Supporting Information

for

Selective modification of a native protein in a patient tissue homogenate using palladium nanoparticles

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Content

S1 GENERAL INFORMATION

Equipements

Infrared (IR) spectra were obtained as solid using a Fourier Transform Bruker Vector 22 spectrometer. Only significant absorptions are listed. The ^{1}H, and ^{13}CNMR were recorded using a Bruker Advance 300 (300 and 75 MHz, respectively) spectrometer. Recognition of methyl, methylene, methine, and quaternary carbon nuclei in ^{13}C NMR spectra rests on the *J*-modulated spin-echo sequence. All chemical shifts are quoted on the δ scale in ppm using residual solvent as the internal standard (^{1}H NMR: $CDCl_3$ = 7.26; D_2O = 4.79; DMSO-d6 = 2.50; D_3COD = 3.33, and ^{13}C NMR: DMSO-d6 = 39.5). Coupling constants (J) are reported in Hz with the following splitting abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet and m = multiplet. Mass spectra were recorded using a LTQ-Velos Pro Thermofisher Scientific spectrometer. The sizes of the obtained Pd nanoformulations were measured using a Malvern particle size analyser [nano ZS (173° scattering angle)]. The morphology of the different palladium nanoformulations was examined by transmission electron microscope (TEM) using a JEOL JEM 100CXII transmission electron microscope at an accelerating voltage of 100 kV and for high resolution image using a JEOL JEM 2010 instrument at 200 kV. Deionized water was used for chemical reactions and Milli-Q purified water for nanoparticle preparation and protein manipulations. Bidistilled MilliQ water was produced using a water purification system (Millipore). Images of protein gels including stain free gels, coumassie SDS-PAGE gel, stain free and western blotting membranes were taken on ChemiDoc MP Imaging System (Bio-Rad) or the MF-ChemiBis system (Berthold, Pforzheim). Proteomic analyses were performed either by Liquid chromatography-mass spectrometry (LC-MS) on a Micromass LCT (ESITOF- MS) coupled to an Agilent 1100 Series HPLC system or by liquid chromatography (nano-UPLC Ultimate 3000 RSLCnano, Thermo Fisher Scientific) combined with mass spectrometry (LTQ-Orbitrap, Thermo).

Cell Culture

The Raw 264.7 cell lines (Tebu-bio) were grown in DMEM (Dulbecco's modified Eagle's medium, from Sigma Aldrich) supplemented with 10% FBS (fetal bovine serum, from GIBCO) and 1% antibiotic antimycotic solution (Sigma Aldrich). The cells were cultured at 37 °C in a humidified air atmosphere containing 5% CO_2. The Raw cells formed a confluent monolayer within 5–7 days.

Primary thyrocytes were prepared according to the protocol described in the Literature[1,2] from thyroid biopsies and obtained from thyroidectomy in patients who gave their informed consent. The biopsies were washed successively with EtOH (10 mL/g of tissue) and twice with calcium- and magnesium free phosphate-buffered saline (PBS) (10 mL/g of tissue) before minced into small pieces. To separate connective tissue and thyrocytes, the samples were digested using digestion buffer (10 mL/g of tissue) containing collagenase type I (100.5 U/mL) (Sigma-Aldrich) and dispase II protease (2.35 U/mL) (Sigma-Aldrich) in PBS with glucose (2 mg/mL), pH 7.4, at 37 °C for 45 min. The digested tissues were filtered through sterile Corning™ filter (100 µm and 40 µm). PBS was added and cells were washed by centrifugations at 300 g for 5 min. Thyrocytes were allowed to adhere at 37 °C (in a humidified air atmosphere containing 5% CO_2) in a DMEM-F12 (Sigma Aldrich) culture medium containing 5% of FBS complemented with 6 growth factors: Transferin (6 µg/mL) (Sigma-Aldrich), hydrocortisone (5 nM) (Sigma-Aldrich), thyreostimulin (1 µU/mL), insulin (10 µg/mL) (Sigma-Aldrich), somatostatin (10 ng/mL) (Sigma-Aldrich) and glycine/L-histidine/L-lysine (10 ng/mL) (Sigma-Aldrich). After 24 h, the remaining blood cells were removed with warm PBS. The cells were cultured at 37 °C in a humidified air atmosphere containing 5% CO_2. The primary culture cells formed a confluent monolayer within 7–9 days.

The Fisher rat cell lines FRTL and FRTL5 (European Collection of Authenticated Cell Cultures) were grown according to the Ambesi-Impoombato protocol[3] at 37 °C in an humidified air atmosphere containing 5% CO_2 in DMEM-F12 culture medium containing 5% of FBS supplemented with glutamine (Sigma Aldrich) (10 µg/mL), insulin (10 µg/mL), (Sigma Aldrich), hydrocortisone (10 nM) (Sigma Aldrich), transferrin (5 µg/mL) (Sigma Aldrich), somatostatin (10 ng/mL) (Sigma Aldrich), glycyl-L- histidyl-L-lysine acetate (10 ng/mL) (Sigma Aldrich) and thyreostimulin (10 mUnits/mL) (Sigma Aldrich). For FRTL cell line, the medium was completed with 30 µM or 1 mM of NaI.

The prostate cancer-3 (PC-3) cell line (European Collection of Authenticated Cell Cultures) was grown in Coon′s modified Ham′s F12 medium (Sigma Aldrich) containing 5% FBS supplemented L ascorbic acid (45 mg/L) (Sigma Aldrich), Inositol (18 mg/L) (Sigma Aldrich), Glutamine (2mM). The cells were cultured at 37 °C in a humidified air atmosphere containing 5% CO_2. The PC-3 cell formed a confluent monolayer within 7–9 days.

Chemicals and Solvents

Chemicals were obtained from Carbosynth Limited (UK), Sigma Aldrich Chemical Co (France) Fluorochem and Alfa Aesar (France) and were used without further purification. Solvents were obtained from VWR. Phosphate Buffer Saline (PBS) pH 7.4 with and without Ca^{2+} and Mg^{2+} were obtained from Sigma Aldrich. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl and CH_2Cl_2 from CaH_2. All reactions involving air- or water-sensitive compounds were routinely conducted in glassware, which was flame-dried under a positive pressure of nitrogen or argon. Analytical thin-layer chromatography was performed on Merck silica gel 60F254 glass precoated plates (0.25 mm layer). Column chromatography was performed on Merck silica gel 60 (230e400 mesh ASTM (American Standard Test Sieve Series).

S2 PREPARATION AND CHARACTERIZATIONS OF Pd-NPs

The influence of the Pd/CPCl ratio, as well as, the reduction of the amount of CPCl by dialysis and ligand exchange on the shape, reactivity and cytotoxicity of the NPs was systematically investigated.

Preparation of the palladium urchin nanoparticles (Pd-Urchin NPs) Pd:CPCl, 1:1, 5:1 or 10:1.

In a 25 mL volumetric flask, cetylpyridinium chloride (CPCl) (850 mg, 2.5 mmol) was dissolved in Milli-Q water to provide a 100 mM stock solution. In a 10 mL glass vial, $(NH_3)_4Cl_2Pd.H_2O$ (131.7 mg, 0.5 mmol) was dissolved in appropriate volume of the CPCl stock solution and Milli-Q water depending on Pd:CPCl molar ratio, ie. 1:1, 5:1 or 10:1. The solution was degassed under nitrogen for 10 min. Palladium was then reduced upon γ-irradiation using the panoramic gamma source of ^{60}Co used for radiolytic synthesis at the maximum dose rate of 4.0 $kGy.h^{-1}$ for 11 h, as described by Ksar et al.[4] to provide a stock solution of Pd-Urchin NPs (100 mM of Pd).

Dialysis of the palladium urchin nanoparticles (Pd-Urchin NPs) 1:1 Pd:CPCl.

500 µL of 1:1 Pd:CPCl nanoparticles were placed in 3.5 KDo Slide-A-Lyzer ® for dialysis against water for 3 days at room temperature (25 °C) with dialysis water changed twice a day. Sample was removed from the cassette to provide a stock solution of dialyzed Pd-Urchin NPs.

Preparation of the palladium PVP nanoparticles (PVP-Pd NPs).

In a 25 mL volumetric flask, polyvinylpyrrolidone (PVP,5 kDa) (125 mg, 0.025 mmol) was dissolved in Milli-Q water to provide a 1 mM PVP stock solution before $(NH_3)_4Cl_2Pd.H_2O$ (131.7 mg, 0.5 mmol) was dissolved in 5 mL of this PVP stock solution. The solution was then degassed under nitrogen for 10 min and the palladium was reduced upon γ-irradiation as described above to provide a stock solution of palladium nanoparticles (100 mM of palladium, molar ratio 100:1 Pd/PVP).

Nanoparticles characterization by dynamic light scattering (DLS)

Mean particle size and polydispersity index were measured at 25 °C by dynamic light scattering with a Nano ZS (Malvern Instrument, 173 scattering angle). The NPs surface charge was investigated by ζ-potential measurement with the Nano ZS instrument at 25 °C after dilution with 1 mM NaCl solution, applying the Smoluchowski equation. Measurements were carried out in triplicate. Colloidal stability in milliQ water at 20 °C was investigated by measuring the Pd NPs mean diameter over a period of 1 month (Fig. S1 F)

Nanoparticles imaging by Transmission Electron Microscopy (TEM)

The morphology of the different palladium nanoformulations was examined by transmission electron microscopy (TEM). Briefly, 5 µL of the Pd NPs suspension (1 mM) were deposited on a Lacey Formvar/carbon 300 mesh copper microscopy grid (Ted Pella). Most of the drop was removed with a blotting filter paper. Images were obtained using a JEOL JEM 100CXII transmission electron microscope at an accelerating voltage of 100 kV and for high resolution image, using a JEOL JEM 2010 instrument at 200 kV (Fig. S1 A-E).

As depicted in Table S1 and Fig.S1 A,B, the initial 1:1 and the 5:1 Pd/CPCl mixtures provided well-defined urchin-shape NPs upon γ–irradiation according to transmission electronic microscopy (TEM). Reduction of the amount of CPCl led to NPs with slightly smaller hydrodynamic diameter but did not affect their overall stability. However, when less than 10% of CPCl were used the nanosuspension became unstable and TEM images revealed

that the urchin-shape was partially lost, giving rise to large islets of aggregated small Pd nanobeads (Fig. S1C). Dialysis of the 1:1 Pd/CPCl NPs did not affect the shape of the NPs (Fig.S1D)

S3. CELL VIABILITY STUDIES ON RAW264.7 AND PRIMARY THYROCYTES

The cytotoxicity of Pd NPs was determined using the 3-[4,5-dimethylthiazol-2-yl]-3,5-diphenyl tetrazolium bromide (MTT) assay, measuring mitochondrial dehydrogenase activity. Thyrocytes (10,000 cells per well in DMEM-F12 with 5% of FBS and 6 hormones) or Raw 264.7 cells (5,000 cells per well in DMEM with 10% FBS) were seeded in a 96-well plates at the appropriate cell concentration. Cells were allowed to adhere for 24 h at 37 °C. Then, fresh medium containing Pd NPs (0.01 μM to 1 mM) was added and incubated for 24 h. MTT cell viability reagent (5 mg/mL; 20μL) was added to each well and the plates were incubated for 24 h at 37 °C. After 2 h incubation, the culture medium was removed, and the formed formazan crystals were dissolved in DMSO (200 μL). Absorbance was measured using microplate reader (Metertech Σ 960, Fisher Bioblock, Illkirch, France) at 570 nm. The percentage of surviving cells was calculated as the absorbance ratio of treated to untreated cells. (See Fig. S1H-I)

S4 SUZUKI–MIYAURA CROSS COUPLING REACTION WITH IODINATED AMINOACIDS CATALYZED BY Pd-NPs

To a suspension of the iodinated aminoacid (0.008 mmol) and boronic acid derivative (0.024 mmol, 3 equiv.) in Milli-Q water (370 μL), was added phosphate buffer pH 8.0, 7.0 or 6.0 (200 mM, 50 μL) and the suspension of Pd NPs (0.00008 mmol Pd, 80 μL, 0.01 eq.). The reaction was mixed and shaken on a thermostated-shaker (bioSan TS-100) at 800 rpm (37 °C). After 2h, the reaction mixture was frozen in liquid nitrogen and freeze-dried. The crude product was taken up in MeOD and analyzed by ^{1}H-NMR. Results with all formulation are depicted in Table S1. Typical ^{1}H NMR spectra of the obtained mixture in comparison with *N*-Boc-4-iodophenylalanine are depicted in Fig. S2 and S3.

S5 SUZUKI-MIYAURA CROSS COUPLING REACTION WITH BOVINE THYROGLOBULIN CATALYSED BY PVP-Pd NPs

Chemical reaction on bovine thyroglobulin catalyzed by palladium NPs.

To a solution of commercially available bovine thyroglobulin (50 µL, 7.4 mg/mL, 1.21 nmol, 1 equiv.) (Sigma Aldrich) in PBS (Sigma Aldrich) pH 7.4, was added a solution of the biotin cyclic trioborate salt derivatives (0.366 mg, 605 nmol, 500 equiv.) in 16 mg/mL aqueous Na_2HPO_4 (7.5 µL). The stock suspension of PVP-Pd NPs (7.5 µL, 8 mM, 60.5 nmol, 50 equiv.) was then added and the mixture was shaken at 37 °C. After 24 h incubation, 30 µL of the reaction mixture were diluted with 30 µL of Laemmli blue 2x (Bio-RadRad), and the sample was heated at 95 °C for 3 min and cooled at 4 °C before separation by SDS-PAGE gels (Fig. S4).

Sample preparation for proteomic analysis

Bovine Tg sample from the reaction described previously was resolved by one dimensional SDS-PAGE (Bio-Rad). The gels were stained with Coomassie Blue (EZ Blue ®) (Sigma Aldrich). Proteins, 2.5 mm top to bottom bands at 250 kDa, were cut and then treated with 100 µL of 100 mM dithiothreitol (DTT) at 56 °C for 45 min to reduce disulfide bonds. After a rapid wash with acetonitrile and a solution of NH_4HCO_3 in MilliQ water (25 mM), a solution of iodoacetamide (100 µL, 55 mM) was added, and the samples were left in the dark for 30 min for cysteine alkylation. Finally, dry gel pieces were rehydrated with 15 µL of 12 ng/µl of Sequencing Grade Modified Chemotrypsin solution (1 mg, Sigma Aldrich) in HCl (1 M) and incubated at 37 °C overnight. Peptides resulting from digestion were extracted with 20 µL of 60% CH_3CN solution enriched with 0.1% HCO_2H. The samples were further analyzed by MS/MS-MS.

Mass spectroscopy of Tg Bovine

4 µL of the peptides from the digested Bovine Tg sample were loaded at 20 µL/min through a C18 enrichment cartridge (PepMap, 75 µm × 15 mm, Thermo Scientific) and a C18 reversed-phase column (Pepmap, 75 µm x 50 mm, Thermo Scientific) kept at 40 °C. Capillary pump flow was set at 0.3 $\mu L.min^{-1}$, with 3% of aqueous solvent A (97.9% H_2O, 2% CH_3CN, 0.1% HCO_2H) and 97% of solvent B (80% CH_3CN, 19.9 %H_2O, 0.1% HCO_2H,). Nano pump flow rate was set at a 300 $nL.min^{-1}$ during the 53 min run. Starting at 1% of solvent B, elution was performed by a two-step linear gradient of solvent B percentage, from 1 to 35% over 47 min,

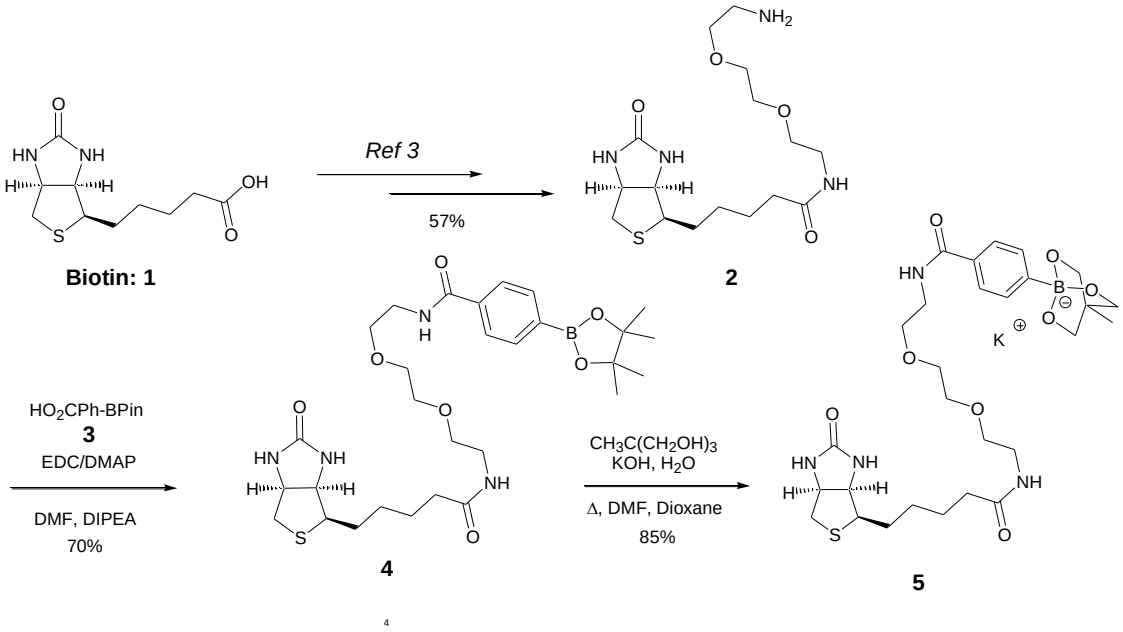
followed by a quick wash of 98% solvent B and next by re-equilibration of the column. Eluted peptides were sprayed by nanospray ion source into the mass spectrometer in positive polarity mode. Peptide ions were analyzed using nano-UPLC. Full-scan mass spectra were measured from m/z 300 to 1400 ±10 ppm. Auto MS2 was activated for charge state +2 and +3 ions, allowing MS/MS scans on the 6 most abundant precursor ions with dynamic exclusion of previously selected ions. The activation type was collision induced dissociation (CID) with MS/MS normalized collision energy set at 35%.

Data processing

Peak lists were generated as mzXML files using MSConvert softaware (ProteoWizard 3.0). Mass spectrometric data were searched using X!TandemPipeline software version 0.2.24. Peptide searches were performed with the following parameters: a mass tolerance of 10 ppm on the parent ion and 0.5 Da for the MS/MS, precursor mass tolerance of ±10 ppm and fragment mass tolerance of 0.5 Da, static modifications of carbamidomethylated cystein (+57.0215), potential modification of oxidized methionine (+15.9949), potential tyrosine post-translational modification: +125.8966 for monoiodotyrosine (MIT); +251.7933 for diiodotyrosine (DIT); +76.0313 for monophenylated-tyrosine; +201.9229 for monoiodo-monophenylated-tyrosine; + 152.0626 for diphenylated-tyrosine. Following chymotrypsin digestion, the maximum number of miss cleavages was set at 3. Bovine (and further rat and human) thyroglobulin sequences were downloaded from UniProt KB: sp|P01267|THYG_BOVIN to allow identification of protein. Under combined analysis mode, all peptides matched with a peptide E-value set at 0.05, allowing identification of each Tg with more 90% coverage.

S6 SYNTHESIS AND CHARACTERIZATION OF THE BIOTIN-CYCLIC TRIOLBORATE PROBE

The synthesis of the cyclic triolborate probe **5** was performed according to the Scheme S1. Briefly, the biotin amine **2** obtained from biotin according to the procedure described by Stewart *et al.*[5] was first coupled with 4-carboxyphenyl boronic acid pinacol ester (**3**) using EDC/DMAP standard coupling conditions. Condensation of boronic acid pinacol ester **4** with 2-(hydroxymethyl)-2-methylpropane-1,3-diol in basic media afforded cyclic triolborate probe **5** in 34% overall yield.



Scheme 1. Synthetic scheme of the biotine cyclic triolborate probe **5**

Synthesis of 5-[(3a*S*,4*S*,6a*R*)-2-oxo-hexahydro-1H-thieno[3,4-d]imidazolidin-4-yl]-N-{2-[2-(2-{[4-(tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]formamido}ethoxy)ethoxy] ethyl} pentanamide (4). To a solution of diisopropylamine (173 µL, 1.06 mmol, 2 equiv.) and ethyl *N*-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (152.4 mg, 0.8 mmol, 1.5 equiv.) in DMF (2 mL) was added dropwise a solution of 4-carboxyphenylboronic pinacol ester (**3**) (156 mg, 0.63 mmol, 12 equiv.) in anhydrous DMF (2 mL). The mixture was stirred for 15 min at room temperature. A solution of biotin-PEG-NH_2 **2** [2] (200 mg, 0.53 mmol, 1 equiv.) and DMAP (6.5 mg, 0.053 mmol, 0.1 equiv.) in DCM (10 mL) was then added and the resulting mixture was stirred at room temperature. After 48h, the solvent was removed under reduced pressure. The residue was washed with diethtyl ether to provide the crude pinacol boronate **4** as a pale yellow pasty solid (223 mg, 70%) which was used directly in the next step. ^{1}H NMR (300 MHz, MeOD) δ: 7.83 (s, 4H, CH_{Ar}), 4.46 (dd, J = 7.5, 4.8 Hz, 1H, HNCONHC*H*), 4.28 (dd, J = 7.8, 4.5 Hz, 1H, HNCONHC*H*), 3.72-3.62 (m, 6H, CH_2O), 3.60 (t, J = 5.4 Hz, 2H, CH_2O), 3.56 (t, J = 5.7 Hz, 2H, CH_2NH), 3.38-3.34 (m, 2H, CH_2NH), 3.24-3.15 (m, 1H, C*H*S), 2.92 (dd, J = 12.8, 5.0 Hz, 1H, CH_2S), 2.71 (d, J = 12.8, 1H, CH_2S), 2.20 (t, J = 7.5 Hz, 2H, CH_2CON), 1.80-1.50 (m, 4H, CH_2CH_2CH_2CH_2CON) 1.47-1.38 (m, 2H, CH_2CH_2CH_2CON), 1.37 (s, 12H, (CH_3)$_2$CC(CH_3)$_2$) ppm (Fig. S6); ^{13}C NMR (75 MHz, MeOD) δ: 174.70 (2C, *C*ON), 136.70 (C, C_{Ar}), 134.33 (CH, *C*H_{Ar}), 126.07 (CH, *C*H_{Ar}), 84.03 (2C, (CH_3)$_2$*C*O), 69.92 (2CH_2, O*C*H_2), 69.20 (2CH_2, O*C*H_2), 61.94 (*C*H, HNCONH*C*H), 60.20 (*C*H, HNCONH*C*H), 55.53 (CH, *C*HS), 39.61 (CH_2, *C*H_2S), 39.5 (CH_2, *C*H_2NCO),

38.87 (CH_2, *C*H_2NCO), 35.32 (CH_2, *C*H_2CON), 28.29 (CH_2, *C*H_2CH$_2$CON), 28.06 (CH_2, *C*H_2CH$_2$CH$_2$CON), 25.37 (CH_2, *C*H_2CH$_2$CH$_2$CH$_2$CON), 23.8 (4CH_3, (*C*H_3)$_2$CC(*C*H_3)$_2$) ppm , the C-B was not detected (Fig. S7); MS (ESI+): *m/z* (%) = 643.2 (100) $[M + K]^+$, 627.3 (10) $[M + Na]^+$, 605.3 (35) $[M + H]^+$, 485.2 (45) $[M - C_6H_{10}N_2OS + K]^+$.

Synthesis of potassium 4-methyl-1-(4-(12-oxo-16-((3a*R*,4*R*,6a*S*)-2-oxohexahydro-1*H*-thieno[3,4-d]imidazol-4-yl)-5,8-dioxa-2,11-diazahexadecyl)phenyl)-2,6,7-trioxa-1-borabicyclo[2.2.2]octan-1-uide (5). To a solution of pinacol boronate **4** (50 mg, 0.089 mmol, 1 equiv) in DMF/DMSO (1:1, 500 µL) was added 2-(hydroxymethyl)-2-methylpropane-1,3-diol (10.7 mg, 0.089 mmol, 1 equiv.), KOH (5 mg, 0.089 mmol, 1 equiv.) and water (2 µL, 0.089 mmol, 1 equiv.). The reaction mixture was stirred at 30 °C under argon atmosphere. After 4h, the mixture was added dropwise to diethyl ether (10 mL) in a Falcon® tube. The precipitate was centrifuged (4000 rpm, 10 min), and after supernatant removal, it was taken up into acetone and centrifuged once more (4000 rpm, 10 min). Acetone was then removed and the solid was dried under vacuum to provide the cyclic triolborate **5** as a white powder (48 mg; 85%). ^{1}H NMR (300 MHz, DMSO-d6) δ 8.14 (t, *J* = 4.8 Hz, 1H, CON*H*), 7.80 (t, *J* = 5.2 Hz, 1H, CON*H*), 7.51 (d, *J* = 7.6 Hz, 2H, CH_{Ar}), 7.39 (d, *J* = 7.6 Hz, CH_{Ar}), 6.38 (s, 1H, NHCON*H*), 6.35 (s, 1H, NHCON*H*), 4.28 (br t *J* = 6.57 Hz, 1H, HNCONHC*H*), 4.11 (m, 1H, HNCONHC*H*), 3.58 (s, 6H, C(C*H*$_2$O)$_3$B), 3.52 (s, 6H, OC*H*$_2$C*H*$_2$O), 3.42-3.32 (m, 4H, C*H*$_2$O, C*H*$_2$NCO), 3.16 (t, *J* = 5.8 Hz, C*H*$_2$NCO), 3.10-3.02 (m, 1H, C*H*S), 2.77 (dd, *J* = 12.3, 7.2 Hz, 1H, C*H*$_2$S), 2.50 (m, 1H, C*H*$_2$S), 2.05 (t, *J* = 7.2 Hz, 2H, C*H*$_2$CONH), 1.70-1.20 (m, 6H, C*H*$_2$C*H*$_2$C*H*$_2$CH$_2$CONH), 0.49 (s, 3H, C*H*$_3$) ppm (Fig. S8); ^{13}C NMR (75 MHz, DMSO-d6) δ 172.1 (C, *C*ON), 167.4 (C, *C*ON), 162.7 (C, N*C*ON), 131.8 (CH, *C*H$_{Ar}$), 130.5 (C, *C*$_{Ar}$), 124. 5 (CH, *C*H$_{Ar}$), 73.1 (3CH_2, C(*C*H$_2$O)$_3$B), 69.5 (CH_2, O*C*H$_2$), 69.4 (CH_2, O*C*H$_2$), 69.1 (CH_2, O*C*H$_2$), 68.9 (CH_2, O*C*H$_2$), 61.0 (CH, OCHN*C*H), 59.2 (CH, OCHN*C*H), 55.3 (CH, *C*HS), 38.4 (CH_2, CH$_2$*C*H$_2$NH), 35.0 (CH_2, CH$_2$*C*H$_2$NH), 34.6 (CH_2, *C*H$_2$CON), 28.1 (CH_2, *C*H$_2$CH$_2$CON), 28.0 (CH_2, *C*H$_2$CH$_2$CH$_2$CON), 25.2 (CH_2, *C*H$_2$CH$_2$CH$_2$CH$_2$CON), 16.2 (CH_3, *C*H$_3$C(CH$_2$O)$_3$B) ppm; the C-B was not detected and both the *C*H$_2$S methylene and the quaternary carbon of the triol were masked by the solvent pics (Fig. S9); MS (ESI$^-$): *m/z* (%) = 605.2 (100) $[M]^-$, 567 (30) ([M - $CH_3C(CH_2OH)_3$+ H_2O+ 2MeOH]$^-$, 5212 (23) ([M - $CH_3C(CH_2OH)_3$+ 2H_2O]$^-$; HRMS (–ESI) calcd for $C_{28}H_{42}BN_4O_8S^-$: 605.2816, found 605.2798; IR (neat, cm^{-1}): 3262, 3120, 2928, 2853, 1654, 1647, 1456, 1356, 1360, 1308, 1221, 1181, 1072, 973, 930, 837, 760.

S7. ANALYSIS OF THE MODIFIED BOVINE THYROGLOBULIN BY WESTERN BLOT

Western Blotting with primary and secondary anti-bodies for the detection of Thyroglobulin

The separated proteins from SDS-PAGE gels, were transferred to nitrocellulose. Membranes were saturated in 5% non-fat milk TRIS buffered saline TBS-Tween 0.1% (Bio-Rad) for one hour at room temperature and incubated with primary antibody (goat Anti-Tg from Santa Cruz BiotechnologyN15 1:200 in 25 mL of blocking solution) overnight at 4 °C. After washing (3 x 20 min TBS-Tween 0.1%), horseradish peroxidase (HRP) secondary antibody (Donkey anti-goat from Santa Cruz Biotechnology 1:5000 25 mL of blocking solution) was added for 1 h at room temperature. After washing (3 x 20 min TBS-Tween 0.1%), detection of the modified Tg was achieved using the Clarity Western ECL Substrate (Bio Rad). Chemiluminescent signals were analyzed using the ChemiDoc™ (Fig. S8-C).

Western Blotting with streptavidin for the detection of cross coupling reaction between Biotin probe and Thyroglobulin

The separated proteins from SDS-PAGE gels were transferred to nitrocellulose. Membranes were saturated in 5% non-fat milk TRIS buffered saline TBS-Tween 0.1% (Bio-Rad) for one hour at room temperature and incubated with streptavidin HRP (from Santa Cruz Biotechnology 1:10000 25 mL of blocking solution) overnight at 4 °C. After washing, detection was achieved using the Clarity Western ECL Substrate (Biorad). Chemiluminescent signals were analyzed using the ChemiDoc™ (Fig. S8-D).

S.8 PRODUCTION OF NATURALLY IODINATED THYROGLOBULIN

Thyroglobulin detection in cellular lysate

3’000’000 FRTL, FRTL5 or PC-3 cells were lysed in 60 µL of RIPA Lysis and Extraction buffer (Thermo Fisher Scientific) with protease inhibitors (Roche Applied Science) at 0 °C for 30 min. The mixture was centrifuged 10 min at 10’000 rpm. The total protein content was determined by bicinchoninic acid BCA assay .The cellular lysate was diluted in PBS buffer 25 µL and 25 µL of Laemmli buffer 2x (Bio-Rad) to provide a 1 µg/µL solution of total proteins and then heated to 95 °C for 3 min. 30 µL of each cellular lysate and rat thyroid homogenate sample (12 µL for Bovine Tg) were separated by SDS-PAGE on 4-15% Bis–Tris polyacrylamide gel (Bio-Rad) with SDS 1x running buffer. The proteins were revealed with

EZ-Blue© (Sigma Aldrich) stain and then extensively washed with water. The separated proteins from SDS-PAGE gels, were transferred to Polyvinylidene difluoride PVDF membranes 0.2 µm (Bio-Rad), and blots were incubated in blocking solution containing 5% milk and 0.1% Tween 20 (Bio-Rad) in Tris-saline buffer, pH 7.6 (TBST) overnight at 4 °C. The membranes were then incubated with primary antibody (1:350) Goat Anti-Tg N15 in 25 mL of blocking solution. Blots were washed five times in TBST at room temperature for 10 min. Afterwards, the membranes were incubated with a secondary antibody (1:5000) Donkey AntiGoat (Ati Tg) Ig conjugated to HRP in 25 mL of blocking solution. Blots were washed five times at room temperature in TBST for 10 min and the blotting proteins were detected after 4 min membrane incubation with the detection solution (Clarity™ Western ECL Substrate). Chemiluminescent signals were analyzed using the MF-ChemiBis system (Fig.S11).

Preparation of Rat Thyroid homogenate

Thyroid rat homogenates were prepared according to the protocol describe by Römisch-Margl, W. *et al.*[6] Frozen samples from thyroid gland arising from three sacrificed rats were placed into 1.5 mL Eppendorf test tubes® containing MilliQ water with Complete™ Protease Inhibitor Cocktail (Roche Complete without EDTA) to a concentration of approximately 6 µL water/mg thyroid. The mixture was homogenized three times using an inox Turbospin at 6000 rpm for 30 s, with interval of 30 s on ice between each homogenization steps. The mixture was then centrifuged at 10000 rpm for 10 min at room temperature and the supernatant was recovered. The total protein concentration was determined by BCA Protein Assay (Thermo Fisher Scientific).

Thyroid homogenization from patient tissue

Human thyroid homogenates were prepared according to the protocol described above for rat thyroid homogenate. Frozen samples from thyroid gland arising from surgical thyroidectomy of three Grave's disease patients (50–150 mg) were placed into 15 mL Falcon tubes® containing MilliQ water with Complete™ Protease Inhibitor Cocktail (Roche Complete without EDTA) to a concentration of approximately 6 µL water/mg thyroid. The mixture was homogenized three times using an inox Turbospin at 6000 rpm for 30 s, with interval of 30 s on ice between each homogenization steps. The mixture was then centrifuged at 10000 rpm for 10 min at room temperature and the supernatant was recovered. The total protein

concentration was determined by BCA Protein Assay and thyroglobulin concentration by (Tg) ELISA Kit.

Sample Preparation for proteomic analysis

Cellular lysate from FRTL and human thyroid homogenate were analyzed by proteomic according the protocol described for bovine Tg.

Data processing

Data processing were performed in a similar manner as for bovine Tg. Rat and human thyroglobulin sequence were downloaded from UniProt KB: sp|P06882|THYG_RAT, sp|P01266|THYG_HUMAN to allow identification of protein. Under combined analysis mode, all peptides matched with a peptide E-value set at 0.05, allowing identification of each Tg species with more 90% coverage.

S9. SUZUKI-MIYAURA CROSS COUPLING OF HUMAN Tg CATALYZED BY PVP-Pd NPs IN PATIENT TISSUE HOMOGENATE

Chemical reaction in human thyroid gland homogenate catalyzed by palladium NPs

To a solution of thyroglobulin from human thyroid homogenate (50 µL, 7.4 mg/mL, 1.21 nmol, 1 equiv.) in pH 7.4 PBS was added a solution of the cyclictrioborate salt derivative (0.147 mg, 605 nmol, 500 equiv.) in 16 mg/mL aqueous Na_2HPO_4 (7.5 µL). Stock suspension of PVP-Pd NPs (7.5 µL, 8 mM, 60.5 nmol, 50 equiv.) was then added and the mixture was shaken at 37 °C. After 24 h incubation, 30 µL of reaction mixture were diluted into 30 µL of Laemmli blue 2x, and the sample was heated at 95 °C for 3 min and cooled at 4 °C before separation by SDS-PAGE gels.

S10. SUPPLEMENTARY TABLES

Table S1. Suzuki-Miyaura cross-coupling reactions in water between iodinated amino acids and phenyl boronic acid derivatives catalyzed by Pd NPs at different pH

N°	Catalyst	RI	Organoboron reagent	pH	%Conv[a]
1	CPCl :Pd ; 1:1	1	3	8.0	48
2	CPCl :Pd ; 1:1	2	3	8.0	70
3	CPCl :Pd ; 1:1	2	4	8.0	90
4	CPCl :Pd ; 1:1	1	4	8.0	98
5	CPCl :Pd ; 1:1	1	4	7.0	76
6	CPCl :Pd ; 1:1	1	4	6.0	53
7	CPCl :Pd ; 1:5	1	4	8.0	98
8	CPCl :Pd ; 1:10	1	4	8.0	98
9	CPCl :Pd, 1 :1; dialyzed	1	4	8.0	98
10	PVP :Pd ; 1 :100	1	4	8.0	98
11	PVP :Pd ; 1 :100	1	4	7.0	98
12	PVP :Pd ; 1 :100	1	4	6.0	98

1 equiv. R-I, 3 equiv. R-B$(OR)_3$, 1 mol% Pd NPs, PBS, 37 °C, 2 h [a] Conversions were determined by ^{1}H NMR spectroscopy on the crude reaction mixture analyzing the C-3 methylene signal of the *N*-Boc amino-acids (Fig. S2 and S3).

Table S2 Iodinated and phenylated tyrosines in bovine Tg

Residues	Iodinated peptides detected	Phenylated peptides detected
Y24	Thyr.	Not detected
Y382	D	MP
Y703	D	MP, DP
Y784	M, D	Not detected
Y809	M, D	MIP, MP, DP
Y839	M, D	MP, DP
Y879	D	DP
Y883	M, D	MP, DP
Y945	M	MP
Y1027	D	Not detected
Y1253	M	M
Y1310	M, D	MP, DP
Y1394	M, D	MP
Y1469	M	M
Y1503	M, D	MP, MIP, DP
Y1679	D	D
Y1952	D	D
Y2040	M, D	M, D
Y2053	M	Not detected
Y2183	M, D	M, D
Y2185	D	DP
Y2336	Not detected	MIP
Y2488	M	MP, DP
Y2541	M, D	M
Y2574	M, D, Tyr	MP, DP
Y2588	M, D	MP, D
Y2618	M, D	MP, D
Y2644	M, D	M, D
Y2767	D, Tyr	D

M: mono-iodinated, D, Diiodinated, MP: monophenylated, DP: diphenylated;
MIP Monoiodinated monophenylated,

Table S3 Iodinated peptides detected in Bovine, Rat and Human thyroglobuline

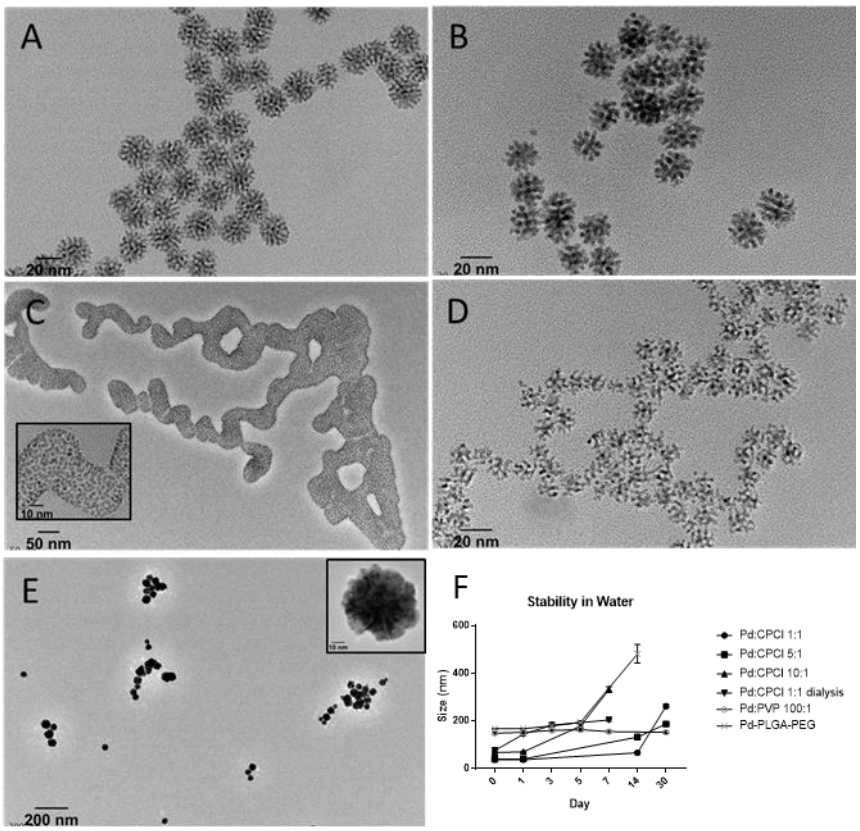
Protein	Iodinated Peptides detected
Tg Bovine (purified)	32
Tg Ratus (from FRTL cells)	1
Tg Human (from homogenate)	19

Table S4 Iodinated and phenylated tyrosines in human Tg

Residues	Iodinated peptides detected	Phenylated peptides detected
Y24	MI, DI	
Y48	Not detected	MIP
Y108	Not detected	DP
Y116	MI	
Y126	MI	
Y149	MIT, DIT	
Y211	Not detected	MP
Y277	Not detected	MP
Y626	MI	
Y785	MI	
Y866	MI	
Y1027	Not detected	DP, MP
Y1445	Not detected	MP
Y1310	MI	
Y1677	MI,	
Y1705	DI	
Y2157	MI	MIP, MP
Y2184	MI	
Y2283	MI	
Y2335	DI	
Y2478	MI	MP
Y2540	MI, DI	DP
Y2637	Not detected	MIP, MP
Y2573	MI, DI	
Y2697	DI	
Y2721	DI	
Y2767	MI, DI	

M: mono-iodinated, D, Diiodinated, MP: monophenylated, DP: diphenylated;
MIP Monoiodinated monophenylated,

S.11 SUPPLEMENTARY FIGURES



G

NPs	Size (nm)	ζ-Potential (mV)	IC_{50} (Raw) µM	IC_{50} (Thyrocytes) µM
Pd/CPCl 1:1	47.6	+19.7	10	10
Pd/CPCl 5:1	38.3	+15.4	20	20
Pd/CPCl 10:1	109	+8.0	40	35
Dialyzed Pd/CPCl 1:1	144	+3.9	>100	50
Pd/PVP 100 :1	70	+2.9	NR	NR

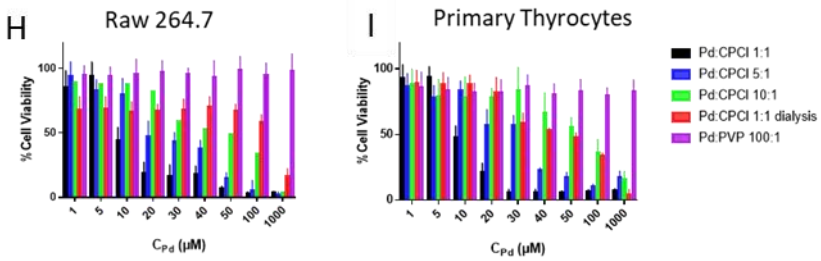


Fig. S1. Characterization and cytotoxicity of Pd-Urchin NPs. The morphological appearance of Pd-Urchin NPs was observed by transmission electronic microscopy (TEM). TEM images of CPCl/Pd (A) 1:1, (B) 1:5, (C) 1:10, (D) 1:1 after dialysis, (E) PVP:Pd 1:100; (F) Evolution of the mean diameter of the different nanoformulations of Pd NPs at 25 °C in milli-Q water, as measured by DLS. The 1:1 Pd:CPCl dialyzed and 10:1 Pd: CPCl samples precipitated to a certain extend after 7 d and 14 d, respectively (G) Table of Size and surface charge (zeta potential) of Pd NPs measured by DLS; the size distributions were highly monodisperse (PdI < 0.250) and only slight modifications of the size were observed when the amount of CPCl was reduced (data presented as mean ± SD); IC50 of Pd NPs

on Raw 264.7 macrophages cell-line and primary culture of human thyrocytes from patient (NR: IC50 was not reached due to absence of cytotoxicity) (H) Cell viability of Pd NPs on Raw 264.7 (I) Cell viability of Pd NPs on primary culture of human thyrocytes from patient tissue after 24h incubation.

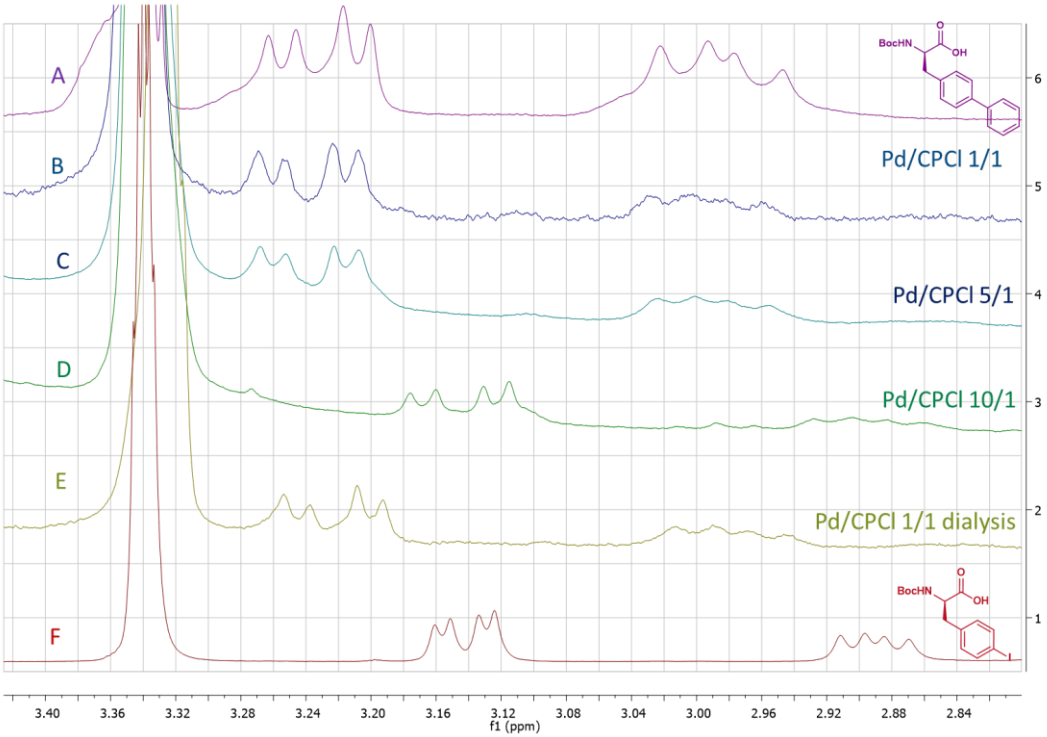


Fig. S2. NMR stacking spectrum of the C-3 methylene protons of phenylamine at 2.90-3.30 ppm with the various catalysts at pH 8. A *N*-Boc-biphenylalanine (purple), B-F Suzuki-Miyaura cross-coupling reaction catalyzed by urchins Pd:CPCl 1:1 (blue), 5:1 (light blue), 10:1 (turquoise), 1:1 after dialysis (green), and **F** *N*-Boc-4-iodophenylalanine (red)

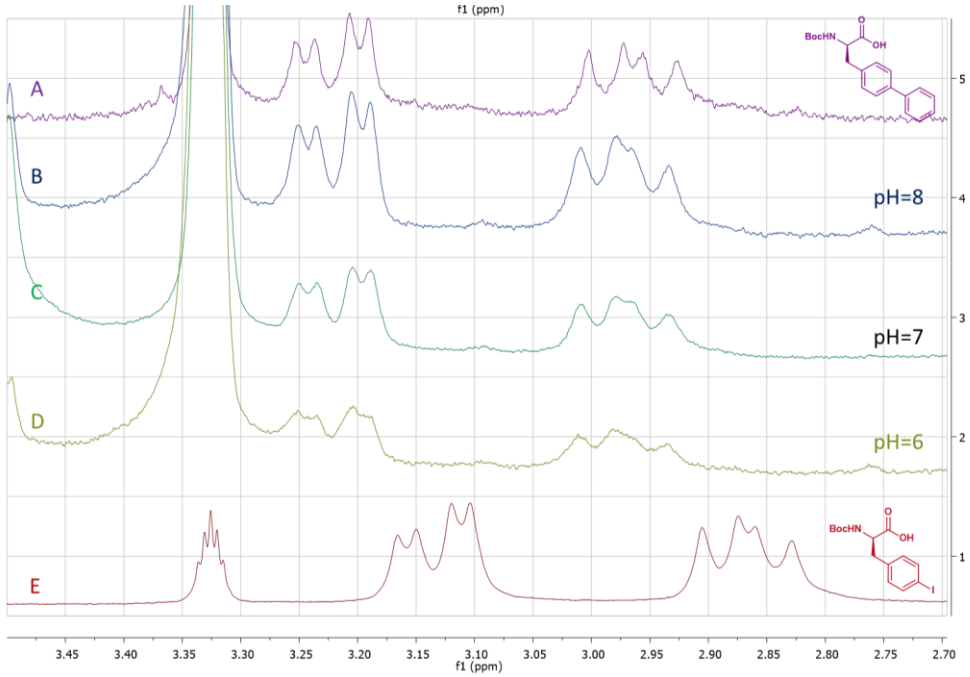


Fig. S3. NMR stacking spectrum of the C-3 methylene protons at 2.90-3.30 ppm with Pd:PVP NPs 100:1 at different pH. A *N*-Boc-biphenylalanine (purple), **B-D** Suzuki-Miyaura cross-

coupling reaction catalyzed by PVP-Pd NPs at pH = 8 (blue), pH = 7 (green), pH = 6 (light green) and *N*-Boc-4-iodophenylalanine (red).

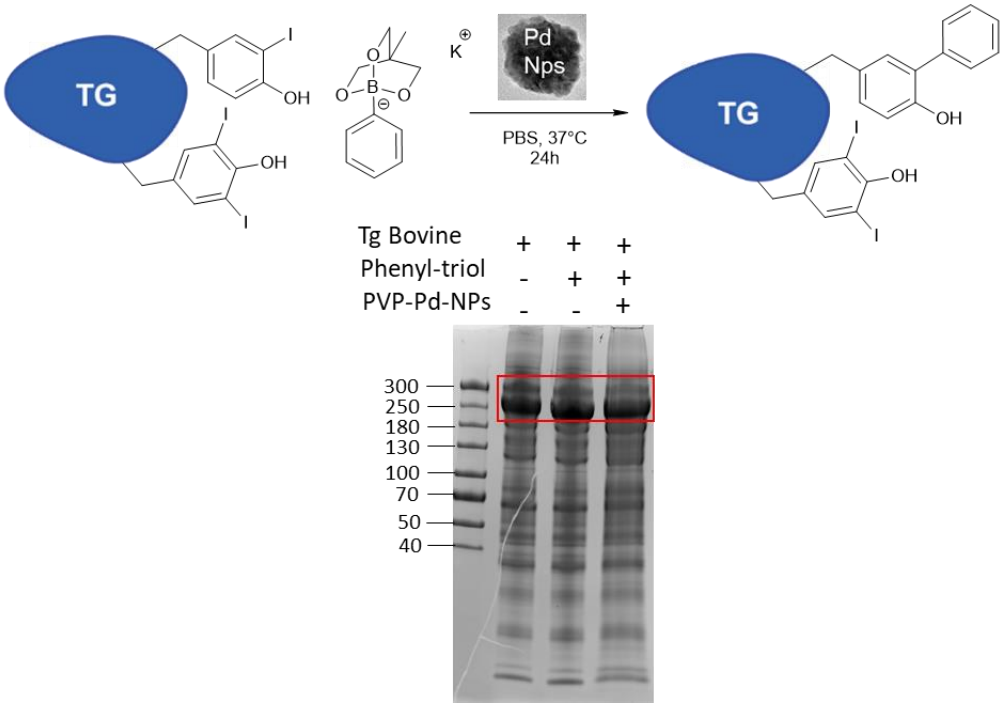


Fig. S4. EZ Blue staining 1D-SDS Page gels of bovine thyroglobulin

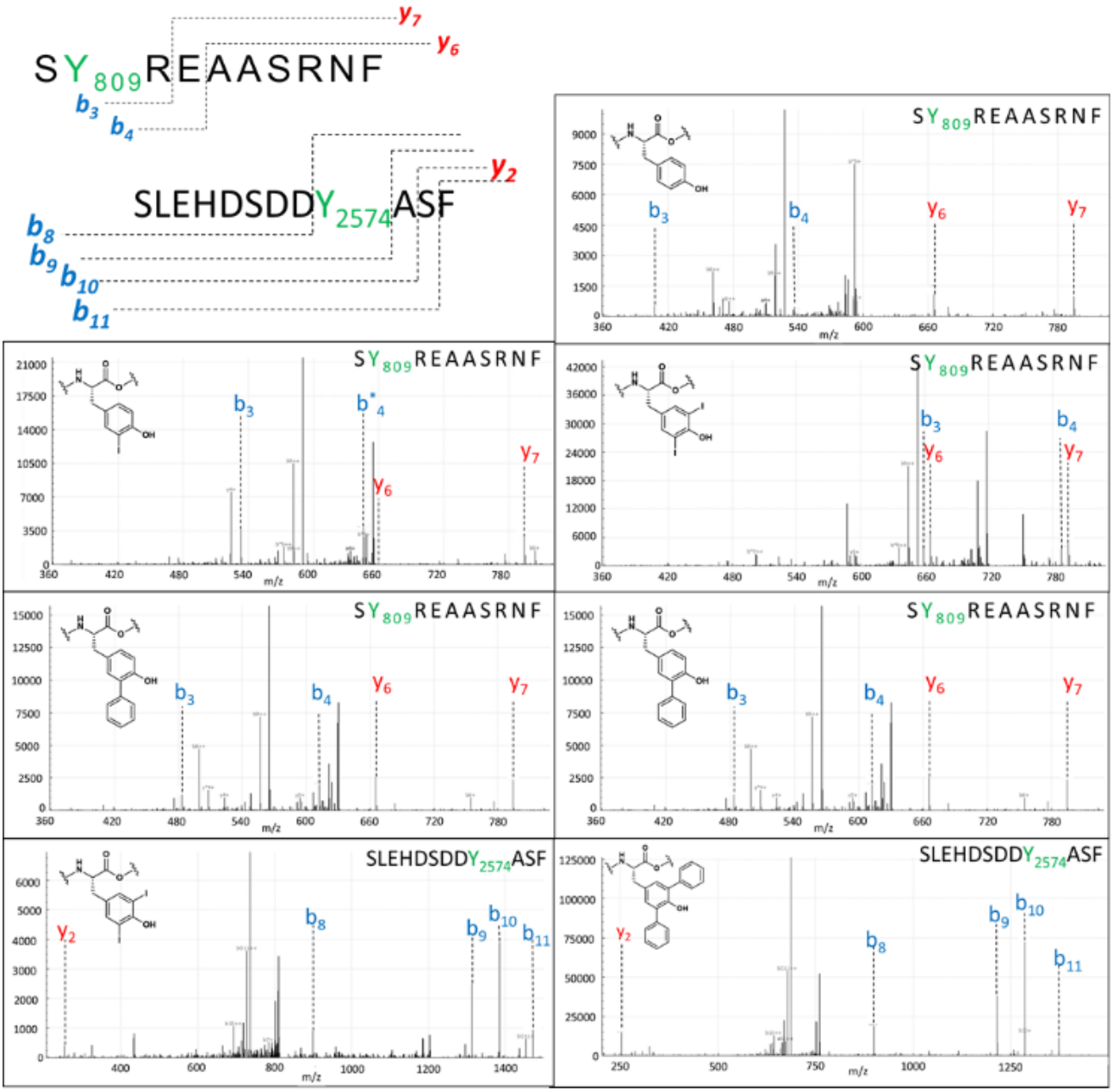


Fig. S5. ESI CID-fragmentation spectra for Tg peptides SYREAASRNF and SLEHDSDDYASF and assignment of Tyr809 and Tyr2574 iodation and phenylation patterns. No-iodinated tyrosine; monoiodinated tyrosine, diiodinated tyrosine, monophenylated tyrosine, monophenylated and iodinated tyrosine, diphenylated tyrosines

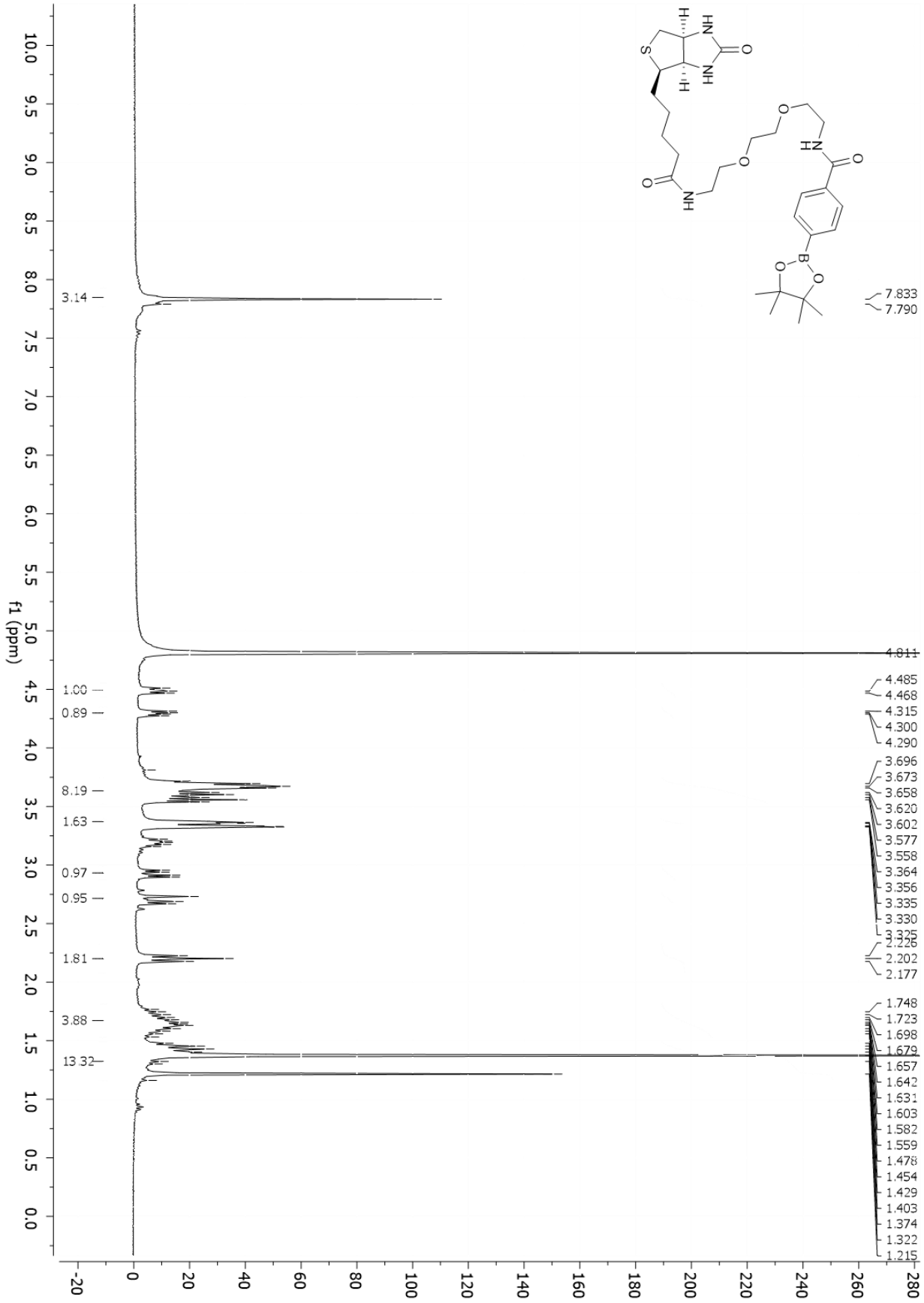


Fig S6. ^{1}H NMR Spectrum of the pinacol boronate biotin compound.

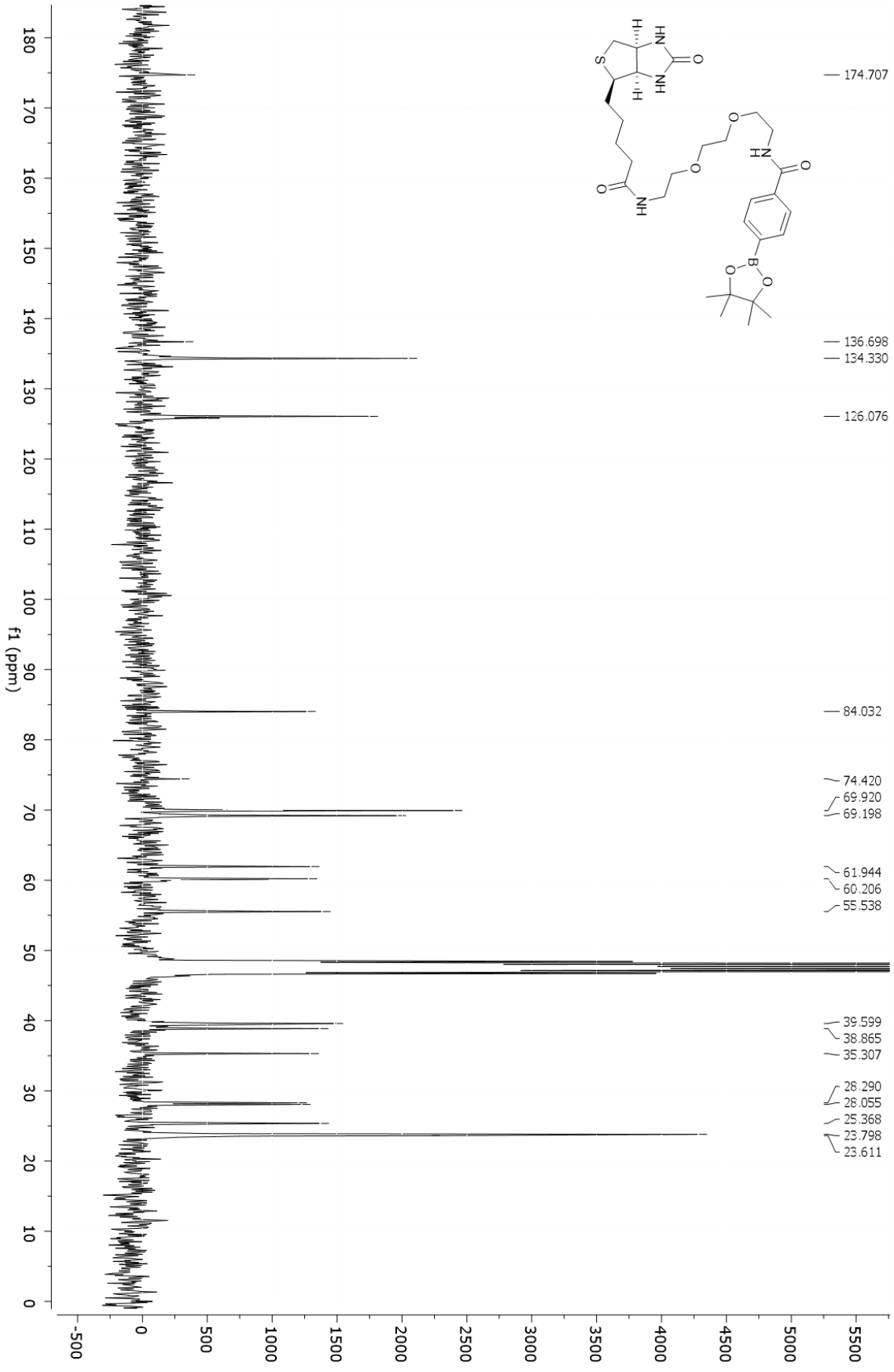


Fig S7. ^{13}C NMR Spectrum of the pinacol boronate biotin compound.

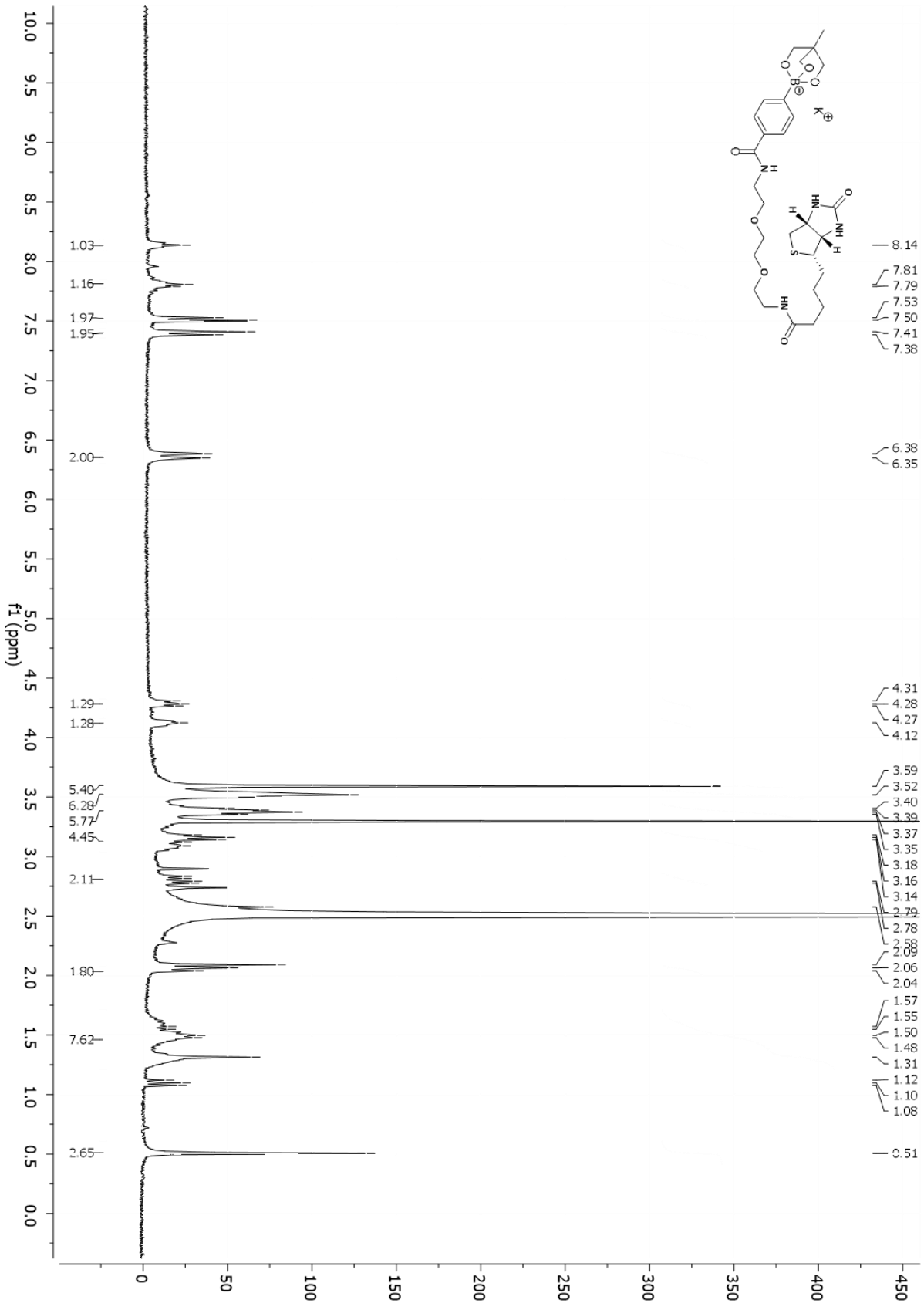


Fig S8. ^{1}H NMR Spectrum of the cyclic triolborate biotin probe.

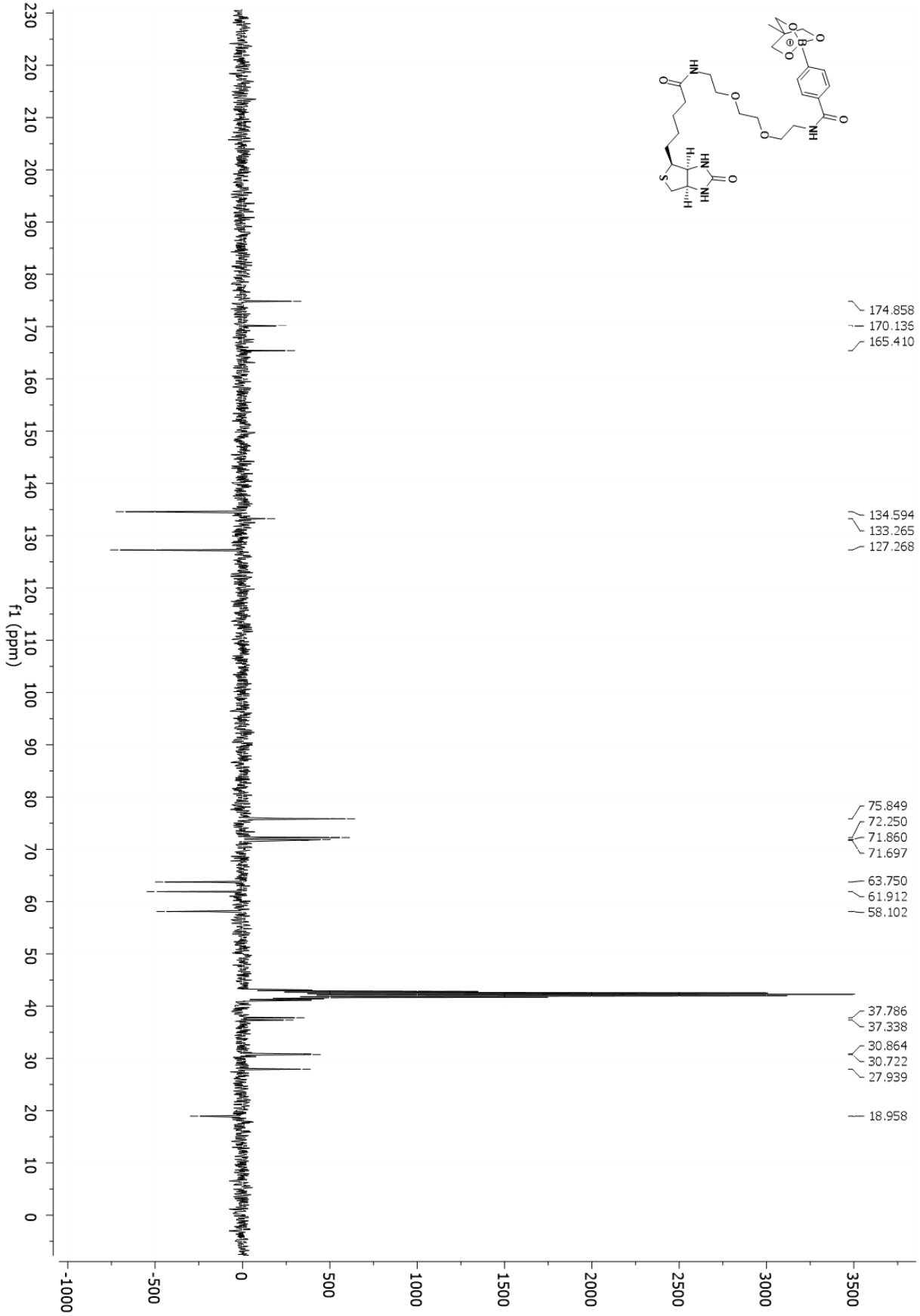


Fig. S9. ^{13}C NMR Spectrum of the cyclic triolborate biotin probe**.**

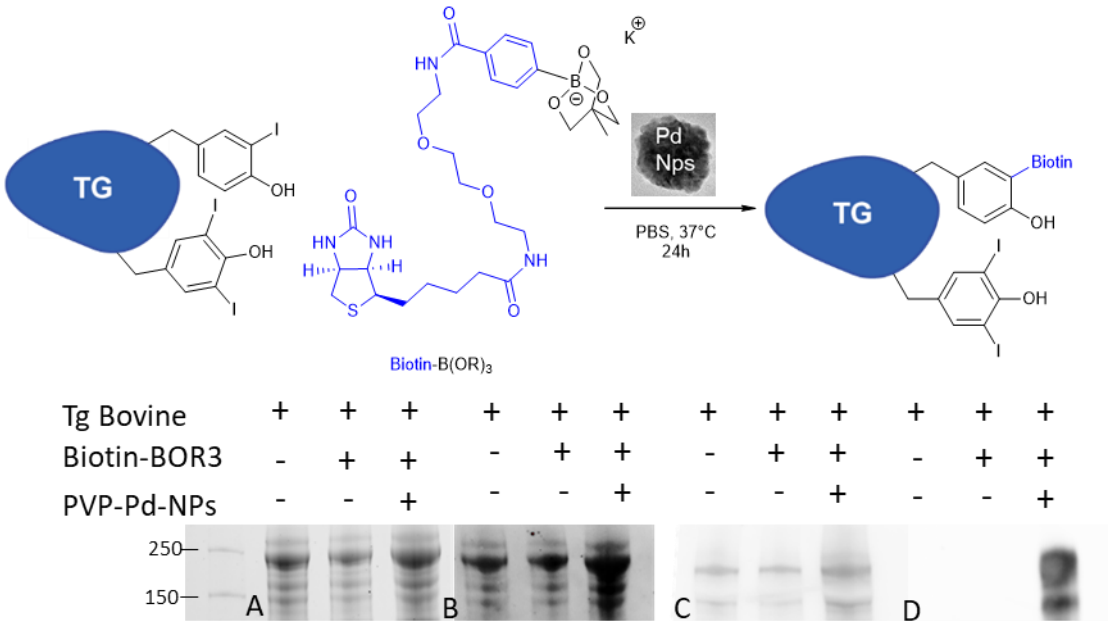


Fig S10. Synthetic scheme of the reaction of the biotin triolborate probe with the iodinated bovine Tg and Western-Blot analysis. (A) Separation of proteins by 1D-SDS Page gel (Stain Free image); (B) Stain free image after protein transfer, (C) Detection of bovine Thyroglobulin on nitrocellulose membrane was probed with 1 goat Anti-Human Thyroglobulin Monoclonal Antibody followed by HRP-conjugated Anti-goat Secondary Antibody. The specific band was detected for Thyroglobulin around 250 kDa; (D) Detection of Tg modification on nitrocellulose membrane probe with streptavidin-HRP.

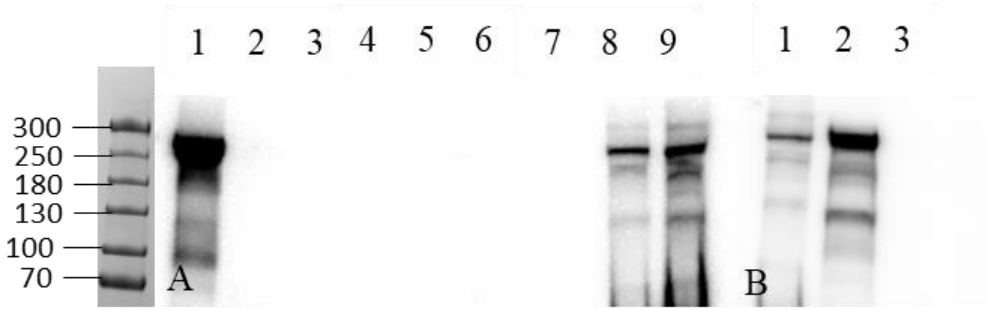


Fig. S11. Detection of thyroglobulin in FRTL and FRTL5 cell lysates (Western Blot). Detection of Thyroglobulin on PVDF membrane was probed with Anti-Thyroglobulin N-15, follow by Donkey anti-goat IgG HRP-conjugated. The specific band was detected with MF-ChemiBis system around 300 kDa for Thyroglobulin**.** (A**)** 1-Rat Thyroid homogenate (positive control) FRTL-5line Lysates: 2-with no inhibitor of Tg (Thunicamycin); 3-Lysate with thunicamycin; 4-Lysate with TSH added after 24h; 5-Lysate with TSH added after 24h and Tunicamycin; 6-Lysate with No TSH and No Thunicamycin; 7-Lysate from Prostate Cancer 3 cell line lysate (negative control); 8-Bovine Tg (16 µg), 9- Bovine Tg (42 µg)). (B**)** 1-Bovine Tg 112µg (positive control); 2-FRTL Cellular lysate; 3-PC-3 Cellular lysate (negative control)

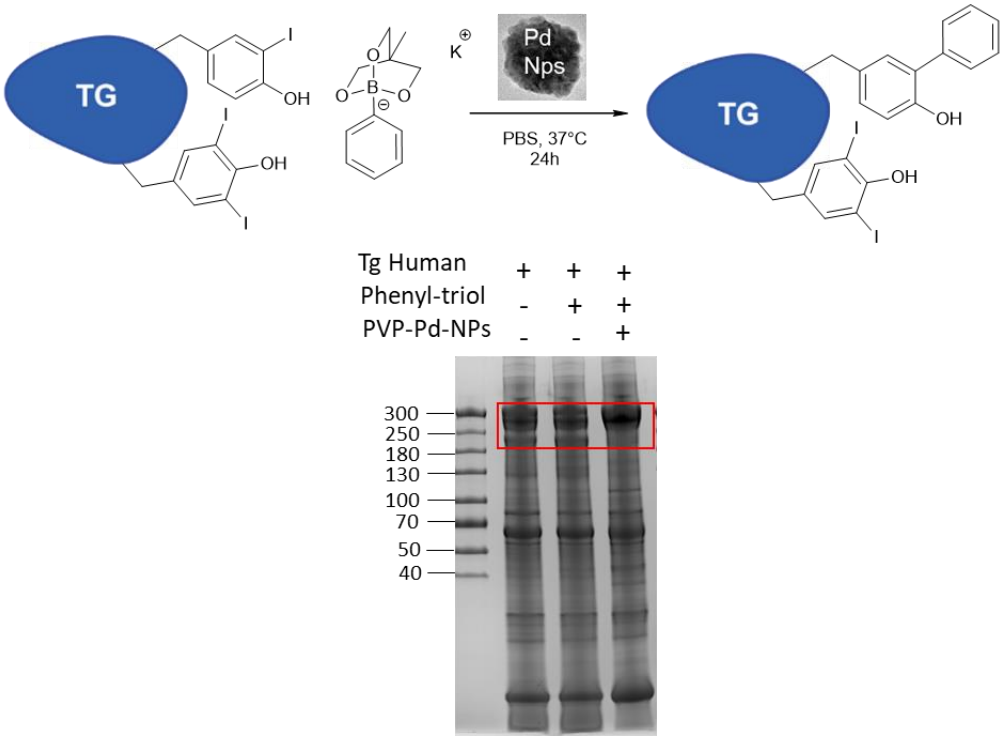


Fig. S12. EZ Blue staining 1D-SDS Pages gels of human thyroid gland homogenate

S12. REFERENCES

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