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Short Communication

A Convenient Synthesis of *N,N*-Bis(2-chloroethyl)-*p*-aminophenylacetic Acid, a Major Metabolite of the Cancer Chemotherapeutic Agent Chlorambucil

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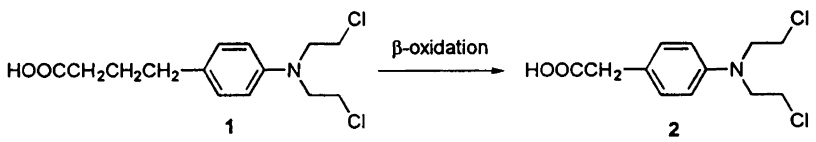
An aromatic nitrogen mustard, *N,N*-bis(2-chloroethyl)-*p*-aminophenylbutyric acid, (chlorambucil; **1**) is an alkylating agent originally synthesized by Everett *et al.* in 1953.[1] Its cytotoxicity is based on its DNA cross-linking properties. It has been in clinical use since 1961, and it is still a front-line drug in the treatment of chronic lympholytic leukemia.[2] The principal route of metabolism for chlorambucil occurs by β-oxidation of the butyric acid side chain giving an intermediate (*E*)-[*N,N*-bis(2-chloroethyl)-*p*-aminophenyl]-3-butenoic acid that is further converted into the final metabolite, *N,N*-bis(2-chloroethyl)-*p*-aminophenylacetic acid[3] (phenylacetic acid mustard; **2**; Scheme 1). The chemistry and pharmacology of both **1** and **2** have been, and are still, intensively studied. Furthermore, **1** and **2** are often needed as reference compounds when new and better chemotherapeutic agents are developed. While chlorambucil is cheap and can be purchased from several chemical suppliers, phenylacetic acid mustard is not commercially available. Thus there is a need for a simple synthetic method for its preparation.

Results and discussion

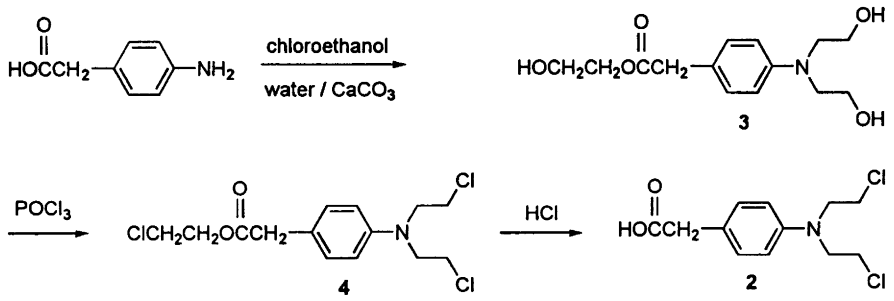
According to the original synthetic strategy[1] phenylacetic acid mustard is prepared in four steps starting from *p*-aminophenylacetic acid, which is initially converted into its ethyl ester and then alkylated with oxirane at high temperature giving rise to ethyl *N,N*-bis(2-hydroxyethyl)-*p*-aminophenylacetate. Chlorination of this, followed by hydrolysis of the ester function yields the title compound. Thus, rather vigorous reaction conditions, special apparatus, and the use of a highly toxic, volatile and carcinogenic reagent, oxirane, is needed.

When it is desired to avoid the use of oxirane *N,N*-bis(2-hydroxyethyl)arylamines can be prepared by two alternative methods: (*i*) from arylamines and 2-chloroethanol in boiling water in the presence of base,[4,5] and (ii) from aryl halides and diethanolamine.[5,6] Both methods are not widely used since bis-hydroxyethylation of arylamines with oxirane gives normally considerably higher yields. Furthermore, method (ii) requires the use of activated aryl halides, such as halonitrobenzenes, and thus it is unsuitable for the present case. By contrast, treatment of *p*-aminophenylacetic acid in a mixture of water and 2-chloroethanol containing calcium carbonate at reflux resulted not only in bis-hydroxyethylation of the amino group, but also esterification of the carboxylic acid function giving rise to the 2-hydroxyethyl ester of *N,N*-bis(2-hydroxyethyl)-*p*-aminophenylacetic acid (**3**; Scheme 2). Hence an initial esterification of *p*-aminophenylacetic acid is not needed, and the product is easily separated from the reaction mixture by extraction into ethyl acetate.



1 **2**

Scheme 1.



Scheme 2.

2-Hydroxyethyl *N,N*-bis(2-hydroxyethyl)-*p*-aminophenylacetate (**3**) can be further converted into **2** by standard literature procedures.[7] Accordingly, treatment of **3** with phosphorus oxychloride gives the 2-chloroethyl ester of *N,N*-bis(2-chloroethyl)-*p*-aminophenylacetic acid (**4**) which is subsequently hydrolysed to **2** with hydrochloric acid. The final product can be obtained in 46% overall yield without need of isolation of the reaction intermediates (**3** and **4**).

Experimental

Adsorption column chromatography was performed on columns packed with Silica gel 60 (Merck). Analytical TLC was carried out on Silica gel 60 F_{254} plates (Merck) with the following solvent systems: system A, CH_2Cl_2–MeOH 9:1 (v/v); system B, CH_2Cl_2. NMR spectra were recorded on a Jeol LA-400 spectrometer operating at 399.8 and 100.5 MHz for ^1H and ^{13}C, respectively. Me_4Si was used as an internal reference. Coupling constants are given in Hz. Electron impact mass spectra were recorded at 70 eV on a VG Analytical MM 7070E instrument. IR spectra were recorded on a Mattson Instruments 6030 Galaxy Series spectrophotometer. Melting points were measured on a Büchi 510 apparatus and are uncorrected. The elemental analyses were performed on a Perkin Elmer 2400 Series II instrument and all compounds prepared gave satisfactory microanalyses.

WARNING. Aromatic nitrogen mustards are known to be carcinogenic. They should be handled in a well-ventilated hood using suitably protective clothes. Wastes generated during synthetic procedures should be destroyed by overnight treatment with aqueous base.

N,N-*Bis(2-chloroethyl)*-p-*aminophenylacetic acid,* **2**. To a suspension of *p*-aminophenylacetic acid (Aldrich; 5.0 g, 33.1 mmol) and calcium carbonate (12 g) in water (30 ml) was added 2-chloroethanol (30 ml) and the mixture was refluxed overnight. The reaction mixture was then allowed to cool, filtered and washed in water. The filtrate was concentrated to an oil which was then stirred vigorously in ethyl acetate with heating. The ethyl acetate layer was decanted off, and the procedure was repeated until TLC analysis showed that all the product [**3**; R_f (A): 0.37] had been transferred into the ethyl acetate. After drying ($MgSO_4$) the solvent was removed *in vacuo*. To the cold residue (ice bath) was added phosphorus oxychloride (15 ml) with stirring. When the evolution of gas had ceased, the resulting solution was allowed to warm to room temperature and then heated at reflux until all **3** had been consumed, and the product appeared [ca. 30 min; R_f **4** (A): 1.0; (B): 0.85]. The solution was allowed to cool and volatile material was removed *in vacuo*. The residue was dissolved in conc. hydrochloric acid (20 ml) and heated at reflux for 1 h. The cooled solution was poured into ice–water saturated with sodium chloride. The resulting solution was extracted with dichloromethane, and the organic phase was dried ($MgSO_4$) and concentrated to give the title compound as a solid (4.2 g; 46%) R_f (A): 0.52. M.p. (benzene–heptane; needles) 105 °C (lit.[1] 105 °C; lit.[8] 104–105 °C; lit.[9] 102–104 °C). Anal. ($C_{12}H_{15}Cl_2NO_2$): C, H, N. ^1H NMR ($CDCl_3$): δ 7.16 (2 H, d, *J* 8.8 arom.), 6.64 (2 H, d, *J* 8.8, arom.), 3.72 (4 H, t, *J* 6.6, 2 × CH_2N), 3.62 (4 H, t, *J* 6.6, 2 × CH_2Cl), 3.55 (2 H, s, PhCH_2COOH). ^{13}C NMR ($CDCl_3$): δ 178.4, 145.2, 130.6, 122.2, 112.1, 53.6, 40.3, 39.9. MS [*m/z* (rel. int.%)]: 277 (8), 275 (12), 228 (33), 226 (100), 164 (4), 132 (5), 118 (24), 90 (5), 77 (3), 63 (8).

Characterization of the reaction intermediates. 2-Hydroxyethyl ester of N,N-*bis(2-hydroxyethyl)*-p-*aminophenylacetic acid,* **3**. Crude **3** obtained as shown above was dissolved in a mixture of dichloromethane and methanol (9:1; v/v) and loaded onto a silica gel column. The column was eluted with the same solvent system. Fractions containing pure **3** were pooled and concentrated to give the title compound as an oil. Anal. ($C_{14}H_{21}NO_5$): C, H, N. ^1H NMR ($CDCl_3$): δ 7.13 (2 H, d, *J* 8.8, arom.), 6.65 (2 H, d, *J* 8.8, arom), 4.21 (2 H, t, *J* 4.6, CH_2OCO), 3.82 (4 H, t, *J* 4.6, 2 × CH_2N), 3.79 (2 H, s, PhCH_2COO), 3.55 (8 H, 3 × CH_2OH and 2 × OH), 2.19 (1 H, br, OH). ^{13}C NMR ($CDCl_3$): δ 172.8, 146.8, 130.0, 121.5, 112.4, 66.1, 60.5, 60.3, 55.0, 39.9. IR/cm^{-1} (neat): 3378 (OH), 1730 (C=O). MS [*m/z* (rel. int.%)]: 283 (12), 252 (100), 194 (11), 164 (4), 118 (29), 45 (8).

2-Chloroethyl ester of N,N-*bis(2-chloroethyl)*-p-*aminophenylacetic acid,* **4**. Crude **4** obtained as described above was dissolved in dichloromethane and washed with water. The organic phase was dried ($MgSO_4$), concentrated and was passed through a funnel loaded with silica gel using dichloromethane as the eluent. The solvent was removed *in vacuo* to yield the title compound as an oil. Anal. ($C_{14}H_{18}Cl_3NO_2$): C, H, N. ^{1}H NMR ($CDCl_3$): δ 7.16 (2 H, d, *J* 8.8, arom.), 6.63 (2 H, d, *J* 8.8, arom.), 4.32 (2 H, t, *J* 5.6, $OCOCH_2$), 3.70 (4 H, t, *J* 6.6, $2 \times CH_2N$), 3.66 (2 H, t, *J* 5.6, CH_2Cl), 3.61 (4 H, t, *J* 6.6, $2 \times CH_2Cl$), 3.56 (2 H, s, $PhCH_2$). ^{13}C NMR ($CDCl_3$): δ 171.6, 145.8, 130.5, 122.4, 111.9, 64.2, 53.3, 41.5, 40.3, 39.8. IR/cm^{-1} (neat): 1740 (C=O). MS [*m/z* (rel. int.%)]: 341 (4), 339 (12), 337 (13), 288 (100), 232 (10), 230 (15), 181 (3), 132 (4), 118 (29), 90 (4), 63 (8).

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