Supporting Information

Shared biosynthesis of the saliniketals and rifamycins in *Salinispora arenicola* is controlled by the *sare1259*-encoded cytochrome P450

Micheal C. Wilson[1], Tobias A. M. Gulder[1], Taifo Mahmud[2], and Bradley S. Moore[1,3]

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093-0204; Department of Pharmaceutical Sciences, Oregon State University, Corvallis, Oregon 97331; and the Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, California 92093

E-mail: bsmoore@ucsd.edu

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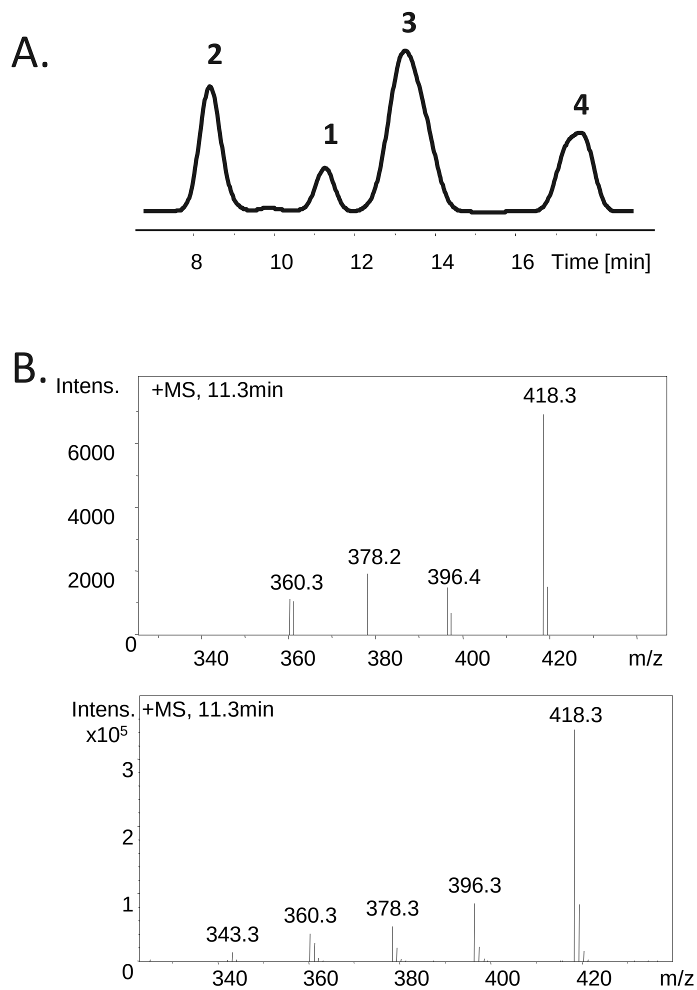

Figure S1. HPLC-MS analysis of *A. mediterranei* S699. (A) Extracted ion chromatogram for all masses corresponding to the m/z of the sodium adducts of the saliniketals (**1**–**2**) and rifamycins (**3**–**8**) produced by *A. mediterranei* S699. (B) MS fragmentation pattern for **1** from *A. mediterranei* S699 (top panel) and authentic standard (bottom panel).

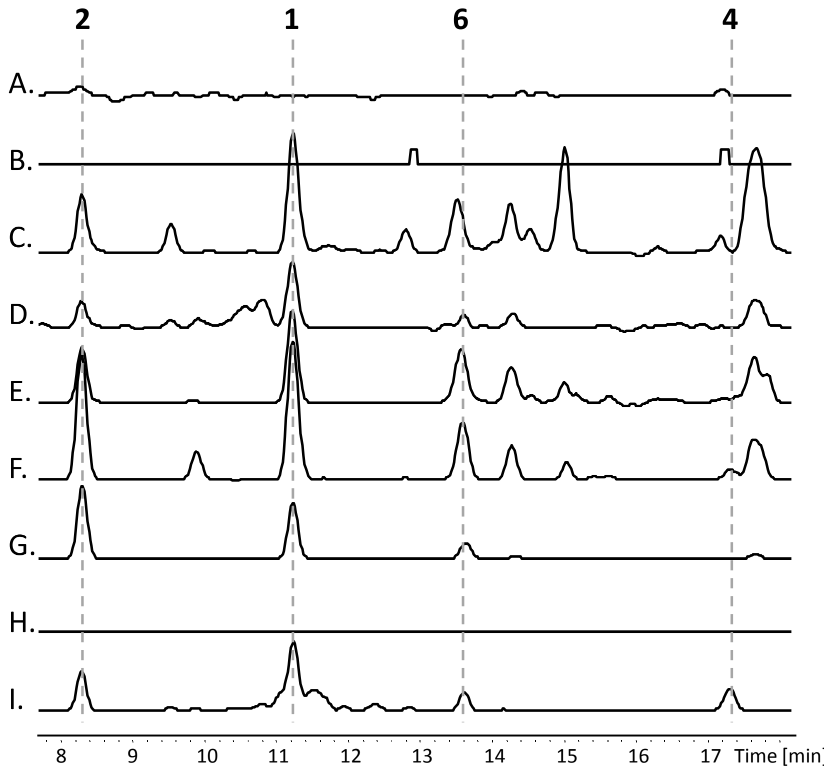

Figure S2. HPLC-MS analysis of SA-*rif* mutants generated in this study. Extracted ion chromatogram for all masses corresponding to the m/z of the sodium adducts of the saliniketals (**1**–**2**) and rifamycins (**3**–**8**) produced by (A) *sare1242::Apr^R*, (B) *sare1243::Apr^R*, (C) *sare1263::Apr^R*, (D) *sare1264::Apr^R*, (E) *sare1265::Apr^R*, (F) *sare1266::Apr^R*, (G) *sare1267::Apr^R*, (H) *sare1272/1273::Apr^R*, and (I) *sare1274::Apr^R*. Unlabeled peaks correspond to non-*rif* related compounds and/or unconfirmed *rif* shunt products.

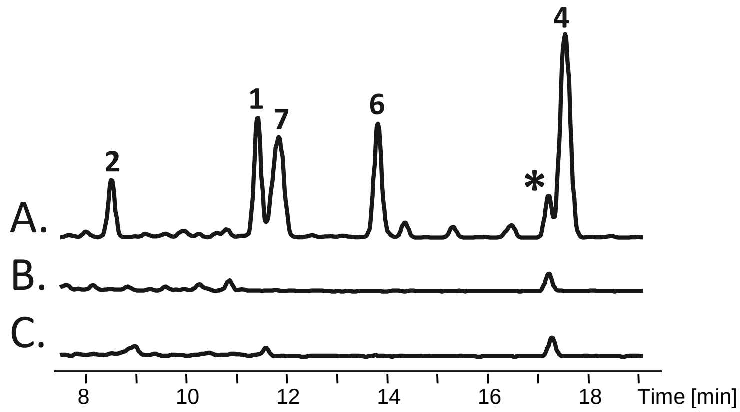

Figure S3. HPLC-MS analysis of the chemical complementation of the *S. arenicola rif-orf0* mutant strain *sare1245::apr*^{R}. EIC for all masses corresponding to the *m/z* of the sodium adducts of the saliniketals (**1**–**2**) and rifamycins (**3**–**8**) for (A) *sare1245::apr*^{R} chemically complemented with **7**, (B) *sare1245::apr*^{R} chemically complemented with AHBA, and (C) *sare1245::apr*^{R} negative control. (* indicates unrelated compounds that share an *m/z* ratio with one of the compounds **1**–**8**)

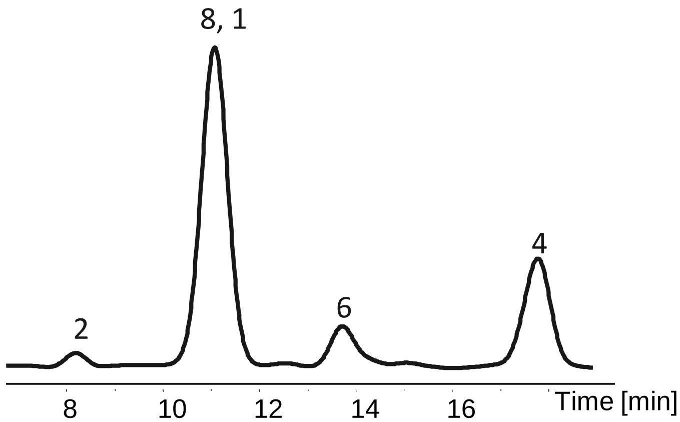

Figure S4. HPLC-MS analysis of the *A. mediterranei rif-orf5* mutant strain MT45025H. EIC for all masses corresponding to the *m/z* of the sodium adducts of the saliniketals (**1**–**2**) and rifamycins (**3**–**8**) produced by MT45025H cultured for 10 days in A1 media at 28°C and 225 rpm.

Figure S5. ^{13}C NMR spectrum (126 MHz, MeOH-d_4) of saliniketal A (**1**) from [U-^{13}C]propionate feeding study.

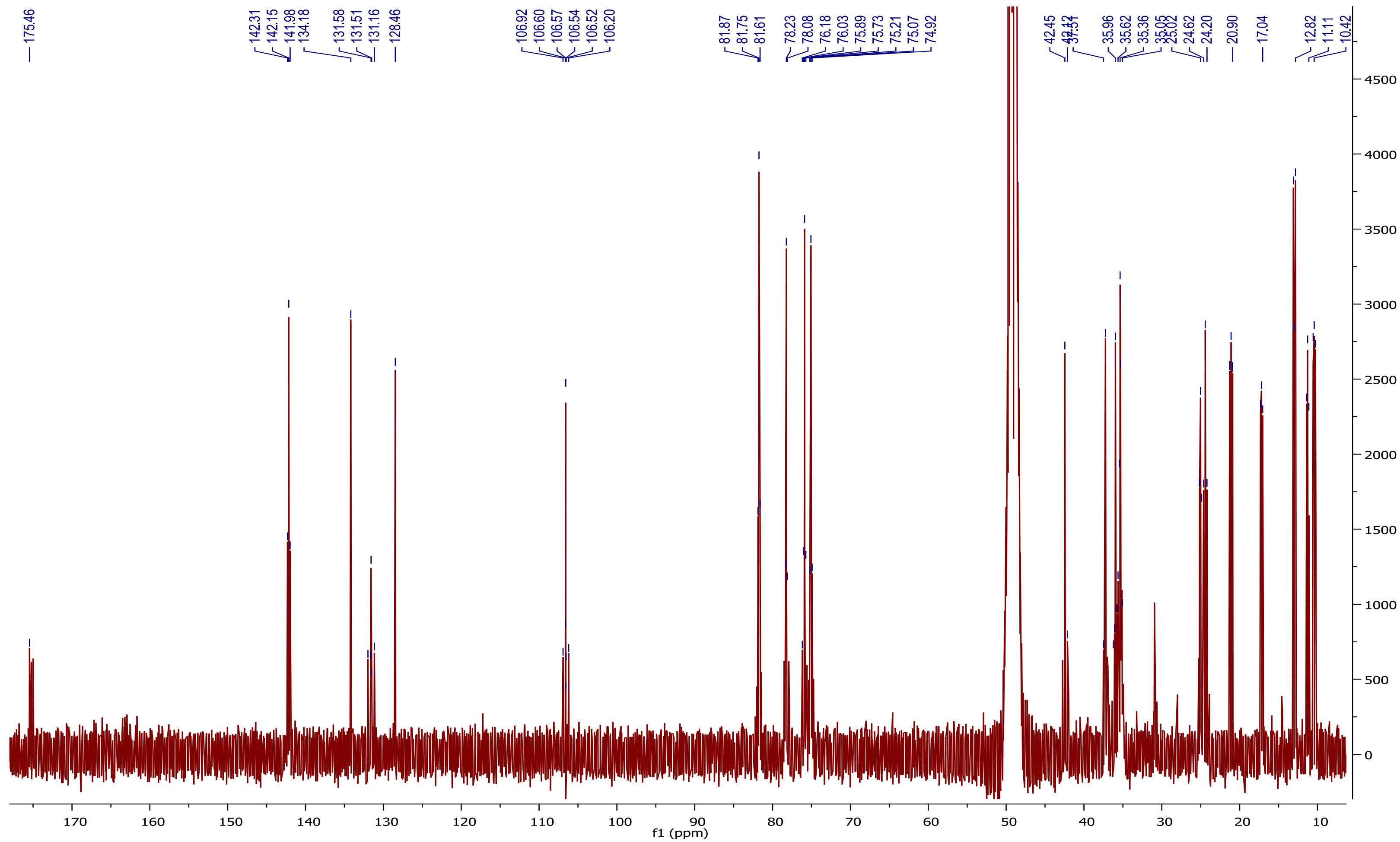

Figure S6. Partial ^{13}C NMR spectrum (126 MHz, MeOH-d_4) of saliniketal A (**1**) from [U-^{13}C]propionate feeding study (127-177 ppm)

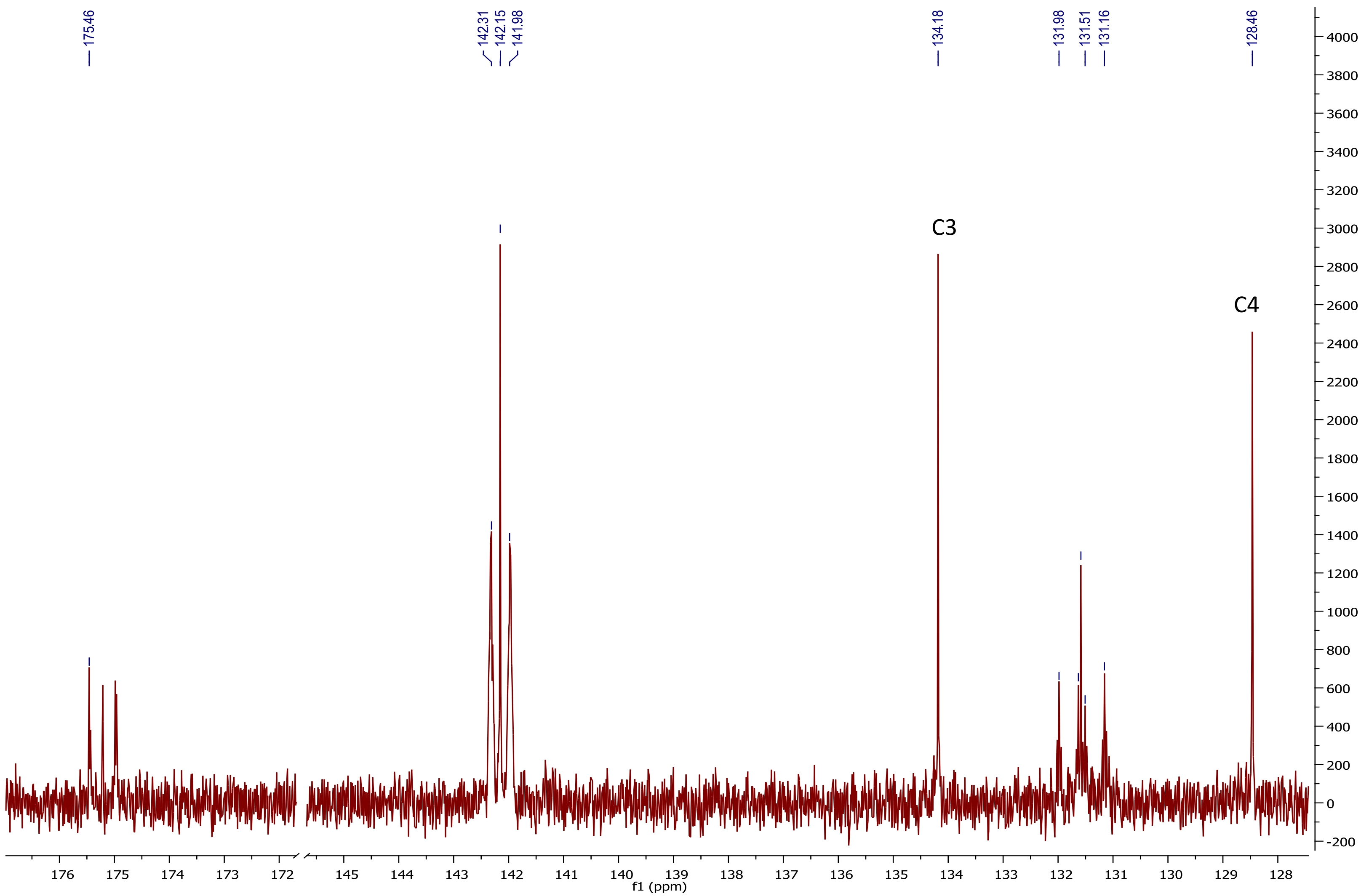

Figure S7. ^{1}H NMR spectrum (600 MHz, DMSO-d_6) of 34a-deoxy-rifamycin W (**7**) from *sare1259::apr*^{R} mutant

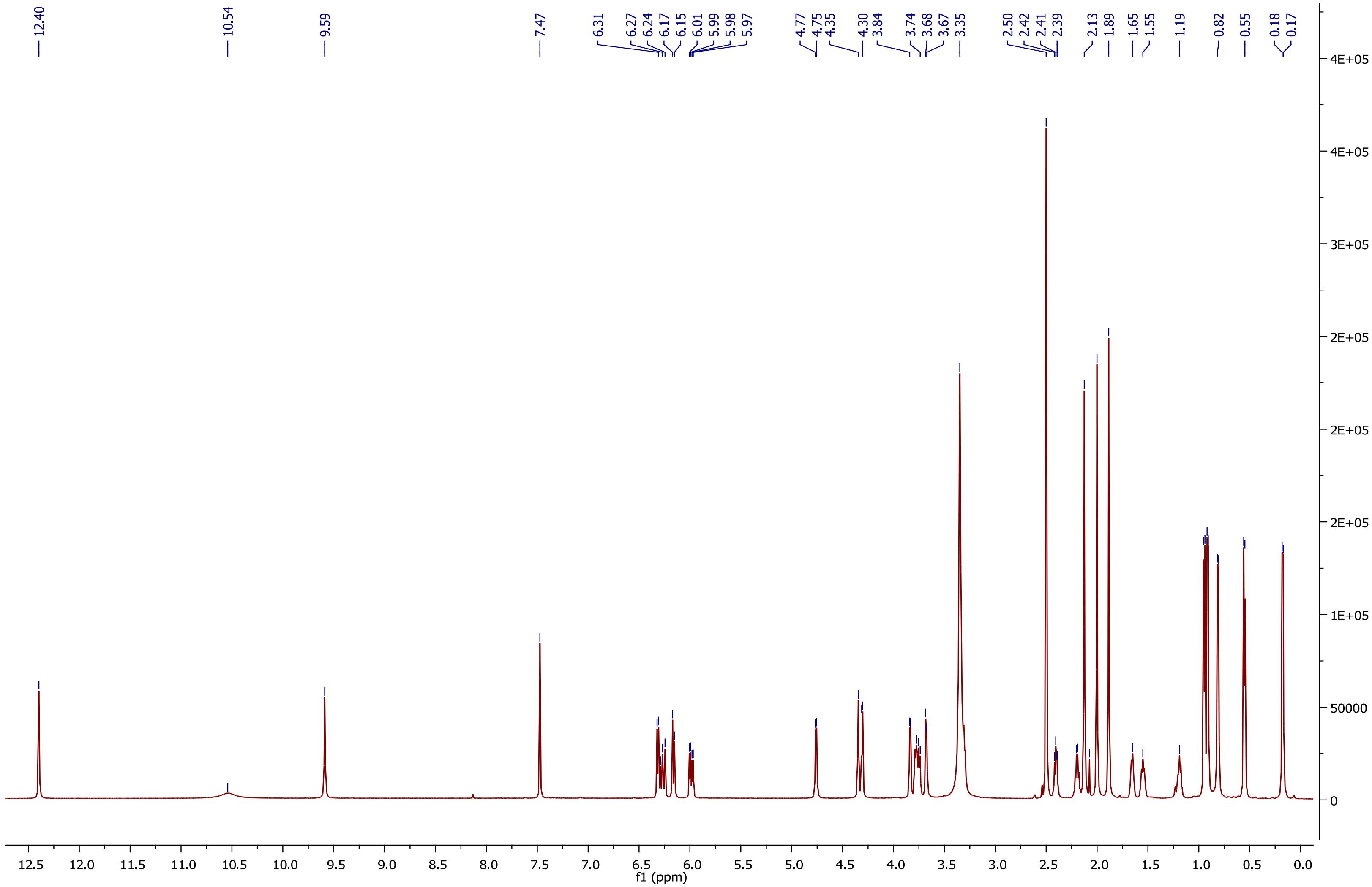

Figure S8. gHSQC spectrum (600 MHz, DMSO-d_6) of 34a-deoxy-rifamycin W (**7**) from *sare1259::apr*^{R} mutant

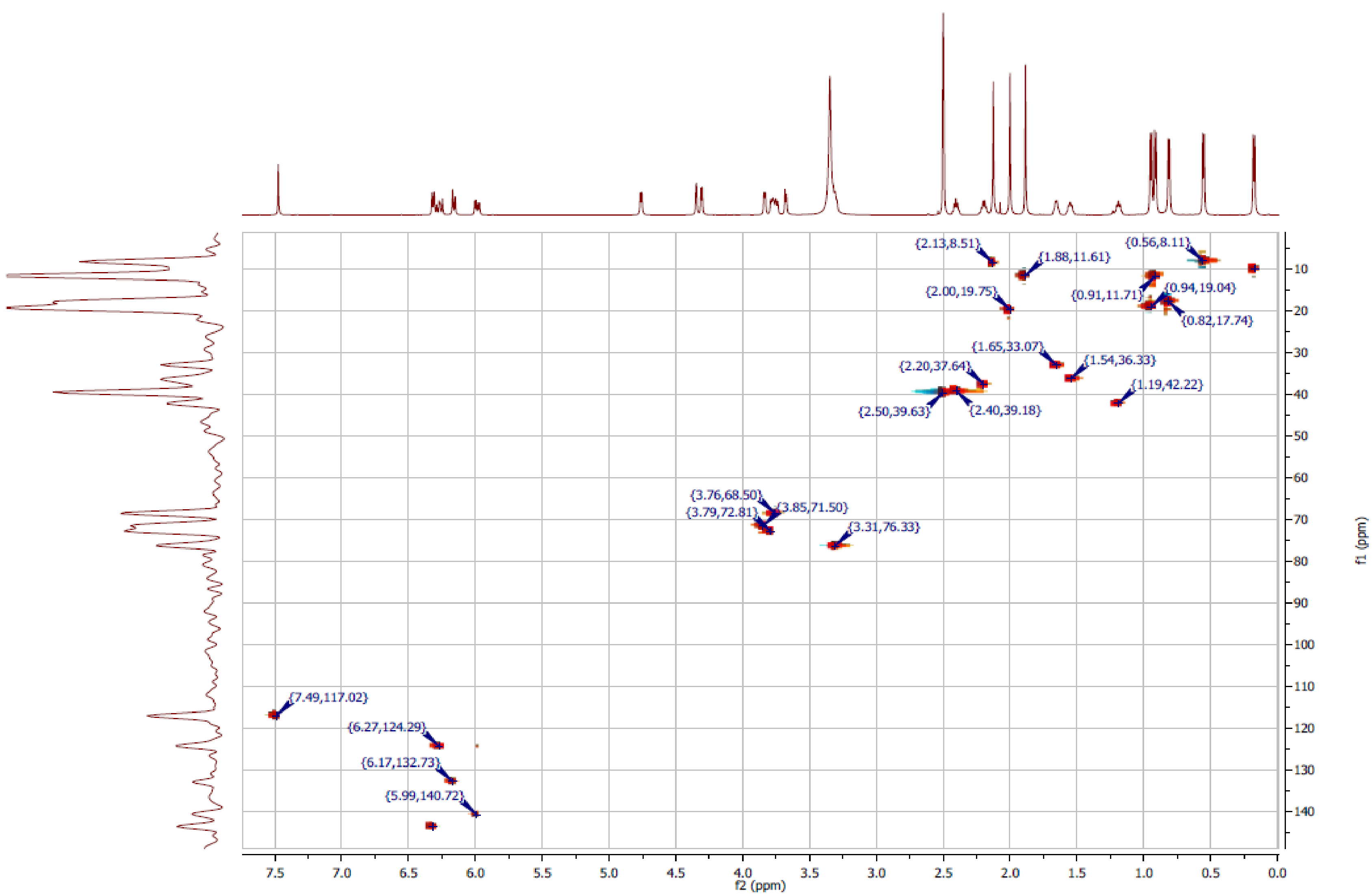

Figure S9. gHMBC spectrum (600 MHz, DMSO-d_6) of 34a-deoxy-rifamycin W (**7**) from *sare1259::apr*^{R} mutant

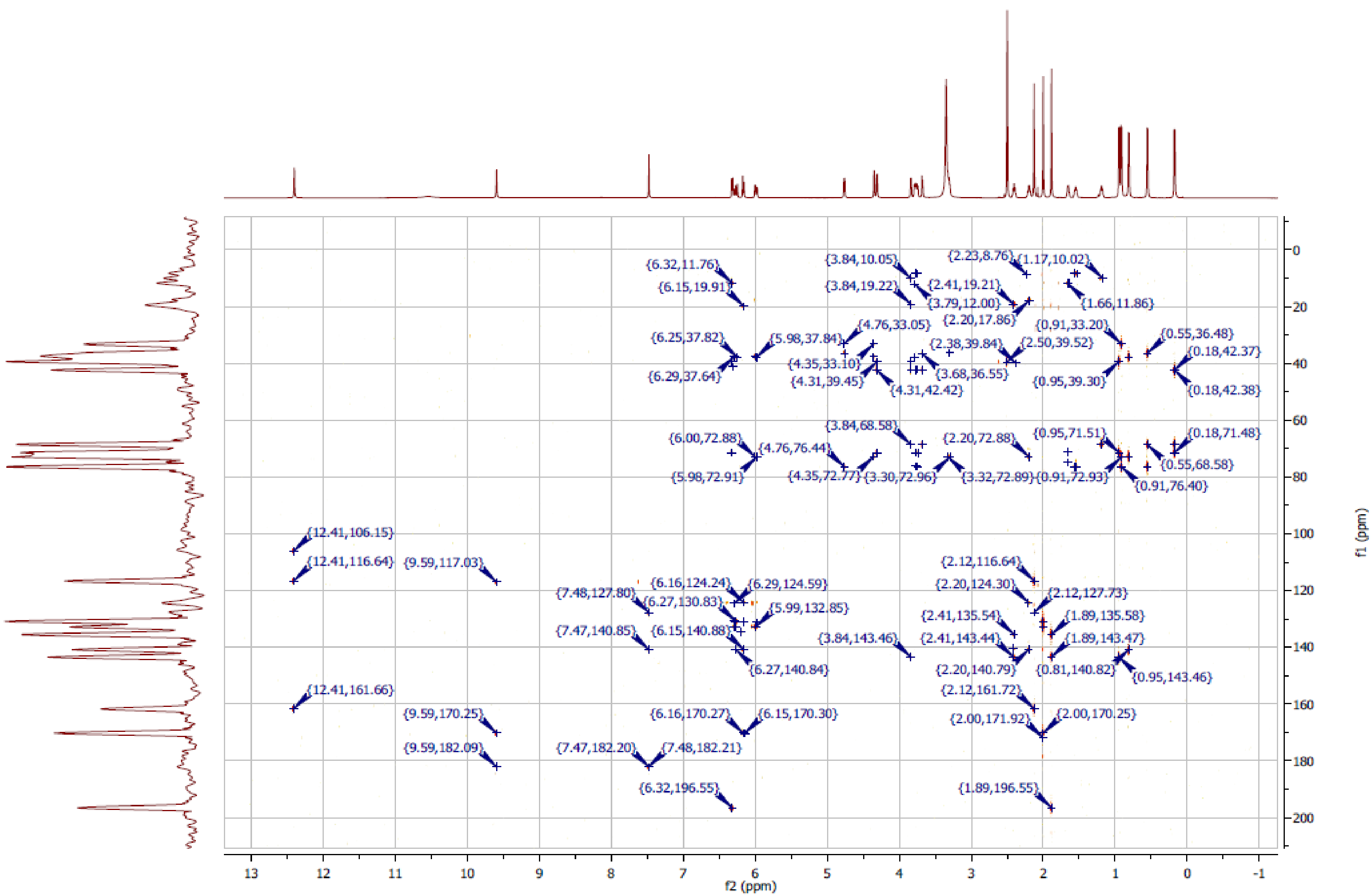

Table S1. Primers synthesized for gene inactivation experiments and verification of double crossover mutants in the SA-*rif* gene cluster.

Primers	Oligonucleotides
sare1242KOF	5'-cgtgggccccgcaggtggaggaagggctggaaagcaatgattccggggatccgtcgacc-3'
sare1242KOR	5'-tcatgcggcgactcctttcaccggcacttccacggcacgtgtaggctggagctgcttc-3'
sare1243KOF	5'-atgagagttgccgtcctgtccccggcgcacgaacgcgccattccggggatccgtcgacc-3'
sare1243KOR	5'-ccgttcgttcctcgcgccggccgggggccggcgccgtcatgtaggctggagctgcttc-3'
sare1245KOF	5'-ctcatccgcacaccatcgccgagtgagaaggatgccatgattccggggatccgtcgacc-3'
sare1245KOR	5'-gcactcagggaagaacgctcaccagcagacgggcaggtttgtaggctggagctgcttc-3'
sare1255KOF	5'-cgttcacgcatttcctcacggagaattcggagagacatgattccggggatccgtcgacc-3'
sare1255KOR	5'-gcccgacgaccgccacccggacgctcatgcgctggccactgtaggctggagctgcttc-3'
sare1258KOF	5'-cacgggcggatcgaccgatccgtgctggcaggcggcatgattccggggatccgtcgacc-3'
sare1258KOR	5'-gcggccagtgcgaccggggatcagtgcgacaggggatcatgtaggctggagctgcttc-3'
sare1259KOF	5'-ggatcgccggcttttcgacacgagagggagggctcatgattccggggatccgtcgacc-3'
sare1259KOR	5'-aggcacctcgggtcagggccagccgcggcggtgtgctcatgtaggctggagctgcttc-3'
sare1260KOF	5'-gtgcgatttgccgcatcagtagagggaggcctcgcgttgattccggggatccgtcgacc-3'
sare1260KOR	5'-cgatgcacacttcccggttcgcgcgcaccctcacggcgttgtaggctggagctgcttc-3'
sare1262KOF	5'-tccaccggacttcgcacgctgattggagaccgattcgtgattccggggatccgtcgacc-3'
sare1262KOR	5'-gcgcacgcggcctgacgccgggcggtacgccgacggtcatgtaggctggagctgcttc-3'
sare1263KOF	5'-gcctcgcagagaacctcacgaccgctgggagacgacttgattccggggatccgtcgacc-3'
sare1263KOR	5'-accccgcctcgccgccgggcccgtgggacggacgcctcatgtaggctggagctgcttc-3'
sare1264KOF	5'-cagcgacagcccacactcgaccgtaggggatacgacatgattccggggatccgtcgacc-3'
sare1264KOR	5'-ctggtcaccgatgcccgagtggaccgaacccacccttcatgtaggctggagctgcttc-3'
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sare1242CKF	5'-ccggcaccggtgaactaccc-3'
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sare1260CKF	5'-gagcgagggatgtgtggccg-3'
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sare1262CKF	5'-acgtcccaccgaccgactgg-3'
sare1262CKR	5'-cagtgctcgtgaccggggct-3'
sare1263CKF	5'-tggtccggctgtttcgtccc-3'
sare1263CKR	5'-gctggtgacgtcggcaagct-3'
sare1264CKF	5'-agcgagttgcggtggctacg-3'
sare1264CKR	5'-gtgctgggcaggtcaggtgc-3'
sare1265CKF	5'-gcccagcacatcaccggagg-3'
sare1265CKR	5'-acagctcagcggtgtgcggg-3'
sare1266CKF	5'-gtcctcgaagtggatcggcc-3'
sare1266CKR	5'-ggcatgtcggccctactctc-3'
sare1267CKF	5'-ggaacccggaacacgagacc-3'
sare1267CKR	5'-tacgagctccggcgccggtg-3'
orf15CKF	5'-tgatcaaccacgacccgctg-3'
orf15CKR	5'-ctcggccgtagcgtcgacc-3'
sare1274CKF	5'-agcaccggcttcgaccggtc-3'
sare1274CKR	5'-tcaggcccgaccagcgaac-3'

Complete reference 30.

Trefzer, A.; Jungmann, V.; Molnar, I.; Botejue, A.; Buckel, D.; Frey, G.; Hill, D. S.; Jorg, M.; Ligon, J. M.; Mason, D.; Moore, D.; Pachlatko, J. P.; Richardson, T. H.; Spangenberg, P.; Wall, M. A.; Zirkle, R.; Stege, J. T. *Appl. Environ. Microbiol.* **2007**, *73*, 4317.