Supporting information for:

Synthesis of a Glycopolymeric Pt^{II} Carrier and its Induction of Apoptosis in Resistant Cancer Cells

Andreas Wild, Krzysztof Babiuch, Marcel König, Andreas Winter, Martin D. Hager, Michael Gottschaldt, Aram Prokop and Ulrich S. Schubert*

Experimental Section

Materials. Pentafluorostyrene (99%, Aldrich) and BlocBuilder® (Arkema) were used as received. 2,3,4,6-Tetra-*O*-acetyl-1-thio-*β*-D-galactopyranose (**4**), 6-([2,2':6',2''-terpyridin]-4'-ylthio)hexane-1-thiol (**2**) and $Pt(COD)Cl_2$ were synthesized as previously reported.[1-3] Triethylamine (TEA) was ordered from Merck (for synthesis, ≥99%), *N*,*N*-dimethylformamide (≥99.5%) and sodium methanolate from Fluka, methanol (anhydrous 99.8%) and phosphazene base P1-*t*-Bu (≥97.0%) from Aldrich.

Instrumentation. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer, and ^{19}F NMR spectra on a Bruker Avance 200 MHz spectrometer in deuterated DMF or $CDCl_3$. The chemical shifts were calibrated with respect to solvent residual peaks. Size exclusion chromatography (SEC) was measured on an Agilent Technologies 1200 Series size exclusion chromatography system equipped with a G131A isocratic pump, a G1329A autosampler, a G1362A refractive index detector, and both a PSS Gram 30 and a PSS Gram 1000 columns in series. 2.1% LiCl solution in DMA was used as eluent at 1 mL·min -1 flow rate at a column oven temperature of 40 °C. The reported number average molar masses were calculated according to polystyrene standards. Dynamic light scattering (DLS) was performed at a scattering angle of 90° on an ALV CGS-3 instrument and a He–Ne laser operating at a wavelength of λ = 633 nm at 25°C. The CONTIN algorithm was applied to analyze the correlation functions obtained. Apparent hydrodynamic radii were calculated according to the Stokes–Einstein equation. All CONTIN plots are number-weighted. Cryo-TEM images were recorded using a Technai G2 Sphera (FEI) TEM with an acceleration voltage of 200 kV. Samples for cryo-TEM were prepared using a FEI Vitrobot system. 3 µL of the sample solution were transferred onto a Quantifoil (R2/2) grid, and blotting was performed at 3 mm and 3.5 s of blotting time. Samples were rapidly transferred into liquid ethane and stored in liquid nitrogen until the measurements were performed using a Gatan cryo holder.

Synthesis of poly(pentafluorostyrene) (1). Pentafluorostyrene (1.538 g, 7.92 mmol), BlocBuilder® (50 mg, 0.13 mmol), DMF (0.602 g, 8.25 mmol) as an internal standard, and tetrahydrofuran (1.5 mL) were added in a 10 mL pressure-resistant round-bottom flask. The mixture was saturated with argon while stirring for at least 30 min. The flask was sealed and placed into a preheated oil bath (110 °C). The reaction was continued for 5 h. Subsequently, the reaction vessel was cooled down with tap water and the viscous reaction mixture was precipitated into cold methanol to remove the residual monomer. The isolated white powder was dried under vacuum for 24 h.

^{1}H NMR (300 MHz, $CDCl_3$): δ 4.35–3.95 (CH_2 initiator), 2.80–2.22 (broad signal, CH backbone), 2.21–1.66 (broad signal, CH_2 backbone), 1.36–0.69 (m, CH_3 initiator).

^{19}F NMR (200 MHz, $CDCl_3$): δ –143.9 (broad signal, *ortho*-PPFS), –154.5 (broad signal, *para*-PPFS), –161.6 (broad signal, *meta*-PPFS).

Synthesis of tpy-grafted poly(pentafluorostyrene) (3). 1 (300 mg, 1.46 mmol of PFS), was dissolved in 15 mL DMF and the solution was bubbled with a flow of argon for 1 h. Subsequently **2** (28 mg, 0.073 mmol) and P_1-*t*-Bu (17 mg, 0.073 mmol) were added and the solution was stirred for 2 h at rt. The reaction mixture was precipitated into methanol several times. **3** was obtained as a brown power (265 mg, isolated yield = 88%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.72–8.54 (m, $H^{6,6''}$, $H^{3,3''}$, 4H), 8.34–8.24 (broad singlet, $H^{3',5'}$, 2H), 7.90–7.78 (broad triplet, $H^{4,4''}$, 2H), 7.26–7.38 (broad triplet, $H^{5,5''}$, 2H), 4.31–3.90 (CH_2 initiator), 3.17 (m, CH_2), 2.58–2.22 (broad signal, CH backbone, CH_2), 2.23–1.67 (broad signal, CH_2 backbone, CH_2), 1.36–0.69 (m, CH_3 initiator, CH_2).

^{19}F NMR (200 MHz, $CDCl_3$δ): δ –134.5 (broad signal, *ortho*-F (tpy substituted)), –142.5 to –144.8 (broad signal, *ortho*-PPFS, *meta*-F (tpy substituted)), –154.6 (broad signal, *para*-PPFS), –161.6 (broad signal, *meta*-PPFS (the unreacted ones)).

Synthesis of 5. **3** (100 mg, 0.53 mmol of PFS) and the protected sugar (0.480 g, 1.32 mmol, 2.5 eq. per PFS unit) were dissolved in 20 mL of dry DMF. TEA (200 µL, 1.44 mmol) was added to the solution. The reaction mixture was stirred for 23 h at 50 ºC, concentrated to approximately 2 mL, and precipitated into 20 mL of cold ethanol. The polymer was purified by reprecipitation from DMF into ethanol (2 times), filtered, and vacuum-dried to afford 230 mg of a brown powder (**5**, isolated yield = 83%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.79–8.65 (m, $H^{6,6''}$, $H^{3,3''}$, 4H), 8.44–8.29 (broad singlet, $H^{3',5'}$, 2H), 7.56–7.45 (m, $H^{5,5''}$, 2H), 6.08–3.65 (multiple broad signals of carbohydrate protons, CH_2 initiator), 3.25 (m, CH_2), 2.64–1.73 (backbone and CH_3 of acetyl groups), 1.38–0.62 (m, CH_3 initiator, CH_2).

^{19}F NMR (200 MHz, DMF-d_7): δ –132.8 (broad signal, *ortho*-F (sugar substituted)), –134.5 (broad signal, *ortho*-F (tpy substituted), –139.9 to –143.3 (broad signal, *meta*-F (tpy substituted), *meta*-F (sugar substituted), –162.2 (broad signal, *meta*-PPFS).

Synthesis of 6. The acetylated polymer (**5**) (173 mg, 0.30 mmol of sugar units) was dissolved in an oven-dried, round-bottom flask in 2 mL of dry DMF. Sodium methanolate (0.3 mL of 0.1 M solution in dry methanol) was added dropwise via a syringe to the solution. The reaction mixture was stirred at room temperature for 1 h, concentrated by evaporation to approximately 0.2 mL, precipitated into cold ethanol, filtered, and vacuum-dried to obtain 95 mg of grey powder, PTFSGlcOH (**6**, isolated yield = 86%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.82–8.61 (m, $H^{6,6''}$, $H^{3,3''}$, 4H), 8.48–8.28 (broad singlet, $H^{3',5'}$, 2H), 7.63–7.48 (m, $H^{5,5''}$, 2H), 5.95–3.05 (multiple broad signals of carbohydrate protons and OH groups of the sugar, CH_2 initiator), 2.45–1.63 (backbone), 1.38–0.65 (m, CH_3 initiator, CH_2).

^{19}F NMR (200 MHz, DMF-d_7): δ –135.4 (broad signal, *ortho*-F (sugar substituted)), –138.4 (broad signal, *ortho*-F (tpy substituted)), –142.6 to –145.3 (broad signal, *meta*-F (tpy substituted), *meta*-F (sugar substituted), –164.2 (broad signal, *meta*-PPFS).

Synthesis of 7. **6** (35 mg, 0.004 mmol of tpy units) was dissolved in a mixture of water (5 mL) and DMF (2 mL) and the solution was bubbled with a flow of argon for 1 h. $Pt(COD)Cl_2$ (3 mg, 0.008 mmol) was added and the mixture was stirred 48 h at 60 °C. Subsequently, the water was evaporated and the polymer precipitated several times into cold methanol and diethyl ether. **6** was obtained as a yellowish powder (30 mg, isolated yield = 83%).

^{1}H NMR (300 MHz, DMF-d_7): δ 9.00 (m, $H^{6,6''}$, 2H), 8.80–8.70 (m, $H^{3,3''}$, 2H), 8.39 (m, $H^{3',5'}$, 2H), 6.05–3.08 (multiple broad signals of carbohydrate protons and OH groups of the sugar, CH_2 initiator), 2.35–1.39 (backbone), 1.38–0.62 (m, CH_3 initiator, CH_2).

^{19}F NMR (200 MHz, DMF-d_7): δ –135.5 (broad signal, *ortho*-F (sugar substituted), broad signal, *ortho*-F (tpy substituted), –142.4 to –146.0 (broad signal, *meta*-F (tpy substituted), *meta*-F (sugar substituted)), –164.6 (broad signal, *meta*-PPFS).

^{195}Pt NMR (600 MHz, DMF-d_7): δ –2707.

NMR and SEC analysis

***Para*-fluorine-substitution.** Determination of the *para*-fluorine substitution content was performed by ^{19}F NMR spectroscopy. ^{19}F NMR spectra allow the calculation of both the efficiency and the selectivity of the substitution reaction of PPFS. As illustrated in **Figure 1**, there are three peaks visible in the starting homopolymer, which correspond to fluoro atoms at the *ortho*(–143 ppm), *meta* (–162 ppm), and *para* (–154 ppm) positions. The conversion of the terpyridine grafting reaction could easily be calculated by integration of the fluorine signals. The new signal, which appeared at –134 ppm, corresponding to the *ortho*-F of the substituted pentafluorophenyl rings in polymer **3**. The integrals indicate a substitution of about 5%, as expected. Besides the shift of the *ortho*-F, also the *meta*-F of the substituted rings shift from about –162 ppm to –143 ppm and overlaps now with the *ortho*-F of the unsubstituted rings. Beside ^{19}F

NMR, also ^{1}H NMR spectroscopy (Figure S1) was used to determine the coupled amount of terpyridine moiety (**2**).

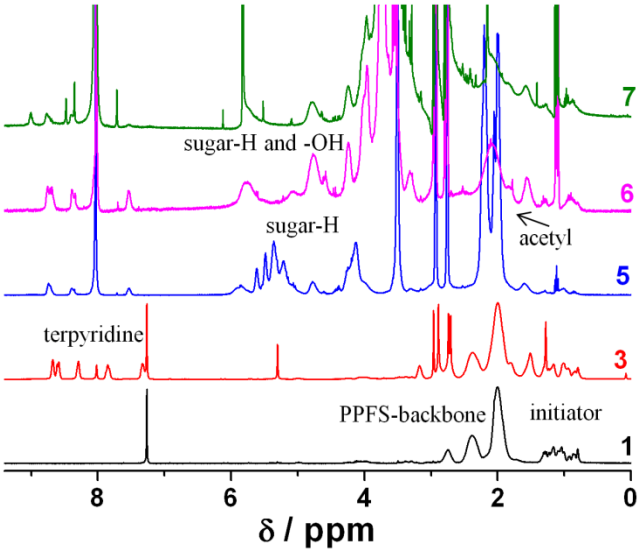

Figure S1. ^{1}H NMR spectra (300 MHz) of **1**, **3** ($CDCl_3$) and **5**, **6**, **7** (DMF-*d*7).

The comparison of the integral of the initiator signal at 1 ppm and the backbone signal at 2 ppm corresponds to a degree of polymerization (DP) of 38, which was in good correlation with the SEC analysis results (Figure 2). Using this DP, we could calculate from ^{1}H NMR (ratio between the integrals at 8.72 to 8.54 ppm and 2.58 to 1.67 ppm corresponding to $H^{6,6''}$, $H^{3,3''}$ of tpy and protons of the backbone, respectively) approximately two terpyridine moieties to be in one polymer chain, what corresponds well to the 5 mol%, calculated by ^{19}F NMR. After grafting the sugar units and deprotection, applying the standard Zemplén procedure for sugar deacetylation, using sodium methanolate, the amount of carbohydrate moieties, as calculated from ^{19}F NMR, remained unchanged (95-99%). Polymers **1**, **3**, **5**, and **6** stayed well-defined (PDI values below 1.15) and the molar masses, obtained from SEC (according to polystyrene calibration), consecutively increased with each grafting step. **6** showed more hydrophilic character. This change of solubility behavior caused the increase in the hydrodynamic volume of the glycopolymer in DMA, as depicted in the SEC measurements (Figure 2) by the shift of the trace toward lower elution volumes (higher molar masses).

Complexation of platinum(II). The complexation of platinum by the terpyridine moiety can be confirmed by ^{1}H NMR spectroscopy. Due to complexation, the terpyridine signals shift significantly, as visible in **Figure S1**, **7**. In particular the 4,4”; 5,5” and 6,6” protons of **7** showed downfielded shifts, in comparison to the uncomplexed polymer **6**, caused by the influence of the metal-ligand bond. The amount of conjugated carbohydrate moieties remained unchanged, as confirmed by ^{19}F NMR spectroscopy. The

final, metal-carrying glycopolymer **7** was well-defined (PDI = 1.12) and its trace in the SEC analysis showed a slight shift toward lower elution volumes, indicating an increase of its molar mass after complexation. In addition, ^{195}Pt NMR proofs the presence of only one Pt-species in the final polymer.

Bioassays

1. *Cell culture*:

Nalm-6 is a human B-cell precursor cell line derived from peripheral blood of a 19-year-old man with acute lymphoblastic leukemia (ALL). The cells are suspension cells. They were provided by the AG Seeger, Charité, Campus Virchow; vincristine and daunorubicine-resistant Nalm-6 cells were generated by Dr. Prokop, Children's Hospital Cologne, Germany. The cells were subcultured every 2-3 days by dilution of the cells to a concentration of 1×10^5 cells/mL. All experiments with suspension cells were performed in RPMI 1640 supplemented with 10% heat inactivated fetal calf serum, 1% penicillin/streptomycin. Adherent cells were cultured in DMEM supplemented with 10% heat inactivated fetal calf serum, 0.4 mg/mL geniticine. Twenty-four hours before the assay setup, suspension cells were cultured at a concentration of 3×10^5 cells/mL to ascertain standardized growth conditions. For apoptosis assays, the cells were then diluted to a concentration of 1×10^5 cells/mL immediately before addition of the different drugs.

2. *Determination of cell concentration and cell viability (proliferation inhibition)*:

Cell viability was determined by CASY® Cell Counter + Analyzer System of Schaerfe System GmbH (Reutlingen, Germany). Settings were specifically defined for the requirements of the cells used. With this system the cell concentration is analyzed simultaneously in three different size ranges: cell debris, dead cells, and viable cells were determined in one measurement. Nalm-6 cells were cultivated in the presence of different concentrations of **7**. The initial cell concentration was adjusted to 1×10^5 cells/mL. After 24 h of incubation with default drug, the cells were re-suspended properly and 100 µL of each sample was diluted in 10 mL CASYton (ready-to-use isotonic saline solution) for an immediate automated count of the cells. Depicted data (**Figure 3**) are mean values of 3 batches and standard deviation. Anti-proliferatory properties of glycopolymer **7** were further confirmed by microscopic observations (Figure S2) of the cells treated with different concentrations of the polymer for 72 h.

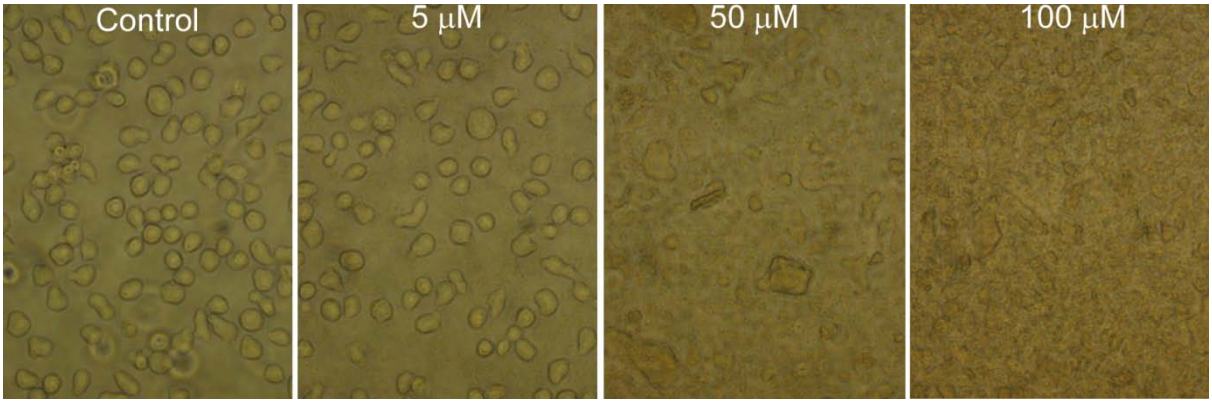

Figure S2. Microscopic pictures of Nalm-6 cells treated with, from left to right, 0 µM, 5 µM, 50 µM and 100 µM of **7** after 72h of incubation, 400× magnification.

3. *Measurement of membrane integrity by LDH-release assay*:

Cytotoxicity of **7** was measured by the release of lactate dehydrogenase (LDH) as described previously.[4] After incubation with different concentrations of the agents for 1 h, the LDH activity released by the Nalm-6 cells was measured in cell culture supernatants using the Cytotoxicity Detection Kit from Boehringer Mannheim (Mannheim, Germany). 20 µL of cell-free supernatants were diluted with 80 µL phosphate-buffered saline (PBS) and 100 µL reaction solution were added. Time-dependent formation of the reaction product was quantified photometrically at 490 nm. The maximum amount of LDH activity released by the cells was determined by lysis of the cells using 0.1% Triton X-100 in culture medium and set as 100% cell death. The results, depicted in **Figure S3**, show no detectable membrane disintegration caused by glycopolymer **7**.

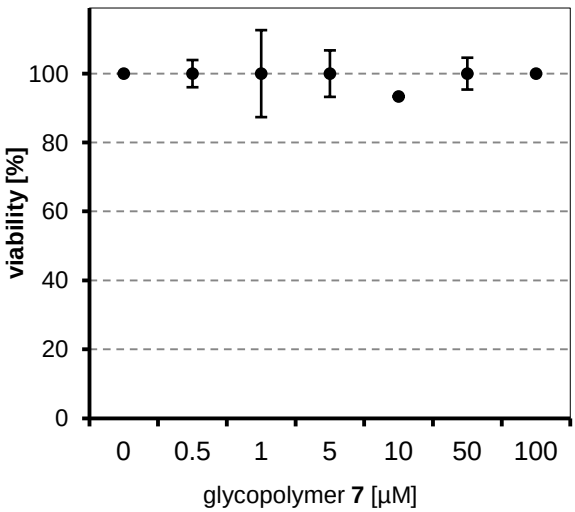

Figure S3. Results of cytotoxicity induction by glycopolymer **7**, measured by LDH-release assay.

4. *Immunization of Nalm-6 cells against Vincristine (VCR) and Daunorubicine (Dau)*:

Leukemia cells (NALM-6) were immunized to the established cytostatics Vincristine and Daunorubicine, respectively, by long term exposure and enrichment under low concentrations of each. Vincristine and Daunorubicine are transported out of the cell because of the expression of P-Glycoprotein after incubation with the cytostatic drugs. The comparison of wild type and Vincristine resistant as well as wild type and Daunorubicine resistant lymphoma Nalm-6 cells builds a model system to analyze the P-Glycoprotein dependent resistance mechanism in new agents.[5][13] A control batch of both resistant and non-resistant cells grown with 20 nM of VCR or 70 nM of Dau shows the resistance of the cells (Figure 5).

5. *Apoptosis induction assays*:

The same procedure was applied for the wild type and immunized Nalm-6. To quantify apoptosis induction a flow cytometric method, first introduced by Nicoletti, has been used.[6] DNA fragmentation measurements were carried out as described.[7] Nuclear DNA fragmentation was then quantified by flow cytometric determination of hypodiploid DNA content. Data were collected and analyzed using a FACScan (Becton Dickinson, Heidelberg, Germany) equipped with the CELLQuest software. Data are given in % hypodiploidy (subG1), which reflects the number of apoptotic cells. After 72 h of cultivation of wild type or immunized Nalm-6 cells in the presence of different concentrations of **7** or respective cytostatics, the apoptosis induction was determined. Three batches per concentration were analyzed. Depicted in Figure 4 and 5 are mean values and standard deviation.

Particle size determination.

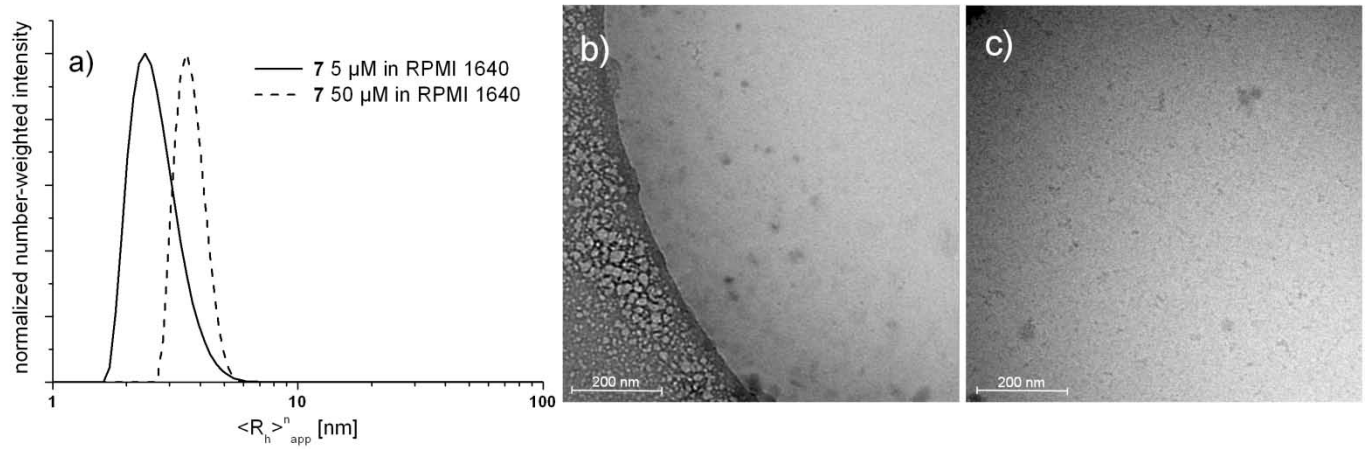

Figure S4. a) Number-weighted size distribution obtained by DLS from a 5 µM and 50 µM solution of **7** in RPMI 1640 medium. Cryo-TEM images obtained from a b) 5 µM and c) 50 µM solution of **7** in RPMI 1640 medium.

References.

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