

**Synthesis and Evaluation of the *Bis-Nor-Anachelin* Chromophore
as Potential Cyanobacterial Ligand**

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Experimental.

General. Boc-Ser(OBn)-OH was purchased from Senn Chemicals, Switzerland. Solvents for work-up and chromatography were distilled from technical quality. Solvents used for chemical transformations were either puriss. quality or dried over columns of dry aluminum oxide. The preparation of nitro-DOPA derivative **4** following a modified procedure of Kolosa and Miller¹ is reported in the Supporting Information.

[1] Kolasa, T.; Miller, M. J. *J. Org. Chem.* **1990**, *55*, 4246-4255.

(2S)-2-allyloxycarbonylamino-3-(3,4-dimethoxy-phenyl)-propionic acid ethyl ester

To a suspension of *L*-DOPA (11 g, 0.056 mol) in MeOH (200 mL) cooled at -40°C was added thionyl chloride (9.32 g, 0.078 mol) dropwise over 1.5 hours. After 1 day at RT the reaction was complete and MeOH was removed under reduced pressure. To the residue suspended in H_2O (100 mL), were added subsequently NaHCO_3 (9.41 g, 0.112 mol) and allylchloroformiate (8.08 g, 0.067 mol) in THF (100 mL). The reaction mixture was then stirred over night at room temperature. The solvent was evaporated and the aqueous solution was extracted 3 x with AcOEt. The combined organic layers were dried over Na_2SO_4 , filtered and evaporated under reduced pressure. To the residue dissolved in absolute ethanol (350 mL) was added anhydrous K_2CO_3 (16.96 g, 0.123 mol), MeI (16.75 g, 0.118 mol) and NaI (0.839 g, 0.0056 mol). The mixture was heated to reflux for 3 hours. It was then poured into water (1 L). The solution was extracted with EtOAc and the combined organic phases were dried over Na_2SO_4 , filtered and evaporated under reduced pressure. FC (AcOEt / Hexane 4:6) gave **3** (16 g, 0.0476 mol, 85%). White solid. $M_p = 63-65^{\circ}\text{C}$. $R_f = 0.54$ (AcOEt/Hexane 5:5). $[\alpha]_D = +37.0$ ($C=1.13$, CH_2Cl_2 , $T = 26^{\circ}\text{C}$). $^1\text{H-NMR}$ (CDCl_3 , 300Mhz) 1.08 (t, 3H, $J = 7.16$), 2.84-2.92 (m, 2H), 3.67 (s, 6H), 4 (q, 2H, $J = 7.16$), 4.38 (d, 2H, $J = 5.3$), 4.4 (m, 1H), 5.03 (dd, 2H, $J_1 = 2.18$, $J_2 = 11.8$), 5.47 (bd, 1H, $J = 8.4$), 5.67-5.73 (m, 1H), 6.53-6.55 (m, 2H), 6.63 (d, 1H, $J = 8.4$). $^{13}\text{C-NMR}$ (CDCl_3 , 75MHz) 14.1, 37.6, 54.9, 55.61, 55.66, 61.2, 65.5, 111, 112.2, 117.3, 121.2, 128.2, 132.5, 147.8, 148.6, 155.34, 171.5. IR 3432 m , 3021 s , 2939 m , 1720 s , 1515 s . MS 360.1 (100, $[\text{M}+\text{Na}]^+$), 338.2 (14, $[\text{M}+\text{H}]^+$), 294.2 (13, $[\text{M}-\text{EtOH}]^+$).HRMS calcd. For $\text{C}_{17}\text{H}_{23}\text{NO}_6\text{Na}$ ($\text{M}+\text{H}$) $^+$: 360.1417 found: 360.1410.

(2*S*)-2-allyloxycarbonylamino-3-(4,5-dimethoxy-2-nitro-phenyl)-propionic acid ethyl ester

A solution of **3** (4.73 g, 14 mmol) in AcOH (30 mL) was cooled to 0°C and nitric acid (10 mL) was then added dropwise. The orange mixture was stirred at RT for 2 hours and poured into water (150 mL). The mixture was filtered, the solvent removed under reduced pressure, and dried under high vacuum to give **4** (3.86 g, 0.010 mol, 72%). Yellow solid. Mp = 114-116 °C. R_f = 0.47 (CH₂Cl₂/MeOH 99:1). [α]_D = 6 (C = 0.62, CH₂Cl₂, T = 25°C). ¹H-NMR (CDCl₃, 300Mhz) 1.25 (t, 3H, *J* = 6.2), 3.32 (dd, 1H, *J*₁ = 8.7, *J*₂ = 13.7), 3.52 (dd, 1H, *J*₁ = 6, *J*₂ = 13.7), 3.9 (s, 3H), 4.12-4.26 (m, 2H), 4.49 (d, 1H, *J* = 5.3), 4.64-4.72 (m, 1H), 5.16-5.27 (m, 2H), 5.47 (d, 1H, *J* = 8.71), 5.77-5.88 (m, 1H), 6.74 (s, 1H), 7.6 (s, 1H). ¹³C-NMR (CDCl₃, 75MHz) 14.1, 35.9, 54.5, 56.3, 56.4, 61.8, 65.8, 108, 113.7, 117.8, 126.6, 152.8, 171.2. IR 3022_m, 1721_s, 1584_w, 1524_s, 1335_m, 1272_s. MS 405.1 (100, [M+Na]⁺), 389.1 (43, [M-EtOH]⁺). HRMS calcd. for C₁₇H₂₂N₂O₈Na (M+Na)⁺: 405.1268, found: 405.1268.

Figure 6: Air oxidation of compound **10** (0.15 mM) at pH = 9.2 in degassed phosphate buffer (50 mM). UV-Vis spectra were measured every 30 seconds and air was added in between each measurement.

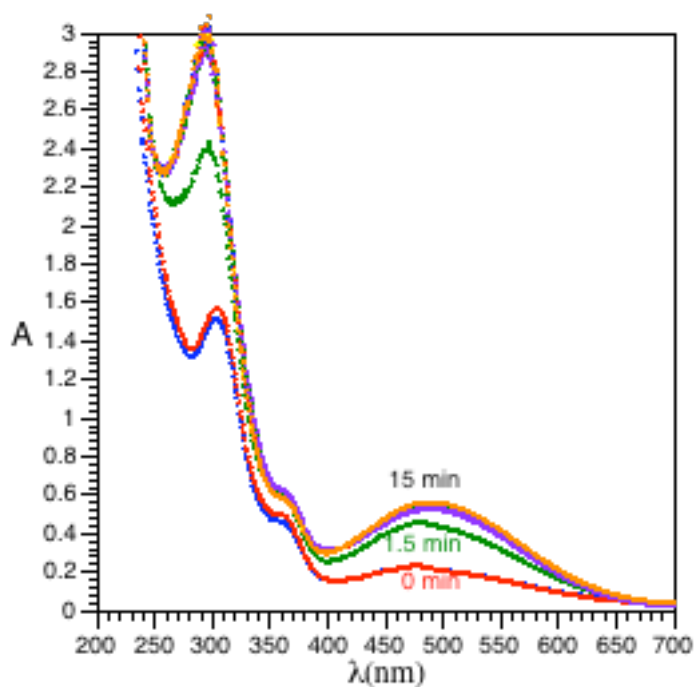


Figure 7: Oxidized form of compound **10** at pH = 4, 6.5, 9.2.

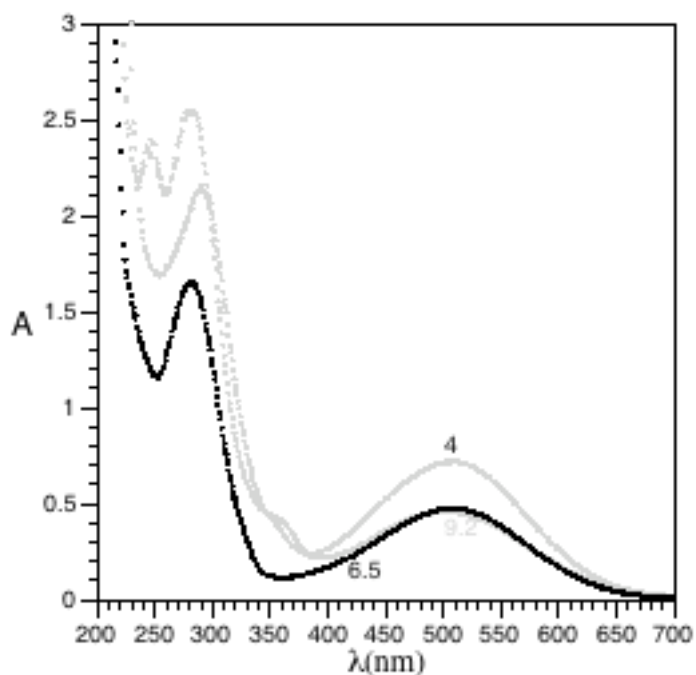


Figure 8: Kinetic of the mushroom tyrosinase oxidation of compound **10**.

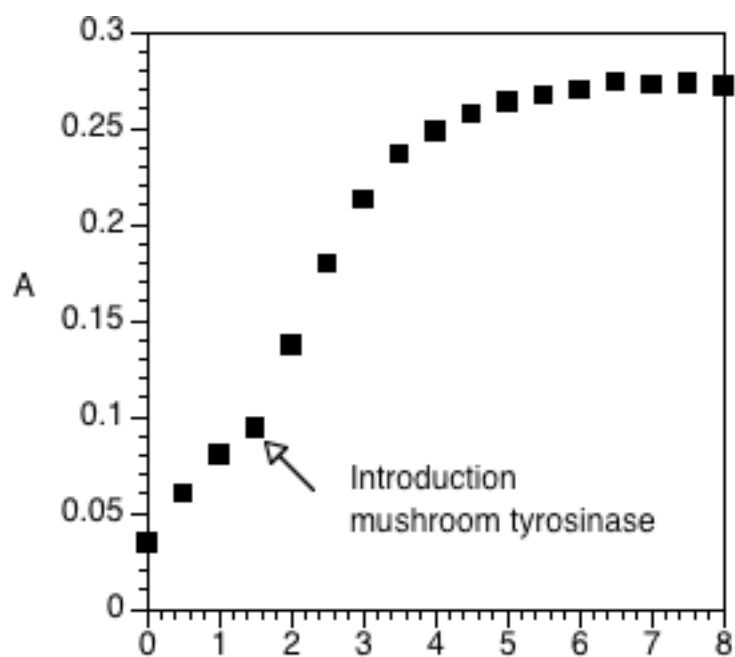
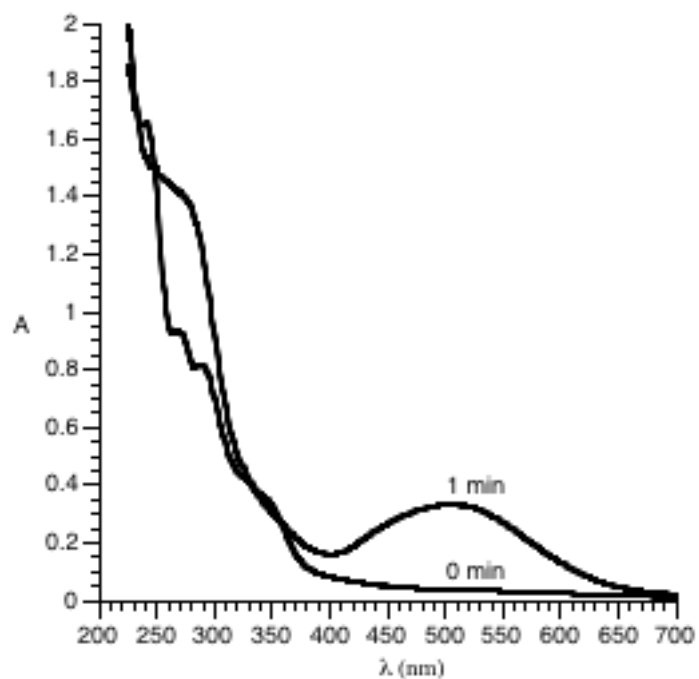


Figure 9: Oxidation of the *bis-nor-anachelin* chromophore **10** with Fe(III) at pH = 4.



STANDARD 1H OBSERVE

File: PROTON

Pulse Sequence: s2pul

Solvent: CDCl3

Ambient temperature

User: ebethu

Mercury-300BB "gem6oc"

Pulse 30.0 degrees

Acq. time 3.138 sec

Width 5099.4 Hz

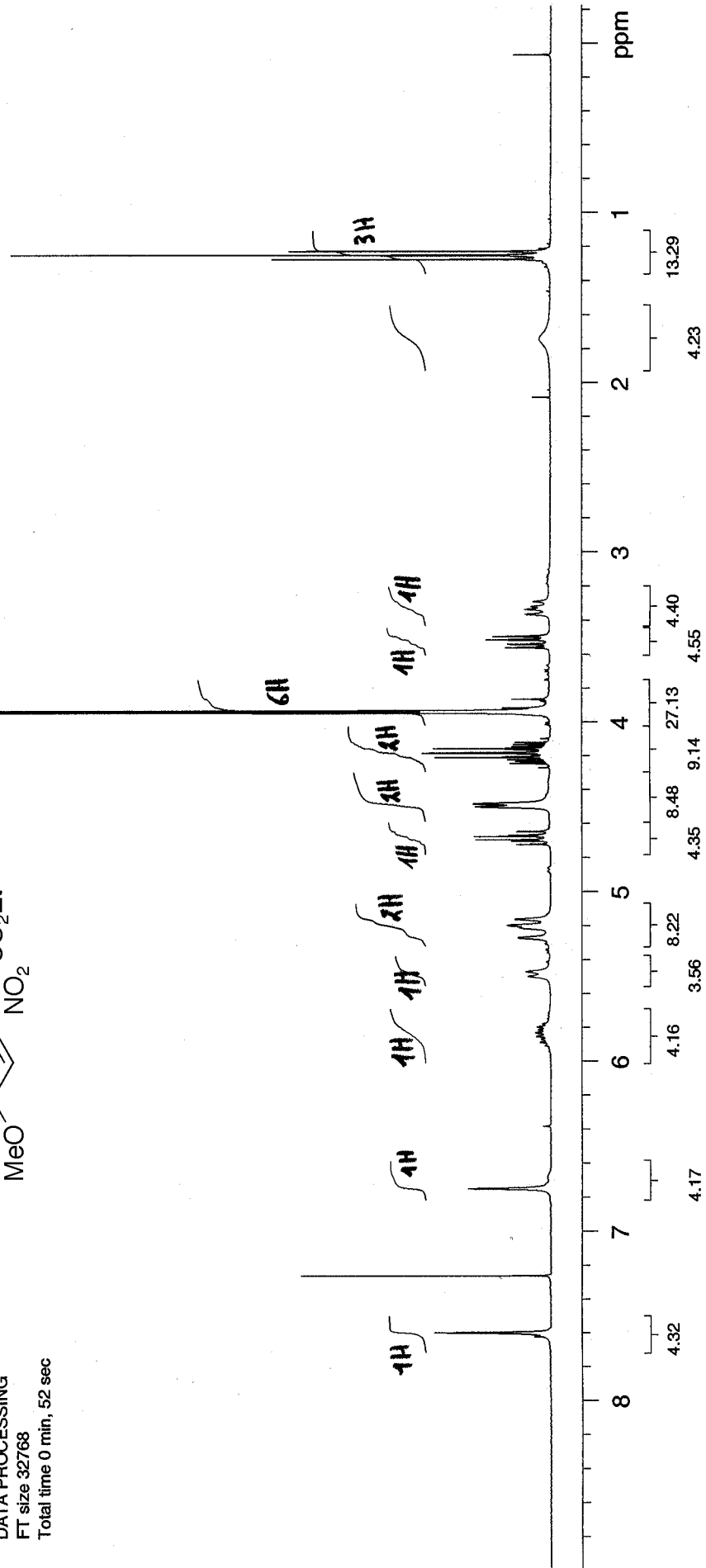
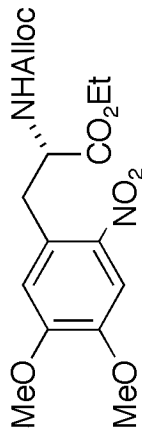
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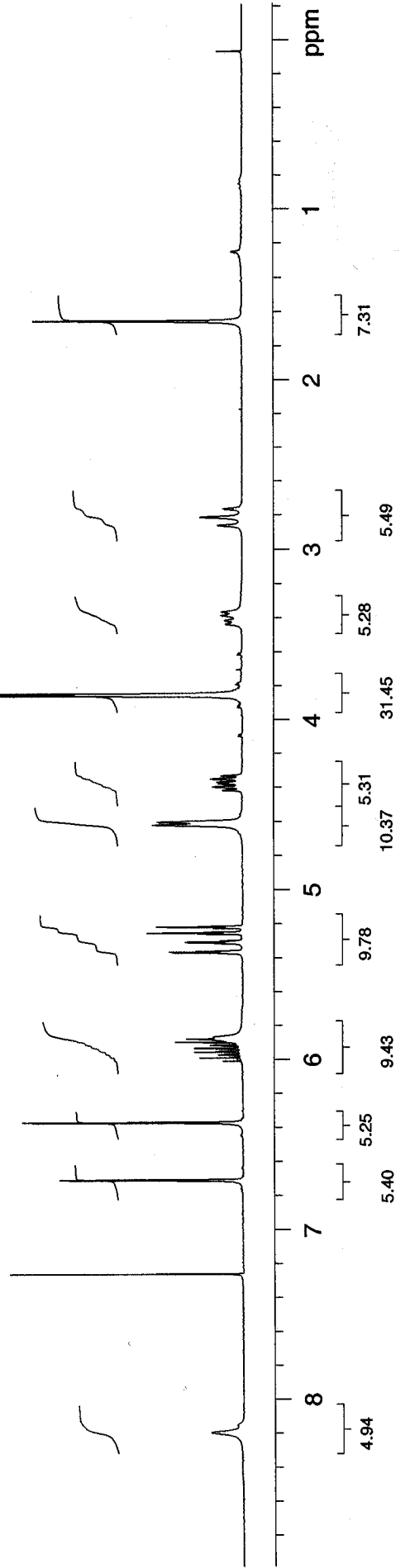
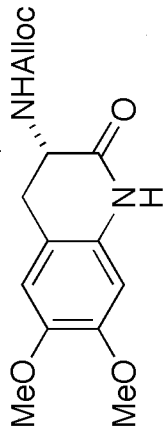
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Ambient temperature
User: ebethu
File: yb0151-chrom
UNITYplus-300 "nmroc"

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Width 5099.4 Hz
16 repetitions
OBSERVE H1, 300.2230602 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 50 sec



STANDARD 1H OBSERVE

File: PROTON

Pulse Sequence: s2pul

Solvent: CDCl3

Ambient temperature

User: ebethu

Mercury-300BB "gem6oc"

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Acq. time 3.138 sec

Width 5099.4 Hz

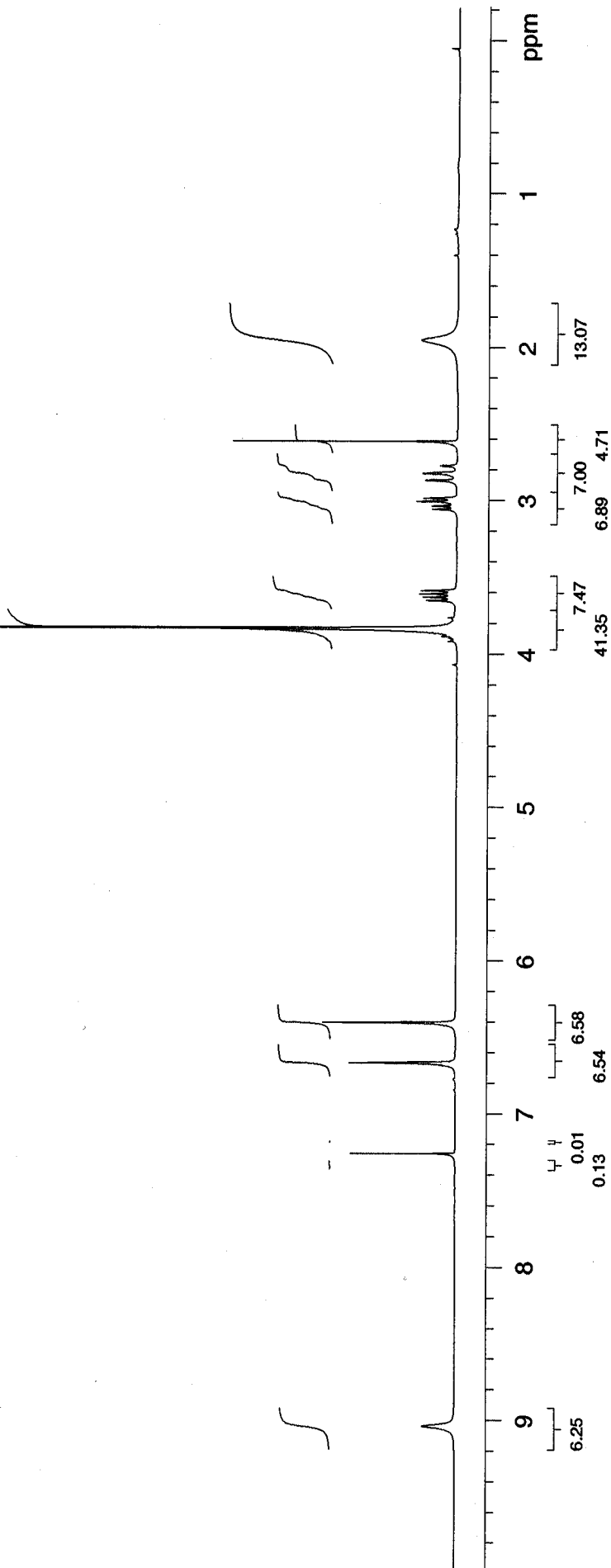
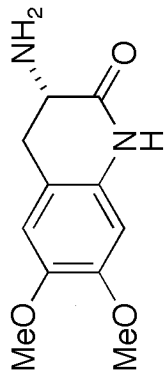
16 repetitions

OBSERVE H1, 300.2230602 MHz

DATA PROCESSING

FT size 32768

Total time 0 min, 52 sec



STANDARD 1H OBSERVE

File: PROTON

Pulse Sequence: s2pul

Solvent: CDCl3

Ambient temperature

User: ebethu

Mercury-300BB "gem6oc"

Pulse 30.0 degrees

Acq. time 3.138 sec

Width 5099.4 Hz

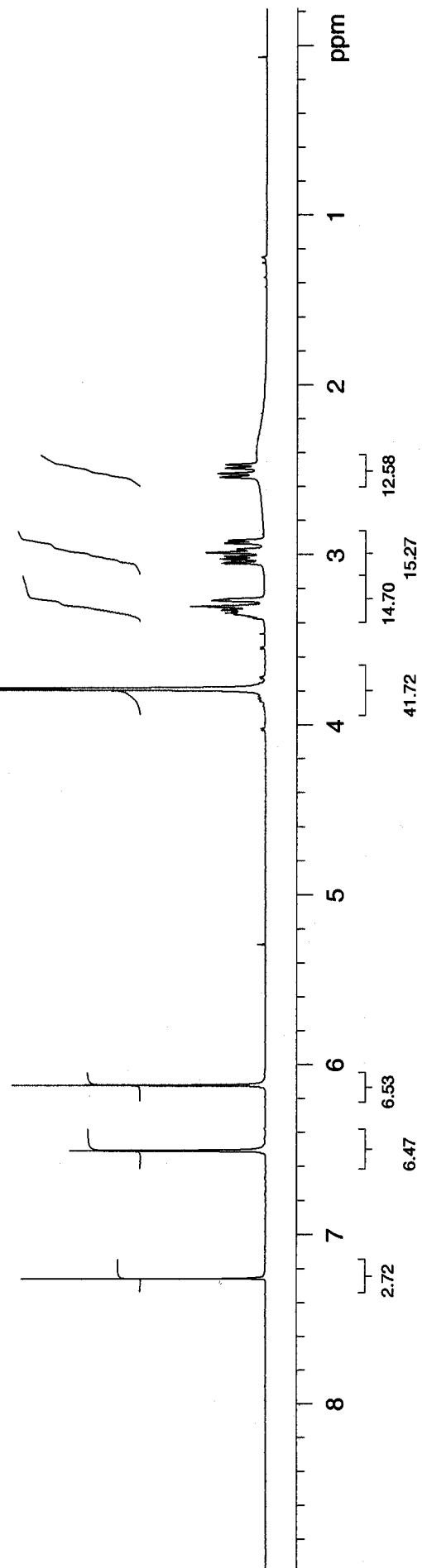
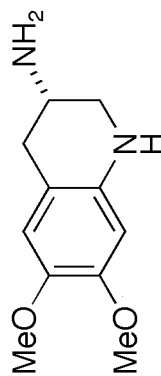
16 repetitions

OBSERVE H1, 300.2230602 MHz

DATA PROCESSING

FT size 32768

Total time 0 min, 52 sec



STANDARD 1H OBSERVE

File: PROTON

Pulse Sequence: s2pul

Solvent: CDCl3

Ambient temperature

User: ebethu

Mercury-300BB "gem6oc"

Pulse 30.0 degrees

Acq. time 3.138 sec

Width 5099.4 Hz

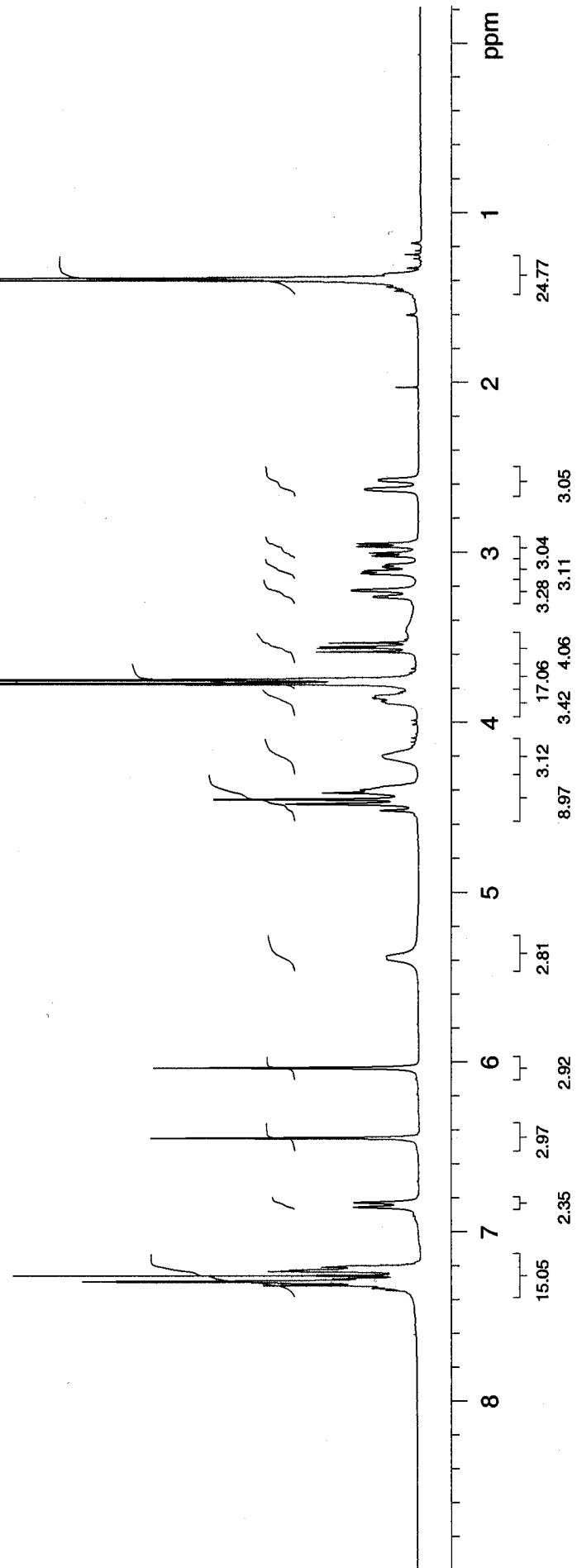
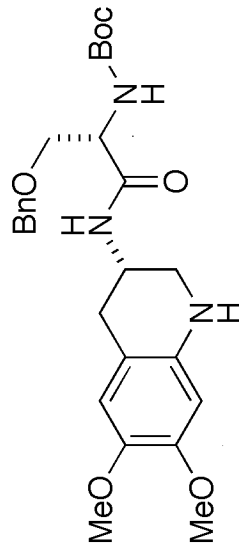
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OBSERVE H1, 300.2230602 MHz

DATA PROCESSING

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Total time 0 min, 52 sec



STANDARD 1H OBSERVE

Pulse Sequence: s2pul

Solvent: CD3OD

Ambient temperature

User: ebethu

File: yb0175-lyo

Mercury-300BB "gem6oc"

Pulse 30.0 degrees

Acq. time 3.138 sec

Width 5099.4 Hz

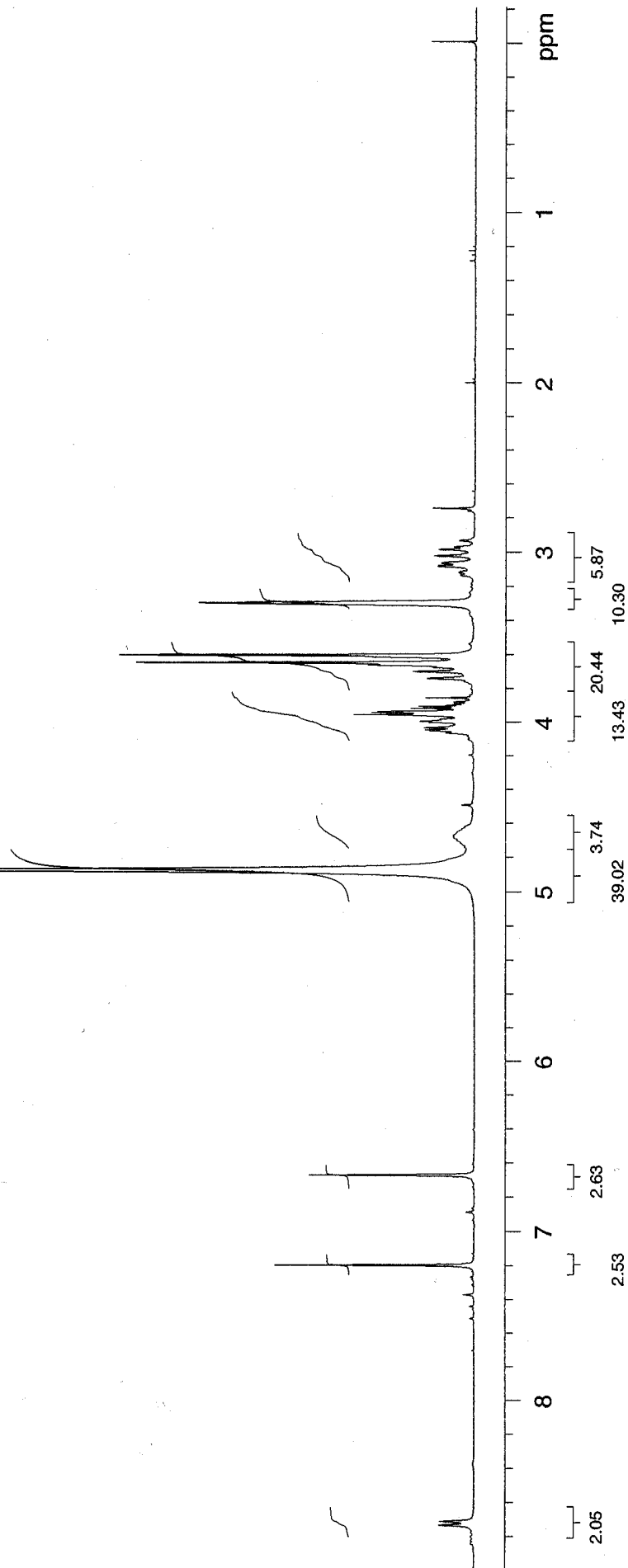
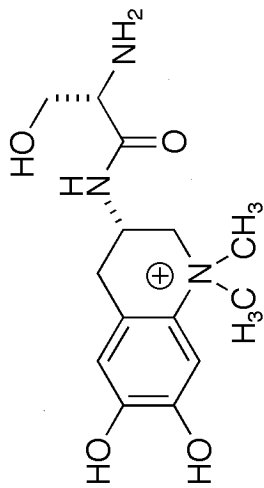
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OBSERVE H1, 300.2242441 MHz

DATA PROCESSING

FT size 32768

Total time 0 min, 52 sec



STANDARD 1H OBSERVE

File: PROTON

Pulse Sequence: s2pul

Solvent: CD3OD

Ambient temperature

User: ebethu

Mercury-300BB "gem6oc"

Pulse 30.0 degrees

Acq. time 3.138 sec

Width 5099.4 Hz

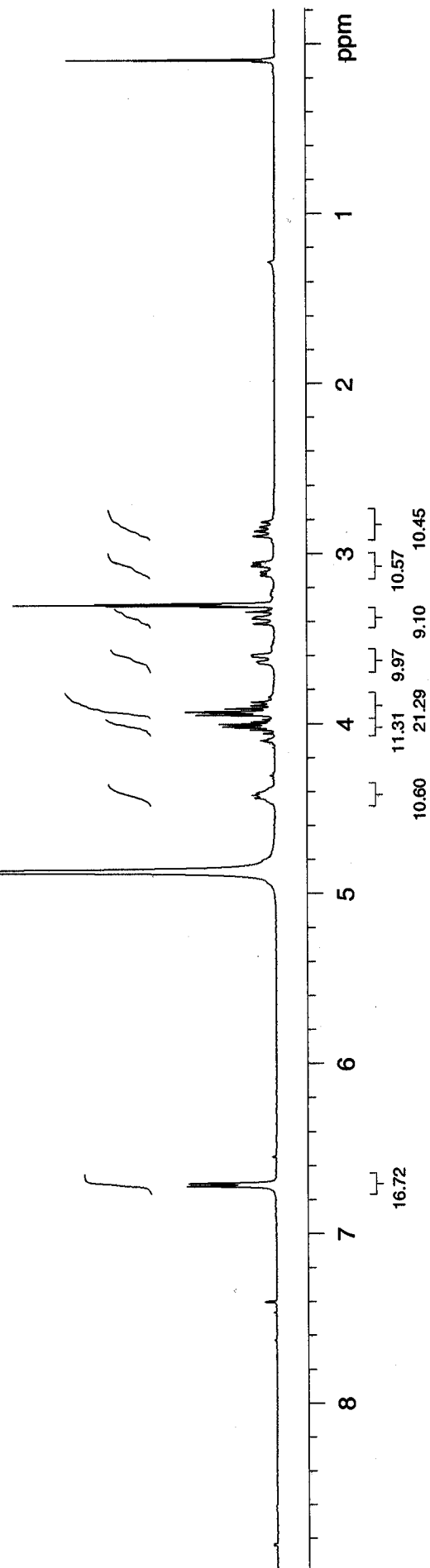
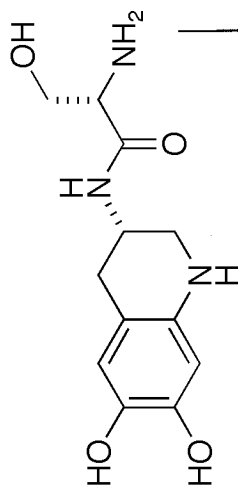
16 repetitions

OBSERVE H1, 300.2242441 MHz

DATA PROCESSING

FT size 32768

Total time 0 min, 52 sec



$M = C_{12}H_{15}N_3O_4$

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 $MNA^+ = 288.0855$ } *ben.*

M_2Na^+
553.2

