CHEMISTRY
A European Journal

Supporting Information

Distinct Spacing Between Anionic Groups: An Essential Chemical Determinant for Achieving Thiophene-Based Ligands to Distinguish β-Amyloid or Tau Polymorphic Aggregates

Therése Klingstedt,[a] Hamid Shirani,[a] Jasmin Mahler,[b, d] Bettina M. Wegenast-Braun,[b, d] Sofie Nyström,[a] Michel Goedert,[c] Mathias Jucker,[b, d] and K. Peter R. Nilsson*[a]

chem_201500556_sm_miscellaneous_information.pdf

Supporting Information:

Table of contents:

Experimental details

Synthesis of LCOs

The synthesis of HS-68 has been published elsewhere[1]. HS-129, HS-136, HS-145 and HS-149 were synthesized as outlined below and in Scheme 1, 2 and 3.

General procedure for bromination

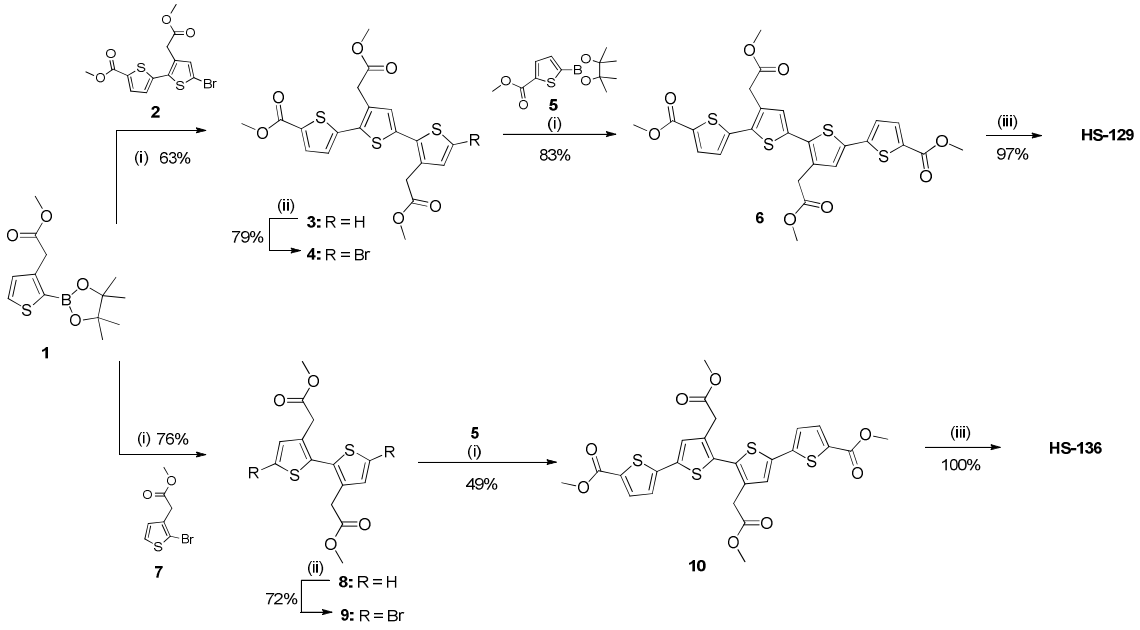
A solution of N-Bromosuccinimide (NBS) (1 or 2 equiv.) in dimethylformamide (DMF) (3 mL/mmol) was added drop wise to desired thiophene derivative in DMF (2 mL/1 mmol) at 0 °C. The mixture was allowed to reach room temperature (r.t.) overnight (16h). Subsequently water was added and the product was extracted with dichloromethane (DCM) (3×30 mL/mmol). The organic phase was washed with water (3×30 mL/mmol), brine (30 mL), dried over $MgSO_4$ and the solvent was evaporated under reduced pressure.

General procedure for Suzuki coupling

PEPPS-IPr (5 mol %) was added to a mixture of the bromo thiophene derivatives (1-2 equiv), K_2CO_3 (3 equiv./bromine), the desired boronic acid or pinacol esters (1-2 equiv./bromine) in 1,4-dioxane/methanol (8 : 2, 8 mL/mmol, degassed). The mixture was heated to 70 °C for 20 min then cooled to room temperature (RT) and pH adjusted to 4 by 1 M HCl. The residue was extracted with DCM (3×30 mL/mmol), washed with water (3×30 mL/mmol), brine (30 mL) and the combined organic phase was dried over $MgSO_4$. The crude product was either subjected to column chromatography or treated with appropriate solvent to give desired products.

General procedure for methylesters hydrolysis

NaOH (1 M, 1.5 equiv./ester) was added to a solution of the oligothiophene in 1,4-dioxane (7 mL/1 mmol) and heated to 50 °C for 5 h. When precipitation appeared, H_2O was added and the solution was lyophilized.



Scheme 1: **Reagents and conditions:** (i) 1,4-dioxane/MeOH, PEPPSI^{TM}-IPr, K_2CO_3, 70 °C, 20 min; (ii) NBS, DMF, 16 h; (iii) NaOH (1M), 1,4-dioxane, 60 °C, 16 h.

Dimethyl 2,2'-(5''-(methoxycarbonyl)-[2,2':5',2''-terthiophene]-3,4'-diyl)diacetate (3)

General procedure of Suzuki coupling was performed starting with compound **1** (0.830 g, 2.9 mmol) and **2** (1.09 g, 2.9 mmol). Purification by column chromatography using *n*-heptane/ethyl acetate (4:1) gave **3** (0.750 mg, 63 %) as yellow oil.

IR (KBr) 1739, 1714, 1521, 1443, 1339, 1290, 1266, 1196, 1170, 1015, 832, 748, 714 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, *J* = 4.0 Hz, 1H), 7.26 (d, *J* = 5.2 Hz, 1H), 7.19 (d, *J* = 4.0 Hz, 1H), 7.13 (s, 1H), 7.05 (d, *J* = 5.2 Hz, 1H), 3.90 (s, 3H), 3.79 (s, 4H), 3.74 (s, 3H), 3.73 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3, 170.9, 162.5, 141.6, 135.2, 134.2, 133.4, 132.9, 132.4, 132.0, 131.0,

130.6, 130.1, 127.2, 125.2, 52.5, 52.0, 52.4, 34.9, 34.8. MALDI-TOF: *m/z* calcd for $C_{20}H_{18}O_6S_3$ $(M+H)^+$: 451.0. Found: 451.0.

Dimethyl 2,2'-(5-bromo-5''-(methoxycarbonyl)-[2,2':5',2''-terthiophene]-3,4'-diyl)diacetate (4)

General procedure of bromination was performed using compound **3** (0.380 g, 0.84 mmol) and NBS (0.150 g, 0.843 mmol). Purification by column chromatography using *n*-heptane/ethyl acetate (4:1) gave compound **4** (0.354 mg, 79 %) as yellow solid.

mp130-131.5 °C. IR (KBr) 1739, 1729, 1703, 1456, 1435, 1412, 1341, 1321, 1269, 1243, 1195, 1174, 1100, 1011, 973, 865, 847, 829, 804, 788, 744, 732 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, *J* = 4.0 Hz, 1H), 7.18 (d, *J* = 4.0 Hz, 1H), 7.09 (s, 1H), 7.03 (s, 1H), 3.90 (s, 3H), 3.78 (s, 2H), 3.74 (s, 3H), 3.72 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 170.8, 162.4, 141.3, 134.1, 133.9, 133.8, 133.6, 133.4, 133.1, 132.1, 131.5, 130.5, 127.4, 112.1, 52.5, 52.5, 52.5, 34.8, 34.6. MALDI-TOF: *m/z* calcd for $C_{20}H_{17}BrO_6S_3$ $(M+H)^+$: 531.0. Found: 531.0.

Dimethyl 3',3''-bis(2-methoxy-2-oxoethyl)-[2,2':5',2'':5'',2'''-quaterthiophene]-5,5'''-dicarboxylate (6)

General procedure of Suzuki coupling was performed starting with compound **4** (0.057 g, 0.108 mmol) and **5** (0.028 g, 0.108 mmol). Trituration with warm MeOH gave compound **6** (0.053 g, 83 %) as orange solid.

mp162 °C. IR (KBr) 1732, 1721, 1716, 1516, 1463, 1450, 1435, 1340, 1311, 1266, 1250, 1236, 1195, 1173, 1096, 845, 799, 742 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 4.0 Hz, 1H), 7.70 (d, *J* = 3.9 Hz, 1H), 7.23 (s, 1H), 7.20 (d, *J* = 4.0 Hz, 1H), 7.18 (s, 1H), 7.15 (d, *J* = 3.9 Hz, 1H), 3.91 (s, 3H), 3.90 (s, 3H), 3.80 (s, 2H), 3.79 (s, 2H), 3.76 (s, 3H), 3.76 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 170.7, 162.4, 162.3, 143.1, 141.2, 135.2, 134.2, 134.2, 134.0, 133.5, 133.2, 132.8,

132.0, 131.9, 131.6, 130.3, 128.3, 127.2, 124.2, 52.4, 52.3, 52.2, 34.7. MALDI-TOF: *m/z* calcd for $C_{26}H_{22}O_8S_4$ $(M+H)^+$: 591.0. Found: 591.

HS-129

Hydrolysis of compound **6** applying general procedure gave **HS-129** as red solid.

mp > 300 °C. IR (KBr) 1575, 1520, 1456, 1386, 1278, 1039, 952, 881, 805, 768 cm^{-1}. ^1H NMR (300 MHz, D_2O) δ 7.54 (d, *J* = 3.9 Hz, 1H), 7.49 (d, *J* = 4.0 Hz, 1H), 7.26 (d, *J* = 3.7 Hz, 1H), 7.24 (s, 1H), 7.21 (d, *J* = 4.0 Hz, 1H), 7.13 (s, 1H), 3.74 (s, 2H), 3.73 (s, 2H). ^{13}C NMR (75 MHz, D_2O) δ 179.1, 179.1, 169.6, 169.6, 140.6, 140.2, 139.5, 139.3, 134.9, 134.6, 134.5, 134.1, 133.6, 131.9, 131.7, 131.4, 129.9, 128.9, 126.1, 124.2, 38.1, 37.9. MALDI-TOF: *m/z* calcd for $C_{22}H_{14}O_8S_4$ $(M+H)^+$: 535.0. Found: 534.9.

Dimethyl 2,2'-([2,2'-bithiophene]-3,3'-diyl)diacetate (8)

General procedure of Suzuki coupling was performed starting with compound **1** (0.300 g, 1.06 mmol) and **7** (0.250 g, 1.06 mmol). Column chromatography using *n*-heptane/ethyl acetate (5:1) yielded the dimer **8** (0.25 g, 76 %) as colorless oil.

IR (KBr) 1738, 1732, 1435, 1414, 1335, 1265, 1197, 1169, 1014, 830, 710 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, *J* = 5.3 Hz, 2H), 7.06 (d, *J* = 5.3 Hz, 2H), 3.65 (s, 6H), 3.54 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 134.1, 129.1, 126.6, 52.1, 34.1. MALDI-TOF: *m/z* calcd for $C_{14}H_{14}O_4S_2$ $(M+H)^+$: 311.0. Found: 311.0.

Dimethyl 2,2'-(5,5'-dibromo-[2,2'-bithiophene]-3,3'-diyl)diacetate (9)

General procedure of bromination was performed using compound **8** (0.120 g, 0.387 mmol) and NBS (0.138 g, 0.773 mmol). Column chromatography using *n*-heptane/ethyl acetate (5:1) yielded the dimer **9** (0.13 g, 72 %) as colorless oil.

IR (KBr) 1747, 1733, 1448, 1435, 1413, 1305, 1250, 1186, 1134, 1124, 1005, 999, 953, 878, 834 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.04 (s, 1H), 3.67 (s, 4H), 3.48 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 135.6, 132.0, 130.5, 114.0, 52.3, 33.9. MALDI-TOF: *m/z* calcd for $C_{14}H_{12}Br_2O_4S_2$ $(M+H)^+$: 468.9. Found: 468.8.

Dimethyl 3'',4'-bis(2-methoxy-2-oxoethyl)-[2,2':5',2'':5'',2'''-quaterthiophene]-5,5'''-dicarboxylate (10)

General procedure of Suzuki coupling was performed starting with compound **9** (0.109 g, 0.233 mmol) and **5** (0.125 g, 0.466 mmol). Column chromatography using *n*-heptane/ethyl acetate (3:1→1:1) yielded the tetramer **10** (0.067 g, 49 %) as orange solid.

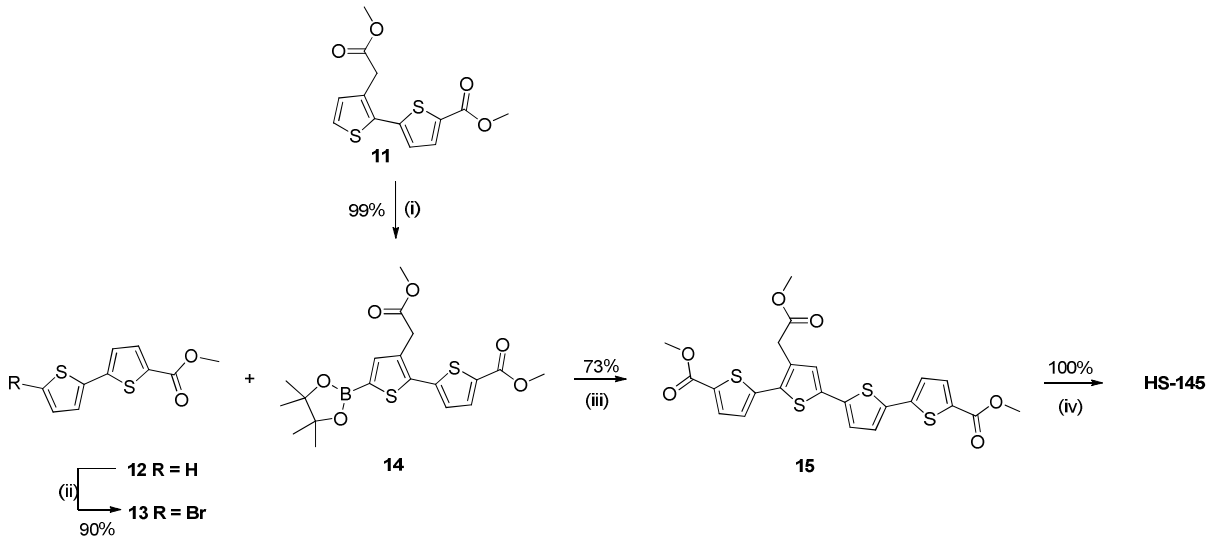
mp 142-144 °C. IR (KBr) 1746, 1732, 1703, 1520, 1464, 1435, 1338, 1290, 1265, 1202, 1160, 1105, 1049, 1018, 927, 888, 856, 849, 821, 815, 755, 751 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (d, *J* = 3.9 Hz, 1H), 7.26 (s, 1H), 7.16 (d, *J* = 3.9 Hz, 1H), 3.90 (s, 3H), 3.70 (s, 3H), 3.58 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 162.5, 143.2, 137.6, 135.7, 134.4, 132.2, 130.1, 127.2, 124.6, 52.4, 52.4, 34.3 MALDI-TOF: *m/z* calcd for $C_{26}H_{22}O_8S_4$ $(M+H)^+$: 591.0. Found: 591.0.

HS-136

Hydrolysis of compound **10** applying general procedure gave **HS-136** as red solid.

mp > 300 °C. IR (KBr) 1575, 1520, 1464, 1387, 1019, 1010, 865, 825, 802, 782 cm^{-1}. ^{1}H NMR (300 MHz, D_2O) δ 7.53 (d, *J* = 4.0 Hz, 1H), 7.35 (s, 1H), 7.29 (d, *J* = 4.0 Hz, 1H), 3.62 (s, 2H). ^{13}C

NMR (75 MHz, D_2O) δ 179.4, 169.6, 140.7, 139.6, 137.8, 136.2, 131.7, 129.2, 127.6, 124.4, 38.7. MALDI-TOF: *m/z* calcd for $C_{22}H_{14}O_8S_4$ $(M+H)^+$: 535.0. Found: 535.4.



Scheme 2. **Reagents and conditions:** (i) $[Ir(OMe)(COD)]_2$, 4,4-di-tert-butyl-2,2-bipyridine, HBpin, THF, 75 °C (ii) NBS, DMF, 16 h; (iii) 1,4-dioxane/MeOH, PEPPSI^{TM}-IPr, K_2CO_3, 70 °C, 20 min; (iv) NaOH (1M), 1,4-dioxane, 60 °C, 16 h.

Methyl [2,2'-bithiophene]-5-carboxylate (12)

This compound was prepared according to the general Suzuki coupling starting with 2-bromothiophene (Sigma Aldrich, St. Louis, MO, USA) (5 g, 30.7 mmol), 2-carboxythiophene-5-boronic acid (Sigma Aldrich) (5.27 g, 30.7 mmol) and K_2CO_3 (17 g, 122.7 mmol) in 1,4-dioxane/methanol (8 : 2, 150 mL). The reaction mixture was extracted with DCM (3×100 mL), washed with water (50 mL) and brine (50 mL). The combined organic phase was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude product, off white solid, was dissolved in MeOH (50 mL), conc. sulfuric acid (15 mmol) was added and the mixture was heated at 70°C for 16 h. The mixture was neutralized by aq. Na_2CO_3 and the aqueous layer was extracted by DCM (3×50 mL). The combined organic phases were washed with water (50

mL) and brine (50 mL) and dried with $MgSO_4$. The solvent was removed under reduced pressure and the residue was subjected to column chromatography using n-heptane/ethyl acetate (9:1) yielded the dimer **12** (4.1 g, 60 %) as white solid. Recrystallization from methanol gave an analytical pure compound.

mp 81-82 °C. IR (KBr) 1706, 1511, 1450, 1421, 1367, 1346, 1321, 1261, 1231, 1194, 1100, 890, 842, 807, 790, 745, 725 cm^{-1}.^{1}H NMR (300 MHz, DMSO-d_6) δ 7.75 (d, *J* = 4.0 Hz, 1H), 7.65 (dd, *J* = 5.0, 1.2 Hz, 1H), 7.52 (dd, *J* = 3.7, 1.2 Hz, 1H), 7.39 (d, *J* = 4.0 Hz, 1H), 7.15 (dd, *J* = 5.0, 3.7 Hz, 1H), 3.83 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.6, 143.5, 135.1, 134.8, 130.4, 128.7, 127.5, 126.2, 124.6, 52.3. MALDI-TOF: *m/z* calcd for $C_{10}H_8O_2S_2$ $(M+H)^+$: 225.0. Found: 224.0.

Methyl 5'-bromo-[2,2'-bithiophene]-5-carboxylate (13)

General procedure of bromination was performed using compound **12** (0.940 g, 4.2 mmol) and NBS (0.746 g, 4.2 mmol). Column chromatography using *n*-heptane/ethyl acetate (10:1) yielded the dimer **13** (0.97 g, 90 %) as white solid.

mp 128-129 °C. IR (KBr) 1703, 1551, 1516, 1455, 1426, 1353, 1300, 1257, 1196, 1102, 971, 954, 873, 813, 790, 749 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67 (d, *J* = 4.0 Hz, 1H), 7.06 (d, *J* = 4.0 Hz, 1H), 7.02 – 6.98 (m, 2H), 3.89 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.5, 143.1, 137.9, 134.3, 131.9, 131.1, 125.4, 124.2, 113.1, 52.4. MALDI-TOF: *m/z* calcd for $C_{10}H_7BrO_2S_2$ $(M+H)^+$: 302.9. Found: 302.9.

Methyl 3'-(2-methoxy-2-oxoethyl)-5'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[2,2'-bithiophene]-5-carboxylate (14)

To a mixture of $[Ir(OMe)(COD)]_2$ (0.0025 equvi.), 4,4'-di-*tert*-butyl-2,2 '-bipyridine (0.0050 equvi.) and the dimer **11**[2] (500 mg, 1.7 mmol) in dry in dry tetra hydro furan (THF) (15 mL) was

added 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (HBPin/Sigma Aldrich) (0.238 g, 1.86 mmol) and heated at 75 ˚C under argon for 18 h. The solvent was evaporated and the residue, dark oil, was purified by column chromatography using *n*-heptane/ ethyl acetate (7:1→3:1) yielding compound **14** as colorless oil (0.71 mg , 99 %).

IR (KBr) 1742, 1716, 1527, 1439, 1382, 1338, 1293, 1267, 1141, 1097, 1027, 957, 852, 749 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, *J* = 3.9 Hz, 1H), 7.54 (s, 1H), 7.20 (d, *J* = 3.9 Hz, 1H), 3.89 (s, 3H), 3.78 (s, 2H), 3.71 (s, 3H), 1.34 (s, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 162.5, 142.2, 140.6, 140.3, 139.1, 134.1, 133.6, 132.6, 127.3, 84.6, 52.4, 52.4, 34.7, 24.9. MALDI-TOF: *m/z* calcd for $C_{19}H_{23}BO_6S$ $(M+H)^+$: 422.1. Found: 422.0.

Dimethyl 3'-(2-methoxy-2-oxoethyl)-[2,2':5',2'':5'',2'''-quaterthiophene]-5,5'''-dicarboxylate (15)

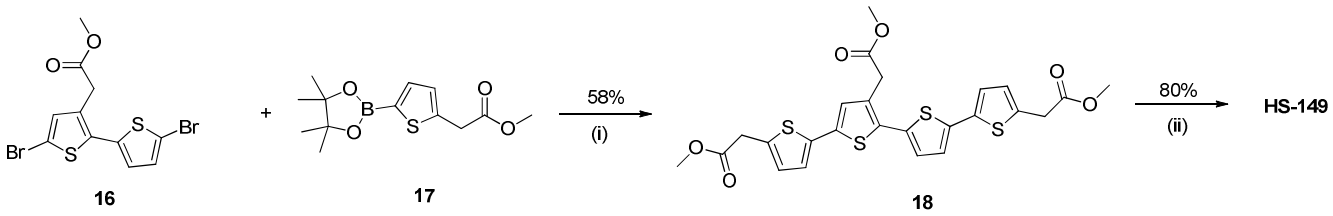
General procedure of Suzuki coupling was performed starting with compound **13** (0.271 g, 0.642 mmol) and **14** (0.195 g, 0.642 mmol). Column chromatography using CH_2Cl_2/MeOH (0.5 %) yielded the tetramer **15** (0.243 g, 73 %) as orange solid.

mp 168 °C. IR (KBr) 1742, 1713, 1544, 1513, 1447, 1418, 1355, 1339, 1293, 1256, 1239, 1192, 1099, 856, 800, 792, 743 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 4.0 Hz, 1H), 7.71 (d, *J* = 4.0 Hz, 1H), 7.20 (d, *J* = 3.8 Hz, 2H), 7.17 – 7.12 (m, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.79 (s, 2H), 3.76 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 162.4, 162.3, 143.5, 141.4, 136.8, 136.3, 135.8, 134.3, 134.0, 133.3, 132.2, 131.7, 131.6, 127.4, 127.0, 126.0, 125.1, 124.0, 52.4, 52.3, 52.2, 34.8. MALDI-TOF: *m/z* calcd for $C_{23}H_{18}O_6S_4$ $(M+H)^+$: 519.0. Found: 519.0.

HS-145

Hydrolysis of compound **15** applying general procedure gave **HS-145** as red solid.

mp > 300 °C. IR (KBr) 1576, 1511, 1453, 1360, 1039, 806, 772 cm^{-1}. ^{1}H NMR (300 MHz, D_2O) δ 7.48 (d, *J* = 3.9 Hz, 1H), 7.43 (d, *J* = 3.8 Hz, 1H), 7.23 (d, *J* = 3.9 Hz, 1H), 7.18 – 7.09 (m, 4H), 3.65 (s, 2H). ^{13}C NMR (75 MHz, D_2O) δ 179.0, 169.5, 169.5, 140.7, 139.9, 139.6, 139.1, 136.1, 135.4, 135.0, 134.5, 131.7, 131.3, 131.0, 128.2, 125.8, 125.5, 124.7, 124.0, 38.1. MALDI-TOF: *m/z* calcd for $C_{20}H_{12}O_6S_4$ $(M+H)^+$: 477.0. Found: 477.0.



***Scheme 3*. Reagents and conditions:** (i) 1,4-dioxane/MeOH, PEPPSI^{TM}-IPr, K_2CO_3, 70 °C, 20 min; (ii) NaOH (1M), 1,4-dioxane, 60 °C, 16 h.

Methyl 2-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiophen-2-yl)acetate (17)

Compound **17** was prepared following the procedure as described for compound **14** starting with methyl 2-thienylacetate (500 mg, 3.2 mmol), prepared according to available procedure[3], 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (HBPin/purchased) (451 mg, 3.5 mmol) in dry THF (15 mL). Column chromatography using *n*-heptane/ ethyl acetate (10:1)] yielded compound **17** as colorless oil, which solidified upon standing (780 mg, 86 %).

IR (KBr) 1745, 1538, 1471, 1360, 1273, 1232, 1204, 1140, 1065, 1016, 1002, 958, 852, 832, 824, 778, 751, 725 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (d, *J* = 3.5 Hz, 1H), 7.03 – 7.00 (m, 1H), 3.87 (s, 2H), 3.72 (s, 3H), 1.33 (s, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 142.4, 137.4,

128.5, 84.2, 52.4, 35.6, 24.9. MALDI-TOF: *m/z* calcd for $C_{13}H_{19}BO_4S$ $(M+H)^+$: 283.1. Found: 283.2.

Trimethyl 2,2',2''-([2,2':5',2'':5'',2'''-quaterthiophene]-3'',5,5'''-triyl)triacetate (18)

General procedure of Suzuki coupling was performed starting with compound **16** (0.075 g, 0.189 mmol) and **17** (0.106 g, 0.377 mmol). Column chromatography using *n*-heptane/ethyl acetate (4:1→1:1) yielded the tetramer **18** (0.067 g, 49 %) as dark red oil.

IR (KBr) 1738, 1435, 1334, 1262, 1203, 1170, 1011, 798 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 7.12 – 7.03 (m, 3H), 7.02 (dd, *J* = 4.7, 3.7 Hz, 2H), 6.85 (ddt, *J* = 3.6, 2.7, 0.9 Hz, 2H), 3.82 (s, 4H), 3.76 (s, 5H), 3.75 (s, 3H), 3.74 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 170.8, 170.7, 138.0, 136.7, 136.5, 136.1, 134.7, 134.7, 133.6, 131.9, 130.9, 127.9, 127.8, 127.5, 126.7, 124.3, 123.8, 123.7, 52.5, 52.4, 35.6, 35.0. MALDI-TOF: *m/z* calcd for $C_{25}H_{22}O_6S_4$ $(M+H)^+$: 546.0. Found: 546.0.

HS-149

Hydrolysis of compound **18** applying general procedure gave **HS-149** as dark red solid.

mp > 300 °C. IR (KBr), as free acid, 1718, 1653, 1517, 1437, 1384, 1273, 1024, 997, 794, 767 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6), as free acid, δ 7.24 (d, *J* = 3.8 Hz, 1H), 7.21 – 7.17 (m, 2H), 7.16 – 7.11 (m, 2H), 6.88 (d, *J* = 3.8 Hz, 2H), 3.78 (s, 4H), 3.69 (s, 2H).^{13}C NMR (75 MHz, DMSO-d_6), as free acid, δ 171.8, 171.7,137.4, 137.4, 136.9, 134.7, 134.7, 134.5, 133.2, 133.1, 132.9, 129.8, 127.5, 127.5, 127.1, 124.2, 123.6, 123.5, 36.0, 35.3. MALDI-TOF: *m/z* calcd for $C_{22}H_{16}O_6S_4$ $(M+H)^+$: 505.0. Found: 505.2.

Mice

Transgenic APPPS1 mice co-expressing KM670/671NL mutated amyloid precursor protein and L166P mutated presenilin 132 were used to model Aβ pathology[4]. For the studies of tau, transgenic mice homozygous for human tau with the P301S mutation[5] were employed. All animal experiments were performed in accordance with the UK Animal (Scientific Procedures) Act 1986 for the welfare of laboratory animals (United Kingdom) or the veterinary office regulations of Baden-Württemberg (Germany) and were approved by the Medical Research Council local animal welfare and ethical review board (United Kingdom) or the local Animal Care and Use Committees (Germany).

LCO staining and spectral analysis of histological samples

Frozen brain sections (10 μm) from transgenic APPPS1 or P301S tau mice sacrificed at different ages were fixed in 96% EtOH, rehydrated in 50% EtOH and dH_2O and then incubated in phosphate buffered saline (PBS, 10 mM phosphate, 140 mM NaCl, 2.7 mM KCl, pH 7.4) for 10 min. The LCOs HS-68, HS-129, HS-136, HS-145 or HS-149 were diluted to 3 μM in PBS and added to the sections. After 30 min, the sections were washed with PBS and mounted with Dako fluorescent mounting medium (Dako Cytomation, Glostrup, Denmark). The mounting medium was allowed to solidify over night before collecting emission spectra of LCOs bound to misfolded Aβ and tau using an inverted LSM 780 confocal microscope (Carl Zeiss, Oberkochen, Germany) using excitation wavelength 405 nm. For the spectral comparison using HS-68, brain sections from 16 APPPS1 mice and 12 P301S tau mice were analyzed collecting spectra from Aβ and tau in the cortex and brain stem, respectively. In each APPPS1 mouse, the LCO emission spectrum from three to five regions in the central compact core of 10-20 Aβ plaques in the brain parenchyma was collected. Aβ-deposits in the vasculature

(cerebral β-amyloid angiopathy) was excluded from the analysis. In the analysis of tau, each time point corresponds to three different mice and, whenever possible, 10-15 neurofibrillary tangles (NFTs) with 3-5 ROIs for each mouse. In two cases, the number of NFTs was lower; however, the total count of emission spectra for a time point was never less than 140. In the spectral graphs, the emission spectrum for one representative Aβ plaque or NFT was chosen to illustrate the spectral signature for each time point and ratio value as indicated in the graphs. In the plots, the ratio of the fluorescence intensity at the specified blue and red-shifted wavelengths has been calculated for each collected emission spectrum. The spectral analysis of HS-129, HS-136, HS-145 and HS-149 in comparison with HS-68 was performed on two young and two aged APPPS1 (148, 245, 457 and 531 days) or three young (2 to 3 month) and three aged P301S tau mouse (6 months). The spectral graphs show the emission spectra for 3-5 ROIs in 15-25 Aβ plaques and 11-21 NFTs.

Immunohistochemistry (LCO and antibody double staining)

Frozen sections (10 µm) from APPPS1 mice aged 156 or 531 days and P301S tau mice aged 2 or 6 months were fixed with acetone at -20°C for 10 min and then allowed to dry. Mouse monoclonal anti-Aβ antibody (6E10, Covance, Princeton, NJ, USA) or mouse monoclonal antibody directed against tau phosphorylated at serine 202 and threonine 205 (AT8, Thermo Fisher Scientific, Waltham, USA) were diluted 1:100 in PBS supplemented with 1% (w/v) bovine serum albumin and added to the sections. After 16 h incubation at 4°C, the sections were washed with PBS 3x5 min and then incubated for 30 min at room temperature (RT) with donkey anti-mouse antibody conjugated with Alexa 647 nm fluorophore diluted 1:200 in PBS. After washing with PBS 3x5 min, the sections were incubated for 30 min with 3 µM HS-68, HS-129, HS-136, HS-145 or HS-149 in PBS at RT. Excessive staining solution was removed by

repetitive washing with PBS and the sections were mounted using Vectashield mounting medium (Vector Laboratories, Burlingame, USA). Fluorescence images were collected on a Leica DM6000 B fluorescence microscope (Leica Microsystems, Wetzlar, Germany) using bandpass filters 405/20 (LP460), 436/20 (LP460), 480/40 (LP515) and 640/30 (BP700/75).

Spectral characterization of LCOs

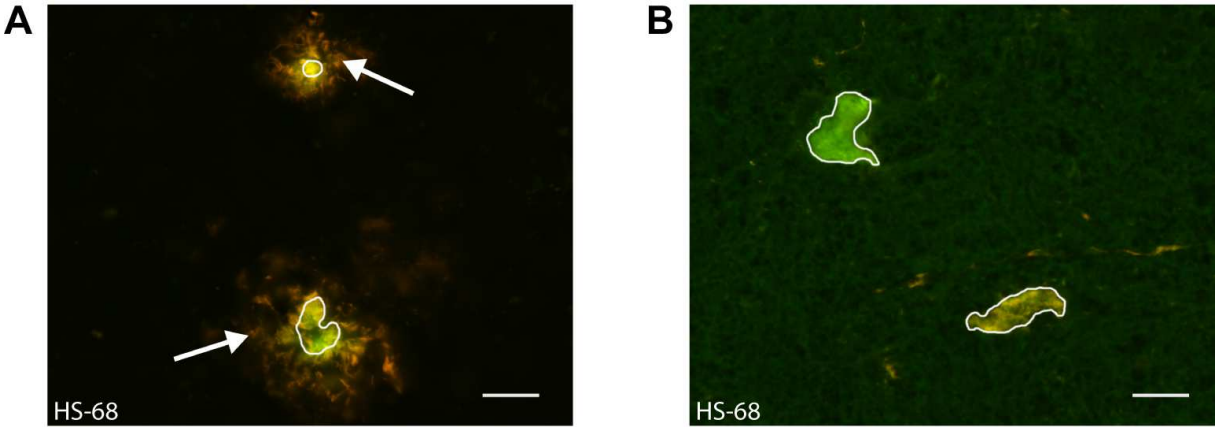
A set of buffers with pH ranging from 3.5 to 7 was prepared by dissolving 20 µM sodium acetate or 20 µM phosphate in water and adjusting the pH to 3.5 to 5 or 6 and 7, respectively. The LCO HS-68, HS-129, HS-136, HS-145 or HS-149 was diluted to 30 µM in each buffer and added to a microtiter plate (Corning Incorporated, Corning, NY, USA) for the collection of absorbance, emission and excitation spectra. Emission and excitation spectra of the LCO solutions were collected at excitation wavelength 375 or 450 nm and emission wavelength 510 or 575 nm, respectively, using a Tecan Saphire^2 microplate reader (Tecan, Männedorf, Switzerland).

Statistical analysis

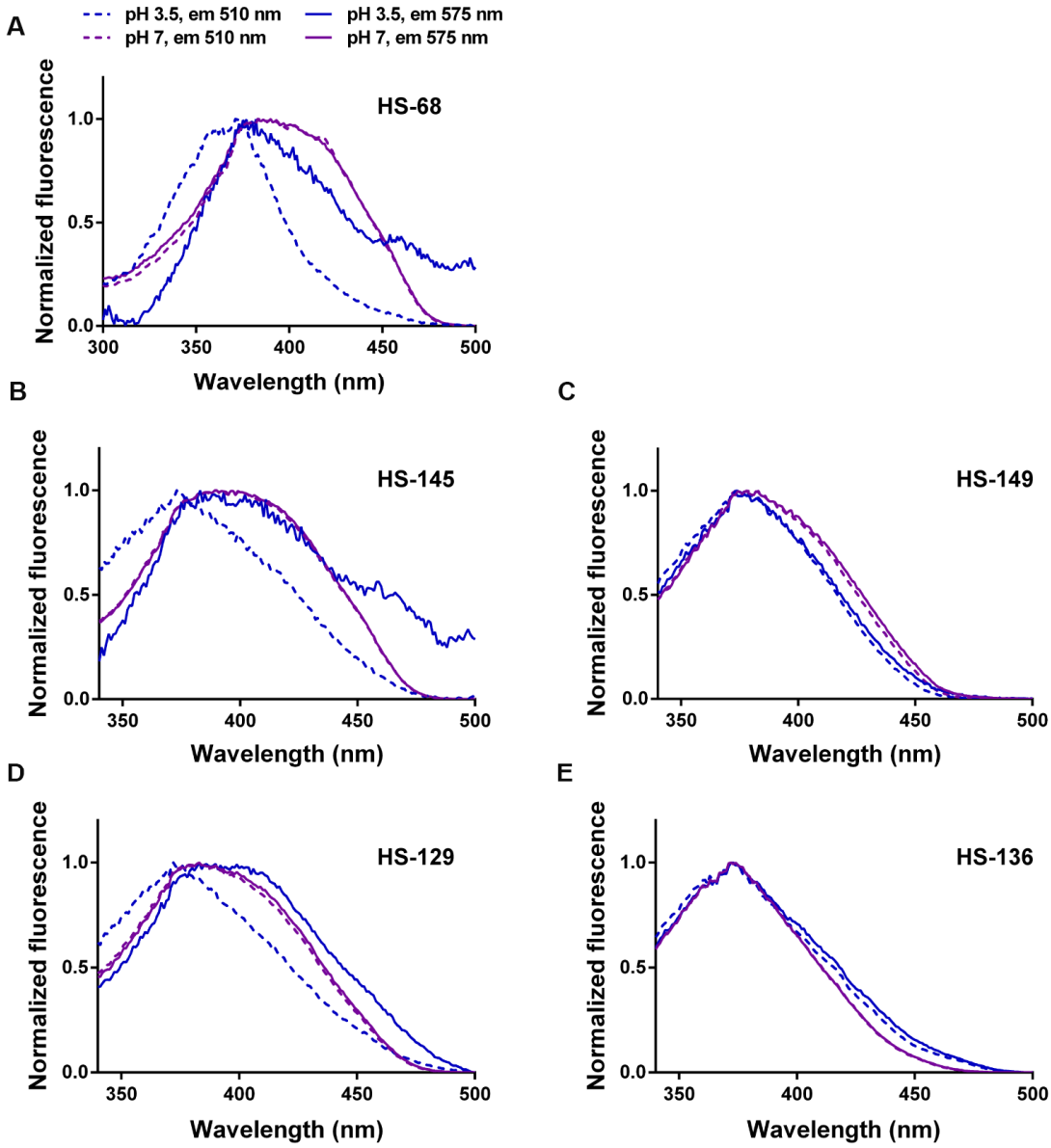
Data were analyzed with one way analysis of variance followed by Tukey´s multicomparison post hoc test (Figure 1 and 2) or with unpaired t test with Welch´s correction (Figure 5) (GraphPad Prism 6, GraphPad Software, CA, USA). The mean and standard error of the mean for each group of values are indicated in red. Significance is represented as *** = $p< 0.005$, **** = $p<0.0001$.

References

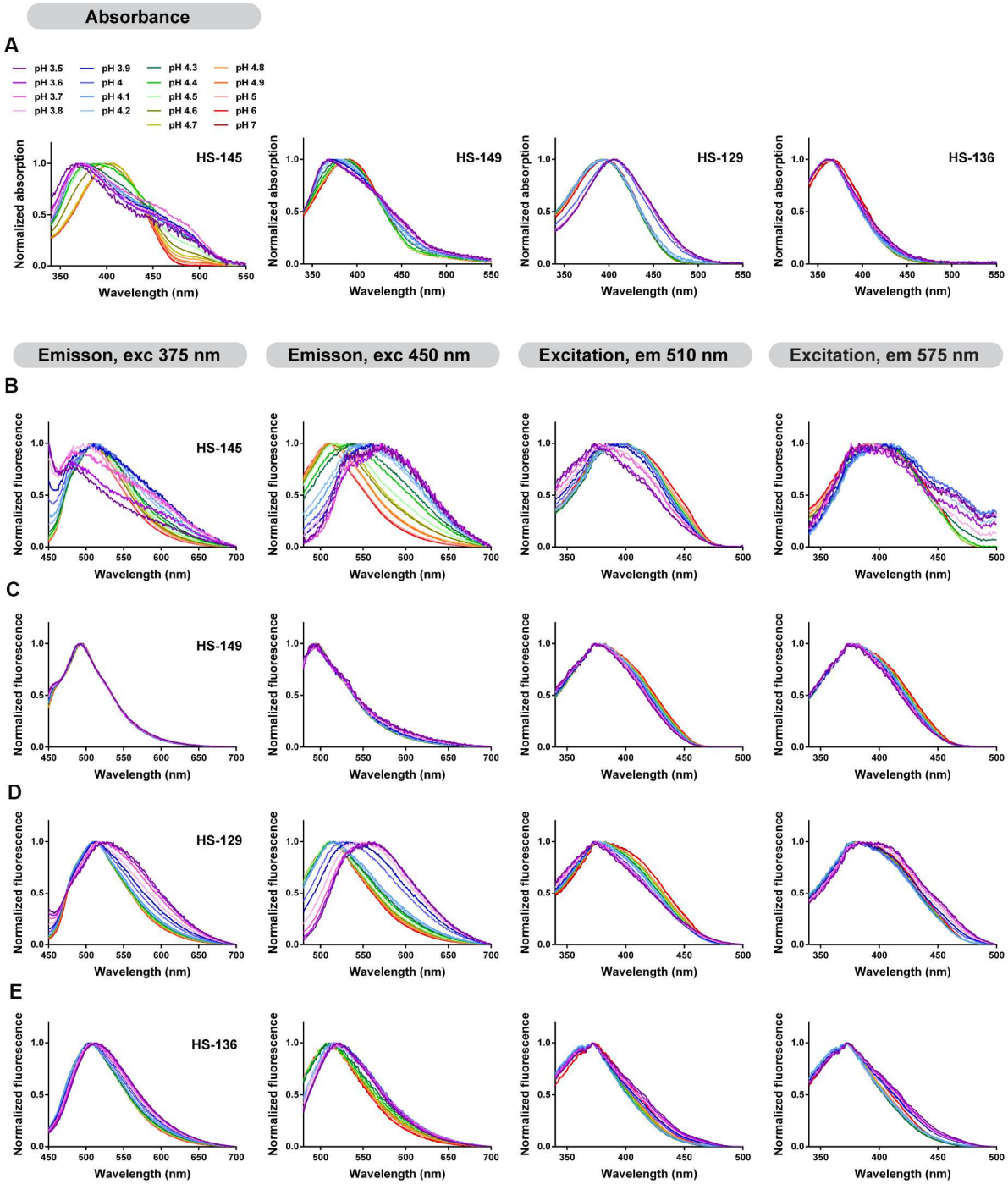
1. Klingstedt, T. *et al. Chemistry* **2013**, *19*, 10179-10192.
2. Simon, R., Bäck, M., Shirani, M., Lindgren, M., Nilsson K. P. R. pH-dependent optical transitions in anionic pentameric oligothiophenes. *Submitted to ChemPhysChem 2015*
3. Terao, Y., Miyamoto, K. & Ohta, H. *Chem. Commun.* **2006**, *34*, 3600-3602.
4. Radde, R., *et. al. EMBO Rep*, **2006**, *7*, 940-946
5. Allen, B., *et al. J. Neurosci.* **2002**, *22*, 9340-9351.



Supplemental Figure 1. Collection of HS-68 emission spectra from Aβ plaques and tau aggregates in transgenic mice. (A) Fluorescence image of Aβ plaques in brain tissue section from APPPS1 mouse sacrificed at 156 days of age stained with HS-68. The emission spectra of HS-68 were collected from the core region of the plaques defined by the white lines in the image. Arrows indicate the surrounding diffuse amyloid structure not included in the spectral analysis. Scale bar represents 20 μm. **(B)** Fluorescence image of tau aggregates in brain tissue section from P301S tau mouse sacrificed at six months of age stained with HS-68. The emission spectra of HS-68 were collected from the cell body region defined by the white lines in the image. Scale bar represents 20 μm.

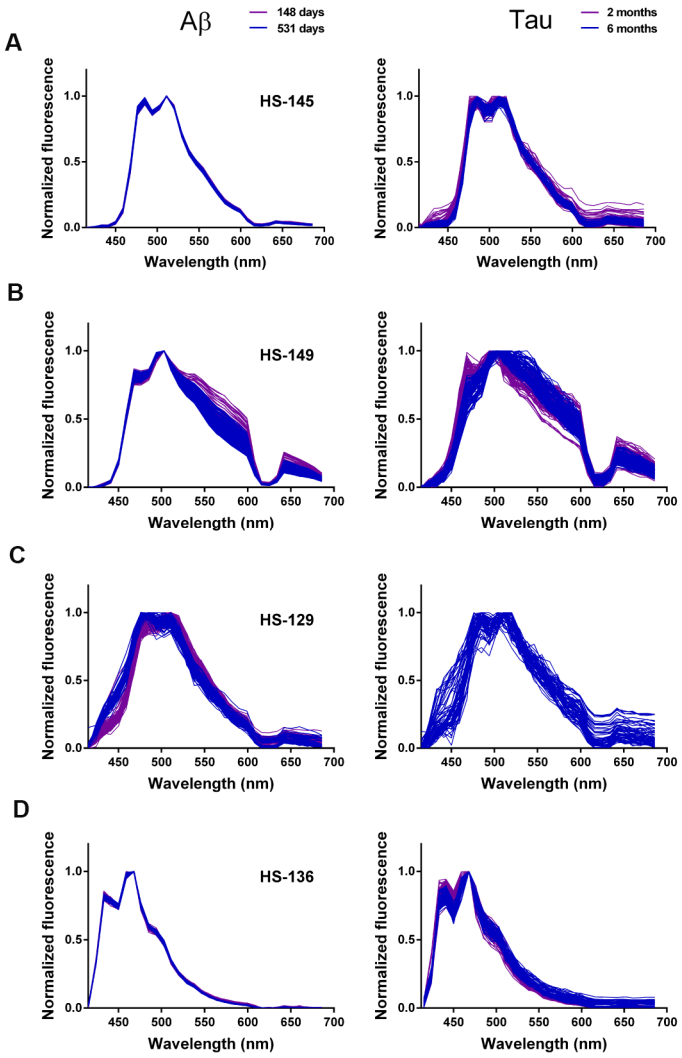


Supplemental Figure 2. The pH effect on the excitation spectrum of HS-68 and its structural analogues. HS-68 (A), HS-145 (B), HS-149 (C), HS-129 (D) or HS-136 (E) was diluted to 30 µM in buffer with pH 3.5 (blue) or pH 7 (magenta). Excitation spectra were collected for each indicated LCO by locking the emission at 510 (dashed line) or 575 (solid line) nm. Depending on the emission wavelength, the excitation spectrum of HS-68, HS-145 and HS-129 shows a pH dependent blue or red shift indicating conformational alterations of the conjugated thiophene backbone or π-stacking between adjacent thiophene chains. HS-149 and HS-136 display minor shifts at shorter and longer wavelengths, respectively, at low pH values.



Supplemental Figure 3. pH-dependent photophysical properties of HS-68 structural analogues. (a) The absorbance spectrum of the indicated HS-68 analogue diluted to 30 µM in buffer solutions ranging from pH 3.5 to 7. The pH effect on HS-145 absorbance properties is similar to what was observed with HS-68 indicating that also this structure forms distinct types of assemblies at low pH values. An analogous pattern of pH induced wavelength shifts is seen with HS-149, but not at all as pronounced as with HS-68 and HS-145. The absorbance properties of HS-136 appear to be pH independent, whereas its structural analogue HS-129

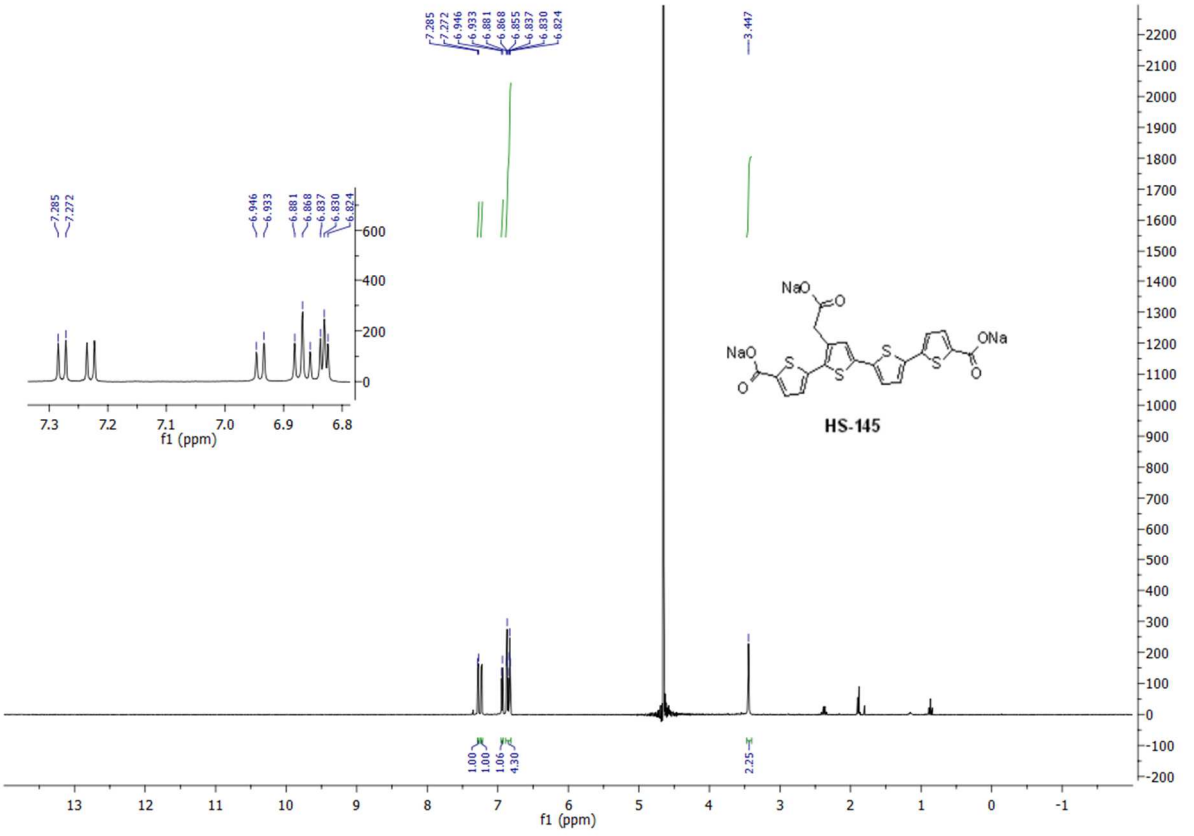
displays a spectral red shift with decreasing pH. The legend defining the pH values in (a) is valid for all graphs in the figure. **(b-e)** Emission (excitation at 375 or 450 nm) and excitation (emission at 510 or 575 nm) spectra of the indicated HS-68 analogue diluted to 30 µM in buffer solutions ranging from pH 3.5 to 7. By choosing the optimal excitation or emission wavelength it is possible to demonstrate the occurrence of both blue and red shifted HS-145 assemblies, similar to the properties of HS-68. The fluorescence spectra of HS-129 do not reveal any signs of blue shifted aggregated species and, judged by their constant spectral signatures, neither HS-136 nor HS-149 can be induced to form assemblies in solution by lowering the pH value.

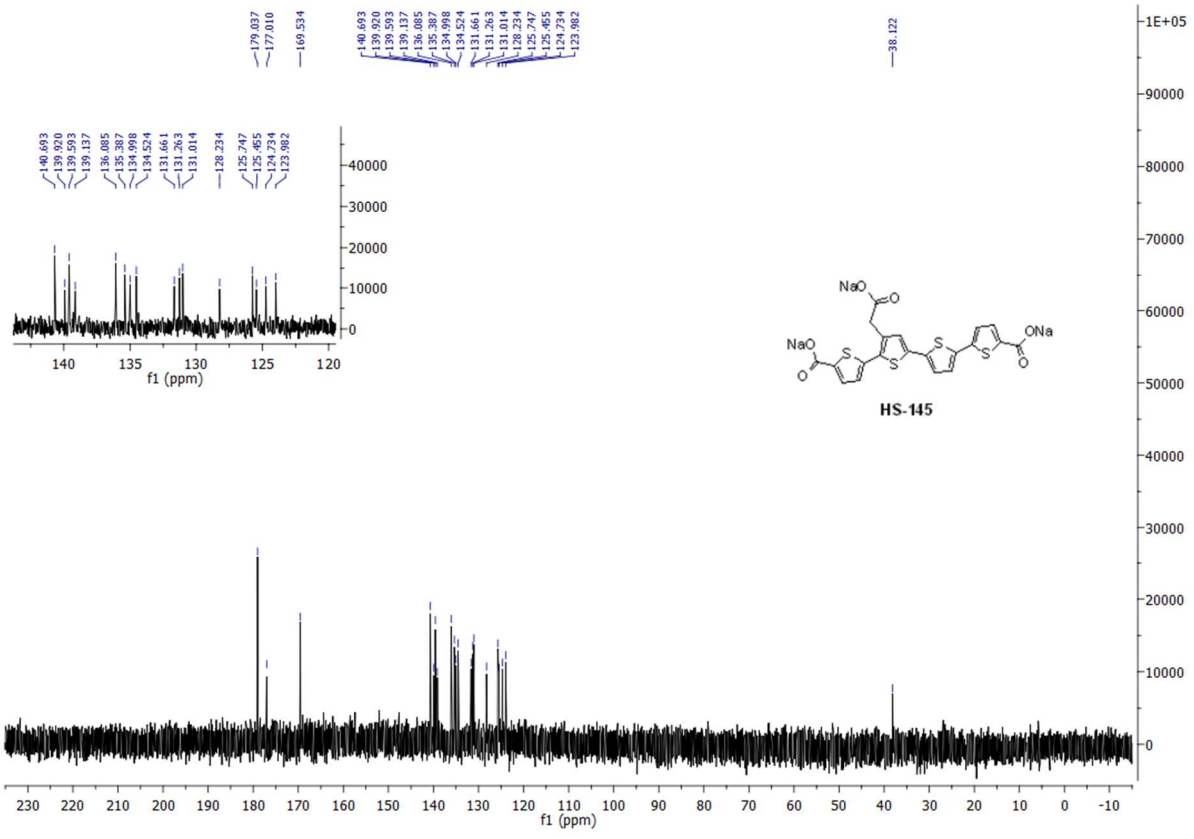


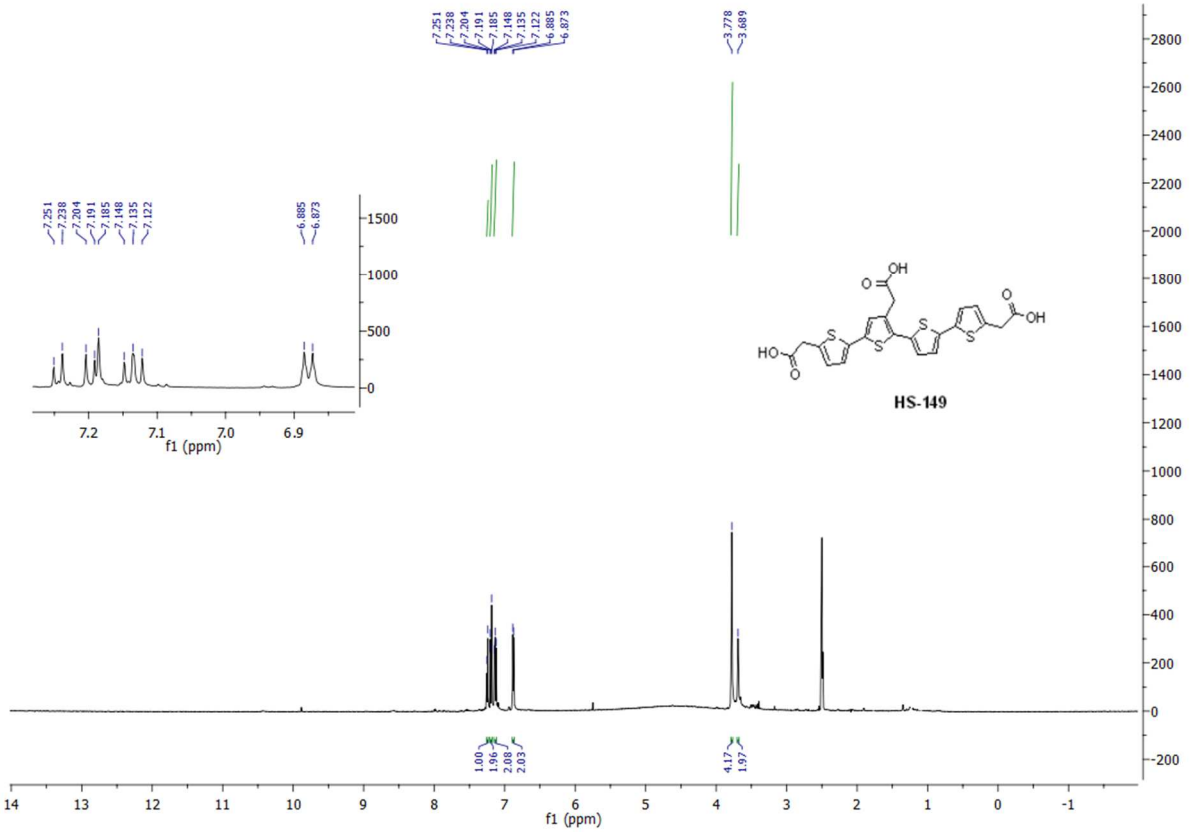
Supplemental Figure 4. Spectral analysis of HS-68 analogues binding to Aβ deposits and aggregated tau. Frozen brain sections from APPPS1 or P301S tau mice sacrificed at the indicated ages were fixed with ethanol and stained with 3 µM of **(a)** HS-145, **(b)** HS-149, **(c)** HS-129 or **(d)** HS-136 in phosphate buffered saline at pH 7.4. Exciting the LCOs at 405 nm and collecting the emission from Aβ plaques (left) or tau aggregates (right) showed that neither HS-145 (a) nor HS-136 (d) was able to spectrally distinguish the protein aggregates dependent on age. In addition, the emission spectra of HS-136 were very well-resolved and blue shifted indicating a twisted and locked conformation of the backbone. For Aβ plaques, both HS-149 (b) and HS-129 (c) showed a blue shift in emission upon age, but not as pronounced as HS-68. HS-149 was demonstrating age-dependent transitions also for tau; however, the emission was red shifted in older mice (b). HS-129 staining of tau aggregates in the young P301S tau mouse was very weak and it was not possible to collect any reliable emission spectra (c).

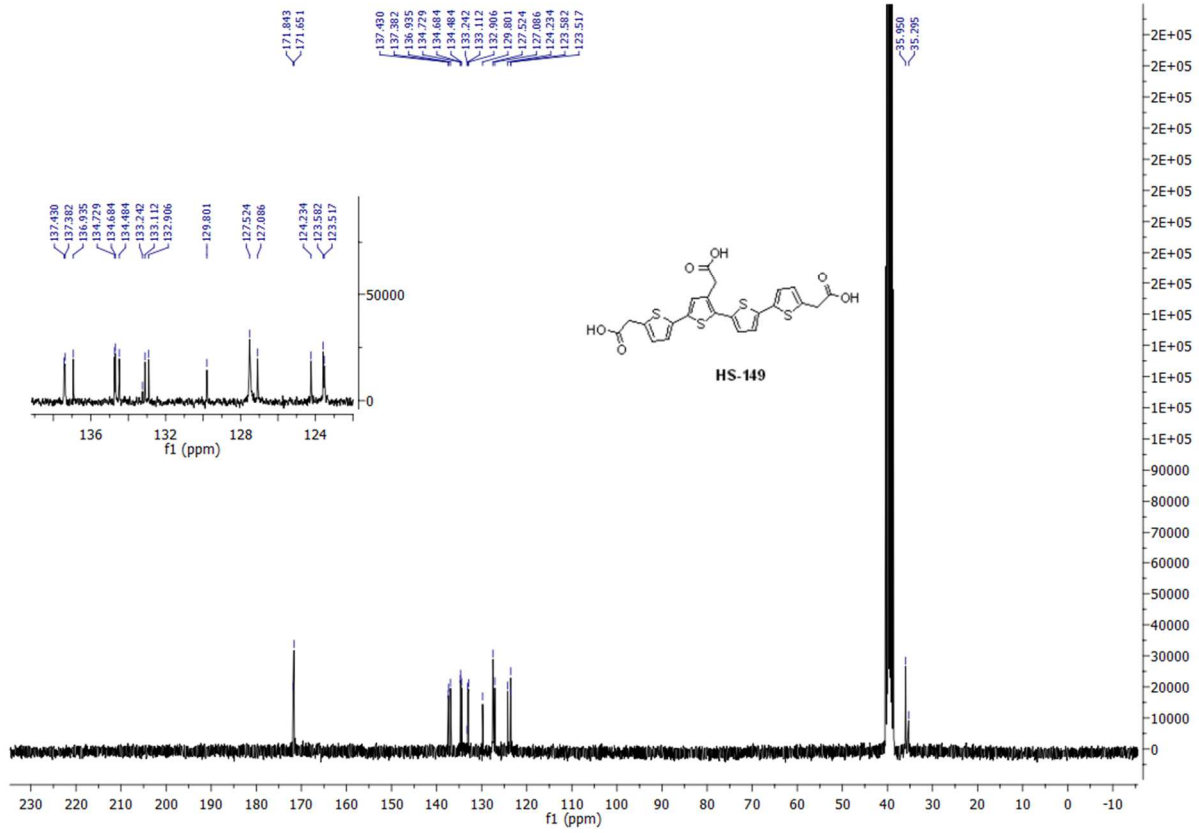
Supplemental Table S1. Statistical analysis (Unpaired t-test with Welch´s correction) of spectral difference between aggregated Aβ in a 245 day old mouse and a 457 day old mouse.

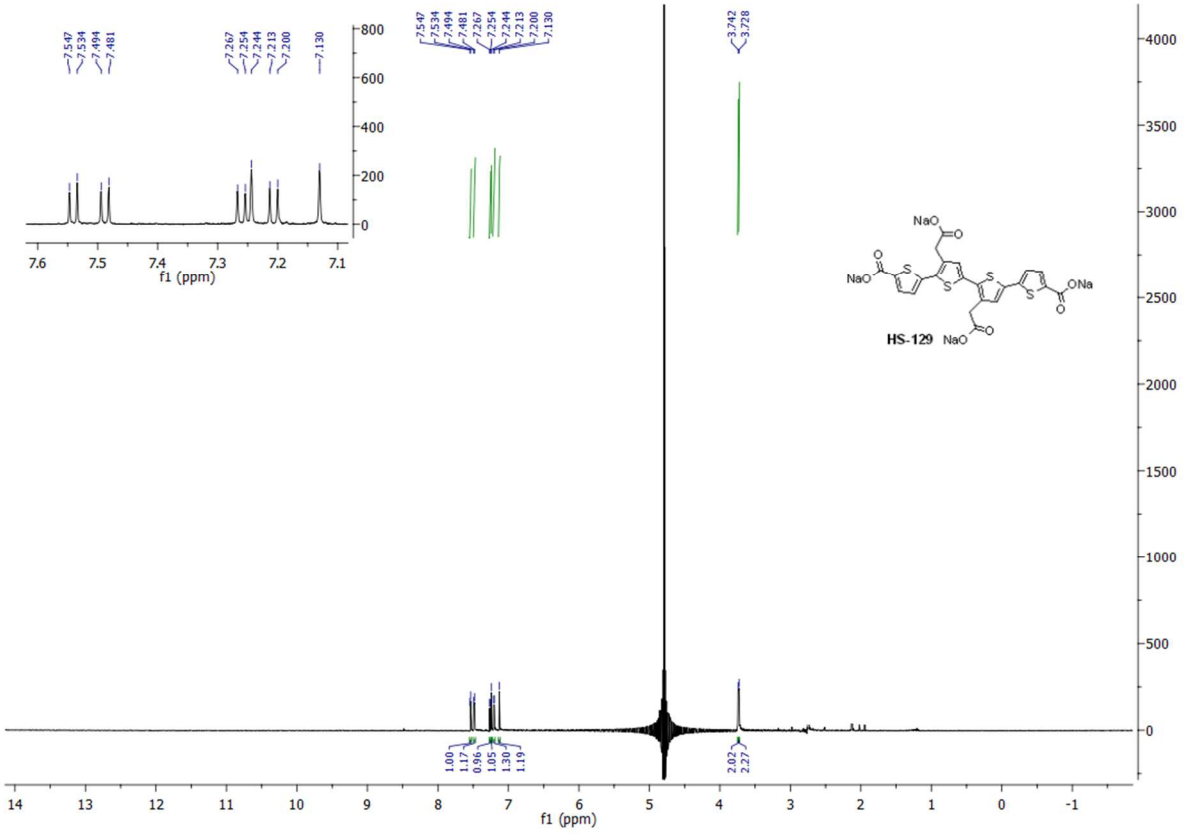
LCO	Difference of the mean	R^2
HS-68	-0.4778 ± 0,02209	0.8327
HS-145	-0.0217 ± 0.0182	0.0325
HS-149	-0.1354 ± 0.0314	0.1213
HS-129	-0.3217 ± 0.0236	0.6283
HS-136	0.0148 ± 0.0025	0.1376

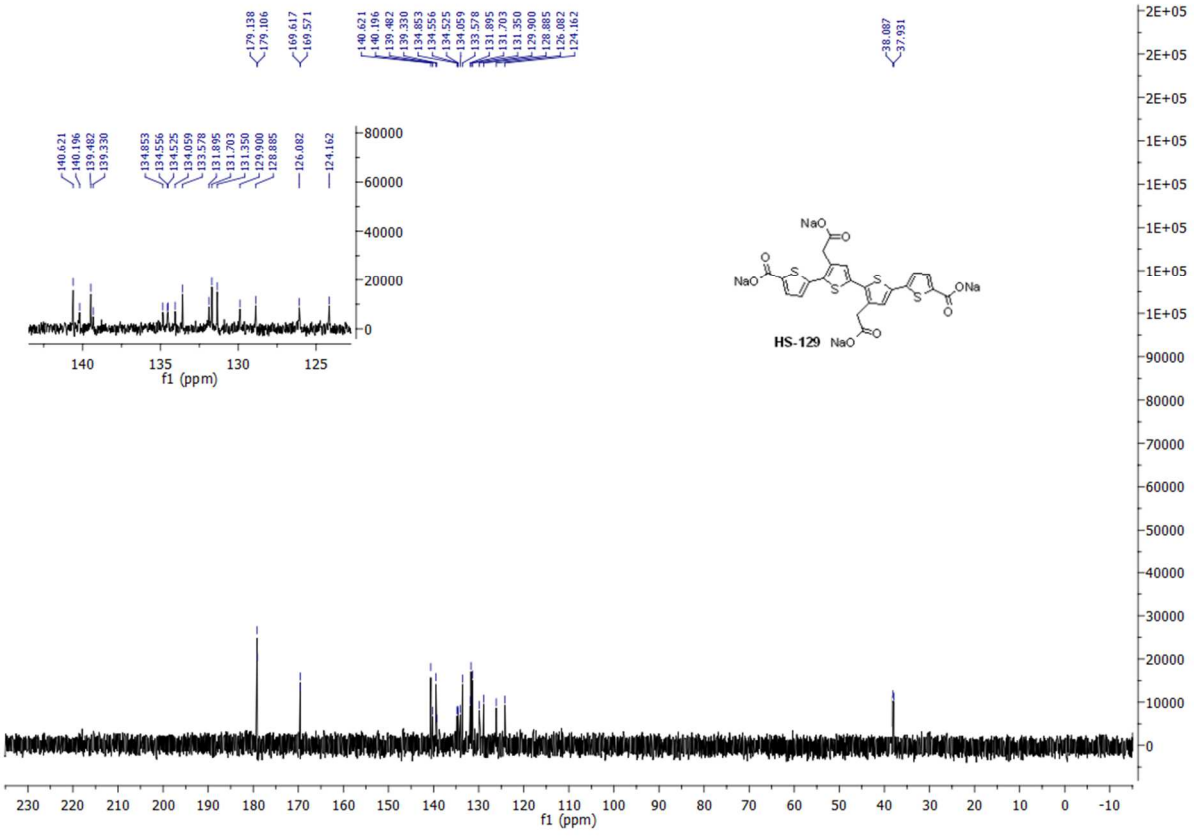

7.285
7.272
6.946
6.933
6.881
6.868
6.855
6.837
6.830
6.824
3.447
NaO
O
NaO
O
S
S
S
ONa
O
HS-145
f1 (ppm)

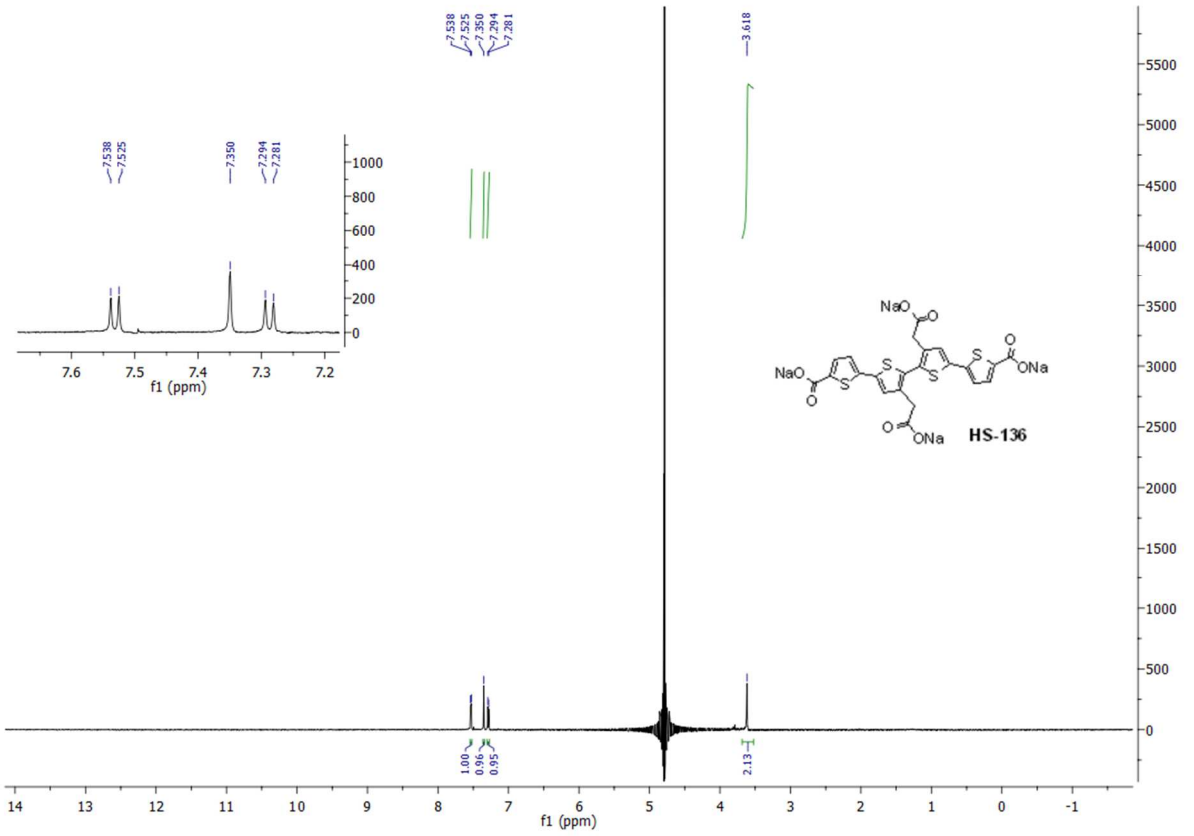

179.037
177.010
169.534
140.693
139.920
139.593
139.137
136.085
135.387
134.998
134.524
131.661
131.263
131.014
128.234
125.747
125.455
124.734
123.982
38.122
NaO
O
NaO
O
S
S
S
ONa
O
HS-145
f1 (ppm)

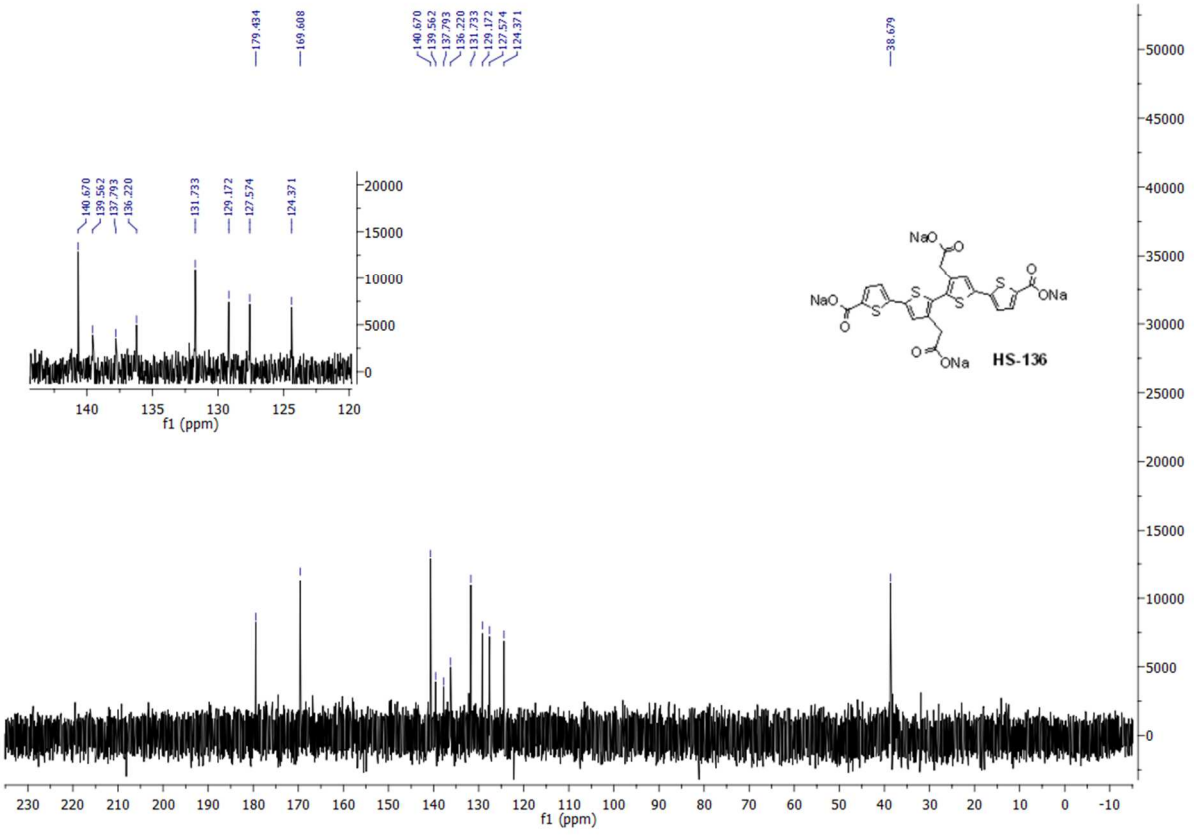

7.251
7.238
7.204
7.191
7.185
7.148
7.135
7.122
6.885
6.873
3.778
3.689
HS-149
f1 (ppm)


171.843
171.651
137.430
137.382
136.935
134.729
134.684
134.484
133.242
133.112
132.906
129.801
127.524
127.086
124.234
123.582
123.517
35.950
35.295
HS-149
f1 (ppm)


7.547
7.534
7.494
7.481
7.267
7.254
7.244
7.213
7.200
7.130
3.742
3.728
1.00
1.17
0.96
1.05
1.30
1.19
2.02
2.27
f1 (ppm)
NaO
NaO
ONa
NaO
HS-129


179.138
179.106
169.617
169.571
140.621
140.196
139.482
139.330
134.853
134.556
134.525
134.059
133.578
131.895
131.703
131.350
129.900
128.885
126.082
124.162
38.087
37.931
f1 (ppm)
NaO
NaO
ONa
NaO
HS-129


7.538
7.525
7.350
7.294
7.281
3.618
1.00
0.96
0.95
2.13
f1 (ppm)
HS-136


179.434
169.608
140.670
139.562
137.793
136.220
131.733
129.172
127.574
124.371
38.679
f1 (ppm)
HS-136