Hypoxia-Selective Antitumor Agents. 12. Nitrobenzyl Quaternary Salts as Bioreductive Prodrugs of the Alkylating Agent Mechlorethamine

Moana Tercel,[†] William R. Wilson,[‡] Robert F. Anderson,[§] and William A. Denny*,[†]

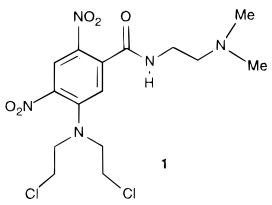
Cancer Research Laboratory and Section of Oncology, Department of Pathology, University of Auckland School of Medicine, Private Bag 92019, Auckland, New Zealand, and Department of Chemistry, University of Auckland, Private Bag 92019, Auckland, New Zealand

Received October 18, 1995[⊗]

A series of benzene-substituted analogues of the novel hypoxia-selective cytotoxin *N,N*-bis(2-chloroethyl)-*N*-methyl-*N*-(2-nitrobenzyl)ammonium chloride (**3a**), together with three corresponding tetrahydroisoquinolinium "cyclic" analogues **21a**−**23a** and two naphthalene derivatives (**19a** and **20a**), have been prepared and evaluated for cytotoxicity in cultured mammalian tumor cells under aerobic and hypoxic conditions. The parent compound **3a** has a one-electron reduction potential of −358 mV and undergoes reductively-induced fragmentation to release the nitrogen mustard mechlorethamine. The compounds were prepared by halogenation ($SOCl_2$) of the corresponding quaternary diols, which in turn were synthesized from *N*-methyldiethanolamine and substituted nitrobenzyl chlorides. The reduction potentials of the benzene-substituted compounds were generally well-predicted by Hammett substituent relationships. All of the compounds were much more toxic toward repair-deficient UV4 cells than the corresponding wild-type AA8 cells, as expected if the active cytotoxic species is a DNA alkylating agent. They were also more toxic toward the human cell lines EMT6 and FME compared to AA8, but the reasons for this are not known. Analogues of **3a** substituted in the phenyl ring with electron-donating substituents provided compounds with widely differing selectivities for hypoxic AA8 cells, ranging from no selectivity for the 3-Me compound **9a** to 3000-fold (at least as great as that of the parent **3a**) for the 4-OMe compound **14a**. The naphthalene derivatives **19a** and **20a** and the tetrahydroisoquinolinium compounds **21a**−**23a** showed no hypoxic selectivity. Selective chemical reduction of **22a** and **23a** with nickel boride resulted in isolation of the corresponding stable amino derivatives, indicating that reduction of these compounds does not result in fragmentation. The reason(s) for the marked differences in hypoxic selectivity of the nitrobenzyl quaternary mustards is unknown, but may reflect differences in radical chemistry, cell uptake, or sensitivity to enzymatic reduction.

Compounds which are selectively toxic to hypoxic cells (hypoxia-selective cytotoxins; HSCs) are of current interest as potential anticancer drugs, because of increasing evidence that the small proportion of hypoxic cells which occur in many human solid tumors are resistant to both radiotherapy[1−3] and some chemotherapeutic drugs.[4]

The majority of HSCs studied can be considered as prodrugs, capable of undergoing selective metabolism (usually reductive) under hypoxic conditions to generate a much more cytotoxic species.[4] Conceptually, they consist of three domains: a "trigger" which undergoes this hypoxia-selective (oxygen-inhibited) reduction, an "effector" which is activated following reduction of the trigger, and a "linker" which connects the other two domains and communicates the change.[5] We have outlined a number of reasons why aliphatic (and activated aromatic) nitrogen mustards are particularly suitable as effector units,[5] and have been exploring chemistries which utilize these units in HSCs. Examples have included nitro-deactivated aniline mustards (e.g., **1**),[6] cobalt(III) complexes of aliphatic mustards (e.g., **2**),[7] and nitrobenzyl quaternary mustards (e.g., **3a**).[8]



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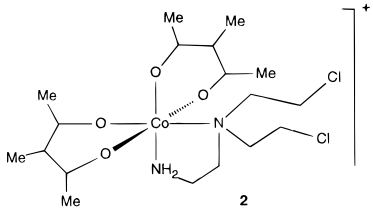

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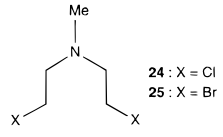
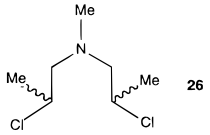


24 : X = Cl
25 : X = Br



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The concept of nitrobenzyl quaternary mustards as HSCs arose from the fact that nitrobenzyl species bearing a variety of leaving groups are known to undergo fragmentation following one-electron reduction. In nitrobenzyl halides, which have been studied extensively, initial formation of the radical anion is followed by fragmentation, with expulsion of the halide anion and generation of a reactive benzyl radical (Scheme 1).[9−11] Evidence for this general mechanism includes cyclic voltammetry studies[12] and direct observation of the absorption spectra of both the transient radical anion and resulting benzyl radical, following pulse radiolysis of aqueous solutions of the nitrobenzyl halides.[9]

The cytotoxic effects of nitrobenzyl halides have been attributed to the reactive benzyl radicals formed by reduction and subsequent fragmentation. Under aero-

[†] Cancer Research Laboratory.
[‡] Section of Oncology.
[§] Department of Chemistry.
[⊗] Abstract published in *Advance ACS Abstracts,* February 1, 1996.

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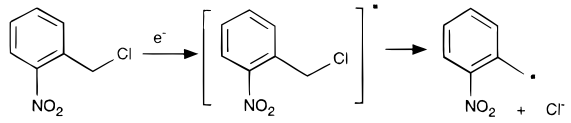
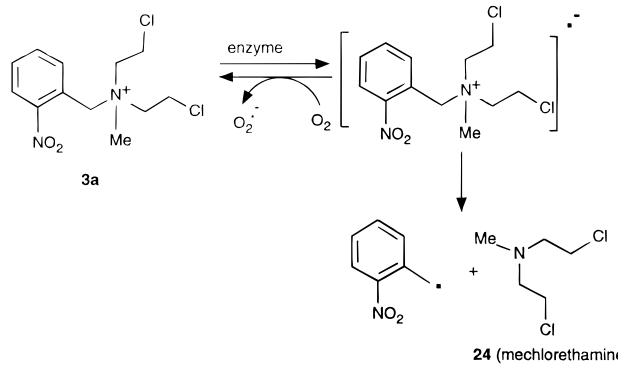
Scheme 1. Reductive Fragmentation of Nitrobenzyl Halides



Scheme 2. Reductive Fragmentation of Nitrobenzyl Quaternary Mustards

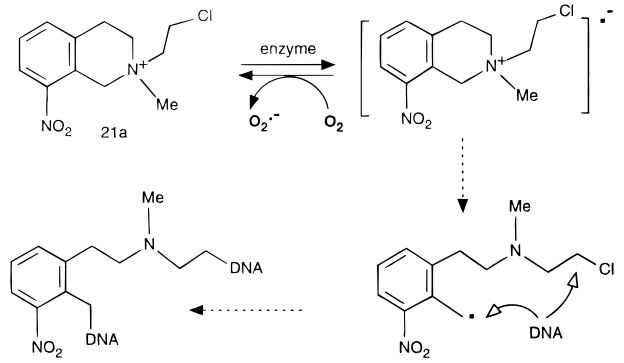


bic conditions, microsomal reduction of 2- and 4-nitrobenzyl chloride leads to oxygen consumption and the formation of superoxide in a futile cycle, while under hypoxic conditions benzyl radicals resulting from fragmentation have been detected.[13] Some nitrobenzyl halides have been evaluated as hypoxia-selective cytotoxins, and evidence for selective killing of hypoxic EMT6 cells was obtained,[14,15] although the differentials between aerobic and hypoxic conditions were small.

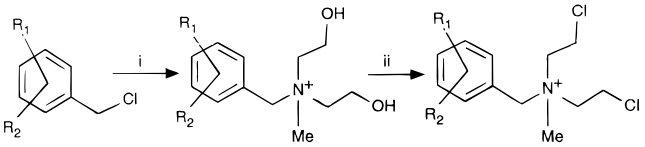
Nitrobenzyl quaternary ammonium salts are known to undergo similar fragmentation on reduction, with the leaving group in this case being the neutral tertiary amine. No rates have been reported, but there is evidence that the radical anions of these quaternary ammonium salts are more stable than those of the corresponding chlorides.[16,17] We have adapted this reaction in our development of nitrobenzyl quaternary mustards (Scheme 2) and have shown that simple analogues such as **3a** show very large hypoxic selectivities *in vitro*.[8,18] This compound also shows much greater cytotoxicity against intact compared with dissociated EMT6 spheroids,[18] presumably because of release of mechlorethamine (**24**) in the hypoxic core of the spheroids as a diffusible cytotoxin. The novel activity of **3a** prompted us to extend our studies in this class, and in the present paper we explore structure−activity relationships for hypoxic selectivity among a series of substituted 2-nitrobenzyl quaternary mustards and related compounds.

The cytotoxicity of **3a** appears to result from release of a bifunctional mustard rather than the concomitantly-formed benzyl radical.[8,18] However, the relatively high cytotoxicity of simple nitrobenzyl halides has been attributed to formation of this benzyl radical and its subsequent reactions.[14,15] The related tetrahydroisoquinoline quaternary salts (**21a**−**23a**) were therefore also prepared, in an attempt to exploit this reactive species. Provision of a second, noncleavable linking chain between the nitrobenzyl chromophore and the quaternary leaving group in **21a**−**23a** ensures that the released mustard and the benzyl radical remain linked (Scheme 3). Although only a monofunctional mustard will be generated, this is expected to be compensated

Scheme 3. Proposed Mechanism of DNA Alkylation by Tetrahydroisoquinolium Mustands



Scheme 4[a]



[a] (i) *N*-Methyldiethanolamine/MeCN/reflux/6 h; (ii) $SOCl_2$/20 °C/72 h.

for by the simultaneous generation of a linked reactive center generated from the attached benzyl radical; the tetrahydroisoquinoliniums thus retain a potential for bifunctional reaction (Scheme 3).

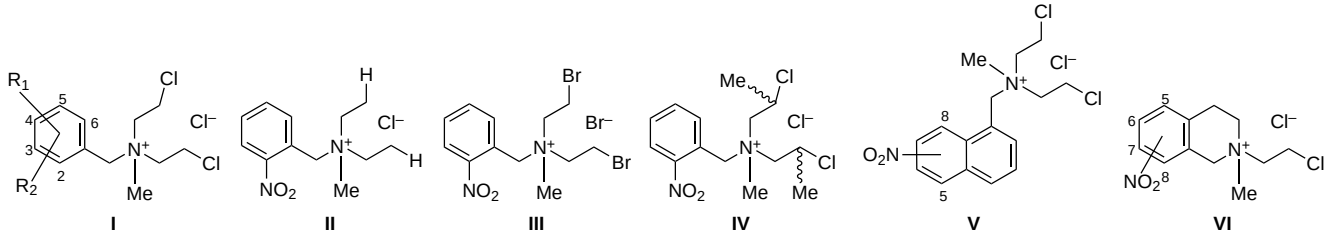
Chemistry

The desired quaternary mustards (**3a**−**23a**) (Table 1) were prepared by halogenation of the corresponding quaternary diols (**3b**−**23b**) (Table 2), typically with $SOCl_2$ at room temperature. The diols **3b**−**23b** were prepared by treatment of the appropriate nitrobenzyl chlorides with *N*-methyldiethanolamine in CH_3CN (Scheme 4). Slight modifications to this procedure gave the diethyl derivative **4a** (by displacement of 2-nitrobenzyl chloride with $MeNEt_2$) and the bromo compound **5a** (by treatment of **5b** with $SOBr_2$). The methyl-substituted diol **6b**, resulting from reaction of 2-nitrobenzyl chloride with *N*-methyldi(propan-2-ol)amine, was obtained as a mixture of diastereoisomers, from which a single diastereoisomer could be obtained by fractional crystallization. This diastereoisomer yielded a crystalline mustard (**6a**) on chlorination.

This general route was unsuccessful for the synthesis of more activated dinitrobenzyl derivatives. While the 3,4-dinitrobenzyl diol **17b** was stable, the corresponding mustard derivative could be not be obtained in a pure form. The 2,3- and 2,5-dinitrobenzyl diols could not even be prepared, as the parent benzyl bromides underwent spontaneous intramolecular displacement of the *o*-NO_2 group when treated with *N*-methyldiethanolamine, giving the oxazepinium derivatives **27** and **28** (Scheme 5). The synthesis was also unsuccessful with the sterically hindered 6-methyl-2-nitrobenzyl chloride. Although this gave an isolable quaternary diol (**12b**), subsequent treatment with $SOCl_2$ caused fragmentation back to the parent nitrobenzyl chloride.

All but one of the nitrobenzyl halides required for the syntheses of the diols of Table 2 were either commercially available or previously reported: **9b**,[19] **10b**,[20] **12b**,[21] **13b**,[22] **14b**,[23] **16b**,[24] **18b**,[25] **19b**,[26] and **20b**.[26] 5-Methoxy-2-nitrobenzyl chloride was prepared by chlo-

Table 1. Structures and Physicochemical Properties of Nitrobenzyl Quaternary Mustards

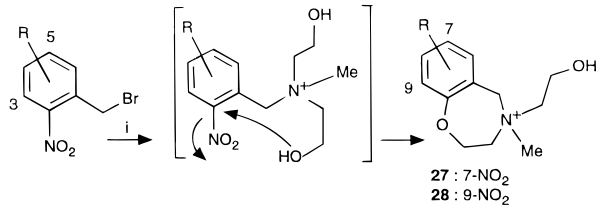


I II III IV V VI

no.	form	R_1	R_2	yield (%)	mp (°C)	solvent[a]	formula	analyses[b]	$E(1)$ (mV)[c]
3a	I	2-NO_2[d]	H	87	152−153.5	A	$C_{12}H_{17}Cl_3N_2O_2$	C,H,N,Cl	−358 ± 10
4a	II	2-NO_2	H	78	167−168	B	$C_{12}H_{19}ClN_2O_2$	C,H,N,Cl	−386 ± 8
5a	III	2-NO_2	H	15	150−155	C	$C_{12}H_{17}Br_3N_2O_2$	C,H,N,Br	−361 ± 8
6a	IV	2-NO_2	H	50	147−148	B	$C_{14}H_{21}Cl_3N_2O_2\cdot H_2O$	C,H,N	−357 ± 8
7a	I	3-NO_2	H	92	157−158	A	$C_{12}H_{17}Cl_3N_2O_2\cdot H_2O$	C,H,N	−407 ± 7
8a	I	4-NO_2[d]	H	45	175−177	A	$C_{12}H_{17}Cl_3N_2O_2$	C,H,N,Cl	−380 ± 7
9a	I	2-NO_2	3-Me	66	167−168	D	$C_{13}H_{19}Cl_3N_2O_2$	C,H,N	−440 ± 7
10a	I	2-NO_2	4-Me	73	151−152	D	$C_{13}H_{19}Cl_3N_2O_2$	C,H,N,Cl	−375 ± 7
11a	I	2-NO_2	5-Me	51	133−134	E	$C_{13}H_{19}Cl_3N_2O_2$	C,H,N,Cl	−384 ± 7
13a	I	2-NO_2	3-OMe	72	175−176	D	$C_{13}H_{19}Cl_3N_2O_3$	C,H,N	−415 ± 7
14a	I	2-NO_2	4-OMe	34	142−143	F	$C_{13}H_{19}Cl_3N_2O_3$	C,H,N,Cl	−349 ± 7
15a	I	2-NO_2	5-OMe	47	126−127	F	$C_{13}H_{19}Cl_3N_2O_3\cdot 0.5H_2O$	C,H	−400 ± 13
16a	I	3-NO_2	5-NO_2	84	201−202	G	$C_{12}H_{16}Cl_3N_3O_4$	C,H,N,Cl	−283 ± 8
18a	I	4-SO_2Me	H	55	179−180	C	$C_{13}H_{20}Cl_3NO_2S\cdot 0.5H_2O$	C,H	−733 ± 11
19a	V	5-NO_2		64	152−153.5	B	$C_{16}H_{19}Cl_3N_2O_2$	C,H,N,Cl	−438 ± 8
20a	V	8-NO_2		37	103−104	H	$C_{16}H_{19}Cl_3N_2O_2\cdot H_2O$	C,H,N,Cl	−371 ± 11
21a	VI	8-NO_2		60	225−226	D	$C_{12}H_{16}Cl_2N_2O_2$	H,N,Cl; C[e]	−452 ± 8
22a	VI	7-NO_2		42	202−203	E	$C_{12}H_{16}Cl_2N_2O_2\cdot 0.25H_2O$	C,H,N,Cl	−435 ± 8
23a	VI	6-NO_2		87	nc[f]		$C_{12}H_{16}Cl_2N_2O_2\cdot H_2O$	H,N,Cl; C[e]	−439 ± 9

[a] Recrystallization solvent: A, EtOH−Et_2O; B, *i*-PrOH−Et_2O; C, EtOH−*i*-PrOH; D, *i*-PrOH; E, MeCN; F, *i*-PrOH−EtOAc; G, EtOH; H, Me_2CO. [b] Analytical results within ±0.4% of the theoretical value. [c] $E(1)$ values determined by pulse radiolysis in 10 mM phosphate buffer, pH 7 (see text). [d] Compounds reported in ref 36 as the bromide salts, but no structural characterization. [e] C out by 0.5%. [f] Not crystalline.

Scheme 5[a]

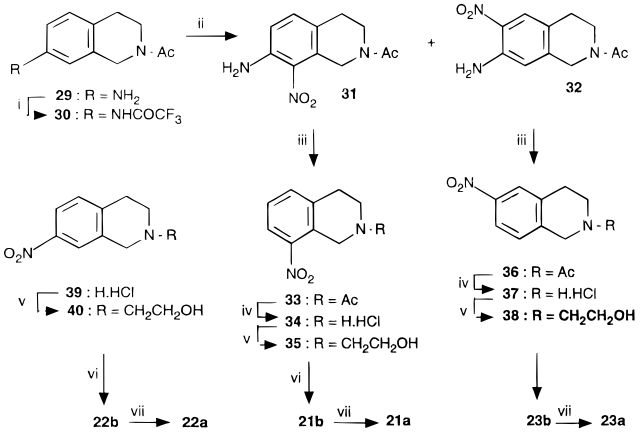


[a] (i) *N*-Methyldiethanolamine/MeCN/reflux/1.5 h.

rination of the known[27] 5-methoxy-2-nitrobenzyl alcohol. In addition to these literature procedures, a convenient preparation for many of the nitrobenzyl chlorides was by reduction of the corresponding nitrobenzoic acid with BH_3·DMS/$B(OMe)_3$[28] (yields 60−90%), followed by chlorination with $SOCl_2$ or MsCl/Et_3N (70−90%). *N*,*N*-Bis(2-chloroethyl)-*N*-methylamine (mechlorethamine; **24**), *N*,*N*-bis(2-bromoethyl)-*N*-methylamine (**25**),[29] and *N*,*N*-bis(2-chloropropyl)-*N*-methylamine (**26**)[30] (Table 3) are also known compounds.

While the 6-methyl-2-nitro diol **12b** could not be converted to the corresponding mustard, the tetrahydroisoquinolinium mustard **21a**, together with the isomers **22a** and **23a**, was prepared from the appropriate nitro-1,2,3,4-tetrahydroisoquinolines by the route shown in Scheme 6. The 7-nitro isomer **39** has been reported previously,[31] but the 6- and 8-nitro derivatives were unknown. These were prepared from *N*-acetyl-7-amino-1,2,3,4-tetrahydroisoquinoline (**29**)[31] by N-protection with TFAA, followed by nitration with H_2SO_4/KNO_3 and hydrolysis of the trifluoroacetamide, giving a mixture of the 8- and 6-NO_2 compounds (**31** and **32**), which were separated by chromatography on silica gel (Scheme 6). Deamination of the corresponding diazo compounds with hypophosphorous acid followed by hydrolysis of the acetamides gave the required 8- and 6-nitro-1,2,3,4-tetrahydroisoquinolines (**34** and **37**). All three nitro-1,2,3,4-tetrahydroisoquinolines were converted to the corresponding *N*-hydroxyethyl derivatives (**35**, **38**, and **40**) with 2-bromoethanol, followed by methylation with dimethyl sulfate, ion exchange chromatography, and chlorination with $SOCl_2$ to give the desired quaternary salts (**21b**−**23b**).

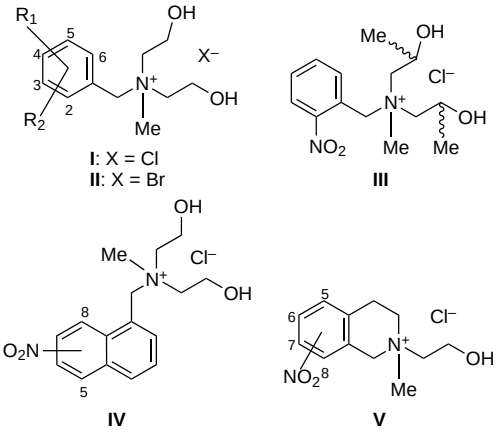
Scheme 6[a]



[a] (i) TFA/TFAA/CH_2Cl_2/reflux/10 min; (ii) concentrated H_2SO_4/KNO_3/ca. 5 °C/70 min, then concentrated HCl/MeOH/reflux/45 min; (iii) $NaNO_2$/HCl, then H_3PO_2/4 °C/18 h; (iv) concentrated HCl/MeOH/reflux/18 h; (v) $BrCH_2CH_2OH/Et_3N$/THF/reflux/18 h; (vi) DMS/MeCN/20 °C/16 h, then ion exchange chromatography; (vii) $SOCl_2$.

Pulse radiolysis experiments were performed on a modified Dynaray 4 (4 MeV) linear accelerator (200 ns

Table 2. Structures and Physicochemical Properties of Nitrobenzyl Quaternary Diols



no.	form	R_1	R_2	yield (%)	mp (°C)	solvent[a]	formula	analyses[b]
3b	I	2-NO_2	H	77	148−149	A	$C_{12}H_{19}ClN_2O_4$	C,H,N,Cl
5b	II	2-NO_2	H	80	152−153[c]	B	$C_{12}H_{19}BrN_2O_4$	C,H,N,Br
6b	III	2-NO_2	H	36[d]	193−194	C	$C_{14}H_{23}ClN_2O_4$	C,H,N,Cl
7b	I	3-NO_2	H	77	130−131	B	$C_{12}H_{19}ClN_2O_4$	C,H,N,Cl
8b	I	4-NO_2	H	56	96−97	C	$C_{12}H_{19}ClN_2O_4 \cdot 2H_2O$	C,H,N
9b	I	2-NO_2	3-Me	89	120−121	f	$C_{13}H_{21}ClN_2O_4$	C,H,N,Cl
10b	I	2-NO_2	4-Me	86	131−132	f	$C_{13}H_{21}ClN_2O_4$	C,H,N
11b	I	2-NO_2	5-Me	90	nc[e]		$C_{13}H_{21}ClN_2O_4$	HRMS[g]
12b	I	2-NO_2	6-Me	63	125−126	f	$C_{13}H_{21}ClN_2O_4$	C,H,N
13b	I	2-NO_2	3-OMe	89	144−145	A	$C_{13}H_{21}ClN_2O_5$	C,H,N,Cl
14b	I	2-NO_2	4-OMe	78	142−143	C	$C_{13}H_{21}ClN_2O_5$	C,H,N,Cl
15b	I	2-NO_2	5-OMe	73	143−144	C	$C_{13}H_{21}ClN_2O_5$	C,H,N,Cl
16b	I	3-NO_2	5-NO_2	57	206−207	B	$C_{12}H_{18}ClN_3O_6$	C,H,N,Cl
17b	I	3-NO_2	4-NO_2	40	100−105	f	$C_{12}H_{18}ClN_3O_6 \cdot 1.5H_2O$	C,H,N,Cl
18b	I	4-SO_2Me	H	78	130−131	B	$C_{13}H_{22}ClNO_4S$	C,H,N
19b	IV	5-NO_2		78	134−135	A	$C_{16}H_{21}ClN_2O_4$	C,H,N,Cl
20b	IV	8-NO_2		70	nc		$C_{16}H_{21}ClN_2O_4$	HRMS
21b	V	8-NO_2		73	103.5−107	C	$C_{12}H_{17}N_2O_3 \cdot CH_3O_4S$	C,H,N,S
22b	V	7-NO_2		90	122−124.5	C	$C_{12}H_{17}N_2O_3 \cdot CH_3O_4S$	C,H,N,S
23b	V	6-NO_2		48	156−157.5	D	$C_{12}H_{17}N_2O_3 \cdot 0.25H_2O$	C,H,N,Cl

[a] Recrystallization solvent: A, EtOH−Et_2O; B, EtOH; C, *i*-PrOH; D, MeCN. [b] Analytical results within ±0.4% of the theoretical value. [c] Literature mp 153−154 °C (ref 50). [d] Yield from first recrystallization, 2:1 mixture of diastereoisomers; second recrystallization gives single diastereoisomer (6% overall yield), mp 198−199 °C. [e] Not crystalline. [f] Crude material analytically pure. [g] High-resolution mass spectrum of ammonium ion by fast atom bombardment.

pulse length and a custom-built optical radical detection system (new facility at the University of Auckland). One-electron reduction potentials [$E(1)$] for the compounds were determined in aqueous solutions containing 2-propanol (0.1 M) buffered at pH 7.0 (10 mM phosphate) by measuring the equilibrium constant[32] for electron transfer between the radical anions of the compounds and the appropriate viologen reference standard. Data were obtained at three or more different concentrations, and are presented in Table 1 (see Supplementary Material for additional details). Aqueous solubilities (in water and culture medium) were determined by UV spectrophotometry.

Biological Evaluation. Aerobic cytotoxicities of the quaternary mustards (**3a**−**23a**) 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,[33] and are given in Table 3, together with similar data for the mustard effector units (**24**−**26**). The UV4 cell line is a repair-defective mutant hypersensitive to agents whose cytotoxicity is due to bulky DNA adducts or cross-links.[34] The hypoxic selectivities of the compounds were determined by clonogenic assay of stirred EMT6 cells, continually gassed with 5% CO_2 in air or N_2, as described previously.[35] As noted previously for **3a**,[18] the product of the drug concentration and exposure time to produce 10% cell survival (CT_{10}) was essentially constant under aerobic conditions, but decreased progressively under hypoxia. In the present study a fixed 3 h drug exposure time was used, and hypoxic selectivity was quantitated as the ratio of concentrations required to reduce cell survival to 10% of controls (C_{10}) under aerobic and hypoxic conditions (Table 3).

Results and Discussion

Physicochemical Properties. Structures and physicochemical data for the quaternary mustards are given in Table 1. The 2- and 4-nitrobenzyl derivatives (**3a** and **8a**) have been reported previously by Papanastassiou *et al.*,[36] who evaluated them as potential prodrugs of mechlorethamine which would be activated *in vivo* by nucleophilic attack. However, we later showed that these compounds were stable to thiols under typical cell culture conditions.[8] The 4-isomer **8a** was reported to have some activity against experimental rodent tumors,[36] but has not been considered as an HSC.

As a consequence of the permanent cationic charge, all of the compounds showed very high aqueous solubility (> 40 mM for all the analogues and >400 mM at 20 °C for **3a**). The benzylic quaternary salt is a very

Table 3. Biological Activity in Cell Culture of the Quaternary Mustards of Table 1

	growth inhibition				clonogenic assay, EMT cells	
		hypersensitivity factor[b]				
no.	IC_{50}[a] (mM) AA8	UV4	EMT6	FME	aerobic C_{10}[c] (mM h)	air/N_2[d]
3a	2.70 ± 0.13	300 ± 70	40 ± 12	24 ± 8	20	230−2500[e]
4a	3.20 ± 0.80	1.4 ± 0.7	12 ± 3	23 ± 10	12	0.11
5a	14.6 ± 2.5	17 ± 7	17 ± 2	56 ± 13	13	>200
6a	14.8 ± 1.5	2.6 ± 0.2	11 ± 2	118 ± 27	10	5.6
7a	20.9 ± 2.8	95 ± 84	16 ± 3	72 ± 48		
8a	6.14 ± 1.67	2900 ± 800	121 ± 30	22 ± 3		
9a	3.00 ± 1.16	730 ± 110	400 ± 200	330 ± 80	0.2	1.0
10a	3.30 ± 0.87	470 ± 68	120 ± 45	54 ± 2	0.29	240
11a	0.69 ± 0.04	200 ± 10	68 ± 16	19 ± 12	0.13	10
13a	10.5 ± 3.1	1560	58 ± 37	32 ± 7	0.97	3.3
14a	7.76 ± 0.13	435 ± 56	54 ± 17	27	6.7	3000
15a	8.81 ± 2.21	300 ± 160	45 ± 15	26 ± 2	2.9	10−500[e]
16a	0.13 ± 0.05	530 ± 85	41 ± 8	65 ± 49	0.08	1.3
18a	13.5 ± 1.17	195 ± 23	91 ± 10	26 ± 7	>30	ca. 1[f]
19a	5.25 ± 0.48	17 ± 7	11 ± 4	7.4 ± 1.2	3.2	0.5−2.0[e]
20a	1.38 ± 0.10	37 ± 6	12 ± 7	16 ± 7	1.3	1.5
21a	7.22 ± 0.64	2.5 ± 0.6	1.8 ± 0.3			
22a	10.90 ± 5.2	1.8 ± 1.3	1.4 ± 0.8			
23a	8.70 ± 0.16	1.5 ± 0.2	2.3 ± 0.1		10.7	1.8
24	0.002 ± 0.0002	42 ± 12	16 ± 3	9.1 ± 1.0		
25	0.0025 ± 0.0005	100 ± 30	12 ± 4			
26	0.030 ± 0.007	47 ± 2	4 ± 2			

[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 ± SEM for separate determinations. 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 to reduce cell survival to 10% of control values under hypoxic conditions (3 h drug exposure). Values are the average of at least two separate determinations, which did not vary by more than 15%. [d] Ratio aerobic C_{10}/hypoxic C_{10}. [e] Hypoxic cytotoxicity variable; see text. For compounds where a range is not given, the figure quoted is the average of at least two separate determinations which did not vary by more than 15%. [f] Survival ca. 20% at 30 mM under either aerobic or hypoxic conditions.

powerful electron-withdrawing group ($CH_2N^+(CH_3)_3$ has Hammett σ_p and σ_m values of 0.67 and 0.68, respectively],[37] and this is reflected in the high $E(1)$ values (e.g., −358 mV for **3a** compared with ca. −500 mV for 2-nitroaniline mustard[38]). The 3-nitro analogue **7a**, where the nitro group is not in conjugation, had a lower $E(1)$ (−407 mV) as expected. Substitution of **3a** with methyl or methoxy groups not adjacent to the nitro group produced changes in $E(1)$ consonant with the electronic properties of the groups; thus for compounds **3a**, **10a**, **11a**, and **14a**−**16a**, $E(1)$ values correlate well with Hammett σ values[39] (eq 1):

$$E(1)\ (\text{mV}) = 111\sigma - 362 \qquad (1)$$

$$n = 6 \qquad r = 0.996$$

The value for the 3,5-dinitro derivative **16a** is fitted well by this equation because the *meta* and *para* electronic effects of the benzylammonium group are equal.[37] However, both of the 3-substituted derivatives **9a** and **13a** have much lower $E(1)$ values than predicted, presumably because of steric interactions twisting the nitro group out of plane, resulting in deconjugation.[40] The SO_2Me derivative **18a** was evaluated as an analogue of similar electronic properties to **3a**, without being a substrate for the reductase enzymes.[41] However, **18** had a very low $E(1)$ (−733 mV), even though the SO_2Me and NO_2 groups have similar Hammett σ values.

While the 8-nitronaphthalene derivative **20a** had a reduction potential (−371 mV) similar to that of **3a**, that of the 5-nitro derivative **19a** was lower (−438 mV). The reason for this is not clear; ^{17}O and UV measurements on related compounds[10,42,43] suggest that the nitro group in **20a** would be out of plane (and thus deconjugated) to the greater extent, but this is not reflected in the $E(1)$ values. However, both $E(1)$ values are high enough to expect reduction of the compounds in cells. The 8-nitrotetrahydroisoquinolinium derivative (**21a**) had an $E(1)$ value of −452 mV. Although the related 6-methyl benzyl analogue could not be made (see above), the reduction potential of **21a** is still 70 mV lower than predicted by substituent electronic effects alone, suggesting that the structural constraint of ring formation lessens the electron-withdrawing ability of the quaternary salt. The isomeric nitrotetrahydroisoquinoliniums (**22a** and **23a**) had very similar $E(1)$ values (ca. −440 mV).

Biological Activity. We have previously reported[8] preliminary biological data for the three nitro positional isomers **3a**, **7a**, and **8a**. The 3-isomer **7a**, with the nitro group in an unconjugated position, was less cytotoxic than the other isomers, consistent with the known[9] much lower rates of fragmentation of related 3-substituted compounds. However, all three isomers were much less cytotoxic (1300−10000-fold) than mechlorethamine (**24**), indicating the powerful deactivating effect of the permanent cationic charge.

In the present studies, all the compounds were evaluated for cytotoxicity in a range of cell lines, including repair-proficient AA8 cells and a derived line (UV4) deficient in the repair of DNA adducts. Drugs showing much higher toxicity in UV4 cells (quantified by the hypersensitivity factor, HF = IC_{50} AA8/IC_{50} UV4) are considered to act via DNA alkylation. All of the quaternary mustards showed such selective toxicity for UV4 cells, but with HF values much higher than those for conventional DNA alkylators. For example, **3a** had an HF of 300 and **8a** one of 2900, compared with a (more typical) HF ratio of 42 for mechlorethamine (**24**) (Table

3). This could be a consequence of carrier-mediated uptake, which would introduce a nonlinear relationship between intracellular and extracellular drug concentration and amplify the differential sensitivity between the cell lines. Preliminary studies (X. Xu and W. R. Wilson, unpublished) show that **3a** and other analogues inhibit the uptake of [^{14}C]choline, suggesting that a major mechanism for uptake of these compounds may be via the choline transport pathway rather than by passive diffusion, as is the case for mechlorethamine itself.[44] The HF ratios for the quaternary mustards in the EMT6 and FME cell lines, while less dramatic, are similarly elevated over those of mechlorethamine, for unknown reasons. For the latter two lines it does not appear to be due to a lack of crosslink repair via the pathway which is defective in UV4 cells, since large HFs are observed for compounds to which UV4 is not markedly hypersensitive (e.g., **4a**, **6a**).

The nonalkylating analogue **4a**, while having the expected low HF value against UV4 cells, is almost as toxic as **3a** in aerobic AA8 cell cultures, suggesting that mechlorethamine release is not a major contributor to the cytotoxicity of the quaternary mustards under aerobic conditions in the repair-proficient cell line. However, **4a** was unique in the series in showing much greater cytotoxicity (9-fold) under aerobic compared with hypoxic conditions, suggesting that its toxicity is due to redox cycling rather than the reductive release of fragmentation products such as the nitrobenzyl radical. Reactive oxygen species may therefore make a major contribution to the aerobic cytotoxicity of the nitrobenzyl quaternary mustards in aerobic AA8 cell cultures.

Compounds **5a** and **6a** possess different mustard effectors and were prepared in the expectation that the use of more reactive mustards would enhance cytotoxicity, as is the case with aniline mustards.[38,45] However, the aliphatic bromo mustard **25** was found to be no more cytotoxic than mechlorethamine (IC_{50} 2.5 compared with 1.8 μM against the AA8 cell line), while the α-methyl derivative **26** was considerably less effective (IC_{50} 30 μM) (Table 3), and the corresponding quaternary mustards were less potent than **3a** in aerobic AA8 cells.

We have previously reported that **3a** shows excellent hypoxic selectivity in UV4[8] and EMT6[18] cell cultures, and the present investigation confirms the high hypoxic differential of **3a** in EMT6 cultures (Table 3). However, repeat testing over four experiments indicated a wide range in air/N_2 ratios (230−2500) as a result of considerable variability in the hypoxic (but not aerobic) C_{10} values (Figure 1). A similar lack of reproducibility was observed with the 5-OMe derivative **15a** and (to a lesser extent) with the naphthalene derivative **19a**. The air/N_2 ratios for these compounds are therefore shown as ranges in Table 3. Investigation of the causes of this variability failed to demonstrate any consistent effects of the age of the culture medium, concentration of ascorbate, serum batch, cell density, or culture history. Although this variability is unexplained, the other compounds gave more consistent data, and there were clearly large, structure-related differences in hypoxic selectivity between different analogues.

Compounds **9a**−**15a** comprise a series of analogues of **3a** bearing various substituents at different benzene ring positions, where $E(1)$ values vary in a predictable way (see above). Most of these compounds showed

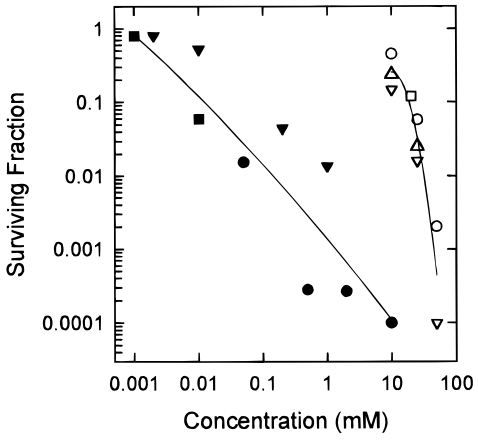


Figure 1. Cytotoxicity of **3a** in aerobic (open symbols) and hypoxic (closed symbols) EMT6 cells in a clonogenic assay, with a 3 h drug exposure (see text). Different symbols denote separate experiments under identical conditions, illustrating the variability of the hypoxic data.

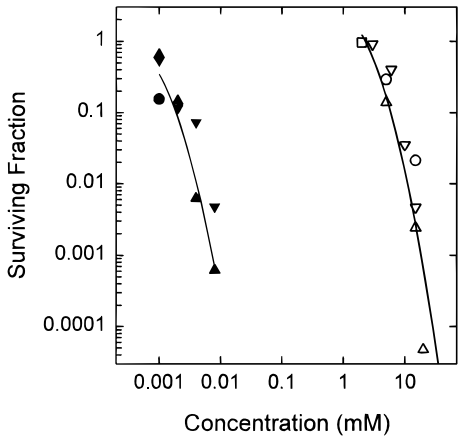
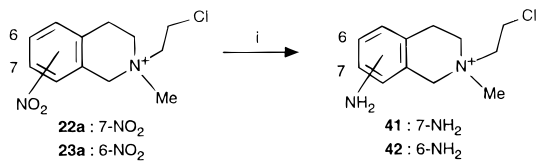


Figure 2. Cytotoxicity of **14a** in aerobic (open symbols) and hypoxic (closed symbols) EMT6 cells in a clonogenic assay, with a 3 h drug exposure, demonstrating the large differential cytotoxicity (ca. 3000-fold).

aerobic cytotoxicities similar to that of **3a** (3−10 mM), with only the 5-methyl derivative **11a** being slightly more potent (Table 3). The 3,5-dinitro derivative **16a** was much more toxic (IC_{50} 130 μM) but not hypoxic selective, presumably as a consequence of enhanced redox cycling due to its higher reduction potential. The 4-methanesulfonyl derivative (**18a**), where there is little possibility of reductive release of the mustard (reduction potential −733 mV), was only 2-fold less cytotoxic than the corresponding 4-nitro compound (**8a**) under aerobic conditions.

The methyl derivatives **9a**−**11a** all showed lower hypoxic selectivities, with the 4-Me analogue **10a** being the best at 240-fold. The 3- and 5-OMe derivatives (**13a** and **15a**) also showed low selectivity. In contrast the 4-OMe compound **14a** showed selectivity at least as great as **3a** (CT_{10} air/N_2 ratio of ca. 3000-fold; Figure 2). The 3,5-dinitro derivative **16a** had only low selectivity, while the 4-methanesulfonyl compound **18a** was not cytotoxic under either aerobic or hypoxic conditions. The naphthalene-based analogues **19a** and **20a** were prepared as more lipophilic derivatives with potentially improved rates of passive uptake, and also to provide a variation in the lifetimes of the radical anions; previous work with 8- and 5-(chloromethyl)nitronaphthalene has shown that the corresponding radical anions have

Scheme 7[a]



[a] (i) Ni_2B/HCl/MeOH/reflux/4 h.

lifetimes approximately 10× and 100× respectively, that of 4-(chloromethyl)nitrobenzene.[10] Both of the naphthalene quaternary mustards had aerobic cytotoxicities similar to that of **3a** and lower HF values (similar to that of mechlorethamine), but showed essentially no hypoxic selectivity in the clonogenic assay.

As noted above, the tetrahydroisoquinolinium compounds **21a**−**23a** were prepared as "tethered" analogues of the corresponding isomeric nitrobenzyl mustards (**3a**, **7a**, and **8a**, respectively), in order to explore the consequences of retaining the released mustard species in the same molecule as the concomitantly-generated benzyl radical (Scheme 3). These compounds possessed cytotoxicities equivalent to the corresponding nitrobenzyl derivatives and showed similar trends (for example, the 3-nitro isomers **22a** and **7a** were the least cytotoxic in each series). However, the tetrahydroisoquinolinium compounds showed essentially no differential cytotoxicity for the UV4 cell line (HF values 1.5−2.5) and no hypoxic selectivity in the clonogenic assay, suggesting that these compounds do not act as alkylating agents, despite their appropriate reduction potentials. To ascertain whether these compounds do undergo fragmentation following reduction, **22a** and **23a** were subjected to chemical reduction with nickel boride (Scheme 7).[46] Both compounds gave moderate yields of the corresponding amino compounds (**41** and **42**, respectively), which were stable under both neutral and mildly basic conditions.

The parent compound **3a,** at 75% of the MTD, showed only weak activity against the hypoxic subfraction of cells in KHT tumors *in vivo* when combined with radiation[18] but is active against the MDAH-MCa-4 tumor when combined with 5,6-dimethylxanthenone-4-acetic acid, an agent which selectively inhibits tumor blood flow[47] and thus induces additional tumor hypoxia (W. R. Wilson, unpublished results). Comparison of analogues of **3a** in these models is in progress.

Conclusions

Nitrobenzyl quaternary mustards have a number of unique properties which make them of interest for development as possible HSCs. These include the potential for activation by one-electron reduction, an opportunity to control the stability and lifetime of the intermediate radical anion, and the release of various reactive aliphatic mustards (*e.g.*, mechlorethamine) upon activation. Following our demonstration[8,18] of the outstanding *in vitro* hypoxic selectivity of **3a**, the present work sought to study the effects of nuclear substitution and other structural changes on physicochemical and biological properties in this class. The results show that substitution with methyl or methoxy groups not adjacent to the nitro group alter reduction potentials in the expected manner, such that $E(1)$ is tightly correlated with Hammett σ constants. In contrast, substitution in the 3-position (*ortho* to the nitro group) resulted in compounds with much lower $E(1)$ values, presumably due to deconjugation of the sterically crowded nitro group. Except in a few cases (e.g., **16a** and **18a**), the reduction potentials are in the range expected to be suitable for oxygen-inhibited bioreduction.

However, despite well-behaved physicochemical properties, the hypoxic selectivities of the substituted analogues varied greatly with structure (from no differential for the 3-Me analogue **9a** to ca. 3000-fold for the 4-OMe analogue **14a**). This variation cannot be explained by alterations in reduction potential, and further work is required to see whether it might be related to factors such as radical lifetimes or the ability of the drugs to be transported into cells by the choline carrier pathway. The tetrahydroisoquinolinium compounds **21a**−**23a** had $E(1)$ values consonant with efficient reduction, but showed low HF values for UV4. Compound **23a** was examined under hypoxic conditions but showed no selectivity. Chemical reduction of the latter compound resulted in a moderate yield of the corresponding amino compound, suggesting that fragmentation following reduction does not occur.

Experimental Section

Chemistry. Analyses were carried out in the Microchemical Laboratory, University of Otago, NZ. Melting points were determined using an Electrothermal Model 9200 digital melting point apparatus and are as read. NMR spectra were obtained on a Bruker AM-400 spectrometer (Me_4Si). Mass spectra were obtained on a Varian VG 7070 mass spectrometer at nominal 5000 resolution.

5-Methoxy-2-nitrobenzyl Chloride. MsCl (1.9 mL, 24 mmol) was added to 5-methoxy-2-nitrobenzyl alcohol (1.48 g, 8.1 mmol) and Et_3N (4.5 mL, 32 mmol) in CH_2Cl_2 (80 mL), and the mixture was heated under reflux for 12 h. The solution was cooled, washed with dilute HCl (2N, 2 × 50 mL), dried (Na_2SO_4), concentrated, and filtered through a short column of silica (eluting with 9:1 petroleum ether/EtOAc) to give 5-methoxy-2-nitrobenzyl chloride as a yellow liquid (1.55 g, 95%): ^{1}H NMR ($CDCl_3$) δ 8.17 (d, J = 9.1 Hz, 1 H, H-3), 7.20 (d, J = 2.8 Hz, 1 H, H-6), 6.93 (dd, J = 9.1, 2.8 Hz, 1 H, H-4), 5.03 (s, 2 H, $ArCH_2Cl$), 3.93 (s, 3 H, $ArOCH_3$); ^{13}C NMR δ 163.7 (C-2), 140.7, 135.5, 128.2, 116.5, 113.7 (C-1,3,4,5,6), 56.1 (OCH_3), 43.7 (CH_2Cl); MS (EI) m/z 201 [70, M^+ (^{35}Cl)], 184 (100), 166 (60, M − Cl), 156 (50), 106 (70); HRMS calcd for $C_8H_8ClNO_3$ 201.0193, found 201.0181.

Preparation of *N*,*N*-Bis(2-hydroxyethyl)-*N*-methyl-*N*-(2-nitrobenzyl)ammonium Chloride (3b). Example of the General Method. A solution of 2-nitrobenzyl chloride (5.00 g, 29 mmol) and *N*-methyldiethanolamine (3.47 g, 29 mmol) in CH_3CN (100 mL) was heated under reflux for 6 h. The resulting red suspension was allowed to cool to 20 °C, diluted with Et_2O (150 mL), and stirred overnight. The resulting precipitate was filtered off and dried to give *N*,*N*-bis(2-hydroxyethyl)-*N*-methyl-*N*-(2-nitrobenzyl)ammonium chloride (**3b**) (6.52 g, 77%), suitable for the next step. A sample crystallized from EtOH/Et_2O as plates: mp 148−149 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.16 (dd, J = 7.9, 1.5 Hz, 1 H, H-3 or H-6), 8.03 (dd, J = 7.6, 1.4 Hz, 1 H, H-3 or H-6), 7.90 (td, J = 7.5, 1.5 Hz, 1 H, H-4 or H-5), 7.85 (td, J = 7.7, 1.5 Hz, 1 H, H-4 or H-5), 5.59 (t, J = 5.0 Hz, 2 H, 2 × OH), 5.15 (s, 2 H, $ArCH_2N$), 3.94−3.83 (m, 4 H, NCH_2CH_2O), 3.69−3.61 (m, 2 H, NCH_2CH_2O), 3.45−3.37 (m, 2 H, NCH_2CH_2O), 3.01 (s, 3 H, NCH_3); ^{13}C NMR δ 151.0 (C-2), 136.5, 133.7, 132.2, 125.7 (C-3,4,5,6), 121.2 (C-1), 63.5, 61.5 ($ArCH_2NCH_2$), 54.9 (CH_2OH), 47.8 (NCH_3). Anal. ($C_{12}H_{19}ClN_2O_4$) C, H, N, Cl.

Preparation of *N*,*N*-Bis(2-chloroethyl)-*N*-methyl-*N*-(2-nitrobenzyl)ammonium Chloride (3a). Example of the General Method. *N*,*N*-Bis(2-hydroxyethyl)-*N*-methyl-*N*-(2-nitrobenzyl)ammonium chloride (**3b**) (3.00 g, 10.3 mmol) was added in portions to $SOCl_2$ (15 mL) with stirring, and the

resulting pale yellow solution was stirred at 20 °C with protection from moisture for 3 days. The excess $SOCl_2$ was evaporated at 20 °C, and the resulting pale yellow solid recrystallized from EtOH/Et_2O to give *N*,*N*-bis(2-chloroethyl)-*N*-methyl-*N*-(2-nitrobenzyl)ammonium chloride (**3a**) as pale yellow needles (2.95 g, 87%): mp 152−153.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.18 (dd, J = 7.9, 1.3 Hz, 1 H, H-3 or H-6), 8.03 (d, J = 7.5 Hz, 1 H, H-3 or H-6), 7.92 (td, J =7.5, 1.2 Hz, 1 H, H-4 or H-5), 7.87 (td, J =7.7, 1.2 Hz, 1 H, H-4 or H-5), 5.19 (s, 2 H, $ArCH_2N$), 4.25−4.10 (m, 4 H, NCH_2CH_2Cl), 3.99−3.90 (m, 2 H, NCH_2CH_2Cl), 3.81−3.73 (m, 2 H, NCH_2CH_2Cl), 3.13 (s, 3 H, NCH_3); ^{13}C NMR δ 150.9 (C-2), 136.6, 133.8, 132.6, 126.0 (C-3,4,5,6), 120.4 (C-1), 61.5, 61.1 ($ArCH_2NCH_2$), 47.1 (NCH_3), 36.0 (CH_2Cl). Anal. ($C_{12}H_{17}Cl_3N_2O_2$) C, H, N, Cl.

Reaction of 2,5-Dinitrobenzyl Bromide with *N*-Methyldiethanolamine: Formation of 4-(2-Hydroxyethyl)-4-methyl-7-nitro-2,3,4,5-tetrahydrobenzo[*f*][1,4]oxazepin-4-ium Chloride (27). A solution of 2,5-dinitrobenzyl bromide (0.66 g, 2.53 mmol) and *N*-methyldiethanolamine (1.5 g, 13 mmol) in CH_3CN (30 mL) was heated under reflux for 90 min. The red-brown solution was cooled and diluted with Et_2O, causing a brown oil to separate. The supernatant was decanted and the CH_3CN/Et_2O dissolution/precipitation cycle repeated several times until TLC (EtOAc/petroleum ether, 1:2) of the oil showed only baseline material. Crystallization from EtOH gave 4-(2-hydroxyethyl)-4-methyl-7-nitro-2,3,4,5-tetrahydrobenzo[*f*][1,4]oxazepin-4-ium bromide (**27**) as a tan crystalline solid (0.43 g, 51%): mp 198−199 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.45 (d, J = 2.8 Hz, 1 H, H-6), 8.32 (dd, J = 8.9, 2.8 Hz, 1 H, H-8), 7.32 (d, J = 8.9 Hz, 1 H, H-9), 5.40 (br s, 1 H, OH), 4.97 (AB coupling, J = 13.9 Hz, 2 H, $ArCH_AH_BN$), 4.71−4.60 (m, 2 H of $ArOCH_2CH_2NCH_2CH_2O$), 4.12−4.04 (m, 1 H of $ArOCH_2CH_2NCH_2CH_2O$), 4.00−3.88 (m, 3 H of $ArOCH_2CH_2NCH_2CH_2O$), 3.68−3.52 (m, 2 H of $ArOCH_2CH_2NCH_2CH_2O$), 3.25 (s, 3 H, NCH_3); ^{13}C NMR δ 164.1, 142.8 (C-7,11), 129.0, 127.3, 121.9 (C-6,8,9), 121.3 (C-10), 65.6, 64.2, 64.0, 63.2 (4 × CH_2), 54.6 (CH_2OH), 49.3 (NCH_3); MS (FAB) m/z 253 (100, M^+ for ammonium ion). Anal. ($C_{12}H_{17}BrN_2O_4$) C, H, N.

Similar reaction with 2,3-dinitrobenzyl bromide gave 4-(2-hydroxyethyl)-4-methyl-9-nitro-2,3,4,5-tetrahydrobenzo[*f*][1,4]-oxazepin-4-ium bromide (**28**) (16%): mp (EtOH/Et_2O) 182−183 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.03 (dd, J = 8.2, 1.5 Hz, 1 H, H-6 or 8), 7.83 (dd, J = 7.6, 1.4 Hz, 1 H, H-6 or 8), 7.42 (t, J = 7.9 Hz, 1 H, H-7), 5.37 (t, J = 4.8 Hz, 1 H, OH), 5.02−4.93 (m, 2 H, $ArCH_AH_BN$), 4.66−4.54 (m, 2 H of $ArOCH_2CH_2NCH_2CH_2O$), 4.12−3.85 (m, 4 H of $ArOCH_2CH_2NCH_2CH_2O$), 3.67−3.52 (m, 2 H of $ArOCH_2CH_2NCH_2CH_2O$), 3.26 (s, 3 H, NCH_3); ^{13}C NMR δ 151.5, 142.7 (C-9,11), 137.3, 126.3, 124.6 (C-6,7,8), 124.8 (C-10), 66.4, 64.3, 64.2, 63.8 (4 × CH_2), 54.6 (CH_2OH), 49.2 (NCH_3). Anal. ($C_{12}H_{17}BrN_2O_4$) C, H, N, Br.

Preparation of *N*-(2-Chloroethyl)-*N*-methyl-8-nitro-1,2,3,4-tetrahydroisoquinolinium Chloride (21a) (Scheme 6). A mixture of *N*-acetyl-7-amino-1,2,3,4-tetrahydroisoquinoline[31] (**29**) (9.7 g, 51 mmol), trifluoroacetic anhydride (12.2 mL, 86 mmol), and trifluoroacetic acid (6.6 mL, 86 mmol) in CH_2Cl_2 (150 mL) was stirred under reflux for 10 min, cooled, and evaporated, and the residue was chromatographed on silica gel. Elution with EtOAc gave *N*-acetyl-7-(trifluoroacetamido)-1,2,3,4-tetrahydroisoquinoline (**30**) (13.3 g, 91%): mp (MeOH) 182.5−185 °C; ^{1}H NMR [$(CD_3)_2SO$], a 3:2 mixture of amide conformers doubling most signals, δ 11.22 (br s, 1 H, NH), 7.57−7.40 and 7.23−7.19 (m, 3 H, H-5,6,8), 4.64 (s, 0.8 H, $ArCH_2N$), 4.58 (s, 1.2 H, $ArCH_2N$), 3.65 (t, J = 6.0 Hz, 2 H, $ArCH_2CH_2N$), 2.85 (t, J = 5.9 Hz, 1.2 H, $ArCH_2CH_2N$), 2.73 (t, J = 5.9 Hz, 0.8 H, $ArCH_2CH_2N$), 2.09 (s, 1.2 H, $COCH_3$), 2.08 (s, 1.8 H, $COCH_3$); ^{13}C NMR δ 168.7 and 168.6 ($COCH_3$), 154.9, 154.5, 154.2, 153.8 ($COCF_3$), 134.3 and 134.2, 134.0, 132.2 and 132.0, 129.1 and 129.0, 119.4 and 119.3, 118.9 and 118.7 (C-5,6,7,8,9,10), 120.0, 117.2, 114.3, 111.4 (CF_3), 47.1, 43.3, 43.1 (C-1,3), 28.2 and 27.5 (C-4), 21.7 and 21.3 ($COCH_3$). Anal. ($C_{13}H_{13}F_3N_2O_2$) C, H, N.

A solution of **30** (14.2 g, 50 mmol) in ice-cold concentrated H_2SO_4 (100 mL) was treated portionwise with powdered KNO_3 (6.07 g, 60 mmol), keeping the temperature below 8 °C. The mixture was stirred at 0 °C for a further 70 min, then poured into ice-cold water (500 mL), and extracted with CH_2Cl_2, and the extracts were dried (Na_2SO_4) and evaporated. This product contained a 3:2 mixture of the 6-NO_2 and 8-NO_2 products (by ^{1}H NMR). Hydrolysis of the trifluoroacetamides in MeOH (200 mL) and concentrated HCl (50 mL) under reflux for 45 min followed by evaporation, extraction with CH_2Cl_2, and washing with aqueous $NaHCO_3$ gave a mixture of the nitroamines which was chromatographed on silica gel. Elution with EtOAc gave firstly *N*-acetyl-7-amino-8-nitro-1,2,3,4-tetrahydroisoquinoline (**31**) (1.93 g, 17%): mp (EtOAc) 148−149 °C; ^{1}H NMR ($CDCl_3$) showed a 3:2 mixture of amide conformers doubling most signals: δ 7.10 (d, J = 8.5 Hz, 0.6 H, H-5), 7.06 (d, J = 8.5 Hz, 0.4 H, H-5), 6.74 (d, J = 8.5 Hz, 0.6 H, H-6), 6.69 (d, J = 8.5 Hz, 0.4 H, H-6), 5.60 and 5.40 (2 × br s, 2 H, NH_2), 4.92 (s, 0.8 H, $ArCH_2N$), 4.81 (s, 1.2 H, $ArCH_2N$), 3.79 (t, J = 6.1 Hz, 1.2 H, $ArCH_2CH_2N$), 3.68 (t, J = 6.1 Hz, 0.8 H, $ArCH_2CH_2N$), 2.83 (t, J = 6.1 Hz, 0.8 H, $ArCH_2CH_2N$), 2.77 (t, J = 6.1 Hz, 1.2 H, $ArCH_2CH_2N$), 2.16 (s, 3 H, $COCH_3$); ^{13}C NMR δ 169.3 and 169.1 ($COCH_3$), 142.9 and 142.3, 133.7 and 132.7, 130.8 and 129.9, 125.4 and 124.0 (C-7,8,9,10), 135.3 and 134.4, 117.6 and 117.0 (C-5,6), 47.1, 43.5, 42.7, 38.9 (C-1,3), 28.8 and 27.9 (C-4), 21.9 and 21.4 ($COCH_3$). Anal. ($C_{11}H_{13}N_3O_3$) C, H, N.

Continued elution with EtOAc gave *N*-acetyl-7-amino-6-nitro-1,2,3,4-tetrahydroisoquinoline (**32**) (3.64 g, 31%): mp (Me_2CO) 177−178 °C; ^{1}H NMR [$(CD_3)_2SO$], a 2:1 mixture of amide conformers doubling most signals, δ 7.81 (s, 0.66 H, H-5), 7.79 (s, 0.34 H, H-5), 7.32−7.22 (m, 2 H, NH_2), 6.81 (s, 1 H, H-8), 4.59 (s, 0.7 H, $ArCH_2N$), 4.53 (s, 1.3 H, $ArCH_2N$), 3.64−3.57 (m, 2 H, $ArCH_2CH_2N$), 2.79 (t, J = 5.9 Hz, 1.3 H, $ArCH_2CH_2N$), 2.67 (t, J = 5.9 Hz, 0.7 H, $ArCH_2CH_2N$), 2.08 (s, 2 H, $COCH_3$), 2.07 (s, 1 H, $COCH_3$); ^{13}C NMR δ 168.8 and 168.6 ($COCH_3$), 144.5 and 144.4, 142.6 and 142.3, 129.2 and 129.1, 122.6 and 122.5 (C-6,7,9,10), 124.3 and 124.2, 115.6 and 115.5 (C-5,8), 46.9, 43.4, 43.1, 39.0 (C-1,3), 27.3 and 26.7 (C-4), 21.6 and 21.3 ($COCH_3$). Anal. ($C_{11}H_{13}N_3O_3$) C, H, N.

A stirred solution of **31** (0.65 g, 2.8 mmol) in HCl (6 N, 8 mL) was treated dropwise with a solution of $NaNO_2$ (0.23 g, 3.3 mmol) in water (1 mL) at 0 °C. After 100 min at 0 °C, hypophosphorous acid (50% aqueous solution, 2.9 mL) was added dropwise, and the mixture was kept at 4 °C for 18 h, then poured into water, and extracted with CH_2Cl_2. Chromatography on silica gel (eluting with EtOAc) gave *N*-acetyl-8-nitro-1,2,3,4-tetrahydroisoquinoline (**33**) (0.45 g, 73%): mp (MeOH) 151−152 °C; ^{1}H NMR ($CDCl_3$), a 3:2 mixture of amide conformers doubling most signals, δ 8.00 (d, J = 8.1 Hz, 0.6 H, H-5 or 7), 7.93 (d, J = 7.8 Hz, 0.4 H, H-5 or 7), 7.51−7.29 (m, 2 H, 2 × ArH), 5.08 (s, 0.8 H, $ArCH_2N$), 4.98 (s, 1.2 H, $ArCH_2N$), 3.86 (t, J = 6.1 Hz, 1.2 H, $ArCH_2CH_2N$), 3.74 (t, J = 6.1 Hz, 0.8 H, $ArCH_2CH_2N$), 3.08−2.92 (m, 2 H, $ArCH_2CH_2N$), 2.20 (s, 1.8 H, $COCH_3$), 2.19 (s, 1.2 H, $COCH_3$); ^{13}C NMR δ 169.4 and 169.2 ($COCH_3$), 147.9 and 147.2, 138.3 and 136.8, 129.7 and 128.9 (C-8,9,10), 134.9 and 134.2, 127.5 and 127.0, 123.4 (C-5,6,7), 46.4, 43.1, 42.2, 38.5 (C-1,3), 29.8 and 28.9 (C-4), 21.9 and 21.4 ($COCH_3$). Anal. ($C_{11}H_{12}N_2O_3$) C, H, N.

Hydrolysis of **33** (0.32 g, 1.45 mmol) in MeOH (10 mL) and concentrated HCl (5 mL) under reflux for 18 h gave 8-nitro-1,2,3,4-tetrahydroisoquinoline hydrochloride (**34**): mp (MeOH) 249−250 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 9.89 (br s, 2 H, NH_2), 8.04 (d, J = 8.1 Hz, 1 H, H-5 or 7), 7.68 (d, J = 7.6 Hz, 1 H, H-5 or 7), 7.57 (t, J = 7.9 Hz, 1 H, H-6), 4.54 (s, 2 H, $ArCH_2N$), 3.38 (t, J = 6.3 Hz, 2 H, $ArCH_2CH_2N$), 3.17 (t, J = 6.3 Hz, 2 H, $ArCH_2CH_2N$); ^{13}C NMR δ 147.0 (C-8), 135.4, 124.7 (C-9,-10), 135.0, 128.1, 123.4 (C-5,6,7), 41.8, 39.1 (C-1,3), 24.9 (C-4). Anal. ($C_9H_{10}N_2O_2$·HCl) C, H, N, Cl.

A mixture of **34** (0.54 g, 2.5 mmol), Et_3N (3.5 mL, 25 mmol), and 2-bromoethanol (0.89 mL, 12.5 mmol) in THF (50 mL) was stirred at reflux for 18 h, then cooled, and evaporated. The residue was diluted with water and extracted with CH_2Cl_2, followed by chromatography on silica gel (eluting with 2% MeOH in CH_2Cl_2) to give *N*-(2-hydroxyethyl)-8-nitro-1,2,3,4-tetrahydroisoquinoline (**35**) (0.50 g, 89%). Hydrochloride salt: mp (MeOH) 243−244 °C dec; ^{1}H NMR [$(CD_3)_2SO/D_2O$] δ 8.15 (d, J = 8.1 Hz, 1 H, H-5 or 7), 7.69 (d, J = 7.7 Hz, 1 H, H-5 or 7), 7.59 (t, J = 8.0 Hz, 1 H, H-6), 4.88 (br s, 2 H, $ArCH_2N$), 4.06−4.02 (m, 2 H, CH_2), 3.80−3.60 (br m, 2 H, CH_2),

3.56−3.50 (m, 2 H, CH_2), 3.36 (t, J = 6.0 Hz, 2 H, CH_2); ^{13}C NMR δ 147.1 (C-8), 134.9, 124.1 (C-9,10), 134.7, 128.3, 123.3 (C-5,6,7), 57.1, 55.4, 50.5, 47.3 (C-1,3 and NCH_2CH_2OH), 24.9 (C-4). Anal. ($C_{11}H_{14}N_2O_3$·HCl) C, H, N, Cl.

A solution of **35** (0.50 g, 2.3 mmol) and dimethyl sulfate (0.23 mL, 2.5 mmol) in CH_3CN (15 mL) was stirred at 20 °C for 16 h, then diluted with Et_2O, and stirred until the separated oil solidified completely. The solid was filtered off and dried to give *N*-(2-hydroxyethyl)-*N*-methyl-8-nitro-1,2,3,4-tetrahydroisoquinolinium methosulfate (**21b**) (0.57 g, 73%): mp (*i*-PrOH) 103.5−107 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.06 (d, J = 8.0 Hz, 1 H, H-5 or 7), 7.75 (d, J = 7.7 Hz, 1 H, H-5 or 7), 7.64 (t, J = 7.9 Hz, 1 H, H-6), 5.34 (t, J = 5 Hz, 1 H, OH), 4.97 (AB quartet, J = 18 Hz, 2 H, $ArCH_2N$), 3.95−3.86 (m, 2 H, CH_2), 3.81 (t, J = 6.5 Hz, 2 H, CH_2), 3.49 (t, J = 4.8 Hz, 2 H, CH_2), 3.37 (s, 3 H, CH_3OSO_3), 3.36−3.30 (m, 2 H, CH_2), 3.18 (s, 3 H, NCH_3); ^{13}C NMR δ 147.6 (C-8), 132.9, 122.6 (C-9,10), 134.9, 128.6, 123.7 (C-5,6,7), 64.1, 59.6, 55.8, 54.6 (C-1,3 and NCH_2CH_2OH), 52.7 (CH_3OSO_3), 48.4 (NCH_3), 23.2 (C-4). Anal. ($C_{12}H_{17}N_2O_3.CH_3O_4S$) C, H, N, S.

The methosulfate salt of **21b** (0.51 g, 1.5 mmol) was chromatographed on Dowex 50 WX8 ion exchange resin (7 mL), eluting with 6 N HCl (35 mL). The aqueous phase was evaporated, and the residue was extracted with hot *i*-PrOH, filtered, and evaporated to give the chloride salt of **21b** as a yellow solid. This was reacted with $SOCl_2$ as above to give *N*-(2-chloroethyl)-*N*-methyl-8-nitro-1,2,3,4-tetrahydroisoquinolinium chloride (**21a**) (60%): mp (*i*PrOH) 225−226 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.07 (d, J = 8.1 Hz, 1 H, H-5 or 7), 7.75 (d, J = 7.2 Hz, 1 H, H-5 or 7), 7.65 (t, J = 7.9 Hz, 1 H, H-6), 5.05 (AB quartet, J = 16.6 Hz, 2 H, $ArCH_2N$), 4.28−4.17 (m, 2 H, CH_2), 3.96−3.80 (m, 4 H, 2 × CH_2), 3.35−3.29 (m, 2 H, CH_2), 3.21 (s, 3 H, NCH_3); ^{13}C NMR δ 147.3 (C-8), 134.7, 128.5, 123.6 (C-5,6,7), 132.5, 122.0 (C-9, 10), 62.5, 58.8, 55.2 (C-1,3 and N*C*H_2CH_2Cl), 47.5 (NCH_3), 35.7 (CH_2Cl), 22.8 (C-4). Anal. ($C_{12}H_{16}Cl_2N_2O_2$) C, H, N, Cl.

***N*-(2-Chloroethyl)-*N*-methyl-6-nitro-1,2,3,4-tetrahydroisoquinolinium Chloride (23a)**. Deamination of **32** as described above gave *N*-acetyl-6-nitro-1,2,3,4-tetrahydroisoquinoline (**36**) (69%): mp (EtOAc) 117−118 °C; ^{1}H NMR ($CDCl_3$), a 2:1 mixture of amide conformers doubling most signals, δ 8.10−8.00 (m, 2 H, H-5,7), 7.36−7.25 (m, 1 H, H-8), 4.83 (s, 0.7 H, $ArCH_2N$), 4.72 (s, 0.3 H, $ArCH_2N$), 3.87 (t, J = 5.9 Hz, 0.7 H, $ArCH_2CH_2N$), 3.74 (t, J = 5.9 Hz, 1.3 H, $ArCH_2CH_2N$), 3.02 (t, J = 5.9 Hz, 1.3 H, $ArCH_2CH_2N$), 2.96 (t, J = 5.9 Hz, 0.7 H, $ArCH_2CH_2N$), 2.21 (s, 3 H, $COCH_3$); ^{13}C NMR δ 169.5 and 169.3 (*C*OCH_3), 147.4 and 146.5, 140.9 and 139.7, 136.8 and 135.5 (C-6,9,10), 127.7 and 127.0, 124.0 and 123.5, 121.7 and 121.4 (C-5,7,8), 47.9, 44.0, 43.4, 38.8 (C-1,3), 29.4 and 28.5 (C-4), 21.8 and 21.5 (CO*C*H_3). Anal. ($C_{11}H_{12}N_2O_3$) C, H, N.

Hydrolysis of **36** as described above gave 6-nitro-1,2,3,4-tetrahydroisoquinoline hydrochloride (**37**) (87%): mp (MeOH) 256−257 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 10.00 (br s, 2 H, NH_2), 8.16 (d, J = 2.3 Hz, 1 H, H-5), 8.09 (dd, J = 8.5, 2.3 Hz, 1 H, H-7), 7.54 (d, J = 8.5 Hz, 1 H, H-8), 4.38 (s, 2 H, $ArCH_2N$), 3.38 (t, J = 6.2 Hz, 2 H, $ArCH_2CH_2N$), 3.17 (t, J = 6.2 Hz, 2 H, $ArCH_2CH_2N$); ^{13}C NMR δ 146.4 (C-6), 136.9, 134.3 (C-9,-10), 128.3, 123.5, 121.1 (C-5,7,8), 43.2, 39.7 (C-1,3), 24.6 (C-4). Anal. ($C_9H_{10}N_2O_2$·HCl) C, H, N.

Alkylation of **37** with 2-bromoethanol as described above gave *N*-(2-hydroxyethyl)-6-nitro-1,2,3,4-tetrahydroisoquinoline (**38**) (76%). Hydrochloride salt: mp (EtOH) 181−182 °C; ^{1}H NMR (D_2O) δ 8.19 (d, J = 2.0 Hz, 1 H, H-5), 8.13 (dd, J = 8.6, 2.0 Hz, 1 H, H-7), 7.46 (t, J = 8.6 Hz, 1 H, H-8), 4.68 (br s, 2 H, $ArCH_2N$), 4.06 (t, J = 5.2 Hz, 2 H, CH_2), 3.90−3.65 (br m, 2 H, CH_2), 3.54 (t, J = 5.2 Hz, 2 H, CH_2), 3.36 (t, J = 5.9 Hz, 2 H, CH_2); ^{13}C NMR δ 150.0 (C-6), 137.4, 135.4 (C-9,10), 130.8, 126.6, 124.7 (C-5,7,8), 60.0, 57.8, 55.5, 51.9 (C-1,3 and NCH_2CH_2OH), 27.4 (C-4). Anal. ($C_{11}H_{14}N_2O_3$·HCl) C, H, N, Cl.

Quaternization of **38** with dimethyl sulfate, followed by ion exchange chromatography as described above, gave *N*-(2-hydroxyethyl)-*N*-methyl-6-nitro-1,2,3,4-tetrahydroisoquinolinium chloride (**23b**) (48%): mp (from MeCN by ultrasound induction) 156−157.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.24 (d, J = 2.2 Hz, 1 H, H-5), 8.15 (dd, J = 8.5, 2.2 Hz, 1 H, H-7), 7.51 (t, J = 8.5 Hz, 1 H, H-8), 5.65 (t, J = 5.1 Hz, 1 H, OH), 4.90 (AB quartet, J = 16.2 Hz, 2 H, $ArCH_2N$), 3.95−3.90 (m, 2 H, CH_2), 3.87 (t, J = 6.5 Hz, 2 H, CH_2), 3.55−3.50 (m, 2 H, CH_2), 3.38−3.32 (m, 2 H, CH_2), 3.22 (s, 3 H, NCH_3); ^{13}C NMR δ 147.0 (C-6), 134.7, 132.2 (C-9,10), 128.7, 123.7, 121.6 (C-5,7,8), 64.0, 61.3, 56.9, 54.5 (C-1,3 and NCH_2CH_2OH), 48.2 (NCH_3), 23.2 (C-4). Anal. ($C_{12}H_{17}ClN_2O_3$·0.25H_2O) C, H, N, Cl.

Chlorination of **23b** with $SOCl_2$ as described above gave *N*-(2-chloroethyl)-*N*-methyl-6-nitro-1,2,3,4-tetrahydroisoquinolinium chloride (**23a**) as a foam that could not be induced to crystallize. The product was purified by repeatedly dissolving in a polar solvent (CH_3CN or EtOH) and separating the salt by slow addition of a less polar solvent (Et_2O or Me_2CO). Decanting the supernatant at each cycle, and drying, gave a yellow-brown gum (87%): ^{1}H NMR [$(CD_3)_2SO$] δ 8.24 (d, J = 2.3 Hz, 1 H, H-5), 8.16 (dd, J = 8.5, 2.3 Hz, 1 H, H-7), 7.49 (t, J = 8.5 Hz, 1 H, H-8), 4.91 (AB quartet, J = 16.2 Hz, 2 H, $ArCH_2N$), 4.23 (t, J = 6.8 Hz, 2 H, CH_2), 3.98−3.80 (m, 4 H, 2 x CH_2), 3.36−3.30 (m, 2 H, CH_2), 3.19 (s, 3 H, NCH_3); ^{13}C NMR δ 147.1 (C-6), 134.3, 132.1 (C-9,10), 128.8, 123.8, 121.8 (C-5,7,8), 62.9, 60.8, 56.7 (C-1,3 and N*C*H_2CH_2Cl), 47.2 (NCH_3), 35.9 (CH_2Cl), 23.1 (C-4). Anal. ($C_{12}H_{16}Cl_2N_2O_2$·H_2O) C, H, N, Cl.

***N*-(2-Chloroethyl)-*N*-methyl-7-nitro-1,2,3,4-tetrahydroisoquinolinium Chloride (22a)**. Alkylation of 7-nitro-1,2,3,4-tetrahydroisoquinoline hydrochloride[31] (**39**) with bromoethanol as above gave *N*-(2-hydroxyethyl)-7-nitro-1,2,3,4-tetrahydroisoquinoline (**40**) as a brown oil (80%): ^{1}H NMR ($CDCl_3$) δ 7.99 (dd, J = 8, 2 Hz, 1 H, H-6), 7.92 (d, J = 2 Hz, 1 H, H-8), 7.26 (d, J = 8 Hz, 1 H, H-5), 3.78 (s, 2 H, $ArCH_2N$), 3.74 (t, J = 5.3 Hz, 2 H, C*H*$_2$OH), 3.00 (t, J = 5.8 Hz, 2 H, $ArCH_2CH_2N$), 2.86 (t, J = 5.8 Hz, 2 H, $ArCH_2CH_2N$), 2.76 (t, J = 5.3 Hz, 2 H, NC*H*$_2CH_2OH$), 2.35 (br s, 1 H, OH); ^{13}C NMR δ 146.1, 142.2, 136.1 (C-7,9,10), 129.6, 121.7, 121.3 (C-5,6,8), 59.0, 58.2, 55.4, 49.9 (C-1,3 and NCH_2CH_2OH), 29.4 (C-4). Hydrochloride salt: mp (MeOH) 218−219 °C dec; Anal. ($C_{11}H_{14}N_2O_3$·HCl) C, H, N, Cl.

Methylation of **40** with dimethyl sulfate as above gave *N*-(2-hydroxyethyl)-*N*-methyl-7-nitro-1,2,3,4-tetrahydroisoquinolinium methosulfate (**22b**) as a tan solid (3.84 g, 90%): mp (*i*-PrOH) 122−124.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.22−8.15 (m, 2 H, H-6,8), 7.62 (d, J = 8.4 Hz, 1 H, H-5), 5.37 (t, J = 4.9 Hz, 1 H, OH), 4.83 (AB quartet, J = 15.8 Hz, 2 H, $ArCH_2N$), 3.97−3.91 (m, 2 H, CH_2), 3.84 (t, J = 6.5 Hz, 2 H, CH_2), 3.53−3.47 (m, 2 H, CH_2), 3.38 (s, 3 H, CH_3OSO_3), 3.37−3.31 (m, 2 H, CH_2), 3.19 (s, 3 H, NCH_3); ^{13}C NMR δ 146.2, 138.1, 129.0 (C-7,9,10), 130.4, 122.7, 122.4 (C-5,6,8), 63.9, 61.1, 57.1, 54.7 (C-1,3 and NCH_2CH_2OH), 52.8 (CH_3OSO_3), 48.3 (NCH_3), 23.4 (C-4). Anal. ($C_{12}H_{17}N_2O_3$·CH_3O_4S) C, H, N, S.

Conversion of **22b** to the chloride form by ion exchange chromatography on Dowex 50 WX8 resin and reaction of this with $SOCl_2$ as above gave *N*-(2-chloroethyl)-*N*-methyl-7-nitro-1,2,3,4-tetrahydroisoquinolinium chloride (**22a**) (42%): mp (MeCN) 202−203 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.20 (dd, J = 8.5, 2.3 Hz, 1 H, H-6), 8.15 (d, J = 2.3 Hz, 1 H, H-8), 7.63 (d, J = 8.5 Hz, 1 H, H-5), 4.97 (AB quartet, J = 15.7 Hz, 2 H, $ArCH_2N$), 4.30−4.19 (m, 2 H, CH_2), 4.02−3.87 (m, 4 H, 2 × CH_2), 3.41−3.33 (m, 2 H, CH_2), 3.23 (s, 3 H, NCH_3); ^{13}C NMR δ 146.2, 138.0, 128.6 (C-7,9,10), 130.5, 122.7, 122.4 (C-5,6,8), 62.6, 60.5, 56.8 (C-1,3 and N*C*H_2CH_2Cl), 47.2 (NCH_3), 35.8 (CH_2Cl), 23.3 (C-4). Anal. ($C_{12}H_{16}Cl_2N_2O_2$·0.25H_2O) C, H, N, Cl.

Reduction of 22a and 23a with Nickel Boride. A solution of **22a** (39 mg, 0.13 mmol) in MeOH (3 mL) was treated successively with Ni_2B[46] (0.10 g) and aqueous HCl (2 N, 1.5 mL). The suspension was stirred under reflux for 4.5 h, during which time most of the Ni_2B was consumed, giving a green solution. The mixture was evaporated, and the residue was dissolved in MeOH and filtered to remove the last of the Ni_2B. Ion exchange chromatography on Dowex 50 WX8 resin (1 mL), eluting first with 2 N HCl (5 mL) to remove other salts and then with 6 N HCl (5 mL), gave 7-ammonio-*N*-(2-chloroethyl)-*N*-methyl-1,2,3,4-tetrahydroisoquinolinium dihydrochloride (**41**) as a pale green oil (14 mg, 35%): ^{1}H NMR [$(CD_3)_2SO$) δ 7.38 (d, J = 8.2 Hz, 1 H, H-5 or H-6), 7.31 (d, J = 8.2 Hz, 1 H, H-5 or H-6), 7.17 (s, 1 H, H-8), 4.79 (AB quartet, J = 15.6 Hz, 2 H, $ArCH_2N$), 4.22 (t, J = 6.7 Hz, 2 H, CH_2),

3.90−3.73 (m, 4 H, 2 × CH_2), 3.55 (br s, 3 H, NH_3), 3.23−3.10 (m, 2 H, CH_2), 3.15 (s, 3 H, NCH_3); ^{13}C NMR δ 131.4, 128.5, 127.8 (C-5,6,8), 129.9, 122.4, 120.5 (C-7,9,10), 62.6, 60.5, 56.9 (C-1,3 and NCH_2CH_2Cl), 46.6 (NCH_3), 35.6 (CH_2Cl), 22.3 (C-4); MS (FAB, ^{35}Cl) *m/z* 225 (60, ammonium ion $M^{2+} - H^+$), 106 (100); HRMS calcd for $C_{12}H_{18}ClN_2$ 225.1159; found 225.1155.

Similar reduction of **23a** (except that the crude product was treated with dilute NH_4OH for several minutes at 20 °C before ion exchange chromatography) gave 6-ammonio-*N*-(2-chloroethyl)-*N*-methyl-1,2,3,4-tetrahydroisoquinolinium dihydrochloride (**42**) as a pale yellow oil (24% yield): ^{1}H NMR [$(CD_3)_2SO$] δ 7.24 (s, 3 H, H-5,7,8) (appears as multiplet, d 7.30−7.15 on D_2O exchange), 4.76 (AB quartet, J = 15.7 Hz, 2 H, $ArCH_2N$), 4.21 (t, J = 6.5 Hz, 2 H, CH_2), 3.94−3.75 (m, 4 H, 2 × CH_2), 3.25−3.10 (m, 2 H, CH_2), 3.13 (s, 3 H, NCH_3); ^{13}C NMR δ 133.7, 130.9, 128.2, 124.8, 121.9, 121.1 (C-5,6,7,8,9,10), 62.5, 60.7, 56.9 (C-1,3 and NCH_2CH_2Cl), 46.8 (NCH_3), 35.8 (CH_2Cl), 22.9 (C-4); MS (FAB, ^{35}Cl) *m/z* 225 (100, ammonium ion $M^{2+} - H^+$), 106 (90); HRMS Calcd for $C_{12}H_{18}ClN_2$ 225.1159, found 225.1155.

In Vitro Cytotoxicity. Cell lines were maintained as log-phase monolayers in tissue culture flasks using antibiotic-free Alpha MEM with 5% v/v heat-inactivated (56 °C, 40 min) fetal bovine serum. Doubling times were approximately 14 h for AA8, 15 h for UV4, 9 h for EMT6, and 24 h for FME cells. Cultures were tested for mycoplasma contamination frequently, using a cytochemical staining method.[48] Growth inhibition studies were performed as described in detail elsewhere,[33] using 200 AA8, 300 UV4, 50 EMT6, or 1000 FME cells in 0.05 mL per well in 96-well tissue culture dishes. Drugs were added 24 h after initiation of cultures and removed 18 h later. The IC_{50} was determined as the drug concentration needed to inhibit cell protein (measured 72-78 h after drug washout by staining with methylene blue and determining absorbance in a microplate photometer[49]) to 50% of the mean value for eight control cultures on the same 96-well plate.

Clonogenic assays were performed using EMT6 cells derived from multicellular spheroids, which were grown to diameters of approximately 1 mm in spinner flasks and dissociated using pronase (0.5 mg/mL) and DNAase (0.2 mg/mL).[18] Magnetically-stirred single cell suspensions (10 mL at 10^6/mL) were gassed continuously with 5% CO_2 in air or N_2 (hypoxic cultures) as detailed elsewhere.[18,35]

Acknowledgment. The authors thank Karin Tan and Susan Pullen for technical help. This work was carried out under Contract NO1-CM 47019 from the National Cancer Institute, with further support from the Auckland Division of the Cancer Society of New Zealand and the Health Research Council of New Zealand.

Supporting Information Available: Yields, melting points, NMR data for the quaternary mustards **3a**−**23a** of Table 1 and the corresponding quaternary diols **3b**−**23b** of Table 2, and details of the determinations of the *E*(1) values of compounds **3a**−**23a** (10 pages). Ordering information is given on any current masthead page.

References

(1) Bush, R. S.; Jenkin, R. D. T.; Allt, W. E. C.; Beale, F. A.; Bean, H.; Dembo, A. J.; Pringle, J. F. Definitive evidence for hypoxic cells influencing cure in cancer therapy. *Br. J. Cancer* **1978**, *37*, 302−306.

(2) Gatenby, R. A.; Kessler, H. B.; Rosenblum, J. S.; Coia, L. R.; Moldofsky, P. J.; Hartz, W. H. Oxygen distribution in squamous cell carcinoma metastases and its relationship to outcome of radiation therapy. *Int. J. Radiat. Oncol. Biol. Phys.* **1988**, *14*, 831−838.

(3) Hockel, M.; Knoop, C.; Schlenger, K.; Vorndran, B.; Baubmann, E.; Mitze, M.; Knapstein, P. G.; Vaupel, P. Intratumoral pO_2 predicts survival in advanced cancer of the uterine cervix. *Radiother. Oncol.* **1993**, *26*, 45−50.

(4) Wilson, W. R. Tumour hypoxia: challenges for cancer chemotherapy. In *Cancer Biology and Medicine;* Waring, M. J., Ponder, B. A. J., Eds.; Kluwer Academic Publishers: Lancaster, 1992; Vol. 3, pp 87−131.

(5) Denny, W. A.; Wilson, W. R. Bioreducible mustards: a paradigm for hypoxia-selective prodrugs of diffusible cytotoxins (HPDCs). *Cancer Met. Rev.* **1993**, *12*, 135−151.

(6) Palmer, B. D.; Wilson, W. R.; Cliffe, S.; Denny, W. A. Hypoxia-selective antitumor agents. 5. Synthesis of water-soluble nitroaniline mustards with selective cytotoxicity for hypoxic mammalian cells. *J. Med. Chem.* **1992**, *35*, 3214−3222.

(7) Ware, D. C.; Palmer, B. D.; Wilson, W. R.; Denny, W. A. Hypoxia-selective antitumor agents. 7. Metal complexes of aliphatic mustards as a new class of hypoxia-selective cytotoxins. Synthesis and evaluation of cobalt (III) complexes of bidentate mustards. *J. Med. Chem.* **1993**, *36*, 1839−1846.

(8) Tercel, M.; Wilson, W. R.; Denny, W. A. Nitrobenzyl mustard quaternary salts: a new class of hypoxia-selective cytotoxins showing very high *in vitro* selectivity. *J. Med. Chem.* **1993**, *36*, 2578−2579.

(9) Neta, P.; Behar, D. Intramolecular electron transfer in the anion radicals of nitrobenzyl halides. *J. Am. Chem. Soc.* **1980**, *102*, 4798−4802.

(10) Bays, J. P.; Blumer, S. T.; Baral-Tosh, S.; Behar, D.; Neta, P. Intramolecular electron transfer and dehalogenation of nitroaromatic anion radicals. *J. Am. Chem. Soc.* **1983**, *105*, 320−324.

(11) Norris, R. K.; Barker, S. D.; Neta, P. Steric effects on rates of dehalogenation of anion radicals derived from substituted nitrobenzyl halides. *J. Am. Chem. Soc.* **1984**, *106*, 3140−3144.

(12) Andrieux, C. P.; Le Gorande, A.; Saveant, J.-M. Electron transfer and bond breaking. Examples of passage from a sequential to a concerted mechanism in the electrochemical reductive cleavage of arylmethyl halides. *J. Am. Chem. Soc.* **1992**, *114*, 6892−6904.

(13) Moreno, S. N. J.; Schreiber, J.; Mason, R. P. Nitrobenzyl radical metabolites from microsomal reduction of nitrobenzyl chlorides. *J. Biol. Chem.* **1986**, *261*, 7811−7815.

(14) Teicher, B. A.; Sartorelli, A. C. Nitrobenzyl halides and carbamates as prototype bioreductive alkylating agents. *J. Med. Chem.* **1980**, *23*, 955−960.

(15) Kirkpatrick, D. L.; Johnson, K. E.; Sartorelli, A. C. Nitrobenzyl derivatives as bioreductive alkylating agents: evidence for the reductive formation of a reactive intermediate. *J. Med. Chem.* **1986**, *29*, 2048−2052.

(16) Russell, G. A.; Danen, W. C. Electron-transfer processes. VIII. Coupling reactions of radicals with carbanions. *J. Am. Chem. Soc.* **1968**, *90*, 347−353.

(17) Stock, L. M.; Wasielewski, M. R. The electron paramagnetic resonance spectra of α-substituted nitrotoluene anion radicals. The influence of electron-withdrawing substituents on the coupling constants for β-hydrogen atoms. *J. Am. Chem. Soc.* **1975**, *97*, 5620−5622.

(18) Denny, W. A.; Wilson, W. R.; Tercel, M.; Van Zijl, P.; Pullen, S. M. Nitrobenzyl mustard quaternary salts: a new class of hypoxia-selective cytotoxins capable of releasing diffusible cytotoxins on bioreduction. *Int. J. Radiat. Oncol. Biol. Phys.* **1994**, *29*, 317−321.

(19) Chupp, J. P.; Grabiak, R. C.; Leschinsky, K. L.; Neumann, T. L. Substituted benzotrichloride synthesis by phase transfer-catalysed chlorination. *Synthesis* **1986**, 224−226.

(20) Kienzle, F.; Kaiser, A.; Chodnekar, M. S. 1,5-Dihydroimidazoquinazolinones as blood platelet aggregation inhibitors. *Eur. J. Med. Chem.-Chim. Ther.* **1982**, *17*, 547−556.

(21) Beverung, W. N.; Partyka, R. A. 6-Methyl-1,2,3,5-tetrahydroimidazo[2,1-b]quinazolin-2-one, a potent inhibitor of ADP-induced platelet aggregation. *J. Med. Chem.* **1975**, *18*, 224−225.

(22) Saari, W. S.; King, S. W. Synthesis and biological activity of some aporphine derivatives related to apomorphine. *J. Med. Chem.* **1974**, *17*, 1086−1090.

(23) Wakae, M.; Konishi, K. Chloromethylation of 3-substituted chloro- or nitrobenzene. *Yuki Gosei Kagaku Kyokaishi* **1959**, *17*, 697−704; *Chem. Abstr.* **1960**, *54*, 2221a.

(24) Fuchs, R.; Carlton, D. M. Substituent effects in the solvolysis and thiosulphate reactions of 3-, 4- and 3,5-substituted α-chlorotoluenes. *J. Am. Chem. Soc.* **1963**, *85*, 104−107.

(25) Samanen, J. M.; Brandeis, E. The *p*-(Methylsulfinyl)benzyl Group: A TFA-stable carboxyl-protecting group readily convertible to a TFA-labile group. *J. Org. Chem.* **1988**, *53*, 561−569.

(26) Bays, J. P.; Blumer, S. T.; Baral-Tosh, S.; Behar, D.; Neta, P. Intramolecular electron transfer and dehalogenation of nitroaromatic anion radicals. *J. Am. Chem. Soc.* **1983**, *105*, 320−324.

(27) Bowman, R. E.; Islip, P. J.; Lockhart, I. M.; Richards, K. E.; Wright, M. The synthesis of some 3-aminohydrocarbostyrils. *J. Chem. Soc.* **1965**, 1080−1087.

(28) Lane, C. F.; Myatt, H. L.; Daniels, J.; Hopps, H. B. Organic synthesis using borane-methyl sulfide. II. Reduction of aromatic carboxylic acids in the presence of trimethyl borate. *J. Org. Chem.* **1974**, *39*, 3052−3054.

(29) Compound **25a** had mp 145−146 °C (Me_2CO). Sakurai and Izumi (Sakurai, Y.; Izumi, M. *N*-Oxides of β-chloroethylamine derivatives. *Chem. Pharm. Bull.* **1953**, *1*, 297−301) report mp 134 °C. Bobranski, B.; Jakobiec, T.; Prelicz, D. An improved method of obtaining methyl derivatives of aliphatic diamines, β-haloalkylamines, and bis(β-haloalkyl)amines. (*Roczniki Chem.* **1956**, *30*, 623−625; *Chem. Abstr.* **1957**, *51*, 4272i) report mp 145−147 °C.

(30) Kuwada, Y. Studies on carcinostatic substances. XXXI. Antitumor action of 1-dialkylamino-2,3-dichloropropanes and 2-dialkylamino-1,3-dichloropropanes. *Chem. Pharm. Bull.* **1960**, *8*, 807−814.
(31) Ajao, J. F.; Bird, C. W. The preparation and oxidative dimerisation of 2-acetyl-7-hydroxy-1,2,3,4-tetrahydroisoquinoline. A new approach to tetrahydroisoquinoline synthesis. *J. Heterocycl. Chem.*, **1985**, *22*, 329−331.
(32) Patel, K. B.; Willson, R. L. Semiquinone free radicals and oxygen. Pulse radiolysis study of one electron transfer equilibria. *J. Chem. Soc., Faraday Trans.* **1973**, *69*, 814−819.
(33) 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 derivatives. *J. Med. Chem.* **1989**, *32*, 31−38.
(34) 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.
(35) 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.
(36) Papanastassiou, Z. B.; Bruni, R. J.; White, E. Potential carcinolytic agents. VI. New deactivated biological alkylating agents. *Experientia* **1968**, *24*, 325.
(37) Hoefnagel, A. J.; Hoefnagel, M. A.; Wepster, B. M. Substituent effects. 6. Charged groups: a simple extension of the Hammett equation. *J. Org. Chem.* **1978**, *43*, 4720−4745.
(38) Palmer, B. D.; Wilson, W. R.; Pullen, S. M.; Denny, W. A. Hypoxia-selective antitumor agents. 3. Relationships between structure and cytotoxicity against cultured tumor cells for substituted *N,N*-bis(2-chloroethyl)anilines. *J. Med. Chem.* **1990**, *33*, 112−121.
(39) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley-Interscience: New York, 1979.
(40) (a) Hutchings, M. G. The isomerisation of some *ortho*-substituted nitrobenzenes; a structural correlation analysis. *J. Chem. Soc., Perkin Trans. 2* **1982**, 1241−1246. (b) Boyd, M.; Lee, H. H.; Anderson, R. F.; Denny, W. A. Relationships between nitro group reduction potentials and torsion angles in di-*ortho*-substituted nitrobenzenes; a crystallographic and oxygen-17 NMR study. *J. Chem. Soc., Perkin Trans. 2*, **1994**, 291−295.
(41) Kwon, C. H.; Blanco, D. R.; Baturay, N. *p*-(Methylsulfinyl)phenyl nitrogen mustard as a novel bioreductive prodrug selective against hypoxic tumors. *J. Med. Chem.* **1992**, *35*, 2137−2139.
(42) Balakrishnan, P.; Boykin, D. W. Relationship of aromatic nitro group torsion angles with ^{17}O chemical shift data. *J. Org. Chem.* **1985**, *50*, 3661−3663.
(43) Boyd, M., Boyd; P. D. W.; Atwell, G. J.; Wilson, W. R.; Denny, W. A. Crystallographic and oxygen-17 NMR studies of nitro group torsion angles in a series of 4-alkylaminonitroquinolines designed as hypoxia-selective cytotoxins. *J. Chem. Soc., Perkin Trans. 2* **1992**, 579−584.
(44) Goldenberg, G. J.; Vanstoen, C. L.; Bihler, I. Transport of nitrogen mustard on the transport carrier for choline in L5178Y lymphoblasts. *Science* **1971**, *172*, 1148−1149.
(45) Panthananickal, A.; Hansch, C.; Leo, A.; Quinn, F. R. Structure-activity relationships in antitumor aniline mustards. *J. Med. Chem.* **1978**, *21*, 16−26.
(46) Seltzman, H. H.; Berrang, B. D. Nickel boride reduction of aryl aromatic nitro compounds. *Tetrahedron Lett.* **1993**, *34*, 3083−3086.
(47) Cliffe, S.; Taylor, M. L.; Rutland, M.; Baguley, B. C.; Hill, R. P.; Wilson, W. R. Combining bioreductive drugs (SR 4233 or SN 23862) with the vasoactive agents flavoneacetic acid and 5,6-dimethylxanthenoneacetic acid. *Int. J. Radiat. Oncol. Biol. Phys.* **1994**, *29*, 373−377.
(48) Chen, T. R. In situ detection of *Mycoplasma* contamination in cell cultures by fluorescent Hoechst 33258 stain. *Exp. Cell Res.* **1977**, *104*, 255−262.
(49) Finlay, G. J.; Baguley, B. C.; Wilson, W. R. A semiautomated microculture method for investigating growth inhibitory effects of cytotoxic compounds on exponentially growing carcinoma cells. *Anal. Biochem.* **1984**, *139*, 272−277.
(50) Copp, F. C. Phosphorylated quaternary ammonium compounds of improved oral adsorption. U.S. Patent 3,090,725; *Chem. Abstr.* **1964**, *61*, 9434e.

JM9507791