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Research paper

Dehydroabietylamine-substituted trifluorobenzene sulfonamide rhodamine B hybrids as anticancer agents overcoming drug resistance

Niels V. Heise [a], Sven J. Meyer [a], René Csuk [a,*], Thomas Mueller [b]

[a] Organic Chemistry, Martin-Luther University Halle-Wittenberg, Kurt-Mothes-Str. 2, D-06120, Halle (Saale), Germany
[b] University Clinic for Internal Medicine IV, Hematology/Oncology, Medical Faculty, Martin-Luther-University Halle-Wittenberg, Ernst-Grube-Str. 40, D-06120, Halle (Saale), Germany

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ABSTRACT

Attachment of a conjugate assembled from a novel fluorinated carbonic anhydrase inhibitor and rhodamine B onto dehydroabietylamine (DHA) or cyclododecylamine led to first-in-class conjugates of good cytotoxicity; thereby IC_{50} values (from SRB assays; employed tumor cell lines A2780, A2780Cis, A549, HT29, MCF7, and non-malignant human fibroblasts CCD18Co) between 0.2 and 0.7 μM were found. Both conjugates showed similar cytotoxic activity but the dehydroabietylamine derived conjugate outperformed its cyclododecyl analog in terms of tumor cell/non-tumor cell selectivity. Both conjugates accumulate intracellular, and the DHA conjugate was able to overcome drug resistance which is effective independent of the expression status of carbonic anhydrase IX.

1. Introduction

The abietane-like diterpenoid dehydroabietylamine (**DHA**, Fig. 1) has been extensively studied since its first isolation in 1949 from "Amine D" (also known as rosin amine D), a chemical used as a disinfectant in sanitation [1]. Although more than 800 publications have now appeared dealing with **DHA** and its derivatives in one form or another [2–5], the number of publications dealing with the cytotoxicity of **DHA** has remained manageable [6–26].

DHA benzamides oxidized at C-7 in ring B were found to be cytotoxic for PC-3 (prostate carcinoma) and Hey-1B (ovarian carcinoma) cells when a nitro group was simultaneously introduced at position C-12 [8,12], while several benzamides [9] and other amides [17,23], were found to be only slightly cytotoxic but exhibited anti-leishmanial [9,17], or anti-malarial activity [3,11,19]. Different modes of action have been postulated for **DHA** and its derivatives. These ranged from membrane damage [24] and the induction of apoptosis [24] to inhibit lipogenesis [20,21] in PC-3 cells [9], and the induction of an anti-mobility effect by activation of p38 and JNK MAPs in HCC and BC cells by **DHA** [20].

DHA has also been used as a starting material for the synthesis of polyhydrodibenzoxepines and quinazolines holding some cytotoxic activity of L02 and Hep-G2 cell lines. [15], and *N*-acetyl-α-amino acid **DHA** derivatives have been reported for the therapy of hepatocellular carcinoma [16]. DHA ureas and thioureas of good cytotoxicity were shown to act as inhibitors of tyrosyl-DNA phosphodiesterase I; thereby, these compounds enhanced the antitumor effect of temozolomide in glioblastoma cells [13,14].

Cytotoxicity in the nanomolar concentration range was established for constructs obtained by isocyanide-based multi-component reactions [5,22,23]. **DHA** derivatives containing thiophene or pyrazine rings acted by apoptosis [26]. Several reviews cover these topic [2,4,5].

Cancer is the second most common of morbidity and mortality (approximately 22 % in the EU), and chemotherapy remains one of the most important methods for its treatment. Hybrids resulting from merging secondly natural products either with targeting groups or cytotoxic moieties have attracted many scientists especially during the last two decades [27–32]. These natural products are an important source of bioactive compounds, and **DHA** remains an interesting starting material since several derivatives thereof (vide supra) have already shown good to excellent cytotoxic properties.

Despite all these successes, a problem remains since intrinsic tumor hypoxia promotes invasiveness, and tumor hypoxia is soften associated with resistance to chemotherapy and consequently to poor prognosis [33–36]. It seems well established that several human carbonic anhydrases (CAs) especially CA IX are significantly up-regulated by the hypoxia-inducible factor 1a [37] or other micro-environmental factors

* Corresponding author
E-mail address: rene.csuk@chemie.uni-halle.de (R. Csuk).

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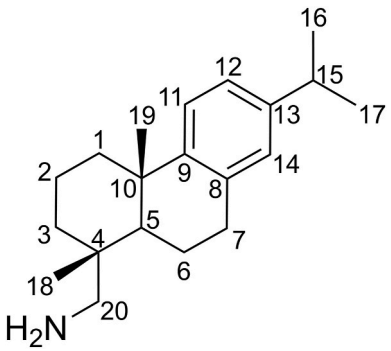


Fig. 1. Structure of dehydroabietylamine (**DHA**) and numbering scheme.

[38–42]. while being limited expressed in normal tissue [43]. Recently Kazokaite et al. [44] have shown that several fluorinated CA IX inhibitors show high affinity to CA IX and hence, some cytotoxicity. Furthermore, several fluorescent probes to determine the affinity of compounds to CA IX have been suggested [45]. Recently, we have demonstrated that especially conjugates of di- or triterpenes with rhodamines (especially rhodamine B or rhodamine 101) exhibited excellent cytotoxic effects for a broad variety of human tumor cell lines, even those being resistant towards established chemotherapeutics [46–48]. The high cytotoxicity was established in classical 2D cell culture but also in 3D spheroid models [49]. Furthermore, it was shown that these compounds targeted the mitochondria [47–50]. Consequently, we decided to synthesize a limited number of **DHA** hybrids consisting of an efficient CA IX inhibitor and rhodamine B in a proof-of-concept study acting as „double targeted molecular biological missile“.

2. Results and discussion

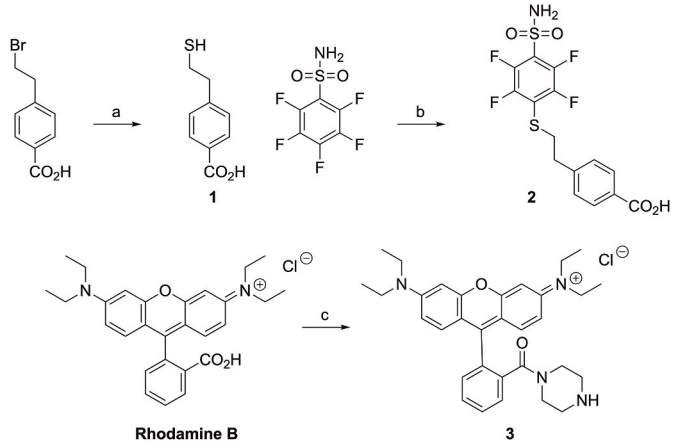
Commercial **DHA** was purified by an acetylation/deacetylation sequence as reported by W. Gottstein and L.C. Cheney [51]. 4-(2-Bromoethyl)-benzoic acid (Scheme 1) was reacted with thiourea/sodium hydroxide [52] to furnish 4-(2-sulfanylethyl)-benzoic acid (**1**) in 90 % isolated yield. Commercial pentafluorobenzene sulfonamide gave upon reaction with **1** compound **2** as a white solid in 83 % yield. The physical and spectroscopic data of this compound matched perfectly with the data published earlier. Furthermore, it is characterized in its ^{19}F NMR spectrum by the presence of two signals at $\delta = -133.0$ ppm (two fluorine substituents in *ortho* position to the sulfonamide moiety) and $\delta = -139.1$ ppm (*para*), respectively.

Rhodamine B was converted into its piperazinyl amide **3** as reported earlier [47–49]. Following the strategy outlined by Kazokaite et al. [44], reaction of **2** with DHA furnished **4** while from the reaction with cyclododecylamine compound **5** was obtained (Scheme 2).

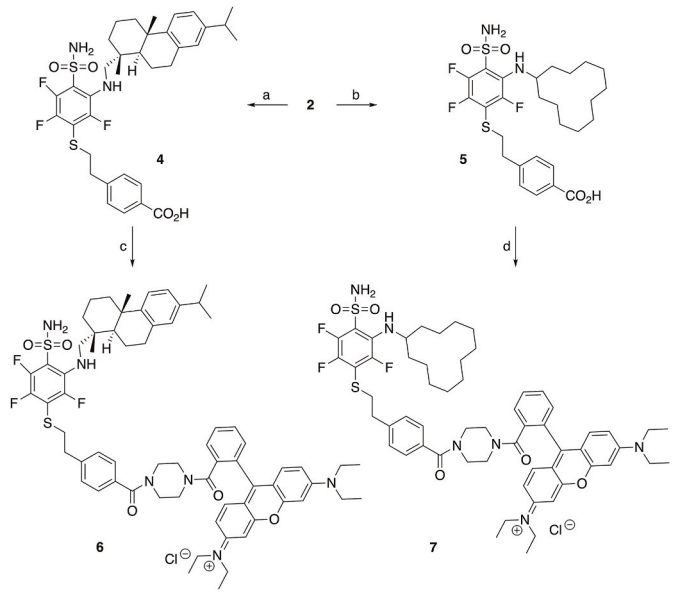
Amide **3** was coupled with **4** and **5** to result in final compounds **6**/**7**, respectively. The purple color of the latter compounds is significant for the cationic rhodamine moiety and its extended conjugated bond system.

Compounds **6** and **7** were subjected to cytotoxic evaluation by SRB assays, the results of which are summarized in Table 1. To this end, we used our cell line panel representing different solid tumor entities and non-malignant human fibroblasts (CCD18Co), including the cell line pair A2780/A2780cis, a well-known model of acquired drug resistance to conventional drugs. These results show them to act as (sub)-micromolar acting cytotoxic agents holding IC_{50} values between 0.2 μM and 0.7 μM. Overall, the compounds **6** and **7** showed similar cytotoxic activity, with **7** being slightly more active. Doxorubicin treatment, used as a positive standard, resulted in an approximately 10-fold difference in the IC_{50} values of A2780 and A2780cis, reproducing the chemoresistance characteristic represented by this model. Both compounds were able to substantially reduce this resistance, resulting in ratios below 2-fold (Table 1, RI). Notably, compound **6** outperformed compound **7** in terms of tumor cell/non-tumor cell selectivity (Tables 1 and SI), suggesting that the **DHA** moiety, renders the whole conjugate more tumor-selective.

To investigate the mechanism of action, we analyzed the cellular accumulation and subcellular localization of compounds employing the fluorescence ability mediated by the rhodamine B moiety. With the aim to analyze the role of the CAIX inhibitor group of compounds, the study was performed in the HT29 cell line, which is known for their ubiquitous expression of cell surface localized CAIX [53]. The imaging agent HypoxiSense 680 (PerkinElmer) targeting CAIX was used as a positive control. As depicted in Fig. 2, both compounds showed direct intracellular accumulation early after start of treatment, with a distinct subcellular localization pattern resembling those of the mitochondria targeting compounds we previously described [47,48,54–57]. This suggests that the main part of the impact of compounds is mediated by the rhodamine B moiety, thereby probably preventing an initial interaction of the CAIX-binding/inhibiting group with CAIX at the cell surface. In conclusion, with **6** we created a tumor-cell selective compound, which overcomes drug resistance, and which is effective independent of



Scheme 1. Reactions and conditions for the synthesis of starting materials: a) H_2N-(C═S)–NH_2, H_2O, reflux, 1 h, them NaOH, 90 %; b) **1** in MeOH, NEt_3, 20 °C, 1d, 83 %; c) $(COCl)_2$, DMF (cat.), then DCM, piperazine, 1 d, 20 °C, 67 %.



Scheme 2. Reactions and conditions: a) DMSO, NEt_3, 70 °C, 36 h, 79 %; b) DMSO, NEt_3, 70 °C, 36 h, 74 %; c) DCM, **3**, NEt_3, EDC, HOBt, 20 °C, 2d, 38 %; d) DCM, **3**, NEt_3, EDC, HOBt, 20 °C, 2d, 50 %.

Table 1
Cytotoxicity of compounds **6** and **7** (IC_{50} in μM, from SRB assays, after 72 h of incubation, values are means from 2 independent experiments with standard deviation lower than 15 %). Human cancer cell lines: A2780 (ovarian adenocarcinoma), A2780cis (resistant derivative of A2780), CCD18Co (non-malignant human fibroblasts), A549 (lung carcinoma), HT29 (colorectal adenocarcinoma), MCF7 (breast adenocarcinoma); Doxorubicin (Dox) has been used as a positive standard. Resistance index (RI): IC_{50} ratio of A2780Cis/A2780, Selectivity index (SI): IC_{50} ratio of CCD18Co/A2780cis.

#	A2780	A2780 cis	A549	HT29	MCF7	CCD18Co	RI	SI
6	0.38	0.46	0.71	0.53	0.41	1.02	1.21	2.22
7	0.24	0.45	0.59	0.32	0.26	0.55	1.87	1.22
Dox	0.010	0.099	0.023	0.086	0.033	0.269	9.9	26.9

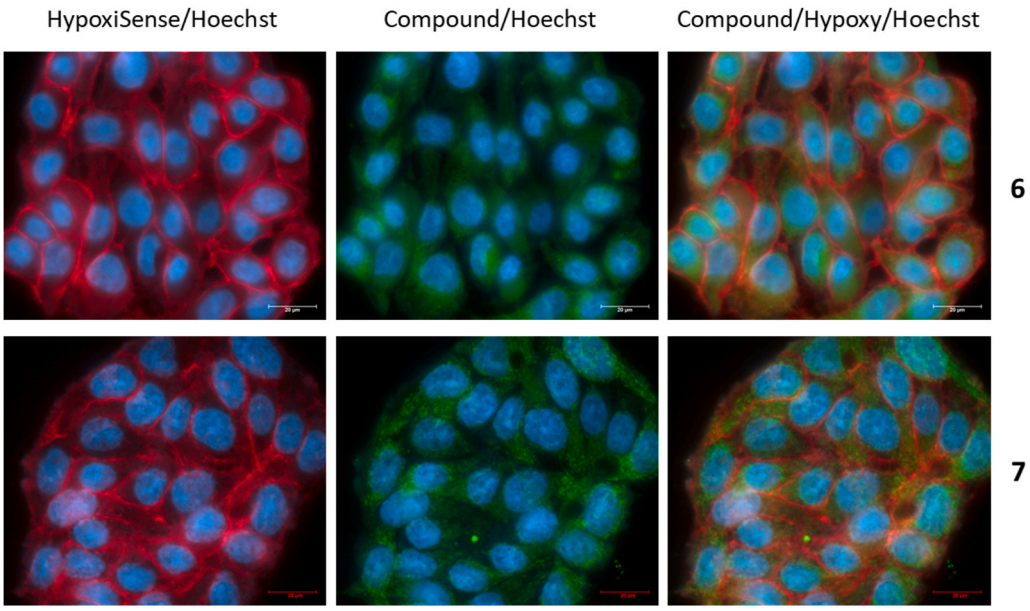


Fig. 2. Analysis of subcellular localization of compounds **6** and **7** after 30 min of treatment, using combined treatment with the CAIX targeting compound HypoxySense 680 and Hoechst 33342 for staining nuclei, shows intracellular accumulation of the compounds without indications of cell membrane staining. Scale bar: 20 μm. The single treatment with the compounds resulted in an identical accumulation pattern, thereby excluding the possibility of interference from bound HypoxySense 680 with the accumulation of the compounds. Pictures of higher resolution are collected in the Supplementary Materials File.

the CAIX expression status.

3. Conclusion

First-in-class conjugates with good cytotoxicity were accessed by attaching a conjugate made of a new fluorinated carbonic anhydrase inhibitor and rhodamine B onto dehydroabietylamine (DHA) or cyclododecylamine. The former compound was synthesized from 4-(2-sulfanylethyl)-benzoic acid and pentafluorobenzene sulfonamide. Coupling of this construct with dehydroabietylamine or cyclododecylamine followed by conjugation with piperazinyl-spacered rhodamine B gave the target compounds that were screened for their cytotoxic activity in SRB assays employing several human tumor cell lines and non-malignant human fibroblast. As a result, IC_{50} values between 0.2 and 0.7 μM were discovered. While the cytotoxic efficacy of both conjugates was comparable, the hybrid generated from dehydroabietylamine exhibited superior selectivity between tumor and non-tumor cells compared to its cyclododecyl analog. Both conjugates accumulate intracellularly, and the DHA conjugate — which is effective regardless of carbonic anhydrase IX expression — was able to overcome drug resistance.

4. Experimental

Equipment and assays were as previously described [47–49]. or updated and briefly listed below. Starting material were obtained from local vendors and used as received; cell lines were obtained from ATCC; solvents were dried according to usual procedures.

4.1. Cell culture

The human cancer cell lines A2780 (ECACC #93112519), A2780Cis (ECACC # 93112517), A549 (ATCC - CCL-185), HT29 (ATCC - HTB-38), MCF7 (ATCC - HTB-22) were cultivated in RPMI1640 medium, non-malignant human fibroblasts CCD18Co (ATCC - CRL-1459) were grown in MEME (both from Sigma-Aldrich, St. Louis, MO, USA). Both media were supplemented with 10 % fetal bovine serum (Biowest, Nuaillé, France) and 1 % penicillin-streptomycin (Sigma-Aldrich).

4.2. SRB assay

Cells were seeded in 96-well plates and after 24 h were treated with serial dilutions of compounds for 72 h. All subsequent steps were performed according to the previously described SRB assay protocol [47, 58]. Dose-response curves and calculation of IC_{50} values including standard deviations were carried out using GraphPad Prism8.

4.2.1. Staining/fluorescence microscopy

Analysis of subcellular localization of compounds was performed in HT29 cells. The imaging agent Hypoxisense 680 (PerkinElmer, Waltham, Massachusetts, United States) was used to prove CAIX expression. Cells were seeded in a μ-Plate 96 Well Black plate (ibiTreat: #1.5 polymer coverslip bottom, ibidi GmbH, Gräfelfing, Germany) at cell density of 20.000 per well. After 48 h, cells were supplemented with RPMI 1640 without Phenol Red (Pan-Biotech GmbH, Aidenbach, Germany) and treated with 500 nM of compounds or HypoxiSense 680

(1:2000), and were examined after 30 min and at additional time points thereafter. For simultaneous analysis of subcellular localization, cells were co-treated with 500 nM of compounds, Hypoxisense 680 (1:2000) and Hoechst 33342 (Sigma-Aldrich), and were analyzed after 30 min. Live cell imaging was performed on an Axio Observer 7 (Carl Zeiss Microscopy Deutschland GmbH, Oberkochen, Germany) using the settings for Ex/Em as followed: Hoechst 33342 (385nm/425 nm), compounds (555nm/592 nm), HypoxiSense 680 (630nm/681 nm). For simultaneous analysis, multiple Z-stacked images were captured and resulting pictures were reconstructed using the ZEN 3.5 pro software (Zeiss).

4.3. Syntheses

4.3.1. 4-(2-Sulfanylethyl)-benzoic acid (1) [930102-58-2]

An aq. solution (30 mL) of 4-(2-bromoethyl)-benzoic acid (2.35 g, 10.34 mmol) and thiourea (2.36 g, 31.0 mmol) was heated under reflux for 1 h, followed by adding an aq. solution of NaOH (5 mL, 10 %); stirring was continued for 1 h. The reaction mixture was cooled to room temperature, and the product was precipitated by acidifying with conc. HCl; the product was filtered off, washed with water and dried in vacuo. Compound **1** (1.7 g, 90 %) was obtained as a white solid; m.p. 165–168 °C (lit. [52]: 156–158 °C); R_F = 0.1 (SiO_2, $CHCl_3$/MeOH, 9:1); UV/Vis (MeOH): λ_{max} (log ε) = 236 nm (4.02); IR (ATR): ν = 2832w, 2666w, 2551w, 1679vs, 1609 m, 1573 m, 1423s, 1316s, 1289vs, 1240 m, 1178 m, 1125 m, 1113w, 1017 m, 935 m, 872 m, 757s, 735 m, 717 m, 629 m, 545s, 502w, 475 m, 1046w, 1038 m, 1029 m, 819w, 750w, 733 m, 717s, 463 m, 449w, 436w, 424 m cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.87 (d, J = 8.3 Hz, 2H, 2-H, 6-H), 7.35 (d, J = 8.3 Hz, 2H, 3-H, 5-H), 2.92 (t, J = 7.5 Hz, 2H, 8-H), 2.84–2.71 (m, 2H, 7-H), 2.28 (s, 1H, *SH*) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 167.2 (C-9), 145.3 (C-4), 129.3 (C-2, 6), 128.9 (C-1), 128.8 (C-3, 5), 39.3 (C-7), 24.7 (C-8) ppm; MS (ESI, MeOH): m/z = 181.1 (100 %, $[M-H]^-$); anal. calcd for $C_9H_{10}O_2S$ (182.24), C 59.32, H 5.53; found: C 59.01, H 5.83.

4.3.2. 4-(2-{[4-(Aminosulfonyl)-2,3,5,6- tetrafluorophenyl]sulfanyl} ethyl)-benzoic acid (2) [3025142-26-8]

To a solution of pentafluorobenzene sulfonamide (1.89 g, 7.7 mmol) and **1** (1.68 g, 15.4 mmol) in MeOH (20 mL), Et_3N (1.56 g, 15.4 mmol) was added, and the reaction mixture was stirred for one day at 20 °C. After acidifying to pH = 5 with conc. hydrochloric acid, the solvent was evaporated under diminished pressure, and the crude product was washed with water; recrystallisation from EtOH gave 2 (2.62 g, 83 %) as a white solid; m.p. 234–236 °C (lit. [44]: 235–236 °C); R_F = 0.1 (SiO_2, $CHCl_3$/MeOH, 9:1); UV/Vis (MeOH): λ_{max} (log ε) = 231 nm (4.24), 282 nm (3.94); IR (ATR): ν = 3396w, 3281w, 2986w, 2945w, 2842w, 1700 m, 1685 m, 1610 m, 1593w, 1576w, 1555 m, 1463vs, 1424 m, 1398 m, 1379 m, 1365s, 1355s, 1319 m, 1292 m, 1263s, 1253 m, 1170s, 1127w, 1019w, 965s, 936 m, 923 m, 863w, 836w, 773w, 760 m, 719 m, 705w, 637 m, 602s, 547 m, 510 m, 503 m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 12.82 (s, 1H, OH), 8.36 (s, 2H, NH_2), 7.84 (d, J = 8.3 Hz, 2H, 3-H, 7-H), 7.35 (d, J = 8.3 Hz, 2H, 4-H, 6-H), 3.36 (t, J = 7.4 Hz, 2H, 9-H), 2.95 (t, J = 7.4 Hz, 2H, 8-H) ppm; ^{19}F NMR (470 MHz, $CDCl_3$): δ = −133.0, −139.1 ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 167.6 (C-1), 146.7 (C-12, C-14), 144.8 (C-5), 142.9 (C-11, C-15), 129.8 (C-3, C-7), 129.3 (C-4, C-6), 122.8 (C-10), 118.7 (C-13), 35.9 (C-8), 34.8 (C-9) ppm; MS (ESI, MeOH): m/z = 408.3 (100 %, $[M-H]^-$); anal. calcd for $C_{15}H_{11}F_4NO_4S_2$ (409.37), C 44.01, H 2.71, N 3.42; found: C 43.78, H 2.95, N 3.17.

4.3.3. N-(6-(Diethylamino)-9-(2-(piperazin-1-carbonyl)phenyl)-3H-xanthen-3-yliden)-N-ethylethanaminium chloride (3)

This compound was prepared as previously reported from rhodamine B (10.0 g, 22.3 mmol), oxalyl chloride (9.0 mL, 47.3 mmol) and one drop of dry DMF followed by the evaporation of all volatiles. The residue was dissolved in dry DCM (300 mL), and this solution was slowly added to a solution of piperazine (10.0 g, 116.0 mmol) in dry DCM (350 mL). After 24 h of stirring at 20 °C, the solvent was removed under reduced pressure followed by chromatographic purification (silica gel, chloroform/methanol, 9:1) of the crude material to yield 3 (7.24 g, 67 %) as a dark purple solid; R_F = 0.14 (chloroform/methanol, 8:2); m.p. > 350 °C; *sp*ectroscopic data as previously reported [47–50]; MS (ESI, MeOH): m/z = 511.5 (100 %, $[M-Cl]^+$).

4.3.4. 4-(2-{[3-(Abieta-8,11,13-trien-18-ylamino)-4-(aminosulfonyl)-2,5,6-trifluorophenyl]sulfanyl}ethyl)benzoic acid (4)

Compound **2** (2.55 g, 6.2 mmol) and **DHA** (2.73 g, 9.4 mmol) were dissolved in DMSO (45 mL), Et_3N (2.38 g, 23.5 mmol) was added, and the reaction mixture was stirred at 70 °C for 36 h. Water (20 mL) was added, and the solution was acidified to pH 5 with aqu. HCl (2 M) The precipitate was filtered off, washed with water and purified by column chromatography ($CHCl_3$/MeOH, 9:1) to yield 4 (3.35 g, 79 %) as a white solid; m.p. >150 °C (slow decomp.); R_F = 0.5 (SiO_2, $CHCl_3$/MeOH, 9:1); $[\alpha]_D^{20}$ = +22.2° (*c* 0.14, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 276 nm (4.16), 341 nm (3.72); IR (ATR): ν = 3366w, 2957 m, 2927 m, 2868w, 1703 m, 1611w, 1574w, 1456vs, 1416 m, 1384w, 1339 m, 1327 m, 1312 m, 1245s, 1178w, 1155s, 1070w, 1014s, 948s, 898w, 824w, 756 m, 720w, 703 m, 606vs, 512 m, 819w, 750w, 733 m, 717s, 463 m, 449w, 436w, 424 m, 413w, cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.83 (d, J = 8.3 Hz, 2H, 31-H, 33-H), 7.32 (d, J = 8.3 Hz, 2H, 30-H, 34-H), 7.15 (d, J = 8.2 Hz, 1H, 11-H), 6.95 (*dd*, J = 8.1, 1.6 Hz, 1H, 12-H), 6.84 (d, J = 1.5 Hz, 1H, 14-H), 6.21 (t, J = 5.6 Hz, 1H, NH), 3.31 (t, J = 7.3 Hz, 2H, 28-H), 3.16 (*dd*, J = 11.9, 4.1 Hz, 1H, 20-H_a), 3.00 (*dd*, J = 11.8, 7.2 Hz, 1H, 20-H_b), 2.92 (t, J = 7.3 Hz, 2H, 27-H), 2.82 (*dt*, J = 14.7, 6.1 Hz, 2H, 7-H), 2.75 (*hept*, J = 6.9 Hz, 1H, 15-H), 2.28 (d, J = 13.0 Hz, 1H, 1-H_b), 1.82–1.69 (m, 2H, 6-H), 1.70–1.56 (m, 2H, 2-H), 1.50 (d, J = 12.2 Hz, 2H, 5-H), 1.48–1.40 (m, 2H, 3-H), 1.24 (*td*, J = 12.9, 3.4 Hz, 1H, 1-H_a), 1.16 (d, J = 2.3 Hz, 6H, 16-H, 17-H), 1.15 (s, 3H, 18-H), 0.93 (s, 3H, 19-H) ppm; ^{19}F NMR (470 MHz, $CDCl_3$): δ = −118.98, −137.45, −145.11 ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 167.8 (C-35), 147.4 (C-9), 146.7 (C-23), 145.4 (C-13), 144.7 (C-29), 144.6 (C-26), 141.6 (C-24), 134.7 (C-8), 134.2 (C-32), 130.3 (C-21), 129.8 (C-31, C-33), 129.2 (C-30, C-34), 126.8 (C-14), 124.6 (C-11), 124.1 (C-12), 119.2 (C-25), 58.0 (C-20), 117.8 (C-22), 45.2 (C-5), 38.4 (C-1), 37.8 (C-4), 37.6 (C-10), 35.9 (C-28), 35.6 (C-3), 34.6 (C-27), 33.3 (C-15), 30.0 (C-7), 25.7 (C-18), 24.4 (C-16, 17), 19.0 (C-19), 18.8 (C-2, C-6) ppm; MS (ESI, MeOH): m/z = 674.0 (100 %, $[M-H]^-$); anal. calcd for $C_{35}H_{41}F_3N_2O_4S_2$ (674.84), C 62.29, H 6.12, N 4.15; found: 61.97, H 6.33, N 3.98.

4.3.5. 4-(2-{[4-(Aminosulfonyl)-3-(cyclododecylamino)-2,5,6-tetrafluorophenyl]sulfanyl}-ethyl)-benzoic acid (5)

A solution of **2** (1.43 g, 3.5 mmol) and cyclododecylamine (1.0 g, 5.5 mmol) in DMSO (10 mL) containing Et_3N (0.89 g, 7.0 mmol) was stirred at 70 °C for 36 h. Water (20 mL) was added, and the solution was acidified to pH 5 with aq. HCl (2 M). The precipitate was filtered off, washed with water and purified by column chromatography ($CHCl_3$/MeOH, 9:1) to yield 5 (1.5 g, 74 %) as a white solid.; m.p.175–177 °C (lit. [44]: 169–170 °C); R_F = 0.5 (SiO_2, $CHCl_3$/MeOH, 9:1); UV/Vis (MeOH): λ_{max} (log ε) = 232 nm (4.32), 277 nm (3.84), 343 nm (3.54); IR (ATR): ν = 3355w, 3005w, 2931 m, 2861 m, 1703 m, 1611w, 1574w, 1457vs, 1416 m, 1334 m, 1312 m, 1244s, 1178w, 1154s, 1119w, 1015s, 948s, 899w, 757 m, 719 m, 702 m, 671w, 606vs, 511 m, 1038 m, 1029 m, 819w, 750w, 733 m, 717s, 463 m, 449w, 436w, 424 m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 12.82 (s, 1H, OH), 8.10 (s, 2H, NH_2), 7.84 (d, J = 8.2 Hz, 2H, 23-H, 25-H), 7.32 (d, J = 8.2 Hz, 2H, 22-H, 26-H), 6.17 (d, J = 9.3 Hz, 1H, NH), 3.68 (s, 1H, 1-H), 3.28 (t, J = 7.5 Hz, 2H, 20-H), 2.91 (t, J = 7.5 Hz, 2H, 19-H), 1.62–1.50 (m, 2H, 2-H_a, 12-H_a), 1.46–1.19 (m, 20H, CH_2) ppm; ^{19}F NMR (470 MHz, $CDCl_3$): δ = −120.56, −137.02, −145.13 ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 167.2 (C-27), 148.7 (C-18), 146.7 (C-15), 144.3 (C-21), 142.2 (C-16), 132.4 (C-13), 129.3 (C-23, 25), 129.2 (C-24), 128.6 (C-22, 26), 119.1 (C-17), 117.2 (C-14),

52.1 (C-1), 35.4 (C-19), 34.2 (C-20), 30.5 (C-2, 12), 23.6 (C-3, 11), 23.2 (C-6, 8), 22.9 (C-5, 9), 22.7 (C-4, 10), 20.8 (C-7) ppm; MS (ESI, MeOH): m/z = 571.8 (100 %, $[M - H]^-$); anal. Calcd for $C_{27}H_{35}F_3N_2O_4S_2$ (572.70), C 56.62, H 6.16, N 4.89; found: C 56.30, H 6.38, N 4.55.

4.3.6. 4-(2-{[4-(Aminosulfonyl)-3-(abieta-8,11,13-trien-18-amino)-2,5,6-tetrafluorophenyl]thio}-ethyl)benzoic acid (4-{2-[3,6-bis (diethylamino)xanthenium-9-yl]-benzoyl}-piperazin-1-yl) amide chloride (6)

To a solution of **4** (0.14 g, 0.2 mmol) in dry DCM (10 mL), Et_3N (0.04 g, 0.4 mmol), EDC (0.06 g, 0.4 mmol) and HOBt (0.05 g, 0.4 mmol) were added. After stirring at 20 °C for 10 min, **5** (0.16 g, 0.3 mmol) was added, and stirring was continued for 48 h; purification by column chromatography ($CHCl_3$/MeOH, 85:15) gave 6 (120 mg g, 50 %) as a dark purple solid; m.p. >300 °C (decomp.); R_F = 0.95 (SiO_2, $CHCl_3$/MeOH, 1:1); UV/Vis (MeOH): λ_{max} (log ε) = 562 nm (5.05); IR (ATR): ν = 2961w, 2925w, 1630 m, 1586vs, 1528 m, 1508w, 1491 m, 1458s, 1431 m, 1412s, 1394 m, 1335s, 1271s, 1261s, 1245s, 1197 m, 1179vs, 1156s, 1132s, 1094 m, 1072s, 1039 m, 1003s, 976w, 950w, 922 m, 820 m, 799 m, 747s, 712w, 683 m, 663w, 605 m, 584w, 524w, 518w cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.73–7.01 (m, 15H, 12-H, 30-H, 31-H, 33-H, 34-H, 43-H, 44-H, 45-H, 46-H, 50-H, 52-H, 53-H), 6.93 (d, J = 8.8 Hz, 1H, 11-H), 6.87 (d, J = 2.0 Hz, 1H, 14-H), 5.93 (s, 1H, NH), 3.73–3.56 (m, 8H, 54-H), 3.41 (s, 8H, 36-H, 37-H, 38-H, 39-H), 3.28 (t, J = 6.8 Hz, 2H, 27-H), 3.05 (s, 2H, 20-H), 3.01 (t, J = 6.4 Hz, 2H, 28-H), 2.80 (hept, J = 13.8, 6.9 Hz, 1H, 15-H), 2.26 (d, J = 13.1 Hz, 1H, 1-H_a), 1.88–1.63 (m, 4H, 2-H, 6-H), 1.60–1.51 (m, 1H, 3-H_a), 1.56–1.49 (m, 1H, 5-H), 1.42 (*td*, J = 13.4, 3.7 Hz, 1H, 3-H_b), 1.37 (*td*, J = 12.9, 3.3 Hz, 1H, 1-H_b), 1.31 (t, J = 7.3 Hz, 12H, 55-H), 1.24 (d, J = 5.9 Hz, 2H, 7-H), 1.20 (d, J = 4.2 Hz, 6H, 16-H, 17-H), 1.19 (s, 3H, 19-H), 0.95 (s, 3H, 18-H) ppm; ^{19}F NMR (470 MHz, $CDCl_3$): δ = −117.84, −138.91, −143.61 ppm ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.9 (C-35), 167.7 (C-40), 157.8 (C-51),156.3 (C-49), 155.8 (C-47), 149.6 (C-23), 147.4 (C-9), 145.6 (C-13), 143.5 (C-26), 140.9 (C-24), 138.4 (C-53), 134.2 (C-32), 134.1 (C-42), 132.6 (C-41), 131.1 (C-21), 130.9 (C-8), 130.5 (C-43), 130.3 (C-44), 129.2 (C-46), 127.7 (C-30, C-34), 127.2 (C-31, C-33), 127.0 (C-14, C-45), 124.3 (C-11), 123.9 (C-12), 121.4 (C-48), 119.1 (C-52), 118.4 (C-25), 113.9 (C-22), 109.8 (C-50), 58.5 (C-20), 48.0 (C-36, C-39), 46.3 (C-54), 45.8 (C-5), 41.9 (C-37, C-38), 38.6 (C-1), 37.9 (C-28), 37.8 (C-10), 37.7 (C-4), 35.8 (C-3), 34.0 (C-27), 33.5 (C-15), 30.3 (C-7), 25.5 (C-19), 24.1 (C-16, 17), 19.1 (C-6), 18.9 (C-2), 18.6 (C-18), 12.8 (C-55) ppm; MS (ESI, MeOH): m/z = 1168.2 (100 %, $[M\text{-H-Cl}]^+$); anal. calcd for $C_{67}H_{78}ClF_3N_6O_5S_2$ (1203.96), C 66.84, H 6.53, N 6.98; found: C 66.65, H 6.89, N 6.64.

4.3.7. N-[9-{2-[(4-2-{[3-(Abieta-8,11,13-trien-18-yl-amino)-4-(aminosulfonyl)-2,5,6-trifluorophenyl]sulfanyl}ethyl)phenyl]carbonyl} piperazin-1-yl)carbonyl]phenyl}7-(diethylamino)-3H-xanthen-3-ylidene]-N-ethyl-ethanaminium chloride (7)

Reaction as described above for **6**, from **5** (286 mg, 0.5 mmol), Et_3N (0.10 g, 1.0 mmol), EDC (0.155 g, 1.0 mmol), HOBt (0.135 g, 1.0 mmol) and **5** (0.41 g, 0.75 mmol) for 72 h followed by purification of the crude material by column chromatography ($CHCl_3$/MeOH, 85:15) 7 (210 mg, 38 %) was obtained as a dark purple solid; m.p. >300 °C (decomp.); R_F = 0.95 (SiO_2, $CHCl_3$/MeOH, 1:1; UV–Vis (MeOH): λ_{max} (log ε) = 561 nm (4.87); IR (ATR): ν = 2927 m, 2860w, 1631 m, 1587vs, 1557 m, 1529w, 1508w, 1461s, 1412s, 1394 m, 1335s, 1272s, 1246s, 1197 m, 1179s, 1157 m, 1132 m, 1095w, 1073 m, 1003 m, 922w, 824w, 749 m, 683 m, 662w, 604w, 820 m, 799 m, 747s, 712w, 683 m, 663w, 605 m, 584w, 524w, 518w cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.74–7.62 (m, 2H, 45-H), 7.55 (d, J = 6.2 Hz, 1H, 35-H), 7.39–7.30 (m, 1H, 36-H), 7.26 (s, 5H, 23-H, 25-H, 38-H, 42-H), 7.22–7.09 (m, 4H, 22-H, 26-H, 44-H), 6.83–6.70 (m, 1H, 37-H), 4.64 (s, 2H, NH2–H), 3.92–3.83 (m, 1H, 1-H), 3.66 (s, 8H, 46-H), 3.46 (s, 8H, 28-H, 29-H, 30-H, 31-H), 3.26 (s, 2H, 19-H), 3.03–2.95 (m, 2H, 20-H), 1.67 (d, J = 82.8 Hz, 4H, 2-H, 12-H), 1.51–1.19 (m, 30H, CH_2, 47-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 172.6 (C-32), 167.8 (C-27), 157.9 (C-39), 155.8 (C-43), 152.1 (C-41), 143.2 (C-15), 141.6 (C-21), 141.5 (C-16), 138.4 (C-18), 134.6 (C-24), 132.4 (C-45), 132.2 (C-33), 130.5 (C-35), 130.4 (C-22, C-26), 130.3 (C-36), 130.3 (C-34), 129.2 (C-37), 128.9 (C-38), 127.8 (C-44), 127.7 (C-23, C-25), 127.1 (C-13), 122.9 (C-40), 117.8 (C-17), 116.8 (C-14), 96.5 (C-42), 57.9 (C-1), 56.5 (C-30), 56.4 (C-29), 53.4 (C-28, C-31), 46.4 (C-46), 37.6 (C-20), 34.0 (C-19), 29.5 (C-2, C-12), 24.5 (C-3, C-11), 24.2 (C-6, C-8), 23.9 (C-5, C-9), 23.3 (C-4, C-10), 22.6 (C-7), 12.8 (C-47) ppm; MS (ESI, MeOH): m/z = 1066.3 (100 %, $[M - Cl]^+$); anal. calcd for $C_{59}H_{72}ClF_3N_6O_5S_2$ (1101.81), C 64.32, H 6.59, N 7.63, S 5.82; found: C 63.97, H 6.84, N 731.

CRediT authorship contribution statement

Niels V. Heise: Writing – review & editing, Writing – original draft, Investigation. **Sven J. Meyer:** Writing – review & editing, Writing – original draft, Investigation. **René Csuk:** Writing – review & editing, Writing – original draft, Validation, Supervision, Conceptualization. **Thomas Mueller:** Writing – review & editing, Writing – original draft, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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