POTENTIAL ANTICANCER AGENTS: PHARMACOLOGICALLY ACTIVE BENZO(b)THIOPHENE DERIVATIVES

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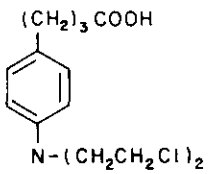
Abstract - The synthesis of potential antitumor compounds namely, 5-bis[2-(chloroethyl)-amino-(3-benzo(b)thienyl)]-α-aminopropionic acid and 5-bis{(2-chloroethyl)amino-benzo-(b)thiophene)]-3-propionic acid, using the key intermediate 5-nitro-3-methylbenzo(b)thiophene, is described.

Alkylating agents of various types probably constitute the largest single group of antitumor agents and of these nitrogen mustards have received most attention. One of the most useful alkylating agents in the clinic[1] is chloroambucil, 4-[bis(2-chloroethyl)amino]phenylbutyric acid (1).[2] Similarly, sarcolysin (II) is very active against the standard three-tumor system as well as against Walker Sarcoma 256.[3]

If one accepts the so-called "carrier" hypothesis as it relates to mechanism of drug action, the observation of Cole and Mathews[4] that labelled tryptophan is localized in a metastatic human tumor to a considerably greater extent than in certain normal tissues, becomes of interest and suggests that the scheme may be used to direct the pharmacodynamic mustard function selectively to malignant cells. Triggle[5] has suggested that the carrier portion of the molecule must fit some part of the



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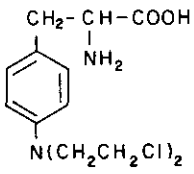
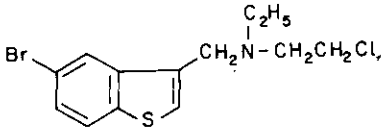


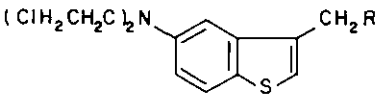
II

site of action of such drugs results from an intially reversible adsorption of the carrier portion of this site. Chapman et al.[6,7] have observed considerable pharmacological activity in a number of



III

benzo(b)thiophenes particularly in 5-bromo-3-(N-2-chloroethyl-N-ethylaminoethyl)benzo(b)thiophene (III). This compound proved significantly active as an antitumor agent, which showed that benzo(b)-thiophene nucleus acts as an effective carrier portion of the molecule. We decided therefore to synthesise the 5-substituted mustard (IV) of benzo(b)thiophene analog of tryptophan and 3-propionic acid derivative (V) for evaluation of their biological activity.



IV, R = $-CH(NH_2)-COOH$

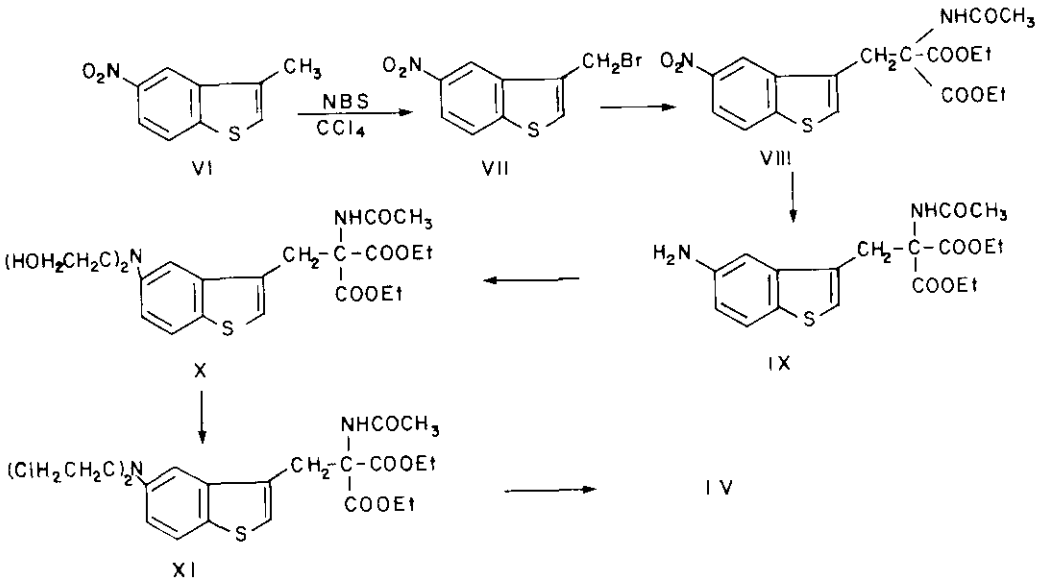
V, R = CH_2COOH

The key intermediate 5-nitro-3-methylbenzo(b)thiophene (VI) was prepared according to the procedure of Chapman et al.[8]

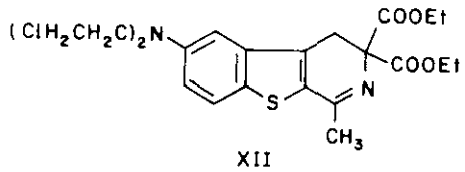
Bromination of (VI) with N-bromosuccinimide in boiling carbon tetrachloride resulted in the formation of 3-bromomethyl compound (VII) which reacted smoothly with diethyl acetamidomalonate in the presence of sodium ethoxide to give the diethyl 5-nitro-3-benzo(b)thienyl acetamidomalonate (VIII) in 80% yield.

The nitro diester (VIII) was reduced with Raney nickel and hydrazine hydrate in ethanol to give the amino diester (IX) as a crystalline solid in 85% yield which was characterized by its i.r. and H^1-n.m.r. spectra.

The amino diester (IX) reacted with ethylene oxide in methanol in the presence of catalytic amount of p-toluenesulphonic acid over a period of 24 h yielding the bis-hydroxyethylated compound (X) as a thick viscous liquid which crystallized from ethanol as a colourless powder in 75% yield. Its infrared spectrum conveyed a strong hydroxyl absorption at 3300 cm^{-1} which distinctly blended with the amino absorption of the acetamido group. The characteristic feature of its H^1-n.m.r. spectrum, recorded in deuterated chloroform, was the appearance of an eight-proton multiplet having a chemical shift in the range δ 3.46-3.83, which was allocated to the bis-hydroxyethyl protons on the basis of the H^1-n.m.r. spectrum of the precursor (IX).

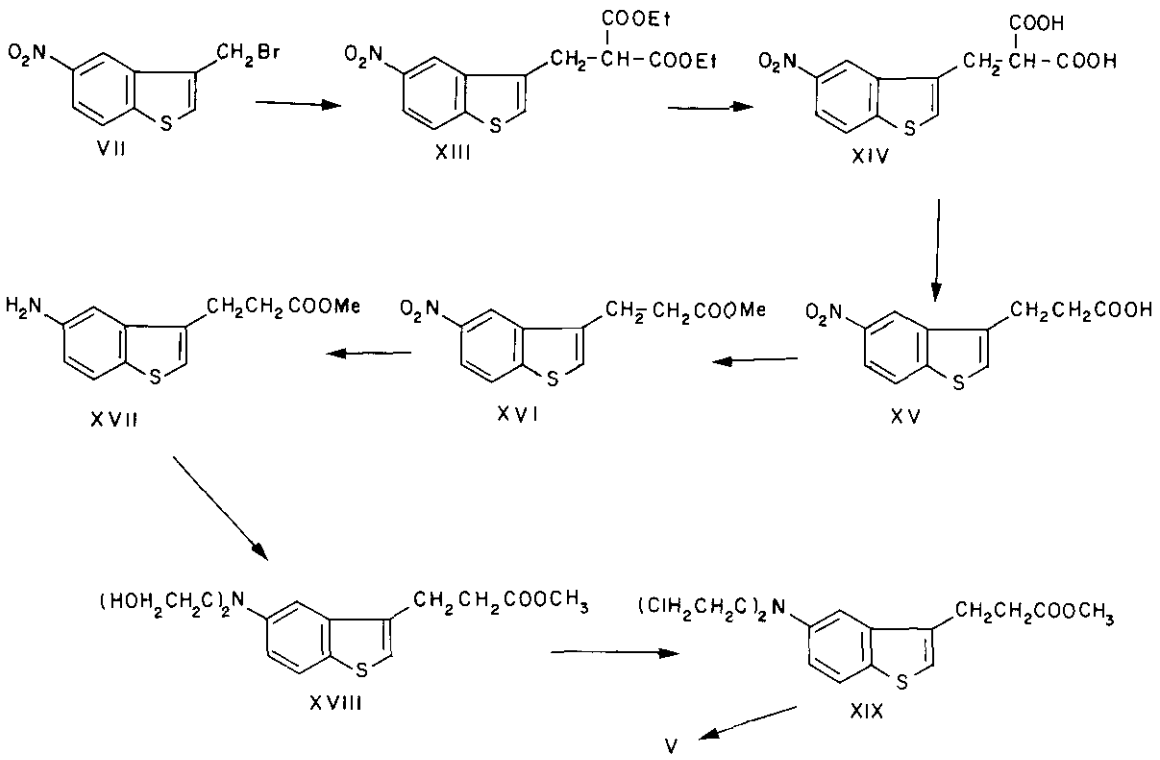


When (X) was allowed to react with freshly distilled thionyl chloride in chloroform, the desired blocked mustard (XI) was otained as a colourless solid. However, due to the extremely hygroscopic nature of the compound, it could not be isolated in pure form. In this reaction no harman type compound (XII) was isolated.



Hydrolysis of the blocking groups with concentrated hydrochloric acid at reflux proceeded very smoothly to afford the mustard (IV) as a crystalline precipitate in low yield. The amino acid was best precipitated by careful adjustment of the hydrolysate to pH 5-6 with saturated sodium hydrogen carbonate solution.

The preparation of the propionic acid mustard (V) started from 5-nitro-3-bromomethylbenzo(b)thiophene (VII). Reaction of (VII) with a large excess of diethyl malonate in alcoholic solution of sodium ethoxide afforded the substituted malonic ester (XIII) as a crystalline solid of mp 95-96°C. The use of smaller amount of diethyl malonate gave diethyl bis(5-benzo(b)thiophene-3-ylmethyl)malonate of mp. 198-199°C as a major product of the reaction. Saponification of XIII followed by acidi-



fication, afforded a solid assumed to be the diacid XIV, which was decarboxylated at 180-190°C to the propionic acid (XV). The overall yield of (XV) from (VII) was 57%.

Conventional esterification followed by reduction with hydrazine hydrate in the presence of Raney nickel, yielded the amino ester (XVII) as a light pink coloured solid in 92% yield. The bis(2-hydroxyethyl)amine (XVIII) was obtained as a viscous oil which did not crystallize. Treatment of XVIII with thionyl chloride in chloroform afforded the bis(2-chloroethyl)amine (XIX) as a crystalline solid in low yield. Use of pyridine as a solvent gave only dark intractable products. Hydrolysis of XIX with concentrated hydrochloric acid followed by adjustment to pH 5-6 with saturated sodium hydrogen carbonate solution gave the required compound (V) as tan coloured crystals in 19.5% yield.

EXPERIMENTAL

Melting points were determined in capillary tubes (electro-thermal melting point apparatus) and are uncorrected. Infrared (IR) spectra (KBr disc or thin film) were measured on Perkin Elmer 520B spectrophotometer. The H^1-NMR spectra were obtained with a 60 MHz Varian T60 A Spectrometer in the indicated solvents. Chemical shifts and coupling constants were measured in ppm (δ) and J (Hz) with respect to TMS. Microanalyses were performed by Prof. Malissa and G. Reuter, Analytisches Laboratorium, BRD.

3-Bromomethyl-5-nitrobenzo(b)thiophene (VII)

Benzoyl peroxide (500 mg) was added to a vigorously stirred solution of 3-methyl-5-nitrobenzo(b)thiophene (VI) (20 g, 0.102 mole) in pure dry carbon tetrachloride (1200 ml). N-Bromosuccinimide (18.2 g, 0.102 mole) was added in small portions to the boiling mixture which was irradiated by two 200 W electric bulbs. The mixture was refluxed for 1.5 h, cooled and filtered. Concentration of the filtrate in vacuo to ca. 50 ml caused most of the product (VII) to precipitate out. Recrystallisation from benzene gave (VII), as yellow needles, mp 162-183°C, yield 16 g (58%), IR (KBr) (νcm^{-1}): 3100 (aromatic C-H), 1340 (NO_2); ^{1}H-NMR ($CDCl_3$): 4.8 (2H, s, $-CH_2-Br$), 7.2-8.4 (4H, m, aromatic protons). Anal. Calcd. for $C_9H_6BrNO_2S$: C, 39.72; H, 2.22; N, 5.14. Found: C, 39.38; H, 2.16; N, 4.95.

Diethyl acetamido(5-nitro-3-benzo(b)thienylmethyl)malonate (VIII)

To a solution of sodium (2.3 g, 0.10 g. atom) in absolute ethyl alcohol (200 ml) was added ethyl acetamidomalonate (21.7 g, 0.10 mole) followed by bromo compound (VII) (27.29 g, 0.10 mole) in small portions. The mixture was refluxed with stirring for 3 h and then poured into cold water (1000 ml). The precipitate was filtered, washed with water and dried. Recrystallisation from benzene gave (VIII), yellow needles, mp 172-173°C, yield 34.6 g (65%); IR (KBr) (νcm^{-1}): 3350 (NH), 1760 (ester C=O), 1670 (amide C=O); ^{1}H-NMR ($CDCl_3$): 1.27 (6H, t, J 7, $(\underline{CH_3}CH_2-)_2$), 2.0 (3H, s, $\underline{CH_3}CONH$), 3.97 (2H, s, Ar-$\underline{CH_2}$-), 4.23 (4H, q, J 7, $(CH_3\underline{CH_2}-)_2$), 7.03 (1H, bs, $CH_3CO\underline{NH}$), 7.3-8.3 (4H, m, aromatic protons). Anal. Calcd for $C_{18}H_{20}N_2O_7S$: C, 52.94; H, 4.90; N, 6.86. Found: C, 52.78; H, 4.93; N, 6.75.

Diethyl acetamido(5-amino-3-benzo(b)thienylmethyl)malonate (IX)

To a solution of the nitro ester (VIII) (20 g, 0.05 mole) in ethyl alcohol (150 ml) was added 85% aqueous hydrazine hydrate (20 ml) followed by Raney nickel catalyst (2g). When the initial vigorous reaction had subsided more catalyst (0.5 g) and hydrazine hydrate (5 ml) were added and the mixture was refluxed for 1 h. The catalyst was filtered off and the remaining solution was treated with charcoal, heated and filtered. Removal of the solvent yielded a viscous oil which on scratching solidified. Recrystallisation from ethyl alcohol gave (IX), colourless plates, mp 125-126°C, yield 17 g (17%); IR (KBr) (νcm^{-1}), 3420, 3330 3250 (NH_2, NH), 1740 (ester C=O), 1660 (amide C=O); ^{1}H-NMR ($CDCl_3$); 1.26 (6H, t, J 7 $(\underline{CH_2}CH_2)_2$), 1.93 (3H, s, $\underline{CH_3}CONH$), 3.70 (2H, bs, $ArN\underline{H_2}$), 3.80 (2H, s, Ar-$\underline{CH_2}$), 4.26 (4H, 1, J 7 $(CH_3\underline{CH_2})$, 6.96 (1H, bs, $CH_3CO\underline{NH}$), 6.5-7.7 (4H, m, aromatic protons). Anal. Calcd for $C_{18}H_{22}N_2O_5S$: C, 57.14; H, 5.82; N, 7.40. Found: C, 57.00; H, 5.80; N, 7.36.

Diethyl acetamido[5-bis(2-hydroxyethyl)amino-3-benzo(b)thienylmethyl)]malonate (X)

To an ice cold suspension of the amino diester (IX) (15.0 g, 0.04 mole) in methyl alcohol (150 ml) containing p-toluenesulphonic acid (150 mg) was added ethylene oxide (30 ml) and the mixture was stirred at 25°C for 24 h. A saturated solution of $NaHCO_3$(10 ml) was added. Evaporation to dryness

in vacuo yielded a residue which was extracted with dichloromethane (50 ml), the extract was washed with water (50 ml) and dried ($MgSO_4$). Removal of solvent gave a gummy residue which after evacuation on an oil pump for 2 h followed by scratching, solidified. Recrystallisation from ethyl alcohol afforded (X), mp 129-130°C, yield 17.3 g (97%); IR (KBr) (ν cm^{-1}), 3300 (NH, OH), 1740 (ester >C=O), 1650 (amide (>C=O); ^{1}H-NMR ($CDCl_3$): 1.06 (6H, t, J 7 ($\underline{CH_3}CH_2$)), 1.86 (3H, s, $\underline{CH_3}CONH$), 3.0-4.0 (2H, br, ($CH_2\underline{CH_2}OH$), 3.46-3.83 (8H, m, $\underline{CH_2}\underline{CH_2}$-OH)$_2$), 3.85 (2H, s, Ar-$\underline{CH_2}$), 4.06 (4H, q, J 7, ($CH_3\underline{CH_2}$)$_2$), 6.90 (1H, bs, $CH_3CO\underline{NH}$), 6.5-7.6 (4H, m, aromatic protons). Anal. Calcd for $C_{22}H_{30}N_2O_7S$: C, 56.65; H, 6.43; N, 6.00. Found: C, 56.52; H, 6.42; N, 5.86.

Diethyl acetamido[5-bis(2-chloroethyl)amino-3-benzo(b)thienylmethyl]malonate (XI)

To a solution of the bishydroxyethyl compound (X) (13.98, 0.03 mole) in dry chloroform (150 ml) was added freshly distilled thionyl chloride (10.7 g, 0.09 mole). The mixture was refluxed for 2 h. Removal of the solvent and excess thionyl chloride left a residue which solidified in dry ether. Recrystallisation from ethyl alcohol gave (XI). The product was extremely hygroscopic and could not be isolated in pure form. The strong hydroxyl absorption apparent in the IR of precursor (X) was absent in the IR spectrum of the product (XI). IR (KBr) (ν cm^{-1}), 3400 (NH), 1740 (ester >C=O), 1670 (amide >C=O). The product was used directly for the next step.

[5-Bis(2-chloroethyl)amino-(3-benzo(b)thienyl)]α-amino-3-propionic acid (IV)

A solution of the blocked mustard (XI) (12.5 g) in concentrated hydrochloric acid (125 ml) was heated under reflux for 5 h. The solution was cooled and its pH adjusted to 5-6 with a saturated $NaHCO_3$ solution. After overnight chilling the solid was filtered, washed with water and dried. The product (IV) was obtained as pink crystals, yield 4.9 g (50%), which had no definite mp. It showed gradual decomposition above 190°C. TLC analysis: single spot in solvent system (isopropyl alcohol 2-N, Hydrochloric acid) identified by ninhydrin spray. A typical Zwitterionic amino acid infrared spectrum was obtained for (IV). IR (KBr) (ν cm^{-1}), 2500-2600 (N^+H_3), 1600 (CO_2^-). Anal. Calcd for $C_{15}H_{17}Cl_2N_2O_2S$: C, 50.00; H, 4.75; N, 7.77; Cl, 19.68. Found: C, 50.40; H, 4.82; N, 7.55; Cl, 19.42.

Diethyl (5-nitrobenzo(b)thiophene-3-ylmethyl)malonate (XIII)

To a solution of sodium (2.3 g, 0.10 g. atom) in absolute ethyl alcohol (100 ml) was added diethyl malonate (64.08 g, 0.4 mole) followed by 3-bromomethyl-5-nitrobenzo(b)thiophene (VII) (27.2 g, 0.10 mole) in small portions. The mixture was refluxed with stirring for 3 h and then poured into cold water. The precipitated solid was filtered, washed with water and dried. Recrystallisation from ethyl alcohol afforded the diethyl ester (XIII) as yellow needles, mp 97-98°C, yield (85%); IR (KBr) (ν cm^{-1}), 1730 (ester >C=O), 1340 (NO); ^{1}H-NMR ($CDCl_3$): 1.26 (6H, t J 7, ($\underline{CH_2}CH_2$)$_2$), 3.23 (2H, d, J 7, Ar-$\underline{CH}$ -CH<), 3.75 (1H, t, J 7, Ar-CH_2-CH<), 7.2-8.4 (4H, m, aromatic protons); Anal. Calcd. for $C_{16}H_{17}N\ O_6S$: C, 54.70; H, 4.84; N, 3.98. Found: C, 54.70; H, 4.73; N, 4.23. When the reaction was

conducted with equimolar quantities of the bromo compound (VII) and diethyl malonate, the major product isolated was a compound, mp 198-199°C, postulated to be diethyl bis-(5-nitrobenzo(b)thienyl-3-methyl)malonate. Anal. Calcd for $C_{25}H_{22}N_2O_8S$: C, 55.30; H, 4.05; N, 5.16. Found: C, 55.11; H, 4.05; N, 5.14.

5-Nitrobenzo(b)thiophene-3-β-propionic acid (XV)

A solution of the malonic ester (XVI) (35.1 g, 0.10 mole) in ethyl alcohol (200 ml) and 10% sodium hydroxide solution (300 ml) was refluxed for 3 h and the alcohol was evaporated in vacuo. The aqueous residue was diluted with water (750 ml) to dissolve the crystalline sodium salt of the product. The resulting solution was made strongly acidic with conc. hydrocloric acid and extracted with ethyl acetate (3 x 500 ml) and dried ($MgSO_4$). Evaporation of the solvent gave yellow crystals presumed to be diacid (XIV). The material was heated at 180-190°C for 0.75 h after which the evolution of CO_2 gas ceased. The melt was extracted with dichloroethane several times. The combined extracts were left standing overnight affording the acid (XV) as light brown plates, mp 199-200°C, yield 20 g (89%); IR (KBr) (ν cm^{-1}): 3340 (broad (OH), 1710 (acid >C=O), 1340 (NO_2); Anal. Calcd for $C_{11}H_9NO_4S$: C, 52.90; H, 3.61; N, 5.57. Found: C, 52.99; H, 3.69; N, 5.39.

Methyl 5-nitrobenzo(b)thiophene-3-β-propionate (XVI)

A solution of the acid (XV) (12.5 g, 0.05 mole) in methyl alcohol (250 ml) containing conc. H_2SO_4 acid (1 ml) was refluxed for 4 h and evaporated to dryness in vacuo. The residue was dissolved in dichloromethane (100 ml), washed with saturated $NaHCO_3$ solution (20 ml) and the organic layer dried ($MgSO_4$). Evaporation yielded a dark coloured residue which was chromatographed on a column of alumina. Elution with ether afforded the desired methyl ester (XVI) which crystallised from ethyl alcohol as pink needles, mp 110-111°C, yield 11 g (80.7%); IR (KBr) (ν cm^{-1}); 1735 (ester >C=O), 1340 (NO_2); ^{1}H-NMR ($CDCl_3$): 2.83 (2H, t, J 7, Ar-$\underline{CH_2}CO_2Me$), 3.26 (2H, t, J 7, Ar$\underline{CH_2}CH_2CO_2Me$), 3.66 (3H, s, $CO_2\underline{Me}$), 7.6-8.4 (4H, m, aromatic protons). Anal. Calcd for $C_{12}H_{11}NO_4S$: C, 54.30; H, 4.18; N, 5.18. Found: C, 54.48; H, 4.33; N, 4.81.

Methyl 5-aminobenzo(b)thiophene-3-β-propionate (XVII)

The preparation of this compound was carried out according to the procedure described previously for compound (IX). The amino compound (XVII) crystallised from ethyl alcohol as pink plates, mp 126-127°C, yield (80%); IR (KBr) (ν cm^{-1}): 3440, 3460 (NH_2), 1724 (ester >C=O), there was no NO_2 band near 1340; ^{1}H-NMR ($CDCl_3$): 2.76 (2H, t, J 7, $ArCH_2$-$\underline{CH_2}CO_2Me$), 3.00 (2H, t, J 7, $Ar\underline{CH_2}CH_2CO_2Me$), 2.7-3.3 (2H, bs, $N\underline{H_2}$), 3.63 (3H, s, -$CO_2\underline{Me}$), 6.6-7.8 (4H, m, aromatic protons). Anal. Calcd for $C_{12}H_{13}NO_2S$: C, 65.73; H, 5.97; N, 6.38. Found: C, 65.61; H, 5.91; N, 6.25.

Methyl [5-bis(2-hydroxyethyl)aminobenzo(b)thiophene]-3-β-propionate (XVIII)

The preparation of this compound followed the procedure described for compound (X) above. Compound (XVIII) was obtained as a yellowish syrup, IR (thin film) (ν cm^{-1}): 3400 (OH), 1739 (ester >C=O). The oil could not be crystallised and was used as such for the next step.

Methyl [5-bis(2-chloroethyl)aminobenzo(b)thiophene]-3-β-propionate (XIX)

This compound was prepared according to the procedure described for compound (XI). Recrystallisation from ethyl alcohol afforded (XIX), colourless needles, mp 136-137°C, yield (55%); IR (KBr) (ν cm^{-1}): 1740 (ester >C=O). Anal. Calcd for $C_{16}H_{19}Cl_2NO_2S$: C, 53.33; H, 5.27; N, 3.66. Found: C, 53.01; H, 5.18; N, 3.68.

[5-Bis(2-chloroethyl)amino(3-benzo(b)thiophene)]-3-β-propionic acid (V)

A suspension of the ester (XIX) (20 g, 0.56 mole) in conc. HCl (100 ml) was heated under reflux with stirring for 5 h. The reaction mixture was cooled overnight and its pH was adjusted to 5-6 with a saturated $NaHCO_3$ solution to give a precipitate which was filtered, washed with water and dried. Recrystallisation from ethyl alcohol yielded the product (V) as a light brown powder, mp 146-147°C, yield 4 g (19.5%); IR (KBr) (ν cm^{-1}): 3400 (broad OH), 1710 (acid >C=O); ^{1}H-NMR (DMSO-d_6): 2.66 (2H, bt, J 7, CH_2-CH_2-CO_2H), 3.05 (2H, bt, J 7, $CH_2CH_2CO_2H$), 3.57-3.83 (8H, m, smeared by DMSO signal, -N(CH_2CH_2Cl)), 6.66-7.75 (4H, m, aromatic protons). Anal. Calcd for $C_{15}H_{17}Cl_2NO_2S$: C, 52.02; H, 4.94; N, 4.04; Cl, 20.50. Found: C, 51.91; H, 4.92; N, 4.10; Cl, 20.20.

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REFERENCES

1. R.W. Renoles, J. Griszles, W.N. Bell, C.C. Corlay, W.E. Frommeyat, H.C. Greenberg, G.W. James, III, R. Riser, Jr., and S.J. Wilson, Am. J. Med., 27, 424 (1959).
2. J.L. Everett, J.J. Roberts, and W.C.K. Rose, J. Chem. Soc., 2386 (1953).
3. Cancer Chemotherapy. Report No. 6, P.61, 1966, published by Cancer Chemotherapy National Services Centre, National Cancer Institute.
4. J.W. Cole, and L.M. Mathews, A.M.R. Arch. Surg., 76, 912 (1958).
5. D.J. Triggle, J. Theoret. Biol., 1, 241 (1964).
6. N.B. Chapman, K. Clarke, and S.D. Saraf, J. Chem. Soc.(C), 1612 (1969).
7. N.B. Chapman, K. Clarke, and I. Iddon, J. Chem. Soc., 774 (1965).
8. N.B. Chapman, K. Clarke, B.A. Gore, and K.S. Sharma, J. Chem. Soc.(C), 915 (1971).

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