Synthesis of BODIPY-labeled sphingosine derivatives by cross-metathesis reaction

Carsten Peters, Andreas Billich, Michael Ghobrial, Klemens Högenauer, Thomas Ullrich and

*Peter Nussbaumer**

Supporting Information:

General procedures	S2
4,4-Difluoro-5,7-dimethyl-4-bora-3a,4a-diaza- <i>s</i> -indacen-3-undec-10-ene 6b	S3
(<i>S</i>)-4-[(<i>E</i>)-(<i>R</i>)-1-Hydroxy-12-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-dodec-2-enyl]-2,2-dimethyl-oxazolidine-3-carboxylic acid <i>tert</i> -butyl ester 10a	S3
(<i>S</i>)-4-[(<i>E</i>)-(<i>R</i>)-1-Hydroxy-7-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza- <i>s</i> -indacen-3)-hept-2-enyl]-2,2-dimethyl-oxazolidine-3-carboxylic acid <i>tert</i> -butyl ester 10b	S4
(<i>E</i>)-(2 <i>S</i> ,3 <i>R</i>)-2-Amino-14-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-tetradec-4-ene-1,3-diol 11a	S4
(<i>E</i>)-(2 <i>S</i> ,3 <i>R</i>)-2-Amino-9-(4,4-Difluoro-5,7-dimethyl-4-bora-3a,4a-diaza- <i>s</i> -indacen-3)-non-4-ene-1,3-diol 11b	S5
¹ HNMR and ¹³ CNMR spectra of compounds 6a-b , 7 , 10 a-d , 11 a-d	S6-S14
Quantitative HPLC-analysis of compounds 11b-d	S14-S16

General. Solvents used were dried over molecular sieves or bought as absolute solvents. Flash-chromatography was performed using Biotage SP4 with cyclohexane/ ethyl acetate in various proportions as eluent. In NMR spectra, multiplicities are given as singlet (s), doublet (d), triplet (t), quadruplet (q), quintet (quin). Coupling constants are given in Hz. All commercially available compounds were used as received.

General procedure cross-metathesis. For cross-metathesis reaction Garner-allylic alcohol (*anti-9*)* and alkenylated label at a stoichiometric ratio of 1:1 to 1:3 were dissolved in dry CH₂Cl₂ and the resulting solution was degassed carefully. Then, under argon-atmosphere, a catalytic amount of 2nd generation Grubbs' catalyst was added and the reaction mixture was allowed to reflux for 2-5 h until TLC indicated no further change in composition of the reaction mixture. Afterwards solvent was removed *in vacuo* and the crude material was purified by flash column chromatography.

General procedure deprotection. For removal of Boc-/acetal protection the product of the cross-metathesis reaction was dissolved at room temperature in an appropriate amount of 4M HCl/dioxane and stirred for approx. 20 min (TLC monitoring). After removal of the solvent the crude material was purified by preparative RP-HPLC and the product containing fractions were pooled and lyophilized.

* this compound was prepared according to reference 11 and separation of diastereomers was performed on normal phase HPLC using a Nucleosil100 Si 5 µm column (250*40 mm) with heptane:ethylacetate 7:3 (v/v) (isocratic) as eluent at a flow rate of 30 ml/min.

4,4-Difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacen-3-undec-10-ene 6b.

By pursuing the same strategy as described for compound **6a**, compound **6b** was obtained as a dark red oil starting from dipyrromethene **5b** (340 mg, 1.05 mmol); yield: 350 mg (0.94 mmol, 89 %); ¹H-NMR (500MHz, CDCl₃) δ 1.24–1.46 (m, 12H), 1.72 (quin, *J*= 7.6 Hz, 2H), 2.03 (q, *J*= 7.4 Hz, 2H), 2.24 (s, 3H), 2.55 (s, 3H), 2.96 (t, *J*= 7.9 Hz, 2H), 4.92 (dd, *J*= 10.2 Hz, 1.1 Hz, 1H), 4.99 (dd, *J*= 17.2 Hz, 1.9 Hz, 1H), 5.81 (ddt, *J*= 16.6 Hz, 6.8 Hz, 6.6 Hz 1H), 6.08 (s, 1H), 6.28 (d, *J*= 4.0 Hz, 1H), 6.90 (d, *J*= 4.0 Hz, 1H), 7.05 (s, 1H); ESI-MS⁺: 373.2 [M+Na]; HRMS: M calcd for C₂₂H₃₂BF₂N₂ 372.2657, found 372.2658.

(S)-4-[(E)-(R)-1-Hydroxy-12-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-dodec-2-enyl]-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester 10a.

According to the general procedure, Garner allylic alcohol (*anti*-**9**) (65 mg, 0.25 mmol) were reacted with alkenylated-NBD dye **2** (100 mg, 0.30 mmol) and 2nd gen. Grubbs' catalyst (21 mg, 0.025 mmol): yield 95 mg (0.17 mmol, 66%); orange oil; ¹H-NMR (400MHz, CDCl₃) δ 1.31 (s, 6H), 1.32–1.68 (m, 12H), 1.49 (s, 9H), 1.81(quin. *J*= 7.8 Hz, 2H), 2.04 (q, *J*= 7.0 Hz, 2H), 3.48 (q, *J*= 7.0 Hz, 2H), 3.76–4.27 (m, 4H), 5.45 (dd, *J*= 15.4 Hz, 6.1 Hz, 1H), 5.45 (td, *J*= 14.1 Hz, 6.4 Hz, 1H), 6.18 (d, *J*= 8.6 Hz, 1H), 6.29 (s, 1H), 8.50 (d, *J*= 8.6 Hz, 1H); ¹³C-NMR (125.7 MHz) δ 26.3, 26.9, 28.4, 28.5, 28.6, 29.0, 29.1, 29.2, 29.3, 29.4, 29.5, 32.3, 44.1, 94.4, 98.5, 123.6, 128.2, 129.0, 133.2, 133.8, 136.5, 143.9, 144.1, 144.3; ESI-MS⁺: 584.4 [M+Na]; HRMS: M calcd for C₂₈H₄₃N₅O₇Na 584.3061, found 584.3054.

(S)-4-[(E)-(R)-1-Hydroxy-7-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacen-3)-hept-2-enyl]-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester 10b.

According to the general procedure, Garner allylic alcohol (*anti*-**9**) (111 mg, 0.43 mmol) were reacted with C6-alkenyl-BDP **7** (65 mg, 0.22 mmol) and 2nd gen. Grubbs' catalyst (18 mg, 0.022 mmol): yield 77 mg (0.14 mmol, 79%); orange oil; ¹H-NMR (400MHz, CDCl₃) δ 1.46–1.50 (m, 15H), 1.53 (quin. *J*= 7.7 Hz, 2H), 1.75 (quin. *J*= 7.7 Hz, 2H), 2.12 (q, *J*= 7.4 Hz, 2H), 2.24 (s, 3H), 2.55 (s, 3H), 2.97 (t, *J*= 7.8 Hz, 2H), 3.78–4.25 (m, 4H), 5.47 (dd, *J*= 15.3 Hz, 6.1 Hz, 1H), 5.74 (td, *J*= 15.3 Hz, 6.1 Hz, 1H), 6.08 (s, 1H), 6.28 (d, *J*= 4.0 Hz, 1H), 6.90 (d, *J*= 4.0 Hz, 1H), 7.06 (s, 1H); ESI-MS⁺: 554.3 [M+Na].

(E)-(2S,3R)-2-Amino-14-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-tetradec-4-ene-1,3-diol 11a.

According to the general procedure fully protected NBD-labeled sphingosine **10a** (50 mg, 0.09 mmol) were treated with 4 ml of 4M HCl/ dioxane and afterwards purified; yield 26 mg (0.06 mmol, 70%); orange lyophilisate; ¹H-NMR (400MHz, d₄-MeOD) δ 1.28–1.33 (m, 16H), 1.75–1.83 (m, 2H), 2.10 (q *J*= 7.4 Hz, 2H), 3.20–3.23 (m, 1H), 3.48–3.53 (m, 1H), 3.45 (dd, *J*= 11.6 Hz, 4.7 Hz, 1H), 3.81 (dd, *J*= 11.6 Hz, 4.0 Hz, 1H), 4.29 (t, *J*= 5.4 Hz, 1H), 5.48 (dd, *J*= 15.2 Hz, 6.0 Hz, 1H), 5.84 (td, *J*= 14.3 Hz, 6.3 Hz, 1H), 6.35 (d, *J*= 8.9 Hz, 1H), 8.55 (d, *J*= 8.9 Hz, 1H); ¹³C-NMR (125 MHz, CDCl₃) δ 26.6, 28.7, 28.9, 29.0, 29.1, 29.2, 31.9, 57.2, 58.0, 69.6, 97.9, 127.0, 135.1, 137.0; ESI-MS⁺: 445.1 [M+Na]; HRMS: M calcd for C₂₀H₃₁N₅O₅Na 444.2223, found 444.2216.

(E)-(2S,3R)-2-Amino-9-(4,4-Difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacen-3)-non-4-ene-1,3-diol 11b.

According to the general procedure, fully protected BDP-labeled sphingosine **10b** (77 mg, 0.14 mmol) was treated with 4 ml of 4M HCl/ dioxane and afterwards purified; yield 35 mg (0.07 mmol, 48%); orange lyophilisate; ¹H-NMR (400MHz, DMSO-d₆) δ 1.53 (quin. *J*= 7.7 Hz, 2H), 1.68 (quin. *J*= 7.7 Hz, 2H) 2.06 (q *J*= 7.3 Hz, 2H), 2.25 (s, 3H), 2.43 (s, 3H), 2.83 (t, *J*= 7.7 Hz, 2H), 2.97–3.06 (m, 1H), 3.45 (dd, *J*= 11.3 Hz, 2.9 Hz, 1H), 3.59 (dd, *J*= 11.3 Hz, 4.1 Hz, 1H), 4.17 (dd, *J*= 9.2 Hz, 4.5 Hz, 1H), 5.03-5.19 (bs, 2H), 5.45 (dd, *J*= 15.3 Hz, 6.6 Hz, 1H), 5.74 (td, *J*= 15.3 Hz, 6.9 Hz, 1H), 6.27 (s, 1H), 6.40 (d, *J*= 3.9 Hz, 1H), 7.10 (d, *J*= 3.9 Hz, 1H), 7.66 (s, 1H), 7.74 (bs, 3H); ¹³C-NMR (100 MHz) δ 11.2, 14.6, 27.8, 27.9, 28.5, 31.4, 57.0, 58.1, 69.0, 117.0, 120.2, 125.3, 128.7, 129.3, 132.9, 133.0, 134.3, 143.8, 158.6, 159.3; ESI-MS⁺: 392.2 [M+H]; HRMS: M calcd for C₂₀H₂₈BF₂N₃O₂Na 413.2171, found 413.2171.

4,4-Difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacen-3-hex-5-ene **6a**

4,4-Difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacen-3-undec-10-ene **6b**

4,4-Difluoro-1,3,5,7-tetramethyl-8-(dec-9-en)-4-bora-3a,4a-diaza-s-indacene **7**

(S)-4-[(E)-(R)-1-Hydroxy-12-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-dodec-2-enyl]-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester **10a**.

(S)-4-[(E)-(R)-1-Hydroxy-7-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacen-3)-hept-2-enyl]-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester **10b**

(S)-4-[(E)-(R)-1-Hydroxy-11-(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene-8)-undec-2-enyl]-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butylester **10d**

(E)-(2S,3R)-2-Amino-14-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-tetradec-4-ene-1,3-diol

11a

(E)-(2S,3R)-2-Amino-9-(4,4-Difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacen-3)-non-4-ene-1,3-diol **11b**

(E)-(2S,3R)-2-Amino-14-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacen-3)-
tetradec-4-ene-1,3-diol **11c**

(E)-(2S,3R)-2-Amino-13-(4,4-Difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene-8)-tridec-4-ene-1,3-diol **11d**

Quantitative HPLC-analysis of compounds **11b-d**

Determination of fluorescence-purity was carried out on a Hewlett-Packard 1090M system using a Phenomenex Gemini C18 100*4 mm; 5 μ m column. Fluorophore was excited at λ = 502 nm and emission was measured at λ = 515 nm. Determination of UV-purity was carried out on a Agilent 1100 system using a XTerra RP18 4.6*50 mm, 3.5 μ m column. In both cases a gradient of acetonitrile in 10 mM phosphate-buffer (adjusted to pH 5) starting from 5% of acetonitrile to 95% within 8 min was used.

HPLC spectra of compound **11b**

Fluorescence detection, excitation wavelength λ = 502 nm; purity 97.9% (according to integration)

UV-detection, $\lambda = 220$ nm, purity 98.2% (according to integration)

HPLC spectra of compound **11c**

Fluorescence detection, excitation wavelength $\lambda = 502$ nm; purity 97.9% (according to integration)

UV-detection, $\lambda = 220$ nm, purity 98.2% (according to integration)

HPLC spectra of compound **11d**

Fluorescence detection, excitation wavelength $\lambda = 502$ nm; purity 96.8% (according to integration)

UV-detection, $\lambda = 220$ nm, purity 97.1% (according to integration)