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# Supporting Information

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# Supporting Information

for

# Pentafluorosulfanyl as a Novel Building Block for Enzyme Inhibitors: Trypanothione Teductase Inhibition and Antiprotozoal Activities of Diarylamines

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## 1. X-ray Crystallography

### a) Figures

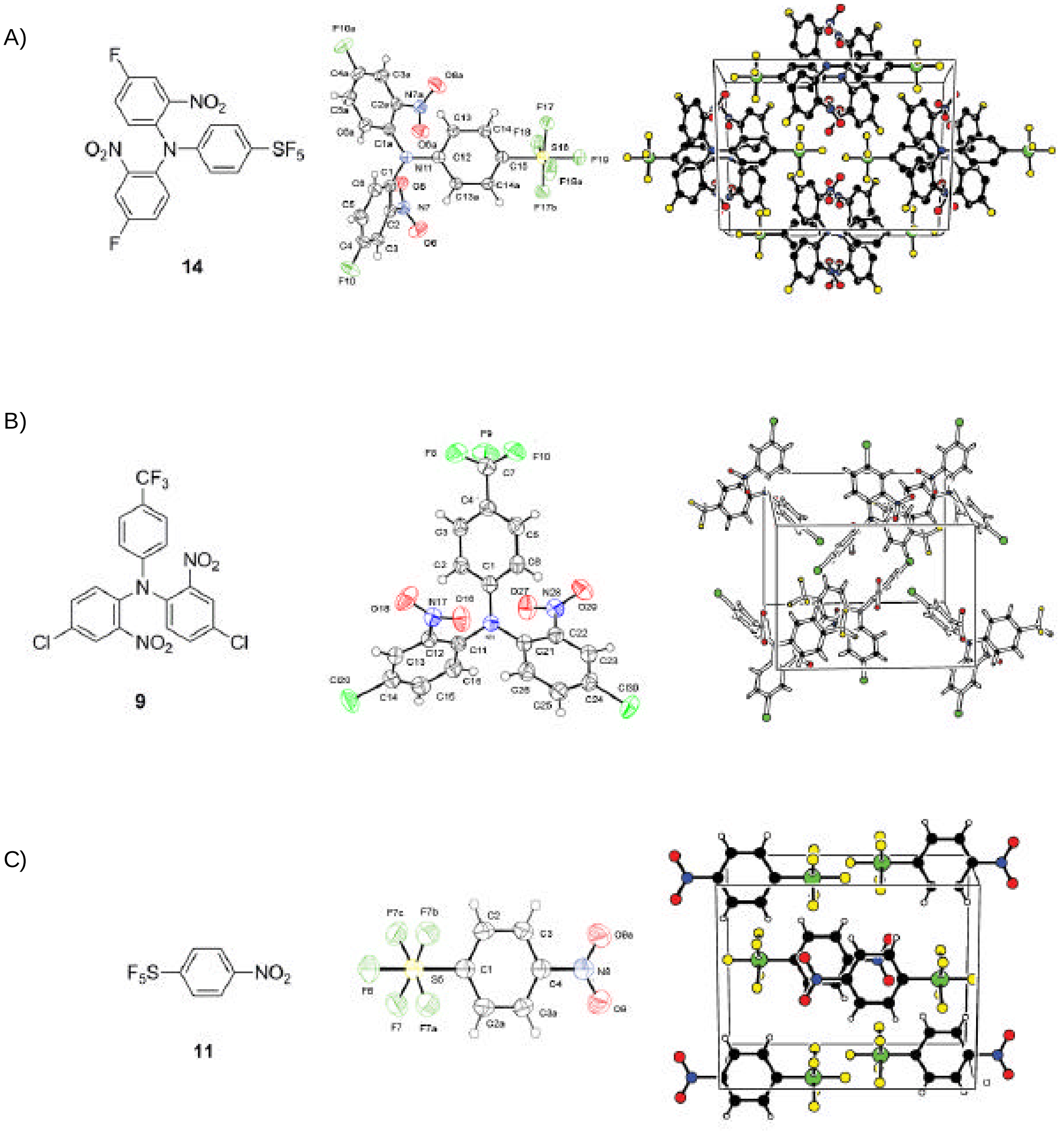

**Figure S1.** ORTEP plots (T = —50 °C **9**, **11**; —40 °C **14**), with vibrational ellipsoids shown at the 50% probability level, and crystal packings for triphenylamines **14** (a), **9** (b), and nitrophenylsulfur pentafluoride (**11**, c). Arbitrary numbering.

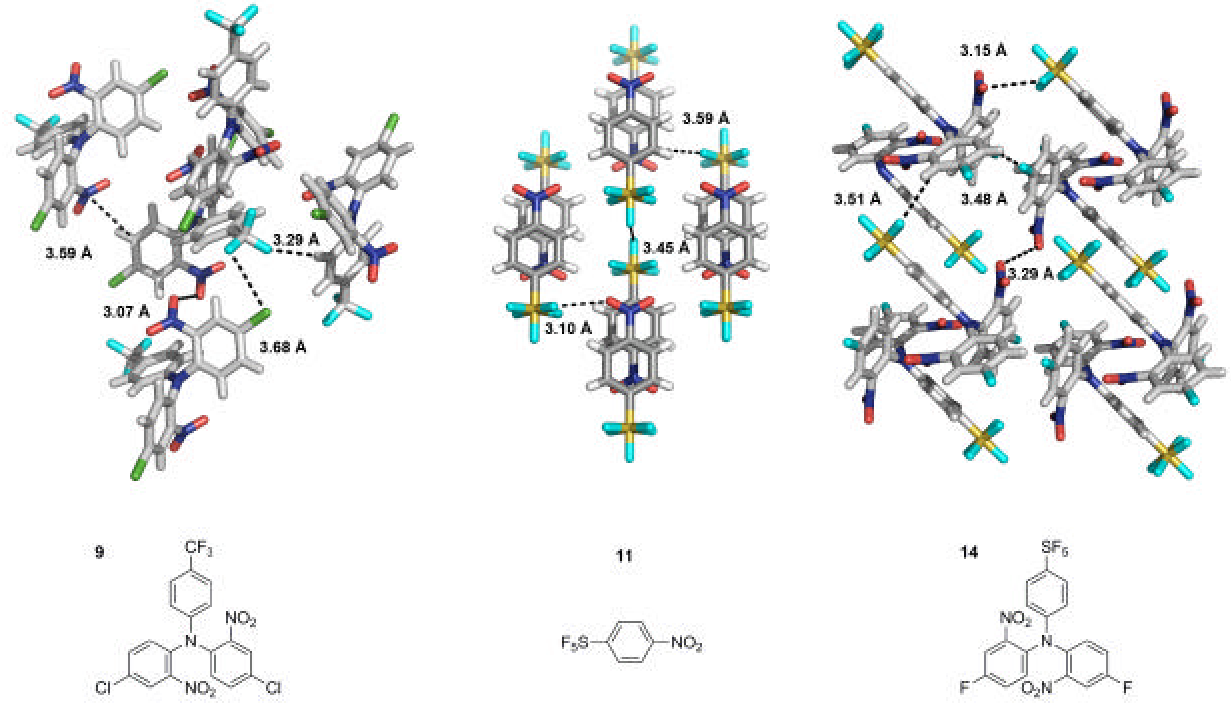

**Figure S2.** Crystal packings of triphenylamines **9** and **14** and p-nitrophenylsulfur pentafluoride **11**.

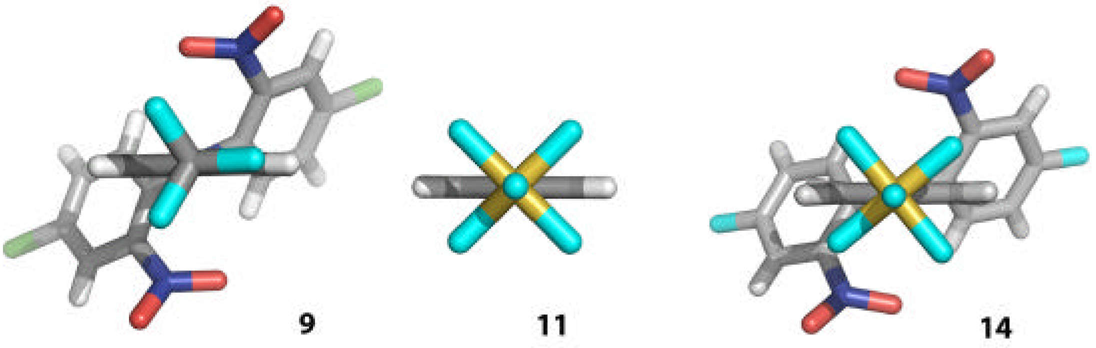

**Figure S3.** Staggered conformation of the $SF_5$ group in the crystal structure of nitrobenzene **11** (center) and triphenylamine **14** (right), compared to the $F_3C$-bearing triphenylamine derivative **9** (left).

### b) Discussion of the X-ray crystal structures

Crystals of the triphenylamine derivatives **9** and **14** were suitable for X-ray diffraction analysis. In both cases, the three phenyl rings are arranged propeller-shaped around the central nitrogen atom (Figure S1 A, B), as reported for related derivatives earlier.[1-3] The pentafluorosulfanyl compound **14** crystallizes in the monoclinic space group C2/c with half a molecule in the asymmetric unit (z' = 0.5). A twofold axis through the sulfur and the amine nitrogen completes the molecule. The crystal packing shows distinct layers along the a-c diagonal of alternating antiparallel $SF_5$-substituted phenyl rings. (Figure S2). In contrast, the closely related trifluoromethyl derivative **9** crystallizes in the monoclinic space group $P2_1/c$ and exhibits no pronounced layers in the crystal packing (Figure S2). The crystal structure of *p*-nitrophenylsulfur pentafluoride (**11**), one of the few organic pentafluorosulfanyl derivatives that are commercially available, was not known to date. By vacuum sublimation, crystals of **11** were grown that allowed X-ray investigation. The compound crystallizes in the orthorhombic space group Cmcm with one quarter of the molecule as asymmetric unit (z' = 0.25). Except of one F-atom, the molecule lies on the mirror plane perpendicular to the c-axis and is completed by the mirror plane perpendicular to the a-axis. This creates layers showing alternating $SF_5$ and nitro substituents in the direction of the a-axis.

The bond lengths of the approximately octahedral $SF_5$ group (**11**: $S\text{-}C_{ipso}$ = 1.812(3) Å, $S\text{-}F_{ax}$ = 1.581(1), $S\text{-}F_{eq}$ = 1.576(2) Å; **14**: $S\text{-}C_{ipso}$ = 1.795(3) Å, $S\text{-}F_{ax}$ = 1.576(2), 1.567(2), $S\text{-}F_{eq}$ = 1.574(2) Å) are almost identical for the two $SF_5$ compounds and are in the same range as the corresponding bond lengths in the few published crystal structures of phenylsulfur pentafluoride derivatives.[4-6] In both cases, the equatorial F atoms of the $SF_5$ substituents adopt a staggered conformation in respect to the phenyl ring, as proposed by Sheppard (Figure S3).[7]

## 2. Experimental Section

## a) X-ray crystal structure determination

Copies of the data can be obtained free of charge on application to Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ ccdc.cam.ac.uk).

Data has been measured on a Bruker Kappa-CCD diffractometer with Mo*K*α radiation, $l$ = 0.71073 Å, graphite monochromator at —50 °C (**14** —40 °C). The structures were solved by direct methods (SIR97).[8] Non-H atoms were refined anisotropically and H atoms isotropically with SHELXL-97.[9]

Compound **9**: monoclinic *C2/c*; $a$ = 12.8804(6), $b$ = 15.7370(8), $c$ = 11.1786(5) Å, $b$ = 123.574(3)°, $V$ = 11887.9(2) $Å^3$ from 4212 reflexions; Z = 4. Final $R(F)$ = 0.0433, $wR(F^2)$ = 0.1522 for 266 parameters, 0 restraints, and 2159 reflections with $I > 2s(I)$ and $2.75 < q < 27.49°$. Deposition No. CCDC 698093.

Compound **11** orthorhombic *Cmcm*; $a$ = 10.0679(6), $b$ = 12.3720(7), $c$ = 6.8861(4) Å, $V$= 857.73(9) $Å^3$ from 965 reflexions; Z = 4. Final $R(F)$ = 0.0379, $wR(F^2)$ = 0.132 for 54 parameters, 0 restraints, and 507 reflections with $I > 2s(I)$ and $2.43 < q < 27.49°$. Deposition No. CCDC 698095.

Compound **14**: monoclinic $P2_1/c$; $a$ = 10.6878(3), $b$ = 15.0810(4), $c$ = 12.8358(4) Å, $b$ = 108.380(1)°, $V$ = 1963.4(1) $Å^3$ from 8734 reflexions; Z = 4. Final $R(F)$ = 0.0455, $wR(F^2)$ = 0.1404 for 312 parameters, 0 restraints, and 3501 reflections with $I > 2s(I)$ and $2.55 < q < 27.49°$. Deposition No. CCDC 698094.

## b) Synthesis.

**General**: Solvents and reagents were purchased reagent-grade and used without further purification. All reactions were carried out under a nitrogen atmosphere unless otherwise stated. $CH_2Cl_2$ and toluene were freshly distilled over $CaH_2$ and sodium, respectively. All products were dried under high vacuum ($10^{-2}$ Torr) before analytical characterisation. TLC: Aluminium sheets coated with $SiO_2$-60 $UV_{254}$ from *Macherey-Nagel*, visualization by UV light at 245 nm and staining with a solution of $KMnO_4$ (1.5 g), $K_2CO_3$ (10 g), 5 % NaOH (2.5 mL) in $H_2O$ (150 mL), or a solution of ninhydrin (0.3 g) in butanol (100 mL) and glacial acetic acid (3 mL). Column chromatography (CC): $SiO_2$-60 (230-400 mesh, 0.040-0.063 mm) from *Fluka*. Melting points (mp): *Büchi-510* apparatus; uncorrected. IR Spectra: *Perkin Elmer Spectrum BX FTIR System* spectrometer (*ATR-unit, Attenuated Total Reflection, Golden Gate*). NMR spectra ($^1$H, $^{13}$C, $^{19}$F): *Varian Gemini-300*, *Bruker ARX-300, AV-400 and DRX-400*; spectra were recorded at 25 °C using the solvent peak as an internal reference. Coupling constants (*J*) are given in Hz. The resonance multiplicity is described as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), quint (quintet), sext (sextet), and m (multiplet). High-resolution mass spectra (HRMS): *IonSpec Ultima FT-ICR* with 3-hydroxypicolinic acid (3-HPA) as matrix (MALDI), *Micromass AutoSpec-Ultima* (EI), *Varian's IonSpec FT-ICR* (ESI). Elemental analyses were performed by the Mikrolabor at the Laboratorium für Organische Chemie, ETH Zürich. The nomenclature was generated with the computer program *ACD/Name* (*ACD/Labs*).

The $SF_5$-substituted aniline **12** has been synthesized as reported earlier.[11]

**General procedure A for the alkylation of *N*-methylpiperazine:** The chloride (1 equiv) and *N*-methyl-piperazine (20 equiv), dissolved in $CH_3CN$ (2-3 mL), were stirred overnight at 50 °C. Concentration in vacuo, redissolving of the obtained residue ($CH_2Cl_2$/*i*-PrOH 67:33), washing (saturated aqueous $NaHCO_3$ solution), extraction of the aqueous phases ($CH_2Cl_2$/*i*-PrOH 67:33), drying of the combined organic phases ($MgSO_4$), filtration, and concentration in vacuo followed by purification by column chro-matography ($SiO_2$, $CH_2Cl_2$/MeOH 97:3) delivered the desired piperazine derivative.

**General procedure B for the quaternization of amines using 3,4-dichlorobenzyl chloride:** The dimethylamine derivative (1 equiv) was suspended in $Et_2O$ (2 mL). Acetone was added, until a clear solution was obtained. Addition of 3,4-dichlorobenzyl chloride (1 equiv), stirring, filtration, followed by washing of the obtained residue (hexane), and drying in vacuo delivered the desired cation.

**General procedure C for the $S_N$Ar-reaction of anilines with 2,5-difluoronitrobenzene:** 2,5-Difluoronitrobenzene (1 equiv) was added to the aniline (1 - 1.2 equiv), dissolved in $Me_2SO$ (5-10 mL), and *t*-BuOK (1 - 1.2 equiv) was added. Stirring of the reaction mixture, followed by dilution (AcOEt), washing (saturated aqueous $NaHCO_3$ and NaCl solution), drying of the organic phases ($MgSO_4$), filtration, con-centration in vacuo, and purification of the obtained residue by column chromatography ($SiO_2$, hexane/ $CH_2Cl_2$ 85:15) delivered the desired diarylamine.

**General procedure D for the reduction of nitrobenzene derivatives and subsequent alkylation of the formed aniline:** The nitrobenzene derivative (1 equiv), Zn powder (20 equiv), and $NH_4Cl$ (20 equiv) were suspended in MeOH, and the mixture was stirred at 65 °C before it was filtered over Celite and concentrated in vacuo. The residue was redissolved in THF, 3-chloropropionyl chloride (1.1 equiv) was added, and the mixture was left to stir at 25 °C before the addition of $BH_3 \cdot THF$ (1 M solution in THF, 7 equiv). The mixture was stirred at 66 °C, cooled to 25 °C, quenched by addition of MeOH, and concentrated in vacuo. Purification by column chromatography ($SiO_2$, hexane/$CH_2Cl_2$ 90:10) delivered the desired chloride.

**General procedure E for the alkylation of dimethylamine:** The appropriate chloride (1 equiv) was dissolved in DMF, $HNMe_2$ (40 % solution in $H_2O$, 50 equiv) was added, and the mixture was stirred overnight at 90 °C. The mixture was diluted (saturated aqueous NaCl solution) and extracted ($CH_2Cl_2$). The combined organic phases were washed (saturated aqueous NaCl solution), dried ($MgSO_4$), filtered, and concentrated in vacuo. The desired dimethylamine was obtained after purification by column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH/$NEt_3$ 98:1:1).

**4-Fluoro-$N^1$-[4-(trifluoromethyl)phenyl]-$N^2$-[3-(4-methyl-piperazin-1-yl)propyl]benzene-1,2-diamine (1)**: General procedure A, starting from chloride **17** (12 mg, 35 µmol) and *N*-methylpiperazine (77 µL, 0.69 mmol) in $CH_3CN$ (1 mL), left to stir for 14 h at 70 °C, delivered amine **1** (13 mg, 92 %) after purification by column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH 99:1) as a pale red oil. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ = 1.76 (quint, *J* = 6.6, 2 H), 2.20 (s, 3 H), 2.35-2.38 (m, 10 H), 3.16 (t, *J* = 6.6, 2 H), 5.21 (br s, 1 H), 5.23 (s, 1 H), 6.30-6.44 (m, 2 H), 6.66 (d, *J* = 8.6, 2 H), 7.02 (dd, *J* = 8.7, 6.3, 1 H), 7.40 ppm (d, *J* = 8.6, 2 H); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ = 25.6, 42.8, 45.9, 53.4, 54.9, 56.9, 98.0 (d, *J* = 27.2), 102.5 (d, *J* = 23.1), 113.9, 120.6 (q, *J* = 33.0), 121.7 (d, *J* = 2.4), 126.7 (q, *J* = 3.8), 127.9 (d, *J* = 10.7), 146.9 (d, *J* = 11.6), 149.4, 162.7 ppm (d, *J* = 241.8) ($CF_3$ signal not visible); $^{19}F$ NMR (282 MHz, $CDCl_3$): $\delta$ = —112.1 - —112.2 (m, 1 F), –61.2 ppm (s, 3 F); IR (neat): $\tilde{n}$ = 2941, 2881, 2806, 1613, 1518, 1447, 1320, 1284, 1159, 1105, 1064, 999, 827, 610 $cm^{-1}$; HRMS (MALDI): calcd for $C_{21}H_{27}F_4N_4^+$ ($MH^+$): 411.2166, found: 411.2156.

**4-Fluoro-$N^1$-{4-(pentafluoro-?$^6$-sulfanyl)phenyl}-$N^2$-[3-(4-methylpiperazin-1-yl)propyl]benzene-1,2-diamine (2)**: General procedure A, starting from chloride **18** (6 mg, 15 µmol) and *N*-methylpiperazine (0.1 mL, 0.90 mmol) in $CH_3CN$ (2 mL), left to stir for 14 h at 60 °C, delivered amine **2** (6 mg, 86 %) after purification by column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH 99:1) as a pale red oil. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ = 1.76 (quint, *J* = 6.6, 2 H), 2.20 (s, 3 H), 2.24-2.40 (m, 10 H), 3.16 (t, *J* = 6.6, 2 H), 5.28 (br s, 2 H), 6.34 (dt, *J* = 8.4, 2.9, 1 H), 6.41 (dd, *J* = 11.1, 2.9, 1 H), 6.59 (d, *J* = 9.1, 2 H), 7.01 (dd, *J* = 8.6, 6.2, 1 H), 7.53 ppm (d, *J* = 9.1, 2 H); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ = 25.6, 42.8, 45.8, 53.3, 54.9, 56.9, 98.1 (d, *J* = 27.0), 102.6 (d, *J* = 23.0), 113.1, 121.2, 127.5 (sext, *J* = 4.5), 128.2 (sext, *J* = 10.6), 147.2 (sext, *J* = 31.1), 147.0 (d, *J* = 11.7), 148.9, 162.8 ppm (d, *J* = 242.9); $^{19}F$ NMR (376

MHz, $CDCl_3$): δ = —113.1 (br s, 1 F), 64.5 (d, *J* = 148.6, 4 F), 87.4 ppm (quint, *J* = 148.6, 1 F); IR (neat): $\tilde{n}$ = 3264, 2940, 2882, 2808, 1600, 1517, 1450, 1290, 1166, 1099, 1005, 818, 632 cm$^{-1}$; HRMS (MALDI): calcd for $C_{20}H_{27}F_6N_4S^+$ (*M*H$^+$): 469.1855, found: 469.1866.

**4-Fluoro-*N*$^{1}$-(4-*tert*-butylphenyl)-*N*$^{2}$-[3-(4-methylpiperazin-1-yl)propyl]benzene-1,2-diamine (3)**: General procedure A, starting from chloride **19** (18 mg, 54 µmol) and *N*-methylpiperazine (0.12 mL, 1.08 mmol) in $CH_3CN$ (1 mL), left to stir for 12 h at 70 °C, delivered amine **3** (20 mg, 93 %) after purification by column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH 99:1) as a pale red solid. Mp: 86 °C; $^{1}$H NMR (300 MHz, $CDCl_3$): δ = 1.27 (s, 9 H), 1.77 (quint, *J* = 6.6, 2 H), 2.18 (s, 3 H), 2.27-2.43 (m, 10 H), 3.16 (t, *J* = 6.6, 2 H), 4.81 (br s, 1 H), 5.32 (br s, 1 H), 6.28-6.41 (m, 2 H), 6.61 (d, *J* = 8.7, 2 H), 7.01 (dd, *J* = 8.4, 6.3, 1 H), 7.20 ppm (d, *J* = 8.7, 2 H); $^{13}$C NMR (75 MHz, $CDCl_3$): δ = 25.8, 31.6, 34.0, 42.7, 45.8, 53.3, 54.9, 56.9, 97.6 (d, *J* = 27.0), 102.2 (d, *J* = 22.7), 114.8, 124.1, 126.2 (d, *J* = 11.9), 126.4, 142.0, 143.8, 146.4 (d, *J* = 11.3), 161.9 ppm (d, *J* = 240.1); $^{19}$F NMR (282 MHz, $CDCl_3$): δ = —115.2 - —115.1 ppm (m, 1 F); IR (neat): $\tilde{n}$ = 3314, 2941, 2861, 2795, 1671, 1518, 1444, 1288, 1142, 1006, 824, 629 cm$^{-1}$; HRMS (MALDI): calcd for $C_{24}H_{35}FN_4^+$ (*M*H$^+$): 399.2919, found: 399.2913.

***N*-(3,4-Dichlorobenzyl)-3-[(5-fluoro-2-{[4-trifluoromethyl)-phenyl]amino}phenyl)amino]-*N,N*-dimethylpropan-1-ammonium chloride (4)**: General procedure B, starting from dimethylamine **20** (18 mg, 49 µmol) and 3,4-dichlorobenzyl chloride (0.10 mL, 0.72 mmol), left to stir for 16 h at 50 °C, delivered cation **4** (15 mg, 55 %) as a red solid. Mp: 61 °C; $^{1}$H NMR (300 MHz, $CDCl_3$): δ = 2.04-2.09 (m, 2 H), 2.97 (s, 6 H), 3.10-3.15 (m, 2 H), 4.04-4.09 (m, 2 H), 4.89 (s, 2 H), 5.13 (br s, 1 H), 6.19 (dd, *J* = 9.9, 2.7, 1 H), 6.36 (dt, *J* = 8.4, 2.7, 1 H), 7.00 (d, *J* = 8.4, 2 H), 7.10 (dd, *J* = 8.7, 6.3, 1 H), 7.30 (d, *J* = 8.4, 2 H), 7.38-7.42 (m, 2 H), 7.59 (s, 1 H), 7.72 ppm (s, 1 H); $^{13}$C NMR (75 MHz, $CDCl_3$): δ = 22.0, 39.8, 49.1, 63.8, 66.4, 97.9 (d, *J* = 26.8), 103.1 (d, *J* = 22.3), 113.4, 113.7, 119.3 (q, *J* = 32.5), 123.2, 126.3 (q, *J* = 3.6), 126.9 (d, *J* = 7.3), 131.4, 132.3, 133.8, 134.4, 136.1, 144.2 (d, *J* = 10.8), 150.0, 161.4 ppm (d, *J* = 241.8) ($CF_3$ signal not visible); $^{19}$F NMR (282 MHz, $CDCl_3$): δ = —60.9 (s, 3 F), —114.5 - —114.6 ppm (m, 1 F); IR (neat): $\tilde{n}$ = 3224, 2963, 1610, 1522, 1471, 1317, 1260, 1158, 1100, 1063, 1034, 824, 671 cm$^{-1}$; HRMS (MALDI): calcd for $C_{25}H_{26}Cl_2F_4N_3^+$ (*M*-Cl$^+$): 514.1434, found: 514.1443.

***N*-(3,4-Dichlorobenzyl)-3-[(5-fluoro-2-{[4-(pentafluoro-?$^{6}$-sulfanyl)phenyl]amino}phenyl)-amino]-*N,N*-dimethylpropan-1-ammonium chloride (5)**: General procedure B, starting from dimethylamine **21** (13 mg, 31 µmol) and 3,4-dichlorobenzyl chloride (80 µL, 0.58 mmol), left to stir for 16 h at 50 °C, delivered cation **5** (12 mg, 67 %) as a brown solid. Mp: 151 °C; $^{1}$H NMR (300 MHz, $CDCl_3$): δ = 2.08-2.20 (m, 2 H), 3.00 (s, 6 H), 3.14-3.19 (m, 2 H), 3.97-4.03 (m, 2 H), 4.90 (s, 2 H), 5.01 (s, 1 H), 6.28-6.33 (m, 1 H), 6.38 (dt, *J* = 8.3, 2.7, 1 H), 6.89 (d, *J* = 8.9, 2 H), 7.07 (dd, *J* = 8.6, 6.0, 1 H), 7.36-7.48 (m, 4 H),7.61 ppm (s, 1 H) ; $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 22.3, 40.4, 49.5, 63.8, 66.5, 98.7 (d, *J* = 27.0), 104.0 (d, *J* = 21.0), 113.1, 122.9, 126.9, 127.3 (sext, *J* = 4.0), 128.0 (d, *J* = 10.0), 131.6, 132.5, 134.0, 134.6, 136.3, 144.3 (sext, *J* = 13.2 ppm), 149.5, 160.7, 163.1 ppm; $^{19}$F NMR (376 MHz, $CDCl_3$): δ = —114.0 - —113.9 ppm (m, 1 F); 64.6 (d, *J* = 149.7, 4 F); 88.2 ppm (quint, *J* = 149.7, 1 F); IR (neat): $\tilde{n}$ = 3349, 3017, 2961, 1596, 1470, 1404, 1355, 1219, 1170, 1136, 1098, 1034, 820, 669, 644 cm$^{-1}$; HRMS (MALDI): calcd for $C_{24}H_{26}Cl_2F_6N_3S^+$ ([*M*-Cl]$^+$): 527.1123, found: 527.1114.

***N*-(3,4-Dichlorobenzyl)-3-({5-fluoro-2-[(4-*tert*-butyl-phenyl)amino]phenyl}amino)-*N,N*-dimethyl-propan-1-ammonium chloride (6)**: General procedure B, starting from dimethylamine **22** (18 mg, 52 µmol) and 3,4-dichlorobenzyl chloride (0.10 mL, 0.72 mmol), left to stir for 16 h at 50 °C, delivered cation **6** (17 mg, 60 %) as a red solid. Mp: 61 °C; $^{1}$H NMR (300 MHz, $CDCl_3$): δ = 1.22 (s, 9 H), 2.13-2.16 (m, 2 H), 3.02 (s, 6 H), 3.09-3.13 (m, 2 H), 3.96-4.00 (m, 2 H), 4.92-5.00 (m, 3 H), 6.25-6.35 (m, 2 H), 6.90 (d, *J* = 8.6, 2 H), 7.11 (d, *J* = 8.6, 2 H), 7.20-7.63 ppm (m, 5 H); $^{13}$C NMR (75 MHz, $CDCl_3$): δ = 22.3, 31.6, 33.9, 40.2, 44.6, 49.1, 63.6, 97.9 (d, *J* = 27.0), 102.8 (d, *J* = 22.3), 115.2, 123.9 (d, *J* = 9.7), 125.9, 127.3, 127.8, 130.7, 131.3, 132.6 (d, *J* = 7.1), 133.5, 135.7, 137.6, 144.2 (d, *J* = 10.5), 143.6, 160.3 ppm (d, *J* = 239.6); $^{19}$F NMR (282 MHz, $CDCl_3$): δ = —116.8 - —117.0 ppm (m, 1 F); IR (neat): $\tilde{n}$ = 3265, 2961, 1611, 1515, 1470, 1403, 1264, 1170, 1134, 1034, 906, 824, 727 $cm^{-1}$; HRMS (MALDI): calcd for $C_{28}H_{35}Cl_2FN_3^+$ ($M$-$Cl^+$): 502.2187, found: 502.2182.

***N*-[(4-Trifluoromethyl)phenyl]-4-chloro-2-nitroaniline (8)**: General procedure C, starting from 4-aminobenzotrifluoride (**7**, 0.08 mL, 0.62 mmol), 2,5-dichloronitrobenzene (0.14 g, 0.75 mmol), and *t*-BuOK (70 mg, 0.62 mmol) in $Me_2SO$ (5 mL), left to stir for 14 h, delivered diarylamine **8** (40 mg, 20 %) as a red solid. Mp: 93 °C; $^{1}$H NMR (300 MHz, $CDCl_3$): δ = 7.30-7.36 (m, 3 H), 7.40 (dd, *J* = 8.7, 2.7, 1 H), 7.66 (d, *J* = 8.4, 2 H), 8.23 (d, *J* = 2.7, 1 H), 9.42 (br s, 1 H); $^{13}$C NMR (75 MHz, $CDCl_3$): δ = 117.8, 121.0 (q, *J* = 280.2), 122.6, 123.4 (q, *J* = 36.0), 126.0, 127.0 (q, *J* = 3.7), 128.1, 134.3, 135.8, 139.7, 141.8; $^{19}$F NMR (282 MHz, $CDCl_3$): δ = —62.2 (s, 3 F); IR (neat): $\tilde{n}$ = 3342, 3094, 1604, 1567, 1484, 1322, 1248, 1174, 1148, 1097, 1061, 1012, 810; HRMS (EI): calcd for $C_{13}H_8ClF_3N_2O_2^+$ ($M^+$): 316.0231, found: 316.0234; anal.: calcd for $C_{13}H_8ClF_3N_2O_2$ C 49.31, H 2.55, N 8.85, found: C 49.08, H 2.74, N 8.67.

The same reaction delivered **4-Chloro-*N*-(4-chloro-2-nitrophenyl)-2-nitro-*N*-[4-(trifluoromethyl)-phenyl]aniline** (**9**, 25 mg, 9 %) as a dark red solid. Mp: 141 °C; $^{1}$H NMR (300 MHz, $CDCl_3$): δ = 6.82 (d, *J* = 8.4, 2 H), 7.35 (d, *J* = 8.7, 2 H), 7.44 (d, *J* = 8.4, 2 H), 7.58 (dd, *J* = 8.7, 2.4, 2 H), 7.86 (d, *J* = 2.4, 2 H); $^{13}$C NMR (75 MHz, $CDCl_3$): δ = 119.8, 126.0, 126.9 (q, *J* = 3.7), 131.3, 132.1, 132.9 (q, *J* = 36.0), 134.4, 136.5, 145.3, 147.4 (one signal not visible); $^{19}$F NMR (282 MHz, $CDCl_3$): δ = —62.2 (*s*, 3 F); IR (neat): $\tilde{n}$ = 3094, 2926, 2858, 1532, 1474, 1321, 1257, 1160, 1117, 1066, 881, 827, 767; HRMS (EI): calcd for $C_{19}H_{10}Cl_2F_3N_3O_4^+$ ($M^+$): 470.9995, found: 470.9998.

***N*-[4-(Trifluoromethyl)phenyl]-4-fluoro-2-nitroaniline (10)**: General procedure C, starting from 4-aminobenzotrifluoride (**7**, 0.10 mL, 0.80 mmol), 2,5-difluoronitrobenzene (0.13 g, 0.80 mmol), and *t*-BuOK (90 mg, 0.80 mmol) in $Me_2SO$ (6 mL), left to stir for 14 h, delivered diarylamine **10** (0.086 g, 35 %) as a red solid. Mp: 85 °C; $^{1}$H NMR (300 MHz, $CDCl_3$): δ = 7.22-7.28 (m, 1 H), 7.33 (d, *J* = 8.4, 2 H), 7.36-7.41 (m, 1 H), 7.65 (d, *J* = 8.4, 2 H), 7.93 (dd, *J* = 8.7, 2.7, 1 H), 9.28 ppm (br s, 1 H); $^{13}$C NMR (75 MHz, $CDCl_3$): δ = 112.4 (d, *J* = 26.3), 118.3 (d, *J* = 7.3), 121.9, 123.9 (q, *J* = 271.3), 124.0 (d, *J* = 23.8), 126.4 (q, *J* = 33.0), 126.9 (d, *J* = 4.3), 134.0 (d, *J* = 7.9), 137.6, 142.3, 154.4 ppm (d, *J* = 242.3); $^{19}$F NMR (282 MHz, $CDCl_3$): δ = —121.6 - —121.7 (m, 1 F), —62.2 ppm (s, 3 F); IR (neat): $\tilde{n}$ = 3345, 3087, 2966, 1584, 1504, 1420, 1325, 1244, 1168, 1131, 1095, 1066, 1012, 946, 873, 815, 793, 756, 726 $cm^{-1}$; HRMS (EI): calcd for $C_{13}H_8F_4N_2O_2^+$ ($M^+$): 300.0516, found: 300.0516.

**4-Fluoro-2-nitro-*N*-[4-(pentafluoro-?$^6$-sulfanyl)phenyl]-aniline (13)**: General procedure C, starting from aniline **12** (50 mg, 0.23 mmol), 2,5-difluoronitrobenzene (36 mg, 0.23 mmol), and *t*-BuOK (26 mg, 0.23 mmol) in $Me_2SO$ (1 mL), left to stir for 3 h, delivered diarylamine **13** (20 mg, 25 %) as a red solid. Mp: 91 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 7.24-7.30 (m, 3 H), 7.42 (dd, $J$ = 9.0, 4.8, 1 H), 7.34-7.78 (m, 2 H), 7.94 (dd, $J$ = 8.7, 3.0, 1 H), 9.23 ppm (br s, 1 H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 112.7 (d, $J$ = 26.7), 118.7 (d, $J$ = 7.2), 121.1, 124.1 (d, $J$ = 23.4), 127.8 (sext, $J$ = 4.5), 134.8 (d, $J$ = 8.8), 137.1, 142.3, 149.5 (sext, $J$ = 20.3), 154.9 ppm (d, $J$ = 243.7); $^{19}$F NMR (376 MHz, $CDCl_3$): $\delta$ = —112.4 - 112.3 ppm (m, 1 F); 64.4 (d, $J$ = 150.5, 4 F); 87.2 ppm (quint, $J$ = 150.5, 1 F); IR (neat): $\tilde{n}$ = 3734, 3361, 2964, 2922, 1583, 1510, 1350, 1243, 1092, 819, 649 cm$^{-1}$; HRMS (EI): calcd for $C_{12}H_8F_6N_2O_2S^+$ ($M^+$): 358.0205, found: 358.0204.

The same reaction delivered **4-fluoro-*N*-(4-fluoro-2-nitrophenyl)-nitro-*N*-[4-(pentafluoro-?$^6$-sulfanyl)phenyl]-aniline** (**14,** 18 mg, 16 %) as a brown solid. Mp: 186 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 6.70 (d, $J$ = 9.2, 2 H), 7.38 (dd, $J$ = 7.2, 2.9, 2 H), 7.47 (dd, $J$ = 9.0, 5.1, 2 H), 7.52-7.54 (m, 2 H), 7.62 ppm (dd, $J$ = 7.5, 3.0, 2 H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 113.5 (d, $J$ = 27.6), 118.0, 121.9 (d, $J$ = 22.6), 127.5 (sext, $J$ = 4.2), 132.4 (d, $J$ = 8.6), 133.8, 145.8 (sext, $J$ = 11.6), 147.5, 159.5 ppm (d, $J$ = 253.0) (one signal not visible); $^{19}$F NMR (376 MHz, $CDCl_3$): $\delta$ = —110.0 - —109.9 ppm (m, 1 F); 63.7 (d, $J$ = 148.6, 4 F); 85.0 ppm (quint, $J$ = 148.6, 1 F); IR (neat): $\tilde{n}$ = 3088, 1533, 1441, 1342, 1213, 1108, 828 cm$^{-1}$; HRMS (EI): calcd for $C_{18}H_{10}F_7N_3O_4S^+$ ($M^+$): 497.0275, found: 497.0275.

***N*-(4-*tert*-Butylphenyl)-4-fluoro-2-nitroaniline (16)**: General procedure C, starting from 4-*tert*-butylaniline (**15**, 0.14 mL, 1.26 mmol), 2,5-difluoronitrobenzene (0.23 g, 1.51 mmol), and *t*-BuOK (0.17 g, 1.51 mmol) in $Me_2SO$ (5 mL), left to stir for 6 h at 50 °C, delivered diarylamine **16** (0.14 g, 38 %) as a red solid. Mp: 72 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.35 (s, 9 H), 7.15-7.45 (m, 6 H), 7.90 (dd, $J$ = 9.0, 2.7, 1 H), 9.34 ppm (br s, 1 H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 31.4, 34.6, 111.7 (d, $J$ = 26.3), 117.6 (d, $J$ = 6.7), 124.0, 124.3 (d, $J$ = 23.9), 126.6, 131.7, 135.7, 140.4, 148.9, 153.3 ppm (d, $J$ = 240.2); $^{19}$F NMR (282 MHz, $CDCl_3$): $\delta$ = —124.7 - —124.9 ppm (m, 1 F); IR (neat): $\tilde{n}$ = 3355, 2960, 2869, 1581, 1507, 1343, 1264, 1232, 1131, 946, 816, 616 cm$^{-1}$; HRMS (EI): calcd for $C_{16}H_{17}FN_2O_2^+$ ($M^+$): 288.1269, found: 288.1269.

***N*$^2$-(3-Chloropropyl)-4-fluoro-*N*$^1$-[4-(trifluoromethyl)phenyl]-benzene-1,2-diamine (17)**: General procedure D, starting from nitrobenzene **10** (0.14 g, 0.46 mmol), $NH_4Cl$ (0.49 g, 9.13 mmol), Zn (0.60 g, 9.13 mmol), 3-chloropropionyl chloride (52 µL, 0.55 mmol), $BH_3 \cdot THF$ (1 M in THF, 3.65 mL, 3.65 mmol), left to stir for 5 h for the first, 1 h for the second, and 4 h under reflux for the third step, yielded chloride **17** (87 mg, 55 %) as a yellow oil. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 2.04 (quint, $J$ = 6.4, 2 H), 3.32 (t, $J$ = 6.4, 2 H), 3.57 (t, $J$ = 6.4, 2 H), 4.40 (br s, 1 H), 5.21 (br s, 1 H), 6.35-6.47 (m, 2 H), 6.63 (d, $J$ = 8.6, 2 H), 7.00-7.05 (m, 1 H), 7.41 ppm (d, $J$ = 8.6, 2 H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 32.0, 40.5, 42.3, 98.1 (d, $J$ = 27.3), 103.2 (d, $J$ = 22.8), 113.6, 120.8 (q, $J$ = 32.7), 121.5 (d, $J$ = 2.4), 124.8 (q, $J$ = 279.4), 126.8 (q, $J$ = 3.7), 128.9 (d, $J$ = 10.5), 146.6 (d, $J$ = 11.6), 149.3, 162.9 ppm (d, $J$ = 243.2); $^{19}$F NMR (282 MHz, $CDCl_3$): $\delta$ = —112.4 - —112.3 (m, 1 F), —61.2 ppm (s, 3 F); IR (neat): $\tilde{n}$ = 3400, 2922, 2857, 1613, 1520, 1447, 1319, 1163, 1107, 1064, 827, 647 cm$^{-1}$; HRMS (EI): calcd for $C_{16}H_{15}ClF_4N_2^+$ ($MH^+$): 346.0854, found: 346.0854.

***N*$^2$-(3-Chloropropyl)-4-fluoro-*N*$^1$-[4-(pentafluoro-?$^6$-sulfanyl)phenyl]benzene-1,2-diamine (18)**: General procedure D, starting from nitrobenzene **13** (60 mg, 0.17 mmol), NH$_4$Cl (0.18 g, 3.35 mmol), Zn (0.22 g, 3.35 mmol), 3-chloropropionyl chloride (19 µL, 0.20 mmol), BH$_3$·THF (1 M in THF, 1.34 mL, 1.34 mmol), left to stir for 1 h for the first, 1 h for the second, and 16 h at 25 °C for the third step, yielded chloride **18** (0.50 g, 74 %) as a yellow oil. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 2.04 (quint, $J$ = 6.0, 2 H), 3.33 (q, $J$ = 6.3, 2 H), 3.56-3.60 (m, 2 H), 4.37 (br s, 1 H), 5.25 (s, 1 H), 6.37 (dt, $J$ = 8.1, 2.7, 1 H), 6.45 (dd, $J$ = 10.8, 2.6, 1 H), 6.56 (s, $J$ = 9.2, 2 H), 7.02 (ddd, $J$ = 8.4, 6.0, 0.8, 1 H), 7.54 ppm (d, $J$ = 9.2, 2 H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ = 31.7, 40.6, 42.3, 98.1 (d, $J$ = 27.3), 103.3 (d, $J$ = 22.8), 112.8, 121.0, 127.5 (sext, $J$ = 4.5), 129.0 (sext, $J$ = 10.6), 145.1 (sext, $J$ = 19.1), 146.6 (d, $J$ = 1.5), 148.8, 163.0 ppm (d, $J$ = 243.6); $^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ = —112.4 - —112.3 ppm (m, 1 F); 64.4 (d, $J$ = 150.5, 4 F); 87.2 ppm (quint, $J$ = 150.5, 1 F); IR (neat): $\tilde{n}$ = 3403, 2923, 1601, 1517, 1301, 1171, 1100, 1005, 816, 633 cm$^{-1}$; HRMS (EI): calcd for $C_{15}H_{15}ClF_6N_2S^+$ ($M^+$): 404.0543, found: 404.0544.

***N*$^2$-(3-Chloropropyl)-4-fluoro-*N*$^1$-(4-*tert*-butylphenyl)benzene-1,2-diamine (19)**: General procedure D, starting from nitrobenzene **16** (0.12 g, 0.43 mmol), NH$_4$Cl (0.46 g, 8.53 mmol), Zn (0.56 g, 8.53 mmol), 3-chloropropionyl chloride (49 µL, 0.51 mmol), BH$_3$·THF (1 M in THF, 3.41 mL, 3.41 mmol), left to stir for 2 h for the first, 1.5 h for the second, and 4 h under reflux for the third step, yielded chloride **19** (89 mg, 62 %) as a yellow oil. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 1.29 (s, 9 H), 2.03 (quint, $J$ = 6.3, 2 H), 3.31 (br s, 2 H), 3.56 (t, $J$ = 6.3, 2 H), 4.50 (br s, 1 H), 4.85 (br s, 1 H), 6.35-6.45 (m, 2 H), 6.58 (d, $J$ = 8.3, 2 H), 7.00-7.04 (m, 1 H), 7.22 ppm (d, $J$ = 8.3, 2 H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ = 31.5, 31.8, 34.0, 40.5, 42.4, 97.8 (d, $J$ = 27.4), 102.8 (d, $J$ = 22.6), 114.4, 123.8, 126.1, 127.6 (d, $J$ = 10.3), 142.2, 143.8, 146.2 (d, $J$ = 11.3), 162.2 ppm (d, $J$ = 241.4); $^{19}$F NMR (282 MHz, CDCl$_3$): $\delta$ = —114.2 ppm (s, 1 F); IR (neat): $\tilde{n}$ = 3383, 2958, 2867, 1612, 1515, 1444, 1264, 1167, 1111, 1010, 823, 790, 615 cm$^{-1}$; HRMS (EI): calcd for $C_{19}H_{24}ClFN_2^+$ ($MH^+$): 334.1607, found: 334.1604.

***N*-[4-(Trifluoromethyl)phenyl]-*N*$^2$-[3-(dimethylamino)propyl]-4-fluorobenzene-1,2-diamine (20)**: General procedure E, starting from chloride **17** (74 mg, 0.21 mmol) in DMF (2 mL) and HNMe$_2$ (40 % solution in H$_2$O, 0.5 mL), left to stir for 14 h at 60 °C, delivered dimethylamine **20** (69 mg, 91 %) as a pale brown solid. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 1.76 (quint, $J$ = 6.3, 2 H), 2.05 (s, 6 H), 2.30 (t, $J$ = 6.3, 2 H), 3.15 (t, $J$ = 6.5, 2 H), 5.31 (br s, 2 H), 6.23-6.41 (m, 2 H), 6.61 (d, $J$ = 8.4, 2 H), 7.00 (dd, $J$ = 8.4, 6.2, 1 H), 7.39 ppm (d, $J$ = 8.4, 2 H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ = 26.1, 43.0, 45.2, 58.4, 97.8 (d, $J$ = 27.0), 102.2 (d, $J$ = 22.9), 113.2, 120.2 (q, $J$ = 32.6), 121.1 (d, $J$ = 2.3), 126.6 (q, $J$ = 3.7), 128.6 (d, $J$ = 10.6), 146.3 (d, $J$ = 11.6), 149.5, 162.9 ppm (d, $J$ = 42.5) (CF$_3$ signal not visible); $^{19}$F NMR (282 MHz, CDCl$_3$): $\delta$ = —113.0 - —112.9 (m, 1 F), —61.1 ppm (s, 3 F); IR (neat): $\tilde{n}$ = 3229, 2928, 2803, 1614, 1522, 1319, 1163, 1108, 826, 631 cm$^{-1}$; HRMS (MALDI): calcd for $C_{18}H_{22}F_4N_3^+$ ($MH^+$): 356.1744, found: 356.1739.

***N*$^2$-[3-(Dimethylamino)propyl]-4-fluoro-*N*$^1$-[4-(pentafluoro-?$^6$-sulfanyl)phenyl]benzene-1,2-diamine (21)**: General procedure E, starting from chloride **18** (40 mg, 99 µmol) in DMF (2 mL) and HNMe$_2$ (40 % solution in H$_2$O, 0.5 mL), left to stir for 16 h at 60 °C, delivered dimethylamine **21** (37 mg, 91 %) as a pale red. Mp: 89 °C; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 1.83 (quint, $J$ = 6.4, 2 H), 2.23 (s, 6 H), 2.53 (t, $J$ = 6.4, 2 H), 3.18 (t, $J$ = 6.4, 2 H), 5.01 (br s, 1 H), 5.74 (s, 1 H), 6.31-6.38 (m, 2 H), 6.61 (d, $J$ = 9.0, 2 H), 7.02 (ddd, $J$ = 9.0, 6.4, 0.9, 1 H), 7.47-7.53 ppm (m, 2 H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 25.3, 42.0, 44.5, 57.5, 97.7 (d, $J$ = 26.9), 102.4 (d, $J$ = 22.7), 112.4, 120.9, 127.1 (sext, $J$ = 4.9), 128.4 (sext, $J$ = 11.0), 144.2 (sext, $J$ = 18.9), 146.3 (d, $J$ = 11.6), 148.8, 162.5 ppm (d, $J$ = 242.6); $^{19}$F NMR (376 MHz, $CDCl_3$): $\delta$ = —113.4 ppm (br s, 1 F), 64.6 (d, $J$ = 150.5, 4 F), 87.7 (quint, $J$ = 150.5, 1 F); IR (neat): $\tilde{n}$ = 3348, 3002, 2924, 1724, 1597, 1498, 1421, 1353, 1259, 1172, 1097, 1021, 819, 653 $cm^{-1}$; HRMS (MALDI): calcd for ($MH^+$) $C_{17}H_{22}F_6N_3S^+$: 414.1433, found: 414.1425.

***N*-(4-*tert*-Butylphenyl)-*N*$^2$-[3-(dimethylamino)propyl]-4-fluorobenzene-1,2-diamine (22)**: General procedure E, starting from chloride **19** (74 mg, 0.22 mmol) in DMF (2 mL) and $HNMe_2$ (40 % solution in $H_2O$, 0.5 mL), left to stir for 14 h at 60 °C, delivered dimethylamine **22** (70 mg, 92 %) as a pale brown. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.28 (s, 9 H), 1.73 (quint, $J$ = 6.7, 2 H), 2.10 (s, 6 H), 2.30 (t, $J$ = 6.7, 2 H), 3.15 (t, $J$ = 6.7, 2 H), 4.89 (s, 1 H), 5.09 (br s, 1 H), 6.30 (dt, $J$ = 8.4, 2.7, 1 H), 6.38 (dd, $J$ = 11.1, 2.9, 1 H), 6.58 (dd, $J$ = 6.6, 2.3, 2 H), 7.00 (ddd, $J$ = 8.4, 6.3, 0.6, 1 H), 7.20 ppm (dd, $J$ = 6.6, 2.3, 2 H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 26.6, 31.6, 34.0, 42.5, 45.3, 57.9, 97.6 (d, $J$ = 27.0), 102.1 (d, $J$ = 22.6), 114.2, 123.5 (d, $J$ = 2.3), 126.0, 127.2 (d, $J$ = 10.4), 141.7, 143.9, 146.7 (d, $J$ = 11.5), 162.2 ppm (d, $J$ = 240.9); $^{19}$F NMR (282 MHz, $CDCl_3$): $\delta$ = —114.9 - —114.8 ppm (m, 1 F); IR (neat): $\tilde{n}$ = 3380, 2953, 2864, 2776, 1612, 1516, 1456, 1290, 1166, 1116, 993, 822, 732 $cm^{-1}$; HRMS (MALDI): calcd for ($M$-$Cl^+$) $C_{21}H_{30}FN_3^+$: 344.2497, found: 344.2496.

## c) Enzymatic assays.

**Materials**. Trypanothione disulfide ($TS_2$) was purchased from *Bachem*. Recombinant *T. cruzi* trypanothione reductase (TR) was prepared following published procedures.[12] NADPH was purchased from *Biomol*.

**Trypanothione reductase assay**. TR activity was measured at 25 °C in a total volume of 1 mL in the presence of 100 μM NADPH and 5-10 mU enzyme in TR assay buffer (40 mM HEPES, 1 mM EDTA, pH 7.5) containing 5 % $Me_2SO$. The reaction was started by adding $TS_2$; NADPH consumption was followed spectrophotometrically at 340 nm. $V_{max}$ was calculated using a $K_m$ value of 18 μM for $TS_2$.[13]

## d) Parasitology: in vitro bioassays, $IC_{50}$ determination.

***Trypanosoma b. rhodesiense***. Minimum Essential Medium (50 µL) supplemented according to Baltz et al.[14] with 2-mercaptoethanol and 15 % heat-inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions were prepared covering a range from 90 to 0.123 µg $mL^{-1}$. Then, $10^4$ bloodstream forms of *T. b. rhodesiense* STIB 900 in 50 µL were added to each well and the plate was incubated at 37 °C under a 5 % $CO_2$ atmosphere for 72 h. 10 µL of Alamar Blue (12.5 mg resazurin dissolved in 100 mL distilled water) was added to each well and the incubation was continued for another 2-4 h. The plate was analysed in a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and emission wavelength of 588 nm.[15] Fluorescence development was measured and expressed as percentage of the control. Data were transferred into the graphic programme Softmax Pro (Molecular Devices) which calculated $IC_{50}$ values.

***Plasmodium falciparum***. Antiplasmodial activity was determined using the K1 strain of *P. falciparum* (resistant to chloroquine and pyrimethamine). A modification of the [$^3$H]-hypoxanthine incorporation assay was used.[16] Briefly, infected human red blood cells in RPMI 1640 medium with 5 % Albumax were exposed to serial drug dilutions in microtiter plates. After 48 h of incubation at 37 °C in a reduced oxygen atmosphere, 0.5 µCi $^3$H-hypoxanthine was added to each well. Cultures were incubated for a further 24 h before they were harvested onto glass-fiber filters and washed with distilled water. The radioactivity was counted using a Betaplate$^{TM}$ liquid scintillation counter (Wallac, Zurich, Switzerland). The results were recorded as counts per minute (CPM) per well at each drug concentration and expressed as percentage of the untreated controls. From the sigmoidal inhibition curves, $IC_{50}$ values were calculated. Assays were run in duplicate and repeated once.

***Trypanosoma cruzi.*** Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates at 2000 cells/well in 100 µL RPMI 1640 medium with 10% FBS and 2 mM L-glutamine. After 24 h, the medium was removed and replaced by 100 µL per well containing 5000 trypomastigote forms of *T. cruzi* Tulahuen strain C2C4 containing the β-galactosidase (Lac Z) gene. After 48 h, the medium was removed from the wells and replaced by 100 µL fresh medium with or without a serial drug dilution. Seven threefold dilutions were used covering a range from 90 µg/mL to 0.123 µg/mL. Each drug was tested in duplicate. After 96 h of incubation, the plates were inspected under an inverted microscope to assure growth of the controls and sterility. Then, the substrate CPRG/ Nonidet (50 µL) was added to all wells. A color reaction developed within 2-6 h and could be read photometrically at 540 nm. Data were transferred into the graphic programme Softmax Pro (Molecular Devices) which calculated $IC_{50}$ values.

***Leishmania donovani* (axenic assay).** Culture medium (50 µL), a 1:1 mixture of SM medium[17] and SDM-79 medium[18] at pH 5.4 supplemented with 10 % heat-inactivated FBS, was added to each well of a 96-well microtiter plate (Costar, USA). Serial drug dilutions in duplicates were prepared covering a range from 30 to 0.041 µg/mL. Then, $10^5$ axenically grown *Leishmania donovani* amastigotes (strain MHOM/ET/67/L82) in 50 µL medium were added to each well and the plate incubated at 37 °C under a 5 % $CO_2$ atmosphere for 72 h. A total of 10 µL of resazurin solution (12.5 mg resazurin dissolved in 100 mL distilled water) were then added to each well and incubation continued for a further 2-4 h. The plate was then read in a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and emission wavelength of 588 nm[15]. Fluorescence development was measured and expressed as percentage of the control. Data were transferred into the graphic programme Softmax Pro (Molecular Devices) which calculated $IC_{50}$ values.

**Cytotoxicity L-6 cells.** The rat skeletal myoblast cell line (L-6 cells) was used to assess cytotoxicity. The cells were grown in RPMI 1640 medium supplemented with 1 % *L*-glutamine (200 nM) and 10 % fetal bovine serum at 37 °C in 5 % $CO_2$ in air. Assays were performed in 96-well microtiter plates, each well receiving 100 µL of culture medium with $4 \cdot 10^4$ cells. After 24 h, the medium was removed from all wells and serial drug dilutions were prepared covering a range from 90 to 0.123 µg $mL^{-1}$.

Each drug was tested in duplicate. After 72 h of incubation, the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. Then, 10 µL of Alamar blue (12.5 mg resazurin dissolved in 100 mL distilled water) was added to each well and the plates were incubated for another 2 h. The plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. $EC_{50}$ values were determined using the microplate reader software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA).

## e) Pharmacokinetics.

**Determination of passive membrane permeability.** The membrane permeability was determined using a parallel artificial membrane permeation assay (PAMPA) as described recently.[19]

**Determination of logD values.** The logarithmic distribution coefficient logD was determined by a high-throughput (HT) screening method as previously described.[20]

**Determination of pKa values.** The determination of pKa values was performed by potentiometric titration following a protocol previously described.[20]

## 3. Literature

[1] Y. Song, C.-A. Di, W. Xu, Y. Liu, D. Zhang, D. Zhu, *J. Mater. Chem.* **2007**, *17*, 4483-4491.

[2] J. E. Field, D. Venkataraman, *Chem. Commun.* **2002**, 306-307.

[3] A. Ito, H. Ino, K. Tanaka, K. Kanemoto, T. Kato*, J. Org. Chem.* **2002**, *67*, 491-498.

[4] R. D. Bowden, P. J. Comina, M. P. Greenhall, B. M. Kariuki, A. Loveday, D. Philp, *Tetrahedron* **2000**, *56*, 3399-3408.

[5] E. L. Nava, A. Jesih, E. Goreshnik, *Acta Crystallogr., Sect. E: - Struct. Rep. Online* **2008**, *64*, o416.

[6] C. Zarantonello, A. Guerrato, E. Ugel, R. Bertani, F. Benetollo, R. Milani, A. Venzo, A. Zaggia, *J. Fluorine Chem.* **2007**, *128*, 1449-1453.

[7] W. A. Sheppard, *J. Am. Chem. Soc.* **1962**, *84*, 3072-3076.

[8] A. Altomare, M. C. Burla, M. Camalli, G. L. Cascarano, C. Giacovazzo, A. Guagliardi, A. G. Polodori, R. Spagna, *J. Appl. Cryst.* **1999**, *32*, 115-119.

[9] G. M. Sheldrick, Program for the Refinement of Crystal Structures. University of Göttingen, Germany, **1997**.

[10] P. R. Gerber, K. Müller, *J. Comput. Aid. Mol. Des.* **1995**, *9*, 251-268.

[11] P. J. Crowley, G. Mitchell, R. Salmon, P. A. Worthington, *Chimia* **2004**, *58*, 138-142.

[12] F. X. Sullivan, C. T. Walsh, *Mol. Biochem. Parasit.* **1991**, *44*, 145-147.

[13] M. C. Jockers-Scherübl, R. H. Schirmer, R. L. Krauth-Siegel, *Eur. J. Biochem.* **1989**, *180*, 267-272.

[14] T. Baltz, D. Baltz, C. Giroud, J. Crockett, *EMBO J.* **1985**, *4*, 1273-1277.

[15] B. Räz, M. Iten, Y. Grether-Bühler, R. Kaminsky, R. Brun, *Acta Trop.* **1997**, *68*, 139-147.

[16] H. Matile, J. R. L. Pink, in *Immunological Methods* (Eds.: I. Lefkovits, B. Pernis), Academic Press, San Diego, **1990**, pp. 221-234.

[17] I. Cunningham, *J. Protozool.* **1977**, *24*, 325-329.

[18] R. Brun, M. Schonenberger, *Acta Trop.* **1979**, *36*, 289-292.

[19] H. Fischer, M. Kansy, A. Avdeef, F. Senner, *Eur. J. Pharm. Sci.* **2007**, *31*, 32-42.

[20] J. Olsen, P. Seiler, B. Wagner, H. Fischer, T. Tschopp, U. Obst-Sander, D. W. Banner, M. Kansy, K. Müller, F. Diederich, *Org. Biomol. Chem.* **2004**, *2*, 1339– 1352.