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Supporting Information

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A novel method for trapping intermediates of polyketide biosynthesis with a non-hydrolysable malonyl-coenzyme A-analogue

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Synthesis

Ethylmalonyl-CoA-analogue 2

To a solution of 100 mg ethyl-4-chloro-3-oxobutanoate (0.74 mM) in 5 ml Li_2CO_3 solution (0.11 M) 200 mg coenzyme A (0.26 mM) were added and stirred for 3 h.[1] Extraction with ethylacetate was used to remove unreacted ethyl-4-chloro-3-oxobutanoate. The combined aqueous layers were neutralised with NH_4Cl, concentrated to 2 ml by freeze drying and directly subjected to preparative HPLC purification (Phenomenex Luna 250 x 20 mm, 10 µm, A: water, B: methanol;from 100 % A to 100 % B in 20 min).

Yield: 183 mg 79 %

^{1}H-NMR (D_2O, 500 MHz): δ?0.76(s, 3H), 0.88 (s, 3H), 1.26 (t, *J* = 7.17, 3H), 2.49 (t, *J* = 6.49, 2H), 2.63 (t, *J* = 6.56, 2H), 3.34 (t, *J* = 6.56, 2H), 3.44-3.52 (m, 2H), 3.55-3.61 (m, 1H), 3.81-3.86 (m, 1H), 4.02 (s, 1H), 4.21 (q, *J* = 7.17, 2H), 4.26 (s, 1H), 4.59 (s, 1H), 4.82-4.87 (m, *J* = 6.71, 1H), 6.17 (d, 1H), 8.24 (s, 3H), 8.54 (s, 3H).

HR-ESI-MS: $C_{27}H_{45}N_7O_{19}P_3S$ $[M+H]^+$ observed 896.1724, calc. 896.1704.

Malonyl-CoA-analogue 3

10-20 mg of ethylmalonyl-CoA-analogue(11-22 µM) **2** in 2 ml 50 mM Hepes buffer (100 mM NaCl, pH 8) were dissolved and 50 µl of pig liver esterase (PLE) were added.[2] The pH was monitored during the reaction and readjusted to pH 8 if necessary. The progression of the reaction was followed by LC-MS and after 6 h the reaction was quenched by addition of $CHCl_3$ in order to precipitate the PLE. After vortexing the mixture was centrifuged to separate the phases. The aqueous layer was collected. Traces of $CHCl_3$ were removed in an argon stream. The reaction mixture was directly used for the bioassays. The amount of **3** was estimated by comparison of its UV signal to a known concentration of pure **2**. In the buffer solution **3** is stable for month if stored at -20 °C.

HR-ESI-MS: $C_{25}H_{40}N_7O_{19}P_3S$ $[M+H]^+$ observed 868.1429, calc.868.1391

Purification of succinyl-CoA:3-ketoacid-transferase (EC 2.8.3.5)[3]

pBR322 containing the gene for succinyl-CoA:3-ketoacid-transferase was a gift from Prof. Dr. Fraser (University of Calgary, Canada). *E. coli* Bl21DE3 was transformed with the plasmid. *E. coli* cells were grown at 37 °C in 1l of LB medium until an A_{600} of about 0.8 was reached. Protein expression was induced with 0.5 mM IPTG at 16 °C, and after 12 h cells were harvested by centrifugation, resuspended in 50 mM Hepes buffer, pH 7.8, and ruptured by sonification. To the cell free extract $(NH_4)_2SO_4$ was added to 50 % saturation to precipitate proteins. After centrifugation to the supernatant further $(NH_4)_2SO_4$ was added to 65 % saturation. Centrifugation at 5000 U/min for 30 min gave a pellet containing the crude succinyl-CoA-transferase. This crude preparation was used in order to generate [$^{13}C_3$]malonyl CoA.

Preparation of labelled [$^{13}C_3$]malonyl-CoA[4]

2 mg succinic anhydride were dissolved in 300 µl acetone, an aqueous solution of 12 mg coenzyme A lithium salt was added quickly and the mixture was vortexed for several minutes to generate succinyl-CoA. Its formation was controlled by LC-MS. The acetone was removed in an argon stream.

[$^{13}C_3$]malonate was dissolved in 500 µl 50 mM Hepes pH 7.6, the pH was readjusted to 7 with the help of a pH-microelectrode. The [$^{13}C_3$]malonate solution was added to the solution containing succinoyl-CoA, the pH was controlled and adjusted to pH 7. Finally, 5-10 mg from the pellet of the crude succinoyl-CoA transferase were added. After 1 h the reaction was quenched by addition of 300 µl $CHCl_3$. The enzyme was precipitated by vortexing for 1 min, the solution was centrifuged and the aqueous solution was collected. The synthesized [$^{13}C_3$]malonyl-CoA was used for the bioassays. The amount of [$^{13}C_3$]malonyl-CoA generated by the enzyme was estimated by comparison of the UV LC-trace with the known concentration of malonyl-CoA.

Purification of stilbene synthase[5]

pET-28a(+) containing the sts gene from cDNA of *Pinus sylvestris* was used as expression vector which provides an *N*-terminal His_6-tagged protein. *E. coli* cells were grown at 37 °C in 1l of LB medium until an A_{600} of about 0.7 was reached. Protein expression was induced with 0.2 mM IPTG at 16 °C, and after 12 h cells were harvested by centrifugation, resuspended in 10 mM imidazole-HCl buffer, pH 7.8 (binding buffer), and ruptured by sonification. The cell-free extract was applied to a Ni^{2+}-NTA resin column which was washed successively with binding buffer and wash buffer (30 mM imidazole-HCl) before eluting the target protein with 100 mM imidazole-HCl. The buffer was exchanged to 50 mM HEPES buffer, pH 7.2 using Millipore centrifugal filters. The relative molecular masses

of purified protein STS was determined by electrospray mass spectrometry (ESI-MS) to be 44760. The recombinant protein is in good agreement with the predicted calculated mass (M: 44892; M-Met: 44761), taking the His_6 tag into consideration. The concentration of the enzyme was measured using the Bradford assay.

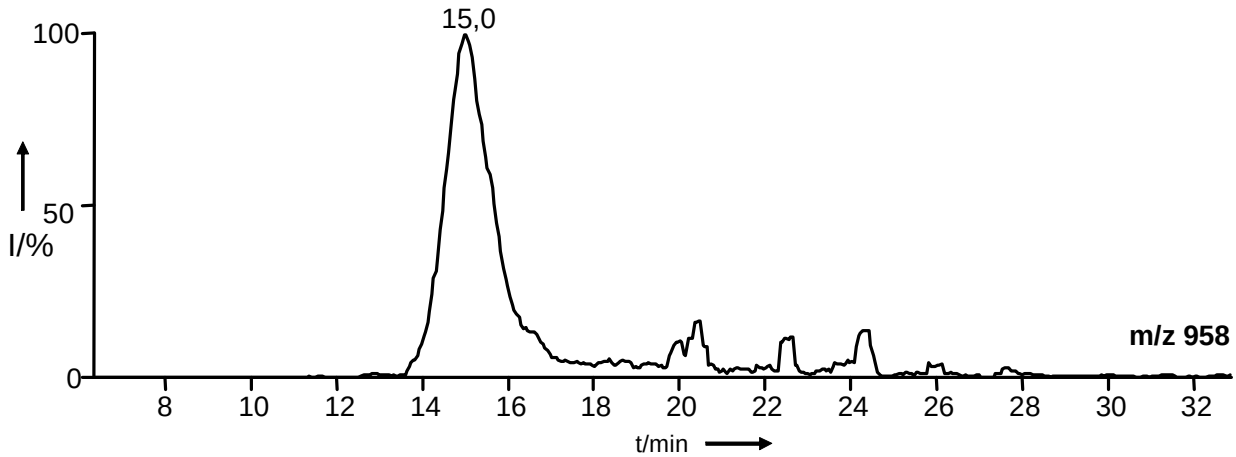
Assay conditions

To a solution of 20 µl starter unit CoA (10 mM), 7 µl malonyl-CoA (30 mM), 40 µl analogue **3** (4 mM) in 50 µl buffer (50 mM Hepes, 100 mM NaCl, pH 7) 10 µl (0.2 µmol) STS were added and the samples were incubated at 30 °C or 42 °C.

After 3 h, 6 h or overnight incubation 10 µl 6 N HCl were added in order to precipitate the enzyme. The sample was centrifuged and the supernatant was directly subjected to LC-MS/MS analysis. Controls were incubated under identical conditions without the STS.

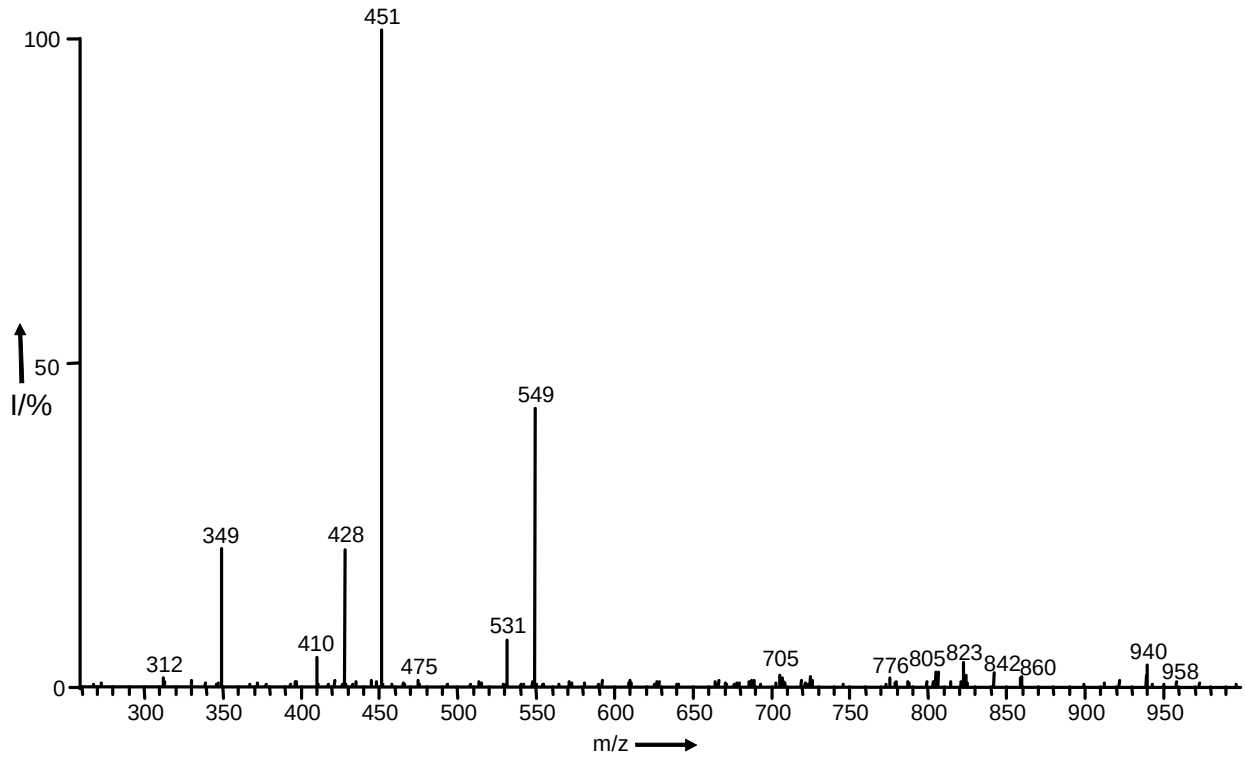
LC-MS conditions: Phenomenex Synergy polar RP-column (150 mm × 2 mm, 4 µm) using gradient elution: 100 % A for 3 min, from 100 % A to 0 % A in 27 min, 100 % B 10 min; A: H_2O 0.1 % TFA, B: MeCN 0.1% TFA; flowrate: 0.3 ml/min, injection volume 50-100 µl; ESI-MS/MS collision energy 25 % - 28 %.

LC-trace of diketide intermediate of the STS (4-hydroxyphenyl-acetyl-CoA and malonyl-CoA-analogue)9

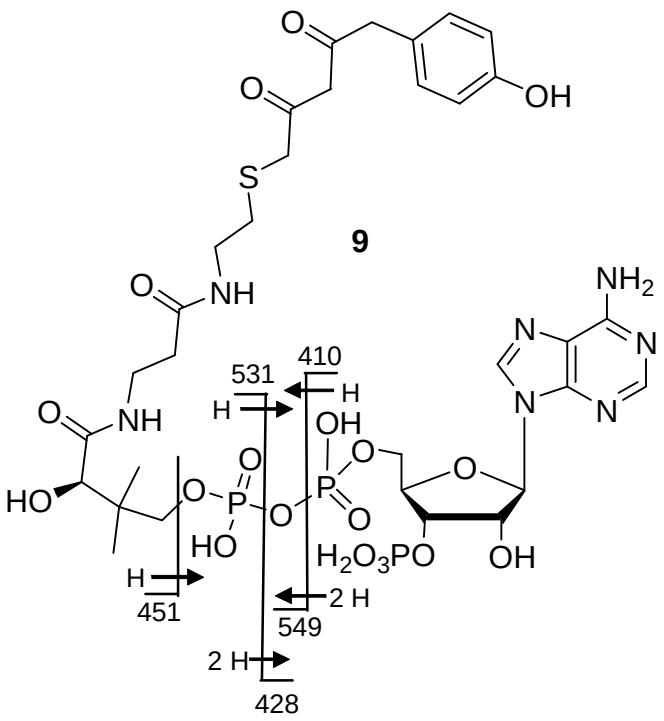


MS/MS of diketide intermediate of the STS (4-hydroxyphenyl-acetyl-CoA and malonyl-CoA-analogue)9

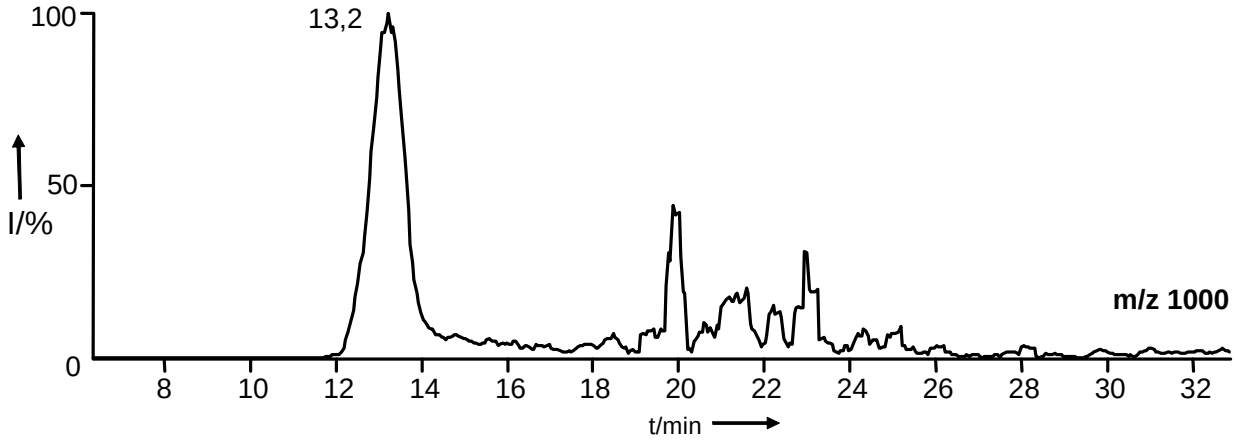
ESI Full ms2 958,00@28,00



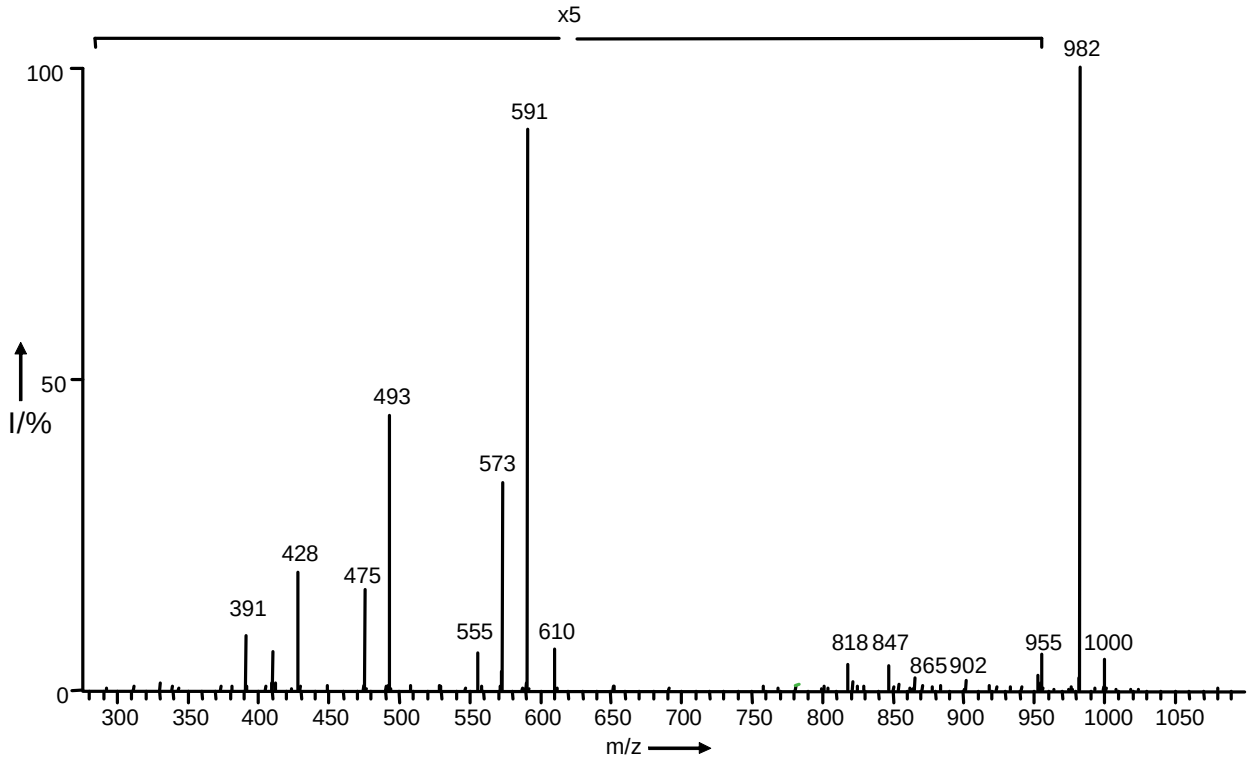
Diketide intermediate of the STS (4-hydroxyphenylacetyl-CoA and malonyl-CoA-analogue)9



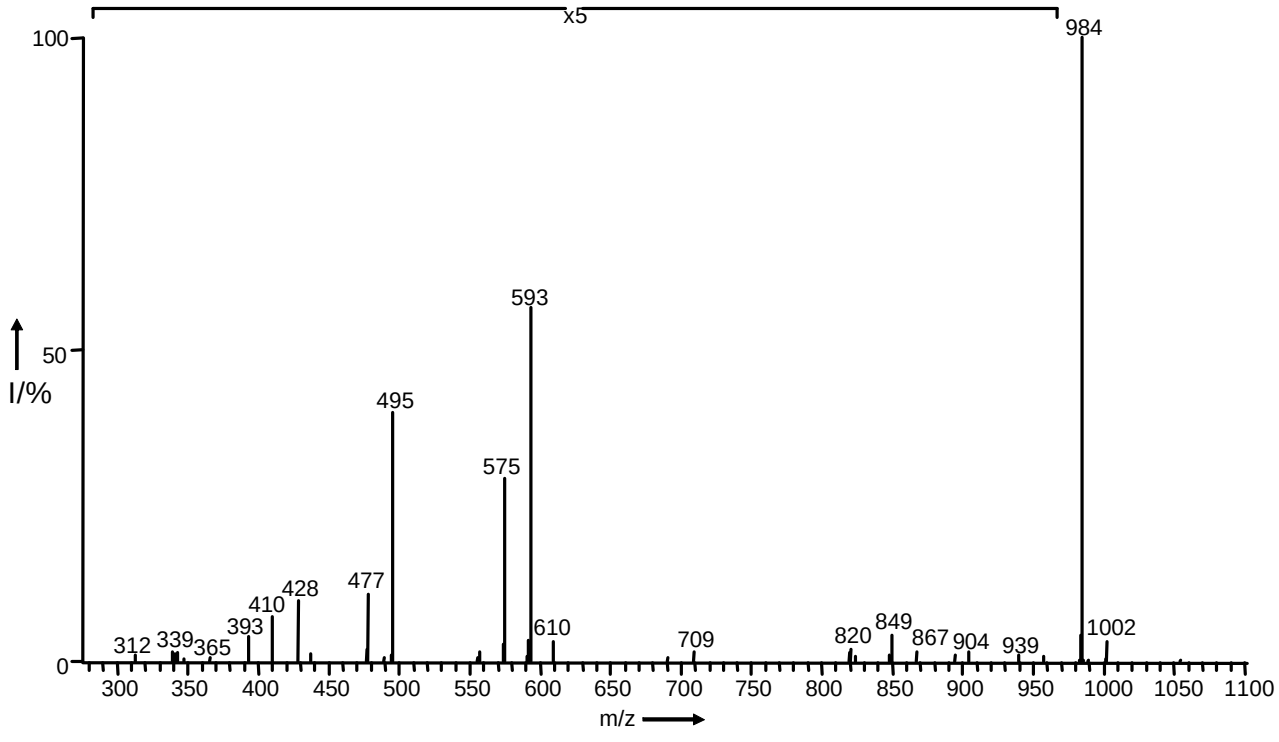
LC-trace of triketide intermediate of the STS (4-hydroxyphenylacetyl-CoA, malonyl-CoA and malonyl-CoA-analogue)10



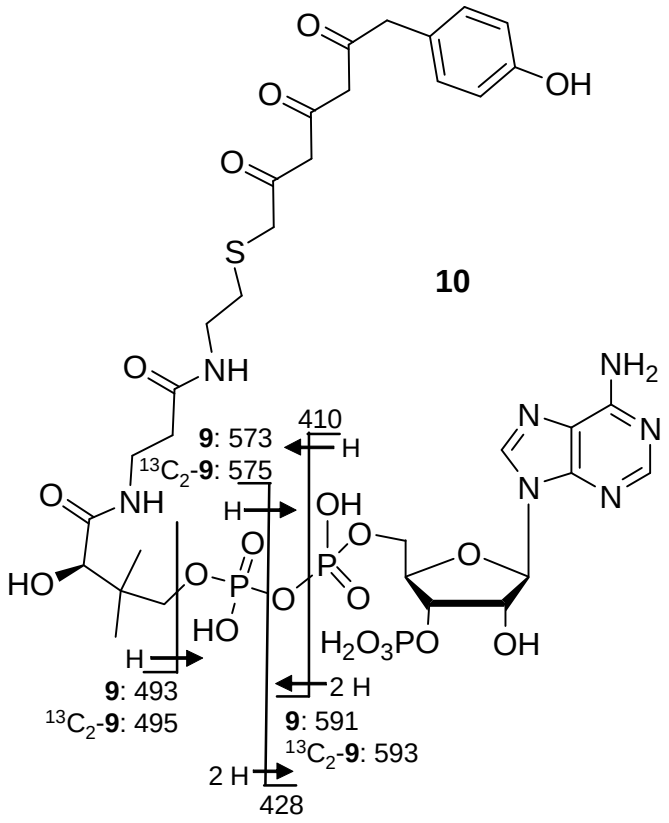
MS/MS of triketide intermediate of the STS (4-hydroxyphenyl-acetyl-CoA, malonyl-CoA and malonyl-CoA-analogue)10



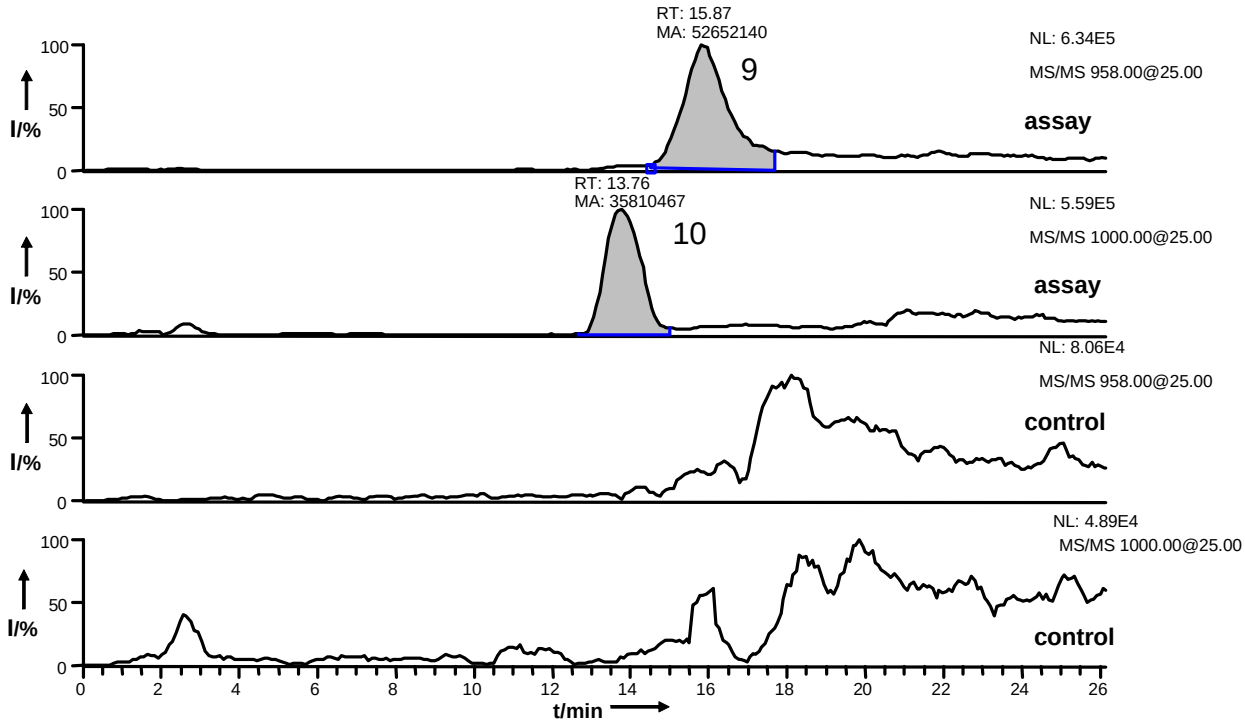
MS/MS of triketide intermediate of the STS (4-hydroxyphenyl-acetyl-CoA, [$^{13}C_3$]malonyl-CoA and malonyl-CoA-analogue)10



Triketide intermediate of the STS (4-hydroxyphenylacetyl-CoA, malonyl-CoA and malonyl-CoA-analogue)10



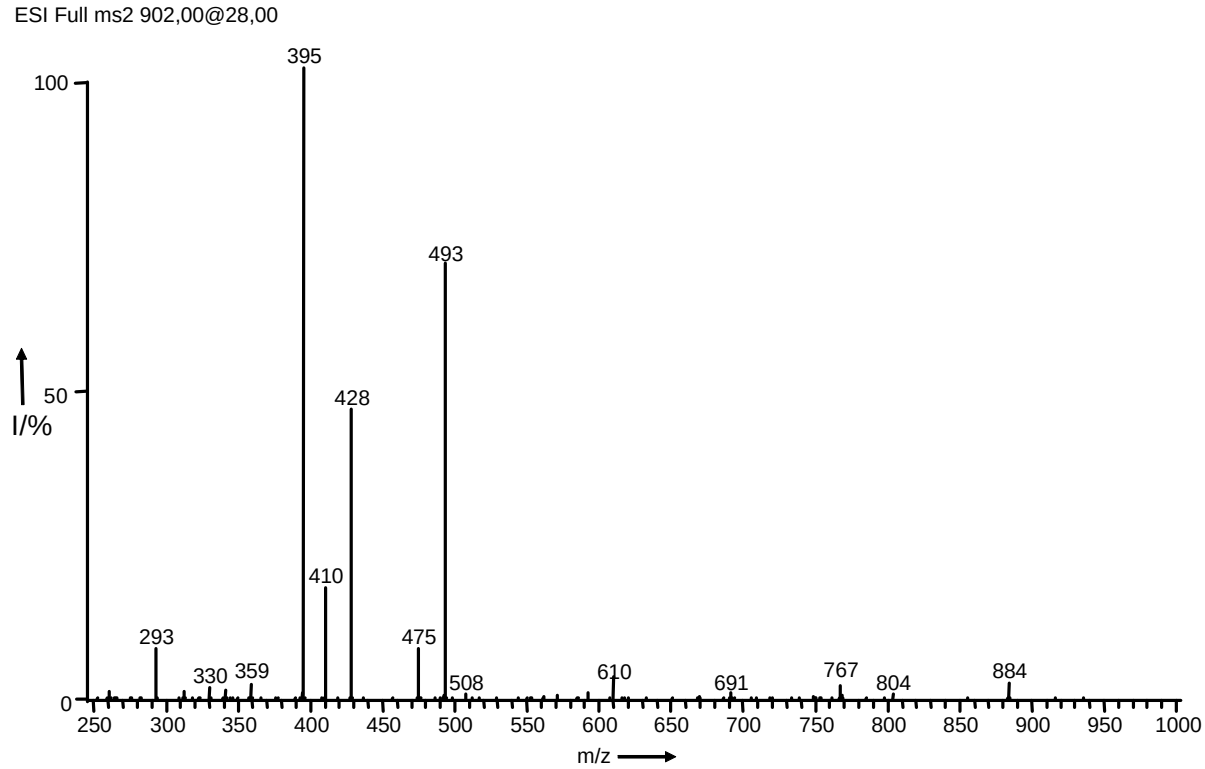
Comparison between the bioassays and control reactions



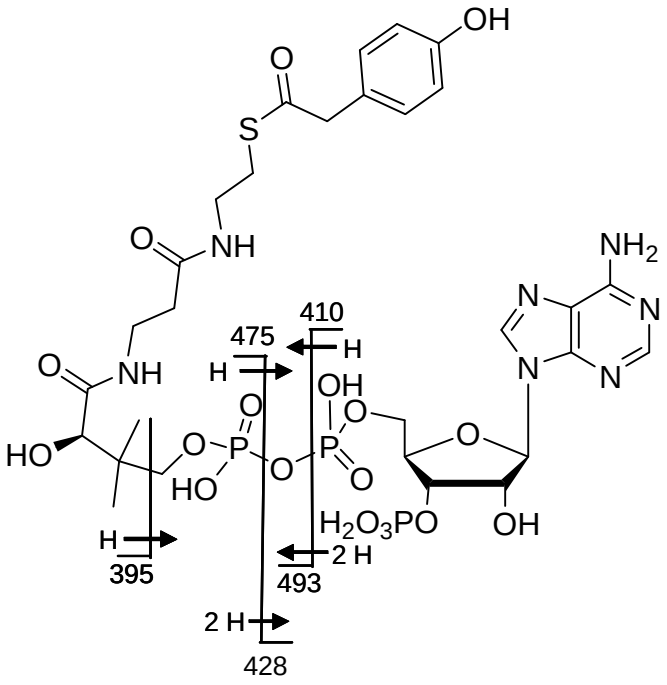
MS/MS 1000 and 958 assay: 4-hydroxyphenylacetyl-CoA, malonyl-CoA and malonyl-CoA-analogue, sts

MS/MS 1000 and 958 control: 4-hydroxyphenylacetyl-CoA, malonyl-CoA and malonyl-CoA-analogue, no sts

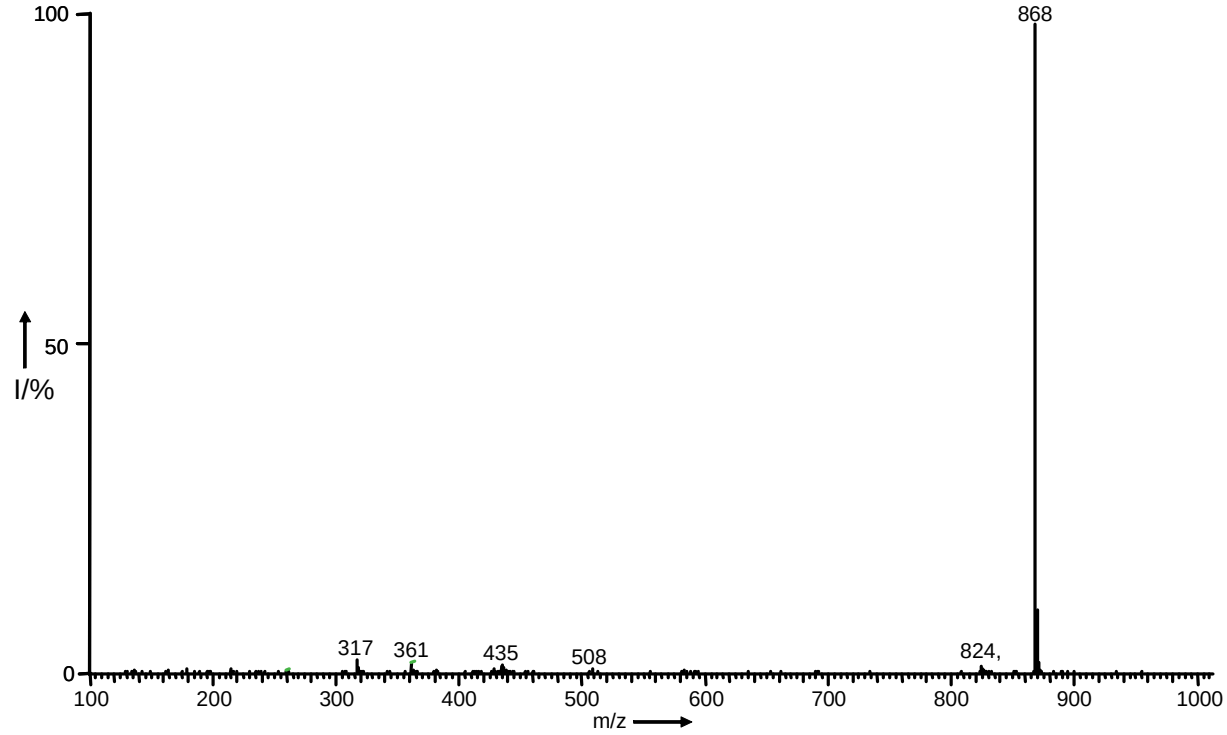
MS/MS 4-Hydroxyphenylacetyl-CoA (5)



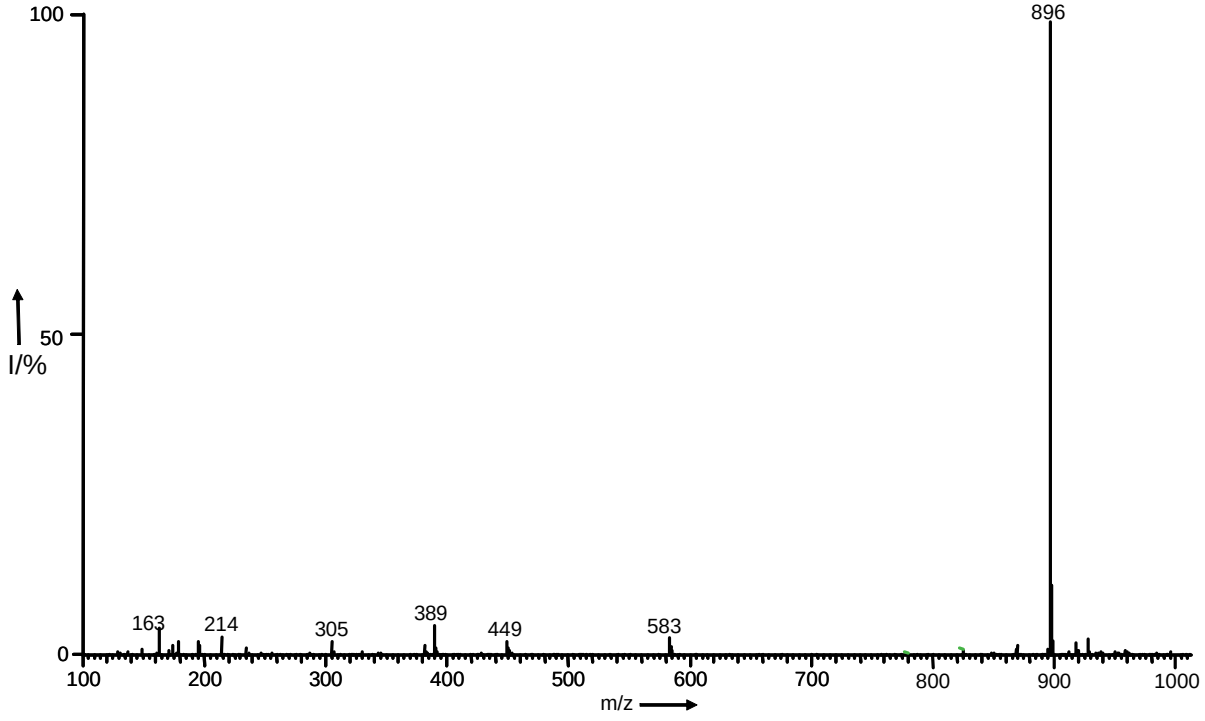
4-Hydroxyphenylacetyl-CoA (5)



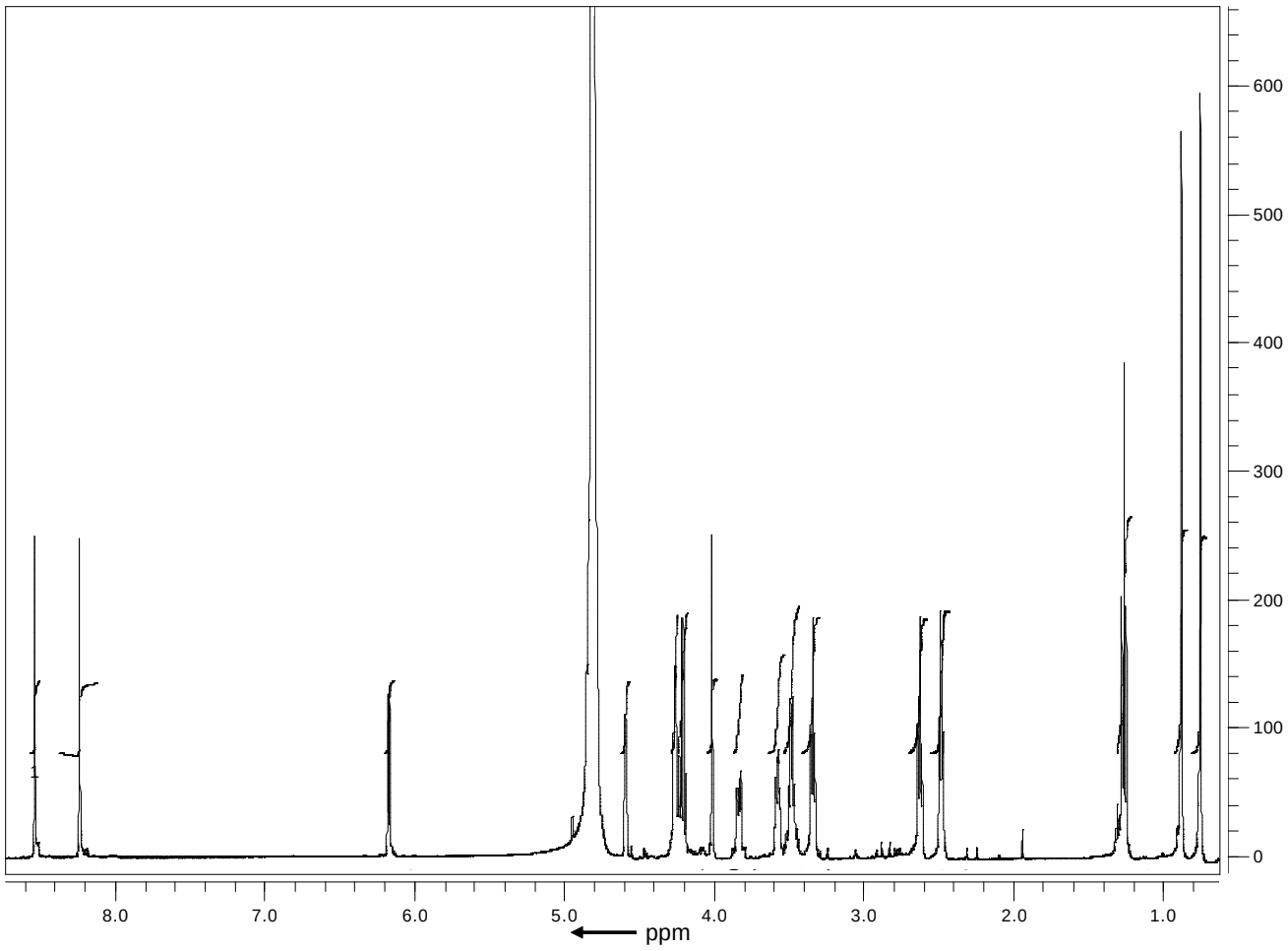
ESI-MS Malonyl-CoA-analogue 3



ESI-MS Ethylmalonyl-CoA-analogue 2



^{1}H-NMR (500 MHz, D_2O) ethylmalonyl-CoA-analogue 2



References

[1] L. A. Paige, G. Q. Zheng, S. A. Defrees, J. M. Cassady, R. L. Geahlen, *J. Med. Chem.* **1989**, *32*, 1665-1667.

[2] W. Boland, C. Frössl, M. Lorenz, *Synthesis* **1991**, 1049-1072.

[3] T. W. Lin, W. A. Bridger, *J. Biol. Chem.* **1992**, *267*, 975-978.

[4] P. M. Jordan, J. B. Spencer, D. L. Corina, *J. Chem. Soc.-Chem. Commun.* **1986**, 911-913.

[5] J. Fliegmann, G. Schröder, S. Schanz, L. Britsch, J. Schröder, *Plant Mol.Biol.* **1992**, *18*, 489-503.