CHEMBIOCHEM

Supporting Information

Unprecedented Utilization of Pelargonidin and Indole for the Biosynthesis of Plant Indole Alkaloids

Anne-Christin Warskulat, Evangelos C. Tatsis, Bettina Dudek, Marco Kai, Sybille Lorenz, and Bernd Schneider*[a]

cbic_201500572_sm_miscellaneous_information.pdf

Table of contents

S1. Assessment of ^{13}C Isotope Abundances by NMR

Assessment of relative and absolute abundances and isotopologue composition in the experiment with [$^{13}C_6$]glucose was performed as previously exemplified in the supporting information describing a pulse-chase experiment with $^{13}CO_2$ under http://dx.doi.org/10.1002/cbic.201402109. [1]

The relative ^{13}C abundances of the biosynthetic nudicaulin samples obtained from specific labeling experiments were determined by comparing the signal intensities with the signals of samples of natural ^{13}C abundance measured under identical spectroscopic conditions. In detail, ^{13}C NMR spectra of nudicaulin I obtained from incubation of sliced petals with ^{13}C-labeled precursors were acquired and the integrals of selected ^{13}C NMR signals were determined. A ^{13}C NMR spectrum of nudicaulin I under the same experimental conditions was also acquired and the integrals of ^{13}C signals of nudicaulin I of natural abundance isotope composition were determined. A signal of a natural abundance ^{13}C atom was used as a reference. The ratio of signal integrals of corresponding carbon atoms in nudicaulin I from feeding experiments and of natural abundance material was then calculated. This ratio is a direct measure of the relative ^{13}C abundance at each position of nudicaulin I from labeling experiments.

[1] E. C. Tatsis, A. Schaumlöffel, A. C. Warskulat, G. Massiot, B. Schneider, G. Bringmann, *Org. Lett*. 2013, *15*, 156-159.

List of figures

List of tables

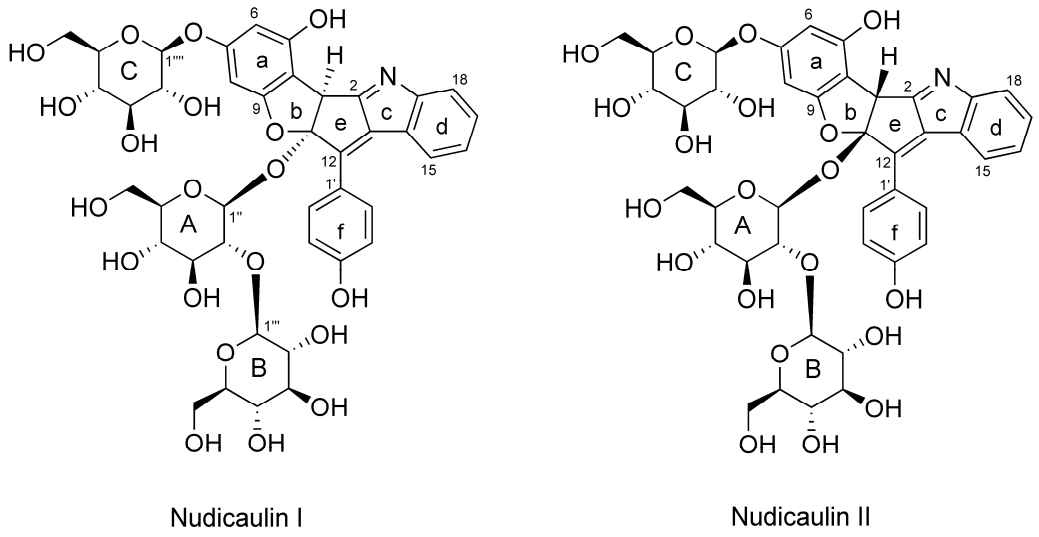

Figure S1. Structures of nudicaulin I (3*S*,11*R*) and nudicaulin II (3*R*,11*S*).

Table S1. ESI-MS data of nudicaulin I of natural abundance isotope composition.
For spectrum, see Figure S2.

m/z	Intensity	Relative intensity
872.26	178896.1	100
873.26	77763.8	43.47
874.26	22778.2	12.73
875.27	4057.7	2.27

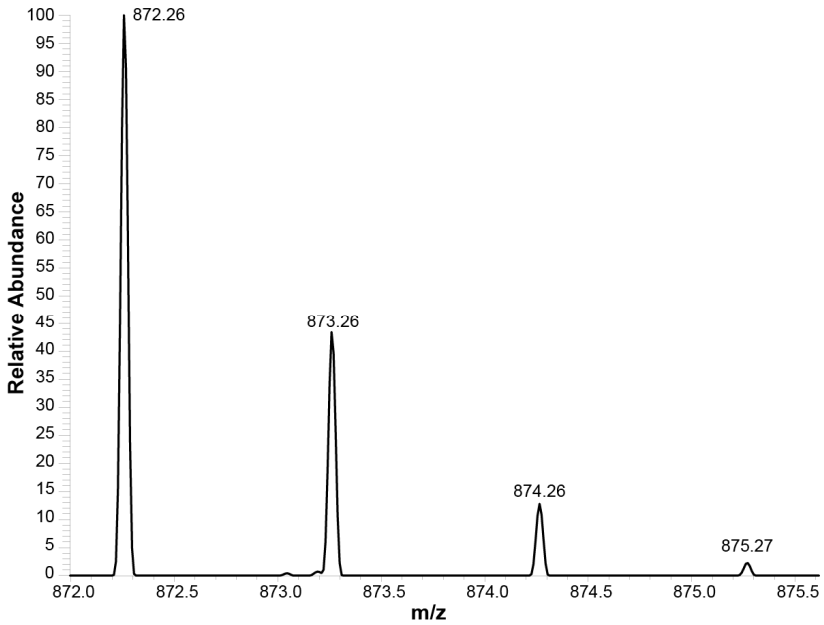

Figure S2. ESI-MS spectrum of nudicaulin I of natural abundance isotope composition.

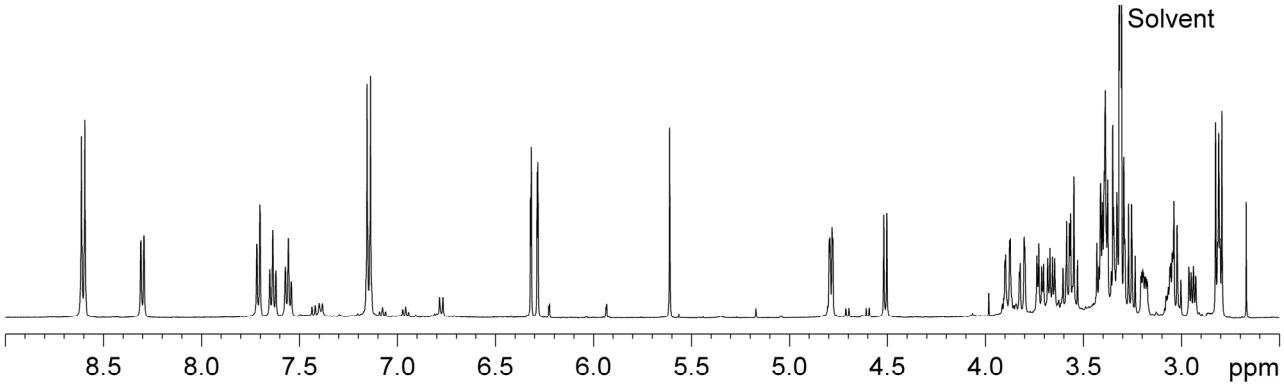

Figure S3. ^{1}H NMR spectrum (500 MHz, CD_3OD) of nudicaulin I of natural abundance isotope composition.

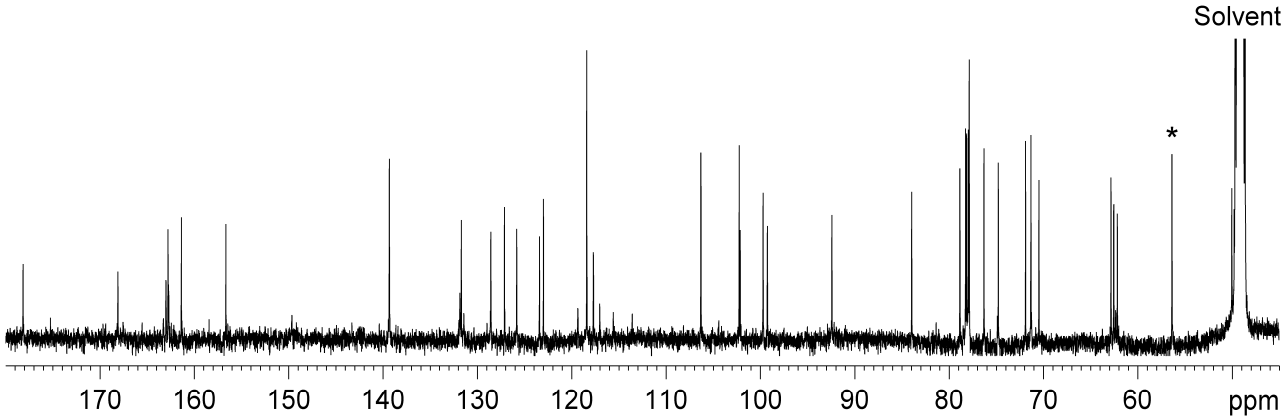

Figure S4. ^{13}C NMR spectrum (125 MHz, CD_3OD) of nudicaulin I of natural abundance isotope composition.

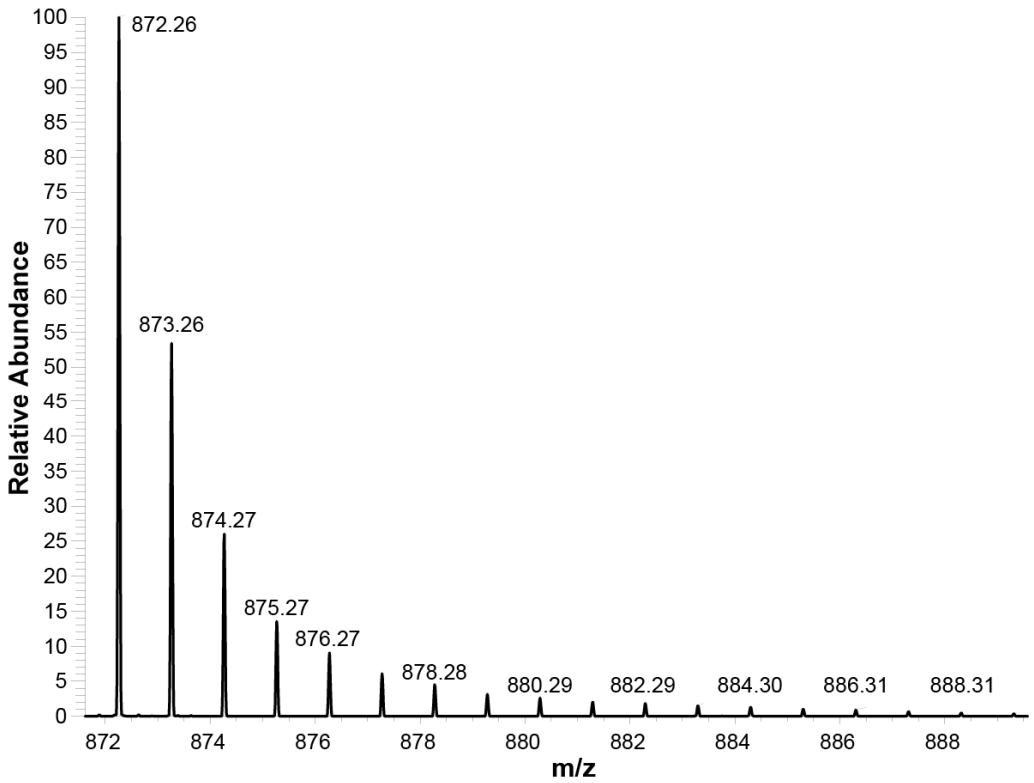

Figure S5. ESI-MS spectrum of nudicaulin I obtained from the labeling experiment with [$^{13}C_6$]glucose as a tracer.

Table S2. ESI-MS data of nudicaulin I obtained from the labeling experiment with [$^{13}C_6$]glucose as a tracer. For spectrum, see Figure S5.

m/z	Intensity	Relative intensity
872.26	2329834.0	100.00
873.26	1241770.8	53.30
874.27	613506.9	26.33
875.27	317167.0	13.61
876.27	210509.4	9.04
877.28	142013.2	6.10
878.28	106431.3	4.57
879.28	73759.5	3.17
880.29	60438.2	2.59
881.29	48049.4	2.06
882.29	42943.4	1.84
883.30	35384.6	1.52
884.30	31021.3	1.33
885.30	23778.1	1.02
886.31	21020.6	0.90
887.31	15379.0	0.66
888.31	11299.6	0.48
889.32	8663.8	0.37
Calculated total ^{13}C enrichment: 3.3%		

Table S3. ^{1}H and ^{13}C NMR spectroscopic data (500 MHz for ^{1}H; 125 MHz for ^{13}C) of nudicaulin I aglycone with ^{13}C abundances obtained from the labeling experiment using [$^{13}C_6$]glucose as a tracer.

No.	^{1}H NMR	^{13}C NMR	Coupling constant	^{13}C and isotopologue abundances		
	δ, mult., *J* (Hz)	δ[a]	J_{CC} (Hz) [coupled C][b]	% ^{13}C [c]	% ^{13}C^{13}C[d] [coupled C][b]	% mol isotopologues [position][e]
Aglycone						
2		177.5	48 [13]	20.5	64.1 [13]	13.1 [2, 13]
3	5.63, *s*	49.9	41 [11]	3.3	60.0 [11]	1.8 [3, 11]
4		101.8	77 [5], 63 [9]	6.3	45.1	2.2 [4, 5] or [4, 9]
5		156.4	77 [4], 66 [6]	4.9	50.3	2.2 [4, 5] or [5, 6]
6	6.33, *d*, 2.0	99.3	66 [5], 71 [7]	5.5	47.6	2.2 [6, 7] or [5, 6]
7		162.6	71 [6], 71 [8]	4.0	40.1	2.2 [6, 7] or [7, 8]
8	6.29, *d*, 2.0	92.4	71 [7], 71 [9]	4.7	48.5	2.2 [8, 9] or [7, 8]
9		161.1	71 [8], 63 [4]	4.3	45.5	2.2 [8, 9] or [4, 9]
11		126.9	41 [3], 53 [12]	5.4	38.4 [3, 12]	2.6 [3, 11, 12]
12		168.5	53 [11]	3.3	53.5 [11]	2.0 [11, 12]
13		131.2	48 [2]	15.8	68.4 [2]	11.3 [2, 13]
14		122.7	59 [19]	16.8	56 [19]	9.4 [14, 19]
15	8.33, *d*, 7.8	125.6	58 [16]	13.3	76.0 [16]	11.3 [15,16] and [15,16,17]
16	7.59, *dd*, 7.8, 7.8	128.5	58 [15], 56 [17]	14.9	75.7 [15 and 17], 12.6 [15 or 17]	9.7 [15, 16, 17], 1.7 [15, 16] and [16, 17]
17	7.66, *dd*, 7.8, 7.8	131.4	56 [16], 56 [18]	11.6	39.9 [16 and 18], 46.5 [16 or 18]	4.8 [16, 17, 18], 5.4 [16, 17] and [17, 18]
18	7.72, *d*, 7.8	117.2	56 [17]	18.8	48.8 [17]	9.2 [17,18] and [16,17,18]
19		148.4	59 [14]	19.3	73.8 [14]	12.3 [14, 19]
1′		123.0	59 [2′]	3.5	66.5 [2′]	2.3[1′, 2′]
2′/6′	8.63, *d*, 9.0	139.3	59 [1′] / 59 [5′]	5.5	52.5 [1′ or 5′]	2.9 [1′, 2′] and [5′, 6′]
3′/5′	7.16, *d*, 9.0	118.3	59 [6′], 61 [4′]	4.9	26.0 [4′ and 6′] 15.7 [4′ or 6′]	1.5 [3′,4′,5′] and [4′, 5′, 6′], 1.2 [3′, 4′] and [5′, 6′]
4′		168.3	61 [3′/ 5′], 9 [6′]	4.3	39.6 [3′ and 5′], 19.7 [3′ or 5′]	2.4 [3′,4′,5′] and [4′, 5′, 6′], 1.2 [3′, 4′] and [5′, 6′]

[a] ^{13}C NMR chemical shifts are from Schliemann et al., *Phytochemistry* **2006**, *67*, 191-201 and may slightly deviate in the spectra obtained from the $^{13}CO_2$ experiment due to residual trifluoroacetic acid remaining from HPLC solvent.
[b] The numbers in parentheses indicate the coupling partners.
[c] ^{13}C abundances of individual carbon atoms (%^{13}C).
[d] Relative contribution of a satellite pair to the overall ^{13}C NMR signal integral of the indexed carbon atom.
[e] Relative molar amounts of isotopologues were calculated as %^{13}C × % $^{13}C^{13}C$. Bracketed numbers indicate ^{13}C atoms of the respective isotopologue.

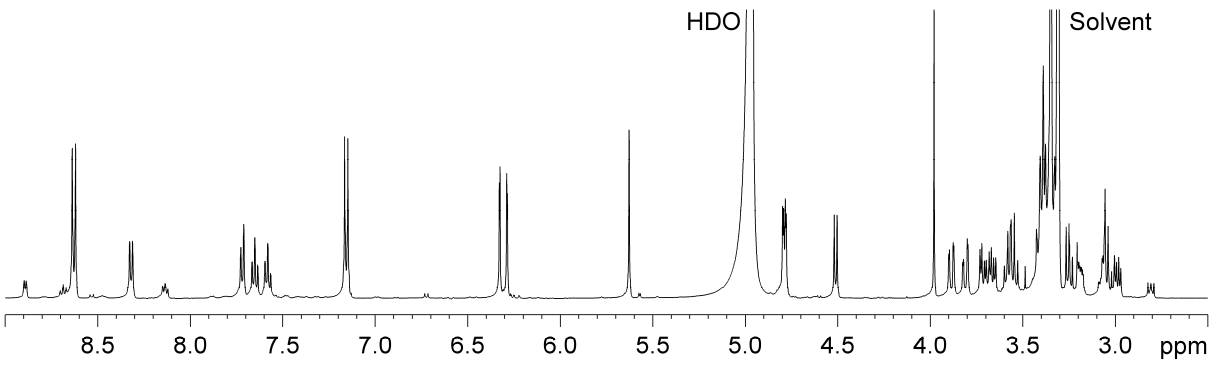

Figure S6. ^{1}H NMR spectrum (500 MHz, CD_3OD) of nudicaulin I obtained from the labeling experiment using [$^{13}C_6$]glucose as a tracer. The spectrum was recorded without solvent suppression.

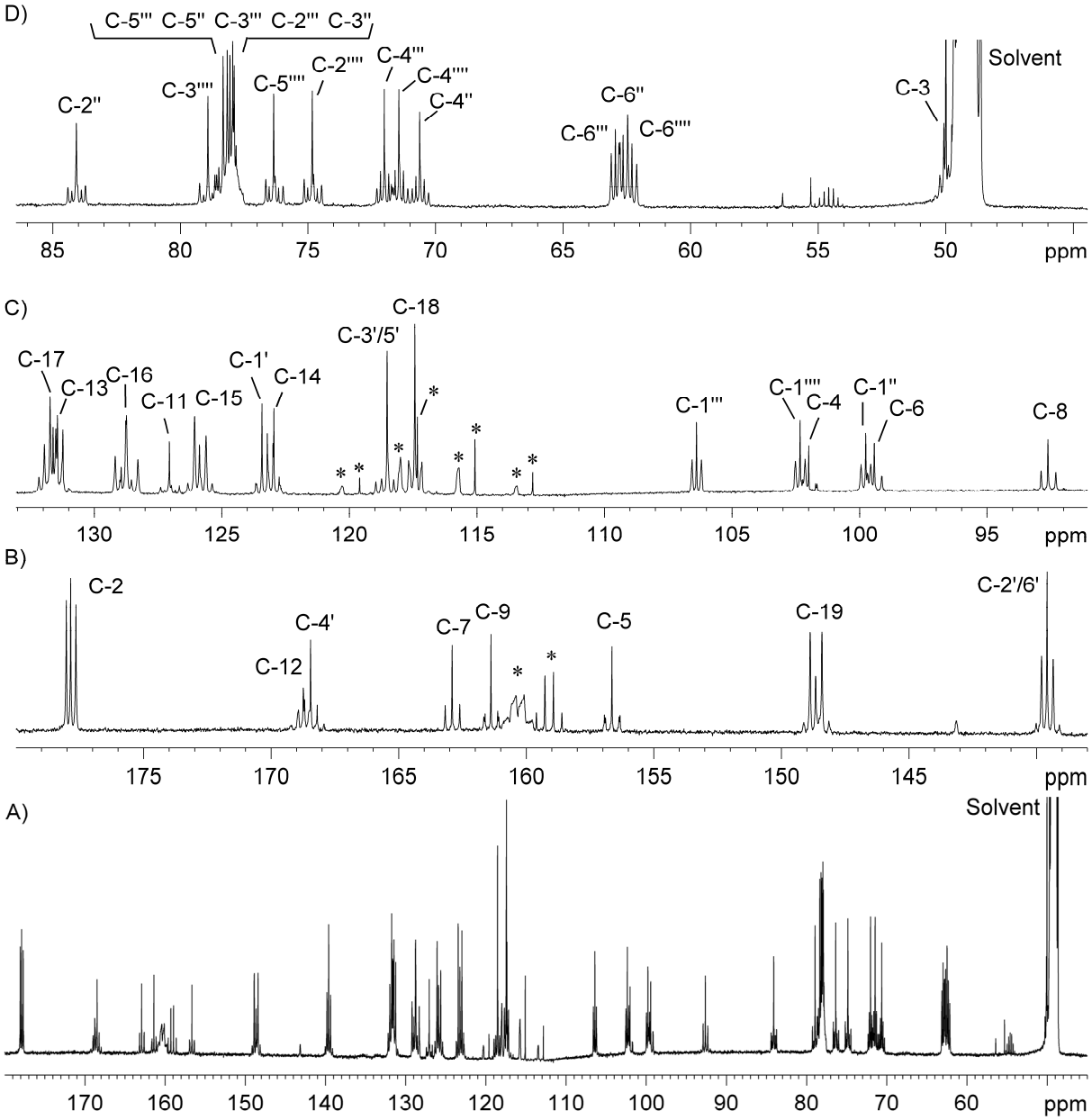

Figure S7. ^{13}C NMR spectra (125 MHz, CD_3OD) of nudicaulin I obtained from the labeling experiment using [$^{13}C_6$]glucose as a tracer. Panel A): Full spectrum; panels B)-D): extended partial spectra with carbon numbering of ^{13}C-enriched signals. For $J_{13C\text{-}13C}$ values, see Table S3. The asterisks (*) indicate the signals of trifluoroacetic acid.

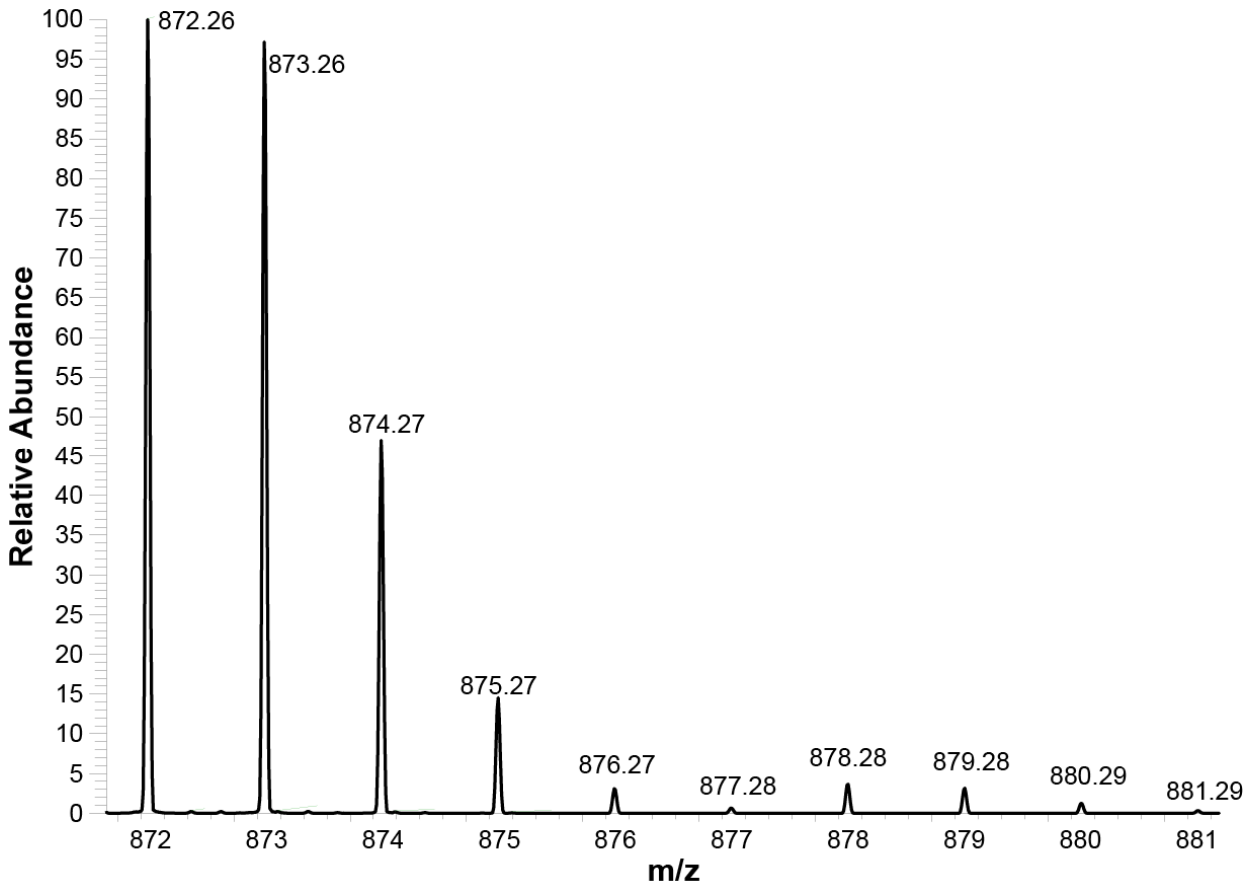

Figure S8. ESI-MS spectrum of nudicaulin I obtained from the labeling experiment with [ring-$^{13}C_6$]anthranilic acid as a tracer.

Table S4. ESI-MS data of nudicaulin I obtained from the labeling experiment with [ring-$^{13}C_6$]anthranilic acid as a tracer. For spectrum, see Figure S8.

m/z	Intensity	Relative intensity
872.26	3683457.5	100.00
873.26	3608827.8	97.97
874.27	1737296.9	47.16
875.27	534059.1	14.50
876.27	115343.2	3.13
877.27	24306.2	0.66
878.27	136494.1	3.71
879.27	116956.3	3.18
880.27	46912.2	1.27
881.27	12064.7	0.33
Calculated total ^{13}C enrichment: 16.9%		

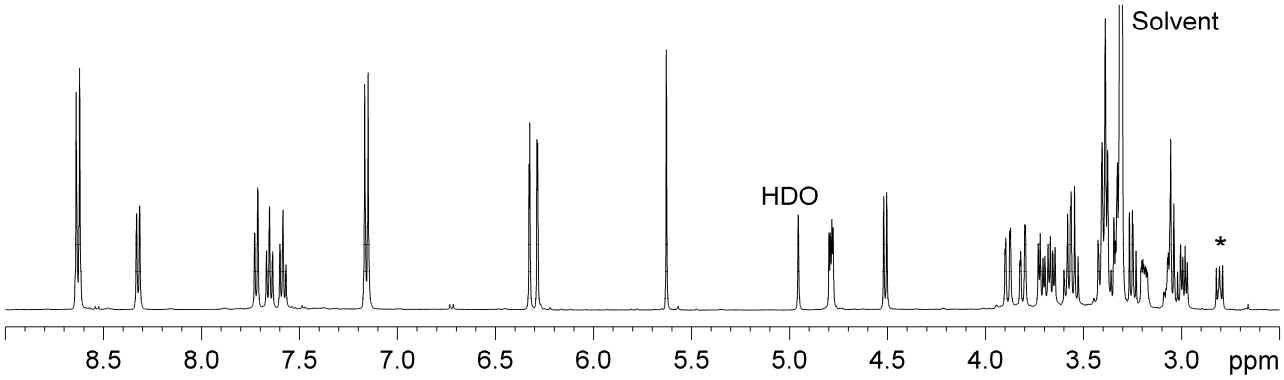

Figure S9. ^{1}H NMR spectrum (500 MHz, CD_3OD) of nudicaulin I obtained from the labeling experiment using [ring-$^{13}C_6$]anthranilic acid as a tracer. The asterisk (*) indicates the signal of a contamination.

Table S5. ^{1}H and ^{13}C NMR spectroscopic data (500 MHz for ^{1}H; 125 MHz for ^{13}C) for ^{13}C-labeled positions of nudicaulin I with ^{13}C abundances obtained from the labeling experiment using [ring-$^{13}C_6$]anthranilic acid as a tracer.

No.	^{1}H NMR	^{13}C NMR	Coupling constant
	δ, mult., J_{HH} (Hz)	δ[a]	J_{CC} (Hz) [coupled C][b]
Aglycone			
14		122.7	59 [19], 56 [15]
15	8.33, *d*, 7.8	125.6	58 [16], 56 [14]
16	7.59, *dd*, 7.8, 7.8	128.5	58 [15], 56 [17]
17	7.66, *dd*, 7.8, 7.8	131.4	56 [16], 56 [18]
18	7.72, *d*, 7.8	117.2	56 [17], 56 [19]
19		148.4	59 [14], 56 [18]

[a] ^{13}C NMR chemical shifts are from Schliemann et al., *Phytochemistry* **2006**, *67*, 191-201 and may slightly deviate in the spectra obtained from the $^{13}CO_2$ experiment due to residual trifluoroacetic acid remaining from HPLC solvent.
[b] The numbers in parentheses indicate the coupling partners.

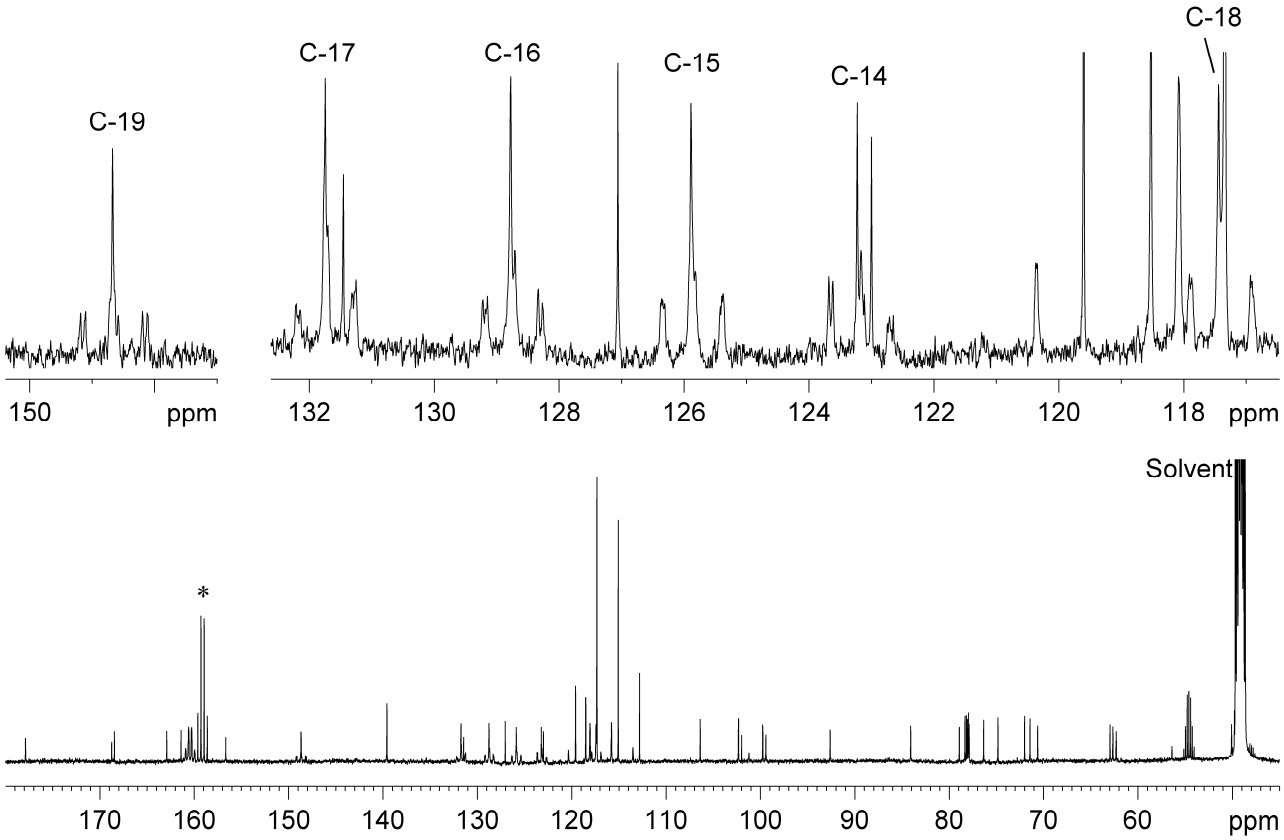

Figure S10. ^{13}C NMR spectrum (125 MHz, CD_3OD) of nudicaulin I obtained from the labeling experiment using [ring-$^{13}C_6$]anthranilic acid as a tracer. Specific ^{13}C-enrichment at C-14 to C-19 were 6-8% (calculated as described in S1, using the signal of C-2'/6' (δ 139.3) as a reference). Below: Full spectrum; above: partial spectra showing signals of ^{13}C-enriched positions. The asterisk (*) indicates the signal of trifluoroacetic acid.

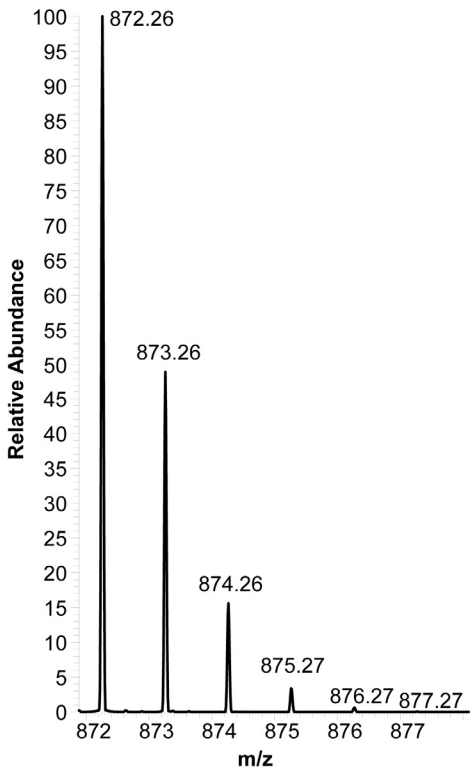

Figure S11. ESI-MS spectrum of nudicaulin I obtained from the labeling experiment with [^{15}N]anthranilic acid as a tracer.

Table S6. ESI-MS data of nudicaulin I obtained from the labeling experiment with [^{15}N]anthranilic acid as a tracer. For spectrum, see Figure S11.

m/z	Intensity	Relative intensity
872.26	4594081.5	100.00
873.26	2249753.0	48.97
874.27	716722.9	15.60
875.27	158632.5	3.45
876.27	28717.1	0.63
Calculated total ^{13}C enrichment: 1.7%		

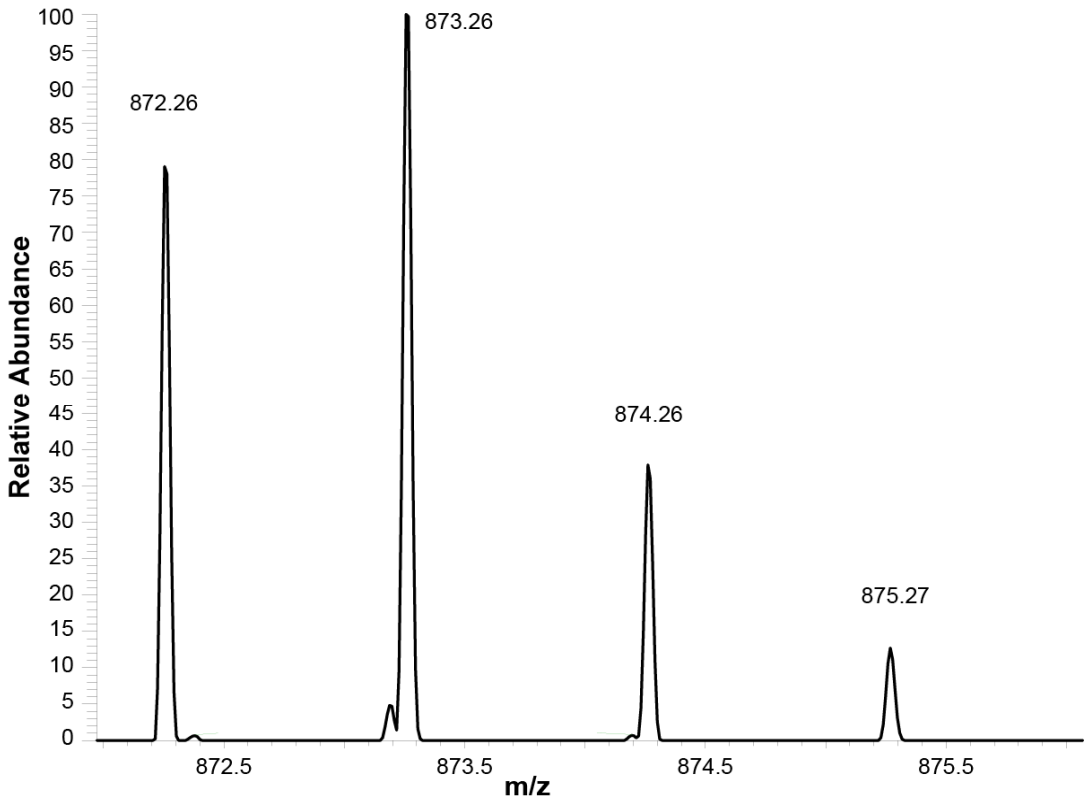

Figure S12. ESI-MS spectrum of nudicaulin I obtained from the labeling experiment with [2-^{13}C]indole as a tracer.

Table S7. ESI-MS data of nudicaulin I obtained from the labeling experiment with [2-^{13}C]indole as a tracer. For spectrum, see Figure S12.

m/z	Intensity	Relative intensity
872.26	16161.2	77.84
873.26	20517.4	100.00
874.27	7642.9	38.59
875.27	2524.8	10.65
Calculated total ^{13}C enrichment: 56.1%		

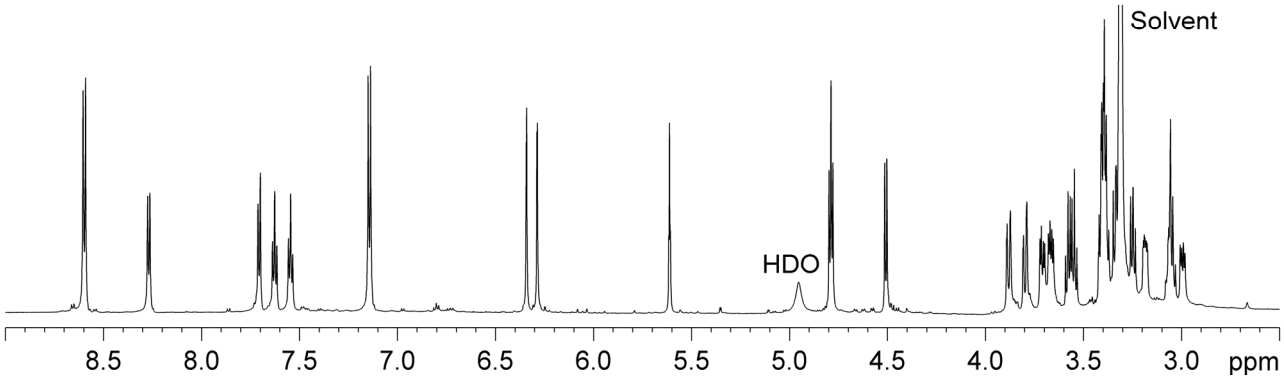

Figure S13. ^{1}H NMR spectrum (700 MHz, CD_3OD) of nudicaulin I obtained from the labeling experiment using [2-^{13}C]indole as a tracer.

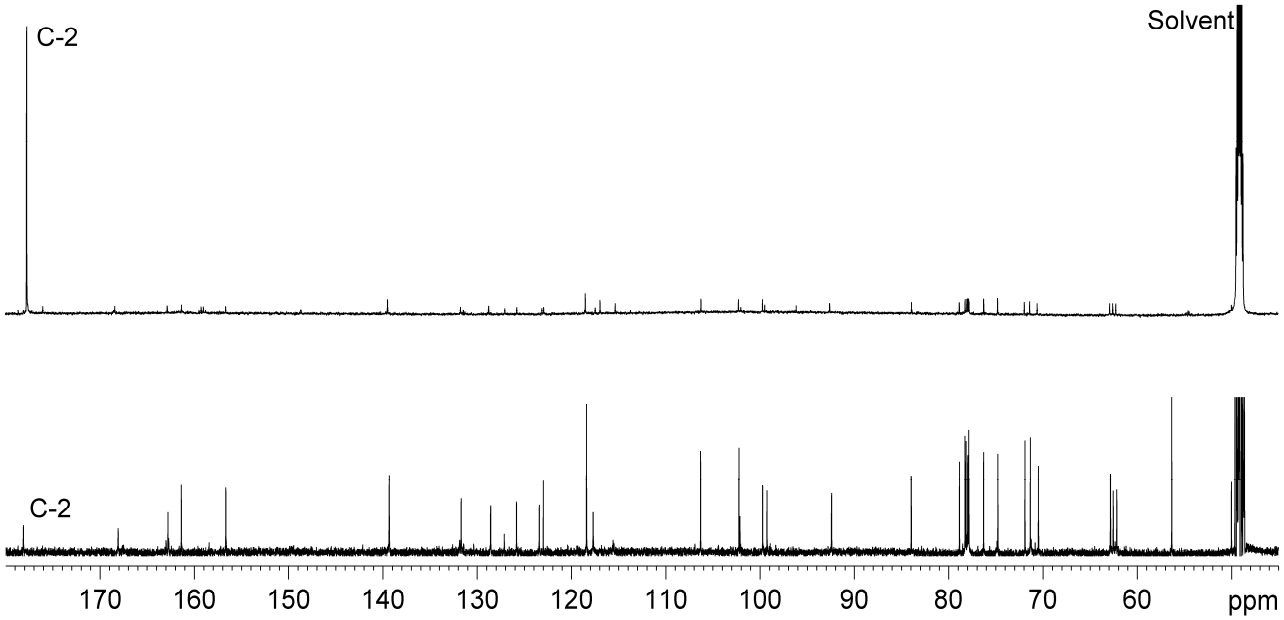

Figure S14. ^{13}C NMR spectra (175 MHz, CD_3OD) of nudicaulin I. Above: spectrum obtained from the labeling experiment using [2-^{13}C]indole as a tracer. Specific ^{13}C-enrichment at C-2 was 57% (calculated as described in S1, using the signal of C-2'/6' (δ 139.3) as a reference). Below: Spectrum of unlabeled nudicaulin I (reference).

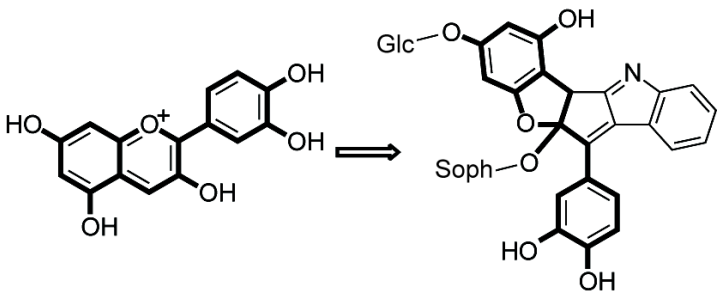

Figure S15. PDB experiment with cyanidin. The cyanidin scaffold is shown in bold;
Glc: β-glucosyl; Soph: β-sophorosyl.

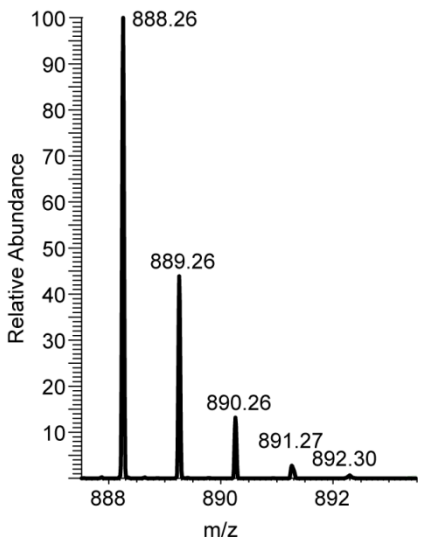

Figure S16. LC-ESI-MS (extracted ion chromatogram) of 3'-hydroxynudicaulin obtained from the PDB experiment with cyanidin.

Table S8. LC-ESI-MS data of 3'-hydroxynudicaulin obtained from the PDB experiment with cyanidin. For spectrum, see Figure S16.

m/z	Intensity	Relative intensity
888.26	522311.6	100.00
889.26	229375.1	43.92
890.26	68888.6	13.19
891.27	14429.4	2.76
892.30	3416.1	0.65

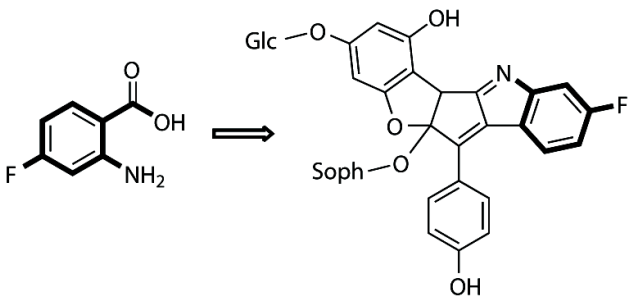

Figure S17. PDB experiment with 4-fluoroanthranilic acid. The 4-fluoroanthranilic acid scaffold is shown in bold; Glc: β-glucosyl; Soph: β-sophorosyl.

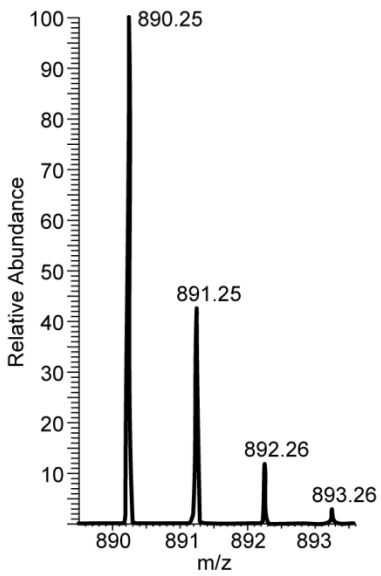

Figure S18. LC-ESI-MS (extracted ion chromatogram) spectrum of 17-fluoronudicaulin obtained from the PDB experiment with 4-fluoroanthranilic acid.

Table S9. LC-ESI-MS data of 17-fluoronudicaulin obtained from the PDB experiment with 4-fluoroanthranilic acid. For spectrum, see Figure S18.

m/z	Intensity	Relative intensity
890.25	127777.4	100.00
891.25	54556.9	42.70
892.26	15495.0	12.13
893.26	3680.5	2.88
894.27	457.7	0.36

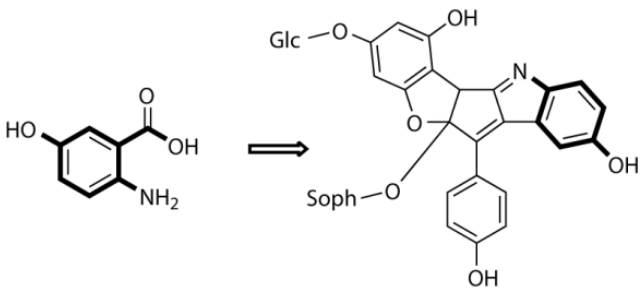

Figure S19. PDB experiment with 5-hydroxyanthranilic acid. The 5-hydroxyanthranilic acid scaffold is shown in bold; Glc: β-glucosyl; Soph: β-sophorosyl.

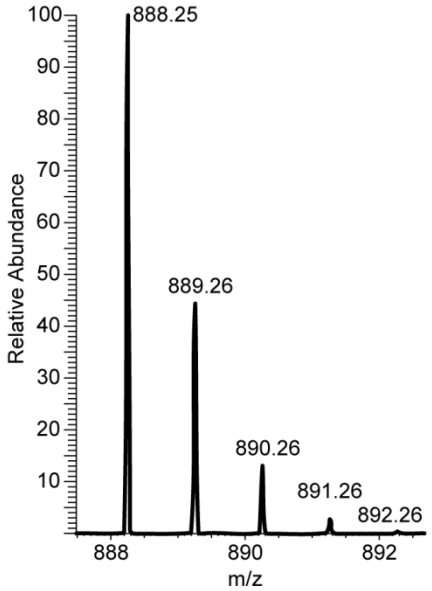

Figure S20. LC-ESI-MS (extracted ion chromatogram) spectrum of 16-hydroxynudicaulin obtained from the PDB experiment with 5-hydroxyanthranilic acid.

Table S10. LC-ESI-MS data of 16-hydroxynudicaulin obtained from the PDB experiment with 5-hydroxyanthranilic acid. For spectrum, see Figure S20.

m/z	**Intensity**	**Relative intensity**
888.25	277447.7	100.00
889.26	122378.8	44.11
890.26	36020.5	12.98
891.26	7544.3	2.72
892.26	1422.6	0.51

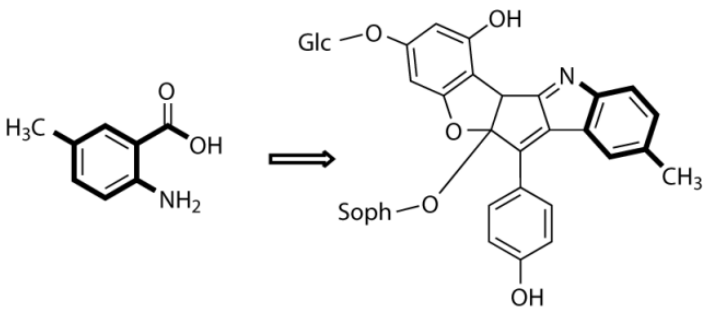

Figure S21. PDB experiment with 5-methylanthranilic acid. The 5-methylanthranilic acid scaffold is shown in bold; Glc: β-glucosyl; Soph: β-sophorosyl.

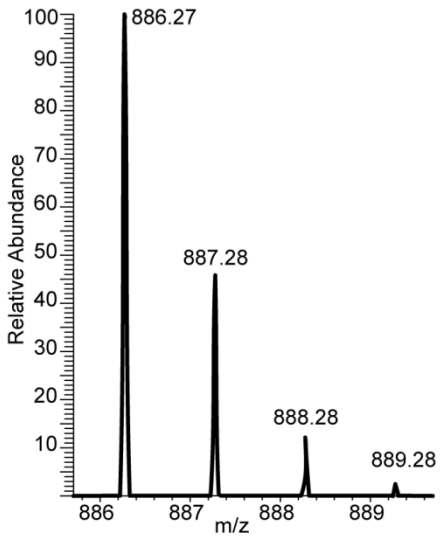

Figure S22. LC-ESI-MS (extracted ion chromatogram) spectrum of 16-methylnudicaulin obtained from the PDB experiment with 5-methylanthranilic acid.

Table S11. LC-ESI-MS data of 16-methylnudicaulin obtained from the PDB experiment with 5-methylanthranilic acid. For spectrum, see Figure S22.

m/z	Intensity	Relative intensity
886.27	35041.1	100.00
887.28	15588.1	44.49
888.28	4191.3	11.96
889.28	858.2	2.45

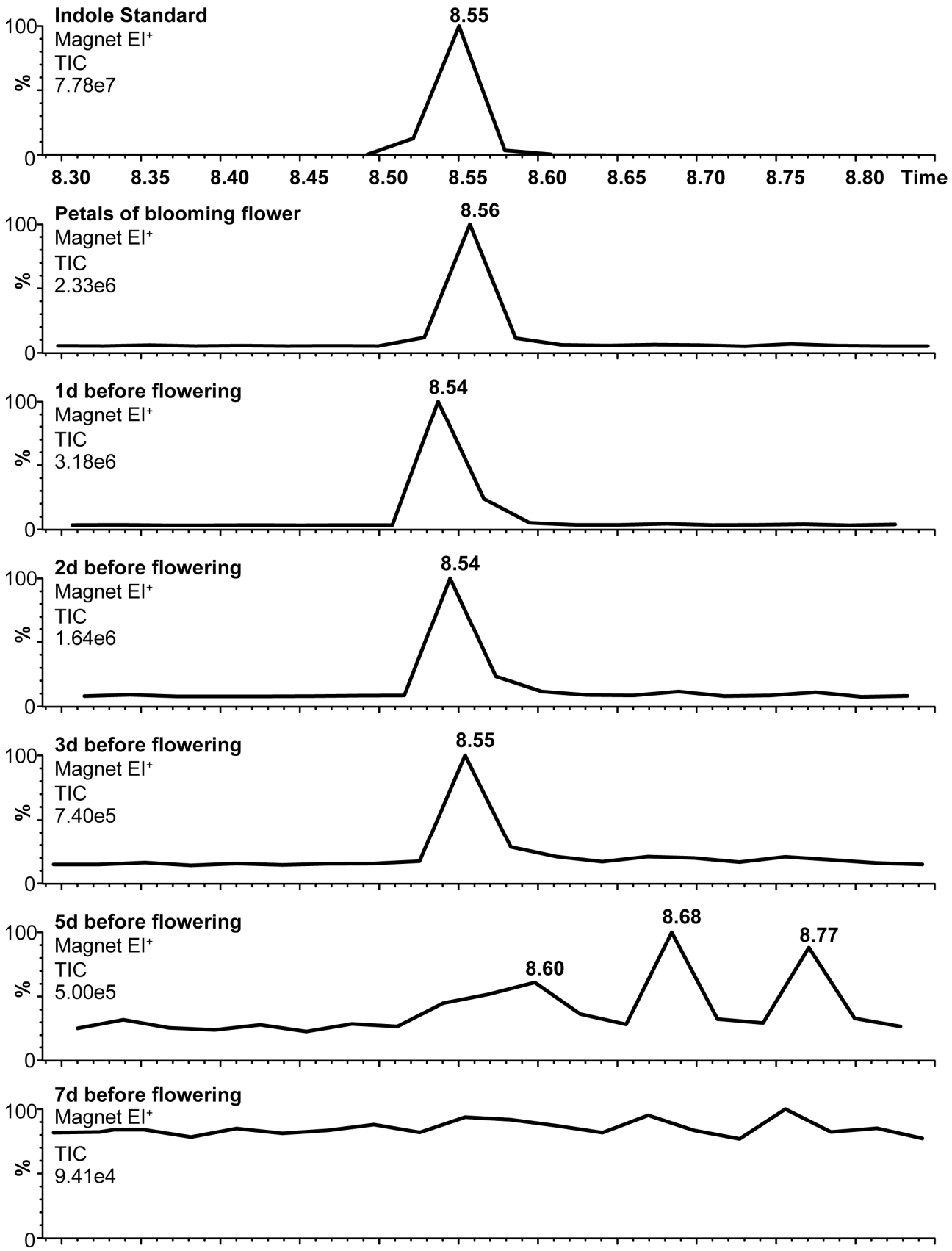

Figure S23. Detection of indole by GC-MS analysis (total ion chromatograms) of the petal extracts of yellow *P. nudicaule*. Petals were collected from buds at days 7, 5, 3, 2, and 1 before flowering and from the blooming flower.

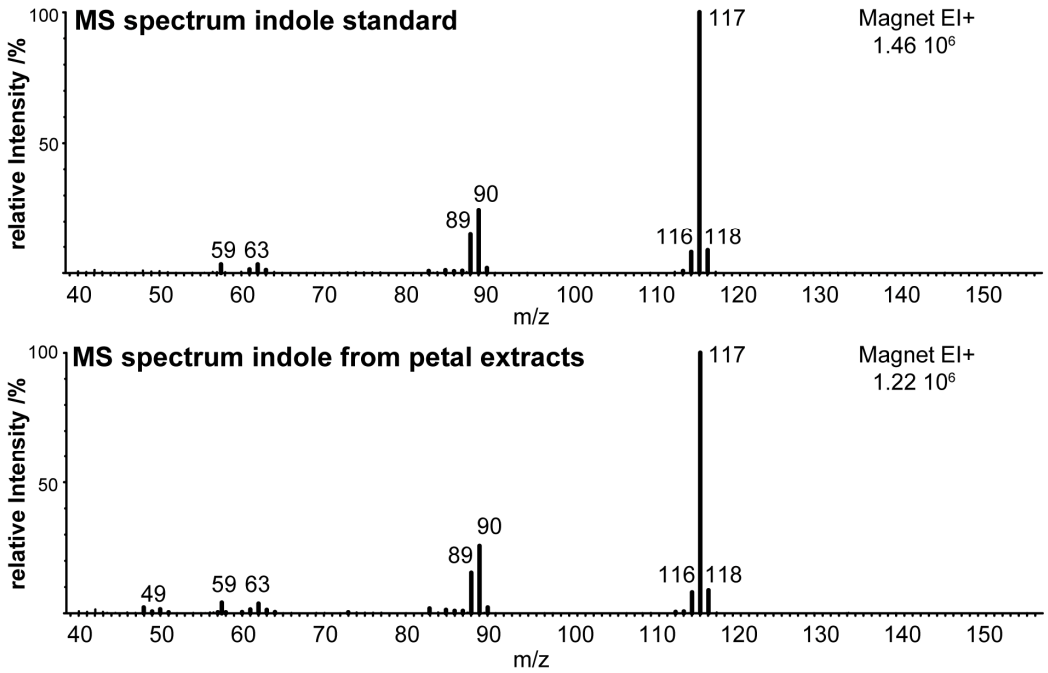

Figure S24. GC-EIMS spectra of indole (positive ionization). Above: Indole standard. Below: Indole from extracts of yellow petals of *P. nudicaule*.

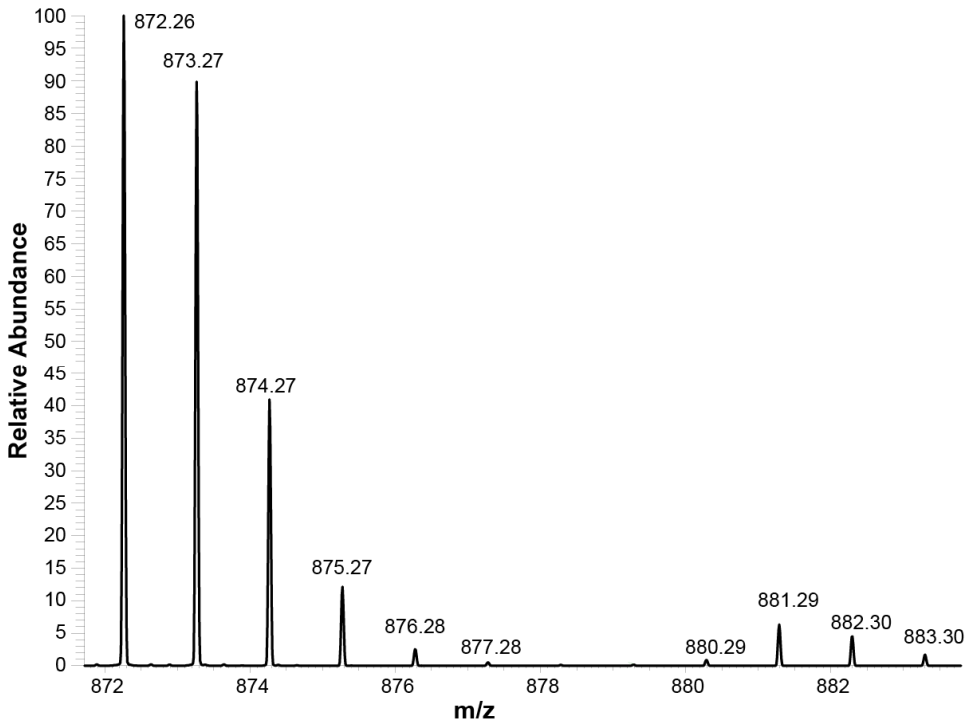

Figure S25. ESI-MS spectrum of nudicaulin I obtained from the labeling experiment with [$^{13}C_9$]L-phenylalanine as a tracer.

Table S12. ESI-MS data of nudicaulin I obtained from the labeling experiment with [$^{13}C_9$]L-phenylalanine as a tracer. For spectrum, see Figure S25.

m/z	Intensity	Relative intensity
872.26	1383446.6	100.00
873.26	1255039.1	90.61
874.27	569636.6	41.13
875.27	169974.2	12.25
876.27	35691.4	2.58
877.28	7088.2	0.51
880.29	12317.0	0.89
881.29	87749.8	6.33
882.29	64220.1	4.65
883.30	23668.8	1.71
Calculated total ^{13}C enrichment: 11.3%		

Table S13. ^{1}H and ^{13}C NMR spectroscopic data (500 MHz for ^{1}H; 125 MHz for ^{13}C) of nudicaulin I aglycone with ^{13}C abundances obtained from the labeling experiment using [$^{13}C_9$]L-phenylalanine as a tracer.

No.	^{1}H NMR	^{13}C NMR	Coupling constant
	δ, mult., J_{HH} (Hz)	δ[a]	J_{CC} (Hz) [coupled C][b]
Aglycone			
3	5.63, *s*	49.9	41 [11], 3 [12]
11		126.9	41 [3], 53 [12]
12		168.5	53 [11], 54 [1′]
1′		123.0	59 [2′/6′], 54 [12]
2′/6′	8.63, *d*, 9.0	139.3	59 [1′], 59 [3′/5′]
3′/5′	7.16, *d*, 9.0	118.3	59 [2′/6′], 61 [4′]
4′		168.3	61 [3′/ 5′], 9 [2′/6′]

[a] ^{13}C NMR chemical shifts are from Schliemann et al., *Phytochemistry* **2006**, *67*, 191-201 and may slightly deviate in the spectra obtained from the $^{13}CO_2$ experiment due to residual trifluoroacetic acid remaining from HPLC solvent.
[b] The numbers in parentheses indicate the coupling partners.

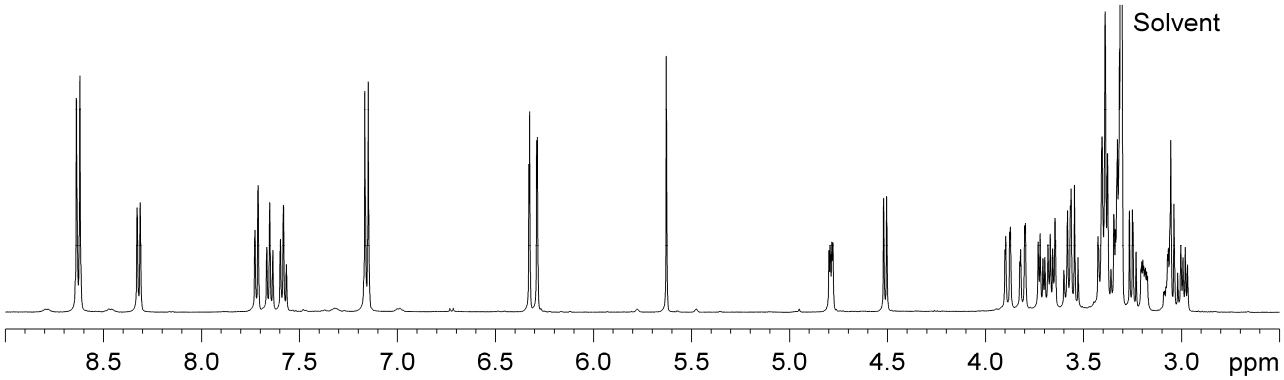

Figure S26. ^{1}H NMR spectrum (500 MHz, CD_3OD) of nudicaulin I obtained from the labeling experiment using [$^{13}C_9$]L-phenylalanine as a tracer.

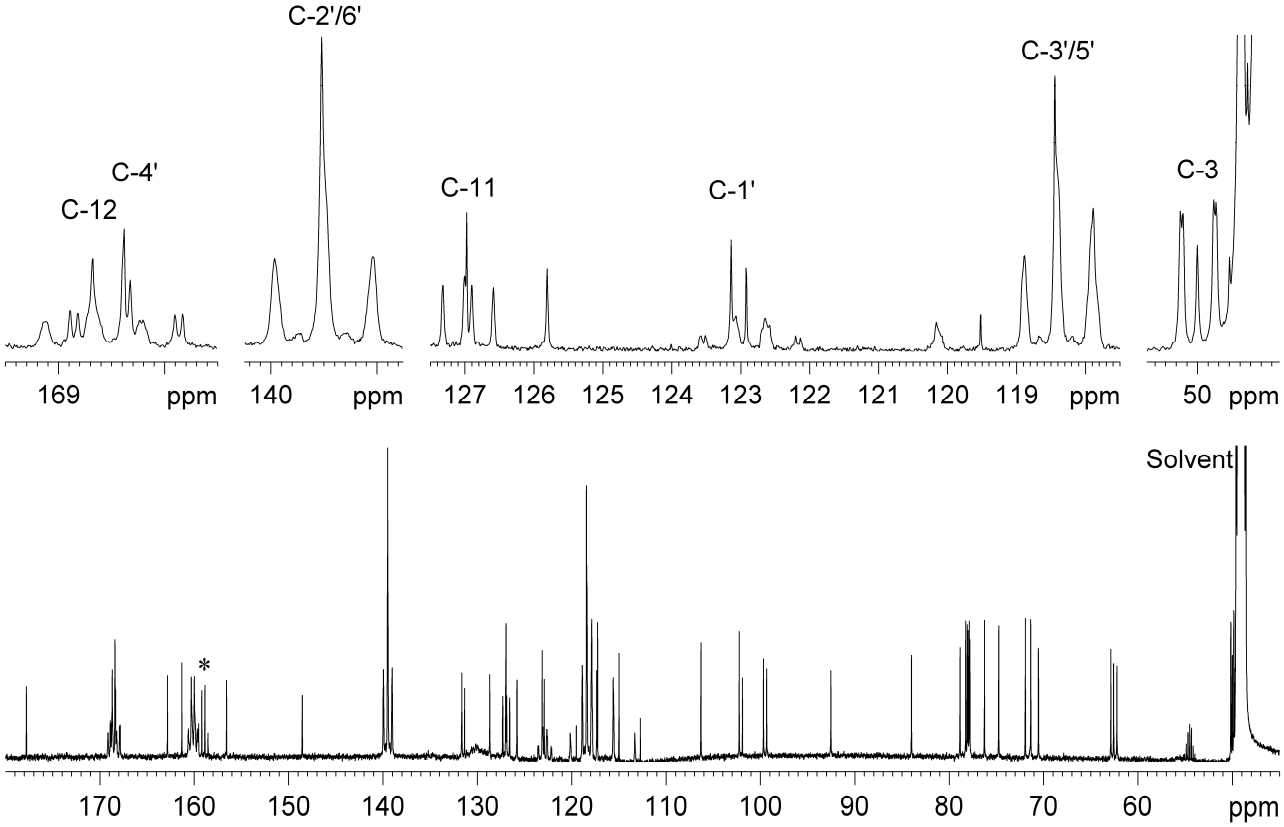

Figure S27. ^{13}C NMR spectrum (125 MHz, CD_3OD) of nudicaulin I obtained from the labeling experiment using [$^{13}C_9$]L-phenylalanine as a tracer. Below: Full spectrum; above: partial spectra showing signals of ^{13}C-enriched positions. Specific ^{13}C-enrichment at C-3, C-11, C-12 and C-1' to C-6' were 4-8% (calculated as described in S2, using the signal of C-15 (δ 125.6) as a reference). The asterisk (*) indicates the signal of trifluoroacetic acid.

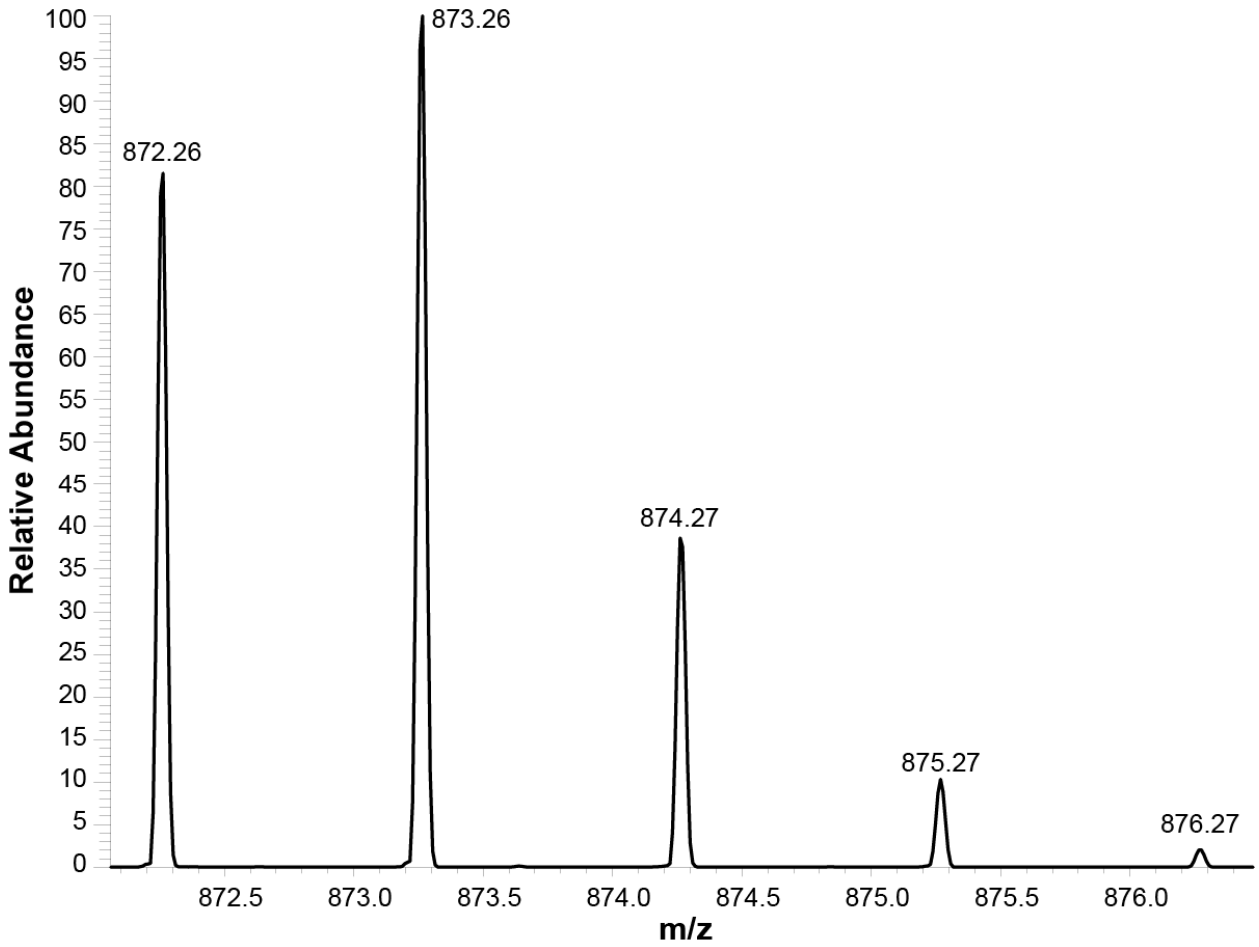

Figure S28. ESI-MS spectrum of nudicaulin I obtained from the labeling experiment with [2-^{13}C]L-tyrosine as a tracer.

Table S14. ESI-MS data of nudicaulin I obtained from the labeling experiment with [2-^{13}C]L-tyrosine as a tracer. For spectrum, see Figure S28.

m/z	Intensity	Relative intensity
872.26	1383446.6	74.65
873.26	1255039.1	100.00
874.27	569636.6	38.06
875.27	169974.2	10.16
876.27	35691.4	2.25
Calculated total ^{13}C enrichment: 56.8%		

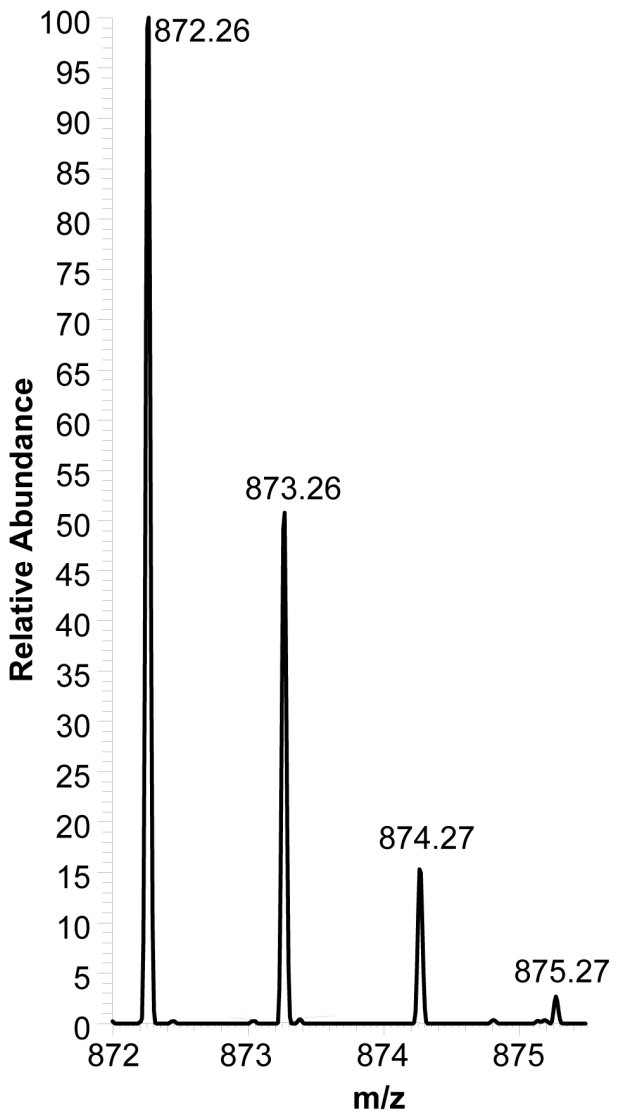

Figure S29. ESI-MS spectrum of nudicaulin I obtained from the labeling experiment with [2-^{13}C]*p*-coumaric acid as a tracer.

Table S15. ESI-MS data of nudicaulin I obtained from the labeling experiment with [2-^{13}C]*p*-coumaric acid as a tracer. For spectrum, see Figure S29.

m/z	Intensity	Relative intensity
872.26	12117.9	100.00
873.26	6466.6	53.36
874.27	1794.7	14.81
875.27	565.3	3.11
Calculated total ^{13}C enrichment: 4.7%		

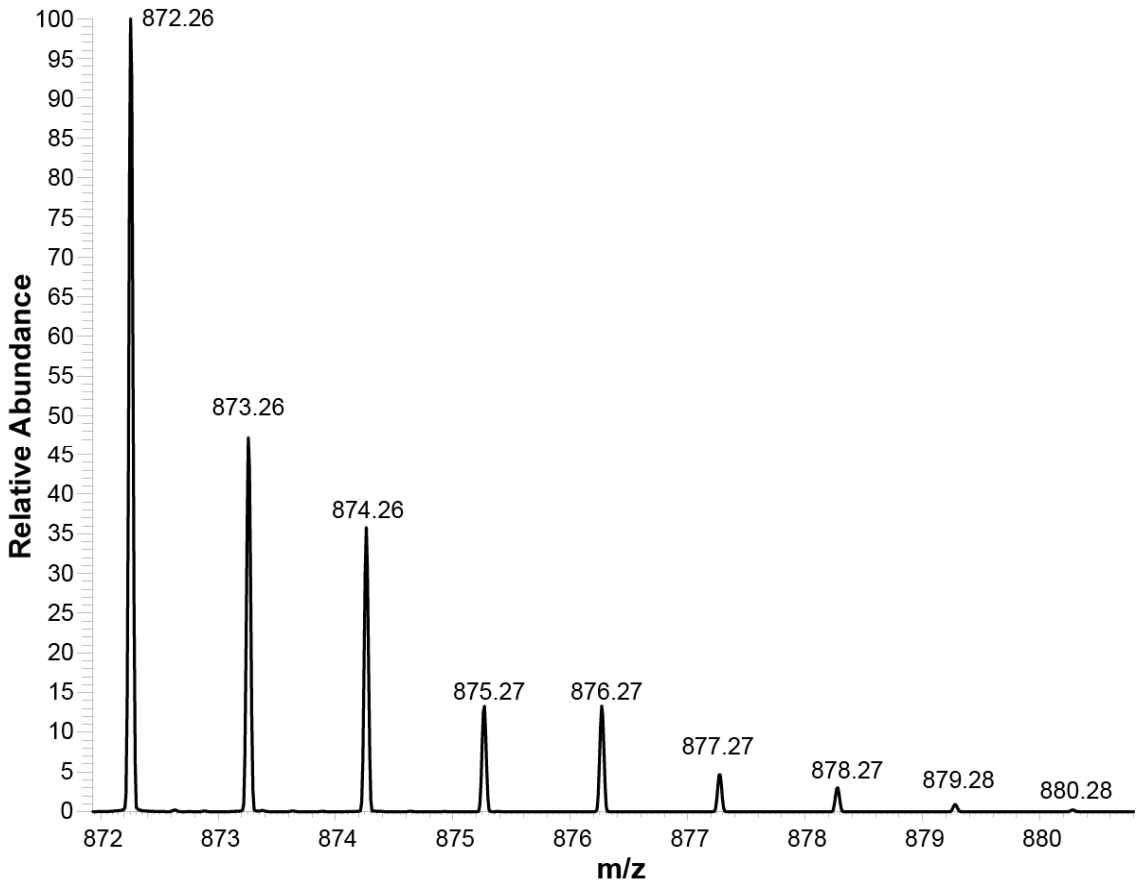

Figure S30. ESI-MS spectrum of nudicaulin I obtained from the labeling experiment with [$^{13}C_2$]acetate as a tracer.

Table S16. ESI-MS data of nudicaulin I obtained from the labeling experiment with [$^{13}C_2$]acetate as a tracer. For spectrum, see Figure S30.

m/z	Intensity	Relative intensity
872.26	2998524.8	100.00
873.26	1426116.8	47.56
874.27	1078839.0	35.98
875.27	402629.3	13.43
876.27	401013.7	13.37
877.27	142641.9	4.76
878.27	95178.7	3.17
879.27	28458.4	0.95
880.27	6861.0	0.23
Calculated total ^{13}C enrichment: 13.4%		

Table S17. ^{1}H and ^{13}C NMR spectroscopic data (500 MHz for ^{1}H; 125 MHz for ^{13}C) of nudicaulin I aglycone with ^{13}C abundances obtained from the labeling experiment using [$^{13}C_2$]acetate as a tracer.

No	^{1}H NMR	^{13}C NMR	Coupling constant
	δ, mult., J_{HH} (Hz)	δ[a]	J_{CC} (Hz) [coupled C][b]
4		101.8	77 [5], 63 [9]
5		156.4	77 [4], 66 [6], 5 [9]
6	6.33, *d*, 2.0	99.3	66 [5], 71 [7]
7		162.6	71 [6], 71 [8]
8	6.29, *d*, 2.0	92.4	71 [7], 71 [9]
9		161.1	71 [8], 63 [4], 5 [5]

[a] ^{13}C NMR chemical shifts are from Schliemann et al., *Phytochemistry* **2006**, *67*, 191-201 and may slightly deviate in the spectra obtained from the $^{13}CO_2$ experiment due to residual trifluoroacetic acid remaining from HPLC solvent.
[b] The numbers in parentheses indicate the coupling partners. Due to alternative cyclization between C-4 and C-5 or between C-4and C-9, bond labeling of C-5/C-6, C-7/C-8, C-9/C-4 and C-4/C-5, C-6/C-7, C-9/C-4 was observed.

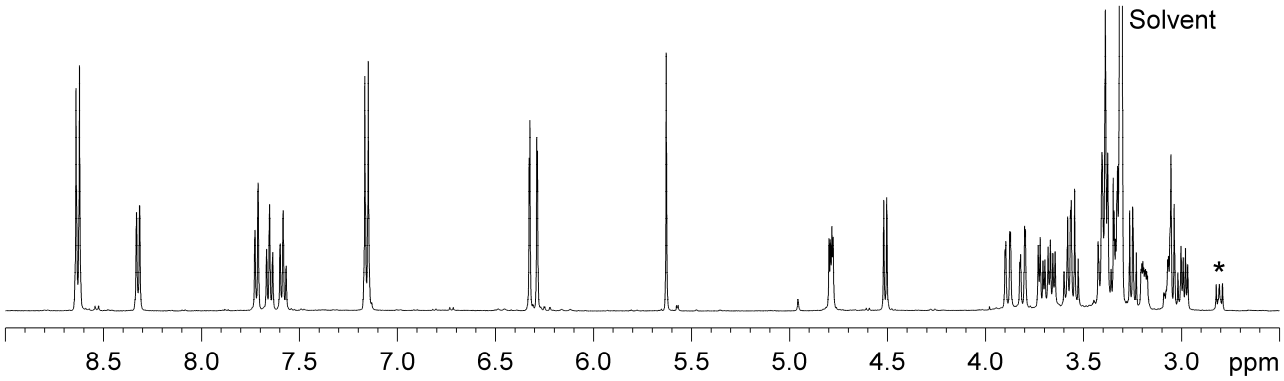

Figure S31. ^{1}H NMR spectrum (500 MHz, CD_3OD) of nudicaulin I obtained from the labeling experiment using [$^{13}C_2$]acetate as a tracer. The asterisk (*) indicates the signal of a contamination.

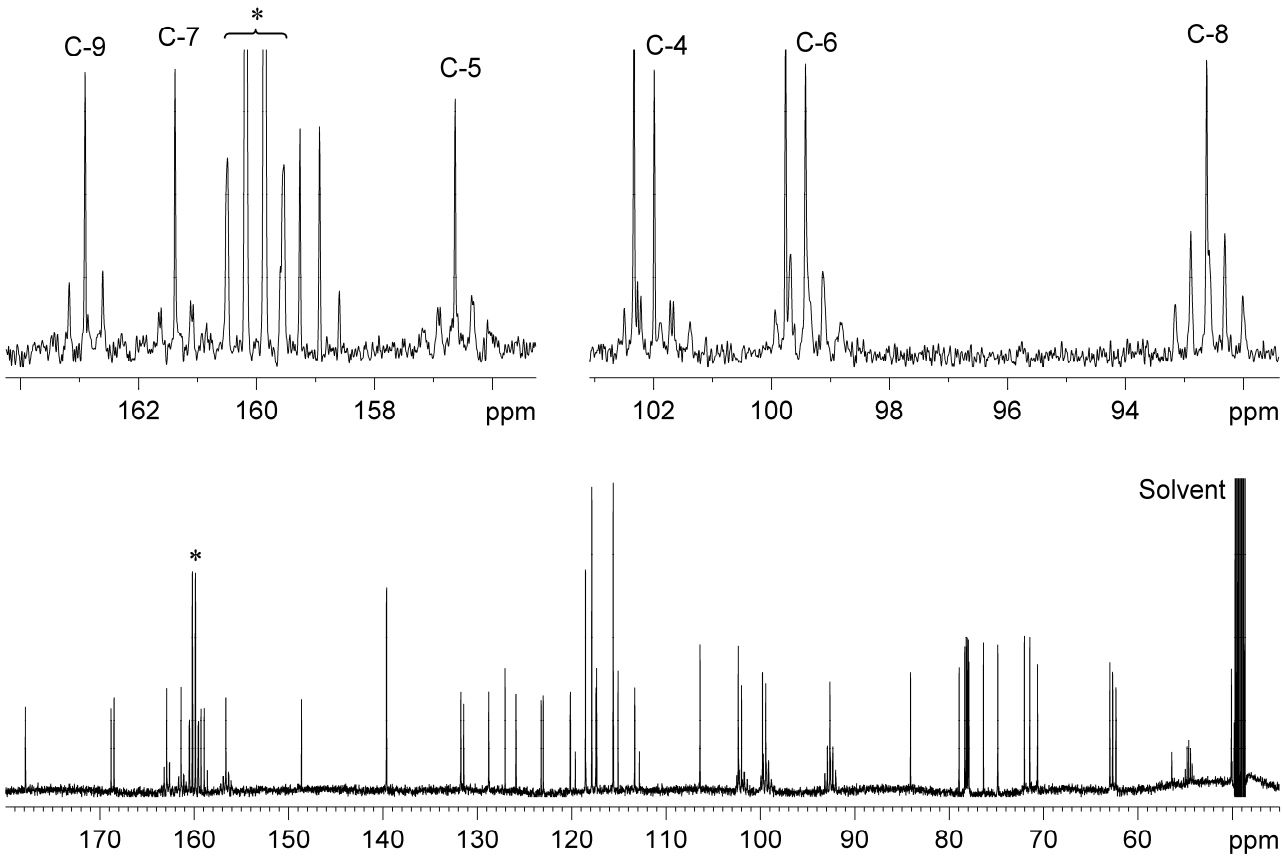

Figure S32. ^{13}C NMR spectrum (125 MHz, CD_3OD) of nudicaulin I obtained from the labeling experiment using [$^{13}C_2$]acetate as a tracer. Full spectrum, see below; partial spectra showing signals of ^{13}C-enriched positions, see above. Specific ^{13}C-enrichment at C-4 - C-9 were 3-5% (calculated as described in S1, using the signal of C-2'/6' (δ 139.3) as a reference). The asterisk (*) indicates the signal of trifluoroacetic acid.

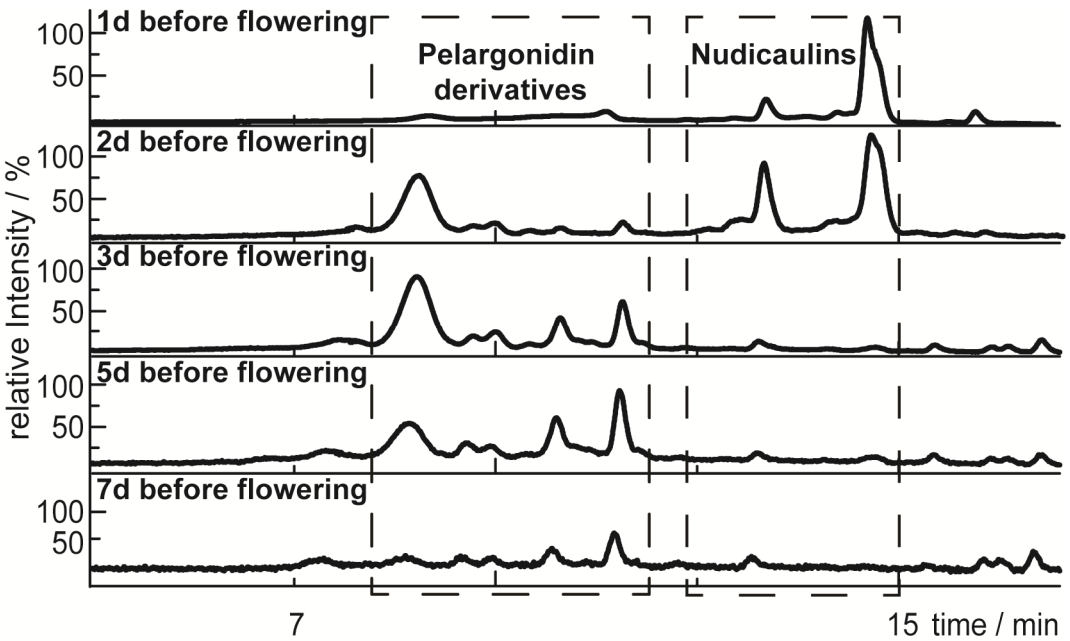

Figure S33. HPLC profiles (UV 460 nm) of petal extracts of yellow *P. nudicaule*. Petals were collected from buds at days 7, 5, 3, 2, and 1 before flowering.