Gliotoxin Analogues as Inhibitors of Reverse Transcriptase. 1. Effect of Lipophilicity

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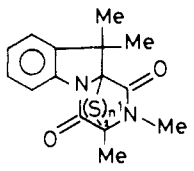
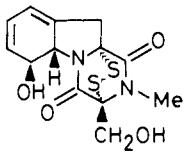
The reaction scheme, developed for the synthesis of the gliotoxin analogue **2**, was found to be of general applicability for analogues with varying substituents at N(1) and C(2). Analogues **11b–g** prepared by this method are inhibitors of reverse transcriptase (RNA-directed DNA polymerase). Their inhibitory activity seems to be related to the lipophilicity of the effector molecules: the most lipophilic compound is the most active inhibitor. The techniques of reversed-phase thin-layer chromatography with silylated, precoated plates as well as reversed-phase high-performance liquid chromatography were used to measure the relative lipophilicities; both techniques gave analogous results.

The discovery of RNA-directed DNA polymerase (reverse transcriptase) activity in RNA tumor viruses[1,2] has stimulated a worldwide search for inhibitors of this enzyme. It was hoped that such inhibitors might lead to the development of drugs that would be of value in the chemotherapy of viral diseases and cancer.[3] To date many compounds have been found capable of inhibiting the reverse transcriptase activity to varying degrees.[4] In preceeding articles we described the synthesis[5] of epipolythiodioxopiperazines **1–4** and studied their antireverse transcriptase activity.[6] These compounds are analogues



1: n = 1 **3**: n = 3
2: n = 2 **4**: n = 4



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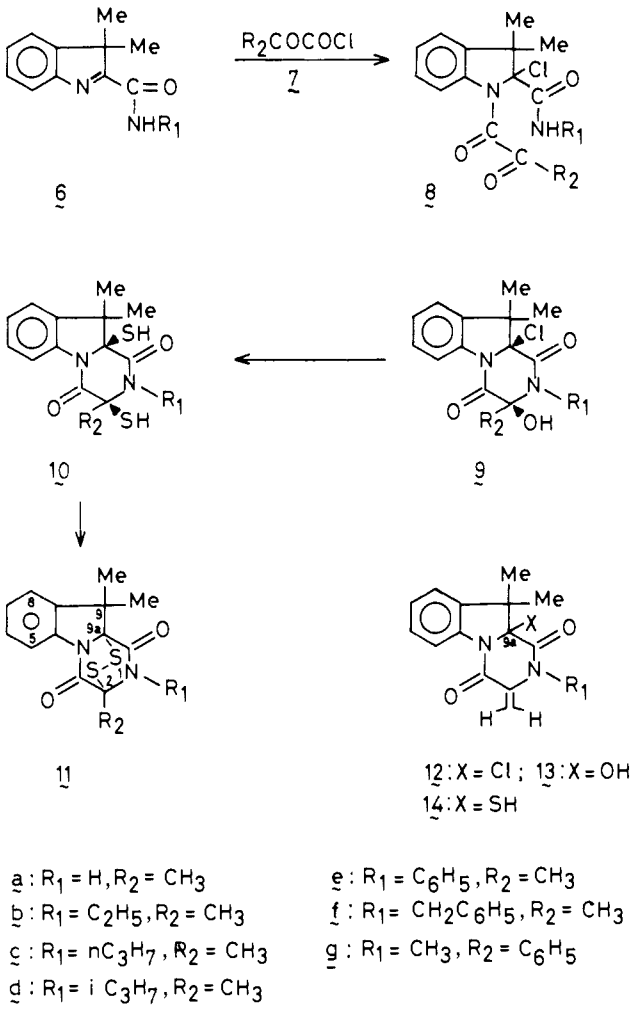
of gliotoxin (**5**),[7] which by itself is an inhibitor of reverse transcriptase.[8] Since that time we have devoted ourselves to further investigating the structure–activity relationship within this class of compounds. Herein we report the synthesis of a series of gliotoxin analogues **11** with varying substituents at N(1) and C(2). Each analogue was tested as an inhibitor of reverse transcriptase. Finally, the techniques of reversed-phase thin-layer chromatography and reversed-phase high-performance liquid chromatography were used to obtain a measure of the relative lipophilicity of these derivatives.

Synthesis of Gliotoxin Analogues 11. The reaction scheme developed[5] for the preparation of **2** was applied after some alterations for the synthesis of the gliotoxin analogues **11a–g** as outlined in Scheme I. All but one of the indolenine carboxamides **6** were prepared from ethyl 3,3-dimethylindolenine-2-carboxylate[9a] and the corresponding amine; **6e** was synthesized from the corresponding indoleninecarboxylic acid and aniline with the aid of $POCl_3$.[9b]

The reaction of **6a–g** with pyruvoyl chloride **7** (R_2 = CH_3)[10] or phenylglyoxylyl chloride **7** (R_2 = C_6H_5)[10] in CCl_4 was performed as described.[5] In general a few hours after mixing of the reagents the ^1H NMR spectra showed the presence of only one stereoisomer of **9**; this diastereoselective ring closure probably gives the cis products.[5] This reaction is strongly influenced by steric and electronic interactions; in the case of the conversions of **8d** and **8e** into **9d** and **9e**, respectively, ring closure was not complete after 7 days at room temperature. In both cases the reaction could be catalyzed by addition of a small amount

Scheme I



a: R_1 = H, R_2 = CH_3 **e**: R_1 = C_6H_5, R_2 = CH_3
b: R_1 = C_2H_5, R_2 = CH_3 **f**: R_1 = $CH_2C_6H_5$, R_2 = CH_3
c: R_1 = nC_3H_7, R_2 = CH_3 **g**: R_1 = CH_3, R_2 = C_6H_5
d: R_1 = i C_3H_7, R_2 = CH_3

of CF_3COOH, which gave a mixture of the alkenes **12** and **13**. The latter apparently arises from the water produced on dehydration. All cyclols **9** but **9g** were converted into a mixture of the corresponding alkenes **12** and **13** in varying ratios when stirring was continued. When **9g** or the alkene mixtures **12a–f** and **13a–f** were allowed to react with liquid H_2S in the presence of a catalytic amount of $ZnCl_2$, the *cis*-dithiols **10a–g** resulted. All of these reactions were found to proceed in this diastereoselective fashion as had been observed and rationalized in the synthesis of **2**.[5,11] Finally, the dithiols could be oxidized to the corresponding disulfides **11** by treatment with I_2 in CH_2Cl_2 in the presence of pyridine under anhydrous conditions. After column chromatography all of these compounds but

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Table I. Reverse Transcriptase Inhibition and Lipophilicity of Gliotoxin Analogues

analogue	log ($I_{50} \times 10^5$)	R_m	log k'
14 ($R_1 = CH_3$)	2.066	0.0132	−1.0670
11d	1.932	0.2769	−0.1291
11b	1.891	0.1636	−0.3400
11e	1.690	0.2892	−0.1127
11c	1.061	0.2717	−0.0817
11g	0.978	0.3007	−0.0527
11f	0.574	0.3514	+0.0994

11a could be isolated in fair to good yields. Material believed to be **11a** decomposed in solution and could not be isolated. For this reason it was not tested for biological activity.

Antireverse Transcriptase Activity and Lipophilicity. The gliotoxin analogues **11b–g** and the mercapto compound **14** ($R_1 = CH_3$)[5] were tested as inhibitors of the RNA-directed DNA polymerase activity associated with murine leukemia virus. In Table I the inhibition values, I_{50}, are given for each derivative; I_{50} is defined as the molar concentration of analogue corresponding to 50% inhibition of the control reverse transcriptase activity (obtained in the presence of Me_2SO). Of all seven gliotoxin analogues tested, **11f** and **11g** can be considered as the most potent inhibitors of reverse transcriptase. Their inhibitory activities correspond to those observed previously for the gliotoxin analogues **2–4**.[6]

However, it should be noted that the activities measured might be a sum of the RNA-dependent DNA polymerase activity and the subsequest DNA-dependent DNA polymerase activity. Therefore, the inhibitory activity of the gliotoxin analogues determined in our assay is the resultant of their effect on both reactions.

As we were interested in a possible correlation between the lipophilicity of the analogues described above and their inhibitory activity on reverse transcriptase, we resorted to the technique of reversed-phase thin-layer chromatography. This technique, which is especially well suited for lipophilic molecules with very low water solubility, as **11b–g** are, has been used as an indication of the relative lipophilicity of a series of analogues. From the R_f values obtained the free-energy related parameter R_m [=log $(1/R_f - 1)$] can be calculated,[12] which is analogous to the π parameter used by Hansch[13] and which has been shown to correlate well with the actions of penicillins[12] and rifamycins.[14] In addition, Calvin and co-workers[15] used this technique to demonstrate a relationship between the antireverse transcriptase activity of rifamycin analogues and their lipophilicity.

When the compounds to be tested were spotted on Merck precoated silica plates, which had been immersed in solutions of silicone gum rubber SE-30 or silicone oil DC-200, no differences in R_f values were observed after development with acetone–water in varying ratios. However, when the plates were immersed in Dow-Corning 704 diffusion pump fluid, small but significant differences in R_f values were found. It was hypothesized that in order to increase these differences in R_f values the plates had to have a high degree of impregnation. Therefore, the plates were immersed first in a solution of $(CH_3)_2SiCl_2$, in order to make the thin layer more lipophilic,[16] before treatment with the Dow-Corning 704 fluid. In doing so, the increase in weight after development with the oil was twice that of the unsilylated plates, and a considerable increase in difference of R_f values was observed. It was found that impregnation with a 5% solution of the oil gave sharp spots after development, whereas a 10% solution gave large, irregular ones. From the R_f values, the R_m values shown in Table I were calculated.

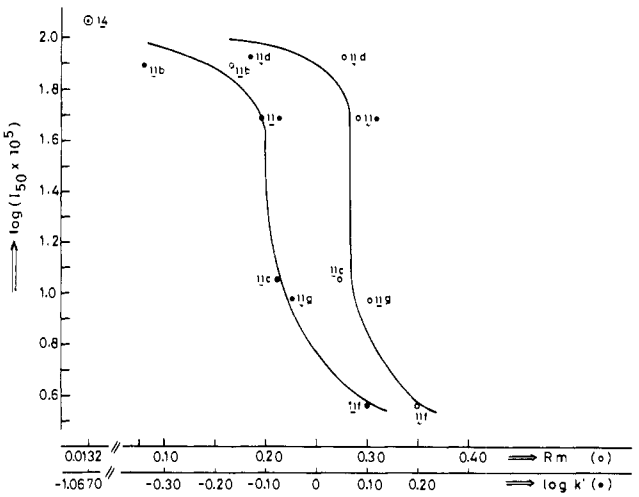

Figure 1. Plot of reverse transcriptase inhibition vs. lipophilicity.

As this procedure is rather tedious, we compared these results with those obtained with the technique of reversed-phase high-performance liquid chromatography, where the analysis time is shorter. Recently McCall[17] has shown that the parameter R_m is analogous to log k', in which k' is $(t_r - t_0)/t_0$ with t_r being the development time of a retained peak and t_0 the elution time of an unretained one. As the stationary chromatographic phase CO:PELL ODS (octadecylsilane) was used, the mobile phase was MeOH–H_2O (2:1). From the retention times, the log k' values shown in Table I were calculated. A plot of inhibition (log I_{50}) as a function of lipophilicity (expressed in R_m as well as in log k') is shown in Figure 1. From this, it may be concluded that the inhibition of reverse transcriptase activity is related to the lipophilicity of the structurally related gliotoxin analogues **11b–g** tested. This figure also shows that both methods used to measure the relative lipophilicities of the gliotoxin analogues give analogous results. The choice of a chromatographic method for further work is merely a matter of convenience now.

Experimental Section

Infrared spectra were measured with a Perkin-Elmer spectrophotometer, Model 257. Proton magnetic resonance spectra were measured on a Varian Associates Model T-60 spectrometer. Chemical shifts are reported as δ values (parts per million) relative to tetramethylsilane as an internal standard; deuteriochloroform was used as solvent unless stated otherwise. Mass spectra were obtained with a double-focusing Varian Associates SMI-B spectrometer. Melting points were taken on a Köfler hot stage (Leitz-Wetzlar) and are uncorrected. Thin-layer chromatography (TLC) was carried out using Merck precoated silica gel F-254 plates, thickness 0.25 mm. Spots were visualized with a UV hand lamp, iodine vapor, and, in the case of sulfur-containing products, by spraying with 2% aqueous $AgNO_3$.[18]

***N*-Substituted 3,3-Dimethylindolenine-2-carboxamides 6. Method I.** A cooled (dry ice–acetone) solution of n mmol of ethyl 3,3-dimethylindolenine-2-carboxylate[9] in dimethoxyethane ($3n$ mL) containing ammonia ($0.5n$ mL) was placed in an autoclave, after which the temperature was raised to 100 °C for 16 h. Evaporation of the solvent and excess reagent gave a crystalline mass. **6a**: yield 88%; mp 153–154 °C (chloroform–hexane). Anal. ($C_{11}H_{12}N_2O$) C, H, N. **6b** (method I was applied while aqueous ethylamine was used): yield 80%; mp 85 °C (methanol–hexane). Anal. ($C_{13}H_{16}N_2O$) C, H, N.

Method II. A solution of n mmol of ethyl 3,3-dimethylindolenine-2-carboxylate[9] and $20n$ mmol of amine in $5n$ mL of dimethoxyethane was refluxed gently for 18 h. Evaporation of the solvent and excess reagent gave the desired amide. **6c**: yield

88%; mp 89–90 °C (methanol). Anal. ($C_{14}H_{18}N_2O$) C, H, N. **6d**: (method II was applied, while a catalytic amount of sodium methoxide was added to the refluxing solution): yield 61%; mp 124–125 °C (methanol). Anal. ($C_{14}H_{18}N_2O$) C, H, N. **6f** (method II): yield 62%; mp 70–71 °C (methanol). Anal. ($C_{18}H_{18}N_2O$) C, H, N. **6g**: as has been described previously.[5] For **6e** the following procedure was used. To a stirred and cooled (−10 °C) solution of 94 mg (0.5 mmol) of 3,3-dimethylindolenine-2-carboxylic acid (prepared in 87% yield from the corresponding ethyl ester by saponification with sodium hydroxide in aqueous dioxane) and 45 mg (0.49 mmol) of aniline in 1.1 mL of dry pyridine was added dropwise a solution of 0.05 mL (0.55 mmol) of $POCl_3$ in 10 mL of CH_2Cl_2. After stirring for 15 min at 0 °C and for 1 h at room temperature, the reaction mixture was concentrated to a final volume of 2 mL after which water was added. Extraction with ethyl acetate and washing of the organic layer with 5% $NaHCO_3$, 1 N HCl, and water until neutral gave, after drying (Na_2SO_4) and evaporation of the solvent, 210 mg (40%) of a crystalline mass, mp 117–119 °C (methanol). Anal. ($C_{17}H_{16}N_2O$) C, H, N.

9,9a-Dihydro-2,9,9-trimethyl-2,9a-epidithio-3,10-diketopiperazino[1,2-*a*]indole (11a) (Attempted Preparation). To a stirred suspension of 552 mg (3 mmol) of **6a** in 6 mL of dry CCl_4 was added dropwise at room temperature 7 mL of a 0.5 M solution of 7 ($R_2 = CH_3$) in dry CCl_4. After stirring for 5 h at room temperature a precipitate had formed (**8a**); after evaporation of the solvent 15 mL of $CHCl_3$ and a trace of trifluoroacetic acid were added, together with enough CH_3OH to give a clear solution. Stirring was continued for 16 h, after which time solid Na_2CO_3 was added, together with Na_2SO_4. Filtration and concentration in vacuo gave the C_{9a}-methoxy analogue of **12a**: NMR δ 8.20 (m, 1 H, C_5-H), 7.30 (m, 3 H, C_{6-8}-H), 6.05 (d, 1 H, C=CH), 5.45 (d, 1 H, C=CH), 3.73 (s, 1 H, NH), 3.50 (s, 3 H, OCH_3), 1.95 (s, 3 H, C_9-$C_\alpha H_3$), and 1.46 (s, 3 H, C_9-$C_\beta H_3$).

This compound was processed as has been described[5] for the preparation of **2** by treatment with liquid H_2S in the presence of $ZnCl_2$, followed by oxidation with I_2 in the presence of pyridine under anhydrous conditions. However, **11a** could not be isolated in a pure form, as it decomposed within a few hours.

9,9a-Dihydro-2,9,9-trimethyl-1-ethyl-2,9a-epidithio-3,10-diketopiperazino[1,2-*a*]indole (11b). To a stirred solution of 324 mg (1.5 mmol) of **6b** in 6 mL of dry CCl_4 was added at room temperature 3.3 mL of a 0.5 M solution of 7 ($R_2 = CH_3$) in dry CCl_4 (1.65 mmol). After stirring for 16 h at room temperature the ring closure product **9b** had formed quantitatively, as could be concluded from the ^1H NMR spectrum (CCl_4): δ 6.45 (s, br, 1 H, OH). After addition of 1 drop of trifluoroacetic acid, the methylene compound **12b** precipitated: NMR δ 6.25 (d, 1 H, C=CH_α), 5.41 (d, 1 H, C=CH_β). After addition of Na_2CO_3 and filtration the solvent was removed in vacuo to yield a crystalline mass. From this the dimercapto compound **10b** was prepared as has been described[5] for the preparation of **2** by treatment with liquid H_2S in the presence of $ZnCl_2$: NMR δ 4.15 (s, 1 H, SH), 3.40 (s, 1 H, SH), and 2.40 (s, 3 H, C_2-CH_3). Oxidation of the dithiol **10b** to the corresponding disulfide **11b** was done with I_2 in the presence of pyridine as has been described.[5] The reaction product was column chromatographed on 70 g of Merck silica gel PF-254 in CH_2Cl_2–CCl_4 (1:1 v/v) under slightly increased pressure (about 10 cmHg) to afford 250 mg (50% overall yield) of **11b** (mp 153–155 °C, CH_3OH) which was homogeneous by TLC (CH_2Cl_2): NMR δ 8.38 (m, 1 H, C_5-H), 7.64 (m, 3 H, C_{6-8}-H), 4.32 (8 lines, 1 H, N-CH_α-CH_3), 3.86 (8 lines, 1 H, N-CH_β-CH_3), 2.53 (s, 3 H, C_2-CH_3), 2.16 (s, 3 H, C_9-CH_3), 1.94 (s, 3 H, C_9-CH_3), and 1.70 (m, 3 H, CH_2CH_3). Anal. ($C_{16}H_{18}N_2O_2S_2$) C, H, N.

9,9a-Dihydro-2,9,9-trimethyl-1-*n*-propyl-2,9a-epidithio-3,10-diketopiperazino[1,2-*a*]indole (11c) was prepared in 35% yield as described for the synthesis of **11b** (after addition of a catalytic amount of trifluoroacetic acid stirring was continued for 23 h, yielding **12c** as intermediate): mp 146–147 °C (CH_3OH); homogeneous by TLC (CH_2Cl_2); NMR δ 8.30 (m, 1 H, C_5-H), 7.70 (m, 3 H, C_{6-8}-H), 4.12 (m, 1 H, N-C-H_α), 3.88 (m, 1 H, N-C-H_β), 2.55 (s, 3 H, C_2-CH_3), 2.0 (s and m, 5 H, CH_2CH_3 and C_9-CH_3), 1.98 (s, 3 H, C_9-CH_3), and 1.40 (t, 3 H, CH_2CH_3). Anal. ($C_{17}H_{20}N_2O_2S_2$) C, H, N.

9,9a-Dihydro-2,9,9-trimethyl-1-isopropyl-2,9a-epidithio-3,10-diketopiperazino[1,2-*a*]indole (11d) was prepared in 29% yield as described for the synthesis of **11b** [after stirring for 160 h ring closure (→**9d**) was not complete; addition of 1 drop of trifluoroacetic acid and stirring for 40 h completed the formation of a mixture of **12d** and **13d**]: mp 131–132 °C (CH_3OH); homogeneous by TLC (CH_2Cl_2); NMR ($CDCl_3$) δ 8.40 (m, 1 H, C_5-H), 7.70 (m, 3 H, C_{6-8}-H), 4.52 (m, 1 H, NCH–), 2.56 (s, 3 H, C_2-CH_3), 2.20 (s, 3 H, C_9-CH_3), 1.98 (s, 3 H, C_9-CH_3), and 1.95 (d, 6 H, CH_3CHCH_3). Anal. ($C_{17}H_{20}N_2O_2S_2$) C, H, N.

9,9a-Dihydro-2,9,9-trimethyl-1-phenyl-2,9a-epidithio-3,10-diketopiperazino[1,2-*a*]indole (11e) was prepared in 37% yield as described for the synthesis of **11d**: mp 166–168 °C (CH_3OH); homogeneous by TLC (CH_2Cl_2); NMR δ 8.31 (m, 1 H, C_5-H), 7.87 (m, 5 H, C_6H_5), 7.67 (m, 3 H, C_{6-8}-H), 2.17 (s, 3 H, C_9-CH_3), 2.09 (s, 3 H, C_2-CH_3), 1.97 (s, 3 H, C_9-CH_3). Anal. ($C_{20}H_{18}N_2O_2S_2$) C, H, N.

9,9a-Dihydro-2,9,9-trimethyl-1-benzyl-2,9a-epidithio-3,10-diketopiperazino[1,2-*a*]indole (11f) was prepared in 40% yield as described for the synthesis of **11b**: mp 133–135 °C (CH_3OH); homogeneous on TLC (CH_2Cl_2); NMR δ 8.35 (m, 1 H, C_5-H), 7.65 (m, 8 H, C_{6-8}-H and C_6H_5), 5.23 (s, 2 H, $CH_2C_6H_5$), 2.37 (s, 3 H, C_2-CH_3), 2.17 and 1.99 (2 s, 3 H each, $2C_9$-CH_3). Anal. ($C_{21}H_{20}N_2O_2S_2$) C, H, N.

9,9a-Dihydro-2-phenyl-9,9-dimethyl-1-methyl-2,9a-epidithio-3,10-diketopiperazino[1,2-*a*]indole (11g) was prepared in 56% yield from **7** ($R_2 = C_6H_5$) and **6** ($R_1 = CH_3$) as described for the synthesis of **11b**; no trifluoroacetic acid was added. Ring closure (→**9g**) was complete after 160 h of stirring: mp 150–152 °C (CCl_4–*n*-hexane); homogeneous on TLC (CH_2Cl_2); NMR (internal Me_4Si) δ 7.80 (m, 1 H, C_5-H), 7.40 (s, 5 H, C_6H_5), 7.11 (m, 3 H, C_{6-8}-H), 2.66 (s, 3 H, NCH_3), 1.78 and 1.60 (2 s, 3 H each, CH_3-C_9-CH_3). Anal. ($C_{20}H_{18}N_2O_2S_2$) H, N; C: calcd, 62.80; found, 61.72.

Reverse Transcriptase Assay. Stock solutions of the gliotoxin analogues were prepared in dimethyl sulfoxide (Me_2SO) at 10 mg/mL. The solutions were stored at 4 °C. Murine leukemia virus (Moloney strain) was supplied by Electron Nucleonics Laboratories (Bethesda, Md.) (Catalog No. 1024; lot no. 719-24-9). The virus stock was derived from the NIH-3T3 cell line infected with Moloney murine leukemia virus. It had been purified by double density gradient zonal centrifugation and contained 10^{11}–10^{12} virus particles (approximately 0.2–1.0 mg of protein) per milliliter of 0.1 M NaCl, 0.01 M Tris-HCl (pH 7), and 0.001 M EDTA. The virus stock was stored at −70 °C until used. Prior to use 8% of glycerol was added. The virus could be stored at 4 °C in the presence of glycerol for several months without appreciable loss of DNA polymerase activity.

(***methyl***-^{3}H)-dTTP was purchased from the Radiochemical Centre, Amersham (England), through the C.E.N. Radioisotopes Department of Mol (Belgium). The specific activity of (*methyl*-^{3}H)-dTTP was 50 Ci/mmol.

DNA Polymerase Activity Assay. The standard assay mixture (250 μL) contained 40 mM Tris-HCl (pH 7.8), 50 mM NaCl, 2 mM $MnCl_2$, 1.6 mM dithiothreitol, 0.0125% (v/v) Triton X-100, 0.64 mM each of dATP, dCTP, and dGTP, 0.8 M (*methyl*-^{3}H)-dTTP, 20 μL of virus stock suspension, and varying concentrations of the compounds. Since stock solutions of the gliotoxin analogues had been prepared in Me_2SO, we also determined the effect of Me_2SO on DNA polymerase activity. At the gliotoxin concentrations used (4, 40, and 400 μg/mL) the corresponding Me_2SO concentrations were 0.2, 2, and 4% (v/v), respectively. The assay mixtures were incubated at 37 °C. At 30, 60, and 120 min 50-μL aliquots were withdrawn and tested for acid-precipitable radioactivity as described previously.[19] The data for three separate determinations were averaged. In Table I the data from incubations for 120 min have been used because the differences in activity between the various gliotoxin analogues were most pronounced at that time.

All inhibition values are reported in terms of an inhibition constant (I_{50}). The I_{50} for each derivative is obtained from a plot of derivative concentration (10^{-5} mol/L) vs. percent control activity and is defined as the molar concentration of the analogue inhibiting the control reverse transcriptase activity by 50%. The control value was the activity obtained in the presence of Me_2SO only.

Reversed-Phase TLC. Merck precoated 20 × 20 cm silica gel F-254 plates, thickness 0.25 mm, were activated at 120 °C and immersed in a 5% solution (v/v) of $(CH_3)_2SiCl_2$ in toluene during

6 h. The solvent was allowed to evaporate and excess reagent was removed by immersing the plates in toluene (15 min) and CH_3OH (1 h). After drying at 120 °C (weight gain 0.40 g) the plates were immersed in a 5% solution (by weight) of Dow-Corning 704 diffusion pump fluid in ether overnight. The solvent was allowed to evaporate, after which the plates were kept at 120 °C for 1 h. By this treatment the plates had gained an additional 1.0 g in weight. The analogues were spotted as 1 μL each of a CH_2Cl_2 solution (3 mg/mL). The plates were developed in an acetone–water solution (3:1 v/v), saturated with the Dow-Corning fluid. Spots were visualized by spraying with 2% aqueous $AgNO_3$. The R_m values reported are averages of five determinations and are calculated by means of the formula: $R_m = \log (1/R_f - 1)$.

Reversed-Phase LC. As stationary phase a CO:PELL ODS (stationary phase, octadecylsilane; Reeve Angel, Clifton, N.J.) was used; column dimensions were 90 cm × 2.1 mm i.d. The mobile phase was MeOH–H_2O (2:1), while the flow rate was kept at 0.4 mL/min. The eluate was monitored by UV absorption (Waters Associates, Model 440). The elution time of CH_3CN was defined as t_0. The log k' values reported are averages of two determinations and are calculated by means of the formula: $k' = (t_r - t_0)/t_0$.

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Gliotoxin Analogues as Inhibitors of Reverse Transcriptase. 2.[1] Resolution and X-ray Crystal Structure Determination

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A novel, simple, and efficient method for the chemical resolution of epidithiodioxopiperazines is reported, which is based upon covalent formation of diastereomers. This method might be a general one for the resolution of chiral cyclic disulfides. Dithiol **5**, prepared from **2** by reduction with $NaBH_4$, was allowed to react with the disulfenyl chloride **8** to yield **9** and **10**, which were separated by short-column chromatography on silica gel. From these, the optically pure enantiomers **11** and **12**, respectively, were obtained by reduction with $NaBH_4$, followed by reoxidation with I_2–pyridine. In this way the precursor **7** of the resolving agent could also be recovered. The absolute configurations of **11** and **12** were derived from CD spectra. Kinetic asymmetric transformation of the gliotoxin analogue **2** with the diphosphine **6** gave a 19% enrichment in one enantiomer of the starting material. Surprisingly, both enantiomers were found to inhibit reverse transcriptase, the RNA-dependent DNA polymerase, to the same degree, indicating that there is no relation between this property of epidithiodioxopiperazines and their bridgehead configurations. From the X-ray crystal structure determination it can be seen that there is a considerable torsional and conformational strain in compound **2**, which might enhance the ease of cleavage of the S–S bond. A possible relationship between this property and the biological activity of **2** is discussed.

Gliotoxin (**1**), the sporidesmins,[2] and chaetocin[3] belong to the class of fungal metabolites containing an epidithiodioxopiperazine ring system. The first two compounds have the *R* configuration at the bridgehead carbons and exhibit selective antiviral properties, whereas the antipodal chaetocin does not show this activity. Recently, we reported a synthesis[4] of a racemic gliotoxin analogue **2**, which inhibits reverse transcriptase,[5] the RNA-dependent DNA polymerase of RNA tumor viruses, and whose activity is of the same order as that of gliotoxin. As the antiviral activity of epidithiodioxopiperazines might be related to their bridgehead configurations, we wanted to examine the

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