Supporting Information

Dark to Light! A New Strategy for Large Stokes Shift Dyes: Coupling of Dark Donor with Tunable High Quantum Yield Acceptors

Dongdong Su,[a] Juwon Oh[c], Sung-Chan Lee,[b] Jong Min Lim[c], Srikanta Sahu,[b] Xiaotong Yu[a], Dongho Kim*,[c] and Young-Tae Chang*,[a, b]

[a]Department of Chemistry and MedChem Program, Life Sciences Institute, National University of Singapore, 3 Science Drive 3, Singapore, 117543;

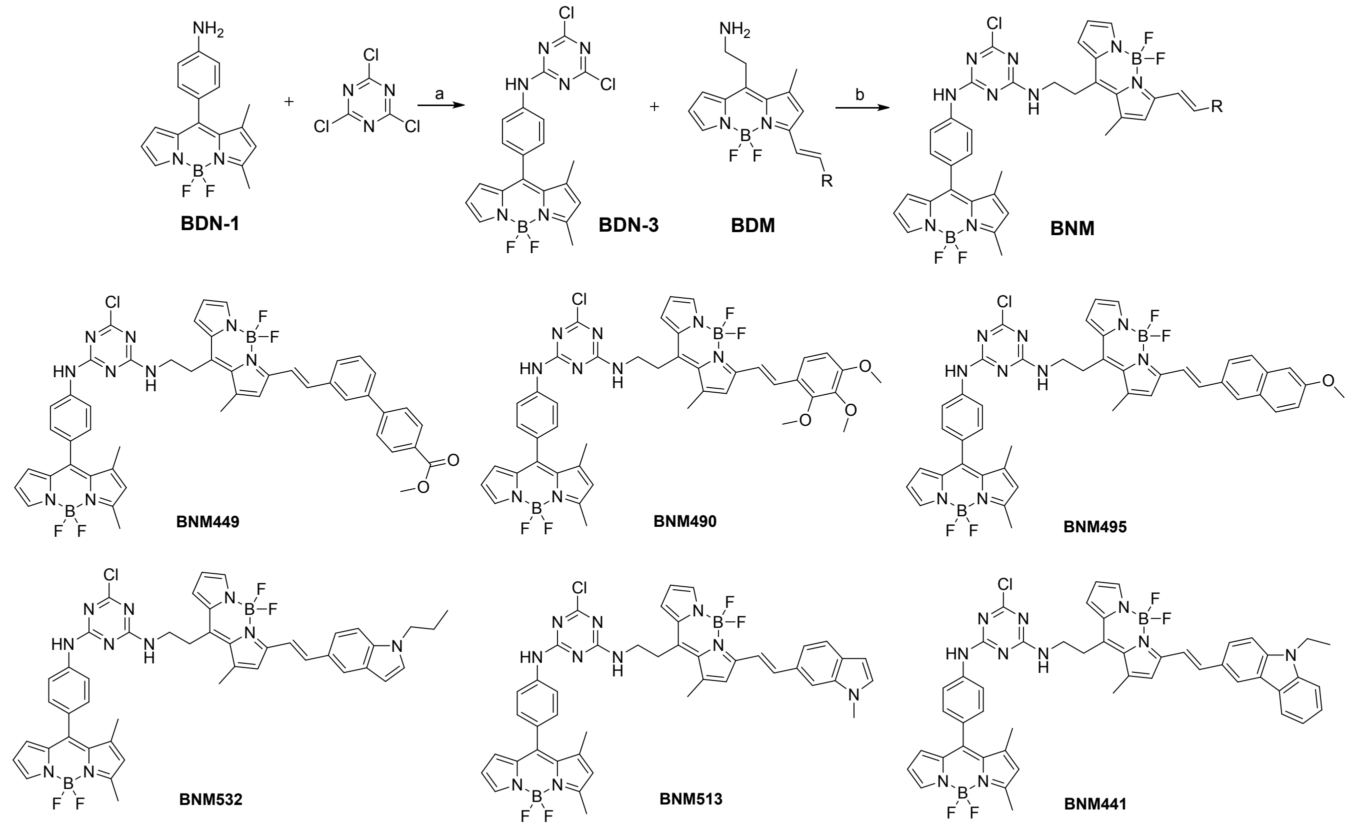
[b]Laboratory of Bioimaging Probe Development, Singapore Bioimaging Consortium (SBIC), 11 Biopolis Way, #02-02 Helios, Agency for Science, Technology and Research (A*STAR), Biopolis, Singapore, 138667;

[c]Department of Chemistry and Spectroscopy of π-Functional Electronic Systems, Yonsei University, Seoul, 120-749, Korea.

List of Contents

List of contents:

Scheme S1. Synthetic scheme and structures of **BNM** compounds[a]



[a]Reagents and conditions: (a) DIEA, THF, 0 ºC., 1 h; (b) DIEA, THF, rt, 2 h.

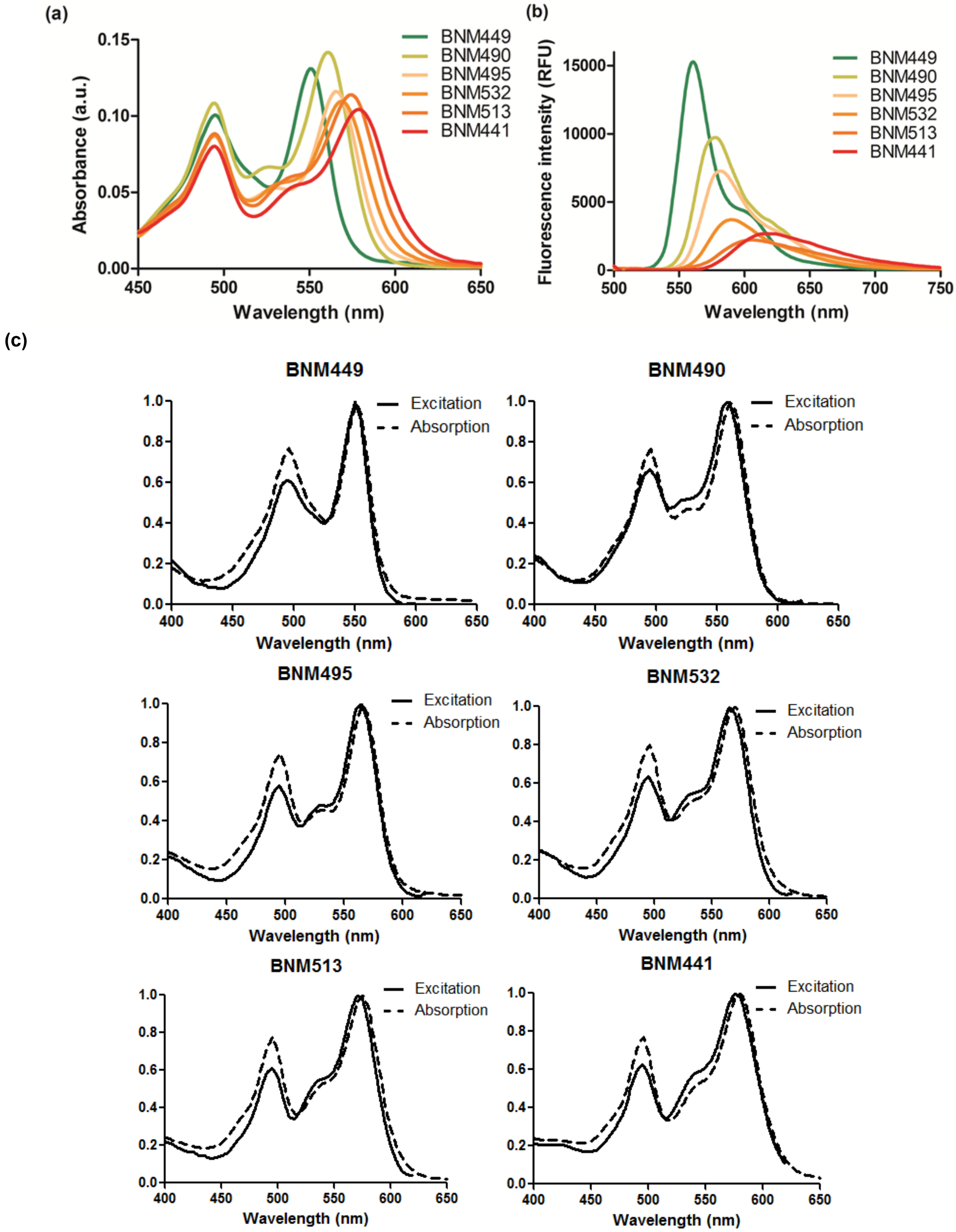


Figure S1. Spectroscopic properties of **BNM** compounds. Absorbance (a), fluorescence spectra (b) and excitation spectra (the emission wavelength was fixed at 680 nm) (c) of the selected **BNM** compounds (10 μM in EtOH, λ_{ex}= 470 nm).

Table S1. Photophysical data of **BNM** compounds.

	λ_{abs} (nm)	Log ε_{max}[a]	λ_{em} (nm)	Φ[b]	$\Delta\lambda$[c]
BNM449	494/549	4.93	560	0.75	66
BNM490	494/554	4.89	570	0.55	76
BNM495	494/563	4.99	580	0.63	86
BNM532	494/566	4.96	590	0.40	96
BNM513	494/572	4.94	603	0.30	109
BNM441	494/577	4.98	617	0.38	123

[a]The maximal absorption of the **BDM** part; [b]Fluorescence quantum yields were measured in EtOH (10 μM, λ_{ex}= 470 nm) using rhodamine B (Φ=0.7 in EtOH) as a standard. [c]Pseudo-Stokes shifts of the selected **BNM** compounds.

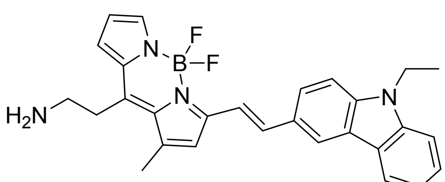
Table S2. The quantum yield of the **BDN** derivatives.

BDN Donor derivatives

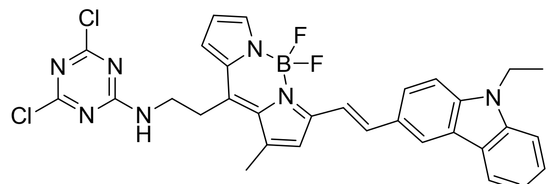
Compound code	**BDN-1**	**BDN-2**	**BDN-3**	**BDN-4**	**BDN-5**	**BNM449**
Quantum yield[a]	0.00084	0.012	0.0085	0.010	0.0036	0.75

[a]Fluorescence quantum yields were measured in EtOH (10 µM, λ_{ex}= 470 nm) using rhodamine B (Φ=0.7 in EtOH) as a standard.

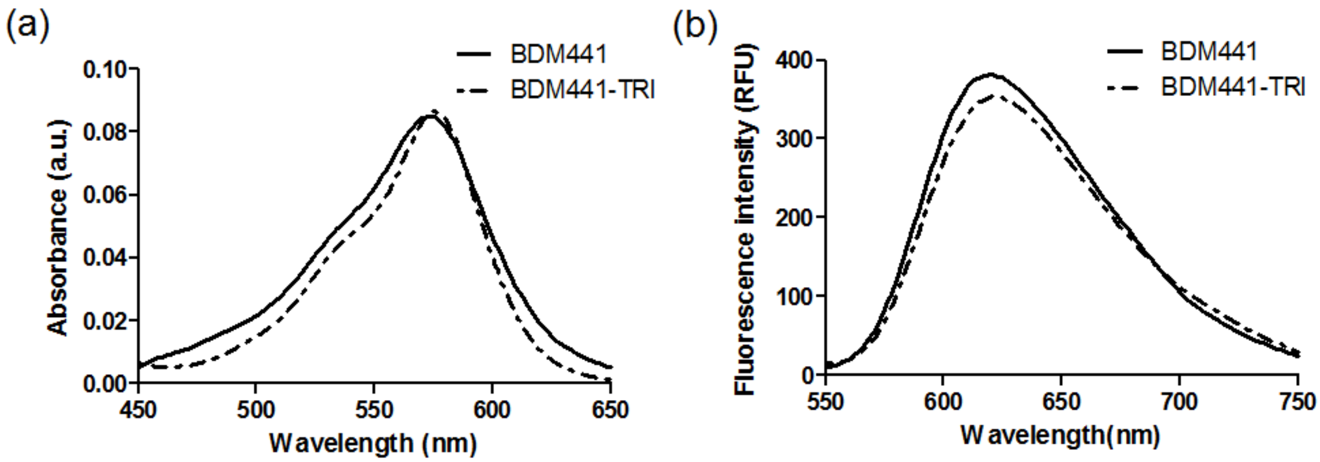
Note: For femtosecond transient absorption measurements and picosecond time-resolved fluorescence measurements, we use **BDM441-Tri** instead of **BDM441** because of its much better stability than free amine version of **BDM441**. The absorption and emission spectra show no difference in the range of 450 nm to 750 nm when tested in EtOH. The structure and spectra can be seen in the following.



BDM441



BDM441-Tri



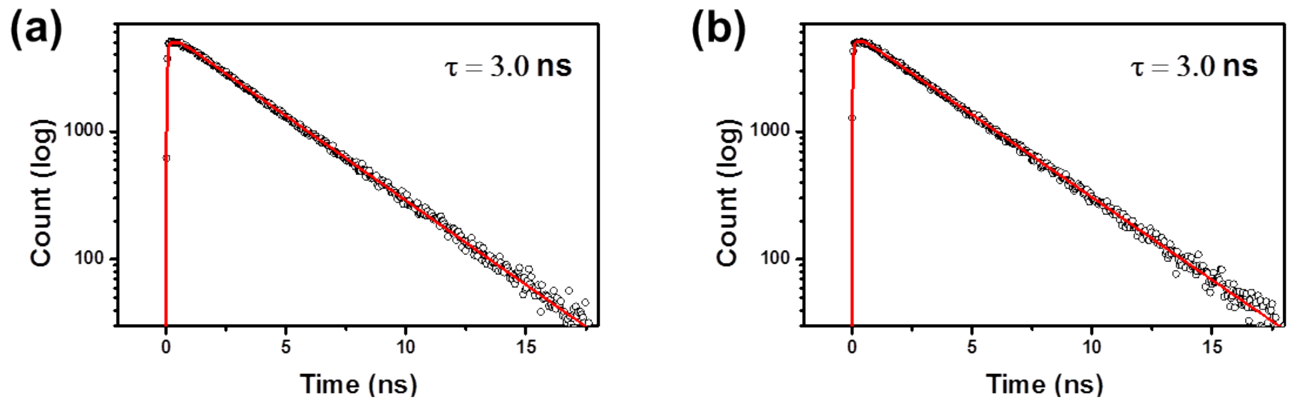


Figure S2. The fluorescence decay profiles of (a) **BDM441-Tri** and (b) **BNM441** in EtOH. The decay profiles are obtained by photoexcitation at 460 nm and monitoring at 650 nm with the time-correlated single photon counting (TSCPC) technique.

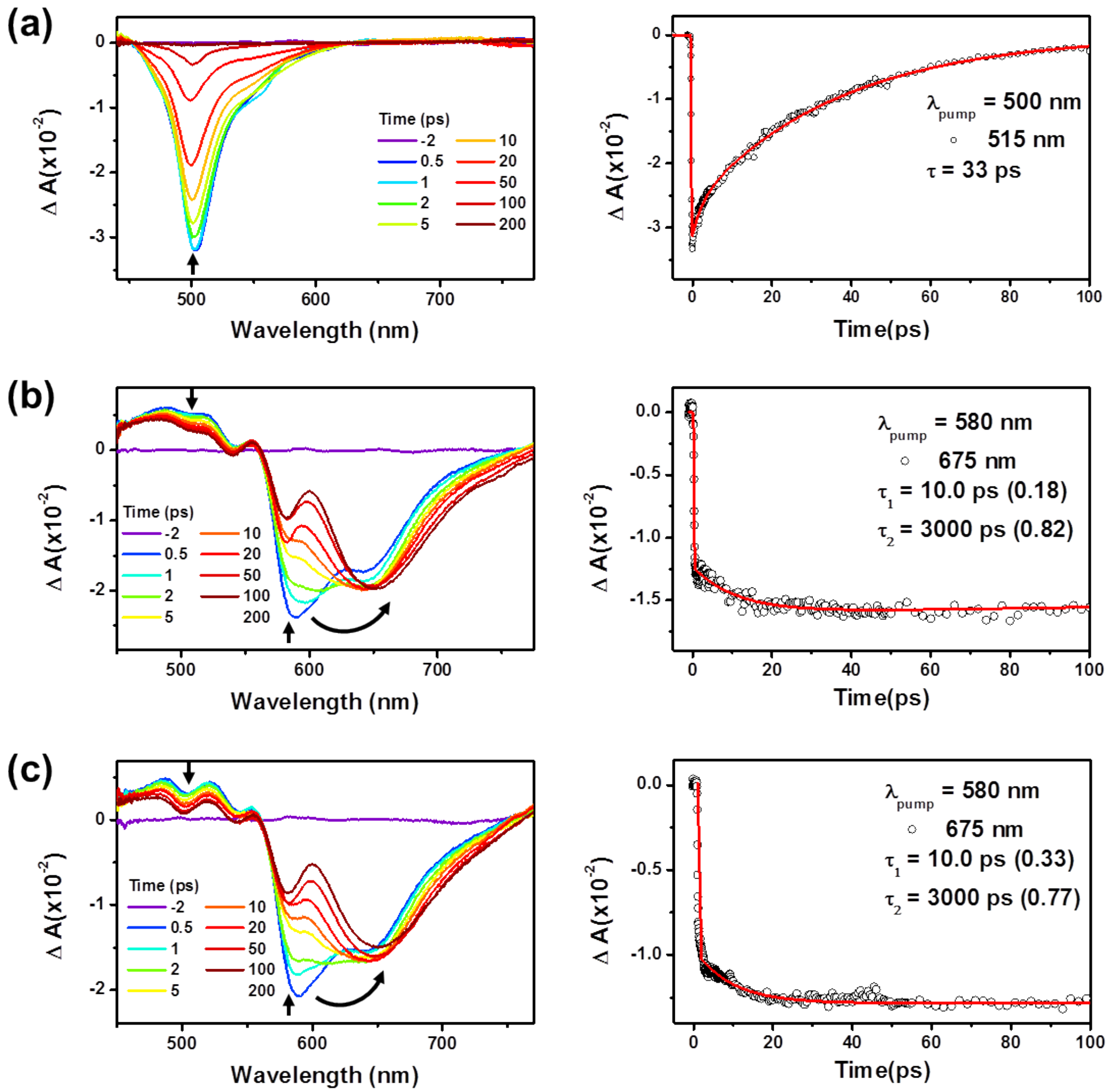


Figure S3. The fs-TA spectra (left) and decay profile (right) of (a) **BDN-3**, (b) **BDM441-Tri** and (c) **BNM441** in EtOH. The 500 nm photoexcitation is employed for **BDN-3** and 580nm for **BDM441-Tri** and **BNM441**. The ns time constants were obtained by the fluorescence lifetime based on the time-correlated single photon counting (TSCPC) technique.

Table S3. List of lifetime of **BDN-3**, **BDM441-Tri** and **BNM441**.

	BDN-3	**BDM441-Tri**	**BNM441**^{a}	**BNM441**^{b}
τ (%)	33 ps	10 ps 3.0 ns	1.2 ps 10 ps 3.0 ns^{c}	10 ps 3.0 ns^{c}

^{a}The time component obtained by the TA decay profile by photoexcitation at 500 nm and monitoring at 515 and 675 nm; ^{b}The time component obtained by the TA decay profile by photoexcitation at 580 nm and monitoring at 675 nm; ^{c}The time component obtained by the fluorescence lifetime based on the time-correlated single photon counting (TSCPC) technique.

Table S4. List of parameters for Förster-type energy transfer rate and efficiency calculation.

	k_F^{a}	J ($\times10^{15}M^{-1}cm^{-1}nm^{4}$)^{b}	κ^{c}	r^{d}(Å)	n^{e}	Eff_{FRET}^{f}
BNM441	1.8×10^{11}~ $5.0\times10^{12}s^{-1}$	1.477	2/3	10 ~ 18	1.36	0.96

^{a}The value obtained by Forster-type energy transfer rate calculation equation $k_F = \frac{8.79\times10^{-5}J\kappa^2\Phi_D}{n^4R(\text{Å})\tau_D}$; ^{b}Spectral overlap value estimated by $J = \int F(\lambda)\varepsilon(\lambda)\lambda^4 d\lambda$; ^{c}Orientation factor which is chosen as randomized value, 2/3, based on non-rigid molecular structure of **BNM441**; ^{d}Maximum and minimum intermolecular distance between donor and acceptor in **BNM441**; ^{e}Refractive index of EtOH; ^{f}RET efficiency is calculated with equation, $Eff_{RET} = k_{RET}/(k_{RET}+k_D)$, where k_D is the excited-state decay rate of donor.

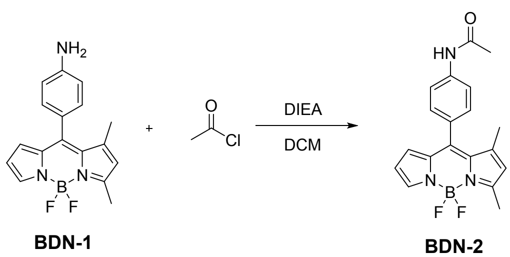
Synthesis and Characterization

Synthesis of the **BDN** derivatives

Synthesis of **BDN-1**: The synthesis of **BDN-1** was following the reported literature.[1]

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.65 (s, 1H), 7.13 (d, J = 8.3 Hz, 2H), 6.75 (d, J = 8.3, 2H), 6.52 (d, J = 3.2 Hz, 1H), 6.37 (d, J = 1.8 Hz, 1H), 6.12 (s, 1H), 2.61 (s, 3H), 1.67 (s, 3H); ^{13}C-NMR (75.5 MHz, $CDCl_3$): 161.00, 147.64, 146.70, 144.44, 138.02, 135.01, 133.69, 130.39,126.92, 123.77, 122.88, 115.74, 114.57, 15.43, 15.03.

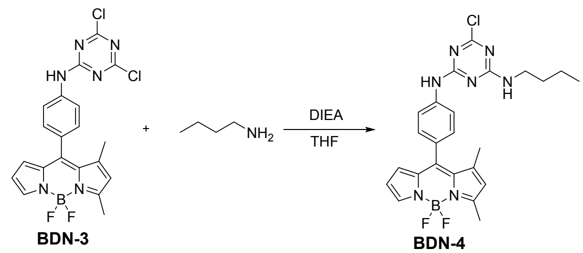
HRMS m/z (C17H16BF2N3) calculated: 311.1405, found: 334.1313 (M+Na).



Synthesis of **BDN-2**: A solution of **BDN-1** (20 mg, 0.064 mmol) and DIEA (12 µL, 0.096 mmol) in CH_2Cl_2 (6 mL) was stirred under N_2 atmosphere at 0 ºC. Acetyl chloride (6 µL, 0.077 mmol) in CH_2Cl_2 (1 mL) was added dropwise and the resulting mixture was stirred at 0 ºC for 1 h. After removal of the CH_2Cl_2, the residue was purified by silica gel chromatography (CH_2Cl_2–MeOH, 95: 5) to give **BDN-2** as a yellow solid (18 mg, 80%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.70 (s, 1H), 7.69 (d, J=8.7 Hz, 2H), 7.34 (d, J=8.5 Hz, 2H), 7.30 (s, 1H), 6.45 (s, 1H), 6.41 (s, 1H), 6.17 (s, 1H), 2.66 (s, 3H), 2.26 (s, 3H), 1.63 (s, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): 161.94, 160.00, 146.85, 139.20, 138.58, 134.71, 129.66, 129.46, 126.95, 123.21, 119.19, 116.01, 24.65, 15.23, 15.11.

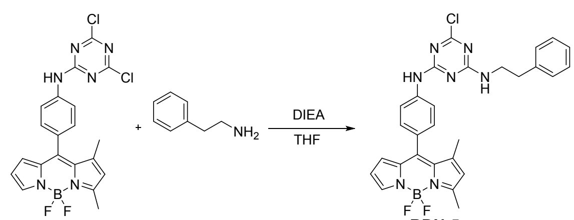
HRMS m/z (C19H18BF2N3O) calculated: 353.1511, found: 376.1418 (M+Na).



Synthesis of **BDN-4**: A solution of **BDN-3** (25 mg, 0.054 mmol) and DIEA (10 µL, 0.081 mmol) in THF (6 mL) was stirred under N_2 atmosphere at rt. 1-Butanamine (8 µL, 0.081 mmol) was added and the resulting mixture was stirred at rt for 2 h. After removal of the THF, the residue was purified by silica gel chromatography (CH_2Cl_2–MeOH, 95: 5) to give BDN-4 as a yellow solid (22 mg, 82%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (d, *J*=8.5 Hz, 2H,), 7.72 (s, 1H), 7.38 (d, *J*=8.5 Hz, 2H), 6.49 (s, 1H), 6.42 (s, 1H), 6.18 (s, 1H), 3.57-3.47 (m, 2H), 2.67 (s, 3H), 1.66 (m, 5H), 1.49-1.41(dd, 2H), 1.03-0.97 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): 163.84, 161.98, 146.72, 142.86, 139.14, 138.67, 134.73, 133.62, 129.66, 129.22, 126.96, 123.25, 119.78, 116.06, 41.15, 31.19, 20.00, 15.27, 15.13, 13.69.

HRMS m/z (C24H25BClF2N7) calculated: 495.1921, found: 518.1837 (M+Na).

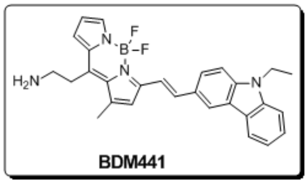


Synthesis of **BDN-5**: Compound **BDN-5** was synthesized as for **BDN-4** and obtained as a yellow solid (61%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (d, *J*=8.5Hz, 2H), 7.74 (s, 1H), 7.41-7.24 (m, 7H), 6.50 (s, 1H), 6.43 (s, 1H), 6.18 (s, 1H), 3.85-3.76 (m, 2H), 2.99 (t, *J*=7.0Hz, 2H), 2.68 (s, 3H), 1.66(s, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): 169.31, 163.81, 162.03, 146.73, 139.06, 138.70, 138.07, 134.73, 129.71, 129.34, 128.81, 128.73, 128.65, 126.98, 126.95, 126.83, 123.26, 119.88, 119.80, 116.11, 42.61, 35.43, 15.28, 15.14.

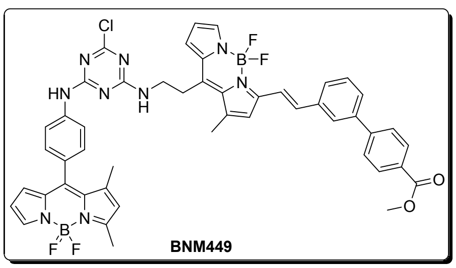
HRMS m/z (C28H25BClF2N7) calculated: 543.1921, found: 542.1876 (M-H).

Synthesis of **BDM441**: The synthesis of **BDM441** was following the reported literature.[2]



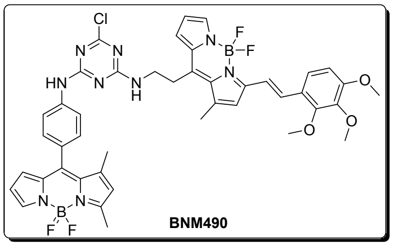
^{1}H NMR (500 MHz, $CDCl_3$-MeOD): δ 8.26 (s, 1H), 8.10 (d, *J*=7.2 Hz, 1H), 7.73 (d, *J*=7.6 Hz, 1H), 7.62 (s, 1H), 7.61 (d, *J*=12.3 Hz, 2H), 7.45 (s, 1H), 7.41-7.36 (m, 2H), 7.32 (s, 1H), 7.25 (s, 1H), 6.87 (s, 1H), 6.48 (s, 1H), 4.50 (s, 2H), 3.39 (s, 2H), 2.52 (s, 3H), 1.41(s, 2H), 1.24(s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$-MeOD): 159.16, 144.20, 142.85, 142.84, 140.95, 140.10, 135.94, 135.12, 133.31, 126.49, 125.94, 125.63, 123.14, 122.44, 121.80, 120.83, 120.20, 120.15, 119.32, 115.37, 114.44, 108.67, 108.58, 40.53, 37.29, 26.45, 15.75, 13.26.

HRMS m/z (C28H27BF2N4) calculated: 468.2297, found: 491.2201 (M+Na).



^{1}H NMR (500 MHz, $CDCl_3$): δ 8.14 (t, *J*=7.8 Hz, 2H), 7.80-7.58 (m, 10H), 7.52-7.40 (m, 2H), 7.33-7.29 (m, 2H), 7.06 (s, 1H), 6.79 (d, *J*=16.9 Hz, 1H), 6.51-6.39 (m, 3H), 6.13 (d, *J*=25.4 Hz, 1H), 3.97 (d, *J*=7.0 Hz, 3H), 3.90 (s, 1H), 3.76 (s, 1H), 3.32 (dd, *J*=14.2, 7.4 Hz, 2H), 2.65-2.52 (m, 6H), 1.60 (d, *J*=20.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 166.92, 166.90, 162.19, 156.74, 146.71, 144.85, 144.73, 144.17, 140.78, 140.69, 139.30, 138.74, 138.65, 138.44, 136.47, 136.35, 135.18, 134.62, 134.55, 134.47, 133.56, 130.18, 129.75, 129.51, 129.25, 128.70, 127.26, 127.10, 127.05, 126.77, 126.66, 124.11, 123.94, 123.36, 120.28, 119.92, 119.79, 119.07, 118.87, 116.40, 116.13, 52.16, 43.10, 42.59, 16.69, 16.53, 15.31.

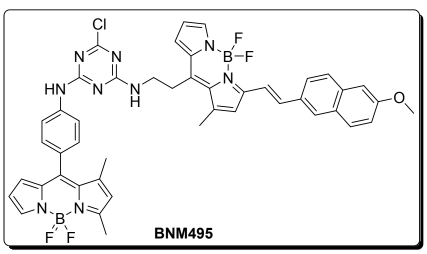
HRMS m/z (C48H40B2ClF4N9O2) calculated: 907.3116, found: 930.3010 (M+Na).



BNM490

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.74-7.70 (m, 3H), 7.67-7.64 (m, 2H), 7.59 (s, 1H), 7.42 (dd, *J*=39.8, 8.9 Hz, 1H), 7.32-7.31 (m, 2H), 7.25 (d, *J*=3.8 Hz, 1H), 7.02 (s, 1H), 6.81 (d, *J*=18.8 Hz, 1H), 6.70 (dd, *J*=27.4, 8.9 Hz, 1H), 6.48-6.39 (m, 3H), 6.14 (d, *J*=10.8 Hz, 1H), 3.98-3.88 (m, 10H), 3.77 (dd, *J*=13.5, 6.5 Hz, 1H), 3.31 (dd, *J*=14.2, 7.3 Hz, 2H), 2.64-2.51 (m, 6H), 1.61 (d, *J*=13.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 165.08, 163.37, 162.11, 158.56, 158.36, 155.52, 155.49, 152.85, 146.74, 144.35, 143.71, 142.67, 142.27, 138.64, 137.04, 135.41, 135.11, 134.91, 134.62, 134.15, 133.54, 129.71, 129.67, 126.97, 123.33, 122.84, 122.70, 120.24, 117.32, 116.10, 115.76, 107.87, 61.57, 60.89, 56.09, 43.08, 42.64, 16.64, 16.52, 15.30.

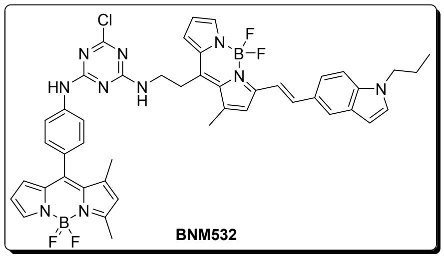
HRMS m/z (C43H40B2ClF4N9O3) calculated: 863.3065, found: 886.2998 (M+Na).



BNM495

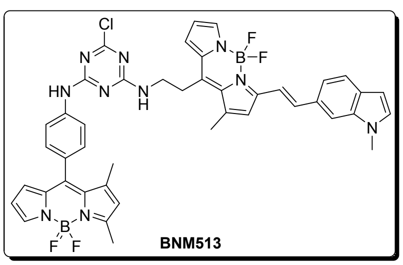
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.87 (d, *J*=29.2 Hz, 1H),7.80-7.64 (m, 7H), 7.50 (d, *J*=16.1 Hz, 1H), 7.35 (d, *J*=8.2 Hz, 1H), 7.32 (d, *J*=8.3 Hz, 1H), 7.27 (d, *J*=3.7 Hz, 1H), 7.20-7.13 (m, 2H), 7.05 (s, 1H), 6.81 (s, 1H), 6.51-6.37 (m, 3H), 6.11 (d, *J*=48.0 Hz, 1H), 3.96 (d, *J*=8.6 Hz, 3H), 3.90 (d, *J*=6.6 Hz, 1H), 3.80 (dd, *J*=12.6, 6.2 Hz, 1H), 3.32 (dd, *J*=14.9, 7.8 Hz, 2H), 2.65-2.52 (m, 6H), 1.60 (d, *J*=32.8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 162.13, 158.90, 158.88, 146.73, 144.16, 140.55, 140.39, 138.74, 138.63, 138.59, 137.64, 135.61, 134.34, 134.32, 133.60, 131.35, 131.23, 130.18, 130.14, 130.03, 129.78, 129.73, 129.15, 128.83, 127.57, 124.52, 124.38, 123.37, 123.34, 123.31, 120.18, 120.13, 120.10, 119.96, 119.47, 116.13, 116.09, 116.05, 106.13, 55.40, 43.29, 31.90, 16.75, 16.61, 15.33.

HRMS m/z (C45H38B2ClF4N9O) calculated: 853.3010, found: 876.2903 (M+Na).



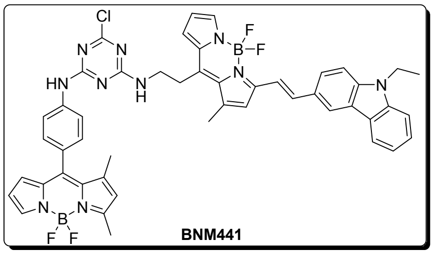
[1]H NMR (500 MHz, $CDCl_3$): δ 7.85 (d, *J*=23.8 Hz, 1H), 7.73-7.46 (m, 7H), 7.37-7.30 (m, 3H), 7.20 (d, *J*=3.9 Hz, 1H), 7.13 (dd, *J*=10.0, 3.1 Hz, 1H), 6.81 (d, *J*=9.1 Hz, 1H), 6.54 (dd, *J*=10.2, 3.0 Hz, 1H), 6.46-6.38 (m, 3H), 6.12 (d, *J*=34.9 Hz, 1H), 4.13-4.07 (m, 2H), 3.91 (s, 1H), 3.80 (s, 1H), 3.31 (dd, *J*=16.0, 8.1 Hz, 2H), 3.65-2.51 (m, 6H), 1.91-1.86 (m, 2H), 1.60 (d, *J*=27.8 Hz, 3H), 0.96 (q, *J*=7.5 Hz, 3H); [13]C NMR (125 MHz, $CDCl_3$): 162.75, 162.17, 158.78, 146.75, 144.26, 143.11, 138.81, 138.64, 137.33, 136.48, 135.54, 135.43, 134.63, 134.00, 133.58, 130.02, 129.77, 129.72, 129.15, 128.98, 127.43, 127.33, 127.00, 126.88, 123.37, 122.62, 121.32, 120.55, 120.25, 120.12, 116.16, 116.12, 115.57, 115.50, 110.10, 102.25, 102.22, 48.25, 43.09, 31.92, 23.60, 22.67, 16.59, 14.09, 11.46.

HRMS m/z (C45H41B2ClF4N10) calculated: 854.3326, found: 877.3241(M+Na).



[1]H NMR (500 MHz, $CDCl_3$): δ 7.71-7.29 (m, 12H), 7.11 (dd, *J*=29.4, 2.9 Hz, 1H), 6.94 (s, 1H), 6.71 (d, *J*=6.2 Hz, 1H), 6.50-6.39 (m, 3H), 6.12 (d, *J*=34.1 Hz, 1H), 3.88 (s, 1H), 3.80 (d, *J*=28.1 Hz, 3H), 3.68 (s, 1H), 3.22 (s, 2H), 2.62 (d, *J*=29.3 Hz, 3H), 2.47 (d, *J*=17.6 Hz, 3H), 1.61 (d, *J*=21.5 Hz, 3H); [13]C NMR (125 MHz, $CDCl_3$): 165.90, 163.95, 162.00, 158.31, 146.74, 144.16, 142.86, 142.60, 142.34, 138.94, 138.86, 138.63, 138.50, 138.39, 136.95, 134.70, 134.62, 134.13, 133.58, 131.45, 131.41, 130.49, 129.73, 129.62, 129.35, 126.94, 126.81, 123.37, 123.28, 122.20, 121.19, 120.15, 120.03, 119.91, 116.04, 115.56, 101.61, 43.00, 32.98, 31.87, 22.65, 16.64, 16.51.

HRMS m/z (C43H37B2ClF4N10) calculated: 826.3013, found: 849.2920 (M+Na).



BNM441

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.27 (d, *J*=25.7 Hz, 1H), 8.15 (dd, *J*=11.8, 7.8 Hz, 1H), 7.77-7.65 (m, 5H), 7.59-7.40 (m, 4H), 7.35-7.30 (m, 3H), 7.21 (s, 1H), 6.97 (s, 1H), 6.76 (d, *J*=32.1 Hz, 1H), 6.48-6.37 (m, 3H), 6.11 (d, *J*=45.8 Hz, 1H), 4.42-4.32 (m, 2H), 3.91 (s, 1H), 3.73 (s, 1H), 3.27 (dd, *J*=13.5, 6.8 Hz, 2H), 2.62 (d, *J*=23.7 Hz, 3H), 2.50 (s, 3H), 1.60 (d, *J*=27.3 Hz, 3H), 1.30-1.27 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 163.27, 162.16, 158.49, 146.72, 144.16, 142.35, 141.21, 140.45, 138.74, 138.59, 136.41, 135.45, 134.64, 134.18, 134.04, 133.56, 129.74, 129.70, 126.97, 126.83, 126.30, 126.01, 125.96, 123.53, 123.50, 123.33, 122.87, 121.12, 120.74, 120.19, 119.74, 116.13, 116.06, 115.56, 115.47, 115.35, 115.09, 109.03, 109.00, 108.89, 108.86, 60.37, 43.00, 37.77, 29.67, 16.72, 16.53, 15.31, 13.83.

HRMS m/z (C48H41B2ClF4N10) calculated: 890.3326, found: 913.3206 (M+Na).

References

1. N. Y. Kang, S. C. Lee, S. J. Park, H. H. Ha, S. W. Yun, E. Kostromina, N. Gustavsson, Y. Ali, Y. Chandran, H. S. Chun, M. Bae, J. H. Ahn, W. Han, G. K. Radda and Y. T. Chang, *Angew. Chem. Int. Edit.*, 2013, **52**, 8557-8560.
2. M. Vendrell, G. G. Krishna, K. K. Ghosh, D. T. Zhai, J. S. Lee, Q. Zhu, Y. H. Yau, S. G. Shochat, H. Kim, J. Chung and Y. T. Chang, *Chem. Commun.*, 2011, **47**, 8424-8426.