PREPARATION OF ALIPHATIC ANALOGUES OF S-ADENOSYL-L-HOMOCYSTEINE AND RELATED COMPOUNDS

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Received April 7th, 1981

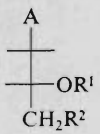
Reaction of 9-((*RS*)-2,3-dihydroxypropyl)adenine (*I*) with *p*-toluenesulfonyl chloride afforded the 3-O-*p*-toluenesulfonyl derivative *II* which on treatment with 2,3-dihydropyran was transformed into the 3-O-*p*-toluenesulfonyl-2-O-tetrahydropyranyl derivative *III*. Reaction of *II* with sodium isobutyl mercaptide in liquid ammonia gave 9-((*RS*)-3-isobutylthio-2-hydroxypropyl)adenine (*IV*). Analogously, compound *III* and disodium salt of L-homocysteine after acid hydrolysis afforded S-((*RS*)-3-(adenin-9-yl)-2-hydroxypropyl)-L-homocysteine (*V*). 9-((2*S*,3*S*)-*threo*-2,3-O--Isopropylidene-4-O-*p*-toluenesulfonyl-2,3,4-trihydroxybutyl)adenine (*VIII*) was transformed in a similar way into the 4-isobutylthio derivative *IX* and the L-homocysteine derivative *X*.

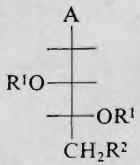
9-Allyladenine (*XII*) on treatment with bromine in dioxane afforded 9-((*RS*)-2,3-dibromopropyl)adenine (*XIII*) and probably 3,9-(2-bromotrimethylene)adeninium bromide (*XIV*). Reaction of compounds *XIII*, *XIV* and 9-((*RS*)-2,3-bis-*p*-toluenesulfonyloxypropyl)adenine (*XI*) with sodium hydrogen sulfide or sodium thioacetate led invariably to polymeric compounds. 4-*p*-Toluenesulfonyloxymethyl-2,2-dimethyl-1,3-dithiolane (*XVa*) reacted with sodium salt of adenine to give 9-(*RS*)-2,2-dimethyl-1,3-dithiolane-4-ylmethyl)adenine (*XVIa*); analogously, 4-*p*-toluenesulfonyloxymethyl-2-phenyl-1,3-dithiolane (*XVb*) afforded the 2,3-S-benzylidene derivative *XVIb* and 1-*p*-toluenesulfonyloxy-2,3-bis(benzylthio)propane (*XIXb*) gave 9-((*RS*)-2,3-bis-(benzylthio)propyl)adenine (*XIXc*). Acetolysis of *XVIa* or reduction of *XVIb* with sodium in liquid ammonia led to 9-((*RS*)-2,3-dimercaptopropyl)adenine (*XVIII*) and the corresponding episulfide *XVII*.

The discovery of antiviral action of 9-((*S*)-2,3-dihydroxypropyl)adenine (*I*) gave impetus to a systematic study of aliphatic nucleoside analogues, mainly hydroxyl--containing alkyladenine derivatives[1,2]. Recently, when studying possible mechanism of action of compound *I*, we found that this compound is a strong reversible inhibitor of S-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1.; see[3]), an enzyme which plays a crucial role in regulation of methylation processes. This finding initiated studies of structure–activity relationship between aliphatic nucleoside analogues and the mentioned enzyme[4]. Among these compounds, 9-alkyladenine derivatives with sulfur-containing substituents bonded to the alkyl chain, have a special position. S-Adenosyl-L-homocysteine hydrolase which catalyzes the hydrolysis of S-adenosyl--L-homocysteine to adenosine and L-homocysteine (as well as the reverse reaction)

* Part II in the series Studies on S-Adenosyl-L-homocysteine Hydrolase; Part I: This Journal *45*, 3039 (1980).

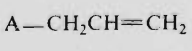
is inhibited by 5′-deoxy-5′-isobutylthioadenosine[5,6] (SIBA). In this communication which describes synthesis of adenine derivatives with three- and four-carbon chain, containing one or two sulfur-containing groups, we are therefore paying particular attention to aliphatic analogues of the substrate and inhibitor, *i.e.* L-homocysteine and the S-isobutylthio derivatives, derived from compound *I* (in the racemic series) and from the homologous compound, 9-((2*S*, 3*S*)-*threo*-2,3,4-trihydroxybutyl)-adenine (*VI*); in the latter case the compound *VI* was chosen from the four possible



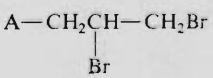


I, $R^1 = H$, $R^2 = OH$
II, $R^1 = H$, $R^2 = OTs$
III, R^1 = tetrahydropyran-2-yl, $R^2 = OTs$
IV, $R^1 = H$, $R^2 = SCH_2CH(CH_3)_2$
V, $R^1 = H$, $R^2 = SCH_2CH_2CH(NH_2)COOH$
XI, $R^1 = Ts$, $R^2 = OTs$

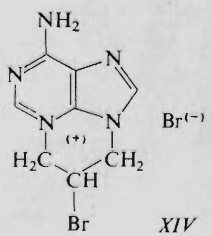
VI, $R^1 = H$, $R^2 = OH$
VII, $R^1 = (CH_3)_2C$<, $R^2 = OH$
VIII, $R^1 = (CH_3)_2C$<, $R^2 = OTs$
IX, $R^1 = H$, $R^2 = SCH_2CH(CH_3)_2$
X, $R^1 = H$, $R^2 = SCH_2CH_2CH(NH_2).COOH$



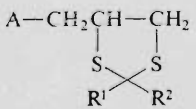
XII

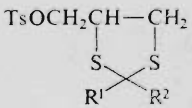


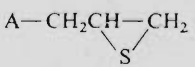
XIII



XIV



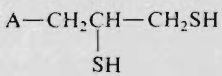




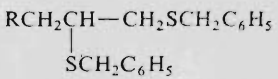
XVIa, $R^1 = R^2 = CH_3$
XVIb, $R^1 = H$, $R^2 = C_6H_5$

XVa, $R^1 = R^2 = CH_3$
XVb, $R^1 = H$, $R^2 = C_6H_5$

XVII



XVIII



XIXa, R = OH
XIXb, R = OTs
XIXc, R = A

A = adenin-9-yl, Ts = *p*-toluenesulfonyl residue

stereoisomers, since it exhibited also an antiviral[2] as well as enzyme-inhibitory activity[4].

Both the derivatives of *I* were synthesized starting from 9-((*RS*)-3-*p*-toluenesulfonyloxy-2-hydroxypropyl)adenine (*II*) which in turn was prepared by reaction of compound *I* (ref.[7]) with *p*-toluenesulfonyl chloride in pyridine.The *p*-toluenesulfonyl group in compound *II* was substituted by reaction with sodium isobutyl mercaptide in liquid ammonia. In the preparation of the S-adenosyl-L-homocysteine analogue, the *p*-toluenesulfonyl derivative *II* was first treated with dihydropyran to give the 2-O-tetrahydropyranyl-3-O-*p*-toluenesulfonyl derivative *III*; its reaction with disodium salt of L-homocysteine (obtained by *in situ* reaction of L-homocystine with sodium in liquid ammonia), followed by acid hydrolysis afforded the pure product *V*.

Protection of the free hydroxyl in compound *II* proved to have no decisive effect on the course of substitution reactions in liquid ammonia. Since conversion into the pyranyl derivative *III* gave relatively low yields, it was better to use directly the compound *II*. The homologues *IX* and *X* were prepared in the similar manner, the 2,3-O-isopropylidene derivative *VII*, described in one of our previous communications[8] being the starting compound. It was transformed into the 4-O-*p*-toluenesulfonyl derivative *VIII* which was treated with sodium salts of isobutyl mercaptan or L-homocysteine in liquid ammonia, similarly as described above. After hydrolysis with dilute sulfuric acid the products were isolated by chromatography on an ion exchange resin.

Compounds *IV*, *V*, *IX* and *X*, obtained in this way, were chromatographically as well as electrophoretically homogeneous and gave positive reaction for sulfur; compounds *V* and *X* were moreover ninhydrine-positive. Spectra of all the four derivatives agreed with the 9-alkyladenine structure.

Synthesis of the simple sulfur analogue of *I*, 9-((*RS*)-2,3-dimercaptopropyl)adenine (*XVIII*) encountered extraordinary difficulties. The first pathway consisting in nucleophilic reaction of the di-*p*-toluenesulfonate[9] *XI* with sodium hydrogen sulfide, triethylammonium hydrogen sulfide or sodium benzyl mercaptide in dimethylformamide at elevated temperature, led to a profound destruction of the starting compound. Also the reaction of *XI* with sodium thioacetate in dimethylformamide was unsuccessful, adenine being the only isolated product.

The second procedure started from 9-allyladenine (*XII*), prepared by a modified reaction of adenine sodium salt with allyl bromide[10]. Treatment of compound *XII* with an equimolar amount of bromine in dioxane afforded, in addition to the desired 9-((*RS*)-2,3-dibromopropyl)adenine (*XIII*), an isomeric, dioxane-insoluble compound of ionic character. According to elemental analysis, UV-spectrum and electrophoretical behaviour, indicating a positively charged adenine grouping, this compound was assigned the structure *XIV*. On treatment with triethylammonium hydrogen sulfide in dimethylformamide at room temperature, the compound *XIV* did not react whereas at elevated temperature it underwent decomposition. On the contrary, com-

pound *XIII* reacted quantitatively already at room temperature but the product was a polymeric material of amphoteric character, insoluble at neutral pH in water or ethanol. Obviously, the polymerization took place after intermediate formation of the episulfide *XVII*; since the polymer did not change on treatment with 2-mercaptoethanol, it contained no disulfide bonds.

Another attempted preparation of compound *XVIII* consisted in condensation of reactive derivatives of 2,3-dimercaptopropanol (BAL) in which the sensitive thioglycol grouping was protected either with a group of 1,3-dithiolane type or with S-benzyl groups. The 1,3-dithiolane derivative[11], obtained from 2,3-dimercaptopropanol and acetone, was transformed into 4-O-*p*-toluenesulfonyloxymethyl-2,2-dimethyl-1,3-dithiolane (*XVa*) by reaction with *p*-toluenesulfonyl chloride in pyridine. Treatment of *XVa* with sodium salt of adenine afforded the chemically stable 9-((*RS*)-2,2-dimethyl-1,3-dithiolane-4-ylmethyl)adenine (*XVIa*). However, this compound did not change in boiling 90% formic acid or 2M-H_2SO_4 and was recovered unchanged even after treatment with a mixture of trifluoroacetic anhydride and trifluoroacetic acid. Acetolysis in a mixture of acetic anhydride and sulfuric acid gave three compounds, the main product being again the mentioned amphoteric insoluble polymer. Further, a non-polar crystalline product was isolated which, according to its analysis and mass spectrum, was the episulfide *XVII*. The third product was weakly acidic and its properties corresponded to the desired 2,3-dimercaptopropyl derivative *XVIII*.

A more facile removal of the protecting group should be accomplished using the S-benzylidene derivative: 2,3-dimercaptopropanol on treatment with benzaldehyde afforded 2-phenyl-1,3-dithiolanyl-4-methanol[11] which was transformed into the 4-O-*p*-toluenesulfonyl derivative *XVb*. Condensation with sodium salt of adenine converted *XVb* into the derivative *XVIb* which, however, was also acid-stable. Removal of the S-benzylidene protecting group by titration with sodium in liquid ammonia afforded 9-allyladenine (*XII*) as product of elimination, the desired derivative *XVIII*, adenine and, similarly to the previous preparations, also the mentioned polymer. Preparation of compound *XVIII* by this route was somewhat more advantageous than its synthesis *via* the derivative *XVIa*.

We modified the latter method by using the S-benzyl protecting groups: 2,3-bis(benzylthio)propanol (*XIXa*; see ref.[12]) was converted into the 1-O-*p*-toluenesulfonyl derivative *XIXb* which on reaction with sodium salt of adenine afforded 9-((*RS*)-2,3-bis(benzylthio)propyl)adenine (*XIXc*). However, removal of the S-benzyl groups from the compound *XIXc* afforded no product *XVIII* at all. In addition to the polymeric material we identified in the reaction mixture only benzyl mercaptan, 9-allyladenine (*XII*) and adenine. The action of sodium in liquid ammonia on compounds *XVIb* and *XIXc* led thus to elimination of the sulfur-containing substituents.

It follows from the presented material that the main obstacle to successful preparation of *XVIII* and analogous compounds is the extraordinary propensity of the thioglycol or episulfide groupings to autocondensation reactions.

The synthesized derivatives were used in the study of substrate and inhibitory activity of S-adenosyl-L-homocysteine hydrolase. These results will be described in other communication of this Series.

EXPERIMENTAL

Unless stated otherwise, the solutions were taken down at 40°C and 2 kPa and the products dried at 13 Pa over phosphorus pentoxide. Melting points were determined on a Kofler block and are uncorrected. Paper chromatography was performed on a Whatman No 1 paper (descendant arrangement) in the systems: S1 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2), S2 butanol–acetic acid–water (10 : 1 : 3), S3 2-methyl-1-propanol–formic acid–water (6 : 1 : 1). Thin-layer chromatography was performed on Silufol UV_{235} plates in the systems: S4 tetrachloromethane, S5 chloroform, S6 chloroform–ethanol (95 : 5), S7 chloroform–ethanol (9 : 1), S8 chloroform–methanol (4 : 1), S9 chloroform–methanol (7 : 3). Preparative chromatography on silica gel was carried out on loose layers (30 × 16 × 0·3 cm) of silica gel, containing a fluorescence indicator (Service Laboratories of the Institute) or on a column of dry silica gel, according to Pitra (30—50 μ). Chromatography on cellulose was performed on an 80 × 4 cm column of cellulose powder (Macherey-Nagel) in 70% aqueous 2-propanol (20 ml/h) with continuous measurement of the eluate absorption on a Uvicord LKB instrument. Paper electrophoresis was carried out on a paper Whatman No 3 MM (20 V/cm) for 1 h in the system E1 0·1M triethylammonium hydrogen carbonate, pH 7·5, E2 1M-acetic acid. The UV absorption spectra were measured in aqueous solutions on a Specord UV-VIS instrument (K. Zeiss, Jena, GDR).

9-((*RS*)-3-*p*-Toluenesulfonyloxy-2-hydroxypropyl)adenine (*II*)

9-((*RS*)-2,3-Dihydroxypropyl)adenine (*I*; see ref.[7]) (15·4 g; 75 mmol) was codistilled with pyridine (2 × 100 ml) *in vacuo*. The residue was dissolved in pyridine (150 ml) and stirred with with *p*-toluenesulfonyl chloride (16·2 g; 85 mmol) and 4-dimethylaminopyridine (1 g) at room temperature for 48 h. A small amount of the unreacted *I* was filtered off, the filtrate was taken down *in vacuo*, the residue dissolved in chloroform (500 ml) and washed with water (3 × 100 ml). The chloroform solution was dried over magnesium sulfate, filtered, taken down *in vacuo* and the residue crystallized from ethanol (ether added until the solution became turbid), affording 7·1 g (18%) of the di-*p*-toluenesulfonyl derivative *XI*, m.p. 145°C, identical with an authentic material[9]; R_F 0·37 (S7). The mother liquor after crystallization of *XI* which contained practically pure compound *II* was taken down and chromatographed on a column of silica gel (200 g) in chloroform–ethanol (95 : 5).

The product-containing fractions were combined, taken down and the residue was crystallized from ethanol (with addition of ether), yielding 7·7 g (28%) of compound *II*, m.p. 243—244°C; R_F 0·11 (S7). For $C_{15}H_{17}N_5O_4S$ (363·4) calculated: 49·57% C, 4·72% H, 19·28% N, 8·82% S; found: 49·84% C, 5·02% H, 18·98% N, 8·86% S.

9-((*RS*)-3-*p*-Toluenesulfonyloxy-2-(tetrahydropyran-2-yloxy)propyl)adenine (*III*)

A mixture of the compound *II* (3·63 g; 10 mmol), dioxane (50 ml) 2,3-dihydropyran (6 ml) and trifluoroacetic acid (1 ml) was stirred at room temperature for 24 h, neutralized with triethylamine and taken down *in vacuo*. The residue was taken up in chloroform (200 ml), washed with water (2 × 25 ml), dried over magnesium sulfate and taken down *in vacuo*. The residue was chromato-

graphed on two thin layers of silica gel in the system S7. Elution of the band of R_F 0·40 with methanol (500 ml), followed by evaporation of the solvent and drying *in vacuo*, afforded 1·3 g (39%) of the compound *III* as an amorphous foam which was used in further reaction.

9-((*RS*)-3-Isobutylthio-2-hydroxypropyl)adenine (*IV*)

Sodium hydride (0·24 g; 10 mmol) was added to a solution of isobutyl mercaptan (0·90 g; 10 mmol) in dimethylformamide (20 ml) and the mixture was stirred under exclusion of moisture for 30 min. Compound *II* (2·5 g; 7 mmol) was added and the mixture was heated to 100°C for 8 h under exclusion of moisture. After evaporation *in vacuo* at 40°C/13 Pa, the residue was dissolved in water (20 ml) and the solution neutralized with acetic acid. The precipitate was filtered, washed with cold water (20 ml) and crystallized from water (charcoal), affording thus 1·0 g (51%) of compound *IV*, m.p. 159—160°C; R_F 0·36 (S8), 0·82 (S1); for *II* R_F 0·30 (S8). For $C_{12}H_{18}N_5OS$ (280·4) calculated: 51·40% C, 6·47% H, 24·98% N, 11·43% S; found: 51·64% C, 6·01% H, 25·25% N, 11·58% S. Positive reaction with potassium permaganate solution.

S-(3-(*RS*)-Adenin-9-yl-2-hydroxypropyl)-L-homocysteine (*V*)

Sodium was added to a refluxing and stirred suspension of L-homocystine (500 mg; 1·86 mmol) in liquid ammonia (30 ml; freshly distilled from sodium) until the blue colour persisted for 3 min. The mixture was decolorized with a crystal of ammonium sulfate and this solution was added to a solution of the evaporation residue of *III* (1·3 g; 2·9 mmol) in dry ammonia (10 ml). The reaction mixture was stirred under reflux for 2 h and evaporated. The residue was degassed, taken into water (50 ml) and adjusted to pH 1·4 with sulfuric acid. After standing for 2 days at room temperature the mixture was neutralized with a barium hydroxide solution (pH 7·0), filtered through Celite and taken down. The residue was dissolved in water (5 ml; pH 8·5) and applied on a column of Dowex 1X2 (acetate; 25 ml).The column was washed with water till the UV absorption dropped and then with 0·5M acetic acid. The second UV-absorbing eluate was taken down and chromatographed on a silica gel plate in the system S3. The product band of R_F 0·15 (ninhydrine-positive) was eluted with 50% methanol, the eluate was taken down *in vacuo* and the residue chromatographed on a column of Dowex 50X8 (H^+-form) (10 ml). The column was washed first with water and then with 1 : 10 aqueous ammonia. The ammonia eluate was taken to dryness *in vacuo* and the residue precipitated from methanol (5 ml) with ether (50 ml), affording 300 mg (31·7%) of the compound *V*, R_F 0·29 (S1), 0·15 (S2), E_{Up} 0·05 (E1), —2·15 (E2). UV spectrum (at pH 2): λ_{max} 262 nm (ε_{max} 14 600).

9-((2*S*,3*S*)-*threo*-4-Isobutylthio-2,3-dihydroxybutyl)adenine (*IX*)

Sodium hydride (0·65 g; 26·6 mmol) was added to a solution of isobutyl mercaptan (2·4 g; 26·6 mmol) in dimethylformamide (20 ml) and the mixture was stirred for 20 min. The compound *VIII* (ref.[8]; 1·35 g; 3·1 mmol) was added and the mixture was heated to 100°C for 14 h with stirring and exclusion of moisture. After evaporation of the solvent at 40°C/13 Pa the dry residue was dissolved in water (25 ml) and neutralized with acetic acid. The precipitated product was collected on a filter, suspended in water (50 ml), the suspension adjusted to pH 1·3 with sulfuric acid and set aside for 2 h at room temperature. The mixture was neutralized with barium hydroxide, warmed to 70°C, filtered through Celite and the filtrate was taken down *in vacuo*. Crystallization of the residue from water afforded 0·70 g (72·5%) of *IX*, m.p. 138—140°C; $[\alpha]_D^{20}$ —6·3° (*c* 0·5, 1M-HCl); R_F 0·15 (S7), 0·28 (S8). For $C_{13}H_{21}N_5O_2S$ (311·4) calculated: 50·14% C, 6·80% H, 22·49% N, 10·29% S; found: 50·73% C, 6·92% H, 22·31% N, 10·05% S.

S-((2*S*,3*S*)-4-(Adenin-9-yl)-2,3-dihydroxybutyl)-L-homocysteine (*X*)

A solution of the disodium salt of L-homocysteine was prepared from L-homocystine (270 mg; 1 mmol) in liquid ammonia (50 ml) in the same way as described for the compound *V*. To this solution compound *VIII* (ref.[8]; 0·65 g; 165 mmol) was added and the mixture was stirred for 2 h under reflux. The ammonia was evaporated, its last traces were removed *in vacuo*, the residue was dissolved in water (50 ml), the solution adjusted to pH 1·5 with sulfuric acid and set aside for 20 h at room temperature. The mixture was neutralized with barium hydroxide and filtered through Celite which was then washed with water. The filtrate was taken down *in vacuo*, the residue dissolved in water (5 ml) and applied to a column of Dowex 50X8 (H^+-form; 20 ml). After washing with water (100 ml) the product was eluted with a dilute (1 : 10) ammonia solution (100 ml), the eluate was taken down and the residue chromatographed on one layer of silica gel in the system S3. The product band was eluted with 50% aqueous methanol, taken down and the residue applied on a column of Dowex 1X2 (acetate; 20 ml). The product was eluted with water, the UV-absorbing eluate was concentrated *in vacuo* and freeze-dried, affording 213 mg (40%) of the chromatographically pure compound *X*, R_F 0·22 (S1), 0·12 (S2), E_{Up} 0·05 (E1), —2·0 (E2). Positive ninhydrine and potassium permanganate reaction.

9-Allyladenine (*XII*) (ref.[10])

A stirred mixture of adenine (13·5 g; 0·1 mol), potassium carbonate (15·2 g; 0·11 mol), dimethylformamide (200 ml) and allyl bromide (8·8 ml; 0·1 mol) was refluxed with exclusion of moisture (calcium chloride tube) for 16 h, filtered while hot, the solid washed on the filter with dimethylformamide and the filtrate taken to dryness *in vacuo*. The residue was treated with hot chloroform (100 ml), filtered, the solid washed with chloroform (50 ml) and the filtrate was chromatographed on a column of silica gel (200 g). The product was eluted with a chloroform–ethanol (9 : 1) mixture and crystallized from hot ethanol with addition of 5 parts of ether; yield 6·8 g (39%) of compound *XII*, m.p. 163°C (stated[10] m.p. 163°C). R_F 0·69 (S1), 0·30 (S7), 0·58 (S8).

9-((*RS*)-2,3-Dibromopropyl)adenine (*XIII*) and 3,9-(2-Bromotrimethylene)adeninium Bromide (*XIV*)

A solution of bromine (1·15 ml; 20 mmol) in dioxane (50 ml) was added dropwise to a stirred solution of compound *XII* (3·5 g; 20 mmol) in dioxane (100 ml) in the course of 40 min. After stirring for an additional 1 h the separated product was collected on filter, washed with dioxane and ether and dried *in vacuo*, yielding 1·80 g (27%) of *XIV*, not melting under 260°C. R_F 0·60 (S1), 0 (S8); E_{Up} —0·9 (E1). UV spectrum: λ_{max} 270 nm (pH 2, 7), 336 nm (pH 12), λ_{min} 237 nm (pH 2, 7). For $C_8H_9N_5Br_2$ (335·1) calculated: 28·68% C, 2·71% H, 47·70% Br, 20·91% N; found: 29·00% C, 2·80% H, 47·83% Br, 20·56% N.

The filtrate from the compound *XIV* was taken to dryness and the residue crystallized from 90% ethanol (ether added until the solution became turbid), affording 3·30 g (49%) of the compound *XIII* which did not melt under 260°C. R_F 0·68 (S1), 0·42 (S7); E_{Up} 0 (E1). UV spectrum: λ_{max} 263 nm (pH 2, 7, 12), λ_{min} 232 nm (pH 2, 7, 12). For $C_8H_9N_5Br_2$ (335·1) calculated: 28·68% C, 2·71% H, 47·70% Br, 20·91% N; found: 29·41% C, 2·92% H, 47·65% Br, 20·30% N.

9-((*RS*)-2,2-Dimethyl-1,3-dithiolane-4-ylmethyl)adenine (*XVIa*)

A mixture of 2,3-dimercaptopropanol (50 ml; 62·5 g; 0·5 mol), acetone (150 ml), 2,2-dimethoxypropane (70 ml) and 6M-HCl in dimethylformamide (3 ml) was set aside overnight, then neutralized with triethylamine and taken down *in vacuo*. The residue was dissolved in ether (200 ml),

washed with water (2 × 50 ml), dried over magnesium sulfate, taken down and the residue distilled *in vacuo*, affording 60·9 g (75%) of 2,2-dimethyl-1,3-dithiolan-4-ylmethanol, b.p. 82 to 84°C/13 Pa. This product was dissolved in pyridine (150 ml) and cooled to 0°C. A solution of *p*-toluenesulfonyl chloride (57·2 g; 0·3 mol) in pyridine (70 ml) was added dropwise under stirring during 30 min. The mixture was stirred at 0°C for 4 h and then kept at room temperature overnight. Water (20 ml) was added and after 1 h the mixture was taken down *in vacuo*. The residue was taken up in ethyl acetate (500 ml), the solution washed with water (3 × 100 ml), dried over magnesium sulfate and taken down. After drying *in vacuo*, the *p*-toluenesulfonyl derivative *XVa* (36·8 g; 33%) was obtained as a pinkish oil.

Sodium hydride (2·4 g; 0·1 mol) was added to a suspension of adenine (13·5 g; 0·1 mol) in dimethylformamide and the mixture was stirred at 60°C for 1 h under exclusion of moisture. A solution of the *p*-toluenesulfonate *XVa* (33·6 g; 0·106 mol) in dimethylformamide (50 ml) was then added. The mixture was stirred for 14 h at 100°C, taken down *in vacuo* (60°C/14 Pa) and the residue taken into chloroform (100 ml) and chromatographed on a column of silica gel (200 ml). The product was eluted with a mixture of chloroform and ethanol (95 : 5). Crystallization from ethanol (ether added) afforded 13·1 g (46·6%) of the compound *XVIa*, m.p. 218°C; R_F 0·78 (S8). Mass spectrum: M^+ 281 ($C_{11}H_{15}N_5S_2$), 135 (BH), 136 (BH_2). UV spectrum: λ_{max} 262 nm (pH 2, 7, 12). For $C_{11}H_{15}N_5S_2$ (281·4) calculated: 46·95% C, 5·37%H , 24·89% N, 22·78% S; found: 46·57% C, 5·15% H, 24·84% N, 22·58% S.

A 5% solution of the compound *XVIa* in 80% acetic acid, 90% formic acid or 1M sulfuric acid remained unchanged after 5 h at 100°C.

9-((*RS*)-2-Phenyl-1,3-dithiolane-4-ylmethyl)adenine (*XVIb*)

A mixture of 2,3-dimercaptopropanol (12·4 g; 0·1 mol), benzene (30 ml), benzaldehyde (10·6 g; 0·11 mol) and hydrochloric acid (two drops) was stirred for 30 min, diluted with benzene (100 ml), treated with magnesium sulfate (25 g) and stirred for 3 h. The mixture was filtered, the solid washed with benzene, the filtrate taken down and the residue stirred with light petroleum (200 ml). The separated product was collected on filter, washed with light petroleum and dried *in vacuo*; yield 19·8 g (93%) of 3-phenyl-1,3-dithiolan-4-ylmethanol.

This product (10·9 g; 51·4 mmol) was dissolved in pyridine (70 ml) and a solution of *p*-toluenesulfonyl chloride (10·5 g; 55·1 mmol) in pyridine (50 ml) was added dropwise at 0°C during 40 min to the stirred solution. The mixture was set aside overnight at 0°C, diluted with water (10 ml) and after 1 h taken down *in vacuo*. The residue was dissolved in ethyl acetate (200 ml), washed with a saturated solution of sodium hydrogen carbonate and water (50 ml each), dried over magnesium sulfate, filtered and taken to dryness *in vacuo*, leaving the product *XVb* (10·4 g; 55%) as a yellowish oil.

Sodium hydride (0·72 g; 30 mmol) was added to a suspension of adenine (4·05 g; 30 mmol) in dimethylformamide and the mixture was stirred at 60°C for 1 h under exclusion of moisture. A solution of the *p*-toluenesulfonate *XVb* (10·4 g; 28 mmol) in dimethylformamide (20 ml) was then added. After stirring for 14 h at 100°C, the mixture was evaporated at 40°C/13 Pa, the residue taken up in hot chloroform (100 ml) and filtered. The solid on the filter was washed with chloroform (200 ml) and the filtrate was taken down *in vacuo*. The residue was chromatographed on a silica gel column (100 g) in chloroform, containing 5% of ethanol. Crystallization of the product from ethyl acetate (light petroleum added) afforded 2·05 g (22·8%) of the compound *XVIb*, m.p. 168—169°C. Mass spectrum: M^+ 329, 136 (BH), 137 (BH_2), R_F 0·54 (S7). For $C_{15}H_{15}N_5S_2$ (329·4) calculated: 54·68% C, 4·59% H, 21·26% N, 19·46% S; found: 55·11% C, 4·59% H, 21·43% N, 19·55% S. The product *XVIb* did not change on boiling with 80% acetic acid or 90% formic acid for 5 h or in 1M-H_2SO_4 at 37°C for 24 h.

1-*p*-Toluenesulfonyloxy-2,3-bis(benzylthio)propane (*XIXb*)

Sodium hydroxide (4·5 g; 112·5 mmol) was added to a solution of 2,3-dimercaptopropanol (6·2 g; 50 mmol) in water (30 ml). Benzyl chloride (12·6 ml; 13·8 g; 103 mmol) was added dropwise under nitrogen in the course of 20 min. After stirring for 1 h at room temperature and for 90 min at 100°C under reflux condenser, the mixture was cooled with ice, extracted with ether (2 × 50 ml), the extract was washed with water (2 × 25 ml), dried over magnesium sulfate and taken down. The residue was coevaporated with pyridine (2 × 25 ml) *in vacuo*, dissolved in pyridine (50 ml) and a solution of *p*-toluenesulfonyl chloride (10 g; 52·5 mmol) in pyridine (50 ml) was added dropwise with ice-cooling and stirring. After stirring for 2 h at 0°C the mixture was set aside for two days at room temperature, treated with water (5 ml) and taken down *in vacuo*. The residue was mixed with ethyl acetate (300 ml), washed with water, dilute (1 : 10) hydrochloric acid (to acid reaction), saturated sodium hydrogen carbonate solution and again water (50 ml each), dried over magnesium sulfate and taken down. The residue was chromatographed on a column of silica gel (200 g) with tetrachloromethane as eluant, the product-containing fractions (R_F 0·28, S4) were combined and taken down *in vacuo*, yield 5·1 g (22%) of *XIXb* as a yellow oil with a positive reaction with potassium permanganate. The product was used immediately in the further reaction.

9-((*RS*)-2,3-Bis(benzylthio)propyl)adenine (*XIXc*)

A suspension of adenine (0·54 g; 4 mmol) in dimethylformamide (10 ml) was stirred with sodium hydride (0·10 g; 4 mmol) at 60°C for 1 h with exclusion of moisture. A solution of the compound *XIXb* (1·7 g; 3·7 mmol) in dimethylformamide (5 ml) was added and the mixture was heated to 100°C for 16 h, again with exclusion of moisture. After evaporation at 60°C/13 Pa the residue was extracted with boiling chloroform (200 ml), the extract was taken down *in vacuo* and the residue chromatographed on two layers of silica gel in the system S6. The bands of the product *XIXc* (R_F 0·33 in S6) were eluted with methanol (500 ml), yielding 0·57 g (37·5%) of the chromatographically pure product as an amorphous foam. UV spectrum (pH 2): λ_{max} 262 nm (ε_{max} 14 800).

Acetolysis of Compound *XVIa*

Concentrated sulfuric acid (4 ml) was added in portions to a suspension of compound *XVIa* (5·6 g; 20 mmol) in acetic anhydride (80 ml) in the course of 4 h. During the reaction the compound dissolved and crystallized again. The suspension was stirred at room temperature overnight, poured into ice-cold water (500 ml) and mixed with a saturated solution of crystalline barium acetate (20·5 g; 75 mmol). After filtration of the suspension through Celite, the filtrate was concentrated *in vacuo* to about 50 ml. This solution was chromatographed on a column of Dowex 50X8 (H^+; 300 ml). Elution with water afforded two UV-absorbing fractions. The first was neutralized with aqueous ammonia, taken down and the residue chromatographed on a column of Sephadex G-10 (medium; 500 ml) in 0·02M triethylammonium hydrogen carbonate (30 ml/h, 15 ml fractions). The UV-absorbing fraction was taken down and the residue was chromatographed on a cellulose column in 70% aqueous 2-propanol and fractions containing the product *XVIII* were combined, taken down *in vacuo* and the compound was precipitated from methanol (2 ml) with ether (50 ml). Yield 0·32 g (6·7%) of *XVIII*. For $C_8H_{11}N_5S_2$ (241·3) calculated: 39·81% C, 4·60% H, 21·02% N, 26·57% S; found: 40·06% C, 4·57% H, 19·40% N, 27·02% S. UV spectrum (pH 2): λ_{max} 262 nm (ε_{max} 15 000). R_F 0·34 (S1), E_{Up} 0·50 (E_1). The second fraction from the chromatography on Dowex 50 on evaporation and crystallization from water afforded 1·20 g (29%) of compound *XVII*, not melting below 260°C. Mass spectrum: M^+ 207 ($C_8H_9N_5S$), 174 (;M—SH), 135 (B), 136 (BH). UV spectrum (pH 2): λ_{max} 263 nm (ε_{max} 14 500).

R_F 0·58 (S1), 0·15 (S9), E_{Up} 0·10 (E1). For $C_8H_9N_5S$ (207·3) calculated: 46·36% C, 4·38% H, 33·80% N, 15·47% S; found: 46·52% C, 4·12% H, 33·56% N, 15·90% S.

Reductive Cleavage of Compound *XVIb*

Metallic sodium was added to a stirred and refluxing solution of compound *XVIb* (1·90 g; 5·78 mmol) in distilled ammonia (200 ml) until the red colouration of the mixture changed into black (stable for 3 min). Ammonium chloride had been added till the colour changed again into red. Ammonia was evaporated, finally *in vacuo*, the residue was dissolved in water (100 ml), neutralized with acetic acid, filtered and the filtrate extracted with ether (2 × 25 ml), removing thus benzyl mercaptan, identified by comparison with an authentic sample by thin-layer chromatography in the system S4. The aqueous layer was applied on a column of Dowex 50X8 (H^+; 100 ml). The aqueous eluate did not contain any UV-absorbing material; elution with dilute (1 : 10) ammonia afforded a UV-absorbing fraction which was taken down and chromatographed on a column of cellulose in 70% aqueous 2-propanol. Three chromatographically pure fractions were obtained which had identical UV spectra (λ_{max} 262 nm at pH 2). The first gave 200 mg of *XII*; R_F 0·67 (S1), m.p. 163°C. The second compound, R_F 0·53 (S1), 0·25 (S8) (120 mg) was identical with adenine (S1, S8 and E1). The third fraction (250 mg; 18%) was identical with compound *XVIII* (according to S1 and E1).

Reductive Cleavage of Compound *XIXc*

A solution of compound *XIXc* (0·57 g; 1·39 mmol) in redistilled ammonia (200 ml) was treated in the same way as described for the cleavage of compound *XVIb*. Neutralization with acetic acid afforded a white insoluble product which was collected on filter, washed with water and ethanol and dried. In S1 spot from the start, in E1 immobile. The filtrate after deionization on Dowex 50 showed only traces of *XII*. The insoluble portion, when stirred in a mixture of water (10 ml) and 2-mercaptoethanol (1 ml) for 6 days, did not undergo any change (S1, E1).

Reaction of Compounds *XIII* and *XIV* with Triethylammonium Hydrogen Sulfide

A 1M solution (30 ml) of triethylammonium hydrogen sulfide in dimethylformamide was added to a solution of compound *XIII* or *XIV* (1·05 g; 3 mmol) in dimethylformamide (10 ml) and the mixture was stirred at room temperature. Compound *XIV* was unchanged even after 3 days, whereas compound *XIII* reacted quantitatively after 24 h (according to thin-layer chromatography in S1). The mixture was stirred at 40°C and diminished pressure to remove hydrogen sulfide and the resulting mixture was taken down *in vacuo*. The residue was dissolved in water (10 ml), neutralized with acetic acid and the separated product collected on filter. The filtrate contained no UV-absorbing material. The compound of R_F 0·58 (corresponding to *XVII*), originally present in the reaction mixture, disappeared completely.

Reactions of Compound *XI*

A) The reaction mixture consisted of compound *XI* (1 mmol), triethylammonium hydrogen sulfide (3 mmol) and dimethylformamide (5 ml). After 24 h, chromatography in S1 revealed only a polymeric product (see cleavage of *XIXc*).

B) A mixture of *XI* (1 mmol) and sodium hydrogen sulfide (3 mmol) in dimethylformamide (5 ml) was kept at 70°C. After 6 h the reaction mixture contained (according to thin-layer chromatography in S1, S3 and S8) only adenine and no *XI*.

C) A mixture of *XI* (1 mmol) and sodium thioacetate (5 mmol) in dimethylformamide (5 ml) was treated and worked up in the same manner as described under *B*). After evaporation *in vacuo* the mixture was mixed with 0·1M sodium methoxide in methanol and after standing for 3 h neutralized with Dowex 50X8 (H^+-form) and filtered. The filtrate was shown (S1, S2, S7, S8) to contain only adenine in addition to the starting compound *XI*.

The author is indebted to Dr I. Rosenberg for carrying out some analyses and to Dr J. Kohoutová for the measurement and interpretation of mass spectra. The excellent technical assistance of Mrs B. Nováková is gratefully acknowledged.

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Translated by M. Tichý.