

S-Functionalized Cysteine: Powerful Ligands for the Labelling of Bioactive Molecules with Triaquatricarbonyltechnetium-99m(**1**+) ($[\text{}^{99\text{m}}\text{Tc}(\text{OH}_2)_3(\text{CO})_3]^+$)

by Dave R. van Staveren^{a)} ^{b)}, Paul D. Benny^{a)}, Robert Waibel^{b)}, Philipp Kurz^{a)}, Jae-Kyoung Pak^{a)}, and Roger Alberto^{*a)}

^{a)} Institute of Inorganic Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich (phone: +41-1-635-4631; fax: +41-1-635-6803; e-mail: ariel@aci.unizh.ch)

^{b)} Paul Scherrer Institut, Center for Radiopharmaceutical Sciences, CH-5232 Villigen PSI

Dedicated to Professor *André Merbach* on the occasion of his 65th birthday

S-Alkylated cysteines are used as efficient tridentate N,O,S-donor-atom ligands for the *fac*- $[\text{M}(\text{CO})_3]^+$ moiety ($\text{M} = {}^{99\text{m}}\text{Tc}$ or Re). Reaction of $(\text{Et}_4\text{N})_2[\text{ReBr}_3(\text{CO})_3]$ (**3**) with the model *S*-benzyl-L-cysteine (**2**) leads to the formation of $[\text{Re}(\text{2}')(\text{CO})_3]$ (**4**) as the exclusive product (**2'** = C-terminal anion of **2**). The tridentate nature of the alkylated cysteine in **4** was established by X-ray crystallography. Compound **2** reacts with $[\text{}^{99\text{m}}\text{Tc}(\text{OH}_2)_3(\text{CO})_3]^+$ under mild conditions (10^{-4} M, 50°, 30 min) to afford $[\text{}^{99\text{m}}\text{Tc}(\text{2}')(\text{CO})_3]$ (**5**) and represents, therefore, an efficient chelator for the labelling of biomolecules. L-Cysteine, *S*-alkylated with a 3-aminopropyl group ($\rightarrow$ **7**), was conjugated *via* a peptide coupling sequence with *Coa*-[α -(5,6-dimethyl-1*H*-benzimidazolyl)]-*Co* β -cyanocobamic *b*-acid (**6**), the *b*-acid of cyanocob(III)alamin (vitamin B₁₂) (*Scheme 3*). More convenient was a one-pot procedure with a derivative of vitamin B₁₂ comprising a free amine group at the *b*-position. This amine **15** was treated with NHS (*N*-hydroxysuccinimide)-activated 1-iodoacetic acid **14** to introduce an I-substituent in vitamin B₁₂. Subsequent addition of unprotected L-cysteine resulted in nucleophilic displacement of the I-atom by the *S*-substituent, affording the vitamin B₁₂ alkylated cysteine fragment **17** (*Scheme 4*). The procedure was quantitative and did not require purification of intermediates. Both cobalamin–cysteine conjugates could be efficiently labelled with $[\text{}^{99\text{m}}\text{Tc}(\text{OH}_2)_3(\text{CO})_3]^+$ (**1**) under conditions identical to those of the model complex **5**. Biodistribution studies of the cobalamin conjugates in mice bearing B10-F16 melanoma tumors showed a tumor uptake of $8.1 \pm 0.6\%$ and $4.4 \pm 0.5\%$ injected dose per gram of tumor tissue after 4 h and 24 h, respectively (*Table 1*).

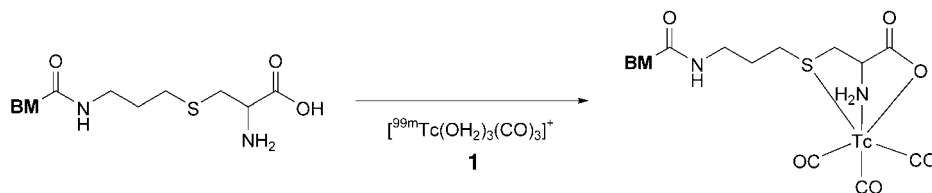
Introduction. – The labelling of biologically active molecules with ${}^{99\text{m}}\text{Tc}$ is a field of intense research, since ${}^{99\text{m}}\text{Tc}$ is one of the most widely employed radionuclides for imaging in nuclear medicine [1–3]. Besides the usual precursors, which are based on the $[\text{Tc}=\text{O}]^{3+}$ moiety, the organometallic complex $[\text{}^{99\text{m}}\text{Tc}(\text{OH}_2)_3(\text{CO})_3]^+$ (**1**) has attracted much attention since it offers a viable pathway to ${}^{99\text{m}}\text{Tc}$ -labelled compounds. The approach has been critically discussed recently [4][5]. The interest is based on several issues. Complexes comprising the $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3]^+$ core are highly robust *in vitro* and *in vivo* and display a distinct affinity for a large variety of donor atoms. The starting complex $[\text{}^{99\text{m}}\text{Tc}(\text{OH}_2)_3(\text{CO})_3]^+$ (**1**) is conveniently prepared, and GMP-compliant kits for the preparation of $[\text{}^{99\text{m}}\text{Tc}(\text{OH}_2)_3(\text{CO})_3]^+$ are commercially available (*Isolink*® *Tyco-Mallinckrodt Med. B.V.*). The wide variety of chelators appropriate for stabilizing the *fac*- $[\text{Tc}(\text{CO})_3]^+$ moiety enables fine-tuning of labelled compounds and systematic drug-finding for radiopharmaceuticals. Basic and applied studies with a number of ligands have been described [6–11].

Among the chelators efficiently coordinating to the *fac*-[Tc(CO)₃]⁺ moiety, tridentate ligands are favorable since labelling at low concentrations, as required for receptor-targeting agents, is feasible, and complexes of high biological stability are obtained. However, tridentate ligands demand protecting-group chemistry that is selectively conjugated to anchoring groups on biomolecules. In earlier studies, picolylamine-diacetic acid (= *N*-(carboxymethyl)-*N*-(pyridin-2-ylmethyl)glycine) and L-histidine proved to be particularly interesting ligands [6][12][13]. The former one can directly be coupled to biomolecules but the resulting complexes are relatively lipophilic. L-Histidine on the other hand affords a more hydrophilic complex, but its selective functionalization requires a multi-step synthetic procedure [8].

We have recently described different methods for the selective *N*^ε-functionalization of L-histidine with an acetate and an alkylamine group [8][13]. These amino acid derivatives were coupled to targeting molecules such as biotin, vitamin B₁₂ and the neuropeptide leucine-enkephalin and labelled with ^{99m}Tc. Biodistribution studies with several vitamin B₁₂-L-histidine conjugates in tumor-bearing mice showed promising results [14].

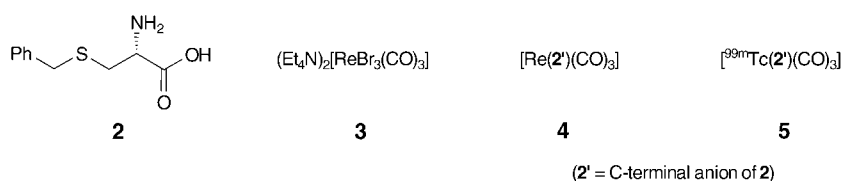
Despite the availability of tripodal L-histidine, it remains a challenge to introduce further chelators smaller in size, with different physicochemical properties and by a versatile synthetic procedure. L-Methionine is a reasonably good chelator and forms one seven-, one six-, and one five-membered chelate ring upon coordination with [^{99m}Tc(OH₂)₃(CO)₃]⁺ (**1**). However, bifunctionalization at the N–C(α) of methionine would yield four diastereoisomers after coordination to **1**. Replacing methionine by an *S*-alkylated cysteine reduces this number to two. In addition, coordination affords here two five- and one six-membered ring, thus, a thermodynamically more-stable complex. Accordingly, we present in this study the synthesis of bifunctional *S*-alkylated L-cysteine, its conjugation to vitamin B₁₂ *via* two different synthetic routes, and labelling experiments along with biodistribution studies. *S*-Alkylated L-cysteine represents a small tridentate N,O,S-ligand of comparable affinity for the [Tc(CO)₃]⁺ moiety than histidine (*Scheme 1*). The concept is general and can be applied to various types of biomolecules.

Scheme 1. Biomolecule (BM) Derivatized with a Tripodal *S*-Alkylated L-Cysteine Ligand and Labelling with [^{99m}Tc(OH₂)₃(CO)₃]⁺ (**1**)



Results and Discussion. – To assess the authenticity of ^{99m}Tc complexes, it is common to prepare macroscopic amounts of the corresponding rhenium analogue first. Comparison of its retention time in HPLC with the corresponding microscopic amounts of ^{99m}Tc complex establishes the mutual identity. For these basic coordination chemistry experiments with *S*-alkylated L-cysteines, the model *S*-benzyl-L-cysteine (**2**)

was chosen. The reaction of **2** with the complex $(\text{Et}_4\text{N})_2[\text{ReBr}_3(\text{CO})_3]$ (**3**) was investigated. Dissolution of **3** in water yielded the solvated precursor $[\text{Re}(\text{OH}_2)_3(\text{CO})_3]^+$. The subsequent reaction with **2** proceeded straightforward, and, after heating equimolar amounts of **2** and **3** in water at 70° for several hours in the presence of NaHCO_3 as a base, the complex $[\text{Re}(\mathbf{2}')(\text{CO})_3]$ (**4**; $\mathbf{2}'$ = C-terminal anion of **2**) was obtained as an analytically pure white solid after filtration. HPLC Analysis of **4** shows the presence of one single peak. Since the coordinating thioether S-atom represents a prochiral center, one would expect two diastereoisomers that should be distinguishable, provided that the corresponding retention times are not identical. The presence of one well-behaved peak implies either the preferential formation of one isomer or the formation of two isomers with identical retention times. X-Ray-quality crystals of **4** were obtained by slow evaporation of a $\text{H}_2\text{O}/\text{MeOH}$ solution. An ORTEP presentation of **4** is given in Fig. 1. As anticipated, the ligand **2** acts as a tridentate N,O,S-donor ligand, coordinating to the Re^{I} center in a facial arrangement. Bond lengths and angles are within the expected range. As evident from Fig. 1, the complex comprises one six- and two five-membered rings.



The complex formation of **2** with the $^{99\text{m}}\text{Tc}$ precursor $^{99\text{m}}\text{Tc}(\text{OH}_2)_3(\text{CO})_3^+$ (**1**) proceeded analogously. To obtain a measure for the efficiency of a particular ligand, concentration range and temperature have to be screened systematically. Conditions under which quantitative complex formation was achieved give a hint as to what can be expected when using the corresponding ligand coupled to a biomolecule. Heating at 50° for 30 min and at concentrations of **2** between 10^{-4} and 10^{-6} M ($^{99\text{m}}\text{Tc}$ 10^{-7} – 10^{-8} M) gave complete conversion of **1** and **2** to $^{99\text{m}}\text{Tc}$ -complex **5** according to radiochromatographic analysis. As with the Re-complex **4**, the radiochromatogram depicted one single peak with a retention time equal to that of **4** (Fig. 2). The labelling efficiency was high and compared well with that of L-histidine. In a competition experiment, L-methionine (Met–OH) and **2** were added in equimolar amounts, which resulted in the formation of the two complexes $^{99\text{m}}\text{Tc}(\mathbf{2}')(\text{CO})_3$ (**5**) and $^{99\text{m}}\text{Tc}(\text{Met–O})(\text{CO})_3$ in a 4:1 ratio. Accordingly, S-alkylated cysteines are *ca.* four times as efficient as L-methionine towards the $[\text{Tc}(\text{CO})_3]^+$ moiety.

Ligand **2** represents obviously a strong chelator, and its conjugation to a biomolecule will allow labelling at low concentrations and moderate temperature, conditions required for future routine use. Furthermore, the complex is neutral and small, and, thus, expected not to affect the biological behavior of the carrier molecule to a significant extent. We selected cyanocob(III)alamin or vitamin B₁₂ for conjugation to L-cysteine derivatives for the following reasons. Vitamin B₁₂ is of high interest for tumor imaging, since rapidly proliferating cells are high vitamin B₁₂ consumers, and

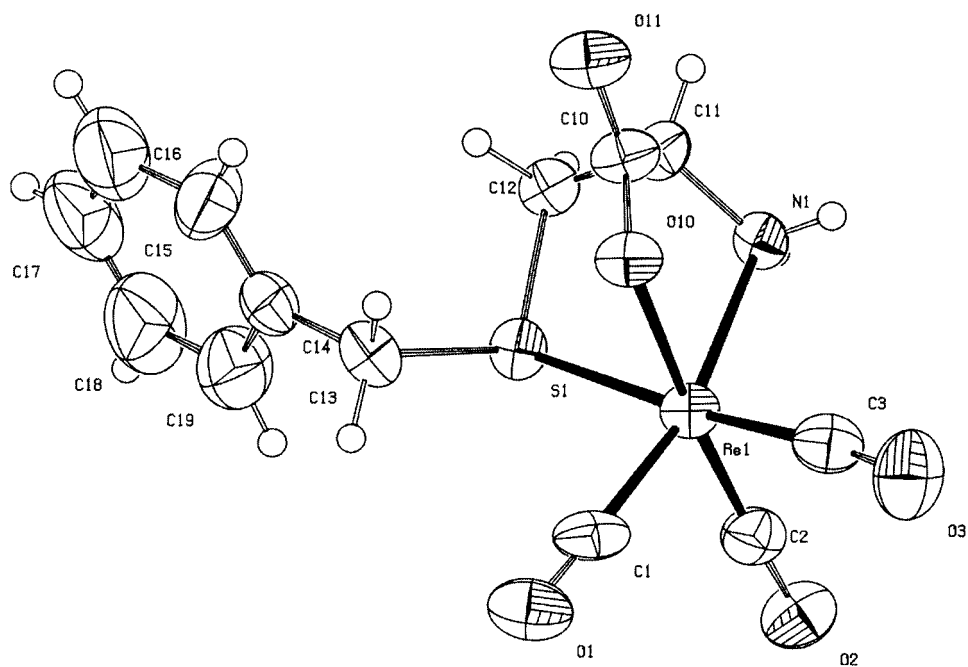


Fig. 1. ORTEP Projection of $4 \cdot \frac{1}{2} \text{H}_2\text{O}$, with thermal ellipsoids at 50% probability level. The uncoordinated half water molecule is omitted for clarity. Selected bond lengths [Å]: Re(1)–O(10), 2.169(7); Re(1)–N(1), 2.218(8); Re(1)–S(1), 2.484(3); Re(1)–C(1), 1.917(13); Re(1)–C(2), 1.909(13); Re(1)–C(3), 1.920(13).

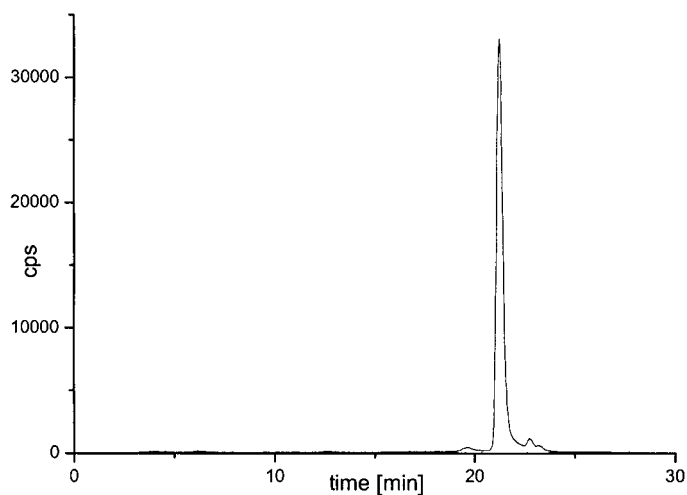


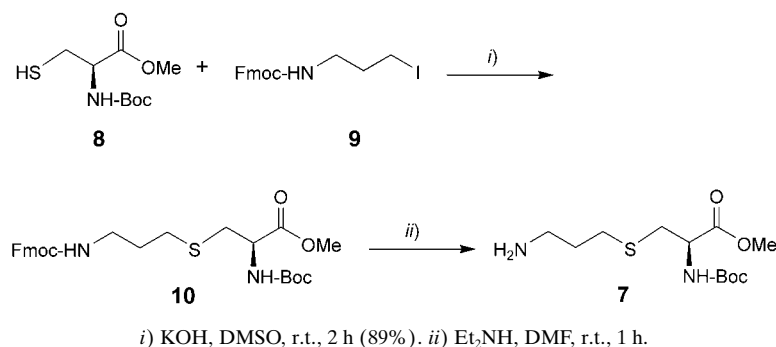
Fig. 2. Radio trace of the labelling of S-benzyl-L-cysteine (**2**) with $[^{99\text{m}}\text{Tc}(\text{OH}_2)_3(\text{CO})_3]^+$ (**1**). $[\mathbf{2}] = 10^{-4} \text{ M}$, 50° , 30 min.

strong accumulation of radioactivity can be expected with vitamin B₁₂ as a carrier molecule [15][16]. Conjugation and subsequent labelling of vitamin B₁₂ is challenging because it contains a variety of potential donor atoms that might compete with the alkylated L-cysteine ligand for ^{99m}Tc-coordination. To circumvent unspecific binding, high labelling selectivity at the ligand site is required.

Vitamin B₁₂ does not possess functionalities available for direct coupling to L-cysteine or L-cysteine derivatives. An ‘activated’ precursor of choice is vitamin B₁₂ in which one of the pendant amide functionalities at the corrin macrocycle has been hydrolyzed to the corresponding carboxylate. The preparation of such precursors has been described, and so called *Coα*-[α -(5,6-dimethyl-1*H*-benzimidazolyl)]-*Coβ*-cyanocobamic *b*-acid (abbreviated as cyanocob(III)alamin-*b*-acid; **6**) can be prepared conveniently in reasonable yields [17]. *S*-Alkylated L-cysteines can then be conjugated to vitamin B₁₂ along two principle routes. An additional amino group in derivatized L-cysteine **7** can directly be coupled to **6** by amide formation according to procedures known from peptide chemistry or, alternatively, an alkyl halide group is previously introduced in vitamin B₁₂, and subsequent direct alkylation with protected or unprotected L-cysteine results then in the tridentate ligand attached to vitamin B₁₂. The first approach requires derivatization of L-cysteine, whereas, for the second method, derivatization of vitamin B₁₂ is necessary. As will be discussed below, both strategies are feasible.

The introduction of a primary amino group in fully N,O-protected cysteine was achieved according to the procedure outlined in *Scheme 2*. The reaction of Fmoc-NH-(CH₂)₃-I (**9**; Fmoc = (9*H*-fluoren-9-ylmethoxy)carbonyl), prepared as published previously [13], with *N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester (**8**) afforded *S*-{3-[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}propyl)-*N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester (**10**) in good yield. The Fmoc protecting group was cleaved by treatment with Et₂NH in DMF, which afforded *S*-(3-aminopropyl)-*N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester (**7**) quantitatively. Purification of **7** at this stage was not necessary because the fulvene by-products were removed during the prep. HPLC purification of the final vitamin-B₁₂ product.

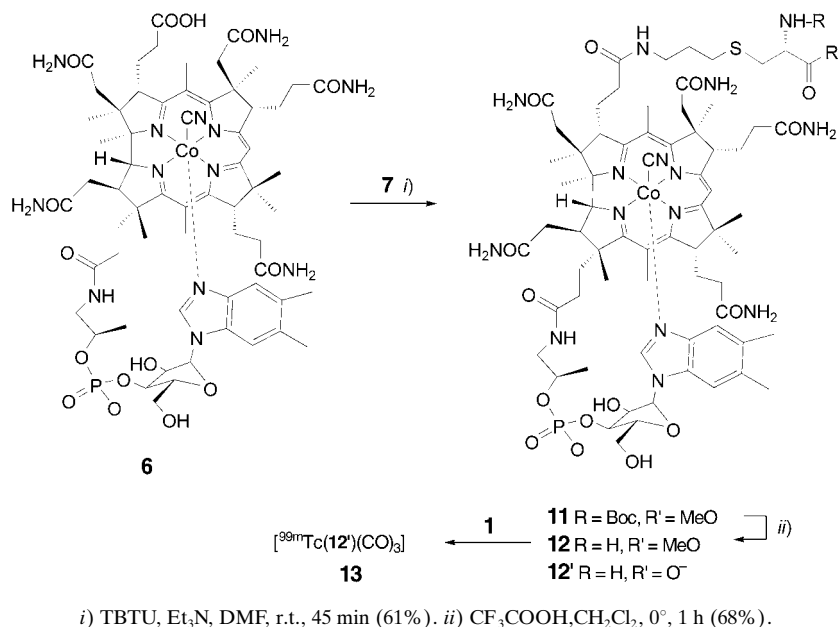
Scheme 2. Synthesis of *S*-Alkylated L-Cysteine Derivative **7**



Coupling of **7** with cyanocob(III)alamin-*b*-acid (**6**) in DMF by employing TBTU (*O*-(1*H*-benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate) as the

coupling reagent afforded **11** in good yield after purification by prep. HPLC (Scheme 3). The TBTU coupling procedure is very mild and required only stirring of the reactants for 45 min at room temperature. Evidence for the proposed constitution of **11** was obtained from the ESI-MS ($[M + H]^+$ at m/z 1630.4). Further confirmation was obtained by comparing the ^1H - and ^{13}C -NMR spectra of **7** in CD_3OD with those of cyanocob(III)alamin-*b*-acid (**6**) [13]. Additional resonances could be assigned to the pendant ligand of the conjugate¹⁾.

Scheme 3. Preparation of Conjugates **11** and **12**



The Boc-protecting group has to be removed to obtain the final vitamin-B₁₂ derivative with a pendant tridentate ligand. Reacting **11** in $\text{CH}_2\text{Cl}_2/\text{CF}_3\text{COOH}$ (4:1) for 1 h at 0° resulted in quantitative cleavage of the Boc group and gave the product **12**. The ESI-MS ($[M + H]^+$ at m/z 1530.5) and the ^1H - and ^{13}C -NMR spectra confirmed the proposed structure¹⁾. The disappearance of the signals at δ 150.8, 81.0, and 28.9 in the ^{13}C -NMR spectrum indicated complete removal of the Boc group.

Compound **12** represents a vitamin-B₁₂ derivative with a pendant tridentate L-cysteine chelator that can be labelled with the *fac*- $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3]^+$ moiety. The C-terminus of the L-cysteine moiety in **12** is still protected as a methyl ester, but this is cleaved concomitantly during the reaction of **12** with **1**, and no yield effect in labelling the free ligand or its ester was found. Presumably, the *Lewis* acid metal center mediates intra- or intermolecular hydrolysis of the methyl ester **12**, to the corresponding carboxylate **12'**, which is then prone to coordination. It is now of interest to determine whether labelling occurs site specifically or if unspecific coordination to other donating

¹⁾ ^1H - and ^{13}C -NMR Spectra of **11**, **12**, and **17** are available from the senior author (R.A.).

groups in the vitamin B₁₂ moiety takes place. The 1*H*-benzimidazole in the backloop of **12'**, which can be released intermediately, would be available for coordination in particular.

The reaction of **12** with [^{99m}Tc(OH₂)₃(CO)₃]⁺ (**1**) occurred very similarly to the model reaction of ligand **2**. After 30 min at 50° and at concentrations between 10^{−4} and 10^{−5} M, **1** had bound quantitatively to **12** and one single new peak was observed (Fig. 3) representing the conjugate [^{99m}Tc(**12'**)(CO)₃] (**13**). The longer retention time of the complex **13** as compared to unlabelled **12** is in agreement with the general observation that the introduction of [^{99m}Tc(OH₂)₃(CO)₃]⁺ (**1**) in a vitamin B₁₂ derivative increases the lipophilicity of the resulting labelled conjugate. The efficient and site-directed labelling indicates that the presence of the bulky vitamin B₁₂ moiety with its potentially competing ligand sites does not significantly affect the reaction.

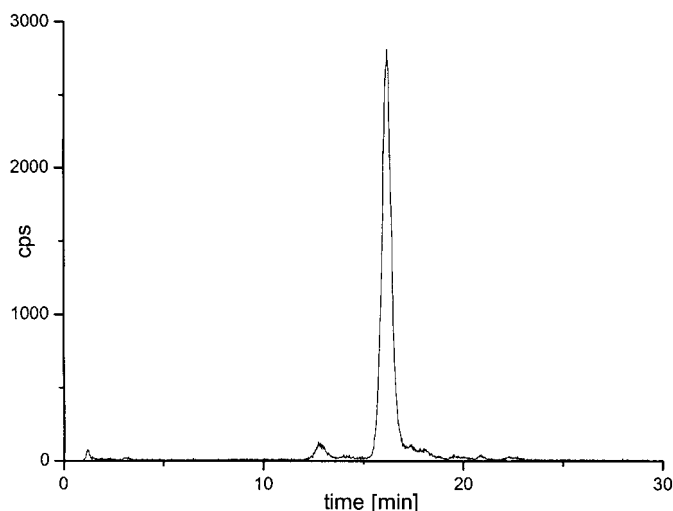
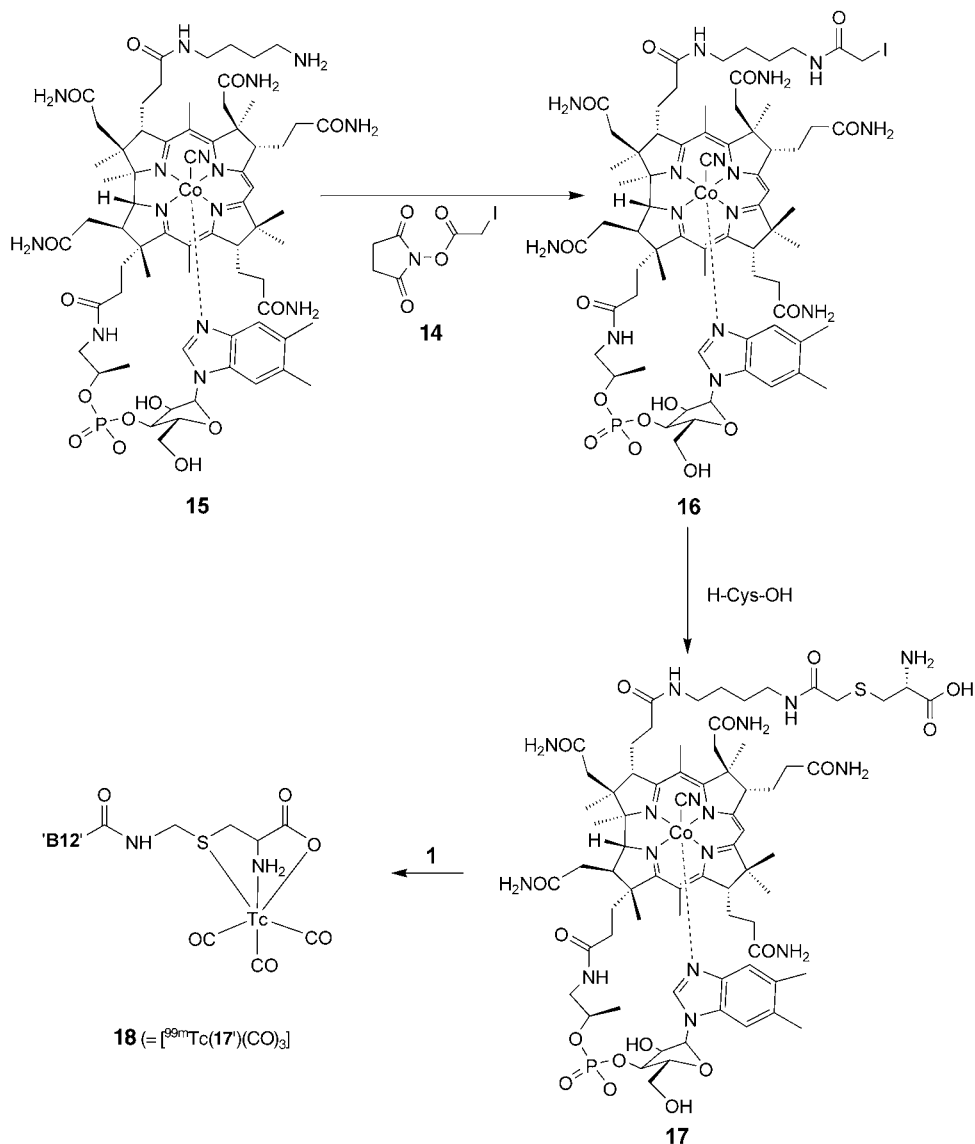


Fig. 3. Radio trace of the labelling of **12** with [^{99m}Tc(OH₂)₃(CO)₃]⁺ (**1**). [**12**] = 10^{−4} M, 50°, 30 min.

The L-cysteine derivative **7** represents a direct precursor for a tridentate ligand that can not only be introduced to vitamin B₁₂ but also to other biomolecules with an available carboxylic acid function. Thus, the strategy represented above is general, and **7** can be considered a versatile precursor for the labelling of many other bioactive molecules. Still, deprotection of Boc is required, which might not be feasible for sensitive molecules. Therefore, we attempted to explore a second approach, which is employing *N*-unprotected L-cysteine.

The introduction of an activated alkyl halide in vitamin B₁₂ followed by nucleophilic attack of the thiol moiety of L-cysteine would result directly in the third coordinating function, the thioether group. According to strategies known from protein derivatizations, we selected NHS (*N*-hydroxysuccinimide)-activated iodoacetic acid **14** [18]. For the introduction of the iodoacetic acid moiety into cyanocob(III)alamin-*b*-acid, the presence of a primary amino group in **6** was mandatory. Thus, butane-1,4-diamine was linked to the *b*-acid **6** by amide formation to afford compound **15** (Scheme 4), following a procedure previously reported for the synthesis of the corresponding dodecane

Scheme 4. Stepwise Assembly of Conjugate **17** from **15** and Labelling with $[^{99m}\text{Tc}(\text{OH}_2)_3(\text{CO})_3]^+$ (**1**)

compound [17]. Subsequent reaction of **15** with 5 equiv. of commercially available NHS-activated iodoacetic acid **14** in phosphate buffer at pH 7.4 for 30 min afforded clean conversion to one single new product **16** according to HPLC. Without isolation or purification of **16**, excess L-cysteine (10 equiv.) was added to the reaction mixture, and stirring was continued for another 30 min, resulting in complete conversion to **17**. The presence of one single peak on HPLC analysis indicated that no competing alkylation at $\text{NH}_2\text{-C}(\alpha)$ of L-cysteine took place and implied that the iodo group reacted

exclusively with the thiol function. Compound **17** was isolated and purified by means of an *18ec* cartridge (see *Exper. Part*). Evidence for the proposed constitution of **17** was obtained from the ESI-MS ($[M + H]^+$ at m/z 1601.5). The ^{13}C -NMR spectrum of **17** showed the expected number of extra resonances with respect to the free *b*-acid [13]. The ^{13}C - and ^1H -NMR spectra of **17** confirmed the structure¹⁾.

The reaction sequence from **15** to **17** is versatile since no purification of the intermediate **16** was required. The reaction conditions are mild, which is important in the context of biomolecules such as vitamin B_{12} . The direct availability of an alkylated L-cysteine ligand without any protecting group is ideal with respect to later labelling. We emphasize that the second procedure can also be applied to other biomolecules, thus representing again a general approach for introducing a tridentate L-methionine-type chelator to a bioactive molecule.

Labelling of **17** with **1** was performed under identical conditions to that of **12** (10^{-4} M, 50° , 30 min). The reactivity of **17** was similar to that of **12**, and no starting material could be observed after the reaction. One major difference in the labelling between **17** and **12** is the observation that **17** yielded a complex **18**, which exhibited two peaks of about equal intensities in the HPLC (Fig. 4) instead of one as observed for the complex **13** obtained from **12**. As discussed in the context of the model ligand **2** and its complex **4**, the two HPLC peaks of **18** can probably be explained by the presence of two diastereoisomers. The isolated complex is always a pair of enantiomers since the group attached to the S-donor can adopt two orientations. In the case of complex **4**, evidence was obtained that one pair is thermodynamically preferred. If the S-donor is attached to a chiral vitamin B_{12} derivative, formation of a 1:1 mixture of two diastereoisomers is thus expected and obviously observed in the case of **18** (Scheme 4). In the case of **13** (Scheme 3), the long linker ($\text{CH}_2\text{CH}_2\text{CH}_2$) between the cyanocob(III)alamin *b*-acid moiety and the complexing moiety makes this pendant latter moiety structurally flexible, thus resulting in similar polarity of the two diastereoisomers; this is likely to

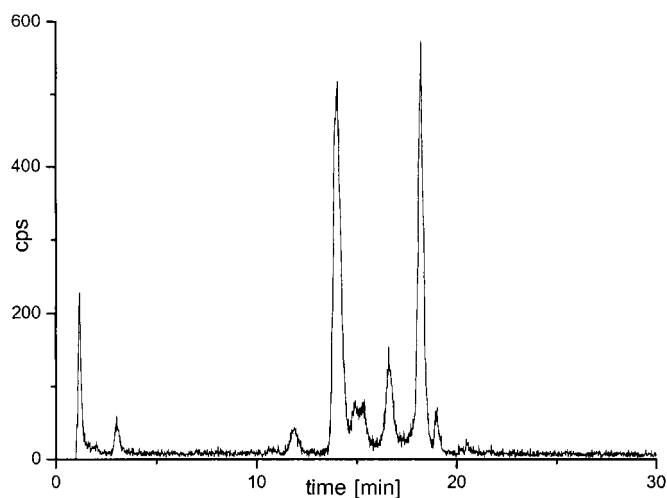


Fig. 4. Radio trace of the labelling of **17** with $[^{99\text{m}}\text{Tc}(\text{OH}_2)_3(\text{CO})_3]^+$ (**1**). $[\textbf{17}] = 10^{-4}$ M, 50° , 30 min.

exclude the observation of the two diastereoisomers in the HPLC. The short spacer (CH_2) in **17**, however, renders the conjugate much more rigid, leading to hindered rotation of the complexing moiety which in turn allows the chromatographic observation of the two diastereoisomers of **18**.

We emphasize at this place that the presence of a Me ester at the C-terminus of the S-alkylated L-cysteine does not influence the labelling yield and reaction conditions. Regardless of the presence of such an ester, both C-terminal acid **17** and C-terminal ester **12** can be labelled with $^{99\text{m}}\text{Tc}(\text{OH}_2)_3(\text{CO})_3]^+$ (**1**) under identically mild conditions.

*Biodistribution Studies of $^{99\text{m}}\text{Tc}(\text{12}')(\text{CO})_3$ (**13**).* – Vitamin B_{12} (=cyanocob(III)-alamin) is an essential cofactor for the proper function of living cells. It is utilized in intracellular metabolic pathways, and its chemistry and biochemistry has comprehensively been reviewed [15][16]. Methylcob(III)alamin (Me-Cbl) is the cofactor for methionine synthase, whereas (5'-deoxy-5'-adenosyl)cob(III)alamin (Ado-Cbl) is involved in methylmalonyl-CoA mutase, the rearrangement of methylmalonyl-CoA to succinyl-CoA. Cob(III)alamin is also involved in the reductive conversion of ribonucleotides to deoxyribonucleotides to generate DNA. Hyperproliferative cells, such as many cancer cells, require an increased uptake of vitamin B_{12} . It is a broader aspect of this study to find a $^{99\text{m}}\text{Tc}$ -labelled vitamin B_{12} derivative that allows, *via* high accumulation in tumors, radiodiagnostic imaging. We, therefore, investigated the biodistribution of $^{99\text{m}}\text{Tc}(\text{12}')(\text{CO})_3$ -(**13**) in mice with B16–F10 melanoma tumors. Despite many research efforts in that direction, no vitamin B_{12} based appropriate radiopharmaceutical is available to date [19–21].

Groups of three mice were injected with 0.5–1 ng of **13** (specific activity 10 mCi/ μg), and the organ distribution of **13** in comparison to native cyano ^{57}Co]cob(III)-alamin (^{57}Co] vitamin B_{12}) was studied at 4-h and 24-h post-injection. The organ distribution is listed in *Table 1*.

Four-hours postinjection shows a relatively large accumulation of **13** in the tumor but also a substantial amount in blood, liver, and kidney. All data points are comparable to those of native vitamin B_{12} at this stage. After 24 h, however, significant differences are observed. The activity in blood in particular decreased by a factor of 5 for **13**, but the activity in the tumor decreased as well by a factor two. The labelled conjugate **13** does not show improved tumor enrichment at this time point with respect to ^{57}Co]vitamin B_{12} . This does not come as a surprise, since modification of biomolecules is generally accompanied by a decrease in receptor affinity. Of more interest are the differences in the relative organ distributions. The three most important organs/fluids from a radiopharmaceutical point of view are liver, kidney, and blood. High liver and kidney uptake increases the dose burden to these organs and leads to a low target to non-target ratio. Therefore, low kidney and liver values are crucial for a good imaging or therapeutic agent. At 24 h, **13** displays a similar kidney uptake as ^{57}Co]vitamin B_{12} , whereas its liver uptake is decreased substantially by about 30%. Especially for imaging – for which Tc-radiopharmaceuticals are used – low blood values are mandatory because high blood values will result in high background noise. As can be seen from *Table 1*, the activity in the blood of **13** after 24 h is six-fold less than that of ^{57}Co]vitamin B_{12} , and the tumor-to-blood ratio is, therefore, much better for **13** than for ^{57}Co]vitamin B_{12} . The ratio is *ca.* 5, which is considered to be at the lower limit to

achieve good resolution images. Still, the high kidney and liver accumulations are a matter of concern, and further derivatizations with other chelators and/or spacers are required to improve the radiolabelled targeting agent. Recently, we have studied the biodistribution on several vitamin B₁₂–L-histidine conjugates. Comparing the results from **13** with the best L-histidine derivative, the latter appears to be more promising [14]. The tumor-to-blood ratios of the two derivatives are similar, but the latter has better tumor-to-kidney and tumor-to-liver ratios. If this is due to the different chelator or the different spacer types remains to be determined.

Table 1. Biodistribution of [^{99m}Tc(**12'**)(CO)₃] (**13**) at 4 h and 24 h Post-Injection in Mice Bearing Syngeneic B16–F10 Melanoma Tumors. Values are given as percentage injected dose per gram of tissue (average of 3 ± σ).

Organ	[^{99m} Tc(12')(CO) ₃] (13)		[⁵⁷ Co]vitamin B ₁₂ ^{a)}
	4 h	24 h	24 h
Blood	5.0 ± 0.3	0.9 ± 0.1	6.3 ± 0.5
Spleen	5.9 ± 0.9	3.1 ± 0.5	8.0 ± 1.6
Kidney	33.7 ± 2.7	26.2 ± 2.8	29.0 ± 1.8
Liver	22.6 ± 4.1	18.2 ± 1.3	12.4 ± 1.4
Muscle	1.4 ± 0.2	0.6 ± 0.1	2.5 ± 0.0
Tumor	8.1 ± 0.6	4.4 ± 0.5	9.9 ± 0.3

^{a)} From [14].

Conclusions. – *S*-Alkylated L-cysteine derivatives are excellent ligands for the [^{99m}Tc(CO)₃]⁺ moiety and its Re-surrogate. By variation of the *S*-alkylation agent, different additional coupling functionalities such as a carboxylic acid or a primary amine group can be introduced. This allowed the coupling of the protected L-cysteine derivative *via*, *e.g.*, amide formation to the biomolecule (*cf. Scheme 3*). A second and more straightforward method for conjugation of L-cysteine to a biomolecule is also presented. Due to the highly selective reaction of thiols with iodoalkanes, the derivatization of a biomolecule with NHS-(*N*-hydroxysuccinimide)-activated iodoacetic acid and subsequent coupling with unprotected L-cysteine gave one single product under mild conditions (*cf. Scheme 4*). Both approaches were exemplified with vitamin B₁₂ and represent a general strategy for introducing highly efficient and stable tripodal chelators in biomolecules for subsequent labelling with ^{99m}Tc. The labelling of either vitamin B₁₂ derivatives with [^{99m}Tc(OH₂)₃(CO)₃]⁺ was quantitative and occurred under mild conditions.

We thank *Solidago* for financial support. *Christine De Pasquale* is kindly acknowledged for performing the animal studies.

Experimental Part

General. The complex (Et₄N)₂[ReBr₃(CO)₃] (**3**) [22], Fmoc-NH-(CH₂)₃-I(**9**) [13], and *Coa*-[α-(5,6-dimethyl-1*H*-benzimidazol-1-yl)]-*Coβ*-cyanocob(III)amic *b*-acid (= cyanocob(III)alamin-*b*-acid; **6**) were prepared according to [13][17]. All other chemicals were obtained from commercial sources. Anal. HPLC: *Merck-Hitachi L-7000* system, equipped with a *EG&G-Berthold LB-508* radiometric detector; *Waters XTerra-RP8* columns (5 μm particle size, 1 × 100 mm); flow rate 0.5 ml/min or 1 ml/min; detection at 250 and 360 nm, eluant

$A = \text{NaOAc}$ buffer, prepared by mixing AcOH (2.9 ml) and 2M NaOH (4.55 ml) in H_2O (900 ml) and MeOH (100 ml); eluent $B = \text{MeOH}$. Prep. HPLC: *Varian Prostar* system, equipped with two *Prostar-215* pumps and a *Prostar-320* UV/VIS detector; *Waters XTerra-Prep-RP8* column (5 μm particle size, $30 \times 100\text{ mm}$); flow rate 30 ml/min. After prep. HPLC purification, the cob(III)alamin derivatives were desalted by applying an aqueous soln. of the compound to a *Chromafix RP18ec* cartridge, followed by thorough rinsing with H_2O . The desalted product was then eluted with MeOH , the solvent evaporated, and the product dried under high vacuum. IR Spectra: $\tilde{\nu}$ in cm^{-1} . ^1H -, ^{13}C -, and ^{31}P -NMR Spectra: *Varian Gemini-2000* (^1H at 300.08 MHz) and *Bruker DRX-500* (^1H at 500.25 MHz) spectrometer; $\delta(\text{H})$ and $\delta(\text{C})$, referenced to SiMe_4 , by using the $\delta(\text{C})$ or residual $\delta(\text{H})$ of the deuterated solvents as internal standards; $\delta(\text{P})$ referenced against $0.1\text{M H}_3\text{PO}_4$ in H_2O ; δ in ppm, J in Hz. Electrospray-ionization (ESI) MS: *Merck-Hitachi M-8000* spectrometer; positive-ion mode; MeOH as solvent; in m/z . Elemental analyses: *Leco CHNS-932* elemental analyzer.

(OC-6-44)(*S*-Benzyl-L-cysteinato- $\kappa\text{N},\kappa\text{O},\kappa\text{S}$)tricarbonylrhenium(I) (**4**). A mixture of $[\text{Et}_4\text{N}]_2[\text{ReBr}_3(\text{CO})_3]$ (**3**; 100 mg, 0.130 mmol) and *S*-benzyl-L-cysteine (**2**; 28 mg, 0.132 mmol) in 1M aq. NaHCO_3 (10 ml) was heated at 70° for 14 h. During this period, a white precipitate formed, which was filtered off, washed with H_2O , and dried *in vacuo*: 53 mg (85%) of **4**. X-Ray-quality crystals were grown by slow evaporation of a $\text{H}_2\text{O}/\text{MeOH}$ 1:4 (v/v) soln. IR (KBr): 2030s, 1898s (br.). ^1H -NMR (300.08 MHz, CD_3OD): 7.37 (s, Ph); 4.28 (s, PhCH_2); 4.02 (m, $\text{H}-\text{C}(\alpha)$); 2.61 (m, $\text{CH}_2(\beta)$). ^{13}C -NMR (75.47 MHz, CD_3OD): 195.5, 195.0, 193.4 (3 CO), 181.3 (C=O); 135.2, 131.2, 130.4, 130.4, 130.2, 109.9 (PhCH_2); 59.3; 41.6; 30.5. (CO). ESI-MS: 481.7 ($[\text{M} + \text{H}]^+$). Anal. calc. for $\text{C}_{13}\text{H}_{12}\text{O}_5\text{ReS}$: C 32.49, H 2.52, N 2.92; found: C 32.86, H 2.89, N 2.83.

S-[3-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]propyl]-*N*-[(tert-butoxy)carbonyl]-L-cysteine Methyl Ester (**10**). To a soln. of KOH (56 mg, 1.0 mmol) in DMSO (10 ml) was added a solution of *N*-[(tert-butoxy)carbonyl]-L-cysteine methyl ester (**8**; 235 mg, 1.0 mol) in DMSO (2 ml). To this mixture was added a soln. of $\text{Fmoc-NH}-(\text{CH}_2)_3\text{I}$ (**9**; 406 mg, 1.0 mmol) in DMSO (2 ml). The mixture was stirred at r.t. for 4 h. Then 2M NaHCO_3 (ca. 50 ml) was added, the mixture extracted with AcOEt ($3 \times 40\text{ ml}$), the combined org. extract washed with sat. NaCl soln. ($3 \times 30\text{ ml}$), dried (Na_2SO_4), and evaporated, and the residue purified by column chromatography ($\text{AcOEt}/\text{hexane}$): 426 mg (83%) of **10**. White solid. ^1H -NMR (300.08 MHz, CD_3CN): 7.82 ('d', 2 arom. H); 7.63 ('d', 2 arom. H); 7.41 ('t', 3 arom. H); 7.33 ('d', 2 arom. H); 5.68 (br., NH); 4.32 (m, CH_2CH , $\text{H}-\text{C}(\alpha)$), 4.20 (d, $^3J(\text{H,H}) = 6.3$, CH); 3.63 (s, MeO); 3.13 (m, 2 H, CH_2CH_2); 2.90 (m, 1 H-C(β)); 2.81 (m, 1 H-C(β)); 2.51 (m, 2 H, CH_2CH_2); 1.68 (m, $\text{CH}_2\text{CH}_2\text{CH}_2$); 1.39 (s, 'Bu). ^{13}C -NMR (75.47 MHz; CDCl_3): 171.8 (COOMe); 156.6, 155.4 (2 C=O); 144.1, 141.4, 127.8, 127.1, 125.1, 120.0 (6 arom. C); 80.2 (quat. C); 66.5, 53.0, 52.5, 47.2, 39.4, 34.6, 29.5, 29.1, 28.2 (Me_3C). ESI-MS: 514.4 (M^+), 537.2 ($[\text{M} + \text{Na}]^+$), 1050.1 ($[\text{2M} + \text{Na}]^+$). Anal. calc. (%) for $\text{C}_{27}\text{H}_{34}\text{N}_2\text{O}_6\text{S}$: C 63.01, H 6.66, N 5.44; found: C 63.28, H 6.41, N 5.49.

N-[(tert-Butoxy)carbonyl]-*S*-[3-[cyanocob(III)alamin- N^b -yl]propyl]-L-cysteine Methyl Ester (**11**). A soln. of **7** (51.4 mg, 0.1 mmol) in $\text{DMF}/\text{Et}_3\text{NH}$ 2:1 (3 ml) was stirred at r.t. for 1 h. Thereafter, the solvent was evaporated. To the residue was added a soln. of cyanocob(III)alamin-*b*-acid (**6**; 20 mg, $14.8\text{ }\mu\text{mol}$) in DMSO (0.5 ml) and DMF (4 ml). Subsequently were added Et_3N (1 ml) and TBTU (32 mg). After stirring at r.t. for 45 min, the mixture was evaporated. Purification by prep. HPLC afforded **11** (14 mg, 61%). Red solid. ^{13}C -NMR (125.8 MHz, CD_3OD): 181.7; 180.3; 177.8; 177.7; 176.8; 175.7; 175.5; 174.8; 174.8; 174.5; 173.5; 167.3; 167.0; 158.0; 143.6; 138.4; 135.8; 133.9; 131.6; 118.0; 112.7; 108.9; 105.3; 95.8; 88.1; 86.6; 83.9; 83.8 (d, $J(\text{P,C}) = 5.9$); 81.0; 76.5 (d, $J(\text{P,C}) = 3.4$); 75.6; 73.7 (d, $J(\text{P,C}) = 6.0$); 70.9; 62.8; 60.5; 57.8; 57.1; 55.2; 53.1; 52.7; 49.9; 46.9 (d, $J(\text{P,C}) = 4.4$); 44.1; 43.2; 40.3; 39.6; 37.0; 35.5; 34.8; 33.7; 33.2; 33.2; 32.5; 30.7; 30.3; 29.8; 28.9; 27.7; 27.6; 21.2; 20.7; 20.5; 20.3 (d, $J(\text{P,C}) = 2.9$); 20.1; 17.7; 17.2; 16.5; 16.2. ^{31}P -NMR (202.5 MHz, CD_3OD): 0.57. ESI-MS: 1630.4 ($[\text{M} + \text{H}]^+$, $\text{C}_{75}\text{H}_{109}\text{CoN}_{15}\text{O}_{16}\text{PS}^+$; calc. 1629.7).

S-[3-[Cyanocob(III)alamin- N^b -yl]propyl]-L-cysteine Methyl Ester (**12**). Compound **11** (14 mg, $8.5\text{ }\mu\text{mol}$) was dissolved in $\text{CH}_2\text{Cl}_2/\text{CF}_3\text{COOH}$ 4:1 (5 ml) 0° . After stirring for 1 h at 0° , the mixture was evaporated. The residue was purified by prep. HPLC: **12** (9 mg, 68%). Red solid. ^{13}C -NMR (125.80 MHz, CD_3OD): 181.7; 180.3; 177.8; 177.7; 176.8; 175.7; 175.7; 175.5; 175.1; 174.8; 174.4; 167.3; 167.1; 143.6; 138.4; 135.8; 133.9; 131.6; 117.7; 112.7; 108.8; 105.3; 95.8; 95.8; 88.1; 86.5; 83.8; 76.5; 75.6; 73.7; 70.8; 62.8; 60.5; 57.7; 57.0; 55.2; 54.9; 52.9; 52.7; 50.0; 48.5; 46.9; 44.0; 43.1; 40.2; 39.5; 37.0; 36.9; 35.5; 33.6; 33.1; 32.5; 32.4; 30.8; 30.4; 30.1; 29.8; 27.7; 27.5; 21.1; 20.6; 20.5; 20.4; 20.3; 20.0; 17.6; 17.2; 16.5; 16.2. ^{31}P -NMR (202.5 MHz, CD_3OD): 1.24. ESI-MS: 1530.5 ($[\text{M} + \text{H}]^+$, $\text{C}_{70}\text{H}_{101}\text{CoN}_{15}\text{O}_{16}\text{PS}^+$; calc. 1529.6).

S-[2-[[4-[Cyanocob(III)alamin- N^b -yl]butyl]amino]-2-oxoethyl]-L-cysteine (**17**). N^b -(4-Aminobutyl)cyanocob(III)alamin (**15**) (23 mg, $16.3\text{ }\mu\text{mol}$), prepared by the method of Pathare *et al.* [17] for the dodecane analogue [18], was dissolved in a phosphate buffer at pH 7.4. Iodoacetic acid succinimid-*N*-yl ester (= 1-[(iodoacetyl)oxy]pyrrolidine-2,5-dione; 14 mg; $50\text{ }\mu\text{mol}$) were added, and the mixture was stirred for 45 min at r.t. Subsequently, L-cysteine (H-Cys-OH ; 18 mg, 0.15 mmol) was added, and the mixture was stirred for 45 min. The mixture was

purified by prep. HPLC: **17** (22 mg, 82%). Red solid. ^{13}C -NMR (125.80 MHz, CD_3OD): 181.6; 180.3; 177.7; 177.7; 176.8; 175.7; 175.7; 175.6; 174.8; 174.8; 174.5; 172.8; 172.6; 167.3; 167.0; 143.7; 138.4; 135.7; 133.9; 131.6; 118.0; 115.3; 112.8; 109.4; 108.9; 105.3; 95.8; 88.2; 86.6; 83.9; 76.6; 75.6; 73.8; 70.8; 62.8; 60.6; 57.7; 57.1; 55.5; 55.2; 52.8; 50.0; 49.8; 48.5; 46.9; 44.2; 43.1; 40.7; 40.4; 40.3; 37.0; 36.4; 35.5; 35.4; 33.8; 33.2; 33.1; 32.9; 32.5; 29.8; 28.0; 27.8; 27.6; 21.2; 20.6; 20.5; 20.5; 20.3; 17.8; 17.3; 16.4; 16.1. ^{31}P -NMR (202.5 MHz, CD_3OD): 1.20. ESI-MS: 1601.5 ($[\text{M} + \text{H}]^+$, $\text{C}_{72}\text{H}_{104}\text{CoN}_{17}\text{O}_{17}\text{PS}^+$; calc. 1600.7).

Labelling with $^{99\text{m}}\text{Tc}(\text{OH}_2)_3(\text{CO})_3]^+$ (1**): General Procedure.** A soln. of the alkylated L-cysteine derivative **2**, **12**, or **17** (10^{-3} or 10^{-4} M in H_2O , 200 μl) was added to a vial, which was then sealed and degassed with a stream of N_2 for 10 min. A soln. of $^{99\text{m}}\text{Tc}(\text{OH}_2)_3(\text{CO})_3]^+$ (**1**; 1800 μl) [23][24] was added to the vial via a syringe, and the vial was heated to 50° for 30 min to yield the $^{99\text{m}}\text{Tc}(\text{CO})_3$ -labelled (OC-6-44)-tricarbonyl[S-{3-[cyanocob(III)amin-N^b-yl]propyl}-L-cysteinato- $\kappa\text{N},\kappa\text{O},\kappa\text{S}}$] $^{99\text{m}}\text{Tc}$ technetium (**13**) and (OC-6-44)-tricarbonyl[S-{2-[[4-[cyanocob(III)amin-N^b-yl]butyl]amino]-2-oxoethyl}-L-cysteinato- $\kappa\text{N},\kappa\text{O},\kappa\text{S}}$] $^{99\text{m}}\text{Tc}$ technetium (**18**), which was demonstrated by HPLC (radioactive detection; RP8 column; NaOAc buffer (described above) = eluent A and MeOH = eluent B; gradient B/A: $0 \rightarrow 15\%$ B with in 30 min, then $\rightarrow 100\%$ B with in 15 min.).

Biodistribution Studies of **13.** The biodistribution of $^{99\text{m}}\text{Tc}$ labelled **13** as well as of ^{57}Co cyanocob(III)-alamin (= ^{57}Co vitamin B₁₂) [14] for reference purposes was studied in mice bearing B16–F10 melanoma tumors. Female balb/c mice (10–12 weeks old), which were kept on folate- and vitamin B₁₂ deficient food, were injected subcutaneously in the flank with 10^6 B16–F10 mouse melanoma tumor cells (ATCC CRL-6475). At 2 weeks post-inoculation, mice bearing B16-F10 tumors were injected with 0.5–1 ng of **13** (specific activity 10 mCi/ μg) or 1 ng of ^{57}Co vitamin B₁₂ as control (specific activity 0.2 mCi/ μg) via the tail vein. Groups of three mice per compound were sacrificed and dissected at 4 h and 24 h post-injection. Organs were weighed and counted in a gamma scintillation counter. Experiments were carried out in compliance with Swiss laws related to the conduct of animal experimentation.

Table 2. Crystal Data and Refinement for **4** · $\frac{1}{2}$ H_2O

Empirical formula	$\text{C}_{13}\text{H}_{13}\text{NO}_{5.50}\text{ReS}$
M_r	489.50
Crystal size [mm]	$0.45 \times 0.05 \times 0.05$
T [K]	183(2)
λ [Å] (MoK α)	0.71073
Crystal system	tetragonal
Space group	$P4_3$
a [Å]	16.9849(14)
b [Å]	16.9849(14)
c [Å]	5.4378(5)
V [Å ³]	1568.7(2)
Z	4
ρ_{cal} [g/cm ³]	2.073
μ [mm ⁻¹]	7.901
$F(000)$	932
θ range [$^\circ$]	2.68 to 29.96
Reflections measured	18257
Independent reflections	4566 ($R(\text{int}) = 0.1548$)
Reflections observed	2961
Completeness to $\theta = 29.96^\circ$	99.8%
Max. and min. transmission	0.7351 and 0.4058
Refinement method	full-matrix least-squares on F^2
Data/restraints/parameters	4566/34/199
Goodness-of-fit on F^2	0.871
Final R indices ($I > 2\sigma(I)$)	$R_1 = 0.0485$, $wR_2 = 0.1067$
R indices (all data)	$R_1 = 0.0804$, $wR_2 = 0.1150$
Absolute structure parameter	–0.04(2)
Largest diff. peak and hole [$\text{e} \cdot \text{\AA}^{-3}$]	1.643 and –2.172

X-Ray-Structure Determination of $4 \cdot \frac{1}{2} H_2O$. Crystal data and details of data collection and structure refinement are given in Table 2. Data were collected on a *Stoe IPDS* diffractometer by using graphite-monochromated MoK_α irradiation (λ 0.71073 Å) at 183 K. A ϕ -oscillation scan was performed with a ϕ increment of 1.1°. Preliminary unit-cell parameters were obtained from five frames. Final unit-cell parameters were determined by refinement of reflections from the integration of the complete data set. The *IPDS* software package [25] was used for the collection of data frames, the determination of lattice parameters, and the indexation and integration of reflections. Space-group determination, data reduction, and absorption correction were performed with the aid of X-RED [25]. The structure was solved with SHELXS-97 [26], structure refinement was carried out with SHELXL-97 [27]. Molecular graphics were created with the aid of ORTEP-3 [28].

CCDC-253751 contains the supplementary crystallographic data for **4**. These data can be obtained free of charge via <http://www.ccdc.cam.ac.uk/conts/retrieving.html> (or from the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB2 1EZ, UK; fax +44 1223 336033; email: deposit@ccdc.cam.ac.uk).

REFERENCES

- [1] S. S. Jurisson, J. D. Luxdon, *Chem. Rev.* **1999**, 99, 2205.
- [2] R. Alberto, U. Abram, in 'Nuclear Chemistry', Ed. F. Rösch, Kluwer Academic Publisher, Dordrecht, 2003, Vol. 4, p. 211.
- [3] J. R. Dilworth, S. J. Parrott, *Chem. Soc. Rev.* **1998**, 27, 43.
- [4] M. Welch, *Eur. J. Nucl. Med. Mol. Imaging* **2003**, 30, 1302.
- [5] R. Alberto, *Eur. J. Nucl. Med. Mol. Imaging* **2003**, 30, 1299.
- [6] R. Schibli, R. La Bella, R. Alberto, E. Garcia-Garayoa, K. Ortner, U. Abram, P. A. Schubiger, *Bioconjugate Chem.* **2000**, 11, 345.
- [7] H. J. Wester, M. Schottelius, M. Schwaiger, *J. Nucl. Med.* **2001**, 42, 115.
- [8] J. K. Pak, P. Benny, B. Spingler, K. Ortner, R. Alberto, *Chem.-Eur. J.* **2003**, 9, 2053.
- [9] S. Seifert, J.-U. Künstler, A. Gupta, H. Funke, T. Reich, H.-J. Pietzsch, R. Alberto, B. Johannsen, *Inorg. Chim. Acta* **2001**, 322, 79.
- [10] R. Alberto, in 'Comprehensive Coordination Chemistry II, Technetium', Ed. J. Dilworth, Elsevier Pergamon, Oxford, 2003; Vol. 5, p. 127.
- [11] S. Mundwiler, M. Kündig, K. Ortner, R. Alberto, *Dalton Trans.* **2004**, 1320.
- [12] R. Alberto, R. Schibli, A. Egli, U. Abram, T. A. Kaden, P. A. Schubiger, *J. Am. Chem. Soc.* **1998**, 120, 7987.
- [13] D. R. van Staveren, S. Mundwiler, U. Hoffmanns, J. K. Pak, B. Spingler, N. Metzler-Nolte, R. Alberto, *Org. Biomol. Chem.* **2004**, 2, 2593.
- [14] D. R. van Staveren, W. R., S. Mundwiler, P. A. Schubiger, R. Alberto, *J. Organomet. Chem.* **2004**, 689, 4803.
- [15] R. Banerjee, 'Chemistry and Biochemistry of B12', Wiley-Interscience, New York, 1999.
- [16] K. Bernhauer, H. Vogelmann, F. Wagner, *Hoppe-Seyler's Z. Physiol. Chem.* **1968**, 349, 1281.
- [17] P. M. Pathare, D. S. Wilbur, S. Heusser, E. V. Quadros, P. McLoughlin, A. C. Morgan, *Bioconjugate Chem.* **1996**, 7, 217.
- [18] E. S. Rector, K. S. Tse, A. H. Sehon, R. J. Schwenk, *J. Immunol. Methods* **1978**, 24, 321.
- [19] D. A. Collins, H. P. C. Hogenkamp, *J. Nucl. Med.* **1997**, 38, 717.
- [20] D. A. Collins, H. P. C. Hogenkamp, M. W. Gebhard, *Mayo Clin. Proc.* **1999**, 74, 687.
- [21] D. A. Collins, H. P. C. Hogenkamp, M. K. O'Connor, S. Naylor, L. M. Benson, T. J. Hardyman, L. M. Thorson, *Mayo Clin. Proc.* **2000**, 75, 568.
- [22] R. Alberto, U. Abram, K. Hegetschweiler, V. Gramlich, P. A. Schubiger, *J. Chem. Soc., Dalton Trans.* **1994**, 2815.
- [23] R. Alberto, R. Schibli, A. P. Schubiger, U. Abram, H. J. Pietzsch, B. Johannsen, *J. Am. Chem. Soc.* **1999**, 121, 6076.
- [24] R. Alberto, K. Ortner, N. Wheatley, R. Schibli, A. P. Schubiger, *J. Am. Chem. Soc.* **2001**, 123, 3135.
- [25] X-RED, IPDS version 2.87, *Stoe & Cie*, GmbH, Darmstadt, Germany, 1998.
- [26] G. M. Sheldrick, *Acta Crystallogr., Sect. A* **1990**, 46, 467.
- [27] G. M. Sheldrick, University Göttingen, 1997.
- [28] L. J. Farrugia, *J. Appl. Crystallogr.* **1997**, 30, 565.

Received October 30, 2004