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Multi-metal and multi-cavity systems based on the coordination properties of terpyridine functionalized cyclodextrin polymers were synthesized and characterized. Nanoparticles decorated with terpyridine derivatives *via* metal coordination showed high anti-proliferative activity in tumor cells.

Cyclodextrins (CDs) are cyclic oligosaccharides of $\alpha(1 \rightarrow 4)$ glucose, characterized by their toroidal shape.^{1,2} CDs and their conjugates with different molecules provide desirable systems for building multifunctional systems devoted to supramolecular, bioinorganic, organic, cosmetic, pharmaceutical and materials chemistry applications.³⁻⁹

Moreover, the functionalization of CDs is a powerful strategy to obtain CD metal complexes.¹⁰⁻¹² The CD cavity can confer water solubility to the metal complex, and the cavity can cooperate with the metal center to bind non-coordinating substrates or selectively recognize coordinating substrates.¹³

More recently, the interest in CDs has been extended to the production of nanoparticles (NP).¹⁴ A variety of NPs based on CDs has been synthesized, ranging from supramolecular NPs,¹⁵⁻¹⁷ to hybrid NPs (Au, Ag, ferrite, silica).^{18,19} Some NPs based on CD polymers have also been synthesized and chemically modified for improving their properties.²⁰⁻²⁴

Some metal ligands have also been covalently linked to CD-based NPs.²⁵⁻²⁸

Terpyridine (tpy) is among the N-heterocycles with a very high binding affinity towards transition metal ions and this feature has been widely used to build supramolecular systems, mainly soluble in organic solvents.²⁹⁻³³ In the last years, the

anticancer activity of tpy and its coordination compounds has also been reported.³⁴⁻³⁹

Herein, we report a new multi-cavity and multi-metal system based on the coordination of tpy functionalized CD polymers. Particularly we functionalized a cross-linked β -CD polymer with tpy moieties to exploit the coordination ability of tpy in an aqueous solution. The polysaccharide system confers water solubility to the tpy-based system and may extend the application of tpy chemistry. We exploited the coordination chemistry to decorate the CD polymer with biotin by coordinating a tpy-biotin (tpybio) conjugate (Fig. 1). Biotin has widely been used as a targeting unit.^{40,41} Exogenous biotin is taken up *via* a high-affinity biotin transporter and sodium-dependent multivitamin transporter (SMVT) overexpressed in specific cancer cells.³⁸ We compared the systems decorated with biotin with the analogous systems without biotin moieties.

The iron and copper ternary complexes of pCDtpy were tested as antiproliferative agents compared to the binary copper and iron complexes of tpyNH₂ and tpybio.

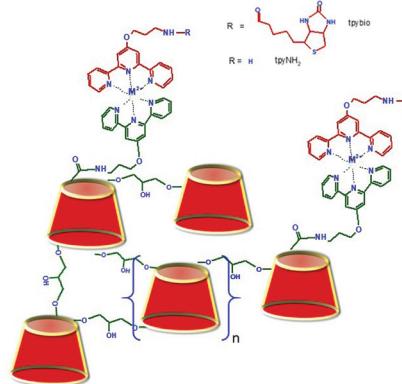


Fig. 1 Schematic structure of pCDtpy and M^{II} ternary complex with the tpy derivatives (tpybio or tpyNH₂).

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[†] Electronic supplementary information (ESI) available: Synthesis procedures, UV Vis spectra, MS spectra. See DOI: 10.1039/d2dt00613h



We synthesized 3-([2,2':6',2"-terpyridin]-4'-yloxy)propan-1-amine (tpyNH₂) for the functionalization of the cross-linked CD polymer carboxylate (pCDCOOH) (Fig. S1†).

The NMR spectra of pCDtpy confirmed the functionalization of pCDCOOH with tpyNH₂. ¹H NMR spectrum shows broad signals as typically found for the CD polymers. From integral ratios of the signal of Hs-1 of CD and the aromatic protons, we determined an average of about 21 tpy units functionalizing pCDtpy.

The metal complexes of tpybio and tpyNH₂ with Cu²⁺ and Fe²⁺ were investigated using High-Resolution Mass Spectrometry (HRMS). Fe²⁺ forms a unique complex species [Fe(tpyBio)₂]²⁺ whereas both [Cu(tpyBio)₂]²⁺ and other complex species were detected (Table S1, Fig. S3 and S4†). The same trend has been observed in the case of tpyNH₂ ligand. Also in this case, [Fe(tpyNH₂)₂]²⁺ was identified whereas peaks due to both [Cu(tpyNH₂)₂]²⁺ and [Cu(tpyNH₂)Cl]⁺ were found.

The UV-vis spectra of all tpy derivatives and their Fe²⁺ and Cu²⁺ complexes were carried out (Fig. 3 and S5†). The spectra of the ligands showed bands at 241 and 280 nm with a shoulder at 310 nm due to π - π^* transitions, as reported for tpy.³² The UV-Vis spectra of complexes showed a band at 313 nm due to the conformational change from the *trans/trans* to *cis/cis* conformation of tpy in the metal complexes. Spectra of Fe²⁺ complexes showed a characteristic band due to the metal-ligand charge transfer (MLCT) band at about 560 nm.

To study the complexation properties of pCDtpy we carried out the titrations of pCDtpy with M²⁺ salt solutions (M = Fe, Cu). The UV-Vis spectra of pCDtpy are reported in Fig. 3. The UV-Vis spectra upon the stepwise addition of Fe²⁺ (Fig. 3) showed a change at 313 nm due to the conformational change of tpy ring and the characteristic band due to the metal-ligand charge transfer (MLCT) band at 558 nm. The color of the aqueous solution turned to be violet with Fe²⁺.

The spectra changed up to a ratio Fe/pCDtpy of 11. The spectral changes occurred at metal/ligand (tpy units) ratios below 0.5, indicating two tpy moieties can bind Fe²⁺ in a ML₂-like coordination in a similar way to free tpy ligand. A similar

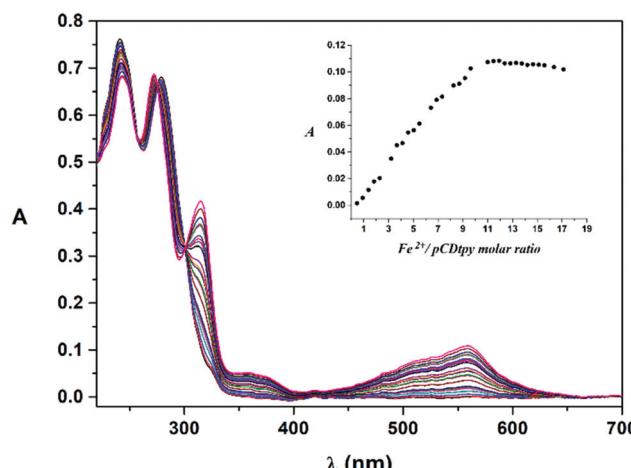


Fig. 3 UV-Vis spectra of the titration of pCDtpy with Fe²⁺ solution in phosphate buffer pH = 7.4. Inset: Absorbance (A) at 558 nm.

trend was found when Cu²⁺ were added to the pCDtpy solution (pH = 7.4). Similar coordinative polymers have been reported elsewhere.⁴²

Ternary complexes were also studied by titrating a mixture of pCDtpy and the tpy ligand (tpybio or tpyNH₂) 1:20 molar ratio with Fe²⁺ or Cu²⁺ solution. Data are reported in Fig. S6 and S7.† Spectral changes occurred at metal/pCDtpy/tpy below 20:1:20 molar ratio (metal/tpy unit ratio below 0.5), indicating the formation of ternary complexes in ML₂-like coordination as a sketch in Fig. 1.

The ¹H NMR spectra of Fe²⁺ complexes of pCDtpy were also acquired (Fig. 2 and S8†). Signals were very broad, as in the case of the ligand pCDtpy. Upon complexation, shifts of the signals due to the tpy protons can be observed in the spectra. The H-6 and H-6" were upfield shifted at about 7 ppm and H-3' and H-5' downfield shifted in keeping with the pyridine rings flipping from an antiperiplanar into a synperiplanar conformation as reported for other tpy derivatives. As for ternary systems, spectra were carried out at Fe/pCDtpy/tpy 10:1:20 and 20:1:20 molar ratios. Spectra showed at 10/1/20 molar ratio the signals of metal complexes and tpy ligand, while at 20:1:20 molar ratio the signals of the main species are evident in the aromatic region (Fig. S6†).

Hydrodynamic volume of pCDtpy was measured by DLS (Table S2†). Data showed that pCDtpy forms NPs with the Z average of about 67 nm. In the presence of the metal ion (M/pCDtpy 20:1), the Z average value increased, and this trend suggested the aggregation of NPs assisted by the metal coordination as reported for other polymeric ligands.^{25,43}

When tpyNH₂ or tpybio was added to pCDtpy-Fe²⁺ or pCDtpy-Cu²⁺, Z average value decreased, suggesting the formation of the ternary complex pCDtpy-M²⁺-tpybio (or pCDtpy-M²⁺-tpyNH₂). Tpy can form complex with M²⁺ and disfavors the intermolecular complexation of other tpy units in the polymer. Ternary complexes with tpyNH₂ showed higher Z-average values.

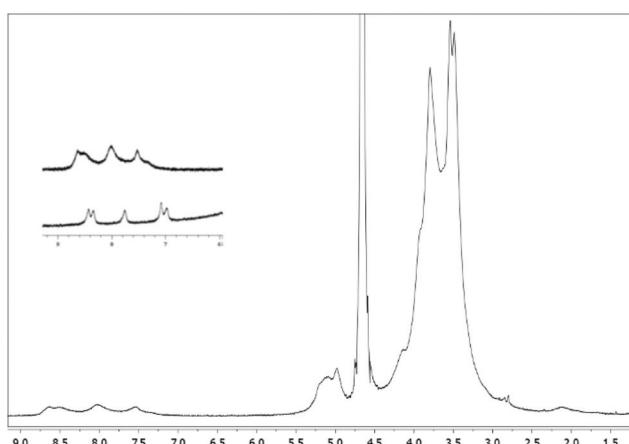


Fig. 2 ¹H NMR spectra (500 MHz, D₂O) of pCDtpy. Inset: Aromatic region of pCDtpy (up) and pCDtpy-Fe (bottom).



Table 1 IC₅₀ values (nM) of tpy, tpybio, pCDtpy and their Cu^{II} and Fe^{II} complexes (Dox is doxorubicin)

	A2780	A549	MDA-MB-453	SkHep1
tpyNH ₂	513 ± 75	178 ± 32	723 ± 217	501 ± 218
tpybio	508.4 ± 14.8	88.5 ± 10.0	858.5 ± 187.4	568.8 ± 63.4
Fe-tpyNH ₂	1211 ± 272	501.1 ± 56.5	3951 ± 1144	1858 ± 184
Cu-tpyNH ₂	292.9 ± 53.6	413.9 ± 140.0	5621 ± 481	1417 ± 438
Fe-tpybio	241.1 ± 29.9	117.9 ± 34.0	952.8 ± 85.4	506.1 ± 116.2
Cu-tpybio	484.1 ± 44.8	194.9 ± 33.5	903.5 ± 19.9	627.7 ± 33.8
CDtpy	684.8 ± 205.8	540.5 ± 70.2	1062 ± 122	338 ± 55
pCDtpy-Fe	12 072 ± 2948	≥30 000	≥30 000	≥30 000
pCDtpy-Cu	2869 ± 638	≥30 000	2739 ± 922	≥30 000
pCDtpy-Fe-tpyNH ₂	38.1 ± 6.1	51.4 ± 19.3	30.9.0 ± 11.3	9.9 ± 2.1
pCDtpy-Cu-tpyNH ₂	26.3 ± 14.2	24.9 ± 15.2	1.2 ± 0.5	29.4 ± 16.9
pCDtpy-Fe-tpybio	8.02 ± 3.27	8.23 ± 3.42	2308 ± 1172	141.4 ± 34.9
pCDtpy-Cu-tpybio	9.25 ± 4.09	4.67 ± 1.25	100.2 ± 15.6	60.1 ± 7.2
Dox	7.7 ± 3.6	58.4 ± 13.8	178 ± 26	51 ± 7

The antiproliferative activity of pCDtpy and its metal complexes were studied in A2780, A549, MDA-MB-453, and SkHep1 cancer cells. Cell proliferation assay was performed for pCDtpy, tpyNH₂ and tpybio and their Cu²⁺ and Fe²⁺ complexes (Table 1). Doxorubicin (Dox) is also reported in Table 1 for a comparison. The ligand tpyNH₂ and tpybio showed a significant activity with IC₅₀s always lower than 1 μM.

The effect of the biotin moiety is evident only in A549 cells with a 50% reduction of the IC₅₀ (178.4 ± 31.9 nM vs. 88.5 ± 10.0 nM, *p* < 0.0001). As for Fe-tpyNH₂ or Cu-tpyNH₂ complexes, the antiproliferative activity decreased in comparison to the free ligands in all cell lines except in the case of Cu-tpyNH₂ in A2780 cells (292.9 ± 53.6 nM vs. 513.7 ± 74.8 nM, *p* < 0.0001). Generally, copper complexes are more active than iron complexes. Interestingly, in the case of tpybio metal complexes, the antiproliferative activities were significantly higher than the analogous complexes of tpyNH₂, except for Cu-tpybio vs. Cu-tpyNH₂ in A2780 cells (484.1 ± 44.8 vs. 292.9 ± 53.6 nM, *p* < 0.0001; Table 1).

When the polymer pCDtpy was assayed, its antiproliferative activity was generally lower than that of the free tpyNH₂ (not significant only in SkHep1 cells, Table 1). The Fe or Cu binary complexes of pCDtpy showed a much lower antiproliferative activity in comparison to pCDtpy or tpy alone.

It is noteworthy that pCDtpy-M-tpyNH₂ and pCDtpy-M-tpybio systems showed significantly higher antiproliferative activity than the tpybio or tpyNH₂ complexes in cell lines tested. More interestingly, in A549 cells, both the antiproliferative activity of pCDtpy-Fe-tpybio and pCDtpy-Cu-tpybio ternary complexes was higher than Dox, an anticancer drug highly active in these cell lines. Moreover, pCDtpy-Fe²⁺-tpyNH₂ and pCDtpy-Cu²⁺-tpyNH₂ antiproliferative activity was significantly higher than that of the analogous pCDtpy-M-tpybio systems in SkHep1 and especially in MDA-MB-453 cells.

Further experiments may clarify these data; nevertheless, as widely reported, the different selectivity of the cell lines could correlate to the number of biotin transporters present on the membrane cell lines³⁸ and/or to other properties of biotin free NPs, such as the presence of amino groups charged at physiological pH.

To conclude, we functionalized a cross-linked beta-cyclodextrin polymer to exploit the coordination chemistry for their modification. We found that the Cu^{II} and Fe^{II} ternary complexes of pCDtpy with the tpy derivatives show antiproliferative activity (nM range) like the well-known anticancer agent Dox, with dependence on the cell line. The presence of biotin makes the CD nanoparticles much more active than those without the biotin moiety in A2780 and A549 cells. Next *in vivo* experiments may highlight the potential of NPs for the Enhanced Permeability Retention (EPR) effect of tumor tissues.

Furthermore, the approach may be extended to other tpy derivatives by modulating the properties of the final system. Water-soluble tpy based nanoparticles can also extend the application of tpy derivatives in water.

Conflicts of interest

There are no conflicts to declare.

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