# Supporting Information

# Succinylated octopamine ascarosides and a new pathway of biogenic amine metabolism in *C. elegans*[*]

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## 1. Supporting Figures

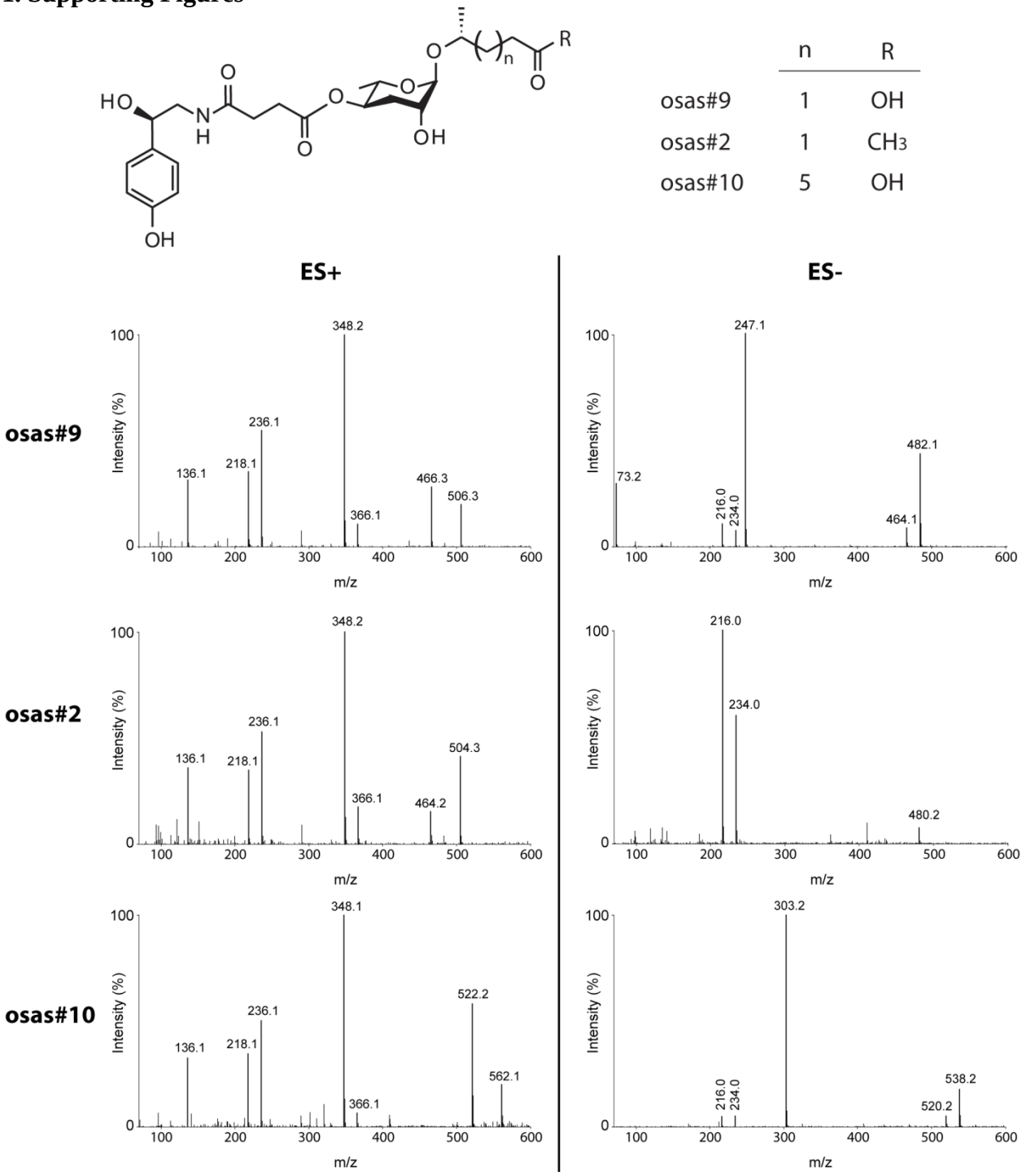



**Figure S1**. Electron spray ionization MS spectra of **osas#9**, **osas#2**, and **osas#10** in both positive and negative ion modes. Fragmentation in ES+ yields the same ions for all three osas ascarosides (*m/z* 136, 218, 236, 348, 366) resulting from fragmentation of octopamine succinyl ascarylose moiety as well as $[M+H-H_2O]^+$ and $[M+Na]^+$. Fragmentation of **osas#9** and **osas#10** in ES- produces **ascr#9** and **ascr#10** ions, respectively, but this fragmentation pathway is absent in **osas#2**. All three osas ascarosides also produce ions at *m/z* 216 and 234 in ES- resulting from octopamine succinyl moiety, in addition to molecular ions $[M-H]^-$.

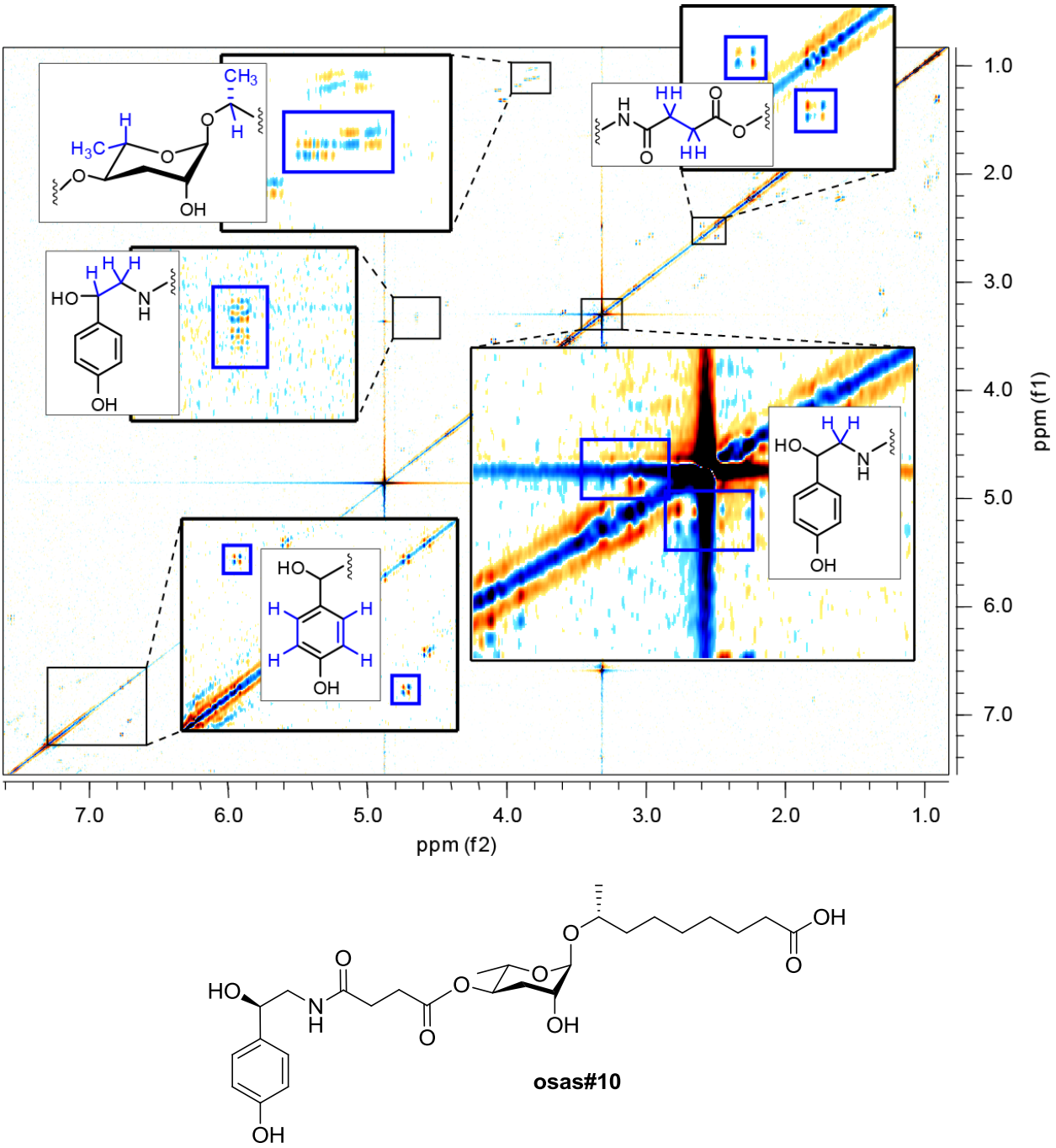



**Figure S2**. dqfCOSY of enriched natural fraction containing **osas#10** (m/z = 538). Peaks highlighted represent specific structural features associated with this ascaroside. For stereochemical assignments, see Figure S4.

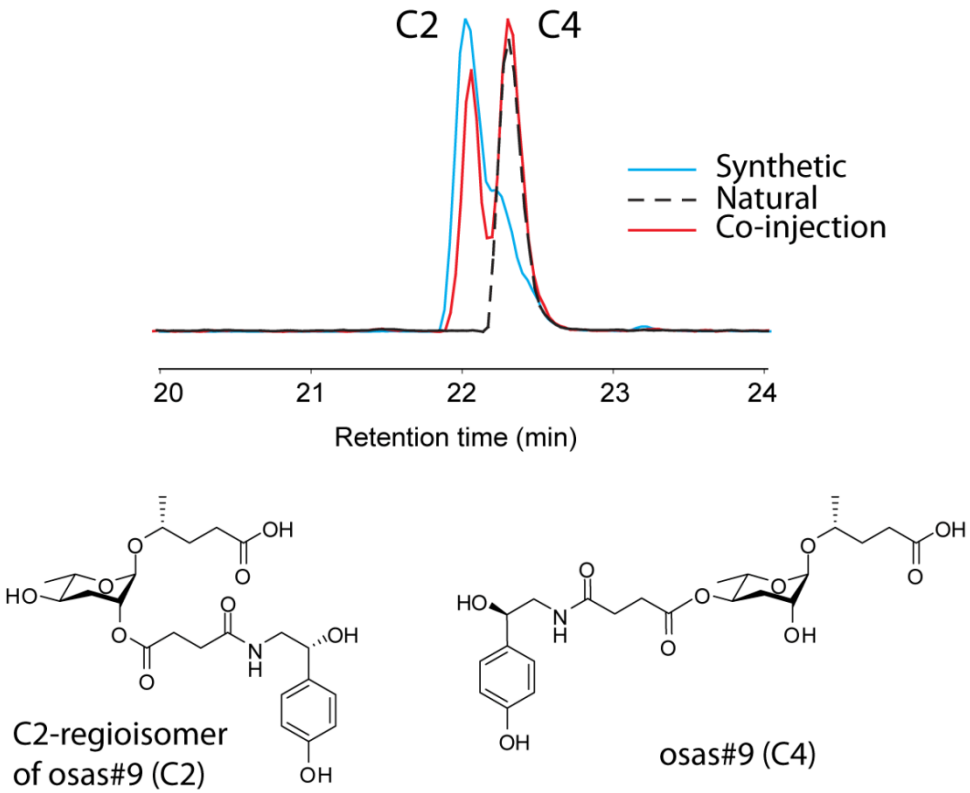



**Figure S3**. HPLC-MS co-injection of natural **osas#9** with two regioisomers confirms that the ascarylose ring of the natural compound is modified on the 4 position.

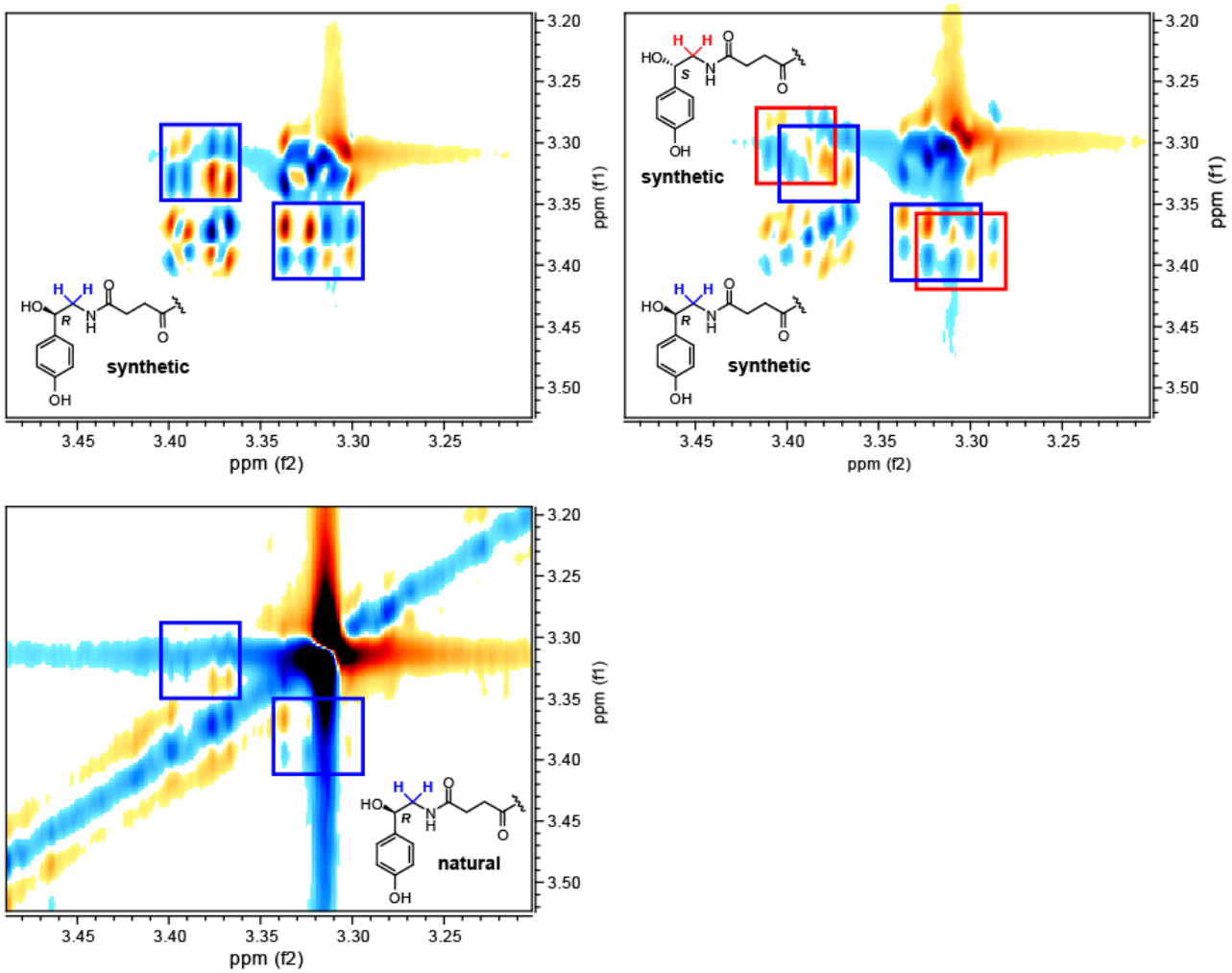



**Figure S4**. 2D NMR spectroscopic analysis reveals that the two **osas#9** diastereomers derived from *R*- and *S*-octopamine have different chemical shift values for the indicated methylene protons. Comparison with the spectrum of the natural sample confirms *R* stereochemistry.

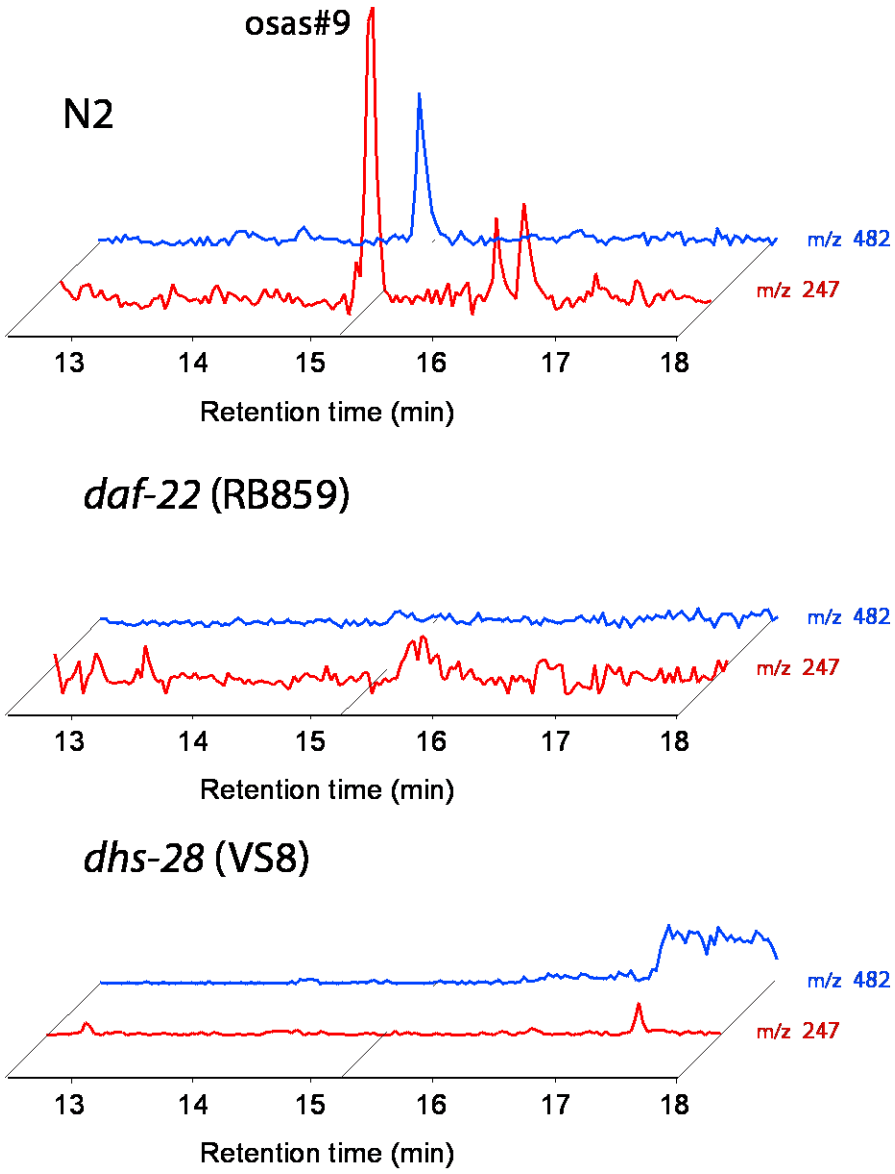



**Figure S5**. Mutants defective in peroxisomal β-oxidation do not produce **osas#9**.

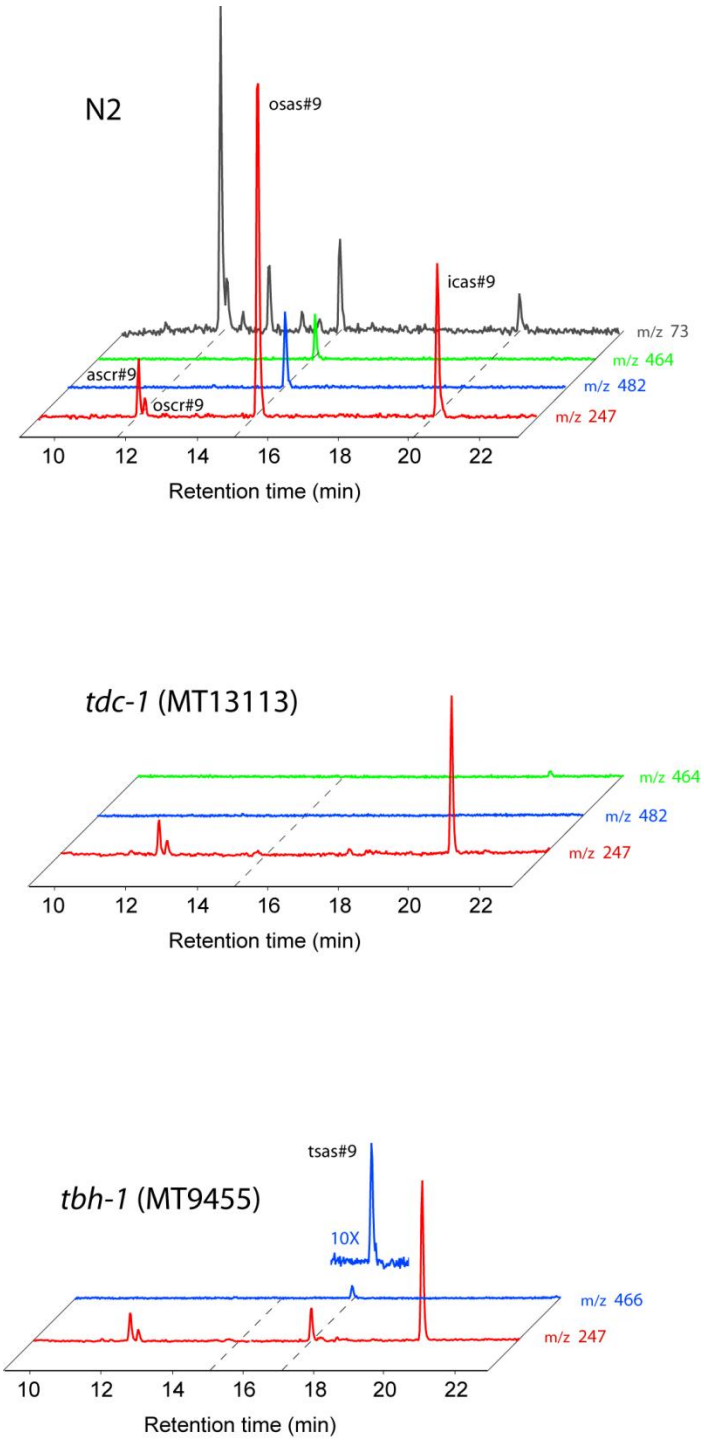



**Figure S6**. Starved L1 larvae from octopamine biosynthesis mutants still produce **icas#9** and **ascr#9** but not **osas#9**. Notably, *tbh-1* mutant strain incorporates tyramine to produce **tsas#9** (see materials and method for full characterization).

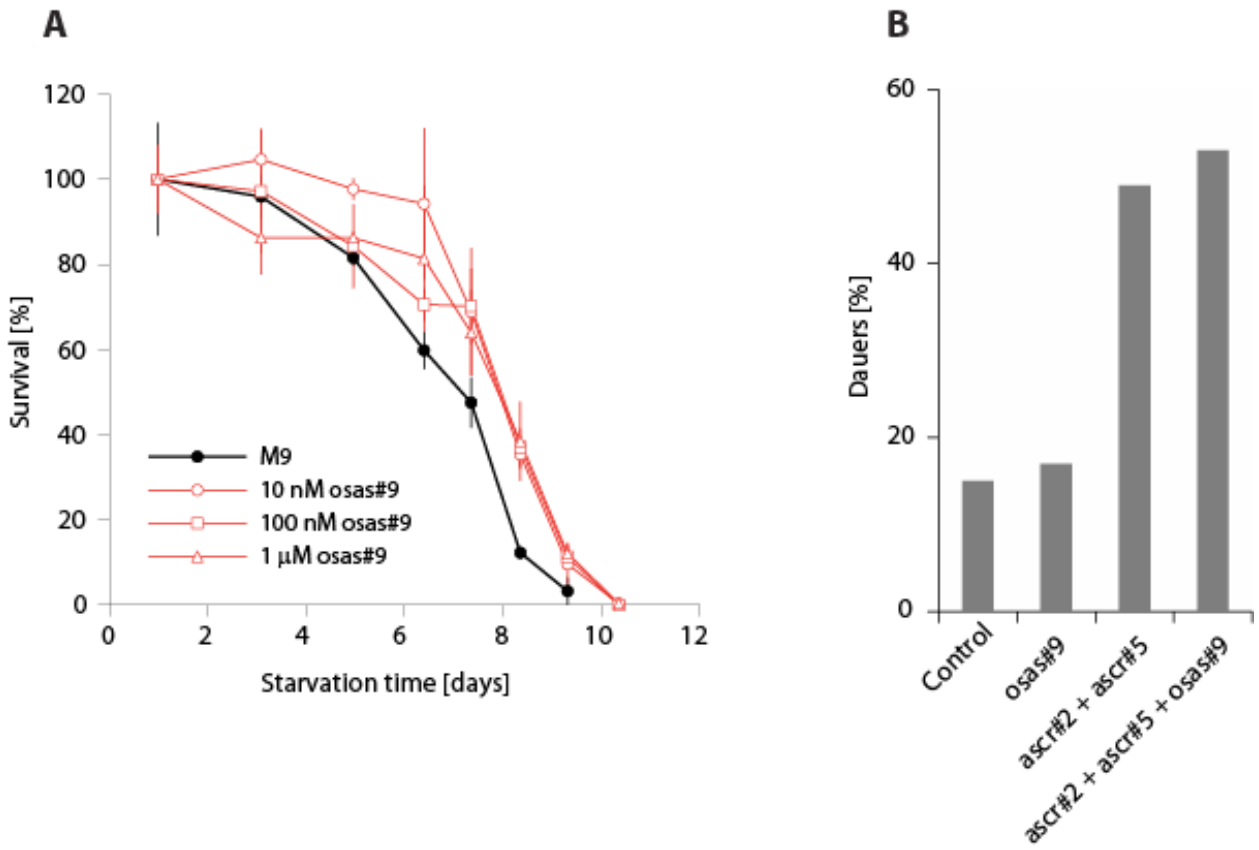



**Figure S7**. **osas#9** has no significant effect on L1 starvation survival (A) and dauer formation (B). Dauer formation was assessed either for **osas#9** alone or in addition to a mixture of **ascr#2** and **ascr#5**, which are known to induce dauer formation.

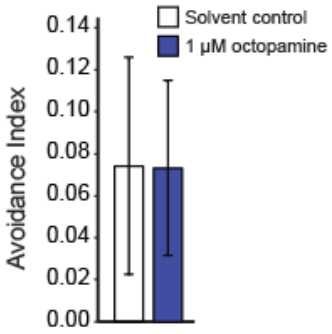



**Figure S8**. Octopamine does not elicit avoidance behavior in adult *C. elegans.*

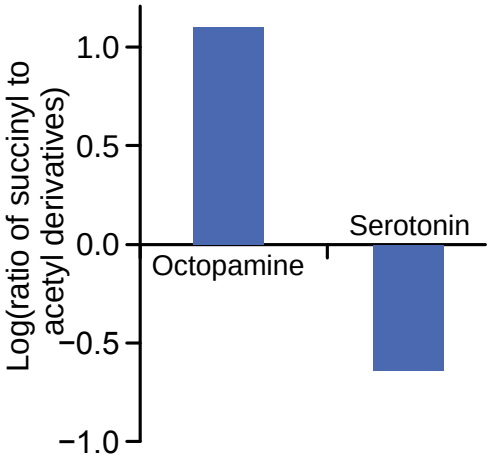



**Figure S9.** Ratio of N-succinyl to N-acetyl derivatives of serotonin and octopamine in a worm pellet from a mixed stage wild type (N2) culture.

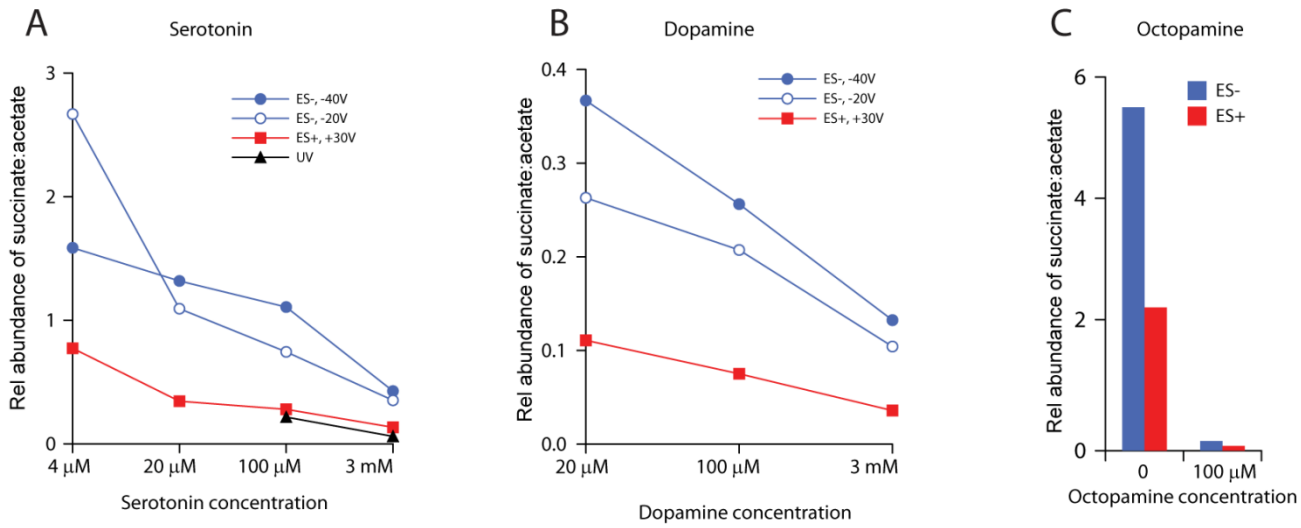



**Figure S10.** A. Ratio of N-succinyl serotonin to N-acetyl serotonin in L1 medium as a function of exogenously added serotonin. B. Ratio of N-succinyl dopamine to N-acetyl dopamine in L1 medium as a function of exogenously added dopamine. C. Ratio of N-succinyl octopamine to N-acetyl octopamine in L1 medium as a function of exogenously added octopamine.

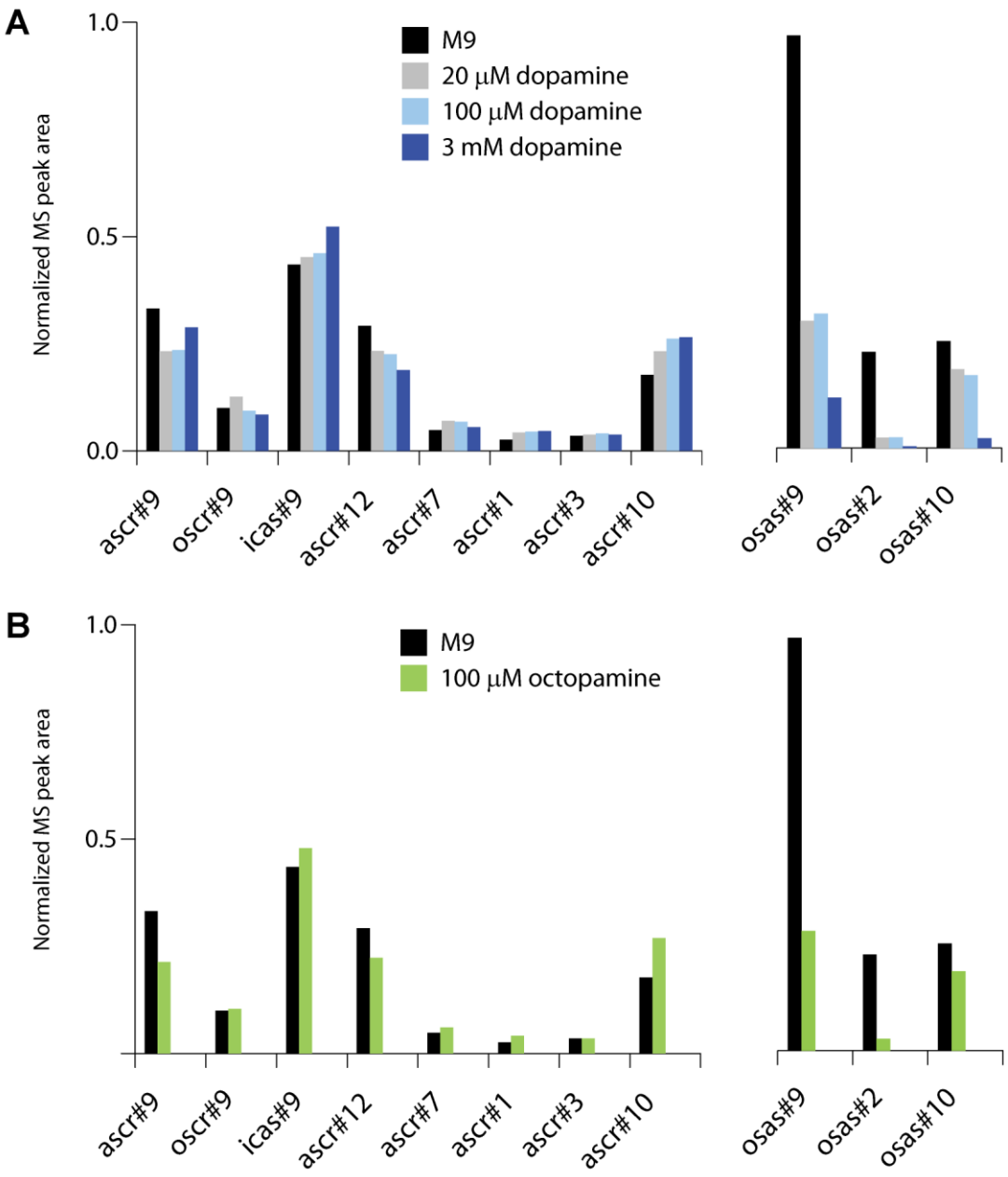



**Figure S11.** Concentrations of osas ascarosides strongly decrease in response to exogenously added dopamine (A) or octopamine (B), whereas levels of all other ascarosides are only marginally affected..

## 2. Supporting Methods

Unless stated otherwise, reagents were purchased from Sigma-Aldrich and used without further purification. *N*,*N*-dimethylformamide (DMF), dichloromethane (DCM), and tetrahydrofuran (THF) were dried over 4 Å molecular sieves prior to use.

NMR spectra were recorded on Varian INOVA 600 (600 MHz) or Varian INOVA 500 (500 MHz) spectrometers in Cornell University's NMR facility. $^{1}$H NMR chemical shifts are reported in ppm (δ) relative to residual solvent peaks (3.31 ppm for methanol-$d_4$). NMR-spectroscopic data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (Hz), and integration. $^{13}$C NMR chemical shifts are reported in ppm (δ) relative to $CH_3OH$ (δ 49.0) in methanol-$d_4$).

Optical rotations were measured on a Perkin Elmer 241 polarimeter. Solvent used for taking optical rotations (methanol) was not further purified prior to use.

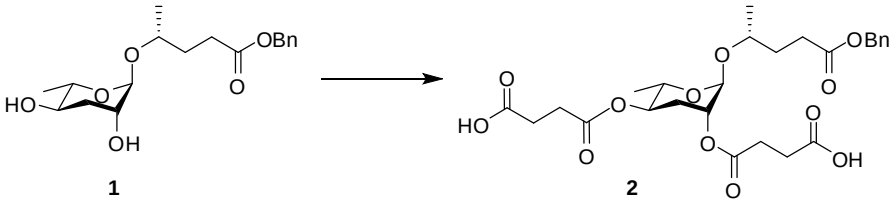
Thin-layer chromatography (TLC) was performed using J. T. Baker Silica Gel IB2-F.

Flash chromatography was performed using Teledyne Isco CombiFlash systems and Teledyne Isco RediSep Rf silica columns.

Purification of synthetic **osas#9** and **tsas#9** by preparative HPLC was performed using an Agilent 1100 Series HPLC system equipped with an Agilent Eclipse XDB-C18 column (9.4 x 250 mm, 5 μm particle diameter). A 0.3% acetic acid-methanol solvent gradient was used at a flow rate of 3.6 mL/min, starting with a methanol content of 10% for 5 min which was increased to 63% over a period of 29 min. The methanol content was increased to 100% over the next 3 min and was held at 100% for 8 min.

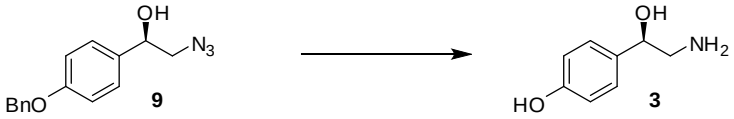
## 3. Synthesis of osas#9

3.1 Synthesis of 4,4'-(((2*R*,3*R*,5*R*,6*S*)-2-(((*R*)-5-(benzyloxy)-5-oxopentan-2-yl)oxy)-6-methyltetrahydro-2H-pyran-3,5-diyl)bis(oxy))bis(4-oxobutanoic acid) (**2)**:



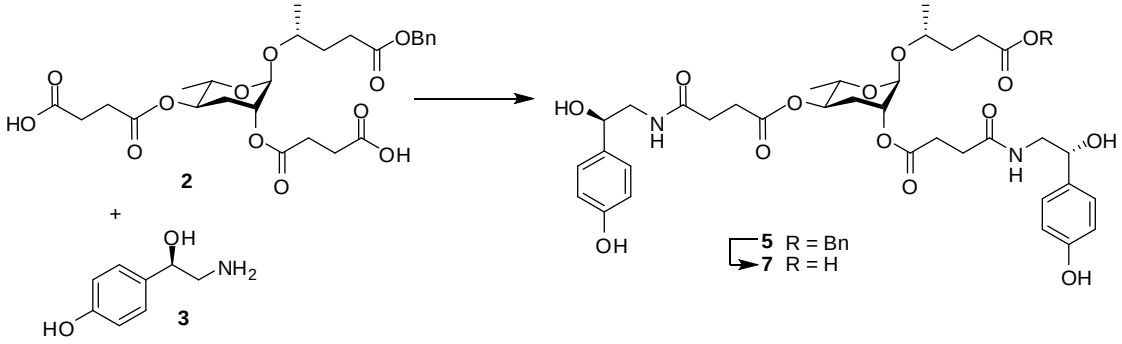

To a solution of **1**[1] (12 mg, 36 µmol) in dry dichloromethane (300 µL), succinic anhydride (15 mg, 150 µmol) in dry dimethylformamide (200 µL) was added with stirring. After 5 min, DIEA (25 µL) and 4-dimethylaminopyridine (8 mg, 65 µmol) was added and the mixture was allowed to stir. After stirring for 2 h, the reaction was quenched with sat. $KHSO_4$ (500 µL), extracted with ethyl acetate, dried over $NaSO_4$, and concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-15% methanol in dichloromethane containing 0.25% glacial acetic acid afforded **2** (16 mg, 30 µmol, 83%) as a white powder. $^1$H NMR (500 MHz, methanol-$d_4$): δ (ppm) 7.39-7.28 (m, 5H), 5.13 (s, 2H), 4.81-4.78 (m, 1H), 4.78-4.70 (m, 1H), 4.75 (br s, 1H), 3.88-3.80 (m, 2H), 2.73-2.46 (m, 10H), 2.12-2.05 (m, 1H), 1.96-1.79 (m, 3H), 1.15 (d, $J$ = 6.1 Hz, 3H), 1.11 (d, $J$ = 6.3 Hz, 3H). $^{13}$C NMR (125 MHz, methanol-$d_4$): δ (ppm) 175.92, 175.88, 174.8, 173.3, 164.8, 137.6, 129.6, 129.21, 129.20, 94.6, 72.3, 72.1, 71.2, 68.2, 67.3, 33.1, 31.3, 30.25, 30.22, 30.1, 29.74, 29.72, 19.1, 18.0.

3.2 Synthesis of (*R*)-4-(2-amino-1-hydroxyethyl)phenol (**3)**:



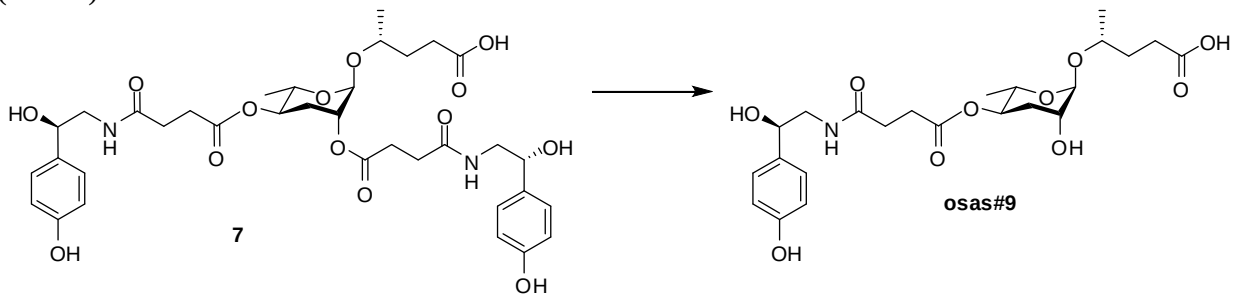

A solution of Pd/C (300 mg, 10%, *w/w*) in 8 mL methanol and 770 µL of 1M aqueous HCl was first flushed with argon for 5 minutes and subsequently with a moderate flow of $H_2$ gas. To this stirring solution was added a solution **9**[2] (105 mg, 390 µmol) in 2 mL methanol. After 8 min, the reaction was filtered over a pad of silica and concentrated *in vacuo* to afford **3** (29 mg, 150 µmol, 39%) as a white powder and used in the next step without further purification. NMR spectroscopic data was in agreement with literature values.[2]

3.3 Synthesis of (*R*)-4-(((2*R*,3*R*,5*R*,6*S*)-3,5-bis((4-(((*R*)-2-hydroxy-2-(4-hydroxyphenyl)ethyl)amino)-4-oxobutanoyl)oxy)-6-methyltetrahydro-2H-pyran-2-yl)oxy)pentanoic acid (**5)**:



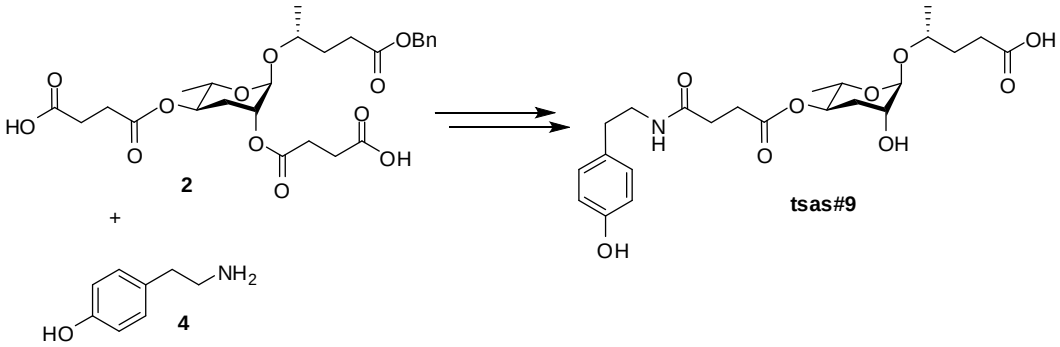

A solution of **2** (16 mg, 30 µmol) in 500 µL dry dichloromethane was treated with 4-dimethylaminopyridine (33 mg, 270 µmol) and EDC hydrochloride (34 mg, 180 µmol). After stirring for 30 minutes, **3** (17 mg, 89 µmol) in 500 µL dry dichloromethane was added to the mixture and stirred. After 3 h, the reaction was quenched with sat $KHSO_4$ (400 µL), extracted with ethyl acetate, dried over $NaSO_4$, and concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-15% methanol in dichloromethane containing 0.25% glacial acetic acid afforded **5** (13.7 mg, 17 µmol, 56%) as a white powder. A solution of Pd/C (18 mg, 10%, *w/w*) in 500 µL methanol was first flushed with argon for 5 minutes and subsequently with a moderate flow of $H_2$ gas. To this stirring solution was added a solution **5** (13.7 mg, 17 µmol) in 500 µL methanol. After 30 min, the reaction was filtered over a pad of silica and concentrated *in vacuo* to afford **7** (11.2 mg, 16 µmol, 92%) as a colorless oil and used in the next step without further purification. $^1$H NMR (500 MHz, methanol-$d_4$): δ (ppm) 7.23-7.18 (m, 4H), 6.79-6.74 (m, 4H), 4.83-4.80 (m, 1H), 4.79-4.72 (m, 1H), 4.78 (br s, 1H), 4.68-4.63 (m, 2H), 3.96-3.89 (m, 1H), 3.89-3.82 (m, 1H), 3.41-3.31 (m, 4H), 2.66 (t, *J* = 6.8 Hz, 2H), 2.59 (t, *J* = 6.7 Hz, 2H), 2.54-2.46 (m, 4H), 2.42-2.35 (m, 2H), 2.13-2.07 (m, 1H), 1.99-1.92 (m, 1H), 1.87-1.77 (m, 2H), 1.17 (d, *J* = 6.1 Hz, 3H), 1.16 (d, *J* = 6.2 Hz, 3H). $^{13}$C NMR (125 MHz, methanol-$d_4$): δ (ppm) 178.5, 173.04, 173.02, 172.1, 172.0, 156.67, 156.65, 133.22, 133.20, 127.08, 127.06, 114.66, 114.65, 93.1, 71.88, 71.84, 71.2, 70.8, 69.9, 66.8, 46.8, 46.7, 32.4, 31.0, 29.95, 29.85, 29.17, 29.15, 28.9, 17.7, 16.6.

3.4 Synthesis of (*R*)-4-(((2*R*,3*R*,5*R*,6*S*)-3-hydroxy-5-((4-(((*R*)-2-hydroxy-2-(4-hydroxyphenyl)ethyl)amino)-4-oxobutanoyl)oxy)-6-methyltetrahydro-2H-pyran-2-yl)oxy)pentanoic acid (**osas#9**):




A solution of **7** (4.2 mg, 5.8 µmol) in THF : dioxane : $H_2O$ (10:20:3, *v*/*v*/*v*, 660 µL) was stirred and heated to 65 °C. To this mixture, a solution of LiOH (56 µg) in $H_2O$ (75 µL) was added and the reaction allowed to stir. After 1.7 h, the reaction was acidified to pH = 6 with glacial acetic acid and concentrated *in vacuo*. HPLC afforded **osas#9** (600µg, 1.2 µmol, 21%) as a white powder. $^1$H NMR (600 MHz, methanol-$d_4$): δ (ppm) 7.21-7.18 (m, 2H), 6.78-6.74 (m, 2H), 4.88-4.84 (m, 1H), 4.68 (br s, 1H), 4.64 (dd, *J* = 7.8 Hz, 4.9 Hz, 1H), 3.88-3.81 (m, 2H), 3.73-3.71 (m, 1H), 3.38 (dd, *J* = 13.7 Hz, 5.2 Hz, 1H), 3.34-3.29 (m, 1H), 2.59 (t, *J* = 6.8 Hz, 2H), 2.49 (t, *J* = 6.8 Hz, 2H), 2.45-2.32 (m, 2H), 2.06-2.01 (m, 1H), 1.88-1.77 (m, 3H), 1.16 (d, *J* = 6.2, 3H), 1.14 (d, *J* = 6.3 Hz, 3H). $^{13}$C NMR (151 MHz, methanol-$d_4$): δ (ppm) 178.9, 174.5, 173.4, 158.1, 134.6, 128.5, 116.1, 97.4, 73.3, 71.9, 71.6, 69.4, 68.4, 48.2, 33.9, 32.9, 32.3, 31.3, 30.6, 19.0, 18.0. $\alpha_D^{20}$ = -45.0 (*c* 0.06, methanol).

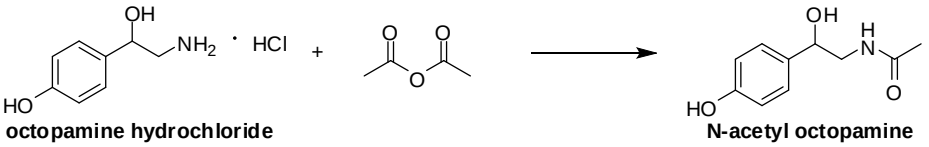
3.5. Synthesis of (*R*)-4-(((2*R*,3*R*,5*R*,6*S*)-3-hydroxy-5-((4-((4-hydroxyphenethyl)amino)-4-oxobutanoyl)oxy)-6-methyltetrahydro-2H-pyran-2-yl)oxy)pentanoic acid (**tsas#9**):




**tsas#9** was prepared following analogous reaction steps as for **osas#9** using **4**. $^1$H NMR (500 MHz, methanol-$d_4$): δ (ppm) 7.05-7.01 (m, 2H), 6.72-6.69 (m, 2H), 4.88-4.84 (m, 1H), 4.68 (br s, 1H), 3.89-3.80 (m, 2H), 3.74-3.70 (m, 1H), 3.35-3.31 (m, 2H), 2.68 (t, *J* = 7.5 Hz, 2H), 2.59 (t, *J* = 6.9 Hz, 2H), 2.45 (t, *J* = 6.9 Hz, 2H), 2.40-2.28 (m, 2H), 2.06-2.00 (m, 1H), 1.89-1.78 (m, 3H), 1.15 (d, *J* = 6.2 Hz, 3H), 1.14 (d, *J* = 6.3 Hz, 3H).$^{13}$C NMR (125 MHz, methanol-$d_4$): δ (ppm) 180 (br), 174.1, 173.4, 156.9, 131.3, 130.7, 116.2, 97.3, 72.1, 71.6, 69.4, 68.4, 42.4, 35.7, 34.3, 33.3, 32.9, 31.4, 30.7, 19.0, 18.0.

## 4. Synthesis of N-acetyl octopamine, N-succinyl octopamine, and N-succinyl serotonin

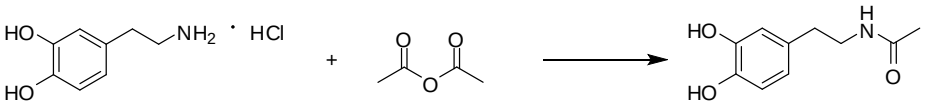
### 4.1 Synthesis of **N-acetyl octopamine**




**octopamine hydrochloride** **N-acetyl octopamine**

To a solution of **octopamine hydrochloride** (120 mg, 0.63 mmol) in water (1.1 mL), acetic anhydride (75μL) was added. Subsequently, sodium acetate (64 mg) was added and the reaction was stirred. After 4 h, the reaction was quenched with acetic acid (15 μL) and the mixture was concentrated *in vacuo*. **N-acetyl octopamine** was used without further purification. $^{1}$H NMR (500 MHz, acetone-$d_6$): δ (ppm) 7.21-7.17 (m, 2H), 6.80-6.76 (m, 2H), 4.66 (dd, $J$ = 8.1 Hz, $J$ = 4.0 Hz, 1H), 3.44 (dd, $J$ = 13.6 Hz, $J$ = 4.0 Hz, 1H), 3.22 (dd, $J$ = 13.6 Hz, $J$ = 8.1 Hz, 1H), 1.90 (s, 3H). $^{13}$C NMR (125 MHz, acetone-$d_6$): δ (ppm) 171.3, 157.4, 135.0, 128.0 (2C), 154.6 (2C), 73.2, 48.4, 22.7.

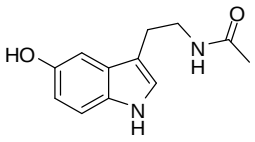
### 4.2 Synthesis of **N-acetyl dopamine**




**dopamine hydrochloride** **N-acetyl dopamine**

To a solution of **dopamine hydrochloride** (105 mg, 0.55 mmol) in water (1.0 mL), acetic anhydride (80 μL) was added. Subsequently, sodium acetate (60 mg) was added and the reaction was stirred. After 4 h, the reaction was quenched with acetic acid (15 μL) and the mixture was concentrated *in vacuo*. **N-acetyl dopamine** was used without further purification. NMR spectroscopic data of **N-acetyl dopamine** was in agreement with literature values.[3]

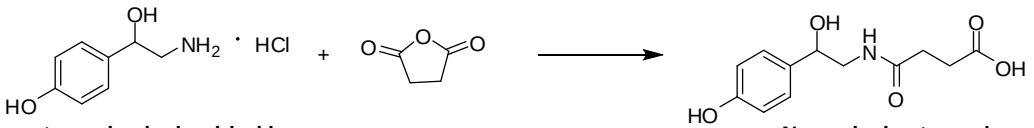
### 4.3 **N-acetyl serotonin**




**N-acetyl serotonin**

**N-acetyl serotonin** was purchased from Sigma Aldrich.

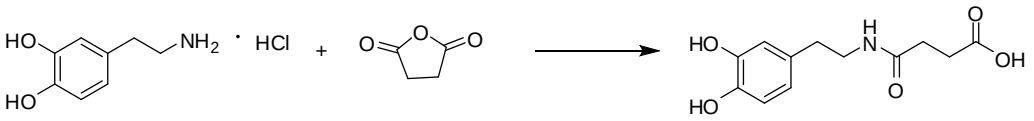
4.4 Synthesis of **N-succinyl octopamine**




**octopamine hydrochloride** **N-succinyl octopamine**

To a solution of **octopamine hydrochloride** (570 mg, 3 mmol) in DCM (3 mL) and DMF (1 mL) and DIEA (1.4 mL), succinic anhydride (270 mg, 2.7 mmol) in DMF (2 mL) was added at 0 °C with stirring. After stirring for 7 h, the reaction was quenched with $K_2CO_3$ (140 mg) in $H_2O$ (200 μL) and 1.8 mL acetic acid, and concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-30% methanol in dichloromethane containing 0.25% glacial acetic acid afforded **N-succinyl octopamine** (630 mg, 2.5 mmol, 83%).[1]H NMR (500 MHz, methanol-$d_4$): δ (ppm) 7.22-7.17 (m, 2H), 6.78-6.73 (m, 2H), 4.65 (dd, $J$ = 7.9 Hz, $J$ = 4.9 Hz, 1H), 3.39, (dd, $J$ = 13.6 Hz, $J$ = 4.9 Hz, 1H), 3.31, (dd, $J$ = 13.6 Hz, $J$ = 7.9 Hz, 1H), 2.59-2.53 (m, 2H), 2.49-2.43 (m, 2H). $^{13}$C NMR (125 MHz, methanol-$d_4$): δ (ppm) 176.7, 174.9, 158.0, 134.6, 128.4 (2C), 116.1 (2C), 73.3, 48.2, 31.7, 30.6.

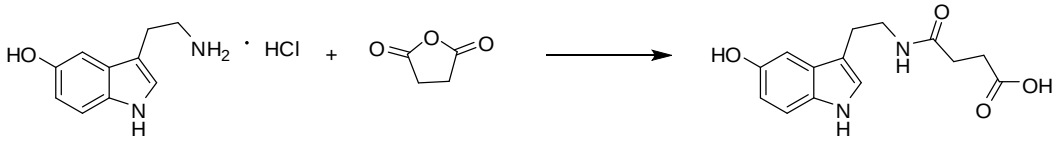
4.5 Synthesis of **N-succinyl dopamine**




**dopamine hydrochloride** **N-succinyl dopamine**

To a solution of **dopamine hydrochloride** (10 mg, 53 μmol) in DCM (300 μL) and DMF (100 μL) and DIEA (15 μL), succinic anhydride (5 mg, 50 μmol) in DMF (100 μL) was added at 0 °C with stirring. After stirring for 30 min, the reaction was concentrated *in vacuo* and used without further purification. NMR spectroscopic data of **N-succinyl dopamine** was in agreement with literature values.[4]

4.6 Synthesis of **N-succinyl serotonin**

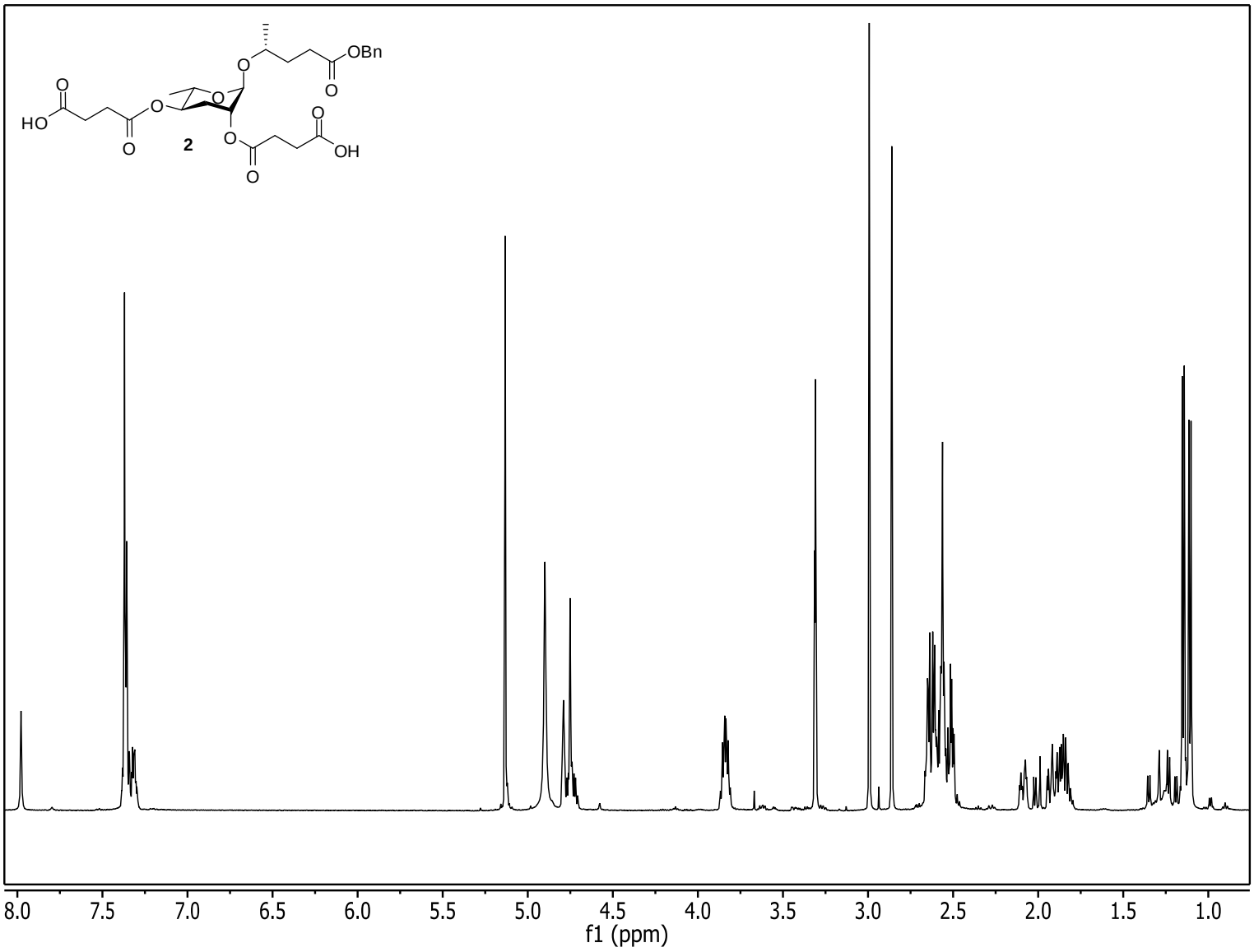



**serotonin hydrochloride** **N-succinyl serotonin**

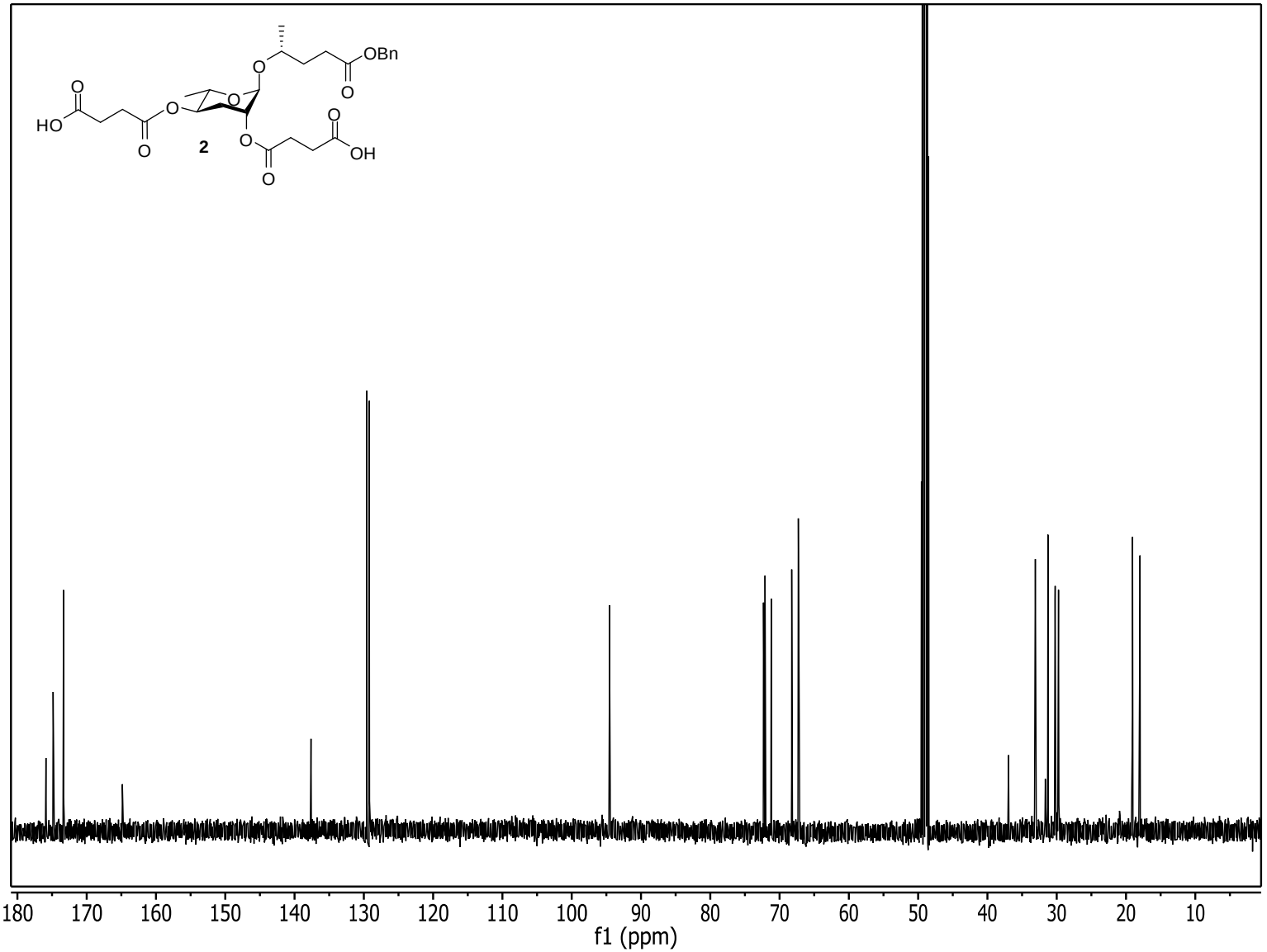
To a solution of **serotonin hydrochloride** (10 mg, 47 μmol) in DCM (300 μL) and DMF (100 μL) and DIEA (18 μL), succinic anhydride (6 mg, 60 μmol) in DMF (100 μL) was added at 0 °C with stirring. After stirring for 30 min, the reaction was concentrated *in vacuo* and used without further purification. NMR spectroscopic data of **N-succinyl serotonin** was in agreement with literature values.[5]

## 5. NMR Spectra of intermediates, osas#9, and tsas#9

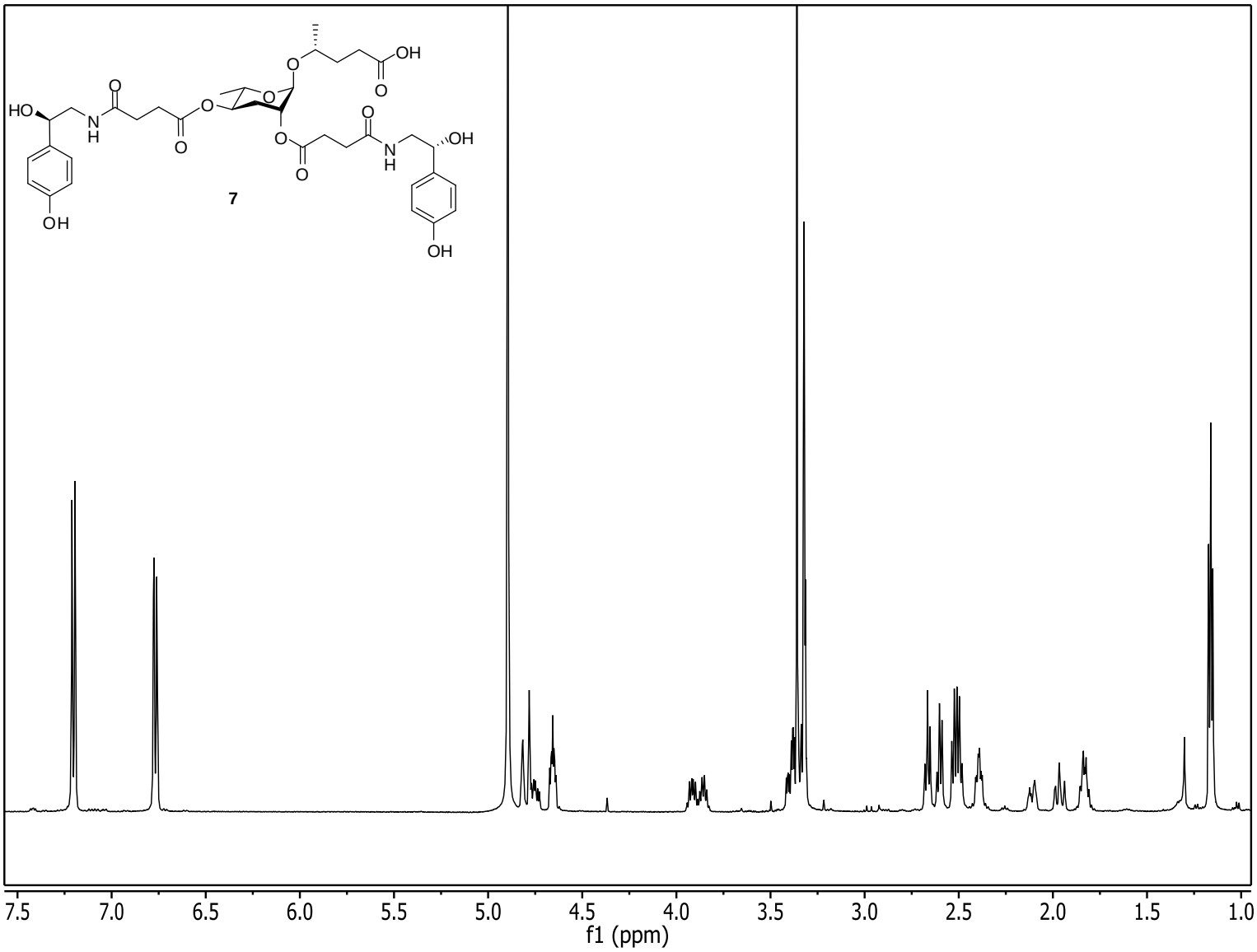
$^{1}$H NMR Spectrum (500 MHz, methanol-$d_4$) of **2**



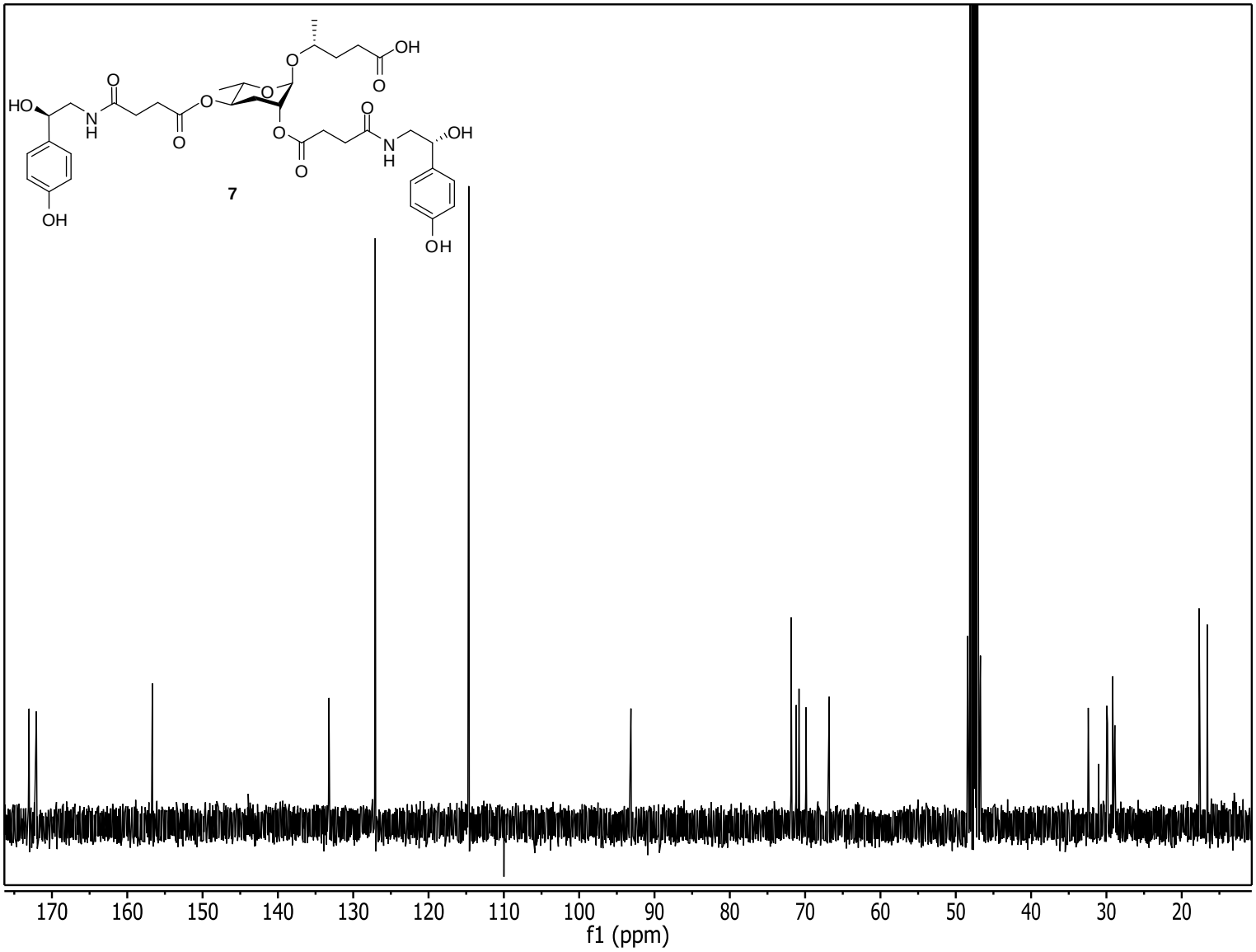
$^{13}$C NMR Spectrum (125 MHz, methanol-$d_4$) of **2**



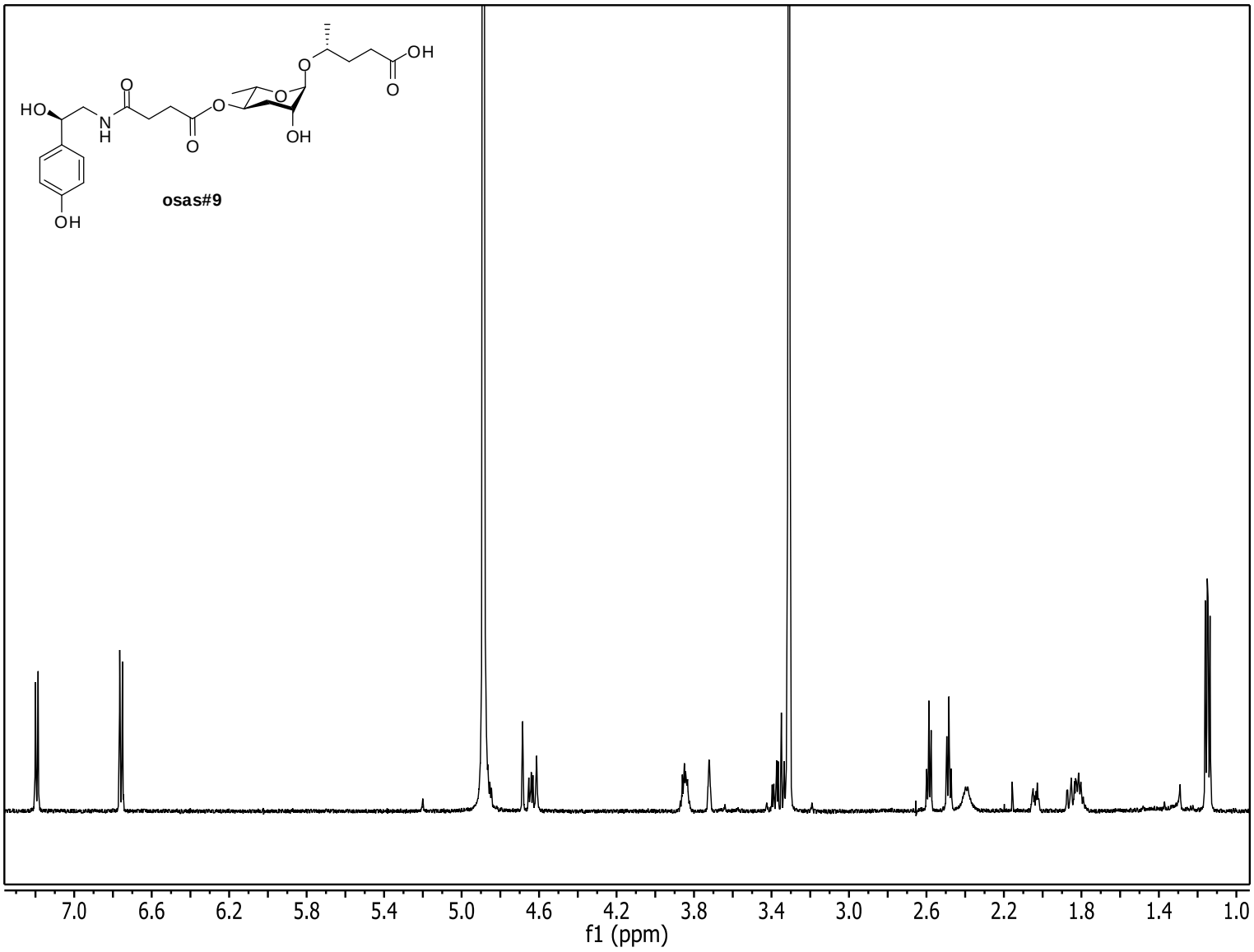
$^{1}$H NMR Spectrum (500 MHz, methanol-$d_4$) of **5**



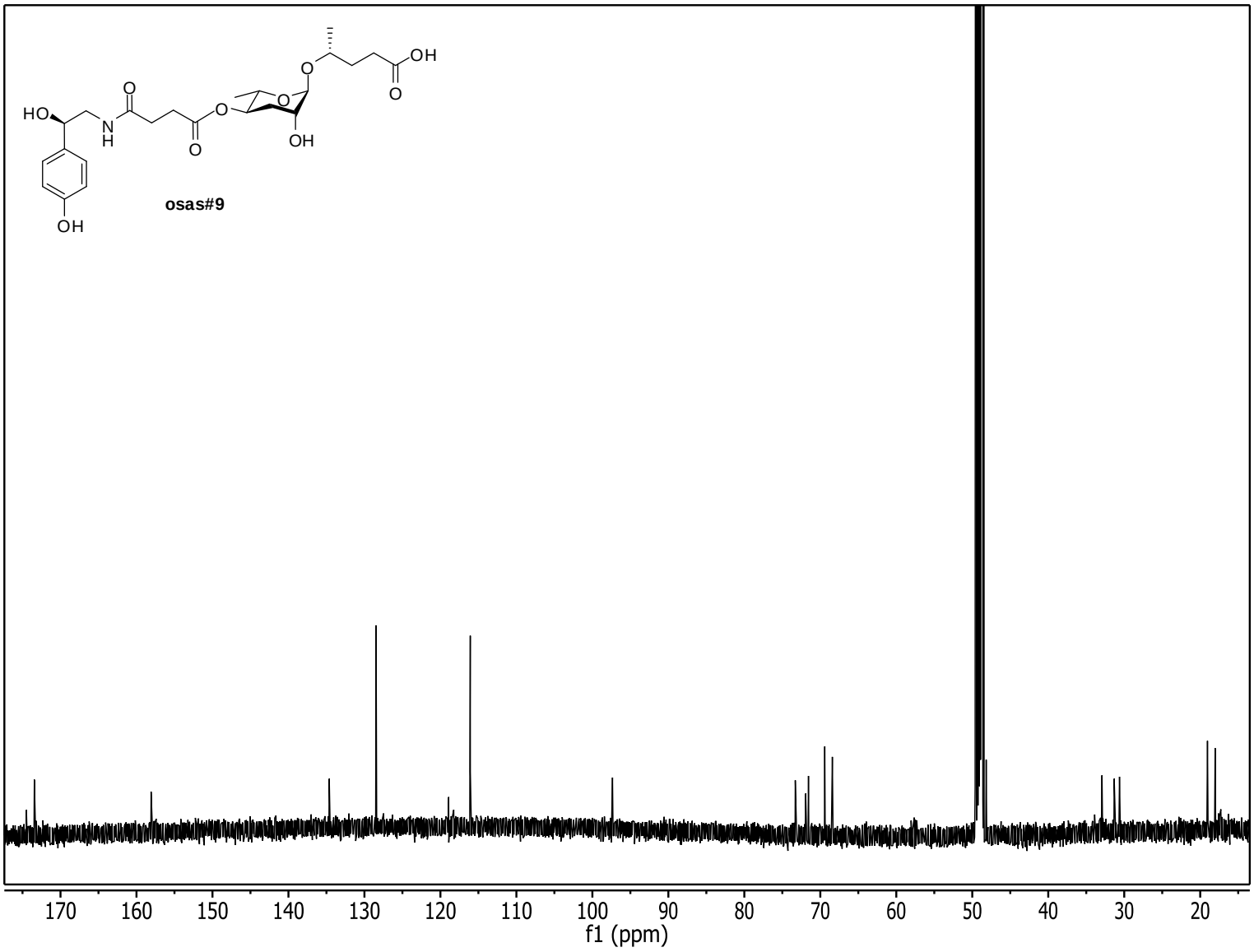
$^{13}$C NMR Spectrum (125 MHz, methanol-$d_4$) of **5**



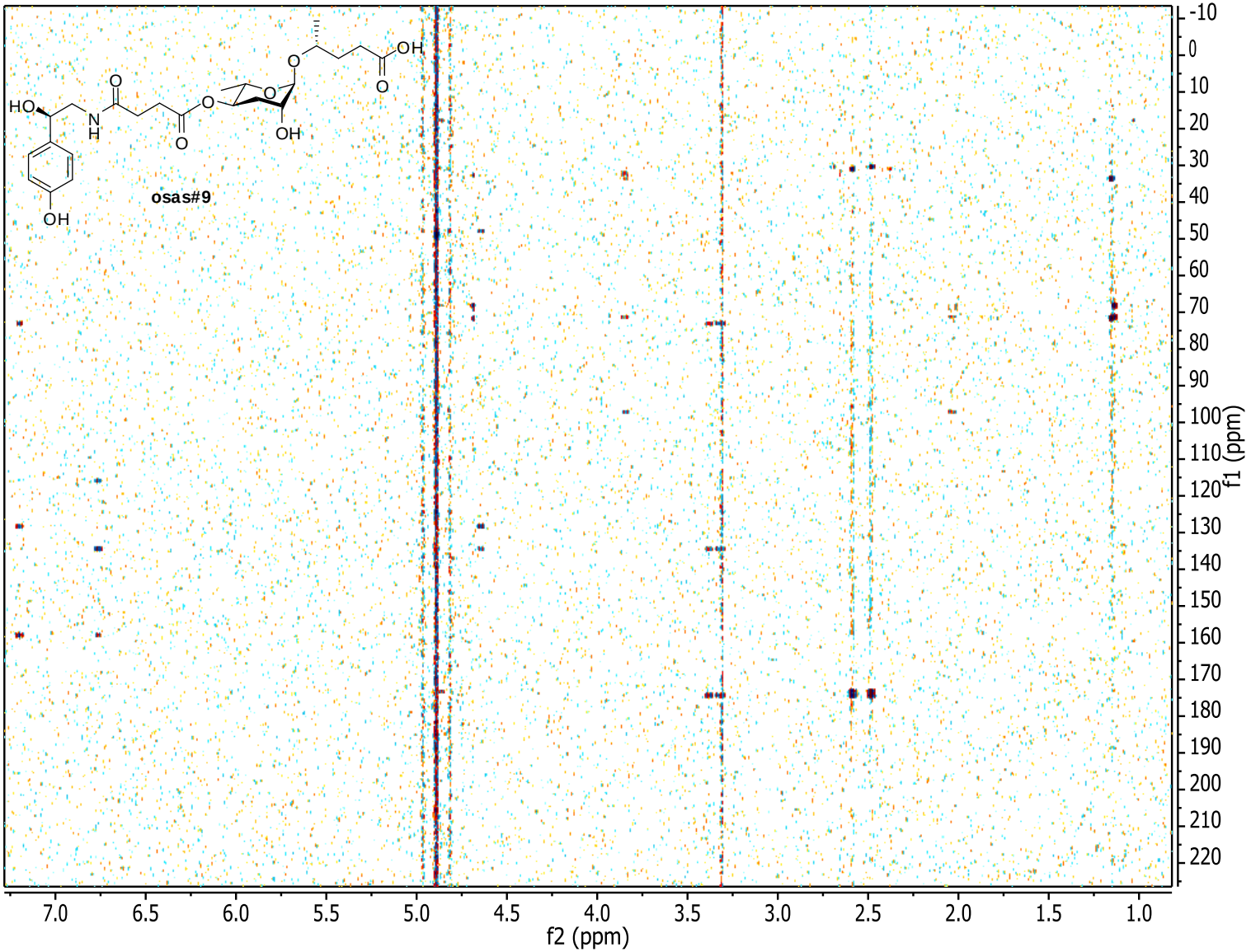
$^{1}$H NMR Spectrum (600 MHz, methanol-$d_4$) of **osas#9**



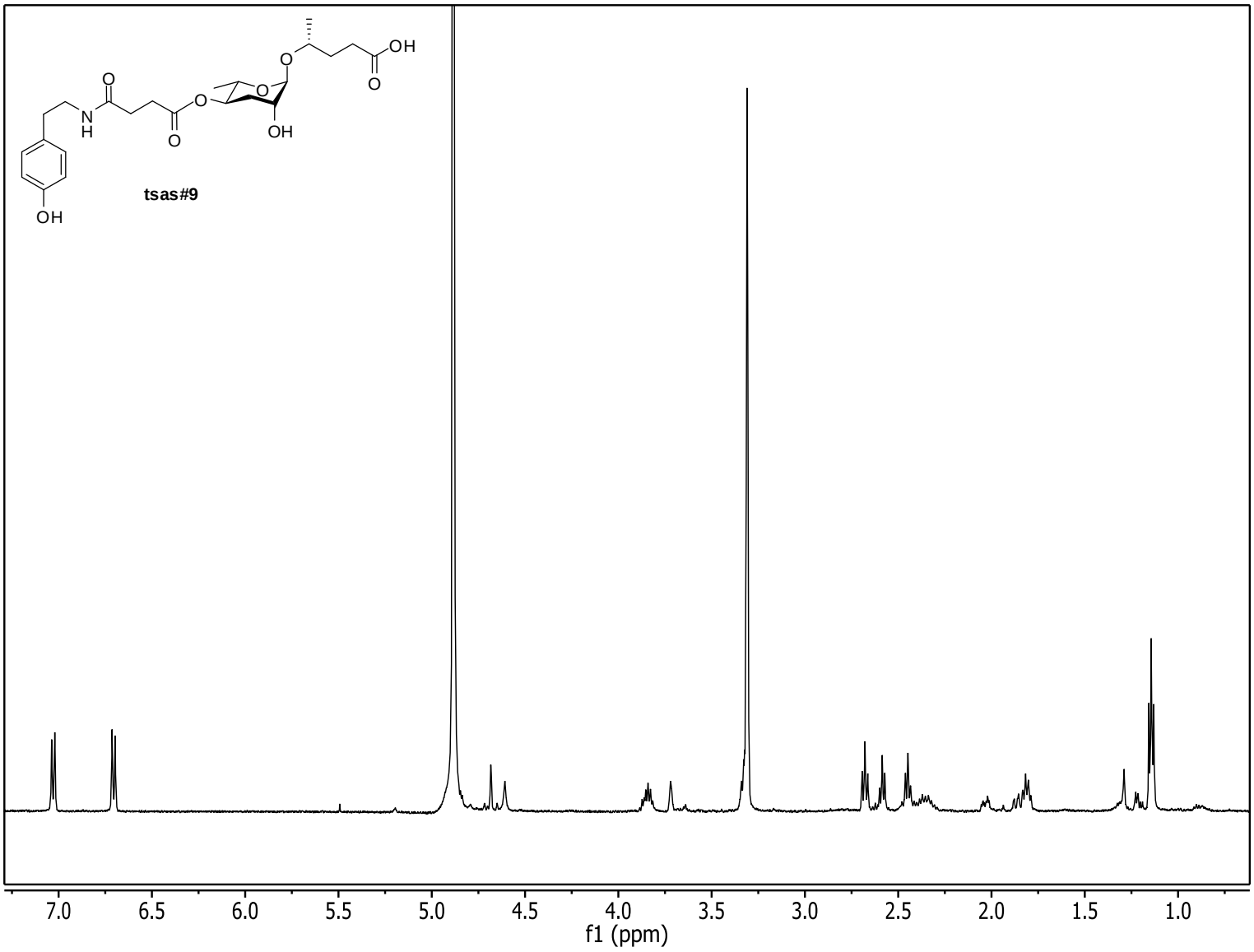
$^{13}$C NMR Spectrum (125 MHz, methanol-$d_4$) of **osas#9**



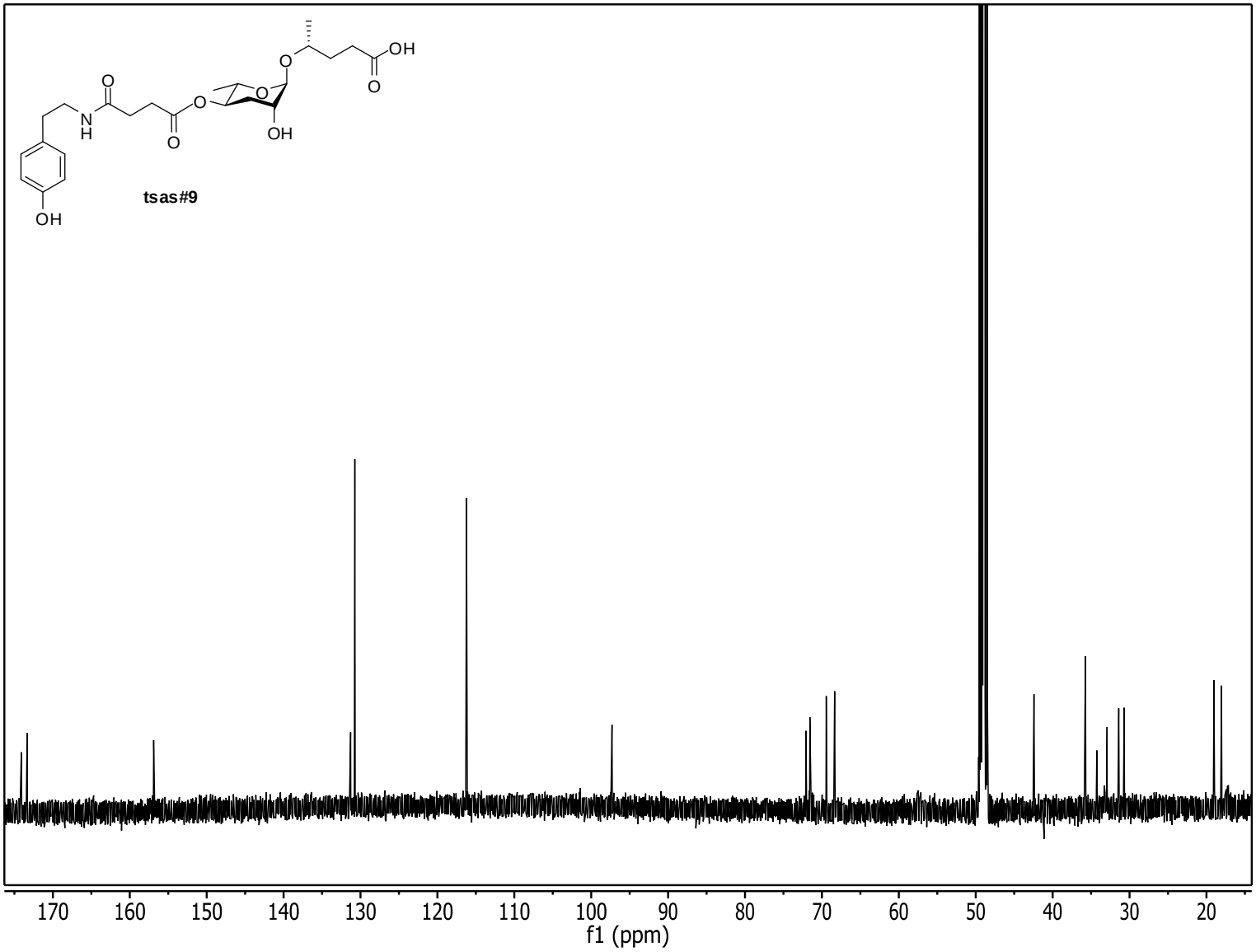
HMBC Spectrum (600 MHz for $^{1}H$, 151 MHz for $^{13}C$, methanol-$d_4$) of **osas#9**



$^{1}$H NMR Spectrum (500 MHz, methanol-$d_4$) of **tsas#9**



$^{13}$C NMR Spectrum (125 MHz, methanol-$d_4$) of **tsas#9**



## 5. References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