was removed under reduced pressure. Distillation gave a colorless oil: 25 g (48%); bp 94–95° (0.1 mm). *Anal.* ($C_{11}H_{19}NO_4S$) C, H, N, S.

The other carbamates **10–12** were prepared in a similar manner. Their absorption spectra were consistent with the assigned structures.

Bis[2-(1-ethoxycarbonylprop-1-en-2-yl)aminoethyl] Disulfide (13). On overnight exposure to the atmosphere, or on standing in a closed container for a longer period of time, **1** gave **13** which was recrystallized from ligroine (bp 66–75°): mp 81–82.5°; ir (KBr) 3250 (w, N-H), 1645 (s, C=O), 1600 cm^{-1} (vs, C=C); pmr ($CDCl_3$) δ 1.24 (t, 6, J = 7.2 Hz, CH_3CH_2O), 1.96 (s, 6, CH_3C=C), 2.82 ("A_2B_2" m, 4, SCH_2CH_2N), 3.59 ("A_2B_2X" m, 4, SCH_2CH_2NH), 4.12 (q, 4, J = 7.2 Hz, CH_3CH_2O), 4.50 (s, 2, CH=C), 8.75 ppm (broad, 2, NH). *Anal.* ($C_{16}H_{28}N_2O_4S_2$) C, H.

13 gave the 2,4-dinitrophenylhydrazone of ethyl acetoacetate, mp 94°, mmp (with an authentic sample) 93°.

Acknowledgments. The authors are indebted to Mr. G. A. Grant of the Defence Research Board of Canada, Defence Research Establishment, Ottawa, for the evaluation of radioprotective activity of **6** and **8** against γ-radiation and to Mr. D. G. Smith of the National Research Council of Canada, Atlantic Regional Laboratory, for determining 100-MHz spectra of **12**.

References

(1) V. G. Yakovlev in "Chemical Protection of the Body Against Ionizing Radiation," V. S. Balabukha, Ed., Pergamon Press, New York, N. Y., 1963, p 11.
(2) F. Yu. Rachinskii, A. S. Mozzhukhin, N. M. Slavachevskaya, and L. I. Tank, *Usp. Khim.*, **28**, 1488 (1959); *Chem. Abstr.*, **54**, 13424 (1960).
(3) A. Kaluszyner, P. Czerniak, and E. D. Bergmann, *Radiat. Res.*, **14**, 23 (1961).
(4) J. F. Thomson, "Radiation Protection in Mammals," Reinhold, New York, N. Y., 1962, p 84.
(5) D. V. Mangind, *Radiobiologiya*, **3**, 240 (1963); *Chem. Abstr.*, **59**, 11860 (1963).
(6) F. Yu. Rachinskii, M. S. Kushakovskii, B. V. Matveev, N. M. Slavachevskaya, and L. I. Tank, *Radiobiologiya*, **4**, 266 (1964); *Chem. Abstr.*, **61**, 4677 (1964).
(7) W. Shapiro, M. F. Tansy, and S. Elkin, *J. Pharm. Sci.*, **57**, 1725 (1968).
(8) B. J. Sweetman, M. Bellas, and L. Field, *J. Med. Chem.*, **12**, 888 (1969).
(9) P. S. Farmer, C.-C. Leung, and P. J. Foreman, *Progr. Antimicrob. Anticancer Chemother., Proc. Int. Congr. Chemother., 7th*, 1971, in press.
(10) R. Tondeur, R. Sion, and E. Deray, *Bull. Soc. Chim. Fr.*, **10**, 2493 (1964).
(11) G. DeStevens, B. Smolinski, and L. Dorfman, *J. Org. Chem.*, **29**, 1115 (1964).
(12) F. Korte and K. Trautner, *Chem. Ber.*, **95**, 295 (1962).
(13) A. G. Sanchez and M. T. Aldava, *J. Chem. Soc. C*, 2570 (1968).
(14) R. Granger, H. Orzalesi, Y. Robbe, J. P. Chapat, A. Terol, M. Randon, J. Bitoun, and F. Simon, *Bull. Chem. Ther.*, 439 (1971).
(15) E. J. Mills and M. T. Bogert, *J. Amer. Chem. Soc.*, **62**, 1173 (1940).

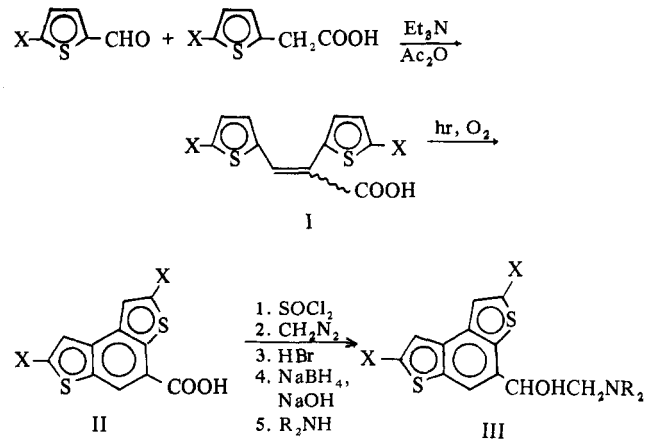
Naphthothiophenes. 2. Benzo[1,2-*b*:4,3-*b'*]dithiophenemethanols as Isosteres of Naphthothiophenes

Bijan P. Das and David W. Boykin, Jr.*

Department of Chemistry, Georgia State University, Atlanta, Georgia 30303. *Received September 27, 1972*

Recently we have been engaged in the synthesis of naphthothiopheneethanolamines for evaluation as antimalarial agents.[1] In connection with this program we have also prepared some closely related isosteric benzo[1,2-*b*:4,3-*b'*]dithiopheneethanolamines and this report deals with the synthesis and antimalarial activity of these compounds.

Scheme I



The synthesis of the benzodithiophenes is similar to that described for the naphthothiopheneethanolamines[1] and is outlined in Scheme I. The key step in this synthesis involves the photooxidative cyclization of dithienylethylenes to the benzodithiophenes. This method has been reported previously for the cyclization of dithienylethylenes in which the thiophene rings were unsubstituted.[2,3] The dithienylacrylic acids (I) were obtained by condensation of thiophene-2-carboxaldehydes and thiophene-2-acetic acids under modified Perkin reaction conditions.[4] No attempt was made to isolate the geometric isomers from the Perkin reaction since it was found that either isomer **1** or **2** underwent photooxidative cyclization upon exposure to 2537-Å light to yield the desired benzodithiophenecarboxylic acid. The structure of the photocyclization product was demonstrated by combustion analysis, by characteristic uv absorptions,[2] and by decarboxylation to yield a compound which has the properties reported in the literature for benzo[1,2-*b*:4,3-*b'*]dithiophene.[2,5] Photocyclization of the dithienylacrylic acids was achieved with **1**, **2**, and with **3** in poor yields. This observation may be worthy of note in view of the continuing interest in cyclization of stilbene analogs, particularly those which fail to cyclize.[6,7] The classical five-step procedure described by Lutz, *et al.*,[8] was employed to convert the benzo[1,2-*b*:4,3-*b'*]dithiophenecarboxylic acids (II) into the desired ethanolamines III.

The antimalarial activity of the benzodithiopheneethanolamines was assessed against *Plasmodium berghei* in mice by the method of Rane, *et al.*,[9] and the results are given in Table I. Table I also contains, for comparison, test data on selected isosteric phenanthreneethanolamines and quinine sulfate. Among the compounds tested 2-chloro-α-(*n*-dibutylaminomethyl)-4-benzo[1,2-*b*:4,3-*b'*]dithiophenemethanol hydrochloride (**9**) showed the most significant activity effecting cures at a dosage of 160 mg/kg. As noted upon comparison with **11**, **12**, and **13**, the activity of these benzodithiophenes is considerably superior to that of quinine but they are not superior to their phenanthrene isosteres. In addition to their *in vivo* antimalarial activity, these benzodithiophenes have been shown to bind *in vitro* to calf thymus DNA.†

Experimental Section

Melting points were obtained on a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were per-

†J. W. Panter, D. W. Boykin, Jr., and W. D. Wilson, unpublished results.

formed by Atlantic Microlab, Atlanta, Ga. Satisfactory uv and ir spectra were recorded for each compound listed in Tables I–III. The expected nmr spectra were obtained in $CDCl_3$ (Me_4Si) on compounds **7–10.**

α,β-Bis(2-thienyl)acrylic Acids (I).[4] See Table II. In a typical example, a mixture of 2-thienylacetic acid (14.2 g), thiophene-2-carboxaldehyde (11.2 g), 20 ml of Ac_2O, and 10 ml of distilled Et_3N was refluxed 8 hr. The mixture was poured into *ca.* 2 l. of H_2O made alkaline with KOH and boiled with charcoal. The solution was filtered, cooled, and acidified with HCl. The precipitate was filtered and washed with H_2O and dried. The solid was triturated with Et_2O. The Et_2O-insoluble material (10.1 g) on crystallization from EtOH–Et_2O gave mp 240–241° which is in accord with that reported for the cis isomer (lit. mp 240°).[4] The Et_2O soluble material (6.9 g) on crystallization from Et_2O–petroleum ether melted at 170–172° in accord with the reported melting point for the trans isomer (lit. mp 174°).[4]

Benzo[1,2-*b*:4,3-*b*′]dithiophene-4-carboxylic Acids (II). See Table III. In a representative procedure a solution of 2 g of α,β-bis-(2-thienyl)acrylic acid (either isomer) and 0.05 g of I_2 in 1.6 l. of EtOH was irradiated for 24 hr in a Rayonett reactor fitted with 2537-Å sources. During the entire irradiation period, air was passed through the solution. The reactions were monitored by periodic scanning of the uv spectrum of the solution (the band at *ca.* 255 nm was used). The EtOH was removed under reduced pressure, and the residue was washed with Et_2O and crystallized from EtOH: yield 1.2 g; mp 290–291°.

Irradiation of **3** required 48 hr and as reported in Table II the yield was considerably poorer than for **1** and **2.** We were unable to isolate material from irradiation of **4** that could be characterized as the expected benzodithiophene.

4-(2-*n*-Dialkylamino-1-hydroxyethyl)benzo[1,2-*b*:4,3-*b*′]dithiophenes (III). See Table III. In a typical procedure 1.3 g of **5** in 20 ml of $SOCl_2$ was refluxed for 4 hr and the excess $SOCl_2$ was removed under reduced pressure. The crude acid chloride was washed with anhydrous petroleum ether, dried *in vacuo*, and dissolved in 50 ml of CH_2Cl_2. The acid chloride solution (0°) was added to an Et_2O solution (0°) of CH_2N_2 prepared from 5.0 g of nitrosomethylurea.[10] The solution was stirred at 0° for 2 hr and then at room temperature overnight. The reaction product was treated with 10 ml of 48% HBr, extracted with Et_2O, washed (H_2O), and dried ($CaSO_4$), and the Et_2O was removed under reduced pressure to yield 1.7 g of crude bromo ketone. The bromo ketone was dissolved in a mixture of 100 ml of C_6H_6 and 50 ml of EtOH and was allowed to react for 15 min with 0.4 g of $NaBH_4$ at room temperature. To the reaction mixture was added 25 ml of 10% KOH and solution was stirred for 10 min at room temperature. The solvent was removed under reduced pressure to yield a residue which was treated with H_2O, extracted with Et_2O, washed (H_2O), and dried ($CaSO_4$), and the Et_2O was removed under reduced pressure to yield an oil, whose nmr was consistent with that expected of the epoxide,[1] which was allowed to react with *n*-dibutylamine without purification. When impure epoxide was obtained, it was readily purified by chromatography over Al_2O_3 employing low boiling petroleum ether as the eluent.

The epoxide was mixed with 3 ml of *n*-dibutylamine and refluxed for 1.5 hr and the excess amine was removed by vacuum distillation. The crude amino alcohol was purified by chromatography over Al_2O_3. The pure amino alcohol (Et_2O eluent; determined by tlc) was dissolved in 50 ml of anhydrous C_6H_6 and dry HCl was passed through until the solution was saturated. Refluxing the solution employing a Dean-Stark trap removed H_2O. The C_6H_6 was removed and the residue was recrystallized from Et_2O–EtOH to yield 1.1 g, mp 144–145° dec.

Decarboxylation of Benzo[1,2-*b*:4,3-*b*′]dithiophene-4-carboxylic Acid. The acid (0.5 g) was dissolved in 15 ml of quinoline and refluxed for 5 min; 5 g of Cu powder was added and the solution was refluxed for 0.5 hr. The mixture was cooled and filtered, and the filtrate was poured in H_2O and acidified with HCl. The acidified solution was extracted (Et_2O), and the Et_2O layer was washed (H_2O), dried ($CaSO_4$), and evaporated to yield a solid (0.4 g). Crystallization from petroleum ether gave 0.35 g of solid, mp 117–118° (lit. mp 117–118°).[2] The picrate was made from an EtOH solution and upon recrystallization from EtOH melted at 149–149.5° (lit. mp 149–150°).[2]

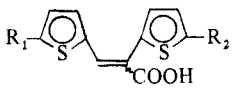
Acknowledgments. We acknowledge the U. S. Army Medical Research and Development Command under Con-

Table I. Antimalarial Activity[a]

Compd	ΔMST (days) after a single mg/kg dose				
	40	80	160	320	640
7	0.3	0.3	0.5	0.5	3.5
8	2.5	4.3	7.7	11.1	13.5
9	1.1	6.9	2C	5C	5C
10	3.3	3.7	5.9	9.3	13.7
11[b]	1.5	2.3	4.7	6.1	7.1
12[c]	4.2	5.2	9.4	10.4	3C
13[d]	6.7	1C	3C	4C	4C

[a]Test data, supplied by Walter Reed Army Institute of Research against *P. berghei* in mice. Increase in mean survival time (MST) in days of the test group is reported. The mean survival time of untreated mice is 6.1 days. A compound is active if ΔMST exceeds 6.1 days and curative (C) if one or more of the five tested mice lived 60 days postinfection. [b]Quinine sulfate. [c]α-(*n*-Diheptylaminomethyl)-9-phenanthrenemethanol hydrochloride; data from W. T. Colwell, V. Brown, P. Christie, J. Lange, C. Reece, K. Yamamoto, and D. W. Henry, *J. Med. Chem.*, **15**, 771 (1972). [d]α-(*n*-Diheptylaminomethyl)-6-bromo-9-phenanthrenemethanol hydrochloride. This compound is considered to be the "standard" for the phenanthrenemethanol series; see E. A. Nodiff, K. Tanabe, C. Seyfried, S. Matsuura, Y. Kondo, E. H. Chen, and M. P. Tyagi, *ibid.*, **14**, 921 (1971).

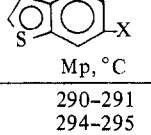
Table II. Dithienylacrylic Acids



Compd	R_1	R_2	Mp, °C	Yield, %	Recrystn solvent	Formula
1[a]	H	H	240–241	44	EtOH–Et_2O	$C_{11}H_8O_2S_2$
2[a]	H	H	170–172	30	Et_2O–petroleum ether	$C_{11}H_8O_2S_2$
3	H	Cl	194–195	54	Et_2O–petroleum ether	$C_{11}H_7ClO_2S_2$[b]
4	Cl	Cl	208–210	56	Et_2O–EtOH	$C_{11}H_6Cl_2O_2S_2$[b]

[a]**1** and **2** are isomers; see Experimental Section. [b]Analyzed within ±0.4% for C and H.

Table III. Benzo[1,2-*b*:4,3-*b*]dithiophenes



Compd	R	X	Mp, °C	Yield, %	Recrystn solvent	Formula
5[a]	H	COOH	290–291	57	EtOH	$C_{11}H_6O_2S_2$
6[b]	Cl	COOH	294–295	15	EtOH	$C_{11}H_5ClO_2S_2$
7[c]	H	$CHOHCH_2N(n\text{-}Bu)_2\cdot HCl$	144–145	75	EtOH	$C_{20}H_{28}ClNOS_2$
8[a]	H	$CHOHCH_2N(n\text{-heptyl})_2\cdot HCl$	110–112	40	EtOH–Et_2O	$C_{26}H_{40}ClNOS_2$
9[c]	Cl	$CHOHCH_2N(n\text{-}Bu)_2\cdot HCl$	194–195	75	EtOH–Et_2O	$C_{20}H_{27}Cl_2NOS_2$
10[c]	Cl	$CHOHCH_2N(n\text{-heptyl})_2\cdot HCl$	154–155	61	EtOH–Et_2O	$C_{26}H_{39}Cl_2NOS_2$

[a]Analyzed within ±0.4% for C, H, and S. [b]Analyzed within ±0.4% for C and H. [c]Analyzed within ±0.4% for C, H, and N.

tract DADA17-68-C-8035 for support of this work. This is Contribution No. 1058 from the Army Research Program on Malaria. The authors wish to thank Dr. Edgar A. Steck of WRAIR for helpful discussions.

References

(1) B. P. Das, J. A. Campbell, F. B. Samples, R. A. Wallace, L. K. Whisenant, R. W. Woodard, and D. W. Boykin, Jr., *J. Med. Chem.*, **15**, 370 (1972).
(2) C. E. Loader and C. J. Timmons, *J. Chem. Soc. C*, 1677 (1967).
(3) G. De Luca, G. Martelli, P. Spalgnolo, and M. Tiecco, *ibid.*, 2504 (1970).
(4) G. M. Badger, J. H. Elix, and G. E. Lewis, *Aust. J. Chem.*, **18**, 70 (1965).
(5) D. S. Rao and B. D. Tilak, *J. Sci. Ind. Res., Sect. B*, **16**, 65 (1957); *Chem. Abstr.*, **51**, 13, 841 (1957).
(6) D. D. Morgan, S. W. Horgan, and M. Orchin, *Tetrahedron Lett.*, 1789 (1972).
(7) W. H. Laarhoven, Th. J. H. Cuppen, and R. J. F. Nivard, *Recl. Trav. Chim. Pays-Bas*, **87**, 687 (1968).
(8) R. E. Lutz, *et al.*, *J. Amer. Chem. Soc.*, **68**, 1813 (1946).
(9) T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).
(10) A. I. Vogel, "Practical Organic Chemistry," 3rd ed, Longmans, Green and Co., London, 1964, p 969.

Studies on the Specificity of Antibodies Produced by Linear Antigenic Polypeptides of a Known Primary Structure. Synthesis and Use of Poly(L-tyrosyl-L-aspartyl-L-alanylglycyl)glycine Methyl Ester†,‡

Brian J. Johnson* and Charles Cheng

Laboratory of Molecular Biology, Department of Biochemistry and Department of Microbiology, Division of Clinical Immunology, University of Alabama Medical School, Birmingham, Alabama 35233. *Received October 16, 1972*

It has been previously reported that the antibodies produced by rabbits against the polypeptide poly(Tyr-Glu-Ala-Gly)Gly-1-^{14}C Et ester[1-3] (**1**) are most probably dependent upon the conformation of the antigen.[4-7] Also, these antibodies have been shown to possess a specificity for the phenolic hydroxyl group and the aromatic moiety of the tyrosyl residue.[8] However, no such specificity has been shown for the alanyl residue.[9] The next phase of this work has been to study the specificity of these antibodies pertaining to the role of the glutamyl residue. In this paper we wish to report the characterization of the specificity of the antisera produced by rabbits against the antigen **1** as studied by cross reactions and absorption studies. For this purpose the following polymer was prepared and used, poly(Tyr-Asp-Ala-Gly)Gly (**2**).

Chemistry. The synthesis of the polymerizing unit *O-tert*-Bu-Tyr-*β-tert*-Bu-Asp-Ala-Gly pentachlorophenyl ester · HCl (**6**) and the necessary intermediates for its preparation are outlined in the Experimental Section. The polymerization of **6** was performed by the procedure which has been shown to produce linear high-molecular-weight polypeptides[1,2,4-8] to give poly(*O-tert*-Bu-Tyr-*β-tert*-Bu-Asp-Ala-Gly)Gly Me ester. The protecting *tert*-Bu groups were removed by the use of 90% $F_3C \cdot CO_2H$ to yield the polymer **2**. After extensive dialysis, the polymer was fractionated by successive diafiltrations through Diaflo membranes into four different molecular weight fractions: $>5 \times 10^4$; $2\text{-}5 \times 10^4$; $1\text{-}2 \times 10^4$; and $<1 \times 10^4$.

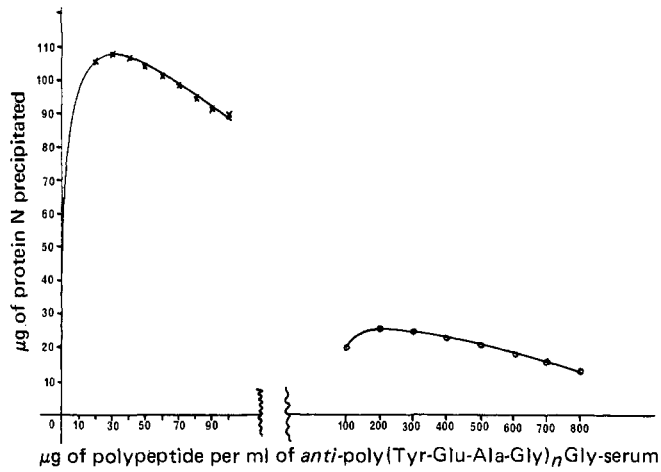


Figure 1. Relative precipitin curves for poly(Tyr-Glu-Ala-Gly)$_n$Gly (**1**, X) and poly(Tyr-Asp-Ala-Gly)$_n$Gly (**2**, O).

Immunochemistry. Eight rabbits were immunized against poly(Tyr-Glu-Ala-Gly)Gly-1-^{14}C Et ester (**1**) using the previously reported protocol.[3] It was found that each serum gave a positive precipitin reaction with the homologous polymer **1**. The serum from each animal was pooled, it being assumed that in this time interval that each rabbit had responded to the same antigenic determinants.[10] Incremental amounts of the polypeptide **2** were added to 1-ml aliquots of this pooled antisera and it was observed that a cross reaction occurred. The relative precipitin curves for the polypeptides **1** and **2** are shown in Figure 1. It was noted that the heterologous polypeptide **2** does not precipitate as much antibody as the homologous polypeptide **1**. In order to quantitate the amount of antibody not precipitated by polymer **2**, a separate experiment was performed. A quantity equal to the equivalent point amount of the heterologous polypeptide **2** was allowed to react with the pooled sera. After removal of the precipitate, 30 μg of the homologous polypeptide **1** was added to the resulting supernatant liquid. Further precipitation was obtained and quantitated by analysis for N (Kjeldahl) as shown in Table I.

Conclusions

It has been found that the heterologous polypeptide **2** cross reacts with *anti*-**1**-sera, precipitating less antibody than the homologous antigen **1**. Also, the amount of the heterologous polymer **2** required to attain the equivalent point is larger than that necessary for the homologous material. It would appear from these cross reactions that the conformation of polymer **2** is similar to that of the homologous antigen **1**; however, these results indicate a lower affinity of the antibody-combining sites for the heterologous polypeptide **2**. Using the rationale that the determinants of the heterologous polypeptide are in the same orientation as those of the antigen **1**, it is suggested that the observed differences in the binding ability of the heterologous polymer **2** are due only to the modification of the glutamyl residue. Thus, it would appear that the antibody-combining sites have a high affinity for the γ-carboxyl group of the glutamyl residue.

Experimental Section

Z-*β-tert*-Bu-Asp-Ala-Gly Me Ester (3).§ To a solution of 11.1 g (0.0565 mol) of Ala-Gly Me ester · HCl and 5.6 g (0.056 mol) of

†All amino acids are of the L variety.

‡Presented in part at the 164th National Meeting of the American Chemical Society, New York, N. Y., 1972.

§ Z = benzyloxycarbonyl.