Electronic Supplementary Information

Synthesis and bioconjugation of first alkynylated poly(dithieno[3,2-*b*:2',3'-*d*] pyrroles)

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Physical measurements and instrumentation:

Nuclear magnetic resonance spectra were recorded on a *Bruker* AMX 500 spectrometer (^{1}H NMR: 500 MHz, ^{13}C-NMR 125 MHz), a *Bruker* Avance 400 (^{1}H NMR: 400 MHz, ^{13}C NMR: 100 MHz) at room temperature unless otherwise noted. Chemical shift values (δ) are given in parts per million using residual solvent protons (^{1}H NMR: δ_H = 7.26 for $CDCl_3$, δ_H = 2.49 for DMSO-d_6; δ_H = 3.33 for MeOD-d_4, δ_H = 1.94 for CD_3CN, δ_H = 5.32 for CD_2Cl_2. ^{13}C NMR: δ_C = 77.0 for $CDCl_3$, δ_C = 49.1 for MeOD-d_4, δ_C = 54.0 for CD_2Cl_2, and 39.43 for DMSO-d_6) as internal standard. The splitting patterns are described as follows: (s) singlet, (d) doublet, (t) triplet, qr (quartet), q (quintet), m (multiplet). Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) measurements were carried out on a *Bruker* Daltonik Reflex III mass spectrometer with the following matrices: HCCA (α-cyano-4-hydroxy cinnamic acid), 1,2,3-trihydroxyanthracene (dithranol), 2,5-dihydroxybenzoic acid (DHB) and T-2- (3-(4-t-Butyl-phenyl)-2-methyl-2-propenylidene) malononitrile (DCTB). Methane chemical ionization (CI) mass spectra were detected with a Finnigan MAT, SSQ-7000 Single-Stage-Quadrupol-System, Absorption spectra were recorded on a *Perkin Elmer* Lambda 19 spectrometer and fluorescence emission spectra on a *Perkin Elmer* LS 55 spectrometer using 1 cm cuvettes. All spectra are corrected. All reactions were monitored by TLC (aluminium plates, pre-coated with silica gel, *Merck* Si60 F254). Fluorescence microscopy was performed using a *Leica* DM5000B microscope equipped with a *Leica* DFC350FXR2 camera. Samples were imaged using a 5× or a 10× PLAN objective. Excitation and emission of samples was conducted using *Leica* GFP (green fluorescent protein) filter settings.

Cyclic voltammetry experiments were performed with a computer-controlled Autolab PGSTAT30 potentiostat in a three-electrode single-compartment cell with a platinum working electrode, a platinum wire counter electrode, and an Ag/AgCl reference electrode. All potentials were internally referenced to the ferrocene/ferrocenium couple.

Indium tin oxide coated glass slides with 8-12 Ω/sc surface resistivity were used as ITO-electrodes. Melting points were determined using a *Büchi* B-545 apparatus.

Fluorescence microscopy images: Photoshop was used for adjustment of brightness, contrast or color balance.

Chemicals: Dichloromethane and toluene (Merck) were dried over CaH_2 and distilled; tris(dibenzylideneacetone)dipalladium(0)-chloroform adduct, tris-tert-butylphosphonium tetraflouroborate, *N*,*N*-diisopropylamine and bis(triphenylphosphine) palladium(II)chloride were purchased from Merck. For purification by column chromatography silica gel 60 (0.040-0.063 mm) from *Machery & Nagel* was used. Solvents were distilled prior to use.

Abbreviations: Calcd: Calculated; ACN: Acetonitrile, DCM: Dichloromethane; DIPA: Diisopropylamine; DMEM: Dulbecco's Modified Eagle Medium; DMSO: Dimethylsulfoxide; DPBS: Dulbecco's Phosphate-Buffered Saline; $CDCl_3$: Chloroform; HCCA: MeOH: Methanol; Mtr: 4-Methoxy-2,3,6-trimethylphenylsulfonyl, $Pd(dba)_2$: Tris(dibenzylideneacetone) dipalladium; BINAP: (±)-2,2'-Bis(diphenylphosphino)-1,1'-binaphthalene; rt: room temperature; TCNE: tetracyanoethylene; THF: tetrahydrofurane; Toluene (VWR) was dried under reflux over CaH_2 (Merck). DCM, THF (Sigma Aldrich), DMF (Merck), and diethyl ether (Merck) were dried and purified by a MB SPS-800 (MBraun). *n*-Hexane, Petrolether, ACN and acetone were purchased from VWR. Sodium *tert*-butoxide, and sodium bicarbonate were purchased from Merck. $Pd(dba)_2$ and BINAP were purchased from Sigma Aldrich, *n*-BuLi (1.6 N in hexane) from Acros Organics, and 1,1'-bis(diphenylphosphino)ferrocene from Frontier Scientific. $ZnCl_2$ (VWR) was dried in high vacuum at high temperature. Cu $(CH_3CN)_4$ PF_6, Cu, 4-((trimethylsilyl)ethynyl)aniline and the 4-Methoxy-2,3,6-trimethylphenylsulfonyl (Mtr)-protected arginine were purchased by Sigma Aldrich. TFA·Gly-L-Asp(OMe)OMe,[1] the α-azido acid (N_3-Arg(Mtr), [2] methyl 4-azidobutanoate **3b**,[3] N-(2-azidoethyl) phtalimide **3c**, [4] azidomethylferrocene **3d**, [5] the azido-ethyl mannoside **3e**, [6] and 3,3'-dibromo-2,2'-bithiophene **4** [7] were prepared as previously described.

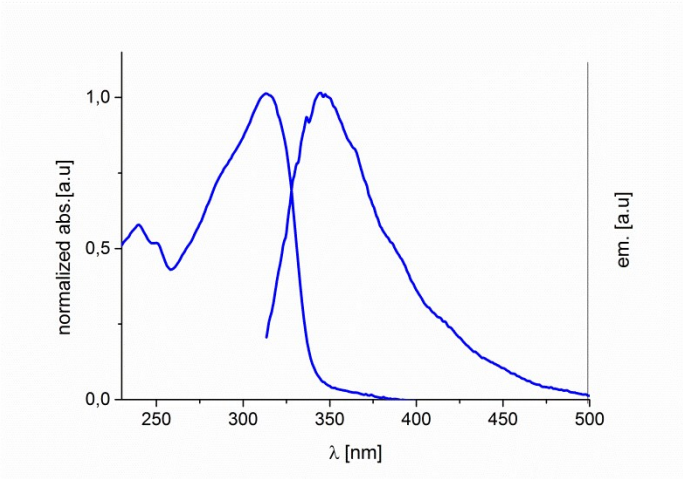


Figure S1. Normalized absorption- and emission spectra of the TMS-protected DTP-monomer **6**.

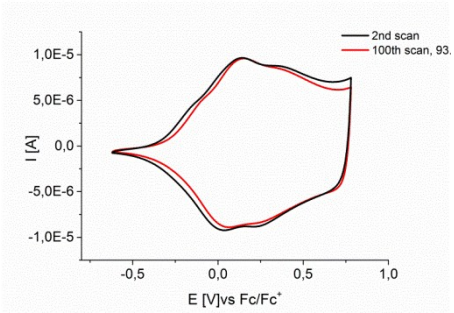
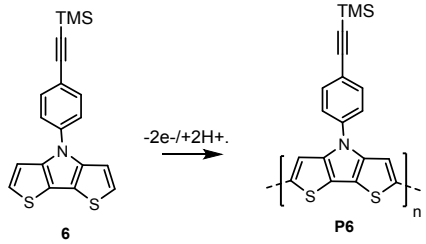


Figure S2. Electrochemical characterization of a polymeric film of **P7** measured in monomer-free DCM solution (Pt electrode, $TBAPF_6$).



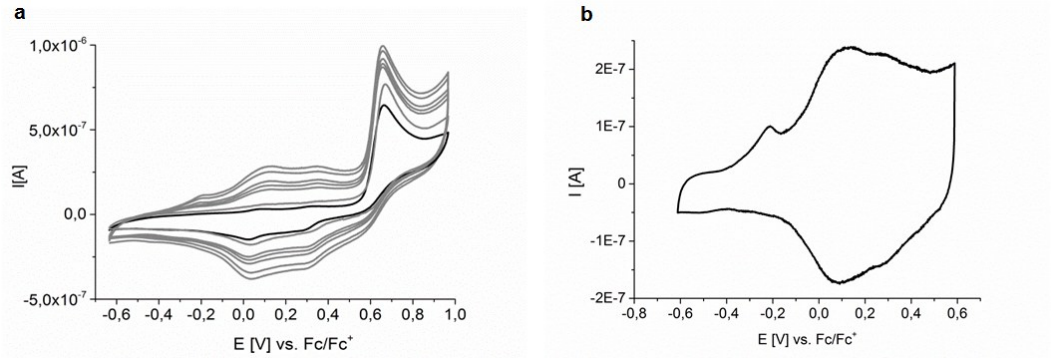


Figure S3 a) Electrochemical polymerization of the TMS-protected monomer **6** (10^{-3} M) measured in DCM. b) CV of the resulting polymeric film (**P6**) in monomer-free solution (Pt electrode, $TBAPF_6$).

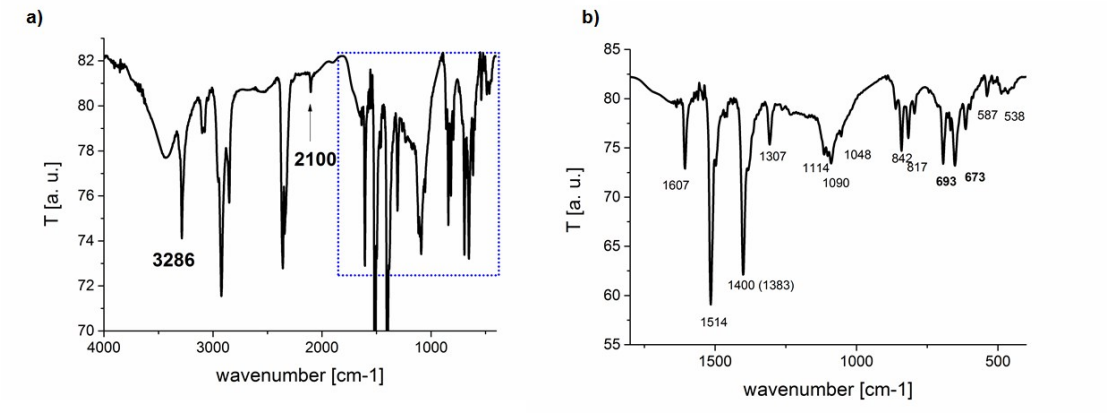


Figure S4. a) FT-IR spectrum of **7** b) enlarged region of the FT-IR spectrum of **7** (KBr).

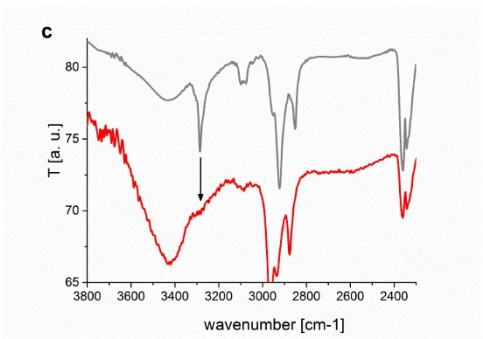


Figure S4. c) Comparison of the FT-IR spectra of **7** (grey) to **P7** (red). Arrow indicates absorption at 3286 cm^{-1} of the terminal C-C triple bond (KBr).

Figure S5

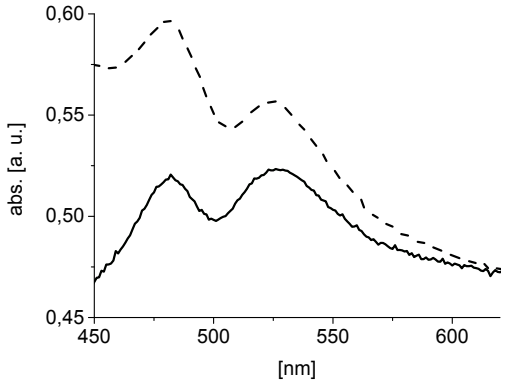
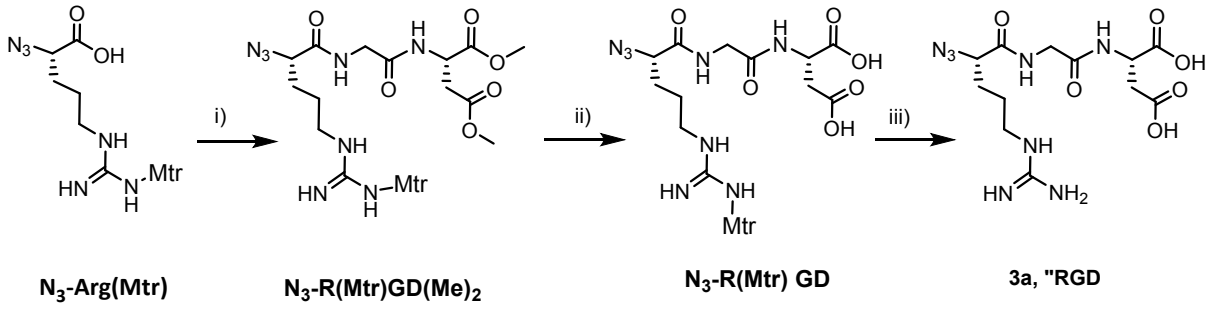


Figure S5: UV-spectra of the *p*DTP **P7** (solid line) and the click-modified counterpart **P7e** (dashed line)

Synthesis of the tripeptide **3a**



Synthesis of **3a** i) TFA·Gly-L-Asp(OMe)OMe, 1 eq. pentafluorphenol, 1.2 eq. 1-Ethyl-3-(3-dimethyl aminopropyl) carbo diimide (EDC), 1.2 eq. Et_3N, ethylacetate, 16 h rt ii) NaOH, THF, 12 h, rt iii) TFA, rt, 10 h.

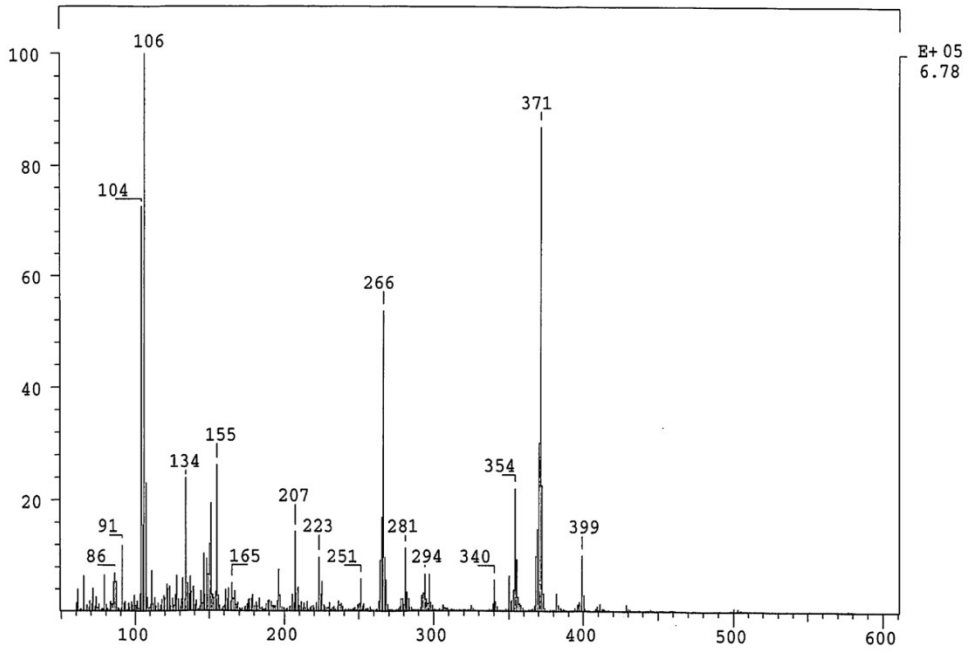
Synthesis of N_3-R(Mtr)GD(Me)$_2$. 103 mg (0.25 mmol) of N_3-Arg(Mtr) were dissolved in 20 ml Ethyl acetate and ice-cooled. 40.4 mg (0.26 mmol) EDC and 47 mg (0.26 mmol) pentafluorphenol were added followed by stirring for 1h. Subsequently, the TFA adduct of the esterprotected dipeptide TFA·Gly-L-Asp(OMe)OMe and 46 µl (0.26 mmol) Et_3N were added and reaction mixture was allowed to warm to room temperature and to stir overnight. The reaction was diluted by addition of H_2O and Ethylacetate and the phases were separated. The organic phase was washed with saturated $NaHCO_3$-solution and subsequently dried over Na_2SO_4. The solvent was removed in *vacuo*. Further purification of the crude product via column chromatography (silicagel, eluent: DCM) yielded 110.0 mg (0.18 mmol, 71 %) of the azido-functionalized, still protected peptide as colorless solid.

HRMS: (Maldi-TOF) calcd. monoisotopic mass for $C_{24}H_{36}N_8O_9S$ 613.239, found m/z: 613.240 [M+1]. ^{1}H-NMR (400 MHz, CD_3CN) δ [ppm]: 7.65, 1H$_{NH}$, 6.93, 1H$_{NH,}$ 6.65, d (br), 2H$_{NHGdn,}$, 7.5 Hz, 6.51, s, 1H$_{ArH}$, 5.87, s (br), 1H$_{NH}$, 4.64, m, 1H$_{\alpha CHAsp}$, 3.78, d, 2H$_{CH2Gly}$, 5.9 Hz, 3.86, m, 1H$_{CHArg}$, 3.67, s, 3H$_{ArOCH3}$, 3.53 and 3.50, 2 x s 6H$_{2xCOOCH3}$, , 3.34-3.45, m, 2H$_{\delta CH2Arg}$, 2.69, dd, 2H$_{\beta CH2Asp}$, 2.49 and 2.42, 2s, 2 x 3H$_{ArCH3}$, 1.97, s, 3H$_{ArCH3}$, 1.55-1.9, m, 2H$_{\alpha CH2Arg\ and}$ 2H$_{\beta CH2Arg}$.

Synthesis of 3a (**"RGD**): 500 µl of a 1 M aqueous NaOH solution was added to a 1:1 (v/v) mixture (2ml) of THF and MeOH. 100 mg of the protected tripeptide **N_3-R(Mtr)GD(Me)$_2$** was dissolved in the mixtures and the solution was allowed for stirring for 4h at room temperatures. Subsequently, the reaction was ice-cooled and then neutralized with 3 ml of 1 M HCl under vigorous stirring. The aqueous solutions were extracted three times with ethyl

acetate; the organic layers were collected, dried over magnesium sulfate and filtered. After removal of the solvent the crude **N_3-R(Mtr)GD** was isolated in a 50% yield (estimated from ^{1}H-NMR analysis). Maldi-TOF MS: calc. monoisotopic mass for $C_{22}H_{32}N_8O_9S$: m/z 584.2, found m/z 585.4 [M+1]. ^{1}H-NMR (400 MHz, MeOD) δ [ppm]: 6.68, s, 1H_{ArH}, 4.43, m, 1$H_{\alpha CHAsp}$, 3.94, m, 2H_{CH2Gly}, 3.85, s, 3H $_{ArOCH3}$, 3.15, m, 2H_{CHArg}, 2.77, m, 1H_{CHArg} 2.70 and 2.63, 2s, 2 x 3H_{ArCH3}, 2.59, m, 2$H_{\delta CH2Asp}$, 2.14, s, 3H_{ArCH3}, 1.64-1.90, m, 4$H_{\alpha CH2Arg\ and\ \beta CH2Arg}$.

Subsequent removal of the Mtr-group was accomplished by dissolving 50 mg of **N_3-R(Mtr)GD** in 10 ml TFA. After stirring 8 h at room temperature the acid was removed and the solid **N_3-RGD** was isolated (yield: n. d.). MS (CI): m/z calcd. for $C_{12}H_{20}N_8O_6$: 372.2, found: 371 [M-1] and m/z = 399 [M-1+28]. Complete deprotection of the arginine residue was indicated by the absence of higher mass peaks. IR spectrum: ν_{N3}: 2108 cm^{-1}.



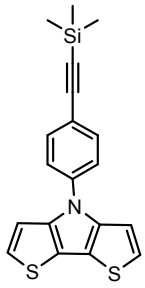
Post-functionalization of **P7** *with azides*:

Electrodes coated with **P7** obtained from 15 repetitive cycles were dipped into a 0.8 mMol solution of the respective azide and $Cu(CH_3CN)_4PF_6$ (5 mol %) in 1.5 ml ACN to which copper powder was added. After a reaction time of 3 days at room temperatures the coated electrodes were rinsed with acetonitrile, DCM and diethylether and dried in vacuum.

Post-functionalization of **P7** *with TCNE:*

Electrodes coated with **P7** obtained from 15 repetitive cycles were dipped into a 0.6 mMol solution of TCNE in 2 ml dichloroethane. After a reaction time of 2 days at 45°C the coated electrodes were rinsed with DCM and diethylether and dried in vacuum.

Synthesis of 4-(4-((trimethylsilyl) ethynyl) phenyl)-4H-dithieno[3,2-b:2',3'-d]pyrrole **6**



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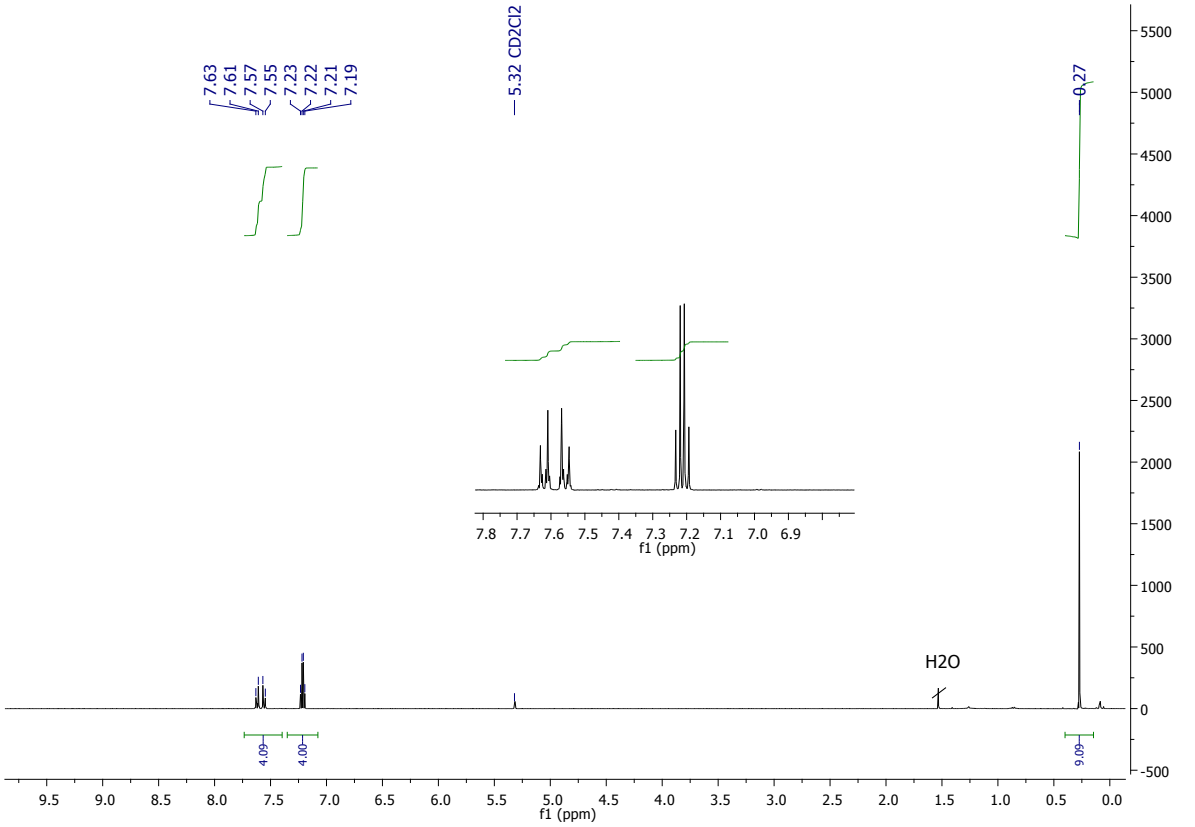
In a Schlenk tube 160 mg (0.5 mmol) 3,3'-dibromo-2,2'-bithiophene **4**, 107 mg (1.12 mmol) sodium tert. butanolate, 8 μmol Pd_2dba_3 and 30 μmol BINAP were dissolved in dry toluene (50 mL) and purged with argon for 20 min. Subsequently, 100.0 mg (0.5 mmol) 4-((trimethylsilyl) ethynyl) phenyl **5** was added and the mixture was stirred for 12 h at 78°C under an argon atmosphere. After cooling to room temperature, again 8 μmol Pd_2dba_3 and 30 μmol BINAP were added and the mixture was again reacted for 10 hours. Subsequently, water was added and the layers were separated. The water phase was extracted three times with diethylether. The combined organic layers were washed twice with water, dried over $MgSO_4$ and the solvent was removed in vacuum. The crude compound was further purified by column chromatography (silica; eluent: PE) to give the alkynylated DTP as transparent resin (115 mg, 0.33 mmol, 65 % yield). MW = 351.057 EI (CI) 352.

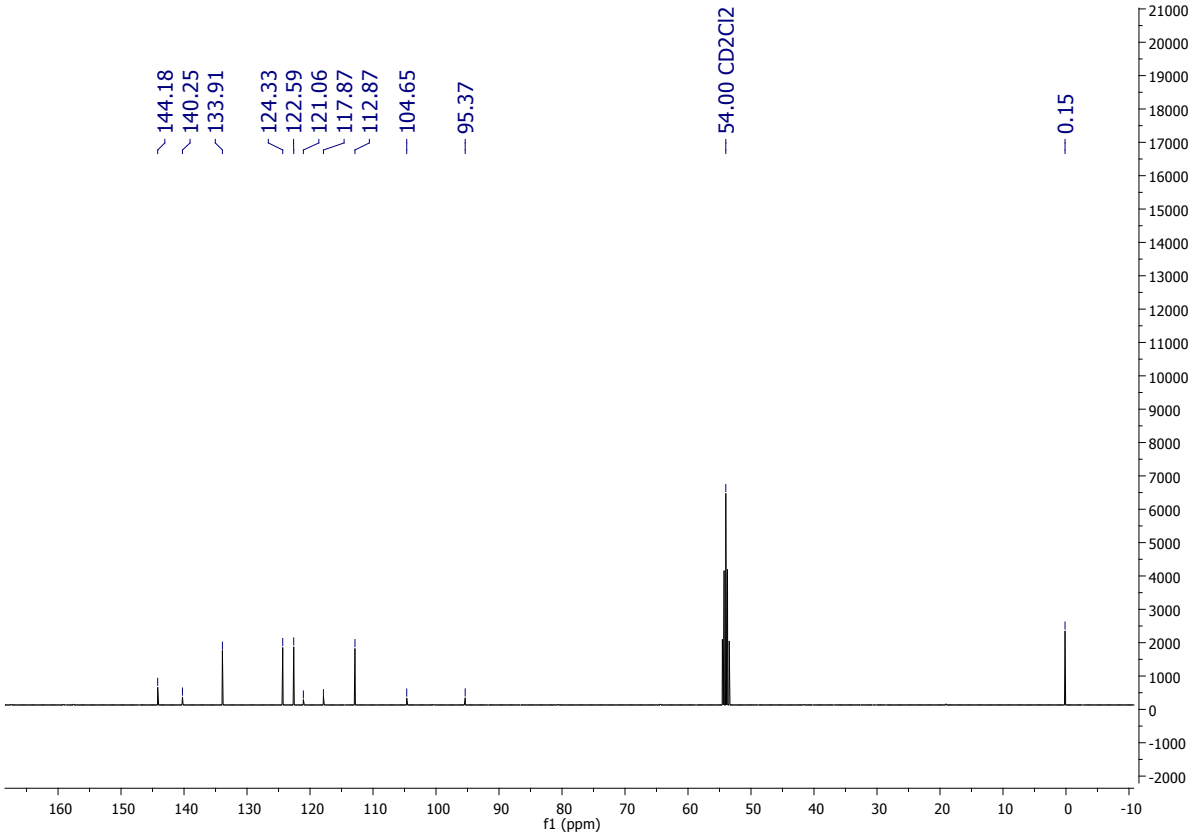
^{1}H-NMR (CD_2Cl_2, 400 MHz): δ = 7.62 (d, *J* = 8.8 Hz, 2H, 3,3'phe), 7.56 (d, *J* = 8.8 Hz, 2H, 2,2'phe), 7.22 (d, *J* = 5.3 Hz, 2H, 2,2'thiophene), 7.20 (d, *J* = 5.3 Hz, 2H, 3,3'thiophene), 0.27 (s, 9H) ppm.

^{13}C-NMR (CD_2Cl_2, 101 MHz): δ = 144.18, 140.25, 133.91, 124.33, 122.59, 121.06, 117.87, 112.87, 104.65, 95.37, 0.15.

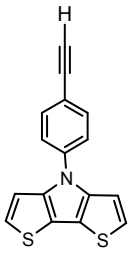
^{1}H-NMR (CD_2Cl_2, 400 MHz) of **6**:



^{13}C-NMR (CD_2Cl_2, 101MHz) of **6**:



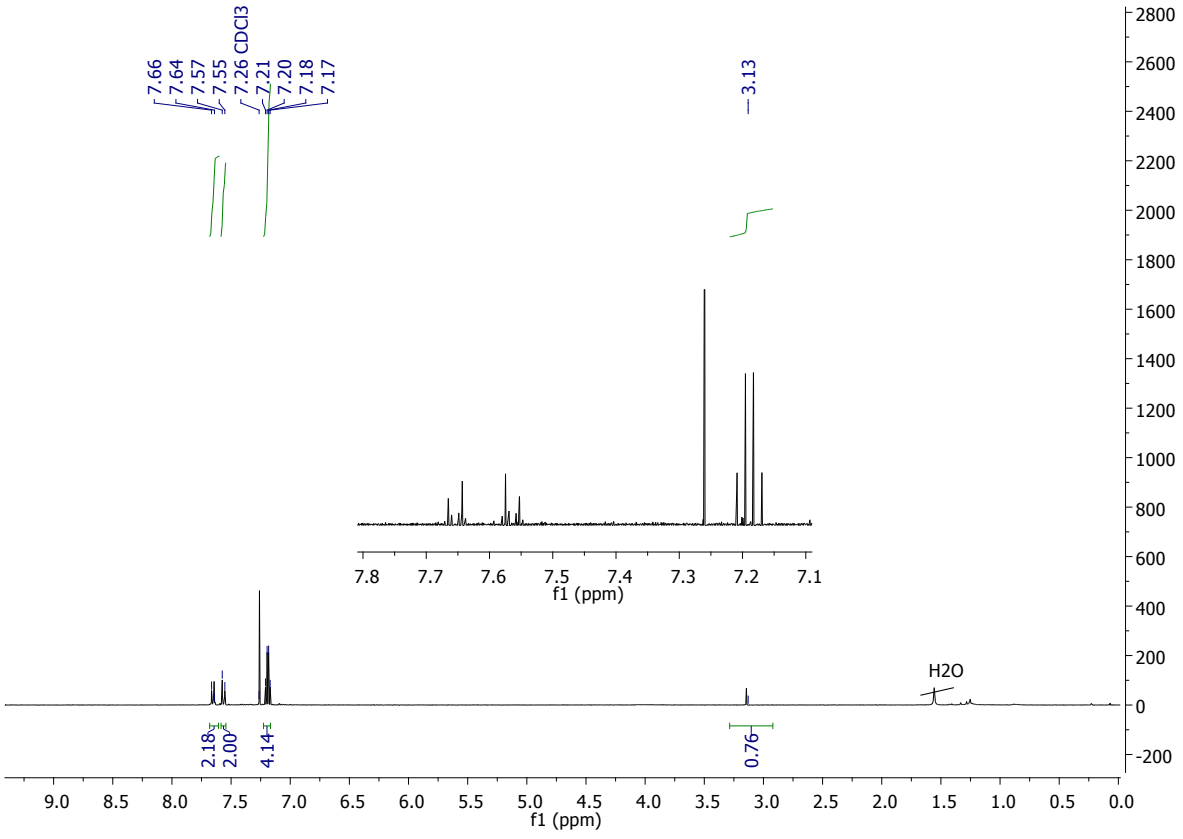
Synthesis of 4-(4-ethynylphenyl)-4H-dithieno[3,2-b:2',3'-d]pyrrole **7**



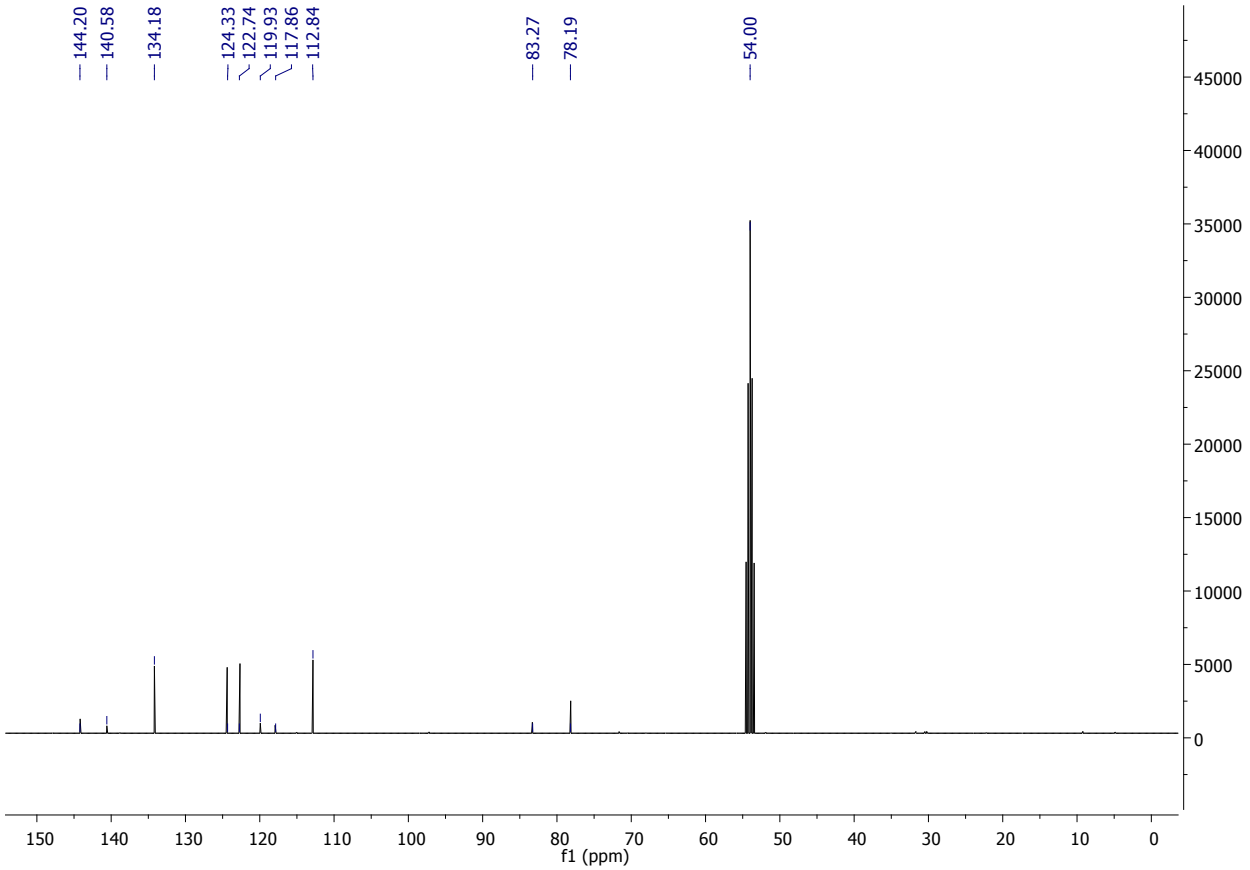
7

To a stirred solution of the TMS-protected **6** (30 mg, 90 µmol) in THF (2 ml) KOH (11 mg, 180 µmol) in methanol (1 ml) added. The solution was allowed to react at room temperature for 12 hours. For working up the mixture were removed and the residue was repeatedly extracted with dichloromethane. The organic phase was washed with brine and subsequently dried over Na_2SO_4 and concentrated. The crude product was purified by flash chromatography (petrolether: DCM, 8:1, *v/v* as the eluent) and provided **7** as colorless solid in a yield to 81 %. Mp. 75-155°C (decomp.); m/z (GC-MS) 279.1 [M^+]; **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ = 7.68 (d, *J* = 8.8 Hz, 2H, 3,3'phe), 7.59 (d, *J* = 8.8 Hz, 2H, 2,2'phe), 7.23 (d, *J* = 5.3 Hz, 2H, 2,2'thiophene), 7.20 (d, *J* = 5.3 Hz, 2H, 3,3'thiophene), 3.17 (s, 1H) ppm. **^{13}C-NMR** (101 MHz, CD_2Cl_2) δ = 144.05, 140.43, 134.03, 124.18, 122.59, 119.77, 117.71, 112.69, 83.12, 78.03.

^{1}H-NMR ($CDCl_3$, 400 MHz) of **7**:



^{13}C-NMR (CD_2Cl_2, 101MHz) of **7**:



A549 Cell Culture

A549 cells, a human alveolar basal epithelial carcinoma cell line (obtained from DSMZ, German Collection of Microorganisms and Cell Cultures, Braunschweig) were cultivated in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Darmstadt, Germany) supplemented with 10 % heat-inactivated (30 min at 56 °C) fetal calf serum (FCS, Gibco, Darmstadt, Germany), 1 % MEM non-essential amino acid solution (Sigma-Aldrich Chemie GmbH) as well as 1 % penicillin (100 U mL^{-1}) and streptomycin (100 mg mL^{-1}) (Sigma-Aldrich Chemie GmbH) at 37 °C under a humidified atmosphere with 5 % CO_2. Cells were reseeded at least twice weekly.

Live-cell imaging using fluorescence microscopy

For the viability assay, the ITO surfaces were placed into ibidi multiwall slides (1µ Slide 8well ibiTreat, ibidi GmbH, Martinsried, Germany) and sterilized via UV for 90 min. The pre-cultured A549 cells were then trypsinated, washed with DMEM and seeded on the respective ITO surfaces with a density of 20,000 cells per well in 300 µL medium, followed by overnight (15 h) incubation in the fully supplemented DMEM medium (1 % MEM, 1 % penicillin-streptomycin) at 37 °C under a humidified atmosphere with 5 % CO_2. The cell viability was evaluated by Calcein-AM staining. Therefore, the cell-culture medium was replaced by 300 µL fully supplemented DMEM medium containing 1 µL calcein-AM solution (1 mg/mL solution in DMSO, BioReagent, suitable for fluorescence, ≥96.0% (HPLC) obtained from Sigma-Aldrich Chemie GmbH, Munich, Germany) and cells were incubated at 37°C for 20 min. Subsequently, the staining solution was removed and the cells were rinsed twice with 4°C DPBS (Sigma-Aldrich Chemie GmbH). Cells were fixed using a 4 % para-formaldehyde solution in DPBS (300 µL) with subsequent incubation for 20 min at RT. Finally, the para-formaldehyde solution was replaced by DPBS and cells were stored at 4 °C under exclusion of light.

For qualitative comparison of the cell morphology, representative images were taken under 10× magnification. To quantify the calcein-AM stained viable cells, multiple (at least three) independent and representative visual fields per well were taken under 5× magnification. The image processing software Image J 1.51f (Image J Software, Wayne Rasband, National Institutes of Health, USA) was used to quantify the number of fluorescent cells and the respective % area. Hereby, the entire image was set as the region of interest (ROI). The

sample size was as follows: ITO (N=6, n=24), ITO coated with non-modified polymer P7 (N=5, n=17), ITO coated with RGD modified polymer P7a (N=4, n=13), ITO coated with mannosidic polymer P7e (N=4, n=12) with "N" being the number of analyzed (modified) ITO glass slides and "n" the total number of analyzed visual fields. The results are presented as a mean ± standard deviation (SD). Evaluation of all data was conducted using the statistics software GraphPad Prism version 5.01 (GraphPad Software, San Diego, CA, USA). Subsequent to evaluation of normality using the Shapiro-Wilk normality test, Kruskal-Wallis with Dunn's post-hoc test was applied for data analysis of **P7** vs **P7a** or **P7e** (Fig 5A and 5B). The level of significance was set to $P<0.05$.

References

[1] T. Jakusch, S. Marcao, L. Rodrigues, I. Correia, J. C. Pessoa and T. Kiss, *Dalton Trans*., 2005, 3072-3078.

[2] J. T. Lundquist, IV and J. C. Pelletier, *Org. Lett*., 2001, **3**, 781-783.

[3] N. Khoukhi, M. Vaultier and R. Carrier, *Tetrahedron*, 1987, **43**, 8, 1811-1822.

[4] M. T. da Silva, R. N. de Oliveira, W. O. Valença, F. C. G. Barbosa, M. G. da Silva and C. A. Camara, *Braz. Chem. Soc*., 2012, **23**, 10, 1839-1843.

[5] J. M. Casas-Solvas, E. Ortiz-Salmerón, J. J. Giménez-Martínez, L. García-Fuentes, L. F. Capitán-Vallvey, F. Santoyo-González, A. Vargas-Berenguel, *Chem. Eur. J.*, 2009, **15**, 710-725.

[6] T. K. Lindhorst and C. Kieburg, *Angew. Chem. Int. Ed*., 1996, **35**, 1953–1956; *Angew*. *Chem*. 1996, **108**, 2083–2086.

[7] K. Ogawa and S. C. Rasmussen, *J. Org. Chem.*, 2003, **68**, 2921-2928.