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Ferrocenyl Polyamines as Agents for the Chemoimmunotherapy of Cancer

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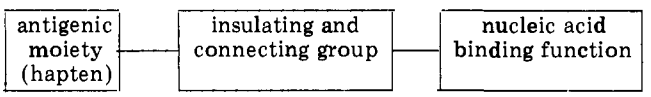
A series of ferrocenyl polyamines, compounds intended to bind to the tumor cell surface nucleic acid and elicit an immune response, was synthesized and screened for antitumor activity. Target ferrocenyl polyamines **1a,b**, **2**, and **3**, bearing 2-, 3-, and 4-amino groups, respectively, were readily obtained in yields of 31–58% from their corresponding ferrocenyl polyamides (**5a–d**) by reduction with diborane in THF; lithium aluminum hydride was not an effective reducing agent in this case. Although the target compounds failed to prolong the life of mice with P-388 lymphocytic leukemia, three of the intermediate amides (**5b–d**) did exhibit low but significant activity (T/C = 123, 132, and 120%, respectively).

The rationale and modus operandi for the development of chemoimmunotherapeutic agents has recently been described by Soloway et al.[1] Basically, the approach calls for the preparation of tagging haptens which could bind to the cell membrane of neoplastic cells, eliciting the generation of cytotoxic antibodies.[1] Chemoimmunotherapeutic agents, by selectively acting at the cell surface of the weakly antigenic tumor cells, would represent an advantage over the conventional antitumor agents which need to be delivered in high, and often toxic, levels to interfere with intracellular processes.

During the course of his work with the platinum–pyrimidine blues, Rosenberg uncovered evidence suggesting the presence of nucleic acids on the cell surface of tumor cells; none was found on normal cells.[2] In an attempt to exploit the apparent surface nucleic acid difference between tumor cells and normal cells, we have synthesized and evaluated the antitumor properties of a series of three-component compounds with the generalized structure



The intent was to produce materials that would interact strongly at site C with the tumor surface nucleic acid, thus anchoring the compounds to the cell. The hapten portion of the molecule, once conjugated with cell surface protein, would then, hopefully, stimulate antibody formation. Since the polyamines such as putrescine [$NH_2(CH_2)_4NH_2$], spermidine [$NH_2(CH_2)_3NH(CH_2)_4NH_2$], and spermine [$NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$] are ubiquitous in nature, pose no specific toxicity problems, and are known to interact strongly with nucleic acids,[3] they were considered good candidates for the nucleic acid binding function. Of the various haptens available, ferrocene was chosen due to its demonstrated ability to elicit a strong antigenic response when conjugated to polypeptides.[4] As an insulating and connecting group, a methylene chain of at least four carbon atoms was used. The resulting ferrocenyl polyamine target compounds (**1a,b–3**) are shown in Chart I.

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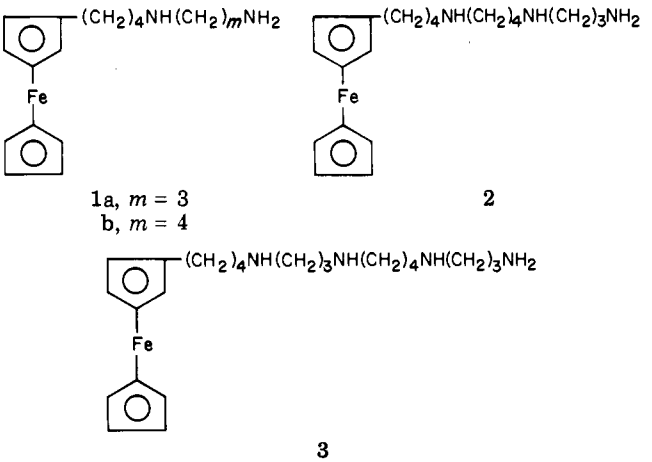
Chart I

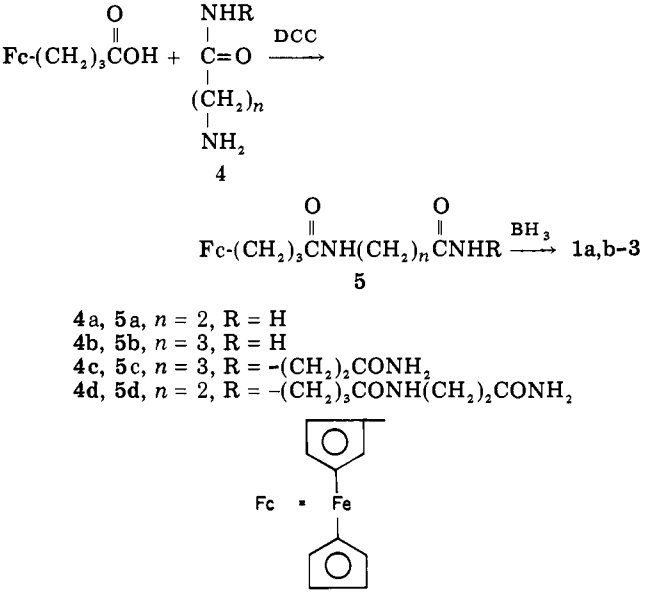


1a, $m = 3$
b, $m = 4$

2

3

Scheme I



4a, **5a**, $n = 2$, R = H
4b, **5b**, $n = 3$, R = H
4c, **5c**, $n = 3$, R = $-(CH_2)_2CONH_2$
4d, **5d**, $n = 2$, R = $-(CH_2)_3CONH(CH_2)_2CONH_2$

Chemistry. Target polyamines **1a,b–3**, isolated as the hydrobromide salts, were readily obtained in moderate

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yields (31–58%) by reduction of their precursor polyamides **5a–d** with diborane in THF (Scheme I); lithium aluminum hydride was ineffective as reducing agent, yielding numerous side products in addition to small amounts of the desired amines. The required polyamides were synthesized by condensation of 4-ferrocenylbutyric acid with the appropriate amino amide in the presence of dicyclohexylcarbodiimide (DCC). Amino diamide **4c** was prepared by catalytic hydrogenolysis of *N*-carbobenzyloxy-γ-aminobutyryl-β-alanine amide which was obtained by condensation of *N*-carbobenzyloxy-γ-aminobutyric acid with β-alanine amide in the presence of 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EDDQ); amino triamide **4d** was similarly prepared by condensation of **4c** with *N*-carbobenzyloxy-β-alanine, followed by hydrogenolysis. Amino amides **4a** and **4b** were commercially available.

Biological Results and Discussion. Compounds **1a,b–3**, **5a–d**, and **4d** were subjected to screening for antitumor activity against lymphocytic leukemia P-388 according to standard protocols of the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health.[5] The compounds were administered to the test mice intraperitoneally with either water or water–Tween 80 as vehicle in doses of 12.5, 25, 50, 100, 200, and 400 mg/kg; compounds were considered active if they gave T/C activity values in the P-388 system equal to or greater than 120%, where T/C represents the ratio of the median survival times of the treated animals over those of the control animals, expressed as a percentage.

All of the target amines, screened as the polyhydrobromides, were inactive; in fact, they were generally toxic in doses above 12.5 mg/kg. In order to ascertain whether the toxicity of the targets was in some way related to the presence of the hydrogen bromide, compound **1b** was tested as the free base; the free diamine displayed only a slight decrease in toxicity (toxic in doses above 25 mg/kg instead of 12.5 mg/kg) and no significant antitumor activity.[6]

Surprisingly, however, diamide **5b** (at 25 mg/kg), triamide **5c** (50 mg/kg), and tetraamide **5d** (400 mg/kg) exhibited low, yet significant, antitumor activity (T/C = 123, 132, and 120%, respectively). While not active according to the previously defined criterion (T/C = 120%), 4-ferrocenylbutyramide, prepared and screened subsequent to the findings for the active polyamides, displayed a borderline T/C value of 117% at 50 mg/kg. Reduction of the above monoamide to 4-ferrocenyl-1-aminobutane yielded an inactive compound. Compound **4d**, which bears structural resemblance to the active tetraamide **5d** but which lacks the ferrocene nucleus, was inactive to 200 mg/kg and toxic at higher doses. It would appear, therefore, that the incorporation of the ferrocene group into an appropriate polyamide carrier might provide an agent with enhanced antitumor activity, and it is hoped that the synthesis and screening of additional ferrocenyl polyamides will yield a more comprehensive structure–activity relationship.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Amino acids and amino amides were purchased from Vega-Fox Biochemicals or ICN Biochemicals. 4-Ferrocenylbutyric acid was prepared according to the procedure of Rinehart et al.[7] The petroleum ether used had a boiling point range of 30–60 °C. Hydrogenations were performed under atmospheric conditions.

***N*-Carbobenzyloxy-γ-aminobutyryl-β-alanine Amide (4c, *N*-Cbz-).** Triethylamine (4.6 mL, 32.8 mmol) was added to a mixture of β-alanine amide hydrochloride (4.1 g, 32.8 mmol) in 160 mL of DMF containing 5 mL of H_2O. *N*-Carbobenzyloxy-γ-aminobutyric acid (7.8 g, 32.8 mmol) was added, followed by EEDQ (8.9 g, 36.1 mmol), and the solution was allowed to stir at room temperature for 4 days. The solvent was evaporated and the remaining solid triturated with EtOAc. The solid material was collected and crystallized from H_2O, yielding 4.1 g (41%) of pure *N*-carbobenzyloxydiamide, mp 183–186 °C. Anal. ($C_{15}H_{21}N_3O_4$) C, H, N.

***N*-Carbobenzyloxy-β-alanyl-γ-aminobutyryl-β-alanine Amide (4d, *N*-Cbz-).** The above protected diamide (4.4 g, 14.2 mmol) was hydrogenated in 200 mL of EtOH in the presence of 650 mg of Pd/black. Following complete uptake of H_2, the catalyst and solvent were removed, leaving a white solid. *N*-Carbobenzyloxy-β-alanine (3.2 g, 14.2 mmol) and EEDQ (7.0 g, 28.3 mmol) were then added to the free amino diamide in 80 mL of DMF containing 6 mL of H_2O, and the reaction mixture was allowed to stir at room temperature for 5 days. The solvent was evaporated and the residue triturated with EtOAc, yielding 4.5 g of solid. Crystallization from H_2O then afforded 4.1 g (77%) of pure product, mp 220–223 °C. Anal. ($C_{18}H_{26}N_4O_5$) C, H, N.

***N*-(4-Ferrocenylbutyryl)-β-alanine Amide (5a).** 4-Ferrocenylbutyric acid (5.0 g, 18.4 mmol), β-alanine amide hydrochloride (2.3 g, 18.4 mmol), and 1-hydroxybenzotriazole hydrate (2.5 g, 18.4 mmol) were dissolved in 50 mL of CH_3CN containing 1 mL of H_2O. Triethylamine (2.6 mL, 18.4 mmol) was added, followed by DCC (4.2 g, 20.2 mmol), and the reaction mixture was allowed to stir overnight at room temperature. The solid was removed by filtration and washed several times with EtOAc. The combined filtrate was removed and EtOAc was added to the residue, yielding 6.4 g of solid. The crude product was purified with column chromatography (silica, 90:9:1 $CHCl_3$–MeOH–HOAc), affording 2.9 g (46%) of **5a** from EtOAc–petroleum ether: mp 137–138 °C. An analytical sample obtained by preparative TLC had mp 145–147 °C. Anal. ($C_{17}H_{22}FeN_2O_2$) C, H, N.

***N*-(4-Ferrocenylbutyryl)-γ-aminobutyramide (5b).** Triethylamine (2.6 mL, 18.4 mmol), DCC (4.2 g, 20.2 mmol), 4-ferrocenylbutyric acid (5.0 g, 18.4 mmol), 1-hydroxybenzotriazole hydrate (2.5 g, 18.4 mmol), and γ-aminobutyramide hydrochloride (2.6 g, 18.4 mmol) were combined and allowed to react as described for **5a**. On removal of the reaction solvent, the solid was thoroughly triturated with EtOAc and removed by filtration. The filtrate was washed with 1 N HCl, saturated $NaHCO_3$, and saturated NaCl and dried ($MgSO_4$). The solvent was evaporated and the residue crystallized from EtOAc–petroleum ether, yielding 5.2 g (79%) of **5b**, mp 110.5–113 °C. Anal. ($C_{18}H_{24}FeN_2O_2$) C, H, N.

***N*-(4-Ferrocenylbutyryl)-γ-aminobutyryl-β-alanine Amide (5c).** Protected diamide **4c** (5.6 g, 18.4 mmol) was hydrogenated in 200 mL of EtOH in the presence of 2.0 g of Pd/black. The white solid obtained on removal of the catalyst and solvent was mixed with 1-hydroxybenzotriazole hydrate (2.5 g, 18.4 mmol), 4-ferrocenylbutyric acid (5.0 g, 18.4 mmol), DCC (4.2 g, 20.2 mmol), and 200 mL of DMF. After stirring overnight at room temperature, the mixture was filtered, the solvent evaporated, and the residue triturated with EtOAc to yield 15.0 g of solid. The solid was purified with column chromatography (silica, 90:9:1 $CHCl_3$–MeOH–HOAc), affording, on crystallization from H_2O, 3.9 g (49%) of **5c**, mp 165–167 °C. Anal. ($C_{21}H_{29}FeN_3O_3$) C, H, N.

***N*-(4-Ferrocenylbutyryl)-β-alanyl-γ-aminobutyryl-β-alanine Amide (5d).** Blocked triamide **4d** (4.0 g, 10.6 mmol) in 300 mL of 20% aqueous EtOH was hydrogenated in the presence of 600 mg of Pd/black. The white solid obtained on removal of the catalyst and solvent was combined with 4-ferrocenylbutyric acid (2.8 g, 10.6 mmol), DCC (2.4 g, 11.1 mmol), and 1-hydroxybenzotriazole hydrate (1.4 g, 10.6 mmol) in 200 mL of DMF. The reaction was allowed to proceed as described for **5c**, and purification with column chromatography afforded 2.9 g (56%) of desired tetraamide from H_2O: mp 178–180 °C. Anal. ($C_{24}H_{34}FeN_4O_4$) C, H, Fe, N.

Preparation of Ferrocenyl Polyamines 1a,b–3. *N*-(4-Ferrocenylbutyl)-1,3-diaminopropane Dihydrobromide (1a). A mixture of **5a** (1.1 g, 3.1 mmol) in 20 mL of THF was added dropwise to a chilled solution of 1 N BH_3 in THF (16 mL, 16 mmol). When solution was obtained, the temperature of the

reaction was brought to reflux for 1.5 h. The solution was allowed to cool to room temperature, and 4 mL of 32% HBr–HOAc was slowly added. After stirring for 45 min, the mixture was filtered to yield 342 mg of crude product; an additional crop of 791 mg was obtained by triturating the mother liquor with petroleum ether. The two crops were crystallized from MeOH–ether, affording 721 mg (49%) of **1a**, no definitive melting point. Anal. ($C_{17}H_{26}FeN_2 \cdot 2HBr$) C, H, Fe, N.

Compounds **1b**, **2**, and **3** were prepared as described above for **1a** in yields of 31, 58, and 42%, respectively; none of the compounds, isolated and analyzed as the hydrobromides, had a definitive melting point. The number of equivalents of BH_3 used to reduce the polyamides was determined by adding the following: 2.4 for each secondary amide present and 3.2 for each primary. In the preparation of tetraamine **3**, HBr treatment did not yield a solid precipitate; however, the addition of petroleum ether did give rise to solid material which crystallized from MeOH–ether.

4-Ferrocenylbutyramide. 4-Ferrocenylbutyric acid (2.7 g, 10.0 mmol), 1-hydroxybenzotriazole hydrate (1.4 g, 10.0 mmol), and DCC (2.2 g, 11.0 mmol) were combined in 150 mL of ether and allowed to stir for 2 h at room temperature. The solid was removed by filtration and washed with ether. The combined ether filtrate was chilled in an ice bath and stirred while NH_3 was bubbled in during 1 h. The solid which formed was removed by filtration, and the filtrate was washed with 0.5 N NaOH and saturated NaCl. The dried ($MgSO_4$) ether was evaporated leaving a solid which crystallized from EtOH–H_2O to yield 2.3 g (85%) of the amide, mp 72–79 °C. An analytical sample obtained by recrystallization had mp 77.5–80 °C. Anal. ($C_{14}H_{17}FeNO$) C, H, N.

4-Ferrocenyl-1-aminobutane Hydrobromide. 4-Ferrocenylbutyramide (500 mg, 1.9 mmol) was reduced with 1 N BH_3 in THF (5.9 mL, 5.9 mmol) as described for **1a**. After acidification with 1.25 mL of 32% HBr–HOAc, petroleum ether was added to separate the product as a green oil. The solvent was decanted and the oil dried in a vacuum desiccator over P_2O_5. Crystallization of the oil from EtOH–ether afforded 56 mg of a slightly crude product; concentration of the mother liquor and crystallization of the resultant oil from acetone–ether yielded another 137 mg of product, elevating the yield to 31%. Repeated recrystallization from EtOH–ether afforded an analytical sample, mp 119–122 °C. Anal. ($C_{14}H_{19}FeN \cdot HBr$) C, H, N.

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2,3-Dihydroxy-9-amino-9,10-dihydrophenanthrene, a Rigid Congener of Dopamine and Isoapomorphine

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A rigid dopamine congener, 2,3-dihydroxy-9-amino-9,10-dihydrophenanthrene, was synthesized and tested for the ability to dilate the renal artery in dogs. The compound was found to be inactive in this assay at molar doses 1000 times greater than required for dopamine. This result is similar to isoapomorphine. The title compound was also examined for its ability to stimulate rat striatal adenylate cyclase. In concentrations of 10–100 μM no effect was observed. The phenanthrene also showed no ability to block dopamine-induced stimulation of adenylate cyclase.

Cannon et al.[1] have reported the synthesis and preliminary biological evaluation of 3,4-dihydroxy-9-*N*,*N*-dimethylamino-9,10-dihydrophenanthrene, a congener of apomorphine. This compound, however, possessed relatively low emetic activity and was completely devoid of apomorphine-like activity on heart and blood pressure. On the basis of the NMR spectra of this and related compounds, Cannon concluded that the lack of activity could be due to the fact that the dimethylamino group exists primarily in the pseudoaxial conformation. This conclusion is in agreement with a later study dealing with conformational preferences of 9-substituted 9,10-dihydrophenanthrenes.[2]

Isoapomorphine (**1**) has little or no central emetic action and does not elicit a gnawing response in rats at ten times the median effective dose of apomorphine.[3] However, Pinder et al.[4] found that the simpler aminotetralin congener of isoapomorphine, 6,7-dihydroxy-2-aminotetralin (ADTN, **2**), was more potent than dopamine in hyperpolarization and inhibition of neurons in the snail, *Helix aspersa*. Most important was the finding that ADTN possessed potent vasodilator activity in the kidney.[5] Isoapomorphine (**1**), however, does not produce renal vasodilation.

We decided to prepare 2,3-dihydroxy-9-amino-9,10-dihydrophenanthrene (**3**) in order to complete the de-

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