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Design and Synthesis of Mixed Oligomers with Thiophenes, Dithienothiophene *S*,*S*-Dioxides, Thieno[3,4]pyrazines and 2,1,3-Benzothiadiazoles: Flipper Screening for Mechanosensitive Systems

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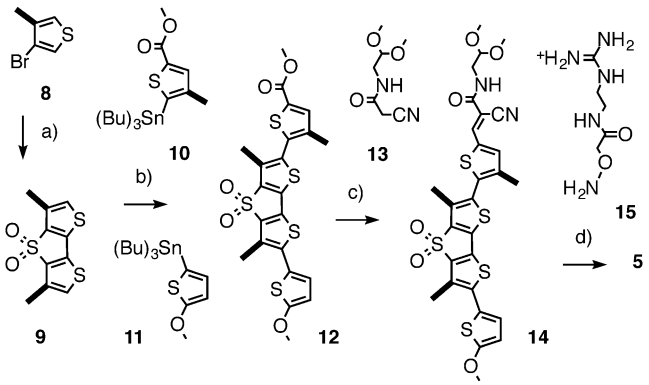
Monomers with large surface area and high quantum yield, that is fluorescent flippers, have been engineered into twisted push–pull oligomers to create membrane probes with high mechanosensitivity and long fluorescence lifetime. Here, the synthesis and characterization of thieno[3,4]pyrazines and 2,1,3-benzothiadiazoles are described in comparison with the original dithienothiophene *S*,*S*-dioxides. Dithienothiophene *S*,*S*-dioxide flippers are confirmed as the best reported so far, and poor results with single flipper probes support that two flippers are needed for the probe to really "swim", that is, for high mechanosensitivity.

The concept of planarizable push–pull probes has been introduced recently as a conceptually innovative approach to image lipid bilayer membranes (A in Figure 1).[1] This combination of polarization and planarization in the ground state is attractive because it applies lessons from nature,[1] is complementary to excited-state planarization in molecular rotors[2] and thus reports changes in excitation rather than emission as most conventional membrane probes.[2,3] However, the original approach with push–pull oligothiophenes suffered from increasing loss in fluorescence upon ground-state twisting, and red shifts in excitation were limited to about +40 nm. To increase mechanosensitivity[4] and fluorescence lifetime (τ), monomers with large surface areas and high quantum yields (φ) were engineered into the twisted oligomers.[5] Twisted push–pull probe **1** with two fluorescent flippers, an electron-rich dithienothiophene and a poorer dithienothiophene *S*,*S*-dioxide,[6] gave quantum yields above 80% also in fully deplanarized form, and shifts up to +80 nm and lifetimes up to 4.8 ns upon planarization.[5] Here, we report synthetic efforts towards oligomers **2**–**7**, designed to vary the number of dithienothiophene flippers per probe and secure access to complementary flippers such as thieno[3,4]pyrazines[7] or 2,1,3-benzothiadiazoles.[8]

The synthesis of dithienothiophene probes **1**–**4** has been described previously (Figure 1).[5] Probe **5** was prepared following the procedure used for probe **4** with some modifications (Scheme 1). Bromothiophene **8** was converted into dithienothiophene following reported procedures, and a successive oxidation with *meta*-chloroperoxybenzoic acid (mCPBA) gave the electron-poor *S*,*S*-dioxide **9**.[5] Two consecutive Stille couplings, first with **10** and finally with **11**, gave push–pull chromophore **12**. Ester reduction to the primary alcohol, oxidation with Dess–Martin periodinane to the aldehyde, and Knoevenagel condensation with cyanoamide **13** afforded acetal **14** as the pure *trans* isomer. The final amphiphile, **5**, was obtained by aldehyde deprotection and oxime formation with alkoxyamine **15**.



Scheme 1. *Reagents and conditions*: a) 1. $S(SnBu_3)_2$, $Pd(PPh_3)_4$, toluene, 130 °C, 12 h, 46%, 2. *n*BuLi, $CuCl_2$, Et_2O, 0 °C→rt, 57%,[6] 3. mCPBA, $CHCl_3$, 40 °C, 12 h, 64%; b) 1. NBS, CH_2Cl_2/AcOH (6:4), 1 h, rt, 45%, 2. **10**, $Pd(PPh_3)_4$, toluene, 130 °C, 12 h, 52%, 3. NBS, CH_2Cl_2/AcOH (6:4), 1 h, rt, 1 h, 96%, 4. $Pd(PPh_3)_4$, **11**, toluene, 130 °C, 12 h, 65%; c) 1. DIBAL, CH_2Cl_2, −78 °C, 4 h, 2. DMP, CH_2Cl_2, rt, 1 h, 96% (2 steps), 3. **13**, piperidine, CH_3CN, 70 °C, 6 h, 42%; d) 1. *p*-TsOH·H_2O, CH_2Cl_2, rt, 75 min, 86%, 2. **15**, DMSO, 60 °C, 90 min, 58%.

Oligothiophene **6** was synthesized starting with the stannylation of thiophene **16** for Stille coupling with thiophene **17** (Scheme 2). The obtained terthiophene **18** was reduced to the unstable diamine and then directly coupled with 2,3-butanedione to give thieno[3,4]pyrazine **6**''.[7] An electron-withdraw-

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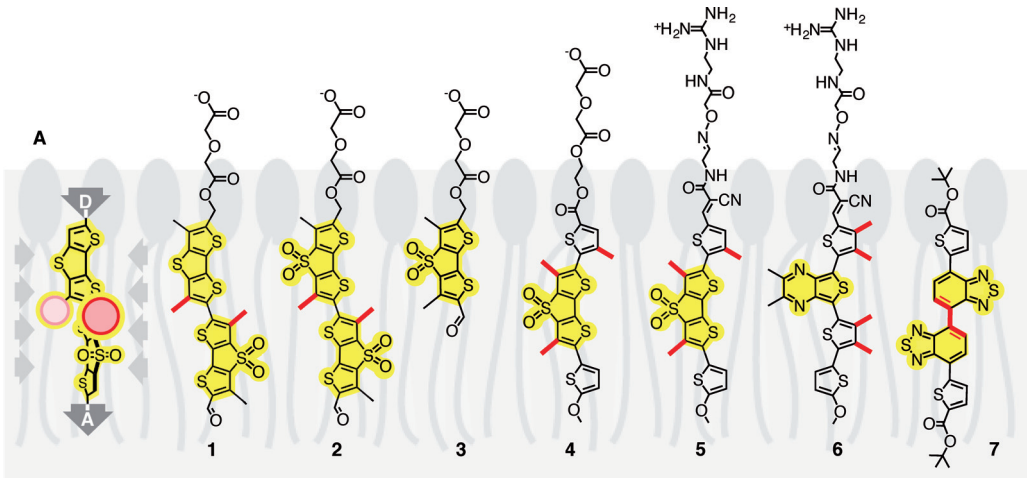
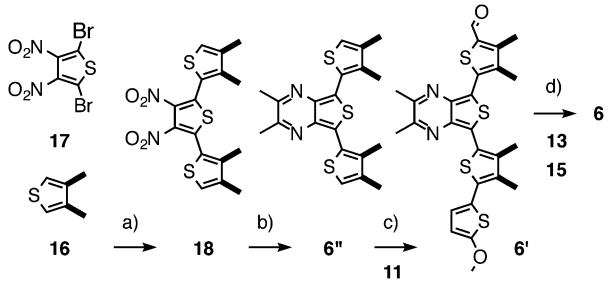
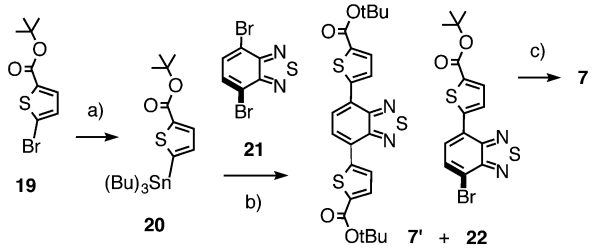


Figure 1. Planarizable push–pull probes (A) and controls with fluorescent dithienothiophene *S,S*-dioxide flippers compared to thieno[3,4]pyrazines or 2,1,3-benzothiadiazoles.



Scheme 2. *Reagents and conditions*: a) 1. **16**, *n*BuLi, Et_2O, 20 min, −78 °C, 20 min, to rt, 1 h, 2. Bu_3SnCl, Et_2O, −78 °C, 5 min, to rt, 12 h; 3. **17**, $Pd(PPh_3)_4$, THF, 66 °C, 12 h, 56% (3 steps); b) 1. $N_2H_4{\cdot}H_2O$, Pd/C (10%), EtOH, rt, 2 h, 2. 2,3-butanedione, EtOH, rt, 12 h, 60% (2 steps); c) 1. $POCl_3$, DMF, CH_2Cl_2, 40 °C, 12 h, 65%, 2. NBS, CH_2Cl_2, rt, 2 h, 60%, 3. **11**, $Pd(PPh_3)_4$, DMF, 80 °C, 12 h, 50%; d) 1. **13**, CH_3CN, piperidine, DMF, 70 °C, 48 h, 55%, 2. *p*-TsOH·H_2O, CH_2Cl_2, rt, 30 min, 35%, 3. **15**, AcOH, DMSO, 60 °C, 10 h, 28%.



Scheme 3. *Reagents and conditions*: a) 1. *n*BuLi, THF, −78 °C, 20 min, 2. Bu_3SnCl, THF, 15 h, rt, 61%; b) **20**, $Pd(PPh_3)_4$, DMF, 80 °C, 15 h, 38% (**22**), 15% (**7′**); c) **22**, $Pd(OAc)_2$, K_2CO_3, PEG4000, DMF, 120 °C, 5 h, 58%.

ing aldehyde group was added through a Vilsmeier–Haack reaction. Subsequent bromination with *N*-bromosuccinimide (NBS) and Stille coupling with **11** afforded push–pull oligomer **6′**. A Knoevenagel condensation with **13**, followed by aldehyde deprotection and oxime formation with **15** gave final amphiphile **6** with a thieno[3,4]pyrazine flipper in the push–pull scaffold (cf. Scheme 1).

Oligomer **7** with 2,1,3-benzothiadiazole flippers was prepared from thiophene **19** (Scheme 3). Stille coupling between **20** and benzothiadiazole **21** gave monoester **22** and diester **7′**.[8] Final homocoupling of **22** gave the target molecule **7**.

As stated in the introduction, the original double-flipper probe (**1**) with one dithienothiophene *S,S*-dioxide acceptor and one dithienothiophene donor excelled with a quantum yield of 83% and a fluorescence lifetime of 2.2 ns already in twisted form (Table 1).[5] Significant solvatochromism was evidenced by the obtained variation of the permanent dipole moment upon excitation ($\Delta\mu$) value of 14.3 D.[5] Ground-state planarization in solid-ordered (S_o) lipid bilayer membranes caused a red shift of the excitation maximum from a λ_{ex} value of 435 nm up to

Table 1. Optoelectronic properties of mixed oligomers with fluorescent flippers.

Compd[a]	λ_{ex} [nm][b]	λ_{em} [nm][c]	$\Delta\mu$ [D][d]	φ [%][e]	$\Delta\lambda_{ex}$ [nm][f]
1	435	530	14.3	83	45–80[g]
2	418	485	11.0	66	0/71[h]
3	398	478	1.9	79	–
4	434	582	10.1	32	12
5	447	620	13.7	2	31[i]
6	550	651	0	3	8[i]
6′	509	610	–	5	–
6″	467	624	–	20	–
7	451	545	–	28	–
7′	444	555	–	99	–

[a] For structures, see Figure 1 and Schemes 1, 2 and 3. Excitation maxima (λ_{ex}), permanent dipole moments upon excitation ($\Delta\mu$) and quantum yields (φ) of **1**–**6** were measured with hydrophobic precursors. For **1**–**4**, φ and $\Delta\lambda_{ex}$ values are taken from Ref. [5]. [b] Determined in $CHCl_3$. [c] Emission maximum (λ_{em}) determined in $CHCl_3$. [d] Variation of $\Delta\mu$, from Lippert analysis of solvatochromism (Figure S4–S7 in the Supporting Information). [e] Determined in $CHCl_3$; calculated using Rhodamine 6G in EtOH as a reference (92%). [f] λ_{ex} in dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) large unilamellar vesicles (LUVs) minus λ_{ex} in 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) LUVs at 25 °C. [g] Broad maximum in DPPC.[5] [h] Two maxima in DPPC.[5] [i] Figure S3 in the Supporting Information.

a $\Delta\lambda_{ex}$ value of +80 nm.[5] Reduction of the push–pull macrodipole in probe **2** with two dithienothiophene *S*,*S*-dioxides resulted in decreased fluorescence ($\varphi = 66\%$), blue-shifted excitation ($\lambda_{ex} = 418$ nm), a decreased $\Delta\mu$ value of 11.0 D in twisted form, and incomplete planarization in S_o membranes (two maxima).[5] The shortened, planar single-flipper control (**3**) had a hypsochromic excitation at a λ_{ex} value of 398 nm, weak solvatochromism but strong fluorescence ($\varphi = 79\%$). This control was dysfunctional because it did not partition into lipid bilayers.[5]

Application of the same evaluation procedure to elongated single-flipper probe **4** with strongly twisted flanking thiophenes exhibited, compared with original **1**, acceptable fluorescence ($\varphi = 32\%$), preserved λ_{ex} value of 434 nm but clearly a weaker $\Delta\mu$ value of 10.1 D in twisted form, and very poor response to planarization in S_o membranes ($\Delta\lambda_{ex} = +12$ nm, Table 1).[5] New single-flipper probe **5** with a most powerful acceptor showed inacceptable fluorescence ($\varphi = 2\%$) but bathochromic excitation ($\lambda_{ex} = 447$ nm) in twisted form in solution. The presence of strong push–pull macrodipoles in **5** was indicated by the $\Delta\mu$ value of 13.7 D obtained from significant solvatochromism. Supporting their importance for mechanosensitivity, the response of single-flipper probe **5** to planarization in S_o membranes ($\Delta\lambda_{ex} = +31$ nm) was much better than with otherwise identical single flipper **4** ($\Delta\lambda_{ex} = +12$ nm, $\Delta\mu =$ 10.1 D) but still much less impressive than that with original double flipper **1** (up to $\Delta\lambda_{ex} = +80$ nm, $\Delta\mu = 14.3$ D).

Probe **6** with a single thieno[3,4]pyrazine flipper embedded within three thiophenes was not really fluorescent ($\varphi = 3\%$), but had nicely red-shifted excitation ($\lambda_{ex} = 550$ nm) without any solvatochromism of the emission maximum (Table 1). Irresponsiveness to planarization in S_o membranes ($\Delta\lambda_{ex} = +8$ nm) suggested that **6** is already almost planar in solution. This disappointing finding implied that N–S attraction overcompensated CH_3–S repulsion between thieno[3,4]pyrazines and thiophenes.[9] For completion, we add that fluorescence was already weak without cyano acceptors in **6′** ($\varphi = 5\%$, $\lambda_{ex} =$ 509 nm) and shorter oligomers **6″** ($\varphi = 20\%$, $\lambda_{ex} = 467$ nm).

Oligomer **7** with two central 2,1,3-benzothiadiazole flippers was reasonably fluorescent ($\varphi = 28\%$) and absorbed in the region of original **1** ($\lambda_{ex} = 451$ nm). Solvatochromism and planarization in S_o membranes were not measured because oligomer **7**, designed for a different purpose,[10] is not a push–pull amphiphile. However, benzothiadiazoles turned out to deserve attention in the context of fluorescent flipper screening because the fluorescence of shortened oligomers **7′** is almost quantitative ($\varphi = 99\%$) and preliminary crystal structures of **7** revealed a torsion angle between two benzothiadiazole flippers of 19° even in the solid state with rather tight face-to-face π stacking (3.69 Å). The benzothiadiazole–thiophene torsion angles were nearly negligible under the same conditions (8°).

In summary, the synthesis of a series of mixed oligothiophenes with dithienothiophenes, dithienothiophene *S*,*S*-dioxides, thieno[3,4]pyrazines and 2,1,3-benzothiadiazoles has been accomplished. As far as their potential use in twisted push–pull mechanophores is concerned, their comparative evaluation is complicated by the complexity of the systems of interest. Nevertheless, some noteworthy trends emerged. Namely, dithienothiophenes and their *S*,*S*-dioxides are the best flippers reported so far, strong push–pull macrodipoles seem beneficial, and it looks like two flippers are needed to really "swim", that is, achieve high mechanosensitivity. In the explored systems, the red-shifted thieno[3,4]pyrazine flippers were disappointing because of poor fluorescence and mechanosensitivity. The twist noted between two adjacent benzothiadiazoles could deserve further attention, but dithienothiophene flippers in oligomers with perfect push–pull dipole and twist certainly invite for the highest expectations with regard to the imaging of membrane order,[1–3,5] potential[3c,d,11] and tension.[12]

Experimental Section

For details of the materials and methods, and full protocols and characterization data for the compounds reported, see the Supporting Information available on the WWW under http://dx.doi.org/10.1002/open.201402139.

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