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# SYNTHESIS OF AN N-MUSTARD PRODRUG

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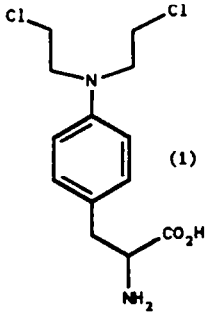
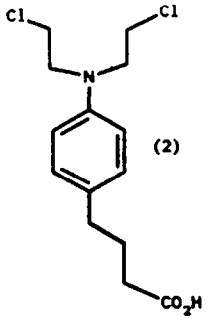
Abstract: We describe the synthesis of the novel N-mustard prodrug 4-[(2-chloroethyl)(2-mesyloxyethyl)amino]benzoyl-L-glutamic acid, which is designed for activation by tumour localising antibody-carboxypeptidase conjugates.

Nitrogen mustards were first used as agents for cancer chemotherapy in the 1940s, but although compounds like melphalan (1) and chlorambucil (2) are effective against a wide range of tumour types, they have little selective toxicity[1]. This means that normal cells as well as tumour cells are damaged which is not only inefficient but also leads to toxic side-effects for the patient.

One possible strategy for ensuring more efficient tumour localisation of a cytotoxic drug involves the use of an antibody (specific for a particular tumour cell type) conjugated to an enzyme that will activate a relatively inactive prodrug. The antibody-enzyme conjugate is designed to localise at the tumour prior to administration of the prodrug which will only be activated once it reaches this site. This method of drug targeting has been shown to be effective in animal models[2], and our aim was to design a viable synthesis of the prodrug (3) which can be activated by treatment with the bacterial enzyme carboxypeptidase G2[3].




(1)

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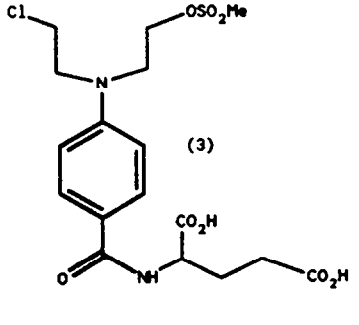
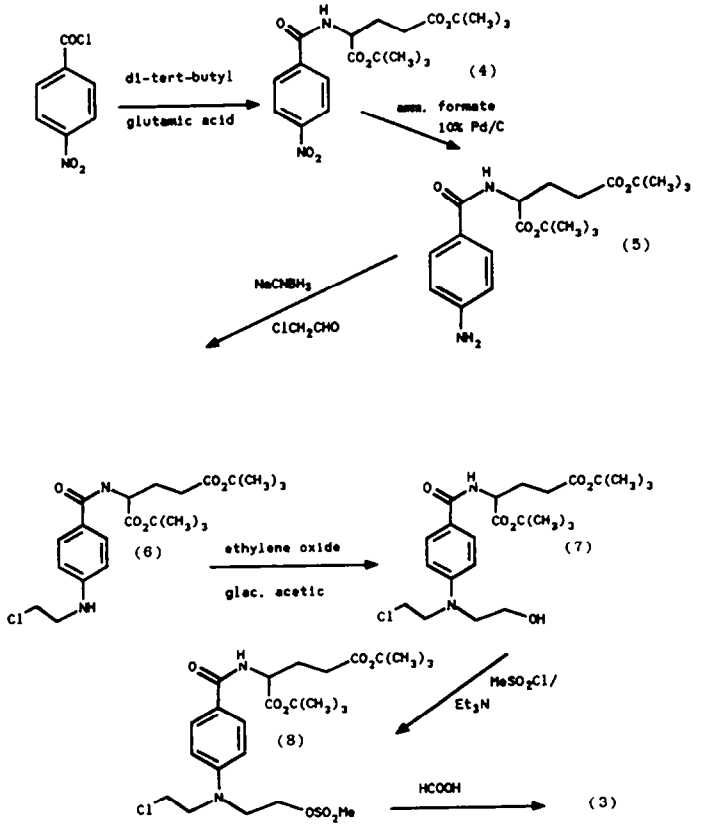



(3)

The synthetic route is shown in the SCHEME, and several points are worthy of note. Firstly, reduction of the nitro compound (4) using transfer hydrogenation[4] was particularly efficient. Secondly, the reductive amination involving amine (5) and chloroethanal allowed a facile monofunctionalisation of the amine, thus providing access to the differentially substituted N-mustards (7) and (8). The stereochemical purity of (7) was established through formation of its 2-methoxy-2-trifluoromethyl acetate (Mosher's ester)[5]. Finally, treatment of compound (8) with formic acid furnished the desired N-mustard prodrug (3). This compound is more chemotherapeutically effective than either the bis-chloroethyl derivative or the bis-mesyloxyethyl derivative, and is more stable than the latter compound.

This synthetic route has now been used (with minimal modification) for the production of prodrug (3) on the kilogramme scale, and this compound is presently undergoing phase I clinical evaluation.



## EXPERIMENTAL

I.r. spectra were recorded with a Perkin-Elmer 881 double beam grating spectrophotometer; n.m.r. spectra were recorded with a Perkin-Elmer R34 (220MHz) instrument or with a Varian T-60 (60MHz) instrument, using tetramethylsilane as internal standard; flash chromatography was performed using Crosfield Sorbsil C60 (40-60 m); solvents were purified according to Perrin, and petrol refers to petroleum ether b.pt. range 40-60°.

Di-tert-butyl 4-nitrobenzoyl-L-glutamate (4)

Triethylamine (3.5ml,25mmol) was added to a cooled (ice/NaCl) solution of 5.3g (20 mmol) glutamic acid-di-tert-butyl ester in 70 ml dry dichloromethane. At that temperature 3.7g (20 mmol) p-nitrobenzoylchloride in 60 ml dry dichloromethane was added dropwise and the solution was stirred overnight at room temperature.

The solution was washed with water (5x), dried over magnesium sulphate and evaporated to form an orange oil.

$^1$H-NMR ($CDCl_3$, 60MHz): δ = 1.43 (s, $3CH_3$), 1.5 (s, $3CH_3$), 1.87-2.6 (m, 4H, $CH_2$), 4.4-4.83 (m, 1H, N-C-H), 7.2-7.63 (d, broad, 1H, N-H), 7.8-8.33 (m, 4H, arom. H) ppm.

Di-tert-butyl 4-aminobenzoyl-L-glutamate (5)

Palladium on charcoal (1.1g of 10%) and 6.6g (0.105 mol) ammoniumformate were added to the cooled (ice-water) solution of the crude compound 4 in 60 ml dry methanol (exothermic reaction). The reaction mixture was stirred at room temperature for 1/2 h. During that time the product precipitated. Dichloromethane was added to dissolve the precipitate and the catalyst was removed by filtration through a celite pad. The filtrate was evaporated and the residue was taken up in water and dichloromethane. The phases were separated, and the organic phase was washed with water, dried over magnesium sulphate and evaporated to give 3 as colourless precipitate. Yield 6.55g (87%) of 5, mp. 130°C (after recrystallisation from ethanol/petrolether (40-60°C).

$^1$H-NMR ($CDCl_3$, 60 MHz): δ = 1.41 (s, $3CH_3$), 1.48 (s, $3CH_3$), 2.0-2.6 (m, 4H, $CH_2$), 3.8-4.13 (s, broad, 2H, exchangeable, $NH_2$), 4.47-4.9 (m, 1H, N-C-H), 6.4-6.87 (m, 3H, 1N-H, 2 arom. H), 7.6 (d, J = 8Hz, 2H, arom. H) ppm.

Di-tert-butyl 4-[(2-chloroethyl)amino]benzoyl-L-glutamate (6)

A 1:1 mixture of 6 N aqueous hydrochloric acid and methanol (1.5ml), chloroacetaldehyde as a 45% aqueous solution (1.5ml,10 mmol), and

0.554g (9 mmol) sodium cyanoborohydride were added successively to a solution of 3.05g (8 mmol) dipeptide 5 in 60 ml dry methanol. The reaction mixture was stirred at room temperature for 5 days then acidified with concentrated hydrochloric acid to pH 1-2 and evaporated. The residue was taken up in dichloromethane and water, the organic layer was separated, and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with water (2x) and 10% aqueous sodium bicarbonate solution, dried over magnesium sulphate and evaporated to give the crude 6.

Another batch was purified by flash chromatography ($R_F$ = 0.44, $SiO_2$, ether/petrolether 2:1) on silica gel with ether/petrolether (40-60°C) (2:1) as eluant and recrystallization with a little dichloromethane, ether and petrolether (40-60°C) to afford 6 as colourless crystals (mp. 144-145°C).

IR ($CHCl_3$): 3356 (broad, N-H), 3010 (C-H), 1712 (C=O), 1610, 1500, 1437, 1148 $cm^{-1}$.

$^1$H-NMR ($CDCl_3$, 220MHz): δ = 1,43 (s, 9H, $CH_3$), 1.50 (s, 9H, $CH_3$), 1.95-2.54 (m, 4H, $CH_2$), 3.54 (t, J=5.3Hz), 2H, $CH_2$), 3.7 (t, J=5.3Hz, 2H, $CH_2$), 4.5-4.82 (m, 2H, 1N-H, exchangeable, 1 N-C-H), 6.6 (d, J=8.8Hz, 2H, arom.H), 6.85 (d, J=8.4Hz, 1H, O=C-N-H), 7.68 (d, J=8.8Hz, 2H, arom.H), ppm.

$^{13}$C-NMR ($CDCl_3$): δ = 27.72 ($1CH_2$), 28.03 ($6CH_3$), 31.70 ($1CH_2$), 42.93 ($1CH_2$), 44.87 ($1CH_2$), 52.62 (1 N-C-H), 80.65 (1 O-C-), 82.17 (1 O-C-), 111.96 (2 arom.C-H), 122.70 (1 arom. C-C=O), 128.90 (2 arom.C-H), 150.10 (1 arom. C-N), 166.73 (1C=O), 171.62 (1 O-C=O), 172.5 (1 O-C=O)ppm.

MS: m/e = 440 ($M^+$), 182 (100%).

Microanalysis: found C, 59.98%, H, 7.67%, N, 6.30%, calculated for $C_{22}H_{33}ClN_2O_5$ C, 59.92%, H, 7.54%, N, 6.35%.

Di-tert-butyl 4-[(2-chloroethyl)(2-hydroxyethyl)amino]benzoyl-L-glutamate (7)

Gaseous ethylene oxide was passed through a solution of the crude 6 in 50 ml glacial acetic acid at room temperature for 3/4 h. The solution was then stirred in a stoppered flask at room temperature for 2 days, prior to dilution with 60 ml of water, and extraction with dichloromethane (3x). The combined organic phases were washed with water, dried over magnesium sulphate and evaporated in vacuo. The residue was purified by flash chromatography on silica gel with ether as eluant ($R_F$ = 0.22, $SiO_2$, ether) to afford 2.537g (65%) 7 as colourless precipitate. 759mg 7 were recrystallized from a little dichloromethane, ether and

petrolether (40-60°C) to give 483 mg 7 as colourless crystals (mp. 97-99°C).
IR ($CHCl_3$): 3429 (broad, NH, OH), 3009 (C-H), 1719 (C=O), 1607, 1498, 1437, 1150 $cm^{-1}$.
$^1$H-NMR ($CDCl_3$, 220MHz): δ = 1.42 (s, 9H, $CH_3$), 1.49 (s, 9H, $CH_3$), 1.92-2.52 (m, 4H, $CH_2$), 2.65 (s, broad, 1H, exchangeable, OH), 3.54-3.72 (m, 4H, $CH_2$), 3.72-3.86 (m, 4H, $CH_2$), 4.60-4.80 (m, 1H, N-C-H), 6.68 (d, 2H,J=8.8Hz,arom.H), 6.88 (d, 1H, J=8.4Hz, N-H), 7.68 (d, 2H, J=8.8Hz, arom.H), ppm.
MS: m/e = 484 ($M^+$), 448 ($M^+$-HCl), 190 (100%).
Microanalysis: found C, 59.32%, H, 7.71%, N, 5.65%, calculated for $C_{24}H_{37}ClN_2O_6$ C, 59.43%, H, 7.69%, N, 5.78%.

Di-tert-butyl 4-[(2-chloroethyl)(2-mesyloxyethyl)amino]benzoyl-L-glutamate (8)

Triethylamine (1.5 ml) and 0.5 ml (6.5 mmol) of methane-sulfonylchloride were added to 2.605g (5.67 mmol) 7 in 50ml dichloromethane at 5°C. After stirring for 1h at 5°C the reaction mixture was poured into 300 ml of water. The phases were separated and the aqueous phase extracted with dichloromethane (2x). The combined organic phases were washed with water (3x), dried over magnesium sulphate and evaporated. The crude product 8 was crystallized from ether/petrolether (40-60°C) to give 2.336g (75%) as colourless crystals (mp. 74.5-75.5°C).
IR ($CHCl_3$): 3430 (N-H), 3009 (C-H), 1722 (C=O), 1648, 1608, 1496, 1368 ($SO_2$-O), 1175, 1153 $cm^{-1}$.
$^1$H-NMR ($CDCl_3$, 220MHz): δ = 1.44 (s, 9H, $CH_3$), 1.51 (s, 9H, $CH_3$), 1.94-2.52 (m, 4H, $CH_2$), 2.95 (s, 3H, $CH_3$), 3.68 (t, J=5.6Hz, 2H, $CH_2$), 3.76-3.9 (m, 4H, $CH_2$), 4,39 (t, J=5 Hz, 2H, $CH_2$), 4.63-4.74 (m, 1H, N-C-H), 6,71 (d, J=8 Hz, 2H, arom. H), 6.82 (d, J=6.8Hz, 1H, N-H), 7.76 (d, J=8 Hz, 2H, arom. H) ppm.

4-[2-chloroethyl)(2-mesyloxyethyl)amino]benzoyl-L-glutamic acid (3)

Formic acid (98%, 600ml) was added to 8 (3.00g, 5.33 mmol) at 10°C. After stirring for 48h at 10°C the reaction mixture was transferred into vials and frozen in liquid nitrogen, prior to lyophilization on a freeze dryer. When all the acid had been removed, the vials were capped while still under vacuum on the freeze dryer. The deprotection was quantitative and gave 2.40g of the dicarboxylate 3 as a white powder.

$^1$H-NMR($Me_2SO$-$d_6$, 250MHz): 1.98 (m, 2H, $\underline{CH_2}CH_2CO_2H$), 2.34 (t, J=7.3Hz, 2H, $CH_2\underline{CH_2}CO_2H$), 3.16 (s, 3H, $CH_3SO_3$), 3.77 (s, 4H, $ClCH_2CH_2$), 3.83 (t, J=5.4Hz, 2H, $CH_3SO_3CH_2\underline{CH_2}$), 4.33 (m, 3H, $CH_3SO_3\underline{CH_2}CH_2$ and CH), 6.82 (AB q, J=8.9Hz, 2H, arom H-3,5), 7.77 (AB q, 2H, arom H-2,6), 8.27 (d, J=7.8Hz, 1H, NH), ppm.

Mass spectrum FAB m/z 451 ([M+H$^+$], 17%)
401(M-$ClCH_2$, 7%), 304 (M-$NHCH(CO_2H)CH_2CH_2CO_2H$, 100%).

Found: C, 44.89%, H, 5.41%, N, 5.78%, Cl, 7.83%, S, 6.97%.

Calculated for $C_{17}H_{23}N_2O_8ClS$-O.$2H_2O$
C, 44.92%, H, 5.19%, N, 6.17%, Cl, 7.79%, S, 7.05%.

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