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Metalla Derivatives of Amino Acids and Peptides. 2. Rhena β-Keto Imine Derivatives of Several Amino Acid Esters

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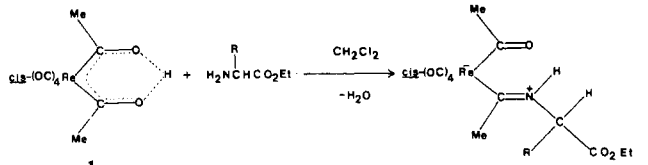
The preparation and characterization of 16 rhena β-keto imine derivatives of 12 amino acids are reported. These rhena-labeled amino acids are prepared by a Schiff-base condensation of a rhenaacetylacetone molecule with the appropriate amino acid free base as the methyl or ethyl ester. Heteroatomic substituents of the amino acid including hydroxyl, phenolic, sulfhydryl, mercaptyl, ester, or basic amino groups do not prevent rhena β-keto imine formation. Distal rhena labeling of the ε-amino group of several L-lysine derivatives is reported also.

Introduction

In the first paper of this series, we reported the preparation of rhena β-keto imine derivatives of ethyl glycinate, ethyl L-alaninate, and ethyl glycylglycinate.[1] The X-ray structure of the rhena ethyl L-alaninate complex was reported also along with selected chemical reactivity studies.

The rhena β-keto imine derivatives of ethyl glycinate and ethyl L-alaninate are prepared by Schiff-base condensation of a rhenaacetylacetone molecule (**1**) with the free base form of



1

2, R = H
3, R = Me

the amino acid esters, as shown. Both *intra* and *inter* isomers of **2** are obtained. These geometrical isomers are distinguished by the relative orientation of the iminium methyl substituent and the iminium hydrogen atom, and they are related by a very slow rotation about the C–N double bond. These isomers are readily identified by ^{1}H NMR.[2,3] The rhena β-keto imine derivative of ethyl glycylglycinate is prepared from **2** and ethyl glycinate by normal peptide coupling procedures utilizing dicyclohexylcarbodiimide as a coupling agent.

We now report the preparation and characterization of 16 new rhena β-keto imine derivatives of 12 additional amino acids (as esters). This study demonstrates the general utility of the Schiff-base condensation of a rhenaacetylacetone molecule with a variety of amino acid esters. The amino acid esters chosen contain a variety of unprotected functional groups appended to the methine carbon atom of the amino acid ester. These functional groups include alkyl substituents, aliphatic or phenolic hydroxyl groups, sulfhydryl or mercaptyl substituents, an ester group, or a basic amino-containing substituent. In each case, the rhena β-keto imine derivative is formed.

These rhena β-keto imine derivatives of amino acids or peptides have potential applications (1) as a new class of N-terminus-protecting group for peptide synthesis, (2) as a heavy-atom label of N termini for X-ray structural determinations, (3) in effecting unusual transport and tissue distribution properties, and (4) as a convenient labeling probe for detection and isolation. For this latter application, the prepartion of the technetium analogues would be particularly important.

Chart I

[Re]-L-Val(OMe) (**4**): CH_2Cl_2; 2 h; 36%; −2.02° (0.42, Silanor-C)
[Re]-L-Leu(OEt) (**5**): CH_2Cl_2; 5 h; 35%; −5.54° (0.41, Silanor-C)
[Re]-L-Phe(OEt) (**6**): CH_2Cl_2; 2.5 h; 82%; −5.28° (1.63, Silanor-C)
[Re]-L-Ser(OEt) (**7**): CH_2Cl_2; 13 h; 40%; +14.48° (0.14, Silanor-C)
[Re]-L-Tyr(OEt) (**8**): DMF; 72 h; 57%; −14.79° (0.015, Silanor-C)
[Re]-L-Cys(OEt) (**9**): CH_2Cl_2; 13 h; 65%; −6.23° (0.77, Silanor-C)
[Re]-L-Cys-Cys(OMe)$_2$ (**10**): CH_2Cl_2; 48 h; −27.99° (0.50, Silanor-C)
[Re]-L-Met(OEt) (**11**): CH_2Cl_2; 13 h; 77%; −6.23° (3.07, Silanor-C)
[Re]-D,L-Asp(OMe)$_2$ (**12**): CH_2Cl_2; 24 h; 46%
[Re]-L-His(OMe) (**13**): CH_2Cl_2; 11 h; 74%; +5.05° (0.38, Silanor-C)
[Re]-L-Trp(OEt) (**14**): CH_2Cl_2; 34 h; 81%; −16.48° (1.93, Silanor-C)
N^{α}-[Re]-L-Lys(OEt) (**15**): CH_2Cl_2; 2.5 h
N^{ϵ}-[Re]-L-Lys(OEt) (**16**): CH_2Cl_2; 2.5 h; 84% combined yield of **15** and **16**; +2.11° (1.42, Silanor-C)
N^{α}-Ac-N^{ϵ}-[Re]-L-Lys(OEt) (**17**): CH_2Cl_2; 30 h; 70%; +24.56° (2.16, Silanor-C)
N^{α}-*t*-BOC,N^{ϵ}-[Re]-L-Lys(OEt) (**18**): CH_2Cl_2; 76 h; 48%; +0.16° (1.28, Silanor-C)
N^{α}-CBZ,N^{ϵ}-[Re]-L-Lys(OEt) (**19**): CH_2Cl_2; 4 h; 74%; +7.25° (0.80, Silanor-C)

Experimental Section

All reactions were performed under dry, prepurified nitrogen at 25 °C, unless stated otherwise, even though the organometallic reactant and products are air stable for at least several days. Solvent purification and spectroscopic procedures including instrument specifications have been published previously.[1] Dimethylformamide, DMF, was dried over molecular seives. Microanalysis was performed by Galbraith Laboratories, Inc., Knoxville, TN.

Complex **1**, *cis*-[(OC)$_4$Re(CH$_3$CO)$_2$]H, was prepared by a literature procedure.[4,5] Amino acids were purchased from Sigma Chemical Co., usually as the hydrochloride salts of the ethyl or methyl esters. Deprotonation to afford the free-base form of the amino acid esters was accomplished by treatment with ca. 0.90 equiv of base to avoid racemization. When necessary, amino acid esters were prepared by alkylating amino acids with the appropriate trialkyloxonium salt.

General Preparation of the Rhena β-Keto Imine Derivatives of Amino Acid Esters. To 0.15–0.40 g of **1** in 3–15 mL of CH_2Cl_2 or DMF was added a slight excess of the amino acid ester as free base. The reaction solution was stirred for 1–76 h. Excess free base and other impurities were removed, usually by passing the product residue through a short Florisil/CH_2Cl_2 column. In selected examples, excess free base was removed by protonation with HCl/ether. Products were

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(2) Lukehart, C. M.; Zeile, J. V. *J. Am. Chem. Soc.* **1978**, *100*, 2774–2778.
(3) Lukehart, C. M.; Zeile, J. V. *Inorg. Chem.* **1978**, *17*, 2369–2374.
(4) Lukehart, C. M.; Zeile, J. V. *J. Am. Chem. Soc.* **1976**, *98*, 2365–2367.
(5) Darst, K. P.; Lukehart, C. M.; Warfield, L. T.; Zeile, J. V. *Inorg. Synth.* **1980**, *20*, 200–204.

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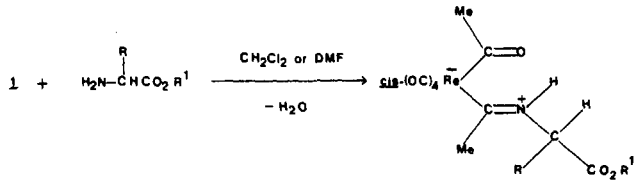
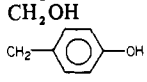
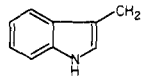
Chart II



compd	designation	R	R¹
4	[Re]-L-Val(OMe)	*i*-Pr	Me
5	[Re]-L-Leu(OEt)	CH_2CHMe_2	Et
6	[Re]-L-Phe(OEt)	CH_2Ph	Et
7	[Re]-L-Ser(OEt)	CH_2OH	Et
8	[Re]-L-Tyr(OEt)		Et
9	[Re]-L-Cys(OEt)	CH_2SH	Et
10	[Re]-L-Cys-Cys(OMe)$_2$	$(-SCH_2-)_2$	Me
11	[Re]-L-Met(OEt)	CH_2CH_2SMe	Et
12	[Re]-D,L-Asp(OMe)$_2$	CH_2CO_2Me	Me
13	[Re]-L-His(OMe)		Me
14	[Re]-L-Trp(OEt)		Et
15	N^{α}-[Re]-L-Lys(OEt)	$(CH_2)_4NH_2$	Et
16	N^{ϵ}-[Re]-L-Lys(OEt)	see text	Et
17	N^{α}-Ac-N^{ϵ}-[Re]-L-Lys(OMe)	see text	Me
18	N^{α}-*t*-BOC,N^{ϵ}-[Re]-L-Lys(OEt)	see text	Et
19	N^{α}-CBZ,N^{ϵ}-[Re]-L-Lys(OEt)	see text	Et

isolated as yellow oils by fractional precipitation from CH_2Cl_2/hexane solution at −15 °C. A listing of the specific reaction conditions of solvent, reaction time, yield, and specific optical rotation, $[\alpha]^{25}_D$, at (*c*, solvent) for each product complex is provided in Chart I. The abbreviated nomenclature used is defined in the text. Tabulations of satisfactory C, H, and N microanalytical data and IR and ^{1}H NMR spectroscopic data are provided as supplementary material. In most cases, a mixture of *intra* and *inter* isomers was obtained. Although these geometrical isomers can normally be separated by column chromatography, this separation was not performed for each product.

Results

The 16 new rhenaacetylacetone imine derivatives of amino acid esters (Chart II) are prepared by a direct Schiff-base condensation of the rhenaacetylacetone molecule (**1**) with the appropriate amino acid ester in the free base form.

For complexes **4–15**, the rhena β-keto imine group has condensed with the α-amino group of the amino acid ester. For complexes **16–19**, the α-amino residue of ethyl L-lysinate either remains unprotected or is protected by acetyl, *t*-BOC,[6] or CBZ[6] groups, so that complex **1** condenses at the ε-amino group of the lysine side chain.

The complexes **4–19** are isolated as yellow oils in crude yields of up to 84% and in analytically pure yields of up to 70%. Purification by fractional precipitation generally reduces the yield of pure compound considerably, and no attempt was made to optimize the yield of the analytically pure products.

Condensation of **1** with ethyl L-tyrosinate in CH_2Cl_2 solution proceeds very slowly and in low yield. However, this condensation proceeds much more rapidly in DMF solution to afford a 57% yield of pure product. Presumably, the greater solvating and hydrogen-bonding ability of DMF inhibits an interaction between the relatively acidic phenolic proton and the free amino group. The Schiff-base condensation requires a relatively basic free amino group.

The infrared spectra of complexes **4–19** in CH_2Cl_2 solution show the expected ν(CO) bands at ca. 2075 (m), 1980 (vs), 1960 (vs), and 1940 (m) cm^{-1} for a *cis*-$(OC)_4Re$ moiety.[2,3] Ester carbonyl stretching frequencies occur in the normal range of 1710–1740 cm^{-1}, and the ν(C=N) and ν(C=O) stretching frequencies of the rhena β-keto imine moieties occur in the usual range of 1540–1560 cm^{-1}.[2,3]

The ^{1}H NMR data of complexes **4–19** are summarized best by considering the organometallic and amino acid moieties separately. For the *inter* isomer, where the amino acid residue is trans to the iminium methyl substituent, the acetyl ligand methyl resonance always appears at higher field than does the iminium ligand methyl resonance. The acetyl methyl singlet appears at an average chemical shift of δ 2.52 (within a range of δ 2.40–2.63), and the iminium methyl singlet appears at an average chemical shift of δ 2.85 (within a range of δ 2.60–2.97). The average anisochronism[7] between these methyl singlets of 0.33 ppm is characteristic of the *inter* isomers of *N*-alkyl rhena β-keto imines.[3] As expected, the iminium ligand NH resonance appears at an average chemical shift of δ 9.50 (within a range of δ 8.50–10.05).

For the *intra* isomers, where the amino acid residue is cis to the iminium methyl substituent, the iminium NH resonance appears, as expected, at a much lower chemical shift. The average value for this resonance is δ 13.33 (within a range of δ 12.70–13.85). Except for complexes **6**, **8**, **13**, and **14**, the acetyl methyl singlet appears at an average chemical shift of δ 2.63 (within a range of δ 2.52–2.69) and the iminium methyl resonance appears at lower field with an average chemical shift of δ 2.75 (within a range of δ 2.70–2.82). The observed smaller anisochronism of 0.12 ppm between these rhenaacetylacetone imine methyl resonances for the *intra* isomers as compared to that observed for the *inter* isomers is an expected trend.[3]

For the *intra* isomers of complexes **6**, **8**, **13** and **14**, the acetyl methyl resonance appears at the expected chemical shift (average value is δ 2.62 within a range of δ 2.52–2.69). However, the iminium methyl resonances move to higher field than the acetyl methyl resonances, thus reversing the normal pattern, and appear at an average chemical shift of δ 2.27 (within a range of δ 2.10–2.52). This pattern reversal is always observed for *intra* isomers that contain an aromatic substituent on the carbon β to the iminium nitrogen atom, as discovered previously.[8]

The ^{1}H NMR resonances for protons within the amino acid ester residues of complexes **4–19** are generally shifted to lower field from the corresponding resonances of the amino acid ester free base. This low-field shift is greatest for the amino protons and the methine proton on the dissymmetric carbon atom for the α-labeled compounds, as illustrated by the methyl valinate complex (**4**). For methyl L-valinate free base, the amino protons and the methine proton on the dissymmetric carbon have resonances at δ 1.44 (relatively sharp singlet) and δ 3.24 (doublet), respectively, in $CDCl_3$ solution. In the *intra* and *inter* isomers of **4**, the N–H resonance shifts to δ 13.42 and 9.22 (broad singlets), respectively, and the methine proton resonance shifts to δ 4.40 and 4.70, respectively, in $CDCl_3$ solution. The methine proton resonances appear as a doublet of doublets because of coupling to both the iminium NH proton and the isopropyl methine proton. The observed low-field shift of the methine proton resonance by over 1 ppm and the additional coupling of this resonance to the iminium NH proton are diagnostic indications of the formation of a rhena β-keto imine derivative of an amino acid residue.

Discussion

The successful formation of complexes **4–19** demonstrates a general method for preparing heavy-atom-labeled derivatives of amino acid residues. Schiff-base condensation of **1** with primary amino groups occurs with (1) ethyl glycinate or al-

(6) The abbreviations *t*-BOC and CBZ refer to the amino-protecting groups *tert*-butoxycarbonyl and carbobenzoxy, respectively.

(7) Jennings, W. B. *Chem. Rev.* **1975**, *75*, 307–322.
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kyl-substituted α-amino acid esters to give complexes **2–6**, (2) the hydroxylated amino acid esters Ser(OEt) and Tyr(OEt) to give **7** and **8**, (3) the mercaptan Cys(OEt) to give **9**, (4) the disulfide and sulfide Cys-Cys$(OMe)_2$ and Met(OEt) to give **10** and **11**, (5) the carboxy amino acid diester Asp$(OMe)_2$ to give **12**, and (6) the basic amino acids His(OMe), Trp(OEt), and various lysine derivatives to give complexes **13–19**. Of these diverse functional groups, only the acidic hydroxyl group of tyrosine interfered with the Schiff-base condensation in CH_2Cl_2 solution. However, in DMF solution, the condensation of Tyr(OEt) proceeds in good yield. Complexes **2–16** are now available as α-amino-protected amino acid residues, which can be incorporated into larger peptides by normal coupling procedures.[1]

Condensation of **1** with ethyl L-lysinate affords a 1:1 mixture of **15** and **16** where either the α- or ε-amino groups have reacted to form a rhena β-keto imine. An important objective of our effort to label biologically important molecules with heavy atoms is the type of distal labeling shown in complexes **16–19**. In these compounds, the rhena β-keto imine label is appended onto the amino acid side chain, R, thereby not interfering with chemical modifications at the N or C termini of the amino acid residue. Compounds **16–19** have the C terminus protected as the ethyl ester, while the N terminus is either unprotected or protected by acetyl, *t*-BOC, or CBZ groups.

Since peptides such as N^{α}-Ac-N^{ϵ}-Ac-L-Lys-D-Ala-D-Ala are known to bind to antibiotics such as vancomycin (presumably through the D-Ala-D-Ala moiety[9]), the distal-labeled tripeptide N^{α}-Ac-N^{ϵ}-[Re]-L-Lys-D-Ala-D-Ala is expected to bind similarly. Work on these more ambitious projects is in progress.

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Registry No. **1**, 59299-78-4; *inter*-**4**, 87681-84-3; *intra*-**4**, 87727-40-0; *inter*-**5**, 87681-85-4; *intra*-**5**, 87727-41-1; *inter*-**6**, 87681-86-5; *intra*-**6**, 87727-42-2; *inter*-**7**, 87681-87-6; *intra*-**7**, 87758-26-7; *inter*-**8**, 87681-88-7; *intra*-**8**, 87727-43-3; *intra*-**9**, 87681-89-8; *intra*-**10**, 87681-90-1; *inter*-**11**, 87681-91-2; *intra*-**11**, 87727-44-4; *inter*-**12**, 87681-92-3; *intra*-**12**, 87758-27-8; *intra*-**13**, 87681-93-4; *inter*-**14**, 87681-94-5; *intra*-**14**, 87727-45-5; *intra*-**15**, 87681-95-6; *inter*-**16**, 87681-96-7; *intra*-**16**, 87727-46-6; *inter*-**17**, 87681-97-8; *intra*-**17**, 87727-47-7; *inter*-**18**, 87681-98-9; *inter*-**19**, 87696-30-8; *intra*-**19**, 87758-28-9; L-valine methyl ester, 4070-48-8; L-leucine ethyl ester, 2743-60-4; L-phenylalanine ethyl ester, 3081-24-1; L-serine ethyl ester, 4117-31-1; L-tyrosine ethyl ester, 949-67-7; L-cysteine ethyl ester, 3411-58-3; L-cystine dimethyl ester, 1069-29-0; L-methionine ethyl ester, 3082-77-7; DL-aspartic acid dimethyl ester, 40149-67-5; L-histidine methyl ester, 1499-46-3; L-tryptophan ethyl ester, 7479-05-2; L-lysine ethyl ester, 4117-33-3; N^2-acetyl-L-lysine methyl ester, 6072-02-2; N^2-[(1,1-dimethylethoxy)carbonyl]-L-lysine ethyl ester, 87681-83-2; N^2-[(phenylmethoxy)carbonyl]-L-lysine ethyl ester, 52396-41-5.

Supplementary Material Available: Tables of microanalytical data and IR and ^1H NMR spectroscopic data for compounds **4–19** (5 pages). Ordering information is given on any current masthead page.

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Axial Ligand Derivatives of a Zinc(II) Tetraimine Macrocyclic Complex

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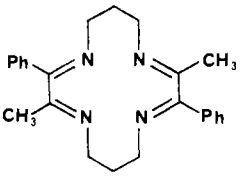
The synthesis of axial ligand derivatives of the macrocyclic complex (2,9-dimethyl-3,10-diphenyl-1,4,8,11-tetraazacyclotetradeca-1,3,8,10-tetraene)zinc(II) with one axial ligand (Cl^-, Br^-, I^-, pyridine, or dimethylbenzimidazole) or two axial ligands (both I^-) is reported. The compounds were characterized by elemental analyses, conductivity measurements, and spectral measurements (infrared, UV–visible, proton and carbon-13 NMR, and mass spectra). The major difference between the zinc complexes and the iron, cobalt, and nickel complexes of the same macrocyclic ligand is the reduced importance of metal–ligand π back-bonding. With the exception of the imine carbon resonances, the NMR spectra of the derivatives are insensitive to changes in axial ligation and coordination number. The imine carbon chemical shifts, however, span a range of 5 ppm and are affected most by coordination number and to a lesser extent by axial ligand.

Introduction

The template condensation of α-diketones with 1,3-diaminopropane in the presence of divalent iron, cobalt, nickel, or copper leads to 14-membered-ring macrocyclic complexes containing two α-diimines. A number of complexes of this class, derived from biacetyl,[1-3] 1-phenyl-1,2-propanedione,[4-6] benzil,[7,8] and substituted benzils[4] have been prepared. Under the template reaction conditions with 1-phenyl-1,2-propanedione as ketone, zinc(II) behaves differently, causing rapid polymerization of the reagents and leading ultimately to deep red polymers but no macrocyclic complex. Therefore, a nontemplate method similar to that reported by Gagné[9] was used by Coltrain[6] to prepare the zinc complex of MePhTIM.



MePhTIM

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