Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting information

Photochromic peptidic NPY-receptor ligands

D. Lachmann[a], A. Konieczny[b], M. Keller[b] and B. König[a]

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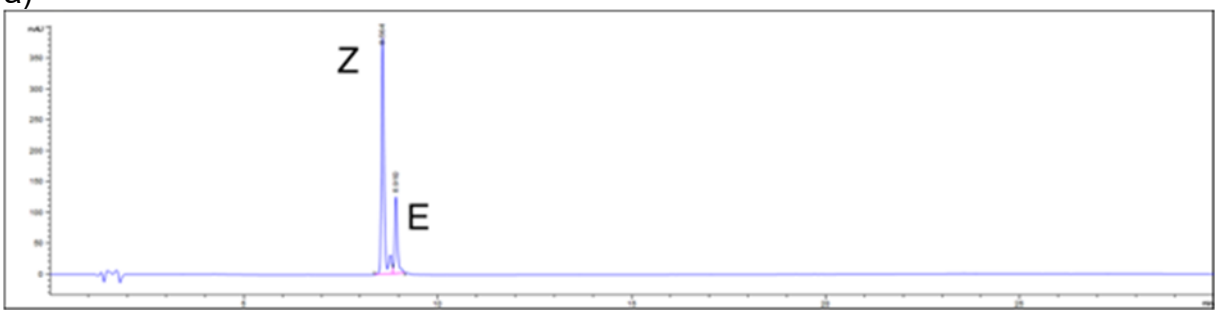
1. PSS determination via HPLC

1.1 Azobenzene **2**

The photochemical isomerization of compound **2** is depicted in **Figure S1**. The *E*→*Z* isomerization was performed with a 365 nm LED (SSC VIOSYS, 700 mA, 1250 mW) and the *Z*→*E* isomerization with a 455 nm LED (OSRAM Oslon SSL 80 blue, 1000 mA, 1480 mW). Conditions analytical HPLC: column *Phenomenex Luna*, 3µ C18(2) 100A, 150 x 2.0 mm, 100 Å, 15 °C, solvent A: H_2O (0.05% TFA), solvent B: MeCN; gradient A/B: 0-20 min: 90/10, 20-30 min: 2/98.

a)



b)

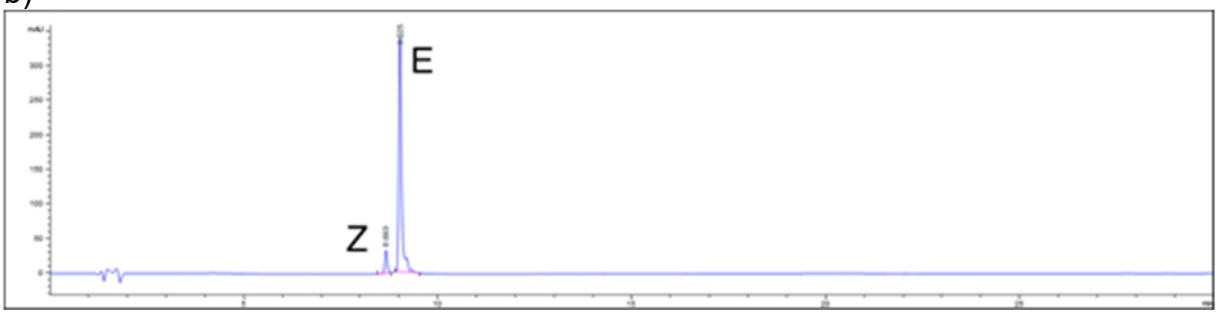
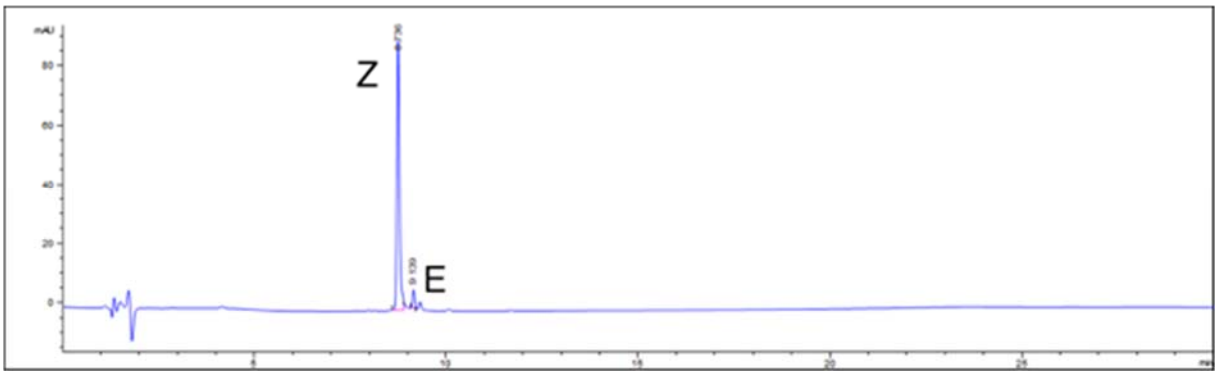


Figure S1. PSS determination via HPLC measurements at the isosbestic point of azobenzene **2** ($t_{R(E)}$ = 9.0 min, $t_{R(Z)}$ = 8.6 min). a)*E*→*Z* isomerization. b)*Z*→*E* isomerization.

1.2 Azopyrazole **3**

The photochemical isomerization of compound **3** is depicted in **Figure S2**. The *E*→*Z* isomerization was performed with a 365 nm LED (SSC VIOSYS, 700 mA, 1250 mW) and the *Z*→*E* isomerization with a 528 nm LED (OSRAM Oslon SSL 80 green, 500 mA, 34 mW). Conditions analytical HPLC: column *Phenomenex Luna*, 3µ C18(2) 100A, 150 x 2.0 mm, 100 Å, 15 °C, solvent A: H_2O (0.05% TFA), solvent B: MeCN; gradient A/B: 0-20 min: 90/10, 20-30 min: 2/98.

a)



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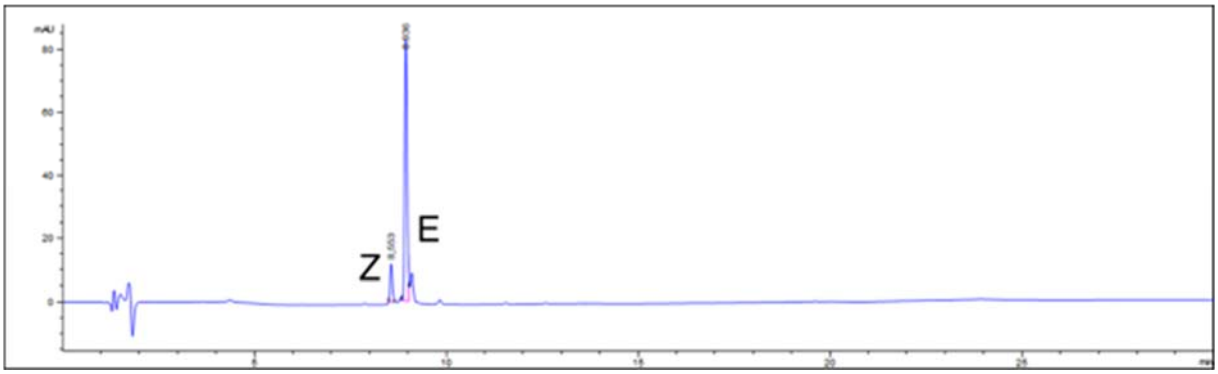
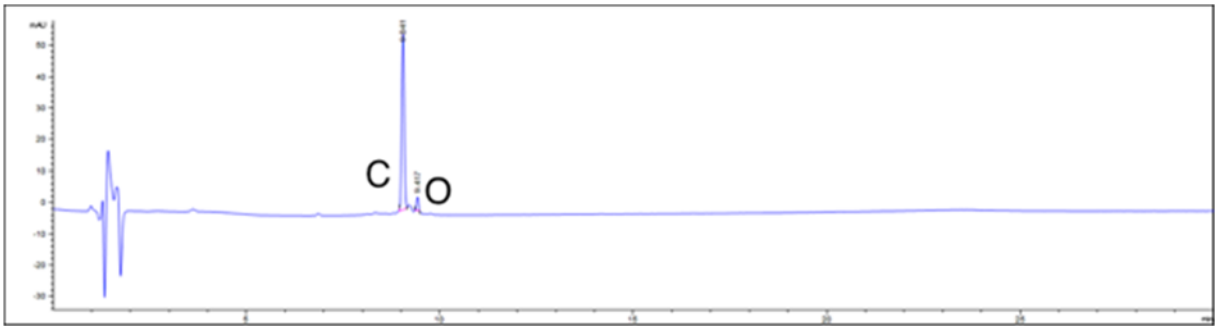


Figure S2. PSS determination via HPLC measurements at the isosbestic point of azopyrazole **3** ($t_{R(E)}$ = 8.9 min, $t_{R(Z)}$ = 8.6 min). a) *E*→*Z* isomerization. b) *Z*→*E* isomerization.

1.3 Dithienylethene **4**

The photochemical isomerization of compound **4** is depicted in **Figure S3**. The *O*→*C* isomerization was performed with a 312 nm tube lamp (Herolab hand-held lamp UV-6 M, 6 W) and the *C*→*O* isomerization with a 528 nm LED (OSRAM Oslon SSL 80 green, 500 mA, 34 mW). Conditions analytical HPLC: column *Phenomenex Luna*, 3µ C18(2) 100A, 150 x 2.0 mm, 100 Å, 25 °C, solvent A: H_2O (0.05% TFA), solvent B: MeCN; gradient A/B: 0-20 min: 90/10, 20-30 min: 2/98

a)



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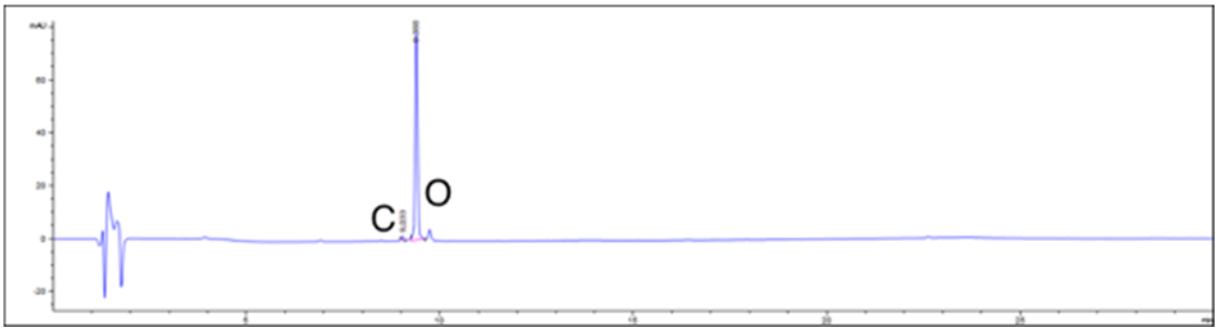
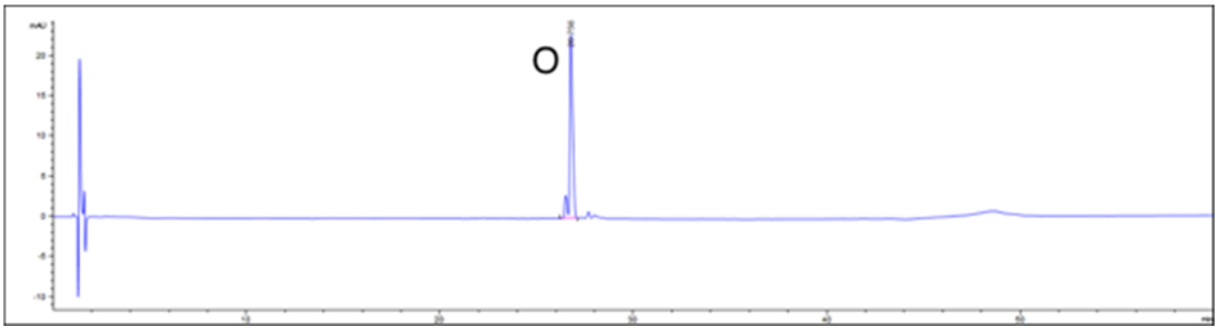


Figure S3. PSS determination via HPLC measurements at the isosbestic point of compound **4** ($t_{R(O)}$ = 9.4 min, $t_{R(C)}$ = 9.0 min). a) *O*→*C* isomerization. b) *C*→*O* isomerization.

1.4 Fulgimide **5**

The photochemical isomerization of compound **5** is depicted in **Figure S4**. The *O*→*C* isomerization was performed with a 365 nm LED (SSC VIOSYS, 700 mA, 1250 mW) and the *C*→*O* isomerization with a 528 nm LED (OSRAM Oslon SSL 80 green, 500 mA, 34 mW). Conditions analytical HPLC: column *Phenomenex Luna*, 3µ C18(2) 100A, 150 x 2.0 mm, 100 Å, 15 °C, solvent A: H_2O (0.05% TFA), solvent B: MeCN; gradient A/B: 0-40 min: 90/10, 40-50 min: 55/45, 50-60 min: 2/98).

a)



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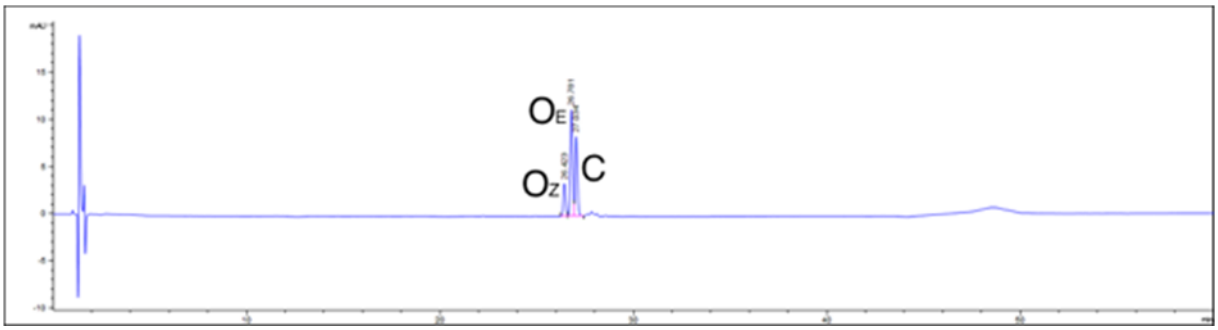
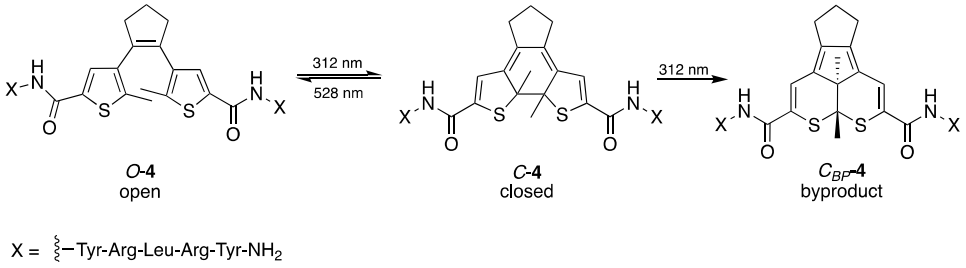


Figure S4. PSS determination via HPLC measurements at the isosbestic point of compound **5** ($t_{R(Z)}$ = 26.4 min, $t_{R(E)}$ = 26.8 min, $t_{R(C)}$ = 27.0 min). a) *O*→*C* isomerization. b) *C*→*O* isomerization.

2. Byproduct formation of compound **4**



Scheme S1. Formation of an irreversible byproduct C_{BP}-**4** upon irradiation with light of 312 nm (Herolab hand-held lamp UV-6 M, 6 W) after 5 min.

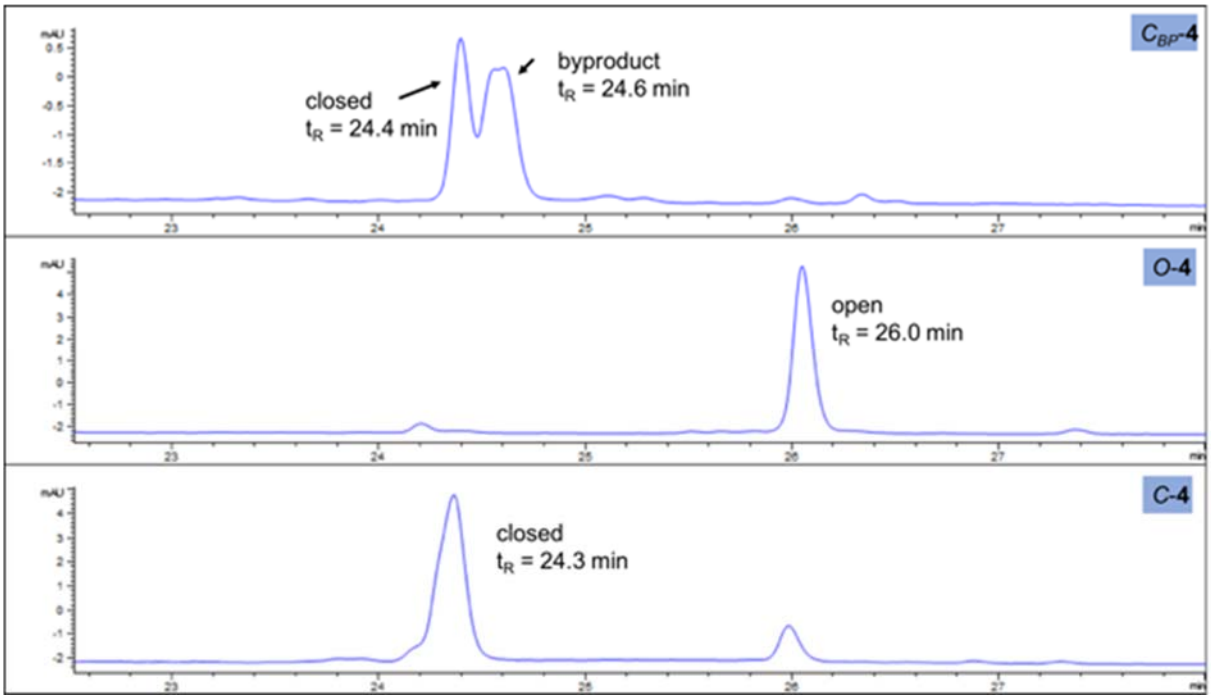


Figure S5. HPLC measurements, the three chromatograms are depicted to show the byproduct formation. Chromatogram C_{BP}-**4** was measured after 5 min irradiation with 312 nm

light. (Method HPLC: column *Phenomenex Luna*, 3µ C18(2) 100A, 150 x 2.0 mm, 100 Å, 25 °C, solvent A: H_2O (0.05% TFA), solvent B: MeCN; gradient A/B: 0-40 min: 90/10, 40-50 min: 55/45, 50-60 min: 2/98)

3. Thermal half-life of compound **2** and **3**

To determine the thermal half-lives, the samples were first irradiated until the photostationary state (PSS) was reached. The samples were left for thermal relaxation at 25 °C and the recovery of the absorbance of the *E*-isomer at λ_{max} was measured. The calculation of the thermal half-life was done by fitting the data with an exponential function (**Figure S6**).

a) b)

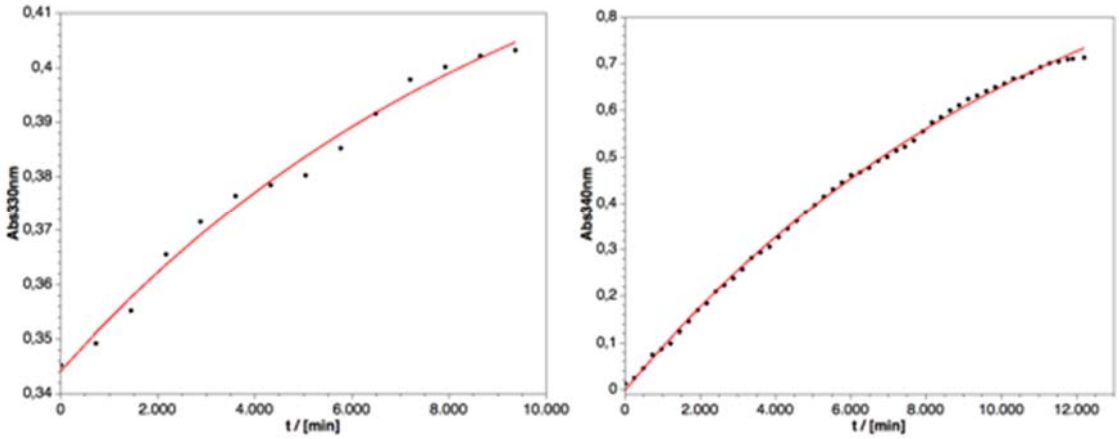


Figure S6. Half-life determination of the azo based compounds. a) Azobenzene **2**; b) Azopyrazole **3**.

4. Biological characterization: displacement curves from radioligand competition binding assays and concentration-effect curves from functional assays

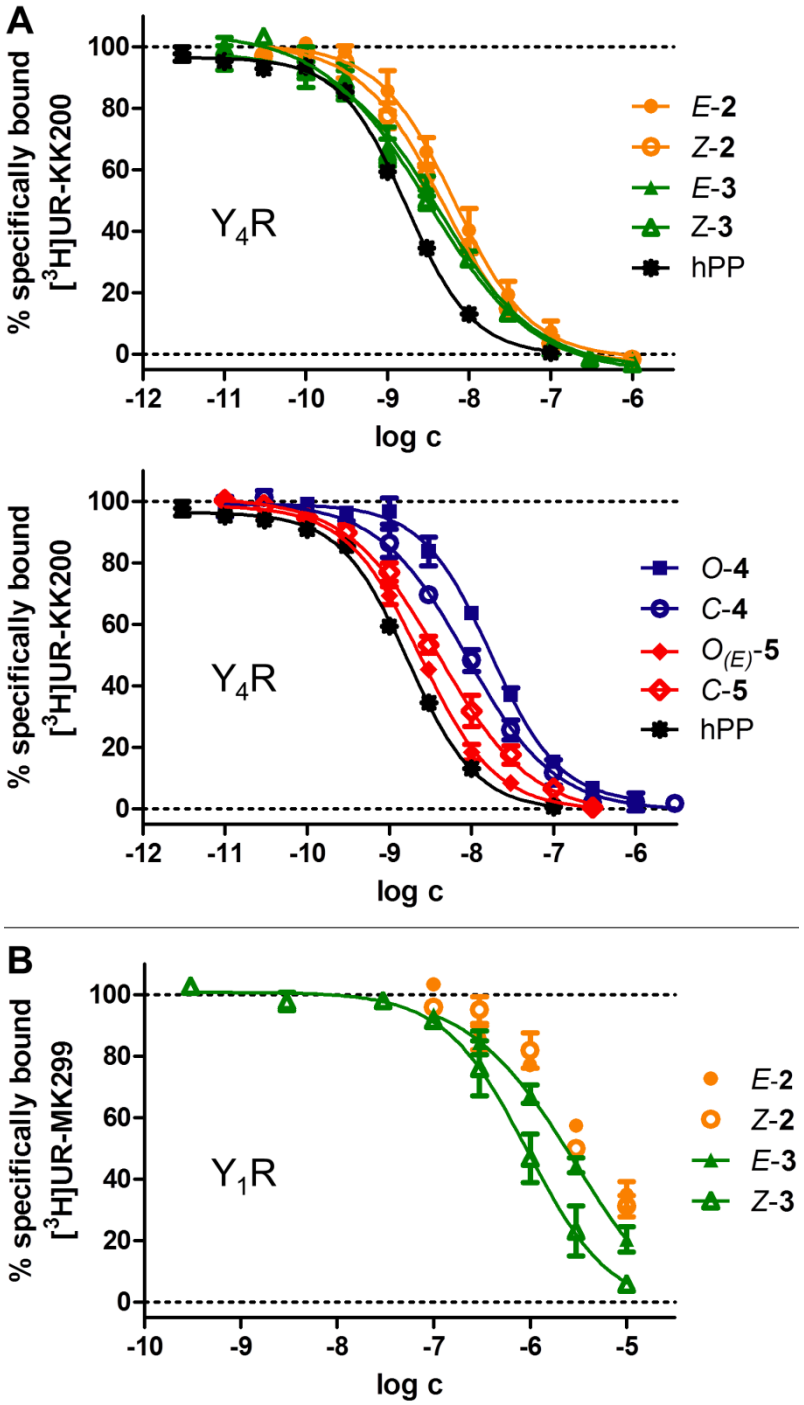


Figure S7. A: Displacement curves obtained from competition binding experiments with the Y_4R radioligand [^{3}H]UR-KK200 (K_d = 0.67 nM, c = 1 nM) and *E*/*Z*-**2***, *E*/*Z*-**3***, *O*/*C*-**4*** and $O_{(E)}$/*C*-**5*** performed at CHO-hY_4R-mtAEQ-G_{qi5} cells. B: Displacement curves obtained from competition binding experiments with the Y_1R radioligand [^{3}H]UR-MK299 (K_d = 0.044 nM, c = 0.15 nM) and *E*/*Z*-**2*** and *E*/*Z*-**3*** performed at SK-N-MC neuroblastoma cells. Data (A, B) represent means ± SEM from three or four independent experiments performed in triplicate. Data were analyzed by four parameter logistic fits (GraphPad Prism 5.0). In B, data of *E*/*Z*-**2** did not allow an analysis by a four parameter logistic fit.

*For an approximation of achieved *E*/*Z* and *O*/*C* ratios (PSS) see **Tables 1** and **2** (main article).

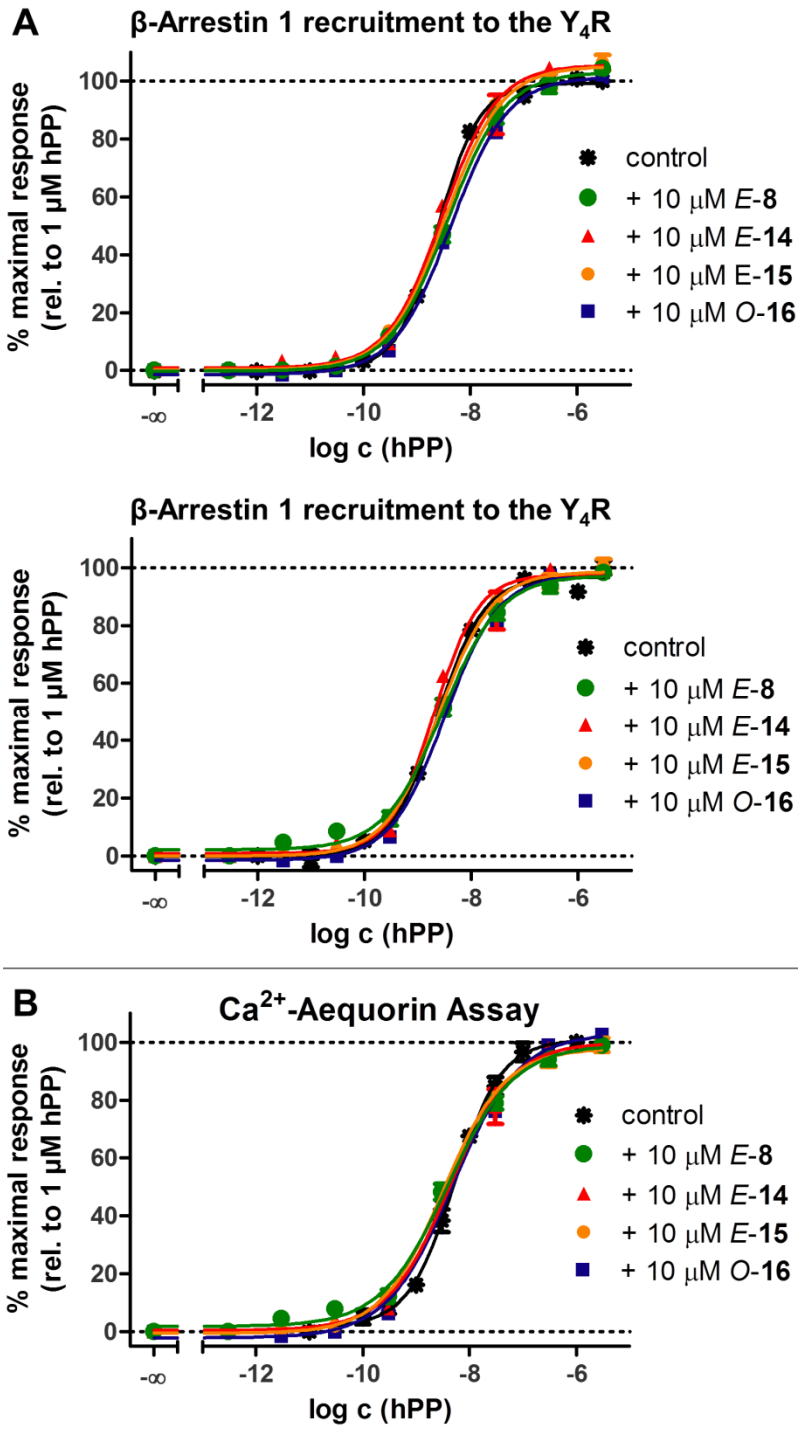


Figure S8. Control experiments to investigate whether the photochromic core structures **8**, **14**, **15** and **16** influence the readout of the functional Y_4R aequorin and arrestin assay. A: Concentration-response curves of hPP (β-arrestin 1 and 2 recruitment assay using HEK293T-ARRB1-Y_4R or HEK293T-ARRB2-Y_4R cells) in the absence and presence of *E*-**8**, *E*-**14**, *E*-**15** and *O*-**16**. B: Concentration-response curves of hPP (Ca^{2+}-aequorin assay using CHO-hY_4R-mtAEQ-G_{qi5} cells) in the absence and presence *E*-**8**, *E*-**14**, *E*-**15** and *O*-**16**. Data (A, B) represent means ± SEM from three or four independent experiments performed in triplicate. Data were analyzed by four parameter logistic fits (GraphPad Prism 5.0). Cellular responses were normalized to the effect of hPP elicited at a concentration of 1 µM. In both assays, *E*-**8**, *E*-**14**, *E*-**15** and *O*-**16** did not affect the assay readout.

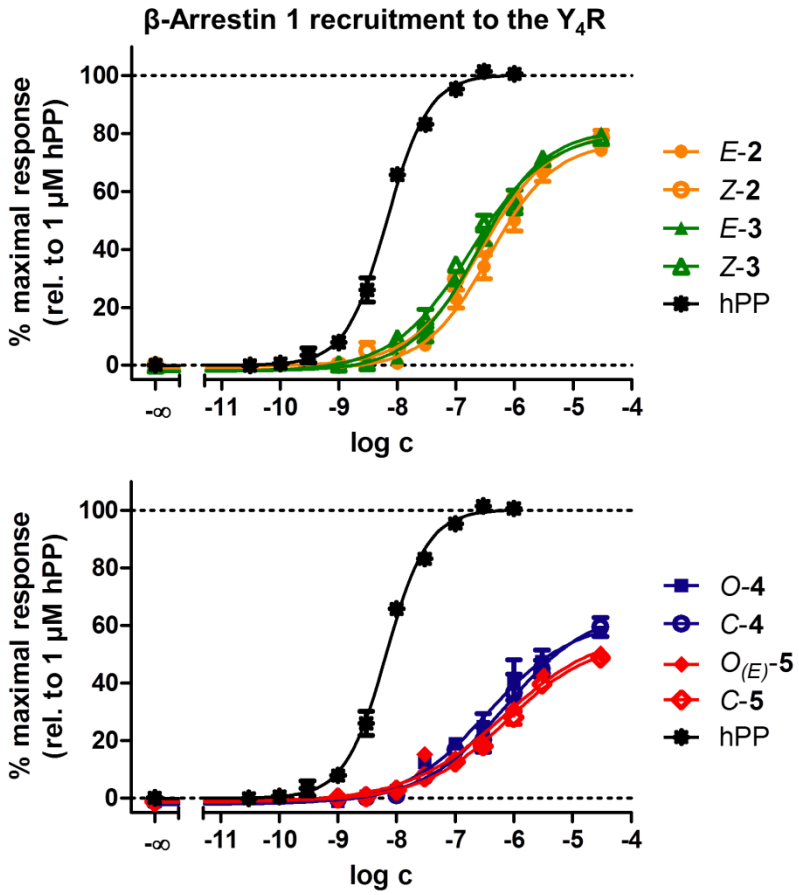


Figure S9. Y_4R functional activities (concentration-response curves) of *E*/*Z*-**2***, *E*/*Z*-**3***, *O*/*C*-**4*** and $O_{(E)}$/*C*-**5*** and the endogenous agonist hPP determined by measuring β-arrestin 1 recruitment to the Y_4R using HEK293T-ARRB1-Y_4R cells. Data represent means ± SEM from three or four independent experiments performed in triplicate. Data were analyzed by four parameter logistic fits (GraphPad Prism 5.0). Cellular responses were normalized to the effect of hPP elicited at a concentration of 1 µM.
*For an approximation of achieved *E*/*Z* and *O*/*C* ratios (PSS) see **Tables 1** and **2** (main article).

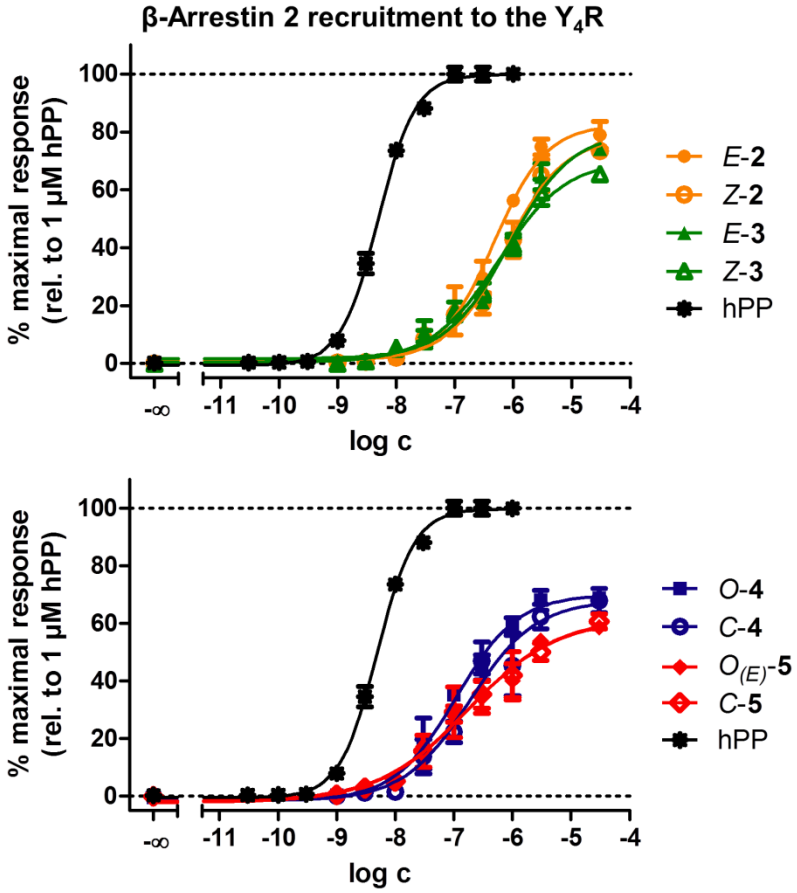


Figure S10. Y_4R functional activities (concentration-response curves) of *E*/*Z*-**2***, *E*/*Z*-**3***, *O*/*C*-**4*** and $O_{(E)}$/*C*-**5*** and the endogenous agonist hPP determined by measuring β-arrestin 2 recruitment to the Y_4R using HEK293T-ARRB2-Y_4R cells. Data represent means ± SEM from three or four independent experiments performed in triplicate. Data were analyzed by four parameter logistic fits (GraphPad Prism 5.0). Cellular responses were normalized to the effect of hPP elicited at a concentration of 1 µM.
*For an approximation of achieved *E*/*Z* and *O*/*C* ratios (PSS) see **Tables 1** and **2** (main article).

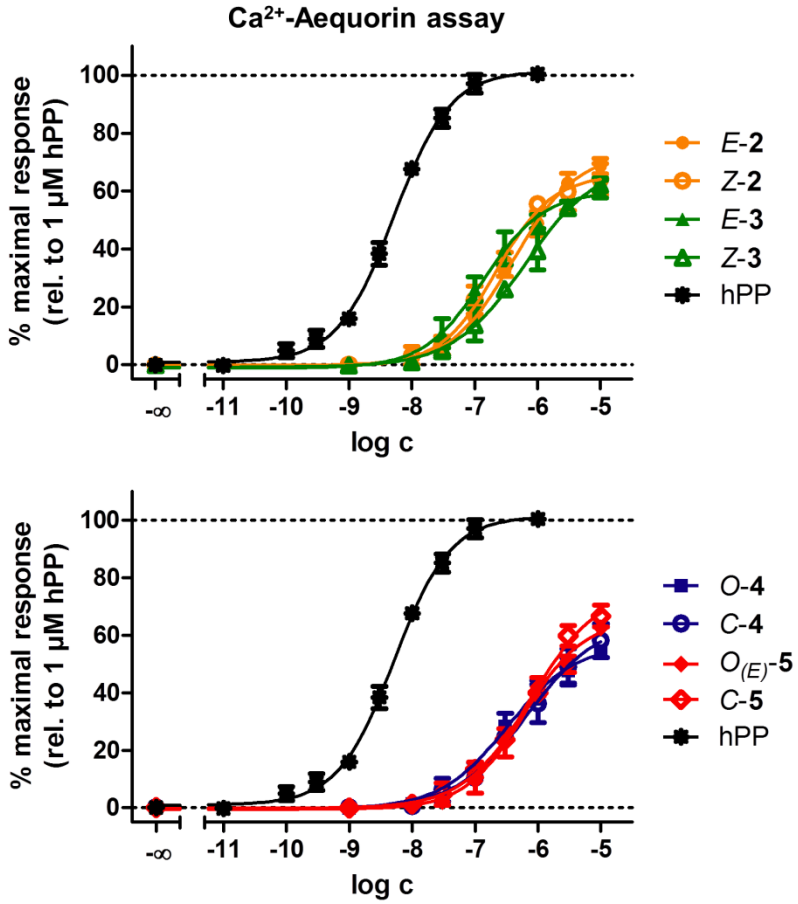
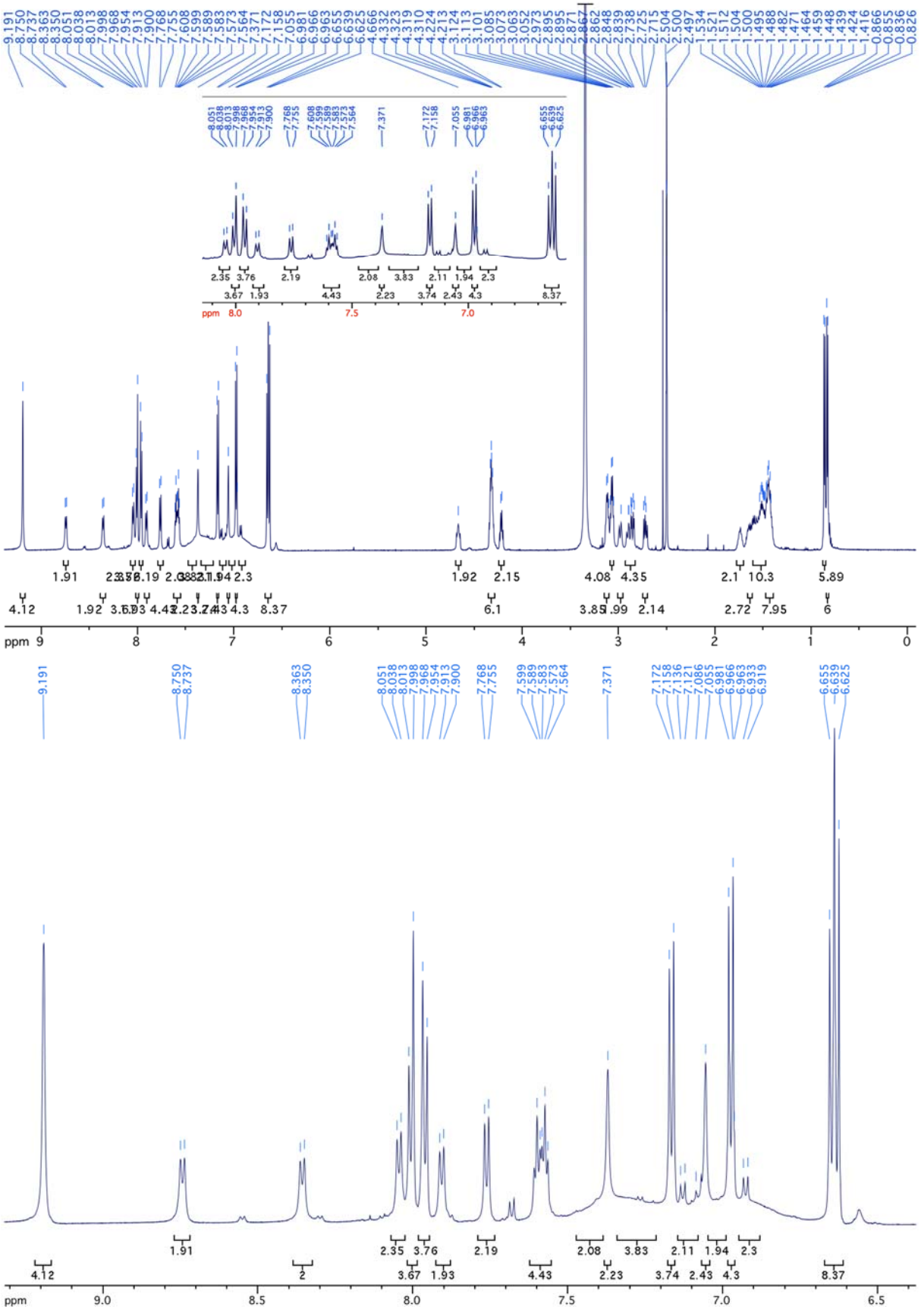


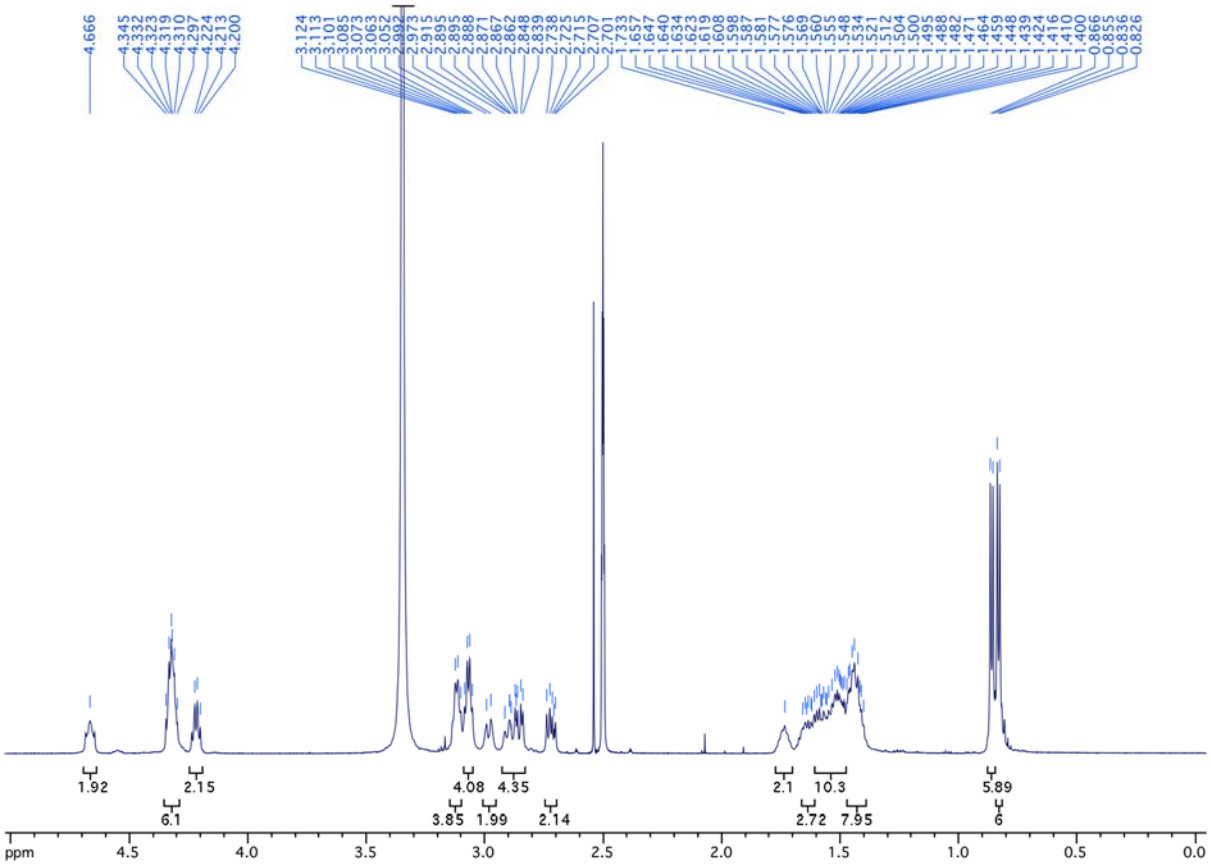
Figure S11. Y_4R functional activity (concentration-response curve) of *E*/*Z*-**2***, *E*/*Z*-**3***, *O*/*C*-**4*** and $O_{(E)}$/*C*-**5*** and the endogenous agonist hPP determined by measuring the intracellular Ca^{2+} mobilization in an aequorin assay using CHO-hY_4R-mtAEQ-G_{qi5} cells. Data represent means ± SEM from three or four independent experiments performed in triplicate. Data were analyzed by four parameter logistic fits (GraphPad Prism 5.0). Cellular responses were normalized to the effect of hPP elicited at a concentration of 1 µM.
*For an approximation of achieved *E*/*Z* and *O*/*C* ratios (PSS) see **Tables 1** and **2** (main article).

5. NMR-spectra

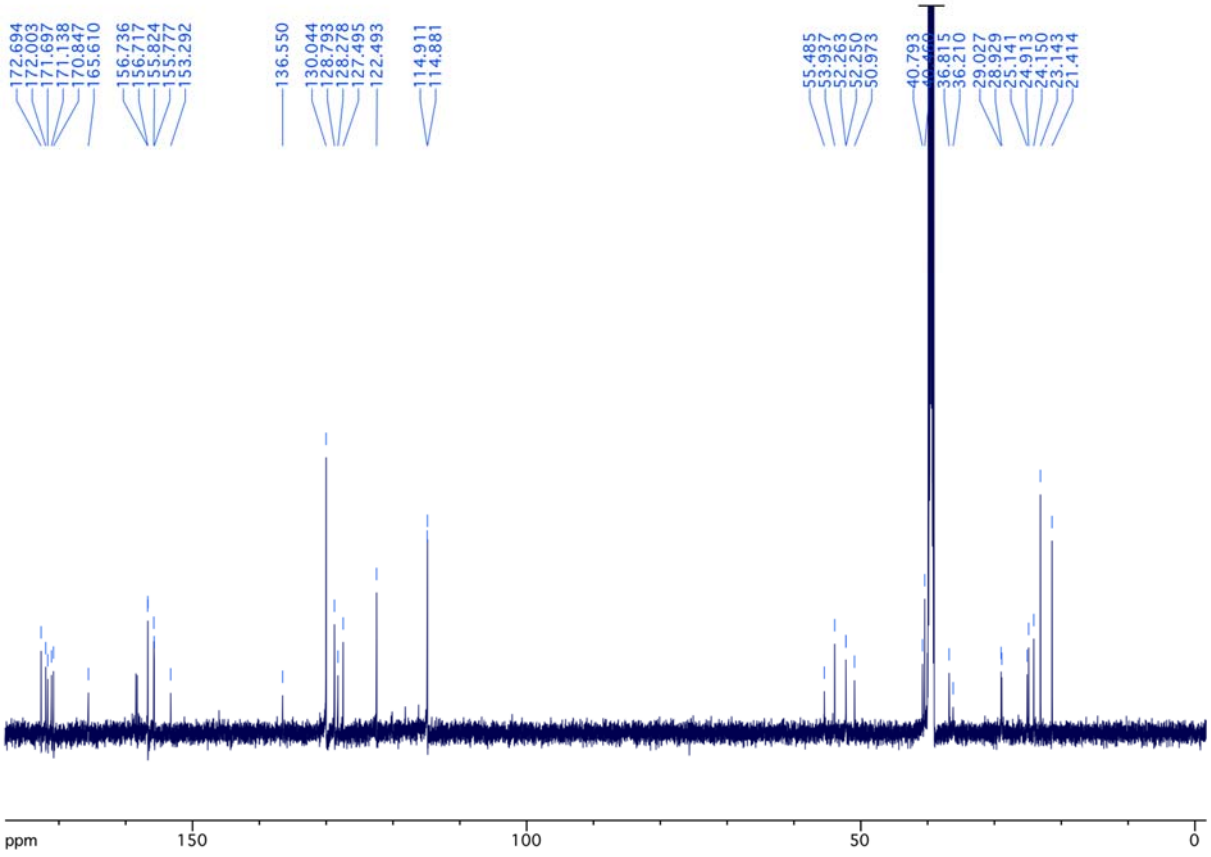
5.1 Compound **2**

^{1}H (D_6-DMSO, 600 MHz)



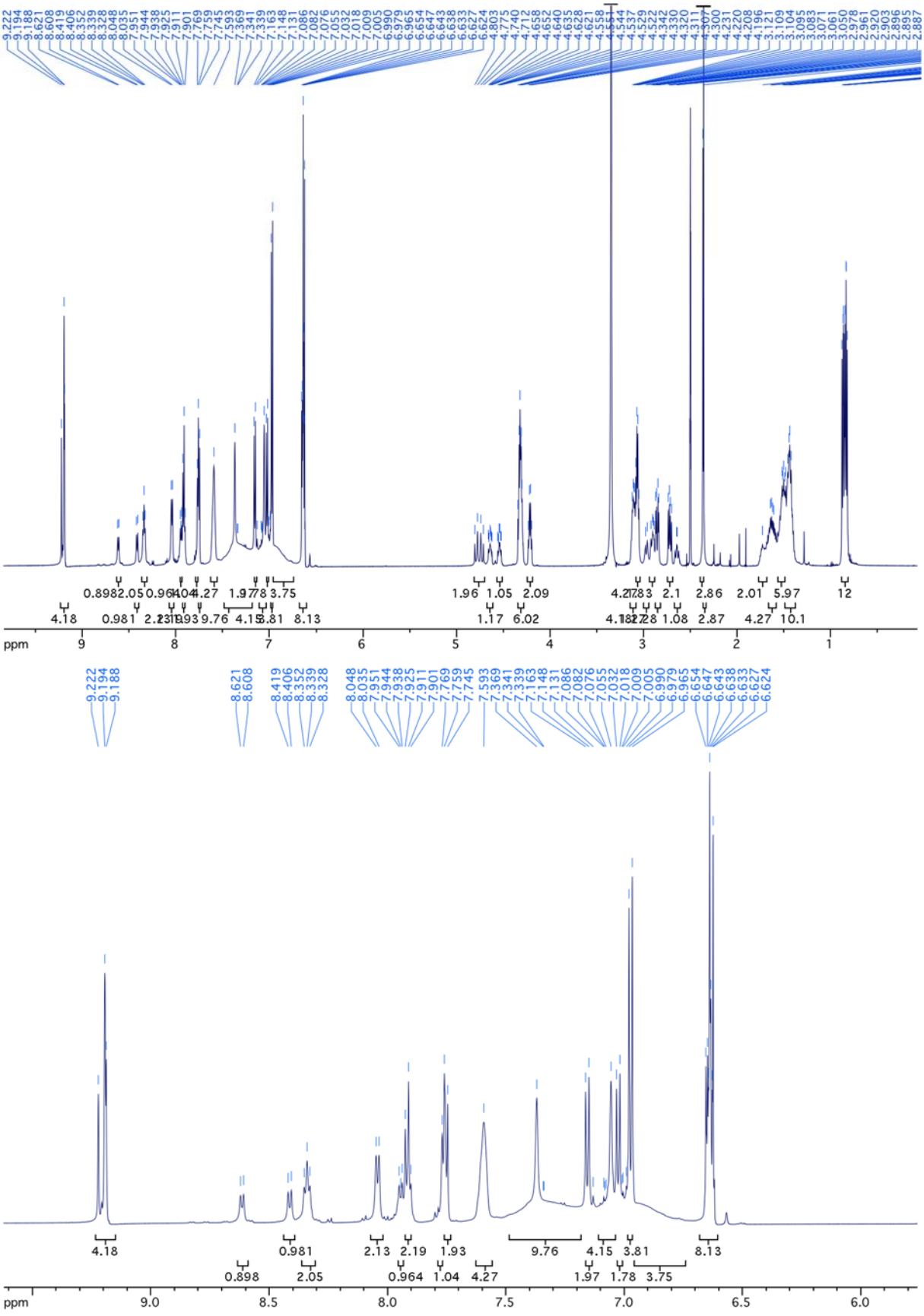


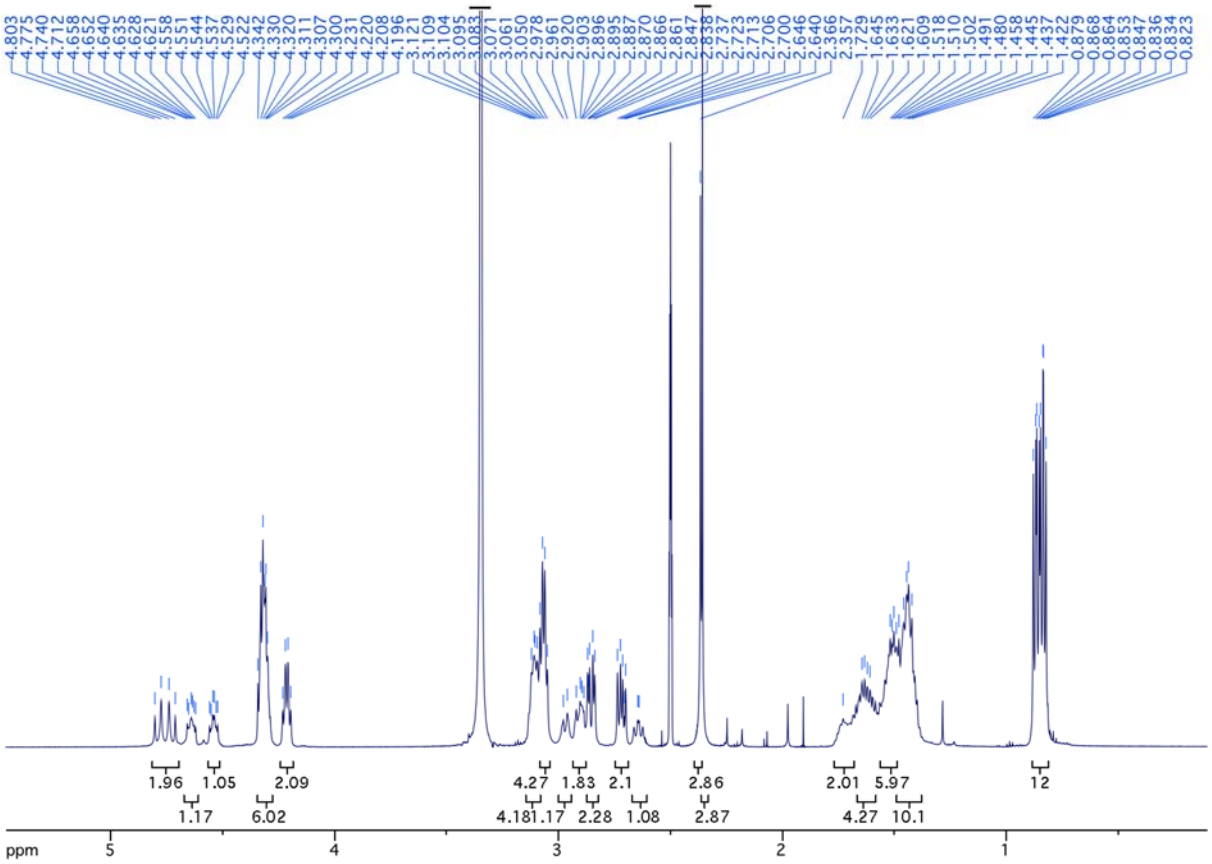
13C (D_6-DMSO, 151 MHz)



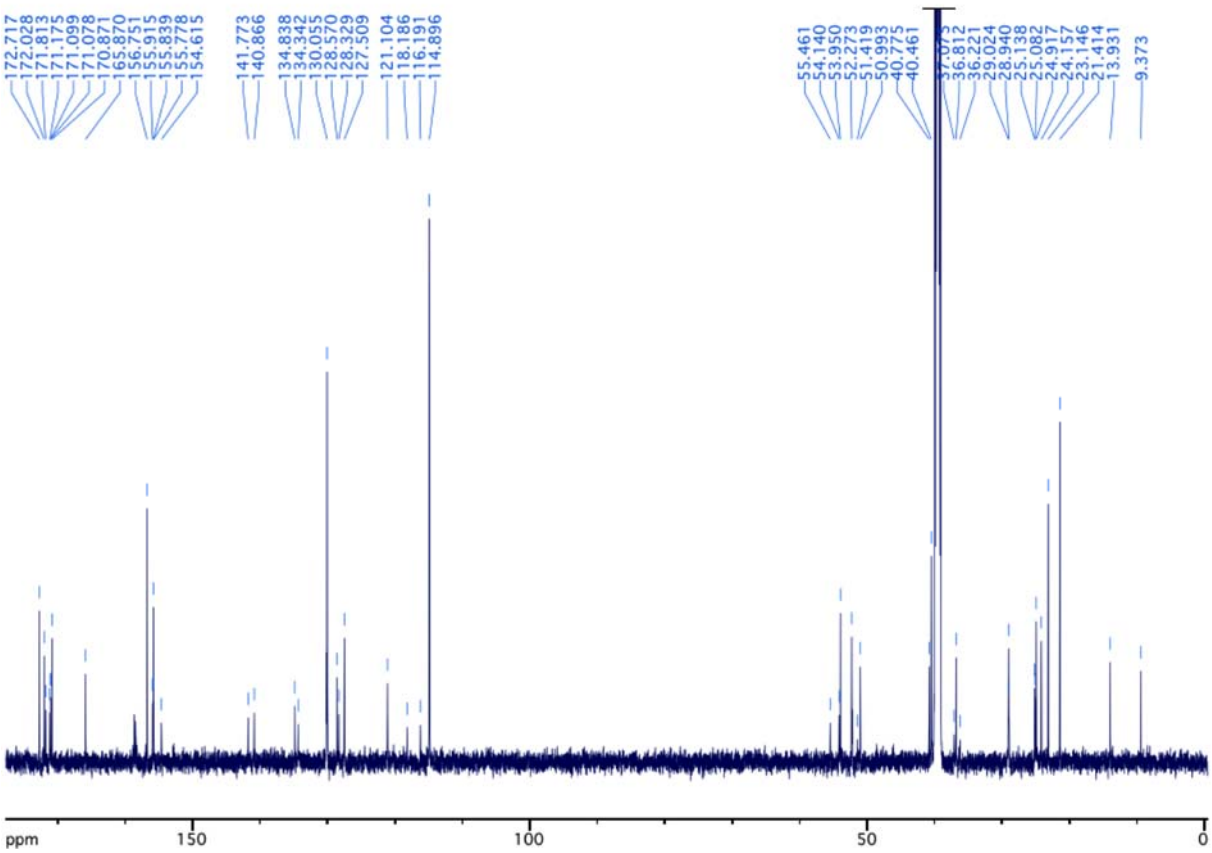
5.2 Compound **3**

^{1}H (D_6-DMSO, 600 MHz)



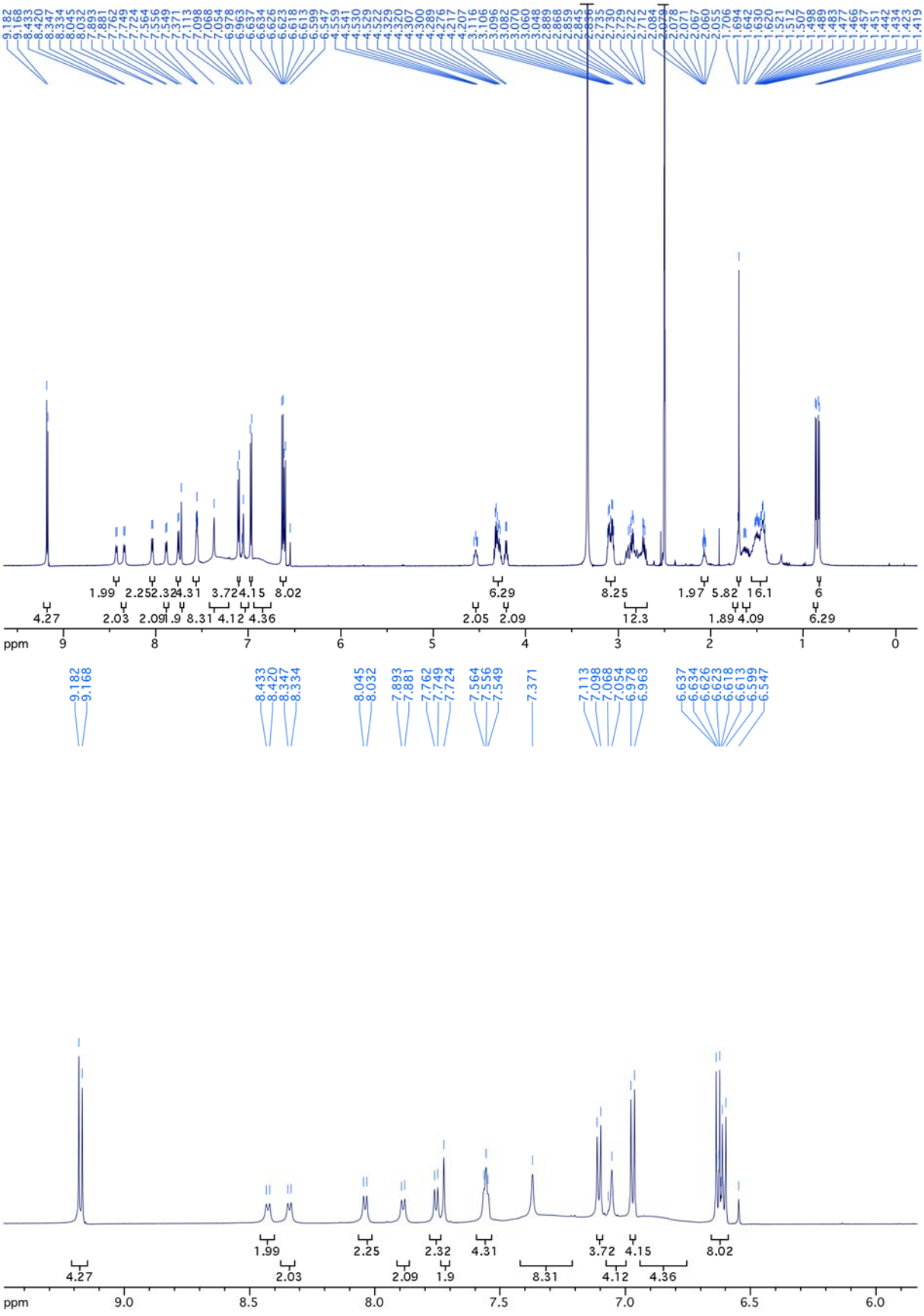


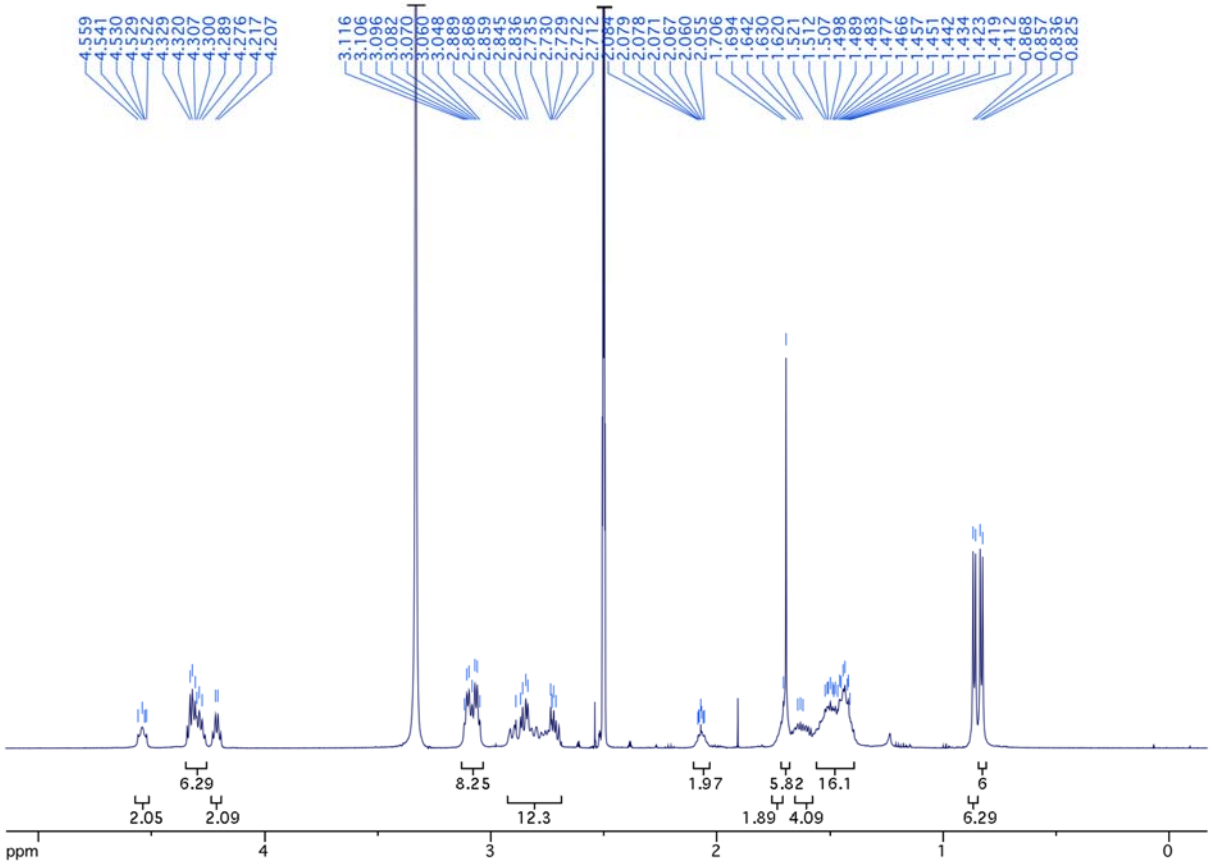
^{13}C (D_6-DMSO, 151 MHz)



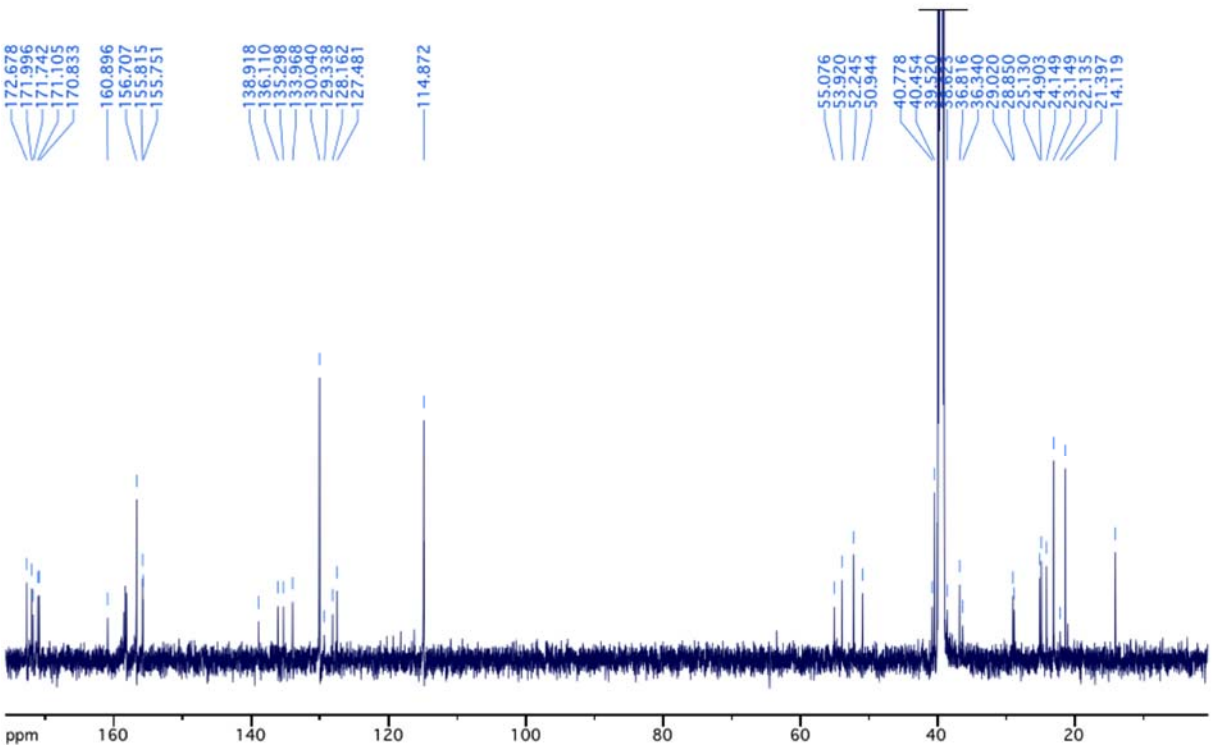
5.3 Compound **4**

^{1}H (D_6-DMSO, 600 MHz)





^{13}C (D_6-DMSO, 151 MHz)



5.4 Compound **5**

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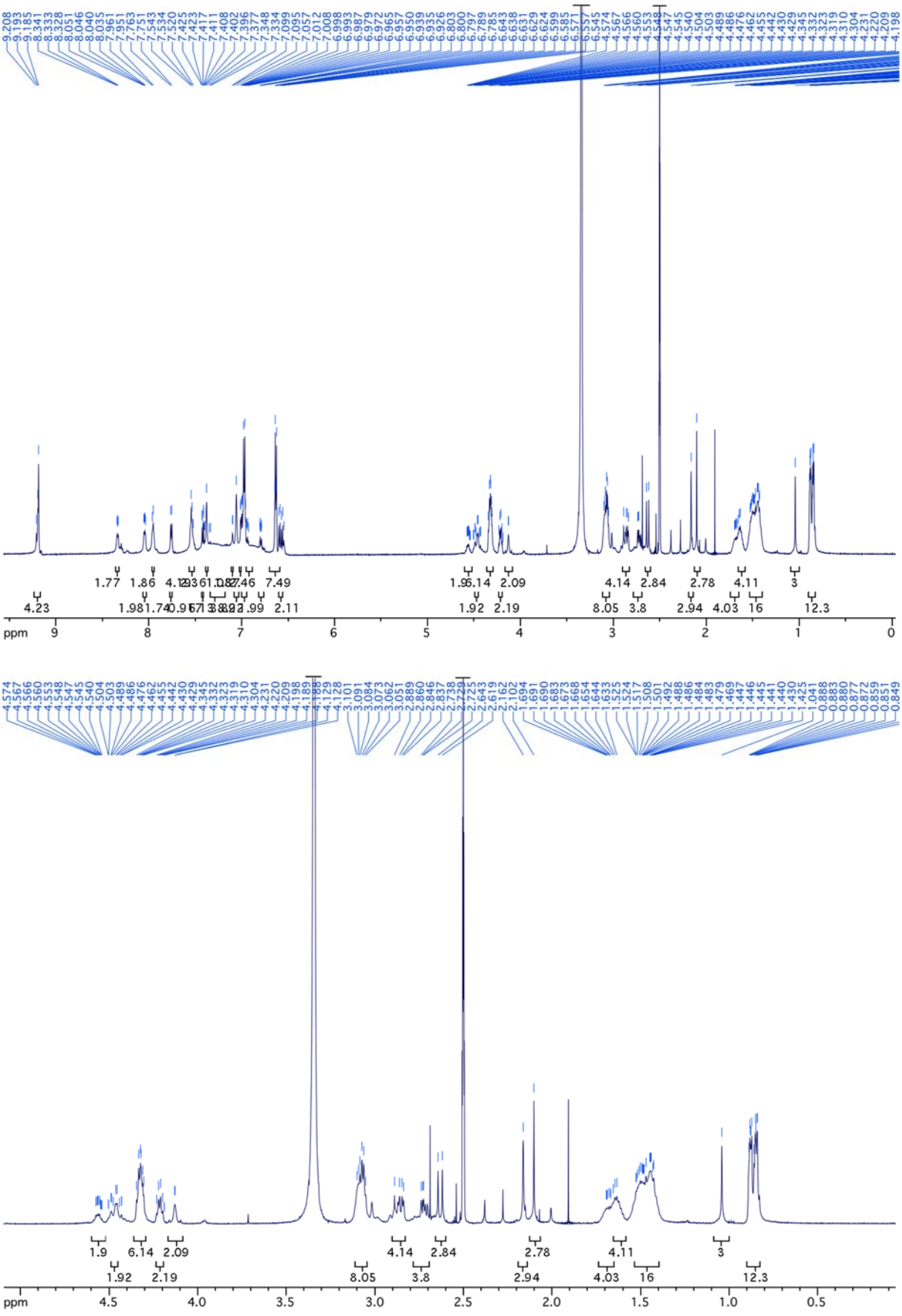
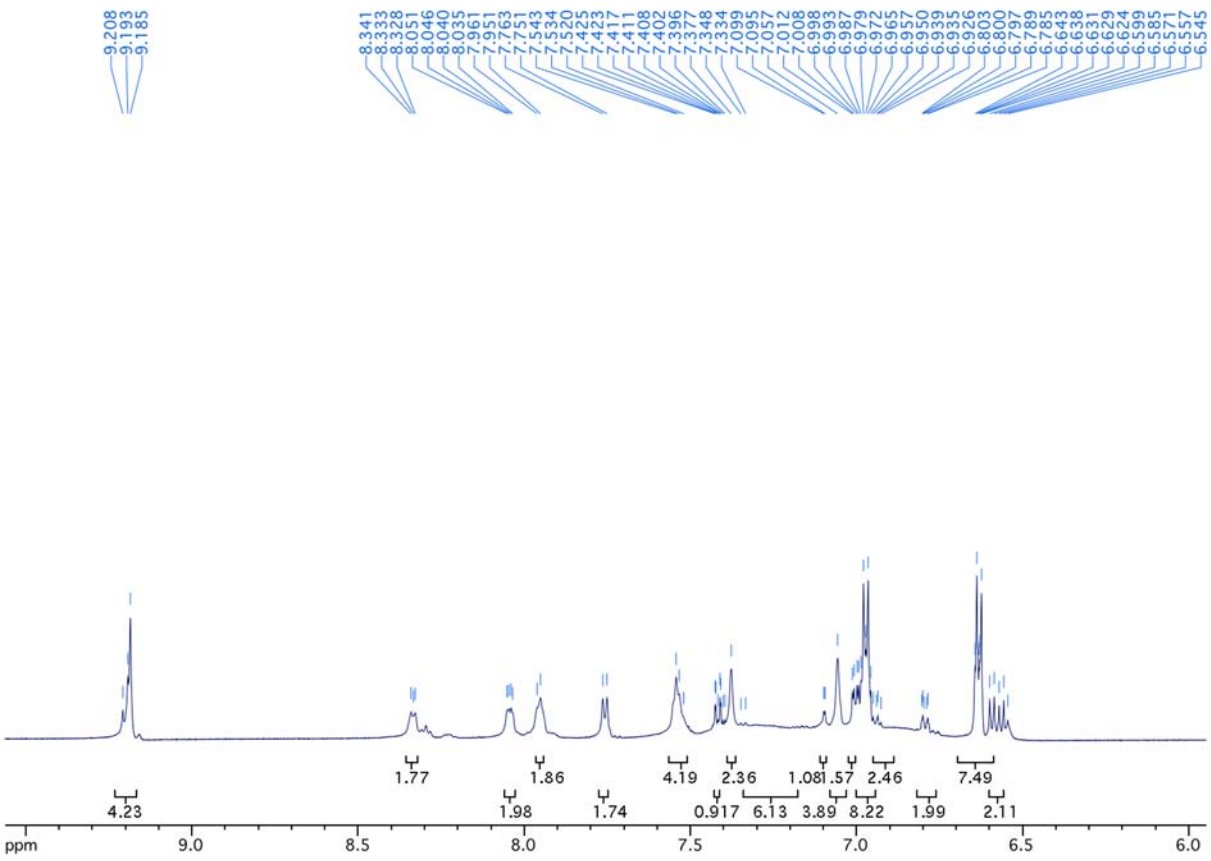
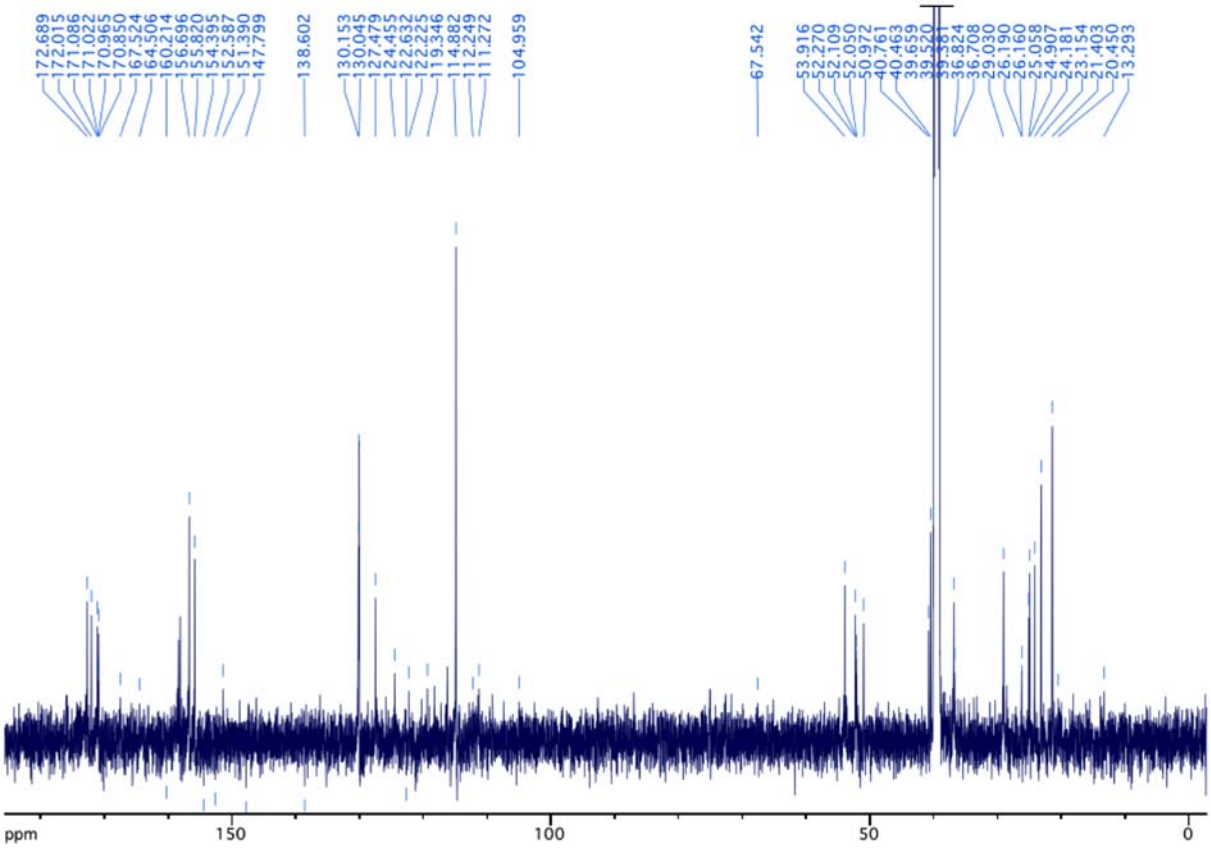




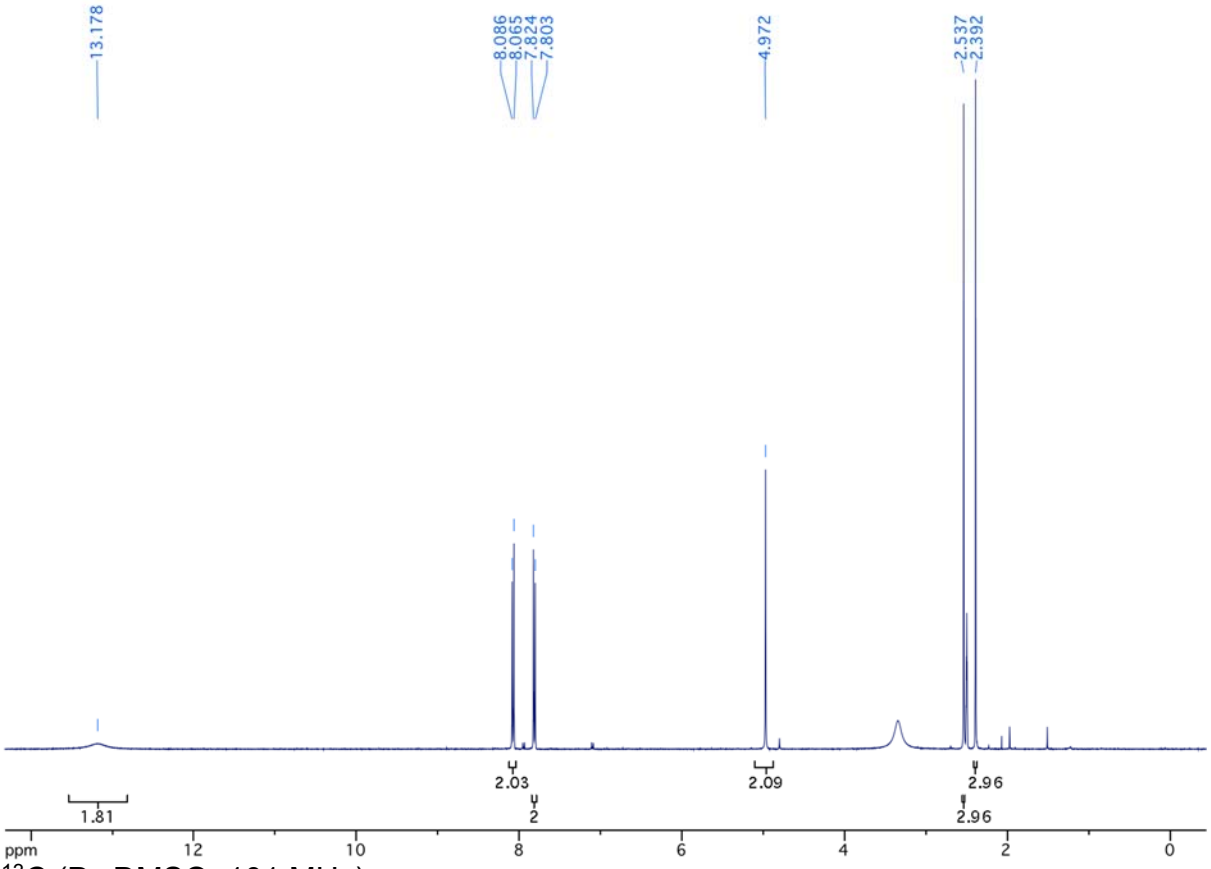
^{13}C (D_6-DMSO, 151 MHz)*



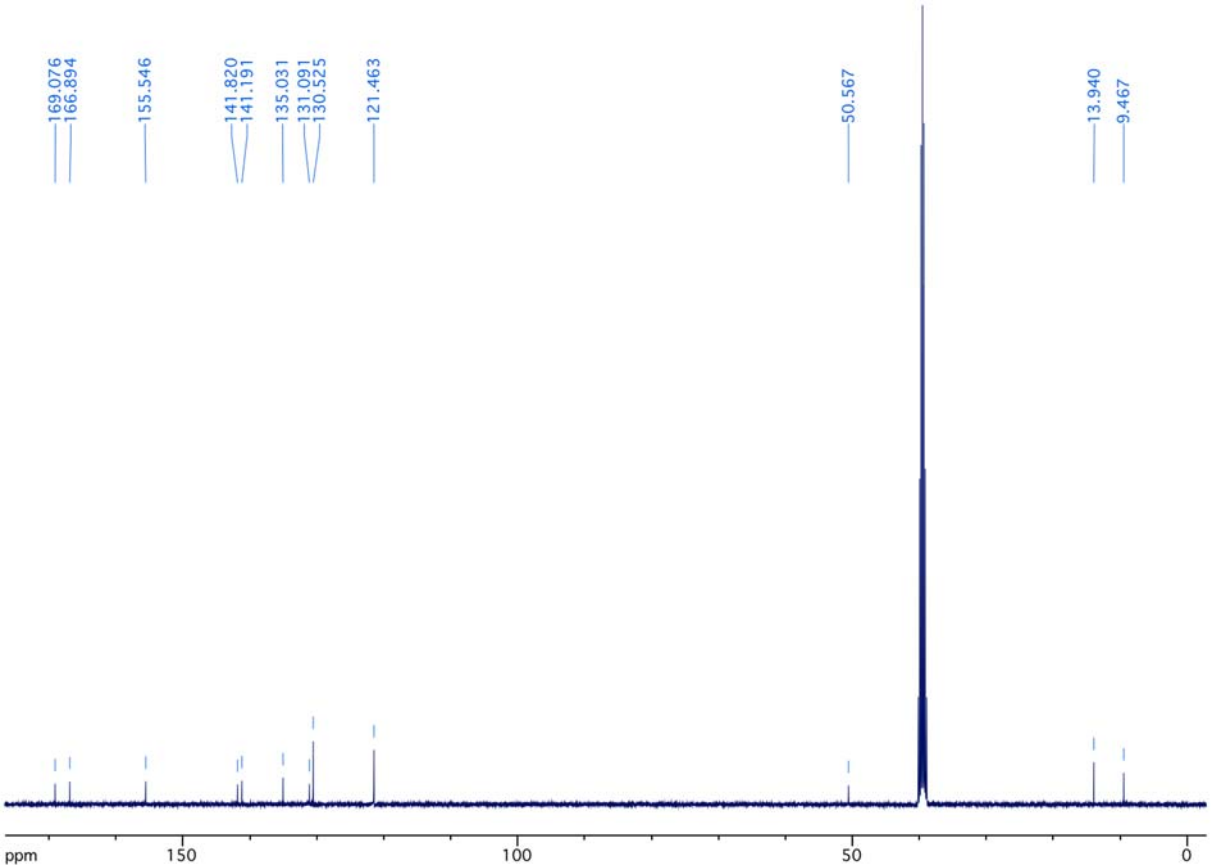
*some signals were determined indirectly by HMBC

5.5 Compound **8**

^{1}H (D_6-DMSO, 400 MHz)

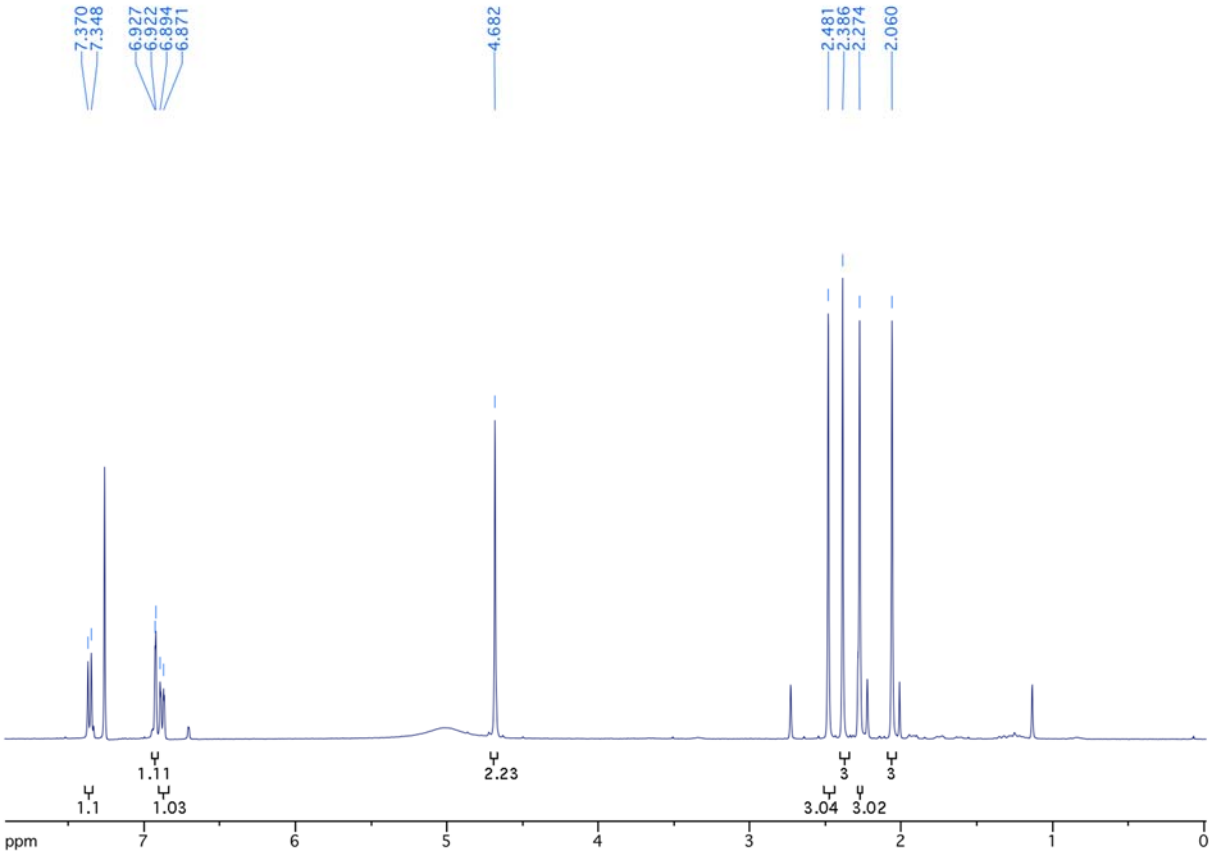


^{13}C (D_6-DMSO, 101 MHz)

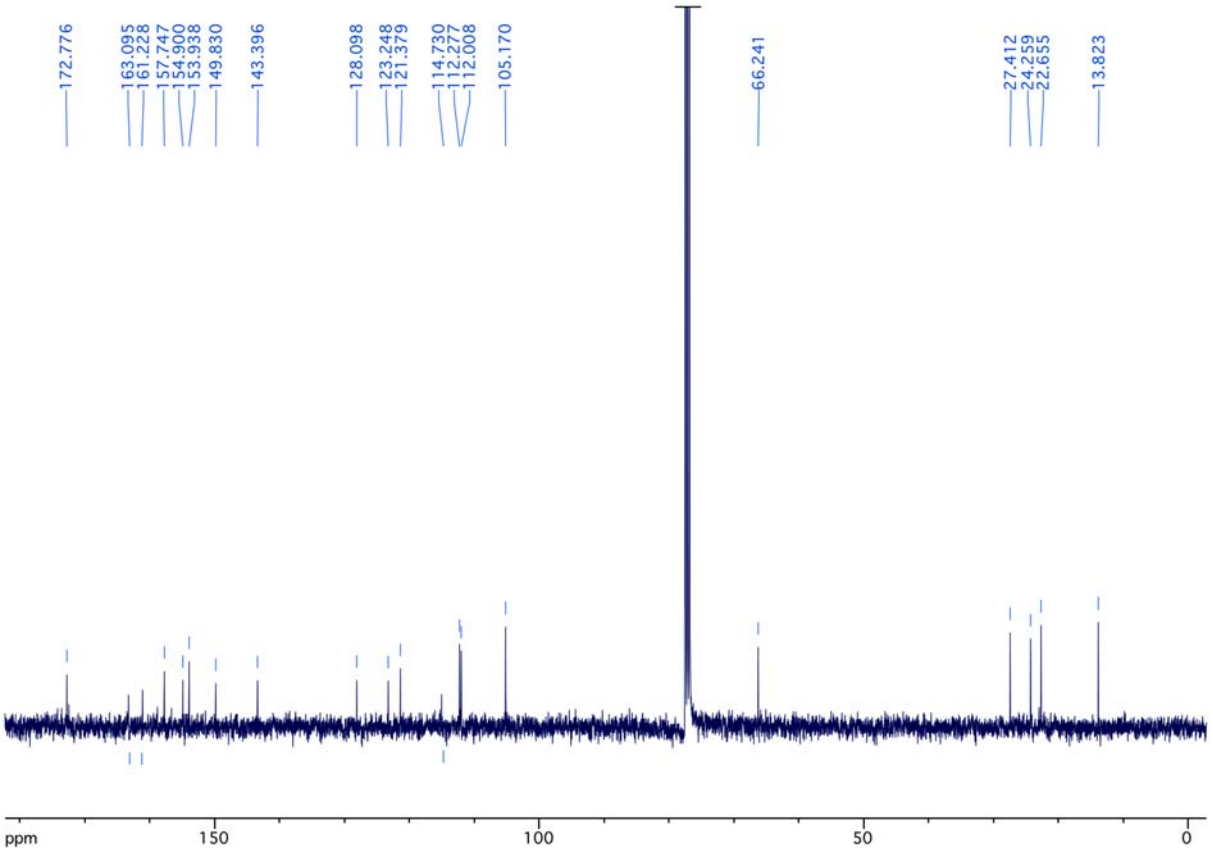


5.6 Compound **11**

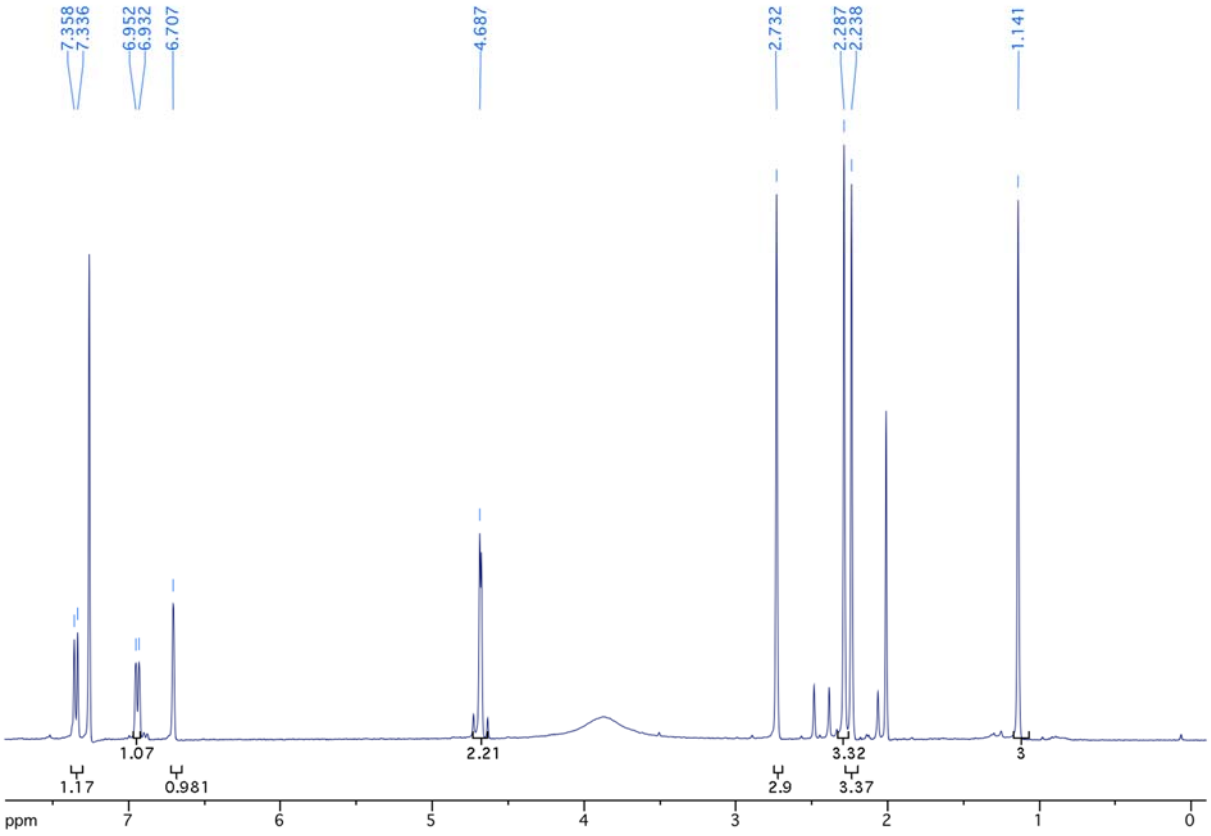
^{1}H ($CDCl_3$, 400 MHz) *E*-isomer



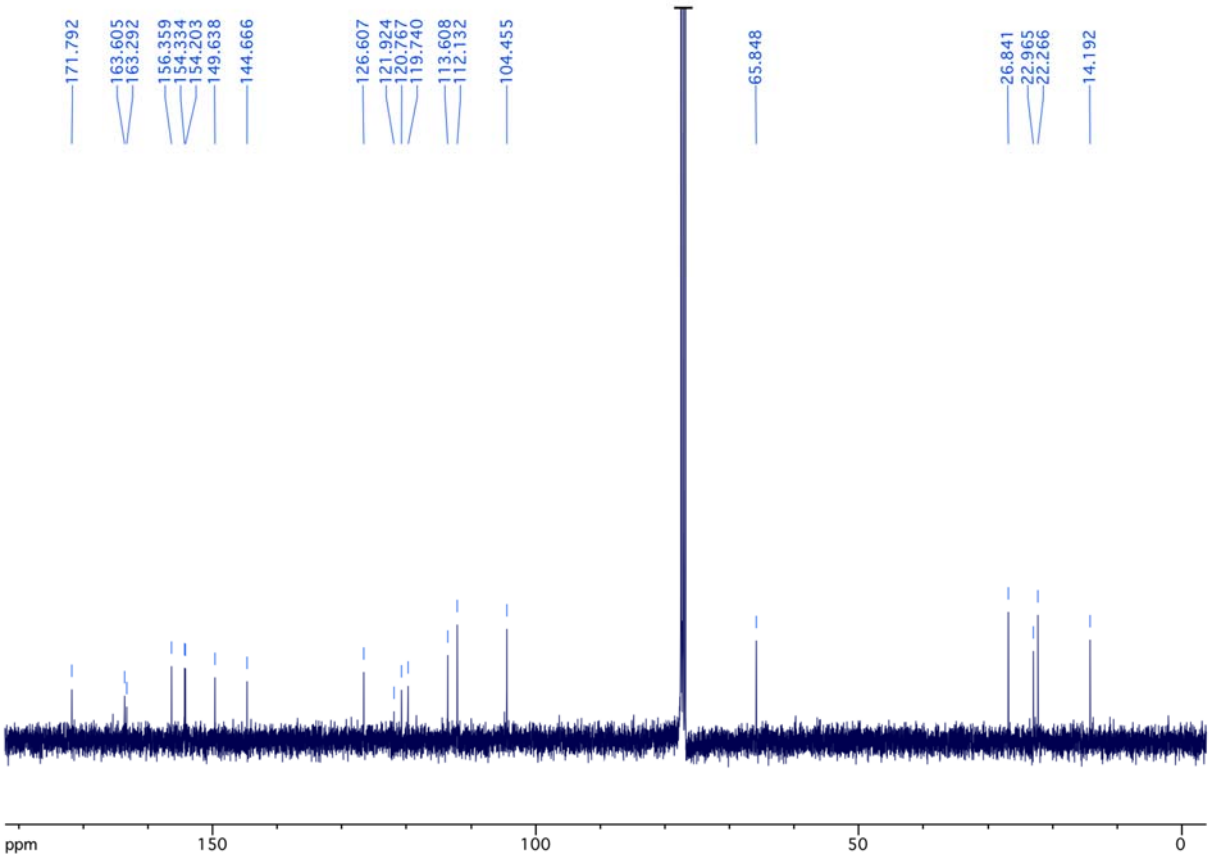
^{13}C ($CDCl_3$, 101 MHz) *E*-isomer



^{1}H ($CDCl_3$, 400 MHz) *Z*-isomer

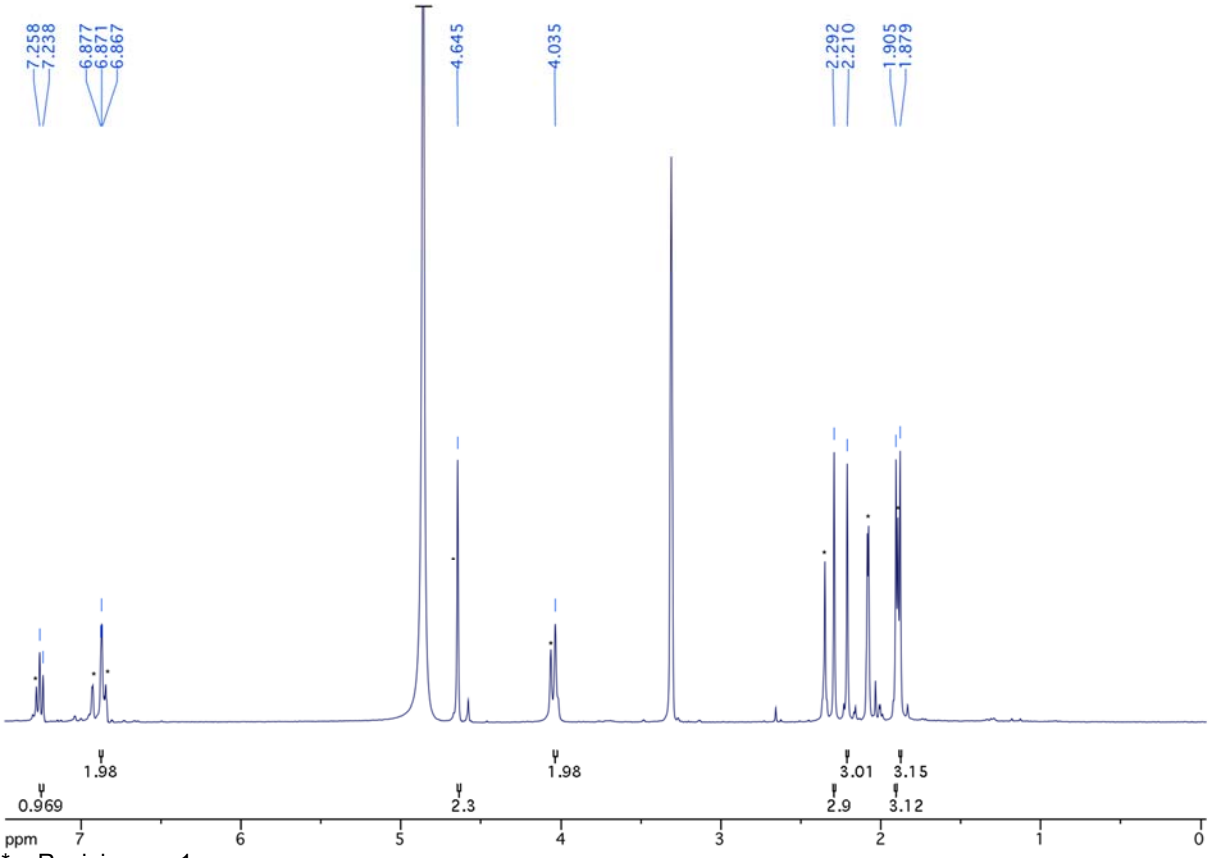


^{13}C ($CDCl_3$, 101 MHz) *Z*-isomer



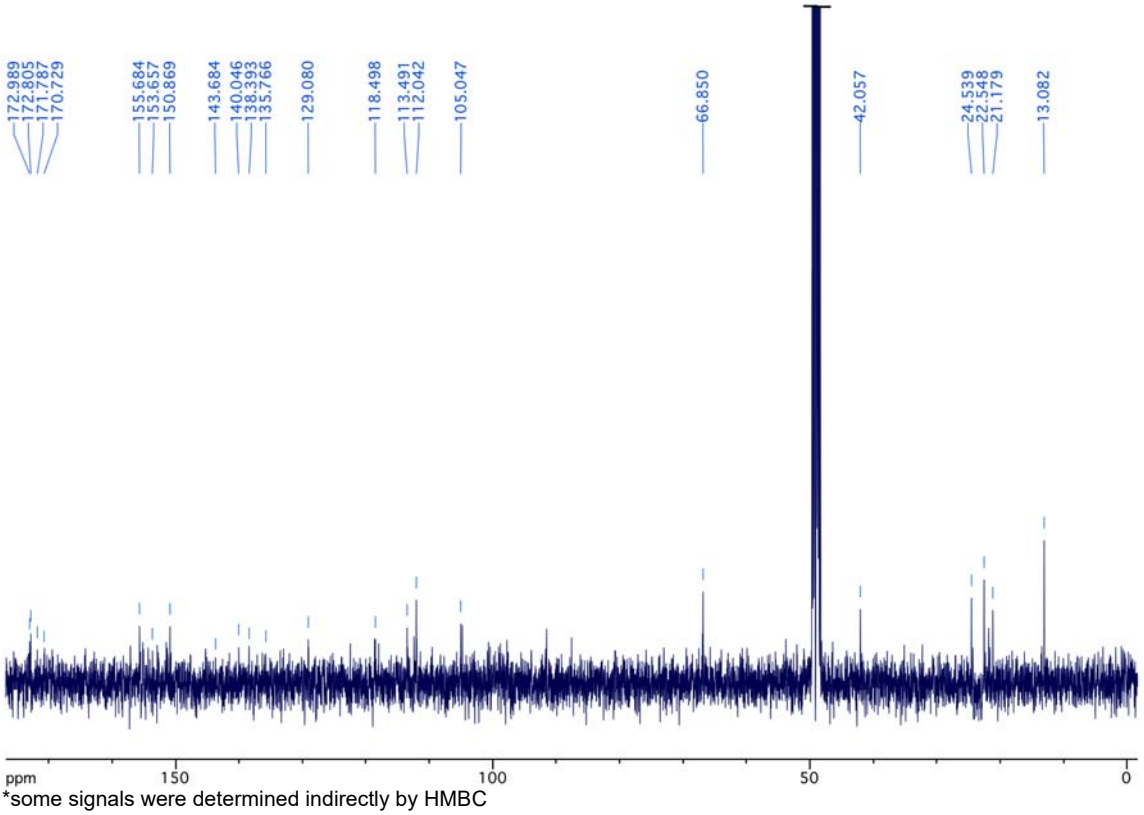
5.7 Compound **13**

^{1}H (MeOD, 400 MHz) Regioisomer mixture, peak picking at Regioisomer 2



* = Regioisomer 1

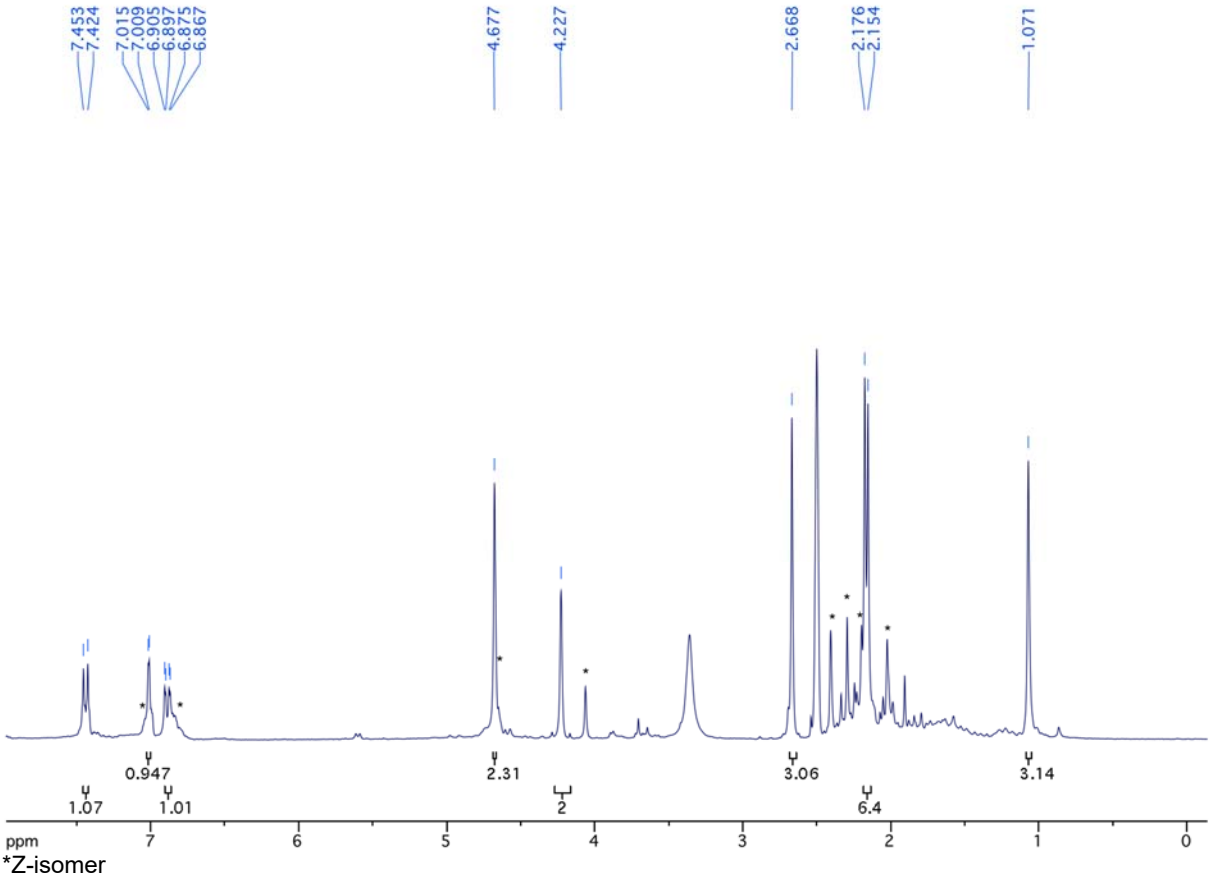
^{13}C (MeOD, 101 MHz)*



*some signals were determined indirectly by HMBC

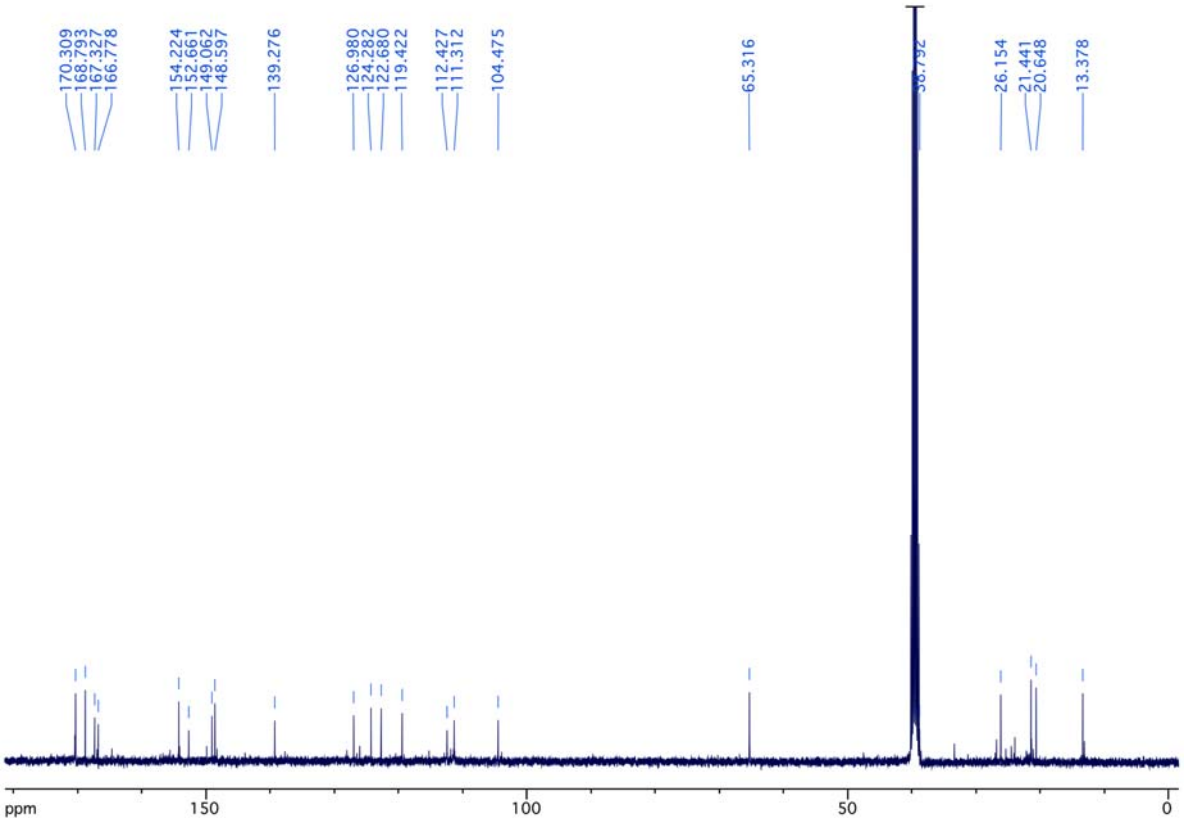
5.8 Compound *E*-**14**

^{1}H (D_6-DMSO, 400 MHz)



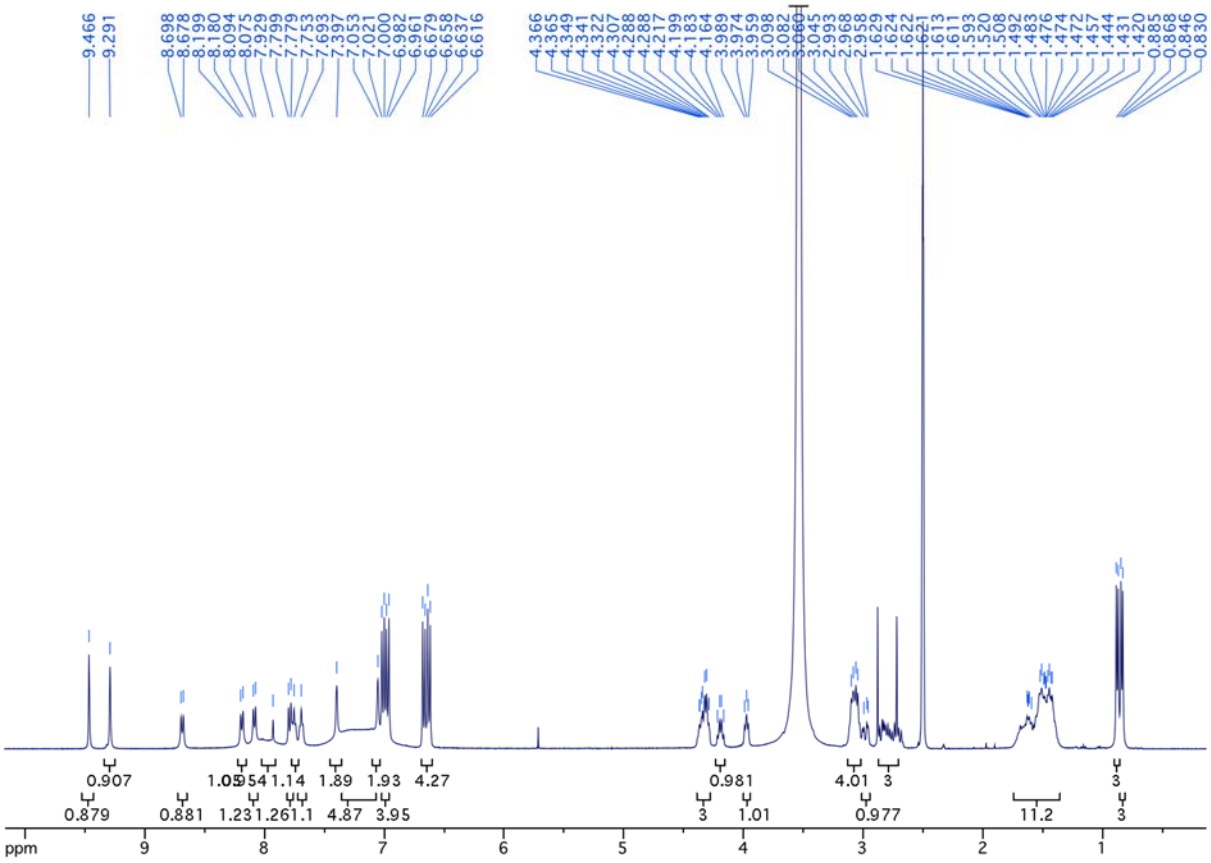
*Z-isomer

^{13}C (D_6-DMSO, 101 MHz)



5.8 Compound **17**

^{1}H (D_6-DMSO, 400 MHz)



^{13}C (D_6-DMSO, 101 MHz)