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# Synthesis of N-α-Aminoacyl Derivatives of Melphalan for Potential Use in Drug Targeting

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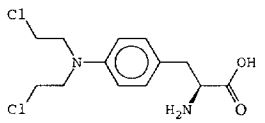
**Abstract:** N-L- and D-alanyl derivatives (**9a**, **9b**) of melphalan (**1**) have been synthesized in eight steps (**9a**, 22%; **9b**, 18% overall yield) from *p*-nitro-L-phenylalanine. 

Melphalan (**1**) (4-*bis*(2-chloroethyl)amino-L-phenylalanine) is a cytotoxic drug used widely in the chemotherapy of various solid tumours such as breast and ovarian cancer, as well as multiple myeloma.[1] As with most cytotoxic drugs, the toxicity of melphalan towards rapidly reproducing normal cells is dose-limiting. In this paper we report a general synthesis of N-α-aminoacyl derivatives of melphalan for potential use in antibody directed enzyme pro-drug therapy (ADEPT).[2] In this approach to drug targeting in cancer chemotherapy, a cytotoxic agent is chemically modified to yield a non-cytotoxic 'pro-drug'. This modification is such that it is reversible by an enzyme linked to a monoclonal antibody specific for a particular tumour. The enzyme - antibody conjugate is first administered to the patient and the pro-drug, subsequently administered, is enzymatically converted to the cytotoxic species at the tumour site.

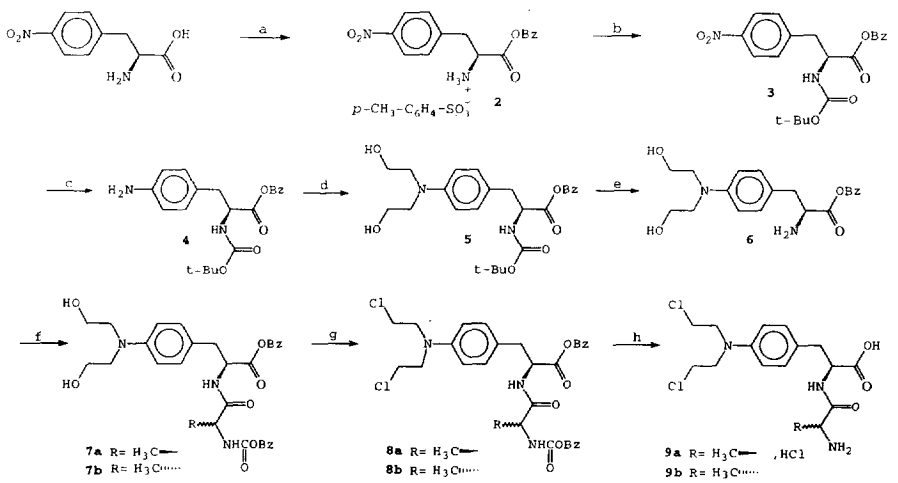


**1**

Melphalan was synthesized in the 1950's as one of a series of nitrogen mustard derivatives.[3] In melphalan, the cytotoxic nitrogen mustard moiety is linked to L-phenylalanine designed to act as a physiological carrier. It has been shown that the free amino group of phenylalanine is essential for cellular uptake and hence cytotoxicity of melphalan.[4] An N-α-aminoacyl derivative of melphalan should therefore behave as a relatively non-cytotoxic pro-drug which may be enzymatically activated by aminopeptidases. Aminopeptidases have been shown previously in this laboratory to activate relatively non-cytotoxic N-α-aminoacyl derivatives of the cytotoxic drug methotrexate.[5]

Previous synthesis of N-α-aminoacyl derivatives of melphalan involved reacting a melphalan ethyl ester with an N-protected α-amino acid.[6] Little attempt was made to remove the ester group since the N-mustard moiety is highly reactive to nucleophiles. The only synthesis of an unesterified N-α-aminoacylmelphalan derivative (L-valylmelphalan) involved a procedure which is not of general applicability.[7] In this communication we describe a general method for synthesizing unesterified N-α-aminoacylmelphalan derivatives, in which the reactive N-mustard group is introduced near the end of the reaction sequence.

The synthetic pathway is outlined overleaf. The carboxylic and amino functional groups of *p*-nitro-L-phenylalanine are first protected by formation of the benzyl ester and then the N-t-butyloxycarbonyl derivative yielding **2**[8] and then **3**.[9] The method of Han *et al.*[10] utilizing hydrazine and graphite was used to reduce the aromatic nitro group to the corresponding amine **4**, in 76% yield and with minimal hydrazinolysis of the benzyl ester. Two 2-hydroxyethyl side-chains were then introduced to the amino group of **4** using ethylene oxide in 50% aqueous acetic acid, following the method of Bergel and Stock.[3] From product **5** the N-t-butyloxycarbonyl group was removed using 4 M HCl in dioxane. The deprotected species **6** was then reacted in separate reactions with the N-hydroxysuccinimide active esters of N-benzyloxycarbonyl-L- and -D-alanine to provide the respective protected alanyl derivatives **7a** and **7b**, with overall yields (three steps) of 76% and 73% respectively. The 2-hydroxyethyl side-chains were chlorinated with thionyl chloride in pyridine yielding **8a** and **8b** (66% and 71% yields respectively). Finally, both the benzyl ester and the N-benzyloxycarbonyl group were removed simultaneously by catalytic hydrogenolysis over palladium-charcoal. L-Alanylmelphalan (**9a**) was isolated as the crystalline hydrochloride salt (63% yield), while D-alanylmelphalan (**9b**) was obtained as the crystalline free base (50% yield). The structures of **9a** and **9b**, as well as those of the intermediates, were consistent with their spectral characteristics and microanalysis data.[11]



a) $C_6H_5CH_2OH$, *p*-$CH_3$-$C_6H_4$-$SO_3H$ (1.1 equiv.), $C_6H_6$ , 120°C, 12 h (98%). b) t-Butylcarbonate, $CHCl_3$, 65°C, 2 h (94%). c) $N_2H_4.H_2O$/graphite, dioxane, 105°C, 3-5 h (76%). d) $(CH_2)_2O$, HOAc/$H_2O$, 25°C, 24 h. e) 4 M HCl in dioxane, 25°C, 2 h. f) N-Benzyloxycarbonyl-L- or -D-alanine N-hydroxysuccinimido ester (1.1-1.2 equiv.), dioxane, 25°C, 1.5 h (**7a**, 76%; **7b**, 73%). g) $SOCl_2$/pyridine, $CHCl_3$, 0°C, then 25°C for 1 h, then 40-45°C for 2 h (**8a**, 66%; **8b**, 71%). h) **9a**, $H_2$/Pd-C, 1 M HCl (1 equiv.), MeOH, 25°C, 2 h (63%); **9b**, $H_2$/Pd-C, MeOH, 25°C, 2 h (50%).

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11. All new compounds were characterized by $^1H$ and $^{13}C$ NMR and $CH_4$ CI-MS. Other data are given below.

| Cmpd. | m.p. (°C) | $[\alpha]_D^{25}$ (deg.) | Mol. Formula |
|---|---|---|---|
| **4** | 89-90 | -6.6 [a] | $C_{21}H_{26}N_2O_4$ [d] |
| **5** | *f* | -4.3 [a] | $C_{25}H_{34}N_2O_6$ [e] |
| **7a** | 124-125 | -18.3 [b] | $C_{31}H_{37}N_3O_7$ [d] |
| **7b** | 111-112 | +10.3 [b] | $C_{31}H_{37}N_3O_7$ [d] |
| **8a** | 128-129 | -14.0 [b] | $C_{31}Cl_2H_{35}N_3O_5$ [d] |
| **8b** | 128-129 | +6.3 [b] | $C_{31}Cl_2H_{35}N_3O_5$ [d] |
| **9a** | 126-128 | +7.9 [c] | $C_{16}Cl_2H_{21}N_3O_3$,HCl [d] |
| **9b** | 209-211 | +12.2 [c] | $C_{16}Cl_2H_{23}N_3O_3$ [d] |

*a* *c* = 1 (EtOH)
*b* c = 1 (MeOH)
*c* *c* = 1 (1M HCl)
*d* With elemental analysis results of C, H and N within 0.3% of calculated values.
*e* With molecular weight, determined by high resolution $CH_4$ CI-MS, within 0.001 a.m.u of calculated value.
*f* Noncrystalline.

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