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PREPARATION OF NEW CHLORAMBUCIL ANALOGS BY *BIS*-$S_{RN}1$ METHODOLOGY

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ABSTRACT

Three series of chlorambucil analogs were prepared by an original reaction of 1-(3-chloro-2-chloromethylpropenyl)-4-nitrobenzene with various nitronate anions under electron transfer reaction conditions.

Chronic lymphocytic leukaemia is the most common form of leukaemia in Western countries. Its chemotherapy has been based on the use of *N*,*N*-*bis*(2-chloroethyl)-*p*-aminophenylbutyric acid (chlorambucil) for more than 30 years.[1] Chlorambucil is a *bis*-alkylating agent that is known to bind covalently to many types of cellular molecules, such as DNA, RNA, and proteins.[2] Its mode of action has not, as yet, been entirely clarified. Previously researchers attributed chlorambucil's cytotoxicity solely to its inhibitors effects on DNA synthesis.[3] More recent studies have revealed

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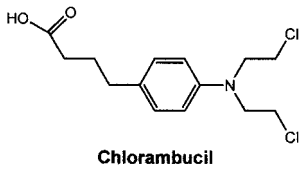
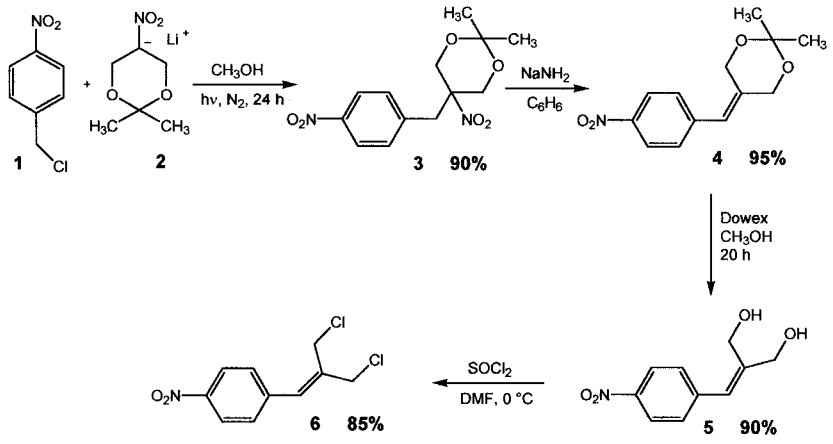


Chlorambucil

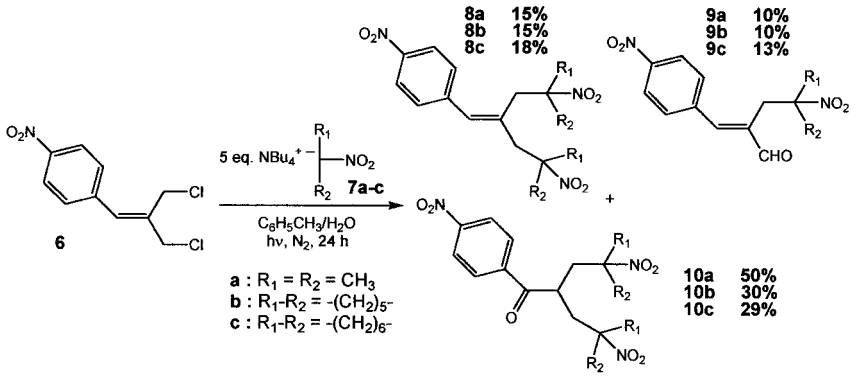
that chlorambucil indirectly affects the synthesis of DNA.[4] Biochemical studies have found that chlorambucil affects other cellular constituents as well,[5] especially nuclear proteins.[6]

To go further into our survey of bioreductive alkylating agents in electron transfer reactions[7] and into the development of new analogs of chlorambucil as anticancer agents, we report herein the synthesis of a *p*-nitrobenzyl *bis*-alkylating agent **6** and the study of its reactivity with various nitronate anions under $S_{RN}1$ reaction conditions.

The *bis*-chloride **6** was prepared in a four-step reaction. Firstly, we have realized an $S_{RN}1$ reaction between *p*-nitrobenzyl chloride **1** and the dioxane salt **2**, as described previously,[8] leading to the *C*-alkylated derivative **3** in 90% yield. The treatment of the latter compound **3** with sodium amide induced a nitrous acid elimination to give the ethylenic derivative **4** in 95% yield. Ring opening of compound **4** was easily effected in refluxing methanol in the presence of an ion exchange resin (Dowex 50x8-50) to give the corresponding diol **5** in 90% yield.[9] Finally, we obtained the required *bis*-alkylating agent **6** in 85% yield by treating the diol **5** with an excess of thionyl chloride in dimethylformamide at 0°C (Scheme 1).



Scheme 1.



Scheme 2.

The *bis*-chloride **6** reacted with 5 equivalents of the nitronate anion **7a–c** under $S_{RN}1$ reaction conditions (inert atmosphere, light catalysis) and in phase-transfer system (40% tetrabutylammonium hydroxide in water and toluene) for 24 h to give three products: the *bis*-*C*-alkylated product **8a–c**, the aldehyde **9a–c** and the ketone **10a–c** (Scheme 2).

As reported previously in the nitroimidazole series,[10] derivatives **8a–c** resulted from a classical *bis*-$S_{RN}1$ mechanism, whereas **9a–c** were formed by a consecutive $S_{RN}1$ (*C*-alkylation) and S_N2 (*O*-alkylation) reaction, while **10a–c** were obtained by an $S_{RN}1$ reaction followed by an S_N2' and a Michael addition of nitronate anion.

In conclusion, we have demonstrated an original synthesis of new analogs of chlorambucil by *bis*-$S_{RN}1$ methodology. The study of the antitumor activity of these compounds is under active investigation.

EXPERIMENTAL

Melting points were determined on Büchi B-540 and are uncorrected. Elemental analyses were performed by the Centre de Microanalyses of the University of Aix-Marseille 3. Both ^1H and ^{13}C NMR spectra were determined on Bruker ARX 200 spectrometer. The ^1H chemical shifts were reported as part per million downfield from tetramethylsilane (Me_4Si), and the ^{13}C chemical shifts were referenced to the solvent peaks: $CDCl_3$ (76.9 ppm). The following adsorbent was used for column chromatography: silica gel 60 (Merck, 230–400 mesh). Thin-layer chromatography was performed with silica gel Merck 60F-254 (0.25 mm layer thickness).

2,2-Dimethyl-5-[nitro-(4-nitrophenyl)-methyl]-1,3-dioxane **3**, 2,2-dimethyl-5-(4-nitrobenzylidene)-1,3-dioxane **4** and 2-(4-nitrobenzylidene)propane-1,3-diol **5** were prepared as previously described.[9] Nitrocyclohexane was commercially available, nitrocycloheptane was prepared by oxidation of the corresponding amine with *m*-chloroperbenzoic acid in refluxing 1,2-dichloroethane by Gilbert and Borden procedure.[11]

1-(3-Chloro-2-chloromethylpropenyl)-4-nitrobenzene (6): Thionyl chloride (5.27 ml, 63 mmol) was added dropwise to a solution of **5** (2.51 g, 12 mmol) in dimethylformamide (50 ml) in a round-bottomed flask equipped with reflux condenser surmounted by a calcium chloride drying tube. After stirring at 0°C for 24 h, the reaction mixture was evaporated under reduced pressure. The residue was dissolved in benzene (25 ml), washed with water (3×20 ml), dried over $MgSO_4$ and the solvent was removed under reduced pressure. Recrystallization from ethanol gave 2.51 g (85% yield) of 1-(3-chloro-2-chloromethylpropenyl)-4-nitrobenzene (**6**)–pale yellow needles, m.p. 65°C. ^{1}H NMR ($CDCl_3$): δ 4.28 (s, 2H), 4.35 (s, 2H), 6.84 (s, 1H), 7.52 (d, $J = 8.8$ Hz, 2H), 8.26 (d, $J = 8.8$ Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 39.6, 46.4, 123.9, 129.5, 131.7, 137.1, 141.5, 147.3. Anal. calcd for $C_{10}H_9NO_2Cl_2$: C, 48.81; H, 3.69; N, 5.69; Cl, 28.81. Found: C, 48.85; H, 3.70; N, 5.75; Cl, 28.75.

General procedure for *bis*-$S_{RN}1$ reaction with aliphatic and cyclic nitronate anions: Under nitrogen atmosphere, a solution of tetrabutylammonium hydroxide (40% in water, 6.7 mol, 10 mmol) was treated with nitroalkane (10 mmol) for 1 h. A solution of 1-(3-chloro-2-chloromethylpropenyl)-4-nitrobenzene **6** (0.50 g, 2 mmol) in toluene (20 ml) was added and the mixture was irradiated with a 300 W sun lamp for 24 h at room temperature under an inert atmosphere. The organic layer was separated and the aqueous layer was extracted with toluene (3×10 ml). The combined organic layers were washed twice with water (30 ml), dried over $MgSO_4$ and removed under reduced pressure. Purification by chromatography on silica gel eluting with chloroform and recrystallization from isopropanol led to the corresponding products **8a–c**, **9a–c**, **10a–c**.

1-[4-Methyl-2-(2-methyl-2-nitropropyl)-4-nitropent-1-enyl]-4-nitrobenzene (8a): White needles, m.p. 80°C. ^{1}H NMR ($CDCl_3$): δ 1.37 (s, 6H), 1.65 (s, 6H), 2.60 (s, 2H), 2.92 (s, 2H), 6.53 (s, 1H), 7.29 (d, $J = 8.5$ Hz, 2H), 8.20 (d, $J = 8.5$ Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 26, 26.2, 40.9, 46.3, 87.6, 88.7, 123.8, 129.6, 133.9, 134.2, 143.5, 146.8. Anal. calcd for $C_{16}H_{21}N_3O_6$: C, 54.70; H, 6.02; N, 11.96. Found: C, 54.65; H, 6.04; N, 12.02.

2-(2-Methyl-2-nitropropyl)-3-(4-nitrophenyl)-propenal (9a): White needles, m.p. 116°C. ^{1}H NMR ($CDCl_3$): δ 1.40 (s, 6H), 3.24 (s, 2H), 7.25 (s, 1H), 7.55 (d, $J = 8.2$ Hz, 2H), 8.25 (d, $J = 8.2$ Hz, 2H), 9.60 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 26, 33.5, 87.5, 124, 129.5, 139.7, 140.4, 148, 150.6, 193.7. Anal.

calcd for $C_{13}H_{14}N_2O_5$: C, 56.11; H, 5.07; N, 10.07. Found: C, 56.07; H, 5.12; N, 10.02.

4-Methyl-2-(2-methyl-2-nitropropyl)-4-nitro-1-(4-nitrophenyl)-pentan-1-one (10a): White needles, m.p. 134°C. ^{1}H NMR ($CDCl_3$): δ 1.43 (s, 6H), 1.59 (s, 6H), 2.10 (dd, $J = 4.3$ Hz and $J = 15.2$ Hz, 2H), 2.43 (dd, $J = 7.1$ Hz and $J = 15.2$ Hz, 2H), 3.30 (tt, $J = 4.3$ Hz and $J = 7.1$ Hz, 1H), 8.04 (d, $J = 8.7$ Hz, 2H), 8.33 (d, $J = 8.7$ Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 26, 26.8, 38.2, 43, 87.2, 124.2, 129.6, 140, 150.7, 199.8. Anal. calcd for $C_{16}H_{21}N_3O_7$: C, 52.31; H, 5.76; N, 11.44. Found: C, 52.76; H, 5.95; N, 11.70.

1-Nitro-4-[3-(1-nitrocyclohexyl)-2-(1-nitrocyclohexylmethyl)-propenyl]-benzene (8b): White needles, m.p. 112°C. ^{1}H NMR ($CDCl_3$): δ 1.20 (m, 6H), 1.30 (m, 2H), 1.45 (m, 2H), 1.60–180 (m, 6H), 2.30 (m, 2H), 2.45 (s, 2H), 2.55 (m, 2H), 2.75 (s, 2H), 6.47 (s, 1H), 7.30 (d, $J = 8.2$ Hz, 2H), 8.20 (d, $J = 8.2$ Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 22, 22.3, 24.2, 24.5, 34.5, 41.6, 47.3, 91, 92.3, 123.8, 129.8, 132.9, 133.7, 143.7, 146.7. Anal. calcd for $C_{22}H_{29}N_3O_6$: C, 61.24; H, 6.77; N, 9.74. Found: C, 61.30; H, 6.80; N, 9.80.

2-(1-Nitrocyclohexylmethyl)-3-(4-nitrophenyl)-propenal (9b): White needles, m.p. 115°C. ^{1}H NMR ($CDCl_3$): δ 1.60 (m, 6H), 2.40 (m, 4H), 3.20 (s, 2H), 7.40 (s, 1H), 7.60 (d, $J = 8.2$ Hz, 2H), 8.30 (d, $J = 8.2$ Hz, 2H), 9.70 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 22.1, 24.2, 34.2, 34.4, 90.8, 123.9, 129.5, 139.3, 140.3, 148, 150.8, 193.6. Anal. calcd for $C_{16}H_{18}N_2O_5$: C, 60.37; H, 5.70; N, 8.80. Found 60.35; H, 5.72; N, 8.85.

3-(1-Nitrocyclohexyl)-2-(1-nitrocyclohexylmethyl)-1-(4-nitrophenyl)-propan-1-one (10b): White needles, m.p. 120°C. ^{1}H NMR ($CDCl_3$): δ 1.25–1.45 (m, 8H), 1.50–1.70 (m, 8H), 1.90 (dd, $J = 4.5$ Hz and $J = 15.1$ Hz, 2H), 2.25 (m, 2H), 2.30 (dd, $J = 6.8$ Hz and $J = 15.2$ Hz, 2H), 2.50 (m, 2H), 3.60 (tt, $J = 4.5$ and $J = 6.8$ Hz, 1H), 8.05 (d, $J = 8.2$ Hz, 2H), 8.35 (d, $J = 8.2$ Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 22.1, 24.4, 34.3, 34.9, 36.3, 42.8, 90.3, 124.1, 129.6, 139.9, 150.7, 193.2. Anal. calcd for $C_{22}H_{29}N_3O_7$: C, 59.05; H, 6.53; N, 9.39. Found: C, 59.07; H, 6.60; N, 9.50.

1-Nitro-4-[3-(1-nitrocycloheptyl)-2-(1-nitrocycloheptylmethyl)-propenyl]-benzene (8c): White needles, m.p. 96°C. ^{1}H NMR ($CDCl_3$): δ 1.30–1.50 (m, 16H), 1.80 (dd, $J = 8.5$ Hz and $J = 15.3$ Hz, 4H), 2.40 (dd, $J = 8.5$ Hz and $J = 15.3$ Hz, 4H), 2.51 (s, 2H), 2.80 (s, 2H), 6.51 (s, 1H), 8.03 (d, $J = 8.1$ Hz, 2H), 8.32 (d, $J = 8.1$ Hz, 2H). Anal. calcd for $C_{24}H_{33}N_3O_6$: C, 62.73; H, 7.24; N, 9.14. Found: C, 62.68; H, 7.30; N, 9.20.

2-(1-Nitrocycloheptylmethyl)-3-(4-nitrophenyl)-propenal (9c): White needles, m.p. 100°C. ^{1}H NMR ($CDCl_3$): δ 1.30–1.50 (m, 8H), 1.80 (dd, $J = 8.5$ Hz and $J = 15.3$ Hz, 2H), 2.40 (dd, $J = 8.5$ Hz and $J = 15.3$ Hz, 2H), 3.20 (s, 2H), 7.40 (s, 1H), 7.50 (d, $J = 8.2$ Hz, 2H), 8.30 (d, $J = 8.2$ Hz, 2H), 9.61 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 22.6, 29.3, 34.3, 36.8, 94.8, 123.9, 129.4, 139.8,

140.4, 148, 150.4, 193.7. Anal. calcd for $C_{17}H_{20}N_2O_5$: C, 61.44; H, 6.07; N, 8.43. Found: C, 61.50; H, 5.92; N, 8.52.

3-(1-Nitrocycloheptyl)-2-(1-nitrocycloheptylmethyl)-1-(4-nitrophenyl)-propan-1-one (10c): White needles, m.p. 102°C. ^{1}H NMR ($CDCl_3$): δ 1.30–1.61 (m, 16H), 1.90 (dd, $J = 8.2$ Hz and $J = 15.1$ Hz, 4H), 1.95 (dd, $J = 4.3$ Hz and $J = 15.2$ Hz, 2H), 2.30 (dd, $J = 8.2$ Hz and $J = 15.1$ Hz, 4H), 2.40 (dd, $J = 4.3$ Hz and $J = 15.2$ Hz, 2H), 3.50 (tt, $J = 4.3$ Hz and $J = 8.2$ Hz, 1H), 8.05 (d, $J = 8.1$ Hz, 2H), 8.35 (d, $J = 8.1$ Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 22.7, 29.5, 36.9, 37.5, 37.7, 43.8, 94.6, 124.1, 129.5, 140.2, 150.6, 199.8. Anal. calcd for $C_{24}H_{33}N_3O_7$: C, 60.62; H, 6.99; N, 8.84. Found: C, 60.61; H, 7.04; N, 8.81.

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