Supporting Information for

Evidence for Tunneling in Base Catalyzed Isomerization of Glyceraldehyde to Dihydroxyacetone by Hydride Shift under Formose Conditions

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1. MATERIAL AND METHODS

Solvents, organic/inorganic reagents were purchased from commercial venders either with a guarantee certificate for absence of the following enzyme traces: DNase, RNase, proteases and phosphatases or of highest purity available, and were used without further purification unless otherwise mentioned. Solvents for HPLC were purchased from Thermo Fisher Scientific of "HPLC" grade and filtered after mixing through MicroLiter 0.2 μm Nylon filters.

NMR spectra were obtained on a Bruker Avance III 400 (400 MHz) spectrometer.

Analytical HPLC was run on a Waters 600 liquid chromatograph equipped with a pumping system, an autosampler (717 plus), a photodiode array UV–Vis detector (2996), and a Waters SunFire™ end–capped C_{18} reverse–phase analytical column (4.6 mm internal diameter × 150 mm length, particle size: 5 nm, particle shape: spherical, pore size: 100 Å, carbon load: 16 %). The reaction solution was analyzed with UV–Vis detection at 365 nm. The elution was 40 % CH_3CN in water at a flow rate of 1.0 $mL{\cdot}min^{-1}$.

The hydrolysis and incubation procedures were performed using VWR Analog Dry Block Heaters equipped with VWR Modular Heating Blocks. Temperature stabilities were kept at ± 0.5 °C (for the hydrolysis at 40 °C and incubation at 37 °C) and ± 1 °C (for the hydrolysis at 80 °C). Hydrolysis at 0 °C was conducted using an ice-water complex.

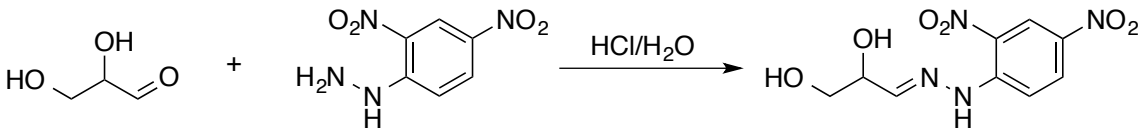
D-[2-^{2}H]glyceraldehyde (2-deuteroglyceraldehyde) was purchase from Omicron Biochemicals, Inc. as an aqueous solution of 0.115 M (98 atom% ^{2}H).

Abbreviations:

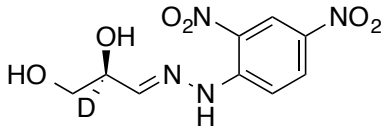
GLA	Glyceraldehyde
DHA	Dihydroxyacetone
2,4-DNP	2,4-Dinitrophenylhydrazone
GLA-2,4-DNPH	Glyceraldehyde 2,4-dinitrophenylhydrazone
DHA-2,4-DNPH	Dihydroxyacetone 2,4-dinitrophenylhydrazone
Ac-2,4-DNPH	Acetone 2,4-dinitrophenylhydrazone

2. PREPARATION OF CARBONYL HYDRAZONES

2.1 Preparation of GLA-2,4-DNPHs [1]

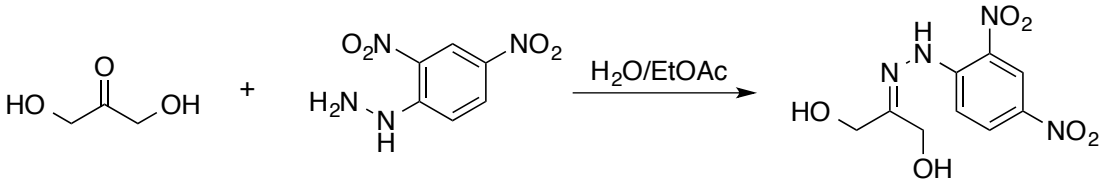


A solution of 0.4 g (4.4 mmol) of D/L-GLA dimer in 2.5 mL of water at 0 °C was added to a solution of 0.8 g (4 mmol) of 2,4-DNP in 48 mL of 2N hydrochloride solution at 0 °C. After the mixture was stirred at 0 °C for 6 hours, the precipitate was filtrated, washed with 2N hydrochloride and water, yield 1.045 g (97 %) as a yellow solid, which contains only D/L-GLA-2,4-DNPH. ^{1}H NMR (DMSO-d_6) δ 3.57-3.58 (2H, J = 5.6 Hz, HO-CH_2), 4.18-4.23 (dd, 1H, J = 5.6 Hz, 11.6 Hz, HO-CH), 7.92-7.98 (m, 2H, NH-C-CH-CH, N-CH), 8.35-8.38 (dd, 1H, J = 2.4 Hz, 9.6 Hz, CH-CH-C-NO_2), 8.846-8.853 (d, 1H, J = 2.8 Hz, O_2N-C-CH-C-NO_2), 11.39 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 64.5 (HO-CH$_2$-CH), 72.1 (HO-CH-C), 117.0 (NH-C-CH-C), 123.4 (O_2N-C-CH-C-NO_2), 129.6 (O_2N-C-CH-C-NH), 130.2 (NH-C-CH-CH), 137.3 (NH-C-CH-CH-C-NO_2), 145.3 (NH-C), 155.1 (N-C-CH_2).



The deuterated hydrazone D-[2-^{2}H]-GLA-2,4-DNPH was isolated after the HPLC assay by preparative TLC. ^{1}H NMR (DMSO-d_6) δ 3.53-3.55 (2H, J = 5.2 Hz, HO-CH_2), 4.83-4.86 (t, 1H, J = 5.7 Hz, HO-CH_2), 5.39 (s, 1H, HO-CD), 7.88-7.94 (m, 2H, NH-C-CH-CH, N-CH), 8.31-8.33 (d, 1H, J = 7.6 Hz, CH-CH-C-NO_2), 8.83-8.84 (d, 1H, J = 2.4 Hz, O_2N-C-CH-C-NO_2), 11.42 (s, 1H, NH).

2.2 Preparation of DHA-2,4-DNPH [2]



DHA-2,4-DNPH was prepared according to the literature starting from 1,3-DHA dimer. ^{1}H NMR (DMSO-d_6) δ 4.11-4.12 (d, 2H, J = 6.0 Hz, C-CH_2-O), 4.56 (s, 2H, C-CH_2-O), 5.27-5.30 (t, 1H, J = 6.0 Hz, CH_2-OH), 6.37 (s, 1H, CH_2-OH), 7.94-7.97 (d, 1H, J = 9.6 Hz, NH-C-CH-CH), 8.36-8.39 (dd, 1H, J = 2.4 Hz, 9.6 Hz, CH-CH-C-NO_2), 8.88-8.89 (d, 1H, J = 2.4 Hz, O_2N-C-CH-C-NO_2), 13.15 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 60.8 (C-CH_2-OH), 63.8 (C-CH$_2$-OH), 116.2 (NH-C-CH-C), 123.6 (O_2N-C-CH-C-NO_2), 129.5 (O_2N-C-CH-C-NH), 130.3 (NH-C-CH-CH), 137.1 (NH-C-CH-CH-C-NO_2), 145.2 (NH-C), 158.8 (N-C-CH_2).

3. HPLC ASSAY [3–6]

Reaction solutions were prepared by combining appropriate amount of GLA and $Ca(OH)_2$ to give a reaction mixture. A representative reaction solutions were prepared by combining D-GLA (10 μL, 100 mM), $Ca(OH)_2$ (30 μL, 20 mM) and water (960 μL). The final concentration was D-GLA 1 mM, $Ca(OH)_2$ 0.6 mM, pH ~10.

GLAs and their isomerization products DHAs were derivatized as the hydrazones (GLA-2,4-DNPH, DHA-2,4-DNPH) using excess 2,4-DNP at 37 °C for 1 hour. To carry out these reactions, samples were removed from the block heater (40 °C/80 °C) or ice (0 °C) at appropriate intervals, immediately mixed with CH_3CN (40 μL) and 5 μL of 2,4-DNP (5 mM in 20 % $HClO_4/CH_3CN$). The tube was capped, vigorously mixed, and submerged in a thermally equilibrated block heater at 37 °C. After incubating for 1 hour, the samples were removed and the solution pH was adjusted to ~7 by adding HEPES buffer (15~20 μL, pH 10) to prevent potential degradation of the formed hydrazones. Ac-2,4-DNPH (analytical standard purchased from Sigma-Aldrich) was added as the internal standard. Precipitated 2,4-DNP was removed by filtration, and then the samples were analyzed by HPLC (λ^{max} 365 nm). A representative HPLC spectrum was as following (Figure S1):

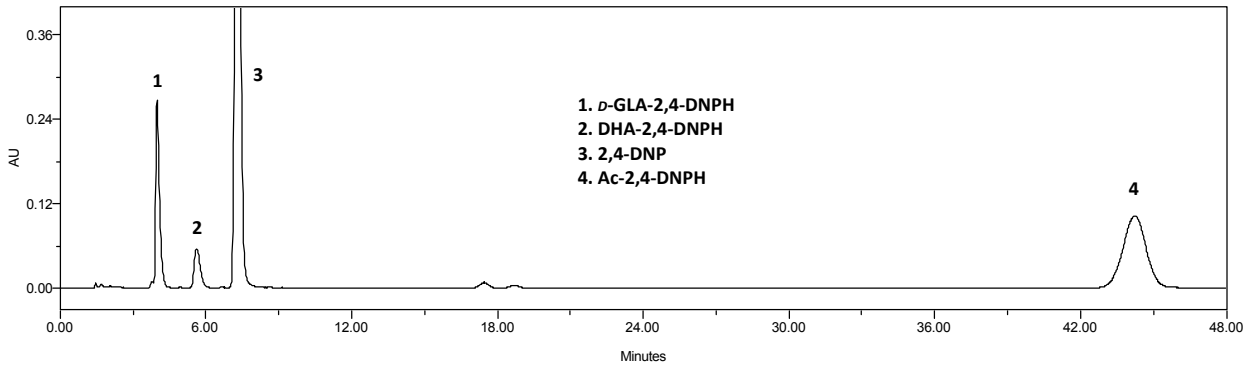


Figure S1. HPLC chromatogram of a reaction solution

A comparison of DHA-2,4-DNPH and Ac-2,4-DNPH was used to confirm that derivatization reactions were completed within 1 hour (Figure S2).

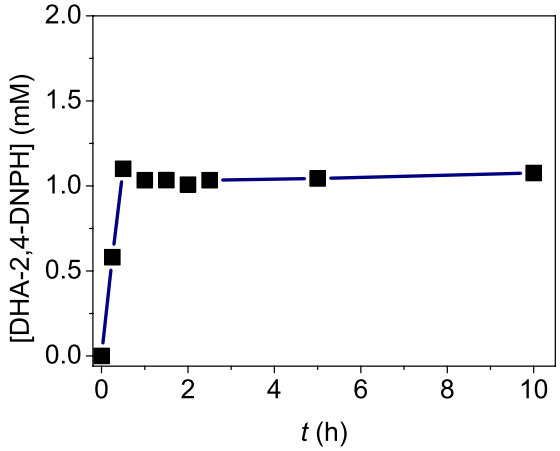


Figure S2. Derivatization of DHA with excess 2,4-DNP is completed within 1 hour at 37 °C.

The stabilities of DHA-2,4-DNPH and Ac-2,4-DNPH in the final solution after the adjustment of pH to ~7 were also determined (Figure S4).

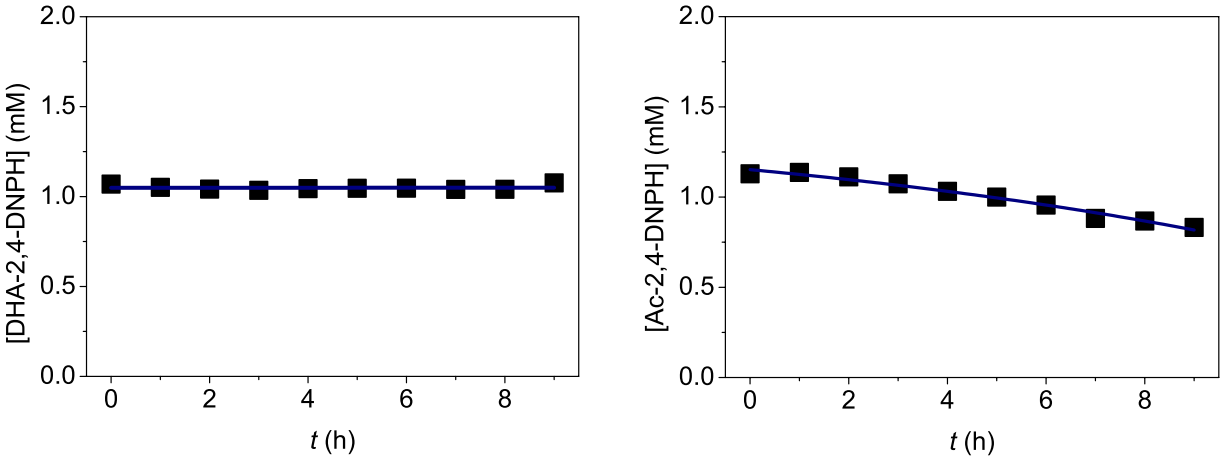


Figure S3. Stability of DHA-2,4-DNPH and Ac-2,4-DNPH after adjustment of pH to ~7. Less than 5% and 15 % depletion were observed over 9 hours, for DHA-2,4-DNPH and Ac-2,4-DNPH, respectively.

A comparison of ^{1}H NMR spectra of D-[2-^{2}H]-GLA-2,4-DNPH (before and after 4 hours' isomerization at 40 °C) was used to confirm that no significant deuterium was lost in the isomerization or during the derivatization. Before isomerization, 98 atom% ^{2}H. After isomerization, 95 atom% ^{2}H (Figure S5).

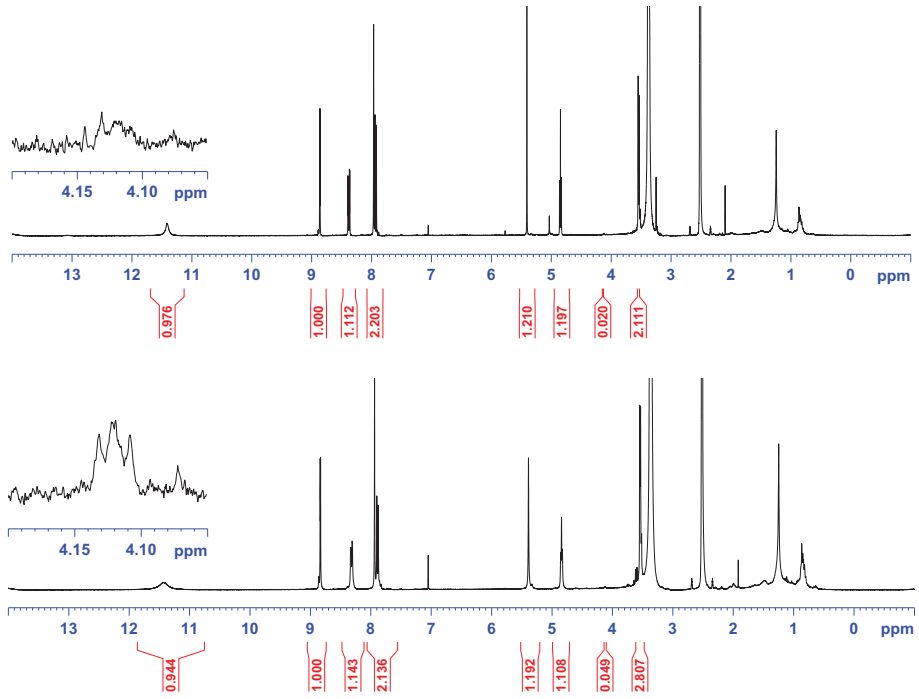


Figure 5. Comparison of deuterium content in the hydrazone derived from D-[2-^{2}H]-GLA before (up) and after the isomerization (down).

Upon each day of chromatography, constants *m* and *b* of the Equation (S1)

$$[\text{DHA-2,4-DNPH}] = m \times [\text{Ac-2,4-DNPH}] \times \frac{\%\text{Area}_{\text{DHA-2,4-DNPH}}}{\%\text{Area}_{\text{Ac-2,4-DNPH}}} + b \qquad \text{(S1)}$$

were determined by a linear fit of the data derived from the injection of at least five calibration standards. These calibration standards were each fixed concentration in internal standard [Ac-2,4-DNPH] and varied in DHA-2,4-DNPH concentration [DHA-2,4-DNPH]. A representative standard sample result was as following (Table S1, Figure S5):

Table S1. Determination of *m* & *b*

No.	[Ac-2,4-DNPH] (mM)	[DHA-2,4-DNPH] (mM)	$\%\text{Area}_{\text{Ac-2,4-DNPH}}$	$\%\text{Area}_{\text{DHA-2,4-DNPH}}$	[Ac-2,4-DNPH] × $\%\text{Area}_{\text{DHA-2,4-DNPH}}$ / $\%\text{Area}_{\text{Ac-2,4-DNPH}}$ (mM)
1	1	1	49.09	50.91	1.04
2	1	2	38.01	61.99	1.63
3	1	3	27.04	72.96	2.70
4	1	4	20.96	79.04	3.77
5	1	5	17.73	82.27	4.64

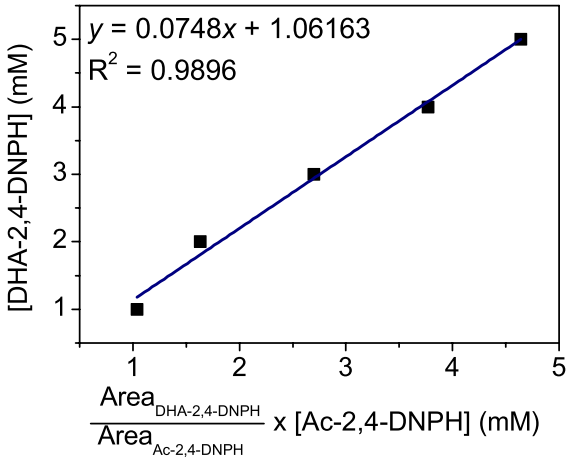


Figure S5. Determination of *m* and *b*.

DHA-2,4-DNPH concentration in the reaction samples [DHA-2,4-DNPH] were obtained from the above equation [Equation S1] using the concentration of the standard [Ac-2,4-DNPH] in the final solution. The pseudo–first–order rate constant k_{obs} for the isomerization were obtained from dividing the slope of a plot of [DHA-2,4-DNPH] *vs.* time by the initial concentration of GLA used [Equation S2]. A minimum of five data points was used to calculate rate constants. Each point was the average result of three parallel samples at the same interval. The calculation was processed with Origin program (OriginPro 8, OriginLab Corporation).

$$k_{obs} = \frac{d[\text{DHA-2,4-DNPH}]/dt}{[\text{GLA}]} \quad \text{(S2)}$$

A representative procedure for the hydrolysis and the data processing were as following (Table S2, Figure S6):

To a solution containing 30 μL of $Ca(OH)_2$ (20 mM) and 960 μL of water (pH ~10) was added 10 μL of D-GLA (1 mM). The tube was then put in a thermally equilibrated block heater at 80 °C. Three samples (5 μL each) were retrieved at fixed interval (1 min), mixed with 2,4-DNP (5 μL, 5 mM in 20 % $HClO_4/CH_3CN$) and CH_3CN (40 μL). The tube was capped, vigorously mixed, and submerged in a thermally equilibrated block heater at 37 °C. After an incubation of 1 hour, the derivation was quenched by adding HEPES buffer (15 μL, 1 M, pH ~10), and then mixed with Ac-2,4-DNPH (15 μL, 0.5 mM) and CH_3CN (80 μL). The precipitation was filtrated and the sample was then subjected to the HPLC analysis.

Table S2. A representative data processing.

No.	Time (min)	[DHA-2,4-DNPH] (mM)[a]
1	1	0.135 ± 0.002
2	2	0.143 ± 0.007
3	3	0.159 ± 0.025
4	4	0.169 ± 0.021
5	5	0.176 ± 0.003
6	6	0.191 ± 0.029
7	7	0.193 ± 0.008
8	8	0.223 ± 0.015
9	9	0.218 ± 0.036
10	10	0.246 ± 0.036

[a] Results ± SEM are the average of three parallel samples.

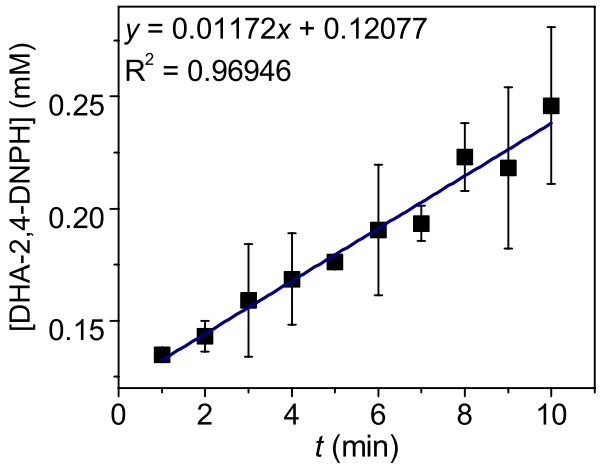


Figure S6. A representative data processing (Error bars represent SEM of three parallel samples)

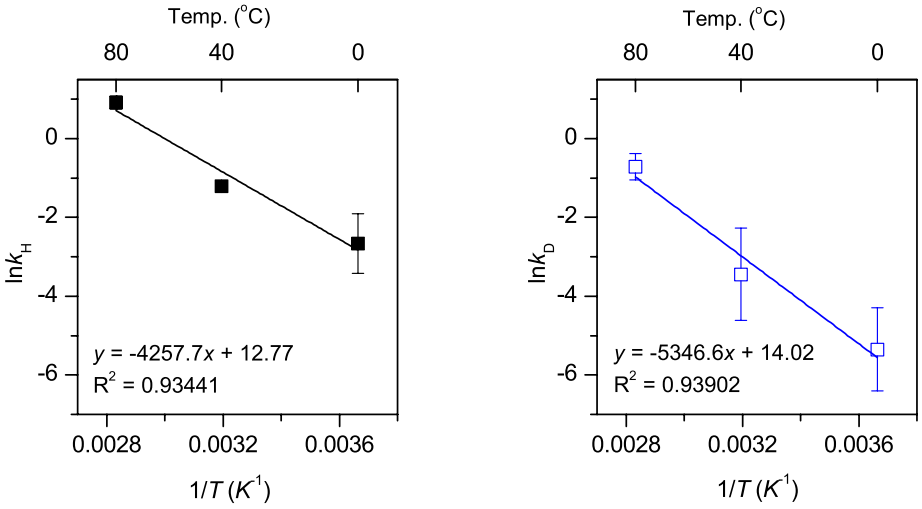
Arrhenius plots [Equation S3, Table S3, Figure S7] of D-GLA and D-[2-^{2}H]-GLA were as following:

$$\ln k = \ln A - \frac{E_a}{R} \times \frac{1}{T} \quad \text{(S3)}$$

Table S3. First-order rate constants for glyceraldehydes to dihydroxyacetone, and the KIE of the isomerization reaction[a]

Temp. (°C)	$T(K)$	$1/T(K^{-1})$	k_H $(h^{-1})^b$	$\ln k_H$	k_D $(h^{-1})^b$	$\ln k_D$	KIE $(k_H/k_D)^c$
0	273	0.0366	0.07 ± 0.02	-2.66 ± 0.76	0.005 ± 0.001	-5.35 ± 1.06	14.9 ± 4.0
40	313	0.0319	0.30 ± 0.02	-1.20 ± 0.08	0.03 ± 0.01	-3.44 ± 1.17	9.3 ± 2.6
80	353	0.0283	2.52 ± 0.44	0.92 ± 0.16	0.49 ± 0.23	-0.71 ± 0.33	5.1 ± 1.8

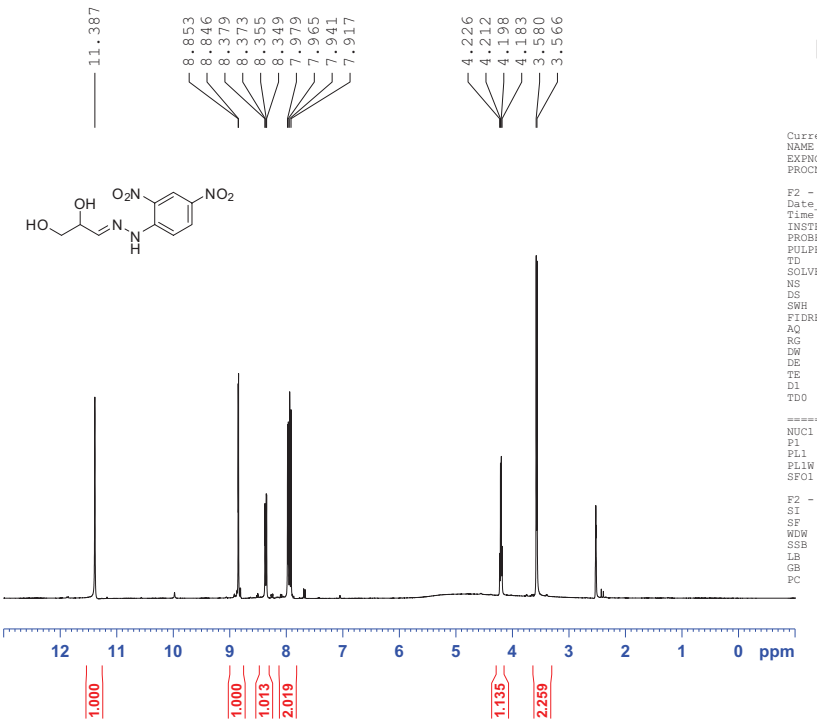
[a] Conditions: glyceraldehyde 1 mM, $Ca(OH)_2$ 0.6 mM, pH ~10.
[b] Results ± SEM are the average of at least three independent experiments.
[c] KIE values were calculated based on unrounded rate constants.



Slope $[-E_{a(H)}/R]$ (K)	−4258 ± 786	Slope $[-E_{a(D)}/R]$ (K)	−5347 ± 838
Intercept $[\ln A_H]$	12.8 ± 2.6	Intercept $[\ln A_D]$	14.0 ± 2.7
$E_{a(H)}$ $(kJ \cdot mol^{-1})$	35.4 ± 6.5	$E_{a(D)}$ $(kJ \cdot mol^{-1})$	44.5 ± 7.0
A_H	97.9 ± 19.6	A_D	344.2 ± 66.6

Figure S7. Arrhenius plots of D-GLA and D-[2-^{2}H]-GLA (Error bars represent SEM of at least three independent experiments. Results were presented as mean ± SEM)

4. NMR SPECTRA



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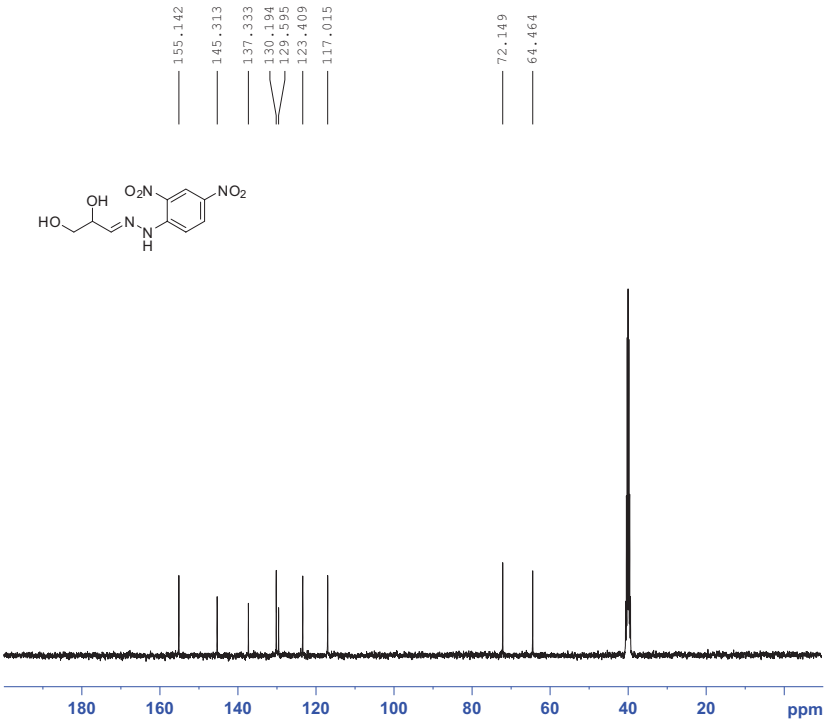

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D11                       0.03000000 sec
TD0                                  1

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NUC1                               13C
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PL1                           -4.60 dB
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PL12                          21.88 dB
PL13                         120.00 dB
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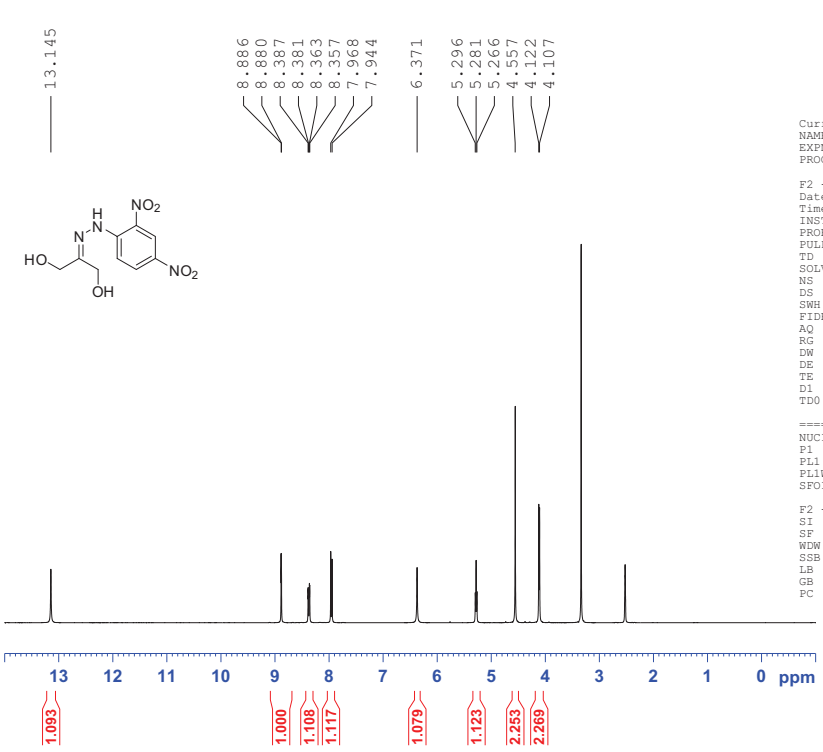

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PROCNO                               1

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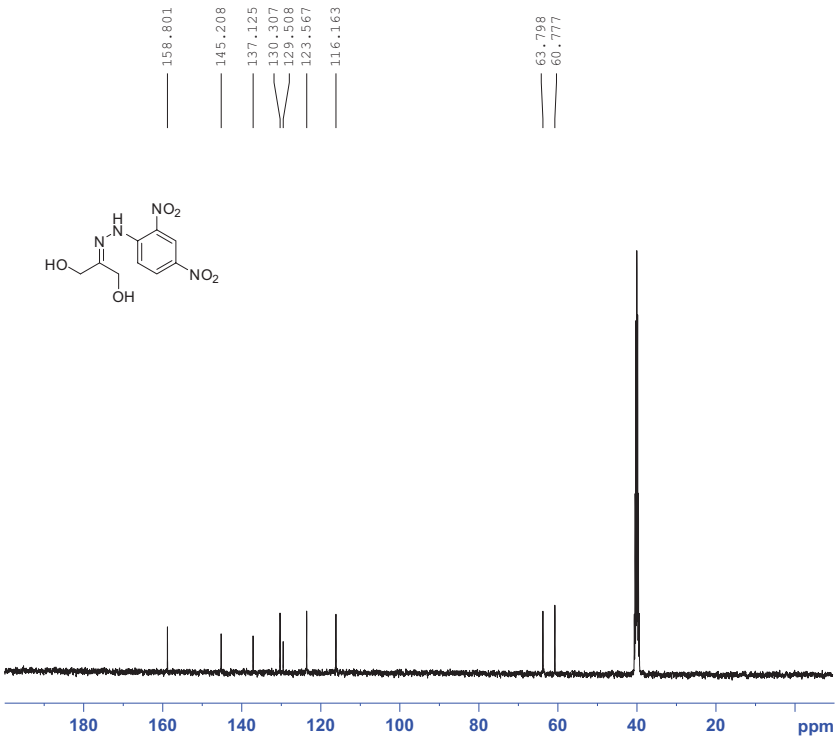

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PROCNO                               1

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D11                       0.03000000 sec
TD0                                 1

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PL1                           -4.60 dB
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SFO1                    100.5710256 MHz

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GB                                  0
PC                             1.40
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Full Polyrate reference 28

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Electronic structure calculations

Geometries (Å) and energies of reactant **1**, product **2**, and saddle point are listed below. Gaussian 09 route directives are listed (excluding those required by Gaussrate). Reaction energetics written by Polyrate are on the next page.

Reactant – Ca-coordinated glyceraldehyde alkoxide

b3lyp/6-31++g** int=ultra scrf(solvent=water) freq=noraman
E = -1020.5904786 au

	X	Y	Z
C	0.162332	1.621778	-0.126068
O	-1.026229	1.622753	0.194528
C	0.898914	0.365228	-0.508591
O	0.128970	-0.768715	-0.482068
C	2.140977	0.188147	0.419687
O	2.678417	-1.104256	0.158455
H	0.720026	2.575773	-0.150426
H	1.302950	0.591476	-1.524464
H	1.829352	0.261301	1.472983
H	2.916177	0.935340	0.224888
H	1.876624	-1.625017	-0.060424
Ca	-2.105386	-0.689403	0.117998

Product – Ca-coordinated dihydroxyacetone alkoxide

b3lyp/6-31++g** int=ultra scrf(solvent=water) freq=noraman
E = -1020.5878834 au

	X	Y	Z
C	0.084063	1.570883	0.039663
O	-1.271954	1.400906	-0.002513
C	0.873585	0.274085	-0.005873
O	0.302101	-0.823647	-0.001867
C	2.389015	0.291699	-0.069307
O	2.940993	-1.006178	0.062320
H	0.448275	2.079230	0.962215
H	0.491983	2.194321	-0.787083
H	2.781985	0.942065	0.721359
H	2.678433	0.739486	-1.031805
H	2.196561	-1.630043	-0.005918
Ca	-2.222317	-0.685685	-0.005459

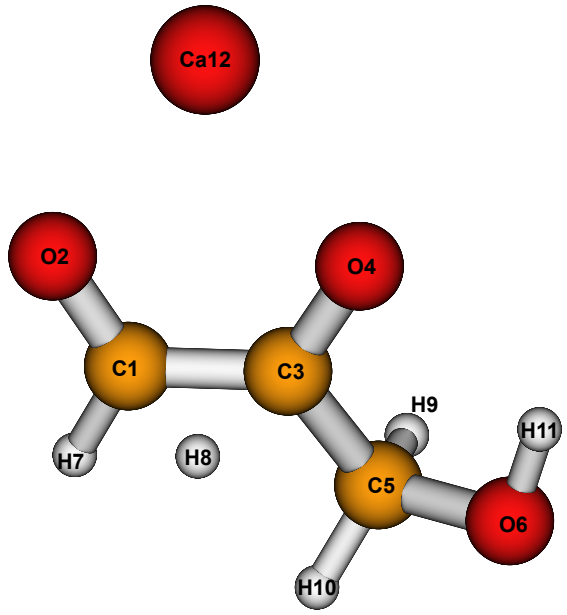
Saddle point

b3lyp/6-31++g** int=ultra scrf(solvent=water) freq=noraman
E = -1020.5611184 au

	X	Y	Z
C	0.102929	1.504782	-0.047077
O	-1.181071	1.538191	-0.092184
C	0.829410	0.256431	0.022242
O	0.205706	-0.875142	-0.001839
C	2.312828	0.226245	0.357520

O	2.938747	-0.889053	-0.268751
H	0.687340	2.430939	0.048141
H	0.766353	0.989363	-1.161103
H	2.396202	0.148167	1.452184
H	2.829904	1.133705	0.036811
H	2.272814	-1.598514	-0.256769
Ca	-2.206534	-0.661018	0.039341

Saddle point



Energetics of saddle point relative to reactant and product (**H isotopomer**)
(V = classical energy, ZPE = zero point energy)

	hartrees	eV	cm**-1	kcal
V w/re reactants V	0.02676	0.72832	5874.24	16.7953
V w/re product V	0.02936	0.79894	6443.82	18.4238
V+ZPE w/re reactant V	0.10588	2.88120	23238.29	66.4416
V+ZPE w/re product V	0.10848	2.95182	23807.87	68.0701
V+ZPE w/re reactant V+ZPE	0.02277	0.61955	4996.98	14.2871
V+ZPE w/re product V+ZPE	0.02644	0.71959	5803.80	16.5939
V+ZPE w/re saddle point V	0.07912	2.15289	17364.05	49.6463

Analogous data for **D isotopomer**

	hartrees	eV	cm**-1	kcal
V w/re reactants V	0.02676	0.72832	5874.24	16.7953
V w/re product V	0.02936	0.79894	6443.82	18.4238
V+ZPE w/re reactant V	0.10379	2.82419	22778.41	65.1268
V+ZPE w/re product V	0.10638	2.89481	23347.99	66.7553
V+ZPE w/re reactant V+ZPE	0.02394	0.65142	5254.00	15.0219
V+ZPE w/re product V+ZPE	0.02752	0.74892	6040.39	17.2703
V+ZPE w/re saddle point V	0.07702	2.09587	16904.18	48.3315

H: ZPE loss from reactant to TS = 14.2871 - 16.7953 = -2.5082 kcal/mol
D: ZPE loss from reactant to TS = 15.0219 - 16.7953 = -1.7734 kcal/mol
Difference in ZPE loss (H – D): 0.7348 kcal/mol

ISPE: MEP corrected by CCSD(T)/6-31++G**, frequencies not corrected.

Energetics of saddle point relative to reactant and product (**H isotopomer**)
(V = classical energy, ZPE = zero point energy)

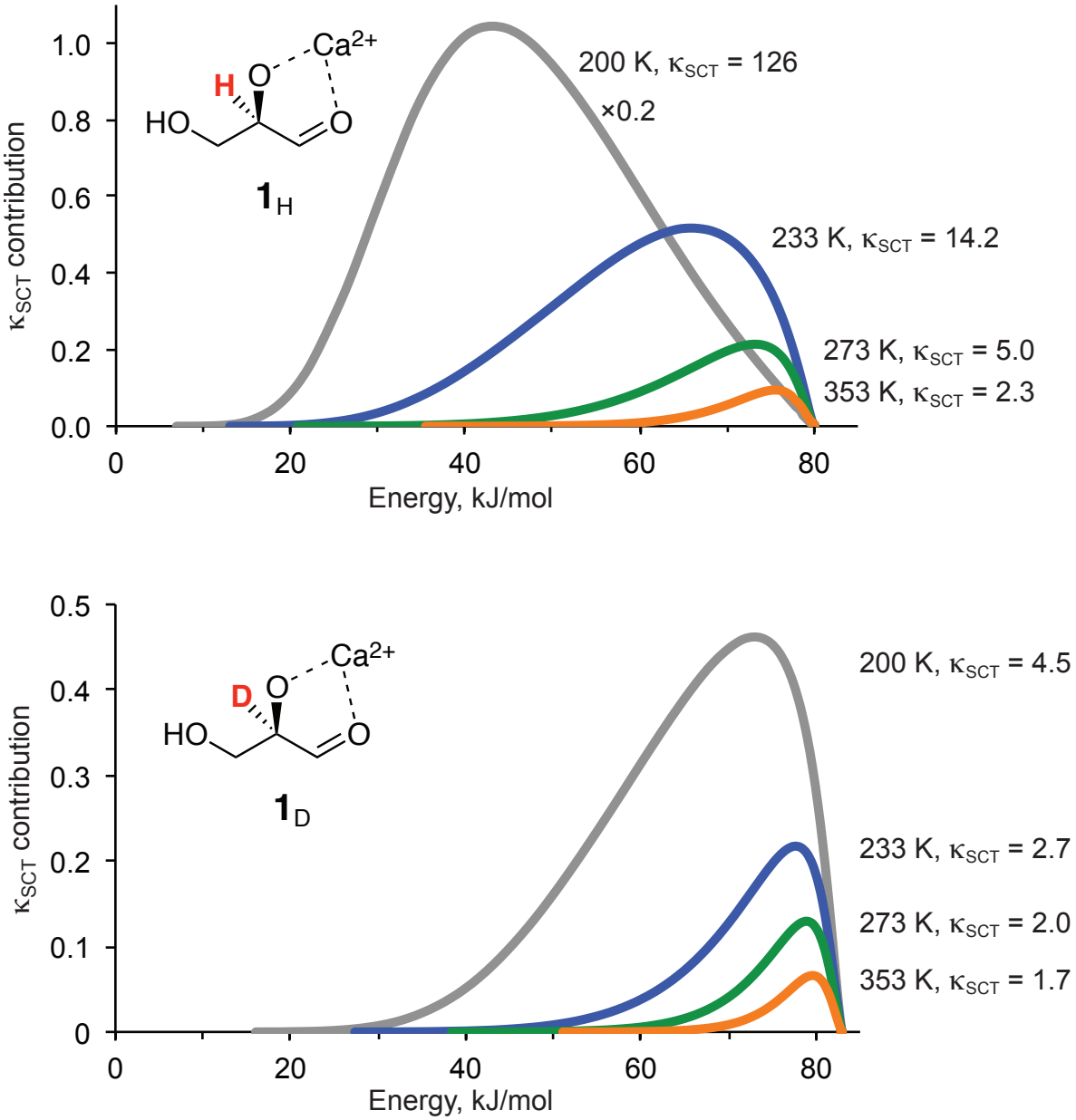
	hartrees	eV	cm**-1	kcal
V w/re reactants V	0.03449	0.93849	7569.40	21.6420
V w/re product V	0.03344	0.91009	7340.31	20.9870
V+ZPE w/re reactant V	0.11361	3.09138	24933.45	71.2883
V+ZPE w/re product V	0.11256	3.06298	24704.36	70.6333
V+ZPE w/re reactant V+ZPE	0.03049	0.82973	6692.14	19.1338
V+ZPE w/re product V+ZPE	0.03053	0.83074	6700.29	19.1571
V+ZPE w/re saddle point V	0.07912	2.15289	17364.05	49.6463

Data for **D isotopomer**

	hartrees	eV	cm**-1	kcal
V w/re reactants V	0.03449	0.93849	7569.40	21.6420
V w/re product V	0.03344	0.91009	7340.31	20.9870
V+ZPE w/re reactant V	0.11151	3.03436	24473.57	69.9735
V+ZPE w/re product V	0.11047	3.00596	24244.49	69.3185
V+ZPE w/re reactant V+ZPE	0.03166	0.86159	6949.16	19.8687
V+ZPE w/re product V+ZPE	0.03161	0.86007	6936.88	19.8335
V+ZPE w/re saddle point V	0.07702	2.09587	16904.18	48.3315

H/D ZPE loss is based on B3LYP frequencies, see above

Figure S8. Contribution to the transmission coefficient $\kappa_{SCT}(T)$ for the reaction **1** → **2** as a function of energy at 200-353 K, for $\mathbf{1}_H$ and $\mathbf{1}_D$, using the CCSD(T) corrected ISPE procedure. The integral of each curve gives $\kappa_{SCT}(T)$ - 1, for the $\kappa_{SCT}(T)$ values shown. Contributions go to zero at the top of the barrier, where transmission and reflection cancel.



Procedure for Polyrate calculations

Reaction paths were computed by the Page-McIver method with cubic first step. Second derivatives were computed at every step (*inh*=1). The initial step size (*fsize*) was 0.002 Å-amu$^{1/2}$ within ±0.4 Å-amu$^{1/2}$ of the saddle point in either direction. Thereafter, the step size (*sstep*) was 0.02 Å-amu$^{1/2}$. The length of the MEP (IRC) was determined by *percentdown*, which was set to include at least 90% of the barrier.

For the D isotopomer, the entire path was recomputed with atom 8 given a mass of 2.0141.

ISPE procedure (interpolated single-point energies along the MEP):
Single point energies of 12 MEP points were computed with CCSD(T)/6-31++G**. These were used in the VTST-ISPE interpolation procedure to correct the classical B3LYP potential energies along the MEP, retaining the frequencies previously calculated with B3LYP. A separate set of CCSD(T) single-point energies were computed for H and D isotopomeric MEPs.

Polyrate dat file for H isotopomer

```
*General

  TITLE
   Formose reaction:
   glyceraldehyde anion + Ca++  -> dihydroxyacetone + Ca++
    b3lyp/6-31++g** , Page-McIver LQA   Input in Å
  END

  ATOMS
    1    C
    2    O
    3    C
    4    O
    5    C
    6    O
    7    H
    8    H
    9    H
   10    H
   11    H
   12    Ca
  END

  NOSUPERMOL
  writefu31

*SECOND

  HESSCAL    HHOOK

*OPTIMIZATION

  OPTTS      OHOOK
  OPTMIN     OHOOK

*REACT1
```

```
   status  2

  GEOM
    1    0.162332     1.621778    -0.126068
    2   -1.026229     1.622753     0.194528
    3    0.898914     0.365228    -0.508591
    4    0.128970    -0.768715    -0.482068
    5    2.140977     0.188147     0.419687
    6    2.678417    -1.104256     0.158455
    7    0.720026     2.575773    -0.150426
    8    1.302950     0.591476    -1.524464
    9    1.829352     0.261301     1.472983
   10    2.916177     0.935340     0.224888
   11    1.876624    -1.625017    -0.060424
   12   -2.105386    -0.689403     0.117998
  END

  SPECIES   NONLINRP

*PROD1
   status  2

  GEOM
    1    0.084063     1.570883     0.039663
    2   -1.271954     1.400906    -0.002513
    3    0.873585     0.274085    -0.005873
    4    0.302101    -0.823647    -0.001867
    5    2.389015     0.291699    -0.069307
    6    2.940993    -1.006178     0.062320
    7    0.448275     2.079230     0.962215
    8    0.491983     2.194321    -0.787083
    9    2.781985     0.942065     0.721359
   10    2.678433     0.739486    -1.031805
   11    2.196561    -1.630043    -0.005918
   12   -2.222317    -0.685685    -0.005459
  END

  SPECIES   NONLINRP

*START
  status  2

  GEOM
    1    0.102929     1.504782    -0.047077
    2   -1.181071     1.538191    -0.092184
    3    0.829410     0.256431     0.022242
    4    0.205706    -0.875142    -0.001839
    5    2.312828     0.226245     0.357520
    6    2.938747    -0.889053    -0.268751
    7    0.687340     2.430939     0.048141
    8    0.766353     0.989363    -1.161103
    9    2.396202     0.148167     1.452184
   10    2.829904     1.133705     0.036811
   11    2.272814    -1.598514    -0.256769
   12   -2.206534    -0.661018     0.039341
  END

  SPECIES   NONLINTS
```

```
 *PATH

     dlx3         0.001
    SCALEMASS      1.0
    SSTEP          0.02
    INH            1
    NSTEPS         99999
    CURV           dhess
    RPM            pagem
    FIRSTSTEP      cubic
    SIGN           product

    SRANGE
       slp    5.
       slm   -5.
    END

   prsaverp

   specstop
    percentdown  92.
   end

   sfirst
    nfstep  200
    fsize  0.002
   end

 *TUNNEL

    ZCT
    SCT
   SCTOPT
     lagrange  4
   END

*RATE
  TST
  CVT

 Temp
  150.
  175.
  200.
  233.
  273.
  313.
  353.
 End
```

Results for B3LYP/6-31++G** without ISPE

Polyrate $\kappa_{SCT}(T)$ and forward rates (H isotopomer)

		Forward rates (s^{-1})	
Temp	κ_{SCT}	CVT	CVT+SCT
200.00	2.5869E+01	1.0068E-03	2.6046E-02
233.00	7.2555E+00	1.8788E-01	1.3632E+00
273.00	3.5653E+00	1.9867E+01	7.0830E+01
313.00	2.4627E+00	6.4684E+02	1.5930E+03
353.00	1.9712E+00	9.6550E+03	1.9032E+04

Polyrate $\kappa_{SCT}(T)$ and forward rates (D isotopomer)

		Forward rates (s^{-1})	
Temp	κ_{SCT}	CVT	CVT+SCT
200.00	6.3561E+00	1.5788E-04	1.0035E-03
233.00	3.3295E+00	3.8231E-02	1.2729E-01
273.00	2.2482E+00	5.0920E+00	1.1448E+01
313.00	1.8035E+00	1.9678E+02	3.5489E+02
353.00	1.5700E+00	3.3525E+03	5.2634E+03

Results for CCSD(T)/6-31++G**//B3LYP/6-31++G** ISPE

Polyrate $\kappa_{SCT}(T)$ and forward rates (H isotopomer)

		Forward rates (s^{-1})	
Temp	κ_{SCT}	CVT	CVT+SCT
200.00	1.2584E+02	5.0868E-09	6.4012E-07
233.00	1.4232E+01	5.3398E-06	7.5995E-05
273.00	4.9711E+00	2.6176E-03	1.3012E-02
313.00	3.0356E+00	2.6696E-01	8.1040E-01
353.00	2.2865E+00	9.6362E+00	2.2033E+01

Polyrate $\kappa_{SCT}(T)$ and forward rates (D isotopomer)

		Forward rates (s^{-1})	
Temp	κ_{SCT}	CVT	CVT+SCT
200.00	1.1887E+01	7.9612E-10	9.4634E-09
233.00	4.4973E+00	1.0850E-06	4.8796E-06
273.00	2.6735E+00	6.7018E-04	1.7917E-03
313.00	2.0275E+00	8.1139E-02	1.6451E-01
353.00	1.7106E+00	3.3433E+00	5.7191E+00