Synthesis and Biological Evaluation of [^{125}I]- and [^{123}I]-4-Iododexetimide, a Potent Muscarinic Cholinergic Receptor Antagonist

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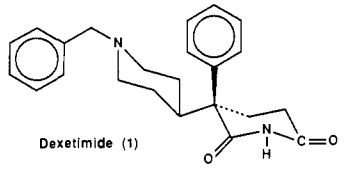
A series of halogenated racemic analogues of dexetimide (1) was synthesized and their affinity for the muscarinic cholinergic receptor measured. One analogue, 4-iododexetimide (21), was efficiently labeled with ^{125}I and ^{123}I at high specific activity. In vitro binding studies and in vivo biodistribution studies suggest that ^{123}I-labeled 21 may be useful for imaging muscarinic cholinergic receptors in the living human brain with single photon emission computed tomography.

Much of the recent interest in imaging muscarinic cholinergic receptors (m-AChR) has been prompted by observations of changes in the regional distribution of m-AChR in some neurodegenerative disorders as measured by postmortem assay.[1] At present only 4-iodoquinuclidinyl benzylate (4-IQNB), labeled with ^{123}I, has been used as a high specific activity radiotracer for the noninvasive imaging of m-AChR in human studies by single photon emission computed tomography (SPECT).[2] However, the yield of 4-IQNB, prepared by the acid-induced triazene decomposition method,[3] is relatively low (18%).[4] Given the current cost of SPECT quality ^{123}I (>$30/mCi), higher yields are necessary for extensive clinical trials to be economically feasible.

The objective of the present work was to design a high-affinity, selective m-AChR antagonist which could be rapidly labeled with ^{123}I in high yield and at high specific activity. Dexetimide ((*S*)-(+)-3-phenyl-3-[(1-phenylmethyl)-4-piperidinyl]-2,6-piperidinedione) (1) is a known



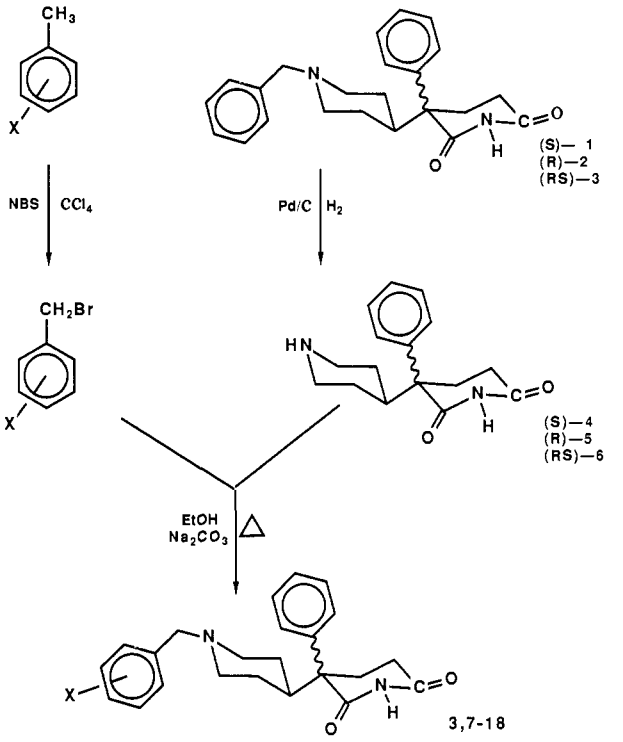
potent m-AChR antagonist which exhibits an appropriate distribution in vitro and in vivo.[5] In vivo binding was shown to be saturable, displaceable, and stereospecific,[6] characteristic of receptor–ligand interactions.[7] Its enantiomer, levetimide (2), has a 1000-fold lower affinity for the m-AChR.

A series of monohalogenated derivatives of racemic dexetimide (i.e., benzetimides) was prepared (Table I) and screened to determine if any analogues, capable of being radiohalogenated, possessed sufficient affinity for the m-AChR to be useful in vivo. Our findings led us to choose the 4-iodinated compound (7) for which we developed two efficient methods of radiolabeling with either ^{125}I or ^{123}I.

Chemistry

Scheme I outlines the synthetic approaches used to prepare the benzetimide derivatives. Norbenzylbenzetimide (6), the precursor to all halogenated analogues, was prepared by the catalytic (Pd/C) hydrogenolysis of benzetimide.[5] The substituted analogues (7–18) were synthesized by N-alkylation of this secondary amine functionality with the appropriate benzyl bromide in ethanol. The benzyl bromides were either obtained from

Scheme I



commercial sources or prepared by benzylic bromination of the appropriately substituted toluene by *N*-bromosuccinimide in CCl_4. Yields (N-alkylation), melting points (mps), HPLC capacity factors (k'), and IC_{50}'s are shown in Table I. 4-(Trimethylsilyl)dexetimide (23) and 4-(trimethylsilyl)levetimide (24) were also prepared according to Scheme I with 4 and 5, respectively, with 4-(tri-

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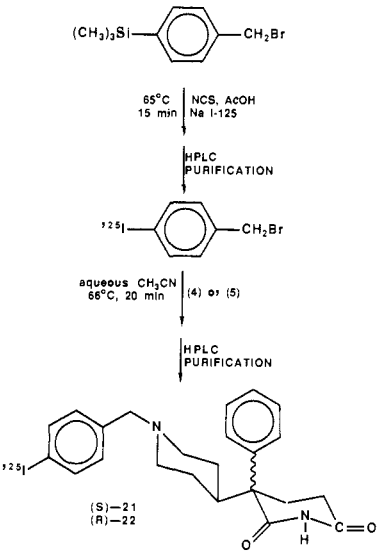
Table I. Analytical and Binding Data for the Substituted Benzetimides[a]

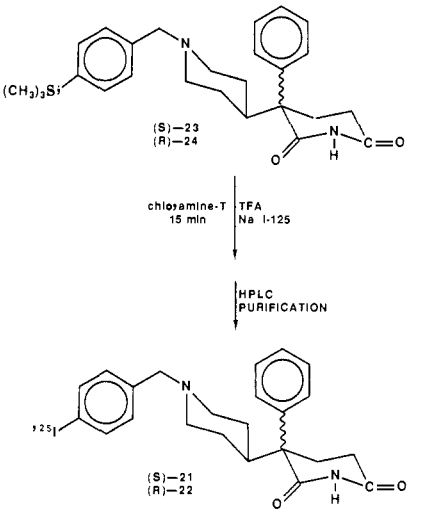
compd	X	mol formula	yield, %	mp,[b] °C	HPLC k'[c]	IC_{50},[d] nM
3	H	$C_{23}H_{27}N_2O_2Cl$	71	281–283[e]	2.2	15
7	4-I	$C_{23}H_{26}N_2O_2ClI \cdot 0.75H_2O$	71	244–246	7.3	17
8	3-F	$C_{23}H_{26}N_2O_2ClF$	97	278–280	2.7	21
9	3-I	$C_{23}H_{26}N_2O_2ClI$	63	305–306	8.4	22
10	3-Br	$C_{23}H_{26}N_2O_2ClBr$	84	297–301	6.9	24
11	3-Cl	$C_{23}H_{26}N_2O_2Cl_2$	63	296–299[f]	5.0	26
12	4-Cl	$C_{23}H_{26}N_2O_2Cl_2 \cdot 0.25H_2O$	65	253–256[g]	4.7	27
13	2-F	$C_{23}H_{26}N_2O_2ClF$	62	278–281	3.3	32
14	4-Br	$C_{23}H_{26}N_2O_2ClBr$	68	275–280	5.7	38
15	4-F	$C_{23}H_{26}N_2O_2ClF$	77	246–249	2.4	98
16	2-Cl	$C_{23}H_{26}N_2O_2Cl_2$	38	279–282	6.0	460
17	2-Br	$C_{23}H_{26}N_2O_2ClBr \cdot H_2O$	80	288–290	9.5	500
18	2-I	$C_{23}H_{26}N_2O_2ClI$	93	275–277	14.7	500

[a] All new compounds gave satisfactory elemental analyses (C, H, N). [b] Of the HCl salts. [c] See Experimental Section for details. [d] Versus [^{3}H]-*N*-methylscopolamine using homogenized rat brain tissue. [e] Literature[21] mp 299–301 °C. [f] Literature[21] mp 296–298 °C. [g] Literature[21] mp 253–256 °C.

Scheme II



Scheme III



methylsilyl)benzyl bromide (**19**) as the alkylating agent.

Radiochemistry

Two methods were used in the synthesis of ^{125}I- and ^{123}I-labeled **21**. The first route (Scheme II) involved the treatment of [^{125}I]- or [^{123}I]-4-iodobenzyl bromide (**20**) with norbenzyldexetimide (**4**) in aqueous CH_3CN at 60 °C, followed by purification by reverse-phase HPLC. Labeled **20** was prepared from **19** as described previously[8] by electrophilic iododesilylation, with sodium [^{125}I]- or [^{123}I]iodide and *N*-chlorosuccinimide in AcOH.[9] An overall radiochemical yield of 50–65% with specific activities of over 600 mCi/μmol was obtained for both ^{125}I and ^{123}I in ca. 2.5 h.

A more direct approach to radiolabeled **21** involves the electrophilic substitution by the radionuclide for the trimethylsilyl group of **23**. Normal conditions[9] were ineffective for this process, however, but the reaction could be carried out with the harsher conditions of trifluoroacetic acid (TFA) with chloramine-T as oxidizing agent (Scheme III). After HPLC purification of the reaction mixture, this convenient one-step procedure gave 65–80% isolated radiochemical yields of [^{125}I]-**21** and somewhat lower yields (55–70%) of [^{123}I]-**21** in a synthesis time of 1 h. Specific activities of between 1000 and 2000 mCi/μmol (^{125}I) and >3000 mCi/μmol (^{123}I) were achieved.

Radiolabeled **21** produced by this method had identical chromatographic properties (HPLC, TLC) with material synthesized via Scheme II and to authentic [^{127}I]-**21**. In addition, essentially indistinguishable biological characteristics (K_{DS}, biodistribution) were found with material from both methods. With the appropriate enantiomeric precursors, ^{125}I- and ^{123}I-labeled 4-iodolevetimide (**22**) were synthesized in identical fashion by both methods.

Potential chemical impurities which could be biologically active, thereby reducing the effective specific activity of the product, include the 4-chloro analogue (**12**) (from chlorodesilylation) and dexetimide itself (from protodesilylation). Potential radiolabeled impurities include the regioisomers **9** and **18**. All could be resolved by the analytical HPLC system (Table I) and none were detected in the final purified product, although dexetimide was a major byproduct in the crude reaction mixture.

Biological Evaluation

Binding assays of the 13 compounds in Table I were carried out in triplicate versus [^{3}H]-*N*-methylscopolamine with mouse brain homogenate tissue. Nonspecific binding (typically 10–15% of total binding) was determined by measuring binding in the presence of 1.0 μM atropine. Details are provided in the Experimental Section. Figure 1 demonstrates the specific binding of [^{125}I]iododexetimide (**21**) as a function of increasing concentrations of unlabeled **21**. Scatchard analysis[10] (Figure 1 insert) yielded a dis-

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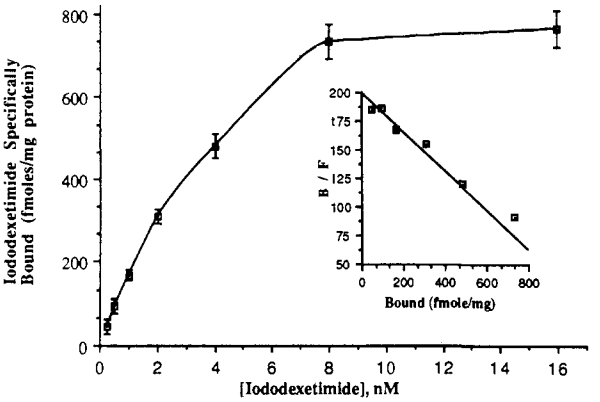

Figure 1. Specific [^{125}I]iododexetimide (**21**) binding as a function of increasing amounts of iododexetimide in mouse brain homogenate (minus cerebellum). Data shown are the mean of two separate experiments performed in triplicate. Errors shown are standard errors. Insert: Scatchard analysis of specifically bound [^{125}I]iododexetimide.

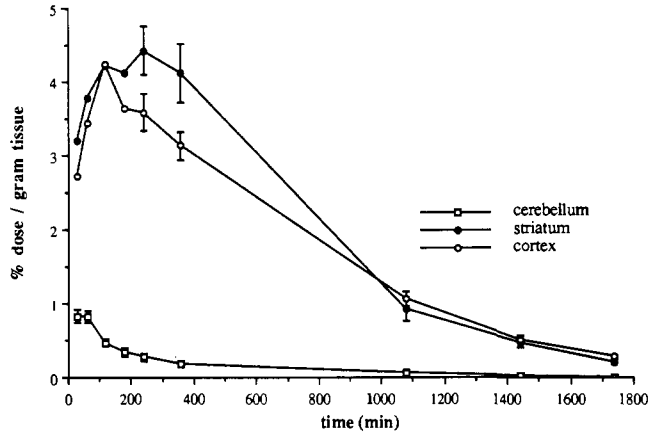

Figure 2. Biodistribution of radioactivity in selected brain regions of mice following administration of [^{125}I]iododexetimide (**21**) (10 μg/kg) as a function of time. Values are the means of four to six experiments. For the sake of clarity, error bars are not shown for the early time points. Errors shown are standard errors.

Table II. [^{125}I]Iododexetimide (**21**) Inhibition Studies

drug	type	IC_{50}, nM
dexetimide	muscarinic	3.3
pirenzepine	muscarinic	750
levetimide	muscarinic	8900
spiperone	dopaminergic	35000
prazosine	adrenergic	13000
ketanserin	seretonergic	130000
naloxone	opioid	500000

sociation constant (K_D) of 5.8 nM and a maximum number of binding sites (B_{max}) of 1.2 pmol/mg of protein.

The selectivity of **21** for the m-AChR was demonstrated by inhibition studies using a variety of drugs with well-characterized neuroreceptor binding properties (Table II). Those ligands possessing affinity for other receptor types (e.g., spiperone) were ineffective in reducing [^{125}I]iododexetimide binding at the concentrations required for specific receptor occupancy.

Biodistribution studies were carried out in male CD-1 mice following literature procedures.[11] Following the intravenous administration of [^{125}I]iododexetimide (**21**) (10

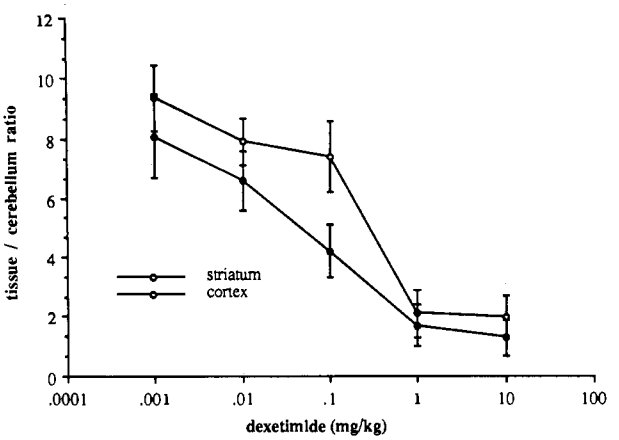

Figure 3. Effect of increasing concentration of dexetimide upon striatum/cerebellum and cortex/cerebellum ratios of radioactivity concentrations. Mice were coinjected with dexetimide and [^{125}I]iododexetimide (**21**) and regions dissected after 120 min. Values are the means of three experiments. Errors shown are standard errors.

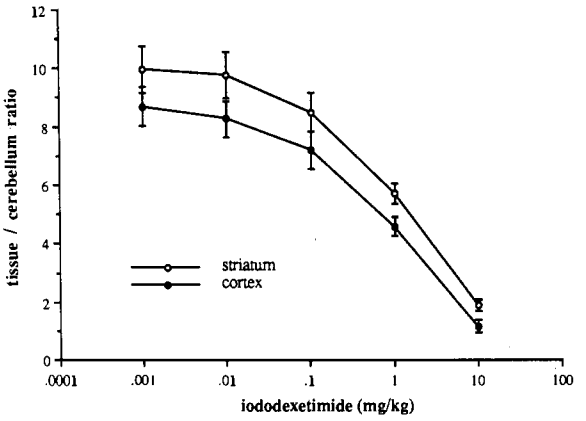

Figure 4. Effect of increasing concentration of iododexetimide upon striatum/cerebellum and cortex/cerebellum ratios of radioactivity concentrations. Mice were coinjected with iododexetimide and [^{125}I]iododexetimide (**21**) and regions dissected after 120 min. Values are the means of three experiments. Errors shown are standard errors.

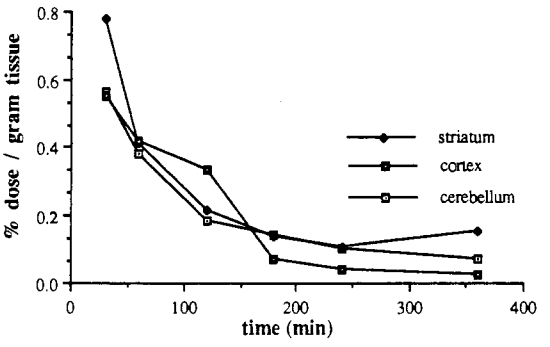

Figure 5. Biodistribution of radioactivity in selected brain regions of mice following administration of [^{125}I]iodolevetimide (**22**) (10 μg/kg) as a function of time. Values are the means of three experiments. For the sake of clarity error bars are not shown; standard errors of ca. 10% were found for all values.

μg/kg), high radioactivity concentrations in the striatum and cerebral cortex and a low radioactivity concentration in the cerebellum were observed as early as 30 min after injection (Figure 2); this distribution corresponds to the known distribution of m-AChR in the brain. Binding in the striatum and the cerebral cortex could be decreased to near cerebellar levels by pretreatment with dexetimide

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(11) Burns, H. D.; Dannals, R. F.; Langstrom, B.; et al. *J. Nucl. Med.* **1984**, *25*, 1222. Frost, J. J.; Smith, A. C.; Kuhar, M. J.; Dannals, R. F. Wagner, H. N., Jr. *Life Sci.* **1987**, *40*, 987.

(Figure 3) but not by levetimide (data not shown), demonstrating pharmacological specificity of **21** binding in vivo.

The saturability of the iododexetimide binding is shown in Figure 4. Pretreatment of mice with increasing amounts of iododexetimide reduced binding in the striatum and cortex regions in a dose-dependent manner.

The stereoselectivity of the m-AChR is demonstrated by the low and uniform radioactivity concentrations found in the brain following the administration of [^{125}I]-iodolevetimide (**22**) (Figure 5). This distribution presumably represents nonspecific binding. Ratios of **21** to **22** (based on cpm/mg of tissue) were 7.5, 8.5, and 1.5 at 1 h postinjection (cerebral cortex, striatum, cerebellum); at 4 h, ratios of 30, 40, and 1.8 were obtained.[12]

Discussion

Of the radiohalogens, ^{123}I is probably closest to ideal for SPECT imaging purposes on the basis of its photon energy, availability, half-life (13 h), and chemical reactivity. Although positron emission tomography (PET) offers superior spatial resolution at present, the use of ^{18}F ($t_{1/2}$ 110 min) requires close proximity between cyclotron and PET scanner. Thus, it was fortuitous that **7** displayed the best binding properties of all the analogues save dexetimide itself. Somewhat surprisingly, the ortho- and parafluorinated analogues (**13**) and (**15**) displayed only moderate affinity for the m-AChR, while **8** with the fluorine in the meta position had only slightly diminished affinity compared with 4-iodobenzetimide and benzetimide itself. This would suggest that interactions between the receptor and the benzyl portion of these molecules are based on more than simple hydrophobic "pocket" effects.

The two-step method, utilizing [^{125}I]- and [^{123}I]-4-iodobenzyl bromide (Scheme II), was a logical entry point to obtaining labeled iododexetimide given our previous work with labeled iodobenzyl bromide[8] and the synthetic route used to obtain iodobenzetimide (Scheme I). However, once the interesting properties of **21** became apparent, the shortcomings of this method were exaggerated. Direct introduction of no carrier added radioactive electrophilic iodine into nonactivated aromatic rings is often achieved by radioiododemetalation using tin, germanium, silicon, or some other ring-bonded metal to activate the aromatic ring and to direct the site of substitution.[13,14] In this context silicon has been slighted as being only mildly activating, requiring forcing conditions[13] to obtain adequate radiochemical yields, and failing when deactivating groups are attached to the aromatic ring.[15] However this lack of reactivity allows more facile handling and incorporation of trialkylsilyl groups into complex organic molecules.[16] Thus 4-(trimethylsilyl)dexetimide (**23**) was readily prepared in good yield by the method shown in Scheme I and has an extended shelf-life (>15 months) as the hydrochloride salt.

Previous conditions (AcOH, *N*-chlorosuccinimide) for radioiododesilylation[8,9] failed when applied to **23**, reflecting the mildly deactivating effect of the protonated methyleneamino group.[17] Near-quantitative incorporation of radioactive iodine could be achieved in minutes at ambient temperature by using chloramine-T as oxidizing agent and the more acidic trifluoroacetic acid as solvent (Scheme III). Although both methods of radiosyntheses are satisfactory, the one-step procedure improves upon radiochemical yield, time of synthesis, and specific activity. Moreover, this method minimizes manipulation of and exposure to radioactive materials as well as lending itself to automation.

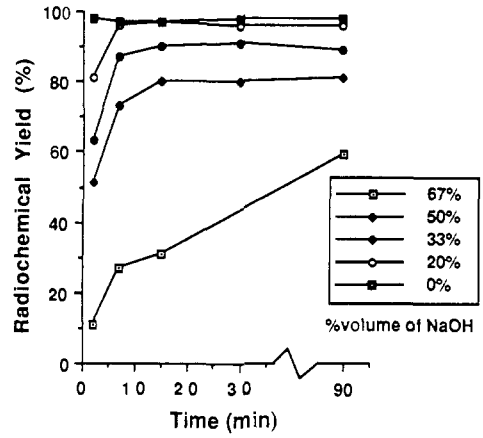

Figure 6. Effect of added 0.1 N aqueous NaOH on radiochemical yield of [^{125}I]iododexetimide (**21**).

[^{123}I]Iodide is most commonly shipped in aqueous solution, usually 0.1 N NaOH or NH_4OH. For many purposes this medium is intolerable and either the water or base or both must be removed prior to reaction. Thus time and often recoverable activity are lost before the chemistry commences. The effect of aqueous 0.1 N NaOH on the radiochemical yields from Scheme III is shown in Figure 6. The reaction proved to be only moderately sensitive to the addition of substantial amounts of aqueous base. Even with a 50/50 (v/v) mixture, yields were reduced only by 15–20%. Thus it is possible in most cases to use the shipped ^{123}I solution directly without further manipulation.

Many parameters determine the usefulness of a radiotracer for neuroreceptor binding studies using emission computed tomography. The excellent target to nontarget ratios and high brain uptake obtained over several hours with [^{125}I]- and [^{123}I]-**21** (Figure 2), together with its convenient preparation, suggest that this compound will be useful in imaging and quantifying levels of m-AChR in the living human brain with SPECT. Iododexetimide demonstrates appropriate pharmacology (Table II), in vivo receptor saturability (Figure 4) and stereospecificity, lending additional support.

An additional advantage of [^{123}I]iododexetimide is the availability of its labeled inactive enantiomer. An estimation of nonspecific binding in neuroreceptor imaging studies of human brain has been done with either areas of the brain which are known to contain very few pertinent neuroreceptors[18] or by blocking studies.[19] Neither approach is totally viable in the study of m-AChR since they are widespread throughout the brain (even the cerebellum has some[20]) and m-AChR antagonists have pronounced pharmacological action, precluding blocking studies. As **21** and **22** are enantiomers and labeled **22** shows only uniform nonspecific binding in the brain, the nonspecific binding component of [^{123}I]iododexetimide (**21**) may be

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(14) Hanson, R. N.; Blumberg, J. B.; Poddubiuk; Davis, M. A.; Holman, B. L. *Int. J. Appl. Radiat. Isot.* **1981**, *32*, 429.
(15) Moerlein, S. M.; Coenen, H. N. *J. Chem. Soc., Perkin Trans. 1* **1985**, 1941.
(16) Habich, D.; Effenberger, F. *Synthesis* **1979**, 841. Chan, T. H.; Fleming, I. *Synthesis* **1979**, 761.
(17) Cornish, A. J.; Eaborn, C. *J. Chem. Soc., Perkin Trans. 2* **1975**, 874.
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closely approximated by measuring the binding of ^{123}I-labeled iodolevetimide (22).

Experimental Section

All purifications and analyses of radioactive mixtures were performed on an HPLC system composed of a Rheodyne 7125 injector, two Waters 510 EF pumps, a UV detector (Waters 481), and an Ortec flow radioactivity detector. The analytical column was a Waters C18 Novapak (15 cm × 3.9 mm) while semipreparative purifications used a Whatman Partisil ODS-3 RAC column (10 cm × 10 mm). Peak areas were measured with a Hewlett-Packard 3390A recording integrator. Isolated radiochemical yields were determined with a dose calibrator (Capintec CRC-7). [^{125}I]Iodide was obtained from Amersham Corp. (IMS.30) and [^{123}I]iodide was supplied by Crocker National Laboratories. NMR spectra were obtained on an IBM NR/80 using $(CH_3)_4Si$ as an internal standard, and IR spectra were recorded on a Perkin-Elmer 399B instrument. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA. All new compounds gave satisfactory analyses (C, H, N, ±0.4%). Melting points are uncorrected.

(*S*)-(+)-3-Phenyl-3-(4-piperidinyl)-2,6-piperidinedione (4). A solution of dexetimide (1) (1.0 g, 2.76 mmol) in CH_3OH (20 mL) and concentrated aqueous HCl (0.5 mL) was treated with 5% Pd/C (0.5 g) and then shaken at room temperature in a Parr apparatus under 60 psi of H_2 for 3 days. Upon removal of the catalyst by filtration, the filtrate was evaporated to dryness and the residue partitioned between H_2O (20 mL) and $CHCl_3$ (20 mL). The aqueous layer was made basic with NH_4OH and extracted with $CHCl_3$ (20 mL). The combined organic extracts were washed with H_2O (30 mL), dried (Na_2SO_4), and filtered. Evaporation of the organic solution left 4 as a white powder, 0.70 g (93%): mp 186–189 °C (lit.[5] mp 181–183 °C). Anal. ($C_{16}H_{20}N_2O_2$) C, H, N.

(*R*)-(−)-3-Phenyl-3-(4-piperidinyl)-2,6-piperidinedione (5) was prepared in an identical manner to 4 from 2: mp 188–191 °C (lit.[5] mp 180.5–182 °C). Anal. ($C_{16}H_{20}N_2O_2$) C, H, N.

(*R,S*)-3-Phenyl-3-(4-piperidinyl)-2,6-piperidinedione (6) from 3: mp 228–230 °C (lit.[21] mp 230–232 °C). Compounds 4–6 were extremely hygroscopic.

(*S*)-3-Phenyl-3-[1-[[4-(trimethylsilyl)phenyl]methyl]-4-piperidinyl]-2,6-piperidinedione (23). A mixture of 4 (0.50 g, 1.83 mmol) and Na_2CO_3 (0.25 g, 23.6 mmol) in EtOH (10 mL) was stirred at reflux while a solution of 19 (0.45 g, 1.87 mmol) in EtOH (10 mL) was added dropwise over 1 h. Upon stirring for a further 0.5 h the mixture was cooled and the solvent removed. The residue was taken up in $CHCl_3$ (20 mL) and washed with H_2O (20 mL) and aqueous HCl (1 M, 20 mL). The organic solution was dried (Na_2SO_4), filtered, and evaporated to dryness to leave a white powder (0.96 g). This material was triturated with petroleum ether and then dissolved in boiling THF (10 mL) containing sufficient MeOH to clarify the solution. Upon cooling, hexane (25 mL) was added to precipitate a white solid which was collected by vacuum filtration and washed with ice-cold THF. After drying in vacuo 0.597 g (69%) of 23 (HCl salt) was obtained: mp 278–280 °C; IR (KBr) 3355, 1715, and 1695 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.25 (s, 9 H), 1.2–3.7 (m, 13 H), 4.19 (s, 2 H), 7.33 (s, 5 H), 7.54 (s, 4 H), 10.69 (br s, 2 H). Anal. ($C_{26}H_{35}N_2O_2SiCl$) C, H, N.

(*R*)-3-Phenyl-3-[1-[[4-(trimethylsilyl)phenyl]methyl]-4-piperidinyl]-2,6-piperidinedione (24) was prepared in a similar fashion to that for 23 with 5 as precursor: mp 277–279 °C (HCl salt). Spectral characteristics were identical with those of 23. Anal. ($C_{26}H_{35}N_2O_2SiCl$) C, H, N.

(*S*)-3-Phenyl-3-[1-[(4-iodophenyl)methyl]-4-piperidinyl]-2,6-piperidinedione (21). A mixture of 4 (25 mg, 0.092 mmol), 4-iodobenzyl bromide (20) (27.3 mg, 0.092 mmol), and potassium carbonate (12.7 mg, 0.092 mmol) in ethanol (4 mL) was heated to reflux for 3 h and then cooled. The mixture was filtered and solvent removed from the filtrate to leave a yellow solid. This was treated with boiling ether (20 mL) and filtered. The filtrate was washed with water, dried (Na_2SO_4), and filtered. HCl gas was passed through the filtrate and the resultant white precipitate collected by vacuum filtration, dissolved in methanol (5 mL), and filtered and the methanol removed to yield 21 as the HCl salt (43.0 mg, 89%): mp 283–284 °C; IR (KBr) 3205, 1695 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.33–3.15 (m, 13 H), 4.40 (s, 2 H), 7.35–8.00 (m, 9 H), 10.95 (s, 1 H), 11.35 (br s, 1 H). Anal. ($C_{23}H_{26}N_2O_2ClI$) C, H, N.

(*R*)-3-Phenyl-3-[1-[(4-iodophenyl)methyl]-4-piperidinyl]-2,6-piperidinedione (22) was prepared in a similar manner to that for 21 with 5 as precursor: mp 286–287 °C. Anal. ($C_{23}H_{26}N_2O_2ClI$) C, H, N. Spectral characteristics were as for 21.

Halogenated benzetimides (7–18) were prepared in a similar manner with the racemic norbenzylbenzetimide (6) as the precursor and the appropriately substituted benzyl bromides. All new compounds gave satisfactory elemental analyses (C, H, N). HPLC k's were determined with the analytical column with 60/40 CH_3CN/H_2O (0.1 N $NH_4CH_3CO_2$) as mobile phase at a flow of 2 mL/min. These conditions were also used to analyze all purified radiochemical products.

4-Iodobenzyl Bromide (20). A mixture of 4-iodotoluene (23.3 g, 107 mmol), *N*-bromosuccinimide (22.8 g, 127 mmol), and benzoyl peroxide (1.16 g, 4.8 mmol) in dry CCl_4 (35 mL) was stirred and heated to reflux for 3.5 h and then cooled and filtered. The red filtrate was washed with saturated sodium thiosulfate solution (20 mL), dried, and filtered. The solvent was removed to leave a green solid (21.0 g) which was taken up in boiling hexane, filtered, and cooled to precipitate an off-white solid (15.8 g, 49.7%): mp 78–79.5 °C (lit.[22] mp 78.5–79.5 °C).

(*S*)-3-Phenyl-3-[1-[(4-[^{125}I]iodophenyl)methyl]-4-piperidinyl]-2,6-piperidinedione (^{125}I-21). Method A. [^{125}I]-4-Iodobenzyl bromide (20) was prepared by an electrophilic substitution on 4-(trimethylsilyl)benzyl bromide (19) with *N*-chlorosuccinimide in acetic acid as described previously.[8] After purification by HPLC, a solution of 20 (0.02–2 mCi) in 55% aqueous CH_3CN (3–4 mL) was treated with 7.0 mg of 4 and heated to 66 °C for 20 min. The mixture was evaporated to dryness, taken up in 1 mL of HPLC buffer, and applied to the preparative HPLC column eluted with 55/45 CH_3CN/H_2O (0.1 N $NH_4CH_3CO_2$) at 6 mL/min (t_R of 21 9.9 min). The desired fraction was collected, evaporated to dryness, and taken up in 10% aqueous EtOH (5 mL) for binding studies. Isolated yields of 70–80% were obtained (56–68% from [^{125}I]iodide) with specific activities of 600–1200 mCi/μmol.

Method B. A freshly prepared solution of 23 (1.0 mg) and chloramine-T (1.5 mg) in trifluoracetic acid (TFA) (100 μL) was added to a conical vial containing aqueous sodium [^{125}I]iodide (5 μL, 0.5 mCi) and stirred at 20 °C. After 15 min a mixture of concentrated aqueous NH_4OH (70 μL) and HPLC buffer (200 μL) was added to neutralize the TFA. The total contents were then injected onto the preparative HPLC column. The conditions and workup were as described for method A. The isolated radiochemical yields were 65–80% with specific activities between 1000 and 2000 mCi/μmol. [^{125}I]-4-Iodolevetimide (22) was prepared by both methods using the appropriate enantiomeric precursors with similar results.

(*S*)-3-Phenyl-3-[1-[(4-[^{123}I]iodophenyl)methyl]-4-piperidinyl]-2,6-piperidinedione (^{123}I-21) was prepared by method A from ^{123}I-labeled 20.[8] Yields and specific activities were similar to ^{125}I runs.

Method B. The procedure for ^{125}I-labeled 21 (method B) was followed with 12–64 μL of sodium [^{123}I]iodide in 0.1 N NaOH (2–15 mCi). Upon evaporation of the collected HPLC fraction, the residue was taken up in 7 mL of 10% aqueous EtOH and passed through a Millipore filter into a sterile, pyrogen-free bottle. Isolated radiochemical yields of 55–70% at specific activities of above 3000 mCi/μmol were obtained.

Radioiodinations with Added Base. A freshly prepared solution of 23 (1.0 mg) and chloramine-T (1.5 mg) in TFA (100 μL) was added to a solution of sodium [^{125}I]iodide (2 μL, 0.2 mCi) and aqueous 0.1 N NaOH (0–200 μL) in a conical vial. The mixture was stirred at 20 °C, and aliquots (5 μL) were removed periodically and quenched with 5% aqueous $Na_2S_2O_5$ solution. HPLC analyses of the quenched solutions were carried out with the analytical column eluted with 60/40 CH_3CN/H_2O (0.1 N

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$NH_4CH_3CO_2$) at 2 mL/min and peak areas measured. Yields (Figure 1) are given as area (product)/area (all peaks).

Determination of Specific Activities and Radiochemical Purities. An aliquot of the solution was transferred to a thin glass vial and its radioactivity measured with a dose calibrator. Another aliquot (of the same volume) was removed by the same syringe and injected onto the analytical HPLC column and the UV peak area of the product measured. The mass of the product was calculated by comparison to a standard curve. The sensitivity of the analyses was maximized by setting the wavelength of the UV detector to 239 nm—the λ_{max} of **21**. The specific activity in mCi/μmol was then calculated by dividing the number of millicuries in the aliquot by the number of micromoles in the same aliquot. The radiochemical purity of the final product was always >99%; some hydrophilic radioactive impurities (13%) were detected in ^{125}I-labeled **21** following storage for 3 months at −10 °C.

Binding Assays. Male CD-1 mice were killed, and the whole brain minus cerebellum was rapidly homogenized in 50 mM phosphate-buffered saline (pH = 7.4, 37 °C). The tissue homogenate (8 mg/mL buffer) was then incubated with 1 nM [^{3}H]-*N*-methylscopolamine (New England Nuclear, 70 Ci/mmol) alone or in the presence of 1.0 μM atropine and in the presence of increasing concentrations of the compounds listed in Table I. Samples were incubated for 30 min at 37 °C in a shaking water bath to achieve equilibrium. All samples were incubated in triplicate. Samples containing atropine defined nonspecific binding.

Following the incubation period samples were rapidly filtered over Whatman glass fiber filters under vacuum and rapidly washed with 15 mL of ice-cold phosphate-buffered saline. Filters were then counted in the presence of 10 mL of Formula 963 scintillation fluid (New England Nuclear) by using standard techniques at efficiencies of ca. 40%. Radioactivity in the presence of atropine (nonspecific binding) was subtracted from that in other samples to compute net specific binding. Net specific binding at each drug concentration was then expressed as percent of control specific binding. Log-logit analysis was then used to compute the 50% inhibitory concentrations (IC_{50}) for each test compound. Dexetimide was always studied in parallel with other compounds to assure experimental reproducibility. The values shown in Table I represent average results from two to three experiments; values varied less than 20%. Saturation experiments using [^{125}I]- and [^{123}I]iododexetimide were carried out in a similar fashion using 10^{-6} M atropine to define nonspecific binding.

Biodistribution studies were carried out on male CD-1 mice according to literature procedures.[11]

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Registry No. (*S*)-**1**, 21888-98-2; (*R*)-**2**, 21888-99-3; (*R,S*)-**3**, 119391-55-8; (*R,S*)-**3**·HCl, 6767-69-7; (*S*)-**4**, 115216-87-0; (*R*)-**5**, 115216-88-1; (*R,S*)-**6**, 119477-43-9; (*R,S*)-**7**, 119391-56-9; (*R,S*)-**7**·HCl, 119391-73-0; (*R,S*)-**8**, 119391-57-0; (*R,S*)-**8**·HCl, 119391-74-1; (*R,S*)-**9**, 119391-58-1; (*R,S*)-**9**·HCl, 119391-75-2; (*R,S*)-**10**, 119391-59-2; (*R,S*)-**10**·HCl, 119391-76-3; (*R,S*)-**11**, 119391-60-5; (*R,S*)-**11**·HCl, 119391-77-4; (*R,S*)-**12**, 119391-61-6; (*R,S*)-**12**·HCl, 119391-78-5; (*R,S*)-**13**, 119391-62-7; (*R,S*)-**13**·HCl, 119391-79-6; (*R,S*)-**14**, 119391-63-8; (*R,S*)-**14**·HCl, 119391-80-9; (*R,S*)-**15**, 119391-64-9; (*R,S*)-**15**·HCl, 119391-81-0; (*R,S*)-**16**, 119391-65-0; (*R,S*)-**16**·HCl, 119391-82-1; (*R,S*)-**17**, 119391-66-1; (*R,S*)-**17**·HCl, 119391-83-2; (*R,S*)-**18**, 119413-94-4; (*R,S*)-**18**·HCl, 119391-84-3; **19**, 17903-42-3; **20**, 16004-15-2; **20** (^{125}I), 105644-30-2; **20** (^{123}I), 105644-31-3; **21**, 119478-57-8; ^{125}I-**21**, 119391-69-4; ^{123}I-**21**, 119391-70-7; **22**, 119477-44-0; ^{125}I-**22**, 119391-71-8; ^{123}I-**22**, 119391-72-9; (*S*)-**23**, 119391-67-2; (*R*)-**24**, 119391-68-3; 4-iodotoluene, 624-31-7; 3-fluorobenzyl bromide, 456-41-7; 3-iodobenzyl bromide, 49617-83-6; 3-bromobenzyl bromide, 823-78-9; 3-chlorobenzyl bromide, 766-80-3; 4-chlorobenzyl bromide, 622-95-7; 2-fluorobenzyl bromide, 446-48-0; 4-bromobenzyl bromide, 589-15-1; 4-fluorobenzyl bromide, 459-46-1; 2-chlorobenzyl bromide, 611-17-6; 2-bromobenzyl bromide, 3433-80-5; 2-iodobenzyl bromide, 40400-13-3.

Aromatic Dienoyl Tetramic Acids. Novel Antibacterial Agents with Activity against Anaerobes and Staphylococci[1]

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Streptolydigin (1) and tirandamycin A (2) are typical members of the naturally occurring class of 3-dienoyl tetramic acids. These compounds, which possess potent antibacterial activity particularly against anaerobes, have been shown to inhibit bacterial RNA polymerase. In contrast, tenuazonic acid (5), which lacks a complex dioxabicyclononane moiety and diene chromophore present in 1 and 2, exhibits essentially no antimicrobial activity and has no effect on bacterial RNA polymerase, suggesting that one or both of these structural features may be critical for antibacterial activity. In this paper, we report on a novel series of synthetic dienoyl tetramic acids that lack a complex dioxabicyclononane unit. Several of these compounds, particularly **8T–W**, exhibit potent antimicrobial activity against Gram-positive and Gram-negative anaerobes as well as staphylococci. We will discuss the structure–activity relationship for this series of compounds which, in contrast to their natural counterparts, do not inhibit significantly RNA polymerase. We will also discuss preliminary results on the biochemical and microbiological properties of this series of compounds, several of which moderately inhibit supercoiling by DNA gyrase isolated from *E. coli* H560, although this enzyme has not been established as their target in whole cells. Compound **8W**, which is not cross-resistant with DNA gyrase subunit A or B inhibitors or tirandamycin, has also been demonstrated to be rapidly bactericidal.

Streptolydigin (**1**),[2] tirandamycin A (**2**),[3] BU2313A (**3**), and BU2313B (**4**)[4] are members of the naturally occurring class of 3-dienoyl tetramic acids. These compounds possess potent antibacterial activity, particularly against anaerobes and some Gram-positive aerobes. Streptolydigin and tirandamycin A have been shown to be inhibitors of bacterial RNA polymerase.[3,5] In contrast, tenuazonic acid

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