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Conjugation of a novel histidine derivative to biomolecules and labelling with $[^{99m}Tc(OH_2)_3(CO)_3]^+$†

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The new histidine derivative 3-{1-[3-(9*H*-fluoren-9-ylmethoxycarbonylamino)-propyl]-1*H*-imidazol-4-yl}-2-(3-trimethylsilanyl-ethylcarboxyamino)-propionic acid methyl ester (**7**) has been prepared *via* alkylation of the histidine urea derivative (7*S*)-5,6,7,8-tetrahydro-7-(methoxycarbonyl)-5-oxoimidazo-[1,5-*c*]-pyrimidine (**2**) with Fmoc-protected 3-iodopropyl-amine, followed by ring opening with 2-trimethylsilylethanol. After Fmoc cleavage by $HNEt_2$, the histidine amine derivative was coupled to biotin, to the pentapeptide leucine-enkephalin and to Vitamin B12-b-acid by amide formation, employing TBTU as the coupling reagent. In order to make the histidine accessible for labelling, the teoc protecting group was removed by either NBu_4F (for the biotin conjugate) or by TFA (for the enkephalin and B12 conjugates). Reaction of a 10^{-4} M solution of the bioconjugates with $[^{99m}Tc(H_2O)_3(CO)_3]^+$ at 50 °C for 30 min led to the formation of one single new peak in the HPLC radiochromatogram in each case, confirming quantitative labelling of the respective biomolecules. To assess the nature of the labelled compounds, the rhenium analogues with $Re(CO)_3$ were also synthesised and similar retention times confirmed the identity with the ^{99m}Tc labelled conjugates.

Introduction

The labelling of biologically active molecules with ^{99m}Tc is a field of intense research,[1–4] as ^{99m}Tc is one of the most widely employed isotopes for imaging in nuclear medicine. This is due to the favourable properties of this isotope: the emission of a 140 keV γ-ray with an abundance of 89% and a half-life of 6.02 h. In the past compounds with ^{99m}Tc in an almost exclusively +V oxidation state have been used for labelling purposes but recently the $^{99m}Tc(CO)_3^+$ core in which the oxidation state is +1 has attracted much attention. Three reasons account for this interest: 1) the robustness of complexes with this core, 2) the high affinity of the Tc(I) ion to a large variety of donor atoms and 3) the easy preparation of the precursor $[^{99m}Tc(H_2O)_3(CO)_3]^+$. In fact, GMP produced kits for preparing $[^{99m}Tc(H_2O)_3(CO)_3]^+$ are now commercially available (Isolink from Malinckrodt, Inc.), which is expected to furnish the way towards commercial applications.[5]

To avoid dissociation of the metal ion from the bioconjugate in biological systems, the ligand covalently attached to the biomolecule should bind as tight as possible to the "*fac*-$[^{99m}Tc(CO)_3]^+$" moiety. On the basis of thermodynamic considerations a tridentate ligand is preferred. It forms highly robust complexes and also prevents cross-metalation to ligands present in blood plasma, in particular to those with sulfur or aromatic nitrogen donors. A $^{99m}Tc(CO)_3$ complex with a tridentate ligand is less prone to undergo such a undesired reaction due to its coordinative saturatation and because thermodynamic factors strongly disfavour the dissociation of one donor atom from the tridentate ligand.

It is often important for the biological activity of a labelled compound that the overall charge does not change, hence a monoanionic tridentate ligand is required for the *fac*-$[^{99m}Tc(CO)_3]^+$ core. Systematic investigations about the *in vitro* and *in vivo* properties of a variety of complexes containing the $Tc(CO)_3$ and $Re(CO)_3$ groups[6,7] showed that histidine in particular forms complexes of high (biological) stability.[8–10] Furthermore, histidine is very attractive for labelling with $[^{99m}Tc(H_2O)_3(CO)_3]^+$, since short reaction times (30 min) at relatively low temperatures (50 °C) and ligand concentration result in quantitative labelling yield, making it ideally suited for radiopharmaceutical applications. Another virtue of the histidine ligand is its chirality, which implies that the formed $Tc(His–N^{\varepsilon}–R)(CO)_3$ complex moiety will be enantiomerically pure if enantiomerically pure histidine is used.

In contrast, other favourable mono-anionic ligands with a NNO donor set such as the pama-ligand ([pyridine-2-ylmethyl)-amino]-acetic acid) will always lead to a racemic complex.[11] In combination with chiral biomolecules, mixtures of diastereomers will result, which should be avoided for pharmaceutical applications in particular.

The favourable coordination properties of histidine to the different organometallic Mo(η-allyl)$(CO)_2$ core, and the facile derivatisation of Mo(His)(η-allyl)$(CO)_2$ by alkylation of the imidazole N^{ε}-atom have been previously reported by van Staveren and Metzler-Nolte in a series of papers.[12–15] In addition it was shown that the Mo(η-allyl)$(CO)_2$ complex can be attached to the N-terminus of a peptide as a last step of a solid phase synthesis protocol.[14]

Recently we reported the carboxylic acid histidine derivative methyl 1-(2-*tert*-butoxy-2-oxoethyl)-*N*-(9*H*-fluoren-9-ylmethoxycarbonyl)histidinate (**1**) which was coupled to the amino group of phenylalanine by employing standard peptide chemistry methods.[16] After deprotection histidine served as an efficient ligand for $^{99m}Tc(CO)_3$. Since it is often desired to attach the bifunctional chelator to the C-terminus of *e.g.* a peptide we extend this study by introducing an amino group for coupling to the carboxylate of biomolecules. We report in the present work the synthesis of a protected histidine derivative with an amino group attached on an alkyl chain to the N^{ε}-atom (**7**). Although seemingly straightforward, the introduction of such a linking group is not routine due to the reactivity of the histidine precursor. The α-amine protecting group can be removed either under acidic or Lewis basic conditions which is important in case of sensitive biomolecules. To exemplify the versatility of this approach we show the convenient coupling of this compound to vitamin B12, enkephalin and biotin, biomolecules of varying complexity. Furthermore, we demonstrate that the derivatised biomolecules can be efficiently labelled with $[^{99m}Tc(OH_2)_3(CO)_3]^+$ under mild conditions.

† Electronic supplementary information (ESI) available: complete ^1H and ^{13}C NMR spectra of **14**, **15**, **16** and **19**. See http://www.rsc.org/suppdata/ob/b4/b405575f/

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Results and discussion

Synthesis

Histidine as a very efficient ligand is the focus of our interest and our aim was a derivative with an additional amine group linked by an alkyl chain to the N^{ε}-atom in the imidazole side chain. This amine group enables ligand conjugation to a carboxylate in biomolecules. The approach is complementary to the one employing **1**, but the N-terminus of a peptide is usually buried in the core of the protein. With the new method, conjugation of histidine to the C-terminus, which is usually on the exterior of the protein, is possible.

A general structure of our target compound as well as a retrosynthetic analysis is shown in Scheme 1. The α-amine and the amine moiety on the alkyl-chain carry orthogonal protecting groups. In analogy with the synthesis of **1**, we planned alkylation of the N^{ε}-atom in the urea-protected derivative **2**, yielding a cationic intermediate. Subsequent ring opening of the urea moiety with an alcohol will then give the desired but still protected compound (Scheme 1). Although this synthetic pathway seems straightforward we encountered a number of unexpected difficulties, which will be discussed in brief below.

Originally we envisaged to perform the ring opening of the cationic urea derivative with (9*H*-fluoren-9-yl)-methanol to yield an α-amine Fmoc protected derivative. Therefore we concentrated on alkylation of the N^{ε} with protected iodo-alkylamines that would resist the conditions required for Fmoc cleavage. In the light of later biomolecule coupling, a short linker between histidine and the biomolecule is preferable and we started with an ethyl spacer. The iodide CBz–NH–CH_2CH_2I (**4**) was synthesised from the corresponding alcohol **3** by using triphenylphosphine, imidazole and iodine (see Scheme 2). Subsequently histidine–urea derivative **2** was reacted with **4**, followed by urea-opening with (9*H*-fluoren-9-yl)-methanol. Surprisingly, the compound isolated after work-up was not the expected CBz derivative, but compound **5** (see Scheme 2). The constitution of the molecule was clearly established from the mass spectrum as well as from the ^{1}H and ^{13}C NMR spectroscopic data. In addition it was possible to coordinate the ligand to $Tc(CO)_3$ and to elucidate the X-ray structure. An ORTEP presentation is given in Fig. 1, confirming the molecular structure of the ligand.

During the reaction or column-chromatographic purification over silica elimination of CO_2 and formation of a benzyl-cation (or tropylium ion) occurred. This cation presumably reacts with the N^{ε}-atom to form compound **5**. The benzyl group is known to have a preference for the His-N^{ε} atom and, in fact, this benzyl moiety is a common protecting group for the N^{ε} of His in peptide chemistry.[17–19]

To circumvent the CBz protecting group, we examined other N-alkylation reagents with ethyl and propyl spacers and with protecting groups compatible with Fmoc deprotection of N^{α}. The compounds Boc-NHCH_2CH_2I, Trt-NHCH_2CH_2I and the propyl derivatives Boc-NH-$CH_2CH_2CH_2$Br and Trt-NH-$CH_2CH_2CH_2$I were prepared from the corresponding alcohols but all alkylation reac-

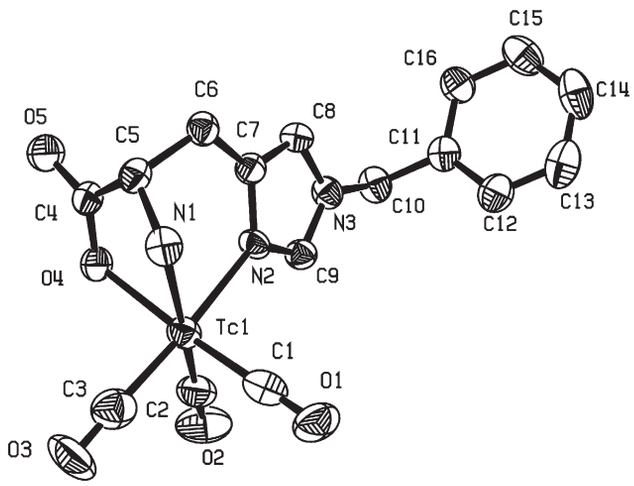
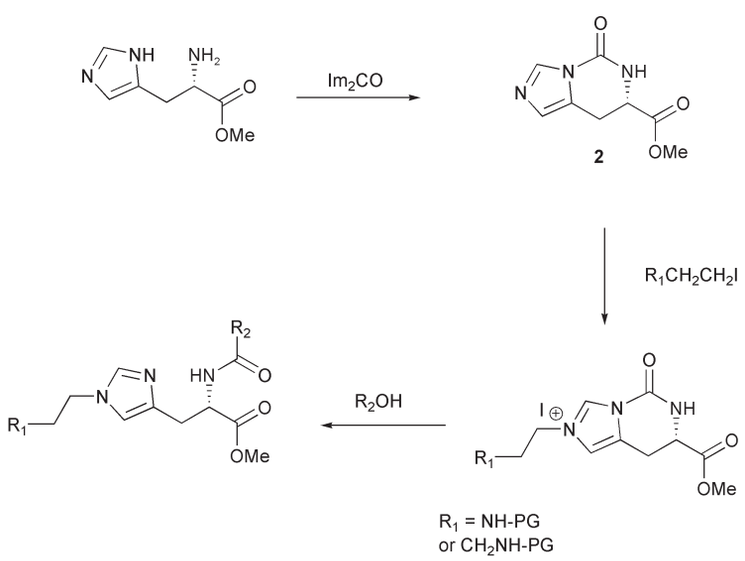
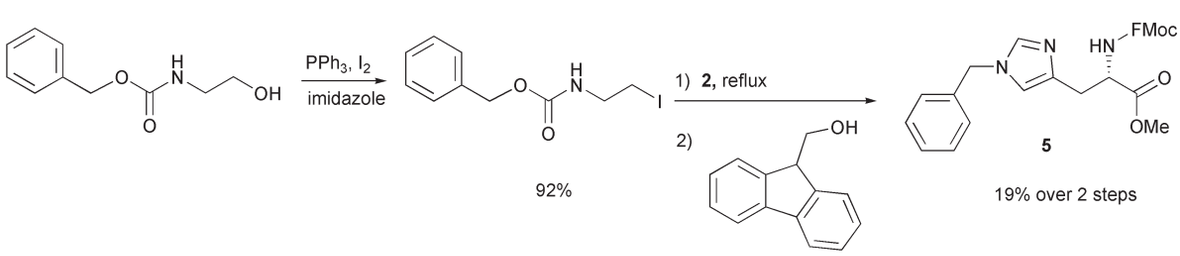


Fig. 1 ORTEP projection of Tc(His-N_{α}-benzyl)$(CO)_3 \cdot H_2O$ at 50% probability level. Hydrogen atoms as well as the H_2O molecule are omitted for clarity. Selected bond lengths (Å): Tc(1)–C(1) 1.895(6), Tc(1)–C(2) 1.916(5), Tc(1)–C(3) 1.929(5), Tc(1)–O(4) 2.165(3), Tc(1)–N(1) 2.200(3), Tc(1)–N(2) 2.204(3).



Scheme 1



Scheme 2

tions with **2** were unsuccessful. After heating at reflux for several days, no new compounds could be identified.

To increase reactivity, we then switched to iodo-alkyl amines protected by an Fmoc group, well aware that this strategy would require a different alcohol for ring opening of the cationic urea intermediate in order to ensure orthogonality between the two amine protecting groups. The alcohol 2-trimethylsilylethanol fulfils this condition ideally since the 2-trimethylsilylethylcarbonyl (teoc) protecting group is stable under basic conditions and can be cleaved both under Lewis basic conditions employing fluoride ions or under acidic conditions by using trifluoroacetic acid.[20]

The iodo derivatives Fmoc-NH-CH_2CH_2I and Fmoc-NH-$CH_2CH_2CH_2I$ (**6**) were prepared from the corresponding primary alcohols. Upon reaction of **2** with Fmoc-NH-CH_2CH_2I, only small amounts of a new product could be observed after a reflux period of 5 days but over 2 weeks **2** decomposed slowly. In contrast, refluxing **6** with **2** already led to the formation of a significant amount of a new product after a reflux period of only 24 h. The reaction was complete after 4.5 days. Compound **6** represents the only alkylating agent giving a clean reaction with **2** and affording the introduction of the desired $–NH_2$ group. Subsequent ring opening of the cationic urea derivative with 2-trimethylsilylethanol afforded overnight the fully protected histidine amine derivative **7** in a 53% yield (two steps) as shown in Scheme 3. Cleavage of the Fmoc group in **7** could be achieved by treatment with $HNEt_2$ in DMF (Scheme 4) and pure **8** could be obtained after purification with preparative HPLC. Principally, conjugation of **8** to biomolecules can be performed after *in situ* preparation of **8** from **7** but sometimes purification of the resulting bioconjugate is complicated by the presence of fulvene derivatives from the Fmoc cleavage.

This synthetic approach now allows the preparation of large amounts of histidine amine derivative **7** or **8** respectively. The next step consisted of the selection of carboxylic acid-containing biomolecules interesting for labelling from a radiopharmaceutical point of view. To show that histidine can be introduced in a variety of biomolecules we selected diverse biomolecules to demonstrate the general versatility of the coupling method with the histidine derivative. Biotin was chosen since its labelling is of ongoing interest in radiopharmacy, enkephalin since it represents a less known biomolecule from the general class of peptides and vitamin B12 as a more complex system with integrated potentially coordinating functions. As for biotin, labelled vitamin B12 is also of strong interest for cancer diagnosis and therapy in nuclear medicine.

D-(+)-Biotin, also called vitamin H, was conveniently coupled to **8** by using TBTU. The purification *via* an extractive work-up proceeded smoothly, affording compound **9** in pure form. Removal of the teoc protecting group was achieved by the reaction of **9** with NBu_4F in THF/DMF. These conditions hydrolyzed also simultaneously but unexpectedly the methyl ester and afforded **10** (Scheme 4) in good yield as derived from the MS and NMR spectroscopic data. Since the fluoride ion is a strong Lewis base, it is conceivable that small amounts of water in combination with reaction temperatures of 70 °C lead to cleavage of the methyl ester. This concerted cleavage is ideal because both protecting groups are released in the same synthetic step, making the histidine now accessible for labelling.

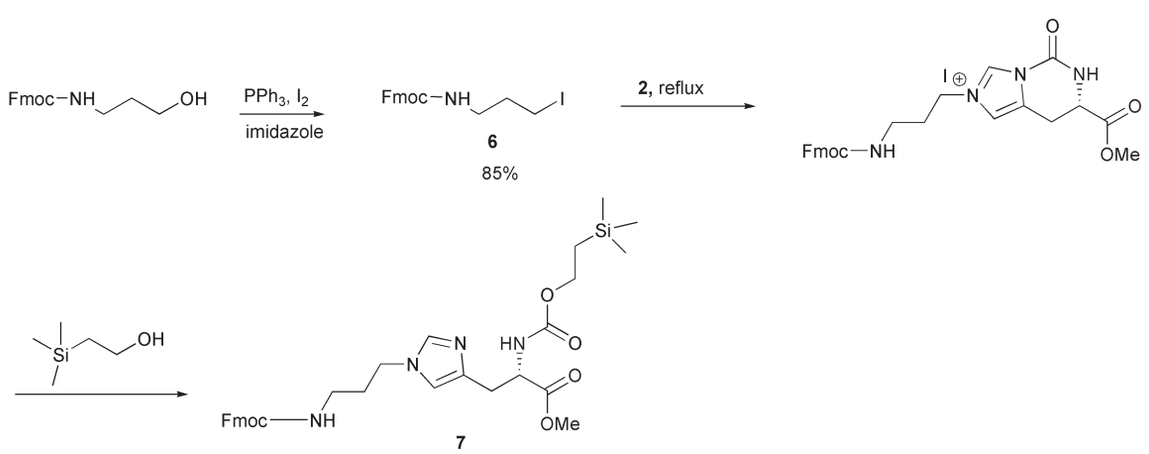
To more generalize the approach we moved on to the attachment of **8** to a receptor-binding peptide. Leucine–Enkephalin, a pentapeptide with the sequence H-Tyr-Gly-Gly-Leu-Phe-OH was chosen. This pentapeptide was first isolated as a mixture with its position 4 methionine analogue by Hughes *et al.* in 1975 from pig brain.[21] The presence of this peptide was later also demonstrated in various human tissues, such as the human brain, the human spinal fluid and in blood plasma. It has an action similar to morphine, exhibiting a high affinity for the opioid receptor and acting as a natural pain killer.[22]

The protected derivative Boc-(Tyr-OtBu)-Enk-OH (**11**) was prepared on a resin with a 2-Cl-trityl linker *via* standard peptide solid phase techniques. The peptide can be cleaved under mildly acidic conditions from the resin, leaving the acid sensitive side-chain protecting groups unaffected. In a next step compound **8** was reacted with **11** employing TBTU as the coupling reagent, which furnished in a clean reaction the expected conjugate **12** after preparative HPLC purification (Scheme 5). The Boc, *tert*-butyl ether and the teoc group were then simultaneously removed in one step by the reaction with TFA in CH_2Cl_2, to afford the fully deprotected peptide **13** in higher than 97% purity. The HRMS and NMR spectroscopic data are consistent with the proposed constitution.

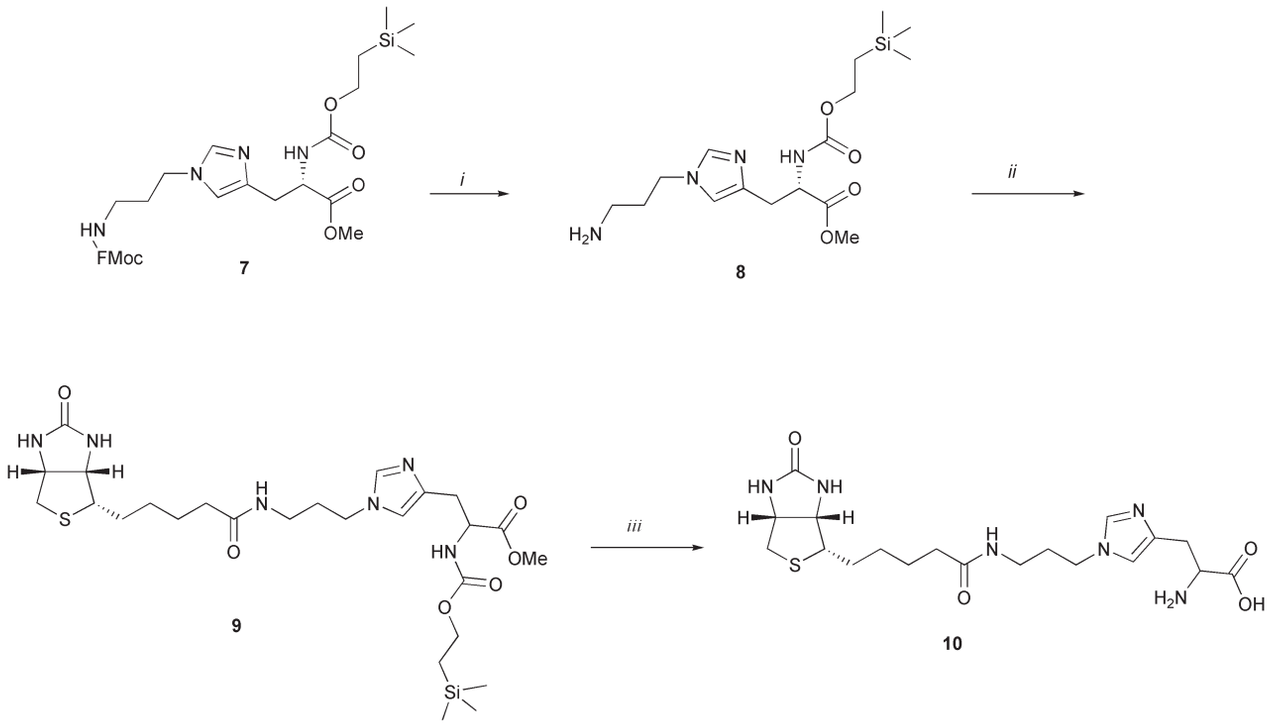
As an example for a more complex and biologically important molecule belonging to a different category, we selected vitamin B12 or cobalamin. Vitamin B12 is necessary for the proper function of the nervous system and for the metabolism of carbohydrates, proteins and fat and it is also involved in the reductive conversion of ribonucleotides to deoxyribonucleotides to generate DNA.[23,24] Vitamin B12 comprises amides, benzimidazole, phosphate esters and other groups which are potentially coordinating for the $[Tc(CO)_3]^+$ moiety. Strong ligands are required in order to yield one well-defined product.

Cobalamin contains five terminal amide groups, but no free carboxylate as required for its coupling to the amine in **8**. According to a literature procedure aqueous acid hydrolysis of vitamin B12 yields a mixture of mono and di-acids. The vitamin B12 compound in which the b-amide is hydrolyzed represents the main hydrolysis product in this reaction (Scheme 6).[25–27] The *b*-acid can be separated by means of ion-exchange column chromatography and preparative HPLC in approximately 15% yield.

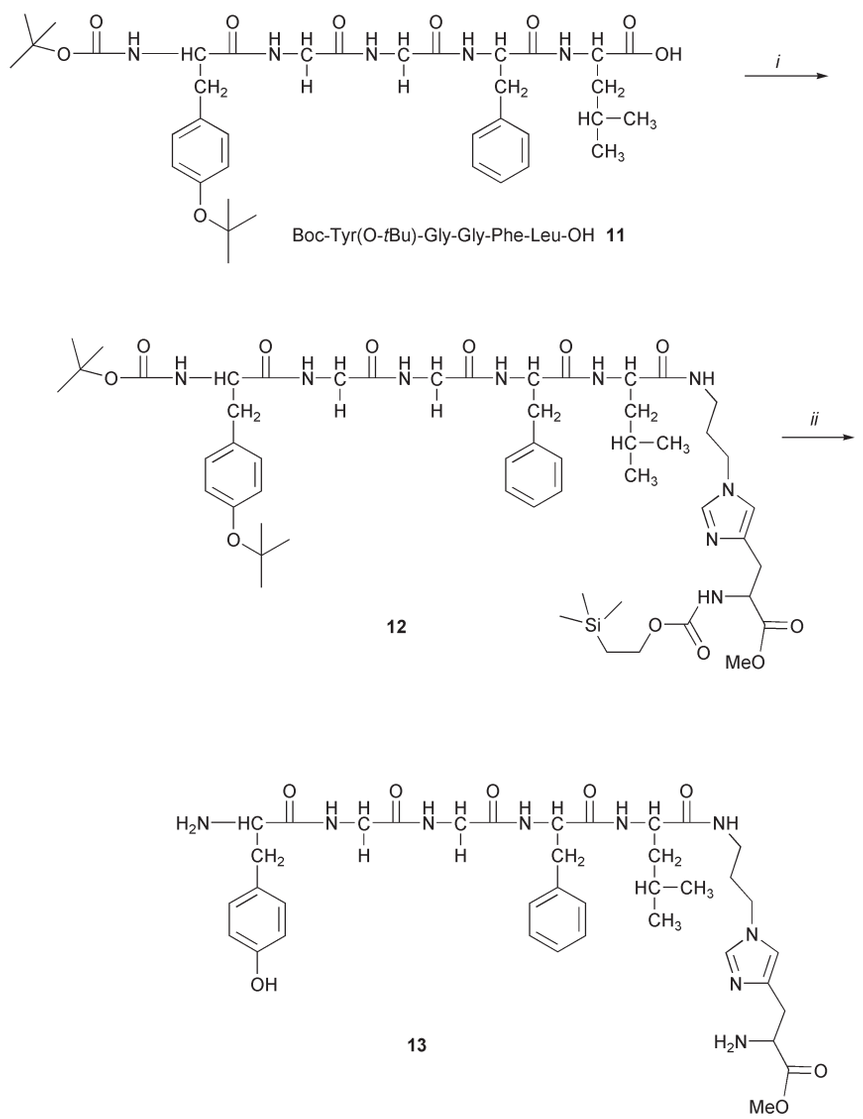
The coupling of the amine histidine derivative **8** with vitamin B12-*b*-acid **14** does not require pure **8**. Instead freshly deprotected **7** was reacted with **14** in a DMSO/DMF mixture with TBTU as the coupling reagent. With preparative HPLC the teoc-protected B12-histidine conjugate **15** (Scheme 6) was conveniently separated from the side products that originated from the Fmoc cleavage. These



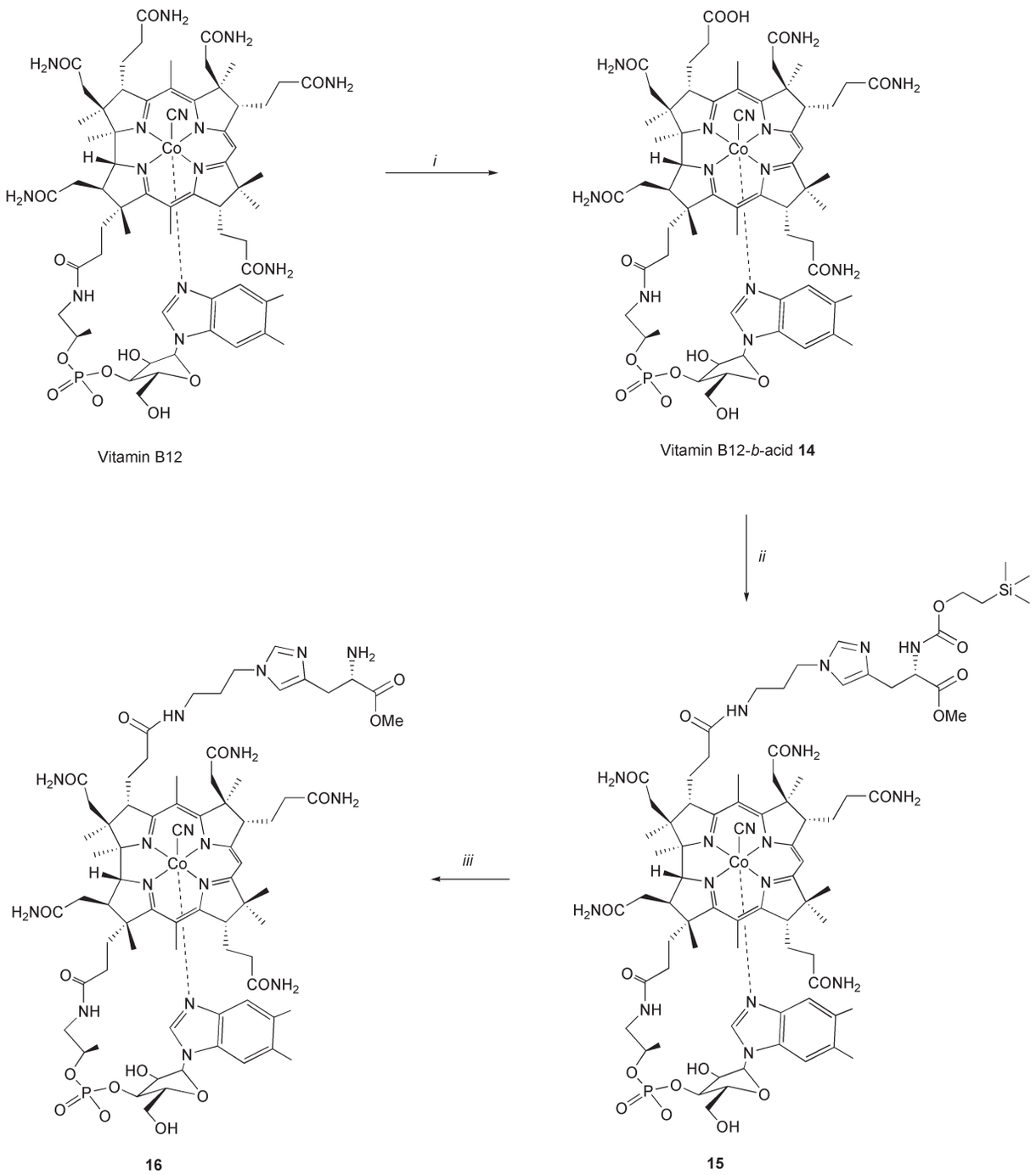
Scheme 3



Scheme 4 *Reagents and conditions*: *i*) NEt_2, DMF, RT, 91%; *ii*) biotin, TBTU, DMF, RT, 78%; *iii*) NBu_4F, 70 °C, 79%.



Scheme 5 *Reagents and conditions*: *i*) **8**, DMF, $HNEt_2$, TBTU, RT, 49%; *ii*) TFA, CH_2Cl_2, RT, 88%.



Scheme 6 *Reagents and conditions*: *i*) 0.1 M HCl, RT, 15%; *ii*) **8**, TBTU, DMF/DMSO, NEt_3, RT, 67%; *iii*) TFA, CH_2Cl_2, 0 °C, 64%.

results show that the TBTU coupling procedure is even suitable for derivatizing highly functional molecules such as vitamin B12-*b*-acid.

To make the histidine moiety accessible for labelling with $[^{99m}Tc(H_2O)_3(CO)_3]^+$ the teoc protecting group had to be removed. The fluoride method, *e.g.* the reaction with NBu_4F or CsF, was not successful and stirring of **15** in the presence of either fluoride source in DMF at RT decomposed B12, as is obvious from an irreversible colour change from red to purple. Quantitative cleavage of the teoc group could only be achieved by stirring **15** in a TFA/CH_2Cl_2 (4/1) mixture at 0 °C for 4.5 h. HPLC analysis indicated the clean conversion of **15** to **16** despite the strongly acidic conditions.

Evidence for the proposed constitution of **15** and **16** was obtained from the ESI-positive mass spectra by the $[M + H]^+$ peaks at m/z = 1710.4 for **15** and m/z = 1565.2 for **16**. The ^1H NMR spectra of **15** and **16** in non-protic solvents display a very complicated appearance, depicting many overlapping resonances. The spectra are, however, much clearer in protic solvents such as CD_3OD and D_2O, as a result of H/D exchange of the amides. When recorded in these solvents, the coupling reaction can be evaluated in the region between 6 and 8.5 ppm. This region consists of four singlets and one doublet for **14** with an intensity of 1H for each signal. In the spectra of **15** and **16**, two additional resonances are observed at around 6.95 and 7.64 ppm, owing to the imidazole hydrogen atoms. For illustration, the corresponding region between 6 and 8.5 ppm of the ^1H NMR spectra for **14** and **15** is shown in Fig. 2. The complete ^1H and ^{13}C NMR spectra of **14**, **15** and **16** are given in the electronic supplementary information.†

In addition, the ^{13}C NMR spectra provide clear evidence for the formation of **15** and **16**. The region between 185 and 100 ppm consists of 22 resonances for compound **14** and five or four additional signals are observed in this region for **15** and **16**, respectively, owing to the amide and imidazole C-atoms. In the region between 100 and −5 ppm extra signals belonging to the ligand part of the conjugates **15** and **16** with respect to **14** can be identified.

Comparison of the NMR spectra of the B12-conjugates with those of the starting material, the vitamin B12-*b*-acid **14**, gives clear evidence for the formation of the expected species.

Table 1 Retention times for the biotin, enkephalin and vitamin B12 conjugates

Biomolecule	Retention time/min	Conjugate	Retention time/min	Conjugate with $^{99m}Tc(CO)_3$	Retention time/min	Conjugate with $Re(CO)_3$	Retention time/min
D-(+)-Biotin	8.9[a]	**10**	4.1[a]	**10**-$^{99m}Tc(CO)_3$	16.5[a]	**18**	14.1[a]
Enkephalin	16.4[a]	**13**	16.1[a]	**13**-$^{99m}Tc(CO)_3$	19.5[a]	**13**-$Re(CO)_3$	18.2[a]
14	6.7[b]	**16**	7.2[b]	**16**-$^{99m}Tc(CO)_3$	14.0[b]	**19**	13.5[b]

[a] C8-column, buffer a) 0.1% TFA, standard TFA-labelling gradient (see experimental section). [b] C8-column, buffer a) NaOAc buffer, standard NaOAc gradient (see experimental section).

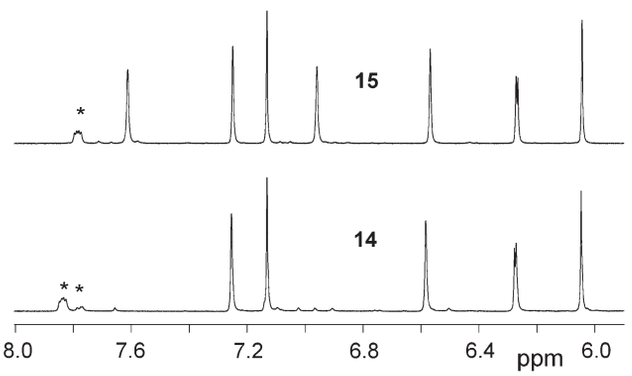
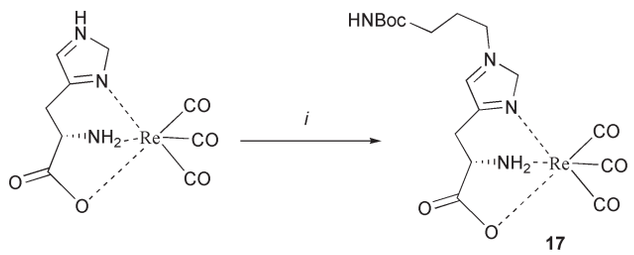


Fig. 2 Selected parts from the ^1H NMR spectra of **14** and **15** in MeOH-d_4. Resonances marked with an asterisk constitute amide protons that have not completely exchanged for D yet.

These three different examples of derivatizing biomolecules demonstrate the convenience of introducing compound **8** to different biologically active structures. Due to the possibility of deprotecting the N^{α} either by Lewis acids or by Brønsted acids, the proper method can be chosen without affecting the authenticity of the original molecule.

Labelling. To identify labelled compounds, it is common in radiopharmaceutical chemistry to compare the retention time in the HPLC radiochromatogram with the UV/Vis trace of the corresponding non-radioactive Re congener. Since ^{99m}Tc labelled compounds are present in a concentration of about 10^{-7} M, radiochromatography is the only way of characterization. Correspondingly, before labelling the bioconjugates with $[^{99m}Tc(OH_2)_3(CO)_3]^+$ the "cold" $Re(CO)_3$ analogues were synthesized and accurately characterized. In particular, our interest concerned the shift in retention times in the HPLC chromatograms between the conjugates with and without the $Re(CO)_3$ moiety. The Re analogues can principally be prepared along two different pathways. The bioconjugates **10**, **13** or **16** are either simply reacted with $[Re(OH_2)_3(CO)_3]^+$ or the complex with the linking moiety is prepared first and then conjugated to the corresponding biomolecule. Obviously, the same product should be received for both methods. We adapted the former method for Re labelling of **13** only, since the latter method lead to a mixture of compounds difficult to separate. Correspondingly, the complex Re(His-N^{ε}-$(CH_2)_3$-NHBoc)$(CO)_3$ (**17**) (Scheme 7) was used as the starting material for preparation of the Re(His)$(CO)_3$ derivatized biotin and vitamin B12 conjugates. Compound **17** was synthesised *via* a straightforward alkylation of Re(His)$(CO)_3$ with Boc-NH-$CH_2CH_2CH_2$-Br in DMF in the presence of solid Cs_2CO_3. This method has been previously reported for the alkylation of Mo(His)(allyl)$(CO)_2$ and Re(His)$(CO)_3$.[12,13,16] The Boc-protecting group was then removed by stirring **17** in a 2/1 CH_2Cl_2/TFA mixture at 0 °C for 1 h. To avoid decomposition, the temperature should not exceed 0 °C and removing the solvent *in vacuo* should be performed without external heating.

The biotin-His-$Re(CO)_3$ conjugate (**18**) was synthesised by reacting deprotected **17** with biotin in DMF in the presence of TBTU and purified by column chromatography. Accordingly, the vitamin B12 conjugate **19** was synthesised and purified by preparative HPLC. The constitution of **19** was established by the $[M + H]^+$ peak at m/z = 1822.1 in the ESI-positive mass spectra, the presence



Scheme 7 *Reagents and conditions*: *i*) Br-$CH_2CH_2CH_2$NHBoc, Cs_2CO_3, DMF, 40 °C, 97%.

of the *fac*-$Re(CO)_3$ group in the IR spectrum with the ν_{CO} vibrations at 2020 and 1901 cm^{-1} and the ^{13}C NMR resonances at 199.0, 197.6 and 197.3 ppm. Furthermore, the aromatic region of the ^1H NMR spectrum contains two additional signals with respect to the spectrum of **14**, which are assigned as the imidazole-^1H resonances. With respect to the ^{13}C NMR spectrum of **14**, the ^{13}C NMR spectrum of **19** shows additional signals owing to the carbon atoms of the histidine ligand and N^{ε}-attached chain. Attempts to isolate the $Re(CO)_3$ conjugate of the enkephalin derivative **13** were unsuccessful. Upon reaction of **13** with $(NEt_4)_2[ReBr_3(CO)_3]$ in 1 M $NaHCO_3$ at 60 °C for 1 h we observed a new peak with a retention time slightly higher than the starting material (see Table 1). The ratio between the new peak and the starting material was about 3/1 and unfortunately they could not be separated by preparative HPLC.

Subsequently we investigated the labelling efficiency of the coupled histidine ligand under various conditions with ^{99m}Tc. Model reactions with histidine showed that quantitative complex formation can be achieved at histidine concentrations as low as 10^{-6} M after 30 min at 95 °C. Heating is required to accelerate the reaction at such a high dilution. It is characteristic for the thermal stability of carbonyl complexes that no decomposition is found even after prolonged boiling in phosphate buffer. However, since some biomolecules do not thermally resist such conditions, more moderate conditions and, correspondingly, reactions at higher concentrations or longer time have to be employed.

Labelling of the conjugates **10**, **13** and **16** was performed with a solution of $[^{99m}Tc(OH_2)_3(CO)_3]^+$, prepared as previously reported.[28,29] The concentration of the bioconjugates was chosen as 10^{-4} M, whereas the concentration of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ is determined by the generator eluate and is typically in the range 10^{-6}–10^{-7} M depending on the "age" of the $^{99}Mo/^{99m}Tc$ generator. After 30 min at 50 °C $[^{99m}Tc(OH_2)_3(CO)_3]^+$ had disappeared completely and the formation of one single new peak could be observed in the radiochromatogram. The labelling yield was quantitative for all bioconjugates. The retention times for the native biomolecules, the unlabelled conjugates, the "cold" $Re(CO)_3$ conjugates and the ^{99m}Tc labelled molecules are given in Table 1. The retention times will be discussed below.

It should be emphasized at this point that the efficiency of labelling the histidine ligand in its carboxylic acid form (**10**) and its methyl ester form (**13** and **16**) is comparable. After 30 min at 50 °C all three bioconjugates did react completely with the abundant $[^{99m}Tc(OH_2)_3(CO)_3]^+$ and the difference, if any, might only manifest itself at lower reaction times or lower temperatures. Consequently for practical purposes the methyl ester form and the carboxylic acid form are equally suited for labelling. Probably, hydrolysis of the ester is metal mediated. Once the Tc centre is bidentate coordinated, the ester is activated towards hydrolysis due to its close proximity

to the metal and the preorientation for tridentate coordination. This is an important finding, because: 1) it saves a reaction step in some synthetic sequences and 2) it permits the labelling of biomolecules that do not withstand strongly alkaline conditions, such as the B12-conjugate **16**.

For the most sterically demanding conjugate, the vitamin B12 compound **16**, we investigated the accessibility of the histidine moiety for labelling as well. Steric accessibility of the chelators in general in **16** might be a limiting factor in the labelling process. Labelling yields with **16** at 10^{-5} M were therefore compared with those for free histidine at the same concentration. Histidine converts completely in the complex $^{99m}Tc(His)(CO)_3$ after 30 min at 50 °C and the behaviour observed for a 10^{-5} M solution of **16** was identical, complete conversion after a period of 30 min at 50 °C. These results demonstrate that histidine attached to the bulky vitamin B12 is not significantly different in its reactivity than free histidine.

The chromatographic behaviour of unlabelled and ^{99m}Tc or Re-labelled conjugates as shown in Table 1 represents important information about hydro-/lipophilicity of biologically active compounds. The comparable retention times of "cold" $Re(CO)_3$ and $^{99m}Tc(CO)_3$ derivatives confirm the identity of the compounds. The differences between $Re(CO)_3$ and $^{99m}Tc(CO)_3$ derivatives are explained by the separation of the UV/Vis and the radiodetector. It is common to all three labelled compounds that their retention times are larger than those of the native molecules. Unless the overall charge is changed, this is a common feature observed in labelling and not related to the *fac*-$[^{99m}Tc(CO)_3]^+$ moiety alone. It indicates increased lipophilicity which is distinct in case of biotin (8.9 *vs.* 16.5 min) and less significant in case of enkephalin and vitamin B12. Since molecular size is also relevant to understanding the delayed retention times and biotin is small in comparison to enkephalin and vitamin B12, the delay should not exclusively be interpreted in terms of lipophilicity. We emphasize that labelled and unlabelled enkephalin and vitamin B12 are not very different and that their *in vivo* behaviour should therefore be similar.

Conclusion

Histidine represent an important ligand for the labelling of biomolecules with the $[^{99m}Tc(CO)_3]^+$ moiety. Its introduction as a tripodal ligand causes difficulties due to the various functional groups present in the basic structure. We have demonstrated that the new N^{ε}-functionalised fully protected L-histidine derivative **7** can conveniently be prepared and, after Fmoc deprotection, easily and quantitatively coupled to carboxylate groups in a variety of different types of biomolecules. Since the biomolecules stem from peptides and vitamins, we emphasize that the method can be generalized to other biomolecules as well. After deprotection of N^{α}, the biomolecule comprises a highly potent tridentate ligand that can quantitatively be labelled with $[^{99m}Tc(OH_2)_3(CO)_3]^+$ under mild conditions (10^{-4} M–10^{-5} M bioconjugate, 50 °C, 30 min). Importantly, there is no difference between labelling the free carboxylic acid form (**10**) and the methyl ester form (**13** and **16**) of histidine and, thus, the ligand and the procedure is also versatile for labelling thermally and base-sensitive biomolecules such as vitamin B12. One and only one product was observed, demonstrating the potency of this ligand since the biomolecule of each of the conjugates contains potentially coordinating donor atoms

The methods presented herein for conjugation of **8** and subsequent labelling with $[^{99m}Tc(OH_2)_3(CO)_3]^+$ are currently being extrapolated to other biomolecules. The amine derivative **8** and the previously reported carboxylic acid derivative **1** constitute a complementary pair of histidine derivatives. With these, biomolecules can be derivatised at either a carboxylic acid or an amine moiety. Currently we are investigating the biological *in vivo* properties of the labelled derivatives presented herein.

Experimental

IR spectra were recorded on a Bio-Rad FTS-45 spectrometer with the samples in compressed KBr pills. Electrospray ionisation mass spectra (ESI-MS) were recorded on a Merck Hitachi M-8000 spectrometer in the positive ion mode using methanol as the solvent. Fast Atom Bombardment (FAB, nitrobenzylalcohol as matrix)) mass spectra, both at low and high resolution, were recorded on a Jeol JMS-700 mass spectrometer. As reference compounds for the high resolution FAB mass spectra PEG's of a suitable mass were used. For the Re compounds, only the values of the ^{187}Re isotope are reported in the low resolution mass spectra, whereas in high resolution mass spectra peaks originating from both the ^{185}Re isotope and the ^{187}Re isotope are reported. Elemental analyses were performed on a Leco CHNS-932 elemental analyzer. ^1H, ^{13}C and ^{31}P NMR spectra were recorded on a Varian Gemini-2000 (^1H at 300.08 MHz), a Bruker 360 MHz (^1H at 360.13 MHz) and a Bruker DRX-500 (^1H at 500.25 MHz) spectrometer. ^1H and ^{13}C NMR spectra were referenced to TMS, using the ^{13}C or residual protio signals of the deuterated solvents as internal standards. ^{31}P NMR spectra were referenced against 0.1 M H_3PO_4 in H_2O.

HPLC analyses were performed on a Merck-Hitachi L-7000 system equipped with a EG&G Berthold LB 508 radiometric detector, using Waters XTerra RP8 columns (5 µm particle size, 1 × 100 mm) and a flow rate of either 0.5 ml min^{-1} or 1 ml min^{-1}. Chromatograms were recorded at 220, 250 and 360 nm. Sodium acetate buffer a) was prepared by mixing 2.9 ml acetic acid and 4.55 ml sodium hydroxide 2 M in 900 ml water and 100 ml methanol. Tris buffer a) was prepared by dissolving tris(hydroxymethyl)aminomethane (6.05 g) in water, adding HCl 2 M to reach a pH of 8.2, adjusting the volume to 1000 ml, and adding acetonitrile (10 ml). TFA buffer a) was prepared by dissolving 1.0 ml of CF_3COOH in 1000 ml of water. Buffer b) was always methanol. Preparative HPLC separations were performed on a Varian Prostar system equipped with two Prostar 215 pumps and a Prostar 320 UV/Vis detector, using a Waters XTerra Prep RP8 column (5 µm particle size, 30 × 100 mm) or a Macherey-Nagel Nucleosil C-18ec column (7 µm particle size, 100 Å pore size, 40 × 250 mm). Flow rates were 30 ml min^{-1} for the RP8 30 × 100 mm column and 40 ml min^{-1} for the C-18ec column. After the preparative HPLC purification, cobalamin derivatives were desalted by applying an aqueous solution of the compound to a Chromafix RP18ce cartridge, followed by thoroughly rinsing with water. The desalted product was then eluted with methanol, the solvent was removed *in vacuo*, and the product was dried under high vacuum. The biotin and enkephalin derivatives purified by preparative HPLC were isolated by removing the methanol portion of the eluent *in vacuo*, followed by lyophilisation.

General procedure for labelling with $[^{99m}Tc(OH_2)_3(CO)_3]^+$

A solution of the bioconjugate **10**, **13** or **16** (10^{-3} or 10^{-4} M in H_2O, 200 µl) was added to a vial with a septum, which was then sealed and degassed with a stream of nitrogen gas for 10 min. A solution of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ (1800 µl)[28,29] was added to the vial *via* a syringe and the vial was heated to 50 °C for 30 min to yield the $[^{99m}Tc(CO)_3]$ labelled bioconjugates, which was demonstrated by HPLC with radioactive detection. For the HPLC analysis of labelled **10** or **13**, the TFA buffer as buffer a) (described above) was used with the C8 column. The following program was used: from 0 to 3 min only the TFA buffer. At 3.1 it jumped to 25% MeOH, which was then kept constant for 6 min. At 9 min a jump to 33% MeOH is followed by a linear gradient to 100% MeOH from 9.1 min to 20 min. For the analysis of labelled **16**, the NaOAc buffer (described above) was used as buffer a) with the C8 column. The following program was used: from 0% to 15% MeOH in 30 min, then the MeOH content increased to 100% over 10 min.

Synthesis

Compounds **2**,[16,30] Cbz-NH-CH_2CH_2OH,[31] Boc-NH-CH_2CH_2I,[32] Trt-NH-CH_2CH_2OH·HCl,[33] Boc-NH-$CH_2CH_2CH_2$-Br,[34] Re(L-His)$(CO)_3$[35] and $(NEt_4)_2[ReBr_3(CO)_3]$[36] were prepared according to methods in the literature.

Trt-NH-$CH_2CH_2CH_2OH$·HCl. The compound Trt-NH-$CH_2CH_2CH_2OH$·HCl was prepared analogously to the method that Maltese reported for the preparation of Trt-NH-CH_2CH_2OH·HCl.[33]

To a solution of 3-aminopropanol (4.0 ml; 3.93 g; 15.7 mmol) in MeCN (20 ml) was added chlorotriphenylmethane (2.0 g; 7.2 mmol). After the mixture was stirred for 30 min at RT, it was poured into 200 ml of H_2O and stirred for 10 min. During this time a white precipitate formed, which was isolated by filtration and dried *in vacuo*. The white solid was dissolved in 60 ml anhydrous diethyl ether and gaseous HCl was bubbled through the solution, resulting in the formation of a finely divided white precipitate. It was isolated by filtration, washed with Et_2O and air-dried. Yield: 2.15 g; 85%.

Found: C, 74.7; H, 6.9; N, 3.9; Calc. for $C_{22}H_{24}NOCl$: C, 74.7; H, 6.8; N, 4.0%.

Fmoc-NH-CH_2CH_2OH and Fmoc-NH-$CH_2CH_2CH_2OH$. The compounds Fmoc-NH-CH_2CH_2OH and Fmoc-NH-$CH_2CH_2CH_2OH$ were prepared according to the method of Jönsson and Undén.[37]

Fmoc-NH-CH_2-CH_2OH. Found: C, 72.3; H, 6.3; N, 4.8 Calc. for $C_{17}H_{17}NO_3$: C, 72.1; H, 6.1; N, 4.9%; ν_{max} (KBr)/cm^{-1} 3333s NH, 1692vs C=O; δ_H (300 MHz; dmso-d_6) 7.89 (2H, pseudo-d, 2 × Ar*H*), 7.69 (2H, pseudo-d, 2 × Ar*H*), 7.40 (2H, pseudo-t, 2 × Ar*H*), 7.32 (2H, pseudo-t, 2 × Ar*H*), 7.25 (1H, t, *J* 5.7, N*H*), 4.65 (1H, t, *J* 5.7, O*H*), 4.29 (2H, d, *J* 6.3, OC*H*$_2$CH), 4.20 (1H, t, *J* 6.3, OCH$_2$C*H*), 3.38 (2H, q, *J* 6.0, 2H, HO-C*H*$_2$-CH$_2$), 3.05 (2H, q, *J* 6.0, NH-C*H*$_2$-CH$_2$); δ_C (75.47 MHz; dmso-d_6) 156.5 (*C*=O), 144.1, 140.9, 127.8, 127.2, 125.4, 120.3 (Ar-*C*), 65.4, 60.0, 46.7, 43.0; *m*/*z* (ESI-pos.; MeOH) 283 [M + H]$^+$, 305 [M + Na]$^+$.

Fmoc-NH-$CH_2CH_2CH_2OH$. Found: C, 73.0; H, 6.5; N, 4.8; Calc. for $C_{18}H_{19}NO_3$: C, 72.7; H, 6.4; N, 4.7%; ν_{max} (KBr)/cm^{-1} 3324s NH, 1689vs C=O; δ_H (300 MHz; dmso-d_6): 7.87 (2H, pseudo-d, 2 × Ar*H*), 7.68 (2H, pseudo-d, 2 × Ar*H*), 7.40 (2H, pseudo-t, 2 × Ar*H*), 7.32 (2H, pseudo-t, 2H, 2 × Ar*H*), 7.25 (1H, t, *J* 5.7, NH), 4.42 (1H, t, *J* 5.4, OH), 4.28 (2H, d, *J* 6.3, OC*H*$_2$CH), 4.21 (1H, t, J *6.3*, OCH$_2$C*H*), 3.41 (2H, q, *J* 6.0, HO-C*H*$_2$-CH$_2$), 3.04 (2H, q, *J* 6.6, NH-C*H*$_2$-CH$_2$), 1.56 (2H, pentet, *J* 6.6, CH$_2$-C*H*$_2$-CH$_2$); δ_H (75.47 MHz; dmso-d_6): 156.4 (*C*=O), 144.1, 140.9, 127.8, 127.2, 125.3, 120.3 (Ar-*C*), 65.4, 58.4, 46.8, 37.5, 32.7; *m*/*z* (ESI-pos.; MeOH) 297 [M + H]$^+$, 319 [M + Na]$^+$.

General synthesis of the iodide derivatives 4, Trt-NH-CH_2CH_2I, Trt-NH-$CH_2CH_2CH_2I$, Fmoc-NH-CH_2CH_2I and 6

To a solution of triphenylphosphine (786 mg; 3.0 mmol) and imidazole (204 mg; 3.0 mmol for the synthesis of **4**, Fmoc-NH-CH_2CH_2I and **6**; 408 mg; 6.0 mmol for the synthesis of Trt-NH-CH_2CH_2I and Trt-NH-$CH_2CH_2CH_2I$) in dry CH_2Cl_2 (50 ml) under N_2 was added in small portions I_2 (837 mg; 3.0 mmol) over a period of 5 min. The mixture was stirred for 15 min at RT and subsequently cooled to 0 °C. After the corresponding alcohol derivative was added as a solid, the suspension was stirred at 0 °C for 15 min and then stirred at RT for 1 h. The mixture was filtered to remove a white solid, followed by evaporation of the solvent under reduced pressure. After purification by column chromatography (silica; hexane/EtOAc 2/1), the iodide was isolated in a 75–92% yield as a colourless solid.

4. Yield: 840 mg; 92%. Found: C, 39.6; H, 4.1; N 4.6; Calc. for $C_{10}H_{12}NO_2I$: C, 39.4; H, 3.9; N, 4.6%; ν_{max} (KBr)/cm^{-1} 3304s NH, 1679vs C=O. δ_H (300 MHz; CD_3CN) 7.35 (5H, 5 × Ar*H*), 5.93 (1H, br s, NH), 5.06 (2H, s, O-C*H*$_2$), 3.41 (2H, q, *J* 6.6, N-C*H*2-CH2), 3.24 (2H, t, *J* 6.6, N-C*H*$_2$-CH$_2$); δ_H (75.47 MHz; CD_3CN): 157.5 (*C*=O), 138.5 (Ar*C*$_q$), 129.7, 129.2, 129.0 (Ar-*C*H), 67.2 (O-*C*H$_2$), 44.4 (N-*C*H$_2$), 6.0 (I-*C*H$_2$); *m*/*z* (ESI-pos.; MeOH) 305 [M + H]$^+$, 327 [M + Na]$^+$.

Trt-NH-CH_2CH_2I. Yield: 989 mg; 80%.Found: C, 60.0; H, 5.0; N, 3.4; Calc. for $C_{21}H_{20}NI$: C, 59.9; H, 5.0; N, 3.5%; ν_{max} (KBr)/cm^{-1} 3301m NH; δ_H (300.08 MHz; dmso-d_6) 7.41 (6H, pseudo-d, 6 × Ar*H*), 7.30 (6H, pseudo-t, 6 × Ar*H*), 7.19 (3H, pseudo-t, 6 × Ar*H*), 3.26 (2H, pseudo-t, HN-C*H*$_2$), 3.07 (1H, br s, NH), 2.29 (2H, t, *J* 6.5, I-CH$_2$); δ_H (75.47 MHz; dmso-d_6) 146.1, 128.5, 128.0, 126.4 (ArC), 70.2 (C$_q$), 46.4 (N-CH$_2$), 7.9 (I-CH$_2$); *m*/*z* (FAB$^+$, NBA) 413.1 [M$^+$], 243.1 [C(C$_6$H$_5$)$_3^+$].

Trt-NH-$CH_2CH_2CH_2I$. Yield: 978 mg; 76%.

Found: C, 60.9; H, 5.2; N, 3.27; Calc. for $C_{21}H_{22}NI$: C, 60.7; H, 5.3; N, 3.4%; ν_{max} (KBr)/cm^{-1} 3297m NH; δ_H (300.08 MHz; dmso-d_6) 7.41 (6H, pseudo-d, 6 × Ar*H*), 7.28 (6H, pseudo-t, 6 × Ar*H*), 7.17 (3H, pseudo-t, 3 × Ar*H*), 3.34 (2H, q, *J* 6.0, NH-C*H*$_2$-CH$_2$), 2.76 (1H, t, *J* 7.5, N*H*), 2.1–1.9 (overlapping m, 4H, I-C*H*$_2$-C*H*$_2$). δ_H (75.47 MHz; dmso-d_6): 146.3, 129.0, 128.6, 127.8 (Ar*C*), 70.3 (*C*$_q$), 43.7 (N-*C*H$_2$), 34.0 (*C*H$_2$-CH$_2$-CH$_2$) 7.0 (I-*C*H$_2$); *m*/*z* (FAB$^+$; NBA): 427.1 [M$^+$], 243.1 [C(C$_6$H$_5$)$_3^+$].

Fmoc-NH-CH_2CH_2I. Yield: 940 mg; 80%.

Found: C, 52.2; H, 4.2; N, 3.58; Calc. for $C_{17}H_{16}NO_2I$: C, 51.9; H, 4.10; N, 3.6%; ν_{max} (KBr)/cm^{-1} 3336m NH, 1690s C=O. δ_H (300.08 MHz; CD_3CN): 7.83 (2H, pseudo-d, 2 × Ar*H*), 7.65 (2H, pseudo-d, 2 × Ar*H*), 7.41 (2H, pseudo-t, 2 × Ar*H*), 7.33 (2H, pseudo-t, 2 × Ar*H*), 5.96 (1H, br s, NH) 4.33 (2H, d, *J* 6.3, O-C*H*$_2$), 4.23 (1H, t, *J* 6.9, O-CH$_2$-C*H*), 3.39 (2H, q, *J* 6.3, HN-C*H*$_2$-CH$_2$), 3.23 (2H, t, *J* 6.3, I-C*H*$_2$); δ_H (75.47 MHz; dmso-d_6): 156.2 (*C*=O), 144.0, 140.9, 127.8, 127.3, 125.3, 120.3 (Ar*C*), 65.5 (*C*H-FMoc), 46.7, 43.0 (N-*C*H$_2$, *C*H$_2$-FMoc), 6.0 (I-*C*H$_2$); *m*/*z* (ESI-pos., MeOH) 179, 216, 393 [M + H]$^+$, 415 [M + Na]$^+$.

6. Yield: 1036 mg; 85%. Found: C: 53.2; H, 4.5; N, 3.5; Calc. for $C_{18}H_{18}NO_2I$: C, 53.0; H, 4.5; N, 3.4%; ν_{max} (KBr)/cm^{-1} 3336m NH, 1690s C=O; δ_H (dmso-d_6; 300.08 MHz) 7.86 (2H, pseudo-d, 2 × Ar*H*), 7.66 (2H, pseudo-d, 2 × Ar*H*), 7.42–7.49 (4H, overlapping-m, 4 × Ar*H*), 4.30 (2H, d, *J* 6.6, CH$_2$-Fmoc), 4.19 (1H, t, *J* 6.6, C*H*), 3.19 (2H, t, *J* 6.9, C*H*$_2$-I), 3.02 (2H, m, NC*H*$_2$), 1.87 (2H, m, CH$_2$-C*H*$_2$-CH$_2$), NH not detected; δ_H (dmso-d_6; 75.47 MHz) 156.4 (C=O), 144.1, 140.9 (2 × Ar*C*$_q$), 127.8, 127.2, 125.3, 120.3 (4 × Ar*C*H), 65.3 (*C*H-Fmoc), 46.8 (*C*H$_2$-Fmoc), 40.8, 33.2, 4.9 (CH$_2$); *m*/*z* (ESI-pos., MeOH) 179, 230, 407 [M + H]$^+$, 430 [M + Na]$^+$.

3-(1-Benzyl-1*H*-imidazol-4-yl)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-propionic acid methyl ester (5)

A mixture of **2** (196 mg; 1.0 mmol) and **4** (610 mg; 2.0 mmol) in MeCN (40 ml) was heated at reflux for 16 h under an atmosphere of N_2. TLC showed that the starting urea derivative had completely reacted. The mixture was concentrated *in vacuo*, resulting in a white solid. This solid was washed with dry Et_2O (3 × 20 ml) to remove excess **4**. The solid material was redissolved in MeCN (40 ml) and 9-fluorenylmethanol (588 mg; 3.0 mmol) and dipea (581 mg; 4.5 mmol) were added. The resulting mixture was stirred at RT under N_2 for 16 h. The solvent was removed *in vacuo*, followed by purification by column chromatography (silica; CH_2Cl_2/MeOH 2/1). Yield: 274 mg (19% over two steps) of a foamy colourless solid. ν_{max} (KBr)/cm^{-1} 3426s NH, 1746s, 1722vs C=O. δ_H (300.08 MHz; CD_3CN): 7.83 (2H, pseudo-d, 2 × Ar*H*), 7.62 (2H, pseudo-d, 2 × Ar*H*), 7.51 (1H, s, N$_2$C*H*$_{His}$), 7.41 (2H, pseudo-t, 2 × Ar*H*), 7.32 (5H, m, 5 × Ar*H*), 7.17 (2H, pseudo-d, 2 × Ar*H*), 6.78 (s, 1H, C*H*$_{imidazole}$), 6.67 (1H, d, *J* 8.1 Hz, NH), 5.06 (2H, s, Nε-C*H*$_2$), 4.34 (3H, overlapping m, Cα*H* + O-C*H*$_2$), 4.21 (1H, t, *J* 6.6, O-CH$_2$C*H*), 3.56 (3H, s, OC*H*$_3$), 2.91 (2H, pseudo-d, C$_β$*H*$_2$). δ_C (75.47 MHz; CD_3CN) 173.6 (*C*=O$_{ester}$), 157.4 (*C*=O$_{amide}$), 145.4, 142.4, 138.9, 138.7, 138.6, 130.1, 129.2, 129.0, 128.6, 128.4, 126.4, 121.3, 118.4 (13 × Ar*C*), 67.4, 55.5, 52.8, 51.2, 48.1 (N^ε-*C*H$_2$, O*C*H$_3$, O-*C*H$_2$-*C*H, *C*$_α$H), 30.6 (C$_β$); *m*/*z* (ESI-pos.; MeOH): 481.9 ([M + H]$^+$, $C_{29}H_{28}N_3O_4$ requires 482.2 g mol^{-1}).

^{99}Tc(His-N$_ε$-benzyl)(CO)$_3$

A solution of 40.4 mg (0.084 mmol) **5** was dissolved in CH_2Cl_2 (2 ml) and TFA (1.5 ml) was added. The mixture was stirred for 1.5 h followed by removal of the solvent *in vacuo*. The residue was dissolved in H_2O (2 ml) and $(NEt_4)_2[TcBr_3(CO)_3]$ was added. The pH was adjusted to 7 by carefull addition of a 1 M NaOH solution. The mixture was allowed to stir overnight, during which time a white/creamy precipitate formed. The solid was isolated by filtration, washed with H_2O and air dried. Yield: 26 mg (72%). HPLC

with radiodetection showed one single peak. Crystals suitable for X-ray diffraction were grown by slow evaporation of a 1/1 EtOH/H_2O mixture.

3-{1-[3-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propyl]-1*H*-imidazol-4-yl}-2-(3-trimethylsilanyl-ethylcarboxyamino)-propionic acid methyl ester (7)

A mixture of **2** (196 mg; 1.0 mmol) and **8b** (1.22 g; 3.0 mmol) in MeCN (40 ml) was heated at reflux for 4.5 days under an atmosphere of N_2. TLC showed that the starting urea derivative had completely reacted. The mixture was concentrated *in vacuo*, resulting in a white solid. The solid material was redissolved in MeCN (40 ml) and 2-trimethylsilylethanol (355 mg; 3.0 mmol) and dipea (259 mg; 2.0 mmol) were added. The resulting mixture was stirred at RT under N_2 for 16 h. The solvent was removed *in vacuo*, followed by purification by column chromatography (silica; EtOAc). Yield: 316 mg (53% over two steps) of a foamy colourless solid

Found: C, 62.1; H, 6.2; N, 9.5; Calc. for $C_{31}H_{40}N_4O_6Si$: C, 62.8; H, 6.8; N 9.5%; ν_{max} (KBr)/cm^{-1} 3329br NH, 1730s, 1698vs C=O; δ_H (300.8 MHz; CD_3CN) 7.82 (2H, pseudo-d, 2 × Ar*H*), 7.64 (2H, pseudo-d, 2 × Ar*H*), 7.38 (3H, m, 2 × Ar*H* + N_2CH_{His}), 7.32 (2H, pseudo-t, 2 × Ar*H*), 6.79 (1H, s, CH_{His}), 6.55 (1H, d, *J* 7.5, NH), 5.68 (1H, br s, NH), 4.35 (3H, overlapping m, OCH_2-Fmoc + $C_\alpha H$), 4.22 (1H, t, *J* 6.6, OCH_2CH-Fmoc), 4.07 (2H, m, CH_2), 3.87 (2H, m, CH_2), 3.60 (3H, s, OCH_3), 2.99 (2H, m, CH_2), 2.90 (2H, m, $C_\beta H_2$), 1.85 (2H, m, CH_2), 0.93 (2H, m, CH_2), 0.01 (s, 9H, Si-$(CH_3)_3$). δ_C (CD_3CN; 75.47 MHz) 173.7 ($C=O_{ester}$), 157.7, 157.6 (2 × $C=O_{amide}$), 145.5, 142.2 (2 × ArC_q), 138.4 (C_{His}), 128.9, 128.3 (2 × Ar*C*H), 126.4 (C_{His}), 121.2, 118.6 (2 × Ar*C*H), 118.1 (C_{His}), 66.9, 63.7, 55.3, 52.7, 48.3, 44.9, 38.6, 32.1, 30.1, 18.3, 1.4 ($Si(CH_3)_3$); *m*/*z* (ESI-pos., MeOH) 343, 371, 533, 593 $[M + H]^+$.

3-[1-(3-Amino-propyl)-1*H*-imidazol-4-yl]-2-(3-trimethylsilanyl-ethylcarboxyamino)-propionic acid methyl ester (8)

Compound **7** (255 mg; 0.43 mmol) was dissolved in a 1/1 DMF/NEt_2 mixture (8 ml). After the mixture was stirred for 1 h at RT, the solvent was removed *in vacuo.* Purification by preperative HPLC (C-18ec column; TFA buffer) afforded **8** as a colourless foamy solid as its trifluoroacetate salt. Yield: 190 mg (91%).

δ_H (300.08 MHz; CD_3CN) 8.59 (1H, s, N_2CH_{His}), 7.95 (3H, br, NH_3^+), 7.26 (1H, s, CH_{His}), 6.56 (1H, d, *J* 8.4 Hz, NH), 4.43 (1H, m, $C_\alpha H$), 4.24 (2H, t, *J* 6.9 Hz, CH_2), 4.05 (2H, m, CH_2), 3.68 (3H, s, OCH_3), 3.22 (1H, m, C_βH), 3.06 (1H, m, C_βH), 2.95 (2H, t, *J* 6.9, CH_2), 2.21 (2H, m, CH_2), 0.89 (2H, m, CH_2), 0.00 (9H, s, Si-$(CH_3)_3$); δ_C (75.47 MHz; CD_3CN): 172.7 ($C=O_{ester}$), 162.2 (q, $J_{C,F}$ 34.6, CF_3), 157.7 ($C=O_{amide}$), 135.9, 132.4, 120.7 (3 × C_{His}), 64.0, 54.6, 53.2, 47.0, 37.3, 28.6, 27.6, 18.2 (OCH_3, C_α, C_β + 5 × CH_2), 1.5 (Si-$(CH_3)_3$); *m*/*z* (ESI-pos.; MeOH): 343.1, 370.8 ($[M + H]^+$, $C_{16}H_{30}N_4O_4Si$ requires 371.2) 762 $[2M + Na]^+$.

Biotin conjugate 9

D-(+)-Biotin (35 mg; 0.14 mmol) was dissolved in a 4/1 (v/v) mixture of DMF/NEt_3 (2.5 ml). To this mixture a solution of **8** (91 mg; 0.19 mmol) was added in DMF (2 ml), followed by addition of TBTU (46 mg; 0.14 mmol). After stirring for 45 min at RT, it was evaporated to dryness *in vacuo.* The residue was taken up in 2 M $NaHCO_3$ (20 ml) and extracted with CH_2Cl_2 (3 × 20 ml). The combined organic layers were washed with 0.5 M HCl (20 ml), H_2O (20 ml) and brine (2 × 20 ml). After removal of the solvent, **9** was obtained as a colourless foamy solid. Yield: 67 mg (78%; relative to biotin).

δ_H (300.08 MHz; CD_3OD) 7.60 (1H, s, N_2CH_{His}), 6.95 (1H, s, CH_{His}), 4.48 (1H, m), 4.41 (1H, m), 4.29 (1H, m), 4.09 (2H, m), 3.98 (2H, m), 3.69 (3H, s, OCH_3), 3.16 (3H, overlapping-m, CH_2 + *H*), 2.95–2.78 (5H, overlapping-m, 2 × CH_2 + H), 2.70 (1H, m), 2.19 (2H, m, CH_2), 1.93 (2H, m, CH_2), 1.63 (4H, m, 2 × CH_2), 1.43 (2H, m, CH_2), 0.94 (2H, m, CH_2), 0.01 (9H, s, Si-$(CH_3)_3$; δ_C (75.47 MHz; CD_3OD): 176.4, 174.3 ($C=O_{ester}$ + $N_2C=O$), 166.3, 158.9 ($C=O_{amide}$), 138.5, 138.3, 118.7 (ArC_{His}), 64.2, 64.4, 61.7, 57.1, 55.7, 52.8, 45.7, 41.1, 37.4, 36.8, 31.9, 31.1, 29.8, 29.5, 26.8, 18.6 (10 × CH_2, Cα, Cβ, OCH_3, 3 × CH), 1.5 (Si-$(CH_3)_3$; *m*/*z* (FAB^+; NBA) 597.2898 (M^+, $C_{26}H_{45}N_6O_6SiS$ requires 597.2891).

Biotin conjugate 10

To a solution of **9** (100 mg; 0.17 mmol) in DMF (5 ml) was added 1 ml of a 1 M NBu_4F solution in THF. After heating the mixture at 70 °C for 1.5 h, the solvent was removed *in vacuo.* Purification by preparative HPLC (C18ec column; TFA gradient) afforded 72 mg (79%) of the trifluoro acetate salt of **10** as a colorless foamy solid.

δ_H (500.25 MHz; CD_3OD): 8.94 (1H, s, N_2CH_{His}), 7.54 (1H, s, CH_{His}), 4.50 (1H, m), 4.30 (2H, m), 4.22 (2H, t, *J* 6.6, N_ε-CH_2), 3.45 (1H, m), 3.33 (1H, m), 3.20 (3H, m), 2.91 (1H, dd, ^3J 4.9, ^2J 12.6, $C_\beta H$), 2.67 (1H, d, ^2J 12.6, $C_\beta H$), 2.24 (2H, t, *J* 7.3, O=C-NH-CH_2), 2.07 (2H, m, $CH_2CH_2CH_2$), 1.66 (4H, m), 1.45 (2H, m). δ_C (125.8 MHz; MeOH-d_4) 176.7, 179.6, 166.3 (3 × C=O), 162.9 (q, $J_{C,F}$ 36, CF_3COO), 137.2 ($ArCH_{His}$), 129.4 (ArC_{His}), 122.5 ($ArCH_{His}$), 119.3 (q, $J_{C,F}$ 292, CF_3), 63.6, 61.8, 57.2, 53.2 (4 × *C*H), 48.3, 41.2, 36.8, 36.8, 31.3, 29.9, 29.6, 26.9, 26.9 (9 × CH_2); *m*/*z* (FAB^+; NBA) 439.2151 ($[M + H]^+$, $C_{19}H_{31}N_6O_4S$ requires 439.2127).

Boc-(Tyr-tBu)-Gly-Gly-Phe-Leu-OH (11)

The synthesis of Boc-Tyr(tBu)-Gly-Gly-Phe-Leu-OH was performed *via* solid phase peptide synthesis methods, adapting the Fmoc strategy. The H-Leu-2-ClTrt resin was obtained from Advanced ChemTech and had a loading of 1.3 mmol g^{-1} with a mesh-size of 100–200. All coupling reagents and amino acids were purchased from Novabiochem and used without further purification.

Coupling reactions were performed with 5 equivalents of amino acid and HOBt and 4.9 equivalents of TBTU for 30 min in DMF. Deprotection was performed twice for 5 min using 20% piperidine in DMF. After complete assembly, the product was cleaved from the resin by 1% TFA in DCM, containing 3% of triisopropylsilane as a scavenger. After evaporating the solvents the crude product was repeatedly washed with cold ether and subsequently lyophilised.

δ_H (500.25 MHz; CD_3OD): 7.26 (4H, m, 4 × ArH_{Phe}), 7.18 (1H, m, ArH_{Phe}), 7.10 (2H, pseudo-d, 2 × ArH_{Tyr}), 6.89 (2H, pseudo-d, 2 × ArH_{Tyr}), 4.68 (1H, m, $C_\alpha H$), 4.43 (1H, m, $C_\alpha H$), 4.25 (1H, m, $C_\alpha H$), 3.88 (2H, m, CH_2), 3.72 (2H, m, CH_2), 3.18 (1H, m, $C_\beta H$), 3.10 (1H, m, $C_\beta H$), 2.92 (1H, m, $C_\beta H$), 2.81 (1H, m,$C_\beta H$), 1.71 (1H, m, $C_\gamma H_{Leu}$), 1.63 (2H, m, $C_\beta H_{2\text{-}Leu}$), 1.35 (9H, s, Tyr-$OC(CH_3)_3$), 1.29 (9H, s, Boc-$(CH_3)_3$), 0.94 (3H, d, *J* 6.4, Leu-CH_3), 0.90 (3H, d, *J* 6.4, Leu-CH_3); δ_C (125.8 MHz; CD_3OD;) 175.8, 175.3, 173.6, 172.3, 171.3, 158.2 (5 × *C*=O), 155.4, 138.6, 133.9, 131.8, 130.6, 129.6, 127.9, 125.4 (10 × Ar*C*), 81.0, 79.7 (2 × $C(CH_3)_3$), 58.1, 56.0, 52.3, 44.0, 43.5, 41.8, 39.0, 38.4 (CH and/or CH_2), 29.4, 29.0 (2 × $C(CH_3)_3$), 26.1, 23.7, 22.1; *m*/*z* (FAB^+; NBA): 734.2 ($[M + H]^+$, $C_{37}H_{53}NaN_5O_9$ requires 734.4).

Enkephalin conjugate 12

To a solution of purified **8** (39 mg; 0.08 mmol) and **11** (85 mg; 0.08 mmol) in DMF (1.5 ml) and NEt_3 (0.5 ml) was added TBTU (25 mg; 0.08 mmol). The mixture was stirred for 45 min at RT and concentrated to dryness *in vacuo.* The compound was purified by two consequetive HPLC runs (run 1: C8-column; 50 mM TRIS buffer; run 2: C8-column; TFA buffer). Yield: 42 mg (49%) of a glassy solid.

δ_H (500.25 MHz; CD_3OD) 8.77 (1H, s, N_2CH_{His}), 7.43 (1H, s, CH_{His}), 7.28 (4H, m, 4 × Ar*H*), 7.20 (1H, m, Ar*H*), 7.12 (2H, pseudo-d, 2 × Ar*H*), 6.90 (2H, pseudo-d, 2 × Ar*H*), 4.52 (1H, m, C_αH), 4.49 (1H, m, C_αH), 4.22–4.09 (7H, overlapping m, 2 × C_αH + 2 × CH_2), 3.85 (2H, m, CH_2-Gly), 3.78 (2H, m, CH_2-Gly), 3.73 (3H, s, OCH_3), 3.25–3.02 (9H, overlapping m, 2 × CH_2 + 3 × C_βH), 2.84 (1H, m, C_βH), 2.04 (1H, m, CH_2-CH_2-CH_2), 1.71 (1H, m, C_γH-Leu), 1.56 (2H, m, $C_\beta H_2$), 1.36 (9H, s, $OC(CH_3)_3$), 1.28 (9H, s, $OC(CH_3)_3$), 0.94 (3H, d, *J* 6.3, Leu-CH_3), 0.89 (3H, d, *J* 6.3, Leu-CH_3); δ_C (90.5 MHz; CD_3OD) 175.5, 175.1, 174.2 172.9, 172.6, 158.7, 158.2

(C=O, 7 signals detected whereas 8 are expected, probably two signals overlap), 155.4, 138.3, 136.4, 133.6, 130.9, 130.2, 129.6, 128.0, 125.2, 121.2 (all Ar*C*), 81.0, 79.6 (C_q), 64.5, 58.0, 57.5, 54.4, 53.2, 47.6, 44.1, 41.0, 38.1, 38.0, 36.3, 30.8, 29.2, 28.7, 28.2, 25.9, 23.5, 21.7, 18.6, −1.5 (CH, CH_2 and CH_3; 20 signals detected whereas 21 are expected. Possibly two signals overlap or a signal is obscured by the MeOH signal); *m*/*z* (FAB$^+$; NBA)1064.5789 ([M + H]$^+$, $C_{53}H_{82}N_9O_{12}Si$ requires 1064.5852).

Enkephalin conjugate 13

Compound **12** (42 mg; 0.04 mmol) was dissolved in 3 ml of a mixture of CH_2Cl_2/TFA (1/1 v/v). After the mixture was stirred at RT for 2 h, it was concentrated to dryness *in vacuo*. Purification by preparative HPLC (C18ec-column; TFA buffer) afforded 29 mg (88%) of the trifluoroacetate salt of **13** as a colourless solid.

δ_H (500.25 MHz; CD_3OD) 8.91 (1H, s, N_2CH_{His}), 7.54 (1H, s, CH_{His}), 7.27 (4H, m, 4 × ArCH_{Phe}), 7.21 (1H, m, ArCH_{Phe}), 7.10 (2H, pseudo-d, 2 × ArCH_{Tyr}), 6.76 (2H, pseudo-d, 2 × ArCH_{Tyr}), 4.53 (1H, m, $C_\alpha H$), 4.45 (1H, m, $C_\alpha H$), 4.21 (3H, m, $C_\alpha H$ + Nε-CH_2), 4.08 (1H, m, $C_\alpha H$), 3.93 (2H, m, CH_2-Gly), 3.85 (3H, s, OCH_3), 3.79 (2H, m, CH_2-Gly), 3.15 (4H, overlapping m, $C\beta H_2$ + NH-CH_2), 2.99 (2H, m, $C\beta H_2$), 2.07 (2H, m, N_ε-CH_2-CH_2), 1.67 (1H, m, $C_\gamma H_{Leu}$), 1.58 (2H, m, $C_\beta H_2$), 0.91 (3H, d, *J* 6.2, Leu-CH_3), 0.89 (3H, d, *J* 6.2, Leu-CH_3); δ_C (125.8 MHz; CD_3OD) 175.3, 174.3, 172.5, 172.1, 170.9, 169.6 (6 × C=O), 163.0 (q, $J_{C,F}$ 35, CF_3COOH), 158.5, 138.3, 137.5, 131.8, 130.4, 129.3, 129.8, 128.2, 126.2, 122.7, 117.0 (All Ar*C*), 57.4, 56.3, 54.3, 54.3, 53.2, 47.8, 43.8, 43.7, 41.2, 38.7, 37.9, 36.5, 30.8, 27.9, 26.9, 24.2, 21.9; *m*/*z* (FAB$^+$; NBA) = 764.4128 ([M + H]$^+$, $C_{38}H_{54}N_9O_8$ requires764.4095).

Vitamin B12-*b*-acid; cyanocobalamin *b*-monocarboxylic acid (14)

Vitamin B12 (1.88 g, 1.39 mmol) was hydrolyzed in HCl 0.1 M (190 ml) as described in the literature.[25–27] The purification was modified in the following way: the Dowex column allowed, after desalting by phenol extractions, the isolation of three fractions, one containing exclusively *d*-acid, a second one consisting of a mixture of *b*-acid and *d*-acid, and a third fraction consisting of a mixture of *b*-acid and *e*-acid. The mixture of *b*-acid and *d*-acid was separated by preparative HPLC (column: Waters XTerra Prep RP8, 5 μm, 30 × 100 mm; buffer a: acetate buffer; gradient: 0.5% min^{-1} starting from 100% buffer a). The mixture of *b*-acid and *e*-acid was separated analogously but using the Tris buffer as buffer a. Cyanocobalamin-*b*-acid was isolated in a yield of 280 mg (15%).

δ_C (125.80 MHz; CD_3OD): 181.8, 180.2, 177.6, 176.8, 176.7, 175.7, 175.6, 175.5, 174.7, 174.4, 174.3, 167.2, 166.9, 143.5, 138.3, 135.7, 134.0, 131.5, 118.3, 112.6, 108.8, 105.2, 95.7, 88.0, 86.5, 83.7 (d, $J_{C,P}$ 5.7), 76.4, 75.5, 73.7 (d, $J_{C,P}$ 5.8), 62.8, 62.1, 61.1, 60.5, 57.6, 56.9, 55.1, 52.6, 49.9, 46.8, 43.9, 43.3, 40.2, 35.6, 35.4, 33.5, 33.1, 33.0, 32.5, 32.4, 29.7, 27.5, 27.1, 21.0, 20.6, 20.4, 20.3 (d, $J_{C,P}$ 2.7), 20.0, 17.7, 17.2, 16.6, 16.2. δ_P (202.5 MHz; CD_3OD)1.24.

Vitamin B12-*b*-acid conjugate 15

Compound **7** (44 mg; 0.07 mmol) was dissolved in DMF (1.5 ml) and Et_2N (1.5 ml). The mixture was stirred for 1 h at RT. Subsequently the mixture was evaporated to dryness *in vacuo*. In another flask, **14** (20.0 mg, 14.8 μmol) was dissolved in 4.5 ml of a DMSO/DMF (1/5 v/v) mixture. This mixture was transferred to the flask containing the deprotected histidine derivative. Subsequently NEt_3 (1.0 ml) and TBTU (32.1 mg; 0.1 mmol) were added. The mixture was stirred for 45 min at RT, after which period it was concentrated to dryness *in vacuo*. Purification by preparative HPLC (C8-column; acetate buffer; gradient: 2.0% per min, starting from buffer a) afforded 16 mg (67%) of a red solid.

δ_C (125.80 MHz; CD_3OD) 181.7, 180.3, 177.8, 177.7, 176.8, 175.8, 175.7, 175.5, 174.8, 174.8, 174.4, 174.2, 167.3, 167.1, 158.8, 143.7, 138.6, 138.5, 138.4, 135.8, 133.9, 131.7, 118.6, 118.0, 116.9, 112.7, 108.9, 105.3, 95.8, 88.1, 86.6, 83.9 (d, $J_{C,P}$ 5.9), 76.5, 75.7, 73.7 (d, $J_{C,P}$ 5.9), 70.9, 64.4, 62.9, 60.5, 57.7, 57.2, 55.8, 55.3, 52.9, 52.7, 50.0, 48.6, 47.0, 45.7, 44.1, 43.1, 40.3, 37.7, 36.7, 35.5, 33.6, 33.2, 33.1, 32.5, 32.5, 32.1, 31.4, 29.8, 27.6, 27.5, 21.1, 20.7, 20.5, 20.5, 20.4 (d, $J_{C,P}$ 2.6), 18.8, 17.7, 17.3, 16.6, 16.3, −1.3 (Si-(CH_3)$_3$); δ_P (202.5 MHz; CD_3OD)1.24; *m*/*z* (MeOH; ESI-pos.): 1710.4 ([M + H]$^+$, $C_{79}H_{115}CoN_{17}O_{18}PSi$ requires 1709.9), 855.0 [M + 2H]$^{2+}$, 866.7 [M + Na + H]$^{2+}$.

Vitamin B12-*b*-acid conjugate 16

To a suspension of **15** (19 mg; 11.1 μmol) in CH_2Cl_2 (4 ml) at 0 °C under an atmosphere of N_2 was added CF_3COOH (1 ml), resulting in a clear red solution. The mixture was stirred for 4 h at 0 °C followed by evaporation of the solvent *in vacuo*. Purification by preparative HPLC (RP8 30 × 100 mm column; acetate buffer; gradient: 1.0% per min, starting from buffer a), yielded 11 mg (64%) of a red solid.

δ_C (CD_3OD; 125.8 MHz): 181.7, 180.3, 177.7, 177.7, 176.7, 175.7, 175.6, 175.5, 175.5, 174.8, 174.8, 174.3, 167.3, 167.0, 143.6, 138.8, 138.4, 138.2, 135.7, 133.9, 131.6, 118.9, 118.0, 112.7, 108.8, 105.3, 95.8, 88.1, 86.5, 83.8 (d, $J_{C,P}$ 5.6), 76.5, 75.6, 73.7 (d, $J_{C,P}$ 6.1), 70.9, 62.8, 60.5, 57.6, 57.1, 55.3, 55.2, 52.8, 52.7, 50.0, 46.9, 45.7, 44.0, 43.1, 40.3, 37.7, 36.7, 35.5, 33.6, 33.6, 33.1, 32.5, 32.5, 32.0, 29.8, 27.5, 27.5, 21.1, 20.7, 20.5, 20.3, 20.1, 17.7, 17.2, 16.5, 16.2; δ_P (202.5 MHz; CD_3OD)1.24; *m*/*z* (ESI-pos.; MeOH): 1565.2 ([M + H]$^+$, $C_{73}H_{104}CoN_{17}O_{16}P$ requires 1565.6), 1587.2 [M + Na]$^+$, 783.4 [M + 2H]$^{2+}$, 794.1 [M + Na + H]$^{2+}$.

Re complex 17

To a solution of Re(His)$(CO)_3$ (178 mg; 0.4 mmol) and Boc-NH-$CH_2CH_2CH_2Br$ (100 mg; 0.4 mmol) in DMF (4 ml) was added Cs_2CO_3 (140 mg; 0.4 mmol). The resulting suspension was heated at 40 °C for 16 h. After the solvent was removed *in vacuo*, the residue was taken up in CH_2Cl_2 (25 ml) and H_2O (20 ml). The phases were separated and the aqueous solution was extracted with CH_2Cl_2 (25 ml). The CH_2Cl_2 were combined and washed with brine (2 × 20 ml) and dried ($MgSO_4$). Yield: 230 mg (97%) of a colourless solid.

Found: C, 35.4; H, 3.9; N, 9.8. Calcd. for $C_{17}H_{23}N_4O_7Re$: C, 35.1; H, 4.0; N, 9.6%; ν_{max} (KBr)/cm^{-1} 3436m NH, 2020s, 1894s, CO; δ_C (500.25 MHz; CD_3CN): 7.94 (1H, s, N_2CH_{His}), 6.92 (1H, s, CH_{His}), 5.45 (1H, br, N*H*-Boc), 4.61 (1H, m, $C_\alpha H$) 3.96 (2H, t, *J* 7.0, N_ε-CH_2), 3.88 (1H, m, Re-N*H*), 3.83 (1H, m, Re-N*H*), 3.20 (1H, m, $C_\beta H$), 2.99 (3H, m, $C_\beta H$ + Boc-NH-CH_2), 1.87 (2H, m, CH_2-CH_2-CH_2), 1.40 (9H, s, $OC(CH_3)_3$). δ_C (75.47 MHz; CD_3CN): 199.5 (CO), 197.9 (2 × CO), 182.8 (His-CO_2), 163.8 (Boc-*C*O), 142.5, 135.8, 119.7 (ArC_{His}), 79.5 (C_q-Boc), 52.6, 46.2, 38.0, 31.7, 28.8 (3 × CH_2 + Cα + Cβ) 28.6 (Boc-CH_3); *m*/*z* (ESI-pos.; MeOH) 483 [M − Boc + 2H]$^+$, 527 [M − C_4H_9 + 2H]$^+$, 582 [M + H]$^+$, 605 [M + Na]$^+$, 1165 [2M + H]$^+$, 1187 [2M + Na]$^+$.

Biotin conjugate 18

To a solution of complex **17** (98 mg; 0.17 mmol) in CH_2Cl_2 (4 ml) at 0 °C was added TFA (2 ml). The mixture was stirred for 1 h at 0 °C, followed by removal of the solvent *in vacuo*. In a separate flask D-(+)-biotin (41 mg; 0.17 mmol) was dissolved in a 4/1 (v/v) DMF/NEt_2 solution. This mixture was added to the Re complex and TBTU (55 mg; 0.17 mmol) was added. After it had been stirred at RT for 45 min, the mixture was evaporated to dryness *in vacuo*. The sticky residue was washed with EtOAc (2 × 50 ml) and H_2O (2 × 10 ml to remove some side-products. The residue was dissolved in a minimum amount of MeOH and subsequently purified by column chromatography (EtOH). Yield: 47 mg (40%) of a white colourless solid.

ν_{max} (KBr)/cm^{-1} 3309w NH, 2019vs CO, 1884vs br, CO, 1687s, 1636s, C=O; δ_H (500.25 MHz; CD_3OD) 8.09 (1H, s, N_2CH_{His}), 6.92 (1H, s, CH_{His}), 5.73 (1H, m, Re-N*H*), 5.09 (1H, Re-N*H*), 4.48 (1H, m), 4.29 (1H, m), 4.02 (3H, overlapping m, $C_\alpha H$ + N_ε-CH_2), 3.17 (5H, overlapping m, $C\beta H_2$ + C*H*-biotin + CH_2-biotin), 2.90 (1H, dd, *J* 5.0, ^2J 12.8, S-C*H*), 2.71 (1H, d, *J* 12.8 Hz, S-C*H*), 2.20 (2H, *J* 7.3 Hz, biotin-CH_2), 1.94 (2H, m, Nε-CH_2-CH_2),

1.62 (4H, overlapping m, 2 × CH_2), 1.42 (2H, m, biotin CH_2); δ_C (125.80 MHz; CD_3OD): 198.9, 197.7, 197.4 (3 × CO), 185.3, 176.5, 166.3 (3 × C=O), 142.9, 136.1, 120.1 (ArC_{His}), 63.5, 61.8, 57.2, 53.5, 46.7, 41.2, 37.4, 36.9, 31.8, 29.9, 29.6, 29.0, 26.9; *m*/*z* (FAB^+; NBA): 707.1434 (M^+, $C_{22}H_{30}N_6O_7{}^{185}ReS$ requires 707.1429), 709.1522 (M^+, $C_{22}H_{30}N_6O_7{}^{187}ReS$ requires 709.1454).

Vitamin B12-*b*-acid conjugate 19

To a solution of complex **17** (40 mg; 0.07 mmol) in CH_2Cl_2 (2 ml) at 0 °C was added TFA (1 ml). The mixture was stirred for 1 h at 0 °C, followed by removal of the volatiles *in vacuo*. In a different flask **14** (20.0 mg, 14.8 μmol), was dissolved in DMSO (0.8 ml). Subsequently were added DMF (2 ml) and NEt_3 (0.1 ml). The mixture was transferred to the flask containing the Re complex and TBTU (32.1 mg; 0.1 mmol) was added. After stirring at RT for 45 min, the solvent was removed *in vacuo*. The residue was purified by preparative HPLC (acetate system, gradient: 2.0% min^{-1} starting from 100% buffer a) to afford 7 mg (26%) of **19**.

ν_{max} (KBr)/cm^{-1} 2020s, 1901vs br CO, 1664vs br C=O.

δ_C (125.8 MHz; CD_3OD) 199.0, 197.6, 197.3 (3 × CO), 185.3, 181.7, 180.3, 177.7, 176.8, 175.7, 175.7, 175.1, 174.9, 174.8, 174.4, 167.3, 167.1, 143.6, 143.1, 138.4, 136.2, 135.8, 133,9, 131.6, 120.1, 118.0, 112.7, 108.9, 105.3, 95.8, 88.1, 86.6, 83.9, 76.5, 75.7, 73.8, 70.9, 62.8, 60.5, 83.8, 76.5, 75.7, 73.8, 57.6, 57.1, 55.2, 53.4, 53.3, 52.7, 50.0, 46.7, 46.6, 44,1, 43.2, 40.3, 37.5, 36.6, 35.5, 33.6, 33.1, 32.5, 29.8, 28.9, 27.6, 27.4, 21.1, 20.6, 20.5, 20.3, 20.1, 17.7, 17.2, 16.5, 16.2; δ_P (202.5 MHz; CD_3OD): 1.25; *m*/*z* (ESI-pos.; MeOH): 911.6 $[M + 2H]^{2+}$, 923.2 $[M + H + Na]^{2+}$, 933.9 $[M + 2Na]^{2+}$, 1822.1 ($[M + H]^+$, $C_{75}H_{101}CoN_{17}O_{19}PRe$ requires 1820.6), 1845.6 $[M + Na]^+$.

X-Ray crystal structure determination of ^{99}Tc(His-N_ε-benzyl)$(CO)_3 \cdot H_2O$

Crystal data. $C_{16}H_{16}N_3O_6Tc$, $M = 957.12$, orthorhombic, $a = 9.0094(5)$, $b = 13.0282(7)$, $c = 15.5661(12)$ Å, $V = 1827.1(2)$ $Å^3$, $T = 183(2)$ K, space group $P2_12_12_1$, $Z = 4$, μ(Mo-Kα) = 0.825 mm^{-1}, 28804 reflections measured, 4425 unique ($R_{int} = 0.0502$) which were used in all calculations. The final $R1$ was 0.0501 and the final $wR(F^2)$ was 0.0915 (all data). The Flack parameter was −0.03(5), in good agreement with the known stereochemistry (L) of the histidine.

CCDC reference number 236282. See http://www.rsc.org/suppdata/ob/b4/b405575f/ for crystallographic data in .cif or other electronic format.

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