New hybrid imine reductases based on Vancomycin for the asymmetric reduction of cyclic imines in aqueous buffer

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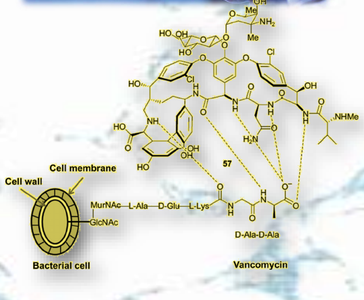
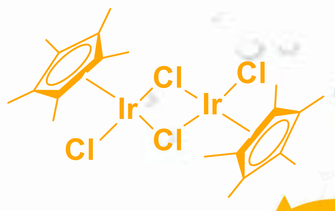
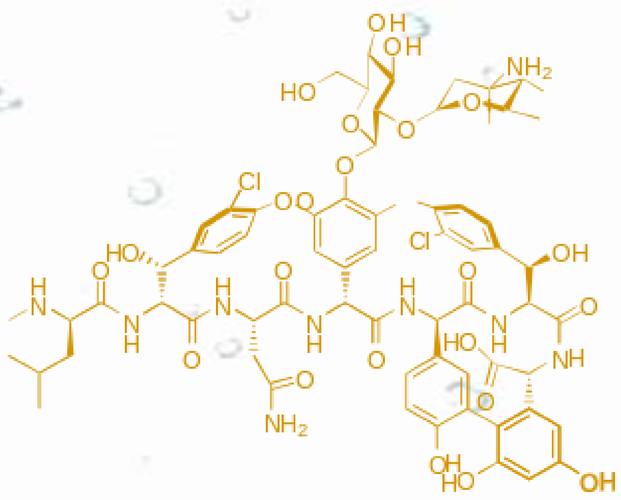
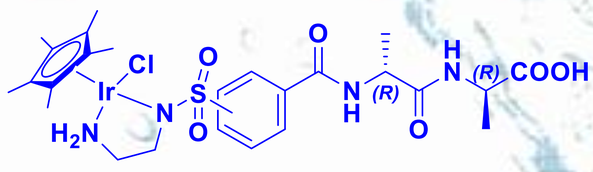
Vancomycin (Van) is a glycopeptide antibiotic active against Gram positive infections. Recently, it was found that several biological effects of Van are also related to its ability to bind both Cu(II) and Zn(II) metal ions under physiological/neutral conditions[1]. Its biological activity is due to a selective binding to D-Ala-D-Ala terminus of peptidoglycan precursor hampering the formation of the bacterial cell wall. Starting from these two different interaction modes of Van, *i.e.* by the direct interaction with $[IrCp^*Cl_2]_2$ or by "trojan-horse" strategy exploiting the D-Ala-D-Ala anchoring system[2] and alternative to the classical biotin/(strept)avidin[3-4], we focused our attention on the possibility to obtain two different hybrid imine reductases, *i.e.* Metallo Peptides (*M-Ps*) and Artificial Metalloenzymes (*Ar-Ms*) to be used in the ATH of cyclic imines[5].





M-Ps

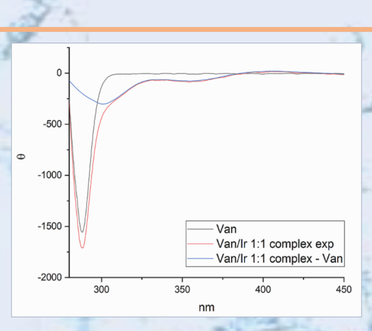




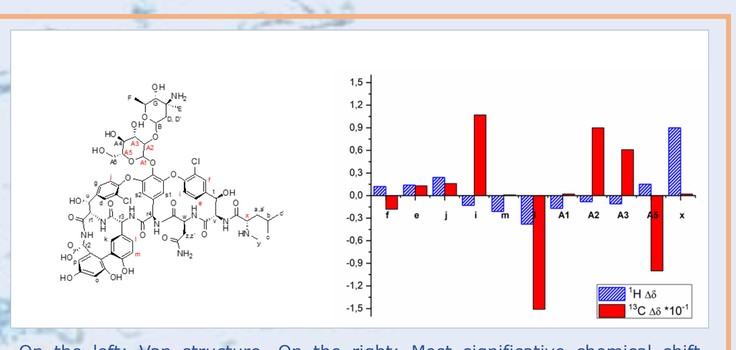
[IrCp*(*m*,*p*-I)Cl]Cl

Ar-Ms

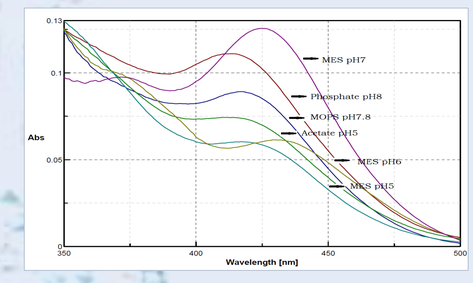
Characterization of the Ir(III)-based hybrid systems:



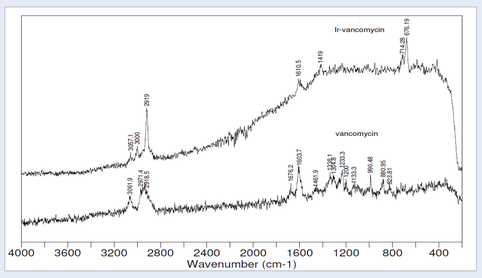
CD spectra of free Van (black line) and Van/Ir 1:1 complex (red line) recorded in acetate buffer (pH 5, 2.5 mM concentration of Ir). The blue line is the obtained spectrum by subtracting Van contribution from 1:1 complex



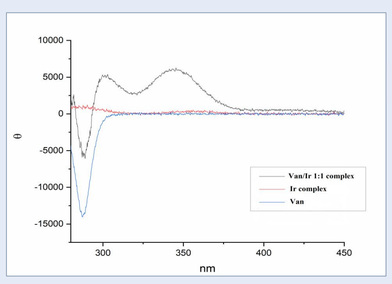
On the left: Van structure. On the right: Most significative chemical shift variations (ppm) of ^{1}H and ^{13}C resonances between free Van and [Ir(Cp*)(Van)Cl] complex in the NMR spectra



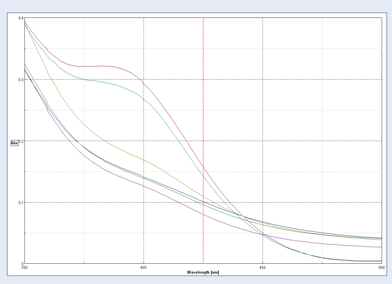
UV spectra of Van/$[IrCp^*Cl_2]_2$ complex (250 μM, 1:0.5 ratio) at different pH values



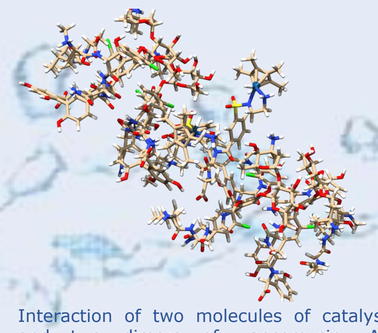
Raman spectra for overlay of vancomycin alone (the band at 1603 cm^{-1}, attributed to carbonyl group; the band at 1338 cm^{-1} attributed to CH_3 bending, the band at 990 cm^{-1} represents breathing of the aromatic ring and the band at 880 cm^{-1} represents the stretching of the C-C bond) and of [Ir(Cp*)(Van)Cl] complex



CD spectra of free Van (blue line), Ir complex bearing the D-Ala-D-Ala anchor (red line) and Van/Ir 1:1 complex (black line) recoded in water (2.5 mM concentration of Ir).



UV-vis of [IrCp*(*m*-I)Cl]Cl (red line), [IrCp*(*p*-I)Cl]Cl (light blue line), [IrCp*(*m*-I)Cl]Cl/Van=1:1 (blue line), [IrCp*(*m*-I)Cl]Cl/Van=1:2 (olive green line), [IrCp*(*p*-I)Cl]Cl/Van=1:1 (purple line) and [IrCp*(*p*-I)Cl]Cl/Van=1:2 (green line) in water (1% DMSO), at the concentration of 250 μM.



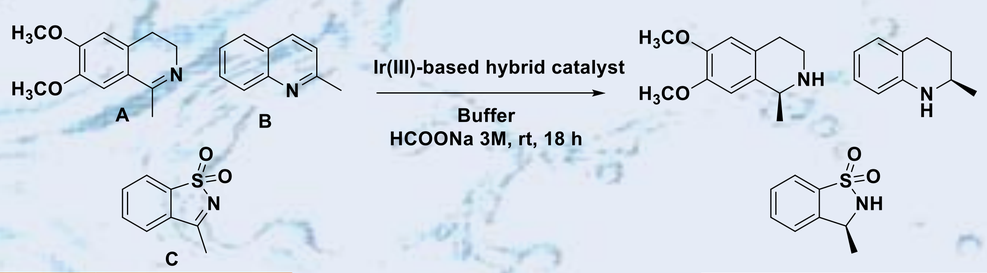
Interaction of two molecules of catalyst and two dimers of vancomycin. As suggested by preliminary QM:MM calculations, the most stable of the four possible configuration is shown.
Optimization with G16: ONIOM B3LYP/(6-31+G*/lanl2dz:UFF) with explicit water molecule

	Diffusion coefficient	Hydrodynamic radius
Van	1,642E-10	1,48859E-09
[IrCp*(m-I)Cl]Cl	3,725E-10	8,26251E-10
[IrCp*(m-I)Cl]Cl⊂Van (1:1)	2,442E-10	1,43534E-09
[IrCp*(m-I)Cl]Cl⊂Van (1:2)	3,126E-10	2,06418E-09

2D DOSY-1H-NMR : [sample]= 33,6 mM in D_2O (1.3% d_6-DMSO), little delta: 5.000m, big delta 149.900m.

Considering the calculated hydrodynamic radius, the presence of aggregates was confirmed for Van alone in water. When [IrCp*(*m*-I)Cl]Cl⊂Van was present, the organization of the structure around the Ir-catalyst was also confirmed.

Application in the asymmetric reduction of cyclic imines:



Buffer	A Conv. % (e.e.%)	B Conv. % (e.e.%)	C Conv. % (e.e.%)
Phosphate 0.1 M pH 8	56 (20, *R*)[a]	30 (36, *R*)	92 (42, *R*)
MOPS 1.2 M pH 7.8	34 (*rac*)	40 (46, *R*)	64 (*rac*)
MES 1.2 M pH 7	82 (4, *S*)	30 (9, R)	60 (4, *R*)
MES 1.2 M pH 6	40 (4, *S*)	67 (12, *R*)	25 (*rac*)
Acetate 0.1 M pH 5	34 (3, *S*)	20 (21, *R*)	30 (*rac*)
MES 1.2 M pH 5	75 (5, *S*)	35 (61, *R*)	20 (30, *S*)

Reaction conditions: substrate concentration 16 mM, 4 mol % Van, 1 mol % $[IrCp^*Cl_2]_2$, buffer, HCOONa 3 M, 18 h and at 25°C. [a] substrate 16 mM, 8 mol % Van, 1 mol % $[IrCp^*Cl_2]_2$, buffer, HCOONa 3 M, 18 h and at 25°C.

The asymmetric reduction performed by the Ir/Van reductase of substrate **A**, proceeded with an appreciable conversion up to 82% although a poor enantioselectivity. The best result in terms of enantioselectivity in the ATH of quinaldine **B** was achieved by performing the reaction in a MES 1.2 M buffer at pH 5 with a significant 61% (*R*) *e.e.*. Interestingly, an inversion of configuration was observed in the ATH of 3-methylbenzo[*d*]isothiazole 1,1-dioxide **C** along with a good 42%(*R*) *e.e.* by changing the buffer and its pH.

Buffer	A Conv. % (e.e.%)	B Conv. % (e.e.%)	C Conv. % (e.e.%)[a]
Phosphate 0.1 M pH 8	16 (*rac*)	64 (34, *S*)	50 (17, *R*)
MOPS 1.2 M pH 7.8	64 (12, *S*)	32 (32, *S*)	52 (12, *R*)
MES 1.2 M pH 7	60 (41, *S*)	85 (51, *S*)	47 (27, *R*)
MES 1.2 M pH 6	96 (31, *S*)	96 (53, *S*)	52 (31, *R*)
Acetate 0.1 M pH 5	>99 (48, *S*)	36 (70, *S*)	48 (34, *R*)

Reaction conditions: substrate concentration 16 mM, 2 mol % Van, 1 mol % $[IrCp^*(m\text{-}I)Cl]Cl$, buffer, HCOONa 3 M, 18 h and at 25°C. [a] substrate 16 mM, 4 mol % Van, 1 mol % $[IrCp^*(m\text{-}I)Cl]Cl$, buffer, HCOONa 3 M, 18 h and at 25°C.

In this context, aminoethylbenzensulfonamide ligands (I) functionalized with the D-Ala-D-Ala dimer at different positions of the phenyl ring were employed for the synthesis of the hybrid catalysts. While the [IrCp*(*p*-I)Cl]Cl⊂Van resulted inactive, by using the [IrCp*(*m*-I)Cl]Cl⊂Van , acetate buffer 0.1 M at pH 5 resulted as the best reaction medium: an encouraging 48% (*S*) e.e. was obtained with the salsolidine precursor **A** while a 70% (*S*) e.e. was obtained in the reduction of quinaldine **B**. For substrate **C** MES buffer 1.2 M afforded the product in a modest 34% (*R*) e.e..

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New Trends in Organic Synthesis

November 25th 2019, Milan