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SYNTHESIS AND HYPOXIA-SELECTIVE CYTOTOXICITY OF A 2-NITROIMIDAZOLE MUSTARD

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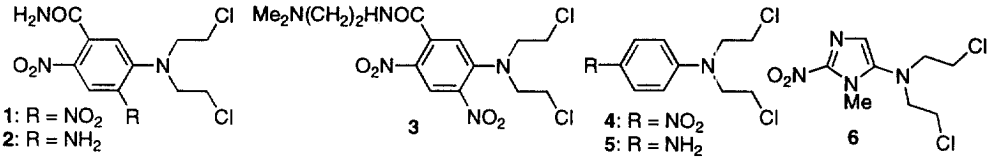
Abstract: A four-step synthesis of 5-[*N,N*-bis(2-chloroethyl)amino]-1-methyl-2-nitroimidazole from 1-methyl-2-nitroimidazole is described. This compound showed similar hypoxia-selective cytotoxicity to the dinitrobenzamide mustard SN 23862 in UV4 cells (ca. 40-fold), and superior selectivity (>7-fold) in repair-competent AA8 cells.

Nitroaromatic mustards have been explored extensively[1] as prodrugs, designed to undergo selective activation by bioreduction in hypoxic cells,[2] for cancer chemotherapy. Selectivity is achieved through initial one-electron reduction by cellular nitroreductases to the nitro radical anion, a process that is efficiently reversed by molecular oxygen in aerobic cells. In the absence of oxygen, further metabolism to hydroxylamines and/or amines occurs, resulting in a large increase of electron density in the aromatic ring, and consequent activation of nitrogen mustard alkylating moieties in resonance positions.[3] For example, the dinitrobenzamide mustard **1** (SN 23862), with a one-electron reduction potential of -421 mV,[4] shows substantial (40- to 60-fold) selectivity for hypoxic UV4 cells in culture, and lesser but significant selectivity for other cell lines under hypoxia.[5,6] The soluble analogue **3** showed similar selectivity in the sensitive, repair-inhibited[7] UV4 line, but lower selectivity (~ 3-fold) in the repair-competent AA8 parent line.[8]



A major limitation with nitrobenzene mustards is the low intrinsic reduction potential of the nitrobenzene system. Thus 4-nitroaniline mustard **4** shows very little hypoxic selectivity, attributed to a reduction potential too low for efficient cellular reduction.[9] Addition of two further electron-withdrawing groups (as in **1**) are required to raise the reduction potential to within the desired range of -300 to -450 mV.[3] Such compounds then remain significantly electron-deficient even following nitro reduction, resulting in "activated" mustard species of rather low cytotoxicity. Thus the major stable metabolite of **1**, the 4-amine **2**, has an IC_{50} of only 180 μM in AA8 cells,[6] compared with 6 μM for the 4-aminoaniline mustard **5**.

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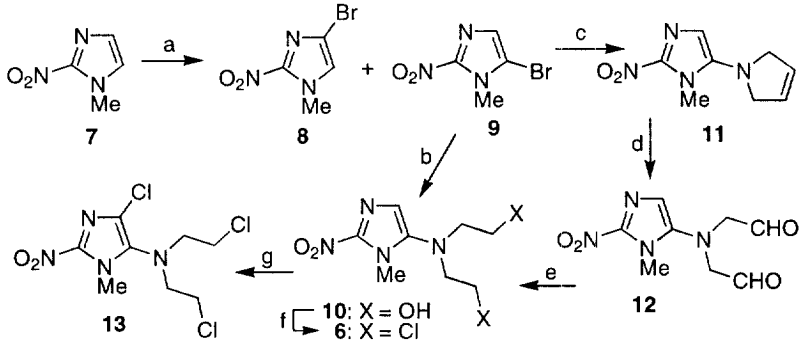
2-Nitroimidazoles have significantly higher intrinsic one-electron reduction potentials (e.g., 2-nitroimidazole; -418 mV, compared with nitrobenzene; -486 mV),[10] and are also known to undergo selective reduction in the hypoxic regions of tumors.[11] In this paper we report the synthesis of 5-[*N*,*N*-bis(2-chloroethyl)amino]-1-methyl-2-nitroimidazole (**6**), and its evaluation as a hypoxia-selective cytotoxin.

Scheme



Reagents: (a) Br_2 (10-15% of **9**) (b) $HN(CH_2CH_2OH)_2$/KF/18-crown-6 (30%) (c) 3-pyrroline, Et_3N (72%) (d) OsO_4, $NaIO_4$ (crude) (e) BH_3.THF (15% from **11**) (f) MsCl, Et_3N, then NaCl/LiCl, DMF (64%) (g) $SOCl_2$, DMF(trace) (17%)

Bromination of 1-methyl-2-nitroimidazole **7** by the method of Farah and McClelland[12] gave a mixture of the 4- and 5-bromo derivatives (**8** and **9**), together with small amounts of the 4,5-dibromo derivative (Scheme 1). The desired **9** was formed in 10-15% yield, and could be isolated by careful chromatography on silica gel. Previous attempts to prepare **9** by lithiation methods were unsuccessful.[13] Reaction of **9** with diethanolamine in the presence of KF/18-crown-6 for 3 days at 70-80 °C gave the diol **10** in 30 % yield (55% based on recovered starting material). Because of the relatively low yield in the displacement with diethanolamine, an alternative route to **10** was sought via reaction of **9** with the much more reactive 3-pyrroline followed by oxidative ring opening. This approach has been used previously by us for the synthesis of heteroaromatic mustards.[14] Conversion of **9** to the corresponding pyrroline **11** proceeded in 72% yield (88% based on recovered starting material). Oxidation of this with OsO_4/periodate gave the crude dialdehyde **12**, which was reduced with borane-THF to the diol **10**, but only in 15% overall yield. Chlorination of **10** to give the mustard **6**[15] proved to be a sensitive reaction. Treatment with electrophiles such as $SOCl_2$ gave a low yield of the 4-chloro derivative **13**,[16] which was also formed during mesylation of **10** followed by mesylate displacement with NaCl in the presence of DMSO. It was found essential to remove all the DMSO solvent from **10** by careful chromatography, and to conduct the mesylate displacement by chloride in DMF, in order to achieve preparation of pure **6** in 64% yield.

The cytotoxicities of the compounds were determined (IC_{50} values for 18 h exposures) in aerobic cultures of two Chinese hamster lines (AA8 and UV4) and the human ovarian cancer line SKOV3, using a growth inhibition microassay[17] (Table). The UV4 cell line is a repair-defective mutant of AA8 which is hypersensitive to alkylating agents whose cytotoxicity is due to bulky DNA adducts or cross-links.[7]

Table. Aerobic and hypoxic cytotoxicities of dinitrobenzamide and 2-nitroimidazole mustards

no.	growth inhibition (aerobic IC_{50}, μM)[a]			clonogenic assay AA8		clonogenic assay UV4	
	AA8	UV4	SKOV3	CT_{10}[b]	ratio[c]	CT_{10}[b]	ratio[c]
1	1450±110	870±63	>1000[d]			95±33	42±20
3	220±33	3.1±0.6		1300	3.1	400	44
6	>990[d]	915±114	1300	1026±120	>7	190±24	31±1.3
13	200±22	123±20	>250[d]			97±10	>20

[a]IC_{50} values determined against aerobic cells (pH 7.4), using an exposure time of 4 h. Values are means ± SEM. Values without SEM are for a single determination only. [b]CT_{10}: the drug concentration (μM) required to reduce cell survival to 10% of controls under hypoxic conditions, using the indicated cell line at 10^6/mL in the clonogenic assay (see text). [c]Ratio of C_{10} values in air and N_2 [C_{10}(air)/C_{10}(N_2)]. [d]Nontoxic at the solubility limit.

The 2-nitroimidazole mustard **6** had approximately the same aerobic cytotoxicity as the dinitrobenzamide mustard **1** in the UV4 line (IC_{50}s ~ 1 mM), suggesting a similar degree of deactivation of the mustard. Both were somewhat more cytotoxic in the repair-deficient UV4 line than in the corresponding wild-type AA8 line, consistent with a mechanism of cytotoxicity involving DNA alkylation. Hypoxic selectivities were determined by clonogenic assay of stirred plateau-phase cultures of AA8 or UV4 cells, continuously gassed with 5% CO_2 in air or N_2, as described previously.[18,19] Cytotoxic potency was determined as CT_{10}, the concentration of drug required for 10% cell survival after a 1 h exposure. While **1** has a hypoxic selectivity of ~ 40-fold in the more sensitive UV4 line,[4,8] it was too insoluble to evaluate in the repair-competent AA8 line (against which it has lower potency). However, a soluble analogue of **1** (compound **3**) shows much lesser hypoxic selectivity in AA8 cells (~ 3-fold).[8]

The 2-nitroimidazole mustard **6** showed comparable hypoxic selectivity in UV4 cells to the dinitrobenzamide mustard **1** (30- to 40-fold), and was also significantly hypoxia-selective in repair-competent AA8 cells (>7-fold, accurate determination limited by solubility) respectively. The 4-chloro analogue **13** was also selective. These data suggest that more soluble 4-substituted analogues of **6** would be of interest.

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15. Compound **6**: mp (EtOAc/petroleum ether) 79-80 °C; ^{1}H NMR ($CDCl_3$) δ 6.97 (s, 1, H-4), 3.94 (s, 3 H, NCH_3), 3.57 (t, J = 5.7 Hz, 4 H, CH_2Cl), 3.49 (t, J = 5.7 Hz, 4 H, CH_2N). Mass spectrum: Found; M^+ 270.0278, 268.0298, 266.0330. Calculated for $C_8H_{12}Cl_2N_4O_2$: M^+ 270.0278, 268.0308, 266.0337. Anal. ($C_8H_{12}C_{l2}N_4O_2$) C, H, N.

16. Compound **13**: mp (EtOAc/petroleum ether) 75-77 °C; ^{1}H NMR ($CDCl_3$) δ 4.00 (s, 3 H, NCH_3), 3.55 (br s, 8 H, CH_2N and CH_2Cl). Mass spectrum: Found; M^+ 305.9860, 303.9881, 301.9912, 299.9941. Calculated for $C_8H_{11}Cl_3N_4O_2$: M^+ 305.5899, 303.9888, 301.9918, 299.9947. Anal. ($C_8H_{11}Cl_3N_4O_2$) C, H, N.

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