René Csuk,
Christian Raschke,
Gunnar Göthe,
Stefan Reißmann

Institut für Organische Chemie,
Martin-Luther-Universität
Halle-Wittenberg,
D-06120 Halle (Saale),
Germany

Synthesis of Dimeric Trifluoromethoxyacridine-derived Pathogen-inactivating Nucleic Acid Intercalators

A series of antiviral active compounds consisting of an intercalating acridine-derived part, a spacer region and a reactive EDTA-derived conjugate was synthesized in an easy sequence. Thus, suitably mono-protected 1,ω-alkyldiamines gave, upon reaction with 9-chloro-2-trifluoromethoxyacridine, followed by deprotection and reaction with EDTA dianhydride, the target molecules. Incorporation of their Fe(II) complexes in the presence of ascorbate gave a reduction of the phage titer of MS2 phages by several logarithmic decades.

Keywords: Pathogen Inactivation; Nucleic Acid Intercalators; Acridine

Received: June 11, 2004; accepted: September 2, 2004 [FP907]
DOI 10.1002/ardp.200400907

Introduction

The safety of blood and plasma is the subject of an ongoing concern among the public, underlining the need for the development of efficient analytical systems as well as of anti-pathogenic drugs. Life-threatening diseases can be the result of accepting blood or plasma donations if they carry viral infectious agents. The potential bioburden of the donated blood has to be minimized, but this goal cannot completely be achieved by simple testing of blood and plasma donations for the presence of infectious agents, since it is not possible to perform assays against all known and unknown biological threats. Thus, the development of anti-pathogenic measures seems to be called for.

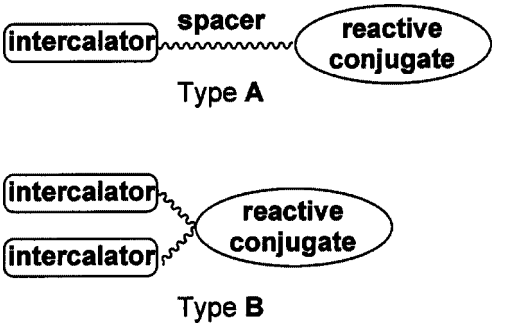


Figure 1. Types of anti-pathogenic compounds.

Results and discussion

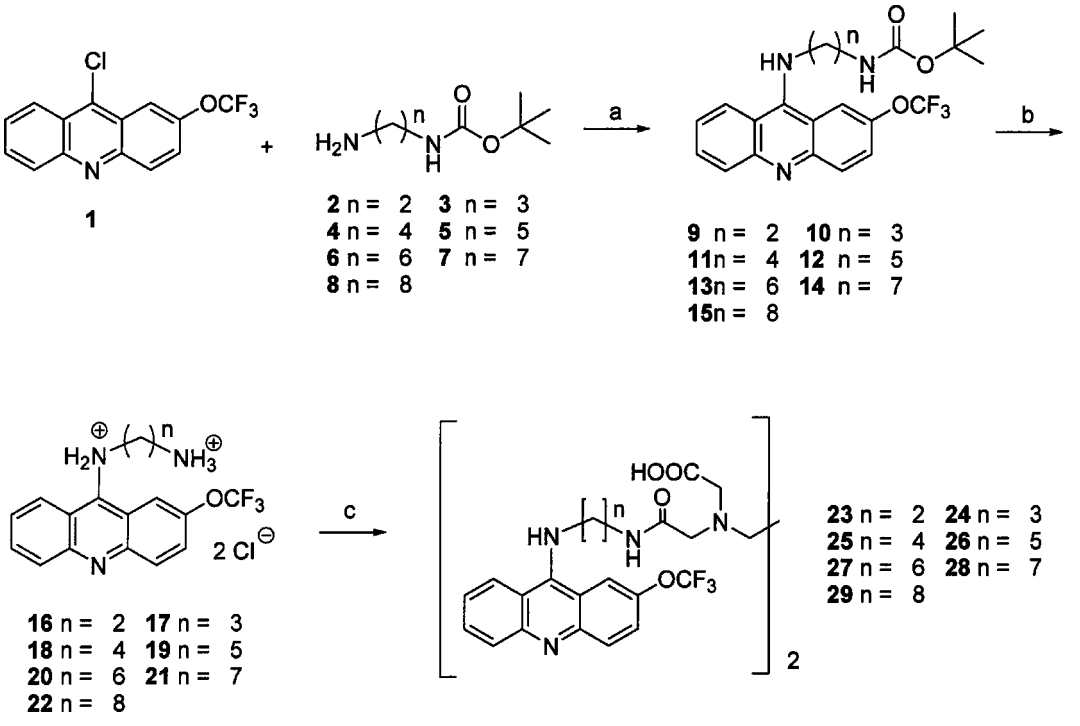
Quite recently [1], a new type of pathogen-inactivating agents (type **A**; Figure 1) was introduced, consisting of an intercalator that binds to the nucleic acid of pathogens combined with a conjugate that subsequently destroys the nucleic acid via a Fe(II)-mediated Fenton mechanism. The latter process accomplishes damage to the nucleic acids by producing OH radicals. Since the metal attached to the conjugate can change between two levels of oxidation, a cycle can finally be constructed in the presence of suitable reduction agents (*e.g.* ascorbic acid) that increases the damage to the biological molecules. Thus, the reactivity of the molecules can be triggered by the presence and the concentration of a more or less harmless agent.

Of paramount importance is the interaction of the intercalator with the nucleic acids. Thus, it can safely be assumed that dimeric structures (type **B**; Figure 1) should provide a better intercalation and thus a higher anti-pathogenic activity. From preliminary modelling studies we deduced that the presence of a 2-trifluormethoxy moiety in the acridine ring should result in an improved interaction between the intercalator and the nucleic acids.

From a palladium-assisted Buchwald-Hartwig amination reaction between methyl 2-iodobenzoate and 4-trifluoromethoxyaniline [2], methyl 2-[4-(trifluoromethoxy)amino]benzoate [3] was obtained. Saponification followed by a $POCl_3$-mediated cyclization furnished the starting material for our sequence,

Correspondence: René Csuk, Institut für Organische Chemie, Martin-Luther-Universität Halle-Wittenberg, Kurt-Mothes-Straße 2, D-06120 Halle (Saale), Germany; Phone: +49 345 5525660, Fax: +49 345 5527030, e-mail: csuk@chemie.uni-halle.de

Full Paper



Scheme 1. Syntheses of the target molecules. Reagents: a) phenol; b) aq. HCl; c) EDTA dianhydride.

9-chloro-2-trifluoromethoxyacridine (**1**) (Scheme 1). Reaction of **1** with monoprotected diamines, 1-amino-x-tert-butyloxycarbonylaminoalkanes **2** [4], **3** [5], **4** [6], **5** [7], **6** [8], **7** [9] or **8** [10] in phenol [11, 12] gave the acridinyl carbamates **9**–**15**, respectively. These acridinyl carbamates were deprotected using aqueous hydrochloric acid to afford the corresponding bishydrochlorides **16**–**22**. These compounds were allowed to react with EDTA dianhydride to yield the target compounds **23**–**29** (Scheme 1).

For biological screening, the compounds were loaded with three equivalents of Fe(III) and then incubated in Tris buffer with MS2 phages [13], in the presence of sodium ascorbate. Whereas almost no anti-pathogenic acivity was observed in the absence of ascorbate, in the presence of ascorbic acid the phage titer of the MS2 phages was reduced by several logarithmic decades (Figure 2). As compared to monomeric, unsubstituted acridine analogues [1] or to the photoinactivation by porphyrins [14], an approximately 100-fold higher anti-pathogenic activity could be achieved.

Modifications in the structure of these compounds, as well as an extended biological screening, are presently performed in our laboratories.

Acknowledgments

Thanks for helpful discussion and parts of the biological screening are due to Dr. H. Knoller and Dr. H.-J.

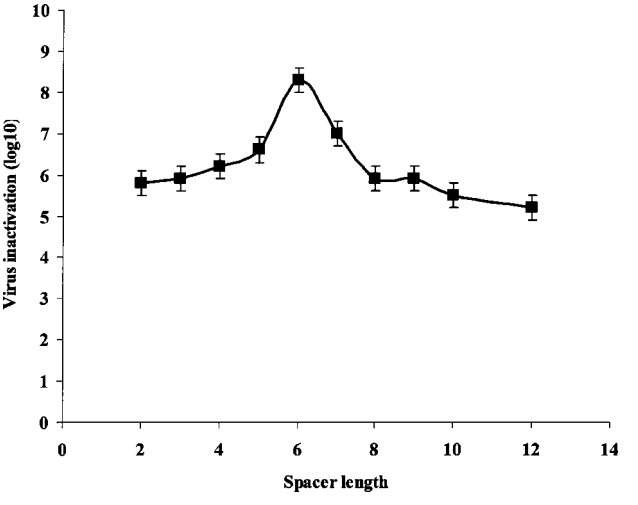


Figure 2. Inactivation of phage MS2 as a function of spacer length [20 °C, 5 mmol Na-ascorbate, 100 μM of compounds **23**–**29** (Fe^{3+} loaded, 3 equiv.)].

Neumann (Fresenius Hemocare); additional screening has been performed by Bioscreen Ltd. Financial support, by the European Communities (SC1-CT92-0780) and the Fonds der Chemischen Industrie, is gratefully acknowledged. We like to thank Dr. D. Ströhl for numerous NMR spectra, Dr. R. Kluge for the MS spectra, Mr. T. Brezesinski and Ms. B. Janeska for her help in the preparation of starting materials.

Experimental

General

Melting points are uncorrected (Leica hot-stage microscope). NMR spectra were recorded using the Varian spectrometers Gemini 200, Gemini 2000 or Unity 500 (δ given in ppm, J inHz, internal Me_4Si); IR spectra (film or KBr pellet) were recorded on a Perkin-Elmer FT-IR spectrometer Spectrum 1000; MS spectra were taken on a Intectra GmbH AMD 402 (electron impact, 70 eV) or on a Finnigan MAT TSQ 7000 instrument (electrospray, voltage 4.5 kV, sheath gas nitrogen). For elemental analysis, a Foss-Heraeus Vario EL instrument was used. TLC was performed on silica gel (Merck 5554); detection by UV absorption or by treatment with a solution of 10% sulfuric acid, ammonium molybdate and cerium$^{(IV)}$ sulfate, followed by gentle heating. The solvents were dried according to usual procedures.

Bacteriophages and screening

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 propagation were grown in tryptic soy broth (TSB, Sigma Chemicals) on an orbital shaker at room temperature, until turbid. Before use, 100 μL 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 bacteria 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 it was incubated overnight at 37 °C. The resulting phage culture was filtered through a 0.2-μm cellulose acetate syringe filter to remove host bacteria and then stored at 4 °C. Typical yields were 1×10^{10} pfu/mL. Culturable counts of phage were performed by mixing 100 μL of phage suspension and 100 μL 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 1×10^9 pfu/mL. Dilutions were prepared in phosphate-buffered saline solution. Initial as well as 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.

*tert-Butyl N-{2-[9-(2-trifluoromethoxyacridinyl)amino]ethyl} carbamate (**9**)*

A mixture of 9-chloro-2-trifluoromethoxyacridine (**1**) (2.00 g, 6.7 mmol) and phenol (10.0 g, 0.1 mol) was stirred at 90 °C for 30 min; **2** (1.18 g, 7.4 mmol) was added, and stirring at this temperature continued for another 15 min. The product was purified by chromatography (silica gel, methanol/ethyl acetate 1 : 4) to afford **9** (1.47 g, 52%) as a red, highly viscous oil. UV-vis (methanol): λ_{max} (log ε) = 284 nm (4.68). IR (film): ν = 3273*w*, 2981*m*, 1703*m*, 1652*m*, 1634*m*, 1592*s*, 1533*s*, 1407*m*, 1368*m*, 1334*w*, 1261*s*, 1215*s*, 1166*s*, 1044*w*, 1012*w* cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.14 (*d*, $^3J_{H,H}$ = 9.55 Hz, 1H, H-C(4)), 8.12 (*s*, 1H, H-C(1)), 7.91 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(8)), 7.75 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(5)), 7.41–7.36 (*m*, 2H, H-C(3,6)), 7.11 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.74 Hz, 1H, H-C(7)), 6.33 (*br s*, 1H, NH), 4.02–3.98 (*m*, 2H, CH_2(1′)), 3.68–3.62 (*m*, 2H, CH_2(2′)), 1.45 (*s*, 9H, ^{t}Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 157.8 (quart.), 155.1 (C=O), 143.8 (quart.), 142.0 (quart.), 140.1 (quart.), 132.9 (CH), 127.0 (CH), 124.6 (CH), 123.8 (CH), 123.2 (CH), 121.6 (CH), 120.5 (*q*, $^1J_{C,F}$ = 257.4 Hz, OCF_3), 116.2 (CH), 113.0 (quart.), 112.8 (quart.), 80.4 (quart., ^{t}Bu), 51.2 (CH_2(1′)), 40.4 (CH_2(2′)), 28.4 (CH_3, ^{t}Bu). ^{19}F NMR (188 MHz, $CDCl_3$): δ = −58.7 (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 366 (10%) [(M-^{i}butene)H]$^+$, 422 (100%) [MH]$^+$. Analysis for $C_{21}H_{22}F_3N_3O_3$ (421.42): C 59.85; H 5.26; N 9.97; found: C 59.64; H 5.37; N 9.81.

*tert-Butyl N-{3-[9-(2-trifluoromethoxyacridinyl)amino]propyl} carbamate (**10**)*

As described for **9**, from **1** (2.0 g, 6.7 mmol), phenol (10.0 g, 0.1 mol) and **3** (1.42 g, 8.2 mmol), **10** (1.23 g, 42%) was obtained as a red, highly viscous oil. UV-vis (methanol): λ_{max} (log ε) = 284 nm (4.71). IR (film): ν = 3441*s*, 2980*m*, 1690*m*, 1630*m*, 1568*s*, 1524*m*, 1444*w*, 1392*w*, 1368*m*, 1336*w*, 1286*s*, 1262*s*, 1216*m*, 1169*m*, 1084*w*, 1020*w* cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.21 (*d*, $^3J_{H,H}$ = 9.55 Hz, 1H, H-C(4)), 8.10 (*s*, 1H, H-C(1)), 8.08 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(8)), 8.07 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(5)), 7.66 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.64 Hz, 1H, H-C(6)), 7.52 (*dd*, $^3J_{H,H}$ = 9.55 Hz, $^4J_{H,H}$ = 2.08 Hz, 1H, H-C(3)), 7.39 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.64 Hz, 1H, H-C(7)), 6.55 (*br s*, 1H, NH), 4.78 (*br s*, 1H, NH), 3.78–3.75 (*m*, 2H, CH_2(1′)), 3.44–3.41 (*m*, 2H, CH_2(3′)), 1.92–1.86 (*m*, 2H, CH_2(2′)), 1.50 (*s*, 9H, ^{t}Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 157.2 (quart.), 152.1 (C=O), 148.9 (quart.), 147.5 (quart.), 144.1 (quart.), 130.1 (2 × CH), 124.5 (2 × CH), 123.5 (CH), 122.8 (CH), 120.5 (*q*, $^1J_{C,F}$ = 256.7 Hz, OCF_3), 117.1 (quart.), 116.5 (quart.), 114.2 (CH), 80.1 (quart., ^{t}Bu), 46.5 (CH_2(1′)), 37.4 (CH_2(3′)), 32.1 (CH_2(2′)), 28.4 (CH_3, ^{t}Bu). ^{19}F NMR (188 MHz, $CDCl_3$): δ = −58.5 (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 380 (20%) [(M-^{i}butene)H]$^+$, 436 (100%) [MH]$^+$. Analysis for $C_{22}H_{22}F_3N_3O_3$ (435.45): C 60.68; H 5.56; N 9.65; found: C 60.41; H 5.74; N 9.51.

*tert-Butyl N-{4-[9-(2-trifluoromethoxyacridinyl)amino]butyl} carbamate (**11**)*

As described for **9**, from **1** (2.0 g, 6.7 mmol), phenol (10.0 g, 0.1 mol) and **4** (1.26 g, 6.7 mmol), **11** (2.09 g, 69%) was obtained as a red, highly viscous oil. UV-vis (methanol): λ_{max} (log ε) = 286 nm (4.61). IR (film): ν = 3380*m*, 2979*m*, 1699*s*, 1649*m*, 1596*s*, 1535*s*, 1407*m*, 1366*m*, 1260*s*, 1212*s*, 1162*s*, 1005*m* cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(8)), 7.96 (*s*, 1H, H-C(1)), 7.83 (*d*, $^3J_{H,H}$ = 9.55 Hz, 1H, H-C(4)), 7.74 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(5)), 7.45 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.64 Hz, 1H, H-C(6)), 7.36 (*dd*, $^3J_{H,H}$ = 9.55 Hz, $^4J_{H,H}$ = 2.08 Hz, 1H, H-C(3)), 7.15 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.64 Hz, 1H, H-C(7)), 4.93 (*br s*, 1H, NH), 3.84–3.79 (*m*, 2H, CH_2(1′)), 3.23–3.16 (*m*, 2H,

$CH_2(4')$), 1.97–1.88 (*m*, 2H, $CH_2(2')$), 1.71–1.63 (*m*, 2H, $CH_2(3')$), 1.43 (*s*, 9H, ^{t}Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.2 (quart.), 155.3 (C=O), 143.5 (quart.), 140.7 (quart.), 139.3 (quart.), 133.2 (CH), 127.1 (CH), 124.6 (CH), 123.1 (CH), 122.6 (CH), 120.5 (*q*, $^1J_{C,F}$ = 258.6 Hz, OCF_3), 120.4 (CH), 116.6 (CH), 112.6 (quart.), 112.3 (quart.), 79.2 (quart., ^{t}Bu), 48.3 ($CH_2(1')$), 39.9 ($CH_2(4')$), 28.4 (CH_3, ^{t}Bu), 27.4 (CH_2), 27.2 (CH_2). ^{19}F NMR (188 MHz, $CDCl_3$): δ = −58.7 (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 394 (14%) [(M-^{i}butene)H]$^+$, 450 (100%) [MH]$^+$. Analysis for $C_{23}H_{26}F_3N_3O_3$ (449.48): C 61.46; H 5.83; N 9.35; found: C 61.32; H 5.99; N 9.21.

*tert-Butyl N-{5-[9-(2-trifluoromethoxyacridinyl)amino]pentyl} carbamate (**12**)*

As described for **9**, from **1** (2.0 g, 6.7 mmol), phenol (10.0 g, 0.1 mol) and **5** (1.49 g, 7.4 mmol), **12** (2.12 g, 68%) was obtained as a red, highly viscous oil. UV-vis (methanol): λ_{max} (log ε) = 282 nm (4.69). IR (film): ν = 3439*s*, 2934*w*, 1630*m*, 1508*w*, 1367*w*, 1261*s*, 1218*m*, 1165*m* cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.07 (*d*, $^3J_{H,H}$ = 9.55 Hz, 1H, H-C(4)), 8.05 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(8)), 8.03 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(5)), 7.92 (*d*, $^4J_{H,H}$ = 2.08 Hz, 1H, H-C(1)), 7.67 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.64 Hz, 1H, H-C(6)), 7.51 (*dd*, $^3J_{H,H}$ = 9.55 Hz, $^4J_{H,H}$ = 2.08 Hz, 1H, H-C(3)), 7.40 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.64 Hz, 1H, H-C(7)), 5.05 (*br s*, 1H, NH), 4.53 (*br s*, 1H, NH), 3.79–3.74 (*m*, 2H, $CH_2(1')$), 3.15–3.08 (*m*, 2H, $CH_2(5')$), 1.84–1.76 (*m*, 2H, $CH_2(2')$), 1.54–1.42 (*m*, 4H, 2 × $CH_2(3',4')$), 1.41 (*s*, 9H, ^{t}Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.9 (C=O), 151.2 (quart.), 149.1 (quart.), 147.5 (quart.), 144.0 (quart.), 131.6 (CH), 130.1 (CH), 129.4 (CH), 124.4 (CH), 123.9 (CH), 121.9 (CH), 120.5 (*q*, $^1J_{C,F}$ = 256.6 Hz, OCF_3), 116.7 (quart.), 115.9 (quart.), 113.9 (CH), 79.2 (quart., ^{t}Bu), 50.7 ($CH_2(1')$), 40.2 ($CH_2(5')$), 31.3 (CH_2), 29.9 (CH_2), 28.4 (CH_3, ^{t}Bu), 24.1 (CH_2). ^{19}F NMR (188 MHz, $CDCl_3$): δ = −58.5 (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 408 (12%) [(M-^{i}butene)H]$^+$, 464 (100%) [MH]$^+$. Analysis for $C_{24}H_{28}F_3N_3O_3$ (463.50): C 62.19; H 6.09; N 9.07; found: C 62.00; H 6.23; N 8.95.

*tert-Butyl N-{6-[9-(2-trifluoromethoxyacridinyl)amino]hexyl} carbamate (**13**)*

As described for **9**, from **1** (2.0 g, 6.7 mmol), phenol (10.0 g, 0.1 mol) and **6** (1.60 g, 7.4 mmol), **13** (1.24 g, 39%) was obtained as a red, highly viscous oil. UV-vis (methanol): λ_{max} (log ε) = 287 nm (4.70). IR (film): ν = 3358*m*, 3009*m*, 2934*s*, 2860*m*, 1692*s*, 1619*m*, 1561*s*, 1509*s*, 1476*s*, 1392*s*, 1366*s*, 1258*s*, 1167*s*, 1022*m* cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.08 (*d*, $^3J_{H,H}$ = 9.55 Hz, 1H, H-C(4)), 8.06 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(8)), 8.05 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(5)), 7.94 (*s*, 1H, H-C(1)), 7.67 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.64 Hz, 1H, H-C(6)), 7.51 (*dd*, $^3J_{H,H}$ = 9.55 Hz, $^4J_{H,H}$ = 2.08 Hz, 1H, H-C(3)), 7.39 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.64 Hz, 1H, H-C(7)), 5.15 (*br s*, 1H, NH), 4.51 (*br s*, 1H, NH), 3.79–3.74 (*m*, 2H, $CH_2(1')$), 3.13–3.05 (*m*, 2H, $CH_2(6')$), 1.81–1.73 (*m*, 2H, $CH_2(2')$), 1.50–1.32 (*m*, 15H, ^{t}Bu, 3 × $CH_2(3',4',5')$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.9 (C=O), 151.2 (quart.), 149.2 (quart.), 147.5 (quart.), 143.9 (quart.), 131.6 (CH), 130.2 (CH), 129.5 (CH), 124.4 (CH), 123.8 (CH), 121.9 (CH), 120.6 (*q*, $^1J_{C,F}$ = 254.6 Hz, OCF_3), 116.8 (quart.), 115.8 (quart.), 113.9 (CH), 79.1 (quart., ^{t}Bu), 50.5 ($CH_2(1')$), 40.2 ($CH_2(6')$), 31.6 (CH_2), 30.1 (CH_2), 28.5 (CH_3, ^{t}Bu), 26.4 (CH_2), 26.3 (CH_2). ^{19}F NMR (188 MHz, $CDCl_3$): δ = −58.5 (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 378 (4%) [(M-Boc)H]$^+$, 422 (12%) [(M-^{i}butene)H]$^+$, 478 (100%) [MH]$^+$. Analysis for $C_{25}H_{30}F_3N_3O_3$ (477.53): C 62.88; H 6.33; N 8.80; found: C 62.71; H 6.43; N 8.75.

*tert-Butyl N-{7-[9-(2-trifluoromethoxyacridinyl)amino]heptyl} carbamate (**14**)*

As described for **9**, from **1** (2.0 g, 6.7 mmol), phenol (10.0 g, 0.1 mol) and **7** (1.70 g, 7.4 mmol), **14** (1.59 g, 48%) was obtained as a red, highly viscous oil. UV-vis (methanol): λ_{max} (log ε) = 286 nm (4.71). IR (film): ν = 3425*s*, 2930*s*, 2856*s*, 1690*s*, 1632*s*, 1591*s*, 1566*s*, 1528*s*, 1502*s*, 1391*m*, 1365*m*, 1276*m*, 1247*s*, 1170*s*, 1030*m* cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.09 (*d*, $^3J_{H,H}$ = 9.55 Hz, 1H, H-C(4)), 8.06 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(8)), 8.03 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(5)), 7.92 (*d*, $^4J_{H,H}$ = 2.08 Hz, 1H, H-C(1)), 7.68 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.64 Hz, 1H, H-C(6)), 7.52 (*dd*, $^3J_{H,H}$ = 9.55 Hz, $^4J_{H,H}$ = 2.08 Hz, 1H, H-C(3)), 7.41 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.64 Hz, 1H, H-C(7)), 5.00 (*br s*, 1H, NH), 4.47 (*br s*, 1H, NH), 3.80–3.75 (*m*, 2H, $CH_2(1')$), 3.11–3.03 (*m*, 2H, $CH_2(7')$), 1.81–1.73 (*m*, 2H, $CH_2(2')$), 1.47–1.25 (*m*, 17H, ^{t}Bu, 4 × $CH_2(3'–6')$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.9 (C=O), 151.2 (quart.), 149.3 (quart.), 147.7 (quart.), 144.0 (quart.), 131.9 (CH), 130.1 (CH), 129.8 (CH), 124.4 (CH), 123.9 (CH), 121.8 (CH), 120.5 (*q*, $^1J_{C,F}$ = 256.4 Hz, OCF_3), 116.7 (quart.), 115.9 (quart.), 113.8 (CH), 79.0 (quart., ^{t}Bu), 50.8 ($CH_2(1')$), 40.5 ($CH_2(7')$), 31.7 (CH_2), 30.0 (CH_2), 29.0 (CH_2), 28.5 (CH_3, ^{t}Bu), 26.8 (CH_2), 26.6 (CH_2). ^{19}F NMR (188 MHz, $CDCl_3$): δ = −58.5 (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 392 (8%) [(M-Boc)H]$^+$, 436 (15%) [(M-^{i}butene)H]$^+$, 492 (100%) [MH]$^+$. Analysis for $C_{26}H_{32}F_3N_3O_3$ (491.56): C 63.53; H 6.56; N 8.55; found: C 63.41; H 6.71; N 8.39.

*tert-Butyl N-{8-[9-(2-trifluoromethoxyacridinyl)amino]octyl} carbamate (**15**)*

As described for **9**, from **1** (2.0 g, 6.7 mmol), phenol (10.0 g, 0.1 mol) and **8** (2.71 g, 11.1 mmol), **15** (3.09 g, 61%) was obtained as a red, highly viscous oil. UV-vis (methanol): λ_{max} (log ε) = 286 nm (4.76). IR (film): ν = 3346*m*, 2930*s*, 2857*m*, 1694*s*, 1633*m*, 1564*s*, 1520*s*, 1392*m*, 1366*s*, 1255*s*, 1165*s*, 1041*m* cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.05 (*d*, $^3J_{H,H}$ = 9.55 Hz, 1H, H-C(4)), 8.03 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(8)), 8.02 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(5)), 7.92 (*d*, $^4J_{H,H}$ = 2.08 Hz, 1H, H-C(1)), 7.65 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.64 Hz, 1H, H-C(6)), 7.50 (*dd*, $^3J_{H,H}$ = 9.55 Hz, $^4J_{H,H}$ = 2.08 Hz, 1H, H-C(3)), 7.38 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.64 Hz, 1H, H-C(7)), 4.49 (*br s*, 1H, NH), 3.80–3.75 (*m*, 2H, $CH_2(1')$), 3.10–3.03 (*m*, 2H, $CH_2(8')$), 1.82–1.74 (*m*, 2H, $CH_2(2')$), 1.46–1.38 (*m*, 13H, ^{t}Bu, 2 × $CH_2(3',7')$), 1.33–1.23 (*m*, 6H, 3 ± $CH_2(4',5',6')$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.9 (C=O), 151.2 (quart.), 149.3 (quart.), 147.6 (quart.), 144.0 (quart.), 131.8 (CH), 130.1 (CH), 129.7 (CH), 124.5 (CH), 123.9 (CH), 121.8 (CH), 120.7 (*q*, $^1J_{C,F}$ = 256.7 Hz, OCF_3), 116.7 (quart.), 115.9 (quart.), 113.8 (CH), 79.0 (quart., ^{t}Bu), 50.8 ($CH_2(1')$), 40.6 ($CH_2(8')$), 31.7 (CH_2), 30.1 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 28.5 (CH_3, ^{t}Bu), 26.8 (CH_2), 26.7 (CH_2). ^{19}F NMR (188 MHz, $CDCl_3$): δ = −58.5 (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 406 (4%) [(M-Boc)H]$^+$, 450 (10%) [(M-^{i}butene)H]$^+$, 506 (100%) [MH]$^+$. Analysis for $C_{28}H_{34}F_3N_3O_3$ (517.60): C 64.98; H 6.62; N 8.12; found: C 64.81; H 6.85; N 7.99.

*N^1-[9-(2-Trifluoromethoxyacridinyl)-ethane-1,2-diamine bis-hydrochloride (**16**)*

A solution of **9** (1.41 g, 3.3 mmol) in methanol (50 mL) containing aq. hydrochloric acid (10%, 25 mL) was stirred at room temperature for 16 h. The solvents were removed under reduced pressure, and **16** (1.29 g, 98%) was obtained as a yellow, amorphous solid. UV-vis (methanol): λ_{max} (log ε) = 284 nm (4.59). IR (KBr): ν = 3423*s*, 1592*m*, 1534*m*, 1474*m*, 1259*m*, 1165*m* cm^{-1}. ^1H NMR (400 MHz, D_2O): δ = 8.12 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(8)), 8.07 (*d*, $^4J_{H,H}$ = 1.97 Hz, 1H, H-C(1)), 7.82 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.95 Hz, 1H, H-C(7)), 7.75 (*dd*, $^3J_{H,H}$ = 9.34 Hz, $^4J_{H,H}$ = 1.97 Hz, 1H, H-C(3)), 7.61 (*d*, $^3J_{H,H}$ = 9.34 Hz, 1H, H-C(4)), 7.53 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(5)), 7.44 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.95 Hz, 1H, H-C(6)), 4.34–4.29 (*m*, 2H, CH_2(1′)), 3.47–3.43 (*m*, 2H, CH_2(2′)). ^{13}C NMR (100 MHz, D_2O): δ = 157.5 (quart.), 144.3 (quart.), 139.3 (quart.), 137.1 (quart.), 136.0 (CH), 129.4 (CH), 124.8 (2 ± CH), 120.7 (CH), 120.3 (*q*, $1J_{C,F}$ = 257 Hz, OCF_3), 118.4 (CH), 116.2 (CH), 112.2 (quart.), 111.5 (quart.), 45.8 (CH_2(1′)), 38.5 (CH_2(2′)). ^{19}F NMR (188 MHz, D_2O): δ = −58.8 (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 322 (100%) $[MH]^+$.

*N^1-[9-(2-Trifluoromethoxyacridinyl)]-propane-1,3-diamine bis-hydrochloride (**17**)*

As described for **9**, from **10** (1.18 g, 2.7 mmol), **17** (1.04 g, 94%) was obtained as a yellow, amorphous solid. UV-vis (methanol): λ_{max} (log ε) = 280 nm (4.55). IR (KBr): ν = 3418*s*, 2928*s*, 1628*s*, 1594*s*, 1537*m*, 1482*s*, 1400*m*, 1367*m*, 1262*s*, 1204*s*, 1165*s* cm^{-1}. ^1H NMR (400 MHz, D_2O): δ = 7.96 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(8)), 7.89 (*s*, 1H, H-C(1)), 7.70 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.95 Hz, 1H, H-C(7)), 7.64 (*d*, $^3J_{H,H}$ = 9.34 Hz, 1H, H-C(3)), 7.40 (*d*, $^3J_{H,H}$ = 9.34 Hz, 1H, H-C(4)), 7.33 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.95 Hz, 1H, H-C(6)), 7.32 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(5)), 3.95–3.89 (*m*, 2H, CH_2(1′)), 3.02–2.97 (*m*, 2H, CH_2(3′)), 2.17–2.09 (*m*, 2H, CH_2(2′)). ^{13}C NMR (100 MHz, D_2O): δ = 157.0 (quart.), 144.0 (quart.), 139.0 (quart.), 137.1 (quart.), 136.8 (CH), 129.3 (CH), 124.5 (CH), 124.1 (CH), 120.5 (CH), 120.3 (*q*, $1J_{C,F}$ = 258 Hz, OCF_3), 118.2 (CH), 116.4 (CH), 111.8 (quart.), 111.4 (quart.), 45.9 (CH_2(1′)), 36.9 (CH_2(3′)), 27.0 (CH_2(2′)). ^{19}F NMR (188 MHz, D_2O): δ = −58.8 (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 336 (100%) $[MH]^+$.

*N^1-[9-(2-Trifluoromethoxyacridinyl)]-butane-1,4-diamine bis-hydrochloride (**18**)*

As described for **9**, from **11** (1.50 g, 3.3 mmol), **18** (1.39 g, 99%) was obtained as a yellow, amorphous solid. UV-vis (methanol): λ_{max} (log ε) = 285 nm (4.52). IR (KBr): ν = 3427*s*, 2929*s*, 1631*s*, 1596*s*, 1535*m*, 1477*m*, 1366*w*, 1261*s*, 1207*s*, 1162*s* cm^{-1}. ^1H NMR (400 MHz, D_2O): δ = 8.08 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(8)), 7.99 (*s*, 1H, H-C(1)), 7.77 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.95 Hz, 1H, H-C(7)), 7.70 (*d*, $^3J_{H,H}$ = 9.34 Hz, 1H, H-C(3)), 7.55 (*d*, $^3J_{H,H}$ = 9.34 Hz, 1H, H-C(4)), 7.48 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.95 Hz, 1H, H-C(6)), 7.37 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(5)), 3.95–3.90 (*m*, 2H, CH_2(1′)), 2.92–2.87 (*m*, 2H, CH_2(4′)), 1.86–1.78 (*m*, 2H, CH_2(2′)), 1.68–1.58 (*m*, 2H, CH_2(3′)). ^{13}C NMR (100 MHz, D_2O): δ = 155.8 (quart.), 143.8 (quart.), 138.4 (quart.), 136.7 (quart.), 135.7 (CH), 129.1 (CH), 124.6 (CH), 120.3 (CH), 120.3 (*q*, $1J_{C,F}$ = 258 Hz, OCF_3), 117.8 (CH), 110.8 (quart.), 48.1 (CH_2(1′)), 39.2 (CH_2(4′)), 26.3 (CH_2), 24.3 (CH_2). ^{19}F NMR (188 MHz, D_2O): δ = −58.8 (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 350 (100%) $[MH]^+$.

*N^1-[9-(2-Trifluoromethoxyacridinyl)]-pentane-1,5-diamine bis-hydrochloride (**19**)*

As described for **9**, from **12** (2.02 g, 4.4 mmol), **19** (1.86 g, 98%) was obtained as yellow, amorphous solid. UV-vis (methanol): λ_{max} (log ε) = 284 nm (4.49). IR (KBr): ν = 3418*s*, 2966*s*, 1629*m*, 1594*s*, 1538*m*, 1485*s*, 1363*w*, 1258*s*, 1207*s*, 1165*s* cm^{-1}. ^1H NMR (400 MHz, CD_3OD): δ = 8.55 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(8)), 8.51 (*s*, 1H, H-C(1)), 7.99 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.95 Hz, 1H, H-C(7)), 7.95–7.92 (*m*, 2H, 2 ± H-C(4,5)), 7.85 (*d*, $^3J_{H,H}$ = 9.34 Hz, 1H, H-C(3)), 7.60 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.95 Hz, 1H, H-C(6)), 4.21–4.17 (*m*, 2H, CH_2(1′)), 2.98–2.94 (*m*, 2H, CH_2(5′)), 2.10–2.00 (*m*, 2H, CH_2(2′)), 1.81–1.70 (*m*, 2H, CH_2(4′)), 1.61–1.54 (*m*, 2H, CH_2(3′)). ^{13}C NMR (125 MHz, CD_3OD): δ = 159.4 (quart.), 146.1 (quart.), 141.1 (quart.), 138.9 (quart.), 136.9 (CH), 130.3 (CH), 129.9 (CH), 129.2 (CH), 126.3 (CH), 122.0 (*q*, $1J_{C,F}$ = 257 Hz, OCF_3), 122.1 (CH), 119.7 (CH), 113.2 (quart.), 113.0 (quart.), 50.2 (CH_2(1′)), 40.5 (CH_2(5′)), 29.9 (CH_2), 28.1 (CH_2), 24.7 (CH_2). ^{19}F NMR (188 MHz, CD_3OD): δ = −60.1 (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 364 (100%) $[MH]^+$.

*N^1-[9-(2-Trifluoromethoxyacridinyl)]-hexane-1,6-diamine bis-hydrochloride (**20**)*

As described for **9**, from **13** (1.19 g, 2.5 mmol), **20** (1.09 g, 98%) was obtained as a yellow, amorphous solid. UV-vis (methanol): λ_{max} (log ε) = 284 nm (4.60). IR (KBr): ν = 3445*s*, 2936*s*, 1629*m*, 1595*s*, 1538*m*, 1484*m*, 1363*w*, 1260*s*, 1208*s*, 1163*s* cm^{-1}. ^1H NMR (400 MHz, D_2O): δ = 7.96 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(8)), 7.85 (*s*, 1H, H-C(1)), 7.72 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.95 Hz, 1H, H-C(7)), 7.66 (*d*, $^3J_{H,H}$ = 9.34 Hz, 1H, H-C(3)), 7.44 (*d*, $^3J_{H,H}$ = 9.34 Hz, 1H, H-C(4)), 7.37 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(5)), 7.33 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.95 Hz, 1H, H-C(6)), 3.79–3.73 (*m*, 2H, CH_2(1′)), 2.88–2.82 (*m*, 2H, CH_2(6′)), 1.76–1.68 (*m*, 2H, CH_2(2′)), 1.57–1.50 (*m*, 2H, CH_2(5′)), 1.34–1.26 (*m*, 4H, CH_2(3′,4′)). ^{13}C NMR (125 MHz, D_2O): δ = 156.5 (quart.), 135.6 (CH), 129.1 (CH), 120.3 (CH), 120.2 (*q*, $1J_{C,F}$ = 258 Hz, OCF_3), 122.1 (CH), 118.0 (CH), 48.5 (CH_2(1′)), 39.3 (CH_2(6′)), 28.7 (CH_2), 26.6 (CH_2), 25.4 (CH_2), 25.2 (CH_2). ^{19}F NMR (188 MHz, D_2O): δ = −58.8 (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 378 (100%) $[MH]^+$.

*N^1-[9-(2-Trifluoromethoxyacridinyl)]-heptane-1,7-diamine bis-hydrochloride (**21**)*

As described for **9**, from **14** (1.54 g, 3.1 mmol), **21** (1.41 g, 97%) was obtained as a yellow, amorphous soild. UV-vis (methanol): λ_{max} (log ε) = 286 nm (4.62). IR (KBr): ν = 3421*m*, 2930*s*, 1629*m*, 1596*s*, 1540*m*, 1477*m*, 1367*w*, 1257*s*, 1212*s*, 1156*s* cm^{-1}. ^1H NMR (400 MHz, D_2O): δ = 7.95 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(8)), 7.84 (*s*, 1H, H-C(1)), 7.72 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.95 Hz, 1H, H-C(7)), 7.66 (*d*, $^3J_{H,H}$ = 9.34 Hz, 1H, H-C(3)), 7.45 (*d*, $^3J_{H,H}$ = 9.34 Hz, 1H, H-C(4)), 7.37 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(5)), 7.33 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.95 Hz, 1H, H-C(6)), 3.77–3.72 (*m*, 2H, CH_2(1′)), 2.87–2.82 (*m*, 2H, CH_2(7′)), 1.74–1.66 (*m*, 2H, CH_2(2′)), 1.55–1.48 (*m*, 2H, CH_2(6′)), 1.30–1.22 (*m*, 6H, 3 × CH_2(3′–5′)). ^{13}C NMR (100 MHz, D_2O): δ = 156.3 (quart.), 135.6 (CH), 129.1 (CH), 120.4 (CH), 120.2 (*q*, $1J_{C,F}$ = 257

Hz, OCF_3), 118.0 (CH), 48.6 ($CH_2(1')$), 39.6 ($CH_2(7')$), 29.0 (CH_2), 28.0 (CH_2), 27.8 (CH_2), 25.9 (CH_2), 25.7 (CH_2). ^{19}F NMR (188 MHz, D_2O): $\delta = -58.9$ (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 392 (100%) $[MH]^+$.

*N^1-[9-(2-Trifluoromethoxyacridinyl)]-octane-1,8-diamine bishydrochloride (**22**)*

As described for **9**, from **30** (2.99 g, 5.9 mmol), **22** (2.6 g, 92%) was obtained as a yellow, amorphous solid. UV-vis (methanol): λ_{max} (log ε) = 285 nm (4.56). IR (KBr): ν = 3424*m*, 2928*s*, 1629*m*, 1597*s*, 1540*m*, 1487*s*, 1366*w*, 1256*s*, 1212*s*, 1162*s* cm^{-1}. ^1H NMR (400 MHz, CD_3OD): $\delta = 8.53$ (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(8)), 8.47 (*s*, 1H, H-C(1)), 7.99 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.95 Hz, 1H, H-C(7)), 7.95 (*d*, $^3J_{H,H}$ = 9.34 Hz, 1H, H-C(4)), 7.92 (*dd*, $^3J_{H,H}$ = 9.34 Hz, $^4J_{H,H}$ = 2.28 Hz, 1H, H-C(3)), 7.85 (*d*, $^3J_{H,H}$ = 8.72 Hz, 1H, H-C(5)), 7.59 (*dd*, $^3J_{H,H}$ = 8.72 Hz, $^3J_{H,H}$ = 6.95 Hz, 1H, H-C(6)), 4.17–4.12 (*m*, 2H, $CH_2(1')$), 2.93–2.88 (*m*, 2H, $CH_2(8')$), 2.05–1.97 (*m*, 2H, $CH_2(2')$), 1.70–1.62 (*m*, 2H, $CH_2(7')$), 1.54–1.38 (*m*, 6H, 4 × $CH_2(3'–6')$). ^{13}C NMR (100 MHz, CD_3OD): δ = 159.2 (quart.), 136.7 (CH), 130.2 (CH), 125.2 (CH), 122.0 (CH), 121.9 (*q*, $1J_{C,F}$ = 257 Hz, OCF_3), 119.6 (CH), 50.5 ($CH_2(1')$), 40.8 ($CH_2(8')$), 30.5 (CH_2), 30.0 (2 ± CH_2), 28.5 (CH_2), 27.7 (CH_2), 27.4 (CH_2). ^{19}F NMR (188 MHz, CD_3OD): $\delta = -60.4$ (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 406 (100%) $[MH]^+$.

*2-((2-{[2-(9-{2-Trifluoromethoxyacridinyl}amino)ethyl]amino}-2-oxoethyl){2-[(2-{[2-(9-{2-trifluoromethoxyacridinyl}amino)-ethyl]amino}-2-oxoethyl)(carboxymethyl)amino]ethyl}amino) acetic acid (**23**)*

A solution of **16** (304 mg, 0.77 mmol) in dry DMF (10 mL) containing triethylamine (3 mL) was stirred at room temperature for 10 min; EDTA dianhydride (99 mg, 0.39 mmol) was added, and stirring was continued at room temperature for another 12 h. After addition of acetone (10 mL) and hexane (40 mL), the crude product was filtered off and dissolved in methanol (15 mL) containing aq. hydrochloric acid (10%, 0.5 mL). The solvents were removed *in vacuo*, and the product was purified by chromatography (silica gel, methanol/acetonitrile/ammonia 70 : 25 : 5) to afford **23** (80 mg, 23%) as an amorphous solid. UV-vis (methanol): λ_{max} (log ε) = 280 nm (4.91). IR (KBr): ν = 3266*m*, 1590*s*, 1479*m*, 1435*m*, 1403*m*, 1332*m*, 1255*s*, 1216*s*, 1160*s* cm^{-1}. ^1H NMR (500 MHz, DMSO-D_6/D_2SO_4): $\delta = 8.51$ (*s*, 2H, 2 × H-C(1)), 8.50 (*d*, $^3J_{H,H}$ = 8.64 Hz, 2H, 2 × H-C(8)), 8.00–7.95 (*m*, 6H, 6 × H-C(3,4,7)), 7.83 (*d*, $^3J_{H,H}$ = 8.64 Hz, 2H, 2 × H-C(5)), 7.53–7.49 (*m*, 2H, 2 ± H-C(6)), 4.16–4.13 (*m*, 4H, 2 × $CH_2(1')$), 3.87 (*s*, 4H, 2 × $CH_2(2'')$), 3.79 (*s*, 4H, 2 × $CH_2(3'')$), 3.66–3.61 (*m*, 4H, 2 × $CH_2(2')$), 3.25 (*s*, 4H, 2 × $CH_2(1'')$). ^{13}C NMR (125 MHz, DMSO-D_6/D_2SO_4): δ = 169.7 (C=O), 168.48 (C=O), 168.44 (quart.), 157.5 (quart.), 157.4 (quart.), 143.7 (quart.), 139.5 (quart.), 138.2 (quart.), 135.7 (CH), 129.0 (CH), 124.6 (CH), 121.44 (CH), 121.35 (CH), 120.2 (*q*, $^1J_{C,F}$ = 257 Hz, OCF_3), 118.8 (CH), 118.7 (CH), 109.7 (quart.), 55.3 ($CH_2(3'')$), 54.2 ($CH_2(2'')$), 50.8 ($CH_2(1'')$), 48.6 ($CH_2(1')$), 38.2 ($CH_2(2')$). ^{19}F NMR (188 MHz, DMSO-D_6): $\delta = -57.7$ (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 469 (100%) $[MHK]^{2+}$, 899 (14%) $[MH]^+$, 837 (27%) $[MK]^+$. Analysis for $C_{42}H_{40}F_6N_8O_8$ (898.83): C 56.13; H 4.49; N 12.47; found: C 55.97; H 4.61; N 12.32.

*2-((2-{[3-(9-{2-Trifluoromethoxyacridinyl}amino)propyl]-amino}-2-oxoethyl){2-[(2-{[3-(9-{2-trifluoromethoxyacridinyl}-amino)propyl] amino}-2-oxoethyl)(carboxymethyl)amino]-ethyl}amino) acetic acid (**24**)*

As described for **23**, from **17** (0.5 g, 1.23 mmol) and EDTA dianhydride (141 mg, 0.55 mmol), **24** (220 mg, 43%) was obtained as an orange, amorphous solid. UV-vis (methanol): λ_{max} (log ε) = 280 nm (4.68). IR (KBr): ν = 3281*s*, 1652*s*, 1574*s*, 1404*m*, 1258*s*, 1117*m* cm^{-1}. ^1H NMR (500 MHz, DMSO-D_6/D_2SO_4): $\delta = 8.50–8.42$ (*m*, 2H, 2 × H-C(8)), 8.43 (*s*, 2H, 2 × H-C(1)), 8.04–7.93 (*m*, 6H, 6 × H-C(3,4,7)), 7.85 (*d*, $^3J_{H,H}$ = 8.92 Hz, 2H, 2 × H-C(5)), 7.55–7.49 (*m*, 2H, 2 × H-C(6)), 4.12–4.06 (*m*, 4H, 2 × $CH_2(1')$), 3.91 (*s*, 4H, 2 × $CH_2(2'')$), 3.81 (*s*, 4H, 2 × $CH_2(3'')$), 3.32 (*s*, 4H, 2 × $CH_2(1'')$), 3.26–3.21 (*m*, 4H, 2 × $CH_2(3')$), 2.08–2.02 (*m*, 4H, 2 × $CH_2(2')$). ^{13}C NMR (125 MHz, DMSO-D_6/D_2SO_4): δ = 172.3 (C=O), 169.8 (C=O), 169.3 (quart.), 166.8 (quart.), 157.6 (quart.), 157.5 (quart.), 135.6 (quart.), 138.2 (quart.), 129.0 (CH), 123.9 (CH), 121.4 (CH), 121.3 (CH), 120.2 (*q*, $^1J_{C,F}$ = 258 Hz, OCF_3), 118.8 (CH), 118.7 (CH), 55.4 ($CH_2(3'')$), 54.4 ($CH_2(2'')$), 50.7 ($CH_2(1'')$), 46.7 ($CH_2(1')$), 36.3 ($CH_2(3')$), 28.7 ($CH_2(2')$). ^{19}F NMR (188 MHz, DMSO-D_6): $\delta = -57.7$ (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 483 (100%) $[MHK]^{2+}$, 927 (10%) $[MH]^+$, 965 (50%) $[MK]^+$. Analysis for $C_{44}H_{44}F_6N_8O_8$ (926.88): C 57.02; H 4.79; N 12.09; found: C 56.91; H 4.93; N 11.88.

*2-((2-{[4-(9-{2-Trifluoromethoxyacridinyl}amino)butyl]amino}-2-oxoethyl){2-[(2-{[4-(9-{2-trifluoromethoxyacridinyl}-amino)butyl]amino}-2-oxoethyl)(carboxymethyl)amino]-ethyl}amino) acetic acid (**25**)*

As described for **23**, from **18** (0.48 g, 1.14 mmol) and EDTA dianhydride (131 mg, 0.51 mmol), **25** (60 mg, 12%) was obtained as an orange, amorphous solid. UV-vis (methanol): λ_{max} (log ε) = 281 nm (4.99). IR (KBr): ν = 3261*m*, 2929*m*, 1652*s*, 1566*s*, 1478*m*, 1395*m*, 1256*s*, 1217*s*, 1160*s* cm^{-1}. ^1H NMR (500 MHz, DMSO-D_6/D_2SO_4): $\delta = 8.50–8.45$ (*m*, 2H, 2 ± H-C(8)), 8.05–7.90 (*m*, 8H, 8 × H-C(1,3,4,7)), 7.87 (*d*, $^3J_{H,H}$ = 8.73 Hz, 2H, 2 × H-C(5)), 7.55–7.50 (*m*, 2H, 2 × H-C(6)), 4.09–4.04 (*m*, 4H, 2 × $CH_2(1')$), 4.07 (*s*, 4H, 2 × $CH_2(2'')$), 3.95 (*s*, 4H, 2 × $CH_2(3'')$), 3.55 (*s*, 4H, 2 × $CH_2(1'')$), 3.16–3.11 (*m*, 4H, 2 × $CH_2(4')$), 1.92–1.85 (*m*, 8H, 4 × $CH_2(2',3')$), 1.56–1.50 (*m*, 8H, 4 × $CH_2(2',3')$). ^{13}C NMR (125 MHz, DMSO-D_6/D_2SO_4): δ = 170.6 (C=O), 168.2 (C=O), 165.1 (quart.), 158.1 (quart.), 157.6 (quart.), 143.8 (quart.), 139.5 (quart.), 138.2 (quart.), 129.5 (CH), 124.9 (CH), 124.8 (CH), 121.9 (CH), 121.5 (CH), 120.3 (*q*, $^1J_{C,F}$ = 257 Hz, OCF_3), 119.2 (CH), 118.9 (CH), 111.8 (quart.), 111.6 (quart.), 55.5 ($CH_2(3'')$), 54.3 ($CH_2(2'')$), 50.3 ($CH_2(1'')$), 48.6 ($CH_2(1')$), 38.5 ($CH_2(4')$), 26.3 (CH_2), 26.1 (CH_2). ^{19}F NMR (188 MHz, DMSO-D_6): $\delta = -57.7$ (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 497 (100%) $[MHK]^{2+}$, 955 (4%) $[MH]^+$, 993 (15%) $[MK]^+$. Analysis for $C_{46}H_{48}F_6N_8O_8$ (954.93): C 57.86; H 5.07; H 11.73; found: C 57.62; H 5.23; N 11.63.

*2-((2-{[5-(9-{2-Trifluoromethoxyacridinyl}amino)pentyl]-amino}-2-oxoethyl){2-[(2-{[5-(9-{2-trifluoromethoxyacridinyl} amino)pentyl]amino}-2-oxoethyl)(carboxymethyl)amino]-ethyl}amino) acetic acid (**26**)*

As described for **23**, from **19** (0.5 g, 1.15 mmol) and EDTA dianhydride (132 mg, 0.52 mmol), **26** (80 mg, 16%) was obtained as an orange, amorphous solid. UV-vis (methanol): λ_{max} (log ε) = 284 nm (5.01). IR (KBr): ν = 3406*m*, 2933*m*, 1565*s*, 1523*m*, 1478*m*, 1436*m*, 1397*m*, 1330*w*, 1257*s*,

1217*s*, 1160*m* cm^{-1}. ^1H NMR (500 MHz, DMSO-D_6/D_2SO_4): δ = 8.69−8.40 (*br m*, 4H, 4 × H-C(1,8)), 8.01−7.95 (*m*, 6H, 6 × H-C(3,4,7)), 7.87 (*d*, $^3J_{H,H}$ = 8.94 Hz, 2H, 2 × H-C(5)), 7.56−7.51 (*m*, 2H, 2 × H-C(6)), 4.09 (*s*, 4H, 2 × CH_2(2″)), 4.06−4.01 (*m*, 4H, 2 × CH_2(1′)), 3.97 (*s*, 4H, 2 × CH_2(3″)), 3.56 (*s*, 4H, 2 × CH_2(1″)), 3.13−3.08 (*m*, 4H, 2 × CH_2(5′)), 1.91−1.84 (*m*, 4H, 2 × CH_2(2′)), 1.51−1.43 (*m*, 4H, 2 × CH_2(4′)), 1.42−1.34 (*m*, 4H, 2 × CH_2(3′)). ^{13}C NMR (125 MHz, DMSO-D_6/D_2SO_4): δ = 169.1 (C=O), 168.2 (C=O), 165.0 (quart.), 157.6 (quart.), 157.5 (quart.), 143.8 (quart.), 139.5 (quart.), 135.7 (CH), 129.1 (CH), 124.8 (CH), 121.53 (CH), 121.43 (CH), 120.4 (*q*, $^1J_{C,F}$ = 257 Hz, OCF_3), 118.96 (CH), 118.85 (CH), 111.8 (quart.), 111.6 (quart.), 55.5 (CH_2(3″)), 54.3 (CH_2(2″)), 50.3 (CH_2(1″)), 49.0 (CH_2(1′)), 38.7 (CH_2(5′)), 28.5 (2 × CH_2), 23.7 (CH_2). ^{19}F NMR (188 MHz, DMSO-D_6): δ = −57.7 (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 511 (100%) $[MHK]^{2+}$, 1021 (9%) $[MK]^+$. Analysis for $C_{48}H_{52}F_6N_8O_8$ (982.99): C 58.65; H 5.33; N 11.40; found: C 58.51; H 5.56; N 11.21.

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

As described for **23**, from **20** (0.5 g, 1.11 mmol) and EDTA dianhydride (128 mg, 0.5 mmol), **27** (122 mg, 24%) was obtained as an orange, amorphous solid. UV-vis (methanol): λ_{max} (log ε) = 283 nm (4.92). IR (KBr): ν = 3423*s*, 2935*m*, 1647*s*, 1593*s*, 1485*m*, 1401*m*, 1364*m*, 1259*s*, 1208*s*, 1163*s* cm^{-1}. ^1H NMR (500 MHz, DMSO-D_6/D_2SO_4): δ = 8.55−8.37 (*br m*, 4H, 4 × H-C(1,8)), 8.02−7.89 (*m*, 6H, 6 × H-C(3,4,7)), 7.85 (*d*, $^3J_{H,H}$ = 8.57 Hz, 2H, 2 × H-C(5)), 7.52−7.47 (*m*, 2H, 2 × H-C(6)), 4.11 (*s*, 4H, 2 × CH_2(2″)), 4.04−3.96 (*m*, 4H, 2 × CH_2(1′)), 3.99 (*s*, 4H, 2 × CH_2(3″)), 3.62 (*s*, 4H, 2 × CH_2(1″)), 3.07−3.01 (*m*, 4H, 2 × CH_2(6′)), 1.86−1.79 (*m*, 4H, 2 × CH_2(2′)), 1.42−1.23 (*m*, 12H, 6 × CH_2(3′−5′)). ^{13}C NMR (125 MHz, DMSO-D_6/D_2SO_4): δ = 168.0 (C=O), 164.7 (C=O), 158.2 (quart.), 157.6 (quart.), 144.0 (quart.), 139.7 (quart.), 138.4 (quart.), 135.9 (CH), 129.3 (CH), 125.1 (CH), 121.73 (CH), 121.63 (CH), 120.6 (*q*, $^1J_{C,F}$ = 257 Hz, OCF_3), 119.15 (CH), 119.05 (CH), 112.0 (quart.), 111.8 (quart.), 55.7 (CH_2(3″)), 54.6 (CH_2(2″)), 50.4 (CH_2(1″)), 49.1 (CH_2(1′)), 39.0 (CH_2(6′)), 28.9 (2 × CH_2), 26.31 (CH_2), 26.24 (CH_2). ^{19}F NMR (188 MHz, DMSO-D_6): δ = −57.8 (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 525 (100%) $[MHK]^{2+}$, 1049 (20%) $[MK]^+$. Analysis for $C_{50}H_{56}F_6N_8O_8$ (1011.04): C 59.40; H 5.59; N 11.08; found: C 59.32; H 5.78; N 10.94.

*2-((2-{[7-(9-{2-Trifluoromethoxyacridinyl}amino)heptyl]-amino}-2-oxoethyl){2-[(2-{[7-(9-{2-trifluoromethoxyacridinyl}-amino)heptyl amino}-2-oxoethyl)(carboxymethyl)amino]-ethyl}amino) acetic acid (**28**)*

As described for **23**, from **21** (0.5 g, 1.08 mmol) and EDTA dianhydride (124 mg, 0.48 mmol), **28** (63 mg, 13%) was obtained as an orange, amorphous solid. UV-vis (methanol): λ_{max} (log ε) = 281 nm (5.03). IR (KBr): ν = 3323*s*, 2936*m*, 1650*s*, 1593*s*, 1488*m*, 1399*m*, 1364*m*, 1260*s*, 1211*s*, 1160*s* cm^{-1}. ^1H NMR (500 MHz, DMSO-D_6/D_2SO_4): δ = 8.57−8.38 (*br m*, 4H, 4 × H-C(1,8)), 8.07−7.95 (*m*, 6H, 6 × H-C(3,4,7)), 7.87 (*d*, $^3J_{H,H}$ = 8.75 Hz, 2H, 2 × H-C(5)), 7.57−7.51 (*m*, 2H, 2 × H-C(6)), 4.09−4.02 (*m*, 4H, 2 × CH_2(1′)), 4.07 (*s*, 4H, 2 × CH_2(2″)), 3.94 (*s*, 4H, 2 × CH_2(3″)), 3.53 (*s*, 4H, 2 × CH_2(1″)), 3.09−3.03 (*m*, 4H, 2 × CH_2(7′)), 1.89−1.82 (*m*, 4H, 2 × CH_2(2′)), 1.42−1.18 (*m*, 16H, 8 × CH_2(3′−6′)). ^{13}C NMR (125 MHz, DMSO-D_6/D_2SO_4): δ = 168.2 (C=O), 164.7 (C=O), 157.4 (quart.), 129.3 (2 × CH), 121.0 (2 × CH), 120.0 (CH), 119.15 (2 × CH), 56.0 (CH_2(3″)), 55.0 (CH_2(2″)), 50.5 (CH_2(1″)), 49.0 (CH_2(1′)), 40.3 (CH_2(7′)), 28.7 (CH_2), 28.4 (2 × CH_2), 26.2 (2 × CH_2). ^{19}F NMR (188 MHz, CD_3OD/TFA): δ = −78.3 (OCF_3). MS (ESI, 4.1 kV, 8 μL/min, N_2, methanol): 539 (40%) $[MHK]^{2+}$, 1077 (100%) $[MK]^+$. Analysis for $C_{52}H_{60}F_6N_8O_8$ (1039.10): C 60.11; H 5.82; N 10.78; found: C 59.96; H 5.95; N 10.61.

*2-((2-{[8-(9-{2-Trifluoromethoxyacridinyl}amino)octyl]amino}-2-oxoethyl){2-[(2-{[8-(9-{2-trifluoromethoxyacridinyl}amino)-octyl]amino}-2-oxoethyl)(carboxymethyl)amino]ethyl}amino) acetic acid (**29**)*

As described for **23**, from **22** (0.5 g, 1.05 mmol) and EDTA dianhydride (120 mg, 0.47 mmol), **29** (62 mg, 12%) was obtained as an orange, amorphous solid. UV-vis (methanol): λ_{max} (log ε) = 280 nm (4.93). IR (KBr): ν = 3260*m*, 3077*m*, 2930*s*, 2856*m*, 1648*s*, 1592*s*, 1478*m*, 1399*m*, 1362*m*, 1330*w*, 1258*s*, 1216*s*, 1162*s* cm^{-1}. ^1H NMR (500 MHz, DMSO-D_6/D_2SO_4): δ = 8.51−8.40 (*br m*, 4H, 4 × H-C(1,8)), 7.99−7.91 (*m*, 6H, 6 × H-C(3,4,7)), 7.85 (*d*, $^3J_{H,H}$ = 8.46 Hz, 2H, 2 × H-C(5)), 7.53−7.47 (*m*, 2H, 2 × H-C(6)), 4.10 (*s*, 4H, 2 × CH_2(2″)), 4.03−3.95 (*m*, 4H, 2 × CH_2(1′)), 3.97 (*s*, 4H, 2 × CH_2(3″)), 3.59 (*s*, 4H, 2 × CH_2(1″)), 3.06−3.00 (*m*, 4H, 2 × CH_2(8′)), 1.86−1.77 (*m*, 4H, 2 × CH_2(2′)), 1.38−1.15 (*m*, 20H, 10 × CH_2(3′−7′)). ^{13}C NMR (100 MHz, DMSO-D_6/D_2SO_4): δ = 169.7 (C=O), 166.8 (C=O), 157.2 (2 × quart.), 146.3 (quart.), 135.4 (CH), 128.7 (CH), 125.1 (CH), 121.1 (CH), 121.2 (CH), 120.6 (*q*, $^1J_{C,F}$ = 257 Hz, OCF_3), 118.6 (CH), 118.5 (CH), 112.6 (2 × quart.), 55.6 (CH_2(3″)), 54.1 (CH_2(2″)), 51.0 (CH_2(1″)), 48.9 (CH_2(1′)), 38.7 (CH_2(6′)), 28.8 (CH_2), 28.7 (CH_2), 28.6 (2 × CH_2), 26.3 (CH_2), 26.1 (CH_2). ^{19}F NMR (188 MHz, DMSO-D_6): δ = −57.8 (OCF_3). MS (ESI, 4.1 kV, 8 μlL/min, N_2, methanol): 553 (100%) $[MHK]^{2+}$, 1065 (18%) $[MK]^+$. Analysis for $C_{54}H_{64}F_6N_8O_8$ (1067.15): C 60.78; H 6.05; N 10.50; found: C 60.61; H 6.23; N 10.43.

References

[1] H.-J. Neumann, H. Knoller, PCT WO 02/32875A1 [Chem. Abstr. **2002**, 136, 319358].

[2] R. Csuk, A. Barthel, C. Raschke, *Tetrahedron* **2004**, *60*, 5737−5750.

[3] E. S. Endel'man, V. S. Danilenko, F. P. Trinus, P. A. Yufa, A. G. Fadeicheva, I. I. Muravov, Y. A. Fialkov, L. M. Yagupol'skii, *Pharm. Chem. J. (Engl. Transl.)* **1973**, *7*, 755−759.

[4] P. Xu, T. Zhang, W. Wang, X. Zou, X. Zhang, Y. Fu, *Synthesis* **2003**, 1171−1176.

[5] G. Byk, C. Gilon, *J. Org. Chem.* **1992**, *57*, 5687−5692.

[6] C. Haskell-Luevano, A. Rosenquist, A. Sovers, K. C. Khong, J. A. Ellman, R. D. Cone, *J. Med. Chem.* **1999**, *42*, 4380−4387.

[7] M. Takeyama, A. Otaka, N. Fuji, *Chem. Pharm. Bull.* **1992**, *40*, 2199−2201.

[8] Y. Okada, Y. Tsuda, M. Toda, K. Wanaka, U. Okamoto, A. Hijikato-Okunomija, S. Okamoto, *Chem. Pharm. Bull.* **2000**, *48*, 1964−1972.

[9] M. Macchia, F. Salvetti, S. Bertini, V. di Bussolo, L. Gattuso, M. Gesi, M. Hamdan, K.-N. Klotz, T. Laragione, A.

Lucacchini, F. Minuto, *Bioorg. Med. Chem. Lett.* **2003**, *11*, 3023–3026.

[10] B. G. Jimenez, T. Klimkait, I. H. Gilbert, C. Simons, *Bioorg. Med. Chem.* **2003**, *11*, 87–94.

[11] D. J. Dupre, F. A. Robinson, *J. Chem. Soc.* **1945**, *2*, 549–551.

[12] E. T. Martins, H. Beruah, J. Kramarczyk, G. Saluta, *J. Med. Chem.* **2001**, *25*, 4492–4496.

[13] R. Young, *Microbiol. Rev.* **1992**, *56*, 430–481.

[14] M. D. Sobsey, A. Gold, M. J. Casteel, PCT WO 02/02153A1 [Chem. Abstr. **2002**, 136, 90904].