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Syntheses of bifunctional 2,3-diamino propionic acid-based chelators as small and strong tripod ligands for the labelling of biomolecules with ^{99m}Tc†

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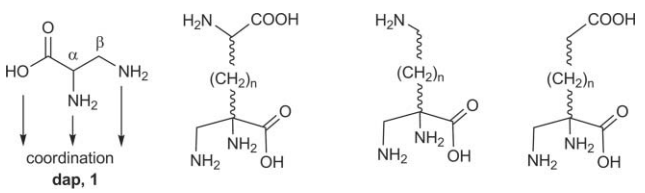
The labelling of targeting biomolecules requires small and hydrophilic complexes in order to not affect the binding properties of the vectors. 2,3-Diamino propionic acid (dap) is a small and strong, albeit scarcely used, tripod ligand for the *fac*-[^{99m}Tc(CO)$_3$]$^+$ moiety. We have introduced at the α-carbon atom in the basic dap structure various second functionalities such as carboxylato, amino and α-amino acid groups *via* various spacers in order to yield bifunctional chelators. These dap derivatives can be coupled to targeting molecules for application in molecular imaging. Full characterizations of the bifunctional chelators, X-ray structures of intermediates and of one rhenium complex, as well as labelling studies with ^{99m}Tc, are presented.

Introduction

Molecular imaging has become a major diagnostic tool in medicine which relies on labelled compounds targeting specific biological events on a molecular level.[1] Gene expression, receptors up-regulation, messenger molecules or enzymes such as tyrosine kinase are prominent molecular examples in current research.[2–4] Chemists and biologists aim to find and explore labelled molecules for imaging with highest specificity and sensitivity. Among other examples of imaging modalities are Magnetic Resonance Imaging (MRI), Single Photon Emission Computer Tomography (SPECT) or Positron Emission Tomography (PET) and combined methods. Probably the most developed example is the molecular targeting of hexokinase with ^{18}FDG visualizing increased cell proliferation related to oncology.[5] Active transport of ^{18}FDG into cells with high glucose (energy) demand and intracellular trapping results in substantial signal amplification for imaging. All molecular imaging modalities finally rely on affinity ligands which confer molecular or cellular specificity for the target of interest.

A basic chemical challenge to targeting vectors for imaging is the combination of the signaling compound with the affinity ligand since the latter should not interfere with the former. The combination of a signaling PET or SPECT metal with a targeting vector is particularly challenging since metals must be stably bound to chelators. Numerous examples of successful compounds have been described but few have entered clinical application so far.[6–11] The radionuclide ^{99m}Tc is a very favorable radionuclide but combination with targeting molecules is not routine. Labelling approaches are based on only a few fragments, namely [Tc=O]$^{3+}$, [Tc≡N]$^{2+}$ and *fac*-[Tc(CO)$_3$]$^+$. Our ongoing attempts to optimize ligands for the *fac*-[Tc(CO)$_3$]$^+$ core with respect to size, hydrophilicity and labelling efficiency brought 2,3-diamino propionic acid **1** (dap) into our interest. Compound **1** (Scheme 1) is ubiquitous in the natural world and has been found in the *Bombyx* larva[12] on earth as well as on the *Murchison* meteorite from the sky.[13] Consequently, **1** and its derivatives have stimulated both chemists and biochemists through the years to find the biological significance of its unique structural roles from chemistry to peptide synthesis[14] and the origin of life.[15] From an inorganic point of view, **1** is a classical tripod ligand of very low molecular weight; still, its coordination chemistry has not been explored in great detail. In the context of radiolabelling, our group has demonstrated that **1** is an efficient tripod ligand for the *fac*-[Tc(CO)$_3$]$^+$ moiety.[10,16] The resulting complex *fac*-[Tc(**1**)(CO)$_3$] is small, forms at low concentration and is highly hydrophilic. We have also shown that **1** conjugated by the α-C to a spacer and an α-amino acid group was recognized and transported by the L-type amino acid transporter LAT1 (Scheme 1).[10]



Scheme 1 Basic building blocks for bifunctional chelators containing the **dap** ligand (**1**).

Due to the very favorable properties of the dap ligand, it would be desirable to derivatize it at the α-carbon for coupling to different targeting biomolecules with specific receptor binding properties for going beyond perfusion agents.[17,18] We present in this synthetic and labelling study the preparation of α-carbon derivatives of **1**, carrying pendant amino acid groups for integration as artificial amino acids into peptide chains, and with a simple carboxylate or

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† Electronic supplementary information (ESI) available: ^{13}C NMR data for all new compounds, labelling yields for **20** and **25**, and crystallographic data for **4**. CCDC reference numbers 761460 (**4**), 761459 (**5**), 761461(**6**), 751390 (**19**), 751391 (**26**) and 761462 (**28**). For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c002796k

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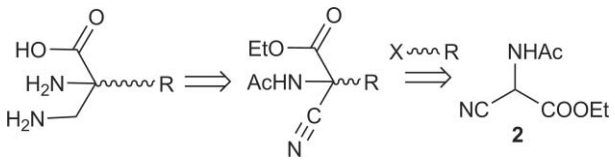
amine group for coupling to the corresponding functionalities in other biomolecules (Scheme 1). The unique tripod structure of dap and the properties of its ^{99m}Tc complexes renders it a basic building block in future development of vectors for molecular imaging.

Results and discussion

The syntheses of the building blocks shown in Scheme 1 requires α-carbon derivatization. To be general, the synthetic approach should be flexible with respect to the spacer between the two functions. A retro-synthetic analysis revealed that derivatization at the α-carbon of the latter dap ligand is best achieved by starting from commercially available *N*-acetyl-3-nitriloalanine (**2**), a useful starting material for α-amino acids (Scheme 2). Alkylation of **2** with *e.g.* diethyl 2-acetamido-2-(4-bromobutyl)malonate (**4**), prepared by alkylation of diethyl malonate with 1,4-dibromobutane[19] will ultimately lead to dap-based compounds (Scheme 3). The key step in the retro-synthetic analysis is the functional group interconversion step, involving the reduction of the nitrile (–CN) to the amine ($–CH_2NH_2$). While there are many catalysts for –CN to $–CH_2NH_2$ conversion, it was important to find a selective one for this transfer in the presence of other functional groups. It should be emphasized that the retro-synthetic analysis as presented in Scheme 2 will not be stereospecific at the α-carbon. After labeling, two diastereomeric complexes will be received. However, since the label is distant and does not interact with the target, enantiopure metal complexes are not crucial at this point.



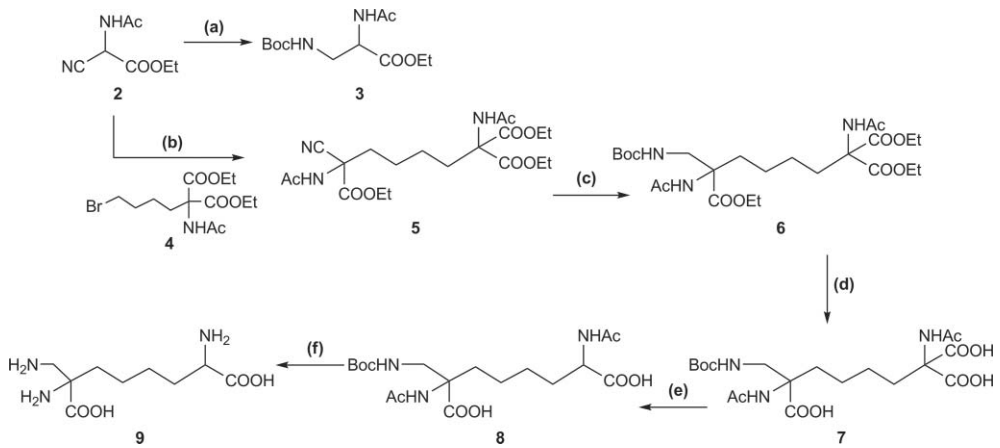
Scheme 2 Retrosynthetic analysis of the target compound with $(CH_2)_4$ spacer (protection groups are omitted for clarity during analysis).

Syntheses

The crucial step in the preparation of the bifunctional chelators is the conjugation of the latter tripod building block **2** to the 2nd von hochgesellt function, the conjugation group to the targeting molecule or an amino acid. The mild reducing agent $NaBH_4$ does not reduce –CN groups alone at ambient temperature. However, it has been reported that a combination of $NaBH_4$ and $NiCl_2$,[20] $CoCl_2$[21] or I_2[22] does reduce –CN to $–CH_2NH_2$ efficiently. The respective metal cations coordinate to nitriles, thereby activating them for reduction. This method was applied to **2** alone to test if simple dap can be received. The combination of $NaBH_4$ and $NiCl_2$ catalytically reduced the –CN group in **2** and *in situ* protection with Boc_2O gave the protected dap derivative **3** in 80% yield (Scheme 3). It is interesting to note that both the –NHAc and –COOEt did not interfere with the reduction and no *trans*-esterification or reduction was observed.

Thus, the reductive conversion of **2** to **3** provides a straightforward and convenient method for the synthesis of protected dap. Some syntheses of dap as reported are multi-step or use rather expensive reagents/catalyst.[23] The Hoffman rearrangement of asparagines, for example, is probably the most convenient approach to dap reported so far, requires costly and not routine trivalent iodine from bis(trifluoroacetoxy)iodobenzene.[24] The conjugation of an α-amino acid *via* an alkyl spacer of various lengths to **2** was performed as shown in Scheme 3. Compound **2** was deprotonated with NaOEt and then alkylated with **4**, a typical precursor in the *Sorensen* method of amino acid synthesis, to give **5** in 79% yield. The same –CN to $–CH_2NH_2$ reduction as before was employed for compound **5**, and **6** was isolated in 73% yield. Despite some steric crowd around the –CN group, the smooth conversion of **5**→**6** demonstrated that the alkylation of **2** had little effect on its reduction. This implied that the procedures illustrated in Scheme 3 provide a general and efficient way for the synthesis of dap derivatives functionalized at the α-carbon position. The same syntheses in comparable yields were applied for pentyl and hexyl spacers, underscoring the general synthetic principle. The X-ray structures of **5** and **6** could be elucidated and ORTEP presentations are given in Fig. 1.

Both **5** and **6** crystallized in racemic form in the space groups *Pbca* and $P2_1/n$, respectively. Data collection and structure solution can be found in Table 1. The bond length 1.138(2) Å of C(10)–N(3) in **5** is typical for a triple bond. The two AcNH– groups in compound **5** are not equal. The bond length of C(19)–N(2)



Scheme 3 (a) 1) MeOH, $NaBH_4$, $NiCl_2$, Boc_2O, r.t. 2) diethylenetriamine, r.t., 80.0%; (b) EtOH, NaOEt, diethyl 2-acetamido-2-(4-bromobutyl)malonate (**4**), reflux, 79.0%; (c) 1) MeOH, $NaBH_4$, $NiCl_2$, Boc_2O, r.t. 2) diethylenetriamine, r.t., 73.0%; (d–f) *in situ*, 1 M NaOH 80 °C, conc. HCl, 8 h (35%).

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Table 1 Crystallographic data for **5**, **6**, **19**, **26** and **28**

Compound	**5**	**6**	**19**	**26**	**28**
Empirical formula	$C_{20}H_{31}N_3O_8$	$C_{25}H_{43}N_3O_{10}$	$C_{13}H_{25}N_3O_5$	$C_7H_{13}ClN_2O_3$	$C_{10}H_{13}N_2O_7Re$
Formula weight	441.48 g mol^{-1}	545.62 g mol^{-1}	303.36 g mol^{-1}	208.64 g mol^{-1}	459.42 g mol^{-1}
Diffractometer	IPDS Stoe	IPDS 2T Stoe	Bruker-AXS APEX	Bruker-AXS APEX	Oxford Diffraction Xcalibur Ruby
Wavelength	0.7107 Å	1.5418 Å	0.7107 Å	0.7107 Å	0.7107 Å
Crystal system	Orthorhombic	Monoclinic	Orthorhombic	Triclinic	Triclinic
Space group	*Pbca*	$P2_1/n$	$Pca2_1$	$P\bar{1}$	$P\bar{1}$
Unit cell dimensions	$a = 17.2196(7)$ Å $b = 27.2811(13)$ Å $c = 10.2859(4)$ Å	$a = 8.8810(11)$ Å $b = 20.9682(19)$ Å $c = 16.241(2)$ Å $\beta = 101.327(10)°$	$a = 10.1904(8)$ Å $b = 17.6150(12)$ Å $c = 9.4373(5)$ Å	$a = 6.4348(2)$ Å $b = 8.0509(2)$ Å $c = 9.6331(3)$ Å $\alpha = 74.2580(10)°$ $\beta = 101.327(10)°$ $\gamma = 78.232(2)°$	$a = 6.7581(10)$ Å $b = 8.7934(2)$ Å $c = 11.4890(2)$ Å $\alpha = 86.1958(16)°$ $\beta = 79.7708(16)°$ $\gamma = 83.8740(17)°$
Volume	4832.0(4) Å^3	2965.5(6) Å^3	1694.0(2) Å^3	469.81(2) Å^3	667.28(2) Å^3
Z	8	4	4	2	2
Density (calculated)	1.214 Mg m^{-3}	1.222 Mg m^{-3}	1.189 Mg m^{-3}	1.475 Mg m^{-3}	2.287 Mg m^{-3}
Absorption coefficient	0.094 mm^{-1}	0.787 mm^{-1}	0.091 mm^{-1}	0.385 mm^{-1}	0.913 mm^{-1}
F(000)	1888	1176	656	220	436
Crystal size	0.57 × 0.21 × 0.20 mm^3	0.2 × 0.18 × 0.08 mm^3	0.25 × 0.10 × 0.06 mm^3	0.50 × 0.10 × 0.04 mm^3	0.2 × 0.2 × 0.1 mm^3
θ range	2.42–27.10°	3.49–58.99°	3.16–25.02°	2.99–25.68°	2.88–27.99°
Index ranges	$-22 \le h \le 22, -34 \le k \le 34, -13 \le l \le 13$	$-9 \le h \le 9, -21 \le k \le 23, -16 \le l \le 16$	$-12 \le h \le 8, -15 \le k \le 19, -11 \le l \le 11$	$-7 \le h \le 7, -9 \le k \le 9, -11 \le l \le 11$	$-8 \le h \le 8, -11 \le k \le 11, -15 \le l \le 15$
Reflections collected	43838	30674	4537	3354	16418
Independent reflections	5312 [R(int) = 0.0577]	4152 [R(int) = 0.0957]	2321 [R(int) = 0.0545]	1781 [R(int) = 0.0171]	3211 [R(int) = 0.0385]
Completeness to θ	99.7% (27.10°)	97.4% (58.99°)	96.1% (25.02°)	99.7% (25.68°)	99.9% (27.99°)
Max. and min. transmission (rel. for **28**)	0.9843 and 0.9618	0.9372 and 0.8557	0.9945 and 0.9775	0.9848 and 0.8310	1.0000 and 0.2585
Data/restraints/parameters	5312/0/304	4152/1/362	2321/1/215	1781/0/134	3211/0/182
Goodness-of-fit on F2	1.102	1.107	0.985	1.057	1.064
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0604$, $wR_2 = 0.1629$	$R_1 = 0.0660$, $wR_2 = 0.1751$	$R_1 = 0.0552$, $wR_2 = 0.0931$	$R_1 = 0.0282$, $wR_2 = 0.0708$	$R_1 = 0.0177$, $wR_2 = 0.0469$
R indices (all data)	$R_1 = 0.0720$, $wR_2 = 0.1686$	$R_1 = 0.0984$, $wR_2 = 0.2198$	$R_1 = 0.0870$, $wR_2 = 0.1011$	$R_1 = 0.0324$, $wR_2 = 0.0729$	$R_1 = 0.0192$, $wR_2 = 0.0474$
Extinction coefficient		0.0049(9)			
Largest diff. peak and hole	0.327 and –0.194 e Å^{-3}	0.305 and –0.292 e Å^{-3}	0.206 and –0.208 e Å^{-3}	0.305 and –0.177 e Å^{-3}	0.899 and –0.623 e Å^{-3}

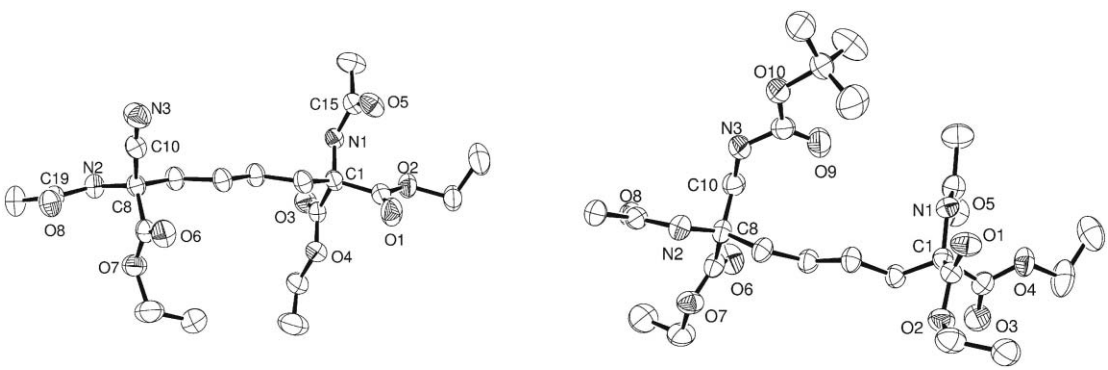

Fig. 1 ORTEP drawing of compounds **5** and **6** (H atoms and minor conformations of disordered ethyl (**5**) and methyl groups (**6**) were omitted for clarity) in 50% probability.

(1.355(2) Å) is significantly longer than C(15)–N(1) (1.338(2) Å). This bond length difference of 0.017 Å was also indirectly reflected by the difference of ^{1}H-NMR shift of AcNH– groups (δ 6.80 and 6.26).

In situ hydrolysis of the ethyl ester groups in **6** gave the tricarboxylic acid **7** and subsequent decarboxylation gave compound **8** which, after deprotection, afforded the final α-amino acid **9** conjugated to the dap ligand in D- and L-forms.

With respect to later incorporation of artificial amino acids in peptide syntheses or to use them as labelled amino acids, it is important to have the L-form only. This could be achieved by starting directly from amino acids such as lysine (Scheme 4). The ε-NH_2 group was oxidized to the –OH group (**10**) as reported[25] and activated with mesitylchloride to give compound **11**. Proceeding as described above finally gave compound **14** bearing an enantiomerically pure L-form amino acid.

Besides the direct conjugation of bioactive fragments such as amino acids to dap as described above, the bifunctional chelator (BFC) concept is very important in radiopharmaceutical chemistry. To prepare bifunctional chelators comprising the dap

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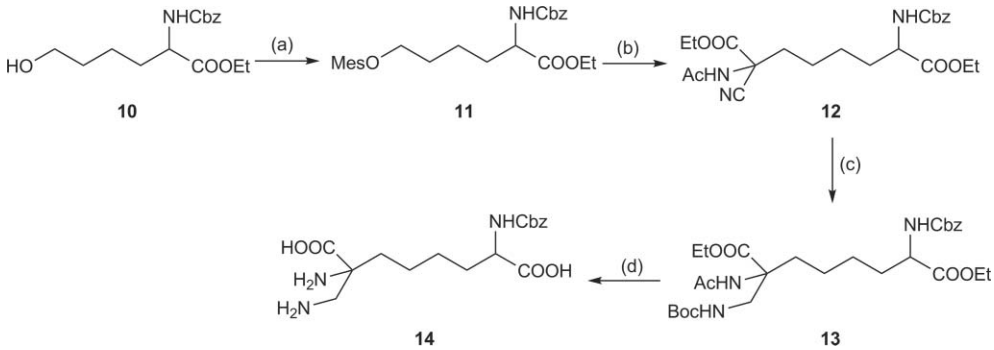
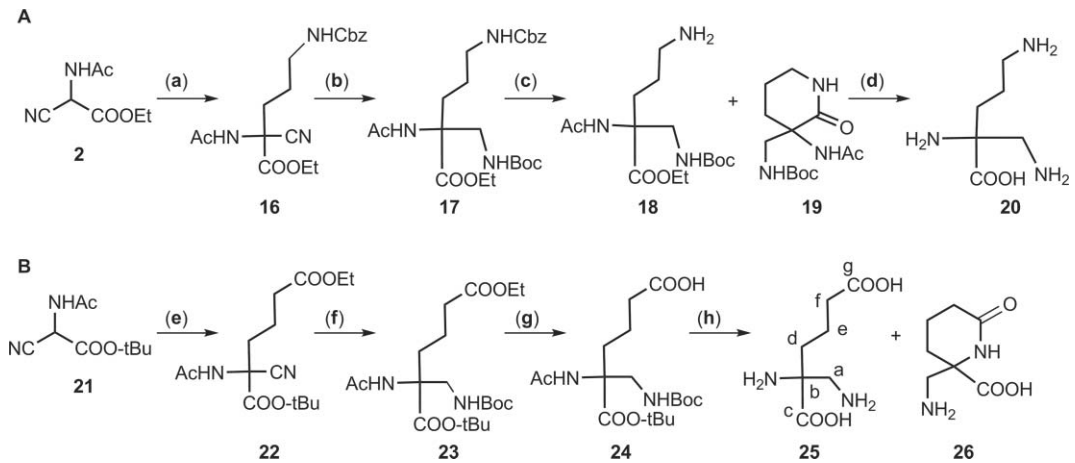


Scheme 4 (a) MesCl, Et_3N, CH_2Cl_2, −78 °C, 96%; (b) EtOH, NaOEt, **2**, reflux, (c) 1) MeOH, $NaBH_4$, $NiCl_2$, Boc_2O, r.t. 2) diethylenetriamine, r.t.; (d) 1 M NaOH 80 °C, then conc. HCl, 8 h.



Scheme 5 *Reagents and conditions*: **A**: (a) EtOH, NaOEt, benzyl-*N*-(3-bromopropyl)carbamate (**15**), reflux, 41.2%; (b) 1) MeOH, $NaBH_4$, $NiCl_2$, Boc_2O, r.t. 2) Diethylenetriamine, r.t., 40.3%; (c) EtOH, H_2, Pd/c, r.t., 89.9%; (d) HCl 4 M, reflux, 92.6%. **B**: (e) DMF, NaH, ethyl-4-bromobutyrate, reflux, 51.1%; (f) 1) MeOH, $NaBH_4$, $NiCl_2$, Boc_2O, r.t. 2) Diethylenetriamine, r.t., 97.0%; (g) MeOH/NaOH 2 M, r.t., 67.9%; (h) HCl 4 M, reflux, 52.0%. (*numbering scheme for NMR assignments is displayed for* ***25*** *as an example*).

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coordination motif together with a single functionality for conjugation to biomolecules of interest, we proceeded to dap-based compounds derivatised with a primary amine or a carboxylic acid. With the scope of subjecting these compounds to derivatization of biomolecules, the conjugating functions should be unprotected but the **dap** unit be protected. The synthetic strategy is similar as before and the reaction sequences are shown in Scheme 5.

The dap derivatives **20** and **25** have been prepared starting from **2** or *tert*-butyl acetamidocyanoacetate **21**, respectively. The precursor **21** was synthesized following a similar procedure as described for **2** but starting from *tert*-butyl cyanoacetate. Deprotonation of the α-C–H in **2** with NaOEt and subsequent reaction with benzyl-*N*-(3-bromopropyl)carbamate (**15**), prepared by bromination of benzyl-*N*-(3-hydroxypropyl)carbamate with CBr_4 and PPh_3, gave **16** in moderate yield (Path **A** in Scheme 5). The reduction of the –CN group in **16** and *in situ* protection with Boc_2O led to **17**. Deprotection of the Cbz group in **17** to give **18** was accomplished almost quantitatively by catalytic hydrogenation, using an optimized amount of catalyst (Pd/C, Pd content 10%). When a large excess of Pd/C catalyst was used, compound **18** was always obtained in a lower yield (51.4%) due to the formation of a side product, which was formulated as **19** (18.7% yield) based on multinuclear NMR spectroscopy, ESI-MS and X-ray diffraction analysis (see experimental section and Fig. 3). The formation of **19** was due to *in situ* cyclisation from the terminal amine with the ethyl ester. The goal compound **20** finally was obtained in good yield by full deprotection of **18** under acidic conditions. The preparation of **22** by alkylation of **21** with ethyl-4-bromobutyrate in the presence of NaOEt/EtOH was unsuccessful due to a *trans*-esterification reaction (path **B** in Scheme 5). Compound **22** could only be obtained using NaH as a base in DMF. Compound **23** was obtained in almost quantitative yield starting with **22** and using the same conditions as described for **17**. Sequential removal of the ethyl ester and protecting groups of the **dap** unit in **23** gave **24**, and then the desired product **25**, respectively. As for **18**, the product of this reaction was always a mixture of the goal compound **25** and a second species formulated as a lactam (**26**). Compounds **25** and **26** were obtained in 60 and 40% yield, respectively, based on ^{1}H-NMR data. All the attempts to minimize the formation of the undesired lactam **26** remained unsuccessful. At higher pH, **25** even converted almost quantitatively to **26**. The identification of **25** and **26** in the reaction mixture was based on NMR. In fact, 1D and 2D ^{1}H- and ^{13}C-NMR experiments at different pH values allowed a complete assignment of the resonances for each species (see experimental section). For illustration, Fig. 2 shows the ^{1}H-NMR spectra of a mixture of **25** and **26** immediately after BOC

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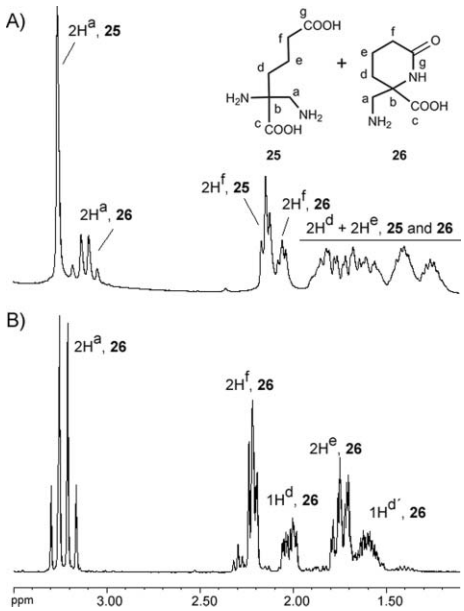


Fig. 2 ^{1}H-NMR spectra (D_2O) of the mixture **25** + **26** at pH 1.4 (**A**) and 4.3 (**B**).

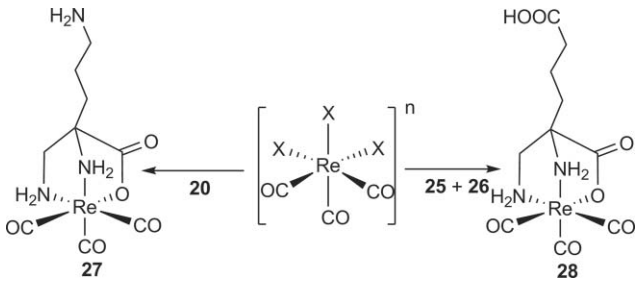
deprotection, at pH 1.4 (Fig. 2, **A**). The same mixture at pH 4.3 (Fig. 2, **B**) exhibits almost complete lactamisation of **25** → **26**.

The main differences in the ^{1}H-NMR spectra of **25** and **26** are related to the splitting and/or chemical shifts of H^a and H^d protons. In **25**, the $2H^a$ protons appear as a sharp singlet at δ 3.17, while in the lactam **26** these protons appear as a quartet centered at δ 3.03. The two $CH_2{}^d$ protons in **26** become diastereotopic due to the rigidity imposed by cyclisation. Accordingly, two resonances at δ 2.00 and δ 1.60 appeared, each integrating for one proton. The assumed structure of **26** could be confirmed by recrystallization of a CH_2Cl_2–EtOH solution containing mainly **26**. Single crystals suitable for X-ray diffraction analysis were obtained. Fig. 3 (right) shows an ORTEP diagram of **26**, confirming its lactam authenticity. The ESI-MS of the mixture **25** + **26** presented two main peaks at m/z values which agree with the expected values for **25** ($[M + H]^+$, 191.0) and **26** ($[M + H]^+$, 172.9).

Synthesis and characterization of the Re(I) complexes *fac*-[Re(k^3-L)(CO)$_3$] 27 and 28

In order to characterize the ^{99m}Tc complexes at very high dilution, it is common to prepare and fully characterize the macroscopic rhenium homologues and to compare their HPLC retention time with those of the corresponding ^{99m}Tc complex.

Reaction of **20** with the precursor *fac*-$[Re(H_2O)_3(CO)_3]^+$ in refluxing water afforded the rhenium complex *fac*-[Re(**20**)$(CO)_3$] (**27**) in 79% yield (Scheme 6). The analogue reaction of *fac*-$[ReBr_3(CO)_3]^{2-}$ with a mixture of **25** + **26** overnight at pH ~ 4 gave a white precipitate. After washing with water and warm CH_2Cl_2, the solid obtained was dried under vacuum. Based on multinuclear NMR and IR spectroscopy, and ESI-MS the complex was formulated as *fac*-[Re(**25**)$(CO)_3$] (**28**). X-ray structure analysis confirmed this composition (Fig. 4).



Scheme 6 Reaction pathways to Re(I) complexes with the tripod ligands **20** and **25**. X = Br, n = –2; X = H_2O, n = +1.

After precipitation of **28** from the reaction mixture, HPLC analysis of the solution still revealed small amounts of dissolved **28** (R_t = 9.9 min), unreacted *fac*-$[ReBr_3(CO)_3]^{2-}$ precursor (R_t = 6.5 min) and the lactam **26** (R_t = 5.5 min). To force the reaction to completion, the pH of the solution was increased to 8 and the mixture refluxed overnight. After this time, HPLC analysis of the solution indicated no residual *fac*-$[ReBr_3(CO)_3]^{2-}$, the presence of a small amount of **28** (R_t = 9.9 min), and the formation of a defined new species (R_t = 13.2 min), which could be isolated by chromatography. Based on multinuclear NMR and IR spectroscopy, and ESI-MS we tentatively formulated this complex as "*fac*-[Re(**26**)$(CO)_3$]".

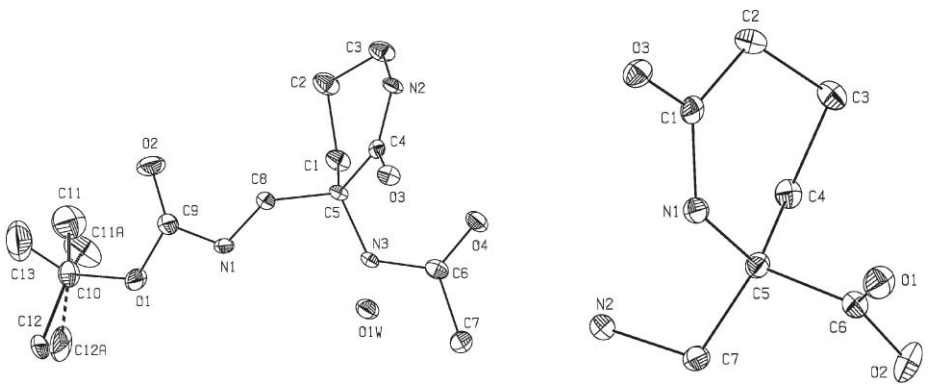


Fig. 3 ORTEP drawing of compounds **19** (left) and **26** (right) in 50% probability (H atoms were omitted for clarity). Selected bond lengths (Å) and angles (°): For **19**: C(5)–C(1), 1.528(4) Å; C(5)–C(4), 1.545(4) Å; C(5)–C(8), 1.550(5) Å; C(5)–N(3), 1.453(4) Å; C(1)–C(5)–C(4), 112.9(3)°; N(3)–C(5)–C(8), 107.5(3)°. For **26**: C(5)–N(1), 1.4656(19) Å; C(5)–C(4), 1.536(2) Å; C(5)–C(7), 1.544(2) Å; C(5)–C(6), 1.553(2) Å; N(1)–C(5)–C(4), 110.10(12)°; C(7)–C(5)–C(6), 107.83(12)°.

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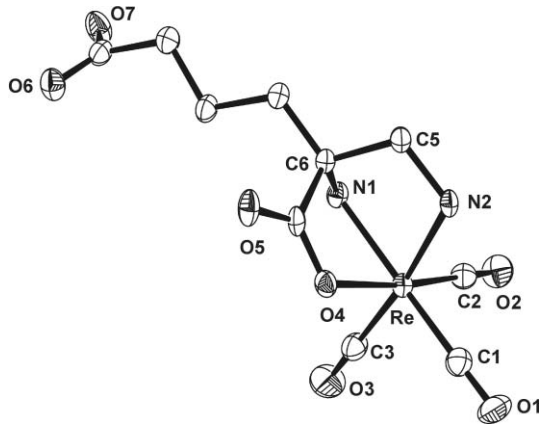

Fig. 4 ORTEP view of complex **28**; thermal ellipsoids are drawn at the 40% probability level. Selected bond lengths (Å) and angles (°): Re1–C1, 1.915(3) Å; Re1–C2, 1.906(3) Å; Re1–C3, 1.914(3) Å; Re1–N1, 2.218(2) Å; Re1–N2, 2.206(2) Å; Re1–O4: 2.154(2) Å; C1–Re1–N1, 168.48(10)°; C3–Re1–N2, 172.90(11)°; C2–Re1–N1, 100.11(12)°; C1–Re1–O4, 96.25(11)°; C3–Re1–C1, 89.67(14)°; C3–Re1–C2, 87.28(14)°; O4–Re1–N1, 74.32(8)°; N2–Re1–N1, 76.49(9)°; O4–Re1–N2, 77.96(8)°.

The infrared spectra of **27** and **28** showed strong CO stretching bands in the 2028–1906 cm^{-1} range, which is in coincidence with a *fac*-$[Re(CO)_3]^+$ motif for both complexes. The ESI-MS spectra showed dominant single peaks with the expected isotopic pattern and correct *m/z* values (**27**, $[M + H]^+ = 431.8$ *m/z*; **28**, $[M + Na]^+ = 482.9$ *m/z*). The ^{1}H-NMR spectra of **27** and **28** (D_2O) were very similar. The most interesting features are four broad multiplets in the range δ 5.32–4.31, integrating for 1 H each (assigned to the diastereotopic protons of the coordinated NH_2 groups), and two other resonances, integrating for 1 H each due to the CH_2[a] protons, which became diastereotopic upon coordination of the dap derivatives to the metal (**27**, δ 2.79 and 2.51; **28**, δ 2.76 and 2.59). Together with the ^{13}C-NMR spectra, these data are consistent with a tridentate coordination of the chelators in **27** and **28**. The structure was confirmed by an X-ray structure analysis of **28** (Fig. 4).

Labelling studies: syntheses of *fac*-[^{99m}Tc(20)(CO)$_3$] and *fac*-[^{99m}Tc(25)(CO)$_3$]

For the labelling of targeting biomolecules, quantitative labelling at the lowest possible concentrations is crucial. A high amount of unlabelled biomolecules entails purification which is not feasible in clinical routine. High hydrophilicity is desirable since it prevents accumulation of the radiosensor in non-targeted organs, such as the liver or the lungs. Our labelling studies focused on these two aspects to reveal the advantages of the dap ligand and its derivatives. The synthesis of the precursor complex $[^{99m}Tc(OH_2)_3(CO)_3]^+$ was done as previously reported.[26] The ^{99m}Tc(I)-complexes *fac*-[^{99m}Tc(**20**)(CO)$_3$] and *fac*-[^{99m}Tc(**25**)(CO)$_3$] were prepared in high radiochemical yield (>95%) by reaction of *fac*-$[^{99m}Tc(H_2O)_3(CO)_3]^+$ with **20** or a mixture of **25** + **26**, respectively. The reaction with **20** at 8×10^{-5} M and with **25** + **26** at 1×10^{-4} M was completed after 30 min at 100 °C and pH = 7.4. The lowest concentration at which quantitative labelling after ≈ 60 min was achieved was 10 μM. At lower temperature, higher concentrations are required. However, since this ligand is very useful for small hydrophilic biomolecules such as peptides, ligands with higher labelling rates might be more suitable for proteins. The radiochemical purity of the complexes has been determined by RP-HPLC analysis, and the chemical identity ascertained by comparing their HPLC traces with the corresponding HPLC traces of the Re congeners **27** and **28**. All complexes could be kept in solution for at least 18 h at 37 °C without any noticeable decomposition (RP-HPLC analysis). A table with the radiochemical labelling yields with **20** and **25** at 75 and at 100° C and at different concentrations is given in the supplementary information.†

It is worth mentioning that the reaction of a mixture of **25** + **26** with *fac*-$[^{99m}Tc(H_2O)_3(CO)_3]^+$ gave only *fac*-[^{99m}Tc(**25**)(CO)$_3$], most probably for kinetic reasons. Incubation of *fac*-[^{99m}Tc(**25**)(CO)$_3$] with a large excess (100×) of **26** (2 h at 100 °C) revealed its high stability since no *trans*-chelation could be observed. Interestingly, if the pure complex "*fac*-[^{99m}Tc(**26**)(CO)$_3$]" was incubated (2 h at 100 °C) with a large excess (100×) of pure unsubstituted **dap** amino acid, *trans*-chelation occurred with formation of "*fac*-[^{99m}Tc(**dap**)(CO)$_3$]" in *ca.* 70% radiochemical yield.

The complex *fac*-[^{99m}Tc(**20**)(CO)$_3$] is very hydrophilic and eluted in the standard gradient 0.1% CF_3COOH–MeOH with the same retention time as the precursor *fac*-$[^{99m}Tc(H_2O)_3(CO)_3]^+$. A different gradient was, thus, required in order to differentiate between the two complexes. This was achieved by replacing the 0.1% CF_3COOH aqueous solution with Et_3N–CH_3COOH [2.1 : 2.8 (v/v)] which might be of useful help in characterizing such hydrophilic radiocomplexes (Fig. 5).

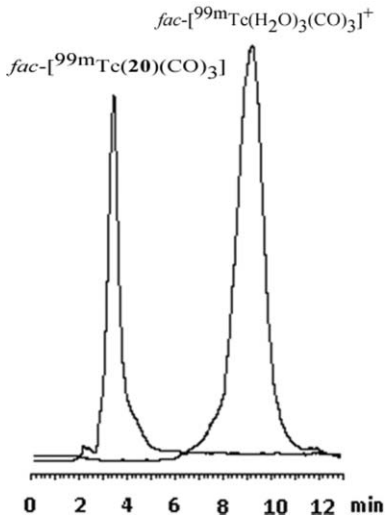

Fig. 5 RP-HPLC radioactive traces of *fac*-[^{99m}Tc(**20**)(CO)$_3$] and *fac*-$[^{99m}Tc(H_2O)_3(CO)_3]^+$ obtained using a gradient with Et_3N–CH_3COOH [2.1 : 2.8 (v/v)] and MeOH.

Conclusions

It has been shown that $NaBH_4/NiCl_2$ reagent system can be adopted as a good catalyst for the facile synthesis of dap-containing compounds starting from ethyl or *tert*-butyl acetamidocyanoacetate. The synthetic strategy reported herein provides an efficient method for the preparation of α-positioned dap derivatives which involves carbon–carbon bond formation. Therefore, it has been possible to prepare in a straightforward way an

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artificial amino acid (**9**) combining a pendant amino acid group and a dap chelating unit, which can be incorporated into peptide chains for (radio)metallation after introduction of orthogonal protecting groups in the amino acid and chelating moieties. It has also been possible to prepare new versatile bifunctional chelators for radiopharmaceutical applications comprising a dap coordinating unit and a pendant amine (**20**) or carboxylate (**25**) group for conjugation to relevant biomolecules. Such chelators react efficiently with the organometallic moiety "$M(CO)_3$" (M = Re, ^{99m}Tc), yielding well-defined complexes of the type *fac*-[M(k^3-L)$(CO)_3$] (L = **20** and **25**). In these complexes the dap unit acts as tridentate chelator through the *N*,*N*,*O* donor atom set, without any interference of the amine or carboxylate functional groups.

Experimental section

General

Chemicals and solvents of reagent grade were purchased from Aldrich and used without further purification. Ethyl-*N*-acetyl-3-nitriloalaninate (**2**), $(Et_4N)_2[ReBr_3(CO)_3]$ and $[Re(H_2O)_3(CO)_3]Br$ were prepared according to published methods.[27–29] All reactions were carried out under N_2. NMR spectra were recorded on Varian Mercury 200 MHz, Varian Gemini 300 MHz or Bruker 500 MHz instrument. ^{1}H and ^{13}C chemical shifts were referenced with the residual solvent resonances relative to TMS. The spectra were fully assigned with the help of 2D experiments (^{1}H–^{1}H correlation spectroscopy, gCOSY and ^{1}H–^{13}C heteronuclear single quantum coherence, HSQC). Assignments of the ^{1}H and ^{13}C NMR resonances are given in accordance with the identification system displayed for compounds **25** and **28**. Electron impact mass spectra were taken using Merck Hitachi M-8000 LCMS. HPLC analyses were performed on a Perkin Elmer LC pump 200 coupled to a Shimadzu SPD 10AV UV/Vis and to a Berthold-LB 509 radiometric detector, using an analytic Macherey-Nagel C18 reversed-phase column (Nucleosil 100-10, 250 × 4 mm) with a flow rate of 1 mL min^{-1}. Purification of the rhenium compounds was achieved on a semi-preparative Macherey-Nagel C18 reversed-phase column (Nucleosil 100-7, 250 × 8 mm) or on a preparative Waters μ Bondapak C18 (150 × 19 mm) with a flow rate of 2.0 and 5.5 mL min^{-1}, respectively. UV detection: 254 or 220 nm. Method 1—Eluent A was aqueous 0.1% CF_3COOH and eluent B was MeOH. For complex *fac*-[^{99m}Tc(**20**)$(CO)_3$], an alternative method was used: Method 2—Eluent A was aqueous Et_3N–CH_3COOH [2.1 : 2.8 (v/v)] solution and eluent B was MeOH. The HPLC gradient was the same for both methods: t = 0–3 min, 0% B; 3–3.1 min, 0→25% B; 3.1–9 min, 25% B; 9–9.1 min, 25→34% B; 9.1–20 min, 34→100% B; 20–25 min, 100% B; 25–25.1 min, 100→0% B; 25.1–30 min, 0% B.

Na[$^{99m}TcO_4$] was eluted from a ^{99}Mo/^{99m}Tc generator, using 0.9% saline. The radioactive precursor *fac*-[$^{99m}Tc(H_2O)_3(CO)_3]^+$ was prepared using a IsoLink® kit (Malinckrodt, Med B.V.).

Ethyl-2-acetamido-3-(*tert*-butoxycarbonylamine) propanoate 3

To a MeOH (10 mL) solution of **2** (0.170 g, 1 mmol) cooled with an ice bath, was added $(Boc)_2O$ (0.440 g, 2 mmol) and $NiCl_2{\cdot}6H_2O$ (25 mg, 0.1 mmol) to afford a green solution. To this solution was added $NaBH_4$ (0.230 g, 6 mmol) in small portions with stirring. The purple mixture was allowed to warm to 20 °C slowly and stirred overnight and then diethylenetriamine (0.2 mL) was added. The reaction was stirred for 1 h before the volatile part of the mixture was removed by vacuum. The residue was partitioned between EtOAc and saturated $NaHCO_3$ solution. The organic phase was dried with $MgSO_4$. Removal of organic solvent gave a colorless residue, which was purified by silica chromatography (hexane–EtOAc) to give a colourless oil of **3** (0.220 g, 80%). ^{1}H-NMR (200 MHz, $CDCl_3$, ppm) δ_H 6.71 (1H, s, AcN***H***-), 4.96 (1H, s, BocN***H***-), 4.58 (1H, m, C***H***), 4.20 (2H, q, -OC***H***$_2$CH$_3$), 3.69 (1H, br m, BocNHC***H***$_2$-), 3.31 (1H, br m, BocNHC***H***$_2$-), 2.02 (3H, s, -COC***H***$_3$), 1.44 (9H, s, -C(C***H***$_3$)$_3$), 1.21 (3H, t, -OCH$_2$C***H***$_3$). Anal. Calcd. for $C_{12}H_{22}N_2O_5$: C, 52.54; H, 8.08; N, 10.21. Found: C, 52.83; H, 8.02; N, 10.27%.

Triethyl-1,6-diacetamido-6-cyanohexane-1,1,6-tricarboxylate 5

To a flask containing absolute ethanol (20 mL) was added sodium (0.023 g, 1 mmol) with stirring. After complete dissolution of sodium, **2** (0.170 mg, 1 mmol) was added and warmed up to 60 °C for 30 min. After cooling down to room temperature, **4**[10] (0.352 mg, 1 mmol) was added to the solution in one portion. The reaction mixture was refluxed overnight and then the solvent was removed under reduced pressure. The resulting residue was treated with H_2O (20 mL) and was extracted with EtOAc (2 × 50 mL). The organic phase was washed with brine and dried over $MgSO_4$. After filtration, the organic phase was evaporated to give a colorless gel, which was recrystallised from EtOAc–hexane to yield colorless crystals (0.350 g, 79%). ^{1}H-NMR (200 MHz, $CDCl_3$, ppm) δ_H 6.80 (1H, br s, N***H***), 6.26 (1H, br s, N***H***), 4.31 (6H, m, -OC***H***$_2$CH$_3$), 2.09 (3H, s, -COC***H***$_3$), 2.06 (3H, s, -COC***H***$_3$), 2.02 - 2.39 (4H, m), 1.20 - 1.51 (13H, m). Anal. Calcd. for $C_{20}H_{31}N_3O_8$: C, 54.41; H, 7.08; N, 9.52. Found: C, 54.57; H, 7.02; N, 9.44%.

Triethyl-1,6-diacetamido-7-(*tert*-butoxycarbonylamino)heptane-1,1,6-tricarboxylate 6

To the MeOH (10 mL) solution of **5** (0.220 g, 0.5 mmol) cooled with an ice bath, was added $(Boc)_2O$ (0.218 g, 1 mmol) and $NiCl_2{\cdot}6H_2O$ (0.012 g, 0.05 mmol) to give a green solution. To this solution was added $NaBH_4$ (0.152 g, 4 mmol) in portions with stirring. The purple mixture was stirred overnight at 20 °C and then diethylenetriamine (0.06 mL) was added. The reaction was stirred for 1 h before the volatile part of the mixture was removed by vacuum. The residue was partitioned between EtOAc and saturated $NaHCO_3$ solution. The organic phase was dried with $MgSO_4$. Removal of organic solvent gave a colorless residue, which was re-crystallized with EtOAc–hexane to yield colorless crystals of **6** (0.200 g, 73%). ^{1}H-NMR (500 MHz, $CDCl_3$, ppm) δ_H 6.74 (1H, s, N***H***), 6.48 (1H, s, N***H***), 4.83 (1H, s, BocN***H***-), 4.24 (m, 6H, -OC***H***$_2$CH$_3$), 3.85 (1H, br m, BocNHC***H***$_2$-), 3.62 (1H, br m, BocNHC***H***$_2$-), 2.05 (3H, s, -COC***H***$_3$), 2.03 (3H, s, -COC***H***$_3$), 1.75 - 2.29 (4H, m), 1.42 (9H, s, -C(C***H***$_3$)$_3$), 1.03 - 1.32 (13H, m). Anal. Calcd. for $C_{25}H_{43}N_3O_{10}$: C, 54.02; H, 7.95; N, 7.70. Found: C, 55.21; H, 7.76; N, 7.99%.

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2,7-Diamino-2-(aminomethyl)octanedioic acid 9

This compound was directly prepared from **6** without isolation of the intermediates **7** and **8**. Compound **6** (430 mg, 0.81 mmol) was dissolved in a mixture of 4 ml 1 M NaOH and MeOH. The mixture was heated to 80° C overnight causing de-esterification and decarboxylation. After neutralization with HCl, the solution was brought to dryness and the residue re-dissolved in 2 ml conc. HCl. Heating to 90° C for 8 h resulted in complete removal of the amine protecting groups. Slow reduction of the volume to about 1/3 caused precipitation of a white solid. (Yield 0.090 g, 35%). The residual solution contains more product but could not be separated from NaCl. Anal. Calcd. for $C_9H_{17}N_3O_4 \cdot 3HCl$: C, 31.85; H, 5.95; N, 12.39. Found: C, 30.72; H, 6.17; N, 11.69%.

(*S*)-2-Benzyloxycarbonylamino-6-methanesulfonyloxy-hexanoic acid ethyl ester 11

Compound **10** (0.804 g, 2.6 mmol) and methanesulfonylchloride (0.22 mL, 2.86 mmol) were dissolved in 5 mL CH_2Cl_2 and cooled to −78 °C under nitrogen. Triethylamine (0.38 mL, 2.86 mmol) was added dropwise to the stirred solution at −78 °C. The solution was then allowed to warm up to room temperature and stirred for 2 h. The reaction mixture was then diluted by 5 mL CH_2Cl_2, and washed with water and brine. The organic phase was dried with Na_2SO_4 and evaporated under reduced pressure. The product was obtained as a yellow oil (0.962 g, 96%) and was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ_H 7.36 (5H, m, Cbz), 5.45 (1H, br m, CbzN***H***), 5.11 (2H, s, Cbz), 4.37 (1H, m), 4.20 (4H, m), 2.99 (3H, s, C***H***$_3$), 2.10 - 1.40 (6H, m), 1.29 (3H, t, -OCH$_2$C***H***$_3$).

Diethyl-2-acetamido-7-(benzyloxycarbonylamino)-2-cyanooctanedioate 12

Compound **12** was obtained by the same coupling conditions as described for **5** but with different purification. After evaporation of the solvent, the reaction mixture was loaded on a silica gel column and eluted with an EtOAc–hexane gradient. The product **12** was obtained as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ_H 7.38 (5H, m, Cbz), 6.30 (1H, m, AcN***H***-), 5.40 (1H, CbzN***H***) 5.14 (s, 2H, Cbz), 4.20 - 4.37 (5H, m), 2.10 (3H, s, -COC***H***$_3$), 2.30 - 1.40 (8H, m), 1.30 (6H, m, -OCH$_2$C***H***$_3$). Anal. Calcd. for $C_{23}H_{31}N_3O_7$: C, 59.86; H, 6.77; N, 9.10. Found: C, 60.03; H, 6.84; N, 9.16%.

Diethyl-2-acetamido-7-(benzyloxycarbonylamino)-2-((*tert*-butoxycarbonylamino)methyl) octanedioate 13

Compound **13** was obtained by the same reduction conditions as described for **6**. Instead of recrystallization, the residue was loaded on silica gel and purified with EtOAc–hexane gradient. The product of **13** was obtained as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.38 (5H, m, Cbz), 6.30 (1H, br s, AcN***H***-), 5.40 (1H, CbzN***H***-) 5.14 (2H, s, Cbz), 4.87 (1H, m, BocN***H***-), 4.20 - 4.37 (5H, m), 3.86 (1H, m, BocNHC***H***$_2$-), 3.62 (1H, m, BocNHC***H***$_2$-), 2.07 (3H, s, -COC***H***$_3$), 1.42 (9H, s, -C(C***H***$_3$)$_3$), 1.50 - 1.03 (14H, m). Anal. Calcd. for $C_{28}H_{43}N_3O_9$: C, 59.45; H, 7.66; N, 7.43. Found: C, 59.63; H, 7.72; N, 7.50%.

2-Amino-2-(aminomethyl)-7-(benzyloxycarbonylamino)octanedioic acid 14

Compound **14** was obtained as described for **9**, alkaline de-esterification followed by conc. HCl for Boc and acetate protecting group removal.

Benzyl-*N*-(3-bromopropyl)carbamate 15

A solution of triphenylphosphine (9.4 g, 28.60 mmol) and carbon tetrabromide (7.5 g, 28.60 mmol) in dry THF was added dropwise (30 mL) to a solution of benzyl-*N*-(3-hydroxypropyl)carbamate (3.0 g, 14.30 mmol) in the same solvent (30 mL). After 48 h of stirring at room temperature, the solution was filtrated to remove an insoluble solid. After evaporation of filtrate, the residue was dissolved in CH_2Cl_2 and the solution washed with H_2O. The organic layer was dried over $MgSO_4$. Removal of the solvent yielded an oily residue, which was purified by column chromatography (CH_2Cl_2 to MeOH). After evaporation of the solvents, benzyl-*N*-(3-bromopropyl)carbamate was obtained as an orange oil (3.2 g, 86.4%). R_f (silica gel, $CHCl_3$) = 0.5. ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ_H 7.32 (5H, m, Cbz), 5.15 (1H, br s, CBzN***H***-), 5.06 (2H, s, Cbz), 3.39 (2H, t), 3.30 (2H, q), 2.06–1.96 (2H, m).

Ethyl-2-acetamido-5-(benzyloxycarbonylamino)-2-cyanopentanoate 16

Metallic sodium (0.141 g, 5.88 mmol) was added to dry EtOH under stirring. After complete dissolution of sodium, ethyl acetamidocyanoacetate (**2**) (1.0 g, 5.88 mmol) was added and the solution warmed up to 60 °C for 30 min. After cooling down to room temperature, **15** (1.6 g, 5.88 mmol) was added to the solution in one portion. The reaction mixture was refluxed overnight and, after this time, turned deep brown from the initially orange colour. Evaporation of the solvent gave a dark residue which was treated with H_2O and extracted three times with EtOAc. The organic phases were collected and washed with brine, and dried over $MgSO_4$. After filtration, the organic phase was evaporated to give an orange oil (0.841 g, 41.2%), which was purified by column chromatography (EtOAc to MeOH). R_f (silica gel, hexane–EtOAc 80%) = 0.45. ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ_H 7.98 (1H, s, AcN***H***-), 7.34 (5H, m, Cbz), 5.42 (1H, t, CbzN***H***-), 5.09 (2H, s, Cbz), 4.28 (2H, q, -OC***H***$_2$CH$_3$), 3.26 (2H, m, -CH^f-), 2.09 (2H, m, -CH^d-), 2.04 (3H, s, -COC***H***$_3$), 1.81 (1H, m, -CH^e-), 1.68 (1H, m, -CH$^{e'}$-), 1.28 (3H, t, -OCH$_2$C***H***$_3$). Anal. Calcd. for $C_{18}H_{23}N_3O_5$: C, 59.82; H, 6.41; N, 11.63. Found: C, 59.60; H, 6.40; N, 11.63%. Retention time (analytic RP-HPLC, 220 nm): 23.4 min.

Ethyl-2-acetamido-5-(benzyloxycarbonylamino)-2-((*tert*-butoxycarbonylamino)-methyl)pentanoate 17

Compound **17** was prepared by using the same reduction conditions described above for **6**. An excess of $(Boc)_2O$ (1.015 g, 4.65 mmol), $NiCl_2 \cdot 6H_2O$ (0.055 g, 0.23 mmol), $NaBH_4$ (0.700 g, 18.61 mmol) and diethylenetriamine (0.239 g, 2.32 mmol) was added to **16** (0.841 g, 2.32 mmol). Compound **17** was purified by column chromatography (EtOAc to MeOH) and obtained as a yellow pale oil (0.437 g, 40.3%). R_f (silica gel, hexane–EtOAc 80%) = 0.50. *Isomer a*: ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ_H 7.35

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(5H, m, Cbz), 6.53 (1H, s, AcN***H***-), 5.09 (2H, s, Cbz), 4.88 (2H, m, CbzN***H***- + BocN***H***-), 4.24 (2H, q, -OC***H***$_2$CH$_3$), 3.85 (1H, m, BocNHC***H***^{a}-), 3.61 (1H, m, BocNHC***H***$^{a'}$-), 3.17 (2H, m, -CH^f-), 2.23 (2H, m, -CH^d-), 2.01 (3H, s, -COC***H***$_3$), 1.82 (1H, m, -CH^e-), 1.72 (1H, m, -CH$^{e'}$-), 1.41 (9H, s), 1.27 (3H, t, -OCH$_2$C***H***$_3$). *Isomer b*: ^{1}H-NMR (300 MHz, CDCl$_3$, ppm) δ_H 7.58 (1H, s, AcN***H***-), 7.37 (5H, m, Cbz), 5.12 (2H, s, Cbz), 4.88 (2H, m, CbzN***H***- + BocN***H***-), 4.36 (2H, q, -OC***H***$_2$CH$_3$), 3.85 (1H, m, BocNHC***H***^{a}-), 3.61 (1H, m, BocNHC***H***$^{a'}$-), 3.30 (2H, m, -CH^f-), 2.23 (2H, m, -CH^d-), 2.08 (3H, s, -COC***H***$_3$), 1.82 (1H, m, -CH^e-), 1.72 (1H, m, -CH$^{e'}$-), 1.41 (9H, s), 1.31 (3H, t, -OCH$_2$C***H***$_3$). ESI-MS (+) (*m*/*z*): 488.1 [M+Na]$^+$; calcd for $C_{23}H_{35}N_3O_7Na$ = 488.2. Anal. Calcd. for $C_{23}H_{35}N_3O_7$: C, 59.34; H, 7.58; N, 9.03. Found: C, 59.00; H, 7.64; N, 8.99%. Retention time (analytic RP-HPLC, 220 nm): 27.1 min.

Ethyl-2-acetamido-5-amino-2-((*tert*-butoxycarbonylamino)methyl)pentanoate 18

Compound **17** (0.437 g, 0.94 mmol) was dissolved in dry EtOH (15 mL) and Pd/C (Pd content 10%, 0.215 g) was added. The solution was bubbled with H_2 at room temperature for 8 h and left overnight under H_2 atmosphere. The catalyst was filtered through Celite, the filtrate was evaporated and the obtained residue purified by column chromatography (CH_2Cl_2 to EtOH–NH_4OH 5%). Compound **18** was obtained as yellow viscous oil (0.280 g, 89.9%). R_f (silica gel, CH_2Cl_2–EtOH 20%) = 0.20. ^{1}H-NMR (300 MHz, CD_3OD, ppm) δ_H 4.15 (2H, q, -OC***H***$_2$CH$_3$), 3.57 (2H, s, BocNHC***H***$_2^a$), 2.87 (2H, t, -CH^f-), 1.94 (3H, s, -COC***H***$_3$), 1.82 (2H, m, -CH^d-), 1.63 (2H, m, -CH^e-), 1.42 (9H, s), 1.24 (3H, t, -OCH$_2$C***H***$_3$). ESI-MS (+) (*m*/*z*): 331.4 [M+H]$^+$; calcd for $C_{15}H_{29}N_3O_5$ = 331.2. Retention time (analytic RP-HPLC, 220 nm): 15.2 min.

When the reaction is performed using a large excess of Pd/C catalyst (1 : 1 ratio of Pd/C), two compounds, formulated as **18** and **19**, were obtained in 51.4 and 18.7% yield, after column chromatography. **19**: R_f (silica-gel, CH_2Cl_2–EtOH 20%) = 0.70. ^{1}H-NMR (300 MHz, CDCl$_3$, ppm) δ_H 7.16 (1H, s, AcN***H***-), 6.32 (1H, br s, N***H*-amide**), 5.64 (1H, br m, BocN***H***-), 3.64 (1H, m, BocNHC***H***^{a}-), 3.50 (2H, m, -CH$_2^f$-), 3.31 (1H, m, BocNHC***H***$^{a'}$-), 2.38 (1H, m, -CH^d-), 2.09 (1H, m, -CH$^{d'}$-), 2.01 (3H, s, -COC***H***$_3$), 1.89 (2H, m, -CH^e-), 1.50 (9H, s). ESI-MS (+) (*m*/*z*): 308.1 [M+Na]$^+$; calcd for $C_{13}H_{23}N_3O_4Na$ = 308.1. Single crystals suitable for X-Ray diffraction analysis were grown by slow evaporation of a EtOH–CH_2Cl_2 solution at room temperature.

2,5-Diamino-2-(aminomethyl)pentanoic acid 20

Compound **20** was obtained directly by hydrolysis of the protecting groups of **18** (0.047 g, 0.14 mmol) with a 4 M HCl solution (5 mL). The reaction mixture was refluxed overnight, cooled down to room temperature and washed with CH_2Cl_2. Compound **20** was recovered as a colorless oil, after drying the aqueous phase under vacuum (0.035 g, 92.6%, calcd. for $C_6H_{15}N_3O_2Cl_3$). IR (KBr, cm^{-1}): 1787 m, 1619 s and 1606 s. ^{1}H-NMR (300 MHz, D_2O, ppm) δ_H 3.32 (2H, s, NH$_2$C***H***$_2^a$-), 2.88 (2H, t, -CH^f-), 2.00–1.84 (1H, m, -CH^d-), 1.82–1.78 (1H, m, -CH$^{d'}$-), 1.78–1.66 (1H, m, -CH^e-), 1.66–1.47 (1H, m, -CH$^{e'}$-). ESI-MS (+) (*m*/*z*): 162.0 [M+H]$^+$; calcd for $C_6H_{15}N_3O_2$ = 161.2. Anal. Calcd. for $C_6H_{15}N_3O_2.3Cl$: C, 26.90; H, 5.65; N, 15.70. Found: C, 27.20; H, 6.00; N, 16.00%. Retention time (analytic RP-HPLC, 220 nm): 2.4 min.

tert-Butyl acetamidocyanoacetate 21

To a stirred solution of *tert*-butyl cyanoacetate (5.6 g, 40.0 mmol) and 45% aq HOAc (50 mL) at 0 °C was added portion-wise $NaNO_2$ (8.3 g, 120.0 mmol) over 1.5 h. After the addition was completed, the stirring was continued at room temperature for 3 h. The reaction mixture was extracted with Et_2O (3×). The ethereal solution containing *tert*-butyl isonitrosocyanoacetate was immediately mixed with Ac_2O (10 mL, 105.0 mmol) and HOAc (28 mL, 500.0 mmol). With vigorous stirring, zinc powder (8.0 g, 125.0 mmol) was added in small portions and the stirring was then continued for 6 h. After filtration, the solvent was evaporated at reduced pressure to give a pale yellow oil, which was purified by column chromatography (hexane–EtOAc 25–100%). Compound **21** was recovered as yellowish oil (0.761 g, 35.0%). R_f (silica gel, hexane–EtOAc 50%) = 0.30. ^{1}H-NMR (300 MHz, CDCl$_3$, ppm) δ_H 6.39 (1H, s, AcN***H***-), 5.40 (1H, d, -C***H***-), 2.11 (3H, s, -COC***H***$_3$), 1.55 (9H, s);

1-*tert*-Butyl-6-ethyl-2-acetamido-2-cyanohexanedioate 22

Compound **22** was obtained by reaction of **21** (0.740 g, 3.73 mmol) with NaH (0.150 g, 3.73 mmol) in DMF, followed by addition of ethyl-4-bromobutyrate (539 µL, 3.73 mmol), using a procedure similar to that described for **5**. Purification by column chromatography (hexane–EtOAc 25–100%) gave **22** as a colourless oil (0.630 g, 54.1%). R_f (silica gel, hexane–EtOAc 50%) = 0.40. ^{1}H-NMR (300 MHz, CDCl$_3$, ppm) δ_H 6.94 (1H, s, AcN***H***-), 4.15 (2H, q, -OC***H***$_2$CH$_3$), 2.37 (2H, m), 2.26 (1H, m), 2.08 (3H, s, -COC***H***$_3$), 2.01 (1H, m), 1.82 (2H, m), 1.54 (9H, s), 1.27 (3H, t, -OCH$_2$C***H***$_3$); ESI-MS (+) (*m*/*z*): 335.2 [M+Na]$^+$; calcd for $C_{15}H_{24}N_2O_5Na$ = 335.2. Anal. Calcd. for $C_{15}H_{24}N_2O_5$: C, 57.68; H, 7.74; N, 8.97. Found: C, 57.50; H, 7.80; N, 8.81%. Retention time (analytic RP-HPLC, 220 nm): 20.9 min.

1-*tert*-Butyl-6-ethyl-2-acetamido-2-((*tert*-butoxycarbonylamino)methyl)hexanedioate 23

Compound **23** was prepared by using the same reduction conditions described above for **6**. An excess of $(Boc)_2O$ (1.762 g, 8.08 mmol), $NiCl_2·6H_2O$ (0.040 g, 0.40 mmol), $NaBH_4$ (1.216 g, 32.32 mmol) and diethylenetriamine (0.416 g, 4.04 mmol) was added to **22** (0.630 g, 2.02 mmol) to force reaction to completion. Compound **23** was purified by column chromatography (hexane to EtOAc) and obtained as a yellow pale oil (0.817 g, 97.0%). R_f (silica-gel, hexane–EtOAc 50%) = 0.45. ^{1}H-NMR (300 MHz, CDCl$_3$, ppm) δ_H 6.60 (1H, s, AcN***H***-), 4.85 (1H, m, BocN***H***-), 4.13 (2H, q, -OC***H***$_2$CH$_3$), 3.85 (1H, br m, BocNHC***H***^{a}-), 3.70 (1H, br m, BocNHC***H***$^{a'}$-), 2.29 (m, 2H), 2.08 (3H, s, -COC***H***$_3$), 1.74 (1H, m), 1.60 (1H, m), 1.49 (9H, s), 1.44 (2H, m), 1.41 (9H, s), 1.22 (3H, t, -OCH$_2$C***H***$_3$); Anal. Calcd. for $C_{20}H_{36}N_2O_7$: C, 57.67; H, 8.71; N, 6.73. Found: C, 57.50; H, 8.60; N, 7.00%.

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5-Acetamido-6-*tert*-butoxy-5-((*tert*-butoxycarbonylamino)methyl)-6-oxohexanoic acid 24

The protected intermediate **23** (0.600 g, 1.440 mmol) was dissolved in H_2O–MeOH, and an excess of NaOH (0.144 g, 3.6 mmol) was added. The obtained solution was refluxed overnight. The solution was neutralized with 1 M HCl at 0 °C and extracted with CH_2Cl_2. The organic phases were collected, dried over $MgSO_4$, filtered and the solvent evaporated. The crude product was purified by column chromatography (CH_2Cl_2–EtOH 0–30%). Compound **24** was obtained as a colorless oil (0.380 g, 67.9%), which crystallizes upon standing. R_f (silica-gel, CH_2Cl_2–EtOH 10%) = 0.40. ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ_H 6.79 (1H, s, AcN***H***-), 4.94 (1H, m, BocN***H***-), 3.83–3.65 (2H, m, BocNHC***H***^{a}- and BocNHC***H***$^{a'}$-), 2.33 (2H, m), 2.06 (3H, s, COC***H***$_3$), 1.74 (1H, m), 1.55 (3H, m), 1.48 (9H, s), 1.41 (9H, s). ESI-MS (-) (*m*/*z*): 387.1 [M − H]$^-$, 423.1 [M+Cl]$^-$; calcd for $C_{18}H_{32}N_2O_7$= 388.2. Anal. Calcd. for $C_{18}H_{32}N_2O_7$: C, 55.66; H, 8.30; N, 7.21. Found: C, 55.03; H, 8.10; N, 6.96%. Retention time (analytic RP-HPLC, 220 nm): 18.9 min.

2-Amino-2-(aminomethyl)hexanedioic acid 25

Compound **25** was obtained directly by hydrolysis of the protecting groups of the **dap** unit of **24** (0.230 g, 0.592 mmol) with a 4 M HCl solution (5 mL). After refluxing for 18 h, the solvent was evaporated to dryness. The oily residue was thoroughly washed with CH_2Cl_2 and dried. The deprotection reaction afforded compound **25** (60% by ^{1}H-NMR) together with a side product which was formulated as the lactam **26** (40% by ^{1}H-NMR). After appropriate work-up, 0.080 g of the mixture (**25** + **26**) was obtained. Mixture of **25** + **26**: IR (KBr, cm^{-1}): 1746 vs, 1605 vs, 1211 m, 1179 m and 1144 m. ^{1}H-NMR (300 MHz, D_2O, ppm) δ_H 3.18 (2H, s, $NH_2C\boldsymbol{H}_2{}^a$-, **25**), 3.03 (2H, q, $NH_2C\boldsymbol{H}_2{}^a$-, **26**), 2.09 (2H, t, -$CH_2{}^f$-, **25**), 1.98 (2H, t, -$CH_2{}^f$-, **26**), 1.89–1.20 (8H, m, -CH^d- + -CH^e-, **25** + **26**). ESI-MS (+) (*m*/*z*): 172.9 [M+H]$^+$ and 191.0 [M+H]$^+$; calcd for $C_7H_{12}N_2O_3$ = 172.0 (*m*/*z* for **26**) and for $C_7H_{14}N_2O_4$ = 190.0 (*m*/*z* for **25**). Retention time (analytic RP-HPLC, 220 nm): 3.9 min. At pH > 4, **25** converted almost quantitatively to **26**: ^{1}H-NMR (300 MHz, D_2O, ppm) δ_H 3.17 (2H, q, $NH_2C\boldsymbol{H}^a$-), 2.24 (2H, m, -$CH_2{}^f$-), 2.00 (1H, m, -CH^d-), 1.71 (2H, m, -CH^e-), 1.60 (1H, m, -$CH^{d'}$-). ESI-MS (+) (*m*/*z*): 172.9 [M+H]$^+$; calcd for $C_7H_{12}N_2O_3$ = 172.0. Anal. Calcd. for $C_7H_{13}N_2O_3$·HCl: C, 40.30; H, 6.28; N, 13.43. Found: C, 39.60; H, 6.68; N, 12.85%. Single crystals suitable for X-ray diffraction analysis were grown by slow evaporation of a EtOH–CH_2Cl_2 solution at room temperature.

Syntheses of the Re complexes 27 and 28

Precursor *fac*-[$Re(H_2O)_3(CO)_3$]Br (0.026 g, 0.065 mmol) reacted with **20** (0.017 g, 0.065 mmol) in refluxing water for 18 h (pH 7). After evaporation of the solvent the resulting residue was purified by preparative RP-HPLC. Compound **27** was obtained as a colourless oil (0.028 g, 79.2%, calcd for $C_9H_{14}N_3O_5Re$·TFA). IR (KBr, cm^{-1}): 2028 s, ~1911 s (C≡O), ~1682 m, 1207 m and 1135 m. ^{1}H-NMR (300 MHz, D_2O, ppm) δ_H 5.08 (1H, m, $N\boldsymbol{H}_2C^b$-), 4.74 (2H, m, $N\boldsymbol{H}_2C^b$- + $N\boldsymbol{H}_2C^a$-, overlapped with H_2O peak; assigned from gCOSY spectrum), 4.31 (1H, m, $N\boldsymbol{H}_2CH_2{}^a$-), 2.88 (2H, m, -$CH_2{}^f$-), 2.79 (1H, m, -$CH_2{}^a$-), 2.51 (1H, m, -$CH_2{}^{a'}$-), 1.69 (2H, m, -$CH_2{}^d$-), 1.51 (2H, m, CH^e). ESI-MS (+) (*m*/*z*): 431.8 [M+H]$^+$; calcd for $C_9H_{15}N_3O_5Re$ = 432.0. *Anal.* Calcd. for $C_9H_{14}N_3O_5Re$·3TFA: C, 23.32; H, 2.21; N, 5.44. Found: C, 23.00; H, 2.39; N, 5.80%. Retention time (analytic RP-HPLC, 220 nm): 5.5 min.

Synthesis of *fac*-[Re(k^3-25)$(CO)_3$] (28)

Precursor $(Et_4N)_2[ReBr_3(CO)_3]$ (0.050 g, 0.194 mmol) reacted with a mixture of **25** + **26** (0.146 g, 0.194 mmol) in refluxing water for 18 h (pH 4). After cooling to room temperature and concentration of the solvent a white insoluble solid was obtained. This precipitate was isolated by centrifugation and washed several times with water and warm CH_2Cl_2 to remove excess [NEt_4]Br. The spectral data of this complex were in accordance with the formulation proposed for **28**. Analytic RP-HPLC analysis of the supernatant obtained after precipitation of **28** revealed still the presence of **26**, Re precursor and a small amount of **28**. By refluxing overnight at pH 8, the rhenium precursor was consumed and a mixture of **28** and a second new species, formulated as "*fac*-[Re(**26**)$(CO)_3$]", was obtained. Complexes **28** and "*fac*-[Re(**26**) $(CO)_3$]" were isolated by RP-HPLC purification. *fac*-[Re(k^3-**25**)$(CO)_3$] (**28**): Yield: 0.049 g, 54.9% (calcd for $C_{10}H_{13}N_2O_7Re$). IR (KBr, cm^{-1}): 2021 s, 1907 s, 1884 s, 1693 m (C=O) and 1647 m (C=O). ^{1}H-NMR (300 MHz, CD_3OD, ppm) δ_H 5.32 (1H, m, $N\boldsymbol{H}_2C^b$-), 4.95 (1H, m, $N\boldsymbol{H}_2C^a$-), 4.75 (1H, m, $N\boldsymbol{H}_2CH_2{}^b$-), 4.64 (1H, m, $N\boldsymbol{H}_2CH_2{}^a$-), 2.76 (1H, m, -$CH_2{}^a$-), 2.59 (1H, m, -$CH_2{}^{a'}$-), 2.33 (2H, m, -$CH_2{}^f$-), 1.78 (2H, m, -$CH_2{}^d$-) 1.67 (2H, m, CH^e). ESI-MS (+) (*m*/*z*): 482.9 [M+Na]$^+$; calcd for $C_{10}H_{13}N_2O_7ReNa$ = 483.0. *Anal.* Calcd. for $C_{10}H_{13}N_2O_7Re$: C, 26.14; H, 2.85; N, 6.10. Found: C, 26.07; H, 2.93; N, 6.25%. Retention time (analytic RP-HPLC, 220 nm): 9.9 min. Single crystals suitable for X-ray diffraction analysis were grown by slow evaporation of a EtOH–CH_2Cl_2 solution at room temperature. *fac*-[Re(**26**)$(CO)_3$]: Yield: 0.025 g, 29.1% (calcd for $C_{10}H_{11}N_2O_6Re$). IR (KBr, cm^{-1}): 2022 s, ~1918 s (C≡O), 1678 m, 1630, 1585, and 1416 m. ^{1}H-NMR (300 MHz, CD_3OD, ppm) δ_H 4.78 (1H, m, $N\boldsymbol{H}_2CH_2{}^a$-, overlapped with H_2O peak; assigned from gCOSY spectrum), 4.63 (1H, m, $N\boldsymbol{H}_2CH_2{}^a$-), 2.86 (1H, m, -$CH_2{}^a$-), 2.55 (1H, m, -$CH_2{}^{a'}$-), 2.45 (2H, m, -$CH_2{}^f$-), 2.20 (1H, m, -$CH_2{}^d$-), 1.76 (2H, m, -$CH_2{}^e$-) 1.58 (1H, m, $CH^{d'}$). ^{1}H-NMR (300 MHz, DMSO, ppm) δ_H 4.93 (1H, m, $N\boldsymbol{H}_2CH_2{}^a$-), 4.80 (1H, m, $N\boldsymbol{H}_2CH_2{}^a$-), 2.87 (1H, br s, -$CH_2{}^a$-), 2.71 (1H, br s, -$CH_2{}^{a'}$-), 2.36 (2H, br m, -$CH_2{}^f$-), 1.98 (1H, m, -$CH_2{}^d$-), 1.70–1.40 (3H, m, -$CH_2{}^e$- + -$CH^{d'}$-). ESI-MS (+) (*m*/*z*): 464.9 [M+Na]$^+$; calcd for $C_{10}H_{11}N_2O_6ReNa$ = 465.0. ESI-MS (-) (*m*/*z*): 440.9 [M − H]$^-$; calcd for $C_{10}H_{11}N_2O_6Re$ = 442.0. Retention time (analytic RP-HPLC, 220 nm): 13.2 min.

Synthesis of the ^{99m}Tc(I)complexes (*fac*-[^{99m}Tc(**20**)$(CO)_3$]and *fac*-[^{99m}Tc(**25**)$(CO)_3$])

General method. In a nitrogen-purged glass vial, 100 μL of an aqueous solution of the compounds (**20** and **25**; the concentrations are discussed in the text and ESI† [ ] = 10^{-3}–10^{-4} M) were added to 900 μL of a solution of the organometallic precursor *fac*-[^{99m}Tc$(H_2O)_3(CO)_3]^+$ (1–2 mCi) in saline (pH 7.4). The reaction mixture was then heated to 100 °C for 30–60 min, cooled on an H_2O bath and the final solution analyzed by RP-HPLC, yielding the complexes *fac*-[^{99m}Tc(**20**)$(CO)_3$] and *fac*-[^{99m}Tc(**25**)$(CO)_3$]). Retention times: 4.29 min (*fac*-[^{99m}Tc(**20**)$(CO)_3$],

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method 2) and 10.0 min (*fac*-[^{99m}Tc(**25**)(CO)$_3$], **method 1**). Reaction of the pure lactam **26** with *fac*-[^{99m}Tc(H$_2$O)$_3$(CO)$_3$]$^+$ under the same conditions (30 min, 100 °C, 1×10^{-4} M, pH 7.4) gave the complex "*fac*-[^{99m}Tc(CO)$_3$(**26**)]". Retention time: 13.7 min.

X-Ray crystallography

Single crystals were grown by slow evaporation of n-hexane–EtOAc (**5** and **6**) and EtOH–CH_2Cl_2 (**19**, **26** and **28**) solutions of the compounds at room temperature. Crystallographic data of **5** were collected at 183 K on an IPDS Stoe diffractometer. A maximum of eight thousand reflections distributed over the whole limiting sphere were selected by the program SELECT and used for unit cell parameter refinement with the program CELL.[30] Crystallographic data of **4** and **6** were collected at 183 K on a Stoe IPDS 2T diffractometer with Cu-Kα radiation. Reflection data was processed with the help of the program X-Area.[31] Data were corrected for Lorentz and polarisation effects as well as for absorption (numerical). Crystallographic data of **28** were collected on an Oxford Diffraction Xcalibur system with a Ruby detector. The program suite CrysAlis^{Pro} was used for data collection, semiempirical absorption correction, and data reduction.[32] Crystallographic data of **19** and **26** were collected at 150 K on a Bruker-AXS APEX-CCD area-detector diffractometer. Empirical absorption correction was carried out using SADABS.[33] Data collection and data reduction were done with the SMART and SAINT programs.[34] The structures were solved by direct methods with SIR97[35] and refined by full matrix least-squares analysis with SHELXL97[36] using the WINGX suite of programs. All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were placed in calculated positions. Molecular graphics were prepared using ORTEP3.[37] Significant crystal data collection and refinement parameters are listed in Table 1 and in the Supporting Information† for **4**.

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