Supporting Information

SI Materials and Methods

Bacterial strains, plasmids and growth conditions. *Streptomyces* strains were cultivated on SFM, in YEME and on or in HT media and manipulated as described previously (1). Growth curves, RNA extractions, LC-MS and HPLC analysis were obtained from cultures grown in or on MP5 medium (2). *Escherichia coli* strains were cultivated in LB and SOB liquid medium (3). When needed these antibiotics were added into the cultures: ampicillin, apramycin, kanamycin, spectinomycin at 50µg/ml, chloramphenicol and nalidixic acid at 25µg/ml. For λ *red* genes induction, 10mM of filtered L-arabinose was added to the culture.

DNA manipulation. Plasmid and BAC DNA were extracted from *E. coli* by alkaline lysis method (3). Genomic DNA extraction of *Streptomyces* and pulsed-field gel electrophoresis were performed as already described (4). Southern blots were performed with a Hybond-N nylon membrane (Amersham-Pharmacia) and a vacuum transfer system (BioRad), as previously described (5). Amplification of DNA fragments by PCR was performed with *Taq* DNA polymerase (NEB) or Takara polymerase (Fermentas), according to the manufacturer's instructions. PCR products and restriction fragments were purified from agarose gel with the High Pure PCR product purification kit (Roche).

Overexpression of the TCS. The two component system-encoding sequence was amplified by PCR from *S. ambofaciens* ATCC23877 genomic DNA with the primers OE468-F and OE469-R (see Table S10 for sequences). The PCR conditions, the cloning steps into pIB139 and the integration of the recombinant plasmid (pOE-4689, Table S9) in *S. ambofaciens* ATCC23877 were as described for the overexpression of the LAL regulator (see Materials and Methods).

Construction of the glycosyltransferase mutant strain. The REDIRECT system was used to make an in-frame deletion of *samR0481* in *S. ambofaciens* ATCC/OE484 as already described for the deletion of *samR0467* (see Materials and Methods). However, in this case the *aac(3)IV + oriT* mutagenesis cassette was used as template in the PCR reaction with the primers D481-F and D481-R (Table S10) and the BAC of interest used was BBC (Table S9).

Antimicrobial and antiproliferative tests. The antibacterial activities of the purified stambomycin compounds were first analysed by loading a drop of compounds (mixture of stambomycin A and B or stambomycin C and D; from 1 µg up to 12.5 µg) onto a lawn of *Bacillus subtilis* ATCC6633 and *Micrococcus luteus* used as indicator strains. The IC50 and IC90 values were then determined only for

the mixture of stambomycin C/D (the most active fraction) against *Bacillus subtilis* BGSC 1A72, *Mycobacterium smegmatis* ATCC700084 and against clinical isolates of *Enterococcus faecalis* and *Staphylococcus aureus* (strains from Libragen, Toulouse, France). Analyses were also performed against Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*, two clinical isolates from Libragen), against yeast (*Candida albicans* IHEM8060) and filamentous fungi (*Aspergillus fumigatus* GASP4707 and *Fusarium oxysporum* DSM2018).
For the antiproliferative activities and cytotoxity, the IC50 values were determined for stambomycin A/B and stambomycin C/D using human adenocarcinoma (HT29), breast (MCF7), lung (H460) and prostate (PC3) cancer cell lines and ovary sane cell line from an adult Chinese hamster (CHO-K1), respectively. The cell viability was determined by measuring the cellular concentration of ATP using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega, G7573) according to the recommendation of the manufacturer.

Identification of stambomycins by LC-MS comparative metabolic profiling. *Streptomyces ambofaciens* ATCC23877, ATCC/OE484 and ATCC/OE484/Δ467 spores were stored in 25% (v/v) aqueous glycerol at -20 °C. 10µl of spores from the stocks of the ATCC23877, ATCC/OE484 and ATCC/OE484/Δ467 strains were used separately to inoculate MP5 liquid medium and the resulting cultures were grown for 3-4 days at 30 °C and 180 rpm. The cultures were centrifuged for 10 minutes at 4000 rpm and 4°C. The mycelia were collected, extracted with 5 ml of methanol and sonicated for 10 minutes. The resulting suspension was filtered through a spin filter (0.4 µm) and the filtrate was analysed by LC-ESI-TOF-MS. A Sigma Ascentis C18 column (100 x 2.1mm, 2.7 µm) connected to Dionex 3000 RS-HPLC coupled with a Bruker MaXis UHR-Q-TOF mass spectrometer was used. The flow rate was 0.2 ml/min. Water/0.1% formic acid (solvent A) and methanol/0.1% formic acid (solvent B) were used as mobile phases. The elution profile was: 80% solvent A/20% solvent B for 5 minutes, gradient to 100% solvent B for 10 minutes, 100% solvent B for 5 minutes, equilibrate back to 80% solvent A/20% solvent B. The mass spectrometer was calibrated at the beginning of each run with 10 mM sodium formate and the following settings in positive ESI mode were used. Full scan: 50-2000 m/z, End plate offset: -500 V, Capillary: -4500 V, Nebulizer gas (N_2): 1.6 bar, Dry gas (N_2): 8 L/min, Dry Temperature: 180 °C.

ORF	Product size (aa)	% identity / similarity	Species	Putative function	Proposed role
samR0465	8154			Type I modular PKS	Polyketide biosynthesis
samR0466	3661			Type I modular PKS	Polyketide biosynthesis
samR0467	5771			Type I modular PKS	Polyketide biosynthesis
samR0468	217	84/88	*S. griseus* (SGR874)	Response regulator	Regulation
samR0469	442	77/84	*S. griseus* (SGR875)	Histidine kinase	Regulation
samR0470	261	87/93	*S. griseus* (SGR876)	Putative permease protein	Resistance
samR0471	312	88/93	*S. griseus* (SGR877)	Putative ABC transporter ATP-binding protein	Resistance
samR0472	244	55/68	*S. erythraea*	N-dimethyltransferase	D-Mycaminose biosynthesis
samR0473	185	46/59	*S. fradiae*	Isomerase	D-Mycaminose biosynthesis
samR0474	6333			Type I modular PKS	Polyketide biosynthesis
samRCDS1	3556			Type I modular PKS	Polyketide biosynthesis
samR0475	3157			Type I modular PKS	Polyketide biosynthesis
samR0476	3565			Type I modular PKS	Polyketide biosynthesis
samRCDS2	1569			Type I modular PKS	Polyketide biosynthesis
samR0477	5447			Type I modular PKS	Polyketide biosynthesis
samR0478	414	43/62	*S. fradiae*	Cytochrome P450	Polyketide hydroxylation
samR0479	401	41/56	*R. castenholzii*	Cytochrome P450	Polyketide hydroxylation
samR0480	369	74/82	*Streptomyces* sp. TP-A0274	Aminotransferase	D-Mycaminose biosynthesis
samR0481	418	48/63	*M. griseorubida*	Glycosyltransferase	D-Mycaminose attachment
samR0482	595	64/75	*S. hygroscopicus*	Acyl-CoA synthetase	Unknown
samR0483	532	62/75	*Micromonopora* sp. ATCC39149	Carboxyl transferase	Precursor biosynthesis
samR0484	958	35/47	*S. venezuelae* (PikD)	Transcriptional activator (LAL)	Regulation
samR0485	255	87/94	*S. griseus* (SGR200)	Type II thioesterase	PKS editing
samR0486	329	77/84	*S. tenebrarius* (AprE)	dTDP-glucose-4,6- dehydratase	D-Mycaminose biosynthesis
samR0487	290	74/85	*S. avermitilis* (AveBIII)	Glucose-1-phosphate thymidyltransferase	D-Mycaminose biosynthesis

Table S1. Properties of genes within the stambomycin biosynthetic cluster. Putative functions of the encoded proteins were deduced from analyses with the BlastP program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The % identity/similarity for the protein in the database with the highest end-to-end similarity is indicated. The stambomycin cluster is located in the right terminal region of the chromosome which has been fully sequenced (AM238664).

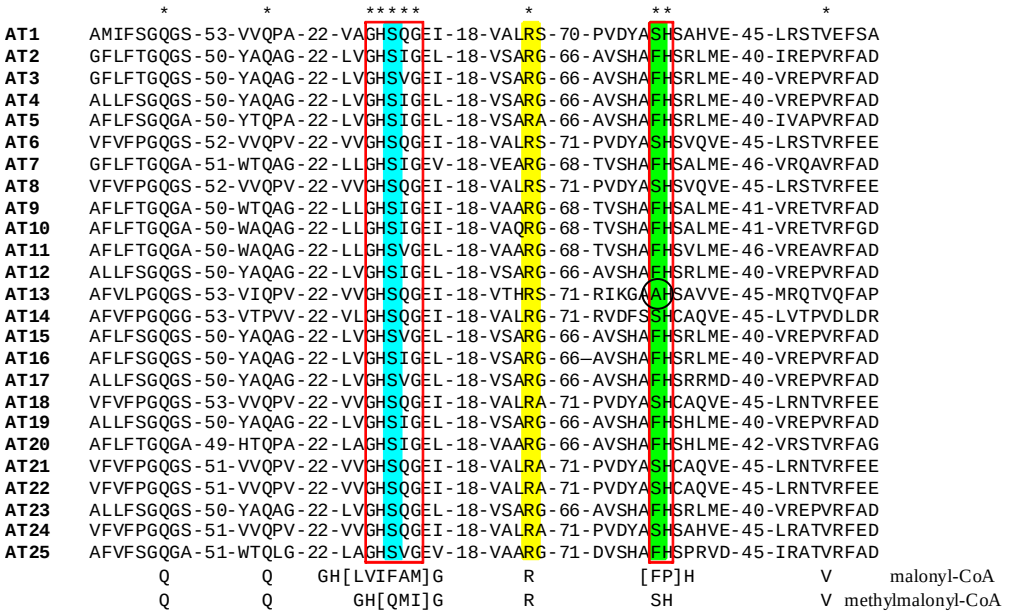

Fig. S1. Sequence alignment of the PKS AT domains. Highly conserved active site residues are marked with an asterisk (6). The boxed residues are diagnostic of substrate specificity, in particular the amino acid underlined in green. The arginine residue underlined in yellow interacts with the carboxyl group of the substrates. The catalytic serine residue is underlined in blue. The consensus sequences for malonyl-CoA and methylmalonyl-CoA-specific AT domains are shown below the alignment. The substrate for AT13 could not be predicted because the circled specificity-conferring residue is different from that found in malonyl- and methylmalonyl-CoA-specific AT domains.

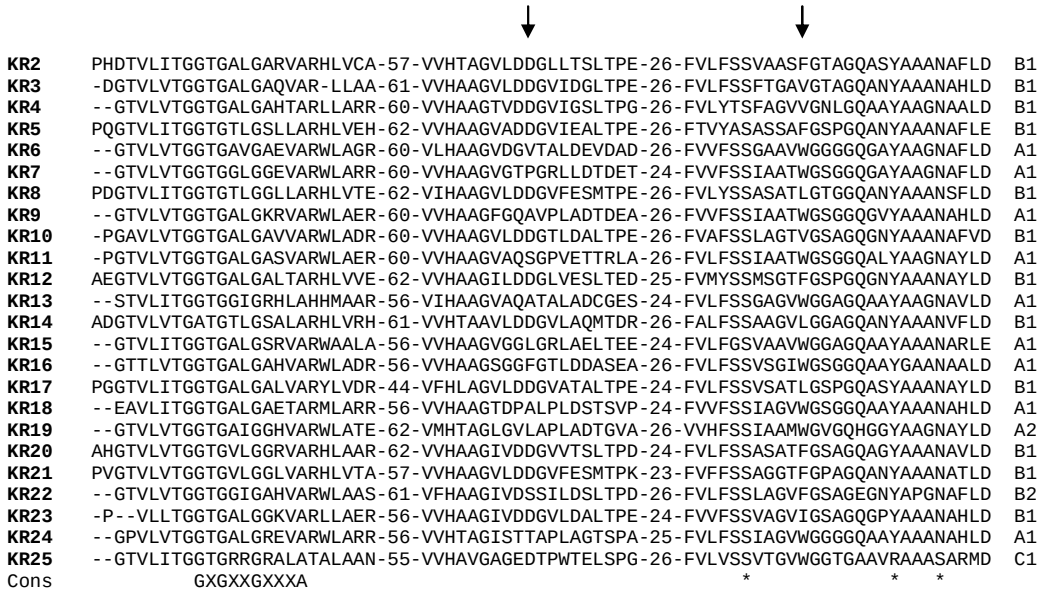

Fig. S2. Sequence alignment of the PKS KR domains. The GXGXXGXXXA consensus sequence in the N-termini is proposed to bind NAD(P)H (7). The residues belonging to the catalytic triad are marked with asterisks. The Y to R and N to S mutations of the catalytic residues in KR25 indicate that it is not active. The arrows indicate the residue predictive of B and A-type alcohol stereochemistry, respectively. The predicted configurations of the α- and β-stereocenters generated by each KR, according to the model of Keatinge-Clay (8), are indicated to the right of the alignment. A1 = 2*R*, 3*S*; A2 = 2*S*, 3*S*; B1 = 2*R*, 3*R*; B2 = 2*S*, 3*R*; C1 = 2*R*.

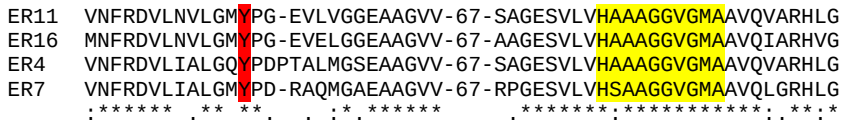

Fig. S3. Sequence alignment of the PKS ER domains. The highly conserved putative NADPH binding motif is highlighted in yellow. The residue that is predictive of stereochemistry is highlighted in red. A tyrosine residue in this position predicts the 2*S* configuration. Another residue (usually Val or Ala) in this position predicts the 2*R* stereochemistry (9).

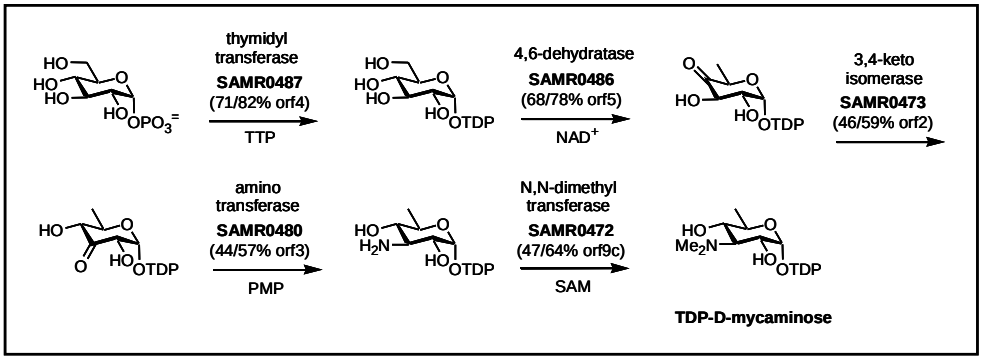

Fig. S4. TDP-D-mycaminose biosynthetic pathway. Proposed pathway for biosynthesis of TDP-D-mycaminose from α-D-glucose-1-phosphate by putative aminodeoxysugar biosynthetic enzymes encoded by genes within the cluster. For each enzymatic step, the gene product is indicated in bold. The identity/similarity with the corresponding enzymes involved the biosynthesis of the β-D-mycaminose residue of spiramycin are indicated in brackets (10). TTP, thymidine triphosphate; TDP, thymidine diphosphate; NAD^+, nicotinamide adenine dinucleotide; PMP, pyridoxamine 5´-phosphate; SAM, *S*-adenosyl-L-methionine.

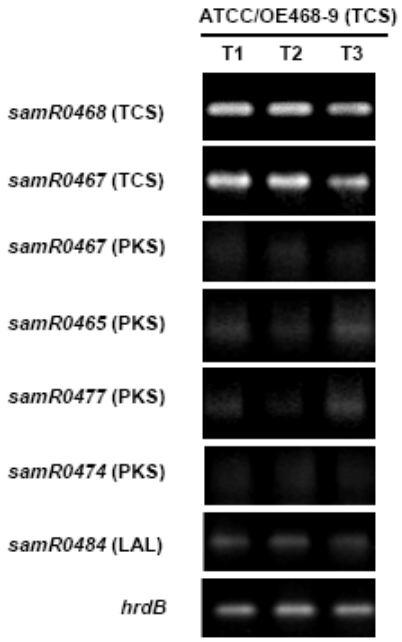

Fig. S5. Transcriptional analysis of the strain overexpressing the two component system (mutant strain ATCC/OE468-9) and grown in MP5 medium. The expression of four biosynthetic genes (*samR0467*, *samR0465*, *samR0477*, *samR0474*), together with the expression of the regulatory genes *samR0468*, *samR0469* and *samR0484*, were analyzed by RT-PCR using 4µg of total RNA. The constitutively-expressed *hrdB* gene, coding for the major sigma factor, was used as a positive control. Experiments carried out on three separate occasions gave the same result. T1= exponential phase; T2= transition phase; T3= stationary phase.

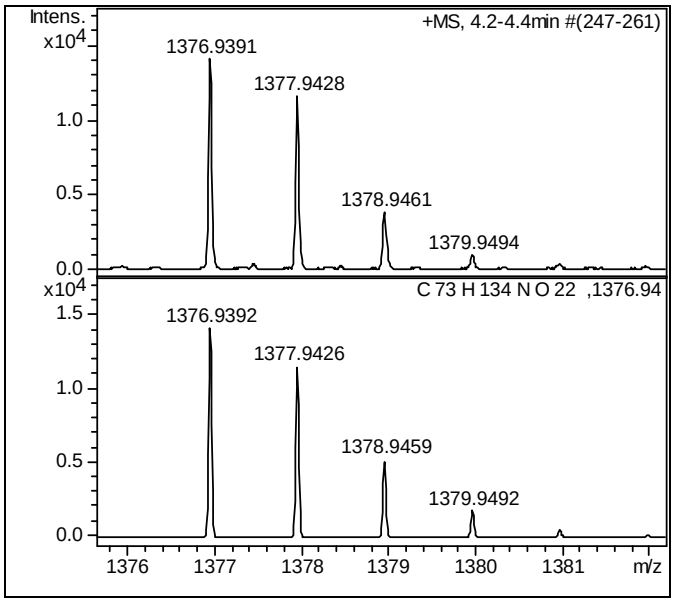

Fig. S6. High resolution mass spectrometry analysis of stambomycins A/B. Measured spectrum of stambomycins A/B (top panel) and simulated spectrum for $C_{73}H_{134}N0_{22}^{+}$ (bottom panel).

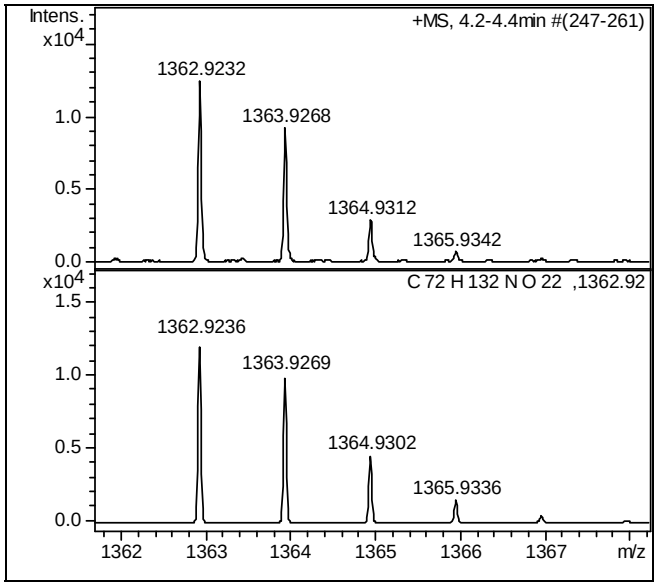

Fig. S7. High resolution mass spectrometry analysis of stambomycins C/D**.** Measured spectrum of stambomycins C/D (top panel) and simulated spectrum for $C_{72}H_{132}N0_{22}^{+}$ (bottom panel).

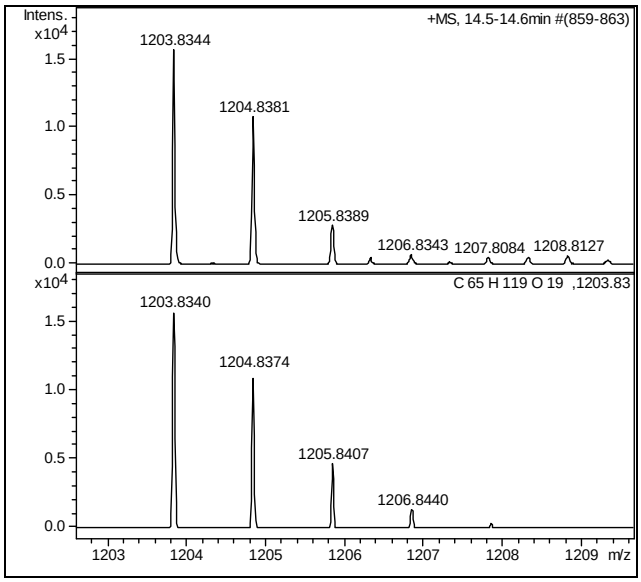

Fig. S8. High resolution mass spectrometry analysis of stambomycin A/B aglycones. Measured spectrum of stambomycin A/B aglycones (top panel) and simulated spectrum for $C_{65}H_{119}0_{19}^{+}$ (bottom panel).

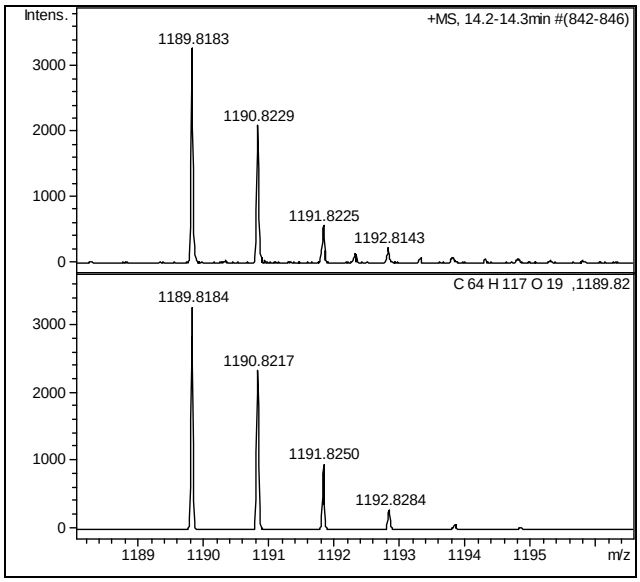

Fig. S9. High resolution mass spectrometry analysis of stambomycin C/D aglycones**.** Measured spectrum of stambomycin C/D aglycones (top panel) and simulated spectrum for $C_{64}H_{117}0_{19}^{+}$ (bottom panel).

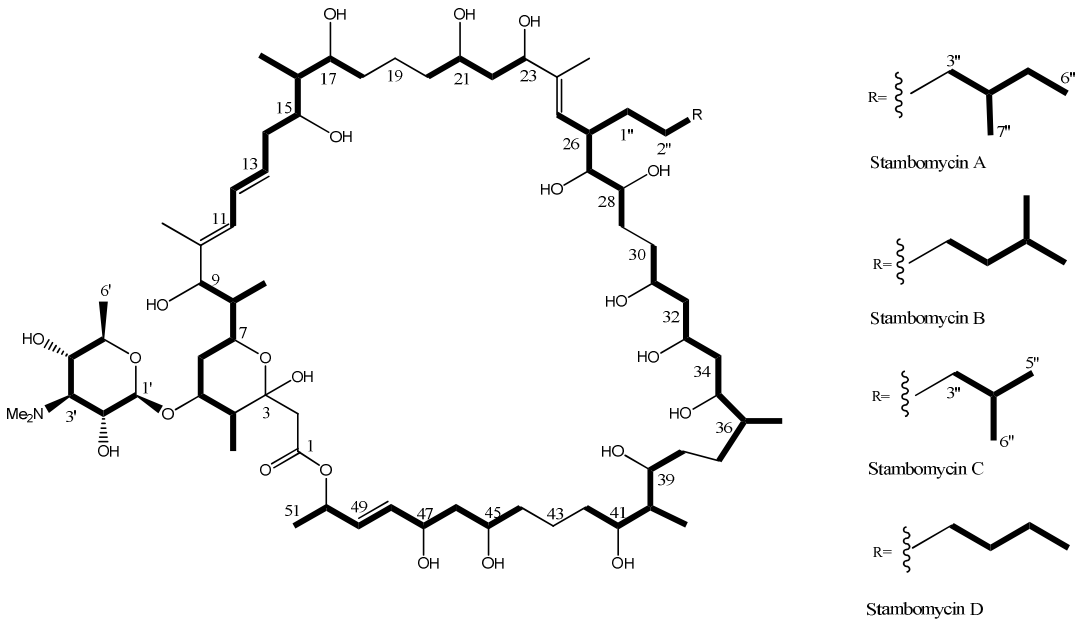

Fig. S10. Summary of COSY NMR correlations observed for stambomycins A-D.

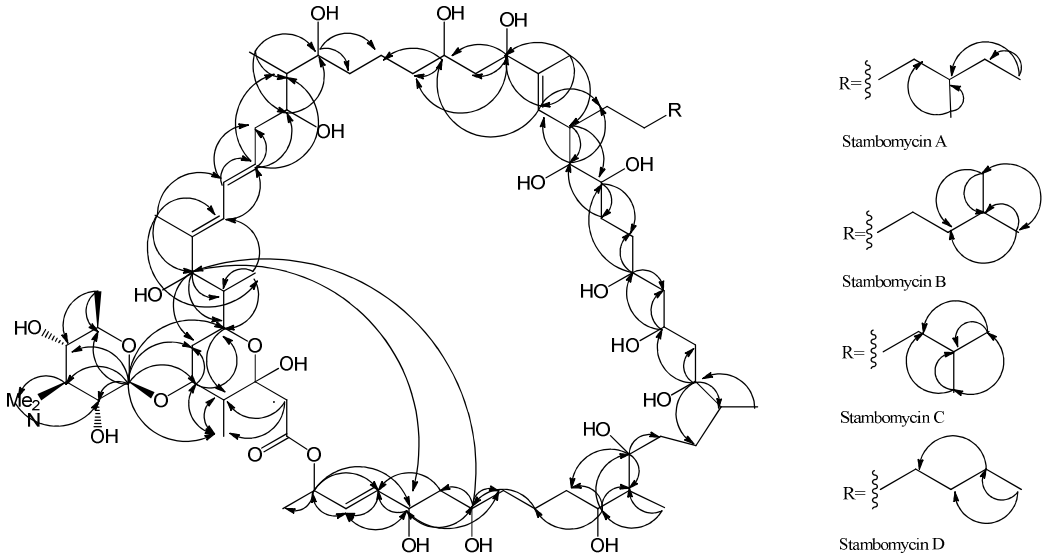

Fig. S11. Summary of NOESY NMR correlations observed for stambomycins A-D.

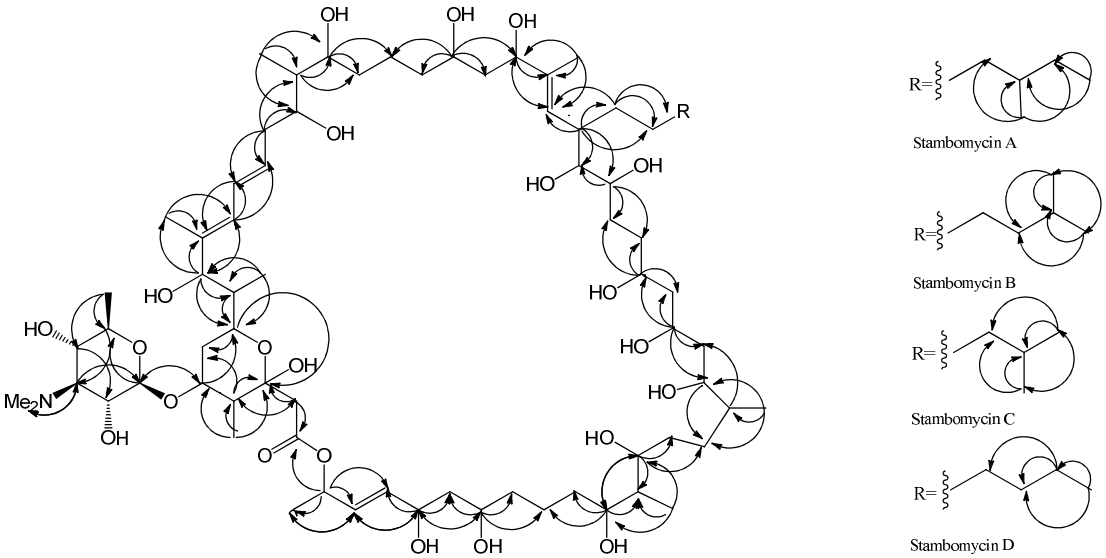

Fig. S12. Summary of HMBC NMR correlations observed for stambomycins A-D in d_4-MeOH.

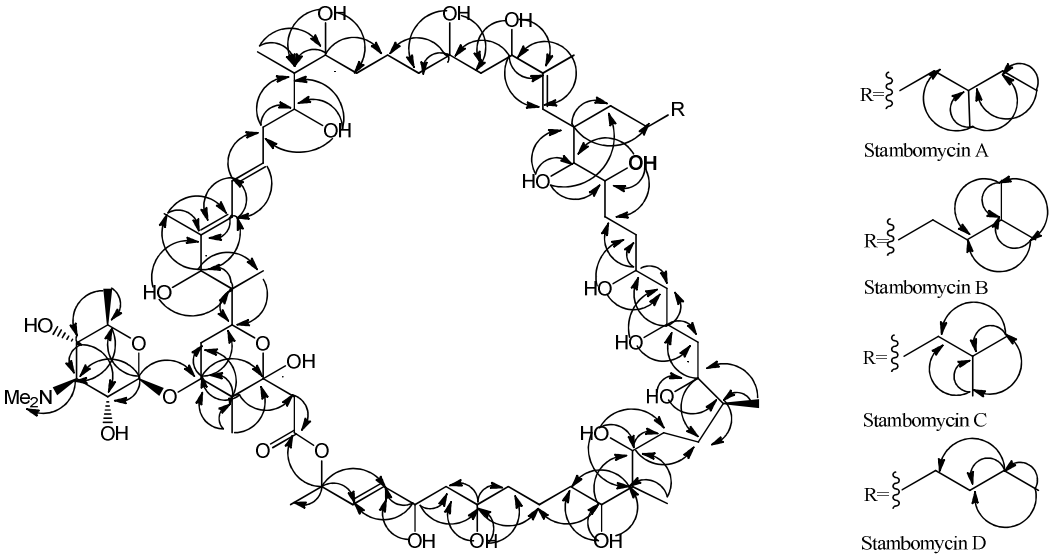

Fig. S13. Summary of HMBC NMR correlations observed for stambomycins A-D in d_6-DMSO.

Table S2. Assignments of ^{1}H and ^{13}C NMR signals for stambomycins A and B. Carbon atom numbering is shown in Fig. S10. Signals for C-1″ to C-7″ of stambomycin B are in brackets.

position	δ_C (ppm)	δ_H (ppm)	J_{H-H} (Hz)
1	173.3		
2	44.3	2.52, 2.83	15.0
3	100.0		
4	46.2	1.56	
4-Me	12.4	1.04	
5	81.0	3.66	
1′	104.6	4.44	7.3
2′	70.0	3.54	7.3, 10.5
3′	72.0	3.23	10.5, 10.5
4′	70.8	3.38	9.8, 10.5
5′	74.0	3.42	9.8, 6.2
6′	18.1	1.29	
3′-NMe_2	42.2	3.03	
6	39.1	1.61, 1.90	
7	70.0	3.83	
8	40.8	1.56	
8-Me	9.3	0.93	
9	79.9	3.90	7.6
10	137.1		
10-Me	12.4	1.65	
11	128.0	6.04	11.0
12	129.9	6.35	15.0, 11.0
13	131.7	5.80	15.0, 6.9
14	39.8	2.34	6.9
15	75.8	3.77	
16	42.6	1.52	
16-Me	7.4	0.94	
17	75.5	3.72	
18	36.1	1.48	
19	23.5	1.33, 1.34	
20	38.6	1.48	
21	71.1	3.55	
22	43.1	1.61, 1.67	
23	77.5	4.23	7.0
24	139.2		
24-Me	12.2	1.64	
25	129.3	5.23	10.5
26	41.9	2.45	
1″	32.9 (32.5)	1.19, 1.77 (1.19, 1.78)	
2″	25.7 (26.6)	1.35 (1.42)	
3″	38.3 (31.8)	1.13, 1.29 (1.29)	
4″	35.7 (40.2)	1.30 (1.19, 1.50)	
5″	30.8 (29.1)	1.13 (1.30)	
6″	12.1 (23.0)	0.86 (0.87)	
7″	19.6 (23.0)	0.85 (0.87)	
27	79.7	3.36	
28	74.2	3.47	
29	27.8	1.43, 1.68	
30	36.1	1.43, 1.70	
31	70.1	3.83	
32	46.4	1.50, 1.57	
33	66.8	4.06	
34	42.0	1.48, 1.50	
35	72.9	3.74	

36	40.8	1.48	
36-Me	15.6	0.92	
37	29.8	1.28, 1.11	
38	36.0	1.50	
39	76.3	3.72	
40	42.3	1.51	
40-Me	7.0	0.91	
41	75.9	3.71	
42	33.8	1.43, 1.62	
43	22.9	1.43	
44	38.8	1.45, 1.46	
45	70.6	3.67	
46	44.8	1.60, 1.65	
47	71.1	4.28	6.3, 12.9
48	136.7	5.80	15.3, 6.3
49	130.8	5.72	6.3, 15.3
50	72.8	5.43	6.3,
51	20.8	1.33	6.3

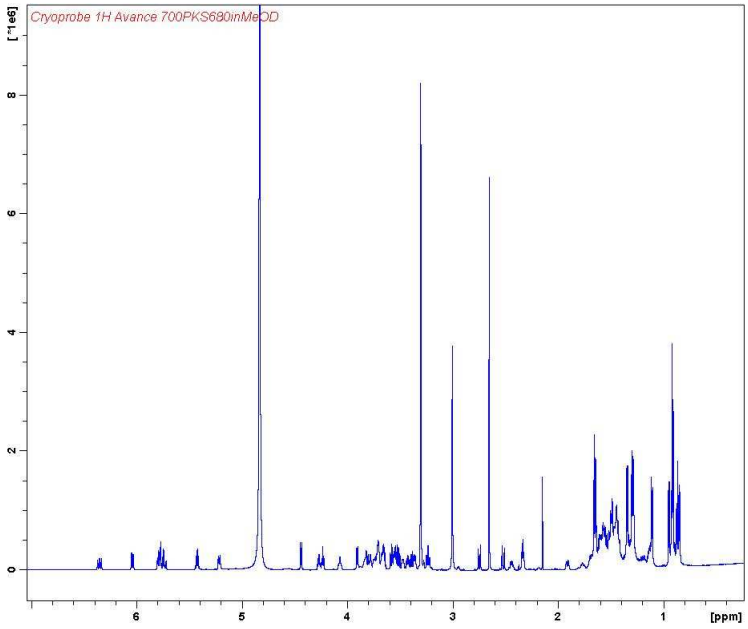

Fig. S14. ^{1}H NMR spectrum (700 MHz, d_4-MeOH) of stambomycins A/B.

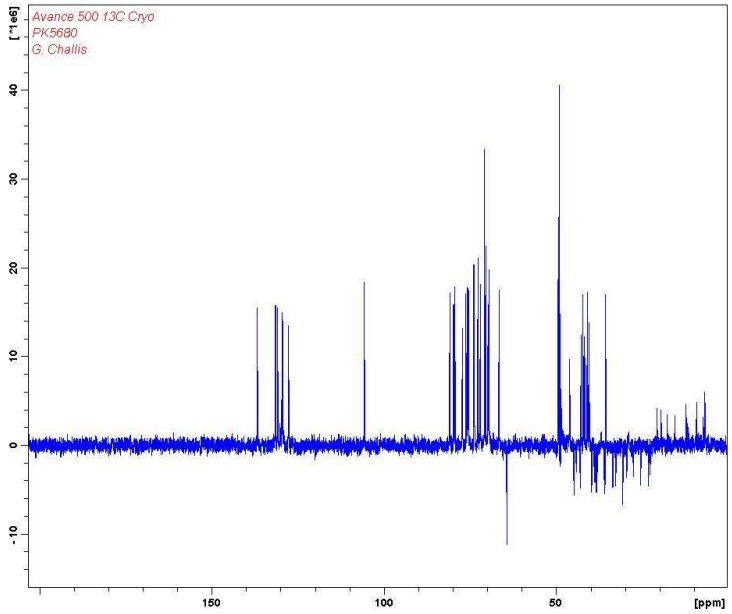

Fig. S15. ^{13}C PENDANT NMR spectrum (125 MHz, d_4-MeOH) of stambomycins A/B.

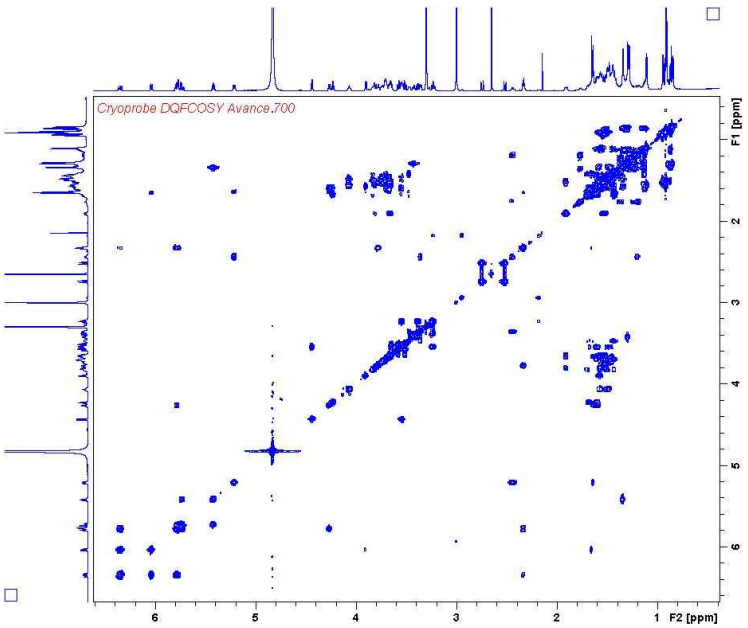

Fig. S16. DQFCOSY NMR spectrum (700 MHz, d_4-MeOH) of stambomycins A/B.

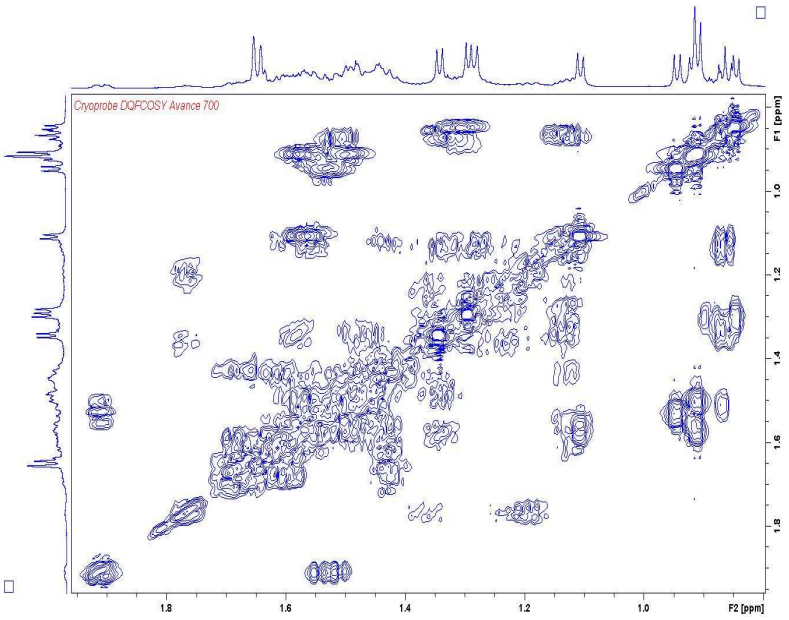

Fig. S17. Expansion of methyl/methylene region of DQFCOSY spectrum of stambomycins A/B.

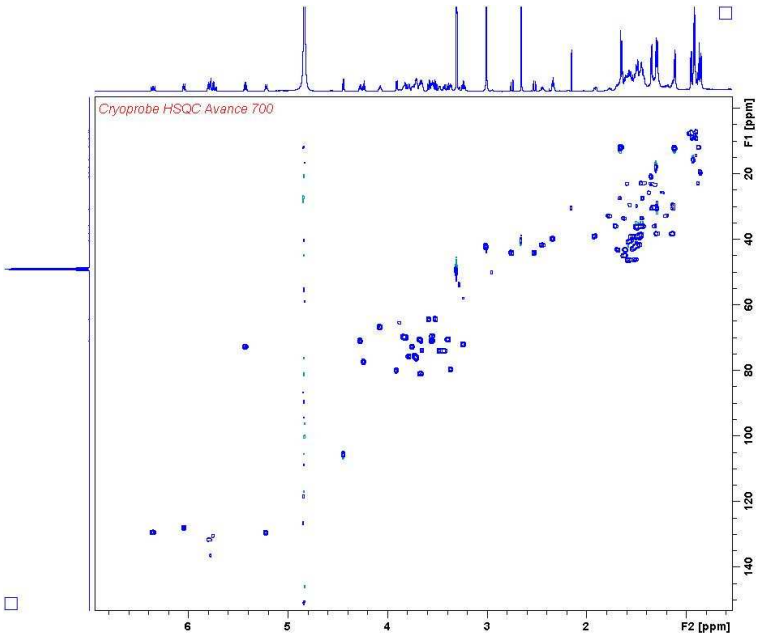

Fig. S18. HSQC NMR spectrum (700 MHz/175 MHz, d_4-MeOH) of stambomycins A/B.

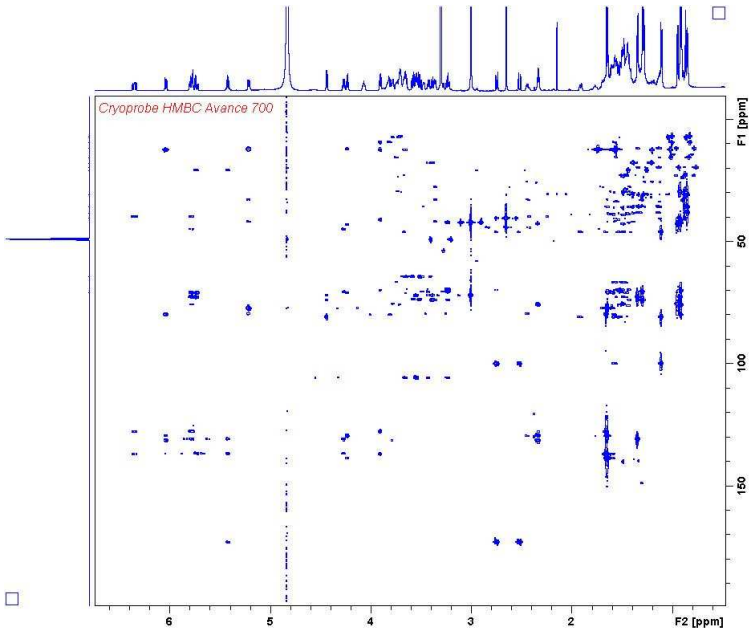

Fig. S19. HMBC NMR spectrum (700 MHz/175 MHz, d_4-MeOH) of stambomycins A/B.

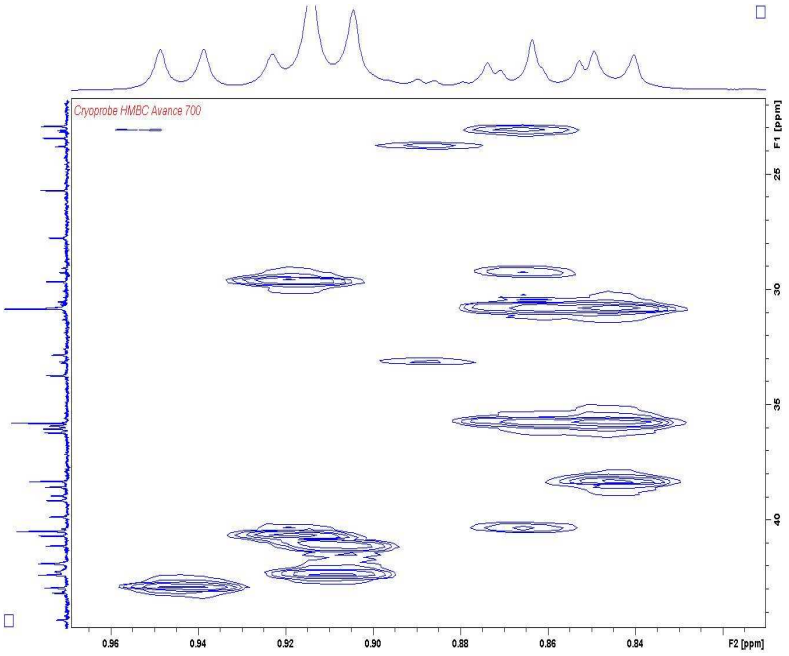

Fig. S20. Expansion of methyl region of HMBC spectrum of stambomycins A/B.

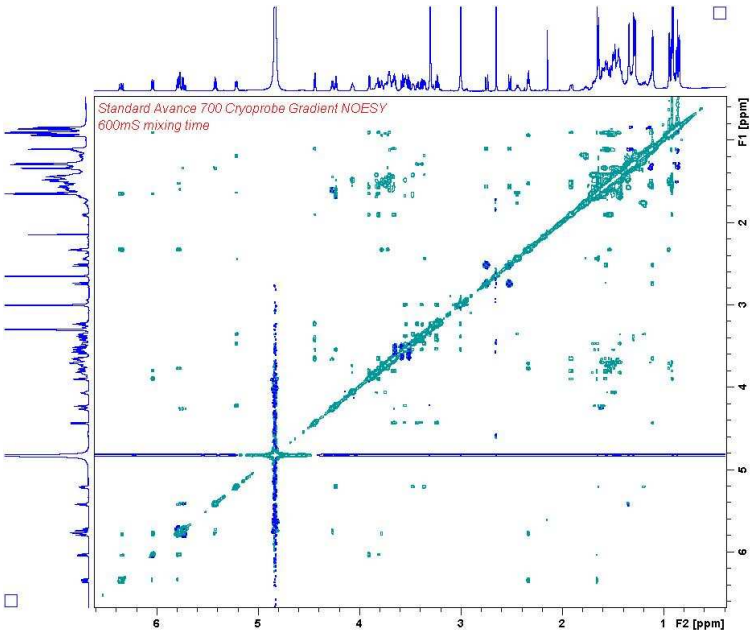

Fig. S21. NOESY NMR spectrum (700 MHz, d_4-MeOH) of stambomycins A/B.

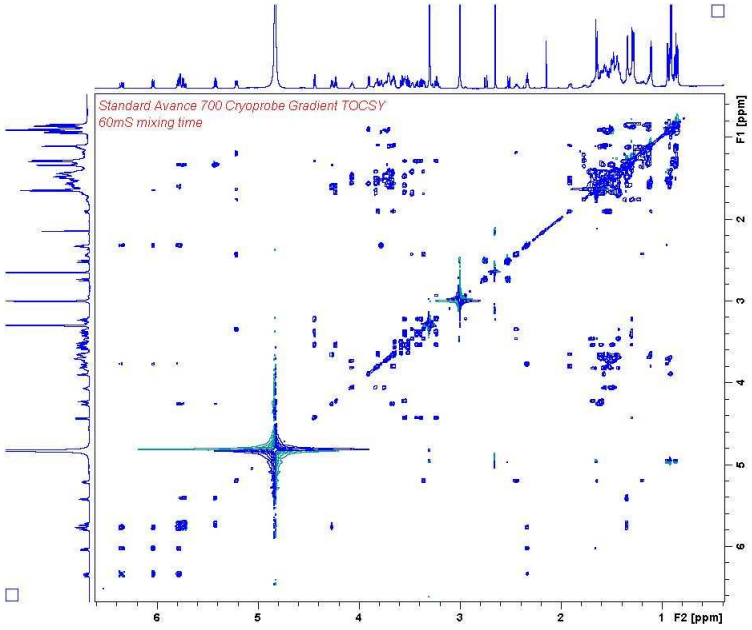

Fig. S22. TOCSY NMR spectrum (700 MHz, d_4-MeOH) of stambomycins A/B.

Table S3. Assignments of ^{1}H and ^{13}C NMR signals for stambomycins C/D. Carbon atom numbering is shown in Fig. S10. Signals for C-1″ to C-6″ of stambomycin D are in brackets.

position	δ_C (ppm)	δ_H (ppm)	J_{H-H} (Hz)
1	173.3		
2	44.3	2.52, 2.83	15.0
3	100.0		
4	46.2	1.56	
4-Me	12.4	1.04	
5	81.0	3.66	
1′	104.6	4.44	7.3
2′	70.0	3.54	7.3, 10.5
3′	72.0	3.23	10.5, 10.5
4′	70.8	3.38	9.8, 10.5
5′	74.0	3.42	9.8, 6.2
6′	18.1	1.29	
3′-NMe_2	42.2	3.03	
6	39.1	1.61, 1.90	
7	70.0	3.83	
8	40.8	1.56	
8-Me	9.3	0.93	
9	79.9	3.90	7.6
10	137.1		
10-Me	12.4	1.65	
11	128.0	6.06	11.0
12	129.9	6.35	15.0, 11.0
13	131.7	5.81	15.0, 6.9
14	39.8	2.34	6.9
15	75.8	3.77	
16	42.6	1.52	
16-Me	7.4	0.95	
17	75.5	3.72	
18	36.1	1.48	
19	23.5	1.32, 1.34	
20	38.5	1.47	
21	71.1	3.55	
22	43.1	1.61, 1.67	
23	77.5	4.23	6.9
24	139.4		
24-Me	12.2	1.64	
25	129.3	5.23	6.5
26	41.9	2.45	
1″	32.5 (32.4)	1.18, 1.79 (1.18, 1.79)	
2″	25.8 (26.6)	1.38 (1.37)	
3″	40.3 (35.8)	1.13, 1.29 (1.31, 1.44)	
4″	29.9 (34.0)	1.30 (1.43, 1.49)	
5″	23.1 (29.2)	0.87 (1.29)	
6″	23.1 (11.5)	0.87 (0.87)	
27	79.7	3.36	
28	74.2	3.47	
29	27.8	1.43, 1.68	
30	36.1	1.43, 1.70	
31	70.1	3.83	
32	46.4	1.50, 1.57	
33	66.8	4.06	
34	42.0	1.48, 1.50	

35	72.9	3.74	
36	40.8	1.48	
36-Me	15.6	0.92	
37	29.8	1.28, 1.12	
38	36.0	1.50	
39	76.3	3.72	
40	42.3	1.51	
40-Me	7.0	0.91	
41	75.9	3.71	
42	33.8	1.43, 1.62	
43	22.9	1.43	
44	38.8	1.45, 1.46	
45	70.6	3.67	
46	44.8	1.60, 1.65	
47	71.1	4.28	6.2, 12.9
48	136.7	5.80	15.0, 6.2
49	130.8	5.72	6.2, 15.0
50	72.8	5.43	6.2
51	20.8	1.33	6.2

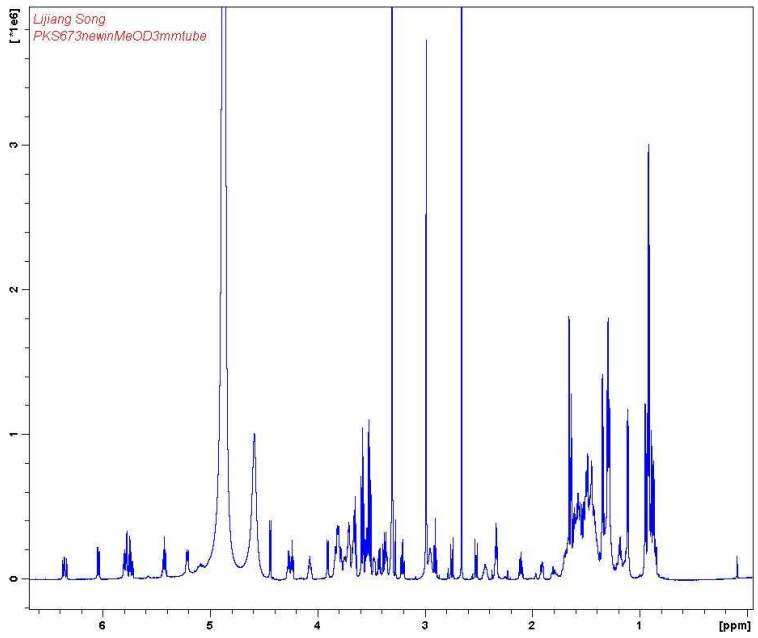

Fig. S23. ^{1}H NMR spectrum (700 MHz, d_4-MeOH) of stambomycins C/D.

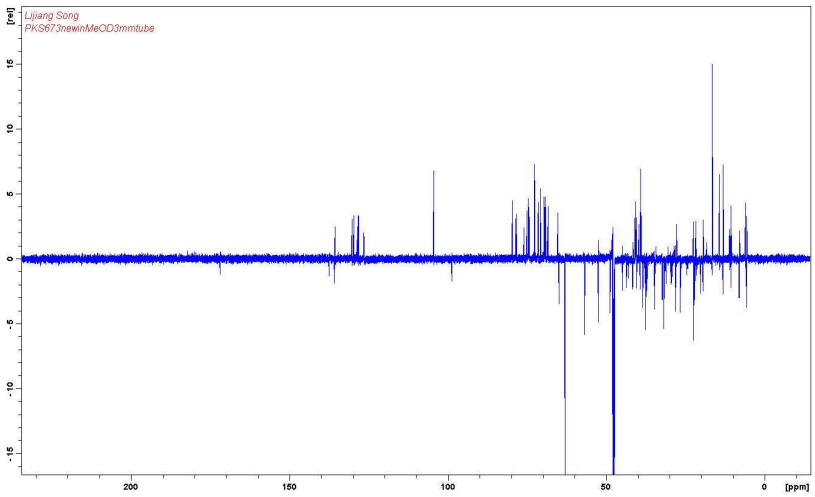

Fig. S24. ^{13}C PENDANT NMR spectrum (175 MHz, d_4-MeOH) of stambomycins C/D.

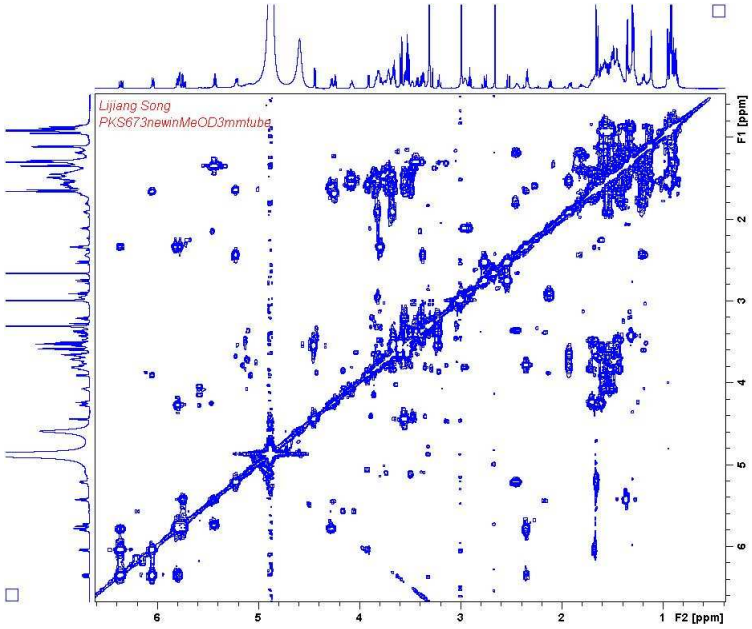

Fig. S25. DQFCOSY NMR spectrum (700 MHz, d_4-MeOH) of stambomycins C/D.

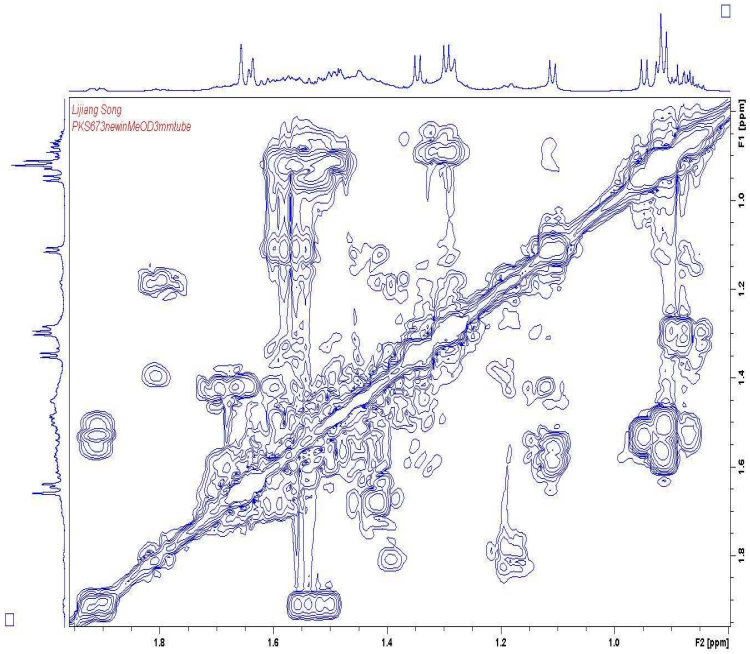

Fig. S26. Expansion of methyl/methylene region of DQFCOSY spectrum of stambomycins C/D.

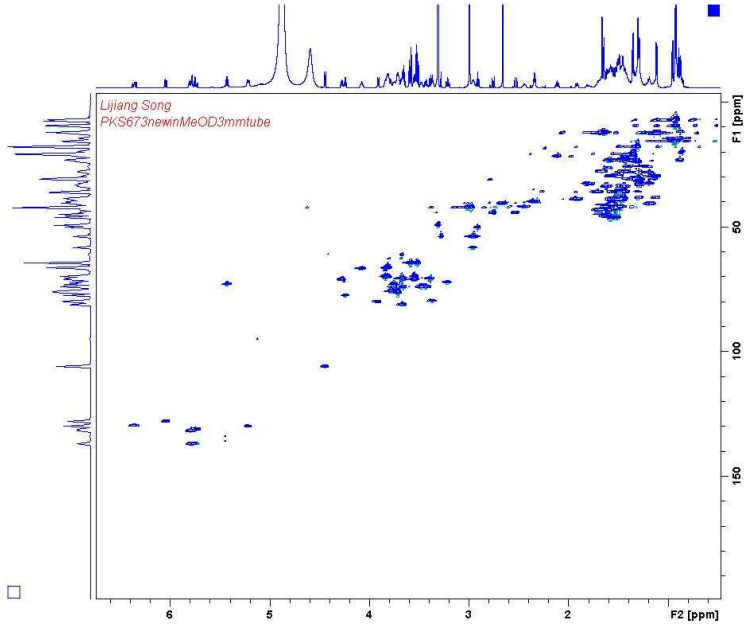

Fig. S27. HSQC NMR spectrum (700 MHz/175 MHz, d_4-MeOH) of stambomycins C/D.

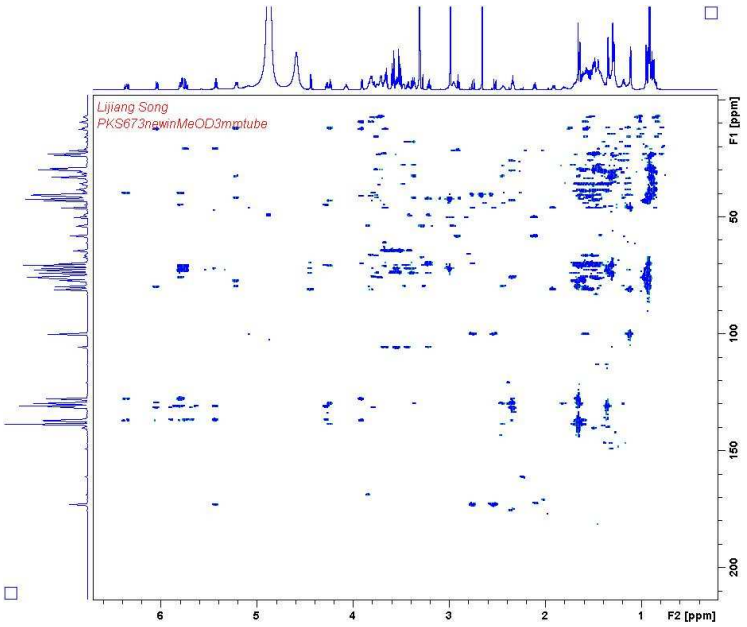

Fig. S28. HMBC NMR spectrum (700 MHz/175 MHz, d_4-MeOH) of stambomycins C/D.

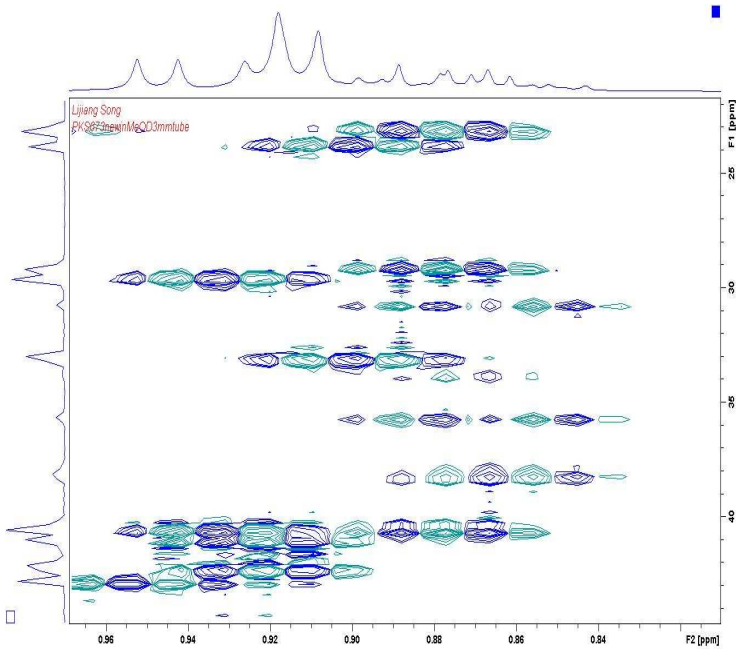

Fig. S29. Expansion of methyl region of HMBC spectrum of stambomycins C/D.

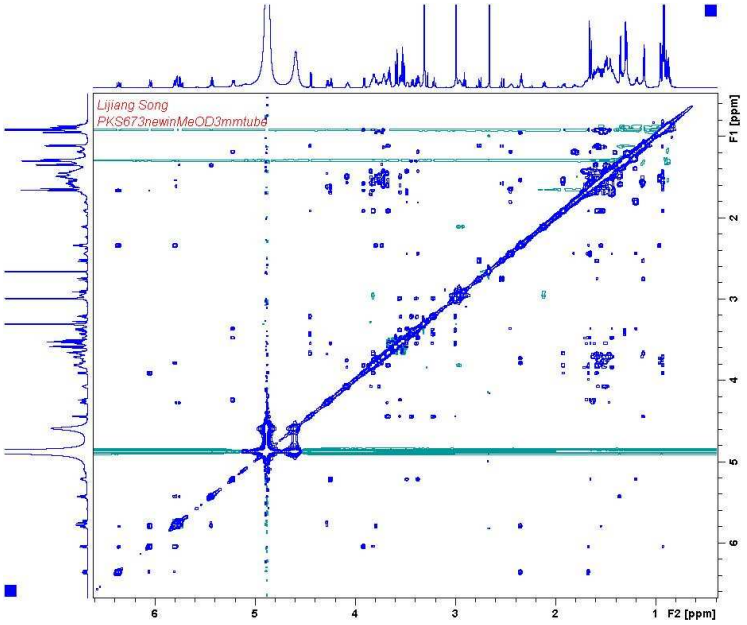

Fig. S30. NOESY NMR spectrum (700 MHz, d_4-MeOH) of stambomycins C/D.

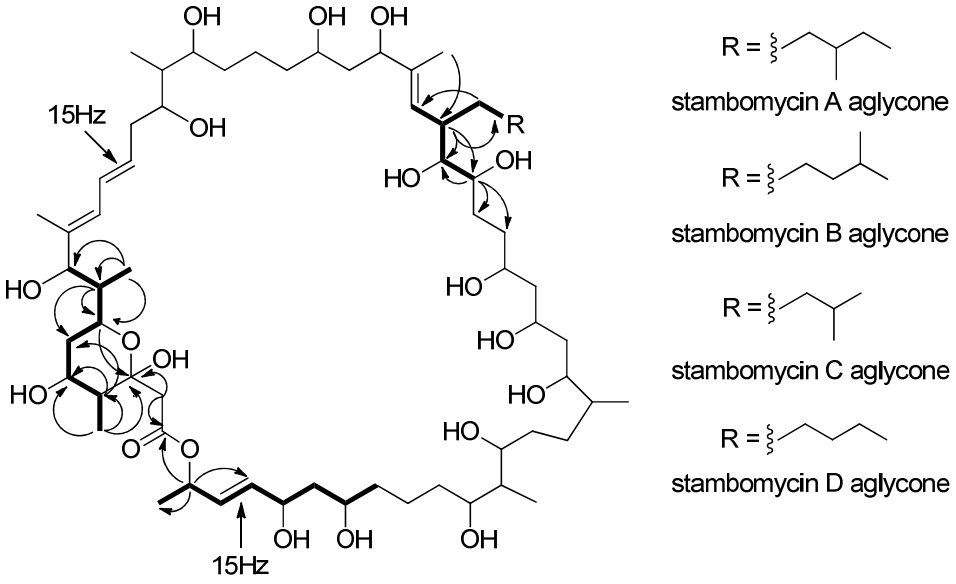

Fig. S31. Summary of key COSY (bold lines) HMBC (arrows) and coupling constant data observed for stambomycin aglycones.

Table S4. Assignments of ^{1}H and ^{13}C NMR signals for the aglycones of stambomycins A/B. Carbon atom numbering is shown in Fig. S10. Signals for C-1″ to C-7″ of stambomycin B are in brackets.

position	δ_C (ppm)	δ_H (ppm)	J_{H-H} (Hz)
1	173.3		
2	44.2	2.51, 2.75	14.6
3	99.9		
4	47.2	1.36	
4-Me	12.3	1.06	
5	70.1	3.60	
6	39.3	1.45, 1.73	
7	70.1	3.81	
8	40.9	1.60	
8-Me	9.3	0.93	
9	79.6	3.92	7.4
10	136.9		
10-Me	11.9	1.65	
11	127.8	6.05	11.2
12	129.5	6.36	15.1, 11.2
13	131.2	5.79	15.1, 6.8
14	39.8	2.34	6.8
15	75.6	3.78	
16	42.7	1.53	
16-Me	7.4	0.95	
17	75.2	3.72	
18	35.9	1.48	
19	23.3	1.33	
20	38.4	1.47	
21	70.8	3.54	
22	42.9	1.61, 1.67	
23	77.2	4.23	7.3
24	138.7		
24-Me	12.2	1.67	
25	129.6	5.22	10.2
26	41.8	2.45	
1”	32.7 (32.6)	1.19, 1.77 (1.17, 1.81)	
2”	25.4 (26.4)	1.37 (1.36)	
3”	38.1 (32.6)	1.14, 1.29 (1.33)	
4”	35.6 (40.1)	1.30 (1.19, 1.48)	
5”	30.6 (29.6)	1.13 (1.32)	
6”	11.6 (22.9)	0.86 (0.88)	
7”	19.5 (22.9)	0.85 (0.88)	
27	79.4	3.36	
28	73.8	3.48	
29	27.6	1.44, 1.67	
30	35.8	1.45, 1.70	
31	69.6	3.83	
32	46.0	1.50, 1.57	

33	66.6	4.07	
34	41.6	1.48, 1.50	
35	72.5	3.75	
36	41.2	1.48	
36-Me	15.4	0.92	
37	30.4	1.14, 1.29	
38	35.9	1.51	
39	76.1	3.71	
40	42.2	1.51	
40-Me	6.8	0.92	
41	75.6	3.71	
42	33.4	1.43, 1.63	
43	22.7	1.44	
44	38.9	1.45, 1.48	
45	70.5	3.68	
46	44.7	1.59, 1.64	
47	70.9	4.28	6.3, 12.8
48	136.5	5.78	15.0, 6.3
49	130.8	5.73	6.4, 15.0
50	72.9	5.43	6.4,
51	20.6	1.36	6.4

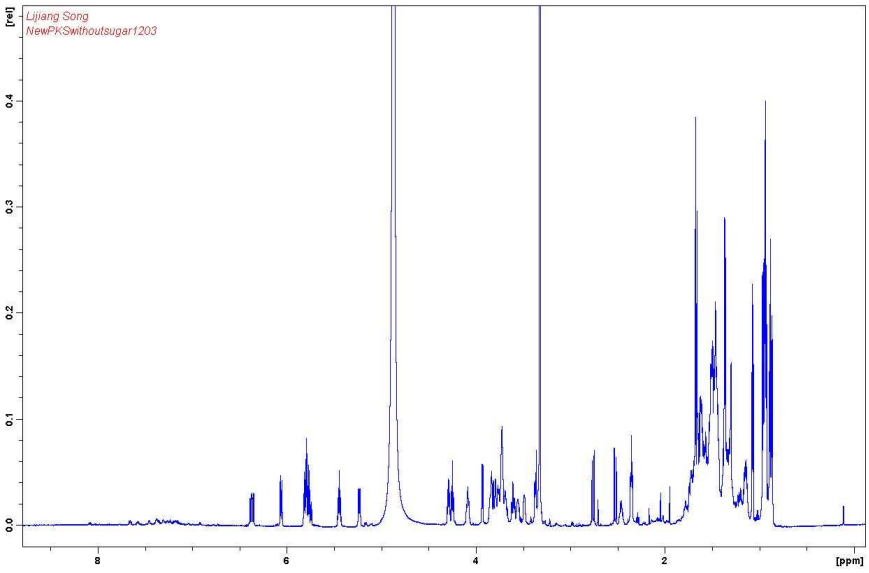

Fig. S32. ^{1}H NMR spectrum (700 MHz, d_4-MeOH) of stambomycin A/B aglycones.

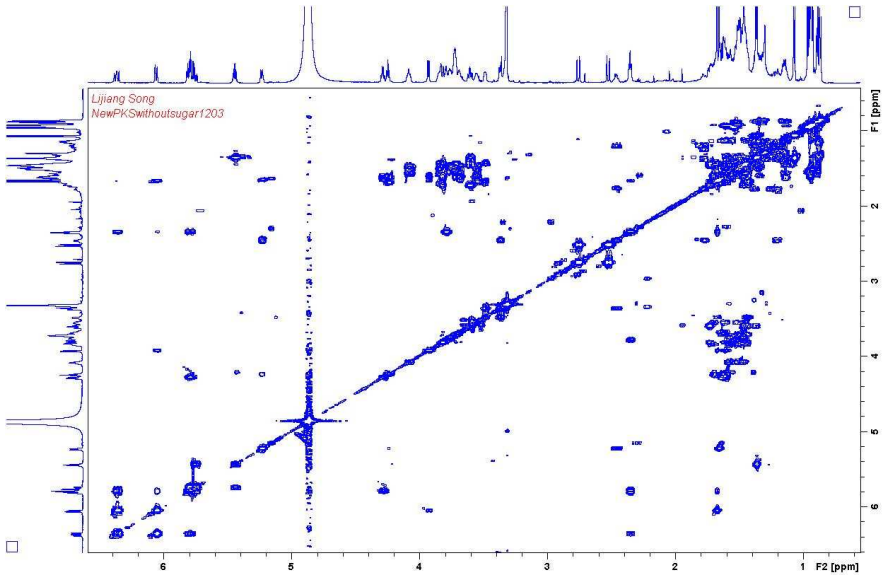

Fig. S33. DQFCOSY NMR spectrum (700 MHz, d_4-MeOH) of stambomycin A/B aglycones.

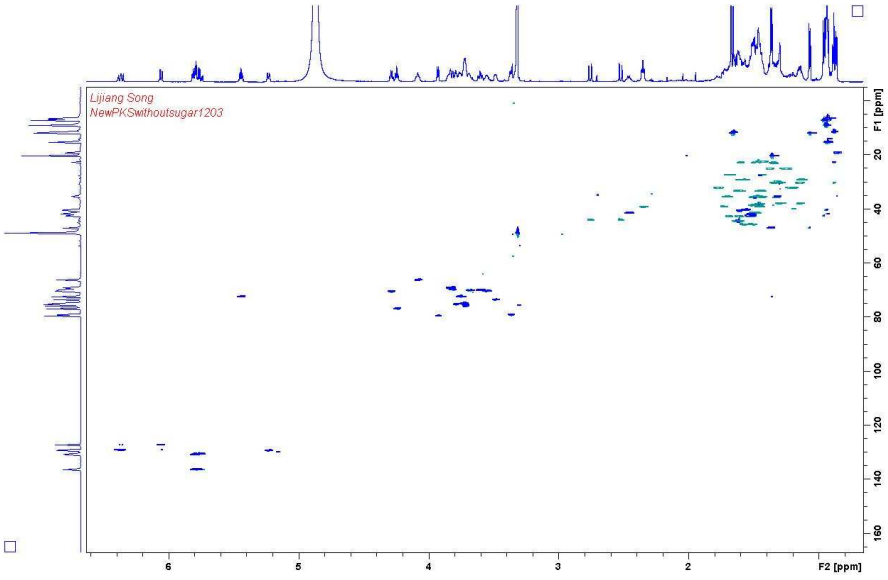

Fig. S34. HSQC NMR spectrum (700 MHz/175 MHz, d_4-MeOH) of stambomycin A/B aglycones.

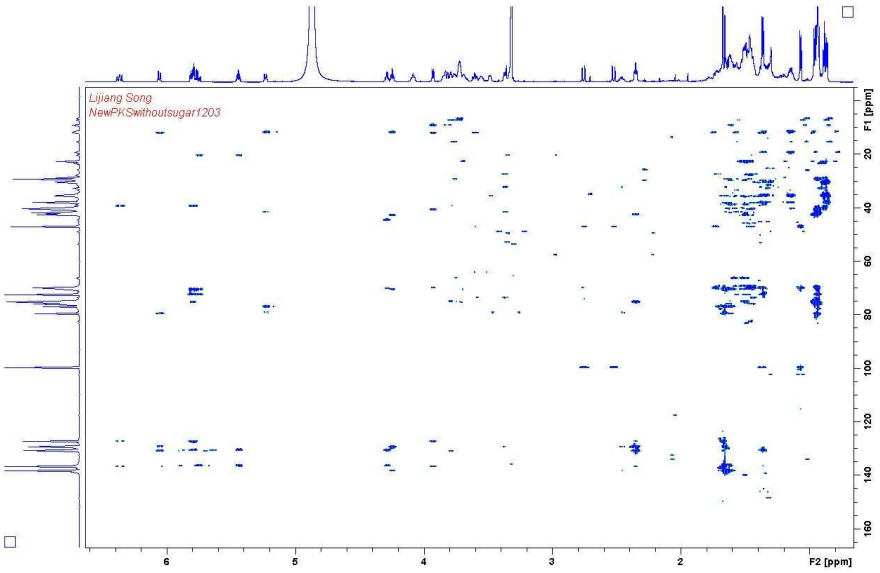

Fig. S35. HMBC NMR spectrum (700 MHz/175 MHz, d_4-MeOH) of stambomycin A/B aglycones.

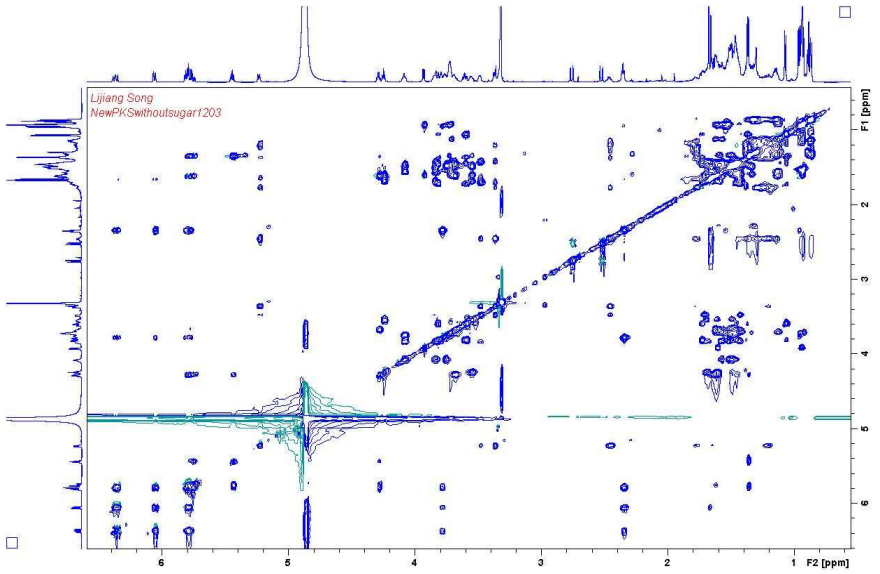

Fig. S36. TOCSY NMR spectrum (700 MHz, d_4-MeOH) of stambomycin A/B aglycones.

Table S5. Assignments of ^{1}H and ^{13}C NMR signals for the aglycones of stambomycins C/D. Carbon atom numbering is shown in Fig. S10. Signals for C-1″ to C-6″ of stambomycin D are in brackets.

position	δ_C (ppm)	δ_H (ppm)	J_{H-H} (Hz)
1	172.9		
2	44.2	2.51, 2.75	14.6
3	99.9		
4	47.2	1.36	
4-Me	12.3	1.06	
5	70.1	3.59	
6	39.4	1.45, 1.71	
7	70.0	3.81	
8	40.8	1.61	
8-Me	9.2	0.93	
9	79.6	3.92	7.5
10	136.7		
10-Me	11.9	1.66	
11	127.5	6.05	11.1
12	129.3	6.36	15.0, 11.1
13	131.2	5.79	15.0, 6.8
14	39.6	2.34	6.8
15	75.4	3.78	
16	42.7	1.53	
16-Me	7.3	0.95	
17	75.3	3.72	
18	36.0	1.48	
19	23.1	1.32	
20	38.4	1.45	
21	70.6	3.54	
22	42.8	1.61, 1.67	
23	77.1	4.23	7.3
24	138.6		
24-Me	12.1	1.66	
25	129.4	5.22	10.0
26	41.6	2.44	
1”	32.5 (32.4)	1.19, 1.77 (1.19, 1.80)	
2”	25.6 (27.1)	1.39 (1.41)	
3”	30.6 (32.9)	1.29, 1.29 (1.45)	
4”	40.4 (32.4)	1.21 (1.44)	
5”	22.9 (29.5)	0.88 (1.26)	
6”	22.9 (11.6)	0.88 (0.87)	
27	79.4	3.36	
28	73.7	3.47	
29	27.6	1.44, 1.67	
30	35.7	1.43, 1.71	
31	69.5	3.83	
32	46.0	1.51, 1.57	
33	66.4	4.07	

34	41.6	1.47, 1.51	
35	72.6	3.75	
36	41.6	1.49	
36-Me	15.6	0.93	
37	30.5	1.13, 1.29	
38	35.9	1.50	
39	76.0	3.71	
40	42.1	1.51	
40-Me	6.8	0.91	
41	75.5	3.71	
42	33.4	1.44, 1.62	
43	22.7	1.45	
44	39.1	1.47, 1.51	
45	70.4	3.68	
46	44.7	1.60, 1.63	
47	70.7	4.28	6.4, 12.5
48	136.5	5.78	15.0, 6.4
49	130.7	5.75	6.4, 15.0
50	72.6	5.43	6.4,
51	20.6	1.35	6.4

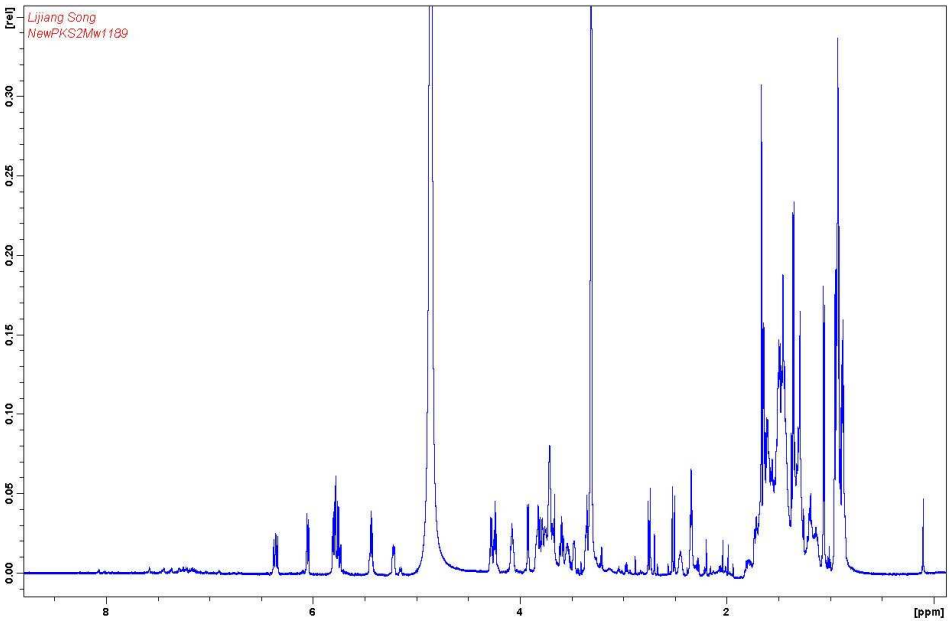

Fig. S37. ^{1}H NMR spectrum (700 MHz, d_4-MeOH) of stambomycin C/D aglycones.

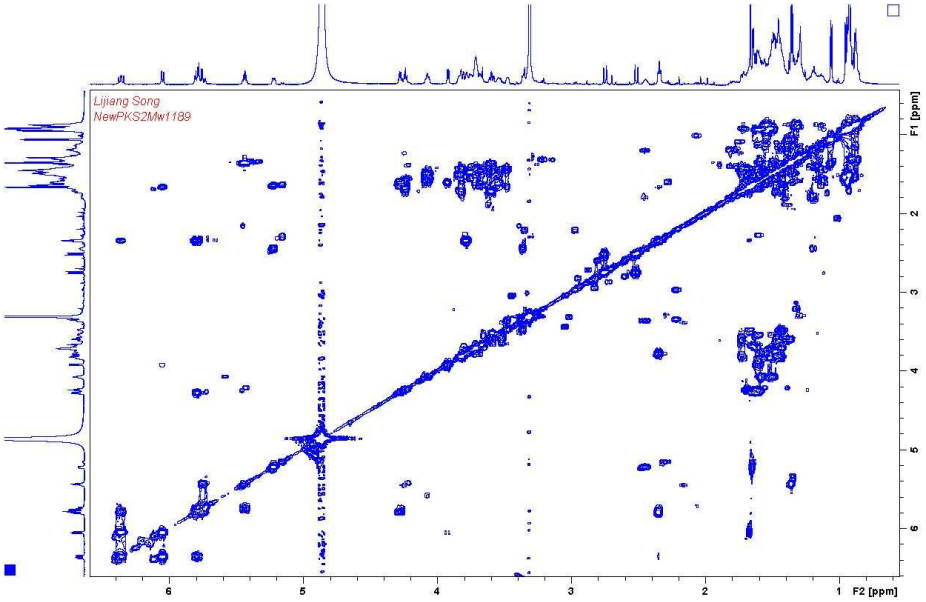

Fig. S38. DQFCOSY NMR spectrum (700 MHz, d_4-MeOH) of stambomycin C/D aglycones.

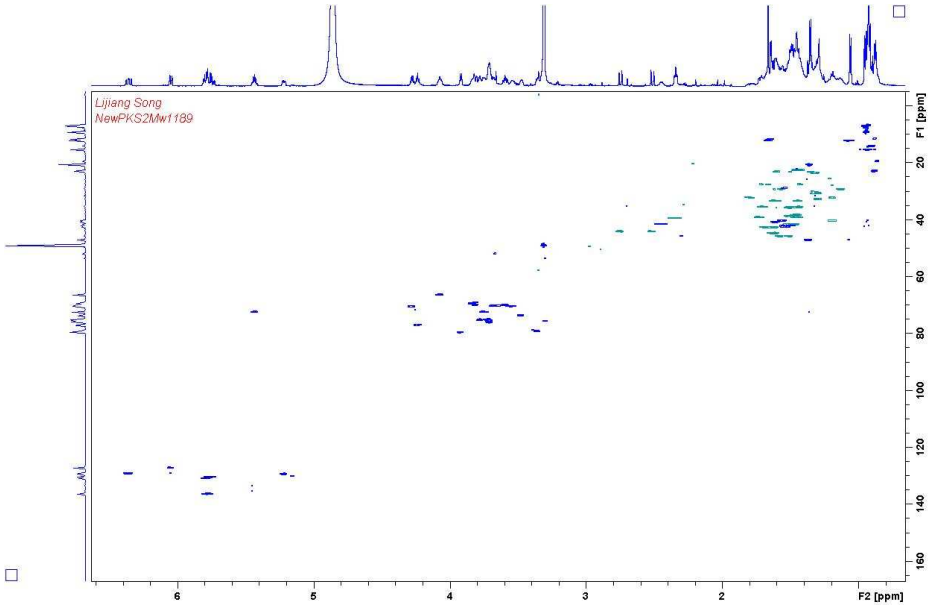

Fig. S39. HSQC NMR spectrum (700 MHz/175 MHz, d_4-MeOH) of stambomycin C/D aglycones.

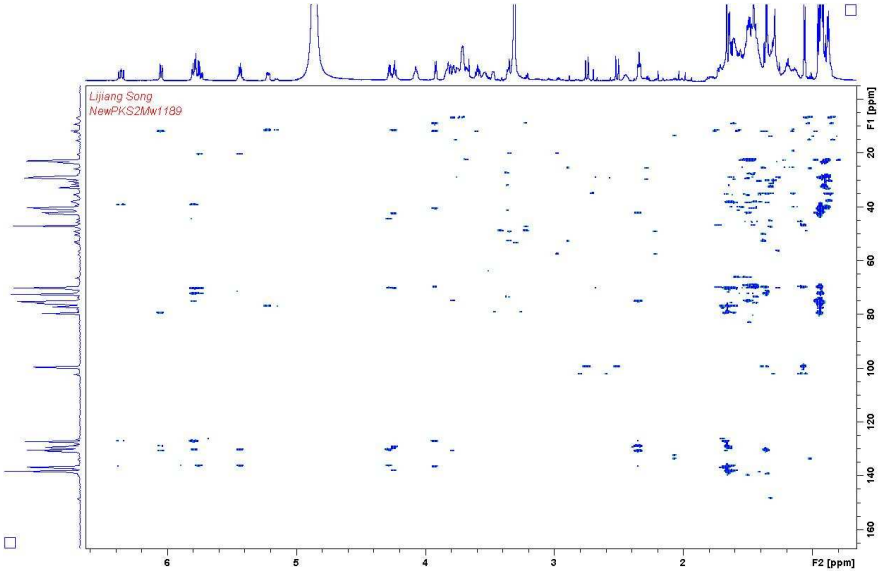

Fig. S40. HMBC NMR spectrum (700 MHz/175 MHz, d_4-MeOH) of stambomycins C/D aglycones.

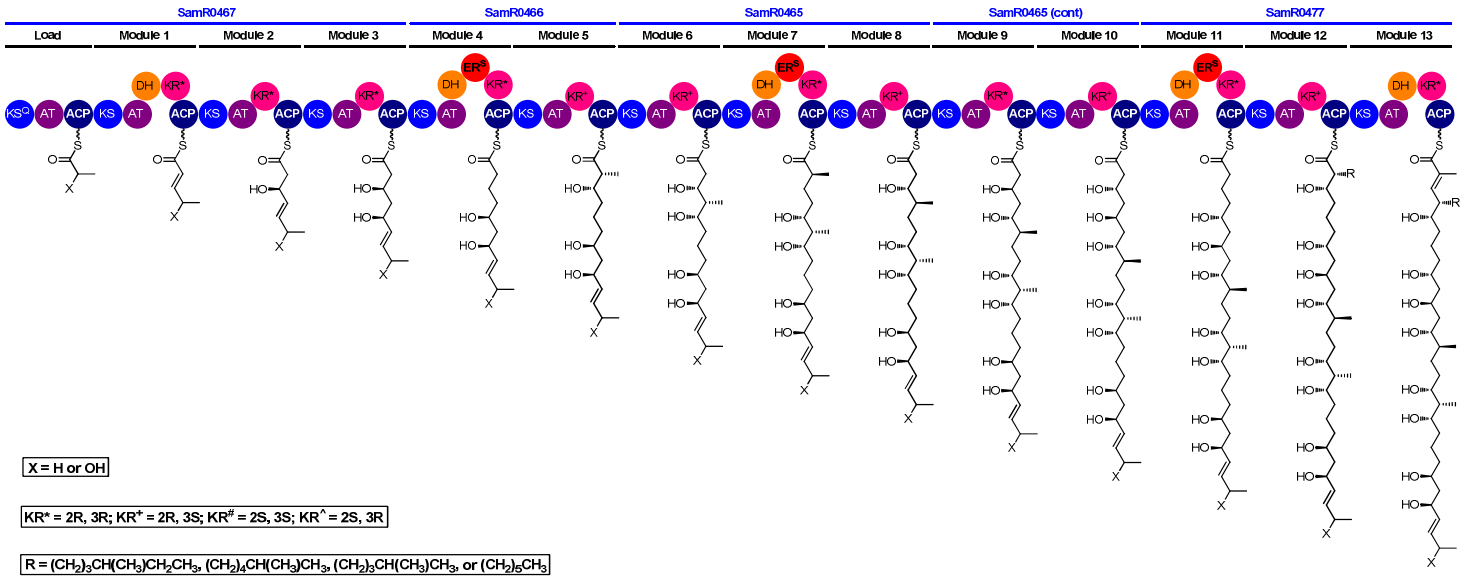

Fig. S41. Model for stambomycin biosynthesis.

The proposed structures of the intermediates assembled by each PKS module are shown attached to the respective ACP domains. The timing of hydroxylation of the propionyl starter unit is unknown and could occur on any of the ACP-bound intermediates, but the hydroxyl group introduced is required for release of the fully-assembled polyketide chain via macrolactonzation.

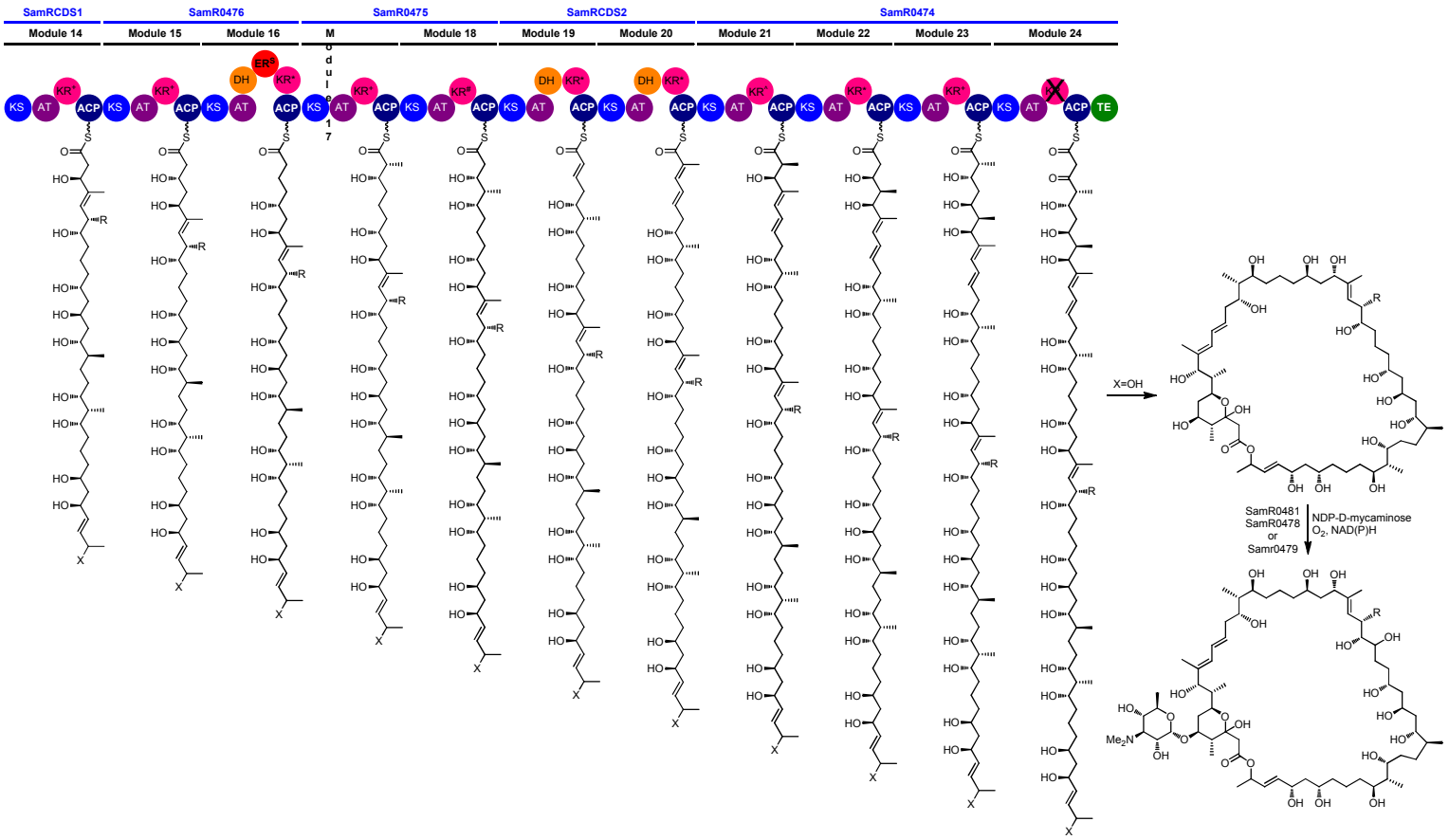
SamRCDS1
SamR0476
SamR0475
SamRCDS2
SamR0474
Module 14
Module 15
Module 16
Module 17
Module 18
Module 19
Module 20
Module 21
Module 22
Module 23
Module 24
KS
AT
ACP
KR*
DH
ER*
TE
X=OH
SamR0481
SamR0478
or
Samr0479
NDP-D-mycaminose
O_2, NAD(P)H

Fig. S41 cont.

Organism	stambomycins		vancomycin	Compounds of reference
	A/B	C/D		
Antibacterial activity (IC_{90}, µg/ml):				
Bacillus subtilis BGSC 1A72	ND	33.53 +/- 0.86	0.425+/-0.008	
Enterococcus faecalis LG40	ND	8.65 +/- 0.49	0.970+/-0.003	
Staphylococcus aureus LG21	ND	33.95 +/- 0.16	0.952+/-0.007	
Antiproliferative activity (IC_{50}, µM):				
HT29	1.77 +/- 0.04	1.74 +/- 0.04		1.32 +/- 0.08[a]
H460	1.49 +/- 0.03	1.30 +/- 0.13		1.51 +/- 0.12[b]; 5.21 +/- 0.22[c]
MCF7	>5.34	3.51+/- 0.16		3.69 +/- 0.11[d]; 0.40 +/- 0.03[e]
PC3	3.39 +/- 0.16	2.79 +/- 0.18		5.64 +/- 0.53[d]; 15.44 +/- 0.77[f]
Cytotoxicity (IC_{50}, µM):				
CHO-K1	8.47 +/- 0.67	8.46 +/- 0.52		1.99 +/-0.25[a]

Table S6. Biological activities of stambomycins A/B and C/D. IC_{90} and IC_{50} indicate the concentrations needed to inhibit the growth of 90% and 50% of cells in the population, respectively. HT29: human colon adenocarcinoma cell line; H460: lung cancer cell line; MCF7: breast cancer cell line; PC3: prostate cancer cell line; CHO-K1: adult Chinese hamster ovary sane cell line. ND: the IC_{90} values for stambomycins A/B were not determined, but these compounds were shown to possess antibacterial activity. Vancomycin was used as positive control in the antibacterial assays. The compounds used as reference for antiproliferative activity or cytotoxicity tests are doxorubicin[a] for HT29 and CHO-K1, mycophenolic acid[b] and 5-fluorouracyl[c] for H460, mitoxantrone[d] and CD437[e] for MCF7 and mitoxantrone and vinorelbin[f] for PC3.

Species	Protein	Function	Reference
S. venezuelae	PikD*	Pikromycin biosynthesis	(11)
S. hygroscopicus	RapH*	Rapamycin biosynthesis	(12)
S. hygroscopicus	Orf6	Type I PKS cluster	(13)
S. noursei	NysRI, NysRII, NysRIII*	Nystatin biosynthesis	(14)
S. cinnamonensis	MonH	Monensis biosynthesis	(15)
S. avermitilis	AveR*	Avermectin biosynthesis	(16)
Streptomyces sp. CK4412	TmcN*	Tautomycetin biosynthesis	(17)
S. albus	SalRI, SalRII	Salinomycin biosynthesis	(18)
S. natalensis	PimR*	Pimaricin biosynthesis	(19)
S. hygroscopicus 17997	GdmRI, GdmRII*	Geldanamycin biosynthesis	(20)
S. hygroscopicus var.	FkbN	FK520 biosynthesis	(21)
Streptomyces sp. FR-008	FscRII, FscRIII, FscRIV	FR-008/candicidin	(22)
S. nodosus	AmphRI, AmphRII,	Amphotericin biosynthesis	(23)
S. platensis Mer-11107	PldR	Pladienolide biosynthesis	(24)
S. ambofaciens	SAMR0484*	Stambomycin biosynthesis	This work
S. narbonensis	NbmM	Narbomycin biosynthesis	AAM88362.1
S. avermitilis	OlmRII, OlmRI	Oligomycin biosynthesis	(25)
*Streptomyces sp*307-9	TamH[#]	Tirandamycin biosynthesis	(26)
S. nanchangensis	NlmRI	Type I PKS cluster	AAS46340.1
S. aizunensis	Orf30	ECO-02301 biosynthesis	(27)
S. nanchangensis	MeiR	Meilingmycin biosynthesis	(28)
S. sp MP39-85	MlaH	ML-449 biosynthesis	(29)
Streptomyces sp	BecH	BE-14106 biosynthesis	(30)
Nonomuraea	Dbv3	A40926 biosynthesis	(31)
S. hygroscopicus	HbmRII, HbmRI	Herbymicin biosynthesis	(32)
S. lydicus	SlgR2[#]	Streptolydigin biosynthesis	(33)
S. neyagawaensis	Orf3	Concanamycin A	(34)
Amycolatopsis orientalis	Orf4	ECO-0501 biosynthesis	(35)
S. carzinostaticus	Orf2	Neocarzilin biosynthesis	(36)
S. griseus	Sgr6177	Type I PKS cluster	(37)
S. cyaneogriseus	NemR	Nemadectin biosynthesis	BAF85834.1
S. hygroscopicus	FkbN	FK520 biosynthesis	(21)
S. antibioticus	IdmG	Indanomycin biosynthesis	(38)
S. lasaliensis	Lsd8	Lasalocid biosynthesis	(39)

Table S7. List of 44 LAL proteins from actinomycetes species found in literature involved in known secondary metabolite biosynthesis (of modular PKS). *LAL regulators that have been characterized experimentally. [#]LAL regulators of PKS/NRPS clusters.

Species	Genes
S. albus J1074	fscRII, fscRIII, fscRIV
S. roseosporus NRRL 15998	SSGG_00417; SSGG_05502
S. hygroscopicus ATCC 53653	SSOG_00531; SSOG_00533; SSOG_05729; SSOG_07879
Micromonospora carbonacea ATCC 39149	MCAG_00565; MCAG_05134
Streptomyces sp. AA4	SSMG_05933
S. clavuligerus ATCC 27064	SSCG_03496
Streptomyces sp. C	SSNG_07480
S. viridochromogenes DSM 40736	SSQG_00945; SSQG_00966
Kutzneria sp. 744	KUTG_07746; KUTG_08953; KUTG_09296
S. roseosporus NRRL 11379 (v4)	SSIG_05739
Micromonospora sp. M42	MCBG_00014; MCBG_00015
Streptomyces sp. Mg1	SSAG_08104
Streptomyces sp. E14	SSTG_03938

Table S8. List of putative LAL regulatory genes involved in the regulation of a secondary metabolite gene cluster. The Blastp analysis was obtained using SamR0484 as bait and using the actinomycetes genome database of the Broad Institute (http://www.broadinstitute.org/annotation/genome/streptomyces_group/MultiHome.html). The genes retained were found nearside a modular PKS (except for SSTG_03938 located within a hybrid PKS-NRPS cluster) and the encoding proteins contain the typical domains of a LAL regulator.

Strains, BAC or plasmid	Principal characteristics[a]	Reference
Strains:		
S. ambofaciens		
ATCC23877	wildtype	(40)
ATCC/pIB139	empty vector integrated in the *att*B site	This work
ATCC/OE484	Overexpression of the LAL regulator	This work
ATCC/OE468-9	Overexpression of the two component system	This work
ATCC/OE484/Δ467	Overexpression of the LAL regulator and the gene *sam*R0467 replaced by a kanamycin resistance cassette	This work
ATCC/OE484/Δ481	Overexpression of the LAL regulator and the gene *sam*R0481 replaced by an apramycin resistance cassette	This work
E.coli		
DH5α	General cloning strain	(41)
ET12567/pUZ8002	Nonmethylating strain with mobilization plasmid for conjugation with *Streptomyces*	(42)
BW25113/pKD20	Strain used for the PCR-targeting mutagenesis (*gam, bet, exo, bla*)	(43)
BAC or plasmids:		
BBB	BAC from the genomic library of *S. ambofaciens* (*cat*)	(44)
BBC	BAC from the genomic library of *S. ambofaciens* (*cat*)	(44)
BBB/Δ467::*neo+oriT*	*sam*R0467 replaced by a neomycin cassette in BBB (*cat, neo*)	This work
BBBspec/Δ467::*neo+oriT*	*cat* of BBB/Δ467::*neo+oriT* replaced by a *aadA* cassette	This work
BBC/Δ481::*aac(3)IV+oriT*	*sam*R0481 replaced by an apramycin cassette in BBC (*cat, neo*)	This work
BBCspec/Δ481::*aac(3)IV +oriT*	*cat* BBC/Δ481::*aac(3)IV+oriT* replaced by a *aadA* cassette	This work
pIJ776	*oriT, neo*	(45)
pIJ778	*oriT, aadA*	(45)
pGEMT-easy	PCR cloning vector, *bla*	Promega
pGEMT-0484	pGEMTeasy + *sam*R0484 without promoter region	This work
pIB139	Conjugative and integrative plasmid (*oriT attP*$_{\phi C31}$ *int* $_{\phi C31}$ *aac*(*3*)*IV ermEp**)	(46)
pOE-0484	pIB139 + *sam*R0484	This work
pOE-4689	pIB139 + *sam*R0468-9	This work

Table S9. List of strains, plasmids and BACs used in this work. [a] *bla*, ampicillin resistance gene; *neo*, kanamycin resistance gene; *aac(3)IV*, apramycin resistance gene; *oriT*, origin of transfer; *aadA*, spectinomycin/streptomycin resistance gene; *gam*, inhibitor of the host exonuclease V; *bet*, single-stranded DNA binding protein; *exo*, exonuclease promoting recombination along with *bet*; *cat*, chloramphenicol resistance gene; *attP*$_{\phi C31}$, ϕC31 attachment site from the ϕC31 phage; *int*$_{\phi C31}$, integrase gene of ϕC31.

Primers	Nucleotide sequence (5′→3′)
Overexpression:	
OE484-F	CATATGCTGGTCCATCGAGACGAAC
OE484-R	TCTAGACTCTGCTCTCTCCAAGGCT
OE468-F	AGGTCTAGAGTCAGCCGAGGAAAC
OE469-R	CATATGACGAACGTGTCACGCGCGC
Deletion:	
D467-F	**GGCGATCTCGCGCCGTCAAGTGATTTCGGGGCATTCATG**TGTAGGCTGGAGCTGCTTC
D467-R	**CAGAGCGACTCCCAGCGGGCAGGACCGTACATGGCGTCA**ATTCCGGGGATCCGTCGACC
D481-F	**GGCGGGCTCCGGGCCGCGCGGGGCCGCGACCCTCCCGT**CTGCCTCTTCGTCCCGAAGCA
D481-R	**ACTGTTTCCGTCGGCGGGCCCGCCTGGAGGACGACA**ATGGCGCGCGCTTCGTTCGGGAC
Transcriptional analysis:	
RT-467-F	GTCGCCGGATCACCGAGGAA
RT-467-R	AGGTCGCGGAACGCCTTGTC
RT-465-F	TGCCTGCGGTGCTCCACCAA
RT-465-R	CGTCGTCTTCTCCTCCATCG
RT-477-F	GGAACAGCTCGCCGTACTCC
RT-477-R	CCGAACTCGTCGGCGTATGG
RT-474-F	ACCGCGCCGGAGGTGAGACA
RT-474-R	GCTGCTCGCCTGCGTGGACA
RT-484-F	CTGGAGACCTTCGGGGAGTG
RT-484-R	TGCCCGAGCACTCCGAAATG
RT-468-F	GTGGGTCAGGTGCGTCTTGAC
RT-468-R	ACTGCAAGTGCTGGAGCACG
RT-469-F	GAGGACGGGAGGGCTGAAGC
RT-469-R	GCGTGGCATCCGACGCGACCC
HrdB-F	CGCGGCATGCTCTTCCT
HrdB-R	AGGTGGCGTACGTGGAGAAC

Table S10. Oligonucleotide primers used in this work. The underlined nucleotides are *Nde*I and *Xba*I restriction sites. The bold nucleotides are identical to the sequences at the extremities of the *samR0467* (D467-F, -R) and *samR0481* (D481-F, -R) genes, which were deleted.

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