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3,3-DIFLUOROCHLORAMBUCIL

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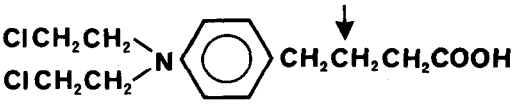
SUMMARY

A difluoro-derivative of the anti-cancer drug chlorambucil has been made in 7 stages from 4-nitrophenylacetic acid. The acid chloride and ethyl malonate/butyl lithium afforded ethyl 4-(4'-nitrophenyl)-3-oxobutanoate, of which the 3-oxo-function was converted to CF_2 by sulphur tetrafluoride/hydrogen fluoride at room temperature. The nitro-group was reduced to amino, which was alkylated to bis(hydroxyethyl)amino using oxirane in acetic acid. Conversion to bis(chloroethyl)amino was by carbon tetrachloride/triphenylphosphine. Hydrolysis of the ester group by hydrochloric acid then gave the target product: 4-[4'-bis(2"-chloroethyl)aminophenyl]-3,3-difluorobutanoic acid (3,3-difluorochlorambucil). Other, unsuccessful, approaches were also attempted.

INTRODUCTION

Among the noteworthy developments in cancer chemotherapy [see, inter alia, 1,2], has been the use of the drug chlorambucil:



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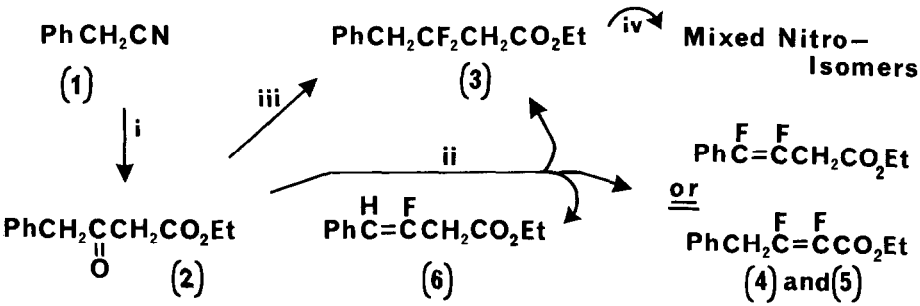
Synthesised by Ross et al. [3] it has been in clinical use for many years [4], mainly in the treatment [5] of conditions associated with the proliferation of white blood cells (e.g. lymphatic leukaemia, malignant lymphomas, Hodgkins disease). It is thought to act mainly by the alkylating activity of the tertiary nitrogen centre [6]. Studies on the metabolism of chlorambucil in animals [7], and clinical studies in man [8], showed that the primary pathway involved is oxidation at the β-carbon atom (arrowed in the formula above), the principal metabolite being the corresponding derivative of phenylacetic acid [9], the process probably involving an initial dehydrogenation stage [10]. Deuteration at the β-carbon, whilst giving identifiable changes to the metabolic pathway, did not significantly alter the therapeutic activity [10].

The aim of the present work was to prepare an analogue of chlorambucil in which the position β to the carboxyl group was CF_2, in the hope that blocking the normal β-oxidation metabolic pathway would improve the therapeutic index. This approach of modification by fluorine substitution has been attempted also with the anti-tumour drug CCNU [11]. It was recognised that the increased acidity of the carboxyl group of the difluoro-derivative might affect the transportation properties relative to those of chlorambucil, but the alkylating properties of the tertiary nitrogen should not be altered appreciably. Also, though β-oxidation to butenoic acids should be blocked as a metabolic pathway, dehydro-fluorination to such species might well be possible.

RESULTS AND DISCUSSION

The approach envisaged for the fluorination stage was conversion of C=O to CF_2 using either sulphur tetrafluoride [12], particularly in the presence of excess hydrogen fluoride [13], or diethylaminosulphur trifluoride (DAST) [14]. The first synthetic sequence investigated was fluorination of an aryl β-keto-ester, followed by nitration of the aryl group to introduce a nitrogen function.

Section 1. Attempts at fluorination followed by nitration



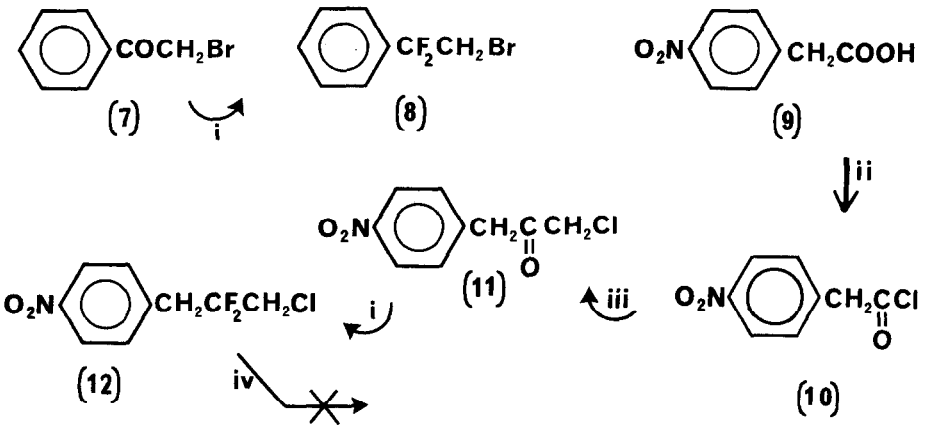
Reagents: i, Zn/$BrCH_2COOEt$; ii, DAST; iii, SF_4/HF; iv, HNO_3/$(CF_3CO)_2O$.

SCHEME 1

Phenylacetonitrile (1) underwent a smooth Reformatsky-type reaction with ethyl bromoacetate and zinc to give ethyl 3-oxo-4-phenylbutanoate (2), previously prepared in other ways [15]. ^1H nmr spectra in carbon tetrachloride showed a keto:enol ratio of about 3:1. Fluorination of compound (2) using DAST afforded a mixture, separable by column chromatography. The major component was the desired ethyl 3,3-difluoro-4-phenylbutanoate (3), but it was accompanied by three minor products, all unsaturated. That present in greatest quantity (6) almost certainly arose by loss of hydrogen fluoride from compound (3), but the others (4 and 5) could not be completely characterised, though both contained a difluorovinyl group (CF=CF), one with cis and the other with trans stereochemistry. Sulphur tetrafluoride and hydrogen fluoride at room temperature was a much better reagent for converting keto-ester (2) into difluoride (3): the conversion was higher and by-products (4-6) were not detected.

Nitration of difluoride (3) proceeded smoothly using nitric acid/trifluoroacetic anhydride [16] to give a clean mono-nitrated product, but unfortunately a mixture (5:3:12) of isomers was formed, and though the major one was the para it could not be separated. Presumably the β-CF_2 group causes sufficient deactivation of the aryl nucleus to enhance ortho- and meta-substitution. At this stage the approach was abandoned: attempts to carry out a Reformatsky-type reaction on 4-nitrophenyl-acetonitrile were unsuccessful.

Section 2. Attempts via an α-halo-ketone

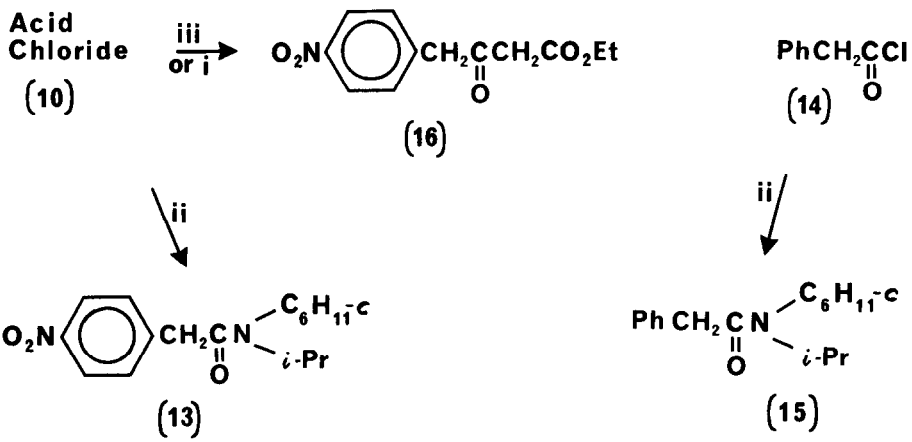


Reagents: i, DAST; ii, PCl_5; iii, CH_2N_2; iv, NaCN.

SCHEME 2

A model reaction of 1-bromo-2-phenylethan-2-one (7) and DAST having afforded 1-bromo-2,2-difluoro-2-phenylethane (8), an attempt was made to synthesise a difluorochloro-compound via a halo-ketone, and thence to insert the acid function by chlorine exchange. The chosen starting point was 4-nitrophenylacetic acid (9). Conversion of this to the acid chloride (10) originally presented difficulties, probably due to its decomposition to a ketene by loss of hydrogen chloride. Eventually, a modification of an old method [17] using phosphorus pentachloride in a rapid reaction with minimal manipulation, gave acid chloride (10) in quantitative yield, and sufficiently pure for further use. Acid chloride (10) was treated with diazomethane by an existing method [18], but using alcohol-free reagents, to give an improved yield of 1-chloro-3-(4'-nitrophenyl)propan-2-one (11). Reaction of this with DAST gave 1-chloro-2,2-difluoro-3-(4'-nitrophenyl)propane (12). No success at all was achieved in exchanging chlorine for cyano using sodium cyanide under various conditions. The approach was abandoned therefore.

Section 3. Synthesis of ethyl 4-(4'-nitrophenyl)-3-oxobutanoate (16)



Reagents: i, CH_3COCH_2COOEt/NaH then NH_4OH; ii, c-C_6H_{11}NH Pr(i)/n-BuLi/ CH_3COOEt; iii, $EtOOCCH_2COOH$/n-BuLi.

SCHEME 3

It was decided to use the readily-available p-nitrophenylacetyl chloride (10) as a starting material to make the 4-nitrophenyl-β-keto-ester (16). Attempts to react (10) with diethyl malonate using several different bases were unsuccessful.

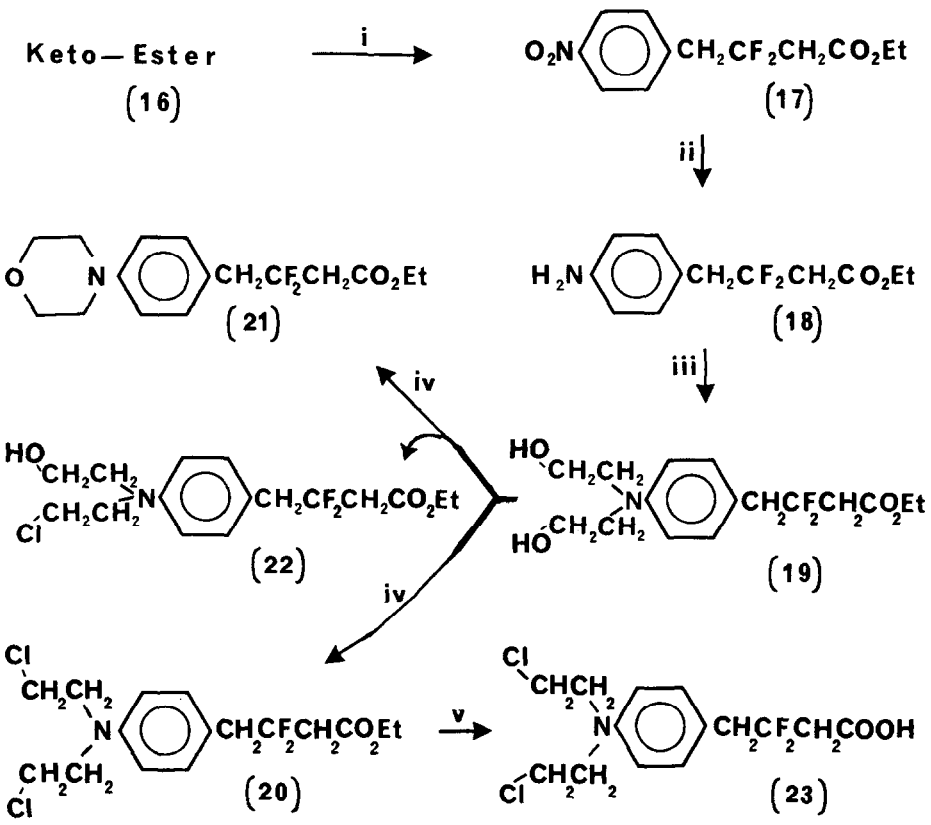
Ethyl acetoacetate had been condensed with (10) in earlier work [19] with sodium ethoxide as base and had given compound (16), but in low yield. The reaction was now carried out using sodium hydride as base, but again yields of product (16) were low (ca. 25%).

n-Butyl lithium and N-cyclohexyl-N-(1-methylethyl)amine give the corresponding amino-lithium derivative, which reacts with ethyl acetate to yield ethyl lithio-acetate, and this has given good yields of β-keto-esters from some acid chlorides [20]. When the reaction was applied to acid chloride (10), the product was the substituted amide of p-nitrophenylacetic acid (13). The same reagent system and phenylacetyl chloride (14) also afforded the corresponding phenylacetamide (15), though spectroscopic examination indicated that the crude product did contain a little of the expected β-keto-ester (2) in this case. Preferential reaction with the amine system clearly occurred with these acid chlorides.

A good synthesis of β-keto-ester (16) was finally achieved by a method [21] employing the di-lithium salt of ethyl propanedioate. The reaction

was spontaneous and, after hydrolysis, a good yield of the desired intermediate (16) was isolated. The proton nmr spectrum in deuterochloroform solution showed a keto:enol ratio of about 4:1.

Section 4. Synthesis of 3,3-difluorochlorambucil



Reagents: i, SF_4/HF; ii, H_2/Pd; iii, oxirane/CH_3COOH; iv, CCl_4/Ph_3P; v, conc. HCl.

SCHEME 4

Fluorination of ß-keto-ester (16) using DAST gave a similar result to that of its analogue (2). Five components were detected by chromatographic analysis and the reaction was not pursued. A smooth fluorination occurred with sulphur tetrafluoride/hydrogen fluoride however, and ethyl 3,3-difluoro-4-(4'-nitrophenyl)butanoate (17) was isolated in good yield. The nitro-group was then converted stepwise into the required tertiary amino-function. Catalytic hydrogenation afforded the relatively stable amine (18), which was alkylated by oxirane in acetic acid [3,22] to give ethyl 4-[4'-bis(2"-hydroxyethyl)aminophenyl]-3,3-difluorobutanoate (19). Though a pure sample was isolated, the synthetic sequence could be followed using crude material.

Triphenylphosphine in carbon tetrachloride has been recommended [23] as a good reagent for exchange of Cl for OH under mild conditions. Applied to compound (19), a mixture was obtained. Fortunately, it was separable by column chromatography, and the major product was the desired bis(chloroethyl) derivative (20). Other minor components isolated were the half-converted species, the N-(chloroethyl)-N-(hydroxyethyl) derivative (22), and the N-morpholinyl derivative (21) presumably formed by ring-closure of the latter (22).

Hydrolysis of the dichloride (20) by warm hydrochloric acid gave the target product, 4-[4'-bis(2"-chloroethyl)aminophenyl]-3,3-difluorobutanoic acid (3,3-difluorochlorambucil) (23) as crystalline plates, m.p. 86-87°C. Its activity as an anti-cancer drug will be discussed elsewhere [26].

The structures of all the compounds made followed from their elemental analyses, and their ^{1}H and ^{19}F nmr spectra (see Table). Infrared and ultraviolet spectra offered little conclusive structural information, but were in conformity with the structures allocated.

EXPERIMENTAL

General

All reactions involving organometallic reagents were done in apparatus dried at 110°C overnight, and flushed with dried (H_2SO_4) oxygen-free nitrogen after assembly. Solvents were dried by standard text-book procedures. Concentration of an organic solution was carried out at reduced pressure on a rotary evaporator, minimal heat being applied.

Organic solutions and extracts were dried using anhydrous magnesium sulphate and filtered.

Gas liquid chromatographic (glc) analysis was done on a Pye 104 machine with glass columns (2m x 4mm) packed with silicone gum rubber E301 (SE30) on Universal B (1:40) (packing A) or Silicone gum rubber E301 (SE30) on Celite 1:10 (packing B). Quoted are the packing, column temperature and carrier gas pressure.

Column (adsorption) chromatography was done using silica gel (Kieselgel 60, 70-230 mesh ASTM, type 7734 - Merck AG) for all separations. Columns were prepared from a slurry of the packing in the eluent used. Separations were monitored by thin layer chromatography.

SECTION 1

Ethyl 3-oxo-4-phenylbutanoate (2)

Ethyl bromoacetate (36.5 cm^3) in dry benzene (125 cm^3) was added during 1.5 hours to a stirred refluxing mixture of freshly purified zinc (26.6 g; AnalaR grade powder was washed successively with 2% hydrochloric acid, water, ethanol, acetone and dry diethyl ether, and then heated to 100°C in vacuo for a short time: cf [24]), mercury (II) chloride (30 mg), phenylacetonitrile (1) (31.2 cm^3) and dry benzene (280 cm^3). The mixture was refluxed for a further 1 hour, and cooled, dilute sulphuric acid (100 cm^3, 2M) was added and the whole stirred for 2 hours. The organic layer was separated, the aqueous phase extracted with ether, and the combined organic layers washed with water (50 cm^3) and saturated sodium bicarbonate solution (50 cm^3). After concentration, distillation in vacuo afforded:- (i) phenylacetonitrile (1) (6.0 g); (ii) ethyl 3-oxo-4-phenylbutanoate (2) (24.0 g) b.p. 98-100°C/0.1 mm (Found: C, 69.8; H, 7.1. Calc. for $C_{12}H_{14}O_3$: C, 69.9; H, 6.8%). Cited [15], b.p. 154-156°C/9 mm.

Reaction of compound (2) with DAST

DAST (1 cm^3) was added to a solution of compound (2) (1.56 g) in dry benzene (5 cm^3) in an atmosphere of dry nitrogen. After being stirred at 20°C for 50 hours, the solution was poured into water (10 cm^3). After ether extraction and water washing and drying, evaporation of the ether left a red liquid (1.7 g). Glc analysis (A, 160°C, 9) showed 4 major

peaks (ratio 75:4:6:15). Separation by adsorption chromatography (column 60 x 2.4 cm, benzene eluent) gave:- (i) glc peak 2, compound (4) (0.07 g) (Z-ethyl 3,4-difluoro-4-phenylbut-3-enoate or Z-ethyl 2,3-difluoro-4-phenylbut-2-enoate), λ_{max} 225; (ii) unknown (0.02 g) under glc peak 1; (iii) glc peak 1, ethyl 3,3-difluoro-4-phenylbutanoate (3) (0.95 g), b.p. 90-91°C/0.3 mm (decomp.); (iv) glc peak 3, compound (5) (0.08 g) (E-ethyl 3,4-difluoro-4-phenylbut-3-enoate or E-ethyl 2,3-difluoro-4-phenylbut-2-enoate), λ_{max} 231; (v) 4th glc peak, compound (6) (0.2 g) (probably Z-ethyl 3-fluoro-4-phenylbut-3-enoate), b.p. 100-108°C/0.3 mm, λ_{max} 246.

Distillation of the crude product gave an increased proportion of compound (6) at the expense of (3).

Ethyl 3,3-difluoro-4-phenylbutanoate (3)

Sulphur tetrafluoride (5 cm^3) was slowly distilled into a small autoclave, held at -78°C and containing hydrogen fluoride (10 cm^3) and compound (2) (4.0 g). The autoclave was sealed, shaken for 4 hours at 15°C, cooled to -78°C, and the contents then poured into a cooled plastic beaker. When volatile materials had evaporated, water was added, the contents extracted with chloroform and the extracts dried and concentrated to leave a red liquid (2.8 g). Glc analysis (A, 170°C, 9) showed only 1 peak. The product in benzene was purified by chromatography (column 15 x 2 cm) to give compound (3), nc (2.6 g) (Found: C, 63.5; H, 6.1; F, 16.3. $C_{12}H_{14}F_2O_2$ requires C, 63.2; H, 6.2; F, 16.6%), identical to fraction (iii) above.

Nitration of compound (3)

Fuming nitric acid (0.2 cm^3) was added during 15 sec. to a stirred solution of compound (3) in trifluoroacetic anhydride (0.38 cm^3) at -30°C. After being stirred for 10 min, the mixture was poured into aqueous sodium bicarbonate and worked up in the usual way. The pale yellow liquid product (0.26 g) on glc analysis (B, 160°C, 8) showed 3 peaks (ratio 5:3:12). The nmr spectrum indicated mixed mono-nitro compounds, with the major component the para-nitro-isomer, but it could not be isolated pure. Nitration at higher temperatures gave di-nitro compounds.

SECTION 2

1-Bromo-2,2-difluoro-2-phenylethane (8)

DAST (2 cm^3) was added to a solution of 1-bromo-2-phenylethan-2-one (7) (3.0 g) in dry benzene (20 cm^3) in an atmosphere of dry nitrogen. After being stirred at 65°C for 48 hours, the mixture was poured into water. Extraction, washing, drying, and evaporation left a red liquid, separated by adsorption chromatography (column 38 x 2.4 cm; eluent benzene) to give:- (i) a colourless liquid, compound (8), nc (1.5 g) (Found: C, 44.2; H, 3.1; Br, 35.8; F, 16.7. $C_8H_7BrF_2$ requires C, 43.5; H, 3.2; Br, 36.1; F, 17.2%); (ii) recovered (7), (0.8 g).

4-Nitrophenylacetyl chloride (10)

4-Nitrophenylacetic acid (9) (30.0 g) and phosphorus pentachloride (38.0 g) were mixed in a dry atmosphere and then warmed at 100°C for 10 min. The volatile products were distilled off in vacuo, and the acid chloride (10) was decanted from the residual PCl_5 into a dry flask. The yield was quantitative, and the purity was sufficient for synthetic work and not improved by recrystallization. After rapid distillation in vacuo, a sample had b.p. 140°C/0.05 mm, m.p. 47-48°C (cited [25], 46-47°C).

1-Chloro-3-(4'-nitrophenyl)propan-2-one (11)

A solution of diazomethane (4.2 g) in ethanol-free diethyl ether (300 cm^3) was distilled into ether (40 cm^3) containing acid chloride (10) (10.0 g). The solution was stirred for 2 hours, and a stream of dry hydrogen chloride was passed through until no more nitrogen was evolved: It was then washed, neutralised with sodium bicarbonate solution, dried and evaporated to give a solid (10.5 g). Recrystallisation from aqueous ethanol afforded compound (11) as cream needles (6.4 g), m.p. 94-95°C (cited [18], 91-92°C).

1-Chloro-2,2-difluoro-3-(4'-nitrophenyl)propane (12)

DAST (2.2 cm^3) was added to a solution of propanone (11) (3.7 g) in dry benzene (20 cm^3) in an atmosphere of dry nitrogen. The mixture was stirred at 65°C for 48 hours, and then poured into water (25 cm^3).

Isolation as before left a red solid which was recrystallized from light petroleum (b.p. 40-60°C) to give compound (12), nc (2.3 g), m.p. 63°C (Found: C, 46.5; H, 3.4; Cl, 15.2; F, 15.7; N, 6.0. $C_9H_8ClF_2NO_2$ requires C, 45.9; H, 3.4; Cl, 15.1; F, 16.1; N, 6.0%).

Reaction of compound (12) with sodium cyanide

Compound (12) (1.2 g) was added to a suspension of dry sodium cyanide (0.4 g) in dry DMSO (4.0 cm^3), which was heated at 90°C for 40 min. Isolation in the usual way left a brown residue yielding no useful product, and showing no fluorine or nitrile peaks by ^{19}F nmr or ir.

SECTION 3

Reaction of acid chloride (10) with ethyl lithioacetate

Freshly dried and distilled ethyl acetate (3.5 g) in dry tetrahydrofuran (20 cm^3) was added dropwise during 5 min to a stirred solution of N-cyclohexyl-N-(1-methylethyl)amino-lithium [from the amine (11.3 g) and n-butyllithium (50 cm^3, 1.6M) in dry THF (60 cm^3)] at -78°C in a nitrogen atmosphere. The mixture was stirred for 10 min, and then added (under nitrogen) to 4-nitrophenylacetyl chloride (10) (8.0 g) in THF (20 cm^3) also at -78°C. After being stirred for 10 min, the mixture was quenched by careful addition of hydrochloric acid (15 cm^3, 20%). Isolation as usual afforded a yellow solid which was recrystallized from light petroleum (b.p. 40-60°C) to give white plates of N-(cyclohexyl)-N-(1'-methylethyl)-4-nitrophenylacetamide (13), nc (5.5 g), m.p. 76°C (Found: C, 67.4; H, 7.9; N, 9.5. $C_{17}H_{24}N_2O_3$ requires C, 67.1; H, 7.9; N, 9.2%).

Reaction of phenylacetyl chloride (14) with ethyl lithioacetate

Performed as above using ½ quantities and phenylacetyl chloride (14) (3.1 g) the reaction afforded a pale yellow liquid (5.8 g). Nmr spectra showed the presence of a little of the desired product (2). After distillation in vacuo, the major fraction (4.1 g) solidified. Recrystallization from light petroleum (b.p. 40-60°C)/carbon tetrachloride afforded N-(cyclohexyl)-N-(1'-methylethyl)phenylacetamide (15), nc, m.p. 110-112°C (Found: C, 78.7; H, 9.7; N, 5.2. $C_{17}H_{25}NO$ requires C, 78.7; H, 9.7; N, 5.4%).

Ethyl 4-(4'-nitrophenyl)-3-oxobutanoate (16)

(i) From ethyl 3-oxobutanoate

4-Nitrophenylacetyl chloride (10) (15.0 g) in dry 1,4-dioxan (50 cm^3) was added to the sodio-derivative from ethyl 3-oxobutanoate (10.5 cm^3) and sodium hydride (2.0 g) in dry dioxan (70 cm^3), the mixture being vibro-stirred. It was then heated at 100°C for 1.5 hours, cooled, and acidified with 60% acetic acid. The mixture was extracted with ether, and the extracts washed with water and concentrated. At 0°C, ammonium hydroxide solution (80 cm^3, s.g. 0.88) was added, and after being stirred for 30 min, the mixture was acidified with 4M hydrochloric acid and extracted with chloroform. The extracts were washed with aqueous sodium bicarbonate, dried, and distilled in vacuo to give ethyl 3-oxobutanoate (4.0 g). The residue (7.8 g) solidified on cooling, and was recrystallized from carbon tetrachloride/light petroleum (b.p. 40-60°C) to give pale yellow crystals (4.5 g) of compound (16), m.p. 80°C, identical to the product from method (ii).

(ii) From ethyl propanedioate

n-Butyl lithium in hexane (250 cm^3; 1.6M) was added slowly with stirring in an atmosphere of nitrogen to a solution, cooled to -70°C, of ethyl propanedioate [15] (22.5 g; dried over molecular sieves) in dry tetrahydrofuran (500 cm^3) containing 2,2'-bipyridyl (10 mg). The temperature was allowed to rise slowly throughout and the end-point when a pink colour persisted, was reached at -5°C. The system was then recooled to -70°C, and freshly prepared acid chloride (10) (21.0 g) in dry THF (60 cm^3) was added during 2 min. The temperature rose to -55°C and a light red colour developed. Stirring was continued for 10 min further, and the temperature fell back to -70°C. The mixture was poured slowly into a stirred and cooled mixture of ether (800 cm^3) and hydrochloric acid (400 cm^3; 1M). The organic phase was separated, washed, dried and the ether evaporated off to leave a pale yellow solid (28.0 g). Recrystallization from aqueous ethanol gave ethyl 4-(4'-nitrophenyl)-3-oxobutanoate (16) (21.0 g), m.p. 78-79°C, sufficiently pure for conversion to (17). Further recrystallization gave white needles m.p. 81.0-81.5°C (cited [19], 82°C) (Found: C, 57.4; H, 5.1; N, 5.6. Calc. for $C_{12}H_{13}NO_5$: C, 57.4; H, 5.2; N, 5.6%).

SECTION 4

Ethyl 3,3-difluoro-4-(4'-nitrophenyl)butanoate (17)

Compound (16) (19.0 g), hydrogen fluoride (21 cm^3) and sulphur tetrafluoride (21 cm^3) were shaken together for 2.5 hours, as in the synthesis of compound (3). The crude red liquid product (19.0 g) was distilled in vacuo to give a colourless liquid (b.p. 131°C/0.08 mm) which solidified (m.p. 45-46°C). Recrystallization from light petroleum (b.p. 40-60°C)/ carbon tetrachloride gave compound (17), nc (16.4 g), m.p. 47-48°C (Found: C, 52.8; H, 4.7; F, 13.9; N, 5.2. $C_{12}H_{13}F_2NO_4$ requires C, 52.7; H, 4.8; F, 13.9; N, 5.1%). An unidentified by-product was present in the mother-liquors.

Ethyl 4-(4'-aminophenyl)-3,3-difluorobutanoate (18)

Nitro-compound (17) (15.2 g) in ethanol (250 cm^3) containing 10% palladised carbon (2.6 g) was shaken at 5°C in an atmosphere of hydrogen at room pressure. When the theoretical volume of hydrogen had been consumed, the solution was filtered and concentrated. Water (25 cm^3) was added, and the system ether-extracted, concentrated and distilled in vacuo to give compound (18), nc (12.0 g), b.p. 127-129°C/0.05 mm (Found: C, 59.0; H, 6.1; F, 15.6; N, 5.6. $C_{12}H_{15}F_2NO_2$ requires C, 59.2; H, 6.2; F, 15.6; N, 5.8%). Hydrogen chloride passed through a solution in ether, afforded the hydrochloride, m.p. 181-184°C.

Ethyl 4-[4'-bis(2"-hydroxyethyl)aminophenyl]-3,3-difluorobutanoate (19)

Oxirane (8.1 cm^3) was added to a solution of amine (18) (13.8 g) in acetic acid (75 cm^3; 16M) at 0°C. The system was allowed to warm to 15°C, and was then stirred for 72 hours. The solution was concentrated, poured into excess bicarbonate solution and ether extracted. Concentration of the extracts left a sticky yellow solid (17.5 g) used in the next stage. A portion (2.0 g) was recrystallized from carbon tetrachloride/light petroleum (b.p. 40-60°C) to give compound (19), nc (1.5 g), white needles m.p. 66.0-66.5°C. (Found: C, 57.7; H, 7.0; F, 11.3; N, 4.5. $C_{16}H_{23}F_2NO_4$ requires C, 58.0; H, 7.0; F, 11.5; N, 42.%).

Reaction of compound (19) with carbon tetrachloride and triphenylphosphine

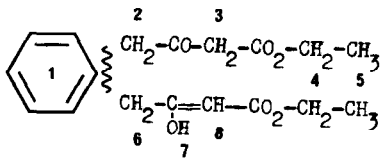
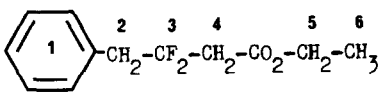
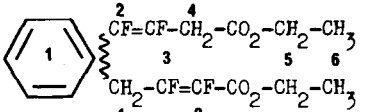
A solution of crude compound (19) (15.5 g) in dry carbon tetrachloride (50 cm^3) was added to a stirred solution of triphenylphosphine (24.7 g) in dry carbon tetrachloride (50 cm^3) in an atmosphere of dry nitrogen. After 3 hours of refluxing, a light brown precipitate had formed: this was filtered off from the hot solution, and washed with ether (50 cm^3). The combined organic solutions were concentrated and the residue extracted with refluxing ether (2 x 100 cm^3). The combined extracts were cooled, filtered and concentrated to half volume. This solution was refluxed for a short while, cooled, filtered and the filtrate concentrated to a viscous oil (17.6 g). Adsorption chromatography (column 51 x 2.5 cm; solvent benzene/diethyl ether 9:1) afforded: (i) ethyl 4-[4'-bis(2"-chloroethyl)aminophenyl]-3,3-difluorobutanoate (20), nc (9.6 g); (ii) unknown, contaminated with (20) (0.4 g); (iii) ethyl 3,3-difluoro-4-(4'-morpholinylphenyl)butanoate (21), nc (2.3 g); (iv) ethyl 4-[4'-(2"-chloroethyl)(2'''-hydroxyethyl)aminophenyl]-3,3-difluorobutanoate (22), nc (1.1 g); (v) triphenylphosphine oxide. Short path distillations in vacuo of fractions (i), (iii) and (iv), respectively, gave analytical samples of compound (20), b.p. 160°C/0.02 mm (Found: C, 52.5; H, 5.8; Cl, 19.0; F, 10.2; N, 4.0. $C_{16}H_{21}Cl_2F_2NO_2$ requires C, 52.2; H, 5.8; Cl, 19.3; F, 10.3; N, 3.8%). compound (21), b.p. 150°C/0.02 mm (Found: C, 61.2; H, 6.8; F, 11.8; N, 4.6. $C_{16}H_{21}F_2NO_3$ requires C, 61.3; H, 6.8; F, 12.1; N, 4.5%); and compound (22), b.p. 160°C/0.02 mm (Found: C, 55.2; H, 6.3; Cl, 10.4; F, 10.7; N, 4.3. $C_{12}H_{22}ClF_2NO_3$ requires C, 54.9; H, 6.3; Cl, 10.1; F, 10.9; N, 4.0%); all were colourless viscous liquids.

3,3-Difluorochlorambucil (23)

The dichlorinated ester (20) (4.68 g) and concentrated hydrochloric acid (35 cm^3) were stirred together at 75°C for 1½ hours. The cooled solution was diluted with water (120 cm^3) and ether-extracted. The crude solid product (4.4 g) was recrystallized from carbon tetrachloride/light petroleum (b.p. 40-60°C) to give colourless plates of 4-[4'-bis(2"-chloroethyl)aminophenyl]-3,3-difluorobutanoic acid (3,3-difluorochlorambucil) (23), nc (3.1 g), m.p. 86-87°C (Found: C, 49.4; H, 5.1; Cl, 20.5; F, 11.5; N, 4.4. $C_{14}H_{17}Cl_2F_2NO_2$ requires C, 49.4; H, 5.0; Cl, 20.8; F, 11.2; N, 4.1%). This acid was somewhat sensitive to light and it decomposed slowly on being heated above 50°C.

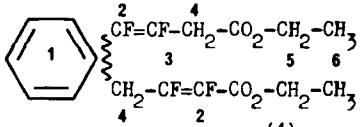
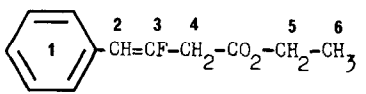
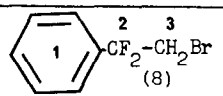
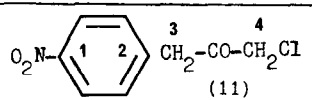
TABLE

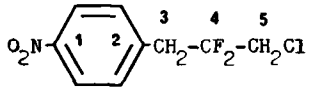
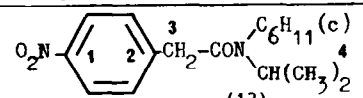
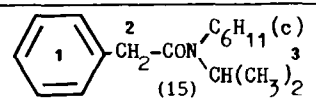
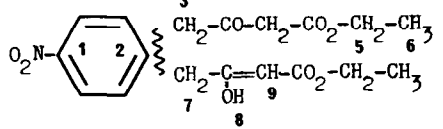
Nmr spectra

Formula, Compound Number, Solvent	Positional Assignment	Signal	Relative Intensity	Chemical Shift
 (2) CCl_4	1	bs	5	7.17
	2	s	1.53	3.68
	3	s	1.53	3.27
	4	q,J=7	2	4.04
	5	t	3	1.17
	6	s	0.47	3.40
	7	bs	0.24	12.2
	8	s	0.24	4.81
 (3) CCl_4	1	bs	5	7.28
	2	t,J=16	2	3.36
	4	t,J=14	2	2.69
	5	q,J=7	2	4.14
	6	t	3	1.25
	3	tt	-	91.9
 (4) CCl_4	1	bs	5	7.27
	4	dd,J=3	2	4.07
	5	q,J=7	2	4.31
	6	t	3	1.37
	2	dt,J_d=0-4	1	153.6
	3	dt,J_t=26	1	107.0

(Continued)

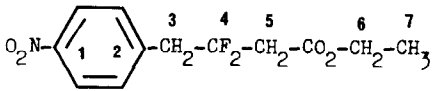
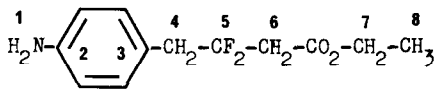
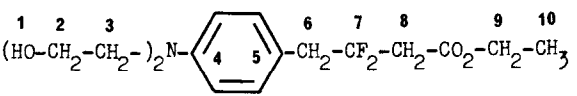
(TABLE (Cont.)

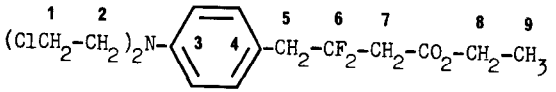
Formula, Compound Number, Solvent	Positional Assignment	Signal	Relative Intensity	Chemical Shift
 (4) $CDCl_3$	1	bs	5	7.24
	4	dd,J=6	2	3.68
	5	q,J=7	2	4.15
	6	t	3	1.29
	2	dt,J_d=130	1	167.7
	3	dt,J_t=23	1	145.1
 (6) CCl_4	1	cm	5	7.1-7.6
	2	d,J=38	1	5.58
	4	d,J=19	2	3.25
	5	q,J=7	2	4.15
	6	t	3	1.27
	3	dt	-	101.0
 (8) Neat	1	bs	5	7.18
	3	t,J=14	2	3.42
	2	t	-	97.9
 (11) $CDCl_4$	1	AA'BB',J=9	1	8.18
	2	AA'BB'	1	7.41
	3	s	1	3.75
	4	s	1	4.45

 (12) CCl_4	1	AA'BB',J=8	1	8.13
	2	AA'BB'	1	7.50
	3	t,J=16	1	3.41
	5	t,J=12	1	3.51
	4	tt	-	100.6
 (13) $CDCl_3$	1	AA'BB',J=9	1	8.20
	2	AA'BB'	1	7.44
	3	s	1	3.78
	4	cm	9	0.8-4.0
 (15) CCl_4	1	bs	5	C7.2
	2	s	2	3.56
	3	cm	18	0.8-4.0
 (16) $CDCl_3$	1	AA'BB',J=9	2	8.19
	2	AA'BB'	2	7.41
	3	s	1.6	4.01
	4	s	1.6	3.53
	5	q,J=7	2	4.19
	6	t	3	1.26
	7	s	0.4	3.60
	8	-	-	-
	9	s	0.2	4.98

(Continued)

TABLE (Cont.)

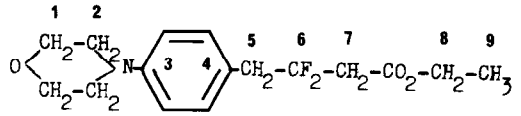
Formula, Compound Number, Solvent	Positional Assignment	Signal	Relative Intensity	Chemical Shift
 (17) $CDCl_3$	1	AA'BB',J=9	2	8.19
	2	AA'BB'	2	7.52
	3	t,J=16	2	3.53
	5	t,J=14	2	2.85
	6	q,J=7	2	4.23
	7	t	3	1.29
	4	tt	-	92.7
 (18) $CDCl_3$	1	bs	2	4.05
	2	AA'BB',J=9	2	6.63
	3	AA'BB'	2	7.08
	4	t,J=16	2	3.23
	6	t,J=15	2	2.78
	7	q,J=7	2	4.18
	8	t	3	1.26
	5	tt	-	92.9
 (19) $CDCl_3$	1	bs	2	4.33
	2	AA'BB',J=4	4	3.76
	3	AA'BB'	4	3.45
	4	AA'BB',J=9	2	6.95
	5	AA'BB'	2	7.10
	6	t,J=17	2	3.20
	8	t,J=14	2	2.77
	9	q,J=7	2	4.15
	10	t	3	1.25
	7	tt	-	93.2



(20)

$CDCl_3$

1,2	cm	8	C3.65
3	AA'BB',J=9	2	6.62
4	AA'BB'	2	7.18
5	t,J=17	2	3.25
7	t,J=14	2	2.78
8	q,J=7	2	4.18
9	t	3	1.28
6	tt	-	93.0



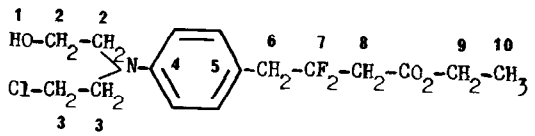
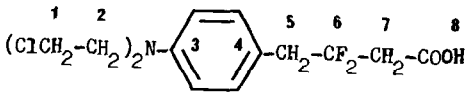
(21)

$CDCl_3$

1	m	4	C3.82
2	m	4	C3.10
3	AA'BB',J=9	2	6.88
4	AA'BB'	2	7.21
5	t,J=16	2	3.28
7	t,J=14	2	2.75
8	q,J=7	2	4.18
9	t	3	1.25
6	tt	-	92.8

(Continued)

TABLE (Cont.)

Formula, Compound Number, Solvent	Positional Assignment	Signal	Relative Intensity	Chemical Shift
 (22) $CDCl_3$	1	bs	1	2.19
	2	m	4	C3.55
	3	cm	4	C3.63
	4	AA'BB',J=9	2	6.69
	5	AA'BB'	2	7.14
	6	t,J=17	2	3.23
	8	t,J=15	2	2.79
	9	q,J=7	2	4.17
	10	t	3	1.27
	7	tt	-	93.1
 (23) CD_3COCD_3	1,2	cm	8	3.75
	3	AA'BB',J=9	2	6.77
	4	AA'BB'	2	7.19
	5	t,J=17	2	3.30
	7	t,J=15	2	2.96
	8	bs	1	7.5
	6	tt	-	92.7

Spectroscopy

Ultraviolet spectra were recorded on a Unican SP800 instrument, all samples being dissolved in spectroscopic ethanol.

Infrared spectra were recorded on a Perkin Elmer 257 instrument as nujol mulls or liquid films.

Nuclear magnetic resonance spectra were measured on a Perkin Elmer R12B spectrometer, ^{1}H spectra at 60 MHz using tetramethyl silane as internal reference, and ^{19}F spectra at 56.4 MHz using trichlorofluoromethane as internal standard. Signals are denoted by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), AA'BB', c (complex), and b (broad). C before the chemical shift value indicates the centre of an involved multiplet. Where a coupling is indicated for a peak, the corresponding coupling was present also in the other appropriate peaks.

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