Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

Novel polymer-based elemental tags for sensitive bio-assays

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EXPERIMENTAL DETAILS

Instrumentation

Polymer molar mass and mass distribution was measured with a Viscotek gel-permeation chromatograph equipped with a Viscotek VE3210 UV/vis detector and a VE3580 reflective index detector and Viscotek GMHHR-M Viscogel™ GPC column was used. The flow rate was maintained at 0.5 mL min-1 using a Viscotek VE1121 GPC pump. 1-Methyl-2-pyrrolidinone containing 0.2 wt% of lithium chloride was used as the eluent. The molecular weights were determined as polystyrene equivalents. ^{1}H NMR spectra were recorded at 400 MHz using a Varian XL400 spectrometer, using deuterated chloroform or deuterium oxide as solvent.

Elemental analysis of metal-tagged samples was carried out with a commercial Inductively Coupled Plasma Mass Spectrometer (ICP-MS) ELAN DRCPlus™ (PerkinElmer SCIEX) described elsewhere[1-3] and operated under normal plasma conditions. The sample uptake rate was 150 μl/min. A MicroFlow PFA-ST concentric nebulizer (Elemental Scientific, Inc) was used in all instances with standard cyclonic spray chamber. Experiments were performed using an autosampler (Perkin Elmer AS 91) modified for operation with Eppendorf 1.5 ml tubes. Sample size was 150 μl. Standards were prepared from 1000 μg/mL PE Pure single-element standard solutions (PerkinElmer, Shelton, CT) by sequential dilution with high-purity deionized water (DIW) produced using a Elix/Gradient (Millipore, Bedford, MA) water purification system. The analyte detector signal was normalized to the detector signal of the internal standard 1 ppb of Ir added during sample preparation to eliminate a long term instrumental drift. This signal referenced to the internal standard is referred to in the text as the “normalized response”.

Cell labelling procedures

Materials. KG-1a, human acute myelogenous leukemia cell line, and THP-1, a human acute monocytic leukemia cell line, were purchased from ATCC. Cells were grown in alpha-MEM, supplemented with 10% FBS (HyClone) and 2 mM l-glutamine (Invitrogen), in a humidified incubator at 37 °C and 5% CO_2. Cells were split every 3–4 days and viability was checked with Trypan blue (90% viable). Monoclonal antibodies CD33, CD34, CD38, CD45, CD54 were purchased from BD Biosciences. Mouse immunoglobulins were from Biomeda Inc.

Antibody labelling. Antibodies were labeled with DOTA-containing polymer-tags **5** according to the following protocol. Prior to conjugation with the tag, antibodies were reduced using TCEP (tris(2-carboxyethyl) phosphine hydrochloride; Pierce) in 100 mM phosphate buffer (pH7.2) with 2.5 mM EDTA. After 30 min incubation at 37 °C, the antibody was separated from reducing agent with 30K centrifugal filter (Pall Nanosep), resuspended in 20 mM Tris-buffered saline (TBS, pH 7.0) at 1 mg/ml and combined with a 10-fold molar excess of polymer **5**. The antibody-tag conjugate was subsequently washed with 20 mM ammonium acetate (pH 6.0) buffer in the spin filters, combined with 5 mM $LnCl_3$ (Sigma-Aldrich) and incubated for 30 min at 37 °C. Specifically, CD33-polymer tag conjugate was incubated with Pr^{3+}; CD34 - with Tb^{3+}; CD38 - with Ho^{3+}; CD45 - with Eu^{3+}; CD54 - with Tm^{3+}; TBS was used for extensive washing of the antibody at the final stage. Mouse immunoglobulins (IgG) were labeled with polymer-tags and lanthanides as the primary antibodies to serve as indicators of non-specific background binding of mouse antibodies to live cells. Elemental-tagged antibodies were stored in TBS at a final concentration 0.5 mg/ml at 4 °C.

Antigen detection with elemental-tagged antibodies. Live cells were washed in phosphate buffered saline (PBS; pH 7.2), counted in a hemocytometer and distributed into triplicate eppendorf tubes at 1×10^6 cells per tube. Antibodies were diluted to 2-5 µg/ml in 100 µl PBS/10% serum and added to cell suspensions. After 30 min incubation at room temperature, the cells were washed several times with PBS by low speed centrifugation. Cells were fixed in 3.7% formaldehyde/PBS and stained with Rh^{3+}-containing DNA metallointercalator[16] for cell number normalization. Finally, washed cells were spun down and the cellular pellet was dissolved in ultra pure concentrated HCl (35%, Seastar Chemicals, Inc.). An equal volume of internal standard 1 ppb Ir/10%HCl solution was added to each tube, and samples were analyzed by ICP-MS.

Polymer Preparation

All chemicals were purchased from Sigma and used without further purification. The protected amino-functionalized DOTA monoamide derivative **2** was synthesized on the gram

scale via the procedure of André et al.[4] The 2,2'-(Ethylenedioxy)bis(ethylmaleimide) was obtained according to the procedure provided by literature procedures.[5]

Polymer synthesis. *N*,*N*-dimethylacrylamide (DMA) was distilled under reduced pressure to remove the inhibitor. *N*-acryloxysuccinimide (NAS) was synthesized as described in ref 6. *tert*-Butyl Dithiobenzoate (*t*-BDB) was synthesized by a one-step process as described in reference 7. Polymerization reactions were carried out on a Schlenck line. To prepare P(DMA-*co*-NAS) with a target composition of 60 mol % NAS groups, NAS (2.46 g, 14.5 mmol), DMA (0.96 g, 9.7 mmol), AMBN (16 mg, 0.09 mmol), *t*-BDB (54 mg, 0.26 mmol) were added into 45 mL of anhydrous DMF. The solution was degassed with three freeze-pump-thaw cycles and heated with continuous stirring at 60 °C for 16 h. Then the solution was cooled and precipitated in 400 mL diethyl ether. The collected solid was redissolved in 1,4-dioxane and precipitated in diethyl ether and then dried under reduced pressure to remove residual solvent. The yield of polymer was 87%.

Characterization of NAS groups. 50 mg (ca. 0.3 mmol eq. of NAS) of polymer **1** were disolved in of dichloromethane (2 mL) and reacted with of benzyl amine (64 mg , 0.6 mmol) at room temperature. The light pink color from the original solution disapeared and turned yellow indicating the cleavage of the end thiobenzoate groups. A white pecipitate was observed which corresponded to the N-hydroxysuccinimide (NHS) groups being released into solution. The organic layer was washed with dilute HCl (6 mL, 1.0 M) and with distilled water (6 mL) to remove excess benzylamine and NHS. The solution was concentrated under reduced pressure, and the polymer was precipitated with 3 mL of diethyl ether. The polymer was dried overnight at 60 °C under reduced pressure and characterized by ^{1}H-NMR in deuterated dichloromethane.

The ^{1}H-NMR spectrum for the purified polymer is shown in Figure S1. In this material, the t-butyl end groups appear as a broad doublet at δ 0.6-0.9 ppm (A). [In $CDCl_3$ (and in D_2O for water-soluble polymer derivatives), the t-butyl group appears as a relatively sharp singlet (c.f. Figure 3 of the text). In $CDCl_2$ and in DMSO-d_6, we observe the broader peaks seen in Figure S1.] The broad peak from δ 1.0-3.1 ppm corresponds to the protons from the backbone and those from the methyl groups of DMA. The broad peak between δ 3.75-4.65 ppm (B) and 6.50-7.75 ppm (C) correspond to the methylene groups and the benzene ring from the benzylamine pendant groups. The ratio of the integration for B and C with respect to A provides a measure of the average number of active esters per polymer. The calculations were performed assuming complete substitution of NAS groups by benzyl amine and one *t*-butyl end group per polymer chain. The average value obtained for the integration ratios B/A and C/A indicates 33 NAS groups per

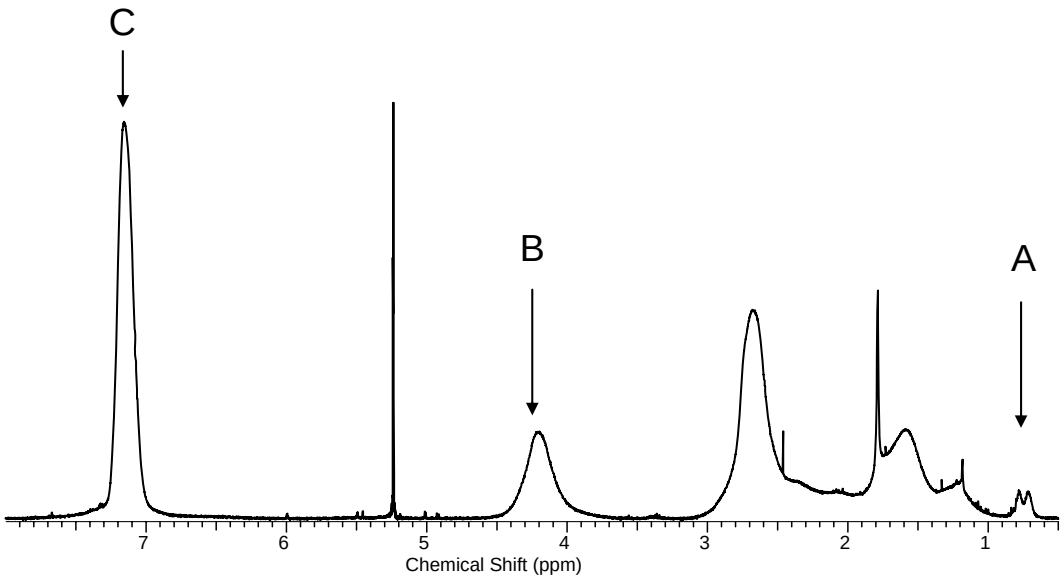

Figure S1. 1H-NMR of p(DMA-*co*-NAS) reacted with excess benzyl amine. Broad peak A corresponds to *t*-butyl end-group. Broad peaks B and C correspond to the methylene and benzylic protons, respectively, of benzyl amine. The number of active ester groups was derived from the ratio of the integration values for B/A and C/A.

polymer chains, which corresponds to 63 mol%. This value is consistent with ratio in the original monomer feed.

Polymer transformation by ligand attachment **6.** To a stirred solution of the co-polymer **1** (100 mg) in DMF (3 mL) and triethylamine (1 mL) was added a solution of amine pendant ligand **2** (363mg, 0.590 mmol) in DMF (2 mL). The reaction mixture was stirred overnight under nitrogen at room temperature. After the solvent was removed under vacuum, the residue **4** was dissolved in neat trifluoroacetic acid (3 mL) and stirred overnight at room temperature. The solution was evaporated, and the residue was taken up in water and dialyzed by repeated washings with deionized water (6x 5 ml) in an Amicon centrifugal filter (5K MW C. O.). The solution remaining in the filter device (ca. 0.8 mL) was concentrated to give a yellow solid **5** (179 mg).

The entire sample of polymer-ligand conjugate **5** was dissolved in 50 mM phosphate buffer (pH 8.5, 2 mL) containing 20 mM DL-dithiothreitol, and the reaction mixture was stirred for 1 h at 50^{o}C. After this time, the mixture was acidified to pH 4 with acetic acid, and washed in an Amicon centrifugal filter (5 K MW C. O.) with aqueous acetic acid (5 mM, 5×5 mL). The solution left in the filter device (0.8 mL) was then transferred to a small reaction flask containing phosphate buffer (100 mM, pH 8.5, 5 mL). A solution of 2,2'-

(Ethylenedioxy)bis(ethylmaleimide) (191 mg, 0.619 mmol) in DMF (2 mL) was added to the flask and the reaction mixture was stirred for 1 hour at room temperature. Water (3 mL) was added into the flask and the solid was removed by filtration. The resulting clear solution was again washed with deionized water (5X 5 mL) using an Amicon centrifugal filter (5K MW C. O) and the supernatant was purified by Sephadex G-50 Column with HPLC system using water as an eluent. The fraction was collected and lyophilized to give the final conjugated polymer **6** (165.0 mg).

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