89Zr-Cobalamin PET Tracer: Synthesis, Cellular Uptake, and Use for Tumor Imaging

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Supporting Information

ABSTRACT: Vitamin B12, or cobalamin (Cbl), is an essential nutrient. Acquisition, transport, and cellular internalization of Cbl are dependent on specific binding proteins and associated receptors. The circulating transport protein transcobalamin (TC) promotes cellular uptake via binding to specific receptors such as CD320, a receptor upregulated in several cancer cell lines. In this study, we report the successful synthesis of 89Zirconium-labeled Cbl that was derivatized with desferrioxamine (89Zr-Cbl). We document the purity of the tracer and its binding to TC compared with that of unmodified cyano-Cbl (CN-Cbl). In vitro studies employing the CD320 receptor-positive breast cancer cell line MDA-MB-453 showed a 6- to 10-fold greater uptake of 89Zr-Cbl when compared with the uptake in the presence of 200-fold excess of CN-Cbl at 37 °C. We used nude mice with MDA-MB-453 tumors to study the feasibility of employing the tracer to visualize CD320 positive tumors. In vivo positron emission tomography images displayed a clear visualization of the tumor with 1.42 ± 0.48 %ID/g uptake (n = 3) at 4 h after injection (p.i.) with the tracer retained at 48 h p.i. Ex vivo biodistribution studies using 89Zr-Cbl exhibited the highest uptake in kidney and liver at 48 h p.i. Results document the feasibility of synthesizing a Cbl-based tracer suitable for both in vivo and ex vivo studies of Cbl trafficking and with the potential to visualize tumors expressing TC receptors, such as CD320.

INTRODUCTION

Vitamin B12 (cobalamin, Cbl) is a critical nutrient that is physiologically required to maintain cell growth and differentiation.1–4 Cbl is involved in the biosynthesis of nucleic acids, lipids, and proteins, and its deficiency leads to a reduction in functional methionine synthase and metabolism of methylmalonic acid in humans, leading to megaloblastic anemia and/or various neurological disorders.5

Cbl gains entry into cells upon binding to transport proteins and subsequent receptor mediated transport. Cbl in blood is bound to the transport protein transcobalamin (TC) (holo-TC), which, in turn, is recognized by specific receptors such as CD320.1–4 Uptregulation of CD320 receptors has been reported in several malignancies including breast, prostate, thyroid, cervical, colorectal, and stomach cancers.5 The important role of Cbl in cellular proliferation and the upregulation of CD320 in tumor cells has made Cbl uptake an attractive candidate for tumor imaging, mainly using single-photon emission computed tomography (PET) imaging agent, labeled with 64Cu (t1/2 ~ 12.7 h), has also been reported.4

Herein, the utility of Cbl as a vector was explored for delivering the PET radionuclide 89Zr (t1/2 ~ 3.27 days). We hypothesized that 89Zr would (1) retain the sensitivity of PET imaging and (2) provide a longer visualization window by providing a greater signal-to-noise ratio compared with prior tracers reported, allowing for an improved tumor targeting and imaging. Following radiosynthesis, we evaluated the in vitro uptake and in vivo pharmacokinetics of the CD320-positive MDA-MB-453 breast cancer in athymic nude mice.

RESULTS

Synthesis of Cbl-Desferrioxamine (Cbl-DFO). Cbl-desferrioxamine (Cbl-DFO) was synthesized by forming a carbamate linkage between the 5′-hydroxyl of deoxyribose moiety in Cbl and the amine group of DFO (Scheme 1, Figure

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Cbl was activated using 1,1′-carbonyl-di-(1,2,4-triazole) (CDT), followed by the addition of DFO, which links through the primary amine. Puriﬁcation and characterization conﬁrmed that the conjugate was of ≥97% purity (Figures S2–S4). The yield of Cbl-DFO was 40 ± 5% based on the Cbl content in the starting material. Calculated mass (m/z): 1942 [M]; observed: 972 [M + 2H]^2+ and 648 [M + 3H]^3+.

Radiolabeling of Cbl with ⁸⁹Zr. Cbl-DFO was labeled with ⁸⁹Zr (⁸⁹Zr-Cbl) using a previously established protocol (Scheme 1). A radiolabeling efﬁciency of ~97% was determined by instant thin-layer chromatography (iTLC, Figure S5a). The speciﬁc activity of the tracer was determined by titrating ⁸⁹Zr⁴⁺ and Cbl at different mole ratios with an
achieved optimum specific activity of 250 ± 20 mCi/μmol (mean ± standard deviation, n = 3).

**In Vitro Stability of \(^{89}\text{Zr}-\text{Cbl} \).** Stability of the tracer was analyzed by incubating \(^{89}\text{Zr}-\text{Cbl}\) in saline and in human serum at 37 °C. Bound versus unbound radio metal was analyzed at 0, 4, 24, and 48 h after incubation using iTLC (Figure 1a,b). The intact tracer was located closer to the origin (40−80 mm), whereas unbound tracer was found at 100−140 mm. After 48 h of incubation, free \(^{89}\text{Zr}\) was <1% in both saline and serum.

**TC Binding Studies.** Mouse TC binding of \(^{91}\text{Zr}-\text{Cbl} \) was studied by radiometric chase assay using \(^{57}\text{Co}-\text{labeled Cbl employing a previously described design.}^{17} \(^{91}\text{Zr}-\text{Cbl} \) was synthesized similar to \(^{89}\text{Zr}-\text{Cbl}, \) but with \(^{91}\text{ZrCl}_4\). Mouse TC binding of \(^{91}\text{Zr}-\text{Cbl} \) displaced \(^{57}\text{Co}-\text{labelled Cbl in a manner comparable to that of CN-Cbl (Figure 1c), indicating that the modification of Zr-Cbl did not compromise binding to TC. The same results were obtained for binding to human intrinsic factor (data not shown).}

**In Vitro Uptake.** An internalization assay was performed to test the uptake of \(^{89}\text{Zr}-\text{Cbl} \) on CD320 receptor cell line MDA-MB-453 at 4 and 37 °C (Figure 1d). The internalized fractions of \(^{89}\text{Zr}-\text{Cbl}\) were expressed as counts per minute (cpm) normalized to \(10^7\) cells (cpm/\(10^7\) cells). Internalization of \(^{89}\text{Zr}-\text{Cbl}\) was higher at 37 °C versus 4 °C at all time points with 144 ± 20 versus 36 ± 12 cpm/\(10^7\) cells at 1 h (\(p < 0.0001\)), 210 ± 64 versus 30 ± 9 cpm/\(10^7\) cells at 4 h (\(p = 0.01\)), and 304 ± 25
versus 83 ± 15 cpm/10^5 cells at 24 h \((p < 0.0001)\). Competitive assays using excess Cbl at 37 °C displayed lower binding at all time points \((p < 0.01)\). All of the data are reported as mean ± standard deviation of four independent measurements.

**PET Imaging.** PET imaging was performed after the administration of ~1 nmol/mouse (200–250 μCi, 7.4–9.3 MBq) of ^99m^Zr-Cbl to nude mice bearing a CD320 positive MDA-MB-453 tumor \((n = 3)\). The image showed visualization of the tumor with tracer uptake of 1.42 ± 0.48 %ID/g at 4 h p.i. with retention observed up to 48 h p.i. (Figure 2a). Cohorts \((n = 3)\) that were co-injected with 200-fold excess unlabeled Cbl (Figure 2b) showed significantly less uptake in the tumors \((0.20 ± 0.05 \%ID/g) at 24 h p.i. \((p ≤ 0.001)\). Other tissues that displayed high tracer uptake were the kidney and liver with 8.92 ± 1.45, 8.80 ± 1.06, and 8.10 ± 0.58 %ID/g for kidney and 4.27 ± 0.51, 4.48 ± 0.65, and 4.47 ± 0.69 %ID/g for liver at 4, 24, and 48 h p.i., respectively (Figure 2c). Tumor-to-muscle ratio \((~3:1)\) did not change significantly over 48 h p.i., indicating that the maximum tumor-to-background ratio was achieved at 4 h p.i. (Figure 2d). All percent injected dose per gram of tissue \((%ID/g)\) values are reported as mean ± standard deviation.

**Ex Vivo Tissue Analysis.** Biodistribution data obtained from tumor-bearing mice injected 0.1 nmol (25 microcuries, 0.9 MBq) of ^99m^Zr-Cbl showed 5.11 ± 0.58 %ID/g \((p = 0.77)\) for kidney and 4.16 ± 1.09 %ID/g \((p = 0.32)\) for liver at 4, 24, and 48 h p.i., respectively (Figure 3a, Table S1). The kidneys showed the highest uptake of the tracer with 94.42 ± 4.27, 103.33 ± 11.50, and 72.74 ± 8.41 %ID/g and the liver showed the second highest uptake with 20.15 ± 3.42, 16.75 ± 1.44, and 17.99 ± 2.54 %ID/g at 4, 24, and 48 h p.i., respectively. Administration of a 200-fold excess of unmodified Cbl (as CN-Cbl) together with the tracer \((n = 4 \text{ mice})\) resulted in an approximately 100-fold decrease \((0.04 ± 0.01 \%ID/g)\) in tracer uptake in tumors at 48 h and also a decreased uptake in the kidney \((1.39 ± 0.18 \%ID/g)\) and the liver \((0.08 ± 0.01 \%ID/g)\). These results are consistent with a Cbl-specific uptake of ^99m^Zr-Cbl (Figure 3b, Table S1) and all of the %ID/g values were reported as mean ± standard deviation.

**DISCUSSION**

In this proof-of-concept study, we report the successful production of a Cbl-derived ^99m^Zr tracer suitable for use in PET studies. Studies on nude mice bearing a human breast cancer cell tumor allow us to demonstrate the use of the tracer for PET visualization of the tumor.

In agreement with previous data \(^{17,18}\), we found that ^99m^Zr-Cbl bound to mouse TC in a manner comparable to that of CN-Cbl (Figure 1c). Next, an in vitro assay was performed in the breast cancer cell line MDA-MB-453 to demonstrate a specific uptake of ^99m^Zr-Cbl. \(^{-}^{19}\) We demonstrated a greater than 40-fold uptake of ^99m^Zr-Cbl at 37 °C versus 4 °C; blocking with 200-fold excess unlabeled Cbl had a similar reduced uptake (Figure 1d). These results support the idea that the targeting properties of ^99m^Zr-Cbl in MDA-MB-453 cells rely on a Cbl-dependent internalization mechanism, likely through the CD320 receptor.

In vivo imaging with ^99m^Zr-Cbl showed an uptake in MDA-MB-453 tumors with 1.42 ± 0.48 %ID/g, whereas ex vivo tissue distribution studies showed a tumor uptake of 5.11 ± 1.33 %ID/g at 4 h p.i. (Figures 2 and 3). Notable uptake was also observed in the liver and kidneys with 4.27 ± 0.51 and 8.92 ± 1.45 %ID/g at 4 h p.i., respectively (Figure 2c). An in vivo block using 200-fold excess of unradiolabeled Cbl showed a significantly reduced uptake \((p < 0.001)\) of the tracer, indicating that the in vivo tumor initialization is Cbl dependent, supported in the in vitro internalization assay. Muscle-to-tumor ratio showed that the maximum tumor-to-background ratio was achieved at 4 h p.i.

To compare the uptake values with those described in the literature, the biodistribution data will be used for a more accurate comparative analysis for the rest of the discussion. Tumor uptake persisted throughout the 4–48 h imaging period. In agreement with previous data \(^{17,18}\), we found that ^91^Zr-Cbl produced lower binding at all time points \((p ≤ 0.001)\). Muscle-to-tumor ratio \((~3:1)\) did not change significantly over 48 h p.i., indicating that the maximum tumor-to-background ratio was achieved at 4 h p.i. (Figure 2d). All percent injected dose per gram of tissue \((%ID/g)\) values are reported as mean ± standard deviation.

**CONCLUSIONS**

We have successfully developed and evaluated the first ^99m^Zr-labeled Cbl tracer as a viable tool for visualizing TC-mediated Cbl uptake into a CD320 positive tumor. ^99m^Zr-Cbl displayed retained tumor uptake up to 48 h p.i., allowing for a longer imaging window. Our data paves the road for future studies to understand the kinetics of Cbl transport and to study the use as a tool for visualizing tumors capable of accumulating Cbl.

**EXPERIMENTAL METHODS**

**General.** Reagents listed below were purchased and used without further manipulations: dimethyl sulfoxide (DMSO, 99%, Sigma), vitamin B12 (Cbl, ≥98%, Sigma), 1,1'-carbonyl-di-(1,2,4-triazole) (CDT, ≥90%, Fluka), and acetonitrile (MeCN, 99.8%, Pharmaco-Aaper). Compounds were confirmed to be
>96% pure by high-performance liquid chromatography (HPLC), proton nuclear magnetic resonance (1H NMR), and/or inductively coupled plasma.

Proton nuclear magnetic resonance (1H NMR) was performed using 400 MHz Bruker spectrometer with the residual solvent peak as an internal standard. Electrospray ionization (ESI) mass spectrometry analyses were carried out on a Shimadzu LCMS-8100. Breast cancer cells (MDA-MB-453) were obtained from the American Type Culture Collection. Charcoal stripped fetal bovine serum (FBS) and Dulbecco’s modified Eagle’s medium (DMEM) were purchased from Sigma and KD medicals, respectively. Penicillin−streptomycin solution with 10,000 units of penicillin and 10 mg/mL streptomycin in 0.9% NaCl was obtained from Corning.

Analysis of the radiotracer was performed using instant thin-layer chromatography (iTLC, Eckert & Ziegler Mini Scan) with an ethylenediaminetetraacetic acid (EDTA) (50 mM) mobile phase.

**Synthesis of Cbl-DFO.** Cbl-DFO was synthesized through the activation of the S′-ribose-hydroxyl group with CDT. CDT (34 mg, 0.261 mmol, 7.2 equiv) was added with cyanolo-Cbl (50 mg, 0.0368 mmol, 1 equiv) in anhydrous DMSO (3 mL) at 40 °C for 2 h. DFO (208 mg, 0.313 mmol, 7.4 equiv) was added to the reaction mixture and mixed overnight. Purification of Cbl-DFO was done using reversed-phase (RP)-HPLC (Agilent 1200) with a C18 column (Agilent Eclipse XDB-C18 5 μm, 4.6 mm × 150 mm) at a flow rate of 1 mL/min. Detection was done using a UV−vis detector at 360 nm. RP-HPLC method: (A) 0.1% trifluoroacetic acid water and (B) MeCN as solvents with the following gradient: 1% B to 70% B over 15 min, (tR = 9.4 min). Purity was ≥97% via RP-HPLC. Yield: 30 mg (0.261 mmol, 7.2 equiv) was added with cyano-Cbl (50 mg, 0.261 mmol, 7.2 equiv) and/or inductively coupled plasma.

**99mTc Radiochemistry.** Optimum conditions for radiolabeling of Cbl-DFO were tested by titrating with 99mTc and analyzing the incubated solution using iTLC. Optimum labeling activity was found to be 250 ± 20 mCi/μmol (9250 ± 740 MBq/micromole). Approximately 1 mCi (37 MBq) of 99mTc(C2O4)₂⁻ (3D Imaging, LLC) was diluted with 0.9% saline and the pH was adjusted to 7−7.5 by adding 1 M Na2CO3. A solution of Cbl-DFO (0.004 μmol, 10.8 μg) was added to the pH-adjusted 99mTc solution and incubated for 20 min at ambient temperature (Scheme 1). Radiolabeling efficiency of >97% was determined by iTLC using silica iTLC strips and EDTA mobile phase (Figure S5a). The identity of the tracer was characterized via matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) analysis using Cbl-DFO labeled with cold Zr⁴⁺ as standard. Expected: 2030.2 [M⁺]; observed: 2005.2 [M⁺−CN⁻]⁺ (Figure S5b).

**In Vitro Stability of 99mTc-Cbl.** Stability of 99mTc-Cbl was tested by incubating the tracer (200 μCi, 7.4 MBq, 100 μL) in saline (0.9% NaCl) and 50:1 (1:1 serum/saline) human serum (Sigma) at 37 °C, and the solutions were analyzed at 0, 4, 24, and 48 h intervals using iTLC (Eckert & Ziegler Mini Scan).

**Mouse TC Binding to 99mTc-Cbl.** Nonradioactive 99mTc-Cbl was synthesized for TC binding studies by reacting Cbl-DFO with 99mTcCl₄ as described above. The conjugate was characterized by ESI-MS (data not shown). Mouse TC binding of 99mTc-Cbl was confirmed by radiometric chase assay using 57Co-labeled Cbl and compared with free Cbl (cyanocobalamin) employing a previously described protocol.27 Mouse TC was derived as previously described.28

**In Vitro Vitruc.** Modified internalization assay was performed. MDA-MB-453 cells were cultured in Cbl-free media (DMEM with 10% charcoal stripped FBS) and plated in six-well plates. Each well contained 200,000 cells plated and incubated overnight. To each well, 99mZr-Cbl (0.1 μCi, 3.7 KBq, 0.4 pmol of Cbl per well) was added. For the blocking experiment, unmodified Cbl was added (40 pmol per well). Plates were incubated for 1, 4, and 24 h intervals at either 37 or 4 °C. At the end of each time point, wells were serially washed with phosphate-buffered saline (1×), acid (1 mM acetic acid and 1 mM glycine), and base (1 M NaOH, 1 mL, 5 min). Each wash was collected and measured for bound activity using a γ counter (Perking Elmer 2480 WIZARD). Control wells were trypsinized and counted using a cell counter (Contessa II). Internalized activity was normalized to 10⁵ cells.

**Cell Lines and Small Animal Xenografts.** All of the animal handling and manipulations were conducted in accordance with the guidelines set by WSU Animal Care and Use Committee (IACUC). For imaging and in vivo uptake experiments, female nude mice (Envigo) were kept under Cbl−deficient diet (Teklad Cbl-free custom diet, Envigo) for 3 weeks. Cells were subcutaneously implanted on the shoulder with MDA-MB-453 cancer cells (5 × 10⁶ cells/mouse) after 2 weeks of Cbl-free diet. Cells were injected in 1:1 media/matrigel (Corning LLC) at a volume of 200 μL. The tumor volume until was calculated using the formula length × width² × 0.52. Mice with tumors of 100−200 mm³ dimensions were used for imaging experiments.

**PET Imaging Experiment.** 99mZr-Cbl was intravenously administered (200−250 μCi/mouse, 7.4−9.3 MBq, 0.8−1 nmol) in sterile saline in mice bearing MDA-MB-453 xenografts. PET imaging was done using a μPET scanner (Concord) at 4, 24, and 48 h p.i. time points, while the mice were anesthetized with 1−2% isoflurane. Images were reconstructed using filtered back projection algorithm. ASIPro VMTM software version 6.3.3.0 (Concord) was used to analyze the images to acquire volumes-of-interest expressed as percent injected dose per gram of tissue (%ID/g). Competitive inhibition was done by co-injecting ~200-fold excess of unmodified Cbl (200 nmol) with the radiotracer.

**Ex Vivo Distribution and Competitive Saturation.** The tissue distribution of 99mZr-Cbl was studied by administering (20−250 μCi/mouse, 0.37−0.93 MBq, 0.04−0.1 nmol) of the tracer on the lateral tail vain of the rodent. For the competitive saturation assay, ~20 nmol/mouse of cold CN-Cbl was co-injected with 99mZr-Cbl. Euthanasia was performed via CO₂ asphyxiation at 4, 24, and 48 h p.i.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01180. Synthesis of Cbl-DFO, LC−MS characterization of Cbl-DFO, 1H NMR characterization of Cbl-DFO, 99mTc characterization of Cbl-DFO, characterization of 99mZr-Cbl, %ID/g values from ex vivo analysis (PDF)
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Author Contributions
R.P.D. and N.V-V conceived of the project and oversaw and designed the experiments. A.N.W.K-W. performed the stability testing, radiolabeling, in vitro, in vivo, and ex vivo radio experiments. J.L.W. synthesized and characterized Cbl-DFO, performed radiolabeling, and conducted in vivo radio experiments. E.N. performed TC binding assays. All of the authors analyzed the data, and the manuscript was written through contributions of all of the authors. All of the authors have given approval to the final version of the manuscript.

Notes
The authors declare the following competing financial interest(s): R.P.D. sits on the scientific advisory board of Xeragenx LLC.

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ABBREVIATIONS
Cbl, vitamin B12 (cyanocobalamin); DFO, desferrioxamine; TC, transcobalamin; CD320, holo-transcobalamin receptor; 89Zr, 89-Zirconium isotope; PET, positron emission tomography; FBS, fetal bovine serum; iTLC, instant thin-layer chromatography; MALDI-MSI, matrix-assisted laser desorption ionization mass spectrometry imaging; %ID/g, percent injected dose per gram; DMEM, Dulbecco’s modified Eagle’s medium

REFERENCES
(1) Nielsen, M. J.; Rasmussen, M. R.; Andersen, C. B. F.; Nexo, E.; Moestrup, S. K. Vitamin B12 transport from food to the body’s cells—a sophisticated, multistep pathway. 

Rev. Gastroenterol. Hepatol. 2012, 9, 345–354.

(2) Green, R.; Allen, L. H.; Bjørke-Monsen, A-L.; Brito, A.; Guéant, J-L.; Miller, J. W.; Molloy, A. M.; Nexo, E.; Stabler, S.; Toh, B-H.; Ueland, P. M.; Yajnik, C. Vitamin B12 deficiency. 

Nat. Rev. Dis. Primers 2017, 3, No. 17040.

(3) Gherasim, C.; Loqmern, M.; Barnerjee, R. Navigating the B12 Road: Endocytic Receptors. 

Nat. Rev. Mol. Cell Biol. 2013, 14, 6319–6334.

(4) Quadros, E. V.; Sequeira, J. M. Cellular Uptake of Cobalamin: Transcobalamin and the TCb1R/CD320 Receptor. 

Biochimie 2013, 95, 1008–1018.

(5) Suel, A. M.; Valli, V. E.; Nagle, R. B.; Bauer, J. A. Immunohistochemical Quantification of the Vitamin B12 Transport Protein (TCbII), Cell Surface Receptor (TCbII-R) and Ki-67 in Human Tumor Xenografts. 

Anticancer Res. 2013, 33, 4203–4212.

(6) Zelder, F.; Alberto, R. The Porphyrin Handbook; Elsevier Science: San Diego, 2012; Vol. 25, pp 83–130.

(7) Collins, D. A.; Hogenkamp, H. P. C.; Gebhard, M. W. Tumor Imaging Via Indium 111–Labeled DTPA—Adenosylcobalamin. 

Mayo Clin. Proc. 1999, 74, 687–691.

(8) Ruiz-Sánchez, P.; Mundwiler, S.; Medina-Molner, A.; Spangler, B.; Alberto, R. Liodination of Cisplatin Adduct of Vitamin B12 ([B12]-CN-{cis-PtCl(NH3)2}]+. 

J. Organomet. Chem. 2007, 692, 1358–1362.

(9) Kunze, S.; Zobi, F.; Kurz, P.; Spingler, B.; Alberto, R. Vitamin B12 as a Ligand for Technetium and Rhenium Complexes. 

Angew. Chem., Int. Ed Engl. 2004, 43, 5025–5029.

(10) Sah, B. R.; Schibli, R.; Waibel, R.; von Bohmer, L.; Blaese, J.; Nexo, E.; Johayem, A.; Fischer, E.; Miller, E.; Soyka, J. D.; et al. 

Tumor Imaging in Patients with Advanced Tumors Using a New 99mTc-Radiolabeled Vitamin B12 Derivative. 

J. Nucl. Med. 2014, 55, 43–49.

(11) Baldoni, D.; Waibel, R.; Blaese, J.; Galli, F.; Iodice, V.; Signore, A.; Schibli, R.; Trampuz, A. Evaluation of a Novel Tc-99m Labelled Vitamin B12 Derivative for Targeting Escherichia coli and Staphylococcus aureus In Vitro and in an Experimental Foreign-Body Infection Model. 

Mol. Imaging Biol. 2015, 17, 829–837.

(12) Zelder, F. Recent Trends in the Development of Vitamin B12 derivatives for medicinal applications. 

Chem. Commun. 2015, 51, 14004–14017.

(13) Waibel, R.; Treichler, H.; Schaefer, N. G.; van Staveren, D. R.; Mundwiler, S.; Kunze, S.; Küenzi, M.; Alberto, R.; Niesch, J.; Knuth, A.; et al. New Derivatives of Vitamin B12 Show Preferential Targeting of Tumors. 

Cancer Res. 2008, 68, 2904–2911.

(14) Iktoven, O. F.; Marquez, B. V.; Fazen, C. H.; Kalkoska, A. R.; Doyle, R. P.; Lapi, S. E. Investigation of a Vitamin B12 Conjugate as a PET Imaging Probe. 

ChemMedChem 2014, 9, 1244–1251.

(15) McEwan, J. F.; Veitch, H. S.; Russell-Jones, G. J. Synthesis and Biological Activity of Ribose-5’-Carbamate Derivatives of Vitamin B12, Bioconjugate Chem. 1999, 10, 1131–1136.

(16) Perk, L. R.; Vosjan, M. J. W. D.; Visser, G. W. M.; Odde, M.; Jurek, P.; Kiefer, G. E.; van Dongen, G. A. M. S. 

Effect of molecular charges on renal uptake of111In-DTPA-conjugated peptides. 

Nucl. Med. Biol. 1999, 26, 2224–2236.

(17) Stuppers, E.; Nexo, E. Effect of the cobalt N coordination on the cobamide recognition by the human vitamin B12 binding proteins intrinsin factor, transcobalamin and haptocorrin. 

Eur. J. Biochem. 1991, 199, 299–303.

(18) Bonaccorso, R. L.; Chepurny, O. G.; Becker-Paule, C.; Holz, G. Z.; Doyle, R. P. Enhanced Peptide Stability Against Protease Digestion Induced by Intronic Binding of a Vitamin B12 Conjugate of Exendin-4. 

Mol. Pharm. 2015, 12, 3502–3506.

(19) Mutti, E.; Ruetz, M.; Birn, H.; Kräutler, B.; Nexo, E. 4-Ethylbenzyl-Cobalamin Impairs Tissue Uptake of Vitamin B12 and Causes Vitamin B12 Deficiency in Mice. 

PLoS One 2013, 8, No. e75312.

(20) Christensen, E. I.; Birn, H. Megalin and Cubilin: Multifunctional Endocytic Receptors. 

Nat. Rev. Mol. Cell Biol. 2002, 3, 256–268.

(21) Christensen, E. I.; Willnow, T. E. Essential Role of Megalin in Renal Proximal Tubule for Vitamin Homeostasis. 

J. Am. Soc. Nephrol. 1999, 10, 2224–2236.

(22) Akizawa, H.; Arano, Y.; Mifune, M.; Iwado, A.; Saito, Y.; Mukai, T.; Uehara, T.; Ono, M.; Fujikoa, Y.; Ogawa, K.; Kiso, Y.; Saji, H. Effect of molecular charges on renal uptake of 111In-DTPA-conjugated peptides. 

Nucl. Med. Biol. 2001, 28, 761–768.

(23) Ferdani, R.; Stigers, D. J.; Flammengo, A. L.; Wei, L.; Li, B. T. Y.; Golen, J. A.; Rheingold, A. L.; Weisman, G. R.; Wong, E. H.; Anderson, C. J. Synthesis, Cu(II) complexation, Cu-labeling and biological evaluation of cross-bridged cyclam chelators with phosphonate pendant arms. 

Dalton Trans. 2012, 41, 1938–1950.

(24) Korneup, L. S.; Fedosov, S. N.; Juul, C. B.; Greibe, E.; Heegaard, C. W.; Nexo, E. Tissue Distribution of Oral Vitamin B12 Is
Influenced by B12 Status and B12 Form: An Experimental Study in Rats. *Eur. J. Nutr.* 2017, 1–11.

(25) Hygum, K.; Lildballe, D. L.; Greibe, E. H.; Morkbak, A. L.; Poulsen, S. S.; Sorensen, B. S.; Petersen, T. E.; Nexo, E. Mouse transcobalamin has features resembling both human transcobalamin and haptocorrin. *PLoS One* 2011, 6, No. e20638.