Self-association as solubility limiting factor of riboflavin in aqueous medium

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9 Supplementary Material

9.1 Calibration curves for riboflavin, lumichrome and riboflavin 5'-monophosphate sodium salt

Table A1: Calibration curve for riboflavin in water at the UV-Vis spectrophotometer at 449 nm

Slope (AU·kg·mmol^{-1})	Intercept (AU)	Correlation coefficient R^2
12.353	0.0028	0.9999
12.394	0.0057	0.9999
12.133	0.0025	0.9998
averaged	**averaged**	
12.3 ± 0.1	0.004 ± 0.001	

Table A2: Calibration curve for lumichrome in water at the UV-Vis spectrophotometer for at 386 nm

Slope (AU·kg·mmol^{-1})	Intercept (AU)	Correlation coefficient R^2
12.5	0.019	0.9784
11.6	0.068	0.9937
11.0	0.081	0.9957
averaged	**averaged**	-
11.7 ± 0.6	0.05 ± 0.03	-

Table A3: Calibration curve for RF-PO_4 in water at the spectrophotometer for at 445 nm

Slope (AU·kg·mmol^{-1})	Intercept (AU)	Correlation coefficient R^2
11.02	0.003	0.9998
11.01	0.005	0.9999
11.21	0.002	0.9998
averaged	**averaged**	-
11.08 ± 0.003	0.09 ± 0.001	-

9.2 Riboflavin crystal obtained in presence of 3,4-dimethoxycinnamic acid

(The CheckCif report of this riboflavin crystal contains a Level B alert, because the ribityl chain is rather flexible in the solvent prior to crystallization, which apparently leads to a slightly distinct position of the hydroxy groups on each riboflavin molecule in the final crystal. However, Single Crystal X-Ray Diffraction Analysis enables only the determination of the average position of the entire ribityl chain and needle shaped crystals come along with higher uncertainties.

Still, in this case, a Level B alert is not critical. Of major importance for the message of our article is not the exact position of the hydroxy groups on the ribityl chain, but the global direction of the ribityl chain in relation to the directly attached riboflavin molecules, i.e. the syn-orientation of the ribityl chains of directly neighbored riboflavin molecules and π-stacking. As we still cannot just ignore a Level B alert, we present another crystal structure of riboflavin in section 9.3.)

$C_{17}H_{20}N_4O_6$, M_r = 376.37, orthorhombic, $P2_12_12_1$ (No. 19), a = 5.31333(5) Å, b = 15.11045(13) Å, c = 20.03449(17) Å, $\alpha = \beta = \gamma = 90^\circ$, V = 1608.51(2) Å^3, Z = 4, Z' = 1, μ(Cu K_α) = 1.009, 16921 reflections measured, 3236 unique (Rint = 0.0260) which were used in all calculations. The final wR2 was 0.0812 (all data) and R1 was 0.0292 (I≥2 σ(I)).

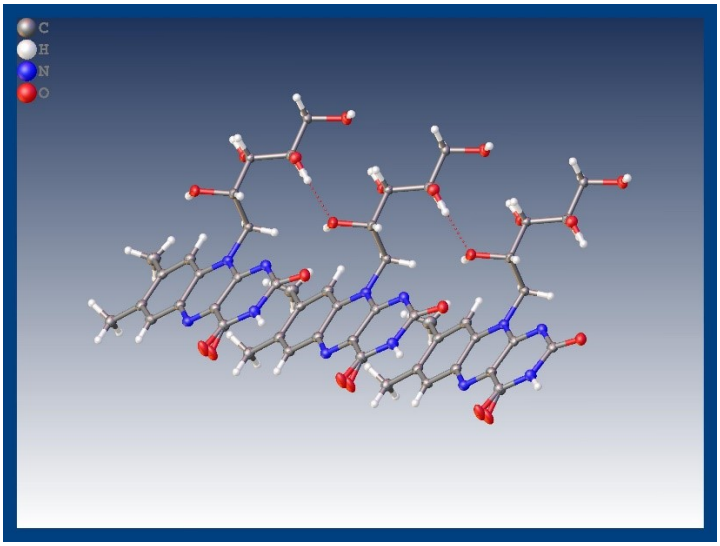

Figure A1: Crystal structure of pure RF needles obtained via the liquid diffusion method from a water/acetonitrile (50/50 (w/w)) solution in presence of 3,4-dimethoxycinnamic acid

Table A4: Compound characterization of the riboflavin crystal obtained from a water/acetonitrile (50/50 (w/w)) solution saturated with 3,4-dimethoxycinnamic acid and riboflavin.

Parameter	Obtained value	Parameter	Obtained value
Formula	C17H20N4O6	**Z**	4
$\rho_{calc.}$ (g·cm^{-3})	1.554	**Z'**	1
μ·mm^{-1}	1.009	**λ (Å)**	1.54184
Formula Weight	376.37	**Radiation type**	Cu K_α
Color	Clear yellow	**θ_{min} (°)**	3.664
Shape	Prism-shaped	**θ_{max} (°)**	75.054
Size (mm^3)	0.28×0.04×0.03	**Measured Refl's.**	16921
T (K)	123.01(10)	**Indep't Refl's**	3236
Crystal System	orthorhombic	**Refl's I≥2 σ(I)**	3105
Flack Parameter	0.2(2)	**R_{int}**	0.0260
Hooft Parameter	0.06(5)	**Parameters**	328
Space Group	P212121	**Restraints**	6
a (Å)	5.31333(5)	**Largest Peak**	0.424
b (Å)	15.11045(13)	**Deepest Hole**	-0.335
c (Å)	20.03449(17)	**GooF**	1.038
α (°)	90	**wR_2 (all data)**	0.0812
β (°)	90	**wR_2**	0.0801
γ (°)	90	**R_1(all data)**	0.0309
V (Å^3)	1608.51(2)	**R_1**	0.0292

Table A5: Fractional Atomic Coordinates (×10^4) and Equivalent Isotropic Displacement Parameters (Å^2×10^3) for the riboflavin crystal obtained from a water/acetonitrile (50/50 (w/w)) solution saturated with 3,4-dimethoxycinnamic acid and riboflavin. U_{eq} is defined as 1/3 of the trace of the orthogonalized U_{ij}.

Atom	x	y	z	U_{eq}
O4	6079(3)	5757.2(9)	6020.6(7)	18.4(3)
O6	12784(3)	6098.0(10)	4901.2(7)	20.2(3)
O2	13984(3)	8843.7(10)	7656.8(7)	22.6(3)
O5	10762(3)	7489.1(9)	5706.8(7)	19.7(3)
O3	4133(3)	7269.8(11)	6725.0(7)	23.4(3)
O1	8930(90)	9300(30)	9390(20)	28(4)
N1	7216(3)	6998.0(11)	7856.4(8)	16.8(4)
N4	10559(3)	7959.7(11)	7710.7(8)	18.6(4)
N3	11227(4)	9133.6(11)	8492.9(9)	19.7(4)
N2	5906(3)	7867.2(11)	9041.9(8)	18.0(4)
C12	8595(4)	7708.8(13)	8064.6(10)	16.7(4)
C9	7771(4)	8132.0(13)	8674.9(10)	17.7(4)
C15	7070(4)	6632.5(13)	5964.2(10)	16.0(4)
C11	12002(4)	8644.7(13)	7936.7(10)	19.2(4)
C1	5224(4)	6680.3(13)	8238.5(9)	17.1(4)
C10	9159(4)	8943.7(14)	8881.4(10)	21.1(4)
C13	7891(4)	6592.2(13)	7207.8(10)	17.6(4)
C2	3839(4)	5919.3(14)	8072.8(10)	20.0(4)
C5	2621(4)	6828.0(14)	9240.0(10)	18.7(4)
C4	1265(4)	6087.0(13)	9078.2(10)	19.5(4)
C14	6757(4)	7129.3(13)	6628.7(10)	17.1(4)
C3	1934(4)	5617.0(13)	8485.2(10)	20.1(4)
C6	4618(4)	7135.4(13)	8838.8(10)	17.9(4)
C17	10153(4)	6181.6(14)	5052.9(10)	18.0(4)

C16	9821(4)	6600.1(13)	5739.8(10)	16.4(4)
C8	-828(4)	5762.0(14)	9519.2(11)	22.0(4)
C7	582(5)	4778.3(14)	8306.0(11)	25.6(5)
O1A	8430(40)	9426(13)	9339(8)	27(2)

Table A6: Anisotropic displacement parameters (×10⁴) for the riboflavin crystal obtained from a water/acetonitrile (50/50 (w/w)) solution saturated with 3,4-dimethoxycinnamic acid and riboflavin. The anisotropic displacement factor exponent takes the form: $-2^2[h^2a^{*2} \times U_{11} + ... + 2hka^* \times b^* \times U_{12}]$

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O4	16.7(7)	19.7(7)	18.7(7)	-0.8(5)	-1.8(6)	-2.0(6)
O6	17.5(7)	21.2(7)	21.7(7)	1.5(6)	4.6(6)	1.2(6)
O2	18.2(8)	27.6(7)	22.1(7)	2.4(6)	2.5(6)	1.8(6)
O5	19.3(7)	18.5(7)	21.3(7)	1.4(5)	-1.7(6)	-2.3(6)
O3	17.4(8)	33.8(8)	19.1(7)	-0.8(6)	0.5(6)	7.6(6)
O1	28(8)	27(7)	30(5)	-12(5)	12(6)	-1(6)
N1	18.7(8)	18.4(8)	13.4(7)	-1.8(6)	-1.2(7)	3.5(7)
N4	18.7(9)	20.6(8)	16.6(7)	0.2(6)	0.5(7)	3.3(7)
N3	19.4(9)	19.0(8)	20.8(8)	-1.9(7)	1.3(7)	-2.5(7)
N2	19.2(9)	18.6(8)	16.2(7)	-0.5(6)	-0.9(7)	1.3(7)
C12	17.6(10)	16.9(9)	15.7(9)	0.9(7)	-1.0(8)	5.5(8)
C9	19.5(10)	18.5(9)	15.1(8)	-0.6(7)	-1.2(8)	2.8(8)
C15	15.9(9)	17.4(9)	14.6(9)	0.0(7)	-1.9(8)	0.3(8)
C11	19.5(10)	21.0(9)	16.9(9)	3.6(7)	-0.5(8)	4.0(8)
C1	17.4(9)	18.8(9)	15.2(8)	1.5(7)	-1.8(8)	3.6(8)
C10	21.4(10)	20.9(9)	21.0(9)	-1.2(7)	1.0(9)	-1.5(8)
C13	19.3(10)	20.0(9)	13.4(9)	-2.1(7)	-0.1(8)	3.0(8)
C2	22.5(11)	19.6(9)	18.0(9)	-1.7(7)	-2.7(8)	2.9(9)
C5	21.6(10)	19.9(9)	14.6(9)	-0.8(7)	-0.7(8)	2.0(8)
C4	18.0(10)	21.9(9)	18.7(9)	3.6(7)	-4.0(8)	1.4(8)
C14	15.5(10)	19.7(8)	16.2(9)	-0.7(7)	-0.5(8)	3.0(8)
C3	21.3(11)	20.0(9)	18.9(9)	1.2(7)	-6.3(8)	1.8(8)
C6	19.7(10)	17.9(8)	16.2(9)	0.5(7)	-3.1(8)	2.4(8)
C17	15.6(10)	20.4(9)	18.2(9)	0.9(7)	0.7(8)	0.8(8)
C16	17.0(9)	15.9(8)	16.3(9)	2.5(7)	-1.1(8)	1.1(8)
C8	20.1(11)	23.2(10)	22.6(10)	3.1(8)	-1.1(9)	-1.4(9)
C7	28.6(13)	22.9(10)	25.3(10)	-2.7(8)	-3.2(10)	-2.9(9)
O1A	23(4)	25(4)	32(2)	-13(2)	11(3)	-6(3)

Table A7: Bond lengths in Å for the riboflavin crystal obtained from a water/acetonitrile (50/50 (w/w)) solution saturated with 3,4-dimethoxycinnamic acid and riboflavin

Atom	Atom	Length (Å)	Atom	Atom	Length (Å)
O4	C15	1.428(2)	C12	C9	1.448(3)
O6	C17	1.436(3)	C9	C10	1.490(3)
O2	C11	1.230(3)	C15	C14	1.537(3)
O5	C16	1.435(2)	C15	C16	1.530(3)
O3	C14	1.423(3)	C1	C2	1.405(3)
O1	C10	1.16(4)	C1	C6	1.422(3)
N1	C12	1.366(3)	C10	O1A	1.234(16)
N1	C1	1.392(3)	C13	C14	1.539(3)
N1	C13	1.481(2)	C2	C3	1.384(3)
N4	C12	1.317(3)	C5	C4	1.370(3)
N4	C11	1.365(3)	C5	C6	1.410(3)
N3	C11	1.399(3)	C4	C3	1.429(3)
N3	C10	1.377(3)	C4	C8	1.503(3)
N2	C9	1.297(3)	C3	C7	1.500(3)
N2	C6	1.363(3)	C17	C16	1.525(3)

Table A8: Bond angles for the riboflavin crystal obtained from a water/acetonitrile (50/50 (w/w)) solution saturated with 3,4-dimethoxycinnamic acid and riboflavin.

Atom	Atom	Atom	Angle (°)	Atom	Atom	Atom	Angle (°)
C12	N1	C1	120.76(16)	N3	C10	C9	114.19(17)
C12	N1	C13	117.62(17)	O1A	C10	N3	123.2(6)
C1	N1	C13	121.61(17)	O1A	C10	C9	122.5(6)
C12	N4	C11	118.97(17)	N1	C13	C14	110.38(17)
C10	N3	C11	125.10(18)	C3	C2	C1	120.80(18)
C9	N2	C6	117.70(17)	C4	C5	C6	122.01(19)
N1	C12	C9	116.30(18)	C5	C4	C3	118.16(19)
N4	C12	N1	119.15(17)	C5	C4	C8	121.14(19)
N4	C12	C9	124.55(19)	C3	C4	C8	120.69(19)
N2	C9	C12	125.00(19)	O3	C14	C15	107.23(16)
N2	C9	C10	118.35(18)	O3	C14	C13	111.11(17)
C12	C9	C10	116.64(18)	C15	C14	C13	110.66(16)
O4	C15	C14	110.10(15)	C2	C3	C4	120.94(19)
O4	C15	C16	110.25(16)	C2	C3	C7	119.08(19)
C16	C15	C14	111.93(16)	C4	C3	C7	120.0(2)
O2	C11	N4	120.96(19)	N2	C6	C1	122.09(19)
O2	C11	N3	119.1(2)	N2	C6	C5	118.38(18)
N4	C11	N3	119.97(19)	C5	C6	C1	119.54(19)
N1	C1	C2	123.41(18)	O6	C17	C16	109.88(16)
N1	C1	C6	118.06(18)	O5	C16	C15	108.45(15)
C2	C1	C6	118.50(19)	O5	C16	C17	107.85(15)
O1	C10	N3	119(2)	C17	C16	C15	112.88(16)
O1	C10	C9	125(2)				

Table A9: Torsion angles for the riboflavin crystal obtained from a water/acetonitrile (50/50 (w/w)) solution saturated with 3,4-dimethoxycinnamic acid and riboflavin.

Atom	Atom	Atom	Atom	Angle (°)
O4	C15	C14	O3	-68.0(2)
O4	C15	C14	C13	53.3(2)
O4	C15	C16	O5	-178.47(15)
O4	C15	C16	C17	62.1(2)
O6	C17	C16	O5	65.5(2)
O6	C17	C16	C15	-174.68(16)
N1	C12	C9	N2	2.6(3)
N1	C12	C9	C10	-176.04(18)
N1	C1	C2	C3	177.67(19)
N1	C1	C6	N2	0.6(3)
N1	C1	C6	C5	-179.61(17)
N1	C13	C14	O3	-51.6(2)
N1	C13	C14	C15	-170.59(17)
N4	C12	C9	N2	-176.88(19)
N4	C12	C9	C10	4.5(3)
N2	C9	C10	O1	11(3)
N2	C9	C10	N3	175.07(19)
N2	C9	C10	O1A	-8.6(15)
C12	N1	C1	C2	-175.80(18)
C12	N1	C1	C6	2.1(3)
C12	N1	C13	C14	-80.6(2)
C12	N4	C11	O2	172.90(18)
C12	N4	C11	N3	-7.3(3)
C12	C9	C10	O1	-171(3)
C12	C9	C10	N3	-6.2(3)

C12	C9	C10	O1A	170.1(15)
C9	N2	C6	C1	-1.6(3)
C9	N2	C6	C5	178.65(18)
C11	N4	C12	N1	-177.01(17)
C11	N4	C12	C9	2.5(3)
C11	N3	C10	O1	167(3)
C11	N3	C10	C9	1.7(3)
C11	N3	C10	O1A	-174.5(15)
C1	N1	C12	N4	176.01(17)
C1	N1	C12	C9	-3.5(3)
C1	N1	C13	C14	98.4(2)
C1	C2	C3	C4	2.2(3)
C1	C2	C3	C7	-177.19(19)
C10	N3	C11	O2	-174.96(19)
C10	N3	C11	N4	5.3(3)
C13	N1	C12	N4	-5.0(3)
C13	N1	C12	C9	175.49(18)
C13	N1	C1	C2	5.3(3)
C13	N1	C1	C6	-176.86(17)
C2	C1	C6	N2	178.58(18)
C2	C1	C6	C5	-1.6(3)
C5	C4	C3	C2	-2.3(3)
C5	C4	C3	C7	177.09(19)
C4	C5	C6	N2	-178.67(19)
C4	C5	C6	C1	1.5(3)
C14	C15	C16	O5	-55.6(2)
C14	C15	C16	C17	-174.99(16)
C6	N2	C9	C12	-0.1(3)
C6	N2	C9	C10	178.55(18)
C6	C1	C2	C3	-0.2(3)
C6	C5	C4	C3	0.4(3)
C6	C5	C4	C8	178.93(19)
C16	C15	C14	O3	168.99(16)
C16	C15	C14	C13	-69.7(2)
C8	C4	C3	C2	179.19(19)
C8	C4	C3	C7	-1.4(3)

Table A10: Hydrogen fractional atomic coordinates (×10⁴) and equivalent isotropic displacement parameters (Å²×10³) for the riboflavin crystal obtained from a water/acetonitrile (50/50 (w/w)) solution saturated with 3,4-dimethoxycinnamic acid and riboflavin. U_{eq} is defined as 1/3 of the trace of the orthogonalized U_{ij}.

Atom	x	y	z	U_{eq}
H3	3475.96	6808.06	6880.5	35
H14	7628.6	7714.53	6598.9	21
H17A	9480(40)	5600(16)	5020(11)	11(5)
H17B	9350(50)	6539(16)	4699(12)	19(6)
H13A	7210(50)	5974(17)	7200(11)	18(6)
H5A	2300(50)	7120(18)	9621(13)	20(6)
H7A	1370(60)	4520(20)	7921(15)	37(8)
H6	13400(60)	6600(20)	4765(16)	41(9)
H16	10720(50)	6301(15)	6040(12)	14(6)
H8A	-2430(60)	5700(20)	9279(14)	33(8)
H8B	-1160(60)	6150(20)	9876(15)	37(8)
H2	4230(50)	5581(16)	7656(12)	19(6)
H13B	9780(60)	6606(18)	7191(12)	25(6)
H5	11890(70)	7470(20)	6053(18)	51(10)
H3A	12160(60)	9550(20)	8626(14)	30(7)

H15	6130(40)	6962(15)	5633(11)	12(5)
H8C	-460(60)	5180(20)	9730(14)	36(8)
H7B	730(60)	4360(20)	8680(15)	36(8)
H7C	-1210(80)	4890(20)	8252(17)	50(9)
H4	5020(60)	5720(20)	5777(15)	31(8)

9.3 Riboflavin crystal obtained in presence of cinnamic acid

$C_{17}H_{20}N_4O_6$, M_r = 376.37, orthorhombic, $P2_12_12_1$ (No. 19), a = 5.30940(10) Å, b = 15.11770(10) Å, c = 20.0373(2) Å, $\alpha = \beta = \gamma = 90^{\circ}$, V = 1608.31(4) Å^3, Z = 4, Z' = 1, μ(Cu K_α) = 1.009, 14730 reflections measured, 3253 unique (R_{int} = 0.0222) which were used in all calculations. The final wR_2 was 0.0664 (all data) and R_1 was 0.0263 (I≥2 σ(I)).

Table A11: Compound characterization for the riboflavin crystal obtained from a water/acetonitrile (50/50 (w/w)) solution saturated with cinnamic acid and riboflavin.

Parameter	Obtained value	Parameter	Obtained value
Formula	$C_{17}H_{20}N_4O_6$	**Z**	4
$\rho_{calc.}$ **(g·cm^{-3})**	1.554	**Z'**	1
μ·mm^{-1}	1.009	λ **(Å)**	1.54184
Formula Weight	376.37	**Radiation type**	Cu K_α
Color	clear yellow	θ_{min} **(°)**	3.663
Shape	needle-shaped	θ_{max} **(°)**	74.975
Size (mm^3)	0.22×0.03×0.02	**Measured Refl's.**	14730
T (K)	123.00(10)	**Indep't Refl's**	3253
Crystal System	orthorhombic	**Refl's I≥2 σ(I)**	3077
Flack Parameter	-0.22(7)	**R_{int}**	0.0222
Hooft Parameter	-0.25(6)	**Parameters**	324
Space Group	$P2_12_12_1$	**Restraints**	0
a (Å)	5.30940(10)	**Largest Peak**	0.191
b (Å)	15.11770(10)	**Deepest Hole**	-0.152
c (Å)	20.0373(2)	**GooF**	1.037
α (°)	90	**wR_2 (all data)**	0.0664
β (°)	90	**wR_2**	0.0652
γ (°)	90	**R_1(all data)**	0.0288
V (Å^3)	1608.31(4)	**R_1**	0.0263

Table A12: Fractional Atomic Coordinates (×10⁴) and Equivalent Isotropic Displacement Parameters (Å²×10³) for the riboflavin crystal obtained from a water/acetonitrile (50/50 (w/w)) solution saturated with cinnamic acid and riboflavin. U_{eq} is defined as 1/3 of the trace of the orthogonalized U_{ij}.

Atom	x	y	z	U_{eq}
O4	6068(3)	5756.7(8)	6020.8(7)	15.0(3)
O6	12776(3)	6097.7(9)	4900.2(7)	16.5(3)
O2	13988(3)	8844.8(9)	7657.9(7)	18.6(3)
O5	10763(3)	7487.5(8)	5707.0(7)	15.6(3)
O3	4120(3)	7264.1(9)	6725.1(7)	19.8(3)
O1	8528(3)	9401.8(10)	9348.5(8)	30.2(4)
N1	7218(3)	6997.8(10)	7856.4(7)	13.7(3)
N4	10558(3)	7960.9(10)	7710.8(8)	15.0(3)
N3	11231(3)	9134.5(10)	8492.7(8)	16.2(3)
N2	5908(3)	7869.0(9)	9042.0(8)	14.2(3)
C12	8597(4)	7709.7(11)	8066.0(9)	13.3(4)
C9	7775(4)	8133.5(12)	8673.7(9)	14.5(4)
C15	7075(3)	6631.9(11)	5965.1(9)	12.4(3)
C13	7893(4)	6595.0(12)	7207.4(9)	13.9(4)
C11	11999(4)	8645.7(12)	7937.0(9)	15.7(4)
C1	5226(4)	6680.9(11)	8238.9(9)	13.7(4)
C2	3837(4)	5920.4(12)	8074.7(9)	15.9(4)
C4	1269(4)	6087.0(12)	9077.6(9)	15.6(4)
C10	9160(4)	8945.2(12)	8881.8(9)	17.2(4)
C6	4620(4)	7135.3(11)	8837.2(9)	14.3(4)
C14	6760(4)	7125.6(12)	6628.9(9)	13.6(4)
C3	1933(4)	5617.8(12)	8485.9(10)	16.3(4)
C5	2620(4)	6828.0(12)	9240.0(9)	15.3(4)
C17	10153(3)	6180.9(12)	5053.6(9)	14.3(4)
C16	9816(3)	6599.1(11)	5739.4(9)	12.9(3)
C8	-830(4)	5760.5(13)	9519.6(10)	17.6(4)
C7	585(4)	4777.8(12)	8304.6(10)	21.5(4)

Table A13: Anisotropic Displacement Parameters (×10⁴) for the riboflavin crystal obtained from a water/acetonitrile (50/50 (w/w)) solution saturated with cinnamic acid and riboflavin. The anisotropic displacement factor exponent takes the form: $-2^2[h^2a^{*2} \times U_{11} + ... + 2hka^* \times b^* \times U_{12}]$

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O4	13.1(6)	16.1(6)	15.8(6)	-1.1(5)	-1.0(5)	-1.8(5)
O6	14.0(7)	16.6(6)	18.9(7)	1.6(5)	4.5(5)	1.4(5)
O2	14.9(7)	23.9(6)	17.0(6)	1.6(5)	2.0(5)	1.9(6)
O5	14.7(7)	14.8(6)	17.3(6)	1.9(5)	-1.3(6)	-2.6(5)
O3	14.8(7)	30.4(7)	14.1(6)	-3.2(5)	-0.4(6)	8.5(6)
O1	33.0(9)	28.1(7)	29.5(8)	-16.2(6)	14.7(7)	-12.3(7)
N1	15.8(8)	14.4(7)	11.0(7)	-1.3(5)	-0.7(6)	2.9(6)
N4	14.9(8)	16.7(7)	13.5(7)	0.1(6)	-0.2(6)	3.6(6)
N3	15.8(8)	16.0(7)	16.7(8)	-2.7(6)	2.0(6)	-1.9(7)
N2	14.5(8)	14.7(7)	13.4(7)	-0.9(5)	-1.4(6)	1.1(6)
C12	14.0(9)	13.0(8)	12.8(8)	0.2(6)	-1.3(7)	4.6(7)
C9	16.3(9)	15.3(8)	11.9(8)	-0.4(7)	-1.2(7)	2.2(7)
C15	13.4(8)	13.1(8)	10.6(8)	1.2(6)	-2.0(7)	0.4(7)
C13	16.0(9)	15.4(8)	10.3(8)	-2.0(6)	-1.0(7)	2.3(7)
C11	17.0(9)	17.3(9)	12.7(9)	2.7(6)	-1.0(7)	4.5(7)
C1	14.1(8)	16.0(8)	11.0(8)	1.6(6)	-2.2(7)	2.6(7)
C2	19.1(10)	15.0(8)	13.5(8)	-2.0(6)	-3.0(7)	1.6(8)
C4	14.5(9)	17.6(8)	14.7(9)	3.4(7)	-3.2(7)	1.7(7)
C10	17.7(9)	17.5(8)	16.5(9)	-1.6(7)	0.9(8)	-1.0(7)

C6	16.1(9)	14.6(8)	12.1(8)	-0.4(6)	-3.3(7)	1.9(7)
C14	11.0(9)	16.0(8)	13.9(9)	-0.3(6)	-0.6(7)	2.4(7)
C3	17.2(10)	15.5(8)	16.2(9)	0.9(7)	-5.7(8)	1.2(7)
C5	17.1(9)	16.2(8)	12.5(8)	-0.6(7)	-1.2(7)	2.2(7)
C17	12.4(9)	17.0(8)	13.4(8)	1.4(7)	0.6(7)	1.4(7)
C16	12.9(8)	12.1(8)	13.6(8)	2.3(7)	-1.1(7)	-0.1(7)
C8	16.0(10)	18.9(9)	17.9(9)	2.1(7)	-1.5(8)	-1.9(8)
C7	24.6(11)	18.5(9)	21.5(10)	-2.7(7)	-3.3(9)	-4.6(8)

Table A14: Bond Lengths for the riboflavin crystal obtained from a water/acetonitrile (50/50 (w/w)) solution saturated with cinnamic acid and riboflavin.

Atom	Atom	Length (Å)	Atom	Atom	Length (Å)
O4	C15	1.431(2)	C12	C9	1.444(2)
O6	C17	1.432(2)	C9	C10	1.490(3)
O2	C11	1.232(2)	C15	C14	1.534(2)
O5	C16	1.436(2)	C15	C16	1.525(2)
O3	C14	1.430(2)	C13	C14	1.533(3)
O1	C10	1.210(2)	C1	C2	1.405(3)
N1	C12	1.368(2)	C1	C6	1.419(2)
N1	C13	1.480(2)	C2	C3	1.382(3)
N1	C1	1.391(2)	C4	C3	1.426(3)
N4	C12	1.317(2)	C4	C5	1.369(3)
N4	C11	1.365(2)	C4	C8	1.507(3)
N3	C11	1.397(2)	C6	C5	1.412(3)
N3	C10	1.378(3)	C3	C7	1.502(3)
N2	C9	1.299(2)	C17	C16	1.523(2)
N2	C6	1.366(2)			

Table A15: Bond Angles for the riboflavin crystal obtained from a water/acetonitrile (50/50 (w/w)) solution saturated with cinnamic acid and riboflavin.

Atom	Atom	Atom	Angle (°)	Atom	Atom	Atom	Angle (°)
C12	N1	C13	117.65(16)	C3	C2	C1	121.00(17)
C12	N1	C1	120.58(15)	C3	C4	C8	120.58(17)
C1	N1	C13	121.76(16)	C5	C4	C3	118.36(18)
C12	N4	C11	118.83(16)	C5	C4	C8	121.05(17)
C10	N3	C11	125.02(16)	O1	C10	N3	122.71(17)
C9	N2	C6	117.47(16)	O1	C10	C9	123.31(18)
N1	C12	C9	116.51(16)	N3	C10	C9	113.98(16)
N4	C12	N1	118.95(16)	N2	C6	C1	122.23(17)
N4	C12	C9	124.54(17)	N2	C6	C5	118.15(16)
N2	C9	C12	124.99(17)	C5	C6	C1	119.62(17)
N2	C9	C10	118.11(16)	O3	C14	C15	107.16(15)
C12	C9	C10	116.89(17)	O3	C14	C13	111.06(16)
O4	C15	C14	109.95(14)	C13	C14	C15	111.00(14)
O4	C15	C16	110.47(14)	C2	C3	C4	120.79(17)
C16	C15	C14	112.15(15)	C2	C3	C7	118.96(17)
N1	C13	C14	110.75(15)	C4	C3	C7	120.24(18)
O2	C11	N4	120.99(17)	C4	C5	C6	121.81(17)
O2	C11	N3	118.85(17)	O6	C17	C16	110.15(15)
N4	C11	N3	120.16(17)	O5	C16	C15	108.50(14)
N1	C1	C2	123.49(17)	O5	C16	C17	107.84(14)
N1	C1	C6	118.12(16)	C17	C16	C15	113.15(15)
C2	C1	C6	118.36(17)				
C3	C2	C1	121.00(17)				

Table A16: Torsion Angles for the riboflavin crystal obtained from a water/acetonitrile (50/50 (w/w)) solution saturated with cinnamic acid and riboflavin.

Atom	Atom	Atom	Atom	Angle/°
O4	C15	C14	O3	-67.61(18)
O4	C15	C14	C13	53.8(2)
O4	C15	C16	O5	-178.56(14)
O4	C15	C16	C17	61.81(18)
O6	C17	C16	O5	65.28(18)
O6	C17	C16	C15	-174.71(14)
N1	C12	C9	N2	2.8(3)
N1	C12	C9	C10	-176.05(16)
N1	C13	C14	O3	-51.5(2)
N1	C13	C14	C15	-170.61(15)
N1	C1	C2	C3	177.66(17)
N1	C1	C6	N2	0.5(3)
N1	C1	C6	C5	-179.54(16)
N4	C12	C9	N2	-177.02(17)
N4	C12	C9	C10	4.2(3)
N2	C9	C10	O1	-5.2(3)
N2	C9	C10	N3	175.02(17)
N2	C6	C5	C4	-178.62(17)
C12	N1	C13	C14	-80.7(2)
C12	N1	C1	C2	-175.82(16)
C12	N1	C1	C6	2.1(2)
C12	N4	C11	O2	172.62(16)
C12	N4	C11	N3	-7.4(2)
C12	C9	C10	O1	173.67(19)
C12	C9	C10	N3	-6.1(2)
C9	N2	C6	C1	-1.4(3)
C9	N2	C6	C5	178.61(17)
C13	N1	C12	N4	-5.0(2)
C13	N1	C12	C9	175.21(16)
C13	N1	C1	C2	5.4(3)
C13	N1	C1	C6	-176.61(15)
C11	N4	C12	N1	-177.03(16)
C11	N4	C12	C9	2.7(3)
C11	N3	C10	O1	-177.96(19)
C11	N3	C10	C9	1.8(3)
C1	N1	C12	N4	176.18(16)
C1	N1	C12	C9	-3.6(2)
C1	N1	C13	C14	98.09(19)
C1	C2	C3	C4	2.1(3)
C1	C2	C3	C7	-177.16(17)
C1	C6	C5	C4	1.4(3)
C2	C1	C6	N2	178.59(16)
C2	C1	C6	C5	-1.5(3)
C10	N3	C11	O2	-174.86(17)
C10	N3	C11	N4	5.2(3)
C6	N2	C9	C12	-0.2(3)
C6	N2	C9	C10	178.57(16)
C6	C1	C2	C3	-0.3(3)
C14	C15	C16	O5	-55.52(18)
C14	C15	C16	C17	-175.15(14)
C3	C4	C5	C6	0.4(3)
C5	C4	C3	C2	-2.2(3)
C5	C4	C3	C7	177.12(17)
C16	C15	C14	O3	169.05(15)
C16	C15	C14	C13	-69.53(19)
C8	C4	C3	C2	179.21(17)

C8	C4	C3	C7	-1.5(3)
C8	C4	C5	C6	178.99(17)

Table A17: Hydrogen Fractional Atomic Coordinates (×10⁴) and Equivalent Isotropic Displacement Parameters (Å²×10³) for the riboflavin crystal obtained from a water/acetonitrile (50/50 (w/w)) solution saturated with cinnamic acid and riboflavin. U_{eq} is defined as 1/3 of the trace of the orthogonalized U_{ij}.

Atom	x	y	z	U_{eq}
H17A	9360(50)	6552(14)	4717(12)	21(6)
H5A	2300(40)	7174(14)	9646(12)	12(5)
H15	6140(40)	6960(14)	5646(10)	13(5)
H17B	9500(40)	5579(13)	5028(10)	6(5)
H13A	7270(50)	5980(16)	7202(11)	20(6)
H13B	9740(50)	6610(14)	7183(11)	14(5)
H14	7650(50)	7710(15)	6599(11)	18(6)
H8A	-1110(50)	6135(16)	9887(13)	29(6)
H16	10800(40)	6236(13)	6077(11)	18(5)
H2	4250(40)	5610(13)	7664(11)	14(5)
H6	13360(50)	6584(17)	4776(13)	28(7)
H8B	-2390(50)	5660(15)	9280(13)	27(6)
H3A	12200(50)	9597(18)	8631(13)	33(7)
H8C	-450(50)	5191(16)	9738(13)	29(6)
H3	4000(60)	7654(17)	7030(15)	38(8)
H7A	1410(50)	4485(15)	7898(13)	27(6)
H7B	-1230(60)	4889(17)	8224(13)	32(7)
H7C	800(50)	4333(17)	8691(14)	41(8)
H5	11920(60)	7515(18)	6042(16)	44(8)
H4	4960(60)	5699(17)	5728(14)	37(8)

9.4 NMR experiments with riboflavin in deuterated dimethyl sulfoxide

The assignment of atom numbers to RF for NMR analysis is given in Figure 2 in the Introduction. The ^{13}C- and ^{1}H-NMR spectrum of RF in DMSO-d_6 are shown in Figure A2 and A3 below. The attribution to the protons and carbons is given in Table A18 and A19. The protons H1, H13/H14 were attributed to the atoms via the chemical shift. The protons H5, H8, H4, H6, H7 and H9 were assigned via the splitting and H11 with via its integral. COSY, NOESY, HSQC and HMBC measurements were used to verify the attribution, see Figure A4-A7. Moreover, the chemical shift was used to assign the atoms to the signals.[62]

The HSQC spectrum allowed to assign C17 to H14 and C12 to H13. Protons of the sugar chain were attributed via HSQC as followed: H12 to C5', H11 to C5'/C3'/C4', H10 to C2' and H8/5 to C1'. H2 and H3 coupled with C9 and C6 respectively. With the help of HMBC, the OH-groups of RF comprising H9, H7, H6 and H4 could be attributed.

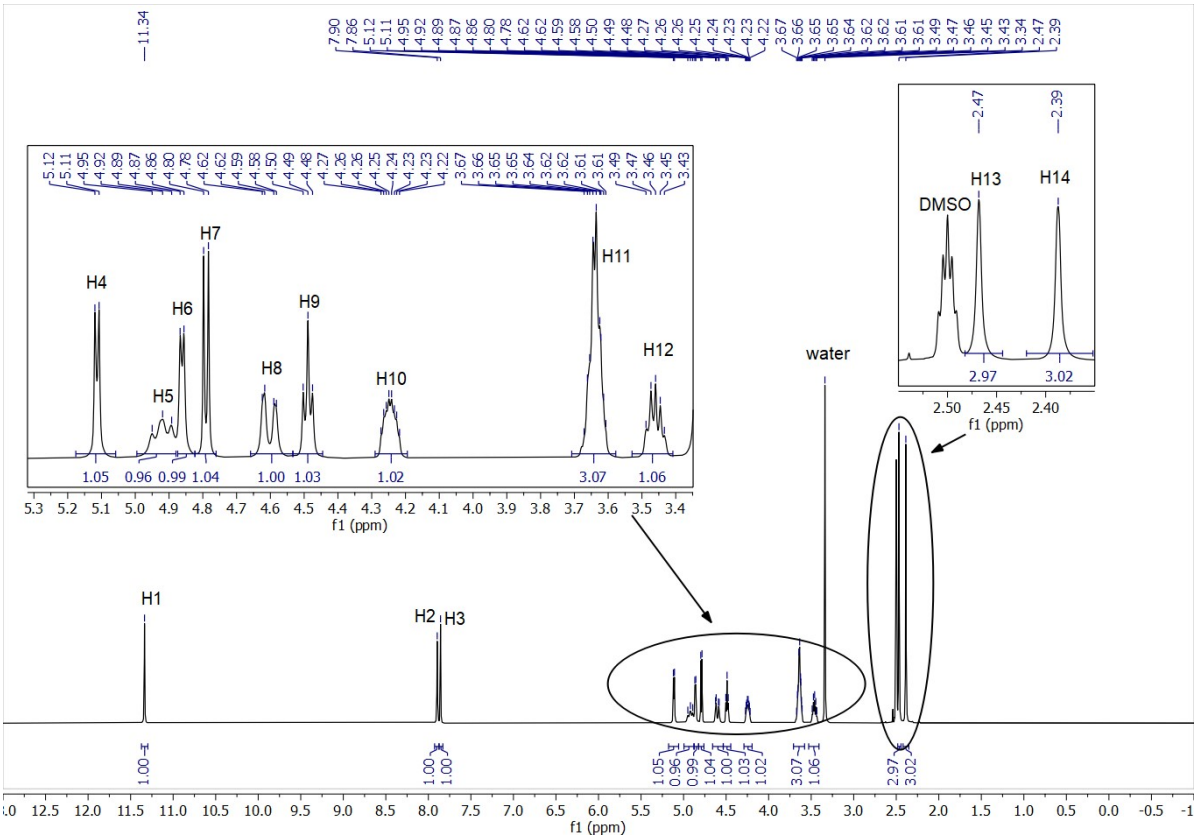

Figure A2: ^{1}H-NMR spectrum of riboflavin in DMSO-d_6 (saturation)

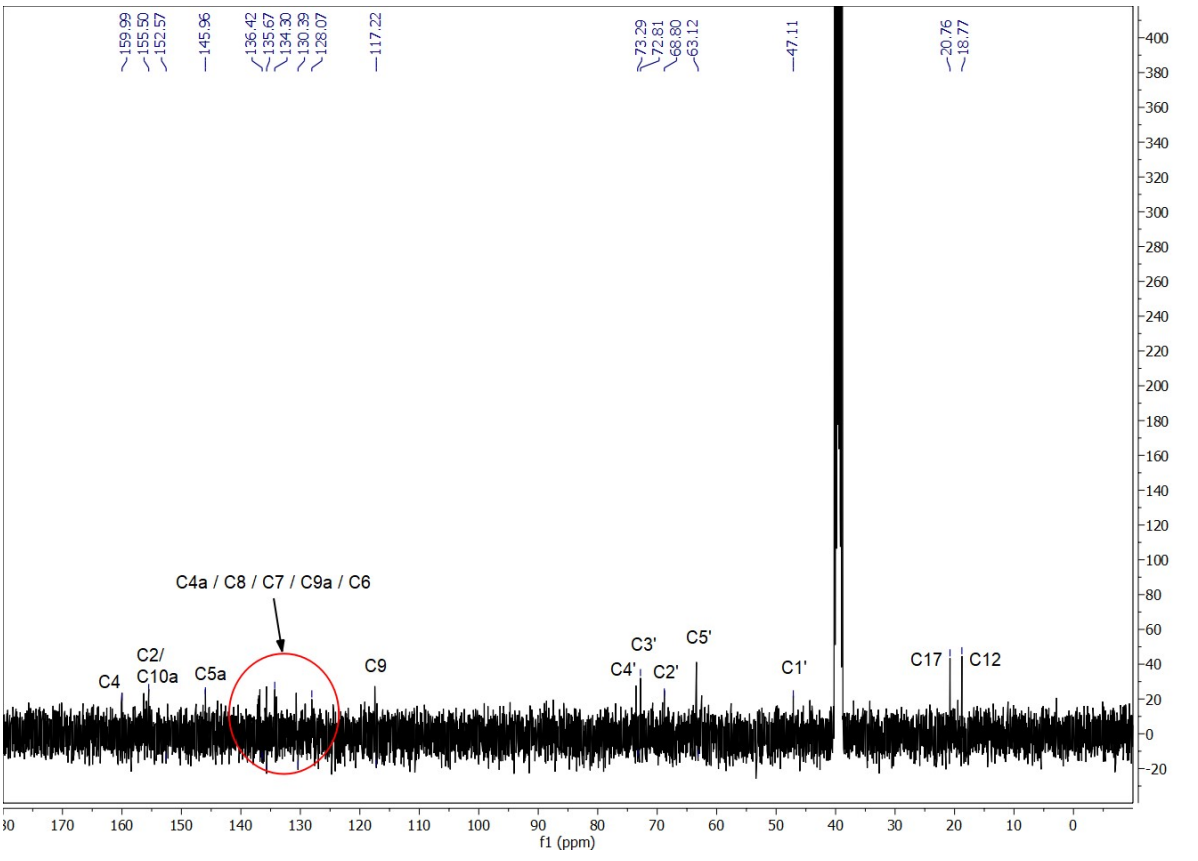

Figure A3: ^{13}C-NMR spectrum of riboflavin in DMSO-d_6 (saturation)

Table A18: Attribution of ^{1}H-NMR signals to riboflavin's atoms, see Figure A2. The COSY, NOESY, HSQC and HMBC spectrum were used for validation of the attribution see Figure A4-A7. s = singlet, d = doublet, t = triplet, m = multiplet.

Proton	δ (ppm)	Number of protons	Splitting	Coupling (Hz)	Attribution via
1	11.34	1	s	-	Shift
2	7.90	1	s	-	HSQC
3	7.86	1	s	-	HSQC
4 (OH)	5.11	1	d	^{3}J(4-11) = 4.95	HMBC
5/8	4.92	1	t	^{2}J(5-8) = 10.51	HSQC, Integral, Shift
6 (OH)	4.86	1	d	^{3}J(6-11) = 4.68	HMBC
7 (OH)	4.79	1	d	^{3}J(7-10) = 5.84	HMBC
8/5	4.60	1	d	^{2}J(8-5) = 13.65	COSY
9 (OH)	4.49	1	t	^{2}J(9-11) = 5.68	Splitting/HMBC
10	4.25	1	m	Not resolved	COSY
11	3.64	3	m	Not resolved	COSY
12	3.46	1	m	Not resolved	COSY
13	2.47	3	s	-	HSQC
14	2.39	3	s	-	HSQC

Table A19: Attribution of ^{13}C-NMR signals to riboflavin's carbon atoms from Figure A3. The atoms attributed via HSQC are indicated in brackets, see Figure A5. All other atoms were attributed via the chemical shift.[62] *C9a was not certain, as the noise was too strong.*

Carbon	δ (ppm)	Carbon	δ (ppm)
4	159.99	9	117.22 (HSQC)
10a	155.50	3‘	72.81 (HSQC)
2	152.57	4‘	73.29(HSQC)
5a	145.96	2‘	68.80 (HSQC)
4a	136.42	5‘	63.12 (HSQC)
8	135.67	1‘	47.11 (HSQC)
7	134.30	12	20.76 (HSQC)
9a*	134.01/128.07	17	18.77 (HSQC)
6	130.39 (HSQC)		

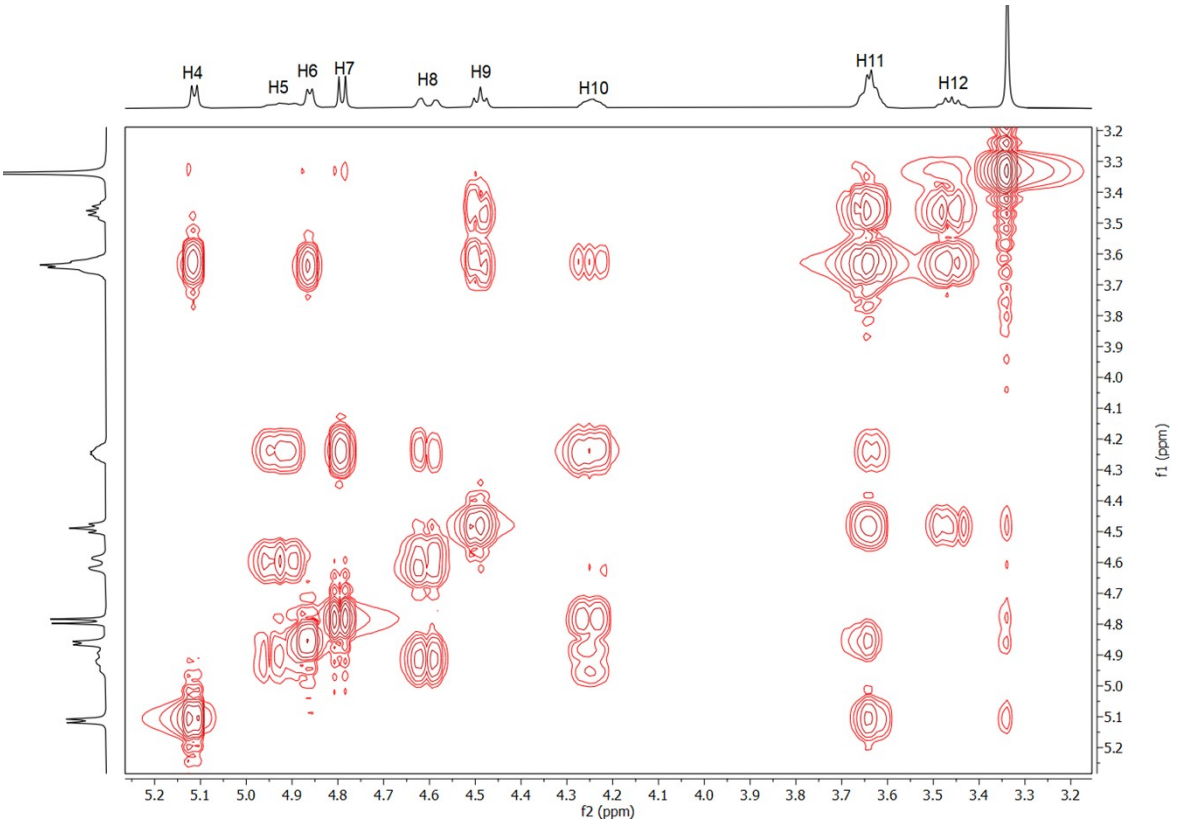

Figure A4: COSY spectrum of riboflavin in DMSO-d_6 (saturation). The following interactions were visible: H12 with H9/11; H11 with H6/H4/H10/H12/H9; H10 with H8/H5/H11; H8 with H5/H10.

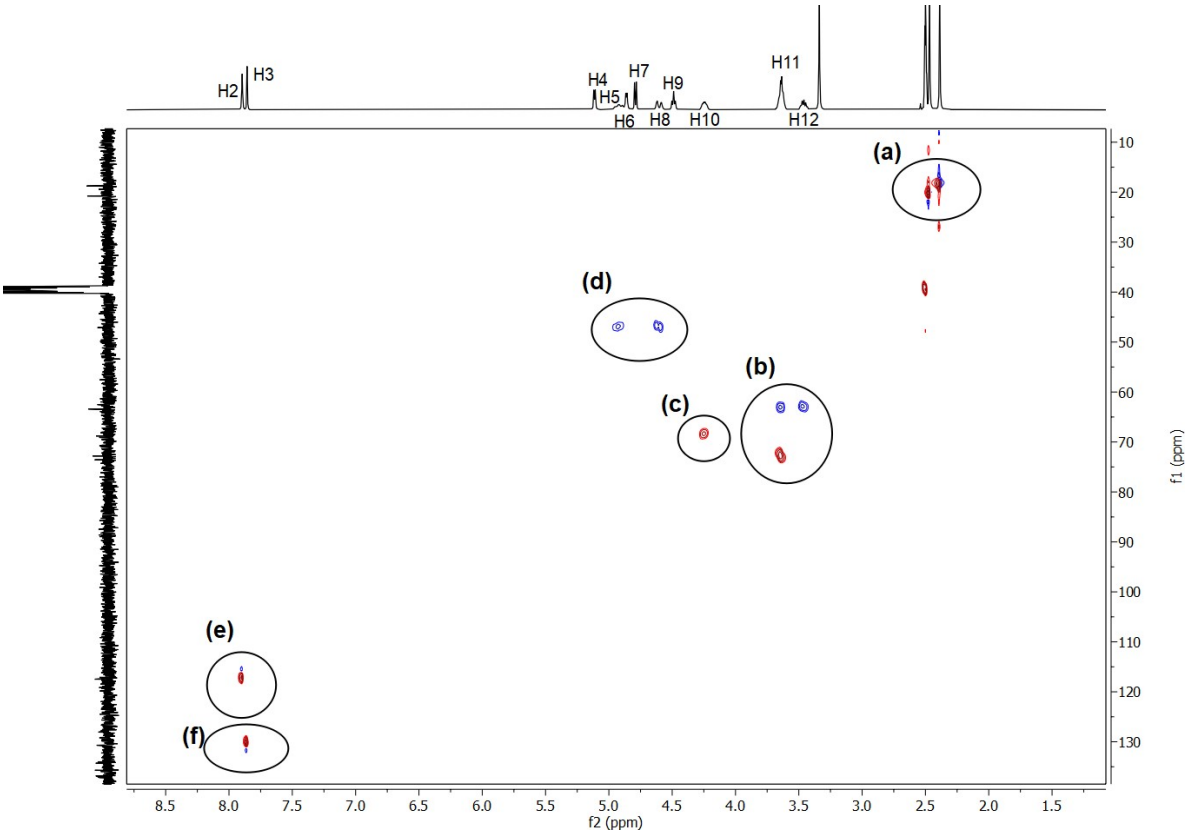

Figure A5: HSQC spectrum of riboflavin in DMSO-d_6 (saturation). The protons could be attributed to the following carbons atoms: (a) C17 to H14 and C12 to H13 - (b) C5' to H12 and C5'/C3'/C4' to H11 - (c) C2' to H10 - (d) C1 to H8/5 - (e)C6 to H3 - (f)C9 to H2

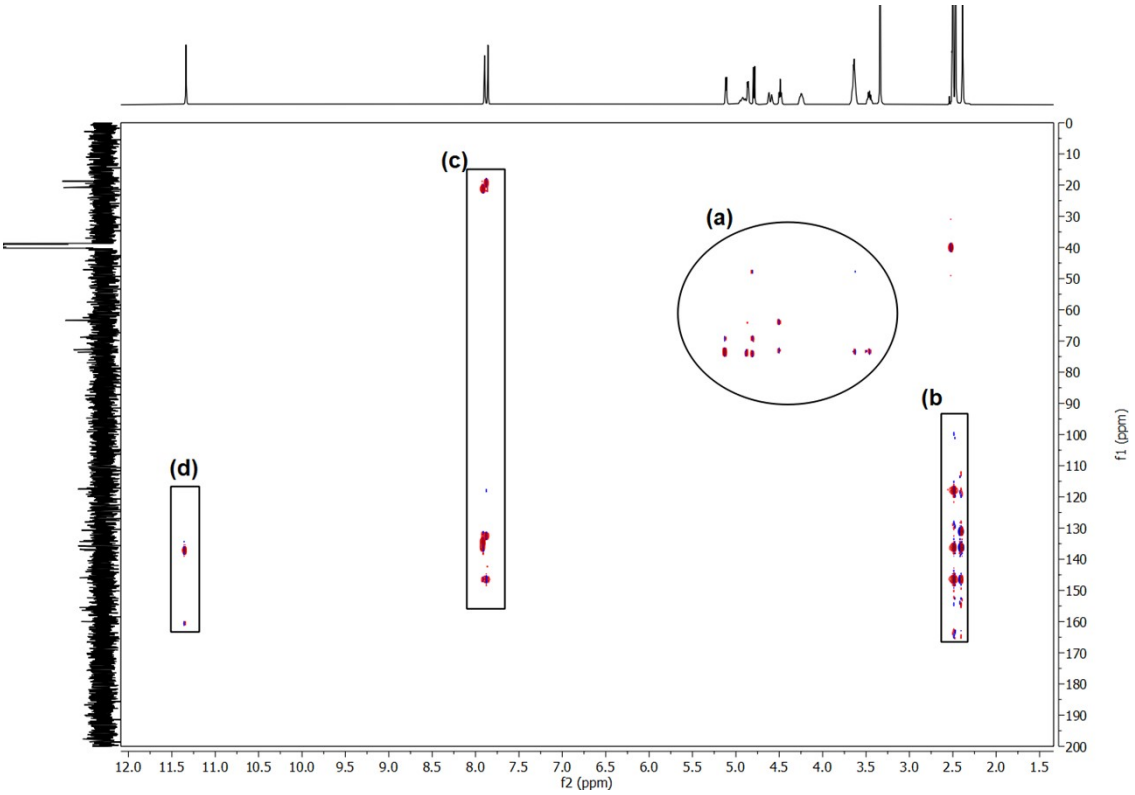

Figure A6: HMBC spectrum of riboflavin in DMSO-d_6 (saturation). The following cross peaks are visible: (a) C3'/C4'-H12 and C3'/C4'-H11 and C5'/C3'/C4'-H9 and C4'/C2'/C1'-H7 and C4'-H6 and C4'/C2'/C3'-H4 – (b) C9/C6/C9a/C7/C8/C5a/C4a-H13/14 – (c) C12/C17/C6/C9a/C7/C8/C5a/C4a/C9-H2/3 – (d) C4a/C4-H1.

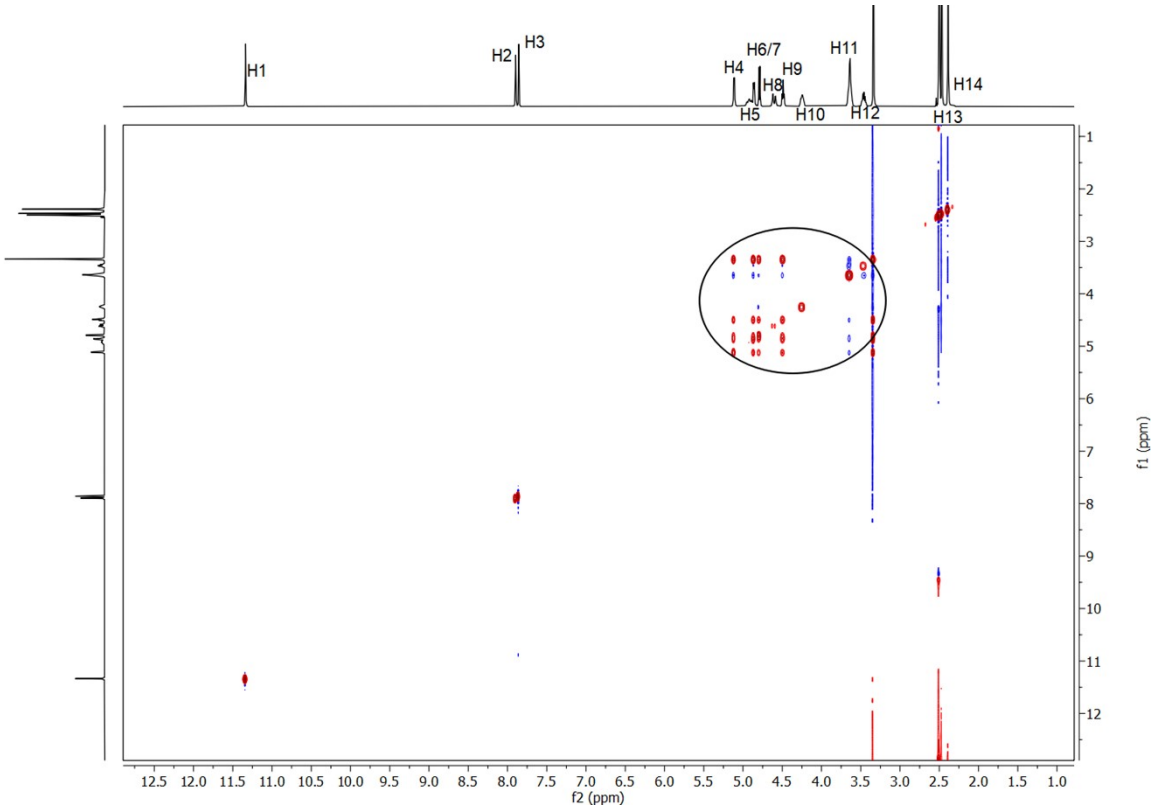

Figure A7: NOESY spectrum of riboflavin in DMSO-d_6 (saturation). The interactions of the sugar chain were marked with a circle. The interactions, which were depicted in -Figure A4 above, are visible in the NOESY spectrum, too. Additionally, a cross-peak between H10 and H7 was found. Therefore, H7 belonged to the OH-group nearest to the isoalloxazine ring.

9.5 NMR experiments with riboflavin 5'-monophosphate sodium salt in deuterium oxide

The ^{1}H-NMR and ^{13}C-NMR spectrum is given in Figure A8 and A9. The protons were attributed via the COSY, NOESY, HSQC and HMBC spectrum and via comparison with the peak attribution of the riboflavin protons.

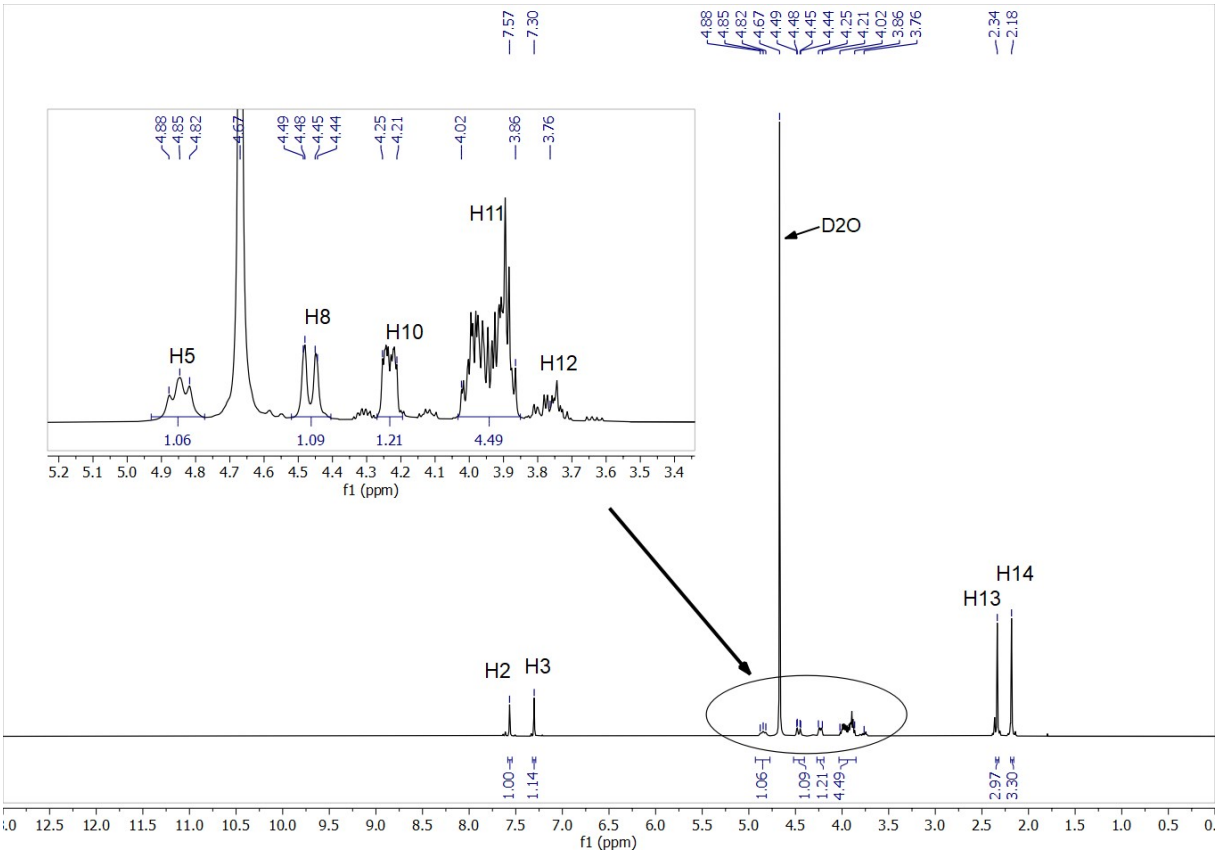

Figure A8: ^{1}H-NMR spectrum of riboflavin 5'-monophoshpate sodium salt in deuterium oxide (saturation).

Table A20: Attribution of ^{1}H-NMR signals from Figure A8 to RF-PO$_4$ protons via the chemical shift δ, the integrals, the splitting and coupling constants. The right column shows the interactions observed from the NOESY spectrum in Figure 7 in section 3.4. vis. = visible, s = singlet, d = doublet, t = triplet, m = multiplet.

Proton	δ (ppm)	Number of protons	Splitting	Coupling (Hz)	NOESY
1	Not vis.	1	-	-	
2	7.57	1	s	-	H13, H10 H8, H5
3	7.30	1	s	-	H14
4 (OH)	Not vis		-	-	
5	4.85	1	t	^{2}J(5-8) = 12.72	H2
6 (OH)	Not vis	-	-	-	
7 (OH)	Not vis	-	-	-	
8	4.46	1	d	^{2}J(8-5) = 14.60	H2

10	4.23	1	m			H2
11	3.94	3	m			
12	3.76	1	m			
13	2.33	3	s	-		H2
14	2.18	3	s	-		H3

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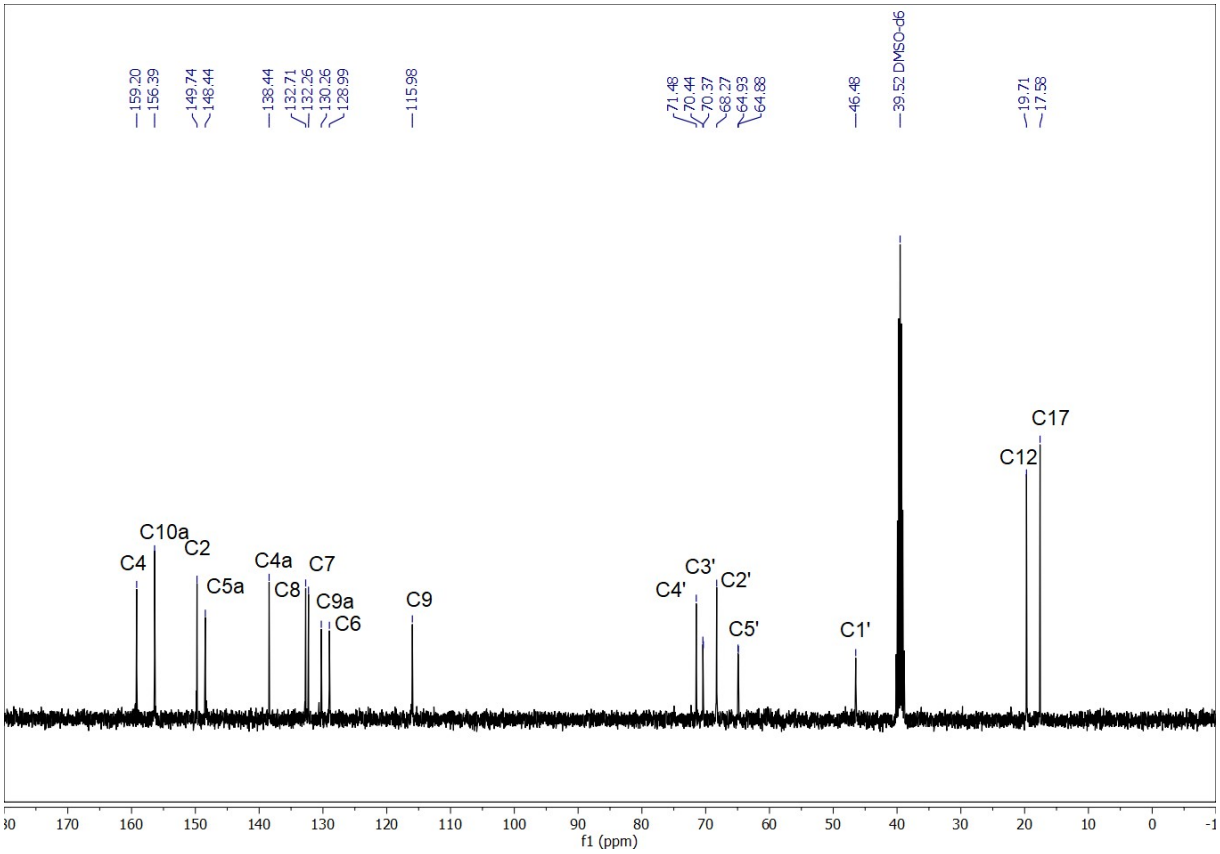


171

172 *Figure A9: ^{13}C-NMR spectrum of riboflavin 5'-monophosphate sodium salt in deuterium oxide*
173 *(saturation). To have a reference an NMR tube with DMSO-d_6 was inserted*

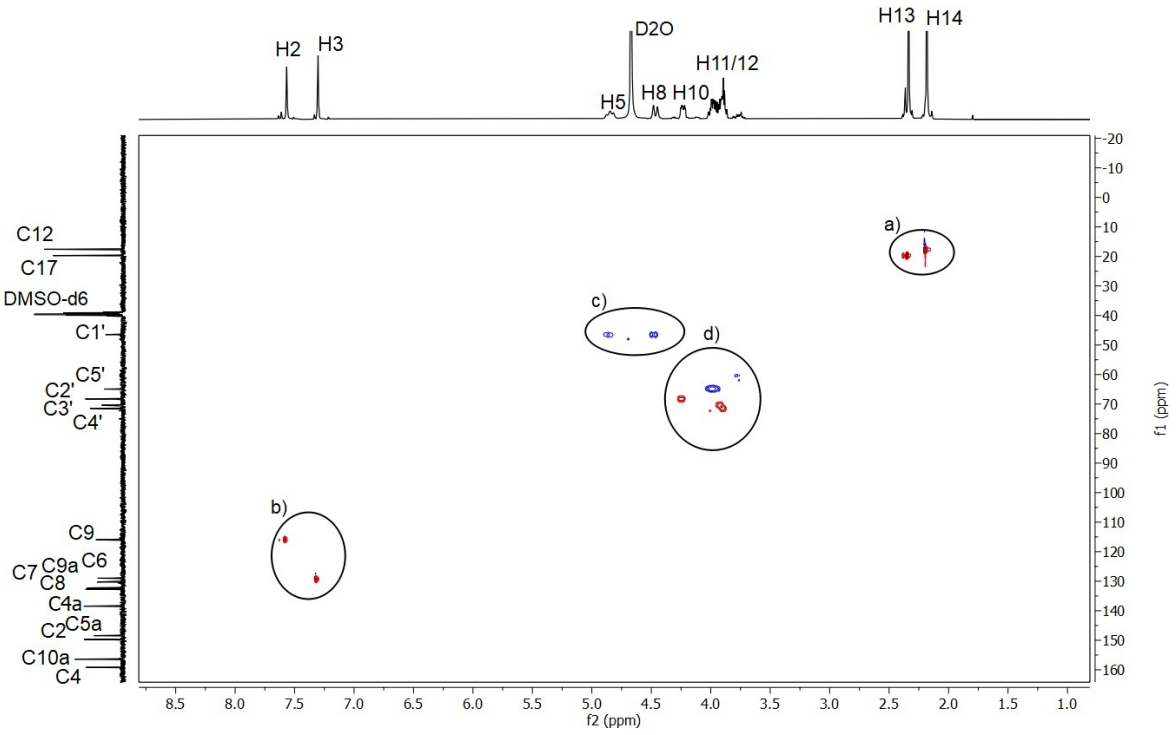
174

175 *Table A21: Attribution of ^{13}C-NMR signals from Figure A9 to RF-PO_4 carbons via the chemical shift δ,*
176 *the HSQC and HMBC spectrum from Figure A10 and A11. C3' and C5' yielded in two signals probably*
177 *due to different degrees of protonation and different conformations of the ribityl chain.*

Carbon	**δ (ppm)**	**HSQC**	**HMBC**
4	159.20		
2	156.39		H3, H14, H13
10a	149.75		
4a	138.44		H2, H13, H14
5a	148.44		
8	132.70		H2, H13
7	132.26		H3, H14
9a	130.26		
6	128.99	H3	
9	115.98	H2	H13
4‘	71.48	H11/12	H11, H12

3‘	70.44/70.37	H11/12	
2‘	68.27	H10	
5‘	64.93/64.88	H11/12	H12
1‘	46.48	H8 / H5	
12	19.71	H13	H2, H14
17	17.58	H14	H3, H13

178



179

180 *Figure A10: HSQC NMR spectrum of riboflavin 5’-monophosphate in deuterium oxide (saturation). To*
181 *have a reference in ^{13}C-NMR tube with DMSO-d_6 was inserted. ^{1}H-NMR was conducted in deuterium*
182 *oxide only. The following cross peaks are present: a) H14/H13 with C17/C12, b) H2 with C9; H3 with*
183 *C6, c) H5 and H8 with C1’, d) H11 with C5’, C3’, C4’ and H10 with C2’.*

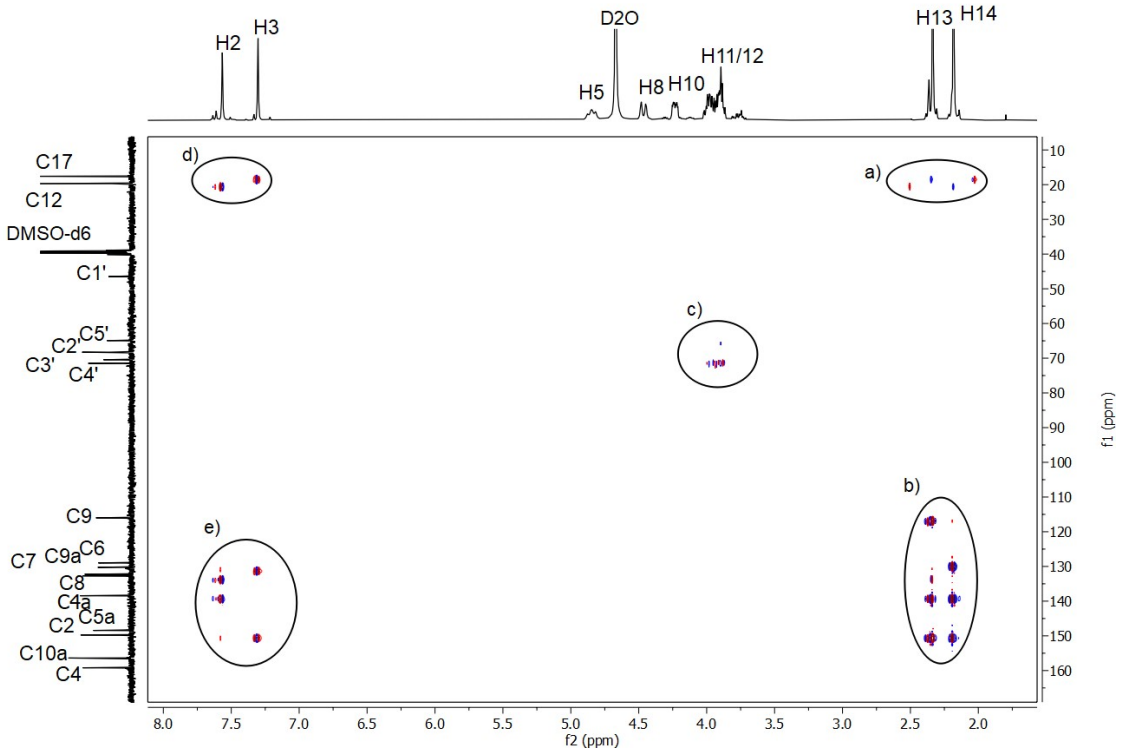


Figure A11: HMBC NMR spectrum of riboflavin 5'-phosphate sodium salt in D_2O (saturation). To have a reference in ^{13}C-NMR tube with DMSO-d_6 was inserted. ^{1}H-NMR was conducted in deuterium oxide only. The following cross peaks are present: a) H14 with C12 and H13 with C17, b) H13 with C9, C8, C4a, C2 and H14 with C7, C4a, C2, c) H12 with C5' and H11/H12 with C4', d) H3 with C17 and H2 with C12, e) H3 with C7, C2 and H2 with C8, C4a.

9.6 Computational Details

9.6.1 Molecular dynamics

A) B)

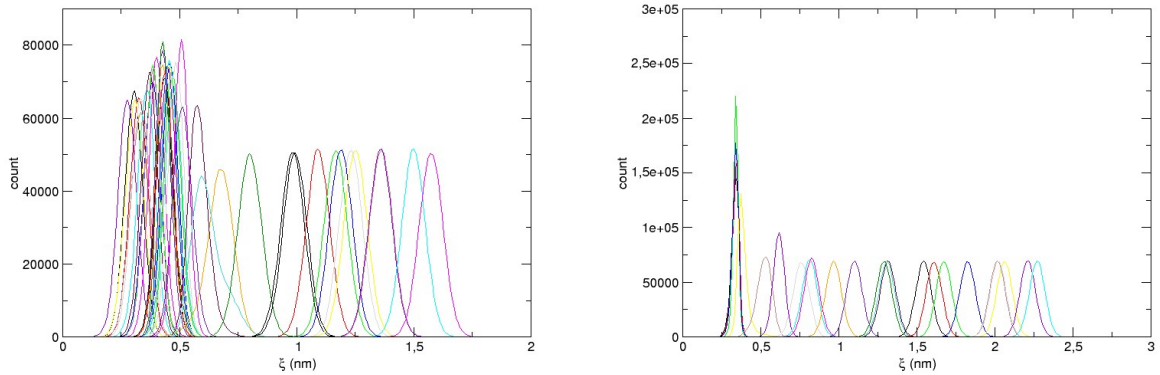


Figure A12. Histogram of the Umbrella sampling for the separation of 2 RF (A) and 2 LC (B) molecules.

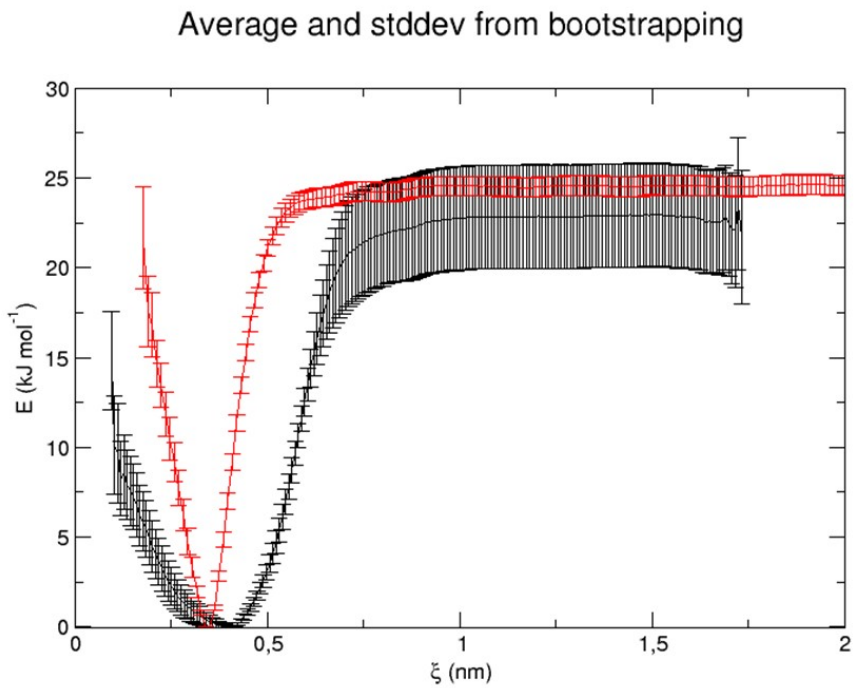


Figure A13. Standard error from bootstrapping of the umbrella sampling. Red: average PMF and standard error for LC; black: average PMF and standard error for RF.

9.6.2 COSMO-RS calculation

Conformational sampling of riboflavin was done using COSMOconf 2021.[63] All together 20 unique conformations were obtained by the procedure. The conformations were be classified according to the internal hydrogen bond constructed between N1/O2 and various OH-groups of the ribityl chains. Figure A14 depicts superimposed flavin ring of RF molecules for each class. Subsequently, solubility calculation of averaged molecule and separated conformations was performed using COSMOtherm 2022 using non-iterative procedure.[64–67]

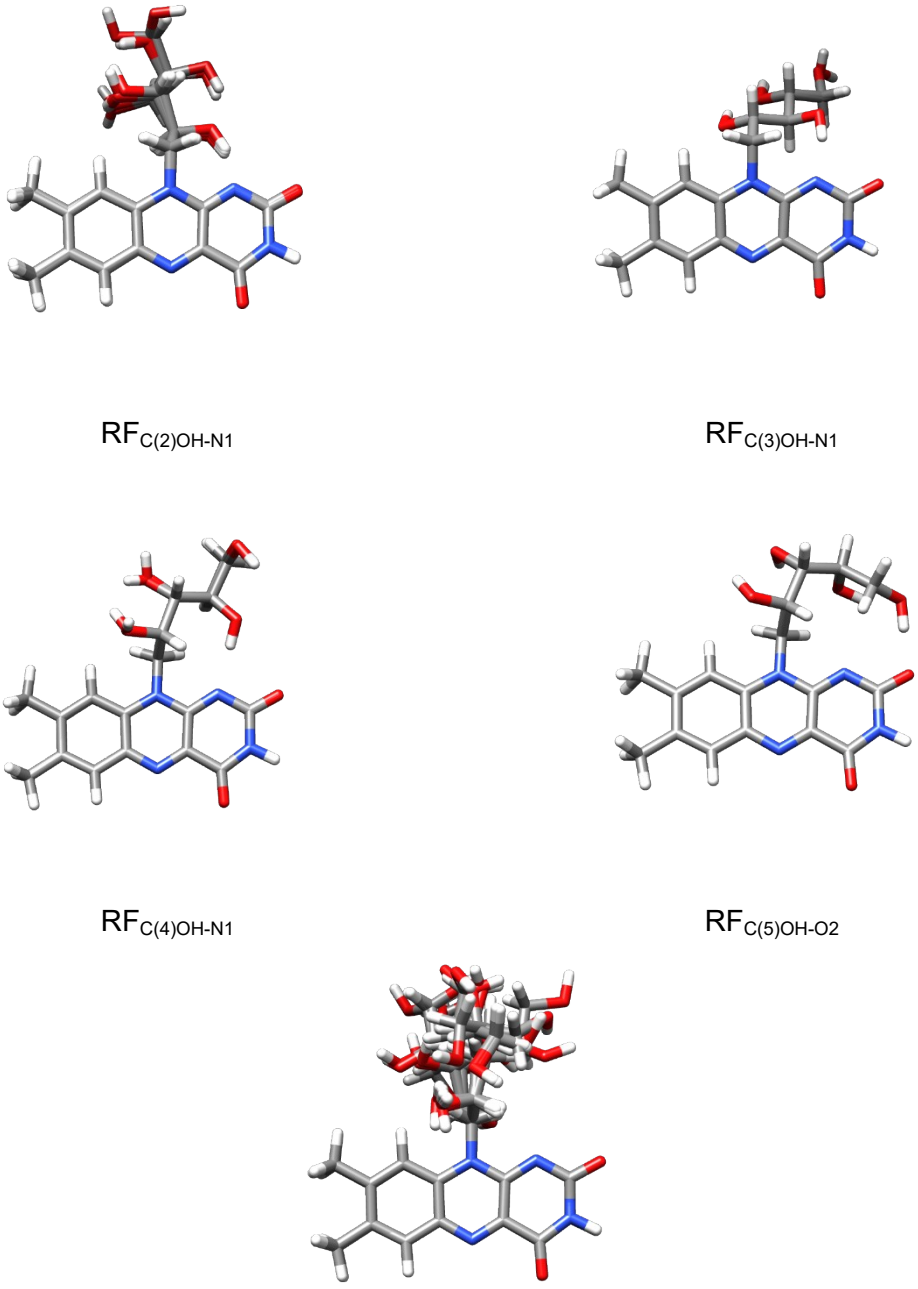


Figure A14. Conformational space calculated by COSMOconf.[63]