A Blue Excitable Charge-Transfer Fluorescent Probe and Its Fluorogenic Derivative

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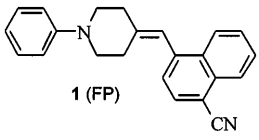
Structural modification of the highly fluorescent donor-bridge-acceptor molecule "Fluoroprobe" (FP) is shown to extend the excitation window to longer wavelengths. The resulting "Fluorotrope" (FT) shows appreciable absorption in the 350–420-nm range, so that visible (blue) light can be used for excitation. Further functionalization with a maleimide group results in the novel fluorogenic reagent MaleimidoFluorotrope (MFT) which yields fluorescent adducts with amines, thiols and other reactive groups that add to the double bond of the maleimide. The fluorescence wavelength of these adducts is extremely sensitive to the polarity and mobility of the medium.

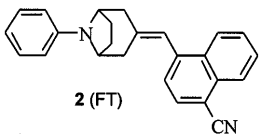
Introduction

Donor-bridge-acceptor systems like the interesting fluorescent molecule "Fluoroprobe" (**1**) have already been studied extensively[1][2][3][4][5], and used as fluorescent probes[6][7][8][9][10]. Fluoroprobe shows a characteristic intramolecular charge-transfer fluorescence that depends strongly on the polarity and polarizability of the medium; the fluorescence maximum shifts from 407 nm in a nonpolar environment like *n*-hexane to 697 nm in the polar acetonitrile. The main disadvantage of the Fluoroprobe system lies in its poor absorption characteristics. Fluoroprobe, has no significant absorption above 350 nm, which strongly reduces its applicability in (bio)polymer experiments.



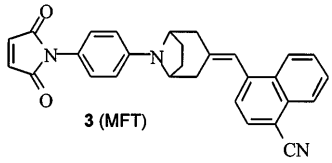
1 (FP)

In the present study enhancement of the absorption characteristics is sought by changing the structure of the hydrocarbon bridge, connecting the aniline electron donor and vinylcyanonaphthalene electron acceptor. In particular we decided to change this bridge in such a way that the phenyl ring of the donor would be forced from its predominantly equatorial orientation in FP towards an axial orientation. This is predicted[11][12] to enhance the through-bond interaction between donor and acceptor, and as a result significant long-wavelength charge-transfer absorption might be expected to appear, thereby expanding the excitable region towards the visible. On the other hand the fluorescent and solvatochromic properties could stay virtually unchanged, because the emissive CT state will retain a planar radical cation geometry around the aniline nitrogen irrespective of whether the phenyl group is equatorial or axial in the ground state. In order to induce an axial orientation of the aniline phenyl group, it was decided to change the piperidine ring of FP to the bicyclic tropane unit, yielding structure **2**, which was dubbed ″Fluorotrope″ (FT).



2 (FT)

Furthermore, in view of the reported maleimide derivatization of Fluoroprobe[13][14], it was decided also to functionalize FT. Adding the maleimide moiety yields compound **3**, MFT, which can readily be conjugated to other (macro)molecules, by addition to the maleimide double bond. Moreover, as anticipated from other maleimide dyes[15], MFT is likely to have the highly desirable fluorogenic property: it does not fluoresce until the double bond of the maleimide group has reacted.



3 (MFT)

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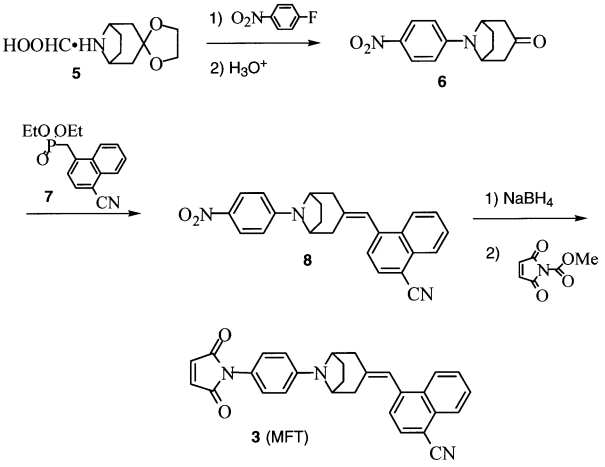
Results and Discussion

Synthesis of Fluorotrope (FT) and Maleimidofluorotrope (MFT): Fluorotrope can be synthesized by means of a Wittig condensation (Wadsworth−Emmons variation) of 8-phenyl-8-azabicyclo[3.2.1]octan-3-one (*N*-phenyltropanone) and phosphonate **7**, derived from 4-methyl-1-naphthalenecarbonitrile.

The synthesis route for MFT (see Experimental Section) is shown in Scheme 1.

Once a protected tropanone (**4**) is made, it can be converted into **5** by nucleophilic substitution with *p*-nitrofluorobenzene, followed by deketalization. Reaction with **7,** as in the synthesis of FT, yields **8**. Reduction of **8** followed by condensation with *N*-(methoxycarbonyl)maleimide gives MFT(**3**).

Scheme 1



Absorption Characteristics: Electronic spectra of FT and MFT are shown in Figure 1 and for comparison that of FP is displayed as well. In the context of the present study the most significant aspect is of course the dramatic increase of absorption in the long-wavelength region, displayed by both FT and MFT as compared to FP, which is connected with the presence of a new broad absorption maximum around 350 nm. The charge transfer (CT) character of this absorption[12] is supported by the fact that it disappears when the aniline donor is disabled by protonation. From the difference in absorption between FT and protonated FT (inset Figure 1) the molar absorption of the charge-transfer band is estimated to be $\varepsilon \approx 4700\ M^{-1}\ cm^{-1}$ at 350 nm. Another indication for the much stronger donor/acceptor interaction in FT (and MFT) as compared to FP is provided by the blue shift and intensity decrease of the acceptor chromophore absorption around 300 nm (see Figure 1). This phenomenon has been observed before in other strongly through-bond coupled D/A systems and has been shown to emerge from significant mixing between the CT state and locally exited states that results in intensity transfer from local transitions to the CT transition[16].

Fluorescence of FT: Fluorescence maxima, quantum yields and decay times of FT in various solvents are compiled in Table 1, which also contains data of FP for comparison. Representative emission spectra of FT in various solvents are displayed in Figure 2 and demonstrate the enormous red shift upon increasing polarity so typical for the charge-transfer fluorescence of rigidly extended donor-bridge-acceptor systems. Figure 2 also contains the excitation spectrum, which − like the absorption spectrum − shows only minor solvent dependence. The excitation spectrum closely matches the absorption spectrum and thus also contains the CT absorption ($\lambda_{max} \approx 350$ nm), which extends the excitable region of FT to beyond 400 nm.

Figure 1. Absorption of FP (A), FT (B) and MFT (C) in dichloromethane. The inset shows the absorption difference between FT and protonated FT as obtained by addition of a few drops of trifluoroacetic acid

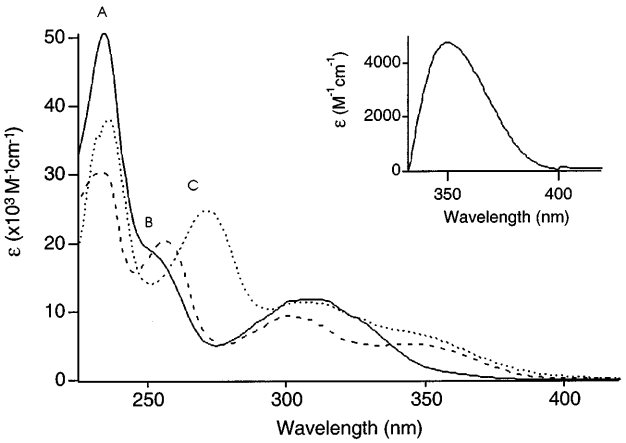
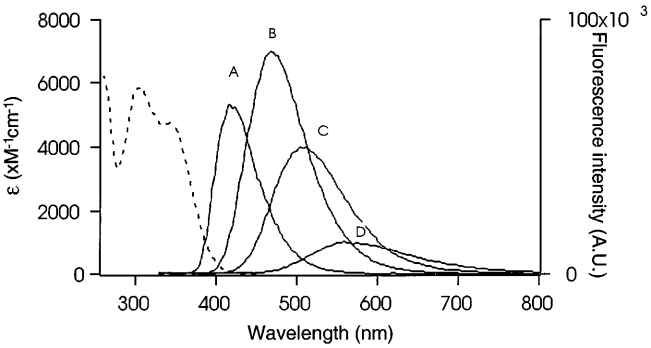


Figure 2. Emission spectra of FT in various solvents: cyclohexane (A), dipentyl ether (B), diethyl ether (C), ethyl acetate (D); the dashed spectrum shows the excitation spectrum (in dichloromethane)



While FT and FP show a clear similarity with respect to fluorescence position and quantum yield as a function of solvent (see Table 1) there are a few differences which deserve comment. Both FT and FP display their strongest fluorescence (ϕ around 80%!) in solvents of moderate polarity but the decrease in quantum yield at both higher and lower polarity appears to be less pronounced for FT than for FP. This is especially so in the non-polar regime. For FP ϕ drops to about 20% in satured alkane solvents, while FT still retains $\phi \geq 50\%$ in such media. The rather sharp decrease of ϕ in non-polar media has been attributed[2][16] to increased mixing between the CT state and a locally excited state of the acceptor, which opens a non-radiative pathway

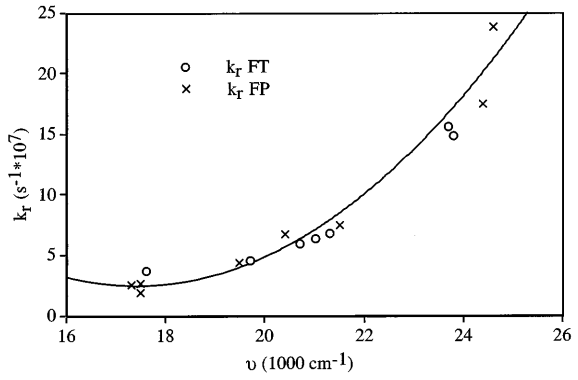
Table 1. CT Fluorescence maxima (ν_{ct} in 10^3 cm^{-1}) and quantum yields of FT (**2**) and FP (**1**) in various solvents

Solvent	FT ν_{ct}	FT ϕ	FT τ (ns)	FP ν_{ct}	FP ϕ	FP τ (ns)
n-hexane	23.8	0.55	3.7	24.6	0.20	0.84
cyclohexane	23.7	0.61	3.9	24.4	0.21	1.2
toluene	20.8					
di-*n*-pentyl ether	21.3	0.77	11.2			
di-*n*-butyl ether	21	0.74	11.5	21.5	0.85	11.4
di-*n*-propyl ether	20.7	0.72	12	20.4	0.78	11.6
diethyl ether	19.7	0.71	15.7	19.5	0.58	13.4
ethyl acetate	17.6	0.25	6.7	17.5	0.19	7.3
tetrahydrofuran	17.5			17.5	0.16	8.7
dichloromethane	17.1			17.3	0.21	8.3
acetone	15.4					

connected to twisting of the vinylic double bond of the acceptor. Fluorescence maxima of FT and FP in non-polar media indicate that in the former the CT state is at slightly lower energy and thus less prone to mix with locally excited states, which might explain the smaller contribution of radiationless decay in FT evident from comparison of the ϕ and τ data for FP and FT in saturated alkanes.

A very important conclusion to be drawn from the data in Table 1 is that − as visualized in Figure 3 − for any given position of the CT fluorescence maximum the radiative rate constants of FT and FP are virtually identical and both display the quadratic dependence on the energy of the emission maximum expected[4][17] for a situation in which intensity borrowing from higher energy local transitions is dominant.

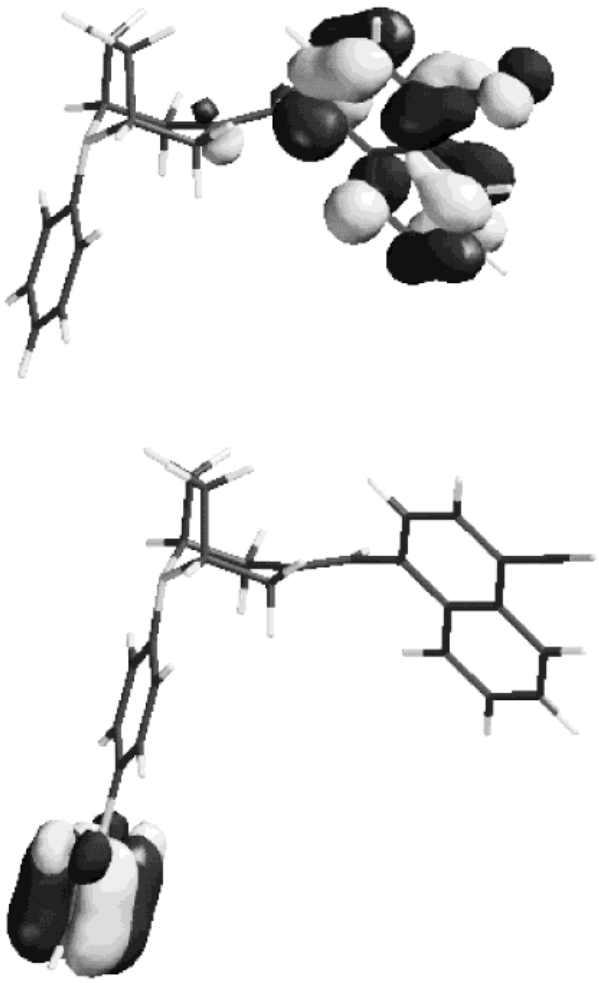
Figure 3. Radiative rate constants ($k_r = \Phi/\tau$) as a function of the emission maximum for FT (o) and FP (x). The drawn curve represents a quadratic fit to the FP data



It may seem contradictory that FT and FP display similar radiative CT emission probabilities, while the CT absorption is much stronger for FT than for FP. However, as already noted in the introduction, in contrast to their difference in ground-state conformation at the aniline nitrogen, this is expected to adopt a planar trigonal conformation in the emissive state of both systems, because of the radical cation character it attains. This implies that through-bond interaction between donor and acceptor is much stronger for FT than for FP in the ground state, but quite similar in the emissive state.

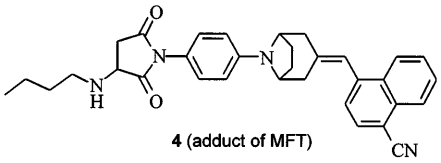
Fluorogenic Behaviour of MFT: In sharp contrast to the strongly fluorescent nature of FT, its maleimide derivative MFT is completely non-fluorescent in all solvents investigated. This behaviour is in fact analogous to that of various other maleimide derivatives of fluorescent dyes[15] including that of FP[13][14]. It has been suggested[13] that the quench-

Figure 4. Representation of the LUMO of FT (top) and MFT (bottom)



ing effect of the maleimide group might be related to a low energy n→π* state, but especially in the present case an alternative explanation may be provided by the strongly electron-accepting properties of the maleimide group. Semi-empirical (AM1, RHF) calculations indicate that the lowest unoccupied MO of MFT is localized in the maleimide group. It implies that a (non-emissive) CT state is available in MFT with an energy below that of the emissive CT state of FT, for which the LUMO is localized in the cyanonaphthalene part (see Figure 4). Irrespective of the quenching mechanism, this can be disabled when the double bond of the maleimide unit is saturated by an addition reaction.

For MFT fluorogenity was now tested by studying the effect of addition of the primary amine *n*-butylamine. The resulting adduct **4** (see Experimental Section) indeed displays CT fluorescence at a position quite similar to FT (a minor blue shift occurs, see Table 2) thus demonstrating that MFT can be employed as a fluorogenic probe.



4 (adduct of MFT)

More detailed data, including quantum yields and lifetimes of fluorescence of MFT adducts will be the subject of future investigations. As will be a number of applications in fluorescent probing (of biomolecules and polymers), that have now come within reach thanks to the red shifted absorption spectrum of Fluorotrope.

Table 2. CT Fluorescence maxima (n_{ct} in 10^3 cm^{-1}) of the *n*-butylamine/MFT adduct **4** in various solvents (FT-value between brakkets)

Solvent	ν_{ct}
cyclohexane	23.8 (23.7)
toluene	21.1 (20.8)
diethyl ether	19.9 (19.7)
ethyl acetate	17.9 (17.6)
dichloromethane	17.4 (17.1)

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Experimental Section

Electronic Absorption and Fluorescence Measurements: Electronic absorption spectra were recorded on a HP 8453 diode array spectrophotometer. The samples were contained in 1-cm rectangular quartz cuvettes. − Fluorescence spectra and quantum yields were measured on a Spex Fluorolog 2 spectrofluorimeter in a right-angle geometry. The spectra were corrected for the detector response. Samples were diluted to *A* (1 cm) ≤ 0.2 at 308 nm. Quantum yields were determined − after deoxygenation by purging with argon − relative to quinine bisulfate (ϕ = 0.546 in 1 M H_2SO_4).[18] − Lifetimes of the fluorescence (λ_{exc} = 320 nm) were measured by time-correlated single photon counting using a set-up described extensively elsewhere[19] after deoxygenation by purging with argon.

3-[(4-Cyano-1-naphthyl)methyl]-8-phenyl-8-azabicyclo[3.2.1]-octane (FT, **2**): 0.40 g (1.9 mmol) of *N*-phenyltropanone[20] and 0.60 g (1.9 mmol) of the phosphonate **7** (see Scheme 1) were dissolved in dry THF under N_2 atmosphere. While cooling the mixture in an ice bath, 0.21 g of NaH (50−60% dispersion in oil) was added. The reaction mixture was stirred overnight. The brown suspension was poured into water and extracted with $CHCl_3$. The organic layers were dried over Na_2SO_4 and evaporated. The product was purified by column chromatography (silica, ethyl acetate/CH_2Cl_2/petroleum-ether 1:1:1). Recrystallization from ethanol yielded yellow needles. Yield: 0.41 g (66%); m.p. 103−104°C. − IR ($CHCl_3$): $\tilde{\nu}$ = 3020 cm^{-1} (m), 2959 (m), 2223 (m) (CN), 1597 (s), 1567 (m), 1497 (s). − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 (d, *J* = 8.3 Hz, 1 H), 7.84 (d, *J* = 7.4 Hz, 1 H), 7.70 (dd, *J* = 6.9, 8.1 Hz, 1 H), 7.62 (dd, *J* = 6.9, 8.3 Hz, 1 H), 7.27 (m, *J* = 8.0, 7.5, 3.38 Hz, 3 H), 6.85 (d, *J* = 8.0, 2.1 Hz, 2 H), 6.77 (t, *J* = 7.5 Hz, 1 H), 6.75 (s, 1 H), 4.43 (m, 1 H), 4.18 (m, 1 H) 2.97 (d, *J* = 13.3 Hz, 1 H), 2.55 (d, *J* = 14 Hz, 1 H), 2.27 (d, 1 H), 2.12 (m, 2 H), 1.96 (m, 2 H), 1.57 (m, 1 H).

Formic Acid Adduct **5**: 15.9 g (73.8 mmol) of *N*-benzyltropanone (Aldrich) was refluxed with 4.6 g (74 mmol) of ethanediol and 15.1 g (79.4 mmol) of *p*-toluenesulfonic acid (TSOH) in 200 ml of toluene using a Dean-Stark apparatus. After 5 hours of azeotrope distillation the mixture was cooled and washed with 200 ml of 5% $NaHCO_3$. IR showed disappearance of the carbonyl absorption, indicating a transformation of the ketone. The organic layers were dried over Na_2SO_4 and evaporated. This yielded 13.4 g (52 mmol, 70%) of a brown solid, the ethylene ketal of *N*-benzyltropanone. ^{1}H NMR: (200 MHz, $CDCl_3$) δ = 7.3 [m, 5 H], 3.95 [t, 2 H], 3.8 [t, 2 H], 3.6 [s, 2 H] 3.25 [br.m, 2 H], 2.0 [m, 6 H], 1.6 [d, 2 H]. − 11.8 g (45.5 mmol) of the ethylene ketal of *N*-benzyltropanone was dissolved in 200 ml of ethanol and was put under 50 ψ H_2 in the Parr-apparatus after addition of 75 mg of Pd/C and 2 ml of formic acid. After 80 hours the catalyst was filtered off, and the solvent evaporated. ^{1}H NMR showed that only 85% of the product was formed. After another 60 hours with new catalyst, ^{1}H NMR showed almost complete disappearance of the benzyl group. Yield: 7.9 g of a colourless solid (39.3 mmol, 86%). − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.8 (br.s, 1 H), 3.95 (t, 2 H), 3.8 (t+s, 4 H), 2.0 (m, 8 H).

8-(p-Nitrophenyl)-8-azabicyclo[3.2.1]octan-3-one (**6**): 6.8 g (33.8 mmol) of **5** was dissolved in 100 ml of DMF. 7.0 g (50 mmol) of K_2CO_3 was added under N_2. Then 4.77 g (33.8 mmol) *p*-fluoronitrobenzene in 20 ml of DMF was added. The reaction mixture was stirred during 24 h at 100°C and poured into 400 ml of 0.1 M NaOH solution. The aqueous solution was extracted (3 x) with ethyl acetate. The organic layers were combined, washed (4 x) with water, dried on Na_2SO_4. Evaporation yielded an oil. The oil was dissolved in CH_2Cl_2 , washed three times with water, dried on Na_2SO_4 and evaporated. This yielded 10.2 g of a yellow/brown solid. This was purified on a column of silica with CH_2Cl_2 as eluent, yielding 5.7 g (19.7 mmol, 58%) of yellow intermediate product: the ketal of *p*-nitrophenyltropanone. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.1 (d, *J* = 9.3 Hz, 2 H), 6.7 (d, *J* = 9.3 Hz, 2 H), 4.35 (s, 2 H), 4.0 (t, *J* = 6.4 Hz, 2 H), 3.8 (t, *J* = 6.3 Hz, 2 H), 2.26 (m, 2 H), 2.02 (m, 4 H), 1.78 (d, *J* = 13.8 Hz, 2 H). − 5.7 g (19.7 mmol) of the ketal of *p*-nitrophenyltropanone was dissolved

in 400 ml of 4 M HCl. The mixture was stirred overnight. The solution was made alkaline (pH 8−9) with $NaHCO_3$ and NaOH and extracted with CH_2Cl_2 (3 x). The organic layers were collected, dried over Na_2SO_4 and evaporated. Yield: 4.16 g (17 mmol, 87%) of yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.2 (d, *J* = 9.3 Hz, 2 H), 6.84 (d, *J* = 9.3 Hz, 2 H), 4.6 (s, 2 H), 2.62 (dd, *J* = 15.7, 4.3 Hz, 2 H), 2.43 (d, *J* = 15.5 Hz, 2 H), 2.25 (m, 2 H), 1.87 (m, 2 H).

3-[(4-Cyano-1-naphthyl)methylene]-8-(p-nitrophenyl)-8-azabicyclo[3.2.1]octane (**8**): 2.43 g (10 mmol) of the tropanone **6** and 3.02 g (10 mmol) of the phosphonate **7** were dissolved in 150 ml of dry THF. The solution was put under N_2 and 1.1 g of NaH (55−60% dispersion in oil) was added slowly. The reaction mixture was stirred overnight, then 200 mg of additional NaH was added, and the mixture was left to stirr for 5 hours more. The mixture was poured into 250 ml of water and extracted (4 x) with CH_2Cl_2 The organic layers were collected, dried over Na_2SO_4 and evaporated. The product was separated by flash column-chromatography (silica, CH_2Cl_2). Yield: 2.2 g (5.6 mmol, 56.5%) of fine yellow flakes. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.27 (d, *J* = 8.4 Hz, 1 H), 8.16 (d, *J* = 9.22 Hz, 2 H), 8.03 (d, *J* = 8.3 Hz, 1 H), 7.86 (d, *J* = 7.4 Hz, 1 H), 7.7 (dd, *J* = 7.0, 8.4 Hz, 1 H), 7.6 (dd, *J* = 7.0, 8.3 Hz, 1 H), 7.26 (d, 1 H), 6.85 (s, 1 H), 6.76 (d, *J* = 9.28 Hz, 2 H), 4.53 (m, 1 H), 4.28 (m, 1 H), 2.88 (d, *J* = 13.8 Hz, 1 H), 2.44 (dd, 2 H), 2.25 (d+m, 2 H), 2.04 (m, 2 H), 1.6 (m, 1 H).

3-[(4-Cyano-1-naphthyl)methyl]-8-(p-maleimidophenyl)-8-azabicyclo[3.2.1]octane (MFT, **3**): 1.4 g (3.5 mmol) of **8** was dissolved in 200 ml of ethanol (dried on mole sieves). 4.6 g (20.3 mmol) of $SnCl_2 \cdot 2\,H_2O$ was added under N_2. The temperature was raised to 60°C and a solution of 130 mg of $NaBH_4$ in 100 ml of ethanol was slowly added. The mixture was allowed to stirr for 48 hours, becoming less yellow. Then the solution was made alkaline (pH = 8) and extracted (4 x) with CH_2Cl_2 . The organic layers were dried on Na_2SO_4 and evaporated. Recrystallization from ethanol yielded 0.95 g (2.6 mmol, 74%) of orange flakes, "aminofluorotrope" (= compound **8** with reduced nitro group). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.25 (d, *J* = 8.3 Hz, 1 H), 8.05 (d, *J* = 8.4 Hz, 1 H), 7.84 (d, *J* = 7.4 Hz, 1 H) 7.7 (dd, *J* = 8.3, 7.0 Hz, 1 H) 7.6 (dd, *J* = 8.4, 7.0 Hz, 1 H), 7.26 (d, *J* = 7.4 Hz, 1 H), 6.75 (m, 5 H), 4.32 (m, 1 H), 4.09 (m, 1 H) 3.01 (d, *J* = 13.48 Hz, 1 H), 2.59 (d, *J* = 14.0 Hz, 1 H), 2.24 (d, *J* = 13.9 Hz, 1 H), 2.13 (m, 2 H), 1.94 (m, 2 H), 1.53 (m, 1 H). − 466 mg (3.05 mmol) of *N*-methoxycarbonylmaleimide was dissolved in 50 ml of dry DMF and put under N_2 atmosphere. The temperature was raised to 70°C and a solution of 550 mg (1.52 mmol) of the aminofluorotrope in 50 ml of dry DMF was added dropwise. The temperature was raised to 120°C and the mixture was stirred overnight. The DMF was removed in vacuo and the product was purified over a silica column (flash chromatography) with CH_2Cl_2 as eluent, secondly over a silica column with ethyl acetate. Temperature plays an important role in this synthesis. First the synthesis was done at 90°C and a stable intermediate product was formed, which could be isolated. It is thought (from IR and NMR) this is an open-chain structure in which ring closure at the nitrogen still has to occur. Yield: 250 mg (0.56 mmol, 37%) of a yellow/orange solid. IR ($CHCl_3$): $\tilde{\nu}$ = 2959 cm^{-1} (s), 2930 (s), 2859 (m), 2222 (w) (CN); 1715 (vs) (C=O), 1600 (m), 1516 (s) 1364 (m), 1280 (s). − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.26 (d, *J* = 8.2 Hz, 1 H), 8.05 (d, *J* = 8.3 Hz, 1 H), 7.85 (d, *J* = 7.4 Hz, 1 H), 7.7 (dd, *J* = 8.2, 7.0 Hz, 1 H), 7.6 (dd, *J* = 8.3, 7.0 Hz, 1 H), 7.26 (d, *J* = 7.4 Hz, 1 H), 7.18 (d, *J* = 8.9 Hz, 2 H), 6.87 (d, *J* = 8.9 Hz, 2 H), 6.83 (s, 2 H), 6.76 (s, 1 H), 4.41 (m, 1 H), 4.15 (m, 1 H), 2.94 (d, *J* = 13.7 Hz, 1 H), 2.53 (d, *J* = 14 Hz, 1 H), 2.34 (d, *J* = 13.8 Hz, 1 H), 2.12 (m, 2 H), 2.04 (m, 2 H), 1.6 (m, 1 H). − ^{13}C NMR (APT, 100 MHz, $CDCl_3$): δ = 145.9, 140.8, 140.6, 134.0 (2 C), 132.5, 132.0, 131.7, 128.3 (2C), 127.7, 127.41, 125.7, 125.6, 25.5, 124.1, 117.9, 115.1 (2 C), 108.7, 89.0 (2 C), 55.1, 54.7, 38.6, 32.9, 28.4 (2 C).

MFT/n-Butylamine Adduct **4**: Equimolar amounts of MFT and *n*-butylamine were dissolved in dichloromethane. The mixture was heated at 40°C over night. The solvent was evaporated in vacuo and the product was used for preliminary measurements without further purification.

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