Supporting Information

Biochemical and structural analysis of aminoglycoside acetyltransferase Eis from *Anabaena variabilis*

Rachel E. Pricer,[a,b] Jacob L. Houghton,[a,c] Keith D. Green,[a] Abdelrahman S. Mayhoub,[a£] and Sylvie Garneau-Tsodikova*[a,b,c]

[a]*Life Sciences Institute,* [b]*Chemical Biology Doctoral Program University of Michigan, and* [c]*Department of Medicinal Chemistry in the College of Pharmacy, 210 Washtenaw Ave, Ann Arbor, MI 48109, USA.* [£]*On leave from Faculty of Pharmacy, Al-Azhar University, Cairo, 11884, Egypt.*

Correspondence to: *Fax: +1 734-615-5521; Tel: +1 734-615-2736; E-mail: sylviegt@umich.edu.*

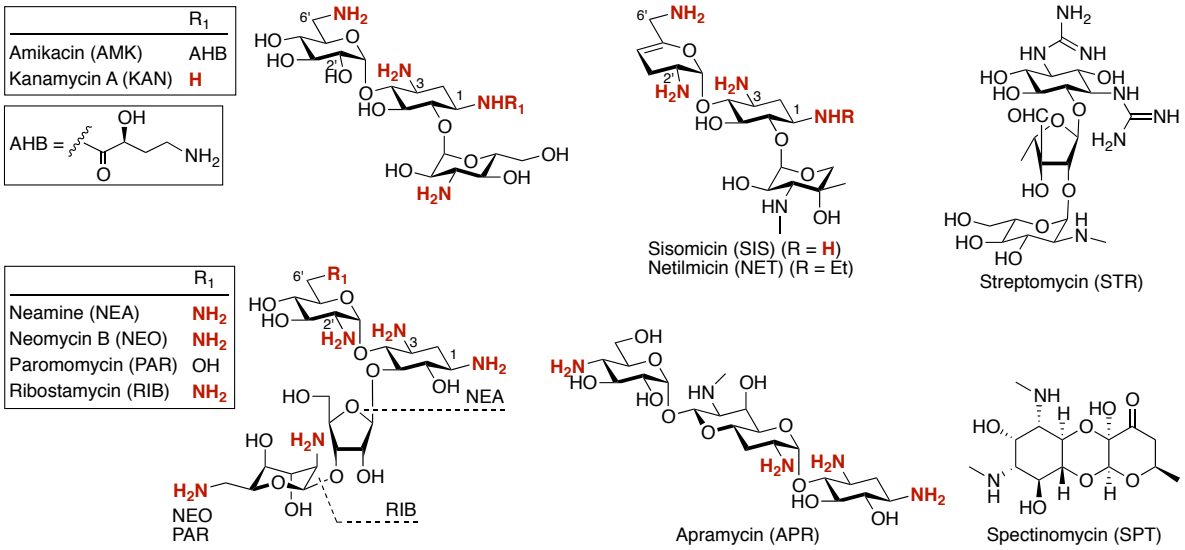

Fig. S1. Structures of aminoglycosides (AGs) tested in this study. The amine functionalities that could potentially be modified by Eis proteins are highlighted in bold red.

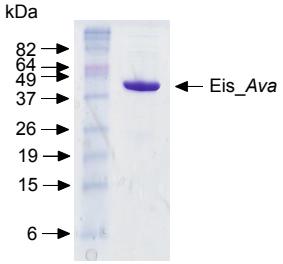

Fig. S2. 15% Coomassie blue-stained SDS-PAGE of purified Eis_*Ava*.

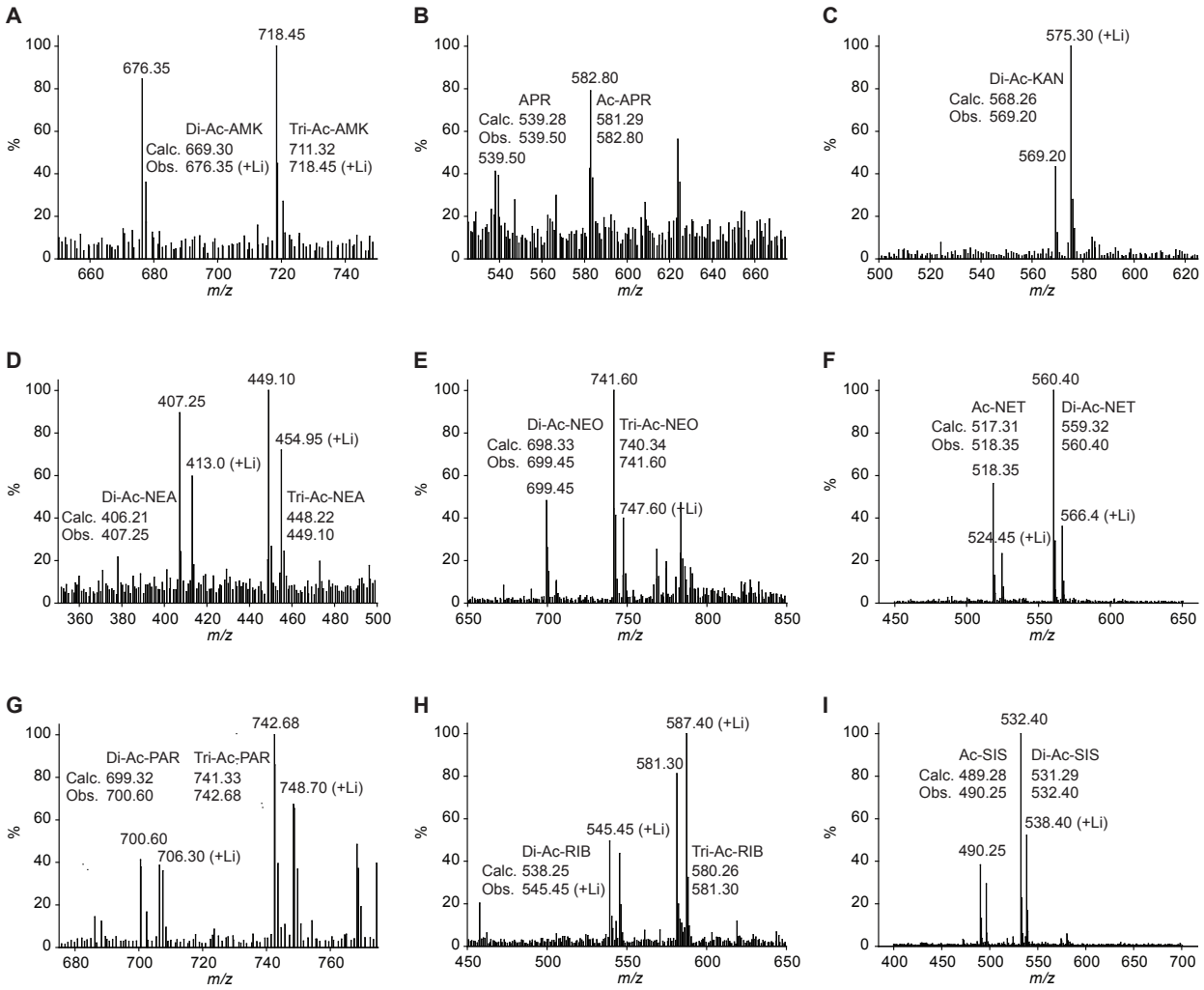

Fig. S3. Mass spectra of AGs multi-acetylated by Eis_*Ava*.

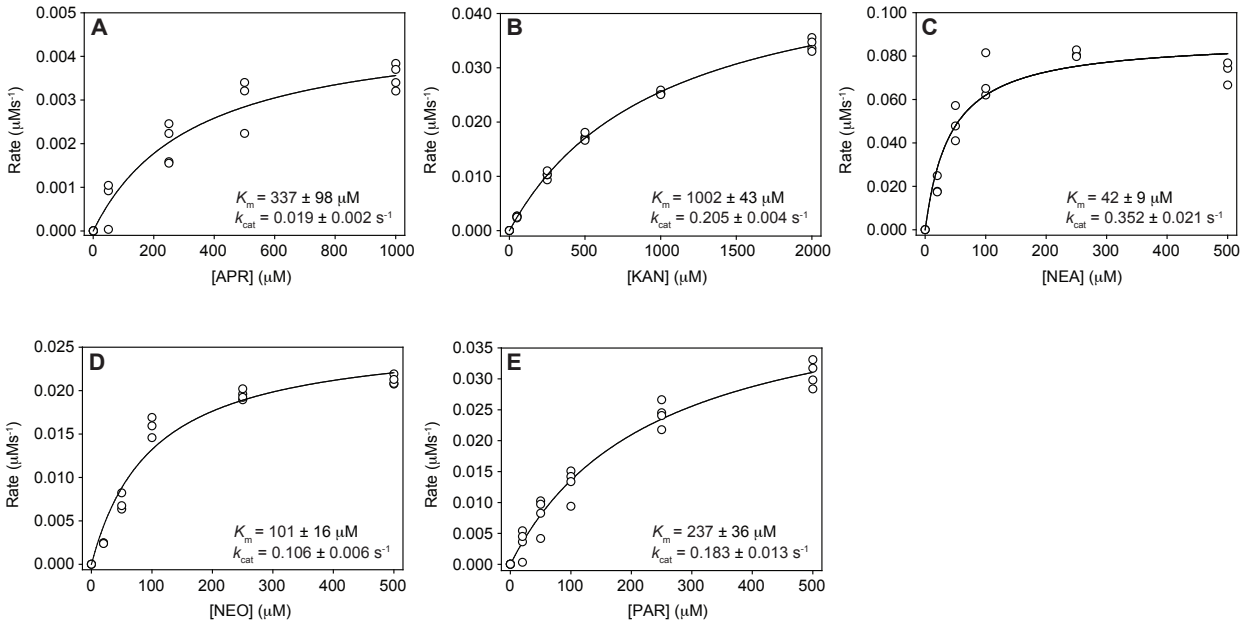

Fig. S4. Michaelis-Menten analysis of the Eis_*Ava* catalyzed acetylation of **A.** APR, **B.** KAN, **C.** NEA, **D.** NEO, and **E.** PAR.

Table S1. Proton chemical shifts determined for NEA and 1,2'-di-acetyl-NEA.[a]

Ring	H position	NEA	1,2'-di-acetyl-NEA	Δppm
II	1	2.91-2.88[b] (m) [2.89][d]	3.70-3.84 (m) [3.77]	**0.88**
	2_{ax}	1.33 (ddd (app. q), $J_{2ax,2eq} = J_{2ax,1} = J_{2ax,3} = 12.0$ Hz)[c]	1.72 (ddd (app. q), $J_{2ax,2eq} = J_{2ax,1} = J_{2ax,3} = 13.0$ Hz)	0.39
	2_{eq}	2.08 (ddd (app. dt), $J_{2eq,2ax} = 12.0$ Hz, $J_{2eq,1} = J_{2eq,3} = 4.0$ Hz)	2.25 (ddd (app. dt), $J_{2eq,2ax} = 13.0$ Hz, $J_{2eq,1} = J_{2eq,3} = 4.1$ Hz)	0.17
	3	2.96[b] (ddd, $J_{3,2eq} = 4.0$ Hz, $J_{3,2ax} = 12.0$ Hz, $J_{3,4} = 9.5$ Hz)	3.33-3.41 (m) [3.38]	0.42
	4	3.35[e] (dd, (app. q) $J_{4',3'} = J_{4',5'} = 9.5$)	3.91-3.79 (m) [3.82]	0.45
	5	3.59-3.55 (m) [3.57]	3.66-3.58 (m) [3.62]	0.05
	6	3.29-3.23[e] (m) [3.26]	3.51-3.37 (m) [3.42]	0.16
I	1'	5.42 (d, $J_{1',2'} = 4.0$ Hz)	5.76 (d, $J_{1',2'} = 3.8$ Hz)	0.34
	2'	2.87 (dd, $J_{2',1'} = 4.0$ Hz, $J_{2',3'} = 10.5$ Hz)	4.01-3.93 (m) [3.98]	**1.11**
	3'	3.63 (dd (app. t), $J_{3',4'} = 9.5$ Hz, $J_{3',2'} = 10.5$ Hz)	3.92-3.85 (m) [3.89]	0.26
	4'	3.35 (dd, (app. q) $J_{4',3'} = J_{4',5'} = 9.5$)	3.51-3.37 (m) [3.44]	0.09
	5'	3.91 (ddd, $J_{5',4'} = 9.5$ Hz, $J_{5',6'a} = 7.5$ Hz, $J_{5',6'b} = 3.0$ Hz)	4.01-3.93 (m) [3.96]	0.05
	$6'_a$	3.04 (dd, $J_{6'a,6'b} = 14.0$ Hz, $J_{6'a,5'} = 7.5$ Hz)	3.22 (dd, $J_{6'a,6'b} = 13.0$ Hz, $J_{6'a,5'} = 7.5$ Hz)	0.18
	$6'_b$	3.29-3.23 (m) [3.25]	3.51-3.37 (m) [3.49]	0.24
Acetyl	N<u>H</u>-2'	×	8.30 (d, $J_{NH,2'} = 8.0$ Hz)	
	N<u>H</u>-1	×	8.24-8.14 (m) [8.20]	
	C<u>H</u>$_3$C=O on 2'	×	2.05 (s)	
	C<u>H</u>$_3$C=O on 1	×	1.97 (s)	

[a]The chemical shift were established based on ^{1}H, zTOCSY, gCOSY, and gHSQC NMR (500 MHz). [b]Could be analogous position of the 2-deoxystreptamine (DOS) ring. [c]Multiplicity and *J* are given in (). [d]The numbers in [] were determined from gCOSY and/or zTOCSY. [e]Could be analogous position of the DOS ring. ×Indicates that the acetyl moiety is not present in the molecule.

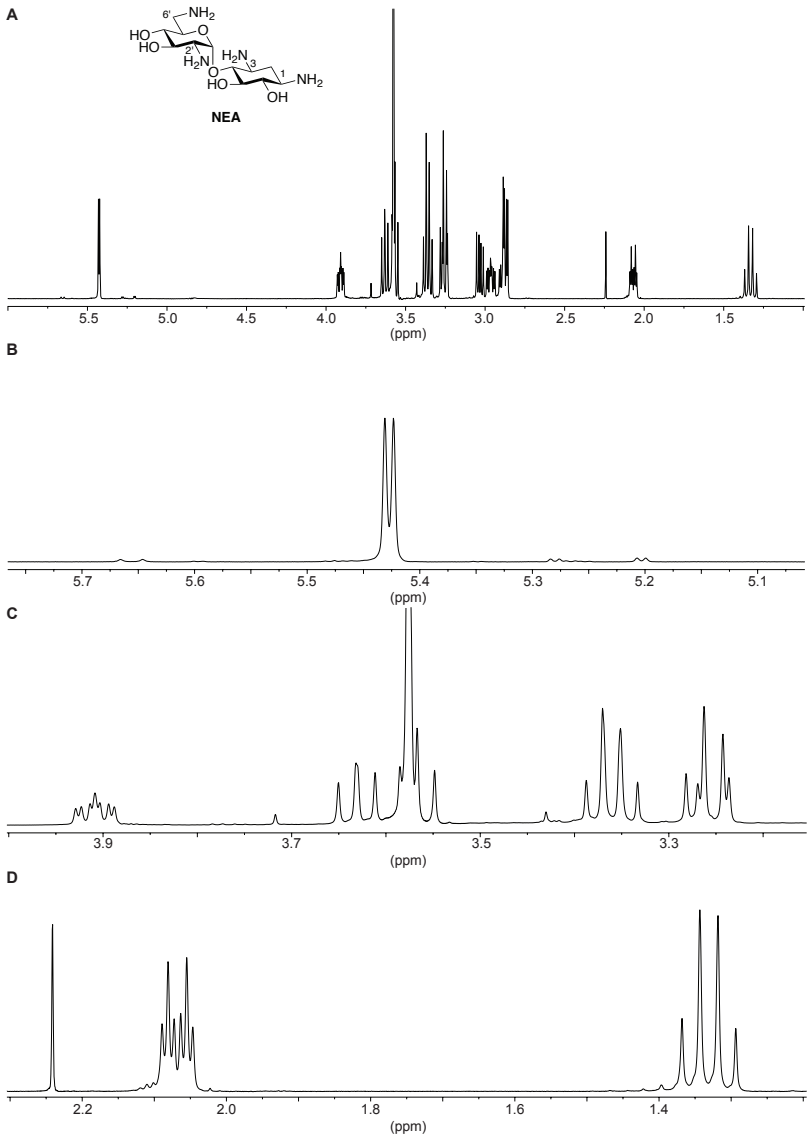

Fig. S5. ^{1}H NMR of NEA in 1.2:8.8/D_2O:H_2O at pH 7.5 (500 MHz). The full spectrum is shown in panel **A** and the expansions in panels **B**-**D**.

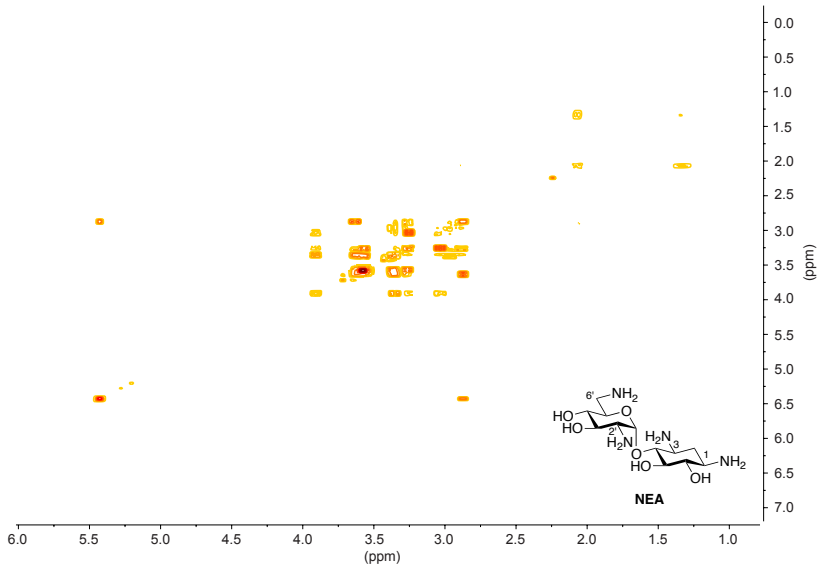

Fig. S6. gCOSY of NEA in 1.2:8.8/D_2O:H_2O at pH 7.5 (500 MHz).

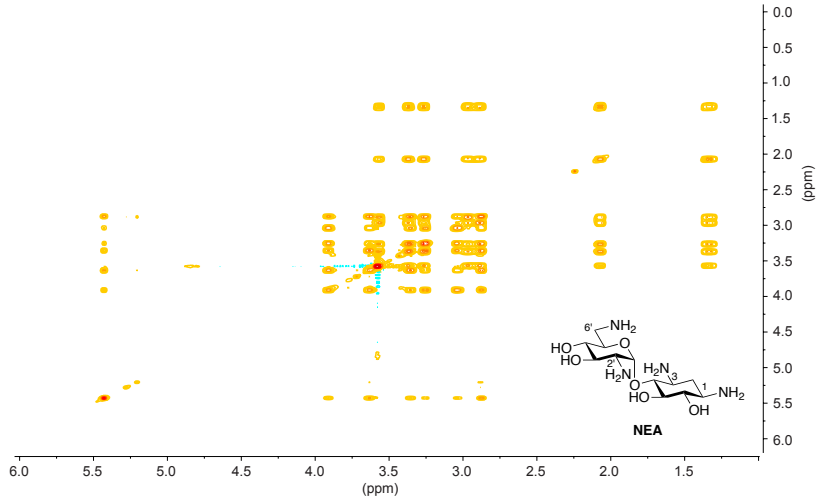

Fig. S7. zTOCSY of NEA in 1.2:8.8/D_2O:H_2O at pH 7.5 (500 MHz).

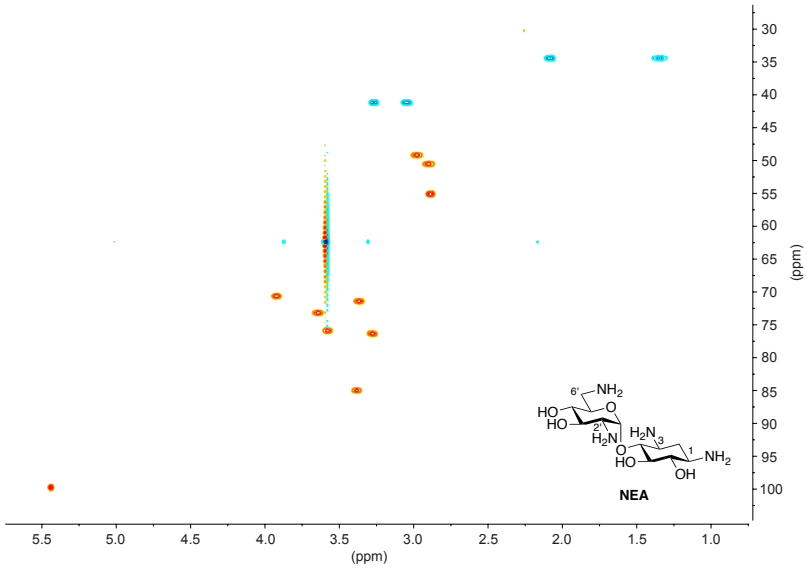

Fig. S8. gHSQC of NEA in 1.2:8.8/D_2O:H_2O at pH 7.5 (500 MHz).

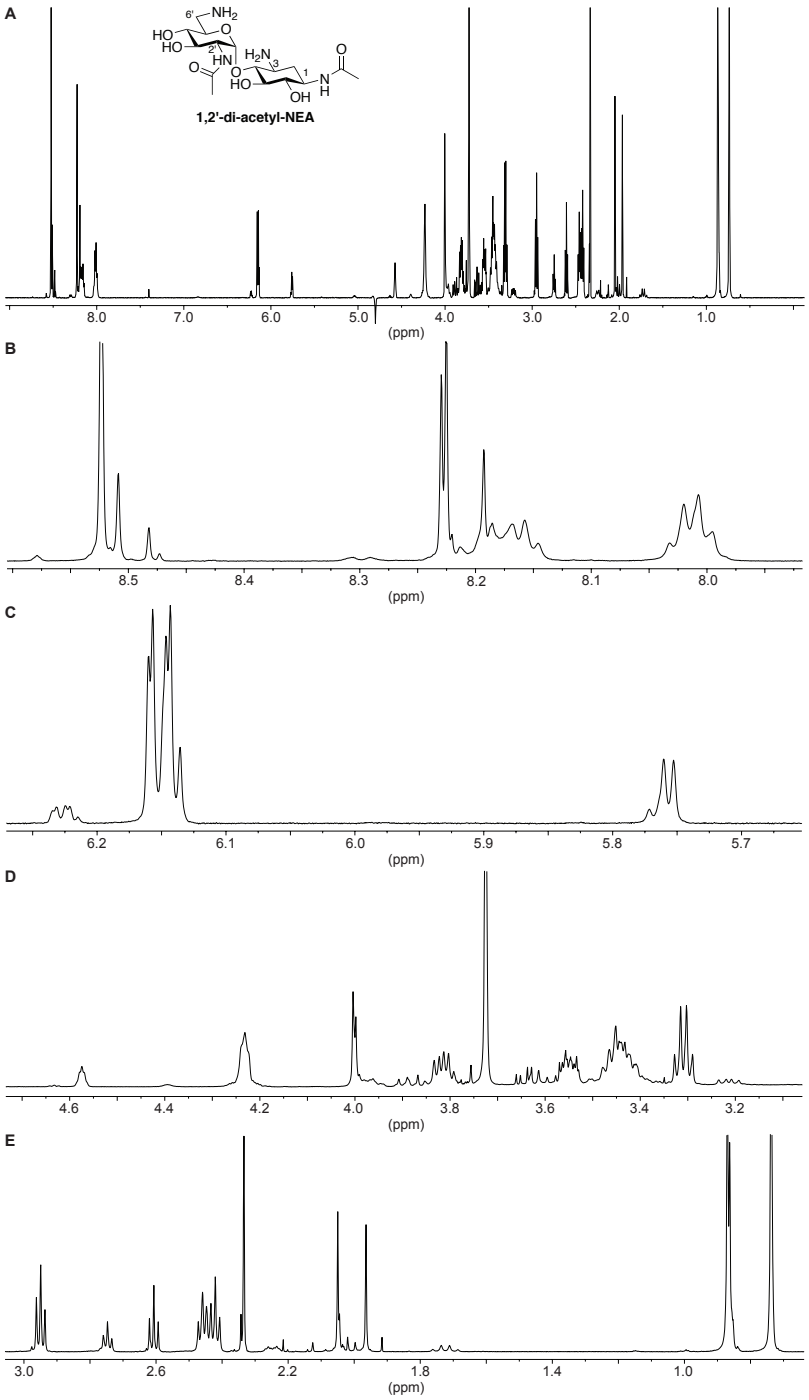

Fig. S9. ^{1}H NMR of 1,2'-di-acetyl-NEA in 1.2:8.8/D_2O:H_2O at pH 7.5 (500 MHz). The full spectrum is shown in panel **A** and the expansions in panels **B**-**D**.

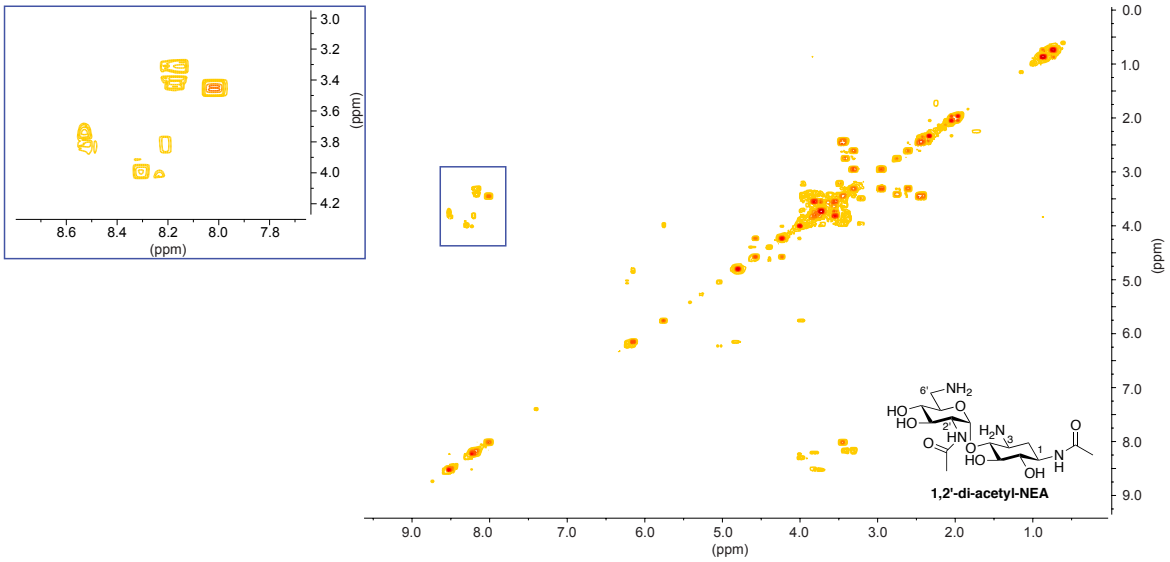

Fig. S10. gCOSY of 1,2'-di-acetyl-NEA in 1.2:8.8/D_2O:H_2O at pH 7.5 (500 MHz).

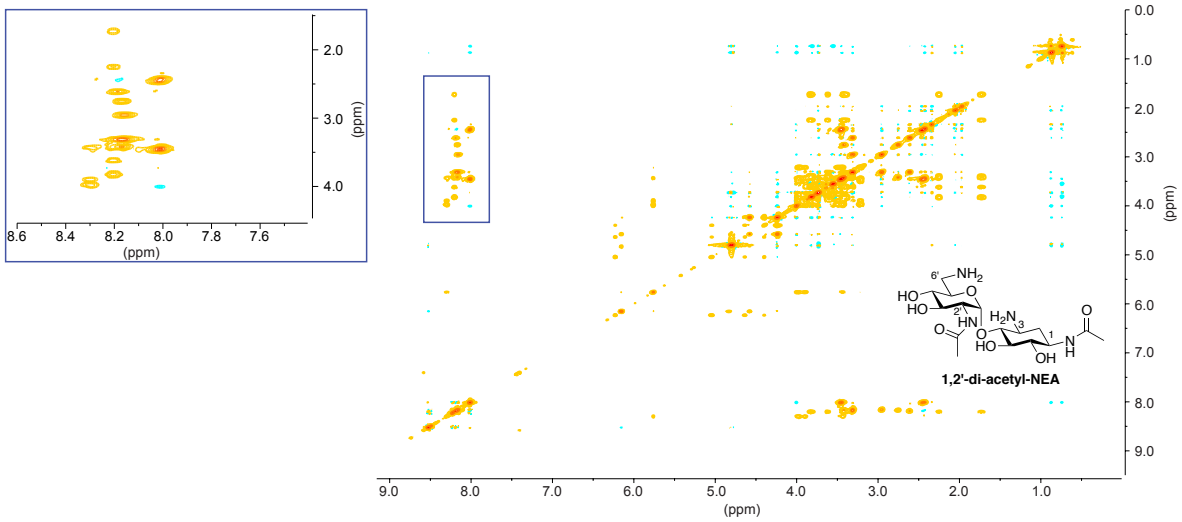

Fig. S11. zTOCSY of 1,2'-di-acetyl-NEA in 1.2:8.8/D_2O:H_2O at pH 7.5 (500 MHz).

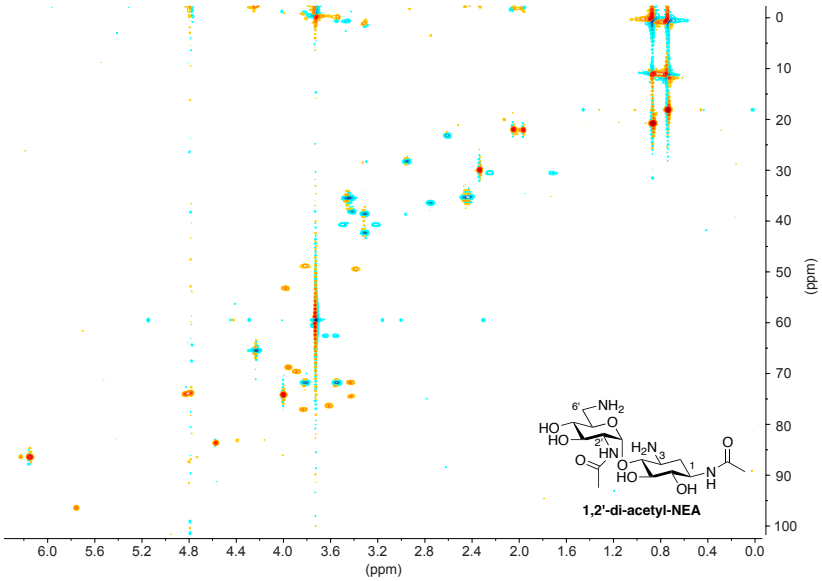

Fig. S12. gHSQC of 1,2'-di-acetyl-NEA in 1.2:8.8/D_2O:H_2O at pH 7.5 (500 MHz).