Motility counts were converted to scores as follows: Score 0 was given to animals showing 0–49 counts, score 1 to animals showing 50–149 counts (range of spontaneous activity of normal untreated rats), and score 2 to rats with counts above 150, indicating hyperactivity. ED_{50} was calculated by log Probit analysis as the dose inducing half-maximal score.

Antagonism of Apomorphine-Induced Stereotypy in Rats. The rats were placed in wire mesh cages the day before the experiment. Test drug or saline was injected sc 30 min before apomorphine (8.2 μmol = 2.5 mg/kg, sc), and the occurrence of oral stereotyped behavior (licking, biting) was observed at 15-min intervals for 90 min. ED_{50} values were calculated on the basis of the observations made 30 min after apomorphine administration, where activity was maximal in the control group.

Acknowledgment. We thank P. Bregnedal, H. Nyegaard, T. Halborg, B. Smedegaard, and S. Jacobsen for excellent technical assistance. Dr. J. Perregaard is thanked for valuable discussions regarding the NMR data. Dr. K. E. Larsen is thanked for analytical assistance. Dr. N. Gawadi and S. Henriksen are thanked for their help with correcting and preparing the manuscript, respectively.

Registry No. 1, 104779-11-5; 2, 27257-46-1; 3, 2407-98-9; 4, 10447-21-9; 5a, 104779-12-6; 5e, 104779-27-3; 6a, 53072-37-0; 6e, 57120-85-1; 7, 104779-13-7; 8, 46946-53-6; 9, 104779-13-7; 10, 57120-90-8; 11, 104779-15-9; 11 (8–8a olefinic isomer), 104779-35-3; 12, 104779-16-0; 13a, 104779-17-1; 13e, 104779-28-4; 14a, 57120-80-6; 14e, 57120-75-9; 14e·HCl, 104779-37-5; 15a, 104779-18-2; 15a·oxalate, 104779-36-4; 15e, 104779-29-5; 16a, 104779-19-3; 17a, 104779-39-7; 17a·HBr, 104779-20-6; 17e·HBr, 104779-30-8; 18a, 104779-21-7; 18a·oxalate, 104779-22-8; 18e, 104779-41-1; 18e·HBr, 104779-31-9; 19a, 104779-42-2; 19a·HBr, 104779-23-9; 19e, 104779-43-3; 19e·HBr, 104779-32-0; 19e·(s)-BNPA, 104972-84-1; (+)-19e, 104870-69-1; (+)-19e·HCl, 104972-77-2; (−)-19e, 104870-70-4; (−)-19e·HCl, 104972-78-3; 20a, 104779-44-4; 20a·HBr, 104779-24-0; 20e·HBr, 104779-33-1; 21, 104779-25-1; 22a, 104779-26-2; 22e, 104779-34-2; ethyl 2β-pyrrolidinyl-1-[(ethoxycarbonyl)methyl]propionate, 104779-38-6; ethyl 2-pyrrolepropionate, 55490-37-4; ethyl bromoacetate, 105-36-2; 3-bromoanisole, 2398-37-0; acetic anhydride, 108-24-7.

Supplementary Material Available: Bond distances and angles and positional and thermal parameters (2 pages) and observed and calculated structure factors (21 pages). Ordering information is given on any current masthead page.

Structural Alterations That Differentially Affect the Mutagenic and Antitrichomonal Activities of 5-Nitroimidazoles

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Two approaches have been used to develop nonmutagenic 5-nitroimidazoles. Both approaches are based on knowledge of the likely mechanisms by which this class of compounds cause mutagenicity. The first approach involved incorporating readily oxidizable gallate derivatives into the molecule. In one case, a very weakly mutagenic active antitrichomonal agent was obtained. The second approach involved incorporating a substituent at the C_4 position of the ring. This generally resulted in a large reduction in mutagenicity and a lowering of antitrichomonal activity in vitro. In certain cases, however, mutagenicity was dramatically reduced while moderate antitrichomonal activity was retained. For example, 1,2-dimethyl-4-(2-hydroxyethyl)-5-nitroimidazole (**5**) showed good antitrichomonal activity in vitro (ED_{50} = 2 μg/kg) while possessing only 4% of the mutagenicity of metronidazole.

The 5-nitroimidazoles are a well-established group of protozoal and bactericidal agents. While several drugs in this group are currently available and in wide use,[1,2] as a group they suffer from the property of being mutagenic. Although some hypotheses exist,[3,4] active research in this area has not produced a comprehensive mechanism for the mutagenic and therapeutic activities of these compounds. A body of indirect evidence suggests, however, that nitro group reduction plays a key role in the overall activity of these agents.[5] It is generally believed that nitro reduction is also responsible for the expression of mutagenicity[6] and drug residue formation;[7] although again, with few exceptions,[7-9] the nature of the mutagenic metabolite or its mechanism of formation is unknown. It is presumed, therefore, that the separation of protozoal and genotoxic activities is not feasible, both of these properties being mediated through a common metabolic intermediate.[10] A nitroimidazole possessing good pharmacological activities with no mutagenicity would, therefore, be of great interest not only from a safety point of view but would also provide a basis for further investigations of the mode of action and mechanism of expression of mutagenicity.

We are aware of only a single attempt to differentiate among the structural elements contributing to mutagenicity and antitrichomonal activities as a basis for the rational design of safer nitroimidazoles.[11] It appeared from the structure that this compound might possess antioxidant activity and, although no such property was described, that this property might be the basis for the absence of mutagenicity of this compound. Conceivably, enzymatic one electron nitro reduction to the radical anion could provide an intermediate that would transfer an electron to molecular oxygen, providing a pathway for the production of superoxide and hydroxyl radicals. The latter agent is capable of effecting DNA damage.[12] An anti-

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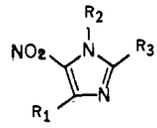
Table I. Structure of 5-Nitroimidazoles



compd	R_1	R_2	R_3
1	CH_3	CH_3	CH_2OCONH_2
2	CH_3	CH_3	4-F-Ph
3	CH_3	CH_2CH_2OH	CH_3
4	CH_3	CH_3	CH_3
5	CH_2CH_2OH	CH_3	CH_3
6	CH_2CH_2OH	CH_3	CH_2OH
7	CH_2CH_2OH	CH_3	4-F-Ph
8	CH_2CH_2OCO-*t*-Bu	CH_3	CH_3
9	$CH_2CH_2OCOCH_3$	CH_3	CH_3
10	Br	CH_3	CH_3
11	F	CH_3	CH_3
12	F	CH_3	H
13	H	$CH_2CH_2OCOR_4$[a]	CH_3
14	H	$CH_2CH_2OCOR_4$[a]	4-F-Ph
15	H	$CH_2CH_2OCOR_5$[b]	CH_3
16	H	CH_3	CH_2OCOR_4[a]
17	H	CH_3	CH_3
18	H	CH_3	CH_2CH_2OH
19	H	CH_3	CH_2OH
20[c]	H	CH_3	CH_2OCONH_2
21	H	CH_3	4-F-Ph
22	CH_3	CH_3	CH_2CH_2OH
23	H	CH_2CH_2OH	CH_3

[a] R_4 = 3,4,5-triacetoxygallyl. [b] R_5 = 3,4,5-trimethoxygallyl. [c] Ronidazole.

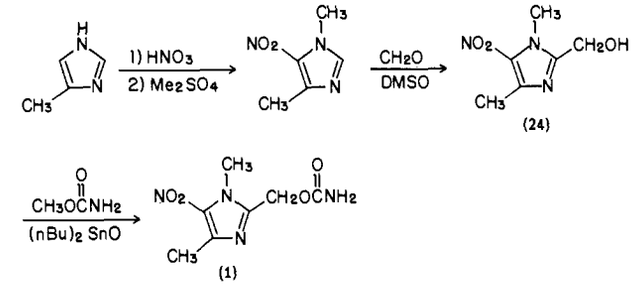
oxidant group might offset this effect and explain the low mutagenic activity. We have, consequently, prepared a number of gallate ester derivatives as potential antioxidant containing nitroimidazoles.

During the course of our work directed at elucidating the mechanism of covalent binding of ronidazole [(1-methyl-5-nitroimidazol-2-yl)methyl carbamate] to tissue constituents,[7,8] it became apparent that the C_4 position of the imidazole ring played a central role in mutagenicity and in disposing this drug to electrophilic addition to protein nucleophiles. It appeared that nitro reduction activated this position to nucleophilic attack, resulting in the generation of an electrophilic species that was responsible for the protein-binding event.[8,9] This observation is not without precedent; the susceptibility of the C_4 position to attack by water is implicated by the nature of the metabolites of metronidazole.[13] To examine this further, the 4-methyl analogue (1) of ronidazole was prepared and indeed found to give only 15% of the protein alkylation and 2% of the mutagenicity of ronidazole, **20**.[8] More interestingly, the in vitro antitrichomonal activity against *Trichomonas foetus* was found to be reduced by only about 50%, demonstrating the partial separation of mutagenic and protein alkylating properties from the antitrichomonal activity of a 5-nitroimidazole. Encouraged by this observation, we have prepared a number of 4-substituted 5-nitroimidazoles in order to more fully determine if these activities can be differentiated.

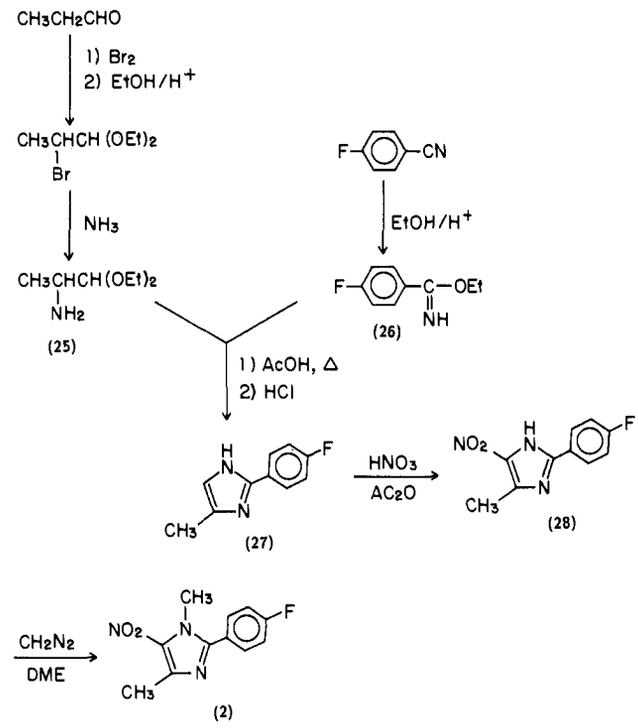
Chemistry

The 4-substituted 5-nitroimidazoles that were prepared had either a methyl, halogen, or 2-hydroxyethyl derivative at the C_4 position (Table I). The syntheses of the 4-methyl derivatives 1 and 2 are outlined in Schemes I and II. These procedures are based on the syntheses of ronidazole, **20**, and 1-methyl-2-(4-fluorophenyl)-5-nitroimidazole, **21**.[14,15] Compound 1 was prepared from 1,4-dimethyl-5-nitroimidazole via condensation with formaldehyde in Me_2SO to give intermediate **24**, which was heated with methyl carbamate and a catalytic amount of di-*n*-butyltin oxide to give 1 in 45% yield (Scheme I).

Scheme I



Scheme II



Compound 2 was prepared from propanal and 4-fluorobenzonitrile as outlined in Scheme II. Condensing the imidate **26**[15] with 2-aminopropionaldehyde diethyl acetal, **25**,[16] in glacial acetic acid[17] gave 4-methyl-2-(4-fluorophenyl)imidazole, **27**. Concentrated HNO_3 and acetic anhydride gave nitration exclusively on the imidazole ring to give **28**. Methylation of **28** with diazomethane in 1,2-dimethoxyethane (DME) gave the 5-nitroimidazole **2**. The position of the nitro group was verified by comparison of the NMR spectrum of the 4-nitro isomer that was obtained through the methylation of **28** with methyl iodide and potassium *tert*-butoxide.

The 4-(2-hydroxyethyl) derivatives **5–9** were prepared by condensing the corresponding 4-methyl derivative with

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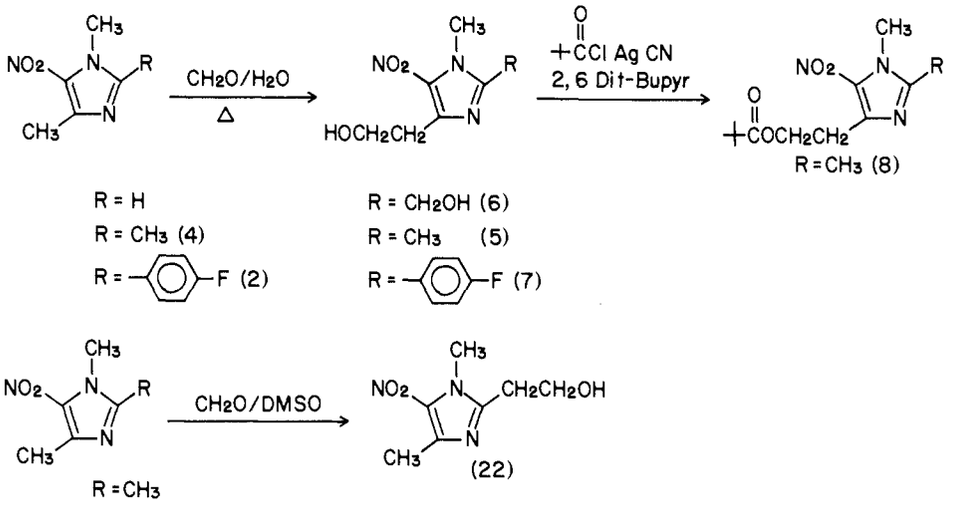
Scheme III

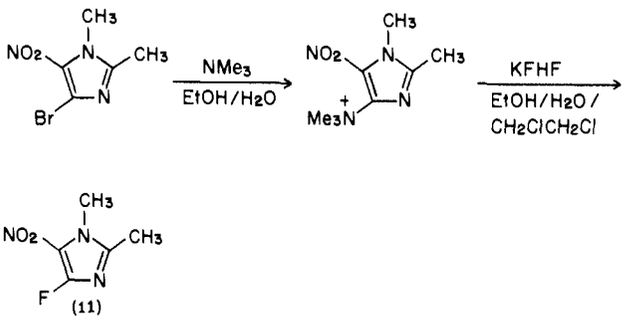


paraformaldehyde (Scheme III). In all cases, the isolated yields were low. In the case of **5**, where condensation with 1,2,4-trimethyl-5-nitroimidazole, **4**,[18] could lead to reaction at either the 2- or 4-positions, it was found that running the reaction in H_2O resulted in reaction predominantly at the C_4-methyl, whereas the use of solvents such as Me_2SO, CH_3CN, or dioxane resulted in reaction predominantly at the C_2-methyl. These products were distinguished by long-range heteronuclear coupling differences in the gated ^{13}C NMR spectra.[19] In most cases, it was found necessary to terminate the reaction at 50% consumption of the reactants since decomposition of the products was observed if the reaction times were prolonged.

The pivaloyl ester **8** was prepared by silver cyanide catalysis[20] in the presence of 2,6-di-*tert*-butylpyridine as an acid scavenger. The acetate **9** was prepared by standard procedures.

Although there are many examples in the literature of ring-halogenated nitroimidazoles containing bromo,[21,22] chloro,[23] or iodo[24,25] substituents, to our knowledge there have been no reports of ring-fluorinated nitroimidazoles. Fluorinated imidazoles are available via photolysis of imidazole diazonium fluoroborates.[26,27] Although nitration might lead to the nitrofluoroimidazoles, a more direct halogen-exchange process on the nitroimidazole seemed feasible. Since direct displacement of bromide from bromonitroimidazoles using metallic fluorides was reported to be unsuccessful,[28] a two-step procedure similar to that described for the synthesis of fluorinated purines[29] was adopted to prepare compounds **11** and **12**. When 1,2-dimethyl-4-bromo-5-nitroimidazole[21] was treated with excess trimethylamine in aqueous ethanol, the 4-trimethylammonium bromide salt was obtained (Scheme IV). Reaction of this product with potassium hydrogen fluoride in aqueous ethanol initially gave the fluoro derivative, but as the reaction time was extended a second product, identified as the 4-ethoxy derivative,[30] began to appear. This latter product presumably arises by displacement of fluoride ion, since it is only seen at longer reaction times with concomitant decrease in the amount of fluoro derivative. This problem was overcome by the addition of dichloroethane to the reaction mixture prior to fluorination in order to continuously extract the fluorinated product from the aqueous ethanol reaction phase. The use of solvents other than aqueous ethanol for the fluorination always resulted in much lower yields of fluorinated products. In all these reactions, the 4-dimethylamino deriva-

Scheme IV



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(30) ^1H NMR ($CDCl_3$) δ 1.45 (3 H, t, J = 7 Hz), 2.36 (3 H, s), 3.83 (3 H, s), 4.50 (2 H, q, J = 7 Hz); MS, m/z 185 (58), 157 (35), 149 (100).

Table II. In Vitro Biological Activity of 5-Nitroimidazoles

compd	*T. foetus*: ED_{50},[a] μg/mL	mutagenicity: % of **20**[b]	safety index[c]
1	1.0	2.0	20
2	10.0	15	0.27
3	21.5	0.3	6.2
4	32.0	0.4	3.1
5	2.0	0.28	71
6	20.0	1.0	2.0
7	7.7	8.0	0.65
8	11.0	2.6	1.4
9	2.8	1.0	14
10	0.75	8	0.65
11	14.5	ND[d]	
12	3.6	16	0.7
13	6.6	0.23	26
14	3.9	17	0.6
15	1.35	12	2.5
16	2.35	26	0.6
17	0.3	15	8.9
18	*e*	19	
19	1.6	16	1.6
20	0.4	100	1
21	0.25	300	0.53
22	*e*	0.8	
23	0.4	7.0	14.3

[a] The ED_{50} values are the mean of two or three determinations. The SD were within 15% of the mean values. [b] The mutagenicity was determined as described in the Experimental Section and are expressed as the mean value for three determinations relative to the mean value for **20**. The SD were within 20% of the mean for all values. [c] Safety index calculated as [(ED_{50} of **20**) × (mutagenicity of **20**)]/ED_{50} × mutagenicity. [d] Not detectable (less than twice background) to 100 μg/plate. Above 100 μg/plate, the compound was toxic to the *Salmonella*. [e] Value not determined.

tive[31] is a major byproduct and largely accounts for the low overall yields of the desired products.

The gallate ester derivatives **12–16** were prepared via standard procedures through reaction of 3,4,5-triacetoxygalloyl chloride and the corresponding alcohol in either pyridine or methylene chloride with 4-pyrolidinopyridine (DMAP) catalysis[32] or dicyclohexylcarbodiimide (DCC) mediated esterification with the free acid. The low yields observed for the triacetyl derivatives **13**, **14**, and **16** are due to two factors. Firstly, the acetate esters are major side products arising from transfer of the acetyl group instead of benzoylation. Secondly, these triacetate derivatives are somewhat unstable and undergo some decomposition during isolation. In contrast, the trimethoxy derivative **15** was obtained in good yield.

Results and Discussion

The in vitro antitrichomonal and mutagenic activities of **1–23** are summarized in Table II. The mutagenic activities of these compounds are expressed relative to ronidazole. The safety index is the reciprocal of the relative mutagenicity for a compound at its ED_{50} value relative to the same measure for ronidazole. The greater the value, the higher the relative safety (reduction in mutagenicity compared to ronidazole, **20**) at the ED_{50} dose. The data in Table II summarize the range in mutagenic and antitrichomonal activities observed for these compounds and illustrate the value of the safety index as a parameter for discriminating among these compounds. For example, compounds **6** and **8** are much less mutagenic than ronidazole, **20**, but are also proportionally less active against *T. foetus*. Consequently, the safety indexes for these compounds are comparable to ronidazole. Compound **5** gave the largest value for the safety index of the compounds examined.

The data reveal two major points of interest. Firstly, in all cases, addition of a 4-substituent to the imidazole ring significantly reduced or eliminated the mutagenicity. This is apparent by comparing the following compounds: **21** vs. **2** or **7**; **20** vs. **1**; **23** vs. **3**; **17** vs. **4**, **5**, **8**, or **9**, and **19** vs. **6**. Secondly, although a 4-substituent always reduced the antitrichomonal activity, in vitro, a marked separation in the reduction of mutagenicity and antitrichomonal activity could be demonstrated. Thus, the index values extend over a 300-fold range, demonstrating an absence of correlation between antitrichomonal and mutagenic activities. This suggests that the structural features that confer good antitrichomonal activity do not always result in a mutagenic compound, and consequently, the expression of these two activities may not be mediated through a common chemical species. It is also evident from the data, however, that simply blocking the 4-position of the imidazole does not always eliminate mutagenicity. For example, compounds containing the 2-(4-fluorophenyl) substituent had greater mutagenicity than those without this substituent (e.g., **2** vs. **4**, **7** vs. **5** or **6**, and **14** vs. **13**). Thus, the degree of reduction of mutagenicity by addition of a 4-substituent is also influenced by the 2-substituent.

The approach of incorporating a latent antioxidant functionality into the nitroimidazole gave one very weakly mutagenic compound, **13**. This 3,4,5-triacetoxybenzoyl ester of metronidazole was found to be greater than 35-fold less mutagenic than the parent compound, metronidazole (**23**). From the ease with which the acetate groups could be solvolyzed, formation of the gallate ester would be anticipated under the assay conditions employed. This could reduce the potential biological damage occurring as a consequence of the nitro anion radical. This hypothesis was strengthened by the observations that compound **13** is less than 2% as mutagenic as **15** and **14** is only 6% as mutagenic as **21**. Moreover, replacement of the labile acetate groups, **13**, with the stable methoxyl groups, **15**, would prevent the formation of an active gallate derivative. Accordingly, **15** would be anticipated to be more mutagenic than **13**, as is observed. This approach does not appear to be general, however, since **14** and **16** are mutagenic. In addition, **16**, the triacetoxygallate ester of **19**, is actually more mutagenic than **19**.

In conclusion we have presented two approaches for developing nonmutagenic 5-nitroimidazoles based on a knowledge of the likely mechanisms by which reactive intermediates are metabolically formed. These approaches are the incorporation of a substituent at the C_4 position of the imidazole ring and the addition of readily oxidizable functionalities into the molecule. The degree to which mutagenicity has been reduced while retaining at least moderate antitrichomonal activity documents the feasibility of separating these activities and offers promise in the development of potentially safer nitroimidazoles.

Experimental Section

Synthetic Methodology. Mass spectra were recorded on an LKB 9000 mass spectrometer. ^1H NMR were obtained on either a Varian T-60 or XL-200 instrument. Infrared spectra were obtained on a Beckman 421 spectrophotometer. Elemental analyses were obtained on all final products and agreed to within 0.4% of the theoretical values.

1,4-Dimethyl-2-(hydroxymethyl)-5-nitroimidazole (24). 1,4-Dimethyl-5-nitroimidazole[33] (1.3 g, 10 mmol) and paraform-

(31) For example, 1,2-dimethyl-4-(dimethylamino)-5-nitroimidazole could be isolated in 30% yield. ^1H NMR ($CDCl_3$) δ 2.33 (3 H, s), 3.10 (6 H, s), 3.80 (3 H, s). This material, initially obtained as yellow crystals from ether/petroleum ether, was found to substantially decompose during 3 days at room temperature.

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aldehyde (1.24 g, 42 mmol) were heated in Me_2SO (10 mL) in a sealed tube at 125 °C for 67 h. The reaction mixture was evaporated to dryness in vacuo and the residual oil dissolved in 50 mL of H_2O. This was made basic with concentrated NH_4OH and extracted with 5 × 40 mL of ethyl acetate. Drying over Na_2SO_4 and evaporation gave 1.8 g of residue. Recrystallization from THF/hexane gave **24** (1.2 g, 76%): NMR (Me_2SO-d_6) δ 2.53 (3 H, s), 3.96(3 H, s), 4.56 (2 H, d, J = 4 Hz), 5.66 (1 H, t, J = 4 Hz); MS, m/z 171 (100), 154 (18), 141 (30), 125 (16); mp 117–117.5 °C. Anal. ($C_5H_9N_3O_3$) C, H, N.

1-Methyl-2-(hydroxymethyl)-4-(2-hydroxyethyl)-5-nitroimidazole (6). Compound **6** was prepared as described for **24**. The material was isolated by preparative HPLC using a 1-in. Whatman Partisil PXS column and 9% methanol/methylene chloride mobile phase. Crystallization from methyl ethyl ketone gave **6** in 4% yield: NMR (Me_2SO-d_6) δ 3.02 (2 H, t, J = 7 Hz), 3.70 (2 H, q, J = 7 Hz), 3.88 (3 H, s), 4.55 (2 H, d, J = 6 Hz), 4.69 (1 H, t, J = 6 Hz), 5.67 (1 H, t, J = 6 Hz); MS, m/z 201 (6), 184 (3), 171 (100), 154 (8), CH_3Si derivative 345 (23), 330 (23), 329 (100); IR (Nujol) 3400, 3300, 1545, 1320, 1165, 1140 cm^{-1}; mp 123.5 °C. Anal. ($C_7H_{11}N_3O_4$) C, H, N.

1,4-Dimethyl-2-[(carbamoyloxy)methyl]-5-nitroimidazole (1). 1,4-Dimethyl-2-(hydroxymethyl)-5-nitroimidazole (**24**; 1.2 g, 7 mmol), methyl carbamate (7.5 g, 90 mmol), and di-*n*-butyltin oxide (50 mg, 0.26 mmol) were combined and heated at 150 °C under a slow stream of N_2 for 5.5 h. The reaction mixture was diluted with water (50 mL) and stirred overnight at 4 °C. The resulting precipitate was filtered and crystallized from 2-propanol to give the carbamate in 45% yield. The analytical sample was obtained after two recrystallizations from H_2O: NMR (Me_2SO-d_6) δ 2.5 (3 H, s), 3.91 (3 H, s), 5.10 (2 H, s), 6.73 (2 H, s); MS, m/z 214 (17), 171 (100), 170 (59), 125 (36); IR ($CHCl_3$) 3540, 3420, 1735, 1475, 1305 cm^{-1}; mp 165–166 °C. Anal. ($C_7H_{10}N_4O_4$) C, H, N.

2-Aminopropionaldehyde Diethyl Acetal (25).[17] 2-Bromopropionaldehyde diethyl acetal[34] (34 g, 160 mmol) was placed in a glass-lined bomb and ammonia (480 mL) was condensed in. This was then heated at 120 °C for 6 h. After evaporation of the excess ammonia, the residue was taken up in 0.7 L of saturated $NaHCO_3$ solution. This was continuously extracted with an equal volume of Et_2O overnight. After drying over Na_2SO_4, the ether layer was concentrated and the residue fractionally distilled to give 2-aminopropionaldehyde diethyl acetal, **25** (11.4 g, 48%): bp 50–55 °C (30 mmHg); NMR ($CDCl_3$) δ 1.07 (3 H, d, J = 7 Hz), 1.11 (3 H, t, J = 7 Hz), 1.13 (3 H, t, J = 7 Hz), 2.88 (1 H, quintet, J = 7 Hz), 3.60 (4 H, m), 4.10 (1 H, d, J = 6 Hz); GC/MS, 2 m 3% OV-17, 75 °C; MS, m/z 130 (1.5), 103 (62), 75 (43), 47 (100), 44 (91).

Ethyl 4-Fluorobenzimidate (26). HCl gas (32 g, 0.877 mol) was condensed into ethanol (150 mL). 4-Fluorobenzonitrile (15 g, 0.124 mol) was then added and the mixture was stirred briefly to dissolve the nitrile. The reaction was allowed to stand at 20 °C for 6 days. The reaction mixture was then concentrated to 100 mL and was added to H_2O (200 mL). After neutralization with solid $NaHCO_3$, the biphasic system was extracted into Et_2O (3 × 100 mL) and was dried over Na_2SO_4. Evaporation gave the imidate (14 g, 67%): NMR ($CDCl_3$) δ 1.4 (3 H, t, J = 7 Hz), 4.33 (2 H, q, J = 7 Hz), 7.06 (2 H, t, J = 8 Hz), 7.76 (2 H, dd, J = 8 Hz, 5 Hz); MS, m/z 167 (3), 166 (12), 139 (37), 123 (100), 122 (100).

4-Methyl-2-(4-fluorophenyl)imidazole (27). 2-Aminopropionaldehyde diethyl acetal (5.0 g, 34 mmol) and ethyl 4-fluorobenzimidate (5.7 g, 34 mmol) were combined with glacial acetic acid (4.0 mL) and heated at 100 °C for 2 h. HCl (5 N, 14 mL) was then added. Heating was continued at 100 °C for a further 30 min and then the reaction mixture was diluted with H_2O (150 mL). The products were extracted into Et_2O (3 × 150 mL), and the ether was dried over Na_2SO_4 and was evaporated to give 3.5 g of residue. This was combined with the crude product obtained from a second reaction of the same scale and was recrystallized from CH_3CN to give **27** (4.7 g, 39%): mp 185–187 °C; NMR (Me_2SO-d_6) δ 2.23 (3 H, s), 6.83 (1 H, s), 7.27 (2 H, t, J = 9 Hz), 7.95 (2 H, dd, J = 9 Hz, 6 Hz); MS, m/z 176 (100), 175 (50), 149 (20), 122 (30). Analysis after two recrsytallizations from CH_3CN ($C_{10}H_9F$) C, H, N.

4(5)-Methyl-5(4)-nitro-2-(4-fluorophenyl)imidazole (28). Compound **27** (2.0 g, 11.4 mmol) was dissolved in acetic anhydride (22 mL) and nitric acid (70%, 0.8 mL, 0.012 mol) added dropwise with cooling in an ice bath. The reaction was then heated at 100 °C for 15 min. After cooling to room temperature, the flask was cooled in an ice bath, and the resulting crystals were collected and washed with ether to give **23** (1.31 g, 52%). The product could be recrystallized from ethyl acetate, but generally the crude product was of sufficient purity for further reactions. NMR (Me_2SO-d_6) δ 2.63 (3 H, s), 7.33 (2 H, t, J = 9 Hz), 7.93 (2 H, dd, J = 6 Hz, 9 Hz); MS, m/z 221 (100), 175 (20), 148 (58), 122 (70).

1,4-Dimethyl-2-(4-fluorophenyl)-5-nitroimidazole (2). A solution of **28** (2.0 g, 9.0 mmol) in DME (100 mL) was cooled in an ice bath and excess diazomethane in Et_2O was added. After the mixture was stirred for 1 h, acetic acid (1 mL) was added and the solution stirred for an additional 45 min. The reaction mixture was added to H_2O (300 mL) and was extracted with ethyl acetate (3 × 300 mL). The ethyl acetate layers were dried over Na_2SO_4, treated with Norit A, filtered through Celite, and evaporated to give a crystalline residue. After two recrystallizations from 1-propanol, **2** was obtained (1.64 g, 77%). HPLC analysis showed 3.5% of the 4-nitro isomer present. A fraction of this material (168 mg) was purified by preparative TLC (2 × 1 mM SiO_2, 70% THF/hexane) to remove the 4-nitro isomer impurity. After elution from the plates, the material was recrystallized from 1-propanol to give pure **2** (114 mg): NMR (Me_2SO-d_6) δ 2.51 (3 H, s), 3.81 (3 H, s), 7.26 (2 H, t, J = 9 Hz), 7.66 (2 H, dd, J = 9 Hz, 6 Hz); MS, m/z 235 (100), 189 (15), 148 (100), 122 (20); IR (Nujol) 1595, 1575, 1545, 1525, 1340, 1225, 1200, 1150 cm^{-1}; mp 138–139 °C. Anal. ($C_{11}H_{10}N_3O_2F$) C, H, N.

1,5-Dimethyl-2-(4-fluorophenyl)-4-nitroimidazole (29). Compound **28** (50 mg, 0.22 mmol) was dissolved in 1 mL of ethanol in a screw-cap tube and potassium *tert*-butoxide (32 mg, 0.28 mmol) was added, followed by methyl iodide (55 mg, 0.39 mmol). The tube was capped and heated at 100 °C for 6 h. The reaction mixture was then diluted with H_2O and extracted with ethyl acetate (3 × 1.5 mL). The combined ethyl acetate layers were dried over Na_2SO_4 and evaporated to give 44 mg of residue. This was purified twice by preparative TLC using 1 mm 20 × 20 cm SiO_2 plates and eluting with 5% methanol/$CHCl_3$ and then 70% THF/hexane. With the latter solvent system, the slower moving band corresponded to the 4-nitro isomer while the faster eluting band corresponded to the 5-nitro isomer. The lower R_f band was eluted to give **29** (26 mg, 49%): NMR (Me_2SO-d_6) δ 2.63 (3 H, s), 3.60 (3 H, s), 7.23 (2 H, t, J = 9 Hz), 7.60 (2 H, dd, J = 9 Hz, 6 Hz); MS, m/z 235 (100), 147 (28), 123 (31); mp 133.5 °C. Analysis after two recrystallizations from 2-propanol ($C_{12}H_{12}N_3O_3F$) C, H, N.

1-Methyl-2-(4-fluorophenyl)-4-(2-hydroxyethyl)-5-nitroimidazole (7). Compound **2** (600 mg, 2.55 mmol) was combined with 37% aqueous formalin (6.5 mL), H_2O (1.0 mL), and Me_2SO (2.0 mL) in a glass tube, which was sealed and heated in a bomb at 140 °C for 40 h. The cooled reaction mixture was filtered and the filtrate extracted with ethyl acetate (3 × 30 mL). The combined ethyl acetate layers were dried over Na_2SO_4 and were evaporated to give an oil (507 mg). This material was chromatographed on silica gel (45 g) and eluted with $CHCl_3$ (350 mL) to remove starting material and then with 1% methanol/$CHCl_3$ to give the 4-hydroxyethyl product (66 mg). Further purification on 2 × 1 mm silica gel plates, using 3% methanol/ethyl acetate, gave **7** (50 mg, 7.5%): NMR (Me_2SO-d_6) δ 3.10 (2 H, t, J = 7 Hz), 3.73 (2 H, q, J = 7 Hz), 3.87 (3 H, s), 4.70 (1 H, t, J = 7 Hz), 7.42 (2 H, t, J = 9 Hz), 7.78 (2 H, dd, J = 9 Hz, 7 Hz); MS, m/z 265 (35), 248 (8), 235 (100), 218 (30), 178 (26), 123 (60); IR (Nujol) 3320, 1545, 1420, 1400, 1355, 1335, 1175, 1040 cm^{-1}; mp 169.5–171 °C. Analysis after recrystallization from ethyl acetate ($C_{12}H_{12}N_3O_3F$) C, H, N.

1,2-Dimethyl-4-(2-hydroxyethyl)-5-nitroimidazole (5). 1,2,4-Trimethyl-5-nitroimidazole (**4**;[18] 5.0 g, 32 mmol) and 37% aqueous formalin (23 mL, 0.31 mol) were combined with H_2O (10 mL) in a glass-lined bomb and were heated at 125 °C for 24 h. After cooling, the reaction mixture was extracted with ethyl acetate (5 × 75 mL). The ethyl acetate layers were dried over Na_2SO_4 and were evaporated to dryness. The residue (7.1 g) was chromatographed on silica gel (350 g) and was eluted with 4%

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methanol/ethyl acetate. The starting material eluted first followed by a small quantity of 1,4-dimethyl-2-(2-hydroxyethyl)-5-nitroimidazole, **22**, and finally **5** (0.61 g). Crude **5** was combined with another batch (0.42 g) and recrystallized from methyl ethyl ketone/hexane to give pure **5** (0.784 g, 7.5%): ^{1}H NMR (acetone-d_6) δ 2.44 (3 H, s), 3.10 (2 H, t, J = 6 Hz), 3.85 (2 H, q, J = 6 Hz), 3.88 (3 H, s); ^{13}C NMR (Me_2SO-d_6) δ 15.0 (q), 24.8 (t), 25.4 (q), 61.6 (t), 138.4 (s), 147.4 (s), 152 (s); MS, m/z 185 (8), 168 (6), 155 (100), 138 (18); IR (Nujol) 3320, 1545, 1495, 1420, 1400, 1355, 1335, 1175, 1040 cm^{-1}; mp 95.5–97 °C. Anal. ($C_7H_{11}N_3O_3$) C, H, N.

1,4-Dimethyl-2-(2-hydroxyethyl)-5-nitroimidazole (22). 1,2,4-Trimethyl-5-nitroimidazole (590 mg, 3.8 mmol) and paraformaldehyde (571 mg, 19 mmol) were combined in Me_2SO (4.2 mL) and heated in a sealed tube at 140 °C for 24 h. After evaporation of the Me_2SO, the product was isolated as described for **5**. The material was purified by HPLC using a Whatman ODS-3 column, eluting with 25% methanol/water, to give **22** as an oil, which slowly crystallized (105 mg, 15% yield): ^{1}H NMR (acetone-d_6) δ 2.43 (3 H, s), 2.88 (2 H, t, J = 6 Hz), 3.86 (5 H, s and q, J = 6 Hz); ^{13}C NMR (Me_2SO-d_6) δ 17.2 (q), 32.0 (t), 35.2 (q), 60.8 (t), 138 (s), 146.4 (s), 153.5 (s); MS, m/z M$^+$ 185 (17), 168 (11), 155 (100), 109 (16); mp 64.5–65.5 °C. Anal. ($C_7H_{11}N_3O_3$) C, H, N.

2,4-Dimethyl-1-(2-hydroxyethyl)-5-nitroimidazole (3).[35] 2,4(5)-Dimethyl-5(4)-nitroimidazole (0.75 g, 5.3 mmol) and ethylene glycol monotosylate[36] (1.8 g, 8.3 mmol) were combined and were heated under N_2 for 3.5 h. The cooled reaction mixture was then partitioned between 1 N HCl and $CHCl_3$. The aqueous phase was made basic with concentrated NH_4OH and extracted with ethyl acetate (5 × 25 mL). The ethyl acetate layers were dried over Na_2SO_4 and evaporated to give 0.71 g of residue. This was dissolved in hot methyl ethyl ketone (10 mL) and cooling gave crystalline starting material (0.12 g), while additional cooling overnight gave a second crop of crystals comprised of **3** and some (15%) starting material. Purification by HPLC using a 1-in. Whatman ODS-3 column and 25% methanol/H_2O) mobile phase gave **3** (0.209 g, 21%): mp 123.5 °C (lit.[37] mp for 4-nitro isomer, 169 °C); ^{1}H NMR (Me_2SO-d_6) δ 2.42 (3 H, s), 2.45 (3 H, s), 3.65 (2 H, q, J = 6 Hz), 4.53 (2 H, t, J = 6 Hz), 5.00 (1 H, t, J = 6 Hz); MS, m/z 185 (65), 168 (10), 139 (31), 138 (56); UV λ_{max} (0.01 N HCl) 290 nm (6400), (0.01 N NaOH) 327 (10400).[38] Anal. ($C_7H_{11}N_3O_3$) C, H, N.

1,2-Dimethyl-4-(2-hydroxyethyl)-5-nitroimidazole Pivalate (8) and Acetate (9). Compound **5** (120 mg, 0.65 mmol), silver cyanide (112 mg, 0.84 mmol) and 2,6-di-*tert*-butylpyridine (0.16 mL, 0.7 mmol) were combined in benzene (10 mL), and the mixture was stirred while pivaloyl chloride (0.1 mL, 0.9 mmol) was added dropwise. The reaction was then refluxed under N_2 for 3 h. A further 0.03 mL (0.3 mmol) of pivaloyl chloride was added and reflux was continued for a further 2 h. The cooled reaction mixture was filtered through Celite and the Celite was washed with ethyl acetate. The combined filtrates were washed with saturated $NaHCO_3$ solution and H_2O and were dried over Na_2SO_4. Evaporation gave 214 mg of residue. The crude ester was purified by preparative TLC (2 × 1 mm SiO_2, 5% methanol/methylene chloride) to give **8** (157 mg, 94%): NMR ($CDCl_3$) δ 1.13 (9 H, s), 2.43 (3 H, s), 3.26 (2 H, t, J = 7 Hz), 3.53 (3 H, s), 4.40 (2 H, t, J = 7 Hz); MS, m/z 269 (2), 254 (4), 168 (37), 167 (100), 122 (23); mp 73–73.5 °C. Analysis after recrystallization from benzene/petroleum ether ($C_{12}H_{19}N_3O_4$) C, H, N. The acetate ester **9** was prepared from **5** in 83% yield with use of acetyl chloride/pyridine: NMR ($CDCl_3$) δ 2.0 (3 H, s), 2.43 (3 H, s), 2.90 (2 H, t, J = 7 Hz), 3.83 (3 H, s), 4.38 (2 H, t, J = 7 Hz); MS, m/z 184 (2), 168 (22), 167 (100); mp 62.0–62.5 °C. Analysis after recrystallization from CCl_4/hexane ($C_9H_{13}N_3O_4$) C, H, N.

1,2-Dimethyl-4-fluoro-5-nitroimidazole (11) and 1-Methyl-4-fluoro-5-nitroimidazole (12). These compounds were prepared from the corresponding 4-chloro or 4-bromo derivatives by the following general procedure. The 4-haloimidazole (2 mmol) was added to H_2O (4.5 mL) and a solution of trimethylamine (6 mmol) in ethanol (4.5 mL) was added. The reaction was stirred overnight at room temperature. The reaction mixture was extracted with methylene chloride (3 × 5 mL) and potassium bifluoride (25 mmol) was added to the aqueous phase. Dichloroethane (8 mL) was also added to the aqueous phase and the two-phase system was refluxed with stirring for 1–2 days. The dichloroethane layer was separated and the aqueous phase was washed with a second volume of dichloroethane. The combined organic layers were dried over Na_2SO_4 and evaporated to dryness. The fluoro derivatives were purified by preparative TLC (SiO_2, 2% methanol/methylene chloride) to give **11** (15%): NMR ($CDCl_3$) δ 2.40 (3 H, s), 3.86 (3 H, s); MS, m/z 159 (100), 129 (30), 72 (100). A small portion was crystallized from methylene chloride/hexane, mp 84–85 °C. Anal. ($C_5H_6FN_3O_2$) C, H, N. Compound **12** was obtained in 19% yield: NMR ($CDCl_3$) δ 4.00 (3 H, s), 7.27 (1 H, s); MS, m/z 145 (100), 116 (27), 115 (34), 72 (77); mp 97.5–98 °C. Anal. ($C_4H_4FN_3O_2$) C, H, N.

2-(2-Methyl-5-nitroimidazol-1-yl)ethyl 3,4,5-Triacetoxybenzoate (13). Metronidazole (**23**; 1.0 g, 5.8 mmol) was combined with 3,4,5-triacetoxybenzoic acid[39] (1.56 g, 5.3 mmol), dicyclohexylcarbodiimide (2.4 g, 12.5 mmol), and 4-pyrrolidinopyridine (120 mg, 0.81 mmol) in methylene chloride (100 mL) and the mixture stirred for 3 h at room temperature. The reaction mixture was then filtered and the filtrate washed with H_2O. The organic layer was dried over Na_2SO_4 and evaporated to give 2.4 g of residue. This was slurried with acetonitrile (25 mL) and filtered, and the filtrate was adsorbed onto silica, which was packed onto a silica column (150 g). The column was eluted with ethyl acetate to give 372 mg of crude **13**. This was chromatographed on a short silica column eluted with methylene chloride/acetone (2:1) to remove polar impurities. Crystallization from toluene gave **13** (0.234 g, 10%): NMR ($CDCl_3$) δ 2.30 (9 H, s), 2.46 (3 H, s), 4.63 (4 H, s), 7.63 (2 H, s), 7.91 (1 H, s); MS, m/z 407 (61), 365 (66), 323 (47), 196 (100); mp 141.5 °C. Anal. ($C_{19}H_{19}N_3O_{10}$) C, H, N.

2-[2-(4-Fluorophenyl)-5-nitroimidazol-1-yl]ethyl 3,4,5-Triacetoxybenzoate (14) and 2-(2-Methyl-5-nitroimidazol-1-yl)ethyl 3,4,5-Trimethoxybenzoate (15). 1-(2-Hydroxyethyl)-2-(4-fluorophenyl)-5-nitroimidazole[40] (500 mg, 2 mmol) was combined with 3,4,5-triacetoxybenzoyl chloride (625 mg, 2 mmol), 4-pyrrolidinopyridine (40 mg, 0.27 mmol) and triethylamine (0.26 mL, 2 mmol) in methylene chloride (12.5 mL) and was stirred at room temperature under N_2 for 2.5 h. The reaction mixture was washed with saturated $NaHCO_3$ solution and water, dried over Na_2SO_4, and evaporated to dryness. The crude product was composed of two major products **14** and the acetate ester of 1-(2-hydroxyethyl)-2-(4-fluorophenyl)-5-nitroimidazole. These products were separated by HPLC using a Whatman ODS-3 M20/50 column eluted with 65% CH_3CN/H_2O at a flow rate of 10 mL/min prior to a step gradient to 85% CH_3CN/H_2O. The later eluting product was collected and rechromatographed, isocratically, with 75% CH_3CN/H_2O to give **14** (234 mg, 22%): NMR (acetone-d_6) δ 2.33 (9 H, s), 4.63 (2 H, t J = 5 Hz), 4.91 (2 H, t, J = 5 Hz), 7.08 (2 H, t, J = 8 Hz), 7.40 (2 H, s), 7.60 (2 H, dd, J = 8 Hz, 6 Hz), 8.03 (1 H, s); MS, m/z 487 (50), 445 (41), 403 (17), 196 (100); IR (Nujol) 1775, 1715, 1600, 1520, 1315 cm^{-1}; mp 139.5–140 °C. Analysis after recrystallization from benzene/petroleum ether ($C_{24}H_{20}N_3O_{10}F$) C, H, N. Compound **15** was prepared in a similar fashion from 3,4,5-trimethoxybenzoyl chloride. The crude product was purified by silica gel chromatography with 5% methanol/ethyl acetate to give **15** (76%): NMR ($CDCl_3$) δ 2.46 (3 H, s), 3.86 (9 H, s), 4.63 (4 H, br s), 7.06 (2 H, s), 7.87 (1 H, s); MS, m/z 365 (100), 212 (11), 195 (68), 149 (18); mp 114.5 °C. Analysis after three recrystallizations from CCl_4 ($C_{16}H_{19}N_3O_7$) C, H, N.

(1-Methyl-5-nitroimidazol-2-yl)methyl 3,4,5-Triacetoxybenzoate (16). Compound **19** (73 mg, 0.46 mmol) was dissolved in 1.0 mL of pyridine and 3,4,5-triacetoxybenzoyl chloride (162

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mg, 0.51 mmol) was added. After the mixture was stirred at room temperature for 1.5 h, the product was isolated in the usual manner. The product was chromatographed on silica gel eluted with 15% acetone/methylene chloride to give crude **16** (167 mg). This was purified further by HPLC using a Whatman ODS-3 M20/50 column eluted with 65% CH_3CN/H_2O to give **16** (110 mg, 54%): NMR ($CDCl_3$) δ 2.33 (9 H, s), 4.03 (3 H, s), 5.50 (2 H, s), 7.83 (2 H, s), 8.03 (1 H, s); MS, *m/z* 393 (100), 351 (80), 309 (43), 140 (100); mp 136–136.5 °C. Analysis after recrystallization from benzene/petroleum ether ($C_{18}H_{17}N_3O_{10}$) C, H, N.

Mutagenicity Assays. The mutagenicity of the nitroimidazoles was determined as described by Maron and Ames[41] with use of *Salmonella typhimurium* strain TA100 without an additional metabolic activating system. The mutagenicity of each compound was calculated as the net number of revertants/plate over the background value for each sample. The values for the mutagenicity/microgram of sample in the linear portion of the dose–response range were averaged and expressed relative to ronidazole.

Antitrichomonal Activity. *Trichomonas foetus* (Ortho strain 2) has been maintained in pure culture at Merck for several years. The organisms were cultured in Diamonds Media containing gentamycin (24 μg/mL) and calf serum (10%). Trichomonads (2×10^6 in 0.1 mL of media) were added to 1.8 mL of media. The test compound, dissolved in either water or Me_2SO, was added to the culture tube over a concentration range appropriate to bracket the dose required to reduce the number of motile organisms by 50% (EC_{50}). If Me_2SO was required to dissolve the sample, the final incubation concentration was never greater than 1% and appropriate solvent control samples were included. The number of surviving motile trichomonads were counted following a 24-h incubation at 37 °C.

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Acknowledgment. We are indebted to Evon Moore for conducting some of the in vitro mutagenicity and antitrichomonal tests.

Registry No. **1**, 104575-24-8; **2**, 104575-25-9; **3**, 104575-26-0; **4**, 18888-57-8; **5**, 104575-27-1; **6**, 104575-28-2; **7**, 104575-29-3; **8**, 104575-30-6; **9**, 104575-31-7; **10**, 21431-58-3; **11**, 104575-32-8; **12**, 104575-33-9; **13**, 104575-34-0; **14**, 104575-35-1; **15**, 104575-36-2; **16**, 104575-37-3; **17**, 551-92-8; **18**, 14766-63-3; **19**, 936-05-0; **20**, 7681-76-7; **21**, 4204-99-3; **22**, 104575-38-4; **23**, 443-48-1; **24**, 104575-39-5; **25**, 55064-41-0; **26**, 52162-47-7; **27**, 104575-40-8; **28**, 104575-41-9; **29**, 104575-42-0; $H_2NCO_2CH_3$, 589-55-0; $H_3CCHBrCHO$, 3400-55-3; $4\text{-}FC_6H_4CN$, 1194-02-1; $(CH_3)_3CCOCl$, 3282-30-2; $3,4,5\text{-}(CH_3CO_2)_3C_6H_2CO_2H$, 6635-24-1; $3,4,5\text{-}(CH_3CO_2)_3C_6H_2COCl$, 70475-59-1; $3,4,5\text{-}(CH_3O)_3C_6H_2COCl$, 4521-61-3; 1,4-dimethyl-5-nitroimidazole, 57658-79-4; 2,4(5)-dimethyl-5(4)-nitroimidazole, 49780-25-8; 1-(2-hydroxyethyl)-2-(4-fluorophenyl)-5-nitroimidazole, 4548-15-6.

Preparation, Receptor Binding, and Fluorescence Properties of Hexestrol–Fluorophore Conjugates: Evaluation of Site of Attachment, Fluorophore Structure, and Fluorophore–Ligand Spacing

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We have undertaken a staged development of certain estrogen–fluorophore conjugates, in order to prepare a fluorescent estrogen suitable for determination of the estrogen receptor content of individual cells. Since non-steroidal estrogens with bulky substituents are often more readily bound by receptor than their steroidal counterparts, we have investigated fluorophore conjugates with derivatives of the non-steroidal estrogen hexestrol ((3*R**,4*S**)-3,4-bis(4-hydroxyphenyl)hexane). On the basis of the receptor-binding affinity of model compounds, we prepared a prototypical set of three ring- and side-chain-substituted fluorescent hexestrol derivatives, whose binding and fluorescence properties ultimately led to the preparation of a series of side-chain-substituted nitrobenzoxadiazole derivatives. The compounds prepared have binding affinities for the estrogen receptors that range from ca. 1% to 5% that of estradiol, and they have very favorable fluorescence characteristics, similar to those of fluorescein.

The presence and concentration of estrogen receptors in breast tumors has been shown to be correlated with patient response to endocrine therapy, so that tumor-receptor content is now routinely assayed and used as a prognostic indicator.[1] However, the correlation between receptor and response is not perfect: The absence or very low titer of receptor is reliably related to a lack of response to hormone treatment, but the presence of receptor only increases the frequency of hormone responsiveness from ca. 30% in untested patients to ca. 60%.[1] Cell heterogeneity in tumors is one hypothesis advanced to explain this discrepancy; accordingly, nonresponsive, receptor-positive tumors might contain mixed populations of receptor-positive and receptor-negative cells (heterogeneous), while responsive, receptor-positive tumors would contain only receptor-positive cells (homogeneous).[1c,d] Whereas these two types of tumors would not be distinguishable by biochemical receptor assay of tumor homogenates, they could, in principle, be distinguished by assay methods that evaluate the receptor content of individual cells. Such considerations have prompted the development of fluorescent estrogens.[2,3]

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0022-2623/87/1830-0156$01.50/0