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Reactions of Coordinated Molecules. 47. Preparation of Rhena-β-ketoimine Derivatives of Iodoacetamide and of an *N*-Hydroxysuccinimide Ester: Heavy-atom Labeling Reagents for Sulfhydryl and Amino Groups

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Abstract

A rhena-β-ketoimine complex containing an iodoacetamide moiety has been prepared as a reagent for effecting heavy-atom labeling of molecules containing sulfhydryl groups. This complex reacts with benzyl mercaptan, ethanethiol, *N*-t-BOC-cystamine, *N*-acetyl-L-cysteine, and *N*-acetyl-DL-penicillamine to give the corresponding rhena-labeled derivatives. A rhena-β-ketoimine containing a free carboxylic acid moiety has been converted to its *N*-hydroxysuccinimide ester. This reagent should be useful in effecting the heavy-atom labeling of molecules containing primary and secondary amino groups.

Introduction

The development of synthetic methods for the heavy-atom labeling of biologically-important molecules is a topic of current interest. Such labeled compounds are particularly useful in the detection and structural analysis of the derivatized molecule. In previous work, we have prepared rhena-β-ketoimine derivatives of several amino acid esters and biologically-important molecules containing 2-ethylamino groups [1–3]. These molecules are labeled with a rhenium organometallic moiety *via* a Schiff-base condensation between a rhena-β-diketone and a primary amino group on the substrate molecule. The resulting rhena-β-ketoimine products represent rhenium-labeled compounds having good chemical stability and a strong covalent link between the rhenium moiety and the substrate molecule. Furthermore, because of the apparent similarity of Re and Tc organometallic chemistry, the possibility of preparing radiolabeled derivatives by a similar method also exists.

To overcome the limitations of choice of solvent and the strong basicity of the primary amine in the above mentioned Schiff-base reactions, we devised a new strategy for preparing rhenium-labeled molecules. In this approach, rhena-β-ketoimine moieties are attached to reagent molecules that are known to react with specific functional groups of substrate molecules, such as sulfhydryl and amino groups. We now report the successful preparation of rhena-β-ketoimine derivatives of iodoacetamide and of an *N*-hydroxysuccinimide ester in non-aqueous media. Haloacetamides have been used to attach spin labels to sulfhydryl groups in biopolymers, such as hemoglobin and nucleotides [4–8]. *N*-hydroxysuccinimide esters have been widely used as reagents in peptide synthesis [9, 10].

Experimental

All reactions and other manipulations were performed under a nitrogen atmosphere. All solvents were dried under a nitrogen atmosphere and were freshly distilled before use. Benzyl mercaptan, chloroacetyl chloride, ethanethiol and triethylamine were distilled before use. *N*-methyl ethylenediamine, *N*-acetyl-L-cysteine and *N*-acetyl-DL-penicillamine were purchased from Aldrich Chemical Company and used without any further purification. Dicyclohexylcarbodiimide (DCC) was purchased from Sigma Chemical Company. The rhenaacetylacetone complex **1** was prepared by a literature method [11].

Infrared spectra were recorded on a Perkin-Elmer 727 spectrophotometer as solutions in 0.10 mm sodium chloride cavity cells using the solvent as a reference and a polystyrene film as a calibration standard. Band frequencies are reported in cm^{-1}.

Routine ^1H NMR spectra were obtained either on a JEOL MH-100 NMR spectrometer or on a JEOL FX-90Q NMR spectrometer using TMS as an internal reference. High resolution 400 MHz ^1H NMR spectra were obtained on a Bruker AM-400 NMR spectrometer. FAB spectra were obtained on a VG 70/250 GC/MS system.

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Preparation of {cis-(OC)$_4$Re[CH$_3$C(O)][CH$_3$CN-(CH$_2$CH$_2$NHMe)(H)]} (2)

To 0.50 g of complex **1** dissolved in 10 ml of methylene chloride was added 1.25 molar equivalents of *N*-methyl ethylenediamine at 25 °C. After stirring the reaction solution for 12 h the solvent was removed at reduced pressure. The reaction residue was extracted three times with 25 ml of 4:1 hexane–methylene chloride solution. After filtration, the solution was concentrated at reduced pressure to afford 0.42 g (73%) of **2** as a golden yellow liquid: IR (C$_6$H$_{12}$)ν(CO) 2075(m), 1980(s), 1970(vs), 1940(s), ν(C···O, C···N) 1560(m); ^{1}H NMR (CDCl$_3$)δ 1.41 (br s, 1, *H*NMe), 2.53 (s, 3, MeN), 2.59 (s, 3, CH$_3$CO), 2.68 (s, 3, CH$_3$CN), 3.04 (t, 2, *CH$_2$*NHMe, J = 7 Hz), 3.60 (q, 2, *CH$_2$*CH$_2$N, J = 7 Hz), 12.68 (br s, 1, $^+$NH). *Anal.* Calc. for C$_{11}$H$_{15}$N$_2$O$_5$Re: C, 29.91; H, 3.43; N, 6.34. Found: C, 29.78; H, 3.54; N, 6.47%.

Preparation of {cis-(OC)$_4$Re[CH$_3$C(O)][CH$_3$CN-(CH$_2$CH$_2$NMeCOCH$_2$Cl)(H)]} (3)

To a solution of 0.34 g of complex **2** in 15 ml of methylene chloride at 0 °C were added 2 molar equivalents of triethylamine and 1 molar equivalent of chloroacetyl chloride. After 30 min the ice–water bath was removed and the reaction mixture was allowed to warm up to room temperature. The solvent was removed at reduced pressure. The reaction residue was extracted with 25 ml of 4:1 hexane–methylene chloride solution and filtered. The filtrate was concentrated to give 0.27 (68%) of **3** as a yellow oil. This oil was dissolved in 3 ml of CH$_2$Cl$_2$ and chromatographed on a short Florisil column (7 cm × 1 cm) prepared in hexane. Complex **3** was eluted from the column with THF: IR (CH$_2$Cl$_2$) ν(CO) 2075(m), 1980(br, vs), 1940(s), ν(C···O, C···N), 1550(m); H^1 NMR (CDCl$_3$)δ 2.59 (s, 3, CH$_3$CO), 2.73 (s, 3, CH$_3$CN), 3.21 (s, 3, MeN), 3.77 (br, 4, CH$_2$CH$_2$), 4.07 (s, 2, CH$_2$Cl), 13.09 (br, 1, $^+$NH). *Anal.* Calc. for C$_{13}$H$_{16}$N$_2$O$_6$ClRe: C, 30.13; H, 3.12; N, 5.41. Found: C, 30.16; H, 3.40; N, 5.18%.

Preparation of {cis-(OC)$_4$Re[CH$_3$C(O)][CH$_3$CN-(CH$_2$CH$_2$NMeCOCH$_2$I)(H)]} (4)

To a solution of 0.16 g of complex **3** in 5 ml of acetone was added 1.1 molar equivalent of NaI in acetone at 0 °C. After an hour the ice–water bath was removed and the reaction mixture was stirred at room temperature for three h. The solvent was removed at reduced pressure to yield complex **4** as a dark yellow oil. The complex **4** is air sensitive and was used *in situ* as a reagent.

Preparation of {cis-(OC)$_4$Re[CH$_3$C(O)][CH$_3$CN-(CH$_2$CH$_2$NMeCOCH$_2$SCH$_2$C$_6$H$_5$(H)]} (5)

The rhenium iodoacetamide complex, **4**, was prepared from 0.16 g of complex **3** using the procedure described above. Complex 4 was dissolved in 5 ml of CH$_2$Cl$_2$ and cooled to 0 °C. To this solution were added 1.1 molar equivalents of triethylamine and 1.1 molar equivalents of benzyl mercaptan. After 10 min the ice–water was removed, and the reaction mixture was stirred at room temperature. After 18 h the solvent was removed at reduced pressure. The reaction residue was extracted with 30 ml of 2:1 hexane–methylene chloride solution and was filtered. The filtrate was concentrated and then chromatographed on a short Florisil column that was packed in hexane. Elution with THF gave 0.096 g (51%) of complex **5** as an oil: IR (CH$_2$Cl$_2$)ν((CO) 2075(m), 1980(br, vs), 1940(s), ν(C···O, C···N), 1550(m); ^{1}H NMR (CDCl$_3$)δ 2.58 (s, 3, CH$_3$CO), 2.72 (s, 3, CH$_3$CN), 3.10 (s, 3, MeN), 3.18 (s, 2, OCCH$_2$S), 3.72 (br, 4, CH$_2$CH$_2$), 3.81 (s, 2, SCH$_2$Ph), 7.37 (m, 5, C$_6$H$_5$), 13.10 (br, 1, $^+$NH). *Anal.* Calc. for C$_{20}$H$_{23}$N$_2$SO$_6$Re: C, 39.64; H, 3.83; N, 4.62. Found: C, 39.51; H, 4.09; N, 4.74%.

Preparation of {cis-(OC)$_4$Re[CH$_3$C(O)][CH$_3$CN-(CH$_2$CH$_2$NMeCOCH$_2$SC$_2$H$_5$)(H)]} (6)

The rhenium iodoacetamide complex, 4, was prepared from 0.20 g of complex **3** using the procedure described above. Complex **4** was dissolved in 5 ml of CH$_2$Cl$_2$ and cooled to 0 °C. To this solution 1.1 molar equivalents of triethylamine and 1.1 molar equivalents ethanethiol were added. After 10 min the ice–water bath was removed, and the reaction mixture was stirred at 25 °C. After 18 h the solvent was removed at reduced pressure. The reaction residue was extracted with 45 ml of 2:1 hexane–methylene chloride solution and filtered. The filtrate was concentrated and chromatographed on a short Florisil column that was packed in hexane. Elution with THF gave 0.13 g (62%) of complex **6** as an oil: IR (CH$_2$Cl$_2$)ν(CO) 2075(m), 1980(br, vs), 1945(s), ν(C···O, C···N) 1560(m); ^{1}H NMR (CDCl$_3$)ν 1.28 (t, 3, CH$_3$CH$_2$, J = 7 Hz), 2.59 (s, 3, CH$_3$CO), 2.65 (q, 2, *CH$_2$*CH$_3$, J = 7 Hz), 2.72 (s, 3, CH$_3$CN), 3.19 (s, 3, MeN), 3.31 (s, 2, SCH$_2$CO), 3.74 (br m, 4, CH$_2$CH$_2$), 13.13 (br, 1, $^+$NH). *Anal.* Calc. for C$_{15}$H$_{21}$N$_2$SO$_6$Re: C, 33.12; H, 3.90; N, 5.15. Found: C, 32.94; H, 4.07; N, 5.25%.

Preparation of {cis-(OC)$_4$Re[CH$_3$C(O)][CH$_3$CN-(CH$_2$CH$_2$NC$_{10}$H$_{19}$SNO$_3$)(H)]} (7)

The rhenium iodoacetamide complex, 4, was prepared from 0.30 g of complex **3** using the procedure described above. Complex **4** was dissolved in 15 ml of CH$_2$Cl$_2$ and cooled to 0 °C. To this solution 1.5 molar equivalents of Et$_3$N and 1.5 molar equivalents of (H$_3$C)$_3$COC(O)NHCH$_2$CH$_2$SH, both dissolved in CH$_2$Cl$_2$, were added. After 20 min the ice–water bath was removed, and the reaction mixture was allowed to stir at 25 °C. After 2.5 h the solvent was removed at reduced pressure. The reaction residue was dissolved in a minimum volume of CH$_2$Cl$_2$ and

chromatographed on a Florisil column that was packed in hexane. Complex 7, contaminated with some impurities, was eluted off the column with THF. Complex 7 was further purified by fractional precipitation. Yield 0.18 g (47%): IR (CH_2Cl_2)ν(CO) 2075(m), 1980(br, vs), 1940(s), ν(C···O, C···N) 1550(m); ^{1}H NMR ($CDCl_3$)δ 1.44 (s, 9, t-Bu), 2.58 (s, 3, CH_3CO), 2.72 (s, 3, CH_3CN), 3.19 (s, 3, MeN), 3.35 (s, 2, SCH_2CO), 3.77 (br, m, 4, $^+NCH_2CH_2N$), 5.05 (br, 1, NH-t-Boc), 12.93 (br, 1, $^+$NH). Complex multiplets at δ 2.80 and δ 3.40 may be due to the SCH_2CH_2 and CH_2CH_2NH-tBoc protons, respectively. *Anal.* Calc. for $C_{20}H_{30}N_3SO_8Re$: C, 36.47; H, 4.59; N, 6.38. Found: C, 37.88; H, 4.70; N, 6.41%.

Preparation of {cis-(OC)$_4$Re[CH$_3$C(O)][CH$_3$CN-(CH$_2$CH$_2$NC$_8$H$_{13}$SNO$_4$)(H)]} (8)

The rhenium iodoacetamide complex, **4**, was prepared from 0.30 g of complex **3** using the procedure described above. Complex **4** was dissolved in 20 ml of CH_2Cl_2 and cooled to 0 °C. To this solution 1.1 molar equivalents of triethylammonium *N*-acetyl-L-cysteinate, dissolved in methylene chloride, was added. After stirring at 0 °C for 3 min, the reaction mixture was stirred at room temperature for 3 h. The solvent was removed at reduced pressure. To the reaction residue were added 50 ml of methylene chloride and 20 ml of deionized water. After stirring vigorously for 3 min, the methylene chloride layer was separated from the aqueous layer and dried over $MgSO_4$. Removal of methylene chloride at reduced pressure and crystallization of the product from a hexane–methylene chloride mixture at −20 °C afforded 0.16 g (49%) of complex **8** as colorless microcrystals: melting point (m.p.) 74–77 °C; IR (CH_2Cl_2)ν(CO) 2075(m), 1990(br, vs), 1940(s), ν(C···O, C···N) 1550(m). ^{1}H NMR ($CDCl_3$)δ 2.09 (s, 3, CH_3CONH), 2.60 (s, 3, CH_3CO), 2.75 (s, 3, CH_3CN), 3.08 (d, 2, SCH_2CH, J = 7 Hz), 3.18 (s, 3, MeN), 3.52 (s, 2, SCH_2CO), 3.80 (br m, 4, CH_2CH_2), 4.81 (q, 1, CH_2CHNHAc, J = 6 Hz), 7.64 (d, 1, CHN*H*Ac, J = 7 Hz), 10.80 (br, 1, COOH), 12.80 (br, 1, $^+$NH). *Anal.* Calc. for $C_{18}H_{24}N_3SO_9Re \cdot 2H_2O$: C, 31.76; H, 4.15; N, 6.17. Found: C, 31.90 H, 3.80; N, 6.18%. FAB mass spectral data: FAB^+, MH^+ = 646; FAB^-, $(M-H)^-$ = 644, plus fragment ion cascade.

Preparation of {cis(OC)$_4$Re[CH$_3$C(O)][CH$_3$CN-(CH$_2$NC$_{10}$H$_{17}$SNO$_4$)(H)]} (9)

The rhenium iodoacetamide complex, **4**, was prepared from 0.30 g of complex **3** using the procedure described above. Complex **4** was dissolved in 20 ml of CH_2Cl_2 and cooled to 0 °C. To this solution 1.1 molar equivalents of the triethylammonium salt of *N*-acetyl-DL-penicillamine, dissolved in methylene chloride, was added. After stirring at 0 °C for about 3 min, the reaction mixture was stirred at room temperature for 2 h. The solvent was removed at reduced pressure. To the reaction residue were added 50 ml of CH_2Cl_2 and 10 ml of deionized water. After stirring vigorously for 3 min, the methylene chloride layer was separated from the aqueous layer and dried over $MgSO_4$. Removal of methylene chloride at reduced pressure and crystallization of the product in a mixture of hexane–methylene chloride at −20 °C gave 0.25 g (64%) of complex **9**: m.p. 160–163 °C; IR (CH_2Cl_2)ν(CO) 2075(m), 1980(br, vs), 1940(s), ν(C···O, C···N), 1550(m); ^{1}H NMR ($CDCl_3$)δ 1.33 and 1.52 (s, *s*, 6, Me_2C), 2.06 (s, 3, CH_3CONH), 2.59 (s, 3, CH_3CO), 2.75 (s, 3, CH_3CN), 3.21 (s, 3, MeN), 3.51 and 3.57 (s, *s*, 2, SCH_2CO), 3.85 (m, 4, CH_2CH_2), 4.73 (d, 1, *CH*NHAc, J = 9 Hz), 7.16 (d, 1, CHN*H*Ac, J = 9 Hz), 12.68 (br, 2, COOH and $^+$NH). *Anal.* Calc. for $C_{10}H_{28}N_3O_9SRe$: C, 35.69; H, 4.20; N, 6.25. Found: C, 35.35; H, 4.04; N, 5.97%.

Preparation of {cis-(OC)$_4$Re[CH$_3$C(O)][CH$_3$CN-(CH$_2$CONC$_4$H$_4$O$_2$)(H)]} (11)

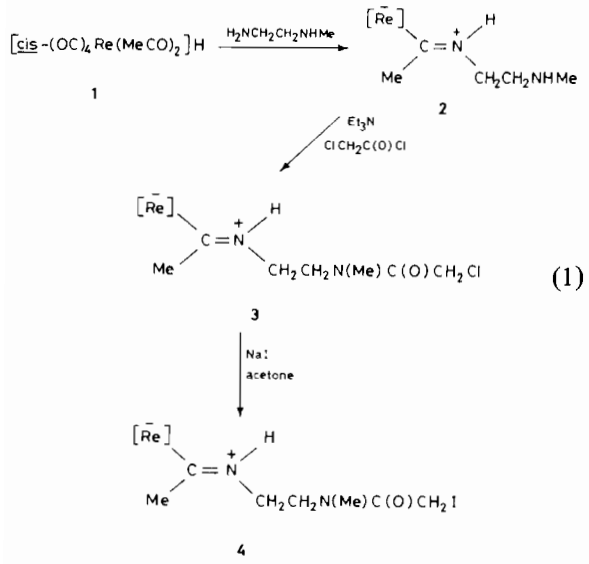
The rhenaacetylacetonimine derivative of acetic acid, **10**, [1] was dissolved in 5 ml of THF and was treated sequentially with DCC and *N*-hydroxysuccinimide following a previously reported procedure [1]. Complex **11** was isolated as a pure yellow oil (40% yield) by precipitation from CH_2Cl_2/hexane solution at −15 °C: IR (CH_2Cl_2)ν(CO) 2075(m), 1980(br vs), 1940(s), ν(C···O, C···N) 1550(m), ν(amide CO) 1740(m); ^{1}H NMR ($CDCl_3$)δ 2.66 (s, 3, CH_3CO), 2.83 (s, 3, CH_3CN), 2.91 (s, 4, $OCCH_2CH_2CO$), 4.73 (d, 2, $^+NCH_2CO_2$), 13.99 (br s, 1, $^+$NH). *Anal.* Calc. for $C_{14}H_{13}N_2O_9Re$: C, 31.15; H, 2.43; N, 5.19. Found: C, 31.44; H, 2.66; N, 5.24%.

High-field ^{1}H NMR spectra were recorded for compounds **3**, **6** and **7**–**9**. The purity of all new complexes was determined by ^{1}H NMR and microanalysis to be greater than 97%.

Results and Discussion

A rhena-β-ketoimine derivative of iodoacetamide is prepared by standard methods beginning with the rhenaacetylacetone complex **1**, eqn. (1); notice that [$\overline{Re}$] = *cis*-(OC)$_4\overline{Re}$(MeCO). Schiff-base condensation of **1** with *N*-methyl ethylenediamine gives **2** in 73% yield. Complex **2** is converted into the chloroacetamide derivative **3** in 68% yield upon treatment with Et_3N and chloroacetyl chloride. A Finkelstein substitution reaction of **3** with NaI gives the rhena-β-ketoimine complex **4** containing an iodoacetamide functional group. Because of the high reactivity of **4**, it is prepared from **3** and then used *in situ*.

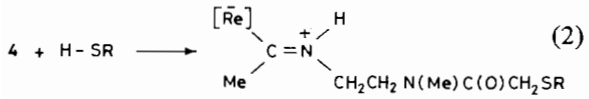
To demonstrate that **4** reacts with sulfhydryl groups to label the sulfur atom with a rhena-β-ketoimine moiety, complex **4** was treated with benzyl mercaptan, ethanethiol, *N*-t-BOC-cystamine, *N*-acetyl-L-cysteine and *N*-acetyl-DL-penicillamine, eqn. (2).



(1)

The corresponding products **5**–**9**, respectively, are isolated as yellow oils (**5**–**7**) or as yellow solids (**8** and **9**) in 47–64% yield.

IR data for the complexes **2**, **3** and **5**–**9** reveal the expected CO stretching bands for a *cis*-$(OC)_4Re$ moiety, and the appropriate C···O, C···N stretching bands for the acyl and iminium ligands. In addition, amide carbonyl stretching bands are observed for compounds **3** and **5**–**9**.



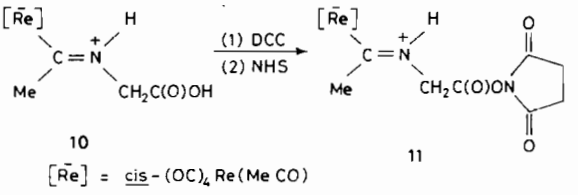
(2)

R	Compound
CH_2Ph	5
Et	6
$CH_2CH_2N(H)CO_2CMe_3$	7
$CH_2CH(CO_2H)N(H)C(O)Me$	8
$CMe_2CH(CO_2H)N(H)C(O)Me$	9

^1H NMR data for compounds **2**, **3** and **5**–**9** are also consistent with the structures shown. For the rhena-β-ketoimine fragments, the acetyl methyl resonances appear as singlets within the range of δ 2.58–2.60, the iminium methyl resonances appear as singlets within the range of δ 2.68–2.75, and the iminium N–H resonances appear as broad singlets within the range of δ 12.68–13.13. These latter chemicals shifts confirm the presence of only the intraisomers, as shown in eqns. (1) and (2) [12]. High-field ^1H NMR spectral data for compounds **3**, **6** and **7**–**9** have been used to determine peak assignments. Complexes **3** and **6** exist as two geometrical isomers due to restricted rotation about the amide C–N bond. Similarly, compounds **7**–**9** exist as more than one amide geometrical isomer because of the presence of two amide groups within these molecules.

Complexes **8** and **9** contain a free acid functionality, and both compounds are hygroscopic. Complex **8** forms a stable dihydrate when exposed to air, while compound **9** forms a monohydrate under similar conditions.

To further extend our strategy of preparing rhena-β-ketoimines containing reactive functional groups capable of labeling specific substituents of substrate molecules, we prepared the rhena *N*-hydroxysuccinimide (NHS) ester **11** from the known rhena acid **10**, eqn. (3), [1]. Complex **11** is isolated as a yellow oil in 40% yield.



NHS esters, like that contained in **11**, are activated toward condensation with primary and secondary amino groups to give amide products [9, 10]. We believe that **11** might be a useful reagent for preparing rhena-β-ketoimine derivatives of biologically-important amines. The stability of rhena-β-ketoimines containing amide substituents has been verified through the preparation of complexes **3**–**9**.

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