Synthesis, structure and cytotoxicity of trimethylsilyl oligothienylcarbaldehydes and their derivatives

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Dedicated to Professor O.M. Nefedov on the occasion of his 70th birthday

Abstract

Trimethylsilyl end-capped bi- and terthiophene carbaldehydes were prepared by reaction of bi- and terthienyl lithium with DMF. Condensation of 5-trimethylsilyl-2,2′-bithiophene-5′-carbaldehyde with dinitrile of malonic acid gave silylbithienyl methylidenedinitrile in good yield, while reaction with hydroxylamine was accompanied by desilylation. The reaction of hydroxylamine with silylbithienyldinitrile leads to the formation of 2-[5-(5′-trimethylsilyl-2,2′-bithienyl)methylidene]malonic acid bisamidoxime. The cytotoxic effect of bi- and terthiophene derivatives was investigated in vitro on two monolayer tumor cell lines: MG-22A (mouse hepatoma) and HT-1080 (human fibrosarcoma). The molecular structure of 5-trimethylsilyl-2,2′-bithiophene-5′-carbaldehyde was studied by X-ray diffraction. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Terthiophene carbaldehydes; Trimethylsilyl; Cytotoxicity

1. Introduction

The increasing interest in π-conjugated thiophene-based materials for exploring their electronic and optical properties has resulted in an extended research in the field of oligothiophenes chemistry [1–5]. However, 2,2′-bithiophenes and 2,2′:5′,2″-terthiophenes are also very important from the biological point of view. These compounds were extracted from various plants, such as *Blumea obliqua* [6], *Tagetes minuta* [7] and *Tagetes* sp. [8] and showed high antibacterial (*Staphylococcus aureus*, *Escherichia coli*) [9], antifungal (*Epidermophyton floccosum*, *Pleurotus ostreatus*) [6], anti-HIV [10] and insecticidal [7,11] activity. For a series of terthiophene derivatives the protein kinase C inhibitory activity was evaluated. Terthiophenes containing aldehyde or hydroxymethyl groups in position 5 were most potent inhibitors ($IC_{50} < 1$ μM) [12,13].

Taking into account the cytotoxic and antitumor activity of 5-silylsubstituted furfurals and thiophene carbaldehydes [14,15], the influence of the α-oligothiophene chain elongation on the cytotoxic effect was investigated in vitro on two monolayer tumor cell lines: MG-22A (mouse hepatoma) and HT-1080 (human fibrosarcoma).

2. Results and discussion

2.1. Synthesis of silylsubstituted bi- and terthiophene carbaldehydes and their derivatives

Scheme 1 outlines the synthetic methods for the preparation of trimethylsilyl end-capped bi- and terthiophene carbaldehydes **3** and **4**. Bithiophene and terthiophene were converted to monosilylsubstituted oligothiophenes (**1** and **2**) by lithiation following silylation. Subsequent metalation of compounds **1** and **2** in ether by *n*-BuLi and treatment with dry DMF afforded aldehydes **3** and **4** in 75 and 61% yields, respectively.

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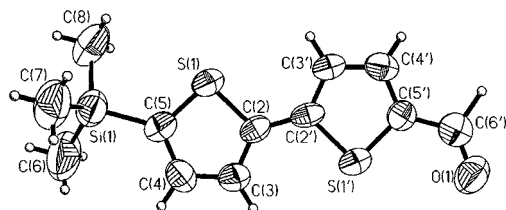*E-mail address:* sinta@osi.lv (E. Lukevics).
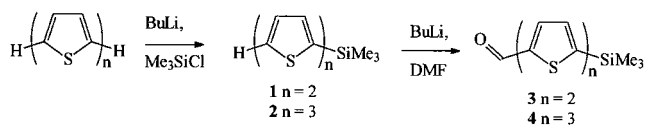
Scheme 1.
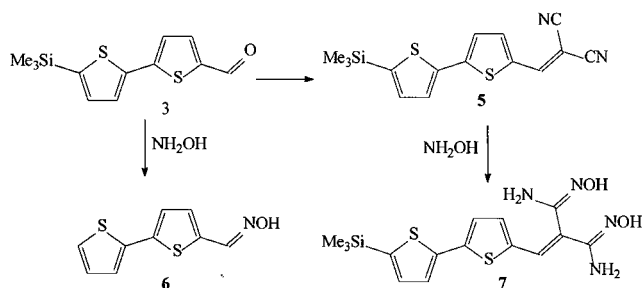
Scheme 2.

Condensation of 5-trimethylsilyl-2,2′-bithiophene-5′-carbaldehyde (**3**) with dinitrile of malonic acid in ethanol at room temperature in the presence of catalytic amount of piperidine gave 1-[5-(5′-trimethylsilyl-2,2′-bithienyl)]-2,2-dicyanoethylene (**5**). The cleavage of the Si–C$_{thiophene}$ bond did not occur. On the contrary, reaction of aldehyde **3** with hydroxylamine in CH$_2$Cl$_2$ proceeded with desilylation to afford 2,2′-bithiophene-5-carbaldoxime (**6**) in 67% yield. Amidoximization of dinitrile **5** was carried out in ethanol with two equivalents of hydroxylamine to yield 2-[5-(5′-trimethylsilyl-2,2′-bithienyl)methylidene]malonic acid bisamidoxime (**7**) containing the silyl group in position 5′ of bithiophene (Scheme 2).

2.2. Molecular structure of 5-trimethylsilyl-(2,2′-bithienyl)-5′-carbaldehyde (3)

The molecular structure of 5-trimethylsilyl-(2,2′-bithienyl)-5′-carbaldehyde (**3**) is presented in Fig. 1. Table 1 gives the values of bond lengths and angles in molecule **3**. As in 5,5′-bis(trimethylsilyl)-2,2′-bithiophene [16] two thiophene heterocycles of aldehyde **3** are almost coplanar (twisting angle 2.6(2)°) and their sulfur atoms lie *anti* to each other. On the other hand, the title structure differs from that of bithiophene carbaldehyde [17] in which the disordered sulfur atoms have a predominantly (80%) *cisoid* structure about the ring junction. The push–pull character of trimethylsilyl and aldehyde substituents leads to increased conjugation and as a result bond lengths C(2)–C(2′) (1.420 Å), C(3)–C(4) (1.399 Å) and C(3′)–C(4′) (1.388 Å) are shortened and C(4)–C(5) (1.376 Å) is elongated [18]. However, the push–pull effect of these groups is weak and only slightly increases the role of the mesomeric structure of semiquinoid type [19].

The presence of trimethylsilyl and aldehyde substituents at the terminal positions of bithiophene **3** plays an important role in the molecular packing of this compound (Figs. 2 and 3). The crystal unit cell contains four molecules of aldehyde **3** ($Z = 4$). In the crystal structure the molecules form rouleaus along crystallographic axis y and lie on a screw axis (2_1). π-Stacking interaction occurs between these molecules (see Fig. 3). The mean distance between π-stacked bithiophene

Fig. 1. Molecular structure of 5-trimethylsilyl-2,2′-bithienylcarb-5′-aldehyde (**3**).

Table 1
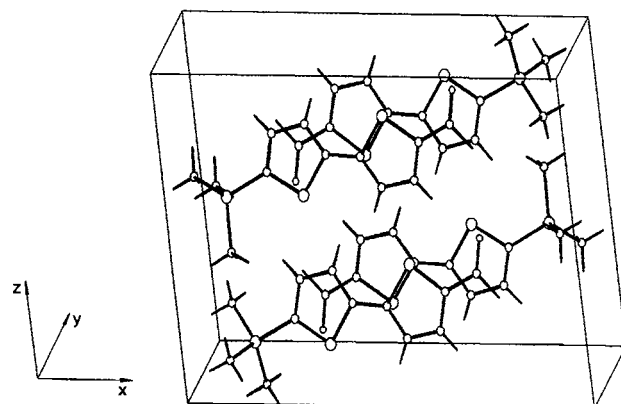Bond lengths (Å) and bond angles (°) for molecule **3**

S(1)–C(5)	1.686(8)	C(5)–S(1)–C(2)	94.2(4)
S(1)–C(2)	1.726(7)	C(3)–C(2)–C(2′)	129.6(6)
C(2)–C(3)	1.352(9)	C(3)–C(2)–S(1)	109.4(6)
C(2)–C(2′)	1.420(9)	C(2′)–C(2)–S(1)	120.9(5)
C(3)–C(4)	1.399(10)	C(2)–C(3)–C(4)	113.1(7)
C(4)–C(5)	1.376(10)	C(5)–C(4)–C(3)	114.5(7)
C(5)–Si(1)	1.876(8)	C(4)–C(5)–S(1)	108.9(6)
Si(1)–C(6)	1.832(11)	C(4)–C(5)–Si(1)	127.6(6)
Si(1)–C(7)	1.860(9)	S(1)–C(5)–Si(1)	123.5(4)
Si(1)–C(8)	1.868(10)	C(5′)–S(1′)–C(2′)	91.9(4)
S(1′)–C(5′)	1.708(7)	C(3′)–C(2′)–C(2)	129.9(6)
S(1′)–C(2′)	1.737(6)	C(3′)–C(2′)–S(1′)	109.1(6)
C(2′)–C(3′)	1.354(9)	C(2)–C(2′)–S(1′)	121.0(5)
C(3′)–C(4′)	1.388(10)	C(2′)–C(3′)–C(4′)	115.4(7)
C(4′)–C(5′)	1.361(9)	C(5′)–C(4′)–C(3′)	111.9(7)
C(5′)–C(6′)	1.456(10)	C(4′)–C(5′)–C(6′)	126.6(7)
C(6′)–O(1)	1.232(9)	C(4′)–C(5′)–S(1′)	111.7(6)
		C(6′)–C(5′)–S(1′)	121.7(6)
		O(1)–C(6′)–C(5′)	123.9(7)

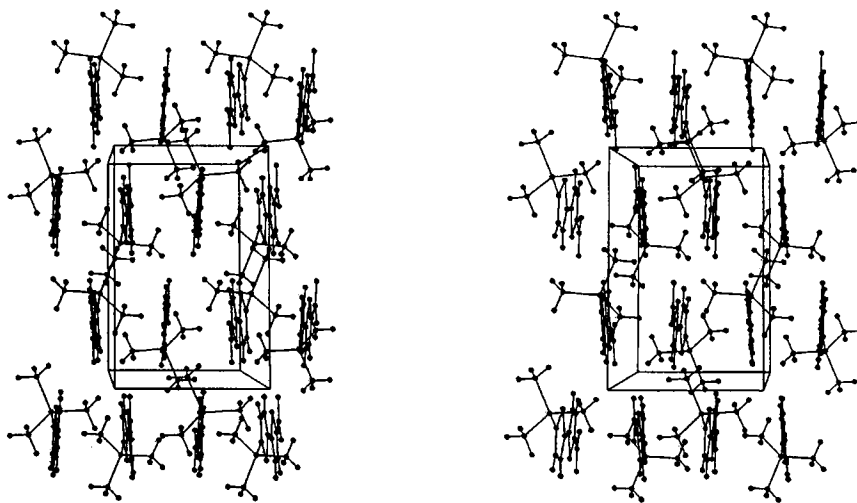Fig. 2. Molecular packing of bithiophene **3**.

Fig. 3. Stereoview of bithiophene **3**.

Table 2
In vitro cell cytotoxicity and the ability of intracellular NO generation caused by oligothiophenes[abc]

Compound	Cell lines					
	HT- 1080			MG- 22A		
	IC_{50} CV [a]	IC_{50} MTT [b]	NO %, CV [c]	IC_{50} CV	IC_{50} MTT	NO % CV
3 Me₃Si...CHO	81	71	233	35	30	260
4 Me₃Si...CHO	>100	>100	2	>100	>100	15
5 Me₃Si...CN,CN	>100	>100	4	>100	>100	12
7 Me₃Si...(NH₂,NOH; H₂N,NOH)	41	8.5	83	36	33	400
8 thiophene...CHO	42	10	350	46	44	400
9 terthiophene...CHO	7	7	64	5	4	100
10 quaterthiophene...CHO	6.2	1	27	2	3	90
11 ...CN,NC	9	9	1200	41	>100	35
6 ...NOH	79	73	300	48	35	175

[a] Concentration (μg/mL) providing 50% cell killing effect (CV: coloration).
[b] Concentration (μg/mL) providing 50% cell killing effect (MTT: coloration).
[c] NO Concentration (%) (CV: coloration).

planes is equal to half of period *b* (3.629(3) Å). As terminal substituents of bithiophene **3** have different electronic effects the π-stacked layers are orientated in such a manner that the aldehyde group of one molecule lies over the trimethylsilyl group of the neighboring molecule. Probably, the mentioned packing type of bithiophene **3** is determined by the dipole–dipole interaction.

2.3. Cytotoxicity of oligothiophenes

Cytotoxic activity of silylsubstituted bi- and terthiophene carbaldehydes and their derivatives was tested in vitro on two monolayer tumor cell lines: MG-22A (mouse hepatoma) and HT-1080 (human fibrosarcoma). The cytotoxic effect of silicon-containing compounds was compared with silicon-free analogs (Table 2), prepared by the Pd^0 catalyzed coupling of thienylstannanes with bromothiophene carbaldehydes [20,21]. 5-Trimethylsilyl-(2,2′-bithienyl)-5′-carbaldehyde (**3**) exhibits higher cytotoxicity than the product of its condensation with dinitrile of malonic acid **5** and analog with terthiophene chain **4**. The effect of bisamidoxime **7** is comparable with aldehyde **3** in MG-22A and more pronounced in the HT-1080 cell line. As compared with desilylated oligothiophene carbaldehydes **8**–**10** organosilicon compounds are less cytotoxic. Monosubstituted ter- and quaterthiophene carbaldehydes **9** and **10** show significant cytotoxic effect ($IC_{50} = 1$–7 μg ml^{-1}).

3. Experimental

3.1. General considerations and materials

All solvents used in the reactions were dried by standard procedures. The following reagents were obtained from commercial sources and used without further purification: 2-bromothiophene, *n*-BuLi (2.5 M hexane solution), trimethylchlorosilane. 2,2′-Bithiophene and α-terthiophene were prepared by catalytic coupling of 2-thienylmagnesium bromide with 2-bromothiophene and 2,5-dibromothiophene, respectively. Column chromatography was carried out using 60–200 mesh silica gel from Acros.

The ^{1}H-, ^{13}C- and ^{29}Si-NMR spectra were recorded on a Varian Mercury 200 spectrometer at 200.06, 50.31 and 39.74 MHz, respectively, at 303 K. The chemical shifts are given relative to Me_4Si from the solvent (CDCl$_3$) signal ($\delta_H = 7.25$ ppm). Mass spectra were recorded on a Hewlett Packard apparatus (70 eV). The melting points were determined on a 'Digital Melting Point Analyzer' (Fisher) and the results are given without correction.

3.1.1. 5-Trimethylsilyl-(2,2′-bithienyl)-5′-carbaldehyde (3)

To a solution of 5-trimethylsilyl-2,2′-bithiophene (0.05 mol) in 30 ml of Et$_2$O was added 20 ml of 2.5 N *n*-BuLi in hexanes (0.05 mol) at room temperature (r.t.). After 1 h, excess (0.08 mol) of DMF was added dropwise. The reaction mixture was refluxed for 2 h, hydrolyzed with a saturated solution of NH$_4$Cl, extracted with Et$_2$O, dried over Na$_2$SO$_4$ and evaporated. The residue was purified on silica gel using CH$_2$Cl$_2$ as eluent. M.p. 85–86°C. Yield 75%. IR (cm^{-1}) $v_{(C=O)} = 1660$. MS–GC: 266. ^{1}H-NMR (CDCl$_3$): δ 0.33 (s, 9H), 7.16 (d, 1H, $J = 3.4$ Hz), 7.24 (d, 1H, $J = 3.4$ Hz), 7.38 (d, 1H, $J = 3.4$ Hz), 7.65 (d, 1H, $J = 3.4$ Hz), 9.85 (s, 1H). ^{13}C-NMR (CDCl$_3$): δ −0.2, 124.3, 127.3, 135.0, 137.4, 140.5, 141.6, 143.4, 147.1, 182.6. ^{29}Si-NMR (CDCl$_3$): δ −5.86. Anal. Found: C, 54.12; H, 5.31; S, 24.11. Calc. for C$_{12}$H$_{14}$OS$_2$Si: C, 54.10; H, 5.30; S, 24.07%.

3.1.2. 5-Trimethylsilyl-(2,2′:5′,2″-terthienyl)-5″-carbaldehyde (4)

M.p. 144–145°C. Yield 61%. ^{1}H-NMR (CDCl$_3$): δ 0.34 (s, 9H), 7.12–7.16 (m, 2H), 7.22 (d, 1H, $J = 4.2$ Hz), 7.26 (d, 2H, $J = 3.6$ Hz), 7.66 (d, 1H, $J = 4$ Hz), 9.85 (s, 1H). ^{13}C-NMR (CDCl$_3$): δ 0.8, 124.9, 125.6, 126.7, 127.9, 135.4, 135.9, 138.3, 140.2, 142.2, 142.5, 147.9, 183.3. ^{29}Si-NMR (CDCl$_3$): δ −6.2. Anal. Found: C, 55.10; H, 4.60; S, 27.53. Calc. for C$_{16}$H$_{16}$OS$_3$Si: C, 55.13; H, 4.63; S, 27.60%.

3.1.3. 1-[5-(5′-Trimethylsilyl-2,2′-bithienyl)]-2,2-dicyanoethylene (5)

M.p. 173–174°C. Yield 95%. MS–GC: 314. ^{1}H-NMR (CDCl$_3$): δ 0.34 (s, 9H), 7.20 (d, 1H, $J = 3.6$ Hz), 7.27 (d, 1H, $J = 3.6$ Hz), 7.46 (d, 1H, $J = 3.4$Hz), 7.62 (d, 1H, $J = 4.2$ Hz), 7.75 (s, 1H). ^{13}C-NMR (CDCl$_3$): δ 0.7, 76.9, 114.4, 115.2, 125.6, 129.3, 134.4, 136.3, 140.8, 141.1, 146.3, 150.4, 151.3. ^{29}Si-NMR (CDCl$_3$): δ −5.53. Anal. Found: C, 57.30; H, 4.52; N, 8.86; S, 20.35. Calc. for C$_{15}$H$_{14}$N$_2$S$_2$Si: C, 57.29; H, 4.49; N, 8.91; S, 20.39%.

3.1.4. 2-[5-(5′-Trimethylsilyl-2,2′-bithienyl)methylidene]-malonic acid bisamidoxime (7)

To a solution of 1-[5-(5′-trimethylsilyl-2,2′-bithienyl)]-2,2-dicyanoethylene (**8**) (0.01 mol) in 10 ml of EtOH was added hydroxylamine (0.02 mol). The reaction mixture was refluxed for 2 h. After cooling the solvent was removed and residue purified on silica gel using EtOAc as eluent, m.p. 128–130°C. Yield 98%. ^{1}H-NMR (CDCl$_3$): δ 0.04 (s, 9H), 5.73 (s, 4H, NH$_2$), 5.93 (s, 2H, OH), 7.03 (d, 1H, $J = 3.4$ Hz), 7.13 (d, 1H, $J = 3.4$ Hz), 7.33 (d, 1H, $J = 4.2$ Hz), 7.37 (d, 1H, $J = 4.2$ Hz), 7.93 (s, 1H). ^{13}C-NMR (CDCl$_3$): δ −0.2, 122.8, 123.6, 125.7, 125.9, 129.3, 130.6, 132.7, 134.8, 134.9, 141.2, 144.8, 163.2. Anal. Found: C, 47.38; H,

Table 3
Crystal data and structure refinement for **3**

Formula weight M_r	266.44
Temperature (K)	293(2)
Wavelength (Å)	0.71073
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	
a (Å)	16.652(8)
b (Å)	7.258(3)
c (Å)	11.499(6)
α (°)	90
β (°)	95.05
γ (°)	90
V (Å^3)	1384.4(11)
Z	4
D_{calc} (mg m^{-3})	1.278
Absorption coefficient (mm^{-1})	0.449
$F(000)$	560
Crystal size (mm)	$0.75 \times 0.25 \times 0.10$
Two-theta (max) (°)	45.0
Index ranges	$-17 \leq h \leq 17$, $-7 \leq k \leq 0$, $0 \leq l \leq 12$
Reflections collected	1939
Independent reflections	1824
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	1824/0/145
Goodness-of-fit on F^2	1.018
Final R factor [$I > 2\sigma(I)$]	0.0725

5.33; N, 14.71; S, 16.80. Calc. for $C_{15}H_{20}N_4O_2S_2Si$: C, 47.35; H, 5.30; N, 14.73; S, 16.85%.

3.2. Crystal structure determination

For X-ray crystal structure analysis a four-circle single-crystal Sintex P2$_1$ diffractometer with graphite-monochromated Mo–K$_\alpha$ ($\lambda = 0.71069$ Å) radiation was used for intensity data collection. Reflection intensities were collected at r.t. using the $\theta/2\theta$ scan technique. Multisolution direct method package SHELX-86 [22] was used for the solution of the structure. SHELXL-93 programs [23] were used for the refinement calculations. Other crystallographic, measurement and refinement data for bithiophene **3** are listed in Table 3.

3.3. In vitro cytotoxicity assay

Monolayer cell lines were cultivated for 72 h in DMEM standard medium without an indicator and antibiotics. The control cells and cells with tested substances in the range of $2-5 \times 10^4$ cell ml^{-1} concentration (depending on line nature) were placed on separate 96 wells plates. Solutions containing the test compounds were diluted and added in wells to give the final concentrations of 50, 25, 12.5, and 6.25 μg ml^{-1}. Control cells were treated in the same manner but only in the absence of test compounds. Plates were cultivated for 72 h. The quantity of cells that survived was determined using crystal violet (CV) or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolinium bromide (MTT) coloration, which was assayed by a multiscan spectrophotometer. The quantity of cells alive on the control plate was taken in calculations for 100% [24,25]. The concentration of NO was determined according to Ref. [24].

4. Supplementary material

Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC no. 157444 for compound **3**. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

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