Supporting Information

Biosynthetic Origin of the Atypical Stereochemistry in the Thioheptose Core of Albomycin Nucleoside Antibiotics

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Table of Contents

Experimental Procedures

Experimental Procedures

S1. General notes

Materials: All chemicals and reagents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA) and were used without further purification unless otherwise specified. Standard genetic manipulations of *Escherichia coli* were performed as described by Sambrook and Russell.[1] DNA sequencing was performed at the core facility of the Institute of Cellular and Molecular Biology, the University of Texas at Austin. Tetrahydrofuran (THF) was distilled from sodium/benzophenone, and dichloromethane (DCM) was distilled from calcium hydride under an argon atmosphere. Oligonucleotide primers were prepared by Integrated DNA Technologies (Coralville, IA). Kits for DNA gel extraction and spin minipreps are products of Qiagen (Valencia, CA). PureLink Genomic DNA Mini Kit was acquired from Invitrogen (Carlsbad, CA). KOD DNA polymerase was purchased from Novagen (Madison, WI). Enzymes and molecular weight standards used in the cloning experiments were obtained from New England Biolabs (Ipswich, MA). Reagents for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were purchased from Bio-Rad (Hercules, CA). Sterile syringe filters were bought from Fisher Scientific. Amicon YM-10 ultrafiltration membranes are products of Millipore (Billerica, MA). Silica gel column chromatography was carried out using SiliaFlash P60 (230–400 mesh, Silicycle).

Bacterial Strains and Plasmids: *Streptomyces* sp. strain ATCC 700974 was obtained from American Type Culture Collection (Manassas, VA). *E. coli* DH5α from Bethesda Research Laboratories (Gaithersburg, MD) was used for routine cloning procedures.[1] The protein overexpression host *E. coli* BL21 star (DE3) was obtained from Invitrogen. Vector pET28b(+) for protein overexpression was purchased from Novagen. Vector pYH7 was used for the gene inactivation experiments.[2] Methylation deficient *E. coli* ET12567/pUZ8002 was used for the intergeneric conjugation.[3] Plasmid pIB139 is a *Streptomyces-E. coli* shuttle vector that can site-specifically integrate into *Streptomyces* chromosomes.[4]

Instrumentation: DNA concentrations were measured using a NanoDrop ND-1000 UV−vis instrument from Thermo Fisher Scientific. High-performance liquid chromatography (HPLC) was performed using a Beckman System Gold 125 Solvent Module with a 166 detector equipped with a C18 reversed-phase column (Microsorb 100-5 C18 250×4.6 mm, Agilent Technologies (Santa Clara, CA)). LC-ESI-TOFMS analysis was performed using an Agilent Technologies HPLC system equipped with a pump (G1311C), an auto sampler (G1329B), and a ToF mass spectrometer (G6230B) with an electrospray ionization (ESI) source. LCMS separations were performed using Poroshell 120 EC-C18 column (2.7 μm, 4.6 × 100 mm) with Eclipse plus C18 guard column (1.8 μm, 2.1 × 5 mm) at a flow rate of 0.4 or 0.5 mL/min using 0.1% formic acid in H_2O (solvent A) and acetonitrile (solvent B). The obtained LCMS data were analyzed using MassHunter software (Agilent Technologies). NMR spectra were recorded using a Varian DirectDrive 600 MHz, a Bruker Avance III HD 500 MHz NMR equipped with CryoProbeTM Prodigy, or a Varian DirectDrive 400 MHz NMR spectrometer at the Nuclear Magnetic Resonance Facility at the University of Texas at Austin. Deuterated solvents were used as internal standards in the NMR spectra unless stated otherwise. Chemical shifts are reported as parts per million (ppm) relative to those of $CDCl_3$, 7.26 ppm for ^1H NMR and 77.16 ppm for ^{13}C NMR, respectively.

S2. *Streptomyces* genetic experiments

S2.1 Gene deletion experiments

The inactivation of *abmH* in *S.* sp. ATCC 700974 was performed by an in-frame deletion method.[5] A 2 kb fragment was amplified using primers, abmHLF and abmHLR. Another 2 kb fragment was amplified using primers, abmHRF and abmHRR. The two fragments were ligated into the *EcoRV* site of linearized pYH7 using Gibson Assembly® Master Mix (NEB) according to the manufacturer's protocol. To transfer the resulting plasmid into *S.* sp. ATCC 700974, conjugation using *E. coli* ET12567/pUZ8002 was carried out following the

standard procedure.[5] The colonies that were apramycin resistant were identified as conjugants. These mutants were cultured in the absence of apramycin, and the resulting apramycin-sensitive colonies were analyzed by PCR. Sequencing of the PCR product confirmed the in-frame deletion of *abmH*. The inactivation of *abmJ* in *S.* sp. ATCC 700974 was also performed based on the same procedure described for the inactivation of *abmH*.

abmHLF: 5′- GCCAGGTTCGGCGATGTCGTAGAACCAGGCGCAGTCGATG -3′
abmHLR: 5′- GAGCGAGAACCGATGACGACGACGGACCAGAACAC -3′
abmHRF: 5′- GTCGTCATCGGTTCTCGCTCGGCACCAGGTTCAGC -3′
abmHRR: 5′- ACGCCGGCTCGCGATACTGGGAAGGGAGCAGAAAGTTGTC -3′

abmJLF: 5′- GCCAGGTTCGGCGATCAGTTCGGTGCTGTCAGCCTCGGTC -3′
abmJLR: 5′- TGGCGGGAGGATCACCGACGAGGTCTCCAGGAGGT -3′
abmJRF: 5′- CGTCGGTGATCCTCCCGCCAGATGTCGCACATCCG -3′
abmJRR: 5′- ACGCCGGCTCGCGATCGTGCTGAGTCACCTGTCCGTCTGG -3′

Tryptic soy broth (TSB) medium (25 mL) was inoculated with the spores of the Δ*abmH* or Δ*abmJ* strain and incubated at 28 °C. After 24 h, 1.5 mL of the TSB seed culture was used to inoculate the albomycin-producing medium (25 mL). The medium was composed of 20 g starch, 5 g L-ornithine·HCl, 1.8 g KH_2PO_4, 8.14 g Na_2HPO_4, 2 g $(NH_4)_2SO_4$, 2 g NaCl, 2 g $MgSO_4{\cdot}7H_2O$, 0.8 g $CaC1_2{\cdot}2H_2O$, 0.28 g $FeSO_4{\cdot}7H_2O$, 0.02 g $ZnSO_4{\cdot}7H_2O$, and 1 L H_2O.[6] The culture was incubated at 28 °C for 96 h and centrifuged at 4,000 × g for 20 min. The cell-free broth was applied to a column packed with Amberlite XAD4 (Sigma-Aldrich Chemical) pre-equilibrated with water. The column was washed with water and eluted with 50% aqueous methanol. The eluted fraction was analyzed by LCMS using Poroshell 120 EC-C18 column (2.7 μm, 4.6 × 100 mm) at a flow rate of 0.5 mL/min using 0.1% formic acid in H_2O (solvent A) and acetonitrile (solvent B) with the following gradient program: 0–10 min 0–30% B, 10–12 min 30–100% B, 12–13 min 100–0% B, 13–18 min 0% B. The obtained data are shown in Figures 2B, S1, and S12. The production of **1** was completely abolished in the Δ*abmH* strain. A similar observation had been made in a previous report.[6b] No intermediates such as **8** were detected in the culture of the Δ*abmH* strain. The culture of the Δ*abmH* mutant strain was also treated with $NaBH_4$ or 2,4-dinitrophenylhydrazine (DNPH); however in LCMS analysis we observed no relevant compounds that could be derived from the proposed aldehyde intermediate.

S2.2 Gene complementation experiments

For complementation of *abmH*, a plasmid containing the *abmH* gene under the control of a constitutive promoter P*ermE** was prepared. The *abmH* gene fragment was amplified by PCR using primers, abmH-complementation-F1 and abmH-complementation-R1, and inserted into pIB139 containing P*ermE** after digestion with *NdeI* and *EcoRI* by the cloning method using seamless ligation cloning extract (SLiCE).[7] Another complementation plasmid containing the *abmH* gene under the control of a native promoter in *S.* sp. ATCC700974 (P*abmJ*) was also prepared. The *abmH* gene fragment was amplified by PCR using primers, abmH-complementation-F2 and abmH-complementation-R2. A fragment containing P*abmJ* (475 bp sequence upstream from the start codon of *abmJ*) was amplified using primers, PabmJ-abmH-F and PabmJ-abmH-R. The two fragments were inserted into pIB139 after digestion with *NisI* and *EcoRI* by the same cloning method. It should be noted that the digestion of pIB139 with *NisI* and *EcoRI* removed the promoter P*ermE** from the vector. The prepared plasmids were then introduced into the Δ*abmH* mutant strain by conjugation based on the standard procedure.[5] The resulting recombinant strain, which is apramycin-resistant, was cultured and the produced metabolites were analyzed as described in Section S2.1 (Figures S1). The complementation of *abmH* led to partial restoration of **1** production in the recombinant strain, which indicated that *abmH* is an essential gene for albomycin biosynthesis.

abmH-complementation-F1: 5′- GTTGGTAGGATCCACATATGGCCGCCCTCTTCGGCG -3′

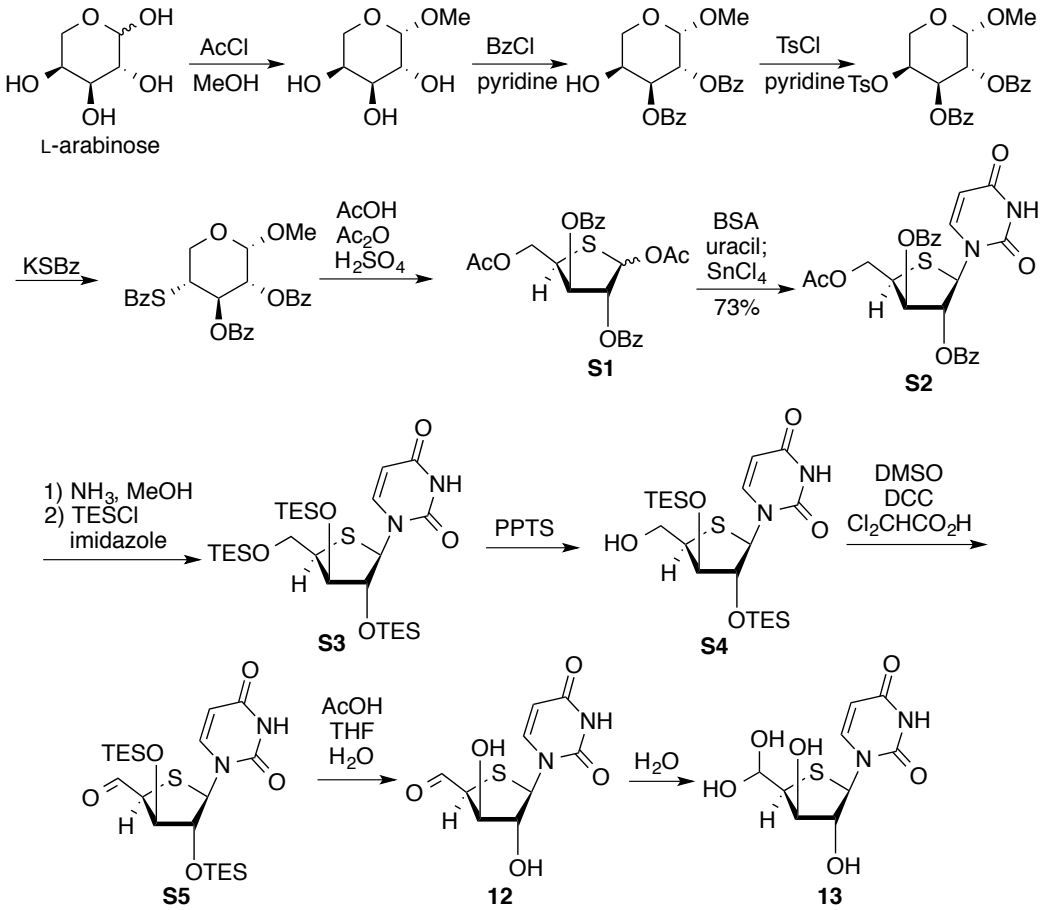
abmH-complementation-R1: 5′- TATGACATGATTACGAATTCTCATCGGTCCTCCCCGTACACGC -3′
abmH-complementation-F2: 5′- GTGGCCGCCCTCTTCGGCGC -3′
abmH-complementation-R2: 5′- TATGACATGATTACGAATTCTCATCGGTCCTCCCCGTACACGC -3′
PabmJ-abmH-F: 5′- AGGTCGACTCTAGTATGCATTCCGCCTCGACCTTGGTCAGTTGC -3′
PabmJ-abmH-R: 5′- GCGCCGAAGAGGGCGGCCACCCGGCACCGGGCGTTGCATC -3′

For complementation of *abmJ*, a plasmid containing the *abmJ* gene under the control of the native promoter P*abmJ* was prepared. The *abmJ* gene fragment with a 475 bp sequence upstream from the start codon of *abmJ* was amplified by PCR using primers, abmJ-complementation-F and abmJ-complementation-R, and inserted into pIB139 containing P*ermE** after digestion with *NisI* and *EcoRI* by the same cloning method. The prepared plasmid was then introduced into the Δ*abmJ* mutant strain by conjugation. The resulting recombinant strain, which is apramycin-resistant, was cultured and the produced metabolites were analyzed as described in Section S2.1 (Figure S13)

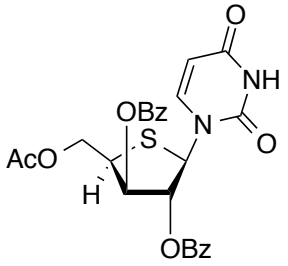
abmJ-complementation-F: 5′- AGGTCGACTCTAGTATGCATTCCGCCTCGACCTTGGTCAGTTGC -3′
abmJ-complementation-R: 5′- TATGACATGATTACGAATTCTCACCGGATCGCCTTGATGGCCAG -3′

S3. Chemical synthesis

S3.1. Synthesis of 1-(4′-deoxy-5′-hydroxy-4′-thio-D-xylofuranosyl)uracil (13)

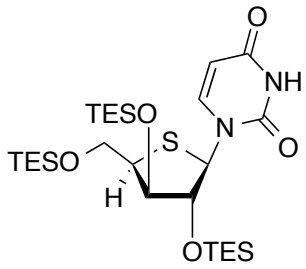


Scheme S1. Synthesis of **13**.



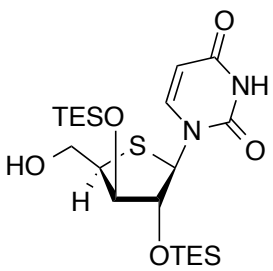
1-(5′-*O*-Acetyl-2′,3′-di-*O*-benzoyl-4′-thio-D-xylofuranosyl)uracil (S2)

To a solution of **S1** (prepared in a 1:1 mixture of α and β isomers as previously described,[8] 1.42 g, 3.1 mmol) and uracil (417 mg, 3.72 mmol) in acetonitrile (18 mL), was added bis(trimethylsilyl)acetamide (2 mL, 8.2 mmol) at room temperature. The mixture was warmed to 60 °C and stirred at 2 h before $SnCl_4$ (1.47 mL, 12.6 mmol) was added at 0 °C. The reaction mixture was warmed to 60 °C, stirred for 30 min, and then quenched with a mixture of saturated aqueous $NaHCO_3$ and ethyl acetate (100 mL/100 mL) at room temperature. The resulting mixture was filtered through Celite to remove white precipitates. The organic phase of the filtrate was separated, and the aqueous phase was extracted with ethyl acetate (200 × 2 mL). The combined organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified using flash chromatography on silica gel ($CHCl_3$ only, $CHCl_3$/MeOH = 100/1, 50/1, then 20/1) to yield **S2** (1.16 g, 73%). The obtained sample was contaminated with inseparable unknown impurities (ca. 30%). The compound was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.09 (1H, d, J = 8.2 Hz, H-6), 7.98–7.34 (10H, m, Bz), 6.46 (1H, d, J = 6.4 Hz, H-1′), 6.06 (1H, dd, J = 6.4 Hz, J = 8.3 Hz, H-2′), 5.89 (1H, d, J = 8.2 Hz, H-5), 5.89 (1H, dd, J = 6.8 Hz, J = 8.3 Hz, H-3′), 4.56 (1H, dd, J = 5.2 Hz, J = 11.9 Hz, H-5′), 4.37 (1H, dd, J = 5.8 Hz, J = 11.9 Hz, H-5′), 4.31 (1H, ddd, J = 5.2 Hz, J = 5.8 Hz, J = 6.8 Hz, H-4′), 1.99 (3H, s, Ac). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 170.1, 165.4, 165.2, 163.5, 150.9, 140.5, 134.0, 133.9, 130.0, 129.8, 128.7, 128.6, 128.4, 128.3, 103.7, 78.0, 74.5, 63.5, 59.6, 43.4, 20.7. ESI-HRMS calcd.for $C_{25}H_{23}N_2O_8S^+$ $[M+H]^+$ 511.1170, found 511.1178.



1-(2′,3′,5′-Tris-*O*-(triethylsilyl)-4′-thio-D-xylofuranosyl)uracil (S3)

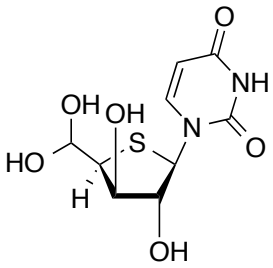
Compound **S2** (1.16 g, 2.28 mmol) was dissolved in methanolic ammonia (7 M, 40 mL) at room temperature. After 23 h, the solvent was evaporated under reduced pressure, and the resulting residue was dissolved in water. The aqueous solution (50 mL) was washed with $CHCl_3$ (50 × 3 mL) and ether (50 × 2 mL). The separated aqueous phase was concentrated. The obtained residue was dissolved in DMF (12 mL) and was added imidazole (1.25 g, 18.2 mmol) and chlorotriethylsilane (2.3 mL, 13.7 mmol). The mixture was stirred at room temperature for 26 h. The reaction mixture was poured into saturated aqueous $NaHCO_3$ and was extracted with ethyl acetate (100 × 3 mL). The combined organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified using flash chromatography on silica gel (hexanes/ethyl acetate = 10/1, 6/1, then 4/1) to yield **S3** (567 mg, 41%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.30 (1H, s, NH), 8.21 (1H, d, J = 8.2 Hz, H-6), 5.74 (1H, s, H-1′), 5.61 (1H, dd, J = 1.0 Hz, J = 8.2 Hz, H-5), 4.17 (1H, s, H-2′), 4.10 (1H, dd, J = 2.8 Hz, J = 3.9 Hz, H-3′), 3.93 (1H, dd, J = 6.2 Hz, J = 8.7 Hz, H-5′), 3.85 (1H, ddd, J = 3.9 Hz, J = 6.2 Hz, J = 6.2 Hz, H-4′), 3.81 (1H, dd, J = 6.2 Hz, J = 8.7 Hz, H-5′), 0.96–0.47 (45H, m, Si-Et). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 164.3, 151.2, 143.9, 100.0, 83.6, 78.0, 70.1, 61.1, 55.6, 6.7, 6.6, 6.6, 4.5, 4.5, 4.3. ESI-HRMS calcd. for $C_{27}H_{55}N_2O_5SSi_3^+$ $[M+H]^+$ 603.3134, found 603.3195.



1-(2′,3′-Bis-*O*-(triethylsilyl)-4′-thio-D-xylofuranosyl)uracil (S4)

To a solution of **S3** (221 mg, 0.367 mmol) in a mixture of MeOH/DCM (1 mL/10 mL), was added *p*-toluenesulfonic acid (46 mg, 0.183 mmol) at 0 °C. The mixture was stirred at 4 °C for 23 h before triethylamine (50 μL) was added at 0 °C. The mixture was concentrated under reduced pressure and purified using flash chromatography on silica gel (hexanes/ethyl acetate = 2/1) to yield **S4** (128 mg, 71%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.11 (1H, s, NH), 8.25 (1H, d, J = 8.2 Hz, H-6), 5.77 (1H, d, J = 1.6 Hz, H-1′), 5.65 (1H, dd, J = 8.2 Hz, H-5), 4.24 (1H, dd, J = 1.6 Hz, J = 2.4 Hz, H-2′), 4.18 (1H, dd, J = 2.4 Hz, J = 3.1 Hz, H-3′), 4.02–3.93 (3H, m, H-4′, H-5′, H-5′), 0.96 (9H, t, J = 8.0 Hz, Me), 0.91 (9H, t, J = 8.0 Hz, Me), 0.67 (6H, q, J = 8.0 Hz, Si-CH_2), 0.57 (6H, q, J = 8.0 Hz, Si-CH_2). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 163.5, 150.9, 143.7, 100.2, 83.5, 79.0, 70.3, 61.4, 55.0, 6.7, 6.7, 4.6, 4.5. ESI-HRMS calcd. for

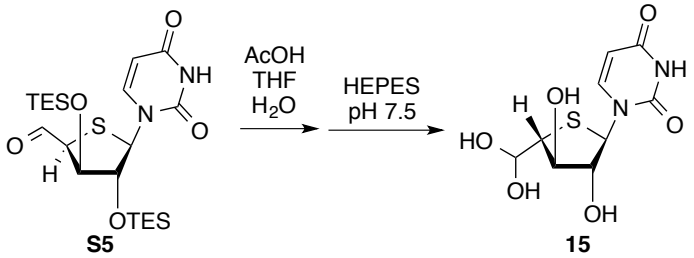
$C_{21}H_{41}N_2O_5SSi_2^+$ $[M+H]^+$ 489.2269, found 489.2291.



1-(5′-Hydroxy-4′-thio-D-xylofuranosyl)uracil (13)

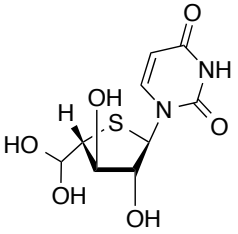
A solution of dichloroacetic acid (43 μL, 0.52 mmol) in dimethyl sulfoxide (DMSO, 0.5 mL) was added to a mixture of **S4** (509 mg, 1.04 mmol) and *N*,*N*'-dicyclohexylcarbodiimide (860 mg, 4.2 mmol) in DMSO (5.2 mL) at room temperature. After 27 h, the resulting white precipitates were removed by centrifugation and washed with ethyl acetate. The filtrate was concentrated and the obtained residue was purified using flash chromatography on silica gel ($CHCl_3$) several times to yield **S5** (300 mg, 59%). Despite multiple times of column purification, the isolated **S5** still contained a small amount of impurities derived from DCC. Compound **S5** was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.76 (1H, d, *J* = 2.7 Hz, H-5′), 8.20 (1H, d, *J* = 8.3 Hz, H-6), 5.84 (1H, d, *J* = 1.5 Hz, H-1′), 5.66 (1H, d, *J* = 8.3 Hz, H-5), 4.53 (1H, dd, *J* = 2.7 Hz, *J* = 4.4 Hz, H-4′), 4.36 (1H, dd, *J* = 2.3 Hz, *J* = 4.4 Hz, H-3′), 4.26 (1H, dd, *J* = 1.5 Hz, *J* = 2.3 Hz, H-2′), 0.95 (9H, t, *J* = 8.0 Hz, Me), 0.86 (9H, t, *J* = 8.0 Hz, Me), 0.67 (6H, q, *J* = 8.0 Hz, Si-CH_2), 0.57 (6H, q, *J* = 8.0 Hz, Si-CH_2). ESI-HRMS calcd. for $C_{21}H_{39}N_2O_5SSi_2^+$ $[M+H]^+$ 487.2113, found 487.2130. Compound **S5** (57.9 mg, 119 μmol) was dissolved in AcOH/THF/H_2O (1.5 mL/1.5 mL/0.5 mL) at room temperature and the solution was stirred at room temperature. After 10 h, the mixture was diluted with water (5 mL). The resulting aqueous solution was washed with $CHCl_3$ (0.5 mL × 3) and ethyl acetate (0.5 mL) before it was lyophilized to dryness. The crude products were separated by HPLC using a semipreparative C18 column (Agilent, ZORBAX, ODS, 5 μm, 9.4 mm x 250 mm). The HPLC column was eluted using 0.1% TFA in H_2O as mobile phase A and 0.1% TFA in acetonitrile as mobile phase B at a flow rate of 4 mL/min with the following gradient program: 0–8 min 0–8% B, 10–14 min 8–80% B, 14–16 min 50% B, 16–18 min 80–0% B, 18–22 min 0% B. Elution of the compounds was monitored by setting the UV-detector to 260 nm. Fractions containing **13** were collected and lyophilized (9.9 mg, 24%). ^{1}H NMR (D_2O, 400 MHz) δ 8.28 (1H, d, *J* = 8.2 Hz, H-6), 5.73 (1H, d, *J* = 8.2 Hz, H-5), 5.72 (1H, d, *J* = 4.8 Hz, H-1′), 5.29 (1H, d, *J* = 5.5 Hz, H-5′), 4.29 (1H, dd, *J* = 4.8 Hz, *J* = 4.8 Hz, H-2′), 4.18 (1H, dd, *J* = 4.8 Hz, *J* = 5.6 Hz, H-3′), 3.62 (1H, dd, *J* = 5.5 Hz, *J* = 5.6 Hz, H-4′). ^{13}C NMR (D_2O, 151 MHz) δ 166.2, 152.2, 144.3, 101.3, 88.8, 80.3, 75.1, 65.3, 55.2. ESI-HRMS calcd. for $C_9H_{13}N_2O_6S^+$ $[M+H]^+$ 277.0789, found 277.0791.

S3.2. Synthesis 1-(4′-deoxy-5′-hydroxy-4′-thio-L-arabinofuranosyl)uracil (15)



Scheme S2. Synthesis of **15**.

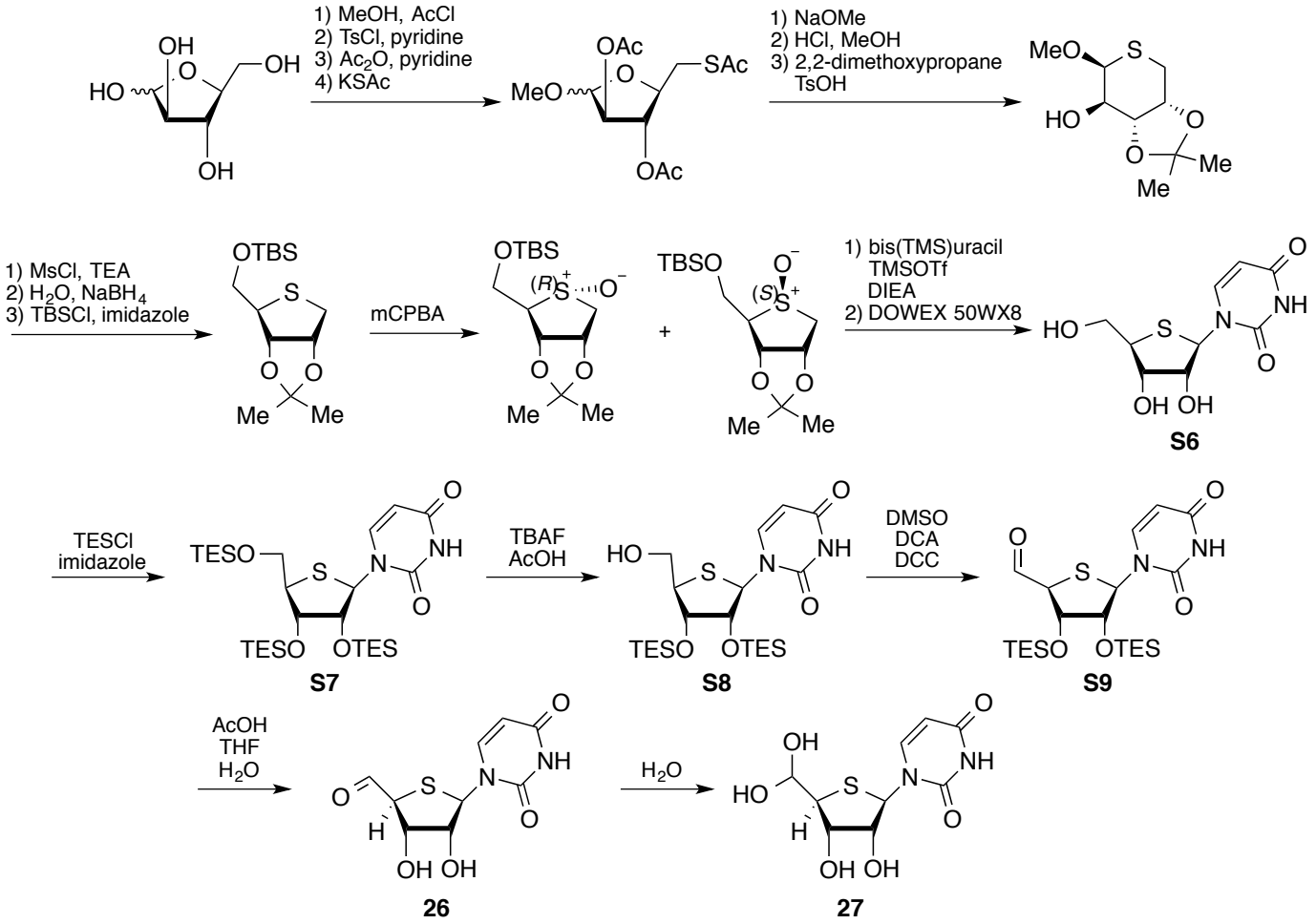
1-(5′-Hydroxy-4′-thio-L-arabinofuranosyl)uracil (15)



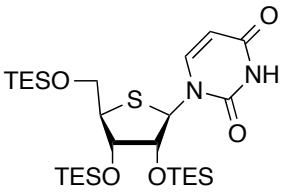
Compound **S5** (50 mg, 0.10 mmol) was dissolved in AcOH/THF/H_2O (0.9 mL/0.9 mL/0.3 mL) at room temperature and the solution was stirred at room temperature. After 7 h, the mixture was concentrated under reduced pressure and diluted with water (5 mL). The resulting aqueous solution was washed with $CHCl_3$ (5 mL × 3) before it was lyophilized to dryness. The residue was redissolved in HEPES buffer (50 mM, pH 7.5) and incubated at room temperature overnight. The mixture was lyophilized and separated by HPLC using the same procedure described for **13**. Fractions containing **15** were collected and

lyophilized (3.1 mg, 12%). ^{1}H NMR (D_2O, 400 MHz) δ 7.94 (1H, d, *J* = 8.1 Hz, H-6), 5.79 (1H, d, *J* = 8.1 Hz, H-5), 5.77 (1H, d, *J* = 8.0 Hz, H-1′), 5.09 (1H, d, *J* = 5.0 Hz, H-5′), 4.03 (1H, dd, *J* = 8.0 Hz, *J* = 8.0 Hz, H-2′), 3.90 (1H, dd, *J* = 8.0 Hz, *J* = 8.0 Hz, H-3′), 3.51 (1H, dd, *J* = 5.0 Hz, *J* = 8.0 Hz, H-4′). ^{13}C NMR (D_2O, 101 MHz) δ 166.0, 152.2, 143.0, 102.7, 90.0, 79.7, 74.8, 60.9, 53.5. ESI-HRMS calcd. for $C_9H_{11}N_2O_6S^-$ $[M–H]^-$ 275.0343, found 275.0340.

S3.3. Synthesis of 4′-deoxy-5′-hydroxy-4′-thiouridine (27)



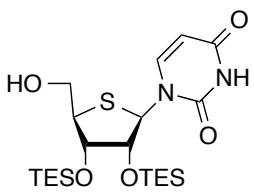
Scheme S3. Synthesis of **27**.



2′,3′,5′-Tris-*O*-(triethylsilyl)-4′-deoxy-4′-thiouridine (S7)

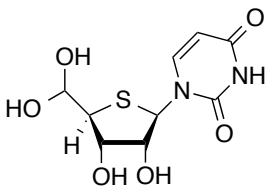
Compound **S6** (prepared as described in the literature,[9] 63 mg, 0.24 mmol) was dissolved in DMF (2 mL) and was added imidazole (100 mg, 1.45 mmol) and chlorotriethylsilane (0.203 mL, 1.21 mmol). The mixture was stirred at room temperature for 11 h. The reaction mixture was diluted with water (20 mL) and extracted with a 1 : 1 mixture of hexanes and ethyl acetate (30 × 3 mL). The combined organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified using flash chromatography on silica gel (hexanes/ethyl acetate = 10/1 then 5/1) to yield **S7** (136 mg, 93%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.00 (1H, br, NH), 8.30 (1H, d, *J* = 8.2 Hz, H-6), 5.79 (1H, d, *J* = 4.0 Hz, H-1′), 5.71 (1H, dd, *J* = 1.8 Hz, *J* = 8.2 Hz, H-5), 4.12 (1H, dd, *J* = 3.2 Hz, *J* = 4.0 Hz, H-2′), 4.08 (1H, dd, *J* = 3.2 Hz, *J* = 5.8 Hz, H-3′), 3.87 (1H, dd, *J* = 2.8 Hz, *J* = 11.1 Hz, H-5′), 3.72 (1H, dd, *J* = 3.8 Hz, *J* = 11.1 Hz, H-5′), 3.39 (1H, ddd, *J* = 2.8 Hz, *J* = 3.8 Hz, *J* = 5.8 Hz, H-4′), 1.00–0.55 (45H, m, Si-Et). ^{13}C NMR ($CDCl_3$,

101 MHz) δ 163.2, 150.6, 142.0, 101.6, 79.3, 73.8, 65.1, 61.3, 52.0, 6.8, 6.7, 6.7, 4.8, 4.7, 4.2. ESI-HRMS calcd. for $C_{27}H_{55}N_2O_5SSi_3^+$ $[M+H]^+$ 603.3134, found 603.3137.



2′,3′-Bis-*O*-(triethylsilyl)-4′-deoxy-4′-thiouridine (S8)

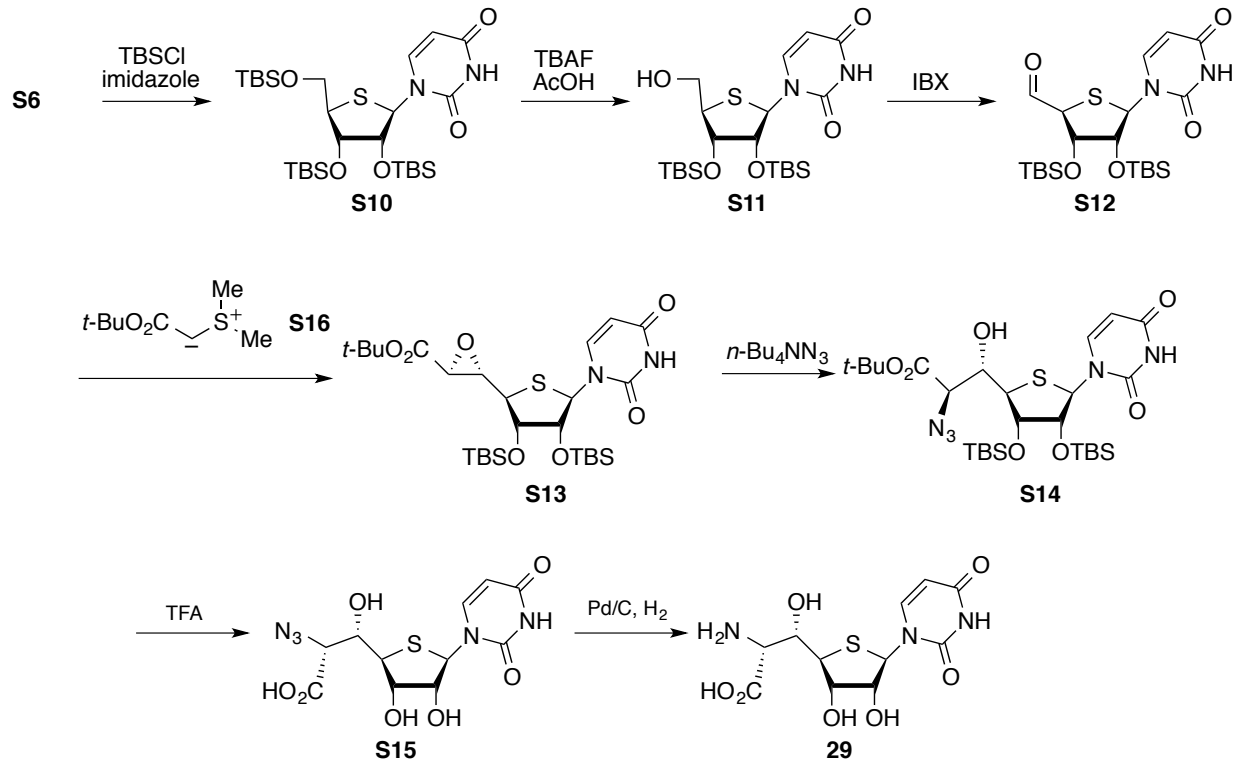
Acetic acid (0.081 mL, 1.41 mmol) and tetrabutylammonium fluoride (1 M solution in THF, 0.941 mL, 0.941 mmol) were added to a solution of **S7** (567 mg, 0.941 mmol) in THF (30 mL) at 0 °C. After 30 min, the mixture was diluted with water (100 mL) and saturated $NaHCO_3$ solution (10 mL), and extracted with DCM (100 × 3 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified using flash chromatography on silica gel (hexanes/ethyl acetate = 2/1) to yield **S8** (361 mg, 78%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.61 (1H, br, NH), 9.99 (1H, d, *J* = 8.0 Hz, H-6), 5.73 (1H, dd, *J* = 8.0 Hz, H-5), 5.72 (1H, d, *J* = 5.4 Hz, H-1′), 4.35 (1H, dd, *J* = 3.9 Hz, *J* = 5.4 Hz, H-2′), 4.07 (1H, dd, *J* = 3.9 Hz, *J* = 4.5 Hz, H-3′), 3.88 (1H, dd, *J* = 3.0 Hz, *J* = 11.5 Hz, H-5′), 3.81 (1H, dd, *J* = 3.3 Hz, *J* = 11.5 Hz, H-5′), 3.41 (1H, ddd, *J* = 3.0 Hz, *J* = 3.3 Hz, *J* = 4.5 Hz, H-4′), 1.00–0.55 (30H, m, Si-Et). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 162.8, 150.3, 142.5, 102.1, 78.4, 74.9, 66.8, 61.9, 52.8, 6.8, 6.7, 4.8, 4.7. ESI-HRMS calcd. for $C_{21}H_{41}N_2O_5SSi_2^+$ $[M+H]^+$ 489.2269, found 489.2225.



4′-Deoxy-5′-hydroxy-4′-thiouridine (28)

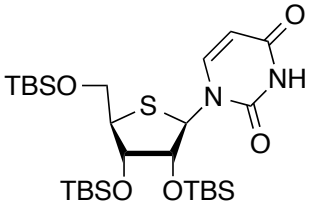
Compound **S9** was synthesized based on the same procedure described for **S5** in 91% yield with a small amount of impurities derived from DCC. Compound **S9** was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.66 (1H, s H-5′), 7.51 (1H, d, *J* = 8.2 Hz, H-6), 6.03 (1H, d, *J* = 6.6 Hz, H-1′), 5.76 (1H, d, *J* = 8.2 Hz, H-5), 4.37 (1H, m, H-3′), 4.17 (1H, dd, *J* = 2.7 Hz, *J* = 6.6 Hz, H-2′), 3.81 (1H, m, H-4′), 1.00–0.55 (30H, m, Si-Et). Compound **28** was synthesized from **S9** based on the same procedure described for **13** in 27% yield. Also produced was the 4′ epimer (20%), which was difficult to separate from **28** by HPLC. The spectral data of **28**: ^{1}H NMR (D_2O, 400 MHz) δ 8.12 (1H, d, *J* = 8.1 Hz, H-6), 5.83 (1H, d, *J* = 5.9 Hz, H-1′), 5.77 (1H, d, *J* = 8.1 Hz, H-5), 5.18 (1H, d, *J* = 4.3 Hz, H-5′), 4.27 (1H, dd, *J* = 4.2 Hz, *J* = 5.9 Hz, H-2′), 4.11 (1H, dd, *J* = 4.1 Hz, *J* = 4.2 Hz, H-3′), 3.35 (1H, dd, *J* = 4.1 Hz, *J* = 4.3 Hz, H-4′). ^{13}C NMR (D_2O, 101 MHz) δ 166.2, 152.2, 144.3, 101.3, 88.8, 80.3, 75.1, 65.3, 55.2. ESI-HRMS calcd. for $C_9H_{11}N_2O_6S^-$ $[M-H]^-$ 275.0343, found 275.0334. The spectral data of the 4′ epimer of **28**: ^{1}H NMR (D_2O, 400 MHz) δ 7.91 (1H, d, *J* = 8.1 Hz, H-6), 5.97 (1H, d, *J* = 8.2 Hz, H-1′), 5.81 (1H, d, *J* = 8.1 Hz, H-5), 5.00 (1H, d, *J* = 8.4 Hz, H-5′), 4.25 (1H, m, H-2′), 4.25 (1H, m, H-3′), 3.35 (1H, dd, *J* = 2.8 Hz, *J* = 8.4 Hz, H-4′). ^{13}C NMR (D_2O, 101 MHz) δ 165.9, 152.3, 142.8, 103.0, 90.2, 78.7, 72.5, 63.5, 52.8.

S3.4. Synthesis of 29



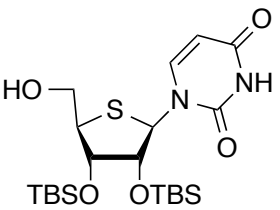
Scheme S4. Synthesis of **29**.

2′,3′,5′-Tris-*O*-(*tert*-butyldimethylsilyl)-4′-deoxy-4′-thiouridine (S10)



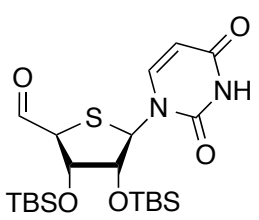
Compound **S6** (prepared as described in the literature,[9] 694 mg, 2.66 mmol) was dissolved in DMF (20 mL) and was added imidazole (1.10 g, 16.0 mmol) and *tert*-butyldimethylsilyl chloride (2.00, 13.3 mmol). The mixture was stirred at room temperature for 11 h. The reaction mixture was diluted with water (200 mL) and extracted with a 1 : 1 mixture of hexanes and ethyl acetate (100 × 3 mL). The combined organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified using flash chromatography on silica gel (hexanes/ethyl acetate = 5/1) to yield **S10** (2.0 g, 80%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.05 (1H, br, NH), 8.28 (1H, d, *J* = 8.2 Hz, H-6), 5.79 (1H, d, *J* = 3.8 Hz, H-1′), 5.72 (1H, dd, *J* = 2.0 Hz, *J* = 8.2 Hz, H-5), 4.09 (1H, dd, *J* = 3.3 Hz, *J* = 3.8 Hz, H-2′), 4.06 (1H, dd, *J* = 3.3 Hz, *J* = 6.2 Hz, H-3′), 3.90 (1H, dd, *J* = 2.9 Hz, *J* = 11.2 Hz, H-5′), 3.78 (1H, dd, *J* = 4.2 Hz, *J* = 11.2 Hz, H-5′), 3.44 (1H, ddd, *J* = 2.9 Hz, *J* = 4.2 Hz, *J* = 6.2 Hz, H-4′), 0.94 (9H, s, Si-*t*-Bu), 0.89 (9H, s, Si-*t*-Bu), 0.89 (9H, s, Si-*t*-Bu), 0.14 (3H, s, Si-Me), 0.13 (3H, s, Si-Me), 0.11 (3H, s, Si-Me), 0.07 (3H, s, Si-Me), 0.06 (3H, s, Si-Me), 0.04 (3H, s, Si-Me). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 164.0, 150.9, 142.1, 101.7, 78.9, 73.1, 65.3, 61.4, 51.7, 26.0, 25.8, 25.7, 18.6, 18.0, 17.9, –4.2, –4.6, –4.8, –4.9, –5.3, –5.5. ESI-HRMS calcd. for $C_{27}H_{55}N_2O_5SSi_3^+$ $[M+H]^+$ 603.3134, found 603.3111.

2′,3′-Bis-*O*-(*tert*-butyldimethylsilyl)-4′-deoxy-4′-thiouridine (S11)



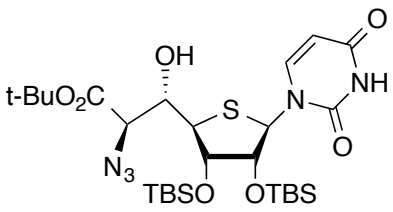
Acetic acid (0.024 mL, 0.41 mmol) and tetrabutylammonium fluoride (1 M solution in THF, 0.273 mL, 0.273 mmol) were added to a solution of **S10** (165 mg, 0.273 mmol) in THF (30 mL) at room temperature. After 1 h, the mixture was diluted with water (30 mL) and saturated $NaHCO_3$ solution (5 mL), and extracted with DCM (30 × 3 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified using flash chromatography

on silica gel (hexanes/ethyl acetate = 3/1) to yield **S11** (361 mg, 78%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.16 (1H, d, *J* = 8.2 Hz, H-6), 5.74 (1H, dd, *J* = 8.2 Hz, H-5), 5.72 (1H, d, *J* = 4.6 Hz, H-1′), 4.30 (1H, m, H-2′), 4.06 (1H, dd, *J* = 3.0 Hz, *J* = 5.3 Hz, H-3′), 3.89 (1H, dd, *J* = 3.4 Hz, *J* = 11.6 Hz, H-5′), 3.83 (1H, dd, *J* = 3.5 Hz, *J* = 11.6 Hz, H-5′), 3.43 (1H, ddd, *J* = 3.0 Hz, *J* = 3.4 Hz, *J* = 3.5 Hz, H-4′), 0.89 (9H, s, Si-*t*-Bu), 0.87 (9H, s, Si-*t*-Bu), 0.08 (3H, s, Si-Me), 0.07 (3H, s, Si-Me), 0.05 (3H, s, Si-Me), 0.04 (3H, s, Si-Me). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 163.9, 150.8, 142.8, 101.9, 78.6, 74.4, 66.5, 61.3, 52.6, 25.8, 25.7, 18.0, 17.9, 4.4, –4.7, –4.7, –4.9. ESI-HRMS calcd. for $C_{21}H_{41}N_2O_5SSi_2^+$ $[M+H]^+$ 489.2269, found 489.2307.



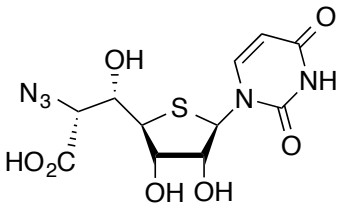
Compound S12

To a solution of **S11** (50 mg, 0.102 mmol) in acetonitrile (2.0 mL) was added 2-iodoxybenzoic acid (prepared according to the literature,[10] 75%, 76 mg, 0.204 mmol) at room temperature. The mixture was stirred at 55 °C. After 5 h, the white precipitates were removed by filtration through Celite and washed with ethyl acetate. The filtrate was concentrated to yield **S12** (purity >95% based on ^{1}H NMR, 48 mg, 97%). Since compound **S12** was not stable, the structure was characterized only by ^{1}H NMR and it was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.72 (1H, d, *J* = 1.6 Hz, H-5′), 8.62 (1H, br, NH), 7.58 (1H, d, *J* = 8.2 Hz, H-6), 6.07 (1H, d, *J* = 6.5 Hz, H-1′), 5.83 (1H, dd, *J* = 2.3 Hz, *J* = 8.2 Hz, H-5), 4.40 (1H, dd, *J* = 3.1 Hz, *J* = 3.5 Hz, H-3′), 4.21 (1H, dd, *J* = 3.1 Hz, *J* = 6.5 Hz, H-2′), 3.87 (1H, dd, *J* = 1.6 Hz, *J* = 3.5 Hz, H-4′), 0.92 (9H, s, Si-*t*-Bu), 0.87 (9H, s, Si-*t*-Bu), 0.11 (3H, s, Si-Me), 0.09 (3H, s, Si-Me), 0.06 (3H, s, Si-Me), 0.01 (3H, s, Si-Me).



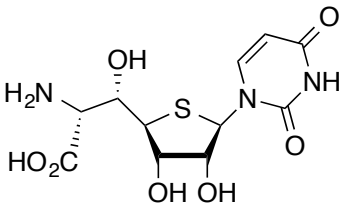
Compound S14

Compound **S12** (48 mg, 0.099 mmol) was dissolved in DCM (1.0 mL) and added another solution of sulfonium ylide **S16** (prepared as described in the literature,[11] 0.31 mmol) in DCM (0.25 mL) at 0 °C. After 2 h, the reaction mixture was diluted with water (10 mL) and extracted with DCM (10 × 3 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was separated using flash chromatography on silica gel (hexanes/ethyl acetate = 10/1, 5/1, then 3/1) to yield a residue containing **S13** (32.5 mg). ESI-HRMS calcd. for $C_{27}H_{47}N_2O_7SSi_2^-$ 599.2648, found 599.2681. The obtained residue contained unknown impurities (approximately 50%). The compound **S13** was used in the next step without further purification. To a solution of **S13** (32 mg) in DMF (3 mL), Amberlyst 15 (hydrogen form, 15 mg) and tetrabutylammonium azide (50 mg, 0.18 mmol) were added at room temperature. After 2 h, the reaction mixture was filtered and the obtained filtrate was diluted with a mixture of ethyl acetate and hexanes (1:1, 10 mL). The organic phase was washed with water (20 mL) and dried over Na_2SO_4, filtered, and concentrated. The crude product was purified using flash chromatography on silica gel (hexanes/ethyl acetate = 10/1, 5/1, 3/1, then 2/1) to yield **S14** (6.1 mg, 10% in 2 steps). The stereoconfiguration at C5′/C6′ was assigned to be (5′*S*,6′*R*) based on that of the analogous compound **S24** (see Section S10 and Figure S27 for the X-ray crystal structure analysis). It has been reported that the formation of **S23** from **S22** is highly diastereoselective.[12] Likewise, we only observed isomer **S14** after the reaction of sulfonium ylide **S16** with **S12** and the ring opening of the resulting epoxide **S13**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.18 (1H, br, NH), 8.10 (1H, d, *J* = 8.2 Hz, H-6), 5.80 (1H, d, *J* = 5.2 Hz, H-1′), 5.75 (1H, dd, *J* = 8.2 Hz, H-5), 4.38 (1H, m, H-2′), 4.08 (1H, dd, *J* = 1.8 Hz, *J* = 8.5 Hz, H-5′), 4.06 (1H, dd, *J* = 3.2 Hz, *J* = 4.7 Hz, H-3′), 3.90 (1H, d, *J* = 8.5 Hz, H-6′), 3.57 (1H, dd, *J* = 1.8 Hz, *J* = 4.7 Hz, H-4′), 0.92 (9H, s, Si-*t*-Bu), 0.88 (9H, s, Si-*t*-Bu), 0.09 (3H, s, Si-Me), 0.07 (3H, s, Si-Me), 0.05 (3H, s, Si-Me), 0.05 (3H, s, Si-Me). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 171.2, 168.8, 162.6, 150.3, 102.2, 78.4, 75.9, 69.1, 65.7, 64.7, 52.8, 28.0, 25.8, 25.7, 18.0, 17.9, –4.4, –4.6, –4.8, –4.8. ESI-HRMS calcd. for $C_{27}H_{48}N_5O_7SSi_2^+$ 642.2818, found 644.2756.



Compound S15

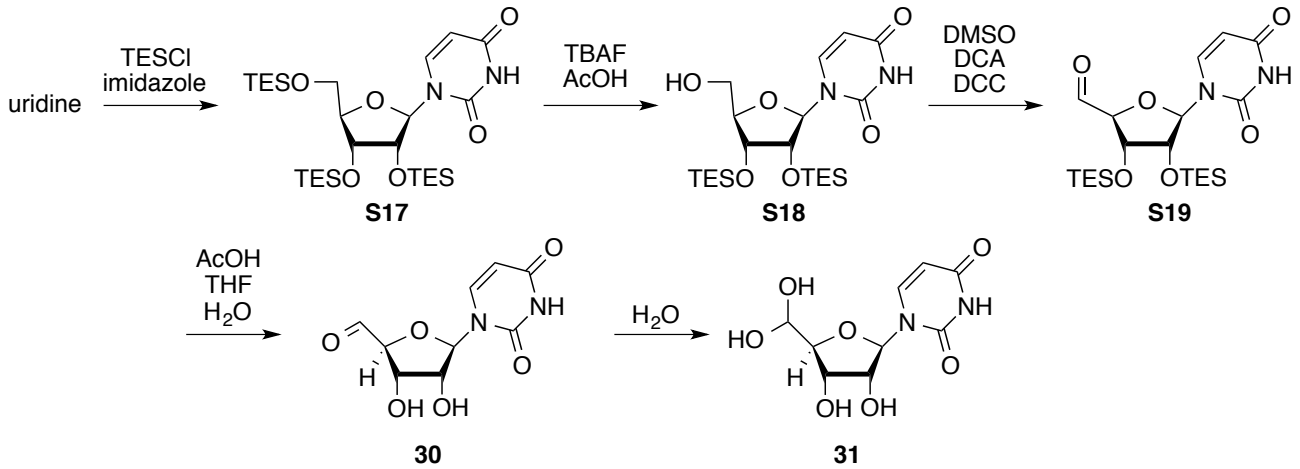
Compound **S14** (6.1 mg, 9.5 μmol) was dissolved in 80% aqueous TFA, and the solution was stirred at room temperature. After 1 h, the mixture was concentrated, and the obtained residue was partitioned between water (3 mL) and ether (3 mL). The separated aqueous phase was concentrated to afford **S15** (2.9 mg, 84%). ^{1}H NMR (D_2O, 400 MHz) δ 8.26 (1H, d, *J* = 8.2 Hz, H-6), 5.75 (1H, dd, *J* = 8.2 Hz, H-5), 5.70 (1H, d, *J* = 3.8 Hz, H-1′), 4.30 (1H, dd, *J* = 2.5 Hz, *J* = 5.5 Hz, H-5′), 4.25 (1H, d, *J* = 5.5 Hz, H-6′), 4.18 (1H, dd, *J* = 3.7 Hz, *J* = 3.8 Hz, H-2′), 4.01 (1H, dd, *J* = 3.7 Hz, *J* = 6.7 Hz, H-3′), 3.39 (1H, ddd, *J* = 2.5 Hz, *J* = 6.7 Hz, *J* = 3.5 Hz, H-4′). ^{13}C NMR (D_2O, 126 MHz) δ 172.0, 166.2, 152.1, 143.3, 101.7, 84.9, 77.0, 74.0, 69.7, 66.5, 65.1, 51.9. ESI-HRMS calcd. for $C_{11}H_{12}N_5O_7S^-$ $[M–H]^-$ 358.0463, found 358.0436.



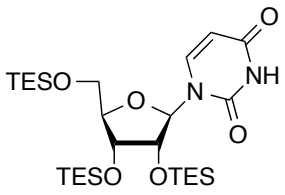
Compound 29

Compound **S15** (2.9 mg) was dissolved in methanol (2.0 mL) and added Pd/C (10%, 10 mg). The mixture was stirred under hydrogen atmosphere at room temperature. After 1 h, the reaction mixture was filtered through Celite and the resulting filtrate was concentrated. The crude product was purified by HPLC using a semipreparative anion-exchange column (Thermo Scientific, Dionex CarboPacPA1, 9.0 mm × 250 mm). The HPLC column was eluted using 0.06% NH_3 in H_2O as mobile phase A and 1 M ammonium acetate in H_2O as mobile phase B at a flow rate of 3 mL/min with the following gradient program: 0–1 min 0% B, 1–3 min 0–15% B, 3–18 min 15–30% B, 18–19 min 30–80% B, 19–23 min 80% B, 23–24 min 80–0% B, 24–30 min 0% B. Elution of the compound was monitored by setting the UV-detector to 260 nm. Fraction containing **29** (0.14 mg) was collected and dried under reduced pressure. ^{1}H NMR (D_2O, 400 MHz) δ 8.20 (1H, d, *J* = 8.1 Hz, H-6), 5.75 (1H, dd, *J* = 8.1 Hz, H-5), 5.74 (1H, d, *J* = 4.4 Hz, H-1′), 4.26 (1H, dd, *J* = 4.3 Hz, *J* = 4.9 Hz, H-5′), 4.26 (1H, dd, *J* = 3.8 Hz, *J* = 4.4 Hz, H-2′), 4.03 (1H, dd, *J* = 3.8 Hz, *J* = 5.4 Hz, H-3′), 3.80 (1H, d, *J* = 4.3 Hz, H-6′), 3.54 (1H, dd, *J* = 4.9 Hz, *J* = 5.4 Hz, H-4′). ^{13}C NMR (D_2O, 126 MHz) δ 171.0, 166.1, 152.1, 143.2, 102.0, 77.2, 74.7, 69.4, 64.8, 57.9, 52.9. ESI-HRMS calcd. for $C_{11}H_{14}N_3O_7S^-$ $[M–H]^-$ 332.0558, found 332.0530.

S3.5. Synthesis of 5′-hydroxyuridine (31)

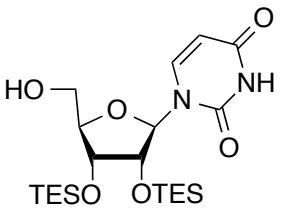


Scheme S5. Synthesis of **31**.



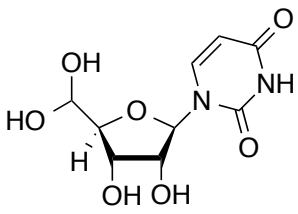
2′,3′,5′-Tris-*O*-(triethylsilyl)uridine (S17)

Compound **S17** was synthesized using uridine based on the same procedure as described for **S7** in 99% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.11 (1H, br, NH), 8.07 (1H, d, *J* = 8.2 Hz, H-6), 5.84 (1H, d, *J* = 3.0 Hz, H-1′), 5.66 (1H, dd, *J* = 8.2 Hz, H-5), 4.12–4.07 (2H, m, H-2′, H-3′), 4.04 (1H, m, H-4′), 3.95 (1H, dd, *J* = 2.2 Hz, *J* = 11.6 Hz, H-5′), 3.73 (1H, dd, *J* = 1.3 Hz, *J* = 11.6 Hz, H-5′), 1.00–0.47 (45H, m, Si-Et). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 164.2, 150.5, 140.3, 101.4, 89.1, 83.8, 76.3, 70.3, 60.8, 6.7, 6.6, 6.6, 4.8, 4.7, 4.1. ESI-HRMS calcd. for $C_{27}H_{55}N_2O_6Si_3^+$ $[M+H]^+$ 587.3362, found 587.3391.



2′,3′-Bis-*O*-(triethylsilyl)uridine (S16)

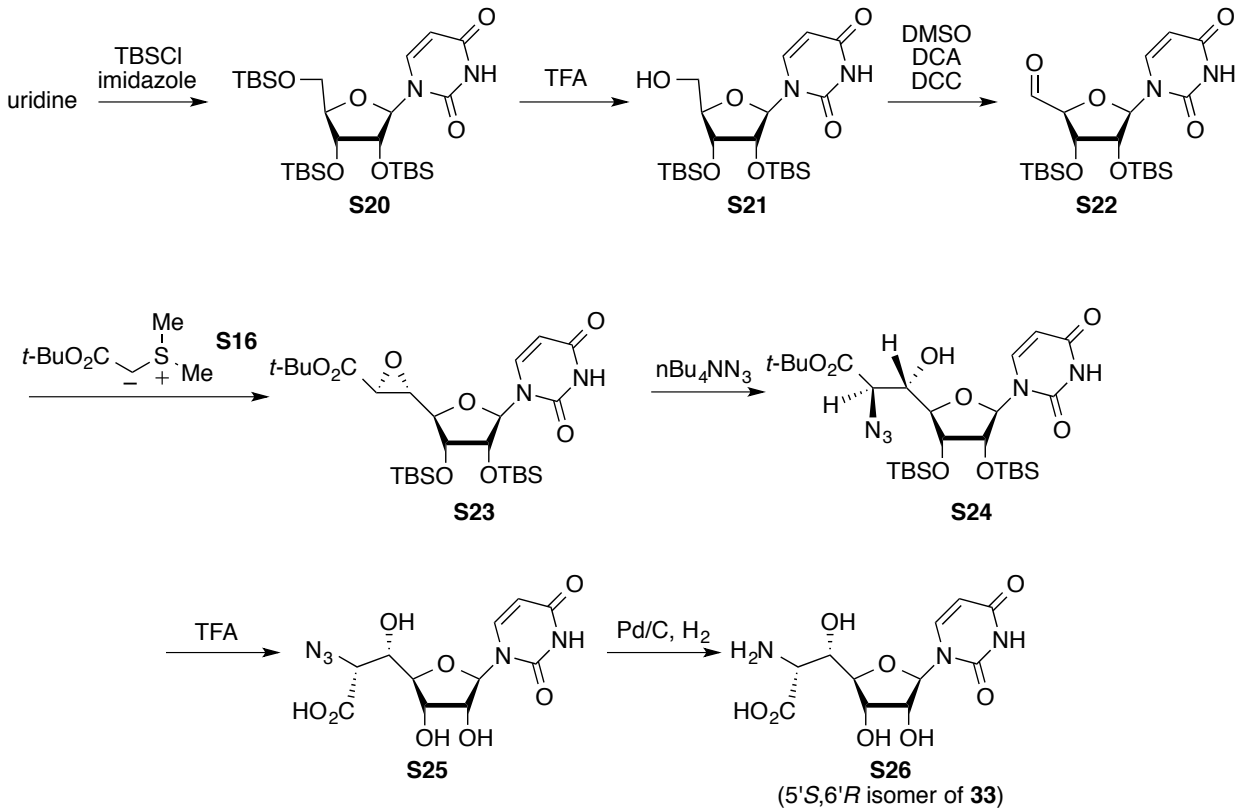
Compound **S16** was synthesized using uridine based on the same procedure as described for **S8** in 48% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.65 (1H, d, *J* = 8.1 Hz, H-6), 5.72 (1H, d, *J* = 8.1 Hz, H-5), 5.51 (1H, dd, *J* = 5.3 Hz, H-1′), 4.54 (1H, dd, *J* = 4.9 Hz, *J* = 5.3 Hz, H-2′), 4.16 (1H, dd, *J* = 3.9 Hz, *J* = 4.9 Hz, H-3′), 4.06 (1H, ddd, *J* = 2.2 Hz, *J* = 2.2 Hz, *J* = 3.9 Hz, H-4′), 3.91 (1H, dd, *J* = 2.2 Hz, *J* = 12.3 Hz, H-5′), 3.71 (1H, dd, *J* = 2.2 Hz, *J* = 12.3 Hz, H-5′), 1.00–0.47 (30H, m, Si-Et). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 163.6, 150.4, 143.0, 102.1, 93.4, 85.9, 73.8, 71.8, 61.6, 6.8, 6.7, 4.8, 4.6. ESI-HRMS calcd. for $C_{21}H_{41}N_2O_6Si_2^+$ $[M+H]^+$ 473.2498, found 473.2590.



5′-hydroxyuridine (31)

Compound **31** was synthesized from **S16** based on the same procedure as described for **13** in 92% yield. ^{1}H NMR (D_2O, 400 MHz) δ 7.77 (1H, d, *J* = 8.1 Hz, H-6), 5.84 (1H, d, *J* = 6.1 Hz, H-1′), 5.77 (1H, d, *J* = 8.1 Hz, H-5), 5.06 (1H, d, *J* = 3.9 Hz, H-5′), 4.26 (1H, dd, *J* = 5.7 Hz, *J* = 6.1 Hz, H-2′), 4.18 (1H, dd, *J* = 3.4 Hz, *J* = 5.7 Hz, H-3′), 3.89 (1H, dd, *J* = 3.4 Hz, *J* = 3.9 Hz, H-4′). ^{13}C NMR (D_2O, 101 MHz) δ 166.1, 151.8, 141.9, 102.5, 88.6, 88.4, 86.2, 73.3, 69.6. ESI-HRMS calcd. for $C_9H_{11}N_2O_7^-$ $[M–H]^-$ 259.0572, found 259.0580.

S3.6. Synthesis of S26



Scheme S6. Synthesis of **S26**.

Compound **S26** was synthesized according to the reported procedure[12] with some modification (Scheme S6). The absolute configuration of **S24** was confirmed by X-ray crystal structure analysis (See Section S10 and Figure S27).

S4. Cloning of *abmH* and *abmD*

The *abmH* gene was PCR-amplified from the genomic DNA using primers carrying engineered *NdeI* and *HindIII* restriction sites. The sequences of the primers are shown bellow. The PCR-amplified gene fragments were purified using Gel Extraction Kit from Qiagen, digested with *NdeI* and *HindIII*, and ligated into pET28b(+) vector, which had also been digested with the same restriction enzymes. The resulting plasmid *abmH*/pET28b(+) was sequenced using the T7 or T7 terminal universal primers. The *abmD* gene was also amplified and cloned into pET28b(+) based on the same procedure described for *abmH*.

abmH-F: 5′- TCTATAACATATGGCCGCCCTCTTCGGCG -3′
abmH-R: 5′- TTACAAGCTTCATCGGTCCTC CCCGTACAC -3′
abmD-F: 5′- TCTATAACATATGACGGTCCTTCCCCTCGGCG -3′
abmD-R: 5′- TTACAAGCTTCAGGAACCGGTGGCAGG -3′

S5. Expression and purification of AbmH and AbmD

The plasmid *abmH*/pET28b(+) was used to transform the *E. coli* BL21 star (DE3) strain for protein expression. An overnight culture of *E. coli* BL21 star (DE3)-*abmH*/pET28b(+) grown in the LB medium (10 mL) containing 50 μg/mL of kanamycin at 37 °C, was used to inoculate 1 L of the same growth medium. The culture was incubated at 37 °C with shaking (220 rpm) until the OD600 reached ~0.6. Protein expression was then induced by the addition of isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.1 mM, and the cells were allowed to grow at 18 °C and 120 rpm for an additional 20 h. The cells were harvested by centrifugation at 4000 × *g* for 10 min and stored at –80 °C until lysis.

All purification steps were carried out at 4 °C. The thawed cells were resuspended in 50 mM HEPES buffer (pH 7.5) buffer containing 10% (v/v) glycerol, 10 mM imidazole, and NaCl (300 mM). After incubation with lysozyme (1 mg/mL) for 30 min, the cells were disrupted by sonication using 10 × 10 s pulses with a 30 s cooling pause between each pulse. The resulting lysate was centrifuged at 20,000 × *g* for 20 min, and the supernatant was subjected to Ni-nitrilotriacetic acid (NTA) resin. Bound protein was eluted using 50 mM HEPES buffer (pH 7.5) buffer containing 10% (v/v) glycerol, 250 mM imidazole, and NaCl (300 mM). The collected protein solution was dialyzed against 3 × 1 L of 50 mM HEPES buffer (pH 7.5) containing 300 mM NaCl and 10% glycerol. The protein solution was then flash-frozen in liquid nitrogen and stored at –80 °C until use. *N*-His_6-AbmD was also expressed and purified based on the same procedure described for AbmH. Protein concentration was estimated by NanoDrop. The yield of *N*-His_6-AbmH was approximately 13 mg from 1 L culture. The yield of *N*-His_6-AbmD was approximately 6 mg from 1 L culture. The molecular mass and purity of the proteins were determined by SDS-PAGE analysis (Figure S2). The proteins were also analyzed by UV (Figure S3).

S6. Detection of PLP from AbmH

The presence of PLP was revealed by UV and confirmed by HPLC analysis upon denaturation of AbmH (see Section S6.2). The purified AbmH exhibits an absorption band at 524 nm (See Section S6.1), which is indicative of a PLP-quinonoid species[13] although the chemical structure could not be determined based on the UV analysis. This species exists without exogenous addition of L-threonine and appears to be stable, because the band at 524 nm remains discernible throughout the purification procedure.

S6.1 UV analysis

Purified AbmH (49 μM) in Tris·HCl buffer (50 mM, pH 7.4, total 0.2 mL) was boiled for 7 min. After centrifugation, the supernatant was analyzed by UV-visible spectroscopy before and after the treatment with $NaBH_4$ (500 mM, 2 μL). The results are shown in Figure S4.

S6.2 HPLC analysis

Purified AbmH (5.0 μM) was boiled for 5 min. After centrifugation, the supernatant was analyzed by HPLC using a C18 reversed-phase column (Microsorb 100-5 C18 250 × 4.6 mm). The HPLC column was eluted using 0.1% TFA in H_2O as mobile phase A and 0.1% TFA in acetonitrile as mobile phase B at a flow rate of 1 mL/min with the following gradient program: 0–20 min 0–85% B, 20–24 min 8–80% B, 24–25 min 80–0% B, 25–30 min 0% B. Elution of the compounds was monitored by setting the UV-detector to 254 nm (Figure S5).

S7. In vitro assays using AbmH and AbmD

S7.1 Typical AbmH assay conditions and procedures

Substrate (i.e., **12**/**13**, **26**/**27**, or **30**/**31**, 0.6 mM) was incubated with AbmH (6.0 μM), L-threonine (5 mM), in HEPES buffer (50 mM, pH 7.5) at room temperature (100 μL total volume). After 8 h, the mixture was filtered through an YM-10 membrane using an Amicon ultrafiltration unit to remove protein. The filtrate was analyzed

by HPLC-UV using a C18 reversed-phase column (Microsorb 100-5 C18 250 × 4.6 mm). The HPLC column was eluted using 0.1% TFA in H_2O as mobile phase A and 0.1% TFA in acetonitrile as mobile phase B at a flow rate of 1 mL/min with the following gradient program: 0–10 min 0–8% B, 10–14 min 8–80% B, 14–18 min 80% B, 18–19 min 80–0% B, 19–26 min 0% B. Elution of the compounds was monitored by setting the UV-detector to 260 nm (Figures 1B, S14, and S21).

To investigate the substrate scope of AbmH, we tested L-threonine, D-threonine, L-*allo*-threonine, L-serine, L-cysteine, and glycine as cosubstrates. HPLC analysis of the reactions indicated that AbmH can accept L-*allo*-threonine in addition to L-threonine (Figure S6).

S7.2 Coupled enzyme assay of AbmH and aldehyde dehydrogenase

The AbmH reaction is expected to produce acetaldehyde byproduct. When the AbmH reaction of **12**/**13** was carried out in the presence of aldehyde dehydrogenase and NAD^+ as described below, consumption of NAD^+ and formation of NADH were observed in the HPLC and UV analysis. Furthermore, when the incubation mixture of AbmH with **12**/**13** was treated with dinitrophenyl hydrazine (DNPH), acetaldehyde-DNPH adduct was formed (see Section S7.3). Together, these results demonstrate that the AbmH reaction with L-threonine produces acetaldehyde as the byproduct.

Compound **13** (0.6 mM) was incubated with AbmH (3.2 μM), L-threonine (5 mM), KCl (100 mM), dithiothreitol (DTT, 1 mM), NAD^+ (2.5 mM), aldehyde dehydrogenase (0.3 U/mL) in HEPES buffer (50 mM, pH 7.5) at room temperature (100 μL total volume). After a certain incubation time, the mixture was filtered through an YM-10 membrane using an Amicon ultrafiltration unit to remove protein. The filtrate was analyzed by HPLC-UV as described in Section S7.1 (Figures S7 and S8).

S7.3 Derivatization of acetaldehyde product

After filtration by the YM-10 membrane filter, the AbmH reaction mixture (50 μL) was acidified to pH 1–2 using 1 N HCl and treated with DNPH (25 mM in acetonitrile, 5 μL) at room temperature for 1 h. After centrifugation, the supernatant was analyzed by HPLC. The sample was eluted using 0.1% TFA in H_2O as mobile phase A and 0.1% TFA in acetonitrile as mobile phase B at a flow rate of 1 mL/min with a gradient program: 0–14 min 10–90% B, 14–18 min 90% B, 18–20 min 90–10% B, 20–26 min 10% B. Elution of DNPH-derivatized compounds was monitored by UV-detector set at 360 nm. The standard aceetaldehyde-DNPH was prepared by treating acetaldehyde (1 mM, 50 μL) with DNPH (25 mM in acetonitrile, 5 μL) at pH 1–2 at room temperature. The obtained sample was analyzed as described above (Figure S9).

S7.4 Assay conditions of the reverse AbmH reaction

Purified **16** (0.22 mM) was incubated with AbmH (6.0 μM), acetaldehyde (5 mM) in HEPES buffer (50 mM, pH 7.5) at room temperature (20 μL total volume). The reaction mixture was analyzed as described in Section S7.1. As shown in Figure S10, consumption of **16** and formation of **13** and **15** were observed. The result demonstrated that the AbmH-catalyzed conversion of **12**/**13** to **16** is a reversible process.

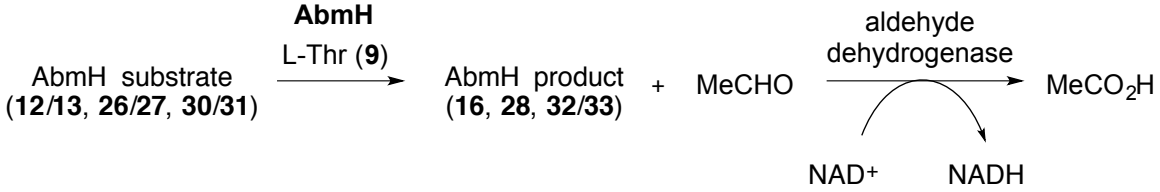
S7.5 UV analysis of the AbmH reaction

Reaction of **12**/**13** with AbmH was monitored by UV. When AbmH (28 μM) was mixed with **12**/**13** (0.3 mM), the absorption at 524 nm corresponding to a quinonoid species remained visible and no other PLP-derived species was found (Figure S11). Even when L-threonine (2 M) was added to the AbmH/**12**/**13** mixture, the UV spectra did not change.

S7.6 Steady-state kinetic analysis of the AbmH reaction

Apparent AbmH steady-state kinetic parameters for the substrate equilibrium mixtures **12**/**13**/4′-epimer, **26**/**27**/4′-epimer or **30**/**31** were determined using a coupled assay with acetaldehyde dehydrogenase based on a reported procedure (Scheme S7).[14] Varied concentrations of total aldehyde/hydrate substrate (i.e., **12**/**13**/4′-epimer, **26**/**27**/4′-epimer, or **30**/**31**) were incubated with AbmH (5.2 μM), potassium activated aldehyde

dehydrogenase (0.3 U/mL), L-threonine (10 mM), NAD^+ (2 mM), DTT (1 mM) and KCl (100 mM) in HEPES buffer (50 mM, pH 7.5). The formation of NADH was monitored by UV absorbance at 340 nm over 10 min in order to determine the initial rate. All measurements were performed in duplicate. The observed rate of the formation of NADH was corrected by subtracting the background increase in UV absorption at 340 nm (1.1×10^{-4} AU·s^{-1}). The apparent steady-state parameters were determined by fitting the Michaelis-Menten equation to the observed initial rates (Figure S24). The results are summarized in Table S1.



Scheme S7. AbmH-aldehyde dehydrogenase coupled assay.

S7.7 Typical AbmD assay conditions and procedures

Compound (5′*S*,6′*S*)-**28** (0.056 mM) was incubated with AbmH (4.6 μM), L-threonine (5 mM), in HEPES buffer (50 mM, pH 7.5) at room temperature (100 μL total volume). After 12 h, the mixture was filtered through an YM-10 membrane using an Amicon ultrafiltration unit to remove protein. The filtrate was analyzed as described in Section 7.1 (Figures S15–S17). The (5′*S*,6′*S*)-**28**/**29** equilibrium constant was determined to be approximately 0.4 favoring **28** based on the integrated area of each peak. Compounds **16**, **32**, and **33** were not accepted as substrate by AbmD.

S7.8 Steady-state Kinetic analysis of the AbmD reaction

The AbmD reaction was analyzed using **29**, because the equilibrium favors the formation of **28**. Compound **29** (39–420 μM) was incubated with AbmD (4.4 μM) and PLP (50 μM) in HEPES buffer (50 mM, pH 7.5). The incubation mixture was filtered using YM10 microcentrifugal filters to stop the reaction at three time points (typically 20–60 min) such that the conversion of **29** was less than 20%. The filtrate was analyzed by HPLC using a C18 reversed-phase column (Microsorb 100-5 C18 250 × 4.6 mm). The HPLC column was eluted using 0.1% TFA in H_2O as mobile phase A and 0.1% TFA in acetonitrile as mobile phase B at a flow rate of 1 mL/min with the following gradient program: 0–2 min 0% B, 2–6 min 0–6%, 6–7 min 6–50% B, 7–11 min 50% B, 11–12 min 50–0% B, 12–17 min 0% B. Elution of **28** and **29** was monitored by setting the UV-detector at 260 nm to determine the extent of the reaction based on the peak areas of **28** and **29**.

The initial, linear portions of the time courses were then fit to obtain the apparent initial rates. As saturation could not be observed under these conditions, the K_m for **29** with AbmD could not be determined; however, it is expected to be greater than 200 μM, and k_{cat} is expected to be greater than 0.2 min^{-1}. The apparent k_{cat}/K_m parameter was found to be $(5.2 \pm 0.3) \times 10^{-4}$ min^{-1}•μM^{-1} (Figure S25).

S8. Isolation and characterization of enzymatic and fermentation products

S8.1 Isolation of AbmH products 16 and (5′*S*,6′*S*)-28

Compound **13** (0.7 mM) was incubated with AbmH (12.0 μM), potassium activated aldehyde dehydrogenase (0.3 U/mL), KCl (100 mM), DTT (1 mM), NAD^+ (2.5 mM), L-threonine (5 mM) in HEPES buffer (50 mM, pH 7.5) at room temperature (30 mL total volume). After 9 h, the mixture was quenched with 60 mL acetonitrile and the resulting white precipitates were removed by centrifugation. The supernatant was concentrated and the product-containing fraction was separated by HPLC using a semipreparative anion-exchange column (Dionex CarboPacPA1, 9.0 mm × 250 mm, Thermo Scientific). The HPLC column was

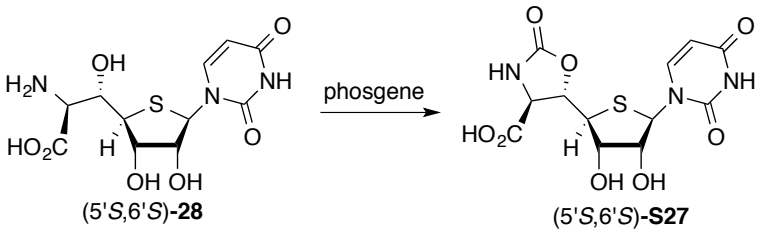
eluted using 0.06% NH_3 in H_2O as mobile phase A and 1 M ammonium acetate in H_2O as mobile phase B at a flow rate of 4 mL/min with the following gradient program: 0–2 min 0% B, 2–17 min 0–30% B, 17–19 min 30–80% B, 19–23 min 80% B, 23–24 min 80–0% B, 24–30 min 0% B. Elution of the compounds was monitored by setting the UV-detector to 260 nm. Fractions containing **16** were collected and lyophilized. The dried residue was dissolved in H_2O (1 mL) and further purified by HPLC using a semipreparative C18 column (ZORBAX, ODS, 5 μm, 9.4 mm × 250 mm, Agilent). The HPLC column was isocratically eluted using 0.1% TFA in H_2O as a mobile phase. Elution of the compounds was monitored by setting the UV-detector to 260 nm. Fractions containing **16** were collected and lyophilized. ^{1}H NMR (600 MHz, D_2O) δ 8.14 (1H, d, *J* = 8.2 Hz, H-6), 5.73 (1H, d, *J* = 2.1 Hz, H-1′), 5.70 (1H, d, *J* = 8.2 Hz, H-5), 4.39 (1H, dd, *J* = 2.6 Hz, *J* = 10.0 Hz, H-5′), 4.30 (1H, m, H-2′), 4.30 (1H, m, H-3′), 3.91 (1H, dd, *J* = 4.0 Hz, *J* = 10.0 Hz, H-4′), 3.97 (1H, d, *J* = 2.6 Hz, H-6′). ^{13}C NMR (D_2O, 151 MHz) δ 169.7, 166.3, 152.0, 144.7, 100.6, 80.6, 75.5, 69.6, 69.0, 57.5, 51.9. ESI-HRMS calcd for $C_{11}H_{16}N_3O_7S^+$ $[M+H]^+$ 334.0703, found 334.0699. A 1:1 mixture of two inseparable minor isomers of **16** were also formed during the AbmH reaction. The stereochemistry of the isomers could not be determined unambiguously because the compounds were obtained in low yields. Isomer 1: ^{1}H NMR (D_2O, 500 MHz) δ 8.36 (1H, d, *J* = 8.2 Hz, H-6), 5.85 (1H, d, *J* = 5.7 Hz, H-1′), 5.83 (1H, d, *J* = 8.2 Hz, H-5), 4.42 (1H, dd, *J* = 5.7 Hz, *J* = 7.3 Hz, H-2′), 4.33 (1H, dd, *J* = 2.9 Hz, *J* = 6.0 Hz, H-5′), 4.16 (1H, dd, *J* = 7.3 Hz, *J* = 7.5 Hz, H-3′), 3.85 (1H, d, *J* = 2.9 Hz, H-6′), 3.70 (1H, dd, *J* = 6.0 Hz, *J* = 7.5 Hz, H-4′). Isomer 2: ^{1}H NMR (D_2O, 500 MHz) δ 8.00 (1H, d, *J* = 8.2 Hz, H-6), 5.92 (1H, d, *J* = 7.2 Hz, H-1′), 5.87 (1H, d, *J* = 8.2 Hz, H-5), 4.55 (1H, dd, *J* = 4.8 Hz, *J* = 5.8 Hz, H-5′), 4.31 (1H, dd, *J* = 6.3 Hz, *J* = 7.9 Hz, H-3′), 4.20 (1H, dd, *J* = 7.2 Hz, *J* = 7.9 Hz, H-2′), 3.90 (1H, d, *J* = 5.8 Hz, H-6′), 3.77 (1H, dd, *J* = 4.8 Hz, *J* = 6.3 Hz, H-4′).

Compound (5′*S*,6′*S*)-**28** was obtained from the reaction of **27** with AbmH based on the same procedure described for **16**. ^{1}H NMR (D_2O, 500 MHz) δ 8.58 (1H, d, *J* = 8.1 Hz, H-6), 5.86 (1H, d, *J* = 4.9 Hz, H-1′), 5.84 (1H, d, *J* = 8.1 Hz, H-5), 4.37 (1H, dd, *J* = 4.3 Hz, *J* = 4.9 Hz, H-2′), 4.30 (1H, dd, *J* = 4.2 Hz, *J* = 6.9 Hz, H-5′), 4.15 (1H, dd, *J* = 4.3 Hz, *J* = 4.7 Hz, H-3′), 3.68 (1H, d, *J* = 6.9 Hz, H-6′), 3.59 (1H, dd, *J* = 4.2 Hz, *J* = 4.7 Hz, H-4′). ^{13}C NMR (D_2O, 126 MHz) δ 171.7, 166.2, 152.1, 143.2, 102.1, 77.7, 74.2, 69.6, 64.7, 58.7, 53.7. ESI-HRMS calcd for $C_{11}H_{14}N_3O_7S^+$ $[M–H]^–$ 332.0558, found 332.0566.

S8.2 Phosgene derivatization of 16 and (5′*S*,6′*S*)-28

To a solution of **16** (0.8 mL, 10 mM), KOH (0.16 mL, 5 N) and phosgene (0.8 mL, 20% solution in toluene) were added at 0 °C. After 30 min incubation with mixing, the aqueous phase was separated and purified by HPLC using a semipreparative C18 column (ZORBAX, ODS, 5 μm, 9.4 mm × 250 mm). The HPLC column was eluted using 0.1% TFA in H_2O as mobile phase A and 0.1% TFA in acetonitrile as mobile phase B at a flow rate of 4 mL/min with the following gradient program: 0–10 min 0–20% B, 10–12 min 20–90% B, 12–16 min 90% B, 16–17 min 90–0% B, 17–23 min 0% B. Elution of the compounds was monitored by setting the UV-detector to 260 nm. Fractions containing **17** (5′*S*,6′*R* or 5′*R*,6′*S*) were collected and lyophilized (1.1 mg). ^{1}H NMR (D_2O, 600 MHz) δ 8.19 (1H, d, *J* = 8.2 Hz, H-6), 5.71 (1H, d, *J* = 2.1 Hz, H-1′), 5.69 (1H, d, *J* = 8.2 Hz, H-5), 5.19 (1H, dd, *J* = 8.2 Hz, *J* = 10.7 Hz, H-5′), 4.28 (1H, m, H-2′), 4.34 (1H, m, H-3′), 3.73 (1H, dd, *J* = 4.4 Hz, *J* = 10.7 Hz, H-4′), 4.33 (1H, d, *J* = 8.2 Hz, H-6′). ESI-HRMS calcd for $C_{12}H_{12}N_3O_8S^–$ $[M–H]^–$ 358.0351, found 358.0346.

A carbamate derivative of (5′*S*,6′*S*)-**28** (**S27**) was obtained by treating (5′*S*,6′*S*)-**28** with phosgene based on the same procedure described for **17** (Scheme S8). ^{1}H NMR (D_2O, 600 MHz) δ 7.98 (1H, d, *J* = 8.2 Hz, H-6), 5.96 (1H, d, *J* = 5.6 Hz, H-1′), 5.87 (1H, d, *J* = 8.2 Hz, H-5), 5.01 (1H, dd, *J* = 2.8 Hz, *J* = 5.1 Hz, H-5′), 4.33 (1H, dd, *J* = 4.1 Hz, *J* = 5.6 Hz, H-2′), 4.26 (1H, dd, *J* = 4.1 Hz, *J* = 4.1 Hz, H-3′), 4.04 (1H, d, *J* = 5.1 Hz, H-6′), 3.73 (1H, dd, *J* = 2.8 Hz, *J* = 4.1 Hz, H-4′). ESI-HRMS calcd for $C_{12}H_{12}N_3O_8S^–$ $[M–H]^–$ 358.0351, found 358.0356.



Scheme S8. Phosgene derivatization of (5′*S*,6′*S*)-**28**.

S8.3 Isolation of AbmD product 29

Compound **27** (0.6 mM) was incubated with AbmH (5.2 μM), AbmD (4.6 μM), and L-threonine (5 mM) in HEPES buffer (50 mM, pH 7.5) at room temperature (40 mL total volume). After 20 h, the mixture was quenched with 80 mL acetonitrile and the resulting white precipitates were removed by centrifugation. The crude product was purified by HPLC using a semipreparative anion-exchange column (Dionex CarboPacPA1, 9.0 mm × 250 mm). The HPLC column was eluted using 0.06% NH_3 in H_2O as mobile phase A and 1 M ammonium acetate in H_2O as mobile phase B at a flow rate of 3 mL/min with the following gradient program: 0–1 min 0% B, 1–3 min 0–15% B, 3–18 min 15–30% B, 18–19 min 30–80% B, 19–23 min 80% B, 23–24 min 80–0% B, 24–30 min 0% B. Elution of the compounds was monitored by setting the UV-detector to 260 nm. Fractions containing **29** were collected and dried under reduced pressure. The spectroscopic data are reported in Section S3.4. Overlaid ^{1}H NMR spectra of the synthetic (5′*S*,6′*R*)-**29** and enzymatically-produced **29** are shown in Figure S70. HPLC analysis of the AbmD product using the synthetic standard of (5′*S*,6′*R*)-**29** is shown in Figure S18.

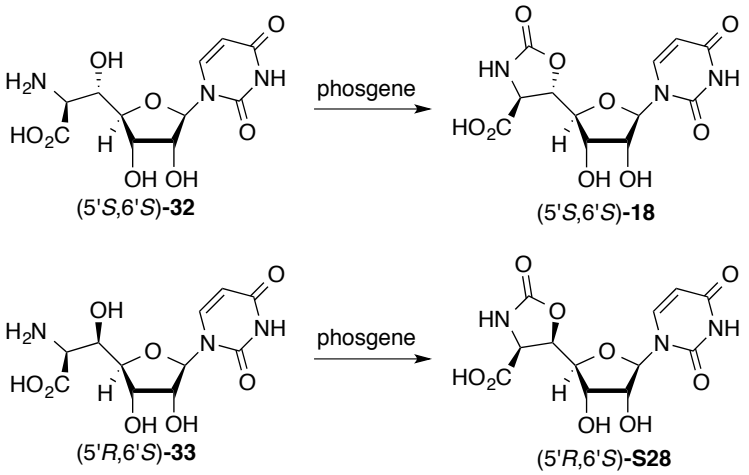
S8.4 Isolation of AbmH products 32 and 33

Compound **31** was incubated with AbmH (10 μM), potassium activated aldehyde dehydrogenase (0.3 U/mL), KCl (100 mM), DTT (1 mM), NAD^+ (1 mM), L-threonine (5 mM) in HEPES buffer (50 mM, pH 7.5) at room temperature (50 mL total volume). After 20 h, the mixture was quenched with 100 mL acetonitrile and the resulting white precipitates were removed by centrifugation. The supernatant was concentrated and the product-containing fraction was separated by HPLC using a semipreparative C18 column (ZORBAX, ODS, 5 μm, 9.4 mm × 250 mm). The HPLC column was eluted using 0.1% TFA in H_2O as mobile phase A and 0.1% TFA in acetonitrile as mobile phase B at a flow rate of 4 mL/min with the following gradient program: 0–7 min 0–6% B, 7–9 min 6–50% B, 9–13 min 50% B, 13–14 min 50–0% B, 14–19 min 0% B. Elution of the compounds was monitored by setting the UV-detector to 260 nm. Compounds **32** and **33** were eluted and collected together and dried under reduced pressure. The obtained residue was further purified by HPLC using a semipreparative anion-exchange column (Dionex CarboPacPA1, 9.0 mm × 250 mm). The HPLC column was eluted using 0.06% NH_3 in H_2O as mobile phase A and 1 M ammonium acetate in H_2O as mobile phase B at a flow rate of 4 mL/min with the following gradient program: 0–2 min 0% B, 2–17 min 0–30% B, 17–19 min 30–80% B, 19–23 min 80% B, 23–24 min 80–0% B, 24–30 min 0% B. Elution of the compounds was monitored by setting the UV-detector to 260 nm. Fractions containing **32** and **33** were separately collected and lyophilized, and the purified products were analyzed by NMR and ESI-MS. Compound **32**: ^{1}H NMR (400 MHz, D_2O) δ 7.96 (1H, d, J = 8.2 Hz, H-6), 5.83 (1H, d, J = 3.3 Hz, H-1′), 5.78 (1H, d, J = 8.2 Hz, H-5), 4.35 (1H, m, H-5′), 4.25 (1H, dd, J = 3.7 Hz, J = 5.7 Hz, H-3′), 4.22 (1H, dd, J = 3.3 Hz, J = 5.7 Hz, H-2′), 4.15 (1H, m, H-4′), 3.87 (1H, d, J = 2.8 Hz, H-6′). ^{13}C NMR (D_2O, 151 MHz) δ 170.8, 166.2, 151.6, 142.0, 102.2, 89.8, 85.1, 73.3, 69.8, 67.0, 58.1. The C7′ signal of **32** is overlapped with the signal of the residual formic acid. ESI-HRMS calcd for $C_{11}H_{14}N_3O_8^-$ $[M–H]^-$ 316.0786, found 316.0796. Compound **33**: ^{1}H NMR (D_2O, 400 MHz) δ 7.66 (1H, d, J = 8.1 Hz, H-6), 5.78 (1H, d, J = 8.1 Hz, H-5), 5.73 (1H, d, J = 4.9 Hz, H-1′), 4.32 (1H, dd, J = 4.9 Hz, J = 5.0 Hz, H-2′), 4.27–4.24 (2H, m, H-3′, H-5′), 4.05 (1H, dd, J = 4.8 Hz, J = 6.8 Hz, H-4′), 3.88 (1H, d, J = 3.4 Hz, H-6′). ^{13}C NMR (D_2O, 151 MHz) δ 170.8, 166.2, 151.5, 142.4, 102.2, 90.0, 82.9, 72.7, 69.8, 69.3, 57.1. The C7′ signal

of **33** is overlapped with the signal of the residual formic acid. ESI-HRMS calcd for $C_{11}H_{14}N_3O_8^-$ [M–H]$^-$ 316.0786, found 316.0798.

S8.5 Phosgene derivatization of 32 and 33

Compounds **32** and **33** were individually derivatized by phosgene (Scheme S9) and the corresponding cyclic carbamates **18** and **S28**, respectively, were purified based on the same method described for **17**. The ^{1}H NMR data of **18** is consistent with the reported data in the literature.[14] **18**: ^{1}H NMR (D_2O, 600 MHz) δ 7.43 (1H, d, *J* = 8.1 Hz, H-6), 5.85 (1H, d, *J* = 4.8 Hz, H-1′), 5.77 (1H, d, *J* = 8.1 Hz, H-5), 4.86 (1H, dd, *J* = 2.5 Hz, *J* = 5.2 Hz, H-5′), 4.31 (1H, dd, *J* = 5.2 Hz, *J* = 5.6 Hz, H-3′), 4.30 (1H, d, *J* = 5.2 Hz, H-6′), 4.27 (1H, dd, *J* = 4.8 Hz, *J* = 5.6 Hz, H-2′), 4.15 (1H, dd, *J* = 2.5 Hz, *J* = 5.2 Hz, H-4′). ESI-HRMS calcd for $C_{12}H_{12}N_3O_9^-$ [M–H]$^-$: 342.0579, found: 342.0576. **S28**: ^{1}H NMR (D_2O, 600 MHz) δ 7.61 (1H, d, *J* = 8.1 Hz, H-6), 5.87 (1H, d, *J* = 7.2 Hz, H-1′), 5.81 (1H, d, *J* = 8.1 Hz, H-5), 5.01 (1H, dd, *J* = 1.9 Hz, *J* = 9.7 Hz, H-5′), 4.48 (1H, d, *J* = 9.7 Hz, H-6′), 4.33 (1H, m, H-4′), 4.20 (1H, m, H-2′), 4.17 (1H, m, H-3′). ESI-HRMS calcd for $C_{12}H_{12}N_3O_9^-$ [M–H]$^-$ 342.0579, found 342.0543.



Scheme S9. Phosgene derivatization of **32** and **33**.

S8.6 Isolation of 25

A 400 mL of TSB medium was inoculated with the spores of the wild type *S.* sp. ATCC700974 and incubated at 28 °C. After 24 h, 50 mL of the TSB seed culture was used to inoculate 6 × 1 L of the albomycin-producing medium.[6] The culture was incubated at 28 °C for 96 h and centrifuged at 4,000 × g. The cell-free broth was applied to a column (5 cm × 10 cm) packed with Amberlite XAD4 pre-equilibrated with water. The column was washed with water and eluted with 50% aqueous methanol. Fractions containing **1** as determined by ESIMS analysis were concentrated under reduced pressure. The obtained residue was then loaded to a Biogel P2 (Bio-Rad) size exclusion column (2.5 cm × 80 cm) using 25 mM NaCl as the running solvent. Fractions containing **1** were collected and concentrated under reduced pressure. The resulting residue was dissolved in a minimum amount of water and loaded on a reverse phase Cosmosil 140C18-OPN (Nacalai USA, Inc. (San Diego, CA)) column (2.5 cm × 17 cm) pre-equilibrated with 1% methanol in water. The column was washed and eluted using 1–25% methanol in water. Evaporation of the elution fractions containing **1** gave a brown residue (44 mg).

The brown residue containing **1** was incubated with leucine aminopeptidase (EC number 3.4.11.2, 0.75 mg) in Tris buffer (50 mM, pH 7.5) at 37 ° for 4 days. After the incubation, the digested compound was purified by HPLC using a semipreparative C18 column (ZORBAX, ODS, 5 μm, 9.4 mm × 250 mm). The HPLC column was eluted using 0.5% ammonium acetate in H_2O as mobile phase A and acetonitrile as mobile phase B at a

flow rate of 4 mL/min with the following gradient program: 0–7 min 0–6% B, 7–9 min 6–50% B, 9–13 min 50% B, 13–14 min 50–0% B, 14–19 min 0% B. Elution of **25** was monitored by setting the UV-detector to 360 nm. During the purification process, partial deamination of **25** was observed. Elution fractions were collected and lyophilized to give **25** (2.9 mg). ^{1}H NMR (D_2O, 600 MHz) δ 8.02 (1H, d, *J* = 8.3 Hz, H-6), 6.02 (1H, d, *J* = 8.3 Hz, H-5), 5.77 (1H, d, *J* = 4.3 Hz, H-1′), 4.41 (1H, dd, *J* = 3.9 Hz, *J* = 6.1 Hz, H-5′), 4.23 (1H, dd, *J* = 4.3 Hz, *J* = 5.7 Hz, H-2′), 4.26 (1H, dd, *J* = 5.7 Hz, *J* = 5.8 Hz, H-3′), 3.86 (1H, dd, *J* = 5.8 Hz, *J* = 6.1 Hz, H-4′), 3.80 (1H, d, *J* = 3.9 Hz, H-6′), 3.17 (3H, s, NMe). ^{13}C NMR (D_2O, 151 MHz) δ 171.0, 167.4, 155.5, 152.2, 138.4, 96.4, 80.4, 75.0, 67.7, 65.7, 57.7, 51.9, 29.8. ESI-HRMS calcd for $C_{13}H_{20}N_5O_7S^+$ $[M+H]^+$ 390.1078, found 390.1077.

S8.7 Phosgene derivatization of 25

To a solution of **25** (0.6 mL, 10 mM), KOH (0.12 mL, 5 M) and phosgene (0.6 mL, 20% solution in toluene) were added at 0 °C. After 10 min, additional KOH (0.12 mL, 5 M) and phosgene (0.6 mL, 20% solution in toluene) were added to the mixture, which was further incubated for 10 min. The aqueous phase was separated and purified by HPLC using a semipreparative C18 column (ZORBAX, ODS, 5 μm, 9.4 mm × 250 mm). The HPLC column was eluted using 0.5% ammonium acetate in H_2O as mobile phase A and acetonitrile as mobile phase B at a flow rate of 4 mL/min with the following gradient program: 0–6 min 0–6% B, 6–7 min 6–50% B, 7–12 min 50% B, 12–13 min 50–0% B, 13–17 min 0% B. Elution of the compounds was monitored by setting the UV-detector to 260 nm. Fractions containing **19** were collected and lyophilized (0.8 mg) ^{1}H NMR (D_2O, 600 MHz) δ 7.65 (1H, d, *J* = 8.3 Hz, H-6), 6.10 (1H, d, *J* = 8.3 Hz, H-5), 5.84 (1H, d, *J* = 7.9 Hz, H-1′), 5.37 (1H, dd, *J* = 2.2 Hz, *J* = 9.9 Hz, H-5′), 4.41 (1H, d, *J* = 9.9 Hz, H-6′), 4.28 (1H, dd, *J* = 7.7 Hz, *J* = 9.4 Hz, H-3′), 4.13 (1H, dd, *J* = 7.9 Hz, *J* = 9.4 Hz, H-2′), 3.77 (1H, dd, *J* = 2.2 Hz, *J* = 7.7 Hz, H-4′), 3.17 (3H, s, NMe). ESI-HRMS calcd for $C_{14}H_{16}N_5O_8S^-$ $[M–H]^-$ 414.0725, found 414.0722.

S8.8 Isolation of 23

A brown residue containing **22** (22 mg) was obtained from 12 × 1 L of the albomycin-producing medium using the Δ*abmJ* strain instead of the wild type strain. ESI-HRMS of **22**: calcd for $C_{37}H_{58}FeN_{12}O_{18}S^+$ $[M+H]^+$: 1046.3057, found: 1046.2985. The residue was incubated with leucine aminopeptidase (EC number 3.4.11.2, 0.75 mg) in Tris buffer (50 mM, pH 7.5) at 37 ° for 4 days. After the incubation, the digested compound was purified by HPLC using a semipreparative C18 column (ZORBAX, ODS, 5 μm, 9.4 mm × 250 mm). The HPLC column was eluted using 0.1% TFA in H_2O as mobile phase A and 0.1% TFA in acetonitrile as mobile phase B at a flow rate of 4 mL/min with the following gradient program: 0–7 min 0–6% B, 7–9 min 6–50% B, 9–13 min 50% B, 13–14 min 50–0% B, 14–19 min 0% B. Elution of the compounds was monitored by setting the UV-detector to 260 nm. Fractions containing **23** were collected and lyophilized. The obtained residue was dissolved in 200 mM ammonium acetate (pH 8.8) and applied to immobilized boronic acid resin (Thermo Fisher Scientific). The resin was washed with 200 mM ammonium acetate (pH 8.8), and **23** was eluted with 0.1 M formic acid. The eluted fraction was lyophilized to afford **23** (2.3 mg). ^{1}H NMR (D_2O, 600 MHz) δ 8.32 (1H, br, s, CO_2H), 8.23 (1H, d, *J* = 8.1 Hz, H-6), 5.85 (1H, d, *J* = 8.1 Hz, H-5), 5.80 (1H, d, *J* = 4.7 Hz, H-1′), 4.30 (1H, dd, *J* = 4.3 Hz, *J* = 4.6 Hz, H-5′), 4.28 (1H, dd, *J* = 3.7 Hz, *J* = 4.7 Hz, H-2′), 4.04 (1H, dd, *J* = 3.7 Hz, *J* = 5.8 Hz, H-3′), 3.83 (1H, d, *J* = 4.3 Hz, H-6′), 3.58 (1H, dd, *J* = 4.6 Hz, *J* = 5.8 Hz, H-4′), 3.16 (3H, s, NMe). ^{13}C NMR (D_2O, 151 MHz) δ 170.9, 165.4, 152.6, 141.0, 101.2, 77.2, 74.6, 69.5, 65.9, 57.9, 52.8, 27.9. ESI-HRMS calcd for $C_{13}H_{20}N_5O_7S^+$ $[M+H]^+$ 348.0860, found 348.0890.

S8.9 Phosgene derivatization of 23

Compound **23** was treated with phosgene to give **24** based on the same procedure described for **19**. ^{1}H NMR (D_2O, 600 MHz) δ 8.16 (1H, d, *J* = 8.2 Hz, H-6), 6.03 (1H, d, *J* = 8.2 Hz, H-5), 5.99 (1H, d, *J* = 5.1 Hz, H-1′), 5.44 (1H, dd, *J* = 1.1 Hz, *J* = 9.7 Hz, H-5′), 4.74 (1H, d, *J* = 9.7 Hz, H-6′), 4.37 (1H, dd, *J* = 4.1 Hz, *J* = 5.1 Hz, H-2′), 4.25 (1H, dd, *J* = 4.1 Hz, *J* = 5.2 Hz, H-3′), 3.81 (1H, dd, *J* = 1.1 Hz, *J* = 5.2 Hz, H-4′), 3.30 (3H, s, NMe). ESI-HRMS calcd for $C_{13}H_{14}N_3O_8S^-$ $[M–H]^-$ 372.0507, found 372.0476.

S9. Disc diffusion assay

An *E. coli* DH5α culture grown overnight (60 μL) was transferred to fresh LB medium (2 mL) and incubated at 37 °C. After 3 h, 60 μL of the culture was transferred to molten LB agar (20 mL) before solidified. After the agar was solidified, **1** and **22** were separately spotted on the plate. The plate was kept at 4 °C for 12 h to allow diffusion of the compounds and then incubated at 37 °C to grow *E. coli.* After 24 h, the bioactivity of **1** and **22** was evaluated by zones of inhibition (Figure S26). The appearance of resistant colonies towards albomycin within the inhibition zone has been reported.[15]

S10. **X-Ray crystal structure analysis**

Crystals of **S24** grew as long, very thin, colorless needles by slow evaporation from DCM/hexanes. The data crystal was cut from a longer needle and had approximate dimensions; 0.31 × 0.025 × 0.016 mm. The data were collected on an Agilent Technologies SuperNova Dual Source diffractometer using a μ-focus Cu Kα radiation source (λ = 1.5418 Å) with collimating mirror monochromators. A total of 600 frames of data were collected using ω-scans with a scan range of 1° and a counting time of 22.5 seconds per frame for frames collected with a detector offset of +/– 42.7° and 87.5 seconds per frame with frames collected with a detector offset of +/– 111.0°. The data were collected at 100 K using an Oxford Cryostream low temperature device. Details of crystal data, data collection and structure refinement are listed in Table S2. Data collection, unit cell refinement and data reduction were performed using Agilent Technologies CrysAlisPro V 1.171.39.46.[16] The structure was solved by direct methods using SHELXT[17] and refined by full-matrix least-squares on F^2 with anisotropic displacement parameters for the non-H atoms using SHELXL-2016/6.[18] Structure analysis was aided by use of the programs PLATON[19] and WinGX.[20] The hydrogen atoms were calculated in ideal positions with isotropic displacement parameters set to 1.2 x Ueq of the attached atom (1.5 x Ueq for methyl hydrogen atoms). The absolute configuration was determined by internal comparison to the known configuration of the furanose ring.

The function, $\Sigma w(|F_o|^2 - |F_c|^2)^2$, was minimized, where $w = 1/[(\sigma(F_o))^2 + (0.0772*P)^2]$ and $P = (|F_o|^2 + 2|F_c|^2)/3$. $R_W(F^2)$ refined to 0.261, with $R(F)$ equal to 0.104 and a goodness of fit, S, = 1.01. Definitions used for calculating $R(F)$, $R_W(F^2)$ and the goodness of fit, S, are given below.[21] The data were checked for secondary extinction effects but no correction was necessary. Neutral atom scattering factors and values used to calculate the linear absorption coefficient are from the International Tables for X-ray Crystallography (1992).[22] The ORTEP structure for compound **S24** is shown in Figure S27.

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Supplementary Tables

Table S1. Apparent steady-state kinetic parameters for the AbmH reaction.

	k_{cat} (min^{-1})	K_m (μM)	k_{cat}/K_m ($min^{-1} \cdot \mu M^{-1}$)
12/13 (+ 4'-epimer)	0.80 ± 0.02	42.8 ± 5.6	0.019
26/27 (+ 4'-epimer)	0.67 ± 0.09	49.2 ± 15	0.014
30/31	0.44 ± 0.03	432 ± 69	0.0010

Table S2. Crystal data and structure refinement for **S24**.

Empirical formula	$C_{27}H_{49}N5O_8Si_2$	
Formula weight	627.89	
Temperature	100(2) K	
Wavelength	1.54184 Å	
Crystal system	monoclinic	
Space group	P 21	
Unit cell dimensions	a = 10.558(4) Å	$\alpha = 90°$
	b = 7.3823(10) Å	$\beta = 100.12(2)°$
	c = 22.505(4) Å	$\gamma = 90°$
Volume	1726.8(8) $Å^3$	
Z	2	
Density (calculated)	1.208 Mg/m^3	
Absorption coefficient	1.355 mm^{-1}	
F(000)	676	
Crystal size	0.310 × 0.025 × 0.016 mm^3	
Theta range for data collection	3.991 to 68.247°	
Index ranges	$-12 \le h \le 11$, $-7 \le k \le 8$, $-22 \le l \le 27$	
Reflections collected	5911	
Independent reflections	4427 [*R*(int) = 0.1183]	
Completeness to theta = 25.242°	98.8 %	
Absorption correction	Gaussian and multi-scan	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4427 / 589 / 393	
Goodness-of-fit on F^2	1.010	
Final *R* indices [$I > 2\text{sigma}(I)$]	*R*1 = 0.1036, *wR*2 = 0.2184	
R indices (all data)	*R*1 = 0.1626, *wR*2 = 0.2609	
Absolute structure parameter	0.10(12)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.493 and –0.386 e.$Å^{-3}$	

Supplementary Figures

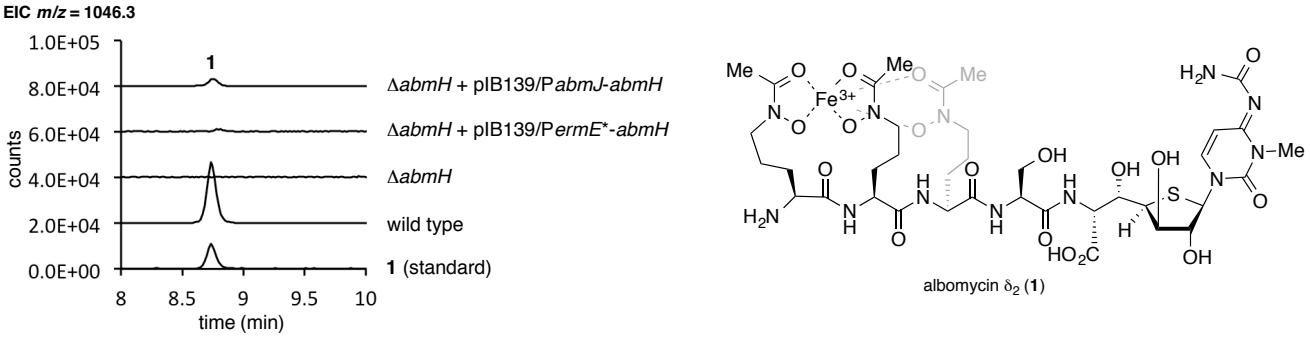


Figure S1. The *abmH* gene deletion and complementation experiments. EIC traces corresponding to the $[M+H]^+$ signal from **1** are shown.

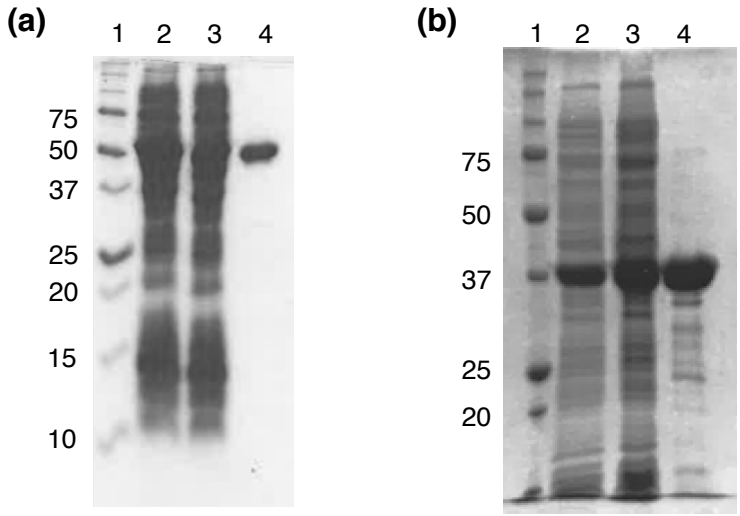


Figure S2. SDS-PAGE of (a) *N*-His_6-AbmH (48.6 kDa) and (b) *N*-His_6-AbmD (38.7 kDa). Lane 1: Protein standards, Lane 2: Whole cell lysate, Lane 3: Supernatant fraction after centrifugation of the lysate, Lane 4: Elution fraction of Ni-NTA affinity chromatography. Minor impurities are possibly resulted from their non-specific interactions with the Ni-NTA resins.

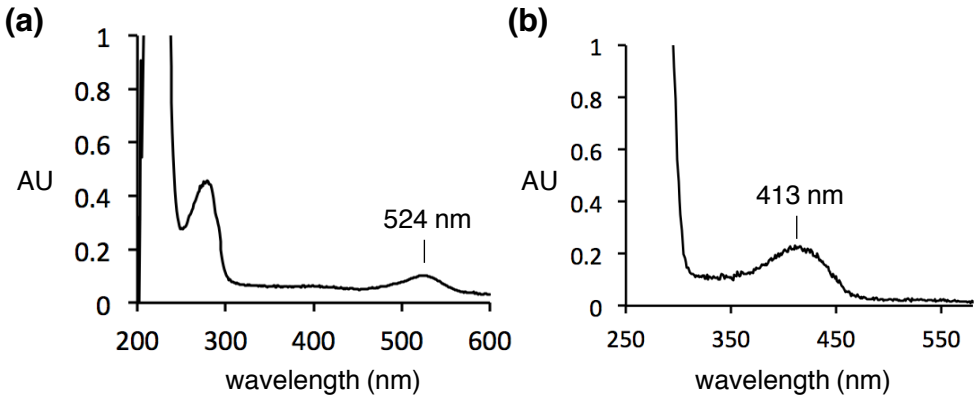


Figure S3. UV-visible spectrum of (a) AbmH and (b) AbmD purified from *E. coli* (50 mM Tris·HCl, pH 7.4).

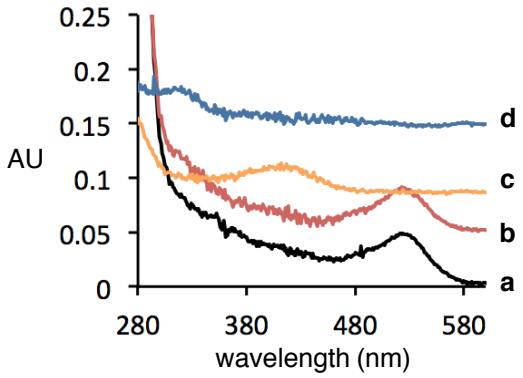


Figure S4. UV detection of PLP from the purified AbmH. (a) No treatment. (b) $NaBH_4$ treatment. (c) 100 °C for 5 min. The absorption at 413 nm corresponds to PLP. (d) 100 °C for 5 min, then $NaBH_4$ treatment. The absorption at 315 nm corresponds to pyridoxine 5′-phosphate.

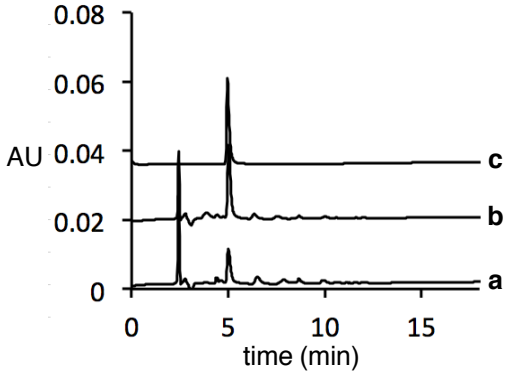


Figure S5. Detection of PLP by HPLC-UV. (a) Supernatant fraction of the denatured protein solution (100 °C for 5 min). (b) Coinjection with the standard PLP. (c) Standard PLP.

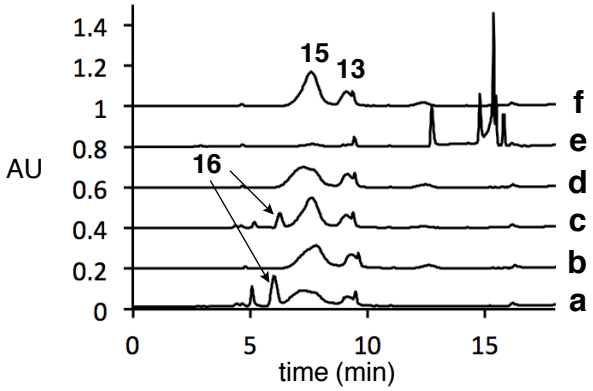


Figure S6. AbmH reaction of **12**/**13** with different cosubstrates. (a) L-Threonine. (b) D-Threonine. (c) L-*allo*-Threonine. (d) L-Serine. (e) L-Cysteine. Peaks observed around 15 min correspond to adducts between cysteine and **12**/**14**, which were non-enzymatically formed. (f) Glycine.

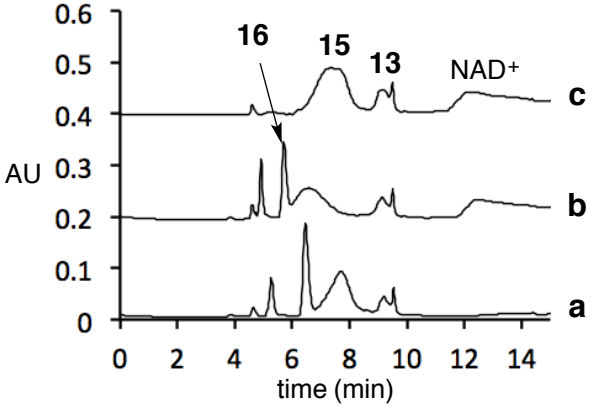


Figure S7. HPLC-UV analysis of the coupled enzyme assay using AbmH and aldehyde dehydrogenase. (a) AbmH and aldehyde dehydrogenase. (b) AbmH only. (c) Aldehyde dehydrogenase only.

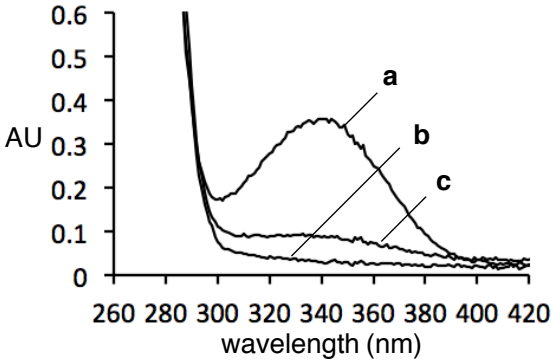


Figure S8. UV analysis of coupled enzyme assay using AbmH and aldehyde dehydrogenase. (a) AbmH and aldehyde dehydrogenase. (b) AbmH only. (c) Aldehyde dehydrogenase only.

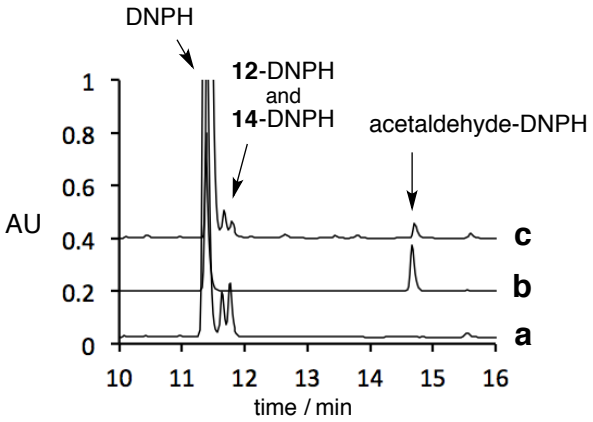


Figure S9. DNPH derivatization of acetaldehyde generated from the AbmH reaction of **12**/**13**. (a) **12**+**13**/DNPH (standard) (b) acetaldehyde /DNPH (standard) (c) DNPH treatment of the AbmH reaction of **13**.

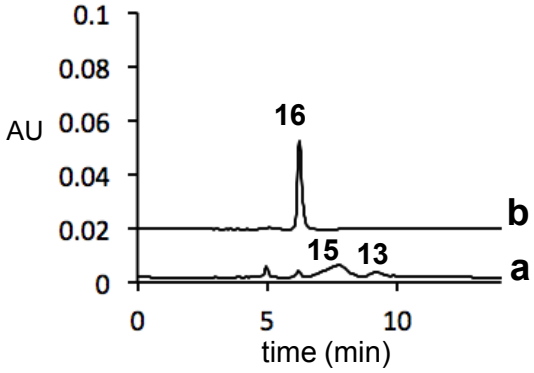


Figure S10. (a) Incubation of purified **16** (a) with AbmH or (b) without AbmH.

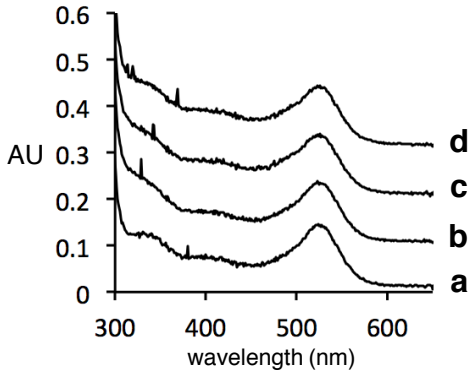


Figure S11. UV analysis of the PLP-derived species during the reaction of AbmH (a) AbmH only. (b) AbmH + **12**/**13** (c) AbmH + **12**/**13** + L-threonine, 3 min. (d) AbmH + **12**/**13** + L-threonine, 30 min.

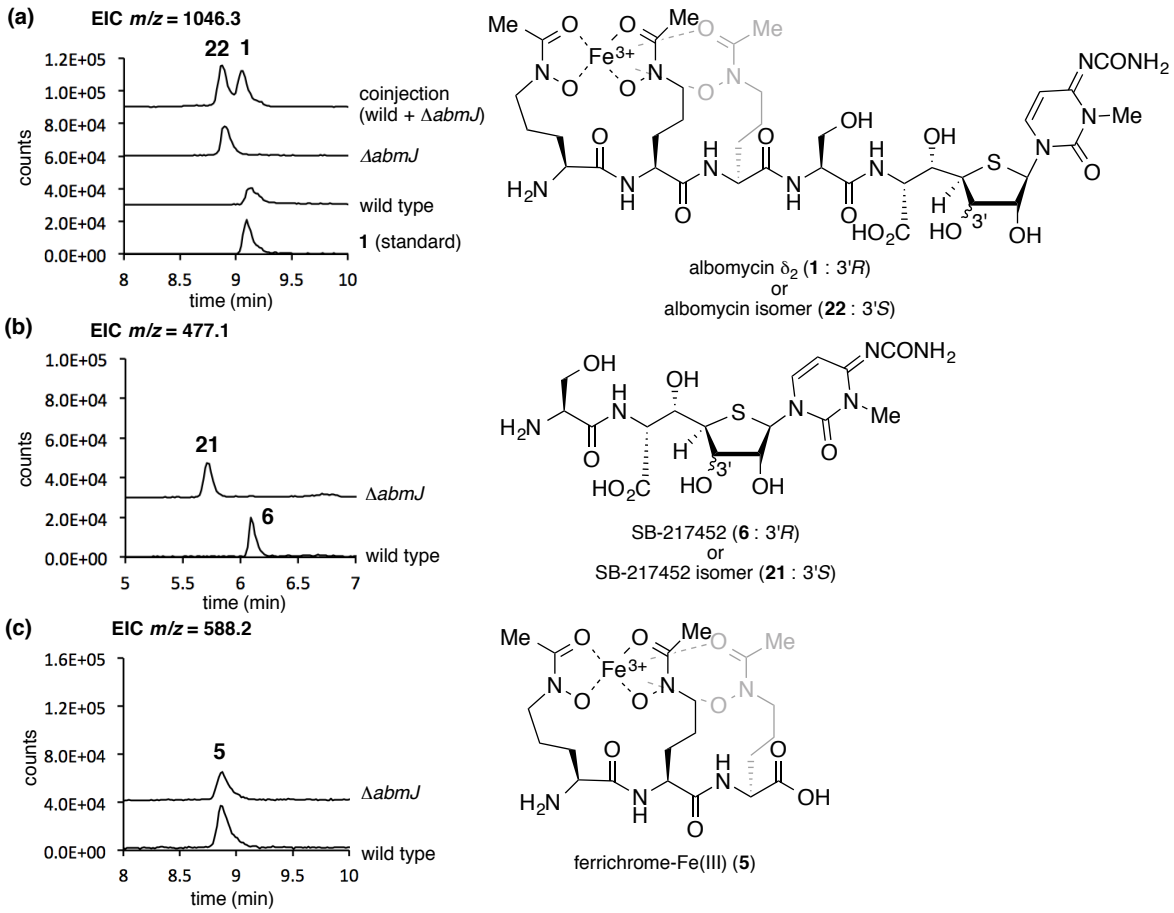


Figure S12. LCMS analysis of the metabolites from the wild type strain and the Δ*abmJ* strain. Extracted ion chromatogram (EIC) traces corresponding to the $[M+H]^+$ signal from each species are shown. (a) Albomycin δ_2 (**1**) and its isomer (**22**). (b) SB-217452 (**6**) and its isomer (**21**). (c) Ferrichrome-Fe(III) (**5**).

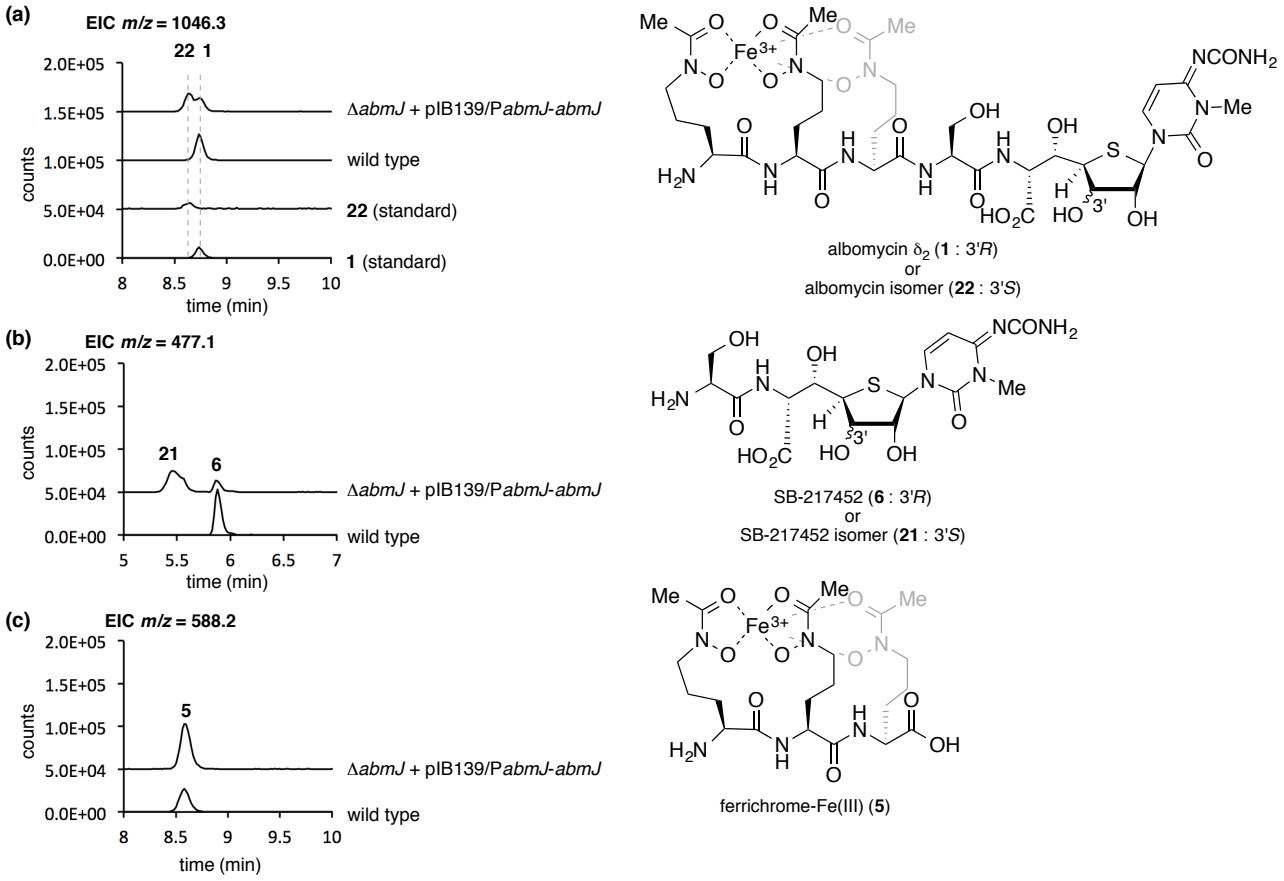


Figure S13. LCMS analysis of the metabolites from the wild type strain and the Δ*abmJ* strain with the *abmJ* gene introduced. Extracted ion chromatogram (EIC) traces corresponding to $[M+H]^+$ signal from each species are shown. (a) Albomycin δ_2 (**1**) and it isomer (**22**). (b) SB-217452 (**6**) and its isomer (**21**). (c) Ferrichrome-Fe(III) (**5**).

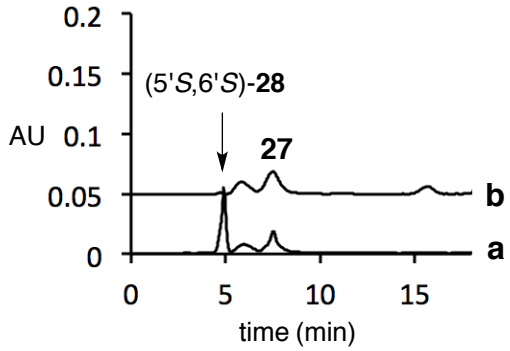


Figure S14. Reaction of **26**/**27** (a) with AbmH and (b) without AbmH. The peak around 6 min corresponds to non-enzymatically generated 4′*R*-epimer of **27**.

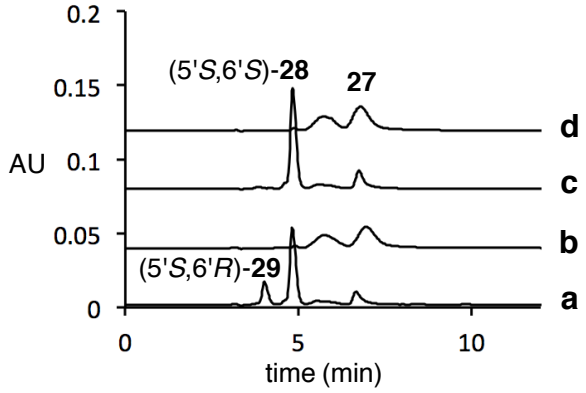


Figure S15. Enzyme assays using **26/27** in the presence of (a) AbmH and AbmD, (b) AbmD, (c) AbmH, and (d) in the absence of the enzymes.

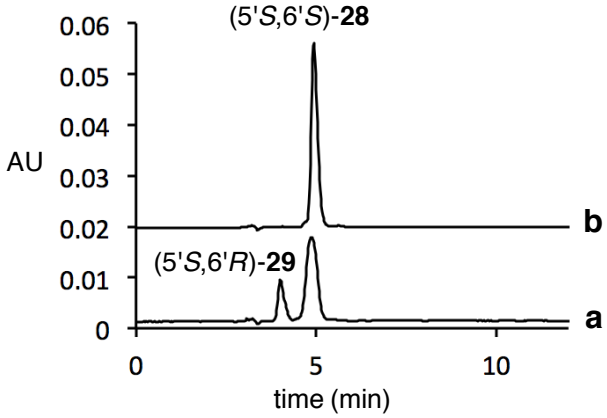


Figure S16. Reaction of (5′*S*,6′*S*)-**28** (a) with AbmD and (b) without AbmD.

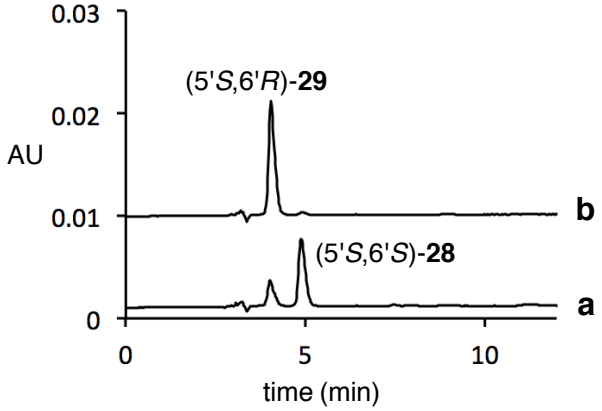


Figure S17. Reaction of (5′*S*,6′*R*)-**29** (a) with AbmD and (b) without AbmD.

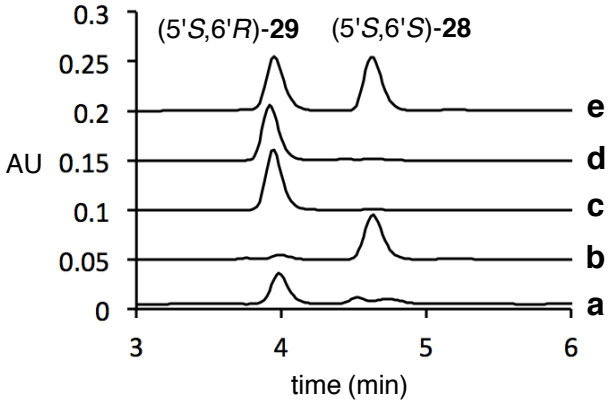


Figure S18. HPLC analysis of the AbmD reaction product from (5′*S*,6′*S*)-**28**. (a) AbmD enzymatic product. (b) (5′*S*,6′*S*)-**28**. (c) Synthetic standard of (5′*S*,6′*R*)-**29**. (d) Co-injection of the AbmD enzymatic product with the standard sample of (5′*S*,6′*R*)-**29**. (e) Co-injection of (5′*S*,6′*S*)-**28** and the standard sample of (5′*S*,6′*R*)-**29**.

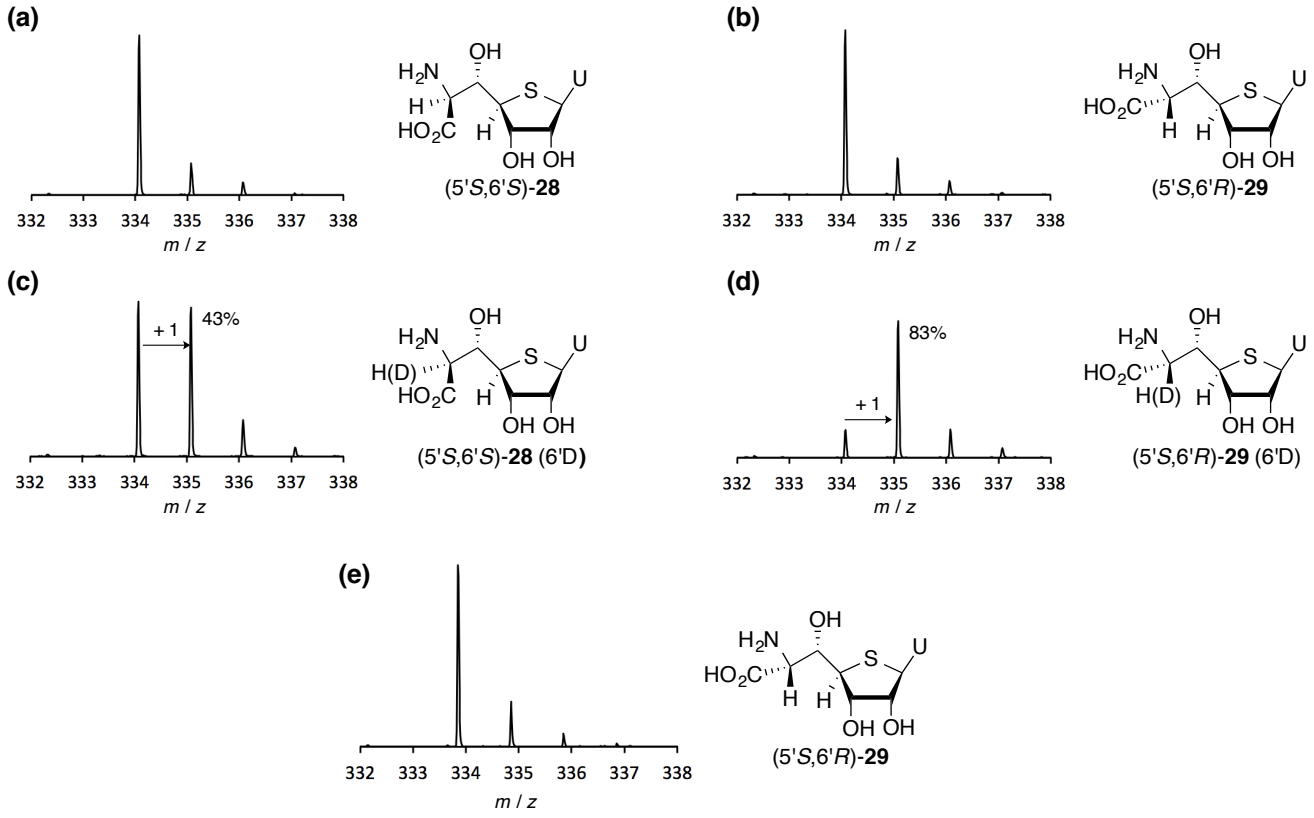


Figure S19. ESI-MS analysis of the reaction of (5′*S*,6′*S*)-**28** with AbmD in H_2O, D_2O (92% D), or $H_2{}^{18}O$ (97% ^{18}O). (a) Residual (5′*S*,6′*S*)-**28** after incubation in H_2O. (b) Product (5′*S*,6′*R*)-**29** formed after incubation of (5′*S*,6′*S*)-**28** in H_2O. (c) Residual (5′*S*,6′*S*)-**28** after incubation in D_2O. (d) Product (5′*S*,6′*R*)-**29** formed after incubation of (5′*S*,6′*S*)-**28** in D_2O. (e) Product (5′*S*,6′*R*)-**29** formed after incubation of (5′*S*,6′*S*)-**28** in $H_2{}^{18}O$.

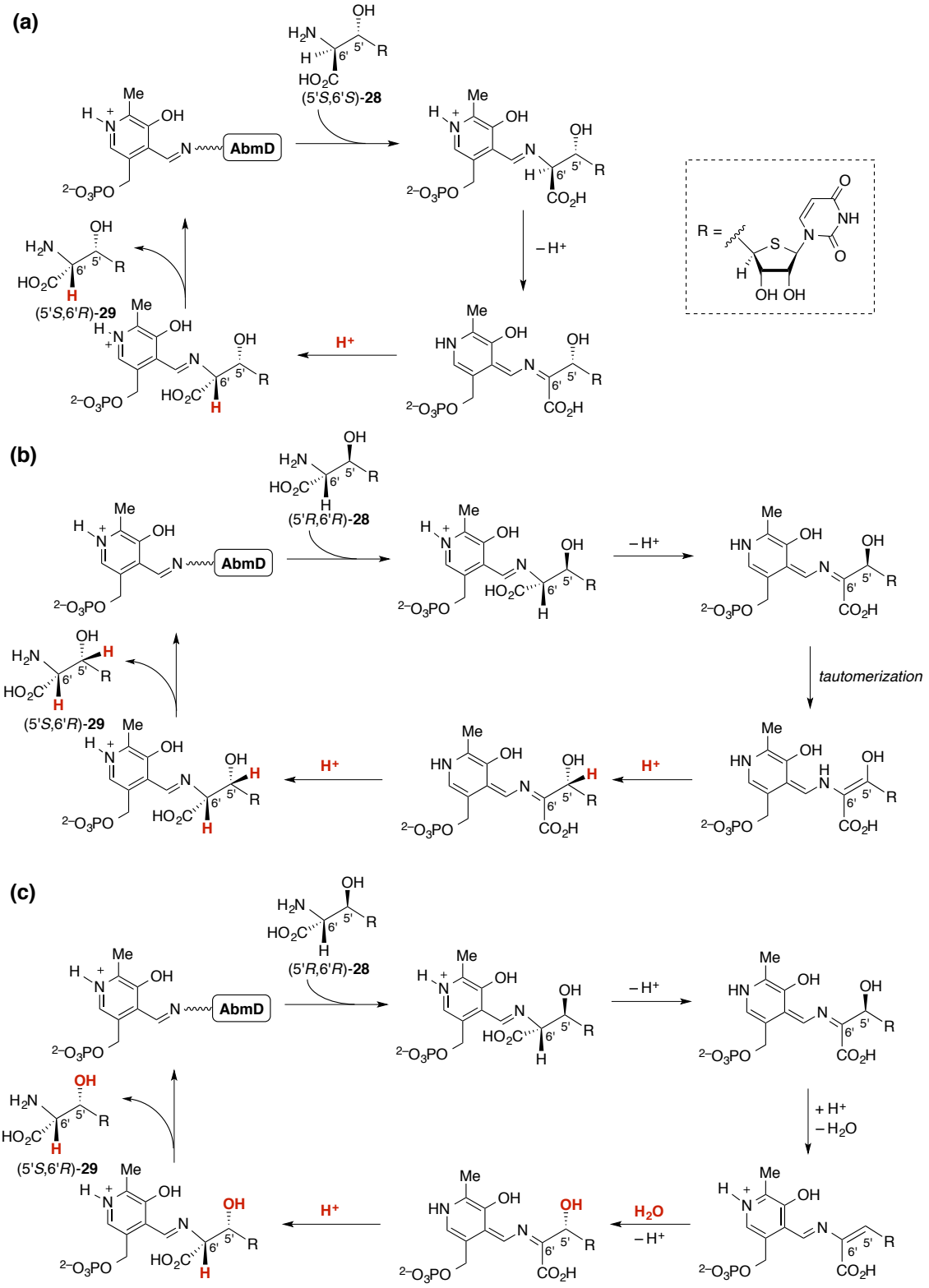


Figure S20. Possible mechanisms for the epimerization of **28** ((5′*S*,6′S)-**28** or (5′*R*,6′*R*)-**28**) to (5′*S*,6′*R*)-**29**. (a) α-Epimerization. (b) β-Epimerization via deprotonation and reprotonation of Cβ. (c) β-Epimerization via β-elimination and rehydration. Hydrogen and oxygen atoms derived from solvent are shown in red.

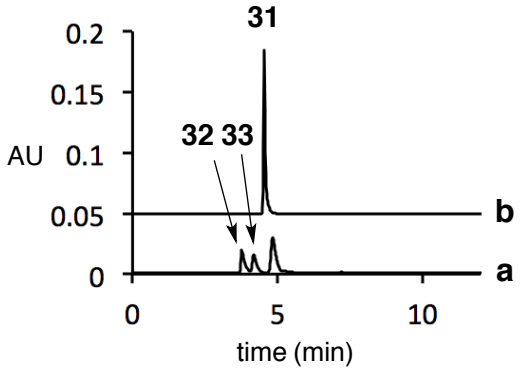


Figure S21. Reaction of **30**/**31** (a) with AbmH and (b) without AbmH.

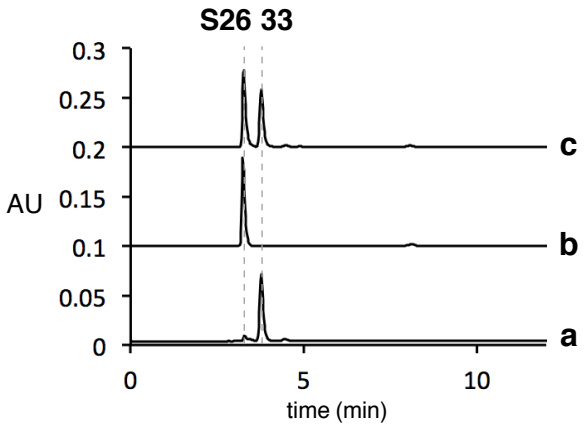


Figure S22. HPLC analysis of the *erythro* AbmH reaction product from **30**/**31**. (a) The *erythro* AbmH enzymatic reaction product. (b) Synthetic standard of **S26** (See Scheme S6). (c) Co-injection of the *erythro* AbmH enzymatic product and the synthetic standard **S26**. The *erythro* AbmH product was characterized to be (5′*R*,6′*S*)-**33** because it does not co-elute with (5′*S*,6′*R*)-**S26**, the other possible *erythro* compound.

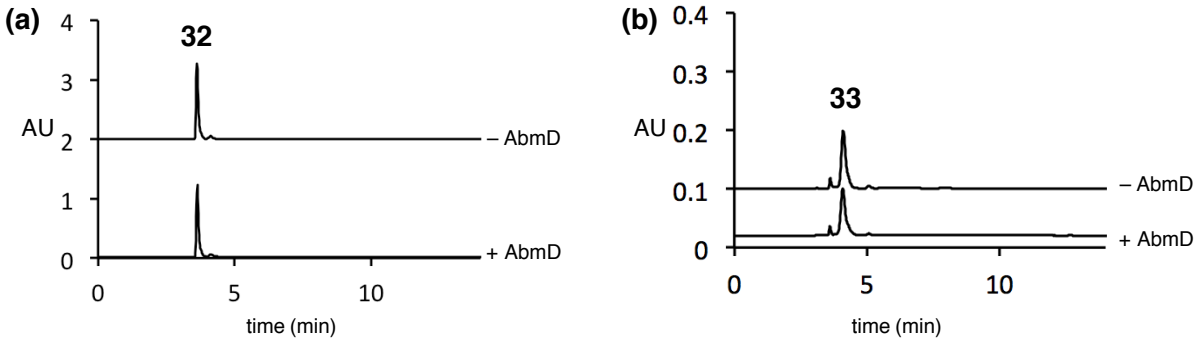


Figure S23. Incubation of AbmD with (a) **32** and (b) **33**.

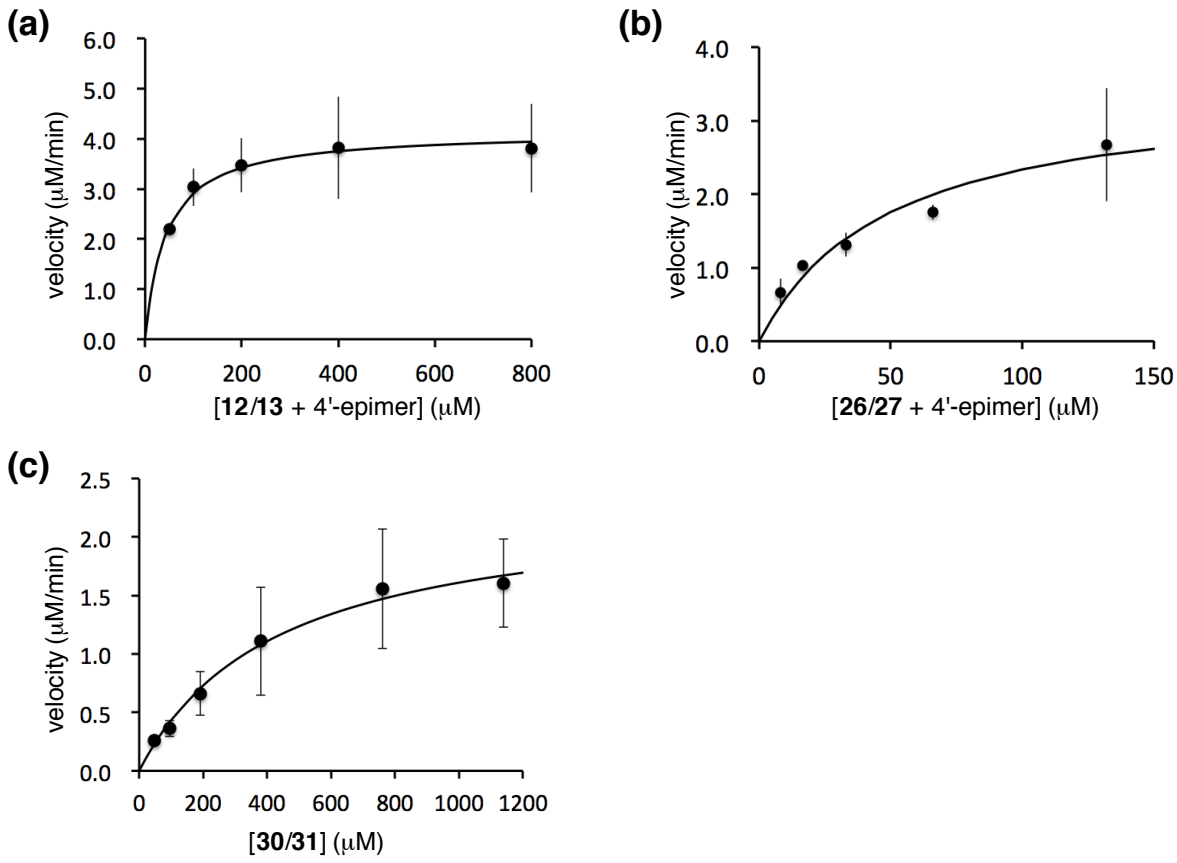


Figure S24. Kinetic analysis of the AbmH reaction with (a) **12**/**13**/4′-epimer, (b) **26**/**27**/4′-epimer, and (c) **30**/**31**.

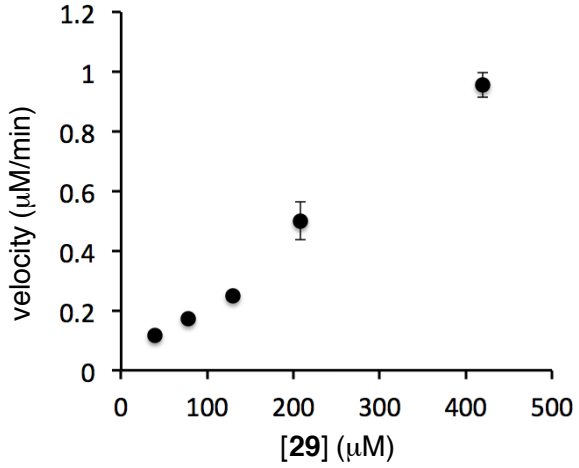


Figure S25. Kinetic analysis of the AbmD reaction with **29**.

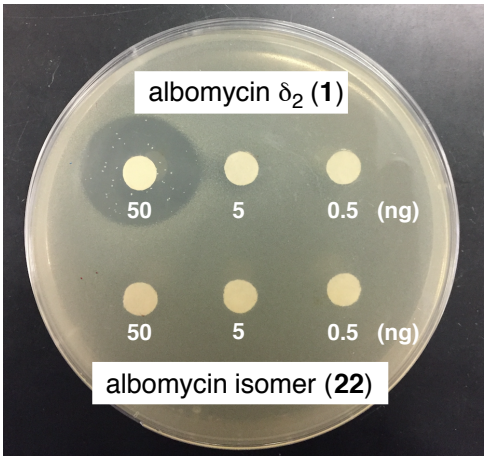
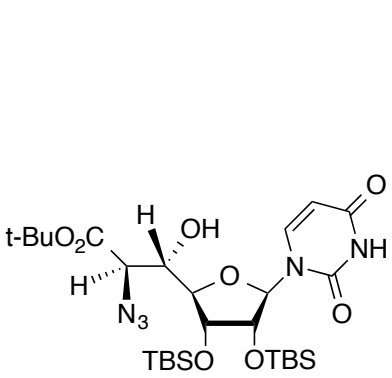


Figure S26. Disc diffusion assay with *E. coli* DH5α using **1** and **22** obtained from the wild type strain and the Δ*abmJ* strain, respectively.



S24

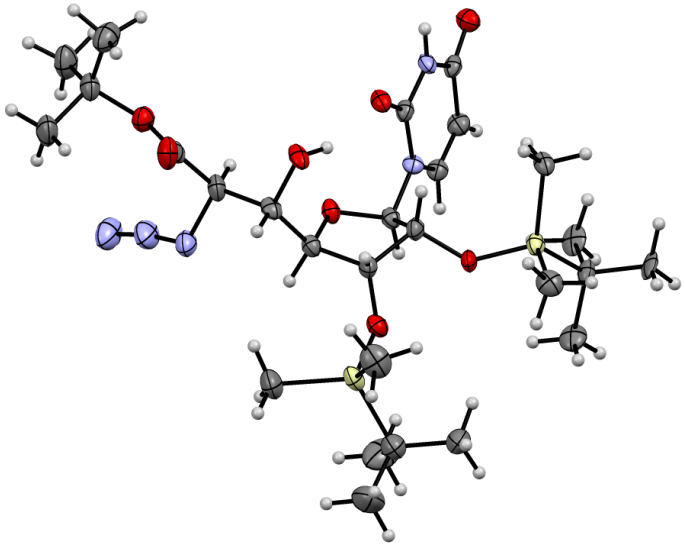
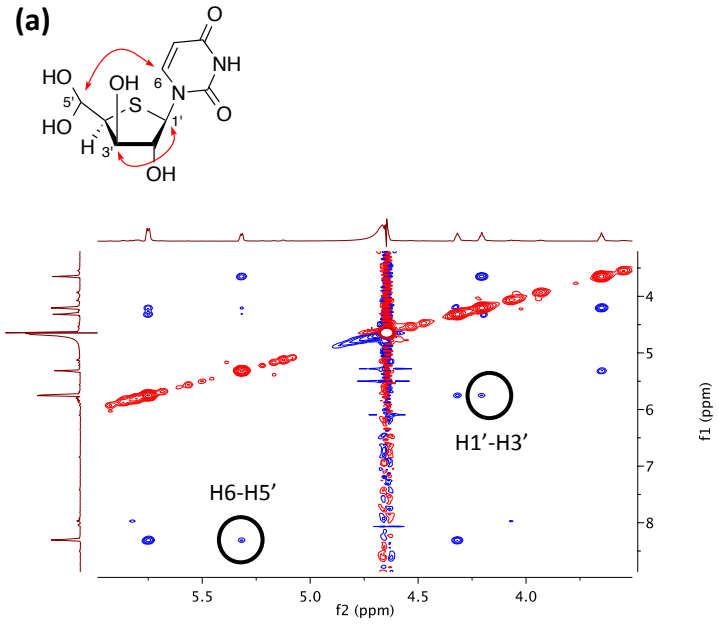
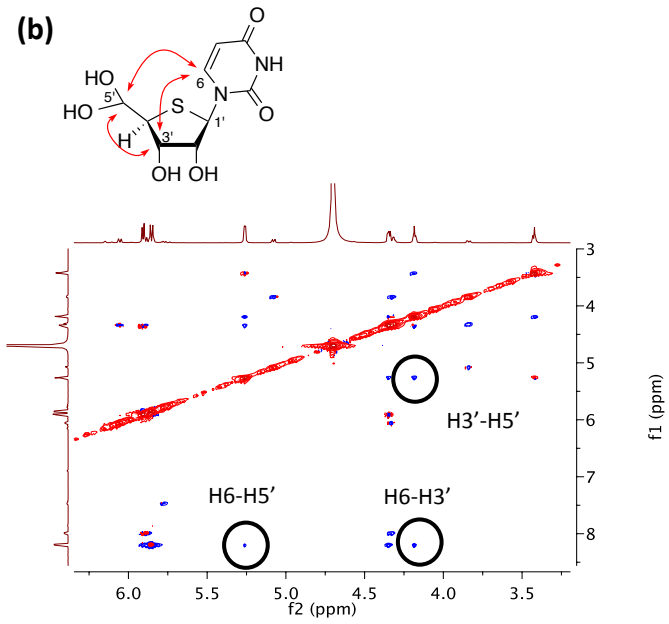
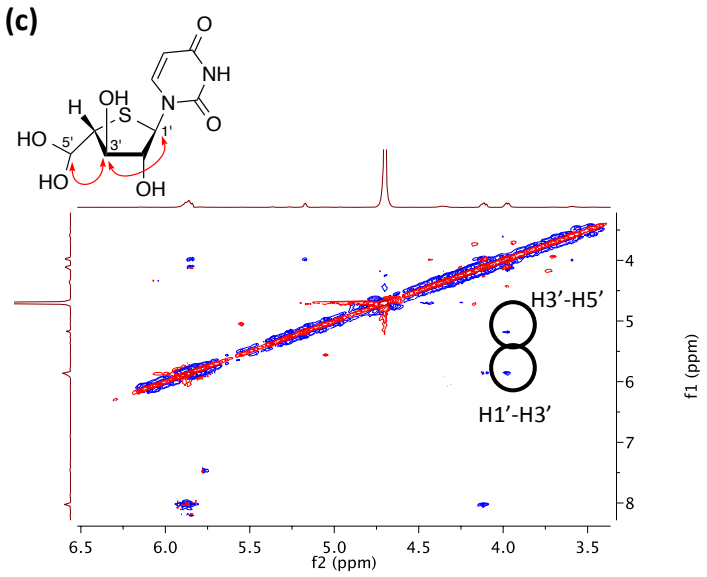
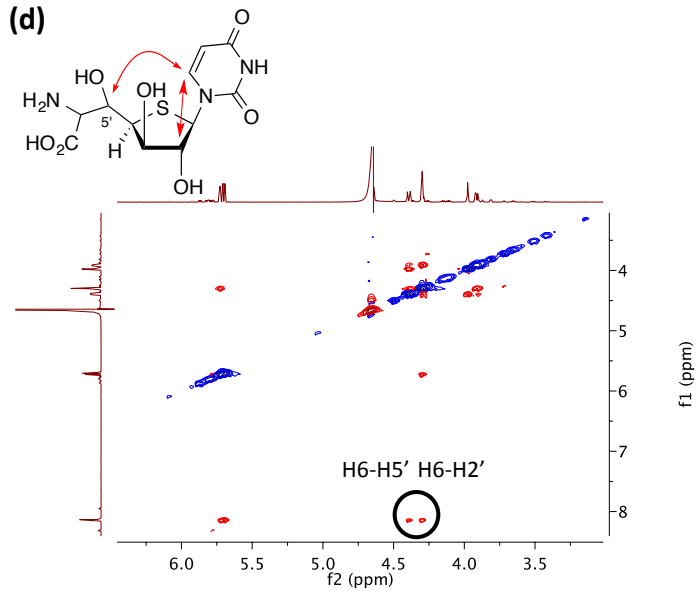


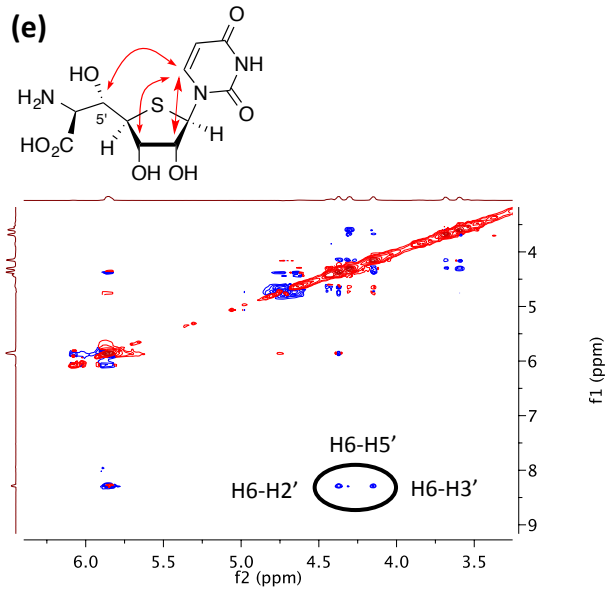
Figure S27. ORTEP drawing of **S24**.


(a)
H1′-H3′
H6-H5′
f2 (ppm)
f1 (ppm)


(b)
H3′-H5′
H6-H5′
H6-H3′
f2 (ppm)
f1 (ppm)


(c)
H3′-H5′
H1′-H3′
f2 (ppm)
f1 (ppm)


(d)
H6-H5′ H6-H2′
f2 (ppm)
f1 (ppm)


(e)
H6-H5′
H6-H2′
H6-H3′
f2 (ppm)
f1 (ppm)

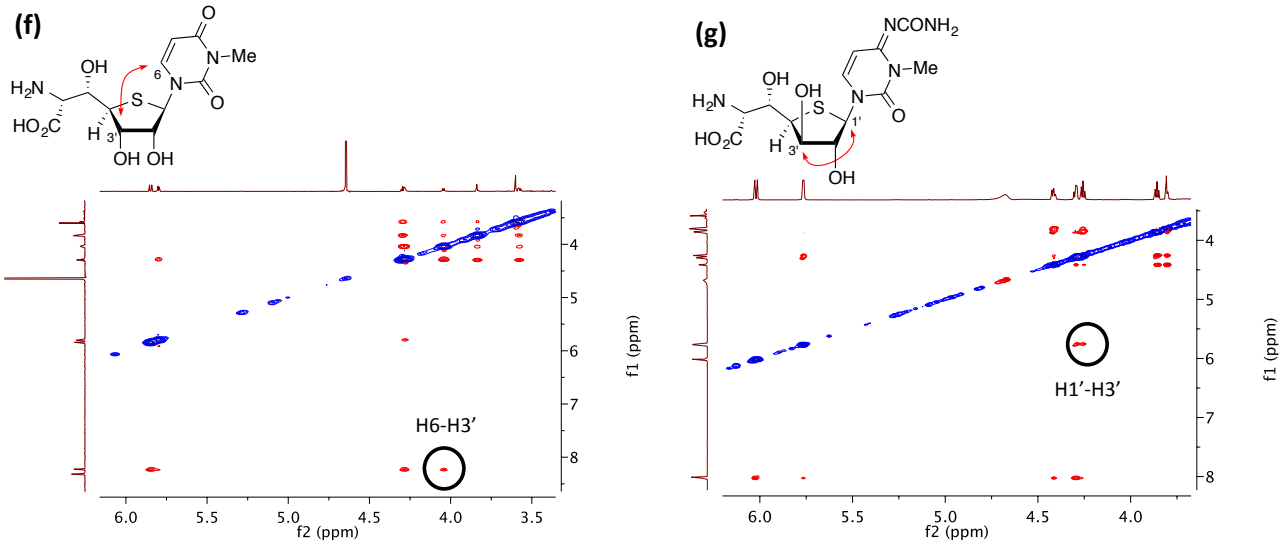


Figure S28. Selected through-space correlations in NOESY spectra. (a) **13**, (b) **27**, (c) **15**, (d) **16**, (e) (5′*S*,6′*S*)-**28**, (f) **23**, (g) **25**. The full NMR spectra are reported in Figures S28–S84.

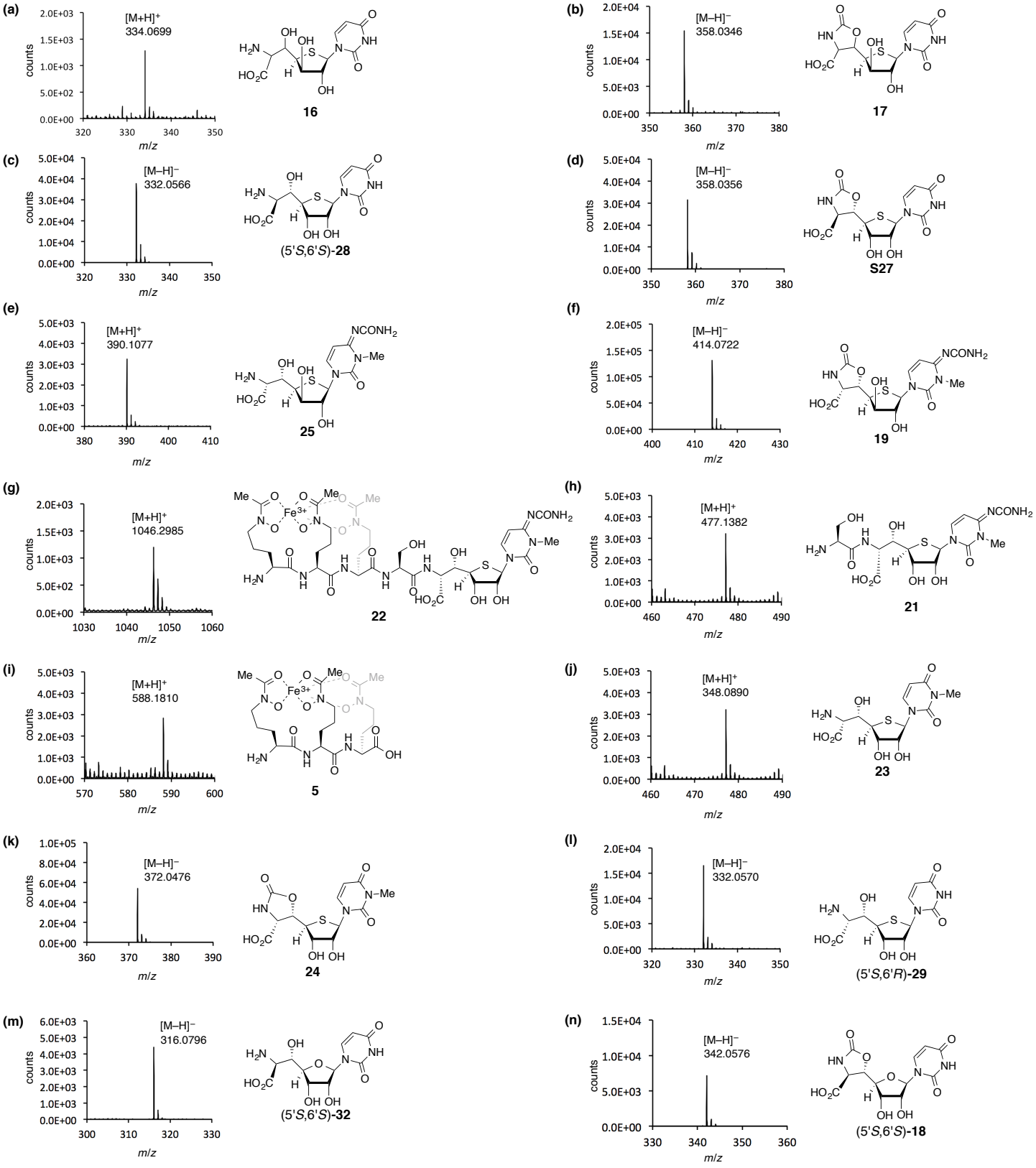

(a)
[M+H]+
334.0699
counts
m/z
16
(b)
[M–H]–
358.0346
17
(c)
[M–H]–
332.0566
(5'S,6'S)-28
(d)
[M–H]–
358.0356
S27
(e)
[M+H]+
390.1077
25
(f)
[M–H]–
414.0722
19
(g)
[M+H]+
1046.2985
22
(h)
[M+H]+
477.1382
21
(i)
[M+H]+
588.1810
5
(j)
[M+H]+
348.0890
23
(k)
[M–H]–
372.0476
24
(l)
[M–H]–
332.0570
(5'S,6'R)-29
(m)
[M–H]–
316.0796
(5'S,6'S)-32
(n)
[M–H]–
342.0576
(5'S,6'S)-18

Figure S29. ESI-MS spectra of enzymatic products, fermentation products, and derivatized compounds. (a) **16**, (b) **17**, (c) (5′*S*,6′*S*)-**28**, (d) **S27**, (e) **25**, (f) **19**, (g) **22**, (h) **23**, (i) **5**, (j) **23,** (k) **24**, (l) **29**, (m) **32**, (n) **18**, (o) **33**, (p) **S28**.

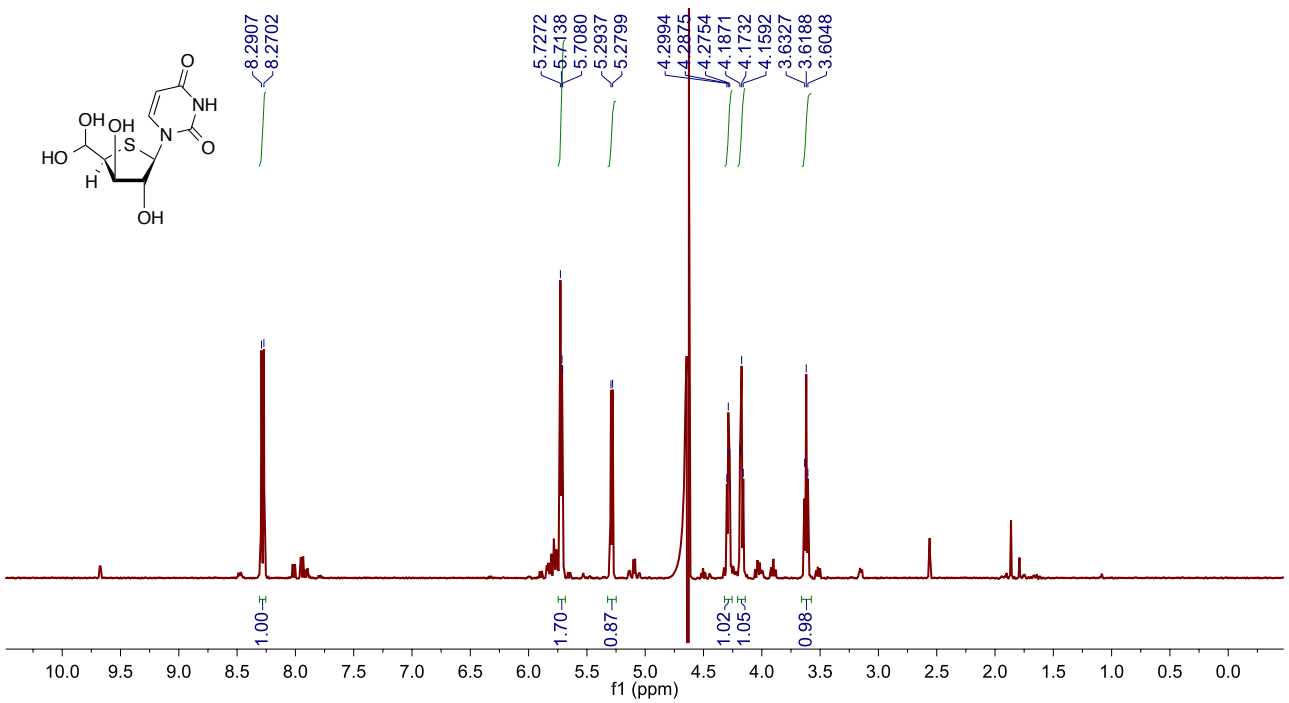


Figure S30. ^{1}H NMR of **13** (400 MHz in D_2O)

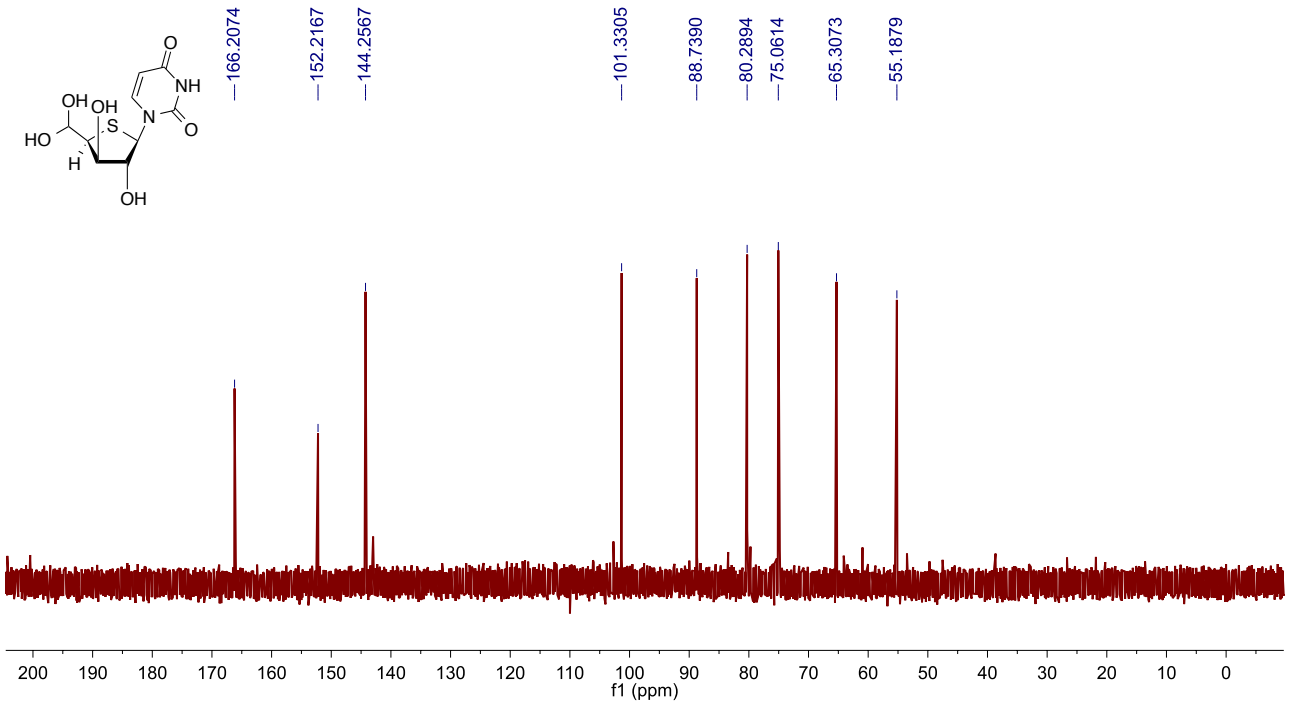


Figure S31 ^{13}C NMR of **13** (101 MHz in D_2O)

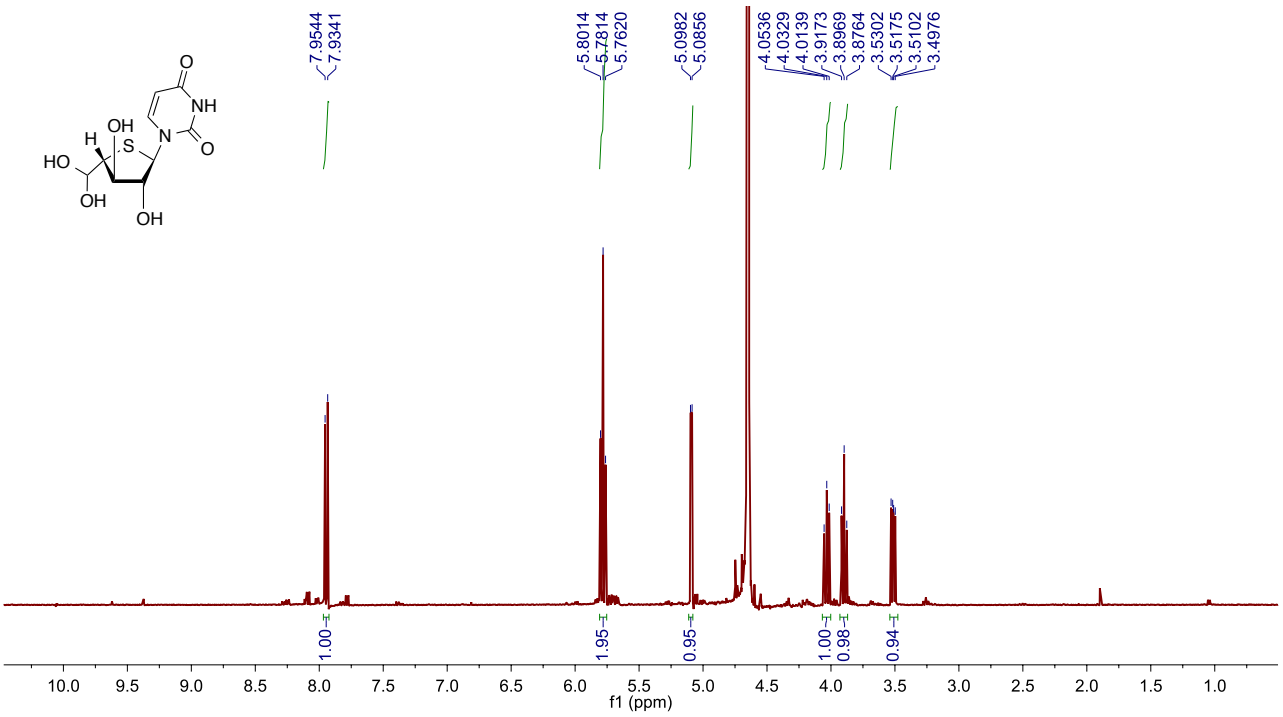


Figure S32. ^{1}H NMR of **15** (400 MHz in D_2O)

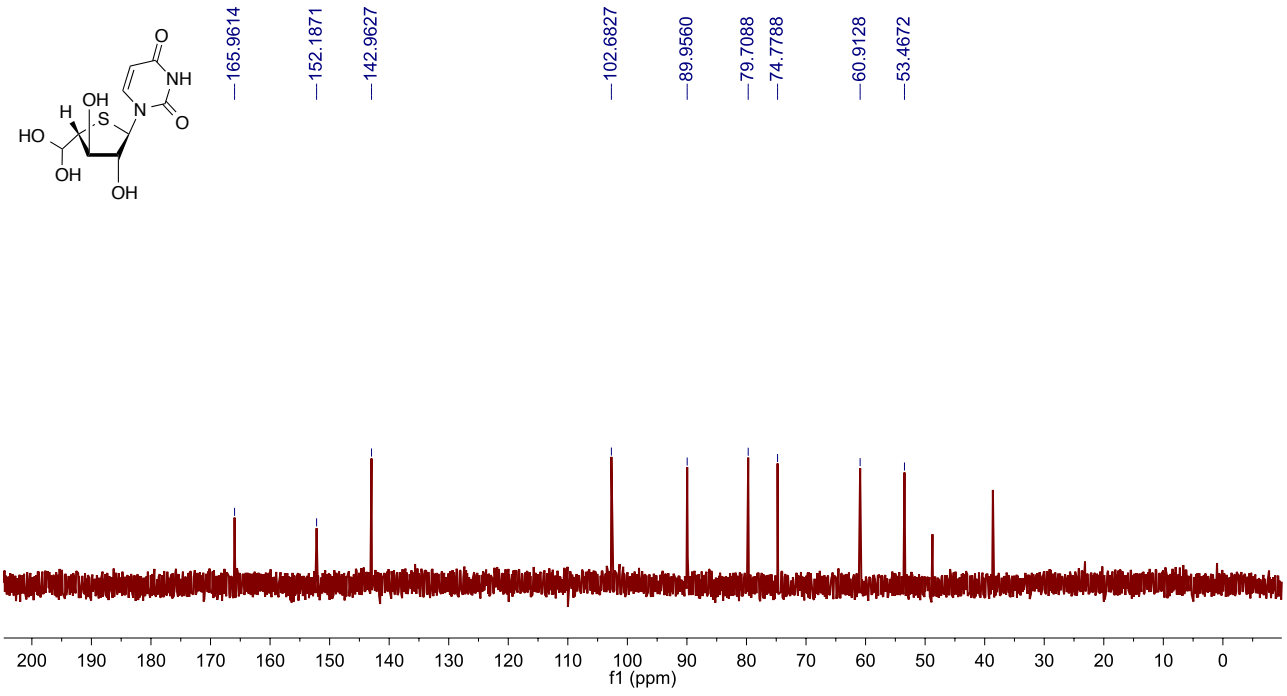


Figure S33. ^{13}C NMR of **15** (101 MHz in D_2O)

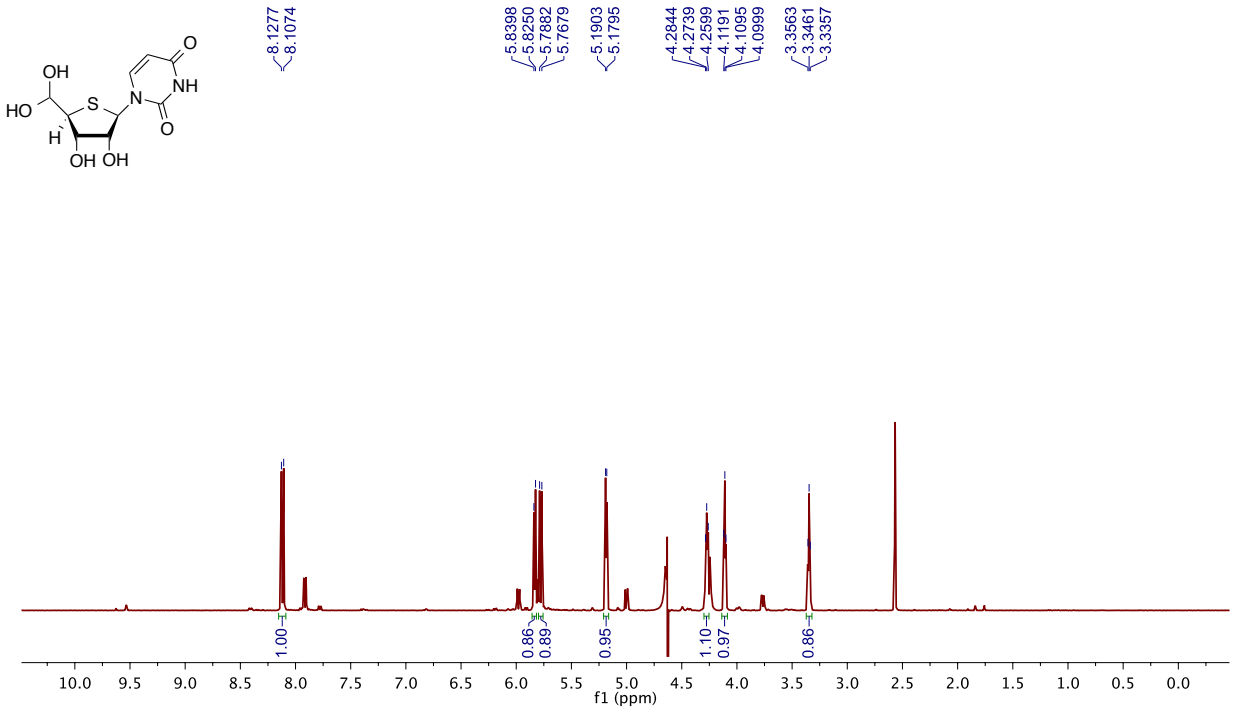


Figure S34. ^{1}H NMR of **27** (400 MHz in D_2O)

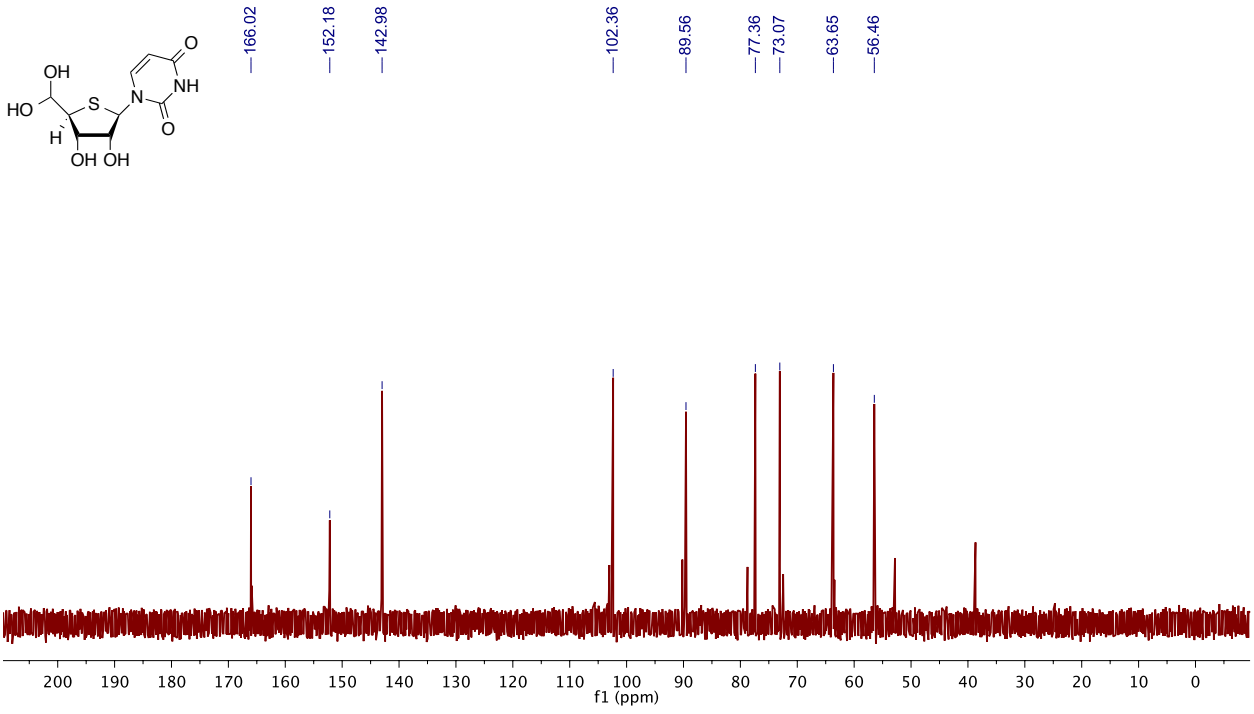


Figure S35. ^{13}C NMR of **27** (101 MHz in D_2O)

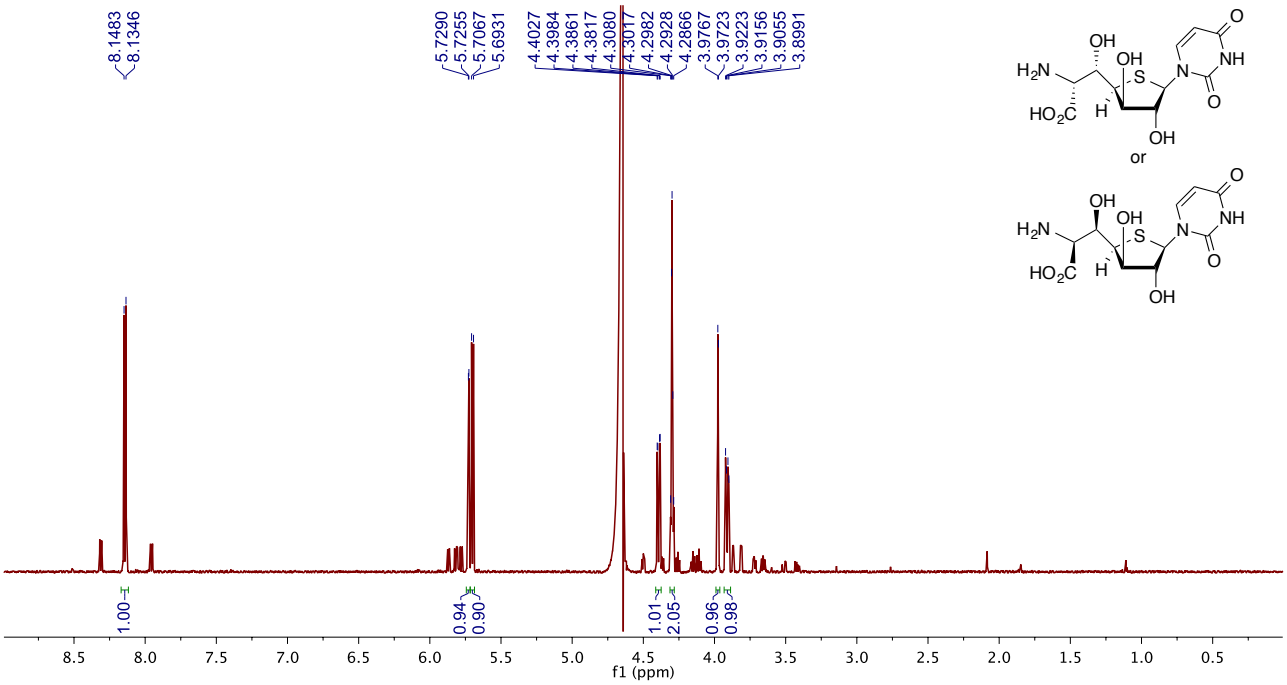


Figure S36. ^{1}H NMR of **16** (600 MHz in D_2O)

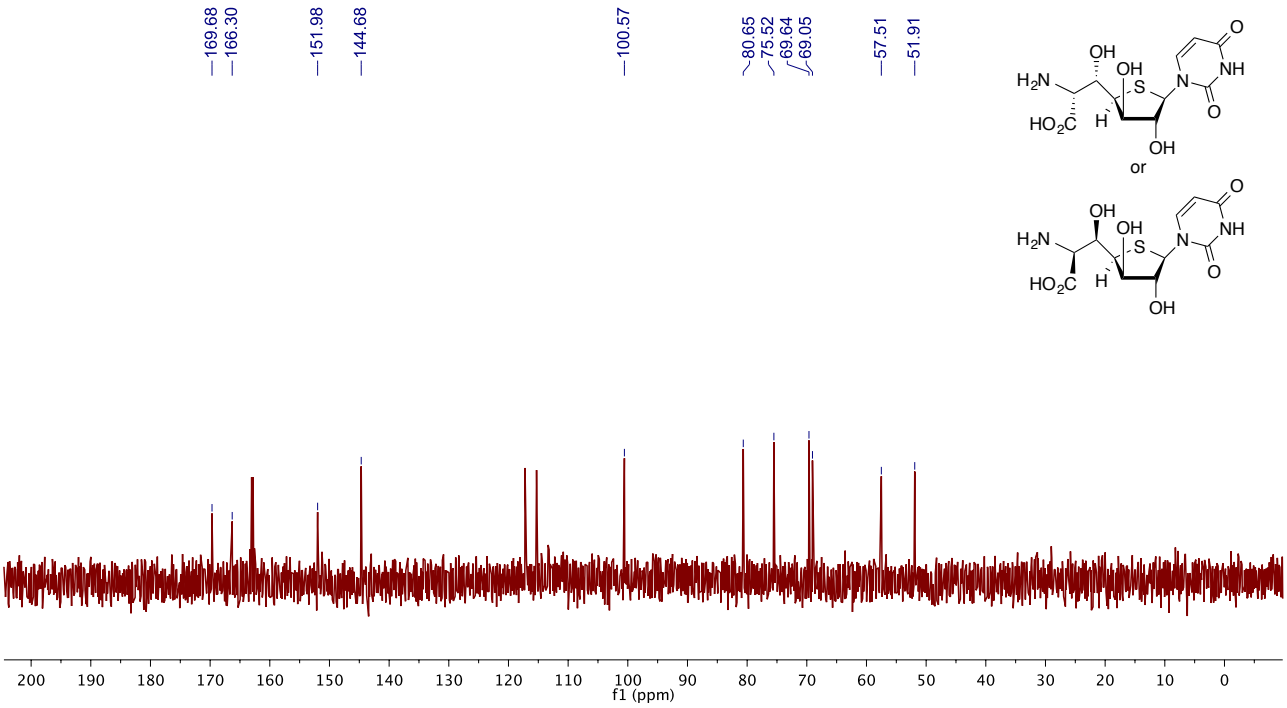


Figure S37. ^{13}C NMR of **16** (151 MHz in D_2O)

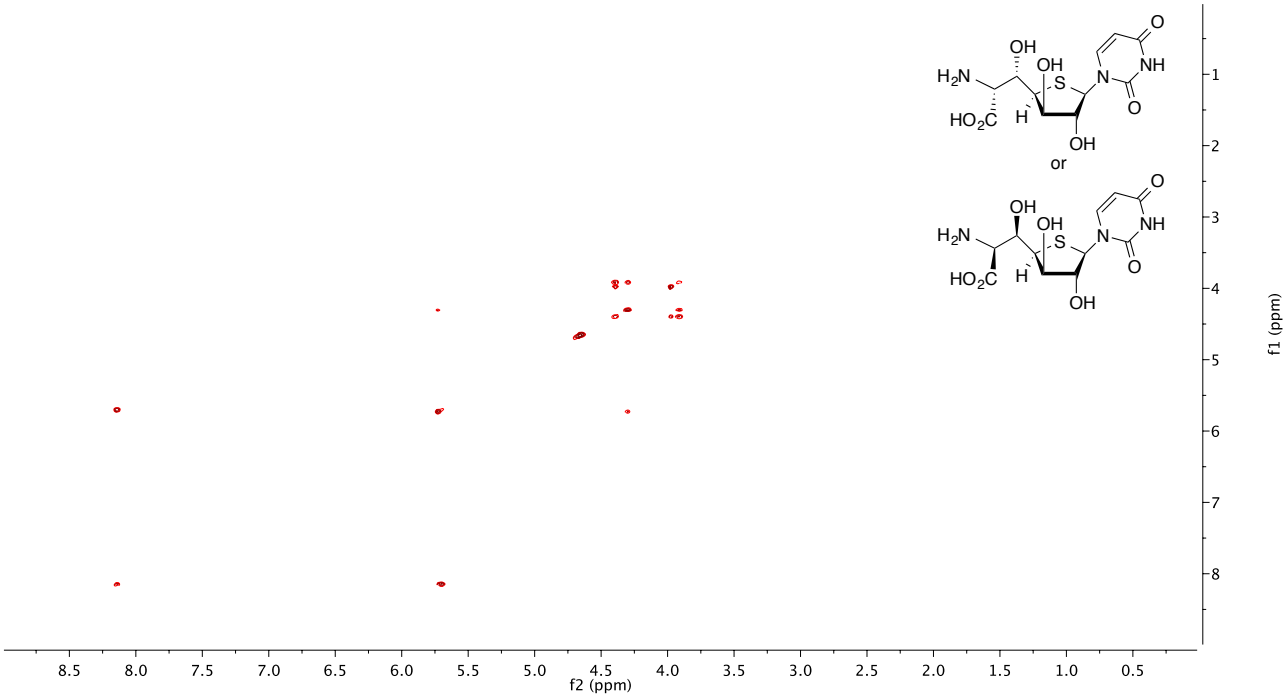


Figure S38. COSY spectrum of **16**

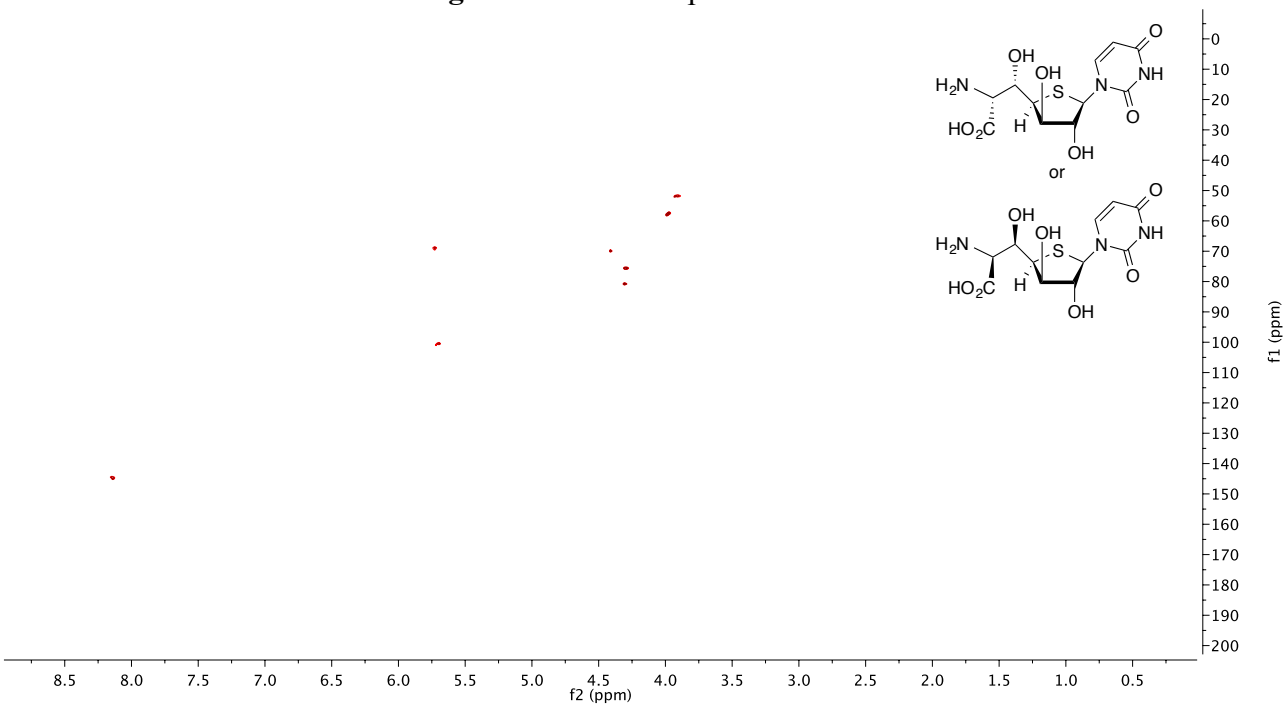


Figure S39. HSQC spectrum of **16**

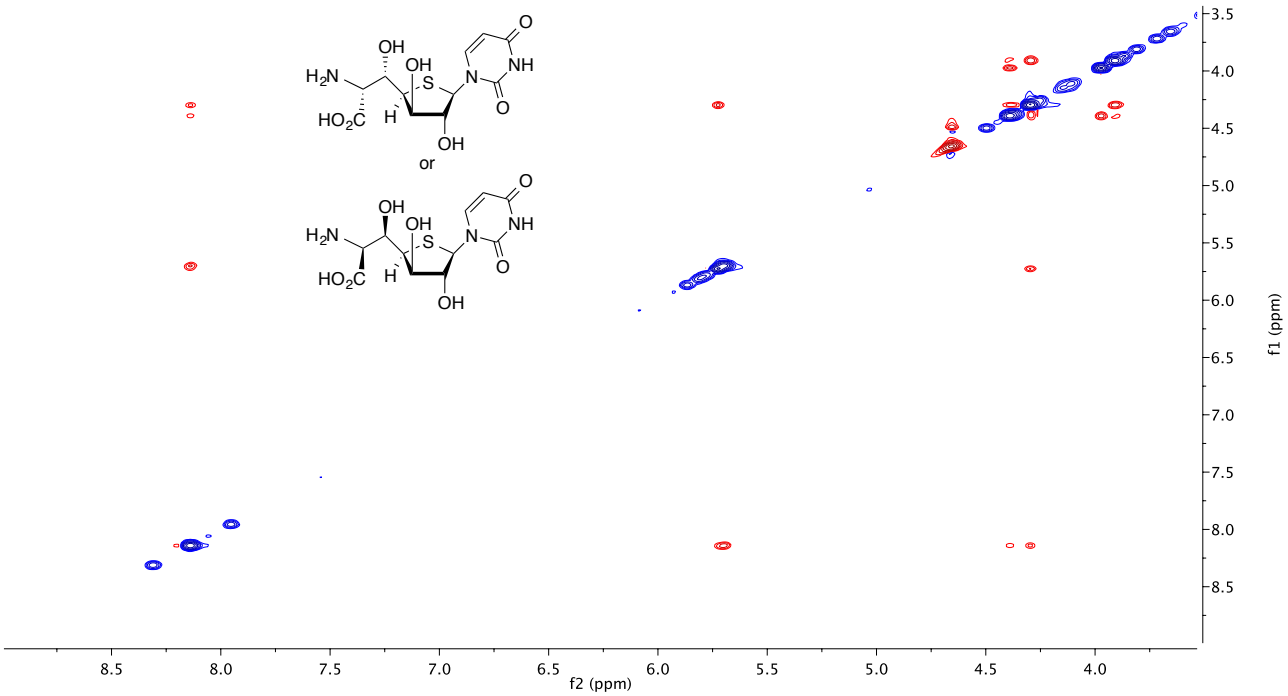


Figure S40. NOESY spectrum of **16**

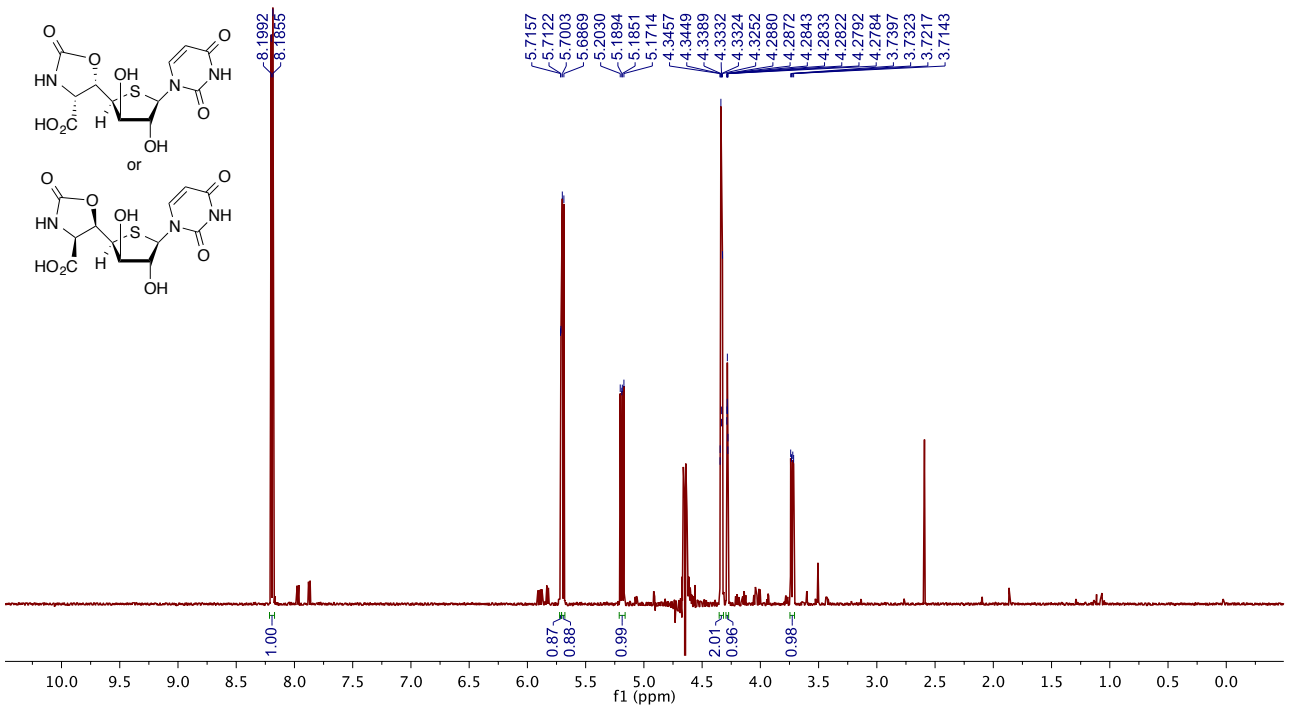


Figure S41. ^{1}H NMR of **17** (600 MHz in D_2O)

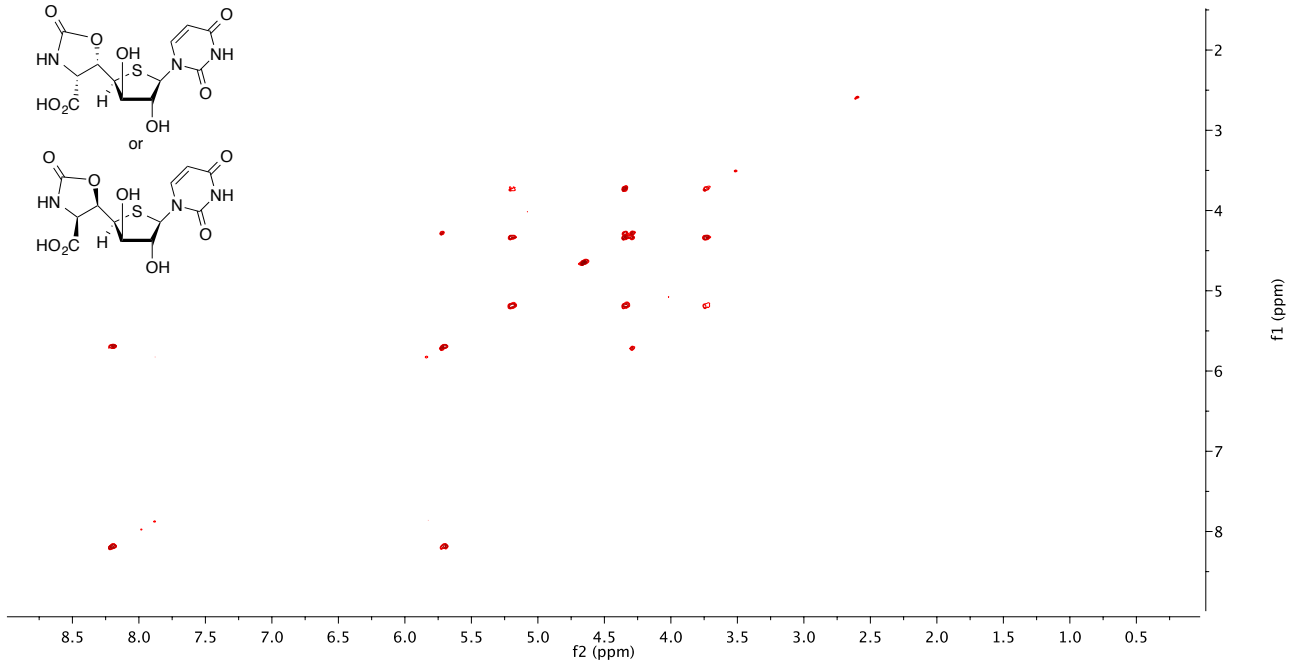


Figure S42. COSY spectrum of **17**

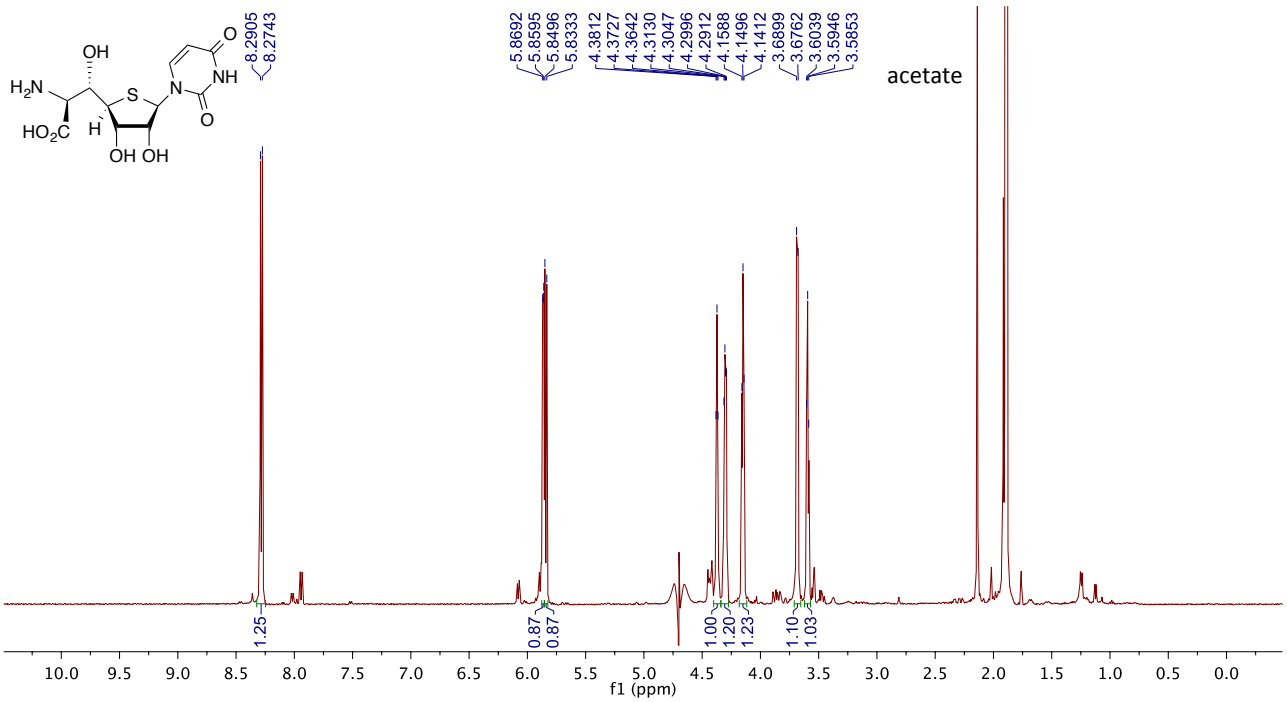


Figure S43. ^{1}H NMR of (5′*S*,6′*S*)-**28** (500 MHz in D_2O)

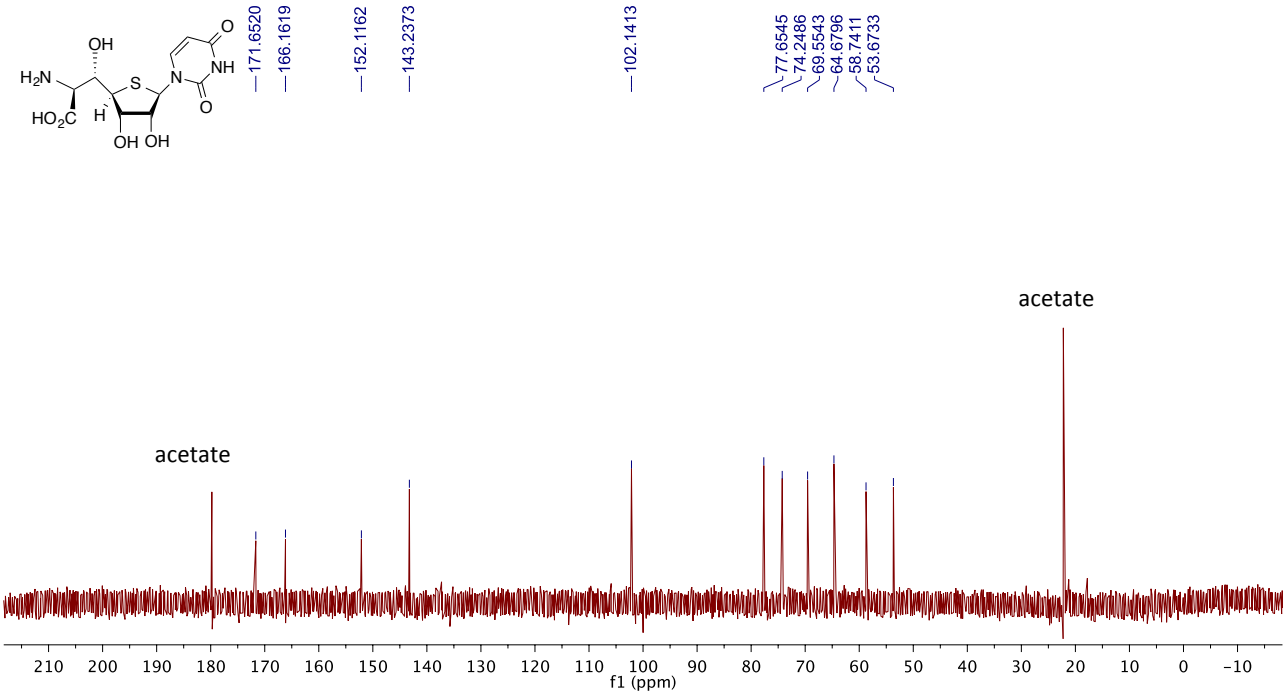


Figure S44. ^{13}C NMR of (5′*S*,6′*S*)-**28** (126 MHz in D_2O)

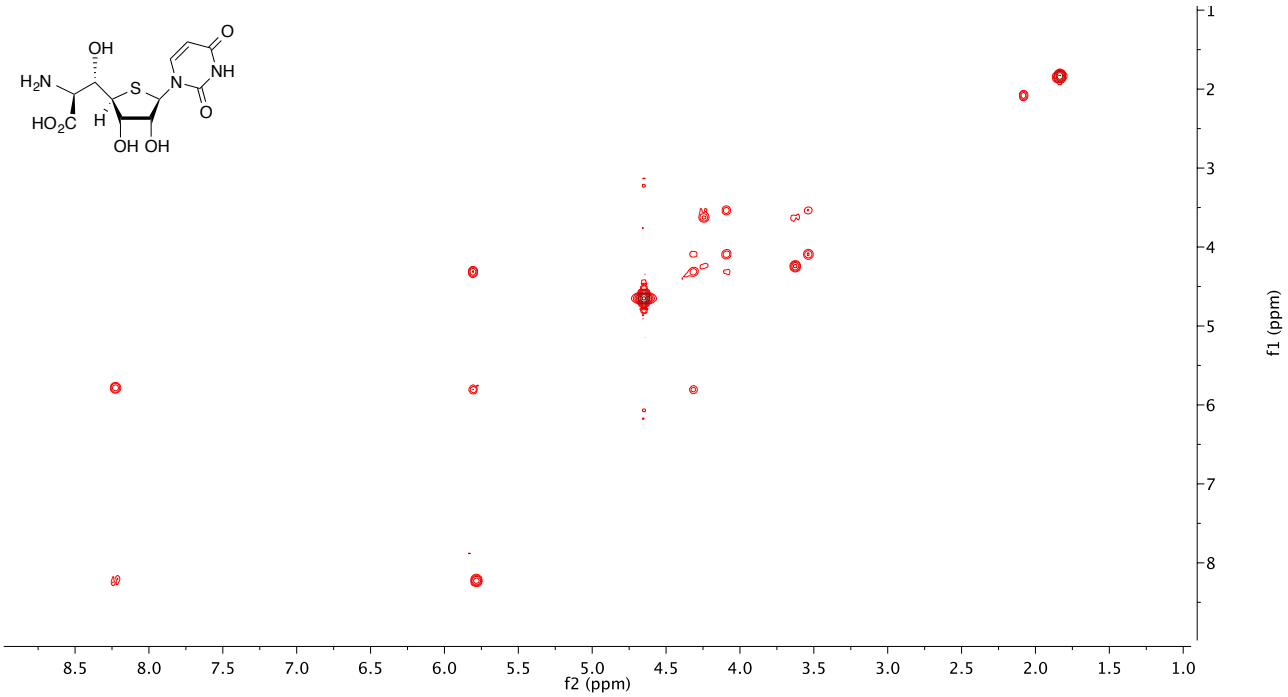


Figure S45. COSY of (5′*S*,6′*S*)-**28**

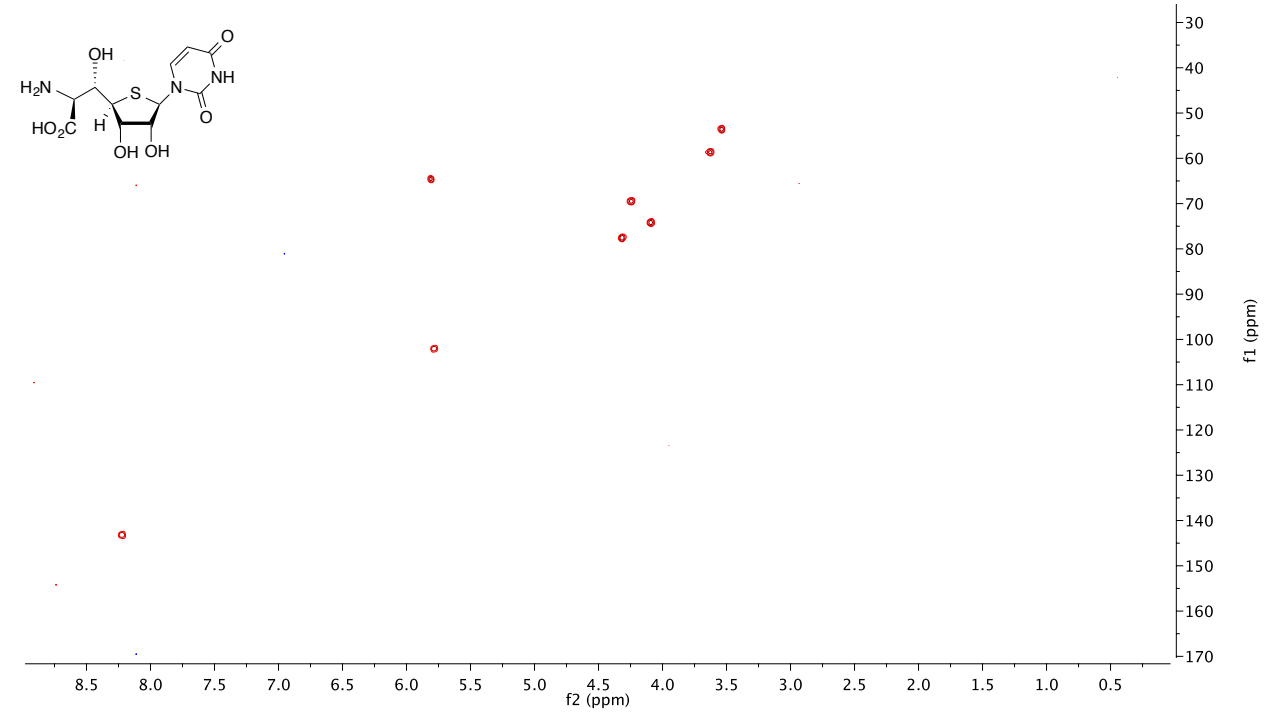


Figure S46. HSQC spectrum of (5′*S*,6′*S*)-**28**

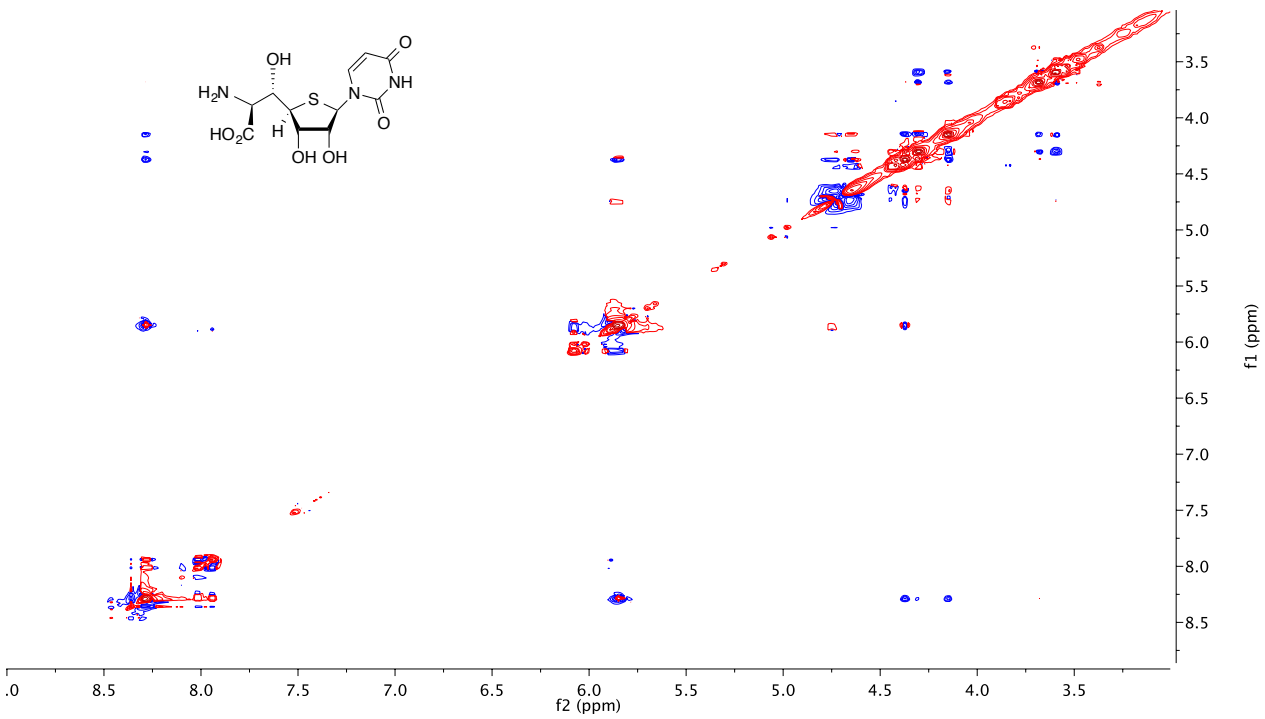


Figure S47. NOESY spectrum of (5′*S*,6′*S*)-**28**

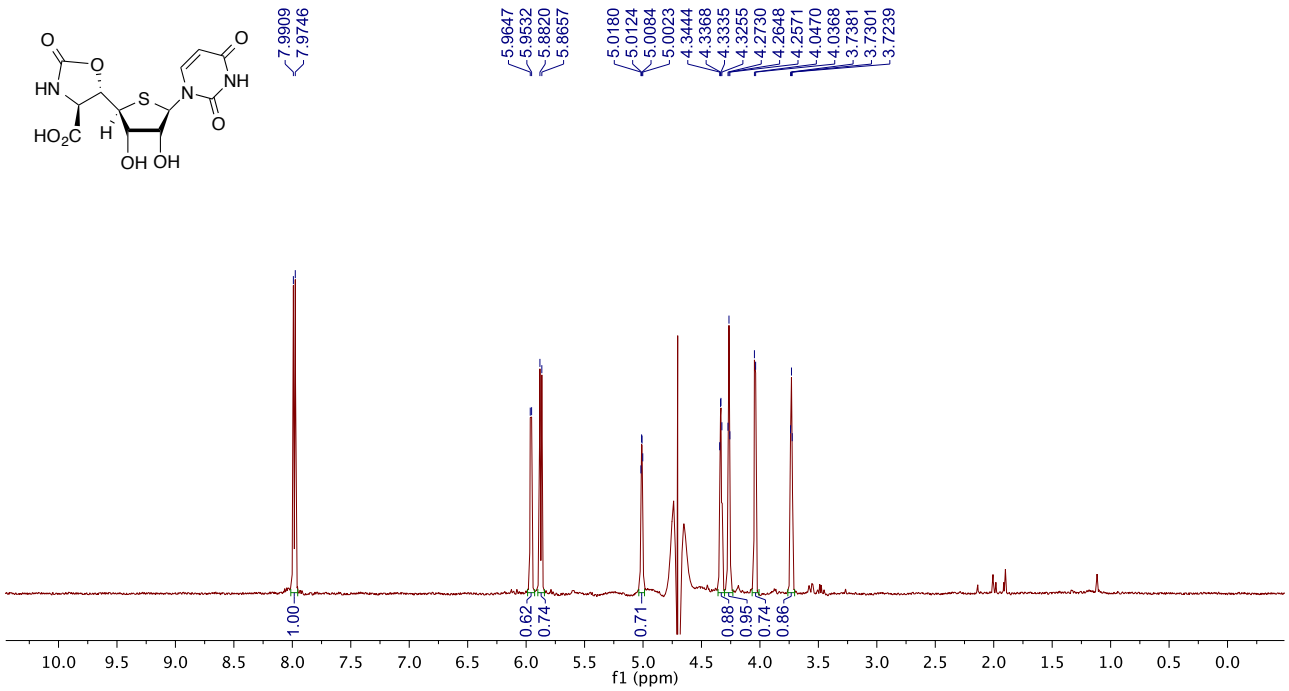


Figure S48. ^{1}H NMR of **S27** (600 MHz in D_2O)

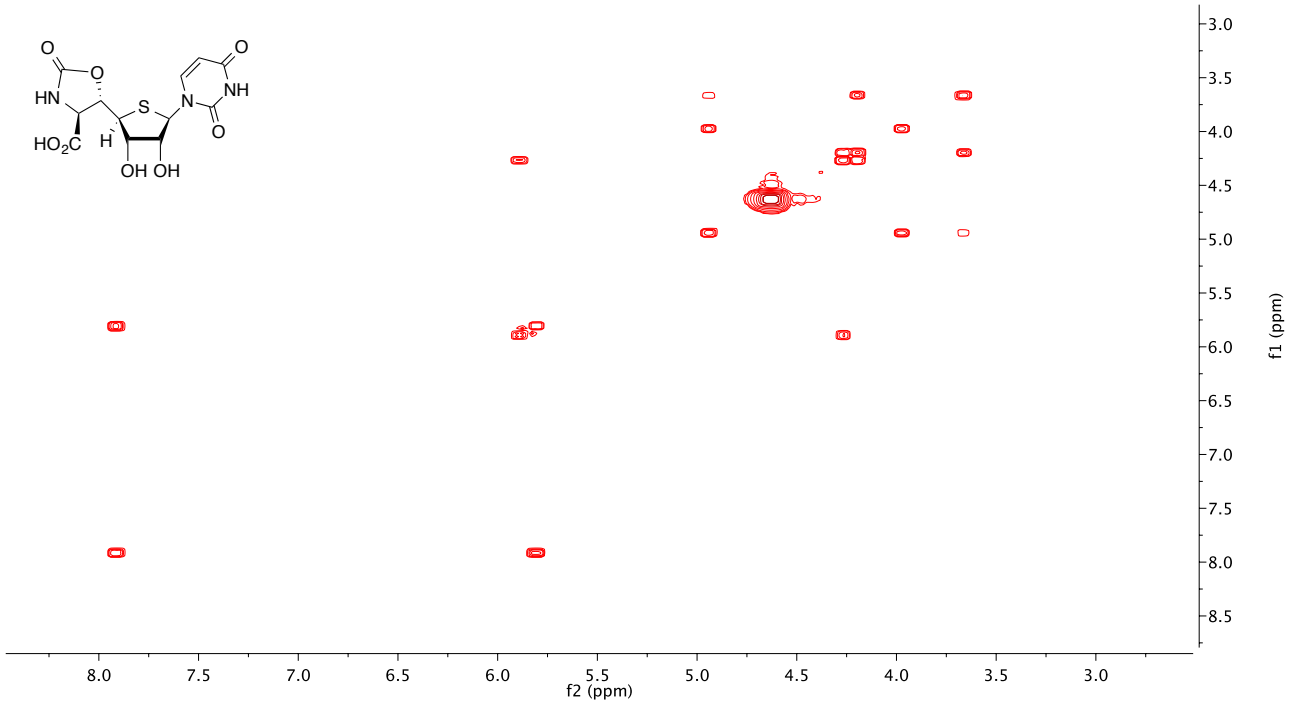


Figure S49. COSY spectrum of **S27**

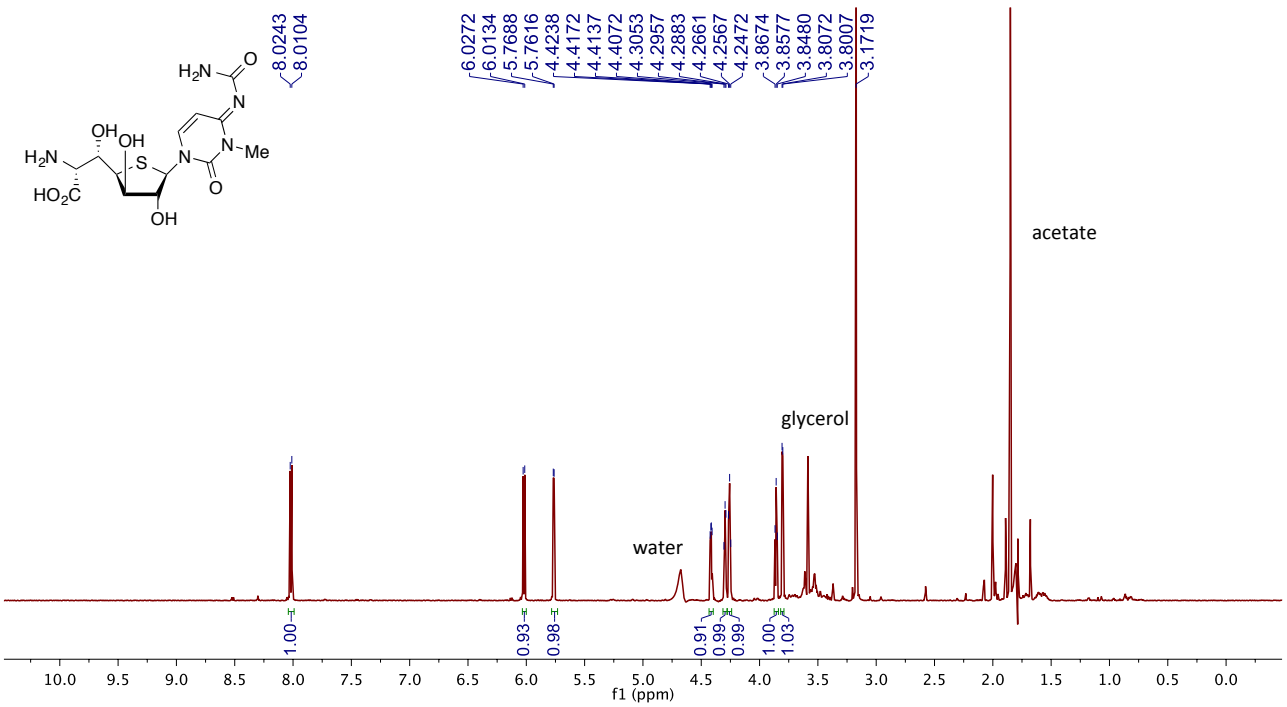


Figure S50. ^{1}H NMR of **25** (600 MHz in D_2O)

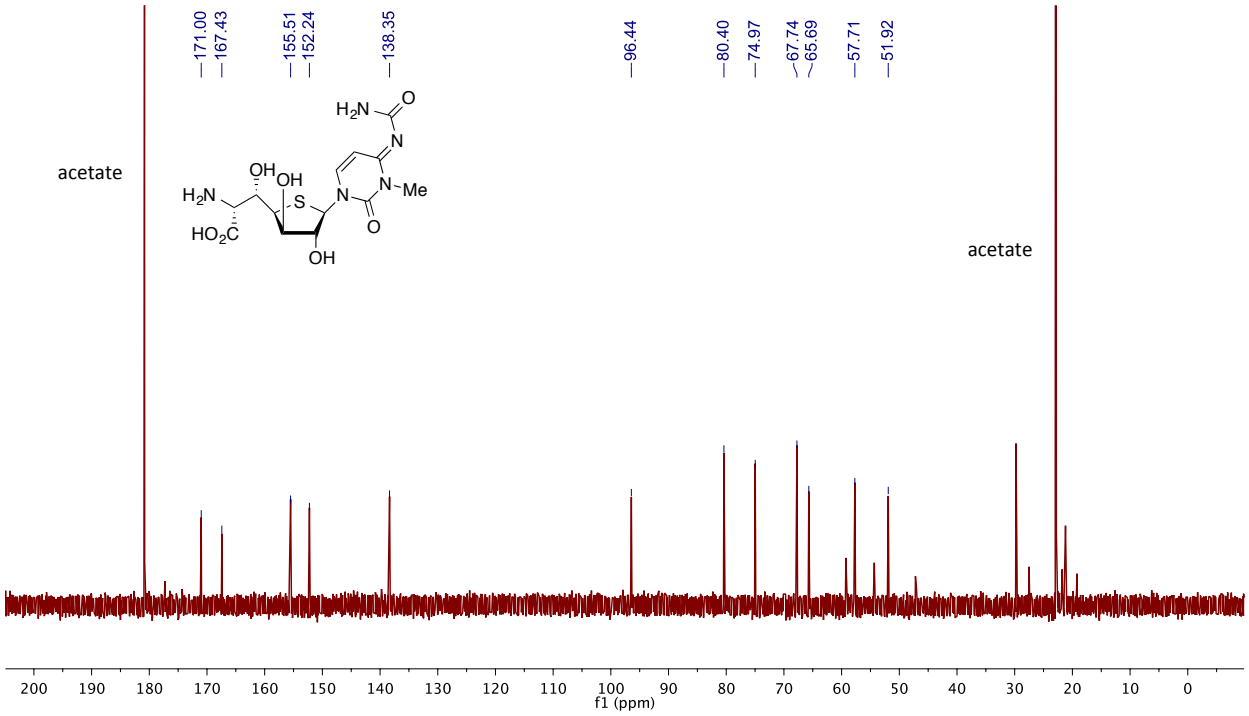


Figure S51. ^{13}C NMR of **25** (151 MHz in D_2O)

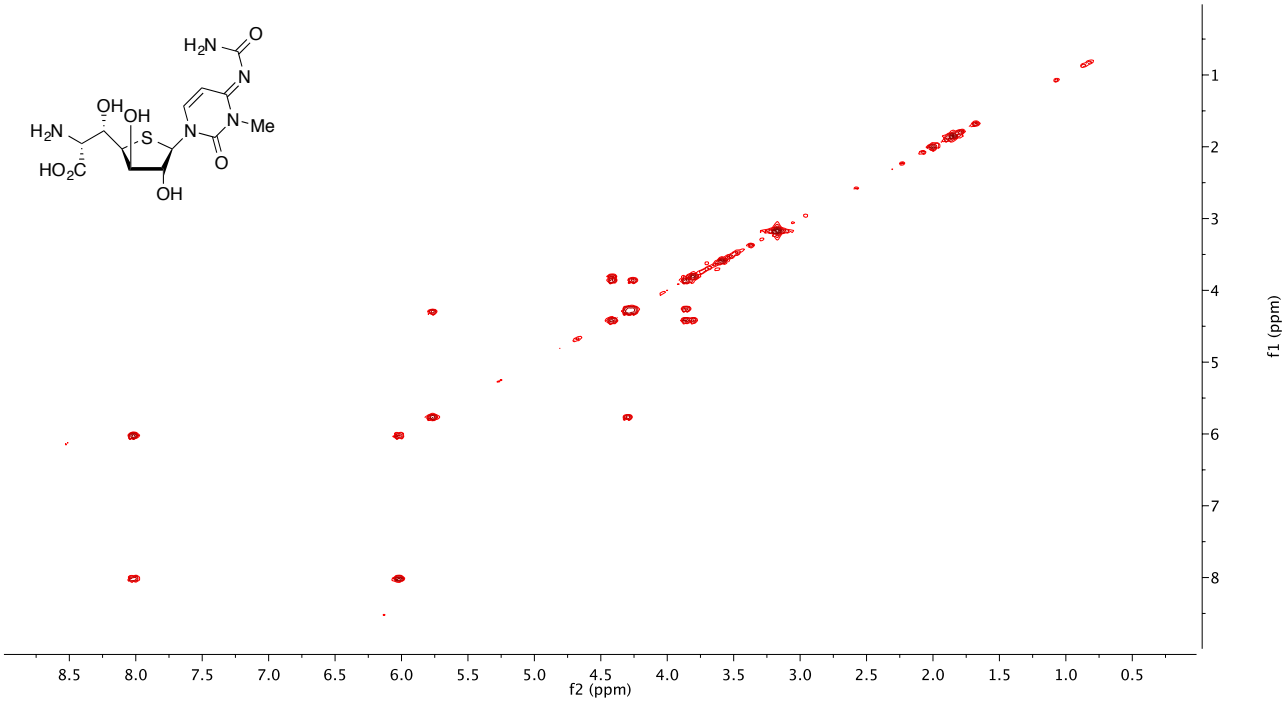


Figure S52. COSY spectrum of **25**

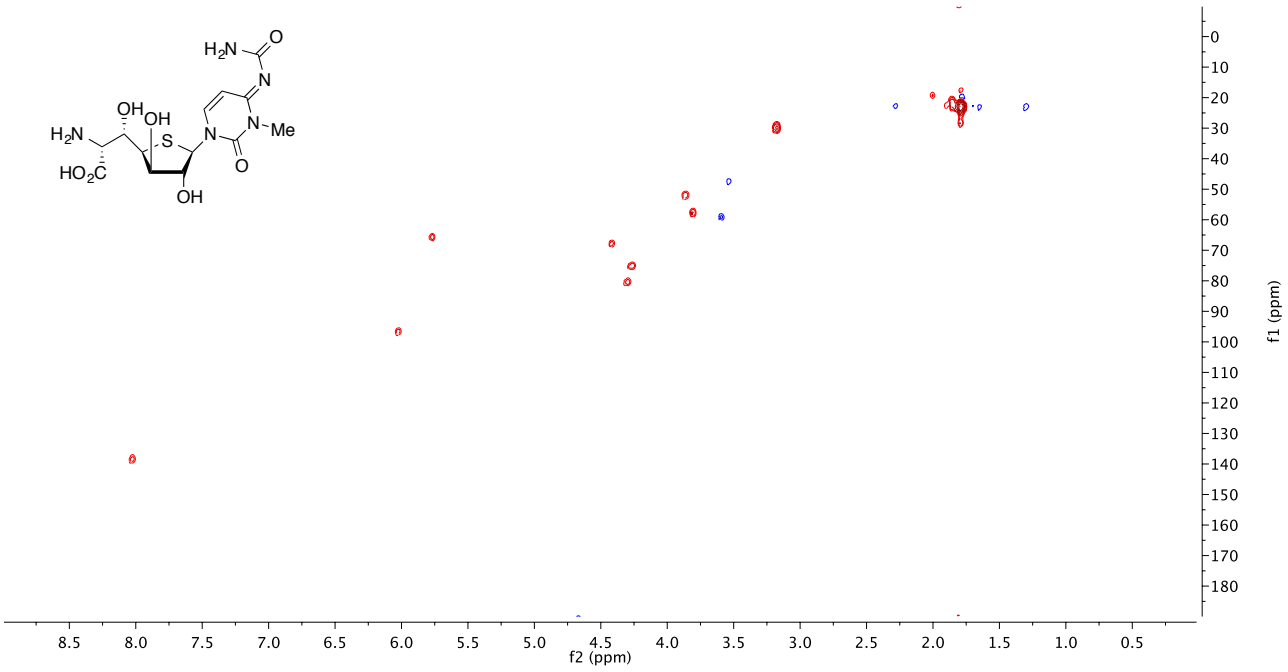


Figure S53. HSQC spectrum of **25**

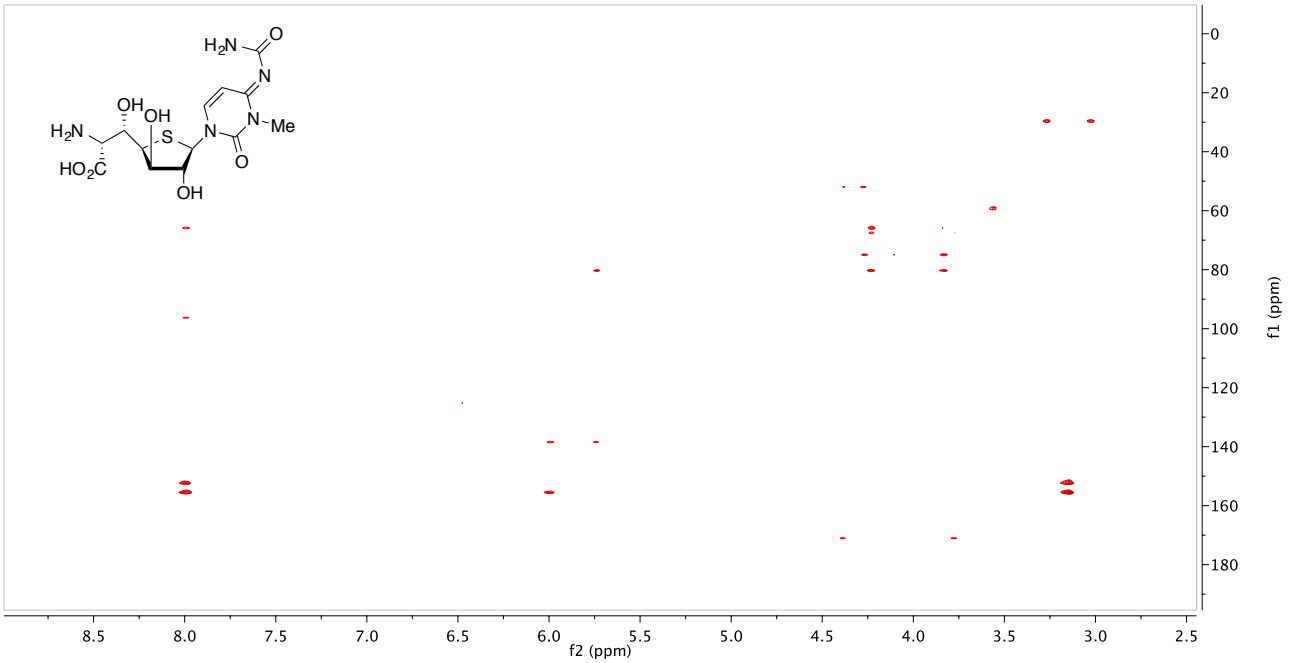


Figure S54. HMBC spectrum of **25**

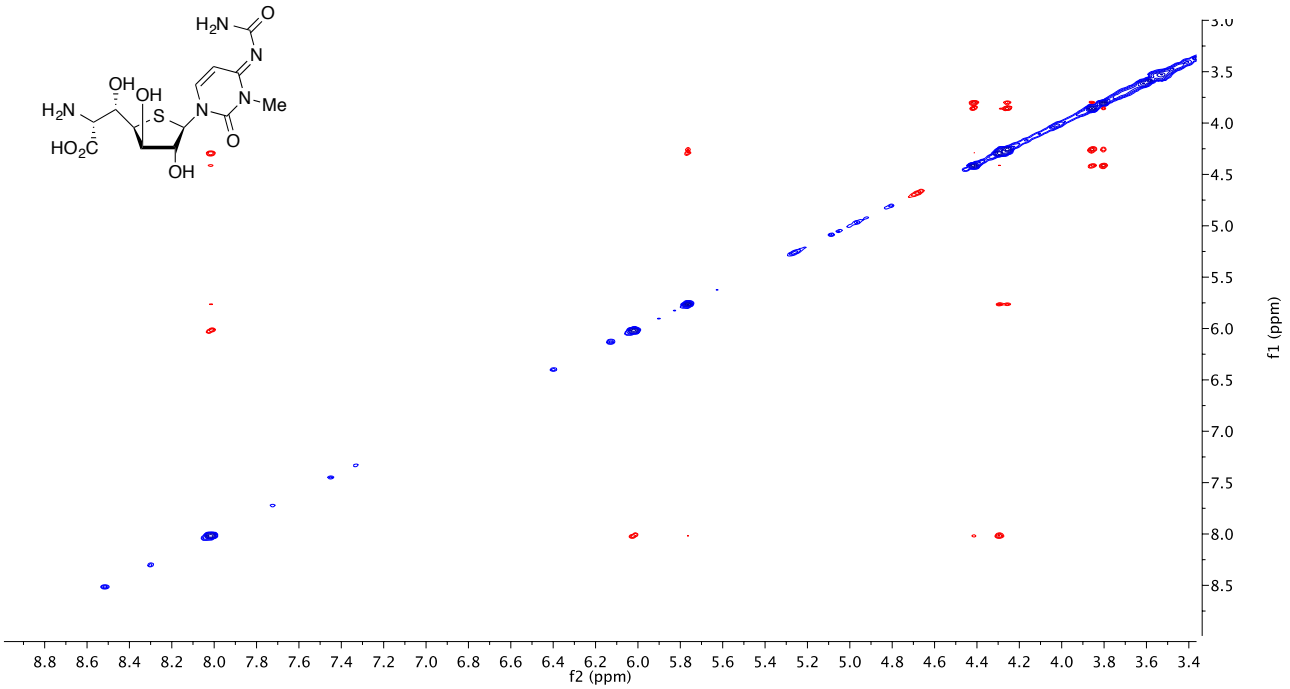


Figure S55. NOESY spectrum of **25**

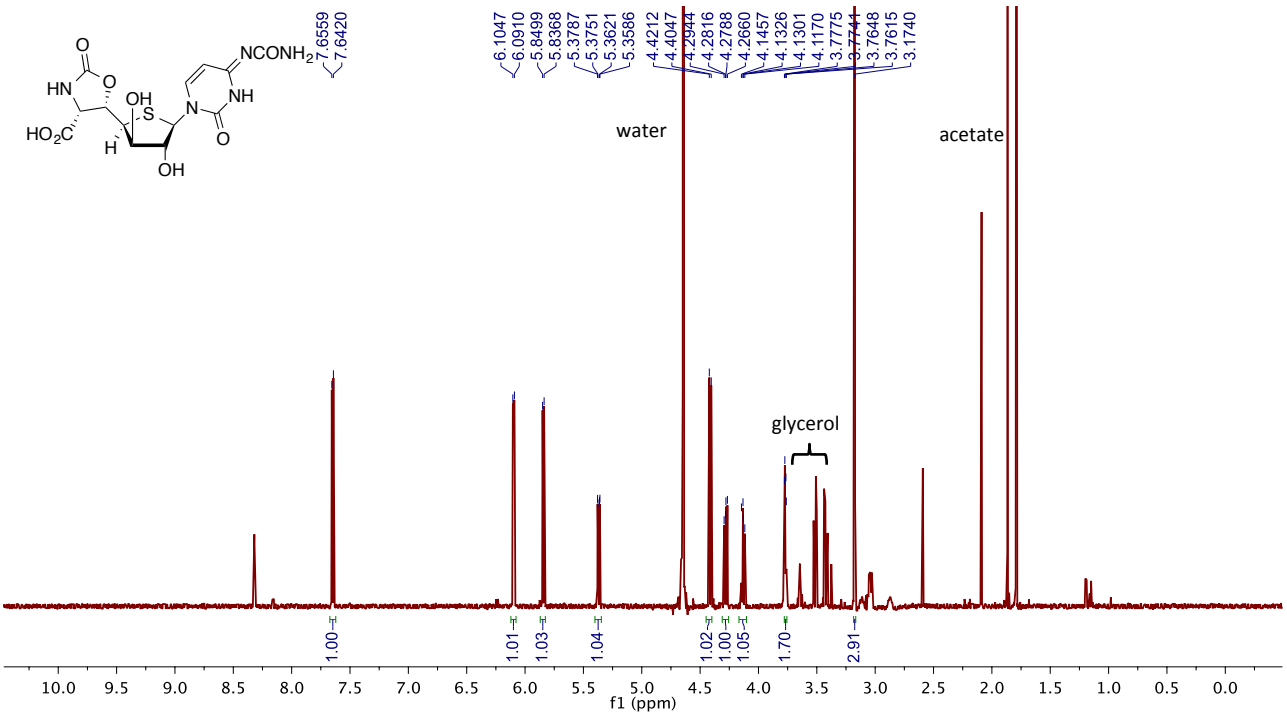


Figure S56. ^{1}H NMR of **19** (600 MHz in D_2O)

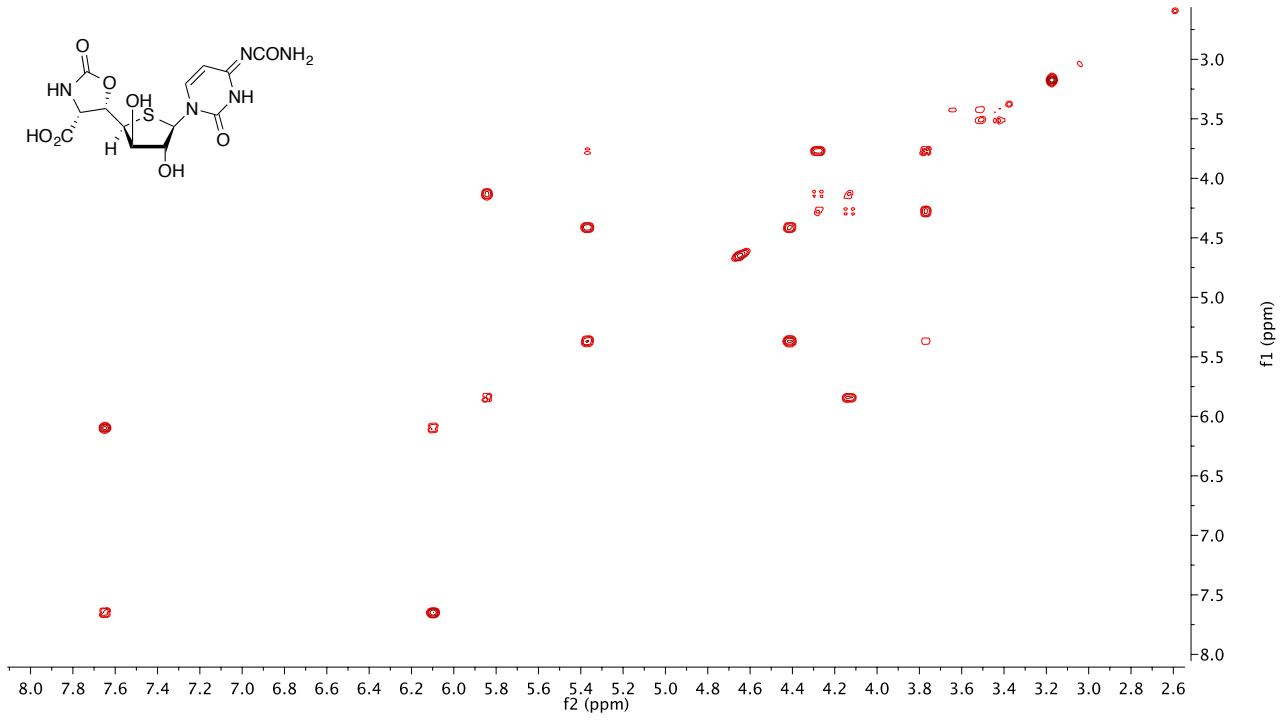


Figure S57. COSY spectrum of **19**

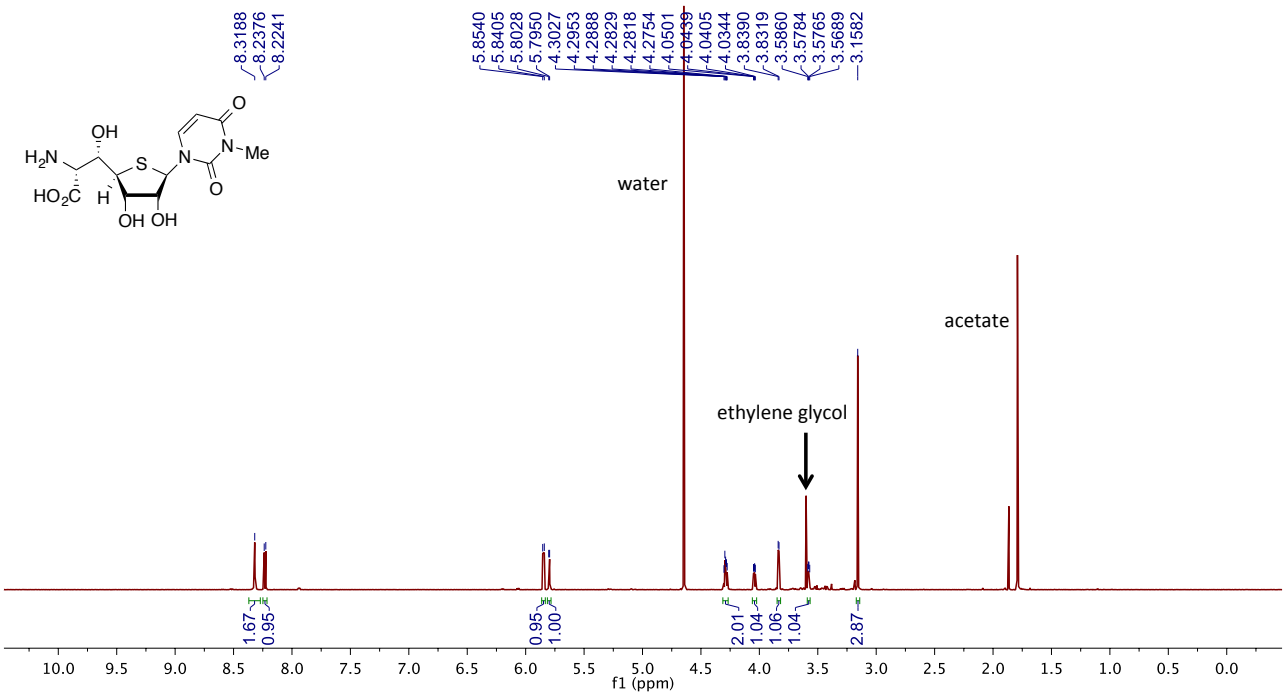


Figure S58. ^{1}H NMR of **23** (600 MHz in D_2O)

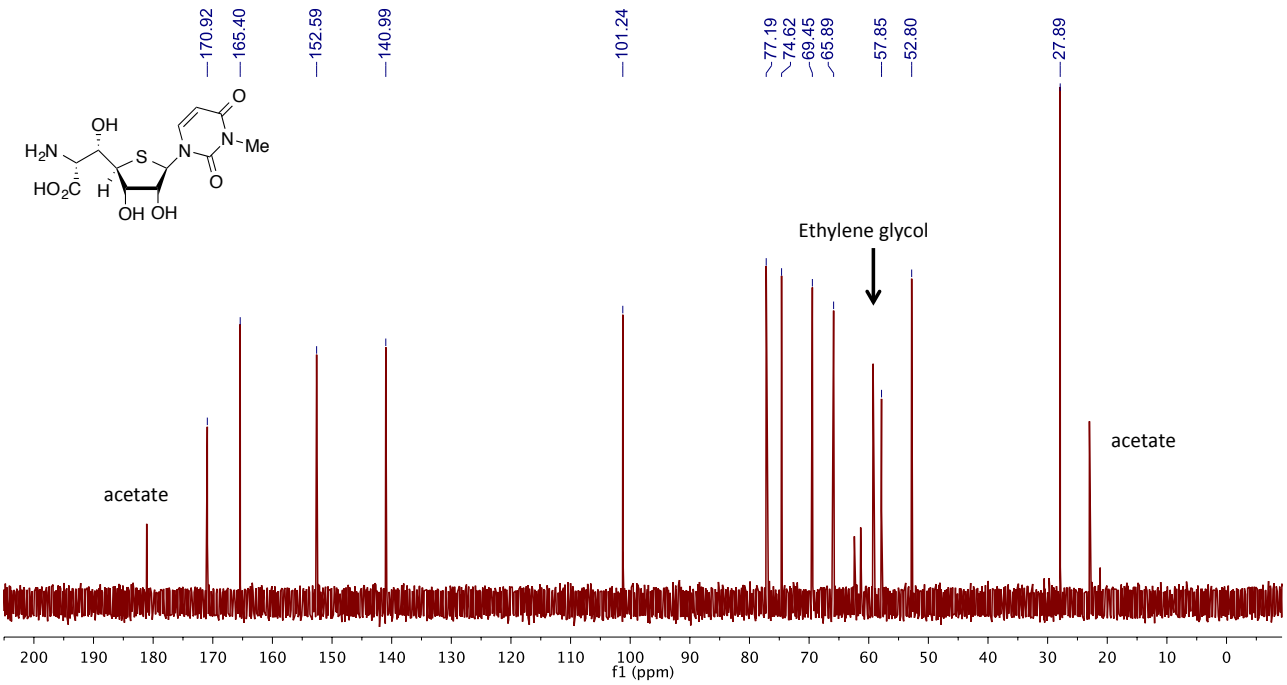


Figure S59. ^{13}C NMR of **23** (151 MHz in D_2O)

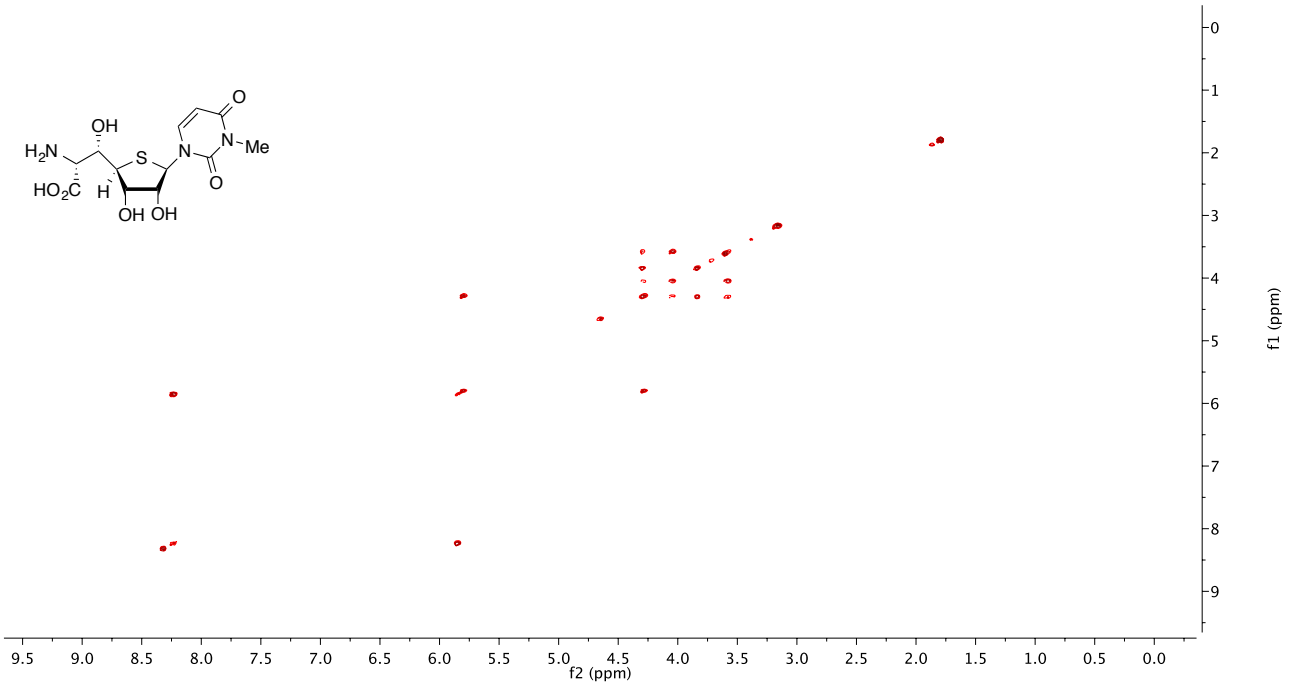


Figure S60. COSY spectrum of **23**

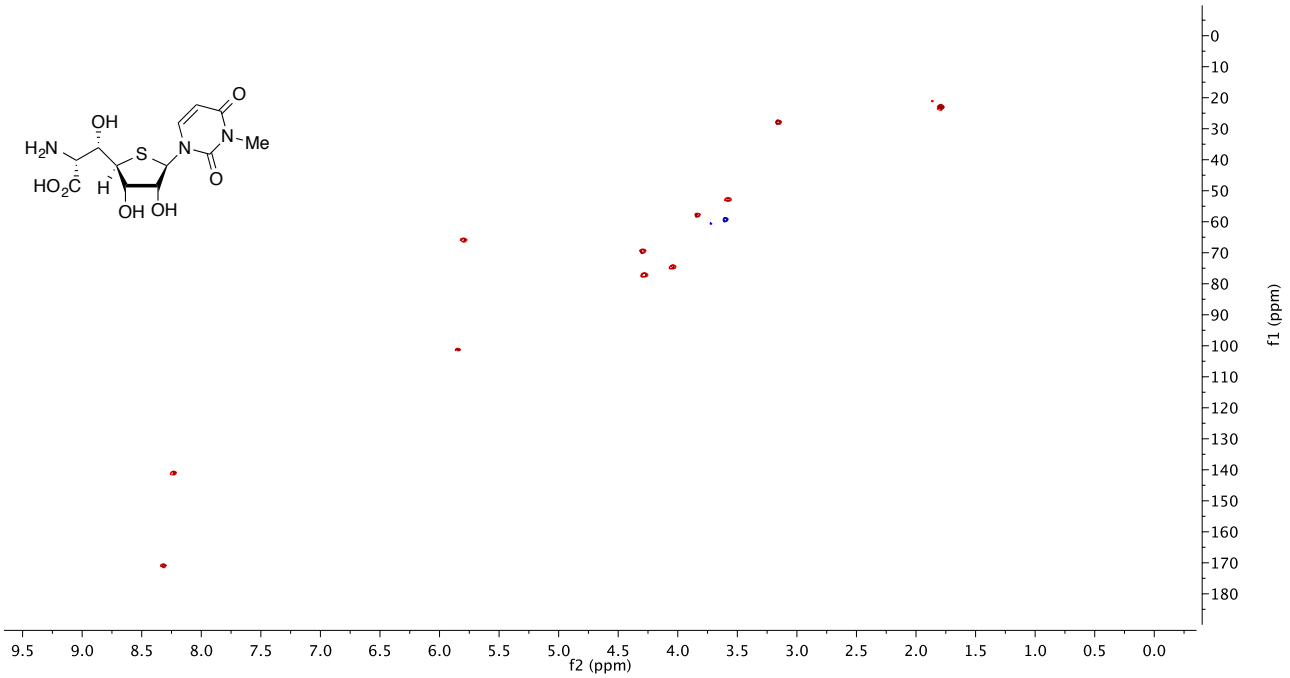


Figure S61. HSQC spectrum of **23**

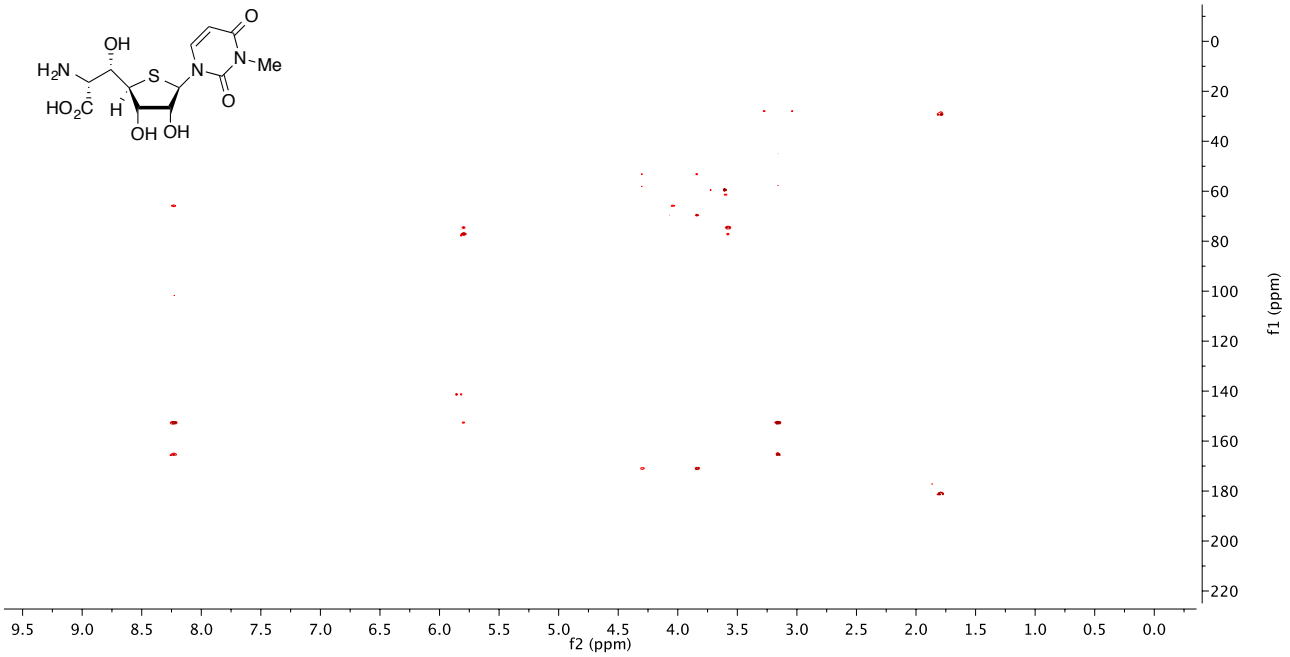


Figure S62. HMBC spectrum of **23**

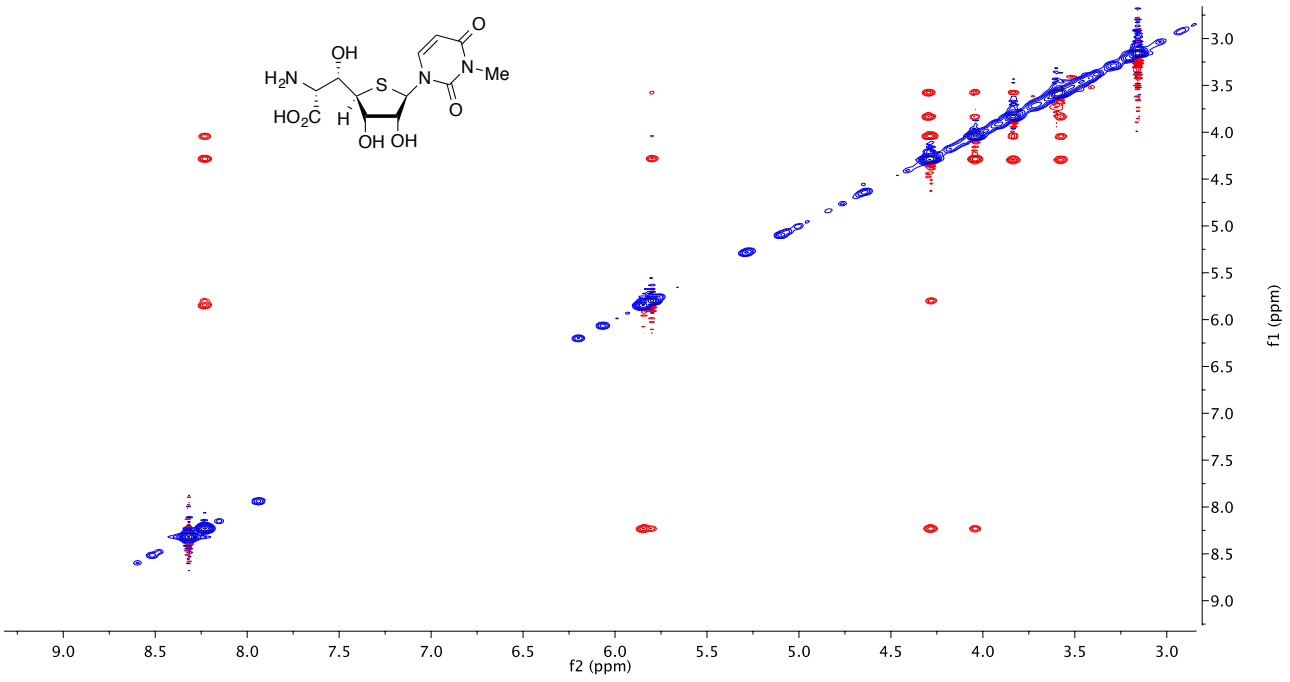


Figure S63. NOESY spectrum of **23**

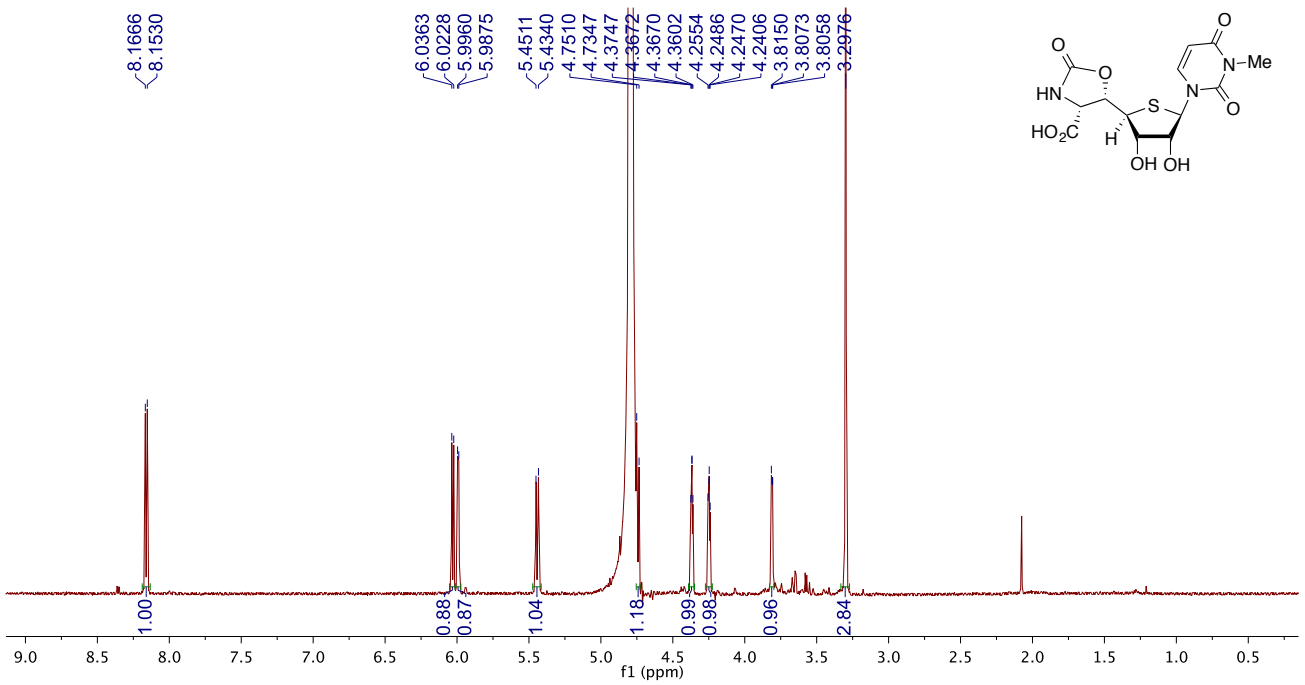


Figure S64. ^{1}H NMR of **24** (600 MHz in D_2O)

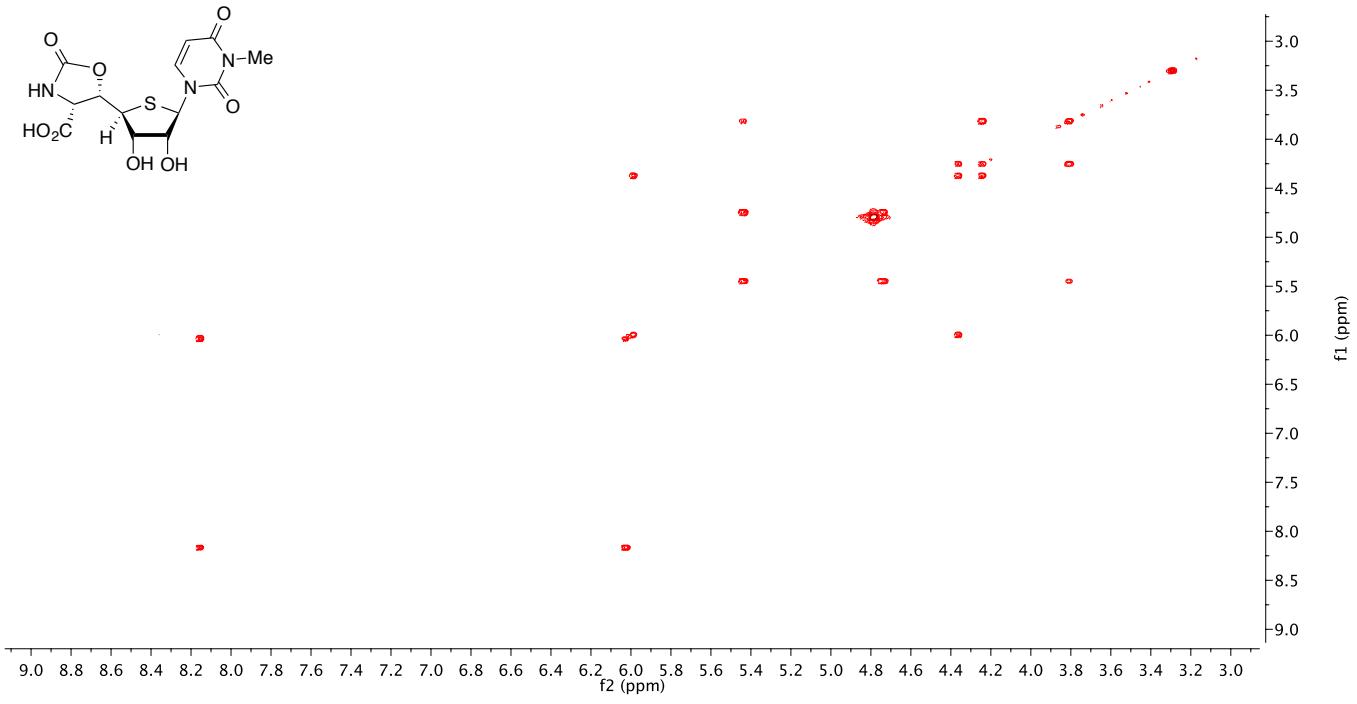


Figure S65. COSY spectrum of **24**

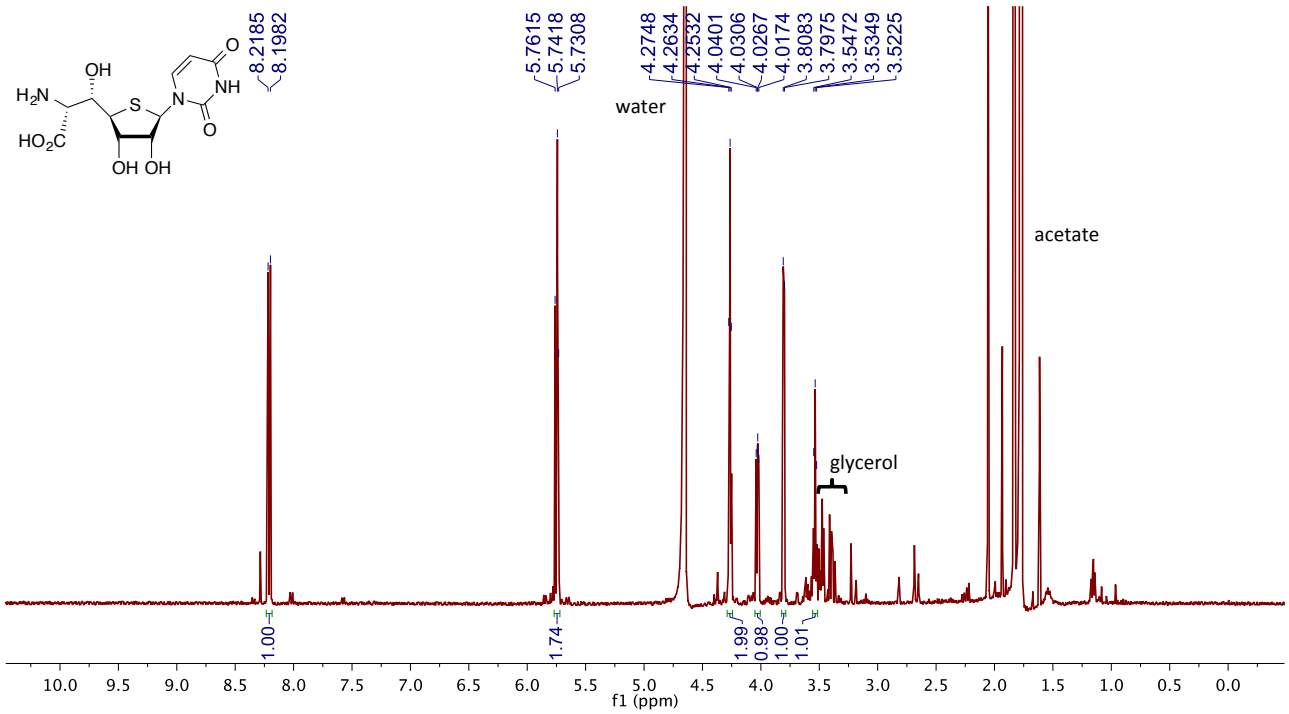


Figure S66. ^{1}H NMR of **29** (400 MHz in D_2O)

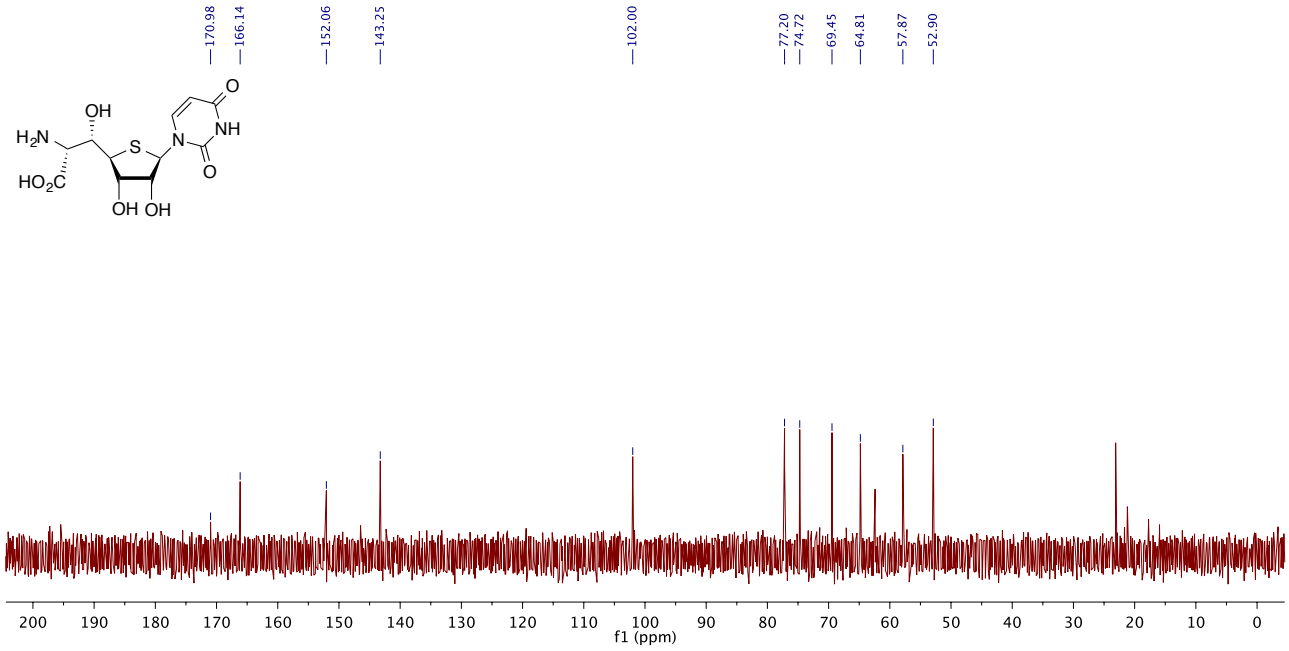


Figure S67. ^{13}C NMR of **29** (151 MHz in D_2O)

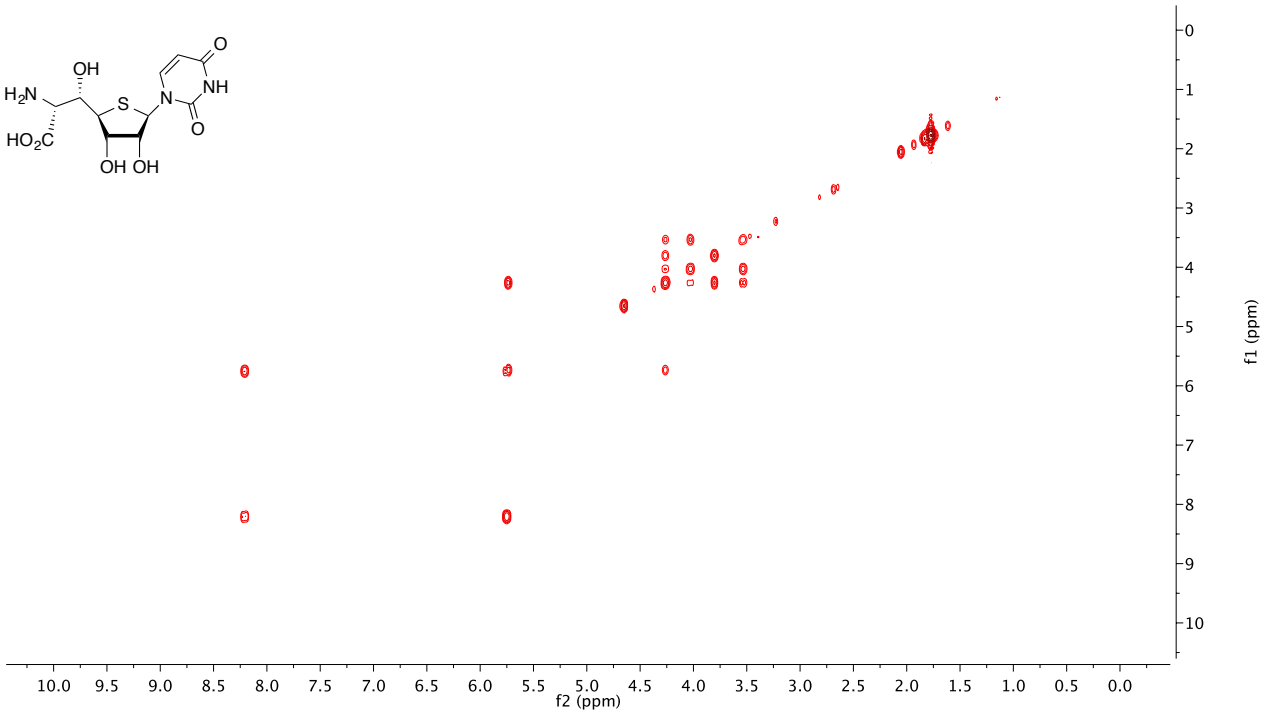


Figure S68. COSY spectrum of **29**

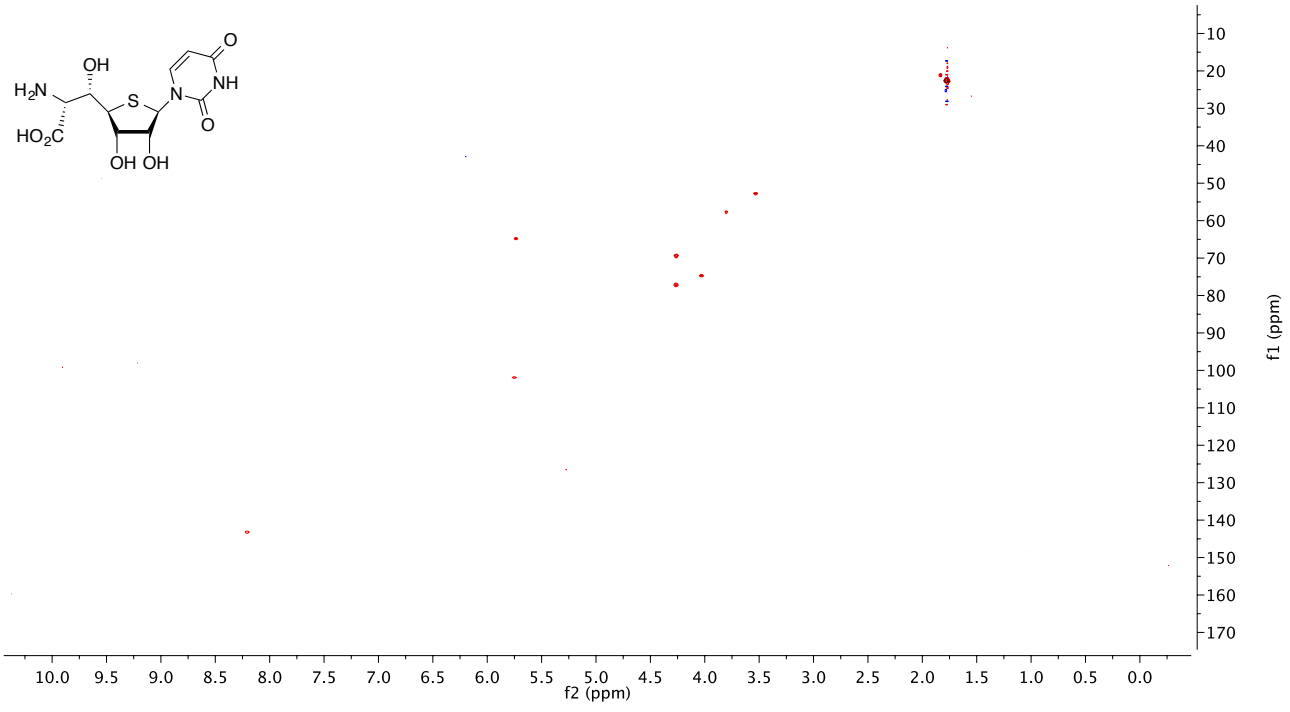


Figure S69. HSQC spectrum of **29**

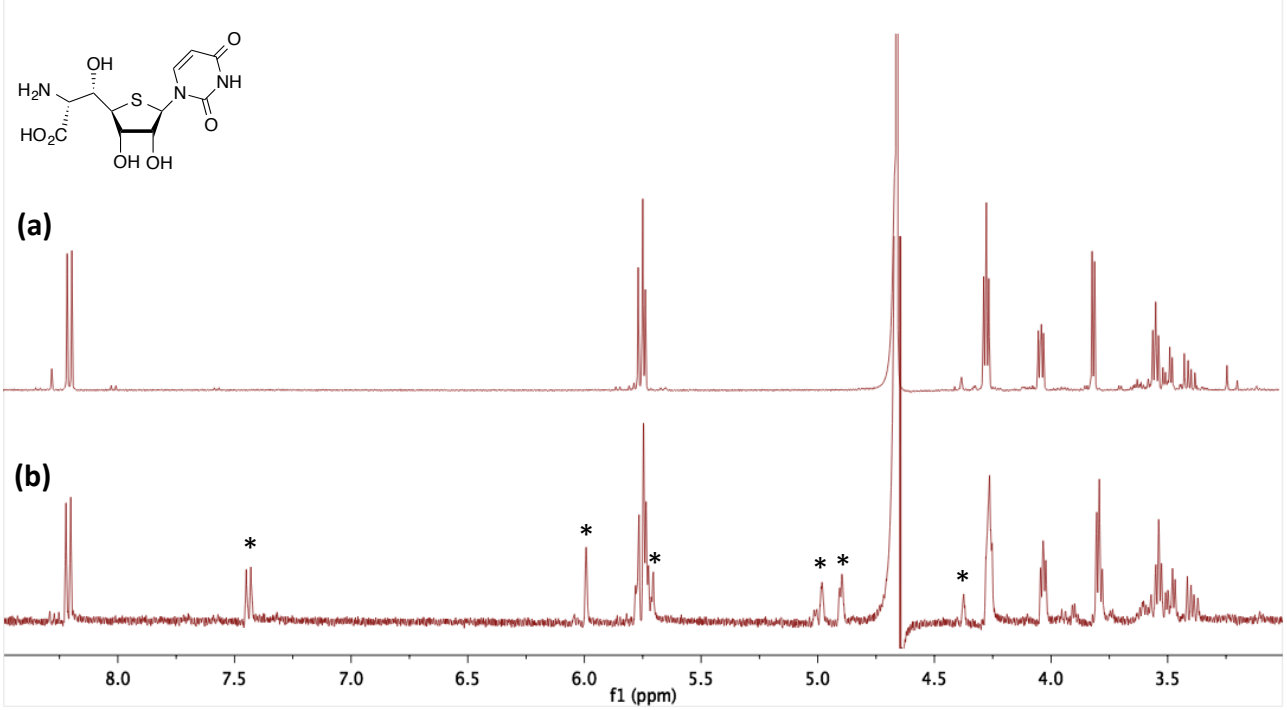


Figure S70. Overlaid ^{1}H NMR spectra of (a) synthetic **29** and (b) enzymatically produced **29**. The sample of enzymatically produced **29** contains minor impurities (indicated by *) that could not separated from **29** during the purification by HPLC.

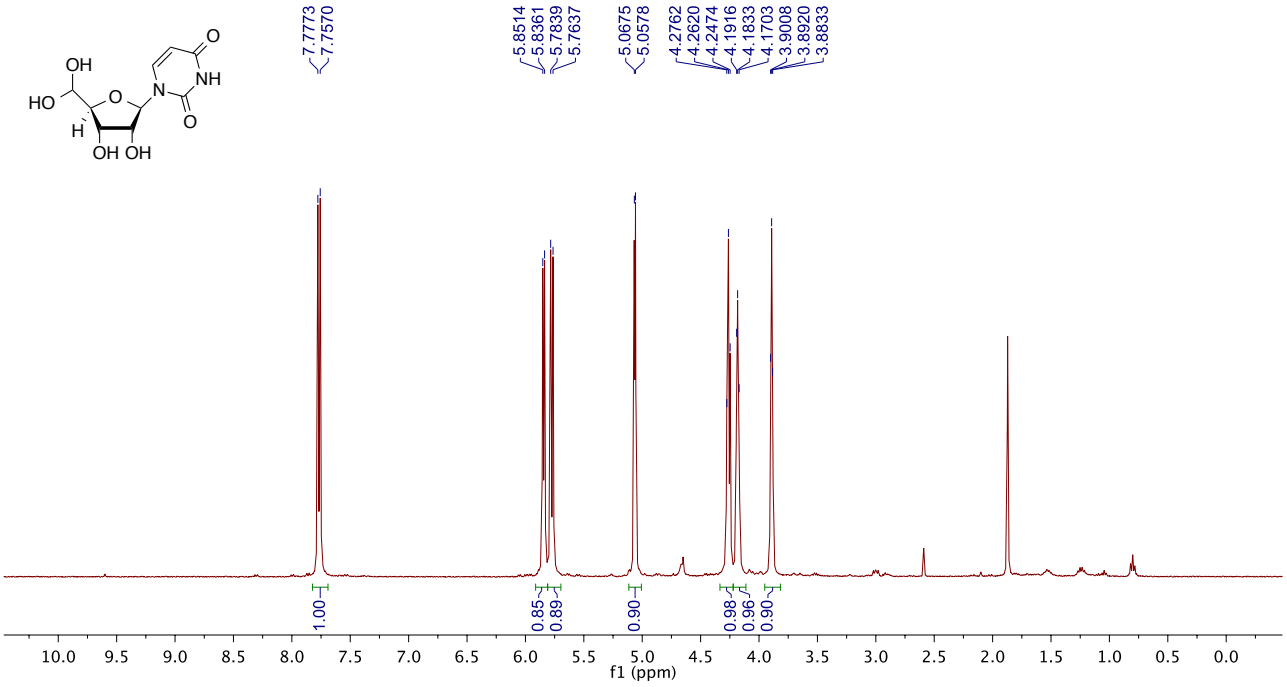


Figure S71. ^{1}H NMR of **31** (600 MHz in D_2O)

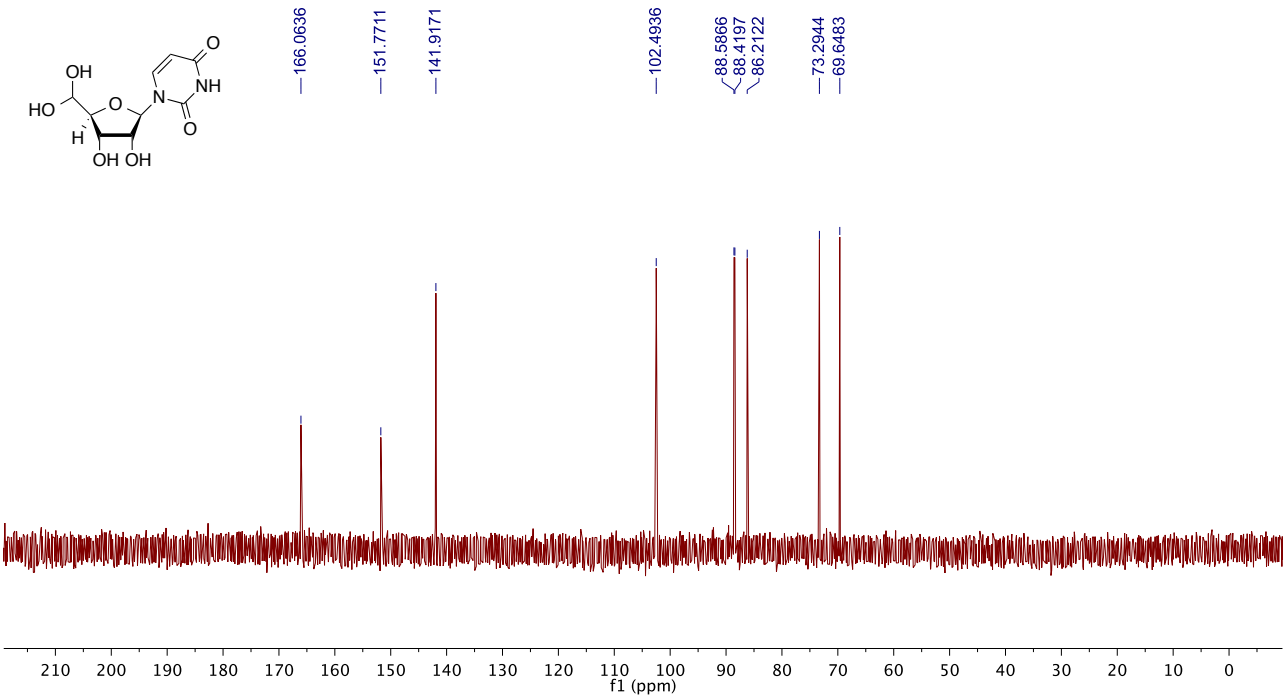


Figure S72. ^{13}C NMR of **31** (151 MHz in D_2O)

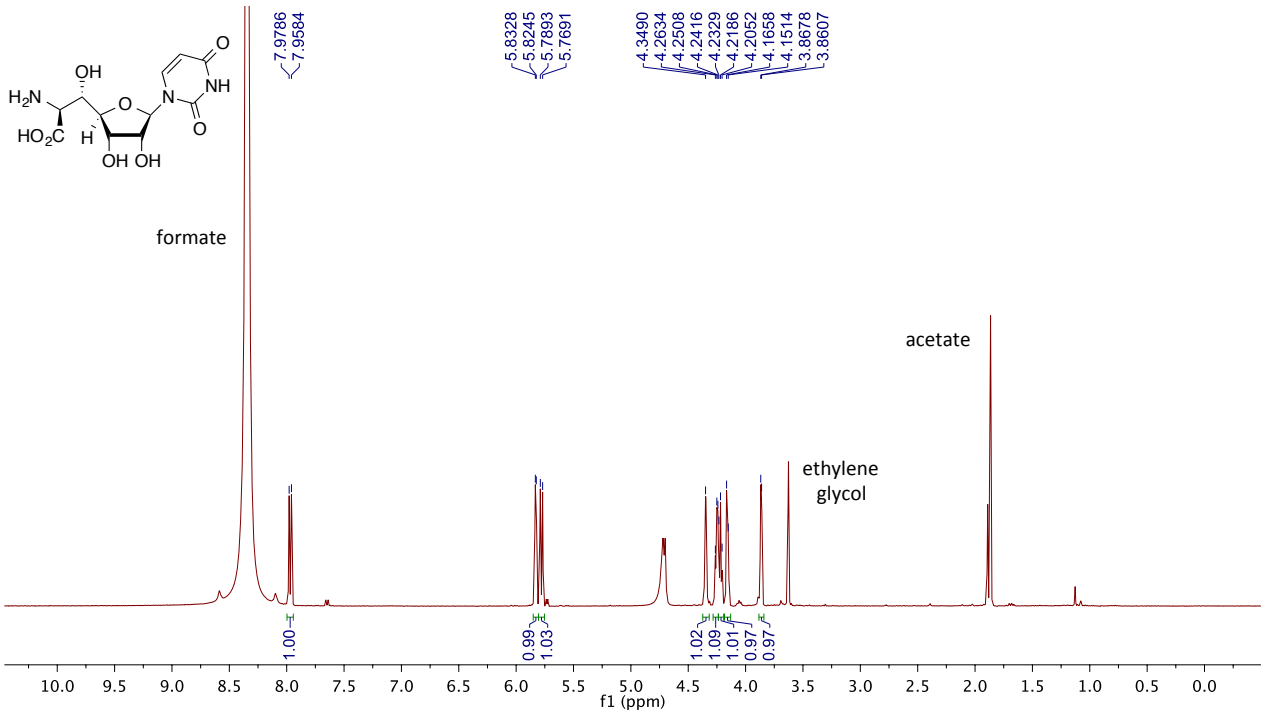


Figure S73. ^{1}H NMR of **32** (600 MHz in D_2O)

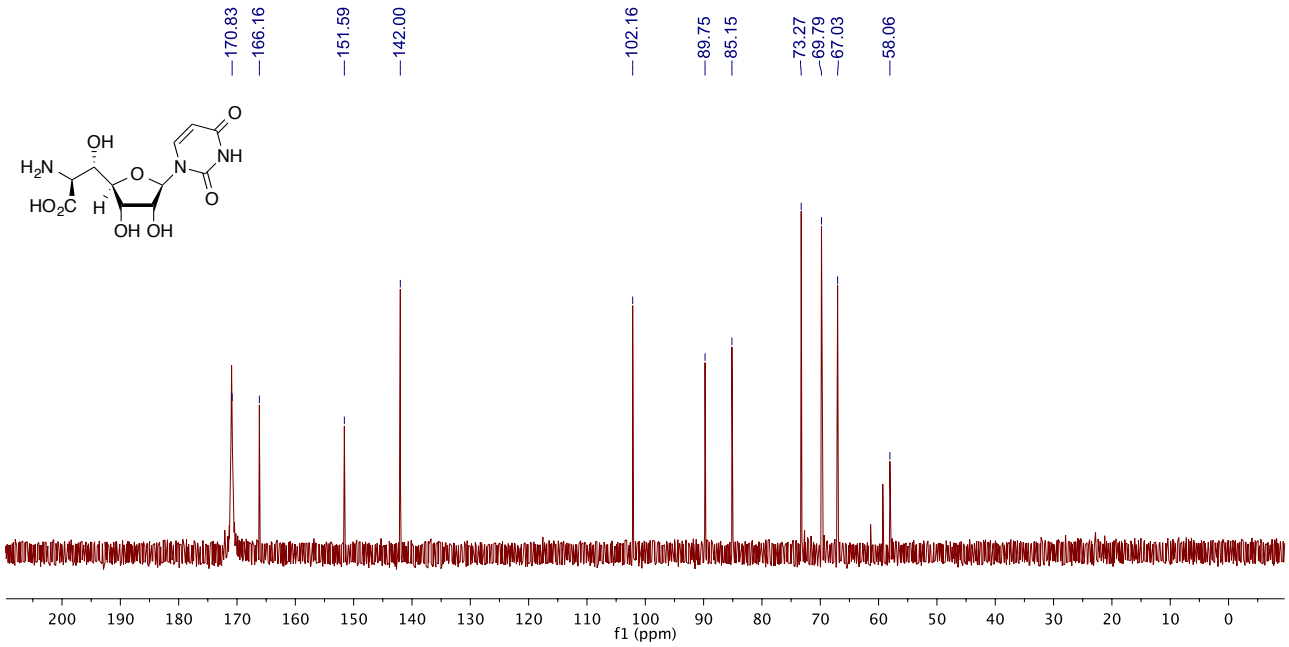


Figure S74. ^{13}C NMR of **32** (151 MHz in D_2O)

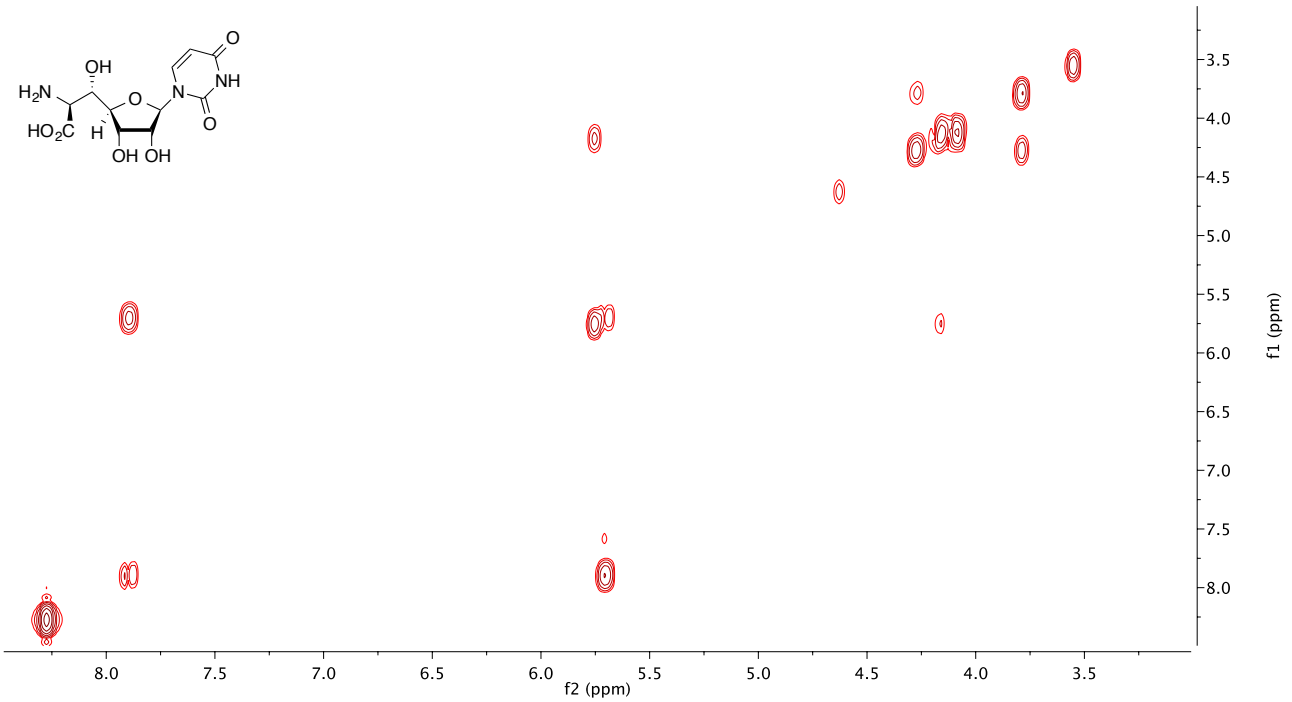


Figure S75. COSY spectrum of **32**

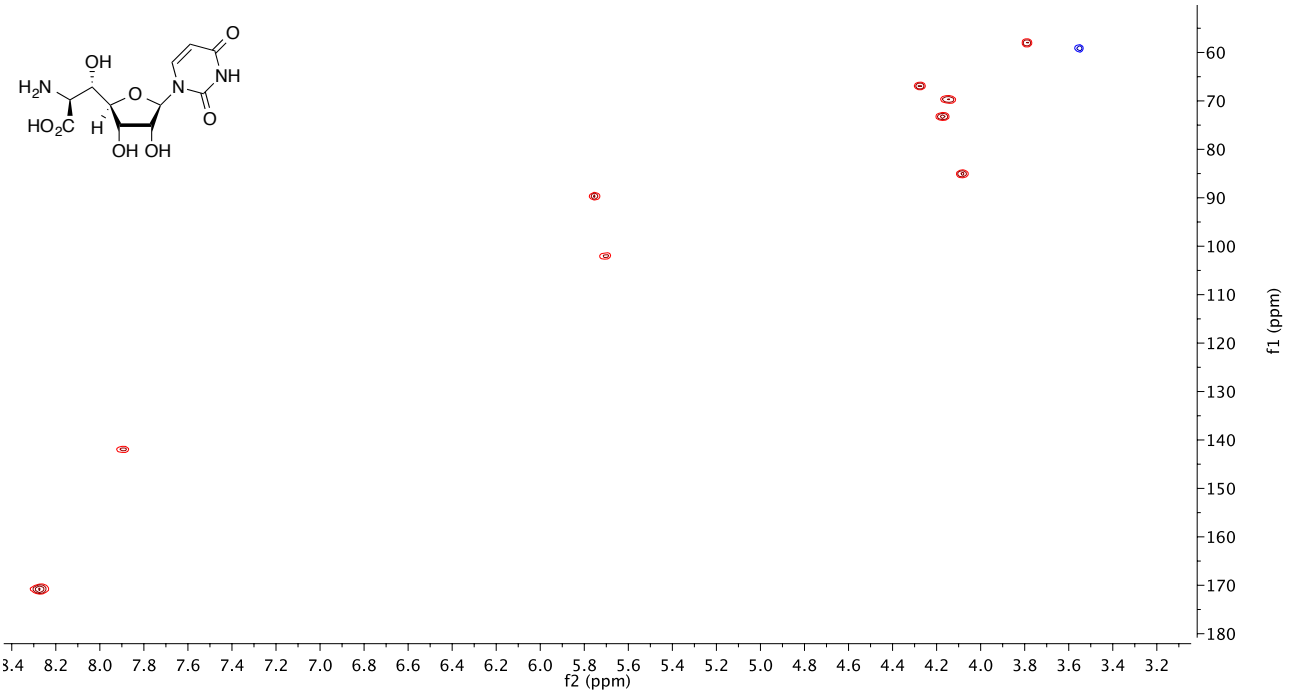


Figure S76. HSQC spectrum of **32**

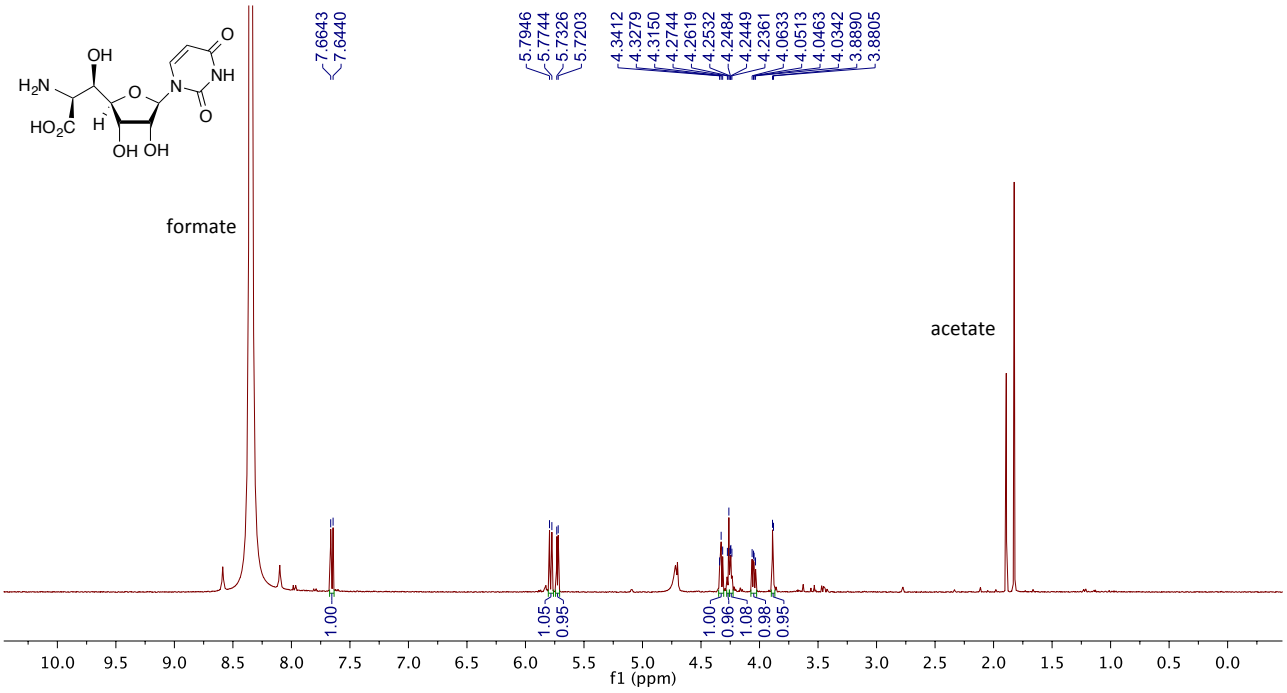


Figure S77. ^{1}H NMR of **33** (600 MHz in D_2O)

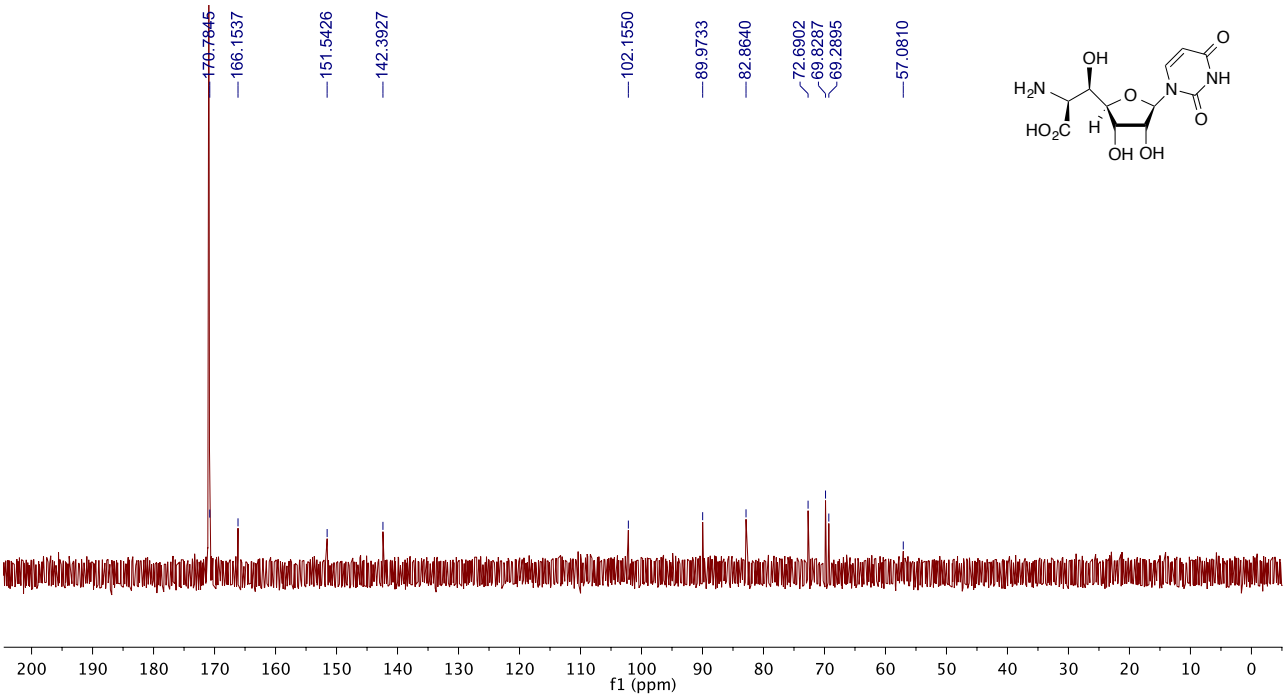


Figure S78. ^{13}C NMR of **33** (151 MHz in D_2O)

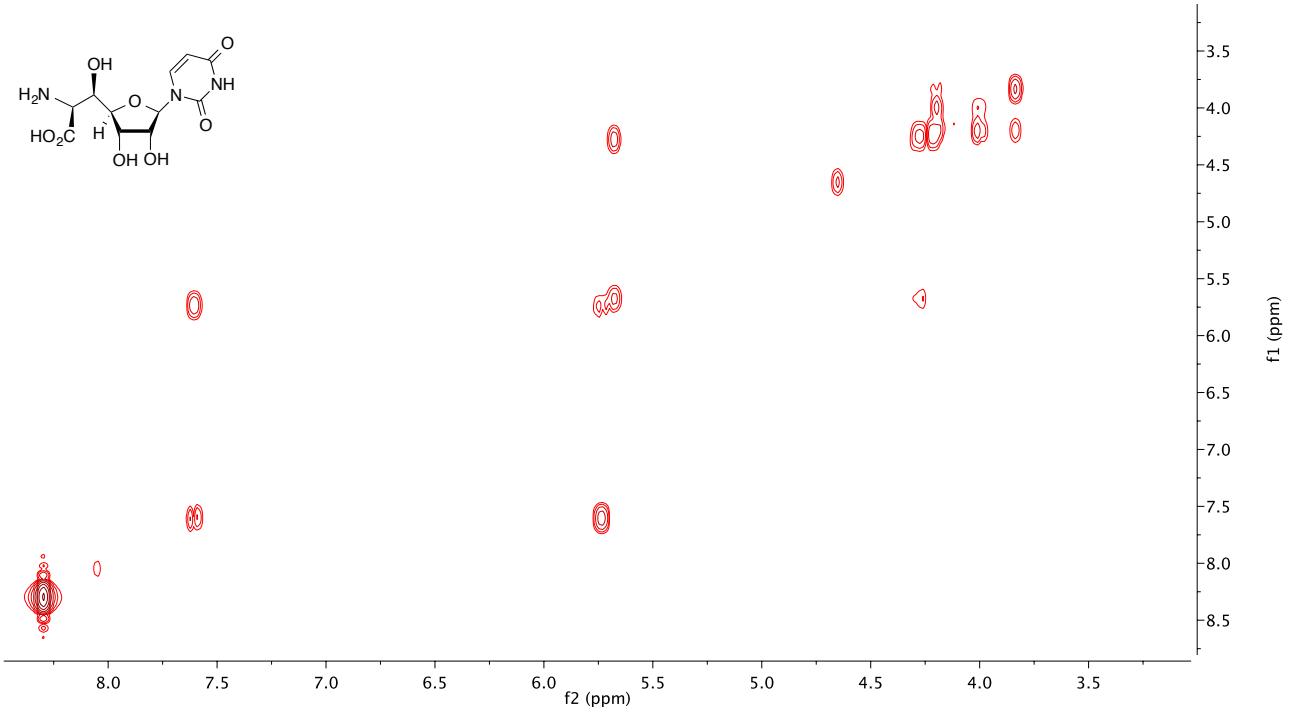


Figure S79. COSY spectrum of **33**

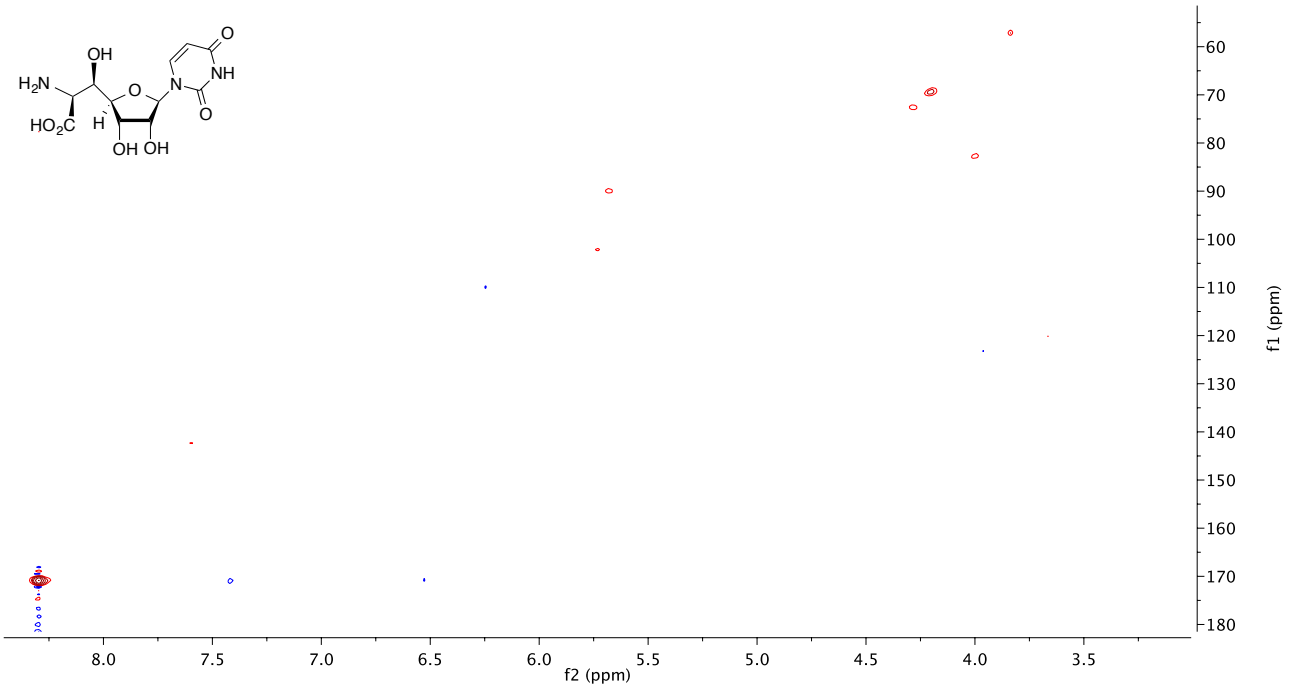


Figure S80. HSQC spectrum of **33**

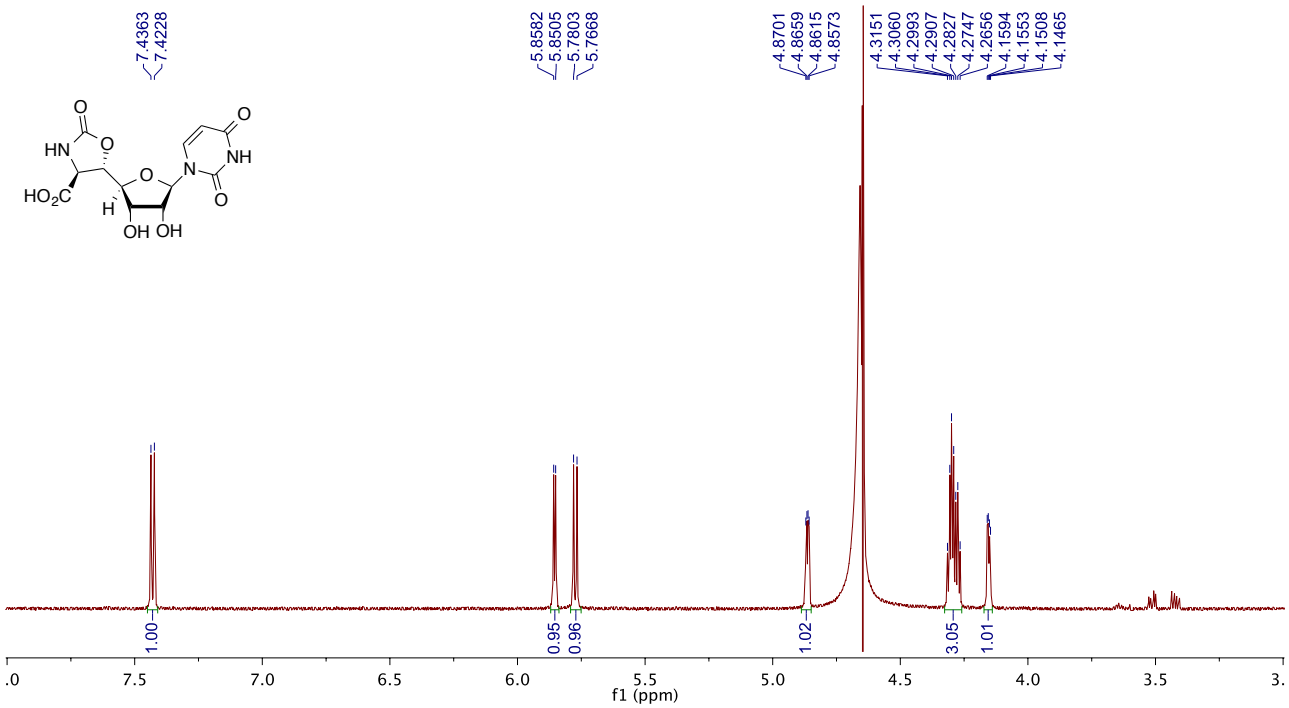


Figure S81. ^{1}H NMR of **S28** (600 MHz in D_2O)

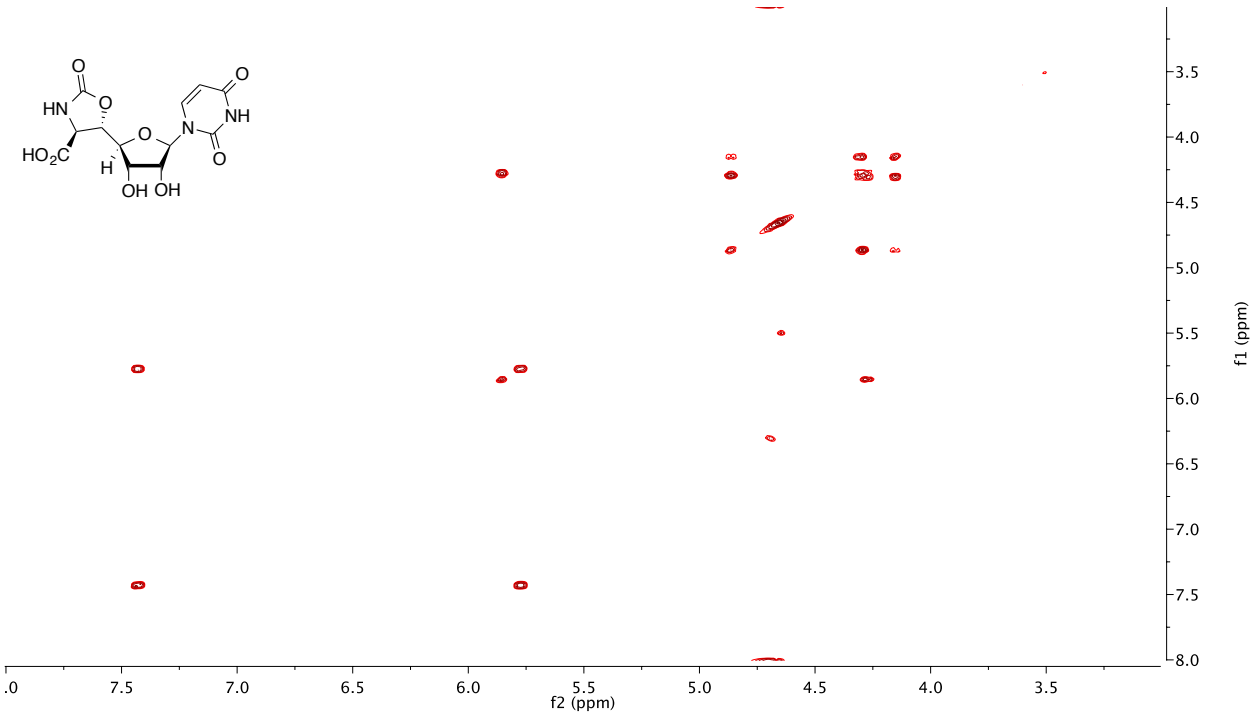


Figure S82. COSY spectrum of **S28**

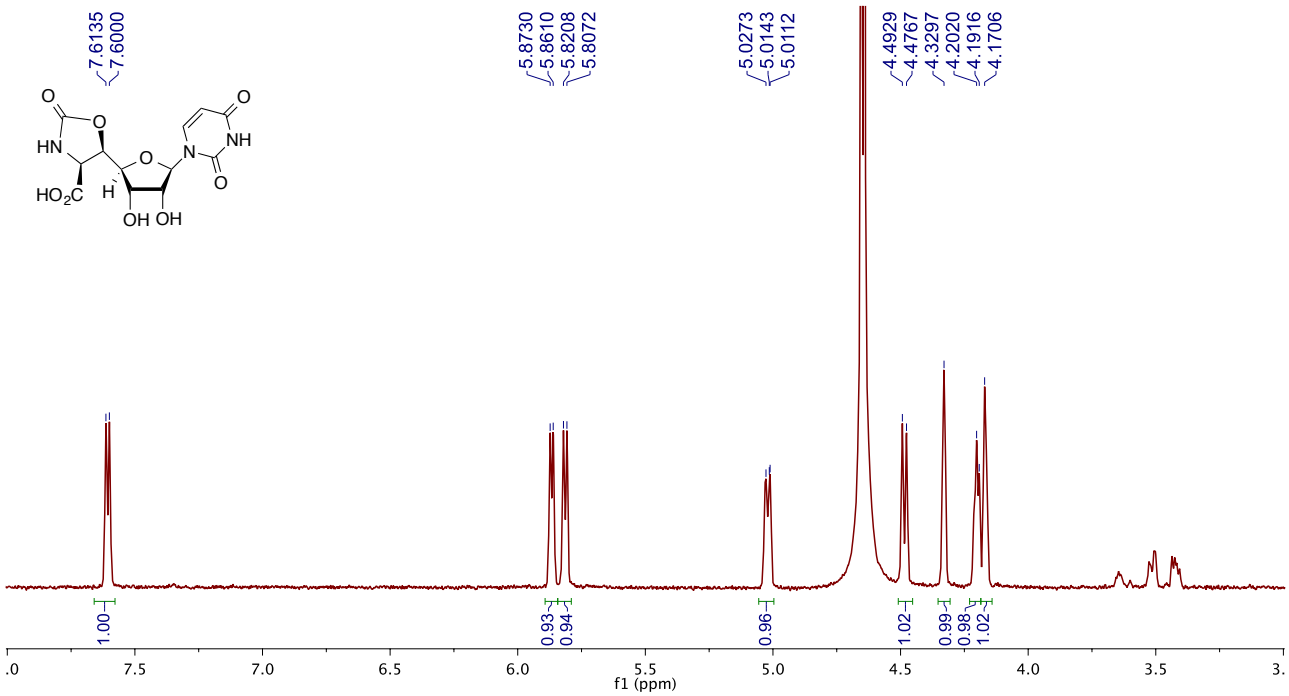


Figure S83. ^{1}H NMR of **S29** (600 MHz in D_2O)

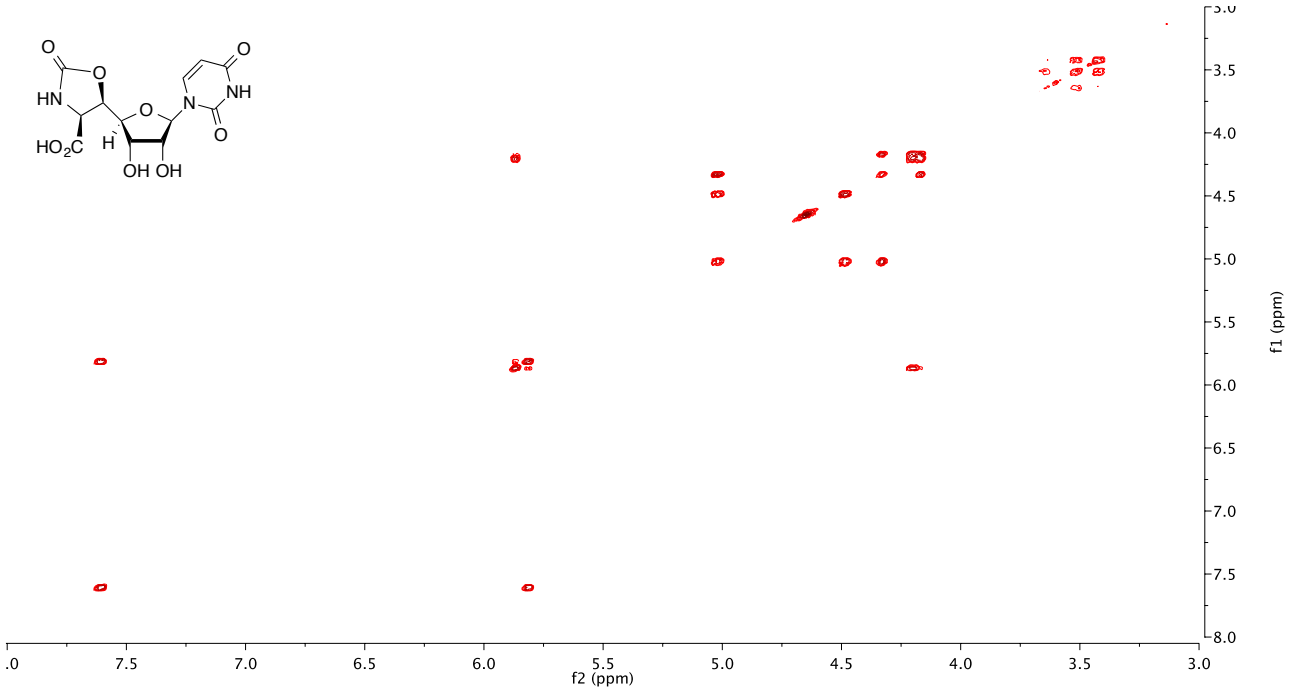


Figure S84. COSY spectrum of **S29**

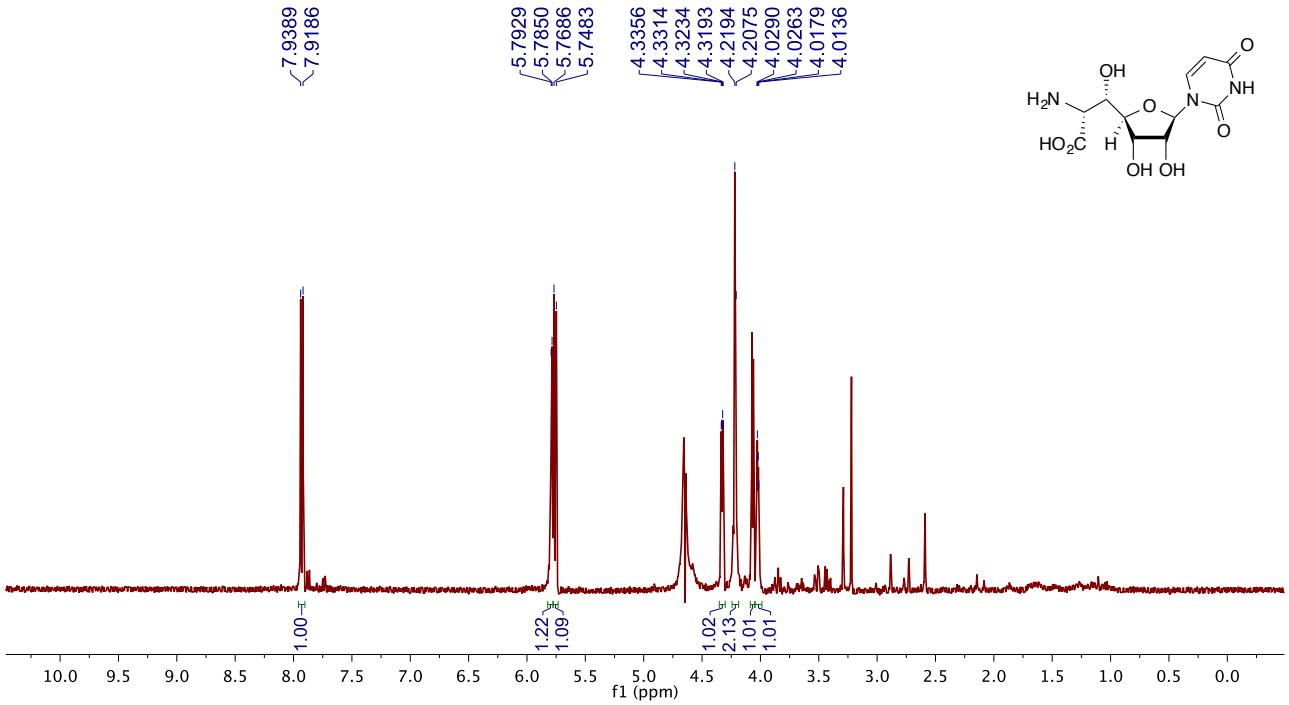


Figure S85. ^{1}H NMR of **S26** (400 MHz in D_2O)

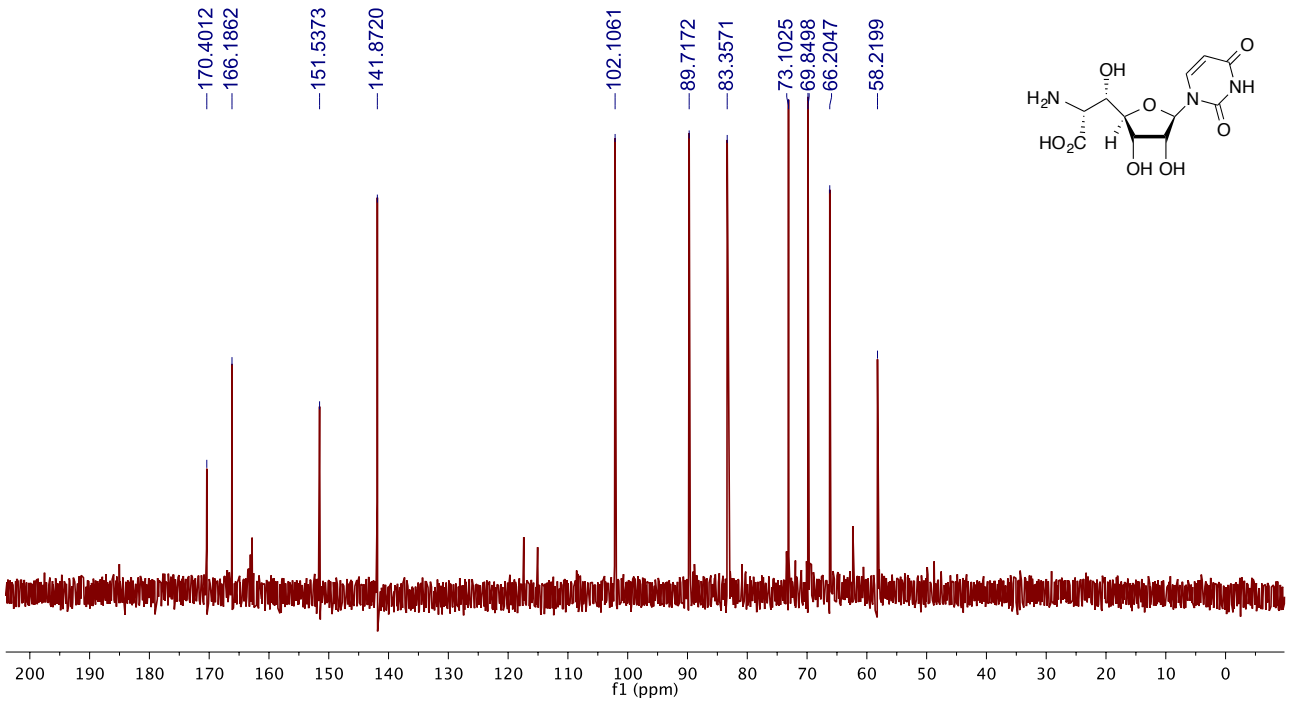


Figure S86. ^{13}C NMR of **S26** (125 MHz in D_2O)