Supporting Information

Polyketide bio-derivatization

using the promiscuous acyltransferase KirCII

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I. Supplementary Material and Methods

1. Media

Medium	Composition	Ingredient amount per 1L H_2O_{deion}	Source*
LB-medium ready for use	LB powder	20 g	Roth
TSB-medium (Bacto™ Tryptic Soy Broth Soybean-Casein Digest) ready for use	TSB powder	30 g	Becton, Dickinson
SGG-medium	Starch (soluble)	10 g	Merck
	Glucose	10 g	Roth
	Glycerol	10 g	Roth
	Cronsteep Powder	2.5 g	Marcor
	BactoPeptone	5 g	Becton, Dickinson
	Yeast extract	2 g	OhlyKAT
	Sodium chloride	1 g	Roth
	Calcium carbonate	3 g	Roth
	pH 7.3		
4xYTP	Tryptone (pancreatic digest of casein)	32 g	Becton, Dickinson Roth
	Yeast extract	20 g	Roth
	Sodium chloride	10 g	Roth
	Sodium dihydrogen phosphate (NaH_2PO_4)	6.87 g	Merck
	Sodium phosphate dibasic (Na_2HPO_4)	11.36 g	Roth
2xYTPG	4xYTP	400 ml of 1l	-
	0.2 M Glucose (autoclave separately)	400 ml of 1l	

*All chemicals from Sigma, except when otherwise indicated.

2. Buffers (for *in vitro* assay)

Buffer	Composition	Concentration
Buffer B (for S12 extract preparation)	Tris/HCl	10 mM
	Magnesium acetate	14 mM
	Potassium glutamate	60 mM
	DTT	1 mM
	pH 8.2	
Buffer A (for S12 extract preparation)	Buffer B	99.95% (v/v)
	β-Mercaptoethanol	0.05% (v/v)
2.5x ivTT-Buffer (for in vitro assay)	Hepes	142.5 mM
	Potassium glutamate	225 mM
	Ammonium acetate	200 mM
	Magnesium acetate	30 mM
	PEG(8000)	1.67% (w/v)
	Dithiothreitol (DTT)	5 mM
	pH 8.2	
10x Energy mix (for in vitro assay)	AMP (100x)	10% (v/v)
	CMP (100x)	10% (v/v)
	GMP (100x)	10% (v/v)
	UMP (100x)	10% (v/v)
	Formyl-FH4 (1000x)	1% (v/v)
	F16BP (20x)	50 % (v/v)
	H_2O_{deion}	9% (v/v)
10x Amino acid mix (AA-Mix, for in vitro assay)	300x AA resolved in H_2O_{deion}	9x 3.33% (v/v)
	H_2O_{deion}	3.33% (v/v)
	300x AA resolved in 1M HCl	9x 3.33% (v/v)
	1M HCl	3.33% (v/v)
	300x AA resolved in 1M KOH	2x 3.33% (v/v)
	1M KOH	26.64 %

3. *In vitro* assay stock solutions (for *in vitro* assay buffers)

Name	Stock solution in H_2O_{deion}	Concentration in H_2O_{deion}
Adenosine 5′-monophosphate sodium salt (AMP)	100x	120 mM
Cytidine 5′-monophosphate disodium salt (CMP)	100x	85 mM
Guanosine 5′-monophosphate disodium salt hydrate (GMP)	100x	85 mM
Uridine 5′-monophosphate disodium salt (UMP)	100x	85 mM
5-Formyl-5,6,7,8-tetrahydropteroyl-L-glutamic acid (Formyl-FH_4)	1000x	34 mg ml^{-1}
D-Fructose 1,6-bisphosphate trisodium salt octahydrate (F16BP)	20x	660 mM
L-Alanine (Ala)	300x	600 mM
L-Arginine monohydrochloride (Arg)	300x	600 mM
Glycine (Gly)	300x	600 mM
L-Histidine monohydrochloride monohydrate (His)	300x	600 mM
L-Lysine (Lys)	300x	600 mM
L-Proline (Pro)	300x	600 mM
L-Serine (Ser)	300x	600 mM
L-Threonine (Thr)	300x	600 mM
L-Valine (Val)	300x	600 mM

Name	Stock solution in 1M HCl	Concentration in 1M HCl
L-Asparagine monohydrate (Asn)	300x	600 mM
L-Aspartic acid (Asp)	300x	600 mM
L-Cysteine (Cys)	300x	600 mM
L-Glutamine (Gln)	300x	600 mM
L-Glutamic acid potassium salt monohydrate (Glu)	300x	600 mM
L-Leucine (Leu)	300x	600 mM
L-Methionine (Met)	300x	600 mM
L-Tryptophan (Trp)	300x	600 mM
L-Tyrosine (Tyr)	300x	600 mM
L-Isoleucine (Ile)	300x	600 mM
L-Phenylalanine (Phe)	300x	600 mM

4. Antibiotics

Antibiotic	Working concentration for *E.coli*	Working concentration for *Streptomyces*	Source
Apramycin	100 µg ml^{-1}	50 µg ml^{-1}	Appli-Chem
Chloramphenicol	34 µg ml^{-1}	-	Serva
Kanamycin	50 µg ml^{-1} (for ET12567, 25 µg ml^{-1})	-	Appli-Chem
Nalidixic acid	20 µg ml^{-1}	20 µg ml^{-1}	Fluka
Tetracycline	12.5-25 µg ml^{-1}	-	Serva

5. Strains

Strain	Specifications	Reference or source
E. coli strains		
NovaBlue	*endA1*, *hsdR17* (r_{K12}^- m_{K12}^+), *supE*44, *thi-1*, *recA1*, *gyrA96*, *relA*1, *lac*, F' [*pro*AB, *lacI*^{q}, *lacZ*ΔM15, Tn*10*]; Tet^R	Novagen
ET12567/pUB307	methylation-deficient *E. coli* strain containing pUB307, F-, *dam*-13::Tn*9*, *dcm*-6, *hsdM*, *hsdR*, *lacY*1, Cam^R, pUB307 is a RP1 derivative; Kan^R, Tet^R	[1-3]
Streptomyces strains		
S. collinus Tü 365	*S. collinus* wild type strain Tü 365; kirromycin producer	[4]
S. collinus pRM4.2-*matB*	*S. collinus* containing the integrative plasmid pRM4.2-*matB* for the expression of MatB (malonyl-CoA synthetase from *Rhizobium leguminosarum*, WP_011650729.1); Apra^R	This study

6. Plasmids

Plasmid	Specifications	Reference or source
pET28-*matB*	plasmid pET28a (Novagen) with engineered matB gene; Kan^R	
pET28-egfp	reporter plasmid (pET28a derivative) for in vitro translation assay; Kan^R	This study
pRM4.2	pSET152ermEp* derivative (ΦC31 integration vector, ermEp* promoter with artificial RBS); Apra^R	[5]
pRM4.2-*matB*	pRM4.2 with engineered *matB* gene; Apra^R	This study

7. Primers and PCRs

Primer name	Sequence (5'→3')	PCR program* (1. and 2. Denaturation 3. Annealing 4. Elongation 5. Final elongation 6. Final hold)
matB-test-up	cacacggacggtaacatc	1. 94°C, 5:00 min 2. 94°C, 1:00 min 3. 55°C, 1:40 min 4. 72°C, 2:30 min 5. 72°C, 8:00 min 6. 4°C, 5 h (Steps 2-4 x 30)
matB-test-low	cacgggtcgttctaaagg	

*PCR reactions were performed in the thermo cycler **PTC-100 Programmable Thermal Controller (MJ Research)**

8. HPLC/ESI-MS analysis of allyl-kirromycin and propargyl-kirromycin extracts

Allyl-kirromycin and propargyl-kirromycin crude extracts obtained from *S. collinus* pRM4.2-*matB* feeding experiments were resolved in 1 ml methanol and analyzed on HPLC-LC/MSD Ultra Trap System XCT 6330, Agilent Technologies. As stationary phase ReproSil-Pur C18 AQ column (5µm, 200 x 2 mm ID, Dr. Maisch) with a precolumn 10 x 2 mm was used (column temperature, 40°C). Solvent A (0.1% formic acid) and solvent B (0.06% formic acid in acetonitrile) were used as mobile phase applying the gradient t_0=47% B, t_{15}= 60% B, t_{16}= t_{20}=100%, posttime 12 min of 47% solvent B (flow rate 400 µl/min; injection volume 2.5 µl). UV signals were detected at: 230 nm, 260 nm, 280 nm, 360 nm and 435 nm. Data analysis was done with the Agilent LC/MSD software ChemStation Rev. B.01.03, Agilent. The following parameters were used for the MS detection, ionization: ESI positive and negative, alternating; mode: Ultra Scan; capillary voltage: 3.5 kV; temperature 350°C; target mass *m/z*=800). Data analyses were performed with the software 6300 Series Trap Control Version 6.1, Bruker Daltonik (Agilent).

9. Testing of kirromycin, allyl-kirromycin and coumarin-kirromycin in an *in vitro* assay

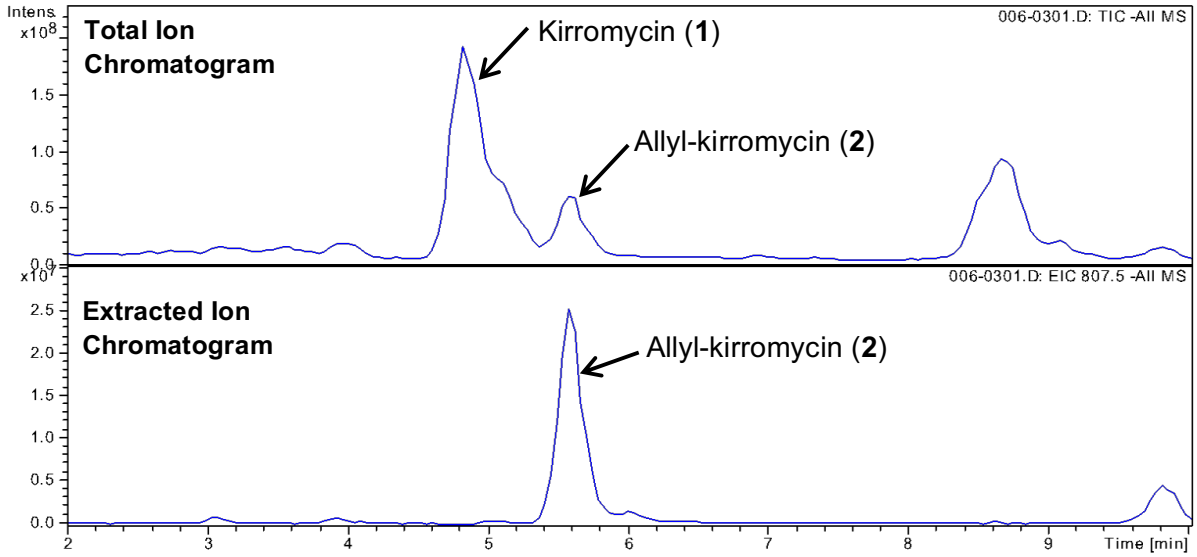
Kirromycin, allyl-kirromycin and coumarin-kirromycin activity was tested in an eGFP-based *in vitro* transcription/translation assay (Supplementary Fig. 6). Therefore, the derivative samples were analyzed by HPLC/ESI-MS and the compound concentration was calculated using kirromycin standards. Because of the residual of kirromycin in the allyl-kirromycin (90% allyl-kirromycin and 10% kirromycin) and coumarin-kirromycin (96% coumarin-kirromycin and 4% kirromycin) sample, the molarity of these samples was calculated for the total amount of kirromycins, when used in the assay (e.g. 5µM [allyl-kirromycin] = 5µM [90% allyl-kirromycin + 10% kirromycin]). To investigate the potential inhibitory effects of the remaining kirromycin molecules, control reactions covering the respective concentration range were included (Supplementary Table 3).

II. Supplementary Results

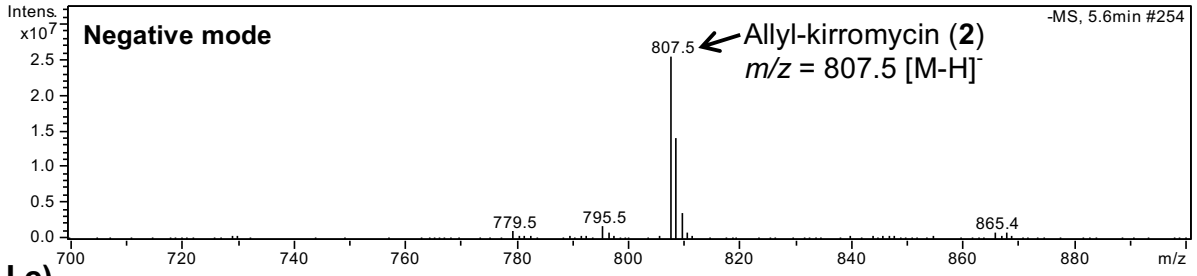
1. Supplementary Figures

Supplementary Figure 1. HPLC-MS analysis of crude extracts from *S. collinus* pRM4.2-*matB* cultures. (**I**) Detection of allyl-kirromycin (**2**) in culture fed with allylmalonic acid. (**II**) Detection of propargyl-kirromycin in culture fed with propargylmalonic acid. (**a**) Total ion chromatogram and extracted ion chromatogram. (**b**) Detection of kirromycin derivtives in negative mode. (**c**) UV/VIS spectrum at 330nm and the ratio of kirromycin and the derivative.

I a)



I b)



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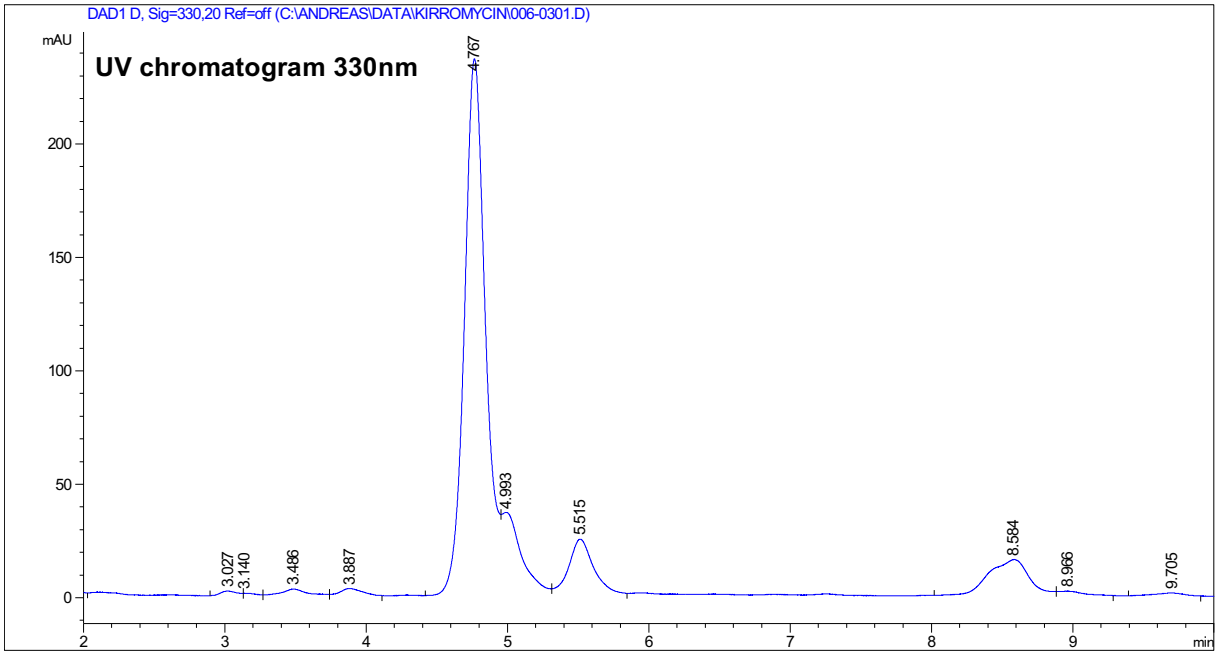
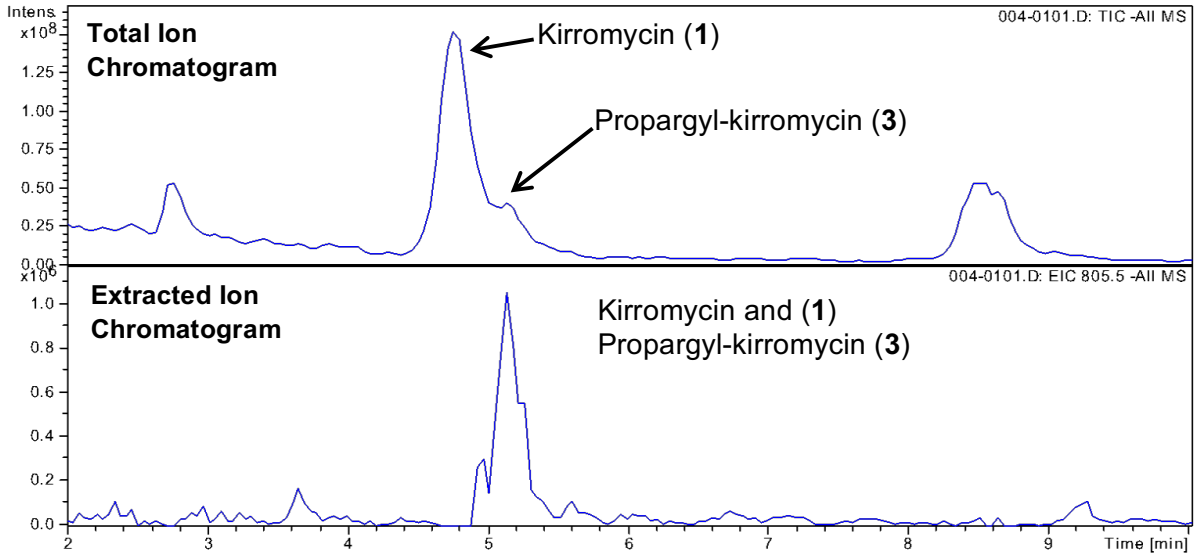
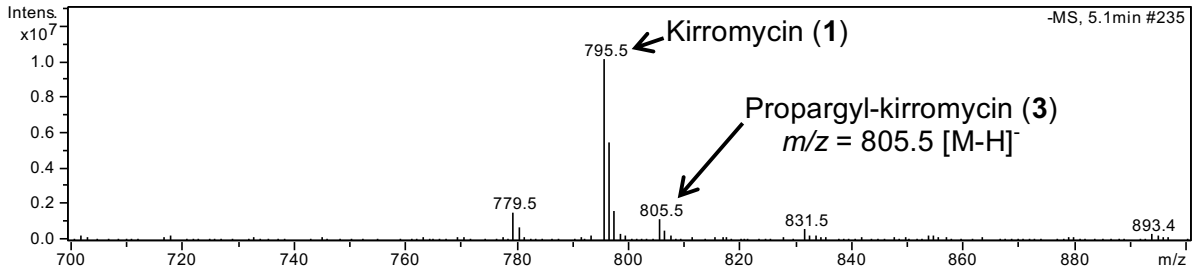


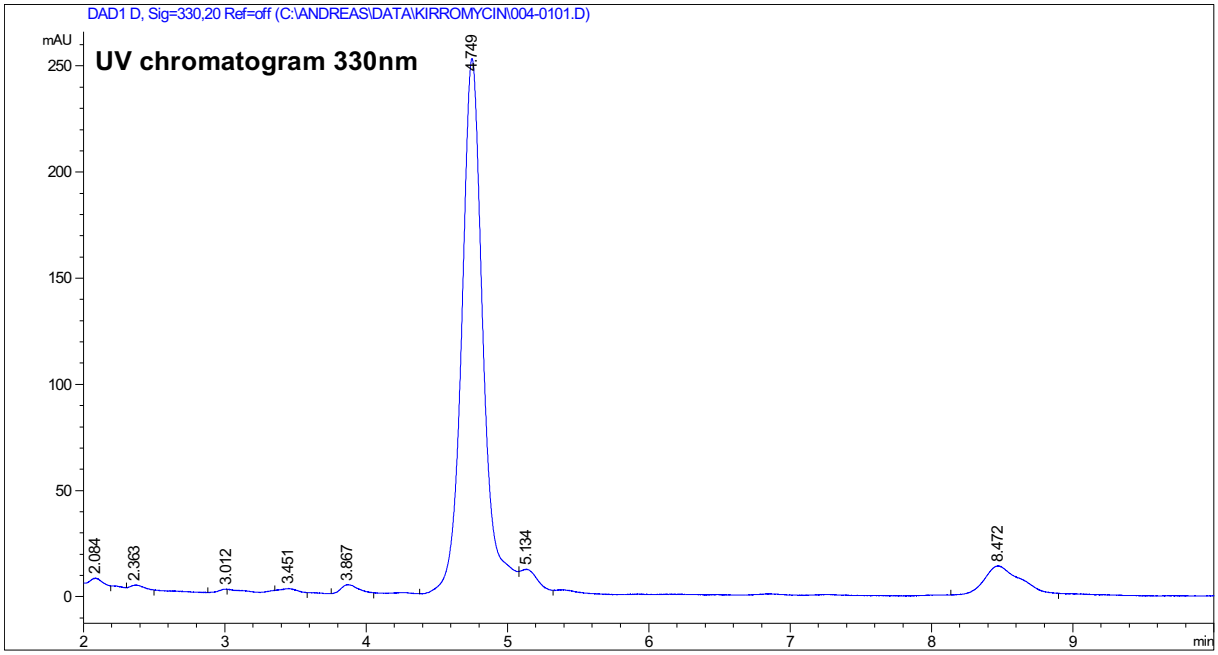
Compound	Retention time [min]	Peak area [mAU]	Percentage* (peak area)	Peak intensity (peak hight) [mAU]	Percentage* (peak hight)
Kirromycin	4.767	2421.8	89.42%	236.5	90.76%
Allyl-kirromycin	5.515	286.8	10.58%	24.1	9.24%

*To calculate the ratio of kirromycin and allyl-kirromycin the signals for peak area or peak intensity were set to 100%.

II a)





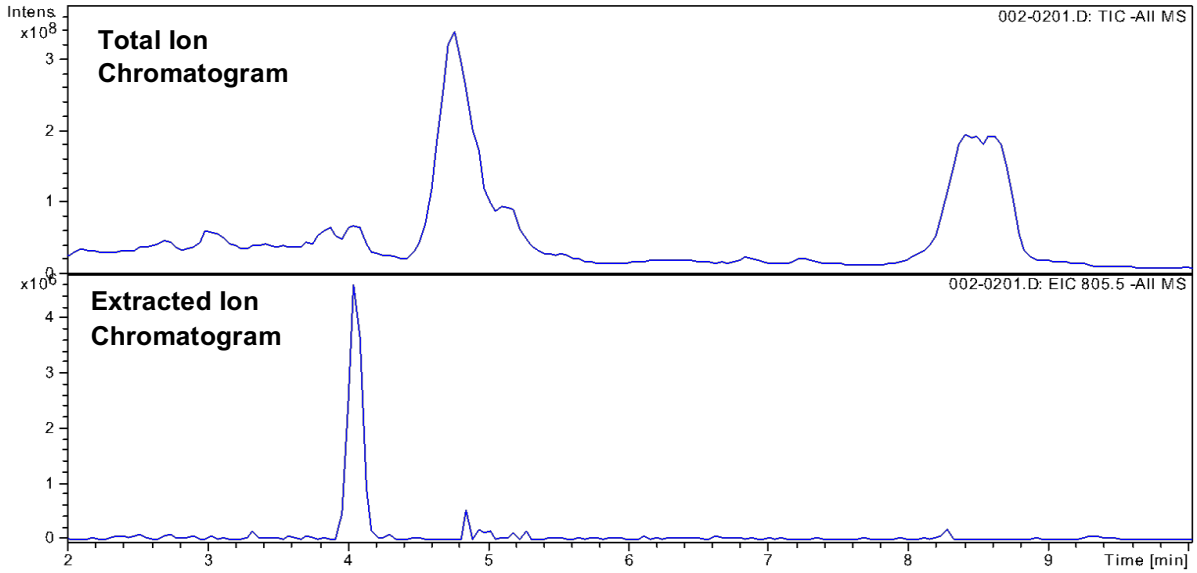


Compound	Retention time [min]	Peak area [mAU]	Percentage* (peak area)	Peak intensity (peak hight) [mAU]	Percentage* (peak hight)
Kirromycin	4.749	2613.8	96.79%	251.8	95.86%
Propargyl-kirromycin	5.134	86.8	3.21%	10.5	4.14%

*To calculate the ratio of kirromycin and allyl-kirromycin the signals for peak area or peak intensity were set to 100%.

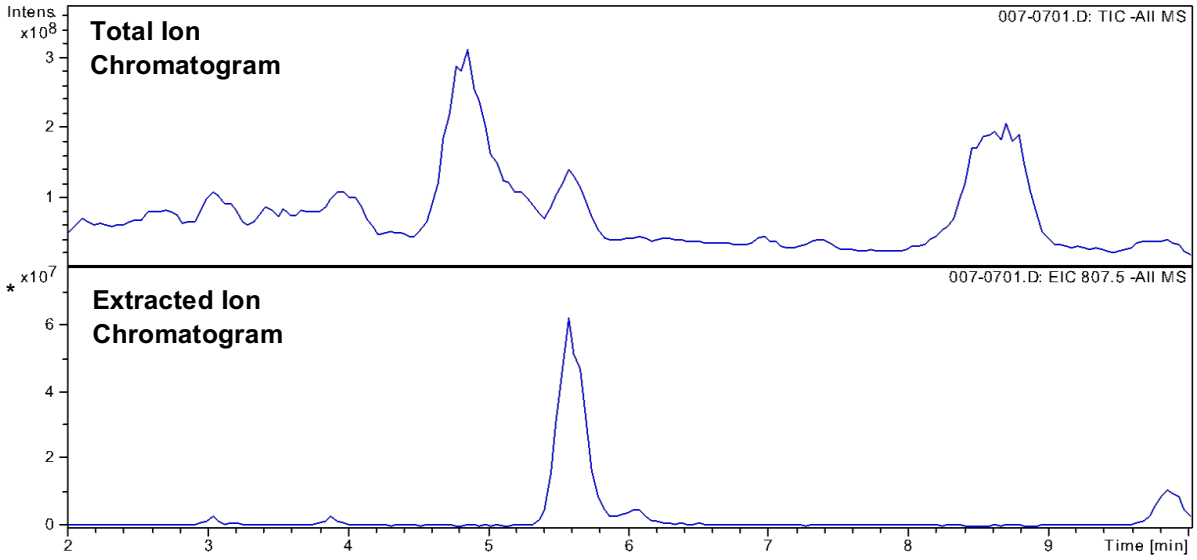
Supplementary Figure 2. HPLC-MS analysis of crude extracts from *S. collinus* Tü 365 cultures. (**I**) *S. collinus* Tü 365 without feeding. (**II**) *S. collinus* Tü 365 fed with allylmalonic acid. (**III**) *S. collinus* Tü 365 fed with propargylmalonic acid. (**a**) Total ion chromatogram. (**b**) Extracted ion chromatogram.

I a)



I b)

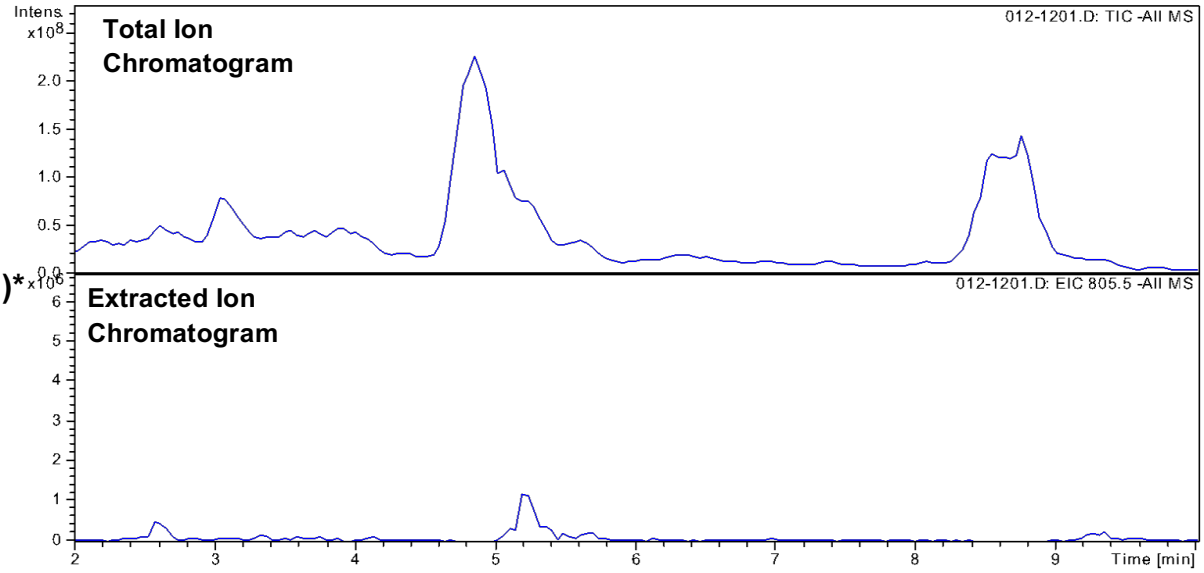
II a) *



II b) *

III a)*

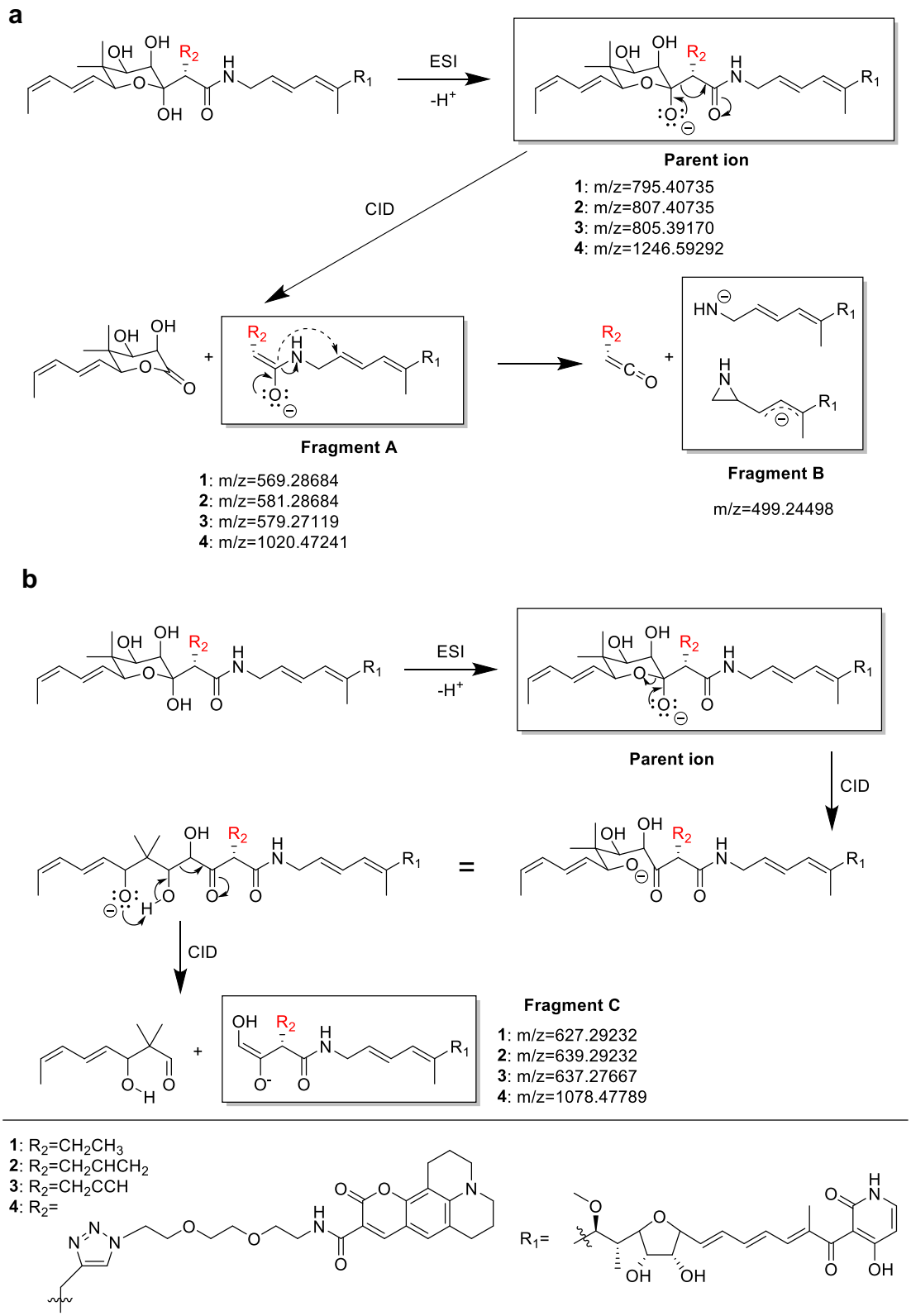
III b)*



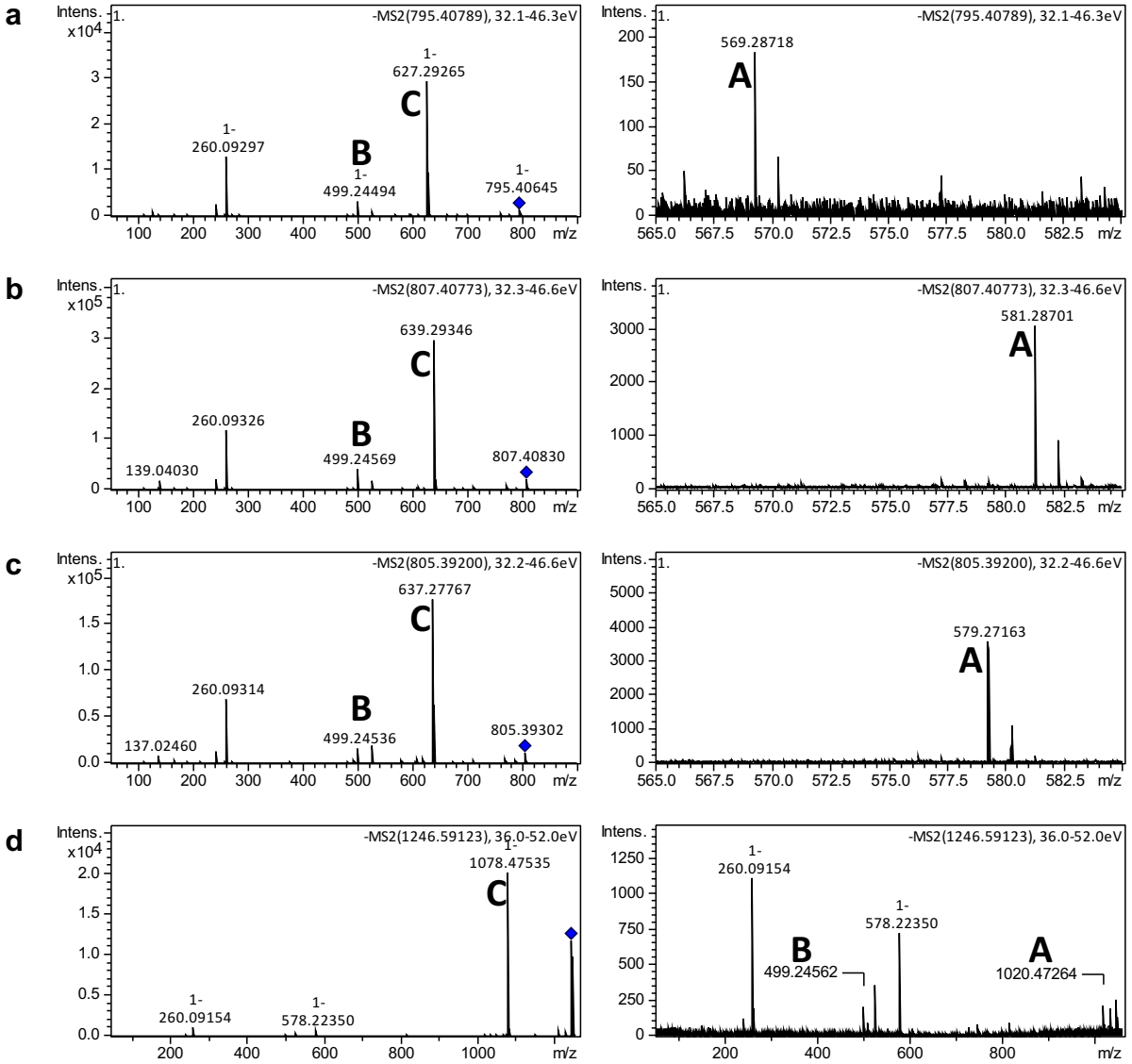
* The MatB-free *S. collinus* Tü365 strain was fed with allyl- or propagyl- malonic acid. The feeding of allyl-malonic acid resulted in the production of allyl-kirromycin, which indicates that the substrate is activated by malonyl-CoA synthetase homologue enzymes, present in *S. collinus* Tü365. Indeed, the sequence analysis using BLAST revealed that the genome of *S. collinus* Tü365 contains several genes, which encode putative acyl-CoA synthetases. This strongly suggests that at least one of the putative acyl-CoA synthetase enzymes can catalyze the activation of allyl-malonic acid, which is subsequently used in the biosynthesis of kirromycin and the derivative allyl-kirromycin. This finding is not surprising, as activity towards allylmalonate has also been described for the *R. trifolii* wild type MatB[6, 7].

Traces of a peak with the mass of *m/z* = 805.5 $[M-H]^-$ were detected also in the culture filtrate of *S. collinus* Tü365 where no substrate was added and in the MatB-free *S. collinus* Tü365, which was fed with propagylmalonic acid. This indicates that the detected mass is likely of diffent origin and that the putative acyl-CoA synthetase(s) present in the wild type strain *S. collinus* Tü365 are not able to efficiently activate propagyl-malonic acid. Only in case where the MatB-engineered *S. collinus* Tü365 was fed with propagyl-malonic acid, significant amounts of propargyl-kirromycin were detected and the product could be enriched.

Supplementary Figure 3. Suggested fragmentation mechanisms for intermediates A-C. (**a**) Fragmentation mechanism of intermediates A and B. (**b**) Fragmentation mechanism of intermediate C. Detected ions are indicated by frames and the calculated *m/z* for fragments of kirromycin (**1**), allyl-kirromycin (**2**), propargyl-kirromycin (**3**) and coumarin-kirromycin (**4**) is provided.



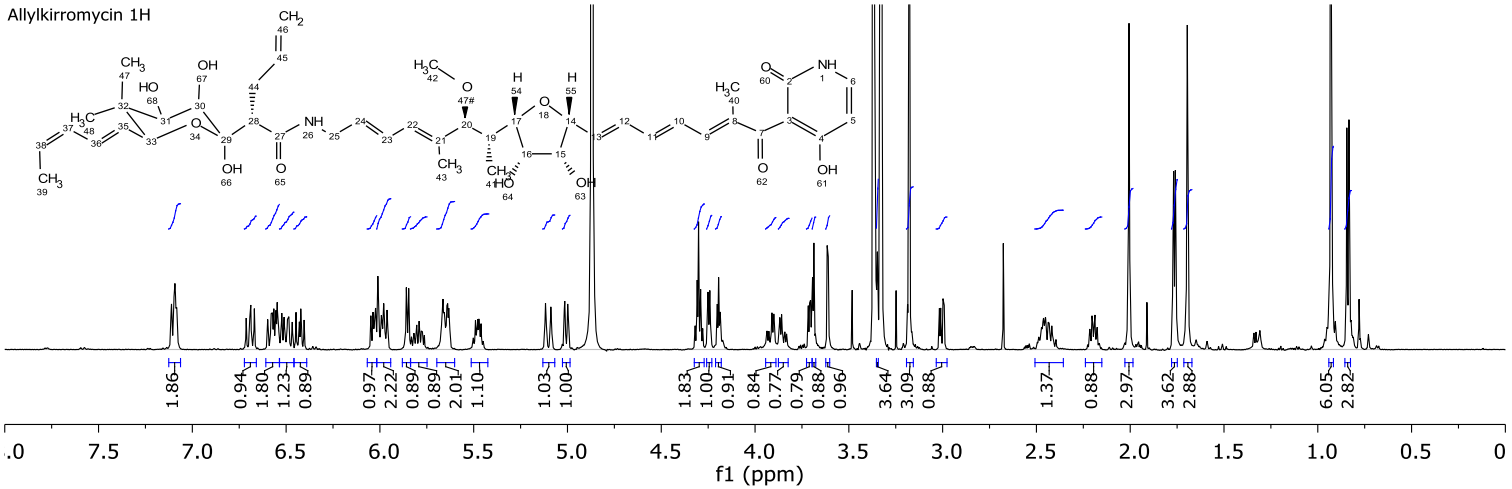
Supplementary Figure 4. MS/MS spectra of (**a**) kirromycin (**1**), (**b**) allylkirromycin (**2**), (**c**) propargyl-kirromycin (**3**) and (**d**) coumarin-kirromycin (**4**) with annotated fragments A, B and C. The parent ions of the intact chemical compounds are indicated by a “blue diamond”.



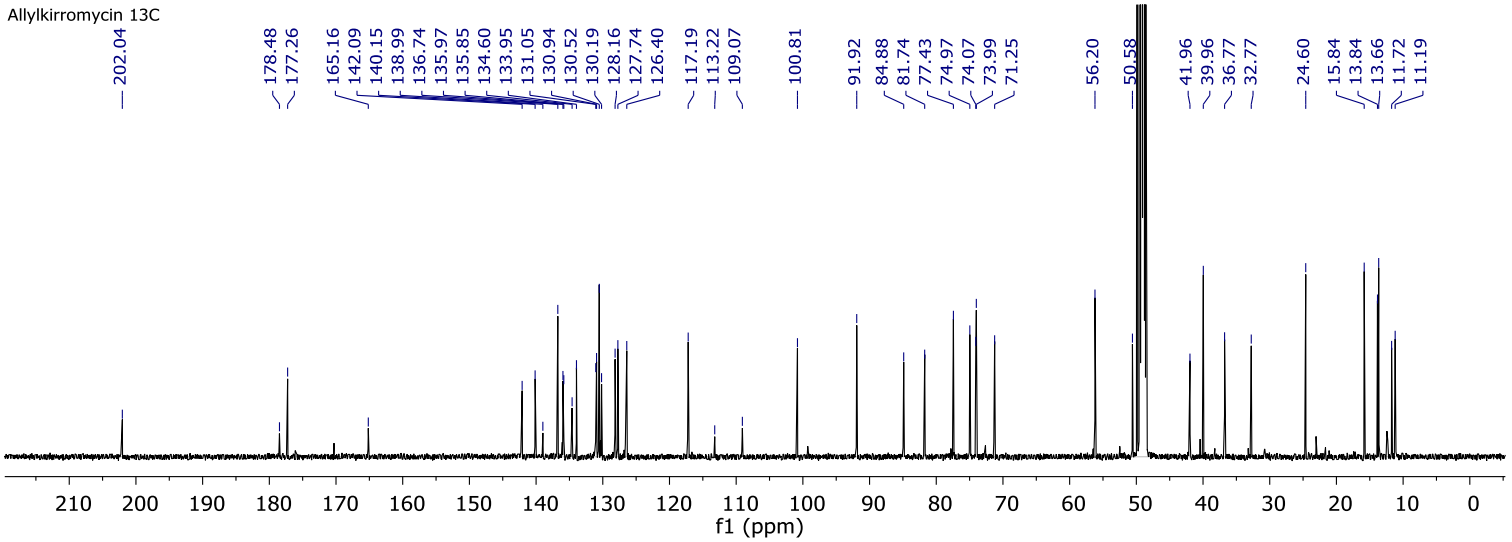
Supplementary Figure 5. 1H NMR spectra and assignment.

(**a**) ^{1}H NMR spectra and (**b**) ^{13}C NMR spectra of allyl-kirromycin (**I,** MeOD, 600 MHz)**,** propargyl-kirromycin (**II,** MeOD, 700 MHz) and coumarin-kirromycin (**III,** MeOD, 700 MHz).

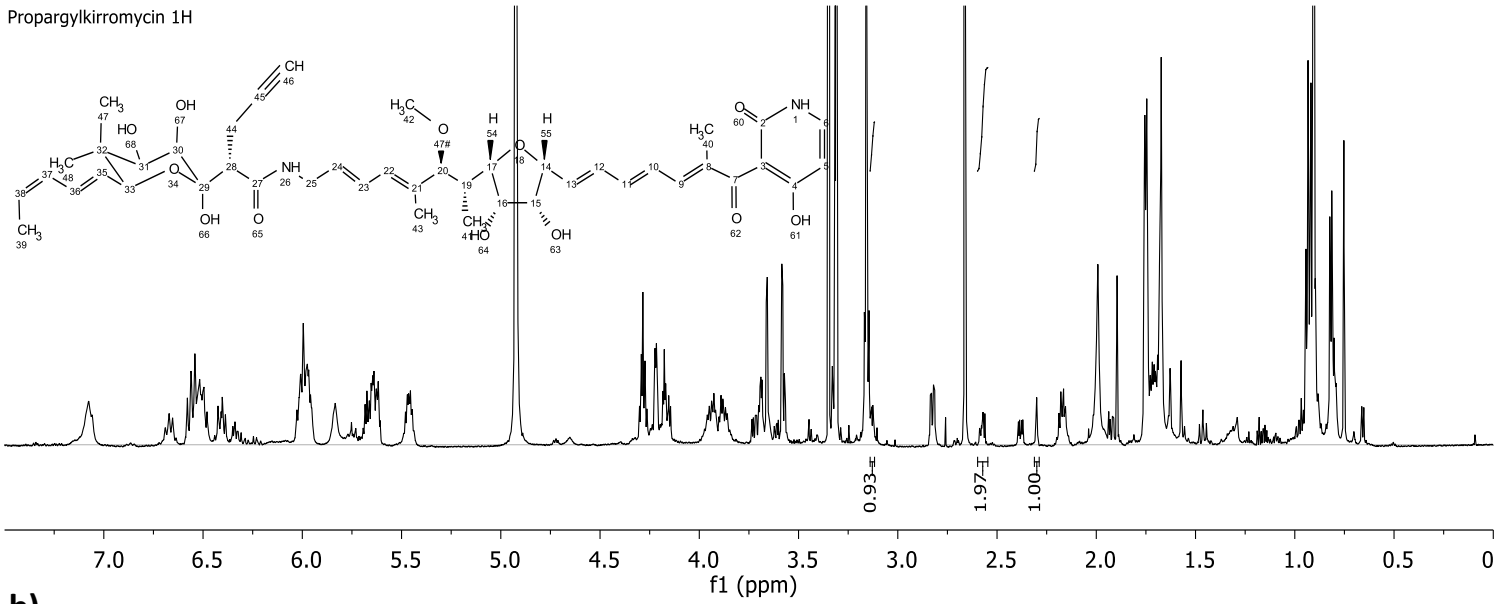
I a)


Allylkirromycin 1H
1.86
0.94
1.80
1.23
0.89
0.97
2.22
0.89
0.89
2.01
1.10
1.03
1.00
1.83
1.00
0.91
0.84
0.77
0.79
0.88
0.96
3.64
3.09
0.88
1.37
0.88
2.97
3.62
2.88
6.05
2.82
f1 (ppm)

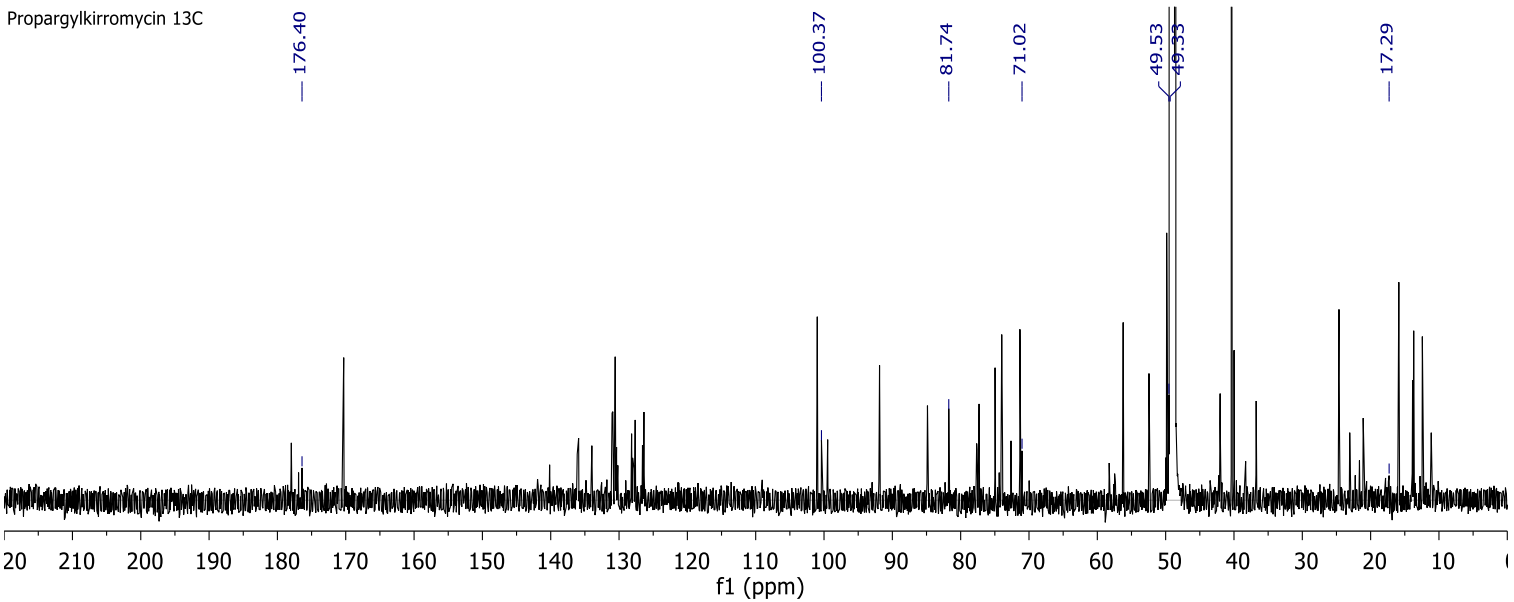
I b)


Allylkirromycin 13C
202.04
178.48
177.26
165.16
142.09
140.15
138.99
136.74
135.97
135.85
134.60
133.95
131.05
130.94
130.52
130.19
128.16
127.74
126.40
117.19
113.22
109.07
100.81
91.92
84.88
81.74
77.43
74.97
74.07
73.99
71.25
56.20
50.58
41.96
39.96
36.77
32.77
24.60
15.84
13.84
13.66
11.72
11.19
f1 (ppm)

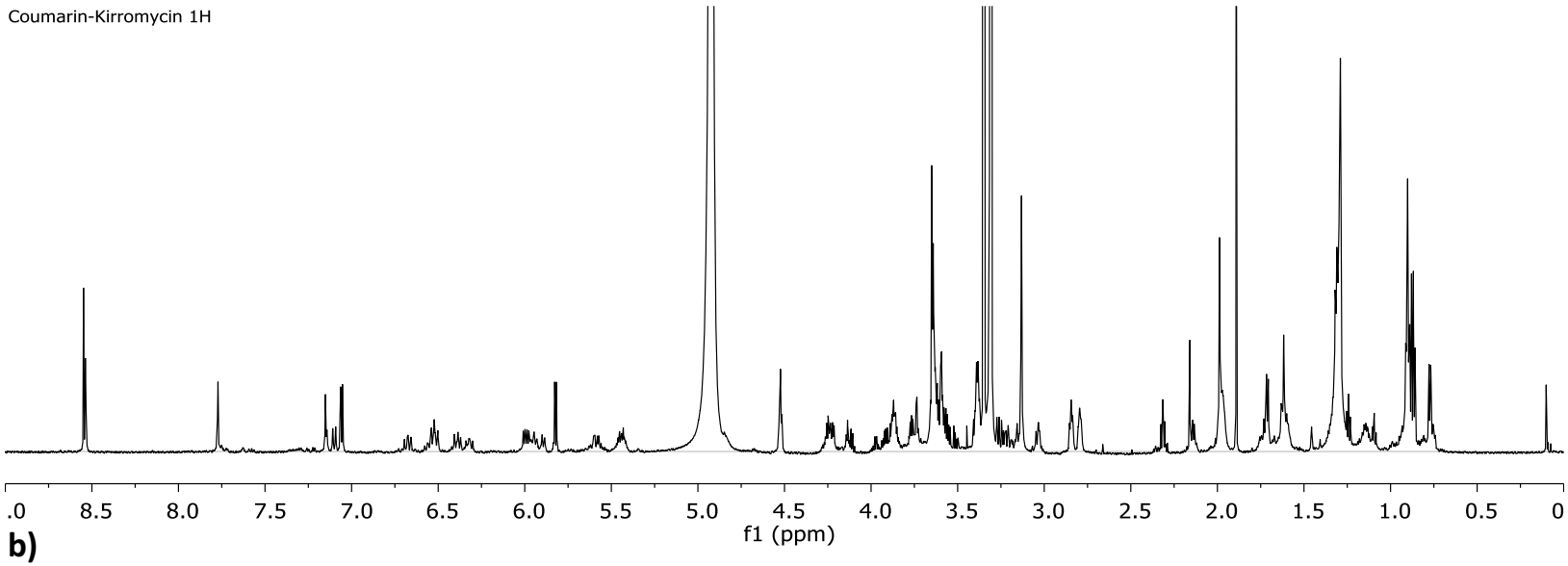
II a)


Propargylkirromycin 1H
0.93
1.97
1.00
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
f1 (ppm)

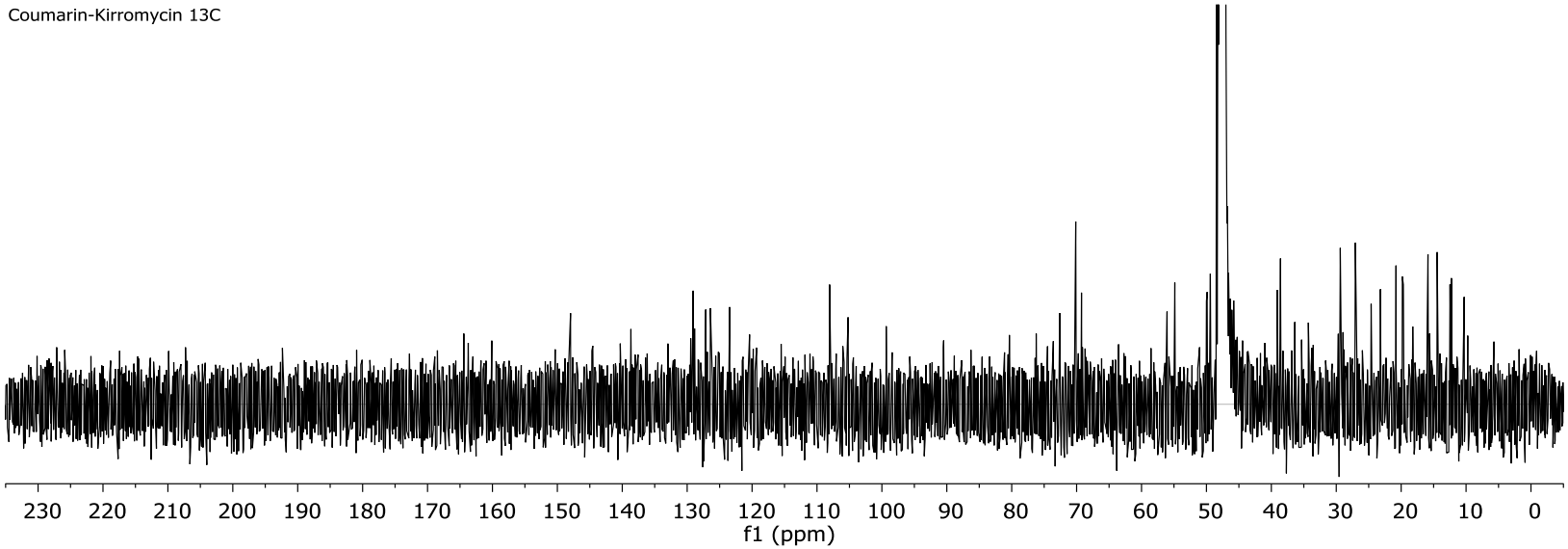
II b)


Propargylkirromycin 13C
176.40
100.37
81.74
71.02
49.53
49.33
17.29
20
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

III a)


Coumarin-Kirromycin 1H
.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
f1 (ppm)

III b)


Coumarin-Kirromycin 13C
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

Supplementary Figure 6. 2D NMR spectra.

(**I**) 2D NMR spectra of allyl-kirromycin (**2**).

I a) 2D NMR spectrum of allyl-kirromycin (**2**), ^{1}H-^{1}H COSY (homonuclear ^{1}H-^{1}H correlation spectroscopy), 600 MHz, CD_3OD, 293 K.

I b) 2D NMR spectrum of allyl-kirromycin (**2**), HSQC (heteronuclear single quantum correlation), 600 MHz, CD_3OD, 293 K.

I c) 2D NMR spectrum of allyl-kirromycin (**2**), HMBC (heteronuclear multiple bond correlation), 600 MHz, CD_3OD, 293 K; key correlations of 44-H_2 (δ_H = 2.44 ppm) are highlighted in green boxes.

(**II**) 2D NMR spectra of propargyl-kirromycin (**3**).

II a) 2D NMR spectrum of propargyl-kirromycin (**3**), ^{1}H-^{1}H COSY (homonuclear ^{1}H-^{1}H correlation spectroscopy), 700 MHz, CD_3OD, 293 K.

II b) 2D NMR spectrum of propargyl-kirromycin (**3**), HSQC (heteronuclear single quantum correlation), 700 MHz, CD_3OD, 293 K.

II c) 2D NMR spectrum of propargyl-kirromycin (**3**), HMBC (heteronuclear multiple bond correlation), 700 MHz, CD_3OD, 293 K; key correlations of 44-H_2 (δ_H = 2.57 ppm) are highlighted in green boxes.

(**III**) 2D NMR spectra of coumarin-kirromycin (**4**), the “click-product”.

^{1}H-^{1}H COSY (homonuclear ^{1}H-^{1}H correlation spectroscopy), 700 MHz, CD_3OD, 293 K.

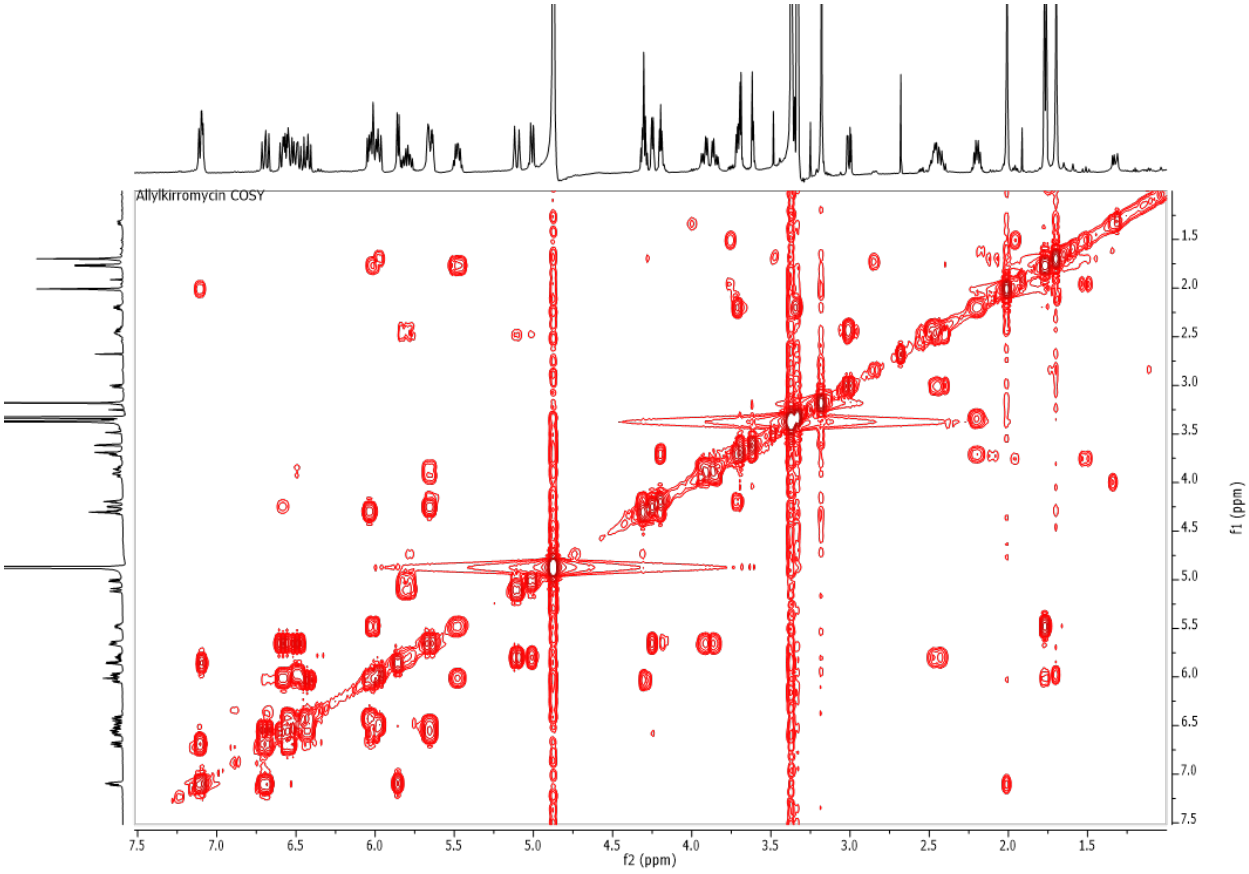
III a1) 2D NMR spectrum of coumarin-kirromycin (**4**), ^{1}H-^{1}H COSY (homonuclear ^{1}H-^{1}H correlation spectroscopy), 700 MHz, CD_3OD, 293 K.

III a2) 2D NMR spectrum of coumarin-kirromycin (**4**), ^{1}H-^{1}H TOCSY (total spin-spin coupling correlation spectroscopy), 700 MHz, CD_3OD, 293 K.

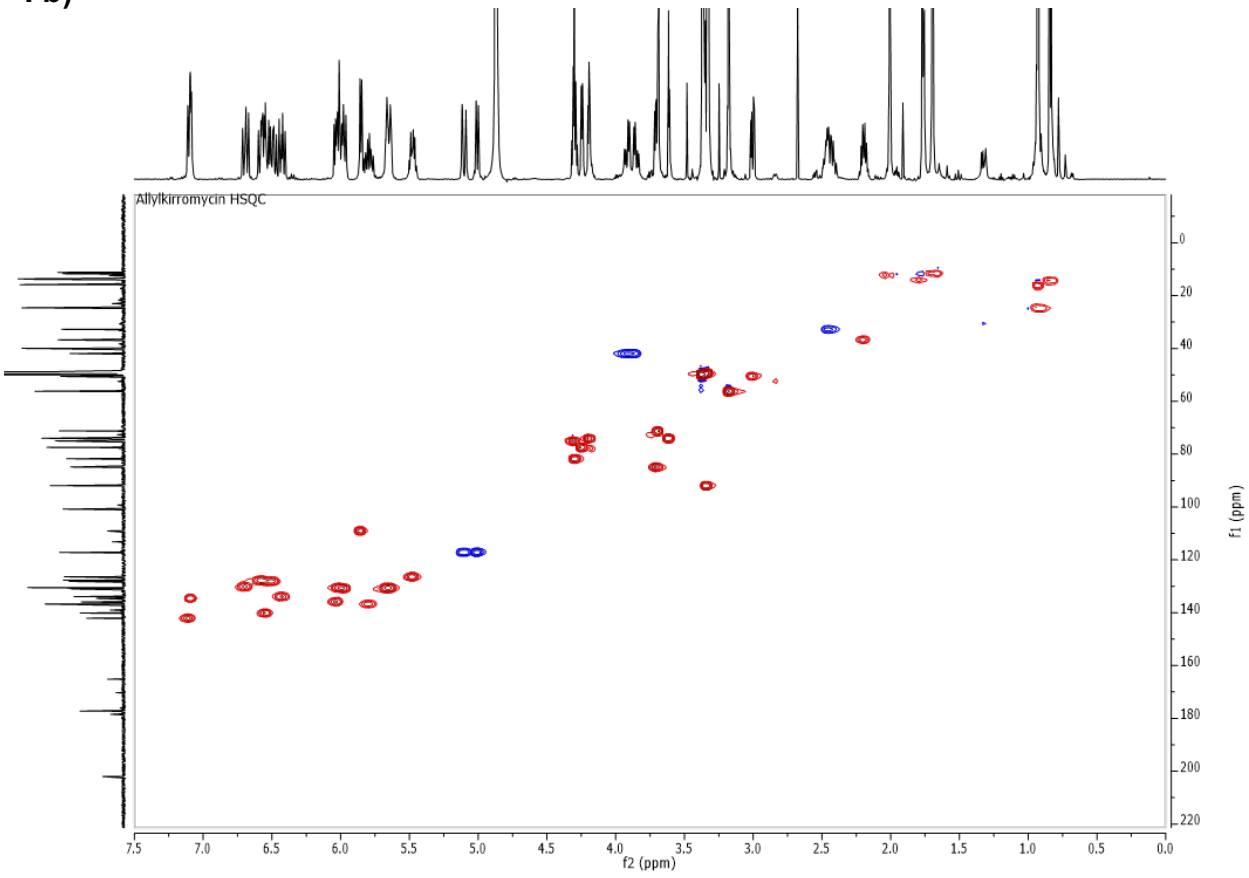
III b) 2D NMR spectrum of coumarin-kirromycin (**4**), HSQC (heteronuclear single quantum correlation), 700 MHz, CD_3OD, 293 K.

III c) 2D NMR spectrum of coumarin-kirromycin (**4**), HMBC (heteronuclear multiple bond correlation), 700 MHz, CD_3OD, 293 K; key correlations of 44-H_2 (δ_H = 3.03 ppm) are highlighted in green boxes.

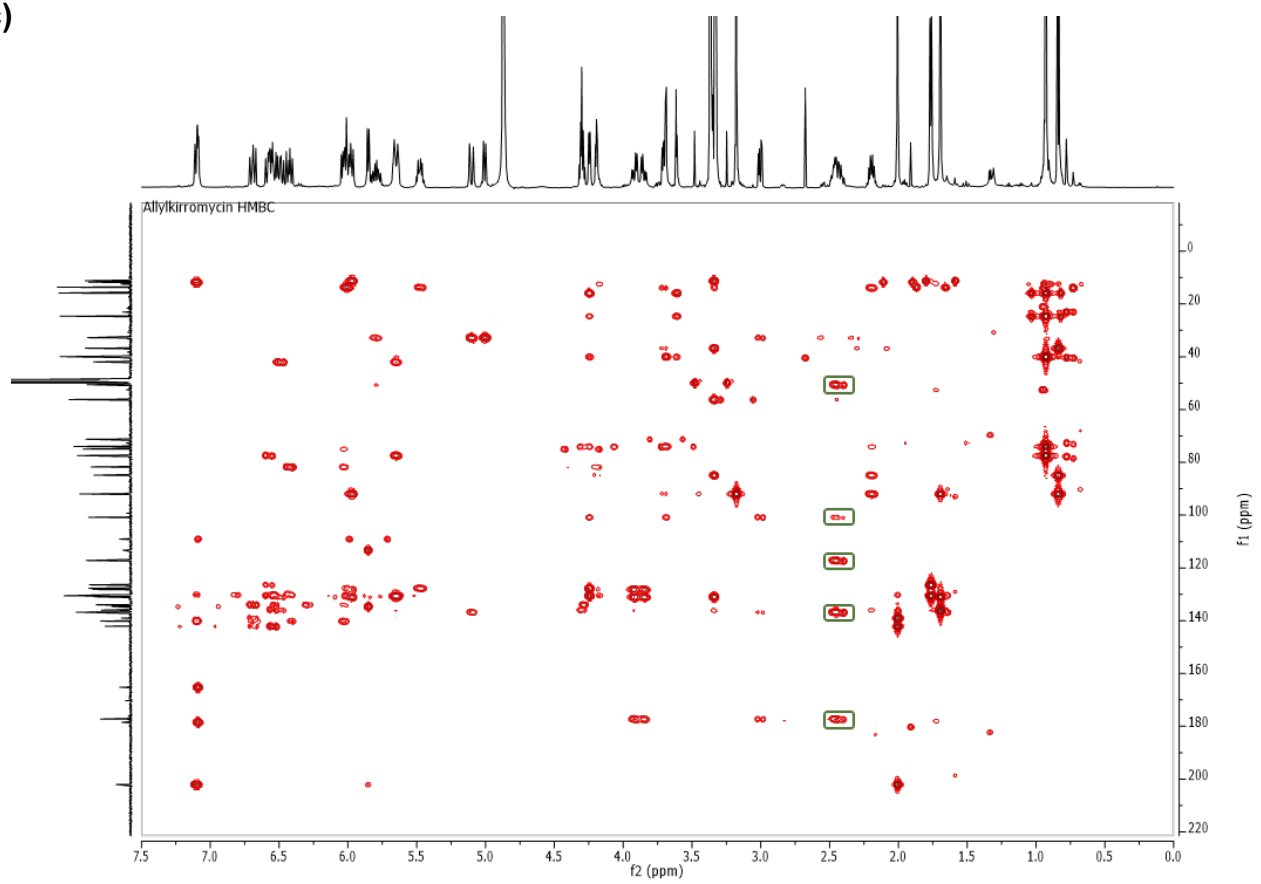
I a)


Allylkirromycin COSY
f2 (ppm)
f1 (ppm)

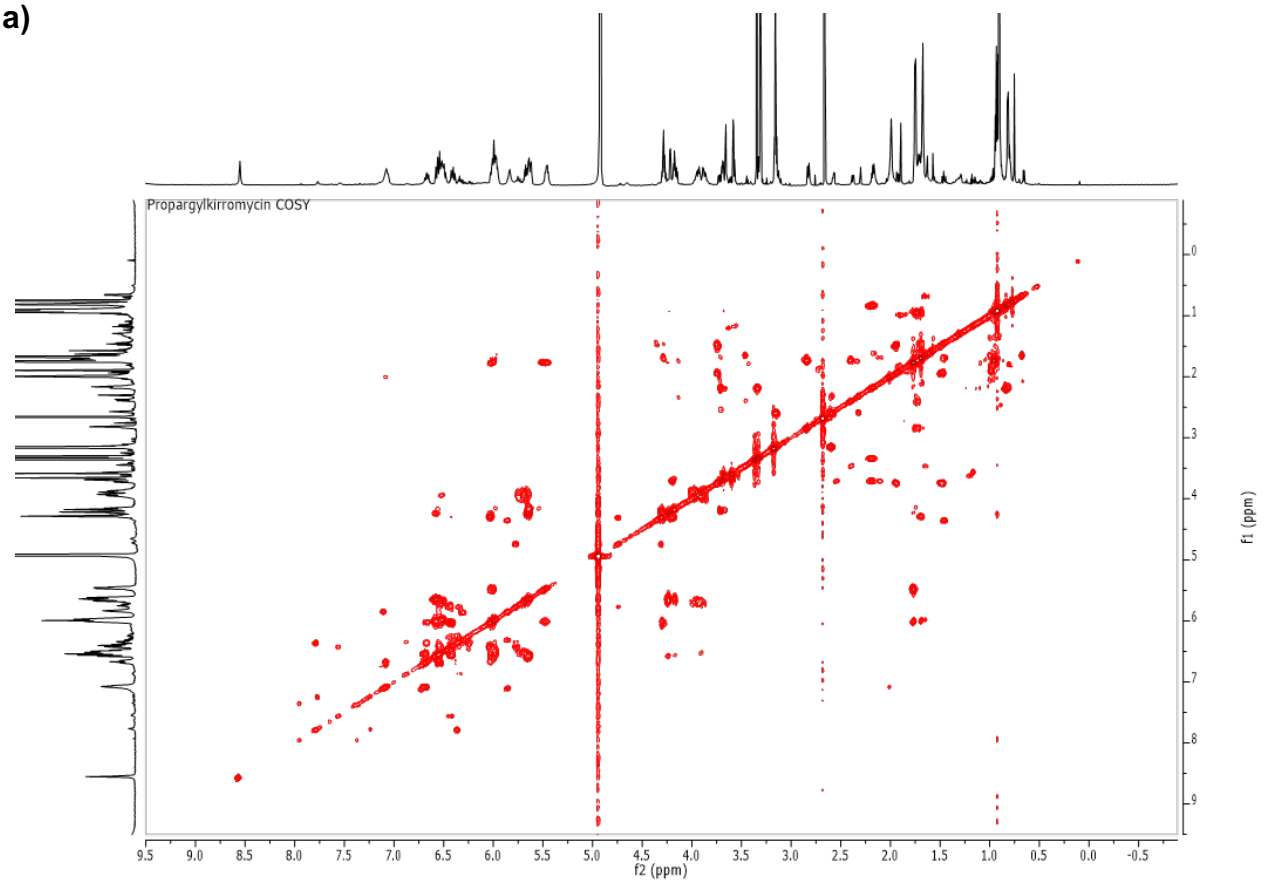
I b)


Allylkirromycin HSQC
f2 (ppm)
f1 (ppm)

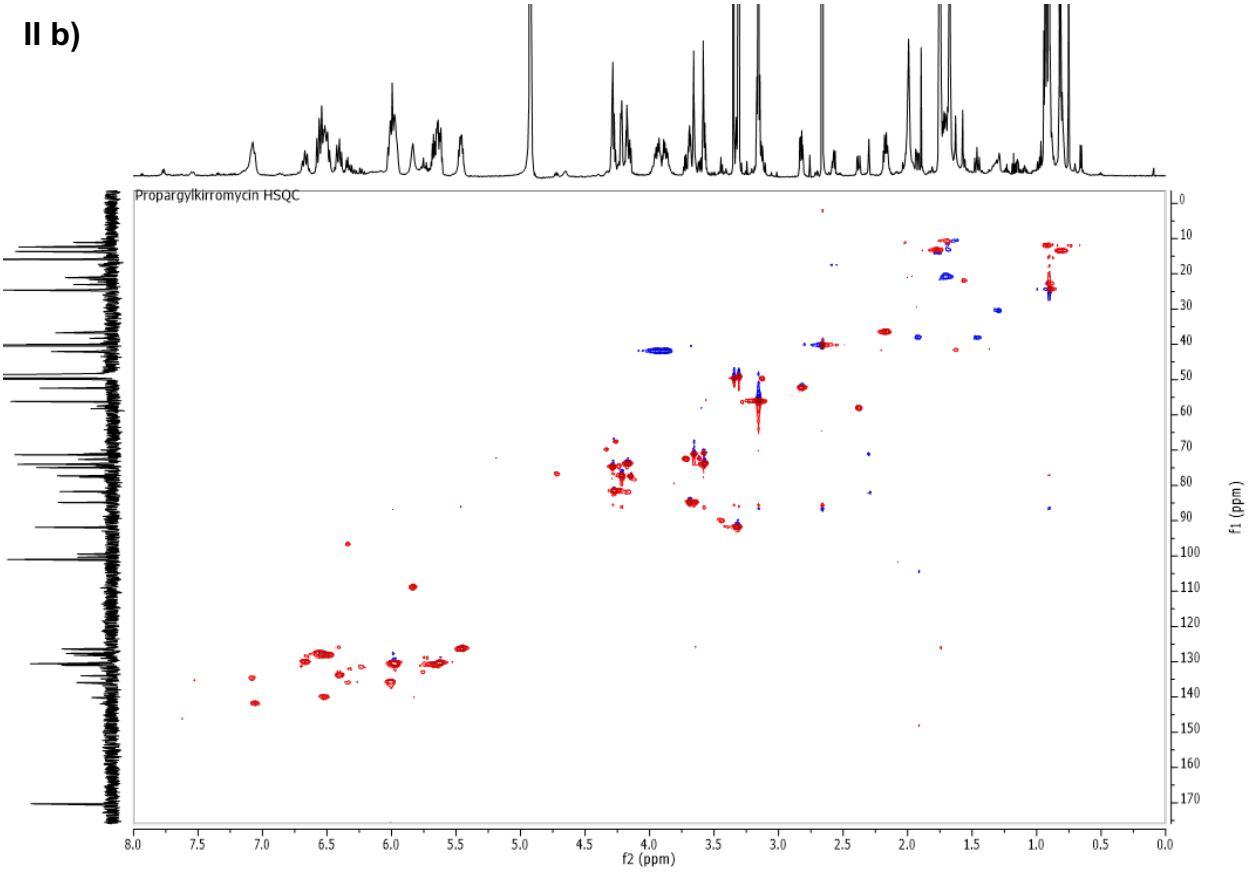
I c)


Allylkirromycin HMBC
f2 (ppm)
f1 (ppm)

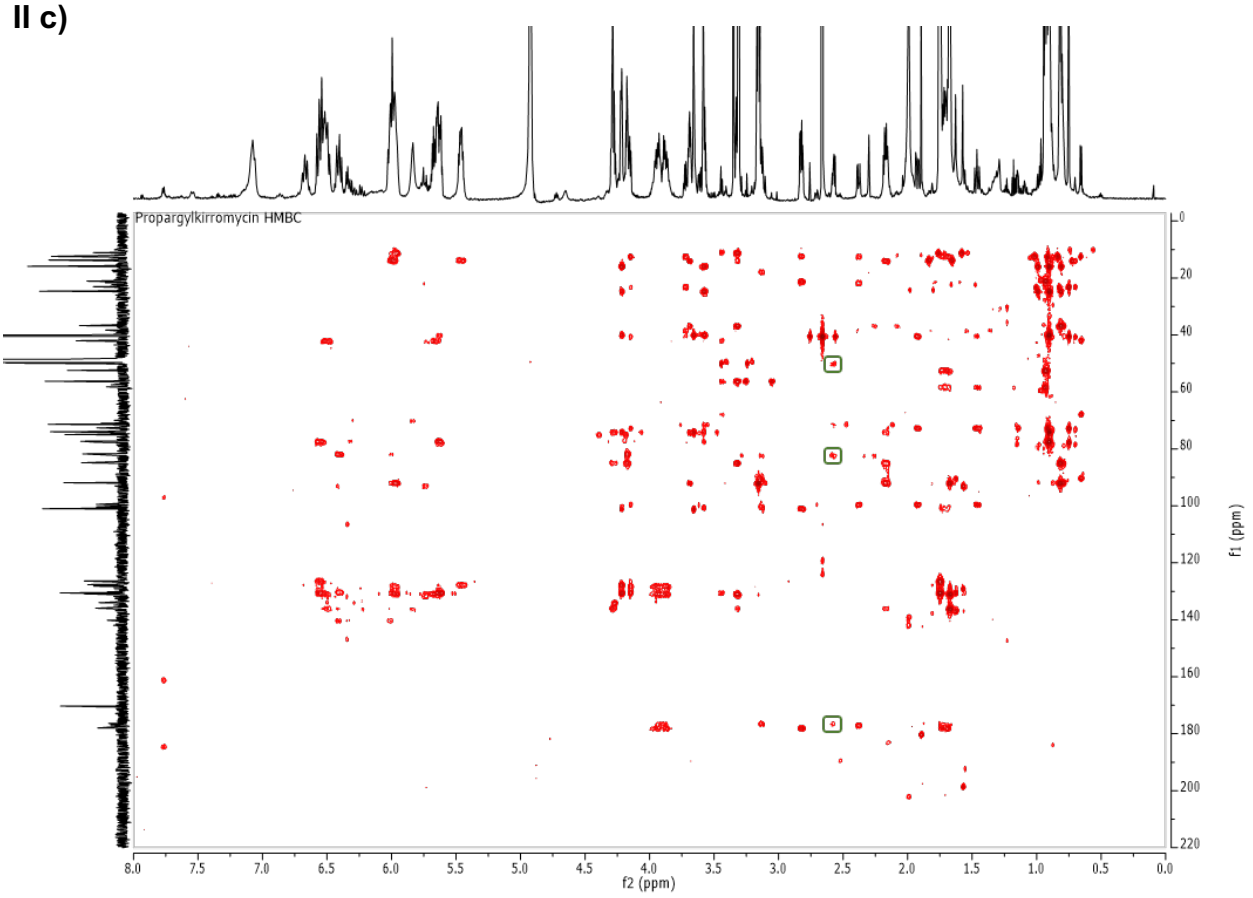
II a)


Propargylkirromycin COSY
f2 (ppm)
f1 (ppm)

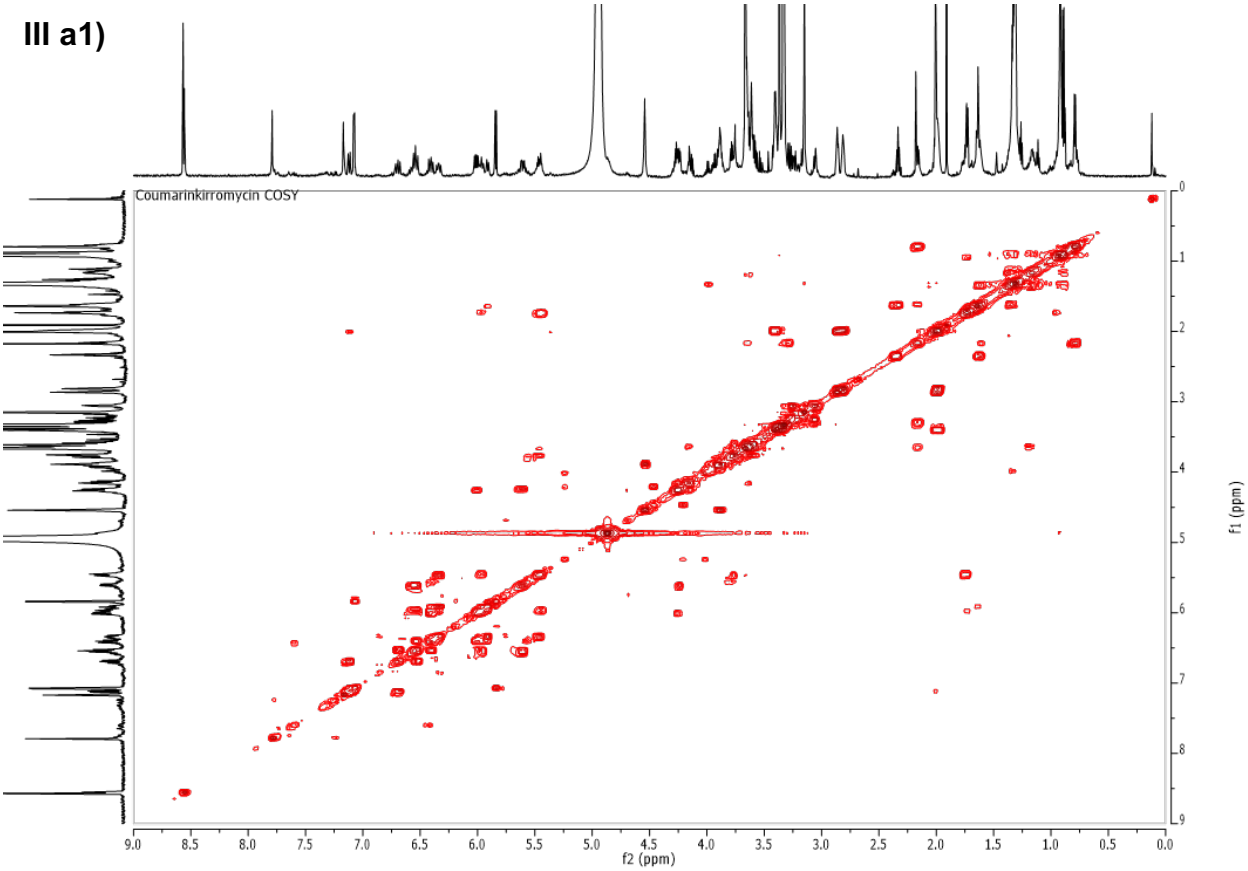
II b)


Propargylkirromycin HSQC
f2 (ppm)
f1 (ppm)

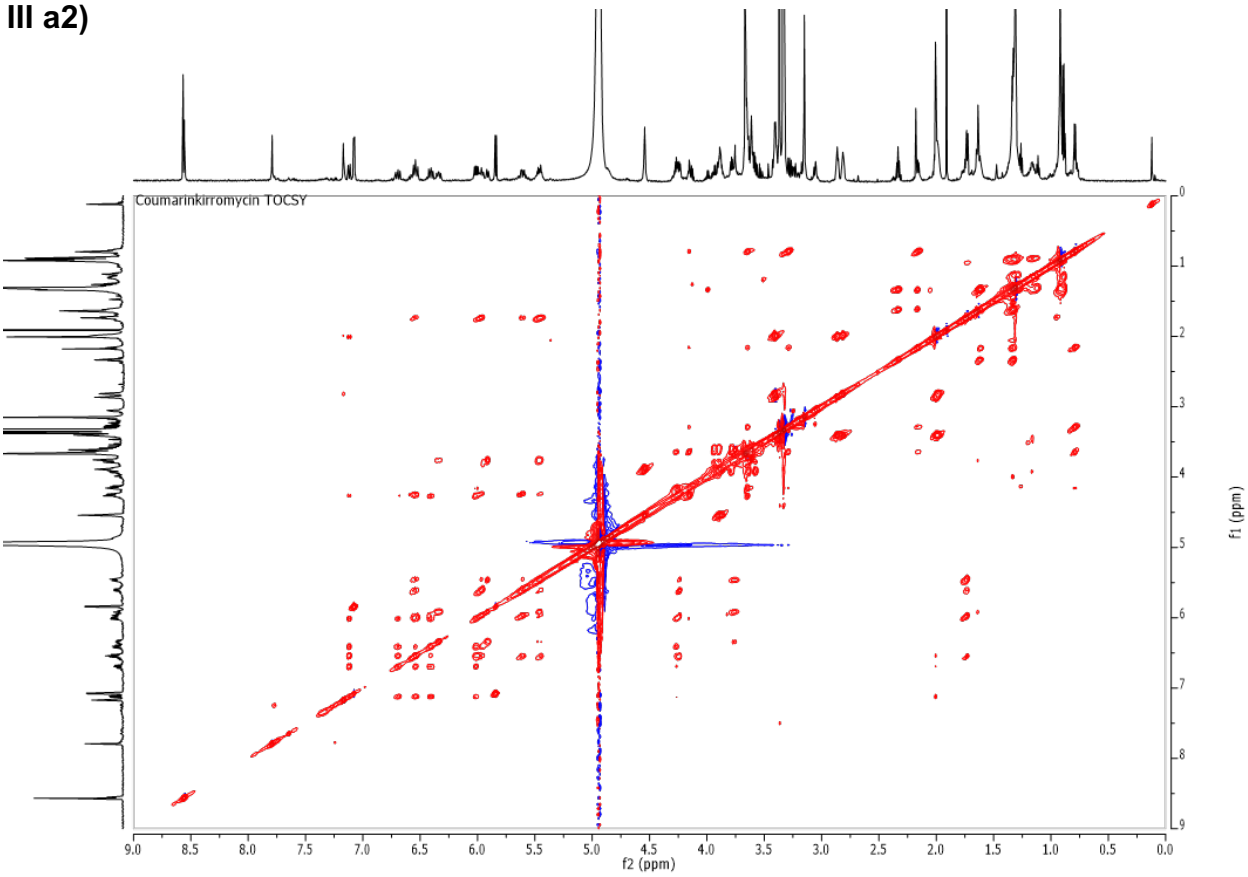
II c)


Propargylkirromycin HMBC
f2 (ppm)
f1 (ppm)

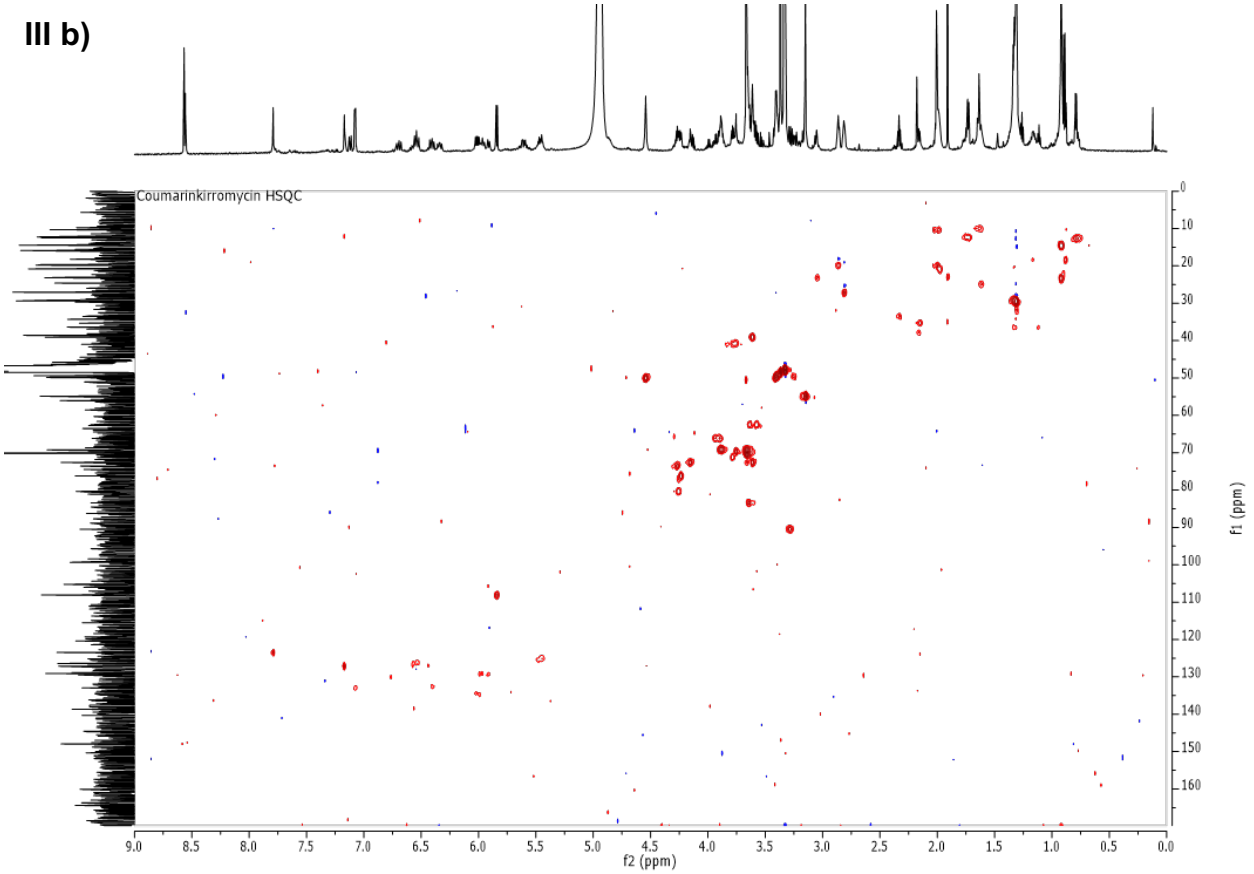
III a1)


Coumarinkirromycin COSY
f2 (ppm)
f1 (ppm)

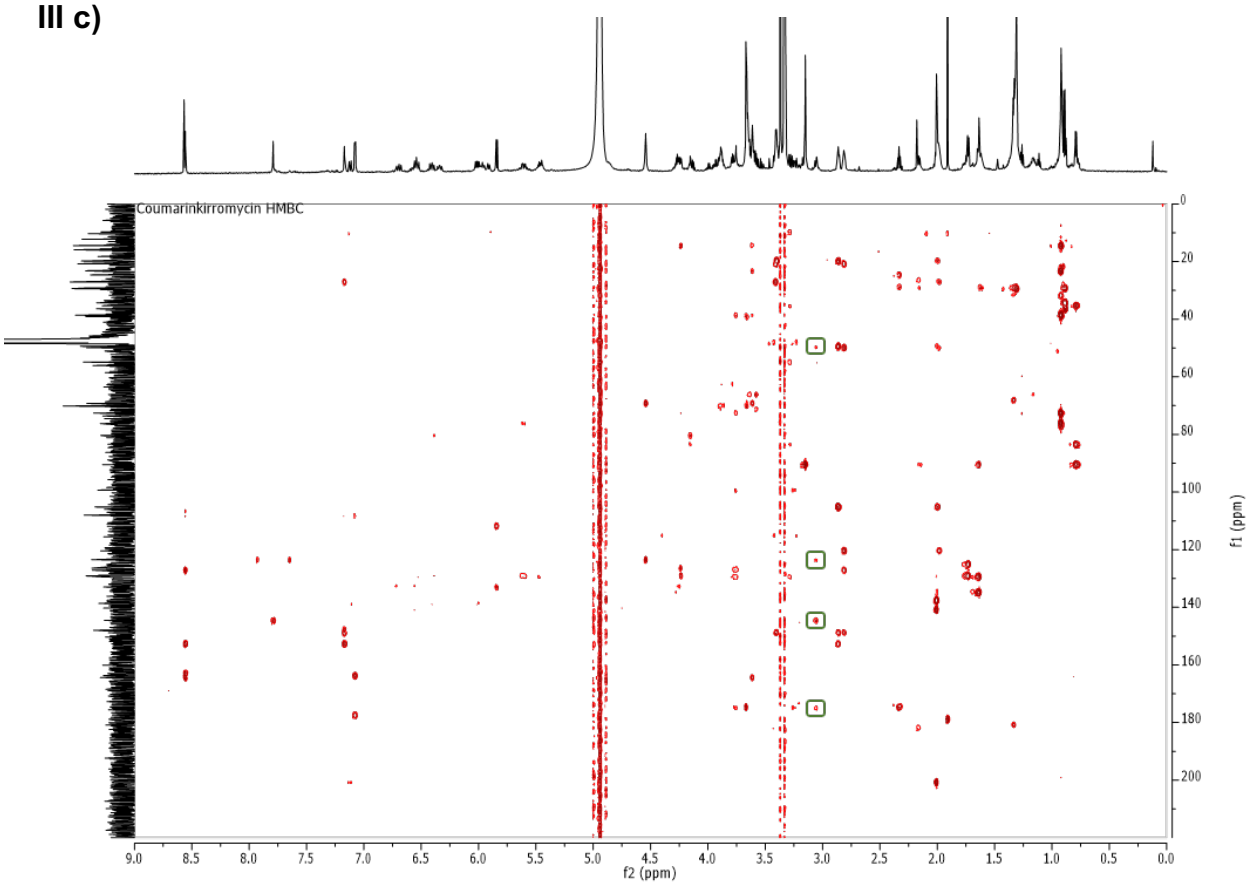
III a2)


Coumarinkirromycin TOCSY
f2 (ppm)
f1 (ppm)

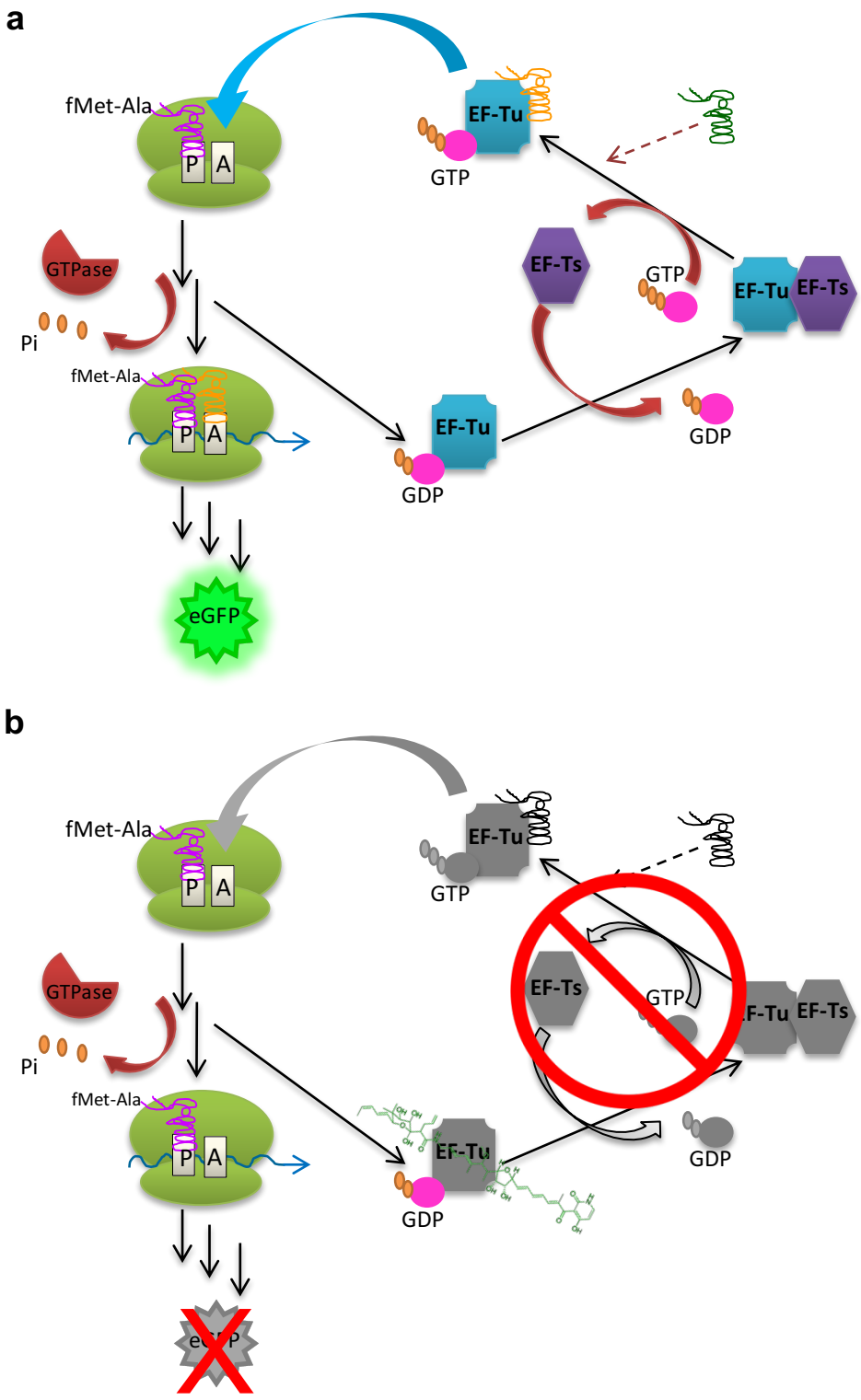
III b)


Coumarinkirromycin HSQC
f2 (ppm)
f1 (ppm)

III c)


Coumarinkirromycin HMBC
f2 (ppm)
f1 (ppm)

Supplementary Figure 7. eGFP based *in vitro* activity assay. (**a**) Schematic *in vitro* translation of eGFP. (**b**) Inhibition of eGFP translation caused by kirromycins.



2. Supplementary Tables

Supplementary Table 1. Calculated and identified masses (*m/z*) of kirromycin (**1**), allyl-kirromycin (**2**), propargyl-kirromycin (**3**) and coumarin-kirromycin (**4**) and their fragmentation products. The chemical shifts, obtained from nuclear magnetic resonance (NMR) spectroscopy analysis are represented by Δ ppm.

Compound		**Parent Ion**	**Fragment A**	**Fragment B**	**Fragment C**
Kirromycin (**1**)	**Formula**	$C_{43}H_{59}N_2O_{12}$	$C_{31}H_{41}N_2O_8$	$C_{27}H_{35}N_2O_7$	$C_{33}H_{43}N_2O_{10}$
	measured	795.40788	569.28718	499.24494	627.29265
	calculated	795.40735	569.28684	499.24498	627.29232
	Δ ppm	0.67	0.60	0.08	0.53
Allyl-Kirromycin (**2**)	**Formula**	$C_{44}H_{59}N_2O_{12}$	$C_{32}H_{41}N_2O_8$	$C_{27}H_{35}N_2O_7$	$C_{34}H_{43}N_2O_{10}$
	measured	807.40773	581.28701	499.24569	639.29346
	calculated	807.40735	581.28684	499.24498	639.29232
	Δ ppm	0.47	0.30	1.44	1.78
Propargyl-Kirromycin (**3**)	**Formula**	$C_{44}H_{57}N_2O_{12}$	$C_{32}H_{39}N_2O_8$	$C_{27}H_{35}N_2O_7$	$C_{34}H_{41}N_2O_{10}$
	measured	805.39200	579.27163	499.24536	637.27767
	calc.	805.39170	579.27119	499.24498	637.27667
	Δ ppm	0.38	0.77	0.77	1.58
Coumarin-Kirromycin (**4**)	**Formula**	$C_{66}H_{84}N_7O_{17}$	$C_{54}H_{66}N_7O_{13}$	$C_{27}H_{35}N_2O_7$	$C_{56}H_{68}N_7O_{15}$
	measured	1246.59124	1020.47264	499.24562	1078.47535
	calculated	1246.59292	1020.47241	499.24498	1078.47789
	Δ ppm	1.35	0.22	1.29	2.35

Supplementary Table 2. ^{1}H and ^{13}C NMR data for kirromycin derivatives.

	Allyl-kirromycin (600 MHz, CD_3OD)		Coumarin-kirromycin (700 MHz, CD_3OD)		Propargyl-kirromycin (700 MHz, CD_3OD)	
Position	**δ_H [ppm] (Integral, Type, J in Hz)**	**δ_C [ppm]**	**δ_H [ppm] (Integral, Type, J in Hz)**	**δ_C [ppm]**	**δ_H [ppm] (Integral, Type, J in Hz)**	**δ_C [ppm]**
1	-	-	-	-		
2	-	165.2	-	n.d.		
3	-	113.2	-	111.8*		
4	-	178.5	-	n.d.		
5	5.85, 1H, d. *J* = 7.4	109.1	5.82, 1H, d, *J* = 7.4	108.1		
6	7.10, 1H, d, *J* = 7.4	134.6	7.06, 1H, d, *J* = 7.4	132.9*		
7	-	202.0	-	200.8*		
8	-	139.0	-	n.d.		
9	7.10, 1H, d, *J* = 11.1	142.1	7.10, 1H, d, *J* = 11.6	n.d.		
10	6.69, 1H, dd, *J* = 14.7, 11.4	130.2	6.68, 1H, dd, *J* = 11.5, 15.0	n.d.		
11	6.55, 1H, m (with 36)	140.2	6.52, 1H, m (with 36)	n.d.		
12	6.43, 1H, dd, *J* = 15.2, 10.7	134.0	6.39, 1H, m	n.d.		
13	6.03, 1H, m (with 22, 37)	135.9	6.00, m	n.d.		
14	4.29, 1H, m (with 15)	81.7	4.24, m	n.d.		
15	4.31, 1H, m (with 14)	75.0	n. a.	n.a.		
16	4.19, 1H, dd, *J* = 4.5, 4.1	74.1	n. a.	n.a.		
17	3.71, 1H, dd, *J* = 7.3, 4.1	84.9	3.64, m	n.d.		
18	-	-	-	-		
19	2.20, 1H, m	36.8	2.14, 1H, m	36.4*		
20	3.35, 1H, m (Überlappung mit MeOD)	91.9	3.27, 1H, m	n.d.		
21	-	136.0	-	n.d.		
22	5.99, 1H, m (with 13, 37)	131.1	5.89, 1H, d, 10.2	n.d.		
23	6.50, 1H, m	128.2	6.32, 1H, m	n.d.		

	Allyl-kirromycin (600 MHz, CD_3OD)		Coumarin-kirromycin (700 MHz, CD_3OD)		Propargyl-kirromycin (700 MHz, CD_3OD)	
Position	**δ_H [ppm] (Integral, Type, J in Hz)**	**δ_C [ppm]**	**δ_H [ppm] (Integral, Type, J in Hz)**	**δ_C [ppm]**	**δ_H [ppm] (Integral, Type, J in Hz)**	**δ_C [ppm]**
24	5.65, 1H, m (with 35)	130.5 (with 37)	5.44, 1H, m (with 38)	n.d.		
25a	3.85, 1H, dd, *J* = 15.8, 5.9	42.0	3.75, m	40.8*		
25b	3.92, 1H, dd, *J* = 15.8, 6.2					
26	-	-	-	-		
27	-	177.3	-	174.9*	-	176.4
28	3.00, 1H, dd, *J* = 11.4, 4.1	50.6	3.23, 1H, dd, *J* = 10.6, 5.2	49.6*	3.13, 1H, m	49.5
29	-	100.8	-	99.3	-	100.4
30	3.69, 1H, d, *J* = 3.6	71.3	n. a.	n. d.		
31	3.61, 1H, d, *J* = 3.6	74.0	n. a.	n. d.		
32	-	40.0	n. a.	n. d.		
33	4.25, 1H, d, *J* = 5.8	77.4	4.24, m	76.1*		
34	-	-	-	-		
35	5.65, 1H, m (with 24)	130.9	5.59, 1H, m	n.d.		
36	6.57, 1H, m (with 11)	127.7	6.54, 1H, m (with 11)	126.4		
37	6.01, 1H, m (with 13, 22)	130.5 (with 24)	5.97, m	129.1*		
38	5.48, 1H, m	126.4	5.44, 1H, m (with 24)	125.0*		
39	1.76, 3H, dd, *J* = 7.2, 1.7	13.7	1.71, 3H, dd, *J* = 1.5, 7.1	12.5		
40	2.01, 3H, s	11.7	1.99, s	10.3		
41	0.83, 2H, d, *J* = 6.9	13.8	0.77, 1H, d, *J* = 6.9	12.3		
42	3.18, 3H, s	56.2	3.13, 3H, s	54.9		
43	1.70, 3H, s	11.2	1.64, 3H, s	9.9		
44	2.44, 2H, m	32.8	3.03, <2H, m	23.0	2.57, 2H, m	17.8
45	5.80, 1H, m	136.7	-	144.6*	-	81.7
46a	5.10, 1H, dm, *J* = 17.3	117.2	7.78, 1H, s	123.5	2.30, 1H, t, *J* = 2.64	71.0
46b	5.01, 1H, dm, J = 10.2					
47	0.93, 3H, s (with 47)	15.8	0.91, s	14.5		

	Allyl-kirromycin (600 MHz, CD_3OD)		Coumarin-kirromycin (700 MHz, CD_3OD)		Propargyl-kirromycin (700 MHz, CD_3OD)	
Position	**δ_H [ppm] (Integral, Type, J in Hz)**	**δ_C [ppm]**	**δ_H [ppm] (Integral, Type, J in Hz)**	**δ_C [ppm]**	**δ_H [ppm] (Integral, Type, J in Hz)**	**δ_C [ppm]**
48	0.93, 1H, s (with 48)	24.6	0.91, s	23.2		
1'			4.52, 2H, t, *J* = 5.1 Hz	50.0		
2'			3.89, m	69.1		
3'			n. a.	n. a.		
4'			n. a.	n. a.		
5'			n. a.	n. a.		
6'			n. a.	n. a.		
7'			-	n. d.		
1''			-	n.d.		
2''			-	n.d.		
3''			8.55, 1H, s	n.d.		
4''			-	n.d.		
5''			7.17, 1H, s	127.1*		
6''			-	120.4		
7''			-	148.8*		
8''			-	105.2		
9''			-	152.7*		
10''			2.79, 2H, m	27.1		
11''			1.99, m (with 14'')	20.8		
12''			3.41, m	49.9		
13''			3.40, m	49.4		
14''			1.99 (with 11'')	19.8		
15''			2.84, 2H, t, 6.3	19.7		

*** ^{13}C shift determined by HSQC or HMBC**

n.d.: not detected

n.a.: not assigned

Supplementary Tables 3 a-b. Raw data of the *in vitro* translation assay. Measurement of eGFP fluorescence by increasing amounts of (**a**) kirromycin (**1**), allyl-kirromycin (**2**) and coumarin-kirromycin (**3**), and (**b**) control reactions with kirromycin (concentration range 1-0.1µM). The measurements were carried out

a

c [µM]	10.00	6.67	4.44	2.96	1.98	1.32	0.88	0.59	0.39	0.26	0.17	0.12	
Fluore-scence signal [arbitrary units]	28748	35372	41078	48216	50508	56118	55326	58603	59447	59217	62123	64231	coumarin-kirromycin
	21311	20285	38406	40247	46745	48831	48754	52139	53625	50911	56702	55784	
	7520	18182	21889	29525	28825	44081	47624	45626	45479	46953	46574	51266	
	167	369	841	3302	6245	12173	18263	26644	30548	32105	35447	33099	kirromycin
	132	266	864	2933	5974	11301	17082	25062	28566	31418	32613	36570	
	126	264	608	2601	5541	10810	16619	25124	26352	31600	33500	35742	
	112	223	1084	3593	8477	16907	21284	29006	34125	35576	37545	39608	allyl-kirromycin
	135	227	920	3036	7583	15023	19906	26513	32219	32965	35424	37641	
	138	213	780	2896	7037	14258	19053	26247	31320	31757	34723	37812	

* Measurement direction: from right (lowest concentration) to the left (highest concentration)

b

c [µM]	1	0.666667	0.444444	0.296296	0.197531	0.131687	0.087791	0.058528	0.039018	0.026012	0.017342	0.011561	
Fluore-scence signal [arbitrary units]	13111	19209	25685	26338	28127	30223	30879	32030	32236	32405	33942	37202	kirromycin
	12709	18909	24899	26486	25716	29784	31266	32572	33097	32663	34258	37331	
	13320	19915	25763	27966	29332	32372	32929	34333	34825	34812	35590	37847	

* Measurement direction: from right (lowest concentration) to the left (highest concentration)

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