

Supporting Information

Insights into Substrate and Metal Binding from the Crystal Structure of Cyanobacterial Aldehyde Deformylating Oxygenase with Substrate Bound

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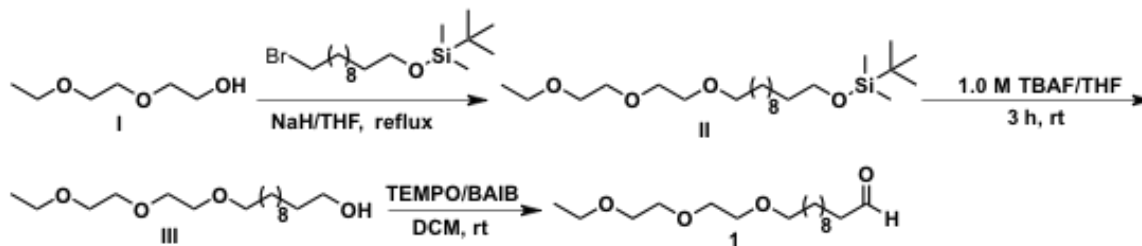
Procedures for the synthesis of 11-(2-(2-ethoxyethoxy)ethoxy)undecanal (**1**) and 2-nonylcyclopropane-1-carbaldehyde (**2'**)

Materials. All reagents were of the purest grade commercially available and used without further purification. Anhydrous solvents such as dichloromethane, n-hexane, ethyl acetate and diethyl ether were used without further distillation. Reactions were monitored by thin-layer chromatography (TLC) with visualization by potassium permanganate (KMnO₄) stains and 2,4-dinitrophenyl hydrazine (DNP) stains. All glassware was oven-dried before use. Column chromatography was performed with silica-gel mesh size 100-200 μm (Fisher Scientific, USA). The removal of solvent and other volatile impurities was performed under reduced pressure using rotatory evaporator in a water bath at < 37 °C. NMR spectra were measured in CDCl₃ at ambient temperature unless otherwise noted.

Synthesis of 11-(2-(2-ethoxyethoxy)ethoxy)undecanal (**1**):

The synthesis of 11-(2-(2-ethoxyethoxy)ethoxy)undecanal (**1**) was carried out in three steps as outlined in Scheme S1:

Scheme S1: 11-(2-(2-ethoxyethoxy)ethoxy)undecanal (**1**)



Synthesis of 2,2,3,3-tetramethyl-4,16,19,22-tetraoxa-3-silatetracosane (II). To a solution of diethyleneglycol monoethyl ether (**I**, 450 mg, 3.35 mmol) were added ((11-bromoundecyl)oxy)(*tert*-butyl)dimethylsilane (1000 mg, 2.74 mmol) and sodium hydride (NaH, 120 mg, 5 mmol) in tetrahydrofuran at room temperature. The reaction was refluxed at 60 °C for overnight. The solvent was removed under reduced pressure using a rotatory evaporator and the crude mixture was applied to a silica-gel column equilibrated in dichloromethane. The column was developed by slowly increasing the polarity of the solvent using a gradient of 0 to 0.5 % ethylacetate in dichloromethane to obtain **II** (1.2 g, 90%). The compound **II** was pure as judged by NMR. ¹H NMR (400 MHz, Chloroform-*d*) δ 3.64-3.42 (m, 10H), 3.35 (t, *J* = 8 Hz, 2H), 3.29 (t, *J* = 8 Hz, 2H), 1.48-1.39 (m, 8H), 1.19-1.09 (m, 13H), 0.80 (m, 13H), 0.80 (s, 9H), -0.05 (s, 6H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 72.55, 71.38, 70.56, 70.53, 70.34, 69.97, 69.81, 69.75, 69.71, 66.55, 66.49, 63.16, 61.53, 33.69, 32.77, 32.76, 29.54, 29.50, 29.49, 29.46, 29.39, 29.38, 29.34, 29.31, 28.67, 28.07, 26.00, 25.87, 25.70, 18.23, 15.05, 14.98, -5.38.

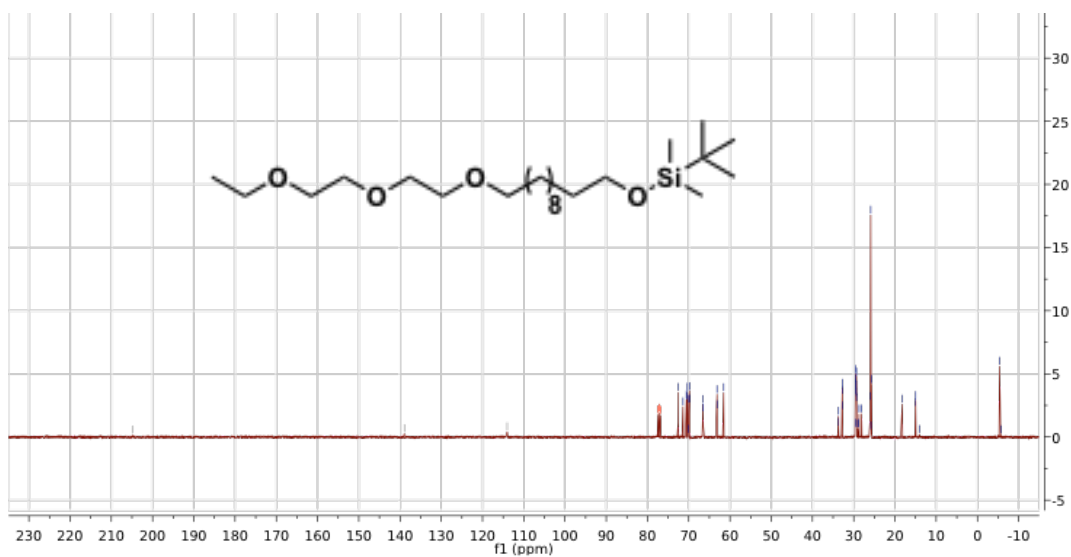
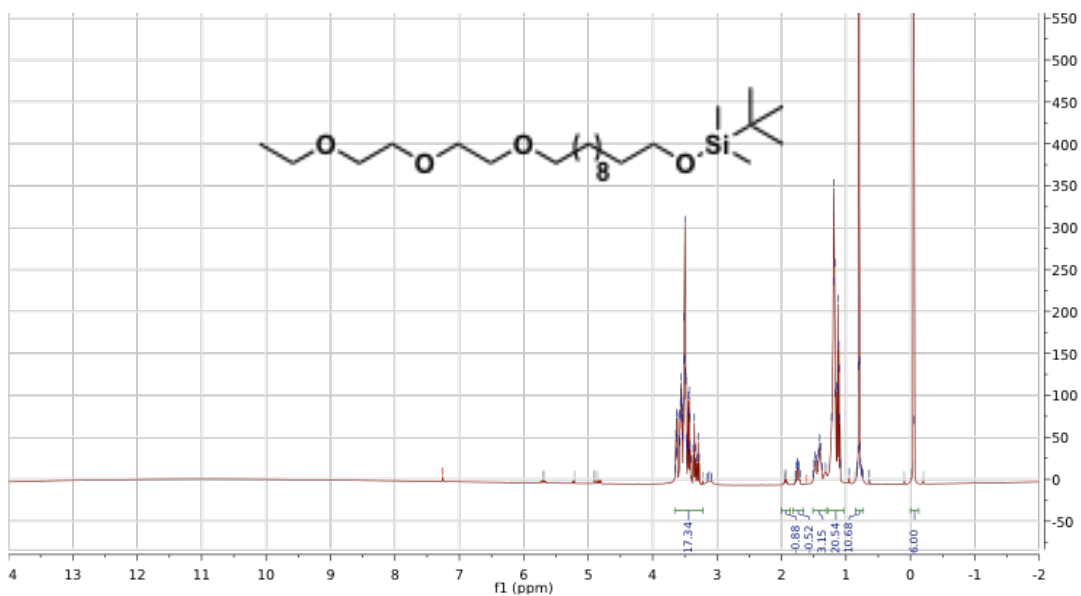


Figure S1. ¹H and ¹³C-NMR of Compound II.

Synthesis of 11-(2-(2-ethoxyethoxy)ethoxy)undecan-1-ol (III). 1 ml of 1.0 M TBAF in THF was added to a solution of **II** (350 mg, 0.86 mmol) in anhydrous THF at room temperature.¹ The reaction mixture was stirred for 3 h under nitrogen. The reaction mixture was diluted with 50 ml dichloromethane and washed with water (2 x 5 ml) and brine (2 x 2 ml). The organic layer was dried over sodium sulphate and concentrated by rotatory evaporation to afford the crude product as yellow oil that was further subjected to silica-gel chromatography using 10% Ethyl acetate/dichloromethane as the eluting solvent and yielded **III** (200 mg, 75%). ¹H NMR (400 MHz, Chloroform-*d*) δ 3.83 – 3.42 (m, 12H), 3.35 (t, *J* = 8 Hz, 2H), 3.04 (s, 1H), 1.44 (m, 8H), 1.17-1.09 (m, 12H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 72.61, 71.41, 70.53, 70.50, 70.24, 69.93, 69.78, 69.71, 66.58, 66.52, 62.52, 61.50, 32.60, 29.51, 29.47, 29.43, 29.37, 25.97, 25.71, 15.05, 14.96.

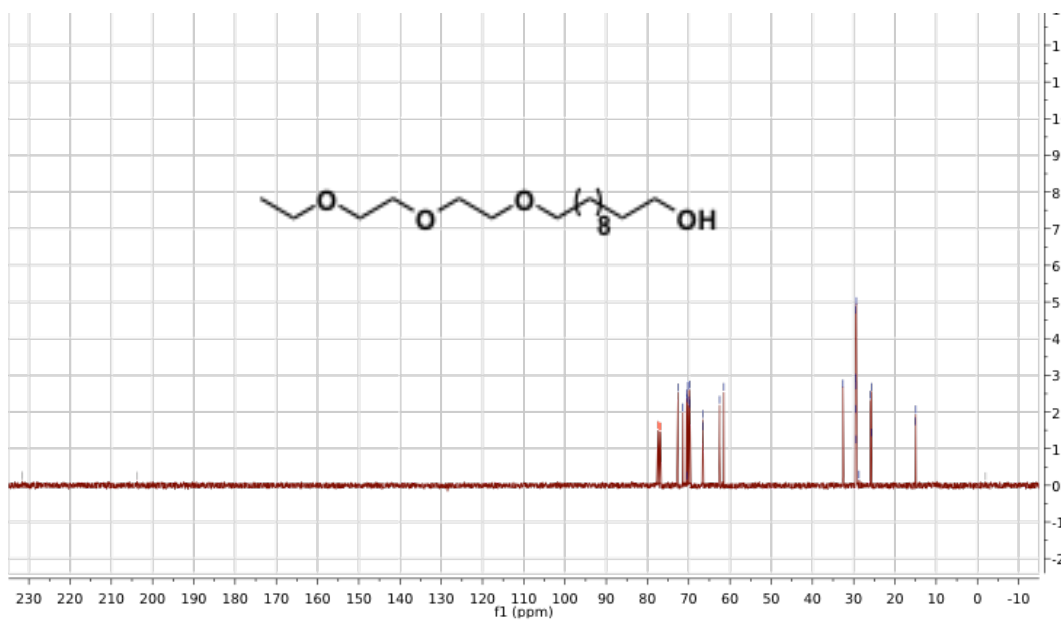
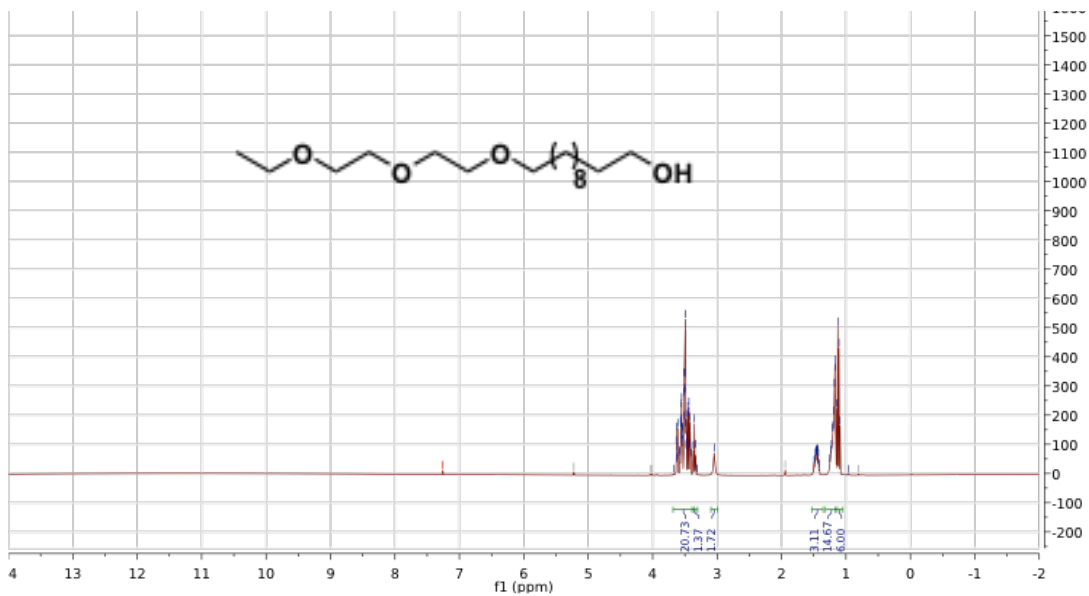


Figure S2. ¹H and ¹³C-NMR of Compound III.

Synthesis of 11-(2-(2-ethoxyethoxy)ethoxy)undecanal (1). To a solution of **III** (200 mg, 0.7 mmol) in dichloromethane at room temperature were added TEMPO (11 mg, 0.07 mol) and BAIB (250 mg, 0.77 mmol).² The reaction was stirred at room temperature for 3 hours under nitrogen. After completion, the crude reaction mixture was concentrated using a rotatory evaporator and subjected to silica-gel column chromatography in dichloromethane in which the gradient of the solvent was gradually increased from neat dichloromethane to 5 % ethylacetate/dichloromethane to obtain **1** (150 mg, 75%). The compound **1** was pure as judged by NMR (Figure S3) and TLC. ¹H NMR (500 MHz, Chloroform-*d*) δ 9.77 (t, $J = 1.9$ Hz, 1H), 3.69 – 3.58 (m, 8H), 3.54 (q, $J = 7.0$ Hz, 2H), 3.45 (t, $J = 6.8$ Hz, 2H), 2.42 (td, $J = 7.4, 1.9$ Hz, 2H), 1.68 – 1.53 (m, 4H), 1.37-1.28 (m, 12H), 1.22 (t, $J = 7.0$ Hz, 3H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 203.02, 71.50, 70.64, 70.61, 70.03, 69.81, 66.63, 43.90, 29.59, 29.48, 29.42, 29.33, 29.32, 29.13, 26.04, 22.05, 15.14.

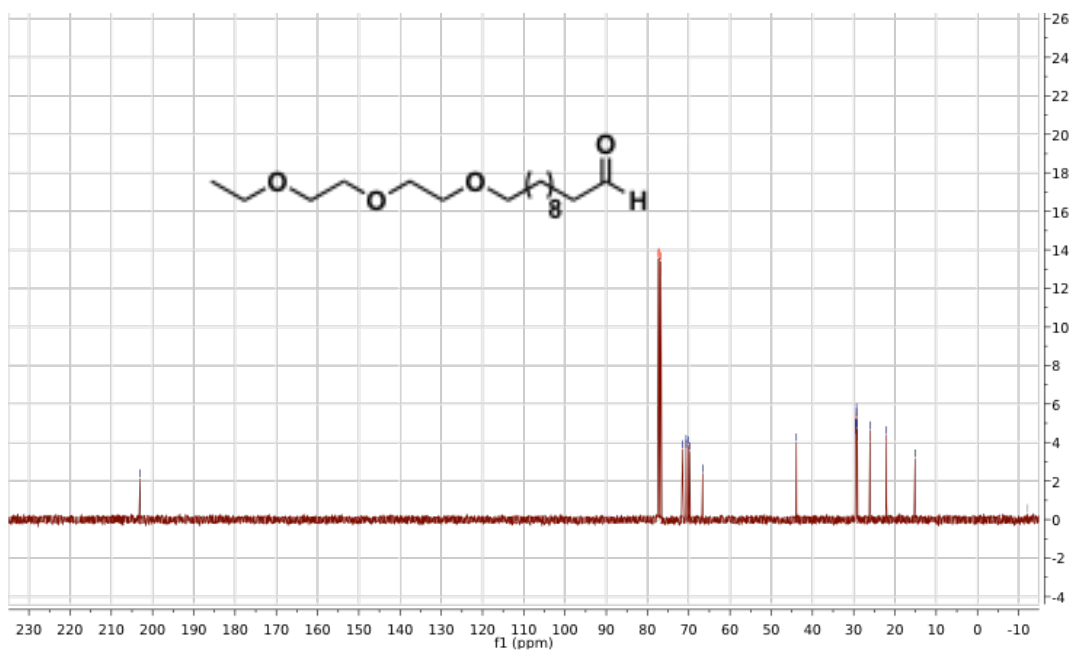
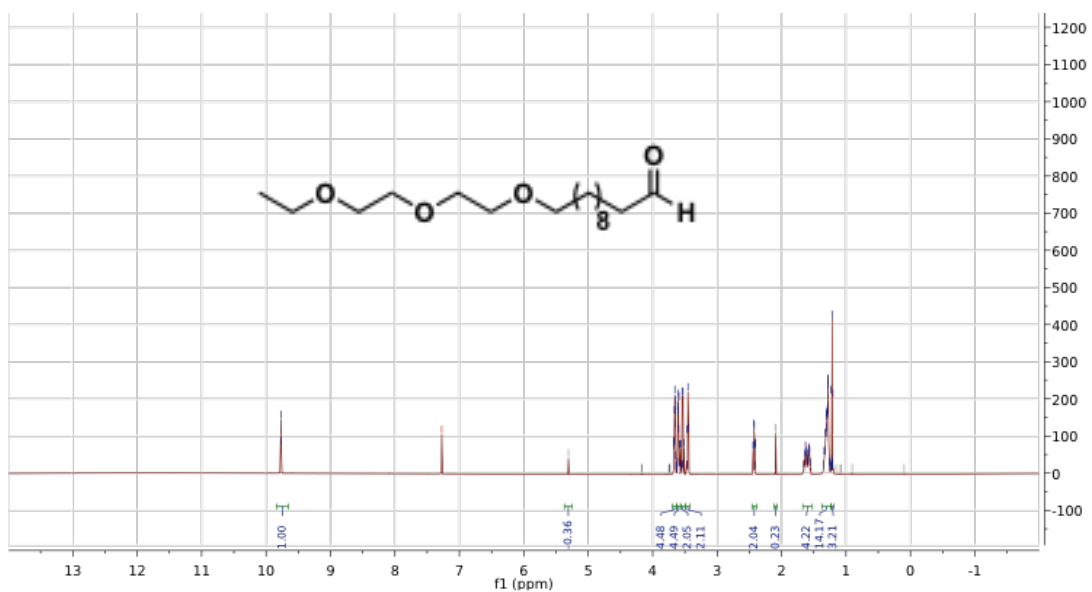
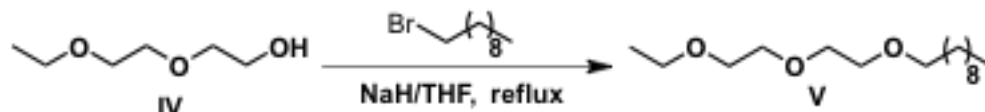


Figure S3. ¹H and ¹³C-NMR of Compound 1.

Synthesis and characterization of 1-(2-(2-ethoxyethoxy)ethoxy)decane (V): (Product standard for reaction of 1 with cDAO)

1-(2-(2-ethoxyethoxy)ethoxy)decane (V) was carried out in one-step as outlined in Scheme S2:

Scheme S2. Synthesis of 1-(2-(2-ethoxyethoxy)ethoxy)decane (V)

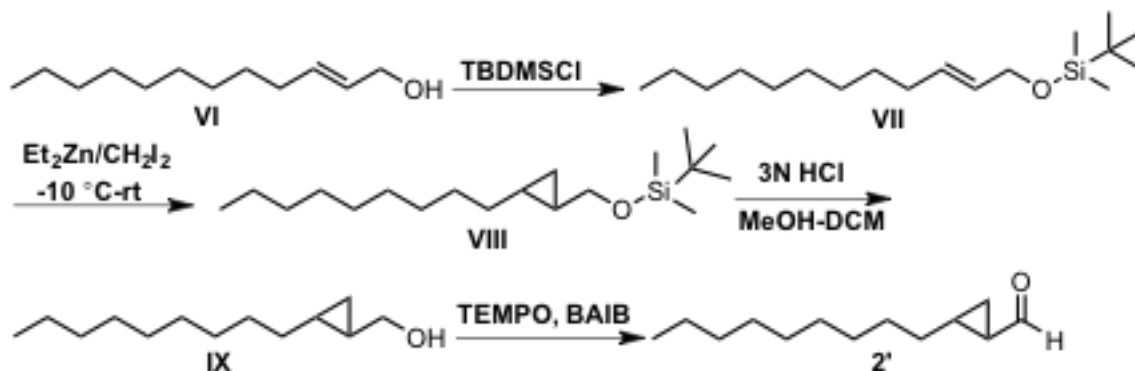


Synthesis of 1-(2-(2-ethoxyethoxy)ethoxy)decane (V). To a solution of IV (268.2 mg, 2 mmol) in anhydrous tetrahydrofuran at room temperature were added 1-bromodecane (372.7, 1.8 mmol) and sodium hydride (72 mg, 3 mmol). The reaction mixture was refluxed at 60 °C and stirred for overnight. Upon completion of the reaction as judged by TLC, the crude mixture was concentrated on a rotatory evaporator. V was purified by silica-gel column chromatography using 1% ethylacetate/dichloromethane as the eluting solvent (390 mg, 80 %). The compound V was pure as judged by NMR (Figure S4) and TLC. ¹H NMR (400 MHz, Chloroform-*d*) δ 3.69-3.58 (m, 8H), 3.52 (q, *J* = 8 Hz, 2H) 3.43 (t, *J* = 6.8 Hz, 2H), 1.59 -1.55 (m, 4H), 1.35-1.19 (m, 14H), 0.87 (t, *J* = 6.7 Hz, 3H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 71.54, 70.65, 70.62, 70.04, 69.82, 66.62, 31.89, 29.61, 29.59, 29.56, 29.48, 29.31, 26.07, 22.67, 15.14, 14.11.

Synthesis and characterization of 2-nonylcyclopropane-1-carbaldehyde (2')

The synthesis of 2-nonylcyclopropane-1-carbaldehyde (2') was carried out in four steps as described in the literature.³

Scheme S3. Synthesis of 2-nonylcyclopropane-1-carbaldehyde (2')



Synthesis of (*E*)-tert-butyl(dodec-2-en-1-yloxy)dimethylsilane (VII). The alcohol functional group in **VI** was protected by *tert*-Butyl-dimethylsilyl group (TBDMS). To a solution of **VI** (500 mg, 2.7 mmol) in anhydrous dichloromethane (DCM) at 0 °C were added imidazole (125 mg, 1.84 mmol), and catalytic amount of DMAP. TBDMS chloride (500 mg, 3.3 mmol) was added to the reaction mixture resulting in the formation of white suspension. The reaction mixture was gradually warmed to room temperature and stirred overnight. The reaction mixture was diluted with DCM (50 ml) and washed with water (2 X 5 ml). The organic layer was separated, dried over anhydrous sodium sulphate and concentrated on a rotatory evaporator. The crude mixture was purified by silica-gel chromatography using 1 % ethylacetate/n-hexane as the eluting solvent to yield **VII** (700 mg, 86%). The compound **VII** was pure as judged by NMR (Figure S5) and TLC. ¹H NMR (500 MHz, Chloroform-*d*) δ 5.65–5.54 (m, 2H), 4.16–4.12 (m, 2H), 2.07-2.03 (m, 2H), 1.45-1.20 (m, 12H), 0.97-0.83 (m, 15H), 0.08 (s, 6H). ¹³C NMR (126 MHz,

Chloroform-*d*) δ 131.57, 129.05, 64.11, 32.21, 31.92, 29.61, 29.53, 29.35, 29.22, 25.99, 22.69, 18.43, 14.12, -5.10.

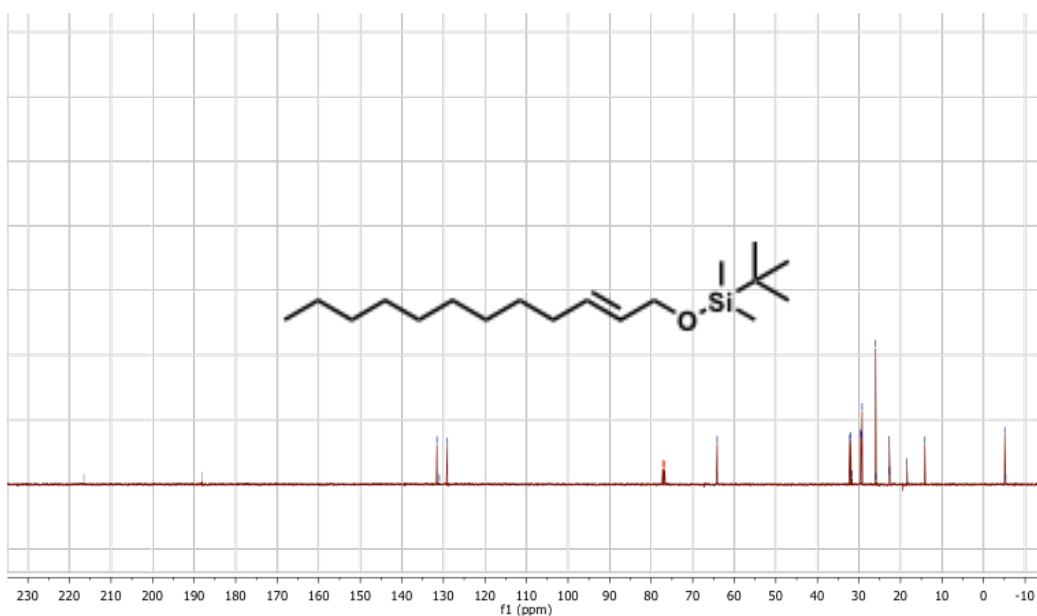
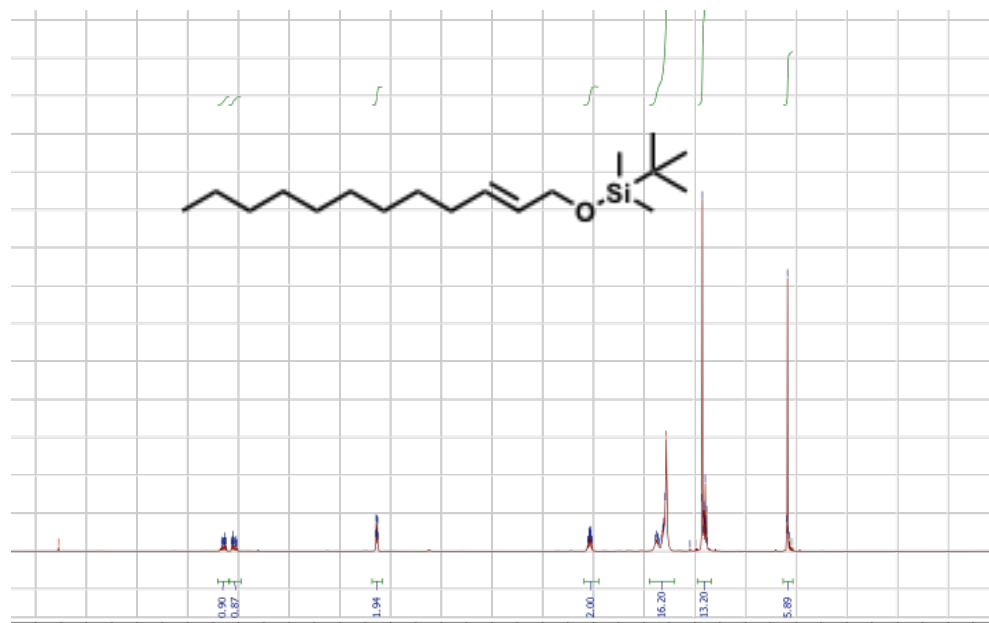


Figure S5. ¹H and ¹³C-NMR of Compound VII.

Synthesis of tert-butyldimethyl((2-nonylcyclopropyl)methoxy)silane (VIII). VII was converted to VIII based on literature procedures. To a solution of VII (100 mg, 0.33 mmol) in anhydrous dichloromethane at 0 °C were added diethyl zinc (80 μ l, 0.78 mmol) and diiodomethane (100 μ l, 1.24 mmol). The reaction mixture was warmed to 40 °C and stirred for overnight. Upon completion of the reaction as judged by TLC, the crude mixture was concentrated on a rotatory evaporator. VIII was purified by silica-gel column chromatography using 1% ethylacetate/n-hexane as the eluting solvent and was obtained as predominant the *trans*-stereoisomer (62 mg, 60%). The compound VIII was > 90% pure as judged by NMR (Figure S6) and TLC. ¹H NMR (401 MHz, Chloroform-*d*) δ 3.53-3.40 (m, 2H), 1.39-1.16 (m, 16H), 0.91-0.77 (m, 15H), 0.78-0.68 (m, 1H), 0.59-0.49 (m, 1H), 0.36-0.28 (m, 1H), 0.26-0.19 (m, 1H), 0.05 (d, *J* = 2.1 Hz, 6H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 67.06, 34.63, 33.75, 31.91, 31.58, 29.70, 29.62, 29.59, 29.52, 29.49, 29.42, 29.36, 29.33, 29.28, 29.13, 25.95, 25.24, 22.67, 22.64, 21.01, 18.36, 16.95, 14.07, 9.78, -5.16, -5.19.

Synthesis of (2-nonylcyclopropyl)methanol (IX). 1 ml of 3N methanolic hydrochloric acid was added to a solution of **VIII** (100 mg, 0.32 mmol) in dichloromethane at 0°C for 4 hours. The reaction mixture was diluted with 50 ml dichloromethane and washed with water (2 x 5 ml) and brine (2 x 2 ml). The organic layer was dried over sodium sulphate and concentrated by rotatory evaporation to afford the crude product as yellow oil that was further subjected to silica-gel chromatography using 10% ethylacetate/n-hexane as the eluting solvent and yielded **IX** as yellow liquid (50 mg, 80%). The compound **IX** was pure as judged by NMR (Figure S7) and TLC. ¹H NMR (400 MHz, Chloroform-*d*) δ 3.52-3.25 (m, 2H), 1.47-1.09 (m, 16H), 0.92-0.71 (m, 4H), 0.62-0.47 (m, 1H), 0.39-0.25 (m, 2H). ¹³C NMR (100 MHz, Chloroform-*d*) δ 67.21, 33.56, 31.87, 29.64, 29.58, 29.41, 29.31, 22.65, 21.16, 17.17, 14.08, 9.89.

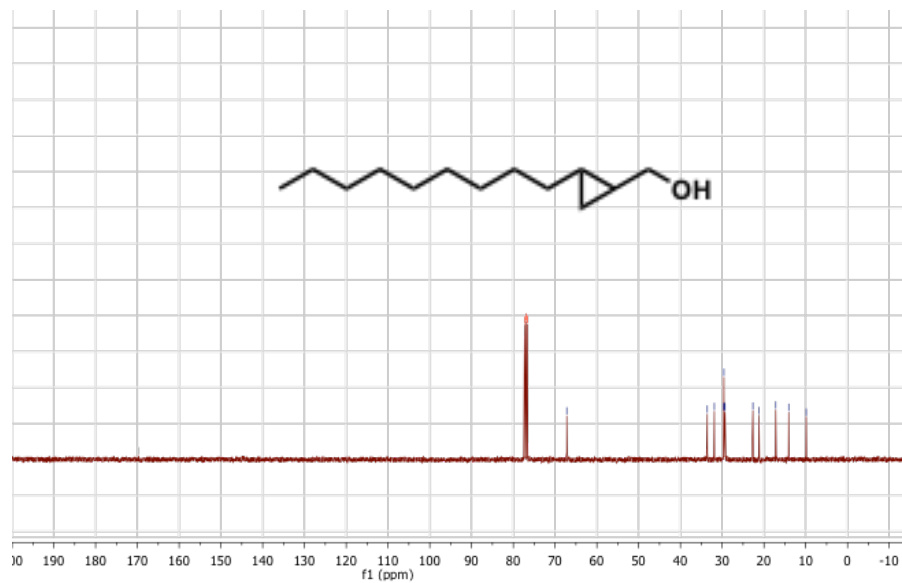
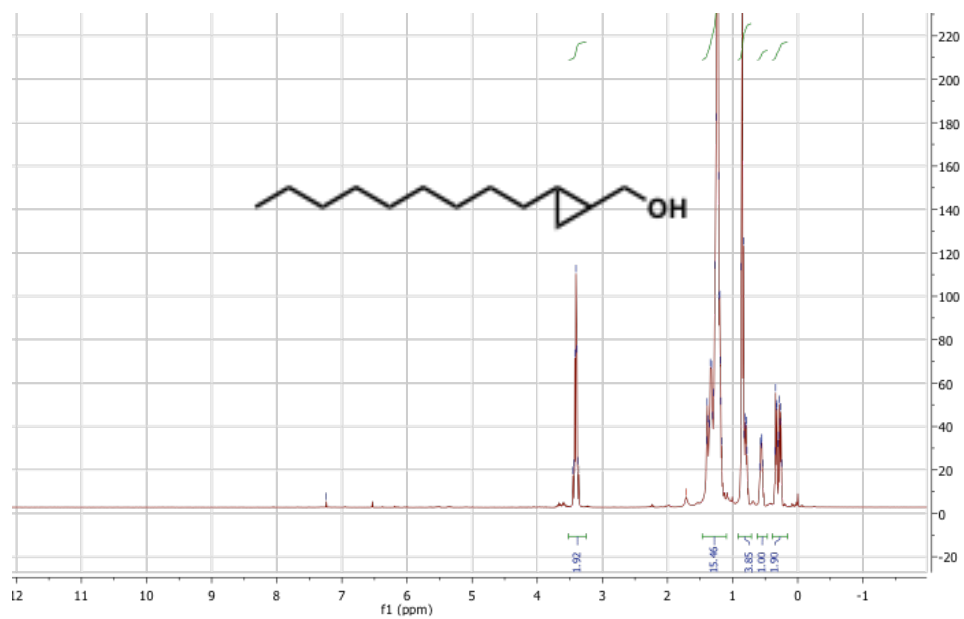


Figure S7. ¹H and ¹³C-NMR of Compound IX.

Synthesis of 2-nonylcyclopropane-1-carbaldehyde (2'). To a solution of **IX** (50 mg, 0.25 mmol) in dichloromethane at room temperature were added TEMPO (4 mg, 0.2 mmol) and BAIB (75 mg, 0.23 mmol). The reaction was stirred at room temperature for 3 hours under nitrogen. After completion, the crude reaction mixture was concentrated using a rotatory evaporator and subjected to silica-gel column chromatography in n-hexane/diethylether in which the gradient of the solvent was gradually increased from neat n-hexane to 5% diethylether/n-hexane to obtain **2'** (30 mg, 40%). The compound **2'** was pure as judged by NMR (Figure S8) and TLC. The identity of the compound was confirmed by high resolution electron-impact MS (m/z): calculated 197.1905; observed 197.1907. ^1H NMR (500 MHz, Chloroform-*d*) δ 9.00 (d, J = 5.6 Hz, 1H), 1.66-1.60 (m, 1H), 1.51-1.45 (m, 1H), 1.42 – 1.23 (m, 18H), 0.91 – 0.87 (m, 5H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 201.02, 32.60, 31.84, 30.50, 29.51, 29.26, 29.22, 29.05, 22.68, 22.63, 14.85, 14.06.

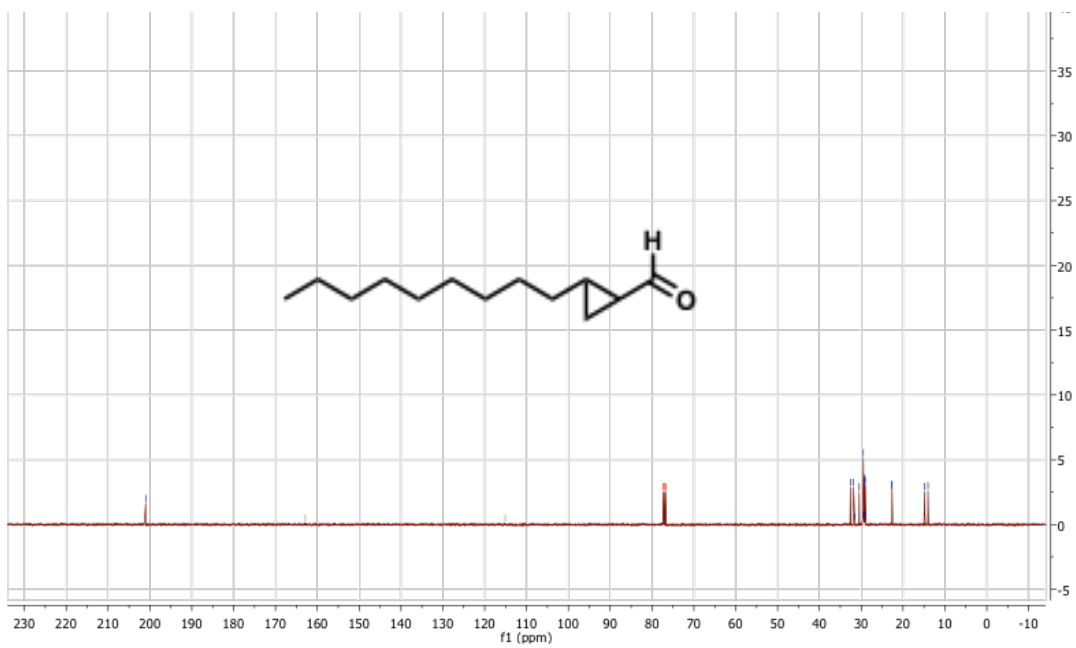
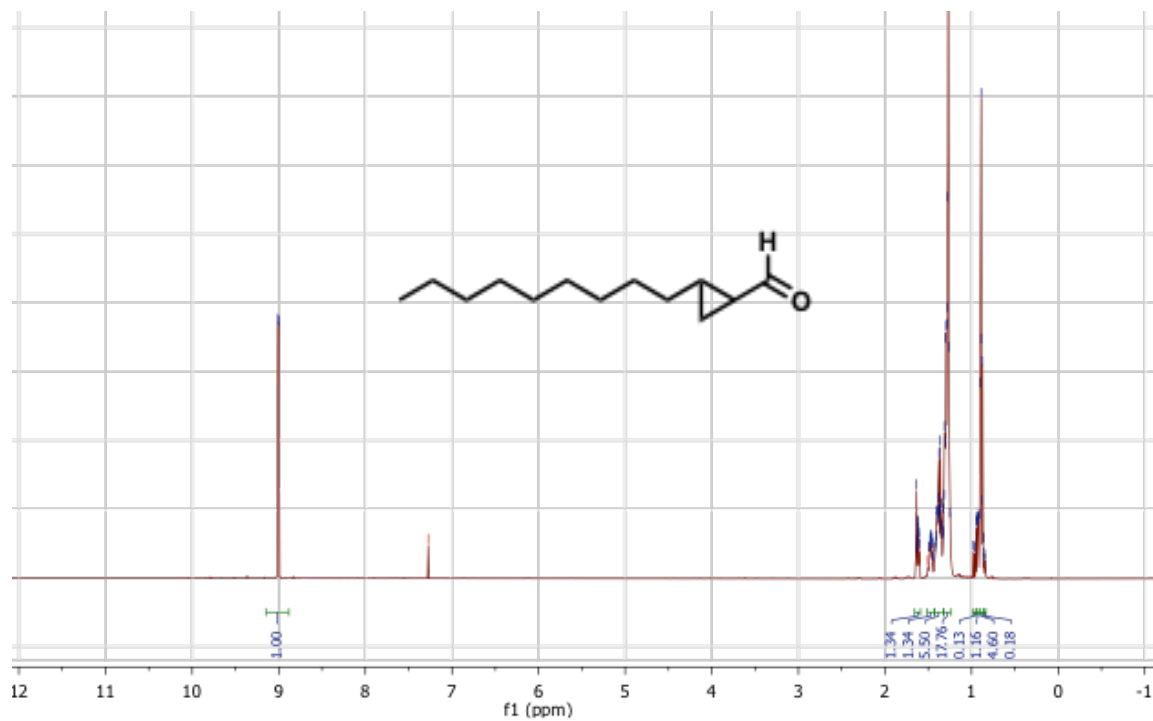


Figure S8. ^1H and ^{13}C -NMR of Compound 2'.

Activity of cADO with compound 2

Enzyme activity assays were performed as described in the main text. Compound **V** was used as a standard to quantify the amount of product formed; a typical set of assay data are shown in Figure S9.

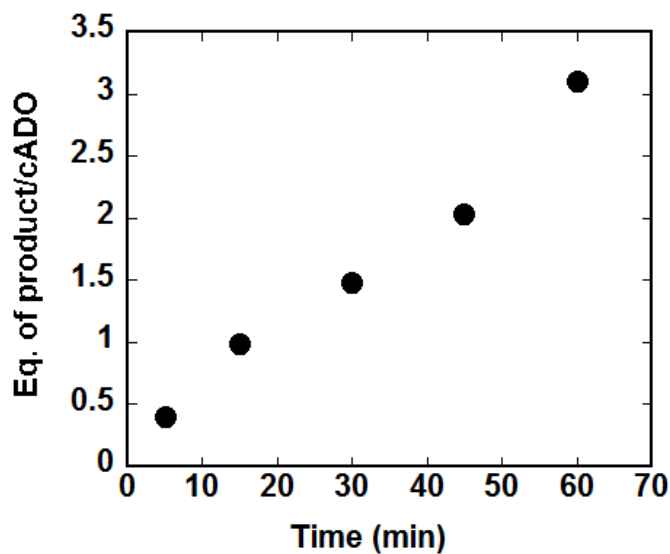


Figure S9. Time course study of cADO deforming 1-(2-(2-ethoxyethoxy)ethoxy)undecanal (**1**) to produce 1-(2-(2-ethoxyethoxy)ethoxy)decane. The rate of product formation from **1** is $0.049 \pm 0.002 \text{ min}^{-1}$.

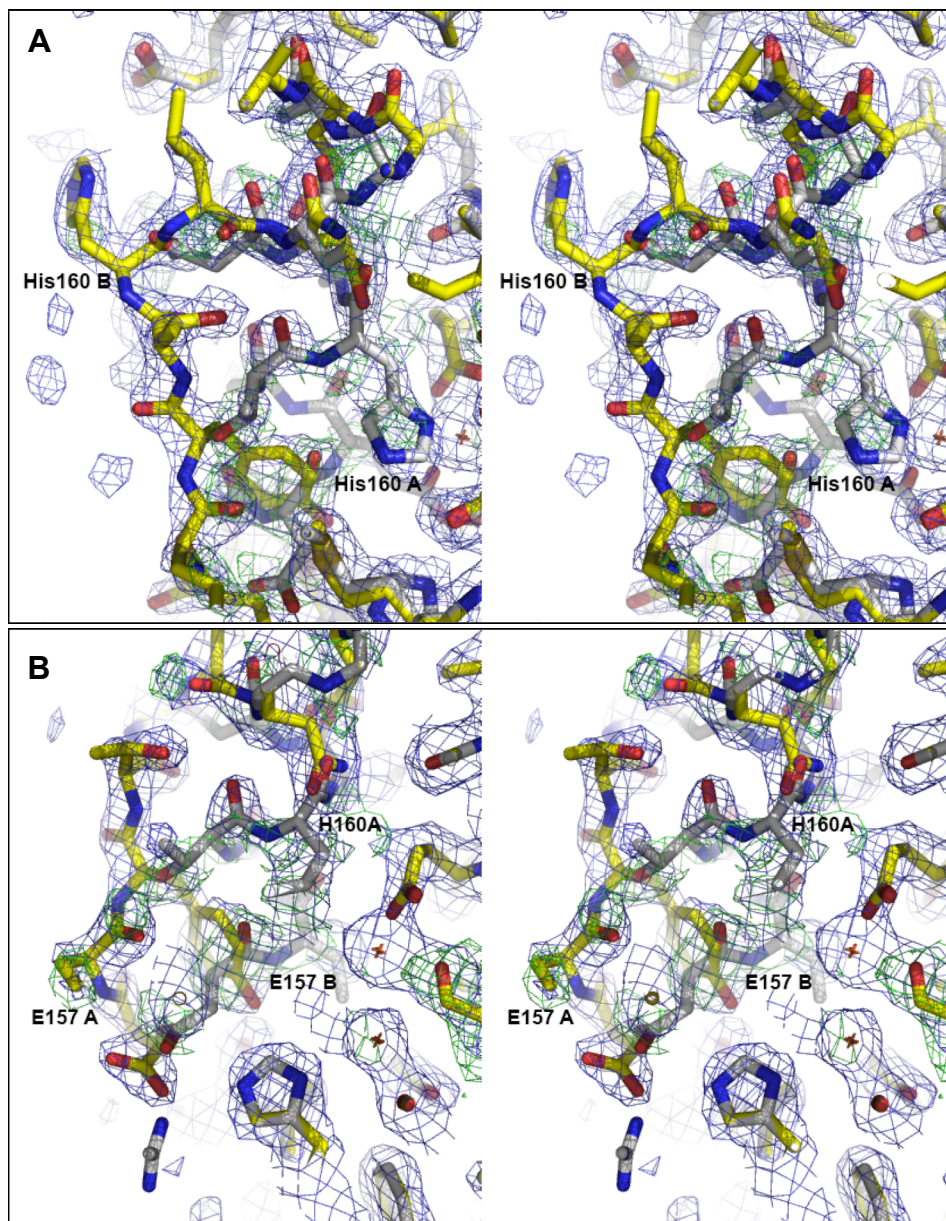


Figure S10. Stereodiagrams of electron density maps resulting from the refinement of the B conformation of helix 5 in the cADOL194A-1 structure. The $2F_o-F_c$ electron density map contoured at 1σ is shown as a blue grid, while the F_o-F_c map contoured at 3σ is shown in green. The alternate positions of His160 and Glu157 are depicted in panels A and B, respectively. Carbon atoms from the A conformation of the helix are shown as gray sticks; B conformation carbon atoms are in yellow. Nitrogens and oxygens of both conformations are shown in blue and red, respectively. Iron atoms positions are depicted with X's.

References:

1. Rajesh, M.; Sen, J.; Parlanti, L.; Srujan, Mukherjee, K. Sreedhar, B., Chaudhuri, A. (2007) Dramatic Influence of the Orientation of Linker between Hydrophilic and Hydrophobic Lipid Moiety in Liposomal Gene Delivery, *J. Am. Chem. Soc.* *129*, 11408–11420.
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3. Paul, B.; Das, D.; Ellington, B.; Marsh, E. N. G. (2013) Probing the mechanism of cyanobacterial aldehyde decarbonylase using a cyclopropyl aldehyde, *J. Am. Chem. Soc.* *135*, 5234–5237.