Benzo[*b*]thiophene Derivatives. XXVIII. 5-Bromo-3-benzo[*b*]thienylalanine and Derivatives [1]

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A potential inhibitor of gelation of sickle hemoglobin, 5-bromo-3-benzo[*b*]thienylalanine, has been synthesized along with several derivatives. Several hydrolytic pathways from the intermediate ethyl 5-bromo-3-benzo[*b*]thienylformamidomalonate have been examined, and that involving alcoholysis to ethyl N-formyl-5-bromo-3-benzo[*b*]thienylalanine shown to be superior.

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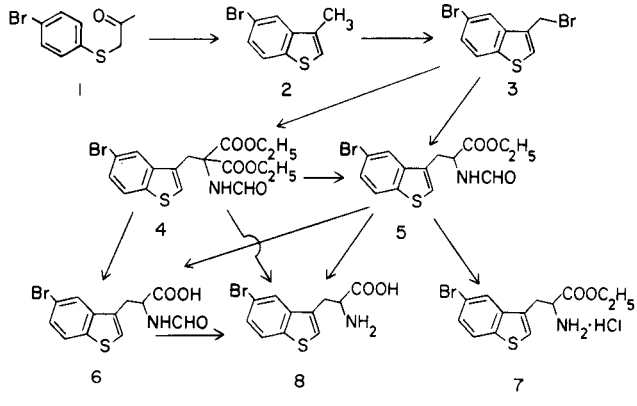
Poillon [2] has evaluated the effects of forty two β-aryl-substituted alanines on the inhibition of gelation of sickle hemoglobin. These included sixteen derivatives and four analogs of tryptophan, the most active group of compounds. The best gelation inhibitor in this study was 5-bromotryptophan, which was five times more effective than phenylalanine, and about twice as effective as tryptophan. Among the most active analogs was 3-benzo[*b*]thienylalanine, about 25% more active than tryptophan. Poillon [2] suggested that by suitable manipulation of the aromatic nucleus it should be possible to increase antigelling activity to a level potent enough to be useful in a therapy for sickle-cell anemia, and a consideration of the data indicated that 5-bromo-3-benzo[*b*]thienylalanine (**8**) should be a likely candidate which might have relatively high antigelling properties [3]. We therefore undertook the preparation of this previously unknown amino acid.

The proposed synthetic scheme is outlined in Scheme I. Cyclization of **1** to 5-bromo-3-methylbenzo[*b*]thiophene (**2**) and its conversion to the 3-bromomethyl derivative **3** have been described in a patent [4]. We repeated these preparations to check yields and record spectral properties. Conversion of 3-bromomethylbenzo[*b*]thiophene derivatives like **3** to the corresponding alanine derivatives *via* formamidomalonate derivatives like **4** has been previously utilized [5]. In the previous reports [5] the arylmethylformamidomalonates were hydrolyzed directly to the amino acids by prolonged refluxing in aqueous or aqueous/ethanolic acid or base, giving yields in the range of 40-50%. We have found that direct hydrolysis of **4** with either acid, base, or a succession of these reagents in one pot, gives yields in the same 40-50% range. We therefore investigated the stepwise hydrolysis of **4**, isolating and characterizing the intermediates **5**, **6** and **7**.

In a preliminary experiment, **3** was converted to **4** using an excess of about 0.3 equivalent of sodium ethoxide in the preparation of the sodium salt of diethyl formamidomalonate. After workup, the product proved to be a mixture of 45% **4** and 55% **5** by tlc. Later we found that **4** could be converted in nearly quantitative yield (88% isolated) to **5** by refluxing with one equivalent of sodium

Scheme I



ethoxide in absolute ethanol. Cope and McElvain [6] observed that disubstituted malonic esters are readily cleaved by sodium ethoxide, producing diethyl carbonate and the disubstituted acetic ester in 60-90% recovered yields. Therefore it was necessary to use exactly one equivalent of sodium ethoxide in preparing the sodium salt of diethyl formamidomalonate, to convert **3** to **4**. Under these conditions, **4** was obtained in 91% isolated yield. Even a slight excess of sodium ethoxide decreased the yield.

The hydrolysis of **5** to the formylalanine derivative **6** was accomplished by mild alkaline hydrolysis of the ester in 70% yield, and refluxing **6** in dilute aqueous/ethanolic hydrochloric acid gave **8** in 85% yield. Hence this route involving isolated intermediates **5** and **6** gave an overall yield from **4** to**8** of 48%, equivalent to earlier results obtained without isolation of intermediates.

Hydrolysis of the formamido ester **5** in dilute alcoholic hydrogen chloride gave the hydrochloride of the amino-ester **7** in 95% yield. Hence we treated **5** with alcoholic hydrogen chloride, then added aqueous sodium hydroxide and continued to reflux, resulting in the isolation of **8** in 87% yield. By this pathway, **8** was obtained from **4** *via* **5** in 70% overall yield. When **4** was refluxed with two equivalents of sodium ethoxide in alcohol for a time, then water added and refluxing continued, **6** was isolated in 82% yield. Since **6** had been converted to **8** in 85% yield

by refluxing in aqueous/ethanolic hydrochloric acid, the overall yield of **8** from **4** *via* **6** was also about 70%. The most convenient preparation of **8** involved converting **3** into **5** using two equivalents of sodium ethoxide, followed by hydrolysis of **5** to **8** in two successive hydrolytic steps, first alcoholic acid, then aqueous base.

The amino acid **8** proved to be very insoluble in water, and soluble only in strong acid or alkali. It, along with its hydrochloride, sodium salt, formyl derivative **6** and ester hydrochloride **7** have been submitted to Dr. W. N. Poillon, at the Center for Sickle Cell Disease at Howard University, Washington DC, 20059, for evaluation of inhibition of sickle hemoglobin gelation.

EXPERIMENTAL

Melting points were determined on a "uni-melt" Thomas Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer Model 137-B infrared spectrometer. Nuclear magnetic resonance spectra were obtained with a Varian T 60A spectrometer using tetramethylsilane as an internal standard. Mass spectral analyses were performed on a Varian MAT CH7 at 70 eV ionization potential. Eastman chromatogram sheets (# 13181 Silica gel with fluorescent indicator # 6060) were used for thin layer chromatography. Elemental analyses were performed by Midwest Microlab, Indianapolis.

4-Bromophenylthiopropanone (**1**).

Pyridine (20 g) was added to a solution of 9.45 g (0.05 mole) of 4-bromothiophenol (Aldrich reagent 95%) in 30 ml of ether, then 4.81 g (0.052 mole) of chloroacetone was added, and the mixture stirred at room temperature for one hour. After adding 70 ml of water, the mixture was extracted three times with ether, and the combined ether extracts washed with water, 3 *N* hydrochloric acid, and then brine. After drying (magnesium sulfate) the ether was removed (rotovap) to leave a brown oily residue which solidified on cooling. Recrystallization from carbon tetrachloride gave 11.2 g (95%) of white crystals melting at 58-61° (Chapman, *et al* [4] reported a melting point of 63-65°, and a 90% yield, running the reaction in aqueous solution); ir (potassium bromide) 1750 cm^{-1} (CO); pmr (deuteriochloroform): δ 2.32 (t, 3H, CH_3), 3.66 (s, 2H, CH_2), 7.31 (q, 4H, ArH).

5-Bromo-3-methylbenzo[*b*]thiophene (**2**).

The cyclizing medium of PPA on Celite, previously described [1] was used to cyclize **1** to **2**. To this fine sand suspended in toluene was added 6.1 g (25 mmoles) of **1** in 40 ml of toluene. The refluxing mixture was stirred for 24 hours, cooled, and the celite-sand collected and washed with toluene. The combined toluene layers were washed with water, sodium bicarbonate solution, and water again, then dried (magnesium sulfate), and evaporated to give 5.2 g (92%) of a yellow oil which solidified on cooling. It melted at 37-38.5° after recrystallization from ethyl acetate: pmr (deuteriochloroform): δ 2.32 (d, J = 1.0, 3H, CH_3), 7.0 (d, J = 1.0, 1H, H-2), 7.17-7.80 (m, 3H, ArH) (7).

5-Bromo-3-bromomethylbenzo[*b*]thiophene (**3**).

A flask containing 6.81 g (30 mmoles) of **2** in 150 ml of dry carbon tetrachloride and 0.3 g of benzoyl peroxide, was stirred and irradiated with two 200 watt light bulbs while refluxing. A total of 5.87 g (33 mmoles) of *N*-bromosuccinimide was added in small portions during the first hour, then refluxing and stirring continued for another hour and a half. After cooling, the suspended succinimide was collected, and the filtrate concentrated. The light brown solid product was recrystallized from a hexane/carbon tetrachloride mixture to give 7.1 g (75%) of pure product melting at 125-127°, in agreement with Chapman, *et al* [4]; pmr (carbon tetrachloride): δ 4.64 (s, 2H, CH_2), 7.44 (q, 1H, H-6), 7.48 (s, 1H, H-2), 7.70 (d, 1H, H-7), 7.95 (d, 1H, H-4).

Ethyl 5-Bromo-3-benzo[*b*]thienylformamidomalonate (**4**).

After 0.43 g (18.7 mmoles) of sodium had completely dissolved in 100 ml of absolute ethanol, 3.8 g (18.7 mmoles) of diethyl formamidomalonate (Aldrich) was added, and after a few minutes, 5.2 g (17 mmoles) of **3** was added, and the solution refluxed for 3 hours. After cooling, the alcohol was removed (rotovap), the residue taken up in water and extracted 3 times with methylene chloride. The combined organic layers were dried (magnesium sulfate) and evaporated to give a crude yellow solid which recrystallized from a mixture of chloroform and carbon tetrachloride to give 5.87 g (91%) of colorless crystals melting at 174-176°: ir (potassium bromide): 3350 (NH), 1776 (CO), 1695, 1670 cm^{-1} (amide CO); pmr (deuteriochloroform): δ 1.3 (t, 6H, CH_3), 3.96 (s, 2H, CH_2), 4.25 (q, 4H, CH_2), 6.92 (broad, 1H, NH), 7.20 (s, 1H, H-2), 7.43 (q, 1H, H-6), 7.74 (d, 1H, H-7), 7.87 (d, 1H, H-4), 8.22 (broad, 1H, CHO).

Anal. Calcd. for $C_{17}H_{18}BrNO_5S$: C, 47.67; H, 4.21; N, 3.27; S, 7.48; Br, 18.67. Found: C, 47.65; H, 4.22; N, 3.10; S, 7.54; Br, 18.81.

Ethyl *N*-Formyl-5-bromo-3-benzo[*b*]thienylalanine (**5**).

A. By Direct Alcoholysis of **4**.

A solution of 2.14 g (5.0 mmoles) of **4** in 50 ml of absolute ethanol containing 4.8 mmoles of sodium ethoxide (0.11 g of sodium metal added) was refluxed for 1 hour, then cooled and concentrated (rotovap). The residue was dissolved in methylene dichloride, then washed with ice-water and dried (magnesium sulfate). The solvent was evaporated, and the residue recrystallized from a mixture of chloroform/carbon tetrachloride to give 1.44 g (88%) of **5**, melting at 125-127°; ir (potassium bromide): 3380 (NH), 1740 (CO, ester), 1660 cm^{-1} (CO, amide); pmr (deuteriochloroform), δ 1.24 (t, 3H, CH_3), 3.38 (d, 2H, CH_2), 4.14 (q, 2H, CH_2), 5.56 (m, 1H, CH), 6.40 (broad, 1H, NH), 7.26 (m, 1H, H-2), 7.45 (q, 1H, H-6), 7.76 (d, 1H, H-7), 7.93 (d, 1H, H-4), 8.23 (broad, 1H, CHO).

Anal. Calcd. for $C_{14}H_{14}BrNO_3S$: C, 47.20; H, 3.93; N, 3.93; S, 8.99; Br, 22.45; MS 355, 357. Found: C, 46.98; H, 3.90; N, 3.87; S, 9.17; Br, 22.65; M^+, 355 (2), 357 (2); M^+-45, 310 (25), 312 (24); M^+-130, 225 (75), 227 (80).

B. In One Step From **3**.

A solution containing 18 mmoles of sodium ethoxide was prepared by dissolving 0.41 g of sodium in 50 ml of absolute alcohol, and then 2.03 g (10 mmoles) of ethyl formamidomalonate was added. Finally, 3.05 g (10 mmoles) of **3** was added, and the mixture was refluxed for 3 hours. It was then worked up as described above, to give 2.8 g (79%) of crystalline **5**, melting at 125-127°.

N-Formyl-5-bromo-3-benzo[*b*]thienylalanine (**6**).

A. By Hydrolysis of Ester **5**.

A solution of 1.5 g (4.2 mmoles) of **5** in a mixture of 50 ml of ethanol and 25 ml of aqueous 5% sodium hydroxide was refluxed for 30 minutes, then concentrated on a rotovap to about one-fifth volume, 50 ml of water added, and the solution treated with activated carbon. The clear filtrate was acidified to give 0.97 g (70%) of white crystals melting at 206-208° dec; ir (potassium bromide): 3350 (NH), 3100-2500 (COOH), 1625 cm^{-1} (amide CO); pmr (deuteriodimethylsulfoxide) δ 3.02-3.55 (m, 2H, CH_2), 4.73 (m, 1H, CH), 7.55 (d, 1H, H-6), 7.60 (s, 1H, H-2), 7.97 (d, 1H, H-7), 8.13 (s, 2H, H-4 and C*H*O), 8.43 (broad, 1H, NH).

Anal. Calcd. for $C_{12}H_{10}BrNO_3S$: C, 43.92; H, 3.05; N, 4.27; S, 9.76; Br, 24.64. Found: C, 43.97; H, 3.17; N, 4.27; S, 9.60; Br, 24.44.

B. By Hydrolysis of **4**.

After dissolving 0.1 g (4.3 mmoles) of sodium in 50 ml of absolute ethanol, 0.86 g (2.0 mmoles) of **4** was added and the mixture refluxed for 1 hour, then concentrated and about 30 ml of water added. The mixture was warmed on a water bath until the initial precipitate was dissolved, then cooled and acidified. The crystals were collected and recrystallized from ethanol to give 0.54 g (82%) of **6**, melting at 205-208°, identical in all respects to that prepared in A, above.

Ethyl 5-Bromo-3-benzo[*b*]thienylalanine Hydrochloride (**7**).

A solution of 1.0 g (2.8 mmoles) of **5** in 20 ml of 0.5 *N* ethanolic hydrogen chloride was refluxed for 1 hour, then concentrated to small volume, cooled, and cold absolute ether added. The white precipitate was collected, washed with cold ether, and recrystallized from an ether/alcohol mixture to give 0.9 g (95%) of white crystals melting at 205-207° dec; pmr (deuterium oxide) δ 1.0 (t, 3H, CH_3), 3.35 (d, 2H, CH_2), 4.05 (q, 2H, CH_2), 4.33 (t, 1H, CH), 4.65 (DHO), 7.38 (q, 1H, H-4), 7.46 (s, 1H, H-2), 7.75 (d, 1H, H-6), 7.80 (s, 1H, H-7).

Anal. Calcd. for $C_{13}H_{15}BrClNO_2S$: C, 42.81; H, 4.21; N, 3.84; S, 8.78; Br, 21.93; Cl, 9.74. Found: C, 42.71; H, 4.37; N, 3.67; S, 8.88; Br, 21.72; Cl, 9.91.

5-Bromo-3-benzo[*b*]thienylalanine (**8**).

A. By hydrolysis of **6**.

A solution of 1.1 g (3.3 mmoles) of **6** in 10 ml of ethanol was acidified with 10 ml of 1 *N* hydrochloric acid and refluxed for 1 hour. After filtering the solution to remove some undissolved material, the *p*H of the solution was adjusted to approximately 4.0 with concentrated ammonium hydroxide. The white precipitate was collected and washed with cold water to give 0.84 g (85%) of **8**, melting at 229-231° dec; ir (potassium bromide): 3450 (NH), 2500-3200 (COOH), 1680 cm^{-1} (CO); pmr (trifluoroacetic acid): δ 3.30-4.10 (m, 2H, CH_2), 4.80 (broad, 1H, CH), 6.90-7.90 (broad, 2H, NH_2), 7.54 (d, 1H, H-6), 7.58 (s, 1H, H-2), 7.79 (d, 1H, H-7), 7.96 (s, 1H, H-4).

Anal. Calcd. for $C_{11}H_{10}BrNO_2S$: C, 44.05; H, 3.33; N, 4.67; S, 10.67; Br, 26.64. Found: C, 43.78; H, 3.27; N, 4.48; S, 10.54; Br, 26.58.

B. By Hydrolysis of **5**.

A solution of 1.0 g (2.8 mmoles) of **5** in 20 ml of 0.5 *N* ethanolic hydrogen chloride was refluxed for 1 hour, then 100 ml of water and 20 ml of 1.0 *N* sodium hydroxide were added, and the aqueous oily suspension stirred on a water-bath (70-90°) until the oil had all dissolved (about 1 hour). The solution was filtered through activated carbon, and cooled. Adjustment of the *p*H to about 4 with dilute hydrochloric acid resulted in the precipitation of **8**, collected and dissolved in warm dilute hydrochloric acid, then cooled and precipitated at *p*H 4 (ammonium hydroxide) to give 0.73 g (87%) of **8**, melting at 229-231° dec.

C. By Hydrolysis of **4**.

A suspension of 1.07 g (2.5 mmoles) of **4** in 20 ml of 10% sodium hydroxide solution was refluxed for 6 hours, then concentrated and 15 ml of concentrated hydrochloric acid added, and the refluxing continued for an additional 5 hours. The mixture was then concentrated to dryness (rotovap) and 150 ml of water added, and heated to dissolve the residue. The solution was filtered and neutralized with ammonium hydroxide to *p*H 4-5, then cooled for several hours. The amorphous white solid was collected, washed with water and dried, to give 0.3 g (42%) of **8**, melting at 229-231° dec.

REFERENCES AND NOTES

[1] For part XXVII of this series, see E. Campaigne and Chung S. Kim. *J. Heterocyclic Chem.*, **20**, 1697 (1983).

[2] W. N. Poillon, *Biochemistry*, **21**, 1400 (1982).

[3] W. N. Poillon, personal communication, May 26, 1982.

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[5a] E. Campaigne and A. Dinner, *J. Med. Chem.*, **13**, 1205 (1970). [b]S. Avakian, J. Moss and G. Martin, *J. Am. Chem. Soc.*, **70**, 3075 (1948).

[6] A. C. Cope and S. M. McElvain, *ibid.*, **54**, 4319 (1932).

[7] Chapman, ref [4] obtained this compound in 70% yield by the use of PPA heated to 120-140° for four hours. He reported it as an oil boiling at 153-158°/12 mm.