Synthesis of a pentathiophene fluorescent probe, 4',3''-bis-carboxymethyl-[2,2';5',2'';5'',2''';5''',2''']quinquethiophene-5,5'

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Method Article

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Abstract

Introduction

Poly and oligo-thiophenes have previously been used for *in vitro*^{1,2}, *ex vivo*^{3,4} and *in vivo*⁵ imaging of protein aggregates. The probe (p-FTAA) has been developed for the purpose of *in vivo* staining of protein aggregates such as amyloid deposits. It effectively passes the blood brain barrier and imaging can be performed live with two-photon imaging or *ex vivo*⁵. The straightforward synthesis of p-FTAA, including two Suzuki couplings, makes it an attractive probe for studies of most diseases involving protein aggregates.

Reagents

- 3-Thiopheneacetic acid (Sigma-Aldrich, cat. no. 220639) - Acetyl chloride (Sigma-Aldrich, cat. no. 00990) - Methanol (Merck, cat. no. 1.08325) - N-bromosuccinimide (NBS) (Sigma-Aldrich, cat. no. B81255) - Chloroform (Prolabo, cat. no. 83626.320) - Acetic acid (Sigma-Aldrich, cat. no. A6283) - Silica Gel for flash column chromatography (Sigma-Aldrich, cat. no. 22716-6) - 2,5-Thiophenediboronic acid (Sigma-Aldrich, cat. no. 470317) - PEPPSI-Ipr® ([1,3-Bis(2,6-Diisopropylphenyl)imidazol-2-ylidene](3-chloropyridyl)palladium(II) dichloride) (Sigma-Aldrich, cat. no. 669032) - Potassium carbonate (Sigma-Aldrich, cat. no. 20961-9) - Toluene (Merck, cat. no. 1.08325) - 5-(Dihydroxyboryl)-2-thiophenecarboxylic acid (Frontier Scientific, cat. no. C1695) - Sodiumhydroxide (Sigma-Aldrich, cat. no. 45291-2) - Deuterated chloroform (Sigma-Aldrich, cat. no. 151823) - Deuterated DMSO (Sigma-Aldrich, cat. no. 151874) - Deuterated D2O (Sigma-Aldrich, cat. no. 151882) - Thin-layer chromatography plates on glass backing, silica gel 60 F254 (Merck) - Ethylacetate (Prolabo cat. no. 23882.321) - Celite® 545 (Prolabo cat. no. 22552.290) - Trifluoroacetic acid (Merck cat. no. 8.08260) - Diethylether (Prolabo cat. no. 23811.292) - HCl (Scharlau cat. no. AC0741) - Dioxane (Prolabo cat. no. 83620.320)

Equipment

- Magnetic hotplate stirrer - Digital temperature probe - Oil bath - 100 and 250 mL round-bottom flasks - Water condenser (to fit neck of flask) - Teflon-coated magnetic stirrer bar - Heat gun - Rotary evaporator (Büchi) - Pyrex chromatographic column (approx. diameter 3 cm) - NMR tubes - VersaFlash® purification system with reversed phase columns (Sigma-Aldrich)

Procedure

Synthesis of (2-bromo-thiophen-3-yl)-acetic Acid methyl ester
1] To a solution at 0 °C with 3-thiopheneacetic acid (1.42 g, 10 mmol) and methanol (20 mL) add acetic acid (1 mL) slowly. 2] Dilute with toluene and wash with NaHCO₃ (sat. aq.) until basic. 3] Dry with MgSO₄, filter and concentrate. 4] Dissolve in 20 mL chloroform/acetic acid (1:1) and cool to 0 °C. 5] Add 1.78 g NBS (10 mmol) 6] After five hours dilute with toluene and wash with NaHCO₃ (sat. aq.) until basic. 7] Dry with MgSO₄, filter and

concentrate. 8] Purify on flush column chromatography (toluene). **Synthesis of (3'-methoxycarbonylmethyl-[2,2';5',2'']terthiophen-3-yl)-acetic acid methyl ester (3)** 1] Dissolve **2** (2.35 g 10 mmol) and 2,5-thiophenediboronic acid (0.781 g, 4.5 mmol) in 20 mL degassed toluene/methanol (1:1). 2] Add 4.14 g (30 mmol) K₂CO₃ and PEPPSI-Ipr (0.204 g, 0.30 mmol) to the solution and heat at 80 °C for 10 minutes. 3] Filter the reaction mixture on celite, dilute with toluene and wash with NaHCO₃ (sat. aq.). 4] Dry with MgSO₄, filter and concentrate. 5] Purify the crude reaction mixture on a reversed phase column (methanol/H₂O 4:1, 0.05 % trifluoroacetic acid) to give the product. **Synthesis of (5,5"-dibromo-3"-methoxycarbonylmethyl-[2,2';5',2'']terthiophen 3-yl)-acetic acid methyl ester (4)** 1] Add NBS (0.460 g, 2.04 mmol) to a solution of the trimer **3** (0.382 g, 0.973 mmol) in chloroform/acetic acid (1:1, 5 mL, 0 °C). 2] Stir for 4 hours before dilution with diethyl ether (20 mL) 3] Wash with NaHCO₃ (sat. aq., 20 mL) and finally water (20 mL). 4] Dry with MgSO₄, filter and concentrate. 5] Purify by flash column chromatography (toluene). **Synthesis of 4',3"-bis-carboxymethyl-[2,2';5',2";5",2"";5""',2""']quinquthiophene-5,5""-dicarboxylic acid (p-FTAA) (5)** 1] Add K₂CO₃ (4.14 g, 30 mmol), PEPPSI-IPr (0.204 g, 0.30 mmol) and 5-(dihydroxyboryl)-2-thiophenecarboxylic acid to a solution of **4** (5.50 g, 10 mmol) in degassed toluene/methanol (1:1, 30 mL). 2] Heat at 80 °C for 10 minutes 3] Add HCl (20 mL, 1 M, aq.) and collect the red precipitate 4] Dissolve the precipitate in boiling dioxane and filter it on celite followed by precipitation by addition of a small amount of water. 5] Dry the precipitate and add two equimolar amounts of NaOH (1 M, aq.) 6] The product is afforded by concentration or freeze drying.

Timing

One week

Critical Steps

During the cross coupling reactions generating product **5** and **3** both temperature and time should be precisely monitored. If available a microwave reactor can be used. The purification of product 3 should be performed on reversed phase column for best result.

Troubleshooting

Low yield: Repeat reaction with fresh reagents.

Anticipated Results

Product 2 Typical isolated yield of **2** should be 2.16 g (92 %). Analytical data: **¹H-NMR** (CDCl₃) δ : 3.63 (s, 2H), 3.71 (s, 3H), 6.30 (d, 1H, J = 5.7 Hz), 7.33 (d, 1H, J = 5.7 Hz) **¹³C-NMR** (CDCl₃) δ : 34.9, 52.2, 111.7, 125.8, 128.7, 133.6, 170.6 **Product 3** Typical isolated yield of **3** should be 1.50 g (85 %) as slightly yellow colored oil. Analytical data: **¹H-NMR** (CDCl₃) δ : 3.72 (s, 4H), 3.79

δ (s, 6H), 7.05 (d, 2H, J = 5.2 Hz), 7.14 (s, 2H), 7.25 (d, 2H, J = 5.2 Hz); ^{13}C -NMR (CDCl₃): 34.7, 52.3, 124.9, 127.5, 130.5, 130.6, 133.0, 135.9, 171.5; **HRMS** m/z calcd. for C₁₈H₁₆O₄S₃: [M]⁺ 392.0211; Found: 392.0228. **Product 4** Typical isolated yield of **4** should be 0.482 g (90 %). Analytical data: ^1H -NMR (CDCl₃): 3.70 (s, 4H), 3.73 (s, 6H), 7.02 (s, 2H), 7.08 (s, 2H); ^{13}C -NMR (CDCl₃): 34.5, 52.4, 111.8, 127.2, 127.8, 131.1, 134.1, 134.8, 170.8 **Product 5** Typical isolated yield of **5** should be 6.00 g (85 %) as red powder. Analytical data: ^1H -NMR (DMSO-D₆): 3.76 (s, 4H), 7.32 (s, 2H), 7.38 (d, 2H, J = 4.10 Hz), 7.45 (s, 2H), 7.66 (d, 2H, J = 4.10 Hz); ^{13}C -NMR (D₂O, 45 °C): 38.4, 124.4, 126.6, 129.1, 131.9, 132.0, 134.4, 134.7, 135.5, 139.6, 140.9, 169.9, 179.4; **HRMS** m/z calcd for C₂₆H₁₅O₈S₅: [M-H]⁻ 614.9371; Found 614.9422

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Figures

Figure 1

Scheme 1. Proposed synthetic route to p-FTAA (5)