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Regioselective synthesis of inhibitors of histone acetyl transferase covalently linking spermidine to the S-terminus of coenzyme A and fragments.

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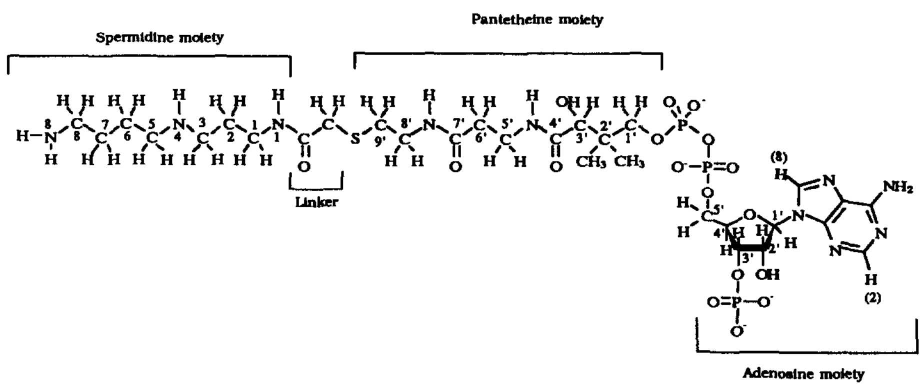
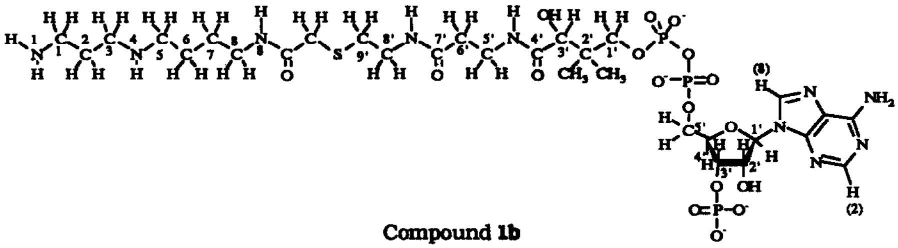
Abstract. The reaction of a bromoacetylthioester $BrCH_2CO$-S-R (R radical in the coenzyme A series) with spermidine (Spd) derivatives is investigated and it is established that the adduct $SpdCOCH_2$-S-R **1** is the product of the reaction. Parallel studies with model compounds show that this is a general reaction of bromoacetylthioesters. The synthesis of analogs of **1** is described and they correspond to inhibitors of the histone acetyltransferase.

In 1982, Cullis et al.[1] introduced the concept of a multisubstrate-type inhibitor interfering with two enzymatic activities, the acetylation of spermidine in the one hand and the acetylation of histones in the other hand, for which acetylcoenzyme A (CoA-S-Ac) acts as the acetyl donor. Such an inhibitor covalently associates the coenzyme A (CoA-SH) with spermidine (Spd) through a carboxymethylene bridge or "linker" corresponding to the general formula CoA-S-CH_2-CO-Spd **1**, with no particular regioselectivity in regard to the nitrogen atom N^1, N^8 or N^4 of the spermidine molecule.

We describe here a regioselective synthesis of the inhibitor, CoA-S-CH_2-CO-Spd, so that the attachment of the spermidine molecule to the CoA moiety is totally controlled and occurs either through its N^1 (compound **1a**) or its N^8 atom (compound **1b**).



Compound **1a** . The CoA numerotation is conform to [7].



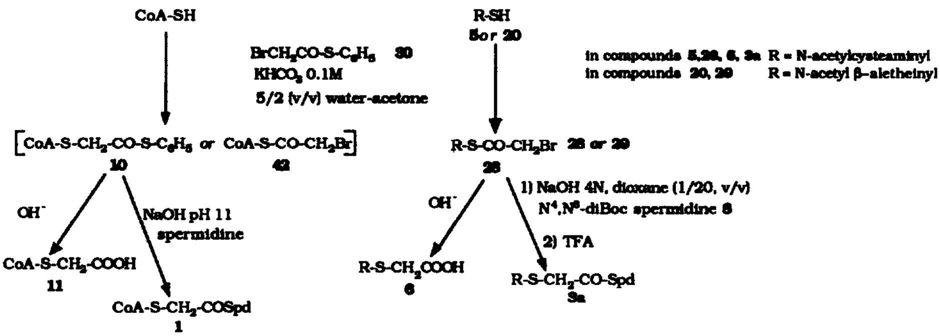
Compound **1b**

Similarly, we give here a full account of the synthesis of two "shortened" inhibitors in which coenzymeA is replaced by its S-terminal β-aletheinyl moiety (inhibitor **2a**) or its S-terminal cysteaminyl moiety (inhibitor **3a**). We have recently shown[2] that **2a** has also an efficient inhibitory effect on the enzymatic acetylation of histones in chromatin.

R-S-CH_2-CONH-$(CH_2)_n$-NH-$(CH_2)_m$-NH_2

1a	R = CoA	n = 3	m = 4
1b	R = CoA	n = 4	m = 3
2a	R = AcNH-$(CH_2)_2$-CONH-$(CH_2)_2$	n = 3	m = 4
3a	R = AcNH-$(CH_2)_2$	n = 3	m = 4

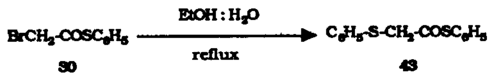
The two-step synthesis of compound **1** (mixture containing presumably **1a** and **1b**) starting from CoA-SH, as proposed by Cullis et al.[1], apparently involves the intermediate derivative **10**, i.e. thiophenylcarboxymethyl-CoA, which is finally reacted with the unprotected spermidine (Scheme 1). The reaction of 2-bromoacetylthiophenol **30** with CoA-SH needs, however, to be considered carefully since two contradictory reports exist in the literature. In their initial study, Chase and Tubbs[3] concluded that the product of the reaction, CoA-SH + **30**, was the bromoacetyl derivative **42** (Scheme 1), as a result of the acylation of CoA-SH. In contrast Clements et al.[4] invoked the formation of the thioester **10**, obtained by alkylation of CoA-SH (Scheme 1). We decided therefore to



Scheme 1

investigate the reaction, CoA-SH + **30**, in more detail under the conditions used by the different authors[1,3,4] by ^1H NMR. As shown in Figure 1, the reaction mixture is characterized by a major product (yield>90%) which can be unambiguously assigned to the bromoacetyl derivative **42**, and not to **10**. Indeed, the methylene α to the bromine atom resonates at 4.17 ppm and practically no phenyl group is present (as expected for **10**).

We have observed that compound **30** must be highly purified for a careful control of the reaction CoASH + **30**. Crystallization of **30** is usually carried out in EtOH-H_2O mixtures[3]. We have observed that an additional product is obtained under such conditions, identified as C_6H_5-S-CH_2-CO-S-C_6H_5 **43**, according to:



Scheme 2

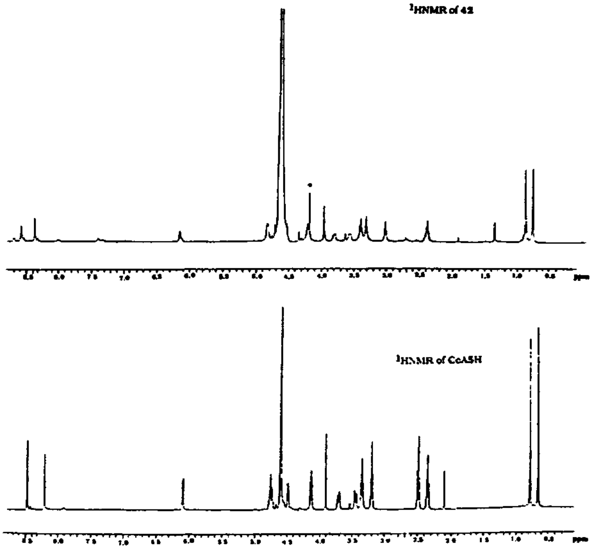


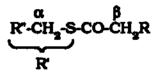
Figure 1. ^1H NMR spectra at 360 MHz of **42** (top) and CoA-SH (bottom) in D_2O solution. (*) corresponds to CO-$\underline{CH_2}$-Br in **42**.

As discussed below, the formation of **43** can be rationalized by an hydrolytic step (with formation of thiophenol) followed by alkylation of the intermediate thiophenol by **30**, in agreement with a previous report describing the formation of **43** from chloroacetylthiophenol in EtOH[6]. A careful control of the purity of **30** can be conveniently carried out by ^1H NMR, since both compounds **30** and **43** display rather different spectra. By crystallizing **30** in benzene-hexane mixtures, a highly purified compound is thus obtained.

We decided to investigate the reaction of **30** with simpler thiols than CoA-SH itself, in order to characterize the corresponding R-S-$COCH_2Br$ derivatives (Scheme 1). Starting from N-acetylcysteamine **5**, the acylated derivative **28** was obtained as practically the unique product of the reaction and was isolated in good yield. The structure of this bromoacetylated derivative is unambiguously established by NMR, as well as by comparison with the product obtained by direct acylation of **5** using bromoacetylbromide (see Experimental Section). Similarly, starting from N-acetyl-β-aletheine **20**, we obtain the corresponding bromoacetylated derivative **29**. As shown in Table 1, very similar ^1H NMR chemical shifts are observed for the CH_2 β group in compounds **28** and **29** (4.15 and 4.10 ppm, respectively) and **42** (4.17 ppm), thus establishing that the occurrence of a two-proton singlet at 4.10-4.17 ppm is characteristic of the S-$COCH_2Br$ methylene group. Furthermore, the downfield shift of about 0.5 ppm of the $CH_2\alpha$ upon formation of the thioester function in **42** (as compared to CoA-SH: $\delta\, CH_2\alpha$= 2.60 ppm), is also observed with compounds **28**, **29**, as well as with **4** and **16** with no bromine atom (Table 1). Both S-bromoacetylthiol compounds, **28** and **29**, therefore provide additional evidence that it is acylation and not alkylation which occurs upon reaction of compound **30** with the different thiols investigated here.

In regard to reaction **42** → **1** (Scheme 1), it was interesting to investigate the reactivity of a simpler S- bromoacetyl derivative, such as **28**, with the N^4,N^8-diBoc derivative **8** of spermidine (Scheme 3). Compound **9** thus obtained displays a carboxymethylene bridge S-CH_2-CO-N, binding the S atom of the cysteamine moiety to the

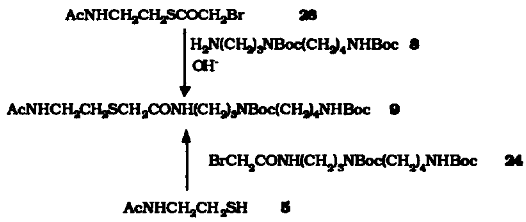
Table 1. ^{1}H NMR chemical shifts (δ in ppm) of the methylene protons in α and β positions of the sulfur atom in:



R'	R	$\delta CH_2\alpha$	$\delta CH_2\beta$	solvent	compound
N-acetylcysteaminyl	Br	3.00 (t) 3.10 (t)	4.15 (s) 4.10 (s)	D_2O $CDCl_3$	**28**
N-acetyl-β-aletheinyl	Br	3.10 (t)	4.10 (s)	$CDCl_3$	**29**
N-acetylcysteaminyl	H	3.00 (t)		$CDCl_3$	**4**
N-acetyl- β -aletheinyl	H	3.05		$CDCl_3$	**16**
CoA-S	Br	3.01	4.17(s)	D_2O	**42**

$\delta CH_2\alpha$= 2.60 ppm for CoA-SH (which corresponds to $C^{9'}H_2$ in Table 3), s = singlet; t = triplet.

N^1 atom of spermidine. This structure would be unexpected if the reaction simply resulted in the alkylation of the free N^1 amino group of **8** with elimination of the bromine atom of **28** (see below Scheme 6 for a more detailed understanding of the reaction **28** → **9** in Scheme 3). The presence of two characteristic resonances at 2.70 ppm ($S-CH_2\alpha$; triplet) and 3.18 ppm ($SCH_2\alpha'$; singlet) in the ^{1}H NMR spectrum of **9** (see Table 2) is in agreement with the proposed structure (Scheme 3). Compound **9** is also obtained by alkylation of the corresponding thiol **5** in the presence of N^1-bromoacetyl derivative N^4,N^8-diBoc spermidine **24** with elimination of the bromine atom of **24**.



Scheme 3

Furthermore it is to be noted that the stability of **30** is such that, in alkaline ethanol medium, this compound leads instantaneously to 2-phenylthioacetic acid **31** (Scheme 4). Similarly **28** leads to the thioacetic acid **6**.

Scheme 4

It is likely that the reactions, **30** → **31** and **28** → **6** , follow the same pathway as postulated when **30** is reacted with a primary amine, under alkaline conditions (see below, Scheme 6). In contrast, Cullis et al.[1] used $KHCO_3$ as a base in the reaction of CoA-SH with **30**. We have not investigated the stability of **30** under such mild alkaline conditions, but it is probable that **30** is not transposed into **31**, and is subject to a nucleophilic attack by CoA-SH to yield the S-bromoacetyl derivative **42** of CoA-SH, as discussed above. It is to be noted that all compounds synthesized in this work, with the $R''-CH_2-S-CH_2COR$ motif, display two characteristic resonances in their

^1H NMR spectra at 2.6-2.7 ppm (S-CH_2 α) and at 3.2-3.3 ppm (S-CH_2 α'), as presented in Table 2.

Table 2. ^1H NMR chemical shifts (δ in ppm) of the methylene protons α and α' in: $R"-CH_2^{\alpha}-S-CH_2^{\alpha'}-COR$ (R" = R')

R'	R	$\delta CH_2\alpha$	$\delta CH_2\alpha'$	solvent	compound
N-acetylcysteaminyl	$NH(CH_2)_3NBoc(CH_2)_4NHBoc$	2.70 (t)	3.18 (s)	$CDCl_3$	**9**
N-acetylcysteaminyl	$NH(CH_2)_3NH(CH_2)_4NH_2$*	2.62 (t)	3.20 (s)	D_2O	**3a**
N-acetylcysteaminyl	OH	2.60 (t)	3.20 (s)	D_2O	**6**
N-acetyl-β-aletheinyl	OH	2.70 (t)	3.32 (s)	D_2O	**21**
N-acetylcysteaminyl	OCH_3	2.70 (t)	3.35 (s)	D_2O	**7**
N-acetyl-β-aletheinyl	OCH_3	2.73 (t)	3.20 (s)	$CDCl_3$	**22**
N-acetyl-β-aletheinyl	$NH(CH_2)_3NBoc(CH_2)_4NHBoc$	2.75 (t)	3.20 (s)	$CDCl_3$	**23**
N-acetyl-β-aletheinyl	$NH(CH_2)_3NH(CH_2)_4NH_2$*	2.60 (t)	3.20 (s)	D_2O	**2a**
CoA-S	$NH(CH_2)_3NBoc(CH_2)_4NHBoc$	2.75 (t)	3.30 (s)	D_2O	**25**
CoA-S	$NH(CH_2)_4NBoc(CH_2)_3NHBoc$	2.57 (t)	3.15 (s)	D_2O	**37**
CoA-S	$NH(CH_2)_3NH(CH_2)_4NH_2$*	2.64 (t)	3.22 (s)	D_2O	**1a**
CoA-S	$NH(CH_2)_4NH(CH_2)_3NH_2$*	2.56 (t)	3.25 (s)	D_2O	**1b**

Radical N-acetylcysteaminyl: $AcNH(CH_2)_2S$. Radical N-acetyl-β-aletheinyl: $AcNH(CH_2)_2CONH(CH_2)_2S$.
(t) = triplet; (s) = singlet.* obtained as a TFA salt .

Furthermore, we tried to obtain an analog of the putative derivative **10** (see Scheme 1) in the case of the simpler radical N-acetylcysteaminyl. The synthesis of such an analog **39** starting from **6** is described in Scheme 5.

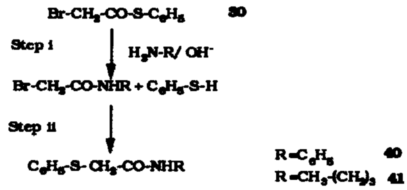
$$AcNH(CH_2)_2\text{-}S\text{-}CH_2\text{-}COOH \xrightarrow[2)\ C_6H_5\text{-}SH]{1)\ Ac_2O} AcNH(CH_2)_2\text{-}S\text{-}CH_2\text{-}CO\text{-}S\text{-}C_6H_5$$

6 → **39**

Scheme 5

On the basis of its ^1H NMR spectrum (see Experimental Section), compound **39** unambiguously corresponds to the expected structure with the thioether-thioester motif R'-S-CH_2-CO-S-R. In this case the resonance of the S-CH_2α' methylene group is significantly displaced to low field, i.e. 3.40 ppm (due to substitution by a thiophenyl group), whereas that of the CH_2 α group is practically unaffected, in compartison to compounds with R non-aromatic (Table 2). We confirm here that, besides the absence of aromatic resonances on the ^1H NMR spectrum of compound **42** (Fig. 1), no triplet at 2.6-2.7 ppm (S-CH_2 α) and no singlet at 3.2-3.3 ppm (S-CH_2α') are observed, thus reinforcing our conclusion that no alkylation of the thiol function is occurring, as previously assumed by Clements et al. [4].

In relation to the formation of **1** from **42**(Scheme 1), the reaction of the bromoacetyl thiol ester **30** with different simple amines, such as aniline and n-butylamine, was studied, thus confirming the formation of the S-CH_2-CO "linker" in the final products (Scheme 6). Such reactions can be accounted for by a two-step pathway: (i) a nucleophilic attack of bromoacetylthiophenol by the primary amine to yield a bromoacetylamide with liberation of thiophenol, and (ii) a nucleophilic attack of the intermediate bromoacetylamide by thiophenol with bromide formation (under alkaline conditions) with elimination of the bromine atom. We note that the reaction of **28** with **8** (Scheme 3) is significantly slower than the reaction of **30** with n-butylamine, which proceeds instantaneously. It is likely that the nucleophilic attack of **30** by n-butylamine to give **41** (Scheme 6), is kinetically fa-

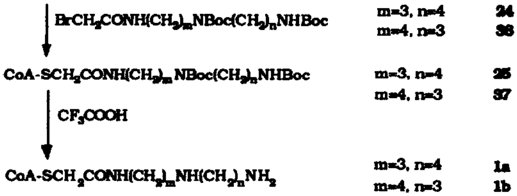


Scheme 6

voured in comparison to **28** +**8** → **9** (Scheme 3), since the leaving group originating from **30** corresponds to thiophenate instead of an aliphatic thiolate in the case of **28**. When **30** is reacted with aniline, instead of n-butylamine, a reduction of the kinetics is observed, likely due to the reduced nucleophilicity of the amino group in aniline. Furthermore, the presence of a bromine atom in α position of the carbonyl group of the thioesters **28** and **30** introduces a favourable condition for a nucleophilic attack of this carbonyl group by the primary amine (first step in scheme 6). Indeed, in the absence of the halogen atom, when acetylthiophenol is reacted with aniline under similar conditions, acetanilide only appears in very weak amounts besides the unreacted acetylthiophenol. If the carbonyl function is displaced to a more distant position from the bromine atom, as in C_6H_5-S-CO-$(CH_2)_2$-CH_2Br, no reaction is detected with aniline for the period of time used for the corresponding high-yield reactions: **28** → **9** (Scheme 3), **30** → **40** and **30** → **41** (Scheme 6). The above mentioned 4-bromobutyric thioester of thiophenol was used to investigate the possibility of a cyclic intermediate (five-membered ring) to account for the "transposition" of the methylene group in the thioesters R-S-CO-CH_2Br to give R-S-CH_2-CONH-R' in the presence of an amine R'-NH_2 under alkaline conditions (putative three-membered ring). The absence of any transposition of the polymethylene chain in C_6H_5-S-CO-$(CH_2)_2$-CH_2Br to give C_6H_5-S-$(CH_2)_3$-CONH-R' appears in favour of the two-step mechanism proposed in scheme 6. Obviously, a more detailed investigation of the mechanism of these reactions is necessary before any conclusion can be firmly drawn.

All together, these results allow us to conclude that the original conditions used by Cullis et al.[1] to obtain the multisubstrate inhibitor **1** (see Scheme 1) lead to the proposed CoA-S-CH_2-CO-Spd structure although the previously assumed intermediate compound **10** (CoA-thiophenylester) apparently is not formed under the conditions used by the authors, but instead CoA-S-bromoacetyl **42** is formed, as demonstrated in this work in agreement with the conclusion of Chase and Tubbs[3].

As noted above, the reaction described in scheme 1 by Cullis et al.[1] does not allow inhibitor **1a** or **1b** to be obtained selectively. So, the main interest of our work consists to propose, as previously reported[2], a new synthetic pathway for preparing the inhibitor **1a** from CoA-SH using the N^1-bromoacetyl derivative **24** of spermidine (Scheme 7). In this work, we show that such a two-step strategy is readily extended to the case of the positional isomer **1b**, which is readily prepared from the N^8-bromoacetyl derivative **36** (Scheme 7). Both compounds **1a** and **1b** are thus obtained in good yields and with a high degree of purity. Table 3 gives the NMR characteristics of **1a** and **1b**, as compared to those of CoA-SH itself.



Scheme 7

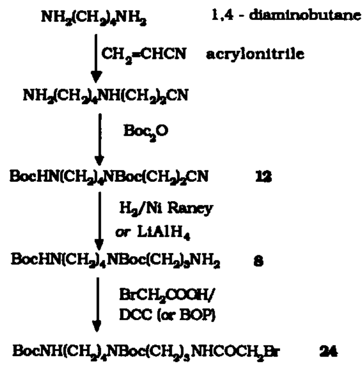
Table 3. ^1H NMR chemical shifts for CoA-SH, **1a** and **1b** in D_2O solution

Adenosine moiety				Pantetheine moiety				Spermidine moiety		
Proton	CoA-SH	1a	1b	Proton	CoASH	1a	1b	Proton	1a	1b
H(1')	6 17	6.17	6.16	H(1')	3.82	3.82	3.82	H(1+1')	3.26	*
H(2')	4.87	4.85	4.85	H(1")	3.55	3.58	3.60	H(2+2')	1.87	2.07
H(3')	4 83	4.77	4.85	CH_3(2')	0.88	0.89	0.89	H(3+3')	3.01	*
H(4')	4.59	4.56	4.56	CH_3(2")	0.76	0.79	0.78	H(5+5')	3.01	*
H(5'+5")	4 23	4.24	4.24	H(3')	4.00	3.97	3.96	H(6+6')	1.72	1.58
H(2)	8.29	8.40	8.38	H(5'+5")	3.46	3.44	3.43	H(7+7')	1.72	1.69
H(8)	8.55	8.60	8.58	H(6'+6")	2.46	2.43	2.48	H(8+8')	3.01	3.21
				H(8'+8")	3.31	3.31	3.28			
				H(9'+9")	2.60	2.64	2.56			

Chemical shifts in ppm *vs*TSP (see Experimental section). The proton labeling follows ref. 7 (see also formulae **1a** and **1b**) The linker CH_2 methylene resonates at 3.22 ppm for **1a** and 3.25 ppm for **1b**. (*) corresponds to one of the overlapping triplets, centered at 3.03, 3.05 and 3.09 ppm respectively, for which no regioselective assignments are given here.

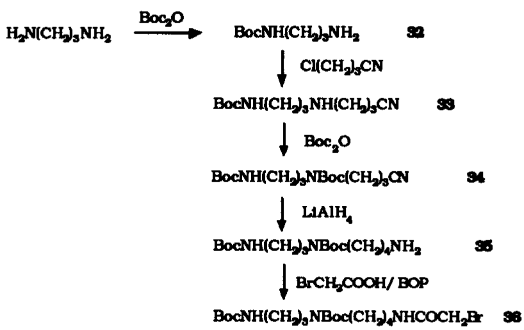
^1H NMR unambiguously establishes the nature of the substitution at the N^1 atom of spermidine in **1a** with the methylene protons $^6CH_2$ and $^7CH_2$ displaying equivalent chemical shifts in the spermidine moiety. In contrast, in **1b** the $^6CH_2$ and $^7CH_2$ groups display distinct resonances (at 1.58 and 1.69 ppm), in agreement with the different environments of N^4 and N^8 (amine and amide, respectively). It is to be noted that in **1a** the spermidine $^2CH_2$ group unexpectedly resonates at higher field (1.87 ppm) than it does in **1b** (2.07 ppm). The former belongs to the motif CO-^{1}NH-$^1CH_2$-$^2CH_2$-$^3CH_2$-^{4}NH-(the $^2CH_2$ group therefore occupies the β position, respectively to the primary amido group ^{1}N and the secondary amino group ^{4}N), whereas the latter belongs to the motif $H_2{}^1N$-$^1CH_2$-$^2CH_2$-$^3CH_2$-^{4}NH- (the $^2CH_2$ group therefore occupies the β position, respectively to both amino groups $^1NH_2$ and ^{4}NH). One would therefore expect, on the basis of inductive effects, that the $^2CH_2$ in **1a** would resonate at lower field than the $^2CH_2$ in **1b**, in contrast with what is observed experimentally (Table 3). As expected, inductive effects are observed for both $^1CH_2$ and $^8CH_2$ when going from **1a** to **1b** (+ 0.2 ppm for $^8CH_2$ and -0.2 ppm for $^1CH_2$). One possibility would be that the *locus* occupied by the $^2CH_2$ in **1a** is affected by ring current shift effects originating from the remote adenosine moiety if the molecule adopts a folded conformation. It must be also noted that the H(2) resonance from the adenine ring has its position significantly altered in comparison to CoA-SH itself, whereas the H(8) resonance is practically not affected (see Table 3). Obviously, care needs to be exerted since intermolecular contacts could occur at the level of the adenine ring, and the differences observed for selective chemical shifts in the CoA-SH, **1a** and **1b** molecules might translate inter- as well as intramolecular effects. A hairpin-like conformation is observed for CoA-SH itself in the crystal of the binary complex CoA-SH/CAT (chloramphenicol acetyltransferase)[8]. A hairpin-folded conformation has also been invoked as a possible conformation in solution for CoA-SH and CoA-SAc on the basis of NMR evidence[7]. It is therefore intriguing to establish if such a folded conformation is also present in solution for compounds **1a** and **1b**, among other possible conformations (NMR work in progress).

We describe now the main steps of the synthesis leading to the inhibitors **1a**, **1b**, **2a** and **3a** of the histone acetyltransferase. The N^1-bromoacetyl N^4,N^8-diBoc spermidine **24**, which is the precursor of **1a**, was synthesized according to scheme 8, starting from 1,4-diaminobutane. The preparation of the diBoc derivative **8** of spermidine closely follows the procedure of Humora and Quick[9], with the exception that $LiAlH_4$ was favourably replaced by H_2/Ni Raney during the reduction of the cyano function of **12** (see Experimental Section). Finally the acylation of the free $^1NH_2$ group in **8** is achieved in high yield through reaction with bromoacetic acid in the presence of DCC or BOP (Scheme 8).



Scheme 8

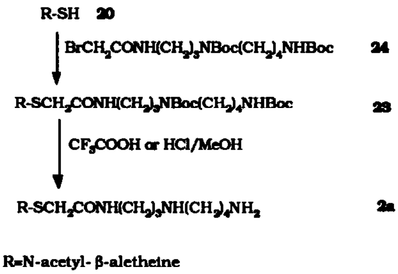
Similarly, N^8-bromoacetyl N^1,N^4-diBocspermidine **36**, which is the precursor of **1b** was prepared according to a novel strategy starting from 1,3-diaminopropane (Scheme 9). The preparation of the monoBoc derivative **32** is only achieved with a low yield (not exceeding 10%: see Experimental Section), whereas the two last steps, i.e. **34** → **35** → **36**, proceed in good yields (about 80% each). The transformations **32** → **33** → **34** are achieved with an overall yield of ca 30%.



Scheme 9

The preparation of selectively protected derivatives of spermidine is crucial for carrying out such regioselective syntheses. In contrast to methods which afford a mixture of different Boc derivatives of spermidine, through partial derivatization by Boc_2O, followed by purification of the corresponding compounds[10], both schemes 8 and 9 for producing N^4, N^8-diBocspermidine **8** (according to Humora and Quick[9]) and N^1, N^4-diBocspermidine **36** (this work) correspond to strict regioselective strategies (the third diBoc derivative N^1, N^8-diBoc spermidine has been also synthesized through a regioselective strategy[10,11]).

In relation to the novel synthetic pathway given in scheme 7, the "shortened " inhibitor **2a** is obtained by replacing CoA-SH by N-acetyl β-aletheine **20** (Scheme 10). The diBoc derivative **23** was treated by TFA in a first time but subsequently it was treated by HCl to give **2a** as an hydrochloride, for preventing TFA effects during *in vivo* inhibitory studies(work in progress). The ^1H NMR spectrum of **2a** unambiguously establishes the validity of our synthetic strategy, in regard to the purity of the final product, as well as to its structure (see NMR parameters in Experimental Section). The observation of both $^6CH_2$ and $^7CH_2$ groups as a superimposed resonance at

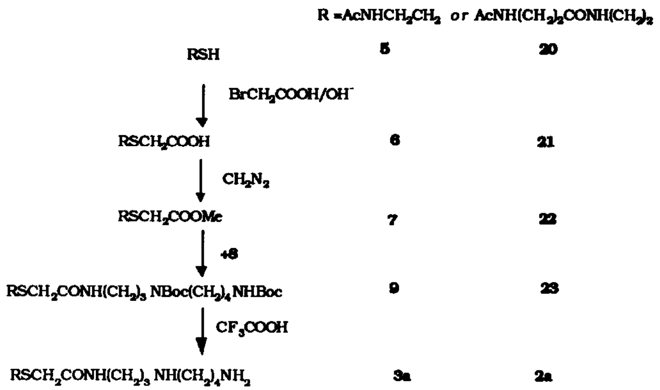


Scheme 10

1.62 ppm clearly establishes the substitution of the Spd N^{1}atom as an amide (compare with **1a** in Table 3). We note here that the Spd polymethylene chain is characterized by ^3J vicinal coupling constants in the 6.8-7.3 Hz range, thus denoting the occurrence of time-averaged rotamers about the different single bonds. The ^3J coupling constants are somewhat lower in the β-aletheine moiety, in the 6.4-6.6 Hz range, and this would translate either selective conformational effects along the chain, or inductive effects acting on the coupling constants. In agreement with our observations with **2a**, CoA-SH itself is characterized by $^3J_{5',6'}$ and $^3J_{8',9'}$ of about 6.6 Hz[7], thus suggesting that the conformation of the β-aletheine moiety is similar in both molecules, **2a** and CoA-SH.

As an alternative way, to obtain the "shortened" inhibitors **2a** and **3a**, we have introduced the carboxymethylene group, or "linker", as the thioacetic derivatives of RSH compounds **5** and **20** by amide formation with the corresponding diBocspermidine derivative **8** (scheme 11).

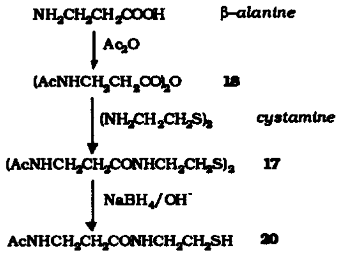
It must be noted that the preparation of the R- S-CH_2-COOH derivatives **6** and **21** can be readily carried out



Scheme 11

by reacting bromoacetic acid itself with the corresponding thiol R-SH[12]. In this respect, **6** and **21** were conveniently prepared from N-acetylcysteamine **5** and N-acetyl-β-aletheine **20**, respectively (Scheme 11). As noted above bromoacetyl derivatives of a thiol such as **30** and **28** (Scheme 4), lead to the same thioacetic acid derivatives **6** and **21**, but such a procedure was not further investigated here since a more direct possibility exists according to Scheme 10 for preparing the two "shortened" inhibitors **2a** and **3a**.

Both compounds **5** and **20** were prepared according to the procedure of Baddiley and Thain[13]. We also report a novel synthesis of **20** (see Experimental Section) through the condensation of the anhydride of β-alanine **18** with cystamine, followed by reduction of the S-S bond (Scheme 12). In comparison to the initial procedure by Baddiley and Thain[13], our three-step synthesis starting from β-alanine appears to be more convenient, although the overall yield is not improved significantly.



Scheme 12

CONCLUSION

We provide here good evidence that the compound formed by reacting CoA-SH with bromoacetylthiophenol **30** is S-bromoacetylCoA **42**, in agreement with the initial report of Chase and Tubbs[3]. Such a conclusion was challenged by Clements et al.[4], who assumed that the reaction yields instead the 2-(S-CoA) acetic acid thiophenylester **10**[1]. These authors indeed observed by treating such a supposedly intermediate compound **10**, under alkaline conditions that the product was 2-(S-CoA) acetic acid **11** (Scheme 1). Such a compound CoA-S-CH_2-COOH would then derive in principle from CoA-S-CH_2-CO-S-C_6H_5 **10**, through saponification of the thioester function. However, we demonstrate in this work that a simpler analog of **42**, i.e.R-S-CO-CH_2Br **28** (including the S-terminal cysteaminyl moiety of coenzymeA) is sensitive to alkaline conditions to yield the corresponding 2-(S-cysteaminyl) acetic acid **6**, R-S-CH_2-COOH, the analog of **11**. Starting from the bromoacetyl derivative, CoA-S-CO-CH_2Br **42** (and not **10**) the formation of **11** is thus explained by a two-step reaction, i.e. saponification of the thioester function followed by a nucleophilic attack of the bromine atom by the intermediarily liberated thiolate anion, thus explaining the "inversion" of the motif S-CO-CH_2- into S-CH_2-CO-. A detailed mechanism of the reaction needs, however, to be established. Furthermore, we unambiguously establish here that the bromoacetyl derivative R-S-CO-CH_2-Br **28** leads, upon treatment with a monoamine derivative (diBoc derivative of Spd), to R-S-CH_2-CO-Spd. This apparently corresponds to a general reactivity of the bromoacetyl derivatives of thiols, as demonstrated by the reaction of bromoacetylthiophenol **30** itself with different nucleophiles (OH^-, primary amine). Such a reaction appears to be novel, and it opens the route to the preparation of compounds R-S-CH_2-COR' from halogeno (X) acetic acid thioesters X-CH_2-CO-S-R'[6]. All together, we suggest that the reaction, as carried out by Cullis et al[1] to prepare CoA-S- CH_2-CO-Spd (through the reaction of CoA-SH with bromoacetylthiophenol **30** followed by the action of spermidine under alkaline conditions), follows the pathway: CoA-SH + **30** $\rightarrow$CoA-S-CO-CH_2Br $\rightarrow$ CoA-S-CH_2-CO-Spd, and not CoA-SH + **30** $\rightarrow$CoA-S-CH_2-CO-S-C_6H_5 $\rightarrow$ CoA-S-CH_2-CO-Spd, as proposed by these authors[1], on the basis of the previous conclusion by Clements et al.[4]. We also conclude that the product of the overall reaction corresponds indeed to the structure **1**, as initially proposed by Cullis et al.[1]. This work provides a novel and regioselective strategy for preparing compounds **1**, i.e. **1a** and **1b**, from CoA-SH by using spermidine substituted by the bromoacetamide function at the N^1 atom and the N^8 atom, respectively. It is to be noted that this strategy differs from that using the S-bromoacetylCoA, as an intermediate, in the sense that the reactive carbon atom, substituted by the halogen, is introduced on the spermidine moiety instead of the CoA moiety.

Finally, this work establishes a versatile and convenient synthetic strategy for preparing a variety of compounds, structurally resembling the multisubstrate inhibitor CoA-S-CH_2-CO-Spd **1**, but differing by their cofactor moieties. As previously established[2], the shortening of the CoA moiety to the level of β-aletheine (compound **2a**) does not affect significantly the inhibitory effect on the histone acetyltransferase. Other analogs including the pantotheinyl radical have now been prepared (to be published). Work is in progress for comparing the inhibitory effects of **1a** and **1b**, as well as of different "shortened" analogs, on the histone and polyamine acetyltransferases.

EXPERIMENTAL SECTION

Commercial products: cystamine dihydrochloride purum (from Fluka, Switzerland); CoA-SH (from Sigma;USA) as the sodium salt: approx. 95% CoA-SH; other products, reagents and solvents of controlled purity. The BOP reagent, i.e.benzotriazolyl N-oxytri-dimethylamino-phosphonium hexafluorophosphate, was prepared as[14]. Elemental analyses were performed by the "Laboratoire de Microanalyses" (ENSCM, Montpellier). ^1H NMR spectra were measured on a Bruker WM 360 WB spectrometer functionning on the FT mode (NMR laboratory, C.C.I.P.E., CNRS-INSERM, Montpellier) using as an internal reference the residual proton signal of the solvent. The NMR chemical shifts δ are given in ppm versus tetramethylsilane (TMS) or trimethylsilylpropionate (TSP). NMR assignments are essentially based on empirical correlations, and involved in some cases double resonance experiments. Coupling constants J in Hz; s = singlet; t = triplet; q = quintuplet. After correction for temperature effects, our NMR,parameters for CoA-SH (at 38°C; see Fig.1 and Table 3) are in complete agreement with those previously reported by Lee and Sarma[7] (at 30.5°C and referred to tetramethylammonium chloride, or TMA, as an internal reference: δ(TMA) = δ (TSP) + 3.20 ppm. Mass spectra (MS) were measured at the "Laboratoire de Mesures Physiques " with a spectrometer JEOL JMS DX300 (U.S.T.L., Montpellier) in Fast Atom Bombardment (Positive), if not stated otherwise, with NBA as a solvent. Analytical thin layer chromatography TLC was performed by using silica gel G60 F_{254} plates and preparative TLC by using Merck TLC G60 F_{254} plates. Revelator: plates sprayed (solution H_2O:H_2SO_4:$SO_4(NH_4)_2$; 100 ml: 4 ml: 20 g) followed of strong heating. The nomenclature concerning the coenzyme A itself and its derivatives with spermidine is given according to ref[7](see also formulae **1a** and **1b**).

As noted by Roberts and Caserio (Basic Principle of Organic Chemistry, 2nd edition), confusion is possible with the names and formulae for the derivatives of the coenzyme A (abbreviated CoASH to emphasize the SH group, whereas the acyl derivatives most often are called acylCoA); throughout this work we systematically included the sulfur atom in all the formulae of the coenzyme A derivatives, i.e. R-S-CoA. The name thioester is systematically used for the different R-S-CO-R' derivatives, in which a thiol R-SH is acylated by a carboxylic acid HOCO-R'.

N^1-[2-(S-coenzyme A) acetyl] spermidine amide ditrifluoroacetate (1a): Scheme 7.

25 (14 mg), dissolved in CF_3COOH (0.5 ml) is left 10 min at room temperature. After evaporation the solution affords **1a** (quantitative yield) as a white solid. ^1H NMR (D_2O): see Table 3.

N^8-[2-(S-coenzyme A) acetyl] spermidine amide ditrifluoroacetate (1b): Scheme 7.

37 (23 mg) is dissolved in CF_3COOH (0.5 ml)and treated as above to yield **1b** (quantitative yield) as a white solid. ^1H NMR (D_2O): see Table 3.

N^1-[2-(S,N-acetyl β-aletheinyl) acetyl] spermidine amide dihydrochloride 2a(Cl): Scheme 10.

23 (1 g, 1.74 mmol) is dissolved in 6 ml of MeOH to which 0.75 ml 12N HCl is added. After standing 8 h, 0.8 ml HCl 12N are added. After 24 h, 0.75 ml 12 N HCl are added . After 48 h, the solution is evaporated. The product, dissolved in minimum MeOH, cristallizes. After addition of ether the product is filtered and dried· **2a**(Cl) (695 mg, yield 96%). An analytical sample is obtained by recristallization in MeOH-EtOH; m.p. 191°C; yield 89%. MS: (m/z) 376 $(M+H)^+$ ($C_{16}H_{33}N_5O_3S$, MW 375.5). pK_1 8.63, pK_2 10.40 (at 25°C). ^1H NMR (D_2O at 46°C) 1.62 (m, 4H, $^6CH_2$, $^7CH_2$); 1.78 (J =7.3, 2H, $^2CH_2$); 1.83 (s, 3H, CH_3CO); 2.30 (t, J= 6.6, 2H, $^{6'}CH_2$); 2.58 (t, J = 6.6, 2H, $^{9'}CH_2$); 2.85-3.0 (m, 6H, $^3CH_2$, $^5CH_2$, $^8CH_2$); 3.16 (s, SCH_2CO); 3.18 (t, J = 6.8, $^1CH_2$: 4H in total); 3.25 (t, J = 6.4, $^{8'}CH_2$); 3.28 (t, J =6.4, $^{5'}CH_2$): 4H in total. ^1H NMR (DMSO-d_6): 7.9 (t, 1H); 8.13 (broad t, 3H, $^8NH_3^+$); 8.34 (t, 1H, amide NH's); 9.12 (broad s, 2H, $^4NH_2^+$). Anal.calcd for $C_{16}H_{35}N_5O_3SCl_2$: $C_{42.85\%}$; $H_{7.87\%}$; $N_{15.62\%}$; $O_{10.71\%}$; $S_{7.15\%}$; $Cl_{15.81\%}$.Found: $C_{42.80\%}$; $H_{7.72\%}$; $N_{15.47\%}$; $S_{6.38\%}$; $Cl_{17.44\%}$.

N^1-[2-(S,N-acetyl β-aletheinyl) acetyl] spermidine amide ditrifluoroacetate 2a(F): Scheme 10.

23 (30 mg, 0.05 mmol) is dissolved in CF_3COOH (0.5 ml). After 1/2 h at room temperature, the solution is evaporated, dissolved in water and washed with CH_2Cl_2. The aqueous layers, lyophilized, lead to **2a**(F). The ^1H NMR spectrum is identical to **2a**(Cl).

N^1-[2-(S,N-acetyl cysteaminyl) acetyl] spermidine amide ditrifluoroacetate (3a): Scheme 11.

9 (73 mg) is dissolved in minimum of CF_3COOH. After 1/2 h at room temperature, the solution is evaporated leading to **3a** (quantitative yield). MS: (m/z) 305(M+H)$^+$($C_{13}H_{29}N_4O_2S$, MW 304.4). ^1H NMR (D_2O) δ 1.65 (m, 4H, $^6CH_2$ + $^7CH_2$); 1.80 (m, 2H, $^2CH_2$); 1.89 (s, 3H, CH_3CO); 2.62 (t, 2H, $^{9'}CH_2$); 2.92-2.97 (m, 6H, $^3CH_2$, $^5CH_2$, $^8CH_2$); 3.20 (s, SCH_2CO); 3.23 (t, $^1CH_2$): 4H in total; 3.30 (t, 2H, $^{8'}CH_2$).

2-(S,N-acetyl cysteaminyl) acetic acid (6).

1) from N-acetyl cysteamine **5**[13]: Scheme 11. A mixture of **5** (1.6 g, 13.4 mmol), KOH 1 N (27 ml) and bromoacetic acid (1.96 g, 14.1 mmol) is left 3 h at room temperature, then evaporated and washed with acetone. The residue acidified and evaporated is dissolved in water and extracted by CH_2Cl_2. The aqueous phase is evaporated under reduced pressure and dried, leading to an oil **6** (1.7 g, yield 71%). CI-MS (Xe) (m/z): 178 (M+H)$^+$($C_6H_{11}NO_3S$, MW 177.2). ^1H NMR (D_2O) δ 1.80 (s, 3H, CH_3CO); 2.60 (t, J=10, 2H, $^{9'}$-CH_2S); 3.20 (s+t, 4H, $^{8'}CH_2N$, S-CH_2COOH).

2) from N-acetyl S-(2-bromoacetyl) cysteamine thioester **28**: Scheme 4. To a solution of **28** (see below in this experimental section) (725 mg, 3 mmol) in EtOH (10 ml) is added a solution of KOH in EtOH (423 mg in10 ml). The solution is evaporated and acidified by HCl and reevaporated. The residue dissolved in water is extracted by CH_2Cl_2. The aqueous phase is evaporated and resuspended in acetone leading to **6** (250 mg, yield 47%).

2-(S,N-acetyl cysteaminyl) acetic acid methyl ester (7): Scheme 11.

6 (300 mg, 1.69 mmol), dissolved in MeOH (15 ml) is treated at room temperature with an etheral solution of CH_2N_2 until the yellow colour remains persistant. After 1 h a drop of acetic acid is added to destroy the excess of CH_2N_2 and evaporation leads to an oil **7** (250 mg, yield 77%). MS: (m/z) 192 (M+H)$^+$ ($C_7H_{13}NO_3S$, MW 191.3). ^1H NMR (D_2O) δ 1.90 (s, 3H, CH_3CO); 2.70 (t, 2H, $^{9'}CH_2S$); 3.3 (t, 2H, $^{8'}CH_2N$); 3.35 (s, 2H, SCH_2CO); 3.7 (s, 3H, OMe).

N^4,N^8-di-tert-butyloxycarbonylspermidine (8): Scheme 8.

N^1,N^4-di-tert-butyloxycarbonyl N^4-(2'-cyanoethyl)1,4-diaminobutane **12** (3.3 g, 9.66 mmol), prepared according to[9] is dissolved in absolute EtOH and stirred under atmospheric pressure of H_2 during 12 days, with Raney Ni. After filtration and evaporation, an oily residue is obtained which is dissolved in $CHCl_3$ and washed several times with a 10% citric acid solution. The aqueous layers are made basic with a saturated NaOH solution. The extraction with CH_2Cl_2 leads to **8** (2 g, yield 60%). MS: (m/z) 346 (M+H)$^+$ ($C_{17}H_{35}N_3O_4S$, MW 345.5). ^1H NMR spectrum identical to that published by Humora and Quick [9].

N^1-[2-(S,N-acetyl cysteaminyl) acetyl] N^4,N^8-di-tert-butyloxycarbonylspermidine amide (9).

1) from the methyl ester **7**: Scheme 11. Compound **7** (100 mg, 0.52 mmol) and **8** (600 mg, 1.74 mmol) are dissolved in MeOH. The solution is evaporated and heated at 100°C, during 10 h, under Ar atmosphere. The mixture treated again as above for eliminating the excess of amine, leads to an oil, which is chromatographed on silica gel with $CHCl_3$ as a solvent. **9** (73 mg, yield 28%) is obtained as an oily residue. MS: (m/z) 505 (M+H)$^+$ ($C_{23}H_{44}N_4O_6S$, MW 504.7). ^1H NMR ($CDCl_3$) δ 1.40, 1.42 (2s, Boc groups at ^4N and ^8N: no regioselective assignment); 1.40-1.70 (m, $^2CH_2$, $^6CH_2$ and $^7CH_2$): 24H in total; 1.95 (s, 3H, CH_3CO); 2.70 (t, 2H, $^{9'}CH_2S$); 3.1-3.45 (m, $^3CH_2$, $^5CH_2$, $^8CH_2$ and $^{8'}CH_2$); 3.18 (s, SCH_2CO):10H in total; 4 7 (broad signals, ^{8}NHBoc); 6.8 and 7.5 (amide NH groups).

2) from N-acetyl cysteamine **5** : Scheme 3. To **5** (109 mg, 0.91 mmol) dissolved in NaOH 1N (2 ml) is added a solution of **24** (464 mg, 1.0 mmol) in MeOH (10 ml). After 4 h at room temperature the solvent is evaporated; the residue is extracted with CH_2Cl_2 and washed with water. The organic layer, dried on Na_2SO_4 leads to a mixture which is chromatographed on silicagel (solvent MeOH-$CHCl_3$ 2:98,v:v). **9** (300 mg, yield 65%) is obtained as an oil. ^{1}H NMR spectrum identical to that of **9** obtained according to procedure *1*.

3) from N-acetyl S-(2-bromoacetyl) cysteamine thioester **28**: scheme 3. To **28** (190 mg, 0.8 mmol) is added, first, a solution of **8** (3.6 g, 10.4 mmol) in dioxane (8 ml) and secondly, NaOH 4N (0.4 ml). After 15 min of stirring at room temperature, dioxane is evaporated. The residue is then extracted with $CHCl_3$, washed with $KHSO_4$ 1M, water and dried on Na_2SO_4, leading to a residue which is dissolved in EtOAc, filtered under vacuum on silicagel G60 Merck (7 g), followed by extensive washings with EtOAc. The organic solution after evaporation yields 460 mg of a residue showing by TLC analysis (MeOH:EtOAc; 20:80; v:v) a major product (coloured with iodine). By preparative TLC of 80 mg from the crude product, pure **9** is obtained, as established by ^{1}H NMR (45 mg, yield 63%).

N,N'-diacetyl-β-alethine (17): Scheme 12.

1) in heterogeneous phase. To a solution of **18** (3 g, 12.3 mmol) in CH_2Cl_2 (25 ml) is added a solution of cystamine dihydrochloride (0.5 g, 2.5 mmol) in H_2O (10 ml) (pH 10 with NaOH 2N). The mixture is stirred overnight and then the organic phase is separated and washed with water. The aqueous phases are acidified with HCl 1N to pH 2.6 and evaporated. The oily residue is stirred with EtOAc. The precipitate thus formed is crystallized two times in MeOH leading to **17** (200 mg, yield 24%); m.p. 209-210°C; [m.p.[15] 207.5-208°C, m.p.[13] 190°C]. MS: (m/z) 379 $(M)^+$ ($C_{14}H_{26}N_4O_4S_2$, MW 378.6). ^{1}H NMR (D_2O) δ 1.90 (s, 2x3H, CH_3CO); 2.35 (t, 2x2H, $^{6'}CH_2CO$); 2.75 (t, 2x2H, $^{9'}CH_2S$); 3.40 (m, 2x4H, $^{5'}CH_2$ and $^{8'}CH_2$).

2) in homogeneous phase . To **18** (120 g, 0.49 mol) is added a solution of 20 g of cystamine (0.13 mol) in 100 ml of pyridine. After one night at room temperature, a solid residue is obtained upon filtration (followed by several washings with EtOAc) and solvent evaporation. Upon crystallization in MeOH, **17** (8 g, yield 16%, m.p. 197-198°C) is thus obtained judged pure by ^{1}H NMR. This product was used successfully for the following synthetic steps.

N-acetyl β-alanyl anhydride (18): Scheme 12.

A suspension of β-alanine (2 g, 22.5 mmol) in acetic anhydride (15 ml) was stirred until it is completely dissolved and concentrated under strong heating. **18** (2.7 g, yield 99%) is obtained as a colourless oil, soluble in CH_2Cl_2 (the corresponding acid is totally insoluble in this solvent).

N-acetyl β-aletheine (20): Scheme 12.

17 (11.9 g, 31 mmol) is dissolved in 29 ml of NaOH 0.5 N to which is added solution of 10.8 g of $NaBH_4$ in 27 ml NaOH 0.5N. The resulting solution is heated on an oil bath (150°C) under nitrogen atmosphere,with magnetic stirring. After 15 min a very strong gas escape is observed. The reaction flask is kept at room temperature during 0.5 hour. After extraction with $CHCl_3$ and evaporation of the organic phase, **20** is obtained as a white solid (11 g, yield 92%), homogeneous by TLC (neutral alumina with MeOH:CH_2Cl_2; 5:95; v:v; coloured yellow by I_2). MS: (m/z) 191$(M+H)^+$ ($C_7H_{14}N_2O_2S$, MW 190.3). ^{1}H NMR (D_2O) δ 1.80 (s, 3H, CH_3CO); 2.30 (t, 2H, $^{6'}CH_2CO$); 2.50 (t, 2H, $^{9'}CH_2S$); 3.30 (m, 4H, $^{5'}CH_2$ and $^{8'}CH_2$).

2-(S,N-acetyl β-aletheinyl) acetic acid (21): Scheme 11.

Bromoacetic acid (200 mg, 1.44 mmol) is added to a solution of **20** (260 mg, 1.38 mmol) in KOH 1 N (3.76 ml). After 12 h at room temperature, the solution is evaporated. The residue is washed three times with acetone and resuspended in water (30 ml). The solution is acidified by concentrated HCl and extracted by CH_2Cl_2. The aqueous layer is evaporated and washed with acetone (5x30 ml). The evaporation of the organic solution leads to an oil **21** (253 mg, yield 74%). MS: (m/z) 249 $(M+H)^+$ ($C_9H_{16}N_2O_4S$, MW 248.3) .^1H NMR (D_2O) δ 1.90 (s, 3H, CH_3CO); 2.35 (t, 2H, $^{6'}CH_2$); 2.7 (t, 2H, $^{9'}CH_2S$); 3.32 (s, SCH_2CO); 3.30-3.40 (m, $^{5'}CH_2$ and $^{8'}CH_2$): 6H in total. In one preparation, a cristallized product **21** was obtained ; m.p.114-116°C. Anal. calcd for $C_9H_{16}N_2O_4S$: $C_{43.53\%}$; $H_{6.49\%}$; $N_{11.28\%}$; $O_{25.78\%}$; $S_{12.91\%}$. Found: $C_{43.13\%}$; $H_{6.53\%}$; $N_{11.19\%}$; $O_{25.49\%}$; $S_{12.62\%}$.

2-(S,N-acetyl β-aletheinyl) acetic acid methyl ester (22): Scheme 11.

A solution of **21** (253 mg, 1.02 mmol) in MeOH (20 ml) is treated as above (preparation of **7**) leading, after chromatography on silicagel (MeOH: CH_2Cl_2) to **22** (88 mg, yield 33%); m.p. 80-81°C. MS: (m/z) 263 $(M+H)^+$ ($C_{10}H_{18}N_2O_4S$, MW 262.3). ^1H NMR ($CDCl_3$) δ 1.89 (s, 3H, CH_3CO); 2.34 (t, 2H, $^{6'}CH_2$); 2.73 (t, 2H, $^{9'}CH_2S$); 3.20 (s, SCH_2CO); 3.40-3.55 (m, 4H, $^{5'}CH_2$ and $^{8'}CH_2N$); 3.67 (s, 3H, OMe); 6.40 (m, 2H, amide NH's). Anal. calcd for $C_{10}H_{18}N_2O_4S$: $C_{45.79\%}$; $H_{6.92\%}$; $N_{10.68\%}$; $O_{24.40\%}$. Found: $C_{45.81\%}$; $H_{7.07\%}$; $N_{10.51\%}$; $O_{24.76\%}$

N^1-[2-(S,N-acetyl β-aletheinyl) acetyl] N^4,N^8-di-tert-butyloxycarbonylspermidine amide (23).

1) from methyl ester **22**: Scheme 11. Compound **22** (80 mg, 0.30 mmol) and **8** (750 mg, 2.17 mmol) are dissolved in MeOH. The solution is evaporated and heated during 24 h to 100°C under Ar and treated as usually: washings with citric acid 10%, $NaHCO_3$ saturated solution and water, leading to 130 mg of an oil. 60 mg of it are chromatographed on preparative TLC with MeOH-acetone, 6:94 (v:v) as solvent. Pure **23** (30 mg, yield 38%) is obtained. MS: (m/z) 576 $(M+H)^+$($C_{26}H_{49}N_5O_7S$, MW 575.8). ^1H NMR ($CDCl_3$) δ 1.50, 1.52 (2s, Boc groups); 1.40-1.70 (m, $^2CH_2$, $^6CH_2$ and $^7CH_2$): 24 H in total; 1.95 (s, 3H, CH_3CO); 2.40 (t, 2H, $^{6'}CH_2$); 2.75 (t, 2H, $^{9'}CH_2S$); 3.10-3.25 (m, 4H, $^3CH_2$, and $^5CH_2$); and 3.20 (s, SCH_2CO): 6H in total; 3.50 (m, 4H, $^{5'}CH_2$ and $^{8'}CH_2$); 4.70, 6.60, 7.10, 7.50 (4m, 4H, NH).

2) from N-acetyl β-alethetne **20**: Scheme 10. Compound **20** (330mg, 1.73 mmol) is dissolved in 4ml NaOH 0.75 N to which **24** (1.05 g, 2.25 mmol) in 7 ml CH_3OH, is added by stirring and kept at room temperature, overnight. This solution is evaporated and the residue is washed with CH_3OH and purified by preparative TLC to give **23** (520 mg, yield 52%). In another preparative assay with 650 mg of **20** the crude product **23** is chromatographed on neutral alumina (EtOAc : hexane, followed by methanol : ethyl acetate mixtures). Finally the pure compound **23** is eluted with MeOH-EtOAc, 5:95 (v:v): 1.54 g; yield 78%.
^1HNMR is identical to procedure 1.

N^1-(2-bromoacetyl) N^4, N^8-di-tert-butyloxycarbonylspermidine amide (24): Scheme 8.

To a solution of **8** (280 mg, 0.81 mmol) in CH_2Cl_2 (50 ml) is added, successively: dicyclohexylcarbodiimide (165 mg), hydroxybenzotriazole (108 mg) and bromoacetic acid (111 mg). A precipitate is formed immediatly and, after 24 h at room temperature, the suspension is filtered. The residue is resuspended in CH_2Cl_2 (10 ml). The filtrate is chromatographed on silicagel. The product is eluted with MeOH-CH_2Cl_2, 1: 99 (v:v). **24** (340 mg, yield 89%) is obtained as a colourles oil. MS: (m/z) 466, 468 $(M+H)^+$ ($BrC_{19}H_{36}N_3O_5$, MW 466.4). ^1H NMR ($CDCl_3$) δ 1.38 and1.42 (2s, Boc groups); 1.30-1.70 (m, $^2CH_2$, $^6CH_2$ and $^7CH_2$): 24H in total; 3.05-3.15 (m, 4H, $^3CH_2$ and $^5CH_2$); 3.15-3.30 (m, 4H, $^1CH_2$ and $^8CH_2$); 3.80 (s, 2H, $BrCH_2CO$).

N^1-[2-(S-Coenzyme A) acetyl] N^4,N^8-di-tert-butyloxycarbonylspermidine amide (25): Scheme 7.

To a solution of CoA-SH (sodium salt; 15 mg, ca 18 μmol) in water (1 ml), is added successively: NaOH 1N (15 μl) and a solution of **24** (13.5 mg, 29 μmol) in THF (135 μl). After 1 h at room temperature, the solution is evaporated, diluted with water and washed several times with CH_2Cl_2. The aqueous layer, after lyophilisation, leads to **25** (14 mg ca 13 μmol; yield ca 70%) as white solid. ^1H NMR (D_2O) δ 1.42, 1.44 (2s, Boc groups); 1.30-1.80 (m, $^2CH_2$, $^6CH_2$ and $^7CH_2$): 24 H in total; 2.75 (t, 2H, $^{9'}CH_2S$); 3.10 (t, 2H, $^8CH_2$); 3.25 (m, 6H, $^1CH_2$, $^3CH_2$ and $^5CH_2$); 3.30 (s, 2H, SCH_2CO).

N-acetyl S-(2-bromoacetyl) cysteamine thioester (28).

1) from 5 with bromoacetylthiophenol **30**: Scheme1. A solution of **5** (90 mg, 0.76 mmol) in some acetone is added to a solution of **30** (2.6 g, 11.3 mmol) in a mixture of acetone (43 ml) and $KHCO_3$ 0.1M (17 ml). After 1 h at room temperature, the solution is acidified with HCl 0.1N to pH 2. The acetone is evaporated under reduced pressure and the suspension extracted by CH_2Cl_2. The organic layers are washed with $KHCO_3$ 3% solution and evaporated. The oily residue is dissolved in C_6H_6 and filtered, under vacuum, on silicagel G60 (Merck)(10 g), this one, washed several times with C_6H_6. The filtrate contains essentially bromoacetyl thiophenol. Then, the silicagel is washed with MeOH-CH_2Cl_2, 20:80 mixture. The filtrate leads, after evaporation, to **28** (130 mg, yield 72%). MS: (m/z) 240, 242 $(M+H)^+$ ($BrC_6H_{10}NO_2S$, MW 240.1). ^1H NMR ($CDCl_3$) δ 2.00 (s, 3H, CH_3CO); 3.10 (t, 2H, CH_2SCO); 3.40 (t, 2H, CH_2N); 4.10 (s, 2H, $COCH_2Br$).

2) from 5 with bromoacetyl bromide. To a solution of **5** (119 mg, 1 mmol) is added, dropwise, bromoacetyl bromide (139 µl). The mixture is stirred under reduced pressure during 5 min, then added of $KHCO_3$ saturated solution and extracted with CH_2Cl_2. **28** (130 mg, yield 54%) is obtained by evaporation of organic phases. ^1H NMR is identical to the product obtained precedently by action of bromoacetyl thiophenol.

N-acetyl S-(2-bromoacetyl) β-aletheine thioester (29): Scheme 1.

A solution of **30** (8 g, 35 mmol) in a mixture of acetone (43 ml) and $KHCO_3$ 0.1M (17 ml) is added to **20** (185 mg, 0.97 mmol). After 1 h at room temperature, the mixture is evaporated and washed with MeOH. This suspension is filtered, evaporated and washed with CH_2Cl_2. The new suspension is filtered and evaporated. The residue is crystallized in EtOAc, then C_6H_6, leading to **29** (80 mg, yield 27%). TLC homogeneous with MeOH: CH_2Cl_2; 8:92 (v/v); m.p. 118-120°C; MS:(m/z) 311, 313 $(M+H)^+$ ($BrC_9H_{15}N_2O_3S$, MW 311.2) . ^1H NMR ($CDCl_3$) δ 2.00 (s, 3H, CH_3CO); 2.30 (t, 2H, CH_2CO); 3.10 (t, 2H, CH_2S); 3.50 (m, 4H, CH_2N); 4.10 (s, 2H, $SCOCH_2Br$); 6.40-6.90 (broad signal, 2H, NH). Anal. calcd for $BrC_9H_{16}N_2O_3S$: $C_{34.74\%}$; $H_{4.86\%}$; $N_{9.00\%}$; $O_{15.43\%}$. Found: $C_{34.87\%}$; $H_{4.98\%}$; $N_{8.81\%}$; $O_{15.59\%}$.

Bromoacetyl thiophenol (30)

Although the synthesis of this compound has been described in the literature[3,5] *we describe here our preparation and emphasize on its purification.* Thiophenol (6 ml, 58.4 mmol) is added in several times to bromoacetyl bromide (7.2 ml). The mixture is maintained stirred, under reduced pressure 60 min at room temperature. The solution is added to saturated solution of $NaHCO_3$ (70 ml). The suspension is extracted with ether. The organic layers are washed two times with diluted solution of $NaHCO_3$. Oil **30** (12 g, yield 89%) crystallizing in cold (m.p. 39°C) is obtained. The crystallization in C_6H_6-hexane leads, after seeding and cooling, in three crops, to **30** (11.2 g, yield 83%), (m.p. 41-42°C) homogeneous in TLC; m.p. 36.3-37.3°C (EtOH/H_2O, yield[3] 57%; m.p. 38-39°C (benzene:petroleum ether), yield[5] 59%). ^1H NMR ($CDCl_3$) δ 4.05 (s, 2H, $BrCH_2CO$); 7.45 (s, 5H, C_6H_5S).

Phenylthioacetic acid (31): Scheme 4.

30 (1g, 4.3 mmol) is added to a solution of KOH (600 mg) in minimum of absolute EtOH. A precipitate is formed instantaneously which, filtered and dried, leads to potassium phenylthioacetate. This salt is dissolved in water and acidified with concentrated HCl, then extracted with CH_2Cl_2. The evaporation of the organic phase leads to **31** (670 mg, yield 93%); m.p. 63-64°C; m.p. [6] 64.5-65.5°C. ^1H NMR ($CDCl_3$) δ 3.66 (s, 2H, CH_2); 7.00-7.40 (m, 5H, C_6H_5); 10.10 (s, 1H, COOH).

N-tert-butyloxycarbonyl 1,3-diaminopropane (32): Scheme 9.

A solution of Boc_2O (8.5 g, 39 mmol) in dioxane (50 ml) is added slowly to a solution of 1,3-diaminopropane (3 g, 40.5 mmol) in water (30 ml). Firstly, there is a precipitation, then redissolution. After 24 h at room temperature, dioxane is evaporated and the suspension extracted by CH_2Cl_2. The organic phase is washed by $KHSO_4$ 1N, and the aqueous phase, basified by saturated NaOH solution, is extracted by CH_2Cl_2. The organic solution is evaporated to give **32** (630 mg, yield 8.9%). TLC homogeneous with $N(Et)_3$: MeOH:EtOAc; 5: 20: 75(v/v/v) . ^{1}H NMR ($CDCl_3$) δ 1.24 (s, 2H, NH_2); 1.41 (s, 9H, Boc); 1.59 (q, 2H, $^2CH_2$); 2.75 (t, 2H, CH_2N); 3.19 (q, 2H, $^1CH_2N$); 3.68 (s, 1H, NHBoc).

N^1-tert-butyloxycarbonyl N^3-(3-cyanopropyl) 1,3-diaminopropane (33): Scheme 9.

To **32** (630 mg, 3.6 mmol) dissolved in n-butanol (30 ml), is added successively: Na_2CO_3 (1.1 g), KI (800 mg) and 4-chlorobutyronitrile (375 µl, 3.8 mmol). After 6 h of reflux, the mixture is filtered and evaporated. The oil is resuspended in $CHCl_3$, washed with water, evaporated, giving crude **33** (840 mg, yield 97%).

N^1,N^3-di-tert-butyloxycarbonyl N^3-(3-cyanopropyl) 1,3-diaminopropane (34): Scheme 9.

To a solution of crude **33** (840 mg, 3.5 mmol) in dioxane (20 ml) is added a solution of Boc_2O (1g) in dioxane (10 ml). After 12 h at room temperature, the solvent is evaporated and the residue, taken by $CHCl_3$ is washed successively by: $KHSO_4$ 1N, a diluted $NaHCO_3$ solution and water. The organic layers lead to an oily residue which is chromatographed on silicagel with ether:hexane, 50:50 (v:v) affording **34** (400 mg, yield 34%). MS: (m/z) 342 $(M+H)^+$ ($C_{17}H_{31}N_3O_4$, MW 341.5). ^{1}H NMR ($CDCl_3$) δ 1.42, 1.45 (2s, 18H, Boc groups); 1.66 (m, 2H, $^2CH_2$); 1.87 (m, 2H, $^6CH_2$); 2.33 (t, 2H, $^7CH_2CN$); 3.08 (q, 2H, $^1CH_2$); 3.2-3.35 (m, 4H, $^3CH_2$ and $^5CH_2$).

N^1,N^4-di-tert-butyloxycarbonylspermidine (35): Scheme 9.

To **34** (735 mg, 2.15 mmol), dissolved in anhydrous ether (40 ml) is added slowly at 0°C, $LiAlH_4$ (550 mg). After 12 h at 0°C, 1 ml of a 15% NaOH solution is added to the suspension and finally 10 ml of water. The extraction gives **35** as an oil (620 mg, yield 84%). MS: (m/z) 346 $(M+H)^+$ ($C_{17}H_{35}N_3O_4$, MW 345.5). ^{1}H NMR ($CDCl_3$) δ 1.20-1.70 (2s+m, Boc groups + $^2CH_2$, $^6CH_2$ and $^7CH_2$):24H in total; 2.69 (t, 2H, $^8CH_2$); 3.00-3.40 (2m, 6H, $^1CH_2$, $^3CH_2$ and $^5CH_2$).

N^8-(2-bromoacetyl) N^1,N^4-di-tert-butyloxycarbonylspermidine amide (36): Scheme 9.

To a solution of **35** (610 mg, 1.8 mmol) in CH_2Cl_2 (70 ml) is added successively: bromoacetic acid (250 mg), N-methylmorpholine (195 µl) and BOP[14] (780 mg). After 24 h at room temperature, the solution is evaporated and extracted by EtOAc, leading to **36** (670 mg, yield 80 %). TLC homogeneous with MeOH: CH_2Cl_2; 2: 98 (v/v).^{1}H NMR ($CDCl_3$) δ 1.40, 1.42 (2s, Boc groups); 1.40-1.70 (m, $^2CH_2$, $^6CH_2$ and $^7CH_2$): 24H in total; 3.10 (q, $^1CH_2$); 3.15-3.25 (m, $^3CH_2$ and $^5CH_2$); 3.35 (q, 2H, $^8CH_2$); 3.75 (s, 2H, CH_2Br).

N^8-[2-(S-coenzyme A) acetyl] N^1,N^4-di-tert-butyloxycarbonylspermidine amide (37): Scheme 7.

To a solution of CoA-SH (Na salt; 15 mg, ca 18 µmol) in water (0.2 ml) is added NaOH 1N (50 µl) and **36** (30 mg, 64 µmol) dissolved in MeOH (500 µl). After 4 h at room temperature, CH_3OH is evaporated and the resulting suspension is diluted with water (10 ml) and repeteadly extracted with portions of 10 ml of ether. The aqueous phase is lyophilized, leading to **37**, as white solid (24 mg). ^{1}H NMR (D_2O) δ 1.20-1.60 (2s+m, 24H, Boc+ $^2CH_2$, $^6CH_2$ and $^7CH_2$); 2.57 (t, 2H, $^{9'}CH_2S$); 2.93 (t, 2H, $^8CH_2$); 3.05-3.10 (m, 6H, $^1CH_2$, $^3CH_2$ and $^5CH_2$); 3.14 (s, $COCH_2S$).

2-(S,N-acetyl cysteaminyl) acetic acid thiophenyl ester (39): Scheme 5.

6 (70 mg, 0.4 mmol) is heated 3/4 h at 100°C with Ac_2O (6 ml). The anhydride in excess is completely removed under vacuum. The residue is dissolved with anhydrous CH_2Cl_2 and 3.6 ml of thiophenol. After 1/2 h, the solution is concentrated until 0.5 ml added with DMAP (10 mg) and left overnight at room temperature. By preparative TLC (UV revelation) the spot of Rf 0.5 (solvent MeOH:CH_2Cl_2, 5:95 (v:v)) leads to **39** as an oil (12 mg, yield 11%). ^{1}H NMR ($CDCl_3$) δ 1.90 (s, 3H, CH_3CO); 2.70 (t, 2H, $^{9'}CH_2S$); 3.30 (m, $^8CH_2$) and 3.40 (s, SCH_2CO):4H in total; 7.50 (s, 5H, C_6H_5S).

2-phenylthioacetanilide (40): Scheme 6.

To aniline (500 µl) is added, successively : dioxane (7ml), water (3 ml), NaOH 1N (1.5 ml) and bromoacetyl thiophenol **30** (231 mg, 1 mmol). The solution is left 10 min at room temperature, then evaporated. The residue, taken with CH_2Cl_2, filtered, leads to 2-phenylthioacetanilide **40** (150 mg, yield 62%); m.p. 70-71°C recrystallized in CH_2Cl_2-hexane giving a pure compound; m.p. 75-76 °C; m.p.[6] 81.5-82.5°C. MS: (m/z) 244 $(M+H)^+$ ($C_{14}H_{13}NOS$, MW 243.3). ^{1}H NMR ($CDCl_3$) δ 3.76 (s, 2H, SCH_2CO); 7.00-7.50 (m, 10H, C_6H_5N and C_6H_5S); 8.52 (s, 1H, NH).

2-phenylthioacetylbutylamide (41): Scheme 6.

To butylamine (200 µl) is added successively: NaOH 4N (375 µl), dioxane (7 ml) and bromoacetyl thiophenol **30** (231 mg, 1 mmol). TLC analysis shows that the reaction is instantaneous. After 10 min at room temperature, the solution is evaporated and the residue taken with $CHCl_3$, filtered and evaporated giving 2-phenylthioacetylbutylamide **41** (197 mg, yield 88%), m.p. 29-31°C recrystallized in acetone-hexane giving a pure compound; TLC homogeneous with AcEt: Hexane; 50: 50 (v/v). m.p. 35 °C. ^{1}H NMR ($CDCl_3$) δ 0.82 (t, 3H, CH_3); 1.20 (m, 2H, CH_2CH_3); 1.85 (m, 2H, NCH_2CH_2); 3.22 (q, 2H, CH_2N); 3.51 (s, 2H, SCH_2-CO); 7.10-7.30 (m, 5H, C_6H_5S). Anal. calcd. for $C_{12}H_{17}NOS$: $C_{64.53\%}$; $H_{7.69\%}$; $N_{6.27\%}$; $O_{7.16\%}$; $S_{14.35\%}$. Found: $C_{64.76\%}$; $H_{7.67\%}$; $N_{6.07\%}$; $O_{7.42\%}$; $S_{14.25\%}$.

CoA-S-(2-bromoacetyl) thioester (42): Scheme 1.

CoA-SH (Na salt; 5 mg, 6 µmol) is dissolved in $KHCO_3$ 0.1M solution (0.2 ml) and acetone (0.5 ml). Bromoacetylthiophenol **30** (0.125 g, 0.54 mmol) [recristallized four times in C_6H_6:hexane (see preparation of **30**)], dissolved in a mixture of $KHCO_3$ 0.1M (0.2 ml) and acetone (0.5 ml) is added to CoA-SH solution. Some additional acetone is added for solubilization. The solution, left at room temperature, 45 min, is acidified with HCl 0.1 N to pH 2. The acetone is evaporated under reduced pressure. The suspension obtained is repeteadly extracted with 10 portions of 10 ml of ether, each organic layer being washed with 5 ml of water. The aqueous layers are lyophilized, leading to a product characterized by NMR (D_2O). As shown in Fig.1. The ^{1}H NMR spectrum does not show any significant signal belonging to a phenyl group but displays at δ 3.01 (t, $^{9'}CH_2S$) and at 4.17 (s, CO-CH_2Br).

Phenyl phenylthiothiolacetate (43)

Bromoacetylthiophenol **30** (1 g, 4.3 mmol) is refluxed with 35 ml ethanol. Water is added until clouding. Reflux is maintened during 5 h. The solution is concentrated until clouding. At room temperature the cristallization is completed by addition of water. The filtration gives **43** (300 mg, yield 31%) as white crystalline material; m.p.66-67°C;m.p.63.5-65°C[6]. ^{1}H NMR is according to the related structure.

REFERENCES

1. Cullis, P.M.; Wolfenden, R.; Cousens, L.S.; Alberts, B.M.; *J. Biol. Chem.* **1982**, *257*, 12165.
2. Parello, J.; Roblot, G.; Wylde, R.; Martin, A.; *C. R. Acad. Sci. Paris* **1990**, *310 série II*, 144.
3. Chase, J.F.A.; Tubbs, P.K.; *Biochem. J.* **1969**, *111*, 225.
4. Clements, P.R.; Wallace, J.C.; Keech, D.B.; *Anal. Biochem.* **1976**, *72*, 326.
5. Dagli, D.J.; Yu,P.S.; Wemple J.; *J. Org. Chem.* **1975**, *40*, 3173.
6. Field, L.; Carlile, C.G.; *J. Org. Chem.* **1961**, *26*, 3170.
7. Lee, C.H.; Sarma, R.H.; *FEBS Letters* **1974**, *43*, 271
8. Leslie, A.G.W.; Moody, P.C.E.; Shaw,W.V.; *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4133.
9. Humora, M.; Quick, J.; *J. Org. Chem.* **1979**, *44*, 1166.
10. Bergeron, R.J.; *Acc. Chem. Res* **1986**, *19*, 105.
11. Ganem, B.; *Acc.Chem.Res* **1982**,*15*, 290; Sundaramoothi, J.L.; Fourrey, J.L.; Das, B.C.; *J. Chem. Soc. Perkin Trans I* **1984**, 2759; Sundaramoothi, J.L.; Marazano, C.; Fourrey, J.L.; Das,B.C. *Tetrahedron Letters* **1984**, *25*, 3191; Lurdes,M.; Almeida, S.; Crehn, L.; Ragnarson,U.; *J. Chem. Soc. Perkin Trans I* ***1988***, 1905.
12. Patai, S.; *"Chemistry of the Thiol Group"*, Wiley NewYork **1974**, *part 2*, 721.
13. Baddiley, J.; Thain, E.M.; *J. Chem. Soc.* **1951**, 3425.
14. Castro, B.; Darmoy, J.R.; Evin, G.; Selve, C.; *Tetrahedron Letters* **1975**, 1219.
15. Tarbell, D.S. and Cameron, B.P.; *J. Am. Chem. Soc.* **1956**,*78*, 2731.