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Synthesis of asymmetric halomesylate mustards with aziridineethanol/alkali metal halides: application to an improved synthesis of the hypoxia prodrug PR-104

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Abstract—Aromatic asymmetric halomesylate mustards are efficiently prepared by reaction of activated aromatic chlorides with aziridineethanol/alkali metal halides, followed by mesylation of the haloalcohol. The reaction conditions are sufficiently mild to be compatible with a range of different substituents and protecting groups, including carboxylate and phosphate esters, and have been used in an improved synthesis of the anticancer bromomesylate mustard PR-104, now in clinical trials.

1. Introduction

(Hetero)aromatic asymmetric mustards have been described for use as prodrugs for hypoxia (e.g., **1**)[1] and for ADEPT (e.g., **2**)[2,3] and GDEPT (e.g., **3**).[4] We have recently reported that halomesylate mustards (e.g., **3**) are more selectively activated than symmetric dihalomustards by cells expressing the *Escherichia coli* nitroreductase NTR.[4] We have been particularly interested in halomesylates as hypoxia prodrugs, and have recently taken the bromomesylate PR-104 (**1**) to clinical trial[5] (Fig. 1).

A key step in the synthesis of **1** is preparation of the bromomesylate (**6**), which is reacted with di-*tert*-butyl diisopropylphosphoramidite to give the phosphate triester (**7**), which is treated with TFA to furnish **1** (Scheme 1).[6] The desired bromomesylate (**6**) can be obtained either by reaction of the bis(mesylate) mustard (**4**) with a limited amount of lithium bromide, or conversely by reaction of the bis(bromo) mustard (**5**) with a limited amount of silver mesylate. These reactions give **6** directly, but the lack of selectivity leads to a mixture of all three possible components **4**–**6** in both cases.[6] The ratios of these can be influenced by the amount of reagent used but careful chromatographic separation is still required, and yields of the desired isolated pure **6** are generally only 30–40%.

In this paper we report a new and more efficient method (Scheme 2) for the preparation of halomesylate mustards (IV) from relatively activated aromatic halides (I), using commercially available aziridineethanol and alkali metal halides (MX), and apply this to an improved synthesis of the clinical drug **1** and analogues. The reaction presumably follows the course shown in Scheme 2, with initial reaction

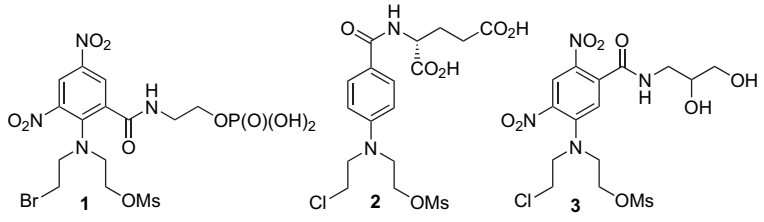

Figure 1.

Keywords: Aniline mustard; Asymmetric mustard synthesis; Aziridineethanol; PR-104.
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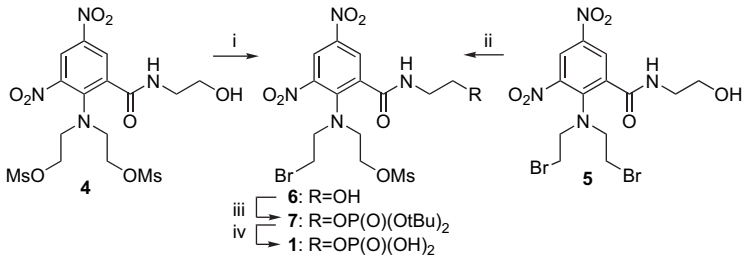
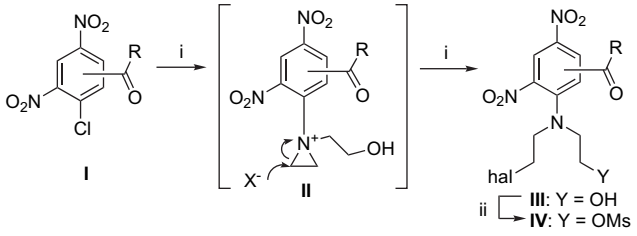


Scheme 1. Reagents: (i) LiBr; (ii) AgOMs; (iii) *i*-$Pr_2NP(O^tBu)_2$; (iv) TFA.



Scheme 2. Reagents: (i) aziridineethanol/M^+X^-; (ii) $(Ms)_2O$ or MsCl.

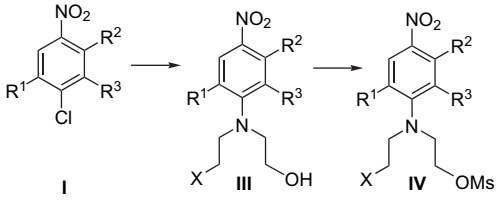
to form an aziridinium intermediate, followed by ring opening with halide anion (X^-). This gives the asymmetric haloalcohols (III) in a single step as the major product in much higher yields, without the need for separation of closely related products. It is compatible with a number of different functional groups, and variation of the halogen in the alkali metal halides (e.g., LiCl, LiBr, NaI) allows the preparation of various haloalcohols from a single precursor and their conversion to halomesylate mustards (IV) with methanesulfonyl chloride or (preferably) methanesulfonic anhydride (which avoids traces of chloro impurities when other halomesylates are prepared).

2. Results and discussion

The aziridineethanol reaction works best with highly activated haloaromatics. Table 1 shows the results of the reaction of a series of chlorodinitrobenzamides and -benzoates with aziridineethanol in the presence of lithium or sodium metal halides. Preferable solvents were DMF, THF, 1,4-dioxane or 3-methyl-2-butanone.

Thus reaction of 2-chloro-3,5-dinitrobenzamide (**8**) with aziridineethanol gave the corresponding haloalcohols (**9a**–**9c**) directly, in yields ranging from 70–90%, and these were converted to the halomesylates (**10a**–**10c**) in good overall yields without the need for chromatographic purification.

Table 1. Preparation of halomesylate mustards (IV) from dinitrobenzamide halides (I)

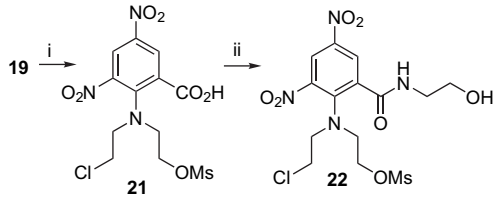


R^1	R^2	R^3	X	I	III	IV	Yield (%)		
							I→III	I→IV	(# steps)[ref]
NO_2	H	$CONH_2$	Cl	**8**	**9a**	**10a**	92	90	15 (3)[1,10]
			Br		**9b**	**10b**	87	74	24 (3)[1,10]
			I		**9c**	**10c**	70	45	
NO_2	H	$CONH(CH_2)_2OTHP$	Cl	**11**	**12a**	**13a**	97/37[a]	95/36[a]	40 (2)[1]
			Br		**12b**	**13b**	80	76	40 (2)[1]
			I		**12c**	**13c**	63	62	19 (2)[6]
NO_2	H	$CONH(CH_2)_2OP(O)(O^tBu)_2$	Cl	**14**	**15**	**16**	90[a]	61[a]	
NO_2	H	$CO_2{}^tBu$	Cl	**17**	**18**	**19**	63	56	
NO_2	$CONH_2$	H	Cl	**20**	**21a**	**22a**	43	32	12 (3)[1,11]
			Br		**21b**	**22b**	~10 (37)[b]	36[b]	
			I		**21c**	**22c**	— (29)[b]	28[b]	
H	CO_2Me	NO_2	Cl	**23**	**24a**	**25a**	63	50	
			Br		**24b**	**25b**	36	28	
			I		**24c**	**25c**	58	53	
H	$CO_2{}^tBu$	NO_2	Cl	**26**	**27**	**28**	51	45	

[a] Using 2-[(2-chloroethyl)amino]ethanol as reagent for the first step.
[b] Subsequent reaction of **21a** with LiBr or NaI (see text).

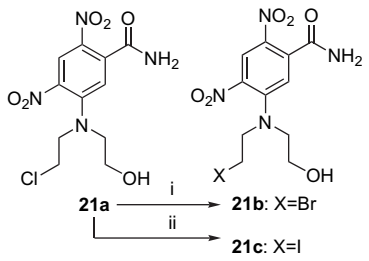
The mild reaction conditions allow the use of a variety of different side chains off the carboxamide. In particular, the THP ether (**11**) gave excellent yields of the haloalcohols (**12a**–**12c**), which could be converted to the halomesylates (**13a**–**13c**) (Table 1), of which **13b** (which can easily be deprotected with catalytic methanesulfonic acid in methanol) is the key intermediate for an improved synthesis of the clinical drug **1**. In both of the above sets, the reactions to form the iodoalcohols (**9c**, **12c**) resulted in the lowest yields, because of greater secondary reaction of the more reactive iodides to form polar impurities. Exploring the limits of the reaction, the phosphate triester (**14**), prepared from 2-chloro-3,5-dinitro-*N*-(2-hydroxyethyl)benzamide[6] with di-*tert*-butyl diisopropylphosphoramidite, did not survive the aziridineethanol reaction but did give a good yield of the chloroalcohol **15** when reacted with the open-chain precursor of aziridineethanol, 2-[(2-chloroethyl)amino]ethanol (Table 1). This reagent is not commercially available, but can be easily prepared by known methods.[7,8] The chloromesylate **16** was prepared from **15** as above (Table 1). But even with 2-[(2-chloroethyl)amino]ethanol, reactions to prepare the corresponding bromo- or iodoalcohols were accompanied by significant loss of the phosphate protecting group.

The *tert*-butyl ester **17** also reacted with aziridineethanol to give the chloroalcohol **18** in 63% yield, followed by mesylation to **19** (Table 1). Cleavage of this with TFA gave the acid **21**, which was activated with oxalyl chloride and coupled with 2-aminoethanol to give the amide **22** (Scheme 3).



Scheme 3. Reagents: (i) TFA; (ii) $(COCl)_2$, then $H_2N(CH_2)_2OH$.

The 5-chloro-2,4-dinitrobenzamide (**20**) was less reactive, giving (in DMF) a moderate (43%) yield of the chloroalcohol **21a**, which could be converted in high yield to the chloromesylate **22a** by reaction with MsCl (Table 1). The overall yield of 32% is compared favourably with previous work,[1] which gave a 12% yield after chromatographic separation. Reaction of **20** in the presence of LiBr gave a much poorer yield of the bromoalcohol **21b** (~10%), and **21b** and the corresponding iodoalcohol **21c** were better obtained by subsequent reaction of **21a** with LiBr or NaI (in 86% and 68% yields, respectively) (Scheme 4). This allowed the preparation of the bromo- and iodomesylates **22b** and **22c** in overall yields of 37% and 29%, respectively, from **20** (Scheme 4 and Table 1).



Scheme 4. Reagents: (i) LiBr; (ii) NaI.

Reaction of 3-chloro-2,6-dinitrobenzamide with aziridineethanol was not successful, but the corresponding methyl ester[12] (**23**) gave the corresponding haloalcohols **24a**–**24c**, which were converted to the halomesylates **25a**–**25c** in good overall yield (Table 1). The corresponding *tert*-butyl ester **26** gave similar yields of the chloroalcohol **27** and the chloromesylate **28**.

3. Conclusions

Aziridineethanol/alkali metal halides offer a straightforward and high-yielding direct preparation of asymmetric halomesylate mustards from activated aromatic halides. This reaction has been used to provide an improved synthesis of the hypoxia prodrug PR-104 (**1**). The reaction conditions are sufficiently mild to be compatible with THP ether and carboxylate ester protecting groups, and a phosphotriester function.

4. Experimental

4.1. General procedures

Analyses were performed by the Microchemical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined using an Electrothermal Model 9200 digital melting point apparatus, and are as read. NMR spectra were measured on a Bruker DRX-400 spectrometer, and referenced to Me_4Si. Mass spectra were recorded on a Varian VG-70SE spectrometer at nominal 5000 resolution. HPLC was carried out using a Bondclone 10 C18 reverse-phase silica gel column, with a Phillips PU4100 M gradient elution pump and a Phillips PU 4120 diode array detector, and eluting with the appropriate ratios of 95% MeCN/5% water (solvent A) and 0.45 M ammonium formate buffer (solvent B; pH 3.45).

4.2. Preparation of starting materials

4.2.1. Di(*tert*-butyl)2-[(2-chloro-3,5-dinitrobenzoyl)-amino]ethyl phosphate (14). A solution of 2-chloro-3,5-dinitro-*N*-(2-hydroxyethyl)benzamide[6] (2.00 g, 6.91 mmol) in dry DMF (8 mL) and THF (12 mL) was treated at 10 °C with 1*H*-tetrazole (0.77 g, 11.0 mmol) followed by di-*tert*-butyl diisopropylphosphoramidite (95%, 2.62 g, 9.44 mmol). The mixture was stirred at room temperature for 2 h under N_2, then treated with a solution of 70% aqueous H_2O_2 (1.8 mL) in THF (2.0 mL) at 10 °C for 30 min. The reaction was diluted with EtOAc, and the solution was washed with water, dried and concentrated under reduced pressure below 30 °C. The residue was purified by chromatography on silica gel, eluting with EtOAc/petroleum ether (4:1), followed by recrystallisation from EtOAc/diisopropyl ether, to give **14** (1.69 g, 51%) as a white solid: mp 98 °C (dec); ^{1}H NMR [$(CD_3)_2SO$] δ 9.05–8.98 (m, 2H), 8.53 (d, J=2.6 Hz, 1H), 4.00 (q, J=6.1 Hz, 2H), 3.53 (q, J=5.6 Hz, 2H), 1.43 (s, 18H); ^{13}C NMR δ 163.0, 148.5, 145.8, 139.8, 128.3, 125.8, 120.7, 81.5 (d, $J_{c\text{-}p}$=7.1 Hz, 2), 61.2 (d, $J_{c\text{-}p}$=6.4 Hz), 39.5

(d, $J_{c\text{-}p}$=8.5 Hz), 29.3 (d, $J_{c\text{-}p}$=4.3 Hz, 6). Anal. Calcd for $C_{17}H_{25}ClN_3O_9P$: C, 42.38; H, 5.23; N, 8.72; P, 6.43. Found: C, 42.67; H, 5.22; N, 9.02; P, 6.36%.

4.2.2. *tert*-Butyl 3-chloro-2,6-dinitrobenzoate (26). A mixture of 3-chloro-2,6-dinitrobenzoic acid[11] (3.00 g, 12 mmol), *tert*-butyl acetate (20 mL, 150 mmol) and 70% $HClO_4$ (0.8 mL) was stirred at 35 °C for 6 h, then poured into excess satd aqueous $NaHCO_3$. The mixture was extracted with CH_2Cl_2 and the organic phase was washed with satd aqueous $NaHCO_3$ and water, dried and evaporated to dryness. The residue was crystallised from hexane to give **26** (2.51 g, 68%) as a white solid: mp 109–110 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.43 (d, J=8.9 Hz, 1H), 8.22 (d, J=8.9 Hz, 1H), 1.49 (s, 9H). Anal. Calcd for $C_{11}H_{11}ClN_2O_6$: C, 43.65; H, 3.66; N, 9.26. Found: C, 43.93; H, 3.68; N, 9.30%.

4.3. Aziridineethanol reaction: general conditions

A slurry of aromatic chloride (1 equiv) and either LiCl (0.5 equiv), LiBr (17 equiv) or NaI (17 equiv) in solvent (3-methyl-2-butanone unless otherwise mentioned for specific products) was cooled to below 5 °C. Aziridineethanol (2.5 equiv) was added over 10 min to the stirred mixture, which was kept below 20 °C overnight. Water was added, and the reaction mixture was extracted three times with EtOAc. The combined organic layers were washed with water and with a 10% aqueous solution of the appropriate sodium halide, dried and concentrated under reduced pressure. The concentrate was passed through a short column of silica gel, eluting with EtOAc/heptane, to provide product. The following compounds were prepared by this method.

4.3.1. 2-[(2-Chloroethyl)(2-hydroxyethyl)amino]-3,5-dinitrobenzamide (9a). From 2-chloro-3,5-dinitrobenzamide[6] (**8**) (in THF) as a yellow solid (92% yield): mp (EtOAc/petroleum ether) 96–100 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.69 (d, J=2.8 Hz, 1H), 8.43 (s, 1H), 8.35 (d, J=2.8 Hz, 1H), 8.10 (s, 1H), 5.15 (t, J=5.6 Hz, 1H), 3.77 (m, 2H), 3.54 (m, 4H), 3.14 (m, 2H); ^{13}C NMR δ 167.6, 146.7, 143.8, 139.7, 134.1, 128.2, 123.1, 57.8, 54.3, 53.6, 41.4. Anal. Calcd for $C_{11}H_{13}ClN_4O_6\cdot 0.5H_2O$: C, 38.66; H, 4.13; N, 16.40. Found: C, 38.37; H, 4.42; N, 15.68%.

4.3.2. 2-[(2-Bromoethyl)(2-hydroxyethyl)amino]-3,5-dinitrobenzamide (9b). From **8** as a yellow solid (87% yield): mp (EtOAc/petroleum ether) 138–140 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.69 (d, J=2.8 Hz, 1H), 8.43 (s, 1H), 8.35 (d, J=2.8 Hz, 1H), 8.10 (s, 1H), 5.15 (t, J=5.6 Hz, 1H), 3.61 (m, 4H), 3.53 (m, 2H), 3.14 (m, 2H); ^{13}C NMR δ 167.6, 146.4, 143.8, 139.8, 134.2, 128.2, 123.0, 57.8, 54.1, 53.6, 29.8. Anal. Calcd for $C_{11}H_{13}BrN_4O_6$: C, 35.0; H, 3.5; N, 14.9. Found: C, 35.0; H, 3.5; N, 14.6%.

4.3.3. 2-[(2-Hydroxyethyl)(2-iodoethyl)amino]-3,5-dinitrobenzamide (9c). From **8** as a yellow solid (70% yield): mp (EtOAc/petroleum ether) 152–155 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.69 (d, J=2.8 Hz, 1H), 8.41 (s, 1H), 8.34 (d, J=2.8 Hz, 1H), 8.10 (s, 1H), 5.13 (t, J=5.6 Hz, 1H), 3.53 (m, 4H), 3.35 (m, 2H), 3.13 (m, 2H); ^{13}C NMR δ 167.5, 146.1, 143.8, 139.8, 134.2, 128.2, 123.0, 57.8, 54.8, 53.7, 2.07. Anal. Calcd for $C_{11}H_{13}IN_4O_6$: C, 31.15; H, 3.09; N, 13.21. Found: C, 31.45; H, 2.99; N, 12.91%.

4.3.4. 2-[(2-Chloroethyl)(2-hydroxyethyl)amino]-3,5-dinitro-*N*-[2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]benzamide (12a). From 2-chloro-3,5-dinitro-*N*-[2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]benzamide[6] (**11**) as a yellow solid (97% yield): mp (EtOAc/petroleum ether) 106–108 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.07 (t, J=5.4 Hz, 1H), 8.70 (d, J=2.8 Hz, 1H), 8.33 (d, J=2.8 Hz, 1H), 5.15 (t, J=5.6 Hz, 1H), 4.62 (t, J=3.7 Hz, 1H), 3.78 (m, 4H), 3.58–3.43 (m, 8H), 3.14 (m, 2H), 1.80–1.40 (m, 6H); ^{13}C NMR δ 165.7, 146.8, 143.7, 139.7, 133.9, 128.4, 123.1, 98.0, 64.8, 61.5, 57.8, 54.3, 53.3, 41.4, 39.5, 30.1, 24.9, 19.1. Anal. Calcd for $C_{18}H_{25}ClN_4O_8$: C, 46.91; H, 5.47; N, 12.16. Found: C, 47.08; H, 5.43; N, 12.21%.

4.3.5. 2-[(2-Bromoethyl)(2-hydroxyethyl)amino]-3,5-dinitro-*N*-[2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]benzamide (12b). From **11** as a yellow solid (80% yield): mp (EtOAc/heptane) 121–123 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.06 (t, J=5.4 Hz, 1H), 8.70 (d, J=2.8 Hz, 1H), 8.33 (d, J=2.8 Hz, 1H), 5.15 (t, J=5.5 Hz, 1H), 4.62 (t, J=3.5 Hz, 1H), 3.78 (m, 2H), 3.63–3.43 (m, 10H), 3.15 (m, 2H), 1.80–1.40 (m, 6H); ^{13}C NMR δ 165.7, 146.6, 143.8, 139.8, 134.0, 128.4, 123.0, 98.0, 64.8, 61.5, 57.9, 54.1, 53.4, 39.5, 30.1, 29.8, 24.9, 19.1. Anal. Calcd for $C_{18}H_{25}BrN_4O_8$: C, 42.79; H, 4.99; N, 11.09. Found: C, 42.82; H, 4.96; N, 11.03%.

4.3.6. 2-[(2-Hydroxyethyl)(2-iodoethyl)amino]-3,5-dinitro-*N*-[2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]benzamide (12c). From **11** as a yellow foam (63% yield); ^{1}H NMR ($CDCl_3$) δ 8.63 (d, J=2.8 Hz, 1H), 8.56 (d, J=2.8 Hz, 1H), 8.24 (br, 1H), 4.54 (m, 1H), 4.48 (m, 1H), 3.93 (m, 2H), 3.84 (m, 1H), 3.70 (m, 6H), 3.54 (m, 1H), 3.30 (m, 2H), 3.18 (m, 2H), 1.90–1.40 (m, 6H); ^{13}C NMR δ 165.7, 146.7, 144.4, 140.9, 134.5, 129.2, 123.5, 101.6, 67.0, 65.4, 58.0, 55.0, 53.7, 40.7, 31.3, 25.1, 21.2, 0.3; HRMS (FAB) calcd for $C_{18}H_{26}IN_4O_8$ $[M+H]^+$ *m/z* 553.0795; found 533.0797.

4.3.7. *tert*-Butyl 2-[(2-hydroxyethyl)(2-chloroethyl)amino]-3,5-dinitrobenzoate (18). From *tert*-butyl 2-chloro-3,5-dinitrobenzoate[9] (**17**) (in DMF) as a yellow solid (63% yield): mp (EtOAc/petroleum ether) 106–107 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.73 (d, J=2.8 Hz, 1H), 8.44 (d, J=2.8 Hz, 1H), 4.58 (t, J=5.4 Hz, 1H), 3.75 (t, J=6.7 Hz, 2H), 3.55 (q, J=5.7 Hz, 2H), 3.46 (t, J=6.7 Hz, 2H), 3.19 (t, J=5.9 Hz, 2H), 1.60 (s, 9H). Anal. Calcd for $C_{15}H_{20}ClN_3O_7$: C, 46.22; H, 5.17; N, 10.78; Cl, 9.10. Found: C, 46.50; H, 5.23; N, 10.66; Cl, 9.05%.

4.3.8. 5-[(2-Chloroethyl)(2-hydroxyethyl)amino]-2,4-dinitrobenzamide (21a). From 5-chloro-2,4-dinitrobenzamide[11] (**20**) (in DMF) as a yellow solid (43% yield): mp (EtOAc/petroleum ether) 131–134 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.48 (s, 1H), 8.10 (s, 1H), 7.75 (s, 1H), 7.35 (s, 1H), 4.76 (vbr s, 1H), 3.83 (t, J=5.9 Hz, 2H), 3.75 (t, J=5.9 Hz, 2H), 3.56 (t, J=5.4 Hz, 2H), 3.36 (t, J=5.5 Hz, 2H); ^{13}C NMR δ 166.3, 147.3, 137.4, 136.9, 135.0, 124.3, 119.8, 58.1, 54.1, 52.5, 41.3. Anal. Calcd for $C_{11}H_{13}ClN_4O_6$: C, 39.71; H, 3.94; N, 16.84. Found: C, 39.79; H, 3.95; N, 16.61%.

4.3.9. Methyl 3-[(2-chloroethyl)(2-hydroxyethyl)amino]-2,6-dinitrobenzoate (24a). From methyl 3-chloro-2,6-dinitrobenzoate[12] (**23**) (in DMF) as a yellow oil (63% yield);

^{1}H NMR [$(CD_3)_2SO$] δ 8.25 (d, J=9.7 Hz, 1H), 7.63 (d, J=9.7 Hz, 1H), 4.79 (t, J=5.2 Hz, 1H), 3.87 (s, 3H), 3.80 (t, J=5.5 Hz, 2H), 3.72 (t, J=5.5 Hz, 2H), 3.54 (q, J=5.3 Hz, 2H), 3.36 (t, J=5.5 Hz, 2H); ^{13}C NMR δ 163.2, 147.8, 136.54, 134.5, 128.0, 126.3, 122.0, 58.1, 54.7, 53.8, 53.8, 41.3. HRMS (FAB) calcd for $C_{12}H_{15}{}^{35}ClN_3O_7$ $[M+H]^+$ m/z 347.0520; found 347.0521.

4.3.10. Methyl 3-[(2-bromoethyl)(2-hydroxyethyl)-amino]-2,6-dinitrobenzoate (24b). From **23** (in DMF) as a yellow oil (36% yield); ^{1}H NMR [$(CD_3)_2SO$] δ 8.24 (d, J=9.4 Hz, 1H), 7.62 (d, J=9.4 Hz, 1H), 4.79 (vbr s, 1H), 3.78 (s, 3H), 3.77 (t, J=6.2 Hz, 2H), 3.65 (t, J=6.1 Hz, 2H), 3.53 (t, J=5.4 Hz, 2H), 3.36 (t, J=5.3 Hz, 2H); ^{13}C NMR δ 163.2, 147.6, 136.4, 134.5, 128.1, 126.3, 121.9, 58.1, 53.8, 53.6, 52.5, 29.7. HRMS (FAB) calcd for $C_{12}H_{15}{}^{79}BrN_3O_7$ $[M+H]^+$ m/z 392.0093; found 392.0093.

4.3.11. Methyl 3-[(2-hydroxyethyl)(2-(iodoethyl)amino]-2,6-dinitrobenzoate (24c). From **23** (in DMF) as a yellow gum (58% yield); ^{1}H NMR [$(CD_3)_2SO$] δ 8.24 (d, J=9.7 Hz, 1H), 7.59 (d, J=9.7 Hz, 1H), 4.79 (t, J=5.2 Hz, 1H), 3.87 (s, 3H), 3.71 (t, J=7.2 Hz, 2H), 3.53 (q, J=5.1 Hz, 2H), 3.40–3.33 (m, 4H); ^{13}C NMR δ 163.2, 147.2, 136.3, 134.5, 128.1, 126.3, 121.8, 58.1, 53.8, 53.5 (2), 2.2. HRMS (FAB) calcd for $C_{12}H_{15}IN_3O_7$ $[M+H]^+$ m/z 439.9955; found 439.9960.

4.3.12. *tert*-Butyl 3-[(2-chloroethyl)(2-hydroxyethyl)-amino]-2,6-dinitrobenzoate (27). From **26** (in DMF) as a yellow gum (51% yield); ^{1}H NMR [$(CD_3)_2SO$] δ 8.20 (d, J=9.6 Hz, 1H), 7.58 (d, J=9.6 Hz, 1H), 4.78 (t, J=5.2 Hz, 1H), 3.81–3.74 (m, 2H), 3.74–3.66 (m, 2H), 3.53 (q, J=5.3 Hz, 2H), 3.34 (t, J=5.5 Hz, 2H), 1.50 (s, 9H); ^{13}C NMR δ 160.9, 147.4, 137.1, 134.8, 127.8, 126.6, 121.7, 84.5, 58.1, 53.8, 52.5, 41.2, 27.0 (3). HRMS (FAB) calcd for $C_{15}H_{21}{}^{35}ClN_3O_7$ $[M+H]^+$ m/z 390.1068; found 390.1063.

4.4. Preparation of di(*tert*-butyl) 2-({2-[(2-chloroethyl)(2-hydroxyethyl)amino]-3,5-dinitrobenzoyl}-amino)ethyl phosphate (15): example of 2-[(2-chloroethyl)amino]ethanol reaction

A suspension of 2-[(2-chloroethyl)amino]ethanol hydrochloride[7,8] (1.0 g, 3.56 mmol), Et_3N (2.0 mL, 14.3 mmol) in 1,4-dioxane (50 mL) was cooled in an ice bath, and **14** (478 mg, 0.99 mmol) was added. The mixture was stirred at room temperature overnight, then water (100 mL) was added and the mixture was extracted with EtOAc (3×100 mL). The combined organic layers were washed with water, dried and concentrated under reduced pressure, then passed through a short column of silica gel, eluting with heptane/EtOAc (gradient from 1:1 to 0:1) to give **15** as a yellow foam (510 mg, 90%); ^{1}H NMR [$(CD_3)_2SO$] δ 8.94 (t, J=5.5 Hz, 1H), 8.71 (d, J=2.8 Hz, 1H), 8.34 (d, J=2.8 Hz, 1H), 5.13 (t, J=5.6 Hz, 1H), 4.02 (m, 3H), 3.77 (m, 2H), 3.55 (m, 6H), 3.14 (m, 2H), 1.42 (s, 18H); ^{13}C NMR δ 266.0, 146.9, 143.7, 139.6, 133.6, 128.3, 123.2, 81.6, 64.0, 59.6, 54.3, 53.3, 41.5, 29.3. HRMS (FAB) calcd for $C_{21}H_{35}{}^{35}ClN_4O_{10}S$ $[M+H]^+$ m/z 569.1779; found 569.1772.

4.5. Preparation of 2-[(2-bromoethyl)-2,4-dinitro-6-({[2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]amino}carbonyl)-anilino]ethyl methanesulfonate (13b): example of mesylation with methanesulfonic anhydride

A solution of **12b** (98% pure) (51.5 g, 102 mmol) in dry CH_2Cl_2 (300 mL) containing dry pyridine (21.4 mL, 265 mmol) was cooled to 0 °C and treated over a 5 min period with a solution of methanesulfonic anhydride (97%, 23.8 g, 137 mmol) in CH_2Cl_2 (80 mL). The mixture was stirred at 20 °C for 1.5 h, then 10% aqueous $KHCO_3$ (200 mL) was added and the mixture was stirred vigorously for 30 min, then concentrated under reduced pressure to remove all of the CH_2Cl_2. The oil that precipitated was separated from the remaining liquid, rinsed with water and dissolved in EtOAc (300 mL). The EtOAc solution was washed with water (2×), dried and filtered through a short column of silica gel. The eluate was concentrated to 100 mL and shaken with excess hexane (250 mL). The precipitated oil was separated from the mother liquor and dried to give **13b** as yellow foam (56.4 g, 95%) (98% pure); ^{1}H NMR ($CDCl_3$) δ 8.62 (d, J=2.8 Hz, 1H), 8.53 (d, J=2.8 Hz, 1H), 7.29 (br, 1H), 4.55 (m, 1H), 4.37 (m, 2H), 3.90 (m, 2H), 3.78 (m, 1H), 3.73–3.49 (m, 9H), 3.01 (s, 3H), 1.90–1.48 (m, 6H); ^{13}C NMR δ 165.0, 145.9, 145.6, 142.1, 136.4, 128.5, 122.8, 100.5, 67.1, 66.3, 64.2, 55.4, 52.1, 40.5, 37.5, 31.0, 29.0, 25.2, 20.6; HRMS (FAB) calcd for $C_{19}H_{28}{}^{79}BrN_4O_{10}S$ $[M+H]^+$ m/z 583.0710; found 583.0712. The following compounds were prepared similarly.

4.5.1. 2-[2-(Aminocarbonyl)(2-bromoethyl)-4,6-dinitro-anilino]ethyl methanesulfonate (10b). From **9b** as a yellow solid (85% yield): mp (EtOAc/petroleum ether) 153–154 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (d, J=2.8 Hz, 1H), 8.33 (d, J=2.8 Hz, 1H), 8.19 (s, 1H), 7.99 (s, 1H), 4.29 (m, 2H), 3.60 (m, 2H), 3.49 (m, 4H), 3.14 (s, 3H); ^{13}C NMR δ 167.11, 145.75, 146.37, 140.92, 136.12, 127.24, 122.20, 67.53, 54.41, 51.16, 36.46, 29.73. Anal. Calcd for $C_{12}H_{15}BrN_4O_8S$: C, 31.72; H, 3.33; N, 12.34; Br, 17.35. Found: C, 32.00; H, 3.41; N, 12.22; Br, 17.71%.

4.5.2. 2-[2-(Aminocarbonyl)(2-iodoethyl)-4,6-dinitroanilino]ethyl methanesulfonate (10c). From **9c** as a yellow solid (64% yield): mp (EtOAc/petroleum ether) 144–147 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.73 (d, J=2.8 Hz, 1H), 8.33 (d, J=2.8 Hz, 1H), 8.16 (s, 1H), 7.97 (s, 1H), 4.28 (m, 2H), 3.49 (m, 4H), 3.32 (m, 2H), 3.14 (s, 3H); ^{13}C NMR δ 167.1, 145.4, 145.3, 140.9, 136.1, 127.2, 122.1, 67.5, 55.6, 50.7, 36.5, 2.6. Anal. Calcd for $C_{12}H_{15}IN_4O_8S$: C, 28.75; H, 3.01; N, 11.16. Found: C, 29.29; H, 3.37; N, 10.73%.

4.5.3. 2-[(2-Iodoethyl)-2,4-dinitro-6-({[2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]amino}carbonyl)anilino]ethyl methanesulfonate (13c). From **12c** as a yellow foam (99% yield); ^{1}H NMR ($CDCl_3$) δ 8.62 (d, J=2.8 Hz, 1H), 8.52 (d, J=2.8 Hz, 1H), 7.21 (br, 1H), 4.55 (m, 1H), 4.35 (m, 2H), 3.90 (m, 2H), 3.80 (m, 1H), 3.70–3.5 (m, 7H), 3.30 (m, 2H), 3.00 (s, 3H), 1.90–1.50 (m, 6H); ^{13}C NMR δ 165.0, 145.7, 142.0, 136.3, 128.2, 122.8, 100.6, 66.9, 66.5, 64.3, 56.7, 51.6, 40.5, 37.6, 31.0, 25.2, 20.6, 0.7; HRMS (FAB) calcd for $C_{19}H_{28}IN_4O_{10}S$ $[M+H]^+$ m/z 631.0571; found 631.0575.

4.5.4. 2-[5-(Aminocarbonyl)(2-bromoethyl)-2,4-dinitroanilino]ethyl methanesulfonate (22b). From **21b** as a yellow solid (96% yield), identical (^{1}H NMR, ^{13}C NMR, HPLC) with compound prepared previously by an alternate route.[11]

4.5.5. 2-[5-(Aminocarbonyl)(2-iodoethyl)-2,4-dinitroanilino]ethyl methanesulfonate (22c). From **21c** as a yellow solid (97% yield), identical (^{1}H NMR, ^{13}C NMR, HPLC) with compound prepared previously by an alternate route.[11]

4.5.6. Methyl 3-[(2-bromoethyl)-[2-[(methylsulfonyl)oxy]ethyl]amino]-2,6-dinitrobenzoate (25b). From **24b** as a yellow oil (77% yield): ^{1}H NMR [$(CD_3)_2SO$] δ 8.24 (d, J=9.4 Hz, 1H), 7.62 (d, J=9.4 Hz, 1H), 4.79 (vbr s, 1H), 3.78 (s, 3H), 3.77 (t, J=6.2 Hz, 2H), 3.65 (t, J=6.1 Hz, 2H), 3.53 (t, J=5.4 Hz, 2H), 3.36 (t, J=5.3 Hz, 2H); ^{13}C NMR δ 163.2, 147.6, 136.4, 134.5, 128.1, 126.3, 121.9, 58.1, 53.8, 53.6, 52.5, 29.7. HRMS (FAB) calcd for $C_{12}H_{15}{}^{79}BrN_3O_7$ [M+H]$^+$ m/z 392.0093; found 392.0093.

4.5.7. Methyl 3-[(2-iodoethyl)[2-[(methylsulfonyl)oxy]ethyl]amino]-2,6-dinitrobenzoate (25c). From **24c** as a yellow gum (92% yield): ^{1}H NMR [$(CD_3)_2SO$] δ 8.30 (d, J=9.6 Hz, 1H), 7.72 (d, J=9.6 Hz, 1H), 4.30 (t, J=5.2 Hz, 2H), 3.88 (s, 3H), 3.71 (t, J=5.2 Hz, 2H), 3.62 (t, J=7.1 Hz, 2H), 3.36 (t, J=7.1 Hz, 2H), 3.14 (s, 3H); ^{13}C NMR δ 162.9, 146.9, 138.0, 136.2, 128.3, 125.9, 123.4, 66.7, 53.9, 53.7, 49.4, 36.6, 2.4. HRMS (FAB) calcd for $C_{13}H_{17}IN_3O_9S$ [M+H]$^+$ m/z 517.9730; found 517.9734.

4.5.8. *tert*-Butyl 3-[(2-chloroethyl)[2-[(methylsulfonyl)oxy]ethyl]amino]-2,6-dinitrobenzoate (28). From **27** as a yellow gum (88% yield): ^{1}H NMR [$(CD_3)_2SO$] δ 8.26 (d, J=9.5 Hz, 1H), 7.70 (d, J=9.5 Hz, 1H), 4.30 (t, J=5.2 Hz, 2H), 3.76 (t, J=6.2 Hz, 2H), 3.68 (t, J=5.2 Hz, 2H), 3.62 (t, J=6.2 Hz, 2H), 3.15 (s, 3H), 1.50 (s, 9H); ^{13}C NMR δ 160.6, 147.0, 138.9, 136.7, 128.0, 126.1, 123.5, 84.8, 66.8, 52.9, 50.0, 41.4, 36.6, 27.0 (3). HRMS (FAB) calcd for $C_{16}H_{23}{}^{35}ClN_3O_9S$ [M+H]$^+$ m/z 468.0844; found 468.0829.

4.6. 2-[(2-Chloroethyl)-2,4-dinitro-6-({[2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]amino}carbonyl)anilino]ethyl methanesulfonate (13a): example of mesylation with MsCl

A stirred solution of **12a** (210 mg, 0.46 mmol) in dry CH_2Cl_2 (10 mL) was cooled in an ice bath at 0 °C and then treated with Et_3N (0.2 mL, 1.44 mmol), followed by MsCl (0.10 mL, 1.29 mmol) dropwise. The mixture was stirred for 10 min at 0 °C, then satd $NaHCO_3$ (10 mL) was added, and after a further 30 min the aqueous phase was extracted with CH_2Cl_2 (2×20 mL). The combined organic phases were dried, concentrated under reduced pressure and filtered through a short column, eluting with EtOAc/petroleum ether (1:1) to give **13a** (240 mg, 98%) as a yellow foam; ^{1}H NMR ($CDCl_3$) δ 8.62 (d, J=2.8 Hz, 1H), 8.55 (d, J=2.8 Hz, 1H), 7.31 (t, J=5.4 Hz, 1H), 4.55 (m, 1H), 4.37 (m, 2H), 3.90 (m, 2H), 3.70 (m, 7H), 3.53 (m, 3H), 3.01 (s, 3H), 1.86–1.45 (m, 6H); ^{13}C NMR δ 165.1, 146.0, 145.6, 142.1, 136.4, 128.7, 122.8, 100.4, 67.2, 66.2, 64.1, 55.2, 52.3, 41.7, 40.5, 37.5, 30.9, 25.2, 20.5; HRMS (FAB) calcd for $C_{19}H_{28}{}^{35}ClN_4O_{10}S$ [M+H]$^+$ m/z 539.1215; found 539.1206. The following compounds were similarly prepared.

4.6.1. 2-[2-(Aminocarbonyl)(2-chloroethyl)-4,6-dinitroanilino]ethyl methanesulfonate (10a). From **9a** (in THF) as a yellow solid (98% yield): mp (EtOAc/petroleum ether) 155–157 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (d, J=2.7 Hz, 1H), 8.34 (d, J=2.7 Hz, 1H), 8.19 (s, 1H), 7.99 (s, 1H), 4.29 (m, 2H), 3.73 (m, 2H), 3.48 (m, 4H), 3.15 (s, 3H); ^{13}C NMR δ 167.11, 145.98, 146.34, 140.84, 136.05, 127.26, 122.22, 67.49, 54.35, 51.34, 41.36, 36.46. Anal. Calcd for $C_{12}H_{15}ClN_4O_8S$: C, 35.1; H, 3.7; N, 13.7; Cl, 8.5. Found: C, 35.7; H, 3.9; N, 13.6; Cl, 8.7%.

4.6.2. 2-[(2-Chloroethyl)-2-(6-*tert*-butoxy-8,8-dimethyl-6-oxido-5,7-dioxa-2-aza-6-phosphanon-1-anoyl)-4,6-dinitroanilino]ethyl methanesulfonate (16). From **15** as a yellow foam (68% yield); ^{1}H NMR [$(CD_3)_2SO$] δ 8.94 (t, J=5.6 Hz, 1H), 8.75 (d, J=2.8 Hz, 1H), 8.34 (d, J=2.8 Hz, 1H), 4.28 (t, J=5.4 Hz, 2H), 4.02 (q, J=6.2 Hz, 2H), 3.74–3.43 (m, 8H), 3.13 (s, 3H), 1.43 (s, 18H); ^{13}C NMR δ 265.6, 146.2, 145.3, 140.8, 135.6, 127.5, 122.4, 81.7, 67.5, 64.2, 54.3, 51.3, 41.4, 36.5, 29.5. HRMS (FAB) calcd for $C_{22}H_{37}{}^{35}ClN_4O_{12}PS$ [M+H]$^+$ m/z 647.1555; found 647.1555.

4.6.3. *tert*-Butyl 2-[(2-chloroethyl)[2-[(methylsulfonyl)oxy]ethyl]amino]-3,5-dinitrobenzoate (19). From **18** as a yellow solid (89% yield): mp (EtOAc/petroleum ether) 95–96 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.80 (d, J=2.8 Hz, 1H), 8.52 (d, J=2.8 Hz, 1H), 4.29 (t, J=5.5 Hz, 2H), 3.73 (t, J=6.8 Hz, 2H), 3.49 (t, J=5.5 Hz, 2H), 3.45 (t, J=6.8 Hz, 2H), 3.12 (s, 3H), 1.60 (s, 9H). Anal. Calcd for $C_{16}H_{22}ClN_3O_9S$: C, 41.07; H, 4.74; N, 8.98; Cl, 7.58. Found: C, 41.25; H, 4.83; N, 8.93; Cl, 7.71%.

4.6.4. 2-[5-(Aminocarbonyl)(2-chloroethyl)-2,4-dinitroanilino]ethyl methanesulfonate (22a). From **21a** as a yellow solid (74% yield), identical (^{1}H NMR, ^{13}C NMR, HPLC) with compound prepared previously by an alternate route.[11]

4.6.5. Methyl 3-[(2-chloroethyl)[2-[(methylsulfonyl)oxy]ethyl]amino]-2,6-dinitrobenzoate (25a). From **24a** as a yellow oil (80% yield); ^{1}H NMR [$(CD_3)_2SO$] δ 8.31 (d, J=9.5 Hz, 1H), 7.74 (d, J=9.5 Hz, 1H), 4.31 (t, J=5.2 Hz, 2H), 3.88 (s, 3H), 3.78 (t, J=6.3 Hz, 2H), 3.70 (t, J=5.2 Hz, 2H), 3.64 (t, J=5.9 Hz, 2H), 3.14 (s, 3H); ^{13}C NMR δ 163.0, 147.5, 138.1, 136.2, 128.3, 125.9, 123.6, 66.7, 54.8, 53.9, 49.8, 36.6. HRMS (FAB) calcd for $C_{13}H_{17}{}^{35}ClN_3O_9S$ [M+H]$^+$ m/z 426.0374; found 426.0372.

4.6.6. 2-[5-(Aminocarbonyl)(2-bromoethyl)-2,4-dinitroanilino]ethyl methanesulfonate (21b). A slurry of **21a** (110 mg, 0.33 mmol) in dry 3-methyl-2-butanone (15 mL) and LiBr (2.0 g) was heated under reflux for 5 h. The reaction mixture was cooled, then water (50 mL) was added, and the mixture was extracted with EtOAc (3×50 mL). The combined organic layer was dried and concentrated under pressure, then passed through a short column of silica gel, eluting with heptane/EtOAc (1:1), to give **21b** (107 mg, 86%) as a yellow solid: mp (EtOAc/petroleum ether) 144–147 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.48 (s, 1H), 8.10 (s, 1H),

7.76 (s, 1H), 7.33 (s, 1H), 4.76 (vbr s, 1H), 3.80 (t, J=5.9 Hz, 2H), 3.69 (t, J=5.9 Hz, 2H), 3.56 (t, J=5.4 Hz, 2H), 3.36 (t, J=5.5 Hz, 2H); ^{13}C NMR δ 166.2, 147.1, 137.8, 137.4, 135.1, 124.3, 119.7, 58.1, 54.0, 52.5, 29.9. Anal. Calcd for $C_{11}H_{13}BrN_4O_6$: C, 35.03; H, 3.47; N, 14.86. Found: C, 39.79; H, 3.95; N, 16.61%.

4.6.7. 2-[5-(Aminocarbonyl)(2-iodoethyl)-2,4-dinitroanilino]ethyl methanesulfonate (21c). A slurry of **21a** (115 mg, 0.35 mmol) in dry 3-methyl-2-butanone (15 mL) and NaI (2.0 g) was heated under reflux for 5 h. The reaction mixture was cooled, then water (50 mL) was added, and the mixture was extracted with EtOAc (3×50 mL). The combined organic layer was dried and concentrated under pressure, then passed through a short column of silica gel, eluting with heptane/EtOAc (1:1), to give **21c** (100 mg, 68%) as a yellow solid: mp (EtOAc/petroleum ether) 144–147 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.47 (s, 1H), 8.10 (s, 1H), 7.76 (s, 1H), 7.30 (s, 1H), 4.76 (t, J=5.3 Hz, 1H), 3.74 (t, J=7.1 Hz, 2H), 3.54 (m, 2H), 3.38 (m, 4H); ^{13}C NMR δ 166.2, 146.7, 137.4, 136.8, 135.0, 124.2, 119.6, 58.1, 53.9, 53.4, 2.5. Anal. Calcd for $C_{11}H_{13}IN_4O_6$: C, 31.15; H, 3.09; N, 13.21. Found: C, 39.79; H, 3.95; N, 16.61%.

4.6.8. 2-[(2-Chloroethyl)-2-[[(2-hydroxyethyl)amino]carbonyl]-4,6-dinitroanilino]ethyl methanesulfonate (22) (Scheme 3). A solution of **19** (646 mg, 1.38 mmol) in TFA (3 mL) was stirred at room temperature for 2 h, then concentrated almost to dryness under reduced pressure. It was then partitioned between EtOAc and water, and the organic phase was dried and evaporated under reduced pressure. Trituration of the oil with i-Pr_2O and recrystallisation of the resulting solid from EtOAc/hexane gave 2-[(2-chloroethyl)-[(2-[(methylsulfonyl)oxy]ethyl]amino]-3,5-dinitrobenzoic acid (**21**) (501 mg, 88%) as a yellow solid: mp 134–135 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 14.2 (vbr, 1H), 8.81 (d, J=2.8 Hz, 1H), 8.60 (d, J=2.8 Hz, 1H), 4.28 (t, J=5.4 Hz, 2H), 3.71 (t, J=6.8 Hz, 2H), 3.53–3.42 (m, 4H), 3.12 (s, 3H). Anal. Calcd for $C_{12}H_{14}ClN_3O_9S$: C, 35.00; H, 3.43; N, 10.20; Cl, 8.61. Found: C, 35.27; H, 3.46; N, 10.07; Cl, 8.89%.

A suspension of **21** (300 mg, 0.73 mmol) in CH_2Cl_2 (5 mL) was treated with oxalyl chloride (0.12 mL, 1.40 mmol) and DMF (one drop), and stirred at room temperature for 1.5 h. Evaporation of the volatiles under reduced pressure below 30 °C, followed by azeotroping with benzene, gave the crude acid chloride. A solution of this in DMF (1 mL) was added dropwise to a stirred solution of 2-aminoethanol (67 mg, 1.10 mmol) and DIPEA (142 mg, 1.10 mmol) in dioxane/THF (1:1) (2 mL) at −5 °C. The mixture was stirred at 0 °C for a further 5 min, then poured into 0.1 N aqueous MsOH (15 mL) and extracted with EtOAc (2×10 mL). The combined organic phase was washed with water, dried and evaporated under reduced pressure. Chromatography on silica gel, eluting with EtOAc, followed by precipitation of the product from a CH_2Cl_2 solution with hexane, gave **22** (272 mg, 82%) as a yellow gum, identical (^{1}H NMR, HPLC) with compound prepared previously by an alternative route.[12]

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