3′-*a*]isoquinoline (18). To a cooled solution (−75 °C) of **3** (830 mg, 1.93 mmol) in dry dichloromethane (150 mL) employing flame-dried glass equipment under an argon atmosphere was added DIBAL (2.9 mL of a 1 M solution in dichloromethane) at such a rate that the temperature remained below −70 °C. After stirring for 30 min, trifluoroacetic acid (1 mL) was added and the reaction mixture was allowed to warm to room temperature. After standing for 30 min at room temperature, all starting material had been consumed and the reaction mixture was successively washed with 10% citric acid, satd $NaHCO_3$ and brine. After drying ($MgSO_4$), the volatiles were evaporated in vacuo and the residue was subjected to column chromatography (EtOAc:hexanes, 1:4) to give 100 mg (13%) of **18** as a white solid; R_f 0.31 (EtOAc:hexanes, 1:2); CIMS (70 eV), *m/z* (relative intensity) 395 ($[M-1]^+$, 0.5), 341 (11), 207 ($[C_{11}H_{13}NO_3]^+$, 100), 57 ($[C_4H_9]^+$, 24); ^{1}H NMR (400 MHz) δ 7.27 (br s, 1H, C(12)H), 6.54 (s, 1H, C(9)H), 5.93 (br d, 1H, J = 9.9 Hz, NH), 5.15 (br AB, 1H, J_{AB} = 10.9 Hz, C(4)Hα), 4.76 (AB, 1H, J_{AB} = 10.9 Hz, C(4)Hβ), 4.58 (br s, 1H, C(1)Hα), 4.01 (s, 1H, C(12b)Hβ), 3.90 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.60 (very br s, 1H, C(7)Hα), 3.43 (br s, 1H, C(2)Hα), 3.09–2.90 (m, 2H, C(7)Hβ and C(8)Hα), 2.73 (dd, 1H, J = 5.1 Hz and J = 14.1 Hz, C(8)Hβ), 2.62 (d, 1H, J = 15.3 Hz, C(2)Hβ), 1.47 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, the marked atoms may be interchanged) δ 154.90 (C=O), 147.70 C(11) and C(10), 127.73 C(12a#), 125.88 C(8a#), 110.39 C(12~), 110.39 C(9~), 79.46 $C(CH_3)_3$, 74.49 C(12b) and C(4), 56.03 OCH_3, 56.2 C(1), 55.81 OCH_3, 53.28 C(7), 33.90 C(2), 29.25 C(8), 28.39 $C(CH_3)_3$. and 360 mg (47%) of **17** as colorless crystals. Recrystallized from EtOAc:hexanes (mp 173–177 °C, a crystal from this batch was used for the X-ray crystal structure determination); R_f 0.21 (EtOAc:hexanes, 1:2); $[\alpha]_D^{20}$ +13.0 (*c* 2.30, CH_2Cl_2:MeOH, 1:1, near racemate); CIMS (70 eV), *m/z* (relative intensity) 397 ($[M+1]^+$, 0.7), 207 ($[C_{11}H_{13}NO_3]^+$, 100), 57 ($[C_4H_9]^+$, 15); ^{1}H NMR (400 MHz, to sharpen up the broadened spectrum recorded at 46 °C) δ 6.75 (s, 1H, C(12)H), 6.53 (s, 1H, C(9)H), 5.23 (br d, 1H, J = 8.1 Hz, NH), 4.91 and 4.79 (AB, 2H, J_{AB} = 9.1 Hz, OCH_2S), 4.63 (br m, 1H, H(1)α), 4.16 (br s, 1H, H(12b)β), 3.86 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.46–3.38 (m, 1H, H(7)β), 3.25 (d, 1H, J = 14.5 Hz, H(2)α), 3.06–2.95 (m, 2H, H(7)α and H(8)β), 2.85 (dd, 1H, J = 14.5 Hz and J = 6.1 Hz, H(2)β), 2.68–2.58 (m, 1H, H(8)α), 1.24 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, the marked atoms may be interchanged) δ 155.30 (C=O), 147.57 C(11*), 147.44 C(10*), 126.42 C(12a#), 125.39 C(8a#), 110.37 C(12~), 109.97 C(9~), 78.98 $C(CH_3)_3$, 71.33 C(12b) and C(4), 55.84 2*OCH_3, 53.40 C(7), 50.69 C(1), 33.08 C(2), 29.28 C(8), 28.17 $C(CH_3)_3$; Anal. calcd for $C_{19}H_{28}N_2O_5S$: C, 57.56; H, 7.12; N, 7.07; S, 8.09. Found: C, 57.52; H, 6.94; N, 6.81; S, 7.66.

***Rac-cis*-1-amino-10,11-dimethoxy-1,2,7,8,12b-pentahydro-[1,6,2]-oxathiazepino-[2′,3′-*a*]isoquinoline (4)**. To a stirred solution of **17** (300 mg, 0.76 mmol) in dry acetonitrile (50 mL) NaI (227 mg, 1.51 mmol) and chlorotrimethylsilane (0.19 mL, 164 mg, 1.51 mmol) were added. After stirring for 4 h all starting material had been consumed and triethylamine (0.5 mL) was added. The volatiles were evaporated in vacuo and the residue was subjected to column chromatography (MeOH:CH_2Cl_2, 2:98) to yield 183 mg (81%) of **4** as a white solid as a near racemate; mp 100–102 °C; R_f 0.21 (MeOH:CH_2Cl_2, 5:95); CIMS (70 eV), *m/z* (relative intensity) 297 ($[M+1]^+$, 11), 280 ($[M-NH_2]^+$, 6), 207 ($[C_{11}H_{13}NO_3]^+$, 100); ^{1}H NMR (400 MHz) δ 6.61 (s, 1H, PhH), 6.59 (s, 1H, PhH), 4.91 and 4.82 (AB, 2H, J_{AB} = 9.2 Hz, OCH_2S), 4.15 (d, 1H, J = 2.1 Hz, H(12b)α), 3.86 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.51–3.43 (m, 2H, H(1)α and H(7)β), 3.33 (d, 1H, J = 14.4 Hz, H(2)α), 3.05–2.95 (m, 2H, H(7)α and H(8)β), 2.87 (dd, 1H, J = 14.4 Hz and J = 6.2 Hz, H(2)β), 2.68–2.58 (m, 1H, H(8)α); ^{13}C NMR (100 MHz, the marked atoms may be interchanged) δ 147.97 C(11*), 147.62 C(10*), 127.99 C(12a#), 126.23 C(8a#), 110.87 C(12~), 108.84 C(9~), 72.57 C(12b), 72.12 C(4), 55.96 (OCH_3), 55.79 (OCH_3), 52.83 C(7), 52.55 C(1), 35.04 C(2), 29.28 C(8); Anal. calcd for $C_{14}H_{20}N_2O_3S$: C, 56.73; H, 6.80; N, 9.45; S, 10.82. Found: C, 56.05; H, 6.47; N, 9.12; S, 10.40.

***Rac-threo*-1-[1-(N-(*tert*-butyloxycarbonyl)amino)-2-(benzylthio)-ethyl]-1,2,3,4-tetrahydro-β-carboline (21)**. To a stirred solution of tryptamine **19** (1.36 g, 8.46 mmol) and *rac*-*S*-benzyl-BOC-cysteinal **20**[9] (2.50 g, 8.46 mmol) in CH_2Cl_2 (30 mL) was added TFA (1.30 mL, 1.92 g, 16.9 mmol). TLC analysis (EtOAc:hexanes, 2:1) after 1 h showed already complete consumption of starting material to give one new spot. On further standing at room temperature overnight no change of the composition was detected. The reaction mixture was washed with 5% $NaHCO_3$ and dried ($MgSO_4$). After evaporation of the volatiles the residue was subjected to column chromatography (EtOAc:hexanes, 2:3) to give 2.0 g (54%) of **21** as a beige solid; R_f 0.34 (EtOAc:hexanes, 2:1); CIMS (100 eV), *m/z* (relative intensity) 438 ($[M+1]^+$, 4), 171 ($[C_{11}H_{11}N_2]^+$, 100), 91 ($[C_7H_7]^+$, 62), 57 ($[C_4H_9]^+$, 34); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (br s, 1H, indole NH), 7.39 (slightly br d, 1H, J = 6.7 Hz, C(8)H), 7.35 (slightly br d, 1H, J = 7.0 Hz, C(5)H), 7.29–7.19 (m, 5H, PhH_5), 7.01 (dt, 1H, J = 6.8 Hz and J = 0.8 Hz, C(6)H), 6.93 (dt, 1H, J = 6.5 Hz and J = 0.8 Hz, C(7)H), 6.25 (br d, 1H, BOCNH), 4.40 (br s, 1H, C(1)H), 4.33–4.28 (m, 1H, C(10)H), 3.64 (s, 2H, SCH_2Ph), 3.28–3.23 (m, 1H, C(3)H), 2.85–2.58 (m, 5H, C(3)H, $C(4)H_2$ and $C(11)H_2$), 1.30 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.3 C=O, 138.6 C(14), 135.8 C(8a), 134.1 C(9a), 129.0 C(15), 128.1 C(16), 126.9 C(4b), 126.6 C(17), 120.4 C(7), 118.1 C(6), 117.2 C(5), 110.8 C(8), 109.1 C(4a), 78.0 C(Me)3, 53.8 C(10), 51.8 C(1), 42.8 C(3), 35.0 C(13), 32.3 C(11), 28.1 $(CH_3)_3$, 22.1 C(4); Anal. calcd for $C_{25}H_{31}N_3O_2S$: C, 68.62; H, 7.14; N, 9.60; S, 7.33. Found: C, 68.40; H, 6.83; N, 9.57; S, 7.13.

***Rac-threo*-1-[1-(N-(*tert*-butyloxycarbonyl)amino)-2-(carboxymethylenethio)]-1,2,3,4-tetrahydro-β-carboline (22)**. Liquid ammonia (100 mL) was condensed into a stirred

and cooled (−30 °C) solution of **21** (1.63 g, 4.0 mmol) in THF (10 mL). To the resulting solution sodium (0.15 g, 6.5 mmol) was added portionwise each time resulting in a deep-blue color for a short moment. After addition of the last portion of sodium, ammonium chloride (1 g) was added. The cooling bath was removed and ammonia was allowed to evaporate, followed by removal of THF under reduced pressure. The residue was subjected to column chromatography (CH_2Cl_2:MeOH:Et_3N, 95:5:0.1) to give 1.14 g (82%) of the thiol as a colorless oil; R_f 0.10 (CH_2Cl_2:MeOH, 95:5); EIMS (70 eV), *m/z* (relative intensity) 348 ($[M+1]^+$, 4), 171 ($[C_{11}H_{12}N_2]^+$, 100), 144 ($[C_{10}H_{10}N]^+$, 9) 57 ($[C_4H_9]^+$, 26); ^{1}H NMR (90 MHz) δ 8.57 (br s, 1H, indole NH), 7.53–7.42 (m, 1H, C(8)H), 7.34–7.00 (m, 3H, C(5)-C(7)H_3), 5.33 (br d, 1H, BOCNH), 4.57 (br s, 1H, C(1)H), 4.34–4.06 (m, 1H, C(10)H), 3.49–2.62 (m, 6H, C(11)H_2 and C(3)-C(4)H_4), 1.74 (br s, 2H, NH and SH), 1.27 (s, 9H, $C(CH_3)_3$).

The heretofore described thiol (0.90 g, 2.59 mmol) was dissolved in benzene (10 mL) and DBU (0.50 g, 3.28 mmol) and methyl chloroacetate (0.21 mL, 0.26 g, 2.40 mmol) were added. After stirring at room temperature for 15 min, work up was accomplished by evaporation of the volatiles in vacuo. The residue was dissolved in EtOAc (100 mL) and washed with two portions of 5% ammonium chloride. After drying ($MgSO_4$) the solvent was evaporated to give the crude product which was further purified by column chromatography (CH_2Cl_2:MeOH, 97:3) to give 0.94 g (87%) of the methyl ester as a colorless oil; R_f 0.24 (CH_2Cl_2:MeOH, 95:5), R_f 0.51 (CH_2Cl_2:MeOH, 9:1): EIMS (70 eV), *m/z* (relative intensity) 420 ($[M+1]^+$, 0.4), 171 ($[C_{11}H_{12}N_2]^+$, 100), 144 ($[C_{10}H_{10}N]^+$, 14) 57 ($[C_4H_9]^+$, 20); ^{1}H NMR (90 MHz) δ 8.74 (br s, 1H, indole NH), 7.53–7.00 (m, 4H, C(5)-C(8)H_4), 5.37 (br d, 1H, J = 9.0 Hz, BOCNH), 4.52 (br d, 1H, $J \approx 3$ Hz, C(1H), 4.47–4.15 (m, 1H, C(10)H), 3.78 (s, 3H, OCH_3), 3.38 (s, 2H, SCH_2CO_2Me), 3.35–2.73 (m, 6H, C(11)H_2 and C(3)-C(4)H_4), 1.87 (br s, NH), 1.31 (s, 9H, $C(CH_3)_3$).

The above obtained methyl ester (0.90 g, 2.15 mmol) was dissolved in dioxane (20 mL) and 7 mL of 2 N NaOH solution was added. The resulting two-phase system was stirred efficiently overnight. The reaction mixture was neutralized by the addition of satd NH_4Cl (150 mL). The water layer was extracted with three portions of EtOAc. The combined organic phases were dried and the solvents were evaporated in vacuo. The residue was subjected to column chromatography (CH_2Cl_2:MeOH, 93:7) to give 0.66 g (77%) of the acid **22** as a colorless gum; R_f 0.16 (CH_2Cl_2:MeOH, 9:1); EIMS (70 eV), *m/z* (relative intensity) 313 ($[M-C_2H_4O_2S]^+$, 0.5), 244 ($[C_{13}H_{12}N_2OS]^+$, 3), 171 ($[C_{11}H_{12}N_2]^+$, 100), 57 ($[C_4H_9]^+$, 34); ^{1}H NMR (90 MHz) δ 10.7 (extremely br s, 1H, COOH), 8.99 (br s, 1H, indole NH), 7.54–7.44 (m, 1H, C(12)H), 7.36–7.01 (m, 3H, C(9)H-C(11)H_3), 6.88 (br s, 1H, J = 10.0 Hz, BOCNH), 5.33 (br d, 1H, $J \approx 3.5$ Hz, C(1)H), 4.90–4.56 (m, 1H, C(10)H), 4.32-4.04 (br q, 1H, C(1)H), 3.58–2.91 (m, 6H), 2.43 (m, 1H, $J \approx 13$ Hz), 1.23 (s, 9H, $C(CH_3)_3$).

***Rac-cis*-1-[[(*tert*-butyloxy)carbonyl]amino]-5-oxo-1,2,4,-7,8,13b-hexahydro-[1,4]-thiazepino[4′,5′:1,2]pyrido-[3,4-*b*]indole (23).** A solution of **22** (0.51 g, 1.26 mmol) in dry DMF (0.5 mL) was added slowly (20 min) at room temperature to an efficiently stirred solution of TBTU (0.45 g, 1.39 mmol) and DIPEA (0.64 mL, 0.49 g, 3.77 mmol) in DMF (25 mL). Monitoring of the reaction by TLC (CH_2Cl_2:MeOH, 95:5) showed an immediate formation of the product. Work up was accomplished by dilution of the reaction mixture with EtOAc (100 mL) and subsequent washings with 5% $KHSO_4$, 5% $NaHCO_3$, and satd NH_4Cl. After drying ($MgSO_4$) the volatiles were evaporated in vacuo followed by purification of the residue by column chromatography (CH_2Cl_2:MeOH, 99:1) to give 0.40 g (82%) of **23** as a white solid; R_f 0.32 (CH_2Cl_2:MeOH, 95:5); mp 154–159 °C; EIMS (70 eV) exact mass calcd for $C_{20}H_{25}N_3O_3S$ *m/z*, 387.1611 ($[M]^+$). Found: 387.1613; *m/z* (relative intensity) 387 ($[M]^+$, 3), 244 ($[C_{13}H_{12}N_2OS]^+$, 100), 169 ($[C_{11}H_9N_2]^+$, 77), 57 ($[C_4H_9]^+$, 14); ^{1}H NMR (400 MHz) δ 9.23 (br s, 1H, indole NH), 7.54–7.44 (m, 1H, C(12)H), 7.19–6.91 (m, 3H, C(9)-C(11)H_3), 5.35 (br d, 1H, $J \approx 9$ Hz, BOCNH), 5.13 (sl br s, 1H, C(13b)H), 5.06–4.79 (m, 2H, C(1H) and C(7)H), 3.76 and 3.33 (AB, 2H, J_{AB} = 14.0 Hz, C(4)H_2), 3.36–3.04 (m, 2H), 3.00–2.58 (m, 3H), 1.28 (s, 9H, $C(CH_3)_3$); Anal. calcd for $C_{20}H_{25}N_3O_3S3/4H_2O$: C, 59.90; H, 6.66; N, 10.48; S, 7.99. Found: C, 59.96; H, 6.40; N, 10.45; S, 7.50.

***Rac-cis*-1-[amino]-1,2,4,5,7,8,13b-heptahydro-[1,4]-thiazepino[4′,5′:1,2]pyrido[3,4-*b*]indole (5).** To a cooled (−20 °C) and stirred solution of **23** (0.32 g, 0.83 mmol) in CH_2Cl_2 (25 mL) DIBAL (4 mL of a 1 M solution in CH_2Cl_2) was added in 10 min. The temperature was allowed to warm up to −5 °C. After 1 h the excess reagent was quenched by the addition of a 10% citric acid solution (20 mL). The resulting two-phase system was stirred until two clear layers appeared. Brine (50 mL) was added and the pH was adjusted to 5 with 4 N NaOH. The water layer was additionally extracted with three portions THF and the combined organic layers were dried ($MgSO_4$). After evaporation of the volatiles in vacuo the residue was subjected to column chromatography (CH_2Cl_2:MeOH, 98.5:1.5) to give 0.16 g (52%) of the amine as a white solid; R_f 0.63 (CH_2Cl_2:MeOH, 95:5); ^{1}H NMR (90 MHz) δ 8.37 (br s, 1H, indole NH), 7.52–7.41 (m, 1H, C(12)H), 7.31–6.98 (m, 3H, C(9)-C(11)H_3), 5.52 (br d, 1H, J = 9.0 Hz, BOCNH), 4.58–4.36 (m, 1H, C(1)H), 4.26 (br d, 1H, $J \approx 2$ Hz, C(13b)H), 3.31–2.65 (m, 10H, C(2)H_2, C(4)-C(5)H_2 and C(7)-C(8)H_4), 1.14 (s, 9H, $C(CH_3)_3$). Also 0.64 g (20%) of the starting material **23** was recovered.

Removal of the BOC protective group was carried out as follows: to a stirred solution of the above described compound (0.16 g, 0.43 mmol) and trimethylsilyl chloride (0.11 mL, 93 mg, 0.86 mmol) in dry acetonitrile (20 mL) was added NaI (0.13 g, 0.87 mmol), to give

immediate precipitation of NaCl. The reaction mixture was stirred overnight followed by the addition of satd $NaHCO_3$ (5 mL). Acetonitrile was removed in vacuo. The aqueous residue was extracted with three portions of CH_2Cl_2 (25 mL). After drying ($MgSO_4$) the volatiles were evaporated in vacuo. The residue was subjected to column chromatography (MeOH:CH_2Cl_2, 5:95) to give 97 mg (83%) of **5** as an off-white solid; R_f 0.12 (MeOH:CH_2Cl_2, 5:95); ^{1}H NMR (400 MHz) δ 8.90 (br s, 1H, indole NH), 7.47 (br d, 1H, J = 8.0 Hz, C(9)H), 7.26 (br d, 1H, $J \approx 8$ Hz, C(12)H), 7.13 (dt, 1H, $J \approx 8$ Hz and J = 2.0 Hz, C(10)H), 7.08 (dt, 1H, $J \approx 8$ Hz and J = 2.0 Hz, C(11)H), 4.04 (br s, 1H, $J \approx 2$ Hz and $J < 2$ Hz, C(13b)H), 3.20 (ddd, 1H, J = 6.0 Hz, J = 4.0 Hz and J = 2.0 Hz, C(1)H), 3.16–3.07 (m, 2H), 2.94 (dd, 1H, J = 4.0 Hz and J = 15.0 Hz, C(2)H), 2.86 (ddd, 1H, J = 14.0 Hz, J = 8.0 Hz and J = 5.0 Hz), 2.81–2.73 (m, 4H), 2.63 (ddd, 1H, J = 14.0 Hz, J = 6.5 Hz and J = 4.0 Hz), 2.60 (dd, 1H, J = 15.0 Hz and J = 6.0 Hz, C(2)H), 2.02 (very br s, 2H, NH_2); ^{13}C NMR (100 MHz) δ 136.43 C(12a), 133.72 C(13a), 126.44 C(8b), 121.60 C(11), 119.35 C(10), 117.90 C(9), 111.62 C(8b), 111.06 C(12), 62.73 C(13b), 55.95 C(1), 53.37 C(7), 38.81 C(5), 34.4 C(2), 31.8 C(4), 21.58 C(8); Anal. calcd for $C_{15}H_{19}N_3S_1$: C, 65.90; H, 7.01; N, 15.38; S, 11.71. Found: C, 65.74; H, 6.97; N, 15.32; S, 11.61.

***Rac-threo*-1-[1-amino-2-(methylthio)-ethyl]-2-methoxy-1,2,3,4-tetrahydro-β-carboline (6) and *rac-erythro*-1-[1-amino-2-(methylthio)-ethyl]-2-methoxy-1,2,3,4-tetrahydro-β-carboline (7).** To a stirred solution of N_b-methoxy-tryptamine **24**[10] (1.48 g, 7.80 mmol) and *rac*-*S*-methyl-*N*-BOC-cysteinal **25**[11] in CH_2Cl_2 (100 mL) TFA (0.5 mL) was added. Monitoring of the reaction by TLC (CH_2Cl_2:MeOH, 99:1) showed complete consumption of the starting materials after 15 min (three new spots). A small aliquot was worked up by washing with 10% Na_2CO_3 followed by drying ($MgSO_4$). NMR analysis showed a 1:1 mixture of the spiro compound(s) **26** and both *threo*/*erythro* β-carbolines **27**/**28** (see the chemistry paragraph). After allowing the reaction mixture to stand for two days at room temperature only one spot was visible on TLC (EtOAc:hexanes, 1:1). Work up was accomplished by washing of the reaction mixture with satd $NaHCO_3$. After drying ($MgSO_4$) the solvent was evaporated in vacuo. The residue was subjected to column chromatography (EtOAc:hexanes, 1:4) to give 1.84 g (60%) of the mixture of **27** and **28**; R_f 0.58 (EtOAc:hexanes, 1:1); both diastereomers appeared as a near single spot.

The stirred solution of the mixture of both diastereomeric β-carbolines **27** and **28** (1.77 g, 4.53 mmol) in dry acetonitrile (50 mL) was added trimethylsilyl chloride (1.15 mL, 0.99 g, 9.10 mmol) and NaI (0.88 g, 5.77 mmol), to give immediate precipitation of NaCl. The reaction mixture was stirred for 4 h followed by the addition of satd $NaHCO_3$ (15 mL). Acetonitrile was removed in vacuo. The aqueous residue was extracted with EtOAc (50 mL). After drying ($MgSO_4$) the volatiles were evaporated in vacuo. 90 MHz ^{1}H NMR analysis of the crude residue revealed a *threo* (**6**):*erythro* (**7**) ratio of 65:35. The residue was subjected to column chromatography (MeOH:$CHCl_3$, 1:99) to give 0.50 g (38%) of **6** (*threo*) as a light-yellow syrup; R_f 0.28 (MeOH:CH_2Cl_2, 5:95); EIMS (70 eV) exact mass calcd for $C_{15}H_{21}N_3OS$ m/z, 291.1405 ($[M]^+$). Found: 291.1402; m/z (relative intensity) 291 ($[M]^+$, 0.5), 201 ($[C_{12}H_{13}N_2O]^+$, 100), 170 ($[C_{11}H_{10}N_2]^+$, 67); ^{1}H NMR (400 MHz) δ 9.84 (br s, 1H, indole NH), 7.45 (d, 1H, J = 7.7 Hz, C(8)H), 7.32 (d, 1H, J = 8.0 Hz, C(5)H), 7.13 (t, 1H, J = 7.4 Hz, C(6)H), 7.06 (t, 1H, J = 7.4 Hz, C(7)H), 4.00 (d, 1H, J = 3.5Hz, C(1)H), 3.78 (dt, 1H, J = 11.4 H and J = 2.8 Hz, C(3)H), 3.65–3.56 (m, 1H, C(10)H), 3.61 (s, 3H, OCH_3), 2.97 (dd, 1H, J = 13.5 Hz and J = 2.1 Hz, C(11)H), 2.92–2.77 (m, 3H, C(3)H and $C(4)H_2$), 2.08 (s, 3H, SCH_3), 2.00 (dd, 1H, J = 13.5 Hz and J = 11.4 Hz, C(11)H), 1.77 (br s, 2H, NH_2); ^{13}C NMR (100 MHz) δ 135.9 C(8a), 132.0 C(9a), 126.0 C(4b), 121.3 C(7), 119.0 C(6), 117.9 C(5), 111.1 C(8), 108.7 C(4a), 67.1 C(10), 60.6 C(15), 52.6 C(3), 50.1 C(1), 35.9 C(11), 20.9 C(4), 15.3 C(13), together with 0.40 g (30%) of **7** (*erythro*) as light-yellow syrup; R_f 0.20 (MeOH:CH_2Cl_2, 5:95); EIMS (70 eV) exact mass calcd for $C_{15}H_{21}N_3OS$ m/z, 291.1405 ($[M]^+$). Found: 291.1405; m/z (relative intensity) 291 ($[M]^+$, 0.6), 201 ($[C_{12}H_{13}N_2O]^+$, 100), 170 ($[C_{11}H_{10}N_2]^+$, 73); ^{1}H NMR (400 MHz, recorded at 58 °C) δ 9.32 (br s, 0.8 H, indole NH), 9.12 (br s, 0.2 H, indole NH), 7.46 (d, 1H, J = 7.7 Hz, C(8)H), 7.29 (d, 1H, J = 8.0 Hz, C(5)H), 7.10 (t, 1H, J = 7.3 Hz, C(6)H), 7.04 (t, 1H, J = 7.3 Hz, C(7)H), 3.93 (d, 0.8 H, J = 8.5 Hz, C(1)H), 3.73 (s, 0.9H, OCH_3), 3.65 (dd, 0.3H, J = 7.3 Hz and J = 5.0 Hz, C(10H), 3.58–3.54 (m, 0.3H), 3.56 (s, 2.1 H,)CH_3), 3.47–3.38 (m, 0.9H), 3.29–3.22 (m, 0.8H), 3.18–3.12 (m, 1.5 H), 2.90–2.61 (m, 3.1H), 2.14 and 2.12 (2*s, 3H, SCH_3), 1.69 (br s, 2H, NH_2); ^{13}C NMR (100 MHz) δ 136.0 C(8a), 132.6 C(9a), 127.0 C(4b), 121.5 C(7), 119.1 C(6), 118.1 C(5), 111.0 C(8), 108.4 C(4a), 65.4 C(10), 59.2 C(15), 53.4 C(1), 48.3 C(3), 41.9 C(11), 17.7 C(4), 16.0 C(13).

Dimethyl *N*-trityl-D-glutamate (29). To cooled (0 °C) dry methanol (35 mL) was added (by stirring) thionyl chloride (12.5 mL, 7.7 g, 64 mmol) over a period of 30 min. D-Glutamic acid (5 g, 34 mmol) was added to the resulting solution in one portion. The resulting white suspension became a clear solution after ca. 1 h. After standing of the reaction mixture overnight the volatiles were evaporated in vacuo. The residue was dissolved in methanol (2.5 mL) and poured into ether:hexanes (30 mL, 1:1). The product was collected by filtration and dried (KOH), to yield 7.0 g (96%) of (*D*)-HCl-Glu(OMe)-OMe as a white crystalline solid, which was then dissolved in dry DMF (40 mL). To this stirred solution, triethylamine (9.1 mL, 6.6 g, 66 mmol) and trityl chloride (9.1 g, 33 mmol) were added. After stirring of the reaction overnight the volatiles were evaporated in vacuo. The residue was dissolved in EtOAc (50 mL) and successively washed with 10% citric acid, satd $NaHCO_3$ and brine. After drying ($MgSO_4$) the solvent was evaporated in vacuo to yield 12.7 g (93%) of **29** as a white solid; R_f 0.53 (EtOAc:hexanes, 1:2); ^{1}H NMR (90 MHz) δ 7.56–7.09 (m, 16H, 3*C_6H_5

and NH), 3.70 (s, 3H, γ-OCH_3), 3.58–3.31 (m, 1H, α-H), 3.16 (s, 3H, α-OCH_3), 2.76–2.13 (m, 4H, CH_2CH_2).

Methyl-(*R*)-5-hydroxy-2-tritylamino-pentanoate (30). To a cooled (0 °C) solution of **29** (5.0 g, 12.0 mmol) in dry ether (100 mL) employing flame-dried glass equipment under an argon atmosphere was added $LiAlH_4$ (0.36 g, 9.6 mmol) in three portions over a period of 10 min. The reaction was monitored by TLC (EtOAc:hexanes, 1:1). After consumption of the starting material (30 min) NaOH (5 mL of a 1 M solution) was added cautiously and the resulting grey suspension was stirred until a white color appeared. The salts were removed by filtration over hyflo and after drying ($MgSO_4$) the solvent was evaporated in vacuo. The residue was subjected to column chromatography (EtOAc:hexanes, 1:1) to yield 3.94 g (85%) of **30** as a white foam; R_f 0.29 (EtOAc:hexanes, 1:1); $[\alpha]_D^{22}$ −53.0 (*c* 2.85, MeOH); ^{1}H NMR (90 MHz) δ 7.56–7.16 (m, 16H, 3*C_6H_6 and NH), 3.64 (t, 2H, *J* = 6.0 Hz, $HOCH_2$), 3.39 (t, 1H, *J* = 5.9 Hz, α-H), 3.18 (s, 3H, $COOCH_3$), 2.28 (very br s, 1H, exchangeable, OH), 1.96–1.40 (m, 4H, CH_2CH_2); CIMS (70 eV), *m/z* (relative intensity) 390 ($[M+1]^+$, 1), 243 ($[CPh_3]^+$, 100), 146 ($M\text{-}CPh_3]^+$, 3.5).

Methyl-(*R*)-5-methylsulfonyloxy-2-tritylamino-pentanoate (31). To a cooled (−20 °C) and stirred solution of **30** (0.5 g, 1.29 mmol) and triethylamine (0.54 mL, 0.39 g, 3.86 mmol) in ether (20 mL) methanesulfonyl chloride (0.25 mL, 0.37 g, 2.57 mmol) was gradually added (over a period of 20 min). After additionally stirring for 15 min, the salts were removed by filtration over hyflo. The resulting solution was then washed with 10% citric acid, satd $NaHCO_3$, and brine. After drying ($MgSO_4$) the solvent was evaporated in vacuo to yield 0.6 g (99%) of **31** as a colorless oil, which decomposed at room temperature. Storage, however, was possible for a few days in the refrigerator ($T < -20$ °C); R_f 0.15 (EtOAc:hexanes, 1:2); ^{1}H NMR (90 MHz) δ 7.54–7.15 (m, 16H, 3*C_6H_6 and NH), 4.31–4.18 (m, 2H, *J* = 6.0 Hz, $MsOCH_2$), 3.34–3.31 (m, 1H, α-H), 3.18 (s, 3H, $COOCH_3$), 3.00 (s, 3H, H_3CSO_3), 1.89–1.76 (m, 4H, CH_2CH_2).

***Rac*-methyl-5-{*N*-[2-(1H-indol-3-yl)-ethyl]-*N*-(2-trimethylsilylethyloxycarbonyl)-aminooxy}-2-(tritylamino)-pentanoate (33).** NaH (35 mg of a 80% oil dispersion, 1.18 mmol) was added in two portions to a stirred solution of **32** (340 mg, 1.07 mmol) in freshly distilled DME (20 mL) employing flame-dried glass equipment under an argon atmosphere. The suspension was stirred until a clear solution appeared (10–30 min) (hydrogen gas evolved). This solution was added dropwise to a stirred solution of **31** (500 mg, 1.07 mmol) in freshly distilled DME (50 mL) at 0 °C. After additional stirring overnight at room temperature EtOAc (50 mL) was added and the reaction mixture was subsequently washed with water and satd NH_4Cl. The organic layer was dried ($MgSO_4$) and the solvent was evaporated in vacuo. The residue was subjected to column chromatography (EtOAc:hexanes, 1:2) to give 740 mg (67%) of **33** as an oil; R_f 0.28 (EtOAc:hexanes, 1:2); CIMS (70 eV), *m/z* (relative intensity) 243 ($[CPh_3]^+$, 29), 144 ($[C_{10}H_{10}N]^+$, 23), 130 ($[C_9H_8N]^+$, 29), 73 ($[Si(CH_3)_3]^+$, 100); ^{1}H NMR (100 MHz) δ 8.02 (br s, 1H, indole NH), 7.71–6.96 (m, 20H, 3*C_6H_5, indole C(2)H and C(4)-C(7)H4), 4.18–3.66 (m, 6H, H_2CNOCH_2 and OCH_2CH_2Si), 3.53–3.29 (m, 1H, α-H), 3.21–3.01 (m, 2H, indole C(3)CH_2), 3.13 (s, 3H, $COOCH_3$), 1.96–1.62 (m, 3H, $NOCH_2CH_2CHH$), 1.33–1.18 (m, 1H, $NOCH_2CH_2CHH$), 1.00–0.76 (m, 2H, $SiCH_2$), 0.00 (s, 9H, $Si(CH_3)_3$).

***Rac*-methyl-2-amino-5-{*N*-[2-(1H-indol-3-yl)-ethyl]-*N*-(2-trimethylsilylethyloxycarbonyl)-aminooxy}-pentanoate (34).** To **33** (0.33 g, 0.48 mmol) in 2,2,2-trifluoroethanol:water (9:1, 10 mL) was added 0.1 N HCl in 2,2,2-trifluoroethanol:water (9:1) in 100 μL portions until the pH remained ca. 3.5. After additional stirring for 1 h the reaction mixture was neutralized with satd $NaHCO_3$ and dichloromethane (50 mL) was added. The organic layer was washed with brine and dried ($MgSO_4$). After evaporation of the volatiles in vacuo the residue was subjected to column chromatography ($MeOH:CH_2Cl_2:Et_3N$, 1:99:0.05) to yield 150 mg (70%) of the amine as a colorless oil; R_f 0.37 ($MeOH:CH_2Cl_2$, 1:9); ^{1}H NMR (90 MHz) δ 8.36 (br s, 1H, indole NH), 7.67–7.56 (m, 1H, indole C(7)H), 7.38–6.99 (m, 4H, indole C(2)H and C(4)-C(6)H_3), 4.16–3.97 (m, 2H, $SiCH_2CH_2$), 3.93–3.68 (m, 5H, H_2CNOCH_2 and α-H), 3.70 (s, 3H, $COOCH_3$), 3.15–2.97 (m, 2H, indole C(3)CH_2), 2.11–1.58 (m, 6H, $NOCH_2CH_2CH_2$ and NH_2), 0.96–0.76 (m, 2H, $SiCH_2$), 0.00 (s, 9H, $Si(CH_3)_3$). The amine (1.23 g, 2.74 mmol) was dissolved in dichloromethane (15 mL) together with di-*tert*-butyl dicarbonate (0.65 g, 2.98 mmol) and five drops of triethylamine. After 2 h the volatiles were evaporated in vacuo and the residue was subjected to column chromatography (EtOAc:hexanes, 1:3) to yield 1.38 g (92%) of of the BOC protected compound as a colorless oil: R_f 0.53 (EtOAc:hexanes, 1:1); CIMS (70 eV), *m/z* (relative intensity) 549 ($[M]^+$, 5), 130 ($[C_9H_8N]^+$, 100), 73 ($[C_3H_9Si]^+$, 73), 57 ($[C_4H_9]^+$, 42); ^{1}H NMR (90 MHz) δ 8.14 (br s, 1H, indole NH), 7.66–7.57 (m, 1H, indole C(7)H), 7.40–7.00 (m, 4H, indole C(2)H and C(4)-C(6)H_3), 5.11 (br d, 1H, *J* = 8.2 Hz, HNBOC), 4.41–3.71 (m, 7H, H_2CNOCH_2, $SiCH_2CH_2$ and α-H), 3.71 (s, 3H, $COOCH_3$), 3.16–2.98 (m, 2H, indole C(3)CH_2), 2.04–1.56 (m, 4H, $NOCH_2CH_2CH_2$), 1.44 (s, 9H, $C(CH_3)_3$), 0.93–0.76 (m, 2H, $SiCH_2$), 0.00 (s, 9H, $Si(CH_3)_3$). To a suspension of the BOC-protected compound (1.38, 2.51 mmol), Bu_4NCl (2.1 g, 7.6 mmol) and $KF{\cdot}2H_2O$ (950 mg, 10.1 mmol) in dry acetonitrile (50 mL) was stirred at 45 °C for 10 h. The solvent was evaporated in vacuo. The residue was dissolved in EtOAc and subsequently washed with water and satd NH_4Cl. The organic layer was dried ($MgSO_4$) and the solvent was evaporated in vacuo. The residue was subjected to column chromatography (EtOAc:hexanes, 1:1) to yield 0.96 g (95%) of **34** as a colorless oil; R_f 0.23 (EtOAc:hexanes, 1:1); CIMS (70 eV), *m/z* (relative intensity) 406 ($[M+1]^+$, 4), 144 ($[C_{10}H_{10}N]^+$, 37), 130 ($[C_9H_8N]^+$, 100), 57 ($[C_4H_9]^+$, 69); ^{1}H NMR (90 MHz) δ

8.08 (br s, 1H, indole NH), 7.71–7.53 (m, 1H, indole C(7)H), 7.43–7.02 (m, 4H, indole C(2)H and C(4)-C(6)H_3), 5.51 (very br s, ONH), 5.14 (br d, 1H, J = 8.0 Hz, HNBOC), 4.42–4.09 (m, 1H, α-H), 3.76–3.64 (m, 2H, $NOCH_2$), 3.73 (s, 3H, $COOCH_3$), 3.33–3.13 (m, 2H, CH_2NO), 3.13–2.91 (m, 2H, indole C(3)CH_2), 1.89–1.60 (m, 4H, $NOCH_2CH_2CH_2$), 1.47 (s, 9H, $C(CH_3)_3$).

***Rac-trans*-1-[[(*tert*-butyloxy)carbonyl]amino]-1,2,3,4,7,-8,13,13b-octahydro-[1,2]-oxazepino-[2′,3′:1,2]pyrido-[3,4-*b*]indole (36) and *cis* (*rac*)-1-[[(*tert*-butyloxy)carbonyl]-amino]-1,2,3,4,7,8,13,13b-octahydro-[1,2]-oxazepino-[2′,3′:1,2]pyrido[3,4-*b*]indole (35) followed by removal of the BOC group in 35 to give *Rac-cis*-1-amino-1,2,3,4,7,8,13,13b-octahydro-[1,2]-oxazepino-[2′,3′:1,2]-pyrido[3,4-*b*]indole (8).** The cyclization reaction was carried out employing flame-dried glass equipment under an argon atmosphere. To a cooled (−75 °C) stirred solution of **34** (820 mg, 2.02 mmol) in dry dichloromethane (100 mL) was added DIBAL (5.0 mL of a 1 M solution in dichloromethane) over a period of 20 min. After completion of the reaction (15 min), as was indicated by TLC (EtOAc:hexanes, 1:4), TFA (0.5 mL) was added. The reaction mixture was allowed to warm to room temperature and then 10% citric acid (20 mL) was added. The organic layer was washed with water and satd $NaHCO_3$:brine, 1:1, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was subjected to column chromatography (EtOAc:hexanes, 1:6) to give 510 mg (71%) of **36** as a white crystalline solid; R_f 0.29 (EtOAc:hexanes, 1:4); mp 173–175 °C (EtOAc:ether); ^{1}H NMR (400 MHz) (to sharpen up the broadened spectrum recorded at 57 °C) δ 9.42 (br d, 1H, indole NH), 7.42 (d, 1H, J = 7.8 Hz, C(12)H), 7.33 (d, 1H, J = 8.0 Hz, C(9)H), 7.11 (t, 1H, J = 7.4 Hz, C(10)H), 7.04 (t, 1H, J = 7.4 Hz, C(11)H), 5.08 (br d, 1H, J = 9.2 Hz, BOCNH), 4.23–4.17 (m, 1H, C(1)Hα), 3.97–3.91 (m, 3H, C(13b)Hβ and C(4)H_2), 3.62–3.52 (m, 1H, C(7)Hβ), 3.03–2.94 (M, 2H, C(7)Hα and C(8)Hβ), 2.76–2.67 (m, 1H, C(8)Hα), 2.21–2.15 (m, 1H, C(3)H), 2.02–1.94 (m, 1H, C(3)H), 1.83–1.73 (m, 2H, C(2)H_2), 1.52 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (100 MHz) (to sharpen up the broadened spectrum recorded at 57 °C) δ 156.29 C=O, 136.65 C(12a), 133.09 C(13a), 126.65 C(8b), 121.47 C(11), 119.22 C(10), 118.07 C(9), 111.31 C(12), 107.77 C(8a), 80.50 CMe3, 72.18 C(4), 71.10 C(13b), 55.34 C(1), 53.91 C(7), 30.28 C(3), 28.49 (CH3)3, 26.65 C(2), 20.52 C(8); Anal. calcd for $C_{20}H_{27}N_3O_3$: C, 67.20; H, 7.61; N, 11.76. Found: C, 67.18; H, 7.39; N, 11.58 together with 9.2 mg (1.3%) of **35** as a white solid; R_f 0.22 (EtOAc:hexanes, 1:4); ^{1}H NMR (400 MHz) δ 8.38 (br s, 1H, indole NH), 7.44 (d, 1H, J = 7.3 Hz, C(12)H), 7.26 (d, 1H, J = 7.4 Hz, C(9)H), 7.10 (t, 1H, J = 7.2 Hz, C(10)H), 7.05 (t, 1H, J = 7.3 Hz, C(11)H), 4.97 (br d, 1H, J = 9.2 Hz, BOCNH), 4.50 (br s, 1H, C(1)Hα), 4.15–4.06 (m, 2H, C(13b)Hα and C(4)H), 3.75 (very br s, 1H, C(4)H), 3.52 (very br s, 1H, C(7)Hβ), 3.11–2.92 (br m, 2H, C(7)Hα and C(8)Hβ), 2.81–2.78 (br m, 1H, C(8)Hα), 2.11–1.98 (br m, 3H, C(2)H and C(3)H2), 1.75 (br s, 1H, C(2)H), 1.17 (s, 9H, $C(CH_3)_3$). Both **35** and **36** are near racemates.

8. To a stirred solution of **35** (8.9 mg, 0.025 mmol) in dry acetonitrile (1 mL) was added NaI (11.2 mg, 0.075 mmol) together with chlorotrimethylsilane (1 mL of a 0.074 M solution in acetonitrile). After stirring for 2 h MeOH:Et_3N (1:1, 0.5 mL) was added to quench the formed HI and the volatiles were evaporated in vacuo. The residue was subjected to column chromatography (MeOH:CH_2Cl_2:Et_3N, 3:97:0.05) to yield 19 mg of contaminated **8**. NMR analysis showed a mixture of Et_3NHI and **8** that could not be separated by extraction (CH_2Cl_2:water) or straight phase (Si60H) chromatography (*in future attempts quenching of HI should be performed with satd $NaHCO_3$*). The salt was removed by preparative reversed phase column chromatography (RP-8, water:methanol, 7:3), followed by another Si60H column (MeOH:CH_2Cl_2:Et_3N, 2:98:0.05) to yield 4,1 mg (64%) of **8** as a white solid; R_f 0.24 (MeOH:CH_2Cl_2, 1:9); $[\alpha]_D^{22}$ −2.9 (*c* 2.05, MeOH:CH_2Cl_2, 1:1); EIMS (70 eV) exact mass calcd for $C_{15}H_{19}N_3O$ *m/z*, 257.1522 ($[M]^+$). Found: 257.1521; CIMS (70 eV), *m/z* (relative intensity) 258 ($[M+1]^+$, 100), 241 ($[M-NH_2]^+$, 8), 171 (29), 144 ($[C_{10}H_{10}N]^+$, 9), 130 ($[C_9H_8N]^+$, 5); ^{1}H NMR (400 MHz) δ 9.07 (br d, 1H, indole NH), 7.46 (d, 1H, J = 7.1 Hz, C(12)H), 7.44 (d, 1H, J = 8.2 Hz, C(9)H), 7.17 (t, 1H, J = 7.3 Hz, C(10)H), 7.11 (t, 1H, J = 7.3 Hz, C(11)H), 3.99 (br s, 1H, C(13b)Hα), 3.86–3.80 (br m, 1H, C(1)Hα), 3.73–3.59 (m, 2H, C(4)H_2), 3.38 (br d, 1H, J = 6.6 Hz, C(7)Hβ), 2.93 (dt, 1H, J = 3.5Hz and J = 8.8 Hz, C(7)Hα), 2.80 (br t, 1H, J = 13.4 Hz, C(8)Hβ), 2.74 (br dt, 1H, J = 2.3 Hz and J = 12.6 Hz, C(8)Hα), 1.94 (m, 1H), 1.76–1.60 (m, 3H).

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References

1. Davidson, B. S. *Chem. Rev.* **1993**, *93*, 1771.

2. (a) Rinehart Jr., K. L.; Kobayashi, J.; Harbour, C. G.; Gilmore, J.; Mascal, M.; Holt, T. G.; Shield, L. S.; Lafargue, F. *J. Am. Chem. Soc.* **1987**, *109*, 3378. (b) Blunt, J. W.; Lake, R. J.; Munro, M. H. G.; Toyokuni, T. *Tetrahedron Lett.* **1987**, *28*, 1825. (c) Lake, R. J.; Brennan, M. M.; Blunt, J. W.; Munro, M. H. G.; Pannell, L. K. *Tetrahedron Lett.* **1988**, *29*, 2255. (d) Lake, R. J.; Blunt, J. W.; Munro, M. H. G. *Aust. J. Chem.* **1989**, *42*, 1201.

3. (a) Hermkens, P. H. H. Thesis, Catholic University of Nijmegen, 1990. (b) Hermkens, P. H. H.; Maarseveen, J. H. van; Ottenheijm, H. C. J.; Kruse, C. G.; Scheeren, J. W. *J. Org. Chem.* **1990**, *55*, 3998. (c) Nakagawa, M.; Liu, J. J.; Hino, T. *J. Am. Chem. Soc.* **1989**, *111*, 2721. (d) Liu, J. J.; Nakagawa, M.; Harada, N.; Tsuruoka, A.; Hasegawa, A.; Ma, J.; Hino, T. **1990**, *31*, 229. (e) Still, I. W. J.; Strautmanis, J. *Tetrahedron Lett.* **1989**, *30*, 1041. (f) Still, I. W. J.; Strautmanis, J. *Can. J. Chem.* **1990**, *68*, 1408.

4. (a) Maarseveen, J. H. van; Hermkens, P. H. H.; De Clercq, E.; Balzarini, J.; Scheeren, J. W.; Kruse, C. G. *J. Med. Chem.* **1992**, *35*, 3223. (b) Recently a paper appeared describing an SAR study of some tetracyclic eudistomin desthia carba derivatives: Kurihara, T.; Sakamoto, Y.; Kimura, T.; Ohishi, H.; Harusawa, S.; Yoneda, R.; Suzutani, T.; Azuma, M. *Chem. Pharm. Bull.* **1996**, *44*, 900.

5. Maarseveen, J. H. van; Kruse, C. G.; Scheeren, J. W. *Tetrahedron* **1993**, *49*, 2325.

6. (a) Tietze, L. F.; Schimpf, R.; Wichmann, J. *Chem. Ber.* **1992**, *125*, 2571. (b) Maryanoff, B. E.; Rebarchak, M. C. *Synthesis* **1992**, 1245.

7. Maurer, P. J.; Miller, M. J. *J. Am. Chem. Soc.* **1982**, *104*, 3096.

8. We also successfully used *O*-TBDMS-*N*-Aloc-hydroxylamine in the Mitsunobu reaction-based approach towards *N*-hydroxy-2(3,4-dimethoxyphenyl)ethylamine derivatives from 2-(3,4-dimethoxyphenyl)ethanol. Similar carbamate-protected hydroxylamines have recently also been used for the Mitsunobu reaction-mediated synthesis of *N*-hydroxy-α-amino acids from α-hydroxy acids; Hanessian, S.; Yang, R-Y. *Synlett* **1995**, 633.

9. McNulty, J.; Still, I. W. J. *Synth.Comm.* **1992**, *22*, 979.

10. Dijkstra, G. D. H.; Tulp, M. Th. M.; Hermkens, P. H. H.; Maarseveen, J. H. van; Scheeren, H. W.; Kruse, C. G. *Recl. Trav. Chim. Pays-Bas* **1993**, *112* , 131.

11. Liu, J.-J.; Nakagawa, M.; Hino, T. *Tetrahedron* **1989**, *45*, 7729.

12. Liu, J.-J.; Nakagawa, M.; Ogata, K.; Hino, T. *Chem. Pharm. Bull.* **1991**, *39*, 1672.

13. The NMR data of the *trans* diastereomer **7** were obtained at 58 °C to give a sharp spectrum. At this temperature two conformers existed in a 4:1 ratio. These conformers result from inversion of the N(2) nitrogen atom. Peak-broadening due to a nitrogen inversion process occurring at the NMR timescale is known for cyclic 1,2-oxaza compounds. See: Riddel, F.G. *Tetrahedron* **1981**, *37*, 849.

14. In the literature two successful approaches to the eudistomin desthia carba skeleton have been described (see also ref 15). During our synthetic investigations towards the *cis* desthia carba derivative, Kurihara and coworkers succeeded in the synthesis using an efficient approach. This strategy included the Meisenheimer [2,3]-sigmatropic rearrangement followed by base-induced epimerization, reduction of the obtained double bond, and a Curtius reaction for introduction of the C(1)-amino group to give the desthia carba eudistomin derivative as a racemate in good yields: Kurihara, T.; Sakamoto, Y.; Matsumoto, H.; Kawabata, N.; Harusawa, S.; Yoneda, R. *Chem. Pharm. Bull.* **1994**, *42*, 475 (see also ref 4b).

15. Kirkup, M. P.; Shankar, N. B.; McCombie, S.; Ganguly, A. K. *Tetrahedron Lett.* **1989**, *30*, 6809.

16. We also attempted the synthesis of **22** by selective reduction of the carboxyl group in (*D*)-Trt-Glu(OH)-OMe. (*D*)-Trt-Glu(OH)-OMe was synthesized in 76% yield by selective saponification of the γ-ester in (*D*)-Trt-Glu(OMe)-OMe **21** following a modified literature procedure; Amiard, G.; Heyms, R.; Velluz, L. *Bull. Soc. Chim. Fr.* **1956**, 97. Selective reduction of the terminal carboxyl group with borane dimethylsulfide surprisingly gave Trt-Pro-OMe in a nearly quantitative yield instead of the anticipated γ-alcohol. This can be explained by the formation of a cyclic iminium ion from the intermediate aldehyde, probably catalyzed by the borane reagent. The highly electrophilic iminium ion is then reduced by the borane dimethylsulfide complex to yield the proline derivative. Substitution of the trityl amino protective group by BOC or Cbz protective groups also gave the prolines in nearly quantitative yields.

17. Attempted transformation of the alcohol into the tosylate also gave Trt-Pro-OMe as the main product (see ref 15). Although the tosylate was visible on TLC as an intermediate, it was not possible to optimize the reaction conditions.

18. Dr B. B. Shankar of Schering Plough Research Institute (New Jersey, U.S.A.) is greatly acknowledged for providing the X-ray crystallography and NMR data of several desthia carba eudistomin analogues.

19. (a) De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R. T.; Jones, A. S.; Torrence, P. F.; Shugar, D. *J. Infect. Dis.* **1980**, *5*, 563. (b) De Clercq, E.; Montgomery, J. A. *Antiviral Res.* **1983**, *3*, 17. (c) Balzarini, J.; Naesens, L.; Herdewijn, P.; Rosenberg, I.; Holy, A.; Pauwels, R.; Baba, M.; Johns, D. G.; De Clercq, E. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 332.

20. The conditions used for measuring antiviral activity and antitumor cell activity were totally different. The antiviral tests were performed in stationary cell monolayers. To measure cytostatic activity, exponentially growing proliferating cells were used. Therefore, it is not fair to compare the IC_{50} value for antitumor activity with the MIC_{50} value for antiviral activity. Even if a compound would not exhibit selectivity based on the comparison of the MIC_{50} and IC_{50} values, this does not exclude its potential use as an antiviral agent as it may be perfectly well applicable as a topical drug (i.e., ointments, drops, aerosol, etc.) in the treatment of virus infections of not actively dividing cells (i.e. on the skin, cornea, respiratory epithelium, etc.). In fact, all antiviral agents that are targeted at cellular enzymes (e.g., ribavirin, neplanocin, pyrazofurin, etc.) may be expected to inhibit both virus replication and cell proliferation. Therefore, the MCC:MIC ratio is a proven parameter for the prediction of clinical antiviral efficacy. The exception to the rule is HIV which replicates in actively dividing cells (i.e., T-lymphocytes). Thus compounds that interfere with the proliferation of the T-cells may not show up as selective anti-HIV agents, as these compounds would be active against HIV only at concentrations that inhibit host cell proliferation as well.

21. (a) De Clercq, E.; Balzarini, J.; Torrence, P. F.; Mertes, M. P.; Schmidt, C. L.; Shugar, D.; Barr, P. J.; Jones, A. S.; Verhelst, G.; Walker, R. T. *Mol. Pharmacol.* **1981**, *19*, 321. (b) Balzarini, J.; De Clercq, E.; Torrence, P. F.; Mertes, M. P.; Park, J. S.; Schmidt, C. L.; Shugar, D.; Barr, P. J.; Jones, A. S.; Verhelst, G.; Walker, R. T. *Biochem. Pharmacol.* **1982**, *31*, 1089.

22. (a) Kepers, Y. P.; Pizao, P. E.; Peters, G. J.; Van Ark-Otte, J.; Winograd, B.; Pinedo, H. M. *Eur. J. Cancer.* **1991**, *27*, 897. (b) Boyd, M. R. *Status of the NCI preclinical antitumor drug discovery screen. Principles and practice of oncology.* **1989**, *3*, 1.

23. De Clercq, E.; Murase, J.; Marquez, V. E. *Biochem. Pharmacol.* **1991**, *41*, 1821.

24. Arx, E. v.; Faupel, M.; Brugger, M. J. *J.Chromatogr.* **1976**, *120*, 224.

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