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Synthesis of several 2,7-dibromoacridine derivatives, including 4-[2-(succinimidyloxycarbonyl)ethyl]phenyl 2,7-dibromo-10-methylacridinium-9-carboxylate trifluoromethanesulfonate

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Dedicated to the memory of Professor Alan R. Katritzky and Professor Charles W. Rees

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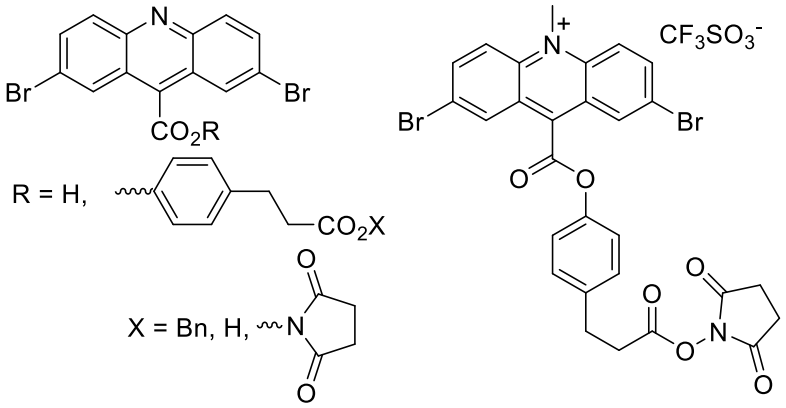
Abstract

The reaction of bis(4-bromophenyl)amine and oxalyl chloride gave *N*-(4-bromophenyl)-5-bromoisatin, which on treatment with potassium hydroxide yielded 2,7-dibromoacridine-9-carboxylic acid. Treatment of acridine-9-carboxylic acid with freshly redistilled thionyl chloride followed by reaction with benzyl 3-(4-hydroxyphenyl)propanoate gave the corresponding benzyl ester. Hydrolysis of the benzyl ester gave the corresponding acid, which, on reaction with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide, gave the corresponding ester. Finally, methylation of the *N*-hydroxysuccinimide ester gave the corresponding 10-methylacridinium trifluoromethanesulfonate.



Keywords: 2,7-Dibromoacridine-9-carboxylic acid; acridinium ester; synthesis; heterocycles; methylation

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Introduction

The global surge in disease prevalence underscores the urgency of developing innovative diagnostic tools, such as the use of chemiluminescent labeling in immunodiagnostics.[1] Chemiluminescent compounds are used for detecting, measuring, and imaging analytes.[2] The synthesis of new chemiluminescent compounds is crucial for discovering novel properties and achieving high sensitivity. Acridinium esters (AEs) act as a key component of labels and have garnered significant attention.[3–8] Our research, therefore, aimed to design and synthesize novel AEs with enhanced performance. Notably, we have recently demonstrated that structural modifications can profoundly influence the chemiluminescent properties of these esters.[9–13] For example, we have recently shown how electron-withdrawing substituents impact the chemiluminescence properties.[13]

An acridinium ester (AE) label consists of four units (Figure 1a): a quaternising group (e.g., methyl) that forms the quaternary nitrogen, which is necessary for encouraging reaction with hydrogen peroxide; an acridine moiety, which ultimately gives rise to the species responsible for the luminescent emission; an aryloxy-leaving group that is eliminated during the reaction with peroxide to create a key dioxetanone intermediate (Figure 1b); and an active moiety (e.g., an *N*-hydroxysuccinimide ester) capable of attaching to a biological molecule (e.g., a protein, oligonucleotide or nucleic acid). Modifications of the basic AE have led to the development of new probes with improved properties for various applications.[14–19]

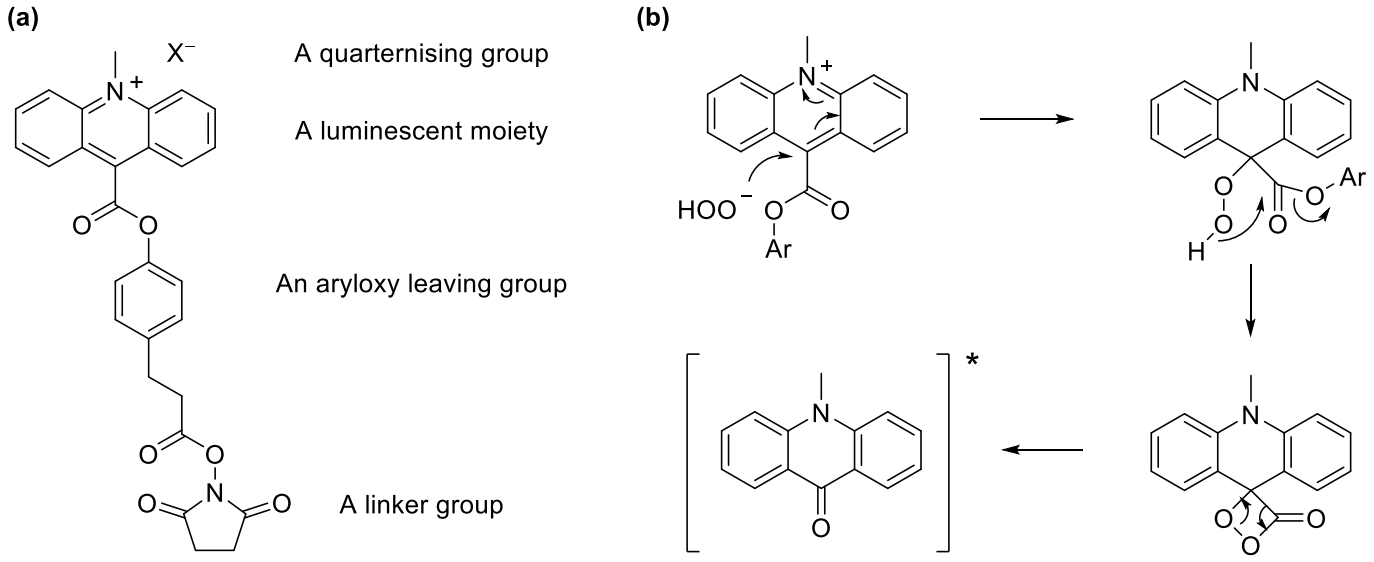


Figure 1. (a) The basic AE for probes production and (b) a typical AE chemiluminescent reaction.

Some years ago, we embarked upon a project aimed at developing a more direct approach to a range of differently-substituted acridinium esters by generating 2,7-dibromoacridine-9-carboxylic acid and using that, or a simple ester, as a common precursor. Substitution of the bromines by many different groups, using techniques such as Br-Li exchange, cross-coupling reactions, and so on, would obviate the need for synthesis of new diarylamines and isatins for every new disubstituted acridine carboxylic acid required. Unfortunately, the project was never completed, although we did generate 2,7-dibromoacridine-9-carboxylic acid and convert it into a single new acridinium ester, 4-[2-(succinimidyloxycarbonyl)ethyl]phenyl 2,7-dibromo-10-methylacridinium-9-carboxylate trifluoromethanesulfonate, i.e., the 2,7-dibromo analogue of the basic AE shown in Figure 1(a). In the intervening period, 2,7-dibromoacridine-9-carboxylic acid has been generated by

another group and converted into several different esters and their 10-methylacridinium counterparts,[20] although not including the ones reported here. Furthermore, no experimental details of the synthetic procedures or characterization details were provided for the products other than the final acridinium esters. Therefore, we believe it is important to provide details of the work that we carried out, and such details are provided in this paper.

Results and Discussion

Scheme 1 represents the synthetic route for 4-[2-(succinimidyloxycarbonyl)ethyl]phenyl 2,7-dibromo-10-methylacridinium-9-carboxylate trifluoromethanesulfonate (**8**).

Benzyl 3-(4-hydroxyphenyl)propanoate (**1**) was prepared in 88% yield using a standard method from the reaction of 3-(4-hydroxyphenyl)propanoic acid and benzyl chloride in the presence of dibenzo-18-crown-6 under basic conditions.[21] The ^{1}H NMR spectrum showed an exchangeable singlet at 6.35 ppm due to the OH proton. It also showed protons due to three different CH_2 groups at 5.17 (singlet), 2.95 (triplet, 7.8 Hz), and 2.72 (triplet, 7.8 Hz). The ^{13}C NMR spectrum of **1** showed the carbonyl carbon at 173.6 ppm. The electrospray (ES^+) mass spectrum of **1** showed a pseudo molecular ion peak (52%) at *m*/*z* = 274, shown by high-resolution mass spectrometry (HRMS) to be due to the $[M + NH_4^+]$ ion.

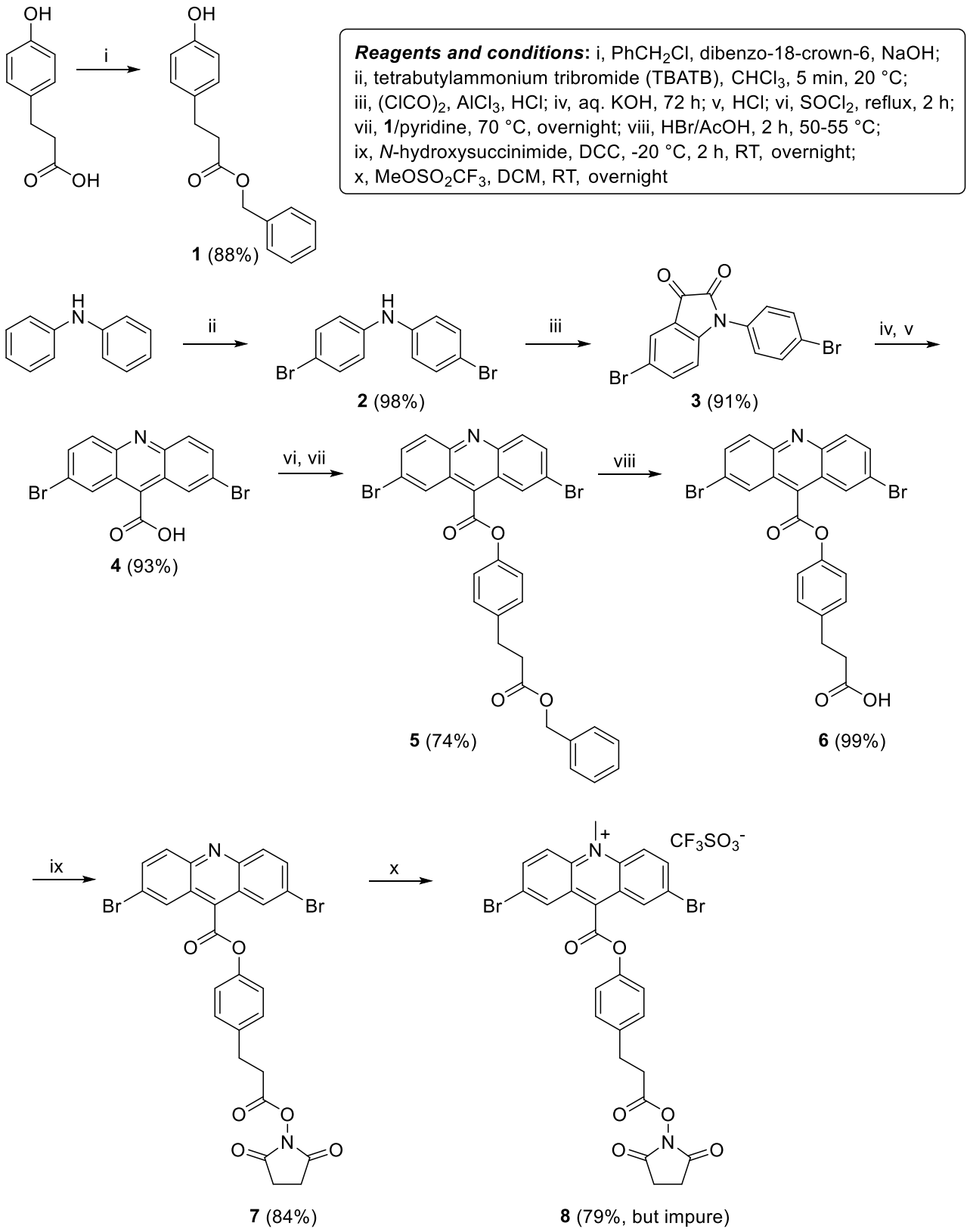
Bis(4-bromophenyl)amine (**2**) was prepared in 98% yield according to a standard literature procedure from bromination of diphenylamine with tetrabutylammonium tribromide (TBATB) in chloroform ($CHCl_3$) at room temperature.[22] NMR and mass spectrometry confirmed the structure of **2**. For example, the ^{1}H NMR spectrum showed an exchangeable singlet at 5.70 ppm due to the NH proton. In addition, it showed the aromatic protons as two doublets (*J* = 8.6 Hz), four protons each, at 7.38 and 6.94 ppm. The ^{13}C NMR spectrum of **2** showed the aromatic carbons at the expected chemical shifts. The atmospheric pressure chemical ionization (APCI) mass spectrum of **2** showed a pseudo molecular ion cluster, with one peak (18%) at *m*/*z* = 326 shown by HRMS measurement to correspond to the $[MH^{79}Br_2]^+$ ion.

Reaction of **2** and oxalyl chloride $[ClCO)_2]$ in dichloromethane (DCM) followed by treatment with aluminum chloride ($AlCl_3$) gave *N*-(4-bromophenyl)-5-bromoisatin (**3**) in 91% yield.[23] The ^{1}H NMR spectrum of **3** showed two doublets (*J* = 8.7 Hz) at 7.80 and 7.42 ppm due to the protons of the 4-bromophenyl ring. The other three aromatic protons appeared at 7.83 (doublet, 2.1 Hz), 7.76 (doublet of doublets, 2.1 and 8.5 Hz), and 6.84 (doublet, 8.5 Hz) ppm. The ^{13}C NMR spectrum showed the carbons at the expected chemical shifts. The electron ionization (EI) mass spectrum of **3** showed a molecular ion cluster, including a peak (12%) at *m*/*z* = 379, shown by HRMS to be due to the $[M^{79}Br_2]^+$ ion.

Treatment of **3** with potassium hydroxide (KOH) under reflux conditions for 72 h gave 2,7-dibromoacridine-9-carboxylic acid (**4**) in 93% yield. The ^{1}H NMR spectrum of **4** showed the aromatic protons as two doublets, two protons each, at 8.32 (2.1 Hz) and 8.18 (9.3 Hz) ppm, and a doublet of doublets at 8.05 ppm (2.1 and 9.3 Hz). It should be noted that the carboxyl proton was not seen in the ^{1}H NMR spectrum. The EI mass spectrum of **4** showed a molecular ion cluster, which included a peak (75%) at *m*/*z* = 383, shown by HRMS to be due to the $([M^{81}Br_2]^+$ ion.

A mixture of **4** and freshly redistilled thionyl chloride ($SOCl_2$) was heated under reflux for 2 h to afford the corresponding acid chloride and allowed to react with **1** in anhydrous pyridine (Scheme 1). The mixture was stirred for 18 h at 70 °C. It was cooled and poured into a dilute hydrochloric acid (HCl) solution to give a yellow solid. Ester **5** was obtained with a yield of 74% after purification through column chromatography. The ^{1}H NMR spectrum of **5** showed protons due to three different CH_2 groups at 5.17 (singlet), 3.08 (triplet, 7.6 Hz),

and 2.78 (triplet, 7.6 Hz) ppm in addition to 15 aromatic protons. The ^{13}C NMR spectrum of **5** showed the presence of two carbonyl carbons at 172.5 and 164.8 ppm. The EI mass spectrum showed a molecular ion cluster, with one peak (3%) at *m/z* = 617, corresponding to the $[M^{79}Br_2]^+$ ion, as shown by HRMS.



Scheme 1. Synthetic route for AE **8**.

A solution of the benzyl ester **5** in a mixture of hydrogen bromide (HBr) and acetic acid (AcOH) was heated for 2 h at 50–55 °C and then poured into water to give **6** in 99% yield. The ^{1}H NMR spectrum of **6** showed the presence of 10 aromatic protons, confirming that hydrolysis had taken place. The ^{13}C NMR spectrum showed the presence of two carbonyl carbons at 174.1 and 164.8 ppm due to the CO_2H and CO_2Ar groups, respectively. The EI mass spectrum showed a molecular ion cluster with a peak (4%) at *m/z* = 527, shown by HRMS to be due to the $[M^{79}Br_2]^+$ ion.

A mixture of *N*-hydroxysuccinimide (NHS), dicyclohexylcarbodiimide (DCC), and **6** was stirred in anhydrous dimethyl formamide (DMF) at –20 °C for 2 h and then overnight at room temperature (RT) to produce **7** in 84% yield. The ^{1}H NMR spectrum of **7** showed the presence of the *N*-hydroxysuccinimide protons as a broad singlet at 2.84 ppm. The EI mass spectrum showed a molecular ion cluster with a peak (4%) at *m/z* = 624, corresponding to the $[M^{79}Br_2]^+$ ion, as confirmed by HRMS.

The reaction of **7** with methyl trifluoromethanesulfonate ($MeOSO_2CF_3$) in anhydrous DCM under nitrogen gave the final product, 4-[2-(succinimidyloxycarbonyl)ethyl]phenyl 2,7-dibromo-10-methylacridinium-9-carboxylate trifluoromethanesulfonate (**8**), along with an impurity, in a combined yield of 79% (Scheme 1). Several attempts were made to purify compound **8** by selective extraction of the impurity, but were unsuccessful. Lack of time at the end of the project prevented further attempts at purification, so characterization depended on the NMR spectrum of the mixture. It was difficult to obtain a good NMR spectrum when DMSO-d_6 was used as a solvent because of the overlap of its signal and that of its water contaminant with those of compound **8**, so the best-resolved spectrum was that in $CDCl_3$. However, the poor solubility of **8** in $CDCl_3$ may have resulted in an exaggerated set of signals for the impurity. Nevertheless, because the recorded spectrum showed an approximate 2:1 ratio of **8**:impurity, it was easily possible to determine which signals belonged to which compound from the ^{1}H NMR spectrum in $CDCl_3$. For example, it showed the following signals for compound **8**: a doublet at 8.78 ppm (9.6 Hz), attributed to H4 and H5 of the acridinium moiety; a doublet with a small coupling constant (2.1 Hz) at 8.45 ppm for the H1 and H8 protons of the acridinium ring; a doublet of doublets (2.1 and 9.6 Hz) at 8.43 ppm corresponding to H3 and H6 of the acridinium ring; a 3H singlet at 5.07 ppm due to the protons of the methyl group attached to the nitrogen of the acridinium ring; multiplets at around 3.1 and 2.9 ppm for the two CH_2 groups of the linker chain; and a singlet at 2.80 ppm for the NHS protons. The HRMS of **8** confirmed one of the pseudo molecular ion peaks ($[M^{79}Br_2 – CF_3SO_3]^+$) as $C_{28}H_{21}N_2O_6Br_2$. Therefore, the ^{1}H NMR and mass spectral data confirmed the structure of **8**. The minor component showed no 3H integral corresponding to an N-Me group, but showed signals corresponding to all other signals of **8**, in the same sequence and with similar chemical shift values, indicating that the minor component almost certainly incorporated a positive charge on nitrogen. The only realistic possibility is that the substituent on nitrogen was a proton. Unfortunately, the proton signal was either too broad to see or fell outside the spectral range recorded, but nevertheless the structure is likely to be a salt of the precursor molecule **7** with CF_3SO_3H present in the $MeOSO_2CF_3$ reagent or formed by its hydrolysis during work-up.

Conclusions

A new acridinium ester, 4-[2-(succinimidyloxycarbonyl)ethyl]phenyl 2,7-dibromo-10-methylacridinium-9-carboxylate trifluoromethanesulfonate, was synthesized in nine steps starting from commercially available starting materials. Various analytical techniques, including NMR spectroscopy and mass spectrometry,

confirmed the structures of all the compounds produced. The product or its intermediates may be useful for the synthesis of a range of other acridine compounds substituted in the acridine ring.

Experimental Section

General. The Aldrich Chemical Company provided the chemicals and reagents. Thionyl chloride and solvents were freshly distilled. Melting point determinations were performed using the open capillary method using a Gallenkamp melting point apparatus. Bruker AV400 or AV500 spectrometers operating at 400 or 500 MHz for ^{1}H and 100 or 125 MHz for ^{13}C measurements were used to record the NMR spectra. Chemical shifts (δ) and coupling constants (*J*) are reported in parts per million (ppm) and Hz, respectively. The ^{13}C multiplicities are based on DEPT signals and are reported as s (C), d (CH), t (CH_2), and q (CH_3). Signal assignments within NMR spectra are based on expected chemical shift values and coupling patterns and have not been rigorously verified. Low and high-resolution electron impact (EI) mass spectra were recorded on a GCT-Premier spectrometer using 70 eV. A Waters LCT Premier XE instrument was used to run atmospheric pressure chemical ionization mass spectra (APCI). Electrospray (ES) analyses were performed on a ZQ4000 spectrometer in positive ionization mode. Fischer Scientific silica 60A (35–70 microns) was used for column chromatography purifications.

Benzyl 3-(4-hydroxyphenyl)propanoate (1). Compound **1** (3.39 g, 13.24 mmol; 88%) was obtained as a colorless oil from 3-(4-hydroxyphenyl)propanoic acid (2.50 g, 15.06 mmol), based on a literature procedure.[21] ^{1}H NMR (500 MHz; $CDCl_3$): 7.43–7.35 (m, 5H, Ph), 7.06 (d, 9.0 Hz, 2H, H3/H5 of Ar), 6.80 (d, 9.0 Hz, 2H, H2/H6 of Ar), 6.35 (s, exch., 1H, OH), 5.17 (s, 2H, C*H*$_2$Ph), 2.95 (t, 7.8 Hz, 2H, C*H*$_2$Ar), 2.72 (t, 7.8 Hz, 2H, CH_2CO). ^{13}C NMR (125 MHz; $CDCl_3$): 173.6 (s, C=O), 154.4 (s, C1 of Ar), 135.8 (s, C1 of Ph), 132.1 (s, C4 of Ar), 129.5 (d, C3/C5 of Ar), 128.6 (d, C3/C5 of Ph), 128.33 (d, C4 of Ph), 128.28 (d, C2/C6 of Ph), 115.5 (d, C2/C6 of Ar), 66.6 (t, *C*H_2Ph), 36.4 (t, CH_2*C*H_2CO_2), 30.2 (t, *C*$H_2CH_2CO_2$). ES$^+$-MS, *m/z* (%): 530 ([2 M + NH_4]$^+$, 100), 274 ([M + NH_4]$^+$, 52) 220 (5). HRMS (ES$^+$): calcd for $C_{16}H_{20}NO_3$ ([M + NH_4]$^+$): 274.1443; found: 274.1438.

Bis(4-bromophenyl)amine (2). Compound **2** was prepared from diphenylamine (3.40 g, 20.01 mmol) based on a standard literature procedure.[22] Compound **2** (6.41 g, 19.6 mmol; 98%) was produced as colourless crystals, Mp 107–108 °C (lit.[22] 106 °C) ^{1}H NMR (500 MHz; $CDCl_3$): 7.38 (d, 8.6 Hz, 4H, H3/H5 of 4-bromophenyl), 6.94 (d, 8.6 Hz, 4H, H2/H6 of 4-bromophenyl), 5.70 (s, exch., 1H, NH). ^{13}C NMR (125 MHz; $CDCl_3$): 141.9 (s, C1 of 4-bromophenyl), 132.3 (d, C3/C5 of 4-bromophenyl), 119.6 (d, C2/C6 of 4-bromophenyl), 113.5 (s, C4 of 4-bromophenyl). APCI-MS: *m/z* (%) = 371 ([M^{81}Br$_2$ + MeCNH]$^+$, 52), 369 ([M^{81}Br^{79}Br + MeCNH]$^+$, 100), 367 ([M^{79}Br$_2$ + MeCNH]$^+$, 52), 330 ([MH^{81}Br$_2$]$^+$, 17), 328 ([MH^{81}Br^{79}Br]$^+$, 32), 326 ([MH^{79}Br$_2$]$^+$, 18), 279 (10), 205 (6), 115 (6). HRMS (APCI): calcd for $C_{12}H_{10}{}^{79}Br_2N$ (MH$^+$): 325.9180; found: 325.9187.

***N*-(4-Bromophenyl)-5-bromoisatin (3).** Compound **3** was prepared from **2** (4.58 g, 14.01 mmol) based on a literature procedure.[23] Compound **3** (4.85 g, 12.73 mmol; 91%) was obtained as orange crystals, Mp 235–236 °C (lit.[22], Mp 235–236 °C). ^{1}H NMR (400 MHz; $CDCl_3$): 7.83 (d, 2.1 Hz, 1H, H4), 7.80 (d, 8.7 Hz, 2H, H2/H6 of 4-bromophenyl), 7.76 (dd, 2.1 and 8.5 Hz, 1H, H6), 7.42 (d, 8.7 Hz, 2H, H3/H5 of 4-bromophenyl), 6.84 (d, 8.5 Hz, 1H, H7). ^{13}C NMR (100 MHz; $CDCl_3$): 181.7 (s, C3), 157.4 (s, C2), 150.1 (s, C7a), 140.1 (d, C6), 133.3 (d, C3/C5 of 4-bromophenyl), 132.9 (s, C1 of 4-bromophenyl), 129.0 (d, C2/C6 of 4-bromophenyl), 127.4 (d, C4), 121.8 (s, C3a), 120.0 (s, C4 of 4-bromophenyl), 115.8 (s, C5), 113.3 (d, C7). EI-MS: *m/z* (%) = 383 ([M^{81}Br$_2$]$^+$, 12), 381 ([M^{81}Br^{79}Br]$^+$, 20), 379 ([M^{79}Br$_2$]$^+$, 12), 274 (93), 272 (100), 246 (18), 244 (19), 165 (22), 112 (24). HRMS (EI): calcd for $C_{14}H_7{}^{79}Br_2NO_2$ (M$^+$): 378.8844; found: 378.8856.

2,7-Dibromoacridine-9-carboxylic acid (4). A mixture of **3** (1.94 g, 5.00 mmol) and KOH (7.00 g, 125 mmol) in water (140 mL) was refluxed for 72 h. The orange solid turned yellow and then dissolved to give a green solution, which turned yellow as the reaction proceeded. The resulting yellow solution, after cooling, was poured into a mixture of HCl (*ca*. 11 M, 15 mL) and ice (60 g). The yellow solid obtained was collected by filtration, washed with water (3 × 40 mL) and methanol (2 × 40 mL), and then dried in a vacuum oven at 60 °C overnight to give **4** (1.81 g, 4.66 mmol; 93%), Mp 311–313 °C. ^{1}H NMR (400 MHz; DMSO-d_6): 8.32 (d, 2.1 Hz, 2H, H1/H8), 8.18 (d, 9.3 Hz, 2H, H4/H5), 8.06 (dd, 2.1 and 9.3 Hz, 2H, H3/H6). ^{13}C NMR (100 MHz; DMSO-d_6): 167.2 (s, C=O), 147.2 (s, C4a/C10a), 143.7 (s, C9), 133.3 (d, C3/C6), 131.1 (d, C4/C5), 129.8 (d, C1/C8), 122.2 (s, C8a/C9a), 118.1 (s, C2/C7). EI-MS: *m/z* (%) = 383 ([$M^{81}Br_2$]$^+$, 75), 381 ([$M^{81}Br^{79}Br$]$^+$, 100), 379 ([$M^{79}Br_2$]$^+$, 87), 366 (6), 364 (13), 362 (8), 339 (42), 337 (88), 335 (44), 303 (22), 301 (23), 274 (12), 272 (13), 257 (33), 255 (32), 246 (19), 244 (20), 221 (11), 207 (14), 177 (61), 150 (23). HRMS (EI): calcd for $C_{14}H_7{}^{79}Br_2NO_2$ (M^+): 378.8844; found: 378.8848.

2,7-Dibromoacridine-9-carbonyl chloride. A mixture of 2,7-dibromoacridine-9-carboxylic acid (1.00 g, 2.63 mmol) and freshly distilled $SOCl_2$ (15 mL) was refluxed under anhydrous conditions for 1 h. The excess $SOCl_2$ was removed under reduced pressure to leave a yellow solid (*ca.* 1.05 g, 2.63 mmol; 100%), which was used straight away for the next reaction.

4-[2-(Benzyloxycarbonyl)ethyl]phenyl 2,7-dibromoacridine-9-carboxylate (5). Benzyl 3-(4-hydroxyphenyl)propanoate (**1**) (0.770 g, 3.00 mmol) in anhydrous pyridine (5 mL) was added to a solution of 2,7-dibromoacridine-9-carbonyl chloride (*ca*. 1.05 g, 2.63 mmol) in anhydrous pyridine (10 mL). The mixture was stirred at 70 °C overnight and then cooled to RT. The mixture was poured into an HCl solution (1 M, 40 mL) to give a greyish-yellow solid. The solid was collected by filtration, washed with water (3 × 30 mL), and dried. Purification of the crude product by column chromatography (silica gel; Et_2O–hexane, 1:2) gave **5** (1.20 g, 1.95 mmol; 74%) as a light yellow solid, Mp 164–165 °C. ^{1}H NMR (400 MHz; $CDCl_3$): 8.41 (d, 2.0 Hz, 2H, H1/H8), 7.18 (d, 9.3 Hz, 2H, H4/H5), 7.90 (dd, 2.0 and 9.3 Hz, 2H, H3/H6), 7.41–7.35 (m, 9H, Ph and Ar), 5.17 (s, 2H, C*H*$_2$Ph), 3.08 (t, 7.6 Hz, 2H, C*H*$_2$Ar), 2.78 (t, 7.6 Hz, 2H, CH_2CO). ^{13}C NMR (100 MHz; $CDCl_3$): 172.5 (s, *C*O_2CH_2Ph), 164.8 (s, *C*O_2Ar), 148.6 (s, C4a/C10a), 146.7 (s, C1 of Ar), 139.3 (s, C4 of Ar), 135.8 (s, C1 of Ph), 134.7 (d, C3/C6), 133.6 (s, C9), 131.4 (d, C4/C5), 129.9 (d, C3/C5 of Ar), 128.6 (d, C3/C5 of Ph), 128.4 (d, C2/C6 of Ph), 127.0 (d, C1/C8 and C4 of Ph), 123.5 (s, C8a/C9a), 123.1 (s, C2/C7), 121.4 (d, C2/C6 of Ar), 66.5 (t, *C*H_2Ph), 35.8 (t, $CH_2CH_2CO_2$), 30.4 (t, *C*$H_2CH_2CO_2$). EI-MS: *m/z* (%) = 621 ([$M^{81}Br_2$]$^+$, 3), 619 ([$M^{81}Br^{79}Br$]$^+$, 9), 617 ([$M^{79}Br_2$]$^+$, 3), 515 (7), 517 (37), 519 (12), 487 (11), 485 (40), 483 (12), 367 (64), 365 (100), 363 (92), 336 (50), 334 (50), 257 (51), 255 (52), 176 (49), 149 (33), 123 (30), 91 (55). HRMS (EI): calcd for $C_{30}H_{21}{}^{79}Br_2NO_4$ (M^+): 616.9837; found: 616.9839.

4-(2-Carboxyethyl)phenyl 2,7-dibromoacridine-9-carboxylate (6). A mixture of the benzyl ester **5** (1.24 g, 2.00 mmol), HBr (12 mL; 48%), and AcOH (24 mL) was stirred for 2 h at 50–55 °C. The solution was poured into water (150 mL), and the yellow solid obtained was filtered, washed with water (3 x 25 mL), and dried under reduced pressure to give **6** (1.06 g, 1.98 mmol; 99%), Mp 245–247 °C. ^{1}H NMR (500 MHz; DMSO-d_6): 8.47 (d, 2.1 Hz, 2H, H1/H8), 8.20 (d, 9.2 Hz, 2H, H4/H5), 8.08 (dd, 2.1 and 9.2 Hz, 2H, H3/H6), 7.52 (d, 8.6 Hz, 2H, H3/H5 of Ar), 7.45 (d, 8.6 Hz, 2H, H2/H6 of Ar), 2.92 (t, 7.6 Hz, 2H, C*H*$_2$Ar), 2.62 (t, 7.6 Hz, 2H, CH_2CO). ^{13}C NMR (125 MHz; DMSO-d_6): 173.7 (s, *C*O_2CH_2), 164.1 (s, *C*O_2Ar), 148.1 (s, C4a/C10a), 146.5 (s, C1 of Ar), 139.7 (s, C4 of Ar), 134.5 (d, C3/C6), 132.6 (s, C9), 131.9 (d, C4/C5), 129.7 (d, C3/C5 of Ar), 126.7 (d, C1/C8), 122.7 (s, C8a/C9a), 122.6 (s, C2/C7), 121.5 (d, C2/C6 of Ar), 35.1 (t, $CH_2CH_2CO_2$), 29.7 (t, *C*$H_2CH_2CO_2$). EI-MS: *m/z* (%) = 531 ([$M^{81}Br_2$]$^+$, 4), 529 ([$M^{81}Br^{79}Br$]$^+$, 11), 527 ([$M^{79}Br_2$]$^+$, 4), 487 (2), 485 (6), 483 (2), 366 (80), 364 (100), 362 (85), 338 (63), 336 (94), 334 (85), 257 (60), 255 (47), 177 (54), 150 (14), 107 (56). HRMS (EI): calcd for $C_{23}H_{15}{}^{79}Br_2NO_4$ (M^+): 526.9368; found: 526.9365.

4-[2-(Succinimidyloxycarbonyl)ethyl]phenyl 2,7-dibromoacridine-9-carboxylate (7). NHS (0.43 g, 0.36 mmol) and **6** (0.19 g, 0.36 mmol) were dissolved in anhydrous DMF (5 mL) and cooled at –20 °C. DCC (0.086 g, 0.42 mmol) was added, and the mixture was kept at -20 °C for 2 h. Following stirring the mixture overnight at RT, the solvent was removed under reduced pressure. The solid obtained was washed with anhydrous DCM (2 × 10 mL) and filtered. The solvent was removed under reduced pressure. Drying the solid obtained under reduced pressure gave **7** (0.19 g, 0.30 mmol; 84%) as a yellow solid, Mp 268–270 °C. ^{1}H NMR (400 MHz; DMSO-d_6): 8.52 (d, 2.1 Hz, 2H, H1/H8), 8.24 (d, 9.3 Hz, 2H, H4/H5), 8.11 (dd, 2.1 and 9.3 Hz, 2H, H3/H6), 7.57 (d, 8.9 Hz, 2H, H3/H5 of Ar), 7.54 (d, 8.9 Hz, 2H, H2/H6 of Ar), 3.15-3.04 (m, 4H, CH_2CH_2), 2.84 (br s, 4H, 2 CH_2 of NHS). EI-MS: *m/z* (%) = 628 ([$M^{81}Br_2$]$^+$, 2), 626 ([$M^{81}Br^{79}Br$]$^+$, 6), 624 ([$M^{79}Br_2$]$^+$, 4), 513 (6), 511 (10), 509 (6), 366 (88), 364 (100), 362 (90), 336 (72), 257 (49), 255 (52), 176 (25), 149 (12), 90 (94). HRMS (EI): calcd for $C_{27}H_{18}{}^{79}Br_2NO_6$ (M^+): 623.9517; found: 623.9532.

4-[2-(Succinimidyloxycarbonyl)ethyl]phenyl 2,7-dibromo-10-methylacridinium-9-carboxylate trifluoromethanesulfonate (8). $MeOSO_2CF_3$ (1.00 mL, excess) was added to a stirred solution of **7** (0.070 g, 0.112 mmol) in anhydrous DCM (8 mL). The mixture was stirred overnight at RT. The yellow solid formed was collected by filtration and washed with Et_2O (10 × 5 mL), then dried under reduced pressure to give impure **8** (0.070 g, 0.089 mmol, 79% if pure), Mp 169–172 °C. ^{1}H NMR ($CDCl_3$): 8.84 (d, 9.6 Hz, 2H, H4/H5), 8.51 (d, 2.1 Hz, 2H, H1/H8), 8.49 (dd, 2.1 and 9.6 Hz, 2H, H3/H6), 7.50–7.38 (m, 4H, H2, H3, H5, H6 of Ar), 5.15 (s, 3H, Me), 3.16 (t, 7.2 Hz, 2H, C*H*$_2$Ar), 2.99 (t, 7.2 Hz, 2H, CH_2CO), 2.86 (br s, 4H, 2 CH_2 of NHS). ES$^+$-MS: *m/z* (%) = 643 ([$M^{81}Br_2 - CF_3SO_3$]$^+$, 52), 641 ([$M^{81}Br^{79}Br - CF_3SO_3$]$^+$, 100), 639 ([$M^{79}Br_2 - CF_3SO_3$]$^+$, 53), 571 (8), 239 (41). HRMS (ES$^+$) calcd for $C_{28}H_{21}N_2O_6Br_2$ ([$M^{79}Br_2 - CF_3SO_3$]$^+$): 638.9766; found: 638.9753.

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Supplementary Material

The Supporting Information is available free of charge and contains the NMR spectra of the synthesized compounds.

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