Formation of Nudicaulins In Vivo and In Vitro and the Biomimetic Synthesis and Bioactivity of *O*-Methylated Nudicaulin Derivatives

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† These authors contributed equally to this work.

Supplementary Materials

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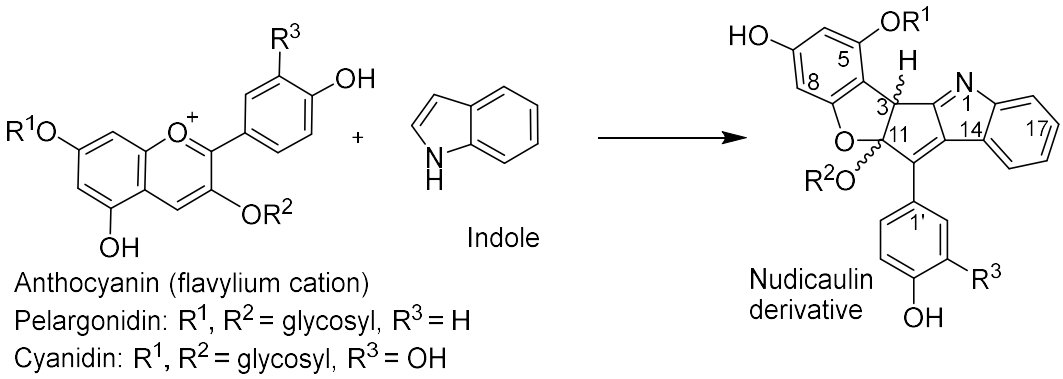
1. Nudicaulin formation



Anthocyanin (flavylium cation)

Pelargonidin: R^1, R^2 = glycosyl, R^3 = H

Cyanidin: R^1, R^2 = glycosyl, R^3 = OH

Scheme S1: Formation of nudicaulin derivatives from pelargonidin glucosides in *P. nudicaule* petals. Cyanidin, which possesses one additional hydroxyl group, can also form nudicaulin derivatives.

2. NMR spectra

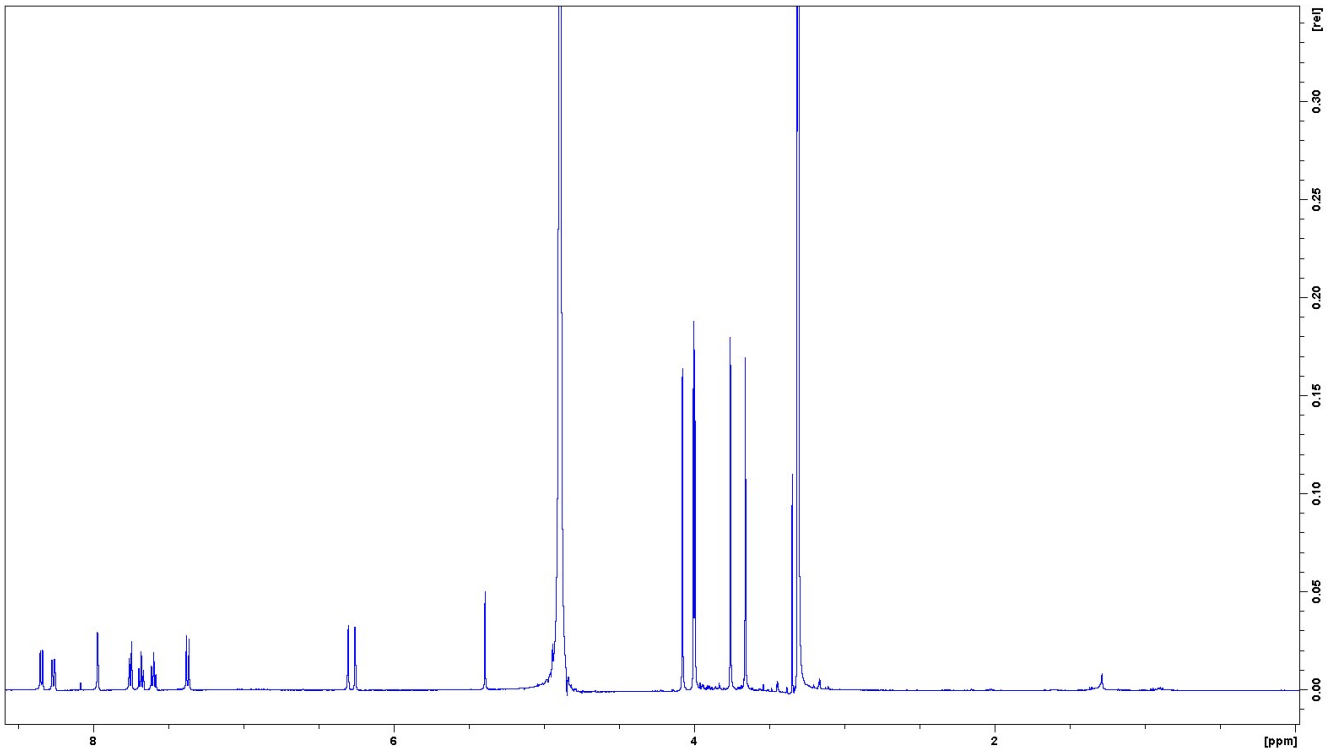


Figure S1: 5,7,11,3‘,4‘-Penta-*O*-methylnudicaulin (**6**), ^{1}H-NMR spectrum (500 MHz, 1% TFA - MeOH-d_4)

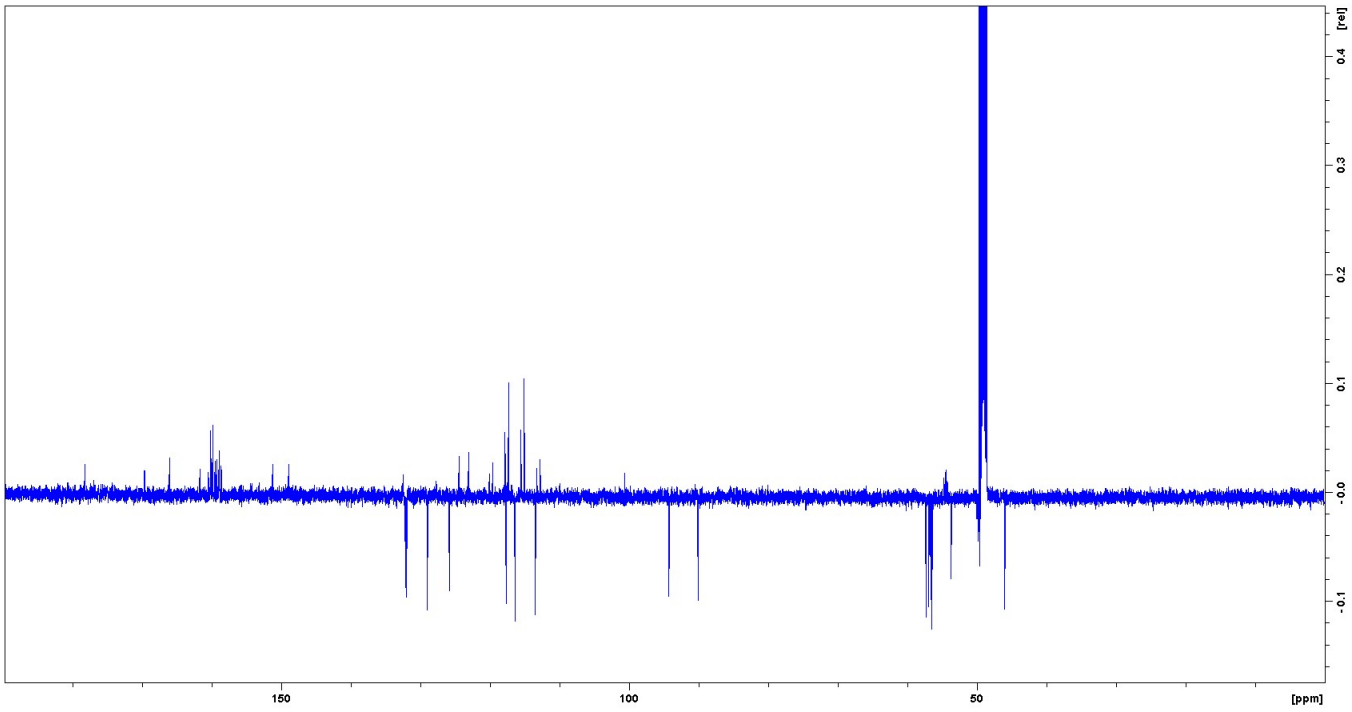


Figure S2: 5,7,11,3‘,4‘-Penta-*O*-methylnudicaulin (**6**), ^{13}C-NMR spectrum (500 MHz, 1% TFA - MeOH-d_4)

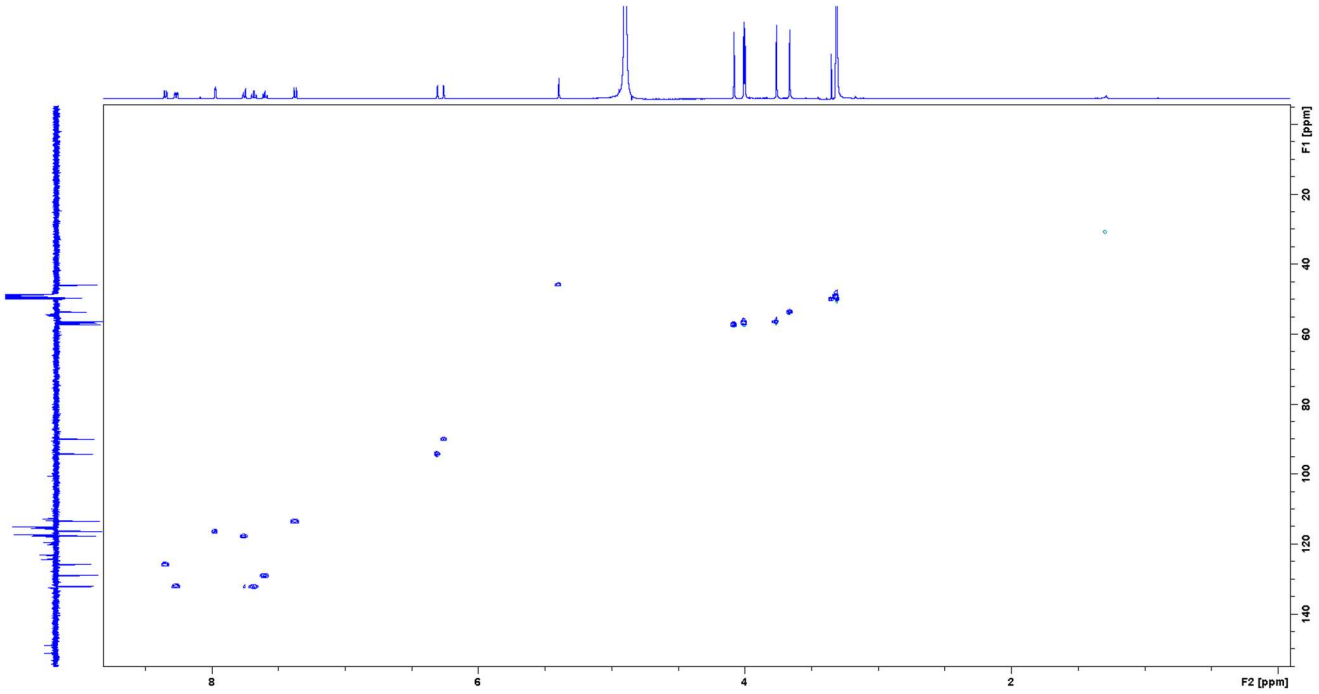


Figure S3: 5,7,11,3‘,4‘-Penta-*O*-methylnudicaulin (**6**), ^{1}H-^{13}C HSQC spectrum (500 MHz, 1% TFA - MeOH-d_4)

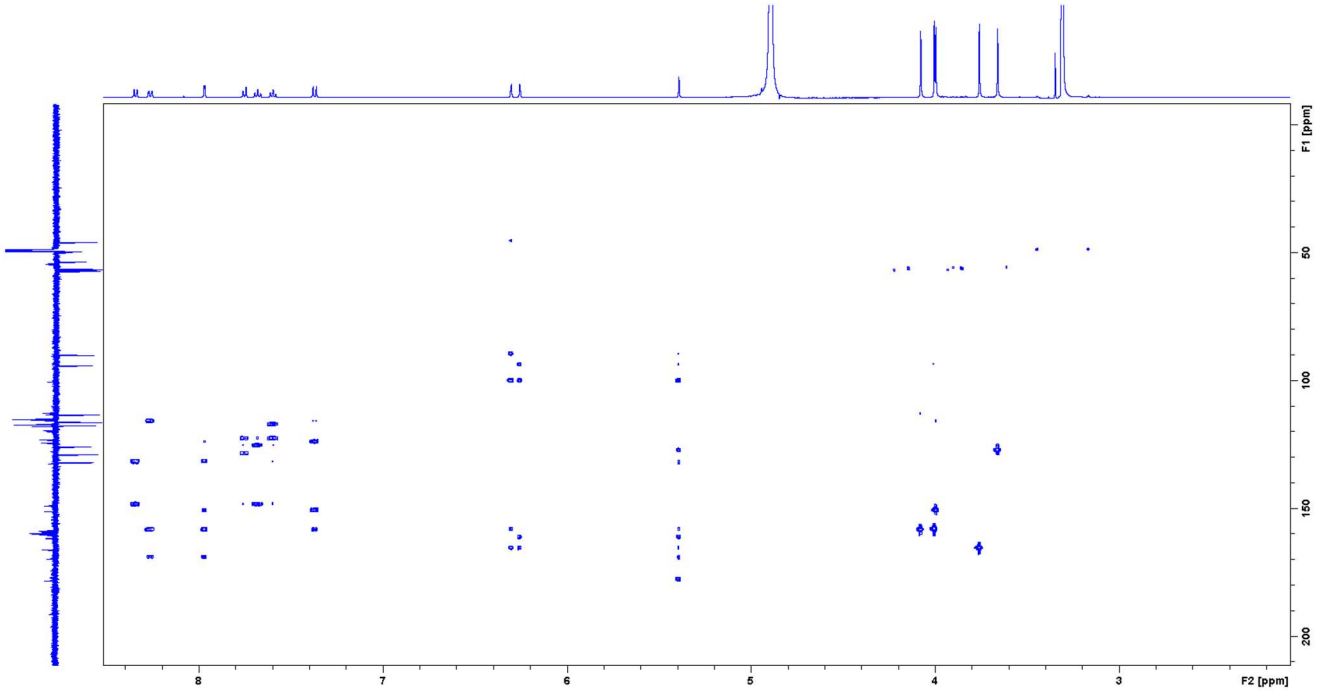


Figure S4: 5,7,11,3‘,4‘-Penta-*O*-methylnudicaulin (**6**), ^{1}H-^{13}C HMBC spectrum (500 MHz, 1% TFA - MeOH-d_4)

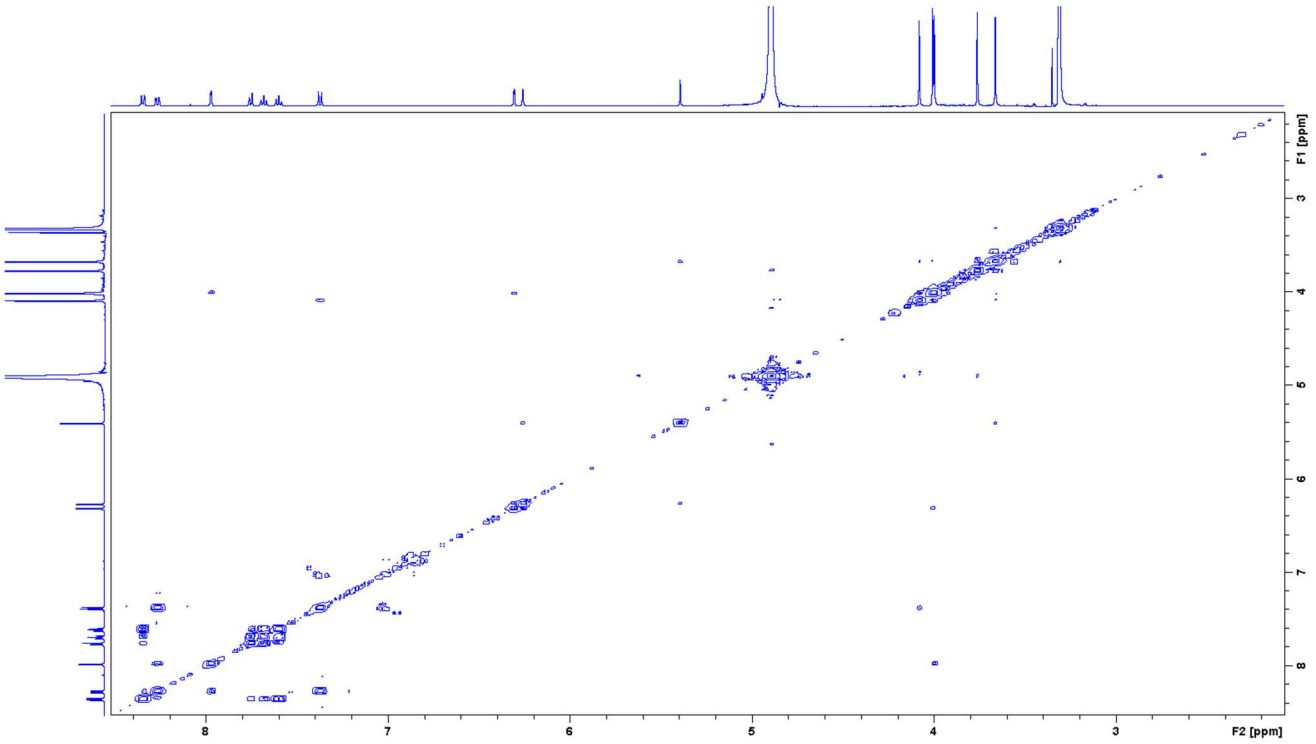


Figure S5: 5,7,11,3‘,4‘-Penta-*O*-methylnudicaulin (**6**), ^{1}H-^{1}H COSY spectrum (500 MHz, 1% TFA- MeOH-d_4)

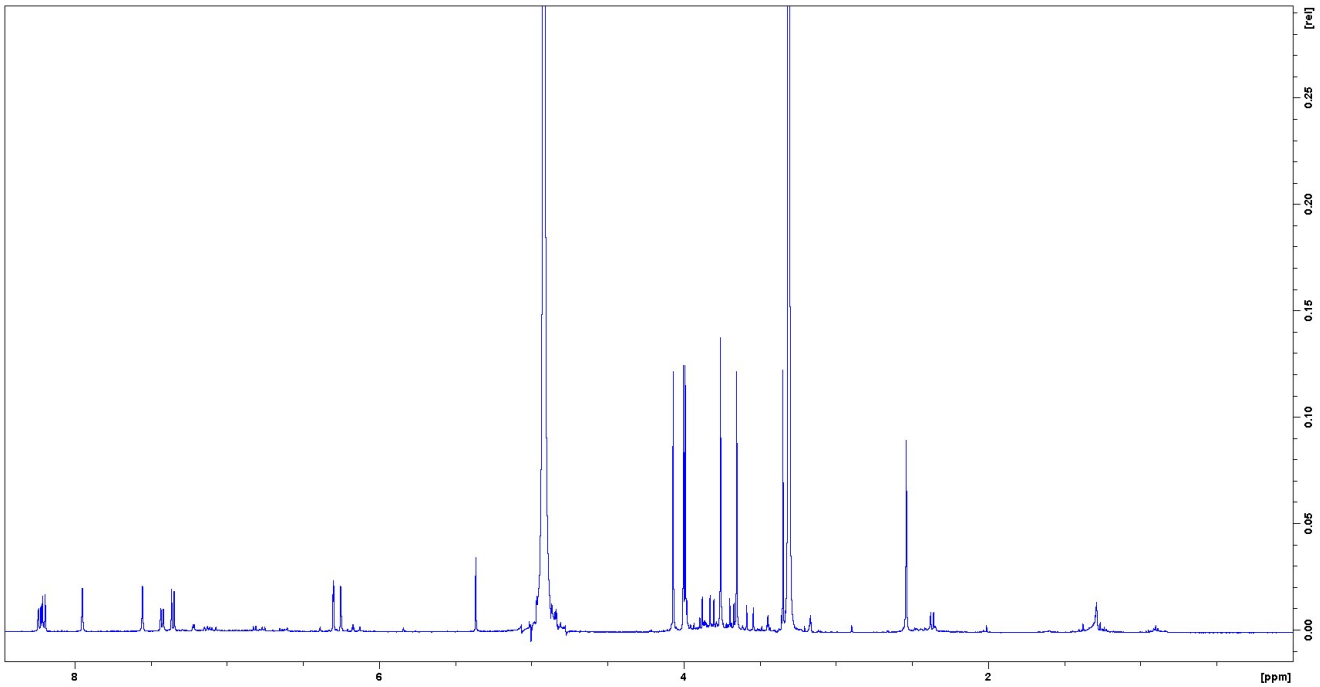


Figure S6: 16-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**7**), ^{1}H-NMR spectrum (500 MHz, 1% TFA - MeOH-d_4)

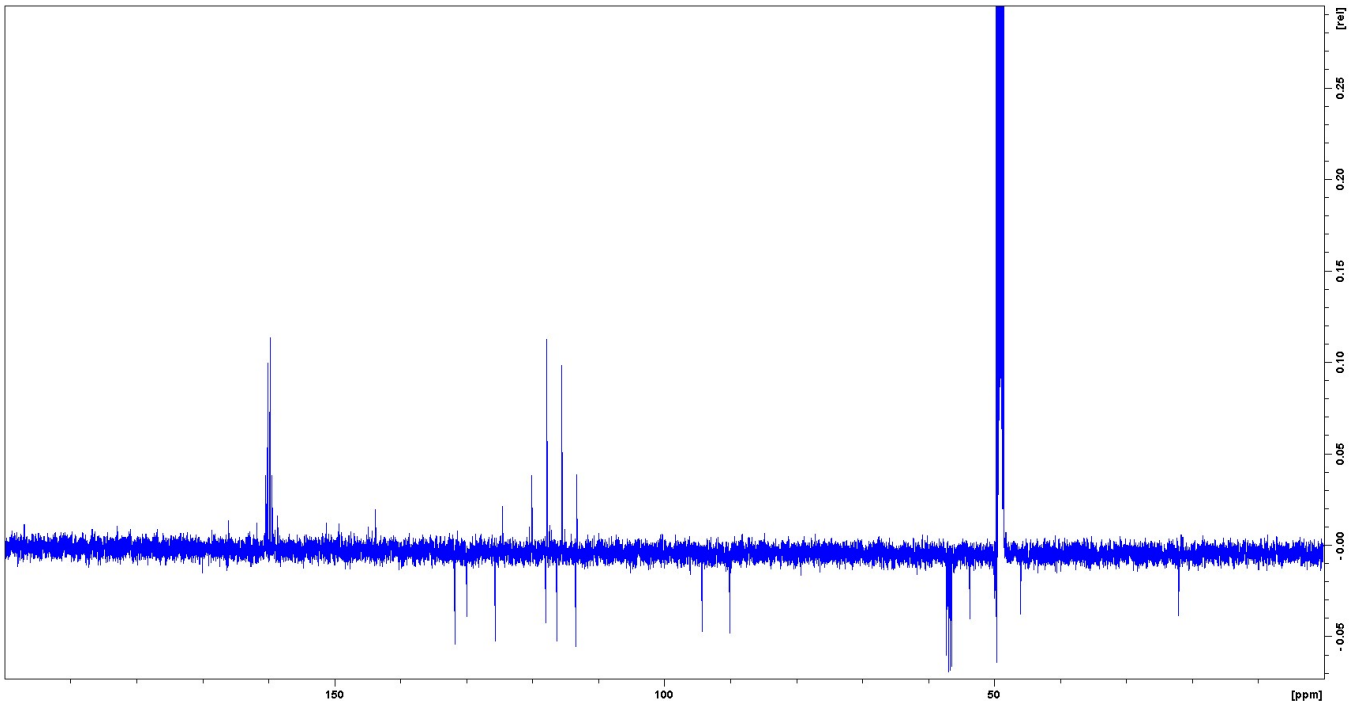


Figure S7: 16-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**7**), ^{13}C-NMR spectrum (500 MHz, 1% TFA - MeOH-d_4)

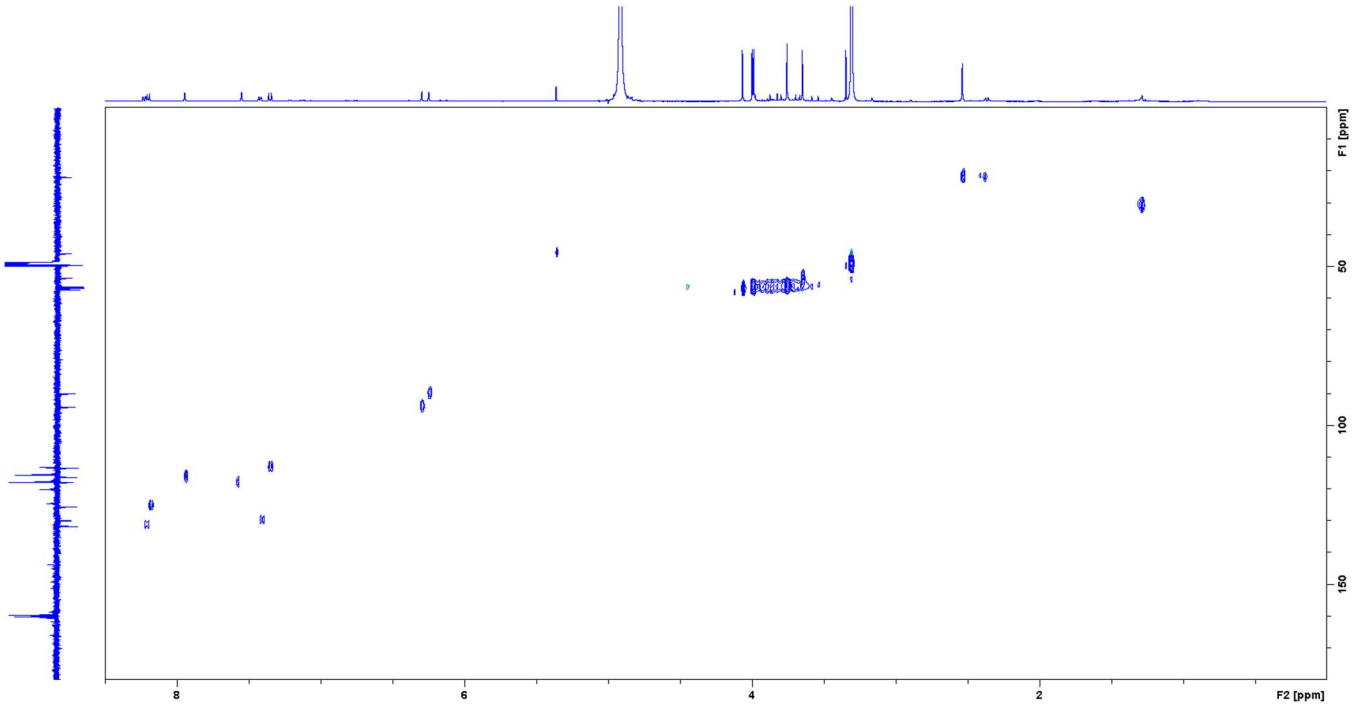


Figure S8: 16-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**7**), ^{1}H-^{13}C HSQC spectrum (500 MHz, 1% TFA - MeOH-d_4)

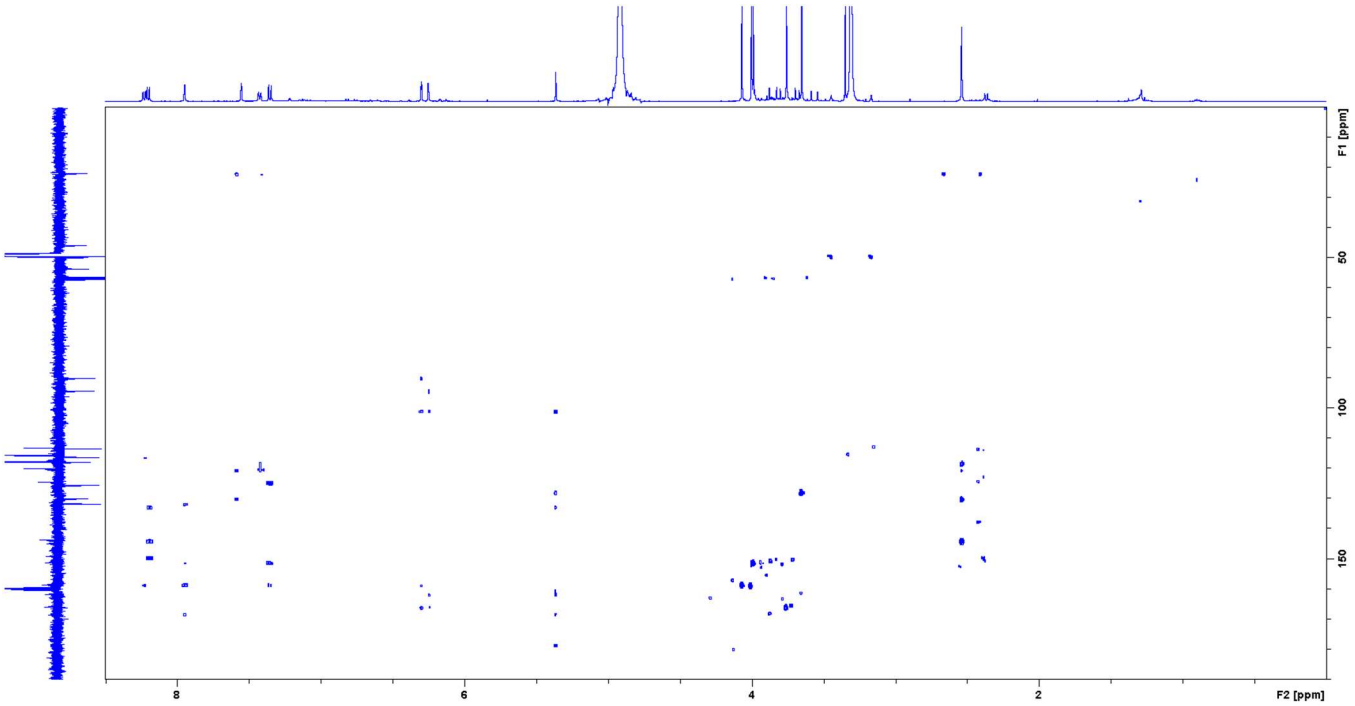


Figure S9: 16-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**7**), ^{1}H-^{13}C HMBC spectrum (500 MHz, 1% TFA - MeOH-d_4)

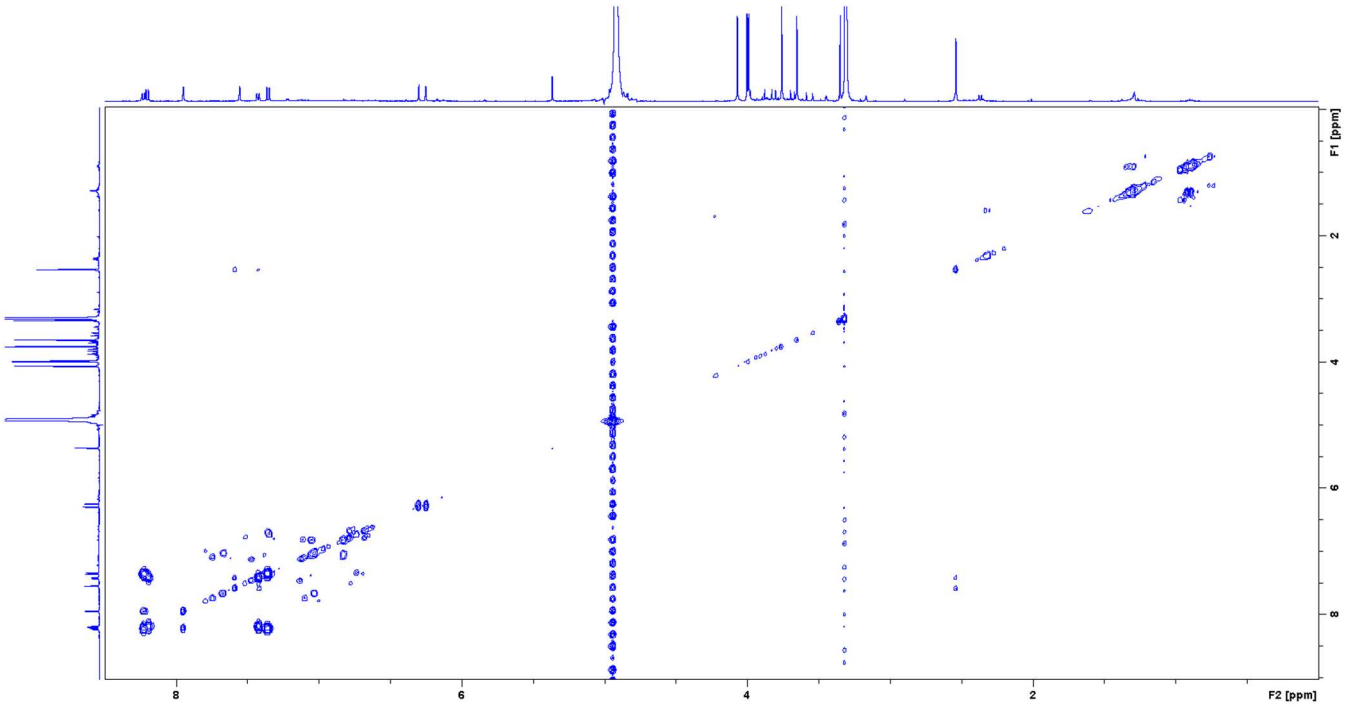


Figure S10: 16-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**7**), ^{1}H-^{1}H COSY spectrum (500 MHz, 1% TFA - MeOH-d_4

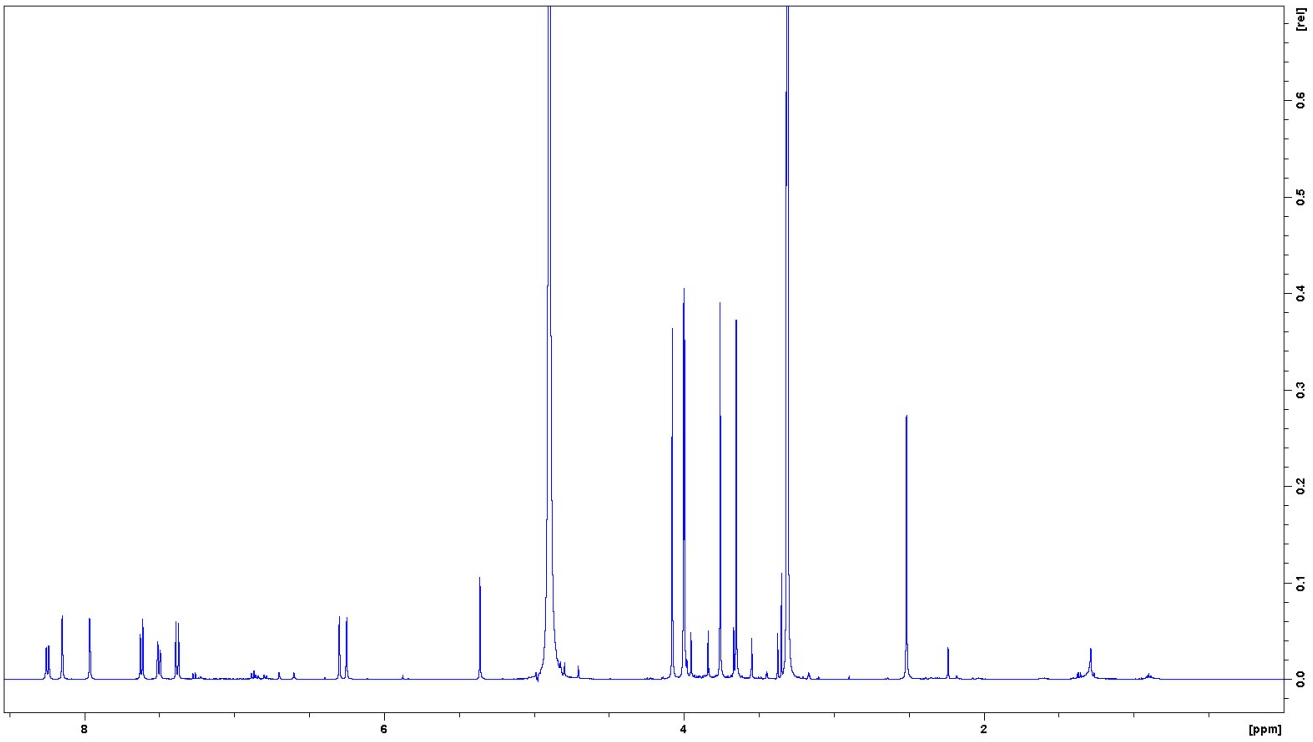


Figure S11: 17-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**8**), ^{1}H-NMR spectrum (500 MHz, 1% TFA - MeOH-d_4)

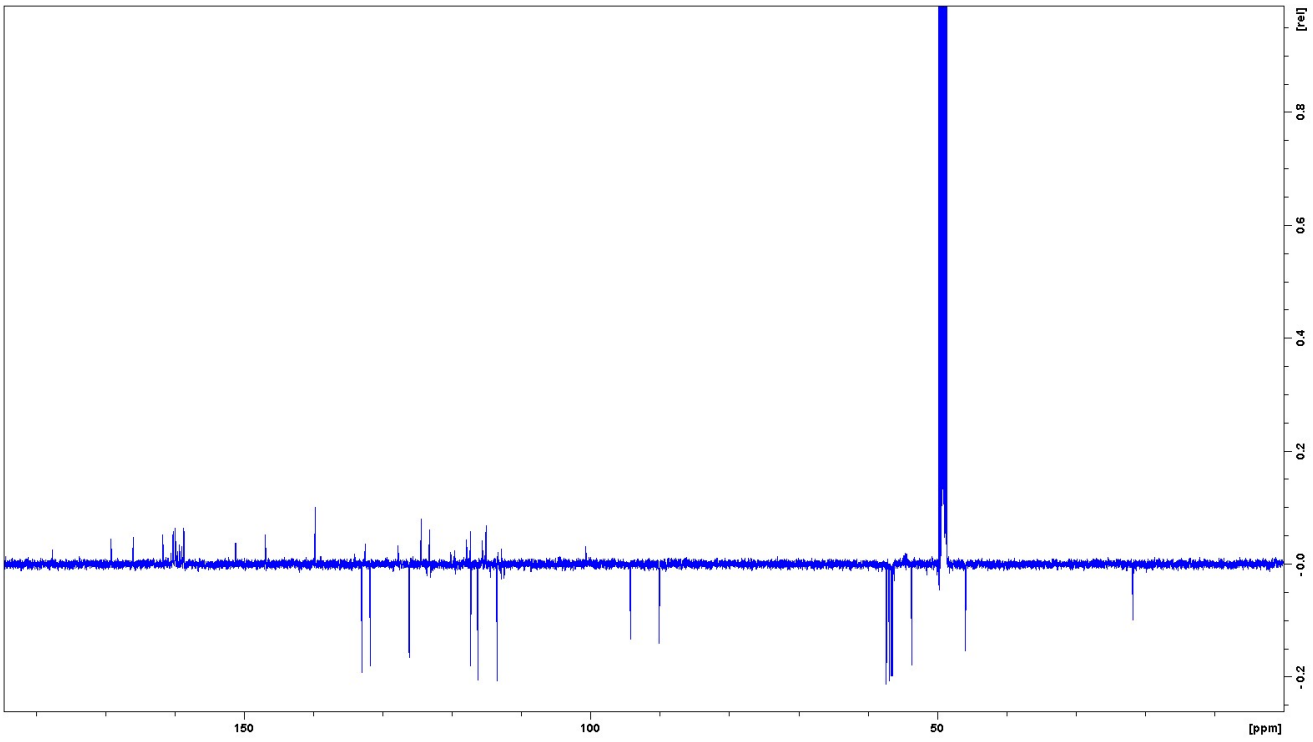


Figure S12: 17-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**8**), ^{13}C-NMR spectrum (500 MHz, 1% TFA - MeOH-d_4)

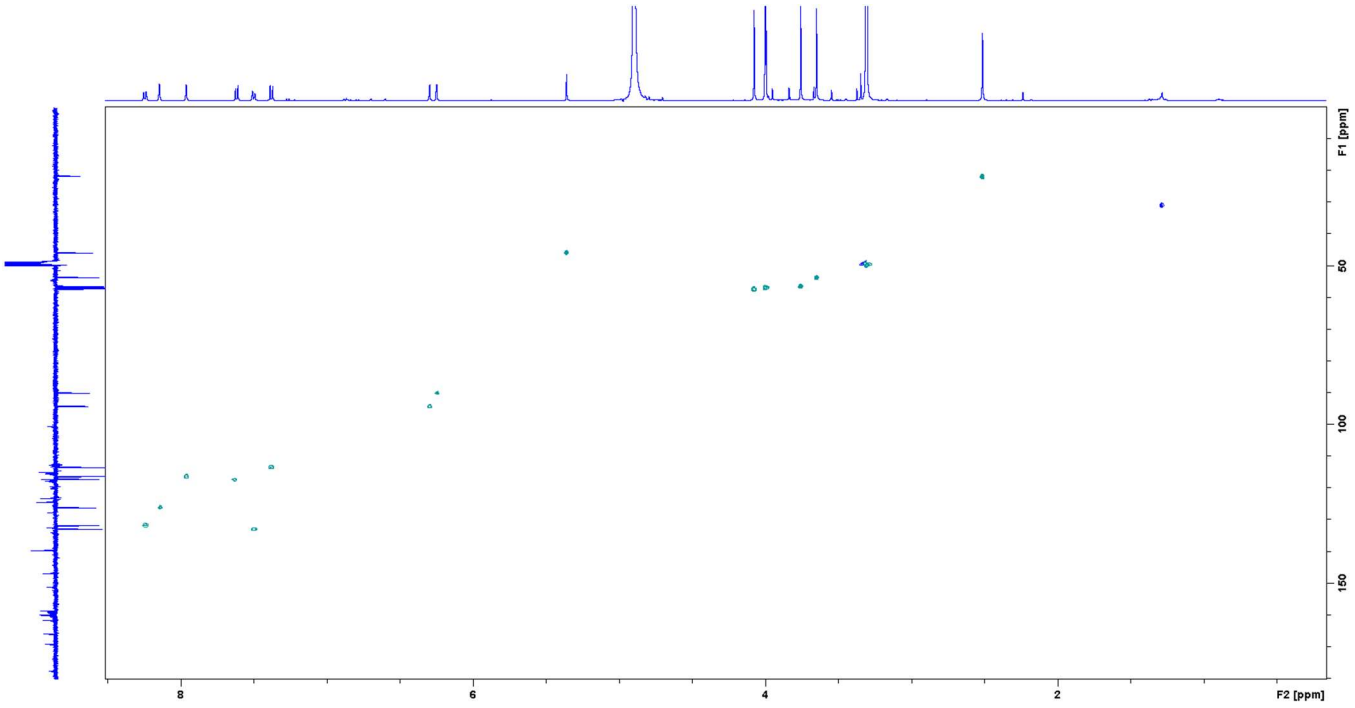


Figure S13: 17-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**8**), ^{1}H-^{13}C HSQC spectrum (500 MHz, 1% TFA - MeOH-d_4)

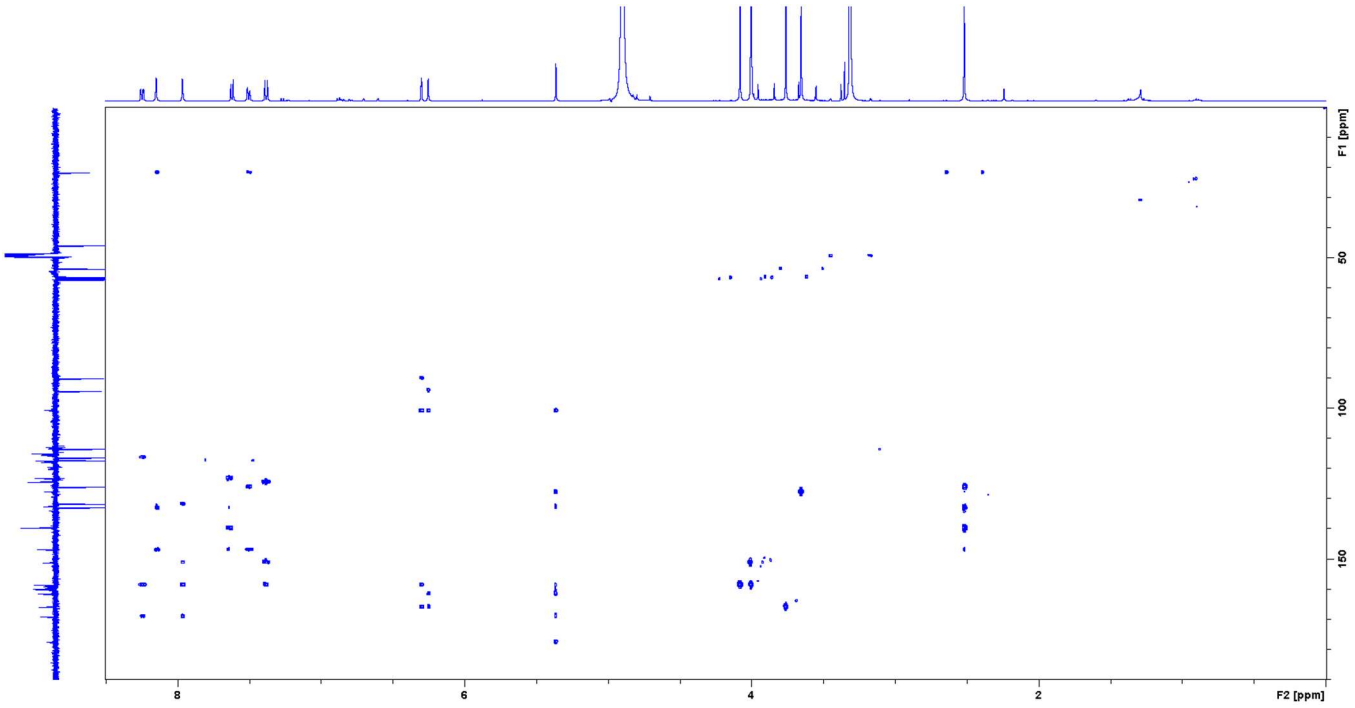


Figure S14: 17-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**8**), ^{1}H-^{13}C HMBC spectrum (500 MHz, 1% TFA - MeOH-d_4)

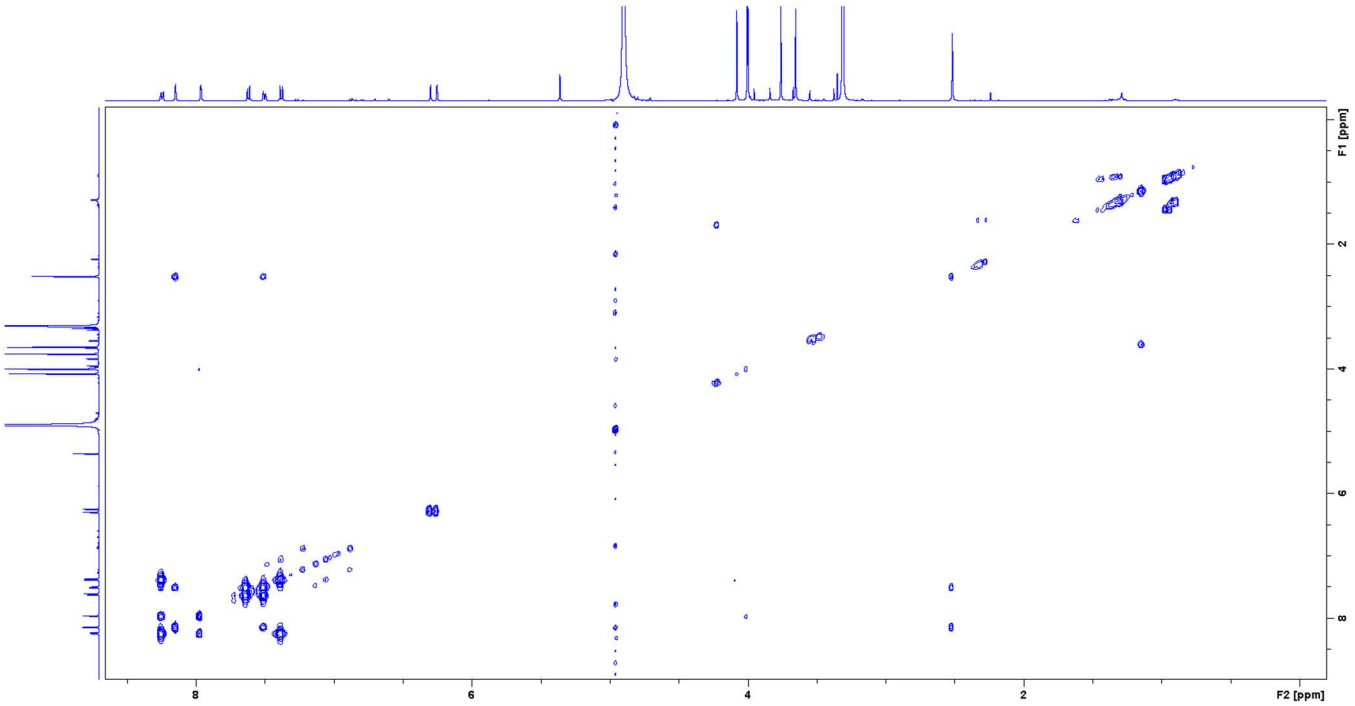


Figure S15: 17-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**8**), ^{1}H-^{1}H COSY spectrum (500 MHz, 1% TFA - MeOH-d_4)

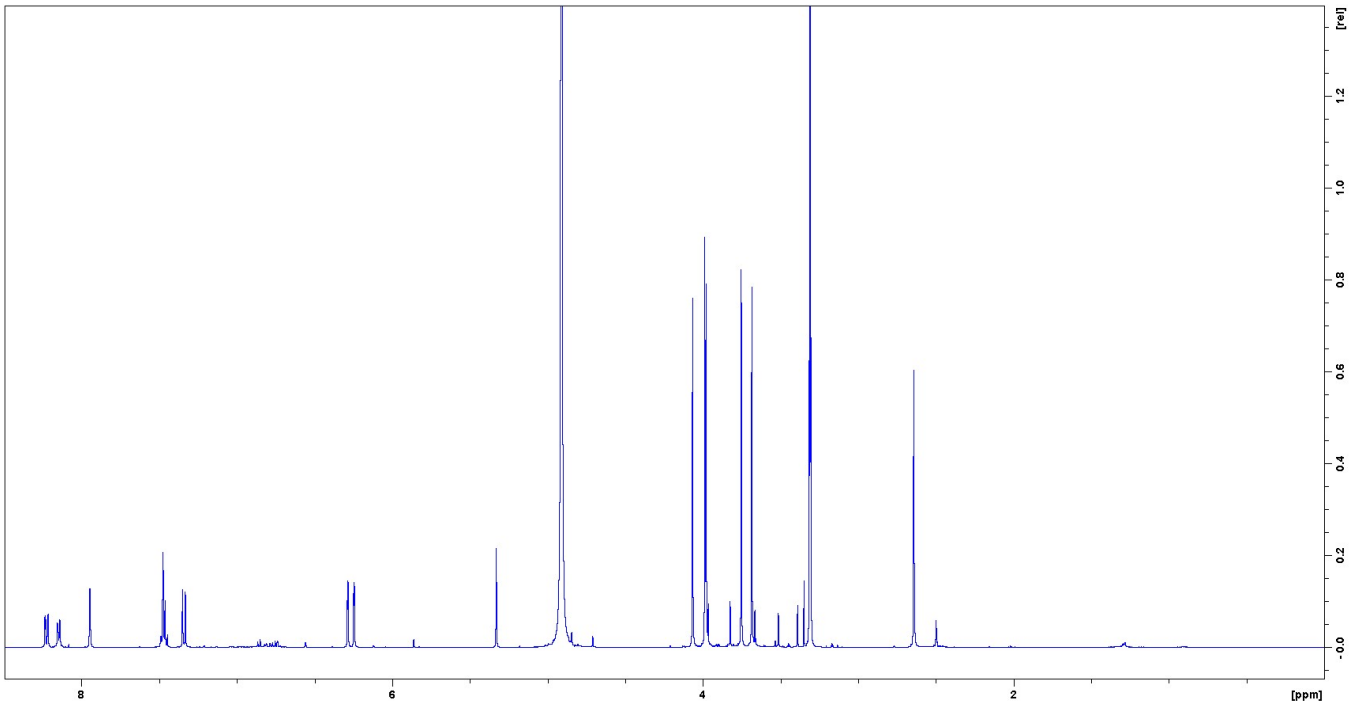


Figure S16: 18-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**9**), ^{1}H-NMR spectrum (500 MHz, 1% TFA - MeOH-d_4)

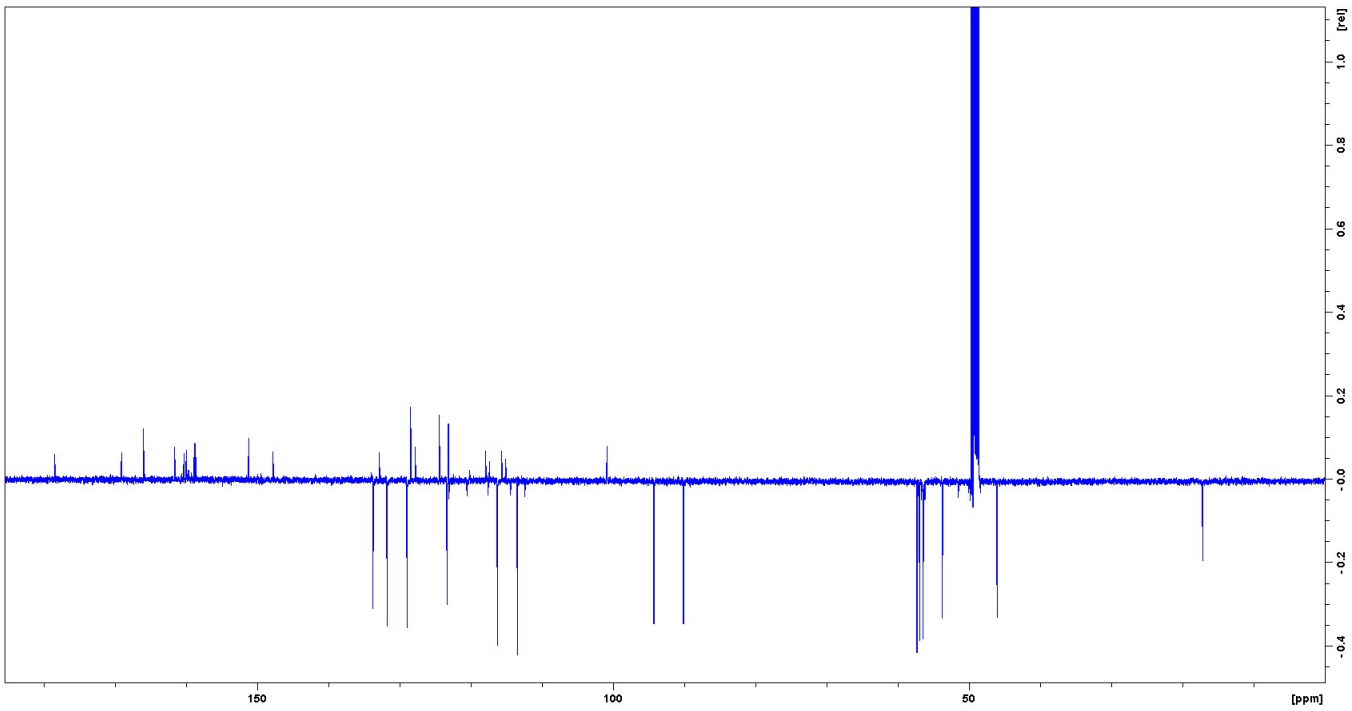


Figure S17: 18-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**9**), ^{13}C-NMR spectrum (500 MHz, 1% TFA - MeOH-d_4)

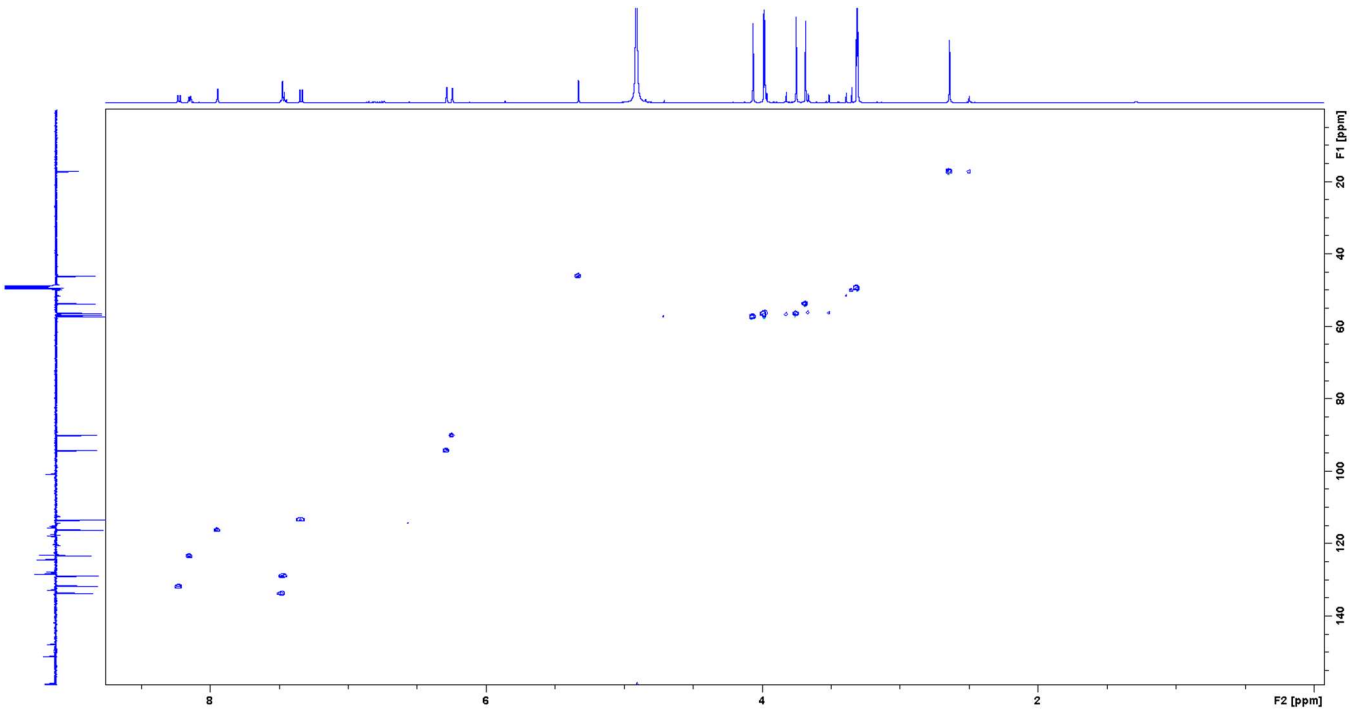


Figure S18: 18-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**9**), ^{1}H-^{13}C HSQC spectrum (500 MHz, 1% TFA - MeOH-d_4)

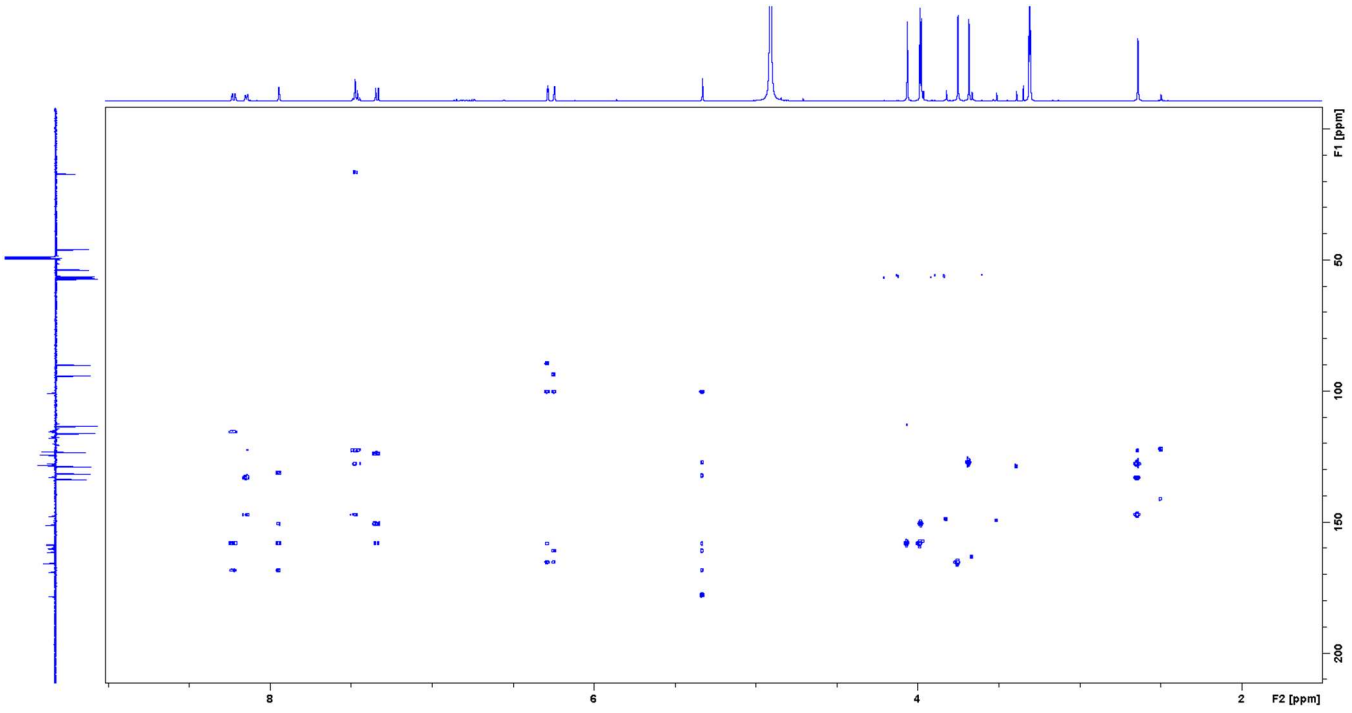


Figure S19: 18-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**9**), ^{1}H-^{13}C HMBC spectrum (500 MHz, 1% TFA - MeOH-d_4)

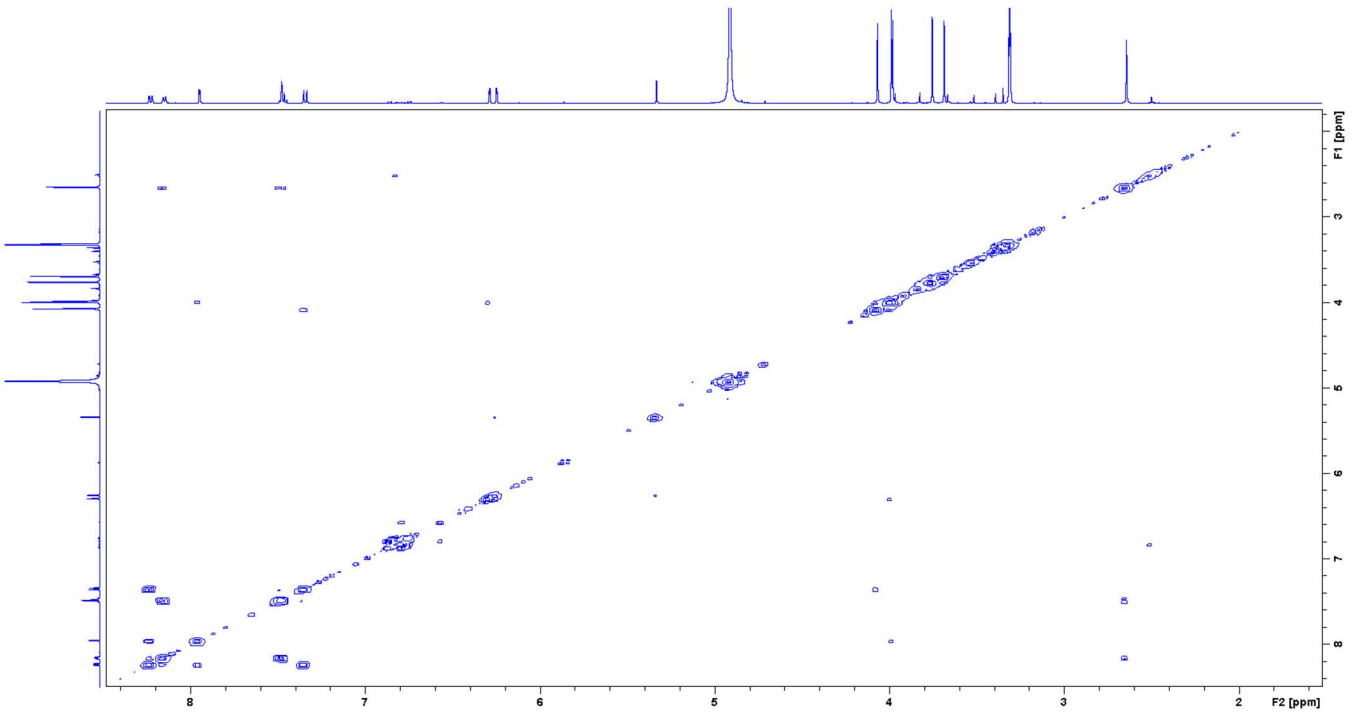


Figure S20: 18-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**9**), ^{1}H-^{1}H COSY spectrum (500 MHz, 1% TFA - MeOH-d_4)

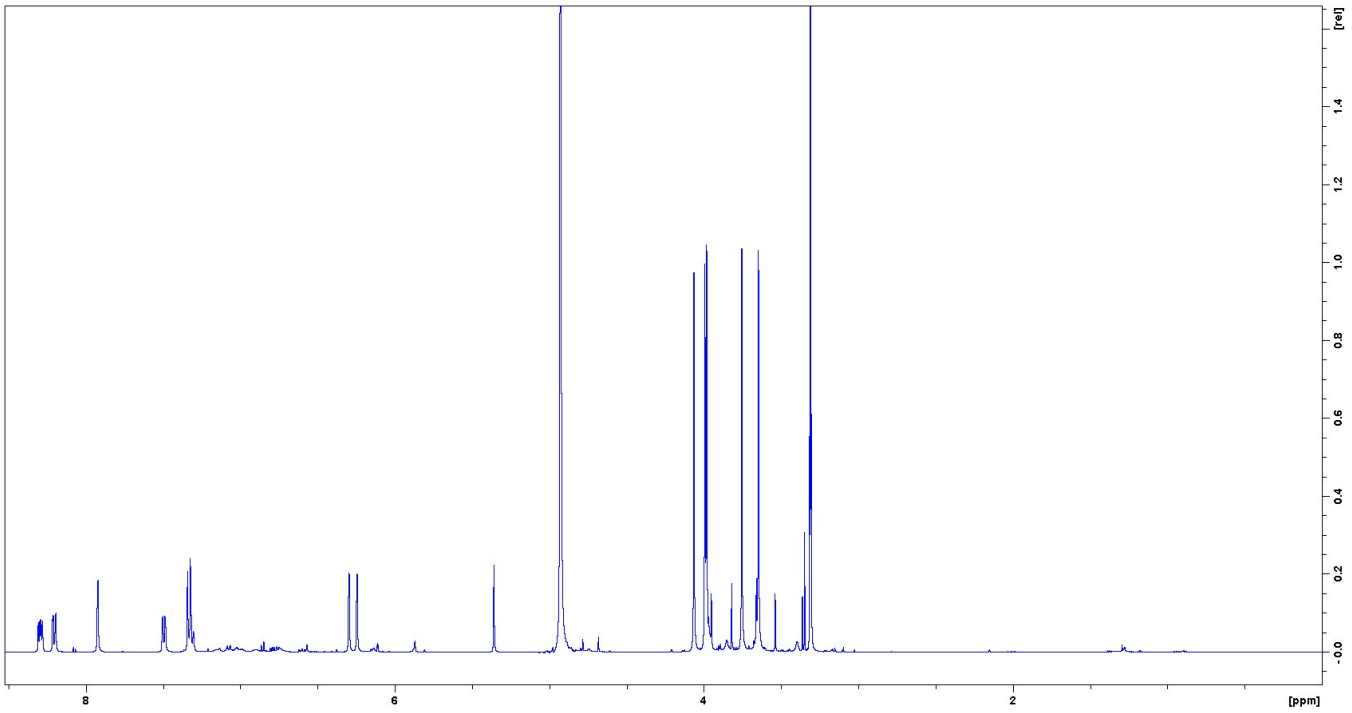


Figure S21: 17-Fluoro-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**10**), ^{1}H-NMR spectrum (500 MHz, 1% TFA - MeOH-d_4)

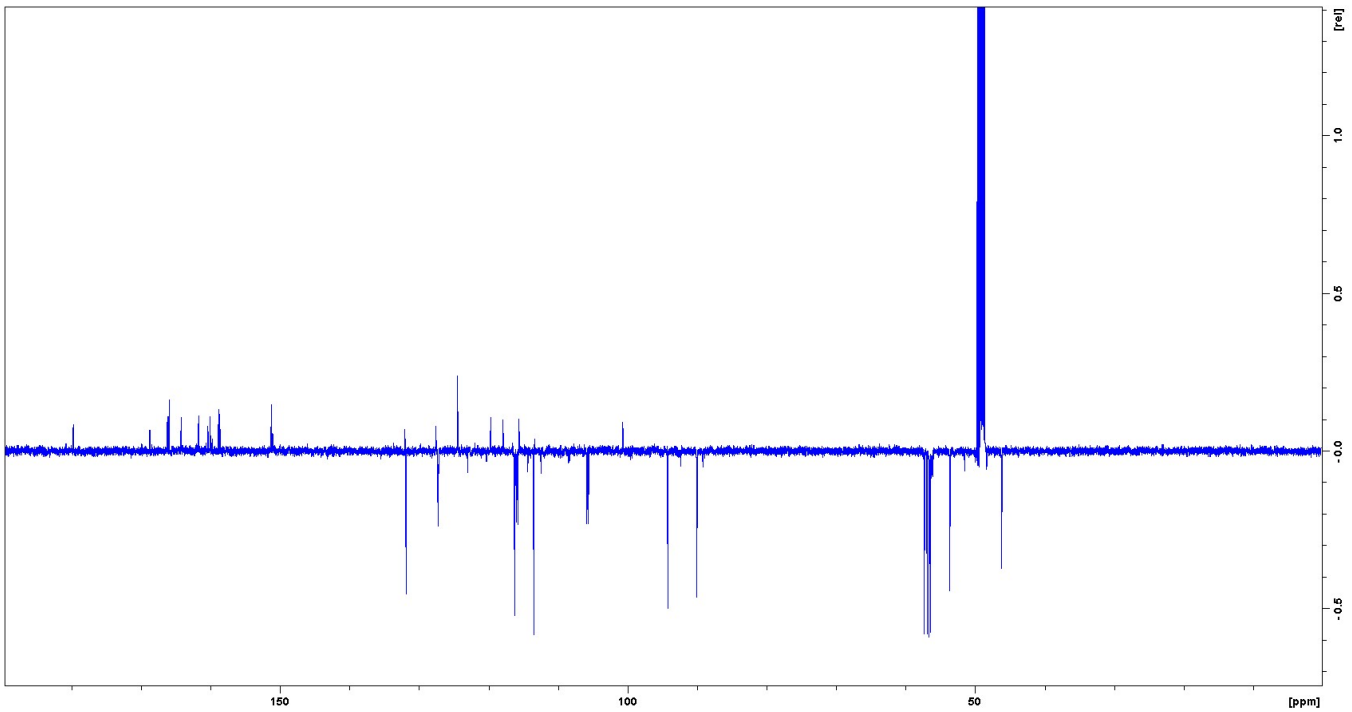


Figure S22: 17-Fluoro-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**8**), ^{13}C-NMR spectrum (500 MHz, 1% TFA - MeOH-d_4)

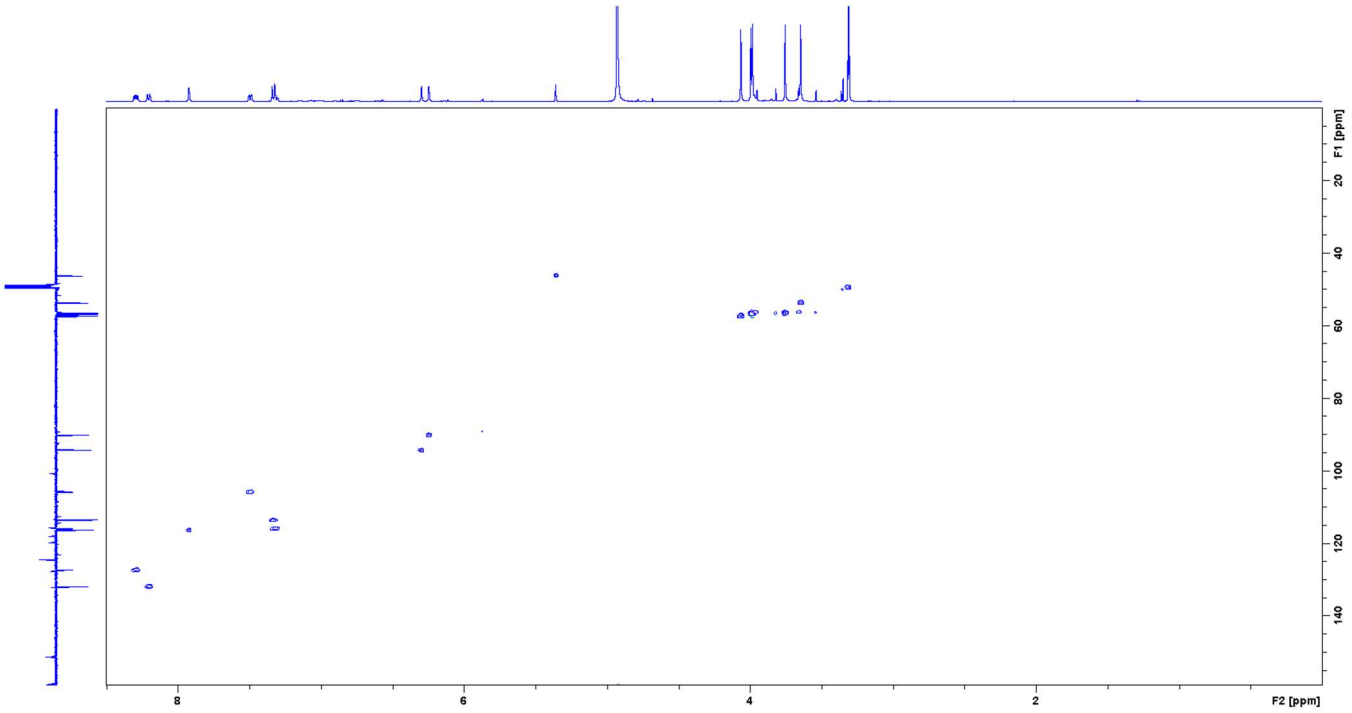


Figure S23: 17-Fluoro-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**8**), ^{1}H-^{13}C HSQC spectrum (500 MHz, 1% TFA - MeOH-d_4)

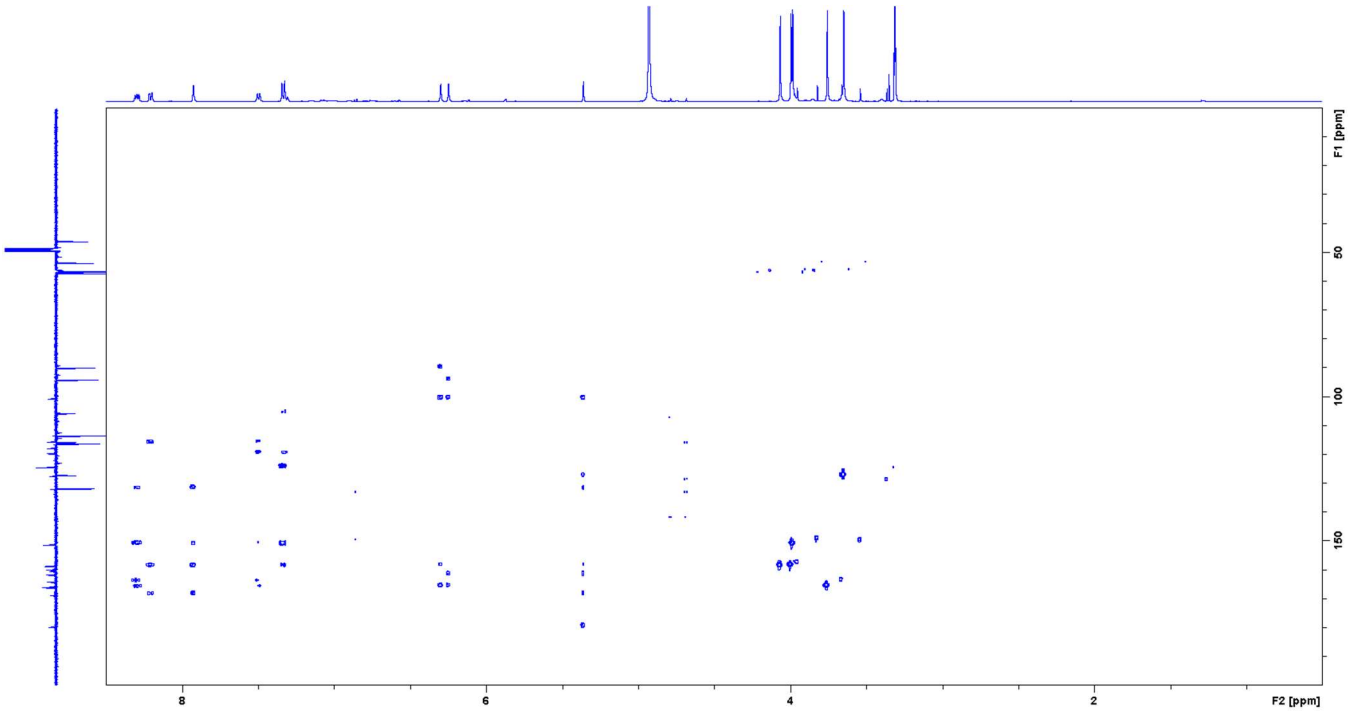


Figure S24: 17-Fluoro-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**8**), ^{1}H-^{13}C HMBC spectrum (500 MHz, 1% TFA - MeOH-d_4)

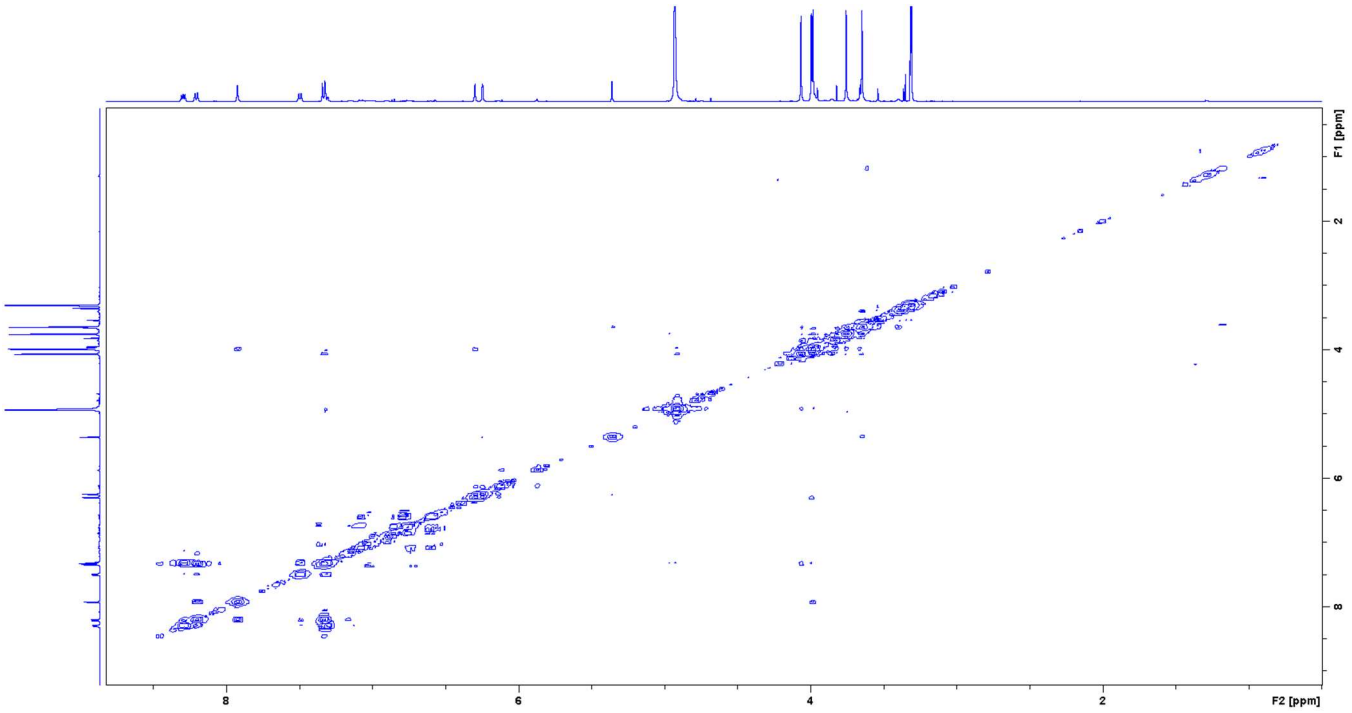


Figure S25: 17-Fluoro-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**10**), ^{1}H-^{1}H COSY spectrum (500 MHz, 1% TFA - MeOH-d_4)

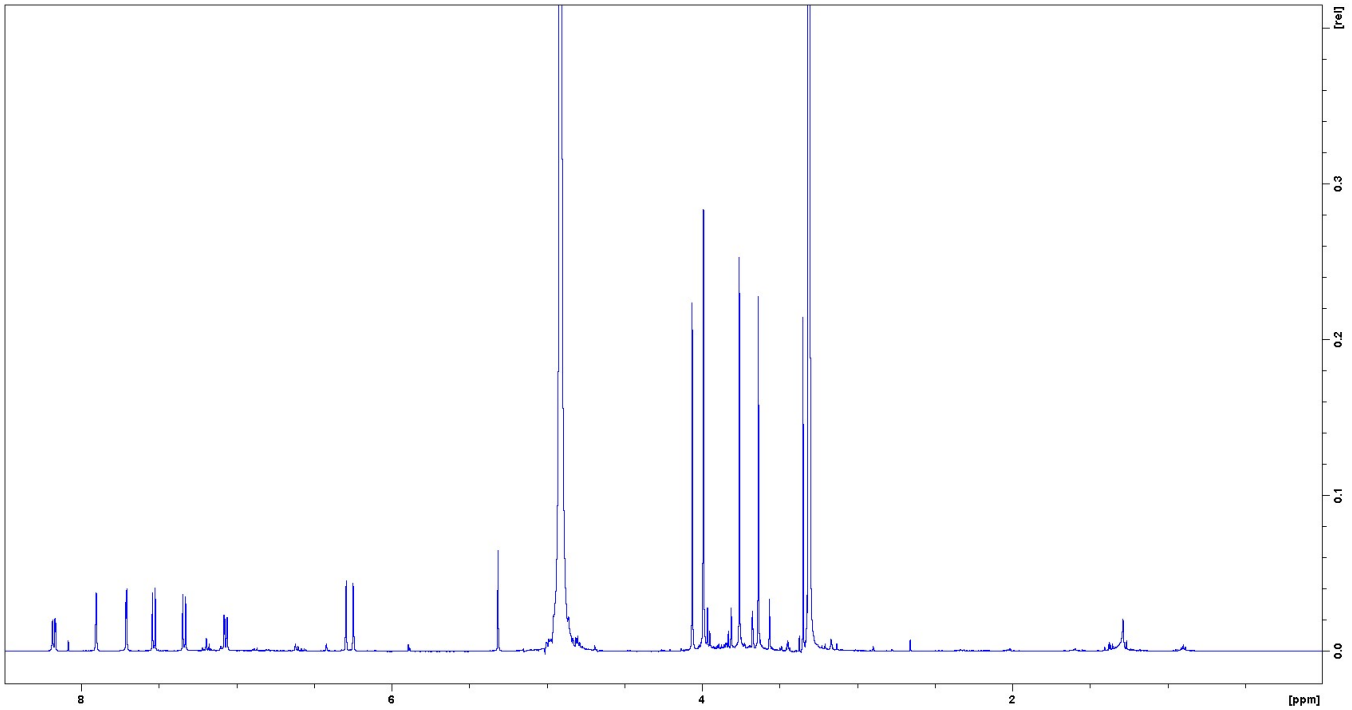


Figure S26: 17-Hydroxy-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**11**), ^{1}H-NMR spectrum (500 MHz, 1% TFA - MeOH-d_4)

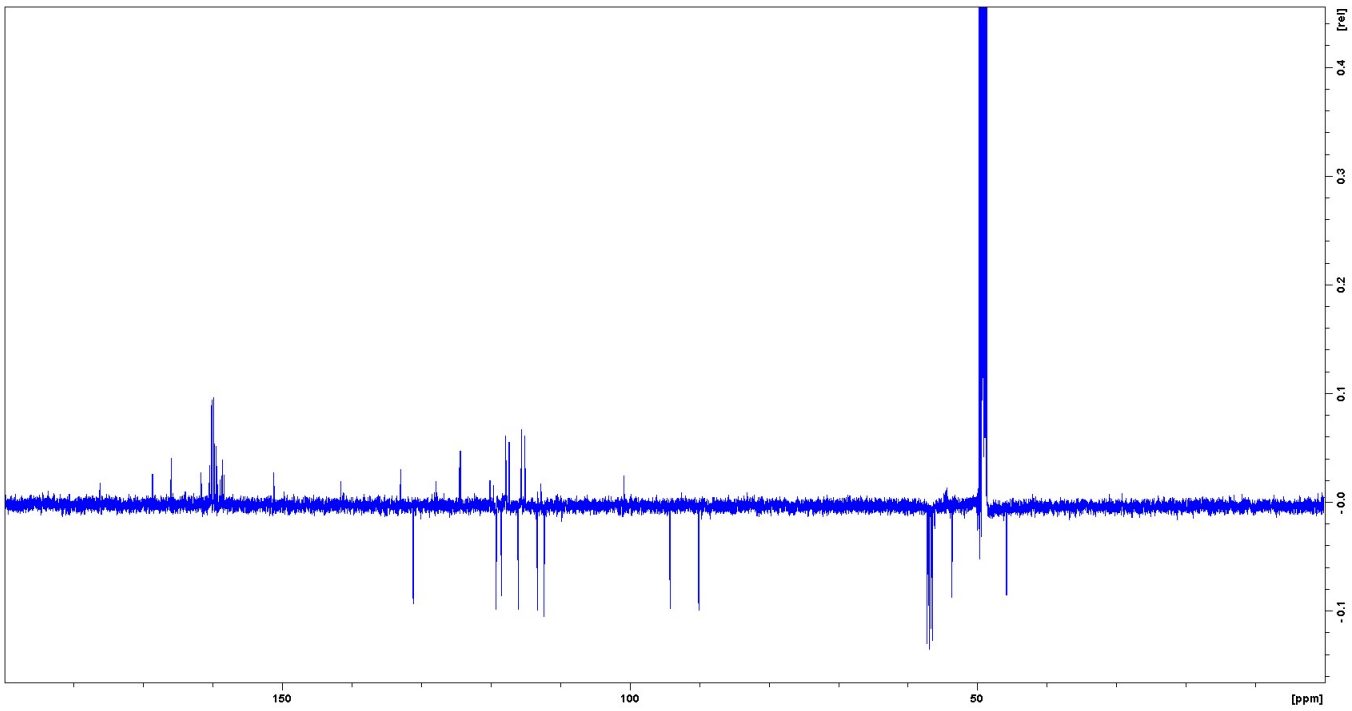


Figure S27: 17-Hydroxy-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**11**), ^{13}C-NMR spectrum (500 MHz, 1% TFA - MeOH-d_4)

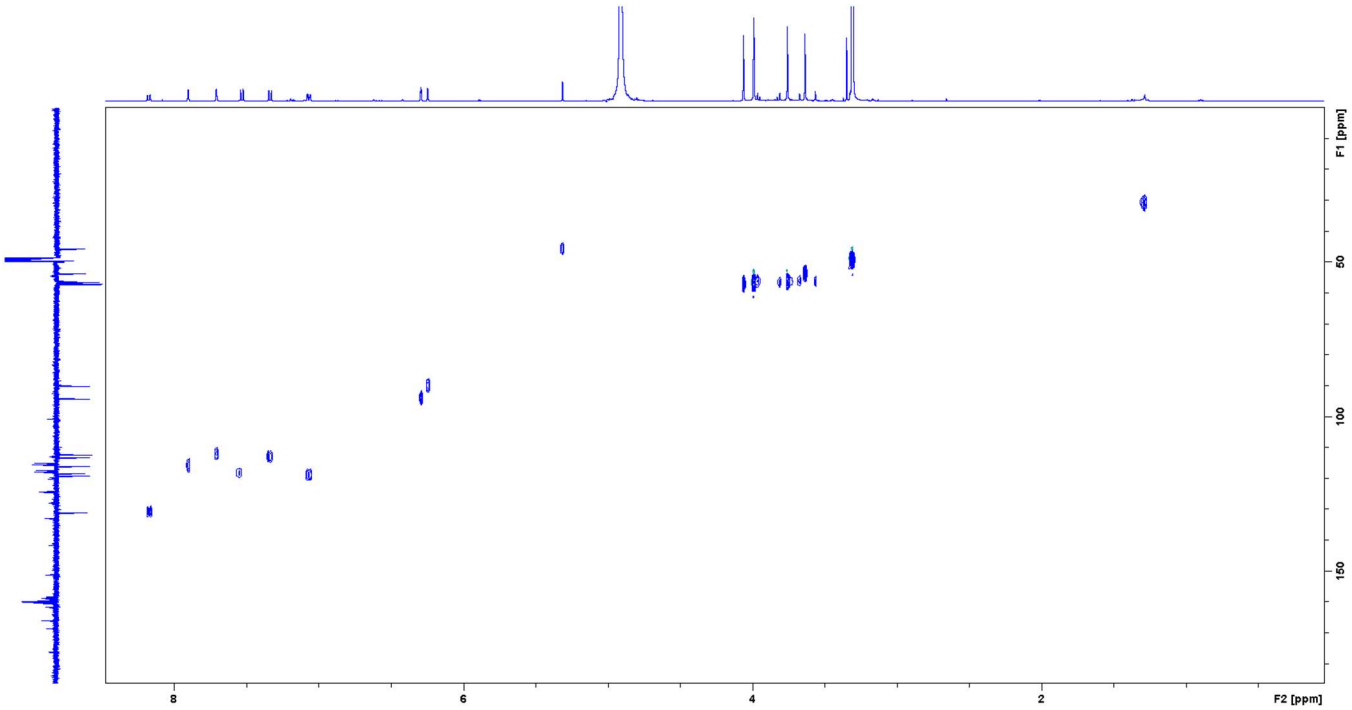


Figure S28: 17-Hydroxy-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**11**), ^{1}H-^{13}C HSQC spectrum (500 MHz, 1% TFA - MeOH-d_4)

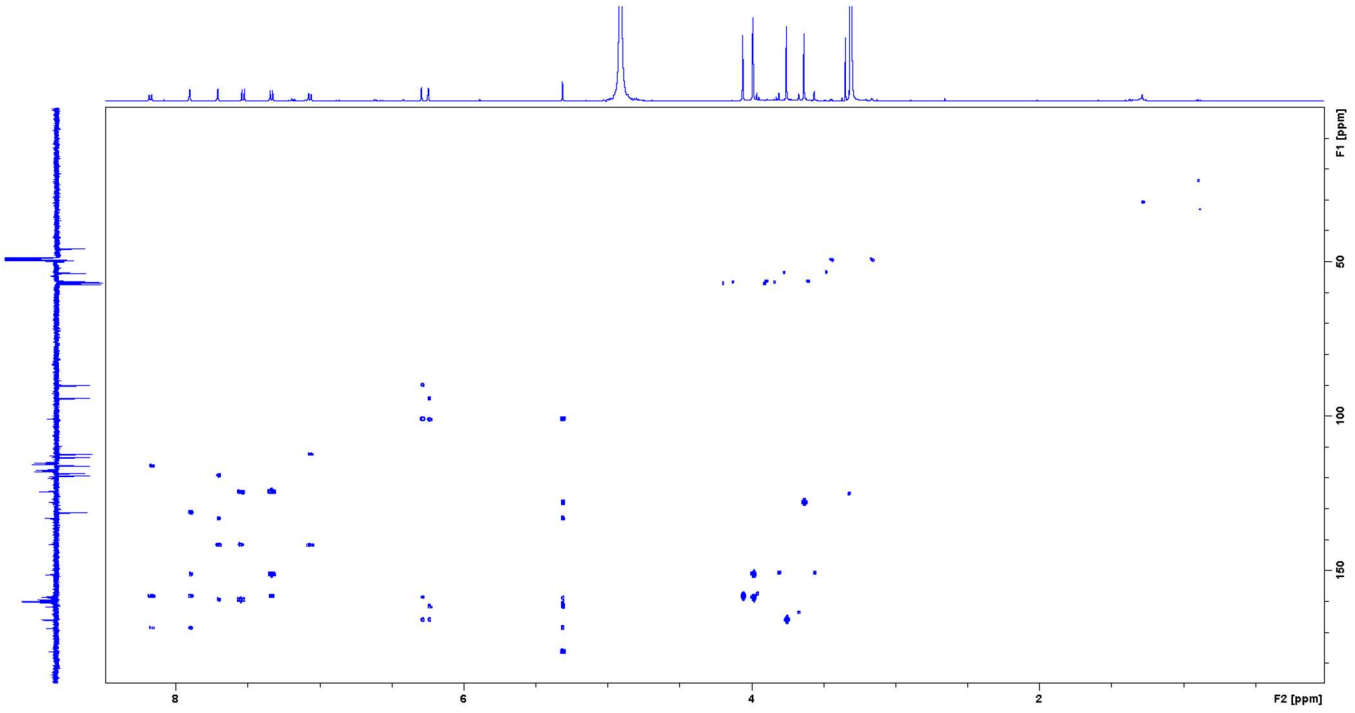


Figure S29: 17-Hydroxy-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**11**), ^{1}H-^{13}C HMBC spectrum (500 MHz, 1% TFA - MeOH-d_4)

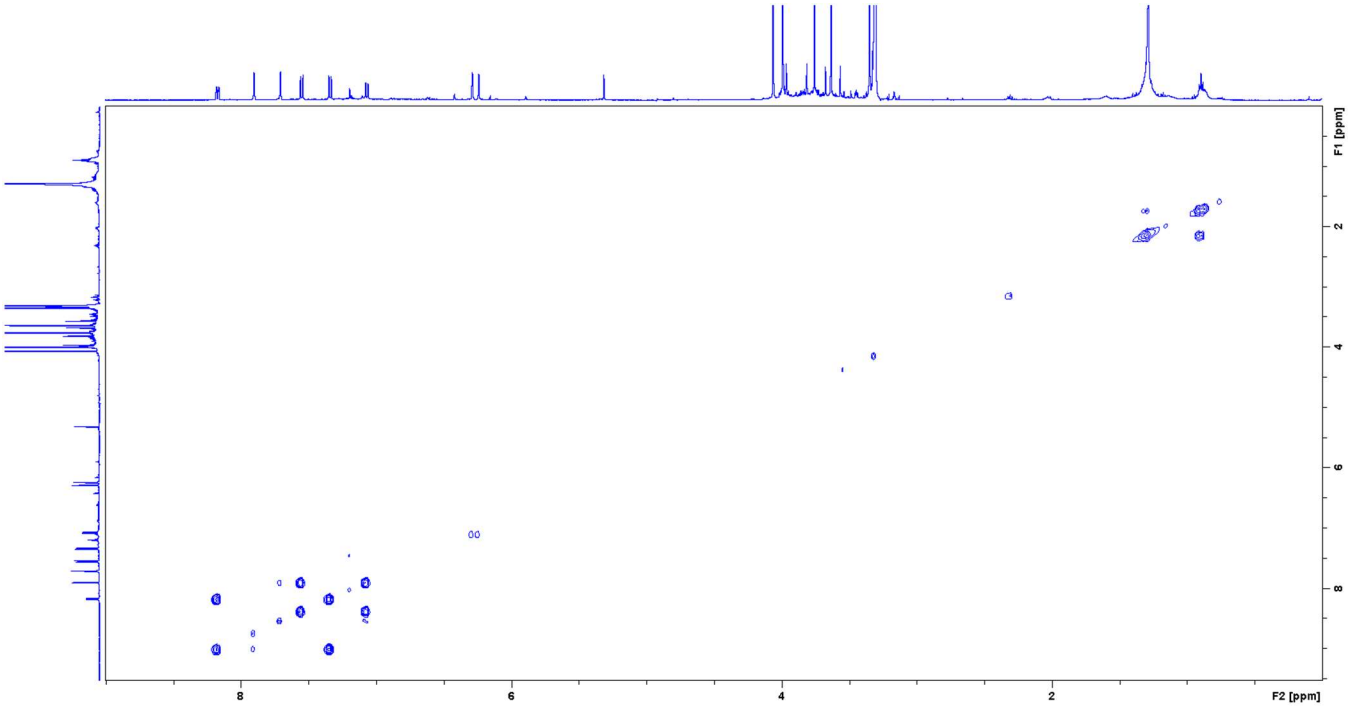


Figure S30: 17-Hydroxy-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**10**), ^{1}H-^{1}H COSY spectrum (500 MHz, 1% - MeOH-d_4)

3. HR-MS

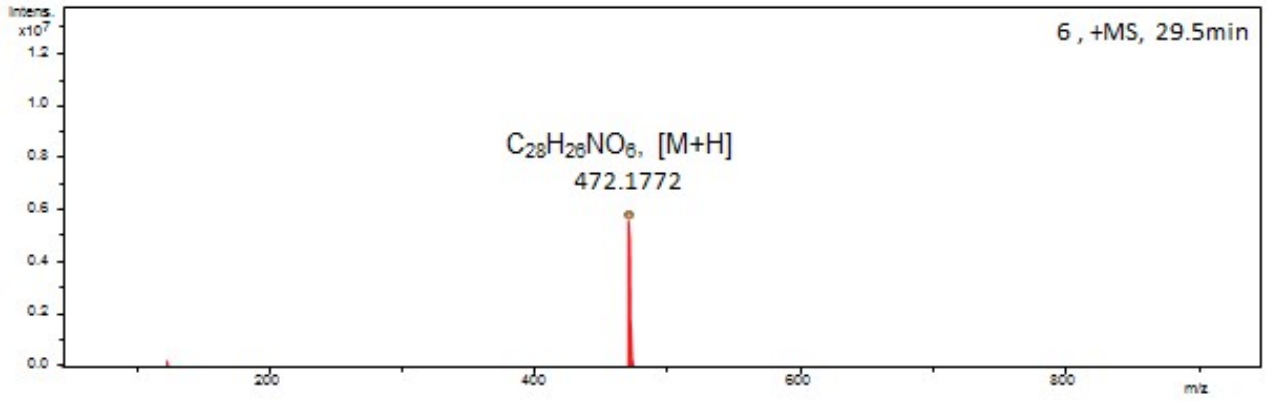


Figure S31: 5,7,11,3‘,4‘-Penta-*O*-methylnudicaulin (**6**), mass spectrum

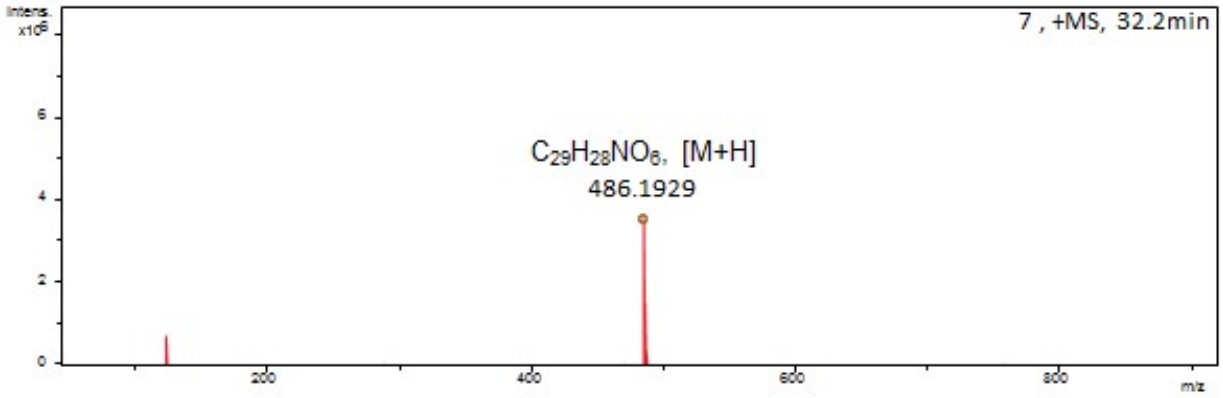


Figure S32: 16-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**7**), mass spectrum

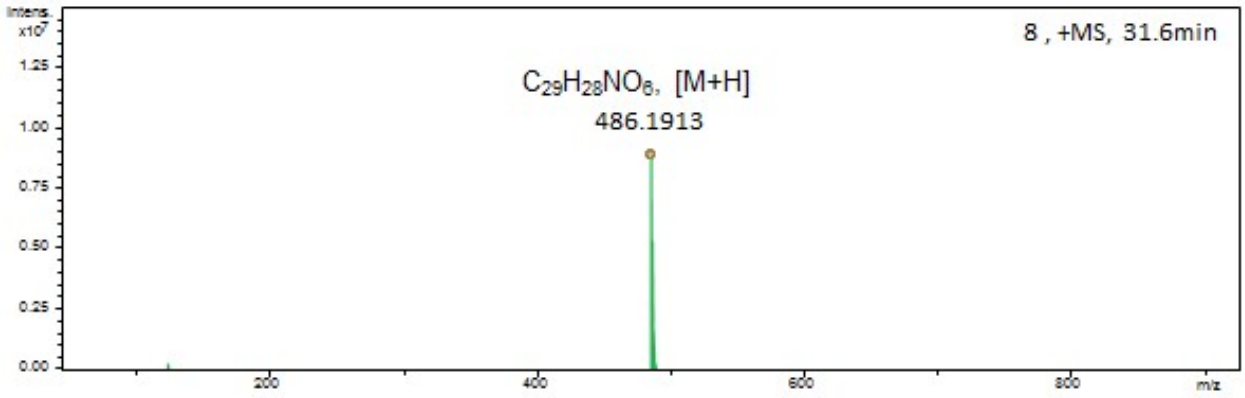


Figure S33: 17-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**8**), mass spectrum

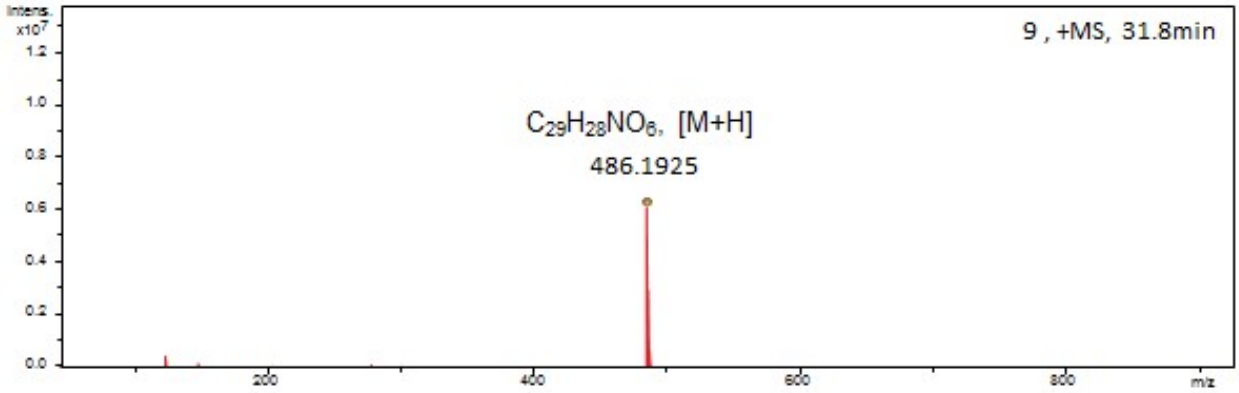


Figure S34: 18-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**9**), mass spectrum

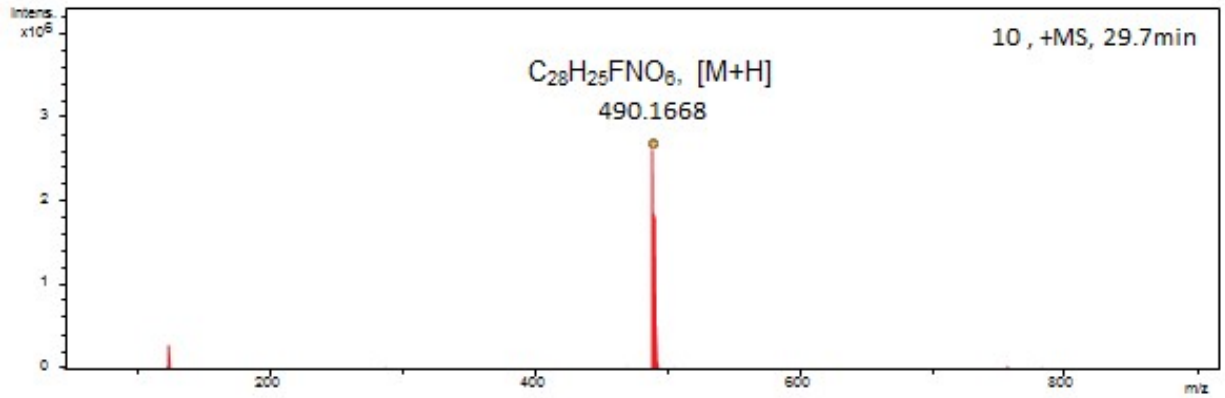


Figure S35: 17-Fluoro-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**10**), mass spectrum

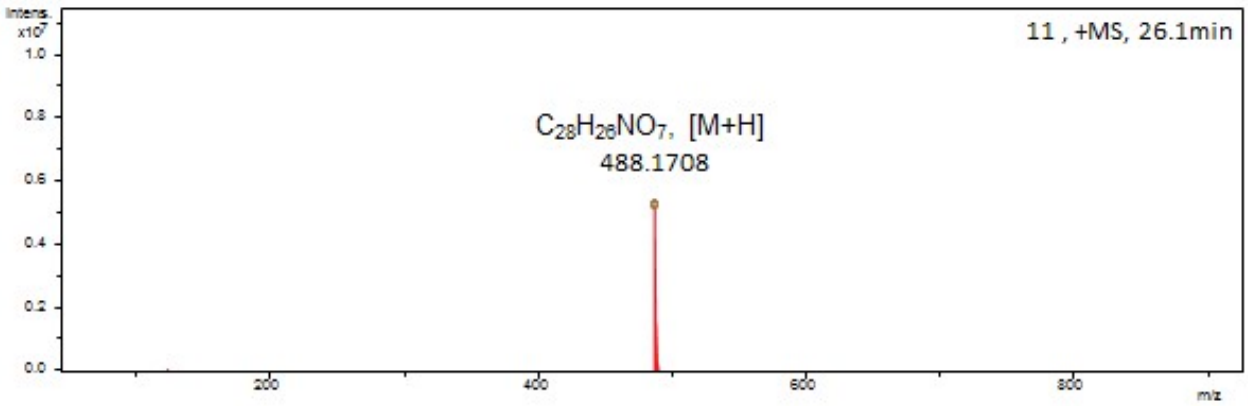


Figure S36: 17-Hydroxy-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**11**), mass spectrum

4. UV/Vis absorption spectra

UV/Vis absorption spectra were acquired with the photodiode array detector (PDA) G1315B, 200-700 nm of *HPLC 1* (Chapter **4.1**).

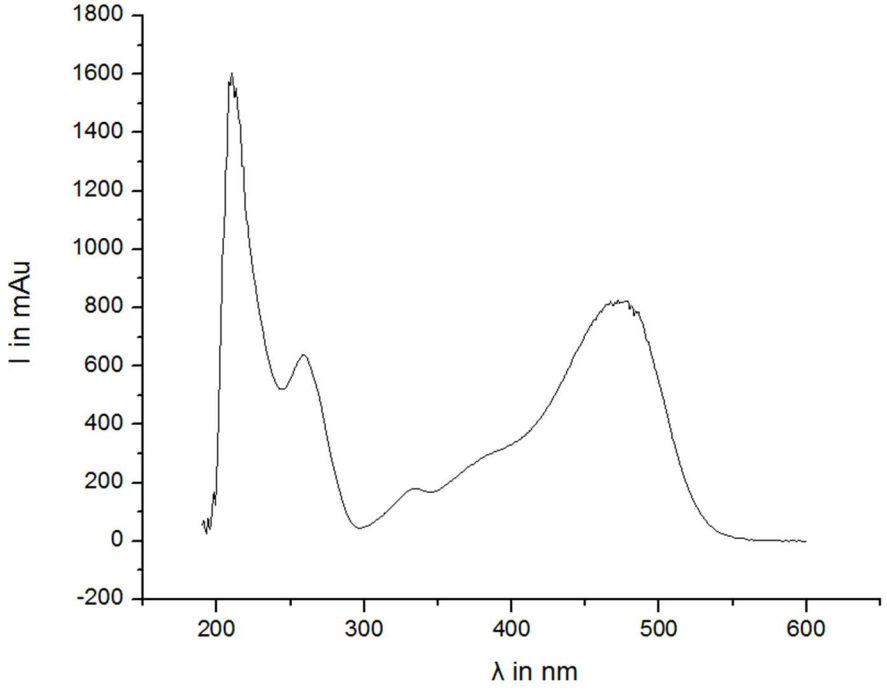


Figure S37: 5,7,11,3‘,4‘-Penta-*O*-methylnudicaulin (**6**), UV/Vis spectrum (0.1 % TFA-MeOH)

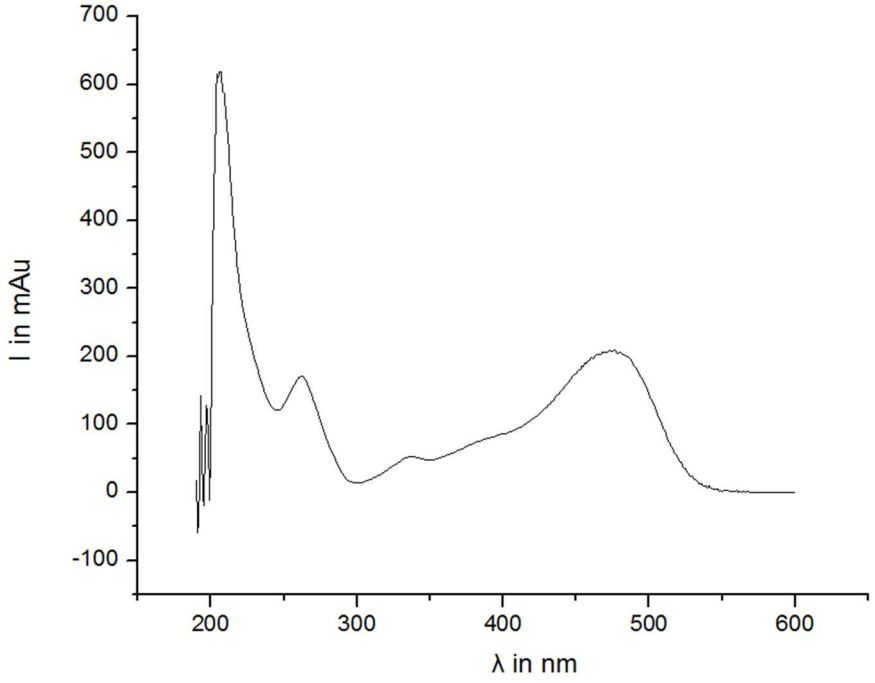


Figure S38: 16-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**7**), UV/Vis spectrum (0.1 % TFA-MeOH)

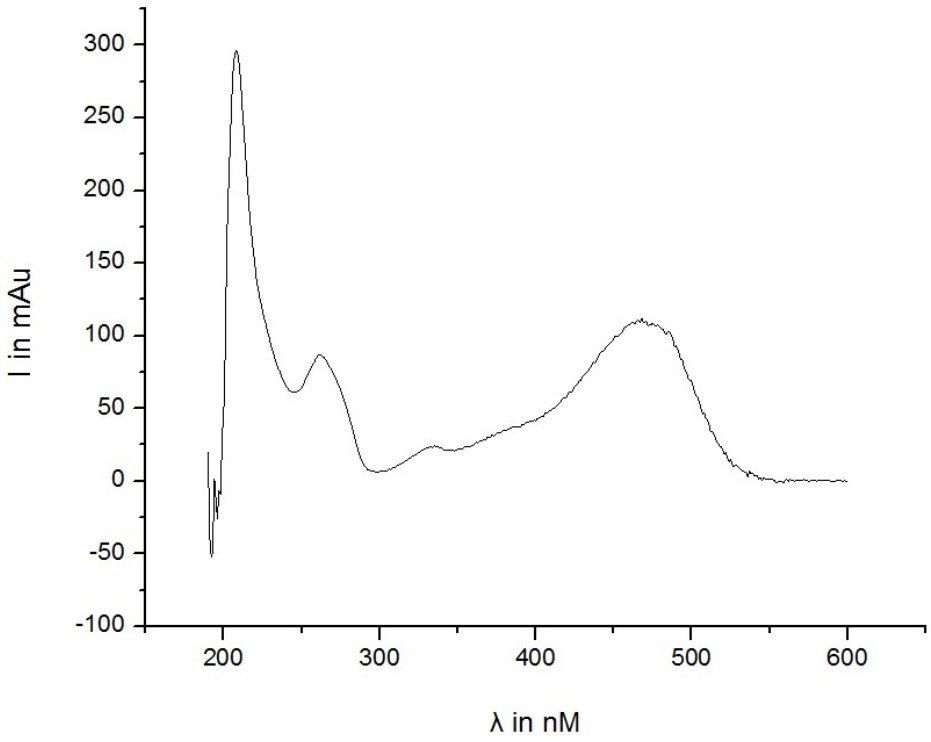


Figure S39: 17-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**8**), UV/Vis spectrum (0.1 % TFA-MeOH)

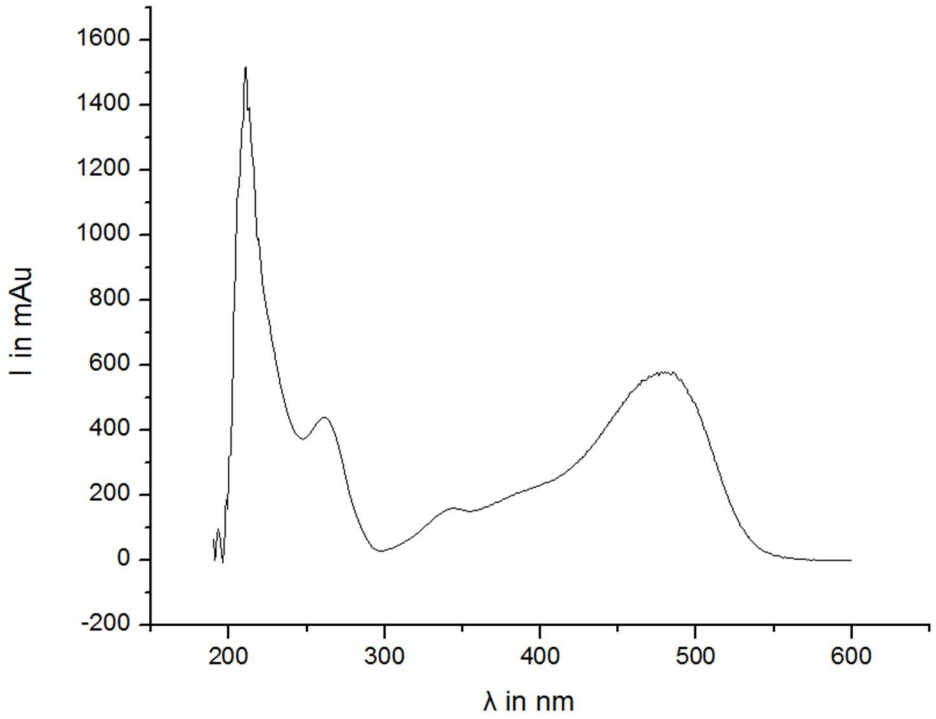


Figure S40: 18-Methyl-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**9**), UV/Vis spectrum (0.1 % TFA-MeOH)

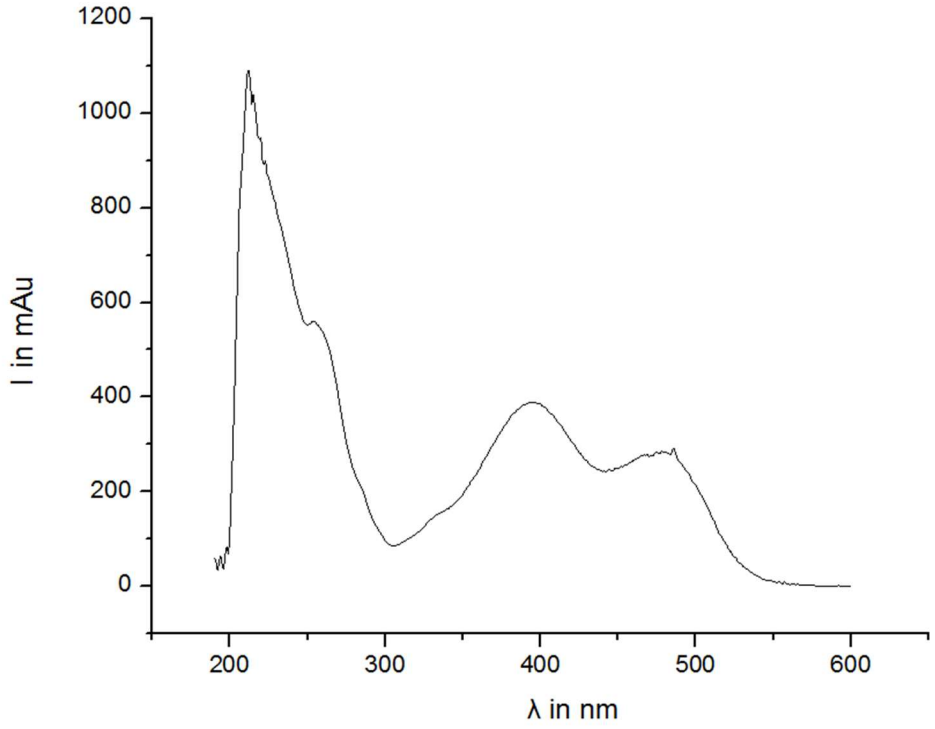


Figure S41: 17-Fluoro-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**10**), UV/Vis spectrum (0.1 % TFA-MeOH)

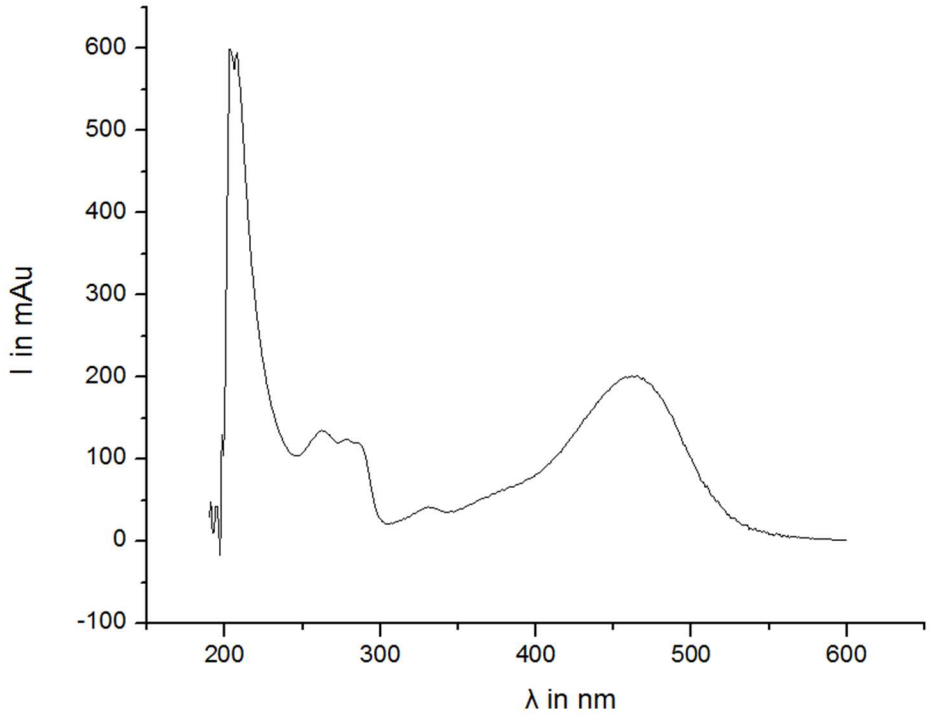


Figure S42: 17-Hydroxy-5,7,11,3‘,4‘-penta-*O*-methylnudicaulin (**11**), UV/Vis spectrum (0.1 % TFA-MeOH)

5. CD spectrum

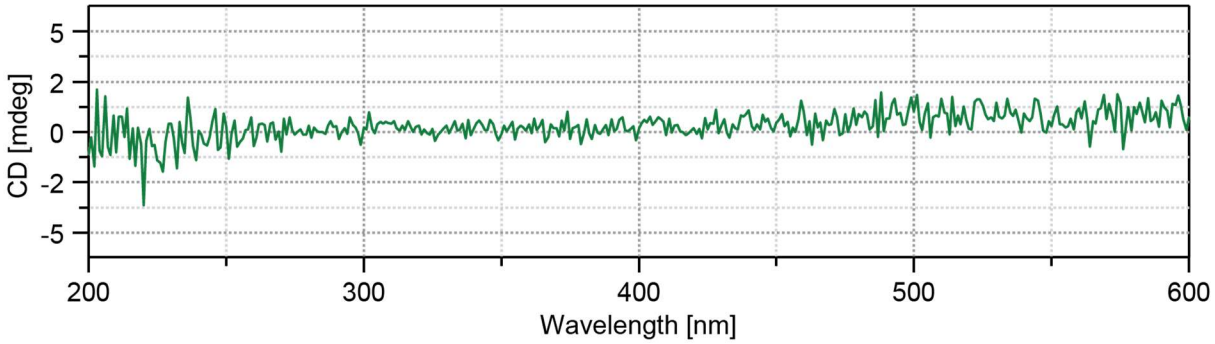


Figure S43: 5,7,11,3‘,4‘-Penta-*O*-methylnudicaulin (**6**), CD spectrum (concentration 0.78 mg ml^{-1} (1.66 mM in MeOH), cuvette width 1 mm)

6. Cell Toxicity

*Table S1: Antiproliferative and cytotoxicity data of compounds **6**-**11***

	Antiproliferative activity		Cytotoxicity
Compound	HUVEC GI_{50} [µmol l^{-1}]	K-562 GI_{50} [µmol l^{-1}]	HeLa CC_{50} [µmol l^{-1}]
6	1.3 (± 0.2)	1.1 (± 0.4)	3.4 (± 0.4)
7	2.5 (± 1.2)	1.4 (± 0.6)	5.8 (± 0.2)
8	5.3 (± 1.9)	4.1 (± 0.6)	16.5 (± 1.4)
9	2.9 (± 0.4)	2.9 (± 0.4)	8.4 (± 1.2)
10	2.2 (± 0.6)	1.0 (± 0.2)	5.7 (± 0.2)
11	2.0 (± 0.4)	1.0 (± 0.4)	5.5 (± 0.2)
Imatinib*	22.1 (± 2.4)	0.2 (± 1.4×10^{-2})	78.6 (± 2.8)
Doxorubicin*	0.2	1.0 (± 1.1)	3.68 (± 1.5)

For structures of compounds **6**-**11**, see Scheme II.

* Chemotherapeutic drugs, values published in [23] Krauth, F.; Dahse, H.-M.; Rüttinger, H.-H.; Frohberg, P. Synthesis and characterization of novel 1,2,4-triazine derivatives with antiproliferative activity. *Bioorg. Med. Chem.* **2010**, *18*, 1816–1821, DOI 10.1016/j.bmc.2010.01.053.

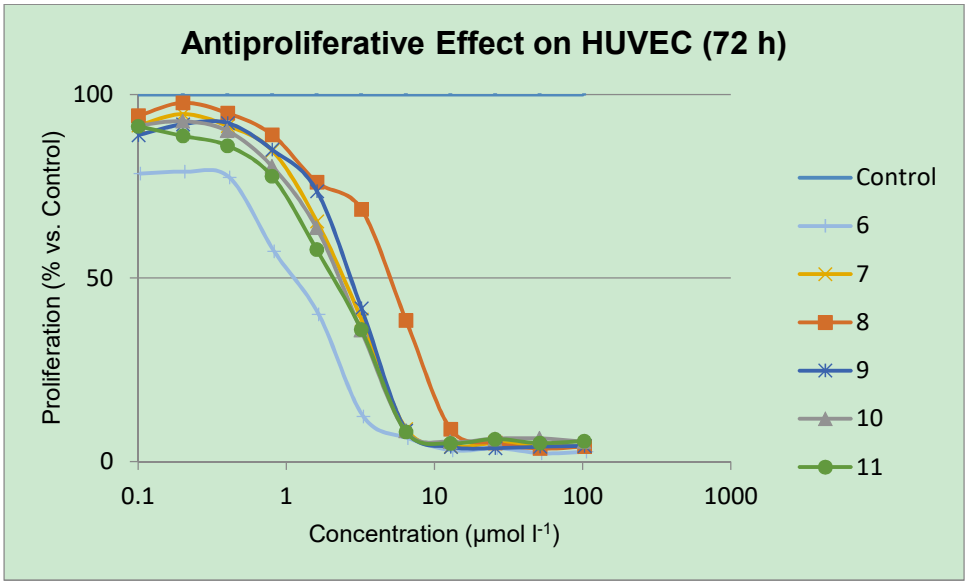


Figure S44: Dose–response relationship for antiproliferative effect on HUVEC cells (72 h), Representative curves are given.

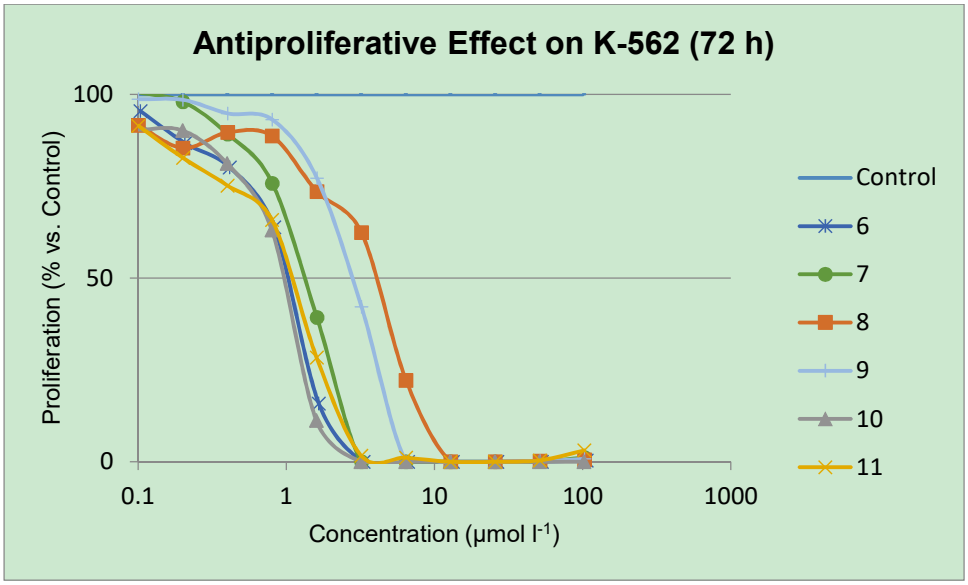


Figure S45: Dose–response relationship values for antiproliferative effect on K-562 cells (72 h), Representative curves are given.

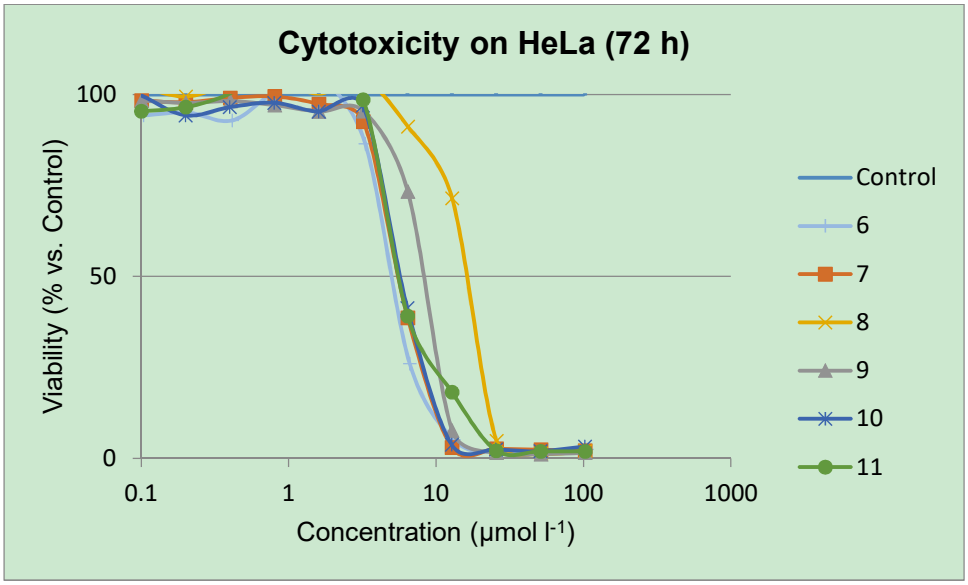


Figure S46: Dose–response relationship values for cytotoxicity on HeLa cells (72 h), Representative curves are given.

7. Antimicrobial activity

*Table S2: Antimicrobial data of compounds **6**-**11***

Compound		6*	7*	8*	9*	10*	11*	Ciprofla-xacin**	MeOH	Amphote-ricin B***
c [mg ml^{-1}]		1	1	1	1	1	1	5*10^{-3}	0	1*10^{-2}
Bacillus subtilis	6633 B1	0	0	12P	0	0	0	28	0	0
Staphylococcus aureus	511 B3	0	0	13P	0	0	0	18	0	0
Escherichia coli	458 B4	0	0	0	0	0	0	24/ 32p	0	0
Pseudomonas aeruginosa	SG137 B7	0	0	0	0	0	0	26	10	0
P. aeruginosa	K799/61 B9	0	0	0	0	0	0	28/37p	0	0
MRSA S. aureus	134/93 R9	0	0	13P	0	0	0	0	0	0
VRE Enterococcus faecalis	1528 R10	0	0	0	0	0	0	17F	0	0
Mycobacterium vaccae	10670 M4	18p	14p	14p	15p	15p	18p	23p	0	0
Sporobolomyces salmonicolor	549 H4	0	0	0	0	0	0	0		18p
Candida albicans	C. alb H8	0	0	0	0	0	0	0	0	21
Penicillium notatum	JP36 P1	0	0	0	0	0	0	0	0	18p

For structures of compounds **6**-**11**, see Scheme II.

p = colonies in the inhibition zone; P = many colonies in the inhibition zone; A = indication of inhibition; F = promotion of growth

* solution in MeOH

** solution in deionized water

*** solution in DMSO/MeOH

MRSA indicates methicillin-resistant *Staphylococcus aureus*

VRE indicates vancomycin-resistant *Enterococcus*