Hypoxia-Selective Antitumor Agents. 10. Bis(nitroimidazoles) and Related Bis(nitroheterocycles): Development of Derivatives with Higher Rates of Metabolic Activation under Hypoxia and Improved Aqueous Solubility

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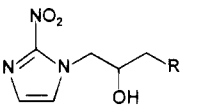
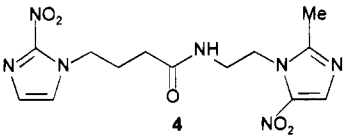
A series of analogues of the previously described compound *N*-[2-(2-methyl-5-nitroimidazol-1*H*-yl)ethyl]-4-(2-nitroimidazol-1*H*-yl)butanamide (**4**), a novel hypoxic cell cytotoxin and radiosensitizer, have been prepared and evaluated for hypoxia-selective cytotoxicity and hypoxic cell radiosensitization *in vitro*. The new derivatives were designed to overcome the low aqueous solubility of **4** and its slow kinetics of killing under hypoxia. The nitroheterocycle unit had a significant effect on solubility, with 3-nitrotriazoles being about 6-fold more soluble than the corresponding 2-nitroimidazoles. Analogues with a range of neutral linker chains (polyhydroxy, alkanesulfonamide, and bisamide) showed only slightly improved solubility and were unable to be fully evaluated. However, a series of analogues with cationic amine linkers had adequate aqueous solubility (up to 280 mM). The amine analogues could not be prepared by direct reduction of precursor amides such as **4** and were most conveniently synthesized by aza-Wittig condensation of the appropriate azide and aldehyde components. The amine-linked compounds were more cytotoxic than **4**, with the symmetrical bis(2-nitroimidazole) derivatives (**13** and **14**) up to 9-fold more potent. They showed hypoxic selectivities comparable to that of **4** (ca. 200-fold) but had much more rapid kinetics of killing under hypoxia, resulting in high hypoxic selectivity at early times in culture. The nature of the mechanism of cytotoxicity of these compounds remains unclear but appears not to be DNA cross-linking, with the compounds showing a lack of hypersensitivity toward repair-deficient UV4 cells. The enhanced solubility and hypoxia-selective cytotoxicity (at early times) of **13** compared with **4** represent significant potential advantages.

There is increasing interest[1–3] in the development of drugs (hypoxia-selective cytotoxins; HSCs) capable of selectively killing the hypoxic cells which are known to be present in many human tumors[4] and which are considered to pose a problem for both the radiotherapy[5] and chemotherapy[6] of cancer. One of the most thoroughly investigated classes of HSCs are those based on 2-nitroimidazoles; these compounds (originally developed as radiosensitizers)[7] undergo enzymic, oxygen-inhibitable, reduction of the nitro group via the nitro radical anion, resulting in preferential metabolism in hypoxic cells to reactive metabolites.[8,9] However, although possessing moderate hypoxic selectivity, these compounds as a class have relatively low cytotoxic potencies. Thus misonidazole (**1**) shows an hypoxic selectivity of ca. 20-fold in AA8 cells in culture[10,11] but has an IC_{50} under hypoxia in the millimolar range. Addition of a cationic side chain as in pimonidazole (**2**) provides higher potency,[12] and more recent compounds of this class (e.g., **3**; RB 6145) employ additional DNA-alkylating functionalities in order to produce toxic bifunctional DNA cross-linking species following reduction.[13] These have proved to be both more cytotoxic and more hypoxia-selective,[14,15] and the *R*-enantiomer of **3** is approaching clinical trial.[16]



1 R = OMe
2 R = piperidine
3 R = $NH(CH_2)_2Br$



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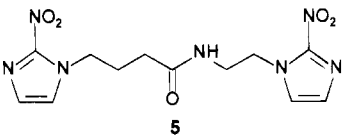

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We have recently proposed an alternative approach in which the "naked" alkylating moiety in **3** is replaced by another bioreductive center capable of producing an alkylating species following reduction and studied a series of bis(nitroimidazoles).[10,17] If reduction of both nitro groups provides a bifunctional cytotoxin with greater cytotoxicity than the monoreduced species, and reduction of each center is independently inhibited by oxygen, then such compounds would act as "bis-bioreductive" agents[18] and enhanced hypoxic selectivity would be expected. These criteria appear to be met by the mixed bis(nitroimidazole) **4**, comprising 2-nitro and 5-nitro units linked by an alkyl carboxamide chain. This compound has greater hypoxic selectivity than the mononitroimidazoles **1**–**3** in AA8 cells (giving a differential potency of up to 200-fold)[10,17] and is active against the KHT[17] and MDAH-MCa-4[10] tumors *in vivo* in combination with radiation. The kinetics of activation of **4** in culture are consistent with the formation of a bifunctional cytotoxic species but, unlike the active

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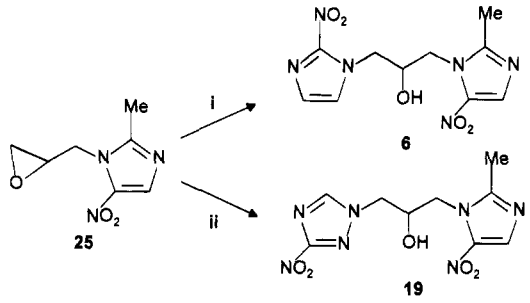
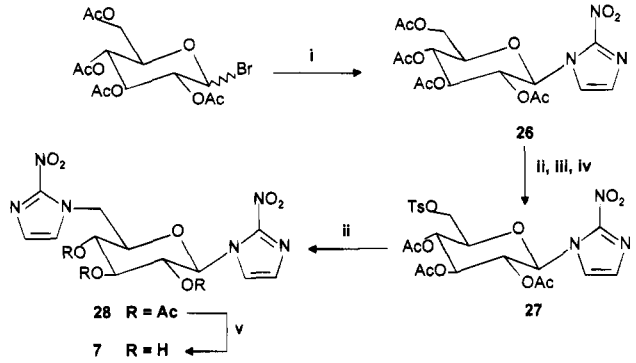
Scheme 1[a]



[a] (i) 2-Nitroimidazole/K_2CO_3/DMF; (ii) 3-nitrotriazole/K_2CO_3/DMF.

Scheme 2[a]



[a] (i) 2-Nitroimidazole/K_2CO_3/DMF; (ii) Et_3N/aqueous MeOH; (iii) TsCl/pyridine; (iv) Ac_2O; (v) NaOMe/MeOH.

intermediate from **3**, this species does not act as a DNA cross-linking agent.[10]

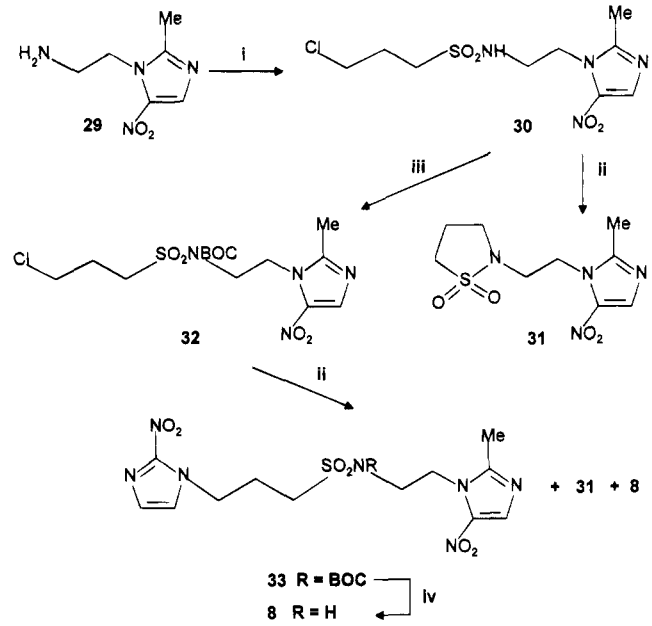
The above investigations identified two limitations of the lead compound (**4**). The dose range which could be evaluated *in vivo* was limited by its low aqueous solubility, and the full hypoxic selectivity of **4** in culture was seen only after exposure times of several hours (presumably because of slow reduction of the less electron affinic 5-nitroimidazole moiety). The corresponding bis(2-nitroimidazole) derivative (**5**) was too insoluble to evaluate. In the present work, we extend the study of bis(nitroheterocycles) as potential bisbioreductive HSCs by the synthesis and evaluation of analogues of **4** and **5** with varying linker chains and nitroheterocyclic units. The objectives of this study are to increase both aqueous solubility and the rate of activation under hypoxic conditions, while retaining the high hypoxia-selective cytotoxicity displayed by **4**.

Chemistry

Compounds containing an isopropyl linker group were synthesized by alkylation of either 2-nitroimidazole or 3-nitrotriazole with 3-(2-methyl-5-nitro-1*H*-imidazolyl)-epoxypropane (**25**)[19] to give the propanols **6** and **19**, respectively (Scheme 1). Alkylation of 2-nitroimidazole with the protected bromoglucopyranose gave **26**; deprotection, tosylation, and acetylation gave the mononitroimidazole **27**. Alkylation of 2-nitroimidazole under basic conditions gave the triacetate **28**, which was deprotected to give the bis(2-nitro-1*H*-imidazolyl)glucopyranose **7** (Scheme 2).

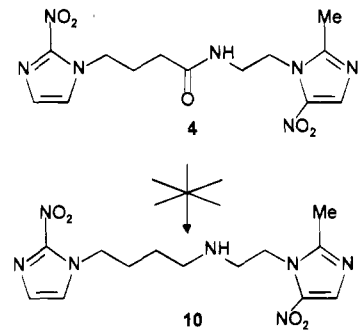
Reaction of 2-(2-methyl-5-nitro-1*H*-imidazolyl)ethylamine[17] (**29**) with 3-chloropropanesulfonyl chloride gave the chloropropanesulfonamide **30** (Scheme 3). However, attempts to alkylate 2-nitroimidazole with **30** under

Scheme 3[a]



[a] (i) $ClSO_2(CH_2)_3Cl/Et_3N/CH_2Cl_2$; (ii) 2-nitroimidazole/$K_2CO_3$/DMF; (iii) $O(CO_2tBu)_2/K_2CO_3$/DMF; (iv) 5 M HCl.

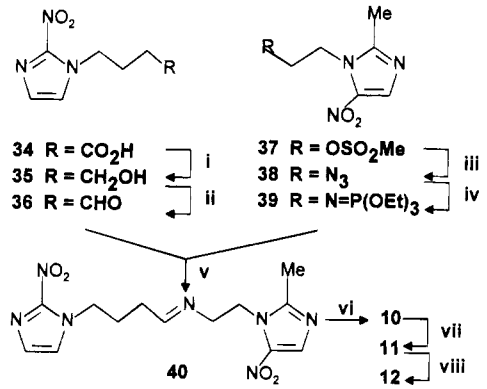
Scheme 4



basic conditions gave only the sultam **31**, and it appeared necessary to protect the sulfonamide nitrogen. Although the *tert*-butyloxycarbonyl (BOC)-protected chloropropanesulfonamide **32** was not entirely stable to the reaction conditions, as evidenced by the formation of **31** and the sulfonamide **8**, this route did provide the BOC-protected bis(nitroimidazole)sulfonamide **33**. The BOC group was readily removed to give **8**. Similarly, reaction of the BOC-protected chloride **32** with 3-nitrotriazole and subsequent deprotection gave **20**.

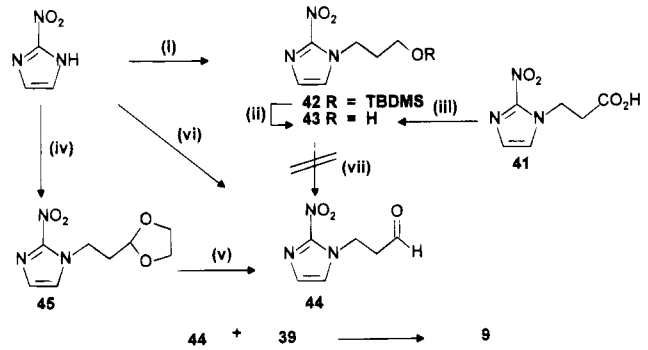
The most direct route to the alkylamino compounds appears to be reduction of the previously-reported alkanecarboxamides.[17] However, while many reagents for such conversions exist, this route (e.g., **4** to **10**; Scheme 4) was not successful. Reduction of **4** with borane dimethyl sulfide (BH_3·DMS) in tetrahydrofuran (THF) gave only starting material. The use of a large excess of BH_3·DMS or high reaction temperature gave complex mixtures in which **10** was present, but from which it could not be isolated. Utilization of Lewis acid catalysis with BF_3·OEt_2 and BH_3·DMS[20] also gave complex mixtures. Reaction of **4** with trimethyloxonium tetrafluoroborate, followed by sodium borohydride ($NaBH_4$) reduction,[21] or with $I_2/NaBH_4$,[22] gave only starting material, and the reactive nitro functionalities in the molecule precluded the use of more forcing conditions.

Scheme 5[a]



[a] (i) BH_3·DMS/THF; (ii) Swern oxidation; (iii) NaN_3/DMF; (iv) $P(OEt)_3/CH_2Cl_2$; (v) CH_2Cl_2/20 °C/16 h; (vi) $NaBH_4$/MeOH; (vii) HCHO/MeCN/$NaBH_3CN$; (viii) Davis' reagent.

Scheme 6[a]

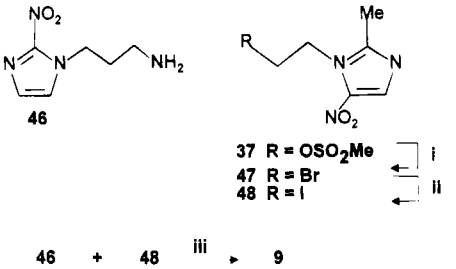


[a] (i) $Br(CH_2)_3OSiTBDM/K_2CO_3$/DMF; (ii) TBAF/THF; (iii) BH_3DMS/THF; (iv) $Br(CH_2)_2CH(O(CH_2)_2O)/K_2CO_3$/DMF; (v) 20% H_2SO_4; (vi) acrolein/KF/alumina/MeCN; (vii) Swern oxidation.

Synthesis of **10** was achieved by a convergent synthesis, employing an aza-Wittig condensation[23] (Scheme 5). The known[17] carboxylic acid **34** was reduced to the alcohol **35** and oxidized under Swern conditions to the aldehyde **36**. The azide **38** (prepared from the known[17] mesylate **37**) was treated with triethyl phosphite to give the iminophosphorane **39**. This reacted with the aldehyde **36** to give the crude imine **40**, which was then reduced efficiently to **10**. The one-pot three-reaction sequence provided the desired unsymmetrical amine **10** from the azide **38** in 81% overall yield.

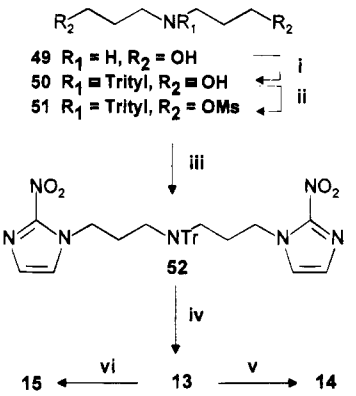
Alkylation of the secondary amine **10** with formaldehyde and sodium cyanoborohydride[24] proceeded efficiently to give the corresponding tertiary amine **11**. Reaction of the tertiary amine **11** with Davis' reagent[25] in dichloromethane converted it selectively to the corresponding aliphatic *N*-oxide **12**. The aza-Wittig method was also used to synthesize the lower homologue **9** (Scheme 6). The known[17] carboxylic acid **41** was reduced to the propanol **43**, although this compound could be prepared in better yield by alkylation of 2-nitroimidazole with a *tert*-butyldimethylsilyl (TBDMS)-protected bromopropanol under basic conditions, followed by deprotection of **42** with tetrabutylammonium fluoride (TBAF). Swern oxidation of **43** gave only acryl aldehyde. This is consistent with the difficulties reported[17] in the synthesis of the corresponding acid **41**. Several other routes to the intermediate aldehyde **44** were then explored. Alkylation of 2-nitroimidazole with 2-(2-bromoethyl)-1,3-dioxolane gave **45**, and subsequent deprotection under acidic conditions provided **44** in 60% yield. Alternatively, alkylation of 2-nitroimidazole with acrolein using KF on alumina[26] as a catalyst gave **44** directly in 67% yield. Preparation of **9** was also achieved by direct alkylation of 3-(2-nitro-1*H*-imidazolyl)propylamine (**46**) with the iodide **48** (derived from the bromide **47**)[27] (Scheme 7), although this method was low-yielding and unreliable.

Scheme 7[a]



[a] (i) NaBr/DMF; (ii) NaI/Me_2CO; (iii) K_2CO_3/DMF.

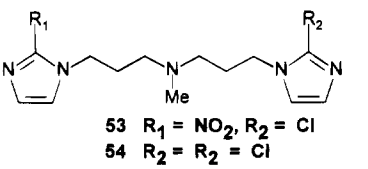
Scheme 8[a]



[a] (i) TrCl/DMAP/Et_3N/DMF; (ii) MsCl/Et_3N/CH_2Cl_2; (iii) 2-nitroimidazole/K_2CO_3/DMF; (iv) HCl/THF; (v) HCHO/MeCN/$BaBH_3CN$; (vi) MeI/K_2CO_3/DMF.

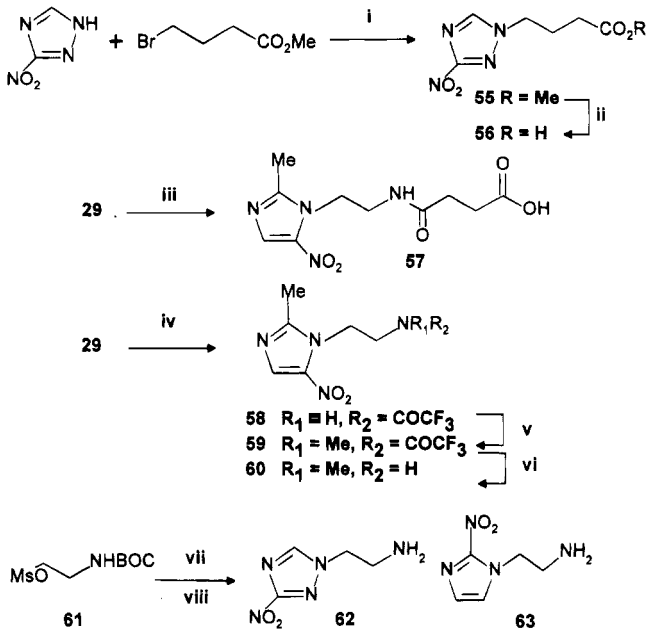
The symmetrical amine **13** was obtained by elaboration of the *N*-trityl diol **50** (Scheme 8). The synthesis of this compound by condensation of 3-aminopropanol and 3-chloropropanol to give the iminodipropanol **49** followed by selective tritylation of the amine has been reported,[28] but with few details. The diol **49** was converted to the bis-mesylate **51**, which was used to alkylate 2-nitroimidazole under basic conditions to give **52**. Deprotection of the amine with acid then gave **13**.

Alkylation of the secondary amine **13** with formaldehyde and sodium cyanoborohydride[24] proceeded efficiently to give the corresponding tertiary amine **14**. Alkylation of the secondary amine **13** with MeI gave the quaternary iodide **15**. The reactive nature of the 2-nitroimidazole units was emphasized by attempts to prepare the hydrochloride salt of **14**. Treatment of the free base with only 2–3 equiv of HCl in methanol gave a hygroscopic oil, which was shown by mass spectrometry to be a mixture of **14** and the mono- and dichloro-substituted bis-imidazoles **53** and **54**.



The 4-(3-nitro-1*H*-triazolyl)butanoic acid (**56**) was prepared from the ester **55** by the method used for

Scheme 9[a]



[a] (i) K_2CO_3/DMF; (ii) concentrated HCl; (iii) succinic anhydride/CH_2Cl_2; (iv) TFAA/Et_3N/CH_2Cl; (v) NaH/MeI/THF; (vi) K_2CO_3/aqueous MeOH; (vii) 2-nitrotriazole/K_2CO_3/DMF; (viii) 5 M HCl/MeOH.

preparation of the corresponding 2-nitroimidazolyl compound **34**.[17] Reaction of 2-(2-methyl-5-nitro-1*H*-imidazolyl)ethylamine (**29**) with succinic anhydride gave the acid (**57**) (Scheme 9). *N*-Methyl-2-(2-methyl-5-nitro-1*H*-imidazolyl)ethylamine (**60**) was synthesized by monoalkylation of the trifluoroacetamide **58** and subsequent deprotection of **59**.[29] The nitrotriazolylamine **62** was prepared by alkylation of 3-nitrotriazole with the known[30] *N*-BOC-protected mesylate **61** and subsequent deprotection (Scheme 9).

On the basis of previous work,[17] the various acids and amines were coupled together with diethyl phosphorocyanidate (DEPC) in the presence of triethylamine (Et_3N) to provide the required amides in good to excellent yields.

Biological Studies

The aerobic cytotoxicities of the compounds were determined (as IC_{50} values) in four cell lines (the Chinese hamster lines AA8 and UV4, the murine mammary carcinoma EMT6, and the human melanoma FME) using a growth-inhibition microassay which has been described previously.[31] The UV4 cell line is a repair-defective mutant, hypersensitive to agents whose cytotoxicity is due to bulky DNA adducts or cross-links.[32] The effect of oxygen on cytotoxicity was determined by clonogenic assay of stirred plateau-phase AA8 cultures, continually gassed with 5% CO_2 in air or N_2, as described previously.[33,34] Drug cytotoxic potency in this assay was determined as CT_{10}, the product of the drug concentration and duration of exposure required for 10% cell survival.[10] As noted previously,[10,17] there is a complex relationship between cell killing, drug concentration, and duration of exposure for compounds of this type. While CT_{10} was essentially constant under aerobic conditions, for some drugs it decreased progressively under hypoxia so that hypoxic selectivity was greater using low drug concentrations and long exposure times. Hypoxic selectivity was measured as CT_{10}(air)/$CT_{10}(N_2)$, and the ranges of this ratio over the drug exposure period 1–8 h are shown in Table 2.

Results and Discussion

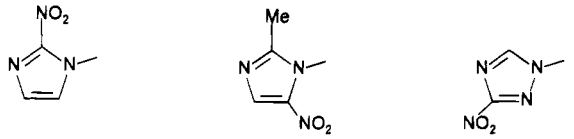
Physicochemical Properties. The structures and physicochemical properties of the bis(nitroheterocycles) (**6**–**24**) are shown in Table 1. Three nitroheterocycles (2-nitroimidazole, 2-methyl-5-nitroimidazole, and 3-nitrotriazole) were used as the bioreductive centers, all linked to the linker unit through the N-1 position via an alkane chain. The $E(1)$ values for these heterocycles were estimated from literature data for related compounds[35–38] as −390, −490, and −330 mV, respectively (assuming complete electronic isolation by the linker chain).

The solubilities of the compounds in the cell culture medium used for *in vitro* studies (α-MEM with 5% fetal calf serum) were determined by spectrophotometry. Compared with the alkanecarboxamide unit used previously,[17] alkanesulfonamide and the hydroxyl-containing linkers were unexpectedly less soluble (cf. **6** and **8** with **3**), precluding full evaluation of their cytotoxicity. The tertiary amide **18** was also less soluble than **3**. Use of a glucopyranosyl linker for two 2-nitroimidazole units (compound **7**) provided a 5-fold improvement in solubility relative to the corresponding alkanecarboxamide **5**. The oxamide-linked compound **16** was relatively insoluble, but the related succinamide **17**, where the two carboxamides are linearly separated, was much more soluble. In terms of high aqueous solubility, the most effective linkers were the alkylamines. Compounds **9**–**15** all show solubilities of >30 mM, with **13** and **14** being 280 and 240 mM, respectively, compared with 5.4 mM for **4**.

Aqueous solubility also depended on the nature of the nitroheterocycles. Replacement of the 2-nitroimidazole in compounds **5** and **6** with a 3-nitrotriazole (compounds **24** and **19**, respectively) provided a 6-fold increase, and a similar trend was seen with the equivalent pair of sulfonamides **8** and **20** (although both **19** and **20** were still too insoluble to evaluate fully). Complex relationships between the solubilizing group, its position in the linking chain, and the adjacent heterocycle were observed for compounds **21**–**24**, because of polar proximity effects, which reduced the solubility of **22** with respect to **23**. This effect appeared to be more important for the interaction of polar groups with a 2-nitroimidazole unit (compound **23**) than for the interaction with the 3-nitrotriazole in **21**.

Aerobic Cytotoxicity. IC_{50} values for the compounds following 18 h exposures in air are given in Table 2. The amino-linked 2-nitro/5-nitro analogues **9**–**11** show increased cytotoxicity (2–7-fold) relative to the carboxamide-linked parent compound **4**, but the corresponding tertiary amine N-oxide **12** was much less potent than **11**. The symmetrical amines **13** and **14** were 6–9-fold more potent than **4**, but the quaternary salt **15** only 2-fold. The increased cytotoxic potency of the amines relative to the analogues with neutral linkers parallels the increase in potency of pimonidazole (**2**) relative to misonidazole (**1**). The succinamide **17** and the tertiary amide **18** also showed only modest increases in toxicity over **4**. Comparison of **10** and **11** with **13**

Table 1. Structural and Physicochemical Data for Bis(nitroimidazoles)



A B C

no.	R_1	side chain	R_2	mp (°C)	formula	anal.	sol[a]
1	A	misonidazole					
2	A	pimonidazole					
3	A	RB-6145					
4	A	$(CH_2)_3CONH(CH_2)_2$	B	ref 17			20
5	A	$(CH_2)_3CONH(CH_2)_2$	A	ref 17			4.2
6	A	$CH_2CH(OH)CH_2$	B	212–213	$C_{10}H_{12}N_6O_5$	C,H,N	1.2
7	A	CH_2-glucopyranosyl	A	141–143	$C_{12}H_{14}N_6O_8$	C,H;N[b]	11.2
8	A	$(CH_2)_3SO_2NH(CH_2)_2$	B	182–184	$C_{21}H_{17}N_7O_6S$	C,H,N	0.13
9	A	$(CH_2)_3NH(CH_2)_2$	B	gum	$C_{12}H_{17}N_7O_4{\cdot}2HCl$	C,H,N,Cl	>46
10	A	$(CH_2)_4NH(CH_2)_2$	B	162.5	$C_{13}H_{19}N_7O_4{\cdot}2HCl$	C,H,N;Cl[c]	>48
11	A	$(CH_2)_4N(Me)(CH_2)_2$	B	oil	$C_{14}H_{21}N_7O_4$	HRMS	33
12	A	$(CH_2)_4N(Me)(O)(CH_2)_2$	B	161–163	$C_{14}H_{21}N_7O_5.2HCl$	C,H	>47
13	A	$(CH_2)_3NH(CH_2)_3$	A	120.5–123	$C_{12}H_{17}N_7O_4.2HCl$	C,H,N,Cl	>48
14	A	$(CH_2)_3N(Me)(CH_2)_3$	A	184	$C_{13}H_{19}N_7O_4.2HClO_4$	H,N;C[d]	>45
15	A	$(CH_2)_3N^+(Me)_2(CH_2)_3$	A	202–204	$C_{13}H_{19}IN_7O_4$	C,H,N,I	29
16	A	$(CH_2)_3NHCOCONH(CH_2)_3$	A	200–200.5	$C_{14}H_{18}N_8O_6$	C,H,N,Cl	0.3
17	A	$(CH_2)_2NHCO(CH_2)_2CONH(CH_2)_2$	B	oil	$C_{16}H_{22}N_8O_6$	C,H,N	45
18	A	$(CH_2)_3CON(Me)(CH_2)_2$	B	112–114	$C_{14}H_{19}N_7O_5$	C,H,N	15
19	B	$CH_2CH(OH)CH_2$	C	171–172	$C_9H_{11}N_7O_5$	C,H,N	7.8
20	C	$(CH_2)_3SO_2NH(CH_2)_2$	B	147–149	$C_{11}H_{16}N_8O_6S$	C,H,N	1.0
21	A	$(CH_2)_3CONH(CH_2)_2$	C	oil	$C_{11}H_{15}N_8O_5$	HRMS	23
22	A	$(CH_2)_2NHCO(CH_2)_3$	C	160–163	$C_{11}H_{14}N_8O_5$	C,H,N	2.3
23	A	$(CH_2)_3NHCO(CH_2)_3$	C	oil	$C_{12}H_{16}N_8O_5$	C,H	>47
24	B	$(CH_2)_2NHCO(CH_2)_3$	C	115–117	$C_{12}H_{16}N_8O_5$	C,H,N	33

[a] Solubility (mM) in culture medium (αMEM + 5% FCS) by spectrophotometry. [b] N out by 0.8%. [c] Cl out by 0.5%. [d] C out by 0.6%.

Table 2. In Vitro Aerobic Cytotoxicity and Hypoxia-Selectivity Data for Bis(nitroimidazoles)

	growth inhibition assay (aerobic)				clonogenic assay (AA8)	
		hypersensitivity factor[b]				
no.	IC_{50}[a] (mM): AA8	UV4	EMT6	FME	hypoxic CT_{10}[c] (mM h)	ratio[d] air/N_2
1[e]	12.0 ± 0.1	1.37 ± 0.12	3.6 ± 0.88	1.94 ± 0.18	16.0–8.3	18–25
2	3.03 ± 0.27	1.37 ± 0.08	6.30 ± 2.45	4.07 ± 1.06	3.5	40
3[e]	0.15 ± 0.21	5.85 ± 0.90	1.55 ± 0.13	1.0	0.4–0.2	20–40
4[e]	7.14 ± 0.79	1.38 ± 0.19	4.64 ± 1.27	3.51 ± 0.80	12.0–0.4	8–200
6	>0.8[f]	>0.85	*f*	>1.11	2.4	>3[g]
7	7.40	0.89	<0.8[f]	2.44	57	>1.5[g]
8	>40.3[f]	*f*	>0.84	>0.91		
9	4.74 ± 1.21	1.42 ± 0.34	4.73	3.61	2.4–0.88	14–38
10	2.00 ± 0.92	1.07 ± 0.52	2.12 ± 0.88	1.27 ± 0.44	2.4–1.4	12–19
11	1.00 ± 0.17	0.98 ± 0.17	1.44 ± 0.19	1.54 ± 0.28	2.0–1.2	8–30
12	20.5 ± 0.72	1.15 ± 0.39	2.05 ± 0.08	2.28 ± 1.14	69	>1
13	1.18 ± 0.37	0.90 ± 0.31	5.37 ± 1.21	2.58 ± 0.76	2.3–0.29	40–200
14	0.81 ± 0.23	0.97 ± 0.07	2.60 ± 0.71	2.55	0.7–0.25	40–130
15	3.65 ± 0.03	0.89 ± 0.04	24.2 ± 5.35	4.85 ± 1.07	23–15	10–15
16	>0.08[f]	*f*	*f*	*f*	>0.7[f]	
17	6.60 ± 1.79	1.00 ± 0.40	7.09	3.49	47	>6[g]
18	4.80 ± 0.93	1.05 ± 0.10	2.09 ± 0.01	2.83 ± 1.80	7.2–4.8	>18
19	2.3 ± 0.19	1.16 ± 0.11	4.85 ± 0.08	3.63 ± 1.41	>47[f]	
20	1.14	<1.35[f]	<1.35[f]	1.19		
21	6.92 ± 0.34	1.19 ± 0.04	10.7 ± 2.83	6.90	17	>6[g]
22	1.71	1.17	1.76	>1.9	15	>1[g]
23	5.28 ± 0.30	1.25 ± 0.23	8.38	5.94	17	3.5–10
24	6.42 ± 1.19	1.2 ± 0.23	4.94 ± 0.51	4.51	100	>1.3[g]

[a] IC_{50}: the concentration of drug required to reduce cell numbers to 50% of controls in a growth inhibition assay (see text). Values are means for three to six separate determinations ± SEM. Where no SEM is provided, results are from a single determination. [b] Hypersensitivity factor; HF = IC_{50} for AA8/IC_{50} for the indicated cell line. [c] Drug concentration × time, μM h) to reduce cell survival to 10% of control values under hypoxic conditions. [d] Ratio aerobic CT_{10}/hypoxic CT_{10}. [e] Data from ref 17, with inclusion of additional independent determinations. [f] Inactive at the solubility limit. [g] No aerobic cytotoxicity at the solubility limit.

and **14** suggests that increasing the reduction potential of the second bioreductive center by replacing the 2-methyl-5-nitroimidazole moiety with another 2-nitroimidazole has little effect on cytotoxicity (although the linker chains are not identical). The 3-nitrotriazole compounds **19**–**24** were 1.1–6.6-fold more potent than **4**. Overall, significant increases in aerobic toxicity were limited to compounds with a protonable amino group in the linker and may be related either to an increased affinity for DNA or improved cell uptake. However,

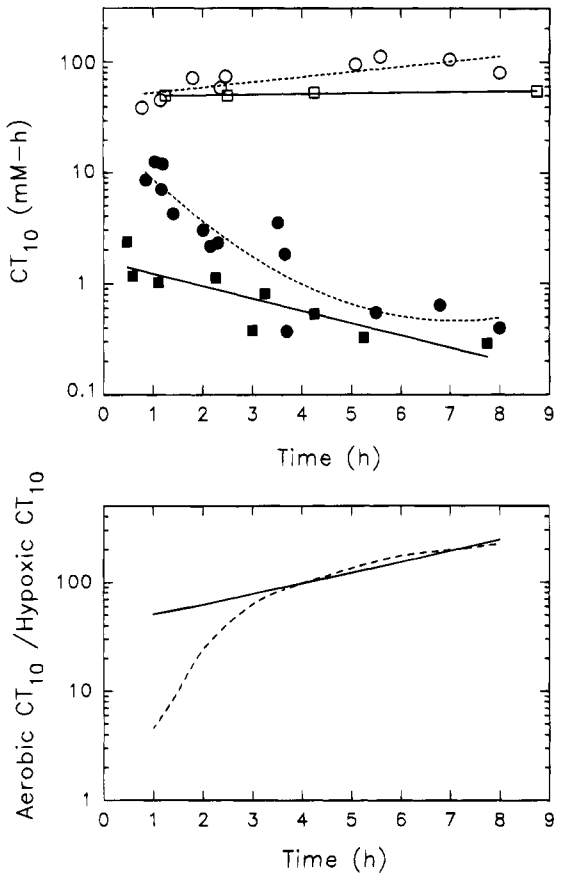


Figure 1. Cytotoxic potency (CT_{10} values) of bisnitroimidazoles **4** (circles) and **13** (squares) in aerobic (open symbols) and hypoxic (filled symbols) cultures of AA8 cells at a range of drug exposure times. The lower panel shows the hypoxic selectivity of these compounds as the ratio of potencies under aerobic and hypoxic conditions at each time.

none of the bis(nitroheterocycles) were as cytotoxic under aerobic conditions as the alkylating derivative **3**.

The latter compound also showed significantly increased potency in the repair-deficient UV4 cell line, with a hypersensitivity factor [HF = IC_{50}(AA8)/IC_{50}(UV4)] of 5.9 in air and ca. 20 under hypoxia,[10] consistent with its reported major mechanism of cytotoxicity being DNA alkylation under aerobic conditions and DNA cross-linking under hypoxia.[13,14] All of the bis(nitroheterocycles) had aerobic HF values close to unity, similar to misonidazole (**1**), suggesting that they do not act as DNA alkylating agents, as shown previously[10] for **4** under both aerobic and hypoxic conditions. The murine breast carcinoma line EMT6 and the human melanoma line FME were generally more sensitive than AA8 to both the mono- and bisnitroheterocycles (Table 2), being most pronounced for the quaternary ammonium salt (**15**) (29-fold more toxic to EMT6 than AA8 cells). Reasons for these differences between cell lines are not known.

Hypoxia-Selective Cytotoxicity. The cytotoxicities of the compounds were compared under aerobic and hypoxic conditions by clonogenic assay of stirred suspensions of AA8 cells (Table 2). For compounds with sufficient solubility, the kinetics of cell killing were assessed at several drug concentrations. Plots of CT_{10} versus time are shown for two representative compounds (**4** and **13**) in Figure 1. In all cases CT_{10} was essentially independent of time under aerobic conditions, but generally decreased with time under hypoxic conditions. The range of hypoxic CT_{10} values obtained for each compound and the corresponding range of hypoxic selectivities (CT_{10} air/CT_{10} N_2) are shown in Table 2.

Four of the eight compounds containing 2-nitro- and 5-nitroimidazole units and neutral linkers (**6**, **8**, **17**, and **18**) showed some selectivity for hypoxic cells, but the extent of the differential could not be determined because of solubility limitations. The readily soluble amines (**9**–**11**) had hypoxic cytotoxic potencies broadly similar to that of **4** but lower hypoxic selectivity at late times than **4** due to greater aerobic cytotoxicity. Compound **12**, the *N*-oxide of the tertiary amine **11**, was of particular interest. Its low aerobic toxicity relative to **11** suggested its potential as a "tris-bioreductive" agent, since reduction of tertiary amine *N*-oxides is known to be inhibited by oxygen.[18,39,40] However, it showed very low potency under hypoxia (CT_{10} 69 mM h), suggesting that either the very polar *N*-oxide slows uptake or is not efficiently metabolized by AA8 cells.

In the bis(2-nitroimidazole) series, the glucopyranosyl-linked analogue (**7**) also showed very low potency under hypoxic conditions, again possibly reflecting limitations on cellular uptake. The readily soluble derivative with a permanently-charged quaternary ammonium linker (**15**) also had very low hypoxic potency and modest hypoxic selectivity (10–15-fold). The soluble secondary and tertiary amine derivatives could be evaluated fully and had similar cytotoxic potencies, with the secondary amine (**13**) being slightly more hypoxia-selective than the tertiary amine (**14**). Most notable (illustrated for **13** in Figure 1) was that the change in hypoxic potency (CT_{10}) with time was less pronounced than for **4**, with high hypoxic selectivity being achieved at early times. For example, after a 1 h exposure the hypoxic selectivity of **4** was only about 5-fold while **13** gave a differential of 40-fold, although at late times both compounds showed a similar ratio (ca. 200-fold). We have previously shown that the time dependence of the hypoxic differential of **4** is not due to slow removal of residual oxygen from the culture system.[10] However, in the case of **13** the small increase in apparent hypoxic potency with time is probably due to such an effect, since in an experiment in which the duration of gassing with 95% N_2/5% CO_2 was extended from 1 to 2 h before mixing drug and cells, the CT_{10} value (2 mM drug concentration) fell from 1.35 to 0.86 mM h.

The lack of hypersensitivity of UV4 cells to bisnitroimidazoles in the IC_{50} assays (Table 2) suggests that they do not act as DNA cross-linking agents under aerobic conditions, but that bifunctional activation under hypoxia might lead to such a lesion. The cytotoxicity of **13** against UV4 cells was investigated under hypoxic as well as aerobic conditions using the clonogenic assay. For UV4 cells the CT_{10} was 1.07 and 0.30 mM h at 1 and 3 h, respectively, under hypoxic conditions, and approximately 24 mM-h under aerobic conditions, indicating a sensitivity indistinguishable to that of AA8 cells (Figure 1).

Five compounds with a nitrotriazole unit replacing one of the nitroimidazole moieties were also examined in the clonogenic assay. The derivatives with a 5-nitroimidazole unit (**19** and **24**) had very low cytotoxic potencies under hypoxia (CT_{10} > 47 mM h) and the

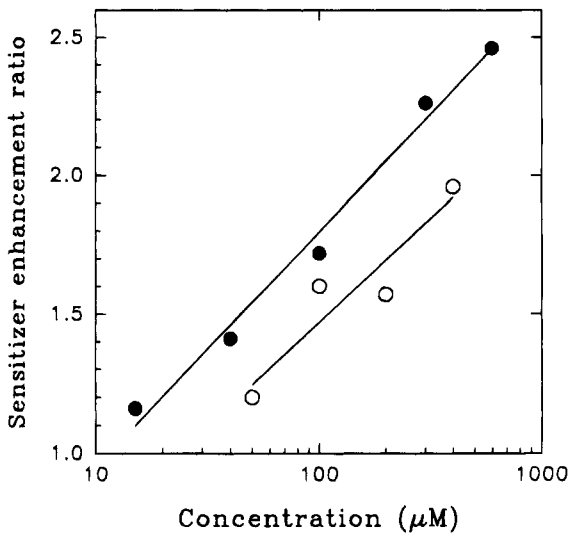


Figure 2. Sensitization of hypoxic AA8 cells to ionizing radiation by compounds **2** (○) and **13** (●) at 37 °C. The sensitizer enhancement ratio is the ratio of radiation doses for 10% survival without and with compound, which was added to the cells 30 min before irradiation.

hypoxic differential could not be determined because of solubility limitations. The three 2-nitroimidazole/nitrotriazole compounds had greater hypoxic potencies (CT_{10} 15–17 mM h). The amide-linked **23** was sufficiently soluble to evaluate fully, but showed only modest hypoxic selectivity.

Hypoxic Cell Radiosensitization. We have previously shown[17] that bis(nitroimidazoles) are hypoxic cell radiosensitizers but that they are not markedly more potent than related mononitroimidazoles. Although our primary intrerest in these compounds was as HSCs, the radiosensitizing potency of **13** was compared with the related cationic mononitroimidazole pimonidazole (**2**) in hypoxic AA8 cultures at 37 °C, with drug added 0.5 h before irradiation (Figure 2). The drug concentrations required to give a sensitizer enhancement ratio of 1.6 ($C_{1.6}$) were 67 and approximately 170 μM for **13** and **2**, respectively, the latter being in good agreement with an earlier determination ($C_{1.6}$ = 80 μM) in this cell line.[12] Thus the bis(2-nitroimidazole) is little more than twice as potent as the corresponding mononitro compound, suggesting that there is no marked enhancement of radiosensitizing potency provided by covalent linkage of the electron affinic units.

Conclusions

Of a number of different linking groups joining the bioreductive units in the bis(nitroheterocycles), only the aminoalkyl provided compounds with sufficient aqueous solubility to evaluate fully in cell culture. For noncharged linkers, polar proximity effects play a major role in determining solubility, which is therefore difficult to predict. It is likely that the increase in aerobic potencies of the aminoalkyl compounds (**9**–**11**, **13**, and **14**) is due to increased cellular uptake and/or increased DNA affinity mediated by the cationic aminoalkyl linker. The reduced time dependency of cytotoxicity under hypoxia of **13** and **14** with respect to **4** is due to more efficient bioreductive activation of the more electron-affinic second 2-nitroimidazole center.

The nature of the mechanism of cytotoxicity of these compounds remains unclear but appears not to be DNA cross-linking as originally conceived.[17] Detailed studies of **4** using alkaline elution techniques have failed to identify DNA cross-links,[10] and, after consideration of the metabolic reduction of metronidazole,[41] a mechanism involving the bioreduction of both nitro groups to generate a locally doubly damaged site on DNA (e.g., a single strand break combined with a monoalkylation site) was proposed.[10] It is not known whether compounds such as **13**, with two 2-nitroimidazole bioreductive units, produce lesions of a similar type; certainly, the low HF values in the UV4 cell line do not suggest DNA alkylation as a major mechanism. The use of nitrotriazoles of even higher reduction potential than 2-nitroimidazole (compounds **21**–**24**) was not successful in providing compounds with higher absolute cytotoxicity.

The enhanced solubility and hypoxia-selective cytotoxicity (at early times) of **13** compared with **4** represent significant potential advantages. Preliminary data show that **13** is active against the KHT tumor *in vivo* in combination with radiation, whether given before or after irradiation. A full *in vivo* evaluation of this compound as an HSC using both single dose and fractionated irradiation (to kill oxygenated cells) in two tumor models is in progress.

Experimental Section

Analyses indicated by symbols of the elements were within ±0.4% of theoretical. Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, NZ. Melting points were determined on an Electrothermal 2300 melting point apparatus. NMR spectra were obtained on a Bruker AC-200 or AM-400 spectrometer and are referenced to Me_4Si for organic solvents or DSS (2,2-dimethyl-2-silapentane-5-sulfonate) for aqueous solvents. Mass spectra were determined on a VG-70SE mass spectrometer using an ionizing potential of 70 eV at a nominal resolution of 1000. High-resolution spectra were obtained at nominal resolutions of 3000, 5000, or 10000 as appropriate. Spectra were obtained using the ionization mode specified, with PFK as the reference unless otherwise stated. Unless otherwise stated, reactions were worked up by drying organic fractions over Na_2SO_4 and removing solvent under reduced pressure. Thin-layer chromatography was carried out on aluminum-backed silica gel plates (Merck 60 F_{254}). Column chromatography was carried out on silica gel (Merck 230–400 mesh). All compounds designated for biological testing were analyzed at >99% purity by reverse phase HPLC using a Philips PU4100 liquid chromatograph, a Phenomenex BondClone 10-C18 stainless steel column (300 mm × 3.9 mm i.d.), and a Philips PU4120 diode array detector. Chromatograms were run using various gradients of aqueous (1 M NaH_2PO_4, 0.75 M heptanesulfonic acid, 0.5 M dibutylammonium phosphate, and MilliQ water in a 1:1:1:97 ratio) and organic (80% MeOH/MilliQ water) phases.

1-(2-Nitro-1*H*-imidazolyl)-3-(2-methyl-5-nitro-1*H*-imidazolyl)propan-2-ol (6). A stirred solution of 3-(2-methyl-5-nitro-1*H*-imidazolyl)-1,2-epoxypropane[19] (**25**) (0.68 g, 3.69 mmol), 2-nitroimidazole (0.44 g, 3.88 mmol), and Et_3N (0.2 mL) in absolute EtOH (25 mL) was heated at reflux temperature for 5 h. The suspension was cooled to 20 °C, and the precipitate was collected and recrystallized from absolute EtOH to give 1-(2-nitro-1*H*-imidazoyl)-3-(2-methyl-5-nitro-1*H*-imidazolyl)propan-2-ol (**6**) (0.67 g, 61%): mp 212–213 °C; ^{1}H NMR (CD_3CN) δ 7.91 (s, 1 H, H-4′), 7.32 (d, J = 1.0 Hz, 1 H, H-5′), 7.10 (d, J = 1.0 Hz, 1 H, H-4′), 4.62 (dd, J = 13.5, 3.1 Hz, 1 H, H-1), 4.54 (dd, J = 14.0, 2.6 Hz, 1 H, H-3), 4.35 (dd, J = 13.5, 8.4 Hz, 1 H, H-1), 4.25–4.31 (m, 1 H, H-2), 4.19 (dd, J = 14.0, 9.1 Hz, 1 H, H-3), 3.72 (br s, 1 H, OH), 2.44 (s, 3 H, CH_3); ^{13}C NMR (CD_3CN) δ 152.4 (C-2″), 145.2 (C-2′), 138.7 (C-5″) 133.4 (C-4″), 127.9 (C-5′)#, 126.7 (C-4′)#, 68.6 (C-2), 52.7

(C-3), 49.3 (C-1), 14.6 (CH_3). (#, assignment interchangeable). Anal. ($C_{10}H_{12}N_6O_5$) C, H, N.

1-(3-Nitro-1,2,4-1*H*-triazolyl)-3-(2-methyl-5-nitro-1*H*-imidazolyl)propan-2-ol (19). A stirred solution of **25** (0.85 g, 4.6 mmol), 3-nitro-1,2,4-triazole (0.53 g, 4.6 mmol), and Et_3N (0.2 mL) in absolute EtOH (25 mL) was heated at reflux temperature for 5 h. The solution was cooled to 20 °C, the solvent removed under reduced pressure, and the residue chromatographed on silica. Elution with a gradient (0–5%) MeOH/EtOAc gave 1-(3-nitro-1,2,4-1*H*-triazolyl)-3-(2-methyl-5-nitro-1*H*-imidazolyl)propan-2-ol (**19**) (0.62 g, 45%): mp (EtOAc) 171–172 °C; ^{1}H NMR [$(CD_3)_2SO$)] δ 8.85 (s, 1 H, H-5′), 8.04 (s, 1 H, H-4″), 5.71 (d, J = 4.5 Hz, 1 H, OH), 4.56 (dd, J = 13.9, 3.0 Hz, 2 H, H-1), 4.41 (dd, J = 13.9, 7.7 Hz, 1 H, H-3), 4.25 (dd, J = 13.8, 9.5 Hz, 1 H, H-3), 4.19 (br s, 1 H, H-2), 2.45 (s, 3 H, CH_3); ^{13}C NMR δ 162.0 (C-3′), 152.1 (C-2″), 147.6 (C-5′), 138.5 (C-5″), 133.0 (C-4″), 67.8 (C-2), 54.0 (C-1), 48.8 (C-3), 14.3 (CH_3). Anal. ($C_9H_{11}N_7O_5$) C, H, N.

2-Nitro-1*H*-[6-(2-nitro-1*H*-imidazolyl)-β-D-glucopyranosyl]-imidazole (7). A mixture of 1-bromo-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (9.5 g, 23.1 mmol), 2-nitroimidazole (2.61 g, 23.1 mmol), and anhydrous K_2CO_3 (3.82 g, 27.7 mmol) in dry DMF (20 mL) was stirred at 60–70 °C for 30 min. The mixture was poured into ice–water, extracted with EtOAc, and worked up. The residue was chromatographed on silica, eluting with 30% EtOAc/petroleum ether, to give crude 2-nitro-1*H*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl]imidazole (**26**) as a pale yellow syrup, which was used directly (3.7 g, 36%): ^{1}H NMR ($CDCl_3$) δ 7.38 (d, J = 1.0 Hz, 1 H, imid-H), 7.20 (d, J = 1 Hz, 1 H, imid-H), 6.43 (d, J = 9.0 Hz, 1 H, H-1), 5.42 (t, J = 9.0 Hz, 1 H, H-2), 5.34 (t, J = 9.0 Hz, 1 H, H-3), 5.23 (dd, J = 10.0, 9.0 Hz, 1 H, H-4), 4.30 (dd, J = 12.7, 5.2 Hz, 1 H, H-6), 4.18 (dd, J = 12.7, 2.1 Hz, 1 H, H-6′), 4.05 (ddd, J = 10.0, 5.2, 2.1 Hz, 1 H, H-5), 2.10, 2.07, 2.03, 1.92 (4 × s, 4 × 3 H, 4 × CH_3).

A solution of **26** (3.4 g, 7.68 mmol) in a mixture of Et_3N (15 mL), MeOH (45 mL), and water (15 mL) was stirred at 20 °C for 90 min. The solvent was evaporated and the residue dissolved in pyridine (15 mL). The stirred solution was treated dropwise at 0 °C with a solution of tosyl chloride (1.76 g, 9.22 mmol) in CH_2Cl_2 (10 mL) and the resulting mixture stirred for 90 min. Ac_2O (15 mL) was added and the mixture stirred at 20 °C for 15 h. The reaction was then poured into ice–water and extracted with EtOAc (3 × 100 mL). The combined organic fraction was washed with cold aqueous $NaHCO_3$ (100 mL) and water (100 mL) and worked up to give crude 2-nitro-1*H*-[6-tosyl-2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl]imidazole (**27**) as a sticky solid which was used directly (4.21 g, 98%): ^{1}H NMR ($CDCl_3$) δ 7.73 (d, J = 8.3 Hz, 2 H, ArH), 7.31 (d, J = 1 Hz, 1 H, imid-H), 7.29 (d, J = 8.3 Hz, 2 H, ArH), 7.18 (d, J = 1.0 Hz, 1 H, imid-H), 6.36 (d, J = 9.5 Hz, 1 H, H-1), 5.36 (t, J = 9.5 Hz, 1 H, H-2), 5.27 (t, J = 9.5 Hz, 1 H, H-3), 5.19 (t, J = 9.5 Hz, 1 H, H-4), 4.22 (dd, J = 11.6, 2.5 Hz, 1 H, H-6), 4.17 (dd, J = 11.6, 4.7 Hz, 1 H, H-6′) 4.03 (ddd, J = 9.5, 4.7, 2.5 Hz, 1 H, H-5), 2.44 (s, 3 H, $C_6H_4CH_3$), 2.03, 2.01, 1.89 (3 × s, 3 × 3 H, 3 × CH_3).

The tosylate **27** (3.89 g, 7.01 mmol) was treated with 2-nitroimidazole (0.79 g, 7.01 mmol) and anhydrous K_2CO_3 (1.11 g, 8.06 mmol) in DMF (20 mL) at 100–110 °C for 4 h. The cooled mixture was poured into ice–water (500 mL), and the precipitate was collected and washed with water (3 × 100 mL). Recrystallization from Me_2CO/CH_2Cl_2 gave 2-nitro-1*H*-[6-(2-nitro-1*H*-imidazolyl)-2,3,4,-tri-*O*-acetyl-β-D-glucopyranosyl]imidazole (**28**) (1.79 g, 51%): mp 234–236 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 7.93 (d, J = 1.0 Hz, 1 H, imid-H), 7.44 (d, J = 1.0 Hz, 1 H, imid-H), 7.28 (d, J = 1.0 Hz, 1 H, imid-H), 7.12 (d, J = 1.0 Hz, 1 H, imid-H), 6.49 (d, J = 9.0 Hz, 1 H, H-1), 5.53 (t, J = 9.0 Hz, 1 H, H-2), 5.48 (t, J = 9.0 Hz, 1 H, H-4), 5.19 (t, J = 9.0 Hz, 1 H, H-3), 4.78 (dd, J = 14.3, 1.7 Hz, 1 H, H-6), 4.56 (dt, J = 9.0, 1.7 Hz, 1 H, H-5), 4.45 (dd, J = 14.3, 9.0 Hz, 1 H, H-6′), 2.10, 1.99, 1.82 (3 × s, 3 × 3 H, 3 × CH_3). Anal. ($C_{18}H_{20}N_6O_{11}$) C, H, N.

A solution of **28** (1.29 g, 2.60 mmol) in MeOH (50 mL) and CH_2Cl_2 (50 mL) was treated with a solution of NaOMe (0.4 g, 7.2 mmol) in MeOH in (10 mL) and stirred at 20 °C for 30 min. Amberlite resin IR-120(H) (5 mL) was added, and the mixture was stirred for 10 min and filtered. Evaporation of the filtrate gave 2-nitro-1*H*-[6-(2-nitro-1*H*-imidazolyl)-β-D-glucopyranosyl]imidazole (**7**) (0.95 g, 99%): mp [$(CH_3)_2SO$] 141–143 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 7.72 (d, J = 1.0 Hz, 1 H, imid-H), 7.50 (d, J = 1.0 Hz, 1 H, imid-H), 7.23 (d, J = 1.0 Hz, 1 H, imid-H), 7.10 (d, J = 1.0 Hz, 1 H, imid-H), 5.80 (d, J = 8.8 Hz, 1 H, H-1), 5.60 (br s, 2 H, OH), 5.47 (br s, 1 H, OH), 4.99 (dd, J = 14.4, 2.5 Hz, 1 H, H-6), 4.41 (dd, J = 14.4, 8.8 Hz, 1 H, H-6′), 3.87 (dt, J = 8.8, 2.5 Hz, 1 H, H-5), 3.53 (t, J = 8.8 Hz, 1 H, H-2), 3.47 (t, J = 8.8 Hz, 1 H, H-4), 3.21 (t, J = 8.8 Hz, 1 H, H-3). Anal. ($C_{12}H_{14}N_6O_8$) C, H, N.

***N*-[2-(2-Methyl-5-nitro-1*H*-imidazolyl)ethyl]-3-(2-nitro-1*H*-imidazolyl)propanesulfonamide (8).** 3-Chloropropanesulfonyl chloride (0.93 mL, 6.66 mmol) was added dropwise to a stirred solution of 2-(2-methyl-5 1*H*-nitroimidazolyl)ethylamine (**29**) (1.03 g, 6.05 mmol) and Et_3N (0.93 mL, 6.66 mmol) in CH_2Cl_2 at 0 °C. The solution was stirred at 20 °C for 16 h, and the solvent was then removed under reduced pressure. Chromatography of the residue, eluting with a gradient (80–100%) of EtOAc/petroleum ether, gave *N*-[2-(2-methyl-5 1*H*-nitroimidazolyl)ethyl]-3-chloropropanesulfonamide (**30**) (1.85 g, 98%): mp (EtOAc/petroleum ether) 125.5–126.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.84 (s, 1 H, H-4″), 6.00 (br s, 1 H, NH), 4.50 (t, J = 5.8 Hz, 2 H, H-2′), 3.67 (t, J = 6.1 Hz, 2 H, H-3), 3.54 (q, J = 6.1 Hz, 2 H, H-1′), 3.20 (t, J = 7.5 Hz, 2 H, H-1), 2.59 (s, 3 H, CH_3), 2.21–2.28 (m, 2 H, H-2); ^{13}C NMR δ 151.6 (C-2″), 138.2 (C-5″), 133.0 (C-4″), 50.1 (C-2′), 47.3 (C-1′), 42.6 (C-1, C-3), 26.7 (C-2), 14.4 (CH_3); EIMS m/z 312 (3%), 310 (M^+, 10), 293 (9), 275 (5), 264 (100); HREIMS m/z calcd for $C_9H_{15}N_4O_4S^{37}Cl/C_9H_{15}N_4O_4S^{35}Cl$ 312.0473/310.0502 (M^+), found 312.0516/310.0502. Anal. ($C_9H_{15}ClN_4O_4S$) C, H, N.

A suspension of **30** (1.54 g, 5.0 mmol), di-*tert*-butyl dicarbonate (2.16 g, 9.9 mmol), and K_2CO_3 (0.75 g, 5.5 mmol) in DMF (30 mL) was stirred at 20 °C for 18 h. The suspension was filtered, and the solvent was removed under reduced pressure. Chromatography of the residue, eluting with 50% EtOAc/petroleum ether, gave *N*-[2-(2-methyl-5-nitro-1*H*-imidazolyl)ethyl-*N*-(*tert*-butyloxycarbonyl)-3-chloropropanesulfonamide (**32**) (1.69 g, 83%): mp (EtOAc/petroleum ether) 135–135.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.97 (s, 1 H, H-4″), 4.56 (t, J = 5.6 Hz, 2 H, H-2′), 4.09 (t, J = 5.6 Hz, 2 H, H-1′), 3.64 (t, J = 6.0 Hz, 2 H, 3-H), 3.57 (t, J = 7.5 Hz, 2 H, H-1), 2.52 (s, 3 H, CH_3), 2.19–2.26 (m, 2 H, H-2), 1.51 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR δ 151.1 (CO_2), 151.0 (C-2″), 139.0 (C-5″), 133.2 (C-4″), 86.2 ($C(CH_3)_3$), 51.4 (C-2′), 46.1 (C-1′), 46.0 (C-1), 42.3 (C-3), 27.9 ($C(CH_3)_3$), 26.4 (C-2), 14.2 (CH_3). Anal. ($C_{14}H_{23}ClN_4O_4S$) C, H, N, Cl.

A suspension of **32** (1.13 g, 2.75 mmol), 2-nitroimidazole (0.47 g, 4.13 mmol), and K_2CO_3 (0.46 g, 3.3 mmol) in DMF (30 mL) was stirred at 100 °C for 4 h. The solvent was removed under reduced pressure and the residue chromatographed. Elution with a gradient (0–10%) of MeOH/EtOAc gave first *N*-*tert*-butyl-*N*-[2-(2-methyl-5-nitro-1*H*-imidazolyl)ethyl]-3-(2-nitro-1*H*-imidazolyl)propanesulfonamide (**33**) (0.50 g, 37%) as an oil which was converted (see below) to **8** without further purification: ^{1}H NMR [$(CD_3)_2SO$] δ 8.00 (s, 1 H, H-4‴), 7.66 (d, J = 1.0 Hz, 1 H, H-5′), 7.19 (d, J = 1.0 Hz, 1 H, H-4′), 4.48–4.51 (m, 2 H, H-2″), 4.45 (t, J = 7.0 Hz, 2 H, H-3), 3.98 (t, J = 5.3 Hz, 2 H, H-1″), 3.53 (dd, J = 8.0, 7.6 Hz, 2 H, H-1), 2.39 (s, 3 H, CH_3), 2.11–2.19 (m, 2 H, H-2), 1.40 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR δ 151.2 (CO_2), 150.6 (C-2‴), 144.5 (C-2′), 138.7 (C-5‴), 132.9 (C-4‴), 127.9 (C-4′), 127.6 (C-5′), 84.6 ($C(CH_3)_3$), 50.1 (C-2″), 47.2 (C-1), 46.1 (C-1″), 45.3 (C-3), 27.2 ($C(CH_3)_3$), 23.6 (C-2), 13.6 (CH_3).

Further elution gave *N*-[2-(2-methyl-5-nitro-1*H*-imidazolyl)-ethyl]-3-propanesultam (**31**) (0.19 g, 25%): mp (MeOH/iPr_2O) 132–132.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.03 (s, 1 H, H-4″), 4.43 (t, J = 5.9 Hz, 2 H, H-2′), 3.32 (t, J = 6.0 Hz, 2 H, H-1′), 3.23 (t, J = 6.7 Hz, 2 H, H-3), 3.14 (dd, J = 7.8, 7.4 Hz, 2 H, H-1), 2.45 (s, 3 H, CH_3), 2.16–2.23 (m, 2 H, H-2); ^{13}C NMR δ 151.6 (C-2″), 138.3 (C-5″), 133.2 (C-4″), 47.7 (C-2′), 45.7 (C-1′), 44.6 (C-3), 44.5 (C-1), 18.8 (C-2), 13.8 (CH_3); EIMS m/z 274 (M^+, 3), 257 (45), 228 (100); HREIMS m/z calcd for $C_9H_{14}N_4O_4S$ 274.0736 (M^+), found 274.0741. Anal. ($C_9H_{14}N_4O_4S$) C, H, N, S.

Further elution gave *N*-[2-(2-methyl-5-nitro-1*H*-imidazolyl)-

ethyl]-3-(2-nitro-1*H*-imidazolyl)propanesulfonamide (**8**) (0.21 g, 20%): mp (MeOH) 218–222 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 8.03 (s, 1 H, H-4‴), 7.65 (d, J = 0.9 Hz, 1 H, H-5′), 7.45 (br t, J = 6.4 Hz, 1 H, NH), 7.19 (d, J = 0.9 Hz, 1 H, H-4′), 4.45 (t, J = 7.1 Hz, 2 H, H-3), 4.33 (t, J = 5.9 Hz, 2 H, H-2″), 3.30 (t, J = 6.2 Hz, 2 H, H-1″), 3.06 (dd, J = 7.9, 7.6 Hz, 2 H, H-1), 2.47 (s, 3 H, CH_3), 2.09–2.16 (m, 2 H, H-2); ^{13}C NMR δ 151.6 (C-2‴), 144.5 (C-2′), 138.3 (C-5‴), 133.7 (C-4‴), 127.9 (C-4′)#, 127.6 (C-5′)#, 48.1 (C-2″)#, 47.6 (C-3)#, 46.4 (C-1″), 41.5 (C-1), 24.1 (C-2), 14.1 (CH_3). (#, assignments interchangeable). Anal. ($C_{12}H_{12}N_7O_6S$) C, H, N, S.

A solution of the *N*-BOC-protected sulfonamide (**33**) (0.48 g) in EtOAc (20 mL) and 5 N HCl (2 mL) was stirred at 20 °C for 16 h to give **8** in quantitative yield, spectroscopically identical to the compound prepared above, giving an overall yield of **8** of 57%.

4-(2-Nitro-1*H*-imidazolyl)butanal (36). Borane dimethyl sulfide complex (2.2 mL, 22.1 mmol) was added dropwise to a stirred solution of 4-(2-nitro-1*H*-imidazolyl)butanoic acid (**34**)[17] (4.0 g, 20.1 mmol) in THF (100 mL) at 0 °C. The solution was stirred at 20 °C for 6 h, and then 5 M HCl (20 mL) was added carefully and the mixture stirred at 20 °C for a further 16 h. The mixture was neutralized with 5 M NaOH and the solution partitioned between EtOAc and water. The organic phase was washed with water (100 mL) and brine (50 mL) and worked up, and the residue was chromatographed. Elution with EtOAc gave 4-(2-nitro-1*H*-imidazolyl)butanol (**35**) as a gum (2.67 g, 72%): ^{1}H NMR ($CDCl_3$) δ 7.15 (s, 1 H, H-5′)#, 7.04 (s, 1 H, H-4′)#, 4.42 (t, J = 7.4 Hz, 2 H, H-4), 3.71 (t, J = 6.1 Hz, 2 H, H-1), 2.67–2.75 (m, 3 H, H-3, OH), 2.34 (dd, J = 6.9, 6.6 Hz, 2 H, H-2); ^{13}C NMR δ 144.7 (C-2′), 128.3 (C-4′), 126.0 (C-5′), 61.9 (C-1), 50.7 (C-4), 29.0 (C-2)#, 27.4 (C-3)# (#, assignments interchangeable); HRDCIMS m/z (NH_3) calcd for $C_7H_{12}N_3O_4$ 186.0879 (MH^+), found 186.0881.

DMSO (1.24 mL, 17.5 mmol) was added dropwise to a solution of oxalyl chloride (0.76 mL, 8.7 mmol) in CH_2Cl_2 (10 mL) at −78 °C under N_2. The clear solution was stirred for 5 min, and a solution of **35** (1.54 g, 8.3 mmol) in CH_2Cl_2 (5 mL) was added dropwise. The solution was stirred for 15 min, Et_3N (5.8 mL, 41.6 mmol) was added dropwise, and the suspension was stirred for 15 min and allowed to warm to 20 °C. The suspension was chromatographed directly on silica, eluting with 80% EtOAc/petroleum ether, to give 4-(2-nitro-1*H*-imidazolyl)butanal (**36**) as an oil (1.29 g, 85%): ^{1}H NMR ($CDCl_3$) δ 9.81 (s, 1 H, CHO), 7.16 (s, 1 H, H-5′), 7.12 (s, 1 H, H-4′), 4.48 (t, J = 7.3 Hz, 2 H, H-4), 2.59 (t, J = 6.8 Hz, 2 H, H-2), 2.16–2.23 (m, 2 H, H-3); ^{13}C NMR δ 200.2 (CHO), 144.8 (C-2′), 128.6 (C-4′), 125.9 (C-5′), 49.1 (C-4), 40.0 (C-2), 23.0 (C-3); EIMS m/z 183 (M^+, 5), 137 (60); HREIMS m/z calcd for $C_7H_9N_3O_3$ 183.0644 (M^+), found 183.0646.

2-(2-Methyl-5-nitro-1*H*-imidazolyl)ethyl azide (38). A stirred solution of 2-(2-methyl-5-nitro-1*H*-imidazolyl)ethyl methanesulfonate (**37**)[17] (24.67 g, 98.9 mmol) and NaN_3 (6.56 g, 0.10 mol) in DMF (100 mL) was heated at 100 °C for 4 h. The solvent was removed under reduced pressure and the residue partitioned between EtOAc/H_2O (500 mL). The organic extract was washed with brine (100 mL) and worked up. The product crystallized from EtOAc/petroleum ether to give 2-(2-methyl-5-nitro-1*H*-imidazolyl)ethyl azide (**38**) (15.14 g, 78%): mp 56–58 °C; ^{1}H NMR ($CDCl_3$) δ 7.98 (s, 1 H, H-4′), 4.45 (dd, J = 5.7, 5.3 Hz, 2 H, H-2), 3.79 (dd, J = 5.6, 5.3 Hz, 2 H, H-1), 2.44 (s, 3 H, CH_3); ^{13}C NMR δ 151.3 (C-2′), 136.7 (C-5′), 133.4 (C-4′), 50.8 (C-1), 45.5 (C-2), 14.5 (CH_3); HREIMS m/z calcd for $C_6H_8N_6O_2$ 196.0709 (M^+), found 196.0707. Anal. ($C_6H_8N_6O_2$) C, H.

***N*-[2-(2-Methyl-5-nitro-1*H*-imidazolyl)ethyl]-4-(2-nitro-1*H*-imidazolyl)butylamine Dihydrochloride (10).** A stirred solution of the azide **38** (1.44 g, 7.34 mmol) in CH_2Cl_2 (20 mL) was treated dropwise with triethyl phosphite (1.26 mL, 7.30 mmol) at 20 °C, and the solution was stirred at 20 °C for 16 h. This solution was then added dropwise to a stirred solution of the aldehyde **36** (1.28 g, 7.0 mmol) in CH_2Cl_2 (20 mL) at 5 °C and stirred at 20 °C for 16 h. The solvent was removed under reduced pressure, the residue was dissolved in anhydrous MeOH, and sodium borohydride (0.29 g, 7.7 mmol) was added carefully. The solution was stirred at 20 °C for 16 h and the solvent removed under reduced pressure. The residue was chromatographed on silica, eluting with a gradient (0–20%) of MeOH in EtOAc containing 1% triethylamine, to give the free amine (1.69 g, 81%), which was treated with HCl in MeOH to give *N*-[2-(2-methyl-5-nitro-1*H*-imidazolyl)ethyl]-*N*-[4-(2-nitro-1*H*-imidazolyl)]butyl]amine dihydrochloride (**10**): mp 162.5 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 7.99 (s, 1 H, H-4‴), 7.66 (d, J = 0.8 Hz, 1 H, H-5′), 7.18 (d, J = 0.8 Hz, 1 H, H-4′), 4.70 (br s, 1 H, NH), 4.27–4.38 (m, 4 H, H-4, H-2″), 2.79 (t, J = 6.1 Hz, 2 H, H-1″), 2.48–2.52 (m, 2 H, H-1), 2.44 (s, 3 H, CH_3), 1.67–1.81 (m, 2 H, H-3), 1.25–1.40 (m, 2 H, H-2); ^{13}C NMR δ 151.4 (C-2‴), (C-2′), 138.3 (C-5‴), 132.8 (C-4‴), 127.7 (C-4′)#, 127.6 (C-5′)#, 49.2, 48.4, 48.2, 45.7 (C-1, C-4, C-1″, C-2″), 27.4 (C-3), 26.0 (C-2), 14.0 (CH_3) (#, assignments interchangeable). Anal. ($C_{13}H_{19}N_7O_4$·2HCl) C, H, N.

***N*-Methyl-*N*-[2-(2-methyl-5-nitro-1*H*-imidazolyl)ethyl]-4-(2-nitro-1*H*-imidazolyl)butylamine (11).** Sodium cyanoborohydride (0.68 g, 10.8 mmol) was added to a stirred solution of **10** (2.81 g, 8.3 mmol) and formaldehyde (37%, 3.4 mL, 41.6 mmmol) in CH_3CN (50 mL), and the mixture was stirred at 20 °C for 30 min. Glacial AcOH was added to keep the pH close to 7, and the mixture was stirred at 20 °C for a further 5 h. The solvent was removed under reduced pressure, the residue was dissolved in water (30 mL), and the pH was adjusted to 10 with 2 M NaOH solution. The solution was extracted with $CHCl_3$ (4 × 50 mL), and workup of the combined organic extracts gave a residue which was chromatographed. Elution with a gradient (0–20%) of MeOH/EtOAc containing 1% Et_3N gave *N*-methyl-*N*-[2-(2-methyl-5-nitro-1*H*-imidazolyl)-ethyl]-4-(2-nitro-1*H*-imidazolyl)butylamine (**11**) as an oil (1.51 g, 52%): ^{1}H NMR [$(CD_3)_2SO$] δ 7.96 (s, 1 H, H-5‴), 7.63 (d, J = 1.0 Hz, 1 H, H-5′), 7.18 (d, J = 1.0 Hz, 1 H, H-4′), 4.27–4.37 (m, 4 H, H-4, H-2″), 2.58 (dd, J = 6.2, 6.1 Hz, 2 H, H-1″), 2.44 (s, 3 H, CH_3), 2.31 (dd, J = 8.1, 7.9 Hz, 2 H, H-1), 2.19 (s, 3 H, N-CH_3), 1.55–1.70 (m, 2 H, H-3), 1.17–1.31 (m, 2 H, H-2); ^{13}C NMR δ 151.3 (C-2‴), 144.5 (C-2′), 138.4 (C-5‴), 132.7 (C-4‴), 127.8 (C-4′)#, 127.6 (C-5′)#, 56.6 (C-1″)#, 56.2 (C-1)#, 49.1 (C-2″), 43.9 (C-4), 42.0 (N-CH_3), 27.2 (C-3), 23.7 (C-2), 13.8 (CH_3) (#, assignments interchangeable); HRDCIMS (NH_3) m/z calcd for $C_{14}H_{22}N_7O_4$ 352.1733 (MH^+), found 352.1729.

***N*-Methyl-*N*-[2-(2-methyl-5-nitro-1*H*-imidazolyl)ethyl]-4-(2-nitro-1*H*-imidazolyl)butylamine *N*-Oxide Hydrochloride (12).** A solution of 2-(phenylsulfonyl)-3-phenyloxaziridine[26] (0.44 g, 1.7 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a stirred solution of **11** (0.57 g, 1.62 mmol) in CH_2Cl_2 (20 mL) at 20 °C, and the solution was stirred for 2 h. The solvent was removed under reduced pressure, and the residue was chromatographed on neutral alumina, eluting with $CHCl_3$, to give *N*-methyl-*N*-[2-(2-methyl-5-nitro-1*H*-imidazolyl)ethyl]-4-(2-nitro-1*H*-imidazolyl)butylamine *N*-oxide (**12**) as a hygroscopic oil (0.48 g, 81%): ^{1}H NMR ($CDCl_3$) δ 8.03 (s, 1 H, H-4‴), 7.73 (d, J = 1.0 Hz, 1 H, H-5′), 7.19 (d, J = 1.0 Hz, 1 H, H-4′), 4.77 (t, J = 6.9 Hz, 2 H, H-2″), 4.41 (t, J = 6.3 Hz, 2 H, H-4), 3.14–3.57 (m, 4 H, H 1, H-1″), 2.93 (s, 3 H, NCH_3), 2.54 (s, 3 H, CH_3), 1.70–1.85 (m, 4 H, H-2, H-3); ^{13}C NMR δ 151.5 (C-2‴), 144.5 (C-2′), 138.4 (C-5‴), 133.0 (C-4‴), 127.8 (C-4′), 127.7 (C-5′), 68.6 (C-1″)#, 65.7 (C-1)#, 55.4 (NCH_3), 49.0 (C-2″), 40.4 (C-4), 27.1 (C-3), 19.5 (C-2), 13.9 (CH_3) (#, assignments interchangeable); FABMS m/z 68 (MH^+, 50%), 211 (50); HRFABMS m/z calcd for $C_{14}H_{22}N_7O_5$ 369.1682 (MH^+), found 368.1687. The hydrochloride salt was crystallized from MeOH/iPr_2O: mp 161–163 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 13.04 (br s, 1 H, HCl), 8.21 (s, 1 H, H-4‴), 7.78 (d, J = 1.0 Hz, 1 H, H-5′), 7.20 (d, J = 1.0 Hz, 1 H, H-4′), 4.86 (dd, J = 7.4,6.9 Hz, 2 H, H-2″), 4.46 (t, J = 6.2 Hz, 2 H, H-4), 4.13 (dd, J = 7.4, 6.7 Hz, 2 H, H-1), 3.73–3.80 (m, 2 H, H-1″), 3.50 (s, 3 H, NCH_3), 2.60 (s, 3 H, CH_3), 1.83–1.90 (m, 4 H, H-2, H-3); ^{13}C NMR δ 151.3 (C-2‴), 144.5 (C-2′), 138.4 (C-5‴), 131.8 (C-4‴), 127.8 (C-4′, C-5′), 66.7 (C-1′), 63.2 (C-1), 52.4 (NCH_3), 48.6 (C-2″), 39.5 (C-4), 26.3 (C-3), 19.2 (C-2), 13.7 (CH_3). Anal. ($C_{14}H_{21}N_7O_5$·2HCl) C, H; N: calcd, 22.3; found, 21.3; Cl: calcd, 16.1; found, 16.7.

3-(2-Nitro-1*H*-imidazolyl)propanol (43). A mixture of (3-bromopropoxy)-*tert*-butyldimethylsilane (7.05 g, 27.9 mmol), 2-nitroimidazole (3.0 g, 26.5 mmol), and K_2CO_3 (4.0 g, 29.2 mmol) in DMF (50 mL) was stirred at 100 °C for 4 h. The

mixture was partitioned between EtOAc and water (200 mL), and the organic fraction was washed with water (50 mL) and brine (50 mL) and worked up. The residue was chromatographed, eluting with a gradient (20–40%) of EtOAc/petroleum ether to give [3-(2-nitro-1*H*-imidazolyl)propoxy]-*tert*-butyldimethylsilane (**42**) as an oil (7.14 g, 94%): ^{1}H NMR ($CDCl_3$) δ 7.12 (s, 1 H, H-5′), 7.10 (s, 1 H, H-4′), 4.53 (t, J = 6.8 Hz, 2 H, H-3), 3.59 (t, J = 5.6 Hz, 2 H, H-1), 1.95–2.08 (m, 2 H, H-2), 0.89 (s, 9 H, $SiC(CH_3)_3$), 0.06 (s, 6 H, $Si(CH_3)_2$); ^{13}C NMR δ 144.7 (C-2′), 128.1 (C-4′), 126.5 (C-5′), 58.7 (C-1), 47.1 (C-3), 32.8 (C-2), 25.8 ($SiC(CH_3)_3$), 18.3 (SiC), −4.0 ($Si(CH_3)_2$); CIMS m/z (NH_3) 286 (MH^+, 100), 270 (5), 228 (30); HRCIMS (NH_3) m/z calcd for $C_{12}H_{24}N_3O_3Si$ 286.1587 (MH^+), found 286.1595.

Tetra-*n*-butylammonium fluoride (27 mL, 27 mmol) was added to a stirred solution of **42** (6.46 g, 22.6 mmol) in THF (100 mL) at 20 °C and the solution stirred for 1 h. The mixture was partitioned between EtOAc and water (200 mL), the aqueous fraction was extracted with EtOAc (4 × 100 mL), and the combined organic fraction was washed with brine (100 mL) and worked up to give 3-(2-nitro-1*H*-imidazolyl)propanol (**43**) (2.95 g, 76%): mp (EtOAc) 100–100.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.17 (s, 1 H, H-5′), 7.14 (s, 1 H, H-4′), 4.59 (t, J = 6.8 Hz, 2 H, H-3), 3.68 (t, J = 5.7 Hz, 2 H, H-1), 2.03–2.16 (m, 2 H, H-2), 1.79 (br s, 1 H, OH); ^{13}C NMR δ 144.8 (C-2′), 128.3 (C-4′), 126.6 (C-5′), 58.5 (C-1), 47.0 (C-3), 32.5 (C-2). Anal. ($C_6H_9N_3O_3$) C, H, N.

Alternative Preparation of Propanol 43. Borane dimethyl sulfide complex (0.71 mL, 7.1 mmol) was added dropwise to a stirred solution of 3-(2-nitro-1*H*-imidazolyl)-propanoic acid (**41**)[17] (1.2 g, 6.5 mmol) in THF (30 mL) at 0 °C. The solution was stirred at 20 °C for 6 h, 5 M HCl (5 mL) was added carefully, and the mixture was stirred at 20 °C for a further 16 h. The reaction was neutralized with 5 M NaOH, and the solution was partitioned between EtOAc and water (100 mL). The organic phase was washed with water (50 mL) and brine (50 mL) and worked up, and the residue was chromatographed, eluting with EtOAc to give **43** (0.24 g, 22%): mp 99–100 °C; spectroscopically identical to the previous sample.

3-(2-Nitro-1*H*-imidazolyl)propanal (44). A stirred suspension of 2-nitroimidazole (1.0 g, 8.84 mmol), acrolein (3 mL, 44 mmol), and KF/alumina[27] (0.25 g) in acetonitrile (20 mL) was heated at reflux temperature for 5 h. The mixture was cooled to 20 °C and filtered, and the solvent was removed under reduced pressure. The residue was chromatographed, eluting with a gradient (50–80%) of EtOAc/petroleum ether to give 3-(2-nitro-1*H*-imidazolyl)propanal (**44**) as an oil (1.0 g, 67%): ^{1}H NMR ($CDCl_3$) δ 9.72 (s, 1 H, CHO), 7.21 (s, 1 H, H-5′), 7.06 (s, 1 H, H-4′), 4.65 (t, J = 6.0 Hz, 2 H, H-3), 3.10 (t, J = 6.0 Hz, 2 H, H-2); ^{13}C NMR δ 198.3 (CHO), 144.5 (C-2′), 128.1 (C-4′), 127.5 (C-5′), 43.6 (C-3)#, 42.9 (C-2)# (#, assignments interchangeable); EIMS m/z 169 (M^+, 5), 123 (90), 97 (60), 40 (100); HREIMS m/z calcd for $C_6H_7N_3O_3$ 169.0487 (M^+), found 169.0486. Anal. ($C_6H_7N_3O_3$) C, H, N.

Alternative Preparation of 44. A mixture of 2-nitroimidazole (3.0 g, 26.5 mmol), 2-(2-bromoethyl)1,3-dioxolane (5.76 g, 31.8 mmol), and K_2CO_3 (3.67 g, 26.5 mmol) in DMF (50 mL) was stirred at 100 °C for 2 h. The solvent was removed under reduced pressure and the residue partitioned between EtOAc and water (200 mL). The organic fraction was washed with water (100 mL) and brine (100 mL) and worked up to give 2-[2-(2-nitro-1*H*-imidazolyl)ethyl]-1,3-dioxolane (**45**) (5.48 g, 97%): mp (EtOAc) 70–72 °C; ^{1}H NMR ($CDCl_3$) δ 7.15 (d, J = 1.0 Hz, 1 H, H-5″), 7.12 (d, J = 1.0 Hz, 1 H, H-4″), 4.90 (t, J = 4.2 Hz, 1 H, H-2), 4.58 (t, J = 7.0 Hz, 2 H, H-2′), 3.92–4.00 (m, 2 H, H-4)#, 3.82–3.90 (m, 2 H, H-5)#, 2.34 (dt, J = 7.0, 4.3 Hz, 2 H, H-1′). (#, assignments interchangeable); ^{13}C NMR δ 145.0 (C-2″), 128.3 (C-4″), 126.3 (C-5″), 65.1 (C-4, C-5), 45.3 (C-2′), 33.9 (C-1′); CIMS m/z (NH_3) 214 (MH^+, 100), 99 (30), 73 (15); HRCIMS (NH_3) m/z calcd for $C_8H_{12}N_3O_4$ 214.0828 (MH^+), found 214.0822. Anal ($C_8H_{11}N_3O_4$) C, H, N.

A suspension of 2-[2-(2-nitro-1*H*-imidazolyl)ethyl]-1,3-dioxolane (**45**) (2.6 g, 12.2 mmol) in 20% aqueous H_2SO_4 (60 mL) was stirred at 20 °C for 16 h. Na_2CO_3 was added carefully until the pH was 6.0, the solution was extracted with EtOAc (3 × 100 mL), and the combined organic fraction was washed with brine (100 mL) and worked up to give 3-(2-nitro-1*H*-imidazol-1*H*-yl)propanal (**44**) as an oil (1.26 g, 61%), spectroscopically identical to an authentic sample.

***N*-[2-(2-Methyl-5-nitro-1*H*-imidazolyl)ethyl]-3-(2-nitro-1*H*-imidazolyl)propylamine (9).** A stirred solution of the above azide **38** (1.17 g, 6.0 mmol) in CH_2Cl_2 (20 mL) was treated dropwise with triethyl phosphite (1.02 mL, 6.0 mmol) at 20 °C, and the solution was stirred at 20 °C for 6 h. This solution was then added dropwise to a stirred solution of the above aldehyde **44** (0.96 g, 5.7 mmol) in CH_2Cl_2 (10 mL) at 5 °C and stirred at 20 °C for 16 h. The solvent was removed under reduced pressure, the residue was dissolved in anhydrous MeOH, and sodium borohydride (0.24 g, 6.2 mmol) was added carefully. The solution was stirred at 20 °C for 16 h, and the solvent was removed under reduced pressure. The residue was chromatographed, eluting with a gradient (0–20%) of MeOH/EtOAc to give *N*-[2-(2-methyl-5-nitro-1*H*-imidazolyl)ethyl]-3-(2-nitro-1*H*-imidazolyl)propylamine (**9**) (0.68 g, 37%). Treatment of this with HCl in MeOH to gave the dihydrochloride salt as a foam: ^{1}H NMR [$(CD_3)_2SO$] δ 8.38 (s, 1 H, H-4‴), 7.87 (s, 1 H, H-5′), 7.22 (s, 1 H, H-4′), 6.85 (br s, 1 H, NH), 4.72 (t, J = 6.5 Hz, 2 H, H-2″), 4.55 (t, J = 6.7 Hz, 2 H, H-3), 3.55–3.40 (m, 2 H, H-1″), 2.90–2.98 (m, 2 H, H-1), 2.65 (s, 3 H, CH_3), 2.22–2.28 (m, 2 H, H-2); ^{13}C NMR δ 151.0 (C-2‴) 144.5 (C-2′), 138.2 (C-5‴), 129.8 (C-4‴), 127.8 (C-5′#), 127.9 (C-4′#), 46.6 (C-2″), 45.4 (C-3), 44.0 (C-1″), 42.5 (C-1), 25.9 (C-2), 13.7 (CH_3) (#, assignments interchangeable). Anal. ($C_{12}H_{17}N_7O_4.2HCl$) C, H, N, Cl.

Alternative Preparation of 9 (Method of Scheme 7). A stirred suspension of the mesylate (**37**)[17] (10.0 g, 40.1 mmol) and NaBr (20.8 g, 0.2 mol) in DMF (100 mL) was heated at 100 °C for 4 h. The mixture was cooled to 20 °C, and the solvent was removed under reduced pressure. The residue was suspended in EtOH (200 mL) and filtered, the solvent was removed under reduced pressure, and the residue was chromatographed. Elution with EtOAc gave an oil which crystallized from EtOAc/petroleum ether to give 2-(2-methyl-5-nitro-1*H*-imidazolyl)ethyl bromide (**47**) (5.86 g, 62%): mp (EtOAc/petroleum ether) 80–81 °C (lit.[27] mp 80–81 °C); ^{1}H NMR ($CDCl_3$) δ 7.99 (s, 1 H, H-4′), 4.69 (t, J = 6.2 Hz, 2 H, H-2), 3.71 (t, J = 6.2 Hz, 2 H, H-1), 2.59 (s, 3 H, CH_3); ^{13}C NMR δ 151.1 (C-2′), 138.2 (C-5′), 133.5 (C-4′), 47.6 (C-2), 29.5 (C-1), 14.8 (CH_3).

A stirred suspension of **47** (7.0 g, 0.03 mol) and NaI (22.4 g, 0.15 mol) in acetone (150 mL) was heated at reflux temperature for 4 h. The suspension was cooled to 20 °C and filtered and the solvent removed under reduced pressure. The residue was partitioned between EtOAc and water and the organic phase worked up. Recrystallization of the residue gave 2-(2-methyl-5-nitro-1*H*-imidazolyl)ethyl iodide (**48**) as tan crystals (3.80 g, 45%): mp (EtOAc/petroleum ether) 107–108 °C; ^{1}H NMR ($CDCl_3$) δ 7.98 (s, 1 H, H-4′), 4.64 (t, J = 7.1 Hz, 2 H, H-2), 3.46 (t, J = 7.1 Hz, 2 H, H-1), 2.58 (s, 3 H, CH_3); ^{13}C NMR δ 150.4 (C-2′), 138.2 (C-5′), 133.4 (C-4′), 47.9 (C-2), 14.8 (CH_3), −0.1 (C-1). Anal. ($C_6H_8IN_3O_2$) C, H, N, I.

A stirred mixture of **48** (1.73 g, 6.2 mmol), 3-(2-nitro-1*H*-imidazolyl)propylamine (**46**)[17] (1.0 g, 5.9 mmol), and K_2CO_3 (0.9 g, 6.5 mmol) in DMF (20 mL) was heated at 110 °C for 2 h. The suspension was cooled to 20 °C and filtered and the solvent removed under reduced pressure. Chromatography of the residue, eluting with 5% MeOH/EtOAc, gave an orange oil which was dissolved in dilute aqueous HCl (1 M, 20 mL). Solvent was removed under reduced pressure, and the residue was crystallized from MeOH to give the dihydrochloride salt of **9** (0.84 g, 40%): mp 198–198 °C; spectroscopically identical to the sample prepared above.

Bis[3-(2-nitro-1*H*-imidazolyl)propyl]amine (13). MsCl (1.3 mL, 16.8 mmol) was added dropwise to a stirred solution of *N*-(triphenylmethyl)-3,3′-iminodipropanol (**50**)[28] (3.0 g, 8.0 mmol) and Et_3N (2.45 mL, 17.6 mmol) in CH_2Cl_2 (100 mL) at 0 °C under N_2. The solution was stirred at 20 °C for 1 h, diluted with CH_2Cl_2 (100 mL), washed with water (3 × 10 mL) and brine (100 mL), and worked up to give [*N*-(triphenylmethyl)-3,3′-iminodipropyl]methane sulfonate (**51**) as a hygroscopic solid (3.97 g, 95%): mp (benzene/petroleum ether) 50–52 °C (lit.[29] mp 51–51.5 °C); ^{1}H NMR ($CDCl_3$) δ 7.44–

7.49 (m, 6 H, H_{ortho}), 7.12–7.31 (m, 6 H, H_{meta}, H_{para}), 4.12 (t, J = 6.3 Hz, 4 H, H-3, H-3′), 2.88 (s, 6 H, 2 × CH_3), 2.47–2.55 (m, 4 H, H-1, H-1′), 1.85–1.99 (m, 4 H, H-2, H-2′); ^{13}C NMR δ 143.5 (C_{ipso}), 129.2 (C_{meta}), 127.7 (C_{ortho}), 126.3 (C_{para}), 79.1 (Ph_3C), 68.1 (C-1, C-1′), 48.8 (C-3, C-3′), 37.4 (SO_2CH_3), 29.7 (C-2, C-2′).

A mixture of **51** (2.67 g, 5.1 mmol), 2-nitroimidazole (1.21 g, 10.7 mmol), and K_2CO_3 (1.55 g, 11.2 mmol) in DMF (30 mL) was stirred at 100 °C for 2 h. The mixture was partitioned between EtOAc and water (400 mL), the organic fraction extracted with water (3 × 100 mL) and brine (100 mL) and worked up, and the residue chromatographed, eluting with (50%) EtOAc/petroleum ether to give *N*-(triphenylmethyl)bis-[3-(2-nitro-1*H*-imidazolyl)propyl]amine (**52**) as an oil (2.56 g, 89%): ^1H NMR ($CDCl_3$) δ 7.37–7.42 (m, 6 H, H_{ortho}), 7.16–7.29 (m, 9 H, H_{meta}, H_{para}), 7.06 (s, 2 H, H-5″, H-5‴), 6.93 (s, 2 H, H-4″, H-4‴), 4.23 (t, J = 7.4 Hz, 4 H, H-1, H-1′), 2.47–2.54 (m, 4 H, H-3, H-3′), 1.87–2.02 (m, 4 H, H-2, H-2′); ^{13}C NMR δ 144.5 (C-2″, C-2‴), 143.2 (C_{ipso}), 128.8 (C_{ortho}), 128.4 (C-4″, C-4‴), 127.8 (C_{meta}), 126.4 (C_{para}), 125.7 (C-5″, C-5‴), 79.0 (Ph_3C), 48.8 (C-3, C-3′)#, 48.6 (C-1, C-1′)#, 30.7 (C-2, C-2′) (#, assignments interchangeable); DCIMS (NH_3) m/z 566 (MH^+, 5%), 324 (30), 277 (100), 243 (80); HRDCIMS (NH_3) m/z calcd for $C_{31}H_{32}N_7O_4$ 566.2516 (MH^+), found 566.2499.

A solution of **52** (2.23 g, 3.94 mmol) in THF (100 mL) and 1 M HCl (10 mL) was stirred at 20 °C for 2 h. The solvent was removed under reduced pressure and the residue dissolved in water (100 mL) and extracted with Et_2O (4 × 50 mL). The aqueous fraction was evaporated to dryness under reduced pressure and the residue dissolved in 1 M HCl (10 mL). The solution was evaporated to dryness under reduced pressure, and the residue was crystallized from methanol to give bis[3-(2-nitro-1*H*-imidazolyl)propyl]amine (**13**) as the dihydrochloride salt (1.21 g, 85%): mp (MeOH) 120.5–123 °C; ^1H NMR [$(CH_3)_2SO$] δ 7.87 (s, 2 H, H-4″, H-4‴), 7.77 (br s, 1 H, NH), 7.21 (s, 2 H, H-5″, H-5‴), 4.54 (t, J = 6.7 Hz, 4 H, H-3, H-3′), 2.82–2.95 (m, 4 H, H-1, H-1′), 2.19–2.26 (m, 4 H, H-2, H-2′); ^{13}C NMR δ 144.5 (C-2″, C-2‴), 127.8 (C-4″, C-4‴, C-5″, C-5‴), 46.6 (C-3, C-3′), 43.6 (C-1, C-1′), 26.0 (C-2, C-2′). Anal. ($C_{12}H_{17}N_7O_4$·2HCl) C, H, N, Cl.

***N*-Methylbis[3-(2-nitro-1*H*-imidazolyl)propyl]amine Perchlorate (14).** $NaBH_3CN$ (0.48 g, 7.6 mmol) was added to a stirred solution of **13** (1.89 g, 5.85 mmol) and formaldehyde (37%, 2.2 mL, 29.2 mmol) in MeCN (80 mL) at 20 °C. The mixture was stirred for 10 min, the pH adjusted to 7 with glacial AcOH, and the suspension was stirred at 20 °C for 4 h, with glacial AcOH being added as required to keep the pH at 7. The solvent was removed under reduced pressure, the residue dissolved in water (50 mL), the pH adjusted to 10, and the aqueous phase extracted with $CHCl_3$ (3 × 50 mL). Workup of the combined organic extracts gave a residue which was chromatographed, eluting with a gradient (0–15%) of MeOH/EtOAc, to give *N*-methylbis[3-(2-nitro-1*H*-imidazolyl)propyl]-amine (**14**) as an oil (1.08 g, 64%): ^1H NMR [$(CD_3)_2SO$] δ 7.67 (d, J = 0.9 Hz, 2 H, H-4″, H-4‴), 7.18 (d, J = 0.9 Hz, 2 H, H-5″, H-5‴), 4.40 (dd, J = 7.4, 7.2 Hz, 4 H, H-3, H-3′), 2.30 (t, J = 6.8 Hz, 4 H, H-1, H-1′), 2.11 (s, 3 H, NCH_3), 1.90–1.93 (m, 4 H, H-2, H-2′); ^{13}C NMR δ 144.6 (C-2″, C-2‴), 127.7 (C-4″, C-4‴, C-5″, C-5‴), 53.7 (C-1, C-1′), 47.7 (C-3, C-3′), 41.0 (NCH_3), 27.2 (C-2, C-2′); DCIMS (NH_3) m/z 338 (MH^+, 100), 291 (90); HRDCIMS (NH_3) m/z calcd for $C_{13}H_{20}N_7O_4$ 338.1577 (MH^+), found 338.1569. A solution of **14** (1.0 g) in water (20 mL) was treated with $HClO_4$ and crystallized from water to give the perchlorate salt: mp 184 °C dec; ^1H NMR [$(CD_3)_2SO$] δ 9.39 (br s, 1 H, NH), 7.73 (d, J = 1.0 Hz, 2 H, H-5″, H-5‴), 7.22 (d, J = 1.0 Hz, 2 H, H-4″, H-4‴), 4.46 (t, J = 7.0 Hz, 4 H, H-3, H-3′), 3.17–3.24 (m, 2 H, H-1, H-1′), 3.07–3.15 (m, 2 H, H-1, H-1′), 2.78 (d, J = 4.6 Hz, 3 H, NCH_3), 2.15–2.27 (m, 4 H, H-2, h 2′); ^{13}C NMR δ 144.5 (C-2″, C-2‴), 127.9 (C-4″, C-4‴)#, 127.7 (C-5″, C-5‴)#, 52.1 (C-3, C-3′), 46.5 (C-1, C-1′), 39.3 (NCH_3), 24.3 (C-2, C-2′) (#, assignments interchangeable). Anal. ($C_{13}H_{19}N_7O_4$.$HClO_4$) H, N; C: calcd, 35.7; found, 35.1; Cl: calcd, 8.1; found, 8.7.

***N*,*N*-Dimethylbis[3-(2-nitro-1*H*-imidazolyl)propyl]ammonium Iodide (15).** Iodomethane (0.9 mL, 14.4 mmol) was added to a stirred suspension of **13** (4.3 g, 13.3 mmol) and K_2CO_3 (2.2 g, 16 mmol) in DMF (40 mL) at 20 °C. The mixture was stirred at 20 °C for 6 h, the solvent was removed under reduced pressure, and the residue was partitioned between $CHCl_3$ and water (400 mL). The aqueous phase was extracted with $CHCl_3$ (3 × 80 mL), and the combined organic fraction was worked up. The residue was chromatographed on neutral alumina, eluting with $CHCl_3$, to give *N*,*N*-dimethylbis[3-(2-nitro-1*H*-imidazolyl)propyl]ammonium iodide (**15**): (2.71 g, 54%): mp ($CHCl_3$) 202–204 °C; ^1H NMR [$(CD_3)_2SO$] δ 7.70 (d, J = 1.0 Hz, 2 H, H-5″, H-5‴), 7.24 (d, J = 1.0 Hz, 2 H, H-4″, H-4‴), 4.44 (t, J = 7.0 Hz, 4 H, H-3, H-3′), 3.35–3.39 (m, 4 H, H-1, H-1′), 3.02 (s, 6 H, $N(CH_3)_2$, 2.20–2.29 (m, 4 H, H-2, H-2′); ^{13}C NMR δ 144.5 (C-2″, C-2‴), 127.9 (C-4, C-4‴), 127.5 (C-5″, C-5‴), 60.0 (C-1, C-1′), 50.25 ($N(CH_3)_2$), 46.3 (C-3, C-3′), 23.0 (C-2, C-2′). Anal. ($C_{14}H_{22}IN_7O_4$) C, H, N, I.

4-(3-Nitro-1*H*-1,2,4-triazolyl)butanoic Acid (56). A stirred suspension of 3-nitro-1*H*-1,2,4-triazole (3.0 g, 26.3 mmol), methyl 4-bromobutanoate (5.71 g, 31.6 mmol), and K_2CO_3 (4.0 g, 28.9 mmol) in DMF (70 mL) was heated at 110 °C for 4 h. The suspension was cooled to 20 °C, the solvent was removed under reduced pressure, and the residue was partitioned between CH_2Cl_2 (150 mL) and water (150 mL). Workup and chromatography of the organic fraction, eluting with a gradient (60–80%) of EtOAc/hexane, gave crude methyl 4-(3-nitro-1*H*-1,2,4-triazolyl)butanoate (**55**) as an oil: ^1H NMR [$(CD_3)_2SO$] δ 8.85 (s, 1 H, H-5′), 3.70 (t, J = 6.9 Hz, 2 H, H-4), 3.56 (s, 3 H, OCH_3), 2.38 (t, J = 7.4 Hz, 2 H, H-2), 2.08 (quintet, J = 7.1 Hz, 2 H, H-3); ^{13}C NMR δ 172.4 (CO_2), 162.1 (C-3′), 146.7 (C-5′), 51.4 (OCH_3), 49.6 (C-4), 30.0 (C-2), 24.3 (C-3). The ester **55** was dissolved in concentrated HCl (100 mL) and stirred at 20 °C for 18 h, after which the solvent was removed under reduced pressure. The residue was dissolved in 2 M KOH (50 mL) and washed with CH_2Cl_2 (2 × 50 mL), and the aqueous fraction was then acidified with concentrated HCl and extracted with CH_2Cl_2. The residue was crystallized to give 4-(3-nitro-1*H*-1,2,4-triazolyl)butanoic acid (**56**) (4.08 g, 77%): mp (MeOH/EtOAc) 92–93 °C; ^1H NMR [$(CD_3)_2SO$] δ 8.87 (s, 1 H, H-5′), 4.36 (t, J = 7.0 Hz, 2 H, H-4), 2.31 (t, J = 7.3 Hz, 2 H, H-2), 2.08 (quintet, J = 7.1 H, 2 H, H-3); ^{13}C NMR δ 173.5 (COOH), 162.0 (C-3′), 146.6 (C-5′), 49.8 (C-4), 30.2 (C-2), 24.3 (C-3). Anal. ($C_6H_8N_4O_4$) C, H, N.

***N*-[2-(2-Methyl-5-nitro-1*H*-imidazolyl)ethyl]succinamidic Acid (57).** A solution of **29** (0.88 g, 5.17 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a solution of succinic anhydride (0.52 g, 5.17 mmol) in CH_2Cl_2 (30 mL) at 0 °C. The resulting suspension was stirred at 20 °C for 18 h, and the precipitate was collected and recrystallized to give *N*-[2-(2-methyl-5-nitro-1*H*-imidazolyl)ethyl]succinamidic acid (**57**) (1.26 g, 90%): mp (MeOH/iPr_2O) 157–157.5 °C; ^1H NMR δ [$(CD_3)_2SO$] δ 8.08 (br t, J = 6.0 Hz, 1 H, NH), 8.02 (s, 1 H, H-4″), 4.30 (t, J = 5.9 Hz, 2 H, H-2′), 3.41 (q, J = 6.0 Hz, 2 H, H-1′), 2.40 (s, 3 H, CH_3), 2.36 (dd, J = 7.0, 6.4 Hz, 2 H, H-2), 2.24 (dd, J = 7.0, 6.4 Hz, 2 H, H-3); ^{13}C NMR δ 173.6 (CO_2H), 171.4 (CONH), 151.3 (C-2″), 138.4 (C-5″), 133.1 (C-4″), 45.3 (C-2′), 37.9, (C-1′), 29.7 (C-1), 28.8 (C-2), 13.7 (CH_3). Anal. ($C_{10}H_{14}N_4O_5$) C, H, N.

***N*-Methyl-2-(2-methyl-5-nitro-1*H*-imidazolyl)ethylamine (60).** Trifluoroacetic anhydride (2.5 mL, 17.8 mmol) was added dropwise to a stirred solution of **29** (2.76 g, 16.2 mmol) and Et_3N (2.7 mL, 19.5 mmol) in CH_2Cl_2 (30 mL) at 0 °C, and the solution was stirred at 20° C for 28 h. The suspension was filtered, the solvent was removed under reduced pressure, and the residue was chromatographed. Elution with EtOAc gave *N*-[2-(2-methyl-5-nitro-1*H*-imidazolyl)ethyl]trifluoroacetamide (**58**) (3.45 g, 80%): mp (EtOAc) 144 °C; ^1H NMR [$(CD_3)_2SO$] δ 9.63 (br s, 1 H, NH), 8.04 (s, 1 H, H-4′), 4.43 (dd, J = 6.0, 5.6 Hz, 2 H, H-2), 3.62 (dt, J = 6.0, 5.9 Hz, 2 H, H-1), 2.40(s, 3 H, CH_3); ^{13}C NMR δ 157.0 (q, J = 36 Hz, CO), 151.3 (C-2′), 138.6 (C-5′), 133.2 (C-4′), 115.7 (q, J = 290 Hz, CF_3), 44.6 (C-2), 38.2 (C-1), 13.6 (CH_3). Anal. ($C_8H_9F_3N_4O_3$) C, H, N.

A solution of **58** (2.0 g, 7.5 mmol) in dry THF (10 mL) was added dropwise to a slurry of NaH (0.43 g, 9.0 mmol; prewashed with petroleum ether) in THF (10 mL) at 0 °C. The mixture was stirred at 0 °C until a red solution formed, then CH_3I (2.4 mL, 38 mmol) was added, and the solution was

stirred at 20 °C for 6 h. The mixture was filtered, the solvent removed under reduced pressure, and the residue chromatographed. Elution with a gradient (50–100%) of EtOAc/petroleum ether gave *N*-[2-(2-methyl-5-nitroimidazol-1*H*-yl)-ethyl]-*N*-methyltrifluoroacetamide (**59**) (1.71 g, 84%): mp (EtOAc/petroleum ether) 95 °C; ^{1}H NMR ($CDCl_3$) δ 7.99 (s, 1 H, H-4′), 4.53 (dd, J = 7.1, 6.4 Hz, 2 H, H-2), 3.77 (dd, J = 7.1, 6.8 Hz, 2 H, H-1), 3.15 (q, J = 1.4 Hz, 3 H, NCH_3), 2.53 (s, 3 H, CH_3); ^{13}C NMR δ 157.9 (q, J = 38 Hz, CO), 151.0 (C-2′), 138.4 (C-5′), 133.5 (C-4′), 116.1 (q, J = 287 Hz, CF_3), 49.6 (C-2), 42.5 (C-l), 36.3 (q, J = 4 Hz, NCH_3), 13.9 (CH_3). Anal. ($C_9H_{11}F_3N_4O_3$) C, H, N.

A solution of **59** (0.46 g, 1.64 mmol) and K_2CO_3 (0.34 g, 2.46 mmol) in aqeous MeOH (20 mL) was stirred at 20 °C for 4 h. The solvent was removed under reduced pressure and the residue partitioned between $CHCl_3$ and water. The residue from the organic fraction was dissolved in MeOH/HCl (10 mL; 1:1) and kept at 20 °C for 1 h. Removal of solvent under reduced pressure and crystallization of the residue gave *N*-methyl-2-(2-methyl-5-nitro-1*H*-imidazolyl)ethylamine (**60**) as the dihydrochloride salt (0.2 g, 56%): mp (MeOH) 230–232 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.57 (br s, 2 H, NH. HCl), 8.37 (s, 1 H, H-4′), 4.69 (t, J = 6.3 Hz, 2 H, H-2), 3.31–3.37 (m, 2 H, H-1), 2.64 (s, 3 H, CH_3), 2.54 (t, J = 5.2 Hz, 3 H, NCH_3); ^{13}C NMR δ 151.1 (C-2′), 138.4 (C-5′), 130.1 (C-4′), 46.9 (C-2), 42.7 (C-l), 32.6 (NCH_3), 13.7 (CH_3). Anal. ($C_7H_{11}Cl_2N_4O_2$) C, N, Cl; H: calcd, 4.36; found, 5.52.

2-(3-Nitro-1*H*-1,2,4-triazolyl)ethylamine (62). A solution of N-(*tert*-butyloxycarbonyl)-2-(methylsulfonyl)ethylamine (**61**)[30] (6.40 g, 28.9 mmol) in DMF (10 mL) was added slowly to a stirred suspension of 3-nitro-1,2,4-triazole (3.00 g, 26.3 mmol) and K_2CO_3 (4.00 g, 28.9 mmol) in DMF (200 mL) at 110 °C. The suspension was stirred at 110 °C for 4 h and then cooled to 20 °C, and the solvent was removed under reduced pressure. The residue was partitioned between EtOAc (300 mL) and water (300 mL), and the aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed with brine (50 mL) and worked up. The residue was dissolved in MeOH (80 mL) and 5 M HCl (20 mL) and stirred at 20 °C for 2 h. Removal of solvent gave 2-(3-nitro-1*H*-1,2,4-triazolyl)ethylamine (**62**) as the hydrochloride salt (3.41 g, 69%): mp (MeOH) 215–215.5 °C; ^{1}H NMR [$(CD_3)_2SO$]) δ 8.99 (s, 1 H, H-5′), 8.50 (br s, 2 H, NH. HCl), 4.71 (t, J = 5.9 Hz, 2 H, H-1), 3.37 (br s, 2 H, H-2); ^{13}C NMR δ 162.1 (C-3′), 147.5 (C-5′), 47.8 (C-2), 37.9 (C-1). Anal. ($C_4H_8ClN_5O_2$) C, H, N, Cl.

***N*-[2-(2-Methyl-5-nitro-1*H*-imidazolyl)ethyl]-3-(3-nitro-1*H*-1,2,4-triazolyl)propanesulfonamide (20).** A suspension of **32** (2.0 g, 6.4 mmol), 3-nitro-1,2,4-triazole (0.81 g, 7.1 mmol), and K_2CO_3 (0.93 g, 6.7 mmol) in DMF (50 mL) was heated at 110 °C for 4 h. The solvent was removed under reduced pressure and the residue chromatographed, eluting with a gradient (0–10%) of MeOH/EtOAc, to give *N*-[2-(2-methyl-5-nitro-1*H*-imidazolyl)ethyl]-3-(3-nitro-1*H*-1,2,4-triazolyl)propanesulfonamide (**20**) (0.72 g, 38%): mp (MeOH/iPr_2O) 147–149 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.83 (s, 1 H, H-5′), 8.04 (s, 1 H, H-4‴), 7.50 (t, J = 6.3 Hz, 1 H, SO_2NH), 4.42 (t, J = 7.0 Hz, 2 H, H-3), 4.33 (t, J = 5.9 Hz, 2 H, H-2″), 3.31 (t, J = 6.2 Hz, 2 H, H-1″), 3.06–3.10 (m, 2 H, H-1), 2.47 (s, 3 H, CH_3), 2.14–2.22 (m, 2 H, H-2); ^{13}C NMR δ 162.1 (C-3′), 151.6 (C-2‴), 146.8 (C-5′), 138.3 (C-5‴), 133.1 (C-4‴), 48.6 (C-2″), 47.8 (C-3), 46.4 (C-1″), 41.5 (C-1), 23.5 (C-2), 14.1 (CH_3). Anal. ($C_{11}H_{16}N_8O_6S$) C, H, N, S.

***N,N′*-Bis[3-(2-nitro-1*H*-imidazolyl)propyl]oxamide (16).** A suspension of **46** (0.92 g, 5.4 mmol) in CH_2Cl_2 (70 mL) and K_2CO_3 (0.74 g, 5.4 mmol) was treated dropwise with oxalyl chloride (0.24 g, 2.8 mmol) at 0 °C, and the mixture was stirred for 2 h. The mixture was partitioned between CH_2Cl_2 and water, the organic fraction was worked up, and the residue was triturated with MeOH to give *N,N′*-bis[3-(2-nitro-1*H*-imidazolyl)propyl]oxamide (**16**) (0.38 g, 36%): mp (MeOH) 200–200.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.89 (br s, 2 H, 2 CONH), 7.73 (s, 2 H, H-5′), 7.18 (d, J = 0.9 Hz, 2 H, H-4′), 4.0 (t, J = 7.0 Hz, 4 H, H-3), 3.18 (q, J = 6.4 Hz, 4 H, H-1), 1.97–2.04 (m, 4 H, H-2); ^{13}C NMR δ 160.0 (2CONH), 144.5 (2C-2′), 127.7 (2C-4′), 127.6 (2C-5′), 47.2 (2C-3), 35.9 (2C-1), 29.2 (2C-2). Anal. ($C_{14}H_{18}N_8O_6$) C, H, N.

***N*-[3-(2-Nitro-1*H*-imidazolyl)propyl]-*N′*-[2-(2-methyl-5-nitro-1*H*-imidazolyl)ethyl]succinamide (17). Example of General Method.** Diethyl phosphorocyanidate (0.88 mL, 5.81 mmol) was added dropwise to a mixture of the acid **57** (1.21 g, 4.47 mmol), the amine **46** (0.76 g, 4.47 mmol), and Et_3N (1.90 mL, 13.4 mmol) in DMF (30 mL) at 0 °C. The solution was stirred at 20 °C for 18 h, solvent was removed under reduced pressure, and the residue was chromatographed. Elution with a gradient (0–20%) of MeOH/EtOAc gave *N*-[3-(2-nitro-1*H*-imidazolyl)propyl]-*N′*-[2-(2-methyl-5-nitro-1*H*-imidazolyl)ethyl]-succinamide (**17**) (1.59 g, 84%): mp (MeOH/iPr_2O) 126–127 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.11 (t, J = 6.0 Hz, 1 H, NH), 8.00 (s, 1 H, H-4‴), 7.95 (t, J = 5.6 Hz, 1 H, NH), 7.70 (d, J = 0.8 Hz, 1 H, H-5″), 7.18 (d, J = 0.8 Hz, 1 H, H-4″), 4.37 (t, J = 7.0 Hz, 2 H, H-3′), 4.30 (t, J = 5.9 Hz, 2 H, H-2‴), 3.41 (q, J = 6.0 Hz, 2 H, H-1‴), 3.05 (q, J = 6.0 Hz, 2 H, H-1′), 2.39 (s, 3 H, CH_3), 2.25 (s, 4 H, H-2, H-3), 1.90 (quintet, J = 6.8 Hz, 2 H, H-2′); ^{13}C NMR δ 171.9 (CONH), 171.3 (CONH), 151.4 (C-2‴), 144.6 (C-2″), 138.5 (C-5‴), 133.1 (C-4‴), 127.9 (C-5″), 127.7 (C-4″), 47.2 (C-3′), 45.4 (C-2‴), 37.9 (C-1′), 35.4 (C-1‴), 30.4 (C-2, C-3), 29.8, (C-2′), 13.7 (CH_3); HRDCIMS (NH_3) m/z calcd for $C_{16}H_{22}N_8O_6$ 423.1741 (MH^+), found 423.1742. Anal. ($C_{16}H_{22}N_8O_6$) C, H, N.

Similar reaction of acid **34** and amine **60** gave *N*-methyl-*N*-[2-(2-methyl-5-nitro-1*H*-imidazolyl)ethyl]-4-(2-nitro-1*H*-imidazolyl)butanamide (**18**) (0.80 g, 88%): mp (MeOH/iPr_2O) 112–114 °C; as a mixture of two rotamers. **Major rotamer**: ^{1}H NMR [$(CD_3)_2SO$] δ 8.00 (s, 1 H, H-4‴), 7.63 (s, 1 H, H-5′), 7.18 (s, 1 H, H-4′) 4.39 (t, J = 6.1 Hz, 2 H, H-2″), 4.35 (dd, J = 7.2, 7.0 Hz, 2 H, H-4), 3.60 (t, J = 6.1 Hz, 2 H, H-1″), 2.89 (s, 3 H, NCH_3 2.39 (s, 3 H, CH_3), 2.31 (dd, J = 7.1, 7.0 Hz, 2 H, H-2), 1.91–1.98 (m, 2 H, H-3); ^{13}C NMR δ 171.5 (CO), 151.1 (C-2‴), 144.6 (C-2′), 138.6 (C-5‴), 133.0 (C-4‴), 127.8 (C-4′), 127.6 (C-5′), 48.9 (C-2″), 46.6 (C-4), 43.3 (C-1″), 35.5 (NCH_3), 29.0 (C-2), 24.9 (C-3), 13.5 (CH_3). **Minor rotamer**: ^{1}H NMR [$(CD_3)_2SO$] δ 8.03 (s, 1 H, H-4‴), 7.61 (s, 1 H, H-5′), 7.17 (s, 1 H, H-4′), 4.46 (t, J = 6.4 Hz, 2 H, H-2″), 3.67 (t, J = 6.5 Hz, 2 H, H-1″), 2.78 (s, 3 H, N-CH_3), 2.40 (s, 3 H, CH_3), 2.14 (dd, J = 7.2, 7.0 Hz, 2 H, H-2), 1.91–1.98 (m, 2 H, H-3); ^{13}C NMR δ 170.6 (CO), 151.1 (C-2‴), 144.6 (C-2′), 138.4 (C-5‴), 133.0 (C-4‴), 127.8 (C-4′), 127.6 (C-5′), 48.2 (C-2″), 46.6 (C-4′), 43.7 (C-1″), 33.4 (NCH_3) 28.0 (C-2), 25.2 (C-3), 13.5 (CH_3). Anal. ($C_{14}H_{19}N_7O_5$) C, H, N.

Similar reaction of acid **34** and amine **62** gave *N*-[2-(3-nitro-1*H*-1,2,4-triazolyl)ethyl]-4-(2-nitro-1*H*-imidazolyl)butanamide (**21**) (0.59 g, 91%): mp 105–107 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.82 (s, 1 H, H-5‴), 8.00 (br t, J = 5.7 Hz, 1 H, NH), 7.63 (d, J = 0.8 Hz, 1 H, H-5′), 7.18 (d, J = 0.8 Hz, 1 H, H-4′), 4.35–4.39 (m, 4 H, H-2″, H-4), 3.49 (q, J = 5.8 Hz, 2 H, H-1″), 2.06–2.10 (m, 2 H, H-2), 1.95–2.01 (m, 2 H, H-3); ^{13}C NMR δ 171.5 (CONH), 162.0 (C-3‴), 147.0 (C-5‴), 144.5 (C-2′), 127.7 (C-4′, C-5′), 50.2 (C-4), 48.8 (C-2″), 38.1 (C-1″), 31.7 (C-3), 25.6 (C-2); HRMS (FAB) m/z calcd for $C_{11}H_{14}N_8O_5$ 339.1165 (MH^+), found 339.1168.

Similar reaction of acid **56** and 2-(2-nitro-1*H*-imidazolyl)-ethylamine (**63**)[17] gave *N*-[2-(2-nitro-1*H*-imidazolyl)ethyl]-4-(3-nitro-1*H*-1,2,4-triazolyl)butanamide (**22**) (1.03 g, 79%): mp (MeOH/iPr_2O) 160–163 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.84 (s, 1 H, H-5′), 7.96 (t, J = 5.8 Hz, 1 H, NH), 7.53 (d, J = 0.8 Hz, 1 H, H-5‴), 7.15 (d, J = 0.8 Hz, 1 H, H-4‴), 4.43 (dd, J = 5.9, 5.4 Hz, 2 H, H-2″), 4.28 (dd, J = 6.7, 6.4 Hz, 2 H, H-4), 3.47 (q, J = 5.9 Hz, 2 H, H-1″), 1.95–2.06 (m, 4 H, H-2, H-3); ^{13}C NMR δ 171.3 (CONH), 162.0 (C-3′), 146.6 (C-5′), 144.7 (C-2‴), 128.3 (C-5‴), 127.6 (C-4‴), 49.9 (C-4), 49.1 (C-2″), 39.0 (C-1″), 31.4 (C-2), 24.7 (C-3). Anal. ($C_{11}H_{14}N_8O_5$) C, H, N.

Similar reaction of acid **56** and amine **46** gave *N*-[3-(2-nitro-1*H*-imidazolyl)propyl]-4-(3-nitro-1*H*-1,2,4-triazolyl)butanamide (**23**) as an oil (2.71 g, 90%): ^{1}H NMR [$(CD_3)_2SO$]) δ 8.86 (s, 1 H, H-5′), 7.95 (t, J = 5.4 Hz, 1 H, NH), 7.70 (s, 1 H, H-5‴), 7.19 (s, 1 H, H-4‴), 4.38 (t, J = 7.0 Hz, 2 H, H-3″), 4.34 (t, J = 6.7 Hz, 2 H, H-4), 3.08 (dt, J = 6.4, 6.0 Hz, 2 H, H-1″), 2.07–2.14 (m, 4 H, H-2, H-2″), 1.92 (quintet, J = 6.8 H, 2 H, H-3); ^{13}C NMR δ 170.9 (CONH), 162.0 (C-3′), 146.7 (C-5′), 144.5 (C-2‴), 127.7 (C-4‴, C-5‴), 50.0 (C-4), 47.3 (C-3″), 35.4 (C-1″), 31.6 (C-2), 29.7 (C-2″), 24.8 (C-3). Anal. ($C_{12}H_{16}N_8O_5$) C, H.

A similar reaction of acid **56** and amine **29** gave *N*-[2-(2-

methyl-5-nitro-1*H*-imidazolyl)ethyl]-4-(3-nitro-1*H*-1,2,4-triazolyl)butanamide (**24**) (1.98 g, 83%): mp (MeOH/iPr_2O) 115–117 °C; ^1H NMR [$(CD_3)_2SO$] δ 8.84 (s, 1 H, H-5′), 8.07 (t, J = 6.0 Hz, 1 H, NH), 8.01 (s, 1 H, H-4‴), 4.26–4.32 (m, 4 H, H-4, H-2″), 3.40 (q, J = 6.0 Hz, 2 H, H-1″), 2.39 (s, 3 H, CH_3), 1.94–2.07 (m, 4 H, H-2, H-3); ^{13}C NMR δ 171.4 (CONH), 162.1 (C-3′), 151.3 (C-2‴), 146.7 (C-5′), 138.5 (C-5‴), 133.1 (C-4‴), 49.9 (C-4), 45.4 (C-2″), 37.8 (C-1″), 31.4 (C-2), 24.8 (C-3), 13.8 (CH_3). Anal. ($C_{12}H_{16}N_8O_5$) C, H, N.

Biological Assays. Methods for growth of the AA8, UV4, EMT6, and FME cell lines and determination of growth inhibition under aerobic conditions using 96-well plates have been described previously.[17] Briefly, exponential-phase cultures (50 μL) were exposed to drugs for 18 h, and subsequent growth was determined 3 days later by staining with methylene blue. The IC_{50} was defined as the drug concentration required to reduce cell density to 50% of the mean value for eight control cultures on the same 96-well plate. In all studies, drug solutions were prepared in αMEM culture medium containing 5% fetal calf serum, and final concentrations were determined by spectrophotometry at 320 nm. In all cases the pH was determined after equilibration under 5% CO_2 at 37 °C and adjusted to 7.4 if necessary. Cytotoxicity under aerobic and hypoxic conditions was compared using well stirred, continuously gassed single cell suspensions ((1–2) × 10^6 AA8 or UV4 cells/mL), with removal of samples for clonogenic assay at intervals, as described in detail elsewhere.[42] Cell suspensions and drug solutions in culture medium were equilibrated separately for 1 h under the appropriate gas phase before mixing. The concentration × time to reduce plating efficiency to 10% of control values (CT_{10}) was determined at each drug concentration, and the hypoxic selectivity calculated as the ratio of aerobic CT_{10}/hypoxic CT_{10} at the same time.

Radiosensitization was assessed by irradiating (cobalt 60) single cell suspensions of hypoxic AA8 cells at 37 °C as detailed previously.[43] Cultures were irradiated 0.5 h after addition of drug. Control experiments showed that the drugs were not cytotoxic in the absence of irradiation under these conditions. At each drug concentration the radiation survival curve was fitted to the linear–quadratic model (ln surviving fraction = $-\alpha D - \beta D^2$, where D is the radiation dose) to interpolate the dose for 10% survival (D_{10}), and the sensitizer enhancement ratio (SER = D_{10} without drug/D_{10} with drug) was determined.

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References

(1) Brown, J. M.; Koong, A. Therapeutic advantage of hypoxic cells in tumors: a theoretical study. *J. Natl. Cancer Inst.* **1991**, *83*, 178–185.
(2) Kennedy, K. A. Hypoxic cells as specific drug targets for chemotherapy. *Anti-Cancer Drug Design* **1987**, *2*, 181–194.
(3) Sartorelli, A. C. Therapeutic attack of hypoxic cells of solid tumors: presidential address. *Cancer Res.* **1988**, *48*, 775–778.
(4) Chapman, J. D. Measurement of tumor hypoxia by invasive and non-invasive procedures: a review of recent clinical studies. *Radiother. Oncol.* **1991**, Suppl. 20, 13–19. .
(5) Gatenby, R. A.; Kessler, H. B.; Rosenblum, J. S.; Coia, L. R.; Moldofsky, P. J.; Harz, W. H. Oxygen distribution in squamous cell carcinoma metasteses and its relationship to outcome of radiation therapy. *Int. J. Radiat. Oncol. Biol. Phys.* **1988**, *14*, 831–838.
(6) Wilson, W. R. Tumor hypoxia; challenges for cancer chemotherapy. In *Cancer Biology and Medicine, Vol. 3*; Waring, M. J., Ponder, B. A. J., Eds.; Kluwer Academic: Lancaster, 1992; pp 87–131.
(7) Adams, G. E.; Stratford, I. J. Hypoxia-mediated nitroheterocyclic drugs in the radio- and chemotherapy of cancer. An overview. *Biochem. Pharmacol.* **1986**, *35*, 71–76.
(8) Rauth, A. M. Pharmacology and toxicology of sensitizers : mechanism studies. *Int. J. Radiat. Oncol. Biol. Phys.* **1984**, *10*, 1293–1300.
(9) Adams, G. E.; Stratford, I. J.; Wallace, R. G.; Wardman, P.; Watts, M. E. Toxicity of nitro compounds towards hypoxic mammalian cells *in vitro*: dependence on reduction potential. *J. Natl. Cancer Inst.* **1980**, *64*, 555–560.
(10) Moselen, J. W.; Hay, M. P.; Denny, W. A.; Wilson, W. R. N-[2-(2-Methyl-5-nitroimidazol-1*H*-yl)ethyl]-4-(2-nitroimidazol-1*H*-yl)-butanamide (SN 24699), a bis-nitroimidazole with enhanced selectivity for hypoxic cells in vitro. *Cancer Res.* **1995**, *55*, 574–580.
(11) Whitmore, G. F.; Guylas, S. Studies on the toxicity of RSU-1069. *Int. J. Radiat. Oncol. Biol. Phys.* **1986**, *12*, 1219–1222.
(12) Denny, W. A.; Roberts, P. B.; Anderson, R. F.; Brown, J. M.; Wilson, W. R. NLA-1: a 2-nitroimidazole radiosensitizer targeted to DNA by intercalation. *Int. J. Radiat. Biol. Phys.* **1992** *22*, 553–556.
(13) Hill, R. P.; Guylas, S.; Whitmore, G. F. Studies on the in vivo and in vitro cytotoxicity of the drug RSU 1069. *Br. J. Cancer* **1986**, *53*, 743–751.
(14) O'Neill, P.; McNeil, S. S.; Jenkins, T. C. Induction of DNA crosslinks in vitro upon reduction of RSU 1069 and RSU 1131. *Biochem. Pharmacol.* **1987**, *36*, 1787–1792.
(15) Jenkins, T. C.; Naylor, M. A.; O'Neill, P.; Threadgill, M. D.; Cole, S.; Stratford, I. J.; Adams, G. E.; Fielden, E. M.; Suto, M. J.; Stier, M. A. Synthesis and evaluation of α-[[(2-haloethyl)amino]-methyl]-2-nitro-1*H*-imidazole-1-ethanols as prodrugs of α-[(1-aziridinyl)methyl)]-2-nitro-1*H*-imidazole-1-ethanol (RSU-1069) and its analogues which are radiosensitisers and bioreductively activated cytotoxins. *J. Med. Chem.* **1990**, *33*, 2603–2610.
(16) Naylor, M. A.; Threadgill, M. D.; Showalter, H. D. H.; Stratford, I. J.; Stephens, M. A.; Fielden, E. M.; Adams, G. E. Synthesis of the enantiomers of the bioreductively-activated cytotoxin RSU-1069 and its prodrug RB6145 and lack of stereoselectivity in their cytotoxicity and radiosensitization in vitro. *Drug Des. Discovery* **1993**, *10*, 249–255.
(17) Hay, M. P.; Wilson, W. R.; Moselen, J. W.; Palmer, B. D.; Denny, W. A. Hypoxia-selective antitumor agents. 8. Bis-(nitroimidazolyl)alkylcarboxamides: a new class of hypoxia-selective cytotoxins and hypoxic cell radiosensitisers. *J. Med. Chem.* **1994**, 37, 381–391.
(18) Wilson, W. R.; van Zijl, P.; Denny, W. A. Bis-bioreductive agents as hypoxia-selective cytotoxins: N-oxides of nitracrine. *Int. J. Rad. Oncol. Biol. Phys.* **1992**, *22*, 693–697.
(19) Hoffer, M.; Grundberg, E. Synthesis and antiprotozoal activity of 1-(3-chloro-2-hydroxypropyl)-substituted nitroimidazoles. *J. Med. Chem.* **1974**, *17*, 1019–1020.
(20) Brown, H. C.; Narasimhan, S.; Choi, Y. M. Improved method for borane-dimethyl sulfide reduction of tertiary and secondary amides in the presence of boron trifloride etherate. *Synthesis* **1981**, 996–997.
(21) Borch, R. F. A new method for the reduction of secondary and tertiary amides. *Tetrahedron Lett.* **1968**, 61–65.
(22) Prasad, A. S.; Kanth, J. V. B.; Periasamy, M. Convenient methods for the reduction of amides, nitriles, carboxylic esters, acids, and hydroboration of alkenes using $NaBH_4/I_2$ system. *Tetrahedron* **1992**, *48*, 4623–4628.
(23) Gadja, T.; Koziara, A.; Osowska-Pacewicka, K.; Zawadzki, S.; Zwierzak, A. A convergent one-pot synthesis of secondary amines via aza-Wittig reaction. *Synth. Commun.* **1992**, *22*, 1929–1938.
(24) Borch, R. F.; Hassid, A. I. A New Method for the Methylation of Amines. *J. Org. Chem.* **1972**, *37*, 1673–1674.
(25) Zajac, W. W.; Walters, T. R.; Darcey, M. G. Oxidation of amines with 2-sulfonyloxaziridines (Davis' Reagent). *J. Org. Chem.* **1988**, *53*, 5856–5860.
(26) Bhujanga Rao, A. K. S.; Gundu Rao, C.; Singh, B. B. Formation of N-alkyl-2-methyl-4-nitro-1*H*-imidazoles via floride-ion-mediated Michael additions. *J. Chem. Res. (S)* **1991**, 350–351.
(27) Kajfez, F.; Sunjic, V.; Kolbah, D.; Fajdiga, T.; Oklobdzija, M. 1-Substitution in 2-methyl-4(5)-nitroimidazole. 1. Synthesis of compounds with potential antitrichomonal activity. *J. Med. Chem.* **1968**, *11*, 167–169.
(28) Cain, B. F.; Denny, W. A. Potential antitumor agents. Part 21. Dialkanolamine dialkanesulfonate esters. *J. Med Chem.* **1977**, *20*, 515–520.
(29) Hendrickson, J. B.; Bergeron, R.; Sternbach, D. D. New 'Gabriel' synthesis of amines. *Tetrahedron* **1975**, *31*, 2517–2521.
(30) Clagett-Dame, M.; Shoenleber, R.; Chung, C.; McKelvy, J. F. Preparation of an affinity chromatography matrix for selective purification of the dopamine D2 receptor from bovine striatal membranes. *Biochim. Biophys. Acta* **1989**, *986*, 271–280.
(31) Wilson, W. R.; Thompson, L. H.; Anderson, R. F.; Denny, W. A. Hypoxia-selective antitumor agents. 2. Electronic effects of 4-substituents on the mechanisms of cytotoxicity and metabolic stability of nitracrine analogues. *J. Med. Chem.* **1989**, *32*, 31–38.

(32) Hoy, C. A.; Thompson, L. H.; Mooney, C. L.; Salazar, E. P. Defective DNA cross-link removal in Chinese hamster cell mutants hypersensitive to bifunctional alkylating agents. *Cancer Res.* **1985**, *45*, 1737–1743.
(33) Wilson, W. R.; Anderson, R. F.; Denny, W. A. Hypoxia-selective antitumor agents. 1. Relationships between structure, redox properties and hypoxia-selective cytotoxicity for 4-substituted derivatives of nitracrine. *J. Med. Chem.* **1989**, *32*, 23–30.
(34) Wilson, W. R.; Denny, W. A.; Twigden, S. J.; Baguley, B. C.; Probert, J. C. Selective toxicity of nitracrine to hypoxic mammalian cells. *Br. J. Cancer* **1984**, *49*, 215–223.
(35) Wardman, P.; Clarke, E. D. One-electron reduction potentials of substituted imidazoles measured by pulse radiolysis. *J. Chem. Soc., Faraday Trans. 1* **1976**, *72*, 1377–1390.
(36) Adams, G. E.; Flockhart, I. R.; Smithen, C. E.; Stratford, I. J.; Wardman, P.; Watts, M. E. Electron-affinic sensitisation. VII. A correlation between structures, one-electron reduction potentials, and efficiencies of 2-nitroimidazoles as hypoxic cell radiosensitisers. *Radiat. Res.* **1976**, *67*, 9–20.
(37) Wardman, P. Some reactions and properties of nitro radical anions important in biology and medicine. *Environ. Health Perspect.* **1985**, *63*, 309–320.
(38) Jenkins, T. C.; Stratford, I. J.; Stephens, M. A. 3-Nitro-1,2,4-triazoles as hypoxia-selective agents. *Anti-Cancer Drug Design* **1989**, *4*, 145–160.
(39) Sugiura, M.; Iwasaki, K.; Kato, R. Reduced nicotinamide adenine dinucleotide-dependent reduction of tertiary amine N-oxide by liver microsomal cytochrome P450. *Biochem. Pharmacol.* **1977**, *26*, 489–495.
(40) Patterson, L. H. Rationale for the use of aliphatic N-oxides of cytotoxic anthraquinones as prodrug DNA binding agents: a new class of bioreductive agent. *Cancer Met. Rev.* **1993**, *12*, 119–134.
(41) Malliaros, D. P.; Goldman, P. Interaction of metronidazole with *Escherichia coli* deoxyribose nucleic acid. *Biochem. Pharmacol.* **1991** *42*, 1739–1744.
(42) Siim, B. G.; Atwell, G. J.; Wilson, W. R. Oxygen dependence of the cytotoxicity and metabolic activation of 4-alkylamino-5-nitroquinoline bioreductive drugs. *Br. J. Cancer* **1994**, *70*, 596–603.
(43) Wilson, W. R.; Siim, B. G.; Denny, W. A.; van Zijl, P. L.; Taylor, M. L.; Chambers, D. M.; Roberts, P. B. 5-Nitro-4-(N,N-dimethylaminopropylamino)quinoline (5-nitraquine), a new DNA-affinic hypoxic cell radiosensitizer and bioreductive agent: comparison with nitracrine. *Radiat. Res.* **1992**, *131*, 257–265.

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