Eur J Med Chem (1995) 30, 659–671

Bimane cyclic esters, possible stereologues of trypanothione as antitrypanosomal agents. Bimanes 29

EM Kosower[1], AE Radkowsky[1], AH Fairlamb[2], SL Croft[2], RA Neal[2,†]

[1]*Biophysical Organic Chemistry Unit, School of Chemistry, Tel-Aviv University, Ramat-Aviv, Tel-Aviv, 69978 Israel;*
[2]*Department of Medical Parasitology, Parasite and Vector Biochemistry Unit, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK*

(Received 5 January 1995; accepted 30 March 1995)

Summary — Tricyclic esters derived from bimanes have been synthesized with ring sizes near or equal to that of trypanothione disulfide ($T(S)_2$), a bis-glutathionylspermidine that is involved in regulating the thiol status of *Leishmania* and other trypanosomatids. Modest activity for many of the compounds against *Leishmania donovani* with a maximum at the $T(S)_2$ ring size suggests that the esters act as $T(S)_2$ surrogates. However, no inhibition of $T(S)_2$-reductase is observed for a number of the compounds. A series of tricyclic bimane amides with structures more closely analogous to $T(S)_2$ are inactive in biological tests. New approaches were developed for the synthesis of the amides. The surprising effectiveness of the cyclic ester synthesis is explained. Acid chloride formation catalyzed by sulfides is briefly described.

trypanothione / antitrypanosomal drug / bimane ester / sulfide catalysis / acid chloride formation / trypanothione stereologue

Introduction

Diseases caused by trypanosomatid parasites and related diseases in tropical and subtropical areas represent a major danger to individuals and thus, to the civic stability and developmental potential of countries in the region. The three major diseases in the class, human African trypanosomiasis, leishmaniasis and Chagas' disease, have no effective common cures [1]. It has been estimated that 12 million new cases of leishmaniases occur each year [2]. Argentina and Brazil are developing countries in which Chagas' disease interferes with the life and the social and economic development of the population. The underdevelopment of parts of Africa, notably Tanzania, has been attributed in part to endemic human African trypanosomiases. The discovery of trypanothione [3–5], an essential metabolic component of the trypanosomatid parasites causing the diseases, has opened the way for new approaches to drugs for treatment. Trypanothione (N^1,N^8-bis-(glutathionyl)spermidine, $T(SH)_2$; disulfide form, $T(S)_2$) appears to control the thiol status of the protozoan parasites responsible for trypanosomiases in the same way [5] that glutathione (γ-glu-cys-gly, GSH; disulfide form, GSSG) controls the thiol status in cells of most higher organisms [6–9].

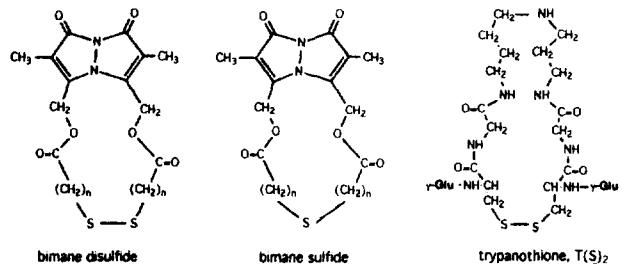
DL-α-Difluoromethylornithine (DFMO) has recently been introduced to replace melarsoprol in the treatment of West African sleeping sickness. Its primary effect is inhibition of ornithine decarboxylase [10] and consequently depletion of spermidine and trypanothione and accumulation of *S*-adenosylmethionine [11]. The subsequent events leading to cell death are the subject of much debate (see reference [5] for a review). Although DFMO blocks growth of *Leishmania* promastigotes grown in polyamine-depleted culture [12, 13], its effects in animal models are suppressive but not curative [13]. DFMO has no effect against *Trypanosoma cruzi* [14]. The synergism between DFMO and melarsoprol in African trypanosomiasis [15, 16] could be due to depletion of trypanothione by inhibition of biosynthesis by DFMO [11] coupled with intracellular complex formation between trypanothione and the arsenical drug [17]. However, melarsoprol-resistant lines do not differ in trypanothione content [18, 19] and resistance is associated with loss of a P-2 adenosine transporter which may be

†Deceased June 1990.

Abbreviations: $T(SH)_2$, reduced trypanothione; $T(S)_2$, trypanothione; GSSG, glutathione disulfide; GSH, glutathione; DFMO, DL-α-difluoromethylornithine.

responsible for drug uptake into the cell [20]. Another possible target for the arsenical drug is dihydrolipoamide dehydrogenase which has different degrees of expression in the procyclic and blood-stream forms of *T brucei* even though there is only one gene [21]. These points emphasize the need for continuing the search for suitable drugs against trypanosomatid diseases, whose urgency is increased by the growing incidence of laboratory-acquired infections [22]. A recent review of drugs used against human African trypanosomiasis offers a somber picture of current drug treatments [23].

We noted several years ago the resemblance of the shape of novel bimane cyclic esters to trypanothione. We hypothesized that the bimane esters may serve as surrogates for the substrates of trypanothione-related enzymes. We therefore designed a series of bimane derivatives as potential inhibitors of enzymes, such as trypanothione reductase in the hope that such compounds would be active as antitrypanosomal drugs. The formulas of the bimane esters (some sulfides, some disulfides) are shown next to the formula for trypanothione in the disulfide form.

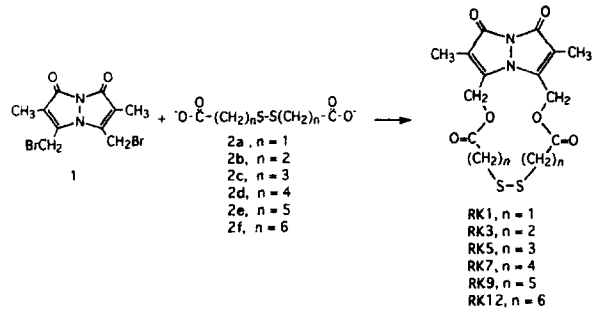


Chemistry

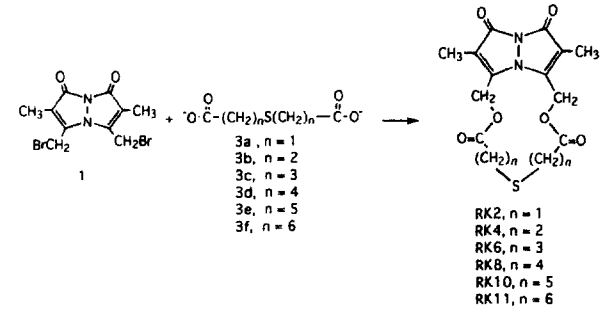
Tricyclic bimane diesters

The syntheses of tricyclic bimane esters involves the synthesis of the ω,ω'-dithio- or ω,ω'-thiodialkanoic acids and reaction of the diacids with *syn*-(bromomethyl,methyl)bimane (bBBr, **1**) [24]. The syntheses and/or sources for the dicarboxylic acids are given in the *Experimental protocols*. The materials for testing as drugs are denoted as **RK *n***, in which the diesters are **RK 1** to **RK 12** (drug candidates are often labeled with an abbreviation for the organization or creator and a number, hence Radkowsky-Kosower or **RK *n***).

To form the **RK 1** to **RK 12** diesters, the dipotassium salts of the dicarboxylic acids **2** or **3** were refluxed in acetonitrile with **1**. Some physical and chemical properties of the esters **RK 1–5** have been reported but the procedures used in the present article are improved over those used previously [25] (see eqs [1] and [2]). Dibenzo-18-crown-6-ether was used to solubilize the potassium salts of the diacids with $n = 1$ and 2. Dimers and oligomers were formed in all the ester syntheses. The chromatographed **RK** esters were recrystallized and characterized by mass spectra, NMR spectra, melting point, and solubility.

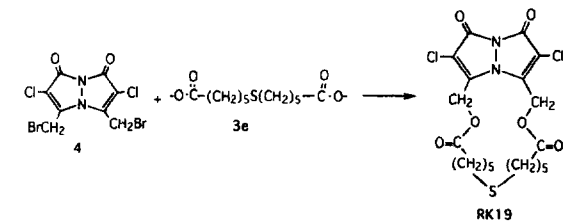


[1]



[2]

An α-chloro tricyclic bimane diester, *syn*-(bromomethyl, chloro)bimane **4**, was prepared by bromination with pure bromine of *syn*-(methyl, chloro)bimane in 67% yield [26]. The diester, **RK 19** [μ-($O_2C(CH_2)_5S$-$(CH_2)_5CO_2$)-*syn*-(CH_2,Cl)B], was prepared from the tetramethylammonium salt of 6,6'-thiodihexanoic acid **3e** and *syn*-(bromomethyl, chloro)bimane **4** in dichloromethane in 25% yield (eq [3]). Although **RK 19** was somewhat unstable on chromatography on silica gel, it proved to be reasonably stable to hydrolysis, with only 12% being lost in a 24 h period on standing in dilute aqueous solution (3.2% CH_3CN).

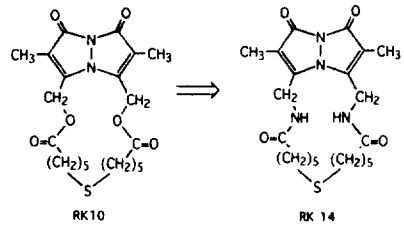


[3]

Tricyclic bimane bis-amides

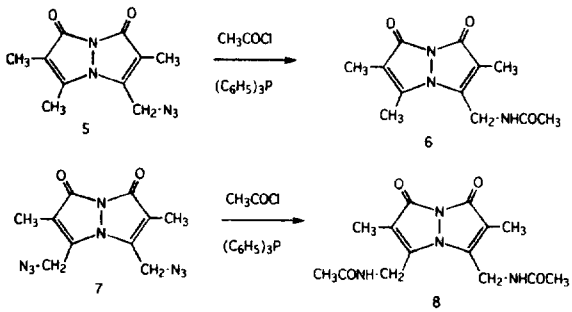
To enhance the similarity of the bimane derivative to trypanothione, we chose as a target the amide

analogue of the most active bimane ester, **RK 10**, a compound designated as **RK 14**. Typically, amides can be prepared by the reaction of a primary amine with an activated carboxylic acid derivative.



Two approaches (acetamide and the Gabriel synthesis) to the synthesis of *syn*-(aminomethyl, methyl)-bimane only yielded the products of *O*-alkylation, either diacetate or diol. The Staudinger reaction of azides seemed a suitable alternative. Our work suggests that heating an azide, a carboxylic acid and triphenylphosphine (Ph_3P) in a low polarity organic solvent [27] to yield an amide apparently proceeds *via* the anhydride.

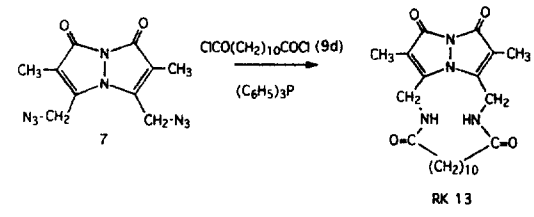
We showed that the Staudinger reaction of acetyl chloride and triphenylphosphine with *syn*-(azidomethyl, methyl)(methyl, methyl)bimane **5** [28] produced *syn*-(acetamidomethyl, methyl)(methyl, methyl)bimane **6**. However, we found that the *syn*-(azidomethyl, methyl)bimane **7** could be converted into *syn*-(acetamidomethyl, methyl)bimane **8** in modest yields only after we changed in the order of addition of the reactants (Shalev *et al*, submitted for publication) (eq [4]). Adding triphenylphosphine to a mixture of the bis-azide **7** and acetyl chloride gave close to the theoretical yield of triphenylphosphine oxide (96%) and increased the yield of the bis-acetamido bimane **8** from 12 to 28%.



[4]

Bis-amide ring formation

A number of α,ω-diacyl dichlorides ($ClOC(CH_2)_n$-$COCl$, n = 3, 4, 8, 10) **9a,b,c,d** were reacted with bBN_3 (**7**) and triphenylphosphine to generate cyclic bis-amides (eq [5] illustrates the reaction of **9d**, n = 10, to form **RK 13**). The yields are compared with those of the size-related esters derived from mono or dithio diacids in table I. Variation in the phosphine or the use of bis-phosphines gave similar or lower yields in most cases.



[5]

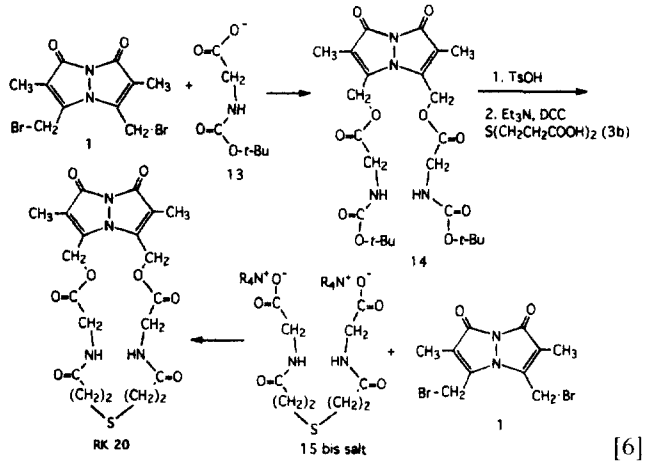
Glutaryl dichloride, n = 3 **9a**, forms the small tricyclic bis-amide **10** easily but adipoyl dichloride, n = 4 **9b**, gives no tricyclic product. The yields of bis-amides fall with the length of the diacyl dichloride in contrast to the yields for the tricyclic bis-esters for which the yield rises with ring size (**RK 5** to **8**). This point will be considered in the *Discussion*.

Triphenylphosphine addition to a mixture of bis-azidobimane **7** and the diacyl chloride, 6,6'-thiodihexanoyl dichloride **12**, in a large amount of acetonitrile gave **RK 14** in 20% yield. The use of a bis-phosphine (one example is given in table I) increased the yield somewhat.

Compound **12**, could be prepared from the acid and thionyl chloride *in situ* without distillation by using the Bosshard (Vilsmeier) reagent [29, 30]. The extremely rapid conversion of the acid **3e** to the acyl chloride suggested catalysis by the sulfide group. A literature search did not reveal any similar finding, and successful catalysis with dibenzothiophene suggested that the phenomenon might be general.

Tricyclic bis-ester bis-amide bimanes

To enhance the resemblance of the surrogates to trypanothione and keep the overall ring size at 24 atoms, **RK 20** was made in two ways. The first involved preparing a bimane bis-ester with *N*-protected glycines, deprotection and formation of the bis-amide. The second started with carboxylate-protected glycines, formation of the bis-amide, deprotection, and reaction of the salt of **15** with dibromobimane **1** (eq [6]). Two criteria for the salt, sufficient solubility in acetonitrile and non-hygroscopicity, had to be satisfied. The results with various salts are summarized in table II. Soluble salts reacted almost instantaneously. However, most of the salts were very hygroscopic and could be kept in a solid form only under a layer of dry THF. The benzyl trimethylammonium salt was the best after drying with THF, giving a 39% yield of **RK 20**. Another difficulty was also that **RK 20** was only soluble in dimethylsulfoxide (DMSO), from which it could be precipitated by the addition of ethyl acetate.



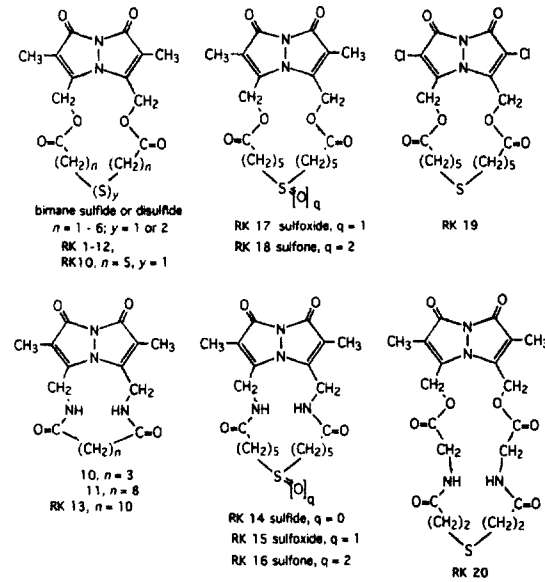
[6]

Sulfoxide and sulfones

In the hope of increasing water solubility of the **RK** compounds, **RK 10** was converted into the sulfoxide **RK 17** and sulfone **RK 18**, and **RK 14** into the sulfoxide **RK 15** and sulfone **RK 16** with 3-chloroperbenzoic acid. The ^1H NMR peaks for the β-methylene protons of **RK 15** indicated two configurations of the molecule. The sulfoxide is much more polar than the sulfone (TLC). The chemical shifts of the methylene protons adjacent to sulfur reflect the oxidation state of the sulfur: 1) esters **RK 10** (S) 2.55, **RK 17** (SO) 2.79, **RK 18** (SO_2) 3.04 ppm; and 2) amides **RK 14** (S) 2.56, **RK 15** (SO) 2.81, and **RK 16** (SO_2) 3.04 ppm.

Pharmacology

The formulas for the tricyclic derivatives of *syn*-bimane, the **RK** ***n*** (Radkowsky-Kosower) compounds, are summarized below.



The biological test results are given in table III. A plot of biological activities for inhibition of *Leishmania donovani* for the ester series (**RK 1** to **RK 12**) against ring size revealed that activity peaked at

Table I. Tricyclic bis-amide and bis-ester yields as a function of chain length (reactants: $ClOC(CH_2)_nCOCl$ or $KOOC(CH_2)_n(S)_q(CH_2)_nCOOK$).

Reaction[a]	*n*	*q*	*Phosphine*[b]	*Product*	*Yield (%)*	*P=O*[c] *(%)*
A	3	0	Ph_3P	**10**	47	99
E	1	1		**RK 2**	31	
A	4	0	Ph_3P	–	0	91.8
E	1	2		**RK 1**	12	
A	8	0	Ph_3P	**11**	17.6	88
E	3	2		**RK 5**	57	
E	3	1		**RK 6**	40	
A	10	0	Ph_3P	**RK 13**	14	89
E	4	2		**RK 7**	61	
E	4	1		**RK 8**	28	
A	5	1	Ph_3P	**RK 14**	19.9	86
A	5	1	BDPE	**RK 14**	26.1	82
E	5	1		**RK 10**	44	

[a]A, amide from bis-azido bimane **7**, phosphine, and diacyl dichloride. E, ester from bBBr **1** and the potassium salts of the diacids. [b]Ph_3P, triphenylphosphine; BDPE, bis-(diphenylphosphinyl)ethane. [c]Yield of phosphine oxide.

Table II. Effectiveness of diacid salts in **RK *n*** formation.

Cation[a]	*Acid*	*Salt*	*Solubility*	*Rate*	*Product*
Me_4N^+	**3e**	Very hygroscopic	Insoluble DMF, CH_3CN	[b]	
Et_4N^+	**3e**	Extremely hygroscopic	Soluble CH_3CN	Fast	**RK 10** with other Et_4N^+ compounds
DIMP	**15**	Very hygroscopic	Sparingly soluble CH_3CN	Very fast	**RK 20** with contamination
$BzMe_3N^+$	**16**	Solid	Soluble CH_3CN	Very fast	**RK13** (42.5% yield)
$BzMe_3N^+$	**15**	Solid	Sparingly soluble CH_3CN	Slow[c]	**RK 20** (39% yield)
$BzMe_3N^+$	**15**	Solid	DMF	Moderate[d]	Some **RK 20**
Li^+	**15**	Solid	Insoluble DMF, CH_3CN	Very slow	Very little **RK 20**

[a]DIMP: *N,N*-dimethylpiperidinium; $BzMe_3N^+$: trimethylbenzylammonium. [b]Not utilized because of insolubility. [c]10 h reflux. [d]3–4 h, rt.

Table III. Trypanothione surrogates[a].

Compound	*Short sample name*	*Mp (°C)*	*MW*	*Activity % inhibition (30 μM)*	*General formula*	*Ring size S = (n, y)*	*Bimane link (E = ester, A = amide)*
RK 1	SS(1)	202–205	370	na[b]	Disulfide, 1	17 (1,2)	E
RK 2	S(1)	225	338	na[b]	Sulfide, 1	16 (1,1)	E
RK 3	SS(2)	195	398	na[b]	Disulfide, 2	19 (2,2)	E
RK 4	S(2)	236	366	na[b]	Sulfide, 2	18 (2,1)	E
RK 5	SS(3)	207–208	426	35	Disulfide, 3	21 (3,2)	E
RK 6	S(3)	236	394	na[b]	Sulfide, 3	20 (3,1)	E
RK 7	SS(4)	188.5	454	78[c]	Disulfide, 4	23 (4,2)	E
RK 8	S(4)	178	422	69	Sulfide, 4	22 (4,1)	E
RK 9	SS(5)	160–162	482	43	Disulfide, 5	25 (5,2)	E
RK 10	S(5)	187	450	92[d]	Sulfide, 5	24 (5,1)	E
RK 11	S(6)	157	478	11	Sulfide, 6	26 (6,1)	E
RK 12	SS(6)	140	510	na[b]	Disulfide, 6	27 (6,2)	E
RK 13	C(10)	? dec	416	42	No sulfur	23	A
RK 14	S(5)	? dec	448	na[e]	Sulfide	24 (5,1)	A
RK 15	SO(5)	? dec	464	na[e]	Sulfoxide	24 (5,1)	A
RK 16	SO_2(5)	? dec	480	na[e]	Sulfone	24 (5,1)	A
RK 17	SO(5)	180–181	466	na[e]	Sulfoxide	24 (5,1)	E
RK 18	SO_2(5)	175–176	482	na[e]	Sulfone	24 (5,1)	E
RK 19	S(5), Cl	164–165	490.7	Active[f]	Sulfide	24	E
RK 20	S(2)-gly	dec	480	29	Sulfide	24	AE

[a]The purity of the compounds was shown by TLC (essentially one spot) (> 99% pure). All compounds are crystalline and have been purified by chromatography and by recrystallization when necessary. All compounds have been characterized by mass spectrometry and NMR. [b]na = not active at 30 μM (0–4% inhibition). [c]20% inhibition of *L donovani* in BALB/c mice subcutaneous 100 mg/kg/d × 5 d. No mortality. [d]44% inhibition of *L donovani* in BALB/c mice subcutaneous 100 mg/kg/d × 5 d. No mortality. [e]na = not active at 30 μM; quantitative data not supplied. [f]68% inhibition at 10 μM; toxic to macrophages and amastigotes at 30 μM.

RK 10, the tricyclic compound with the same number of atoms in the ring as trypanothione (fig 1). Structure–activity relationships with respect to the most active ester of the series (**RK 10**) revealed the following. First, conversion of the sulfide to the sulfoxide (**RK 17**) or the sulfone (**RK 18**) completely abolishes activity. Likewise, replacement of one or both of the ester linkages between the bimane and the sulfur-containing bis-substituent with an amide linkage (**RK 14**) and other modifications (**RK 13, 15, 16**) completely abolished activity except for one ester amide (**RK 20**), which showed modest activity.

Results and discussion

The goal of the present research was to synthesize a series of compounds as surrogates for trypanothione, a trypanosomatid-specific disulfide. We prepared a series of cyclic esters in which the ring size was varied around that corresponding to $T(S)_2$. Many of these had modest activity against the trypanosomatid *L donovani* in tissue culture; *more remarkably, the activity was a function of ring size and reached a maximum at the ring size which corresponded to that of trypanothione* (fig 1). The pattern of activity against *L donovani versus* ring size suggested that a trypanothione enzyme or binding protein is the site of action. We had hoped to inhibit $T(S)_2$ reduction. However, none of the esters, even the most active of the series, inhibited *T cruzi* trypanothione reductase (experiments carried out by Dr Krauth-Siegel) [31]. Thus, this class of active compounds has a target or an action which is as yet unknown; our surmise is that they are *shape surrogates* of $T(S)_2$ and affect some as yet unidentified function of $T(S)_2$. With the positive, albeit modest, therapeutic response of the esters, we anticipated greater activity with further modifications. One such change was the introduction of amide linkages so as to enhance the structural similarity to $T(S)_2$. However the amide derivatives proved to be inactive against *L donovani* in tissue culture.

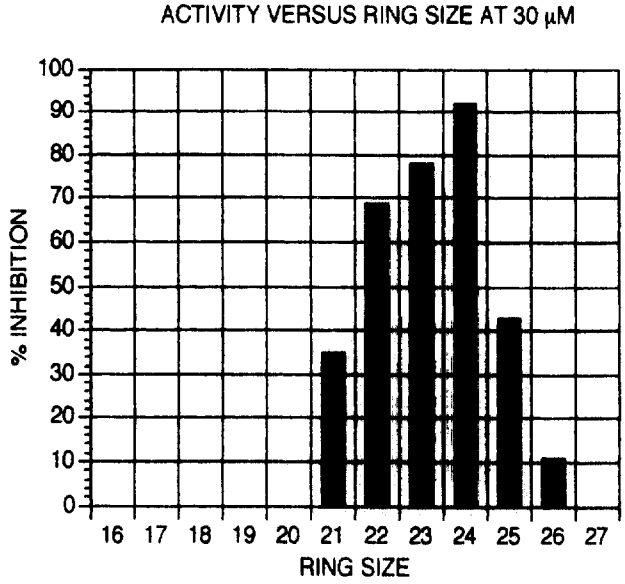


Fig 1. Biological activity of bimane esters. A plot of biological activity (% inhibition of *L donovani*) *versus* ring size of the tricyclic bimane esters, **RK 1–RK 12**.

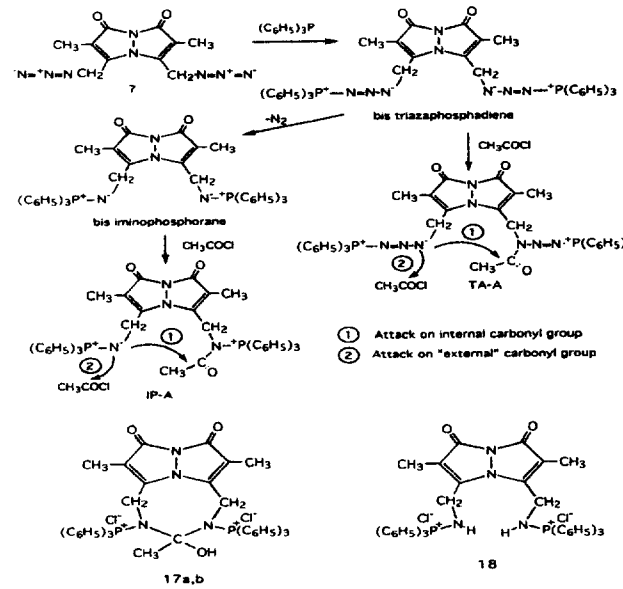
We have discovered a promising new approach to drugs for the treatment of diseases caused by trypanosomatids. Some of the most attractive targets for drug therapy against trypanosomatids are the enzymes or proteins concerned with the parasite-specific compound, trypanothione. Trypanothione reductase from the parasite of an insect trypanosomatid, *Crithidia fasciculata*, has been isolated, purified and crystallized [32–34]. The active site has been modeled and shown to accommodate very well the neuroleptic drug, clomipramine [35]. The *C fasciculata* trypanothione reductase shares some 40% sequence similarity with mammalian glutathione reductase [5, 36]. The crystal structure of a complex of the *C fasciculata* trypanothione reductase with N^1-glutathionylspermidine, a metabolic precursor of trypanothione, has been determined to 2.8 Å resolution, showing that there are no important rearrangements at the binding site by comparison with the previously determined structure of the native enzyme [33]. The trypanothione reductase from *T cruzi* has been crystallized and subjected to X-ray analysis [37, 38]. Many relevant biochemical studies on the $T(S)_2$ reductase and other enzymes have been carried out [31, 39–42]. Were a successful inhibitor of trypanothione reductase to be found, a further difficulty in its application to the organism would be the presence of ovothiol A in substantial amounts at late stages of logarithmic growth [43]. We do not have enough evidence to specify the target or targets of the bimane esters, but preliminary tests for inhibition of trypanothione reductase by RK 10 have been negative.

Our discovery that the order of addition of reagents in Staudinger reaction of azides changed the nature of the products led to an extensive study that will be reported elsewhere. The lessons of that study lead us to compare two intermediates, the first reaction product derived from the bis-iminophosphorane (IP-A) and acetyl chloride and the first reaction product derived from the reaction of the triazaphosphadiene and acetyl chloride (TA-A). This comparison is illustrated in scheme 1. The results demonstrate that internal attack (1) is favored over 'external' attack (2) in the case of IP-A and that the two routes are compa-

rable in the case of TA-A. Delocalization of the negative charge probably decreases the nucleophilicity of the nitrogen in the second triazaphosphadiene group sufficiently to make the 'external' electrophile competitive. The quotes on 'external' cover the cases in which the second acyl group is attached to the first as in a dicarboxylic acid dichloride.

A plausible product of internal attack in the case of IP-A is the tricyclic **17** for which there are two isomers, one with the *N,N*-bridging methyl pointing towards the bimane ring, and one with the methyl group pointing away, at least on the NMR time-scale. A moderately pure fraction of IP-A could be eluted from silica gel with 6% methanol in acetonitrile. The material exhibited three sets of peaks corresponding to the α-methyl (1.57, 1.68 ppm), the bridging methyl (2.10, 2.17 ppm), and the β-methylene (4.55, 4.67 ppm) groups, respectively. Also isolated was a mixture of **17a** and **17b** with the bis-triphenylphosphonio derivative **18**, for which the ^{1}H NMR position of the α-methyl peak is at 1.21 ppm, the furthest upfield for that group in any of the phosphonium-type bimane derivatives we have encountered in our study of the Staudinger reaction. Characteristic low field peaks (7.6–7.97 ppm) are found for the phenyl hydrogens in triphenylphosphonio groups.

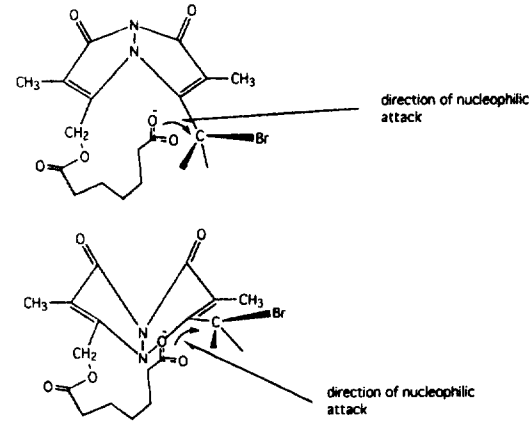
Finally, we consider why the cyclic bimane esters should form so easily, without the use of the high dilution techniques sometimes required for formation of such rings (12–23 atoms in the ring external to the bimanes; the 23-atom ring compound is **RK 12**). The formation of the 13- to 17-membered rings by the simple displacement reaction between bBBr (**1**) and an alkane dicarboxylate is somewhat unexpected. Ring size did not seem to have much effect on the yields (33–72%, except for **RK 1**, for which the procedure was not optimized). The conclusion is that the length of the carboxylic acid chain was of relatively little importance in determining the success of the reaction. The rate of ring closure depends upon an intramolecular route being favored over an intermolecular pathway. If the reactant group is in effect a larger target, the probability of intramolecular reaction would increase. We propose that the flipping of the bimane ring (an experimental fact [44]) causes the reactant group to occupy a larger effective volume, and thus increases the probability of intramolecular reaction (scheme 2).



Scheme 1.

Conclusions

Derivatives of bimanes, a new entity for potential drugs, have been shown to have moderate *in vitro* and *in vivo* activity against *L donovani*. The qualitative resemblance in shape of the cyclic bimane esters to the trypanothione, the natural compound that maintains the thiol status of trypanosomatids, has served as a useful stimulus for the preparation of biologically active materials. The cyclic bimane esters are sufficiently active in biological tests to encourage the synthesis of further variants of tricyclic bimanes. Limitations on the use of similarity as a factor in drug design are illustrated in the case of cyclic bimane amides, which are inactive even though designed to be more similar to trypanothione than the esters. The problems solved in the course of synthesizing the



Scheme 2.

cyclic amides led to (a) new insights into the Staudinger reaction of azides and (b) a new method for catalyzing formation of acid chlorides.

Experimental section

General

The instruments used were as follows. ^1H NMR spectra: Bruker AM-200 spectrometer (chemical shifts are given in δ-values (ppm) downfield from tetramethylsilane as 0.000 or 5.000 (HDO) for samples in D_2O); ultraviolet and visible spectra: Hewlett-Packard 8452A spectrophotometer; fluorescence spectra: Hitachi-Perkin-Elmer MPF-4 fluorescence spectrophotometer; mass spectra: Du Pont 21-491B mass spectrometer; IR spectra: Nicolet 5DX FTIR spectrometer. Certain samples were measured as deposits on a silver halide fiber using infrared fiberoptic spectroscopy with a specially designed cell [45, 46]. Purity was demonstrated in three different chromotography systems.

Chromatography

Flash chromatography on Merck Kieselgel 60 (230–400 mesh) or Merck Cellulose was carried out with elution at the rate of 2.5 cm/30–40 s. TLC separations were done on silica gel (Merck Kieselgel 60 F_{254}) plastic sheets (0.2 mm).

Solvents and materials

Some reaction solvents were dried and distilled, acetonitrile from P_2O_5, tetrahydrofuran and dimethylformamide from CaH_2. Chloroform was purified by passage through a column of alumina. Water was deionized and distilled. Other solvents were used without purification. Buffer, pH 7.3, was made from KH_2PO_4 and Na_2HPO_4. *syn*-(Bromomethyl,methyl)bimane (bBBr, **1**) was prepared by the published procedure [24]. 2,2'-Thiodiacetic acid **3a**, 3,3'-thiodipropionic acid **3b**, 2,2'-dithiodiacetic acid **2a**, and 3,3'-dithiodipropionic acid **2b** were purchased (Aldrich) and used without further purification.

The other thio-(**3c,d,e,f**) and dithiocarboxylic acids (**2c,d,e,f**) were prepared by modifications of known methods [47–59]. Details will be supplied by the authors to anyone encountering difficulties in the syntheses.

Biological tests

In vitro test

Mouse macrophages were isolated from the peritoneal cavity of outbred CD1 mice (Charles Rivers Ltd, UK) and cultured in Labtek eight-well tissue chamber slides in RPMI 1640 medium plus 10% heat-inactivated fetal calf serum (Gibco, UK) at 37°C in a 5% CO_2/air mixture. Macrophages were infected with *L donovani* (MHOM/ET/67/L82) amastigotes, freshly isolated from the spleen of a male golden hamster (Wright's strain). Infected macrophage cultures were incubated in the presence of drug-containing medium for 7 d, with medium being replaced on days 1, 3 and 5 after infection. Compounds were tested in a three-fold dilution series from 30 μM with four replicates at each concentration. The proportion of infected macrophages in Giemsa-stained preparations were determined after a 7 d exposure to the drugs. ED_{50} values were calculated by sigmoidal analysis [60].

In vivo test

Male BALB/c mice were infected iv with 5×10^6 *L donovani* amastigotes freshly isolated from the spleen of a donor hamster. One week after infection mice were randomly assorted into groups of five and treated with compounds ball-milled in 0.25% methyl cellulose. All mice were dosed once a day for 5 d by the subcutaneous route. Three days after the completion of drugging mice were sacrificed, liver smears prepared and Giemsa stained. Drug activity was calculated from the number of amastigotes/500 liver cells × liver weight (mg) in treated and untreated groups.

Syntheses and characterization

***RK 1**: 4,6-(1,10-(2,9-Dioxa-3,8-dioxo-5,6-dithiadecamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [μ-($O_2CCH_2SSCH_2CO_2$)-9,10-dioxa-syn-(CH_2,CH_3)B]*

A suspension of dipotassium 2,2'-dithiodiacetate (from **2a**) (0.780 g, 3.0 mmol) in a solution of *syn*-(bromomethyl, methyl)bimane (bBBr, **1**) (1.050 g, 3.0 mmol) and dibenzo-18-crown-6-ether (1.016 g, 2.91 mmol) in acetonitrile (60 mL) was stirred rapidly and heated at 60–70°C overnight. The solid was filtered off, the filtrate evaporated and the residue flash chromatographed (eluant: dichloromethane with 10–15% ethyl acetate) to give unreacted 1, 0.122 g (37% recovery) and **RK 1**, 0.200 g (18% yield). **RK 1**: Recrystallized from ethyl acetate/diethyl ether, sublimes 190–200°C, ^1H NMR ($CDCl_3$) δ: 1.982 (s, 6H, α-CH_3), 3.649 (s, 4H, CH_2S), 5.157 (s, 4H, β-CH_2) ppm, MS: (M^+) 370 (49%).

***RK 3**: 4,6-(1,12-(2,11-Dioxa-3,10-dioxo-6,7-dithiadodecamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [μ-($O_2CCH_2CH_2SSCH_2CH_2CO_2$)-9,10-dioxa-syn-($CH_2$,$CH_3$)B]*

The procedure described for **RK 1** gave **RK 3** from **2b** in 59% yield after flash chromatography (eluant: dichloromethane containing 10–15% ethyl acetate). **RK 3**: Recrystallized from ethyl acetate/diethyl ether, mp 199–201°C, ^1H NMR ($CDCl_3$) δ: 1.948 (s, 6H, α-CH_3), 2.846 (t, 4H, J = 7 Hz, CH_2CO), 2.983 (t, 4H, J = 7 Hz, CH_2S), 5.137 (s, 4H, β-CH_2) ppm, MS: (M^+) 398 (2%).

***RK 5**: 4,6-(1,14-(2,13-Dioxa-3,12-dioxo-7,8-dithiatetradecamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [μ-($O_2C(CH_2)_3SS(CH_2)_3CO_2$)-9,10-dioxa-syn-($CH_2$,$CH_3$)B]*

The procedure described for **RK 1** gave **RK 5** from **2c**, except that dibenzo-18-crown-6-ether was omitted and the reaction solution was sonicated for a few minutes prior to heating, in 57% yield after flash chromatography (eluant: 3.5% ethyl acetate in dichloromethane). **RK 5**: Recrystallized from ethyl acetate/diethyl ether, mp 207–208°C, ^1H NMR ($CDCl_3$) δ: 1.970 (s, 6H, α-CH_3), 2.070 (q, 4H, J = 7.0 Hz), 2.595 (t, 4H, J = 7.0 Hz, CH_2CO), 2.789 (t, 4H, J = 6.9 Hz, CH_2S), 5.149 (s, 4H, β-CH_2) ppm, MS: (M^+) 426 (100%).

***RK 7**: 4,6-(1,16-(2,15-Dioxa-3,14-dioxo-8,9-dithiahexadecamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [μ-($O_2C(CH_2)_4SS(CH_2)_4CO_2$)-9,10-dioxa-syn-($CH_2$,$CH_3$)B]*

The procedure described for **RK 5** gave **RK 7** from **2d** in 61% yield after flash chromatography (eluant, 3% ethyl acetate in dichloromethane). **RK 7**: Recrystallized from ethyl acetate, mp 188.5°C, ^1H NMR ($CDCl_3$) δ: 1.791 (m, 8H), 1.973 (s, 6H, α-CH_3), 2.474 (t, 4H, CH_2CO), 2.720 (t, 4H, CH_3S), 5.123 (s, 4H, β-CH_2) ppm, MS: (M^+) 454 (100%).

***RK 9**: 4,6-(1,18-(2,17-Dioxa-3,16-dioxo-9,10-dithia-octadecamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [μ-($O_2C(CH_2)_5SS(CH_2)_5CO_2$)-9,10-dioxa-syn-($CH_2CH_3$)B]*
The procedure described for **RK 5** gave **RK 9** from **2e** in 49% yield after flash chromatography (eluant, 5–8% ethyl acetate in dichloromethane). **RK 9**: Recrystallized from ethyl acetate/hexane, mp 160–162°C, ^{1}H NMR ($CDCl_3$) δ: 1.505 (m, 4H), 1.724 (m, 8H) 1.956 (s, 6H, α-CH_3), 2.454 (t, 4H, J = 6.6 Hz, CH_2CO), 2.728 (t, 4H, J = 6.8 Hz, CH_2S), 5.147 (s, 4H, β-CH_2) ppm, MS: (M^+) 482 (100%).

***RK 12**: 4,6-(1,20-(2,19-Dioxa-3,18-dioxo-10,11-dithia-eicosamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [μ-($O_2C(CH_2)_6SS(CH_2)_6CO_2$)-9,10-dioxa-syn-($CH_2CH_3$)B]*
The procedure described for **RK 5** gave **RK 12** from **2f** in 72% yield after flash chromatography (eluant, 7.5% ethyl acetate in dichloromethane). **RK 12**: mp 140°C, ^{1}H NMR ($CDCl_3$) δ: 1.429 (m, 8H), 1.678 (m, 8H), 1.968 (s, 6H, α-CH_3), 2.416 (t, 4H, J = 6.6 Hz, CH_2CO), 2.718 (t, 4H, J = 6.8 Hz, CH_2S), 5.092 (s, 4H, β-CH_2) ppm, MS: (M^+) 510 (100%).

***RK 2**: 4,6-(1,9-(2,8-Dioxa-3,7-dioxo-5-thia-nonamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [μ-($O_2CCH_2SCH_2CO_2$)-9,10-dioxa-syn-(CH_2CH_3)B]*
The procedure described for **RK 1** gave **RK 2** from **3a** in 31% yield after flash chromatography (eluant: 20% ethyl acetate in dichloromethane). **RK 2**: Recrystallized from ethyl acetate/diethyl ether, mp 225°C, ^{1}H NMR ($CDCl_3$) δ: 1.979 (s, 6H, α-CH_3), 3.411 (s, 24H, CH_2S), 5.106 (s, 4H, β-CH_2) ppm, MS: (M^+) 338 (100%).

***RK 4**: 4,6-(1,11-(2,10-Dioxa-3,9-dioxo-6-thia-undecamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [μ-($O_2CCH_2CH_2SCH_2CH_2CO_2$)-9,10-dioxa-syn-($CH_2CH_3$)B]*
The procedure described for **RK 1** gave **RK 4** from **3b** in 50% yield after flash chromatography (eluant: 15% ethyl acetate in dichloromethane). **RK 4**: Recrystallized from ethyl acetate/diethyl ether, mp 232-235°C; ^{1}H NMR ($CDCl_3$) δ: 1.954 (s, 6H, α-CH_3), 2.787 (m, 4H, CH_2CO), 2.904 (m, 4H, CH_2S), 5.084 (s, 4H, β-CH_2) ppm, MS: [M^+] 366 (100%).

***RK 6**: 4,6-(1,13-(2,12-Dioxa-3,11-dioxo-7-thia-tridecamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [μ-($O_2C(CH_2)_3S(CH_2)_3CO_2$)-9,10-dioxa-syn-($CH_2CH_3$)B]*
The procedure described for **RK 5** gave **RK 6** from **3c** in 40% yield after flash chromatography (eluant, 15% ethyl acetate in dichloromethane). **RK 6**: mp 236.5°C, ^{1}H NMR ($CDCl_3$) δ: 1.964 (s, 6H, α-CH_3, m, 4H CH_2), 2.616 (t, 8H, J = 7.1 Hz, CH_2CO, CH_2S), 5.102 (s, 4H, β-CH_2) ppm, MS: [M^+] 394 (100%).

***RK 8**: 4,6-(1,15-(2,14-Dioxa-3,13-dioxo-8-thia-pentadecamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [μ-($O_2C(CH_2)_4S(CH_2)_4CO_2$)-9,10-dioxa-syn-($CH_2CH_3$)B]*
The procedure described for **RK 5** gave **RK 8** from **3d** in 28% yield after flash chromatography (eluant: 10–12% ethyl acetate in dichloromethane). **RK 8**: mp 178°C, ^{1}H NMR ($CDCl_3$) δ: 1.62 (q, 4H), 1.85 (q, 4H), 1.977 (s, 6H, α-CH_3), 2.479 (t, 4H, CH_2CO), 2.513 (t, 4H, CH_2S), 5.151 (s, 4H, β-CH_2) ppm, MS: [M^+] 422 (100%).

***RK 10**: 4,6-(1,17-(2,16-Dioxa-3,15-dioxo-9-thia-heptadecamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [μ-($O_2C(CH_2)_5S(CH_2)_5CO_2$)-9,10-dioxa-syn-($CH_2CH_3$)B]*
The procedure described for **RK 1** gave **RK 10** from **3e** in 44% yield after flash chromatography (eluant: 5–8% ethyl acetate in dichloromethane) and some dimer (6% yield). **RK 10**: Recrystallized from CCl_4, mp 187°C, ^{1}H NMR ($CDCl_3$) δ: 1.56 (m, 4H), 1.718 (q, J = 7.0 Hz, 8H), 1.978 (s, 6H, α-CH_3) 2.435 (t, 4H, J = 6.7 Hz, CH_2CO), 2.552 (t, 4H, J = 6.2 Hz, CH_2S), 5.142 (s, 4H, β-CH_2) ppm, MS: [M^+] 450 (23%).

***RK 11**: 4,6-(1,19-(2,18-Dioxa-3,17-dioxo-10-thia-nonadecamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [μ-($O_2C(CH_2)_6S(CH_2)_6CO_2$)-9,10-dioxa-syn-($CH_2CH_3$)B]*
The procedure described for **RK 1** gave **RK 11** from **3f** in 33% yield after flash chromatography (eluant: 5–10% ethyl acetate in dichloromethane). **RK 11**: Recrystallized from ethyl acetate/hexane, mp 157°C, ^{1}H NMR ($CDCl_3$) δ: 1.426 (m, 8H), 1.644 (m, 8H), 1.972 (s, 6H, α-CH_3), 2.417 (t, 4H, J = 7.1 Hz, CH_2CO), 2.528 (t, 4H, J = 7.2 Hz, CH_2S), 5.092 (s, 4H, β-CH_2) ppm, MS: [M^+] 478 (100%).

Solubility
The solubilities of the **RK *n*** compounds in water were determined using stock solutions in either DMSO and/or acetonitrile. Successive aliquots (10–15 μL) were added to 0.5 mL of water until the first sign of cloudiness was observed, corresponding to the solubility with the specified amount of organic solvent. As examples, (1) **RK 2**: a) DMSO: no cloudiness was observed in an aqueous solution (9% DMSO) at 2.2×10^{-3} M; b) CH_3CN: cloudiness appears at 1.43×10^{-3} M (15% CH_3CN/H_2O). (2) **RK 9**: a) DMSO: precipitate apparent at 1.9×10^{-4} M (1% DMSO/H_2O); b) CH_3CN: cloudy at 1.91×10^{-4} M (3% CH_3CN/H_2O), cloudiness persists when diluted to 0.97×10^{-4} M with water.

***RK 17**: 4,6-(1,17-(2,16-Dioxa-3,15-dioxo-9-oxothia-heptadecamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [μ-($O_2C(CH_2)_5SO(CH_2)_5CO_2$)-9,10-dioxa-syn-($CH_2CH_3$)B]*
3-Chloroperbenzoic acid (0.074 g, 0.428 mmol) in dichloromethane (50 mL) was slowly added with vigorous stirring to **RK 10** (0.155 g, 0.344 mmol) in dichloromethane (100 mL). After an instantaneous reaction, the solution was washed with K_2CO_3 (0.065 g) in a minimum of water, dried ($MgSO_4$), evaporated, and the residue flash chromatographed (eluent, 3–4% isopropyl alcohol in acetonitrile) to give 0.149 g (0.319 mmol) of the sulfoxide ester, **RK 17**, as a light yellow solid (92% yield). **RK 17**: Light yellow product crystallized from ethyl acetate, mp 180–181°C, ^{1}H NMR ($CDCl_3$) δ: 1.902–1.556 (m, 12H, ($-CH_2-$)$_3$), 1.970 (s, 6H, α-CH_3), 2.460 (t, 4H, J = 6.85 Hz, CH_2CO), 2.795 (t, 4H, J = 6.8 Hz, CH_2SO), 5.117 (s, 4H, β-CH_2) ppm, MS: [M^+] 466 (37%). Solubility: (1) DMSO: **RK 17** in DMSO (4×10^{-3} M) did not precipitate at 8.5×10^{-4} M (21.3% DMSO in water); (2) CH_3CN: solubility low; (3) H_2O: 4.1×10^{-4} M solution on warming.

***RK 18**: 4,6-(1,17-(2,16-Dioxa-3,15-dioxo-9-dioxothia-heptadecamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [μ-($O_2C(CH_2)_5SO_2$ $(CH_2)_5CO_2$)-9,10-dioxa-syn-(CH_2CH_3)B]*
A mixture of **RK 10** (0.147 g, 0.328 mmol) and 3-chloroperbenzoic acid (0.168 g, 0.97mmol) in dichloromethane (100 mL) was stirred for several minutes, the solution washed with

K_2CO_3 (0.070 g in 1 mL H_2O), dried ($MgSO_4$), the solvent evaporated and the residue flash chromatographed (silica gel, eluent, 30–50% ethyl acetate in dichloromethane) to give the sulfone ester **RK 18**, which crystallized from ethyl acetate as a light yellow solid, 0.115 g (0.239 mmol) (72% yield). **RK 18**: mp 175–176°C, ^{1}H NMR ($CDCl_3$) δ: 1.916–1.548 (m, 12H, $(-CH_2-)_3$), 1.973 (s, 6H, α-CH_3), 2.468 (t, 4H, J = 6.4 Hz, CH_2CO), 3.037 (t, 4H, J = 7.3 Hz, CH_2SO_2), 5.114 (s, 4H, β-CH_2) ppm, MS: [M$^+$] 482 (65%). Solubility: (1) DMSO: precipitated at 1.64 × 10^{-3} M (23% DMSO in water); (2) CH_3CN: cloudiness at 1.44 × 10^{-3} M (17.4% CH_3CN in water); (3) H_2O: insoluble.

*4,6-Dibromomethyl-3,7-dichloro-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [9,10-dioxa-syn-(CH_2Br,Cl)B] **4***

A modification of a previously reported procedure [26] was used. *syn*-(Methyl,chloro)bimane (3.66 g, 15.7 mmol) and bromine (7 mL) were mixed and stirred for 2 h. The bromine was evaporated at *ca* 15 mm pressure (bromine trapped with cyclohexene in dioxane). Additional bromine (7 mL) was added and the above process repeated. After evaporation of the bromine, dichloromethane was added to the residue and then evaporated, the process being repeated until all the bromine was removed. Alternatively, the reaction mixture can be diluted with dichloromethane and cyclohexene until the color of bromine disappears. The addition of cyclohexane precipitates the product. Flash chromatography (eluent: dichloromethane) of the solid gave 4.079 g, (10.6 mmol) of *syn*-(CH_2Br,Cl)B **4**, crystallized from ethyl acetate/carbon tetrachloride as a bright yellow solid which should be protected from light (67% yield). Including recovered *syn*-(methyl,chloro)bimane and the by-product, *syn*-(bromomethyl,chloro)(methyl,chloro)bimane, 83% of the starting material was accounted for. ^{1}H NMR ($CDCl_3$) δ: 4.634 (s, CH_2Br) ppm.

***RK 19**: 4,6-(1,17-(2,16-Dioxa-3,15-dioxo-9-thiaheptadecamethylene))-3,7-dichloro-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [μ-($O_2C(CH_2)_5S(CH_2)_5CO_2$)-syn-($CH_2$,Cl)B]*

6,6'-Thiodihexanoic acid **3e** (0.897 g, 3.42 mmol) was brought to pH 8 with tetraethylammonium hydroxide and the solution lyophilized to yield the hygroscopic salt. Dichloromethane (600 mL) and *syn*-(CH_2Br,Cl)B **4** (1.335 g, 3.02 mmol) were added. The reaction mixture (which turns black immediately) was refluxed (2 d) until TLC indicated that the starting material had been consumed. The product was purified on a silica-gel column, hydrolysis being avoided by minimizing the height of the column (7 cm) and by eluting rapidly with 10% ethyl acetate in dichloromethane. The material (0.358 g) from the column was crystallized from dichloromethane (1 mL) by addition of CCl_4 (30 mL), followed by dropwise addition of hexane until turbidity developed. After standing overnight, pure **RK 19** (0.200 g, 0.41 mmol) (13.4% yield) was filtered off and dried. **RK 19**: mp 164–165°C, ^{1}H NMR ($CDCl_3$) δ: 1.754–1.507 (m, 12H, $(-CH_2-)_3$), 2.457 (t, 4H, J = 6.67 Hz, CH_2CO), 2.562 (t, 4H, J = 6.09 Hz, CH_2S), 5.242 (s, 4H, β-CH_2) ppm, UV: λ_{max} (ε) (1) CH_3CN: 390 nm (7900), sh 260 (6400), 240 (13 700); (2) H_2O: 394 (7000), sh 264 (8700), 242 (14 000); (3) pH 7.3 aq phosphate buffer: 398 (7400), sh 264 (11 200), 244 (14 800), MS: no parent peak at 490.7 but several fragments showed the presence of two chlorines, 117.0 (100), 119.0 (69.3), 121 (22.4) and 232.1 (6.3), 234.2 (3.4), 236.2 (1.2). Solubility: (1) DMSO: cloudiness apparent at 5 × 10^{-4} M (5.7% DMSO); (2) CH_3CN: cloudiness apparent at 1.8 × 10^{-4} M (2.9% CH_3CN). Only 12% hydrolysis (*ca* 0.4 × 10^{-4} M (3.2% CH_3CN)) over 24 h at rt by the loss of UV absorbance, in both water and aq phosphate, pH 7.3.

In another experiment, *syn*-(CH_2Br,Cl)B **4** (1.254 g, 3.21 mmol) and dipotassium 6,6'-thiodihexanoate (from **3e**) (1.15 g, 3.40 mmol) in acetonitrile (800 mL) were mixed and the mixture refluxed for 2 d. The intensely blue reaction solution left a black residue on evaporation; flash chromatography on silica-gel (5–7% ethyl acetate in dichloromethane) gave 0.0735 g (0.15 mmol) of **RK 19** (4.7% yield). No **RK 19** could be detected by TLC in a reaction carried out in dimethylformamide.

*4,6-Bis-azidomethyl-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [9,10-dioxa-syn-(CH_2N_3,CH_3)B, bBN_3] **7***

A solution of NaN_3 (0.615 g, 9.46 mmol) in water (10 mL) and methanol (48 mL) was added over 30 min to bBBr **1** (1.103 g, 3.15 mmol) in tetrahydrofuran (5 mL) and the whole was stirred for 15 min. The solvents were removed by evaporation at low temperature (14°C) at 15 mmHg, the residual liquid was exhaustively extracted with dichloromethane, and the extract was washed with NaCl solution, and evaporated with silica-gel at low temperature. The solid was dried by azeotroping the water with toluene under reduced pressure and placed on a silica column. Flash chromatography (2% ethyl acetate in dichloromethane) gave 0.591 g (2.16 mmol) of *syn*-(CH_2N_3,CH_3)B **7** (68% yield), mp 164°C, ^{1}H NMR ($CDCl_3$) δ: 1.990 (s, 6H, α-CH_3), 4.455 (s, 4H, β-CH_2) ppm, FTIR (on silver halide fiber) 2106.3 and 740.9 cm^{-1}, N_3 bands, MS: [M$^+$] 274 (100%).

*4,6-(1,9-(2,8-Diaza-3,7-dioxononamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [μ-(HNOC-(CH_2)$_3$CONH)-9,10-dioxa-syn-(CH_2,CH_3)] **10***

A solution of triphenylphosphine (0.187 g, 0.714 mmol) in dry tetrahydrofuran (10 mL) was added to a solution of bis-azido bimane **7** (0.0923 g, 0.402 mmol) and glutaryl dichloride (80 μL, 0.94 mmol) in THF (30 mL). After standing overnight, the solvent was removed, and the residue flash chromatographed to give Ph_3P=O (0.198 g, 0.712 mmol) (100% yield) and the tricyclic diamido bimane **10** (0.0537 g, 0.169 mmol) (47% yield) and some oligomers. Fluorescence (CH_3CN): emission λ_{max} 465 nm (excitation 330 or 401 nm).

*4,6-(1,14-(2,13-Diaza-3,12-Dioxotetradecamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione, [μ-(HNOC(CH_2)$_8$CONH-syn-(CH_2,CH_3)B] **11***

A solution of Ph_3P (0.1896 g, 0.724 mmol) in tetrahydrofuran (8 mL) was added to a solution of **7** (0.0942 g, 0.344 mmol) and sebacoyl dichloride (100 μL, 0.506 mmol) in tetrahydrofuran (10 mL). The reaction residue was flash chromatographed to give Ph_3P=O (0.1770 g, 0.636 mmol) (88% yield) and 0.0221 g (0.061 mmol) of μ-(HNOC(CH_2)$_8$CONH-*syn*-(CH_2,CH_3)B **11** (17.6% yield), a solid with an unusual violet fluorescence. Fluorescence (CH_3CN): emission λ_{max} 442 nm, excitation λ_{max} 378 nm. UV (CH_3CN): λ_{max} 371 nm.

*1,12-Dodecanoyl dichloride, [ClOC(CH_2)$_{10}$COCl] **9d***

1,12-Dodecanedioic acid **16** (2.66 g, 11.6 mmol) was heated in thionyl chloride (2.5 mL) at 50°C for 1.5 h. Excess $SOCl_2$ was removed by evaporation and the 1,12-dodecanoyl dichloride **9d** purified by distillation, $bp_{0.05\ mm}$ 125°C, d 1.21 g/cm^{-3}.

***RK 13**: 4,6-(1,16-(2,15-Diaza-3,14-dioxohexadecamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione, μ-(HNOC(CH_2)$_{10}$CONH)-9,10-dioxa-syn-(CH_2,CH_3)B]*

Ph_3P (0.189 g, 0.721 mmol) in tetrahydrofuran (7 mL, dried over CaH_2) was added to a solution of the bis-azido bimane **7** (0.094 g, 0.344 mmol) and the 1,12-dodecanoyl dichloride **9d**

(125 μL, 0.57 mmol) in tetrahydrofuran (10 mL) and the whole was stirred overnight. After evaporation of the solvent, the residue was flash chromatographed on silica to give triphenylphosphine oxide (0.179 g, 0.64 mmol) (89% yield) and 0.020 g (0.048 mmol) **RK 13** (eluent 20–30% acetonitrile in ethyl acetate) (14% yield). The use of 1,2-bis-(diphenylphosphinyl)ethane (BDPE) in place of Ph_3P increased the yield of **RK 13** to 22.4%. **RK 13** was crystallized from dichloromethane, forming crystals that floated on the surface. **RK 13**: dec 278°C with charring in air, > 450°C in the absence of air. ^1H NMR ($CDCl_3$) δ: 1.355 (broad s, 12H, -$(CH_2)_3$-), 1.711 (m, 4H, J = 6.7 Hz, -CH_2-), 1.857 (s, 6H, α-CH_3), 2.361 (t, 4H, J = 7.0 Hz, CH_2CO), 4.452 (d, 4H, J = 5.4 Hz, β-CH_2), 7.397 (t, 2H, NH) ppm, UV (CH_3CN): λ_{max} 370 nm. Fluorescence (CH_3CN): emission λ_{max} 456 nm, 467.2; excitation λ_{max} 388 nm, 401. Solubility: (1) DMSO: maximum solubility in water 8.4×10^{-4} M (7.4% DMSO); (2) CH_3CN: insoluble.

*6,6'-Thiodihexanoyl dichloride [ClOC$(CH_2)_5$S$(CH_2)_5$COCl] **12***
Using N_2 to exclude moisture, thionyl chloride (120 μL, 1.64 mmol) and dimethylformamide (15 μL, dried over CaH_2), were added to 6,6'-thiodihexanoic acid **3e** (0.131 g, 0.5 mmol) in chloroform (10 mL, purified by passage through alumina) and the mixture stirred [29]. After 5–10 min, all traces of acid had disappeared; the 6,6'-thiodihexanoyl dichloride **12** was used as such after removal of all volatile material at rt at *ca* 15 mmHg. The reaction was done in a flask sufficiently large to accommodate the volumes used in the subsequent reaction.

***RK 14**: 4,6-(1,17-(2,16-Diaza-3,15-dioxo-9-thiaheptadecamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione, [μ-(HNOC$(CH_2)_5$S$(CH_2)_5$CONH)-9,10-dioxa-syn-$(CH_2,CH_3)B$]*
Tetrahydrofuran (*ca* 100 mL) was distilled from CaH_2 into a flask containing the acid chloride **12**; the bis-azide **7** (0.125 g, 0.456 mmol) was then added. A solution of Ph_3P (0.263 g, 1 mmol) in freshly distilled tetrahydrofuran (20 mL), was transferred under nitrogen to an addition funnel and added over 15 min. Stirring at room temperature was continued overnight. Precipitated oligomers (0.128 g) (62% yield) were filtered off; NMR spectra suggested that both bimane and polymethylene fragments were present. Flash chromatography of the residue obtained by evaporation of the filtrate on silica gave Ph_3P=O (0.238 g, 0.857 mmol) (85.7% yield) and **RK 14** (0.041 g, 0.091 mmol) (19.9% yield) (eluant ethyl acetate/acetonitrile (1:3)) which was crystallized from ethyl acetate. **RK 14**: mp > 450°C in the absence of air, ^1H NMR ($CDCl_3$) δ: 1.53–1.74 (m, 12H, -$(CH_2)_3$), 1.821 (s, 6H, α-CH_3), 2.431 (t, 4H, J = 7.36 Hz, -CH_2CO), 2.558 (t, 4H, J = 6.30 Hz, -CH_2S)) 4.403 (d, 4H, J = 5.17 Hz, β-CH_2), 7.424 (t, 2H, NH) ppm. Solubility: (1) DMSO: not cloudy in water at 2.13×10^{-3} M (21.9% DMSO); (2) CH_3CN: insoluble; (3) H_2O: insoluble, 0.49 mg undissolved in 1.45 mL water even after adding 80 μL DMSO.

***RK 15**: 4,6-(1,17-(2,16-Diaza-3,15-dioxo-9-oxothiaheptadecamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [μ-(HNOC$(CH_2)_5$SO $(CH_2)_5$CONH)-9,10,dioxa-syn-$(CH_2,CH_3)B$]*
3-Chloroperbenzoic acid (0.0142 g, 0.082 mmol) in dichloromethane (4 mL) was slowly added with vigorous stirring to **RK 14** (0.0353 g, 0.079 mmol) in dichloromethane (5 mL). After 15 min, $NaHCO_2$ (0.0082 g, 0.1 mmol) was added along with a few drops of water and the layers separated. After evaporation of the solvent, the residue was flash chromatographed on a small amount of cellulose. The order of elution was **RK 14**, the sulfone **RK 16** and the sulfoxide **RK 15** (eluant, 4% methanol in acetonitrile). The **RK 15** fraction was passed through a cellulose column to give 0.027 g of solid (0.057 mmol) (74% yield) and recrystallized with much difficulty from ethyl acetate to yield 8 mg of pure material. **RK 15**: ^1H NMR ($CDCl_3$) δ: 1.59–1.96 (m, 12H, -$(CH_2)_3$-), 1.817 (s, 6H, α-CH_3), 2.422 (t, 4H, J = 6.3 Hz, CH_2CO), 2.776 (t, 4H, J = 6.3 Hz, CH_2S), 4.382 (4H, β-CH_2), 7.698 (t, 2H, NH) ppm.

***RK16**: 4,6-(1,17-(2,16-Diaza-3,15-dioxo-9-dioxothia-heptadecamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [μ-(HNOC$(CH_2)_5SO_2(CH_2)_5$CONH)-9,10-dioxa-syn-$(CH_2,CH_3)B$]*
A mixture of **RK 14** (0.0373 g, 0.083 mmol) and 3-chloroperbenzoic acid (0.0366 g, 0.212 mmol) in dichloromethane (10 ml) was vigorously stirred for 10 min. Sodium bicarbonate (0.019 g, 0.23 mmol) was then added together with a few drops of water. The organic solvent was separated, evaporated, and the residue flash chromatographed twice, first on cellulose to remove very polar impurities and then on silica (eluant acetonitrile/dichloromethane (3:7)) to yield **RK 16** (0.0309 g, 0.064 mmol) (77.5% yield) which was recrystallized from ethyl acetate. **RK 16**: ^1H NMR ($CDCl_3$) δ: 1.55–1.91 (m, 12H, -$(CH_2)_3$-), 1.829 (s, 6H, α-CH_3), 2.432 (t, 4H, J = 7.1 Hz, CH_2CO), 3.037 (t, 4H, J = 7.5 Hz, CH_2SO_2), 4.401 (d, 4H, J = 5.6 Hz, β-CH_2), 7.652 (t, 2H, NH) ppm.

*N-t-Butoxycarbonyl-glycyl diester of 4,6-hydroxymethyl-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [9,10-dioxa-syn-[$(CH_3)_3$COCONH CH_2COOCH_2,CH_3]B] **14***
The acid *t*-BOC-glycine was converted into the potassium salt **13** (1.004 g, 5.73 mmol) by refluxing with potassium bicarbonate (0.629 g, 6.28 mmol) in acetonitrile (dried over P_2O_5). After removal of the solvent, the salt was dried by azeotropic distillation with toluene under reduced pressure. The bis-bromide **1** (1.003 g, 2.86 mmol) in acetonitrile (150 mL) was added and the mixture vigorously stirred and heated (70°C) overnight. After evaporation of the solvent, the residue was flash chromatographed on silica (eluant, ethyl acetate/dichloromethane (1:5)) to give the diester **14** (1.028 g, 1.91 mmol) (66.8% yield). ^1H NMR ($CDCl_3$) δ: 1.435 (s, 18H, CH_3 of *t*-Bu), 1.967 (s, 6H, α-CH_3), 3.963 (d, 4H, J = 5.9 Hz, gly-CH_2) 5.13 (t, 2H, NH), 5.197 (s, 4H, β-CH_2) ppm.

***RK 20**: 4,6-(1,17-(2,16-Dioxa-5,13-diaza-3,6,12,15-tetraoxo-9-thia-heptadecamethylene))-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione [μ-(O_2CCH_2NHCO$(CH_2)_2$S-$(CH_2)_2$CONHCH_2CO_2)-9,10-dioxa-syn-$(CH_2,CH_3)B$]. Carbodiimide condensation*
The diester **14** (0.173 g, 0.322 mmol) was stirred with trifluoroacetic acid (5 mL) for 1 h. The acid was removed under low pressure (vacuum pump). *p*-Toluenesulfonic acid (0.12 g, 0.65 mmol) (dried by azeotropic distillation with toluene) was added, and the trifluoroacetic acid removed by repeated azeotropic evaporation with acetonitrile. When all the TFA had been removed the appearance of the residue changed from a yellow film to a whitish powder. The solid was dissolved in acetonitrile (50 mL), and triethylamine (90 mL, 0.64 mmol), 3,3'-thiodipropionic acid **3b** (0.0574 g, 0.322 mmol), and finally dicyclohexylcarbodiimide (DCC) (0.133 g, 0.644 mmol) were added successively and the whole stirred overnight. After evaporation of the solvent, flash chromatography of the residue on silica gel (eluent, 50–60% ethyl acetate in acetonitrile) gave the tricyclic amide ester **RK 20** (0.031 g, 0.064 mmol) (20% yield). Identification was hindered by the insolubility of the pure compound in solvents suitable for NMR spectra. The R_f

value (TLC) of 0.35 (eluant, 3:7 acetone/acetonitrile) was in accordance with the expected polarity of the compound.

*N,N'-(3,3'-Thiodipropionyl)-bis-glycine [$HOOCCH_2NHCO(CH_2)_2S(CH_2)_2CONHCH_2COOH$] **15***

Via acid chloride. A solution of 3,3'-thiodipropionic acid **3b** (1.34 g, 7.5 mmol), $SOCl_2$ (1.8 mL, 24.7 mmol), DMF (200 mL, dried over CaH_2) in chloroform (30 mL, purified by passage through alumina) was heated to 50°C for 10 min. Volatile material was removed under reduced pressure. Although the smell of sulfide indicated some decomposition, 1.31 g (6.1 mmol) of the acid chloride was successfully distilled from the reaction mixture using a bulb-to-bulb apparatus (80% yield). ^1H NMR ($CDCl_3$) δ: 2.796 (t, J = 7.0 Hz, $-CH_2CO$), 3.141 (t, J = 7.0 Hz, $-CH_2S$). No product could be isolated from a reaction with glycine methyl ester hydrochloride and triethylamine in dry THF.

Via DCC. A mixture of 3,3'-thiodipropionic acid **3b** (1.788 g, 10 mmol), glycine methyl ester hydrochloride (2.589 g, 10 mmol), triethylamine (2.8 mL) and DCC (4.40 g, 21.3 mmol) in dry THF were stirred at 0°C for some time and then at room temperature overnight. After filtration, the solvent was evaporated to dryness. In addition to the bis-amide **15**, the residue contained triethylamine and dicyclohexylurea (DCU). Ester, ^1H NMR ($CDCl_3$) δ: 2.551 (t, 4H, J = 7.1 Hz, $-CH_2CO$), 2.814 (t, 4H, J = 7.1 Hz, $-CH_2S$), 3.662 (s, 6H, ester CH_3), 3.947 (d, 4H, J = 5.4 Hz, gly CH_2), 7.373 (t, 2H, NH) ppm. Sodium hydroxide (1.76 g, 44 mmol) in water (25 mL) and methanol (10 mL) was added, the solution stirred for 1 h, filtered, and extracted with ether to remove triethylamine, brought to pH 7 with HCl, the remaining methanol evaporated, and sufficient HCl added to make the pH 1. On standing overnight, the bis-amide **15** precipitated and was dried in the lyophilizer to yield 1.93 g of product (6.60 mmol)(66% yield). ^1H NMR (D_2O) δ: 2.815 (t, 4H, J = 6.8 Hz, $-CH_2CO$), 3.028 (t, 4H, J = 6.8 Hz, CH_2S), 4.173 (s, 4H, gly CH_2) ppm.

*Synthesis of **RK 20** from **15**.* The bis-amide **15** (0.343 g, 1.173 mmol) in methanol was brought to pH 8.5 with benzyltrimethylammonium hydroxide (Triton B). The methanol was evaporated and the residue lyophilized. The salt was completely dried by treating the solid with dry tetrahydrofuran, decanting most of the solvent and evaporating the remainder. NMR spectra showed that > 95% of acid had been converted to the salt. The bis-bromide **1** (0.410 g, 1.172 mmol) was added together with acetonitrile (600 mL, dry), and the reaction mixture stirred vigorously and refluxed overnight. The solution was directly evaporated with silica gel. Chromatography gave **RK 20** (eluants, ethyl acetate to acetonitile) which precipitated from the eluants (0.143 g, 0.298 mmol) (39% yield). **RK 20** was soluble only in DMSO. Crystallization was effected by dissolution in DMSO, followed by addition of ethyl acetate, yielding 0.081 g of pure **RK 20**. **RK 20**: ^1H NMR (DMSO) δ: 1.862 (s, 6H, α-CH_3), 2.417 (t, 4H, J = 6.6 Hz, $-CH_2CO$), 2.713 (t, 4H, J = 6.4 Hz, $-CH_2S$), 3.891 (d, 4H, J = 5.4 Hz, gly CH_2), 8.457 (t, 2H, NH) ppm. Solubility: (1) DMSO: some cloudiness in water (13% DMSO) at 6.8×10^{-4} M; (2) Water: at *ca* 3×10^{-4} M after some heating and sonication.

Acknowledgments

L Ginodman is thanked for help and advice on several experiments. The support of the World Health Organization and the Wellcome Trust through a grant to the University of London is much appreciated. The investigation also received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. We appreciate the cooperation of L Krauth-Siegel, Institut für Biochemie II, Medizinische Fakultät, Universität Heidelberg, Germany, in carrying out the tests of inhibition of *T cruzi* trypanothione reductase.

References

1 *Science at work* Tropical Disease Research, WHO, Geneva, Switzerland, 29–36
2 Glew RH (1988) *Microbiol Rev* 52, 412–432
3 Fairlamb AH, Blackburn P, Ulrich P, Chait BT, Cerami A (1985) *Science* 227, 1485–1487
4 Fairlamb AH, Cerami A (1985) *Mol Biochem Parasitol* 14, 187–198
5 Fairlamb AH, Cerami A (1992) *Annu Rev Microbiol* 46, 695–729
6 Kosower NS, Kosower EM (1978) *Internat Rev Cytol* 54, 109–160
7 Kosower EM (1989) *In: Coenzymes and Cofactors*, Vol III (Dolphin D, Poulson R, Avramovic O, eds) John Wiley, New York, USA, 103–146
8 Kosower NS, Kosower EM (1989) *In: Coenzymes and Cofactors*, Vol III (Dolphin D, Poulson R, Avramovic O, eds) John Wiley, New York, USA, 319–356
9 Kosower NS, Kosower EM (1995) *In: Biothiols*, Vol 251, Academic Press, Orlando, FL, USA, 123–133
10 Bacchi CJ, Nathan HC, Hutner SH, McCann PP, Sjoerdsma A (1980) *Science* 210, 332–334
11 Fairlamb AH, Henderson GB, Bacchi CJ, Cerami A (1987) *Mol Biochem Parasitol* 24, 185–191
12 Kaur K, Emmett K, McCann PP, Sjoerdsma A, Ullman B (1986) *J Protozool* 33, 518–521
13 Keithly JS, Fairlamb AH (1989) *In: Leishmaniasis: The Current Status and New Strategies for Control* (Hart DT, ed) Plenum Press/NATO ASI Series, Vol 163, New York, USA, 749–756
14 Kierszenbaum F, Wirth JJ, McCann PP, Sjoerdsma A (1987) *Proc Natl Acad Sci USA* 84, 4278–4282
15 Jennings FW (1988) *Trans R Soc Trop Med Hyg* 82, 572–573
16 McCann PP, Bacchi CJ, Nathan HC, Sjoerdsma A (1983) *In: Mechanisms of Drug Action* (Singer TP, Ondarza RN, eds) Academic Press, New York, USA, 159–173
17 Fairlamb AH, Henderson GB, Cerami A (1989) *Proc Natl Acad Sci USA* 86, 2607–2611
18 Fairlamb AH, Carter NS, Cunningham M, Smith K (1992) *Mol Biochem Parasitol* 53, 213–222
19 Yarlett N, Goldbert B, Nathan HC, Garofalo J, Bacchi CJ (1991) *Exp Parasitol* 72, 205–215
20 Carter NS, Fairlamb AH (1993) *Nature (Lond)* 361, 173–175
21 Else AJ, Hough DW, Danson MJ (1993) *Eur J Biochem* 212, 423–429
22 Herwaldt BL, Juranek DD (1993) *Am J Trop Med Hyg* 48, 313–323
23 Wery M (1994) *Int J Antimicrobial Agents* 4, 227–238
24 Kosower EM, Pazhenchevsky B (1980) *J Am Chem Soc* 102, 4983–4993
25 Radkowsky AE, Marciano D, Kosower NS, Zipser J, Shalev DE, Kosower EM (1991) *Heteroatom Chem* 2, 319–333
26 Kosower EM, Zbaida D, Baud'huin M, Marciano D, Goldberg I (1990) *J Am Chem Soc* 112, 7305–7314
27 Garcia J, Urpi F, Vilarrasa J (1984) *Tetrehedron Lett* 25, 4841–4844
28 Shalev DE (1990) MSc Thesis, Tel Aviv University, Israel
29 Bosshard HH, Mory R, Schmid M, Zollinger H (1959) *Helv Chim Acta* 43, 1653–1658
30 Fieser LF, Fieser M (1967) *Reagents for Organic Synthesis*, Vol 1, J Wiley and Sons, New York, USA, 286
31 Jockers-Scherbl MC, Schirmer RH, Krauth-Siegel RL (1989) *Eur J Biochem* 180, 267–272
32 Hunter WN, Smith K, Derewenda Z, Harrop SJ, Habash J, Islam MS, Helliwell JR, Fairlamb AH (1990) *J Mol Biol* 216, 235–237
33 Hunter WN, Bailey S, Habash J *et al* (1992) *J Mol Biol* 227, 322–333

34 Kuriyan J, Kong XP, Krishna TSR *et al* (1991) *Proc Natl Acad Sci USA* 88, 8764–8767
35 Benson TJ, McKie JH, Garforth J, Borges A, Fairlamb AH, Douglas KT (1992) *Biochem J* 286, 9–11
36 Aboagye-Kwarteng T, Smith T, Fairlamb AH (1993) *Mol Microbiol* 6, 3089–3099
37 Krauth-Siegel RL, Sticherling C, Jöst I *et al* (1993) *FEBS Letters* 317, 105–108
38 Lantwin CB, Schlichting I, Kabsch W, Pai EF, Krauth-Siegel RL (1994) *Proteins* 18, 161–173
39 Fairlamb AH, Henderson GB, Cerami A (1986) *Mol Biochem Parasitol* 21, 247–257
40 Fairlamb AH (1989) *Parasitology* 99S, S93–S112
41 Henderson GB, Fairlamb AH (1987) *Parasitology Today* 3, 312–315
42 Fairlamb AH (1989) *In: Leishmaniasis* (Hart DT, ed) Plenum, New York, USA, 487–494
43 Steenkamp DJ, Spies HSC (1994) *Eur J Biochem* 223, 43–50
44 Kosower EM, Pazhenchevsky B, Marciano D *et al* (1990) *J Am Chem Soc* 112, 7315–7319
45 Simhony S, Kosower EM, Katzir A (1986) *Applied Phys Lett* 49, 253–254
46 Simhony S, Katzir A, Kosower EM (1988) *Anal Chem* 60, 1908–1910
47 Kodak (1960) Belgian Patent 593048; *Chem Abstr* (1961) 55, 14142i
48 Schjanberg E (1941) *Ber Deutschen Ges Chimie* 74, 1751–1759
49 Stoner GC, Dougherty G (1941) *J Am Chem Soc* 63, 987–988
50 Minisci F (1959) *Gazz Chim Ital* 89, 2437–2538; *Chem Abs* (1961) 556406g
51 Petrora RG, Freidlina RKh (1958) *Izvest Akad Nauk SSSR, Otd Khim Nauk* 290–295; *Chem Abstr* (1958) 52, 12750c
52 Wardell JL (1974) *In: The Chemistry of the Thiol Groups*, Part 1 (Patai S, ed) John Wiley, New York, USA, 186–195
53 Reid EE (1958) *Organic Chemistry of Bivalent Sulfur*, Vol 1, Chemical Publishing Co, New York, USA, 32–35, 324–331
54 Nesmeyanov AN *et al* (1957) *Chem Tech* (Berlin) 9, 139–150; *Chem Abstr* (1957) 51, 15409a,b
55 Reppe W, Friedrich H (1957) US patent 2795607; *Chem Abstr* (1957) 51, 16254d
56 Nishigori I, Tatsuya B, Murahashi S (1951) *Chem High Polymer (Jpn)* 8, 253–256; *Chem Abstr* (1953) 47, 6345b
57 Harpp DN, Gingras M, Aida T, Chan TH (1987) *Synthesis* 12, 1122–1124
58 Reppe W (1955) *J Liebigs Ann* 596, 197
59 Reppe W, Friedrich H, Albers H (1957) Ger patent 944790; *Chem Abstr* (1957) 51, 464c
60 Neal RA, Croft SL (1984) *J Antimicrobial Chemother* 14, 463–475