Supplementary data

NMR analysis

Samples of compound **3**, **8** and **13** were dissolved in 0.300 ml DMSO, next 0.300 ml of $CDCl_3$ were added and the solution was transferred to a 5 mm NMR tube. NMR spectra were recorded on an Agilent Technologies 400-MR (400/54 Premium Shielded) spectrometer (400 MHz), Bruker Ascend 700 MHz NMR spectrometer or on a Bruker Ascend 600 MHz NMR spectrometer at 300K and at low temperature (260 K) with water suppression by means of the standard Bruker pulse program zgcppr. An inter pulse delay of 10 s was chosen for the ^{1}H spectra to ensure quantitative comparison of signal integrals. All ^{13}C-NMR spectra are 1H-broadband decoupled.

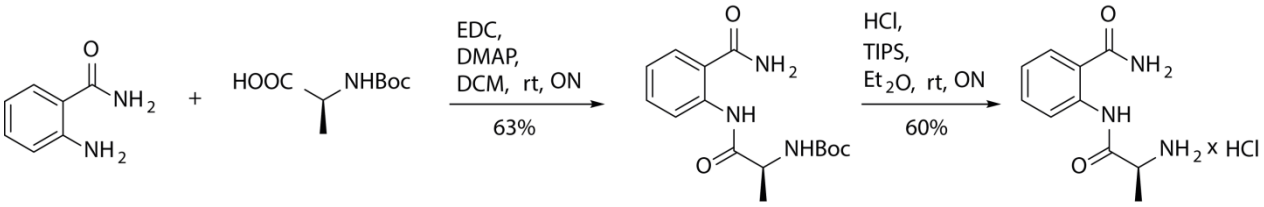
COSY, TOCSY, HSQC and HMBC spectra for assignments of signals were recorded with standard Bruker pulse sequences. Chemical shifts are expressed relative to:

In DMSO-d_6: ^{1}H δDMSO = 2.55, ^{13}C δDMSO = 39.5;

In $CDCl_3$: ^{1}H δTMS = 0.00, ^{13}C $\delta CDCl_3$ = 77.0;

In MeOD: ^{1}H δMeOH = 3.31, ^{13}C δMeOD = 49.0;

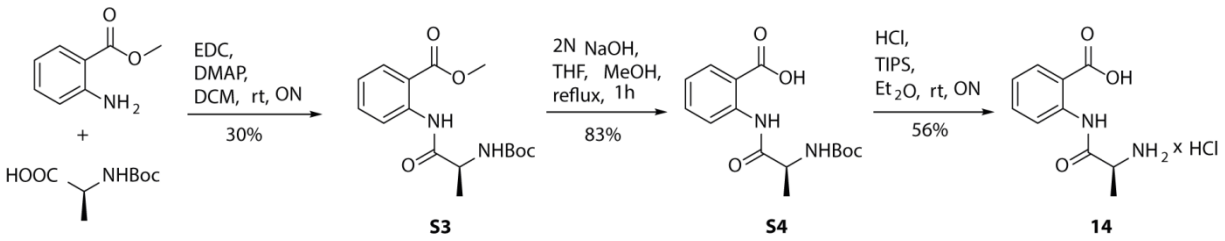
Synthesis of compound 14 and 15



S1: *tert*-Butyl (*S*)-(1-((2-carbamoylphenyl)amino)-1-oxopropan-2-yl)carbamate. A solution of anthranilamide (1.00 mmol, 126 mg), *N*-Boc-L-alanine (1.00 mmol, 189 mg) and dimethylaminopyridine (0.2 mmol, 25 mg) in DCM (4 mL) was stirred at rt. EDC (1.10 mmol, 210 mg) was added in one portion and the stirring continued overnight. The reaction mixture was diluted with ethyl acetate (80 mL) and washed with 1N HCl (3 x 60 mL), sat. aq. $NaHCO_3$ (3 x 60 mL) and brine (60 mL). The organic phase was dried ($MgSO_4$) and the solvent volume was reduced. Addition of pentane resulted in precipitation of product as a white powder (192 mg, 63 %). R_f = 0.56 (pentane / AcOEt, 1:1, v/v); Mp. 76-78 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.26 (d, ^3J = 7.2 Hz, 3H, CH_3CH), 1.39 (s, 9H, $(CH_3)C$), 3.85-3.96 (m, 1H, CH_3CH), 7.09 (app t, ^3J = 7.6 Hz, 1H, ArH), 7.45 (br s, 1H, NH), 7.46 (app t, ^3J = 7.6 Hz, 1H, ArH), 7.56 (br s, 1H, NH), 7.76 (d, ^3J = 8.0 Hz, 1H, ArH), 8.18 (br s, 1H, NH), 8.51 (d, ^3J = 8.4 Hz, 1H, ArH), 11.99 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.8, 28.7, 52.1, 78.8, 120.3 ,120.3 122.8, 129.0, 132.5, 139.8, 155.8, 170.8, 172.6; HRMS (ESI+) calc. for $[M+H]^+$ ($C_{15}H_{22}N_3O_4$): 308.1604, found: 308.1606.

15: (*S*)-2-(2-aminopropanamido)benzamide hydrochloride. To a solution of compound **S1** (0.55 mmol, 170 mg) and tri-*iso*-propylsilane (0.60 mmol, 123 µL) in Et_2O (10 mL) was added 2M HCl in Et_2O (10 mL). The resulting solution was stirred at rt

overnight. A precipitate was formed, which was filtered and washed with excess Et_2O to give, after drying *in vacuo*, a white powder (80 mg, 60%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.63 (d, ^3J = 7.2 Hz, 3H, CH_3CH), 4.16 (q, ^3J = 7.2 Hz, 1H, CH_3CH), 7.21 (app t, ^3J = 7.6 Hz, 1H, ArH), 7.52 (app t, ^3J = 7.6 Hz, 1H, ArH), 8.00 (d, ^3J = 8.0 Hz, 1H, ArH), 8.38 (d, ^3J = 8.0 Hz, 1H, ArH); ^{13}C NMR (100 MHz, CD_3OD): δ 15.7, 49.9, 120.8, 121.2, 123.7, 128.8, 132.2, 138.2, 167.4, 171.9; HRMS (ESI+) calc. for $[M+H]^+$ ($C_{10}H_{14}N_3O_2$): 208.1080, found: 208.1081.



S3 **S4** **14**

S3: Methyl (*S*)-2-(2-((tert-butoxycarbonyl)amino)propanamido)benzoate. A solution of methyl 2-aminobenzoate (2.00 mmol, 302 mg) and *N*-Boc-L-alanine (2.00 mmol, 378 mg) in DCM 8 mL) was stirred at rt. EDC (2.20 mmol, 421 mg) was added in one portion and the stirring continued overnight. The reaction mixture was diluted with ethyl acetate (80 mL) and washed with 1N HCl (3 x 60 mL), sat. aq. $NaHCO_3$ (3 x 60 mL) and brine (60 mL). The organic phase was dried ($MgSO_4$) and the solvent volume was reduced. Addition of pentane resulted in precipitation of product as a white powder (190 mg, 30 %). Mp. 114-115 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (s, 9H, $(CH_3)C$), 1.47 (d, ^3J = 7.2 Hz, 3H, CH_3CH), 3.89 (s, 3H, CH_3O), 4.25-4.40 (m, 1H, CH_3CH), 5.14 (br s, 1H, NHBoc), 7.07 (app t, ^3J = 8.0 Hz, 1H, ArH), 7.52 (app t, ^3J = 8.0 Hz, 1H, ArH), 8.00 (d, ^3J

= 8.0 Hz, 1H, ArH), 8.69 (d, ^3J = 8.4 Hz, 1H, ArH), 11.50 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.9, 28.3, 51.6, 52.3, 80.0, 115.3, 120.3, 122.7, 130.8, 134.6, 141.1, 155.2, 168.4, 171.8; HRMS (ESI+) calc. for $[M+H]^+$ ($C_{16}H_{23}N_2O_5$): 323.1602, found: 323.1599.

S4: (*S*)-2-(2-((tert-butoxycarbonyl)amino)propanamido)benzoic acid. To a solution of compound **S3** (0.50 mmol, 156 mg) in MeOH (12 mL) and THF (12 mL) was added 2N aq. NaOH (4 mL). The resulting mixture was heated at reflux for 1 h. The volatiles were evaporated, the residue was redissolved in with ethyl acetate (30 mL) and washed with 1N HCl (2 x 20 mL) and brine (20 mL). The organic phase was dried ($MgSO_4$) and the solvent volume was reduced. Addition of pentane resulted in precipitation of product as a white powder (128 mg, 83 %). Mp. 154-156 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.28 (d, ^3J = 7.2 Hz, 3H, CH_3CH), 1.38 (s, 9H, $(CH_3)C$), 3.90-4.02 (m, 1H, CH_3CH), 7.12 (app t, ^3J = 7.6 Hz, 1H, ArH), 7.51 (d, ^3J = 6.4 Hz, 1H, NHBoc), 7.57 (app t, ^3J = 7.6 Hz, 1H, ArH), 7.97 (d, ^3J = 8.0 Hz, 1H, ArH), 8.61 (d, ^3J = 8.0 Hz, 1H, ArH), 11.65 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.8, 28.6, 52.2, 78.9, 116.5, 119.8, 123.0, 131.6, 134.6, 141.2, 155.9, 169.6, 172.9; HRMS (ESI+) calc. for $[M+H]^+$ ($C_{15}H_{21}N_2O_5$): 309.1445, found: 309.1440.

14: (*S*)-2-(2-aminopropanamido)benzoic acid hydrochloride. To a solution of compound **S4** (0.39 mmol, 120 mg) and tri-*iso*-propylsilane (0.50 mmol, 108 µL) in Et_2O (5 mL) was added 2M HCl in Et_2O (5 mL). The resulting solution was stirred at rt overnight. A precipitate was formed, which was filtered and washed with excess Et_2O to give, after drying *in vacuo*, a white powder (53 mg, 56%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.66 (d, ^3J = 7.2 Hz, 3H, CH_3CH), 4.23 (q, ^3J = 7.2 Hz, 1H, CH_3CH), 7.21 (app t, ^3J = 7.6

Hz, 1H, ArH), 7.59 (app t, ^{3}J = 7.6 Hz, 1H, ArH), 8.10 (d, ^{3}J = 8.0 Hz, 1H, ArH), 8.51 (d, ^{3}J = 8.4 Hz, 1H, ArH); ^{13}C NMR (100 MHz, CD_3OD): δ 15.7, 50.0, 116.7, 120.3, 123.4, 131.2, 133.9, 140.0 167.6, 169.9; HRMS (ESI+) calc. for $[M+H]^+$ ($C_{10}H_{13}N_2O_3$): 209.0921, found: 209.0916.

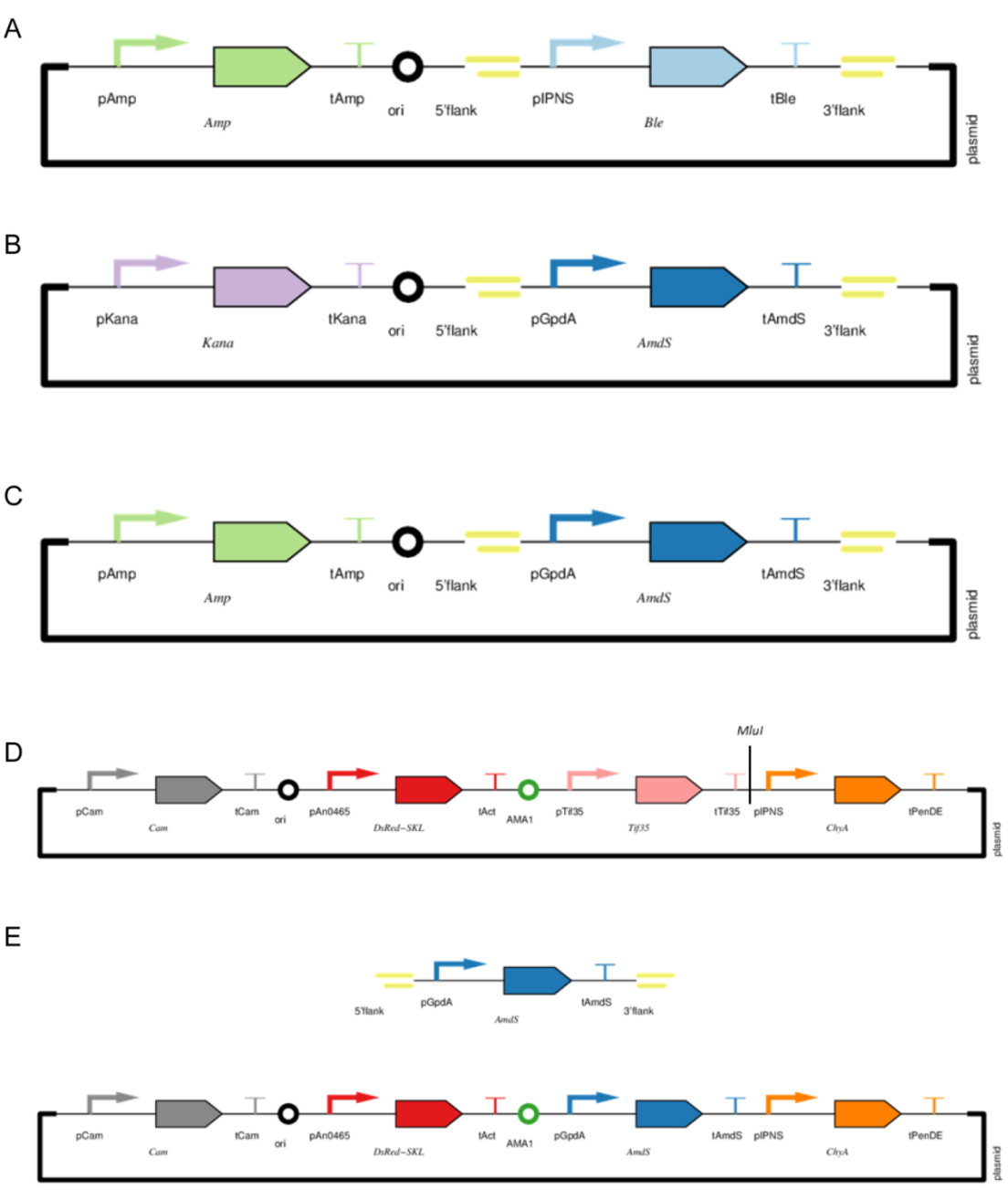


Figure S1. SBOL (Synthetic Biology Open Language) presentation of deletion plasmids for *chyA*, *chyE* (A), *chyC*, *chyD* (B), *chyH*, *chyM* and *Pc21g12640* (C). SBOL presentation of pDSM108_AV1 overexpressing *chyA* and *in vivo* repair cassette (D). *In vivo* recombined plasmid from D (E).

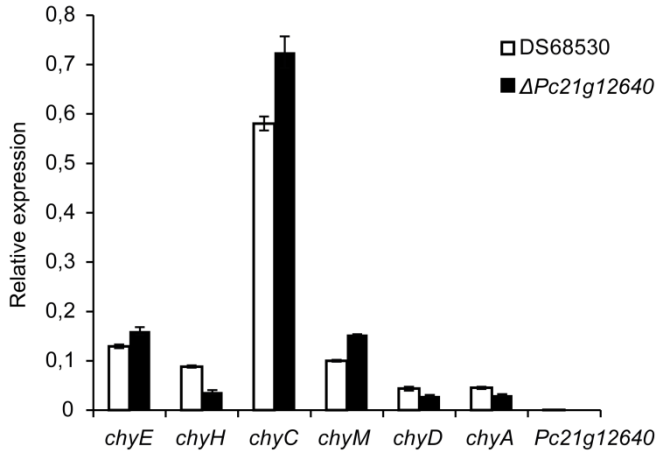


Figure S2 Expression of the putative chrysogine gene cluster in strain DS68530 and *ΔPc21g12640*. RNA was isolated after 48 h of growth in a SMP medium. Data are expressed relative to actin and represented as mean ± SEM.

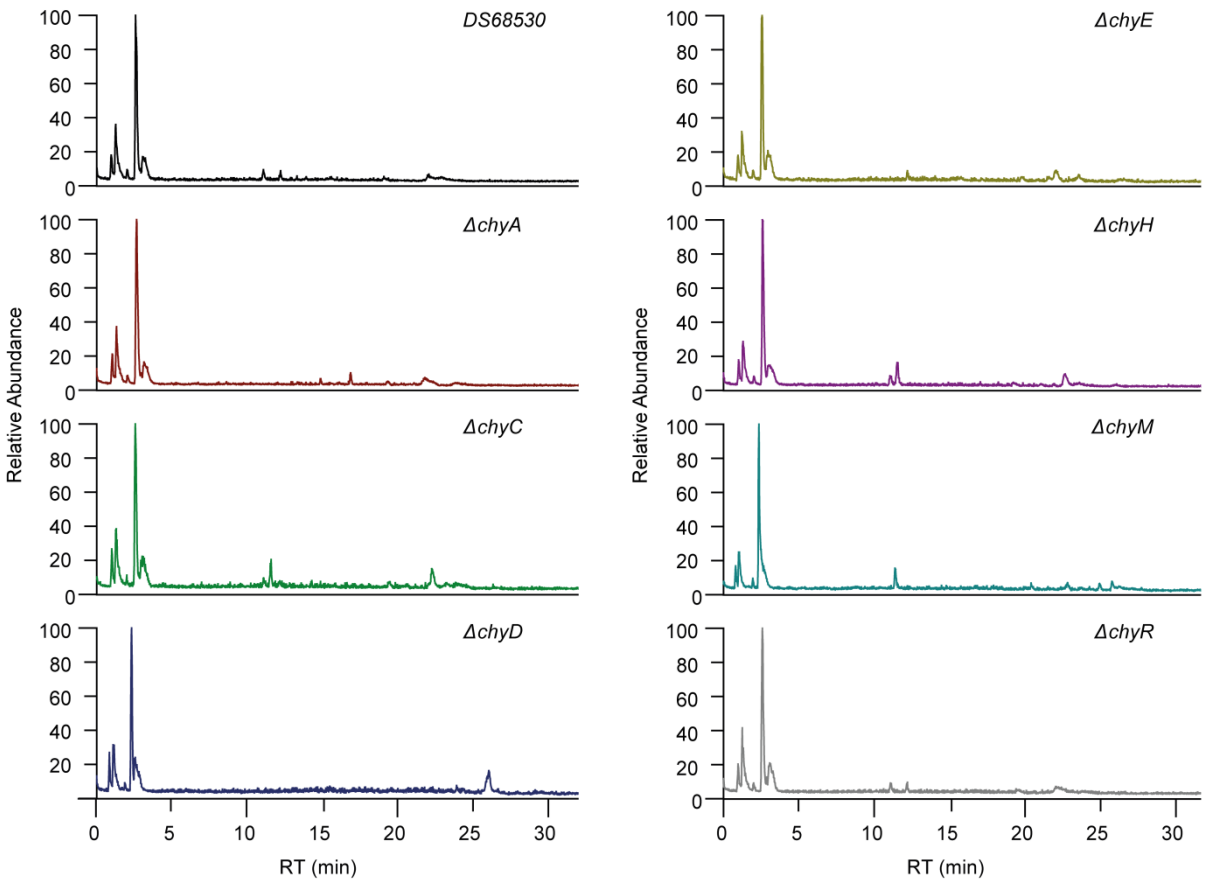


Figure S3. Chromatograms of culture broth from DS68530 (wild-type) and indicated gene deletion strains after 96 h of growth in a SMP medium.

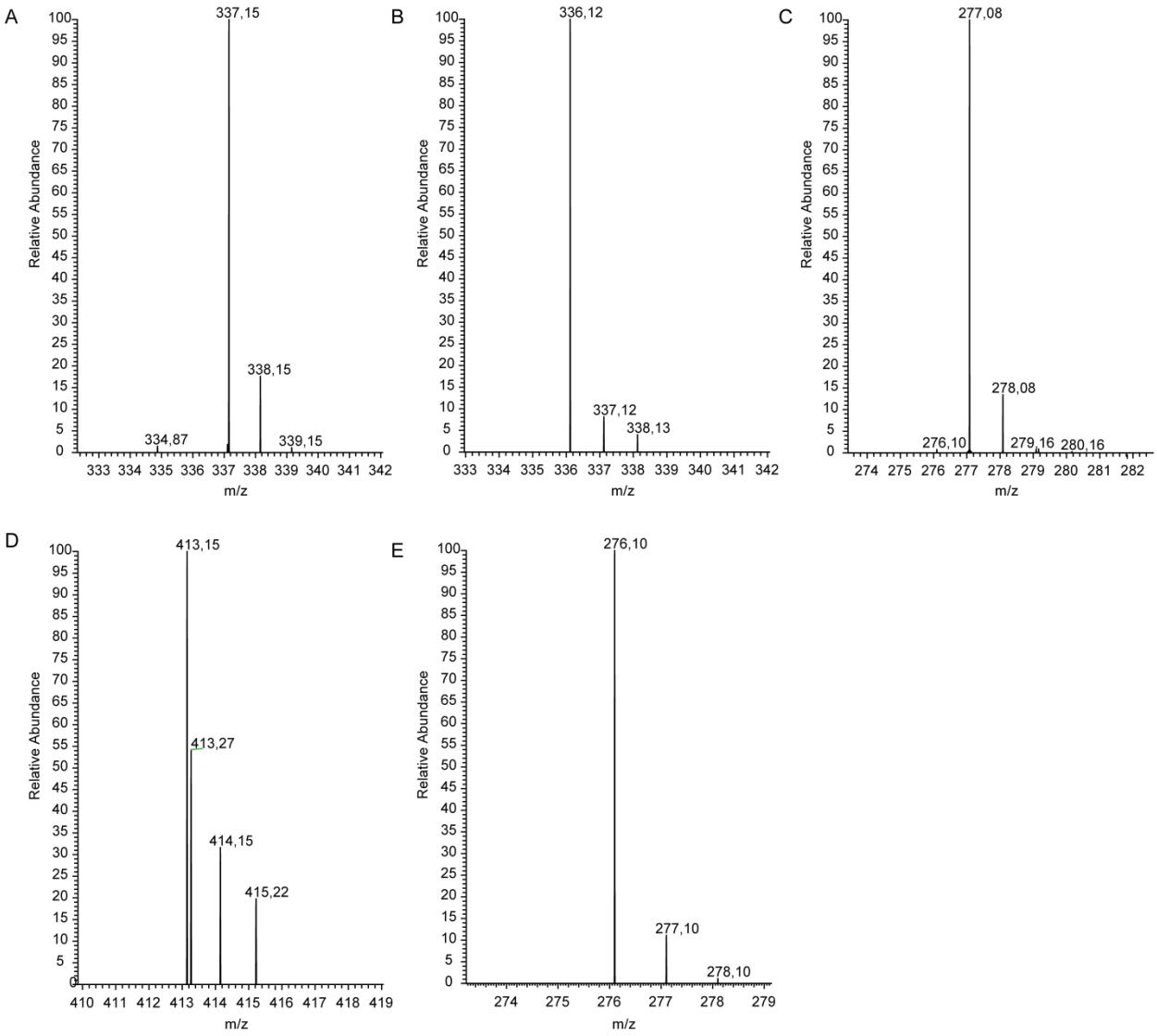


Figure S4. Mass spectra of uncharacterized chrysogine related compounds. Compound **5** (A), compound **6** (B), compound **7** (C), compound **9** and **10** (D), compound **12** (E).

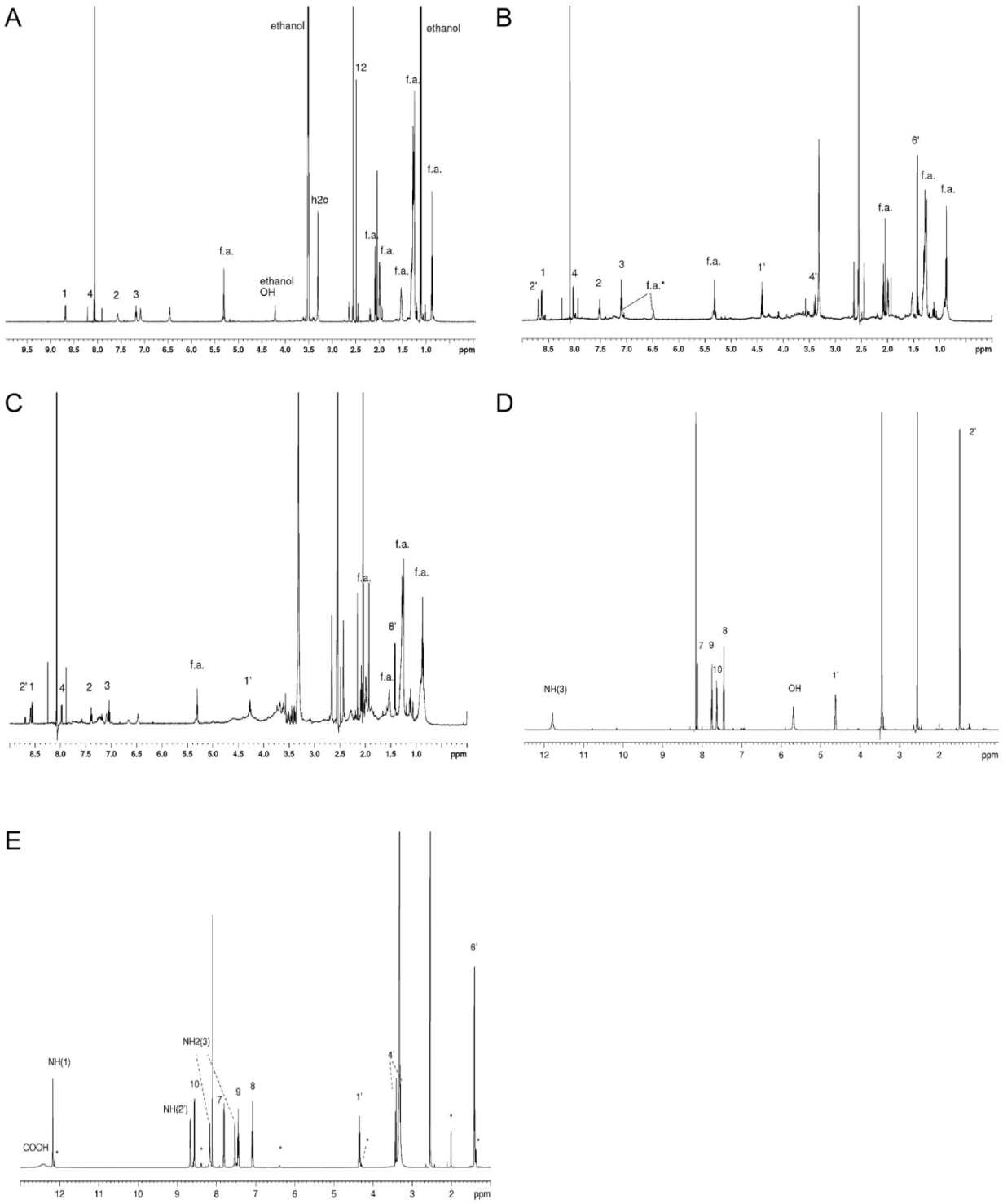


Figure S5. ^{1}H NMR spectrum of compound **3** (A), compound **8** (B), compound **13** (C), compound **1** (D), compound **4** (E). Compound **2** was observed as a minor impurity in this fraction. Signals are labelled with *.

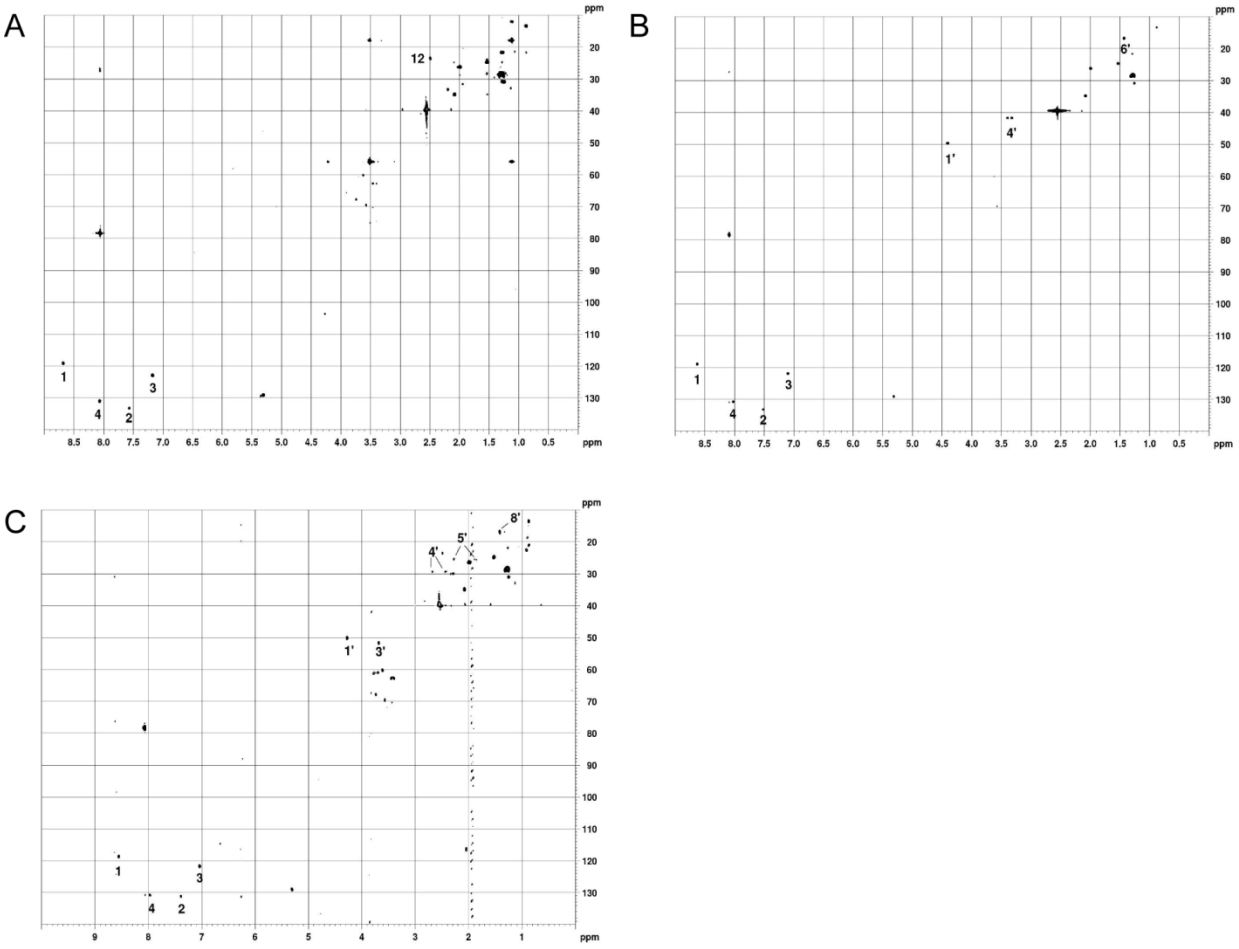


Figure S6. HSQC spectrum of compound **3** (A), compound **8** (B) and **13** (C).

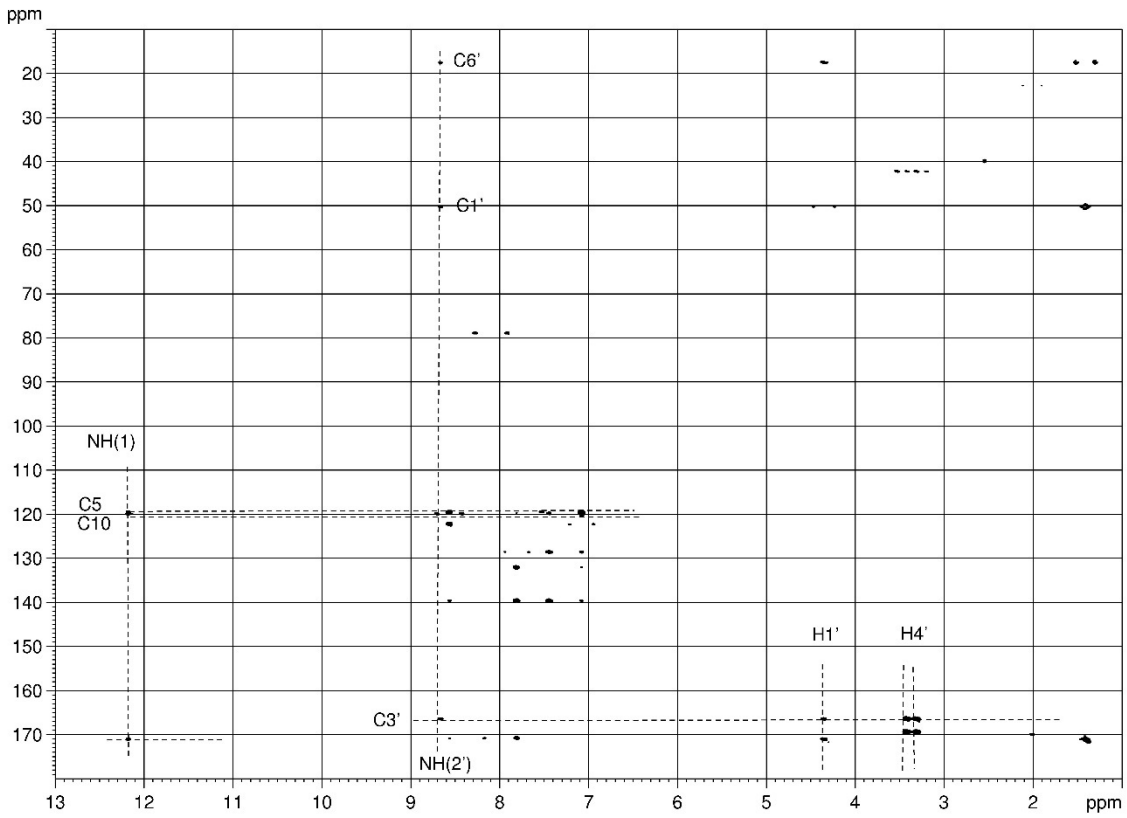


Figure S7. HMBC spectrum of compound **4** and impurity compound **2**.

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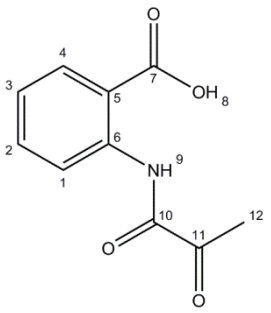
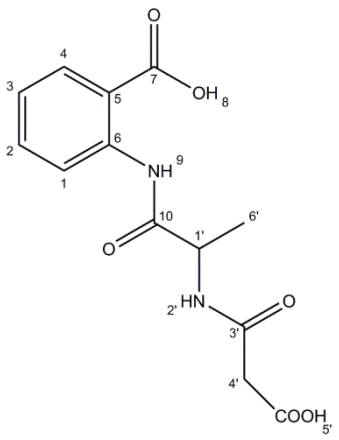


3



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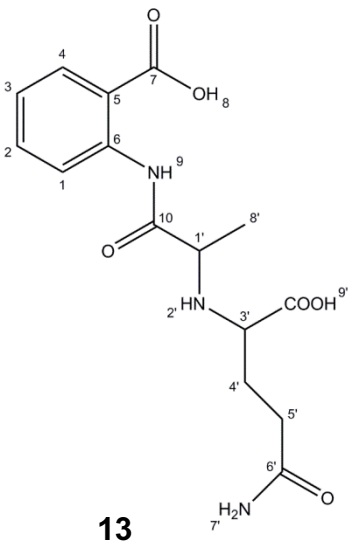

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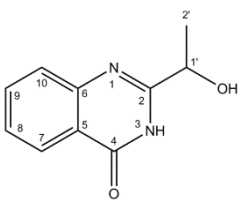
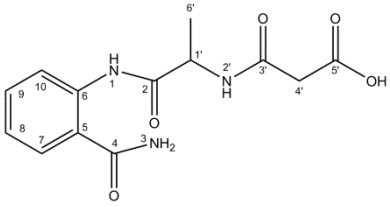
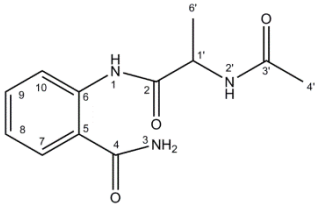
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	8.69	119.1	8.63	118.9	8.56	118.7
2	7.57	133.2	7.52	133.1	7.39	131.2
3	7.18	122.9	7.10	122.0	7.04	121.7
4	8.07	131.0	8.02	130.7	7.97	130.8
5	-	119.5	-	119.4	-	n.o.
6	-	139.1	-	140.4	-	138.8
7	-	168.9	-	169.3	-	168.9
8	n.o.	-	n.o.	-	n.o.	-
9	12.52	-	12.01	-	12.05	-
10	-	158.1	-	170.9	-	171.1
11	-	195.6				
12	2.49	23.6				
1’			4.41	49.8	4.28	50.2
2’			8.69	-	8.59	-
3’			-	166.2	3.69 (*)	51.7
4’			3.39 / 3.33	41.8	2.28 / 1.88 (*)	25.4
5’			-	169.0	2.68 / 2.44 (*)	29.4
6’			1.43	16.8	-	170.9
7’					n.o.	-
8’					1.42	16.9
9’					-	171.8

(*): multiplicities not resolved, -: not applicable, n.o.: not observed

Table S1. Chemical shifts of compound **3**, compound **8** and compound **13** in DMSO/$CDCl_3$ 1/1. δ DMSO = 39.5 / 2.55 ppm. Temperature = 300 K.







1 **4** **2**

	280 K	280 K	300 K		300 K			300 K	
	^{1}H	^{13}C	^{15}N	^{1}H	^{13}C	^{15}N	^{1}H	^{13}C	^{15}N
1	-	-	231.2	12.18	-	120.5	12.13	-	n.o.
2	-	159.7	-	-	171.0	-	-	n.o.	-
3	11.79	-	156.6	8.17 / 7.53	-	108.4	8.15 / 7.48	-	n.o.
4	-	161.7	-	-	170.8	-	-	n.o.	-
5	-	121.3	-	-	119.4	-	-	n.o.	-
6	-	148.5	-	-	139.6	-	-	n.o.	-
7	8.13	125.8	-	7.81	128.2	-	7.80	n.o.	-
8	7.46	126.0	-	7.08	121.9	-	7.07	n.o.	-
9	7.76	134.0	-	7.44	131.6	-	7.43	n.o.	-
10	7.63	126.9	-	8.56	119.5	-	8.59	n.o.	-
1’	4.63	67.2	-	4.35	49.7	-	4.31	n.o.	-
2’	1.48	21.8	-	8.67	-	125.7	8.39	-	125.3
OH	5.69	-	-						
3’				-	166.4	-	-	n.o.	-
				3.42 /		-			-
4’				3.08	41.8		2.01	22.2	
5’				-	169.3	-			
6’				1.41	17.0	-	1.38	n.o.	-
5’ COOH				12.42	-	-			

-: not applicable, n.o.: not observed

Table S2. Chemical shifts of compound **1**, compound **4** and compound **2** in DMSO/$CDCl_3$ 1/1. δ DMSO = 39.5 / 2.55 ppm. Temperature = 280 K and 300 K.