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Synthesis and *in vitro* toxicity of D-glucose and D-fructose conjugated curcumin ruthenium complexes

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Abstract: A series of carbohydrate conjugated bisdemethoxycurcumin (BDC) ligands were synthesized by using the Huisgen copper(I) catalyzed cycloaddition between azide-functionalized D-glucose and D-fructose as well as propargyl-modified BDC. The unprotected sugar ligands were reacted with $Ru(bpy)_2Cl_2$ to form curcumin conjugated Ru-complexes of the general formula $Ru(bpy)_2(L)Cl$. Ligands as well as Ru complexes were analyzed by NMR, IR, UV/Vis and fluorescence spectroscopy, mass spectrometry as well as elemental analysis (EA). Incubation of L929, HepG2 and the breast cancer cell line MDA-MB-231 revealed lower cytotoxicity of all carbohydrate conjugated ligands compared to BDC. The Ru-complexes exhibited higher cytotoxicity as the parent ligands in particular against HepG2 cells, whereas the non-cancerous L929 cell line remained unaffected. Unlike expected, the D-fructose conjugated ligand and its corresponding Ru complex did not show any significant toxicity against MDA-MB-231 cells.

Introduction

In the last decades, medicinal inorganic chemistry has attracted increased attention in desease therapy (e.g. cisplatin for cancer treatment) as well as in desease diagnosis (e.g. ^{99m}Tc in SPECT).[1] Next to platinum-based drugs, also other transition metal compounds offer advantageous properties. Ru-based complexes could overcome resistance problems often linked to platinum containing drugs or could diminish side effects.[2] Further beneficial properties, such as the easy accession to oxidation states +II and +III and the resulting possibility to obtain low reactive prodrugs or the relieved carrying into tumor cells by transferrin, led to early clinical trials of ruthenium based anticancer agents like NAMI-A and promising Ru(III) prodrugs like RAPTA-T.[2] However, to improve the therapeutic index selectivity plays a major role. The active approach aims to target specific cell structures which vary in comparison to healthy cells, e.g. transport proteins, antigens or receptors on the membrane surface and which interact with the potential drug. This is often realized by conjugation of the metal complex to targeting moieties.[3]

Carbohydrates as one of the major energy source and substrates of lipid and protein metabolism are taken up into cells via highly selective transport proteins.[4] Beside transporters for glucose (the major carbohydrate) there are carriers, like GLUT5 for fructose. GLUT5 is one of thirteen members of the known saccharide transporters (GLUTs) and its structure could be determined recently.[5] It is found in the membrane in small intestine and kidney cells, but is also discussed to be overexpressed in 85% of 33 tested breast cancer cell lines,[6] whereas another group reported contradictory results.[7] The latter study concludes that there is no expression of GLUT5 in breast cancer tissue. However, it was shown, that structural modifications of D-fructose at C1 and C6 position seem to be tolerated by the GLUT5 transporter.[8] Based on that, dyes,[9] polymers[10] and nanoparticles[11] were functionalized with D-fructose to successfully target breast cancer cells. Another approach to study the possibility of GLUT5 targeting is the determination of cell internalization of metal complexes modified with fructose residues.[12] For instance, a fructose conjugated Ir(III) complex revealed a 3.6 times higher uptake into MCF-7 cells compared to non-cancerous HEK293T cells, whereas the corresponding non-functionalized Ir(III) complex did not show any significant differences in terms of cell specific uptake.[12a] Another study exhibited the enhanced accumulation of a fructose conjugated Re-complex in breast cancer cells MDA-MB-231 and MCF-7 compared to all other studied cancerous and non-cancerous cell lines. Uptake competition experiments with D-fructose indicated the involvement of the GLUT5 transporter.[12b] Additionally, the hydrophilicity of sugar moieties reduces the toxicity and increases the solubility in water and therewith in the plasma.[13] This offers the possibility to overcome disadvantageous properties of potentially biological active compounds and to enhance the selectivity at the same time.

Besides a large number of other polyphenols, the diarylhepanoid curcumin is one highly bioactive compound that is contained in the roots of the turmeric. It interacts with a large number of molecular targets linked to major diseases of modern societies.[14] It is reported to possess beneficial properties, like antimicrobial, antiinflammatory and chemopreventive effects and low toxicity up to high dosages.[15] Unfortunately, medical applications of curcumin and its derivatives are limited by some major drawbacks, like rapid metabolism and poor solubility in water. In the last decades many strategies were tested to overcome those disadvantages, *e.g.* piperine as concomitant, nanoparticle based systems or micellar formulations.[16] Currently, metal complexes of curcumin and its derivatives are in focus of the scientific community.[17] Curcumin conjugated metal complexes revealed superior properties such as enhanced solubility in water,[18] higher photocytotoxicity[19] or increased cytotoxicity by intercalation.[20] In particular, a RAPTA-type complex of curcumin exhibited outstanding properties like an around 100 times smaller IC_{50} value compared to cisplatin against cisplatin resistant ovarian cancer cells.[18] Furthermore, the attachment of glucose to the curcumin skeleton in an

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oxovanadium complex enhances the solubility in water and the cellular uptake in cancer cells.[21]

A powerful tool to append biomolecules to different structures is the "click" chemistry.[22] It combines a few types of reactions with several advantages including high yields, stereospecificity, easily available starting materials and gentle product isolation.[23] In particular, the Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition (CuAAC) between terminal alkines and azides is used for the synthesis of five-membered heterocyclic systems.[24] Herein, we describe the synthesis, characterization and evaluation of cytotoxicity of two sugar conjugated curcumin ligands and their corresponding $Ru(bpy)_2$-complexes.

Results and Discussion

Synthesis and characterization

The synthesis of altered curcuminoids by using different aldehydes in a double aldol condensation with acetylacetone is well-known and offers the possibility to introduce functionalities to the curcuminoid skeleton (Fig. 1).[25] Compound **1** was obtained using a modified literature procedure: BF_3 etherate promoted the one-pot synthesis with acetylacetonate and 4-(propargyloxy)-benzaldehyde in toluene. Subsequent recrystallization resulted in the desired product in high yield and purity. Compound **1** was characterized by ^1H, ^{13}C and ^{19}F NMR and HR-ESI-MS as well as by elemental analysis to confirm the purity of the compound. The IR spectrum reveals a sharp signal at $\tilde{\nu}$ = 3290 cm^{-1} resulting from the monosubstituted alkine functionality (see Supporting Information, Figure S5). The azide functionalized sugar moieties were synthesized according to literature reports.[25] Bisdemethoxycurcumin (BDC, **2**) was synthesized in high yields like previously reported.[26] Ligands **3a** and **3b** were prepared by the "Huisgen" 1,3-dipolar cycloaddition between compound **1** and two equivalents of the azido-sugars with $CuSO_4$ and sodium ascorbate as catalyst forming pair. The yields of both reactions were relatively low (42% for **3a** and 52% for **3b** respectively). Thin layer chromatography (TLC) revealed byproducts: the copper complex of the ligands, the one site clicked product and the corresponding copper complex of it. Copper could not be removed completely by extraction with EDTA, most probably because of the stability of copper curcumin complexes.[27] Flash column chromatography led to the pure ligands. The ^1H NMR spectra of the ligands clearly reveal the disappearance of the ethinyl singlet of the starting material at δ = 3.63 ppm and the appearance of a signal for the triazole protons at δ = 8.11 ppm for **3a** and δ = 8.20 ppm for **3b** (see Supporting Information, Figures S8 and S14). Furthermore, the cleavage of the BF_2-group was confirmed by the disappearance of the peak at δ = -138.14 ppm in ^{19}F NMR and the strong shift to lower fields of all curcuminoid skeleton related peaks in ^1H NMR spectra. ESI-MS identified the ligands as $[M+X]^+$ (X = H, Na, K). To form ligand **4a** the acetyl groups of compound **3a** were cleaved under basic conditions by using sodium methanolate in dry methanol under argon. The reaction mixture was neutralized with ion exchange resin DOWEX (H^+) and dialyzed in water for one week to remove low molar mass impurities. The product was received in good yield without any side products. The structure of the ligand was established by HR-ESI-MS as $[M+Na]^+$ (error: 1.4 ppm) and elemental analysis. The disappearance of the carbonyl band of the acetyl groups in the IR spectrum as well as of the four singlets (δ = 1.89 to 2.02 ppm) in ^1H- and of the eight signals (δ = 168.97 to 170 ppm, δ = 20.21 to 20.49 ppm) in the ^{13}C NMR confirmed the success of the reaction (see Supporting Information, Figures S20-23). The signals in the NMR spectra are still sharp and well separated. Due to the glycosidic linkage the glucose units are still present in the pyranoide structure and, therefore, no stereoisomers are observable. The cleavage of the isopropylidene groups of compound **3b** was problematic. Neither standard acidic cleavage procedures[28] nor acidic ion exchange resins could be successfully applied.[29] Due to the high sensitivity of the curcuminoid skeleton towards acids (as well as bases and light)[31] the formic acid was chosen to replace the isopropylidene groups under relatively mild conditions.[30] The solution was stirred at room temperature for one week and the progress was monitored by ESI-MS. The absence of all m/z- peaks belonging to the ligands substituted with isopropylidene groups indicated the full conversion. After removing the excess of formic acid *in vacuo* the resulting formic acid esters were cleaved under basic conditions with aqueous 0.1 M NaOH. The crude product was dialyzed to remove the formed sodium formiate. Afterwards, it was analyzed by HR-ESI-MS as $[M+Na]^+$ (error: 4.7 ppm) and

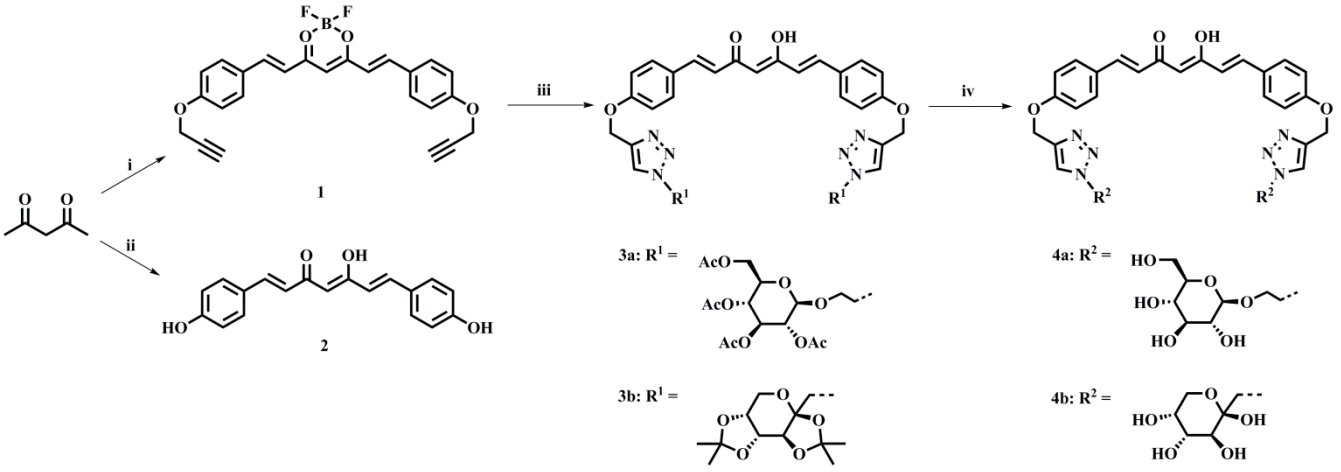

Figure 1. Schematic representation of the ligand synthesis. i) BF_3xEt_2O, *p*-propargyloxy-benzaldehyde, tributyl borate, *n*-butyl amine; N_2, 65 °C, toluol, 18 h, ii) 1. BF_3xEt_2O, *p*-hydroxy-benzaldehyde, tributyl borate, *n*-butyl amine; N_2, 65 °C, toluol, 6 h 2. NaOH, 70 °C, CH_3OH / H_2O, 5 h, iii) azido-sugar, $Cu(II)SO_4$ x 5 H_2O, sodium ascorbate, Ar, 50 °C, THF / H_2O,12 h, iv) 3a → 3b: 1. NaOMe, Ar, rt, MeOH, 0.5 h, 2. DOWEX H^+, 3b → 4b: 1. Formic acid, rt, 1 week, 2. 0.1 M NaOH, rt, THF / H_2O, 1 h.

elemental analysis to confirm the absence of any salts, *e.g.* sodium formiate or NaCl. ^{1}H as well as ^{13}C NMR spectra exhibit the disappearance of the isopropylidene peaks (see Supporting Information, Figures S26-28). The NMR spectra clearly reveal the presence of different ligand species due to the fructose isomers. The interaction between the hydroxyl groups of the sugar units and the curcuminoid enol moieties or sugar hydroxyl groups of neighbored molecules could stabilize different forms of the sugar resulting in wider peaks and a more complex NMR. When measured in deuterated DMSO, broad peaks between δ = 4.5 ppm and δ = 6.5 ppm in ^{1}H NMR appear only coupling with the sugar ring proton signals in 2D-COSY experiments. These signals could be attributed to fructose hydroxyl groups formed during deprotection. Furthermore, there are at least three peaks for each carbon of the fructose residues with different intensities in the ^{13}C NMR of compound **4b**, accentuating the existence of stereoisomers. However, the ESI-MS revealed only two major peaks ([M+Na]$^{+}$ and [M-H+2Na]$^{+}$), what proves the identity of the ligand. Complexes **5a-c** were synthesized in methanol (**5a**) or a mixture of dry methanol and DMF (**5b** and **5c**) with sodium methanolate as a base (Fig. 2). The mixtures were heated under argon at 60 °C for 12 h. An excess of $Ru(bpy)_2Cl_2$ was used to completely react the ligands. For **5a** and **5b** the reaction mixture was evaporated and the remaining excess of precursor was filtered off after re-dissolving the crude product in pure water. The still contained salts were removed from aqueous layer by dialysis against water. HR-ESI-MS showed the pure complex **5a** without signals of precursor or ligand as [M-Cl]$^{+}$ (error: 0.5 ppm) and [M-Cl+Na]$^{2+}$. The ^{1}H NMR shows eight additional peaks in the aromatic region fitting to the bipyridine (bpy) units of the product with a shift to lower ppm values in comparison to the ^{1}H NMR of compound **4a** (see Supporting Information, Figures S32-34). The structure of compound **5b** was challenging to validate due to the instability during mass spectrometric measurements. HR-ESI-MS under soft conditions with an orbitrap mass analyzer combined with LC-MS experiments showed next to product peak [M-Cl]$^{+}$ (error: 1.8 ppm) different fragments *e.g.* without fructose unit(s) or without triazole unit(s). MS/MS experiments of the product ion identified the fragments as a result of cleavage of the parent ion under the applied conditions (see Supporting Information, Figures S41-S42). ^{1}H NMR shows an even higher complexity compared to the ligand. Signals in the aromatic region appear, which can be clearly distinguished from $Ru(bpy)_2Cl_2$ precursor peaks. Furthermore, 2D-COSY and HSQC NMR measurements prove the attachment of fructose. Figure 3 shows the ^{1}H-^{13}C-HSQC experiments of **4b** in comparison to **5b**. The area between 3 and 4 ppm in ^{1}H NMR respectively between 50 and 100 ppm in ^{13}C NMR shows the occurrence of proton and carbon peaks of various forms of fructose units in the ligand as well as in the complex. The observed pattern differs which suggests the presence of different ratios of fructose isomers. It is known, that in aqueous solution of D-fructose at pH = 7 various forms exist and that the percentage of each form is strongly dependent on temperature, salts and other conditions.[31] It was also shown, that certain isomers can be stabilized, e.g., the presence of human serum albumin (HSA) resulted in the stabilization of the open-chained D-fructose by the NH_2 functionalities of the Lys199 residue.[32] In contrast, in a D-fructose decorated glycopolymer, the pyranose form dominates, but also furanose forms were observable.[10]

The synthesis of **5c** was performed as mentioned before, but due to high polarity and low solubility in water of compound **5c** it was neither suitable for normal phase silica chromatography nor convenient for dialysis with water. Therefore, the crude product was purified by size exclusion chromatography using Sephadex LH-20 and methanol as eluent to obtain the pure complex. ^{1}H NMR revealed a shift of all proton signals to lower fields and additional peaks appeared in the aromatic region fitting to the bpy-units of the formed complex (see Supporting Information, Figure S45). HR-ESI-MS confirmed the structure as [M-Cl]$^{+}$ (error: 1.5 ppm).

The absorbance and emission spectra of all compounds were measured in aerated methanol. Due to the polarity of methanol the vibrational structure of the excitation and emission spectra is not visible and the absorption band is bathochromically shifted compared to more unpolar solvents.[33] The cleavage of the boron difluoride group results in an absorption band at λ_{max} = 410 nm for compounds **3a** and **3b**, hypsochromically shifted compared to λ_{max} = 477 nm of **1**. Emission spectra of compound **3a** and **3b** (λ_{max} = 509 nm) and **1** (λ_{max} = 541 nm) revealed the same behavior. The appearing absorption bands for compounds **5a-c** fit to π→π* transition of the bpy-ligands (λ_{max} = 296 nm) and the metal to ligand charge transfer (MLCT) transition (λ_{max} = 517 nm). The complexes revealed fluorescence in methanol in the visible region at λ_{max} = 585 nm when irradiated at λ = 296 nm. Furthermore, a smaller emission maximum is observable around λ = 450 nm, indicating two separate chromophore systems. Like also previously reported,[34] fluorescence of the Ru-complexes was quenched in water and therefore, complexes **5a-c** did not show any emission when measured in aqueous solution.

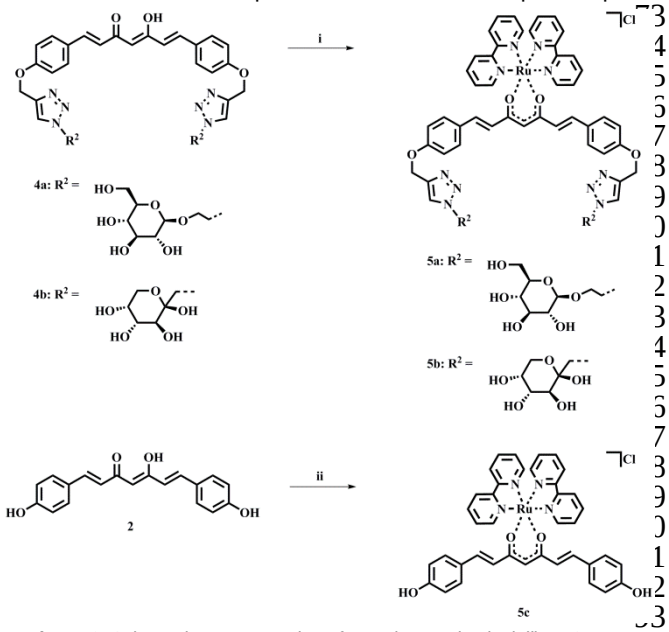

Figure 2. Schematic representation of complex synthesis. i, ii) NaOMe, $Ru(bpy)_2Cl_2$, Ar, MeOH / DMF, 60 °C, 12 h

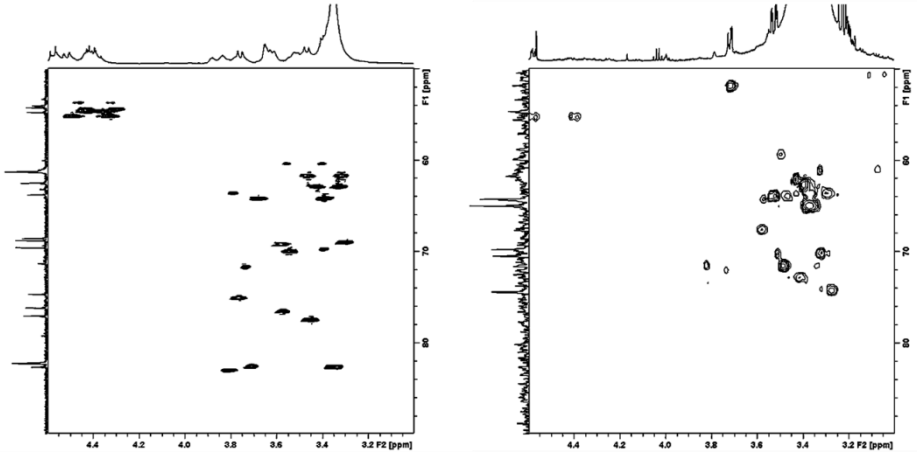

Figure 3. ^{1}H-^{13}C-HSQC spectra of **4b** and **5b** (in DMSO-d_6) focusing area between 3 and 4 ppm in ^{1}H NMR respectively between 50 and 100 ppm in ^{13}C NMR.

Cytotoxicity studies

Recent studies indicated an increased uptake of D-fructose conjugated luminescent metal complexes into breast cancer cells by fluorescence and flow cytometry.[12] Since complexes **5a-c** show no fluorescence in water or cell media due to quenching effects[34] the uptake behavior could not be studied by spectroscopic methods. In order to evaluate the cytotoxicity of the ligands and complexes, the inhibitory effect on the cellular metabolic activity of different cell types was investigated *via* a resazurin based assay (alamarBlue, Thermo Fisher). The non-cancerous cell line L929, the liver cancer cell line HepG2 as well as the breast cancer cell line MDA-MB-231 were treated with ligands **2**, **4a-b** and metal complexes **5a-c** at varying concentrations for 24 h (see Supporting Information, Figures S51-S53). All tested compounds induced no significant reduction in cell viability in non-cancerous cell line L929. Carbohydrate conjugated metal complexes **5a** and **5b** revealed only a slight inhibitory effect on the metabolic activity of MDA-MB-231 cells, which might be attributed to the lipophilic properties of the bipyridyl groups of the complexes, allowing for elevated diffusion through the cell or nucleic membrane in comparison to the more polar ligands **4a** and **4b**. Sugar-decorated ligands **4a** and **4b** had no influence on MDA-MB-231 cells. Fructose–conjugated complex **5b** revealed no specific cytotoxicity against MDA-MB-231 cells. A concentration dependent reduction of cell viability was observed for the sugar-free ligand **2** and complex **5c**, which could be attributed to its increased hydrophobicity due to missing hydrophilic sugar units. As a consequence, a GLUT-5 independent pathway seems to be likely. In contrast to that, HepG2 cells exhibited sensitivity against glucose and fructose conjugated metal complexes independently of tested concentration, as demonstrated by the decrease of cell viability below 50% after 24 h. Whereas, HepG2 remained unaffected after treatment with carbohydrate-conjugated ligands as well as for the metal complex **5c**. Previous studies already revealed a selective uptake of glucose substituted ruthenium complexes in HepG2 cells, what could contribute to the enhanced cytotoxic effects seen in our investigations.[35] Furthermore, increased cytotoxicity of curcumin as well as curcumin-conjugated metal complexes against HepG2 cells is in accordance with literature reports.[36] Correlation between cytotoxicity and specific uptake of D-fructose conjugated compounds requires further investigations.

Conclusions

The conjugation of protected D-glucose and D-fructose to BDC was achieved by click reaction between sugar-azides and propargyl-modified curcumin derivative. Deprotection procedures resulted in carbohydrate-conjugated ligands, which were successfully reacted with $Ru(bpy)_2Cl_2$ to form novel complexes of the general formula $Ru(bpy)_2(L)Cl$. All compounds were extensively analyzed by ^{1}H and ^{13}C NMR, IR, UV/Vis and fluorescence spectroscopy, mass spectrometry as well as elemental analysis. Sugar-decorated ligands and complexes induced a decrease in cell viability of HepG2 and only a slight cytotoxicity for MDA-MB-231. However, BDC based complex **5c** showed an increased cytotoxicity in breast cancer cell line, whereas most HepG2 cells remained unaffected for all tested concentrations, indicating a carbohydrate-independent pathway. Increased cytotoxicity of fructose conjugated compounds in breast cancer cells was not observable in this study.

Experimental Section

Materials and General Experimental Details: All reagents and solvents were commercial products purchased from Aldrich, Sigma, Fluka, Across Organics, Strem, VWR or Alfa Aesar and were used without further purification. Chromatographic separations were performed with NP silica RediSep Cartridges by Teledyne Isco. The progress of reactions was monitored by thin-layer chromatography (TLC) using glass plates precoated with silica gel 60 (Merck). Cell cultivation was performed at 37 °C in a humidified 5% CO_2 atmosphere. L929 (CCL-1, ATCC) and MDA-MB231 (HTB-26, ATCC) cells were cultured in Dulbecco's MEM (DMEM, Lonza) supplemented with 10% fetal calf serum (FCS, Capricorn Scientific), 100 µg mL^{-1} streptomycin and 100 IU mL^{-1} penicillin (Biochrom, Merck). HepG2 cells (HB-8065, ATCC) were routinely cultured in DMEM/ F12 media (Biochrom, Merck Millipore). Consumables for cell culture, like pipettes and cell culture plates (96 well) were obtained from Corning (USA) and Greiner Bio-one (Austria/ Germany).

Instrumentation: ^{1}H NMR and ^{13}C NMR spectra were measured with Bruker spectrometers (600, 300 and 250 MHz). IR spectra were recorded with Nicolet AVATAR 370 DTGS and Bruker Tensor 37 spectrometers. UV/Vis absorption spectra were measured with Thermo Unicam UV500 and analytikjena Specord250 spectrometer and fluorescence was recorded with a Jasco FP 6500 and an Infinite M200 PRO microplate reader (298K, methanol, 1 x 10^{-4} to 2 x 10^{-6} M solutions). High Resolution electron spray ionization mass spectrometry (HR-ESI-MS) was measured

with a Bruker MicroQTof and a Thermo QExactive plus Orbitrap mass spectrometer coupled to an ESI source. Elemental analysis was measured with a Leco CHN-932. The alamarBlue cell viability assay (Thermo Fisher) was performed with an Infinite M200 PRO microplate reader (Tecan) according to supplier's instructions.

Synthesis of the curcuminoid compounds. The synthesis of **1** was carried out according a modified literature procedure and **2** was synthesized as reported.[24]

Synthesis of the azido-sugars: 2-Azidoethyl-2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranoside[25a] and 1-azido-2,3:4,5-di-*O*-isopropyliden-*β*-D-fructopyranosid were synthesized like previously reported.[25b]

1: 0.64 mL (6.2 mmol) of 2,4-pentanedione and 1.16 mL (9.4 mmol) of BF_3xEt_2O were dissolved in 5 mL dry toluene and stirred at 65 °C for 2 h. 2 g (12.5 mmol) of 4-(propargyloxy)-benzaldehyde in 40 mL dry toluene were added to the solution. After the addition of 3.9 mL (15.6 mmol) of tributyl borate the resulting black mixture was stirred at 65 °C for 30 minutes. 0.43 mL *n*-butyl amine (4.34 mmol) were added dropwise until the color of the solution changed to red and stirring was continued at 65 °C overnight. After cooling to room temperature the precipitated solid was collected by filtration and washed with small amounts of cold toluene and water. The crude product was dissolved in acetone and water was added slowly to precipitate 2.575 g (5.96 mmol) of **1** as a red solid, isolated by filtration, washed with water and dried *in vacuo* (yield: 96%). ^{1}H NMR (300 MHz, DMSO-d$_6$): δ = 8.02 (d, ^3J = 15.69 Hz, 2H, H-3), 7.88 (d, ^3J = 8.59, 4H, H-6), 7.12 (m, 6H, H-4, H-7), 6.53 (s, 1H, H-1), 4.92 (s, 4H, H-9), 3.63 (s, 2H, H-11) ppm. ^{13}C NMR (75 MHz, DMSO-d$_6$): δ = 179.33 (C-2), 160.37 (C-8), 146.27 (C-3), 131.61 (C-6), 127.53 (C-5), 119.17 (C-4), 115.61 (C-7). 101.73 (C-1), 78.75 (C-10, C-11), 55.74 (C-9) ppm. ^{19}F NMR (188 MHz, DMSO-d$_6$): δ = .-138.14 ppm. HR-ESI-MS *m/z* calcd. for $C_{25}H_{19}BF_2O_4Na$ $[M+Na]^+$: 455.1237; Found: 455.1219 (error: 4.8 ppm). Elemental analysis calcd. for $C_{25}H_{19}BF_2O_4$ (432.23): C, 69.47; H, 4.43; found: C, 69.44, H, 4.56. IR (KBr): $\tilde{\nu}$ = 667 ($\delta_{\equiv CH}$), 3290 ($\nu_{\equiv CH}$). UV/Vis: λ (ε x 10^{-3} / M^{-1} cm^{-1}): 477 (50.55), 458 (48.1), 254 (11.8) nm. FL (CH$_3$OH, λ_{ex} = 477 nm): λ = 541 nm.

3a: 3.863 g (9.26 mmol) of 2-azidoethyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside and 2 g (4.63 mmol) of **1** were dissolved in 150 mL of degassed THF, 1.5 g (7.57 mmol) sodium ascorbate and 0.24 g (0.96 mmol) $CuSO_4$ in 5 mL of degassed, pure water were added and the mixture was heated at 50 °C under Ar for 12 h. After TLC (Silica NP, EtOAc) indicated that no starting material remained, the solvent was evaporated, the crude product dissolved in $CHCl_3$ and washed thrice with saturated, aqueous EDTA solution and thrice with pure water. The organic layer was dried with Na_2SO_4, the solvent evaporated and the product purified by flash column chromatography (silica NP, EtOAc) to obtain 2.388 g (1.96 mmol) of the pure product (yield: 42%). ^{1}H NMR (600 MHz, DMSO-d$_6$): δ = 8.11 (s, 2H, H-11), 7.71 (d, ^3J = 8.82 Hz, 4H, H-6), 7.62 (d, ^3J = 15.78 Hz, 2H, H-3), 7.12 (d, ^3J = 8.88 Hz, 4H, H-7), 6.82 (d, ^3J = 15.90 Hz, 2H, H-4), 6.10 (s, 1H, H-1), 5.25 – 5.18 (m, 6H, H-3', H-9), 4.92 (t, ^3J = 9.72 Hz, 2H, H-4'), 4.84 (d, ^3J = 8.04 Hz, 2H, H-1'), 4.76 – 4.73 (m, 2H,H-2'), 4.62 – 4.53 (m, 4H, H-1$_{spacer}$), 4.19 (dd, ^2J = 12.30 Hz, ^3J = 5.04 Hz, 2H, H-6'), 4.14 – 4.1 (m, 2H, H-2$_{spacer}$), 4.06 (dd, ^2J = 12.24 Hz, ^3J = 2.28 Hz, 2H, H-6''), 3.99 – 3.92 (m, 4H, H-5', H-2$_{spacer}$), 2.02 – 1.89 (4s, 12H, H-acetyl) ppm. ^{13}C NMR (75 MHz, DMSO-d$_6$): δ = 183.21 (C-2), 170.05 – 168.97 (C=O-acetyl), 159.85 (C-8), 142.18 (C-10), 139.95 (C-3) 130.14 (C-6), 127.67 (C-5), 124.94 (C-11), 122.04 (C-4), 115.19 (C-7), 101.29 (C-1), 99.15 (C-1'), 71.91 (C-3'), 70.66 (C-5'), 70.57 (C-2'), 68.09 (C-4'), 67.41 (C-2$_{spacer}$), 61.65 (C-6'), 61.26 (C-9), 49.34 (C-1$_{spacer}$), 20.49-20.21 (CH$_3$-acetyl) ppm. ESI-MS *m/z* calcd. for $C_{57}H_{66}N_6O_{24}Na$ $[M+Na]^+$ 1241.4; found 1241.3; *m/z* calcd. for $C_{57}H_{67}N_6O_{24}$ $[M+H]^+$ 1219.42; found 1219.41; *m/z* calcd. for $C_{57}H_{66}N_6O_{24}K$ $[M+K]^+$ 1257.38; found 1257.3. Elemental analysis calcd. for $C_{57}H_{66}N_6O_{24}$ (1219.17): C, 56.15, H, 5.46; N, 6.89; found: C, 56.44, H, 5.65, N 6.73. IR (KBr): $\tilde{\nu}$ = 1755 (ν_{CO}), 2887 (ν_{CH}), 2958 (ν_{CH_2}), 3145 ($\nu_{=CH}$). UV/Vis (CH$_3$OH): λ (ε x 10^{-3} / M^{-1} cm^{-1}): 410 (39.25), 243 (13.55) nm. FL (CH$_3$OH, λ_{ex} = 410 nm): λ = 509 nm.

3b: 1.74 g (6.1 mmol) of 1-azido-2,3:4,5-di-*O*-isopropyliden-β-D-fructopyranosid and 1.318 g (3.05 mmol) of **1** were dissolved in 100 mL of degassed THF, 1 g (5.05 mmol) sodium ascorbate and 0.15 g (0.6 mmol) $CuSO_4$ in 5 mL of degassed, water were added and the mixture was heated at 50 °C under Ar for 12 h. After TLC (Silica NP, EtOAc/*n*-hexane, v/v 2:1) indicated that no starting material remained, the solvent was evaporated, the crude product dissolved in $CHCl_3$ and washed thrice with saturated, aqueous EDTA solution and thrice with pure water. The organic layer was dried with Na_2SO_4, the solvent evaporated and the product purified by flash column chromatography (Silica NP, EtOAc/*n*-hexane, v/v 2:1) to obtain 1.503 g (1.57 mmol) of the pure product (yield: 52%). ^{1}H NMR (600 MHz, DMSO-d$_6$): δ (ppm) = 8.20 (s, 2H, H-11), 7.69 (d, ^3J = 8.82 Hz, 4H, H-6), 7.61 (d, ^3J = 15.78 Hz, 2H, H-3), 7.12 (d, ^3J = 8.82 Hz, 4H, H-7), 6.82 (d, ^3J = 15.90 Hz, 2H, H-4), 6.09 (s, 1H, H-1), 5.22 (s, 4H, H-9), 4.66 (m, 6H, H-1', H-1'', H-4'), 4.43 (d, ^3J = 2.26 Hz, 2H, H-3'), 4.27 (d, ^3J = 8.7 Hz, 2H, H-5'), 3.75 (dd, ^4J = 1.59 Hz, ^2J = 12.99 Hz, 2H, H-6'), 3.64 (d, ^2J = 12.84 Hz, 2H, H-6''), 1.41–0.81 (4s, 12H, H-isopropylidene). ^{13}C NMR (75 MHz, DMSO-d$_6$): δ (ppm) = 183.18 (C-2), 159.79 (C-8), 142.10 (C-3), 139.93 (C-10), 130.08 (C-6), 127.60 (C-5), 127.03 (C-11), 121.98 (C-4), 115.23 (C-7), 108.60 (C-isopropylidene), 108.29 (C-isopropylidene), 101.26 (C-1), 100.43 (C-2'), 70.33 (C-3'), 69.82 (C-5'), 69.32 (C-4'), 60.97 (C-9, C-6'), 54.84 (C-1'), 26.02-24.00 (CH$_3$-isopropylidene). HR-ESI-MS *m/z* calcd. for $C_{49}H_{58}N_6O_{14}Na$ $[M+Na]^+$ 977.3903; found: 977.3875 (error: 2.8 ppm). Elemental analysis calcd. for $C_{49}H_{58}N_6O_{14}\cdot 0.5\ H_2O$: C, 61.05; H, 6.17; N, 8.72. Found: C, 61.15; H, 6.18; N 8.71. FT-IR (GA): $\tilde{\nu}$ = 613 ($\nu_{C-isoprpylidene}$), 1087 (ν_{COH}), 1755 (ν_{CO}), 2941 (ν_{CH}), 2987 (ν_{CH_2}). UV/Vis (CH$_3$OH): λ (ε x 10^{-3} / M^{-1} cm^{-1}): 410 (37), 243 (11.95) nm. FL (CH$_3$OH, λ_{ex} = 410 nm): λ = 508 nm.

4a: 400 mg (0.32 mmol) of **3a** were dissolved in 10 mL CH$_3$OH/$CHCl_3$ (v/v 1:2) under Ar and sodium methanolate was added up to reach pH ≈ 9. After 30 min no protected ligand remained (monitored by TLC, silica NP, EtOAc) and DOWEX (H^+) was added to reach pH ≈ 7. The resin was filtered off and the solvent removed. The residue was dissolved in water, filtered and dialyzed for one week to obtain 180 mg (0.20 mmol) of the pure product (yield: 63%). ^{1}H NMR (600 MHz, DMSO-d$_6$): δ = 8.32 (s, 2H, H-3), 7.71 (d, ^3J = 8.10 Hz, 4H, H-6), 7.62 (d, ^3J = 15.78 Hz, 2H, H-3), 7.13 (d, ^3J = 8.58 Hz, 4H, H-7), 6.82 (d, ^3J = 15.12 Hz, 2H, H-4), 6.11 (s, 1H, H-1), 5.20 (s, 4H, H-9), 5.11 (d, ^3J = 4.86 Hz, 2H, HO-2'), 4.98 (d, ^3J = 4.80 Hz, 2H, HO-3'), 4.94 (d, ^3J = 5.34 Hz, 2H, HO-4'), 4.61 (m, 4H, H-1$_{spacer}$), 4.54 (m, 2H, HO-6'), 4.25 (d, ^3J = 7.80 Hz, 2H, H-1'), 4.11 (m, 2H, H-2$_{spacer}$), 3.94 (m, 2H, H-2$_{spacer}$), 3.69 (m, 2H, H-6'), 3.44 (m, 2H, H-6''), 3.17-3.12 (m, 4H, H-3', H-5'), 3.06 (m, 2H, H-4'), 2.99 (m, 2H, H-2') ppm. ^{13}C NMR (75 MHz, DMSO-d$_6$): δ = 183.23 (C-12'), 159.91 (C-6''), 142.12 (C-4''), 139.99 (C-11''), 130.17 (C-8''), 127.66 (C-9''), 125.60 (C-3''), 122.05 (C-10''), 115.26 (C-7''), 102.94 (C-1), 101.34 (C-13''), 77.01 (C-5), 76.62 (C-3), 73.34 (C-2), 70.04 (C-4), 67.34 (C-1''), 61.28 (C-5''), 61.10 (C-6), 49.78 (C-2'') ppm. HR-ESI-MS *m/z* calcd. for $C_{41}H_{50}N_6O_{16}Na$ $[M+Na]^+$: 905.3176; found: 905.3163 (error: 1.4 ppm). Elemental analysis calcd. for $C_{41}H_{50}N_6O_{16}\cdot 2.5H_2O$ (927.9): C, 53.07, H, 5.97, N, 9.06; found: C, 53.25, H, 5.58, N 8.84. IR (KBr): $\tilde{\nu}$ = 3352 (ν_{OH}). UV/Vis (CH$_3$OH): λ (ε x 10^{-3} / M^{-1} cm^{-1}): 408 (18.45), 243 (13.55) nm. FL (CH$_3$OH, λ_{ex} = 408 nm): λ = 509 nm.

4b: 175 mg (0.183 mmol) of **3b** were dissolved in 10 mL formic acid / water (v/v 17:3) and stirred at room temperature for one week. After no isopropylidene groups remained (monitored by ESI-MS), formic acid was coevaporated with water and the residue was dried. The crude product was dissolved in 5 mL of a THF / water mixture (v/v, 1:1) and an aqueous 0.1 M NaOH solution was added to reach pH ≈ 9. The solution was neutralized with 2.5 M HCl, freeze-dried, re-dissolved in water and dialyzed for one week against water to obtain 84 mg (0.106 mmol) of the pure product (yield: 58%). ^{1}H NMR (600 MHz, DMSO-d$_6$): δ (ppm) = 8.09 (s, 1H, H-11), 7.71 (d, ^3J = 8.46 Hz, 4H, H-6), 7.62 (d, ^3J = 15.84 Hz, 2H,

H-3), 7.13 (d, ^3J = 8.52 Hz, 4H, H-7), 6.82 (d, ^3J = 15.84 Hz, 2H, H-4), 6.10 (s, 1H, H-1), 5.22 (m, 4H, H-9), 4.59 – 4.37 (m, 4H, H-1', H-1''), 3.88 – 3.41 (m, 10H, H-3, H-4, H-5, H-6, H-6'). ^{13}C NMR (75 MHz, DMSO-d$_6$): δ (ppm) = 183.24 (C-2), 159.97 (C-8), 141.98 (C-10), 140.01 (C-9), 130.17 (C-6), 127.65 (C-5), 125.94 (C-11), 122.03 (C-4), 115.28 (C-7), 102.70 (C-2'), 101.32 (C-1), 100.24 (C-2'), 96.67 (C-2'), 82.60 (C-3'), 82.21 (C-3'), 77.01(C-5'), 76.10 (C-5'), 74.61 (C-5'), 69.55 (C-4'), 68.76 (C-4'), 68.53 (C-4'), 63.77 (C-6'), 62.49 (C-6'), 61.32 (C-6'), 61.25 (C-9), 54.75 (C-1'), 54.22 (C-1'), 54.04(C-1'). HR-ESI-MS *m/z* calcd. for $C_{49}H_{58}N_6O_{14}Na$ [M+Na]$^+$: 817.2651; found: 817.2613 (error: 4.7 ppm). Elemental analysis calcd. for $C_{37}H_{42}N_6O_{14}\cdot 2.5\ H_2O$: C, 52.92; H, 5.64; N 10.01. Found: C, 52.70; H, 5.24; N 10.04. IR (KBr): $\tilde{\nu}$ = 3352 (ν_{OH}). UV/Vis (CH_3OH): λ (ε x 10^{-3} / M^{-1} cm^{-1}): 409 (8.75), 286 (4.10) nm. FL (CH_3OH, λ_{ex} = 408 nm): λ = 510 nm.

5a: 135 mg of **4a** (0.15 mmol) were dissolved in dry methanol under Ar and 310 µL 0.5 M sodium methanolate solution (0.15 mmol) in methanol were added dropwise. The solution was stirred for 1 h and 81.7 mg [Ru(bpy)$_2$Cl$_2$] (0.17 mmol) in methanol were slowly added. The mixture was heated under Ar and reflux for 12 h at 60 °C. After TLC (Silica NP, CH_3CN/H_2O/sat. aq. KNO_3; v/v 40:4:1) indicated that no starting material remained, the solvent was evaporated. The crude product was dissolved in water, filtered and dialyzed for one week to obtain 191 mg (0.14 mmol) of the pure complex (yield: 93%). ^{1}H NMR (600 MHz, DMSO-d$_6$): δ = 8.80 (d, ^3J = 8.28 Hz, 2H, H-3$_{bpy}$), 8.70 (d, ^3J = 8.22 Hz, 2H, H-3'$_{bpy}$), 8.63 (d, ^3J = 5.28 Hz, 2H, H-6$_{bpy}$), 8.30 (s, 2H, H-11), 8.18 (t, ^3J = 7.80 Hz, 2H, H-4$_{bpy}$), 7.93 (t, ^3J = 7.83 Hz, 2H, H-4'$_{bpy}$), 7.79 (m, 4H, H-6'$_{bpy}$, H-5$_{bpy}$), 7.47 (d, ^3J = 8.76 Hz, 4H, H-6), 7.31 (t, ^3J = 6.69 Hz, 2H, H-5'$_{bpy}$), 7.01 (m, 6H, H-3, H-7), 6.63 (d, ^3J = 15.84 Hz, 2H, H-4), 5.93 (s, 1H, H-1), 5.13 (s, 4H, H-9), 4.58 (m, 4H, H-1$_{spacer}$), 4.24 (d, ^3J = 7.86 Hz, 2H, H-1'), 4.09 (m, 2H, H-2$_{spacer}$), 3.92 (m, 2H, H-2$_{spacer}$), 3.68 (dd, ^2J = 11.76 Hz, ^3J = 1.86 Hz, 2H, H-6'), 3.45 (q, ^3J =5.92, 2H, H-6''), 3.17-3.10 (m, 4H, H-3', H-5'), 3.06 (t, ^3J = 9.18 Hz, 2H, H-4'), 2.98 (t, ^3J = 9.18 Hz, 2H, H-2') ppm. ^{13}C NMR (75 MHz, DMSO-d$_6$): δ (ppm) = 177.63 (C-2), 158.89 (C-2$_{bpy}$), 158.76 (C-2'$_{bpy}$), 157.39 (C-8), 152.84 (C-6'$_{bpy}$), 149.41 (C-6$_{bpy}$), 142.10 (C-10), 136.63 (C-4$_{bpy}$), 135.33 (C-7), 135.00 (C-4'$_{bpy}$), 129.08 (C-6), 128.31 (C-5), 126.79 (C-4), 126.49 (C-5$_{bpy}$), 125.72 (C-5'$_{bpy}$), 125.53 (C-11), 123.44 (C-3$_{bpy}$, C-3'$_{bpy}$), 115.10 (C-3), 102.92 (C-1'), 102.06 (C-1'), 77.03 (C-5'), 76.61 (C-3'), 73.32 (C-2'), 70.02 (C-4'), 67.27 (C-2$_{spacer}$), 61.13 (C-9), 61.05 (C-6'), 49.72 (C-1$_{spacer}$) ppm. HR-ESI-MS *m/z* calcd. for $C_{61}H_{65}N_{10}O_{16}Ru$ [M-Cl]$^+$: 1295.3618; found: 1295.3629 (error: 0.9 ppm). IR (KBr): $\tilde{\nu}$ = 1425 ($\nu_{C=C}$), 768 (δ_{oop}). UV/Vis (CH_3OH): λ (ε x 10^{-3} / M^{-1} cm^{-1}): 517 (9.85), 410 (31.4), 391 (34.7), 296 (46.7), 245 (27.1) nm. FL (CH_3OH, λ_{ex} = 296 nm): λ = 586 nm.

5b: 26 mg of **4b** (32.7 µmol) were dissolved in 5 mL dry DMF under Ar and 75 µL 0.5 M sodium methanolate solution (37.5 µmol) in methanol was added dropwise. The solution was stirred for 1 h and 18 mg [Ru(bpy)$_2$Cl$_2$] (37,2 µmol) in 4 mL dry DMF were slowly added. The mixture was heated under Ar and reflux for 12 h at 60 °C. After TLC (Silica NP, CH_3CN/H_2O/sat. aq. KNO_3; v/v 40:4:1) indicated that no starting material remained, the solvent was evaporated. The crude product was dissolved in water, filtered and dialyzed for one week to obtain 28 mg (22.5 µmol) of the pure complex (yield: 69%). ^{1}H NMR (600 MHz, DMSO-d$_6$): δ = 8.89 (d, ^3J = 5.79 Hz, 2H, H-3$_{bpy}$), 8.78 (d, ^3J = 8.12 Hz, 2H, H-3'$_{bpy}$), 8.63 (d, ^3J = 8.04 Hz, 2H, H-6$_{bpy}$), 8.15 (t, ^3J = 7.81 Hz, 2H, H-4$_{bpy}$), 8.06-7.97 (m, 2H, H-11), 7.85 (t, ^3J = 6.60 Hz, 2H, H-4'$_{bpy}$), 7.79 (t, ^3J = 7.81 Hz, 4H, H-6'$_{bpy}$, H-5$_{bpy}$), 7.63-7.58 (m, 6H, H-6, H-3), 7.39 (m, 2H, H-5'$_{bpy}$), 7.21 (m, 4H, H-7), 6.76 (d, ^3J = 8.63 Hz, 2H, H-4), 6.28-6.22 (m, 1H, H-1), 5.13-4.82 (m, 4H, H-9), 4.59-4.56 (m, 4H, H-1', H-1''), 3.72 (d, ^3J = 2.76 Hz, 2H, H-4'), 3.54-3.51 (m, 4H, H-6', H-6''), 3.30-3.17 (m, 4H, H-3', H-5') ppm. ^{13}C NMR (75 MHz, DMSO-d$_6$): δ (ppm) = 176.03 (C-2), 159.95 (C-8), 158.14 (C-2$_{bpy}$), 157.75 (C-2'$_{bpy}$), 153.10 (C-6'$_{bpy}$), 149.51 (C-6$_{bpy}$), 139.11 (C-10), 137.62 (C-4'$_{bpy}$), 135.75 (C-4$_{bpy}$), 134.11 (C-7), 130.62, 129.18 (C-6), 128.35 (C-5), 126.98 (C-4), 126.17 (C-11), 123.84 (C-3$_{bpy}$), 123.58 (C-3'$_{bpy}$), 115.27 (C-3), 80.12 (C-2'), 74.36 (C-5'), 70.43 (C-4'), 69.69 (C-3'), 64.95 (C-6', C-6''), 64.22 (C-1'), 61.71 (C-1'', C-9) ppm. HR-ESI-Orbitrap-MS *m/z* calcd. for $C_{57}H_{57}N_{10}O_{16}Ru$ [M-Cl]$^+$: 1207.3110; found: 1207.3112 (error: 1.7 ppm). FT-IR (GA): $\tilde{\nu}$ = 3360 (ν_{OH}), 3307 (ν_{OH}), 2920 (ν_{CH}), 2850 (ν_{CH_2}), 1632 ($\nu_{C=C}$), UV/Vis (CH_3OH): λ (ε x 10^{-3} / M^{-1} cm^{-1}): 499 (3.96), 349 (4.89), 294 (29.78), 245 (12.89) nm. FL (CH_3OH, λ_{ex} = 296 nm): λ = 536 nm.

5c: 45 mg of **1** (0.15 mmol) were dissolved in 30 mL dry methanol under Ar and 350 µL 0.5 M sodium methanolate solution (0.17 mmol) in methanol were added. The solution was stirred for 1 h and 71 mg [Ru(bpy)$_2$Cl$_2$] (0.15 mmol) in 30 mL dry DMF were added dropwise. The mixture was heated under Ar for 12 h at 60 °C. After TLC (Silica NP, CH_3CN/H_2O/sat. aq. KNO_3; v/v 40:4:1) indicated that no starting material remained, the solvent was evaporated. The crude product was dissolved in methanol, filtered and purified by size exclusion chromatography (Sephadex LH-20) to obtain 41 mg (0.05 mmol) of the pure complex (yield: 37%). ^{1}H NMR (600 MHz, DMSO-d$_6$): δ = 8.79 (d, ^3J = 8.22 Hz, 2H, H-3$_{bpy}$), 8.69 (d, ^3J = 8.22 Hz, 2H, H-3'$_{bpy}$), 8.64 (d, ^3J = 5.04 Hz, 2H, H-6$_{bpy}$), 8.17 (m, 2H, H-4$_{bpy}$), 7.92 (m, 2H, H-4'$_{bpy}$), 7.78 (m, 4H, H-6'$_{bpy}$, H-5$_{bpy}$), 7.32 (m, 6H, H-6, H-5'$_{bpy}$), 6.93 (d, ^3J = 15.72 Hz, 2H, H-3), 6.72 (d, ^3J = 8.40 Hz, 2H, H-7), 6.52 (d, ^3J = 15.78 Hz, 2H, H-4), 5.86 (s, 1H, H-1) ppm. ^{13}C NMR (150 MHz, DMSO-d$_6$): δ = 177.68 (C-2), 158.97 (C-2$_{bpy}$), 158.80 (C-2'$_{bpy}$), 157.40 (C-8), 152.81 (C-6'$_{bpy}$), 149.40 (C-6$_{bpy}$), 136.55 (C-4$_{bpy}$), 135.92 (C-3), 134.92 (C-4'$_{bpy}$), 129.21 (C-6), 126.45 (C-5), 126.25 (C-5$_{bpy}$), 125.70 (C-5'$_{bpy}$), 125.47 (C-4), 123.43 (C-3$_{bpy}$, C-3'$_{bpy}$), 115.84 (C-7), 101.75 (C-1) ppm. HR-ESI-MS *m/z* calcd. for $C_{39}H_{31}N_4O_4Ru$ [M-Cl]$^+$: 721.1405; found: 721.1383 (error: 1.5 ppm). UV/Vis (CH_3OH): λ (ε x 10^{-3} / M^{-1} cm^{-1}): 516.5 (14.55), 412.5 (48.1), 395 (49.9), 296 (58.35), 245 (34.8) nm. FL (CH_3OH, λ_{ex} = 296 nm): λ = 585 nm.

Determination of cytotoxicity: Cytotoxicity studies were performed with the mouse fibroblast cell line L929, as well as with HepG2 and MDA-MB-231 cells. In detail, cells were seeded at 10^4 cells per well in a 96-well plate and incubated for 24 h. Afterwards, the testing substances (**2**, **4a**, **4b, 5a-c**) at indicated concentrations (25, 50, 100 µM) were added to the cells and the plates were incubated for further 24 h. Subsequently, the medium was replaced by a mixture of fresh culture medium and alamarBlue solution (Thermo Fisher), prepared according to the manufacturer's instructions. After a further incubation of 4 h at 37 °C, the fluorescence was measured at λ_{em} = 570 nm / λ_{em} = 610 nm, with untreated cells on the same well plate serving as negative controls. The negative control was standardized as 0% of metabolism inhibition and referred as 100% viability. Data are expressed as mean ± SD of three independent determinations.

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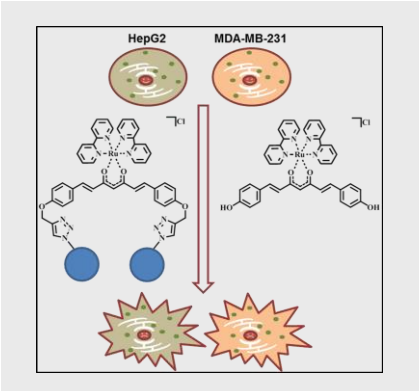
Entry for the Table of Contents

Layout 1:

FULL PAPER

Bisdemethoxy-curcumin (BDC) ligands conjugated with sugar moieties (D-fructose, D-glucose) *via* triazol group have been synthesized and reacted with $Ru(bpy)_2Cl_2$ to form the corresponding complexes. Cytotoxicity assays with MDA-MB-231, HepG2 and L929 cells have been performed. Sugar-decorated compounds showed reduction in cell viability for liver-cancerous cells HepG2, whereas the sugar free complex showed increased toxicity for breast-cancer-cells MDA-MB-231.



Sugar conjugated curcumin ruthenium complexes *

Michael Pröhl, Tanja Buś, Justyna A. Czaplewska, Anja Träger, Henning Weiss, Wolfgang Weigand, Ulrich S. Schubert, Michael Gottschaldt

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Synthesis and in vitro toxicity of D-glucose and D-fructose conjugated curcumin ruthenium complexes