Supplementary Material (ESI) for Chemical Communications

Supporting Information

Self-Immolative Dendritic Probe for Direct Detection of Triacetone Triperoxide

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Supplementary Material (ESI) for Chemical Communications

General methods. All reactions requiring anhydrous conditions were performed under an Argon or N_2 atmosphere. All reactions were carried out at room temperature unless stated otherwise. Chemicals and solvents were either A.R. grade or purified by standard techniques. Thin layer chromatography (TLC): silica gel plates Merck 60 F_{254}: compounds were visualized by irradiation with UV light. Flash chromatography (FC): silica gel Merck 60 (particle size 0.040-0.063 mm), eluent given in parentheses. ^{1}H-NMR spectra were measured using Bruker Avance operated at 400 MHz as mentioned. ^{13}C-NMR spectra were measured using Bruker Avance operated at 100 MHz as mentioned. ^{19}F-NMR spectra were measured using Bruker Avance operated at 188 MHz as mentioned. The chemical shifts are expressed in δ relative to TMS (δ = 0 ppm) and coupling constants *J* in Hz. The spectra were recorded in $CDCl_3$ as solvent at room temperature unless stated otherwise. All general reagents, including salts and solvents, were purchased from Sigma-Aldrich. TATP was prepared according to Internet procedure: (http://web.archive.org/web/20050210062936/http://en.wikibooks.org/wiki/Acetone_peroxide_synthesis).

Abbreviations. DBTL- Dibutyltin dilaurate, DCM- Dichloromethane, DMF- N,N'-Dimethylformamide, DMSO- Dimethylsulfoxide, Et_2O- Diethyl ether, EtOAc- Ethylacetate, HCl- Hydrogen chloride, Hex- n-Hexanes, MeOH- Methanol, , TFA- Trifluoroacetic acid, TFAA- Trifluoroacetic-anhydride, THF- Tetrahydrofurane.

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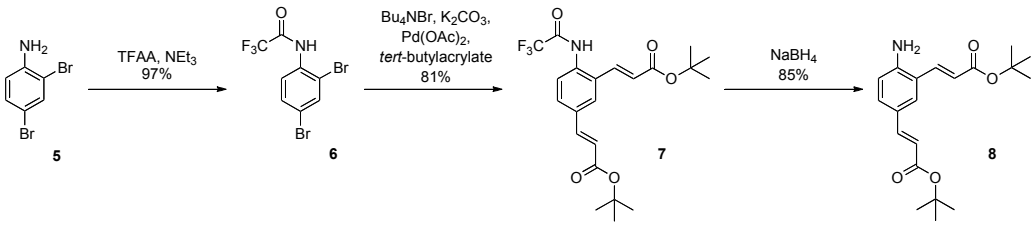

Compound 6. Compound **5**[1] (1 g, 3.98 mmol) was dissolved in DCM, cooled to 0ºC and Triethylamine (0.83 mL, 5.97mmol) was added. A solution of TFAA (0.84 mL, 5.97 mmol) in 2 mL of DCM was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for additional 30 min. The reaction was monitored by TLC (EtOAc/Hex 5:95). After completion, the reaction was diluted with Et_2O and washed with HCl 1N. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/Hex 5:95) to give compound **6** (1.34 g, 97%) as a pale brown powder.

^{1}H NMR (400MHz, $CDCl_3$): δ = 8.39 (1H, brs), 8.19 (1H, d, *J* = 8.8 Hz), 7.75 (1H, d, *J* = 2.7 Hz), 7.50 (1H, dd, *J* = 8.8 Hz, 2.7 Hz). ^{13}C NMR (100MHz, $CDCl_3$): δ = 154.9 (quart, *J* = 37.4 Hz), 135.2, 132.7, 132.1, 123.3, 119.6, 115.8 (quart, *J* = 286.9 Hz), 114.9. ^{19}F NMR (188 MHz, $CDCl_3$): δ = -76.3 (s). MS (ESI): m/z calc. for $C_8H_4Br_2F_3NO$: 346.9; found: 346.9 $[M]^+$.

Compound 7. Compound **6** (1 g, 2.88 mmol) was dissolved in 10 mL dry DMF under Argon atmosphere. Bu_4NBr (2.3 g, 7.2 mmol), K_2CO_3 (3.9 g, 28.8 mmol), $Pd(OAc)_2$ (32 mg, 0.14 mmol) and *tert*-butylacrylate (1.3 mL, 8.64 mmol) were added. The reaction mixture was then heated to 80ºC, stirred for 2 hours and monitored to completion by TLC (EtOAc/Hex 15:85). After cooling to room temperature, the mixture was diluted with EtOAc, washed with saturated solution of NH_4Cl followed by brine. The organic layer dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/Hex 15:85) to give compound **7** (1.02 g, 81%) as a pale yellow powder.

Supplementary Material (ESI) for Chemical Communications

^{1}H NMR (400MHz, $CDCl_3$): δ = 9.04 (1H, brs), 7.89 (1H, d, *J* = 8.4 Hz), 7.78 (1H, d, *J* = 18.0 Hz), 7.77 (1H, d, *J* = 18.0 Hz), 7.56 (1H, dd, *J* = 8.4 Hz, 1.9 Hz), 7.49 (1H, d, *J* = 1.9 Hz), 6.39 (1H, d, *J* = 18 Hz), 6.38 (1H, d, *J* = 18 Hz), 1.53 (9H, s), 1.52 (9H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 166.2, 165.9, 156.2 (quart, *J* = 36.3 Hz), 144.8, 137.1, 134.4, 134.2, 129.8, 129.6, 126.9, 126.2, 123.9, 121.9, 116.1 (quart, *J* = 286.7 Hz), 81.7, 81.3, 28.3, 28.2. ^{19}F NMR (188 MHz, $CDCl_3$): δ = -76.2. MS (FAB): m/z calc. for $C_{22}H_{26}F_3NO_5$: 441.1; found: 441.1 $[M]^+$.

Compound 8. Compound **7** (300 mg, 0.68 mmol) was dissolved in 3 mL MeOH, cooled to 0ºC and $NaBH_4$ (52 mg, 1.36 mmol) was added. After 5 min, another aliquot of $NaBH_4$ (52 mg, 1.36 mmol) was added, and the reaction mixture was stirred for additional 30 min. The reaction was monitored by TLC (EtOAc/Hex 25:75). After completion, the reaction was diluted with EtOAc and washed with saturated solution of NH_4Cl followed by brine. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/Hex 15:85) to give compound **8** (199 mg, 85%) as a yellow powder.

^{1}H NMR (400MHz, $CDCl_3$): δ = 7.66 (1H, d, *J* = 15.7 Hz), 7.46 (1H, d, *J* = 1.8 Hz), 7.44 (1H, d, *J* = Hz), 7.28 (1H, dd, *J* = 8.4 Hz, 1.8 Hz), 6.63 (1H, d, *J* = 8.4 Hz), 6.28 (1H, d, *J* = 15.7 Hz), 6.17 (1H, d, *J* = 15.7 Hz), 4.31 (2H, brs), 1.51 (9H, s) 1.49 (9H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 166.9, 166.4, 147.3, 143.3, 138.3, 130.5, 128.4, 125.2, 121.0, 119.7, 116.6, 116.5, 80.7, 77.2, 28.3, 28.2. MS (FAB): m/z calc. for $C_{20}H_{27}NO_4$: 345.1; found: 345.1 $[M]^+$. Quantum yield calculated with 1-Napthylamine as a reference in ethanol = 0.11 ± 0.01.

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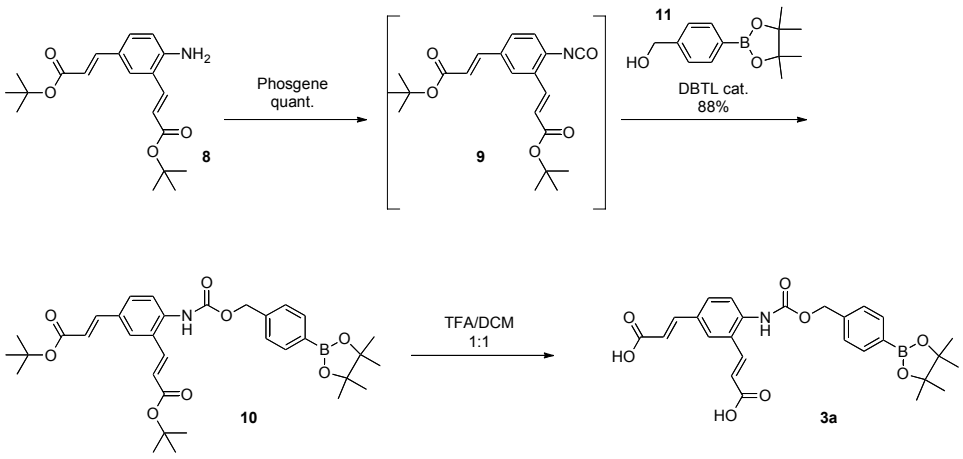


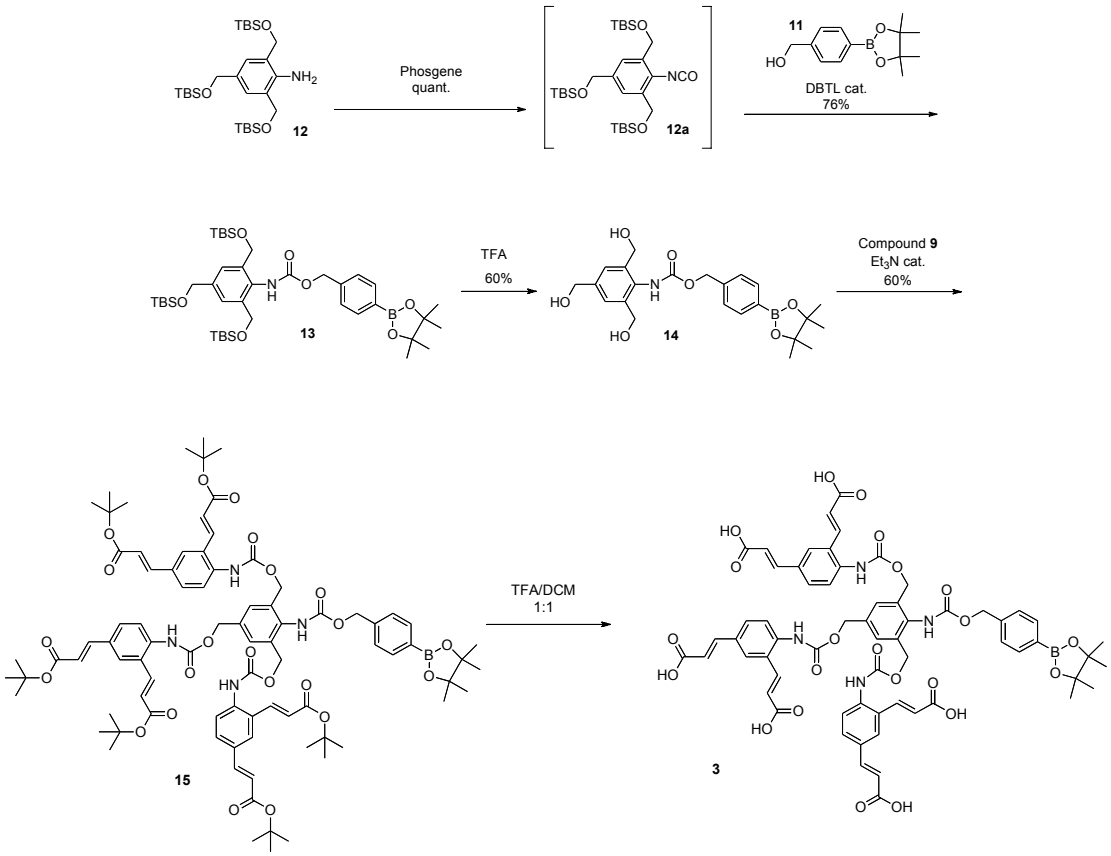
Compound 10. Toluene was heated to reflux (110ºC) under Argon atmosphere and a solution of 20% phosgene in toluene (1.2 mL, 2.3 mmol) was added. Then, a solution of compound **8** (100 mg, 0.28 mmol) in toluene was slowly added dropwise with a syringe. The reaction mixture was stirred for 30 min at reflux and monitored by ^{1}H-NMR. After isocyanate derivative **9** was observed, the solvent was removed under reduced pressure. A solution of compound **11**[2] (50 mg, 0.21 mmol) in dry THF, followed by 0.25 mL of triethylamine, were added to the isocyanate residue. The reaction mixture was heated to 40ºC, stirred for 1 h under Ar atmosphere and monitored by TLC (EtOAc/Hex 25:75). After completion, the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/Hex 20:80) to give compound **10** (112 mg, 88%) as a white powder.

^{1}H NMR (400MHz, $CDCl_3$): δ = 7.94 (1H, d, *J* = 8.4 Hz), 7.83 (2H, d, *J* = 7.9 Hz), 7.68 (1H, d, *J* = 15.8 Hz), 7.59 (1H, d, *J* = 1.8 Hz), 7.51 (1H, d, *J* = 16.0 Hz), 7.50 (1H, dd, *J* = 8.4 Hz, 1.8Hz), 7.41 (2H, d, *J* = 7.9 Hz), 6.82 (1H, s), 6.34 (1H, d, *J* = 15.8 Hz), 6.32 (1H, d, *J* = 16.0 Hz), 5.23 (2H, s), 1.54 (9H, s), 1.53 (9H, s), 1.35 (12H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 166.3, 165.7, 153.3, 142.3, 138.7, 137.4, 137.2, 135.3, 135.2, 130.9, 129.9, 127.6, 127.2, 124.4, 120.2, 84.0, 81.3, 80.7, 67.6, 28.3, 28.2, 24.9. MS (FAB): m/z calc. for $C_{34}H_{44}BNO_8$: 605.5; found: 605.3 $[M]^+$.

Supplementary Material (ESI) for Chemical Communications

Compound 3a. Compound **10** (13 mg, 0.02mmol) was dissolved in 1 mL of a 1:1 mixture of TFA and DCM, stirred for 7 min and the solvents were then removed under reduced pressure. The obtained compound **3a**, as a white solid, was used without further purifications.

^{1}H NMR (400MHz, DMSO-d^6): δ = 9.73 (1H, s), 8.13 (1H, d, *J* = 1.3 Hz), 7.78 (1H, d, *J* = 15.8 Hz), 7.71 (1H, dd, *J* = 8.4 Hz, 1.3 Hz), 7.70 (2H, d, *J* = 7.8 Hz), 7.58 (1H, d, *J* = 16.0 Hz), 7.49 (1H, d, *J* = 8.4 Hz), 7.42 (2H, d, *J* = 7.8 Hz), 6.65 (1H, d, *J* = 15.8 Hz), 6.64 (1H, d, *J* = 16.0 Hz), 5.19 (2H, s), 1.29 (12H, s). ^{13}C NMR (100MHz , DMSO-d^6): δ = 167.7, 167.6, 154.3, 142.9, 140.7, 139.9, 138.9, 138.2, 134.6, 131.4, 129.8, 128.6, 127.0, 125.8, 120.8, 119.5, 116.7, 83.7, 65.8, 24.7. MS (FAB-): m/z calc. for $C_{26}H_{28}BNO_8$: 493.3; found: 492 $[M-H]^-$.



Supplementary Material (ESI) for Chemical Communications

Compound 13. Toluene was heated to reflux (110ºC) under Ar atmosphere and a solution of 20% phosgene in toluene (9.8 mL, 19.0 mmol) was added. Then, a solution of compound **12**[3] (1 g, 1.9 mmol) in toluene was slowly added dropwise with a syringe. The reaction mixture was stirred for 30 min at reflux and monitored by ^{1}H-NMR. After isocyanate derivative **12a** was observed, the solvent was removed under reduced pressure. A solution of compound **11**[2] (489 mg, 2.1 mmol) in toluene, followed by DBTL (40 μL, 0.065mmol), were added to the isocyanate residue. The reaction mixture was heated to reflux (110ºC), stirred for 1 h under Ar atmosphere and monitored by TLC (EtOAc/Hex 20:80). After completion, the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/Hex 5:95) to give compound **13** (1.13 g, 76%) as a white oil.

^{1}H NMR (400MHz, $CDCl_3$): δ = 7.83 (2H, d, *J* = 7.8 Hz), 7.54 (1H, brs), 7.40 (2H, d, *J* = 7.8 Hz), 7.33 (2H, s), 5.20 (2H, s), 4.73 (2H, s), 4.68 (4H, s), 1.35 (12H, s), 0.95 (9H, s), 0.92 (18H, s), 0.10 (6H, s), 0.08 (12H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 154.5, 141.6, 136.4, 135.3, 126.8, 124.9, 122.7, 84.1, 67.1, 65.2, 63.3, 26.3, 26.1, 25.1, 18.7, 18.5, -4.9, -5.0. MS (FAB): m/z calc. for $C_{41}H_{72}BNO_7Si_3$: 785.4; found: 784.3 $[M-H]^-$.

Compound 14. Compound **13** (150 mg, 0.19 mmol) was dissolved in 1mL of a 1:1 mixture of TFA and DCM, stirred for 15 min and the solvents were then removed under reduced pressure. The crude product was purified by column chromatography on silica gel (MeOH/EtOAc 2:98) to give compound **14** (50 mg, 60%) as a white solid.

^{1}H NMR (400MHz, CD_3OD): δ = 7.70 (2H, d, *J* = 7.8 Hz), 7.50 (1H, brs), 7.38 (2H, s), 7.36 (2H, d, *J* = 7.8 Hz), 5.14 (2H, s), 4.54 (6H, s), 3.25 (3H, s), 1.29 (12H, s). ^{13}C NMR (100MHz, CD_3OD): δ = 157.3, 141.8, 141.4, 139.8, 135.9, 131.9, 127.8, 126.3, 125.5, 85.2, 67.8, 64.9, 61.6, 25.2. MS (FAB): m/z calc. for $C_{23}H_{30}BNO_7$: 443.2; found: 442.2 $[M-H]^-$.

Supplementary Material (ESI) for Chemical Communications

Compound 15. Compound **15** was synthesized according to the synthesis of Compound **10**, to give Compound **15** (93 mg, 60%) as a white powder.
^{1}H NMR (400MHz, $CDCl_3$): δ = 7.89-7.69 (10H, m), 7.61-7.48 (10H, m), 7.42-7.32 (5H, m), 6.35-6.25 (6H, m), 5.30 (2H, s), 5.28 (2H, s), 5.21 (4H, s), 1.52 (36H, s), 1.45 (18H, s), 1.28 (12H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 166.5, 157.6, 157.3, 151.4, 150.0, 147.9, 145.2, 144.2, 143.7, 138.2, 133.3, 130.3, 130.0, 128.9, 128.5, 127.1, 121.2, 119.9, 119.0, 115.1, 102.0, 84.2, 80.9, 77.0, 68.3, 66.3, 30.1, 28.5, 25.2.
MS (FAB): m/z calc. for $C_{86}H_{105}BN_4O_{22}Na$: 1580.5; found: 1580.3 $[M+Na]^+$.

Compound 3. Compound **15** (16 mg, 0.01 mmol) was dissolved in 1mL of a 1:1 mixture of TFA and DCM, stirred for 13 min and the solvents were then removed under reduced pressure. The obtained compound **3**, as a white solid, was used without further purifications.
^{1}H NMR (400MHz, DMSO-d^6): δ = 12.37 (6H, brs), 8.07 (2H, s), 7.78-7.70 (4H, m), 7.63-7.45 (16H, m), 7.40-7.33 (3H, m), 6.64-6.53 (6H, m), 5.17 (4H, s), 5.14 (4H, s), 1.24 (12H, s). ^{13}C NMR (100MHz, DMSO-d^6): δ = 168.5, 154.9, 143.8, 139.7, 139.1, 135.4, 131.9, 128.9, 127.8, 127.5, 121.6, 120.2, 84.5, 63.4, 29.8,22.9.

Assay conditions for detection of H_2O_2 by HPLC. Compound **3** [500 μM] and **3a** [500 μM] were treated with H_2O_2 [500 μM], and the kinetics measured with HPLC (grad. 10%ACN to 90%ACN) at wavelength of 278 nm.

Supplementary Material (ESI) for Chemical Communications

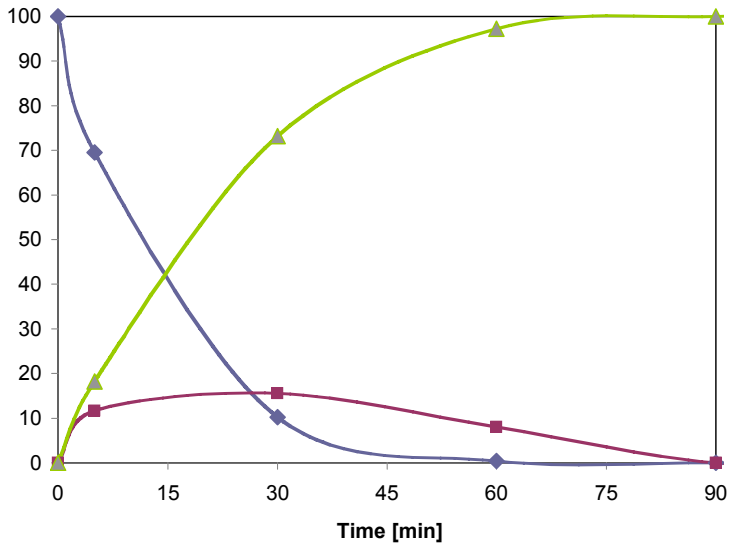


Figure 1. Compound **3** (-◆-) kinetic behavior upon reaction with H_2O_2 to release compound **4** (-▲-) (compound **3** [500 µM] in aqueous $NaHCO_3$ pH=8.3, H_2O_2 [500 µM]), intermediate (-■-)).

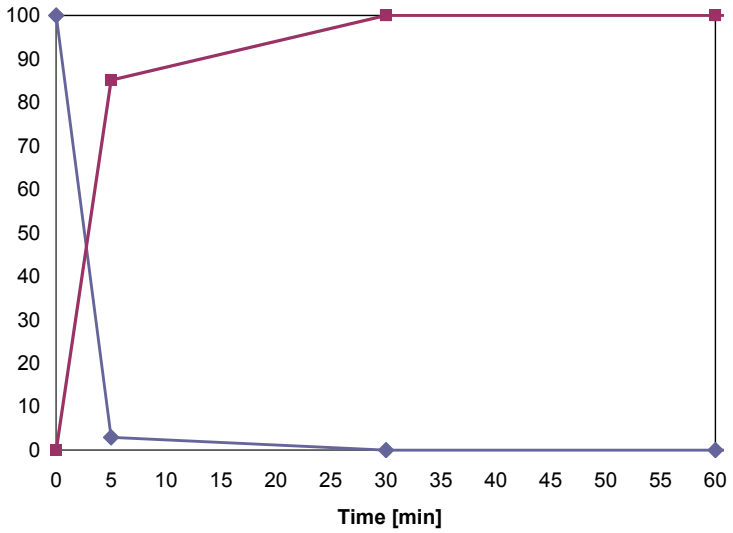


Figure 2. Compound **3a** (-◆-) kinetic behavior upon reaction with H_2O_2 to release compound **4** (-■-) (compound **3a** [500 µM] in aqueous $NaHCO_3$ pH=8.3, H_2O_2 [500 µM]).

Assay conditions for detection of H_2O_2 and TATP by spectrophotometer. Compound **3** [150 µM] and compound **3a** [150 µM] were incubated with various

Supplementary Material (ESI) for Chemical Communications

amounts of H_2O_2, and the fluorescence monitored by with a microplate reader after 90 min (λ_{ex} 270 nm, λ_{em} 560 nm). The same procedure was carried out with various amounts of TATP, and the fluorescence was recorded after 120 min (λ_{ex} 270 nm, λ_{em} 560 nm).

The quantum yield of amine **4** was calculated in comparison to 1-naphtylamine (Φ_F = 0.43[X]) in ethanol. For each compound, the absorbance at λ_{max} and the integrated fluorescence intensity were measured in various concentrations.

For 1-naphtylamine, the absorbance [OD] was measured for λ_{max} = 313 nm and the integrated fluorescence intensity was measured for λ_{ex} = 313 nm at the range of 360-630 nm. For molecule **4**, the absorbance [OD] was measured for λ_{max} = 270 nm and the integrated fluorescence intensity was measured for λ_{ex} = 270 nm at the range of 400-680 nm. The results are plotted in the following Figure 3:

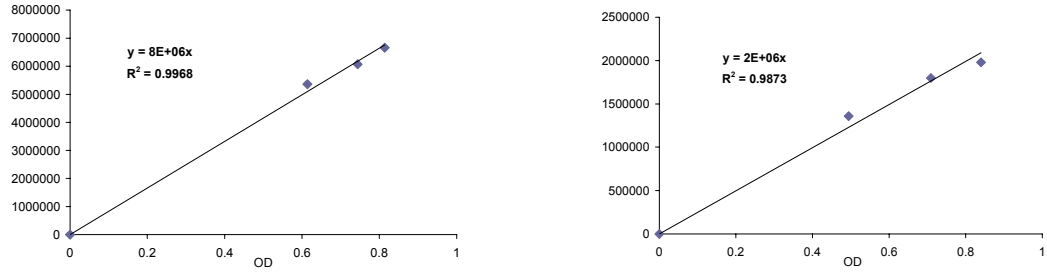


Figure 3. Integrated fluorescence intensity *vs* absorbance at λ_{max} for: 1-naphtylamine (left) and amine **4** (right).

$$\Phi_X = \Phi_{ST} \cdot \left(\frac{Grad_X}{Grad_{ST}} \right) \left(\frac{\eta_X^{\,2}}{\eta_{ST}^{\,2}} \right)$$

Equation 1. The subscript ST and X denote standard and test respectively, Φ is the fluorescence quantum yield, *Grad* the gradient from the plot of integrated fluorescence intensity *vs* absorbance at λ_{max} and η the refractive index of the solvent.

Since ethanol was used as solvent in all measurements, the influence of the refractive index of the solvent was dismissed and the quantum yield was calculated based on the

Supplementary Material (ESI) for Chemical Communications

results presented above using equation 1. The quantum yield found for molecule **4** in ethanol was $\Phi_F = 0.11 \pm 0.01$.

References

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3. A. Sagi, E. Segal, R. Satchi-Fainaro and D. Shabat, *Bioorg. Med. Chem.*, 2007, **15**, 3720-3727.