Synthesis and Radioiodination of Some 9-Aminoacridine Derivatives

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Derivatives of 9-aminoacridine, namely *N*-[ω-(acridin-9-ylamino)alkyl]-3-(trimethylstannyl)benzamides (**1**), where the alkyl group is propyl (**1a**) and octyl (**1b**), and 2-(acridin-9-ylamino)-3-(4-hydroxyphenyl)propionic acid (**2**), have been synthesized with the aim to use them as precursors in the syntheses of radiolabeled DNA intercalators for biological experiments. It was observed that compounds **1a** and **1b** can exist in two isomeric forms at room temperature. Radioiodination of the two benzamides **1a** and **1b** was carried out with the Auger-emitting nuclide ^{125}I by exchange of the trimethylstannyl group. The optimal conditions for radioiodination of the octyl derivative **1b** were established and the labeling yield was found to be as high as 92%, according to TLC analysis in model experiments. Purification of the radioiodinated products gave radiochemical yields of 56% for the propyl and 74% for the octyl compound. The amino acid **2** was directly labeled with ^{125}I at the *ortho* position to the hydroxyl group by taking advantage of the activated ring. The experiment afforded a very high labeling yield (92%).

Introduction

The cytotoxicity of many radionuclides to tumors is highest if the radionuclides are delivered to the nucleus of the cell. This circumstance has given impetus to the development of methods to synthesize DNA-interacting carriers of radionuclides for targeted cancer therapy. The planar structure of acridines allows the molecules to bind strongly to DNA by intercalation. When the acridines have an amino substituent at the 9-position, their binding to a double-stranded DNA has been shown to increase.[1] Radiolabeled 9-aminoacridines are therefore of potential interest.

Here we report the syntheses of two *N*-[ω-(acridin-9-ylamino)alkyl]-3-(trimethylstannyl)benzamides (**1a** and **1b**, where the alkyl groups are propyl and octyl, respectively) and 2-(acridin-9-ylamino)-3-(4-hydroxyphenyl)propionic acid (**2**), which all can act as precursors for radioiodination. 2-(Acridin-9-ylamino)-3-(4-hydroxy-3-iodo-phenyl)propionic acid (**3**), a monoiodinated derivative of **2**, was also prepared for use as a nonradioactive reference substance in the radiolabeling experiments. The radiolabeling of the three compounds with ^{125}I is also presented. Iodine-125 is an Auger-electron-emitting nuclide with a half life of 60 days. The Auger electrons travel along a range similar to the dimensions of the DNA molecule. It has been demonstrated that ^{125}I decaying in the close vicinity of DNA has a much higher cell-killing effect than ^{125}I decaying in the cytoplasm.[2] Because of its relatively long half life and low emission energy, ^{125}I is a very convenient nuclide for laboratory experiments. Biological experiments using these molecules will be reported elsewhere.

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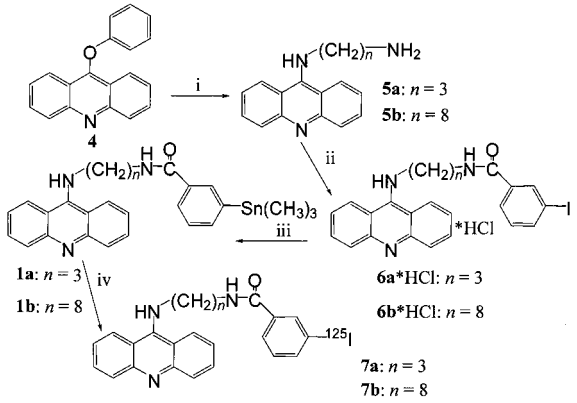
Results and Discussion

Synthesis of *N*-[ω-(Acridin-9-ylamino)alkyl]-3-(trimethylstannyl)benzamides (1a and 1b)

The compounds **1a** and **1b** were prepared as outlined in Scheme 1 starting with the nucleophilic substitution reac-



Scheme 1. *i*) $H_2N(CH_2)_nNH_2$ (n = 3, 8), DMF, 100 °C. *ii*) 3-iodobenzoyl chloride, NaOH, H_2O, chloroform. *iii*) For **1a**: K_2CO_3, $Sn_2(CH_3)_6$, $Pd(PPh_3)_4$, DMF, 100 °C; for **1b**: K_2CO_3, $Sn_2(CH_3)_6$, $PdCl_2(PPh_3)_2$, 1,4-dioxane, 60 °C *iv*) [^{125}I]NaI, MeOH, CAT, 5 min

tion of 9-phenoxyacridine with 1,3-diaminopropane and 1,8-diaminooctane to obtain the products N^1-acridin-9-ylpropyl-1,3-diamine (**5a**) and (N^1-acridin-9-yl)octyl-1,8-diamine (**5b**), respectively. The compounds were treated with 3 M HCl in methanol and then purified by column chromatography to give the hydrochloride salts of **5a** (previously reported as the dihydrogen bromide salt[3]) and **5b** in high yield. The 9-phenoxyacridine was prepared from 9(10*H*)-acridone via 9-chloroacridine by a slight modification of a procedure reported in the literature.[4,5] 9-Chloroacridine was found to be difficult to handle as it is easily hydrolyzed to 9(10*H*)-acridone. It was therefore not advisable to use it for a direct reaction with the diamine. Excess amount of the diamines were used in these reactions to avoid disubstitution.

Several approaches were tried to achieve a simple method of obtaining the benzamides **6a**,**b** in good yield. One way was to couple the amines **5a**,**b** with 3-iodobenzoic acid using dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP). This reaction afforded around 40% of the corresponding benzamides. The coupling reaction of 3-iodobenzoyl chloride with the amines **5a**,**b** provided **6a**,**b**·HCl in 88% and 69% yield, respectively.

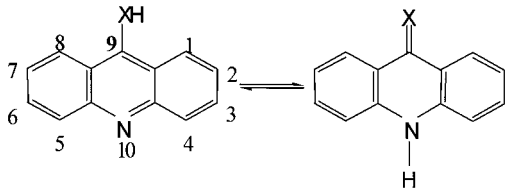
In the last step, the iodine atom in **6a**,**b** was substituted with a trimethylstannyl group using a palladium(0) catalyzed coupling reaction.[6] This reaction in DMF solution gave complete conversion of the starting material in five hours. Interestingly, a palladium(II)-catalyzed reaction in dioxane converted the iodinated compounds to their corresponding stannylated compounds **1** in only one and half hours in similar yields. The obvious advantages of this method over the former one are the lower boiling point of the solvent (dioxane in contrast to DMF), relatively short reaction time and lower reaction temperature (Scheme 1). As the stannylated compounds are sensitive to acidic media, the silica gel used in the chromatographic purification was presaturated with ammonia. For both stannylated compounds **1a** and **1b** two isomers with small differences in retention times were isolated by chromatography. The main difference in the ^{1}H NMR spectra of the two isomers is the chemical shift of the NH proton of the 9-aminoacridine part. These compounds are denoted **1aA**, **1aB**, and **1bA**, **1bB** in the Exp. Sect. The mass spectra of each pair of isomers are virtually the same and give the expected molecular ions with the characteristic isotopic pattern for tin compounds. Compounds **1aB** and **1bB** were the minor products. This issue in relation to literature data for similar compounds will be discussed below.

Tautomerism in the 9-Aminoacridine System

9-Substituted acridines of the general formula **9** can potentially exist in two tautomeric forms (Scheme 2).

It has not been possible to show the existence of 9-hydroxyacridine (**9.1**). The nonaromatic keto structure **10.1** is the only form observed.[7,8] For 9-mercaptoacridine (**9.2**) in DMSO, the thiono form (**10.2**) dominates (85%).[7]

Tautomerism of 9-aminoacridine (**9.3**) and substituted aminoacridines has been studied to some detail using quantum mechanical calculations and spectroscopic data.[9–11] The hydrogens bonded to the exocyclic nitrogen can migrate to the endocyclic nitrogen and result in the two tautomeric forms, **9.3** and **10.3**. Based on the theoretical calculations and spectroscopic investigations, it has been shown[9,10,12] that 9-acridinamine can potentially exist in two tautomeric forms — the amino or imino compounds — in equilibrium with each other at room temperature. The amino tautomer was found to be thermodynamically more stable according to the calculations. The authors have also theoretically determined the difference in energy between the amino and imino compounds to be always positive.



9.1: X = O **10.1**: X = O
9.2: X = S **10.2**: X = S
9.3: X = NH **10.3**: X = NH

Scheme 2. Tautomerism in 9-substituted acridines

For our *N*-aminoacridine derivatives, isomers were observed only for **1a** and **1b**; the amount of the isomers was found to depend on the work-up procedure. The effect of bulky substituents at the 9-position on the tautomeric ratio has been studied before.[13] The crystal structure of 9-(*tert*-butylamino)acridine was determined and it was found that the crystals contained the amino tautomeric form.

No isomers have so far been observed for the 9-aminoacridine hydrochlorides **5a**,**b** and **6a**,**b**, which were purified in the presence of HCl(aq). Elemental analysis of **6a** and **6b** showed these compounds to be singly protonated. It is known in the literature[11,14] that single protonation of both tautomers of 9-aminoacridine actually leads to the same ion. The imino form, which coexists with the amino form in the free-base species, converts to the amino form upon protonation. The tin compounds **1a**,**b** are not stable in the presence of acids and were therefore purified in the presence of a base. In this case, two isomeric forms of the compound were observed, as described earlier. From our observations, it can be said that the relative amounts of the isomers very much depends on the type of solvent and pH of the media.

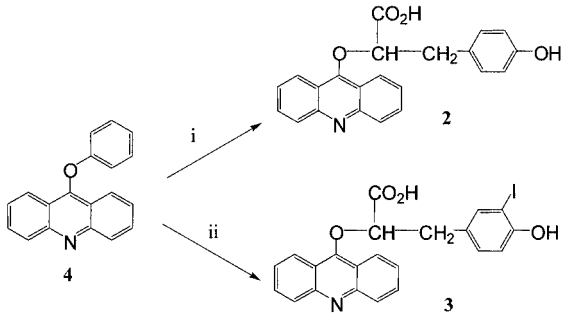
The reason for the different behaviors of our acridine derivatives is not clearly known but the data could be rationalized as follows. In the presence of acid, one of the isomers is dominant, as was the case for **5a**,**b** and **6a**,**b**. In the case of the tin compounds **1a**,**b**, an isomeric mixture is formed during the synthesis and maintained during the purification procedure. This behavior was deduced from NMR and MS experiments. Additionally, when a $CDCl_3$ solution of the minor product (**1bB**) was kept for two days at 4 °C, the spectrum changed, and some nuclear Overhauser effects (NOEs) between the 9-NH proton and the protons in posi-

tions 1(8) and 4(5) of the acridine ring system were observed, indicating that a fast equilibrium is established. Since the NH of the major product could not always be observed by NMR spectroscopy, it was not possible to draw any conclusion from NOE measurements on **1bA**.

It is tempting to suggest that the isomers observed for **1a**,**b** are tautomers but definite proof is so far lacking. This interpretation of the results is, however, supported by a similar observation for nitro-substituted aminoacridines.[15]

Synthesis of 2-(Acridin-9-ylamino)-3-(4-hydroxyphenyl)-propionic Acid (2) and its Iodinated Analog 2-(Acridin-9-ylamino)-3-(4-hydroxy-3-iodophenyl)propionic Acid (3)

Compound **2** (Scheme 3) was obtained by reacting phenoxyacridine and L-tyrosine in molten phenol to give **2** in 98% yield after chromatography and subsequent recrystallization. Similarly, the reaction of phenoxyacridine with 3-iodo-L-tyrosine gave 2-(acridin-9-ylamino)-3-(4-hydroxy-3-iodophenyl)propionic acid (**3**) quantitatively.



Scheme 3. Synthesis of compounds **2** and **3**: *i*) L-tyrosine, phenol, 100 °C, 98%; *ii*) L-3-Iodotyrosine, phenol, 100 °C, 93%

Radioiodination of the *N*-[ω-(Acridin-9-ylamino)alkyl]-3-(trimethylstannyl)benzamides 1a and 1b

In all labeling experiments the major isomers of **1a** and **1b** were used. Compound **1b** was used in experiments for finding optimal labeling conditions with regard to the amounts of substrate and oxidant as well as incubation time. The optimal conditions were found to be 35 μg of compound **1b**, 5 μL of Chloramine-T (CAT) solution (1 mg/mL MeOH) and an incubation time of 5 min. A representative example of a radiochromatogram (radio TLC) is given in Figure 1. Sodium iodide (NaI) was added as a carrier for the stabilization of $Na^{125}I$ against oxidation during chromatography.

During preliminary experiments with **1b** and NaI about 15 different eluent systems were tested (different ratio of CH_2Cl_2/CH_3OH with and without triethylamine, ethyl acetate/hexane, diethyl ether/CH_2Cl_2). The best resolution of the nonradioactive analogs (NaI and **6b**) was achieved when using CH_2Cl_2/CH_3OH (3:1, v:v).

The radio chromatogram (Figure 1) shows a small peak (1) at the start position. This is presumably oxidized iodine, whereas peak 2 is ^{125}I-iodide stabilized with NaI. Two more

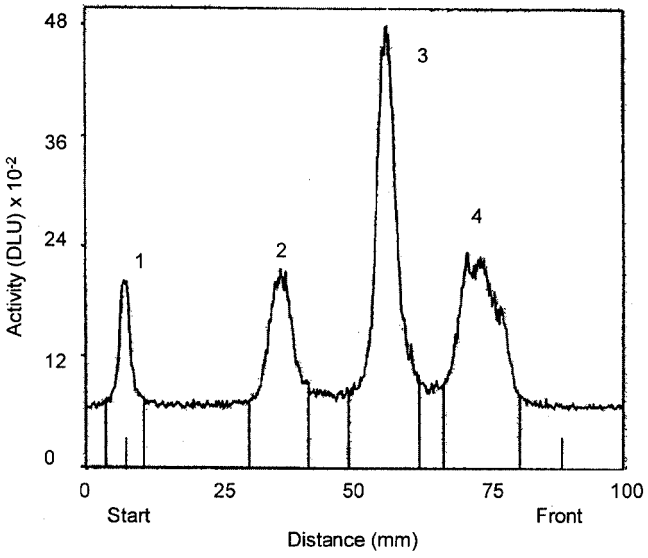


Figure 1. Radiochromatograms of a typical radioiodination with **1b**; Merck silica gel 60 F_{254} TLC aluminum sheets were used and the eluent was CH_2Cl_2/CH_3OH (3:1, v:v)

peaks are seen; peak 3 has the same R_f value as compound **6b** and is thus associated with the labeled compound. The size of peak 4 depends on the amount of CAT in the reaction. The percentage of radioactivity associated with this peak increased as the amount of CAT increased, but completely disappeared when the final optimized labeling conditions where used. The identity of the compound, however, was not determined.

The radioiodination yields obtained using **1b**, under different reaction conditions, are shown in Figure 2. The yield depends strongly on the incubation time and reaches a plateau at an incubation time of 5 min (see a in Figure 2). This result differs from previous findings for iodination of proteins with CAT in which high yields were obtained as soon

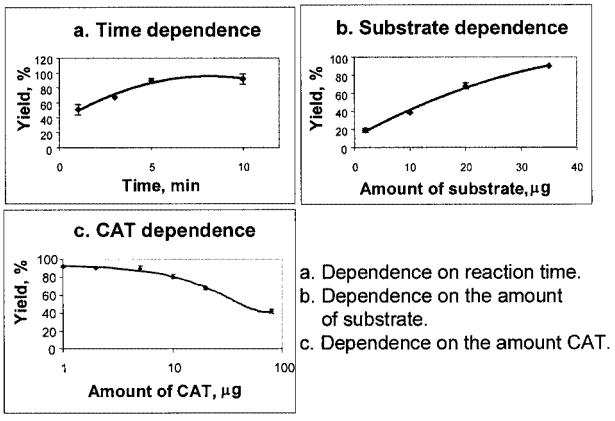


Figure 2. Dependence of radiochemical yields of **1b** on different reaction conditions; experiments were performed in duplicate; error bars were calculated as maximal errors according to the formula: $Er_{max} = (Y_{max} - Y_{min})/2$; total volume of reaction mixture was 70 μL; vessels were vortexed during reaction; the reaction was quenched with 20 μL sodium metabisulfite (3.3 mg/mL MeOH); sodium iodide (5 μL, 10 mg/mL MeOH) was added as carrier before analysis: a) **1b**: 35 μg; CAT: 5 μg.; b) CAT: 5 μg, time: 5 min; c) **1b**: 35 μg, time:5 min

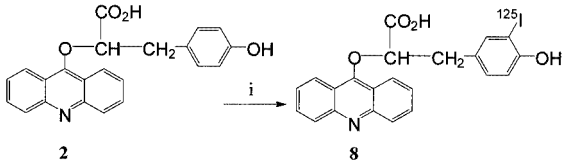
as 30 s after addition of CAT,[16] but agrees well with findings for radioiodinations of similar organotin intermediates.[17,18] For some organotin intermediates the reaction kinetics were even slower and the optimum yield was not reached until 15 min after the start of the reaction.[17–19] The radiolabeling yield decreased dramatically as the amount of acridine decreased (see b in Figure 2). The best yields (up to 92%) were obtained for the reaction mixture containing at least 35 μg of acridine (0.06 μmol). This amount of substrate is larger than that usually required for direct iodination of proteins. For example, small amounts [as little as 5 μg (0.001 μmol)] of epidermal growth factor (EGF) can be labeled efficiently with iodine.[20] When we take into account that one molecule of EGF has five tyrosine residues, the amount of substrate (0.06 μmol) required for successful labeling of acridine is 12 times larger. However, this amount of substrate (0.06 μmol) is at least 10 times smaller than that usually used for tin derivatives of *N*-succinimidyl benzoate.[17,19,21,22]

The dependence of labeling yield on the amount of CAT is given in Figure 2 (c). The best yields were reached using 1–5 μg of CAT (0.045–0.2 μmol), which corresponds to up to a 1:1 molar ratio of CAT to acridine precursor. This result is in agreement with literature data.[19] For comparison we note that the ratio for labeling of EGF was 110:1. Further increases in the amount of CAT decreased the yield of the desired product and increased the amount of the labeled by-product. This suggests that peak 4 in the radio TLC is associated with products of acridine oxidation with CAT. Under optimal labeling conditions the yield of this by-product was reduced to zero.

After optimization of the labeling protocol, the compounds **1a** and **1b** were labeled with ^{125}I and the products of reaction were separated with SepPak® Plus cartridges (5 mL of Elga© water, two times 5 mL of methanol). All fractions were checked by TLC. In both cases (**1a** and **1b**) the water fractions contained unchanged iodine and the labeled acridines **7a** and 7**b** were in the first methanol fractions. The isolated radiochemical yields of the reaction were 54% for **7a** and 75% for **7b**.

Radioiodination of 2-(Acridin-9-ylamino)-3-(4-hydroxyphenyl)propionic Acid (2)

The amino acid **2** was labeled with ^{125}I using the chloroamine-T method (Scheme 4). All parameters were optimized in the same way as used for the tin compounds **1a**,**b**. The iodo derivative **3** was used as a reference in the labeling



Scheme 4. Radioiodination of **2**: i) Na^{125}I, Chloramine-T

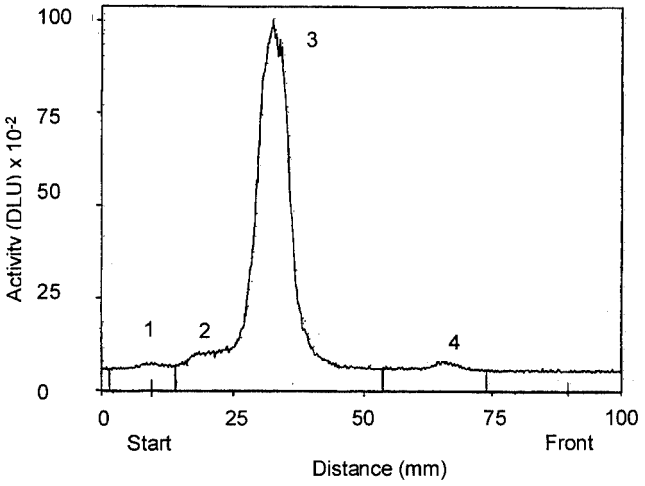


Figure 3. Radio iodination of 2-(acridin-9-ylamino)-3-(4-hydroxyphenyl)propionic acid (**2**); Merck silica gel 60 F_{254} TLC aluminum sheets were used and the eluent was CH_2Cl_2/CH_3OH (4:1, v:v)

experiment. Thin-layer chromatographic analysis (Figure 3) showed that the labeled compound (peak 3) has the same retention factor as the nonradioactive reference compound **3**. Attempts to get a better resolution were not successful although the amount of the unchanged iodide (peak 2) was very low (<2%). The reaction afforded a high labeling yield (96%). An unknown side product was observed (<2%) to come out at a higher retention time (peak 4). Peak 1 corresponds to peak 1 in Figure 1.

Experimental Section

General: ^{1}H and ^{13}C spectra were recorded in $CDCl_3$ (δ = 7.26 ppm ^{1}H; δ = 77.0 ppm ^{13}C), CD_3OD (δ = 3.35 ppm ^{1}H; δ = 49.0 ppm ^{13}C) or DMSO (δ = 2.49 ppm ^{1}H; δ = 39.5 ppm ^{13}C) on a Varian Unity 400 spectrometer operating at 400 and 100.6 MHz, respectively, a Gemini-200 spectrometer operating at 200 and 75.4 MHz, respectively, or a Unity-500 spectrometer operating at 500 and 125 MHz, respectively. For column chromatography Merck silica gel 60 (230–400 mesh) was used. TLC was performed using Merck silica gel 60 F_{254}. DMF was dried according to standard methods. LC/MS in methanol solution was conducted using an AQA mass spectrometer with an electrospray positive ionization method. Elemental analysis was carried out at Mikro Kemi AB, Uppsala. HRMS was conducted at the Organisch Chemisches Institut der Universitaet Muenster, Abt. Massenspektrometrie.

Materials for Radiolabeling: [^{125}I]NaI (3.7 GBq/mL, 644 MBq/μg I) for labeling was obtained from Amersham Int. plc. (Little Chalfont, Buckinghamshire, UK). The radio TLC strips (Merck Silica gel 60 F_{254} TLC aluminum sheets 15 × 100 mm, elution path 80 mm) were developed with CH_2Cl_2/MeOH (3:1) and measured on a Cyclon™ Storage Phosphor System and analyzed using an OptiQuant™ Image Analysis Software (Packard Instrument Company, Inc., USA). Methanol (for HPLC) obtained from Fisher Scientific UK Ltd. (Loughborough, UK) and CH_2Cl_2 (purum) obtained from KEBO lab were used as delivered. Chloramine-T and sodium metabisulfite were obtained from Sigma, and sodium iodide (p.a.) from Merck. High quality Elga© water (resistance higher than 18 MΩ/cm^3) was used for the preparation of aqueous solutions, and fresh solutions were prepared for each experiment.

Radiolabelling experiments were performed in Eppendorf tubes (1.5 mL) at room temperature. Experiments were performed in duplicate. Error bars were calculated as maximal errors according to the formula: $Er_{max} = (Y_{max} - Y_{min})/2$. SepPak® Plus cartridges (sorbent C_{18}) were from Waters Sverige AB (Sollentuna, Sweden).

9-Phenoxyacridine (4): This compound was prepared using a modified literature procedure.[4,5] 9-(10*H*)Acridone (5.00 g, 25.8 mmol) was dissolved in dry benzene. Excess $SOCl_2$ (11 mL) was added and the reaction was allowed to reflux for 5 h in the presence of a few drops of DMF. The solvent was removed under vacuum and the excess thionyl chloride was co-evaporated with benzene to give crude chloroacridine. This product was used in the next reaction without further purification. The resulting 9-chloroacridine, phenol (7.30 g, 77.3 mmol) and K_2CO_3 (30.00 g, 217 mmol) were dissolved in DMF and stirred at 100 °C under inert atmosphere for 24 h. K_2CO_3 was filtered off and the solvent was evaporated. The crude product was purified by column chromatography with CH_2Cl_2/diethyl ether (6:1) as the mobile phase to afford the desired product in 93% yield. ^{1}H and ^{13}C NMR spectra were in accordance with the literature results.[4,5]

(*N*^{1}-Acridin-9-yl)propane-1,3-diamine (5a): A solution of 9-phenoxyacridine (1.90 g, 7.01 mmol) in dry DMF was added to a solution of 1,3-diaminopropane (7.02 mL, 84.1 mmol) in dry DMF. The reaction mixture was stirred at 100 °C under a N_2 atmosphere for 22 h. The solvent was evaporated and the residue was dissolved in 3 M HCl/MeOH and stirred for a few minutes. The mixture was concentrated under vacuum. The resulting crude product was purified by flash chromatography (CH_2Cl_2/MeOH/$HCl_{concd.}$, 7:2:0.7, as the mobile phase) to afford 2.45 g of the hydrochloride salt of **5a** in 97% yield. ^{1}H NMR (CD_3OD): δ = 8.59 (d, J = 8.6 Hz, 2 H, H-4 and H-5 acr), 7.99 (m, 2 H, H-3 and H-6 acr), 7.85 (dd, J = 8.6, 0.73 Hz, 2 H, H-1 and H-8 acr), 7.60 (m, 2 H, H-2 and H-7 acr), 4.31 (t, J = 7.3 Hz, 2 H, -CH_2-NH), 3.15 (t, J = 7.5 Hz, 2 H, -CH_2-NH_3^+), 2.4 (m, 2 H, -CH_2-) ppm. ^{13}C NMR (CD_3OD): δ = 159.5, 141, 136.4, 126.5, 125.2, 119.6, 113.8, 47.2, 38.3, 28.6 ppm. ESI/MS ($C_{16}H_{17}N_3$): *m*/*z* (%) = 252 (100) $[M + H]^+$, 253 (17).

(*N*^{1}-Acridine-9-yl)octane-1,8-diamine (5b): The reaction mixture was prepared using 1,8-diaminooctane as in the procedure described for **5a**. The crude product was purified by column chromatography using CH_2Cl_2/MeOH/$HCl_{concd.}$, 4:1:0.05, as the mobile phase giving the hydrochloride salt of **5b** in 74% yield (1.76 g). ^{1}H NMR (CD_3OD): δ = 8.42 (d, J = 8.7 Hz, 2 H, H-4 and H-5 acr), 7.85 (m, 2 H, H-3 and H-6 acr), 7.78 (dd, J = 8.2 Hz, 0.73 Hz, 2 H, H-1 and H-8 acr), 7.48 (m, 2 H, H-2 and H-7 acr), 4.08 (t, J = 7.6 Hz, 2 H, -NH-CH_2-), 2.81 (t, J = 7.9 Hz, 2 H, -CH_2-NH_3^+), 2.00−1.20 (m, 12 H, -CH_2-) ppm. ^{13}C NMR (CD_3OD): δ = 159.0, 140.9, 136.3, 126.7, 125.0, 119.5, 113.6, 50.6, 40.9, 30.6, 30.0, 30.0, 28.4, 27.7, 27.3 ppm.

***N*-[3-(Acridin-9-ylamino)propyl]-3-iodobenzamide (6a·HCl):** A mixture of 3-iodobenzoic acid (1.50 g, 6.05 mmol) and excess $SOCl_2$ (6 mL) in dry benzene was refluxed for 2.5 h. The excess $SOCl_2$ and solvent were then evaporated. Benzene was added to the crude product and the solvents evaporated. This co-evaporation with benzene was done several times to completely remove traces of thionyl chloride to give 3-iodobenzoyl chloride in quantitative yield. Compound **5a** (1.70 g, 4.71 mmol) in chloroform (45 mL) was treated with NaOH (1.90 g, 47.1 mmol) in H_2O (14 mL). A solution of 3-iodobenzoyl chloride (1.40 g, 5.19 mmol) in chloroform (15 mL) was added to the reaction mixture dropwise (in 10 min) at 0 °C. The reaction was warmed to room temperature and stirred for another hour. The solvent was evaporated and the crude product was converted into its hydrochloride form by treating it with 3 M HCl/MeOH. The resulting salt was purified by column chromatography (CH_2Cl_2/MeOH/$HCl_{concd.}$, 4:1:0.1, as the mobile phase) to afford 2.00 g of compound **6a**·HCl in 88% yield. ^{1}H NMR (CD_3OD): δ = 8.50 (d, J = 8.6 Hz, 2 H, acr), 7.98 (s, 1 H, benz), 7.92 (t, J = 8.6 Hz, 2 H, acr), 7.85 (d, J = 7.8 Hz, 1 H, benz), 7.77 (d, J = 8.6 Hz, 2 H, acr), 7.67 (d, J = 7.8 Hz, 1 H, benz), 7.53 (t, J = 8.6 Hz, 2 H, acr), 7.19 (t, J = 7.8 Hz, 1 H, benz), 4.25 (t, J = 7.8 Hz, 2 H, -NH-CH_2-), 3.55 (t, J = 6.4 Hz, 2 H, CH_2-NH_3), 2.27 (m, 2 H, -CH_2-) ppm. ^{13}C NMR (CD_3OD): δ = 168.7, 160, 141.7, 137.2, 136.98, 136.3, 131.4, 127.4, 126.5 124.9, 119.7, 113.8, 96.9, 94.8, 48.0, 37.9, 30.7 ppm. $C_{23}H_{21}ClIN_3O$: calcd. C 53.3, H 4.1, N 8.1, Cl 6.9; found C 53.3, H 4.4, N 8.0, Cl 7.2.

***N*-[8-(Acridin-9-ylamino)octyl]-3-iodobenzamide Hydrochloride (6b·HCl):** This compound was obtained from **5b** according to the procedure described for **6a**. The crude product was purified by column chromatography using CH_2Cl_2/MeOH/$HCl_{concd.}$, 6:1:0.05, as the mobile phase giving **6b**·HCl in 69% yield. ^{1}H NMR (CD_3OD): δ = 8.37 (d, J = 8.4 Hz, 2 H, acr), 8.02 (s, 1 H, benz), 7.84 (m, 2 H, acr), 7.69 (m, 4 H, acr + benz), 7.40 (t, J = 7.9 Hz, 2 H, acr), 7.08 (t, J = 7.9 Hz, 1 H, benz), 4.02 (t, J = 7 Hz, 2 H, -CH_2-NH-acr), 3.20 (t, J = 7.0 Hz, 2 H, -CH_2-NH-), 2.00−1.08 (m, 12 H, CH_2-CH_2-CH_2) ppm. ^{13}C NMR (CD_3OD): δ = 168.3, 159.5, 141.5, 137.8, 137.3, 136.4, 131.3, 127.5, 126.6, 124.9, 119.6, 113.8, 97.0, 94.7, 50.5, 41.0, 30.5, 30.3, 30.1, 30.1, 27.8, 27.7 ppm. $C_{28}H_{30}ClIN_3O$: calcd. C 57.2, H 5.3, N 7.2; found C 56.6, H 5.5, N 7.4.

***N*-[3-(Acridin-9-ylamino)propyl]-3-(trimethylstannyl)benzamide (1a):** The free base **6a** was obtained by stirring the hydrochloride salt with aqueous K_2CO_3 followed by extraction with CH_2Cl_2. The organic phase was dried over $MgSO_4$. Upon filtration and evaporation of the solvent, the free base was obtained. The free base **6a** (0.415 g, 0.56 mmol) was dissolved in dry DMF (12 mL) and argon was bubbled through the solution for 5 min. Hexamethylditin (1.13 g, 3.44 mmol) was added and argon was bubbled for another 5 min. Tetrakis(triphenylphosphane)palladium(0) (0.20 g, 0.17 mmol) was finally added and the reaction mixture was stirred at 100 °C under argon for 4 h. The mixture was cooled to room temperature and the black catalyst was filtered off through celite. The solution was concentrated. The crude product was purified by column chromatography using CH_2Cl_2/MeOH (8:1) as the mobile phase and afforded two isomeric compounds **1aA** (183 mg) and **1aB** (131 mg) in a total yield of 70%.

Data for 1aA: ^{1}H NMR ($CDCl_3$): δ = 9.58 (br. s, 1 H, acr-NH-), 8.40 (br. s, 1 H, -NHCO-), 8.30 (d, J = 8.5 Hz, 2 H, H-1 and H-8), 8.20 (s with tin satellites, $J_{H,Sn}$ = 22.2 Hz, 1 H, H-2′), 8.00 (d, J = 6.8 Hz, 1 H, H-6′), 7.90 (d, J = 8.5 Hz, 2 H, H-4 and H-5), 7.52 (d, J = 6.8 Hz, 1 H, H-4′), 7.42−7.31 (m, 3 H, H-5′, H-3 and H-6), 7.24 (t, J = 8.5 Hz, 2 H, H-2 and H-7), 4.22 (t, J = 7.8 Hz, 2 H, acr-NH-CH_2-), 3.82 (t, J = 6.4 Hz, 2 H, -CH_2-NHC=O), 2.38 (m, 2 H, -CH_2-), 0.3 [s with tin satellites, $J_{H,Sn}$ = 28.0 Hz, 9 H, $Sn(CH_3)_3$] ppm. ^{13}C NMR (CD_3OD): δ = 169.6 (C=O), 157, 143.0, 139.7, 139.4, 134.7, 134.3, 132.5, 128, 127.3, 124.3, 123.8, 118.8, 113.0, 45.6, 36.9, 30.5, −9.2 [$Sn(CH_3)_3$] ppm. ESI/MS (+ve; $C_{26}H_{29}N_3OSn$): *m*/*z* (%) = 516 (40.5) $[M + H]^+$, 517 (33.1), 518 (74.9), 519 (44.6), 520 (100), 521 (27.9), 522 (16.9). HRMS (*m*/*z*) calcd. for ($C_{26}H_{29}N_3OSn + H^+$) 520.1416; found 520.1413.

Data for 1aB: ^{1}H NMR ($CDCl_3$): δ = 9.70 (br. s, 1 H, acr-NH-), 8.42 (br. s, 1 H, -NHCO-), 8.28 (d, J = 8.4 Hz, 2 H, H-1 and H-8), 8.15 (s with tin satellites, $J_{H,Sn}$ = 22.2 Hz, 1 H, H-2′), 8.00−7.95 (m, 3 H, H-6′, H-4 and H-5), 7.62 (m, 3 H, H-4′, H-3 and H-6),

7.43 (m, 1 H, H-5′), 7.34 (t, J = 8.4 Hz, 2 H, H-2 and H-7), 4.19 (t, J = 7.8 Hz, 2 H, acr-NH-CH_2-), 3.82 (m, J = 6.4 Hz, 2 H, -CH_2-NHC=O), 2.35 (m, 2 H, -CH_2-), 0.32 [s with tin satellites, $J_{H,Sn}$ = 28.0 Hz, 9 H, $Sn(CH_3)_3$] ppm. HRMS (m/z) calcd. for ($C_{26}H_{29}N_3OSn + H^+$) 520.1416; found 520.1433.

***N*-[8-(Acridin-9-ylamino)octyl]-3-(trimethylstannyl)benzamide (1b):** The hydrochloride salt of compound **6b** was converted into the free amine with aqueous K_2CO_3 and extracted with CH_2Cl_2. The organic phase was dried over magnesium sulfate. The solvent was evaporated and this free amine (108 mg, 0.196 mmol) was dissolved in dry 1,4-dioxane. The solution was bubbled with argon for 10 min in order to remove oxygen and carbon dioxide. Hexamethyldistannane (112 mg, 0.342 mmol) was added to the mixture and argon was bubbled for 4 min through the reaction mixture. Finally, bis(triphenylphosphane)palladium(II) dichloride (27.5 mg, 0.039 mmol) was added and the reaction mixture was stirred at 75 °C for 90 min. The reaction mixture was cooled to room temperature and the catalyst was filtered off through celite. After evaporation to dryness the crude mixture was applied to a flash chromatography column and eluted with the mobile phase CH_2Cl_2;MeOH, 6:1, to give two isomeric compounds **1bA** (86.0 mg) and **1bB** (17.0 mg) in a total yield of 89%.

Data for 1bA: (CH_2Cl_2/MeOH, 6:1). ^{1}H NMR ($CDCl_3$): δ = 8.87 (br. s, 1 H, -NH-acr), 8.32 (d, 2 H, H-1 and 8), 8.14 (d, 2 H, H-4 and H-5), 7.94 (s, 1 H, H-2′ with tin satellites), 7.72 (d, 1 H, H-6′), 7.61 (d, 1 H, H-4′), 7.49 (m, 2 H, H-3 and H-6), 7.40 (t, 1 H, H-5′), 7.24 (t, 2 H, H-2 and H-7), 6.44 (t, 1 H, CONH), 4.14 (t, 2 H, acr−NH-CH_2-), 3.48 (q, 2 H, -CH_2-NHCO), 2.07 (m, 2 H, acr-N-CH_2-CH_2-), 1.64 (m, 2 H, -CH_2-CH_2-NHCO-), 1.51 [m, 2 H, -acr-NH-$(CH_2)_2$-CH_2-], 1.50−1.36 (m, 6 H, -CH_2-), 0.30 [s, with tin satellites, 9 H, $Sn(CH_3)_3$] ppm. ^{13}C NMR ($CDCl_3$): δ = 168.5, 156.6, 143.4, 139.6, 139.1, 134.5, 134.4, 128.2, 126.9, 125.6, 123.8, 118.9, 112.4, 48.9, 40.2, 30.2, 29.9, 29.8, 29.0, 26.9, 26.8, −9.1 [$Sn(CH_3)_3$] ppm. HRMS (m/z) calcd. for ($C_{31}H_{39}N_3OSn + H^+$) 590.2200; found 590.2206.

Data for 1bB: (CH_2Cl_2/MeOH, 6:1). ^{1}H NMR ($CDCl_3$): δ = 9.74 (br. s, 1 H, H-10), 8.28 (d, 2 H, H-1 and H-8), 8.00 (d, 2 H, H-4 and H-5), 7.97 (s, with tin satellites, 1 H, H-2′), 7.76 (d, 1 H, H-6′), 7.61 (d, with tin satellites 1 H, H-4′), 7.44 (m, 2 H, H-3 and H-6), 7.38 (t, 1 H, H-5′), 7.20 (t, 2 H, H-2 and H-7), 6.59 (t, 1 H, CONH-), 4.10 (t, 2 H, acr-N-CH_2-), 3.49 (q, 2 H, -CH_2-NHCO-), 2.06 (m, 2 H, acr-N-CH_2-CH_2-), 1.64 (m, 2 H, -CH_2-CH_2-NHCO-), 1.53 [m, 2 H, acr-N-$(CH_2)_2$-CH_2-], 1.50−1.26 (m, 6 H, -CH_2-), 0.36 [s, with tin satellites, 9 H, $Sn(CH_3)_3$] ppm. ^{13}C NMR ($CDCl_3$): δ = 168.4, 157.0, 143.4, 139.0, 134.6, 134.4, 134.0, 128.2, 126.8, 125.4, 123.2, 119.7, 112.5, 48.7, 40.2, 30.3, 29.8, 29.2, 29.1, 26.9, −9.2 [$Sn(CH_3)_3$] ppm. HRMS (m/z) calcd. for ($C_{31}H_{39}N_3OSn + H^+$) 590.2200; found 590.2211.

Data for 1bB after 2 Days at 4 °C in $CDCl_3$ Solution: ^{1}H NMR ($CDCl_3$): δ = 9.70 (br. s, 1 H, NH-), 8.28 (d, 2 H, H-1 and H-8), 8.01 (d, 2 H, H-4 and H-5), 7.97 (s, with tin satellites, 1 H, H-2′), 7.76 (d, 1 H, H-6′), 7.62 (d, with tin satellites 1 H, H-4′), 7.45 (m, 2 H, H-3 and H-6), 7.38 (t, 1 H, H-5′), 7.20 (t, 2 H, H-2 and H-7), 6.59 (t, 1 H, CONH-), 4.10 (t, 2 H, acr-N-CH_2-), 3.48 (m, 2 H, -CH_2-NHCO-), 2.06 (qn, 2 H, acr-N-CH_2-CH_2-), 1.64 (m, 2 H, -CH_2-CH_2-NHCO-), 1.53 [m, 2 H, acr-N-$(CH_2)_2$-CH_2-], 1.50−1.26 (m, 6 H, -CH_2-), 0.32 [s, with tin satellites, 9 H, $Sn(CH_3)_3$].

L-2-(Acridin-9-ylamino)-3-(4-hydroxyphenyl)propionic Acid 2: A mixture of 9-phenoxyacridine (33 mg, 0.12 mmol), L-tyrosine (22 mg, 0.12 mmol) and phenol (2.5 g, 26.6 mmol) was stirred at 100 °C under an inert atmosphere for one hour. The reaction mixture was cooled to room temperature and purified by column chromatography (MeOH/CH_2Cl_2, 1:4, as mobile phase) to give 42 mg (98%) of **2**. The product was converted into its hydrochloride salt by treating it with 3 M HCl in MeOH followed by evaporation to dryness. The analytical sample was obtained by recrystallization from methanol/ethyl acetate. ^{1}H NMR (CD_3OD): δ = 8.30 (d, J = 8.8 Hz, 2 H, H-4 and H-5 acr), 7.88 (t, J = 8.8 Hz, 2 H, H-3 and H-6 acr), 7.71 (d, J = 8.8 Hz, 2 H, H-8 and H-1 acr), 7.48 (t, J = 8.8 Hz, 2 H, H-2 and H-7 acr), 6.8 (d, J = 8.4 Hz, 2 H, benz), 6.39 (d, J = 8.4 Hz, 2 H, benz), 5.10 (m, 1 H, CH), 3.4 (m, 1 H, CH_2), 3.2 (m, 1 H, CH_2) ppm. ^{13}C NMR (CD_3OD): δ = 172.0, 161.0, 157.7, 141, 136.7, 131.3, 127.5, 125.8, 125.4, 119.8, 116.1, 65.4, 53.7 39.2 ppm. MS (ESI+; $C_{22}H_{18}N_2O_3$ free base): m/z (%) = 381 (100) [M + Na]$^+$, 359 [M + H]$^+$. HRMS (m/z) calcd. for ($C_{22}H_{18}N_3OSn + H^+$) 359.1395; found 359.1439; ($C_{22}H_{18}N_3OSn + Na^+$) calcd. 381.1215; found 381.1194.

L-2-(Acridin-9-ylamino)-3-(4-hydroxy-3-iodophenyl)propionic Acid (3): A mixture of 9-phenoxyacridine (33 mg, 0.12 mmol), 3-iodotyrosine (37 mg, 0.12 mmol) and phenol (2.50 g, 26.6 mmol) was stirred at 100 °C under inert atmosphere for one hour. The reaction was cooled to room temperature and purified by column chromatography (4:1, CH_2Cl_2/MeOH was used as the mobile phase). Recrystallization from methanol/ethyl acetate afforded 54 mg (93%) of **3**. ^{1}H NMR (DMSO:D_2O, 4:1): δ = 8.21 (d, 2 H, H-4 and H-5 acr), 7.85 (t, 2 H, H-3 and H-6 acr), 7.69 (d, 2 H, H-1 and H-8 acr), 7.43 (t, 2 H, H-2 and H-7 acr), 6.95 (s, 1 H, benz), 6.81 (d, 1 H, benz), 6.43 (d, 1 H, benz), 4.86 (m, 1 H, CH), 3.15 (m, 2 H, CH_2) ppm. HRMS (m/z) calcd. for ($C_{22}H_{17}N_3IOSn + H^+$) 485.0362; found 485.0403.

Optimization of the Labeling of Benzamide 1bA with ^{125}I: An acridine solution in MeOH (50 μL) and [^{125}I]NaI stock solution in Elga© water (5 μL) were vortexed together. Labeling was started by addition of 5 μL CAT solution in MeOH. The mixture was vortexed for the pre-determined time. Reaction was quenched with 20 μL of sodium metabisulfite solution (1 mg/mL MeOH). NaI (5 μL, 10 mg/mL MeOH) was added as carrier before analysis. Blank experiments were performed following the same protocol but without adding acridine.

[^{125}I]-*N*-[8-(Acridin-9-ylamino)octyl]-3-iodobenzamide (7b): To an Eppendorf tube containing 3 μL of stock solution [^{125}I]NaI (3.7 MBq/μL, 0.13 nmol [^{125}I]NaI) in 15 μL of MeOH was added 35 μL of **1bA** (1 mg/mL in methanol). Reaction was initiated by addition of 5 μL of CAT solution (1 mg/mL in MeOH) and quenched with 6 μL of sodium metabisulfite solution (1 mg/mL) after 5 min of vortexing. TLC analysis of the reaction mixture (with NaI carrier) gave 85% yield of the reaction. The reaction mixture was separated on a SepPak® Plus cartridge eluting with 5 mL of Elga© water followed by two portions of methanol (5 mL). Fractions were checked by TLC. The average yield of [^{125}I]-*N*-[3-(acridin-9-ylamino)octyl]-3-iodobenzamide (**7b**), after separation, over five experiments was 75%. The specific activity was 78 kBq/μg.

[^{125}I]-*N*-[3-(Acridin-9-ylamino)propyl]-3-iodobenzamide (7a): This compound was obtained and purified similarly to **7b** with a yield of 56% and a specific activity of 54 kBq/μg.

Radioiodination of L-2-(Acridin-9-ylamino)-3-(4-hydroxyphenyl)propionic Acid (2): To a solution of 5 μL of Na^{125}I and 50 μL of the precursor **2** (0.98 mg in mixture 650 μL water and 50 μL MeOH) was added 20 μL of chloramine-T (2 mg/mL in water). The mixture was incubated for 3 min. The reaction was then quenched with 20 μL of $Na_2S_2O_5$ (4 mg/mL in water). 10 μL of NaI (10 mg/mL in water) was finally added to stabilize the radioiodine. A blank experiment was performed following the same procedure but with-

out adding the precursor. The reaction mixture was analyzed by radio-TLC (solvent system CH_2Cl_2/MeOH, 4:1) and provided 96% of the labeled compound **8.**

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