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Studies of the fluorescence light-up effect of amino-substituted benzo[*b*]quinolizinium derivatives in the presence of biomacromolecules

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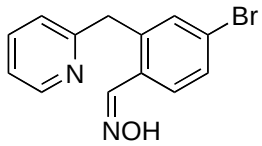
Synthesis



S1

4-Bromo-2-pyridin-2-ylmethyl-benzaldehyde oxime (S1). A solution of hydroxyl-ammonium-hydrochloride (1.03 g, 14.8 mmol) in H_2O (5 ml) was neutralized with an aq. solution of $NaHCO_3$ (5 ml, 3.5 M) and added dropwise at 60 °C to a solution of 9-bromobenzo[*b*]quinolizinium bromide (2.00 g, 5.90 mmol) in water (10 ml). The solution was stirred for 2 h at 60 °C. After cooling the solution to roomtemp. a brown solid precipitated. The solid was filtered off and recrystallized from MeOH to give **S1 (**1.61 g, 5.54 mmol, 94%) as a brown solid; mp 148–150 °C. ^{1}H-NMR (CD_3OD, 400 MHz): δ = 4.32 (s, 2 H, CH_2), 7.16 (d, ^3J = 7 Hz, 1 H, Ar-H), 7.24–7.28 (m, 1 H, Ar-H), 7.42 (d, ^3J = 7 Hz, 2 H, Ar-H), 7.61 (d,

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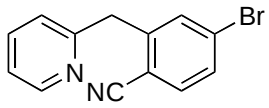
^{3}J = 9 Hz, 1 H, Ar-H), 7.71–7.76 (m, 1 H, Ar-H), 8.29 (s, 1 H, Ar-H), 8.46 (d, ^{4}J = 5 Hz, 1 H, Ar-H). ^{13}C-NMR (CD_3OD, 100 MHz): δ = 41.7 (CH_2), 123.2 (CH), 124.3 (C_q), 124.9 (CH), 130.0 (CH), 131.3 (CH), 132.2 (C_q), 134.9 (CH), 139.0 (CH), 140.7 (C_q), 147.9 (CH), 149.9 (CH), 160.9 (C_q). MS (EI); m/z [%]: 290 (1) (M^+), 260 (100), 194 (76), 167 (51). El. Anal. (%) $C_{13}H_{11}BrN_2O$ (291.14): calc. C 53.63, H 3.81, N 9.62, found C: 53.66, H: 3.73, N: 9.59.



S2

4-Bromo-2-pyridine-2-ylmethyl-benzonitrile (S2). To a solution of **S1** (280 mg, 0.96 mmol) in aq. NaOH (3 ml, 10 %) was added at 60 °C 4-toluenesulfonicacid chloride (220 mg, 1.15 mmol) in small portions. The solution was stirred for 2 h at 60 °C. After cooling to room temp., the solution was extracted with Et_2O (3 × 10 ml). The combined organic layers were dried with $MgSO_4$. After removal of the solvent in vacuo, compound **S2** was obtained as a brown solid (244 mg), which decomposes rapidly, so that it was employed for the subsequent synthetic step without further purification. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 4.53 (s, 2 H, CH_2), 7.42 (td, ^{3}J = 12 Hz, ^{4}J = 6 Hz, 1 H, Ar-H), 7.51 (d, ^{4}J = 1 Hz, 3 H, Ar-H), 7.87-7.92 (m, 2 H, Ar-H), 8.63 (d, ^{3}J = 5 Hz, ^{4}J = 1 Hz, 1 H, Ar-H). ^{13}C-NMR (CD_3OD, 100 MHz): δ = 42.7 (CH_2), 113.0 (C_q), 118.2 (C_q), 123.6 (CH), 125.2 (CH), 129.0 (C_q), 131.9 (CH), 134.9 (CH), 135.5 (CH), 139.0 (CH), 146.1 (C_q), 150.3 (CH), 159.5 (C_q).

6-Amino-9-bromo-acridizinium chloride (1c). A solution of **S2** (1.30 g, 4.76 mmol) in aq. HCl (20 ml, 6 N) was stirred for 2 h at 60 °C. The solution was cooled to room temp. and a yellow solid precipitated, which was filtered off and dried. After recrystallization from MeOH **1c** was obtained as a yellow solid (868 mg, 2.80 mmol, 59%); mp 285–287 °C. ^{1}H-NMR (CD_3OD, 400 MHz): δ = 7.37–7.40 (m, 1 H, Ar-H), 7.46–7.50 (m, 1 H, Ar-H), 7.87 (s, 1 H,

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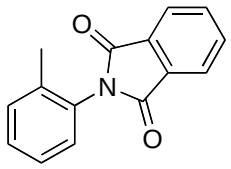
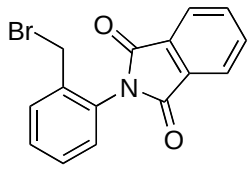
Ar-H), 7.92–7.94 (m, 2 H, Ar-H), 8.34 (s, 1 H, Ar-H), 8.55 (d, ^{3}J = 8 Hz, 2 H, Ar-H). ^{13}C-NMR (CD_3OD, 100 MHz): δ = 111.4 (CH), 114.5 (C_q), 120.2 (CH), 126.0 (CH), 127.6 (CH), 128.2 (CH), 128.6 (CH), 130.3 (C_q), 130.9 (CH), 133.0 (CH), 137.6 (C_q), 138.1 (C_q), 151.7 (C_q). MS (ESI, pos.); m/z (%): 275 (100). El. Anal. (%) $C_{13}H_{10}BrClN_2$ (309.59) calc. C 50.43, H 3.26, N 9.05; found C 50.35, H 3.18, N: 8.92.



S3

N-[2-Methylphenyl]phthalimide (**S3**). A solution of 2-toluidine (11.0 g, 103 mmol) and phthalicacid anhydride (17.6 g, 118 mmol) in acetic acid (200 ml) was stirred for 3 h under reflux. The solution was allowed to cool slowly to room temp. and phthalimide **S3** (21.2 g, 87 %) crystallized as white needles; mp 181-182 °C. - ^{1}H-NMR (200 MHz, $CDCl_3$): δ = 2.21 (s, 3 H, CH3), 7.22-7.39 (m, 4 H, Ar-H), 7.78-7.82 (m, 2 H, Ar-H), 7.94-7.99 (m, 2 H, Ar-H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 21.5 (CH_3), 127.2 (CH), 130.3 (CH), 132.2 (CH), 132.87 (CH), 134.0 (C_q), 134.6 (CH), 135.5 (C_q), 137.7 (CH), 140.0 (C_q), 170.8 (C_q). - El. Anal. (%) $C_{15}H_{11}NO_2$ (237.3): calcd C 75.94, H 4.67, N 5.90; found C 76.27, H 4.78, N 6.09.



S4

N-[2-(Bromomethyl)phenyl]phthalimid (**S4**). A suspension of the phthalimide **S3** (19.3 g, 81.3 mmol), NBS (14.4 g, 80.9 mmol) and a small portion of AIBN in CCl_4 (150 ml) was stirred at reflux for 3 h. The hot suspension was filtered, and the solid was washed with hot water to remove the succinic imide. After drying the remaining solid in vacuo, **S4** was obtained a white powder (16.1 g, 63 %), mp 180-181 °C. ^{1}H-NMR (200 MHz, $CDCl_3$):

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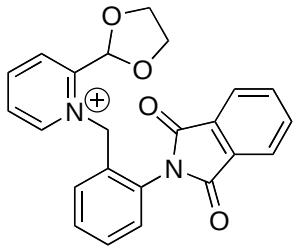
δ = 4.43 (s, 2 H, CH_2), 7.23-7.58 (m, 4 H, Ar-H), 7.78-7.87 (m, 2 H, Ar-H), 7.94-8.02 (m, 2H, Ar-H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 32.9 (CH_2), 127.4 (CH), 133.2 (CH), 133.2 (CH), 133.3 (CH), 134.3 (C_q), 134.5 (CH), 135.3 (C_q), 137.9 (CH), 139.3 (C_q), 170.6 (C_q). – El. Anal. (%) $C_{15}H_{10}BrNO_2$ (316.2): calcd C 56.99, H 3.19, N 4.43; found C 56.96, H 3.16, N 4.51.



S5

2-(1,3-Dioxolan-2-yl)-N-(2-phthalimidylbenzyl)pyridinium bromide (**S5**). Under argon-gas atmosphere a solution of **S4** (5.00 g, 15.8 mmol) and 2-(1,3-dioxolan-2-yl)pyridine[1] (3.00 g, 19.8 mmol) in DMSO (15 ml) was stirred for 7 d at room temp. With rapid stirring, ethyl acetate (300 ml) was added to the solution and a white solid precipitated, which was subsequently separated and washed with ethyl acetate. Recrystallization from methanol yielded **S5** (4.19 g, 60 %) as white, microcystalline solid; mp 165-166 °C (dec.). ^{1}H-NMR (200 MHz, CD_3OD): δ = 3.79-3.97 (m, 4 H, CH_2), 7.45-7.55 (m, 2 H, Ar-H), 7.64-7.71 (m, 2 H, Ar-H), 7.88-7.97 (m, 4 H, Ar-H), 8.07-8.15 (m, 1 H, Ar-H), 8.29 (d, *J* = 10 Hz, 1 H, Ar-H), 8.66 (ddd, *J* = 16 Hz, *J* = 8 Hz, *J* = 1 Hz, 1 H, Ar-H), 8.85 (d, *J* = 8 Hz, 1 H, Ar-H). ^{13}C-NMR (100 MHz, CD_3OD): δ = 60.4 (CH_2), 69.2 (CH_2), 100.8 (CH), 126.9 (CH), 129.2 (CH), 131.6 (CH), 133.8 (CH), 133.9 (CH), 134.0 (CH), 134.4 (CH), 134.4 (C_q), 135.0 (C_q), 135.4 (C_q), 138.1 (CH), 150.1 (CH), 150.5 (CH), 155.7 (C_q), 170.9 (C_q). - El. Anal. (%) $C_{23}H_{19}BrN_2O_4 \times 0.5\ H_2O$ (476.3): C 58.00, H 4.23, N 5.88; found C 58.27, H 4.49, N 5.97.

[1] C. K. Bradsher, J. C. Parham, *J. Org. Chem.* **1963**, *28*, 83–85.

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7-Aminobenzo[b]quinolizinium tetrafluoroborate (**1d**). The pyridinium salt **S5** (1.00 g, 2.14 mmol) was added in small portions to polyphosphoric acid (11 g, 84%) at 80 °C. The highly viscous solution was stirred for 19 h at 90 °C. After careful addition of water (11 ml) the solution was stirred for 30 min at 80 °C (**CAUTION**: The hydrolysis of PPA is strongly exothermic and may start after an induction period!). The solution was cooled to room temp., extracted with chloroform (100 ml, three times), and neutralized with aq. $NaHCO_3$. Subsequently, aq. HBF_4 (50%) was added until a deep red solid precipitated. The solid was separated and crystallized from MeOH to give 7-aminobenzo[b]quinolizinium tetrafluoroborate (**1d**) as deep red needles (189 mg, 32 %); mp 217-218 °C (dec.). ^{1}H-NMR (200 MHz, CD_3OD): δ = 7.07 (d, *J* = 8 Hz, 1 H), 7.48 (d, *J* = 8 Hz, 1 H), 7.65 (ddd, *J* = 8 Hz, J = 7 Hz, J = 1 Hz, 1 H), 7.78-7.91 (m, 2 H), 8.26 (d, *J* = 8 Hz, 1 H), 8.75 (s, 1 H); 8.86 (d, *J* = 7 Hz, 1 H), 10.23 (s, 1 H). ^{13}C-NMR (100 MHz, CD_3OD): δ = 111.3 (CH), 114.7 (CH), 122.1 (CH), 124.5 (CH), 127.6 (C_q), 130.9 (C_q), 134.8 (C_q), 137.8 (CH), 138.8 (C_q). El. Anal. (%)$C_{13}H_{11}BF_4N_2$ (282.1): C 55.36, H 3.93, N 9.93; found C 55.64, H 3.78, N 9.57.

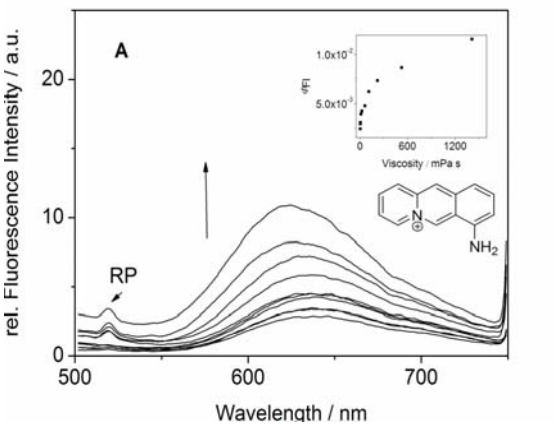

Figure S1. Fluorescence emission spectra of **1d** in glycerol-water mixtures of varied viscosity, (arrow indicates the change of the fluorescence intensity with extended glycerol/ diglyme content).

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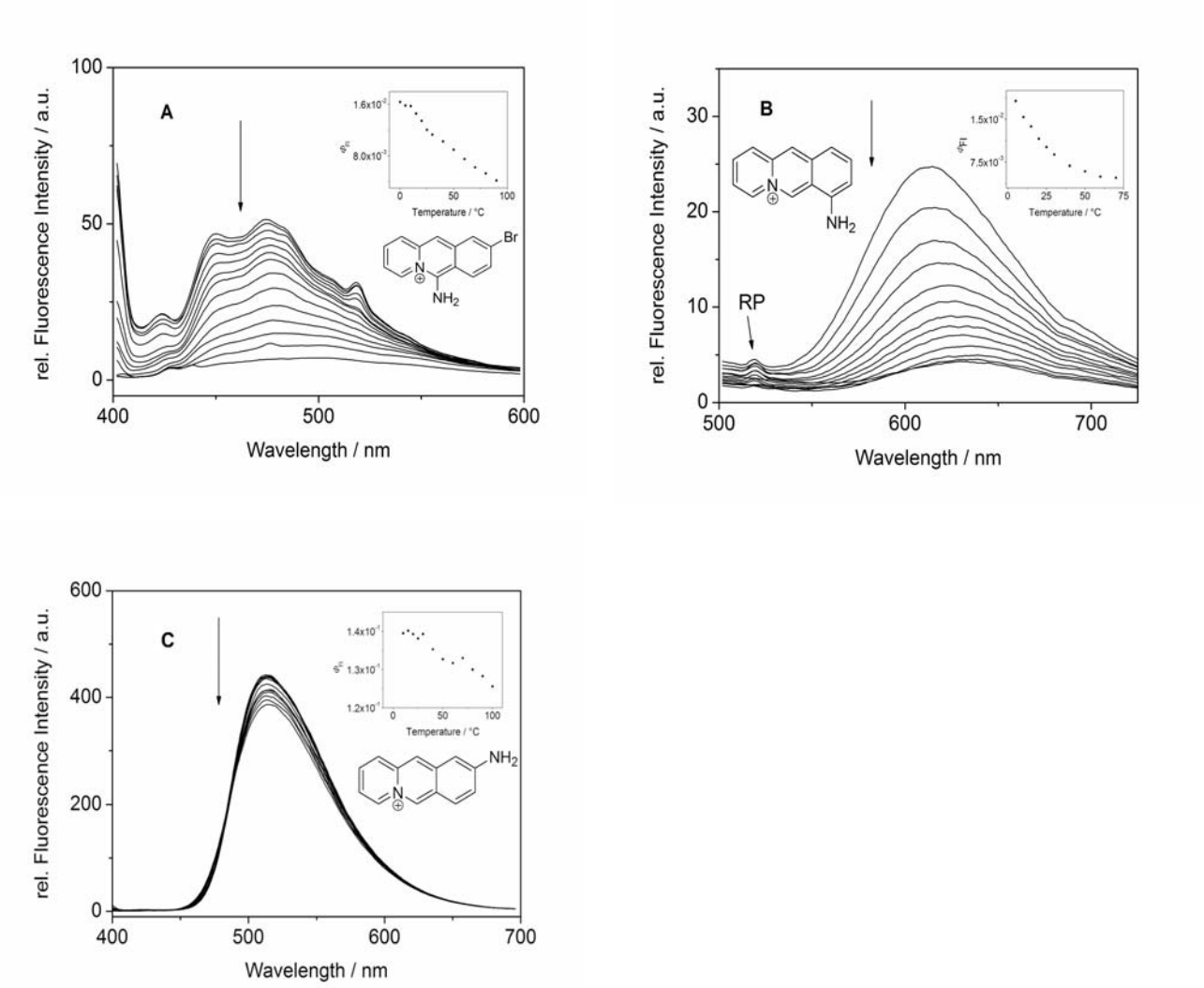

Figure S2. Fluorescence emission spectra of **1c** (A), **1d** (B), and **1e** (C) in glycerol with varied temperature, (arrow indicates the change of the fluorescence intensity with enhanced temperature).

Table S1. Melting temperatures ΔT_m of ct DNA and poly[dA-dT]-poly[dA-dT] in the presence of **1a-e**.

	ct DNA[a]		Poly[dA-dT]-Poly[dA-dT][a]	
	$r = 0.2$	$r = 0.5$	$r = 0.2$	$r = 0.5$
1a	2	3	2	4
1b	3	4	7	13
1c	3	5	7	13
1d	3	4	5	10
1e	5	8	6	11

[a]conditions: c_{DNA} = 40 μmol L^{-1} in bt, in BPE-buffer, c_{Na+} = 16 mmol L^{-1}, estimated error: ΔT_m ± 0.5 °C.

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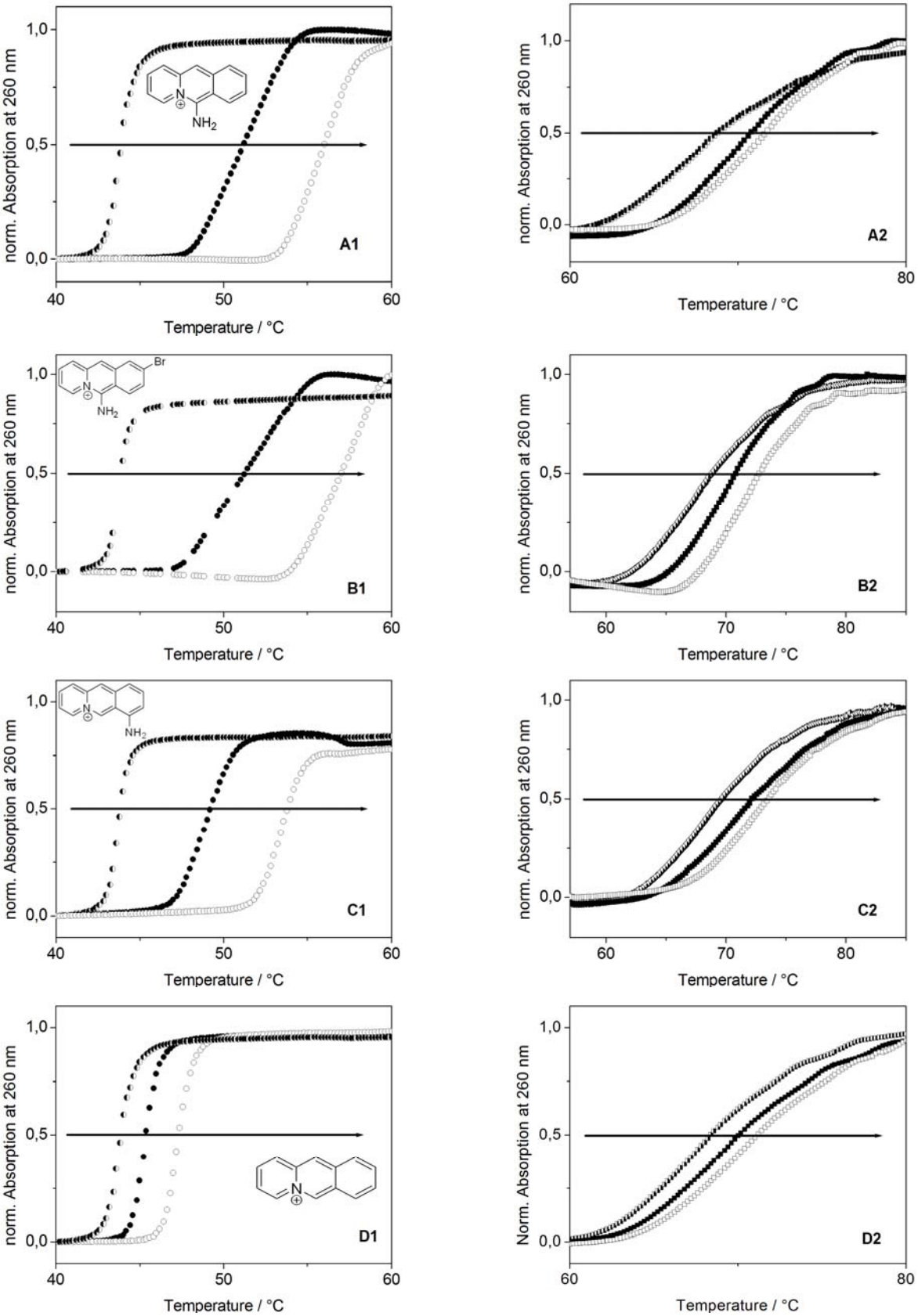

Figure S3. DNA-melting curves of poly[dA-dT]-poly[dA-dT] (1) and ct DNA (2) in the prescence of **1b** (A), **1c** (B), **1d** (C) and **1a** (D) (c_{DNA} = 40 µmol L^{-1}, BPE-buffer) at ligand-to-DNA-ratios of r = 0 (half filled), 0.2 (full) und 0.5 (empty).

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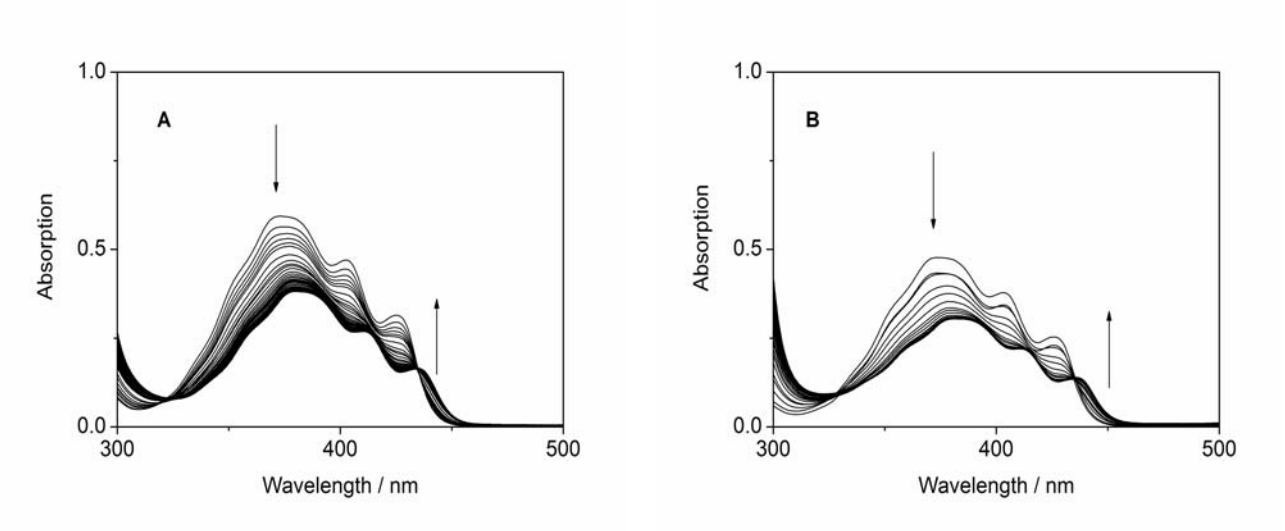

Figure S4. Spectrophotometric titration of poly[dA-dT]-poly[dA-dT] (A) and poly[dG-dC]-poly[dG-dC] (B) to 6-aminoacridizinium (**1b**) ($c = 5 \times 10^{-5}$ mol L^{-1}, phosphate buffer, pH 7.0, $T = 20$ °C).

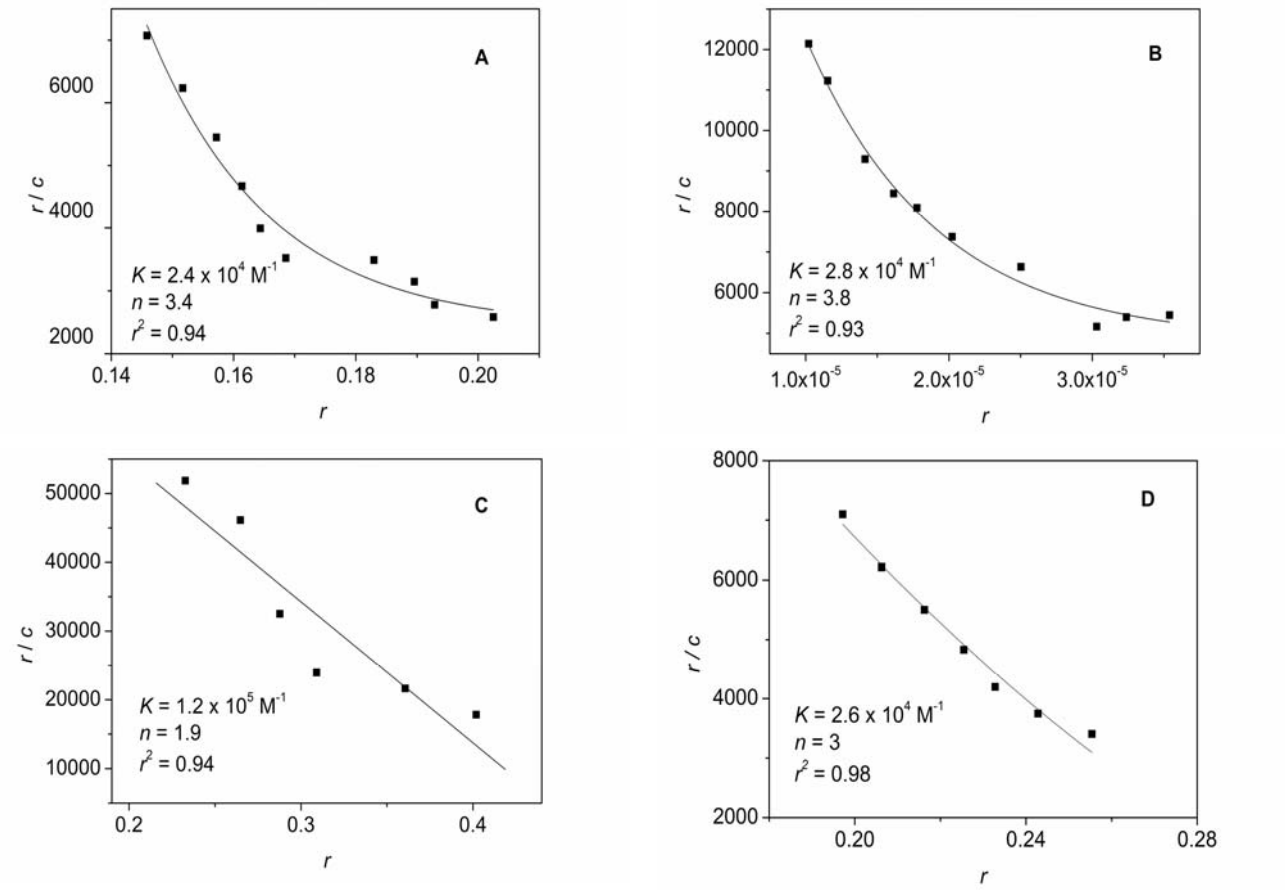

Figure S5. Scatchard plots derived from the spectrophotometric titrations of ct DNA (A), poly[dA-dT]-poly[dA-dT] (B) and poly[dG-dC]-poly[dG-dC] (C) to **1b** and of ct DNA (D) to **1c**.

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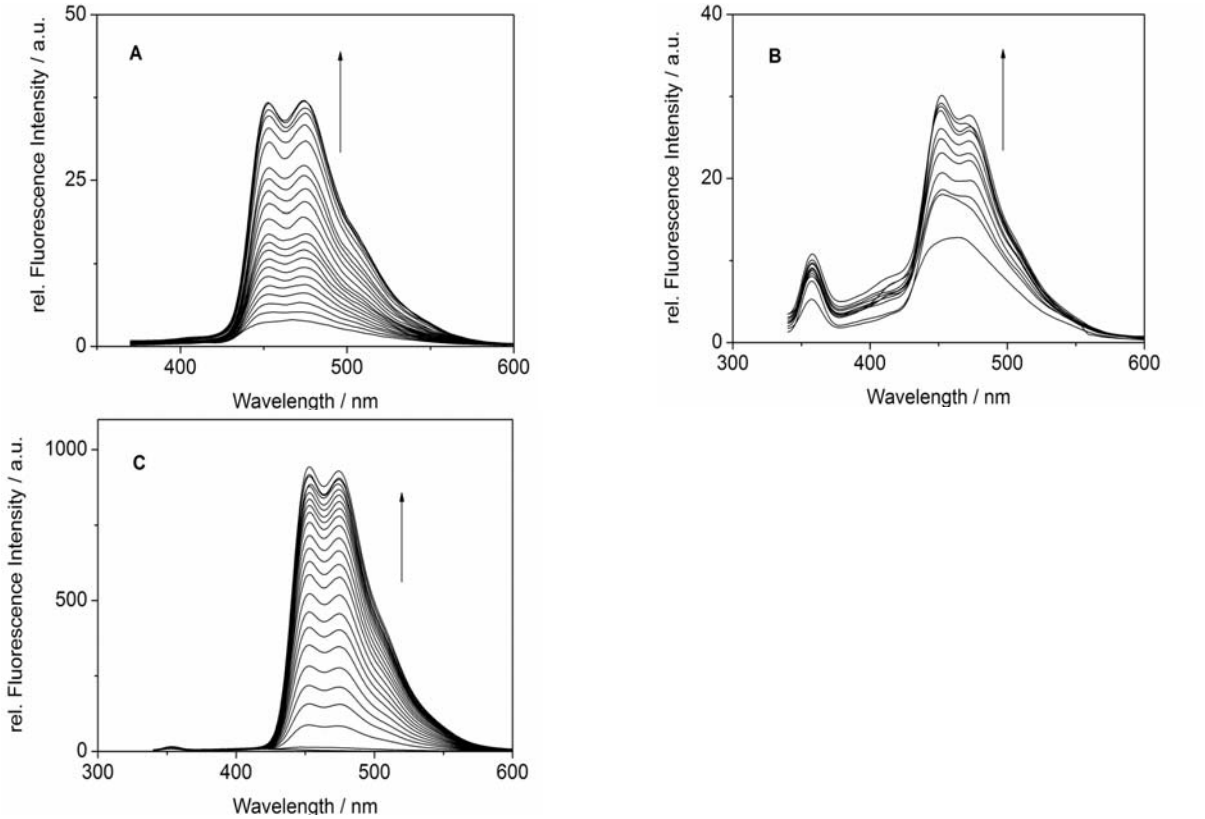

Figure S6. Spectrofluorimetric titration of ct DNA (A), poly[dG-dC]-poly[dG-dC] (B) and poly[dA-dT]-poly[dA-dT] (C) to 6-Aminoacridizinium (**1b**) ($c = 10^{-5}$ mol L^{-1}, phosphate buffer, pH 7.0, $T = 20$ °C).

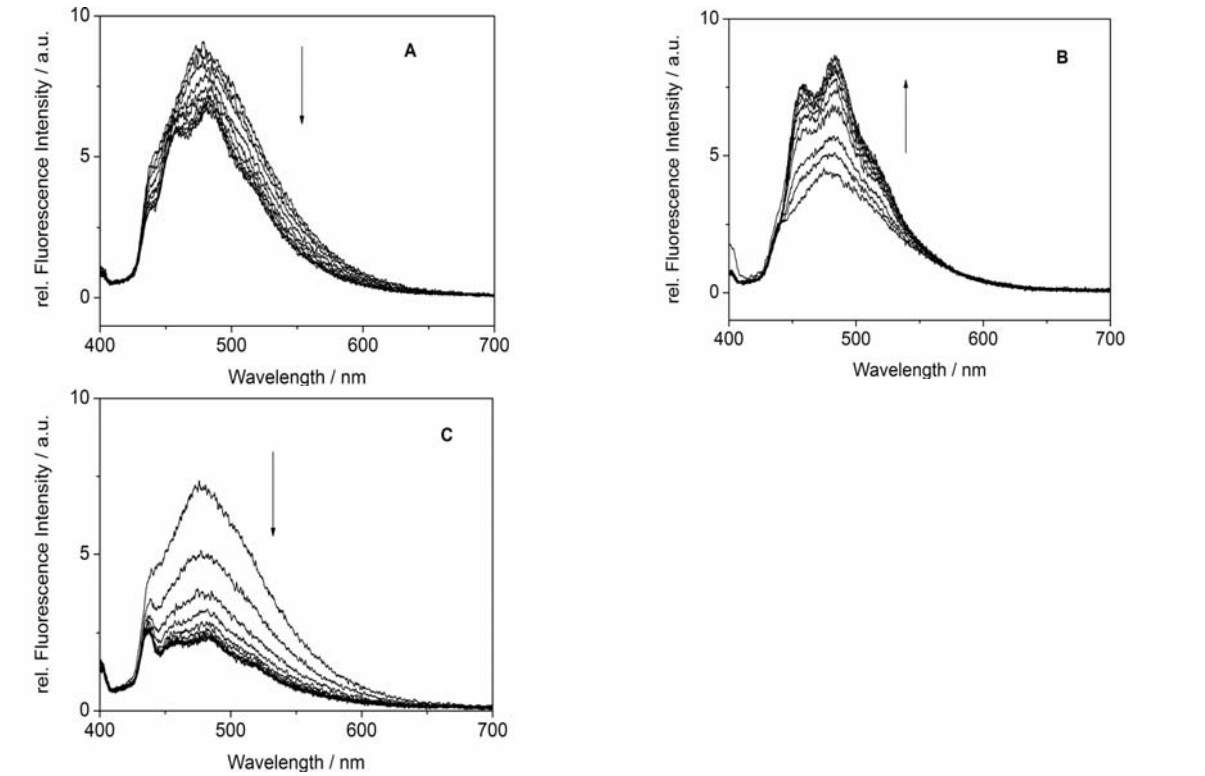

Figure S7. Spectrofluorimetric titration of ct DNA (A), poly[dA-dT]-poly[dA-dT] (B) and poly[dG-dC]-poly[dG-dC] (C) to 6-Amino-9-bromoacridizinium (**1c**) ($c = 10^{-5}$ mol L^{-1}, BPE-Puffer, pH 7.0, $T = 20$ °C).

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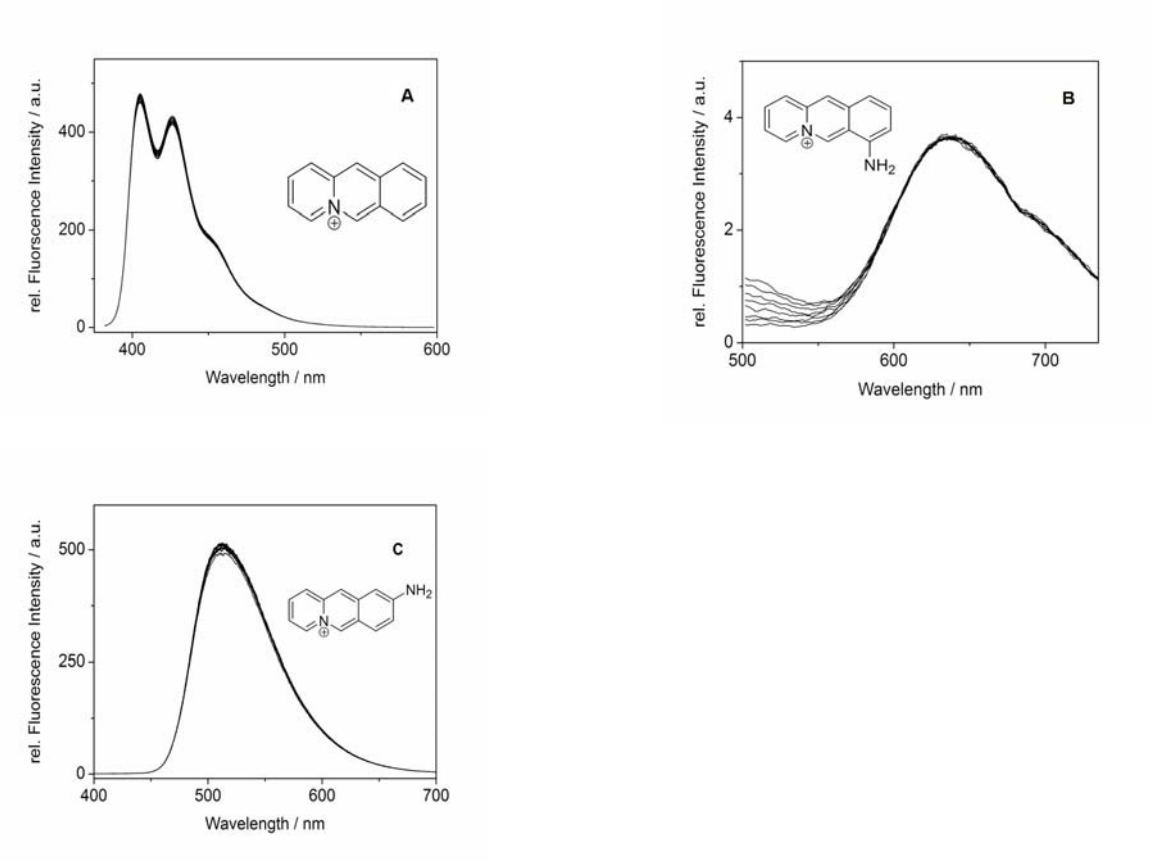

Figure S8. Spectrofluorimetric titration of **1a** (A), **1d** (B), and **1e** (C) with human serum albumin in BPE-buffer (containing 0.05 vol% SDS, $c = 10^{-5}$ M; $\lambda_{ex} = 380$ nm.

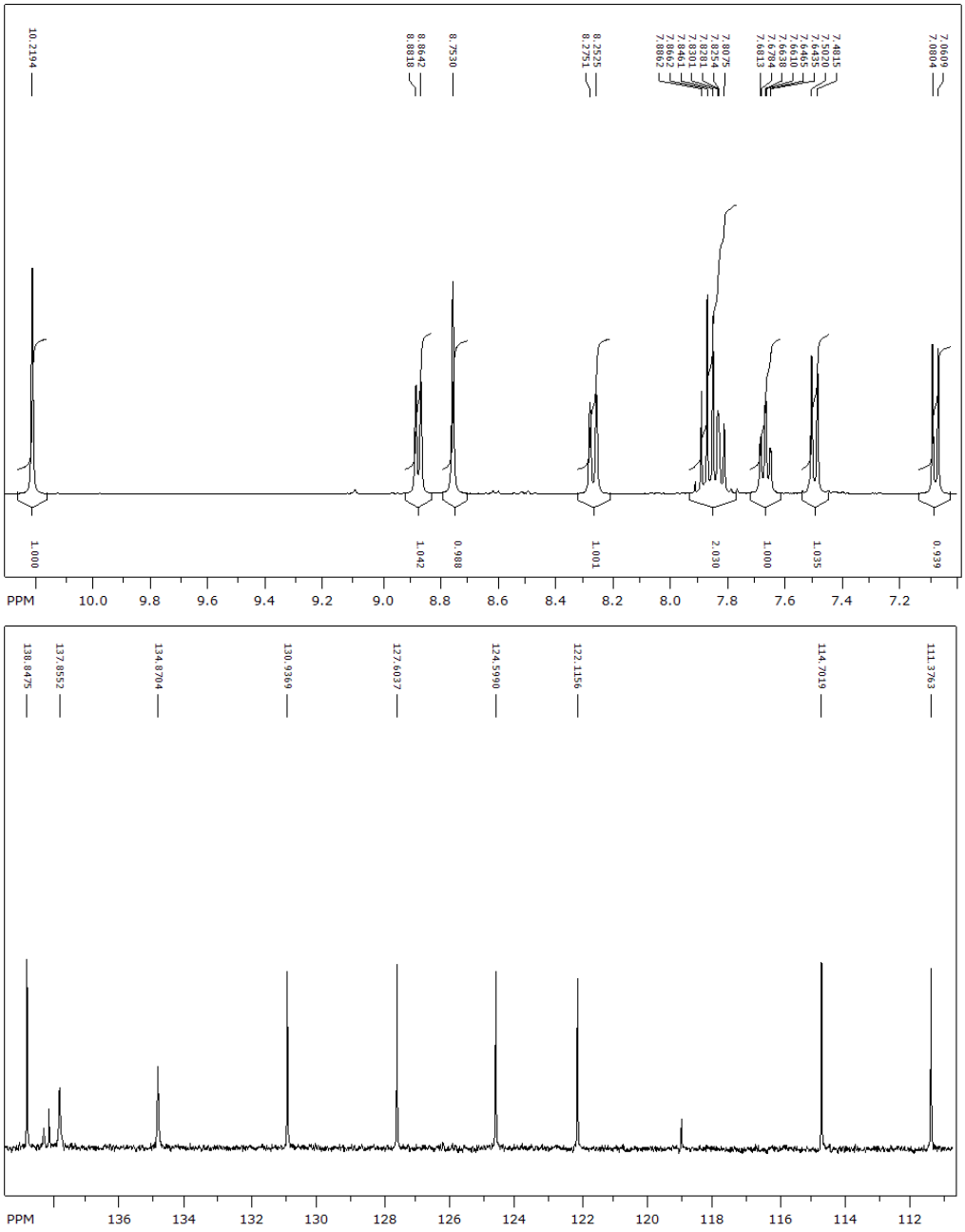

Figure S9. ^{1}H-NMR and ^{13}C-NMR spectrum of 7-Aminobenzo[*b*]quinolizinium **(1d)** in CD_3OD.

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Table S2. Mulliken atomic charges with hydrogens summed into heavy atoms for the electronic ground (S_0) and first excited (S_1) states of cations **1b**, **1d** and **1e** from TD-DFT calculations. The most prominent changes upon excitation ($|\Delta Q| > 0.1$) are printed bold.

Atom	**1b**			**1d**			**1e**		
	S_0	S_1	ΔQ	S_0	S_1	ΔQ	S_0	S_1	ΔQ
C1	−0.011	−0.070	−0.059	0.001	−0.049	−0.050	−0.011	−0.039	−0.028
C2	0.079	0.116	0.037	0.087	0.100	0.013	0.084	0.072	−0.012
C3	0.065	−0.018	−0.083	0.075	0.010	−0.065	0.069	0.048	−0.021
C4	0.095	0.071	−0.024	0.129	0.117	−0.012	0.134	0.111	−0.023
N5	−0.248	−0.233	0.015	−0.175	−0.195	−0.020	−0.176	−0.157	0.019
C6	0.270	0.194	−0.076	**−0.066**	**−0.315**	**−0.249**	**−0.074**	**−0.260**	**−0.186**
C6a	0.219	0.231	0.012	0.246	0.221	−0.025	0.380	0.329	−0.051
C7	−0.101	−0.079	0.022	0.328	0.385	0.057	−0.100	−0.115	−0.015
C8	0.027	0.051	0.024	**−0.159**	**−0.005**	**0.154**	**−0.139**	**−0.241**	**−0.102**
C9	0.059	0.039	−0.020	0.086	0.051	−0.035	0.529	0.569	0.040
C10	−0.117	−0.072	0.045	**−0.190**	**0.010**	**0.200**	**−0.338**	**−0.084**	**0.254**
C10a	0.420	0.406	−0.014	0.467	0.430	−0.037	0.452	0.446	−0.006
C11	−0.200	−0.145	0.055	**−0.169**	**−0.309**	**−0.140**	−0.182	−0.213	−0.031
C11a	0.399	0.437	0.038	0.350	0.370	0.020	0.363	0.371	0.008
NH_2	0.045	0.073	0.028	**−0.009**	**0.180**	**0.189**	**0.008**	**0.161**	**0.153**

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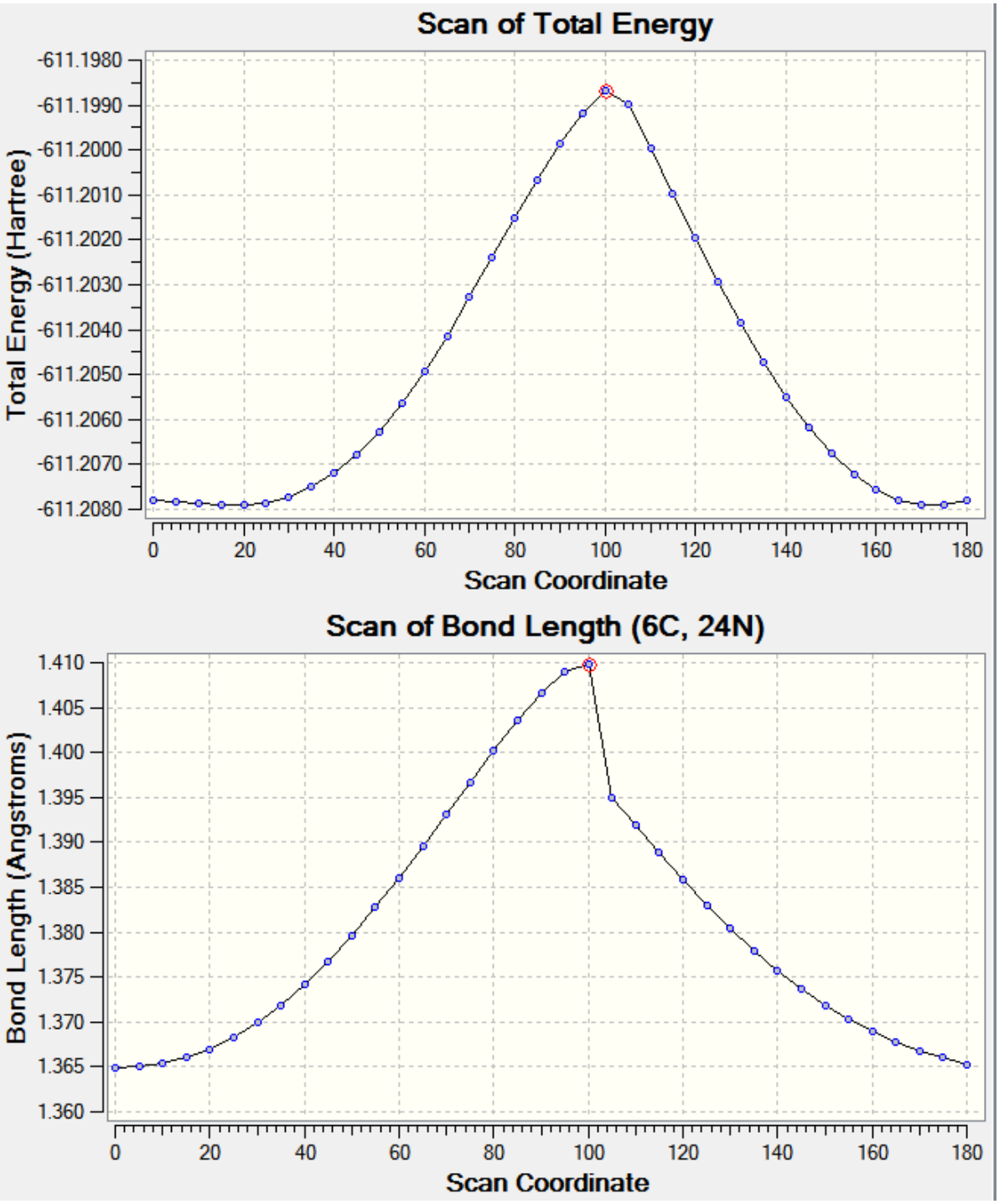

Figure S10. Ground-state potential energy profile and bond length changes (C6−NH_2) during the rotation of the amino group in the cation of **1b** from DFT calculations. The "scan coordinate" is defined as the dihedral angle (C4–C6–N_{exo}–H).