

Vinyl Sulfone Bifunctional Derivatives of DOTA Allow Sulfhydryl- or Amino-Directed Coupling to Antibodies. Conjugates Retain Immunoreactivity and Have Similar Biodistributions

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We have synthesized a bifunctional vinyl sulfone–cysteineamido derivative of DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) that can be conjugated to the sulfhydryls of mildly reduced recombinant antibody (chimeric anti-CEA antibody cT84.66) at pH 7 or to the amino groups of lysine residues at pH 9. The conjugation is sulfhydryl specific at pH 7 (case 1), and amino specific at pH 9 (case 2) as long as the antibody has no free sulfhydryl groups. At a molar ratio of 50 BCA (bifunctional chelating agent) to mAb, the number of chelates conjugated is 0.8 for case 1, and 4.6 for case 2. The resulting conjugates can be radiolabeled with ¹¹¹In to high specific activity (5 mCi/mg) with high efficiency (>95%) at 43 °C in 60 min. The radiolabeled conjugates retained >95% immunoreactivity and are stable in serum containing 1mM DTPA over 3 d. When the radiolabeled conjugates were injected into nude mice bearing LS174T human colon tumor xenografts, over 40% ID/g accumulated in tumors during the period 24–72 h. Tumor-to-blood ratios were 4.5, 3.5, and 2.5 for the sulfhydryl coupled conjugate at 24, 48, and 72 h, respectively, and 2.7, 2.5, and 2.3 for the amino-coupled conjugate at the same time points. For other organs the biodistributions were nearly identical whether the conjugates were attached via sulfhydryl or amino groups. These novel BCAs are easy to synthesize, offer versatile conjugation options, and give equivalent biodistributions that result in high tumor uptake and good tumor-to-blood ratios.

INTRODUCTION

Bifunctional chelating agents (BCAs¹) are routinely conjugated to recombinant antibodies for radiolabeling with radiometals such as ¹¹¹In for radioimmunoinaging and ⁹⁰Y for radioimmunotherapy (1–4). The main advantages of this approach is the ability to radiolabel the conjugate just prior to use and the exceptional stability of the chelated metal, preventing the release of the radionuclide from the conjugate. Starting with BCAs derived from EDTA (5, 6), and then DTPA (7–11), and more recently DOTA (12–16), increasingly more stable radiometal–chelate–antibody conjugates have been crafted. Nonetheless, the approach has certain drawbacks inherent in the biodistribution of the antibody which in the case of intact antibody can be deposited in the liver (17–21), and in the case of antibody fragments, in the kidney (22–25). High uptake in normal tissues is due to the deposition of free antibody or antibody antigen

conjugates in the liver for intact antibody (21) and to renal filtering and reabsorption for antibody fragments (26). In both cases, the exceptional stability of the radiometal–chelate and the lack of metabolizing enzymes prevents the excretion of these end-products from the lysosomal compartment. While it is assumed that the antibodies are completely degraded in the lysosome, releasing radiometal–chelate, it has only been proven in a few cases (27–31). In addition, the site of BCA conjugation to the antibody may play a key role in the fate of the radiolabel in both the tumor and normal organs. Furthermore, it is essential that the BCA be conjugated to the antibody without impairment of immunoreactivity. Since a major goal of the field is to reduce normal tissue uptake without decreasing the tumor uptake, we have investigated new BCA derivatives of DOTA that include the possibility of site-specific conjugation and linker specific cleavage. In previous work we showed that a maleimidocysteineamido derivative of DOTA coupled to sulfhydryl groups of mildly reduced intact antibody underwent pH dependent cleavage at the resulting succinimido groups (32), lowering blood levels while preserving high tumor uptake (33). However, the results were only a marginal improvement over our use of DOTA-active ester conjugated antibody (34, 35). Oxidation of the thioether linker to a sulfone linker further increased the rate of linker bond cleavage, but the rate of blood clearance was too high, preventing good tumor uptake (33). Similar approaches utilizing ester linkers for reducing blood levels have been reported, but also suffer from lower blood levels to the point of reducing

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¹ Abbreviations: BCA, bifunctional chelating agent; CEA, carcinoembryonic antigen; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; DTPA, diethylenetriaminepentaacetic acid; HSA, human serum albumin; sulfo-NHS, *N*-hydroxysulfosuccinimide; TCEP, tris(carboxyethyl)phosphine; VSC-DOTA, vinyl sulfone–cysteineamido-DOTA.

tumor uptake (21, 36–38). A similar problem is found in using antibody fragments, which clear the blood rapidly, but also give low tumor uptake (25). Thus, it is clear that the price of lowering blood levels is the concomitant lowering of tumor uptake, an unacceptable consequence in either radioimmunoimaging or radioimmunotherapy. A possible solution to the problem is the synthesis of BCAs with organ-specific cleavable linkers. Arano and co-workers (26, 39–41) have synthesized C-terminal Lys linkers for a radioiodinated hippuric acid–Fab conjugate that lowered kidney uptake by 50%. Meares and co-workers (42–44) have synthesized DOTA with cathepsin cleavable linkers for use in lowering liver levels. In the work reported here, we have made new derivatives of DOTA that incorporate a vinyl sulfone-based linker that shows versatility in terms of conjugation to either sulfhydryl or amino groups of antibodies. The BCA is easy to synthesize from readily available chemicals and gives high radiolabeling yields when conjugated to either sulfhydryl or amino groups. The biodistributions in tumor-bearing animals is nearly equivalent, suggesting that the site of conjugation has not affected either the tumor or normal organ uptake. The new BCA may be useful in preventing the impairment of immunoreactivity in cases where coupling through amino groups is problematic.

EXPERIMENTAL PROCEDURES

General. DOTA trisodium salt and the free acid were purchased from Parish Chemical Co. (Orem, UT) and Macrocyclics, Inc. (Richardson, TX), respectively. DTPA was obtained from Fluka (Milwaukee, WI), vinyl sulfone from Aldrich (Milwaukee, WI), Chelex 100 from BioRad (Hercules, CA), and L-cysteine from Sigma (St. Louis, MO). Ultrapure water (18 M Ω -cm) was used to make all buffers that were passed over Chelex 100 and pH adjusted if necessary. $^{111}\text{InCl}_3$ was obtained from NEN Life Science products, Inc. (Boston, MA). The anti-CEA chT84.66 has been previously described (35, 45). HPLC and TLC were performed as previously described (32). Mass spectra were recorded on a Finnigan LCQ ion trap mass spectrometer using a custom nanospray interface. NMR spectra were recorded on a Varian Unity Plus 500 MHz spectrometer. Proton chemical shifts are reported relative to HOD (4.80 ppm). Antibody concentrations were calculated at 280 nm (A_{280} , 1 mg/mL = 1.42). UV spectrophotometry was performed on a Shimadzu UV160 instrument using a 1 cm cell. Melting points were recorded on a Thomas-Hoover uni-melt apparatus and are uncorrected. SDS and IEF gel electrophoresis was performed on a Pharmacia PhastGel apparatus according to the manufacturer's instructions.

Vinyl Sulfone–Cysteineamido–DOTA (VSC-DOTA; 2). Cysteineamido-DOTA (**1**) was synthesized as previously reported (46). To a solution of 3.24 mg (6.1 μmol) of **1** in 0.6 mL of 50 mM, pH 7.5 potassium phosphate was added 0.06 mL of 0.1 M DTT (6 mmol) and stirred for 0.5 h at RT under Ar. Vinyl sulfone (6.6 μL (64 μmol) in 0.6 mL of DMF was added with continuous stirring for 5 h at RT. A second aliquot was added and the reaction continued for another 22 h (final vol = 1.2 mL, 5.3 mM **1**, 107 mM vinyl sulfone). The excess vinyl sulfone was extracted with ethyl acetate (2 mL, $\times 3$). The aqueous layer was adjusted to pH 8.4, applied to a BioRad Ag1-X4 column (HCO_2^- form, 1×10 cm), washed with 30 mL of water, and eluted stepwise with 0.05–2 M HCO_2H (5 mL per step). The product (**2**) was found in fractions 8–13 by ESI/MS. The product was repeatedly lyophilized from water to remove excess HCO_2H (yield

3.4 mg). The product had the expected protonated mass by ESI/MS (626.71, expected; 626.73, observed), gave a single spot on TLC, and had a melting point of 140–142 $^\circ\text{C}$. Proton NMR (H_2O): δ 2.92 (m, 2H, $\text{SCH}_2\text{CH}_2\text{SO}_2$), δ 3.10 (m, 2H, $\text{SCH}_2\text{CH}_2\text{SO}_2$), δ 3.10–3.45 [br, 16H, $(\text{CH}_2-\text{NCH}_2) \times 4$], δ 3.53 (dd, 2H, CH_2SO_2), δ 3.45, 3.70, 3.84 (s, 6H, $\text{NCH}_2\text{CO}_2\text{H}$), δ 4.39 (m, 1H, NHCHCO_2H), δ 6.42 (dd, 2H, SO_2CHCH_2), δ 6.88 (dd, 1H, SO_2CHCH_2), δ 8.20 (d, 1H, $\text{CONH}-\text{CH}$).

Preparation of Amino-Conjugated BCA Antibody (VSC-DOTA-NH-chT84.66). chT84.66 (FW = 150000, 1.0 mg, 6 nmol, at 10 mg/mL, 60 μM) was dialyzed vs 20 mM, pH 7.0 sodium phosphate buffer (three changes, $\times 1$ L, 4 $^\circ\text{C}$) and adjusted to pH 9.0 with 200 mM, pH 10 sodium phosphate (final volume, 0.1 mL). VSC-DOTA (0.1 mL of **2**, FW = 625, 0.7 mg, 1.12 μmol at 1.88 mg/mL, 3.1 mM in 50 mM, pH 9.0 sodium phosphate) was added and the solution rotated at 10 rpm for 4 h at 25 $^\circ\text{C}$. The conjugate was dialyzed vs 250 mM NH_4 acetate (three changes, $\times 1$ L, 4 $^\circ\text{C}$).

Preparation of Sulfhydryl-Conjugated BCA Antibody (VSC-DOTA-SH-chT84.66). chT84.66 was dialyzed as above and reduced with TCEP (20 μL of 10 mM in 50 mM sodium phosphate, pH 7.0) under argon for 2.5 h at 25 $^\circ\text{C}$. TCEP was removed on a spin column (1 mL of Sephadex G25–50, 1200 rpm, 2 min) under argon. To 1.0 mg (60 nmol) of reduced chT84.66 in 0.1 mL of 50 mM sodium phosphate, pH 7, was added 0.1 mL of 3 mM (300 nmol) of VSC-DOTA (**2**) in the same buffer. The solution was rotated under argon overnight at 25 $^\circ\text{C}$ and dialyzed vs NH_4 acetate as described above. In parallel, 1 mg of TCEP reduced chT84.66 in 0.15 mL was reacted with 0.1 mL of 3 mM MC-DOTA and run as a control.

Radiolabeling of VSC-DOTA-chT84.66. The conjugates were radiolabeled with $^{111}\text{InCl}_3$ as previously described (47). For the animal studies, the conjugates (0.3 mL of 1 mg/mL in 250 mM NH_4 acetate, pH 5.7) were incubated with 0.3 mCi of ^{111}In (0.15 mL of 0.04 M HCl plus 0.03 mL of 250 mM NH_4 acetate, pH 5.7) for 60 min at 43 $^\circ\text{C}$. Excess ^{111}In was chelated with 1 mM DTPA and the radiolabeled product purified on a TosoHaas TSK–Gel G2000SW column (0.75 $\times$ 30 cm in saline, 0.5 mL/min, 0.5 mL fractions). The protein peak was mixed with 1 drop of 25% (w/v) of HSA. In other test labelings, 1.0 mCi of ^{111}In was added to 0.2 mg of conjugate and the specific activity determined by ITLC (kit 151–770, Biodex, Medical, New York). Determination of the number of chelates per conjugate and the immunoreactivity was performed as previously described (5).

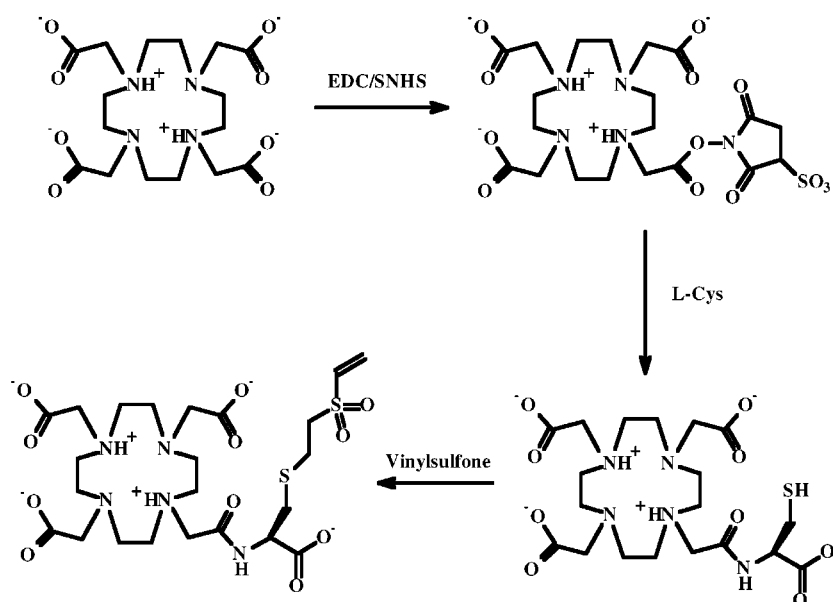
Stability Studies. Aliquots (0.5 mL) of the radiolabeled conjugates in 1% HSA were mixed with 0.05 mL of 10 mM DTPA, stored at 37 $^\circ\text{C}$ and injected at various time points onto a Pharmacia Superose 12HR column (as above) to determine the amount of radiolabel in the conjugate and DTPA.

Animal Biodistributions. Groups of five nude mice (Charles River) bearing LS174T human colon tumor xenografts (ave size = 1.5 mg $\pm$ 0.5 at $t = 0$ h) were injected with 2 μCi (2 μg) of radiolabeled conjugate via tail vein. Animals were sacrificed at 0, 12, 24, 48, 72, and 96 h after injection. Major organs, tumor, and blood were weighed, radioactivity was measured, and the results were expressed as mean %ID/g $\pm$ SD.

RESULTS

Synthesis and Antibody Conjugation. The synthesis of VSC-DOTA (**2**) is shown in Scheme 1. The product gave the expected proton NMR spectrum and protonated

Scheme 1



mass by ESI/MS and was free of DOTA and the bis-derivative. The reactivity of **2** to free L-cysteine was confirmed by ESI/MS (data not shown). At pH 7, only the mono-derivative was formed, suggesting that the thiol and not the α -amino group of L-cysteine was reactive under these conditions. When VSC-DOTA was incubated for 1–7 d in pH 7 PBS and analyzed by RP-HPLC, there was no evidence of chemical instability (data not shown).

Since VSC-DOTA was expected to react with the ϵ -amino group on the lysines of proteins at pH 9–10, we investigated the reaction of VSC-DOTA with antibody over the pH range of 7–9. The degree of conjugation was determined by IEF gel electrophoresis, wherein conjugation of the acidic BCA to antibody results in a dramatic shift of the antibody to a more acidic pI. The results of the conjugation reaction at pH 7, 8, and 9 shown in Figure 1A, lanes 3–5, demonstrate that little or no reaction occurs at pH 7, while substantial amounts of conjugate are formed at pH 9. The results at pH 8 are intermediate, with <20% product formed. The results at 37 °C, pH 9 demonstrate the expected effect of temper-

ature on the extent of reaction (Figure 1A, lane 6). The products show no change in mobility when run on SDS gel electrophoresis under nonreducing conditions (data not shown). On the basis of these results, we reasoned that sulfhydryl specific conjugation could be performed at pH 7 with little or no conjugation at amino groups, while amino group specific conjugation could be performed at pH 9, as long as no free sulfhydryl groups are present. Since the cysteine residues in most antibodies are engaged in disulfide bonds, this criterion is easily met. In addition, under mild reducing conditions (no denaturing agents), only the hinge disulfides of antibodies are reduced, allowing site-specific conjugation at just these sulfhydryl groups (48, 49). The conjugation of VSC-DOTA to mildly reduced chT84.66 at pH 7 shown in Figure 1B, lane 4, demonstrates that >90% of the antibody is conjugated under these conditions. It should be noted that either DTT or TCEP were successfully used as reducing agents. We prefer TCEP because a lower molar excess is required and since removal of the reducing agent by spin columns is not quantitative, better conjugation yields are routinely obtained.

The number of chelates per antibody as determined by trace labeling with ^{111}In in a standard solution of InCl_3 is shown in Table 1. At pH 7 and with a 50:1 molar ratio of VSC-DOTA to mAb, mildly reduced antibody gave 0.8 chelates/mAb out of a theoretical of 8 for a human IgG1 hinge. At pH 9 with the same molar ratio of VSC-DOTA to mAb, unreduced mAb gave 4.6 chelates/mAb. Radiolabeling of these two conjugates with $^{111}\text{InCl}_3$ gave similar percent radiolabeling efficiencies, specific activities, and

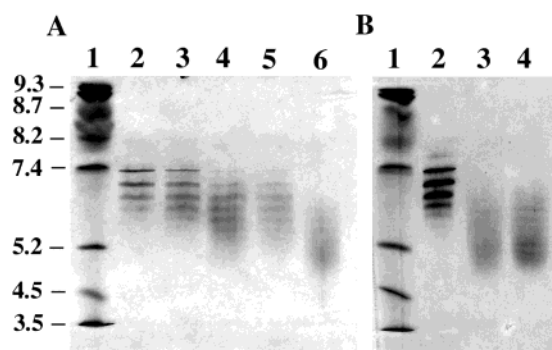


Figure 1. Analysis of VSC-DOTA-mAb conjugates by IEF gel electrophoresis. A: Lane 1: IEF standards; Lane 2: chT84.66 control; Lane 3: 30 μM chT84.66, 1.5 mM VSC-DOTA, pH 7.0, RT, 4h; Lane 4: 30 μM chT84.66, 1.5 mM VSC-DOTA, pH 8.0, RT, 4h; Lane 5: 30 μM chT84.66, 1.5 mM VSC-DOTA, pH 9.0, RT, 4h; Lane 6: 30 μM chT84.66, 1.5 mM VSC-DOTA, pH 9.0, 37 °C, 4h. B: Lane 1: IEF standards; Lane 2: chT84.66 control; Lane 3: 30 μM chT84.66 reduced with 0.5 mM TCEP, the TCEP removed by spin column, and reacted with 1.5 mM MC-DOTA, pH 7.0, RT, 4h; Lane 4: 30 μM chT84.66, reduced with 0.5 mM TCEP, the TCEP removed by spin column, and reacted with 1.5 mM VSC-DOTA, pH 7.0, RT, 4h.

Table 1. Radiometal Labeling and Immunoreactivity of VSC-DOTA-mAb^a

sample	chelates/ mAb	labeling ratio (mCi/mg)	% efficiency	% immuno- reactivity
SH-mAb	0.8	1.0–5.0	>98	>98
NH-mAb	4.5	1.0–5.0	>98	>98

^a VSC-DOTA was coupled to either SH or NH groups in the mAb as described in the text. The number of chelates per mAb was determined by trace labeling with ^{111}In in a standard solution of InCl_3 . The labeling ratio was performed with 0.3 mCi of ^{111}In and 0.3 mg of conjugate in 0.3 mL of 0.25 M ammonium acetate (pH 5.7), or 1.0 mCi of ^{111}In and 0.2 mg of conjugate in 0.2 mL of ammonium acetate (pH 5.7). Immunoreactivity was performed as described in Methods.

Table 2. Biodistribution of ^{111}In -Labeled VSC-DOTA-SH mAb in Nude Mice Bearing LS174T Xenografts^a

	0 h	12 h	24 h	48 h	72 h	96 h
blood	47.97 (2.58)	23.83 (2.43)	15.00 (2.07)	7.33 (3.78)	3.98 (2.51)	2.49 (3.02)
liver	7.30 (0.25)	6.65 (1.94)	10.15 (1.90)	14.91 (6.27)	19.60 (7.19)	18.83 (3.32)
spleen	5.41 (1.22)	5.18 (0.57)	5.93 (0.95)	7.76 (2.54)	6.41 (2.25)	7.69 (2.27)
kidney	6.36 (1.43)	5.62 (0.46)	4.36 (0.56)	3.46 (1.08)	2.82 (0.46)	2.63 (0.43)
lung	14.01 (3.27)	8.59 (1.26)	5.50 (0.72)	2.99 (1.36)	1.87 (0.88)	0.90 (0.59)
tumor	1.59 (0.47)	37.08 (4.09)	43.61 (4.91)	42.01 (12.73)	40.43 (6.96)	39.57 (18.97)
carcass	1.57 (0.15)	2.58 (0.10)	2.15 (0.29)	1.47 (0.48)	1.05 (0.26)	0.96 (0.51)

^a Five animals per group were injected with 4 μCi (4 μg) of VSC-DOTA coupled to chT84.66 via the hinge SH groups and radiolabeled with ^{111}In . The results are reported as mean %ID/g ($\pm$ SD).

Table 3. Biodistribution of ^{111}In -Labeled VSC-DOTA-NH mAb in Nude Mice Bearing LS174T Xenografts^a

	0 h	12 h	24 h	48 h	73 h	96 h
blood	47.61 (2.97)	17.98 (6.29)	17.50 (6.74)	8.98 (3.18)	5.84 (4.14)	3.08 (4.50)
liver	9.16 (0.97)	12.38 (3.77)	13.93 (5.75)	16.27 (4.25)	19.64 (5.36)	18.42 (4.79)
spleen	7.25 (0.68)	6.35 (0.73)	8.09 (2.34)	7.77 (2.00)	10.07 (2.08)	9.06 (2.09)
kidney	7.29 (1.36)	4.24 (1.14)	5.03 (1.77)	3.44 (0.87)	2.60 (0.79)	2.15 (0.76)
lung	13.07 (1.18)	5.63 (1.79)	6.58 (2.58)	3.44 (1.30)	2.58 (1.57)	1.45 (1.63)
tumor	1.26 (0.40)	21.66 (5.02)	38.54 (13.60)	39.27 (10.19)	45.76 (18.25)	33.85 (14.52)
carcass	1.76	2.20	2.64	1.72	1.43	0.89

^a Five animals per group were injected with 4 μCi (3.4 μg) of VSC-DOTA coupled to chT84.66 via NH groups and radiolabeled with ^{111}In . The results are reported as mean %ID/g ($\pm$ SD).

immunoreactivities (Table 1). When the radiolabeled conjugates were incubated in 1% HSA and 1 mM DTPA for 1–7 d, negligible loss of radioactivity was seen to DTPA (Table 1). In addition, their immunoreactivities were >95% when radiolabeled, bound to excess CEA, and analyzed by gel filtration chromatography. Since their immunoreactivities were similar, we used these conjugates for a direct comparison in animal biodistribution studies.

Comparative Animal Biodistributions of [^{111}In]-VSC-DOTA-SH-chT84.66 and [^{111}In]-VSC-DOTA-NH-chT84.66. The two ^{111}In -radiolabeled conjugates were injected into nude mice bearing human colon tumor xenografts and their biodistributions compared (Tables 2 and 3). The overall biodistributions are quite similar, resulting in maximal and sustained tumor uptake of 35–45% ID/g from 24 to 96 h. During the same time period the blood activity drops from about 45% ID/g to 2–3% ID/g. A comparison of tumor to blood curves over the time course suggests that the SH conjugate may be superior to the NH conjugate. Paradoxically, this difference may be due to a slightly slower blood clearance curve for the SH conjugate, resulting in higher uptake. A close analysis of the normal organs reveals nearly identical uptake levels and time courses for the spleen, kidney, and lung, but there are some interesting differences in the liver. The SH conjugate shows lower liver uptake at the earlier time points, suggesting that the NH conjugate accumulates more rapidly in the liver. This result would also account for their observed differences in blood clearance curves.

DISCUSSION

In a search for site-specific BCA derivatives of DOTA we first synthesized a bis-maleimido-hexane derivative of cysteineamido-DOTA (32). Maleimide is a commonly used reagent that undergoes sulfhydryl specific Michael additions that are thought to be chemically stable (50). Although this BCA allowed site-specific conjugation to the sulfhydryl groups of mildly reduced mAb, it proved to be chemically labile, undergoing pH-dependent hydrolysis at its resulting succinimidyl bonds (32). Surprisingly, this phenomenon did not prevent good tumor uptake in animal biodistribution studies (33). As a result, we proposed that further increasing the chemical lability of the linker may be beneficial, since this would result in lower blood levels. Indeed, oxidation of the thioether bond in the linker to a sulfone increased the chemical lability of the BCA (32), but the BCA decomposed too rapidly to allow good tumor uptake. On the basis of these studies, we decided to investigate other Michael addition linkers as candidates for site-specific conjugation to mAbs. We chose vinyl sulfone for its well-documented reaction with sulfhydryl groups (51). However, we realized from the outset that vinyl sulfone can also react with amino groups (52, 53), and thus we were interested in seeing if this reaction could be controlled by manipulation of the reaction pH.

Vinyl sulfone is a viscous liquid with poor solubility in water, but when dissolved in DMF or acetonitrile reacts well with Michael acceptors in aqueous medium. In the synthesis of the title compound (**2**), we demonstrated a facile reaction between vinyl sulfone and cysteineamido-DOTA. Unlike the maleimido derivatives, the vinyl sulfone derivative of cysteineamido-DOTA is stable in PBS at pH 7. When **2** was reacted with chT84.66 at pH 7, no conjugate was formed unless the mAb was mildly reduced prior to reaction. In addition, we showed that the mAb conjugated with **2** at pH 9 to a greater extent (no. chelates/mAb) as it did to reduced mAb at pH 7 using the same molar ratio of **2** to mAb. Thus, we demonstrated that this BCA can be conjugated to the same mAb via different chemical linkages using pH and reducing agents to convey linkage specificity.

Since the biodistributions of radiolabeled mAbs is highly dependent on the mode of attachment of the radiolabel, it is always interesting to compare BCAs conjugated to the same mAb via different chemical linkages. In most cases, it is difficult to perform this type of comparative study. However, in this study, we have an almost ideal model. In one case, the same BCA is conjugated to sulfhydryl groups, and in the other case, amino groups, but the resulting BCA/mAb ratios are different. Even so, the resulting biodistributions are quite similar. When we compare these results to our previous studies in which DOTA was conjugated to chT84.66 by an active ester derivative of DOTA (34, 35), we find that the uptake curves are similar for both tumor and normal tissues. This suggests that linkage of DOTA based BCAs to mAbs via amides, amine, or sulfhydryl bonds results in similar tissue, including tumor, uptakes, and metabolism. Thus, if one wants to manipulate normal or tumor uptake and metabolism of DOTA-based BCAs, it is necessary to explore other modes of chemical linkage or to insert linkers which have built-in chemical or biological lability. Examples of such linkers have been described by Meares (21), Arano (54), and ourselves (32).

When we examined the comparative biodistributions of the SH and NH BCA-chT84.66 conjugates in more detail, we noticed some intriguing trends. They include

the higher blood levels, higher tumor levels, and lower liver levels for the BCA chT84.66 SH conjugate at early time points 0–24 h. These three “organs” are interdependent in their biodistributions as follows: high tumor uptake is dependent on maintaining high blood levels, especially early in a biodistribution. If the blood is cleared by any mechanism, including rapid liver uptake, then blood and tumor levels will fall together. Thus, the lower liver uptake seen at early time points for the SH conjugate leads to higher blood levels and ultimately to higher tumor uptake which is sustained at later time points. While the biological reasons for these differences are unknown at this time, we can speculate that this mode of conjugation will lead to incremental improvements in the human, where most biodistributions are predicted from the animal model.

In summary, we have prepared a new BCA that allows site-specific conjugation to most mAbs either via sulfhydryl or amino groups by manipulating pH and use of reducing agents. The ^{111}In -radiolabeled versions of these conjugates give more or less equivalent animal biodistributions to previously described ^{111}In -radiolabeled BCA-mAbs, including are own version using the active ester of DOTA (34, 35). This approach should have utility, allowing the investigator to chose one type of chemistry over the other depending on the restrictions implied by a given antibody. Finally, the improvement in tumor to blood and tumor-to-liver ratios seen for the SH-specific conjugate may translate into improved performance in the clinic.

ACKNOWLEDGMENT

The authors thank Stefanie Wright for the animal studies. This research was funded by NCI grant CA43904 (A.A.R. and J.E.S.) and the Department of the Army Grant DAMD 17-98-1-8031 (J.E.S.).

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BC015535B