Research Article

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Synthesis, Polymerization and Antioxidant Activity by DPPH Assay of Symmetrically Substituted Thieno[2,3-b]thiophenes

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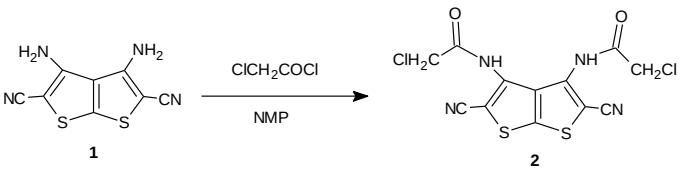
Abstract: *N,N'*-(2,5-dicyanothieno[2,3-*b*]thiene-3,4-diyl)*bis*-(2-chloroacetamide) **2** and 3,4-diaminothieno[2,3-*b*]thiophene-2,5-dicarboxamide **3** were resynthesized via simple and different new methods as bisymmetric monomers. IR, ^{1}H NMR, ^{13}C NMR, and elemental analyses were used to confirm the structure of the two compounds. The resulting bisymmetric compounds are expected to have multiple biological applications such as antioxidant, antitumor, antiviral, antibiotic, antiglaucoma, and antiallergenic, (in vitro) against *E. coli*, and they act as inhibitors for platelet aggregation. They may have multiple industrial applications such as organic light-emitting diodes and the design of novel nonlinear optical (NLO) systems. Also, they can be used as simple monomers for further polymerization. The antioxidant potential of the two compounds was evaluated by the DPPH scavenging activity method. Compound **3** exhibited antioxidant activity, and its DPPH scavenging activity showed a dose-dependant manner, while compound **2** did not. Alkylation polymerization of compound **2** with diamino diphenyl methane **4** at room temperature afforded novel polyacetamide **5**.
Keywords: Bisymmetrical, monomer, alkylation, polymerization, antioxidant activity

1. Introduction

Thieno[2,3-*b*]thiophene compounds played an important role for the synthesis of tetra-condensed-thienothiophenepyrimidines, -triazines, -pyridines, -pyrazoles, -triazolopyrimidines, -imidazopyrimidines, -thiazoles, and -pyrroles [1-4]. The symmetrical thieno[2,3-*b*]thiophenes are used as monomers and co-monomers for polymer syntheses [5], which possess interesting applications such as; organic light emitting diodes [6], smiconductive or conductive materials that can be used in solar cells [6,7], and developement of nonlinear optical devices [8]. Also, diaminothieno[2,3-*b*]thiophenes have different biological activities such as; antibiotic ,antioxidant, antiviral, antitumor, , antiglaucoma, antiallergenic, (in vitro) against E. *coli*, and they act as inhibitors for platelet aggregation [9-11]. Herein, two compounds **2** and **3** were prepared as symmetrical monomers, using 3,4-diaminothieno[2,3- *b*]thiophene-2,5-dicarbonitrile (**1**) [12,13], **(Scheme 1 & 2)**, followed by polymerization of monomer **2** with 4,4'-diaminodiphenylmethane **4, (Scheme 3)**. Also, the antioxidant properties of compound 2 and 3 were estimated. Both of the two methods used to prepare compound 2 and 3 was at room temperature so it energy saving and afforded good yields.



Scheme 1: Synthesis of compound 2**.**

2. Materials and methods

All melting points were uncorrected and recorded through Kofeler melting point apparatus. Any reaction was monitored by TLC plates [silica gel/ UV light (254 nm/365 nm) for visualization]. IR spectra were measured (KBr pellets) on a spectrometer FT-IR spectrophotometer. ^{1}H NMR spectra was recorded at 400 M Hz, and ^{13}C NMR (DMSO-d_6) was recorded at 100 MHz on Bruker Bio Spin AG at Sohag University. Elemental analyses were gaven on a Perkin-Elmer CHN as analyzer model.The antioxidant activity was evaluated by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity.

Synthesis of compound 2:

3 mL of chloroacetyl chloride (37 mmole) was gradually added to 1.04g, 4.72 mmole of compound 1 in 20 mL

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N-methylpyrrolidone NMP. At 0 °C, the reaction mixture was stirred for 1 hr, and then its temperature was raised to room temperature with complete stirring for 11 hrs. After completation of the reaction, the reaction mixture was dispensed into ice water, with the addition of sodium carbonate solution to remove the excess of chloroacetic acid. The formed brown precipitate was collected by filteration and recrystalized from acetone, yield 85 % m.p. = 283 °C, literature 280 ^{0}C [14]. IR (ATR) ν_{max} 3198 (NH), 3058, 3064 (C-H$_{arom.}$), 2873, 2949, 2998 (C-H$_{aliph.}$), 2218 (CN), 1677 (C=O) cm^{-1}, 1547 cm^{-1}, 1460 cm^{-1}, 1388 cm^{-1}, 1326 cm^{-1}, 1200 cm^{-1}, 979 cm^{-1}, 917 cm^{-1}, 820 cm^{-1}, 779 cm^{-1}, 673 cm^{-1}, 570 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (s, 2H, 2NH), 4.38 (4H, 2CH$_2$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.5, 145.2, 136.5, 133.3, 112.9, 106.8, 43.4. *Anal.* Calcd. for $C_{12}H_6Cl_2N_4O_2S_2$ (373.23): C, 38.62; H, 1.62;Cl, 19 N, 15.01; S, 17.18. Found: C, 38.5; H, 1.82; N, 14.66; Cl, 18.70; S, 17.2. *See supporting information*

Synthesis of compound 3:

In a bottomed flask (1 g, 4.5 mmole) of compound **1** in 20 mL of conc. H_2SO_4, was stirred for 3 hours, then the reaction mixture was poured into ice cold water to give greenish white precipitate which was filtered off , washed with ice cold water, dried, and recrystalized from DMF/EtOH (5:1) as white powder yield 80 %, m.p. > 300 ^{0}C.Literature m.p. 280^{0}C [15] IR (ATR) ν_{max} 3152, 3302 (NH$_2$), 3407, 3432 (NH$_2$), 3100 (C-H$_{arom.}$), 1646 (C=O) cm^{-1}, 1615 cm^{-1},1590 cm^{-1}, 1490 cm^{-1}, 1459 cm^{-1} 1394 cm^{-1} 1197 cm^{-1}, 1155 cm^{-1}, 1085 cm^{-1}, 768 cm^{-1} , 735 cm^{-1}, 626 cm^{-1}, 518 cm^{-1}, 460 cm^{-1}, 422 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.89 (s, 4H, 2NH$_2$), 7.02 (s, 4H, 2NH$_2$-amidic); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.9, 147.5, 140.8, 130.5, 102.6. *Anal.* Calcd. for $C_8H_8N_4O_2S_2$ (256.304): C, 37.49; H, 3.15; N, 21.86; S, 25.02. Found: C, 37.8; H, 3.52; N, 21.66; S, 24.80. *see supporting information*

Synthesis of polyacetamide 5

In a round flask under inert atmosphere a monomer **2** (0.94 g, 2.80 mmol) in LiCl/NMP (0.30 g in 20 mL) was stirred at room temperature and a solution diaminodiphenyl methane **4** (0.55 g, 2.80 mmol) in NMP (2.5 mL) was added drop by drop while keeping stirred under inert atmosphere with the addition of few drops of pyridine. The mixture was continuously stirred at about 24 hours. The reaction solution was displended into 30 ml methanol to afford brown fibrous precipitate, that was filtered off, washed with diluted HCl solution, dried and finnaly washed with hot ethanol, yield 75 %. IR (ATR) ν_{max} 3220 (NH), 3051, 3060 (C-H$_{arom.}$), 2215 (CN), 1671 (C=O) cm^{-1},1622 cm^{-1}, 1588 cm^{-1}, 1550 cm^{-1},1510 cm^{-1},1442 cm^{-1}, 1388 cm^{-1}, 1317 cm^{-1}, 1285 cm^{-1}, 1237 cm^{-1}, 1163 cm^{-1} , 844 cm^{-1}, 797 cm^{-1}, 753 cm^{-1}, 692 cm^{-1}, 564 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.05 (NHCO), 8.32 (NHCH$_2$), 6.52-6.86 (CH aromatic), 3.53-3.84 (CH$_2$ of three methylene groups).

Evaluation of antioxidant activity:

Materials and methods:

Antioxidant activity analysis *via* thin layer chromatogram-phy. On TLC plates of silica gel, 2.5 µL of compounds **2** and **3** were loaded, 0.05% DPPH solution in methanol were sprayed on it and examined after 30 minutes. It was observed that compound 3 converted to yellow zone on the purple back ground while compound 2 stilled as it with the comprasion of ascorbic acid and silica gel as positive control.

DPPH radical scavenging activity

Antioxidant activity of compound **3** was detected by DPPH radical scavenging activity. 50 µL of different concentrations of compound **3** dissolved in DMSO were taken and the volume was made uniform to 2mL using methanol, then 2 mL of DPPH solution (0.002 % in methanol) was added. The mixtures were shaken well and left to stand in the dark for 30 minutes at room temperature, then the absorbance was measured at 517 nm using UV *vis* spectrophotometer using methanol as blank. A control experiment was carried out without the test sample using methanol and DPPH radical. Ascorbic acid was used as reference standard [16, 17]. The scavenging activity was calculated according to the following equation:

$$\text{Radical Scavenging Activity (\%)} = \frac{Abs\ control - Abs\ sample}{Abs\ control} x100$$

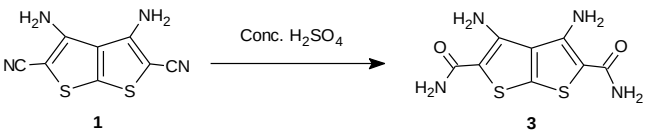
3. Results and Discussion:

3.1. Chemistry

N,*N*′-(2,5-dicyanothieno[2,3-*b*]thiene-3,4-diyl)*bis*-(2-chloro-acetamide) **2** was synthesized *via* acylation of 3,4-diaminothieno[2,3-*b*]thiophene-2,5-dicarbonitrile (**1**) with eight equivalent moles from chloroacetyl chloride in dry *N*-methylpyrrolidone (NMP) as basic solvent, (**Scheme 1**). NMP act as a solvent and a catalyst that absorbs the released HCl gas.

IR spectrum related to **2** showed the fading of distinctive absorption peaks for two -NH$_2$ groups, meanwhile displayed new featured absorption peaks at ῡ 3198, 2949, 1677 and 672 cm^{-1} for the corresponding N-H, aliphatic C-H, amidic C=O, and C-Cl groups, respectively. Its ^{1}H NMR spectrum displayed two singlet signals at δ 10.55 (disappeared by D$_2$O) and 4.38 ppm, which are characteristic for two symmetrical NH and CH$_2$ groups, respectively. Its ^{13}C NMR displayed six signals at δ 165.5, 145.2, 136.5, 133.3, 112.9 and 106.8 ppm, which are assigned to symmetrical carbons of two carbonyl, thiophene rings, and two nitrile groups, while carbons of methylene groups are characterized by signal at 43.4 ppm.

Cyano group of thieno[2,3-*b*]thiophene **1** was easily underwent acid hydrolysis using concentrated sulfuric acid to give the corresponding 3,4-diaminothieno[2,3-*b*]thiophene-2,5-dicarboxamide (**3**), (**Scheme 2**).

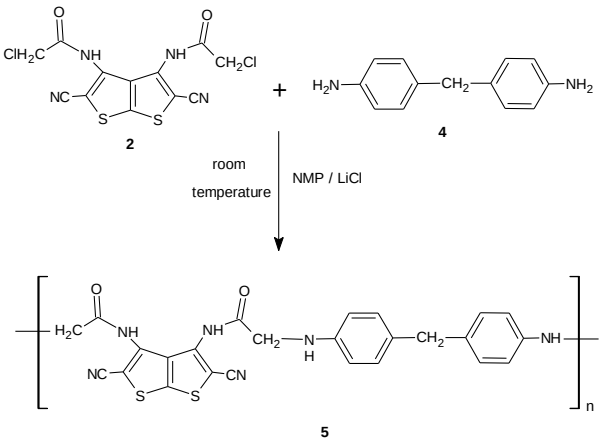


Scheme 2: Synthesis of compound **3.**

IR spectrum of monomer **3** displayed the disappearance of cyano group while exhibited new absorption bands at ῡ 3432, 3407 and 3302 cm^{-1}, which are characteristic for NH$_2$ groups;

and 1646 cm^{-1} due to amidic carbonyl groups. ^1H NMR spectrum of monomer 3 (400 MHz, DMSO-d_6) displayed two singlet signals at δ 7.02 and 6.89 ppm (disappeared by D_2O), which are characteristic for four NH_2 protons. Its ^{13}C NMR spectrum displaayed five signals at δ 166.9 (C=O), 147.5, 140.8, 130.5 and 102.6 ppm (thieophene carbons).

Alkylation polymerization reaction of 4,4'-diamino-diphenylmethane **4** with thieno[2,3-*b*]thiophene **2** (1:1 ratio) using anhydrous lithium chloride in dry *N*-methylpyrrolidone (NMP) at room temperature gave polyacetamide **5**, (**Scheme 3**). ^1H NMR spectrum of polyacetamide **5** was in agree with the repeating unit structure, it displayed the presence of two new broad singlet signals at δ 9.05 and 8.32 ppm, which are characteristic for the corresponding NHCO and N<u>H</u>CH_2 protons; new multiplet signals at δ 6.52-6.86 ppm character-istic of two *p*-phenylene protons; and signals at δ 3.53-3.84 ppm due to three methylene protons. *see supporting information*



Scheme 3: Synthesis of polyacetamide **5**

3.2. Antioxidant Activity

Antioxidant has a significant impact in the preserving the level of antioxidant in the body and could lower the high level of reactive oxygen species (ROS) not balanced by the antioxidative defense system under abnormal physiological conditions [18].

One of the common methods used in measuring the antioxidant activity is the free radical method using DPPH (1,1-diphenyl-2-picrylhydrazyl) due to simplicity, rapidness and independence of the polarity of the sample [19]. In the presence of electron donating compounds or compounds that can donate hydrogen atoms [20], the DPPH violet color is lost within the decrease of the absorbtion wavelengths below 520 nm depending on the number of the paired electrons [21]. The antioxidant activity of compounds **2** and **3** was first determined by thin layer chromatographs [22], as the DPPH is a purple coloured stable free radical, which converted to yellow colour on reduction to diphenylpicrylhydrazine compound. Any antioxidant compound is seen as a yellow spot on a purple background. Ascorbic acid and silica gel were used as positive control. Compound **3** give a yellow spot while compound **2** did not in visible light.

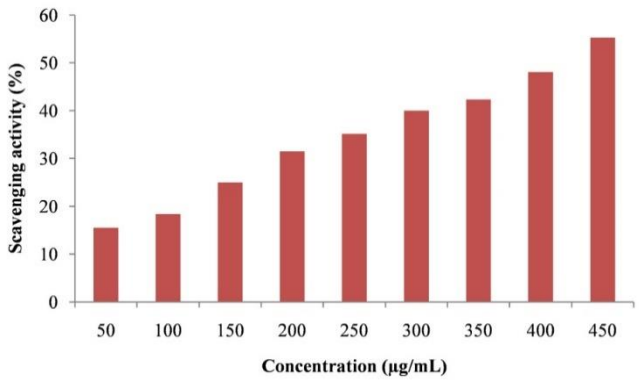


Figure1: DPPH scavenging activity of compound **3**

Antioxidant activity of compound **3** was assessed by DPPH scavenging activity method. Its DPPH scavenging activity showed a dose dependant manner (**Figure1**). The radical scavenging ability of different concentrations, namely 50, 100, 150, 200, 250, 300, 350, 400 and 450 µg/mL of the compound was found to be 15.55, 18.42, 25.00, 31.50, 35.17, 40.00, 42.35, 48.10 and 55.3 %, respectively. However, the compound exhibited much lower antioxidant activity compared to ascorbic acid and the Ec50 for ascorbic acid and the compound **3** was 58 and 420 µg/mL in the same order.

4. Conclusion

N,*N'*-(2,5-dicyanothieno[2,3-*b*]thiene-3,4-diyl)*bis*-(2-chloro-acetamide) **2** and 3,4-diaminothieno[2,3-*b*]thiophene-2,5-dicarboxamide **3** were synthesized *via* simple and different new methods that can be used as symmetric monomers for further polymerization.Monomer 3 exhibted good antioxidant activity

CRediT authorship contribution statement:

Conceptualization, S. M. M, A. H. M., A. M. A. M., and A. K. M..; methodology, , S. M. M, A. H. M., A. M. A. M., N. M. F, and A. K. M.; software, , S. M. M, A. H. M., A. M. A. M., and A. K. M.; validation, , S. M. M, A. H. M., A. M. A. M., and A. K. M.; formal analysis, , S. M. M, A. H. M., A. M. A. M., N. M. F, and A. K. M.; investigation, , S. M. M, A. H. M., A. M. A. M., N. M. F, and A. K. M.; resources, , S. M. M, A. H. M., A. M. A. M., N. M. F, and A. K. M.; data curation, , S. M. M, A. H. M., A. M. A. M., N. M. F, and A. K. M.; writing—original draft preparation, S.M.M..; writing—review and editing, S. M. M. A. H. M., A. M. A. M., and A. K. M..; visualization, A. H. M., A. M. A. M., N. M. F, and A. K. M..; supervision, A. K. M., A. H. M., A. M. A. M..; project administration, A. K. M., A. H. M., A. M. A. M.; Antioxidant activity, N. M. F,. All authors have read and agreed to the published version of the manuscript."

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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