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CEPHALOSPORIN NITROGEN MUSTARD CARBAMATE PRODRUGS FOR "ADEPT".

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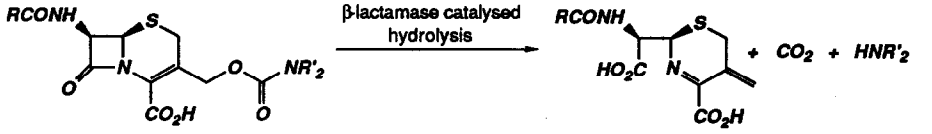
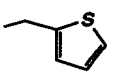
Abstract: A series of cephalosporin carbamates, **(6a-d)** and **(7)**, functionalised with masked nitrogen mustards has been synthesised. These are potential prodrugs for interaction with a monoclonal antibody-β-lactamase tumour targeting system. The β-lactamase catalysed hydrolysis of **(6a-d)** and **(7)** to generate free nitrogen mustards has been characterised.

Conventional anti-cancer chemotherapy is frequently associated with serious side effects. Indeed, a number of promising cytotoxic agents have failed to progress to clinical evaluation due to their lack of selectivity over normal cells. Earlier attempts to capitalise on using enzymes endogenous to tumour tissue to deliver a cytotoxic agent via cleavage of a prodrug did result in tumour regression in animal models [1]. However, many such enzymes are so similar to those of normal tissues that no real benefit emerged. The ready availability of monoclonal antibodies which target specifically to tumour tissue allows the concept of Antibody Directed Enzyme Prodrug Therapy (ADEPT) to be realised [2]. This consists of i) localisation of an antibody-enzyme conjugate or chimeric construct to a tumour; ii) administration of a relatively non-toxic substrate for the enzyme (prodrug) which can liberate on cleavage a cytotoxic agent in the vicinity of the tumour.

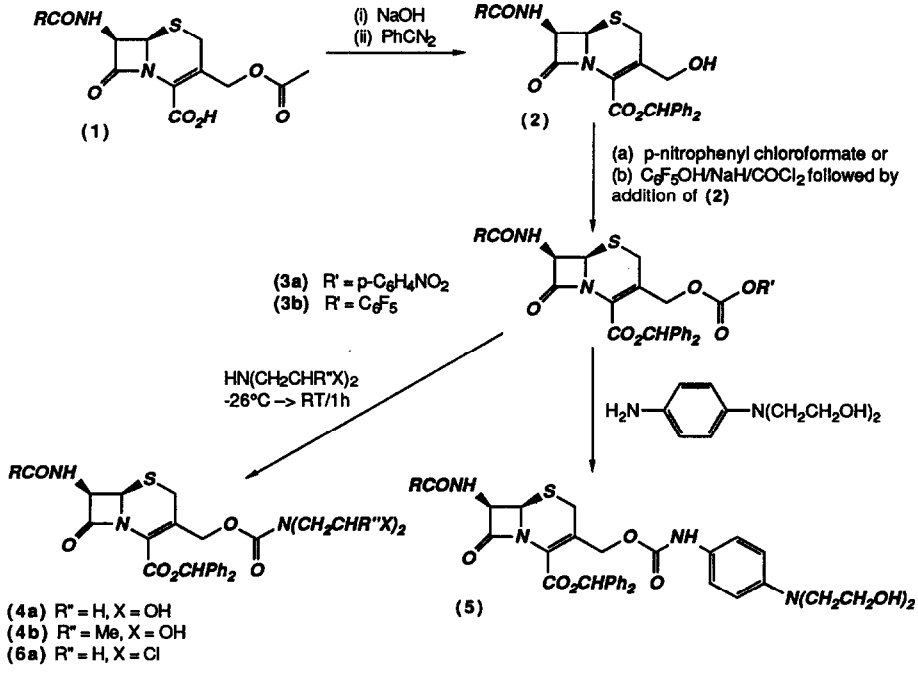
In this context, β-lactamases are of particular interest for the following reasons: i) they are small, soluble monomeric enzymes without a mammalian counterpart; ii) they not only hydrolyse penicillins but also cephalosporins, via a mechanism involving expulsion of a 3' leaving group and iii) the enzyme is particularly tolerant to a wide variety of substituents at this position [3]. Accordingly, we have devised a series of cephalosporin 3' carbamate derivatives which allow the release of a cytotoxic agent on exposure to β-lactamase as shown below. In the case of the nitrogen mustards, which owe their cytotoxicity to alkylation of DNA via



HNR'_2 = $HN(CH_2CH_2Cl)_2$, $HN(CH_2CHMeCl)_2$, $HN(CH_2CH_2Br)_2$, $HN(CH_2CH_2Cl)_2$
$HN(CH_2CHMeBr)_2$, $H_2NC_6H_4N(CH_2CH_2Cl)_2$; R = 

an aziridinium intermediate, such carbamates should be relatively innocuous.

The chemical realisation of such species was not trivial, involving careful choice of protecting groups to prevent lactonisation and reagents which minimised the isomeristion of Δ3 to Δ2 cephalosporins [4]. Having examined both p-nitrobenzyl and t-butyl esters to protect the 4-carboxylate we eventually settled upon the diphenyl methyl ester.

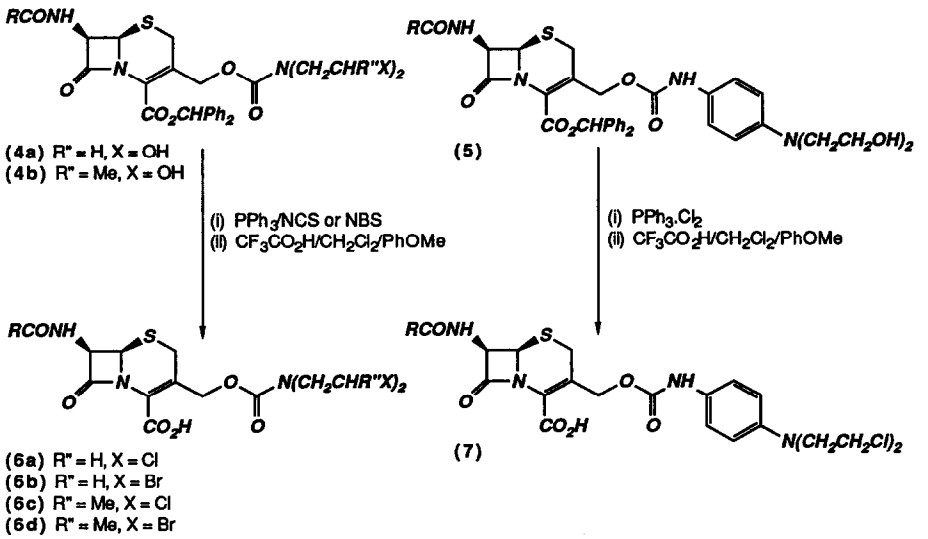


Selective hydrolysis of the 3' acetate of cephalothin (1) [5] followed by esterification with diphenyldiazomethane gave the corresponding 4-ester (2) [6]. Treatment of (2) with carbonyldiimidazole [7], phosgene, diphosgene [8] or triphosgene [9] followed by diethanolamine failed to provide the desired products. However reaction of (2) with p-nitrophenyl chloroformate (1eq., 1eq. pyridine in THF) [10] gave the isolable intermediate carbonate (3a) as a white solid. Upon exposure of (3a) to diethanolamine (1eq. in pyridine at 0°C, 2hrs.) a mixture of Δ2 and Δ3 isomers was formed in favour of the undesired Δ2 isomer (3:1 by 300 MHz.NMR). In order to obtain satisfactory formation of the desired Δ3 carbamate it was necessary to isolate and purify the pentafluorophenyl carbonate (3b). Chloroformate formation in situ ($C_6F_5OH/NaH/COCl_2$ in THF) followed by addition of (2) in the presence of pyridine (1eq.) gave (3b) (70%). Exposure to diethanolamine (1eq., -26°C in DMF) yielded the desired Δ3 isomer (4a) as the major product. The ratios of Δ2 to Δ3 isomers for a variety of amines (best conditions) are summarised in table 1.

Table 1: *Acylation of carbonate (3b) with various secondary amines.*

Amine	*Product*	*Solvent*	*Δ3:Δ2*	*Yield*
$HN(CH_2CH_2OH)_2$	(4a)	DMF	34:1	95%
$HN(CH_2CHMeOH)_2$	(4b)	DMF	26:1	95%
$H_2NC_6H_4N(CH_2CH_2OH)_2$	(5)	$DMSO/C_5H_5N$ (1:1)	16:1	83%
$HN(CH_2CH_2Cl)_2.HCl$	(4c)	C_5H_5N	28:1	77%

Diethanolamine cephems (4a,b) were converted to their corresponding halo derivatives (2.2 eq. PPh_3, NCS or NBS in THF)[11] with complete retention of double bond integrity by 300 MHz. NMR. The arylaminodiol (5) was chlorinated (2.2 eq. $PPh_3.Cl_2$ complex in dry pyridine, 0°C) [12] again retaining the Δ3 double bond. Deprotection (TFA, CH_2Cl_2, Anisole) gave (6a), (6b), (6c), (6c) or (7) after purification by reverse phase HPLC.



Examination of the prodrug principle was achieved by exposure of the cephalosporin mustards to the class C β-lactamase from *Enterobacter clocae* P99 [13]. The kinetic parameters for β-lactamase catalysed hydrolysis of each prodrug are summarised in Table 2. In the case of prodrug (6a), enzyme catalysed hydrolysis was also monitored by ^1H NMR at 300 MHz. Liberation of free *bis*-chloroethylamine was concomitant with β-lactam hydrolysis. The toxicity ratios (mouse lymphoma cells) of these nitrogen mustard prodrugs in the presence and absence of β-lactamase along with other biological data will be reported elswhere.

Table 2: *Michaelis-Menton parameters* [14] *for hydrolysis of cephem carbamate prodrugs by* Enterobacter cloacae *P99 β-lactamase.*

Prodrug	K_M (μM)	k_{cat} (s^{-1})	k_{cat}/K_M ($\mu M^{-1}s^{-1}$)
Ceph-CO-N(CH_2CH_2Cl)$_2$ **(6a)**	108.5	510	4.7
Ceph-CO-N($CH_2CHMeCl$)$_2$ **(6c)**	182	130	0.7
Ceph-CO-N(CH_2CH_2Br)$_2$**(6b)**	71.5	150	2.1
Ceph-CO-N($CH_2CHMeBr$)$_2$ **(6d)**	40	120	3.0
Ceph-CO-HNC_6H_4N(CH_2CH_2Cl)$_2$ **(7)**	142.5	181	1.3

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14. Kinetic measurements were made in 0.1 M phosphate buffer, pH 7.0, 37 °C. Hydrolysis was monitored by absorbance change at 265 nM. Extinction coefficients were determined from absorbance changes after complete hydrolysis. Kinetic parameters were obtained from either complete progress curves using half-time analysis of the integrated Michaelis-Menton equation (C. W. Wharton and R. J. Szawelski, *Biochem. J.*, 203, 351 (1982)) or initial rates over a range of substrate concentrations using the program "Enz Fitter" of Dr. Robin J. Leatherbarrow.
15. For an alternative cephalosporin based approach to "ADEPT" see: T. A. Shepherd, L. N. Jungheim, D. L. Meyer and J. Starling, *BioMed. Chem. Lett.*, 1, 21 (1991)

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