An Insight into The Role of Riboflavin Ligand on the Self-assembly of Poly (lactic-co-glycolic acid)-based Nanoparticles, A Molecular Simulation and Experimental Approach

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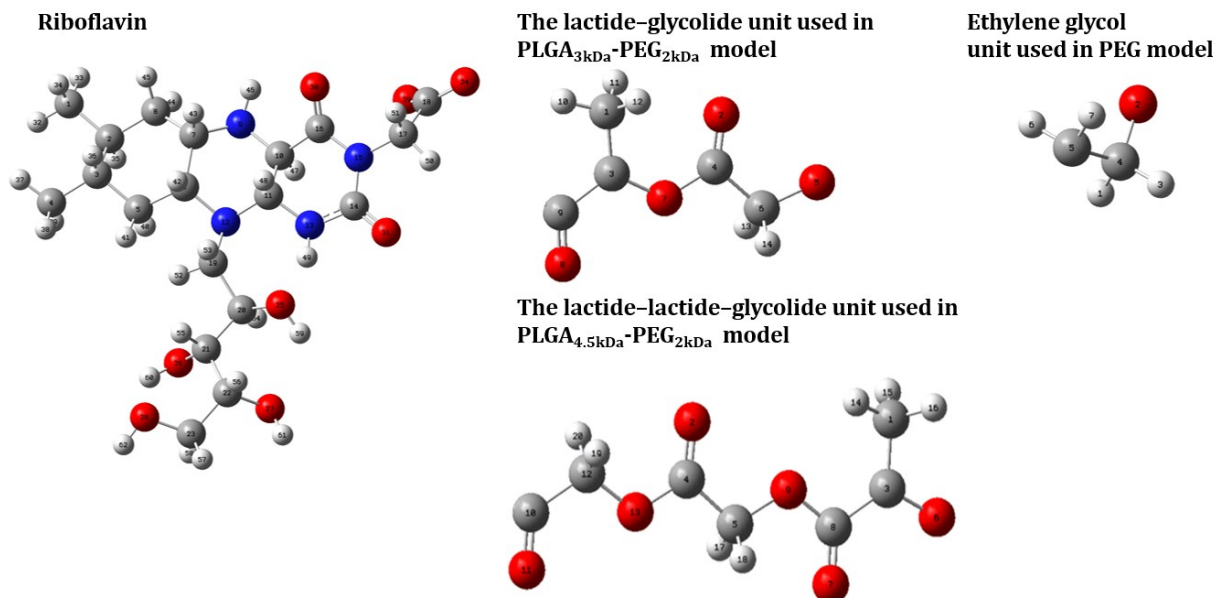Corresponding Authors: moraveji@aut.ac.ir

Riboflavin

The lactide–glycolide unit used in PLGA$_{3kDa}$-PEG$_{2kDa}$ model

Ethylene glycol unit used in PEG model

The lactide–lactide–glycolide unit used in PLGA$_{4.5kDa}$-PEG$_{2kDa}$ model

Figure S1. Atom types of molecules and polymers used in simulations. To mimic the real conditions, the monomer of two PLGA-PEG polymers has a different number of lactide-glycolide units. Details on the type and charge of atoms according to the numbers are provided in Table S1.

Table S1 provides further detail about the molecule structures used in the simulations.

Table S1. *Specification of the OPLS-AA atom types and partial charges used in the simulations.*

Riboflavin			The monomer used in PLGA$_{3kDa}$-PEG$_{2kDa}$			Ethylene glycol		
NO.	Atom	Charge	NO.	Atom	Charge	NO.	atom	charge
1	C00	-0.0041	1	H00	0.3444	1	H00	0.12
2	C01	0.1634	2	O01	-0.5099	2	C01	0.0822
3	C02	-0.3509	3	C02	-0.0017	3	C02	0.0081
4	O03	-0.4288	4	C03	0.3645	4	H03	0.12
5	N04	-0.571	5	H04	0.0785	5	O04	-0.5065
6	C05	0.488	6	H05	0.0785	6	H05	0.0881
7	C06	0.5732	7	O06	-0.2167	7	H06	0.0881
8	N07	-0.652	8	O07	-0.4362			
9	O08	-0.3554	9	C08	-0.4679			
10	C09	0.5054	10	C09	-0.0784			

11	C0A	-0.0361	11	C0A	0.2589
12	O0B	-0.3565	12	H0B	0.0314
13	N0C	-0.1318	13	H0C	0.0314
14	C0D	-0.0472	14	H0D	0.0314
15	C0E	0.1598	15	O0E	-0.545
16	N0F	-0.5311	16	H0F	0.037
17	C0G	-0.0347	The monomer used in PLGA$_{4.5kDa}$-PEG$_{2kDa}$		
18	C0H	-0.1253	1	H00	0.3198
19	C0I	-0.01	2	O01	-0.4672
20	C0J	-0.2623	3	C02	-0.4246
21	C0K	-0.17	4	C03	-0.0879
22	C0M	-0.1595	5	C04	0.3866
23	C0N	-0.0298	6	H05	0.0234
24	C0O	0.1619	7	H06	0.0234
25	C0P	-0.2278	8	H07	0.0234
26	C0Q	-0.191	9	O08	-0.3152
27	C0R	-0.0805	10	O09	-0.5549
28	O0S	-0.5784	11	C0A	0.0221
29	O0T	-0.3829	12	C0B	0.3713
30	O0U	-0.4283	13	H0C	0.1115
31	O0V	-0.4579	14	H0D	0.1115
32	H0W	-0.0227	15	O0E	-0.3035
33	H0X	-0.0227	16	O0F	-0.3926
34	H0Y	0.1532	17	C0G	-0.0544
35	H0Z	0.1532	18	C0H	0.2193
36	H10	0.1523	19	H0I	0.1092
37	H11	0.1667	20	H0J	0.1092
38	H12	0.0865	21	O0K	-0.3189
39	H13	0.0865	22	H0M	0.0887
40	H14	0.0865			
41	H15	0.0799			

42	H16	0.0799
43	H17	0.0799
44	H18	0.1448
45	H19	0.1448
46	H1A	0.1194
47	H1B	0.0556
48	H1C	0.0556
49	H1D	0.3246
50	H1E	0.3779
51	H1F	0.0325
52	H1G	0.4177
53	H1H	0.3927
54	H1I	0.4068

Prior to use polymer chains in the self-assembly process, we calculated the Rg and RMSD fluctuations for individual polymers in an aqueous environment to ensure the reliability of the results. In this regard, Fig.S2 demonstrates the RMSD and Rg for both polymer chains.

Fig. S2 shows the Rg fluctuation diagram for $PLGA_{3kDa}$-PEG_{2kDa} which starts at 0.5 nm and after 40 ns ends at the same Rg value. Results have good agreement with J. Andrews and E. Blaisten-Barojas[1] results. Moreover, the RMSD diagram has good agreement with Ansari et. al[2] report (Figure 3) on the subject as both diagrams have the same initial and final states. In the case of $PLGA_{4.5kDa}$-PEG_{2kDa}, our Rg and RMSD results have good agreements with Rg diagram (Figure 8) and RMSD (Figure 3) respectively, reported by Ansari et. al. These agreements with previous reports declare the reliability of our simulation.
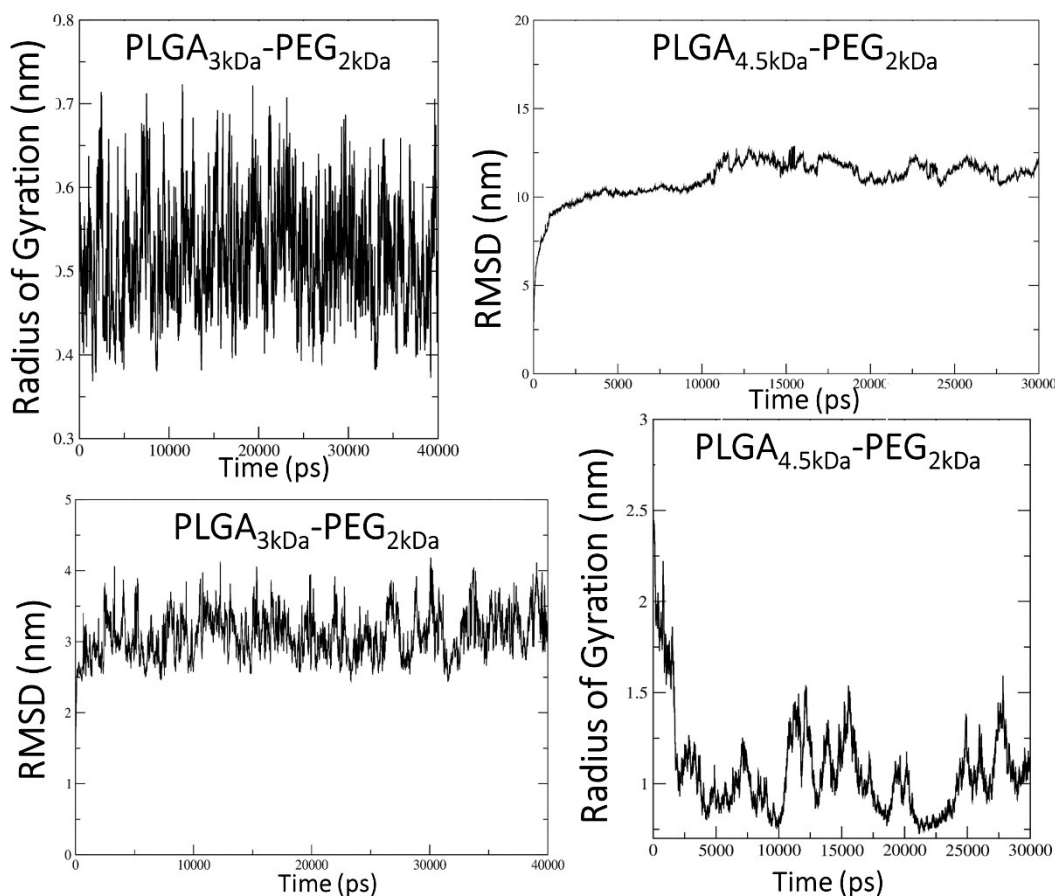

Figure S2. Conformational changes and Gyration radii of individual polymers ($PLGA_{3kDa}$-PEG_{2kDa} and $PLGA_{4.5kDa}$-PEG_{2kDa}) in an aqueous environment.

Table S2. The interaction energy *(kJ/mol.atom*)* of NPs based on both polymers with varying RF-conjugated polymers

PLGA$_{3kDa}$-PEG$_{2kDa}$			
PP:PPR ratio	Electrostatic	vdW	Total
10:0	-1.214861±0.042783	-3.648339±0.161886	-4.8632± 0.061795
9:1	-1.1432584±0.038435	-3.735799±0.131786	-4.8790574±0.053896
8:2	-1.1373482±0.047426	-3.7467901±0.121818	**-4.8841382**±0.060764
7:3	-1.0375081±0.041151	-3.8434537±0.106962	-4.8809617±0.053572
6:4	-0.9402648±0.051421	-3.8641452±0.100563	-4.80441±0.060309
5:5	-0.8474739±0.047234	-3.8246445±0.088762	-4.6721185±0.054706
PLGA$_{4.5kDa}$-PEG$_{2kDa}$			
PP:PPR ratio	Electrostatic	vdW	Total
10:0	-0.9793351±-0.0277	-5.3851223±0.082699	-6.3644574± 0.032
9:1	-0.9511264±-0.03531	-5.4134757±0.09924	**-6.3646021**±0.041439
8:2	-0.8910546±-0.03865	-5.3750609±0.107317	-6.2661154±0.045865
7:3	-0.858731±-0.03371	-5.4013087±0.090762	-6.2600397±0.039297
6:4	-0.8472552±-0.03723	-5.5146676±0.102983	-6.3619227±0.044231
5:5	-0.8212951±-0.03936	-5.5019027±0.10605	-6.3231978±0.047483

* kJ/(mol.atom) is the unit of normalized energy as energy divided by the bonding number.
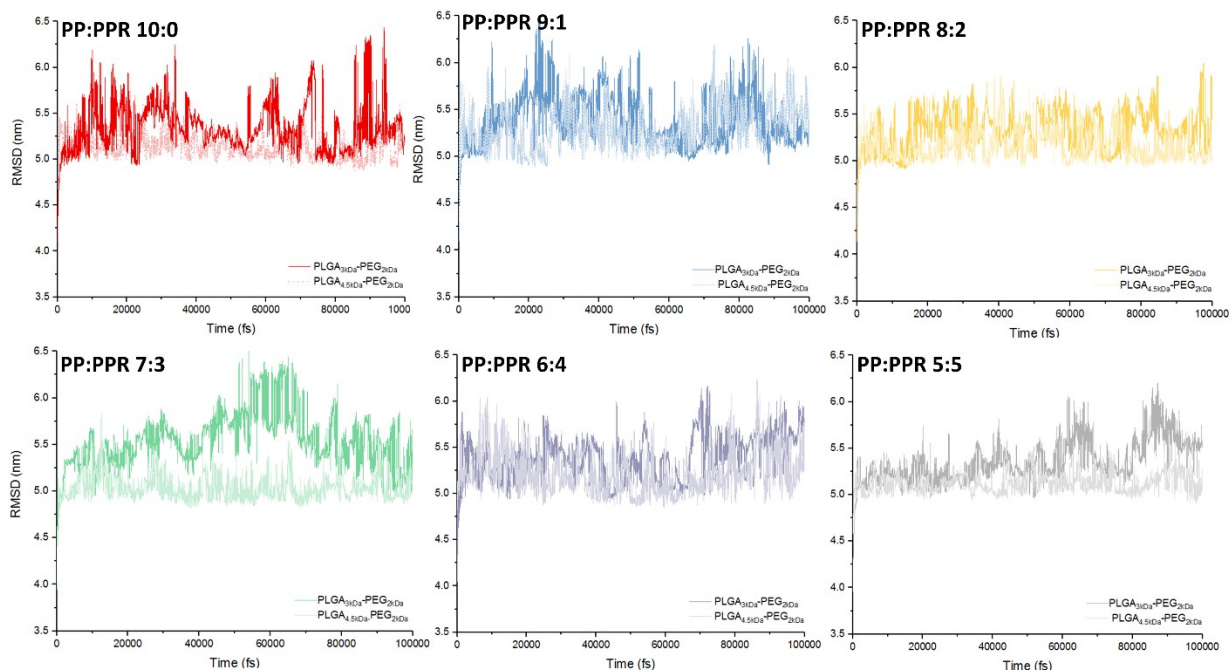
Figure S3. Conformational changes in the PLGA-PEG-RF structure. Solid lines in all diagrams represent RMSD results in the varying PP: PPR ratios for $PLGA_{3kDa}$-PEG_{2kDa} and dots light-colored lines are related to $PLGA_{4.5kDa}$-PEG_{2kDa}. In agreement with previous results, most stable particles are achieved at PP: PPR ratio 8:2 and 9:1 for $PLGA_{3kDa}$-PEG_{2kDa} and $PLGA_{4.5kDa}$-PEG_{2kDa}, respectively.
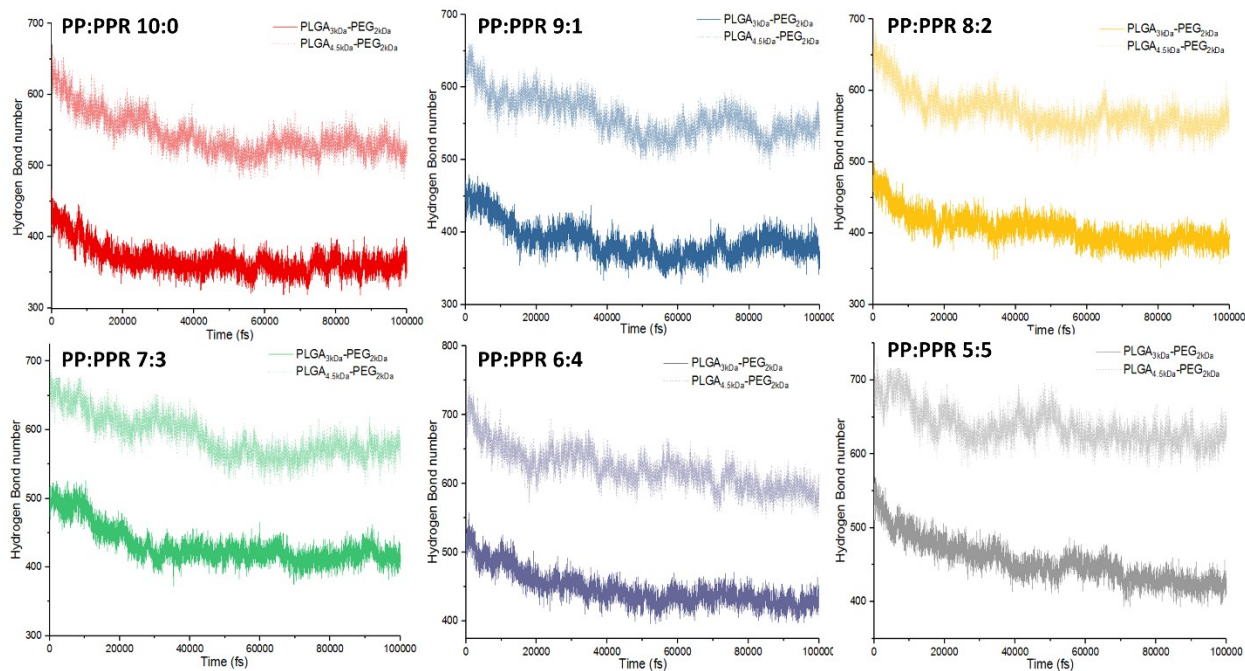

Figure S4. Number of H-bonds formed during 100 ns precipitation process between the NP and water molecules

in the various PP: PPR ratios. Solid color lines represent the H-bond formation for $PLGA_{3kDa}$-PEG_{2kDa} based NPs while the light-colored dot-lines depicts the same parameter for $PLGA_{4.5kDa}$-PEG_{2kDa} based NPs. In all cases, due to the higher number of monomers in the $PLGA_{4.5kDa}$-PEG_{2kDa} polymer chains H-bond counts are about 160-180 more than smaller polymer.
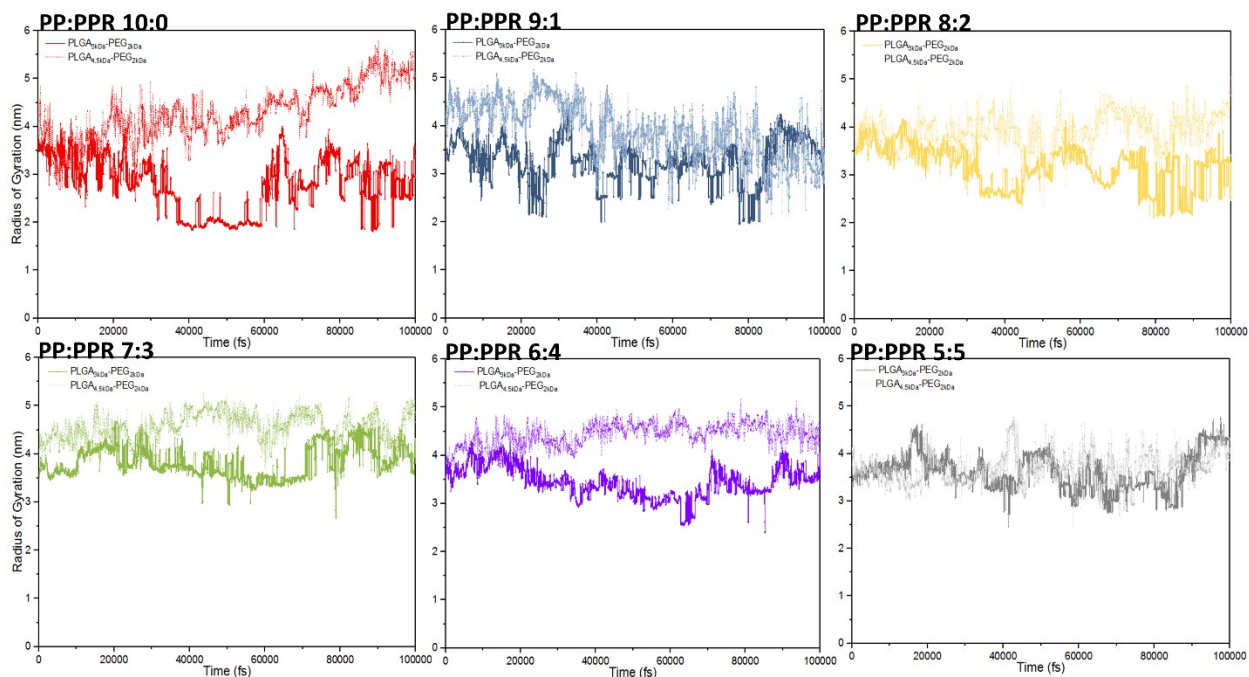

Figure S5. Variation in the radius of gyration in 100 ns. Rg final value and the variation in its values during the simulation are determinant in the stability of particle. The smallest the final Rg and the highest the variation in the Rg the stablest the nanoparticle. Although the $PLGA_{4.5kDa}$-PEG_{2kDa} has the biggest variation in Rg since its values are growing it shows that the particle is unstable.
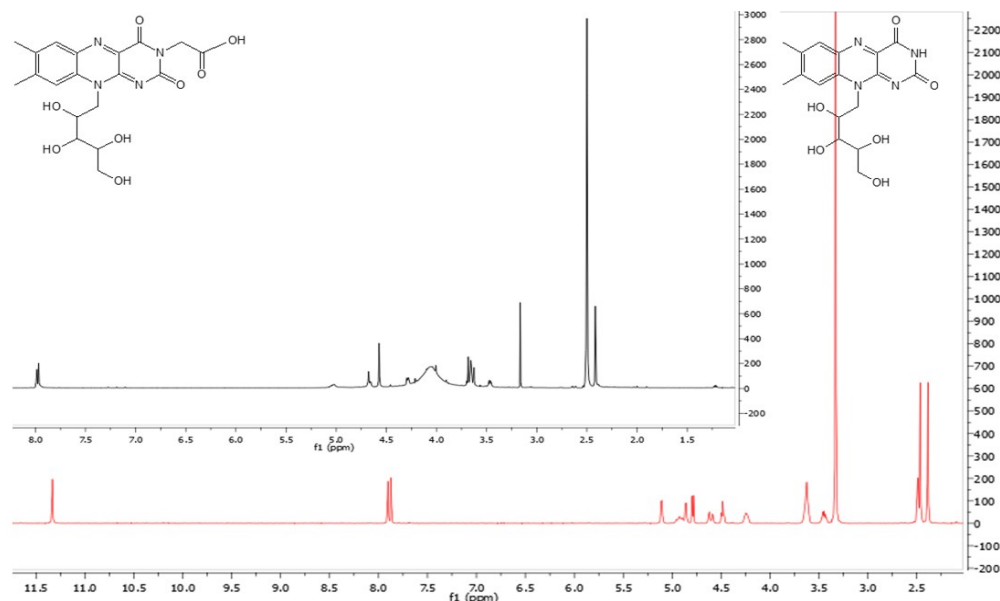
Figure S6. [1] H NMR results in dimethyl sulfoxide-d6 solvent for the initial and final materials in the ligand preparation process.

Details on the NMR spectrums and also for the middle stage are as following:

Protection of hydroxyl groups on the ribitol chain. ^{1}H NMR (300 MHz; CD3OD) δ: 2.04 (s, 3H, CH3), 2.17 (s, 3H, CH3), 2.20 (s, 3H, CH3), 2.21 (s, 3H, CH3), 2.49 (s, 3H, CH3), 2.62 (s, 3H, CH3), 4.35 (ddd, J=18.5, 12.3, 4.6 Hz, 3H, CH2, CH), 4.59 (s, 1H, CH), 5.44 (td, J=3.2, 6.1, 6.1, 1H, CH), 5.44 (td, J=3.2, 6.1, 6.1, 1H, CH), 5.55 (m, 1H, CH), 5.69 (dd, J=12.5, 4.6 Hz, 1H, CH), 7,84 (s 1H, CH), 7.95 (s 1H, CH).

Derivatization of ethoxycarbonylmethyl group on protected riboflavin. ^{1}H NMR (600 MHz; CDCl3) δ: 1.28 (t, J=7.1, 3H, CH3), 1.77 (s, 3H, CH3), 2.08 (s, 3H, CH3), 2.21 (s, 3H, CH3), 2.30 (s, 3H, CH3), 2.45 (s, 3H, CH3), 2.57 (s, 3H, CH3), 4.31 (m, 5H, CH2), 4.82 (dd, J=2.1 Hz, 2H, CH2), 4.42 (dd, J=5.0, 2.6 Hz, 2H, CH2), 5.64 (s, 1H, CH), 7.57 (s 1H, CH), 8.05 (s 1H, CH).

Deprotection of hydroxyl groups. ^{1}H NMR (300 MHz; CD3OD) δ: 2.49 (s, 3H, CH3), δ 2.60 (s, 3H, CH3), 3.67 (m, 1H, CH), 3.83 (m, 3H, CH, CH2), 4.45 (d, J=9.0 Hz, 1H, CH), 4.76 (d, J=7.0 Hz, 2H, CH2), 5.04 (dt, J=22.7, 11.7 Hz, 2H, CH2), 8.05 (s 1H, CH), 8.18 (s 1H, CH).

References

1.   J. Andrews and E. Blaisten-Barojas, *The Journal of Physical Chemistry B*, 2019, **123**, 10233-10244.
2.   M. Ansari, S. Moradi and M. Shahlaei, *Journal of Molecular Liquids*, 2018, **269**, 110-118.