BNL-41800

# DEVELOPMENT OF A NEW RADIOLABEL (LEAD-203) AND NEW CHELATING AGENTS FOR LABELING MONOCLONAL ANTIBODIES FOR IMAGING

Suresh C. Srivastava, Ronnie C. Mease, George E. Meinken,
Leonard F. Mausner, and Zenon Steplewski*



Medical Department
Brookhaven National Laboratory
Upton, New York, USA

BNL--41800

DE89 009530

*The Wistar Institute
Philadelphia, Pennsylvania, USA

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Address Correspondence to:

Suresh C. Srivastava, Ph.D.
Medical Department, Building 801
Brookhaven National Laboratory
Upton, New York 11973, USA

ABSTRACT

High liver uptake and slow body clearance presently limit the usefulness of $^{111}In$ labeled antibodies for tumor imaging. We have investigated $^{203}Pb$ as an alternate and better antibody label. The DTPA and cyclohexyl EDTA (CDTA) conjugates of an anticolon carcinoma antibody, 17-1A were labeled (bicyclic anhydride method) with $^{203}Pb$ and $^{111}In$ with 60 and 90% labeling yields, respectively. The biodistribution of $^{203}Pb$-17-1A conjugates was compared with the corresponding $^{111}In$-labeled preparations and with $^{203}Pb$-DTPA, $^{203}Pb$-nitrate and nonrelevant antibody controls in normal and human tumor (SW948) xenografted nude mice at 24, and 96 hr. Lead-203-labeled CDTA and DTPA antibody conjugates gave similar in vivo distributions. Even though the lead bound to these chelate-antibody conjugates was more labile in serum and in vivo, compared to indium, it cleared much faster from the liver and the whole body. A new series of chelating agents based on the incorporation of a trans-1,2- diaminocyclohexane moiety into the carbon backbone of polyaminocarboxylates is being synthesized. These are expected to provide stronger complexing ability for lead and produce greater in vivo stability. These ligands are also expected to be superior to EDTA and DTPA for labeling antibodies with other radiometals, including indium.

INTRODUCTION

Radiometals bound to antibodies by chelation help avoid the deleterious effects of oxidation experienced in common iodination reactions and also help overcome the problems of in vivo deiodination, particularly when using rapidly internalized antibodies (e.g., T-cell lymphoma). Since most proteins directly bind metals only weakly, the current practice is to covalently attach to the protein a chelating agent (a "bifunctional chelate") which can then bind the radiometal strongly (19,20,21). Among the various chelating agents that have been conjugated to antibodies for labeling with $^{111}In$, the most commonly used is DTPA and the conjugation is generally carried out via the bicyclic DTPA anhydride or mixed DTPA anhydride (11,16,30). While $^{111}In$-labeled antibodies show higher uptake and residence time in tumors compared to iodinated antibodies (15), some of this advantage is lost (3) since a considerable amount of non-antibody-dependent $^{111}In$ is retained in normal tissues including the liver, and its whole body clearance is very slow (14). Satisfactory contrast for imaging is often achieved only 3-6 days after injection of intact or $F(ab')_2$ antibody. Lesions in the liver and surrounding areas are very difficult to localize without SPECT. Also, SPECT imaging is suboptimal due to decreased photon availability at 3-6 days after injection, unless >5 mCi of the labeled preparation is administered.

We are addressing the problem of $^{111}In$ retention in the liver and whole body in two ways. Firstly, we are investigating $^{203}Pb$ as an alternative to $^{111}In$ for monoclonal antibody labeling (28). Secondly, we are undertaking the design, synthesis, and evaluation of new chelates which when coupled to antibodies should yield metal antibody conjugates with increased in vivo

stability (22). This paper presents the results of our preliminary investigations on these two approaches.

LEAD-203 AS AN ANTIBODY LABEL

Lead-203 ($T_{1/2}$, 52 hr) decays by electron capture with a primary (81%) gamma emission of 279 keV and no beta emission. Lead readily forms complexes with ligands that can be conjugated to antibodies; however, the fate of the label once injected into an in vivo system cannot be predicted from in vitro data or from theoretical considerations alone, and has to be determined experimentally.

The anticolon carcinoma antibody 17-1A (IgG2a) was used as a model in these studies. Conjugates of 17-1A with DTPA and cyclohexyl EDTA (CDTA) were prepared using the bicyclic anhydride method. The conjugate of TTHA (triethylenetetraamine hexaacetic acid) was prepared using either the hexa-N-hydroxysuccinimide ester of TTHA or the monocyclic anhydride of TTHA prepared from modifications of the procedures of Najafi and Buckley, respectively (23,24,4,5). The substitution level varied from an average of 3 molecules of DTPA, 3 molecules of CDTA, and 2 molecules of TTHA per molecule of the antibody. The purified conjugates were labeled with $^{111}In$ in an acetate (0.1 M)/citrate (0.02 M) buffer, pH 5, and with $^{203}Pb$ at pH 8 in the presence of 0.02 M citrate/0.1 M $NaHCO_3$. The products were purified by HPLC on a Zorbax GF-250 column with 0.2 M sodium acetate, pH 7, or 0.2 M phosphate buffer pH 7.0, and alternately using Centricon C-30 centrifugation/filtration devices. All $^{203}Pb$-labeled samples were EDTA challenged (10 $\mu$l, 10 mM) prior to HPLC separation in order to assure high radiochemical purity. Average labeling yields were 60% ($^{203}Pb$) and 90% ($^{111}In$). The immunoreactivity of labeled 17-1A conjugates was determined from cell binding assays using specific SW948

colon carcinoma cells and nonspecific WM164 melanoma cells as a control. The results are shown in Table 1. The method of antibody conjugation and the purification of the resulting conjugate both affected the retention of immunoreactivity. In general, the mixture (monomer and oligomer) resulting from the use of the dianhydrides of DTPA and CDTA, gave the highest apparent immunoretention. Antibody conjugates prepared from the monoanhydrides of DTPA and CDTA gave immunoretention values comparable to the HPLC purified monomeric IgG fraction of the corresponding dianhydride coupled antibody. The effect of the different methods of purification on tumor uptake was investigated using human colon tumor (SW948 cells) xenografted nude mice and $^{111}$In labeled 17-1A conjugates purified by either the C-30 Centricon filter which is incapable of separating the mixture of monomeric and oligomeric IgG fractions or HPLC, which separated these fractions. The results are given in Table 2. The monomeric fractions of both DTPA and CDTA conjugates gave the highest tumor uptake, while the oligomeric fractions gave the lowest tumor uptake. The mixture of monomeric and oligomeric IgG gave intermediate results. A comparison of the results from Tables 1 and 2 shows that the oligomeric fractions with high immunoreactivity gave low tumor uptake while the monomeric fractions which had low immunoretention (< 10%) showed higher tumor uptake.

The tissue distribution in human colon tumor (SW948 cells) xenografted nude mice of the $^{203}$Pb and $^{111}$In labeled 17-1A conjugates is given in Table 3. A similarly treated nonrelevant Ig (H-24B5) of the same isotype was used as a control. Other controls included $^{203}$Pb-nitrate and $^{203}$Pb-DTPA. For the $^{203}$Pb labeled preparations, only those using CDTA showed a significant tumor uptake (2-3% at 24 hr). This apparent preference of lead for chelates with a more rigid structure is also seen in serum stability studies where $^{203}$Pb-17-1A-CDTA

was more stable than $^{203}$Pb-17-1A-DTPA (data not shown) which in turn is less stable than $^{111}$In-17-1A-DTPA. In general, the $^{203}$Pb labeled preparations cleared the blood,liver, and whole body faster than the $^{111}$In labeled preparations; however, the tumor uptake was low and the bone and kidney uptakes were higher. By itself, $^{203}$Pb-DTPA gave a distribution corresponding to a rapidly excreted hydrophilic chelate. Since $^{203}$Pb-DTPA does not localize significantly in bone and kidneys, the high uptake of $^{203}$Pb in these organs using $^{203}$Pb labeled 17-1A conjugates is most likely attributable to free lead (dissociated from the conjugated chelate which may bind the lead less strongly than free chelate). The use of conjugates prepared from the decadentate ligand TTHA gave poor results when labeled with lead. When labeled with $^{111}$In, however, they gave results comparable with DTPA as has been previously reported (4). While the semi-rigid CDTA conjugates had some tumor uptake when labeled with lead, indium labeled CDTA conjugates showed tumor uptake similar to DTPA conjugates but much higher liver retention.

The ligands used so far for $^{203}$Pb do not provide tumor uptake or in vivo stability comparable to $^{111}$In-labeled antibody. However, the low liver uptake and increased whole-body clearance of $^{203}$Pb are promising. The data indicate that following initial uptake and/or catabolic processes, particularly in the liver, the expected faster clearance of lead from the liver and the whole body should lead to higher contrast for radioimmunoimaging at earlier time periods. Stronger chelating agents and better conjugation methods will no doubt provide much additional improvement.

Commercially available $^{111}$In and $^{203}$Pb were utilized in these studies. The low specific activity (2-5 $\mu$g/mCi, occasionally more) of commercial $^{203}$Pb

is deemed undesirable for eventual clinical application. No-carrier-added $^{203}Pb$ can be produced in the future at the Brookhaven Linac Isotope Producer (BLIP) or at other high-energy accelerator facilities. An attractive route is the $^{209}Bi(p,2p5n)\ ^{203}Pb$ reaction. With a $^{209}Bi$ target, one can also gain from the indirect reaction $^{209}Bi(p,p6n)\ ^{203}Bi\ \beta\ ^{203}Pb$. The yield will be adequate for diagnostic studies and radiopurity should not be a problem. The only impurity of concern likely to be produced is $^{202m}Pb$, which has a 3.53 hr half-life, significantly shorter than the 52 hr of $^{203}Pb$. The lead-bismuth chemical separation can be performed by anion exchange. These production routes for $^{203}Pb$ are presently being studied.

LIVER UPTAKE OF $^{111}In$

The high accumulation of indium in the liver appears to be due to metabolic trapping that results from the sequestration of indium in liver lysosomes (14). The use of liposome-encapsulated $^{111}In$-$^{14}C$-DTPA showed that the $^{14}C$-DTPA is cleared through the kidney into the urine while the $^{111}In$ remains in the liver (17). This suggests that the retention of indium is due to its transchelation in the liver following its detachment from DTPA, and not because of the inability of the $^{111}In$-DTPA to diffuse from the hepatocytes. This work (17) also suggests that chelates which form stronger complexes with $^{111}In$ will be necessary in order for the $^{111}In$-chelate complex to survive liver catabolism of the antibody-chelate conjugate and be excreted intact through the kidney into the urine. Several attempts have recently been made in this direction by various investigators. EDTA and DTPA analogs which contain a 4-substituted benzyl group on their ethylenediamine backbone have been prepared and conjugated to antibodies using the isothiocyanato or bromoacetamido moiety on the benzyl group (2,12,19). When labeled with

indium, these conjugates are more stable in serum than conjugates prepared from bicyclic DTPA anhydride (7,8). These derivatized EDTA and DTPA chelates have several advantages over DTPA. Firstly, since the binding to the antibody is through the 4-substituted benzyl group, all of the carboxylates remain available for complexing the metal. In the bicyclic DTPA anhydride method, one of the carboxylates is utilized in the coupling to the antibody. Secondly, the steric bulk of the benzyl group may contribute to increased in vivo stability of the chelate by inhibiting transferrin or other in vivo ligands from removing the indium from the complex, and they may be strengthening the nitrogen-metal interaction by sterically hindering the rotation of the nitrogen away from the metal when the metal dissociates, thereby favoring recomplexation. The use of these new ligands, however, has not eliminated the high retention of indium in the liver. A recent study has shown that with p-isothiocyanato benzyl-EDTA conjugates, the indium that remains in the liver is still chelated suggesting that its retention in the liver may not result from transchelation (9). Also, recently a new bifunctional chelate N-(2-hydroxy-3,5-dimethylbenzyl)-N'-2 (hydroxy-5-bromoacetamidobenzyl)-ethylenediamine, a derivative of HBED has been synthesized and conjugated to antibodies (29,31). These HBED derivatives form very stable complexes with trivalent metals including indium (log K for HBED > 40 compared to log K = 29 and 25 for DTPA and EDTA respectively). Indium labeled antibodies prepared using this new ligand still showed considerable liver retention of indium (31), again suggesting that transchelation may not be the sole reason for the retention of indium in the liver.

The attachment of DTPA analogs to the chemically modified Fc carbohydrate region of the antibody has shown reduction in the liver uptake of indium (1,26). This is in spite of the use of 1,6-diaminohexyl-EDDHA or p-amino-aniline-DTPA, where one of the carboxylates is used to conjugate to the antibody. This "site-specific" attachment has not been generally applicable due to the presence in some antibodies of non-Fc carbohydrate residues closer to the binding region (27). In addition, this approach would be less likely to be useful with antibody fragments.

DEVELOPMENT OF NEW LIGANDS - RESULTS TO DATE

Our approach to forming more stable metal complexes in vivo is to incorporate the carbon backbone of the polyaminocarboxylates (EDTA, DTPA, TTHA) in a more rigid molecule such as a cyclohexane ring (22). The benefit of this structural modification of EDTA is seen by the stability constants of trans-1,2-diaminocyclohexane-N,N,N'N'-tetraacetic acid (CDTA) metal complexes being generally 1-3 orders of magnitude higher than the corresponding EDTA complexes (20) and by considerably slower ligand exchange. For example, the exchange of Cu(II) from Cu-CDTA with polyamines is 50,000 times slower than from Cu-EDTA (6). These effects are attributed to the fact that the nitrogens in the equatorial position of the cyclohexane ring are in a fixed position where they can readily complex the metal and do not have to be rotated into position as in EDTA. In vivo, the cyclohexyl group should be as good if not better than the benzyl-EDTA and -DTPA chelates in blocking the approach of competing chelates. The fact that the nitrogens are fixed on the ring should stabilize the nitrogen-metal interaction since the nitrogen cannot rotate away from the metal when the metal dissociates. This effect promises to make this class of chelates of general utility for many metals. In fact, indium-111-

CDTA has been reported to be stable in vivo; however, it was excreted too rapidly for long-term measurements (13). Also, the lack of liver uptake after intravenous injections of $^{62}$Cu-CDTA compared to the substantial liver uptake of $^{62}$Cu-EDTA and -DTPA suggests greater in vivo stability of CDTA complexes (25).

As a first step in this study, we have prepared both the bicyclic and monocyclic anhydrides of CDTA from the dehydration of trans -1,2-diamino-cyclohexane -N,N,N',N'-tetraacetic acid with acetic anhydride and pyridine. In preliminary studies, the use of a 5/1 molar ratio of bicyclic CDTA anhydride/antibody and a 10/1 molar ratio of monocyclic CDTA anhydride/ antibody at an antibody concentration of 15-20 mg/ml produced an average of 3 chelates conjugated per antibody. These anhydrides appear to be either more reactive with the antibody or have a slower rate of hydrolysis than the bicyclic DTPA anhydride since a 10/1 ratio of DTPA dianhydride/antibody is required to conjugate 3-4 chelates per antibody at a similar antibody concentration. This apparent increase in chemical reactivity may become important when working at low antibody concentrations.

In vivo, the $^{203}$Pb-CDTA-17-1A had higher tumor uptake than $^{203}$Pb-DTPA-17-1A. Indium-111 labeled CDTA-17-1A gave tumor uptake comparable to $^{111}$In-DTPA-17-1A but produced much higher liver retention. It is likely that with indium, the stability gained by the cyclohexyl group in CDTA-17-1A is not enough to overcome the loss of one carboxylate used in conjugation to the antibody, so we observe no increase in stability compared to DTPA-17-1A. The effect of the loss of one coordination site of EDTA is seen by a 60-fold enhancement in the rate of dissociation in serum of $^{111}$In from the mono-butylamide of EDTA compared to EDTA (32).

In order to increase the number of coordination sites, we have recently prepared the cyclohexyl derivatives of DTPA and TTHA. The synthesis of CyTTHA is as follows. The nitrogens of the starting material trans-1,2-diaminocyclohexane were acylated using isobutyl chloroformate and t-Boc glycine. The t-Boc protecting groups were hydrolyzed in 3M HCl. Reduction of the amide groups to form the cyclohexyltetramine was accomplished with borane in THF. Amine alkylation with bromoacetic acid yielded the desired CyTTHA. The synthesis of CyDTPA by heating 2-aminocyclohexyl sulfate and ethylenediamine at 180° in an autoclave has been reported (10); however, this reaction is likely to yield a mixture of cis and trans products. A new synthesis of trans-CyDTPA has been developed and is as follows. Monoacylation was achieved by first protecting one of the nitrogens of trans-1,2- diamino-cyclohexane. This was accomplished by reacting an excess of trans-1,2-diaminocyclohexane with di-tert-butyl dicarbonate in THF. Starting with the monoprotected trans-1,2-diaminocyclohexane, the rest of the synthesis is similar to that for CyTTHA.

Currently, we are attempting to prepare the anhydrides of CyDTPA and CyTTHA using either the method of Eckelman (11) or a modification of the method of Buckley (4,5). In the latter method, the N-hydroxysuccinimide of TTHA was prepared in situ by the reaction of excess TTHA with dicyclohexyl-carbodiimide (DCC) in DMSO in the presence of N-hydroxysuccinimide. Using a two-fold molar excess of DCC in DMSO, we have isolated a solid TTHA derivative which has an average of one anhydride/TTHA. Addition of excess N-hydroxy-succinimide to this anhydride in DMSO yields solid TTHA derivatives with an average of one N-hydroxysuccinimide/TTHA (determined by NMR). Both these solids are stable for several months when stored over a desiccant.

Using a 25/1 molar ratio of TTHA monoanhydride/IgG or TTHA mono-N-hydroxysuccinimide ester/IgG in 0.1 M $NaHCO_3$ at a protein concentration of 15 mg/ml, an average of 4 and 4-5 TTHA molecules, respectively, were conjugated to each IgG.

The means of attachment to the antibody is also very important to metal chelate stability. Indium-111 protein-chelate conjugates of EDTA and DTPA where the chelate is conjugated through a substituted benzyl group on ethylenediamine portion have shown greater serum stability (7,8,32) and reduced liver uptake (2,12,19). This type of conjugation to antibodies is preferred over the anhydride or N-hydroxysuccinimide route since it removes the metal complexation site from the antibody conjugation site and does not reduce the number of carboxylates needed for metal complexation. We are currently preparing functionalized cyclohexylpolyaminocarboxylates where the antibody conjugation site and the metal complexing site are on opposite sides of the cyclohexyl moiety.

## CONCLUSION

The in vivo pharmacokinetics of labeled antibodies, including tumor uptake, blood and whole body clearance, maximum tumor-to-normal-tissue ratios, etc., require a better matching with the nuclear and biological properties of the radionuclide so that good contrast and maximum photon yields are achieved for imaging at early time periods after injection. In most cases, these factors are related to the particular antibody or fragment since different antibodies are affected differently by the chemical manipulations involved in the labeling process. To overcome the shortcomings of $^{111}In$, particularly the high liver uptake and slow body clearance, better chelating agents and conjugation methods are necessary. In addition, the use of radiometals that may display more favorable in vivo behavior must be considered. Lead-203 and $^{97}Ru$ are two possible candidates. Preliminary experience with $^{203}Pb$ presented in this study indeed has demonstrated sufficient promise to warrant further investigation.

Systematic investigations on chelation technology including development of new and better ligands (for particular radiometals) must be undertaken in order to produce more effective metal-antibody-chelate conjugates. Since the existing techniques are not optimal, such efforts, some of them described in this paper, may ultimately lead to ideal tumor imaging agents based on monoclonal antibodies.

## ACKNOWLEDGEMENTS

This work was supported by the Office of Health and Environmental Research, U.S. Department of Energy, under Contract No. DE-AC02-7600016.

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TABLE 1

Percent Immunoreactivity Retention of 17-1A Conjugates

Using a Cell Binding Assay [a,b]

| | Average # Chelates/Ab | $^{203}Pb$ | $^{111}In$ |
|---|---|---|---|
| DTPA dianhydride | | | |
| mixture of monomer and oligomer[c] | 3 | 2 | 8-16 |
| monomer[d] | 3 | - | 2 |
| oligomer[d] | 3 | - | 21 |
| DTPA monoanhydride | | | |
| mixture of monomer and oligomer[d] | 3 | 6 | - |
| monomer[d] | 3 | - | 5 |
| TTHA NHS hexaester | | | |
| mixture of monomer and oligomer[e] | 2 | - | 8 |
| TTHA monoanhydride | | | |
| mixture of monomer and oligomer[d] | 3 | 1 | 9 |
| CDTA dianhydride | | | |
| mixture of monomer and oligomer[c] | 3 | 16-30 | 8-28 |
| monomer[d] | 3 | - | 7 |
| oligomer[d] | 3 | - | 24 |
| CDTA monoanhydride | | | |
| mixture of monomer and oligomer[d] | 3 | 2 | 8 |

a) Human anticolon carcinoma cells (SW 948) were used. Binding of unmodified 17-1A ranged between 60-80%.
b) Nonspecific binding (WM 164 melanoma cells) never exceeded 0.5%.
c) Purified by either C-30 Centricon filtration device or Zorbax GF-250 HPLC column.
d) Purified by Zorbax GF-250 HPLC column.
e) Purified by C-30 Centricon filtration device.

Table 2

Effect of $^{111}$In-Antibody-Conjugate Purification on Tissue Distribution[a]

| Compound | IgG | Time, hr | Blood | Liver | Kidney | Bone | Tumor | Whole Body[b] |
|---|---|---|---|---|---|---|---|---|
| 17-1A-DTPA | monomer[d] | 24 | 14.7±2.9 | 10.7±1.0 | 10.6±0.9 | 4.4±0.7 | 17.5±5.0 | 111 |
| | | 96 | 4.3±5.1 | 12.3±6.1 | 12.1±3.4 | 6.4±3.9 | 12.7±5.2 | 71 |
| | monomer & oligomer[c] | 24 | 10.9±4.5 | 9.4±0.6 | 6.9±1.5 | 4.3±1.1 | 7.8±2.4 | 104 |
| | | 96 | 1.8±1.0 | 9.0±0.9 | 7.2±1.0 | 4.1±0.9 | 4.6±1.0 | 76 |
| | oligomer[d] | 24 | 3.7±2.8 | 40.2±15.5 | 9.0±0.9 | 5.8±2.0 | 5.5±3.8 | 116 |
| 17-1A-CDTA | monomer[d] | 24 | 9.5±2.4 | 34.3±2.7 | 8.1±0.8 | 5.1±1.7 | 9.8±1.7 | 124 |
| | | 96 | 1.6±2.4 | 26.2±2.7 | 8.4±1.6 | 5.7±1.8 | 3.5±3.5 | 74 |
| | monomer & oligomer[c] | 24 | 4.8±5.6 | 58.0±13.7[e] | 9.8±1.9 | 11.9±7.7 | 6.5±2.1 | 148[e] |
| | oligomer[d] | 24 | 0.5±0.6 | 106±7[e] | 8.9±0.3 | 5.4±1.0 | 1.4±0.9 | 162[e] |

a) Percent injected dose per g tissue. Nude mice with SW948 tumor xenografts; n=3
b) Percent injected dose retained.
c) Purified by Centricon C-30 filtration
d) Purified by Zorbax GF-250 HPLC column.
e) The use of CDTA dianhydride produced more oligomer than DTPA dianhydride. This may have caused sampling errors due to non-homogeneity of solutions.

Table 3

Tissue Distribution Data (% Dose per g) in Tumor Mice [a,b,c,g]

| Compound | Time,hr | Blood | Liver | Kidney | Bone | Tumor | Whole Body[d] |
|---|---|---|---|---|---|---|---|
| Pb-203 Nitrate | 24 | 7.7±1.5 | 6.0±0.5 | 27.6±1.0 | 12.1±0.7 | 0.4±0.1 | 58 |
| | 48 | 1.8±0.3 | 3.3±0.7 | 15.2±1.7 | 13.3±1.1 | 0.4±0.2 | 61 |
| Pb-203-DTPA | 3 | 1.1±0.1 | 1.2±0.1 | 5.5±0.3 | 1.0±6.1 | - | 11 |
| Pb-203-17-1A-DTPA[e] | 24 | 3.6±0.1 | 5.3±0.1 | 18.6±4.4 | 13.0±1.6 | 1.1±0.2 | - |
| | 96 | 0.9±0.1 | 2.7±0.4 | 6.3±1.5 | 8.1±0.3 | 0.4±0.1 | 61 |
| Pb-203-17-1A-TTHA[h,e] | 24 | 3.9±0.7 | 6.1±1.0 | 19.3±2.5 | 8.2±0.7 | 0.6±0.0 | 56 |
| | 96 | 1.2±0.1 | 2.3±0.3 | 8.0±1.5 | 8.9±1.2 | 0.4±0.1 | 33 |
| Pb-203-17-1A-CyEDTA[e] | 24 | 5.1±0.7 | 9.0±0.7 | 16.6±4.1 | 8.9±0.5 | 2.3±0.2 | 65 |
| | 96 | 1.4±0.2 | 2.5±0.3 | 8.1±0.8 | 9.8±1.1 | 1.1±0.2 | 38 |
| Pb-203-17-1A-F(ab')$_2$-CyEDTA[e] | 24 | 3.9±1.3 | 8.2±1.6 | 19.4±1.3 | 10.1±2.6 | 2.8±0.6 | 67 |
| | 96 | 1.4±0.2 | 2.2±0.7 | 8.2±1.0 | 9.5±0.8 | 0.8±0.1 | 36 |
| In-111-17-1A-DTPA[f] | 24 | 10.9±4.5 | 9.4±0.6 | 6.9±1.5 | 4.3±1.1 | 7.8±2.4 | 104 |
| | 96 | 1.8±1.0 | 9.0±0.9 | 7.2±1.0 | 4.1±0.9 | 4.6±1.0 | 76 |
| In-111-17-1A-TTHA[h,e] | 24 | 2.0±0.6 | 8.4±1.7 | 15.6±1.3 | 5.6±0.6 | 8.1±1.8 | 84 |
| | 96 | 0.3±0.0 | 9.7±2.4 | 10.7±2.3 | 4.8±1.8 | 4.1±0.8 | 62 |
| In-111-17-1A-CyEDTA[f] | 24 | 4.8±5.6 | 58.0±13.7 | 9.8±1.9 | 11.9±7.7 | 6.5±2.1 | 148 |

a) All data normalized to 25g body weight.
b) Nude mice with SW948 tumor xenografts.
c) n=3
d) Percent injected dose retained in whole body.
e) Purified by HPLC (Zorbax GF-250 column); mixture of monomer and oligomer used.
f) Purifed by Centricon C-30 filtration; this contains both the monomer and oligomer.
g) All conjugates prepared by the bicyclic anhydride method except the TTHA conjugate.
h) Prepared by the monoanhydride of TTHA.