Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Light-Controlled DNA Binding of Bisbenzamidines

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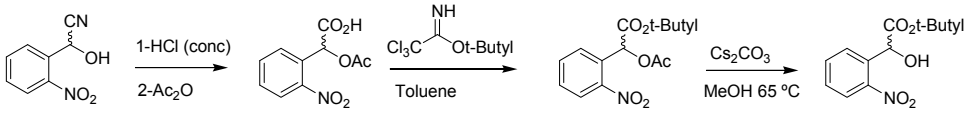
Abbreviations

Abbreviation	Meaning
δ	Chemical Shift
λ	Wavelength
^{t}Bu	Tert-butyl
DIEA	N,N-Diisopropylethylamine
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DMSO-d_6	Deuterated dimethyl sulfoxide
equiv.	Equivalents
ESI	Electrospray ionization
h	Hour
HPLC	High-Performance Liquid Chromatography
MHz	MegaHerz
NMR	Nuclear Magnetic Resonance
RP	Reverse Phase
r.t.	Room temperature
TFA	Trifluoroacetic acid
TLC	Thin Layer Cromatography
UV	Ultraviolet light

SYNTHESIS

All reagents were acquired from commercial sources: DMF and TFA were purchased from *Scharlau*, CH_2Cl_2 from *Panreac* and CH_3CN from *Merck*. The rest of reagents were acquired from *Invitrogen* and *Sigma-Aldrich*. Reactions were followed by analytical RP-HPLC with an *Agilent 1100* series LC/MS using an *Eclipse* XDB-C18 (4.6 x 150 mm, 5 µm) analytical column. Standard conditions for analytical RP-HPLC consisted on a linear gradient from 5% to 95% of solvent B for 30 min at a flow rate of 1 mL/min (solvent A: water with 0.1% TFA, solvent B: acetonitrile with 0.1% TFA). Compounds were detected by UV absorption at 220, 270, 304 and 330 nm. Final products were purified on a Büchi *Sepacore* preparative system consisting on a pump manager C-615 with two pump modules C-605 for binary solvent gradients, a fraction collector C-660, and UV Photometer C-635. Purification was made using reverse phase of water/acetonitrile 0.1% TFA, using a pre-packed preparative cartridge (150 x 40 mm) with reverse phase RP_{18} silica gel (Büchi order number 54863). The fractions containing the products were freeze-dried, and their identity was confirmed by ESI-MS(+). Compound **1a** was synthesized following a protocol similar to that previously described[1]. Compound **5** was prepared according to the following scheme, following previously described protocols.[2,3]



5c **5b** **5a**

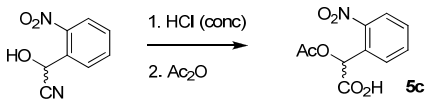
NMR spectra were recorded using Varian Mercury 300 and Brucker DPX 250 spectrophotometers, and processed using the *MestReC*.

[1] S. M. Bakunova, S. A. Bakunov, D. A. Patrick, E. V. K. Suresh Kumar, K. A. Ohemeng, A. S. Bridges, T. Wenzler, T. Barszcz, S. K. Jones, K. A. Werbovetz, R. Brun, and R. R. Tidwell *J. Med. Chem. 52*(7), 2016-2035 (2009).

[2] F. M. Rossi and J. P. Y. Kao *J. Biol. Chem. 272*, 52 3266-3271 (1997).

[3] F. M. Rossi, M. Margulis, C-M Tang and J. P. Y. Kao *J. Biol. Chem. 272*, 52 32933-32939 (1997).

2-Acetoxy-2-[2-nitrophenyl] acetic acid (5c)



5c

A solution of 2-hydroxy-2-[2-nitrophenyl]acetonitrile (2.00 g, 11.40 mmol) was refluxed in concentrated hydricholride acid (50 mL) for 6 h. The solution was cooled to room temperature and extracted with ethyl acetate. The organic layer was dried with Na_2SO_4 and concentrated under low pressure yielding the desire crude product.

o-Nitromandelic acid was refluxed in acetic anhydride (1.50 g, 7.61 mmol) for 1 h. The reaction mixture was cooled to room and diluted with THF (9.5 mL) and water (9.5 mL) . After stirring from 2 h the aqueous layer was removed, and the organic layer was diluted with toluene (20 mL) and extracted with water (2 x 40 mL). The organic layer was dried with Na_2SO_4 and the solvent was evaporated under low pressure.

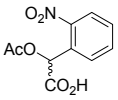
The crude was characterized as the desired product (1.31 g, 5.47 mmol) and was carried into the next reaction without further purification.

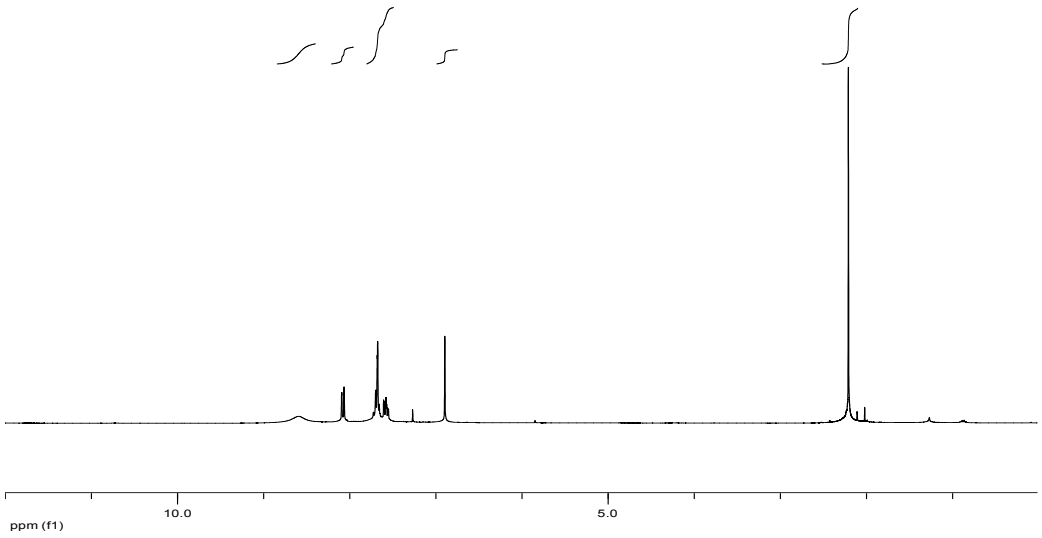
^{1}H RMN δ (300 MHz, *DCCl$_3$*): 2.19 (s, 3H), 6.88 (s, 1H), 7.54-7.71 (m, 3H), 8.07 (d, *J* = 7.9 Hz, 1H), 8.58 (s, 1H).

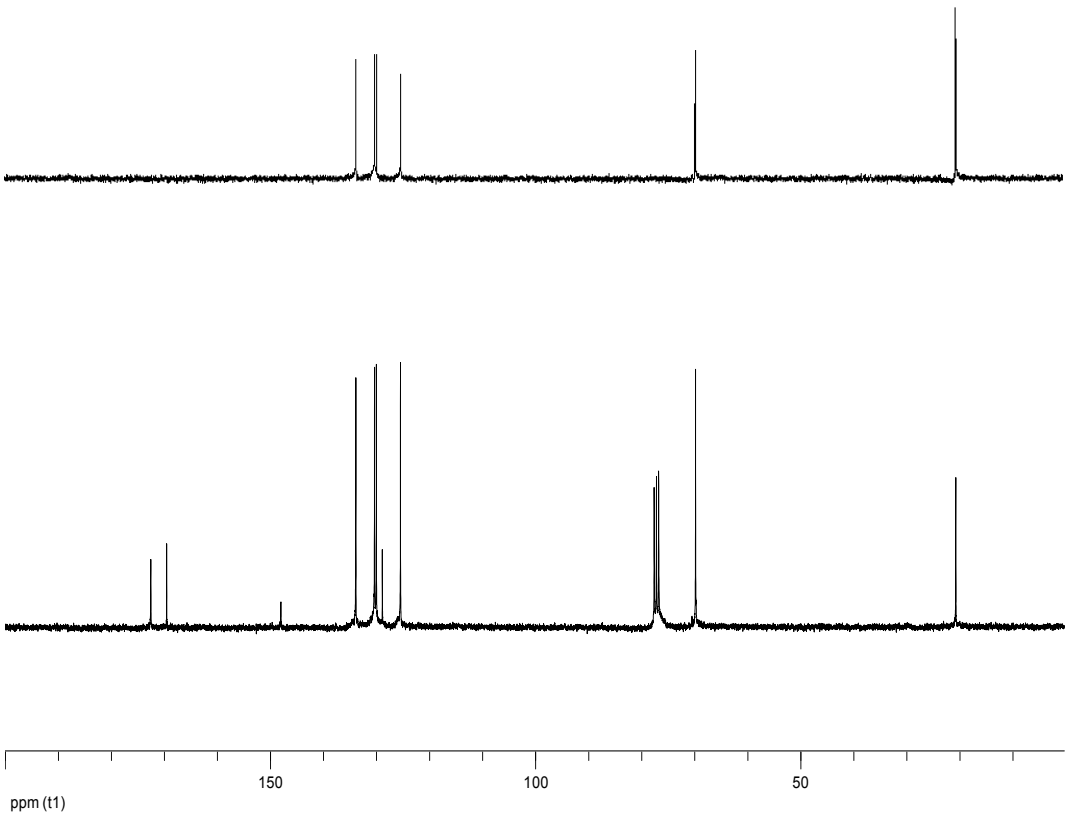
^{13}C RMN δ (300 MHz, *DCCl$_3$*): 20.5 (CH3), 69.6 (CH), 125.3 (CH), 128.7 (C), 129.8 (CH), 130.2 (CH), 133.7 (CH), 147.8 (C), 169.4 (C), 172.4 (C).

ESI-MS: $[M+Na]^+$ calc. for $C_{10}H_9N_1O_6Na_1$ = 262.0322 found 262.0324; $C_{10}H_9N_1O_6$ (M.W. 239,1816)

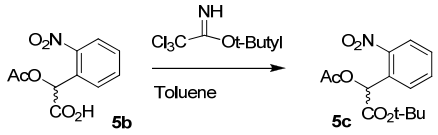
2-Acetoxy-2-[2-nitrophenyl] acetic acid (5c)







***t*-Butyl 2-acetoxy-2-[2-nitrophenyl] acetate (5b)**



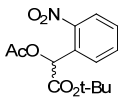
t-Butyltricholoacetimidate (1,20 g, 5,52 mmol) was added to a solution of 2-acetoxy-2-[2-nitrophenyl] acetic acid in toluene (5.02 mmol, 50.2 mL). The reaction was stirred for 1 h, and the solid (white small crystals) formed in the reaction were removed by filtration. The filtrate was concentrated and the residue purified by flash column chromatography on silica gel (20% AcOEt/hexanes) to obtain an oil that was identified as the desired product (0.79 g, 2,66 mmol, 53%).

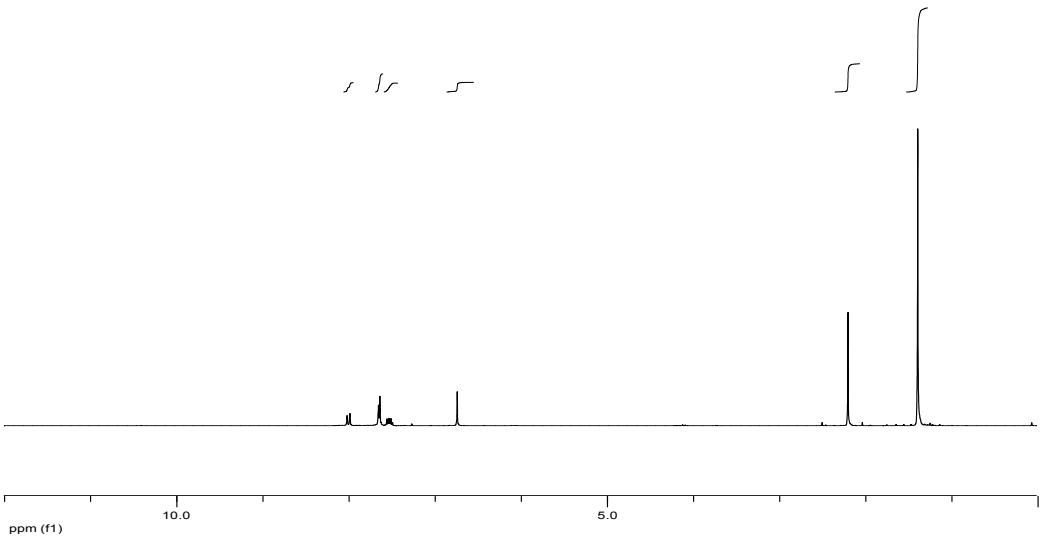
^{1}H RMN δ (300 MHz, *DCCl$_3$*): 1.39 (s, 9H), 2.19 (s, 3H), 6.74 (s, 1H), 7.49-7.55 (m, 1H), 7.63-7.65 (m, 2H), 8.00 (d, *J* = 7.9 Hz, 1H).

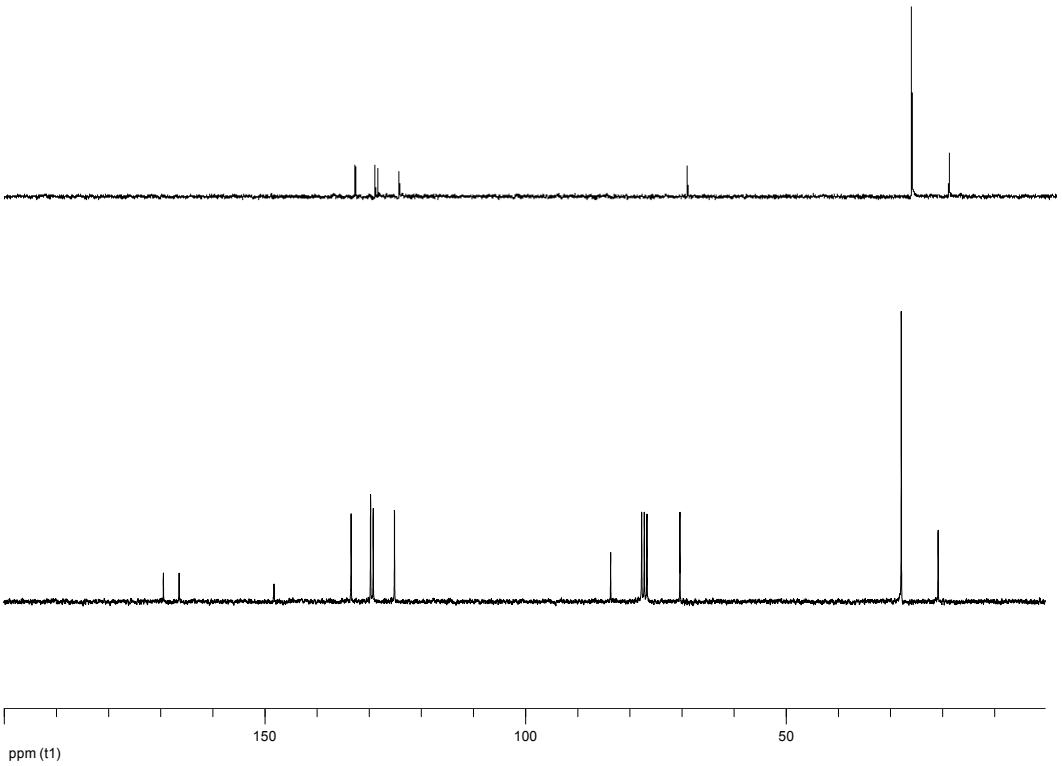
^{13}C RMN δ (300 MHz, *DCCl$_3$*): 20.6 (CH_3), 27.6 (CH_3), 70.2 (CH), 84.0 (C), 125.0 (CH), 129.0 (CH), 129.6 (CH), 129.6 (C), 133.3 (CH), 148.1 (C), 166.4 (C), 169. 4 (C).

ESI-MS: $[M+Na]^+$ calc. for $C_{14}H_{17}N_1O_6Na_1$ = 318.0948 found 318.0940; $C_{14}H_{17}N_1O_6$ (M.W. 295,2879)

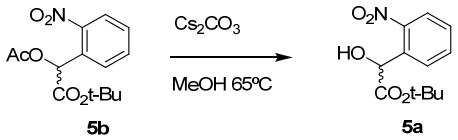
t-Butyl 2-acetoxy-2-[2-nitrophenyl] acetate (5b)







_t_-Butyl 2-hydroxy-2-[2-nitrophenyl]acetate (5a)



5b **5a**

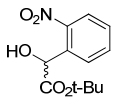
$CsCO_3$ (20.5 mg, 0.06 mmol) was added to a solution of *t*-butyl 2-acetoxy-2-[2-nitrophenyl] acetate (0.50 g, 1,26 mmol) in methanol (12.6 mL). After 1 h, the solution was diluted with ethyl acetate then, silica gel was added to the reaction crude and the solvents were evaporated under low pressure affording the reaction crude adsorbed on silica gel. This silica gel is loaded in to a chromatography column and purified (50% AcOEt/hexanes) to obtain an oil that was identified as the desired product (271 mg, 1.07 mmol, 85%).

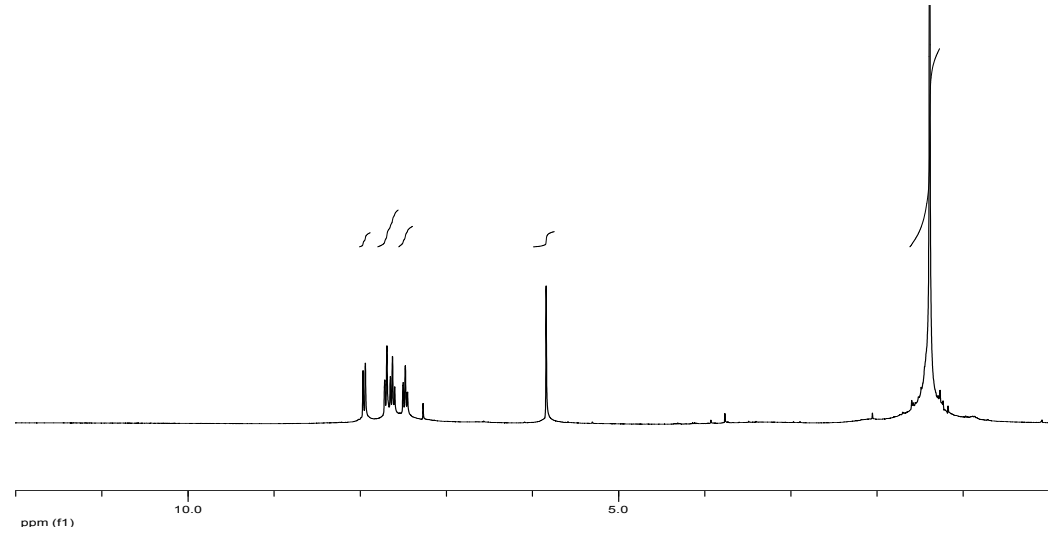
^{1}H RMN δ (300 MHz, $DCCl_3$): 1.36 (s, 9H), 5.82 (s, 1H), 7.46 (t, *J* = 7.7 Hz, 1H), 7.61 (t, *J* = 7.5 Hz, 1H), 7.69 (d, *J* = 7.6 Hz, 1H), 7.94 (d, *J* = 8.1 Hz, 1H).

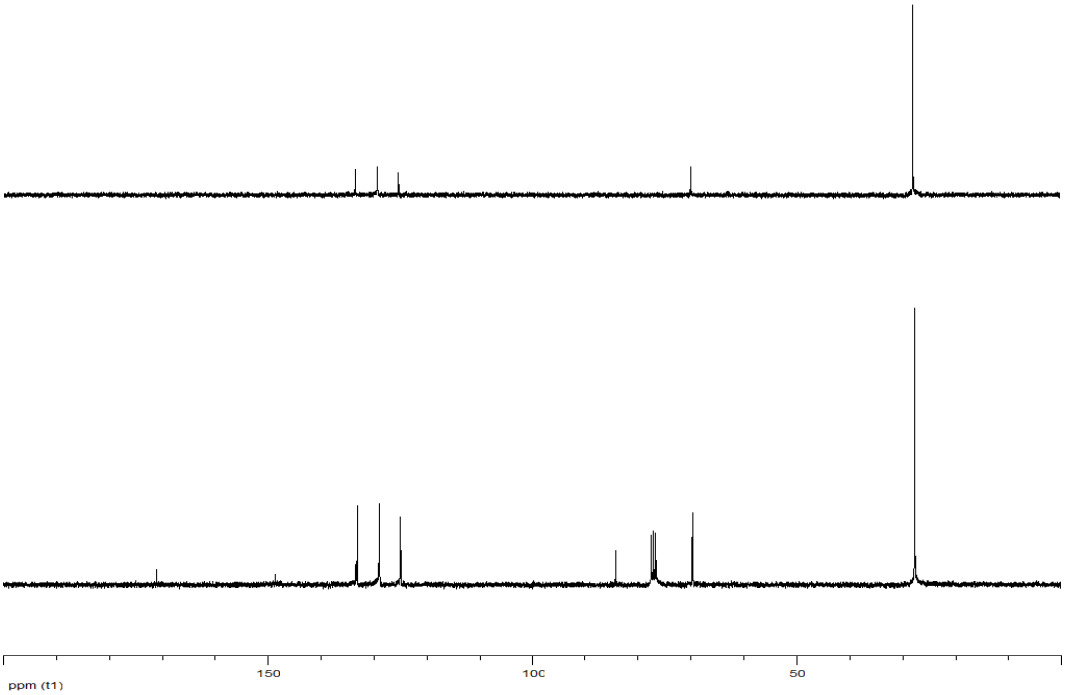
^{13}C RMN δ (300 MHz, $DCCl_3$): 27.7 (CH_3), 69.7 (CH), 84.1 (C), 124.7 (CH), 125.1 (CH), 128.9 (CH), 133.2 (CH), 133.4 (C), 148.1 (C), 171.0 (C).

ESI-MS: $[M+Na]^+$ calc. for $C_{12}H_{15}N_1O_5Na_1$ = 276.0842 found 276.0838; $C_{12}H_{15}N_1O_5$ (M.W. 253,2512)

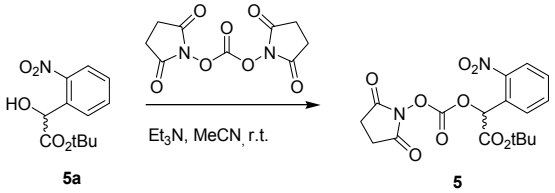
_t_-Butyl 2-hydroxy-2-[2-nitrophenyl]acetate (5a)







tert-Butyl ({[(2,5-dioxo-1-pyrrolidinyl)oxy]carbonyl}oxy)(2-nitrophenyl)acetate (5)



5a **5**

To a solution of alcohol **5a**[2] (200 mg, 0.79 mmol) dissolved in MeCN (8 mL) was added Et_3N (320 mg, 3.16 mmol, 4 equiv), and dissucinimidyl dicarbonate (213 mg, 0.95 mmol, 1.2 equiv). After stirring for 8 h under Ar at rt, TLC analysis showed that the starting material was consumed; the solvent was removed and the residue purified by flash column chromatography on silica gel (60% AcOEt/hexanes) to obtain the desired product (**5**) as a white solid (178 mg, 0.47 mmol, 60%).

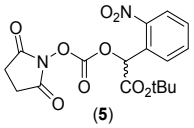
^{1}H-NMR δ (250MHz, *$CDCl_3$*): 1.43 (s, 9H), 2.83 (s, 4H), 6.72 (s, 1H), 7.55-7.62 (m, 1H), 7.70-7.62 (m, 2H), 8.10 (d, 1H, *J* = 8.2 Hz).

^{13}C-NMR δ (250MHz, *$CDCl_3$*): 25.4 (CH_2), 27.6 (CH_3), 75.7 (CH), 84.8 (C), 125.4 (CH), 128.0 (C), 128.8 (CH), 130.3 (CH), 133.9 (CH), 147.6 (C), 150.8 (C), 164.3 (C), 168.2 (C).

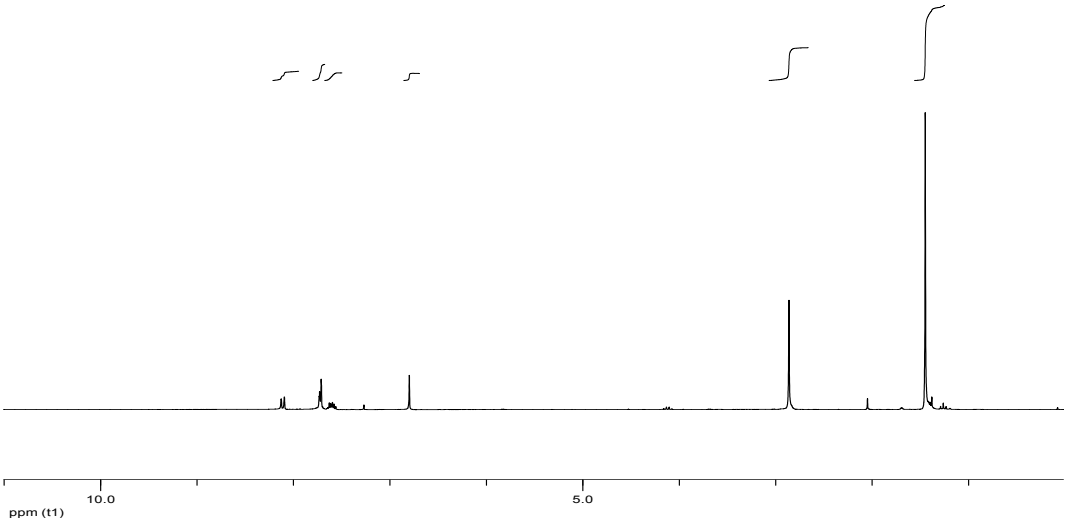
ESI-MS: $[M+Na]^+$, calcd. for $C_{17}H_{18}N_2O_9$ Na =417.0889, found =417.0891. $C_{17}H_{18}N_2O_9$ (M.W. 394,3328)

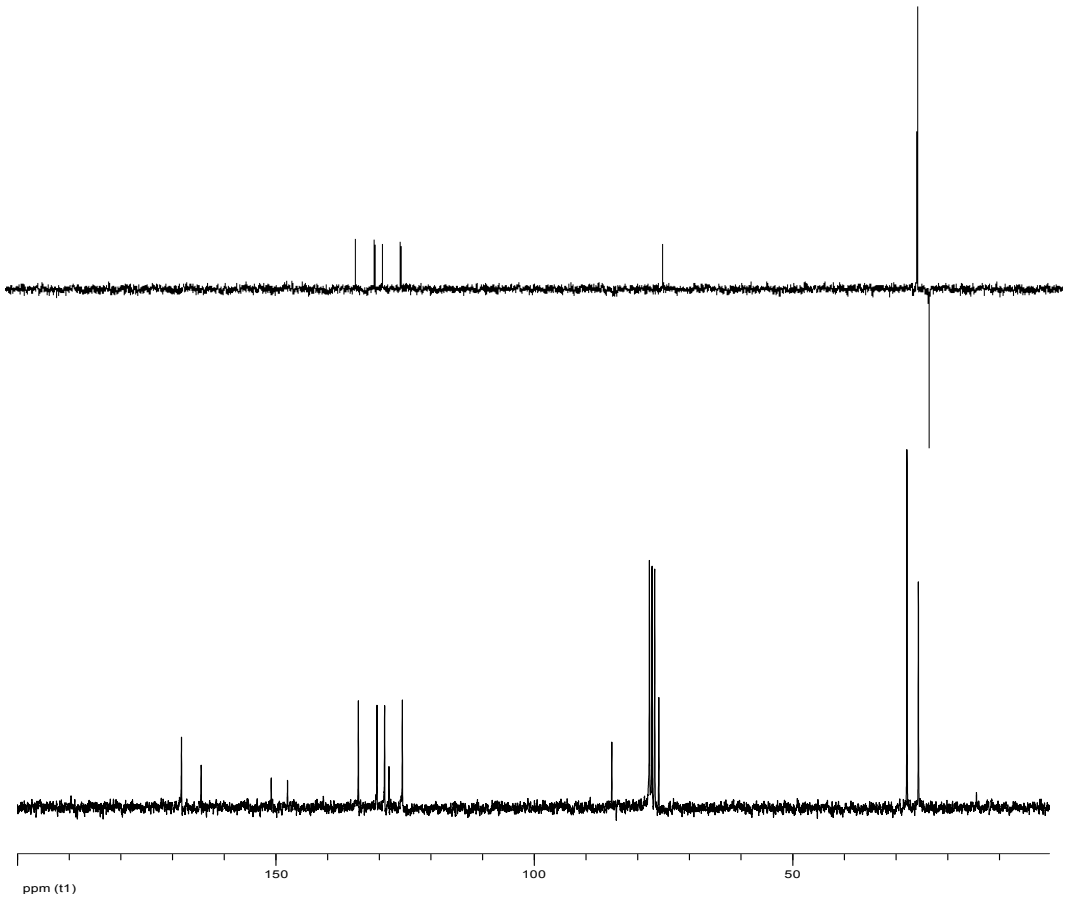
Melting point:: [56.0-59.0] ºC

tert-Butyl ({[(2,5-dioxo-1-pyrrolidinyl)oxy]carbonyl}oxy)(2-nitrophenyl)acetate (5)

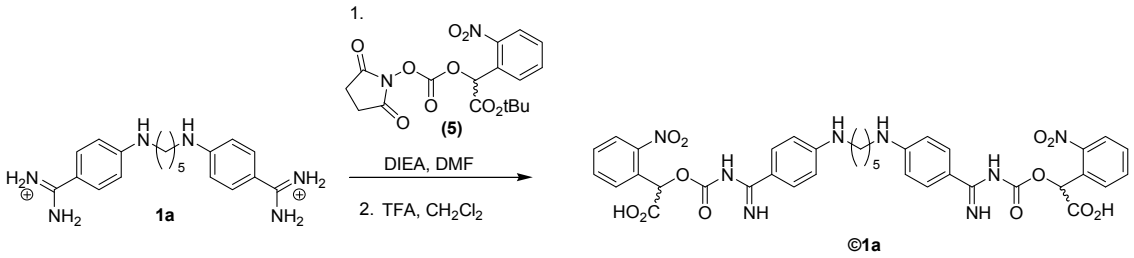


(**5**)





2,2'-{1,5-pentanediylbis[imino-4,1-phenylene(iminomethylene)imino]}bis[(2-nitrophenyl)acetic acid] (Nmoc$_2$-pentamidine, ©1a)



The trifluoroacetic acid disalt of *aza*-pentamidine (**1a**)[1] (50 mg, 0.088 mmol) was dissolved in 1.8 mL of a solution of DIEA/DMF (0.195 M); The nitroderivative **5** was added (75 mg, 0.19 mmol, 2.2 equiv), and the mixture stirred overnight under Ar in the dark. The solvent was removed under reduced pressure, and the residue purified by flash chromatography on silica gel (5% MeOH/CH_2Cl_2) to obtain a pale yellow solid.

This crude sample containing the ^{t}Bu protected derivative was dissolved in CH_2Cl_2 (4.5 mL) and the solution cooled to 0 ºC. TFA (4.5 mL) was slowly added, and the temperature increased to rt during 4 h. The reaction mixture was concentrated under reduced pressure, and the residue purified by preparative reverse-phase chromatography (Büchi Sepacore) (5 min isocratic 5% B, followed by linear gradient from 5% to 95 % B 30 during min). The combined fractions were concentrated and freeze-dried to obtain the desired product **©1a** (pale yellow solid, 63 mg, 0.079 mmol, 89% overall yield).

^{1}H NMR δ (300 MHz, MeOD-*d*$_4$): 1.28-1.30 (m, 2H), 1.69-1.73 (m, 4H), 2.86 (s, 2H, NH), 2.99 (s, 2H, NH), 3.24 (t, *J* = 6.9 Hz, 4H), 6.74 (d, *J* = 9.1 Hz, 4H), 6.99 (s, 2H, CH), 7.67-7.83 (m, 12 H), 8.12 (s, 1H, NH), 8.14 (s, 1H, NH).

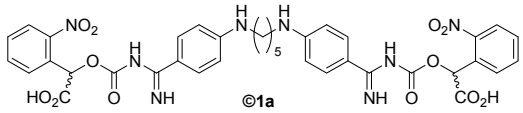
^{13}C NMR δ (300 MHz, MeOD-*d*$_4$): 25.6 (CH_2), 29.7 (CH_2), 43.7 (CH_2), 73.5 (CH), 111.8 (C), 112.9 (CH), 126.4 (CH), 129.7 (C), 131.0 (CH), 131.8 (CH), 132.4 (CH), 135.0 (CH), 149.7 (C), 154.0 (C), 157.0 (C), 165.5 (C), 169.7(C).

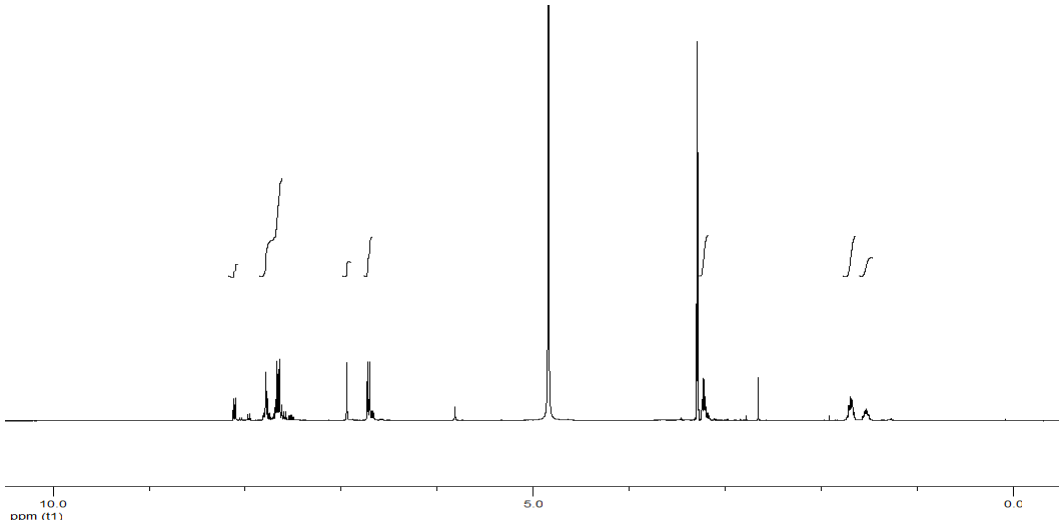
ESI-MS: $[M+H]^+$ calcd. for $C_{37}H_{37}N_8O_{12}$ = 785.2525, found = 785.2527; $C_{37}H_{36}N_8O_{12}$ (MW = 728,6218).

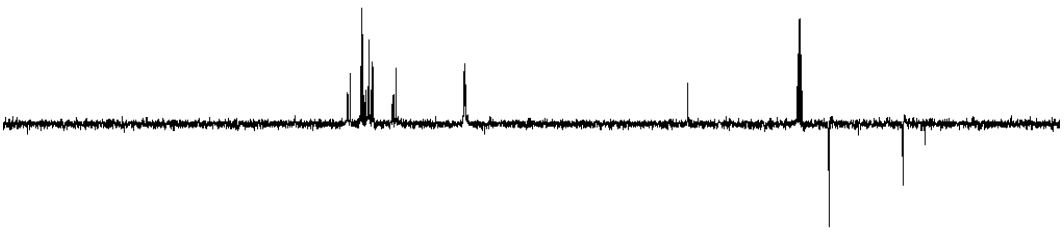
Melting point:: [79.5-84.2] ºC

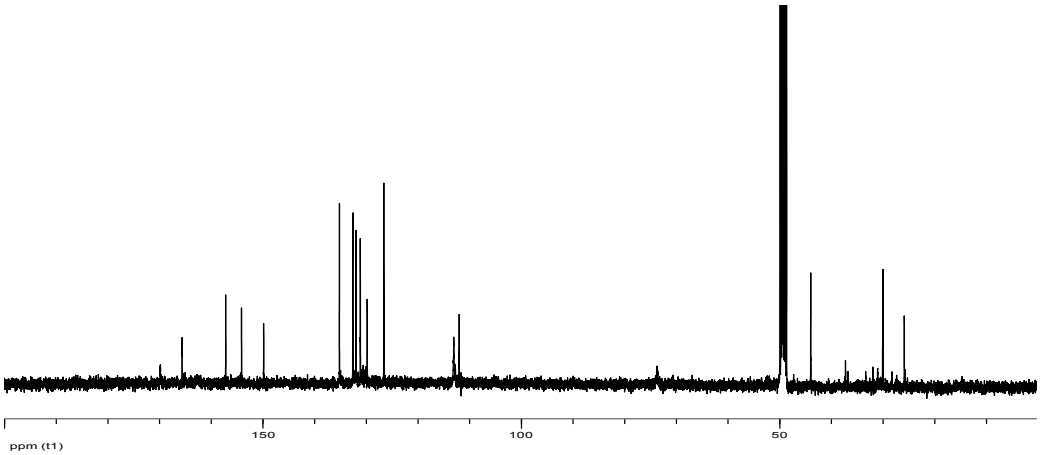
[1] S. M. Bakunova, S. A. Bakunov, D. A. Patrick, E. V. K. Suresh Kumar, K. A. Ohemeng, A. S. Bridges, T. Wenzler, T. Barszcz, S. K. Jones, K. A. Werbovetz, R. Brun, and R. R. Tidwell *J. Med. Chem. 52*(7), 2016-2035 (2009).

2,2'-{1,5-pentanediylbis[imino-4,1-phenylene(iminomethylene)imino]}bis[(2-nitrophenyl)acetic acid] (Nmoc-pentamidine, ©1a)

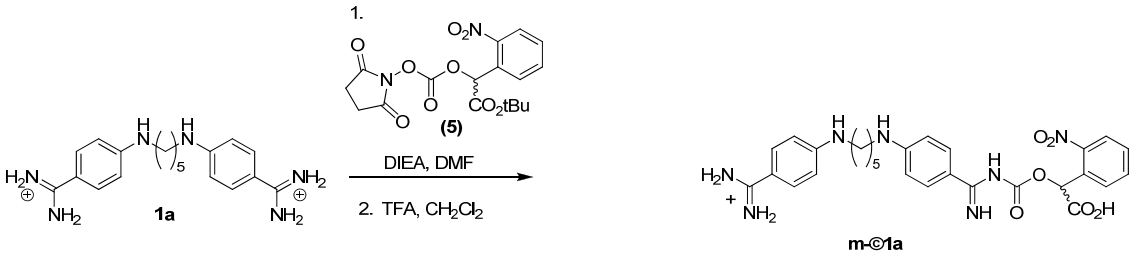








[({[4-({5-[(4-carbamimidoylphenyl) amino]pentyl}amino)phenyl] carboximidoyl} carbamoyl)oxy](2-nitrophenyl)acetic acid, m-©1a



The trifluoroacetic acid disalt of *aza*-pentamidine (**1a**)[1] (80 mg, 0.141 mmol, 3.5 equiv) was dissolved in 1.06 mL of a solution of DIEA/DMF (0.195 M); The nitroderivative **3** was added (15.7 mg, 0.04 mmol), and the m.ixture stirred overnight under Ar in the dark. The solvent was removed under reduced pressure, and the residue purified by flash chromatography on silica gel (5% MeOH/CH_2Cl_2) to obtain a pale yellow solid.

This crude sample containing the ^{t}Bu protected derivative was dissolved in CH_2Cl_2 (4.5 mL) and the solution cooled to 0 ºC, TFA (4.5 mL) was slowly added, and the resulting mixture stirred at room temperature for 4 h. The residue was concentrated and purified by preparative reverse-phase (Büchi Sepacore) (5 min isocratic 5% B, followed by linear gradient from 5% to 95 % B 30 during min). The combined fractions were concentrated and freeze-dried to obtain the desired product **m-©1a** as a pale yellow solid (19.7 mg, 0.03 mmol, 73% overall yield).

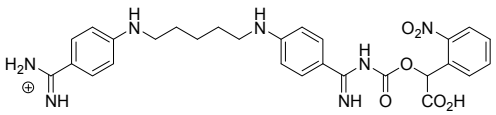
^{1}H NMR δ (300 MHz, DMSO-*d*$_6$): 1.46 (dd, J = 13.8, 7.6 Hz, 2H), 1.55-1.66 (m, 4H), 2.80-3.18 (m, 4H), 6.63-6.72 (m, 4H), 6.76-6.84 (broad signal, s, 1H), 7.63 (d, J = 8.1 Hz, 2H), 7.69-7.80 (m, 3H), 7.84 (d, J = 7.4 Hz, 2H), 8.12 (d, J = 8.2 Hz, 1H), 8.40 (s, 2H), 8.75 (s, 2H).

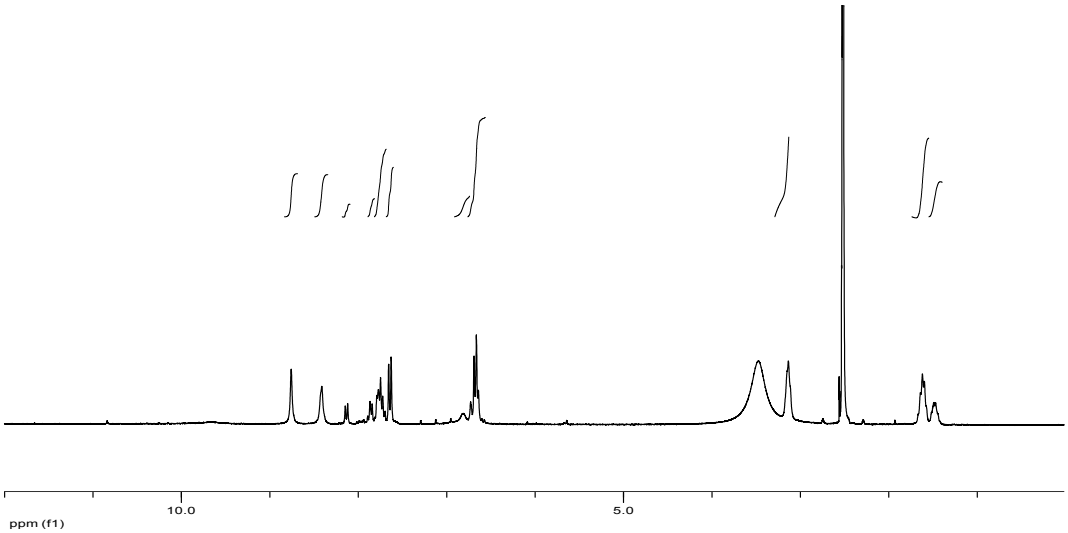
^{13}C NMR δ (300 MHz, DMSO-*d*$_6$): 24.1 (CH_2), 28.1 (CH_2), 42.1 (CH_2), 71.2 (CH), 110.3 (C), 111.0 (CH), 111.9 (C), 125.3 (CH), 128.0 (C), 129.4 (CH), 129.7 (CH), 130.7 (CH), 131.9 (CH), 134.2 (CH), 147.8 (C), 152.3 (C), 153.6 (C), 154.9 (C), 158.9 (C, TFA), 162.8 (C), 164.2 (C), 167.8 (C).

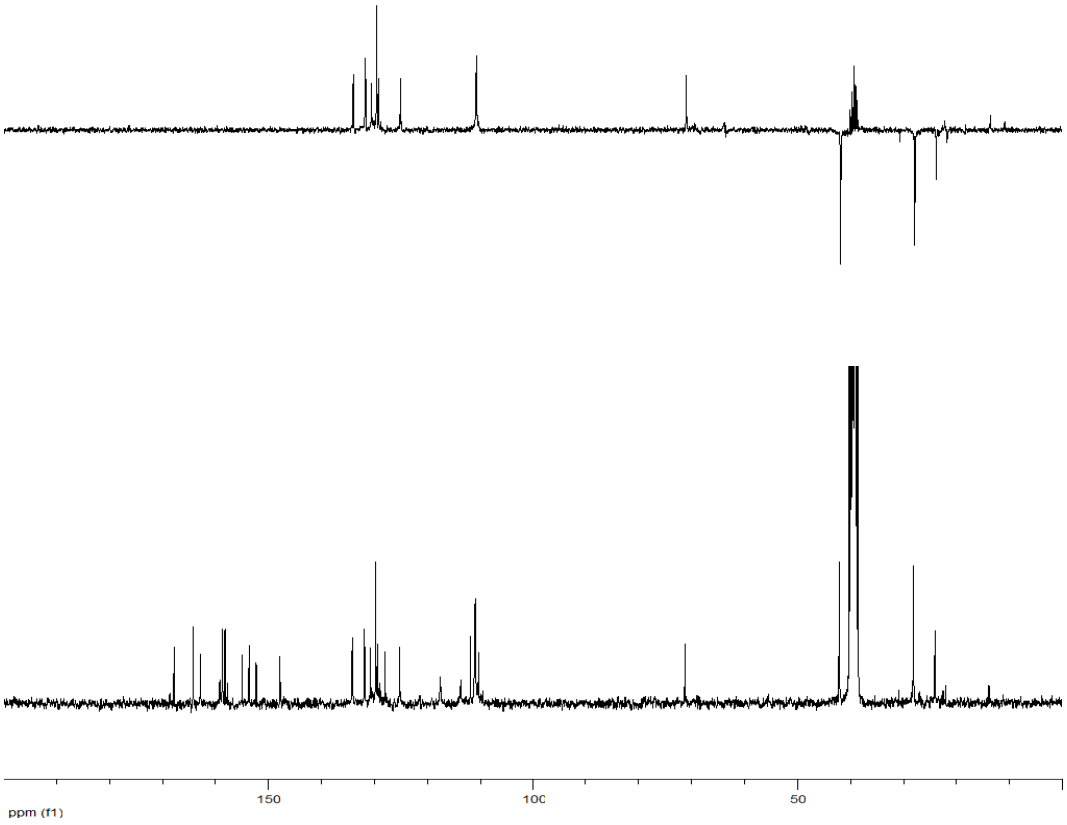
ESI-MS: $[M+H]^+$ calcd. for $C_{28}H_{32}N_7O_6$ = 562,2336, found = 562,2348 ; $C_{30}H_{32}F_3N_7O_8$ (MW = 675,6124).

Melting point: [128.8-132.1] ºC

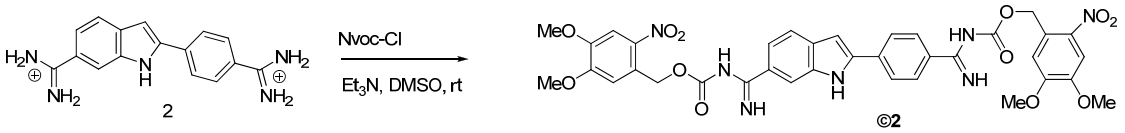
[({[4-({5-[(4-carbamimidoylphenyl) amino]pentyl}amino)phenyl] carboximidoyl} carbamoyl)oxy](2-nitrophenyl)acetic acid, m-©1a







4,5-dimethoxy-2-nitrobenzyl[(4-{6-[({[(4,5-dimethoxy-2-nitrobenzyl)oxy]carbonyl}amino)(imino)methyl]-1H-indol-2-yl}phenyl)(imino)methyl]carbamate (Nvoc$_2$-DAPI, ©2)



To an eppendorf tube containing commercially available 4',6-diamidino-2-phenylindole dihydrochloride (**2**) (12 mg, 0,034 mmol) dissolved in DMSO (0.7 mL), was added Et_3N (30 mg, 41 µL, 0.296 mmol, 8 equiv), and nitroveratryl chloride (NvocCl, 56 mg, 0.204 mmol, 6 equiv). The solution was stirred for 18 h in the absence of light. The reaction crude after concentration was purified by preparative reverse-phase chromatography (Büchi *Sepacore*) (5 min isocratic 5% B, followed by linear gradient from 5% to 95 % B 30 min). The combined fractions were concentrated and freeze-dried to obtain the desired product (**©2**) (14 mg, 0.018 mmol, 54 %).

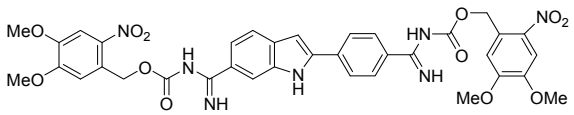
^{1}H NMR δ (500 MHz, MeOH-d_4): 3.93 (s, 3H), 3.94 (s, 3H), 3.99 (s, 3H), 4.00 (s, 3H), 5.76 (s, 2H), 5.78 (s, 2H), 7.27 (s, 1H), 7.33 (d, *J* = 4.5 Hz, 2H), 7.51 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.81 (d, *J* = 2.9 Hz, 2H), 7.87 (d, *J* = 8.4 Hz, 1H), 7.97 (d, *J* = 8.5 Hz, 2H), 8.03 (s, 1H), 8.16 (d, *J* = 8.4 Hz, 2H).

^{13}C NMR δ (300 MHz, MeOH-d_4): 56.9 (CH_3), 57.1 (CH_3), 67.4 (CH_2, Bn), 103.3 (CH), 109.5 (CH), 113.0 (CH), 114.3 (CH), 120.6 (CH), 121.8 (C), 122.9 (CH), 126.0 (C), 126.2 (C), 127.3 (CH), 130.7 (CH), 135.4 (C), 138.3 (C), 138.7 (C), 141.6 (C), 142.4 (C), 150.5 (C), 154.6 (C), 155.2 (C), 167.9 (C), 168.7 (C).

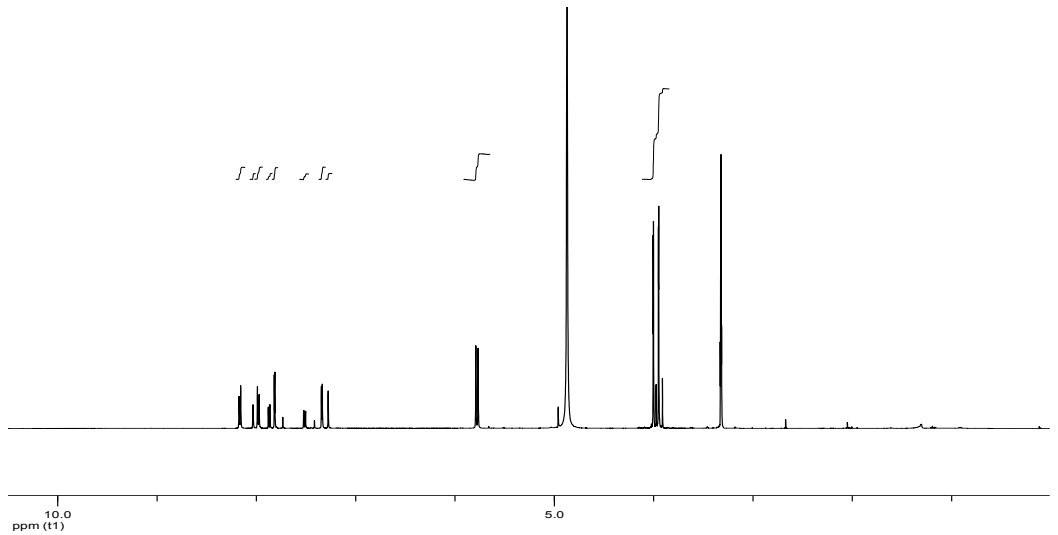
ESI-MS: $[M+H]^+$ calcd. for $C_{36}H_{34}N_7O_{12}$ =756.2260, found = 756.2250; $C_{36}H_{33}N_7O_{12}$ (MW = 755,6869).

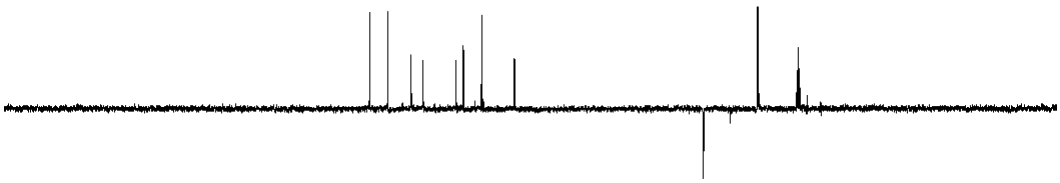
Melting point: [141.2-144.3] ºC

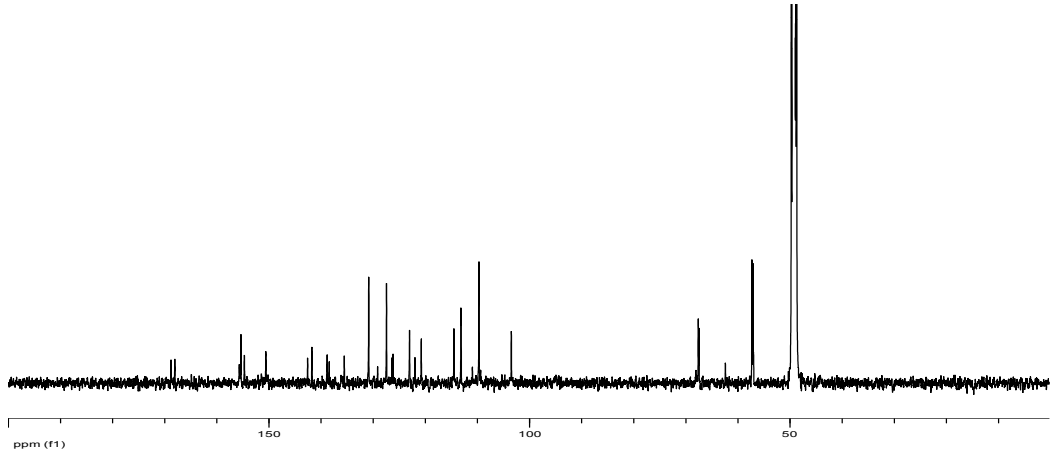
4,5-dimethoxy-2-nitrobenzyl[(4-{6-[({[(4,5-dimethoxy-2-nitrobenzyl)oxy]carbonyl}amino)(imino)methyl]-1H-indol-2-yl}phenyl)(imino)methyl]carbamate (Nvoc$_2$-DAPI, ©2)



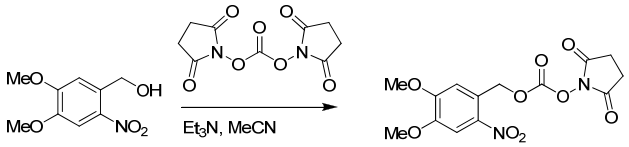
©2







({[(2,5-dioxo-1-pyrrolidinyl)oxy]carbonyl}oxy)-4,5-dimethoxy-2-nitrophenyl (7)



6 7

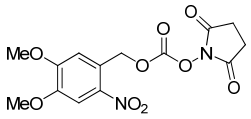
To a solution of alcohol **6** (500 mg, 2.35 mmol) in MeCN (23.5 mL) was added Et_3N (950 mg, 9.4 mmol, 4 equiv), and dissucinimidyl dicarbonate (721 mg, 2.82 mmol, 1.2 equiv). After stirring for 8 h under Ar at r.t., TLC analysis showed that starting material was consumed. The solvent was removed and the residue purified by flash column chromatography on silica gel (60% AcOEt/hexanes) to obtain the desired product (**7**) as a yellow solid (607 mg, 1.71 mmol, 73%).

^{1}H RMN δ (300 MHz, *DCCl$_3$*): 2.85 (s, 4H), 3.96 (s, 3H), 4.05 (s, 3H), 5.78 (s, 2H), 7.04 (s, 1H), 7.75 (s, 1H).

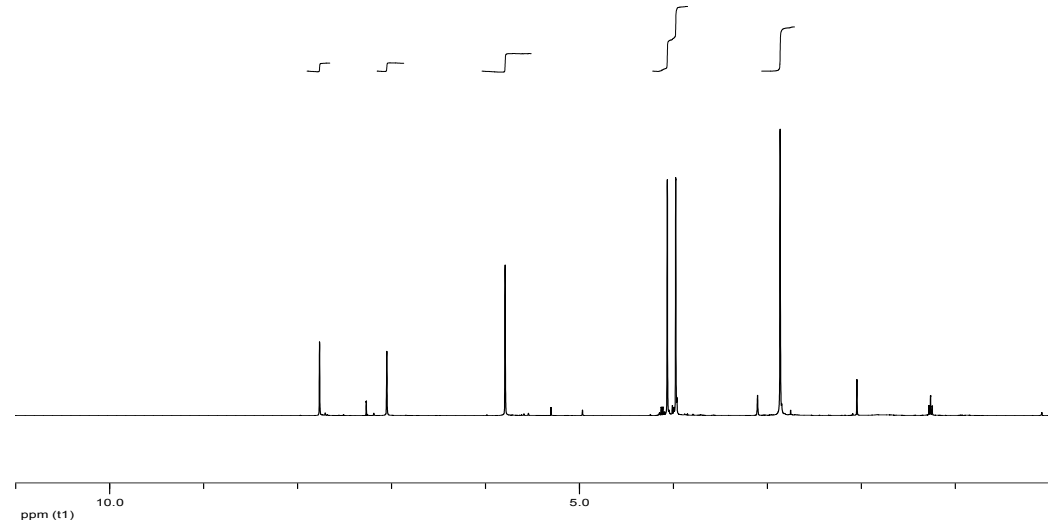
^{13}C RMN δ (300 MHz, *DCCl$_3$*): 25.4 (CH_2), 56.4 (CH_3), 56.6 (CH_3), 69.1 (CH_2), 108.2 (CH), 108.6 (CH), 125.3 (C), 139.1 (C), 148.5 (C), 151.3 (C), 154.1 (C), 168.4 (C).

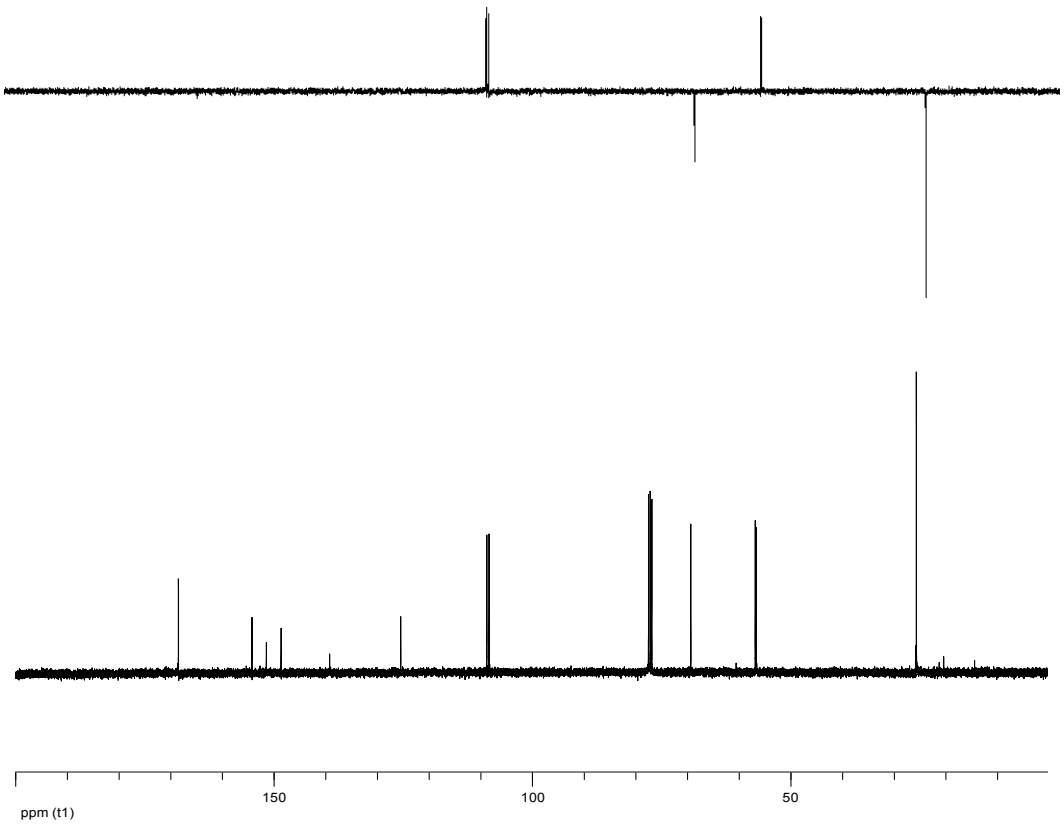
ESI-MS: $[M+Na]^+$ calc. for $C_{14}H_{14}N_2O_9Na_1$ = 377.0592 found 377.0598; $C_{14}H_{14}N_2O_9$ (M.W. 354,2690)

({[(2,5-dioxo-1-pyrrolidinyl)oxy]carbonyl}oxy)-4,5-dimethoxy-2-nitrophenyl (7)

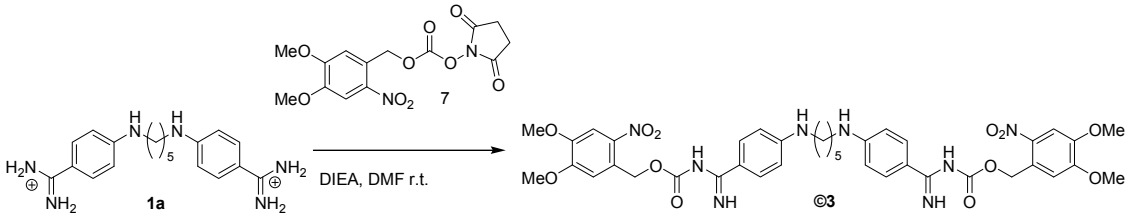


7





(4,5-dimethoxy-2-nitrophenyl)methyl N-[(4-{[5-({4-[({[(4,5-dimethoxy-2-nitrophenyl) methoxy]carbonyl}amino)methanimidoyl]phenyl}amino)pentyl]amino}phenyl)carboximid oyl]carbamate, ©3



The trifluoroacetic acid disalt of *aza*-pentamidine (**1a**)[2] (50 mg, 0.088 mmol) was dissolved in 1.8 mL of a solution of DIEA/DMF (0.195 M); The nitroderivative **7** was added (93 mg, 0.26 mmol, 3.0 equiv), and the mixture stirred overnight under Ar in the dark. The solvent was removed under reduced pressure, and the residue purified by flash chromatography on silica gel (5% MeOH/CH_2Cl_2) to obtain a yellow solid that was identified as the desired product (**©3**) (60 mg, 0.073 mmol, 83%)

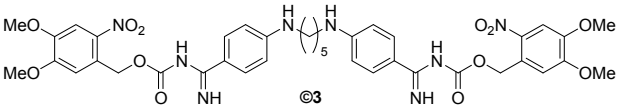
^{1}H NMR δ (400 MHz, DMSO-*d*$_6$): 1.46 (m, 2H), 1.59 (m, 4H), 3.08 (dd, *J* = 12.4, 6.5 Hz, 4H), 3.87 (s, 6H), 3.87 (s, 6H), 5.37 (s, 4H), 6.42 (t, *J* = 5.3 Hz, 2H), 6.57 (d, *J* = 8.9 Hz, 4H), 7.21 (s, 2H), 7.69 (s, 2H), 7.81 (d, *J* = 8.8 Hz, 4H).

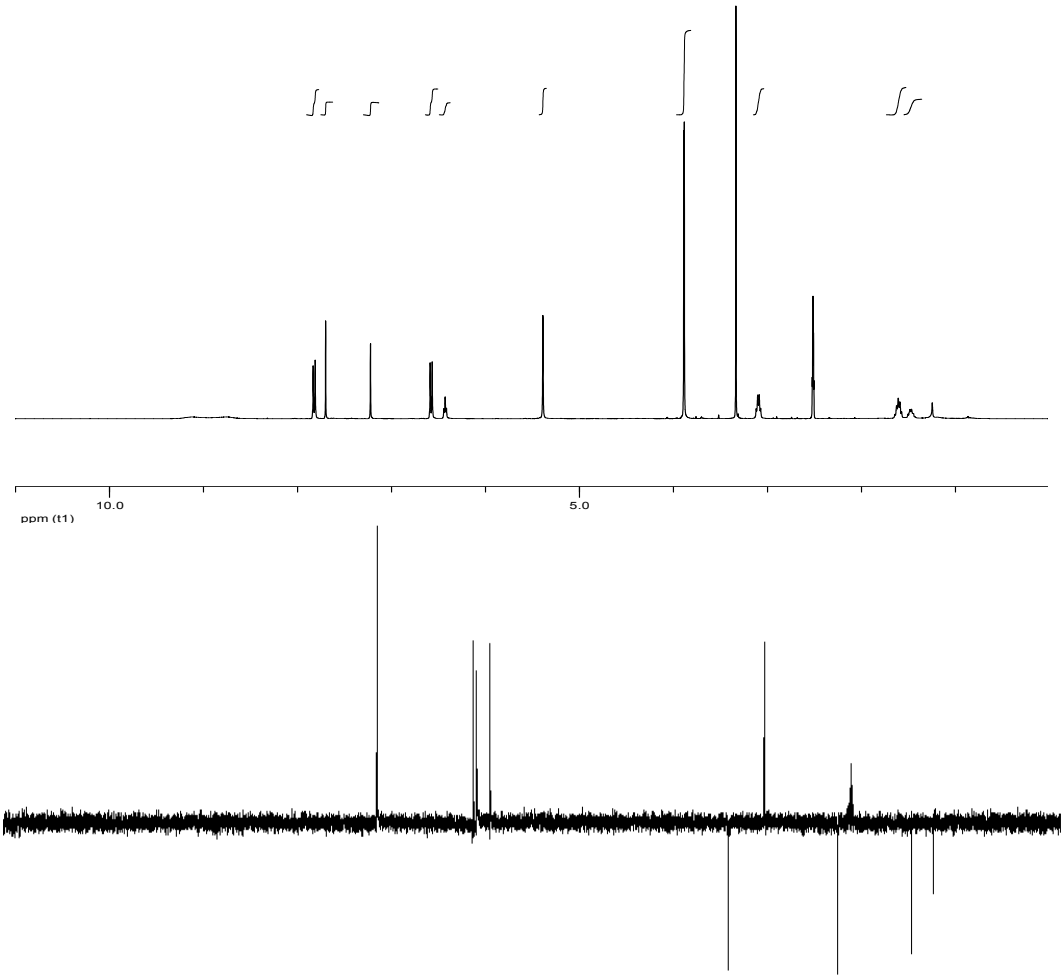
^{13}C NMR δ (300 MHz, DMSO-*d*$_6$): 24.1 (CH_2), 28.2 (CH_2), 42.2 (CH_2), 55.9 (CH_3, OMe), 56.0 (CH_3, OMe), 62.8 (CH_2), 108.0 (CH), 110.5 (CH), 111.1 (CH), 119.2 (CH), 127.7 (C), 129.3 (CH), 139.6 (C), 147.6 (C), 152.3 (C), 153.0 (C), 163.3 (C), 166.8 (C).

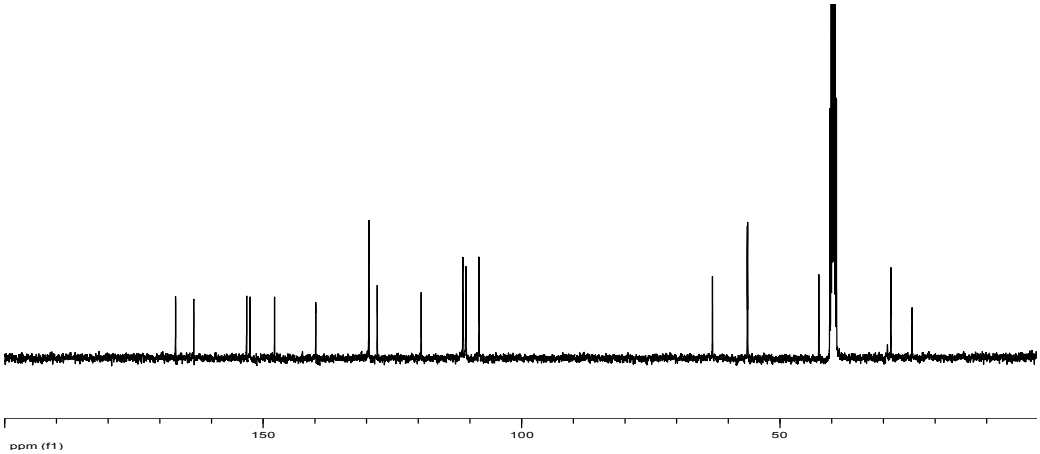
ESI-MS: $[M+Na]^+$ calc. for $C_{39}H_{44}N_8O_{12}Na_1$ = 839.2971 found 839.2965; $C_{14}H_{14}N_2O_9$ (M.W. 816,8131)

Melting point: [171.4-174.8] ºC

(4,5-dimethoxy-2-nitrophenyl)methyl N-[(4-{[5-({4-[({[(4,5-dimethoxy-2-nitrophenyl) methoxy]carbonyl}amino)methanimidoyl]phenyl}amino)pentyl]amino}phenyl)carboximid oyl]carbamate, ©3







FLUORESCENCE SPECTROSCOPY

Measurements were made with a *Jobin−Yvon Fluoromax−3*, (*DataMax 2.20*) coupled to a *Wavelength Electronics LFI−3751* peltier temperature controller. All the data were recorded at 20 ºC.

In vitro experiments with ©1a and m-©1a were made with the following settings: increment: 1.0 nm; integration time: 0.2 s; excitation slit width: 2.0 nm; emission slit width: 4.0 nm; excitation wavelength 329 nm and recorded from 345 nm to 500 nm.

In vitro experiments with ©2 were made with the following settings: increment: 1.0 nm; integration time: 0.25 s; excitation slit width: 2.0 nm; emission slit width: 4.0 nm; excitation wavelength 360 nm and recorded from 380 nm to 600 nm.

Harpin oligonucleotides were supplied by *Thermo Fischer Scientific GmbH*, and their sequences were:

Name	**Full sequence (5' to 3')**
AAATTT	GGC ***AAATTT*** CAG TTTTT CTG ***AAATTT*** GCC
GGCCC	GGCA ***GGCC*** CAGC TTTTT GCTG ***GGCC*** TGCC

Cleavage of the photolabile group was achieved by irradiation with a transilluminator 8 watt lamp (λ_{max} aprox 360 nm).

DNA binding of ©1a

A fluorescence cuvette containing a 0.5 μM solution of **©1a** in Tris-HCl buffer 20 mM; 100 mM NaCl; pH 7.5, and 5 μM DNA oligonucleotide **AAATTT**, was irradiated with UV light (aprox. 360 nm) and fluorescence emission spectra were recorded after different irradiation times, as indicated in the figure. The same experiment was repeated with **©1a** and 5 μM DNA oligonucleotide **GGCCC.**

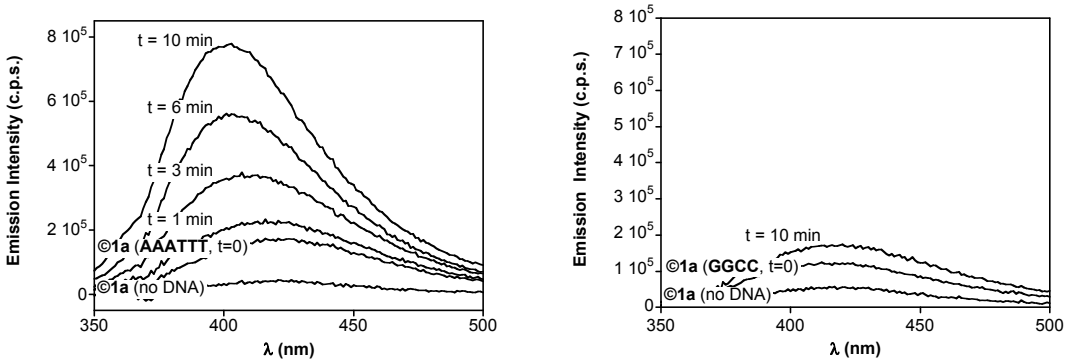


Figure S1. ***Left*** *Irradiation of* ***©1a*** *in the presence of target* **AAATTT** *and non-target oligonucleotide (**Right**)* **GGCCC** *at different irradiation times.* λ_{exc} *329 nm.*

For the competition titrations, aliquots of **©1a** were successively added to a mixture containing 0.5 μM solution of **1a** in Tris-HCl buffer 20 mM; 100 mM NaCl; pH 7.5, and 1.4 μM of the target oligonucleotide **AAATTT**. Fluorescence emission of the DNA/**1a** complex at 390 nm is represented. As can be deduced from the following figure, there is almost not decrease in the fluorescence emission, which means that **©1a** is not able to displace DNA-bound **1a**.

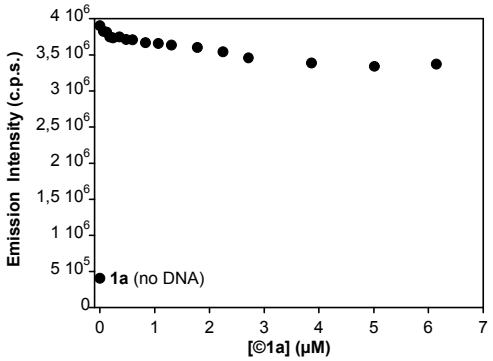


Figure S2. *Competition titration. The emission of* ***1a*** *in the presence of target* **AAATTT** *is monitored at increasing concentrations of* ***©1a*** *. Final point is approximately 12 equivalents of* ***©1a****. The fluorescence emission of* ***1a*** *in absence of DNA is also represented as reference.*

ADDITIONAL CONTROL EXPERIMENT: Azapentamidine (**1a**), 2 μM in Tris-HCl buffer 20 mM; 100 mM NaCl; pH 7.5 was incubated with a DNA hairpin oligonucleotide (**AAATTT**, 2 equiv) containing the target sequence, and the corresponding emission spectrum was recorded. The sample was then irradiated using the standard conditions for 10 min. to simulate the uncaging procedure, and the fluorescence emission was then recorded. As shown in the figure below, the fluorescence emission remains virtually unchanged, demonstrating the negligible effect of the low intensity irradiation on the DNA or the azapentamidine itself.

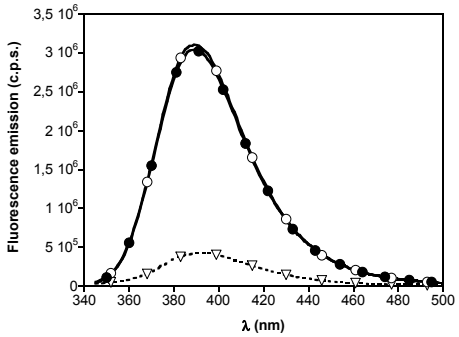


***Figure S3**. Fluorescence emission spectra of azapentamidine (**1a**) (▽), in the presence of target hairpin oligonucleotide (AAATTT) (○), in the presence of target hairpin oligonucleotide (AAATTT) after 10 minutes irradiation (●).λ_{exc} 330 nm.*

DNA binding of ©2

A fluorescence cuvette containing a 2 μM solution of **©2** in Tris-HCl buffer 20 mM; 100 mM NaCl; pH 7.5, and 2.5 μM DNA oligonucleotide **AAATTT** was irradiated with UV light and fluorescence emission spectra were recorded after different irradiation times, as indicated in the figure.

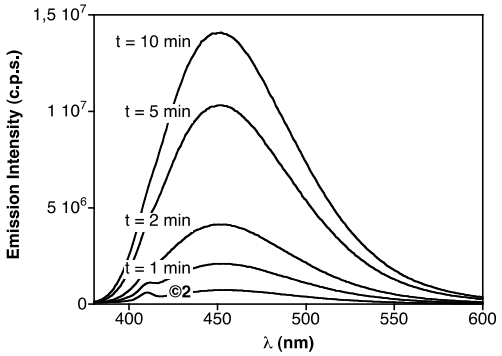


***Figure S4.** Irradiation of **©2** in 20 mM Tris-HCl buffer, 100 mM NaCl in the presence of ds-oligonucleotide **AAATTT** showing the increased emission upon irradiation. λ_{exc} 360 nm.*

For the competition titrations, successive aliquots of **©2** were successively added to a 1 μM solution of **2** in the presence of 2 μM target oligonucleotide **AAATTT**. Emission of the DNA/**3** complex at 456 nm is represented.

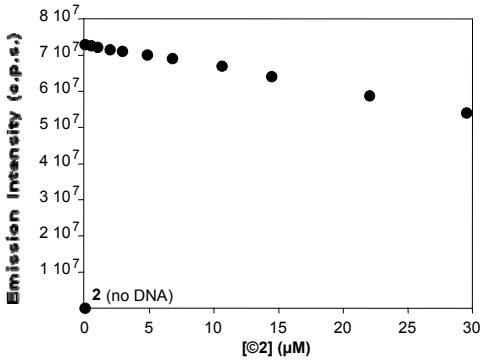


***Figure S5**. Competition titration. The emission of **2** in the presence of target **AAATTT** is monitored at increasing concentrations of **©2** . Final point is approximately 30 equivalents of **©2**. The fluorescence emission of **2** in absence of DNA is also represented as reference.*

DNA binding of m-©1

A fluorescence cuvette containing a 0.5 μM solution of **m-©1** in Tris-HCl buffer 20 mM; 100 mM NaCl; pH 7.5, successive aliquots of target oligonucleotide **AAATTT** were added. Fluorescence emission of the DNA/ **m-©1a** complex at 386 nm is represented. As can be deduced from the following figure, there is binding of the monouncaged benzamidine unit to the AT track which is responsible of the fluorescence increase, however the binding is considerably weaker than that of the parent **1a.**

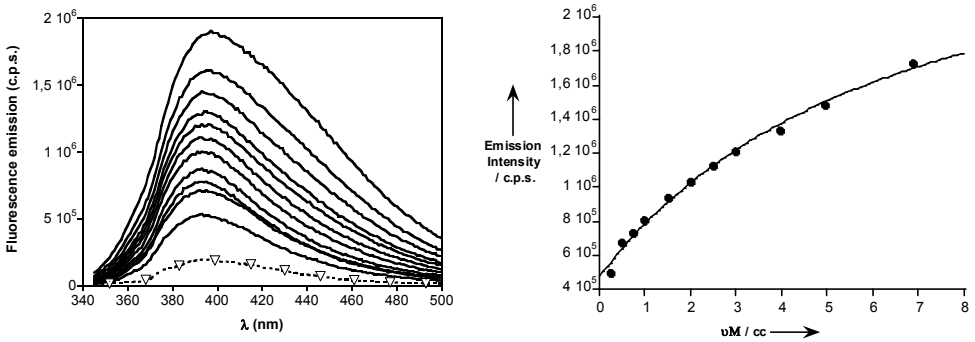


***Figure S6**. **Left**: Fluorescence emission spectra of* **m-©1a**, *in the presence of* successive aliquots of target oligonucleotide **AAATTT**. **Right**. The binding curve represents the best fit to 1:1 binding mode considering the contribution of the DNA to the fluorescence titration. The estimated K_D value is the average of three experiments: K_D aprox 6.3 μM.

To check that this is a specific DNA binding, the same experiment was repeated with **m-©1a** and dsDNA oligonucleotide containing a **GGCCC** sequence. As can be deduced from the following figure the compound is unable to bind this DNA.

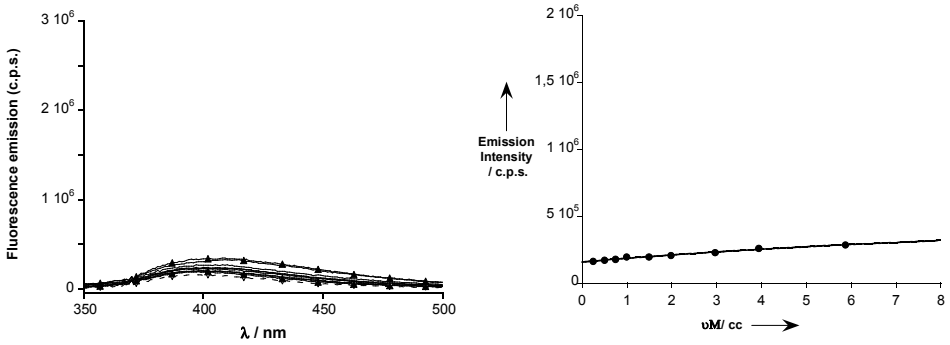


***Figure S7**. Fluorescence emission spectra of* **m-©1a**, *in the presence of* successive aliquots of target oligonucleotide **GGCCC**.The binding curve represents the best fit to 1:1 binding mode considering the contribution of the DNA to the fluorescence titration.

CIRCULAR DICHROISM SPECTROSCOPY

Verification that the process of photolysis does not damage the standar B form of the dsDNA.

Measurements were made with a *Jasco-715 Spectropolarimeter*. All the data were recorded at 20 ºC with the following settings: Sensitivity standard (100 mdeg), data pitch 0.2 nm, scanning mode continuous, scanning speed continuous (50 nm/min), response 1 sec, band with 0.2 nm, accumulation 2.

A circular dichroism cuvette containing a 5.0 μM solution of DNA oligonucleotide **AAATTT** in Tris-HCl buffer 20 mM; 100 mM NaCl; pH 7.5, and 5 μM of **m-©1a**, was irradiated with UV light (365 nm) and circular dichroism spectra were recorded after different irradiation times, as indicated in the figure S8.

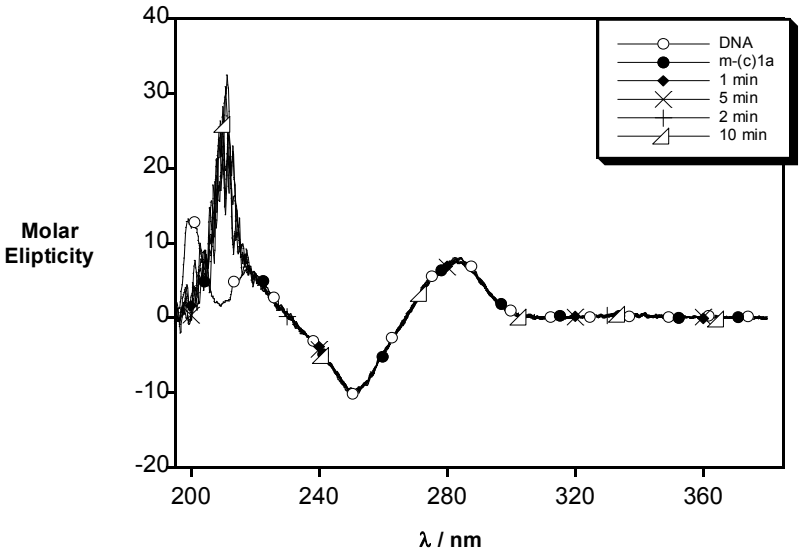


Figure S8. *Circular dichroism spectra of AAATTT hairpin oligonucleotide after successive irradiations under standard uncaging conditions.*

QUANTUM YIELD DETERMINATION

Commercial **Nvoc-GABA** and **m-©1a** were dissolved at appropriate concentrations to have the same absorbance at 329 nm. Both solutions, containing an internal inosine standard were irradiated, and aliquots were taken and different irradiation times, diluted in 200 µL of 1:1 MeCN/H_2O and analyzed by HPLC-MS. Inosine area was used for normalizing the area under each HPLC peak.

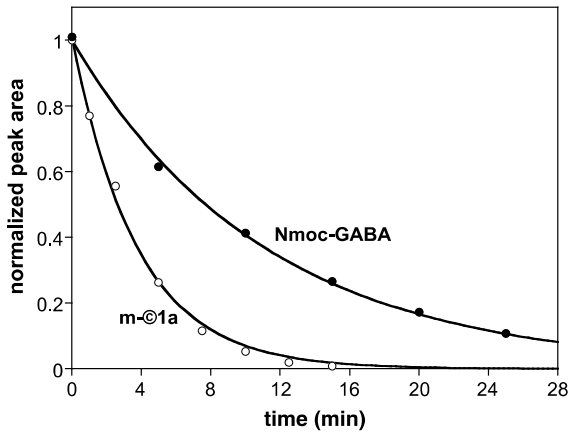


***Figure S9**. Relative uncaging kinetics of **m-©1a** and **Nvoc-GABA**. Best fit to a single exponential is shown.*

DISK DIFFUSION TESTS

Antifungal assay: The antimicrobial activity of the compounds was assayed by the conventional disk diffusion method with *Candida albicans*, following the procedure of the Clinical and Laboratory Standards Institute (CLSI, 2006).

Antimicrobial solution was prepared by using water or DMSO as solvents. Sterile discs of 6mm diameter (Liofilchem, Italy) embedded in the drug, were kept on the surface of Mueller Hinton Agar (Cultimed, Spain) inoculated with the yeast.

The plates were incubated at 37ºC for 24h. Zones of inhibition were measured in millimeters.

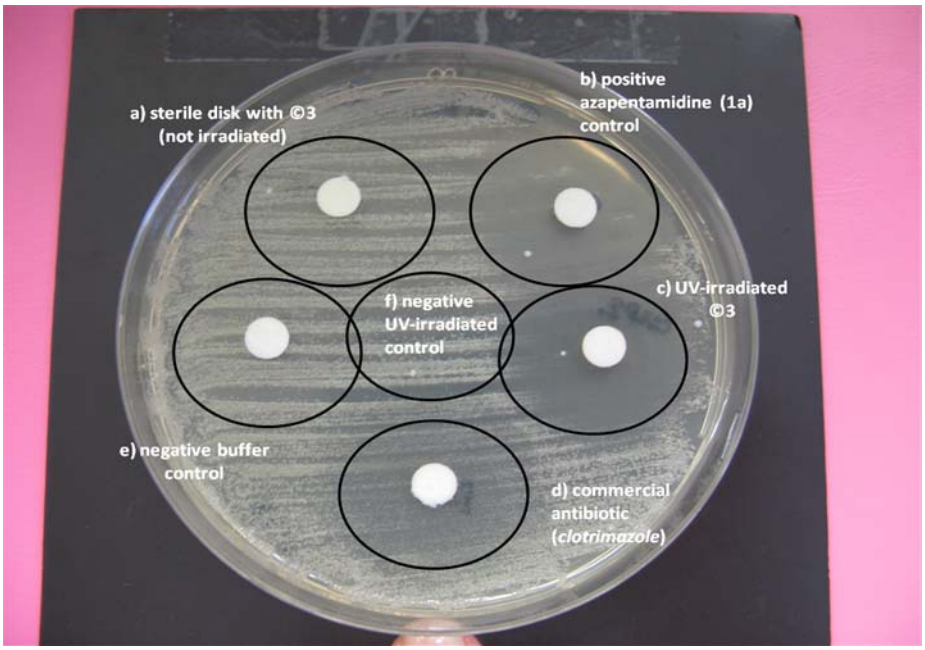


Figure S10. Antifungal assay. We can observe in the picture all the experiments and controls run in the same plate and the consequence on the fungus growth.