Potential Antitumor Agents. 44. Synthesis and Antitumor Activity of New Classes of Diacridines: Importance of Linker Chain Rigidity for DNA Binding Kinetics and Biological Activity

William A. Denny,*† Graham J. Atwell,† Bruce C. Baguley,† and Laurence P. G. Wakelin‡

Cancer Research Laboratory, University of Auckland School of Medicine, Private Bag, Auckland, New Zealand, and Experimental Chemotherapy Unit, Cancer Institute, Melbourne, Victoria 3000, Australia. *Received January 21, 1985*

Four classes of diacridines, joined at the 9-position by linker chains of varying length, rigidity, and polarity, were evaluated for DNA-binding properties and antitumor activity. Diacridines linked by flexible chains of varying polarity show relatively fast chromophore exchange kinetics among DNA binding sites but slower dissociation rates, suggesting the potential for considerable "creeping" of the drug along the helix, and are inactive in vivo. The exchange kinetics can be slowed dramatically by inclusion of positive charges in the side chain, but the resulting polycationic drugs are inactive in vivo, possibly due to poor distribution. Diacridines linked by a rigid, polar but neutral dicarbamoylpyrazole chain retain slow exchange kinetics, have a greatly reduced potential "creep rate", and possess good in vitro potency and significant in vivo antileukemic activity.

Several studies with different classes of DNA-intercalating agents have suggested that antitumor activity is associated with high DNA binding affinity,[1-3] slow drug–DNA dissociation rates,[4] and long drug residence times at individual DNA binding sites.[5] In searching for new classes of compounds possessing these apparently desirable properties, several groups have prepared dimeric molecules, connecting two chromophores by a molecular chain that allows both to intercalate when the ligand is bound to DNA. This approach has many theoretical attractions. In the absence of significant steric or entropic factors, the binding constant of a symmetrical bisintercalator should be approximately the square of that of the monomer.[6] Since complete dissociation of the ligand from the DNA has to involve disengagement of both of the chromophores, drug dissociation rates much slower than those of the monomer can be expected.[7]

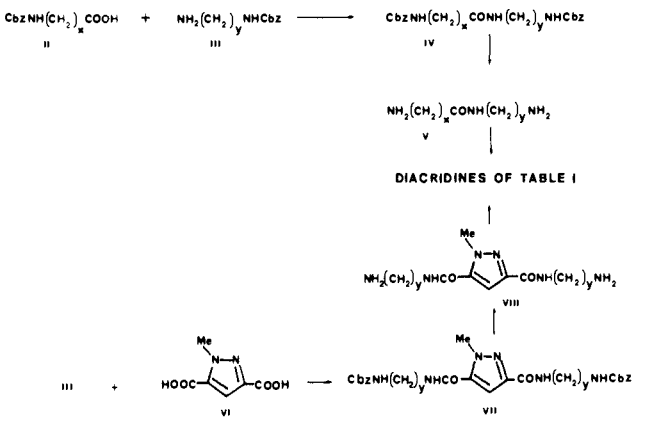
Of several classes of dimeric molecules made as potential bisintercalators, the diacridines have received the most attention. A number of studies of their interaction with DNA have provided information on the acridine substitution patterns and minimum linker chain length necessary for bisintercalative binding to occur.[7-11] While no simple diacridines have binding constants approaching the square of those of the monomer (10^{10}–10^{12} M^{-1}), presumably due to unfavorable entropic and/or geometric effects and to ligand self-stacking interactions, useful increases are seen. Studies of the dissociation kinetics of spermine-linked diacridines have shown some derivatives to have off-rates of the order of 10^{-3} s^{-1}, several orders of magnitude slower than those of the corresponding monomers.[7]

In addition, the much larger binding site size of dimeric compounds compared to their monomeric counterparts greatly increases the potential for DNA sequence-selective binding. Although little attention has been given to this aspect of the binding of bisintercalating agents, positive correlations between antitumor activity and AT/GC binding selectivity have been noted for several series of minor groove binding agents,[12,13] indicating the potential importance of this property.

However, the bisintercalating agents as a class have not produced the superior biological activity expected from the extensive theory underlying their development, and the reasons for this failure of "rational drug design" have not been fully determined. One diacridine, the 1,6-hexanediamine derivative (**3**) has been evaluated for clinical trial as an antitumor agent by the National Cancer Institute (as NSC 219733),[14,15] but soluble formulations displayed

Scheme I

Scheme II

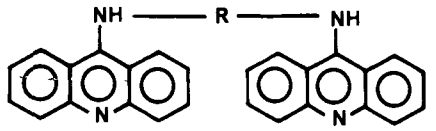


† University of Auckland School of Medicine.
‡ Cancer Institute.

(1) Le Pecq, J.-B.; Xuong, N.-D.; Gosse, G.; Paoletti, C. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 5078.
(2) Baguley, B. C.; Denny, W. A.; Atwell, G. J.; Cain, B. F. *J. Med. Chem.* **1981**, *24*, 520.
(3) Fink, S. I.; Leo, A.; Yamakawa, M.; Hansch, C.; Quinn, F. R. Farmaco. Ed. Sci. **1980**, *35*, 965.
(4) Muller, W.; Crothers, D. M. *J. Mol. Biol.* **1968**, *35*, 251.
(5) Feigon, J.; Denny, W. A.; Leupin, W.; Kearns, D. R. *J. Med. Chem.* **1984**, *27*, 450.
(6) Von Hippel, P. H.; McGhee, J. D. *Ann. Rev. Biochem.* **1972**, *41*, 231.
(7) Capelle, N.; Barbet, J.; Dessen, P.; Blanquet, S.; Roques, P. B.; Le Pecq, J.-B. *Biochemistry* **1979**, *18*, 3354.
(8) Lown, J. W.; Gunn, B. C.; Chang, R.-Y.; Majumdar, K. C.; Lee, J. S. *Can. J. Biochem.* **1978**, *56*, 1006.
(9) Wright, R. G. M.; Wakelin, L. P. G.; Fieldes, A.; Acheson, R. M.; Waring, M. J. *Biochemistry* **1980**, *19*, 5825.
(10) Chen, T.-K.; Fico, R. M.; Canellakis, E. S. *J. Med. Chem.* **1978**, *21*, 868.
(11) King, H. D.; Wilson, W. D.; Gabbay, E. J. *Biochemistry* **1982**, *21*, 4982.
(12) Denny, W. A.; Atwell, G. J.; Baguley, B. C.; Cain, B. F. *J. Med. Chem.* **1979**, *22*, 134.
(13) Denny, W. A.; Cain, B. F. *J. Med. Chem.* **1979**, *22*, 1234.
(14) Goldin, A.; Venditti, M. M.; McDonald, J. S.; Muggia, F. M.; Henney, J. E.; Devita, V. T. *Eur. J. Cancer* **1981**, *17*, 129.
(15) Beltagy, Y. A.; Waugh, W.; Repta, A. J. *Drug. Dev. Ind. Pharmacol.* **1980**, *6*, 411.

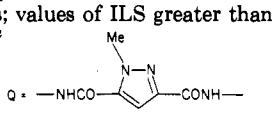
0022-2623/85/1828-1568$01.50/0

Table I. Physicochemical and Biological Data for Diacridines



no.	class[a]	R	x[b]	C_{50} values[c] AT	C_{50} values[c] GC	unwinding angle[d]	Rm[e]	L1210[f] in vitro ID_{50}	P388 in vivo OD[g]	P388 in vivo ILS[h]
	9-aminoacridine			9.6	10.0	16	−0.08	1900	20	*j*
1	A	$(CH_2)_4$	6.3	0.39	0.87	17	−0.21	10800	30	*j*
2	A	$(CH_2)_5$	7.2	0.80	1.5	26	0.02	3700	20	*j*
3	A	$(CH_2)_6$	8.8	0.13	0.29	30	0.15	110	13.3	*j*
4	A	$(CH_2)_7$	10.0	0.26	0.39	34	0.21	50	8.9	*j*
5	A	$(CH_2)_8$	11.3	ND[i]	ND	32	0.28	170	8.9	*j*
6	A	$(CH_2)_9$	12.2	ND	ND	ND	0.40	160	5.9	*j*
7	A	$(CH_2)_{10}$	13.8	0.28	0.28	ND	0.45	280	5.9	*j*
8	B	$CH_2CONH(CH_2)_2$	7.7	1.5	2.2	24	−0.41	>4000	50	*j*
9	B	$CH_2CONH(CH_2)_3$	8.7	0.45	0.71	37	−0.24	>3500	50	*j*
10	B	$(CH_2)_2CONH(CH_2)_2$	8.6	0.81	2.4	38	−0.43	>3500	50	*j*
11	B	$(CH_2)_2CONH(CH_2)_3$	9.8	1.0	1.6	40	−0.28	>3500	15	*j*
12	B	$(CH_2)_3CONH(CH_2)_2$	9.9	0.60	1.2	39	−0.19	>3500	50	*j*
13	B	$(CH_2)_3CONH(CH_2)_3$	11.1	0.70	1.1	35	−0.12	>3500	50	*j*
14	C	$(CH_2)_3NH(CH_2)_3$	9.8	0.14	0.22	35	−0.53	480	5	*j*
15	C	$(CH_2)_3N(Me)(CH_2)_3$	9.8	0.10	0.15	33	−0.65	780	10	*j*
16	C	$(CH_2)_3NH(CH_2)_4$	11.2	0.10	0.14	28	−0.39	29	10	*j*
17	C	$(CH_2)_2NH(CH_2)_2NH(CH_2)_2$	11.3	0.11	0.13	30	−0.90	2900	10	*j*
18	C	$(CH_2)_3NH(CH_2)_2NH(CH_2)_3$	13.5	0.11	0.13	20	−0.86	560	10	*j*
19	C	$(CH_2)_2Y^l(CH_2)_2$	10.1	0.11	0.13	26	−0.77	78	3	*j*
20	C	$(CH_2)_3Y^l(CH_2)_3$	12.0	0.12	0.12	19	−0.72	33	3	*j*
21	C	$(CH_2)_3Y^l(CH_2)_3$	16.1			14	−0.80	530	39	*j*
22	D	$(CH_2)_2Q^k(CH_2)_2$	13.7	0.11	0.14	25	−0.30	13	20	46
23	D	$(CH_2)_3Q^k(CH_2)_3$	15.5	0.20	0.39	36	0.00	55	20	65
24	D	$(CH_2)_4Q^k(CH_2)_4$	17.6	0.11	0.16	29	0.11	24	8.9	26

[a] Class of compound determined by nature of linker chain. See text for discussion. [b] Distance (in Å) between ligands when linker chain is in fully extended conformation estimated from Cortauld models. [c] C_{50}: nanomolar concentration of ligand needed to displace 50% of bound ethidium bromide from either poly(dA-dT) or poly(dG-dC). See ref 24. [d] Unwinding angles determined by viscometry for closed circular DNA from *E. coli* plasmid PML-21, relative to ethidium bromide as 26°. See ref 25. [e] Rm: chromatographic measure of drug lipophilicity, determined for drug cations at pH 1–2 as detailed in ref 29. [f] IC_{50}: the nanomolar concentration of drug that when added to cultures of L1210 cells for a period of 70 h reduces counted cell numbers to 50% of controls (ref 38). [g] OD: highest nontoxic drug dose in milligrams/kilogram per day, administered intraperitoneally as a solution in 0.1 mL of 30% v/v EtOH/water on days 1, 5, and 9 after inoculation of 10^6 P388 leukemia cells. [h] ILS: the percentage increase in lifespan of treated animals over that of control groups of animals injected with tumor alone. The average survival of control mice was 11 days; values of ILS greater than 20% are considered significant. [i] ND: not determined. [j] Compound inactive (ILS < 20%) at all dose levels. [k]



[l] Y = 1,4-piperazinediyl.

CNS toxicity and only marginal activity.[16] This failure to develop a clinical candidate from among the well-defined class of bisintercalating agents is disappointing. The successes over recent years of combination chemotherapy in minimizing the development of tumor resistance during treatment have been encouraging,[17,18] but further advances depend on the availability of new classes of drugs with novel modes of action. Further work on bisintercalating agents thus seems worthwhile, especially since one such derivative (**3**) has shown some experimental activity against intercalator-resistant leukemia sublines.[19]

This paper is a comparative study of the DNA interactions and antitumor activity of four classes of unsubstituted diacridines, two of them novel, where the length and nature of the linker chain are varied.

Chemistry

Many of the required diamines I were commercially available, and those for the preparation of diacridines of classes B and D were made by the methods shown in Scheme II. Alkanediamines were monoprotected with the *N*-benzyloxycarbonyl (Cbz) group by published methods[20] and the corresponding monoamines III coupled with the appropriate *N*-Cbz-protected amino acids II using diethyl phosphorocyanidate[21] to give amides IV. Similar condensation of III with 1-methylpyrazole-3,5-dicarboxylic acid (VI) gave the pyrazolediamides VII. Hydrogenation of compounds IV and VII in MeOH gave the corresponding diamines V and VIII, which were used directly for formation of the diacridines.

The diacridines were prepared from 9-chloroacridine and a stoichiometric amount of the appropriate diamine I in excess phenol at 100 °C. This method, previously developed[10] for the polymethylene derivatives (class A, Table I), did not generally cause significant cleavage of the amide-containing diamines. However, the longer aliphatic amides V ($x = 3$) did undergo some cleavage, leading to release of alkanediamines $NH_2(CH_2)_yNH_2$, which were also

(16) Schepartz, S.; Venditti, J. M.; Plowman, J.; Wolpert, M. K. Report on the Program of the Division of Cancer Treatment, National Cancer Institute, 1981.
(17) Chabner, B. A. In "Pharmacologic Principles of Cancer Treatment"; Chabner, B. A., Ed.; W. B. Saunders Co.: Philadelphia, 1982; p 3.
(18) Liebman, H. A.; Hum, G. J.; Sheehan, W. W.; Ryden, M. V. J.; Bateman, J. R. *Cancer Treat. Rep.* **1983**, *69*, 413.
(19) Johnson, R. K.; Howard, W. S. *Eur. J. Cancer Clin. Oncol.* **1982**, *18*, 179.
(20) Atwell, G. J.; Denny, W. A. *Synthesis* **1984**, 1032.
(21) Sasse, K. *Methoden Org. Chem. (Houben-Weyl)* **1964**, *12*, 298.

Table II. DNA Binding Kinetic Data for Selected Diacridines of Table I

			high-field NMR data[b]			stopped-flow data[c]		
no.	name	class[a]	jump rate,[d] s^{-1}	residence time,[e] ms	temp, °C	av recip time const: τ^{*-1} s^{-1}	time const[g] τ, ms	amplitude[h] A, %
	9-aminoacridine		300	3.0	19	>190	<1	50
							2.4	44
							15	6
5	C_8	A	400–500	2–2.5	21	9.2	63	32
							165	68
7	C_{10}	A	100–200	5–10	29	4.6	157	55
							422	45
13	amide	B	ND[i]	ND	ND	39	6.9	11
							33	73
							116	16
21	BAS	C	<100	>10	27	0.18	1630	18
							7890	43
							26300	39
22	BAPY	D	<50	>20	16	0.44	600	8
							2700	50
							5400	42

[a] As determined in Table I. [b] From ref 27. [c] All determinations at 20 °C. [d] Jump rate: migration of drug molecules (without complete dissociation from the DNA) between DNA binding sites; estimates of upper limit from NMR. [e] Estimate of lower limit of average residence time of drug molecule at a particular binding site, from NMR. [f] Weight-averaged reciprocal time constant, $\tau^{*-1} = \sum(\tau_i^{-1}A_i)/10$. [g] Time constant for the dissociation process(es). [h] Percent of equilibrium absorbance change accounted for by each rate process. [i] ND: not determined.

able to react with 9-chloroacridine to give small amounts of contaminating polymethylene diacridines. These could be removed by chromatographic purification of the free base, followed by crystallization of the dihydrochloride salt.

Results

Table I gives biological and physicochemical data for the 24 diacridines studied, divided into four classes A–D depending on the nature of the linker chain. Class A comprises the well-known[9,10,22,23] class of polymethylene-linked diacridines, where the chain is varied from $(CH_2)_4$ to $(CH_2)_{10}$ in order to cover the interchromophore distance necessary to allow various possible types of intercalation (mono, one-base-pair bis, two-base-pair bis). In class B two CH_2 units of the polymethylene chain have been replaced by a CONH to give more water-soluble derivatives joined by chains of reduced flexibility. Class C comprises a variety of charged linker groups of varying length and rigidity, while group D is a novel class of diacridines containing a neutral, rigid and yet water-soluble dicarbamoylpyrazole moiety.

The interligand distances were estimated from Courtauld models, when the linker chain was in the most fully extended and staggered position, which permitted the chromophores to be on the same side and reasonably parallel to each other; for known derivatives the distances estimated (Table I) agree well with those proposed by others.[9]

Binding of the compounds to DNA was estimated by measurement of C_{50} values, the micromolar concentration of ligand required to displace 50% of previously bound ethidium from either poly(dA-dT) or poly(dG-dC).[24] For monointercalating agents, which compete directly with the ethidium for binding sites, association constants (K) can be determined from C_{50} values by allowing for drug-induced quenching of fluorescence.[24] For other compounds such as the present bisintercalators, determination of K values is not straightforward, but C_{50} values can provide a valuable comparative measure of drug–DNA binding and selectivity.

Unwinding angles were determined with covalently closed circular plasmid PML-21 DNA and the procedures detailed in ref 25.

Table II gives kinetic data for the interaction of 9-aminoacridine and selected diacridines of Table I with DNA, as determined by high-field NMR[27] and stopped-flow spectrophotometry (see Experimental Section).

Class A. The first two compounds (1 and **2**) have a linker chain so short as to preclude their binding by bisintercalation, as shown by the unwinding and DNA-binding data of Table I, and previously by others.[9,22] In addition to the observed 10-fold increase in binding on going from the monomer 9-aminoacridine to the short dimers, a further 4-fold increase occurs when the chain becomes long enough to permit bisintercalation. In agreement with previous statements[10] that bisintercalation is necessary for these compounds to show cytotoxicity, there is an enormous increase in in vitro activity against L1210 leukemia (more than 30-fold) as the chain is lengthened from $(CH_2)_5$ to $(CH_2)_6$, the minimum distance found necessary for bisintercalative binding. However, it should be noted that bisintercalation for the $(CH_2)_6$ compound (**3**), although clearly established by hydrodynamic assays, involves some steric strain (electric dichroism shows an average angle between the chromophores and the DNA base pairs of 10° for compound **3**, compared with zero for higher homologues[22]). Furthermore, this compound on bisintercalation must violate the "nearest-neighbor exclusion" principle[26] by binding with its two chromophores on either side of a base pair. Since high-field NMR studies[27] show no sign of the doubly shifted DNA resonances that would be expected for such a complex, but rather evidence of monointercalation, the binding mode

(22) Wakelin, L. P. G.; Romanos, M.; Chen, T.-K.; Glaubiger, D.; Canellakis, E. S.; Waring, M. J. *Biochemistry* **1978**, *17*, 5057.
(23) Denny, W. A.; Baguley, B. C.; Cain, B. F.; Waring, M. J. In "Molecular Aspects of Anticancer Drug Action"; Neidle, S., Waring, M. J., Eds.; Macmillan: London, 1983; pp 1–34.
(24) Baguley, B. C.; Denny, W. A.; Atwell, G. J.; Cain, B. F. *J. Med. Chem.* **1981**, *24*, 170.
(25) Baguley, B. C.; Ferguson, L. R.; Denny, W. A. *Chem.-Biol. Int.* **1982**, *42*, 97.
(26) von Hippel, P. H.; McGhee, J. D. *Ann. Rev. Biochem.* **1972**, *41*, 231.
(27) Assa-Munt, A.; Leupin, W.; Denny, W. A.; Kearns, D. R. *Biochemistry* **1985**, *24*, 1449.

of this compound under therapeutic conditions in vivo is unclear. The $(CH_2)_7$ derivative (4) is the first member of the series that clearly shows unstrained, bisintercalative binding by both hydrodynamic[22] and NMR[27] techniques, and it is also the most cytotoxic in vitro (ID_{50} = 50 nM). Compounds 5–7, with longer linker chains, also bind by bisintercalation (Table I and ref 22), but cytotoxicity decreases, possibly due to increased lipophilicity.

Several polymethylenediacridines (including compounds 3–7 of Table I) have shown in vivo P388 antileukemic activity when given on a qd 1–9 schedule,[10] whereas the corresponding monointercalator 1 was inactive. On the basis of these tests, the $(CH_2)_6$ derivative (3) underwent preclinical evaluation at NCI but was dropped due to combined problems of insolubility, marginal activity, and CNS toxicity.[16] In agreement with these latter findings, we find no significant in vivo P388 activity for any of the polymethylenediacridines 1–7 (Table I), using a more stringent q4d × 3 protocol and employing solutions rather than suspension of the drug. Recent studies[28] have also shown these compounds to possess very high affinities for α-adrenoreceptors.

Class B. In an effort to increase the water solubility of the diacridines, a series of compounds linked by amide-containing side chains was prepared. The chain lengths (N–N distances measured assuming a coplanar CONH moiety) span the critical distance required for bisintercalative binding in the polymethylene series. Unwinding angles for the longer derivatives were 35–40°; the increase over that seen for polymethylenediacridines (30–33°) (Table I) suggests some additional binding role for the polar amide group. The shortest member of the series (8, N–N distance 7.7 Å) did not appear to bind by bisintercalation in the unwinding assay, and one of the next shortest members (10), although appearing to bis-intercalate under our conditions, has been shown by NMR to monointercalate to the oligomer $d(AT)_5{\cdot}d(AT)_5$.[27] Thus, for both series, unstrained bis-intercalative binding requires a chain length of 10 Å or above.

Measured Rm values for the amide diacridines were much reduced over those for the polymethylene compounds of similar nominal length (an average difference of 0.45 ± 0.07 Rm unit). This corresponds to a difference of 1.65 log P units[29] (compared to a difference of about 2.0 log P units calculated for the replacement of CH_2CH_2 by CONH by the fragment constant method[30] with polar proximity corrections) and is reflected in a large increase in water solubility.

However, the biological activity of these compounds was disappointing. ID_{50} values for in vitro cytotoxicity were over 3000 nM, and none of the compounds showed in vivo activity although many were quite toxic (Table I). It seems unlikely that the low activity was due to rapid cleavage of the amide bond, for related diacridines with two amide linkages in the side chain proved much more potent.[31]

Class C. Since one of the reasons for the low activity of the flexible-linked diacridines was considered to be the unfavorable kinetics of their interaction with DNA (see Discussion and ref 27), a number of diacridines with charged side chains were investigated. The compounds with monocharged side chains (14–16) all had linker distances of about 10–11 Å and were bound as expected by bisintercalation (Table I). Their lipophilicities were comparable to those of the amide-containing diacridines, and the compounds proved very water soluble. As expected for the addition of another cationic charge, binding to DNA is enhanced considerably (about 3-fold) over that of the polymethylene derivatives.

The next four compounds (17–21) contained doubly charged side chains. The first two were relatively flexible linear alkanediamines, while 19 and 20 were the corresponding piperazine derivatives, designed to place the two charges in the same position with respect to the chromophores while providing a more rigid backbone. Finally, the known[32] spermine derivative (21, BAS) was also prepared and included. Interestingly, the affinity for DNA (measured by C_{50} values) was no greater than that for compounds 14–16, in spite of an additional cationic charge. Unwinding angles for compounds 19–21, which contain the $(CH_2)_nY(CH_2)_n$ grouping (Y = 1,4-piperazinediyl; n = 2, 3), were unexpectedly low. However, the binding affinities (as estimated by C_{50} values) were identical with those for the homologues 17 and 19. The lowest unwinding angle (14°) was obtained for BAS (21), but independent NMR studies[27] indicate that this compound does bisintercalate into the oligonucleotide $d(AT)_5{\cdot}d(AT)_5$. This suggests that all four compounds are in fact bisintercalators. In vitro ID_{50} values for these compounds are surprisingly high, and the compounds are on average less potent than the corresponding polymethylene derivatives. None of the compounds proved active in vivo, although all were toxic at or below 10 mg/kg.

Class D. The final class of compound was designed to possess conformationally restricted linker chains with no additional charged functionality yet be sufficiently polar to ensure adequate water solubility for the resulting diacridines. In order to retain the annular geometry required for bis-intercalative binding, various meta-disubstituted benzene derivatives were considered but rejected as likely to provide relatively insoluble derivatives. Finally, the 1-methylpyrazole-3,5-dicarboxylic acid was chosen as having the acceptable geometry and rigidity, while retaining good aqueous solubility. The resulting diacridines (22–24) proved much more water soluble than the corresponding polymethylene compounds of similar chain separation. Thus, compound 22 is 0.75 Rm unit (approximately 3.3 log P units[29]) more hydrophilic than the polymethylene derivative of corresponding chain length (7).

While the unwinding angles of 36 and 29° for the longer derivatives 23 and 24 are indicative of full bis-intercalation, the value of 25° for the shortest analogue 22 is a little low. However, 22 (BAPY) has been shown[27] by NMR to fully bisintercalate to the oligomer $d(AT)_5{\cdot}d(AT)_5$, under conditions that appear to be more severe for bis-intercalation than those of the unwinding assay. Thus, the compound is classified as a bisintercalator.

The pyrazole-linked diacridines show high levels of in vitro cytotoxicity and are the only class of diacridines to show significant, reproducible in vivo P388 activity by the q4d × 3 protocol.

Discussion

The diacridines of Table I all bind very tightly to DNA, the majority by bisintercalation. Although, as noted, it is not possible to determine accurate association constants from C_{50} values for bisintercalating compounds, the mea-

(28) Adams, A.; Jarrot, B.; Elmes, B. C.; Denny, W. A.; Wakelin, L. P. G. *Mol. Pharmacol.* **1985**, *27*, 480.
(29) Denny, W. A.; Cain, B. F. *J. Med. Chem.* **1978**, *21*, 430.
(30) Hansch, C.; Leo, A. J. "Substituent Constants for Correlation Analysis in Chemistry and Biology"; Wiley Interscience: New York, 1979.
(31) Atwell, G. J.; Baguley, B. C.; Wilmanska, D.; Denny, W. A. *J. Med. Chem.*, in press.
(32) Le Pecq, J.-B.; Le Bret, M.; Barbet, J.; Roques, B. P. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2915.

sured values suggest association constants above 10^6 M^{-1}. Although it is to be expected that variations in linker chain will lead to varying degrees of self-stacking of such dimeric ligands, no significant detrimental effects on DNA binding is apparent. At least some members of all four series A–D have lipophilicities within the Rm range of −0.3 to +0.1 covered by the pyrazole compounds **22–24**, but only the latter group show significant in vivo antitumor activity. The main differences in the DNA interactions of members of the different series appear to be kinetic ones.

Table II summarizes various kinetic parameters for 9-aminoacridine and for representative members of each of the series of diacridines A–D. The high-field NMR data[27] and the stopped-flow data reveal quite different aspects of the kinetics of DNA–ligand interaction. The stopped-flow studies, in which the free drug is sequestered by detergent, measure the time courses of processes leading to complete dissociation of the drug from the DNA. By contrast, the NMR measurements provide direct estimates of the average lifetime of the chromophores in the intercalated state. For the compounds investigated here, the stopped-flow studies show that the ligands dissociate by complex mechanisms involving at least two, and mostly three, steps in the pathway from fully bound to free ligand. Lack of information as to whether the observed processes represent steps in sequential or parallel dissociation schemes (e.g., dissociation by disengagement of one chromophore first followed by the other or dissociation by release of both chromophores simultaneously from different sites on the DNA) allows only a limited quantitative interpretation of the data. To provide an approximate comparison with the results of the NMR measurements, the stopped-flow data have been expressed in terms of the weight-averaged reciprocal time constant, which gives a single parameter representing a mean rate of complete dissociation of the ligand.

For the 9-aminoacridine monomer, the weight-averaged reciprocal time constant is comparable to the jump rate between binding sites seen by NMR, suggesting that dissociation of the molecule from a particular intercalation site usually results in complete dissociation from the DNA (and thus in sequestration by detergent in the stopped-flow assay).

Linking two 9-aminoacridine chromophores together by a flexible polymethylene chain long enough to permit bisintercalation (compounds **5** and **7**) increases the average site residence time of each individual chromophore, but only by about 2-fold (Table II). Thus, the kinetic behavior of the individual chromophores is only slightly affected by the flexible linker chain. In contrast, the average dissociation rates of the complete molecules from the DNA are drastically reduced (at least 30-fold). This is presumably because dissociation of *both* chromophores is required before detergent sequestration can occur, and this is a relatively rare occurrence compared to the dissociation of a single chromophore. These data imply there is the potential for significant "creeping" of the flexible diacridines along the DNA, with an average of at least 15 dissociations and reassociations of each individual chromophore for every complete dissociation of the diacridine from the DNA.

Introduction of the polar (and potentially H-bonding) amide into the linker chain affects the geometry of the DNA–ligand complex, as shown by larger helix unwinding angles, reduces binding affinity, and results in faster complete dissociation of the ligand (Tables I and II).

As expected, additional cationic charges in the side chain increase affinity for DNA (Table I), which is reflected in a further large reduction in average dissociation rates (about 25- to 50-fold compared to the polymethylenediacridines, Table II). While a significant reduction in jump rate also occurs, the rate for BAS (**21**) remains so much higher than the dissociation rates that much "creeping" of the diacridine along the DNA may still occur.

In contrast, the rigid pyrazole-linked diacridine (**22**, BAPY) shows a smaller reduction in average dissociation rates compared to the polymethylenediacridines, (only 10- to 20-fold), coupled with a larger reduction in jump rate (up to 6-fold). Thus, the rigid linker chain greatly increases the average residence time of the individual acridine chromophores (the NMR data in Table II provide *minimum* residence times, estimated from the temperature dependence of DNA imino proton shifts in diacridine–oligonucleotide complexes[27]).

Conclusions

The biological and kinetic data discussed above reinforce earlier conclusions[5,23,32] that an important parameter in determining the antitumor activity of intercalating agents is the residence times of the drug on the DNA, rather than the overall binding affinity. The diacridines are thought to act at the cellular level by inhibition of initiation of nucleic acid synthesis.[31,33] The advantage of compounds that possess long residence times at a particular site, thus providing potentially damaging blocks to polymerase binding and progression, is apparent. While the compounds of class C, with cationic side chains, do show somewhat longer residence times, they are toxic but inactive in vivo, probably due to poor distribution and/or uptake associated with their polycationic nature. The pyrazole-linked diacridine (**22**, BAPY) with a linker chain sufficiently rigid to confer desirable DNA binding kinetics and sufficiently polar to ensure good water solubility of the resulting compound, is the most biologically active diacridine yet described. These results suggest directions for future work on developing bis- and polyintercalators as antitumor agents.

Experimental Section

Chemistry. Where analyses are indicated only by symbols for the elements, results obtained for these elements were within ±0.4% of the theoretical. Analyses were carried out at the Microchemical Laboratory University of Otago, Dunedin, New Zealand, under the direction of Professor A. D. Campbell. Melting points were determined on an Electrothermal apparatus using the manufacturer's stem-corrected thermometer and are as read.

1-Methylpyrazole-3,5-dicarboxylic Acid (VI). Pyrazole-3,5-dicarboxylic acid (10.3 g, 66 mmol) was treated with dimethyl sulfate (29 g, 231 mmol) and potassium carbonate (37 g, 264 mmol) in refluxing acetone for 24 h. The cooled mixture was filtered and the filtrate evaporated to small volume. The residue was partitioned between ethyl acetate and 1 N HCl and the organic layer dried and evaporated to yield an orange solid. Washing with water removed much of the color, and the dried residue was crystallized from ethyl acetate–ether as white needles: 6.5 g (50%); mp 72–73 °C (lit.[34] mp 71–72 °C).

Hydrolysis of the diester in refluxing aqueous KOH gave 1-methylpyrazole-3,5-dicarboxylic acid, which crystallized from hot water as prisms, mp 266–268 °C. Anal. ($C_6H_6N_2O_4$) C, H, N.

Preparation of Diamines V (Example of a General Procedure). *N*-(Benzyloxycarbonyl)-1,3-diaminopropane[20] (2.08 g, 10 mmol) and *N*-(benzyloxycarbonyl)-3-aminopropionic acid (2.34 g, 10.5 mmol) were dissolved in dry DMF (12 mL), and diethyl phosphocyanidate[21] (1.95 g, 12 mmol) was added at 0 °C, followed by NEt_3 (1.21 g, 12 mmol). The mixture was stirred for 30 min

(33) Canellakis, E. S.; Bellantone, R. A. *Biochim. Biophys. Acta* **1976**, *418*, 289.
(34) Thomas, A. F.; Marxer, A. *Helv. Chim. Acta* **1958**, *41*, 1898.

Table III. Analytical Details for the New Compounds of Table I

no.	mp, °C	formula	anal.
8	302–303	$C_{30}H_{25}N_5O\cdot 2HCl$	C, H, N, Cl
9	218–219	$C_{31}H_{27}N_5O\cdot 2HCl\cdot 0.5H_2O$	C, H, N, Cl
10	268–269	$C_{31}H_{27}N_5O\cdot 2HCl\cdot 0.5H_2O$	C, H, N, Cl
11	199–202	$C_{32}H_{29}N_5O\cdot 2HCl\cdot 0.5H_2O$	C, H, N, Cl
12	210–213	$C_{32}H_{29}N_5O\cdot 2HCl$	C, H, N, Cl
13	309–311	$C_{33}H_{31}N_5O\cdot 2HCl$	C, H, N, Cl
15	239–242	$C_{33}H_{33}N_5\cdot 3HCl\cdot H_2O$	C, H, N, Cl
18	230–235	$C_{34}H_{36}N_6\cdot 4HCl$	C, H, N, Cl
19	261–263	$C_{34}H_{30}N_6\cdot 4HCl$	C, H, N, Cl
20	299–301	$C_{36}H_{38}N_6\cdot 4HCl$	C, H, N
22	240–243	$C_{36}H_{32}N_8O_2\cdot 2HCl$	C, H, N, Cl
23	211–214	$C_{38}H_{36}N_8O_2\cdot 2HCl$	C, H, N, Cl
24	185–188	$C_{40}H_{40}N_8O_2\cdot 2HCl$	C, H, N, Cl

Table IV. Analytical Details for *N,N'*-Bis(benzoyloxycarbonyl)diamines IV and VII

formula	x	y	mp, °C	formula	anal.
IV	1	2	155–157	$C_{20}H_{23}N_3O_5$	C, H, N
IV	1	3	123–124	$C_{21}H_{25}N_3O_5$	C, H, N
IV	2	2	185–187	$C_{21}H_{25}N_3O_5$	C, H, N
IV	2	3	155–156	$C_{22}H_{27}N_3O_5$	C, H, N
IV	3	2	150–152	$C_{22}H_{27}N_3O_5$	C, H, N
IV	3	3	134–135	$C_{23}H_{29}N_3O_5$	C, H, N
VII		2	211–212	$C_{26}H_{30}N_6O_6$	C, H, N
VII		3	114–115	$C_{28}H_{34}N_6O_6$	C, H, N
VII		4	154–155	$C_{30}H_{38}N_6O_6$	C, H, N

at 20 °C and then 15 min at 90 °C, before being concentrated to half-volume and shaken with aqueous $KHCO_3$. The resulting solid was washed well with aqueous $KHCO_3$ and water and crystallized from aqueous EtOH to provide pure $(Cbz)_2$ derivative IV (x = 2, y = 3) as prisms: mp 155–156 °C; 3.33 g (81%). Anal. ($C_{22}H_{27}N_3O_5$) C, H, N.

Similar reaction of the appropriate *N*-(benzyloxycarbonyl)alkanediamine with the appropriate *N*-(benzyloxycarbonyl)-aminoalkanoic acids[35] gave the other derivatives (Table IV).

The above $(Cbz)_2$ compound IV was hydrogenated in MeOH for 8 h at 60 psi (5% Pd/C). Removal of catalyst and volatiles gave a quantitative yield of the desired diamine V (x = 2, y = 3). This compound (and its congeners) were colorless oils, homogeneous on TLC (development with phosphomolybdic acid), and were used directly.

Preparation of Diamines VIII (Example of a General Procedure). A solution of *N*-(benzyloxycarbonyl)ethylenediamine[20] (2.13 g, 11 mmol) and 1-methylpyrazole-3,5-dicarboxylic acid (0.85 g, 5 mmol) in dry DMF (8 mL) was treated at 0 °C with diethyl phosphorocyanidate (1.9 g, 12 mmol) followed by NEt_3 (1.21 g, 12 mmol). The mixture was stirred at 20 °C for 30 min and then at 90 °C for 1 h, cooled, and shaken with excess aqueous $KHCO_3$. The resulting precipitate was washed with aqueous $KHCO_3$ and water and crystallized successively from aqueous DMF and aqueous EtOH to give the pure $(Cbz)_2$ derivative VII (y = 2): mp 211–212 °C; 1.6 g (61%). Anal. ($C_{26}H_{30}N_6O_6$) C, H, N.

Similar reactions using appropriate amines III gave the homologues VII (y = 3) [mp 114–115 °C (from EtOAc–diisopropyl ether); 51% yield] and VII (y = 4) [mp 154–155 °C (from aqueous EtOH); 62% yield] (Table III).

The above $(Cbz)_2$ derivative VII (y = 2) (3 mmol) was dissolved in MeOH (150 mL) and hydrogenated for 12 h (Pd–C, 60 psi). Removal of catalyst and solvents gave a quantitative yield of the diamine VIII (y = 2), homogeneous by TLC. This compound and its congeners were colorless oils or low-melting solids and were used directly.

Preparation of the Diacridines of Table I (General Procedure). A mixture of diamine V (x = 2, y = 3) (0.73 g, 5 mmol) and freshly crystallized 9-chloroacridine (2.24 g, 10.5 mmol) in dry phenol (6 g) was heated at 125 °C for 2 h. (Temperature control is critical, with higher temperatures resulting in green–black impurities.) The cooled solution was diluted slowly with acetone, and the precipitated solid was collected, washed well with acetone, dissolved in water, and basified with ammonia. The resulting crude free base was purified by chromatography on alumina (activity II–III), eluting with CH_2Cl_2–MeOH. Pure fractions were pooled and crystallized from MeOH–EtOAc. The pure free base was then dissolved in MeOH and treated with 2.1 equiv of 12 N HCl, and EtOAc was added to precipitate the dihydrochloride. Crystallization from MeOH–EtOAc gave pure product dihydrochloride V (x = 2, y = 3) (compound 11 of Table I) as water-soluble yellow crystals: mp 199–202 °C; 1.9 g (60%). Anal. ($C_{32}H_{29}N_5O\cdot 2HCl$) C, H, N, Cl.

Similar reaction conditions and purification techniques were used for the other diacridine derivatives (8–13) of Table I, in yields varying from 47 to 78%.

The use of the pyrazolediamines VIII in similar reactions gave the pyrazole-linked diacridines 22–24 (Table I) as yellow, water-soluble gelatinous dihydrochlorides in 58–78% yields.

Stopped-Flow Spectrophotometry. Dissociation rates of DNA–ligand complexes were measured by using the surfactant-sequestration technique.[36] Measurements were performed with a Dionex D110 stopped-flow spectrophotometer coupled via a dc amplifier and double-beam storage oscilloscope to a 64-kbyte Apple II microcomputer equipped with two floppy disk drives, on an 8-bit analogue-to-digital converter and a high-precision clock. Software was developed to permit collection, storage, editing, and analysis of up to 500 points per data set with a wide choice of sampling frequency.[37] The fastest rate of data collection was 0.1 ms/point. The spectrometer was fitted with a 20-mm light path cuvette, giving the apparatus a dead-time of 2 ms, and was operated in transmittance mode with standard (1:1) drive syringes. Measurements were made at wavelengths of 400 nm for 9-aminoacridine and at 411 nm for the diacridines at 20 °C in a buffer comprised of 94 mM NaCl, 10 μM EDTA, and 2 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid adjusted to pH 7.0. With the exception of 9-aminoacridine, where the DNA and ligand concentrations were doubled, solutions of drug–DNA complexes containing 10 μM ligand and 200 μM DNA (concentration expressed in nucleotide pairs) were mixed with a 20 mM solution of sodium dodecyl sulfate. Control experiments were performed to demonstrate that the detergent fully dissociates the DNA–drug complexes and to show that the kinetic results are independent of sodium dodecyl sulfate concentration. No optical transients were detected when DNA-free drug solutions were mixed with the detergent.

Acknowledgment. The authors thank Georgina Stewart for determining many of the unwinding angles, Antoinette Kernohan, Cherry Grimwade, Julie Harris, and Susan Tapp for carrying out the many biological tests, Sally Hill for preparation of the manuscript, and Dr. Nuria Assa-Munt for many helpful discussions. This work was supported by the Auckland Division of the Cancer Society of New Zealand, the Medical Research Council of New Zealand, and the Australian Research Grants Scheme.

Registry No. 1, 58478-33-4; 2, 61732-86-3; 3, 58903-52-9; 4, 61732-87-4; 5, 57780-57-1; 6, 95480-17-4; 7, 21988-21-6; 8, 97614-70-5; 8·2HCl, 97614-69-2; 9, 97614-72-7; 9·2HCl, 97614-71-6; 10, 94731-71-2; 10·2HCl, 97614-73-8; 11, 94731-73-4; 11·2HCl, 97614-74-9; 12, 94731-72-3; 12·2HCl, 97614-75-0; 13, 94731-74-5; 13·2HCl, 97614-76-1; 14, 91790-15-7; 15, 97614-78-3; 15·3HCl, 97614-77-2; 16, 58478-34-5; 17, 97614-79-4; 18, 97614-81-8; 18·4HCl, 97614-80-7; 19, 97614-83-0; 19·4HCl, 97614-82-9; 20, 97614-84-1; 20·4HCl, 86689-09-0; 21, 58478-35-6; 22, 94731-75-6; 22·2HCl, 97614-85-2; 23, 97614-87-4; 23·2HCl, 97614-86-3; 24, 97614-89-6; 24·2HCl, 97614-88-5; II (x = 1), 1138-80-3; II (x = 2), 2304-94-1; II (x = 3), 5105-78-2; III (y = 2), 72080-83-2; III (y = 3), 46460-73-5; III (y = 4), 62146-62-7; IV (x = 1, y = 2), 97614-90-9; IV (x = 1,

(35) Pettit, G. R. "Synthetic Peptides"; Elsevier: New York, 1976; Vol. 4.

(36) Wakelin, L. P. G.; McFadyen, W. D.; Walpole, A.; Roos, I. A. G. *Biochem. J.* **1984**, *222*, 203.

(37) Roos, I. A. G.; Wakelin, L. P. G.; Hakkennes, J.; Coles, J. *Anal. Biochem.* **1985**, *146*, 287.

(38) Baguley, B. C.; Nash R. *Eur. J. Cancer* **1981**, *17*, 671.

y = 3), 97614-91-0; IV (x = 2, y = 2), 97614-92-1; IV (x = 2, y = 3), 97614-93-2; IV (x = 3, y = 2), 97614-94-3; IV (x = 3, y = 3), 97614-95-4; V (x = 1, y = 2), 84354-31-4; V (x = 1, y = 3), 97614-98-7; V (x = 2, y = 2), 97633-39-1; V (x = 2, y = 3), 97614-99-8; V (x = 3, y = 2), 97615-00-4; V (x = 3, y = 3), 97615-01-5; VI, 75092-39-6; VI (Me5), 33146-99-5; VII (y = 2), 97614-96-5; VII (y = 3), 97614-97-6; VII (y = 4), 97633-83-5; VIII (y = 2), 97615-02-6; VIII (y = 3), 97615-03-7; VIII (y = 4), 97615-04-8; pyrazole-3,5-dicarboxylic acid, 3112-31-0; 9-chloroacridine, 1207-69-8.

Evaluation of the Brain-Specific Delivery of Radioiodinated (Iodophenyl)alkyl-Substituted Amines Coupled to a Dihydropyridine Carrier†,§

M. L. Tedjamulia,‡ P. C. Srivastava,* and F. F. Knapp, Jr.

Nuclear Medicine Group, Health and Safety Research Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831.
Received January 10, 1985

To evaluate the potential usefulness of radioiodinated phenylamines attached to dihydropyridine carriers as a means of brain-specific delivery of radiopharmaceuticals, 1-methyl-3-[*N*-[β-(4-[^{125}I]iodophenyl)ethyl]carbamoyl]-1,4-dihydropyridine ([^{125}I]-**9**) and 1-methyl-3-[*N*-(4-[^{125}I]iodophenyl)carbamoyl]-1,4-dihydropyridine ([^{125}I]-**13**) have been prepared by dithionite reduction of the corresponding pyridinium precursors, [^{125}I]-**8** and [^{125}I]-**12**, respectively. Formation of **8** involved coupling of (*p*-aminophenyl)ethylamine with *N*-succinimidyl (1-methyl-3-pyridinio)formate iodide (**4**) followed by transformation to the corresponding *N*-piperidinyl- (**6**) or (diethylamino)- (**7**) triazines that were converted to **8** by treatment with HI. Alternatively, **12** was prepared by initial conversion of (4-aminophenyl)mercuric acetate (**10**) to 4-iodoaniline (**11**) by treatment with I_2 and then coupling with **4**. The radioiodinated quaternary products, **8** and **12**, showed low brain uptake and low brain to blood ratios, whereas the dihydropyridine analogues, **9** and **13**, showed comparatively good brain uptake and good brain to blood ratios in rats. These data demonstrate that dihydropyridine-coupled radiopharmaceuticals can cross the blood–brain barrier and the technique may be useful for the measurement of cerebral blood perfusion.

The use of iodine-123-labeled radiopharmaceuticals for measurement of regional cerebral blood flow by either planar or single-photon computerized tomographic techniques (SPECT) provides valuable clinical information for the identification and evaluation of brain lesions.[1] Many lipophilic organic compounds cross the intact blood–brain barrier, with the resulting distribution pattern reflecting regional blood flow.[2] After intravenous administration, the delivery of such lipophilic substances to the brain is flow limited, and thus, the amount of activity appearing initially in the brain is proportional to the regional blood flow. After equilibrium is reached, many agents are cleared or "washed out" from the brain tissue at a rate directly proportional to regional blood flow. Lipophilic compounds that have reversible permeability to the blood–brain barrier (freely enter and exit) are not optimal for brain imaging due to their rapid clearance. A variety of strategies have thus been pursued to design agents that are rapidly extracted in the first pass and show rapid blood clearance with resulting good brain to blood ratios. The key feature for such agents is to exhibit prolonged cerebral retention with minimal redistribution. In this manner, imaging technologies that take prolonged acquisition periods such as SPECT can be used to qualitatively and potentially quantitatively determine the regional distribution of the tracer, which reflects blood perfusion.

Strategies that have been pursued include the high cerebral extraction of amphetamines. A variety of structurally modified radioiodinated amphetamines have been screened.[3] These studies have resulted in the development of *p*-[^{123}I]iodo-*N*-isopropylamphetamine (IMP),[4,5] which has been shown to be an excellent agent for SPECT studies in humans, exhibiting high cerebral extraction and slow washout.[5-7] The amphetamines apparently bind strongly to high-affinity nonspecific sites. Another strategy involves the "pH shift" approach using radiolabeled amines that are "trapped" in the brain by the slightly lower cerebral pH in comparison to plasma.[8-10] The extension of this concept has resulted in the development of *N,N,N'*-trimethyl-*N'*-[2-hydroxy-3-methyl-[^{123}I]iodobenzyl]-1,3-propanediamine (HIPDM),[11,12] which also shows excellent properties in human studies.[13,14]

A unique approach for brain-specific sustained release of therapeutic drugs has recently been described by Bodor et al.[15,16] (Figure 1). This approach involves the chemical

§ For reprints contact P.C.S., Nuclear Medicine Group, Oak Ridge National Laboratory, P.O. Box X, Oak Ridge, TN 37831.
† Research supported by the Office of Health and Environmental Research, U.S. Department of Energy, under Contract DE-AC05-840R21400 with Martin Marietta Energy Systems, Inc.
‡ Present address: The Upjohn Co., La Porte, TX 77571.

(1) Budinger, T. F. *J. Nucl. Med.* **1980**, *21*, 279.
(2) Oldendorf, W. H. *Proc. Soc. Exp. Biol. Med.* **1974**, *19*, 1182.
(3) Winchell, H. S.; Baldwin, R. M.; Lin, T. H. *J. Nucl. Med.* **1982**, *21*, 940.
(4) Kuhl, D. E.; Barrio, J. R. Huang, S. C.; Selin, C.; Ackerman, R. F., Lear, J. L.; Wu, J. L.; Lin, T. H.; Phelps, M. E. *J. Nucl. Med.* **1982**, *23*, 196.
(5) Hill, T. C.; Holman, B. L.; Lovett, R.; O'Leary, D. H.; Front, D.; Magistretti, P.; Zimmerman, R. E.; Moore, S.; Clouse, M. E.; Wu, J. L.; Lin, T. H.; Baldwin, R. M. *J. Nucl. Med.* **1982**, *23*, 191.
(6) Winchell, H. S.; Horst, W. D.; Braun, L.; Oldendorf, W. H.; Hattner, R.; Parker, H. *J. Nucl. Med.* **1980**, *21*, 947.
(7) Lee, G. E.; Hill, T. C.; Holman, B. L.; Clouse, M. E. *Radiology* **1982**, *145*, 795.
(8) Lasser, N. A.; Hendriksen, L.; Holm, S. *J. Nucl. Med.* **1983**, *24*, 17.
(9) Kung, H. F.; Blau, M. *J. Nucl. Med.* **1980**, *21*, 147.
(10) Kung, H. F.; Blau, M. *J. Med. Chem.* **1980**, *23*, 1127.
(11) Kung, H. F.; Tramposch, K. M.; Blau, M. *J. Nucl. Med.* **1983**, *24*, 66.
(12) Fazio, F.; Gerundini, P.; Lenzi, G. C. *J. Nucl. Med.* **1983**, *24*, P 5 (abst).
(13) Polak, J. F.: Holman, B. L.; Moretti, J-L.; Eisner, R. C.; Lister-James, J.; English, R. J. *J. Nucl. Med.* **1984**, *25*, 495.
(14) Holman, B. L.; Lee, R. G. C.; Hill, T. C.; Lovett, R. D.; Lister-James, J. *J. Nucl. Med.* **1984**, *25*, 25.