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# Unique photoaffinity probes to study TGFβ signaling and receptor fates

D. Längle,[a,b] F. Wesseler[a,b], D. Flötgen[a], H. Leek[c], A. T. Plowright[d] and D. Schade *[a,b]

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[a] *Department of Pharmaceutical and Medicinal Chemistry, Christian-Albrechts-University Kiel, Gutenbergstr. 76, D-24118 Kiel, Germany. Email: schade@pharmazie.uni-kiel.de*
[b] *Department of Chemistry and Chemical Biology, Technical University Dortmund, Otto-Hahn-Str. 4, D-44227 Dortmund, Germany.*
[c] *Early Product Development, Pharmaceutical Sciences, IMED Biotech Unit, AstraZeneca Gothenburg, Mölndal SE-43183, Sweden*
[d] *Cardiovascular, Renal and Metabolism, IMED Biotech Unit, AstraZeneca Gothenburg, Mölndal SE-43183, Sweden.*

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## 1. Subcellular probe localization (confocal microscopy)

### 1.1. Background fluorescence in FAM-, ROX-, TAMRA-labeling (Figure S1)

Labeling experiments of the mono-functionalized DHP probe **17** (3-alkyne substituent) with FAM-, ROX- and TAMRA-azides demonstrates highest signal/noise ratio, and thus, lowest background fluorescence for FAM- and TAMRA-based protocols (Fig. S1a). As expected, image quality further improved when photoaffinity probe **16** was used: After incubation of HEK293T cells with **16**, cells were irradiated for 5 min at 365 nm and then processed for confocal imaging as described below. Fig. S1b also illustrates a higher background when **16**-treated cells were not irradiated (and washed several times) prior to imaging. In this regard, it should be noted that the ability to perform stringent washing is not only important for imaging applications but also to decrease background in a typical pulldown-proteomics workflow.

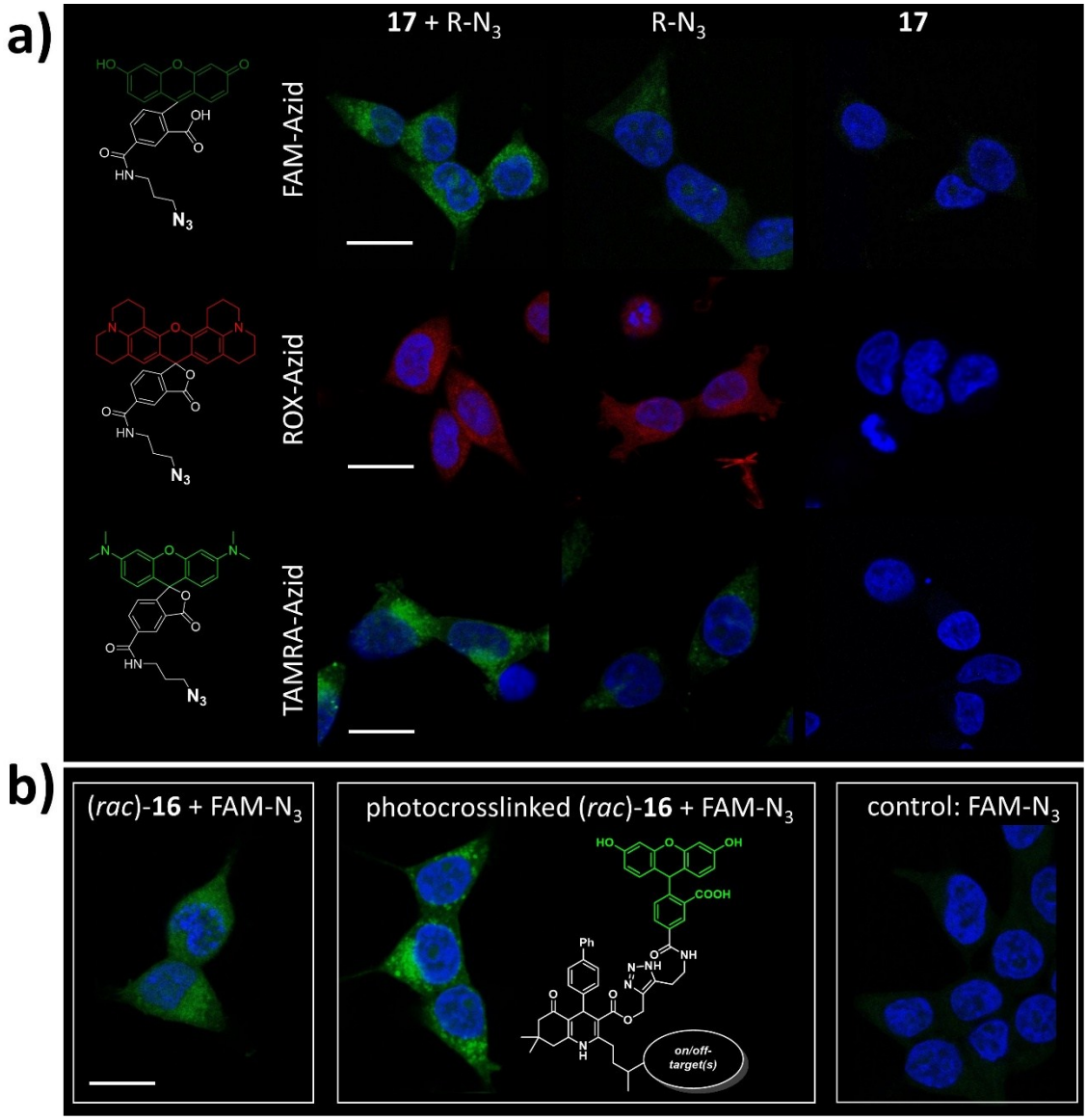



**Figure S1.** Click-labeling of DHP probe **17** (3-alkyne) with different fluorophore azides: FAM (top), ROX (middle), TAMRA (bottom) and evaluation of background fluorescence. **a)** Confocal fluorescence microscopy of HEK293T cells after 1h incubation with **17** (5 µM), followed by fixation and permeabilisation of cells, click-labeling with FAM-, ROX- or TAMRA-azides (green) and staining with DAPI (nuclei, blue). **b)** Confocal fluorescence microscopy as described above, but using photoaffinity probe (*rac*)-**16**: Left panel shows image without photocrosslinking and extensive washing, middle panel including photocrosslinking by 5 min UV-irradiation at 365 nm and several washing steps; Right panel represents a FAM-azide-only control. White bar = 20 µm (Leica SP5, water objective 63x, 1.2 NA).

1.2. Localization of DHP probe (*rac*)-**16** in endoplasmic reticulum vesicles (Figure S2)

Colocalization of photocrosslinked and labeled DHP probe with vesicles of the endoplasmic reticulum (ER marker, calnexin) suggests that the ER is not the primary vesicular system where these DHPs accumulate.

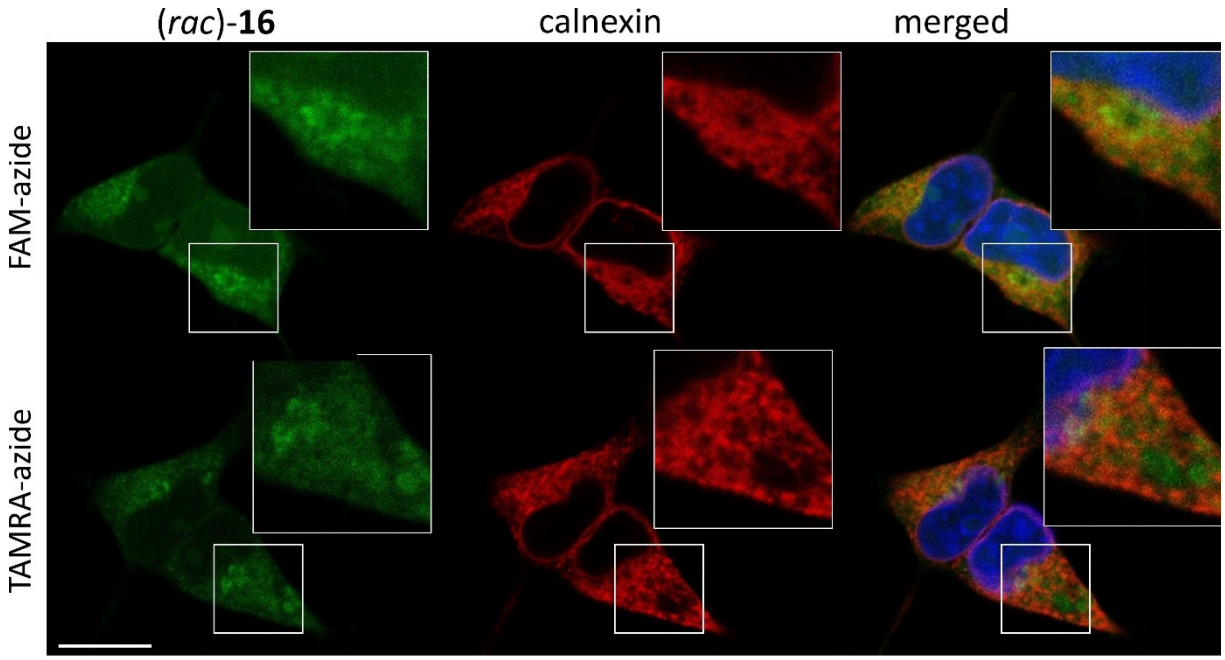
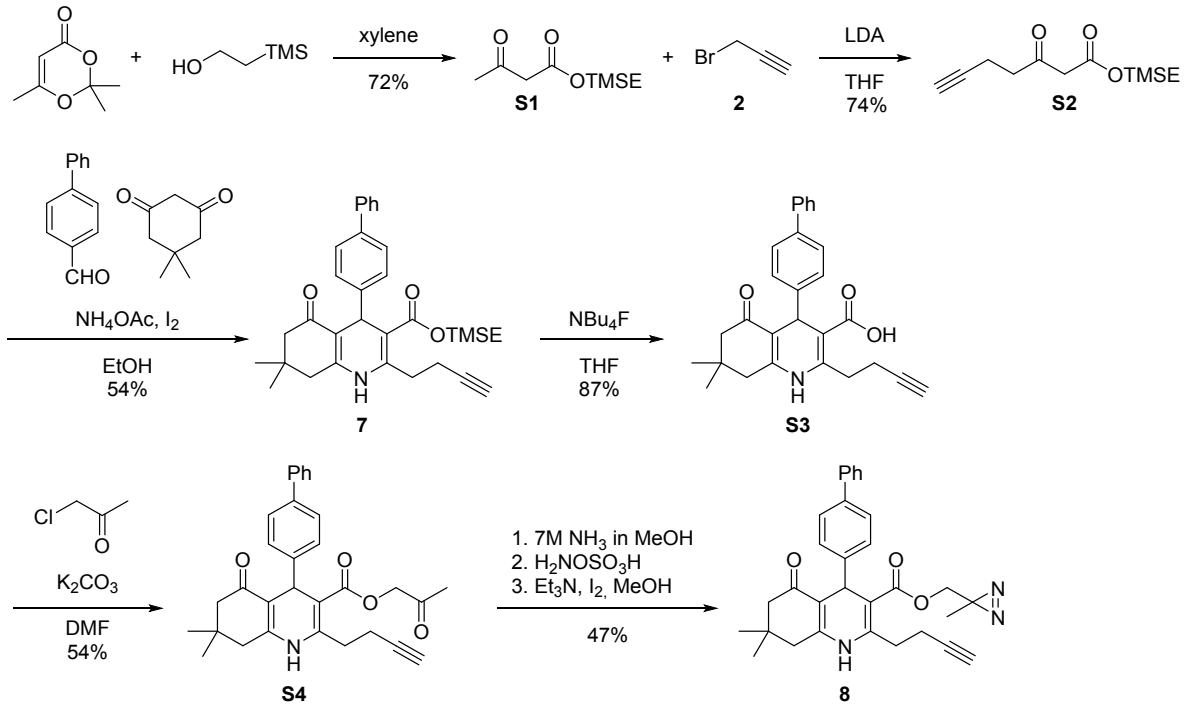



**Figure S2.** Subcellular localization of probe (*rac*)-**16** after photocrosslinking and labeling with FAM- and TAMRA-azides. Confocal fluorescence microscopy of HEK293T cells after 1h incubation with **16** (5 µM), followed by 5 min UV-irradiation at 365 nm (photocrosslinking), fixation and permeabilisation of cells, click-labeling with FAM- or TAMRA-azides (green), stainining with DAPI (nuclei, blue) and immunostaining with 1° (anti-calnexin) and 2° (Alexa-Fluor 647) antibodies (GOLGI, red). White bar = 20 µm (Leica SP5, water objective 63x, 1.2 NA).

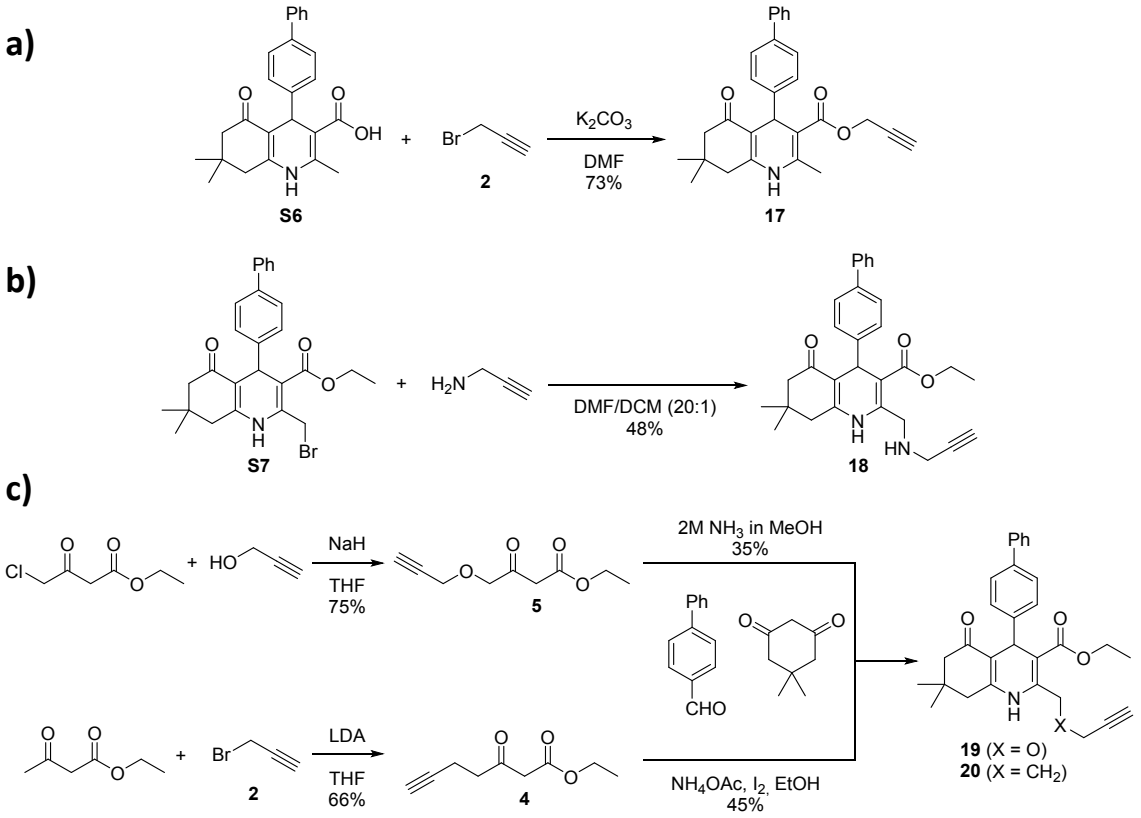
## 2. Complementary schemes on the chemical synthesis of DHP probes

### 2.1. Bifunctionalized DHP probe **8** (Scheme S1)

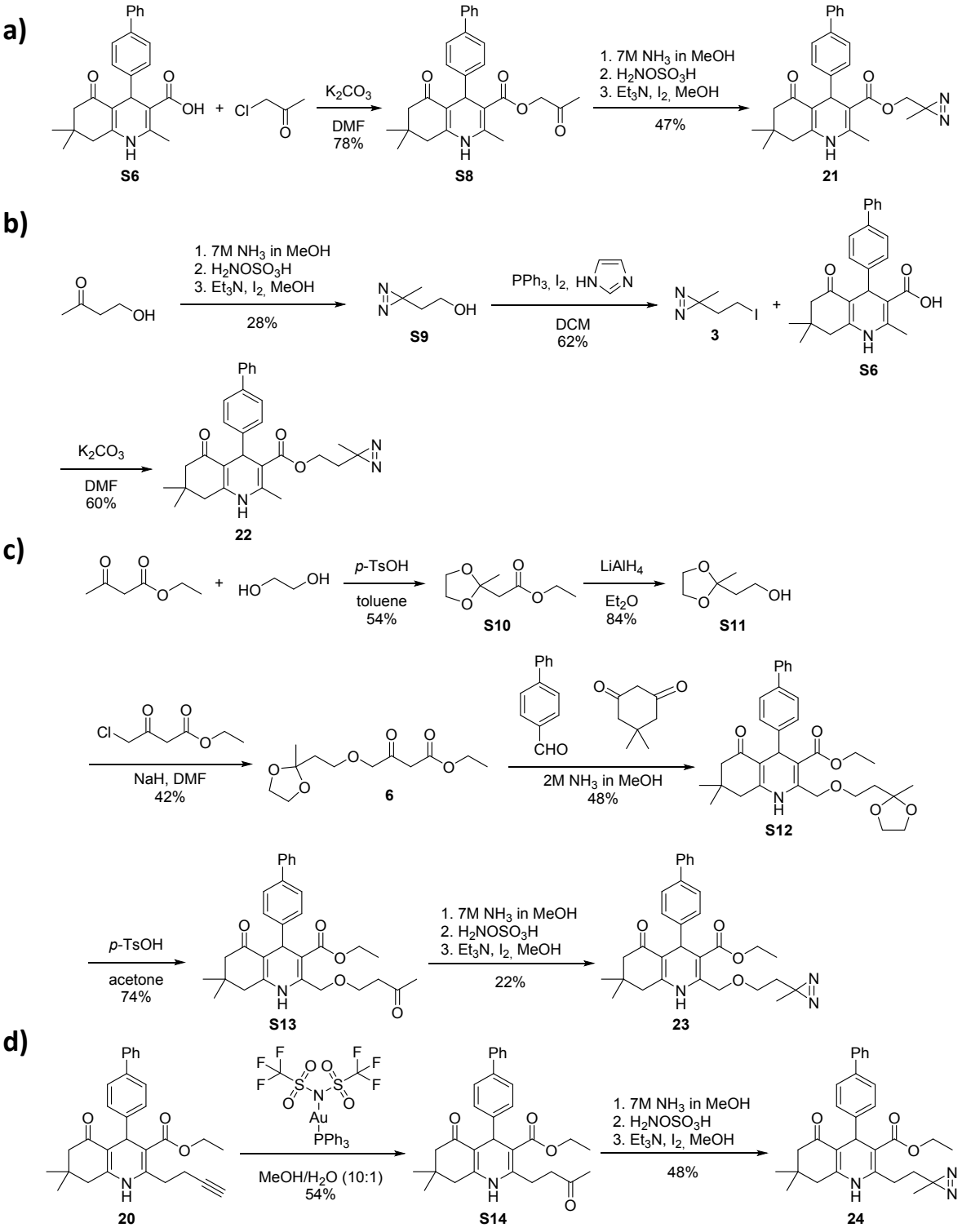



**Scheme S1.** Complete synthetic route to bifunctionalized DHP probe **8**, including key Hantzsch' building block **S2** and intermediates **S3**, **S4**.

2.2. Monofunctionalized DHP probes **17-24** (Schemes S2 and S3)




**Scheme S2.** Summary of synthetic routes to alkyne-substituted DHP probes **17-20** (a-c).




**Scheme S3.** Summary of synthetic routes to diazirine-substituted DHP probes **21-24** (a-d).

## 3. Experimental section – Chemistry

### 3.1. Chemistry – General

Unless otherwise stated, all reagents were obtained from commercially available sources and were used without purification. The reaction process was monitored by TLC with silica gel plates (thickness 250 mm, Indicator F-254) under UV light. Flash column chromatography was performed on a CombiFlash Rf200 (Teledyne ISCO, Lincoln). NMR spectra of compounds were recorded with a Bruker DRX-400 spectrometer, a Bruker DRX-500, a Bruker AV-500 or a Bruker AV-600 spectrometer. Chemical shifts were reported as ppm ($\delta$) relative to the solvent ($CDCl_3$ at 7.26 ppm ($^1H$) and 77.16 ppm ($^{13}C$), DMSO-$d_6$ at 2.50 ppm ($^1H$) and 39.52 ppm ($^{13}C$) or TMS as internal standard. The coupling constants are expressed in Hertz (Hz). Multiplicities are abbreviated as s = singlet, d = doublet, t = triplet, q = quartet, sxt = sextet, sep = septet, m = multiplet, br = broad. Mass spectral data were determined with a Shimadzu System (2x LD-20 AD XR (pumps) with Kinetex RP-18 column (2.6 µm, 50 x 2.10 mm)) using a gradient elution with acetonitrile (0.1% formic acid) and water (0.1% formic acid) at a flow rate of 0.25 mL/ min, coupled to a Shimadzu LC/MS-8030 spectrometer. Purity of final compounds were either determined on a on a Shimadzu system (2x LD-20 AD XR (pumps), SPD-20 AC XR (PDA detector) and a Kinetex RP-18 column, 2.6 µm, 100 x 2.10 mm) using a gradient elution with acetonitrile (0.1% formic acid) and water (0.1% formic acid) at a flow rate of 0.25 mL/ min over 18 min. Purity of all synthetic final products was ≥ 95. High resolution mass spectra were obtained on a Bruker 'compact QTOF' (ESI) via direct injection.

### 3.2. Synthetic procedures (Compounds **S1-S14**, **4-24**)

**2-(Trimethylsilyl)ethyl 3-oxobutanoate (S1).** To a solution of 2,2,6-trimethyl-4-1,3-dioxinone (194.7 µL, 284.3 mg, 2.00 mmol) in xylene (5 mL) 2-trimethylsilylethanol (286.6 µL, 236.5 mg, 2.00mmol) was added and the reaction mixture was heated to 150°C for 1h. After cooling to room temperature the solvent was evaporated and after flash chromatographic purification (cyclohexane/ethyl acetate 85:15) the title compound was obtained as a pale yellow oil (292.1 mg, 1.44 mmol, 72%): $R_f$ = 0.41 (cyclohexane/ethyl acetate 1:1); $^1H$-NMR (500 MHz, $CDCl_3$): $\delta$ = 0.05 (s, 9H), 0.94 - 1.10 (m, 2H), 2.28 (s, 3H), 3.44 (s, 2H), 4.14 - 4.33 ppm (m, 2H).

**2-(Trimethylsilyl)ethyl 3-oxohept-6-ynoate (S2)**. To a solution of diisopropylamine (702.7 µL, 506.0 mg, 5.00 mmol) in THF (5 mL) a 1.6M *n*-butyllithium solution in hexane (3.2 mL, 5.00 mmol) was added dropwise at 0°C. After 30 min a solution of 2-(trimethylsilyl)ethyl 3-oxobutanoate (**S1**) (404.6 mg, 2.00 mmol) in THF (2 mL) was added and stirring was continued for additional 30 min. Then, a 80 wt.% solution of propargyl bromide in toluene (334.1 µL, 356.9 mg, 3.00 mmol) was added slowly and the mixture was stirred at room temperature. After cooling again to 0°C glacial acetic acid (300 µL) was added and the reaction mixture was diluted with a mixture of distilled water (10 mL) and diethylether (10 mL). The aqueous layer was separated and extracted with diethylether (3x 20 mL). The combined organic layers were washed with brine (3x 20 mL), dried over $MgSO_4$ and the solvent was evaporated.

The crude product was purified by flash chromatography (cyclohexane/ethyl acetate 9:1) to give a yellow oil (358.2 mg, 1.49 mmol, 74%): $R_f$ = 0.52 (cyclohexane/ethyl acetate 3:1); $^1$H-NMR (500 MHz, $CDCl_3$): $\delta$ = 0.05 (a, 9H), 0.93 - 1.07 (m, 2H), 1.97 (t, J = 2.7 Hz, 1H), 2.48 (td, J = 7.3, 2.7 Hz, 2H), 2.82 (t, J = 7.3 Hz, 2H), 3.46 (s, 2H), 4.17 - 4.28 ppm (m, 2H).

**2-(Trimethylsilyl)ethyl 4-(biphenyl-4-yl)-2-(but-3-yn-1-yl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexa-hydroquinoline-3-carboxylate (7).** 2-(Trimethylsilyl)ethyl 3-oxohept-6-ynoate (**S2**) (360.6 mg, 1.50 mmol), 4-biphenylcarbaldehyde (273.3 mg, 1.50 mmol), dimedone (315.4 mg, 2.25 mmol), $NH_4OAc$ (231.2 mg, 3.00 mmol) and iodine (114.2 mg, 0.5 mmol) were dissolved in EtOH (1 mL) and the reaction mixture was stirred for 18h at room temperature. Next, the solution was diluted with EtOAc (100 mL) and the organic phase was washed with a saturated $Na_2S_2O_3$ solution (2x 50 mL), distilled water (2x 100 mL) and brine (100 mL), dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate 7:3) to give a yellow solid (343.7 mg, 0.65 mmol, 44%); $R_f$ = 0.46 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta$ = -0.02 (s, 9H), 0.84 (s, 3H), 0.87 - 0.97 (m, 2H), 1.00 (s, 3H), 1.95 - 2.06 (m, 1H), 2.18 (d, J = 16.2 Hz, 1H), 2.26 - 2.36 (m, 1H), 2.37 - 2.47 (m, 3H), 2.80 - 3.02 (m, 3H), 3.94 - 4.15 (m, 2H), 4.92 (s, 1H), 7.25 (m, J = 8.2 Hz, 2H), 7.28 - 7.36 (m, 1H), 7.42 (t, J = 7.8 Hz, 2H), 7.45 - 7.53 (m, 2H), 7.53 - 7.65 (m, 2H), 9.16 ppm (s, 1H); $^{13}$C-NMR (126 MHz, DMSO-$d_6$): $\delta$ = -1.5, 16.9, 17.4, 26.6, 29.2, 30.3, 32.2, 35.4, 50.3, 61.4, 72.1, 83.2, 103.8, 109.8, 126.2, 126.5, 127.1, 128.1, 128.9, 137.7, 140.1, 146.6, 147.5, 149.7, 166.6, 194.3 ppm.

**4-(Biphenyl-4-yl)-2-(but-3-yn-1-yl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carb-oxylic acid (S3).** 2-(Trimethylsilyl)ethyl 4-(biphenyl-4-yl)-2-(but-3-yn-1-yl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahy-droquinoline-3-carboxylate (**7**) (343.7 mg, 0.64 mmol) was dissolved in THF (15 mL) and a 1M solution of $NBu_4F$ in THF (2.0 mL, 2.00 mmol) was added to the reaction mixture. After stirring for 18h at room temperature additional $NBu_4F$ solution (2.0 mL, 2.00 mmol) was added to the flask and stirring was continued for 24h. Then, the solution was diluted with ethyl acetate (100 mL) and the organic layer was washed with a 1M HCl solution (2x 50 mL), followed by brine (100 mL). After drying over $MgSO_4$ the solvent was evaporated and the residue was redissolved in ethyl acetate (50 mL) and extracted with a 1M KOH solution (3x 100 mL). The combined aqueous layers were neutralized with a 1M HCl solution and the precipitated solids were collected by filtration. After extensive washings with distilled water the desired product was isolated as a yellow solid (241.4 mg, 0.56 mmol, 87%); $R_f$ = 0.15 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ = 0.84 (s, 3H), 1.01 (s, 3H), 1.99 (d, J = 16.1 Hz, 1H), 2.18 (d, J = 16.1 Hz, 1H), 2.23 - 2.33 (m, 1H), 2.36 - 2.48 (m, 3H), 2.81 - 3.03 (m, 3H), 4.90 (s, 1H), 7.19 - 7.39 (m, 3H), 7.39 - 7.45 (m, 2H), 7.45 - 7.54 (m, 2H), 7.54 - 7.65 (m, 2H), 9.06 (s, 1 H), 11.87 ppm (br s, 1H); $^{13}$C-NMR (101 MHz, DMSO-$d_6$): $\delta$ = 17.5, 26.6, 29.1, 30.2, 32.2, 35.7, 50.3, 72.0, 83.4, 104.3, 109.7, 126.2, 126.5, 127.1, 128.1, 128.9, 137.6, 140.2, 146.7, 147.1, 149.9, 168.3, 194.4 ppm.

**2-Oxopropyl 4-(biphenyl-4-yl)-2-(but-3-yn-1-yl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (S4).** 4-(Biphenyl-4-yl)-2-(but-3-yn-1-yl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxy-lic acid (**S3**) (260.0 mg, 0.61 mmol) in DMF (20 mL) was added $K_2CO_3$ (126.7 mg, 0.92 mmol) and chloroacetone (73.8 µL, 84.8 mg, 0.92 mmol). After stirring at room temperature for 18h, distilled water (50 mL) was added and the solution was extracted with ethyl acetate (3x 50 mL). The combined organic layers were washed with brine (3x 50 mL), dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate 1:1) to give a pale yellow solid (159.9 mg, 0.33 mmol, 54%): $R_f$ = 0.29 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (600 MHz, DMSO-$d_6$): $\delta$ = 0.85 (s, 3H), 1.02 (s, 3H), 1.99 (s, 3H), 2.02 (d, J = 16.1 Hz, 1H), 2.20 (d, J = 15.8 Hz, 1H), 2.26 - 2.36 (m, 1H), 2.40 - 2.49 (m, 3H), 2.86 - 2.99 (m, 3H), 4.66 (d, J = 16.9 Hz, 1H), 4.73 (d, J = 16.9 Hz, 1H), 4.98 (s, 1H), 7.21 - 7.34 (m, 3H), 7.37 - 7.45 (m, 2H), 7.48 (d, J = 8.4 Hz, 2H), 7.55 - 7.62 (m, 2H), 9.27 ppm (s, 1H); $^{13}$C-NMR (151 MHz, DMSO-$d_6$): $\delta$ = 17.3, 25.8, 26.5, 29.1, 30.4, 32.2, 35.2, 50.3, 68.0, 72.1, 83.2,102.5, 110.1, 126.2, 126.5, 127.1, 128.0, 128.8, 137.7, 140.1, 146.3, 149.0, 149.6, 165.8, 194.4,202.5 ppm.

**(3-Methyl-3*H*-diazirin-3-yl)methyl 4-(biphenyl-4-yl)-2-(but-3-yn-1-yl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (8)**. 2-Oxopropyl 4-(biphenyl-4-yl)-2-(but-3-yn-1-yl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroqui-noline-3-carboxylate (**S4**) (159.9 mg, 0.33 mmol) was suspended in a methanolic 7M $NH_3$ solution (0.33 mL, 2.31 mmol) and stirred at 0°C for 4h. Afterwards, hydroxylamine-*O*-sulfonic acid (37.5 mg, 0.33 mmol) was added and the reaction mixture was stirred for 18h at room temperature. After evaporation of the solvent the residue was suspended in MeOH (10 mL) and filtered. The remaining solid was washed with MeOH (3x 10 mL) and the combined organic phases were evaporated to dryness. This residue was then redissolved in MeOH (2 mL) and the mixture was chilled to 0°C. Then, trimethylamine (92.1 µL, 67.2 mg , 0.66 mmol) was added, followed by the amount of iodine that caused the color of the reaction mixture to stay brown for at least one minute (84.3 mg, 0.33 mmol) and the mixture was stirred for 5h at this temperature. After evaporation of the solvent the residue was dissolved in DCM (25 mL) and the organic phase was washed with distilled water (20 mL) and brine (20 mL), dried over $MgSO_4$ and the solvent was evaporated. After flash chromatographic purification (cyclohexane/ethyl acetate 7:3) the title compound was obtained as a yellow solid (77.0 mg, 0.16 mmol, 47%): $R_f$ = 0.68 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta$ = 0.84 (s, 3H), 0.94 (s, 3H), 1.01 (s, 3H), 2.01 (d, J = 16.1 Hz, 1H), 2.19 (d, J = 16.1 Hz, 1H), 2.28 - 2.36 (m, 1H), 2.36 - 2.49 (m, 3H), 2.76 - 3.00 (m, 3H), 3.81 - 3.87 (m, 1H), 3.87 - 3.94 (m, 1H), 4.89 (s, 1H), 7.20 - 7.35 (m, 3H), 7.38 - 7.45 (m, 2H), 7.46 - 7.54 (m, 2H), 7.55 - 7.67 (m, 2H), 9.25 ppm (s, 1H); $^{13}$C-NMR (126 MHz, DMSO-$d_6$): $\delta$ = 17.0, 17.3, 24.8, 26.5, 29.0, 30.3, 32.2, 35.5, 50.2, 65.1, 72.1, 83.0, 102.6, 110.1, 126.1, 126.4, 127.1, 128.1, 128.8, 137.7, 140.0, 146.4, 148.9, 149.4, 165.7, 194.3. ppm; MS (+ESI) m/z: 494.2 [M+H]; HRMS-ESI m/z [M+H] calc. for $C_{31}H_{32}N_3O_3$: 494.2438, found: 494.2434.

**2-(Trimethylsilyl)ethyl 4-(biphenyl-4-yl)-7,7-dimethyl-5-oxo-2-(3-oxobutyl)-1,4,5,6,7,8-hexahydro-quinoline-3-carboxylate (9)**. To a solution of 2-(trimethylsilyl)ethyl 4-(biphenyl-4-yl)-2-(but-3-yn-1-yl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate **(7)** (500.0 mg, 0.95 mmol) in a mixture of MeOH (80 mL) and destilled water (2 mL) Bis(trifluoromethanesulfonyl)imidate]-2-(dicyclohexyl(2',6'-dimethoxybiphenyl))phosphine gold(I) (2:1 toluene adduct, 17.8 mg, 20.0 µmol) was added and the reaction mixture was stirred at room temperature for 18h.Then, the solvent was evaporated and the residue was dissolved in ethyl acetate (100 mL). The organic phase was washed with brine (50 mL), dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate 7:3) to give a yellow solid (500.2 mg, 0.92 mmol, 97%): $R_f$ = 0.52 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (600 MHz, DMSO-$d_6$): $\delta$ = -0.02 (s, 9H), 0.81 - 0.86 (m, 3H), 0.86 - 0.97 (m, 2H), 1.01 (s, 3H), 1.95 - 2.02 (m, 1H), 2.19 (d, J = 16.1 Hz, 1H), 2.29 (d, J = 17.1 Hz, 1H), 2.43 (d, J = 17.2 Hz, 1H), 2.64 - 2.80 (m, 2H), 2.80 - 2.90 (m, 2H), 3.96 - 4.11 (m, 2H), 4.91 (s, 1H), 7.21 - 7.25 (m, 2H), 7.26 - 7.35 (m, 1H), 7.39 - 7.44 (m, 2H), 7.46 - 7.53 (m, 2H), 7.54 - 7.64 (m, 2H), 9.07 ppm (s, 1H); $^{13}$C-NMR (151 MHz, DMSO-$d_6$): $\delta$ = -1.5, 16.8, 25.8, 26.4, 29.2, 29.5, 32.1, 35.4, 41.7, 50.2, 61.2, 103.5, 109.8, 126.1, 126.4, 127.1, 128.0, 128.8, 137.6, 140.1, 146.7, 148.4, 149.7, 166.6, 194.2, 207.2 ppm.

**2-(Trimethylsilyl)ethyl 4-(biphenyl-4-yl)-7,7-dimethyl-5-oxo-2-(2-(3-methyl-3*H*-diazirin-3-yl)ethyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (11)**. 2-(Trimethylsilyl)ethyl 4-(biphenyl-4-yl)-7,7-dimethyl-5-oxo-2-(3-oxobutyl)-1,4,5,6,7,8-hexahydro-quinoline-3-carboxylate **(9)** (100.0 mg, 0.18 mmol) was suspended in a methanolic 7M $NH_3$ solution (1.3 mL, 9.10 mmol) and stirred at 0°C for 4h. Afterwards, hydroxylamine-*O*-sulfonic acid (20.8 mg, 0.18 mmol) was added and the reaction mixture was stirred for 18h at room temperature. After evaporation of the solvent the residue was suspended in MeOH (5 mL) and filtered. The remaining solid was washed with MeOH (3x 5 mL) and the combined organic phases were evaporated to dryness. This residue was then redissolved in MeOH (3 mL) and the mixture was chilled to 0°C. Then, trimethylamine (37.2 µL, 37.2 mg , 0.37 mmol) was added, followed by the amount of iodine that caused the color of the reaction mixture to stay brown for at least one minute (46.7 mg, 0.18 mmol) and the mixture was stirred for 5h at this temperature. After evaporation of the solvent the residue was dissolved in ethyl acetate (100 mL) and the organic phase was washed with distilled water (25 mL) and brine (25 mL), dried over $MgSO_4$ and the solvent was evaporated. After flash chromatographic purification (cyclohexane/ethyl acetate 4:1) the title compound was obtained as a yellow solid (43.2 mg, 0.078 mmol, 42%): $R_f$ = 0.72 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (600 MHz, DMSO-$d_6$): $\delta$ = -0.01 (s, 9), 0.84 (s, 3H), 0.86 - 0.97 (m, 2H), 1.00 (s, 3H), 1.05 (s, 3H), 1.51 - 1.66 (m, 2H), 1.98 (d, J = 16.1 Hz, 1H), 2.18 (d, J = 16.1 Hz, 1H), 2.23 - 2.34 (m, 1H), 2.41 (d, J = 16.9 Hz, 1H), 2.56 - 2.66 (m, 2H), 3.96 - 4.12 (m, 2H), 4.90 (s, 1H), 7.21 (m, J = 8.4 Hz, 2H), 7.28 - 7.35 (m, 1H), 7.38 - 7.45 (m, 2H), 7.45 - 7.50 (m, 2H), 7.59 (d, J = 7.3 Hz, 2H), 9.07 ppm (s, 1H); $^{13}$C-NMR (151 MHz, DMSO-$d_6$): $\delta$ = -1.5, 16.9, 19.1, 25.9, 26.0, 26.5, 29.1, 32.1, 33.3, 35.5, 50.2, 61.3, 103.6, 109.7, 126.1, 126.4, 127.1, 128.0, 128.8, 137.6, 140.1, 146.6, 147.7, 149.7, 166.6, 194.2 ppm.

**4-(Biphenyl-4-yl)-7,7-dimethyl-5-oxo-2-(2-(3-methyl-3*H*-diazirin-3-yl)ethyl)-1,4,5,6,7,8-hexahydro-quinoline-3-carboxylic acid (13)**. 2-(Trimethylsilyl)ethyl 4-(biphenyl-4-yl)-7,7-dimethyl-5-oxo-2-(2-(3-methyl-3*H*-diazirin-3-yl)eth-yl)-1,4,5,6,7,8-hexahy-droquinoline-3-carboxylate **(11)** (30.0 mg, 0.054 mmol) was dissolved in THF (1.5 mL) and a 1M solution of $NBu_4F$ in THF (270.0 µL, 0.27 mmol) was added to the reaction mixture. After stirring for 18h at room temperature additional $NBu_4F$ solution (270.0 µL, 0.27 mmol) was added to the flask and stirring was continued for 24h. Then, the solution was diluted with ethyl acetate (50 mL) and the organic layer was washed with a 1M HCl solution (2x 20 mL), followed by brine (20 mL). After drying over $MgSO_4$ the solvent was evaporated and the residue was redissolved in ethyl acetate (50 mL) and extracted with a 1M KOH solution (4x 20 mL). The combined aqueous layers were neutralized with a 1M HCl solution and the precipitated solids were collected by filtration. After extensive washings with distilled water the desired product was isolated as a yellow solid (10.9 mg, 0.024 mmol, 44%); $R_f$ = 0.11 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta$ = 0.83 (s, 3H), 1.00 (s, 3H), 1.04 (s, 3H), 1.49 - 1.65 (m, 2H), 1.93 - 2.04 (m, 1H), 2.18 (d, J = 16.2 Hz, 1H), 2.23 - 2.32 (m, 1H), 2.41 (d, J = 16.8 Hz, 1H), 2.57 - 2.71 (m, 2H), 4.88 (s, 1H), 7.21 (m, J = 8.2 Hz, 2H), 7.27 - 7.35 (m, 1H), 7.38 - 7.44 (m, 2H), 7.48 (m, J = 8.5 Hz, 2H), 7.55 - 7.62 (m, 2H), 9.02 (s, 1H), 11.84 ppm (br s, 1H); $^{13}$C-NMR (126 MHz, DMSO-$d_6$): $\delta$ = 19.1, 26.0, 26.0, 26.5, 29.2, 32.2, 33.5, 35.7, 50.3, 104.1, 109.6, 126.2, 126.5, 127.1, 128.1, 128.9, 137.6, 140.2, 146.8, 147.3, 149.9, 168.3, 194.3 ppm.

**Prop-2-yn-1-yl 4-(biphenyl-4-yl)-7,7-dimethyl-5-oxo-2-(2-(3-methyl-3*H*-diazirin-3-yl)ethyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (15)**. To a solution of 4-(biphenyl-4-yl)-7,7-dimethyl-5-oxo-2-(2-(3-methyl-3*H*-diazirin-3-yl)ethyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid **(13)** (10.9mg, 0.024 mmol) in DMF (0.5 mL) was added $K_2CO_3$ (5.0 mg, 0.036 mmol) and a propargyl bromide solution (80 wt.% in toluene, 4.0 µL, 4.3 mg, 0,036 mmol). After stirring at room temperature for 18h distilled water (10 mL) was added and the solution was extracted with ethyl acetate (3x 20 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate 3:2) to give a pale yellow solid (7.3 mg, 0.015 mmol, 62%): $R_f$ = 0.43 (cyclohexane/ethyl acetate 1:1); MS (+ESI) m/z: 494.2 [M+H]; HRMS-ESI m/z [M+H] calc. for $C_{31}H_{32}N_3O_3$: 494.2438, found: 494.2433.

**2-(Trimethylsilyl)ethyl 2-(but-3-yn-1-yl)-4-(4'-chlorobiphenyl-4-yl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (S5)**. 2-(Trimethylsilyl)ethyl 3-oxohept-6-ynoate (**S2**) (1.78 g, 7.38 mmol), 4'-chlorobiphenyl-4-carb-aldehyde (1.6 g, 7.38 mmol), dimedone (1.55 g, 11.09 mmol), $NH_4OAc$ (1.14 g, 14.8 mmol) and iodine (562.8 mg, 2.22 mmol) were dissolved in EtOH (4.8 mL) and the reaction mixture was stirred for 18h at room temperature. Next, the solution was diluted with EtOAc (300 mL) and the organic phase was washed with a saturated $Na_2S_2O_3$ solution (2x 150 mL), distilled water (2x 200 mL) and brine (200 mL), dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate 4:1) to give a yellow solid (2.06 g, 3.67 mmol, 50%); $R_f$ = 0.22 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (600 MHz, DMSO-$d_6$): $\delta$ = -0.02 (s, 9H), 0.84 (s, 3H), 0.86 - 0.98 (m, 2H), 1.00 (s, 3H), 1.99 (d, J = 16.1 Hz, 1H), 2.18 (d, J = 16.1 Hz, 1H), 2.27 -

2.33 (m, 1H), 2.38 - 2.48 (m, 3H), 2.84 - 3.00 (m, 3H), 3.98 - 4.12 (m, 2H), 4.93 (s, 1H), 7.24 - 7.29 (m, 2H), 7.44 - 7.51 (m, 4H), 7.59 - 7.64 (m, 2H), 9.14 ppm (s, 1H); $^{13}$C-NMR (151 MHz, DMSO-$d_6$): $\delta$ = -1.5, 16.8, 17.4, 26.5, 29.1, 30.2, 32.2, 35.5, 50.2, 61.3, 72.0, 83.2, 103.7, 109.7, 126.1, 128.2, 128.8, 131.9, 136.2, 138.9, 147.0, 147.5, 149.7, 166.6, 194.3 ppm.

**2-(Trimethylsilyl)ethyl 4-(4'-chlorobiphenyl-4-yl)-7,7-dimethyl-5-oxo-2-(3-oxobutyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (10)**. To a solution of 2-(trimethylsilyl)ethyl 2-(but-3-yn-1-yl)-4-(4'-chlorobiphenyl-4-yl)-7,7-dimethyl-5-oxo-1,4,5,6,7, 8-hexahydroquinoline-3-carboxylate (**S5**) (1.34 g, 2.39 mmol) in a mixture of MeOH (50 mL) and destilled water (2 mL) Bis(trifluoromethanesulfonyl)imidate]-2-(dicyclohexyl(2',6'-dimethoxybiphenyl))phosphine gold(I) (2:1 toluene adduct, 44.4 mg, 50 µmol) was added and the reaction mixture was stirred at room temperature for 18h.Then, the solvent was evaporated and the residue was dissolved in ethyl acetate (200 mL). The organic phase was washed with brine (100 mL), dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate 65:35) to give a yellow solid (951.8 mg, 1.65 mmol, 69%): $R_f$ = 0.18 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (600 MHz, DMSO-$d_6$): $\delta$ = -0.03 (s, 9H), 0.84 (s, 3H), 0.85 - 0.95 (m, 2H), 1.01 (s, 3H), 1.98 (d, J = 16.1 Hz, 1H), 2.12 (s, 3H), 2.18 (d, J = 16.1 Hz, 1H), 2.24 - 2.32 (m, 1H), 2.43 (d, J = 16.9 Hz, 1H), 2.65 - 2.79 (m, 2H), 2.80 - 2.90 (m, 2H), 3.94 - 4.11 (m, 2H), 4.91 (s, 1H), 7.23 (d, J = 8.4 Hz, 2H), 7.42 - 7.53 (m, 4H), 7.60 - 7.65 (m, 2H), 9.08 ppm (s, 1H); $^{13}$C-NMR (151 MHz, DMSO-$d_6$): $\delta$ = -1.5, 16.8, 25.8, 26.4, 29.2, 29.5, 32.1, 35.5, 41.7, 50.2, 61.3, 103.4, 109.7, 126.1, 128.1, 128.2, 128.8, 131.9, 136.2, 138.9, 147.1, 148.4, 149.7, 166.6, 194.3, 207.2 ppm.

**2-(Trimethylsilyl)ethyl 4-(4'-chlorobiphenyl-4-yl)-7,7-dimethyl-5-oxo-2-(2-(3-methyl-3*H*-diazirin-3-yl)ethyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (12)**. 2-(Trimethylsilyl)ethyl 4-(4'-chlorobiphenyl-4-yl)-7,7-dimethyl-5-oxo-2-(3-oxobutyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate **(10)** (930.0 mg, 1.61 mmol) was suspended in a methanolic 7M $NH_3$ solution (11.5 mL, 80.4 mmol) and stirred at 0°C for 4h. Afterwards, hydroxylamine-*O*-sulfonic acid (181.9 mg, 1.61 mmol) was added and the reaction mixture was stirred for 18h at room temperature. After evaporation of the solvent the residue was suspended in MeOH (30 mL) and filtered. The remaining solid was washed with MeOH (3x 15 mL) and the combined organic phases were evaporated to dryness. This residue was then redissolved in MeOH (25 mL) and the mixture was chilled to 0°C. Then, trimethylamine (412.5 µL, 325.8 mg , 3.2 mmol) was added, followed by the amount of iodine that caused the color of the reaction mixture to stay brown for at least one minute (817.3 mg, 3.22 mmol) and the mixture was stirred for 5h at this temperature. After evaporation of the solvent the residue was dissolved in ethyl acetate (300 mL) and the organic phase was washed with distilled water (150 mL) and brine (150 mL), dried over $MgSO_4$ and the solvent was evaporated. After flash chromatographic purification (cyclohexane/ethyl acetate 4:1) the title compound was obtained as a yellow solid (431.3 mg, 0.73 mmol, 45%): $R_f$ = 0.88 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta$ = -0.02 (s, 9H), 0.83 (s, 3H), 0.86 - 0.96 (m, 2H), 1.00 (s, 3H), 1.05 (s, 3H), 1.47 - 1.63 (m, 2H), 1.92 - 2.03 (m, 1H), 2.17 (d, J = 15.9 Hz, 1H), 2.24 - 2.31 (m, 1H), 2.41 (d, J = 17.1 Hz, 1H), 2.56 - 2.67 (m, 2H), 3.92 - 4.15 (m, 2H), 4.89 (s, 1H), 7.21

(d, J = 8.2 Hz, 2H), 7.41 - 7.54 (m, 4H), 7.58 - 7.66 (m, 2H), 9.09 ppm (s, 1H); $^{13}$C-NMR (126 MHz, DMSO-$d_6$): $\delta$ = -1.5, 16.9, 19.2, 25.9, 26.1, 26.5, 29.2, 32.2, 33.3, 35.5, 50.2, 61.3, 103.5, 109.7, 126.1, 128.1, 128.2, 128.8, 132.0, 136.3, 138.9, 147.1, 147.8, 149.7, 166.5, 194.3 ppm.

**4-(4'-Chlorobiphenyl-4-yl)-7,7-dimethyl-5-oxo-2-(2-(3-methyl-3*H*-diazirin-3-yl)ethyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid (14)**. 2-(Trimethylsilyl)ethyl 4-(4'-chlorobiphenyl-4-yl)-7,7-dimethyl-5-oxo-2-(2-(3-methyl-3*H*-diazirin-3-yl)ethyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate **(12)** (414.3 mg, 0.70 mmol) was dissolved in THF (11.2 mL) and a 1M solution of $NBu_4F$ in THF (3.51 mL, 3.51 mmol) was added to the reaction mixture. After stirring for 18h at room temperature additional $NBu_4F$ solution (3.51 mL, 3.51 mmol) was added to the flask and stirring was continued for 24h. Then, the solution was diluted with ethyl acetate (100 mL) and the organic layer was washed with a 1M HCl solution (2x 100 mL), followed by brine (100 mL), dried over $MgSO_4$ and the solvent was evaporated. After flash chromatographic purification (DCM/ethyl acetate 3:1) the title compound was obtained as a pale yellow solid (250.0 mg, 0.51 mmol, 73%); $R_f$ = 0.32 (DCM/ethyl acetate 3:1); $^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta$ = 0.82 (s, 3H), 1.00 (s, 3H), 1.04 (s, 3H), 1.47 - 1.66 (m, 2H), 1.97 (d, J = 16.2 Hz, 1H), 2.17 (d, J = 16.2 Hz, 1H), 2.22 - 2.32 (m, 1H), 2.40 (d, J = 17.1 Hz, 1H), 2.56 - 2.71 (m, 2H), 4.87 (s, 1H), 7.21 (d, J = 8.5 Hz, 2H), 7.40 - 7.55 (m, 4H), 7.56 - 7.67 (m, 2H), 9.03 (s, 1H), 11.86 ppm (br s, 1H); $^{13}$C-NMR (126 MHz, DMSO-$d_6$): $\delta$ = 19.1, 26.0, 26.0, 26.5, 29.2, 32.2, 33.5, 35.8, 50.3, 104.0, 109.6, 126.2, 128.2, 128.3, 128.8, 132.0, 136.3, 139.0, 147.2, 147.5, 150.0, 168.2, 194.4 ppm.

**Prop-2-yn-1-yl 4-(4'-chlorobiphenyl-4-yl)-7,7-dimethyl-5-oxo-2-(2-(3-methyl-3*H*-diazirin-3-yl)-ethyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (16)**. To a solution of 4-(4'-chlorobiphenyl-4-yl)-7,7-dimethyl-5-oxo-2-(2-(3-methyl-3*H*-diazirin-3-yl)-ethyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid **(14)** (98.0 mg, 0.20 mmol) in DMF (5 mL) was added $K_2CO_3$ (41.5 mg, 0.30 mmol) and a propargyl bromide solution (80 wt.% in toluene, 33.4 µL, 44.6 mg, 0.30 mmol). After stirring at room temperature for 18h distilled water (20 mL) was added and the solution was extracted with ethyl acetate (3x 30 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate 1:1) to give a pale yellow solid (99.8 mg, 0.19 mmol, 95%): $R_f$ = 0.31 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ = 0.85 (s, 3H), 1.01 (s, 3H), 1.06 (s, 3H), 1.47 - 1.65 (m, 2H), 2.00 (d, J = 16.1 Hz, 1H), 2.19 (d, J = 16.1 Hz, 1H), 2.30 (d, J = 17.1 Hz, 1H), 2.43 (d, J = 17.1 Hz, 1H), 2.55 - 2.69 (m, 2H), 3.49 (t, J = 2.7 Hz, 1H), 4.58 - 4.75 (m, 2H), 4.89 (s, 1H), 7.16 - 7.29 (m, 2H), 7.41 - 7.52 (m, 4H), 7.56 - 7.71 (m, 2H), 9.21 ppm (s, 1H); $^{13}$C-NMR (101 MHz, DMSO-$d_6$): $\delta$ = 19.1, 25.9, 26.1, 26.5, 29.1, 32.1, 33.3, 35.3, 50.2, 51.1, 77.4, 78.7, 102.3, 109.9, 126.2, 128.0, 128.2, 128.8, 132.0, 136.4, 138.8, 146.8, 149.2, 149.7, 165.5, 194.4 ppm; MS (+ESI) m/z: 528.2 [M+H]; HRMS-ESI m/z [M+H] calc. for $C_{31}H_{31}N_3O_3{}^{35}Cl$: 528.2049, found: 528.2045.

**Prop-2-yn-1-yl 4-(biphenyl-4-yl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (17)**. To a solution of 4-(biphenyl-4-yl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**S6**)[1] (387.5 mg, 1.00 mmol) in DMF (25 mL) was added $K_2CO_3$ (165.8 mg, 1.20 mmol) and a propargyl bromide solution (80 wt.% in toluene, 99.5 µL, 119.0 mg, 1.00 mmol). After stirring at room temperature for 18h distilled water (40 mL) was added and the solution was extracted with ethyl acetate (3x 20 mL). The combined organic layers were washed with brine (3x 20 mL), dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate 65:35) to give a pale yellow solid (310.6 mg, 0.73 mmol, 73%): $R_f$ = 0.42 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta$ = 0.89 (s, 3 H), 1.03 (s, 3 H), 2.03 (d, J = 16.1 Hz, 1H), 2.21 (d, J = 16.1 Hz, 1H), 2.34 (d, J = 17.2 Hz, 1H), 2.33 (s, 3H), 2.45 (d, J = 17.2 Hz, 1H), 3.48 (t, J = 2.5 Hz, 1H), 4.65 (ddd, J = 18.4, 16.1, 2.7 Hz, 1H), 4.92 (s, 1H), 7.21 - 7.64 (m, 9H), 9.22 ppm (s, 1H); $^{13}$C-NMR (126 MHz, DMSO-$d_6$): $\delta$ = 18.5, 26.6, 29.1, 32.2, 35.3, 50.3, 51.0, 77.3, 79.0, 102.4, 110.1, 126.3, 126.5, 127.1, 128.0, 128.9, 137.8, 140.1, 146.6, 146.6, 149.6, 166.0, 194.5 ppm; MS (+ESI) m/z: 426.1 [M+H].

**Ethyl 4-(biphenyl-4-yl)-7,7-dimethyl-5-oxo-2-[(prop-2-yn-1-ylamino)methyl]-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (18)**. Ethyl 4-(biphenyl-4-yl)-2-bromomethyl-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**S7**)[2] (150.3 mg, 0.30 mmol) was dissolved in a mixture of DCM and DMF (2.0 mL, 1:20 v/v). This solution was cooled to 0°C and propargylamine (57.6 µL, 49.6 mg, 0.90 mmol) was added dropwise. Next, the reaction mixture was warmed to room temperature and stirred for 24h. The solvent was evaporated and the residue was dissolved in DCM (20 mL) and the organic phase was washed with distilled water (2 x 20 mL), dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate 65:35) to give a pale yellow solid (68.0 mg, 0.15 mmol, 48%): $R_f$ = 0.22 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (600 MHz, DMSO-$d_6$): $\delta$ = 0.87 (s, 3H), 1.01 (s, 3H), 1.17 (t, J = 7.2 Hz, 3H), 2.01 (d, J = 16.1 Hz, 1H), 2.19 (d, J = 16.1 Hz, 1H), 2.41 - 2.44 (m, 2H), 3.13 (t, J = 2.6 Hz, 1H), 3.34 (d, J = 2.6 Hz, 2H), 3.82 - 3.85 (m, 2H), 4.01 (q, J = 7.2 Hz, 2H), 4.92 (s, 1H), 7.23 - 7.61 (m, 9H), 8.93 ppm (br, s, 1H); $^{13}$C-NMR (151 MHz, DMSO-$d_6$): $\delta$ = 14.2, 26.6, 29.2, 32.2, 35.9, 37.3, 47.2, 50.3, 59.4, 74.1, 82.5, 103.7, 110.0, 126.2, 126.5, 127.1, 128.1, 128.9, 137.7, 140.1, 146.7, 146.8, 149.6, 166.6, 194.5 ppm; MS (+ESI) m/z: 469.8 [M+H].

**Ethyl 4-(biphenyl-4-yl)-7,7-dimethyl-5-oxo-2-[(prop-2-yn-1-yloxy)methyl]-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (19)**. Ethyl 3-oxo-4-(prop-2-yn-1-yloxy)butanoate (**5**)[3] (330.3 mg, 1.78 mmol), 4-biphenylcarbaldehyde (326.3 mg, 1.78 mmol) and dimedone (251.2 mg, 1.78 mmol) were dissolved in a methanolic 2M $NH_3$ solution (1.34 mL, 2.68 mmol) and heated for 8h under reflux conditions. After cooling to room temperature the solvent and the excess ammonia was evaporated and the residue was redissolved in ethyl acetate (20 mL). The organic phase was washed with distilled water (2x 20 mL) and brine (20 mL), dried over $MgSO_4$ and the solvent was evaporated. After recrystallization from EtOH the crude product was purified by flash chromatography (DCM/methanol 9:1) to give a pale yellow solid (297.1 mg, 0.63 mmol, 35%): $R_f$ = 0.68 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta$ = 0.85 - 0.89 (m, 3 H),1.02 (s, 3H), 1.17 (t, J = 7.1 Hz, 3H), 2.01 (d, J = 16.1 Hz, 1H), 2.20 (d, J = 16.1

Hz, 1H), 2.29 - 2.48 (m, 2H), 3.53 (t, J = 2.3 Hz, 1H), 4.03 (q, J = 7.3 Hz, 2H), 4.24 (d, J = 2.3 Hz, 2H), 4.66 - 4.71 (m, 2H), 4.93 (s, 1H), 7.22 - 7.63 (m, 9H), 8.95 ppm (s, 1H); $^{13}$C-NMR (126 MHz, DMSO-$d_6$): $\delta$ = 15.0, 27.4, 30.0, 33.0, 36.6, 51.1, 58.5, 60.5, 66.6, 78.7, 80.6, 105.4, 110.6, 127.1, 127.3, 128.0, 128.9, 129.7, 138.7, 140.9, 144.8, 147.2, 150.6, 167.2, 195.3 ppm; MS (+ESI) m/z: 470.0 [M+H].

**Ethyl 4-(biphenyl-4-yl)-2-(but-3-yn-1-yl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (20)**. Ethyl 3-oxohept-6-ynoate (**4**)[3] (190.2 mg, 1.11 mmol), 4-biphenyl-carbaldehyde (205.3 mg, 1.11 mmol), dimedone (160.4 mg, 1.11 mmol), $NH_4OAc$ (88.2 mg, 1.11 mmol) and iodine (87.9 mg, 0.34 mmol) in EtOH (3.0 mL) was stirred for 18h at room temperature. Next, the solution was diluted with EtOAc (30 mL) and the organic phase was washed with a saturated $Na_2S_2O_3$ solution (2x 25 mL) and brine (25 mL), dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate 65:35) to give a pale yellow solid (228.2 mg, 0.50 mmol, 45%); $R_f$ = 0.69 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta$ = 0.87 (s, 3H), 1.02 (s, 3H), 1.17 (t, J = 7.3 Hz, 3H), 2.01 (d, J = 16.1 Hz, 1H), 2.19 (d, J = 16.1 Hz, 1H), 2.33 (d, J = 16.1 Hz, 1H), 2.44 (d, J = 16.1 Hz, 1H), 2.46 - 2.49 (m, 2H), 2.85 - 3.02 (m, 2H), 2.89 (t, J = 2.3 Hz, 1H), 4.02 (q, J = 7.3 Hz, 2H), 4.93 (s, 1H), 7.24 - 7.66 (m, 9H), 9.15 ppm (s, 1H); $^{13}$C-NMR (126 MHz, DMSO-$d_6$): $\delta$ = 14.0, 17.4, 26.5, 29.0, 30.2, 32.1, 35.6, 50.3, 59.3, 71.9, 83.1, 103.7, 109.8, 126.1, 126.4, 127.0, 128.1, 128.8, 137.6, 140.1, 146.6, 147.4, 149.6, 166.4, 194.2 ppm; MS (+ESI) m/z: 454.0 [M+H].

**2-Oxopropyl 4-(biphenyl-4-yl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (S8)**. To a solution of 4-(biphenyl-4-yl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**S6**)[1] (387.5 mg, 1.00 mmol) in DMF (25 mL) was added $K_2CO_3$ (165.8 mg, 1.20 mmol) and chloroacetone (80.4 µL, 92.5 mg, 1.00 mmol). After stirring at room temperature for 18h, distilled water (40 mL) was added and the solution was extracted with ethyl acetate (3x 20 mL). The combined organic layers were washed with brine (3x 20 mL), dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate 1:1) to give a pale yellow solid (346.0 mg, 0.78 mmol, 78%): $R_f$ = 0.25 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta$ = 0.87 (s, 3H), 1.03 (s, 3H), 1.98 (s, 3H), 2.02 (d, J = 16.1 Hz, 1H), 2.20 (d, J = 16.1 Hz, 1H), 2.33 (t, J = 17.2 Hz, 1H), 2.33 (s, 3H), 2.43 (d, J = 17.6 Hz, 1H), 4.65 - 4.69 (m, 2H), 4.97 (s, 1H), 7.21 - 7.62 (m, 9H), 9.20 ppm (s, 1H); $^{13}$C-NMR (126 MHz, DMSO-$d_6$): $\delta$ = 18.5, 25.8, 26.5, 29.0, 32.1, 35.2, 50.3, 67.8, 102.3, 110.1, 126.1, 126.4, 127.0, 127.9, 128.8, 137.6, 140.1, 146.5, 146.5, 149.5, 166.2, 194.4, 202.6 ppm; MS (+ESI) m/z: 444.1 [M+H].

**(3-Methyl-3*H*-diazirin-3-yl)methyl 4-(biphenyl-4-yl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (21)**. 2-Oxopropyl 4-(biphenyl-4-yl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxy-late (**S8**) (221.8 mg, 0.50 mmol) was suspended in a methanolic 7M $NH_3$ solution (0.5 mL, 3.50 mmol) and stirred at 0°C for 4h. Afterwards, hydroxylamine-*O*-sulfonic acid (56.5 mg, 0.50 mmol) was added and the reaction mixture was stirred for 18h at room temperature. After evaporation of the solvent the residue was suspended in MeOH (25 mL) and filtered. The remaining solid was washed with MeOH (3x 25 mL) and the combined organic phases were evaporated to

dryness. This residue was then redissolved in MeOH (5 mL) and the mixture was chilled to 0°C. Then, trimethylamine (103.5 µL, 75.5 mg, 0.75 mmol) was added, followed by the amount of iodine that caused the color of the reaction mixture to stay brown for at least one minute (127.0 mg, 0.50 mmol) and the mixture was stirred for 5h at this temperature. After evaporation of the solvent the residue was dissolved in DCM (25 mL) and the organic phase was washed with distilled water (20 mL) and brine (20 mL), dried over $MgSO_4$ and the solvent was evaporated. After flash chromatographic purification (DCM/ethyl acetate 9:1) the title compound was obtained as a pale yellow solid (107.1 mg, 0.24 mmol, 47%): $R_f$ = 0.81 (DCM/ethyl acetate 4:1); $^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta$ = 0.86 (s, 3H), 0.94 (s, 3H), 1.02 (s, 3H), 2.01 (d, J = 16.1 Hz, 1H), 2.19 (d, J = 16.1 Hz, 1H), 2.31 (d, J = 16.1 Hz, 1H), 2.32 (s, 3H), 2.43 (d, J = 16.8 Hz, 1H), 3.84 - 3.88 (m, 2H), 4.88 (s, 1H), 7.24 - 7.62 (m, 9H), 9.19 ppm (s, 1H); $^{13}$C-NMR (126 MHz, DMSO-$d_6$): $\delta$ = 17.0, 18.5, 24.9, 26.5, 29.0, 32.1, 35.5, 50.2, 64.7, 102.4, 110.1, 126.1, 126.4, 127.0, 128.0, 128.8, 137.6, 140.1, 146.5, 146.6, 149.3, 166.1, 194.3 ppm; MS (+ESI) m/z: 456.1 [M+H].

**2-(3-Methyl-3*H*-diazirin-3-yl)ethan-1-ol (S9)**. 4-Hydroxybutan-2-one (1.3 mL, 1.3 g, 15.00 mmol) was dissolved in a methanolic 7M $NH_3$ solution (15 mL, 45.00 mmol) and stirred at 0°C for 4h. Afterwards, hydroxylamine-*O*-sulfonic acid (17.0 mg, 15.00 mmol) was added and the reaction mixture was stirred for 18h at room temperature. After evaporation of the solvent the residue was suspended in MeOH (150 mL) and filtered. The remaining solid was washed with MeOH (3x 75 mL) and the combined organic phases were evaporated to dryness. This residue was then redissolved in MeOH (100 mL) and the mixture was chilled to 0°C. Then, trimethylamine (2.1 mL, 1.5 g, 15.00 mmol) was added, followed by the amount of iodine that caused the color of the reaction mixture to stay brown for at least one minute (1.9 g, 7.50 mmol) and the mixture was stirred for 5h at this temperature. After evaporation of the solvent the residue was dissolved in DCM (150 mL) and the organic phase was washed with distilled water (100 mL) and brine (100 mL), dried over $MgSO_4$ and the solvent was evaporated. After flash chromatographic purification (cyclohexane/ethyl acetate 3:1) the title compound was obtained as a yellow oil (420.5 mg, 4.2 mmol, 28%): $R_f$ = 0.51 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (500 MHz, $CDCl_3$): $\delta$ = 1.10 (s, 3H), 1.49 (br s, 1H), 1.66 (t, J = 6.3 Hz, 2H), 3.56 ppm (t, J = 6.3, 2H); $^{13}$C-NMR (126 MHz, $CDCl_3$): $\delta$ = 20.0, 23.9, 36.7, 57.5 ppm.

**3-(2-Iodoethyl)-3-methyl-3*H*-diazirine (3).** To a solution of triphenylphosphine (865.6 mg, 3.30 mmol) and imidazole (612.7 mg, 9.00 mmol) in DCM (16.5 mL) iodine (913.7 mg, 3.60 mmol) was added slowly at 0°C. After stirring for 15 min a solution of 2-(3-methyl-3*H*-diazirin-3-yl)ethan-1-ol (**S9**) (300.4 mg, 3.00 mmol) in DCM (3.6 mL) was added dropwise and the solution was stirred for additional 4h at this temperature. After warming to room temperature a saturated solution of $Na_2S_2O_3$ (30 mL) was added and the mixture was extracted with ethyl acetate (2x 25 mL). The combined organic layers were washed with brine (2x 25 mL), dried over $MgSO_4$ and the solvent was evaporated. After flash chromatographic purification (cyclohexane/ethyl acetate 92:8) the title compound was obtained as a colorless oil (390.6 mg, 1.86 mmol, 62%): $R_f$ = 0.60 (cyclohexane/ethyl acetate 10:1); $^1$H-NMR (500 MHz, $CDCl_3$): $\delta$ = 1.08 (s, 3H), 2.03 (t, J = 7.6 Hz, 2H), 2.95 ppm (t, J = 7.6 Hz, 2H); $^{13}$C-NMR (126 MHz, $CDCl_3$): $\delta$ = -3.4, 19.6, 39.2, 71.6 ppm.

**2-(3-Methyl-3*H*-diazirin-3-yl)ethyl 4-(biphenyl-4-yl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (22)**. To a solution of 4-(biphenyl-4-yl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**S6**)[1] (387.5 mg, 1.00 mmol) in DMF (25 mL) $K_2CO_3$ (165.8 mg, 1.20 mmol) and 3-(2-iodoethyl)-3-methyl-3*H*-diazirine **(3)** (205.6 mg, 1.00 mmol) was added. After stirring at room temperature for 18h, distilled water (40 mL) was added and the solution was extracted with ethyl acetate (3x 20 mL). The combined organic layers were washed with brine (3x 20 mL), dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate 2:1) to give a pale yellow solid (281.8 mg, 0.60 mmol, 60%): $R_f$ = 0.45 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta$ = 0.87 (s, 3H), 0.96 (s, 3H), 1.02 (s, 3H), 1.61 (td, J = 5.9, 2.9 Hz, 2H), 2.01 (d, J = 16.1 Hz, 1H), 2.20 (d, J = 16.1 Hz, 1H), 2.32 (d, J = 17.2 Hz, 1H), 2.36 (s, 3H), 2.45 (d, J = 17.2 Hz, 1H), 3.86 - 3.89 (m, 2H), 4.98 (s, 1H), 7.22 - 7.65 (m, 9H), 9.17 ppm (s, 1H); $^{13}$C-NMR (126 MHz, DMSO-$d_6$): $\delta$ = 18.6, 19.3, 24.4, 26.6, 29.1, 32.2, 33.1, 35.5, 50.3, 58.5, 103.0, 110.1, 126.2, 126.5, 127.1, 128.1, 128.8, 137.6, 140.2, 145.8, 146.9, 149.5, 166.8, 194.4 ppm; MS (+ESI) m/z: 470.1 [M+H].

**Ethyl 2-(2-methyl-1,3-dioxolan-2-yl)acetate (S10)**. Ethylene glycol (1.6 g 25.00 mmol) and *p*-toluenesulfonic acid (36.5 mg, 0.019 mmol) were added to a stirred solution of ethyl acetoacetate (2.5 g, 19.20 mmol) in toluene (25 mL) and the mixture was heated in a Dean-Stark-apparatus for 5h. After cooling to room temperature the solvent was evaporated and the residue was dissolved in ethyl acetate (35 mL). The organic phase was washed with a 5% $NaHCO_3$ solution (2x 35 mL) and brine (25 mL), dried over $MgSO_4$ and the solvent was evaporated to yield a pale yellow oil (1.8 g, 13.80 mmol, 54%): $R_f$ = 0.43 (cyclohexane /ethyl acetate 1:1); $^1$H-NMR (500 MHz, $CDCl_3$): $\delta$ = 1.27 (t, J = 7.3 Hz, 3H), 1.51 (s, 3H), 2.67 (s, 2H), 3.96 - 4.00 (m, 4H), 4.16 ppm (q, J = 7.3 Hz, 2H); $^{13}$C-NMR (126 MHz, $CDCl_3$): $\delta$ = 24.5, 44.3, 60.9, 64.8, 66.1, 107.6, 109.6, 169.6 ppm; MS (+ESI) m/z: 175.8 [M+H].

**2-(2-Methyl-1,3-dioxolan-2-yl)ethanol (S11)**. Ethyl 2-(2-methyl-1,3-dioxolan-2-yl)acetate (**S10**) (409.7 mg, 3.10 mmol) was added slowly to a stirred suspension of $LiALH_4$ (178.4 mg, 4.65 mmol) in diethyl ether (2.5 mL) at 0°C. Afterwards the mixture was heated to reflux for 90 mins. Next, the reaction mixture was chilled to 0°C and distilled water (5 mL) was added carefully under strong stirring, followed by an addition of a 1M KOH solution (4 mL). The biphasic solution was filtered over a small pad of silica gel which was subsequently washed with diethyl ether (150 mL). The organic layer was separated and dried over $MgSO_4$ and the solvent was evaporated. After flash chromatographic purification (cyclohexane/ethyl acetate 1:1) the product was obtained as a pale yellow oil (348.1 mg, 2.60 mmol, 84%): $R_f$ = 0.26 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (500 MHz, $CDCl_3$): $\delta$ = 1.37 (s, 3H), 1.96 (t, J = 5.4 Hz, 2H), 3.77 (t, J = 5.4 Hz, 2H), 4.00 ppm (s, 4H); $^{13}$C-NMR (126 MHz, $CDCl_3$): $\delta$ = 23.5, 40.1, 58.6, 64.2, 110.1 ppm.

**Ethyl 4-(2-(2-methyl-1,3-dioxolan-2-yl)ethoxy)-3-oxobutanoate (6)**. A solution of 2-(2-methyl-1,3-dioxolan-2-yl)ethanol (**S11**) (307.9 mg, 2.30 mmol) in DMF (2 mL) was added dropwise to a stirred suspension of NaH (242.1 mg, 60% w/w in mineral oil, 10.10 mmol) in DMF (8 mL) at 0°C and the

reaction mixture was stirred for 2h at room temperature. Then, a solution of ethyl chloroacetate (250.1 mg, 1.50 mmol) in DMF (1 mL) was added dropwise. After stirring for 3h at room temperature a 1M HCl solution (1 mL) was added and the mixture was extracted with DCM (3x 15 mL). The combined organic phases were washed with water (3x 20 mL) and brine (20 mL), dried over $MgSO_4$ and the solvent was evaporated. After flash chromatographic purification (cyclohexane/ethyl acetate 3:2) the title compound was obtained as an orange oil (156.2 mg, 0.61 mmol, 42%): $R_f$ = 0.56 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (500 MHz, $CDCl_3$): $\delta$ = 1.28 (t, J = 7.3 Hz, 3H), 1.35 (s, 3H), 2.01 (t, J = 6.9 Hz, 2H), 3.53 (s, 2H), 3.62 (t, J = 6.9 Hz, 2H), 3.90 - 3.98 (m, 4H), 4.11 (s, 2H), 4.20 ppm (q, J = 7.3 Hz, 2H); $^{13}$C-NMR (126 MHz, $CDCl_3$): $\delta$ = 13.8, 24.0, 38.4, 45.7, 61.1, 64.3, 67.6, 75.6, 108.4, 108.5, 166.7, 201.8 ppm; MS (+ESI) m/z: 261.1 [M+H].

**Ethyl 4-(biphenyl-4-yl)-7,7-dimethyl-2-[(2-(2-methyl-1,3-dioxolan-2-yl)ethoxy)methyl]-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (S12)**. Ethyl 4-(2-(2-methyl-1,3-dioxolan-2-yl)ethoxy)-3-oxobutanoate **(6)** (702.9 mg, 2.71 mmol), 4-biphenylcarbaldehyde (493.8 mg, 2.71 mmol) and dimedone (379.9 mg, 2.78 mmol) were dissolved in a methanolic 2M $NH_3$ solution (2.05 mL, 4.10 mmol) and heated for 3h under reflux conditions. After cooling to room temperature the solvent and the excess ammonia was evaporated and the residue was redissolved in ethyl acetate (20 mL). The organic phase was washed with distilled water (2x 20 mL) and brine (20 mL), dried over $MgSO_4$ and the solvent was evaporated. After recrystallization from MeOH the crude product was purified by flash chromatography (cyclohexane/ethyl acetate 3:2) to give a colorless solid (709.4 mg, 1.30 mmol, 48%); $R_f$ = 0.47 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta$ = 0.89 (s, 3H), 1.02 (s,3H), 1.17 (t, J = 7.3 Hz, 3H), 1.27 (s, 3H), 2.02 (d, J = 16.1 Hz, 1H), 2.20 (d, J = 16.1 Hz, 1H), 2.45 (d, J = 2.7 Hz, 2H), 3.55 (td, J = 6.7, 3.8 Hz, 2H), 3.83 - 3.89 (m, 4H), 3.91 - 3.95 (m, 2H), 4.02 (q, J = 7.3 Hz, 2H), 4.58 - 4.61 (m, 2H), 4.93 (s, 1H), 7.22 - 7.61 (m, 9H), 8.84 ppm (s, 1H); $^{13}$C-NMR (126 MHz, DMSO-$d_6$): $\delta$ = 14.0, 23.9, 26.6, 29.0, 32.2, 35.7, 38.0, 50.2, 59.5, 63.9, 66.4, 104.0, 108.2, 109.1, 109.8, 126.1, 126.4, 127.0, 128.0, 128.8, 137.7, 140.0, 144.7, 146.3, 149.6, 166.3, 194.3 ppm; MS (+ESI) m/z: 546.1 [M+H].

**Ethyl 4-(biphenyl-4-yl)-7,7-dimethyl-5-oxo-2-[(3-oxobutoxy)methyl]-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (S13)**. Ethyl 4-(biphenyl-4-yl)-7,7-dimethyl-2-[(2-(2-methyl-1,3-dioxolan-2-yl)-ethoxy)methyl]-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**S12**) (709.4 mg, 1.30 mmol) was dissolved in a mixture of acetone and water (5:1 v/v, 20 mL) and *p*-toluenesulfonic acid (62.8 mg, 0.33 mmol) was added and the reaction mixture was stirred for 48h at room temperature. After evaporation of the solvent the residue was dissolved in ethyl acetate (20 mL) and the organic phase was washed successively with a 10% $NaHCO_3$ solution (2x 15 mL), distilled water (10 mL) and brine (10 mL). After drying over $MgSO_4$ the solvent was evaporated and the crude product was purified by flash column chromatography (cyclohexane/ethyl acetate 4:1) to give a pale yellow solid (482.6 mg, 0.92 mmol, 74%): $R_f$ = 0.47 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta$ = 0.89 (s, 3H), 1.03 (s, 3H), 1.17 (t, J = 7.3 Hz, 3H), 2.03 (d, J = 16.1 Hz, 1H), 2.15 (s, 3H), 2.21 (d, J = 16.1 Hz, 1H), 2.52 (d, J = 3.4 Hz, 2H), 2.80 (t, J = 5.7 Hz, 2H), 3.66 (td, J = 5.7, 1.0 Hz, 2H), 4.02 (q, J = 7.3 Hz, 2H), 4.61 - 4.64 (m, 2H), 4.93 (s, 1H), 7.22 - 7.62 (m, 9H), 8.88 ppm (s, 1H); $^{13}$C-NMR (126 MHz, DMSO-$d_6$): $\delta$ = 14.1, 26.6,

29.1, 32.2, 35.7, 36.2, 42.8, 50.2, 59.5, 65.4, 66.9, 103.7, 110.0, 126.2, 126.4, 127.1, 128.0, 128.8, 137.8, 140.0, 145.0, 146.4, 149.6, 166.3, 194.4, 207.8 ppm; MS (+ESI) m/z: 502.1 [M+H].

**Ethyl 4-(biphenyl-4-yl)-7,7-dimethyl-2-[(2-(3-methyl-3*H*-diazirin-3-yl)ethoxy)methyl]-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (23)**. Ethyl 4-(biphenyl-4-yl)-7,7-dimethyl-5-oxo-2-[(3-oxo-butoxy)methyl]-1,4,5,6,7,8-hexahydro-quinoline-3-carboxylate (**S13**) (445.9 mg, 0.85 mmol) was suspended in a methanolic 7M $NH_3$ solution (0.85 mL, 5.95 mmol) at 0°C and the mixture was stirred for 4h at this temperature. Afterwards, hydroxylamine-*O*-sulfonic acid (96.1 mg, 0.85 mmol) was added and the reaction mixture was stirred for 18h at room temperature. After evaporation of the solvent the residue was suspended in MeOH (10 mL) and filtered. The collected solid was washed with MeOH (3x 10 mL) and the combined organic phases were evaporated to dryness. This residue was then redissolved in MeOH (2 mL) and the mixture was chilled to 0°C. Then, trimethylamine (176.3 µL, 129.0 mg, 1.28 mmol) was added, followed by the amount of iodine that caused the color of the reaction mixture to stay brown for at least one minute (215.7 mg, 0.85 mmol) and the mixture was stirred for 5h at this temperature. After evaporation of the solvent the residue was dissolved in DCM (30 mL) and the organic phase was washed with distilled water (20 mL) and brine (20 mL), dried over $MgSO_4$ and the solvent was evaporated. After flash chromatographic purification (cyclohexane/ethyl acetate 4:1) the title compound was obtained as a yellow solid (96.1 mg, 0.19 mmol, 22%): $R_f$ = 0.77 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (600 MHz, DMSO-$d_6$): $\delta$ = 0.89 (s, 3H), 1.02 (s, 3H), 1.04 (s, 3H), 1.17 (t, J = 7.3 Hz, 3H), 1.65 (t, J = 6.2 Hz, 2H), 2.03 (d, J = 16.1 Hz, 1H), 2.21 (d, J = 16.1 Hz, 1H), 2.47 (s, 2H), 3.38 (s, 2H), 4.03 (q, J = 7.3 Hz, 2H), 4.61 - 4.63 (m, 2H), 4.96 (s, 1H), 7.24 - 7.62 (m, 9H), 8.88 ppm (s, 1H); $^{13}$C-NMR (151 MHz, DMSO-$d_6$): $\delta$ = 14.1, 19.6, 24.7, 26.6, 29.1, 32.2, 33.7, 35.8, 50.3, 59.6, 65.2, 66.3, 104.7, 109.7, 126.2, 126.5, 127.1, 128.1, 128.9, 137.9, 140.0, 146.4, 149.7, 166.4, 194.4 ppm; MS (+ESI) m/z: 514.1 [M+H].

**Ethyl 4-(biphenyl-4-yl)-7,7-dimethyl-5-oxo-2-(3-oxobutyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (S14)**. To a solution of ethyl 4-(biphenyl-4-yl)-2-(but-3-yn-1-yl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexa-hydroquinoline-3-carboxylate **(20)** (100.0 mg, 0.22 mmol) in a mixture of MeOH and destilled water (11 mL, 10:1 v/v) Bis(trifluoromethanesulfonyl)imidate](triphenylphosphine) gold(I) (2:1 toluene adduct, 3.5 mg, 2.20 µmol) was added and the reaction mixture was heated to 70°C for 5h. After cooling to room temperature, the solvent was evaporated and the residue was dissolved in ethyl acetate (20 mL). The organic phase was washed with distilled water (2x 20 mL) and brine (20 mL), dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate 65:35) to give a yellow solid (55.8 mg, 0.12 mmol, 54%): $R_f$ = 0.41 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta$ = 0.86 (s, 3H), 1.02 (s, 3H), 1.14 (t, J = 7.1 Hz, 3H), 2.00 (d, J = 16.4 Hz, 1H), 2.13 (s, 3H), 2.19 (d, J = 16.4 Hz, 1H), 2.30 (d, J = 16.8 Hz, 1H), 2.43 (d, J = 16.8 Hz, 1H), 2.67 - 2.76 (m, 2H), 2.81 - 2.89 (m, 2H), 3.99 (q, J = 7.3 Hz, 2H), 4.90 (s, 1H), 7.19 - 7.65 (m, 9H), 9.08 ppm (s, 1H); $^{13}$C-NMR (126 MHz, DMSO-$d_6$): $\delta$ = 14.0, 25.9, 26.5, 29.1, 29.5, 32.1, 35.6, 41.7, 50.2, 59.2, 103.4, 109.8, 126.1, 126.4, 127.1, 128.0, 128.8, 137.6, 140.0, 146.8, 148.4, 149.7, 166.4, 194.3, 207.2 ppm; MS (+ESI) m/z: 472.2 [M+H].

**Ethyl 4-(biphenyl-4-yl)-7,7-dimethyl-2-(2-(3-methyl-3*H*-diazirin-3-yl)ethyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (24)**. Ethyl 4-(biphenyl-4-yl)-7,7-dimethyl-5-oxo-2-(3-oxobutyl)-1,4,5,6,7,8-hexahydroquinoline-3-carb-oxylate (**S14**) (46.5 mg, 0.10 mmol) was suspended in a methanolic 7M $NH_3$ solution (0.1 mL, 0.70 mmol) and stirred at 0°C for 4h. Afterwards, hydroxylamine-*O*-sulfonic acid (11.3 mg, 0.10 mmol) was added and the reaction mixture was stirred for 18h at room temperature. After evaporation of the solvent the residue was suspended in MeOH (5 mL) and filtered. The remaining solid was washed with MeOH (3x 5 mL) and the combined organic phases were evaporated to dryness. This residue was then redissolved in MeOH (1 mL) and the mixture was chilled to 0°C. Then, trimethylamine (20.7 µL, 15.1 mg, 0.15 mmol) was added, followed by the amount of iodine that caused the color of the reaction mixture to stay brown for at least one minute (25.4 mg, 0.10 mmol) and the mixture was stirred for 5h at this temperature. After evaporation of the solvent the residue was dissolved in DCM (10 mL) and the organic phase was washed with distilled water (5 mL) and brine (5 mL), dried over $MgSO_4$ and the solvent was evaporated. After flash chromatographic purification (cyclohexane/ethyl acetate 2:1) the title compound was obtained as a pale yellow solid (23.2 mg, 0.048 mmol, 48%): $R_f$ = 0.61 (cyclohexane/ethyl acetate 1:1); $^1$H-NMR (600 MHz, DMSO-$d_6$): $\delta$ = 0.87 (s, 3H), 1.02 (s, 3H), 1.07 (s, 3H), 1.17 (t, J = 7.3 Hz, 3H), 1.55 - 1.64 (m, 2H), 2.00 (d, J = 16.1 Hz, 1H), 2.19 (d, J = 16.1 Hz, 1H), 2.31 (d, J = 17.2 Hz, 1H), 2.43 (d, J = 17.2 Hz, 1H), 2.60 - 2.64 (m, 2H), 4.01 (q, J = 7.3 Hz, 2H), 4.90 (s, 1H), 7.20 - 7.63 (m, 9H), 9.09 ppm (s, 1H); $^{13}$C-NMR (151 MHz, DMSO-$d_6$): $\delta$ = 14.1, 19.1, 25.8, 26.0, 26.5, 29.0, 32.1, 33.2, 35.6, 50.2, 59.3, 103.5, 109.7, 126.1, 126.4, 127.0, 128.0, 128.8, 137.6, 140.0, 146.6, 147.7, 149.6, 166.3, 194.2 ppm; MS (+ESI) m/z: 484.1 [M+H].

3.3. Chiral separation of (*rac*)-**16** (Figure S3)

Materials, reagents, instrumentation:
Ethanol (99.5%) was obtained from Kemetyl AB (Haninge, Sweden), 2-propanol from Sigma-Aldrich (Seelze, Germany) and liquid carbon dioxide was purchased from AGA Gas AB (Stenungsund, Sweden). The analytical SFC separations were performed on an ACQUITY UPC$^2$ equipped with a PDA detector and two column ovens with seven column positions each. The chromatographic data was collected using Empower 3 Pro software (Waters, Milford, MA, USA). The preparative SFC separation was run on a SuperSep 150 using the iFix 5.1 software (NovaSep, Pompay, France). The columns used for screening were Chiralpak IA and IC purchased from Chiral Technologies (Illkirch, France), Lux Cellulose 3 and 4 and Lux Amylose 1 from Phenomenex (Torrance, CA, USA), Kromasil CelluCoat from Eka Chemicals (Bohus, Sweden), Chiralart Amylose-SA from YMC (Kyoto, Japan) and Whelk-O1 from Regis Technologies (Morton Grove, IL, USA). The dimensions and particle size for the columns were 150 mm × 4.6 mm I.D. and 3 µm. The preparative column used was purchased as pre-packed from Phenomenex (Torrance, CA, USA)

Isolation of the individual enantiomers from racemate **16** was done by supercritical fluid chromatography (SFC) as follows:

A SFC column screen on (*rac*)-**16** on a broad set of chiral stationary phases (CSPs) resulted in high enantioselectivity on both Chiralpak IA and Lux Amylose 1. After optimization using ethanol and 2-propanol as organic modifiers the most favorable method was identified on Chiralpak IA using ethanol. Unfortunately, due to low solubility of the compound in the supercritical mobile phase this method resulted in peak splitting and co-elution of the enantiomers in the preparative scale. To successfully isolate the enantiomers Lux Amylose 1 was used, also in this case the low solubility caused problems and only 16 mg could be injected each cycle.

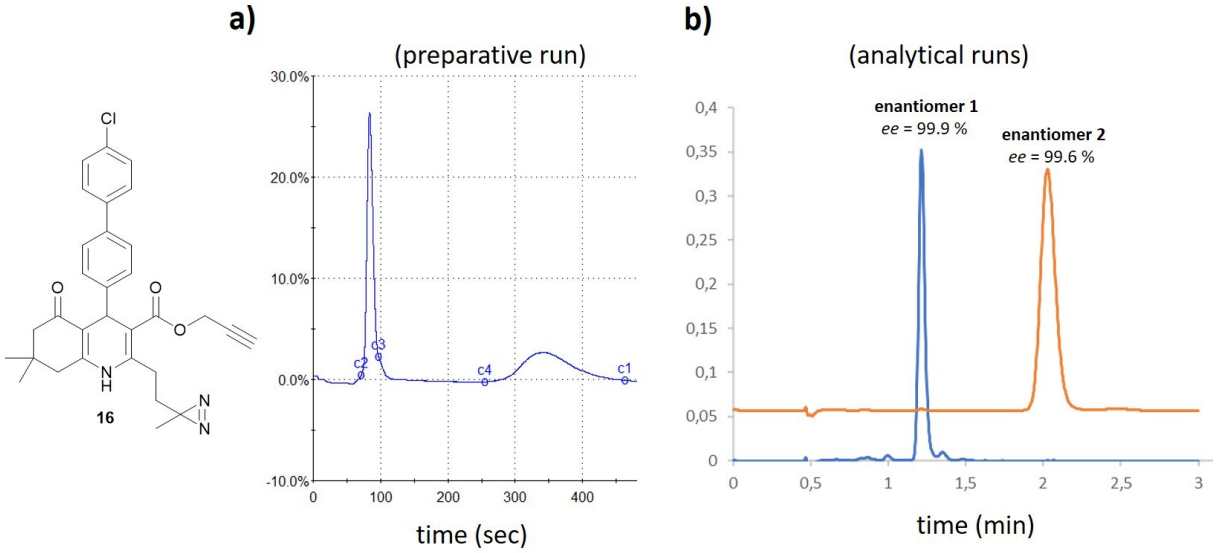



**Figure S3.** Chiral separation of (*rac*)-**16**. **a)** Preparative SFC chromatogram using a Lux Amylose 1 column (250 mm × 20 mm, 5 µm). Mobile phase: 30% ethanol in $CO_2$ at 40 °C, 140 bar at a flow rate of 70 mL/min. The injection amount was limited to 16 mg racemate each cycle (1 mL EtOH) due to low solubility in the supercritical mobile phase. **b)** Analytical SFC chromatogram of the two isolated enantiomers using a Chiralpak IA column (150 mm × 4.6 mm, 3 µm). Mobile phase: 30% ethanol in $CO_2$ at 40 °C, 120 bar at a flow rate of 3.5 mL/min. The compounds were detected at 240 nm.

3.4. TGFβ inhibition assay

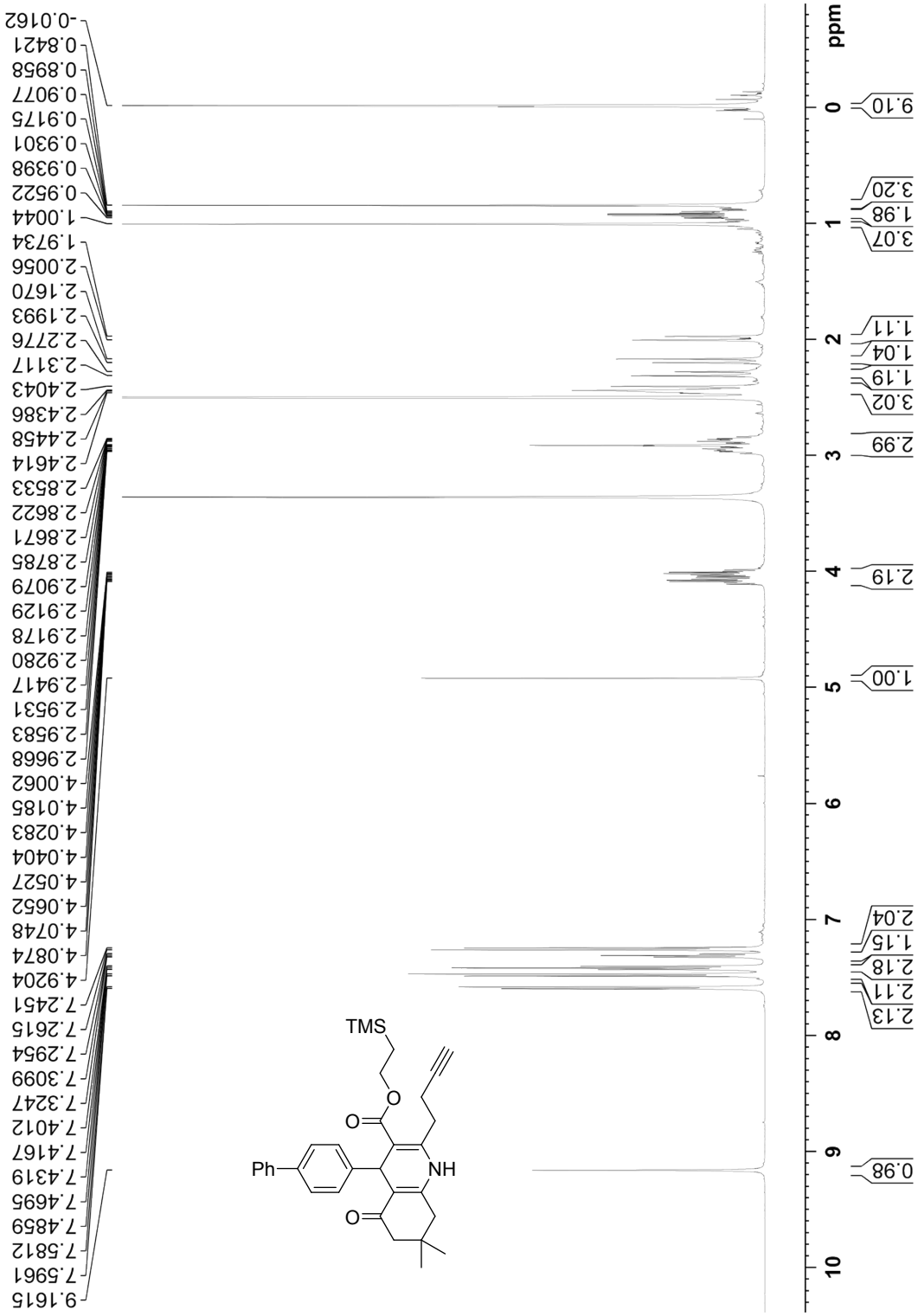
A Smad-4 binding element (SBE-4)-based transient luciferase reporter gene assay was performed in HEK293T cells as described in detail before.[1-2] Briefly, cells were co-transfected with a SBE 4-firefly luciferase and TK-driven renilla luciferase plasmid, replated after 12-14 h on 96-well plates and incubated for 2 hours before addition of test compounds, DMSO and TGFβ-2 (10 ng/mL). Each condition was done in triplicate. After 20-22 h, firefly and renilla luciferase activities were measured on a plate reader (Tecan Infinite M1000) following the instructions of the Dual Glo® Assay Kit (Promega). Presented data was derived from n = 2-4 independent experiments unless otherwise stated. GraphPad Prism 5 was used for data evaluation.

3.5. Subcellular probe localization (confocal microscopy)

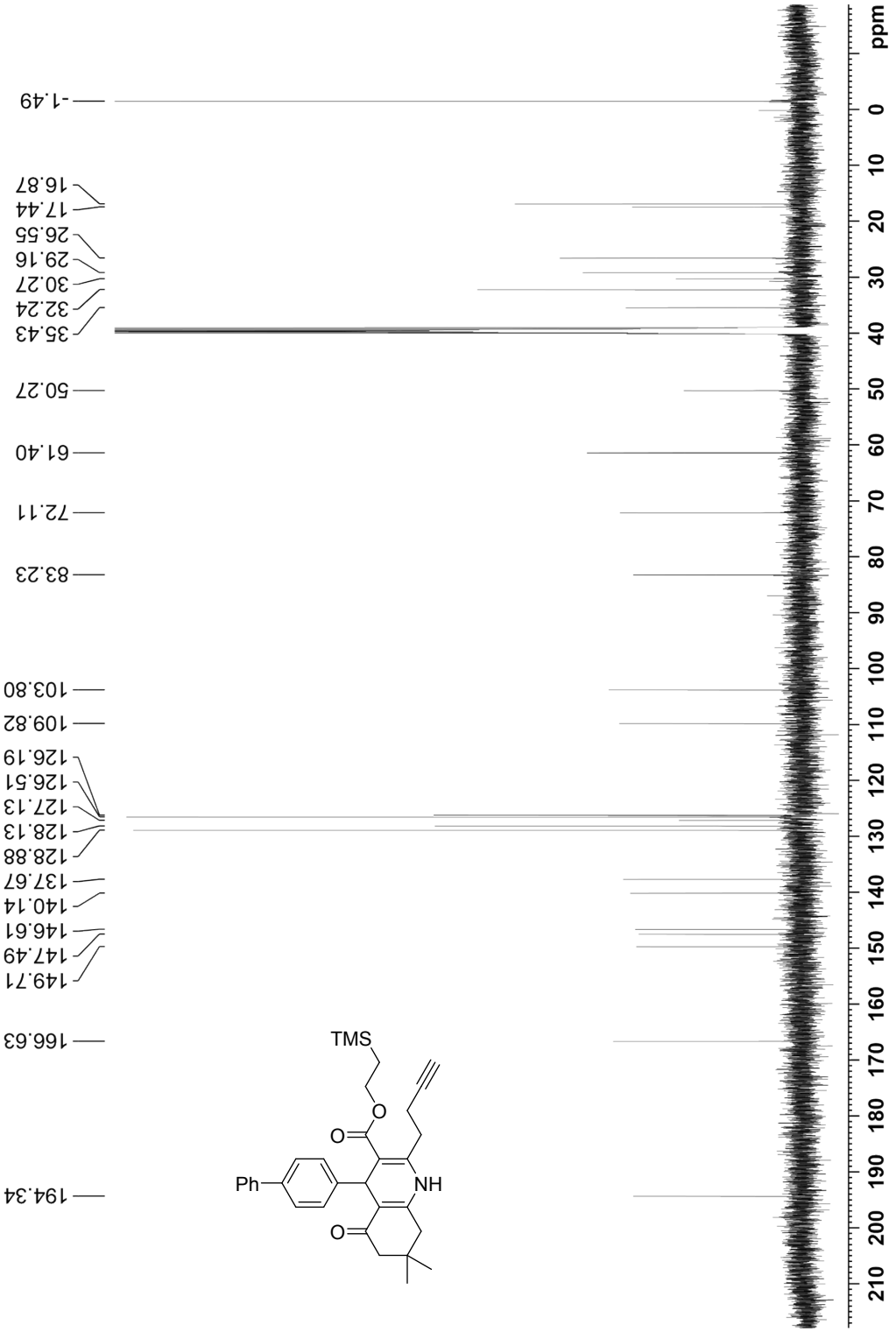
For subcellular localization HEK293T cells were seeded out overnight in DMEM supplemented with 2 % FBS on 384-well plates. After 24 h probes, DMSO and TGFβ-2 (10 ng/mL) were added and incubated for 1 h. Photocrosslinking was performed in a UV-chamber (BioLink DNA-Photocrosslinker, Analytik Jena) for 5 min at 365 nm. Cells were fixed with 4 % PFA for 10 min, followed by washing in DPBS and permeabilisation (0.5 % saponin-solution). Click-labeling with fluorophore-azides was done via copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) for 10 min at r.t. using Click-IT® reagent (Thermo Scientific) according to manufacturer instructions. After reaction, cells were washed with DPBS (5x) and stained with DAPI for visualization of nuclei. For additional co-localization studies, labelled cells were treated with staining buffer (0.2 % Triton-X in DPBS, 5 % FBS) for 20 min. Immunostaining with anti-giantin (Golgi) or anti-calnexin (endoplasmic reticulum) was performed at r.t. for 1 h in the dark, following washing (DPBS, 3x) and addition of Alexa-Fluor 647-labeled secondary antibody. After incubation for 1 h in the dark, cells were washed (3x) and images were taken with a Leica TCS SP5 confocal microscope using a 63 X magnification water objective (HCX PL APO, 1.2 NA). Fluorescence intensity and exposure were adjusted and optimized for each fluorophore. Images were processed with LAS X software and ImageJ.

3.6 $^{1}$H- and $^{13}$C-NMR spectra (Compounds **S3-5, 7, 8, 10, 12, 14, 16**)

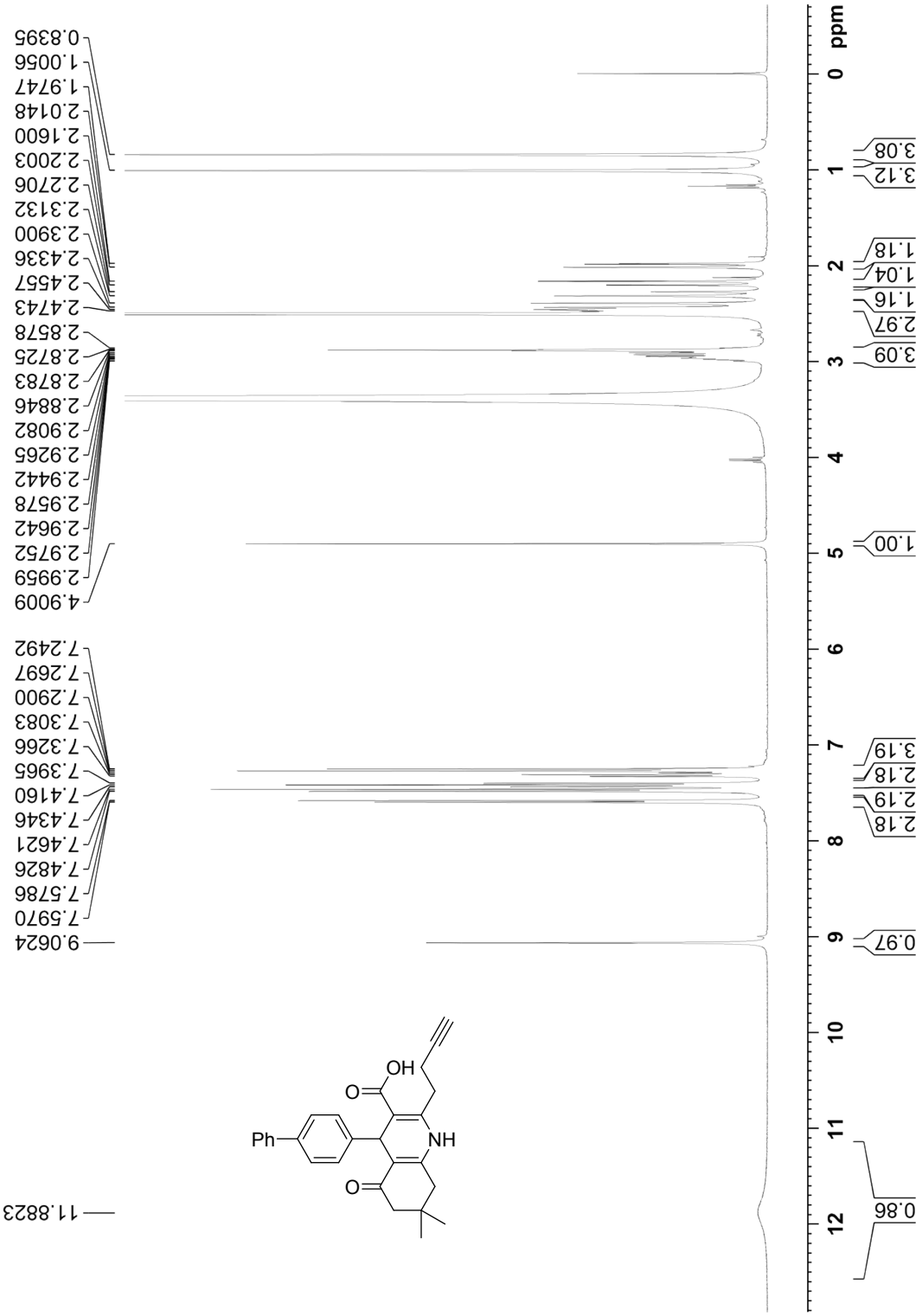
$^{1}$H-NMR-spectrum of Compound **(7)** (500 MHz, DMSO-$d_6$).



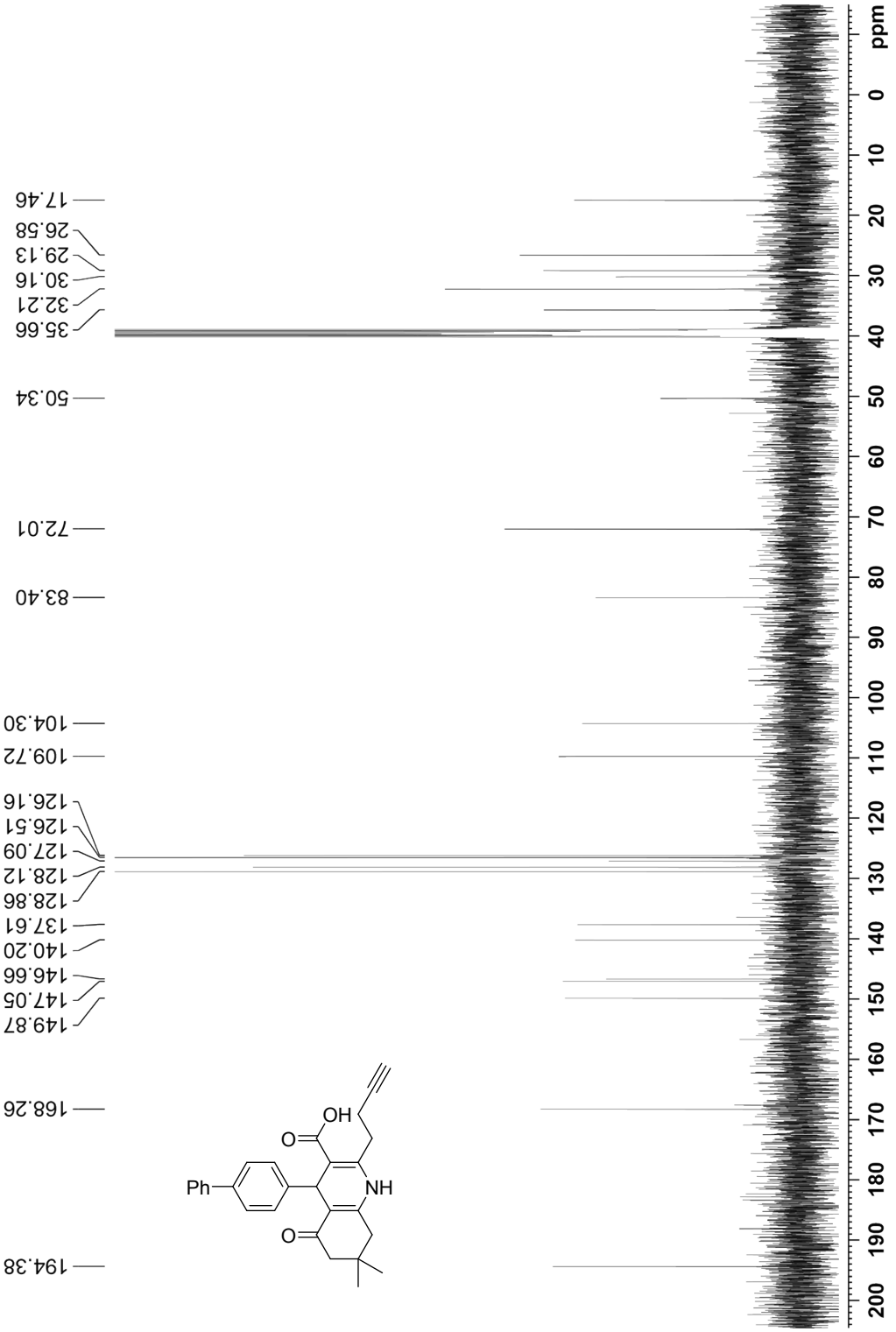
$^{13}$C-NMR-spectrum of Compound **(7)** (126 MHz, DMSO-$d_6$).



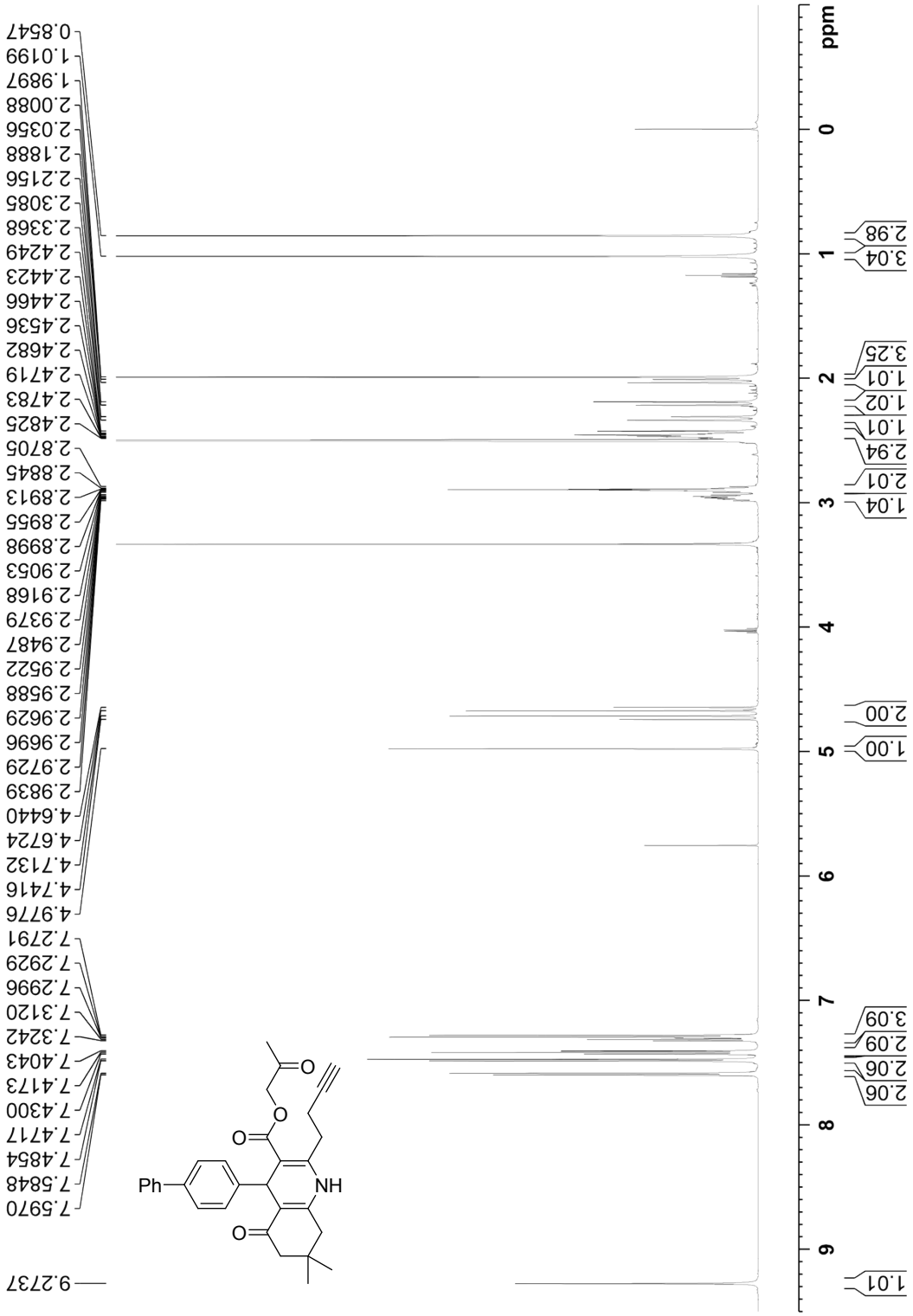
[1]H-NMR-spectrum of Compound **(S3)** (400 MHz, DMSO-$d_6$).



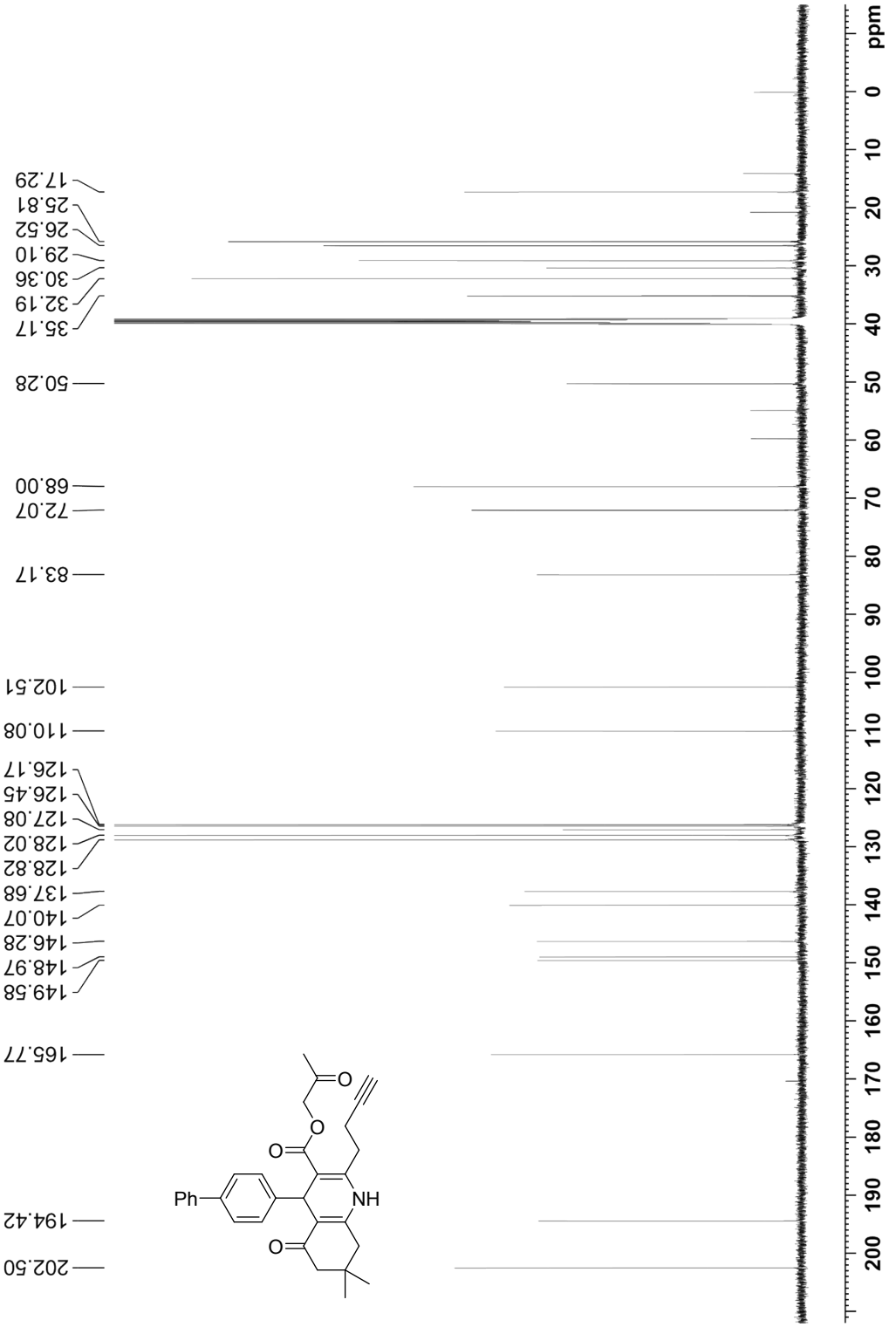
$^{13}$C-NMR-spectrum of Compound **(S3)** (101 MHz, DMSO-$d_6$).



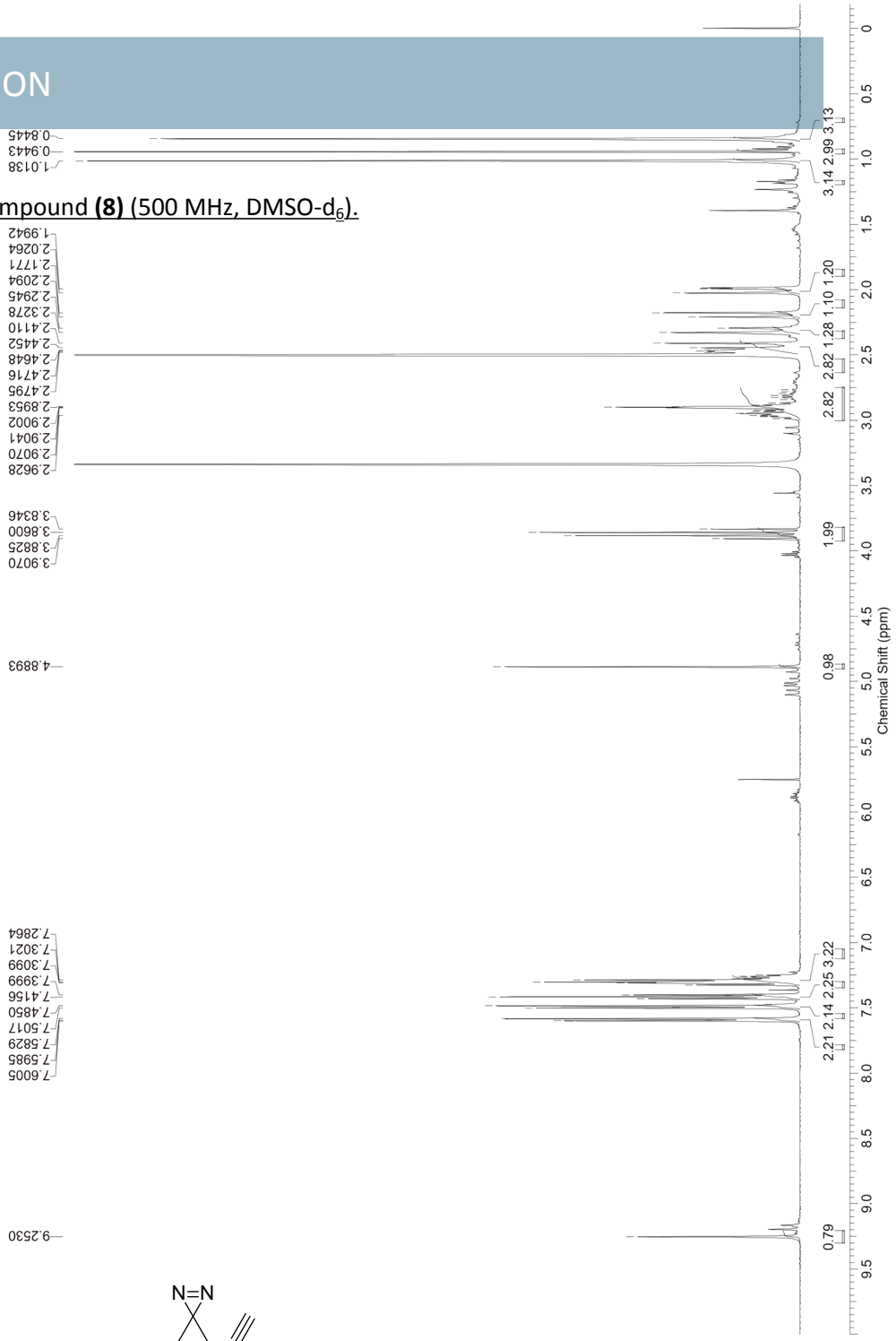
[1]H-NMR-spectrum of Compound **(S4)** (600 MHz, DMSO-$d_6$).

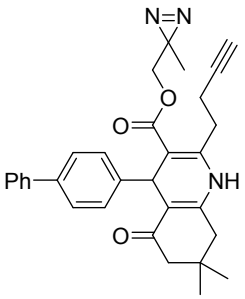


$^{13}$C-NMR-spectrum of Compound **(S4)** (151 MHz, DMSO-$d_6$).

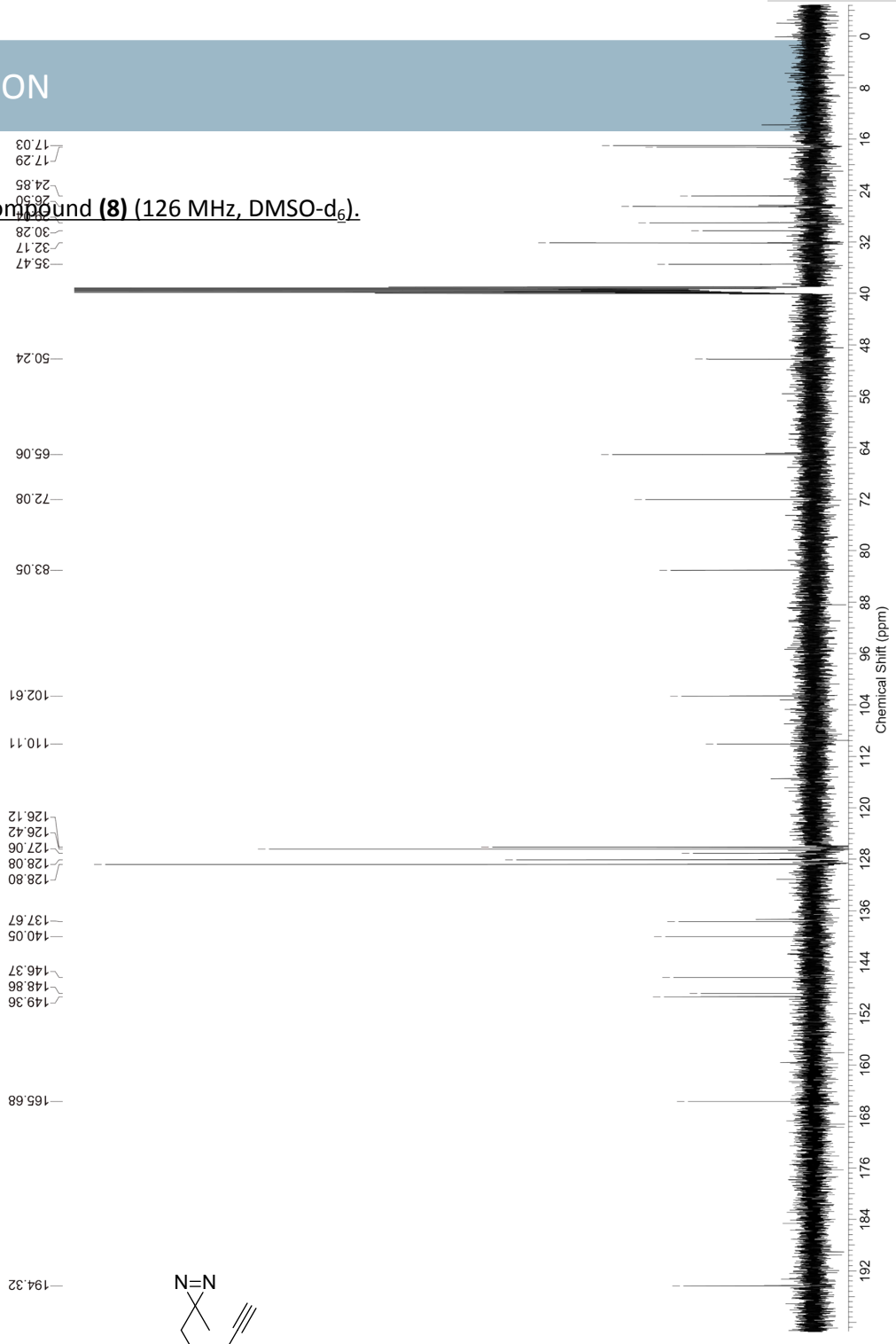


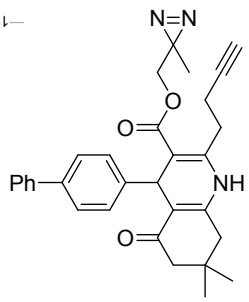
[1]H-NMR-spectrum of Compound **(8)** (500 MHz, DMSO-$d_6$).



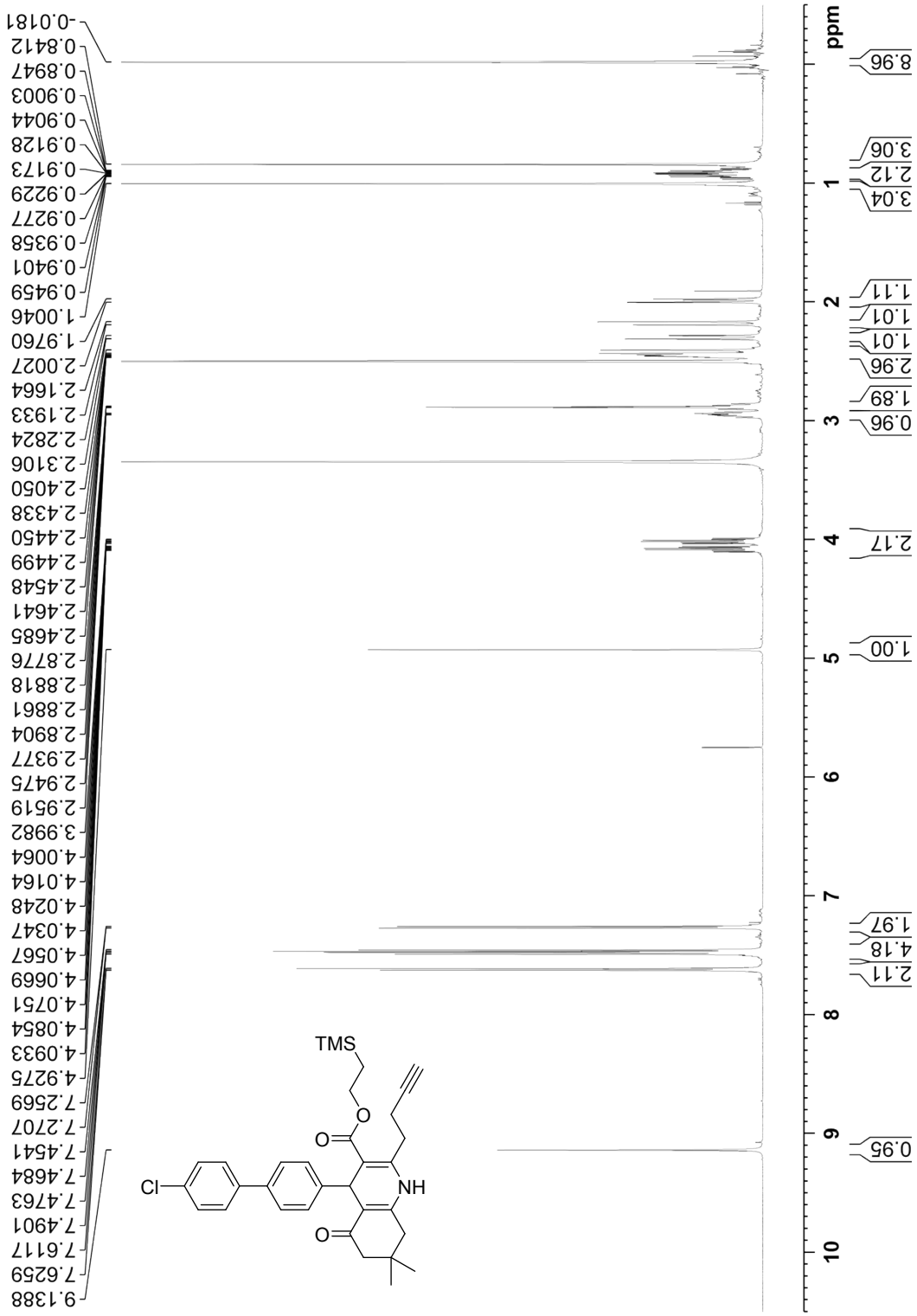



$^{13}$C-NMR-spectrum of Compound **(8)** (126 MHz, DMSO-$d_6$).

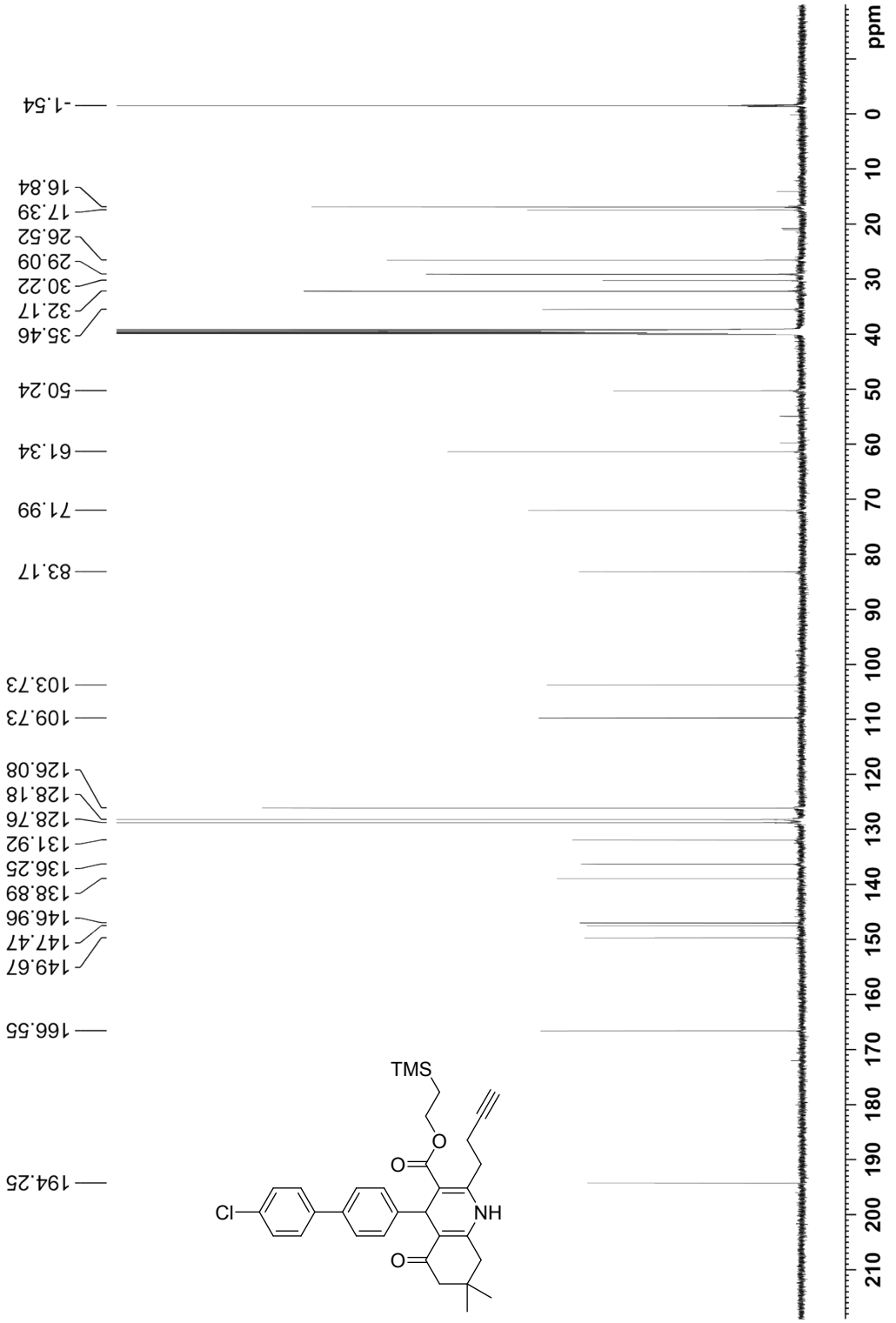


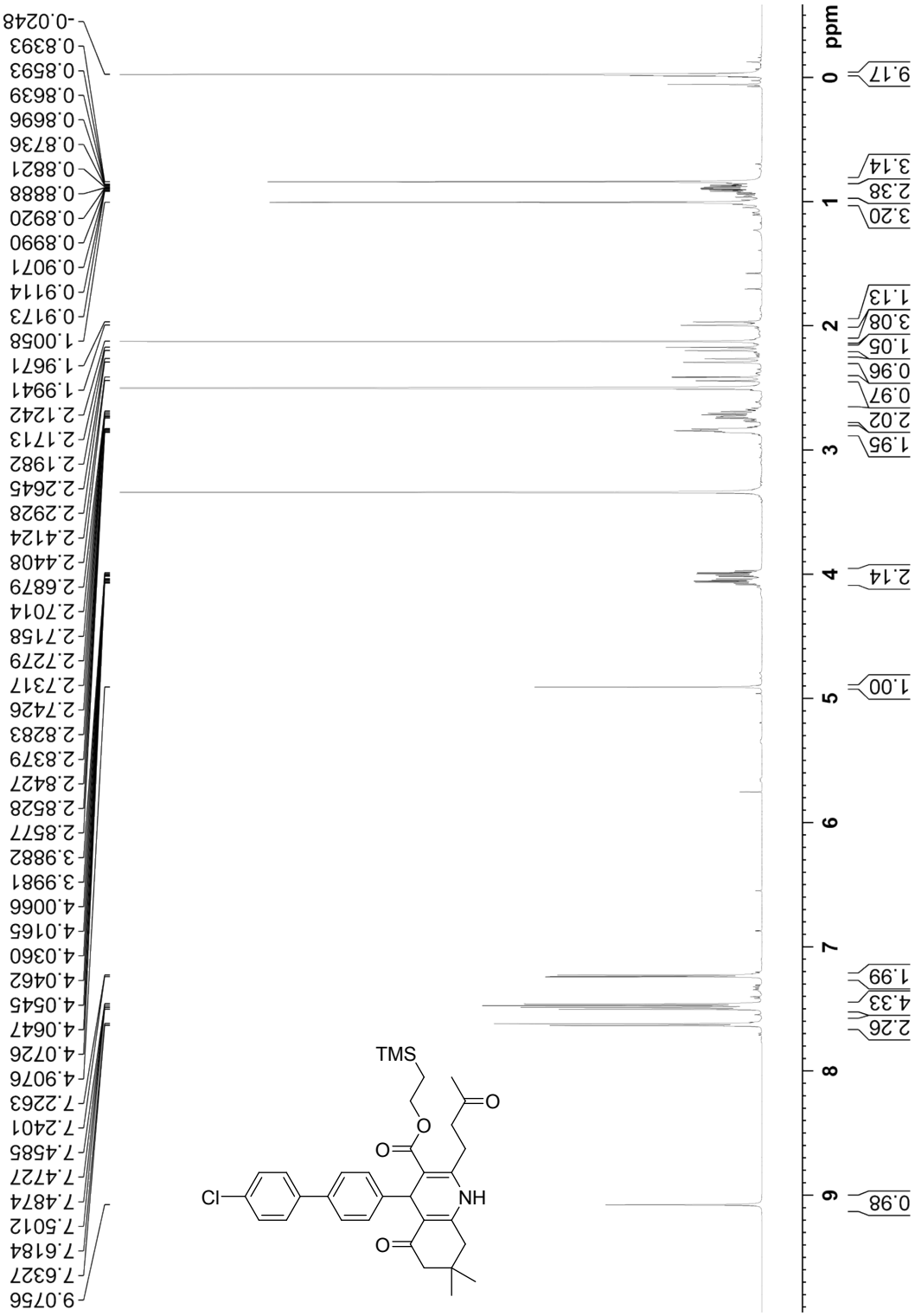
[1]H-NMR-spectrum of Compound **(S5)** (600 MHz, DMSO-$d_6$).



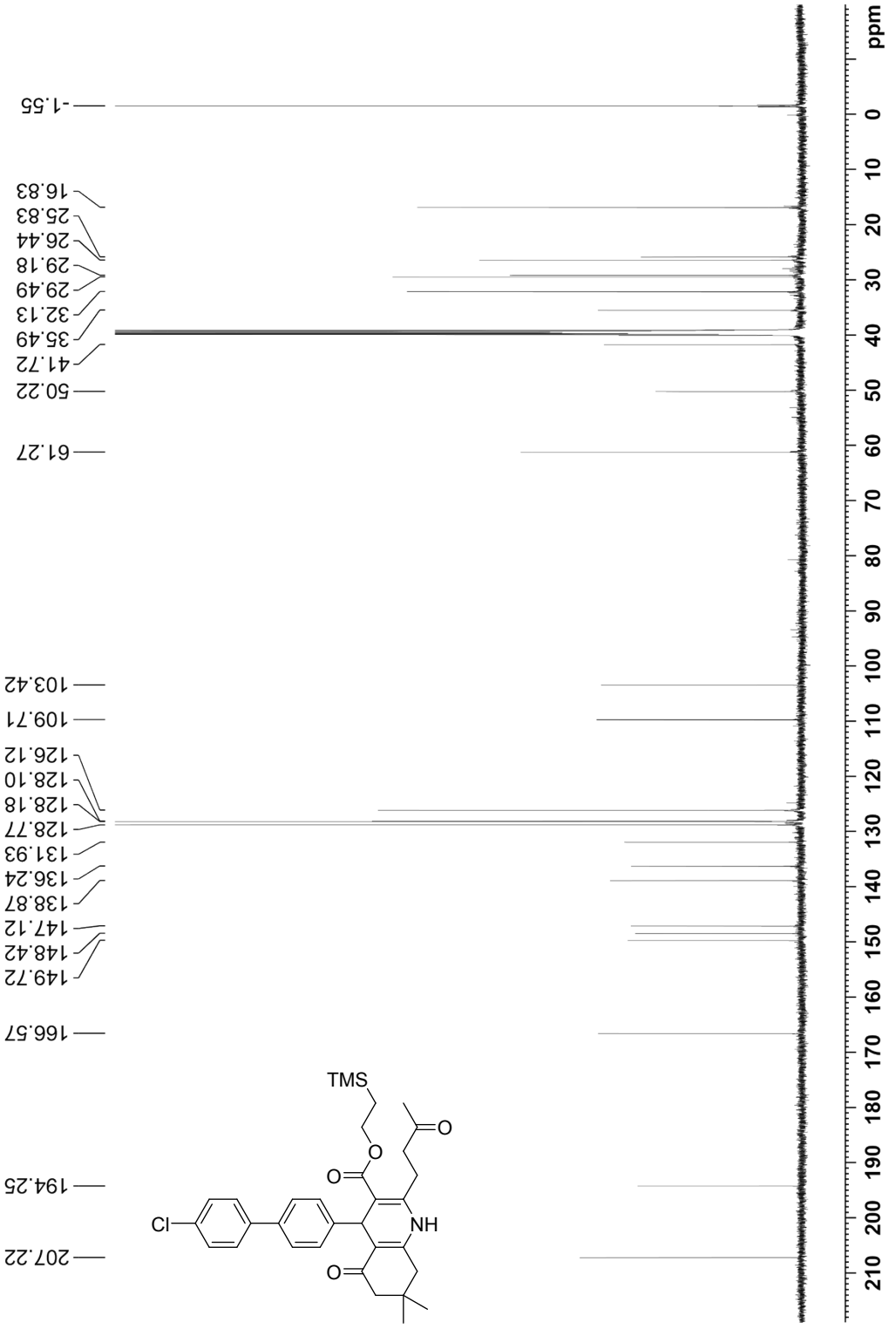
$^{13}$C-NMR-spectrum of Compound **(S5)** (151 MHz, DMSO-$d_6$).



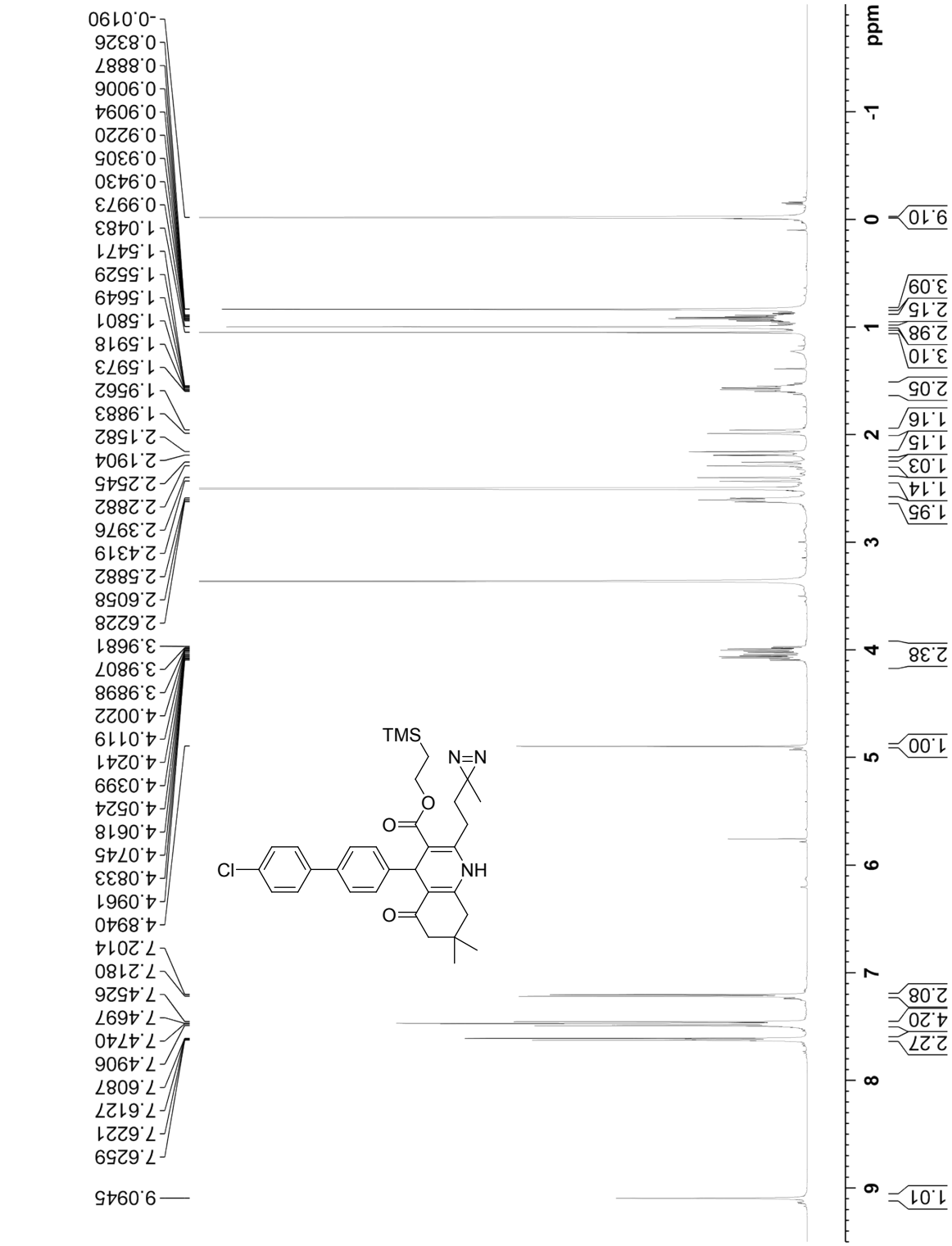
[1]H-NMR-spectrum of Compound **(10)** (600 MHz, DMSO-$d_6$).



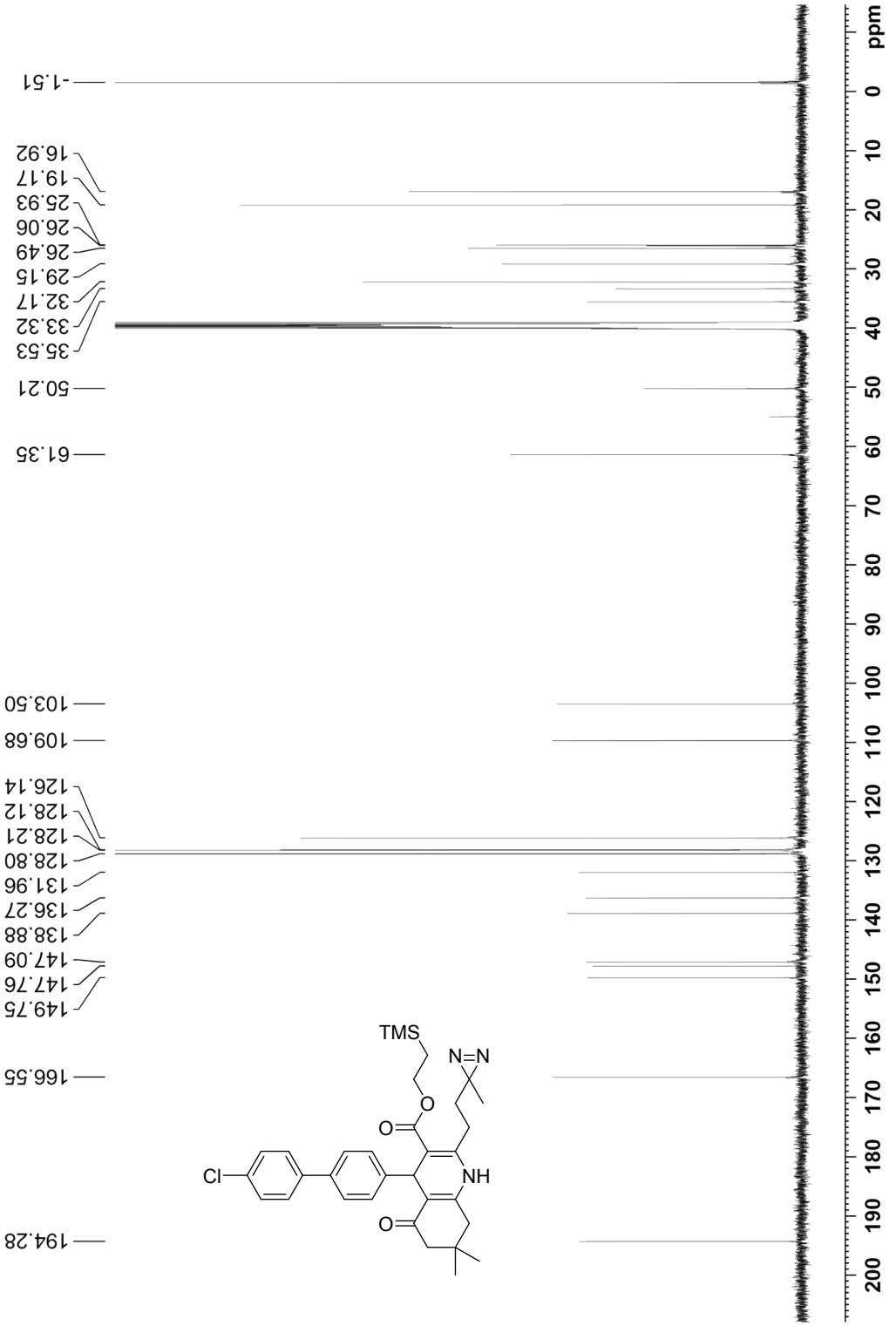
$^{13}$C-NMR-spectrum of Compound **(10)** (151 MHz, DMSO-$d_6$).



[1]H-NMR-spectrum of Compound **(12)** (500 MHz, DMSO-$d_6$).



$^{13}$C-NMR-spectrum of Compound **(12)** (126 MHz, DMSO-$d_6$).



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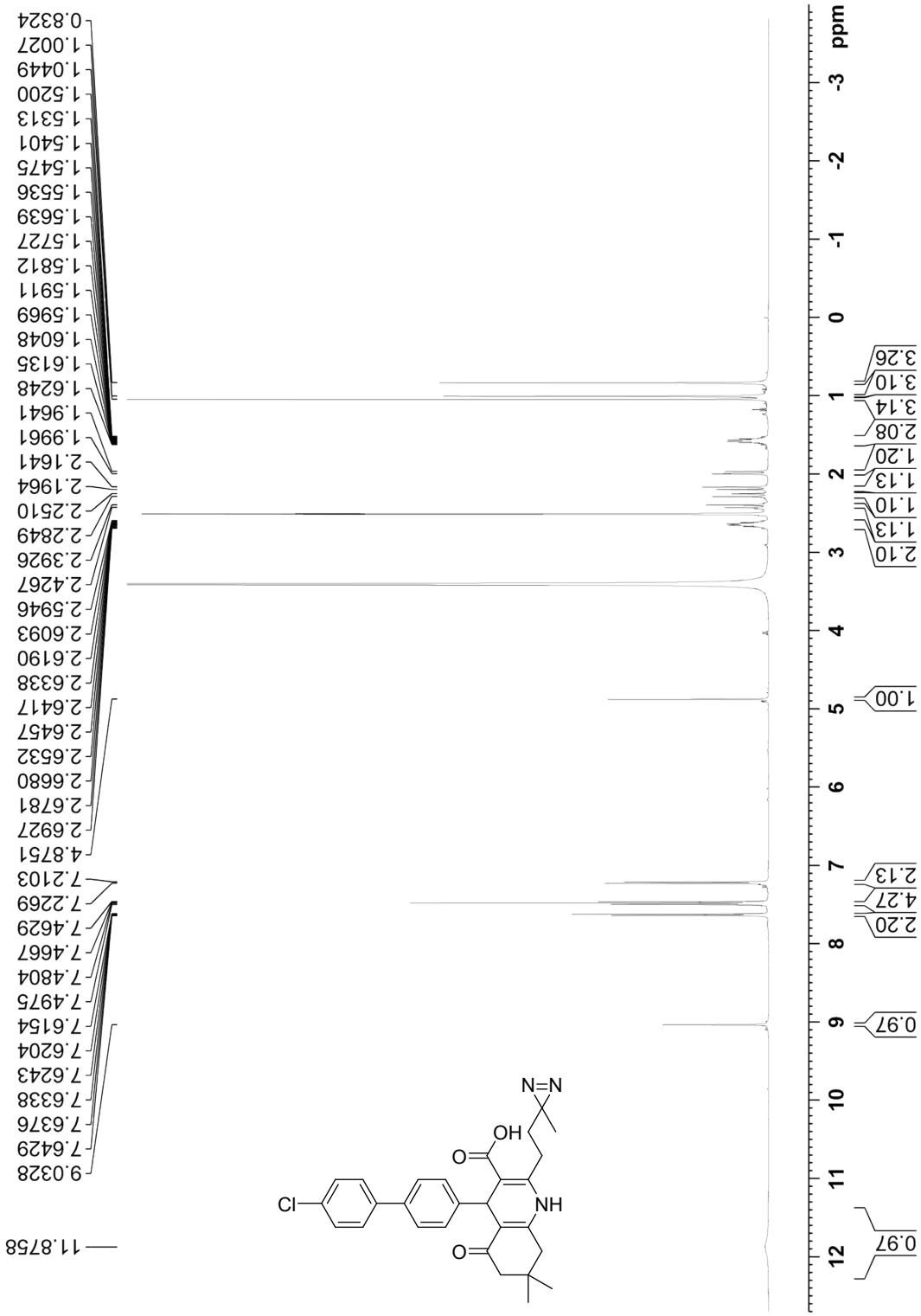
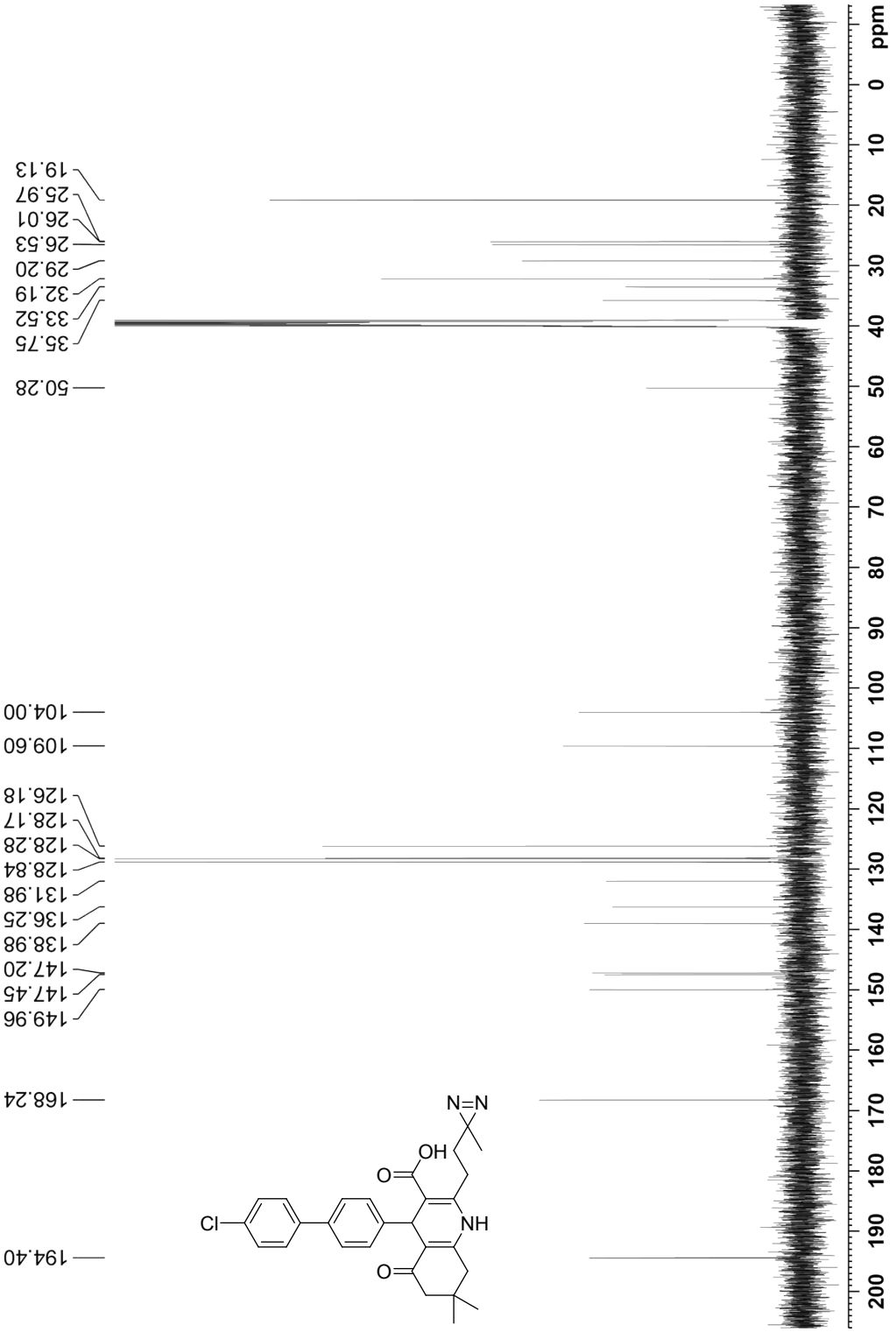
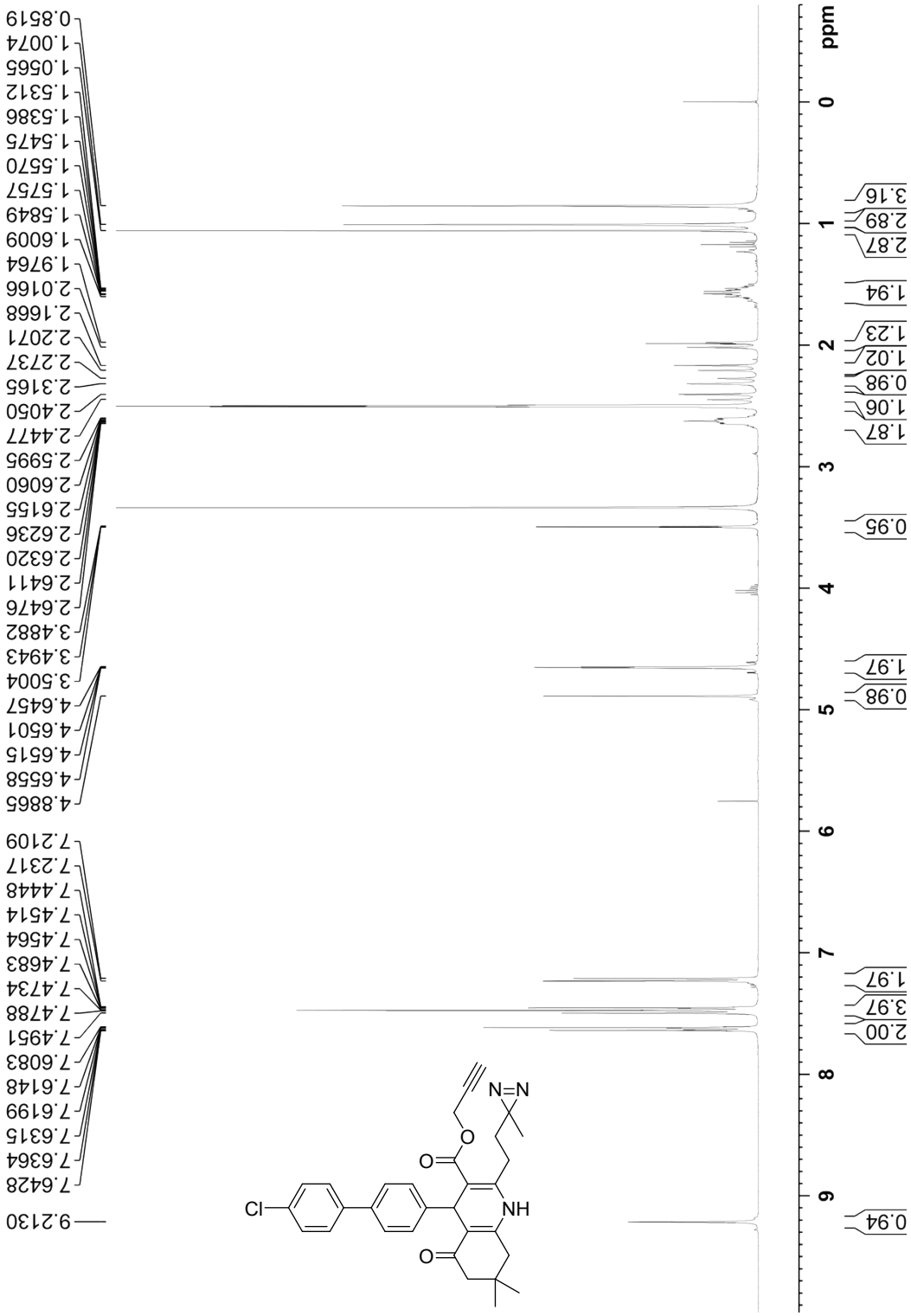
[1]H-NMR-spectrum of Compound **(14)** (500 MHz, DMSO-$d_6$).



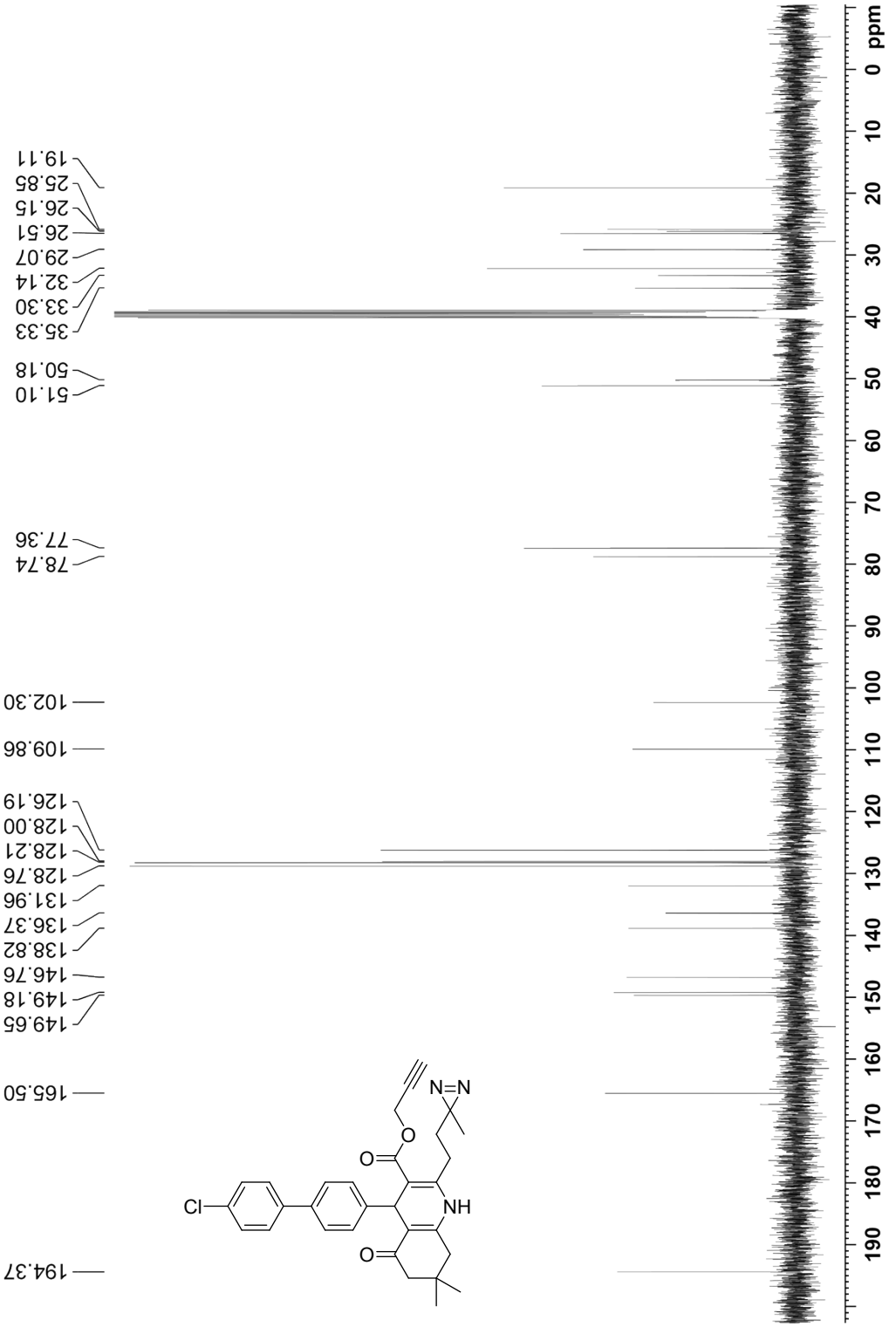
$^{13}$C-NMR-spectrum of Compound **(14)** (126 MHz, DMSO-$d_6$).



$^{1}$H-NMR-spectrum of Compound **(16)** (400 MHz, DMSO-$d_6$).



$^{13}$C-NMR-spectrum of Compound **(16)** (101 MHz, DMSO-$d_6$).



## 4. References

1. Schade, D.; Lanier, M.; Willems, E.; Okolotowicz, K.; Bushway, P.; Wahlquist, C.; Gilley, C.; Mercola, M.; Cashman, J. R., Synthesis and SAR of b-annulated 1,4-dihydropyridines define cardiomyogenic compounds as novel inhibitors of TGFβ signaling. *J. Med. Chem.* **2012,** *55* (22), 9946-9957.
2. Langle, D.; Marquardt, V.; Heider, E.; Vigante, B.; Duburs, G.; Luntena, I.; Flotgen, D.; Golz, C.; Strohmann, C.; Koch, O.; Schade, D., Design, synthesis and 3D-QSAR studies of novel 1,4-dihydropyridines as TGFβ/Smad inhibitors. *Eur. J. Med. Chem.* **2015,** *95*, 249-266.
3. Li, Z.; Hao, P.; Li, L.; Tan, C. Y.; Cheng, X.; Chen, G. Y.; Sze, S. K.; Shen, H. M.; Yao, S. Q., Design and synthesis of minimalist terminal alkyne-containing diazirine photo-crosslinkers and their incorporation into kinase inhibitors for cell- and tissue-based proteome profiling. *Angew. Chem. Int. Ed. Engl.* **2013,** *52* (33), 8551-8556.