Electronic Supplementary Material (ESI) for Inorganic Chemistry Frontiers.

Supporting information

Unexpected In Crystallo Reactivity of the Potential Drug Bis(maltolato)oxidovanadium(IV) with Lysozyme

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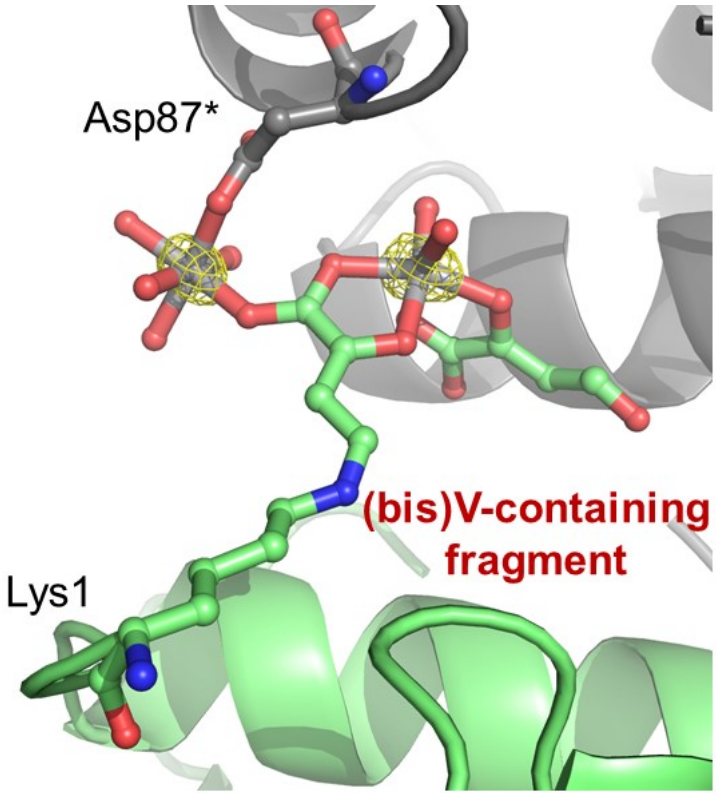

Figure S1 Anomalous difference electron density map (at 3.0 σ level) of the (bis)V-containing fragment. Atoms from a symmetry related molecule are highlighted with the asterisk (*) and coloured in grey.

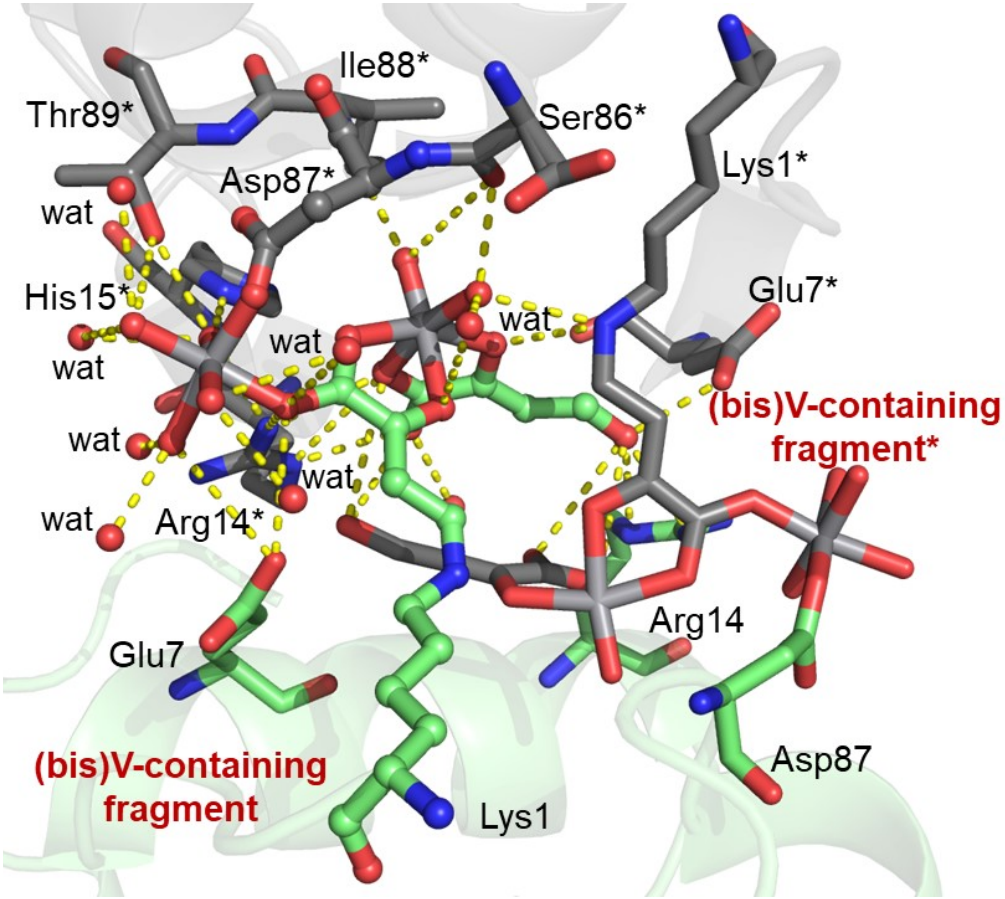

Figure S2 Interaction of the (bis)V-containing fragment bound to the side chain of Lys1. Atoms from a symmetry related molecule are highlighted with the asterisk (*) and coloured in grey. The side chain of Ser86* adopts two difference conformations.

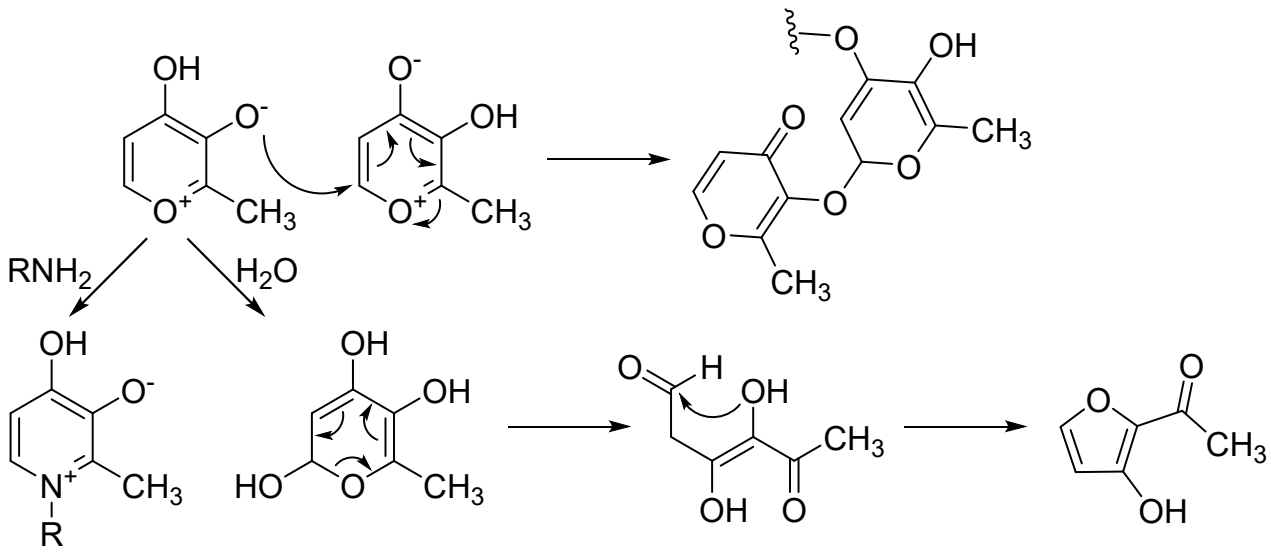

Figure S3 Proposed reactions of pyrylium ions.[1]

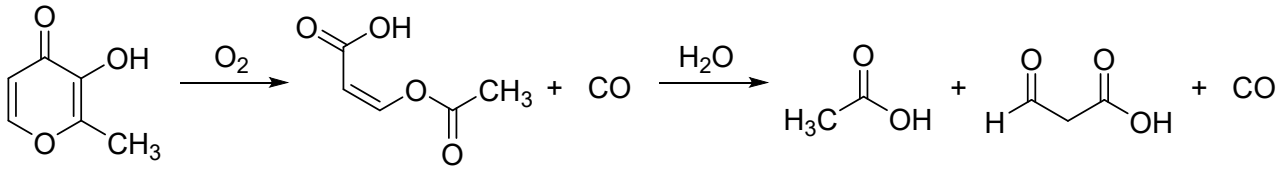

Figure S4 Maltol cleavage by diketone cleaving enzyme via molecular oxygen and subsequent hydrolytic decomposition of the products.[2]

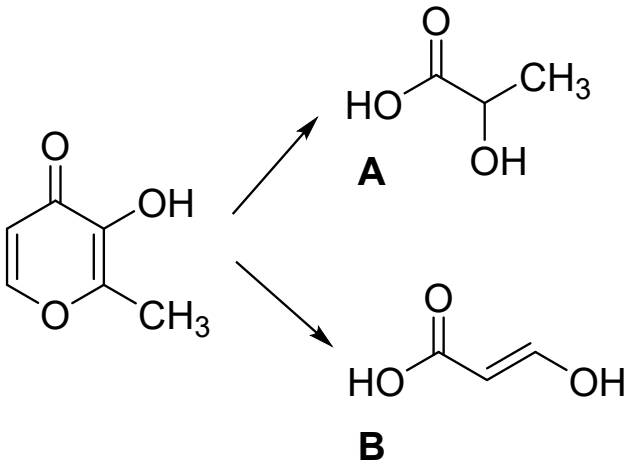

Figure S5 Oxidation products of maltol: A) lactic acid and B) 3-hydroxyacrylic acid.[3]

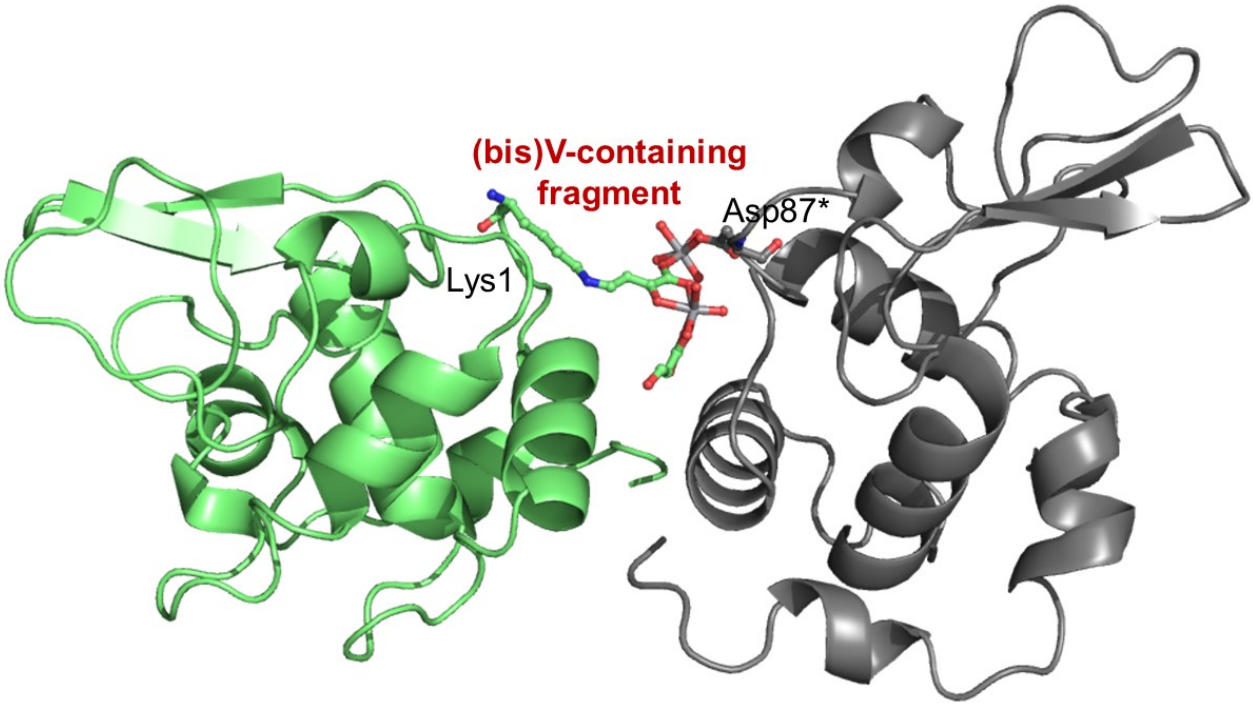

Figure S6 Structure of the cross-linked HEWL dimer formed in the crystals of the protein exposed to BMOV. The symmetry related molecule is coloured in grey. Asp87 from the symmetry-related molecule is highlighted with the asterisk (*).

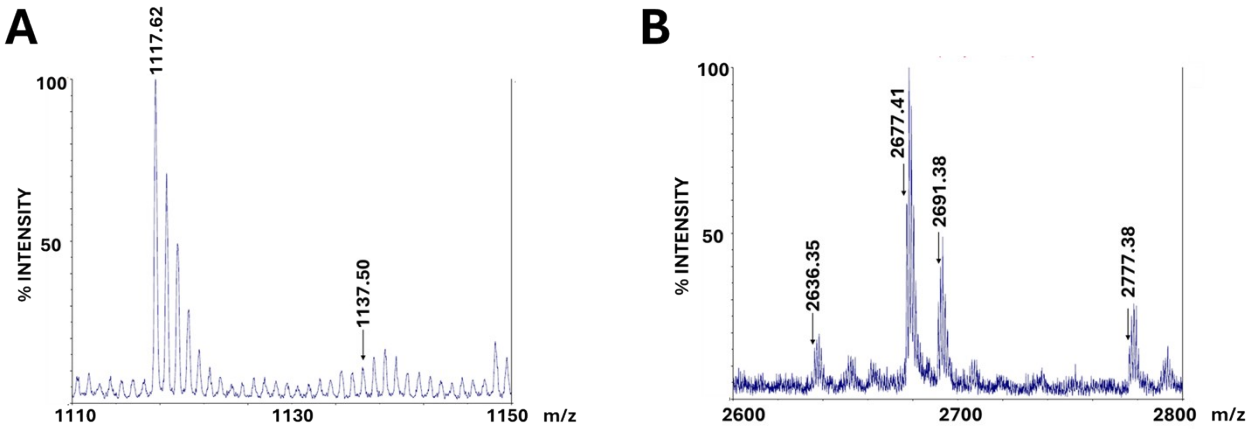

Figure S7 MALDI MS spectra of the peptide mixture obtained from *in situ* hydrolysis of non-metalated HEWL (band 1.1 in Figure 4 of the main text): magnification of 1110-1150 (A) and 2600-2800 (B) m/z ranges.

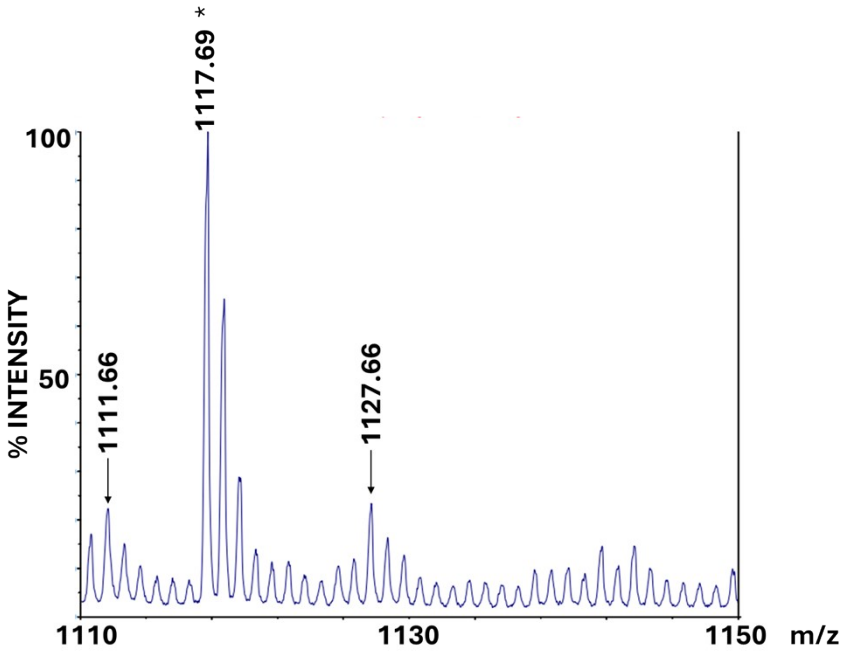

Figure S8 MALDI MS spectra of the peptide mixture obtained from *in situ* hydrolysis of cross-linked HEWL dimer (band 2.1 in Figure 4 of the main text): magnification of 1110-1150 m/z range. The signals present in the reference (not metalated HEWL, band 1.1 in Figure 4 of the main text) are marked with asterisks (*).

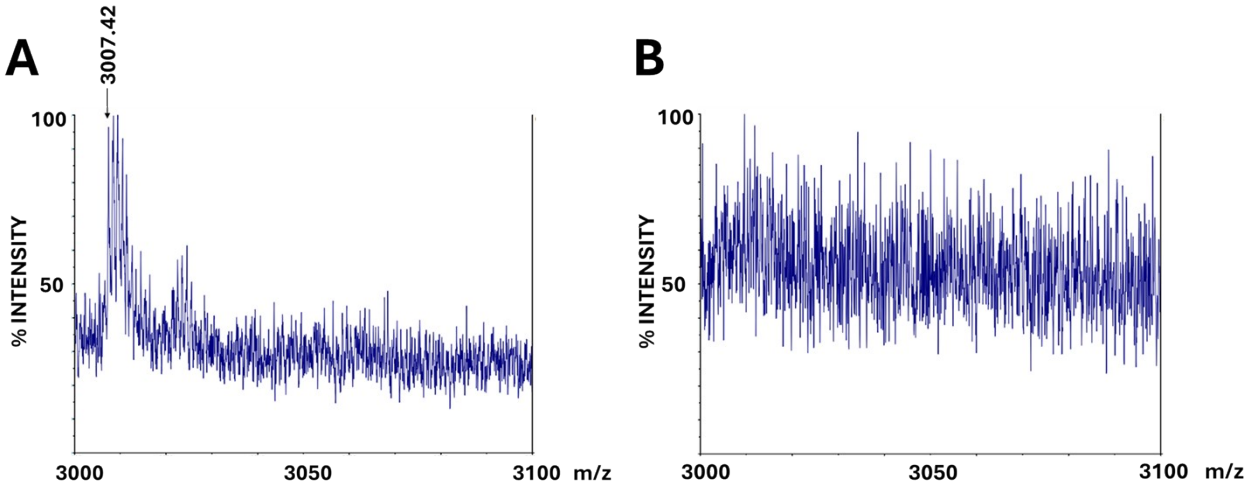

Figure S9 MALDI MS spectra of the peptide mixture obtained from *in situ* hydrolysis of metalated monomeric HEWL (band 2.2 in Figure 4 of the main text) (A) and cross-linked HEWL dimer (band 2.1 in Figure 4 of the main text) (B): magnification of 3000-3100 m/z range.

Table S1 Data collection and refinement statistics.

Data collection	
PDB code	9FMY
Crystallization condition	1.1 M NaCl 0.1M sodium acetate at pH 4.0
Soaking time	21 days
Space group	$P4_3\,2_1\,2$
a (Å)	78.11
b (Å)	78.11
c (Å)	37.21
α/β/γ (°)	90.0/90.0/90.0
Molecules for asymmetric unit	1
Resolution range (Å)	55.23-1.09 (1.11-1.09)
Observations	1112770 (44263)
Unique reflections	48619 (2359)
Completeness (%)	99.9 (99.1)
Redundancy	22.9 (18.8)
Rmerge (%)	0.056 (1.455)
Average I/σ(I)	29.0 (2.2)
$CC_{1/2}$	0.999 (0.822)
Anom. completeness (%)	100.0 (99.5)
Anom. Multiplicity	12.1 (9.7)
Refinement	
Resolution (Å)	55.23-1.09
N° reflections	45192
N° reflections in working set	2841
Rfactor/Rfree	0.129/0.152
N° non-H atoms in the refinement	1272
B-factor overall (Å²)	19.11
Estimated occupancy of V (1)	0.30
Estimated occupancy of V (2)	0.60
Estimated occupancy of $[VO(H_2O)_5]^{2+}$	0.50
Estimated occupancy of (bis)V-containing fragment	0.60
B-factor of V (1) (Å²)	27.47
B-factor of V (2) (Å²)	33.43
B-factor of V $[VO(H_2O)_5]^{2+}$ (Å²)	22.86
B-factor of (bis)V-containing fragment (Å²)	15.4±2.7
Ramachandran values (%)	
Most favoured/Additional allowed	96.52/3.48
Outliers	0.00
R.m.s.d. from ideality	
R.m.s.d. bonds (Å)	1111
R.m.s.d. angles (°)	1515

Table S2 Anomalous difference electron density peaks interpreted as V centers.

Assignment	**Occupancy of the V center in the model**	**Anomalous difference e.d. map peaks σ level**
V (1) of bis(V)-containing fragment	0.60	21.96
V (2) of bis(V)-containing fragment	0.60	16.90
V (1)	0.30	8.31
V (2)	0.60	4.61
V of $[VO(H_2O)_5]^{2+}$	0.50	11.23

Table S3 Specific m/z of signals recorded in MALDI-MS and ESI-LC-MSMS analysis of metalated monomeric HEWL (band 2.2 of SDS-PAGE reported in Figure 4 of the main text) and of cross-linked HEWL dimer (band 2.1 of SDS-PAGE reported in Figure 4 of the main text). Signals are due to peptide mixtures deriving from hydrolysis with Asp-N protease followed by trypsin. Expected Molecular Weight (MW, Da) values are also reported.

Experimental m/z (MALDI-MS) - **Metalated monomeric HEWL**	Experimental m/z (ESI-LCMSMS) - **Metalated monomeric HEWL**	Experimental m/z (MALDI-MS) - **Cross-linked HEWL dimer**	Experimental m/z (ESI-LCMSMS) - **Cross-linked HEWL dimer**	Expected MW (Da)	Interpretation
1111.61	555.76	1111.66	–	1110.39	[62-68] + V + V=O
1127.60	–	1127.66	–	1126.39	[62-68] + 2V=O
2699.31	–	–	–	2699.28	[74-97] K_{96} Ac, (Na^+)
2713.43	–	–	–	2713.28	[74-97] K_{96} Ac (C-propionamide), (Na^+)
2742.37	1371.70, 915.10	–	–	2744.28	[74-97] K_{96} Ac + V=O
2756.36	-	–	–	2575.28	[74-97] K_{96} Ac (C-propionamide) + V=O
2776.35	1389.14, 926.43	–	–	2776.35	[22-45] K_{33} Ac
-	-	4974.19	-	4974.22	[1-17] + [74-96] + (bis)V-containing fragment + VO_3

Notes and references

1 V. A. Yaylayan and S. Mandeville, *J. Agric. Food Chem.*, 1994, **42**, 771–775.

2 C. M. L. Di Giuro, D. Buongiorno, E. Leitner and G. D. Straganz, *J. Inorg. Biochem.*, 2011, **105**, 1204–1211.

3 C. Kanzler, P. T. Haase, H. Schestkowa and L. W. Kroh, *J. Agric. Food Chem.*, 2016, **64**, 7829–7837.