Design, Synthesis, and Testing of Potential Antisickling Agents. 5. Disubstituted Benzoic Acids Designed for the Donor Site and Proline Salicylates Designed for the Acceptor Site

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This paper reports the discovery of a new class of potent antigelling agents. The new compounds, disubstituted benzoic acid derivatives, were designed by using molecular modeling experiments. These molecules contain functional groups positioned to interact with several polar amino acid residues near the Val-6β mutation site (donor site) in HbS. The compounds also contain a hydrophobic group designed to occupy a nonpolar area on the surface of the protein created by several hydrophobic amino acids. The synthesis and testing of these new molecules using a standard solubility assay is reported. A structural comparison is made between one of the most active antigelling agents, compound 13, which has little effect on the oxygen affinity of Hb in solution, and bezafibrate, a known antilipidemic drug that is progelling and has a very potent effect on decreasing Hb oxygen affinity (Perutz, M. F.; Poyart, C. *Lancet* **1983**, *2*, 881–882). We also report the synthesis and testing of a series of proline–salicylate molecules designed to react covalently at the mutation acceptor area. This class of molecules did not show significant activity.

History. Sickle-cell anemia is an inherited disease that is caused by a single mutation in the β subunits of the oxygen-carrying protein hemoglobin (Hb). This one hydrophilic Glu-6β to hydrophobic Val-6β alteration causes the deoxy form of Hb to polymerize and gel inside the erythrocyte, distorting its normal elliptical shape to a sickle shape. Upon oxygenation in the lungs, most of the sickled cells return to their normal shape. Those cells that remain in an abnormal shape regardless of the state of oxygenation of HbS are termed irreversibly sickled cells. Polymer formation is the primary factor underlying the manifestation of the disease. Presently, there are no therapeutic agents available that reverse the sickling process, and medical attention is focused on treating symptoms and side effects. Several efforts have been made by a number of groups to find either covalent or noncovalent inhibitors of gelation (for a review, see Dean and Schechter[2]). More recently we and others have discovered a number of very active halogenated aromatic molecules, which include 5-bromotryptophan,[3] 2-[(3,4-dichlorophenoxy)methyl]-2-imidazoline,[4] [(3,4-dichlorobenzyl)oxy]acetic acid,[5,6] and the potent diuretic drug ethacrynic acid (which binds covalently to HbS).[7] All of these compounds possess high antigelling activity at low concentrations.

This paper reports the synthesis and testing of a new class of highly active molecules: 3,5-disubstituted benzoic acids, which were designed by fitting molecular models to the surface of the protein near the mutation site area. A second class of molecules, proline–salicylates designed to bind at the complementary mutation acceptor area, did not show significant activity.

Design Studies

Compounds Designed for the Donor Site. Our first attempt at designing stereospecific inhibitors of gelation that would bind near the mutation site[8] did not produce compounds with high activity. Originally, we mapped out (for drug design purposes) a binding area that included a trapezoid of polar amino acids that are located near a large number of hydrophobic amino acids on the surface of the protein next to the Val-6β mutation site (Figure 1). We used this site as a template to model a series of rigid molecules that would stereochemically fit the protein surface. The proposed molecules would contain functional groups that would interact with the polar amino acid residues His-2, Thr-4, Glu-7, or Lys-132 (all on the β chain) and place a hydrophobic aromatic ring or alkyl chain near a large number of hydrophobic amino acid residues that appear on the surface of the protein (see the shaded area in Figure 1). The idea was to bind a small molecule near the mutation site in such a fashion that it would prevent the insertion of the β subunit of one Hb molecule containing the Val mutation (donor site) into a hydrophobic pocket of a second Hb molecule as shown in Figure 2. This interaction between two HbS tetramers was discovered by Wishner, Love, and co-workers in their analysis of the HbS crystal structure at 5 Å and verified at 3 Å.[9,10] Other groups have related the paired strands of Hb tetramers in the crystal structure to the formation and structure of the HbS fiber that constitutes the gel.[11-14] The crucial interaction by the mutation site that induces polymer formation results from the insertion of one of the Val-6β methyl groups of one HbS molecule into a hydrophobic "nitch" near Leu-88β and Phe-85β of the second HbS molecule. An important hydrogen bond that also helps stabilize the Val-6β lateral contact between single strands in the crystal is found between Thr-4β of the donor site and Asp-73β of the acceptor site (Figure 2). The representation of the interaction of the mutation site with

(1) Perutz, M. F.; Poyart, C. *Lancet* **1983**, *2*, 881–882.
(2) Dean, J.; Schechter, A. N. *N. Engl. J. Med.* **1978**, *299*, 752–763, 804–811, 863–870.
(3) Poillon, W. N. *Biochemistry* **1982**, *21*, 1400.
(4) Broersma, R. J.; Spittka, G. A., Dow Chemical Co., U.S. Patent 4344958, 1982.
(5) Abraham, D. J., Mehanna, A. S.; Williams, F. L. *J. Med. Chem.* **1982**, *25*, 1015–1017.
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(14) Rosen, L. S.; Magdoff-Fairchild, B. *J. Mol. Biol.* **1982**, *157*, 181–189.

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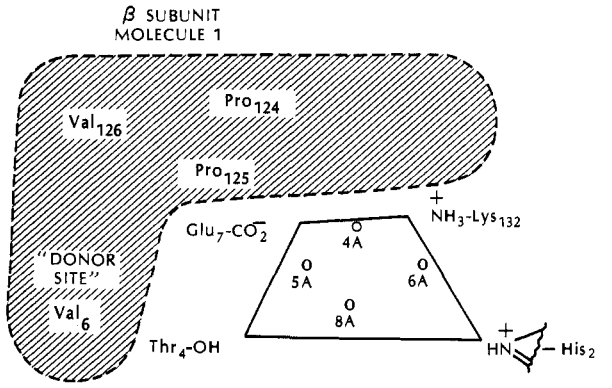

Figure 1. The proposed binding area for the donor site molecules. The trapezoid of polar amino acids is located near a large number of hydrophobic amino acids that appear on the surface of the protein near the mutation Val-6β.

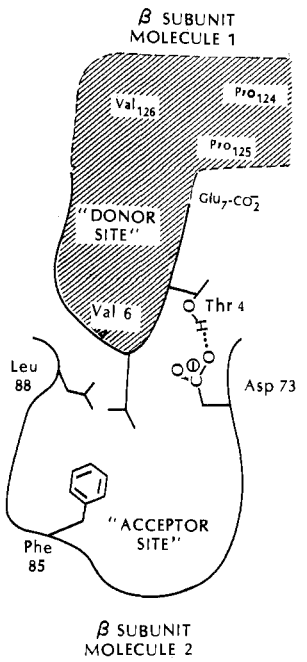

Figure 2. The interaction of two hemoglobin molecules that form the lateral mutation site contact in the crystals is depicted. The hydrophobic environment of Phe-85β and Leu-88β attract one of the Val-6β methyl groups. A hydrogen bond that stabilizes the interaction is found between Thr-4β and Asp-73β.

Leu-88β and Phe-85β and the effect of a potential drug on this area has been illustrated by Ross and Subramanian.[15]

The new series of compounds (3,5-disubstituted benzoic acids) designed to bind to the donor site (Figure 1) were synthesized and tested. Several of the new compounds were found to be extremely active in destabilizing HbS polymer formation. The superposition of the most active compound **15** [5-(3,4-dichlorobenzamido)-α-carboxy-*m*-anisic acid] on the proposed donor binding site is shown in Figure 3 (compare with Figure 1). The hydrophobic tail of the molecule is situated so that it fits into the hydrophobic side-chain area created by several of the nonpolar surface residues including the mutation residue Val-6β. The acid groups are positioned in such a manner that they will interact with His-2β and Lys-132β. The carbonyl oxygen of the amide group is positioned toward Thr-4β to form a hydrogen bond and disrupt the Thr-4β intermolecular interaction with Asp-73β. Since there is a variability in the size and shape of the hydrophobic surface (see the shaded area in Figure 1), we synthesized several molecules with different hydrophobic tails to fit this area. The activity of these compounds is discussed below.

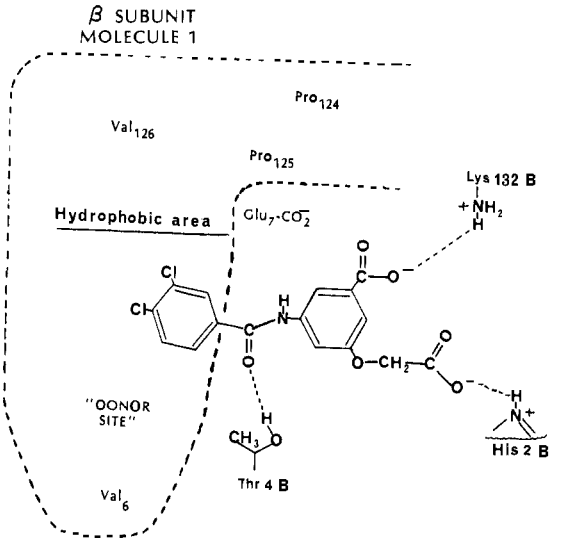

Figure 3. The most active compound **15** is shown superimposed on the donor site binding area depicting possible ionic and hydrophobic interactions.

Compounds Designed for the Acceptor Site. In the following study we report the design of a new class of molecules that were modeled to interact covalently at the acceptor site cavity. Several groups have investigated the covalent binding of potential antisickling agents to Hb in the form of nitrogen mustards,[16,17] cyanates,[18,19] pyridoxal phosphates,[20,21] and imido esters.[22] Although some of these agents have been shown to possess antisickling properties, none have been useful as therapeutic agents.

Another class of antisickling agents that react covalently with Hb, the salicylate esters or aspirin derivatives, have been shown to be effective in the acylation of amine side chains.[23-27] Aspirin itself has been shown to acetylate a variety of sites on both the α and β chains of HbS.[28] The vast majority of acetyl groups were attached at three loci: Lys-59β, Lys-144β, and Lys-90β.

(15) Ross, P. D.; Subramanian, S. *Biochem. Biophys. Res. Commun.* **1977**, *77*, 1217–1223.
(16) Roth, E. F., Jr.; Nagel, R. L.; Bookchin, R. M.; Grayzel, A. I. *Biochem. Biophys. Res. Commun.* **1972**, *48*, 612–618.
(17) Roth, E. F., Jr.; Arnone, A.; Bookchin, R. M.; Nagel, R. L. *Blood* **1981**, *58*, 300–308.
(18) Balcerzak, S. P.; Grever, M. R.; Sing, D. E.; Bishop, J. N.; Segal, M. L. *J. Lab. Clin. Med.* **1982**, *100*, 345–355.
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(22) Lubin, B. H.; Pena, V.; Mentzer, W. C.; Bymun, E.; Bradley, T. B.; Packer, L. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 43–46.
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(28) Shamsuddin, M.; Mason, R. G.; Ritchey, J. M.; Honig, G. R.; Klotz, I. M. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 4693–4697.

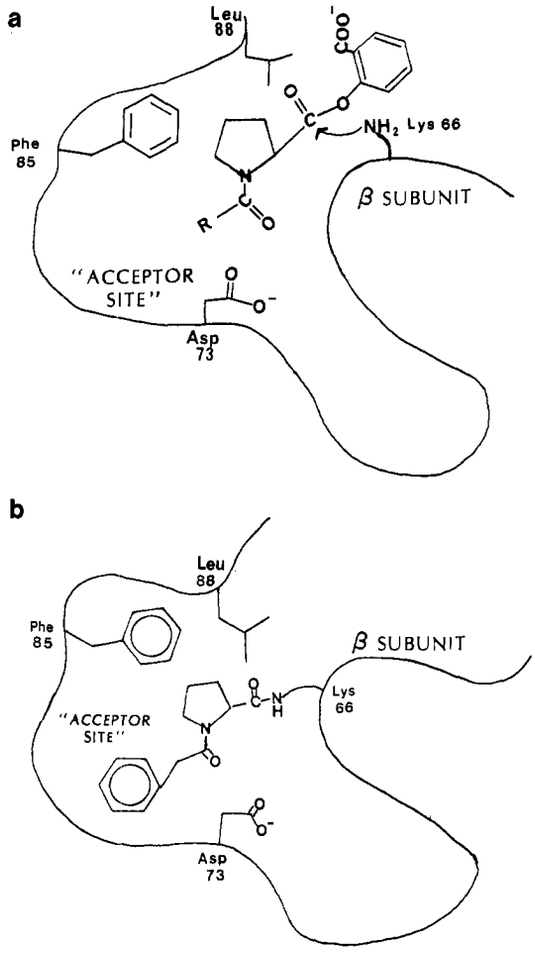

Figure 4. (a) The position of the proposed binding of the proline–salicylates before the attack of Lys-66β of the same subunit. (b) The covalently attached molecule.

With the knowledge that the salicylate moiety is a good leaving group in reactions with Hb, we designed a series of proline–salicylate molecules that were modeled to bind and react covalently at the acceptor cavity (Figure 4). A compound that is bound at this site should sterically inhibit the insertion of the Val-6β methyl group from the donor molecule. Ross and Subramanian[15] had suggested that simple aromatic antigelling agents, such as benzyl alcohol, might act by binding the aromatic ring into the hydrophobic (acceptor) area near Phe-85β and Leu-88β. We have found that a benzyl alcohol derivative (*p*-bromobenzyl alcohol) binds to carbomonoxyhemoglobin (HbCO) at the Trp-14α site.[6] To explore the possibility of binding covalently acting agents to the acceptor site, we attached a variety of hydrophobic groups (R in Figure 4a) to the proline ring and added a salicylate leaving group to the molecule in such a fashion that it would be in a position to undergo nucleophilic attack by Lys-66β (Figure 4a). After displacement of the salicylate ion, the hydrophobic proline portion of the molecule would be covalently attached to Hb (Figure 4b with R = benzyl). Although these compounds were found to be more active than our earlier proline derivatives designed for the donor site,[8] they are outside the range of activity (at low concentrations) necessary for consideration for further study. At present, our most effective covalent acting compounds are ethacrynic acid[7] and one of its nondiuretic analogues.[29]

(29) Abraham, D. J.; Williams, F. L.; Cragoe, E. J., Jr., unpublished results.

Biological Testing

The new compounds were tested for biological activity by using the antigelling assay developed by Hofrichter et al.[30] This assay involves the incubation of the drugs to be tested (at various concentrations) with HbS in EPR tubes in the presence of a deoxygenating substance as previously described.[5,7] Activity is reported as a ratio of the concentration of HbS soluble in the presence of a drug at a given concentration to the HbS solubility in the absence of the drug (control), i.e., [HbS drug]/[HbS control]. The higher the ratio, the higher the activity. Ratios of 1.06–1.17 have been estimated as necessary for decreasing the severity of the disease.[31] A moderate antigelling amino acid, phenylalanine,[32] is used as a drug standard (at 40 mM) in every set of analyses (six tubes). If the ratio [HbS Phe]/[HbS control] is 1.175 ± 0.031 [this is the mean ratio and standard deviation for 173 assays for Phe], the ratios [HbS drug]/[HbS control] obtained from the drug at 5, 10, 20, and 40 mM (four tubes) are considered to be acceptable. The control tube in each set of six for 173 assays shows a mean solubility for HbS of 17.03 g %, ± 0.46.

3,5-Disubstituted Aromatic Acids. The activity of the 3,5-disubstituted aromatic acids are listed in Table I. The compounds are listed in decreasing order of their activity at 5 mM. The first three compounds (**15**, **13**, and **7**) at 5 mM are above the solubility activity ratio of 1.06, a threshold value we have set to select compounds with sufficient activity to warrant further study as potential drugs. Compound **15** represents the most active noncovalently acting molecule that we have tested to date, which includes all of our compounds and the most active halogenated aromatic compounds reported by others[3,4,33] and retested in our laboratory. Considering our work with two other classes of compounds that show multiple binding sites for moderately active compounds,[34] we will refrain from drawing any conclusions about the structure–activity relationships of these new molecules until X-ray binding sites have been mapped out.

Proline Derivatives. The results in Table II (the compounds are listed in decreasing order of their activity at 5 mM) make it evident that the proline–salicylates do not warrant further study at this time. None of the compounds reach the threshold ratio until 40 mM, which is too high a concentration to be considered for drug purposes. Compounds **39** and **31** were tested as the proline acids to see the effect of the loss of the salicylate group. The addition of the salicylate ester to the acid moiety on the proline ring in this series appears to decrease the antigelling activity. Noguchi and Schechter[35] have observed a decrease in HbS solubility with salicylic acid. Salicylate esters, however, react covalently with Hb, as mentioned previously, and prevent polymerization. The production of salicyclic acid as a byproduct in the reaction with HbS by active salicylate esters does not decrease HbS solubility (as might be thought from the studies of Noguchi and Schechter) because the covalently attached portion of the molecule alters tertiary or quaternary structure of HbS,

(30) Hofrichter, J.; Ross, P. D.; Eaton, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 3035–3039.
(31) Sunshine, H. R.; Hofrichter, J.; Eaton, W. A. *Nature (London)* **1978**, *275*, 238–239.
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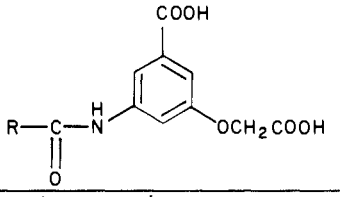
(35) Noguchi, C. T.; Schechter, A. N. *Biochemistry* **1978**, *17*, 5455–5459.

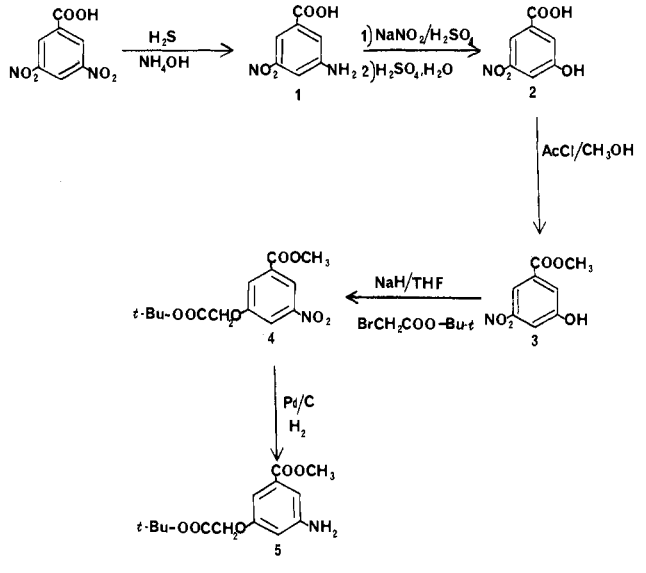
Table I. Aromatic Amides[a]



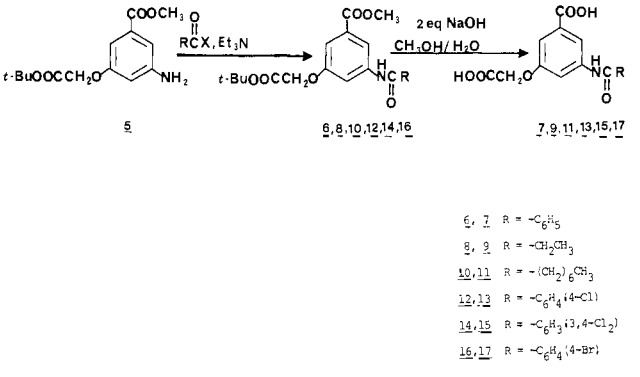
compd[c]	R	[HbS drug]/[HbS control][b] 5 mM	10 mM	20 mM	40 mM	no. of runs	initial HbS concn, g/dL	dHbS control, g/dL
15	$C_6H_3(3,4\text{-}Cl_2)$	1.094	1.192	1.353	1.452	3	24.30, 24.80, 24.32	16.26, 16.85, 16.78
13	$C_6H_4(4\text{-}Cl)$	1.071	1.159	1.339	1.384	1	24.01	17.07
7	C_6H_5	1.061	1.091	1.160	1.368	2	23.83, 23.82	17.22, 17.44
11	$CH_3(CH_2)_6$	1.045 (9.2%)	1.103 (21.1%)	1.228 (46.8%)	1.426 (87.5%)	1	24.29	16.34
17	$C_6H_4(4\text{-}Br)$	1.017	1.077	1.237	1.318	1	24.01	17.44
9	C_2H_5	1.000	1.031	1.075	1.150	1	24.29	16.56
BZF		0.954	0.932	0.981	1.198	3	24.80, 24.81, 24.81	18.24, 17.29, 17.22
Ph		1.036	1.048	1.093	1.178	2	23.74, 24.50	17.32, 17.09
					1.175	173		17.03 (±0.46)

[a] All compounds were prepared as outlined in the Experimental Section except for BZF (bezafibrate), which was generously provided by Dr. M. F. Perutz of Cambridge, England. [b] The ratios are calculated as sol HbS drug (g/dL)/sol HbS control (g/dL). [c] All compounds were dissolved in 0.15 M phosphate buffer, pH 7.4, with 2 equiv of sodium bicarbonate to make the sodium salt, at a concentration of 0.18 M. Appropriate aliquots of this solution (10, 20, 40, and 80 μL) were mixed with buffer to equal 90 μL. The 90-μL solutions were added to 250 μL of HbS (0.15 M phosphate), usually around 35 g %, and then 20 μL of dithionite (1.06 M) was added before the EPR tubes were sealed. Final concentrations of drug were 5, 10, 20, and 40 mM in four separate tubes. A set of six tubes was spun on each run, which included the four drug concentrations, one dHbS control (90 μL of buffer, no acid), and a 40 mM phenylalanine control. The above dilution procedure produces identical HbS initial concentrations for all six tubes. The initial HbS concentrations (in grams per deciliter) for each after addition of the acids and dithionite and the respective solubility of deoxyhemoglobin S (in grams per deciliter) for each control run (no acid) appear in the table above.

Scheme I



Scheme II



thus hindering polymerization.

Chemistry

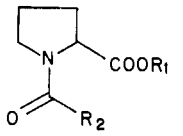
3,5-Disubstituted Aromatics. The common intermediate **5** was required for the synthesis of the amide series, as shown in Scheme II. The synthesis of **5** (Scheme I) was initiated from commercially available 3,5-dinitrobenzoic acid by selective reduction[36] of one of the nitro groups with H_2S/NH_4OH to give **1** in 80% yield. The reaction was monitored by periodic removal of an aliquot and testing its ability to dissolve in concentrated HCl. Some difficulty was encountered in the complete reduction of the nitro group. Replacement of the amino group with a hydroxyl functionality was accomplished by first diazotizing the amine with use of standard conditions[36] followed by hydrolysis with refluxing aqueous sulfuric acid to give **2** in 42% yield. The carboxylate group was protected as the methyl ester by Fischer esterification in methanol to give **3** in an 82.4% yield. Alkylation of the phenolic hydroxyl was accomplished with *tert*-butyl bromoacetate, thus providing diester **4**, which provided us with a versatile intermediate that could be useful for future modification of this series. The presence of the methyl and *tert*-butyl esters would allow for selective removal of one ester, i.e., selective hydrolysis of the methyl ester or selective removal of the *tert*-butyl ester with trifluoroacetic acid. This monoacid could then be converted to an active ester if covalent binding of the drug to Hb was desired. The synthesis of **5** was completed in 86% yield by the catalytic reduction of the nitro group of **4** under hydrogen pressure in a Parr apparatus.

Synthesis of the amide series was relatively straightforward. Treatment of **5** with the corresponding acyl halide and triethylamine in anhydrous THF at room temperature afforded, after chromatography, each of the diester aromatic amides shown in Scheme II. Selective hydrolysis of both esters in the presence of the amides was accomplished with 2 equiv of NaOH, thus affording all of the diacid aromatic amides pictured in Scheme II.

Proline Derivatives. The proline compounds listed in Table II were prepared by using the general procedure

(36) Epstein, E.; Meyer, M. *J. Am. Chem. Soc.* **1955**, *77*, 4059–4061.

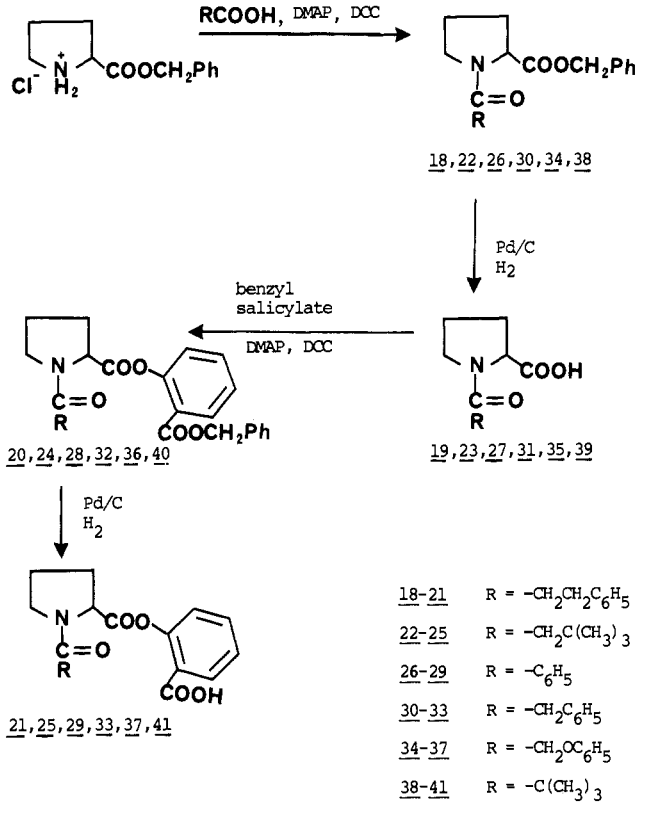
Table II. Proline Derivatives[a]



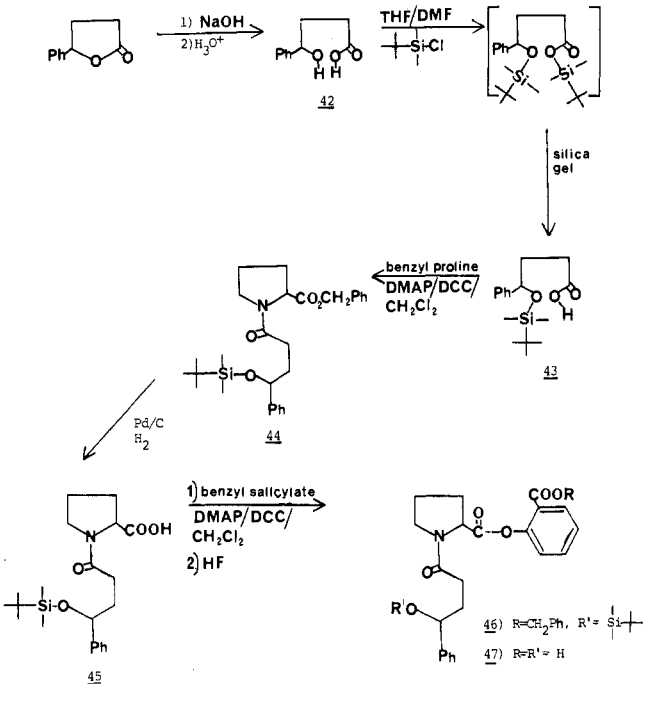
no.[c]	R_1	R_2	[HbS drug]/[HbS control][b] 5 mM	10 mM	20 mM	40 mM	no. of runs	initial HbS concn, g/dL	dHbS control, g/dL
33	salicylic acid	$CH_2C_6H_5$	1.028	1.030	1.052	1.146	1	24.63	17.07
39	H	CMe_3	1.027	1.050	1.052	1.068	1	22.85	16.12
31	H	$CH_2C_6H_5$	1.002	1.014	1.058	1.130	2	24.52, 23.74	17.55, 17.18
29	salicylic acid	C_6H_5	0.999	1.001	1.007	1.027	2	24.52, 23.74	17.22, 16.56
41	salicylic acid	CMe_3	0.993	1.004	1.032	1.083	1	22.85	16.48
25	salicylic acid	CH_2CMe_3	0.991	1.017	0.995	1.055	2	28.26, 23.74	17.62, 17.11
47	salicylic acid	$CH_2CH_2CH(OH)C_6H_5$	0.990	0.980	1.002	1.145	2	24.50, 23.74	17.62, 16.78
37	salicylic acid	$CH_2OC_6H_5$	0.987	1.022	1.018	1.077	1	24.02	17.14
21	salicylic acid	$CH_2CH_2C_6H_5$		0.988	0.983	0.951	1	23.40	

[a] All compounds were prepared as outlined in the Experimental Section. [b] The ratios are calculated as sol HbS drug (g/dL)/sol HbS control (g/dL). [c] All compounds were dissolved in 0.15 M phosphate buffer, pH 7.4, with 1 equiv of sodium bicarbonate to make the sodium salt, at a concentration of 0.18 M. Appropriate aliquots of this solution (10, 20, 40, and 80 μL) were mixed with buffer to equal 90 μL. The 90-μL solutions were added to 250 μL of HbS (0.15 M phosphate), usually around 35 g %, and then 20 μL of dithionite (1.06 M) was added before the EPR tubes were sealed. Final concentrations of drug were 5, 10, 20, and 40 mM in four separate tubes. A set of six tubes was spun on each run, which included the four drug concentrations, one dHbS control (90 μL of buffer, no acid), and a 40 mM phenylalanine control. The above dilution procedure produces identical HbS initial concentrations for all six tubes. The initial HbS concentrations (in grams per deciliter) for each after addition of the acids and dithionite and the respective solubility of deoxyhemoglobin S (in grams per deciliter) for each control run (no acid) appear in the table above.

Scheme III



Scheme IV



outlined in Scheme III. The common starting material for the series was L-proline benzyl ester hydrochloride. Amidation of the nitrogen of the pyrrole ring was accomplished by using the desired carboxylic acid, dicyclohexylcarbodiimide (DCC) as the coupling agent[37,38] and the addition of 4-(dimethylamino)pyridine (DMAP) as a catalyst.[39] When the NMR spectra of **34** was recorded, cis/trans isomerism about the amide bond was observed.[8,40-42] The proline benzyl ester was removed by catalytic hydrogenation to give the corresponding free proline acids. Coupling of the free acid to benzyl salicylate was accomplished by again using DCC and DMAP in methylene chloride. Removal of the salicylate benzyl protecting group was effected by catalytic hydrogenation to give the desired proline–salicylates shown in Scheme III.

The preparation of **47** was somewhat more involved (see Scheme IV) because it necessitated the preparation of the

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(39) Wang, S. S.; Tam, J. P.; Wang, B. S. H.; Merrifield, R. B. *Int. J. Pept. Protein Res.* **1981**, *18*, 459–467.
(40) Thomas, W. A.; Williams, M. K. *J. Chem. Soc., Chem. Commun.* **1972**, 994.
(41) Donan, D. E.; Boney, F. A. *J. Org. Chem.* **1973**, *38*, 2379–2383.
(42) Nagaraj, R.; Venkatachalapathi; Balaram, P. *Int. J. Pept. Protein Res.* **1980**, *16*, 291–298.

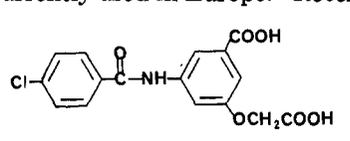
protected 4-hydroxy-4-phenylbutanoic acid **43** before it could be coupled to L-proline benzyl ester. This was accomplished via the base hydrolysis of 4-phenyl-4-butyrolactone. The resulting hydroxy acid **42** was used without further purification (NMR indicated one product) due to its propensity to relactonize. Protection of **42** using *tert*-butyldimethylsilyl chloride[43] resulted in the silylation of both the acid and hydroxy groups. Selective removal of the silyl ester group was accomplished by chromatography on silica gel, affording **43**. This was then coupled with proline benzyl ester followed by removal of the benzyl ester via catalytic hydrogenation to give **45**. The free acid was then esterified with benzyl salicylate by using DCC and DMAP. Finally, the simultaneous removal of both the silyl ether and benzyl ester was accomplished by treatment of **46** with anhydrous HF.

Results and Conclusions

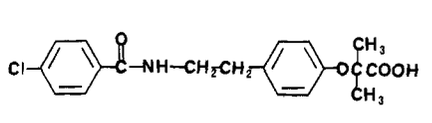
The new class of meta-disubstituted benzoic acid derivatives reported in this paper are among the most promising noncovalent antigelling compounds that we have discovered to date. We have studied one of these derivatives, **13**, in other biological assay systems. It was found to have a positive effect on the rheological characteristics of HbS cells.[44] Early experiments monitoring the effect of **13** on oxygen equilibrium and polymerization of HbS in homozygous red cells indicate that it is active and should be studied further.[45] Stability tests on HbA and HbS solutions with **13** show that it does not denature Hb at concentrations far above that needed for therapy.[46]

The two most active compounds in this study, **15** and **13**, both show saturation effects above 20 mM drug. This can be observed by plotting the data in Table I. Both compounds reduce gel formation by 95% at 40 mM. The other active compounds in Tables I and II appear to have a linear correlation between solubility and concentration of drug.

One of the more striking structure–function comparisons that we have seen in our studies can be made between **13** and the known antilipidemic drug bezafibrate (BZF), which is currently used in Europe. Recently, Perutz and



13



BZF

Poyart[1] reported the profound effect of BZF on reducing the oxygen affinity of Hb. When we compared the structures of BZF and **13**, we thought that BZF would be in the range of antigelling activity shown by **13**. When tested, however, at 5 and 10 mM, BZF actually had progelling properties (see Table I). When **13** was tested for its oxygen equilibrium effect on Hb in solution, it had little or no activity in reducing the affinity of Hb for oxygen. It seems clear that these two somewhat closely related molecules have completely different binding sites that give rise to two different biological effects, one on oxygen equilibrium (BZF) and one on the polymerization of HbS (**13**). Recently we determined the binding site for BZF at 2.4 Å[47] and found it to occupy the central water cavity, yet we have not been able to detect the binding site of **13** in high salt crystals.[48] If the mechanism of antigelling action of **13** is that it binds to the surface site it was designed for, then it makes sense that we did not find it in the first look at the high salt crystal structure because the ionic strength of the crystallization media (over 2 M salt[49]) would interfere with the ionic interactions of the compound with Hb (Figure 3). If **13** is acting by binding to one of the sites we have previously indicated as a potential one for antigelling activity,[34] we should find it when the crystal structure analysis of **13** is completed. Two of our active compounds reported previously, [(*p*-bromobenzyl)oxy]-acetic acid and [(3,4-dichlorobenzyl)oxy]acetic acid,[5,34] have been shown to bind with high occupancy to single sites.[6,48] Secondary binding sites with low occupancy have been observed for [(*p*-bromobenzyl)oxy]acetic acid.[34] If the activities of the two halogenated benzyloxy acids are compared to the high activity of **13** and **15** (at low concentrations), it is probable that they also bind to predominately single sites.

Presently it appears that the meta-disubstituted benzoic acids constitute a class of molecules worth modifying further for detailed biological and molecular study. Designing a drug to bind to the surface of a protein poses some interesting problems since a natural deep cavity that enhances hydrophobic interactions does not exist. The binding interactions we have discovered thus far between drugs and hemoglobin exhibit predominately nonpolar binding that occur in hydrophobic cavities or pockets such as those found with the binding of BZF and clofibric acid.[47,6]

The determination of the binding sites of drug molecules bound to proteins will be crucial to our understanding of true structure–activity relationships. Any general rule for small molecule–large molecule interactions that we can gleen from these Hb studies will be welcome for future rational drug design studies.

Experimental Section

Proton magnetic resonance (NMR) spectra were recorded at 60 MHz on a Hitachi Perkin-Elmer R-24 spectrometer or at 90 MHz on a JEOL FX90Q spectrometer and are reported in ppm (δ) downfield from an internal standard of tetramethylsilane (Me_4Si) or 3-(trimethylsilyl)propionic acid-d_4 sodium salt (TSP). Low-resolution mass spectra were recorded on a Varian-MAT CH-5, LKB 5000 or on a Finnigan 3200 spectrometer. Chromatography was performed on Merck silica gel 60 with a reported solvent and a column size of 1.5 cm × 26 cm. TLC analyses were performed on EM Reagent silica gel 60 (0.20-mm thickness) aluminum-backed plates impregnated with a fluorescent indicator. Spots were visualized either by ultraviolet (UV) light or by charring. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Optical rotations were determined on a Perkin-Elmer 241 automatic polarimeter at the sodium-D line with a cell path of 1 dm. Infrared (IR) spectra were recorded on a Perkin-Elmer 267 grating spectrophotometer and are reported in reciprocal centimeters. Spectra were taken either in solution between two balanced salt plates with a reported solvent or as a KBr pellet. Elemental analyses were performed at Galbraith Laboratories, Inc., Knoxville, TN, and are within 0.4% of calculated values. Intermediate com-

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(49) Perutz, M. F. *J. Cryst. Growth* **1968**, *2*, 54–56.

pounds, where analyses are not reported, were determined to be pure by NMR and TLC. The apparatus for conducting the liquid HF reactions was constructed as outlined earlier.[50,51] Dry THF was obtained by distillation from a purple Na/benzophenone ketyl. Dry triethylamine was obtained by distillation from CaH_2. The petroleum ether used had a boiling point range of 30–60 °C.

1. **Aromatic Amides and Amines. 3-Amino-5-nitrobenzoic Acid** (1). To 20.0 g (9.43×10^{-2} mol) of 3,5-dinitrobenzoic acid were added 27 mL of concentrated ammonium hydroxide and 47 mL of water. After this became homogeneous, hydrogen sulfide was bubbled through the solution until a removed aliquot was completely soluble in concentrated HCl. The mixture was filtered hot, made acidic with glacial acetic acid, and cooled. The precipitate of crude 1 that formed upon acidification was filtered and recrystallized from water to give 12.2 g of a white solid: yield 80%; mp 208–211 °C (lit.[36] mp 208–211 °C); NMR (Me_2SO-d_6 + D_2O + TSP) δ 7.90–7.30 (m, 3 H); IR (KBr) NH stretch at 3410 cm^{-1} and nitro group at 1540 and 1350 cm^{-1}; MS. m/e (relative intensity) 182 (100, M^+).

3-Hydroxy-5-nitrobenzoic Acid (2). To 10.0 g (5.49×10^{-2} mol) of 1 was added 18 mL of water. After this had dissolved, 13 mL of concentrated H_2SO_4 was added followed by the slow addition (~5 min) of 4.20 g (6.18×10^{-2} mol) of sodium nitrite (dissolved in 10 mL of water) until the reaction showed a positive starch–iodide test. The warm reaction mixture was added to a refluxing mixture of 30 mL of water and 40 mL of concentrated H_2SO_4. This was refluxed for 30 min and then diluted with crushed ice, cooled to 0 °C, and filtered, and the crude 2 was recrystallized from 25% HCl to give 3.61 g of a white solid: yield 42%; mp 192–194 °C (lit.[36] mp 192–194 °C) NMR (Me_2SO-d_6 + D_2O + TSP) δ 8.10–6.95 (m, 3 H); IR (KBr) OH stretch 3470 cm^{-1}, carbonyl at 1685 cm^{-1}, nitro group at 1520 and 1340 cm^{-1}; MS, m/e (relative intensity) 183 (100, M^+), 137 (32.2, $M^+ - NO_2$).

Methyl 3-Hydroxy-5-nitrobenzoate (3). To 42 mL of a Fischer solution (40 mL of dry methanol, 2 mL of acetyl chloride) was added 0.35 g (1.91×10^{-3} mol) of 2. This was stirred at room temperature for 18 h before the solvent was removed in vacuo. The contents of the flask were loaded onto a column of 15 g of silica gel and developed with a 25:75 ethyl ether:hexane solvent system. The purified product was recrystallized from water to give 0.31 g of a white solid: yield 82.4%; mp 156–157 °C; NMR (acetone-d_6) δ 8.34–7.64 (m, 3 H), 3.92 (s, 3 H); IR (KBr) OH stretch at 3450 cm^{-1}, carbonyl at 1695 cm^{-1}, nitro group at 1530 and 1340 cm^{-1}; MS, m/e (relative intensity) 198 (4.08, M^+), 166 (100, $M^+ - OCH_3 + H$).

Methyl α-(*tert*-Butoxycarbonyl)-5-nitro-*m*-anisate (4). To 0.250 g (1.26×10^{-3} mol) of 3 was added 2.2 mL of dry THF under a nitrogen atmosphere. To this solution was added 0.295 g (1.51×10^{-3} mol) of *tert*-butyl bromoacetate followed by the addition of 0.073 g (1.5×10^{-3} mol) of NaH (50% oil dispersion). The reaction was refluxed for 8 h before the solvent was removed in vacuo and the crude product loaded onto a column of 15 g of silica gel. This was eluted with a 20:80 ethyl ether:hexane solvent system with 15-mL fractions being collected. This gave 0.317 g of 4 as a pale yellow oil: yield 81%; NMR ($CDCl_3$) δ 8.95 (s, 1 H), 7.85 (s, 2 H), 4.62 (s, 2 H), 3.95 (s, 3 H), 1.50 (s, 9 H); IR (CCl_4) carbonyls at 1755, 1735, and 1685 cm^{-1}, nitro group at 1540 and 1353 cm^{-1}; MS, m/e (relative intensity) 311 (1.16, M^+), 210 (11.69, $M^+ - CO_2C_4H_9$).

Methyl α-(*tert*-Butoxycarbonyl)-5-amino-*m*-anisate (5). To 175 mL of methanol in a Parr hydrogenation bottle was added 0.320 g (1.03×10^{-3} mol) of 4. The solution was deoxygenated with nitrogen and 40 mg of 10% Pd/C was added to the vessel. This was placed on a Parr hydrogenator at 35 psi for 18 h. The solution was filtered and the solvent removed in vacuo. The crude product was loaded onto a column of 15 g of silica gel. The column was eluted with 500 mL of a 20:80 ethyl ether:hexane solvent system with 30 fractions of approximately 15 mL each being collected. The solvent system was changed to 100% ethyl ether with fractions 31–34 containing the product as a white solid. After recrystallization from CCl_4, 0.248 g of 5 was obtained: yield 86%; mp 95–96 °C; NMR ($CDCl_3$) δ 7.01–6.78 (m, 2 H), 6.48–6.36 (m, 1 H), 4.46 (s, 2 H), 3.82 (s, 3 H), 1.48 (s, 9 H); IR (KBr) NH stretch at 3450 and 3360 cm^{-1}, carbonyls at 1740 and 1715 cm^{-1}; MS, m/e (relative intensity) 281 (11.28, M^+), 250 (1.98, $M^+ - OCH_3$).

Methyl α-(*tert*-Butoxycarbonyl)-5-benzamido-*m*-anisate (6). To a flask equipped with a nitrogen balloon apparatus and a septum was added 0.200 g (7.12×10^{-4} mol) of 5, 0.072 g (7.12×10^{-4} mol) of dry triethylamine, and 3 mL of dry THF. To this solution by means of a syringe was added 0.138 g (7.47×10^{-4} mol) of benzoyl bromide. The reaction was stirred for 1.5 h under a nitrogen atmosphere before the solvent was removed in vacuo and the crude product chromatographed on a column of 10 g of silica gel with a 50:50 hexane:ethyl ether solvent system. The collected product was recrystallized from ethyl ether to give 0.243 g of 6: 89% yield; mp 124–125 °C; NMR ($CDCl_3$) δ 8.25–8.00 (s, 1 H), 8.00–7.24 (m, 8 H), 4.55 (s, 2 H), 3.87 (s, 3 H), 1.50 (s, 9 H); IR ($CDCl_3$) NH stretch at 3490 and 3445 cm^{-1}, carbonyls at 1721, 1710, and 1667 cm^{-1}; MS, m/e (relative intensity) 385 (8.81, M^+).

5-Benzamido-α-carboxy-*m*-anisic Acid (7). To 0.200 g (5.19×10^{-4} mol) of 6 was added 6 mL of a 50% solution of water:methanol. After this became homogeneous, 0.0416 g (1.04×10^{-3} mol) of crushed sodium hydroxide was added. The reaction was heated at 60 °C for 2 h. The solvent was then removed in vacuo, and the residue was dissolved in 5 mL of water and extracted with ethyl ether (2 × 10 mL). The aqueous layer was acidified with 1 M $KHSO_4$. The resultant white precipitate was collected by filtration, dissolved in a large volume of boiling THF, and concentrated to several milliliters. Hexane was added dropwise until the solution became turbid. Upon standing this gave 0.137 g of 7: 84% yield; mp 291–293 °C; NMR (Me_2SO-d_6) δ 10.39 (s, 1 H), 8.22–6.24 (m, 8 H), 4.71 (s, 2 H); IR (KBr) NH stretch at 3290 cm^{-1}, carbonyls at 1725, 1705, and 1640 cm^{-1}; MS, m/e (relative intensity) 315 (1.14, M^+), 270 (1.70, $M^+ - CO_2H$). Anal. ($C_{16}H_{13}NO_6$) C, H, N.

Methyl α-(*tert*-Butoxycarbonyl)-5-propionamido-*m*-anisate (8). The title compound 8 was prepared from 5 and propionyl chloride in 88% yield as described for the preparation of 6, mp 78–80 °C. Purification was accomplished by column chromatography on silica gel using a 50:50 mixture of ethyl ether:hexane solvent system followed by recrystallization from ethyl ether:hexane at −70 °C: NMR ($CDCl_3$) δ 8.15 (s, 1 H), 7.67 (m, 2 H), 7.19 (m, 1 H), 4.52 (s, 2 H), 3.82 (s, 3 H), 2.83–2.00 (m, 2 H), 1.60–0.70 (m, 12 H); IR (CCl_4) NH stretch from 3365 to 3320 cm^{-1}, carbonyls at 1755, 1725, and 1700 cm^{-1}; MS, m/e (relative intensity) 337 (5.79, M^+). Anal. ($C_{17}H_{23}NO_6$) C, H, N.

5-Propionamido-α-carboxy-*m*-anisic Acid (9). The title compound 9 was prepared from 8 in 76% yield as described for the preparation of 7: mp 256–257 °C from methanol; NMR (Me_2SO-d_6) δ 7.82 (s, 1 H), 7.54 (s, 1 H), 7.10 (s, 1 H), 4.71 (s, 2 H), 2.37 (q, 2 H), 1.13 (t, 3 H); IR (KBr) OH stretch from 3700 to 2400 cm^{-1}, carbonyls at 1695, 1648, and 1610 cm^{-1}; MS, m/e (relative intensity) 267 (20.91, M^+), 211 (100, $M^+ - C_3H_4O$). Anal. ($C_{12}H_{13}NO_6$) C, H, N.

Methyl α-(*tert*-Butoxycarbonyl)-5-octanamido-*m*-anisate (10). The title compound 10 was prepared from 5 and octanoyl chloride in 94% yield as described for the preparation of 6. Purification was accomplished by column chromatography on silica gel using a 35:65 ethyl ether:hexane solvent system. 10: NMR ($CDCl_3$) δ 8.12 (s, 1 H), 8.04 (s, 2 H), 7.17 (m, 1 H), 4.49 (s, 2 H), 3.82 (s, 3 H), 2.25 (m, 2 H), 1.93–0.30 (m, 22 H); IR (CCl_4) NH stretch from 3380 to 3320 cm^{-1}, carbonyls at 1757, 1727, and 1700 cm^{-1}; MS, m/e (relative intensity) 407 (4.19, M^+), 281 (4.43, $M^+ - C_8H_{14}O$). Anal. ($C_{22}H_{33}NO_6$) C, H. N.

5-Octanamido-α-carboxy-*m*-anisic Acid (11). The title compound 11 was prepared from 10 in 76% yield as described for the preparation of 7: mp 201–202 °C from ethyl ether; NMR (Me_2SO-d_6) δ 9.96 (s, 1 H), 7.79 (s, 1 H), 7.50 (m, 1 H), 7.08 (m, 1 H), 4.17 (s, 2 H), 2.60–0.66 (m, 15 H plus Me_2SO protons); IR (KBr) NH stretch at 3322 cm^{-1}, OH stretch from 3210 to 2700 cm^{-1}, carbonyls at 1705, 1680, and 1635 cm^{-1}; MS, m/e (relative intensity) 337 (2.03, M^+), 210 (100, $M^+ - C_8H_{15}O$). Anal. ($C_{17}H_{23}NO_6$) C, H. N.

Methyl α-(*tert*-Butoxycarbonyl)-5-(*p*-chlorobenzamido)-*m*-anisate (12). The title compound 12 was prepared from 5 and *p*-chlorobenzoyl chloride in 90% yield as described for the preparation of 6, mp 129–130 °C. Purification was ac-

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(51) Mokotoff, M.; Logue, L. W. *J. Med. Chem.* 1981, *24*, 554–559.

complished by column chromatography on silica gel using a 40:60 ethyl ether:hexane solvent system followed by recrystallization from ethyl ether: NMR ($CDCl_3$) δ 10.52 (s, 1 H), 7.71–7.12 (m, 7 H), 4.57 (s, 2 H), 3.88 (s, 3 H), 1.50 (s, 9 H); IR (KBr) NH stretch 3315 cm^{-1}, carbonyls at 1720 (2 carbonyls) and 1670 cm^{-1}; MS, m/e (relative intensity) 421 (10.72, M^+), 419 (31.78, M^+), 390 (1.33, $M^+ - OCH_3$), 388 (3.11, $M^+ - OCH_3$). Anal. ($C_{21}H_{22}NO_6Cl$) C, H, N, Cl.

5-(*p*-Chlorobenzamido)-α-carboxy-*m*-anisic Acid (13). The title compound 13 was prepared from 12 in 86% yield as described for the preparation of 7: mp 269–270 °C after recrystallization from acetone; NMR (acetone-d_6) δ 10.54 (s, 1 H), 8.02 (m, 3 H), 7.48 (m, 4 H), 4.80 (s, 2 H); IR (KBr) OH stretch from 3600 to 2800 cm^{-1}, carbonyls at 1730, 1710, and 1639 cm^{-1}; MS, m/e (relative intensity) 351 (3.84, M^+), 349 (9.38, M^+), 306 (0.47, $M^+ - COOH$), 304 (1.49, $M^+ - COOH$). Anal. ($C_{16}H_{12}NO_6Cl$) C, H, N, Cl.

Methyl α-(*tert*-Butoxycarbonyl)-5-(3,4-dichlorobenzamido)-*m*-anisate (14). The title compound 14 was prepared from 5 and 3,4-dichlorobenzoyl chloride in 87% yield as described for the preparation of 6, mp 155–157 °C. Purification consisted of column chromatography on silica gel using a 33:67 ethyl ether:hexane solvent system followed by recrystallization from ethyl ether: NMR (acetone-d_6) δ 10.49 (s, 1 H), 8.00–7.70 (m, 5 H), 7.35 (m, 1 H), 4.71 (s, 2 H), 3.91 (s, 3 H), 1.50 (s, 9 H); IR (KBr) NH stretch at 3395 cm^{-1}, carbonyls at 1710 (2 carbonyls) and 1675 cm^{-1}; MS, m/e (relative intensity) 401 (12.85, $M^+ - C_4H_8$), 399 (58.77, $M^+ - C_4H_8$), 397 (100, $M^+ - C_4H_8$). Anal. ($C_{21}H_{21}NO_6Cl_2$) C, H, N, Cl.

5-(3,4-Dichlorobenzamido)-α-carboxy-*m*-anisic Acid (15). The title compound 15 was prepared from 14 in 91% yield as described for the preparation of 7: mp 253–254 °C after recrystallization from ethyl acetate:hexane; NMR (Me_2SO-d_6) δ 8.20–7.18 (m, 6 H), 4.70 (s, 2 H); IR (KBr) OH stretch from 3700 to 2550 cm^{-1}, carbonyls at 1730, 1710, and 1640 cm^{-1}; MS, m/e (relative intensity) 387 (9.49, M^+), 385 (61.5, M^+), 383 (100, M^+), 342 (1.05, $M^+ - COOH$), 340 (2.75, $M^+ - COOH$), 338 (3.22, $M^+ - COOH$). Anal. ($C_{16}H_{11}NO_6Cl_2$) C, H, N, Cl.

Methyl α-(*tert*-Butoxycarbonyl)-5-(*p*-bromobenzamido)-*m*-anisate (16). The title compound 16 was prepared from 5 and *p*-bromobenzoyl chloride in 93.2% yield as described for the preparation of 6, mp 128–129 °C. Purification was accomplished by column chromatography on silica gel using a 30:70 ethyl ether:hexane solvent system followed by recrystallization from ethyl ether:hexane: NMR ($CDCl_3$) δ 8.49 (s, 1 H), 7.81–7.25 (m, 7 H), 4.52 (s, 2 H), 3.85 (s, 3 H), 1.52 (s, 9 H); IR (KBr) NH stretch at 3330 cm^{-1}, carbonyls at 1720 (2 carbonyls) and 1675 cm^{-1}; MS, m/e (relative intensity) 409 (2.89, $M^+ - C_4H_8$), 407 (2.54, $M^+ - C_4H_8$). Anal. ($C_{21}H_{22}NO_6Br$) C, H. N, Br.

5-(*p*-Bromobenzamido)-α-carboxy-*m*-anisic Acid (17). The title compound 17 was prepared from 16 in 78% yield as described for the preparation of 7: mp 281–283 °C after recrystallization from methanol; NMR (Me_2SO-d_6) δ 10.46 (s, 1 H), 8.01–7.18 (m, 7 H), 4.71 (s, 2 H); IR (KBr) NH stretch at 3300 cm^{-1}, carbonyls at 1732, 1710, and 1639 cm^{-1}; MS, m/e (relative intensity) 395 (100, M^+), 393 (79.28, M^+), 350 (6.36, $M^+ - COOH$), 348 (7.50, $M^+ - COOH$). Anal. ($C_{16}H_{12}NO_6Br \cdot 0.5H_2O$) C, H, N, Br.

2. Proline Derivatives. 1-Hydrocinnamoyl-L-proline Benzyl Ester (18). To 1.60 g (6.62×10^{-3} mol) of L-proline benzyl ester hydrochloride were added 1.00 g (6.67×10^{-3} mol) of hydrocinnamic acid, 0.448 g (3.67×10^{-3} mol) of 4-(dimethylamino)pyridine (DMAP), and 30 mL of methylene chloride. To this was added 1.51 g (7.33×10^{-3} mol) of DCC dissolved in 10 mL of methylene chloride. After 3 h the DCU was filtered from the reaction and the filtrate was extracted with 10% $NaHCO_3$ (2×20 mL), 1 M $KHSO_4$ (3×40 mL), and water (3×25 mL), dried (Na_2SO_4), and evaporated in vacuo. Chromatography on 15 g of silica gel using 50:50 ethyl acetate:hexane gave 1.98 g of 18 as a clear oil in 89% yield: NMR ($CDCl_3$) δ 7.31 (s, 5 H), 7.20 (s, 5 H), 5.15 (s, 2 H), 4.40 (m, 1 H), 3.65 (m, 2 H), 2.70 (m, 4 H), 1.95 (m, 4 H); IR ($CHCl_3$) carbonyls at 1735 and 1635 cm^{-1}; MS, m/e (relative intensity) 337 (2.11, M^+), 246 (71.22, $M^+ - CH_2C_6H_5$), 202 (11.55, $M^+ - CO_2CH_2C_6H_5$); $[\alpha]^{25}_D$ – 48.5° (c 1.0, Me_2CO).

1-Hydrocinnamoyl-L-proline (19). To a 500-mL Parr bottle were added 9.40 g (2.79×10^{-2} mol) of compound 18, 250 mL of methanol, and 800 mg of 10% Pd/C. This was placed on a Parr hydrogenator at 30 psi for 3 h. The catalyst was filtered from the reaction mixture with Celite and the solvent removed in vacuo to give 6.90 g of a clear oil. This crystallized on standing, which after recrystallization from ethyl ether gave 5.40 g of 19: yield 78%; mp 105–106 °C; NMR ($CDCl_3$) δ 7.25 (s, 5 H), 4.45 (m, 1 H), 3.31 (m, 2 H), 2.75 (m, 4 H), 1.95 (m, 4 H); IR ($CHCl_3$) OH stretch from 3300 to 2600 cm^{-1}, carbonyls at 1745 and 1635 cm^{-1}; MS, m/e (relative intensity) 247 (2.08, M^+), 229 (0.85, $M^+ - H_2O$), 203 (7.18, $M^+ - CO_2$); $[\alpha]^{23}_D$ –13.2° (c 1.0, MeOH). Anal. ($C_{14}H_{17}NO_3$) C, H, N.

1-Hydrocinnamoyl-L-proline 2-Ester with Benzyl Salicylate (20). To 6.90 g (2.79×10^{-2} mol) of 19 were added 6.36 g (2.79×10^{-2} mol) of benzyl salicylate, 6.81 g (5.58×10^{-2} mol) of DMAP, and 150 mL of methylene chloride. In portions 6.91 g (3.35×10^{-2} mol) of DCC, dissolved in 20 mL of methylene chloride, was added. The reaction was stirred overnight at room temperature before the DCU was filtered from the reaction. Ethyl ether (150 mL) was added followed by extraction with 10% $NaHCO_3$ (25 mL), 1 M $KHSO_4$ (2×25 mL), and water (3×25 mL), dried (Na_2SO_4), and concentrated in vacuo. Column chromatography on 30 g of silica gel using 75:25 hexane:ethyl acetate as eluant gave a white solid, which after recrystallization from ethyl ether gave 8.50 g of 20: mp 111–112 °C; yield 67%; NMR ($CDCl_3$) δ 7.95 (d, 1 H), 7.81 (d, 1 H), 7.41 (s, 5 H), 7.22 (s, 5 H), 6.90 (m, 2 H), 5.39 (s, 2 H), 4.50 (m, 1 H), 3.39 (m, 2 H), 2.75 (m, 4 H), 1.85 (m, 4 H); IR ($CHCl_3$) carbonyls at 1765, 1720, and 1640 cm^{-1}; MS, m/e (relative intensity) 457 (0.26, M^+), 230 (7.98, M^+ – benzyl salicylate), 202 (16.85, M^+ – benzyl salicylate, –CO); $[\alpha]^{25}_D$ –64.2° (c 1.0, Me_2CO). Anal. ($C_{28}H_{27}NO_5$) C, H. N.

1-Hydrocinnamoyl-L-proline 2-Ester with Salicylic Acid (21). Deblocking of 20 was accomplished as outlined for 19 to give 21, which was recrystallized from ethyl ether: mp 154–156 °C; yield 55%; NMR (acetone-d_6) δ 8.20 (m, 2 H), 7.65 (m, 2 H), 7.31 (s, 5 H), 4.58 (m, 1 H), 3.40 (m, 2 H), 2.80 (m, 4 H), 2.00 (m, 4 H); IR ($CHCl_3$) OH stretch from 3200 to 2700 cm^{-1}, carbonyls at 1765, 1700, and 1640 cm^{-1}; MS, m/e (relative intensity) 367 (0.08, M^+), 230 (2.31, M^+ – salicylate), 202 (7.98, M^+ – salicylate, –CO, +H); $[\alpha]^{25}_D$ –74.3° (c 1.0, Me_2CO). Anal. ($C_{21}H_{21}NO_5$) C, H, N.

1-(3,3-Dimethylbutyryl)-L-proline Benzyl Ester (22). To 8.00 g (3.31×10^{-2} mol) of L-proline benzyl ester hydrochloride was added 3.86 g (3.33×10^{-2} mol) of 3,3-dimethylbutanoic acid, 0.72 g (5.89×10^{-3} mol) of DMAP, and 200 mL of methylene chloride. To this in portions was added 8.84 g (4.29×10^{-2} mol) of DCC dissolved in 20 mL of methylene chloride. After stirring overnight at room temperature, the reaction mixture was filtered, extracted with 10% $NaHCO_3$ (2×25 mL), 1 M $NaHSO_4$ (2×25 mL), and water (3×25 mL), dried (Na_2SO_4), and evaporated in vacuo. Chromatography on 15 g of silica gel using 30:70 ethyl acetate:hexane as eluant gave 9.24 g of 22 as a clear oil: yield 92%; NMR ($CDCl_3$) δ 7.31 (s, 5 H), 5.12 (s, 2 H), 4.52 (m, 1 H), 3.55 (m, 2 H), 2.19 (s, 2 H), 1.95 (m, 4 H), 1.04 (s, 9 H); IR ($CHCl_3$) carbonyls at 1738 and 1632 cm^{-1}; MS, m/e (relative intensity) 303 (1.58, M^+), 212 (0.78, $M^+ - CH_2C_6H_5$), 168 (10.65, $M^+ - CO_2CH_2C_6H_5$); $[\alpha]^{23}_D$ –49.6° (c 1.0, Me_2CO).

1-(3,3-Dimethylbutyryl)-L-proline (23). Deblocking of 22 was accomplished as outlined for 19 to give 23, which was recrystallized from ethyl ether: mp 127–129 °C; yield 63%; NMR ($CDCl_3$) δ 4.54 (m, 1 H), 3.51 (m, 2 H), 2.22 (s, 2 H), 2.01 (m, 4 H), 1.04 (s, 9 H); IR ($CHCl_3$) OH stretch from 3500 to 2500 cm^{-1}, carbonyls at 1745 and 1650 cm^{-1}; MS, m/e (relative intensity) 213 (3.10, M^+), 169 (5.28, $M^+ - CO_2$), 114 (9.27, $M^+ - C_6H_{11}O$)); $[\alpha]^{23}_D$ –67.2° (c 1.0, Me_2CO). Anal. ($C_{11}H_{19}NO_3$) C, H. N.

1-(3,3-Dimethylbutyryl)-L-proline 2-Ester with Benzyl Salicylate (24). To 0.90 g (4.22×10^{-3} mol) of compound 23 were added 0.96 g (4.21×10^{-3} mol) of benzyl salicylate, 0.59 g (4.84×10^{-3} mol) of DMAP, and 25 mL of methylene chloride. This was stirred at room temperature overnight and then worked up as described for 20. The resulting solid when recrystallized from ethyl ether gave 1.38 g of 24 as a white solid: mp 93–95 °C; yield 77%; NMR (acetone-d_6) δ 8.01 (m, 1 H), 7.55 (m, 8 H), 5.35 (s, 2 H), 4.61 (m, 1 H), 3.60 (m, 2 H), 2.22 (s, 2 H), 2.15 (m, 4 H), 1.05 (s, 9 H); IR ($CHCl_3$) carbonyls at 1765, 1720, and 1630 cm^{-1}; MS, m/e (relative intensity) 197 (0.40, M^+ – benzyl salicylate + H), 91 (100.00, $CH_2C_6H_5$); $[\alpha]^{26}_D$ –73.2° (c 1.0, Me_2CO). Anal. ($C_{25}H_{29}NO_5$) C, H. N.

1-(3,3-Dimethylbutyryl)-L-proline 2-Ester with Salicylic Acid (25). Deblocking of **24** was accomplished as outlined for **19** to give **25**, which was recrystallized from ethyl ether: mp 147–149 °C; yield 61%; NMR (acetone-d_6) δ 7.05 (m, 2 H), 6.65 (m, 2 H), 4.50 (m, 1 H), 3.49 (m, 2 H), 2.19 (m, 2 H), 2.18 (s, 4 H), 1.04 (s, 9 H); IR ($CHCl_3$) carbonyls at 1740 and 1630 cm^{-1}; MS, m/e (relative intensity) 333 (0.04, M^+), 234 (0.07, $M^+ - C_6H_{11}O$), 196 (9.74, M^+ – salicylate); $[\alpha]^{23}_D$ –89.7° (*c* 1.0, Me_2CO). Anal. ($C_{18}H_{23}NO_5$) C, H. N.

1-Benzoyl-L-proline Benzyl Ester (26). To 2.34 g (9.68×10^{-3} mol) of L-proline benzyl ester hydrochloride were added 0.30 g (2.46×10^{-3} mol) of DMAP, 1.19 g (9.75×10^{-3} mol) of benzoic acid, and 20 mL of methylene chloride. This was mixed well before 2.42 g (1.17×10^{-2} mol) of DCC dissolved in 5 mL of methylene chloride was added to the reaction vessel. The mixture was stirred at room temperature overnight and then worked up as described above for **18**. The resulting residue was chromatographed on 25 g of silica gel with a 75:25 hexane:ethyl acetate as eluant to give 2.17 g of **26** (73% yield) as a clear oil: NMR ($CDCl_3$) δ 7.34 (s, 5 H), 7.32 (s, 5 H), 5.20 (s, 2 H), 4.70 (m, 1 H), 3.61 (m, 2 H), 2.01 (m, 4 H); IR ($CHCl_3$) carbonyls at 1730 and 1635 cm^{-1}; MS, m/e (relative intensity) 309 (43.73, M^+), 174 (93.50, $M^+ - CO_2CH_2C_6H_5$), 70 (68.43, C_4H_8N); $[\alpha]^{19}_D$ – 78.5° (*c* 1.0, MeOH).

1-Benzoyl-L-proline (27). Deblocking of **26** was accomplished as outlined for **19** to give **27** as a clear oil: yield 83%; NMR (acetone-d_6) δ 8.71 (br, 1 H), 7.45 (s, 5 H), 4.59 (m, 1 H), 3.60 (m, 2 H), 2.00 (m, 4 H); IR ($CHCl_3$) OH stretch from 3500 to 2400 cm^{-1}, carbonyls at 1730 and 1615 cm^{-1}; MS, m/e (relative intensity) 219 (23.19, M^+), 174 (91.60, $M^+ - CO_2H$), 105 (98.09, COC_6H_5); $[\alpha]^{19}_D$ –26.6° (*c* 1.0, MeOH).

1-Benzoyl-L-proline 2-Ester with Benzyl Salicylate (28). To 1.28 g (5.84×10^{-3} mol) of compound **27** was added 1.34 g (5.88×10^{-3} mol) of benzyl salicylate, 0.18 g (1.48×10^{-3} mol) of DMAP, and 20 mL of methylene chloride. This was stirred well before 1.45 g (7.04×10^{-3} mol) of DCC dissolved in 10 mL of methylene chloride was added to the reaction. After stirring overnight at room temperature, the mixture was worked up as described for **20**. The resulting residue was chromatographed on 20 g of silica gel with 10:90 ethyl acetate:hexane to give 1.70 g of **28** as a clear oil: yield 68%; NMR (acetone-d_6) δ 8.21–6.70 (m, 14 H), 5.38 (s, 2 H), 4.85 (m, 1 H), 3.60 (m, 2 H), 2.69–1.50 (m, 4 H); IR ($CHCl_3$) carbonyls at 1760, 1720, and 1600 cm^{-1}; MS, m/e (relative intensity) 429 (0.10, M^+), 202 (26.33, M^+ – benzyl salicylate), 174 (76.69, $M^+ - CO$ – benzyl salicylate), 91 (51.82, $CH_2C_6H_5$), 70 (14.68, C_4H_8N); $[\alpha]^{19}_D$ –73.5° (*c* 1.0, MeOH).

1-Benzoyl-L-proline 2-Ester with Salicylic Acid (29). Deblocking of **28** was accomplished as outlined for **19** to give **29**, which was recrystallized from ethyl ether: yield 84% as a white solid; mp 150–152 °C; NMR (acetone-d_6) δ 8.01–7.55 (m, 9 H), 4.49 (t, 1 H), 3.65 (m, 2 H), 2.40 (m, 4 H); IR ($CHCl_3$) OH stretch from 3300 to 2600 cm^{-1}, carbonyls at 1760, 1700, and 1620 cm^{-1}; MS, m/e (relative intensity) 202 (0.01, M^+ – salicylate), 175 (0.73, $M^+ - CO$, – salicylate + H); $[\alpha]^{19}_D$ –102.9° (*c* 1.0, Me_2CO). Anal. ($C_{19}H_{17}NO_5$) C, H. N.

1-(2-Phenylacetyl)-L-proline Benzyl Ester (30). To 4.68 g (1.94×10^{-2} mol) of L-proline benzyl ester hydrochloride were added 2.64 g (1.94×10^{-2} mol) of phenyl acetic acid, 0.60 g (4.92×10^{-3} mol) of DMAP, and 40 mL of methylene chloride. After this became homogeneous, 4.84 g (2.35×10^{-2} mol) of DCC dissolved in 10 mL of methylene chloride was added to the reaction. This was stirred overnight and then worked up as described above for **18**. The resulting residue was chromatographed on 15 g of silica gel with 30:70 ethyl acetate:hexane as eluant to give 4.56 g of **30** as a clear oil: yield 73%; NMR ($CDCl_3$) δ 7.21 (s, 5 H), 7.15 (s, 5 H), 5.01 (s, 2 H), 4.40 (m, 1 H), 3.55 (s, 2 H), 3.40 (m, 2 H), 1.85 (m, 4 H); IR ($CHCl_3$) carbonyls at 1740 and 1640 cm^{-1}; MS, m/e (relative intensity) 323 (38.69, M^+), 188 (72.88, $M^+ - CO_2CH_2C_6H_5$), 70 (95.42, C_4H_8N); $[\alpha]^{19}_D$ –60.3° (*c* 1.0, MeOH).

1-(2-Phenylacetyl)-L-proline (31). Deblocking of **30** was accomplished as outlined for **19** to give **31**, which was recrystallized from ethyl ether: mp 150–152 °C; yield 98%; NMR (acetone-d_6) δ 7.29 (s, 5 H), 4.50 (m, 1 H), 3.78 (s, 2 H), 3.62 (m, 2 H), 2.05 (m, 4 H); IR (KBr) carbonyls at 1720 and 1635 cm^{-1}; MS, m/e (relative intensity) 216 (0.24, $M^+ - OH$), 189 (2.08, $M^+ - CO_2$), 119 (100.00, C_8H_7O), 91 (82.24, C_7H_7), 70 (14.38, C_4H_8N); $[\alpha]^{19}_D$ –68.9° (*c* 1.0, MeOH). Anal. ($C_{13}H_{15}NO_3$) C, H. N.

1-(2-Phenylacetyl)-L-proline 2-Ester with Benzyl Salicylate (32). To 2.56 g (1.10×10^{-2} mol) of compound **31** were added 2.51 g (1.10×10^{-2} mol) of benzyl salicylate, 0.34 g (2.78×10^{-3} mol) of DMAP, and 40 mL of methylene chloride. After the reaction became homogeneous, 6.35 g (3.08×10^{-2} mol) of DCC dissolved in 10 mL of methylene chloride was added in portions. After stirring overnight at room temperature, the mixture was worked up as described above for **20**. The resulting residue was chromatographed on 20 g of silica gel with 50:50 hexane:ethyl acetate as eluant to give 4.13 g of a clear oil: yield 85%; NMR (acetone-d_6) δ 8.00–6.98 (m, 14 H), 5.28 (s, 2 H), 4.65 (m, 1 H), 3.70 (s, 2 H), 3.60 (m, 2 H), 2.01 (m, 4 H); IR ($CHCl_3$) carbonyls at 1730 and 1635 cm^{-1}; MS, m/e (relative intensity) 443 (0.19, M^+), 216 (30.89, M^+ – benzyl salicylate), 188 (40.43, M^+ – benzyl salicylate, – CO), 91 (100.00, C_7H_7), 70 (90.43, C_4H_8N); $[\alpha]^{19}_D$ –49.5° (*c* 1.0, MeOH).

1-(2-Phenylacetyl)-L-proline 2-Ester with Salicylic Acid (33). Deblocking of **32** was accomplished as outlined for **19** to give **33**, which was recrystallized from ethyl ether: mp 158–160 °C; yield 53%; NMR (acetone-d_6) δ 8.00 (m, 2 H), 7.65–7.11 (m, 7 H), 4.61 (m, 1 H), 3.72 (s, 2 H), 3.60 (m, 2 H), 2.01 (m, 4 H); IR ($CHCl_3$) carbonyls at 1760, 1695, and 1630 cm^{-1}; MS, m/e (relative intensity) 353 (0.05, $M^+ + H$), 188 (13.11, M^+ – salicylate, – CO), 91 (61.40, C_7H_7), 70 (100.00, C_4H_8N); $[\alpha]^{19}_D$ –91.6° (*c* 1.0, MeOH). Anal. ($C_{20}H_{19}NO_5$) C, H. N.

1-(2-Phenoxyacetyl)-L-proline Benzyl Ester (34). To 4.68 g (1.94×10^{-2} mol) of L-proline benzyl ester hydrochloride were added 2.97 g (1.95×10^{-2} mol) of phenoxyacetic acid, 0.60 g (4.92×10^{-3} mol) of DMAP, and 40 mL of methylene chloride. After this became homogeneous, 5.23 g (2.54×10^{-2} mol) of DCC dissolved in 15 mL of methylene chloride was added to the reaction. After 3 h the reaction was worked up as per the preparation of **18**. The residue was chromatographed on 20 g of silica gel with a solvent system of 20:80 ethyl acetate:hexane to give 4.53 g of a clear oil: yield 69%; NMR ($CDCl_3$) δ 7.31 (s, 5 H), 6.95 (m, 5 H), 5.14, 5.01 geometrical isomers about the amide bond (s, 2 H), 4.65, 4.52 geometrical isomers about the amide bond (s, 2 H), 4.51 (m, 1 H), 3.61 (m, 2 H), 1.95 (m, 4 H); IR ($CHCl_3$) carbonyls at 1740 and 1650 cm^{-1}; MS, m/e (relative intensity) 339 (34.83, M^+), 204 (95.38, $M^+ - CO_2CH_2C_6H_5$), 107 (96.92, C_7H_7O), 91 (100.00, C_7H_7), 70 (39.36, C_4H_8N); $[\alpha]^{21}_D$ –56.1° (*c* 1.0, MeOH).

1-(2-Phenoxyacetyl)-L-proline (35). Deblocking of **34** was accomplished as outlined for **19** to give **35** as a clear oil: yield 91%; NMR (acetone-d_6) δ 7.11 (m, 5 H), 4.76 (s, 2 H), 4.49 (m, 1 H), 3.60 (m, 2 H), 2.02 (m, 4 H); IR ($CHCl_3$) OH stretch from 3500 to 2800 cm^{-1}, carbonyls at 1720 and 1640 cm^{-1}; MS, m/e (relative intensity) 249 (27.00, M^+), 204 (25.07, $M^+ - CO_2H$), 70 (39.36, C_4H_8N); $[\alpha]^{21}_D$ –29.6° (*c* 1.0, MeOH).

1-(2-Phenoxyacetyl)-L-proline 2-Ester with Benzyl Salicylate (36). To 4.55 g (1.83×10^{-2} mol) of compound **35** were added 0.22 g (1.80×10^{-3} mol) of DMAP, 4.16 g (1.82×10^{-3} mol) of benzyl salicylate, and 30 mL of methylene chloride. After this became homogeneous, 4.53 g (2.20×10^{-2} mol) of DCC dissolved in 10 mL of methylene chloride was added to the reaction. After stirring several hours at room temperature, the mixture was filtered and concentrated to a residue, which was then chromatographed on 15 g of silica gel with 75:25 hexane:ethyl acetate to elute the product. This gave 4.25 g of **36** as a clear oil: yield 51%; NMR ($CDCl_3$) δ 8.01 (m, 2 H), 7.35 (s, 5 H), 7.31–6.71 (m, 7 H), 5.27 (s, 2 H), 4.69 (m, 1 H), 4.66 (s, 2 H), 3.65 (m, 2 H), 2.02 (m, 4 H); IR ($CHCl_3$) carbonyls at 1760, 1715, and 1645 cm^{-1}; MS, m/e (relative intensity) 459 (2.09, M^+), 232 (36.44, M^+ – benzyl salicylate), 204 (94.14, M^+ – benzyl salicylate, – CO), 91 (100.00, C_7H_7); $[\alpha]^{21}_D$ –46.3° (*c* 1.0, MeOH).

1-(2-Phenoxyacetyl)-L-proline 2-Ester with Salicylic Acid (37). Deblocking of **36** was accomplished as outlined for **19**, affording **37**, which was recrystallized from ethyl ether: mp 168–170 °C; yield 89%; NMR (acetone-d_6) δ 8.00 (m, 2 H), 7.35 (m, 7 H), 4.61 (s, 2 H), 4.41 (m, 1 H), 3.55 (m, 2 H), 1.85 (m, 4 H); IR ($CHCl_3$) carbonyls at 1750, 1695, and 1600 cm^{-1}; MS, m/e (relative intensity) 369 (0.03, M^+), 232 (0.56, M^+ – salicylate), 204 (3.84, M^+ – salicylate, – CO), 70 (100.00, C_4H_8N); $[\alpha]^{21}_D$ –82.6° (*c* 1.0, MeOH). Anal. ($C_{20}H_{19}NO_6$) C, H, N.

1-(2,2-Dimethylpropionyl)-L-proline Benzyl Ester (38). To 4.70 g (1.94×10^{-2} mol) of L-proline benzyl ester hydrochloride were added 2.00 g (1.96×10^{-2} mol) of trimethylacetic acid, 0.24

g (1.97×10^{-3} mol) of DMAP, and 30 mL of methylene chloride. To this was added 4.85 g (2.35×10^{-2} mol) of DCC dissolved in 10 mL of methylene chloride. This was stirred at room temperature overnight and then worked up as per the procedure for **18**. The crude product was chromatographed on 15 g of silica gel to give 4.37 g of **38** as a clear oil: yield 78%; NMR (acetone-d_6) δ 7.31 (s, 5 H), 5.19 (s, 2 H), 4.61 (m, 1 H), 3.76 (m, 2 H), 2.01 (m, 4 H), 1.28 (s, 9 H); IR ($CHCl_3$) carbonyls at 1735 and 1650 cm^{-1}; MS, *m/e* (relative intensity) 289 (9.64, M^+), 154 (100.00, $M^+ - CO_2CH_2C_6H_5$), 91 (75.03, C_7H_7), 70 (44.77, C_4H_8N); $[\alpha]^{21}_D$ -58.7° (*c* 1.0, MeOH).

1-(2,2-Dimethylpropionyl)-L-proline (39). Deblocking of **38** was accomplished as outlined for **19** to give **39**, which was recrystallized from ethyl ether: mp 135–137 °C; yield 55%; NMR (acetone-d_6) δ 4.45 (m, 1 H), 3.70 (m, 2 H), 2.01 (m, 4 H), 1.21 (s, 9 H); IR ($CHCl_3$) OH stretch from 3200 to 2800 cm^{-1}, carbonyls at 1740 and 1610 cm^{-1}; MS, *m/e* (relative intensity) 199 (2.38, M^+), 155 (29.22, $M^+ - CO_2$), 154 (37.62, $M^+ - CO_2H$), 114 (37.79, $M^+ - C_5H_9O$), 70 (100.00, C_4H_8N); $[\alpha]^{21}_D$ -1.3° (*c* 1.0, MeOH). Anal. ($C_{10}H_{17}NO_3$) C, H. N.

1-(2,2-Dimethylpropionyl)-L-proline 2-Ester with Benzyl Salicylate (40). To 2.03 g (1.02×10^{-2} mol) of compound **39** were added 2.32 g (1.02×10^{-2} mol) of benzyl salicylate, 0.13 g (1.07×10^{-3} mol) of DMAP, and 30 mL of methylene chloride. This was stirred several minutes before 2.53 g (1.23×10^{-2} mol) of DCC dissolved in 10 mL of methylene chloride was added portionwise to the reaction. After 18 h the mixture was filtered and concentrated in vacuo and the crude product chromatographed on 15 g of silica gel developed with 75:25 hexane:ethyl acetate to give 2.67 g of **40** as a clear oil: yield 64%; NMR (acetone-d_6) δ 8.01 (m, 2 H), 7.10–7.02 (m, 7 H), 5.38 (s, 2 H), 4.60 (m, 1 H), 3.69 (m, 2 H), 2.00 (m, 4 H), 1.21 (s, 9 H); IR ($CHCl_3$) carbonyls at 1760, 1720, and 1610 cm^{-1}; MS, *m/e* (relative intensity) 409 (0.14, M^+), 182 (87.90, M^+ – benzyl salicylate), 154 (100.00, M^+ – benzyl salicylate, – CO), 91 (65.40, C_7H_7), 70 (28.76, C_4H_8N); $[\alpha]^{21}_D$ -1.0° (*c* 1.0, MeOH).

1-(2,2-Dimethylpropionyl)-L-proline 2-Ester with Salicylic Acid (41). Deblocking of **40** was accomplished as outlined for **19** to give **41**, which was recrystallized from methylene chloride:diethyl ether, giving 1.00 g; mp 168–169 °C; yield 51%; NMR (acetone-d_6) δ 8.10–7.00 (m, 4 H), 4.48 (m, 1 H), 3.65 (m, 2 H), 2.08 (m, 4 H), 1.11 (s, 9 H); IR ($CHCl_3$) carbonyls at 1760, 1695, and 1605 cm^{-1}; MS, *m/e* (relative intensity) 319 (0.13, M^+), 182 (87.90, M^+ – salicylate), 154 (5.37, M^+ – salicylate, – CO), 120 (21.20, $C_7H_4O_2$), 70 (100.00, C_5H_8N); $[\alpha]^{21}_D$ -1.1° (*c* 1.0, MeOH). Anal. ($C_{17}H_{21}NO_5$) C, H. N.

4-Hydroxy-4-phenylbutanoic Acid (42). To 0.20 g (1.24×10^{-3} mol) of 4-phenyl-4-butyrolactone was added 3 mL of 2 M NaOH. After stirring overnight at room temperature, the reaction mixture was cooled to ice bath temperature, acidified (HCl), extracted (3 × 25 mL) with ice-cold ethyl ether, and dried (Na_2SO_4) and the solvent removed in vacuo to give a viscous oil, wt 0.17 g, yield 76%. Upon standing the oil crystallized, mp 52–54 °C. This was silanized (see below) without further purification because of its propensity toward relactonization. **42**: NMR ($CDCl_3$) δ 7.30 (s, 5 H), 4.69 (t, 1 H), 2.40 (m, 2 H), 1.99 (m, 2 H); IR ($CHCl_3$) OH stretch from 3600 to 2500 cm^{-1}, carbonyl at 1710 cm^{-1}.

4-[(*tert*-Butyldimethylsilyl)oxy]-4-phenylbutanoic Acid (43). To an oven-dried flask equipped with a rubber septum and a nitrogen balloon apparatus was placed 0.20 g (1.11×10^{-3} mol) of **42**. The flask was placed under a nitrogen atmosphere, and to the vessel were added 3 mL of dry THF (distilled from Na/benzophenone), 0.45 g (2.99×10^{-3} mol) *tert*-butyldimethylsilyl chloride, 0.50 g (7.35×10^{-3} mol) of imidazole, and 1 mL of dry DMF (distilled from CaH_2). The reaction was stirred at room temperature for 2 days and filtered and the solvent removed in vacuo to give a clear oil, which was chromatographed on 10 g of silica gel with 80:20 hexane:ethyl acetate as eluant. This gave 0.20 g of a clear oil: yield 61%; NMR ($CDCl_3$) δ 7.31 (s, 5 H), 4.79 (t, 1 H), 2.51 (m, 2 H), 2.20 (m, 2 H), 1.12 (s, 9 H), 0.20 (s, 6 H); IR ($CHCl_3$) OH stretch from 3500 to 2500 cm^{-1}, carbonyl at 1720 cm^{-1}; MS, *m/e* (relative intensity) 294 (1.23, M^+), 277 (11.36, M^+ – OH).

1-[4-[(*tert*-Butyldimethylsilyl)oxy]-4-phenylbutyryl]-L-proline 2-Benzyl Ester (44). To a 100-mL oven-dried flask equipped with a rubber septum and a nitrogen balloon apparatus were placed 0.20 g (6.80×10^{-4} mol) of **43**, 0.12 g (9.84×10^{-4} mol) of DMAP, 0.16 g (6.77×10^{-4} mol) of L-proline benzyl ester hydrochloride, and 15 mL of methylene chloride. This was mixed well before 0.17 g (8.25×10^{-4} mol) of DCC disolved in 5 mL of methylene chloride was added. This was stirred overnight at room temperature before the DCU was filtered from the reaction vessel. The solvent was removed in vacuo to give a clear oil, which after chromatography on 10 g of silica gel with 80:20 hexane:ethyl acetate gave 0.27 g of a clear oil: yield 82%; NMR (acetone-d_6) δ 7.29 (s, 5 H), 7.30 (s, 5 H), 5.25 (s, 2 H), 5.00 (t, 1 H), 4.52 (m, 1 H), 3.62 (m, 2 H), 2.60–1.81 (m, 8 H), 1.06 (s, 9 H), 0.18 (s, 3 H), 0.17 (s, 3 H); IR ($CHCl_3$) carbonyls at 1780 and 1670 cm^{-1}; MS, *m/e* (relative intensity) 481 (12.31, M^+), 346 (23.62, $M^+ - CO_2CH_2C_6H_5$); $[\alpha]^{26}_D$ -30.5° (*c* 1.0, Me_2CO).

1-[4-[(*tert*-Butyldimethylsilyl)oxy]-4-phenylbutyryl]-L-proline (45). Deblocking of **44** was accomplished as outlined for **19** to give **45** as a clear oil: yield 69%; NMR (acetone-d_6) δ 7.05 (s, 5 H), 4.81 (t, 1 H), 4.48 (t, 1 H), 3.50 (m, 2 H), 2.70–1.81 (m, 8 H), 1.01 (s, 9 H), 0.21 (s, 3 H), 0.20 (s, 3 H); IR ($CHCl_3$) OH stretch from 3500 to 2400 cm^{-1}, carbonyls at 1720 and 1630 cm^{-1}; MS, *m/e* (relative intensity) 391 (1.68, M^+), 374 (15.33, M^+ – OH), 346 (29.49, $M^+ - CO_2H$); $[\alpha]^{26}_D$ -35.6° (*c* 1.0, Me_2CO).

1-[4-[(*tert*-Butyldimethylsilyl)oxy]-4-phenylbutyryl]-L-proline 2-Ester with Benzyl Salicylate (46). To 3.95 g (1.01×10^{-2} mol) of **45** were added 2.30 g (1.01×10^{-2} mol) of benzyl salicylate, 0.31 g (2.54×10^{-3} mol) of DMAP, and 25 mL of methylene chloride. This was mixed well before 2.50 g (1.21×10^{-2} mol) of DCC dissolved in 10 mL of methylene chloride was added to the reaction vessel. After stirring overnight at room temperature, the mixture was filtered, concentrated, and chromatographed on 40 g of silica gel with 20:80 ethyl acetate:hexane as eluant to give 3.45 g of **46** as a clear oil: yield 57%; NMR (acetone-d_6) δ 8.00–7.39 (m, 9 H), 7.22 (s, 5 H), 5.45 (s, 2 H), 3.58 (t, 2 H), 2.95–1.95 (m, 8 H), 1.00 (s, 9 H), 0.20 (s, 3 H), 0.15 (s, 3 H); IR ($CHCl_3$) carbonyls at 1765, 1720, and 1635 cm^{-1}; MS, *m/e* (relative intensity) 601 (1.03, M^+), 374 (26.80, M^+ – benzyl salicylate), 346 (14.37, M^+ – benzyl salicylate, – CO); $[\alpha]^{26}_D$ -41.7° (*c* 1.0, Me_2CO).

1-(4-Hydroxy-4-phenylbutyryl)-L-proline 2-Ester with Salicylic Acid (47). Compound **46** (2.00 g, 3.32×10^{-3} mol) was placed in the reaction vessel of a HF apparatus along with 0.70 g (6.48×10^{-3} mol) of anisole. The HF was condensed in the reservoir flask which contained CoF_3 (1.0 g as a drying agent). The HF in the reservoir flask was allowed to distill into the reaction vessel and compound **46** was allowed to stir with the liquid HF for 20 min at room temperature. The HF was removed under vacuo and the crude product chromatographed on 20 g of silica gel with 50:50 hexane:ethyl acetate as eluant. This gave 0.98 g of **47** as a clear oil: 74% yield; NMR (acetone-d_6) δ 8.02–7.11 (m, 9 H), 4.82 (m, 1 H), 4.59 (m, 1 H), 3.81 (m, 1 H), 3.49 (m, 2 H), 2.49–1.39 (m, 8 H); IR ($CHCl_3$) OH stretch from 3300 to 2700 cm^{-1}, carbonyls at 1760, 1700, and 1640 cm^{-1}; MS, *m/e* (relative intensity) 380 (0.11, M^+ – OH), 70 (100.00, C_4H_8N); $[\alpha]^{26}_D$ -43.3° (*c* 1.0, Me_2CO). Anal. ($C_{22}H_{23}NO_6$) C, H. N.

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92010-32-7; 45, 92010-33-8; 46, 92010-34-9; 47, 92010-35-0; HbS, 9035-22-7; 3,5-dinitrobenzoic acid, 99-34-3; *tert*-butyl bromoacetate, 5292-43-3; octanoyl chloride, 111-64-8; *p*-chlorobenzoyl chloride, 122-01-0; 3,4-dichlorobenzoyl chloride, 3024-72-4; *p*-bromobenzoyl chloride, 586-75-4; L-proline benzyl ester hydrochloride, 16652-71-4; hydrocinnamic acid, 501-52-0; benzyl salicylate, 118-58-1; 3,3-dimethylbutanoic acid, 1070-83-3; phenylacetic acid, 103-82-2; phenoxyacetic acid, 122-59-8; trimethylacetic acid, 75-98-9; 4-phenyl-4-butyrolactone, 1008-76-0; *tert*-butyldimethylsilyl chloride, 18162-48-6.

Synthesis and Evaluation of Furan, Thiophene, and Azole Bis[(carbamoyloxy)methyl] Derivatives as Potential Antineoplastic Agents[1]

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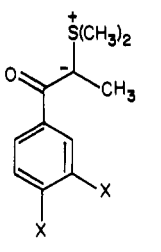
A series of bis(hydroxymethyl)-substituted heterocycles were synthesized and converted to the corresponding bis(methylcarbamate) derivatives. The heterocyclic systems studied were based on 2-phenyl-3-methylfuran (**2–4**), 1-phenylpyrazole (**5–7**), 1-phenyl-5-methylpyrazole (**9–11**), 1-phenyl-5-methylthiophene (**13**), 1-phenyl-1,2,3-triazole (**14**), 3-phenylisoxazole (**15**), 3-phenylisothiazole (**16**), 2-phenylthiazole (**17**), and 2-phenyloxazole (**18**). None of the bis(carbamates) prepared was active against murine P388 lymphocytic leukemia. Pyrrole bis(carbamates) **20** and **21**, which exhibited antileukemic activity, also showed reactivity toward 4-(*p*-nitrobenzyl)pyridine while the inactive bis(carbamates) were unreactive in the 4-(*p*-nitrobenzyl)pyridine assay.

We have recently reported the synthesis and antineoplastic activity of a series of pyrroles and pyrrolizines.[2] The significant reproducible activity that selected agents in these classes have shown against several experimental murine leukemias and solid tumors as well as against human tumor xenografts in the nude mouse has provided a major impetus for continued studies with this group of compounds.[3]

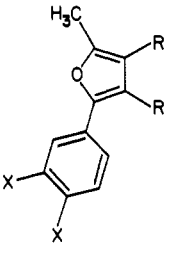
All of the compounds that we have described to date have been based on the pyrrole and pyrrole-fused nuclei where two adjoining pyrrole carbon atoms were substituted with potentially reactive (acyloxy)methyl groups while other positions in the pyrrole nucleus were substituted by groups that could either control the reactivity of the (acyloxy)methyl groups or retard the oxidative decomposition of the pyrrole. This report focuses upon the heteroaromatic nucleus and describes the preparation and antileukemic evaluation of "lead" structures based on furan, thiophene, and azole nuclei.

Chemistry. The furan diesters **2** were prepared from the ylides **1** by treatment with dimethyl acetylenedicarboxylate (DMAD).[4] Reduction of **2** with lithium aluminum hydride gave the diols **3**, which were converted to the bis(methylcarbamates) **4a** and **4b** by treatment with methyl isocyanate.

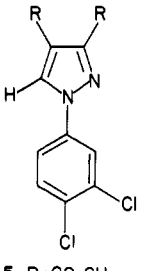
The pyrazole diester **5** was prepared from 3-phenylsydnone by a 1,3-dipolar cycloaddition reaction with DMAD.[5] The diesters **9** were prepared from the appropriate anilines by conversion to the imino chlorides **8**, using the Japp–Klingmann reaction, and subsequent treatment of **8** with the sodium salt of ethyl acetoacetate.[6] The bis(methylcarbamates) **7** and **11a–d** were prepared from the corresponding diesters **5** and **9a–d**, respectively, by reduction (lithium aluminum hydride) and acylation of the resulting diols (**6** and **10a–d**) with methyl isocyanate.



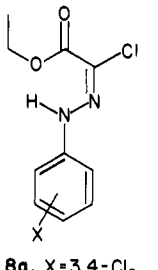
1a, X = H
b, X = Cl



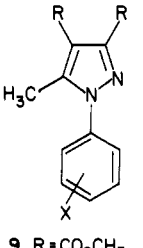
2, R = CO_2CH_3
3, R = CH_2OH
4, R = $CH_2OCONHCH_3$



5, R = CO_2CH_3
6, R = CH_2OH
7, R = $CH_2OCONHCH_3$



8a, X = 3,4-Cl_2
b, X = 4-Cl
c, X = H



9, R = CO_2CH_3
10, R = CH_2OH
11, R = $CH_2OCONHCH_3$

The pyrazole **7** was prepared in order to compare it with **11a** and evaluate the steric effect of the 5-methyl group. The UV spectra of **7** and **11a** showed absorption maxima at 268 (ϵ 25 300) and 255 nm (ϵ 18 000), respectively. The shift to lower wavelength and the reduction of the extinction coefficient of **11a**, compared to **7**, is consistent with

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