Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Supplementary Information

Differently fluorescence-labeled dibenzodiazepinone-type muscarinic acetylcholine receptor ligands with high M_2R affinity

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1. Figures S1-S4

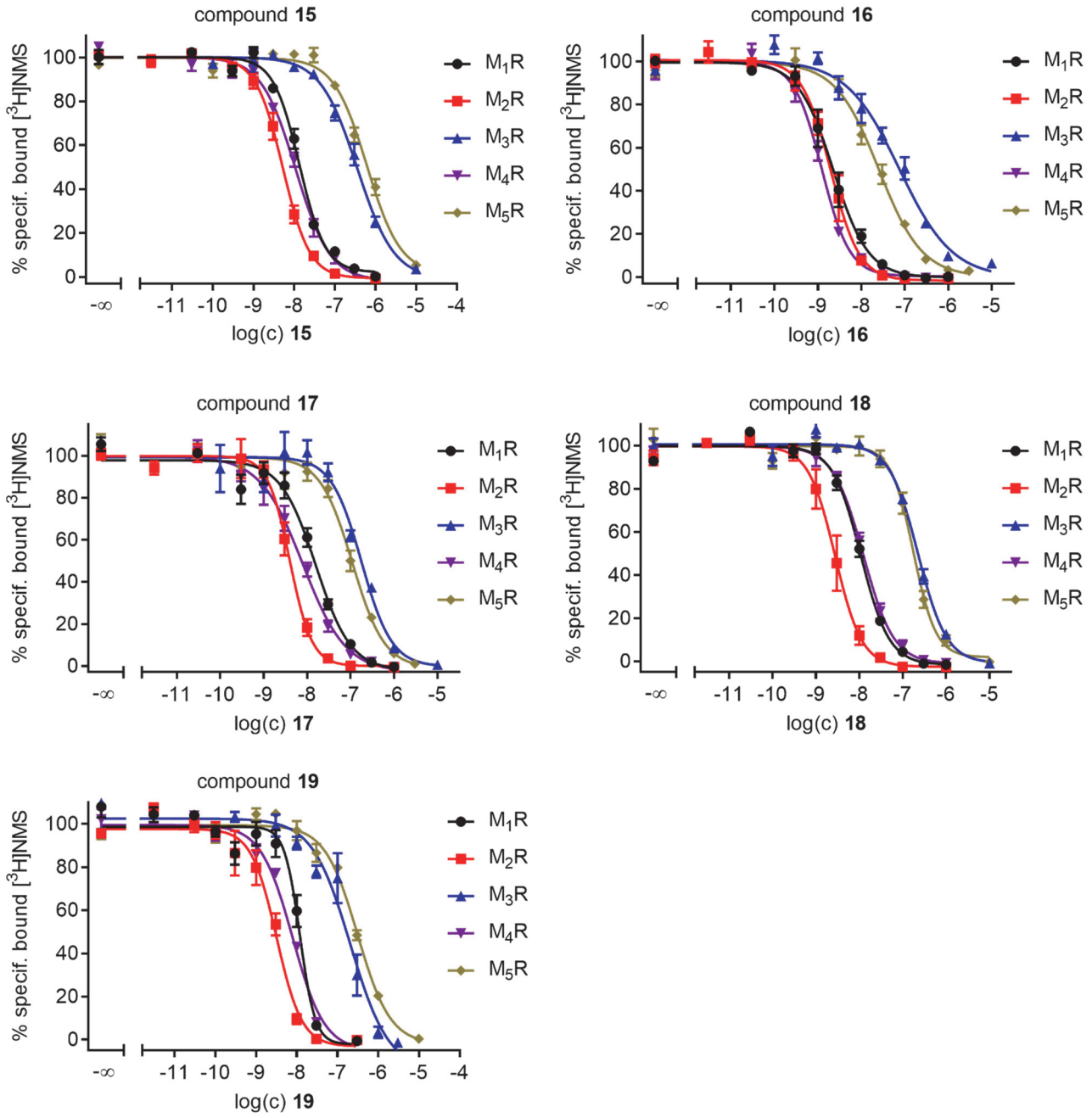


Figure S1. Radioligand displacement curves obtained from competition binding experiments with [^{3}H]NMS (0.2 nM (M_1R, M_2R, M_3R), 0.1 nM (M_4R) or 0.3 nM (M_5R)) and **15-19** at intact CHO-hM_xR cells (x = 1-5). Data represent are mean values ± SEM from at least three independent experiments (each performed in triplicate).

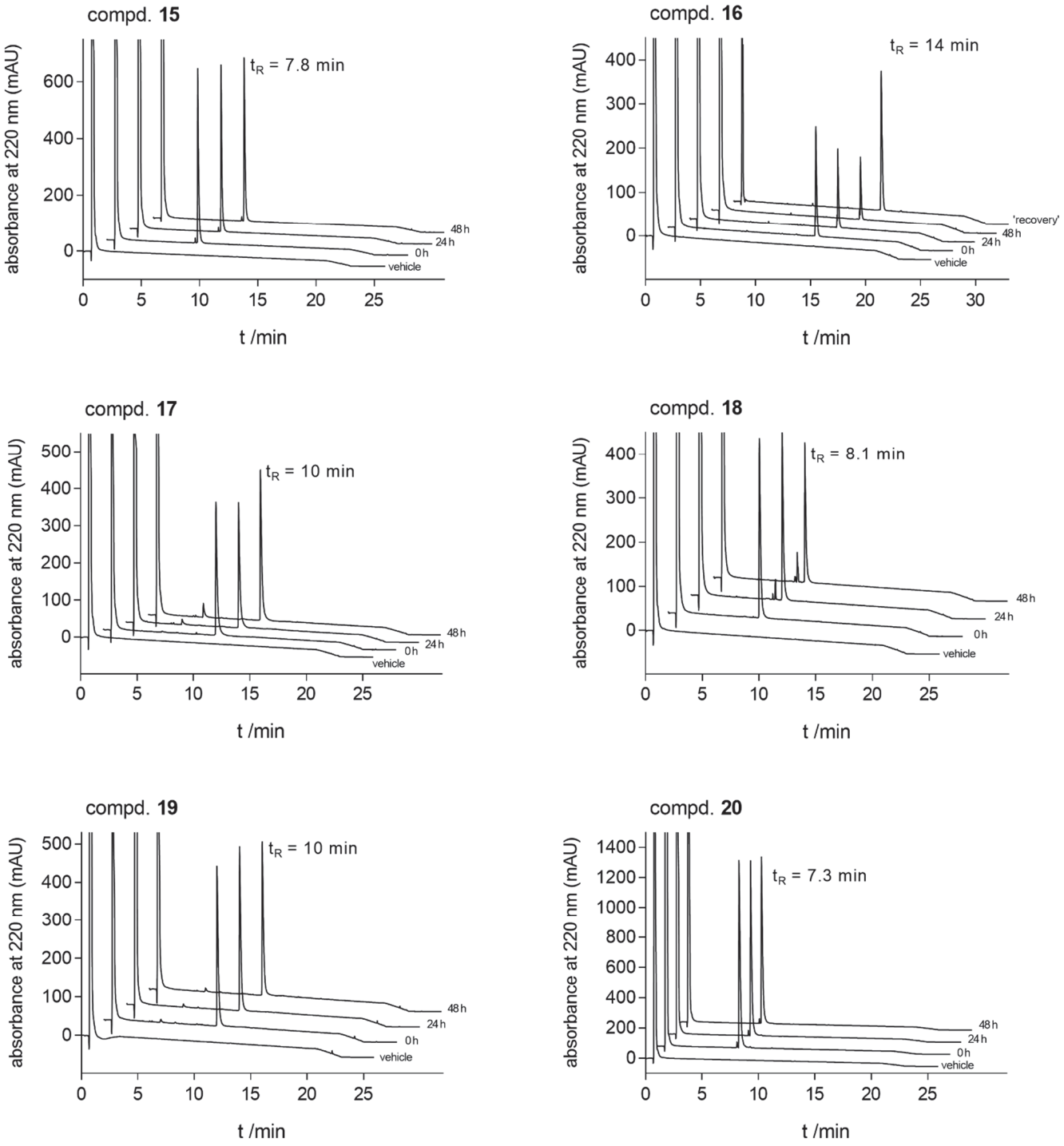


Figure S2. Chromatograms of the HPLC analysis (method see general experimental conditions) of compounds **15**-**20** after incubation in PBS (pH 7.4) at 22 °C for up to 48 h. Whereas compounds **15**, **16**, **19** and **20** showed no decomposition, compounds **17** and **18** showed minor decomposition after 24 h. Compound **16** showed high adsorption to the surface of the polypropylene vessel immediately upon sample preparation. After 48 h, adsorbed **16** was desorbed by replacement of the residual original solution (PBS, pH 7.4) with 0.1% aq TFA/acetonitrile (1:1 v/v, 60 µL) (‘recovery’). For injection, this solution was 1:1 diluted with water.

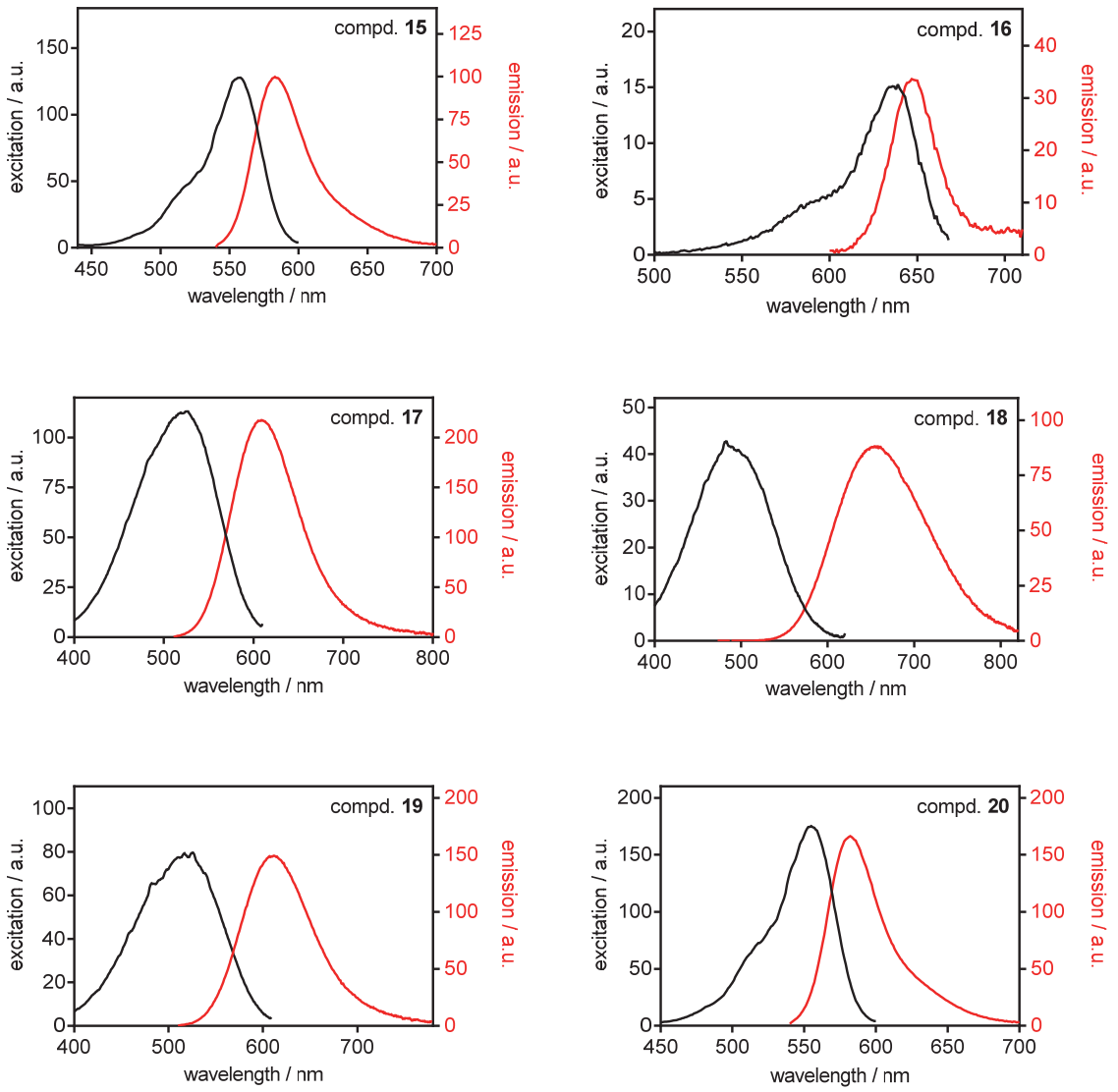
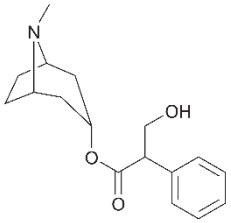


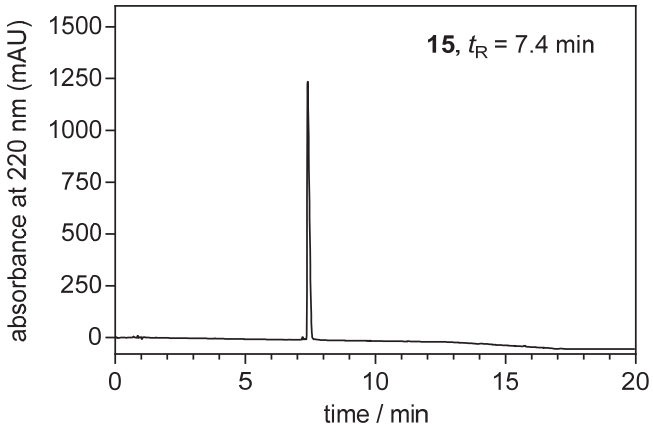
Figure S3. Excitation and corrected emission spectra of fluorescent ligands **15-20** recorded in PBS, pH 7.4, containing 1% BSA, at 22 °C using fluorescent ligand concentrations of 3 µM (**15**, **16**, **20**) or 5 µM (**17**, **18**, **19**).



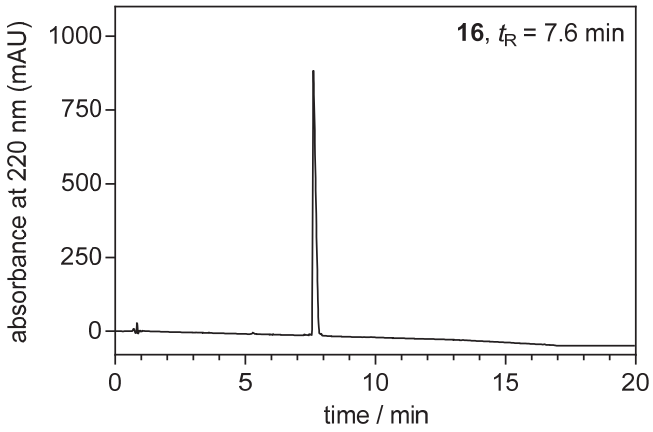
21 (atropine)

Figure S4. Structure of the MR antagonist atropine (**21**).

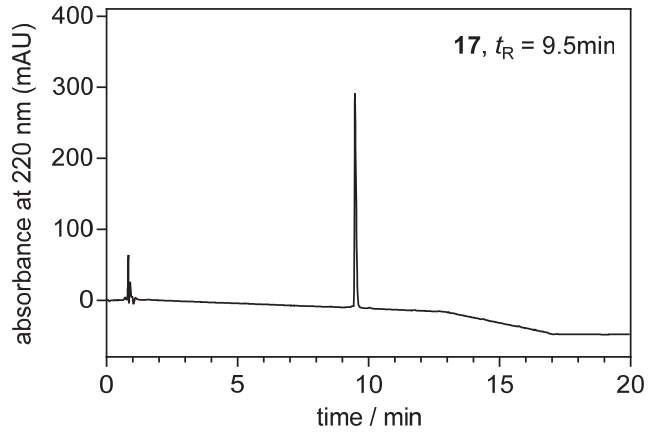
2. RP-HPLC chromatograms of compounds 15-20



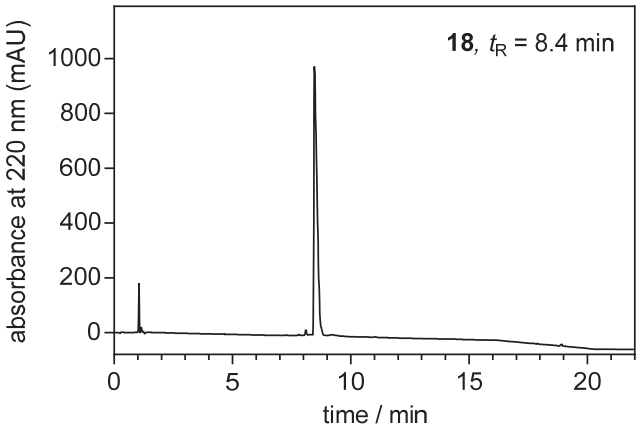
Chromatogram of the RP-HPLC analysis (purity control) of compound **15**



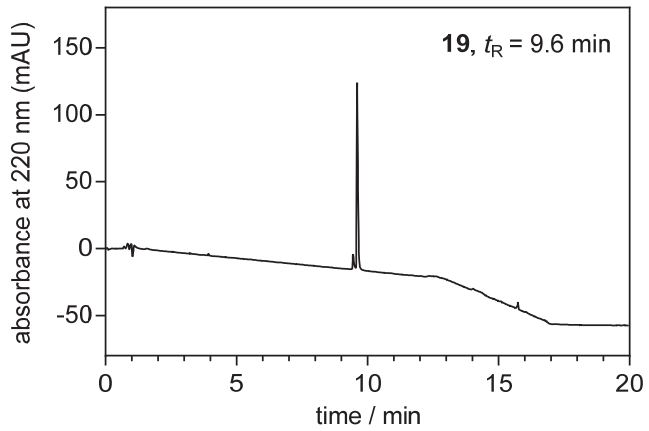
Chromatogram of the RP-HPLC analysis (purity control) of compound **16**



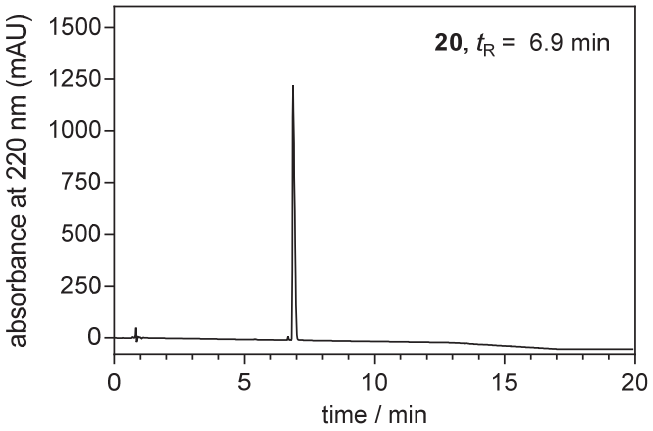
Chromatogram of the RP-HPLC analysis (purity control) of compound **17**



Chromatogram of the RP-HPLC analysis (purity control) of compound **18**

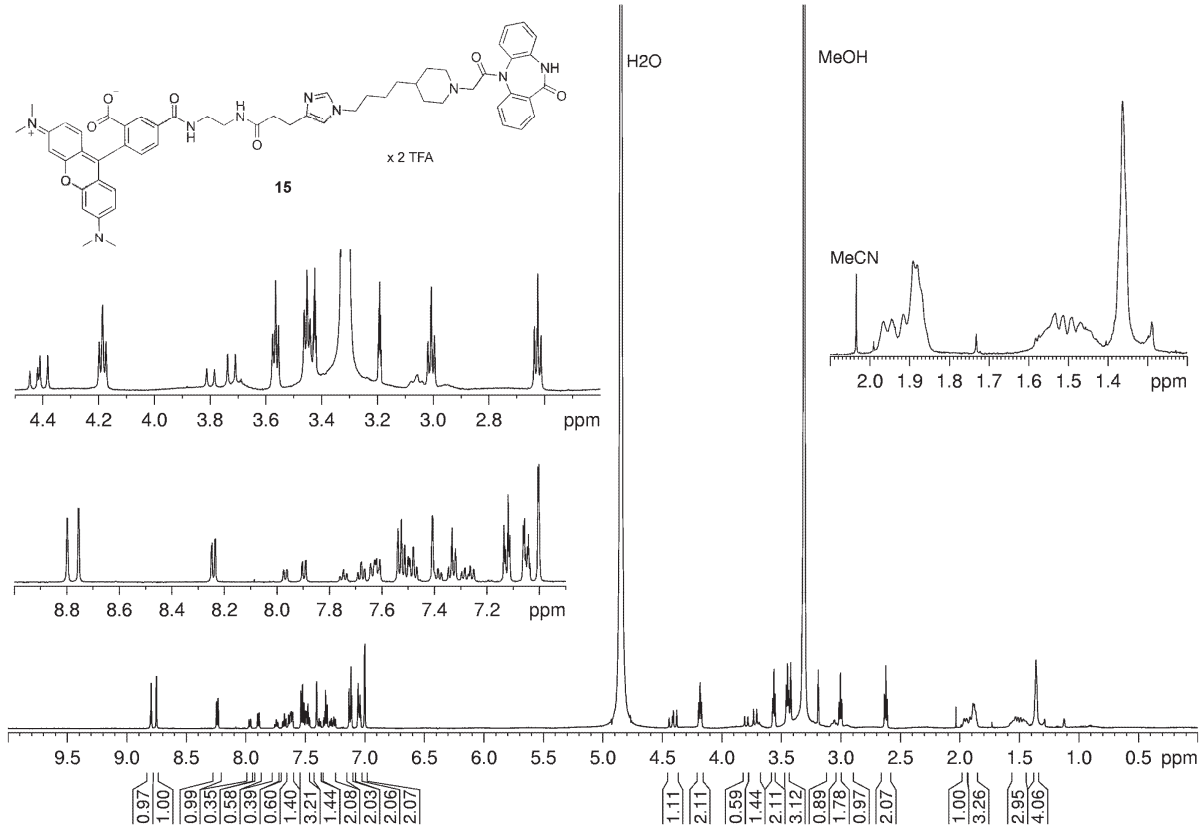


Chromatogram of the RP-HPLC analysis (purity control) of compound **19**

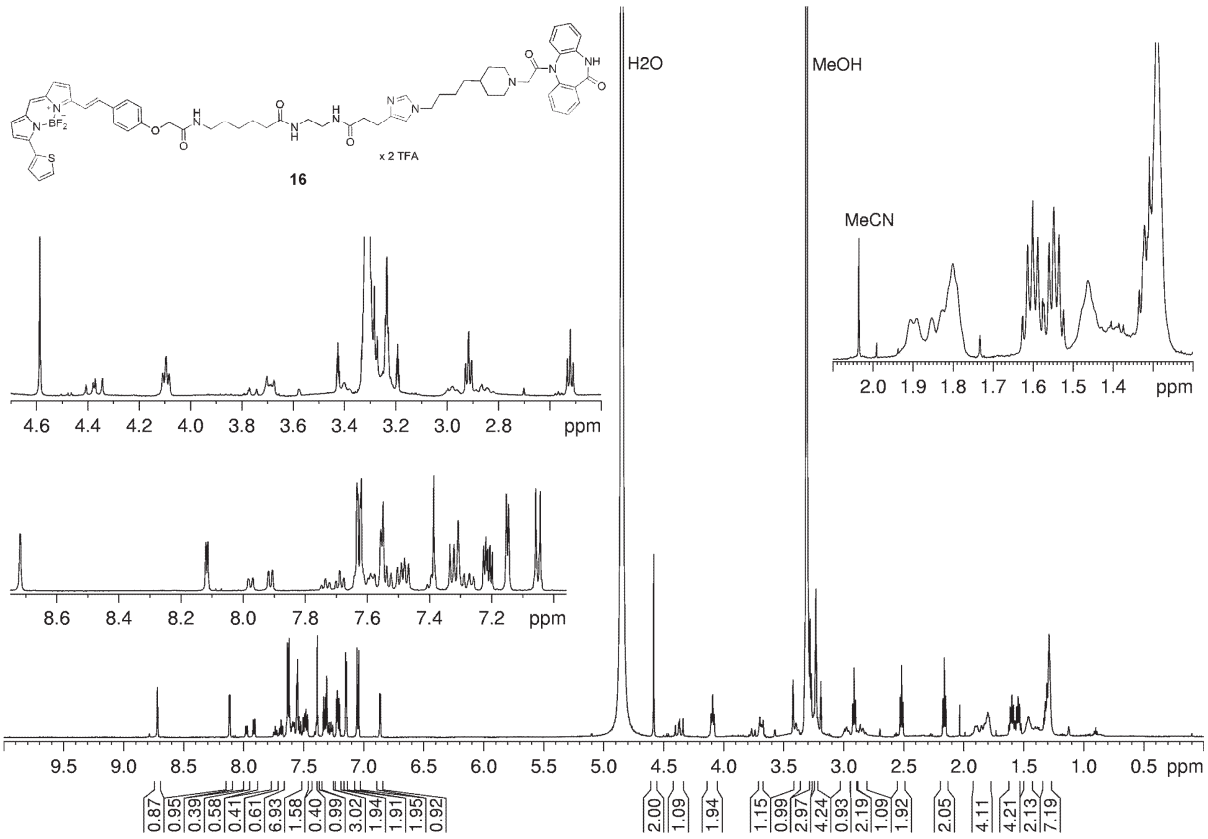


Chromatogram of the RP-HPLC analysis (purity control) of compound **20**

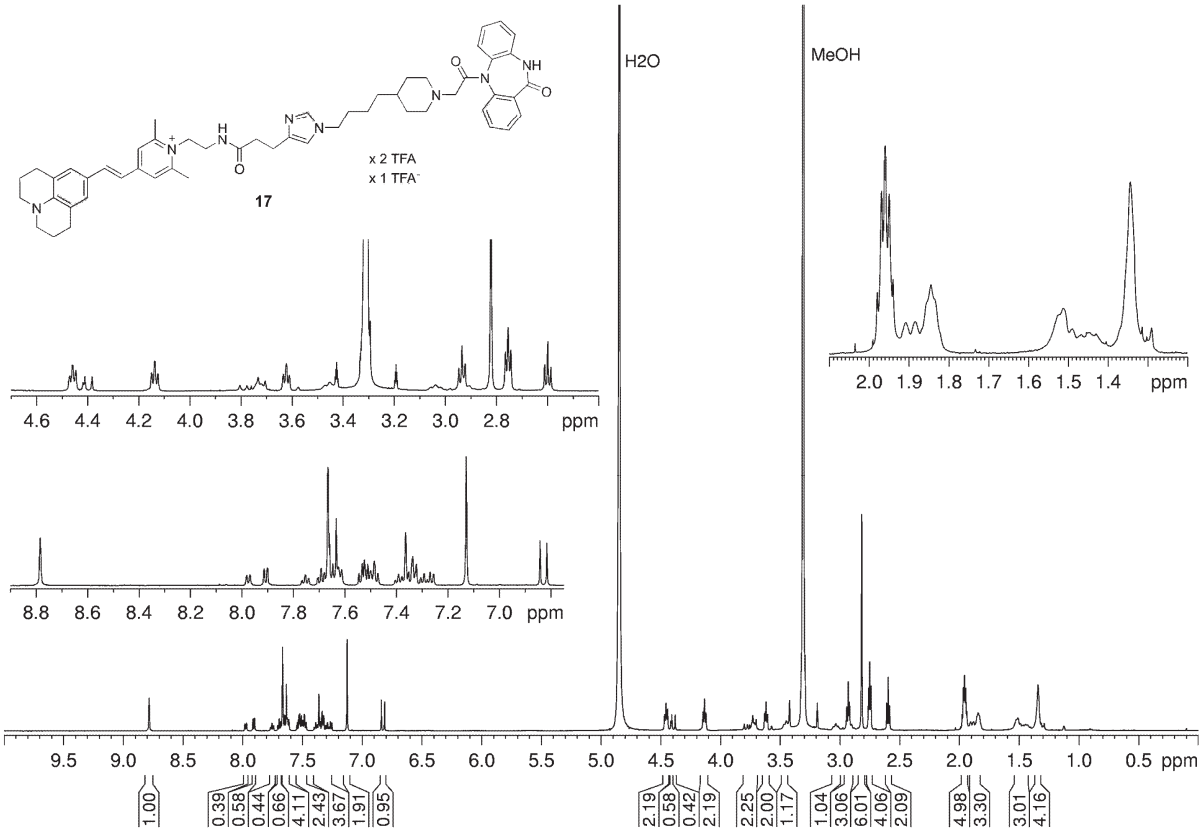
3. ^{1}H-NMR spectra of compounds 15-20



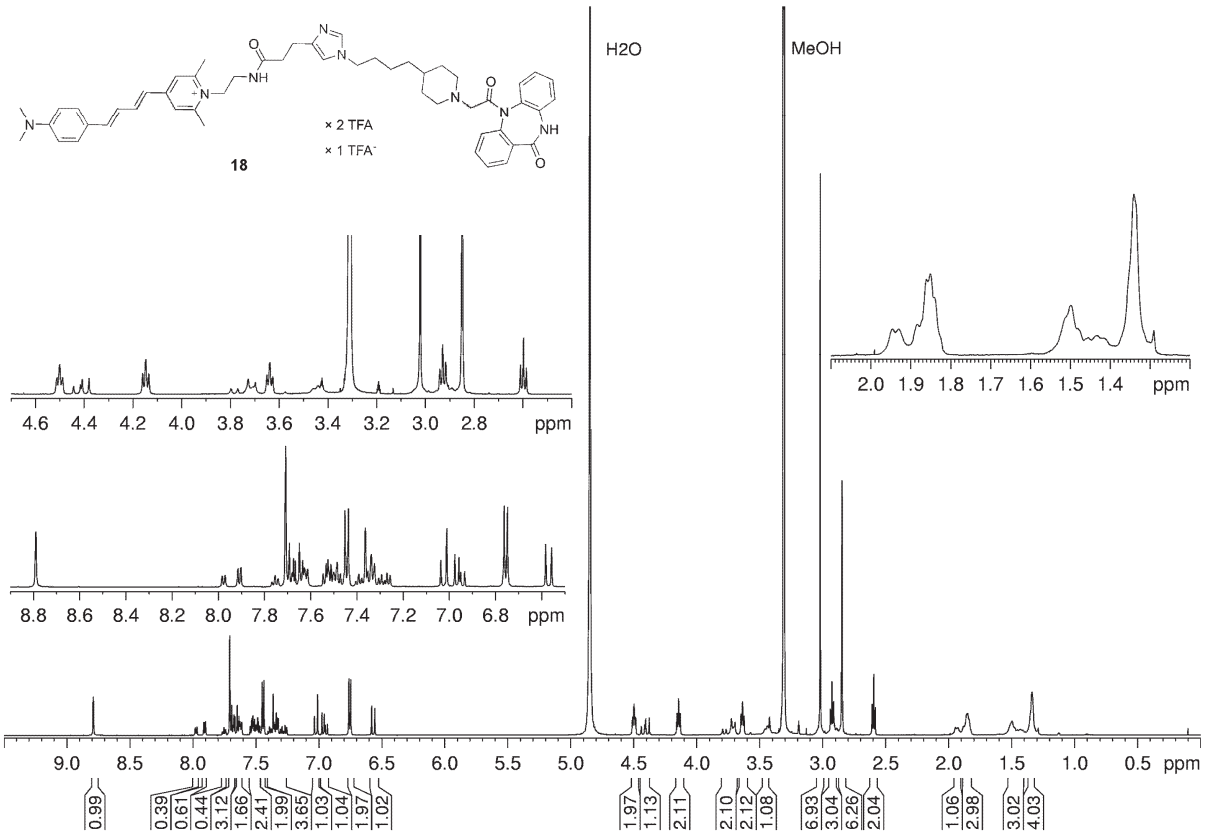
^{1}H-NMR spectrum (600 MHz, MeOH-d_4) of compound **15**



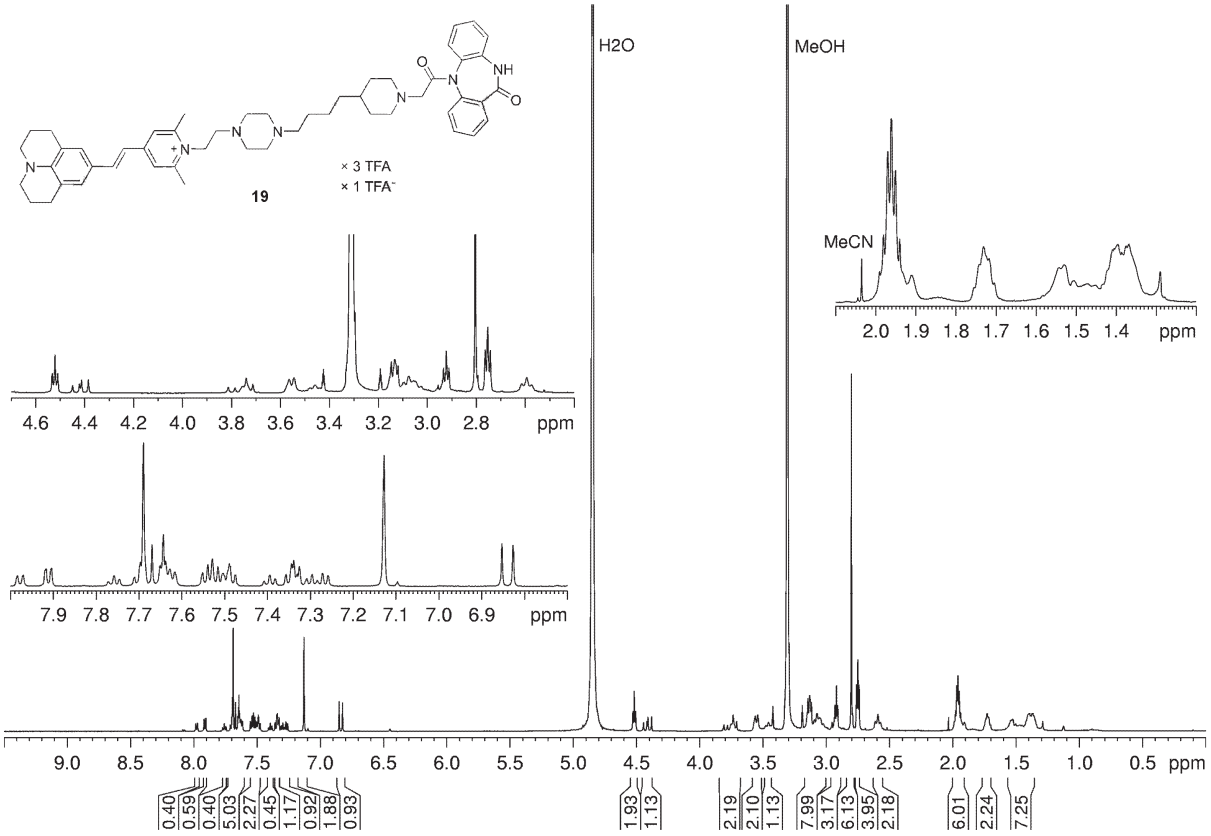
^{1}H-NMR spectrum (600 MHz, MeOH-d_4) of compound **16**



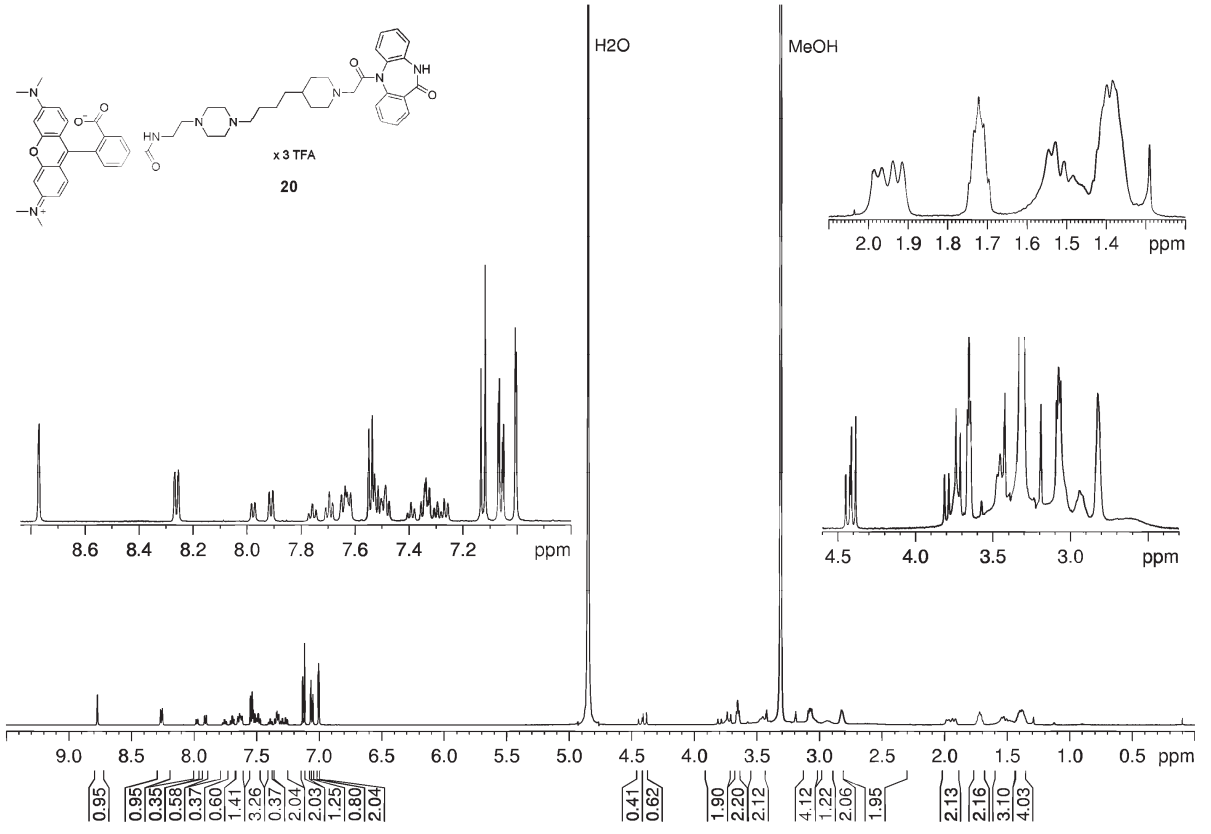
^{1}H-NMR spectrum (600 MHz, MeOH-d_4) of compound **17**



^{1}H-NMR spectrum (600 MHz, MeOH-d_4) of compound **18**



^{1}H-NMR spectrum (600 MHz, MeOH-d_4) of compound **19**



^{1}H-NMR spectrum (600 MHz, MeOH-d_4) of compound **20**