Electronic Supplementary Material (ESI) for Chemical Science

Supporting Information

α,β→β,γ Double Bond Migration in Corallopyronin A Biosynthesis

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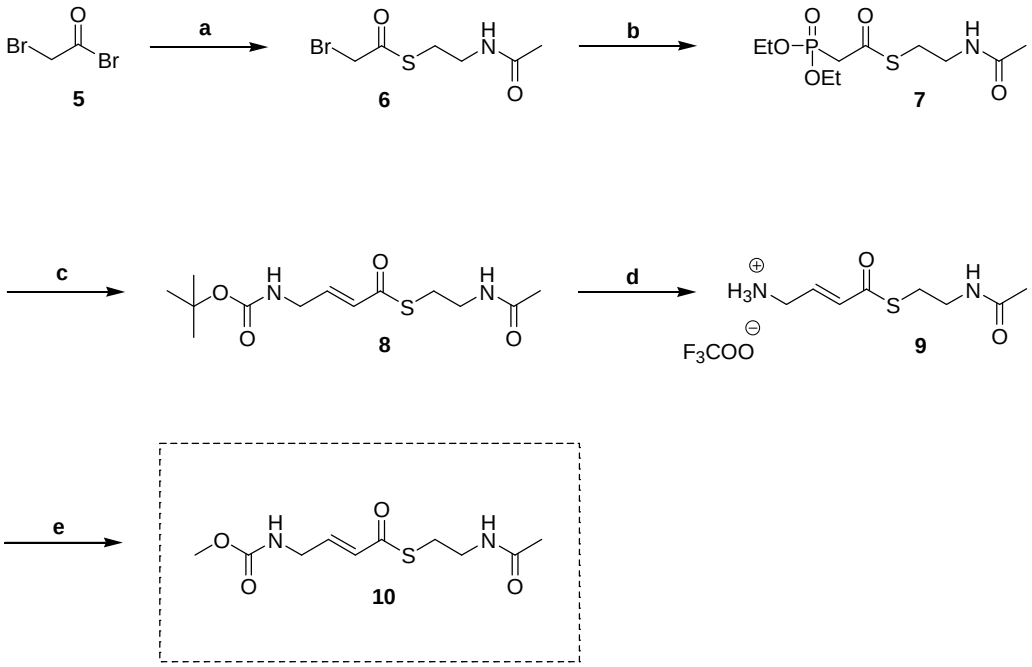
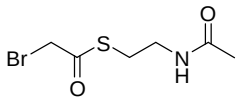
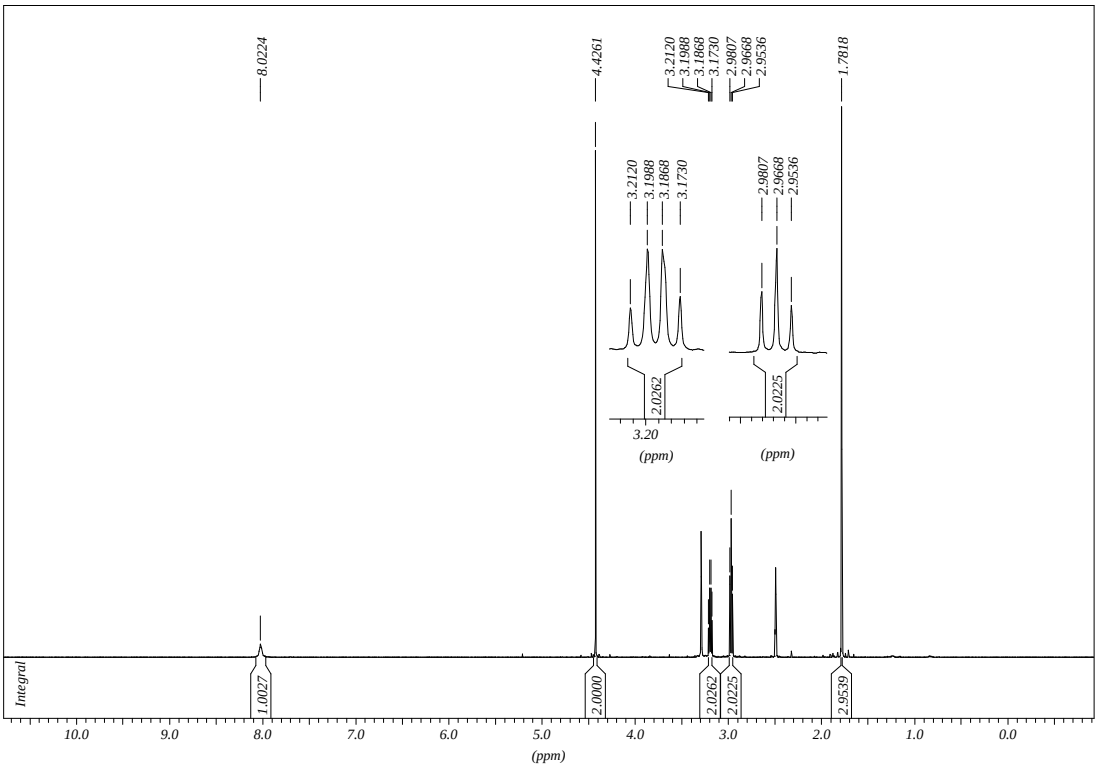


Scheme S1. Synthesis of compound **10** *via* Arbuzov and Horner-Wittig-Emmons reactions. *Reagents and Conditions:* a) Et_3N, *N*-acetylcysteamine, THF, rt; b) triethyl phosphite, THF, 130 °C, sealed tube; c) NaH, Boc-Gly-H, THF, -10°C; d) TFA, CH_2Cl_2, 0°C; e) Et_3N, $ClCO_2Me$, THF, 0°C.

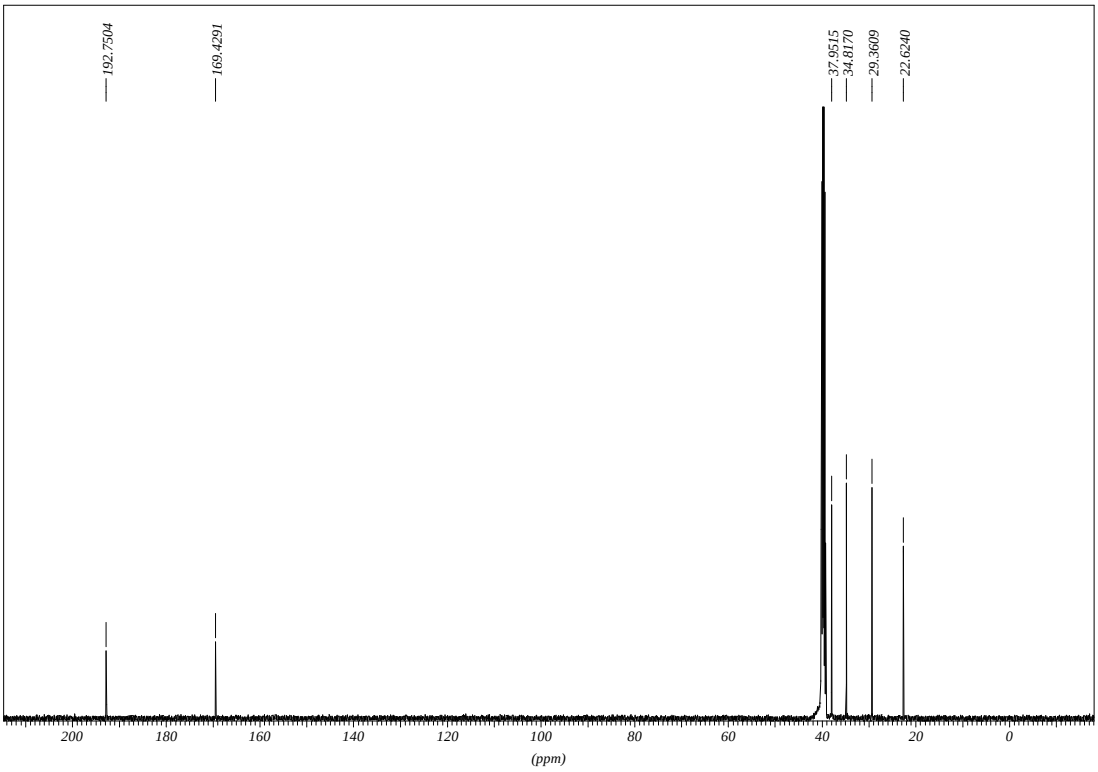
S-2-Acetamidoethyl 2-bromoethanethioate (6)



Compound **6** is known in literature[1] but was generated in a different manner. Bromoacetylbromide (**5**, 1.69 g, 8.37 mmol) was dissolved in THF (20 mL), treated with triethylamine (1.02 g, 10.1 mmol), and *N*-acetylcysteamine (1.00 g, 8.39 mmol) was added dropwise. The resulting reaction mixture was stirred for 1.5 h at room temperature. The solvent was removed and the oily residue was suspended in H_2O. The aqueous suspension was extracted with ethyl acetate (3 × 30 mL), washed with 10% $KHSO_4$ (30 mL), H_2O (30 mL), sat. $NaHCO_3$ (30 mL), and sat. NaCl (30 mL), and dried over Na_2SO_4. The crude product was purified by column chromatography using ethyl acetate as eluent to obtain **6** as a white solid (0.45 g, 22%). NMR data are in accordance with those from literature.[1] ^{1}H NMR (500 MHz, [D_6]DMSO) δ 1.78 (s, 3H, NHCOCH_3), 2.97 (t, ^3J = 6.8 Hz, 2H, SCH_2CH$_2$NH), 3.19 (app. q, 2H, SCH$_2$CH_2NH), 4.43 (s, 2H, BrCH_2CO), 8.02 (bs, 1H, SCH$_2$CH$_2$N*H*); ^{13}C NMR (125 MHz, [D_6]DMSO) δ 22.62 (NHCO*C*H$_3$), 29.36, (S*C*H$_2$CH$_2$NH), 34.82 (SCH$_2$*C*H$_2$NH), 37.95 (Br*C*H$_2$CO), 169.43 (NH*C*OCH$_3$), 192.75 (BrCH$_2$*C*O).

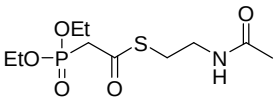


Supplemental figure 1. Compound **6**, ^{1}H NMR (500 MHz, [D_6]DMSO)

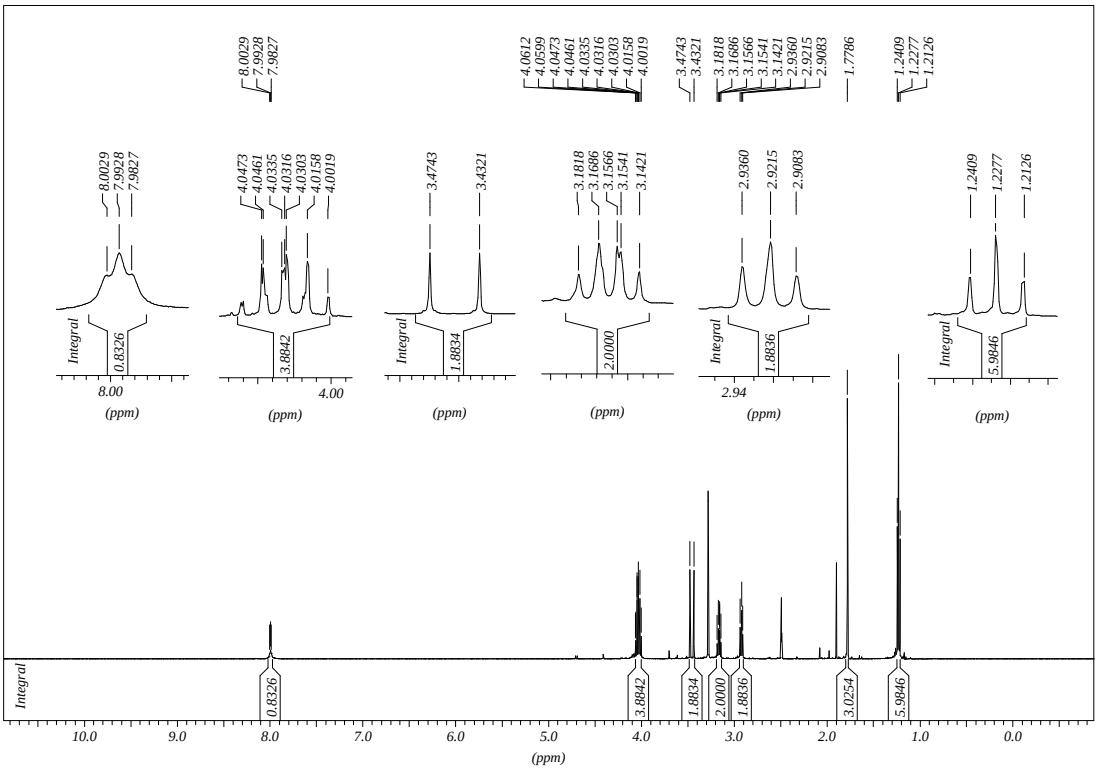


Supplemental figure 2. Compound **6**, ^{13}C NMR (125 MHz, [D_6]DMSO)

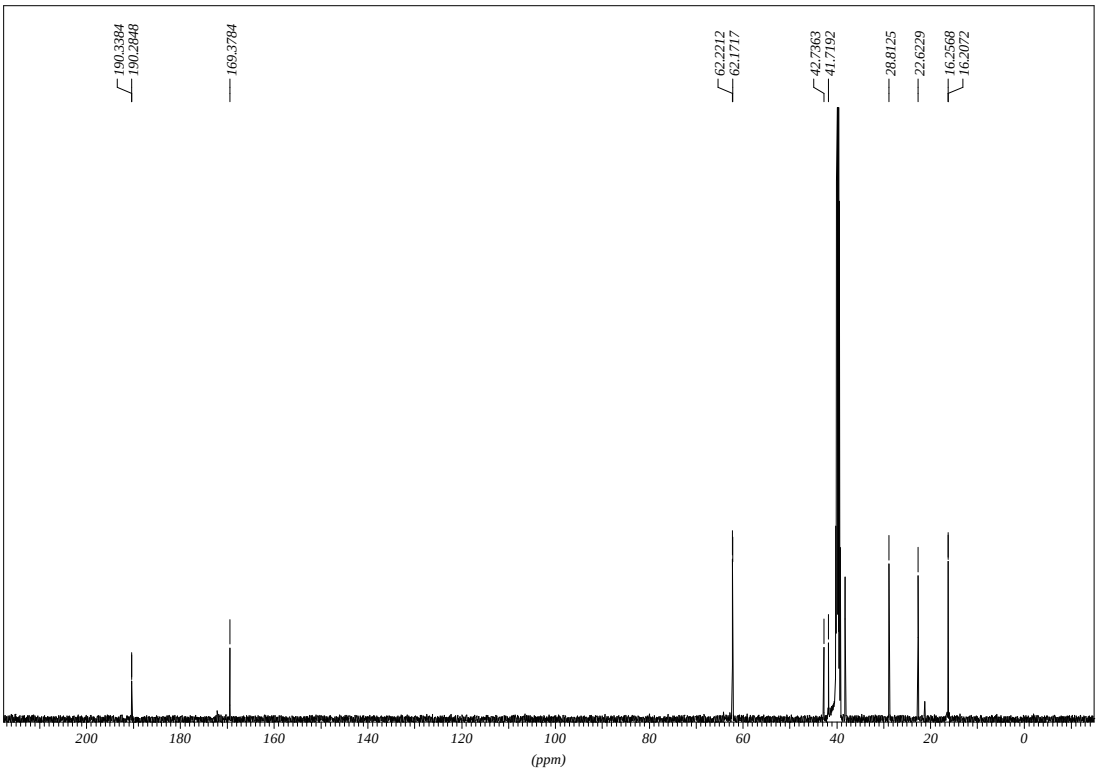
_S_-2-Acetamidoethyl 2-(diethoxyphosphoryl)ethanethioate (7)



Compound **6** (0.43 g, 1.79 mmol) was solved in THF, treated with triethyl phosphite (0.30 g, 1.81 mmol) and stirred at 130 °C in a sealed tube for 3.5 h. The reaction mixture was dissolved in ethyl acetate (60 mL), washed with sat. $NaHCO_3$ (2 × 30 mL) and brine (30 mL) and dried over Na_2SO_4. The crude oily product was purified on column chromatography using ethyl acetate (10 fractions, the volume of each fraction was 50 mL) and additionally ethyl acetate/MeOH (7:3) as eluents to obtain **7** as an oily product (0.16 g, 30%). NMR spectroscopic observations are in agreement with those reported in literature.[2] ^{1}H NMR (500 MHz, [D$_6$]DMSO) δ 1.23 (t, ^3J = 7.1 Hz, 6H, 2 × OCH$_2$C*H*$_3$), 1.78 (s, 3H, NHCOC*H*$_3$), 2.92 (t, ^3J = 6.9 Hz, 2H, SC*H*$_2$CH$_2$NH), 3.14–3.18 (m, 2H, SCH$_2$C*H*$_2$NH), 3.45 (d, $^2J_{PH}$ = 21.1 Hz, 2H, PC*H*$_2$CO), 4.00–4.05 (m, 4H, 2 × OC*H*$_2$CH$_3$), 7.99 (t, ^3J = 5.1 Hz, 1H, N*H*COCH$_3$); ^{13}C NMR (125 MHz, [D$_6$]DMSO) δ 16.21, 16.26 (2 × OCH$_2$*C*H$_3$), 22.62 (NHCO*C*H$_3$), 28.81 (S*C*H$_2$CH$_2$NH), 38.17 (SCH$_2$*C*H$_2$NH), 42.23 (d, $^1J_{PC}$ = 127 Hz, P*C*H$_2$CO), 62.17, 62.22 (2 × O*C*H$_2$CH$_3$) 169.38 (NH*C*OC*H*$_3$), 190.31 (d, $^2J_{PC}$ = 6.7 Hz, PCH$_2$*C*O).

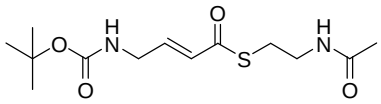


Supplemental figure 3. Compound **7**, ^{1}H NMR (500 MHz, [D_6]DMSO)

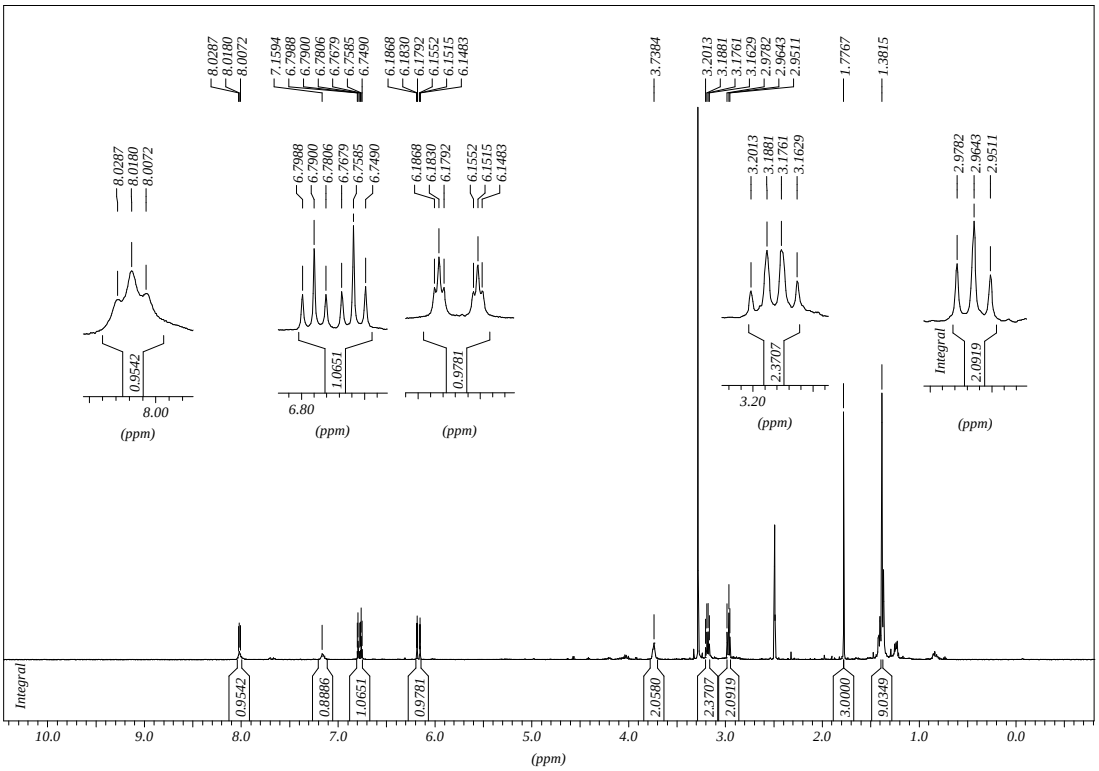


Supplemental figure 4. Compound **7**, ^{13}C NMR (125 MHz, [D_6]DMSO)

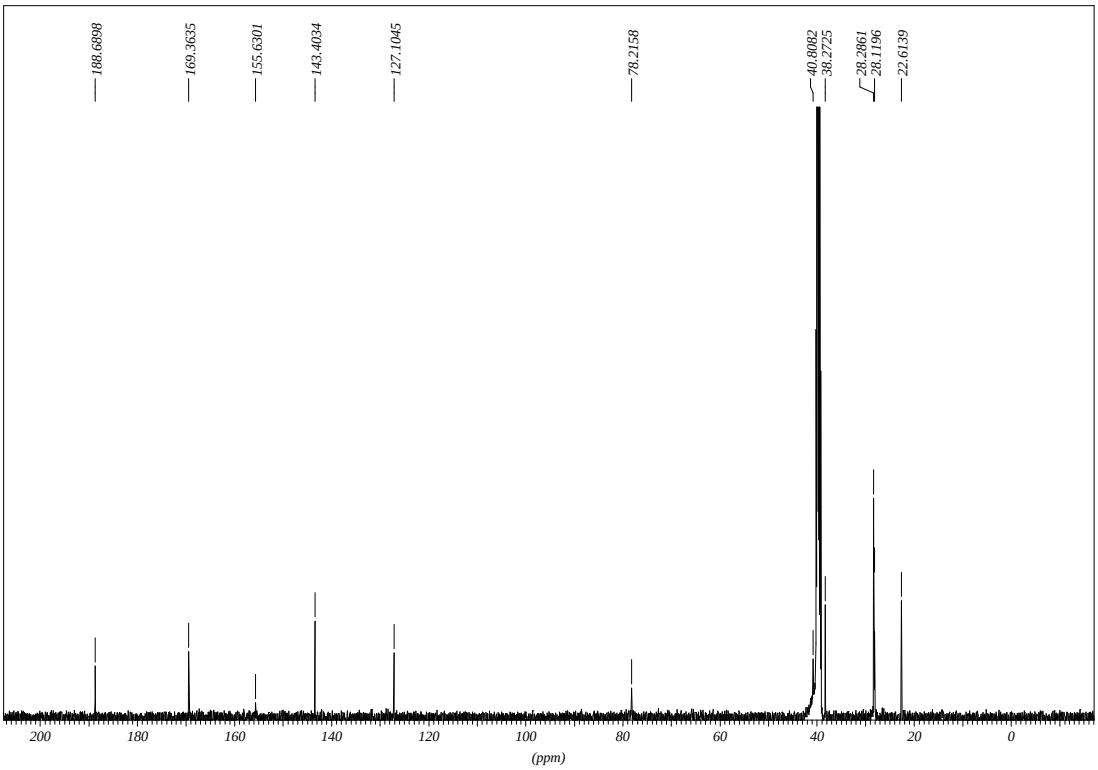
(*E*)-*S*-2-Acetamidoethyl 4-(*tert*-butyloxycarbonylamino)but-2-enethioate (8)



Compound **7** (0.13 g, 0.44 mmol) was dissolved in THF (20 mL). Sodium hydride (18 mg (60% in mineral oil), 0.45 mmol) was added, and the resulting reaction mixture was stirred at -10 °C for 30 min. *N*-(*tert*-Butyloxycarbonyl)glycinal (70 mg, 0.44 mmol) was added, and it was stirred for 1 h at -10 °C. TLC was used for reaction control. THF was removed, and the resulting residue was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine (30 mL) and concentrated *in vacuo*. The precipitated white solid was filtered off. The crude oily product was purified by column chromatography using ethyl acetate as eluent to obtain **8** as an oily product (40.0 mg, 30%). ^{1}H NMR (500 MHz, [D$_6$]DMSO) δ 1.38 (s, 9H, C(CH$_3$)$_3$), 1.78 (s, 3H, NHCOC*H*$_3$), 2.96 (t, ^{3}J = 6.8 Hz, 2H, SC*H*$_2$CH$_2$NH), 3.18 (app. q, ^{3}J = 6.0 Hz 2H, SCH$_2$C*H*$_2$NH) 3.74 (bs, 2H, NHC*H*$_2$CH=CH), 6.17 (dt, ^{3}J = 15.7 Hz, ^{4}J = 1.8 Hz, 1H, NHCH$_2$CH=C*H*), 6.77 (dt, ^{3}J = 15.7 Hz, ^{3}J = 4.6 Hz, 1H, NHCH$_2$C*H*=CH), 7.16 (bs, 1H, N*H*CH$_2$CH=CH), 8.02 (t, ^{3}J = 5.4 Hz, 1H, SCH$_2$CH$_2$N*H*); ^{13}C NMR (125 MHz, [D$_6$]DMSO) δ 22.61 (NHCO*C*H$_3$), 28.12 (S*C*H$_2$CH$_2$NH), 28.29 (C(*C*H$_3$)$_3$), 38.27 (SCH$_2$*C*H$_2$NH), 40.81 (NH*C*H$_2$CH=CH), 78.22 (*C*(CH$_3$)$_3$), 127.10 (NHCH$_2$CH=*C*H), 143.40 (NHCH$_2$*C*H=CH), 155.63 (OCONH), 169.36 (NH*C*OCH$_3$), 188.69 (CH*C*OS).

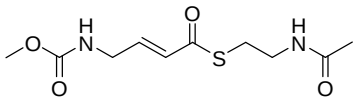


Supplemental figure 5. Compound **8**, ^{1}H NMR (500 MHz, [D$_6$]DMSO)

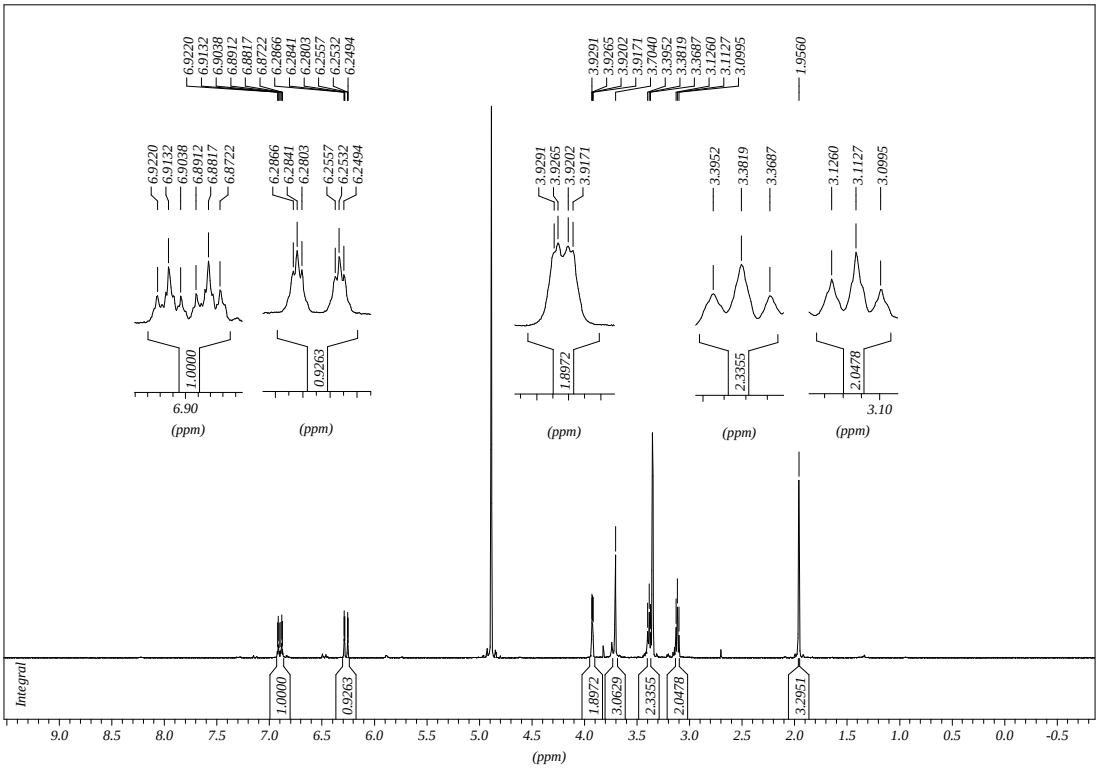


Supplemental figure 6. Compound **8**, ^{13}C NMR (125 MHz, [D$_6$]DMSO)

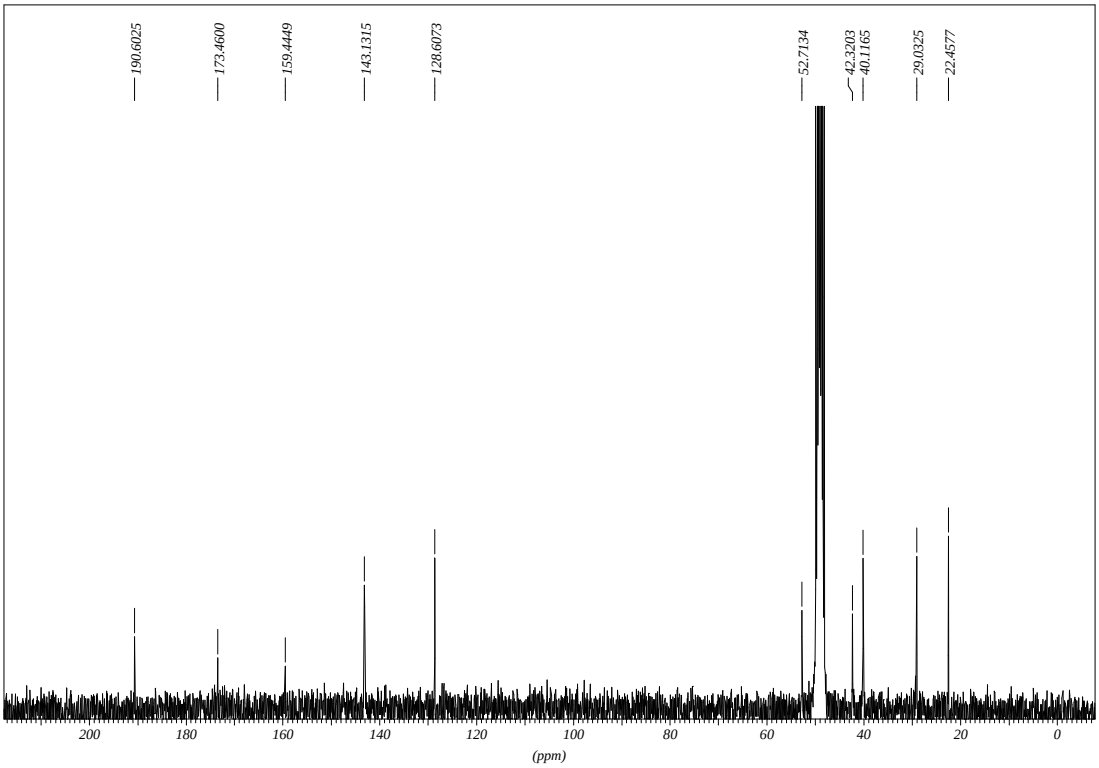
(*E*)-*S*-2-Acetamidoethyl 4-(methoxycarbonylamino)but-2-enethioate (10)



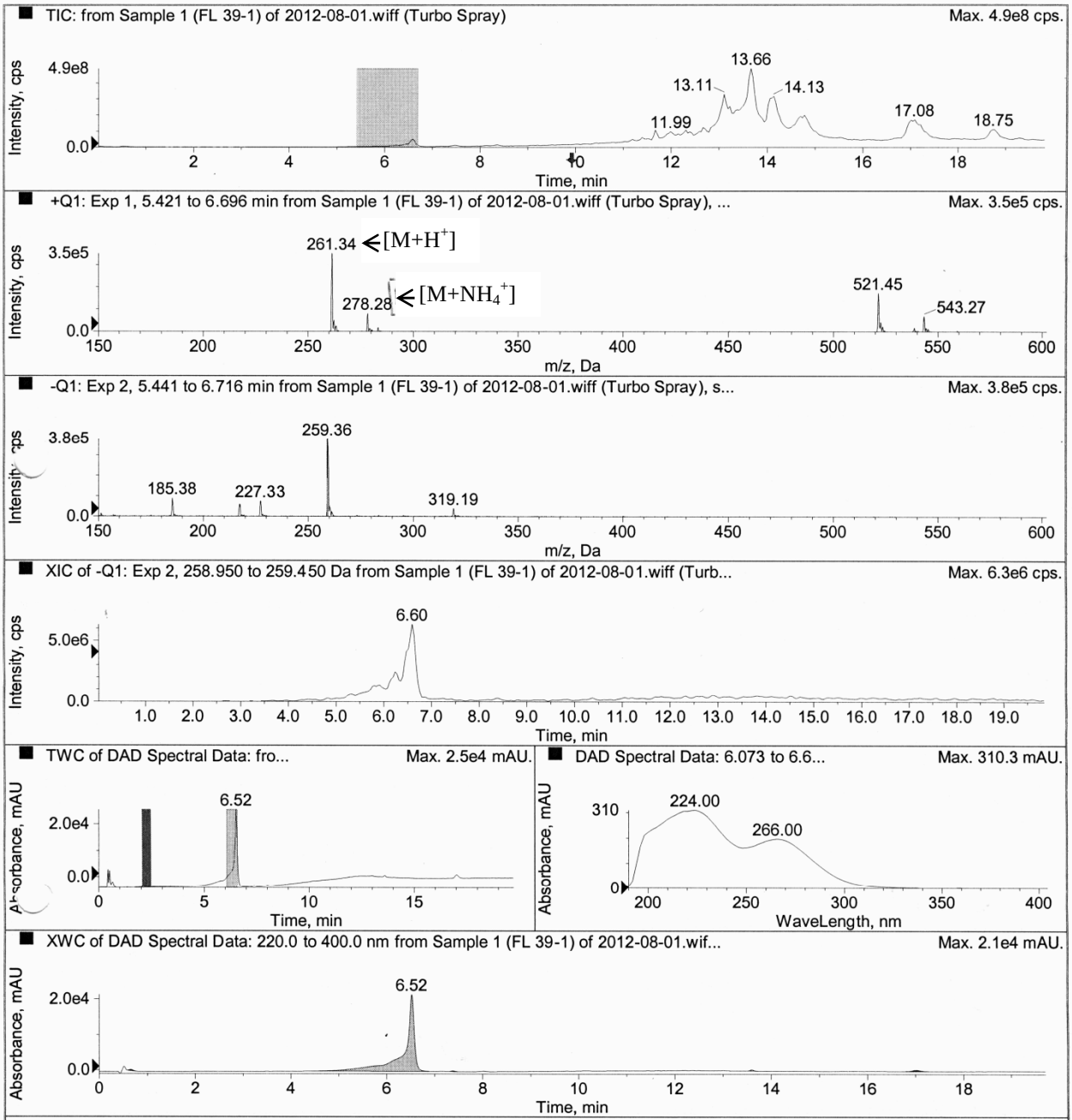
Compound **8** (30 mg, 0.099 mmol) was dissolved in CH_2Cl_2 (30 mL), treated with TFA (5.0 mL) and stirred 30 min at 0 °C. The solvent was evaporated under reduced pressure and the oily residue (compound **9**) was dissolved in 20 mL dry THF. TEA (40 mg, 0.40 mmol) and methyl chloroformate (37 mg, 0.39 mmol) were added, and it was stirred for 2 h at 0 °C. THF was removed. The oily residue was treated with H_2O and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine (30 mL) and concentrated *in vacuo*. The crude oily product was purified by column chromatography using ethyl acetate as eluent to obtain **10** as an oily product (15 mg, 58% from **8**). ^{1}H NMR (500 MHz, [D$_4$]MeOH) δ 1.96 (s, 3H, NHCOC*H*$_3$), 3.11 (t, ^3J = 6.6 Hz, 2H, SC*H*$_2$CH$_2$NH), 3.38 (t, ^3J = 6.6 Hz, 2H, SCH$_2$C*H*$_2$NH), 3.70 (s, 3H, C*H*$_3$OCONH), 3.92-3.93 (m, 2H, NHC*H*$_2$CH=CH), 6.27 (dt, ^3J = 15.5 Hz, ^4J = 1.6 Hz, 1H, NHCH$_2$CH=C*H*), 6.90 (dt, ^3J = 15.5 Hz, ^3J = 4.7 Hz, 1H, NHCH$_2$C*H*=CH); ^{13}C NMR (75.4 MHz, [D$_4$]MeOH) δ 22.46 (NHCO*C*H$_3$), 29.03 (S*C*H$_2$CH$_2$NH) 40.12 (SCH$_2$*C*H$_2$NH), 42.32 (NH*C*H$_2$CH=CH), 52.71 (*C*H$_3$OCONH), 128.61 (NHCH$_2$CH=*C*H), 143.13 (NHCH$_2$*C*H=CH), 159.44 (OCONH), 173.46 (NH*C*OCH$_3$), 190.60 (CH*C*OS). LC-MS(ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220.0–400.0 nm) t_r = 6.52, 97% purity, *m/z* = 261.34 ([M + H]$^+$).



Supplemental figure 7. Compound **10**, ^{1}H NMR (500 MHz, [D$_4$]MeOH)



Supplemental figure 8. Compound **10**, ^{13}C NMR (75.4 MHz, [D$_4$]MeOH)



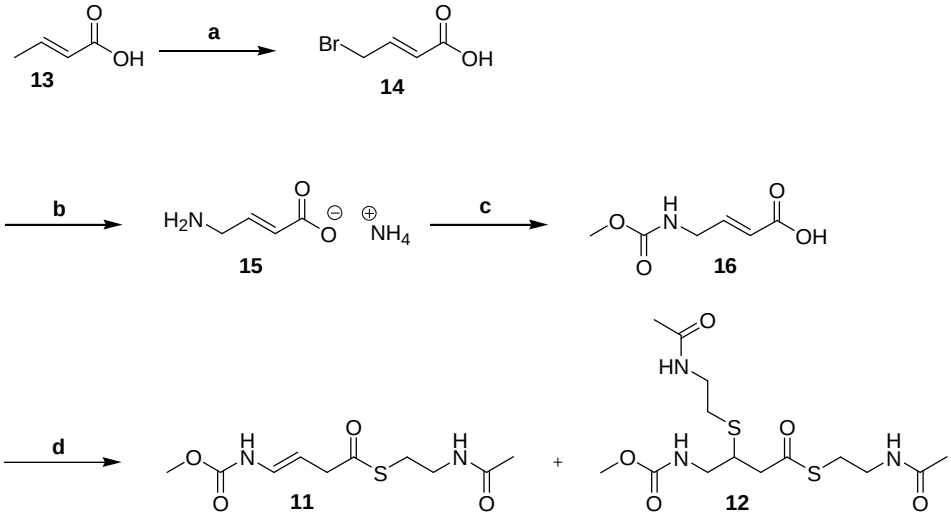
Peak List for "XWC of DAD Spectral Data: 220.0 to 400.0 nm from Sample 1 (FL 39-1) of 2012-08-01.wiff"

	Time (min)	Area (mAU x min)	% Area	Height (mAU)	% Height	Width (min)	Baseline Type
1	0.6529	1840.7338	0.5601	337.3111	1.4675	0.2000	Base to Base
2	6.5219	3.1905e5	97.0834	2.1673e4	94.2949	2.5067	Base to Base
3	7.3776	1260.4707	0.3835	211.5760	0.9205	0.2600	Base to Base
4	13.5914	1880.7158	0.5723	364.2257	1.5846	0.2733	Base to Base
5	17.0138	4603.0664	1.4007	398.1761	1.7324	0.5667	Base to Base

LC Pump Device: Agilent 1100 binary pump

Supplemental figure 9. Compound **10**, LC-MS(ESI) (LC Luna® 3µm C18(2); linear gradient from 90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220.0–400.0 nm).

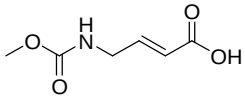
An alternative approach for the synthesis of compound **10** yielded several by-products. Two of them (*i.e.* **11** and **12**) were isolated and their structures were elucidated. Compound **12** represents the corresponding Michael adduct, and **11** the isomer of **10** with the double bond in β,γ position (Supplemental figure 12 and 13: ^{1}H NMR, and ^{1}H/^{1}H COSY 2D–NMR of **11**, respectively).



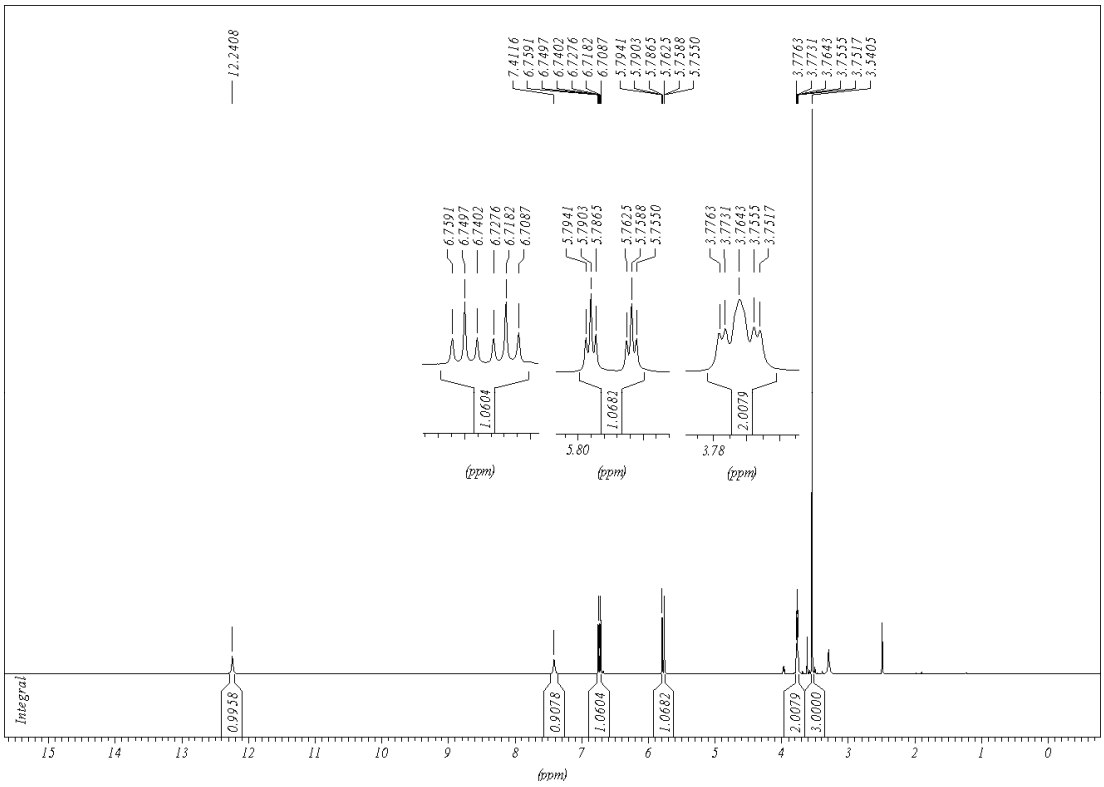
Scheme S2. Alternative approach for the synthesis of **10**, yielding products **11** and **12**

Reagents and Conditions: a) NBS, AIBN, CCl_4, 95°C; b) 25% aq. NH_3, rt; c) $ClCO_2Me$, dioxane/H_2O, $NaHCO_3$, rt; d) EDC, DMAP, *N*-acetylcysteamine, DCM, rt.

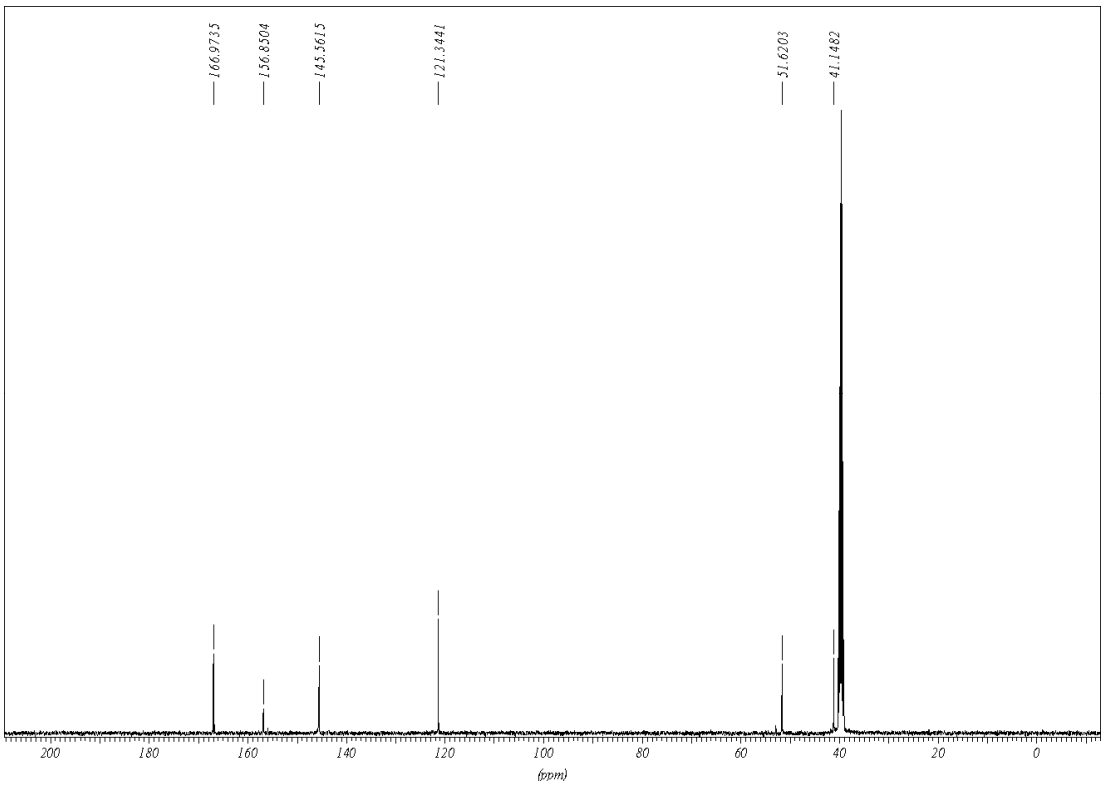
(*E*)-4-Methoxycarbonylaminobut 2-enoic acid (16)[3,4]



For the first step, crotonic acid (**13**) (8.60 g, 100 mmol) was dissolved in CCl_4 (200 mL). Subsequently, *N*-bromosuccinimide (21.4 g, 120 mmol) and AIBN (500 mg, 3.05 mmol) were added and heated under reflux to 95-100 °C for 2 h. After the mixture was cooled down to rt, the solvent was removed and the resulting solid was recrystallized from ethyl acetate/petroleum ether to give (*E*)-4-bromobut-2-enoic acid (**14)** (7.10 g, 43%). Analytical data of **14** were in agreement with those reported in the literature.[3] Secondly, compound **14** (3.20 g, 19.4 mmol) was treated dropwise with 25% aq. NH_3 (15 mL) and the reaction mixture was stirred for 20 h at rt. The solvent and excess ammonia were removed under reduced pressure to obtain a brown solid which was subsequent dissolved in water and passed through a column of acidic resin (Dowex 50W-X8). The column was washed with water and compound **15** was eluted with 5% NH_3. The solution was concentrated and poured into ethanol. The resulting precipitate was filtered off and dried to yield **15** (0.25 g, 11%). Analytical data were in agreement with those reported in the literature.[4] Finally, compound **15** (210 mg, 1.78 mmol) was dissolved in water containing $NaHCO_3$ (0.30 g, 3.57 mmol) to convert it into the corresponding free acid. Thereafter the solvent and remaining NH_3 were evaporated. The residue was dissolved in a dioxane/H_2O mixture (2:1) and treated with methyl chloroformate (0.17 g, 1.8 mmol). After evaporation of the solvent, the residue was suspended in H_2O and the aqueous suspension was adjusted with 10% $KHSO_4$ to pH ~2 and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were concentrated *in vacuo*, the resulting residue was adjusted to pH ~9 using $NaHCO_3$ and again extracted with ethyl acetate (3 × 30 mL) to remove by-products. The aqueous phase was acidified under vigorous stirring with 37% HCl and extracted with ethyl acetate (3 x 30 mL). The combined organic layers were washed with brine. The solvent was removed to obtain compound **16** (200 mg, 71%). ^{1}H NMR (500 MHz, [D_6]DMSO) δ 3.54 (s, 3H, *CH*$_3$OCONH), 3.75–3.78 (m, 2H, NHCH_2CH=CH), 5.77 (dt, ^3J = 15.8 Hz, ^4J = 1.9 Hz, 1H, NHCH$_2$CH=C*H*), 6.73 (dt, ^3J = 15.8 Hz, ^3J = 4.7 Hz, 1H, NHCH$_2$*C*H=CH), 7.41 (bs, 1H, N*H*CH$_2$CH=CH), 12.24 (bs, 1H, COOH); ^{13}C NMR (125 MHz, [D_6]DMSO) δ 41.15 (NH*C*H$_2$CH=CH), 51.62 (*C*H$_3$OCONH), 121.34 (NHCH$_2$CH=*C*H), 145.56 (NHCH$_2$*C*H=CH), 156.85 (OCONH), 166.97 (COOH).

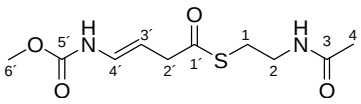


Supplemental figure 10. Compound **16**, ^{1}H NMR (500 MHz, [D_6]DMSO)

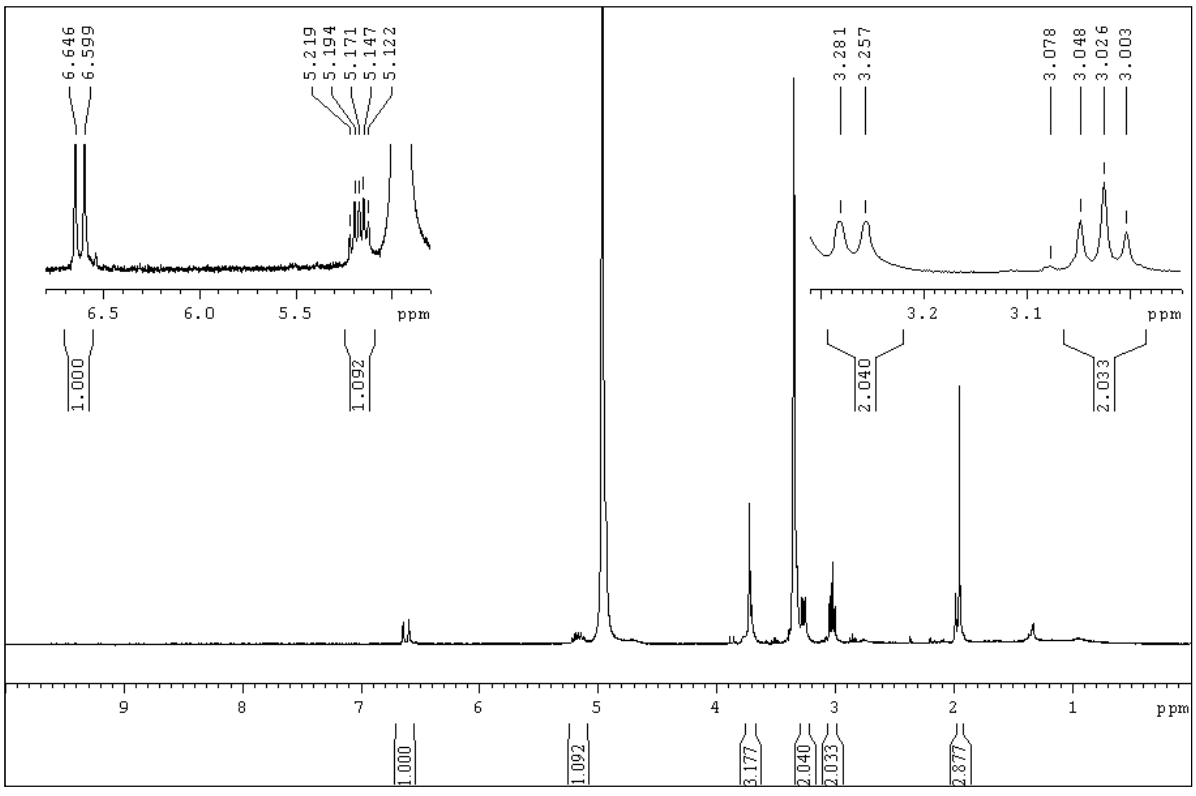


Supplemental figure 11. Compound **16**, ^{13}C NMR (125 MHz, [D_6]DMSO)

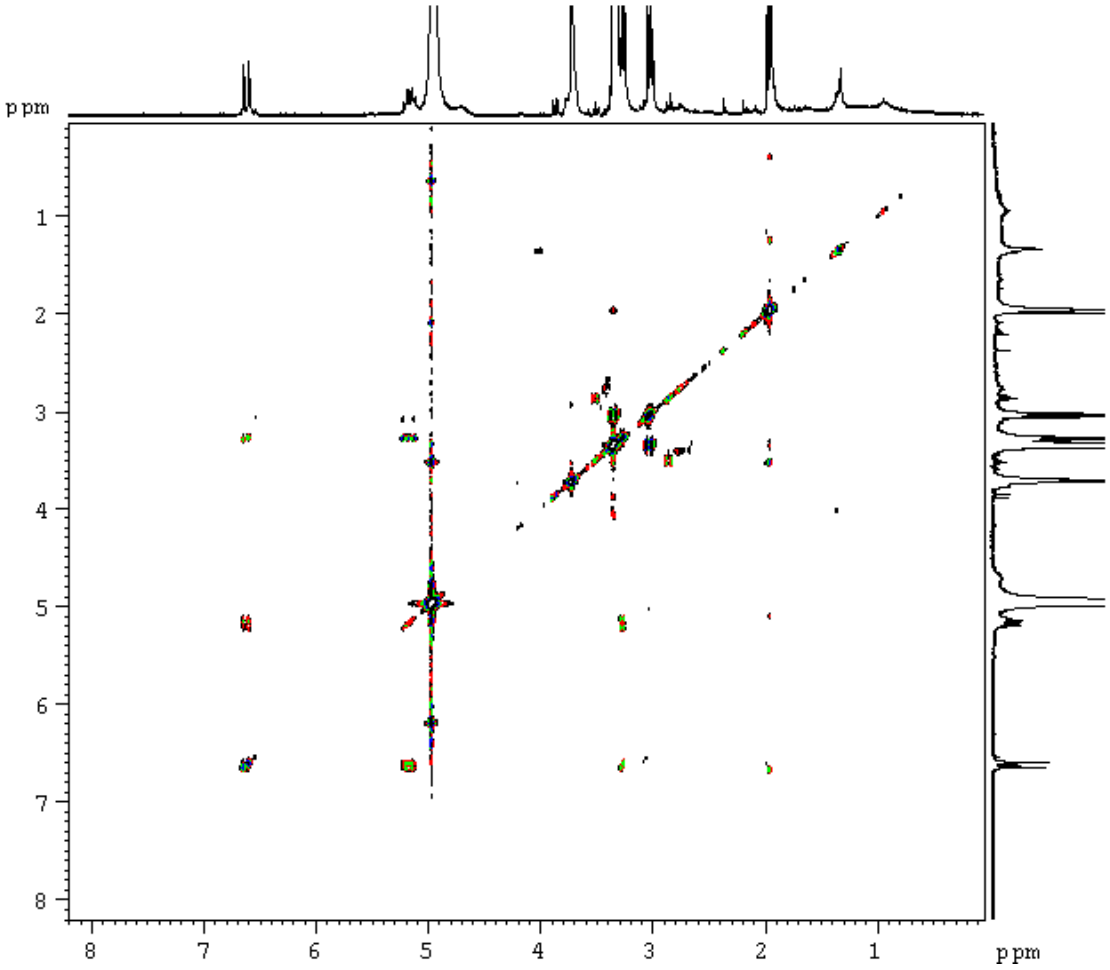
(*E*)-*S*-2-Acetamidoethyl 4-(methoxycarbonylamino)but-3-enethioate (11)



Compound **16** (210 mg, 1.32 mmol) was dissolved in DCM (15 mL) and subsequently treated with DMAP (16 mg, 0.13 mmol) and EDC (230 mg, 1.45 mmol). *N*-acetylcysteamine (160 mg, 1.34 mmol) was dissolved in DCM and added dropwise under ice cooling to the reaction mixture. It was allowed to warm up to rt and stirred over 2 h. After evaporation of the solvent, the residue was suspended in H_2O and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with 10% $KHSO_4$ (10 mL), H_2O (10 mL), sat. $NaHCO_3$ (10 mL), H_2O (10 mL), and brine. The solvent was dried ($NaSO_4$) and removed *in vacuo*. The oily residue was purified by column chromatography on silica gel using ethyl acetate as eluent to obtain **11** (6 mg, 1.7%) and **12** (data not shown). ^{1}H NMR of **11** (300 MHz, [D$_4$]MeOH) δ 1.95 (s, 3H, NHCOC*H*$_3$), 3.03 (t, ^3J = 6.6 Hz, 2H, SC*H*$_2$CH$_2$NH), 3.27 (d, ^3J = 7.3 Hz, 2H, NHCH=CHC*H*$_2$), 3.35 (t, ^3J = 6.6 Hz, 2H, SCH$_2$C*H*$_2$NH), 3.73 (s, 1H, C*H*$_3$OCONH), 5.17 (dt, ^3J = 14.3 Hz, ^3J = 7.3 Hz, 1H, NHCH=C*H*CH$_2$), 6.62 (d, ^3J = 14.3 Hz, 1H, NHC*H*=CHCH$_2$).



Supplemental figure 12. Compound **11**, ^{1}H NMR (300 MHz, [D$_4$]MeOH).



Supplemental figure 13. Compound **11**, $^{1}H/^{1}H$ COSY 2D-NMR (300 MHz, [D_4]MeOH).

Table S1. 1D and 2D-NMR spectroscopic data for compound **11**. [a] [D_4]MeOH, 300 MHz

position	δ_H[a] (*J* in Hz)	COSY[a]
1	3.03 (2H, t, 6.6)	2
2	3.35 (2H, t, 6.6)	1
4	1.95 (3H, s)	
2´	3.27 (2H, d, 7.3)	3´, 4´
3´	5.17 (1H, dt, 14.3, 7.3)	4´, 2´
4´	6.62 (1H, d, 14.3)	3´, 2´
6´	3.73 (3H, s)	

MS^n experiments with the synthesized substrate (*E*)-*S*-2-acetamidoethyl 4-(methoxycarbonylamino)but-2-enethioate (10).

Using a Thermo LTQ Orbitrap Velo coupled with an Advion TriVersaNanoMate enabled a continous electron spray for the MS^n experiments. For the analysis of **10** the parent ion ($M+H^+$) was isolated (window range: 1) and fragmented by applying normalized collision energy between 32 V and 50 V. Fragmentation of the pure compound **10** resulted in the following ions ($M+H^+$):

***m/z* 261.092→ 142.050 → (66.034 and) 110.024 → 66.034**

During the mass spectral measurement of **10**, first the SNAC-part was cleaved, revealing an ion (*m/z* 142.050) which is identical to that of the phosphopantetheine (PPant) bound substrate. This ion ($M+H^+$) *m/z* = 142.050 served as signature ion for the analysis of the substrate bound to the PPant arm of the *holo*-proteins. In addition, it was the parent ion for a further round of fragmentation yielding an ion at *m/z* = 110.024. In the assays performed, the mass of these ions (*m/z* 142.050 to 145.069 and *m/z* 110.024 to 113.043, singly charged) increased in the same way as the mass of the ejection ions (*m/z* 402.169 to 405.188). In contrast, the *m/z* of the PPant part of the ejection ions stayed constant at *m/z* of 261.126.

Bacterial strains, plasmids and culture conditions.

E. coli XL1 Blue was used for plasmid construction. For protein expression experiments either *E. coli* Bap-1, or *E. coli* BL21 were used. Cultivation medium was LB (tryptone 10 g, yeast extract, 5 g, NaCl 10 g, filled with H_2O to 1 L; for solid medium 1.6 % agar was added) supplemented with 60 µg/mL kanamycin for selection and to maintain the constructs.

DNA manipulation.

Either genomic DNA or cosmid DNA served as template for the amplification of DNA-fragments. The generation of these is described in Erol *et al*., 2010.[5] In order to obtain the expression construct for the DH*-ACP didomain a segment harbouring the start of corJ was amplified with the primer pair CorJ_start (5'-TA*GAATTC*ATGACCGTGGAGTCCGACAAGG-3') / corJ_end_ACP (5'-AT*AAGCTT*TAATGCGGGAGGGAGGGCGCGAA-3'), yielding a fragment of 1227 bp. The restriction sites introduced are highlighted in italic. This fragment was ligated into the cloning vector pGEM-T (Promega) and checked for identity by sequencing with standard primers (SP6 and T7). From this plasmid the desired fragment was cleaved out by using the restriction sites *Eco*RI/*Hin*dIII, which were introduced by the primers used. The fragment was ligated in the likewise restricted expression vector pET28a. After ligation *E. coli* XL1 Blue cells were transformed with the ligation mixture and plated on LB supplemented with kanamycin for selection. After identifying positive clones by plasmid

isolation and test restriction the corresponding plasmid was isolated and transferred into the expression host.

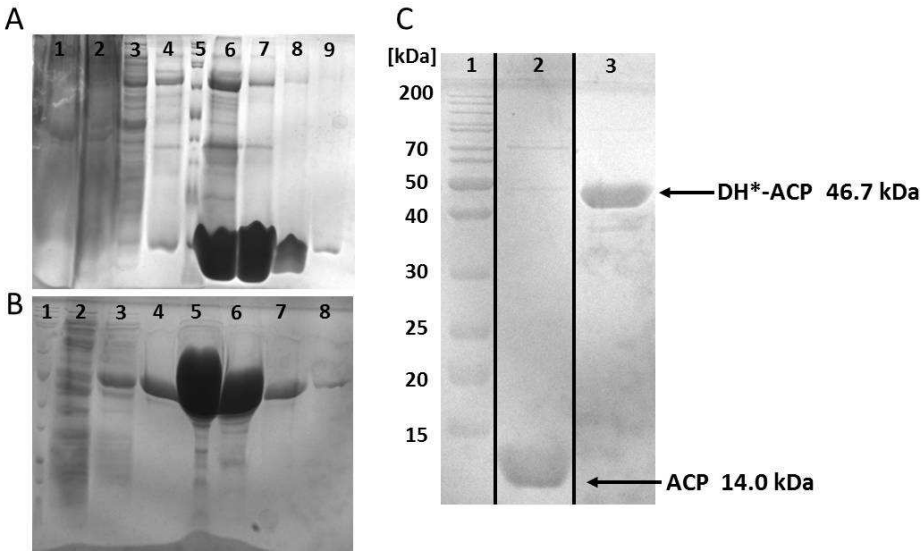
In the same way the construct for the expression of the sole ACP domain was done. The DNA-fragment harboured the sequence coding for the first ACP domain on CorJ. The PCR reaction with the primer pair ACP1_upstream (5'- TA*GAATTC*CCGGTTGCACCGCTCTC -3') / corJ_end_ACP (5'-AT*AAGCTT*TAATGCGGGAGGGAGGGCGCGAA-3'), yielded a fragment of 309 bp. This fragment was ligated into the cloning vector pGEM-T (Promega) and checked for identity by sequencing with standard primers (SP6 and T7). From this plasmid the fragment was cleaved out by also using the restriction sites *Eco*RI/*Hin*dIII, which were introduced by the primers used. The fragment was ligated in the pET28a (*Eco*RI/*Hin*dIII). After ligation *E. coli* XL1 Blue cells were transformed with the ligation mixture and plated on LB supplemented with kanamycin. After identifying positive clones by plasmid isolation and test restriction the corresponding plasmid was isolated and transferred into the expression host.

Amino acid exchange in the DH domain*

To introduce the envisaged point mutations into the enzymes a two-step PCR was applied. Two primers were designed carrying the point mutation. In a first round the point mutation was introduced into the two amplified fragments using (i) the forward primer CorJ_start and the reverse primer carrying the sequence coding for the amino acid exchange, and (ii) the forward primer carrying the sequence coding for the amino acid exchange and the reverse primer corJ_end_ACP. The two fragments obtained by the first round of PCRs served as templates for the second PCR round. First 10 cycles were performed without primers in the sample, then the primers CorJ_start and corJ_end_ACP were added, and 20 further cycles were performed. Primers used for the D264N exchange were D264N_for (5'-CCTCCAGCGCTCCTGAACGGCGTCATCGTCG-3') and D264N_rev (5'-CGACGATGACGCCGTTCAGGAGCGCTGGAGG-3'), respectively; primers used for the H47A exchange were H47A_for (5'-CTGAGGGACCACACCGTCCTCGG-3') and H47A_rev (5'-CCGAGGACGGTGGCGTCCCTCAG-3'), respectively. The fragments obtained by the second round of PCR were ligated into pGEM-T vector, checked for identity by sequencing, cleaved out and ligated into the expression vector pET28 as described above for the wild type protein.

Protein expression and assay.

For the expression of the proteins the corresponding *E. coli* strains carrying either pET28+ACP, or pET28+DH*-ACP were inoculated in 9 mL LB_{Kan} liquid medium and incubated over night at 37° C. With this preculture the main culture was inoculated in LB medium (1 L) at 37 °C until an OD_{600} of 0.4–0.6 was reached. Then the cells were cooled down to 16° C and protein expression was induced with IPTG (final concentration 0.05-0.1 mM). Then growth was continued at 16 °C over night. Cells were harvested by centrifugation, resuspended in lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM Imidazol, pH 8.0), and transferred to 50 mL falcon tubes. The cells were then disrupted by sonification (6 cycles à 10 pulses, and cooling on ice between the cycles). After sonification cell debris was removed by centrifugation for 30-45 min at 8500 rpm at 4° C. The protein containing supernatant was transferred onto gravity flow columns (Qiagen, Germany), which were packed before with 1 mL Ni-NTA agarose. The flow through was collected and back-loaded onto the columns 3 more times. Then 2 washing steps followed (washing buffer 1: 50 mM NaH_2PO_4, 300 mM NaCl, 20 mM Imidazol, pH 8.0, washing buffer 2: 50 mM NaH_2PO_4, 300 mM NaCl, 20 mM Imidazol, pH 8.0). Afterwards the bound protein was eluted with 5 × 500 µL elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, pH 8.0, stepwise increasing Imidazole concentration 1 × 100 mM, 1 × 150 mM, 1 × 200 mM, 2 × 300 mM). The fractions containing the targeted protein were combined and loaded onto a spin filter column (Millipore, 10 kDa exclusion size) for desalting. This column was centrifuged at 5000 rpm at 4° C until only one tenth of the starting volume remained. Then it was re-buffered in deuterated buffer (50 mM Tris, pH 8.0), which was prepared beforehand: 50 mM aqueous Tris-solutions (1 mL each sample) were dried in a SpeedVac completely, and were resolved in the same volume of D_2O. The re-buffered protein solution was concentrated again (final volume ca. 250 µL). This protein was used for the following assay.



Supplemental figure 14. A) SDS-PAGE of ACP purification. Lane 1: flow through; 2: wash step 1; 3: wash step 2; 4: elution fraction 1; 5: size ladder (sizes of the fragments are depicted in C); 6: elution fraction 2; 7: elution fraction 3; 8: elution fraction 4; 9: elution fraction 5. B) SDS-PAGE of DH*-ACP purification. Lane 1: size ladder (sizes of the fragments are depicted in C); 2: wash step 1; 3: wash step 2; 4: elution fraction 1; 5: elution fraction 2; 6: elution fraction 3; 7: elution fraction 4; 8: elution fraction 5. C) SDS-PAGE of the purified proteins. Lane 1: size ladder; 2: ACP; 3: DH*-ACP; sizes are depicted.

Mass-based ejection assay

Assay volume was 100 µL: 10 µL protein solution, 2 µL substrate **10** (equal to 0.2 mM), 38 µL buffer (deuterated Tris 50 mM, pH 8.0). The reaction mixture was incubated at room temperature. In the first reaction step the SNAC-activated substrate should bind to the PPant arm of the *holo*-protein. In the case of the DH*-ACP didomain the shift reaction started directly at this time point (substrate addition was set as time point zero). In the control reaction with the single ACP domain no shift domain was present. To stop the reaction it was directly injected into the HPLC column.

NMR-based assay

After purification the protein (DH*-ACP heterologously expressed in *E. coli* BL21; re-buffered in non-deuterated Tris-buffer) was subsequently incubated with substrate **10** for 16 h. The assay was stopped by adding an equal volume of methanol. The protein was pelleted by centrifugation. Supernatant was transferred to a new vial and dried *in vacuo*. Subsequently, the sample was prepared for adjacent NMR experiments by dissolving it in deuterated methanol. The results of the ^{1}H NMR and the ^{1}H/^{1}H COSY 2D-NMR measurement of the reaction product were compared with the corresponding spectra obtained

for compound **10** (*i.e.* the educt) and **11** (*i.e.* the expected product). The ^{1}H NMR spectrum of the reaction product revealed, *e.g.* a resonance signal at δ_H 6.62 corresponding to H-4´ of the β,γ shifted double bond (as seen for compound **11)**, and lacked resonance signals at δ_H 6.27 and 6.90 , corresponding to H-2´ and H-3´ of a α,β double bond as seen for compound **10**. These results evidence a shift of the α,β double bond to the α,γ position due to the activity of the shift domain (DH*-ACP)

HPLC purification of the proteins for mass spectrometric analysis.

The assay mixture was subjected to semipreparative RP-HPLC (C4 reversed phase column: Symmetry 300™, 5 µM, 4.6 mm × 250 mm, Waters), eluent: water/acetonitrile (70/30) to water/acetonitrile (35/65) over 30 min, flow rate: 1.0 mL/min). The retention time of ACP loaded with substrate **10** was 20.2 min. The retention time of DH*-ACP loaded with substrate **10** was 21.0 min. The peaks were collected manually and deep frozen at –80° C. The deep frozen samples were lyophilized.

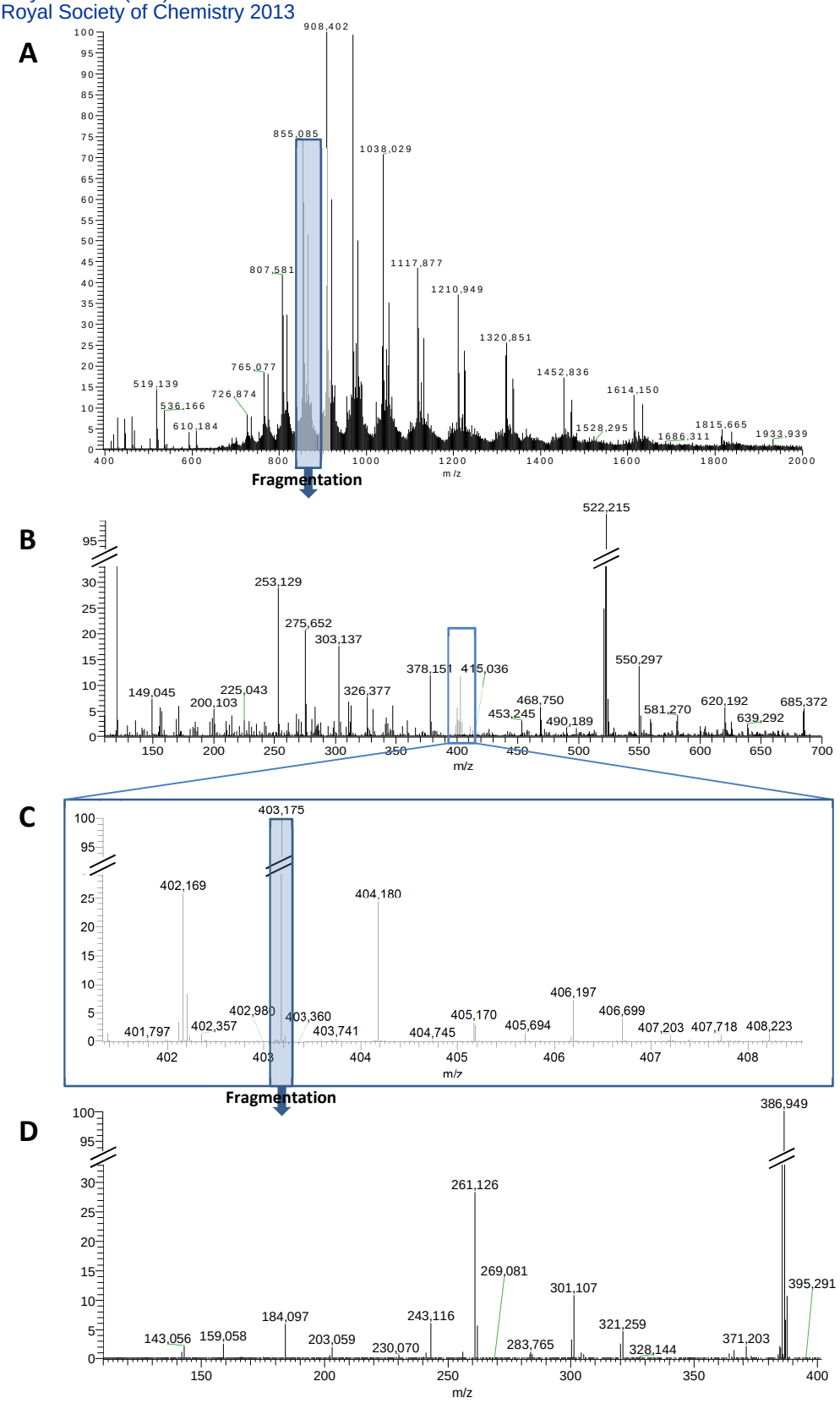
MS^n analysis.

The lyophilized samples were dissolved in an adequate volume of electro spray solution (49.5% H_2O, 49.5% methanol, 1% formic acid). 10 µL samples were loaded onto the 96 well plate of the NanoMate spray robot coupled to the LTQ Orbitrap Velos. A spray chip with 5 µm nozzle diameter was used at a spray voltage of 1.6 kV and 0.3 psi pressure setting. An environmental polysiloxane ion with *m/z* 445.12003 was used as lock mass for internal calibration. Typical mass deviation was <2 ppm. Isolation and fragmentation were performed in the linear ion trap, detection of the final product spectrum was done with the Orbitrap analyzer.

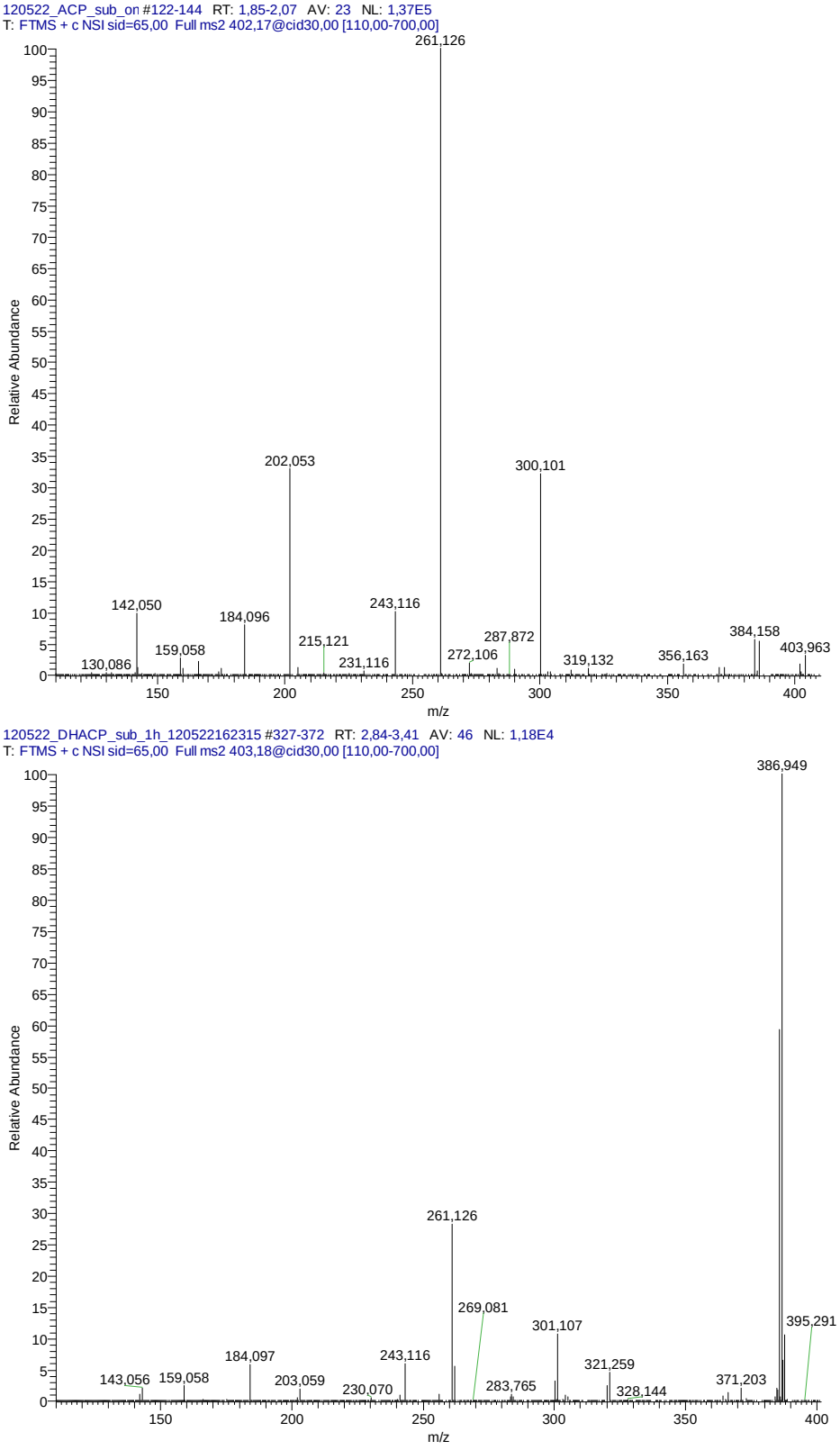
For ACP with a mass of 14 kDa the ejection ions were obtained by either isolating one charge state (charge states isolated were: 16^+ (*m/z* 908.34094) and 17^+ (*m/z* 854.90875), isolation width 7 *m/z*), or by applying in source fragmentation (35 V). For the isolated charge states the ejection ions were obtained by applying normalized collision energy of 30-35% in the linear ion trap. The ejection ions in turn were isolated and subjected to further round(s) of fragmentation. Beside the ejection ions of interest – expected: *m/z* 402.169 (without incorporated deuterium); therefore, the range of *m/z* 400-410 was manually scanned for ions showing a PPant signature – also an ejection ion with *m/z* 303.137 could be detected. This ion represents the phosphopantetheine arm plus an attached acetyl rest. These acetylated PPant arms resulted from the expression of the ACP domain as a *holo*-protein in *E. coli*. Acetyl-CoA, generally present in the cytosol of the *E. coli* cells, can get loaded to the free PPant arms. Such pre-loaded protein variants were not available for the synthesized substrate, and were not further considered.

In the light of the observation of a Michael adduct during synthesis procedure (compound **12**, scheme S2) modifications on the substrate, in the reaction mixture or attached to the PPant residue cannot be excluded, as well as possible further nucleophilic acyl substitutions of the thioester. However, such wrongly loaded PPant arms were also not considered and the latter (acylated variants) would not be available for the envisaged substrate loading.

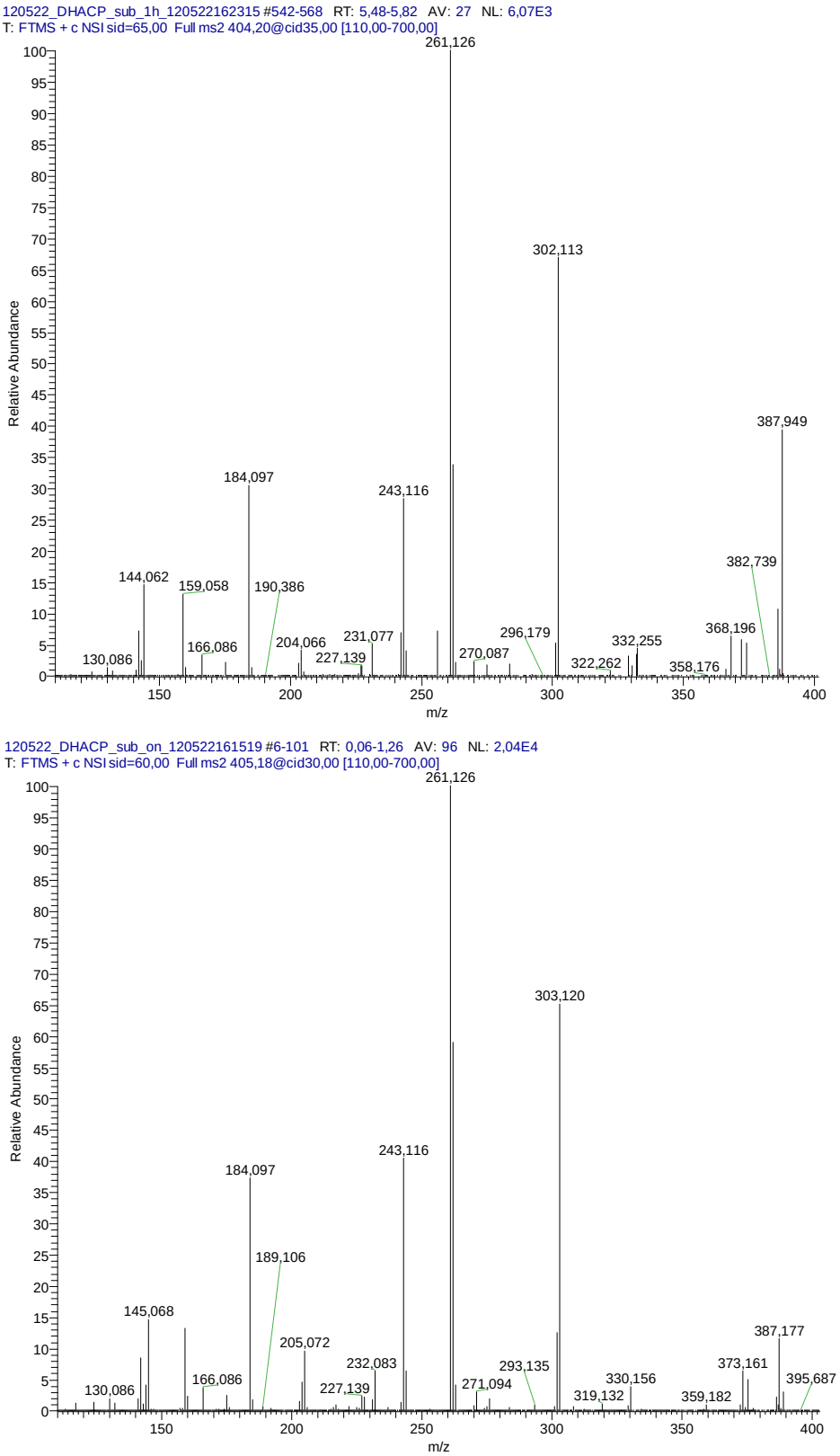
For DH*-ACP with a mass of 46.7 kDa, no single charge states could be isolated. So the ejection ions were obtained by in source fragmentation, applying 50-65 V fragmentation energy. The resulting ejection ions were isolated and subjected to further round(s) of fragmentation.



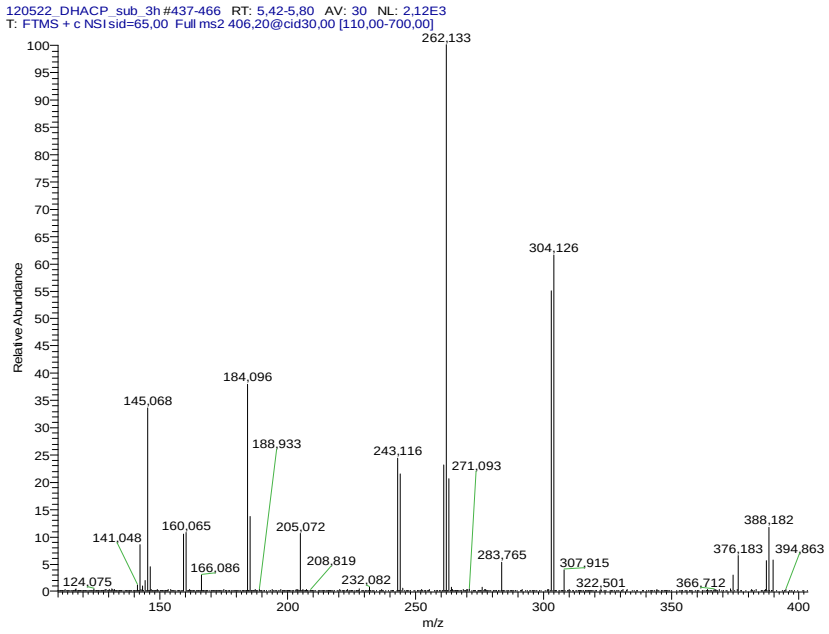
Supplemental figure 15. Overview for a PPant ejection experiment. A) Spectrum of substrate-loaded *holo*-ACP. One charge state (17+, highlighted in blue) was isolated (isolation width 7 *m/z*) and fragmented. This results in spectrum B, where the ejection ions were visible (compare figure S19). The region of the ejection ions of interest, *i.e.* substrate-loaded PPant residues, is magnified in C (compare figure 3 in the manuscript). One single ejection ion was isolated (in this case *m/z* 403.175, highlighted in blue) and subjected to a further round of fragmentation, yielding the respective signature ions[6] depicted in D.



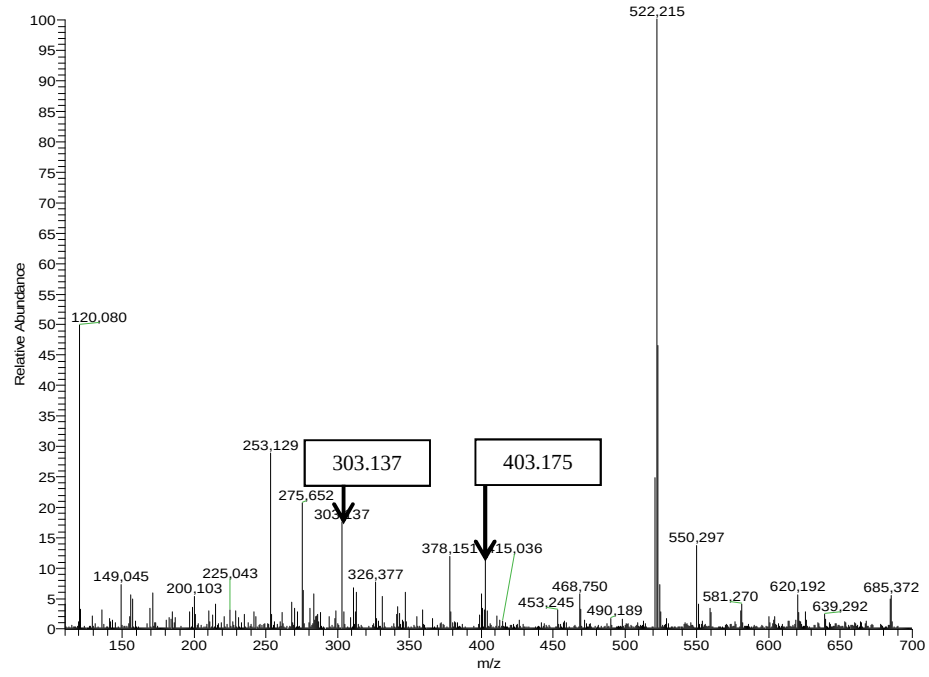
Supplemental figure 16. Top: substrate loaded *holo*-ACP, MS^3 of ion with *m/z* 402.169. Peak at *m/z* 261.126 represents the PPant part, peak at *m/z* 142.050 represents the substrate part. Bottom: substrate loaded *holo*-DH*-ACP MS^3 of ion with *m/z* 403.175. Peak at *m/z* 261.126 represents the PPant part, peak at *m/z* 143.056 represents the substrate part.



Supplemental figure 17. Top: MS^3 of ion with *m/z* 404.182. Peak at *m/z* 261.126 represents the PPant part, peak at *m/z* 144.062 represents the substrate part. Bottom: MS^3 of ion with *m/z* 405.188. Peak at *m/z* 261.126 represents the PPant part, peak at *m/z* 145.068 represents the substrate part.



Supplemental figure 18. MS^3 of ion with *m/z* 406.194. Peak at *m/z* 262.133 represents the PPant part – here with a mass increase of 1 Da due to unspecific deuteration, peak *m/z* 145.068 represents the substrate part.



Supplemental figure 19. Ejection ions of DH*-ACP. It can be seen that many PPant residues were acetylated, yielding a peak with *m/z* 303.137. The peaks near *m/z* 403.175 represent the substrate loaded PPant residues. Due to the overnight incubation in deuterated buffer the dominant Pant+substrate peak is a deuterated variant. (Compare to Figure 3 in the paper). For the other peaks, like the dominant *m/z* 522.215 no PPant signature could be detected. Therefore it is assumed that these represent peptide fragments.

Alignment of the conserved motives of classical DH domains with the DH* domain from the corallopyronin A biosynthesis gene cluster and selected other DH* domains.

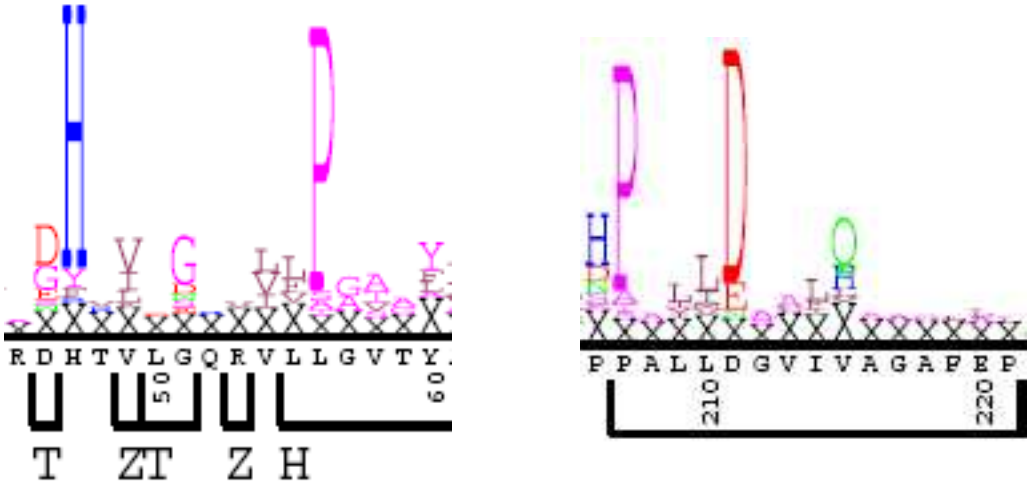
The conserved motives Hx_3Gx_4P and DxxxQ/H are essential for the catalytic activity of a DH as a reductive enzyme. These motives are not intact in the so-called DH* domains which are not catalyzing a reductive dehydration of a hydroxy group.

In DH* from the corallopyronin A biosynthesis gene cluster these motives are mutated to Hx_3Gx_5, and DxxxV, respectively.

```
CorJ_DH*   ...HTVLGQRVLLG...  ...DGVIV...
RhiE_DH*   ...HQFNHRRILLG...  ...NSAFL...
BaeR_DH*   ...HQFSGEPVLVG...  ...NSAYL...
DifK_DH*   ...HLVFGKPALMG...  ...NSCYM...
EryAII_DH  ...HVVGGRTLVPG...  ...DAVAQ...
DH_Cons.   ...HXXXGXXXXPG...  ...DXXXQ/H...
```

Supplemental figure 20. Alignment of DH and DH* amino acid sequences. The conserved catalytic residues for DH domains are highlighted in red. The corresponding accession numbers are ADI59532.1 (CorJ), YP_003748161.1 (RhiE), ABS74065.1 (BaeR), CAJ57411.1 (DifK), AAV51821.1 (EryAII). Cons. = consensus sequence.

Supplemental figure 20 was created by clustalW alignment of representative proteins with a focus on putative shift domains. To generate a multiple alignment of CorJ_DH* the amino acid sequence for the shift domain CorJ_DH* was extracted from the complete CorJ protein sequence. The extracted sequence was submitted in fasta format to Sequence Alignment and Modeling System (SAM, http://compbio.soe.ucsc.edu/sam.html) using SAM_T08_server. This server automatically created an alignment by finding similar protein sequences in the non-redundant protein database (nr proteins of the NCBI database). The sequence logo represents the graphical output, whereby the bigger the letter is, the higher is the abundance of the respective amino acid residue in all similar proteins identified. Supplemental figure 20 shows the same – for the catalytic activity important – residues as Supplemental figure 21.



Supplemental figure 21. Multiple alignment of CorJ DH*. The sequence logo of the regions harboring the two conserved motifs is shown. The numbering is according to CorJ.

Literature

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