SUPPLEMENTARY INFORMATION

Glycosylation engineering of spinosyn analogues containing an L-olivose moiety

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General Methods

E. coli DH10B (GibcoBRL) were grown in 2TY medium as described elsewhere.[1] Vectors pUC19 and Litmus 28 were obtained from New England Biolabs. *E. coli* transformants were selected with ampicillin (100 μg cm^{-3}). *S. erythraea* strains were cultured as described previously[2] unless expressly described otherwise below. Plasmid-containing *S. erythraea* were selected with thiostrepton (40 μg cm^{-3}). DNA manipulations, PCR and electroporation procedures were carried out using standard procedures.[1] Protoplast formation and transformation procedures of *S. erythraea* were as described previously.[2,3]

Construction of pSG146_T28 (see Figure S1)

pSG144[4] was digested with *Spe*I and *Nde*I to remove the *actII*-ORF4 activator and the bi-directional P_{actIII}/P_{actI} promoter. The promoter for resistance to pristinamycin P_{ptr}[5] was self-amplified using the overlapping oligos Pris1 and Pris2 (Table S1), and cloned as a *Spe*I/*Nde*I fragment into the above digested pSG144 to generate pSG146. pSG146 was introduced into the genome of *S. erythraea* via the 'eryRHS' fragment, which is a neutral chromosomal site adjacent to the erythromycin biosynthetic cluster, as described elsewhere.[4]

Biosynthetic gene cassettes were assembled in a manner analogous to that used previously.[4] All DNA generated by PCR amplification was sequenced to confirm the integrity of the genes. Gene 1 (*ermE*) was amplified by PCR (*S. erythraea* NRRL2338 genomic DNA as template, oligos in Table S1) and cloned into pSG146 as an *Nde*I/*Xba*I fragment (this additionally removes *eryBV* present in this plasmid). Gene 2 (*tylAI*) was

amplified by PCR (*S. fradiae* ATCC19609 genomic DNA as template, oligos in Table S1) and cloned into a conversion vector to provide a ribosome binding site up-stream of the start codon, and an *Xba*I site that is methylated when plasmid DNA is isolated from *E. coli* DH10B, but not methylated when plasmid DNA is isolated from a *dam*$^-$ *E. coli* strain such as ET12567.[6] *TylAI* with the upstream RBS is then cloned on an *Xba*I fragment (plasmid DNA being isolated from ET12567) into *Xba*I-digested pSG146ermE. This leaves a single *Xba*I site available for insertion of the next gene. Gene 3 (*tylAII*) was amplified and cloned similarly to *tylAI* (oligos in Table S1).

The genes *spnO* and *spnN* were isolated on a single DNA fragment by PCR amplification using the appropriate oligos (Table S1) and genomic DNA from *Saccharopolyspora spinosa* NRRL18395. This fragment was cloned into pSG146 downstream of the P_{ptr} promoter. A *Spe*I/*Xba*I fragment containing *spnO* and *spnN* along with the upstream P_{ptr} was then isolated and cloned into the single available *Xba*I site that is not methylated downstream from *tylAII*, again leaving a single available *Xba*I (the ligation of *Spe*I to *Xba*I eliminates both sites). Similarly, *spnP* is amplified and cloned (this time as an *Nde*I/*Bgl*II fragment) into pSG146 such that it could then be added as the final gene of the cassette along with the upstream P_{ptr} as a *Spe*I/*Bgl*II fragment to generate pSG146_T28.

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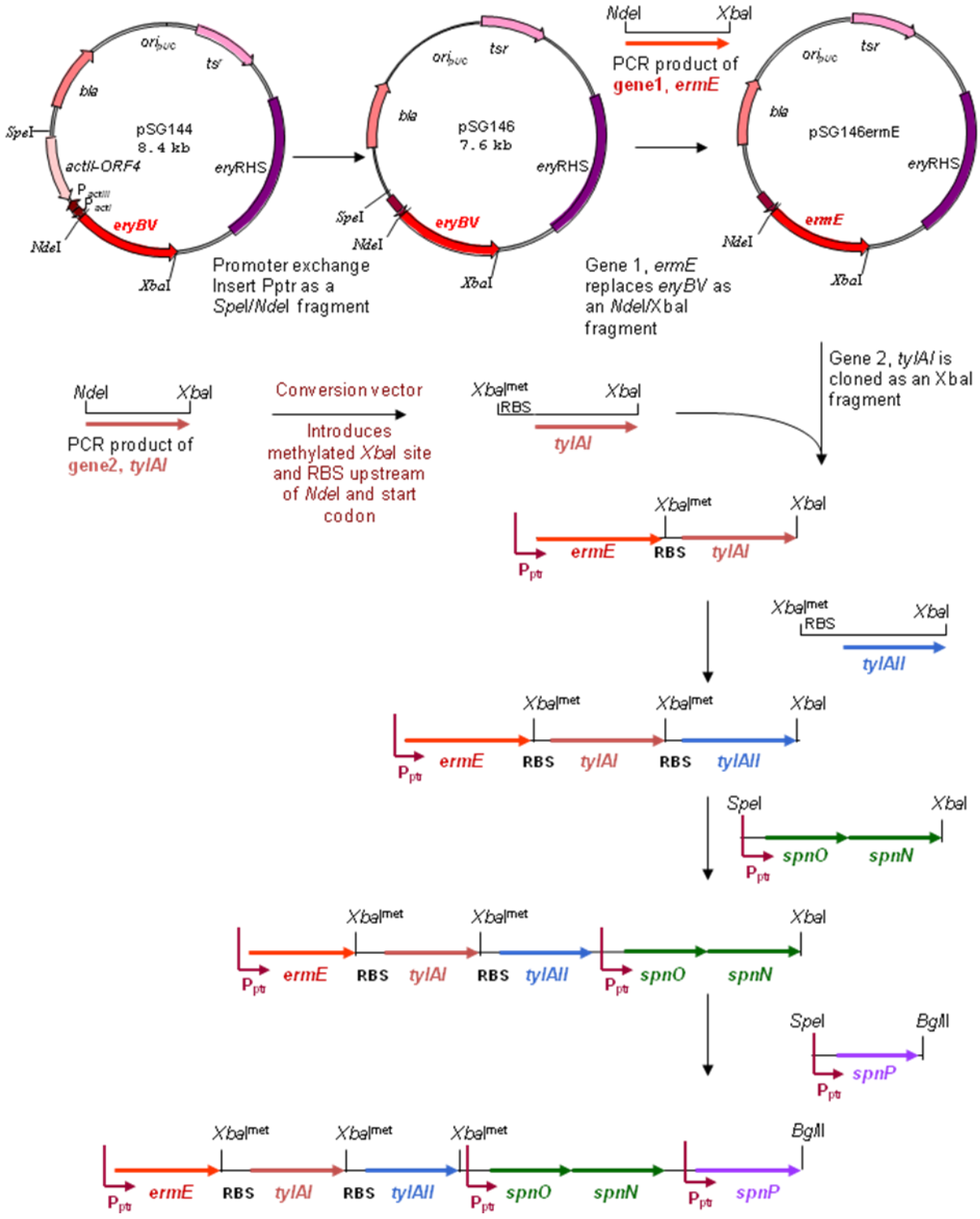

Figure S1

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Table S1

Oligo name	Sequence 5'-3'
Pris1	GGG GAA TTC ACT AGT CCG CGG AGA AAT AGC GCT GTA CAG CGT ATG GGA ATC TCT TGT ACG G
Pris2	GGG GGA TCC CAT ATG GGC TCC TTG TAC GGT GTA CGG GAA GAT ACT CGT ACA CCG TAC AAG AGA TTC CC
ermEfor	GGG CAT ATG AGC AGT TCG GAC GAG CAG CCG CGC CCG CGT
ermErev	GGG GTC TAG AGG TCA CCG CTG CCC GGG TCC GCC GCG TCG CCC TGG
tylAIfor	GGG CAT ATG AAC GAC CGT CCC CGC CGC GCC ATG AAG GG
tylAIrev	CCC CTC TAG AGG TCA CTG TGC CCG GCT GTC GGC GGC GGC CCC GCG CAT GG
tylAIIfor	GGG CAT ATG CGC GTC CTG GTG ACC GGA GGT GCG GGC TTC
tylAIIrev	CCC CTC TAG AGG TCA TGC GCG CTC CAG TTC CCT GCC GCC CGG GGA CCG CTT G
spnO+Nfor	GGG CAT ATG AGC AGT TCT GTC GAA GCT GAG GCA AGT
spnO+Nrev	GGT CTA GAG GTC ATG TGG ACC CGC ACC GAC GGG CCT GAC
spnPfor	TCG GAT CCC ATA TGC GTG TCC TGT TCA CCC CGC T
spnPrev	GTA GAT CTC GGA TGG CCA TCA GAC TGC CCA

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Table S2. NMR data for **9** acquired in $CDCl_3$ at 500 (^1H) and 125 (^{13}C) MHz. ^1H-^1H COSY and ^1H-^{13}C HMBC correlations are given.

Position	δ_C	δ_H (mult., Hz)	H-H COSY	H-C HMBC
1	172.3	-	-	-
2	34.4	3.12 m 2.41 (dd, 13.5, 3.0)	2b 2a	1, 3, 4 1, 3
3	49.6	2.89 (ddt, 10.8, 8.8, 2.6)	4, 13	12, 14
4	41.5	3.45 (dd, 9.3, 3.3)	3, 5, 6, 12	6, 12
5	129.4	5.87 (bd, 9.7)	4, 6	4, 7, 11
6	128.9	5.77 (dt, 9.8, 2.8)	4, 5, 7	3, 4, 7
7	41.1	2.17 m	6, 8, 11	-
8	36.5	1.93 (dd, 13.5, 6.6) 1.34 m	7 -	7, 9, 10 7, 9
9	76.3	4.30 m	8,10a	1', 7
10	37.5	2.25 m 1.32 m	9, 10b, 11 11	7, 8, 11 7, 9
11	46.3	0.89 m	7, 11	7, 10
12	47.6	3.03 m	4, 13	-
13	147.7	6.77 br s	3,12	3, 4, 12, 14, 15
14	147.3	-	-	-
15	202.0	-	-	-
16	46.9	3.29 (dt, 6.9, 2.7)	17, 24	15, 17, 24
17	83.1	3.72 m	16, 18	1''
18	34.4	1.66 m 1.54 m	17	-
19	20.6	1.55 m	20a	20
20	30.8	1.69 m 1.37 m	19 21	 21
21	75.9	4.67 m	20b, 22	-
22	27.9	1.56 m 1.49 (qm 7.3)	21, 23	20, 21, 23 20, 21, 23
23	9.3	0.82 (t, 7.5)	22	21, 22
24	17.3	1.19 (d, 6.9)	16	15, 16, 17
1'	95.7	4.83 (d, 1.7)	2'	9, 3'', 5''
2'	77.8	3.50 m	1'	-
3'	81.1	3.47 m	4'	-
4'	82.3	3.11 m	3', 5'	-
5'	68.0	3.53 m	4', 6'	-
6'	17.8	1.27 (d, 6.3)	5'	4'', 5''
O-CH_3-2'	59.0	3.48 s	-	2'
O-CH_3-3'	57.6	3.49 s	-	3'
O-CH_3-4'	60.8	3.54 s	-	4'
1''	99.9	4.95 (d, 3.6)	2''	17, 3'', 5''

2''	35.4	2.21 (ddd, 14.5, 3.6, 0.8) 1.91 (dd 14.5, 6.9)	1'', 3''	3'', 4''
3''	67.3	3.95d (6.9)	2'', 4''	5''
4''	72.6	3.12 (dd, 9.2, 9.7)	3'', 5''	6''
5''	65.1	3.79 (dq, 9.7, 6.3)	4'', 6''	4''
6''	17.6	1.29 (d, 6.3)	5''	4'', 5''

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