Supporting Information

Development of Photostable Near-Infrared Cyanine Dyes

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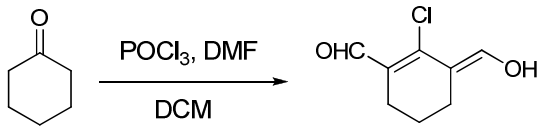
Synthetic Materials and Methods

All the chemicals (building block amines plus others) and solvents were purchased from Sigma Aldrich, Alfa Aesar, Fluka, MERCK or Acros, and used without further purification. ICG-sulfo-OSu was purchased from Dojindo Laboratories. EGFR-IgG_{2a} (sc-120) were supplied by Santa Cruz Biotechnology, Inc. Normal phase purifications were carried out using Merck Silica Gel 60 (particle size: 0.040-0.063mm, 230-400 mesh). Analytical characterization was performed on a HPLC-MS (Agilent-1200 series) with a DAD detector and a single quadrupole mass spectrometer (6130 series) with an ESI probe. Analytical method, unless indicated: eluents: A: H_2O (0.1% HCOOH), B: ACN (0.1% HCOOH), gradient from 5 to 95%B in 6 min; C_{18}(2) Luna column (4.6 x 50mm^2, 5μm particle size). Normal phase purifications of **CyN** and **CyNA** compounds were performed using a 10mL-column, and eluting with DCM-MeOH (ranging from 100:0 to 97:3). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker Avance 300 NMR and 500 NMR spectrometers, and chemical shifts are expressed in parts per million (ppm). High resolution mass spectrometry (HRMS) data was recorded on a Micromass VG 7035 (Mass Spectrometry Laboratory at National University of Singapore (NUS)). Photobleaching irradiation experiments were performed using a UVP Blak-Ray® B-100AP high intensity UV lamp (100W, 365 nm). Spectroscopic and quantum yield data were measured on a SpectraMax M2 spectrophotometer (Molecular Devices), and the data analysis was performed using GraphPrism 5.0.

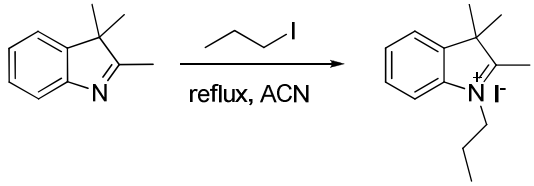
1. Synthesis of 1a



1a

To a chilled solution of dimethylformamide (20 mL, 273 mmol, 5.4 eq.) in 20 mL CH_2Cl_2 under N_2 atmosphere, 20 mL of $POCl_3$ (17.5 ml, 115 mmol, 2.3 eq.) in DCM were added dropwise under an ice bath. After 30 min, cyclohexanone was added (5 g, 50mmol, 1 eq.), and the resulting mixture was refluxed with vigorous stirring for 3 h at 80°C, poured into ice-cold water, and kept it overnight to obtain **1a** as a yellow solid (8.0 g, 92%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.57 (m, 2H), 2.35 (t, 4H, *J*=6.3 Hz), 2.5 (s, 1H), 10.10 (s, 1H). tR: 4.30 min, ESI *m/z* (C8H9ClO2): calc: 172.0; found: 173.1.

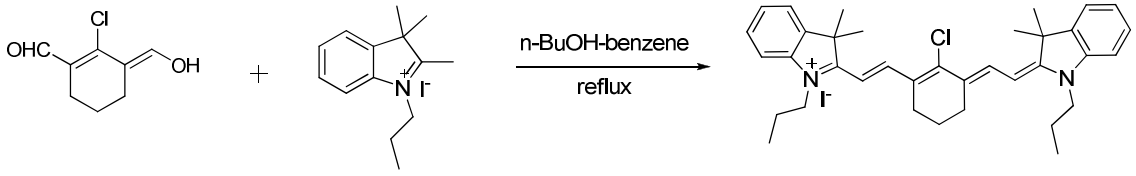
Synthesis of 1b



1b

To a solution of 2,3,3-trimethyl-3*H*-indole (2 g, 12.5 mmol, 1 eq.) in ACN, 1-iodopropane (10.6 mL, 62 mmol, 5 eq.) was added, and refluxed with continuous stirring for 15 h. The mixture was dried in high vacuum and washed by Et_2O. The resulting solid was recrystallized in acetone to obtain **1b** a white solid (3.9 g, 95%). ^{1}HNMR (300 MHz, DMSO-d_6): δ =1.04 (t, 3H, J=7.2), 1.64 (s, 6H), 2.67 (s, 3H), 1.34 (m, 2H), 4.17 (t, 2H, *J*=7.8 Hz), 7.63 (d, 2H), 7.82 (m, 2H). tR: 2.46 min, ESI *m/z* ($C14H20N^+$) calc: 202.4; found: 202.1.

Synthesis of 1



1a **1b** **1**

1a (500 mg, 2.9 mmol, 1eq.) and **1b** (1.91g, 5.81 mmol, 2 eq.) were dissolved in BuOH-benzene (7:3) under N_2 atmosphere, and refluxed at 160°C for 10 h with a Dean-Stark condenser. Afterwards, the

solvent was evaporated, and the resulting green solid mixture was washed with Et_2O and purified by flash chromatography (DCM-MeOH, 50:1) to obtain **1** as a green solid (1.8 g, 96%). ^{1}HNMR (300 MHz, $CDCl_3$) δ=1.06 (t, 6H, *J*=7.5 Hz), 1.31 (m, 4H), 1.64 (s, 12H), 1.95 (m, 2H), 2.73 (m, 4H), 4.15 (t, 4H, *J*=6.9Hz), 6.23 (d, 2H, *J*=14.2 Hz), 7.15-7.72 (m, 8H), 8.19 (d, 2H, *J*=13.8 Hz). tR: 5.64 min, ESI *m/z* ($C36H44ClN2^+$), calc: 539.4; found: 539.1.

2. General procedure for the synthesis of CyN-111, 165, 272, 295

1 (100 mg, 149.5 μmol) was mixed with the corresponding amine building blocks (Chart 1, 598 μmol, 4 eq.), DIEA (38.6 μL, 299 μmol, 2 eq.), and dissolved in ACN. The reaction mixture was heated at 80°C for 20 min, quenched with 0.1 N HCl, concentrated under vacuum, and purified by normal-phase chromatography (DCM-MeOH, 98:2) to render the corresponding **CyN** derivatives.

Characterization of CyN-111, 165, 272, 295

CyN-111 (95 mg, 85%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ=1.03 (t, 6H, *J*=7.5Hz), 1.31 (*m*, 2H), 1.62 (s, 12H), 1.83 (m, 4H), 2.47 (t, 4H, *J*=6.3Hz), 3.38 (t, 2H, *J*=5.7Hz), 3.80 (t, 2H, *J*=7.2Hz), 4.17 (t, 4H, *J*=6.2Hz), 5.63 (d, 2H, *J*= 12.9Hz), 6.85-7.72 (m, 12H), 8.52 (d, 2H, *J*=3.9Hz).

tR: 5.56 min, HRMS ($C_{43}H_{53}N_4^+$), calc: 625.4257; found: 625.4265.

CyN-165 (86 mg, 80%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ=1.05 (t, 6H, *J*=7.5Hz), 1.24 (m, 2H), 1.32 (m, 2H), 1.68 (s, 12H), 1.85 (m, 4H), 2.16 (t, 2H, *J*=5.4 Hz), 2.47 (t, 4H, *J*=6.3 Hz), 3.44 (s, 3H), 3.71 (t, 2H, *J*=5.4Hz), 3.95 (t, 4H, *J*=6.3Hz), 5.61 (d, 2H, *J*=12.9Hz), 6.50-7.72 (m, 8H), 7.64 (d, 2H, *J*=12.9Hz), 7.92 (bs, 1H).

tR: 5.66 min, HRMS ($C_{40}H_{54}N_3O^+$), calc: 592.4255; found: 592.4261.

CyN-272 (93 mg, 83%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ=1.08 (t, 6H, *J*=7.5Hz), 1.31 (m, 2H), 1.45 (m, 4H), 1.54 (m, 2H), 1.68 (s, 12H), 1.85 (m, 4H), 2.51 (t, 4H, *J*=6.3 Hz), 3.44 (t, 6H, *J*=7.7 Hz), 3.81 (t, 4H, *J*=6.9Hz), 4.21 (m, 2H), 5.65 (d, 2H, *J*=12.9Hz), 6.80-7.70 (m, 8H), 7.72 (d, 2H, *J*=12.9Hz).

tR: 5.62 min, HRMS ($C_{43}H_{59}N_4^+$), calc: 631.4740; found: 631.4734.

CyN-295 (88 mg, 87%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ=1.05 (t, 6H, *J*=7.5Hz), 1.56 (t, 3H, *J*= 7.0 Hz), 1.68 (s, 12H), 1.85 (m, 4H), 1.83 (m, 2H), 2.47 (t, 4H, *J*=6.3 Hz), 3.79 (t, 4H, *J*=6.9Hz), 3.96 (m, 2H), 5.62 (d, 2H, *J*=12.9Hz), 6.80-7.28 (m, 8H), 7.72 (d, 2H, *J*=12.5Hz).

tR: 5.66 min, HRMS ($C_{38}H_{50}N_3^+$), calc: 548.3999; found: 548.3999.

CyN-414 (82 mg, 70%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ=1.03 (t, 6H, *J*=7.5Hz), 1.24 (d, 3H, *J*=6.6 Hz), 1.32 (m, 4H), 1.36 (t, 2H, *J*=5.4 Hz), 1.70 (s, 12H), 1.78-1.85 (m, 11H), 2.02-2.16 (m, 4H), 2.47 (t, 4H, *J*=5.4 Hz), 3.76 (t, 2H, *J*=6.0 Hz), 3.93 (t, 2H, *J*=6.0 Hz), 5.58 (d, 2H, *J*=14.1Hz), 6.83 (d, 2H, *J*=14.1Hz), 6.83-7.72 (m, 8H).

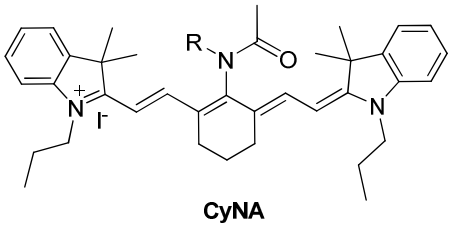
tR: 5.97 min, ESI-MS ($C_{45}H_{63}N_4^+$), calc: 659.5; found: 659.4.

Supplementary Table 1. Absorbance (λ_{abs}) and fluorescence maximum wavelengths (λ_{em}), quantum yields, and extinction coefficients of **CyN-111**, **CyN-165**, **CyN-272**, and **CyN-295**.

compound	λ_{abs}(nm)	λ_{em}(nm)	ϕ*	$\varepsilon(cm^{-1}M^{-1})$
CyN-111	640	760	0.42	$0.538 \cdot 10^5$
CyN-165	640	762	0.37	$0.352 \cdot 10^5$
CyN-272	635	765	0.41	$0.516 \cdot 10^5$
CyN-295	640	760	0.35	$0.519 \cdot 10^5$

* Quantum yields were measured in DMSO, using Cardiogreen as a standard (ϕ : 0.13, in DMSO).[1]

3. General procedure for the CyNA library synthesis



CyNA

For every reaction, **1** (20 mg, 30 μmol, 1 eq.) and the primary amine building block (**Chart S1**) (120 μmol, 4 eq.) were dissolved in ACN, and *N,N*-diisopropylethylamine (DIEA) (7.7 μL, 60 μmol, 2 eq.) was added. The reaction mixture was heated at 80°C for 10-60 min, depending on the reactivity of the amine. The resulting blue color crude mixtures (**CyN**) were neutralized with 0.1 N HCl, and concentrated under vacuum. Resulting **CyN** crudes were dissolved in DCM under N_2 atmosphere, and treated with excess DIEA (96.2 μL, 750 μmol, 25 eq.) and acetyl chloride (11.7 μL, 150 μmol, 5 eq.) at 0°C for 15 min. The final green products were washed with 0.1 N HCl to remove the excess of DIEA, concentrated under vacuum, and purified by a normal-phase silica short column using DCM-MeOH (ranging from 100:0 to 97:3) as the eluting solvent. The characterization of the whole library was performed by HPLC-MS (Table S2), and the compounds studied in detail were also characterized by ^{1}H-NMR and HRMS.

Supplementary Table 2. Absorbance (λ_{abs}) and fluorescence maximum wavelengths (λ_{emis}), quantum yields, LC-MS data, condensation reaction times, and photostability primary evaluation for the whole **CyNA** library.

compound	tR (min)	M^+(calc.)	M^+ (exp.)	λ_{abs}(nm)	λ_{em}(nm)	ϕ*[1]	purity*[2]	reaction time (min)	max RFU	F/F_o*[3]
CyNA-11	2.61	687.4	687.1	802	820	0.11	95.3	20	1250.1	90
CyNA-28	3.42	758.6	758.2	804	818	0.05	93.2	20	23.9	87
CyNA-48*[4]	2.77	783.4	783.0	804	819	0.06	94.6	15	620.9	45
CyNA-49	2.82	696.4	696.1	804	818	0.10	95.3	30	851.3	95
CyNA-55	3.24	744.5	744.2	804	819	0.13	97.1	15	162.3	100
CyNA-80	3.00	688.4	688.2	804	817	0.08	96.1	50	734.9	93
CyNA-92	2.82	646.4	646.1	805	821	0.09	96.3	15	420.2	64
CyNA-95	2.8	604.4	604.2	806	818	0.09	95.6	10	1027.9	91
CyNA-100	2.94	756.4	756.1	804	819	0.08	94.4	15	333.8	63
CyNA-101	2.94	666.4	666.1	806	822	0.08	94.6	25	693.9	86
CyNA-102	2.90	696.4	696.1	805	822	0.14	97.1	15	998.9	63
CyNA-103	2.88	696.4	696.2	804	821	0.14	96.9	20	1106.6	92
CyNA-107	2.35	735.5	735.1	803	820	0.07	93.4	30	275.3	50
CyNA-111	2.68	667.4	667.1	804	818	0.11	98.2	25	565.6	96
CyNA-124	2.86	712.4	712.1	803	820	0.15	98.1	30	1334.2	100
CyNA-131	2.93	694.4	694.1	804	819	0.13	97.3	15	259.8	n.d
CyNA-165	2.78	634.4	634.1	806	821	0.09	98.1	15	775.1	94
CyNA-167	2.82	648.4	648.1	804	818	0.10	94.8	25	1031.8	92
CyNA-180	2.89	630.4	630.1	805	821	0.14	97.5	10	991.3	91
CyNA-181	2.8	712.4	712.1	805	821	0.14	96.5	30	996.8	66
CyNA-185	2.95	632.4	632.1	804	820	0.15	97.4	30	1209.7	91
CyNA-193	3.16	732.5	732.2	806	822	0.12	96.2	15	58.7	n.d
CyNA-201	3.00	694.4	694.1	803	820	0.08	93.2	15	52.6	33
CyNA-218	2.89	670.4	670.1	802	823	0.08	94.2	20	549.9	85
CyNA-219	3.01	672.4	672.1	804	822	0.14	96.4	45	902.7	65
CyNA-220	2.89	686.3	686.1	804	823	0.12	95.3	50	848.2	100
CyNA-221	2.94	687.4	687.1	803	820	0.07	93.2	45	382.6	86
CyNA-230	3.10	674.5	674.2	805	822	0.09	94.3	25	77.7	80
CyNA-262	2.88	712.4	712.1	803	822	0.10	95.1	20	816.5	81
CyNA-266	2.81	726.4	726.1	804	823	0.08	93.5	20	541.8	99
CyNA-272	2.31	673.4	673.1	804	820	0.10	93.8	25	662.1	98
CyNA-274	2.89	662.4	662.1	805	820	0.12	95.6	10	1209.5	99
CyNA-275	2.97	694.4	694.1	804	817	0.14	96.8	45	679.8	62
CyNA-277	3.09	660.4	660.1	804	817	0.11	93.7	15	120.8	n.d
CyNA-282	2.31	675.4	675.1	805	819	0.09	93.5	20	820.1	90
CyNA-295	2.85	590.4	590.1	806	820	0.11	96.4	15	698.6	100
CyNA-319	2.80	712.4	712.1	805	820	0.06	92.1	25	578.6	67
CyNA-329	2.97	732.3	731.9	806	821	0.10	94.5	60	348.8	99
CyNA-335	2.93	680.4	680.1	804	821	0.06	93.2	15	167.9	50
CyNA-336	2.82	742.4	742.1	804	820	0.11	94.5	25	827.7	85
CyNA-341	3.51	732.4	732.1	805	822	0.10	93.7	45	259.2	100
CyNA-346*[5]	4.58	724.4	724.1	804	822	0.07	92.7	25	434.2	69
CyNA-351	4.86	688.4	688.1	805	818	0.10	95.8	35	555.4	52
CyNA-356	4.44	742.4	742.1	804	820	0.12	96.9	20	892.3	83
CyNA-358	4.79	712.4	712.0	805	819	0.14	98.2	30	487.4	100
CyNA-359	4.76	711.4	711.1	805	821	0.11	95.6	20	527.6	85
CyNA-364	5.01	686.3	686.0	804	821	0.05	92.1	60	264.8	98
CyNA-373	4.90	618.4	618.1	805	820	0.07	93.6	15	567.2	76
CyNA-374	4.81	652.4	652.1	806	821	0.14	97.3	15	1224.6	94
CyNA-375	4.84	670.4	670.1	804	821	0.07	93.3	20	533.7	98
CyNA-381	5.12	668.3	668.0	805	821	0.14	96.5	15	1259.2	93
CyNA-382	5.29	658.4	658.1	804	820	0.08	93.4	20	294.6	85
CyNA-387	4.71	682.4	682.1	804	820	0.07	92.9	30	739.9	75
CyNA-388	4.89	682.4	682.0	803	819	0.14	97.8	25	1398.5	97
CyNA-395	4.83	696.4	696.1	804	820	0.07	95.1	20	234.3	95
CyNA-396	5.06	666.4	666.1	804	820	0.09	94.7	30	736.1	96
CyNA-398	5.01	666.4	666.1	804	819	0.07	93.4	25	540.2	99
CyNA-399	4.97	688.4	688.1	804	819	0.10	95.8	60	775.5	95
CyNA-401	5.03	653.4	653.1	805	820	0.10	94.9	35	856.5	91
CyNA-403	4.63	686.3	686.0	805	819	0.05	93.5	30	261.6	45
CyNA-405	4.99	684.3	684.0	806	820	0.06	94.5	20	138.5	83
CyNA-407	5.64	702.3	702.1	805	820	0.08	96.1	20	22.5	n.d.
CyNA-414	3.38	701.5	701.2	804	819	0.13	95.9	30	925.5	100
CyNA-419	5.53	632.4	632.0	804	820	0.13	97.4	10	835.2	91

CyNA-427	3.51	647.3	647.0	805	821	0.14	96.7	45	1247.4	100
CyNA-442	4.97	632.4	632.1	806	822	0.07	94.3	25	424.2	54
CyNA-443	5.11	630.4	630.0	806	821	0.09	95.6	30	583.5	75
CyNA-446	4.79	642.4	642.1	804	821	0.08	94.2	25	709.2	85
CyNA-477	4.91	684.9	684.1	805	819	0.09	93.7	20	169.8	n.d.
CyNA-478	5.03	680.4	680.1	805	821	0.09	95.4	20	121.6	64
CyNA-480	5.01	700.4	700.1	803	817	0.06	92.1	20	154.8	51
CyNA-487	4.98	618.4	618.1	806	823	0.04	91.6	25	45.3	30
CyNA-531	5.21	720.3	720.1	804	820	0.08	97.3	50	187.5	100
CyNA-542	5.01	702.4	702.1	805	821	0.08	94.5	45	442.7	74
CyNA-548	5.01	742.4	742.1	804	820	0.04	92.9	35	67.6	n.d.
CyNA-565	5.20	720.3	720.1	805	821	0.09	95.3	40	335.5	96
CyNA-567	5.69	716.5	716.2	805	820	0.14	98.3	20	279.2	100
CyNA-572	5.21	684.4	684.1	805	818	0.05	93.2	25	58.9	n.d.
CyNA-574	5.01	632.4	632.1	803	818	0.06	93.8	35	321.1	38
CyNA-599	5.20	742.5	742.1	805	819	0.07	97.6	25	105.9	n.d.

*[1] Quantum yields were measured in DMSO, using Cardiogreen as a standard (ϕ: 0.13, in DMSO).[1]
*[2] Purities were determined according to UV absorption at 365 nm.
*[3] Quotients of fluorescent intensities (8h) vs. fluorescent intensities (0h), in a time-course fluorescence measurement using 10 μM (2% DMSO) solutions in HEPES buffer (100 mM, pH 7.4).
*[4] Triacetylated derivative was isolated as the main product.
*[5] Diacetylated derivative was isolated as the main product.
Max. RFU: maximum relative fluorescence units; n.d.: non-determined value due to fluctuation of the experimental data.

Detailed characterization of CyNA-111, 165, 272, 295, 414

CyNA-111 (12 mg, 45%): ^{1}H-NMR (300MHz, $CDCl_3$): δ=1.05 (t, 6H, *J*=7.5Hz), 1.29 (m, 2H), 1.63 (s, 6H), 1.65 (s, 6H), 1.72 (m, 4H), 1.94 (t, 4H), 2.05 (s, 3H), 3.09 (m, 2H), 3.63 (m, 2H), 4.18 (t, 4H, *J*=3.9Hz), 6.07 (d, 0.3H, *J*=13.5Hz), 6.28 (d, 1.7H, *J*=14.1Hz), 7.02-7.72 (m, 12H), 8.43 (d, 0.4H, *J*=14.1Hz), 8.68 (d, 1.6 H, *J*=3.3Hz).

tR: 4.70 min, HRMS ($C_{45}H_{55}ON_4^+$), calc: 667.4370; found: 667.4350.

CyNA-165 (10 mg, 50%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ=1.05 (t, 6H, *J*=7.5Hz), 1.76 (s, 12H), 1.87-1.92 (m, 8H), 2.58 (t, 4H, *J*=6.3 Hz), 3.28 (s, 3H), 3.32 (t, 2H, *J*=5.4Hz), 3.45 (t, 2H, *J*=6.3 Hz), 3.92 (t, 4H, *J*=7.5 Hz), 4.16 (s, 3H), 6.02 (d, 2H, *J*=13.1 Hz), 6.90-7.30 (m, 8H), 7.35 (d, 2H, *J*=4.5Hz).

tR: 6.1 min, HRMS ($C_{42}H_{56}N_3O_2^+$), calc: 634.4367; found: 634.4352.

CyNA-272 (9 mg, 42%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ=1.08 (t, 6H, *J*=7.5Hz), 1.31 (m, 2H), 1.45 (m, 4H), 1.54 (m, 2H), 1.69 (s, 12H), 1.86 (m, 4H), 1.94 (s, 3H), 2.51 (t, 4H, *J*=6.3 Hz), 3.44 (t, 6H, *J*=7.7Hz), 3.81 (t, 4H, *J*=6.9Hz), 4.21 (m, 2H), 5.65 (d, 2H, *J*=12.9Hz), 6.80-7.70 (m, 8H), 7.72 (d, 2H, *J*=12.9Hz).

tR: 4.24min, HRMS ($C_{45}H_{61}N_4O^+$), calc: 673.4840; found: 673.4845.

CyNA-295 (9 mg, 48%): ^{1}H-NMR (300 MHz, $CDCl_3$): δ=1.05 (t, 6H, *J*=7.5Hz), 1.56 (t, 3H, *J*=7.0 Hz), 1.68 (s, 12H), 1.83 (m, 2H),1.85 (m, 4H), 1.94 (s, 3H), 2.59 (t, 4H, *J*=6.3Hz), 3.79 (t, 4H, *J*=6.9Hz), 3.96 (m, 2H), 6.04 (d, 2H, *J*=14.0Hz), 6.80-7.28 (m, 8H), 8.14 (d, 2H, *J*=14.0Hz).

tR: 4.87 min, HRMS ($C_{40}H_{52}N_3O^+$), calc: 590.4105; found: 590.4113.

CyNA-414 (9 mg, 41%): ^{1}H-NMR (300MHz, $CDCl_3$): δ=1.06 (t, 6H, *J*=7.5Hz), 1.24 (d, 3H, *J*=6.6 Hz), 1.23 (m, 2H), 1.58 (s, 6H), 1.66 (s, 6H), 1.83-2.06 (m, 6H), 1.95 (s, 3H), 2.46-2.56 (m, 4H), 2.82 (t, 2H, *J*=5.4 Hz), 2.87 (t, 2H, *J*=5.4 Hz), 3.08 (t, 4H), 2.96-2.98 (m, 2H), 3.08-3.12 (m, 1H), 3.67 (t, 2H, *J*=

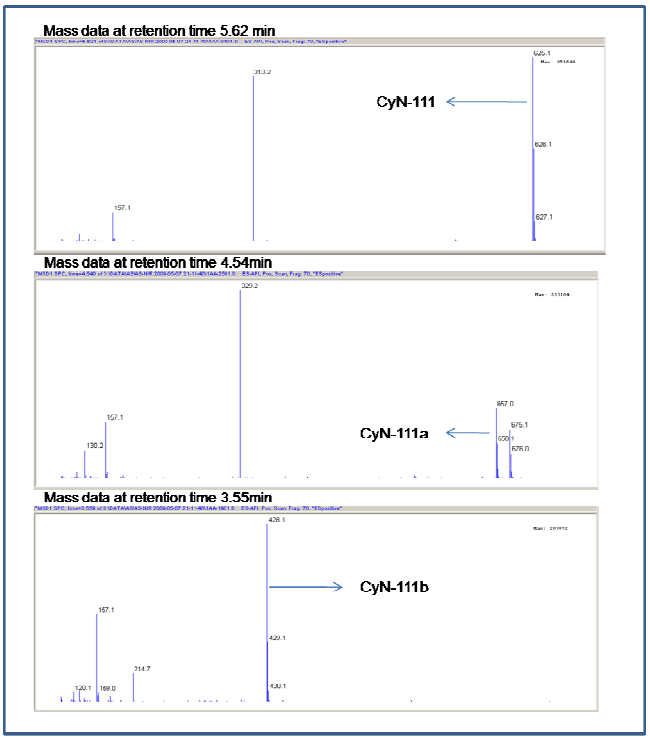
6.0Hz), 4.12 (t, 4H, *J*=7.2Hz), 6.21 (d, 1H, *J*=14.1Hz), 6.26 (d, 1H, *J*=14.1Hz), 7.11 (d, 2H, *J*=7.5 Hz), 7.20 (d, 2H, *J*=6.9 Hz), 7.34 (m, 4H), 7.55 (d, 1H, *J*=14.1 Hz), 7.59 (d, 1H, *J*=14.1Hz).

^{13}C-NMR (75.5MHz, $CDCl_3$): 11.6, 11.7, 20.5, 20.6, 20.8, 20.9, 22.9, 23.7, 25.0, 28.1, 28.2, 28.3, 28.5, 28.6, 29.6, 30.8, 46.2, 46.2, 49.2, 49.3, 102.2, 110.7, 110.8, 122.3, 125.3, 125.4, 128.2, 128.4, 128.6, 140.9, 141.0, 142.2, 154.2, 170.4, 172.1.

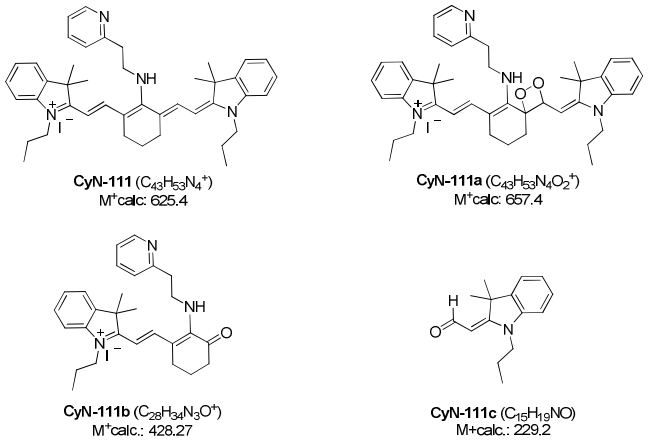
tR: 4.32 min, HRMS ($C_{47}H_{65}N_4O^+$), calc: 701.5153; found: 701.5147.

4. Photodecomposition monitoring of CyN-111

HPLC-MS analysis



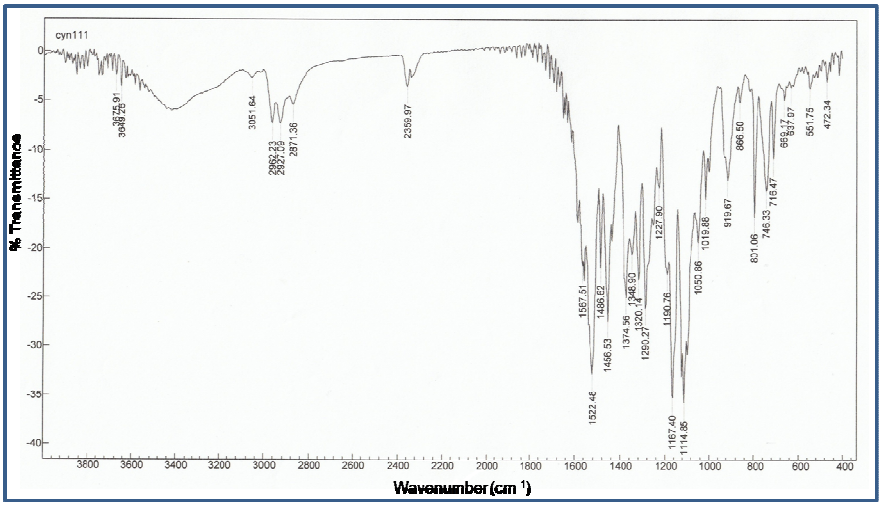
Supplementary Figure 1. MS data for the decomposition monitoring of **CyN-111**.



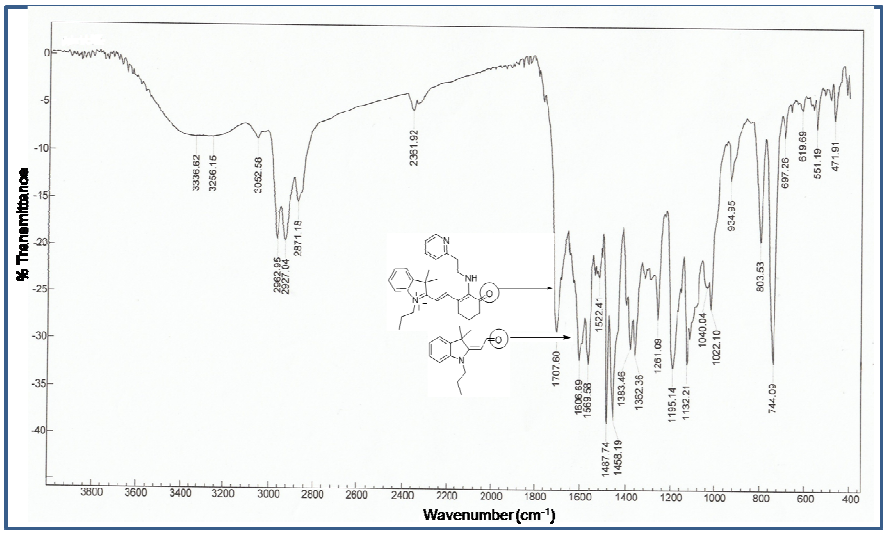
Supplementary Figure 2. Chemical structures of **CyN-111** and decomposition products (**CyN-111a, b, c**).

IR

a)

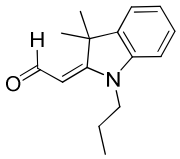


b)



Supplementary Figure 3. IR spectra of the reaction mixture after: a) 0h, b) 6h. The carbonyl bands corresponding to **CyN-111b** (ketone) and **CyN-111c** (aldehyde) are easily distinguishable.

^{1}H-NMR



CyN-111c

^{1}H-NMR (CyN-111c) (300 MHz, $CDCl_3$) δ=0.98 (t, 3H, *J*=7.5Hz), 1.65 (s, 6H), 1.69-1.77 (m, 2H), 3.63(t, 3H, *J*=9.0 Hz), 5.43 (d, 1H, *J*=9.0 Hz), 6.82 (d, 1H, *J*=7.5Hz), 7.04 (t, 1H, *J*=7.5 Hz), 7.22-7.28 (m, 2H), 9.98(d, 1H, J=9.0Hz).

5. Time-course fluorescence measurements

a) CyN-111, 165, 272, 295 vs CyNA-111, 165, 272, 295 and 1

Procedure: 10μM **CyN(A)** solutions in 10mM HEPES buffer (pH 7.4) containing 1% DMSO were placed in a 96-well black plate, and fluorescence intensity measurements were recorded every 10 min for a total period of 10 h (excitation-emission: 640-750 nm for **CyN** derivatives**,** and 790-820 nm for **1** and **CyNA** derivatives). Values are fitted to a non-linear regression one-phase exponential decay (GraphPad Prism 5.0). Detailed statistics:

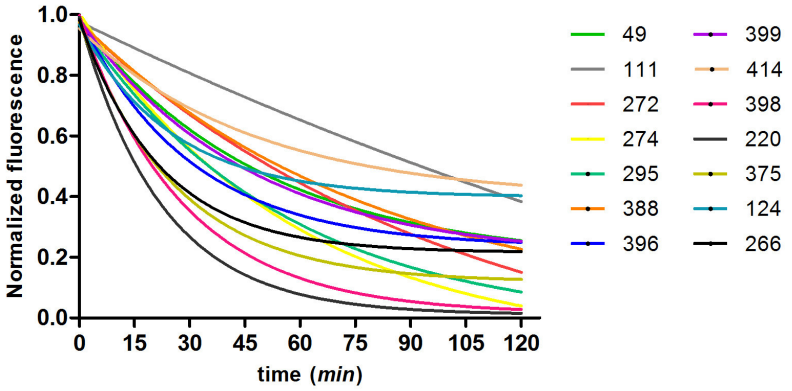
One phase decay									
Goodness of Fit	**CyN-111**	**CyN-165**	**CyN-272**	**CyN-295**	**CyNA-111**	**CyNA-165**	**CyNA-272**	**CyNA-295**	**1**
Degrees of Freedom	57	57	57	57	57	57	57	57	57
R^2	0.9967	0.9988	0.9971	0.9981	0.9736	0.9664	0.9259	0.9418	0.8433
Absolute Sum of Squares	0.008321	0.002994	0.007014	0.00426	0.002442	0.006335	0.004751	0.009447	0.04572
Sy.x	0.01208	0.007247	0.01109	0.008645	0.006546	0.01054	0.00913	0.01287	0.02832
Number of points									
Analyzed	60	60	60	60	60	60	60	60	60

Photodecomposition rate constants were determined by plotting $-ln(F/F_0)$ vs time, and calculated using a *pseudo*-first order rate equation.[2]

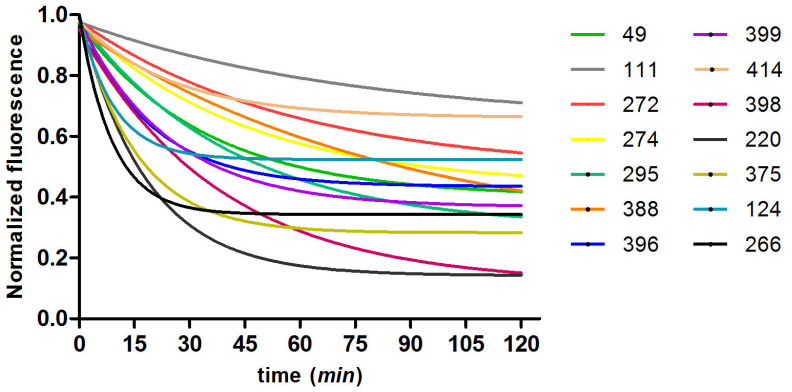
b) CyNA library (primary and secondary screening).

Primary screening: 10μM **CyN(A)** solutions in 100mM HEPES buffer (pH 7.4) containing 2% DMSO were placed in a 96-well black plate, and fluorescence intensity measurements were recorded every 10 min for a total period of 8h (excitation-emission: 790-820 nm). A subset of 14 compounds selected according to their quotients F_{8h}/F_o, quantum yields and maximum RFU values (**Table S2**) was further evaluated on the secondary screening.

Secondary screening: 10μM solutions in 10mM HEPES buffer (pH 7.4) or PBS (pH 7.3) containing 1% DMSO were placed in a 96-well black plate, and irradiated for periods of 15 min (up to 2h) with a high intensity UV lamp (100W, 365 nm) at 2-cm distance. Values are represented as means (n=2), and fitted to a non-linear regression one-phase exponential decay (GraphPad Prism 5.0).



Supplementary Figure 4. Photostability secondary screening of the **CyNA** library in HEPES buffer (10mM, pH 7.4).



Supplementary Figure 5. Photostability secondary screening of the **CyNA** library in PBS buffer (pH 7.3).

Detailed statistics:

For **Supplementary Figure 4**:

One phase decay

Goodness of Fit	**49**	**111**	**272**	**274**	**295**	**388**	**396**
Degrees of Freedom	6	6	6	6	6	6	6
R^2	0.988	0.9892	0.9915	0.998	0.9932	0.9904	0.9775
Absolute Sum of Squares	0.006007	0.00361	0.005435	0.001729	0.005058	0.005089	0.01161
Sy.x	0.03164	0.02453	0.0301	0.01697	0.02903	0.02912	0.04398
Number of points Analyzed	9	9	9	9	9	9	9

Goodness of Fit	**399**	**414**	**398**	**220**	**375**	**124**	**266**
Degrees of Freedom	6	6	6	6	6	6	6
R^2	0.995	0.9465	0.9982	0.99	0.9805	0.9418	0.9843
Absolute Sum of Squares	0.002487	0.01398	0.001551	0.008653	0.01301	0.01779	0.008353
Sy.x	0.02036	0.04827	0.01608	0.03798	0.04656	0.05446	0.03731
Number of points Analyzed	9	9	9	9	9	9	9

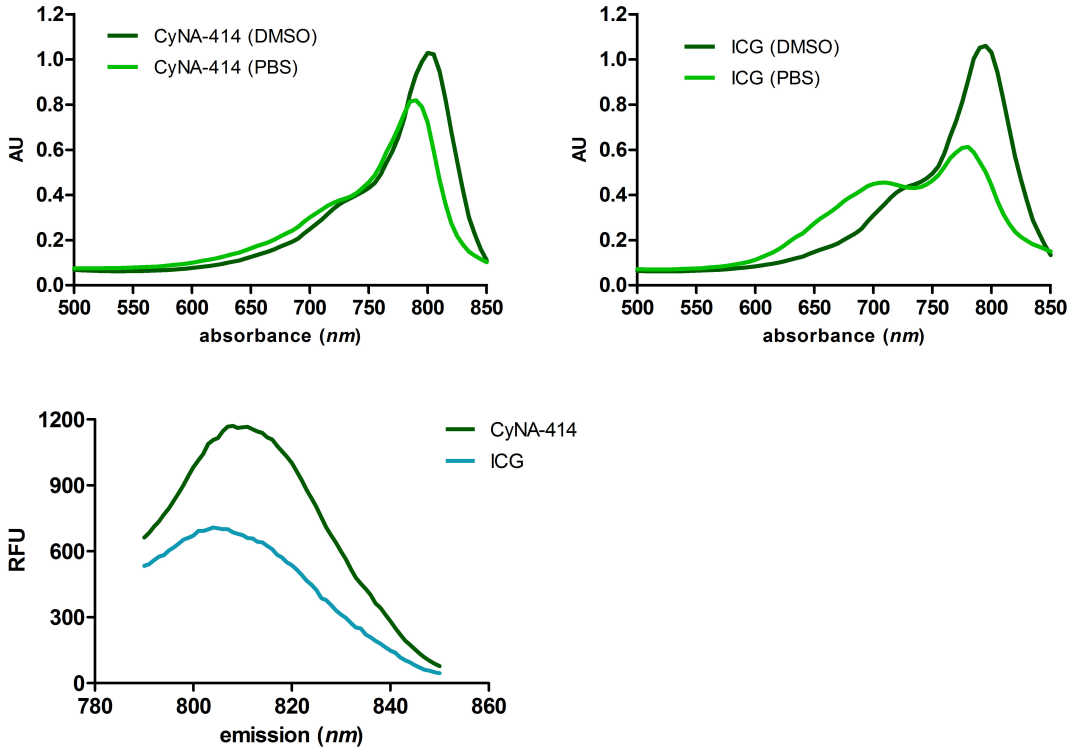
For **Supplementary Figure 5**:

One phase decay							
Goodness of Fit	**49**	**111**	**272**	**274**	**295**	**388**	**396**
Degrees of Freedom	6	6	6	6	6	6	6
R^2	0.9685	0.9472	0.9812	0.9787	0.9695	0.9491	0.9654
Absolute Sum of Squares	0.009142	0.003654	0.003342	0.005172	0.01214	0.0141	0.009859
Sy.x	0.03903	0.02468	0.0236	0.02936	0.04498	0.04847	0.04054
Number of points Analyzed	9	9	9	9	9	9	9
Goodness of Fit	**399**	**414**	**398**	**220**	**375**	**124**	**266**
Degrees of Freedom	6	6	6	6	6	6	6
R^2	0.9788	0.9285	0.9638	0.9933	0.9856	0.9581	0.9928
Absolute Sum of Squares	0.007325	0.006867	0.02214	0.004388	0.006524	0.008531	0.002706
Sy.x	0.03494	0.03383	0.06074	0.02704	0.03298	0.03771	0.02124
Number of points Analyzed	9	9	9	9	9	9	9

6. Comparative spectral properties of CyNA-414 and ICG.

Absorbance spectra: 5µM solutions in PBS (pH 7.3) containing 1% DMSO or in pure DMSO.

Emission spectra: 10µM solutions in PBS (pH 7.3) containing 1% DMSO.



Supplementary Figure 6. Absorbance and emission spectra of **CyNA-414** and **ICG** (for emission spectra, excitation wavelength: 760 nm).

Photostability measurements: 10µM solutions in PBS (pH 7.3) containing 1% DMSO were placed in a 96-well black plate, and irradiated for periods of 10 min (up to 2 h) with a high intensity UV lamp (100W, 365 nm) at 2-cm distance. Values are represented as means (n=6), and fitted to a non-linear regression one-phase exponential decay (GraphPad Prism 5.0).

7. Photobleaching rates of CyNA-414 and CyN-414.

Procedure: 10μM **CyNA-414 and CyN-414** solutions in 10mM HEPES buffer (pH 7.4) containing 1% DMSO were placed in a 96-well black plate, and fluorescence intensity measurements (n=3) were recorded every 10 min for a total period of 12 h (excitation-emission: 640-750 nm for **CyN-414,** and 790-820 nm for **CyNA-414**) under a xenon flash lamp. Values are fitted to a non-linear regression one-phase exponential decay (GraphPad Prism 5.0).

Supplementary Table 3. Photobleaching rates of **CyNA-414** and **CyN-414**.

compound	k (s^{-1})	k_{CyN}/k_{CyNA}
CyN-414	$17.5 \cdot 10^{-6}$	---
CyNA-414	$2.5 \cdot 10^{-6}$	7

References

1. K. Licha, B. Riefke, V. Ntziachristos, A. Becker, B. Chance, W. Semmler. *Photochem. Photobiol.* **2000**, *72*, 392-398.
2. A. Toutchkine, D.V. Nguyen, K.M. Hahn. *Org. Lett.* **2007**, 9 2775-2777.