

Supporting Information

Cosubstrate tolerance of the aminoglycoside resistance enzyme Eis from *Mycobacterium tuberculosis*

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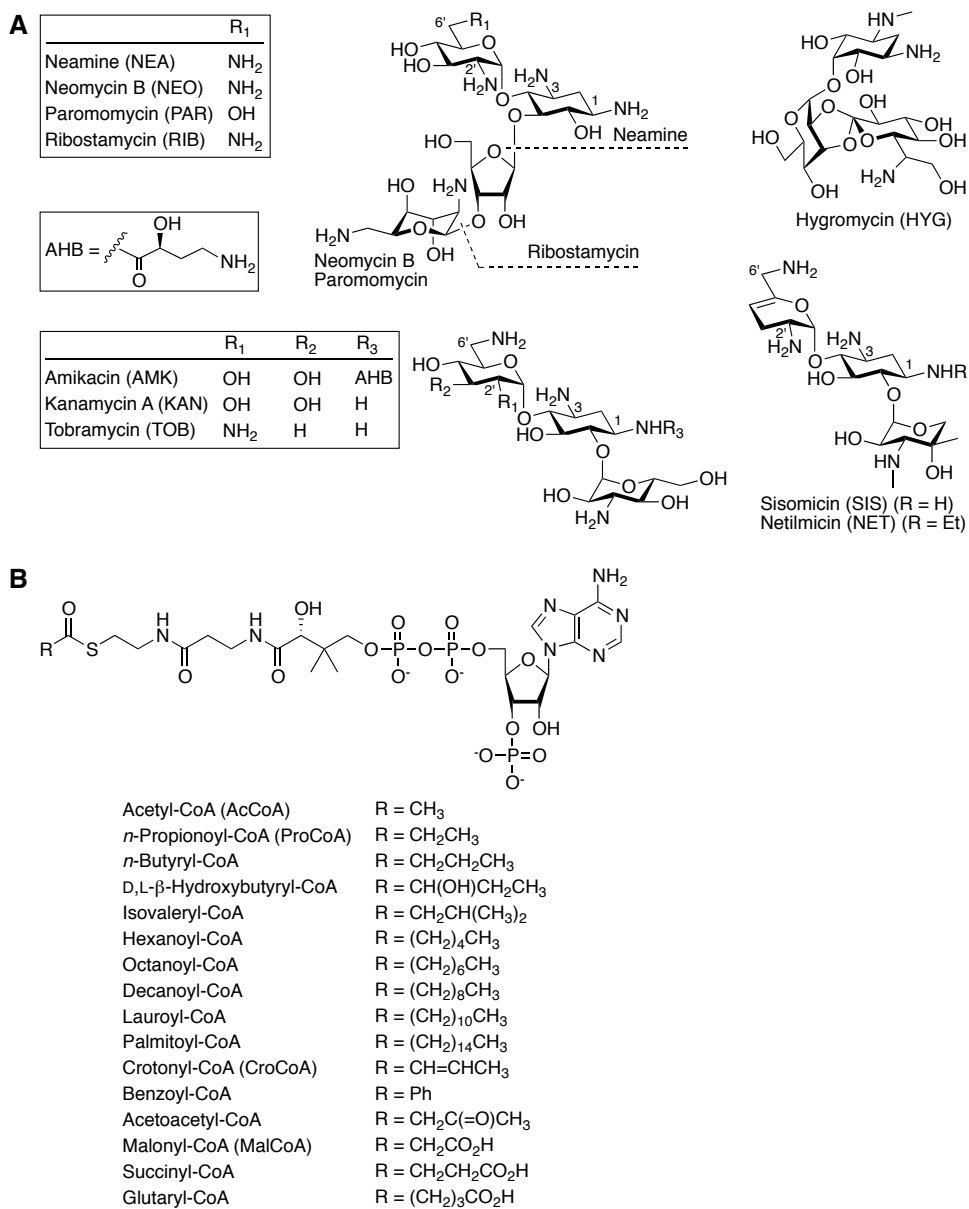


Fig. S1. Structures of **A.** aminoglycosides (AGs) and **B.** acyl-CoAs used in this study.

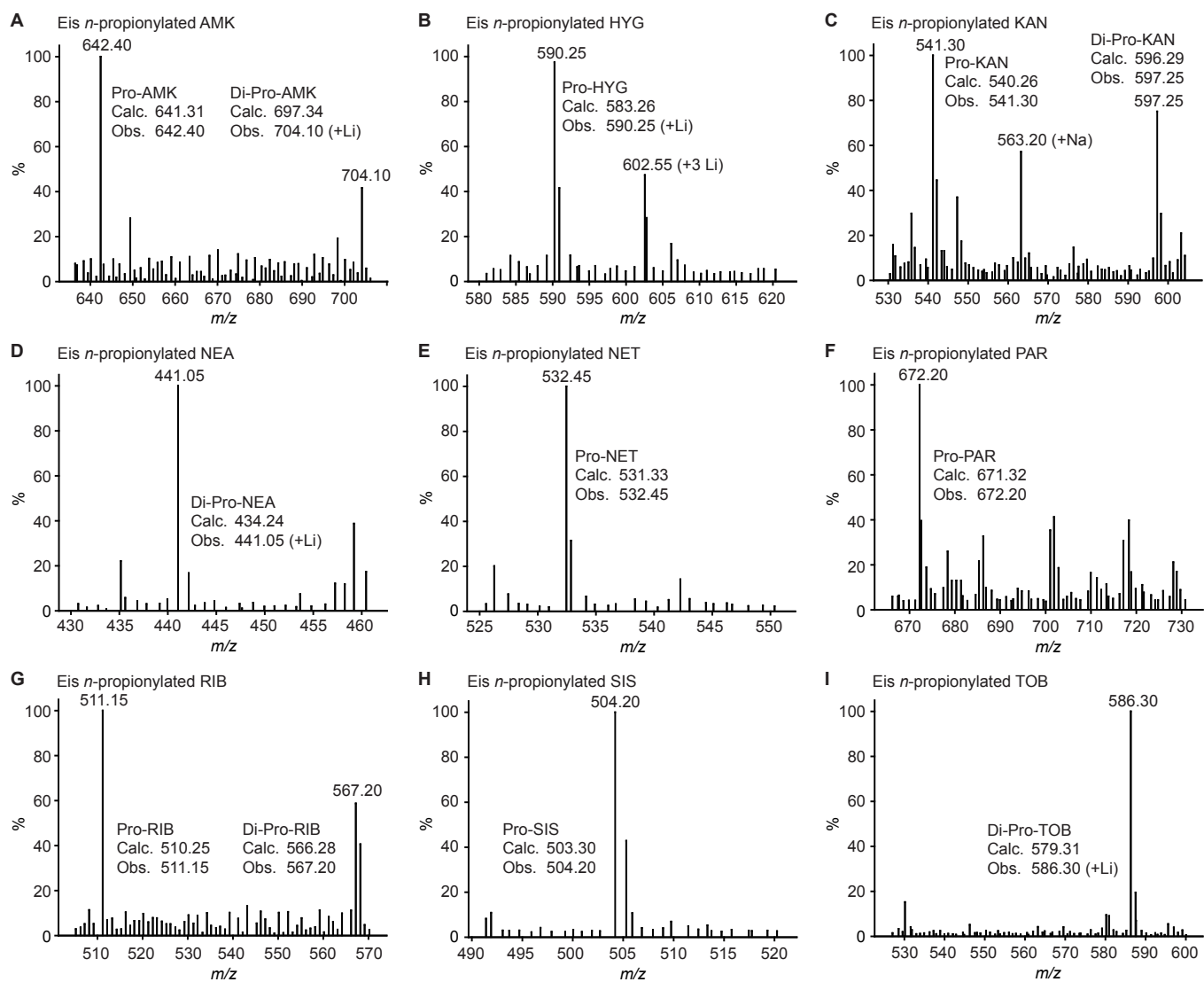


Fig. S2. Mass spectra for mono- and di-*n*-propionylation by Eis of various AGs: **A.** AMK (mono and di), **B.** HYG (mono), **C.** KAN (mono and di), **D.** NEA (di), **E.** NET (mono), **F.** PAR (mono), **G.** RIB (mono and di), **H.** SIS (mono), and **I.** TOB (di).

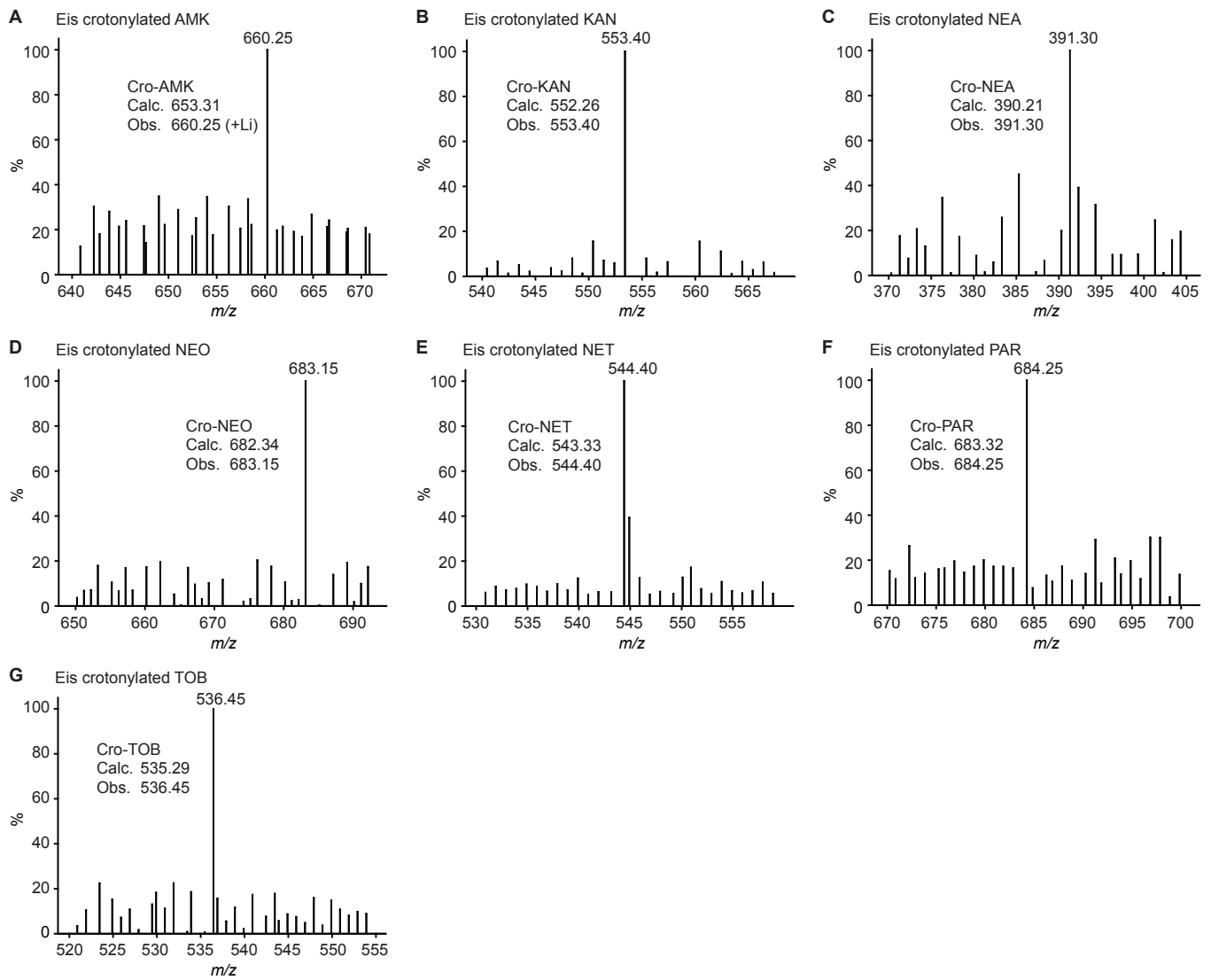


Fig. S3. Mass spectra for mono-crotonylation by Eis of various AGs: **A.** AMK, **B.** KAN, **C.** NEA, **D.** NEO, **E.** NET, **F.** PAR, and **G.** TOB.

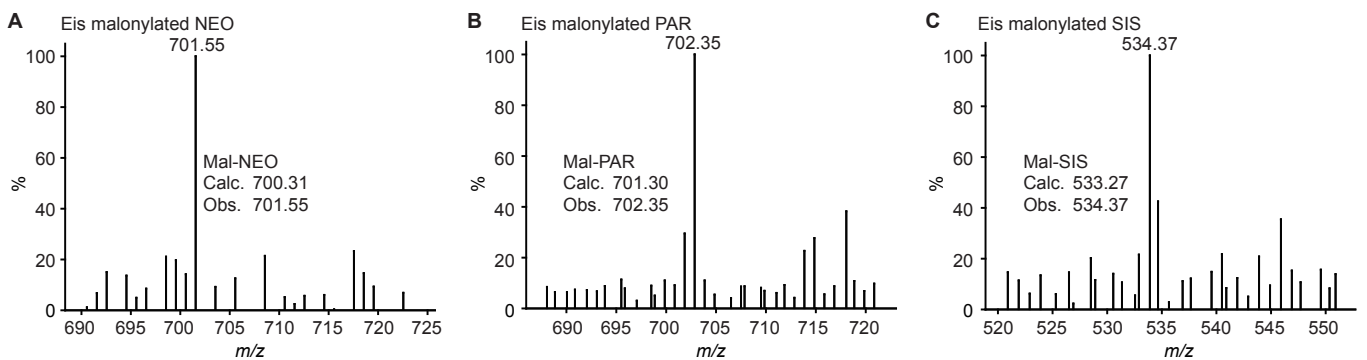


Fig. S4. Mass spectra for mono-malonylation by Eis of various AGs: **A.** NEO, **B.** PAR, and **C.** SIS.

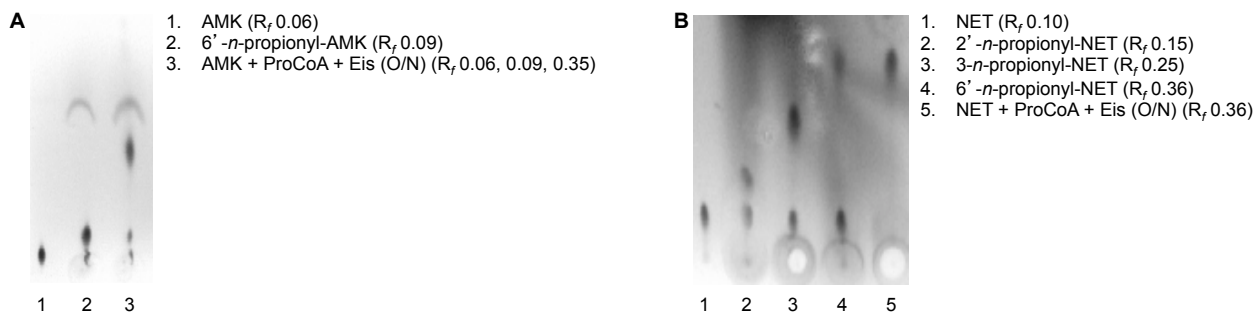


Fig. S5. TLC analysis of the *n*-propionylation of **A.** AMK and **B.** NET.

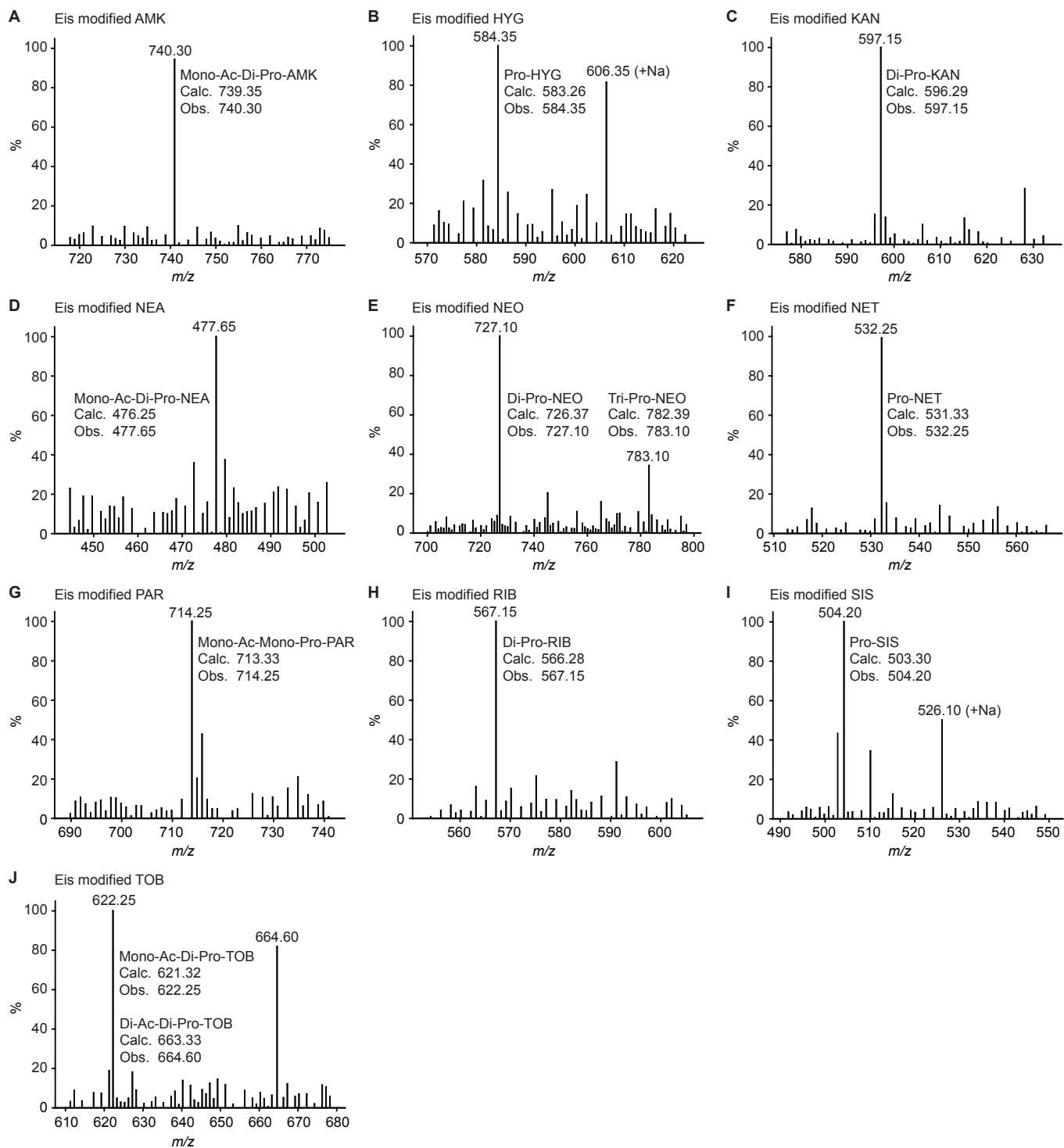


Fig. S6. Mass spectra for sequential reactions monitoring *n*-propionylation followed by acetylation by Eis of various AGs: **A.** AMK, **B.** HYG, **C.** KAN, **D.** NEA, **E.** NEO, **F.** NET, **G.** PAR, **H.** RIB, **I.** SIS, and **J.** TOB.

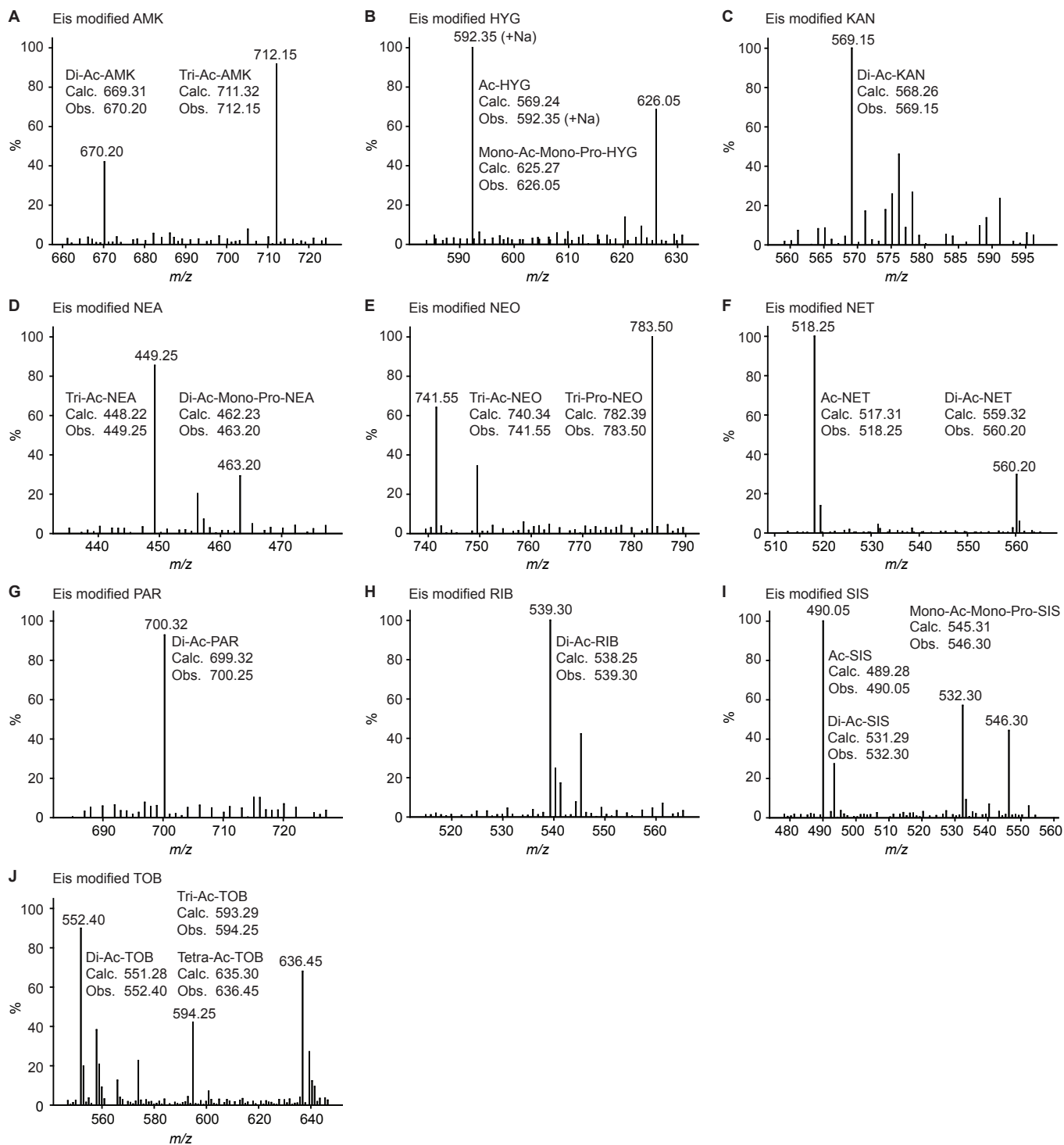


Fig. S7. Mass spectra for reactions monitoring the competition between *n*-propionylation and acetylation by Eis of various AGs: **A.** AMK, **B.** HYG, **C.** KAN, **D.** NEA, **E.** NEO, **F.** NET, **G.** PAR, **H.** RIB, **I.** SIS, and **J.** TOB.

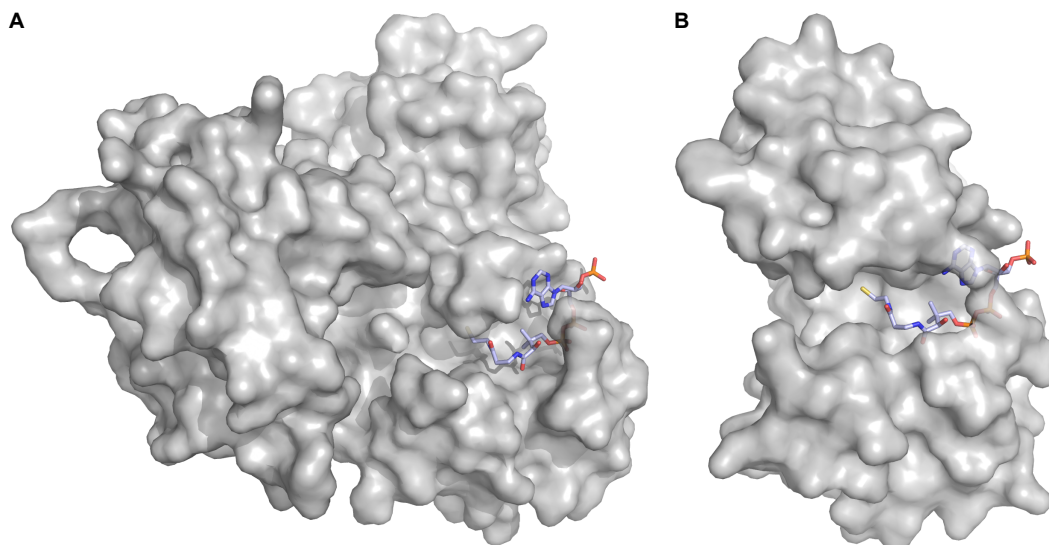


Fig. S8. Surface representations of **A.** *M. tuberculosis* Eis (PDB ID: 3R1K) and **B.** *E. faecium* AAC(6')-Ii (PDB ID: 1N71) monomers in complex with CoA (shown as sticks).

Table S1. R_f values of mono- and di-*n*-propionylated AGs by AAC(2')-Ic, AAC(3)-IV, AAC(6) and Eis proteins.

AG		None	(2) ^c	(3) ^f	(6) ^g	Enzymes utilized			Eis						
						(3) then (2) ^e	(6) then (2) ^e	(6) then (3) ^e	1 min	5 min	10 min	30 min	2 h	O/N	
AMK	Parent	0.06													0.06
	Mono		-- ^b	--	0.09										0.09
	Di					--	--	--							0.35
NEA	Parent ^c	0.11							0.11	0.11	0.11	0.11	0.11		
	Mono ^c		0.13	0.24	0.33				0.13	0.13	0.13	0.13	0.13		
	Di ^d					0.26	0.41	0.53							0.41
NET	Parent	0.10													
	Mono		0.15	0.25	0.36										0.36

^aThe eluent systems used for TLCs were 5:2/MeOH:NH₄OH, 3:0.8/MeOH:NH₄OH, and 12:1/MeOH:NH₄OH for AMK, NEA, and NET, respectively. ^bParent indicates non-modified AGs. ^cMono indicates mono-*n*-propionylated AG product. ^dDi indicates di-*n*-propionylated AG product. ^e(2) indicates that the AG was *n*-propionylated using AAC(2')-Ic. (3) indicates that the AG was *n*-propionylated using AAC(3)-IV. ^f(6) indicates that the AG was *n*-propionylated using the AAC(6') of the AAC(6')/APH(2'') bifunctional enzyme. ^b-- indicates that the AG cannot be *n*-propionylated at this position.

Table S2. Mass analysis of AGs acylated by Eis.

AG		<i>n</i> -Propionylation			Crotonylation			Malonylation	
		Calc [M + H] ⁺	Obs [M + H] ⁺		Calc [M + H] ⁺	Obs [M + H] ⁺		Calc [M + H] ⁺	Obs [M + H] ⁺
AMK	Mono ^a	642.32	642.40	Mono	654.32	660.25 (+Li)	Mono	672.29	-- ^d
	Di ^b	698.35	704.10 (+Li)						
HYG	Mono	584.27	590.25 (+Li)	Mono	569.27	--	Mono	614.24	--
	Mono	541.27	541.30	Mono	553.27	553.40	Mono	571.25	--
NEA	Di	597.30	597.25						
	Mono	379.22	--	Mono	391.22	391.30	Mono	409.19	--
NEO	Di	435.25	441.05 (+Li)						
	Mono	671.35	671.35	Mono	683.35	683.15	Mono	701.32	701.55
NET	Di	727.37	727.40						
	Tri ^c	783.40	783.60						
PAR	Mono	532.33	532.45	Mono	544.33	544.40	Mono	562.31	562.20
	Di	672.33	672.20	Mono	684.33	684.25	Di	648.31	648.20
RIB	Mono	511.26	511.15	Mono	523.26	--	Mono	702.30	702.35
	Di	567.29	567.20					541.24	--
SIS	Mono	504.30	504.20	Mono	516.30	516.25	Mono	534.28	534.37
	Mono	524.29	--	Mono	536.29	536.45	Mono	554.27	--
TOB	Di	580.32	586.30 (+Li)						

^aMono indicates mono-acylation. ^bDi indicates di-acylation. ^cTri indicates tri-acylation. ^d-- indicates that no mass was observed.

Table S3. Mass analysis of ProCoA and AcCoA sequential and competitive reactions with AGs and Eis.

AG		Pro → Ac ^a		Ac + Pro ^b	
		Calc [M + H] ⁺	Obs [M + H] ⁺	Calc [M + H] ⁺	Obs [M + H] ⁺
AMK	Mono ^c -Ac-Di ^d -Pro	740.36	740.30		
				Di-Ac Tri ^e -Ac	670.31 712.32
					670.20 712.15
HYG	Mono-Pro	584.27	584.35 / 606.35 (+Na)		
				Mono-Ac Mono-Ac-Mono-Pro	570.25 626.28
					592.35 (+Na) 626.05
KAN	Di-Pro	597.30	597.15		
NEA	Mono-Ac-Di-Pro	477.26	477.65	Di-Ac	569.27
					569.15
NEO	Di-Pro	727.36	727.10	Tri-Ac Di-Ac-Mono-Pro	449.22 463.24
					449.25 463.20
NET	Tri-Pro	783.40	783.10	Tri-Ac Tri-Pro	741.35 783.40
	Mono-Pro	532.33	532.25		741.55 783.50
PAR	Mono-Ac-Mono-Pro	714.34	714.25	Mono-Ac Di-Ac	518.32 560.33
					518.25 560.20
RIB	Di-Pro	567.29	567.15	Di-Ac	700.32
					700.25
SIS	Mono-Pro	504.30	504.20 / 526.10 (+Na)	Di-Ac	539.26
					539.30
TOB	Mono-Ac-Di-Pro	622.33	622.25	Mono-Ac Di-Ac Mono-Ac-Mono-Pro	490.29 532.30 546.32
	Di-Ac-Di-Pro	664.34	664.60		490.05 532.30 546.30
				Di-Ac Tri-Ac Tetra-Ac	552.29 594.30 636.31
					552.40 594.25 636.45

^aProCoA (5 eq) reactions with Eis (5 μM) and AG (1 eq) were followed by incubation with AcCoA (5 eq) and an additional Eis (5 μM). ^bAcCoA (5 eq) and ProCoA (5 eq) were incubated simultaneously with Eis (10 μM) and AG (1 eq). ^cMono indicates mono-acylation. ^dDi indicates di-acylation. ^eTri indicates tri-acylation.