

1 *Supplementary information*

## 2 **Theranostic aza-BODIPY as vector for enhanced** 3 **Boron Neutron Capture Therapy applications**

4 Ghadir Kalot <sup>1,†</sup>, Amélie Godard <sup>2,†</sup>, Benoît Busser <sup>1,3</sup>, Jacques Pliquett <sup>2</sup>, Mans Broekgaarden <sup>1</sup>,  
5 Vincent Motto-Ros <sup>4</sup>, K. David Wegner <sup>5</sup>, Ute Resch-Genger <sup>5</sup>, Ulli Köster <sup>6</sup>, Franck Denat <sup>2</sup>, Jean-  
6 Luc Coll <sup>1</sup>, Ewen Bodio <sup>2\*</sup>, Christine Goze <sup>2,\*</sup> and Lucie Sancey <sup>1,\*</sup>

7

### 8 **Materials and methods for chemical synthesis and characterization**

9 Reactions were carried out in analytical reagent grade solvents from Carlo Erba under normal  
10 atmosphere. Dry solvents, purchased from Carlo Erba, were non-stabilized and dried using a MB-  
11 SPS-800 (MBraun) or PureSolv-MD-5 (Inert®). All reagents purchased from Sigma Aldrich™,  
12 Thermo Fisher Scientific™ or ACROS Organics™ were used as received without further purification.  
13 Sodium mercaptododecaborate (<sup>10</sup>B) was purchased from Katchem™. Reactions were monitored by  
14 thin-layer chromatography and RP-HPLC-MS. Analytical thin-layer chromatography was performed  
15 with Merck 60 F254 silica gel (precoated sheets, 0.2 mm thick). Column chromatography was carried  
16 out using silica gel (Sigma Aldrich; 40-63 μm 230-400 mesh 60Å). Ion exchange was executed using  
17 an Amberlite™ IRA410Cl ion-exchange resin.

18 (<sup>1</sup>H, <sup>11</sup>B, <sup>13</sup>C, <sup>19</sup>F)-NMR spectra were recorded at 298 or 343 K on Bruker 500 Avance III or 600  
19 Avance III spectrometers. Chemical shifts are given relative to TMS (<sup>1</sup>H, <sup>13</sup>C), BF<sub>3</sub>\*Et<sub>2</sub>O (<sup>11</sup>B, <sup>10</sup>B), CFC<sub>3</sub>  
20 (<sup>19</sup>F), and were referenced to the residual solvent signal. High resolution mass spectra (HR-MS) were  
21 recorded on a Thermo LTQ Orbitrap XL ESIMS spectrometer. NMR and mass-analyses were  
22 performed at the "Plateforme d'Analyse Chimique et de Synthèse Moléculaire de l'Université de  
23 Bourgogne" (PACSMUB).

24 HPLC-MS analyses were obtained from a Thermo-Dionex Ultimate 3000 instrument (pump +  
25 autosampler at 20 °C + column oven at 25 °C) equipped with a diode array detector (Thermo-Dionex  
26 DAD 3000-RS) and a MSQ Plus single quadrupole mass spectrometer equipped with Phenomenex  
27 Kinetex® column (2.6 μm C18 100 Å, LC Column 50 × 2.1 mm).

28 The employed gradient for analyses was as follows:

29

Time [min]	% H <sub>2</sub> O + 0.1% formic acid	% ACN + 0.1% formic acid	Flow [mL/min]
0	95	5	0.5
5	0	100	0.5
6.5	0	100	0.5
6.6	95	5	0.5
8.5	95	5	0.5
8.51	95	5	0.05

30 **Table S1:** HPLC analytical gradient.

31

32

33 Semi-preparative separations were executed on a HPLC-system, from Shimadzu, that was  
 34 equipped with 2 LC-20AT pumps, a SPD-20A UV/Vis detector, a FRC-10A fraction collector, a SIL-  
 35 10AP sampler and a CBM-20A control unit. The column was a Shim-Pack GIST 5  $\mu\text{m}$  C18 10x250 mm  
 36 column obtained from Shimadzu too. The gradient using a mixture of ACN and water with 0.1% TFA  
 37 and a flow rate of 5 mL/min was as follows:

38

39

40

41

42

43

44

45

46

47

Time [min]	% H <sub>2</sub> O + 0.1 %TFA	% ACN + 0.1 %TFA	Flow [mL/min]
0	75	25	5
5	75	25	5
25	0	100	5
28	0	100	5
30	75	25	5

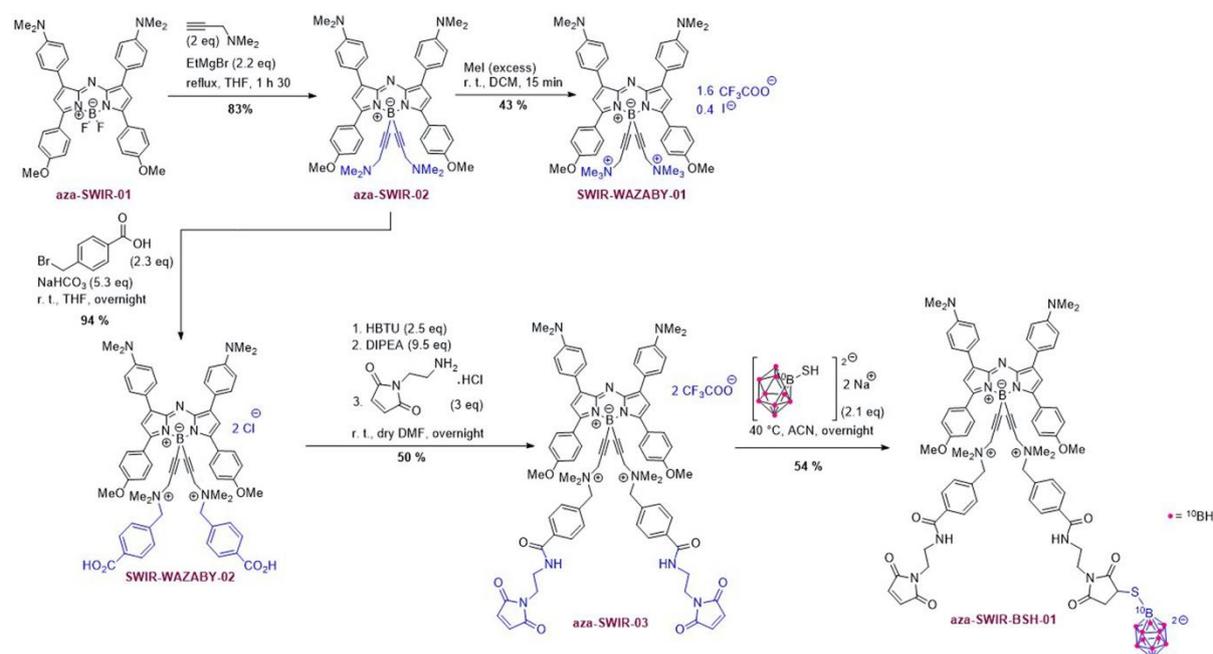
48

Table S2: Detailed of gradients.

49

50

### Synthesis and characterization



51

52

Scheme S1: Synthetic pathways of aza-SWIR-BSH-01.

53 Aza-SWIR-01, aza-SWIR-02, and SWIR-WAZABY-01 were synthesized according to procedures,  
 54 we previously reported [1].

55

56 • Compound **SWIR-WAZABY-02**:

57

58

59

60

61

62

63

64

65

66

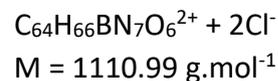
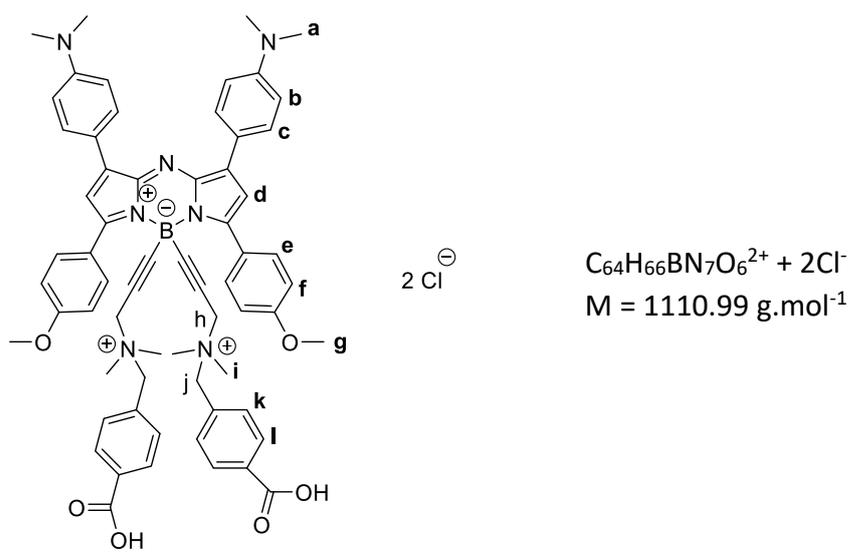
67

68

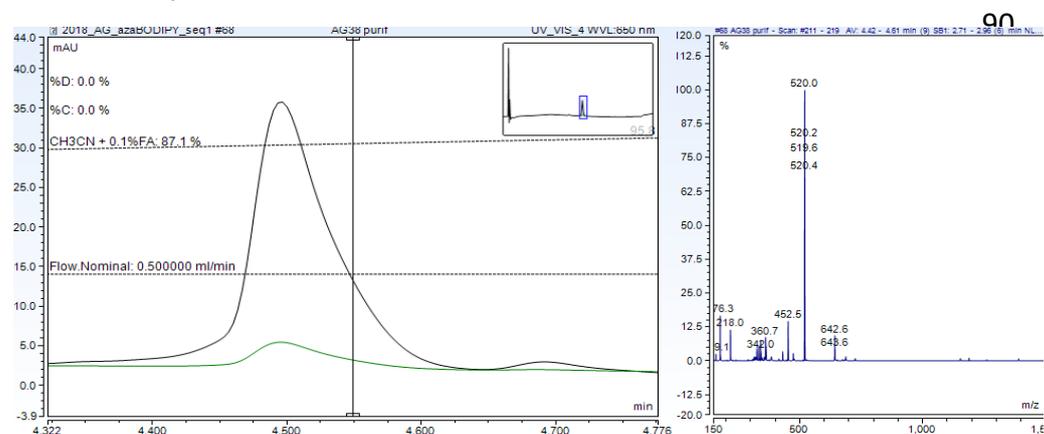
69

70

71



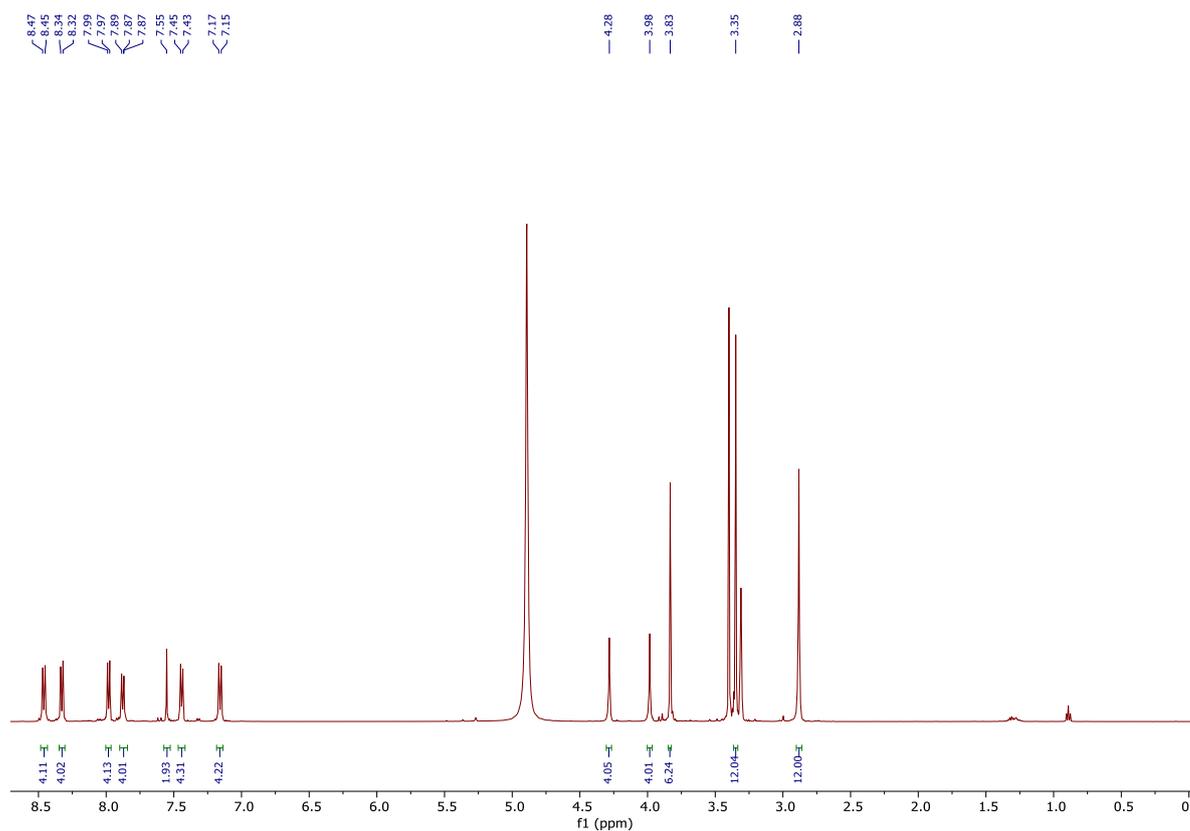
72 **Aza-SWIR-02** (250 mg, 0.3 mmol, 1 eq) was added in a 100 mL round bottom flask and dissolved  
 73 in a mixture of THF and water (50 mL / 8 mL).  $\text{NaHCO}_3$  (137 mg; 1.6 mmol; 5.3 eq) and 4-  
 74 bromomethylbenzoic acid (144 mg; 0.7 mmol; 2.3 eq) were added successively. The resulting solution  
 75 was stirred at room temperature (RT) overnight and transferred to a separating funnel. Then, 90 mL  
 76 of  $\text{Et}_2\text{O}$  and 90 mL of water were added and the two layers were separated. The aqueous layer was  
 77 washed with  $\text{Et}_2\text{O}$  ( $6 \times 60$  mL) to remove the remaining traces of 4-bromomethylbenzoic acid. Then,  
 78 the aqueous layer was evaporated at  $35^\circ\text{C}$  to  $2/3^{\text{th}}$  of the volume and 10 mL of  $\text{HCl}_{\text{aq}}$  (3M) were added  
 79 to precipitate the compound (via protonation of the carboxylate groups). The mixture was  
 80 centrifuged during 5 min at 6 000 RPM. The supernatant was removed and the sediment was  
 81 suspended in  $\text{Et}_2\text{O}$  (15 mL) and centrifuged. The process was repeated 3 times and the solid was dried  
 82 under vacuum to give pure **SWIR-WAZABY-02** as a crystalline dark powder (336 mg; 0.3 mmol; 94%  
 83 yield).  $^1\text{H}$  NMR (500 MHz, 298 K,  $\text{MeOD-d}_4$ )  $\delta$  (ppm) = 2.88 (s, 12H), 3.35 (s, 12H), 3.83 (s, 6H), 3.98 (s,  
 84 4H), 4.28 (s, 4H), 7.16 (d,  $J = 9.0$  Hz, 4H), 7.44 (d,  $J = 8.2$  Hz, 4H), 7.55 (s, 2H), 7.88 (d,  $J = 9.0$ , 4H), 7.98  
 85 (d,  $J = 8.2$  Hz, 4H), 8.33 (d,  $J = 9.0$  Hz, 4H), 8.46 (d,  $J = 9.0$  Hz, 4H).  $^{13}\text{C}$  NMR (125 MHz, 298 K,  $\text{MeOD-d}_4$ )  
 86  $\delta$  (ppm) = 48.0, 50.7, 52.1, 56.7, 57.5, 67.8, 89.6, 116.5, 123.1, 123.3, 125.8, 131.9, 132.2, 133.1, 133.6,  
 87 134.9, 135.2, 135.7, 142.3, 145.1, 145.6, 160.2, 164.9, 169.2.  $^{11}\text{B}$  NMR (193 MHz, 298K,  $\text{MeOD-d}_4$ )  $\delta$  (ppm)  
 88 = -11.63 (bs). HR-MS (ESI):  $m/z$  calculated for  $^{12}\text{C}_{64}^{1}\text{H}_{66}^{11}\text{B}^{14}\text{N}_7^{16}\text{O}_6^{2+}$   $[\text{M}]^{2+}$  519.75783 Th; found  
 89 519.75905 Th. Analytical HPLC: Tr = 4.50 min.



91

**Figure S1:** Analytical HPLC of **SWIR-WAZABY-02**

92

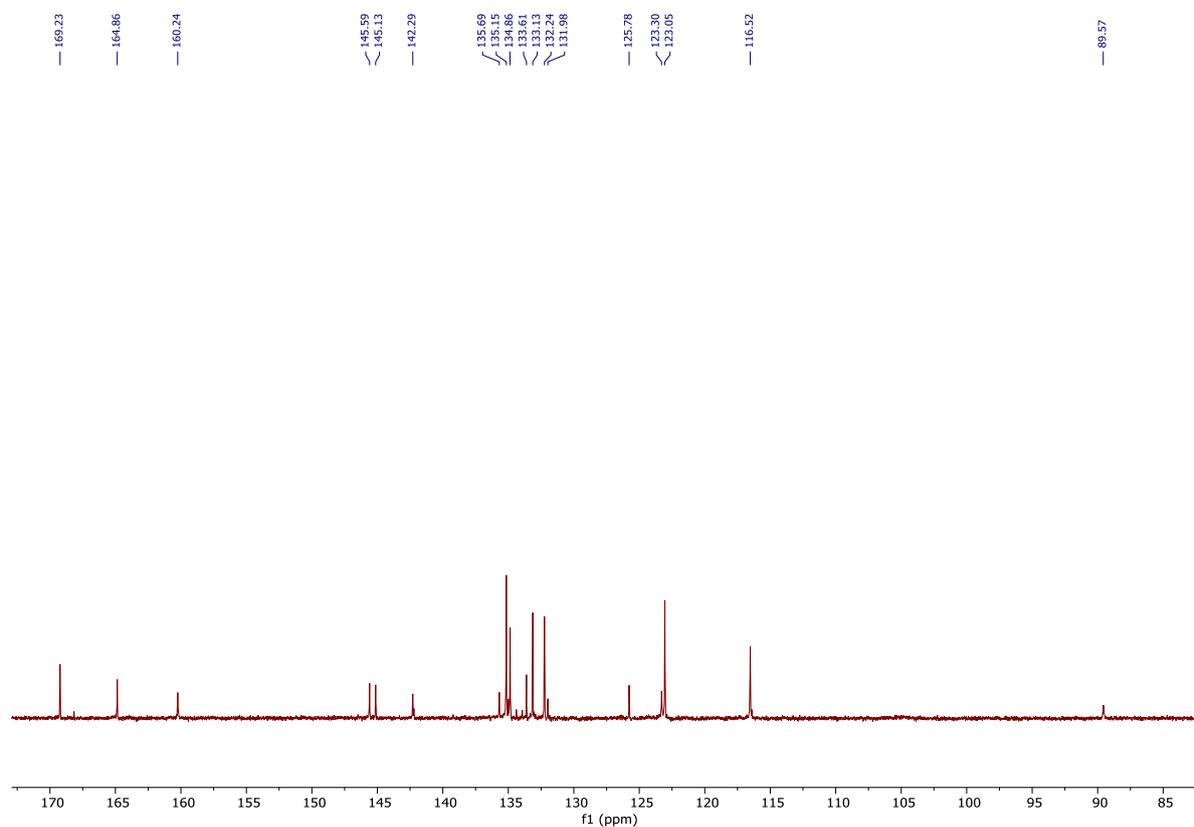


93

94

95

Figure S2: <sup>1</sup>H NMR of SWIR-WAZABY-02 (500 MHz, 298 K, MeOD-d<sub>4</sub>)



96

97

98 **Figure S3:**  $^{13}\text{C}$  NMR of SWIR-WAZABY-02 (125 MHz, 298 K, MeOD- $d_4$ )

99

100 • Compound **aza-SWIR-03**:

101

102

103

104

105

106

107

108

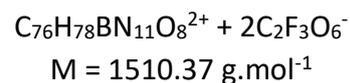
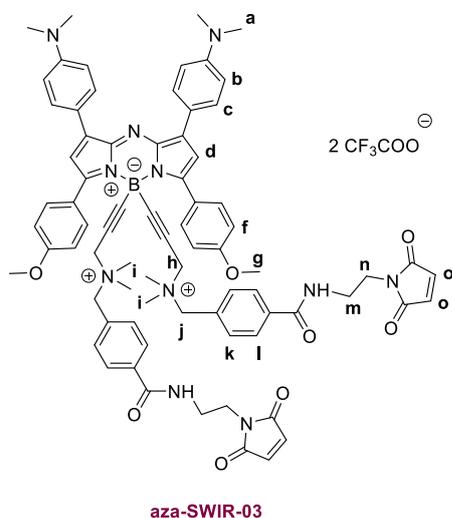
109

110

111

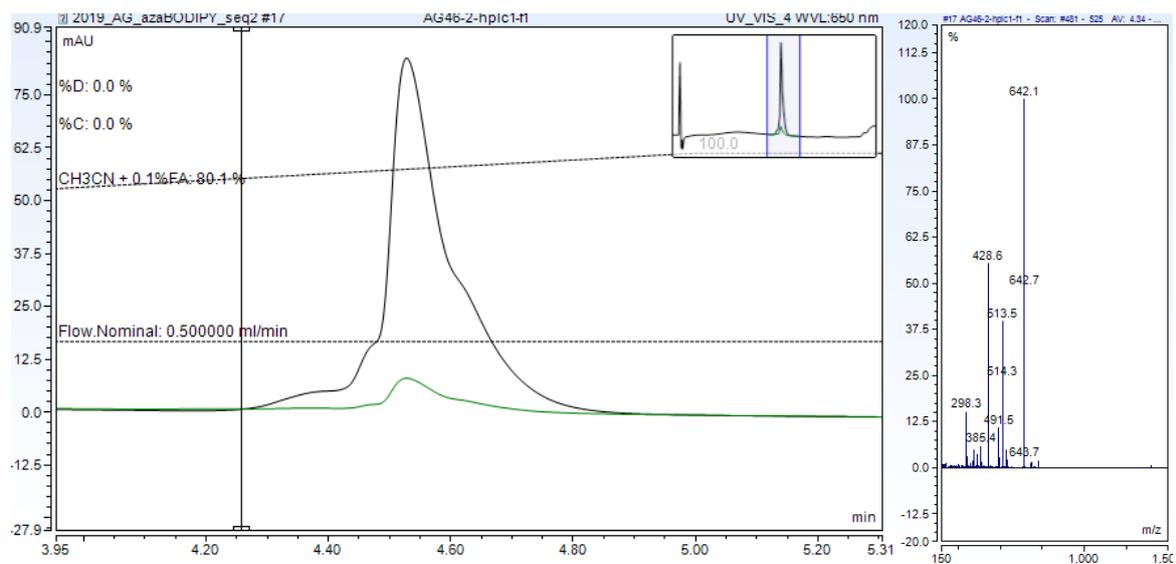
112

113



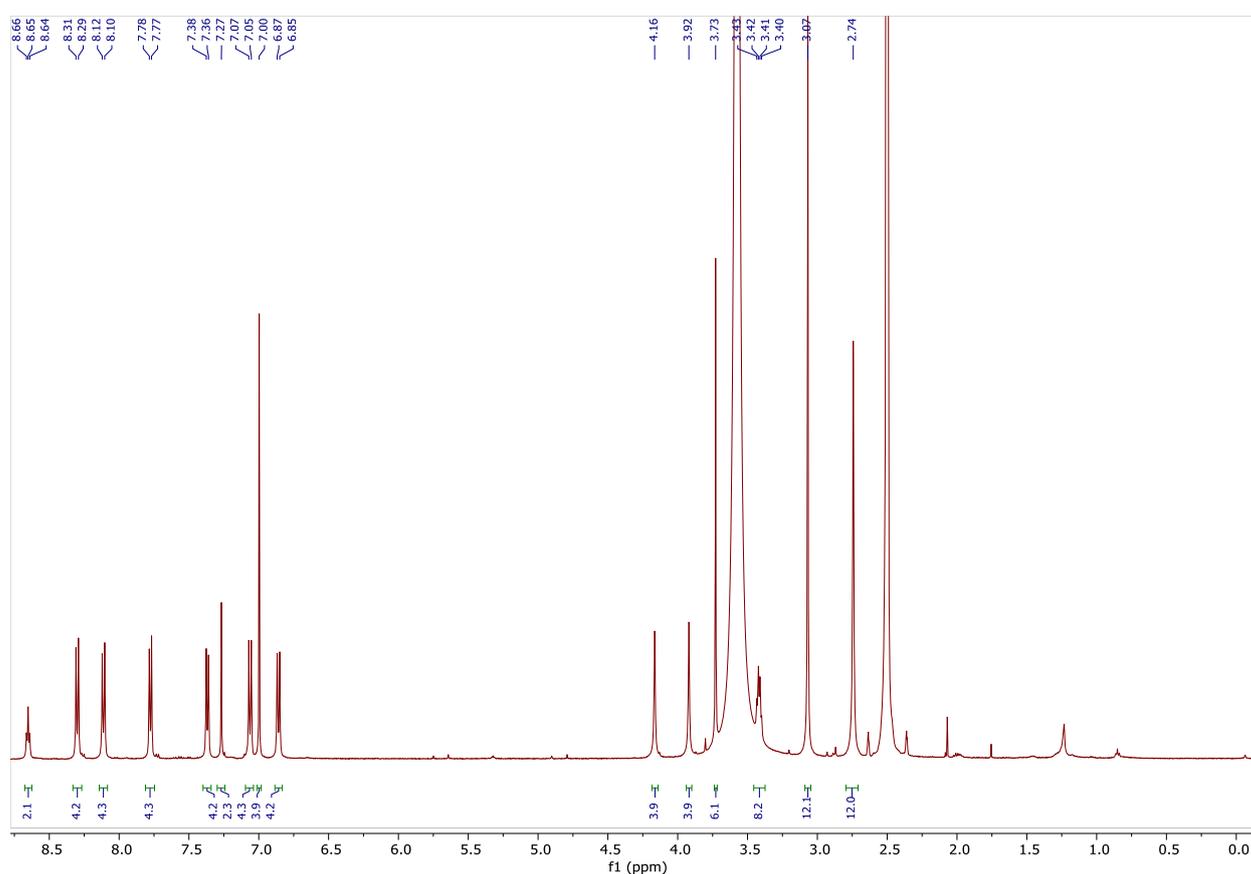
114 **SWIR-WAZABY-02** (250 mg, 0.2 mmol, 1 eq) was dissolved in 10 mL of dry DMF. HBTU (208  
 115 mg; 0.5 mmol; 2.5 eq) solubilized in dry DMF (10 mL) was added to the reaction medium. DIPEA  
 116 (340  $\mu\text{L}$ ; 1.9 mmol; 9.5 eq) was then added and the reaction mixture was stirred at RT for 1 h. *N*-(2-  
 117 aminoethyl)maleimide hydrochloride (0.6 mg, 0.6 mmol, 3 eq) was solubilized in dry DMF (10 mL)  
 118 and added to the medium. The reaction mixture was stirred at RT overnight. The solvent was  
 119 removed at 45°C under vacuum. The crude was purified by semi-preparative HPLC to obtain **aza-**  
 120 **SWIR-03** as a green solid (151 mg, 50 % yield).  $^1\text{H}$  NMR (500 MHz, 298 K, DMSO- $d_6$ )  $\delta$  = 2.74 (s, 12H),  
 121 3.07 (s, 12H), 3.40-3.43 (m, 8H), 3.73 (s, 6H), 3.92 (s, 4H), 4.16 (s, 4H), 6.86 (d,  $J$  = 9.0 Hz, 4H), 7.00 (s,  
 122 4H), 7.06 (d,  $J$  = 9.0 Hz, 4H), 7.27 (s, 2H), 7.37 (d,  $J$  = 8.0 Hz, 4H), 7.78 (d,  $J$  = 8.0 Hz, 4H), 8.11 (d,  $J$  = 9.0  
 123 Hz, 4H), 8.30 (d,  $J$  = 9.0 Hz, 4H), 8.65 (t,  $J$  = 5.9 Hz, 2H).  $^{13}\text{C}$  NMR (150 MHz, 298 K, DMSO- $d_6$ )  $\delta$  (ppm)  
 124 = 37.1, 37.8, 49.1, 54.0, 55.4, 64.7, 86.7, 112.1, 112.8, 113.8, 114.7, 116.0, 116.7, 118.6, 119.8, 124.3, 127.6,  
 125 130.1, 130.5, 132.0, 132.5, 134.6, 136.0, 141.6, 142.1, 151.0, 155.9, 157.9, 158.1, 158.3, 158.6, 160.9, 165.6,  
 126 171.1. HR-MS (ESI):  $m/z$  calculated for  $^{12}\text{C}_{76}^{1}\text{H}_{78}^{11}\text{B}^{14}\text{N}_{11}^{16}\text{O}_8^{2+}$  [M] $^{2+}$  641.80585 Th; found 641.80752 Th.  
 127 Analytical HPLC: Tr = 4.56 min.

128



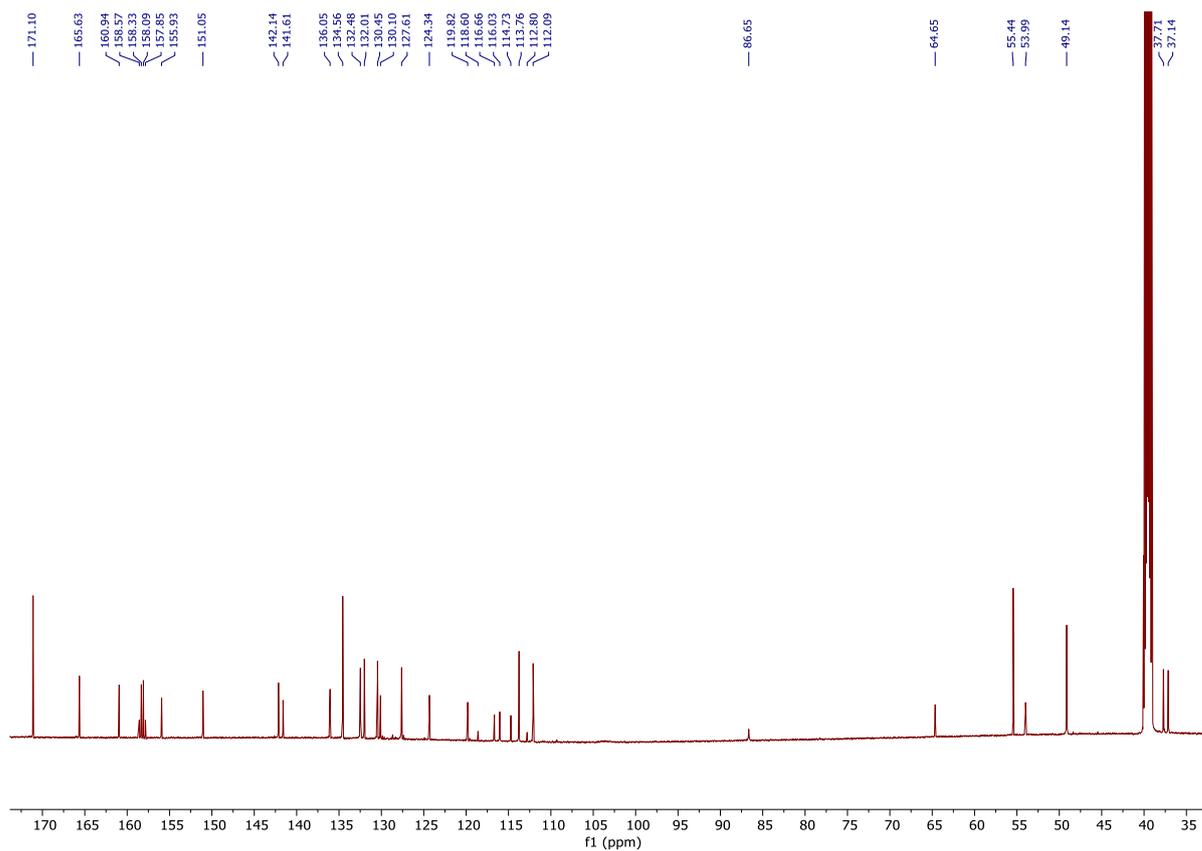
129

Figure S4: Analytical HPLC of aza-SWIR-03



130  
131

Figure S5: <sup>1</sup>H NMR of aza-SWIR-03 (500 MHz, 298 K, DMSO-d<sub>6</sub>)



132

133

134 **Figure S6:**  $^{13}\text{C}$  NMR of aza-SWIR-03 (150 MHz, 298 K, DMSO- $d_6$ )

135

136 • Compound aza-SWIR-BSH-01:

137

138

139

140

141

142

143

144

145

146

147

148

149

150

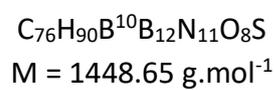
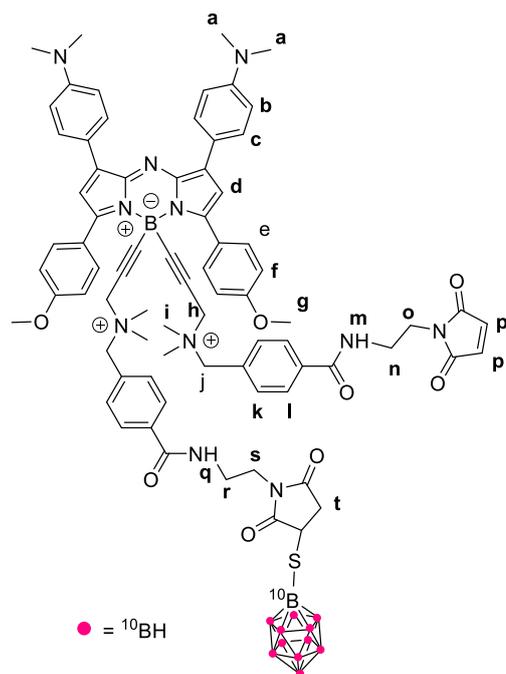
151

152

153

154

155

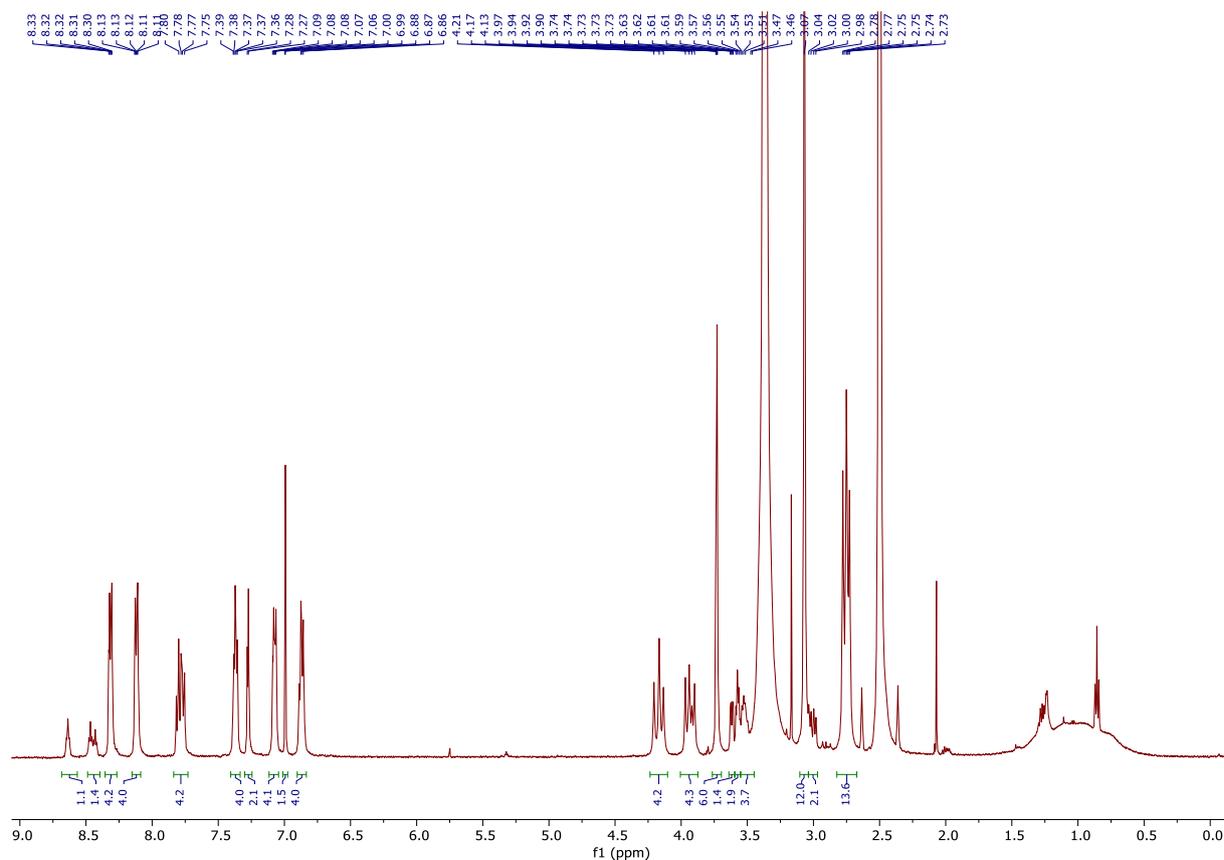


**Aza-SWIR-BSH-01**

156

157 **Aza-SWIR-03** (120 mg, 79  $\mu\text{mol}$ , 1 eq) was dissolved in 4 mL of ACN in a 10 mL round-bottom  
 158 flask. Sodium mercaptoundecahydrododecaborate ( $^{10}\text{B}$ ) (42 mg; 166  $\mu\text{mol}$ ; 2.1 eq) was added and the  
 159 reaction mixture was stirred overnight at 40  $^{\circ}\text{C}$ , leading to the formation of a precipitate. The mixture  
 160 was centrifuged during 5 min at 6 000 RPM. The supernatant was removed and the sediment was  
 161 suspended in ACN (15 mL), the process was repeated three times with ACN, once with DCM (15  
 162 mL), three time with Et $_2$ O (15 mL) and twice with pentane (15 mL) to obtain **aza-SWIR-BSH-01** as a  
 163 green solid (62 mg, 54%).  $^1\text{H}$  NMR\* (500 MHz, 298 K, DMSO- $d_6$ )  $\delta$  = 1.03 (bs, 11H), 2.73–2.78 (m, 14H),  
 164 3.01 (dd,  $J$  = 8.3; 18.4 Hz, 2H), 3.07 (s, 12H), 3.46–3.56 (m, 4H), 3.58 (t,  $J$  = 5.8 Hz, 2H), 3.62 (dd,  $J$  = 8.3;  
 165 3.1 Hz, 1H), 3.73–3.74 (m, 6H), 3.90–3.97 (m, 2H), 4.17 (t,  $J$  = 17.9 Hz, 4H), 6.85–6.89 (m, 4H), 6.99–7.00  
 166 (m, 2H), 7.07–7.09 (m, 4H), 7.26–7.28 (m, 2H), 7.36–7.39 (m, 4H), 7.76–7.82 (m, 4H), 8.10–8.13 (m, 4H),  
 167 8.30–8.33 (m, 4H), 8.43 (t,  $J$  = 5.8 Hz, 1H), 8.64 (t,  $J$  = 5.8 Hz, 1H).  $^{13}\text{C}$  NMR (150 MHz, 343 K, DMSO-  
 168  $d_6$ )  $\delta$  (ppm) = 36.9, 37.0, 37.1, 37.5, 40.7, 40.8, 41.9, 41.9, 49.1, 49.1, 54.2, 54.3, 55.4, 55.4, 64.9, 64.9, 65.0,  
 169 86.2, 111.9, 111.9, 112.0, 113.6, 113.6, 113.6, 115.8, 115.8, 115.8, 119.7, 119.9, 124.1, 124.2, 124.2, 127.4,  
 170 127.5, 127.5, 129.5, 129.6, 129.7, 129.8, 130.2, 130.2, 131.7, 131.7, 132.1, 132.1, 132.2, 132.2, 134.2, 136.1,  
 171 136.2, 136.3, 141.5, 142.0, 150.9, 151.0, 155.9, 156.0, 160.8, 160.8, 160.8, 165.5, 165.5, 165.6, 165.6, 170.7,  
 172 176.9, 178.5, 178.5.  $^{11}\text{B}$  NMR (193 MHz, 343 K, DMSO- $d_6$ )  $\delta$  (ppm) = - 6.92 (bs), - 12.37 (bs), - 14.49 (s),  
 173 - 15.44 (s), - 17.25 (bs).  $^{10}\text{B}$  NMR (64 MHz, 343 K, DMSO- $d_6$ )  $\delta$  (ppm) = - 6.92 (bs), - 14.61 (s), - 15.52 (s).  
 174 HR-MS (ESI):  $m/z$  calculated for  $^{12}\text{C}_{76}^{1}\text{H}_{90}^{11}\text{B}_{10}^{10}\text{B}_{12}^{14}\text{N}_{11}^{16}\text{O}_8^{32}\text{S}_{23}\text{Na}_2^{2+}$   $[\text{M}+2\text{Na}]^{2+}$  746.90622 Th; found  
 175 746.90811 Th. Analytical HPLC: Tr = 5.32 min. NMR spectra seems to indicate the presence of some  
 176 amounts of another product, while there is no sign of it on HPLC chromatogram.

177

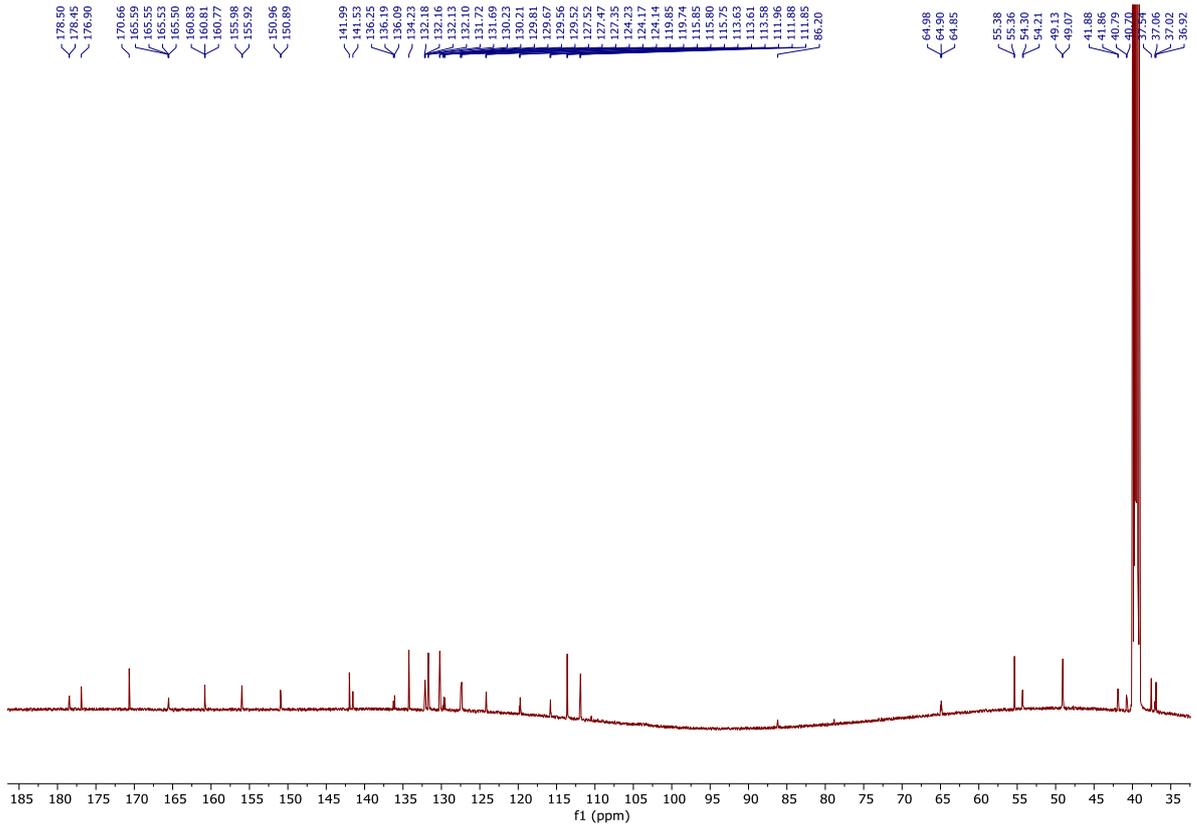


178

179

180

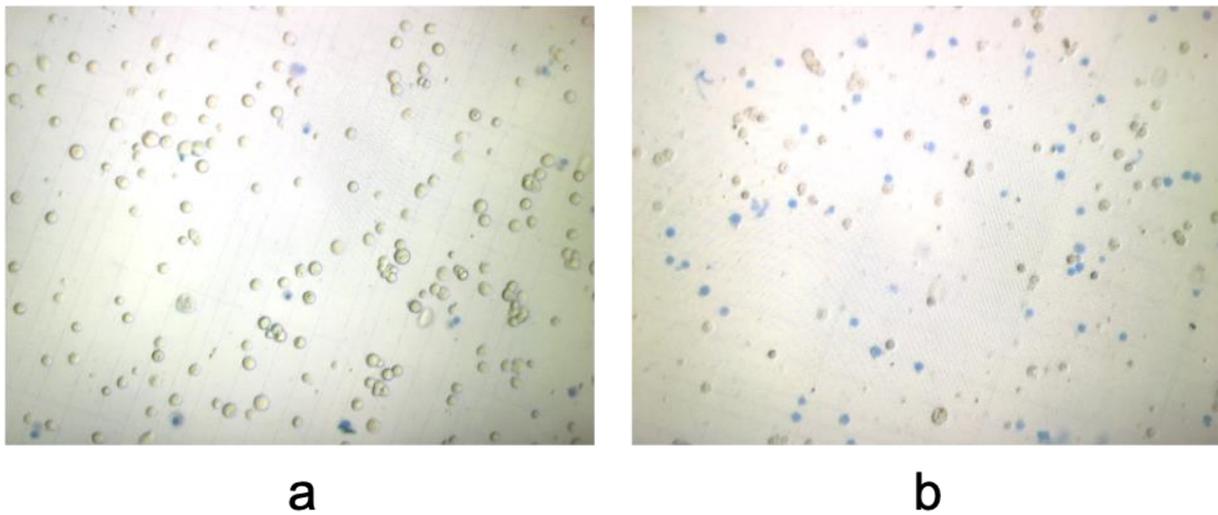
**Figure S7:**  $^1\text{H}$  NMR of **aza-SWIR-BSH-01** (500 MHz, 298 K, DMSO- $d_6$ ).



181  
182

183 **Figure S8:** <sup>13</sup>C NMR of *aza-SWIR-BSH-01* (150 MHz, 343 K, DMSO-d<sub>6</sub>).

184



185

186 **Figure S9:** U-251 cells incubated with *aza-SWIR-BSH-01* before (a) and after (b) 10 min of neutron  
187 exposure. The clichés were taken from microscopy observation. Cells were diluted with Trypan blue  
188 to count the viable cells and exclude the dead cells (in blue) on Malassez slides. The presence of huge  
189 number of blue cells in (b) evidences the strong and immediate impact of the neutron exposure on  
190 cells incubated with *aza-SWIR-BSH-01*.

191

192 **Reference**

193

- 194 1. Godard, A.; Kalot, G.; Pliquett, J.; Busser, B.; Le Guevel, X.; Wegner, K.D.; Resch-Genger, U.;  
195 Rousselin, Y.; Coll, J.L.; Denat, F., et al. Water-Soluble Aza-BODIPYs: Biocompatible  
196 Organic Dyes for High Contrast In Vivo NIR-II Imaging. *Bioconjug. Chem.* **2020**, *31*,  
197 1088-1092, doi:10.1021/acs.bioconjchem.0c00175.

198