# Vicinal benzo[*b*]thiophene-5,6-dicarboxaldehyde in heterocyclic synthesis: a reagent for fluorescence determination of amino acids

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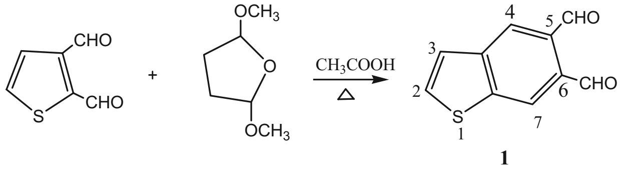
Benzo[*b*]thiophene-5,6-dicarboxaldehyde reacts with dibenzyl ketone, thiodiacetic acid dimethyl ester, hydrazine, *p*-toluidine, *p*-aminoacetophenone and nitromethane to give the corresponding seven, six and five-membered rings condensed to benzo[*b*]thiophene. Reaction of benzo[*b*]thiophene-5,6-dicarboxaldehyde with *p*-toluidine in the presence of 2-mercaptoethanol gives highly fluorescent compounds. Also, benzo[*b*]thiophene-5,6-dicarboxaldehyde was applied to fluorogenic reaction with some amino acids and the obtained data were compared with the reported data in the case of *o*-phthaldehyde. The synthesised compounds were characterised by their elemental analysis, IR, $^1$H NMR and mass spectrometry.

**Keywords:** benzo[*b*]thiophene-5,6-dicarboxaldehyde, thiepine, fluorogenic reaction, *o*-phthaldehyde, amino acids

The reaction of *o*-phthaldehyde and 2-mercaptoethanol with amino compounds has attracted much attention due to the formation of strongly fluorescent compounds.[1-6] This fluorogenic reaction permits the detection of picomole quantities of amino acids,[7-11] peptides,[12] proteins,[13-15] and antibody concentrations[16] in aqueous solutions about hundred times more sensitive than colourimetry.

Our availability of the five-membered heterocyclic *o*-dicarboxaldehydes furan and thiophene moieties prompted us to carry out many of the *o*-dicarboxaldehydes characterisation reactions which were carried out with *o*-phthaldehyde.[17-19]

In a previous work, we have shown that benzo[*b*]thiophene-5,6-dicarboxaldehyde **1** could be prepared in a one-step reaction from thiophene 2,3-dicarboxaldehyde and 2,5-dimethoxy tetrahydrofuran in acetic acid medium as a solvent.[20] (Scheme 1).



**Scheme 1**

Condensation of **1** with dibenzyl ketone, in the presence of sodium methoxide in methanol afforded 6,8-diphenyl benzothieno[3,4-d]cycloheptatrien-7-one **2** in 70% yield. While reaction of **1** with thiodiacetic acid dimethyl ester gave the corresponding thiepine derivative **3** in 50% yield. Similarly benzothieno[3,4-*d*]pyridazine **4** was obtained in 65% yield from reaction of **1** with hydrazine in ethanol as a solvent (Scheme 2).

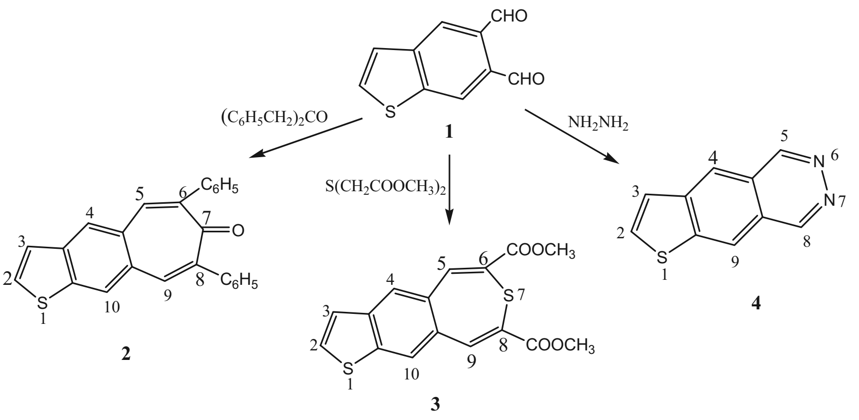
On the other hand, condensation of **1** with some aromatic amines, *e.g. p*-toluidine and *p*- amino acetophenone, gave the compounds N-(4-tolyl)-5-oxo-7*H*-benzothieno[3,4-*c*]pyrrole **5** and N-(4-acetyl phenyl)-5-oxo-7*H*-benzothieno[3,4-*c*]pyrrole **6** (Scheme 3).

It seems likely that condensation and cyclisation followed by 1,3-hydride shift has taken place to produce compounds **5** and **6** according to our previously reported work.[21] Compound **5** could be also obtained in 70% yield by the reaction of **1** with *p*- tolylisocyanate at 170 °C for 2 h.

It was found that reaction of **1** with nitromethane in alcoholic potassium hydroxide gave 6-nitro-5*H*-cyclopenta [*c*]benzothiophen-5-ol **7** in 56% yield (Scheme 4). These results are in accordance with our published results.[22]

Reaction of benzo[*b*] thiophene 5,6 dicarboxaldehyde **1** with *p*-toluidine in the presence of 2-mercaptoethanol in ethanol as a solvent for 30 min gave the corresponding fluorescent adduct **8** in 85% yield, in addition to compound **5** in 15% yield (Scheme 5).

The reaction was applied using some amino acids and 2-mercaptoethanol as reducing agent at room temperature and pH 9.5. The data recorded in Table 1 indicated that the thiophene moiety tends to reduce the fluorescence intensity in most cases. The excitation and emission obtained with compound **1** is around $\lambda_{ex}$ 382 and 480 nm (green emission) compared $\lambda_{ex}$ 325 and 435 nm (blue emission), respectively, where *o*-phthaldehyde was used.



**Scheme 2**

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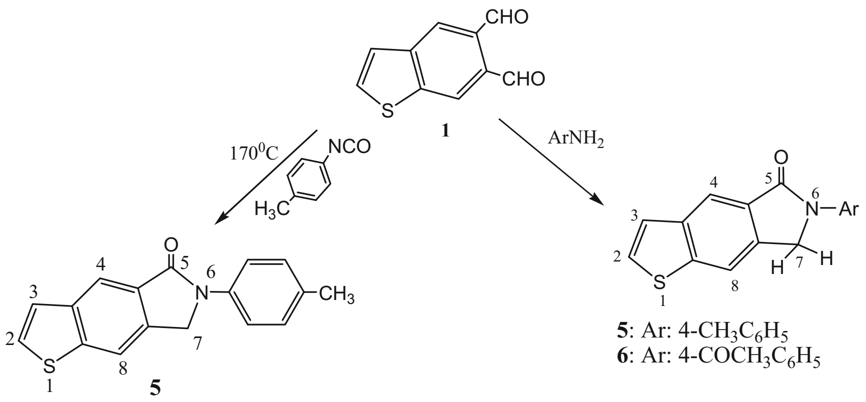
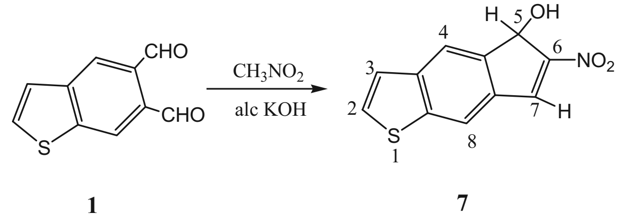


**Scheme 3**



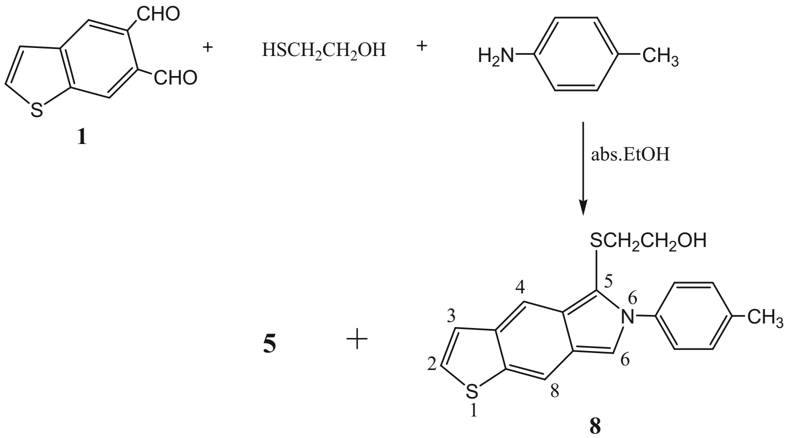
**Scheme 4**

## Experimental

All melting points were measured on a Gallenkamp melting point apparatus and uncorrected. IR spectra were recorded on a Perkin–Elmer1430 spectrophotometer using the KBr disk technique. $^1$H NMR spectra were measured on a Joel-90 MHz spectrometer in $CDCl_3$, chemical shifts were expressed in δ ppm using TMS as an internal standard. Electron impact mass spectra (EI) were obtained using a Finnigan MAT 8222 spectrometer at 70 eV. Elemental analysis were performed on a Perkin-Elmer 240 elemental analyser in Cairo University. Fluorescence was measured on a Shimadzu- UV-3101PC, UV-Vis-NIR scanning spectrophotometer. Progress of reactions was monitored by TLC. Compound **1** was prepared according to the previously reported method, [20] yield (31%), m.p. 186–187 °C.

*6,8-Diphenyl benzothieno[3,4-d]cycloheptatrien-7-one* **(2):** A mixture of **1** (1.9 g, 10.00 mmol) and dibenzyl ketone (3.15 g, 15.00 mmol) in absolute methanol (20 mL), was added dropwise to (20 mmol) sodium methoxide in methanol (20 mL). The reaction mixture was stirred for 3 h at room temperature and refluxed for 1 h. The mixture was hydrolysed in an ice bath, acidified with 2N HCl, extracted with dichloromethane, dried with magnesium sulfate and the solvent was evaporated under reduced pressure. The product was recrystallised from xylene to give **2,** (2.54 g, 70%), M.p. 162–163 °C. IR (KBr) υ cm$^{-1}$:1626 (C=O). $^1$H NMR δ: 7.62 (2H, s, H4,H10), 7.43 (2H, s, H5, H9), 7.20 (1H, d, H2, 4.7 Hz), 7.08 (1H, d, H3, 4.7 Hz), 7.11–7.42 (10H, m, Ph). Anal. Calcd for: $C_{25}H_{16}OS$ (364.46): C, 82.39; H, 4.42; S, 8.80. Found: C, 82.03; H, 4.17; S, 8.3%. MS: *m/z* (%): 364 (M$^+$, 100%), 302 (27%), 249 (38%), 197 (43%), 182 (58%), 165 (90%).

*6,8-Benzothieno[3,4-d]thiepindicarboxylic acid dimethyl ester* **(3):** A mixture of **1** (1.9 g, 10.0 mmol) and thio diacetic acid dimethyl ester (1.88 g, 10.00 mmol) in methanol (20 mL) was added dropwise to methanol (50 mL) containing triethylamine (1 mL). The reaction mixture was stirred for 6 hr at room temperature and refluxed at 60⁰C for 1 h. The mixture was hydrolysed in an ice bath and extracted with dichloromethane, dried with magnesium sulfate and the solvent was



**Scheme 5**

**Table 1**

| Cmpd. | Benzo[b]thiophene 5, 6 dicarboxaldehyde | | | *o*-Phthaldehyde | | |
|---|---|---|---|---|---|---|
| | λ$_{ex.}$/nm | λ$_{fl}$/nm | Fi | λ$_{ex.}$/nm | λ$_{fl.}$/nm | Fi |
| Glycine | 382 | 488 | 179 | 325 | 431 | 128 |
| Serine | 381 | 480 | 219 | 327 | 436 | 295 |
| Glutamine | 384 | 486 | 242 | 326 | 436 | 280 |
| Ph. Alanine | 382 | 482 | 224 | 326 | 434 | 288 |

Λex., Excitation wavelength; λfl., fluorescence maximum; Fi., fluorescence intensity.

evaporated under reduced pressure. The product was recrystallised from ethanol to give **3** (1.66 g, 50%), M.p. 198–200 °C. IR (KBr) υ cm$^{-1}$: 1720 (C=O). $^1$H NMR δ: 7.62 (2H, s, H4,H10), 7.43 (2H, s, H5, H9), 7.20 (1H, d, H2, 4.7 Hz), 7.08 (1H, d, H3, 4.7 Hz), 3.76 (6H, s, 2$CH_3$). Anal. Calcd for: $C_{16}H_{12}O_4S_2$ (332.39), 57.81; H, 3.64; S, 19.29. Found: C, 57.45; H, 3.42; S, 18.9%. MS: *m/z* (%): 332 (M$^+$, 12%), 308 (60%), 229 (30%), 193 (80%), 129 (100%), 91 (40%).

*Benzothieno[3,4-d]pyridazine* **(4):** A mixture of **1** (1.9 g, 10.00 mmol) and (0.5 g, 10.00 mmol) of 98% hydrazine hydrate in ethanol (20 mL) was refluxed for 3 h, the solvent was evaporated under reduced pressure. The product was recrystallised from ethanol to give **4**, (1.21 g, 65%), M.p. 222–223 °C. IR (KBr) υ cm$^{-1}$:1605 (C=N). $^1$H NMR δ: 8.43 (2H, s, H5,H8), 7.62 (2H, s, H4,H9), 7.20 (1H, d, H2, 4.8 Hz), 7.08 (1H, d, H3, 4.8 Hz). Anal. Calcd for: $C_{10}H_6N_2S$ (186.23): C, 64.49; H, 3.25; N, 15.04; S,17.22. Found: C, 64.02; H, 2.9; N, 14.8; S, 16.92%. MS: *m/z* (%): 186 (M$^+$, 35%), 158 (100%), 138 (18%), 91 (62%).

*N-(4-Tolyl)-5-oxo-7H-benzothieno[3,4-c]pyrrole* **(5)***: Method A*. A cold (0 °C) solution of **1** (1.9 g, 10.00 mmol) in absolute ethanol (10 mL) was added to a solution of *p*-toluidine (1.07 g, 10.00 mmol) in *t*-butyl alcohol (10 mL). The reaction mixture was stirred in an ice bath under nitrogen atmosphere for 6 h, the solvent was evaporated under reduced pressure and the residue was chromatographed by silica gel column using ether/hexane 3:1 as eluent. The yellow fraction was obtained and recrystallised from benzene to give **5**, (0.47 g, 17%).

*Method B*. A mixture of **1** (1.9 g, 10.00 mmol) and *p*-methyl phenyl isocyanate (1.33 g, 10.00 mmol) was heated at 160–170 °C under nitrogen atmosphere for 2 h. The residue was chromatographed by using the same eluent. The yellow fraction was obtained and recrystallised from benzene to give **5**, (1.95 g, 70%), M.p 142–144 °C. IR (KBr) υ cm$^{-1}$:1730 (C=O).$^1$H NMR δ: 7.62 (2H, s, H4,H8), 7.42–7.33 (4H, m, Ar), 7.20 (1H, d, H2, 4.8 Hz), 7.08 (1H, d, H3, 4.8 Hz), 4.75 (2H, s, H7), 2.34 (3H, s, $CH_3$). Anal. Calcd for: $C_{17}H_{13}NOS$ (279.36): C, 73.09; H, 4.69; N, 5.01; S, 11.48. Found: C, 72.95; H, 4.52; N, 4.98; S, 11.23%. MS: *m/z* (%): 279 (M$^+$, 100%), 248 (49%), 220 (34%), 163 (60%), 149 (80%), 133 (37%).

*N-(4-Acetyl phenyl)-5-oxo-7H-benzothieno[3,4-c]pyrrole* **(6):** A mixture of **1** (0.95 g, 5.00 mmol) and *p*-aminoacetophenone (0.68 g, 5.00 mmol) in dry xylene (250 mL) was refluxed for 2 h. The reaction mixture was hydrolysed in an ice bath and extracted with dichloromethane, dried with magnesium sulfate and the solvent was evaporated under reduced pressure. The product was recrystallised from benzene to give **6**, (0.38 g, 25%), M.p. 212–214 °C. IR (KBr) υ cm$^{-1}$:1695 (C=O), 1660 ($COCH_3$). $^1$H NMR δ: 7.62 (2H, s, H4,H8), 7.42–7.33 (4H, m, Ar), 7.20 (1H, d, H2, 4.7 Hz), 7.08 (1H, d, H3, 4.7 Hz), 4.8 (2H,s, H5), 2.6 (3H, s, $COCH_3$), 2.34 (3H, s, $CH_3$). Anal. Calcd for: $C_{18}H_{13}NO_2S$ (307.37): C, 70.34; H, 4.26; N, 4.56; S, 10.43. Found: C, 70, 20; H, 4.12; N, 4.32; S, 10.23%. MS: *m/z* (%): 307 (M$^+$, 45%), 264 (100%), 190 (27%), 149 (20%), 133 (30%).

*6-Nitro-5H-cyclopenta[c]benzothiophen-5-ol* **(7):** A mixture of **1** (0.95 g, 5.00 mmol) and nitromethane (0.305 g, 5.00 mmol) in methanol (15 mL) was added dropwise to a cold (0 °C) solution of 10% alcoholic KOH. (5 mL) The reaction mixture was stirred for 2 h, neutralised with 1N HCl. The colourless precipitate was formed, washed with cold water and recrystallised from ethanol to give **7,** (0.65 g, 56%), M.p. 178–179 °C. IR (KBr) υ cm$^{-1}$: 3550 (OH), 1570, 1328 ($NO_2$). $^1$H NMR δ: 7.88 (1H, s, H7), 7.62 (2H, s, H4,H8), 7.20 (1H, d, H2, 4.8 Hz), 7.08 (1H, d, H3, 4.8 Hz), 5.7 (1H, s, H5), 2 (s,1H, OH). Anal. Calcd for: $C_{11}H_7NO_3S$ (233.24): C, 56.64; H, 3.02; N, 6.01; S, 13.75. Found: C, 56.42; H, 2.89; N, 5.85; S, 13.54%. MS: *m/z* (%): 233 (M$^+$, 27%), 204 (100%), 165 (22%), 149 (43%), 90 (79%).).

*N-(4-Tolyl)-2-thioethanol-benzothieno[3,4-c]pyrrole* **(8):** A mixture of **1** (0.95 g, 5.00 mmol), *p*-toluidine (0.35 g, 5.00 mmol) and 2-mercaptoethanol (0.78 g, 10.00 mmol) in absolute ethanol (25 mL) was stirred in an ice bath for 30 min. The reaction mixture was evaporated to give residue which was chromatographed by silica gel column using ether/hexane 3:1 as eluent.The first fraction was collected, evaporated and recrystallised from benzene to give **8** (1.44, 85%), M.p. 143–145 °C, the second fraction gave **5** (0.42 g, 15%). For compound **8**, IR (KBr) υ cm$^{-1}$: 3500 (OH). $^1$H NMR δ: 7.62 (2H, s, H4,H8), 7.42–7.33 (4H, m, Ar), 7.20 (1H, d, H2, 4.7 Hz), 7.18 (1H, s, H7), 7.08 (1H, d, H3, 4.7 Hz), 3.98 (2H, t, $CH_2OH$), 2.58 (2H, t, $SCH_2$), 1.6 (1H, s, OH). Anal. Calcd for: $C_{19}H_{17}NOS_2$ (339.47): C, 67.22; H, 5.05; N, 4.13; S, 18.89. Found: C, 67.20; H, 5.00; N, 4.10; S, 18.80%. MS: *m/z* (%): 339 (M$^+$, 12%), 295 (45%), 248 (83%), 202 (100%), 169 (69%), 79 (83%).

*The determination of florescence data of some amino acids*
*Procedure*: To assay amino acids in aqueous solution, mix 100 µL of the unknown (concentration: approx.10$^{-4}$ M) with buffered reagent (3 mL) [which was prepared by mixing benzo[*b*]thiophene-5, 6-dicarboxaldehydes **1** (1.5 mL) (10 mg/mL in ethanol) with 90 mL of borate buffer of pH 9.5] and 2-mercaptoethanol (1.5 mL) (5 µL/mL ethanol). The reagent is stable one day at room temperature. The fluorescence was measured within 5 and 25 min after mixing. blanks were run without amino acids and a standard solution (5 × 10$^{-4}$ M amino acid) simultaneously.

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## References

1 R.P. Maickel and F.P. Miller, *Anal. Chem.*, 1966, **38**, 1937.
2 S.S. Simons and D.F. Johnson, *Anal. Biochem,* 1977, **82**, 250.
3 S.S. Simons and D.F. Johnson, *J. Org. Chem.*, 1978**, 43** (14), 2886.
4 J.F. Stobaugh, A.J. Repta and L.A. Strernson, *J. Pharmaceut. Biomed. Anal.*, 1986, **4** (3), 341.
5 J.M. Arque, J. Serrano, A.D. Leiva and R. Segura., *Anal. Biochem.*, 1988, **174** (1), 275.
6 G. Campo, B. Gallego and I. Berregi, *Talanta,* 2006, **68**, 1126.
7 M. Roth., *Anal. Chem.*, 1971, **43** (7)**,** 880.
8 K.S Lee and D.G. Drescher, *J. Biol. Chem.*, 1979, **245,** 6248.
9 J.R. Beson and P.E. Hare, *Proc. Nat. Acad. Sci. USA*, 1975, **72**, 619.
10 T.A. Durkin, G.M. Anderson and D.J. Cohen, *J. Chromatogr. B: Biomed. Sci. Appl.,* 1988**, 328**, 9.
11 A. Taga, M. Sugimura and S. Honda, *J. Chromatogr., A.*, 1998, **802**, 243.
12 S. Taylor and A. Tappel, *Anal. Biochem.*, 1973, **56**, 140.
13 E.Weidekamm, D.F. Wallach and R. Fluckiger, *Anal. Biochem.*, 1973, **54**, 102.
14 E. Mendez and J.G. Gavilanes, *Anal. Biochem.*, 1976**, 72**, 473.
15 E.C. Butcher and O.H Lowry, *Anal. Biochem.*, 1976**, 76**, 502.
16 L. Xiaopu, A. Kaveh and S.J. Mentzer, *J. Immunol. Meth.*, 1994, **172**, 141.
17 M. El-Borai and M. Hassanein, *Org. Prep. Proced. Int.*, 1983, **14**, 409.
18 M. El-Borai and M. Hassanein, *J. Prakt. Chem.*, 1983**, 5**, 853.
19 F. Benachenhore, M.A. Mesli, M. El-Borai, B. Hanquet and R.J. Guilard, *J. Heterocycl. Chem.*, 1988**, 25**, 1531.
20 M. El-Borai, M.F. Abdel Megeed, M. Hassanein and M. Fahmy, *Sulfur Lett.*, 1987**, 4,** 99.
21 M. El-Borai, M.F. Abdel Megeed and A.A. El Said, *Sulfur Lett.*, 1987, **7**, 7.
22 M. El-Borai and M. Fahmy, *Heterocycles*, 1987, **26**, 385.