Physicochemical and Structural Investigation of L-Threonine/Glycerol-based Deep Eutectic Solvent Using Experimental and Molecular Modelling Approaches.

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List of Supporting Tables

Table S1. IR wavenumbers and intensities of L-threonine, Glycerol, and four cluster conformers (A, B, C, D) of 1:3 Thr: Gly DES clusters

	Gly	Intensity		Thr	Intensity		A	Intensity		B	Intensity		C	Intensity		D	Intensity
υ (OH-free)	3410	48.21	υ (OH-free)	3306	87.71	υ (OH-free)	3380	55.27	υ (OH-free)	3386	42.54	υ (OH-free)	3363	78.2	υ (OH-free)	3409	41.58
						υ (OH-bond.)	3200	355.86	υ (OH-bond.)	3277	244.8	υ (OH-bond.)	3193	396.88	υ (OH-bond.)	3257	323.26
															υ (OH-bond.)	2912	666.17
			υas (NH)	3156	9.44	υas (NH)	N/A		υas (NH)	3138	29.86	υas (NH)	N/A		υas (NH)	3137	30.94
			υs (NH)	3077	7.29	υs (NH)	3105	76.53	υs (NH)	3076	17.2	υs (NH)	3080	29.32	υs (NH)	3069	61.81
υas (CH)	2867	50.38	υas (CH)	3013	22.66	υas (CH)	2977	28.39	υas (CH)	2976	32.77	υas (CH)	2969	29.76	υas (CH)	2982	28.85
υs (CH)	2920	62.36	υs (CH)	2839	50.48	υs (CH)	2887	43.39	υs (CH)	2891	43.31	υs (CH)	2899	37.46	υs (CH)	2899	43.92
			υ (C=O)	1780	41.77	υ (C=O)	1754	525.27	υ (C=O)	1787	290.8	υ (C=O)	1749	320.12	υ (C=O)	1732	334.11
			S (NH2)	1602	33.71	S (NH2)	1611	45.46	S (NH2)	1624	32.98	S (NH2)	1618	31.85	S (NH2)	1626	20.46
υ(C-C) + υ(C-O) + S (C-C-C)	1059	66.71	υ (C-C)	1048	60.93	υ (C-C)	1440	27.21	υ (C-C)	1408	29.07	υ (C-C)	1390	56.65	υ (C-C)	1417	33.38
			δ (COOH)	1105 - 1384	49.61	δ (COOH)	1157 - 1418	28.07	δ (COOH)	1118 - 1336	29.37	δ (COOH)	1173 - 1397	24.42	δ (COOH)	1214 - 1446	28.76
β (OH)	1033 - 1397	37.51	β (OH)	1048 - 1287	54.78	β (OH)	1013 - 1474	33.15	β (OH)	1023 - 1441	35.79	β (OH)	1017 - 1463	33.69	β (OH)	1024 - 1446	35.07

			υ (C-O) + υ (C-N)	1107	21.08	υ (C-N)	1160	292.06	υ (C-N)	1118	202.94	υ (C-N)	1132	56.657	υ (C-N)	1176	26.65
						υ (C-O)	1052	130.71	υ (C-O)	1076	254.07	υ (C-O)	1060	57.82	υ (C-O)	1066	54.37
			W (NH2)	888	56.21	W (NH2)	847	30.95	W (NH2)	872	25.27	W (NH2)	848	57.1	W (NH2)	882	41.58
γ (OH)	544	55.04	γ (OH)	535-724	58.61	γ (OH)	529-874	74.86	γ (OH)	519-860	79.91	γ (OH)	523-862	77.2	γ (OH)	524-915	61.04

υas = asymmetric stretching; υs = symmetric stretching; β = In-plane bending; γ = out-plane bending; δ = deformation; W = wagging; S = scissoring

Table S2. Gibbs free energy change ΔG and electronic energy change ΔE (in kJ/mol) of L-Threonine, Glycerol and the DES's Conformers Calculated using the ωB97xD/6-311G(d,p) Level of Theory in the Gas Phase and with Standard Conditions

Parameter	Conformer A	Conformer B	Conformer C	Conformer D
ΔG (kJ/mol)	-53.9908	-43.6043	-50.2074	-48.0046
ΔE (kJ/mol)	-241.882	-231.711	-241.129	-224.869

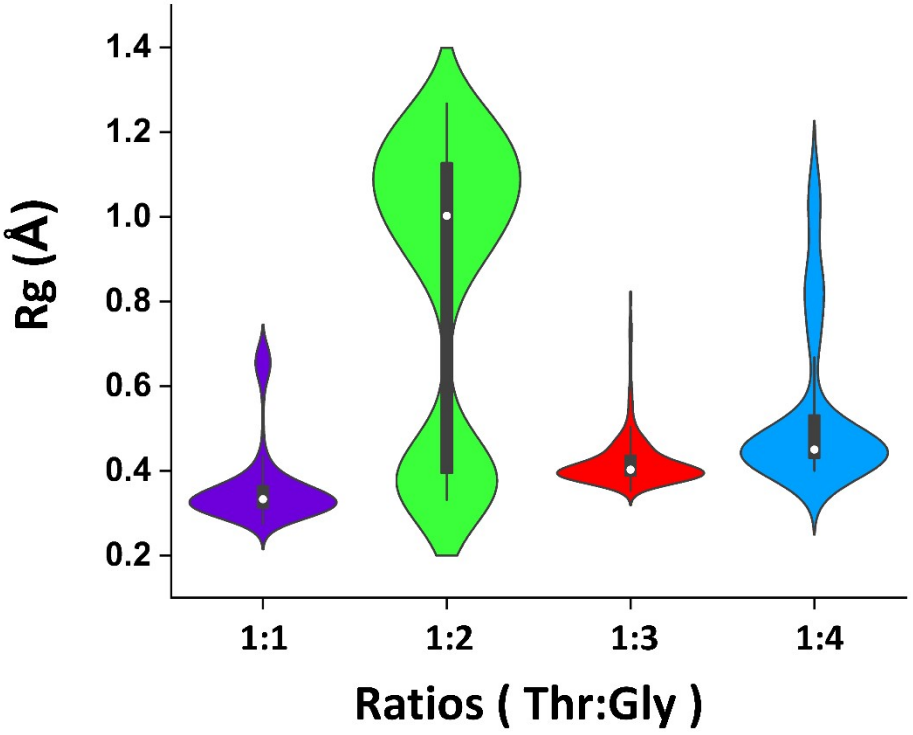
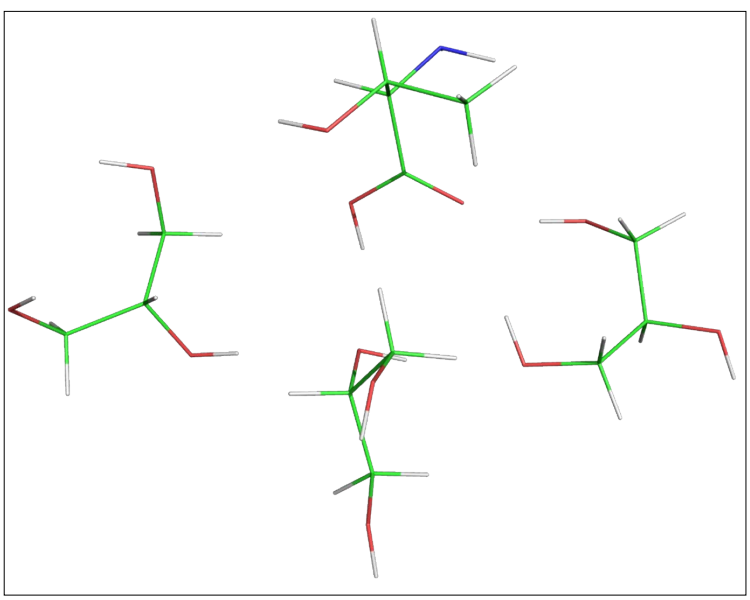
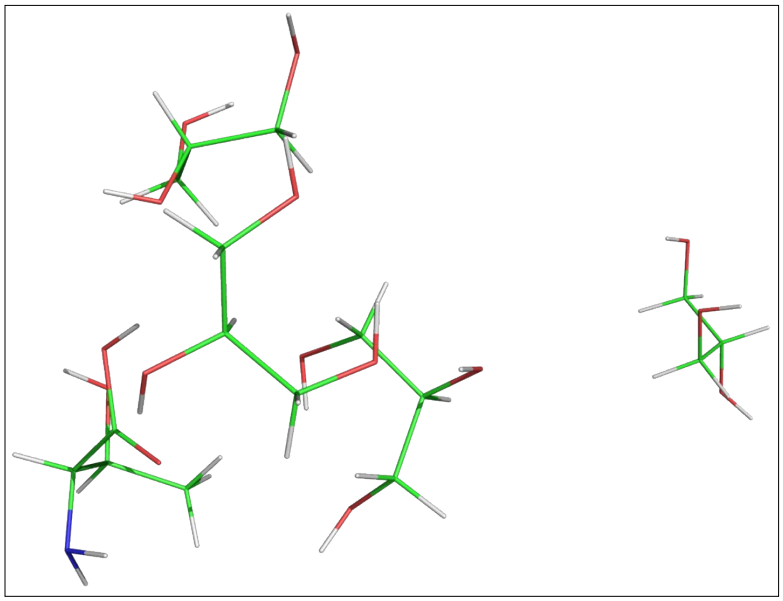


Figure S1. The radius of gyration of different pairs of threonine and glycerol





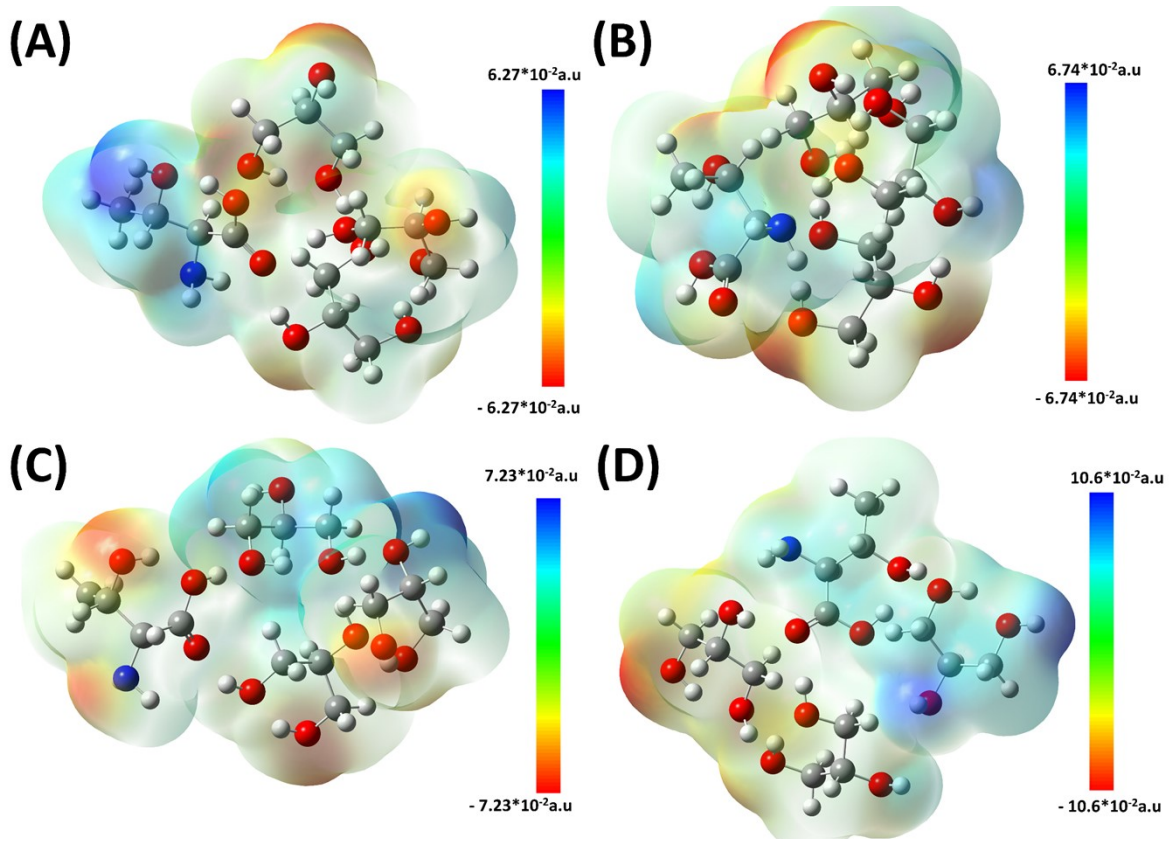


Figure S2. Representative conformers of 1:3 (left) and 1:4 pair (right)

Figure S3. The surface electrostatic potential is created by mapping the total density onto the electrostatic potential of conformers (A-D). The color code determines the electrostatic potential where: red= the most negative, blue = the most positive, and green = zero-potential region.

S1.1 The Surface Electrostatic Potential

The surface electrostatic potential gives information regarding the density of electrons in space around the molecule and eventually plays an important role in unrevealing the prominent non-covalent interaction sites[1,2]. The surfaces were generated and plotted in Gauss View 5. In clusters A and B, the doubly bonded oxygen atom of Threonine is shown in yellow, indicating a strong negative electrostatic potential, while in clusters C and D, it is light yellow, suggesting a moderate negative potential. Nearby hydrogen atoms of Glycerol are very light blue, representing a very low positive potential. This facilitates bonding between these atom pairs from both molecules, which can endure steric hindrance[3]. The hydrogen atoms attached to the oxygen of Threonine are light blue in clusters A, C, and D. In contrast, the oxygens of Glycerol are red in cluster A and very light yellow in the other two clusters, reflecting strong non-bonding interactions in the first type and moderate interactions in the second. In cluster B, the single-bonded oxygen on Threonine is red, and the corresponding hydrogen atoms (attached to carbon) are mostly very light blue, suggesting that the bonding here would be of moderate to low intensity. The nitrogen atom's surface in cluster B is light blue, with nearby hydrogens (attached to O or C) also very light blue, indicating that any potential non-bonding interactions between these atoms would be very weak. For Glycerol bonding interactions, atoms C-H, O-H, and O exhibit various patterns, allowing for a range of non-bonding interactions from low to strong.

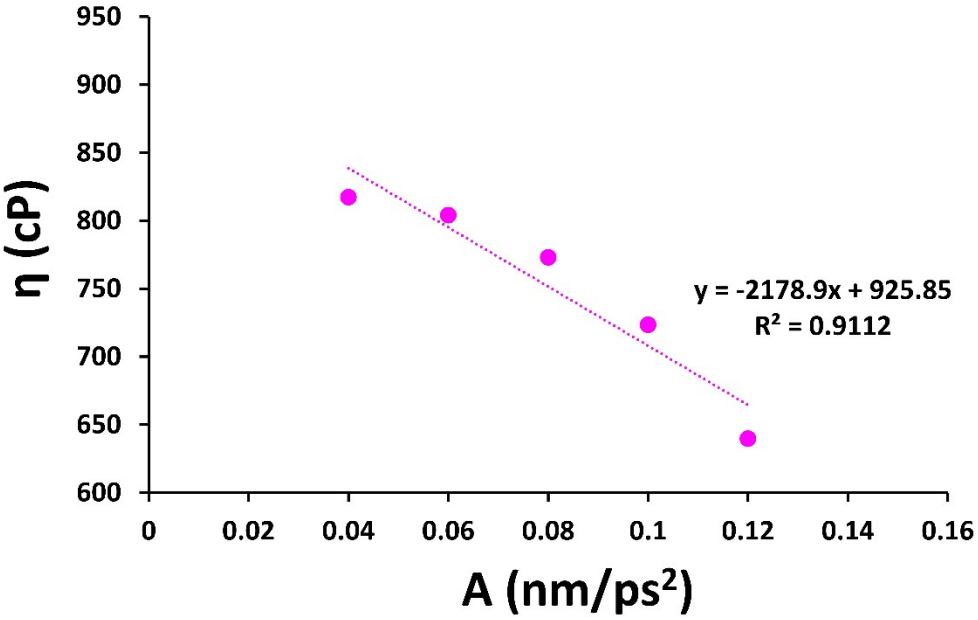


Figure S4. Extrapolated Viscosity of glycerol (NEMD)

Modified Force field Parameters of L-threonine.

;

; GENERATED BY LigParGen Server

; Jorgensen Lab @ Yale University

; Scaled by a factor of 0.8 by The Red-Green Research Center

; rgrcdhakalab@gmail.com

[moleculetype]

; Name nrexcl

THM 3

[atoms]

; nr type resnr residue atom cgnr charge mass

1 opls_800 1 THM C00 1 0.36448 12.0110

2 opls_801 1 THM C01 1 0.07144 12.0110

3 opls_802 1 THM C02 1 0.152 12.0110

4 opls_803 1 THM C03 1 -0.19712 12.0110

5 opls_804 1 THM O04 1 -0.3972 15.9990

6 opls_805 1 THM O05 1 -0.43264 15.9990

7 opls_806 1 THM O06 1 -0.54768 15.9990

8 opls_807 1 THM N07 1 -0.75336 14.0070

9 opls_808 1 THM H08 1 0.28984 1.0080

10 opls_809 1 THM H09 1 0.28984 1.0080

11 opls_810 1 THM H0A 1 0.35144 1.0080

12 opls_811 1 THM H0B 1 0.36208 1.0080

13 opls_812 1 THM H0C 1 0.0808 1.0080

```
   14   opls_813     1    THM   H0D     1     0.0808     1.0080
   15   opls_814     1    THM   H0E     1     0.1208     1.0080
   16   opls_815     1    THM   H0F     1     0.08208    1.0080
   17   opls_816     1    THM   H0G     1     0.0808     1.0080
[ bonds ]
   2    1    1     0.1522 265265.600
   3    2    1     0.1529 224262.400
   4    3    1     0.1529 224262.400
   5    1    1     0.1229 476976.000
   6    1    1     0.1364 376560.000
   7    3    1     0.1410 267776.000
   8    2    1     0.1448 319657.600
   9    8    1     0.1010 363171.200
  10    8    1     0.1010 363171.200
  11    7    1     0.0945 462750.400
  12    6    1     0.0945 462750.400
  13    4    1     0.1090 284512.000
  14    4    1     0.1090 284512.000
  15    2    1     0.1090 284512.000
  16    3    1     0.1090 284512.000
  17    4    1     0.1090 284512.000

[ angles ]
;  ai    aj    ak funct         c0         c1         c2         c3
   1    2    3    1   111.100   527.184
   2    3    4    1   112.700   488.273
   2    1    5    1   120.400   669.440
   2    1    6    1   108.000   585.760
   2    3    7    1   109.500   418.400
   1    2    8    1   111.200   669.440
```

```
   2    8    9    1    109.500    292.880
   2    8   10    1    109.500    292.880
   3    7   11    1    108.500    460.240
   1    6   12    1    113.000    292.880
   3    4   13    1    110.700    313.800
   3    4   14    1    110.700    313.800
   1    2   15    1    109.500    292.880
   2    3   16    1    110.700    313.800
   3    4   17    1    110.700    313.800
  14    4   17    1    107.800    276.144
  13    4   14    1    107.800    276.144
   9    8   10    1    106.400    364.845
   3    2   15    1    110.700    313.800
   7    3   16    1    109.500    292.880
   4    3    7    1    109.500    418.400
  13    4   17    1    107.800    276.144
   3    2    8    1    109.470    470.282
   5    1    6    1    121.000    669.440
   8    2   15    1    109.500    292.880
   4    3   16    1    110.700    313.800

[ dihedrals ]
; IMPROPER DIHEDRAL ANGLES
;  ai    aj    ak    al funct         c0         c1         c2         c3         c4         c5
    6     1     2     5    4       180.000     43.932     2

[ dihedrals ]
; PROPER DIHEDRAL ANGLES
;  ai    aj    ak    al funct         c0         c1         c2         c3         c4         c5
   4    3    2    1      3     -4.960   6.286   1.310  -2.636  -0.000   0.000
```

10	8	2	1	3	-1.268	3.021	1.745	-3.498	-0.000	0.000
9	8	2	1	3	-1.268	3.021	1.745	-3.498	-0.000	0.000
9	8	2	3	3	-1.268	3.021	1.745	-3.498	-0.000	0.000
10	8	2	3	3	-1.268	3.021	1.745	-3.498	-0.000	0.000
15	2	1	5	3	0.000	0.000	0.000	-0.000	-0.000	0.000
15	2	1	6	3	0.000	0.000	0.000	-0.000	-0.000	0.000
16	3	2	1	3	-0.209	-0.628	0.000	0.837	-0.000	0.000
17	4	3	2	3	0.628	1.883	0.000	-2.510	-0.000	0.000
13	4	3	2	3	0.628	1.883	0.000	-2.510	-0.000	0.000
14	4	3	2	3	0.628	1.883	0.000	-2.510	-0.000	0.000
15	2	3	4	3	0.628	1.883	0.000	-2.510	-0.000	0.000
16	3	4	14	3	0.628	1.883	0.000	-2.510	-0.000	0.000
16	3	2	15	3	0.628	1.883	0.000	-2.510	-0.000	0.000
16	3	4	13	3	0.628	1.883	0.000	-2.510	-0.000	0.000
17	4	3	16	3	0.628	1.883	0.000	-2.510	-0.000	0.000
16	3	2	8	3	-4.096	5.088	2.966	-3.958	-0.000	0.000
14	4	3	7	3	0.979	2.937	0.000	-3.916	-0.000	0.000
13	4	3	7	3	0.979	2.937	0.000	-3.916	-0.000	0.000
17	4	3	7	3	0.979	2.937	0.000	-3.916	-0.000	0.000
15	2	3	7	3	0.979	2.937	0.000	-3.916	-0.000	0.000
15	2	8	9	3	0.837	2.510	0.000	-3.347	-0.000	0.000
15	2	8	10	3	0.837	2.510	0.000	-3.347	-0.000	0.000
16	3	7	11	3	0.736	2.209	0.000	-2.946	-0.000	0.000
12	6	1	2	3	26.150	-3.138	-23.012	-0.000	-0.000	0.000
12	6	1	5	3	23.012	0.000	-23.012	-0.000	-0.000	0.000
11	7	3	4	3	-0.444	3.833	0.728	-4.117	-0.000	0.000
11	7	3	2	3	-0.444	3.833	0.728	-4.117	-0.000	0.000
8	2	1	5	3	0.000	0.000	0.000	-0.000	-0.000	0.000
8	2	1	6	3	14.435	-11.004	-3.431	-0.000	-0.000	0.000
8	2	3	4	3	3.335	-1.552	2.820	-4.602	-0.000	0.000

8	2	3	7	3	16.736	-16.736	0.000	-0.000	-0.000	0.000
5	1	2	3	3	0.000	0.000	0.000	-0.000	-0.000	0.000
6	1	2	3	3	5.318	0.732	-2.284	-3.766	-0.000	0.000
7	3	2	1	3	-12.929	12.929	0.000	-0.000	-0.000	0.000

[pairs]

1	4	1
3	5	1
1	7	1
3	6	1
1	9	1
1	10	1
4	8	1
3	9	1
5	8	1
3	10	1
2	11	1
6	8	1
2	12	1
7	8	1
4	11	1
2	13	1
2	14	1
5	12	1
1	16	1
4	15	1
2	17	1
7	13	1
5	15	1
7	14	1

6 15 1

7 15 1

9 15 1

8 16 1

7 17 1

10 15 1

11 16 1

13 16 1

14 16 1

15 16 1

16 17 1

DATA AND SOFTWARE AVAILABILITY

Server/Software	Function	Website
Gaussian09	Optimization, frequency and Gibbs free energy calculation	https://gaussian.com/glossary/g09/
Biovia Discovery Studio 2024	Visualization of compound models and interactions	https://www.3ds.com/products/biovia/discovery-studio/
Chimera X 1.3	Visualization of complexes	https://www.cgl.ucsf.edu/chimerax/download.html
Origin Pro (2021)	Plotting data, PCA	https://www.originlab.com/2021
Microsoft Excel	Plotting data	https://www.microsoft.com/en-us/microsoft-365/microsoft-office
GROMACS 2022.4	Molecular Dynamics Simulation And data analysis	https://manual.gromacs.org/documentation/2022.4/index.html
LigParGen server	Forcefield parameters of L-threonine	https://zarbi.chem.yale.edu/ligpargen
Peer Reviewed Article	Forcefield parameters of Glycerol	https://pubs.acs.org/doi/10.1021/acs.jpcb.8b06647

Travis code	Analyzing RDF, CDF, SDF and MSD	http://www.travis-analyzer.de
VMD 1.9.3	Analyzing interactions and SDF	https://www.ks.uiuc.edu/Research/vmd

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3 M. K. Chaudhary, A. Srivastava, K. K. Singh, P. Tandon and B. D. Joshi, *Comput. Theor. Chem.*, 2020, **1191**, 113031.