Antiviral and Antitumor Structure-Activity Relationship Studies on Tetracyclic Eudistomines

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The in vitro antiviral and antitumor activities of (-)-debromoeudistomin K (**1a**) and 10 structural analogues (**1b-1j** and **1l**) were evaluated. The synthesis was accomplished with an *intra*molecular Pictet-Spengler condensation reaction as the key step. This examination revealed some structural and stereochemical features that are important for both the antiviral and antitumor activities. The most striking points for activity are the necessity to have the correct natural stereochemistry at both C(1) and C(13b) and the presence of the C(1)-NH_2 substituent. As was revealed before with naturally isolated eudistomins a substituent in the indole ring greatly influences the biological activity. The 5-OMe derivative **1h** shows high potency in both antiviral and antitumor models.

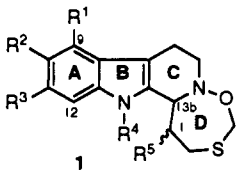
Marine species have been especially important in the field of antiviral compounds, providing some promising leads.[1] Tetracyclic eudistomines **1** (Chart I), which were first isolated out of the colonial tunicate *Eudistoma olivaceum*, possessed very potent antiviral and antitumor activities.[2-5] They belong to the β-carboline class and their most striking structural feature is the 7-membered oxathiazepine D-ring. It has been reported by Rinehart that the activities of eudistomins C, E, K and L against herpes simplex virus-1 (HSV-1), in vitro, are in the range of 25-250 ng/disk.[3] In addition Munro reported high activities for eudistomin K sulfoxide and the indole unsubstituted derivative eudistomin K (debromo) against both HSV-1 and polio vaccine type-1 virus.[4,5] Antitumor activity has also been reported. Eudistomin K gave, in vitro, an IC_{50} for P388 of 0.01 μg/mL. The in vivo assay gave a T/C of 137% at 100 mg/kg. Further in vivo antitumor activities were reported against L1210, A549, and HCT-8 cell lines.[5] No biological activities are known for eudistomin F.

Although two routes toward total synthesis of eudistomines have been developed,[6,7] up to now no structure-activity relationship (SAR) investigation has been published. In this article we report our first results concerning the influence of the stereochemistry at C(1) and C(13b) and of the substituents at C(1), C(10), and N(13) on both the antiviral and the antitumor activity. For this (-)-debromoeudistomin K (**1a**) and 10 structural analogues (**1b-j** and **1l**) were synthesized using the new *intra*molecular Pictet-Spengler condensation recently developed in our laboratory.[6a,8,9] Its usefulness has already been demonstrated by the convenient synthesis of all four debromoeudistomin K stereoisomers **1a-d**,[6a] the C(1) unsubstituted derivative **1g**,[9] and the 10-OMe derivatives **1h,i**.[6a] The synthesis of the remaining derivatives **1e,f** and **1j-l** are described. The inclusion of compound **1a** (debromoeudistomin K) in our biological tests gives the possibility to compare its activity with those reported for the isolated naturally occurring eudistomins.

Chemistry

For the construction of the eudistomin skeleton for the remaining derivatives **1e,f** and **1j-l** the key intermediates are the O-alkylated *N*-oxotryptamine derivatives **12**. Synthesis of these functionalized molecules can be accomplished by nucleophilic coupling of [[2-(trimethylsilyl)ethyl]oxy]carbonyl (Teoc) protected *N*-hydroxytryptamines **7**[8] and **10** with chloromethyl sulfides **6**.

Chart I



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Eudistomin		R^1	R^2	R^3	R^4	R^5	chirality at center: 1	13b
L		H	Br	H	H	NH_2	S	S
K		H	H	Br	H	NH_2	S	S
K(sulfox.)		H	H	Br	H	NH_2	S	S
C		H	OH	Br	H	NH_2	S	S
E		Br	OH	H	H	NH_2	S	S
F		H	OH	Br	H	$NC_2H_4O_2$	S	S
K(debromo)	**1a**	H	H	H	H	NH_2	S	S
	1b	H	H	H	H	NH_2	S	R
	1c	H	H	H	H	NH_2	R	S
	1d	H	H	H	H	NH_2	R	R
	1e	H	H	H	CH_3	NH_2	S	S
	1f	H	H	H	CH_3	NH_2	S	R
	1g	H	H	H	H	H	racemic	
	1h	H	OCH_3	H	H	NH_2	S	S
	1l	H	OCH_3	H	H	NH_2	S	R
	1j	H	H	H	H	OH	S	R
	1k	H	H	H	H	OH	S	S
	1l	H	H	H	H	CH_3	S	S

Chloromethyl Sulfides 6a-c (Scheme I). The chloromethyl sulfides **6** were prepared in a standard

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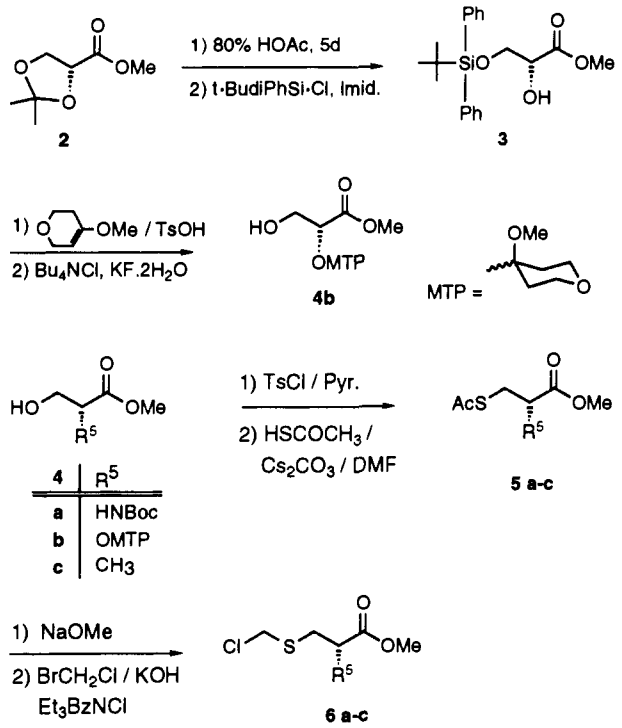
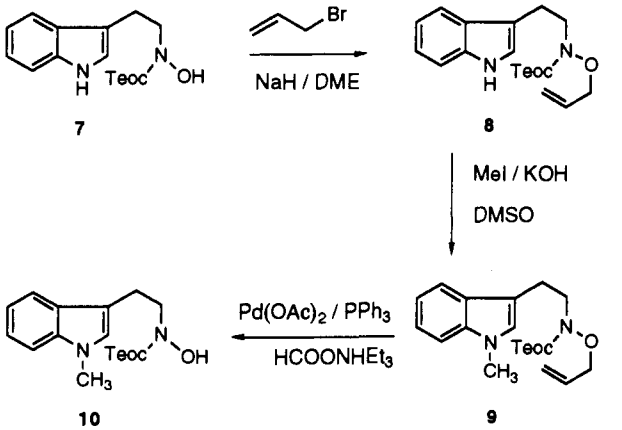
Scheme I



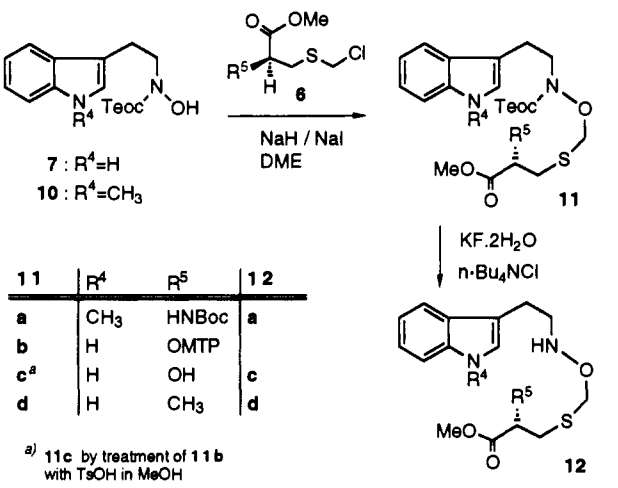
Scheme II



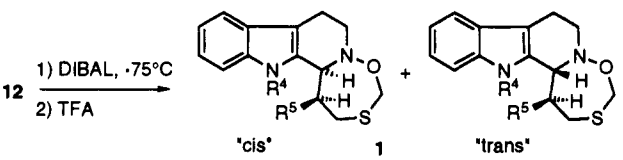
fashion from α-substituted β-hydroxypropanoic acid methyl esters **4**. The synthesis of **4a** has already been published.[6a] For the synthesis of the oxo derivative **4b** methyl α,β-isopropylidene-D-glycerate (**2**) was chosen as the starting compound. After deprotection of the isopropylidene group by stirring in 80% HOAc for 5 days, the terminal primary alcohol was protected with the diphenyl-*tert*-butylsilyl group to give **3**. Protection of the remaining hydroxyl group was carried out by treatment with 5,6-dihydro-4-methoxy-2*H*-pyran and a catalytic amount *p*-toluenesulfonic acid in THF, followed by deprotection of the silyl group to give **4b** in 96% yield. A precursor for the C(1)-methyl derivative was commercially available in optically pure form as (*R*)-(−)-methyl 3-hydroxy-2-methylpropionate (**4c**). The terminal alcohol functionality in **4a–c** was transformed into a thiol group by using the method developed by Kellogg and co-workers.[10] In this paper the alcohols **4a–c** were transformed into the tosylates which gave, after purification followed by treatment with Cs_2CO_3 and thiolacetic acid in DMF, the thioacetates **5a–c**. After liberation of the thiols with sodium methoxide in methanol solution and subsequent alkylation under phase-transfer conditions using bromochloromethane and benzyltriethylammonium chloride/powdered KOH, the chloromethyl sulfides **6a–c** were isolated without further purification in excellent yields.

***N*-Hydroxytryptamines 7 and 10 (Scheme II).** *N*-Teoc-*N*-hydroxytryptamine **7** was prepared as described before.[6a,8] For the synthesis of the indole *N*-methyl derivative **10**, **7** was chosen as the starting compound. To methylate the indole nitrogen selectively it was necessary to protect the more reactive hydroxamic oxygen as an allyl ether. Treatment of **7** with sodium hydride in 1,2-dimethoxyethane (DME) and subsequent addition of allyl bromide gave a quantitative yield of the *O*-allyl derivative of **8**, which was methylated by stirring in DMSO with methyl iodide and powered KOH to give **9**. Deprotection of the allyl group was carried out with a cocktail of pal-

Scheme III



Scheme IV



starting compound	R^4	R^5	Yield (%)	products cis	products trans	ratio cis/trans[a]
12a	CH_3	HNBoc	79	**1e**[b]	**1f**[b]	31/69
12c	H	OH	66	**1j**	**1k**	38/62
12d	H	CH_3	69	**1l**	**1m**	100/0

a) The cis/trans ratio's were determined by analytical HPLC.
b) The yield is based on the Boc protected products. The Boc group is removed with 1 eq. of TMSI in MeCN.

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ladium(II) acetate/triphenylphosphine/triethylammonium formate in refluxing acetonitrile[11] to give **10** in an overall yield of 98%.

Coupling (Scheme III). For selective O-alkylation of the TEOC-protected *N*-hydroxytryptamines **7** and **10** with the chloromethyl sulfides **6**, an efficient method was developed. Compounds **7** and **10** were treated with sodium hydride in freshly distilled DME. This solution of the oxo anion of **7** or **10** is dropped slowly (3–4 h) under an argon atmosphere into a cooled (0 °C) DME solution of the iodomethyl sulfides prepared in situ from **6a–c**. The functionalized tryptamines **11a,b,d** were isolated in yields of 60%, 89%, and 80%, respectively. The free α-hydroxy derivative **11c** was prepared in quantitative yield by deprotection of the MTP group by stirring **11b** in methanol with a catalytic amount of *p*-toluenesulfonic acid. As described in a previous paper,[6a] deprotection of the TEOC group was most effectivily carried out by using "naked" fluoride ion generated by tetrabutylammonium chloride and potassium fluoride dihydrate in acetonitrile at elevated temperature (50 °C), to yield **12a,c,d** from **11a,c,d** in yields of 94%, 79%, and 67%, respectively.

Cyclization Reactions (Scheme IV). As described earlier the intramolecular Pictet–Spengler condensation proceeds smoothly with aldehydes. Because cysteinaldehydes readily racemize, they were prepared in situ by reduction of the methyl esters with diisobutylaluminum hydride at −75 °C. The reaction was monitored by TLC and, when complete, trifluoroacetic acid was added and the reaction mixture was allowed to warm up to room temperature. After workup the product ratio was determined by analytical HPLC (see Scheme IV). The yields were determined gravimetrically after column chromatography. The stereochemistry of the eudistomins was determined by X-ray crystallography (**1i**[14] and **1l**), or by NOE difference NMR techniques at 400 MHz (**1e**, **1f**, **1j**, and **1k**). The NMR data of the synthesized eudistomin derivatives are collected in Table VI. A detailed discussion about the observed diastereoselectivities will be published elsewhere. Deprotection of the Boc group to give **1e** and **1f** was carried out as described earlier with Me_3SiI in acetonitrile.[6]

Antiviral and Antitumor Structure–Activity Relationships

Antiviral Activities. Our first goal in the SAR study of eudistomins was to examine the importance of the stereochemistry as found in natural tetracyclic eudistomins. Besides alterations to the amino group, the introduction of a methoxy group at C(10) in the aromatic indole portion and methylation of the indole nitrogen were included in the SAR study. The eudistomins **1a–j** and **1l** were evaluated for their inhibitory effects on the replication of a number of viruses, including influenza virus A and B in MDCK cells (Table I); respiratory syncytial virus, vesicular stomatitis virus, Coxsackie virus B4, and polio virus-1 in HeLa cell cultures (Table II); parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, and Semliki forest virus in Vero cell cultures (Table III); herpes simplex virus type-1 (HSV-1) (strains KOS, F, McIntyre), HSV-2 (strains G, 196, Lyons), vaccinia virus, vesicular stomatitis virus, and thymidine kinase (TK) deficient (TK^-) HSV-1 (strain B2006) in PRK cell cultures (Table IV); and HIV-1 and HIV-2 in human MT-4 cells. Human immunodeficiency virus (HIV) infection was carried out with the $HTLV\text{-}III_B$ strain. The virus was prepared from the culture supernatant of a persistently $HTLV\text{-}III_B$-infected MT-4 cells. The antiviral assays were based on inhibition of HIV-induced cytopathogenicity in either MT-4, MDCK, HeLa, Vero, or PRK cell cultures. All antiviral tests were carried out following established procedures.[12] Table I shows that against influenza virus A and influenza virus B only synthetically derived debromoeudistomin K (**1a**) and its enantiomer (**1d**) showed significant activities. The MCC/MIC ratios of **1a** and **1d** were 10 and 13, respectively. The activities were considered significant if the MCC/MIC ratio's were >10. Upon further investigation it became clear that the activity of **1d** was due to an enantiomeric impurity,[6a] i.e., **1d** contains 5.5% **1a**. The same effect for **1d** was found with the other viruses. Table II shows that with respiratory syncytial virus, vesicular stomatitis virus, Coxsackie virus B4, and polio virus-1 a similar trend was found for **1a**. With these viruses eudistomin derivatives **1e** and **1h** also showed antiviral acitvity. **1h** in particular had high potency with MCC/MIC ratios ranging between 13 and 67. Table III shows an equivalent activity profile against reovirus-1, Sindbis virus, Coxsackie virus B4, and Semliki forest virus. The most promising activities were achieved against HSV-1 and HSV-2 (Table IV) with **1a**, **1e**, and **1h** with MCC/MIC values ranging from 19 to 125 (**1a**), 13 to 57 (**1e**), and 45 to 294 (**1h**). No significant anti-HIV-1 and anti-HIV-2 activities were found with the synthetically derived eudistomins **1a–l** (data not shown). These data may be misinterpreted because eudistomins are very toxic toward MT-4, MDCK, HeLa, Vero, and PRK cell cultures (see Table V).

Table I. Cytotoxic and Antiviral Activity of the Synthetically Derived Eudistomins in MDCK Cell Cultures

compd	minimum cytotoxic concn (MCC)[a] (μg/mL)	minimum inhib concn[b] (μg/mL) influenza virus A (Ishikawa)	minimum inhib concn[b] (μg/mL) influenza virus B (Singapore)
1a	8	0.8	0.8
1b	15	>8	>8
1c	8	>1.6	>1.6
1d	40	3	3
1g	80	>40	>40
1h	0.8	>0.32	>0.32
1j	3	>1.6	>1.6
ribavirin	>200	15	15

[a] Required to cause a microscopically detectable alteration of normal cell morphology. [b] Required to reduce virus-induced cytopathogenicity by 50%. Virus-induced cytopathogenecity was recorded at 5 days after infection.

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Table II. Cytotoxity and Antiviral Activity of the Synthetically Derived Eudistomins in HeLa Cell Cultures

compd	minimum cytotoxic concn[a] (μg/mL)	minimum inhib concn[b] (μg/mL) respiratory syncytial virus (long)	vesicular stomatitus virus	Coxsackie virus B4	polio virus-1
1a	≥4	0.8	0.2	0.7	0.7
1b	≥10	>8	>10	>10	>10
1c	≥4	>1.6	>1	>1	>1
1d	40	3	7	>10	>10
1e	≥10		7	7	2
1f	≥10		>10	>10	>10
1g	≥40	>8	>10	>10	>10
1h	0.4	0.15	0.02	0.02	0.02
1i	4	>1.6	>1	>1	>1
1j	100		>100	>40	>100
1l	40		>40	>40	>40
BVDU	>400		>400	>400	>400
DHPA	>400		300	>400	>400
ribavirin	>400	3	20	70	70
carbocyclic 3-deazaadenosine	>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.

Table III. Cytotoxity and Antiviral Activity of the Synthetically Derived Eudistomins in Vero Cell Cultures

compd	minimum cytotoxic concn[a] (μg/mL)	minimum inhibn concn[b] (μg/mL) para influenza-3 virus	reo virus-1	Sindbis virus	Coxsackie virus B4	Semliki forest virus
1a	≥4	0.2	0.4	0.2	0.4	0.2
1b	40	>10	>10	>10	>10	>10
1c	≥10	>4	>4	>4 (7)	>4 (7)	>4
1d	40	7	20	20	7	10
1e	≥20	0.7	1	2	0.7	2
1f	≥40	>10	>10	>10	>10	>10
1g	≥40	>10	>10	>10	>10	>10
1h	≥1	0.7	0.07	0.02	0.07	0.07
1i	≥2.5	>1	>1	>1	>1	>1
1j	≥100	>40	>40	20	>40	>40
1i	≥100	>40	>40	20	20	>40
BVDU	>400	>400	>400	>400	>400	>400
DHPA	>400	70	70	150	70	>400
ribavirin	>400	20	70	40	70	40
carbocyclic 3-deazaadenosine	>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.

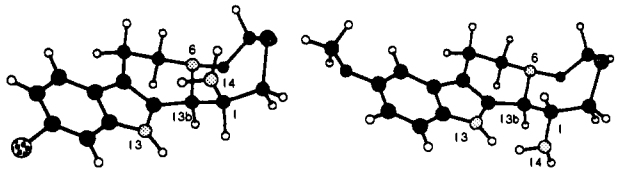
Table IV. Cytotoxic and Antiviral Activity of the Synthetically Derived Eudistomins in Primary Rabbit Kidney (PRK) Cell Cultures

compd	minimum cytotoxic concn (MCC)[a] (μg/mL)	minimum inhibitory concentration (MIC)[b,c] (μg/mL) herpes simplex virus-1 (KOS)	herpes simplex virus-1 (F)	herpes simplex virus-1 (McInyre)	herpes simplex virus-2 (G)	herpes simplex virus-2 (196)	herpes simplex virus-2 (Lyons)	vaccinia virus	vesicular stomatitis virus	herpes simplex virus-1 (TK⁻) (B2006)
1a	≥10	0.7	0.07	0.2	0.2	0.2	0.07	0.1	0.2	0.7
1b	40	>10	>10	>10	>10	>10	>10	>10	>10	>10
1c	10	>4	2	>4	>4	2	2	>4	>4	>4
1d	≥10	2	2	2	2	2	2	2	2	7
1e	≥40	3	2	0.7	0.7	1	0.7	1.5	7	7
1f	40	>10			>10			>10	>10	
1g	≥200	>100	>100	>100	>100	>100	>100	>100	>100	>100
1h	≥1	0.045	0.07	0.02	0.02	0.02	0.04	0.02	0.02	0.04
1i	≥10	>4	>4	>4	>4	>4 (7)	>4	>4	>4	>4
1j	≥100	>100			>100			>100	>100	
1l	≥100	>100			>100			>100	>100	
BVDU	≥400	0.04	0.02	0.02	7	70	70	7	>200	150
DHPA	≥400	>100	>100	>100	>100	>100	>100	20	20	>100
ribavirin	≥400	>200	>200	>200	>200	>200	>200	20	>400	>200
carbocyclic 3-deazaadenosine	≥400	>200	>200	>200	>200	>200	>200	0.7	2	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 generalization we can conclude from this data is that the stereochemistry at C(13)b must be the same as that of the natural eudistomins, indicating at least a three-point interaction at receptor level. Furthermore, to

Chart II



natural Eudistomin K 5-OMe and C(1)-epimer derivative

some structural data of both diastereoisomers	distance (dihedral angle)			
	N(14)-N(6)	N(14)-S	S-N(6)	N(13)-N(6)
Eudistomin K	3,050Å (75.9°)	3.126Å (-55.7°)	2.982Å (57.3°)	3.726Å (-164.5°)
5-OMe and C(1)-epimer derivative	3.750Å (-171.4°)	4.098Å (-166.7°)	3.046Å (60.4°)	3.684 (-159.8°)

Table V. Inhibitory Effects of Eudistomins on the Proliferation of Murine Leukemia Cells (L1210), Human T-Lymphoblast Cells (Molt/4F) and Human T-Lymphocyte (MT-4)

compd	ID_{50}[a] (μg/mL)		
	L1210[b]	Molt-4F	MT-4
1a	0.11 ± 0.01	0.12 ± 0.02	0.075 ± 0.007
1b	17.4 ± 1.0	5.14 ± 0.40	4.5 ± 0.07
1c	1.40 ± 0.15	0.60 ± 0.01	0.77 ± 0.23
1d	1.24 ± 0.35	1.54 ± 0.31	3.58 ± 0.01
1e	0.56 ± 0.003	0.37 ± 0.08	0.69 ± 0.015
1g	24.9 ± 7.3	18.3 ± 0.41	96 ± 31.7
1h	0.005 ± 0.0004	0.0062 ± 0.0006	0.005 ± 0.001
1i	6.54 ± 0.87	0.94 ± 0.12	1.99 ± 1.17

[a] 50% inhibitory dose or dose 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.

obtain a significant activity it seems necessary that the NH_2 group occupies the axial position, as is found in the natural isolated tetracyclic eudistomins. At this point it is interesting to take a closer look at the conformations of the CD-ring systems in both eudistomin diastereoisomers. In Chart II the X-ray structures of eudistomin K[13] (for clearness the *N*(1)-*p*-bromobenzoyl group has been omitted) and **1i**[14] (inverted) are shown. It is obvious that the only difference in both diastereoisomers is the position of the NH_2 group (see data of Chart II). In the natural stereoisomer the NH_2 group points in the same direction as the lone pair on N(6), indicating that both could be involved in the receptor binding process. The observation that replacement of the NH_2 group by H (**1g**) results in loss of activity supports this supposition. Furthermore the complete loss of activity when the NH_2 group is substituted by a hydroxyl group (**1j**) indicates that the amino group acts as an H-bond acceptor, leading to an ionic interaction with the receptor. This is further supported by Rinehart and Munro who found that N-acylation leads to loss of activity.[3,4] The strong influence of substituents on the 6-membered ring in the indole part (see also Table I) demonstrates that the aromatic indole nucleus also contributes to the binding process at the receptor site. Finally, it is interesting to note that a methyl substituent at the indole N in **1e** only moderately lowers the potency.

Cytostatic Activities. For this antitumor SAR study, the inhibitory effects of the compounds **1a–e** and **1g–i** on the proliferation of murine leukemia cells (L1210), human T-lymphoblast cells (Molt/4F), human T-lymphocyte (MT-4), and P388 leukemia cells were evaluated. The antitumor tests were also carried out following established procedures.[15] The results are shown in Table V. For **1a**

Table VI. ^1H NMR Signals for Eudistomins **1e**, **1j**, **1f**, **1k**, and **1l**[a]

	δ, ppm, multiplicity (*J*, Hz)														
	H1[α]	H2[α]	H2[β]	H4[α]	H4[β]	H7[α]	H7[β]	H8[α]	H8[β]	H9	H10	H11	H12	H13bα	N(13)H	misc
							Natural Isomers									
1e	3.61 m	3.35 dd(14.5, 2.2)	2.83 dt(14.8, 3.0)	4.92 AB(9.4)	4.84 AB(9.4)	3.08 m(11.3, 9.5, 4.0)	3.54 m(9.3, 4.2, 2.4)	2.93–2.88 m	2.93–2.88 m	7.29 d(8.1)	7.22 dt(7.8, 1.0)	7.13 dt(7.8, 1.0)	7.49 d(7.8)	4.38 br s		3.69 s, N-CH_3
1j[b]	4.32 br s	3.32 br d(12.5)	2.97 dd(14.4, 6.0)	4.93 AB(8.7)	4.80 AB(9.1)	3.15 m(11.8, 10.2, 4.3)	3.61 m(10.0, 4.3)	2.82 dd(15.2)	2.97 m	7.28 d(8.0)	7.14 dt(8.2, 1.3)	7.08 dt(8.0, 1.1)	7.45 d(7.7)	4.04 br s	7.91 br s	3.39 d(3.5), OH
							Unnatural Isomers									
1f	3.64 m(4.9, 3.4, 1.5)	3.86 dd(14.3, 1.4)	2.54 ddd(14.3, 5.2, 1.7)	5.17 AB (11.2)	4.71 d, AB-(11.2, 1.7)	2.98 ddd(11.7, 9.9, 3.3)	3.47 ddd(9.2, 4.3, 2.0)	2.88 dddd(14.1, 12.0, 4.3, 2.2)	2.75 br d(14.2)	7.28 d(8.2)	7.20 dt(8.2, 1.0)	7.09 dt(7.8, 0.9)	7.45 d(7.8)	4.09 br s		3.91 s, N-CH_3
1k[c]	4.10 m	2.88 dd(14.2, 2.0)	2.77 m	4.99 AB (10.0)	4.95 AB(10.0)	3.23 m	3.60 m	2.98 m	3.10 m	7.32 d(8.0)	7.15 dt(8.1, 1.1)	7.09 dt(7.9, 1.0)	7.47 d(7.7)	4.02 d(7.3)	8.76 br s	2.23 br s, OH
1l	2.55 m	3.37–3.01 m	3.37–3.01 m	5.01 br s	5.01 br s	3.08 m	3.64 br s	2.72 m	3.01 m	7.32 d(7.9)	7.15 dt(8.2, 1.2)	7.10 dt(8.0, 1.0)	7.46 d(7.7)	3.95 m	7.77 br s	1.47 d(3.5), CH_3

[a] All spectra are recorded in $CDCl_3$ at 400 MHz. [b] To sharpen up the broadened spectrum, recorded at 57 °C. [c] To sharpen up the broadened spectrum, recorded at 38 °C.

a marked inhibitory effect could be observed while **1h** appeared to be a very potent cytostatic compound with ID_{50} values down to 0.005 μg/mL for L1210, Molt-4F, MT-4, and P388 cells. For the antitumor activity of eudistomins, roughly the same SAR conclusions can be drawn as for the antiviral activities. This indicates that possibly eudistomins act on a biochemical process that is essential for both virus growth and tumor cell growth.[16]

In conclusion it can be stated that tetracyclic eudistomins may have promise as antiviral and antitumor compounds. Further work is in progress to examine the biological target with which eudistomins interact to achieve their effect on both viruses and tumor cells. Also the influence of the D-ring size and the heteroatoms in the D-ring on the biological activity is under investigation.

Experimental Section

Proton 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 solvent. Mass spectra were obtained with a double-focusing VG 7070E spectrometer. Thin-Layer chromatography (TLC) was carried out by using silica gel F-254 plates (thickness, 0.25 mm). Spots were visualized with a UV hand-held lamp, iodine vapor, or Cl_2–TDM.[17]

Methyl 1-*O*-(*tert*-Butyldiphenylsilyl)-D-glycerate (3). α,β-isopropylidene-D-glyceric acid methyl ester (**2**) (10 g, 62 mmol) was dissolved in $HOAc/H_2O$ = 80/20 (v/v, 25 mL) and kept standing at room temperature for 5 d. The solvent was removed under high vacuum to yield 7.5 g (100%) of D-glyceric acid methyl ester (**2**) as a colorless oil: ^1H NMR (90 MHz) δ 4.32 (t, 1 H, J = 4.0 Hz, *CH*OH), 4.00–3.83 (m, 4 H, 2 OH and CH_2OH), 3.83 (s, 3 H, OCH_3). D-Glyceric acid methyl ester (1.0 g, 8.3 mmol), *tert*-butyldiphenylsilyl chloride (2.5 g, 1.1 equiv), and imidazole (1.7 g, 3 equiv) were dissolved in dry DMF (25 mL) and stirred for 25 h. The reaction mixture was concentrated in vacuo. The residue was dissolved in EtOAc (50 mL) and subsequently washed with 0.1 N HCl and brine. The organic layer was dried ($MgSO_4$) and the solvent was evaporated in vacuo. The residue was subjected to column chromatography (chloroform) to give 2.73 g of **3** as an oil: R_f 0.34 ($CHCl_3$/MeOH = 99.5/0.5, v/v); $[\alpha]^{22}_D$ = −15.2° (c = 2.7, MeOH); CIMS (100 eV), m/z (relative intensity) 359 ($[M + 1]^{+1}$, 0.6), 239 ($[C_{16}H_{19}Si]^+$, 13), 199 (100), 57 ($[C_4H_9]^+$, 7); ^1H NMR (90 MHz) δ 4.30–4.18 (m, 1 H, CH), 3.97 and 3.91 (AB part of ABX spectrum, 2 H, J_{AB} = 11.4 Hz, J_{AX} = 2.8 Hz and J_{BX} = 3.3 Hz, CH_2), 3.77 (s, 3 H, OCH_3), 3.18 (d, 1 H, J = 7.7 Hz, OH).

Methyl 2-*O*-[4-(4-Methoxytetrahydropyranyl)]-D-glycerate (4b). To **3** (2.6 g, 7.3 mmol) and 5,6-dihydro-4-methoxy-2*H*-pyran (1.7 g, 2 equiv) in THF (25 mL) was added a catalytic amount Tos-OH-H_2O (20 mg). After 2 h, the reaction mixture was diluted with EtOAc (50 mL) and washed with 2 portions of saturated $NaHCO_3$/brine = 1/1 (v/v, 25 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was dissolved in dry THF (50 mL) and tetrabutylammonium fluoride (8 mL of a 1 M solution in THF) was added. After completion of the reaction, as was monitored by TLC (EtOAc/*n*-hexane = 1/2, v/v) after 2 h, the reaction mixture was concentrated in vacuo and subjected to column chromatography ($MeOH/CHCl_3$ = 3/97, v/v) to yield 1.64 g (96%) of **4b** as an oil: R_f 0.26 ($MeOH/CHCl_3$ = 3/97, v/v); $[\alpha]^{22}_D$ = +44.9° (c = 4.52, MeOH); CIMS (100 eV), m/z (relative intensity) 235 ($[M + 1]^+$, 1), 203 ($[M - OCH_3]^+$, 53), 115 ($[C_6H_{11}O_2]^+$, 100); ^1H NMR (90 MHz) δ 4.42 (t, 1 H, J = 4.8 Hz, CH), 4.04–3.46 (m, 6H, CH_2 and $-CH_2OCH_2-$), 3.76 (s, 3 H, $COOCH_3$), 3.25 (s, 3 H, OCH_3), 2.48 (br t, 1 H, OH), 1.93–1.75 (m, 4 H, $-CH_2CCH_2-$).

(*S*)-Methyl 2-*O*-[4-(4-Methoxytetrahydropyranyl)]-3-(acetylthio)propanoate (5b). In freshly distilled pyridine (25 mL) **4b** (1.35 g, 5.8 mmol) was dissolved together with tosyl chloride (1.23 g, 1.1 equiv) and the reaction mixture was stirred overnight at 4 °C. The reaction mixture was concentrated to dryness at high vacuum (the temperature was kept at <25 °C). The residue was dissolved in EtOAc and subsequently washed with 0.1 N HCl, saturated $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was subjected to column chromatography ($MeOH/CHCl_3$ = 2/98, v/v) to give 1.72 g (77%) of the tosylate as an oil: R_f 0.56 ($MeOH/CHCl_3$ = 3/97, v/v); $[\alpha]^{22}_D$ = +19.3° (c = 2.18, MeOH); CIMS (100 eV), m/z (relative intensity) 389 ($[M + 1]^+$, 1), 155 ($[C_7H_7SO_3]^+$, 57), 115 ($[C_6H_{11}O_2]^+$, 100); ^1H NMR (90 MHz) δ 7.80 and 7.37 (AB, 2 H, ^2J = 8.1 Hz, C_6H_4), 4.53 (t, 1 H, J = 5.3 Hz, OCH_2CH), 4.20 (d, 2 H, J = 5.3 Hz, OCH_2CH), 3.86–3.41 (m, 4 H, $-CH_2OCH_2-$), 3.69 (s, 3 H, $COOCH_3$), 3.18 (s, 3 H, OCH_3), 2.45 (s, 3 H, p-$C_6H_4CH_3$), 1.81–1.67 (m, 4 H, $-CH_2CCH_2-$). To DMF (25 mL) were subsequently added Cs_2CO_3 (1.0 g, 3.1 mmol) and thioacetic acid (440 mg, 5.8 mmol). The suspension was stirred in the darkness until all Cs_2CO_3 was dissolved. To this solution was added the tosylate (1.72 g, 4.43 mmol) dissolved in DMF (10 mL) and the reaction mixture was allowed to stand overnight at room temperature in the dark. The solvent of the resulting light yellow solution was removed in vacuo. The residue was dissolved in EtOAc (50 mL) and subsequently washed with 0.1 N HCL and brine. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was subjected to column chromatography ($MeOH/CHCl_3$ = 3/97, v/v) to give 1.02 g (79%) of **5b** as an oil: R_f 0.39 ($MeOH/CHCl_3$ = 3/97, v/v); $[\alpha]^{22}_D$ = −14.0° (c = 2.5, MeOH); CIMS (100 eV), m/z (relative intensity) 293 ($[M + 1]^+$, 4), 161 ($[C_6H_9O_3S]^+$, 91), 115 ($[C_6H_{11}O_2]^+$, 100); ^1H NMR (90 MHz) δ 4.39 (t, 1 H, CH_2CH), 3.87–3.48 (m, 4 H, $-CH_2OCH_2-$), 3.74 (s, 3 H, $COOCH_3$), 3.27–3.18 (m, 2 H, $*CH_2CH$), 3.23 (s, 3 H, OCH_3), 2.34 (s, 3 H, $SCOCH_3$), 1.91–1.73 (m, 4 H, $-CH_2CCH_2-$).

(*S*)-Methyl 2-Methyl-3-(acetylthio)propanoate (5c). The same procedure as described for **5b** was followed. (*R*)-(−)-Methyl 3-hydroxy-2-methylpropionate (2.5 g, 21 mmol) and tosyl chloride (4.40 g, 1.1 equiv) gave after column chromatography (EtOAc/*n*-hexane = 35/65, v/v) 5.1 g (89%) of the tosylate as an oil: R_f 0.25 (EtOAc/*n*-hexane = 20/80, v/v); CIMS (100 eV), m/z (relative intensity) 273 ($[M + 1]^+$, 63), 155 ($[C_7H_7SO_3]^+$, 46), 117 ($[M - C_7H_7SO_3]^+$, 46), 91 ($[C_7H_7]^+$, 57), 69 (100); ^1H NMR (90 MHz) δ 7.82 and 7.38 (AB, 4 H, J = 8.4 Hz, C_6H_4), 4.33–3.99 (m, 2 H, CH_2CH), 3.67 (s, 3 H, $COOCH_3$), 3.02–2.64 (m, 1 H, CH_2CHCH_3), 2.45 (s, 3 H, p-$C_6H_4CH_3$), 1.20 (d, 3 H, J = 7.0 Hz, $CHCH_3$). This tosylate (5.10 g, 18.3 mmol) together with Cs_2CO_3 (4.13 g, 0.7 equiv) and thiolacetic acid (2.08 g, 1.5 equiv) gave after column chromatography (*n*-hexane/$CHCl_3$ = 18/85, v/v) 1.74 g (54%[18]) of thioacetate **5c**: R_f 0.41 ($CHCl_3$); ^1H NMR (90 MHz) δ 3.71 (s, 3 H, $COOCH_3$), 3.12 and 3.05 (AB part of ABX spectrum, 2 H, J_{AX} = 7.8 Hz, J_{BX} = 3.5 Hz and J_{AB} = 13.5 Hz, CH_2CH), 2.89–2.51 (m, 1 H, CH_2CH), 2.32 (s, 3 H, $SCOCH_3$), 1.23 (d, 3 H, J = 7.0 Hz, $CHCH_3$).

(*S*)-Methyl 3-(Chloromethylthio)-2-[[4-(4-methoxytetrahydropyranyl)]oxy]propanoate (6b). To dry MeOH (25 mL) was added Na (102 mg, 4.43 mmol). This NaOMe solution was added dropwise to a stirred solution of thioacetate **5b** (1.30 g, 4.45 mmol) in dry MeOH (50 mL). After stirring for 15 minutes,

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(17) Arx, E. von; Faupel, M.; Brugger, M., The 4,4′-tetramethyldiaminodiphenylmethane reagent (TDM). A modification of the chloro-o-tolidine color reaction for thin-layer chromatography. *J. Chromatogr.* **1976**, *120*, 224.

(18) The reason for the moderate yield was the bad quality of the DMF used. The DMF should be purified as described: Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press, New York, 1988; p 157.

saturated NH_4Cl (1 mL) was added and the solvent was evaporated in vacuo. The residue was dissolved in EtOAc (25 mL) and washed with saturated NH_4Cl. The organic layer was dried ($MgSO_4$) and concentrated in vacuo to yield 1.12 g of crude thiol: R_f 0.54 (MeOH/$CHCl_3$ = 3/97, v/v). The crude thiol was dissolved in $BrCH_2Cl$ (50 mL). To this solution triethylbenzylammonium chloride (TEBAC) (101 mg, 0.44 mmol) and powdered KOH (374 mg, 6.70 mmol) were added. The suspension was stirred vigorously for 30 min. The reaction mixture was subsequently washed with NH_4Cl (20 mass %) and brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo to yield 1.32 g (99%) of crude chloromethyl sulfide **6b**: R_f 0.68 (MeOH/$CHCl_3$ = 3/97, v/v); CIMS (100 eV), m/z (relative intensity) 298 ($[M]^+$, 0.1), 183 ($[C_5H_6O_3SCl + 2]^+$, 3), 181 ($[C_5H_6O_3SCl]^+$, 9), 115 ($[C_6H_{11}O_2]^+$, 100); ^1H NMR (90 MHz) δ 4.97–4.65 (AB pattern, 2 H, SCH_2Cl), 4.58 (t, 1 H, J = 5.7 Hz, CH_2CH), 3.92–3.45 (m, 4 H, $-CH_2OCH_2-$), 3.76 (s, 3 H, $COOCH_3$), 3.24 (s, 3 H, OCH_3), 3.09 (d, 2 H, J = 5.7 Hz, CH_2CH), 1.92–1.74 (m, 4 H, $-CH_2CCH_2-$).

(*S*)-Methyl 3-(Chloromethylthio)-2-methylpropanoate (6c). The same procedure as described for **6b** was followed. Thioacetate **5c** (1.7 g, 9.7 mmol) and Na (223 mg, 1 equiv) gave the corresponding thiol (not weighed): ^1H NMR (90 MHz) δ 3.73 (s, 3 H, $COOCH_3$), 2.93–2.53 (m, 3 H, $CHCH_2$), 1.60–1.18 (m, 4 H, $CHCH_3$ and SH). This crude thiol together with $BrCH_2Cl$ (100 mL), TEBAC 313 mg (0.1 equiv) and powdered KOH (815 mg, 1.5 equiv) gave 1.26 g of chloromethyl sulfide **6c** (71% overall from **5c**): CIMS (100 eV), m/z (relative intensity) 147 ($[M - Cl]^+$, 100), 101 ($[C_5H_9O_2]^+$, 29); ^1H NMR (90 MHz) δ 4.75 (s, 2 H, SCH_2Cl), 3.73 (s, 3 H, $COOCH_3$), 3.22–2.63 (m, 3 H, $CHCH_2$), 1.29 (d, 3 H, J = 6.2 Hz, CCH_3).

3-[2-[[[[2-(Trimethylsilyl)ethyl]oxy]carbonyl](allyloxy)amino]ethyl]indole (8). To 7[8] (1 g, 3.1 mmol) in freshly distilled DME (25 mL) was added NaH (125 mg of a 60% oil dispersion, 3.1 mmol) and the suspension was stirred until a clear solution appeared (hydrogen gas evolved). To this solution was added allyl bromide (1.5 g, 12.4 mmol) at once (immediate NaBr formation). After 2 h the suspension was concentrated 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 1.12 g (100%) of allyl ether **8**: R_f 0.63 (EtOAc/*n*-hexane = 1/1, v/v); CIMS (100 eV), m/z (relative intensity) 360 ($[M]^+$, 18), 318 ($[M - C_3H_6]^+$, 54), 158 (100), 144 ($[C_{10}H_{10}N]^+$, 96), 130 ($[C_9H_8N]^+$, 52), 73 (60); ^1H NMR (60 MHz) δ 8.03 (br s, 1 H, indole-NH), 7.70–7.00 (m, 5 H, indole C(2)H and C(4)H-C(7)H), 6.35–5.80 (m, 1 H, CH_2CH=CH_2), 5.40–5.20 (m, 2 H, CH_2CH=CH_2), 4.40–3.70 (m, 6 H, CH_2CH=CH_2, OCH_2CH_2Si, and indole-C(3)CH_2CH_2N), 3.15–3.00 (m, 2 H, indole C(3)CH_2CH_2N), 0.95–0.75 (m, 2 H, OCH_2CH_2Si), 0.00 (s, 9 H, $Si(CH_3)_3$).

1-Methyl-3-[2-[[[[2-(trimethylsilyl)ethyl]oxy]carbonyl](allyloxy)amino]ethyl]indole (9). To DMSO (20 mL) was added 8 (1.2 g, 3.3 mmol), MeI (1.0 g, 7.0 mmol), and powdered KOH (370 mg, 6.6 mmol). The initially dark solution became clearer as the reaction proceeded. After stirring for 1.5 h the reaction mixture was diluted with EtOAc (50 mL) and subsequently washed with 1 N HCl, 3 portions water, and brine. The organic layer was dried ($MgSO_4$) and the solvent was evaporated in vacuo. The residue was subjected to column chromatography (EtOAc/*n*-hexane = 20/80, v/v) to yield 1.22 g (99%) of **9** as an oil: R_f 0.30 (EtOAc/*n*-hexane = 20/80, v/v); ^1H NMR (90 MHz) δ 7.62–7.52 (m, 1 H, indole C(7)H), 7.28–6.96 (m, 3 H indole C(4)-C(6)H), 6.84 (s, 1 H, indole C(2)H), 6.22–5.77 (m, 1 H, CH=CH_2), 5.37–5.17 (m, 2 H, CH=CH_2), 4.34 (d, 2 H, J = 6.0 Hz, CH_2CH=CH_2), 4.16–3.96 (m, 2 H, OCH_2CH_2Si), 3.86–3.69 (m, 2 H, CH_2N), 3.71 (s, 3 H, NCH_3), 3.12–2.96 (m, 2 H, indole C(3)CH_2), 0.94–0.75 (m, 2 H, OCH_2CH_2Si), 0.00 (s, 9 H, $SiC(CH_3)_3$).

1-Methyl-3-[2-[[[2-(trimethylsilyl)ethyl]oxy]carbonyl]-hydroxyamino]ethyl]indole (10). A solution of **9** (980 mg, 2.62 mmol), $Pd(OAc)_2$ (6 mg, 0.03 mmol), PPh_3 (27 mg, 0.1 mmol), and $HCOOHNEt_3$ (3.4 g, 23 mmol) in acetonitrile/water = 80/20, (v/v, 50 mL) was refluxed. After completion of the reaction (30 min), as was monitored by TLC (EtOAc/*n*-hexane = 20/80, v/v), the reaction mixture was diluted with EtOAc (50 mL) and subsequently washed with 2 portions water and brine. The organic layer was dried ($MgSO_4$) and the solvent was evaporated in vacuo. The residue was subjected to column chromatography (MeOH/$CHCl_3$ = 3/97, v/v) to yield 870 mg (99%) of **10** as an oil: R_f 0.13 (EtOAc/*n*-hexane = 20/80, v/v), CIMS (100 eV), m/z (relative intensity) 334 ($[M]^+$, 39), 144 ($[C_{10}H_{10}N]^+$, 100); ^1H NMR (90 MHz) δ 7.67–7.58 (m, 1 H, indole C(7)H), 7.35–7.03 (m, 3 H, indole C(4)-C(6)H), 6.92 (s, 1 H, indole C(2)H), 4.13–3.93 (m, 2 H, OCH_2CH_2Si), 3.84 (t, 2 H, J = 7.0 Hz, CH_2N), 3.73 (s, 3 H, NCH_3), 3.12 (t, 2 H, J = 7.0 Hz, indole C(3)CH_2), 0.87–0.67 (m, 2 H, OCH_2CH_2Si), 0.00 (s, 9 H, $SiC(CH_3)_3$).

1-Methyl-3-[2-[[[[*O*-methyl-*N*-(*tert*-butyloxycarbonyl)-D-cystein-*S*-yl]methyl]oxy][[[2-(trimethylsilyl)ethyl]oxy]carbonyl]amino]ethyl]indole (11a). NaH (175 mg of a 60% oil dispersion, 4.4 mmol) was added to a stirred solution of **10** (1.9 g, 6.7 mmol) in freshly distilled DME (50 mL). The suspension was stirred until a clear solution appeared (10–30 min) (hydrogen gas evolved). This solution was added dropwise (over 4–5 h) to a stirred solution of **6a**[7] (1.9 g, 6.7 mmol) and NaI (990 mg, 6.6 mmol) in freshly distilled DME (250 ml) at 0 °C. After stirring for an additional 1 h saturated NH_4Cl (1 mL) was added and the suspension was concentrated in vacuo. The residue was dissolved in EtOAc (50 mL) and subsequently washed with water and saturated NH_4Cl. The organic layer was dried ($MgSO_4$) and the solvent was evaporated in vacuo. The residue was subjected to column chromatography (EtOAc/*n*-hexane = 1/2, v/v) to yield 1.53 g (60%) of **11a** as an oil: R_f 0.52 (EtOAc/*n*-hexane = 1/1, v/v); CIMS (100 eV), m/z (relative intensity) 581 ($[M]^+$, 32), 158 ($[C_{11}H_{12}N]^+$, 79), 144 ($[C_{10}H_{10}N]^+$, 100), 73 ($[SiC(CH_3)_3]^+$, 75), 57 ($[C(CH_3)_3]^+$, 82); ^1H NMR (90 MHz) δ 7.62–7.53 (m, 1 H, indole C(7)H), 7.31–6.98 (m, 3 H, indole C(4)-C(6)H), 6.89 (s, 1 H, indole C(2)H), 5.60 (br d, 1 H, J = 9.0 Hz, *H*NBoc), 4.87 (s, 2 H, OCH_2S), 4.66–4.46 (m, 1 H, *CH*COOMe), 4.26–3.49 (m, 4 H, OCH_2CH_2Si and NCH_2), 3.69 (s, 6 H, $COOCH_3$ and NCH_3), 3.22–2.98 (m, 4 H, indole C(3)CH_2 and $CHCH_2S$), 1.38 (s, 9 H, $C(CH_3)_3$), 0.91–0.71 (m, 2 H, OCH_2CH_2Si), 0.00 (s, 9 H, $SiC(CH_3)_3$).

(*S*)-Methyl 2-[[4-(4-Methoxytetrahydropyranyl)]oxy]-3-[[[[[[[2-(trimethylsilyl)ethyl]oxy]carbonyl][2-(3-indolyl)ethyl]amino]oxy]methyl]thio]propanoate (11b). The same procedure was followed as for **11a**. Compound **7** (930 mg, 2.9 mmol), NaH (116 mg of a 60% oil dispersion, 2.9 mmol), chloromethyl sulfide **6b** (1.3 g, 4.3 mmol), and NaI (650 mg, 4.3 mmol) in freshly distilled DME (100 ml) gave after column chromatography (chloroform) 1.5 g (89%) of **11b** as an oil: R_f 0.42 (MeOH/$CHCl_3$ = 2/98, v/v); CIMS (100 eV), m/z (relative intensity) 468 ($[M + 1 - C_6H_{11}O_2]^+$, 1), 130 ($[C_9H_8N]^+$, 30), 115 ($[C_6H_{11}O_2]^+$, 55), 73 ($[SiC(CH_3)_3]^+$, 36), 41 (100); ^1H NMR (90 MHz) δ 8.11 (br s, 1 H, indole-NH), 7.67–7.04 (m, 5 H, indole C(2)H and C(4)-C(7)H), 4.98 (s, 2 H, OCH_2S), 4.51 (t, 1 H, J = 6.4 Hz, $CHCH_2$), 4.12–3.39 (m, 8 H, CH_2OCH_2, OCH_2CH_2Si, and NCH_2), 3.73 (s, 3 H, $COOCH_3$), 3.22 (s, 3 H, OCH_3), 3.18–2.95 (m, 4 H, indole C(3)CH_2 and $CHCH_2$), 1.91–1.74 (m, 4 H, CH_2CCH_2), 0.91–0.72 (m, 2 H, OCH_2CH_2Si), 0.00 (s, 9 H, $SiC(CH_3)_3$).

(*S*)-Methyl 2-hydroxy-3-[[[[[[[2-(trimethylsilyl)ethyl]oxy]carbonyl][2-(3-indolyl)ethyl]amino]oxy]methyl]thio]propanoate (11c). To a solution of **11b** (650 mg, 1.12 mmol) in MeOH (30 mL) is added a catalytic amount of Tos-OH·H_2O (20 mg). After completion of the deprotection (50 min), as was monitored by TLC (MeOH/$CHCl_3$ = 2/98, v/v), saturated $NaHCO_3$ (1 mL) was added and the solvent was evaporated in vacuo. The residue was dissolved in EtOAc (50 mL) and subsequently washed with saturated $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$) and the solvent was evaporated in vacuo to yield 515 mg (98%) of crude **11c**: R_f 0.29 (MeOH/$CHCl_3$ = 2/98, v/v), ^1H NMR (90 MHz) δ 8.09 (br s, 1 H, indole NH), 7.68–7.06 (m, 5 H, indole C(2)H and C(4)-C(7)H), 4.98 (s, 2 H, OCH_2S), 4.53 (br s, 1 H, OH), 4.18–4.66 (m, 5 H, $CHCH_2$, OCH_2CH_2Si, and NCH_2), 3.80 (s, 3 H, $COOCH_3$), 3.34–2.86 (m, 4 H, indole C(3)CH_2 and $CHCH_2S$), 0.84–0.65 (m, 2 H, OCH_2CH_2Si), 0.00 (s, 9 H, $SiC(CH_3)_3$).

(*S*)-Methyl 2-Methyl-3-[[[[[[[2-(trimethylsilyl)ethyl]oxy]carbonyl][2-(3-indolyl)ethyl]amino]oxy]methyl]thio]propanoate (11d). The same procedure was followed as for **11a**. Compound **7** (1.3 g, 4.0 mmol), NaH (160 mg of a 60% oil dispersion, 4.0 mmol), chloromethyl sulfide **6c** (1.1 g, 6.0 mmol), and NaI (900 mg, 6.0 mmol) in freshly distilled DME (200 mL) gave after column chromatography (EtOAc/*n*-hexane = 1/2, v/v) 1.50 g (80%) of **11d** as an oil: R_f 0.59 (EtOAc/*n*-hexane = 1/1, v/v); CIMS (100 eV), m/z (relative intensity) 480 ($[M + CH_4]^+$, 1), 4.66

([M]+, 4), 130 ([C_9H_8N]+, 64), 73 ([$SiC(CH_3)_3$]+, 100); ^{1}H NMR (90 MHz) δ 8.16 (br s, 1 H, indole NH), 7.68–7.02 (m, 5 H, indole C(2)H and C(4)-C(7)H), 4.91 (s, 2 H, OCH_2S), 4.14–3.80 (m, 4 H, OCH_2CH_2Si and NCH_2), 3.68 (s, 3 H, $COOCH_3$), 3.18–2.73 (m, 5 H, indole C(3)CH_2 and $CHCH_2$), 1.26 (d, 3 H, J = 6.3 Hz, $CHCH_3$), 0.94–0.74 (m, 2 H, OCH_2CH_2Si), 0.00 (s, 9 H, $SiC(CH_3)_3$).

1-Methyl-3-[2-[[[[*O*-methyl-*N*-(*tert*-butyloxycarbonyl)-D-cystein-*S*-yl]methyl]oxy]amino]ethyl]indole (12a). A solution of **11a** (1.52 g, 2.62 mmol), Bu_4NCl (2.2 g, 7.9 mmol), and KF·2H_2O (991 mg, 10.5 mmol) in dry acetonitrile (50 mL) was stirred at 50 °C for 10 h. The solvent was evaporated in vacuo. The residue was dissolved in EtOAc and subsequently washed with water and saturated NH_4Cl. The organic layer was dried ($MgSO_4$) and the solvent was evaporated in vacuo. The residue gave after column chromatography (EtOAc/*n*-hexane = 1/1, v/v) 1.07 g (93%) of **12a** as an oil: R_f 0.37 (EtOAc/*n*-hexane = 1/1, v/v); CIMS (100 eV), *m/z* (relative intensity) 438 ([M + 1]+, 70), 437 ([M]+, 18), 327 (86), 203 (71), 158 ([$C_{11}H_{12}N$]+, 100), 144 ([$C_{10}H_{10}N$]+, 89), 57 ([C_4H_6]+, 88); ^{1}H NMR (90 MHz) δ 7.63–7.49 (m, 1 H, indole C(7)H), 7.34–7.03 (m, 3 H, indole C(4)-C(6)H), 6.96 (s, 1 H, indole C(2)H), 5.99 (very br d, 2 H, J = 9.0 Hz, HNBoc and ONH), 4.85 (AB, 2 H, OCH_2S), 4.68–4.53 (m, 1 H, $CHCH_2S$), 3.74 (s, 3 H, $COOCH_3$), 3.71 (s, 3 H, indole NCH_3), 3.36–2.86 (m, 6 H, indole C(3)CH_2CH_2 and $CHCH_2S$), 1.42 (s, 9 H, $C(CH_3)_3$).

(*S*)-Methyl 2-Hydroxy-3-[[[[[2-(3-indolyl)ethyl]amino]-oxy]methyl]thio]propanoate (12c). The same procedure was followed as for **11a**. Compound **12c** (540 mg, 1.15 mmol), Bu_4NCL (960 mg, 3.5 mmol), and KF·2H_2O (434 mg, 4.6 mmol) in acetonitrile (25 mL) gave after column chromatography (MeOH/$CHCl_3$ = 1/99, v/v) 296 mg (79%) of **12c**: R_f 0.32 (MeOH/$CHCl_3$ = 3/97, v/v); $[\alpha]^{22}_D$ = +3.9° (*c* = 3.6, MeOH); CIMS (100 eV), m/z (relative intensity) 325 ([M + 1]+, 0.2), 294 ([M – OCH_3]+, 11), 144 ([$C_{10}H_{10}N$]+, 34), 130 ([C_9H_8N]+, 100); ^{1}H NMR (90 MHz) δ 8.15 (br s, 1 H, indole NH), 7.66–7.02 (m, 5 H, indole C(2)H and C(4)-C(7)H), 5.55 (br s, 2 H, NH and OH), 4.90 (AB, 2 H, J = 11.9 Hz, OCH_2S), 3.76 (s, 3 H, $COOCH_3$), 3.42–2.95 (m, 6 H, indole C(3)CH_2CH_2 and $CHCH_2S$).

(*S*)-Methyl 2-Methyl-3-[[[[[2-(3-indolyl)ethyl]amino]-oxy]methyl]thio]propanoate (12d). The same procedure was followed as for **12a**. Compound **11d** (1.5 g, 3.22 mmol), Bu_4NCl (2.7 g, 9.7 mmol), and KF·2H_2O (1.21 g, 12.9 mmol) in acetonitrile (50 mL) gave after column chromatography (EtOAc/*n*-hexane = 1/2, v/v) 693 mg (67%) of **12d** as an oil: R_f 0.45 (EtOAc/*n*-hexane = 1/2, v/v); $[\alpha]^{22}_D$ = –30.4° (*c* = 2.50, MeOH); CIMS (100 eV), *m/z* (relative intensity) 323 ([M + 1]+, 10), 188 ([$C_{11}H_{12}N_2O$]+, 72), 144 [($C_{10}H_{10}N$]+, 81), 130 ([C_9H_8N]+, 100); ^{1}H NMR (90 MHz) δ 8.15 (br s, 1 H, indole NH), 7.63–7.03 (m, 5 H, indole C(2)H and C(4)-C(7)H), 5.91 (very br s, 1 H, NH), 4.86 (s, 2 H, OCH_2S), 3.68 (s, 3 H, $COOCH_3$), 3.40–2.56 (m, 7 H, indole C(3)CH_2CH_2 and $CHCH_2S$), 1.24 (d, 3 H, J = 6.2 Hz, $CHCH_3$).

(1*S*,13b*S*)-1-Amino-13-methyl-1,2,7,8,13,13b-hexahydro-[1,6,2]oxathiazepino[2′,3′:1,2]pyrido[3,4-*b*]indole (1e) and (1*S*,13b*R*)-1-Amino-13-methyl-1,2,7,8,13,13b-hexahydro-[1,6,2]oxathiazepino[2′,3′:1,2]pyrido[3,4-*b*]indole (1f). To a cooled (–74 °C) stirring solution of **12a** (160 mg, 0.37 mmol) in dry dichloromethane (35 mL) in an argon atmosphere was added DIBAL (1.1 mL of a 1 M solution in *n*-hexane, diluted with 10 mL of dichloromethane) in 10 min. After completion of the reaction (15–60 min), as indicated by TLC (EtOAc/*n*-hexane = 1/1, v/v), TFA (0.5 mL) was added in 2 min. After 15 min the reaction mixture was allowed to warm up to room temperature. After additional 20-min stirring at room temperature the reaction mixture was poured into 1 N HCl/Brine = 9/1 (v/v, 50 mL). The organic layer was subsequently washed with 1 N HCl/brine 9/1 (v/v) and brine and dried (Na_2SO_4). The product ratio was determined at this stage by analytical HPLC [waters RCM 8 × 10, reversed-phase C-18 column, acetonitrile/water = 9/1, v/v, flow = 1 mL/min, λ = 282 nm); retention time (min), Boc-protected **1e** (5.6), Boc-protected **1f** (6.5)]. The organic layer was subsequently concentrated in vacuo and subjected to column chromatography (*n*-hexane/$CHCl_3$ = 20/80) to yield 37 mg (25%) of Boc-protected **1e** and 81 mg (57%) of Boc-protected **1f**. Compound **1e**: Boc-protected **1e** (81 mg 0.20 mmol), trimethylsilyl chloride (34 mg, 0.31 mmol), and NaI (47 mg, 0.31 mmol) were dissolved in 10 mL of acetonitrile and stirred for 20 min under an argon atmosphere. The reaction mixture was concentrated to dryness and subjected to column chromatography (EtOAc/*n*-hexane = 1/1, v/v) to yield 41 mg (68%) of **1e** as a white solid: R_f 0.14 (EtOAc/*n*-hexane = 1/1, v/v), 0.55 (MeOH/$CHCl_3$ = 7/93, v/v); $[\alpha]_D$ = –93.9° (*c* = 1.5, MeOH); CIMS (100 eV) exact mass calcd for $C_{15}H_{19}N_3OS$ *m/z* 289.1249 ([M]+), found 289.1248; *m/z* (relative intensity) 290 ([M + 1]+, 3.8), 289 ([M]+, 1.0), 200 ([$C_{12}H_{12}N_2O$]+, 100), 184 ([$C_{12}H_{12}N_2$]+, 28). Compound **1f**: The same procedure was followed as for **1f**. Boc-protected **1f** (34 mg, 0.09 mmol), trimethylsilyl chloride (15 mg, 0.13 mmol), and NaI (20 mg, 0.13 mmol) gave after column chromatography (MeOH/Et_3N/$CHCl_3$ = 1/0.05/98.95) 12 mg (46%) of **1f**: R_f 0.37 (MeOH/$CHCl_3$ = 7/93, v/v); $[\alpha]_D$ = +63.9° (*c* = 1.8, MeOH); CIMS (100 eV) exact mass calcd for $C_{15}H_{19}N_3OS$ m/z 289.1249 ([M]+), found 289.1250; m/z (relative intensity) 289 ([M]+, 0.4), 200 ([$C_{12}H_{12}N_2O$]+, 100), 184 ([$C_{12}H_{12}N_2$]+, 26).

(1*S*-13b*R*)-1-Hydroxy-1,2,7,8,13,13b-hexahydro[1,6,2]oxathiazepino[2′,3′:1,2]pyrido[3,4-*b*]indole (1j) and (1*S*,13b*S*)-1-Hydroxy-1,2,7,8,13,13b-hexahydro[1,6,2]oxathiazepino[2′,3′:1,2]pyrido[3,4-*b*]indole (1k). For cyclization the same procedure as for **1e** and **1f** was followed. **12c** (150 mg, 0.46) gave after column chromatography (EtOAc/*n*-hexane = 1/4, v/v) 21 mg (17%) of **1j** and 62 mg (49%) of **1k**. The product ratio was determined by analytical HPLC [acetonitrile/water = 55/45, v/v, flow = 1 mL/min, λ = 282 nm); retention time (min), **1j** (4.9) and **1k** (5.4)]. Compound **1j**: R_f 0.18 (EtOAc/*n*-hexane = 1/2, v/v), $[\alpha]^{22}_D$ = –75.0° (*c* = 0.4, EtOAc); CIMS (100 eV) exact mass calcd for $C_{14}H_{16}N_2O_2S$ *m/z* 276.0933 ([M]+) found 276.0930; *m/z* (relative intensity), 276 ([M]+, 17), 186 ([$C_{11}H_{10}N_2O$]+, 100). Compound **1k**: R_f 0.18 (MeOH/$CHCl_3$ = 1/99, v/v), 0.24 (EtOAc/*n*-hexane = 1/2, v/v); $[\alpha]^{22}_D$ = –5.8° (*c* = 4.15, MeOH); CIMS (100 eV) exact mass calcd for $C_{14}H_{16}N_2O_2S$ *m/z* 276.0933 ([M]+), found 276.0932; *m/z* (relative intensity) 276 ([M]+, 13), 186 ([$C_{11}H_{10}N_2O$]+, 100). Anal. Calcd for $C_{14}H_{18}N_2O_2S$: C, 60.85; H, 5.84; N, 10.14; S, 11.60. Found: C, 60.68; H, 5.88; N, 10.34; S, 11.55.

(1*S*,13b*R*)-1-Methyl-1,2,7,8,13,13b-hexahydro[1,6,2]oxathiazepino[2′,3′:1,2]pyrido[3,4-*b*]indole (1l). For cyclization the same procedure as for **1e** and **1f** was followed. **12d** (215 mg, 0.67 mmol) gave after column chromatography (EtOAc/*n*-hexane = 1/4, v/v) 125 mg (69%) of **1l** as an amorphous white solid and 13 mg of an unidentified, but by NMR (90 MHz) clearly not cyclized, product. By TLC (EtOAc/*n*-hexane = 1/2, v/v) and analytical HPLC [acetonitrile/water = 4/1, v/v, flow = 1 mL/min, λ = 254 nm; retention time (min, **1l** (6.1)] the other possible formed diasteroisomer could not be detected. **1l** crystallized from CH_2Cl_2/*n*-hexane (mp 135–141 °C); R_f 0.50 (EtOAc/*n*-hexane = 1/2, v/v); $[\alpha]^{22}_D$ = +94.8° (*c* = 1.55, MeOH); CIMS (100 eV), *m/z* (relative intensity) 274 ([M]+, 38), 186 ([$C_{11}H_{10}N_2O$]+, 100), 170 ([$C_{11}H_{10}N_2$]+, 25), 144 ([$C_{10}H_{10}N$]+, 16), 130 ([C_9H_8N]+, 16). Anal. Calcd for $C_{16}H_{18}N_2OS$: C, 65.66; H, 6.61; N, 10.21; S, 11.69. Found: C, 65.60; H, 6.75; N, 9.96; S, 11.65.

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