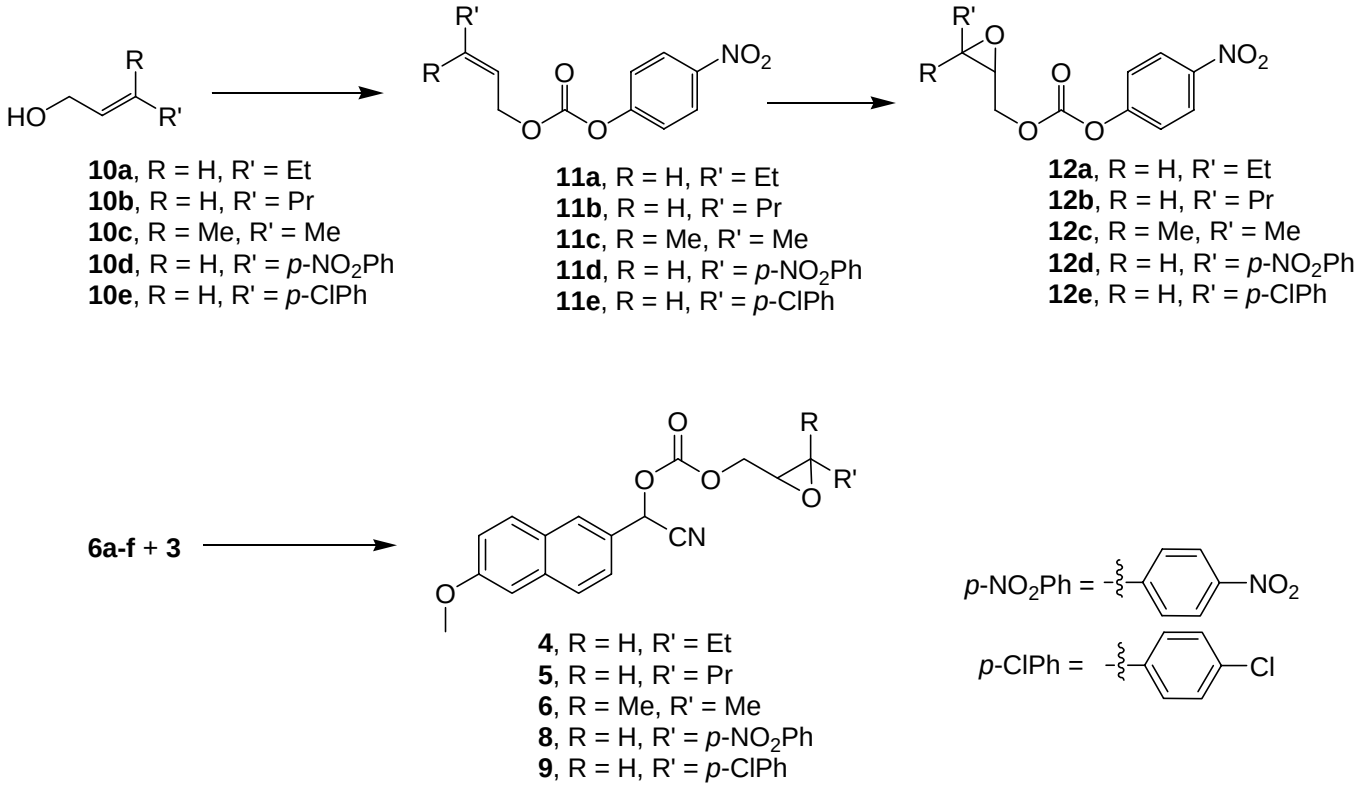
Supplemental Information

Jones et al. **Fluorescent Substrates for Soluble Epoxide Hydrolase and Application to Inhibition Studies**

Synthetic Procedures

Synthetic Route A: Synthesis of carbonates



Scheme S1: Synthetic route for the synthesis of α-cyanocarbonates

4-Nitrophenyl trans-2-pentenyl carbonate (**11a**)**.** Alcohol **10a** (1.0 mL, 9.8 mmol) and TEA (1.37 mL, 9.8 mmol) in THF (5.0 mL) were added dropwise to a 0°C solution of 4-nitrophenylchloroformate (1.98 g, 9.8 mmol) in THF (40 mL) over 30 minutes. The reaction was then stirred for 12 hrs at room temperature. The reaction was then washed repeatedly with 1 M $K_2CO_{3\,(aq)}$ until the aqueous layer was colorless. The organic layer was dried over $MgSO_4$, filtered and evaporated. The resulting residue was chromatographed on SiO_2 with dichloromethane:hexane (1:1) to give compound **11a** as a pale yellow oil (1.54 g, 63%). ^{1}H (300 MHz) δ: 8.31-8.25 (m, 2H), 7.42-7.36 (m, 2H), 5.97 (dt, *J* = 15.3, 6.2 Hz, 1H), 5.65 (dt, *J* = 15.3, 6.7 Hz, 1H), 4.72 (d, *J* = 6.68 Hz, 2H), 2.12 (p, *J* = 7.6 Hz, 2H), 1.04 (t, *J* = 7.5 Hz, 3H). ^{13}C (75 MHz) δ: 155.5, 152.3, 145.3, 140.4, 125.2, 121.7, 121.2, 70.1, 25.2, 12.0.

4-Nitrophenyl trans-((3-ethyloxiran-2-yl)methyl) carbonate **(12a).** Alkene **11a** (1.000 g, 4.0 mmol) was dissolved in dichloromethane (25 mL). To this was added *m*-CPBA (1.000g, 5.8 mmol) and the reaction was stirred for 18 hrs at room temperature. The reaction was washed with $K_2CO_{3(aq)}$ (1M, 3x25 mL) and the organic layer was dried over $MgSO_4$. The solvent was evaporated and the residue chromatographed on SiO_2 (1:1 DCM:hexane) to give compound **12a** as a clear oil (1.104g, 95%). ^{1}H (300 MHz) δ: 8.31-8.25 (m, 2H), 7.42-7.36 (m, 2H), 4.54 (dd, *J* = 12.0, 3.1 Hz, 1H), 4.16 (dd, *J* = 12.0, 6.3 Hz, 1H), 3.15-3.04 (m, 1H), 2.93 (td, *J* = 5.4, 2.1 Hz), 1.75-1.57 (m, 2H), 1.01 (t, *J* = 7.5 Hz, 3H). ^{13}C (75 MHz) δ: 155.1, 152.1, 145.1, 125.0, 121.5, 68.9, 57.2, 53.9, 24.2, 9.4

Cyano-(6-methoxy-naphthalen-2-yl)-methyl trans-((3-ethyl-oxiran-2-yl)methyl) carbonate **(4).** Compound **4** was synthesized via reaction of carbonate **12a** and aldehyde **1** as per compound **7** to give the product as a pale yellow oil (168 mg, 30%). ^{1}H (300 MHz) δ: 7.95 (d, *J* = 1.4 Hz, 1 H), 7.81 (d, *J* = 8.7 Hz, 1H), 7.78 (d, *J* = 9.1 Hz, 1H), 7.54 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.21 (dd, *J* = 8.9, 2.5 Hz, 1H), 7.15 (d, *J* =2.4 Hz, 1H), 6.39 (s, 1H), 4.48 – 4.40 (m, 1H), 4.18- 4.08 (m,1H), 3.93 (s, 3H), 3.04 – 2.99 (m, 1H), 2.89 – 2.84 (m, 1H), 1.65-1.55 (m, 2H), 0.98 (brt, *J* = 7.5 Hz, 3H). ^{13}C (75 MHz) δ: 158.9, 153.4, 135.5, 129.9, 128.2, 128.1, 125.7, 124.8, 120.0, 115.6, 105.6, 69.3, 69.0, 67.0, 57.5, 57.4, 55.3, 54.1, 24.3, 9.6. HRMS (*m/z*): calculated for $C_{38}H_{39}N_2O_{10}$ $[2M+H]^+$: 683.2605, found: 683.2618.

4-Nitrophenyl trans-2-hexenyl carbonate **(11b).** This was synthesized as above starting from *trans*-2-hexene-1-ol (**10b**) and 4-nitrophenylchloroformate as per above giving the product in 63% yield as a pale yellow oil. ^{1}H (300 MHz) δ: 8.29-8.24 (m, 2H), 7.40-7.35 (m, 2H), 5.92 (dt, *J* = 15.3, 6.8 Hz, 1H), 5.66 (dtt, *J* = 15.3, 6.7, 1.4 Hz, 1H), 4.72 (d, *J* = 6.66 Hz, 2H), 2.08 (q, *J* = 7.3, 2H), 1.44 (m, 2H), 0.92 (t, *J* = 7.5 Hz, 3H). ^{13}C (75 MHz) δ: 155.6, 152.3, 145.3, 138.8, 125.2, 122.2, 121.7, 70.1, 34.2, 21.8, 13.5.

4-Nitrophenyl trans-((3-propyloxiran-2-yl)methyl) carbonate **(12b).** Alkene **11b** was oxidized with *m*-CPBA as per above giving the product in 73% yield as a clear oil. ^{1}H (300 MHz) δ: 8.31-8.25 (m, 2H), 7.42-7.36 (m, 2H), 4.56 (dd, *J* = 12.0, 3.2 Hz, 1H), 4.18 (dd, *J* = 12.0, 6.3 Hz, 1H), 3.11-3.07 (m, 1H), 2.97-2.92 (m, 1H), 1.70-1.4 (m, 4H), 0.98 (t, *J* = 7.3 Hz, 3H). ^{13}C (75 MHz) δ: 155.2, 152.2, 145.3, 125.2, 121.6, 69.1, 56.2, 54.3, 33.3, 19.0, 13.7.

Cyano(6-methoxynaphthalen-2-yl)methyl trans-((3-propyloxiran-2-yl)methyl) carbonate **(5).** This was synthesized by reaction carbonate **12b** with aldehyde **1** as per compound **7** giving the product in 42% yield as a pale yellow oil. ^{1}H (300 MHz) δ: 7.92 (br s, 1 H), 7.81 (d, *J* = 8.9 Hz, 1H), 7.77 (d, *J* = 9.0 Hz, 1H), 7.54 (d, *J* = 8.5 Hz, 1H), 7.2 (dd, *J* = 8.9, 2.5 Hz, 1H), 7.14 (br, 1H), 6.39 (s, 1H), 4.48 – 4.40 (m, 1H), 4.18- 4.10 (m,1H), 3.92 (s, 3H), 3.02 – 2.96 (m, 1H), 2.90 – 2.84 (m, 1H), 1.60 – 1.35 (m, 4H), 1.65-1.55 (t, *J* = 7.13 Hz, 3H). ^{13}C (75 MHz) δ: 158.7, 153.1, 135.2, 129.6, 127.9, 127.8, 125.5, 124.5, 119.7, 115.4, 105.4, 69.1, 68.8, 66.7, 56.1, 56.0, 55.0, 54.1, 33.0, 18.8, 13.5. HRMS (*m/z*): calculated for $C_{40}H_{43}N_2O_{10}$ $[2M+H]^+$: 711.2918, found: 711.2911.

4-Nitrophenyl trans-3-methyl-but-2-enyl carbonate **(11c).** This was synthesized as per above starting from 3-methyl-2-butenol (**10c**) and 4-nitrophenylchloroformate, giving the product in 61% yield as a yellow oil. ^{1}H (300 MHz) δ: 8.30 – 8.22 (m, 2H), 7.43 – 7.38 (m, 2H), 5.45 (br t, *J* = 7.3 Hz, 1H), 4.78 (d, *J* = 7.3 Hz, 2H), 1.81 (s, 3H), 1.77 (s, 3H). ^{13}C (75 MHz) δ: 155.5, 152.4, 145.1, 141.4, 125.1, 121.7, 117.0, 65.9, 25.7, 18.0.

3,3-Dimethyl-oxiranylmethyl 4-nitrophenyl carbonate. Alkene (**11c**) was oxidized with *m*-CPBA as described above giving the product as a clear oil in 68% yield. ^{1}H (300 MHz) δ: 8.35 – 8.25 (m, 2H), 7.45 – 7.35 (m, 2H), 4.49 (dd, *J* = 11.9, 4.3 Hz, 1H), 4.31 (dd, *J* = 11.9, 6.8), 3.12 (dd, *J* = 6.8, 4.3, 1H), 1.40 (s, 3H), 1.38 (s, 3H). ^{13}C (75 MHz) δ: 155.2, 152.2, 145.2, 125.1, 121.6, 67.9, 59.5, 58.3, 24.3, 18.7.

Cyano-(6-methoxy-naphthalen-2-yl)-methyl 3,3-dimethyl-oxiranylmethyl carbonate **(6).** This was synthesized as compound **7**, starting from aldehyde **1** and epoxide **12c** to give the product as a pale oil in 15% yield. ^{1}H (300 MHz) δ: 7.95 (d, *J* = 1.4 Hz, 1 H), 7.81 (d, *J* = 8.7 Hz, 1H), 7.78 (d, *J* = 9.1 Hz, 1H), 7.54 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.21 (dd, *J* = 8.9, 2.5 Hz, 1H), 7.15 (d, *J* =2.4 Hz, 1H), 6.39 (s, 1H), 4.45 – 4.20 (m, 2H), 3.93 (s, 3H), 3.00-3.08 (m, 1H), 1.34 (br s, 3H), 1.32 (br s, 3H). ^{13}C (75 MHz) δ: 159.0, 153.5, 135.5, 129.9, 128.2, 128.1, 125.8, 124.8, 120.1, 115.6, 105.7, 69.0, 67.0, 59.6, 59.5, 58.4, 55.3, 24.4, 18.8. HRMS (*m/z*): calculated for $C_{38}H_{39}N_2O_{10}$ $[2M+H]^+$: 683.2605, found: 683.2576.

4-Nitrophenyl trans-(3-(4-nitro-phenyl)-allyl) carbonate **(11d).** Alcohol **10d** was reacted with 4-nitrophenylchloroformate as described above to give the product in 75% yield as an amorphous yellow solid. ^{1}H (300 MHz) δ: 8.30 – 8.26 (m, 2H), 8.21 – 8.19 (m, 2H), 7.60 – 7.54 (m, 2H), 7.42 – 7.39 (m, 2H), 6.84 (d, *J*= 15.9 Hz, 1H), 6.52 (dt, *J*= 15.9, 6.2 Hz, 1H), 4.99 (d, *J*= 6.2 Hz, 2H). ^{13}C (75 MHz) δ: 155.1, 152.0, 147.2, 145.2, 141.8, 132.7, 127.1, 125.9, 125.1, 123.8, 121.5, 68.6.

4-Nitrophenyl trans-((3-(4-nitrophenyl)-oxiranylmethyl))carbonate **(12d).** Alkene **11d** was oxidized with *m*-CPBA as described above. The product was isolated in 40% yield as a waxy white solid. ^{1}H (300 MHz) δ: 8.32-8.29 (m, 2H), 8.25-8.23 (m, 2H), 7.50-7.47 (m, 2H), 7.43-7.40 (m, 2H), 4.72 (dd, *J*= 12.9, 3.2 Hz, 1H), 4.41 (dd, 13.0, 5.3 Hz, 1H), 4.05 (br s, 1H), 3.40 – 3.34 (m, 1H). ^{13}C (75 MHz) δ: 154.9, 152.0, 147.8, 145.3, 142.8, 126.2, 125.1, 123.6, 121.4, 67.4, 58.8, 54.9.

Cyano-(6-methoxynaphthalen-2-yl)methyl trans-((3-(4-nitrophenyl)-oxiran-2-yl)methy)) carbonate **(8).** Substrate **8** was synthesized as above starting from aldehyde **1** and epoxide **12d** as per compound **7**. The product was isolated in 48% yield as a clear oil. ^{1}H (300 MHz) δ: 8.16 (dd, *J* = 8.8, 2.4 Hz, 2H), 7.96 (br s, 1H), 7.81 (d, *J* = 9.6 Hz, 1H), 7.77 (d, *J* = 9.6 Hz, 1H), 7.55 (d, *J* = 8.5 Hz, 1H), 7.38 (br d, *J* = 8.7 Hz, 2H), 7.22 (dd, *J* = 8.9, 2.4 Hz, 1H), 7.15 (br d, *J* = 2.3 Hz, 1H), 6.40 (s, 1H), 4.62 – 4.55 (m, 1H), 4.40 – 4.30 (m, 1H), 3.96 (br s, 4H), 3.29 – 3.23 (m, 1H). ^{13}C (75 MHz) δ: 159.0, 153.2, 147.9, 143.1, 135.5, 129.8, 128.2, 126.3, 125.5, 124.7, 123.7, 120.1, 115.5, 105.6, 67.7, 67.4,

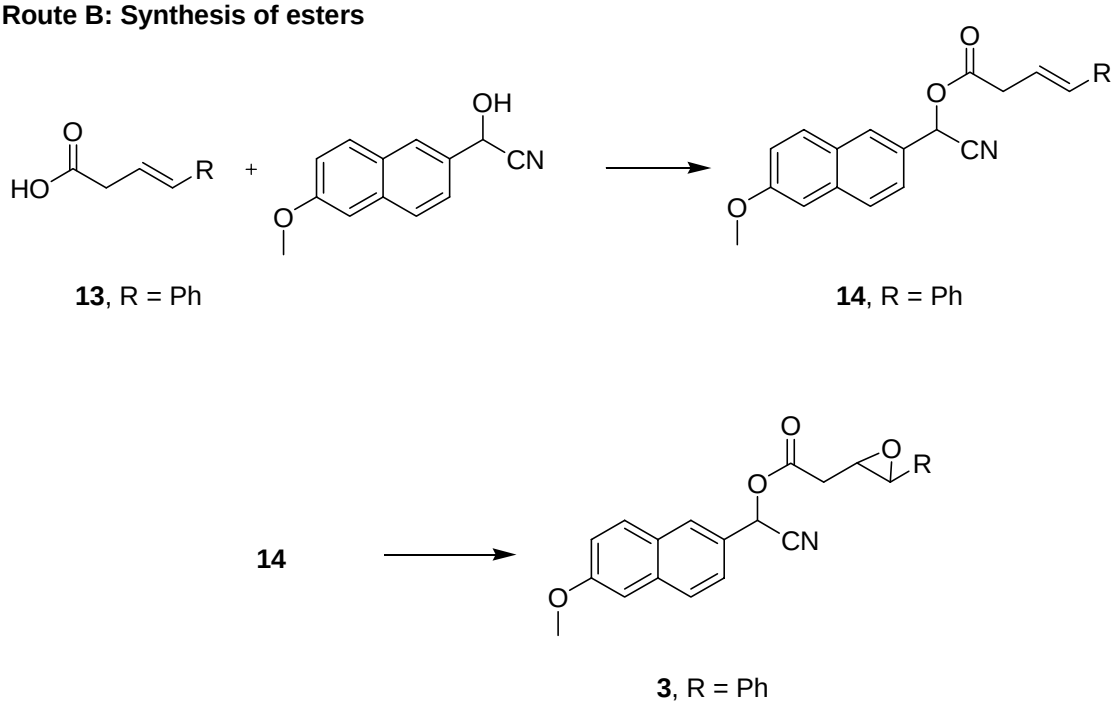
67.2, 58.9, 55.3, 55.2, 55.1. HRMS (*m/z*): calculated for $C_{23}H_{17}N_2O_7$ $[M-H]^-$: 433.1036, found: 433.1060.

4-Chlorophenyl trans-(3-(4-nitrophenyl)-allyl) carbonate **(11e).** Compound **11e** was synthesized as described above by reacting alcohol **10e** with 4-nitrophenylchloroformate. The product was isolated in 71% yield as a yellow amorphous solid. ^{1}H (300 MHz) δ: 8.31-8.26 (m, 2H), 7.42-7.26 (m, 6H), 6.72 (d, *J* = 15.9 Hz, 1H), 6.33 (dt *J* = 15.9, 6.7 Hz, 1H), 4.92 (d, *J*= 6.7 Hz, 2H). ^{13}C (75 MHz) δ: 155.4, 152.3, 145.3, 134.7, 134.2, 134.1, 128.8, 127.9, 125.2, 121.8, 121.7, 69.5.

4-Chlorophenyl trans-((3-(4-nitrophenyl)-oxiranylmethyl))carbonate **(12e).** Compound **12e** was synthesized by oxidation of alkene **11e** with *m*-CPBA as above. The product was isolated in 77% yield as an amorphous white solid. ^{1}H (300 MHz) δ: 8.29-8.26 (m, 2H), 7.40-7.37 (m, 2H), 7.34 – 7.31 (m, 2H), 7.23-7.20 (m, 2H), 4.66 (dd, *J* = 12.1, 3.1 Hz, 1H), 4.33 (dd, *J* = 12.1, 5.6 Hz, 1H) 3.88 (d, *J* = 1.8 Hz, 1H), 3.33 – 3.30 (m, 1H). ^{13}C (75 MHz) δ: 155.2, 152.3, 145.4, 134.5, 134.2, 128.8, 126.9, 125.3, 121.7, 68.1, 58.5, 55.6.

Cyano-(6-methoxy-naphthalen-2-yl)methyl trans-((3-(4-chlorophenyl)-oxiranylmethyl)) carbonate **(9).** Substrate **9** was synthesized as per compound **7** by reaction of aldehyde **1** with epoxide **12e**. The product was isolated in 67% yield as a clear oil. ^{1}H (300 MHz) δ: 7.94 (s, 1H), 7.78 (d, *J* = 9.3 Hz, 1H), 7.76 (d, *J* = 9.3 Hz, 1H), 7.30-7.12 (m, 6H), 6.39 (s, 1H), 4.58 – 4.53 (m, 1H), 4.30 – 4.20 (m, 1H), 3.91 (s, 3H), 3.81 – 3.78 (m, 1H), 3.25 – 3.20 (m, 1H). ^{13}C (75 MHz) δ: 158.9, 153.2, 135.4, 134.3, 134.2, 129.8, 128.7, 128.1, 128.0, 126.9, 125.6, 124.7, 120.0, 115.6, 105.6, 68.2, 67.9, 67.1, 58.4, 55.6, 55.3. HRMS (*m/z*): calculated for $C_{46}H_{37}Cl_2N_2O_{10}$ $[2M+H]^+$: 847.1825, found: 847.1823.

Synthetic Route B: Synthesis of esters



Scheme S2: Synthetic route for the synthesis of α-cyanoesters

Cyano-(6-methoxy-naphthalen-2-yl)methyl trans-((2-(2-Phenylethenyl)))acetate **(14).** Ester **14** was synthesized in a manner similar to cyano-(6-methoxynaphthalen-2-yl)methyl (*trans*-2-(pentenyl))acetate (see main article) starting with hydroxy-(6-methoxy-naphthalen-2-yl)-acetonitrile and *trans*-styrylacetic acid (**13**). The product was isolated as a clear oil in 57% yield. ^{1}H (300 MHz) δ: 7.92 (s, 1H), 7.79 (d, *J* = 8.6 Hz, 1H), 7.74 (d, *J* = 9.0 Hz, 1H), 7.52 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.35 – 7.18 (m, 6H), 7.13 (d, *J* = 2.4 Hz, 1H), 6.58 (s, 1H), 6.49 (d, *J* = 15.8 Hz, 1H), 6.24 (dt, *J* = 15.9, 7.0 Hz, 1H), 3.91 (s, 3H), 3.41-3.26 (m, 2H). ^{13}C (75 MHz) δ: 169.6, 158.8, 136.3, 135.2, 134.4, 129.8, 128.5, 128.1, 128.0, 127.8, 127.7, 126.3, 126.2, 124.8, 119.9, 119.8, 116.1, 105.6, 63.3, 55.3, 37.5.

Cyano(2-methoxynaphthalen-6-yl)methyl trans-(2-(3-phenyloxiran-2-yl))acetate (**3**). Epoxide **3** was synthesized following the procedure for the synthesis of compound **2** to give the product in 20% yield as a clear oil. ^{1}H (300 MHz) δ: 7.94 (br s, 1H), 7.80 (d, *J* = 8.8 Hz, 1H), 7.77 (d, *J* = 10.7 Hz, 1H), 7.54-7.49 (m, 1H), 7.34-7.20 (m, 6H), 7.15 (d, *J* = 2.4 Hz, 1H), 6.61 (br s, 1H), 3.94 (s, 3H), 3.72 – 3.70 (m, 1H), 3.36 – 3.32 (m, 1H), 2.94 – 2.74 (m, 2H). ^{13}C (75 MHz) δ: 168.4, 158.9, 136.1, 135.4, 129.8, 128.5, 128.4,

128.2, 128.1, 128.0, 125.6, 124.8, 120.0, 105.6, 126.2, 115.9, 63.5, 58.0, 57.1, 55.3, 37.3. HRMS (*m/z*): calculated for $C_{46}H_{39}N_2O_8$ $[2M+H]^+$: 747.2706, found: 747.2704.