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SUPPLEMENTARY MATERIAL

Synthesis and Spectroscopic Properties of a Novel Bi-Functional Pyrene Derivative and its Incorporation into OligoDNA

Tomohisa Moriguchi,[A] *Akemi Hida,*[A] *Fumio Yoneda,*[B] *and Kazuo Shinozuka**[A]

[A]Division of Molecular Science, School of Science and Technology, Gunma University, 1-5-1 Tenjin-cho, Kiryu 376-8515, Japan.

[B]Institute of Research and Development, Fujimoto Pharmaceutical Corporation, 1-3-40 Nishiotsuka, Matsubara, Osaka 580-0011, Japan

*Author for correspondence, email address: sinozuka@chem-bio.gunma-u.ac.jp

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Experimental

General

1,6-Dibromopyrene, 1H-benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluoro -phosphate (BOP) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were purchased from Wako Pure Chemical Industries, Ltd. Chloro(dimethyl)vinylsilane was purchased from Sigma-Aldrich. Thin layer column chromatography was carried out on Merck silica gel 60 F254-precoated plate. All synthesized compounds except oligoDNA were purified by column chromatography using silica gel 60 N (neutral, spherical, 63-210 μm). ^{1}HNMR and ^{31}PNMR spectra were recorded on JOEL (JNM-ECF 400) FT NMR SYSTEM at 400 MHz and 161.8 MHz, respectively, using tetramethylsilane as internal standard. ESI-Mass spectra were recorded on a Perkin Elmer API-2000 ESI-MS Spectrophotometer. Uv-vis spectra were recorded on UV-2450 Spectrophotometer (SHIMADZU). Fluorescence spectra were recorded on F-4500 Fluorescence Spectrophotometer (HITACHI). OligoDNA was purified by reversed-phase HPLC (PU-2089 plus, JASCO) attached with uv-vis detector (PU-2075, JASCO) and chromatopac (C-R8A, SHIMADZU) using Wakosil 5C18 Column (ø 4.6 mm x 250mm). The purity of the compounds that were isolated were better than 95 % as evidenced from the chromatography and their NMR spectra (see below).

*1-Bromo-6-(dimethylvinylsilyl)pyrene **2***

1,6-Dibromopyrene (1.3 g, 3.6 mmol) in dry THF (20 ml) was treated with an equimolar amount of *n*-BuLi (THF solution) at -78˚C for 1h followed by chloro(dimethyl)vinylsilane (1 ml, 7.2 mmol) under nitrogen atmosphere. The mixture was stirred at -78˚C for 1 h then allowed to reach room temperature. After evaporation of the mixture to dryness, the residue was dissolved in ethyl acetate (200 ml) and washed with saturated sodium bicarbonate (200 ml). The organic layer was dried over sodium sulfate then evaporated to dryness. The residue was purified by silica gel column chromatography (EtOAc/Hexane = 9:1) to give compound **2** (0.51 g, 38.7 %) as a colorless liquid.

^{1}H NMR ($CDCl_3$) δ 8.45 (1H, d, *J* = 5.5 Hz, PyH), 8.36 (1H, d, *J* = 9.2 Hz, PyH), 8.21-7.89 (6H, m, PyH), 6.56 (1H, dd, *J* = 14.6 Hz, 20.6 Hz, –CH=), 6.14 (1H, dd, *J* = 14.6 Hz, 4.6 Hz, =CH (cis)), 5.88 (1H, dd, *J* = 20.6 Hz, 4.6 Hz, =CH (trans)), 0.64 (6H, s, 2xCH_3).

*1-Formyl-6-(dimethylvinylsilyl)pyrene **3***

Compound **2** (0.51 g, 1.4 mmol) in dr y THF (20 ml) was treated with a slight excess amount of of *n*-BuLi (THF solution, 2.1 mmol) at -78˚C for 1h followed by DMF (3.5 ml) under nitrogen atmosphere. The mixture was stirred at -78˚C for 1 h then at room temperature for 3 h. After evaporation of the mixture to dryness, the residue was dissolved in ethyl acetate (50 ml) and washed with saturated NaCl (50 ml). The obtained organic layer was dried over sodium sulfate then evaporated to dryness. The residue was purified by silica gel column chromatography (CH_2Cl_2/Hexane = 4:1) to give compound **3** (0.11 g, 24.4 %) as a colorless liquid.

^{1}H NMR ($CDCl_3$) δ 10.80 (1H, s, -CHO), 9.43 (1H, d, *J* = 9.1 Hz, PyH), 8.56 (1H, d, *J* = 9.1 Hz, PyH), 8.44 (1H, d, *J* = 9.1 Hz, PyH), 8.30 (1H, d, *J* = 9.1 Hz, PyH), 8.29 (1H, d, *J* = 9.1 Hz, PyH), 8.27 (1H, d, *J* = 9.1 Hz, PyH), 8.12 (1H, d, *J* = 9.1 Hz, PyH), 6.58 (1H, dd, *J* = 20.1 Hz, 14.7 Hz, –CH=), 6.17 (1H, dd, *J* = 14.7 Hz, 3.7 Hz, =CH (cis)), 5.90 (1H, dd, *J* = 20.1 Hz, 3.7 Hz, =CH (trans)), 0.71 (6H, s, 2xCH_3).

*1-Cyano-6-(dimethylvinylsilyl)pyrene **5***

The mixture of compound **3** (0.1 g, 0.34 mmol) and hydroxylamine-*O*-sulfonic acid (0.15 g, 1.3 mmol) in EtOH (10 ml) was stirred at 80˚C for 1 h then evaporated to dryness. The residue was dissolved in CH_2Cl_2 (20 ml) and washed with 0. 5 % HCl (20 ml) followed by saturated sodium bicarbonate (2 x 20 ml). The organic layer was dried over sodium sulfate then evaporated to dryness. The obtained crude material was used for next step without further purification.

The above crude material was dissolved in DMF (7 ml). To the solution was added 1*H*-benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP, 0.08 g, 0.67 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.1 ml, 0.67 mmol) and the whole mixture was stirred for 30 min. at room temperature. After evaporation of the mixture to dryness, the resulting residue was dissolved in EtOH (30 ml) and washed with saturated NaCl (30 ml). The obtained organic layer was dried over sodium sulfate then evaporated to dryness. The residue was purified by silica gel column chromatography (EtOAc/Hexane = 1:9) to give compound **5** (0.89 g, 89.9 % from compound **3**) as a colorless liquid.

^{1}H NMR ($CDCl_3$) δ 8.63 (1H, d, *J* = 9.6 Hz, PyH), 8.49 (1H, d, *J* = 9.2 Hz, PyH), 8.32 (1H, d, *J* = 7.8 Hz, PyH), 8.26 (1H, d, *J* = 6.5 Hz, PyH), 8.22 (1H, d, *J* = 8.3 Hz, PyH), 8.17 (1H, d, *J* = 9.1 Hz, PyH), 8.14 (1H, d, *J* = 7.8 Hz, PyH), 8.07 (1H, d, *J* = 8.7 Hz, PyH), 6.57 (1H, dd, *J* = 20.6 Hz, 14.6 Hz, –CH=), 6.17 (1H, dd, *J* = 14.6 Hz, 3.2 Hz, =CH (cis)), 5.90 (1H, dd, *J* = 20.6 Hz, 3.2 Hz, =CH (trans)), 0.66 (6H, s, 2xCH_3).

*1-Cyano-6-[(2-hydroxyethyl)dimethylsilyl]pyrene **6***

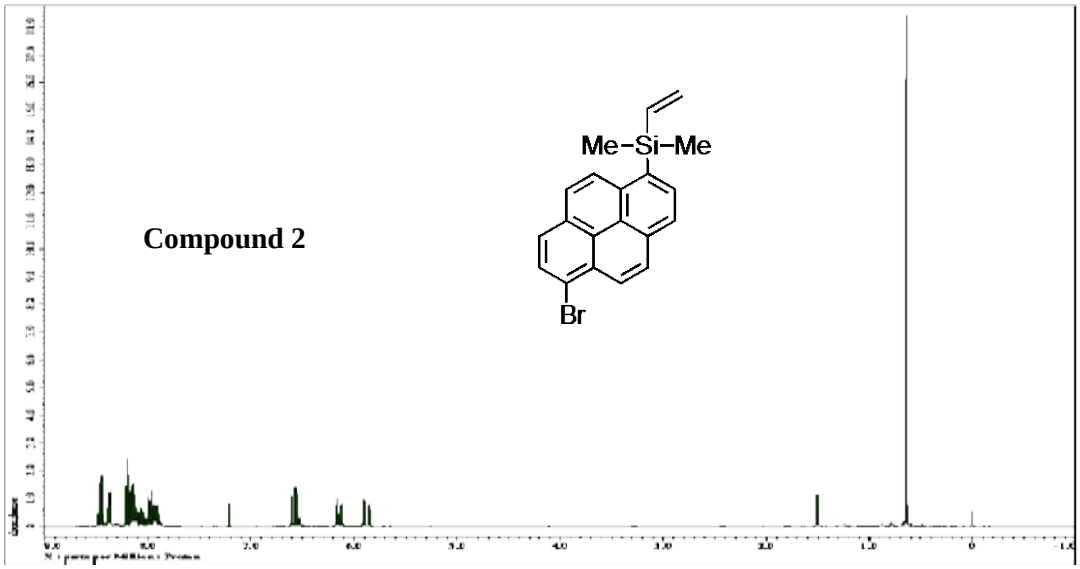
The mixture of compound **5** (0.2 g, 0.64 mmol) and 9-borabicyclo[3.3.1]nonane (9-BBN, 0.5 M THF solution, 2 ml, 1.0 mmol) in 5 ml of dry THF was heated at 50 ˚C for 2 h. After cooling, EtOH (10 ml), 1 M NaOH (3 ml, 3 mmol) and 30 % H_2O_2 (0.6 ml, 6mmol) were added to the mixture. The whole mixture was stirred for overnight under cooling at 0 ˚C then evaporated to dryness. The obtained residue was dissolved in ethyl acetate (20 ml) and washed with saturated NaCl (20 ml). The isolated organic layer was dried over sodium sulfate then evaporated to dryness. The residue was purified by silica gel column chromatography (EtOAc/Hexane = 4:6) to give compound **6** (0.12 g, 55.7 %) as a colorless solid.

mp >125 ˚C decomp; ^{1}H NMR ($CDCl_3$) δ 8.51 (1H, d, *J* = 9.2 Hz, PyH), 8.44 (1H, d, *J* = 9.2 Hz, PyH), 8.28-8.22 (4H, m, PyH), 8.17 (1H, d, *J* = 7.8 Hz, PyH), 8.13 (1H, d, *J* = 9.2 Hz, PyH), 3.79 (2H, t, *J* = 8.2 Hz, CH_2OH), 1.56 (2H, t, *J* = 8.2 Hz, CH_2Si), 0.66 (6H, s, 2xCH_3).

*2-Cyanoethyl (2-((6-cyanopyren-1-yl)dimethylsilyl)ethyl) diisopropylphosphoramidite **7***

To the solution of compound **6** (0.2 g, 0.61 mmol) in dry CH_2Cl_2 (10 ml) was added *N*,*N*-diisopropylethyamine (0.5 ml, 2.86 mmol) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.64 ml, 2.86 mmol) by dropwise under cooling in an ice-bath. The mixture was stirred at 0˚C for 15 min. then at room temperature for 30 min. The excess phosphoramidite reagent was decomposed by the addition of dry-MeOH (1 ml). After evaporation of the mixture, the resulting oil was dissolved in CH_2Cl_2 (30 ml) and washed with sat. $NaHCO_3$ solution (2 x 30 ml), followed by saturated NaCl solution (30 ml). The isolated organic layer was dried over sodium sulfate then evaporated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane/triethylamine = 47.5:47.5:5). The appropriate fractions were collected and evaporated to an oil which was dissolved in small amount of CH_2Cl_2 and dropwised added to rapidly stirring cold hexane (150 ml) to precipitate compound **7** (0.19 g, 57.9 %).

mp >130 ˚C decomp; ^{1}H NMR ($CDCl_3$) δ 8.51 (1H, d, *J* = 9.2 Hz, PyH), 8.45 (1H, d, *J* = 9.2 Hz, PyH), 8.44 (1H, d, *J* = 9.1 Hz, PyH), 8.31-8.28 (2H, m, PyH), 8.25 (1H, d, *J* = 6.8 Hz, PyH), 8.19 (1H, d, *J* = 8.3 Hz, PyH), 8.14 (1H, d, *J* = 9.2 Hz, PyH), 3.74-3.67 (2H, m, CH_2OP), 3.53-3.47 (2H, m, CH_2OP), 2.53 (2H, t, *J* = 6.4 Hz, CH_2CN), 1.63-1.58 (2H, m, CH_2Si), 1.07 (12H, 2d, J = 6.4 Hz, CH_3 of *i*-Pr), 0.66 (6H, s, 2xCH_3); ^{31}P NMR ($CDCl_3$ with external reference of 85 % H_3PO_4) δ 147.0 (singlet).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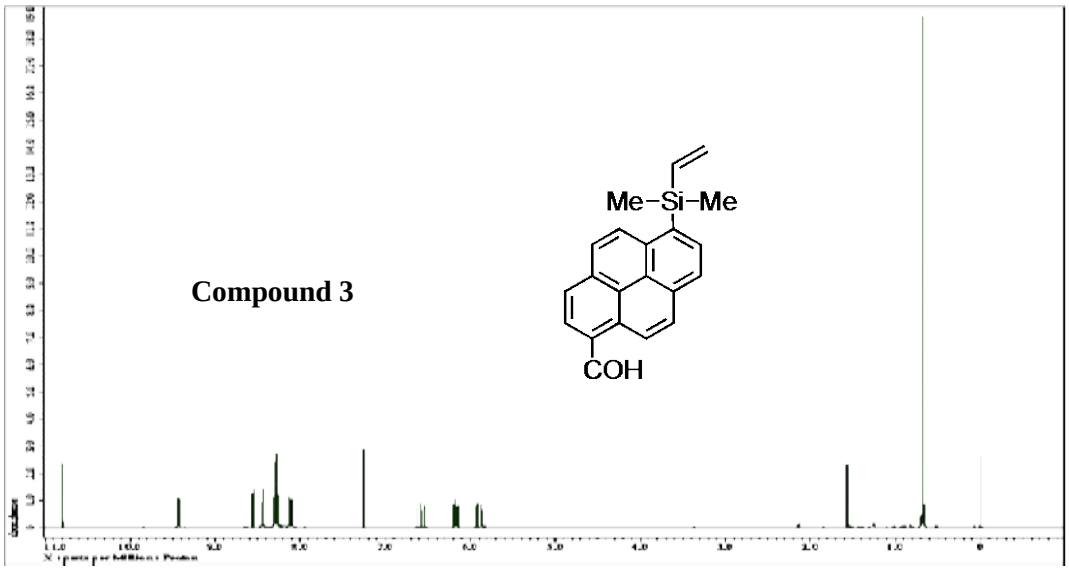
Br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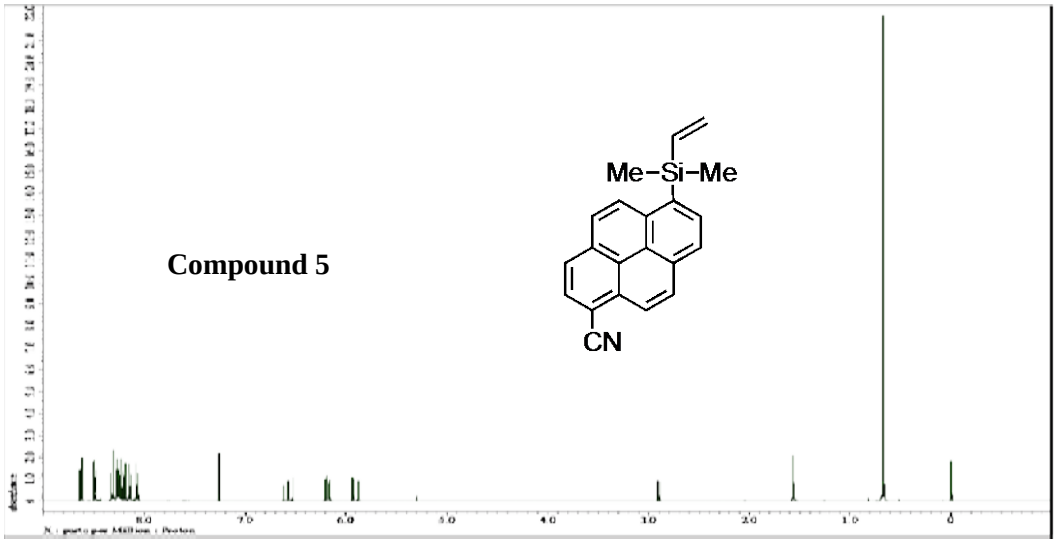
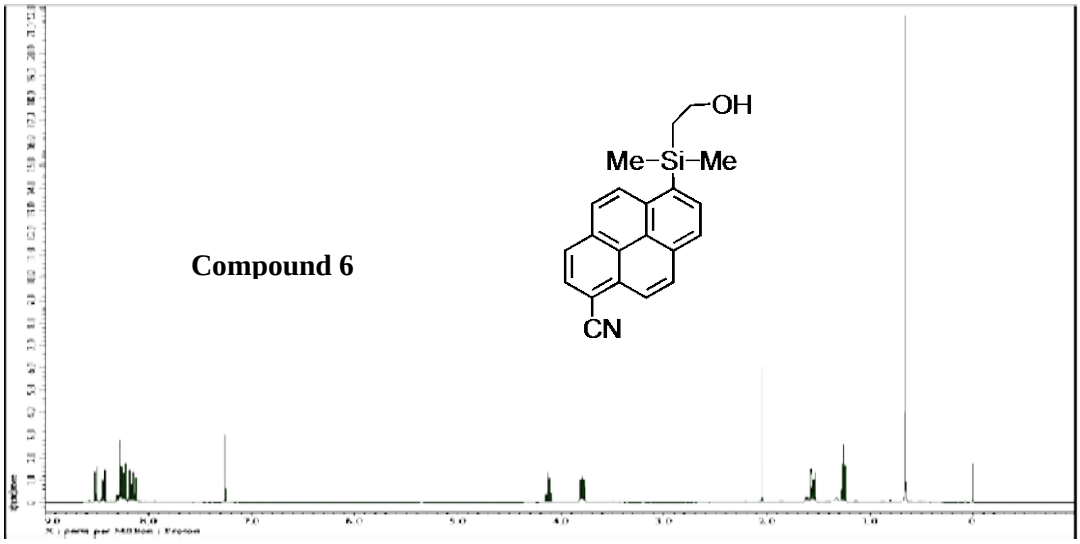

Compound 5
Me–Si–Me
CN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Me–Si–Me
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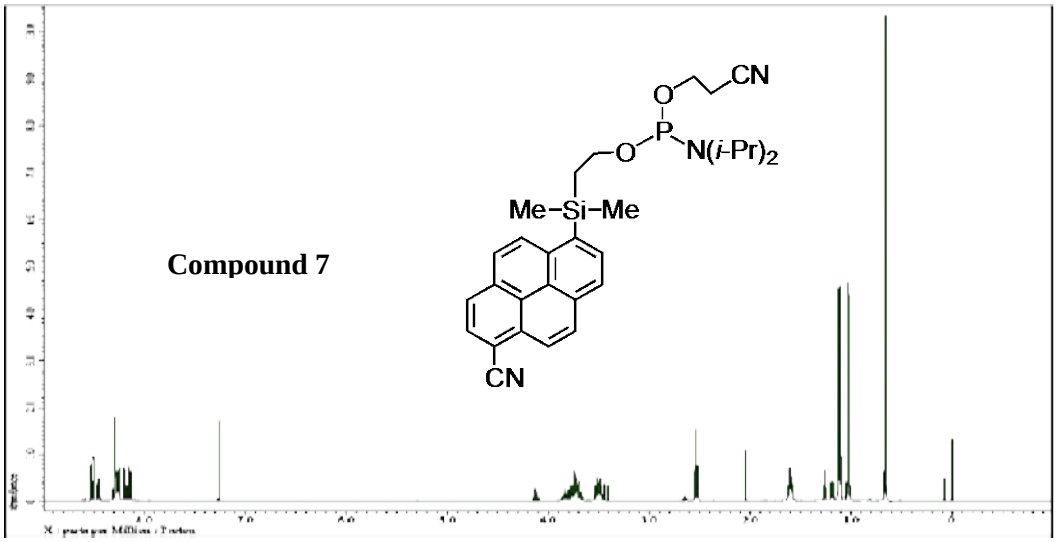
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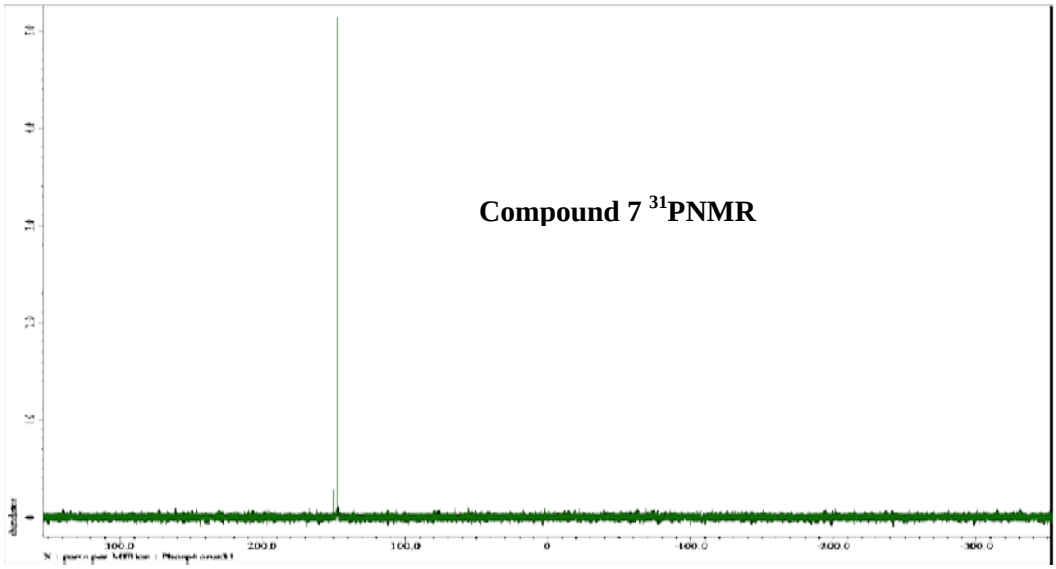
O
P
O
N(i-Pr)2
Me–Si–Me
CN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