# Synthesis of Monomeric Acridine Derived Nucleic Acid Intercalators

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A series of antiviral compounds consisting of an intercalating acridine derived part, a spacer region and a reactive EDTA-derived conjugate was synthesized in an easy sequence. In the presence of ascorbate a reduction of the phage-titer of MS2 phages by several logarithmic decades was achieved.

*Key words:* Acridine, Antivirals, Intercalators, Fenton Mechanism

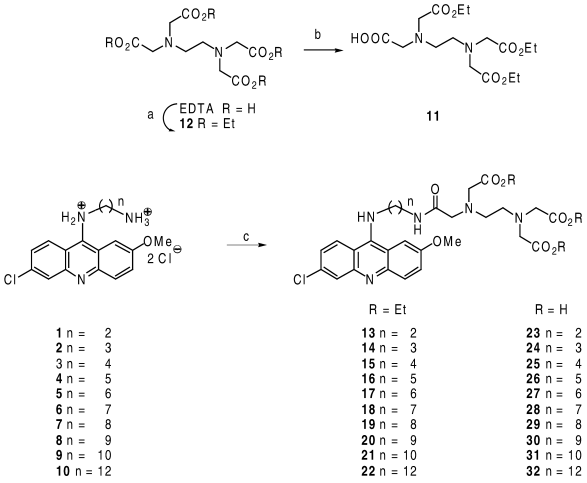
## Introduction

The blood supply in industrialized countries is safer than ever. However, blood is a natural vehicle for the transmission of infectious agents. In recent years, numerous pathogens have emerged in Europe, the United States and worldwide with the potential to affect the safety of the blood supply. Although the movement of transfusable blood and blood components between countries is relatively uncommon, infectious agents can cross international borders, however, through migration or travel. Finally, variant forms of recognized pathogens can potentially affect the safety of the blood supply as well.

To address this target in a more general way, one may think of disrupting the ability of the genetic blueprints in DNA and RNA to be expressed and reproduced. Thus, it seems of interest to synthesize (small) molecules that prevent viruses, bacteria and other pathogens from causing infections and also prevent the proliferation of white blood cells which are associated with a variety of adverse transfusion reactions.

The major blood components used for transfusion – platelets, plasma, and red blood cells – do not contain nuclear DNA or RNA, and therefore retain their biological utility after an inactivation treatment. This may be achieved either by UV mediated crosslinking of the nucleic acid or by damaging the DNA/RNA by the action of radicals. The latter may be the result of an intracellular metal-catalyzed Fenton process [1, 2].

Quite recently, the first compounds consisting of an intercalator and a reactive centre have been investigated [1] and they showed some promising results. In order to improve the intercalating ability of such compounds, we planned to access molecules possessing a substituted acridine-derived intercalating part being attached to an EDTA derived metal complexation region that should be able to bind Fe(II) and Fe(III).




Scheme 1. a) EtOH, $H_2SO_4$; b) PLE; c) NMM, isobutyl chloroformate.

Reaction of the amines **1 – 10** [3] with EDTA-triethylester (**11**) (that was conveniently prepared even on a larger preparative scale from the corresponding tetra-ethylester **12** [4, 5] by selective enzymatic monodeesterification [6] using pig liver esterase (PLE)) using the mixed-anhydride method (isobutyl chloroformate/ *N*-methyl-morpholine *N*-oxide (NMM)) furnished the corresponding triethylesters **13 – 22**. Saponification of the esters was performed by their treatment with aq. sodium hydroxide to afford the

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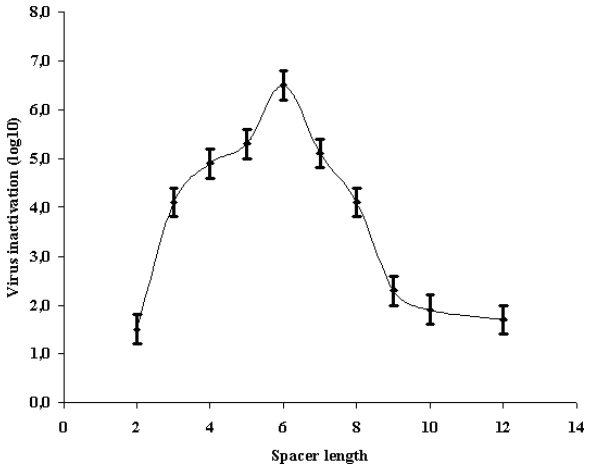


Fig. 1. Inactivation of phage MS2 as a function of spacer length (5 mmol Na ascorbate, 100 $\mu$mol of compounds, 3 equiv. $Fe^{3+}$ loaded, incubation time 4 h, 25 °C).

target compounds **23 – 32**. These target molecules were treated with a threefold molar excess of $Fe^{3+}$, lyophilized and incubated with the phages in Tris-buffer in the presence of sodium ascorbate [1].

As shown in Fig. 1, the length of the spacer exhibits significant effects on the reduction of the phage titer of MS2 phages. Best results were obtained for a spacer length of $n = 6$ giving rise to a reduction of the phage titer of MS2 phages by $> 6$ logarithmic decades. Additional screening revealed that the inactivation of this virus depends both on the concentration and temperature, the time of incubation as well as on the concentration of ascorbate. Increased activity with increased concentration of ascorbate as well as the observation that no activity is associated with these compounds in the absence of ascorbate allows a triggering of the activity by the addition of ascorbic acid. In addition, the phage was reduced by at least 5 – 6 logarithmic levels (27, 100 $\mu$mol, 25 °C, 4 h incubation) in plasma, erythrocyte concentrate, in whole blood and in thrombocyte concentrate. No significant antipathogenic activity, however, was found upon incubating the MS2 phages with acridine, Fe(III)-loaded EDTA in the presence of ascorbate. Additional screening is presently performed in our laboratories.

## Experimental Section

*General*

The melting points are uncorrected (*Reichert* hot stage microscope), NMR spectra (internal Me4Si) were recorded using either a Bruker AM250 or a Varian XL300 instrument ($\delta$ given in ppm, $J$ in Hz), IR spectra (film or KBr pellet) on a Perkin-Elmer 298 instrument, MS spectra were taken either on a MAT311A or a Varian-112S instrument; for elemental analysis a Foss-Heraeus Vario EL instrument was used. TLC was performed on silica gel (Merck 5554, detection by dipping in a solution containing 10% sulfuric acid (400 ml), ammonium molybdate (20 g) and cerium(IV) sulfate (20 mg) followed by heating to 150 °C. All reactions were performed under dry argon. Compounds **13 – 22** and **23 – 32** are members of homologous series and thus similar in their spectra; only representative values are therefore given.

The effect of the compounds on bacteriophages was tested on bacteriophages MS2 grown on its E.coli ATCC15597 host. Log-phase host bacteria for bacteriophage propargation were grown in tryptic soy broth (TSB, Sigma Chemicals) on an orbital shaker at room temperature until turbid. Before use, 100 $\mu$l of culture was inoculated into fresh TSB containing 0.0025% $CaCl_2$. The cultures were incubated in a 37 °C shaking water bath for 4 h until the log-phase was reached. Fresh bacteriophages were cultured from freezer stocks. Log-phase host bateria and phages were mixed at a multiplicity of infection of approximately 1 in 5 ml TSB. The culture was kept on ice for 15 min to facilitate adsorption of the phages to the host cells, then incubated overnight at 37 °C, the phage culture was filtered through a 0.2 $\mu$m cellulose acetate syringe filter to remove host bacteria and then stored at 4 °C. Typical yields were $1 \times 10^{10}$ PFU/ml. Culturable counts of phage were performed by mixing 100 $\mu$l of phage suspension and 100 $\mu$l of host culture in 4 ml molten TSB top agar (containing 0.7% agar). The top agar was vortexed gently, then poured on TSB plates; the plates were incubated at 37 °C. Bacteriophages from freeze stocks were diluted into buffer (30 mM Tris, 150 mM KCl, pH 8.3) to a final population density of approximately $1 \times 10^9$ PFU/ml. Dilutions were prepared in phosphate-buffered saline solution. Initial as well post-exposure culturable counts were performed in triplicate. The counts were divided by the mean unexposed control counts to normalize the data and then log-transformed.

*Ethylenediaminetetraacetic acid triethyl ester (***11***)*

To a solution of EDTA (15.0 g, 51.3 mmol) in ethanol (600 ml) conc. sulfuric acid (10 ml) was added and the mixture was heated under reflux for 6 h. After cooling to room temperature the reaction mixture was neutralized by the careful addition of $NaHCO_3$ and the solvents were removed under reduced pressure. The residue was suspended in water (200 ml) and extracted with chloroform ($2 \times 150$ ml). The combined organic phases were dried ($Na_2SO_4$), the solvent was evaporated and EDTA-tetraethyl ester **12** (14.5 g, 70%) was obtained. M.p. 30 – 32 °C (lit.: 34 °C [4], 32 °C [5]). – IR (KBr): $\nu = 2982s$, 2940*m*, 1736*s*, 1614*w*, 1447*m*, 1421*m*, 1368*s*, 1348*s*, 1190*s*, 1030*s*, 862*w*, 749*m* $cm^{-1}$. – $^1$H NMR

(500 MHz, $CDCl_3$): $\delta = 4.12$ (*q*, $^3J_{H,H} = 7.10$ Hz, 8H, 4×$CH_2$), 3.56 (*s*, 8H, 4×$CH_2$), 2.87 (*s*, 4H, 2×$CH_2$), 1.23 (*t*, $^3J_{H,H} = 7.10$ Hz, 12H, 4×$CH_3$). – $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta = 171.3$ (CO), 60.4 (C-2), 55.2 (C-3), 52.3 (C-4), 14.2 (C-1). – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z = 405$ (19%) $[MH]^+$, 427 (100%) $[MNa]^+$].

To an emulsion of **12** (20.0 g, 49.0 mmol) in water (1500 ml) containing $Na_2HPO_4$ (39.2 g, 276 mmol) and $KH_2PO_4$ (1.16 g, 8.5 mmol) at 27 °C PLE (BioChemica, 2.5 ml (3500 units)) were added and the mixture was stirred for 8 h. After extraction with hexane (2 × 50 ml, discarded) the aqu. layer was extracted with dichloromethane (5 × 100 ml) and the combined organic phases were dried ($MgSO_4$), and the solvent was evaporated to yield **11** (14.5 g, 78%) as a viscous oil [6]. IR (KBr): $\nu = 2983m$, 2938*m*, 1738*s*, 1634*m*, 1378*s*, 1199*s*, 1097*s*, 1028*m*, 865*w*, 734*w*, 588*w* cm$^{-1}$. – $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 4.15$ (*m*, $^3J_{H,H} = 7.10$ Hz, 6 H, 3×$CH_2$), 3.53 (*s*, 4H, 2×$CH_2$), 3.47 (*s*, 2H, $CH_2$), 3.45 (*s*, 2H, $CH_2$), 2.84 (*s*, 4H, $CH_2$), 1.24 (*t*, $^3J_{H,H} = 7.10$ Hz, 9H, 3×$CH_3$). – $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta = 173.0$ (CO), 170.9 (CO), 170.6 (CO), 70.0 (C-7), 60.8 (C-6), 57.0 (C-5), 56.1 (C-4), 54.6 (C-3), 52.5 (C-1), 51.7 (C-2), 14.1 (C-8,9). – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z = 377$ (39%) $[MH]^+$, 399 (100%) $[MNa]^+$, 415 (28%) $[MK]^+$, 791 (93%) $[M_2K]^+$. – HRMS for $C_{16}H_{29}N_2O_8$: calcd. 376.14857; found 376.14859.

*Ethyl 2-((2-{[2-(9-{6-chloro-2-methoxyacridinyl}amino)-ethyl]amino}-2-oxoethyl){2-[bis(2-ethoxy-2-oxoethyl)-amino]ethyl}amino)-acetate (***13***)*

To an ice cold solution of **11** (410 mg, 1.09 mmol) and NMM (1 ml) in DMF/ethyl acetate (1:1, 50 ml) isobutyl chloroformate (147 mg, 1.08 mmol) was added and stirring at this temperature continued for another hour. Then **1** (336 mg, 0.90 mmol) and NMM (2 ml) were added and the mixture stirred for 12 h at room temperature, the solvents were removed under diminished pressure and the residue was subjected to chromatography (silica gel, methanol/ethyl acetate 1:9) to afford **13** (355 mg, 60%) as a highly viscous orange oil. $R_F$ (methanol/ethyl acetate 1:6) 0.63. – UV/vis (methanol): $\lambda_{max}(\log \varepsilon) = 287$ nm (4.64). – IR (film): $\nu = 3305$m, 2981m, 2361w, 1738s, 1634s, 1608m, 1564s, 1523s, 1440s, 1372m, 1347m, 1241s, 1199s, 1030s cm$^{-1}$. – $^1$H NMR (400 MHz, $CDCl_3$): $\delta = 8.83$ (br m, 1H, NH), 8.15 (d, $^3J_{H,H} = 9.32$ Hz, 1H, 8-H), 8.01 (d, $^4J_{H,H} = 2.07$ Hz, 1H, 5-H), 7.95 (d, $^3J_{H,H} = 9.32$ Hz, 1H, 4-H), 7.43 (d, $^4J_{H,H} = 2.59$ Hz, 1H, 1-H), 7.35 (dd, $^3J_{H,H} = 9.32$ Hz, $^4J_{H,H} = 2.59$ Hz, 1H, 3-H), 7.18 (dd, $^3J_{H,H} = 9.32$ Hz, $^4J_{H,H} = 2.07$ Hz, 1H, 7-H), 4.08 – 3.99 (m, 8H, 3×$CH_2$), 3.99 (s, 3H, $OCH_3$), $CH_2$(1')), 3.78 – 3.73 (m, 2H, $CH_2$(2')), 3.37 (s, 4H, 2×$CH_2$(5")), 3.31 (s, 2H, $CH_2$(4")), 3.20 (s, 2H, $CH_2$(3")), 2.66 (s, 4H, 2×$CH_2$(1", 2")), 1.21 – 1.16 (m, 9H, 3×$CH_3$). – $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta = 174.3$ (C=O), 171.0 (C=O), 170.9 (C=O), 156.0 (q), 150.9 (q), 148.2 (q), 146.0 (q), 135.0 (q), 130.1 (CH), 127.0 (CH), 125.6 (CH), 124.6 (CH), 123.2 (CH), 116.5 (q), 113.8 (q), 99.8 (CH), 60.7 ($CH_2$), 60.6 ($CH_2$), 58.5 ($CH_2$(4")), 55.8 (OMe), 55.6 ($CH_2$(5")), 54.6 ($CH_2$(3")), 52.9 ($CH_2$(1")), 52.5 ($CH_2$(2")), 52.3 ($CH_2$(1')), 40.0 ($CH_2$(2')), 14.2 ($CH_3$). – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z = 660$ (100%) $[M(^{35}Cl)H]^+$, 661 (34%) $[M(^{37}Cl)H]^+$. – Analysis for $C_{32}H_{42}ClN_5O_8$ (660.17): calcd. C 58.22, H 6.41, N 10.61; found C 58.01, H 6.55, N 10.42.

*Ethyl 2-((2-{[3-(9-{6-chloro-2-methoxyacridinyl}amino)-propyl]amino}-2-oxoethyl){2-[bis(2-ethoxy-2-oxoethyl)-amino]ethyl}amino)-acetate (***14***)*

As described for **13** from **2** (1.0 g, 2.6 mmol), **11** (1.16 g, 3.1 mmol) and isobutyl chloroformate (423 mg, 3.10 mmol) **14** (730 mg, 42%) was obtained as an orange coloured viscous oil. $R_F$ (methanol/ethyl acetate 1:6) 0.63. – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z = 674$ (100%) $[M(^{35}Cl)H]^+$, 676 (34%) $[M(^{37}Cl)H]^+$. – Analysis for $C_{33}H_{44}ClN_5O_8$ (674.20): calcd. C 58.79, H 6.58, N 10.39; found C 58.51, H 6.78, N 10.14.

*Ethyl 2-((2-{[4-(9-{6-chloro-2-methoxyacridinyl}amino)-butyl]amino}-2-oxoethyl){2-[bis(2-ethoxy-2-oxoethyl)-amino]ethyl}amino)-acetate (***15***)*

As described for **13** from **2** (1.0 g, 2.5 mmol), **11** (1.12 g, 3.0 mmol) and isobutyl chloroformate (408 mg, 2.99 mmol) **15** (1.35 g, 79%) was obtained as a highly viscous, orange-coloured oil. $R_F$ (methanol/ethyl acetate 1:6) 0.63. – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z = 688$ (100%) $[M(^{35}Cl)H]^+$, 690 (45%) $[M(^{37}Cl)H]^+$. – Analysis for $C_{34}H_{46}ClN_5O_8$ (688.23): calcd. C 59.34, H 6.74, N 10.18; found C 59.13, H 6.94, N 10.03.

*Ethyl 2-((2-{[5-(9-{6-chloro-2-methoxyacridinyl}amino)-pentyl]amino}-2-oxoethyl){2-[bis(2-ethoxy-2-oxoethyl)-amino]ethyl amino)-acetate (***16***)*

As described for **13** from **4** (0.8 g, 1.92 mmol), **11** (866 mg, 2.3 mmol) and isobutyl chloroformate (315 mg, 2.31 mmol) **16** (785 mg, 58%) was obtained as a highly viscous, orange-coloured oil. $R_F$ (methanol/ethyl acetate 1:6) 0.63. – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z = 702$ (100%) $[M(^{35}Cl)H]^+$, 704 (55%) $[M(^{37}Cl)H]^+$. – Analysis for $C_{35}H_{48}ClN_5O_8$ (702.25): calcd. C 59.86, H 6.89, N 9.97; found C 59.64, H 7.02, N 9.75.

*Ethyl 2-((2-{[6-(9-{6-chloro-2-methoxyacridinyl}amino)-hexyl]amino}-2-oxoethyl){2-[bis(2-ethoxy-2-oxoethyl)-amino]ethyl)amino)-acetate (***17***)*

As described for **13** from **5** (550 mg, 1.28 mmol), **11** (578 mg, 1.54 mmol) and isobutyl chloroformate (210 mg,

1.54 mmol) **17** (649 mg, 71%) was obtained as a highly viscous, orange-coloured oil. $R_F$ (methanol/ethyl acetate 1:6) 0.63. – UV/vis (methanol): $\lambda_{max}(\log\varepsilon) = 285$ nm (4.66). – IR (film): $\nu = 3307$m, 2934m, 2858m, 1738s, 1667s, 1633s, 1606m, 1521m, 1466m, 1437m, 1372m, 1238s, 1199s, 1137m, 1030m cm$^{-1}$. – $^1$H NMR (500 MHz, $CDCl_3$): $\delta =$ 8.04 (d, $^3J_{H,H} = 9.32$ Hz, 1H, 8-H), 8.04 (d, $^4J_{H,H} = 2.07$ Hz, 1H, 5-H), 8.00 (br m, 1H, NH), 7.96 (d, $^3J_{H,H} = 9.32$ Hz, 1H, 4-H), 7.37 (dd, $^3J_{H,H} = 9.32$ Hz, $^4J_{H,H} = 2.59$ Hz, 1H, 3-H), 7.27 (dd, $^3J_{H,H} = 9.32$ Hz, $^4J_{H,H} = 2.07$ Hz, 1H, 7-H), 7.26 (d, $^4J_{H,H} = 2.59$ Hz, 1H, 1-H), 4.12 (q, $^3J_{H,H} = 7.10$ Hz, 2H, $CH_2$), 4.11 (q, $^3J_{H,H} = 7.10$ Hz, 4H, 2×$CH_2$), 3.95 (s, 3 H, $OCH_3$), 3.74 – 3.70 (m, 2H, $CH_2$(1')), 3.50 (s, 4H, 2×$CH_2$(5")), 3.39 (s, 2H, $CH_2$(4")), 3.27 (s, 2H, $CH_2$(3")), 3.27 – 3.23 (m, 2H, $CH_2$(6')), 2.82 – 2.74 (m, 4H, 2×$CH_2$(1",2")), 1.80 – 1.74 (m, 2H, $CH_2$(2')), 1.56 – 1.44 (m, 4H, 2×$CH_2$(3',5')), 1.40 – 1.35 (m, 2H, $CH_2$(4')), 1.24 – 1.17 (t, $^3J_{H,H} = 7.10$ Hz, 9H, 3×$CH_3$). – $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta =$ 171.33 (C=O), 171.27 (C=O), 170.0 (C=O), 155.9 (q), 150.3 (q), 139.6 (q), 135.2 (q), 131.9 (CH), 125.7 (CH), 124.6 (CH), 124.4 (CH), 124.2 (CH), 117.4 (q), 115.1 (q), 99.7 (CH), 60.7 ($CH_2$), 60.5 ($CH_2$), 58.7 ($CH_2$(4")), 56.0 ($CH_2$(3")), 55.6 (OMe), 54.9 ($CH_2$(5")), 53.0 ($CH_2$(1")), 52.3 ($CH_2$(2")), 50.2 ($CH_2$(1')), 38.6 ($CH_2$(6')), 31.4 ($CH_2$), 29.5 ($CH_2$), 26.35 ($CH_2$), 26.28 ($CH_2$), 14.2 ($CH_3$). – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z = 716$ (100%) [M($^{35}$Cl)H]$^+$, 718 (45%) [M($^{37}$Cl)H]$^+$. – Analysis for $C_{36}H_{50}ClN_5O_8$ (716.28): calcd. C 60.37, H 7.04, N 9.78; found C 59.92, H 7.21, N 9.61.

*Ethyl 2-((2-{[7-(9-{6-chloro-2-methoxyacridinyl}amino)-heptyl]amino}-2-oxoethyl){2-[bis(2-ethoxy-2-oxoethyl)-amino]ethyl}amino)-acetate (***18***)*

As described for **13** from **6** (1.0 g, 2.3 mmol), **11** (1.02 g, 2.7 mmol) and isobutyl chloroformate (369 mg, 2.7 mmol) **18** (710 mg, 43%) was obtained as a highly viscous, orange-coloured oil. $R_F$ (methanol/ethyl acetate 1:6) 0.63. – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z =$ 730 (100%) [M($^{35}$Cl)H]$^+$, 732 (43%) [M($^{37}$Cl)H]$^+$. – Analysis for $C_{37}H_{52}ClN_5O_8$ (730.31): calcd. C 60.85, H 7.18, N 9.59; found C 60.65, H 7.34, N 9.34.

*Ethyl 2-((2-{[8-(9-{6-chloro-2-methoxyacridinyl}amino)-octyl]amino}-2-oxoethyl){2-[bis(2-ethoxy-2-oxoethyl)-amino]ethyl}amino)-acetate (***19***)*

As described for **13** from **7** (330 mg, 0.72 mmol), **11** (325 mg, 0.86 mmol) and isobutyl chloroformate (118 mg, 0.86 mmol) **19** (298 mg, 56%) was obtained as a highly viscous, orange-coloured oil. $R_F$ (methanol/ethyl acetate 1:6) 0.63. – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z =$ 744 (100%) [M($^{35}$Cl)H]$^+$, 746 (63%) [M($^{37}$Cl)H]$^+$. – Analysis for $C_{38}H_{54}ClN_5O_8$ (744.34): calcd. C 61.32, H 7.31, N 9.41; found C 61.05, H 7.55, N 9.31.

*Ethyl 2-((2-{[9-(9-{6-chloro-2-methoxyacridinyl}amino)-nonyl]amino}-2-oxoethyl){2-[bis(2-ethoxy-2-oxoethyl)-amino]ethyl}amino)-acetate (***20***)*

As described for **13** from **8** (247 mg, 0.52 mmol), **11** (235 mg, 0.62 mmol) and isobutyl chloroformate (85 mg, 0.62 mmol) **20** (258 mg, 65%) was obtained as a highly viscous, orange-coloured oil. $R_F$ (methanol/ethyl acetate 1:6) 0.63. – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z =$ 758 (100%) [M($^{35}$Cl)H]$^+$, 760 (43%) [M($^{37}$Cl)H]$^+$. – Analysis for $C_{39}H_{56}ClN_5O_8$ (758.36): calcd. C 61.77, H 7.44, N 9.24; found C 61.57, H 7.64, N 8.96.

*Ethyl 2-((2-{[10-(9-{6-chloro-2-methoxyacridinyl}amino)-decyl]amino}-2-oxoethyl){2-[bis(2-ethoxy-2-oxoethyl)-amino]ethyl}amino)-acetate (***21***)*

As described for **13** from **9** (1.0 g, 2.1 mmol), **11** (930 mg, 2.47 mmol) and isobutyl chloroformate (337 mg, 2.47 mmol) **21** (1.21 g, 76%) was obtained as a highly viscous, orange-coloured oil. $R_F$ (methanol/ethyl acetate 1:6) 0.63. – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z =$ 772 (100%) [M($^{35}$Cl)H]$^+$, 774 (70%) [M($^{37}$Cl)H]$^+$. – Analysis for $C_{40}H_{58}ClN_5O_8$ (772.39): calcd. C 62.20, H 7.57, N 9.07; found C 61.96, H 7.69, N 8.86.

*Ethyl 2-((2-{[12-(9-{6-chloro-2-methoxyacridinyl}amino)-dodecyl]amino}-2-oxoethyl){2-[bis(2-ethoxy-2-oxoethyl)-amino]ethyl}amino)-acetate (***22***)*

As described for **13** from **10** (0.6 g, 1.17 mmol), **11** (528 mg, 1.4 mmol) and isobutyl chloroformate (192 mg, 1.41 mmol) **22** was obtained as a highly viscous, orange-coloured oil. $R_F$ (methanol/ethyl acetate 1:6) 0.63. – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z = 801$ (100%) [M($^{35}$Cl)H]$^+$, 803 (74%) [M($^{37}$Cl)H]$^+$. – Analysis for $C_{42}H_{62}ClN_5O_8$ (800.44): calcd. C 63.02, H 7.81, N 8.75; found C 62.94, H 8.02, N 8.49.

*2-[{2-[(2-{[2-(9-{6-Chloro-2-methoxyacridinyl}amino)-ethyl]amino}-2-oxoethyl)(carboxymethyl)amino]ethyl}-(carboxymethyl)amino] acetic acid (***23***)*

A solution of **13** (335 mg, 0.51 mmol) in ethanol/water 5:1, 50 ml) containing NaOH (142 mg, 3.55 mmol) was stirred at room temperature overnight. After neutralization with aq. hydrochloric acid (10%), the solvents were removed under reduced pressure **23** (485 mg, 43 wt-% NaCl by analysis) was obtained as a red coloured amorphous solid. An analytically pure sample was obtained after dialysis followed by repeated chromatography (RP18, methanol/water and acetonitrile/water). UV/vis (methanol): $\lambda_{max}(\log\varepsilon) =$

286 nm (4.45). – IR (KBr): $\nu$ = 3324s, 1582s, 1526m, 1403s, 1338m, 1256m, 1172w, 1114w, 1027w $cm^{-1}$. – $^1H$ NMR (500 MHz, $D_2O$ / $CD_3OD$): $\delta$ = 7.53 (d, $^3J_{H,H}$ = 9.31 Hz, 1H, 8-H), 7.27 (d, $^3J_{H,H}$ = 9.31 Hz, 1H, 4-H), 7.22 (s, 1H, 5-H), 7.02 (dd, $^3J_{H,H}$ = 9.31 Hz, $^4J_{H,H}$ = 2.59 Hz, 1H, 3-H), 6.88 (dd, $^3J_{H,H}$ = 9.31 Hz, $^4J_{H,H}$ = 2.07 Hz, 1H, 7-H), 6.64 (s, 1H, 1-H), 3.68 (s, 3H, $OCH_3$), 3.56 – 3.53 (m, 2H, $CH_2$(1')), 3.29 – 3.24 (m, 2H, $CH_2$(2')), 2.80 (s, 4H, 2×$CH_2$(5")), 2.72 (s, 2H, $CH_2$(4")), 2.70 (s, 2H, $CH_2$(4")), 2.22 – 2.16 (m, 2H, $CH_2$(1")), 2.10 – 2.04 (m, 2H, $CH_2$(1")). – $^{13}C$ NMR (100 MHz, $CD_3OD$): $\delta$ = 178.6 (C=O), 178.3 (C=O), 174.3 (C=O), 154.6 (q), 150.5 (q), 145.9 (q), 143.4 (q), 135.4 (q), 127.9 (CH), 125.1 (CH), 124.39 (CH), 124.38 (CH), 123.4 (CH), 115.7 (q), 113.2 (q), 99.9 (CH), 58.73 ($CH_2$(5")), 58.72 ($CH_2$(4")), 58.5 ($CH_2$(3")), 55.6 (OMe), 52.0 ($CH_2$(1")), 51.8 ($CH_2$(2")), 48.6 ($CH_2$(1')), 39.9 ($CH_2$(2')). – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z$ = 574 (60%) [M($^{35}$Cl)-H]$^-$, 576 (100%) [M($^{37}$Cl)-H]$^-$. – Analysis for $C_{26}H_{30}ClN_5O_8$ (576.01): calcd. C 54.21, H 5.25, N 12.16; found C 54.01, H 5.50, N 12.00.

*2-[{2-[(2-{[3-(9-{6-Chloro-2-methoxyacridinyl}amino)-propyl]amino}-2-oxoethyl)(carboxymethyl)amino]ethyl}-(carboxymethyl)amino] acetic acid (***24***)*

As described for **23**, from **14** (722 mg, 1.07) **24** (1.04 g, 42 wt-% NaCl) was obtained as a red, amorphous solid. An analytically pure sample was obtained after dialysis followed by repeated chromatography (RP18, methanol/water and acetonitrile/water). – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z$ = 588 (100%) [M($^{35}$Cl)-H]$^-$, 590 (30%) [M($^{37}$Cl)-H]$^-$. – Analysis for $C_{27}H_{32}ClN_5O_8$ (590.04): calcd. C 54.96, H 5.47, N 11.87; found C 54.74, H 5.61, N 11.62.

*2-[{2-[(2-{[4-(9-{6-Chloro-2-methoxyacridinyl}amino)-butyl]amino}-2-oxoethyl)(carboxymethyl)amino]ethyl}-(carboxymethyl)amino] acetic acid (***25***)*

As described for **23**, from **15** (940 mg, 1.37 mmol) **25** (1.34 g, 42 wt-% NaCl) was obtained as a red, amorphous solid. An analytically pure sample was obtained after dialysis followed by repeated chromatography (RP18, methanol/water and acetonitrile/water). – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z$ = 602 (100%) [M($^{35}$Cl)-H]$^-$, 604 (28%) [M($^{37}$Cl)-H]$^-$. – Analysis for $C_{28}H_{34}ClN_5O_8$ (604.06): calcd. C 55.68, H 5.67, N 11.59; found C 55.41, H 5.89, N 11.32.

*2-[{2-[(2-{[5-(9-{6-Chloro-2-methoxyacridinyl}amino)-pentyl]amino}-2-oxoethyl)(carboxymethyl)amino]ethyl}-(carboxymethyl)amino acetic acid (***26***)*

As described for **23** from **16** (773 mg, 1.10 mmol) **26** (1.10 g, 41 wt-% NaCl) was obtained as a red, amorphous solid. An analytically pure sample was obtained after dialysis followed by repeated chromatography (RP18, methanol/water and acetonitrile/water). – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z$ = 616 (100%) [M($^{35}$Cl)-H]$^-$, 618 (55%) [M($^{37}$Cl)-H]$^-$. – Analysis for $C_{29}H_{36}ClN_5O_8$ (618.09): calcd. C 56.36, H 5.87, N 11.33; found C 56.09, H 5.99, N 11.06.

*2-[{2-[(2-{[6-(9-{6-Chloro-2-methoxyacridinyl}amino)-hexyl]amino}-2-oxoethyl)(carboxymethyl)amino]ethyl}-(carboxymethyl)amino] acetic acid (***27***)*

As described for **23**, from **17** (595 mg, 0.83 mmol) **27** (838 mg, 40 wt-% NaCl) was obtained as a red, amorphous solid. An analytically pure sample was obtained after dialysis followed by repeated chromatography (RP18, methanol/water and acetonitrile/water). UV/vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 287 nm (4.56). – IR (KBr): $\nu$ = 3424s, 2932m, 1589s, 1501m, 1404s, 1326m, 1245s, 1178w, 1120m, 1092m, 1031w $cm^{-1}$. – $^1H$ NMR (500 MHz, $CD_3OD$): $\delta$ = 7.27 (d, $^3J_{H,H}$ = 9.31 Hz, 1H, 8-H), 7.03 (d, $^3J_{H,H}$ = 9.31 Hz, 1H, 4-H), 6.96 (d, $^4J_{H,H}$ = 2.07 Hz, 1H, 5-H), 6.94 (dd, $^3J_{H,H}$ = 9.31 Hz, $^4J_{H,H}$ = 2.59 Hz, 1H, 3-H), 6.76 (dd, $^3J_{H,H}$ = 9.31 Hz, $^4J_{H,H}$ = 2.07 Hz, 1H, 7-H), 6.37 (d, $^4J_{H,H}$ = 2.59 Hz, 1H, 1-H), 3.55 (s, 3H, $OCH_3$), 3.31 (br s, 4H, 2×$CH_2$(5")), 3.14 (s, 2H, $CH_2$(4")), 3.12 – 3.07 (m, 2H, $CH_2$(1')), 3.07 – 3.02 (m, 2H, $CH_2$(6')), 3.05 (s, 2H, $CH_2$(3")), 2.85 – 2.77 (m, 2H, $CH_2$(1")), 2.72 – 2.66 (m, 2H, $CH_2$(2")), 1.41 – 1.31 (m, 4H, 2 x $CH_2$(2',5')), 1.15 – 1.07 (m, 4H, 2×$CH_2$(3',4')). – $^{13}C$ NMR (125 MHz, $D_2O$): $\delta$ = 178.8 (C=O), 178.7 (C=O), 173.5 (C=O), 154.0 (q), 151.0 (q), 142.7 (q), 139.5 (q), 136.6 (q), 124.9 (CH), 124.6 (CH), 124.5 (CH), 123.0 (CH), 121.4 (CH), 113.3 (q), 110.7 (q), 100.4 (CH), 58.6 ($CH_2$(5")), 58.3 ($CH_2$(4")), 57.7 ($CH_2$(3")), 55.3 (OMe), 52.1 ($CH_2$(1")), 51.4 ($CH_2$(2")), 48.2 ($CH_2$(1')), 38.9 ($CH_2$(6')), 29.8 ($CH_2$), 28.2 ($CH_2$), 25.7 ($CH_2$), 25.6 ($CH_2$). – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z$ = 630 (45%) [M($^{35}$Cl)-H]$^-$, 632 (100%) [M($^{37}$Cl)-H]$^-$. – Analysis for $C_{30}H_{38}ClN_5O_8$ (632.12): calcd. C 57.00, H 6.06, N 11.08; found C 56.77, H 6.29, N 10.87.

*2-[{2-[(2-{[7-(9-{6-Chloro-2-methoxyacridinyl}amino)-heptyl]amino}-2-oxoethyl)(carboxymethyl)amino]ethyl}-(carboxymethyl)amino] acetic acid (***28***)*

As described for **23**, from **18** (698 mg, 0.96 mmol) **28** (1.03 g, 43 wt-% NaCl) was obtained as a red, amorphous solid. An analytically pure sample was obtained after dialysis followed by repeated chromatography (RP18, methanol/water and acetonitrile/water). – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z$ = 644 (60%) [M($^{35}$Cl)-H]$^-$, 646 (100%) [M($^{37}$Cl)-H]$^-$. – Analysis for $C_{31}H_{40}ClN_5O_8$ (646.15): calcd. C 57.63, H 6.24, N 10.84; found C 57.41, H 6.39, N 10.63.

*2-[{2-[(2-{[8-(9-{6-Chloro-2-methoxyacridinyl}amino)-octyl]amino-2-oxoethyl)(carboxymethyl)amino]ethyl}-(carboxymethyl)amino] acetic acid (***29***)*

As described for **23**, from **19** (252 mg, 0.34 mmol) **29** (351 mg, 40 wt-% NaCl) was obtained as a red, amorphous solid. An analytically pure sample was obtained after dialysis followed by repeated chromatography (RP18, methanol/water and acetonitrile/water). – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z = 658$ (100%) [M($^{35}$Cl)-H]$^-$, 660 (60%) [M($^{37}$Cl)-H]$^-$. – Analysis for $C_{32}H_{42}ClN_5O_8$ (660.17): calcd. C 58.22, H 6.41, N 10.61; found C 57.99, H 6.70, N 10.38.

*2-[{2-[(2-{[9-(9-{6-Chloro-2-methoxyacridinyl}amino)-nonyl]amino}-2-oxoethyl)(carboxymethyl)amino]ethyl}-(carboxymethyl)amino] acetic acid (***30***)*

As described for **23**, from **20** (206 mg, 0.27 mmol) **30** (285 mg, 39 wt-% NaCl) was obtained as a red, amorphous solid. An analytically pure sample was obtained after dialysis followed by repeated chromatography (RP18, methanol/water and acetonitrile/water). – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z = 672$ (90%) [M($^{35}$Cl)-H]$^-$, 674 (100%) [M($^{37}$Cl)-H]$^-$. – Analysis for $C_{33}H_{44}ClN_5O_8$ (674.20): calcd. C 58.79, H 6.58, N 10.39; found C 58.51, H 6.81, N 10.07.

*2-[{2-[(2-{[10-(9-{6-Chloro-2-methoxyacridinyl}amino)-decyl]amino}-2-oxoethyl)(carboxymethyl)amino]ethyl}-(carboxymethyl)amino] acetic acid (***31***)*

As described for **23**, from **21** (669 mg, 0.87 mmol) **31** (922 mg, 39 wt-% NaCl) was obtained as a red, amorphous solid. An analytically pure sample was obtained after dialysis followed by repeated chromatography (RP18, methanol/water and acetonitrile/water). – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z = 686$ (100%) [M($^{35}$Cl)-H]$^-$, 688 (15%) [M($^{37}$Cl)-H]$^-$. – Analysis for $C_{34}H_{46}ClN_5O_8$ (688.22): calcd. C 59.34, H 6.74, N 10.18; found C 59.05, H 6.92, N 9.95.

*2-[{2-[(2-{[12-(9-{6-Chloro-2-methoxyacridinyl}amino)-dodecyl]amino}-2-oxoethyl)(carboxymethyl)amino]ethyl}-(carboxymethyl)amino] acetic acid (***32***)*

As described for **23** from **22** (411 mg, 0.51 mmol) **32** (560 mg, 38 wt-% NaCl) was obtained as a red, amorphous solid. An analytically pure sample was obtained after dialysis followed by repeated chromatography (RP18, methanol/water and acetonitrile/water). – MS (ESI, 4.1 kV, 8 $\mu$l/min, $N_2$, methanol): $m/z = 714$ (100%) [M($^{35}$Cl)-H]$^-$, 716 (30%) [M($^{37}$Cl)-H]$^-$. – Analysis for $C_{36}H_{50}ClN_5O_8$ (716.28): calcd. C 60.37, H 7.04, N 9.78; found C 60.11, H 7.28, N 9.59.

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