Available online at www.sciencedirect.com
ScienceDirect
Journal of Organometallic Chemistry 692 (2007) 1392–1397
Journal of Organometallic Chemistry
www.elsevier.com/locate/jorganchem

The acetyl CoA synthase paradigm for hybrid bio-organometallics: Quantitative measures for resin-bound Ni–Rh complexes

Kayla N. Green [a], William D. James [b], Angelica V. Cantillo [a], Marcetta Y. Darensbourg [a,*]

[a] Department of Chemistry, Texas A&M University, College Station, TX 77845, United States
[b] Center for Chemical Characterization and Analysis, Department of Chemistry, Texas A&M University, College Station, TX 77845, United States

Received 16 October 2006; received in revised form 23 October 2006; accepted 24 October 2006
Available online 3 November 2006

Abstract

The active site of Acetyl CoA Synthase utilizes a square planar NiN_2S_2 complex in the form of $Ni^{II}(CGC)^{2-}$ (CGC = the cysteine–glycine–cysteine tripeptide motif within the protein) to serve as a bidentate sulfur-donor ligand to chelate a second, catalytically active Ni atom responsible for the C–C and C–S coupling reactions for the production of Acetyl CoA. Metalloenzymes, such as this, which house stable catalytic complexes within intricately designed pockets accessible by solvent channels, have inspired design of resin-bound complexes. Through the use of TentaGel S-RAM® resin beads, the **O**-$Ni(CGC)^{2-}$ ligand has been synthesized and derivatized with the $Rh^{I}(CO)_2$ moiety. The identification of the **O**-$Ni(CGC)Rh(CO)_2^{1-}$ adduct on these resin beads is afforded by attenuated total reflectance FTIR spectroscopy in the ν(CO) region and compared to solution analogues. The goal of this study is to establish a quantitative measure of the loading of nickel and rhodium on the tripeptide modified resin beads, **O**-(CGC). The extent of CGC derivatization was determined by Fmoc cleavage of the Fmoc protected **O**-(CGC). Nickel and rhodium loading were determined by Neutron Activation Analysis. This work provides evidence that the TentaGel S-RAM® resin beads greatly decrease the air sensitivity of the Ni–Rh complex as compared to the unsupported solution phase analogue. The derivatized beads have also been studied for their ability to withstand a number of physical stresses, i.e., for leaching.

Keywords: Nickel; Rhodium; Resin beads; TentaGel; Acetyl CoA Synthase; Tripeptide

1. Introduction

Metalloenzymes, evolutionarily optimized natural catalysts, utilize polypeptides as both ligands to the catalytic metal and as superstructures housing the catalytic site, thus creating a system superior to any manmade analogues. As inspired by nature, bioorganometallic hybrids of synthetic catalysts imbedded in proteins have been designed in an attempt to take advantage of substrate selectivity features of the protein [1]. For example, Ward and co-workers have shown that insertion of a rhodium–diphosphine complex directly into the cavity of (Strept)avidin will enable enantioselective hydrogenation chemistry [2]. These investigations coincide with the expanding field of metallopeptide complexes as small molecule mimics of enzyme active sites [3]. A prime example is the recently reported synthesis of $Ni(CGC)^{2-}$, a component in the distal site of the A-cluster found in Acetyl CoA Synthase, ACS (Fig. 1a) [4,5]. Through its cysteinyl sulfurs, $Ni(CGC)^{2-}$ is proposed to serve as a ligand to the catalytically active nickel which mediates the synthesis of Acetyl CoA [6].

We have explored the binding of the $Ni(CGC)^{2-}$ ligand to metal carbonyl fragments in order to establish the relative electron donating ability of the natural metallodithiolate ligand with synthetic NiN_2S_2 *cis*-dithiolates including

Abbreviations: $Ni(ema)^{2-}$, (*N*,*N*′-ethylenebis-2-mercaptoacetamide) nickel(II); Ni(bmedaco) = Ni-1, (*N*-*N*′-bis-2-mercaptoethyl-*N*-*N*′-diazocyclooctane) nickel(II); Fmoc, 9-fluorenylmethoxycarbonyl; Rink linker, trialkoxybenzhydrylamine; Mmt, (4-methoxytrityl).

* Corresponding author. Tel.: +1 979 845 2981; fax: +1 979 8445 0158.
E-mail address: marcetta@mail.chem.tamu.edu (M.Y. Darensbourg).

0022-328X/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jorganchem.2006.10.051

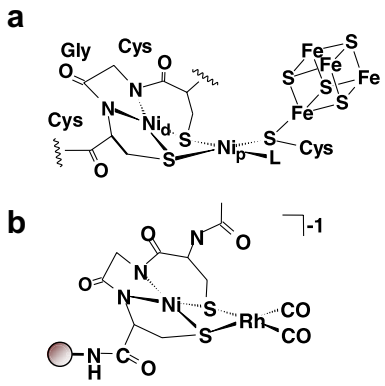


Fig. 1. (a) Representation of the A-Cluster active site of Acetyl CoA Synthase [5] and (b) presumed structure of ACS-inspired complex immobilized on TentaGel resin [8].

the dianionic $Ni(ema)^{2-}$ as well as traditional diphosphine and diimine ligands, with results shown in Fig. 2 [7]. We have also shown that the $[Ni(CGC) \cdot M(CO)_x]^{n-}$ adducts can be produced while still anchored to the TentaGel S-RAM® support utilized for the synthesis of the CGC tripeptide [8]. Preliminary investigations followed the addition of $M(CO)_x$ moieties, $Rh^{I}(CO)_2$ and $W^{0}(CO)_5$, to **O**-$Ni(CGC)^{2-}$ and characterized the adducts by their ν(CO) vibrations established via ATR-FTIR (attenuated total reflectance-FTIR) spectroscopy.

The TentaGel resin beads utilize a cross-linked polystyrene (PS) backbone derivatized with polyethyleneglycol (PEG) [9]. These PEG moieties are responsible for the excellent swelling of the resins in a range of solvents and they also produce channels and micro-porous domains, reminiscent of pockets surrounding enzyme active sites [9a]. The properties of these supports lead to applications that exist beyond peptide synthesis. For example, small enzymes have been shown to permeate and carry out

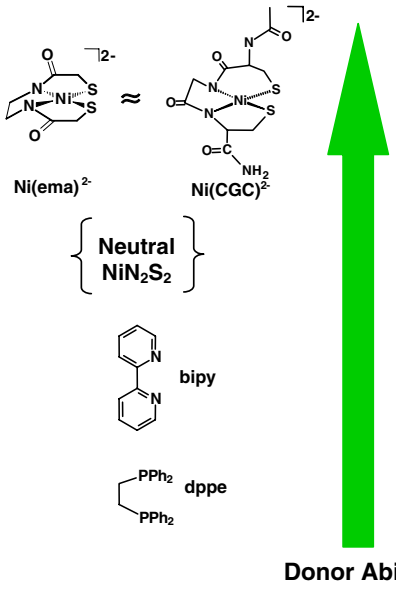


Fig. 2. Electron donating ability of $Ni(CGC)^{2-}$ in comparison to other NiN_2S_2 ligands [7].

catalytic activities on resin-bound substrate inside TentaGel resins [10]. In the case of **O**-$Ni(CGC)^{2-}$ we have noted air stability of the resin-bound complex which contrasts to the air sensitivity of the $Ni(CGC)^{2-}$ in solution. This conclusion was based on color retention in the presence of air, the retrieval of colorless solutions when the beads were swollen, and consistent results when the beads were derivatized with the $M(CO)_x$ fragment of choice.

In order to have a greater knowledge of the extent to which sites are available for incorporating the bioorganometallic hybrids into the resin beads, a quantitative approach to establishing resin-attached CGC, Ni and Rh concentrations has been developed. In the following report we describe a sequential approach to quantification of the loading of the CGC tripeptide, the incorporation of Ni into this tripeptide and the attachment of $Rh(CO)_2^+$ to the **O**-$Ni(CGC)^{2-}$ as well as the stability of the **O**-Ni(CGC) $Rh(CO)_2^+$ adduct. Such studies are a reference point for future explorations of heterogeneous reaction chemistry based on the nickel tripeptide as an anchor for resin-bound bio-inspired organometallic complexes with catalytic potential.

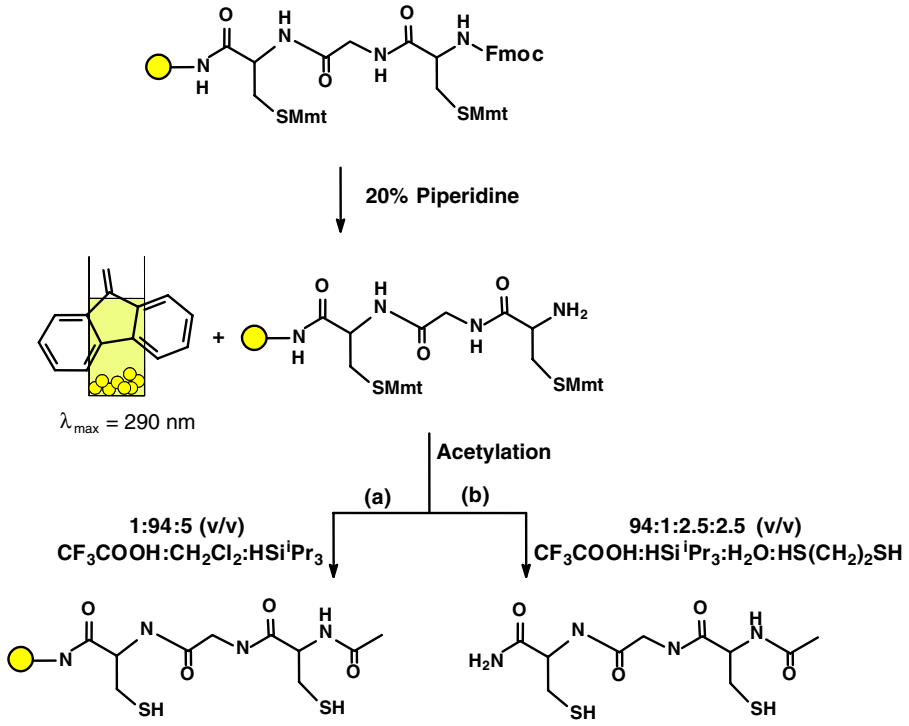
2. Results and discussion

2.1. Loading of Ni on *O*-Ni(CGC)$^{2-}$

The extent to which the resin beads were derivatized with the Cys–Gly–Cys tripeptide, further loaded with nickel(II), and subsequently with rhodium(I) was evaluated according to the following protocol. As shown in Scheme 1, the solid phase synthesis of the tripeptide resulted in Mmt-protected cysteinyl sulfurs and an Fmoc-protected terminal amino group. Deprotection by piperidine induces release of the Fmoc product, dibenzofulvene, which produced a pale yellow solution of characteristic $\lambda_{max} = 290$ nm. Beer's law analysis of this solution yielded a CGC loading average of 0.143 mmol per gram of the resin beads [11].

As shown in Scheme 1, route (a), mild acid conditions selectively cleave the Mmt group and retain the amido-Rink linkage to the resin. Concentrated acid conditions, route (b), lead to Mmt loss as well as cleavage of the tripeptide as H_4CGC. That the H_4CGC was the main component on the beads was confirmed by analysis via $^{+}$ESI-MS. Note that this is the same procedure used previously for preparing solution phase $Ni(CGC)^{2-}$ [4].

Addition of 1.4 mmol $Ni(acac)_2$ and KOH to the resin-bound ligand, **O**-H_4CGC, produced bright orange beads within 10 min. The reaction was allowed to proceed for a further 30 min to ensure completeness. (Extended times (days) gave identical results.) Following extensive washes and drying as described in Section 4, the nickel-loaded beads were analyzed by Neutron Activation Analysis. The average amount of nickel bound to the resin, i.e. the metal loading measured on four separately prepared samples, was determined to be 0.144 mmol of Ni atoms per gram of resin (mmol/g), as given in Table 1. The concurrence of CGC loading as reported in Fig. 3 and the nickel



Scheme 1. Route for determining the CGC loading through Fmoc deprotection to produce dibenzofulvene as well as (a) deprotection of the thiolate sulfurs of **O**-Ni(CGC)$^{2-}$ and (b) paralleled cleavage and deprotection of the H_4CGC tripeptide from the TentaGel beads.

loading is consistent with the uptake of a nickel(II) ion into each **O**-CGC site. This result is also consistent with earlier studies which demonstrated that **O**-Ni(CGC)$^{2-}$ reaction with $M(CO)_x$ reagents, $(THF)W(CO)_5$ and $[Rh(CO)_2Cl]_2$, display ν(CO) IR bands comparable to analogous resin-free complexes of $[Ni(CGC)\cdot W(CO)_5]^{2-}$ and $[Ni(CGC)\cdot Rh(CO)_2]^{1-}$ [8]. To verify this conclusion of selective Ni^{II} binding into the **O**-CGC site, a dichloromethane solution of $Ni(acac)_2$ and KOH was added to the underivatized (no peptide present) beads and showed no color change. Neutron Activation Analysis showed that the amount of nickel in this sample was less than 0.07 mmol/g, thus indicating that the tripeptide is required for binding of the Ni^{II} component.

Table 1
Average loading of **O**-Ni(CGC)$^{2-}$ (mmol Ni/g resin bead) determined via Neutron Activation Analysis on separately prepared samples

Sample #	Loading (mmol/g)
1	0.152
2	0.143
3	0.152
4	0.129
Average	0.144

As a baseline study the number of resin sites accessible for tripeptide synthesis and metallation was also evaluated by UV–Vis analysis of dibenzofulvene produced upon Fmoc deprotection of the Rink amide termini of the peptide-free beads via a 20% piperidine/DMF solution. These studies showed that about 0.227 mmol/g resin of amide sites were available for construction of the tripeptide. As compared to the CGC loading described above, 0.143 mmol/g resin, only 63% of the Rink amide sites were utilized for tripeptide synthesis. This difference can be attributed to solvents used in the synthesis of the tripeptide. *N*,*N* dimethylformamide (DMF) is avoided in peptide synthesis as it

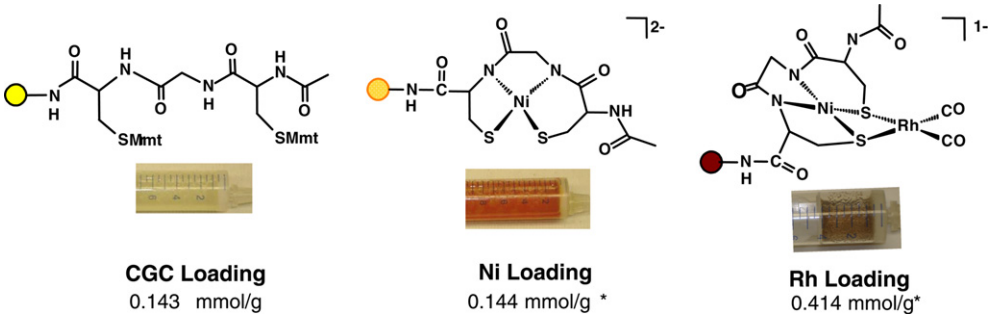


Fig. 3. Loading of Mmt-protected **O**-CGC; **O**-Ni(CGC)$^{2-}$; and **O**-Ni(CGC)Rh(CO)$_2^-$. *Loading may vary up to 0.01 mmol/g per batch.

slows the rate of peptide bond-formation [11]. Hence, dichloromethane was used here. Although often considered a good swelling solvent for PS-PEG systems, dichloromethane is less optimal than DMF. It is likely, therefore, that a number of sites are not accessible during peptide synthesis due to slightly restricted swelling in dichloromethane (as compared to DMF used in the Fmoc analysis above).

2.2. Loading of Rh on *O*-Ni(CGC)Rh(CO)$_2^{1-}$

As shown in Fig. 4, the addition of a dichloromethane solution of $[Rh(CO)_2Cl]_2$ to the pre-swollen **O**-Ni(CGC)$^{2-}$ beads transforms the orange into a purple/brown hue within 5 min. Again mixing time was extended to 30 min to ensure reagent access to all **O**-Ni(CGC)$^{2-}$ sites. The beads were then washed with dichloromethane, methanol and diethyl ether. The signature ν(CO) bands expressed for CH_3CN solution phase $[Ni(CGC)\cdot Rh^{I}(CO)_2]^-$ (2058 and 1986 cm^{-1}) were observed for these Ni/Rh loaded beads at 2067 and 1990 cm^{-1} via ATR-FTIR spectroscopy [8]. Determination of the Ni and Rh loading on such derivatized beads showed that the Ni loading remained about constant, 0.132 mmol/g, and that the Rh loading was 0.414 mmol/g yielding a Rh:Ni ratio of ca. 3 (Table 2).

The addition of $[Rh(CO)_2Cl]_2$ to the beads *sans* nickel-tripeptide induces an immediate color change from yellow to orange/brown. These beads showed ν(CO) bands at 2076s and 1998s cm^{-1} that dissipated after 2 h, a marked difference from the stable **O**-Ni(CGC)Rh(CO)$_2^{-1}$. Metal

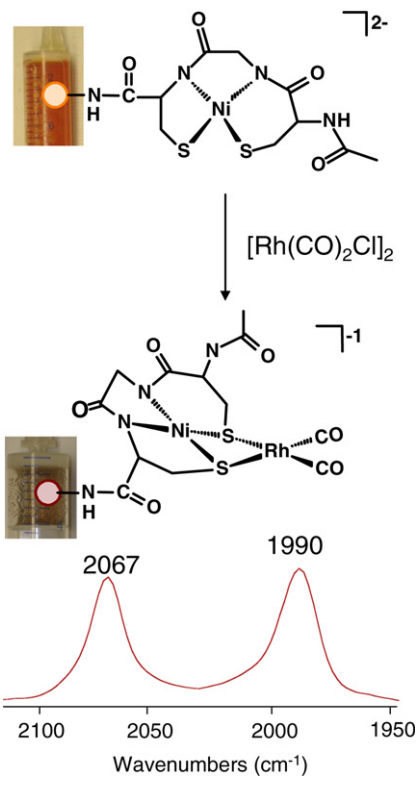


Fig. 4. Synthetic route to **O**-Ni(CGC)Rh(CO)$_2$ and its infrared spectrum obtained on vacuum dried polystyrene beads via ATR-FTIR.

Table 2
Ni and Rh loadings for **O**-Ni(CGC)Rh(CO)$_2^{1-}$ resulting from induced stress conditions

Sample	Ni loading (mmol/g)	Rh loading (mmol/g)	Rh:Ni ratio	Conditions[a]
1a	0.132	0.414	3.13	[b]
1b	0.134	0.375	2.80	–
2	0.123	0.415	3.36	Shake in benzene
3	0.122	0.421	3.44	Bubbled w/air
4	0.126	0.427	3.40	Swell/dry

[a] Conditions discussed in detail may be found in Section 4. Duration of stress in each case was 2 days.
[b] Standard = complex made, washed with CH_2Cl_2, MeOH, and ether, then vacuum dried. Samples 2–4 were derived from batch 1a.

analysis of these beads showed Rh loading to equal 0.221 mmol/g indicating that the PS-PEG polymers bind carbonyl-free rhodium species independent from those of the dithiolate–Rh contact in **O**-Ni(CGC)Rh(CO)$_2^{1-}$. As polyethylene glycol is well known for its ability to form complexes with metal ions, it is assumed to be the site of interaction for the extraneous Rh ions [12]. It should be noted addition of $[Rh(CO)_2Cl]_2$ directly to **O**-CGC gave a color change and transient ν(CO) bands and substantial Rh uptake. We have seen no indication that rhodium displaces nickel in the **O**-Ni(CGC)$^{2-}$ derivatives.

2.3. Stability of *O*-Ni(CGC)Rh(CO)$_2^{1-}$

As complexes bound to solid supports, such as those used for heterogeneous catalysis, are often plagued by leaching, the robustness of the **O**-Ni(CGC)Rh(CO)$_2^{1-}$ complex was investigated under a variety of conditions. As shown in Table 2, and discussed in detail in Section 4, several stresses on the environment of the **O**-Ni(CGC)Rh(CO)$_2^{1-}$ complex were attempted. Samples 2–4 showed no discoloration of the beads or the supernatant solutions used in the experiments or after being washed with ether and dried. Furthermore the amount of nickel and of rhodium remaining on the beads is substantially the same as in the control sample. The supernatant benzene showed no ν(CO) bands and the ether washes were colorless. The FTIR ν(CO) spectrum of the beads showed only a minor decline in intensity. We have taken this preliminary data as indication that the Ni/Rh derivatized beads are largely stable.

3. Conclusion

Neutron Activation Analysis has been used to determine the metal loading of Ni and Rh on TentaGel S-RAM® beads. The 1:1 Ni:CGC^{2-} ratio was confirmed via the use of the UV–Vis spectrum of free Fmoc from deprotection of the terminal amine of the terminal cysteine whose concentration matched that of nickel analysis by Neutron Activation Analysis. Control experiments showed that the CGC ligand was necessary in order to produce the orange, resin-bound species **O**-Ni(CGC)$^{2-}$ and that no PEG-Ni

interaction was evident. In contrast rhodium uptake by the PEG-PS beads was observed, however this interaction was not responsible for the ν(CO) bands attributed to **O**-Ni(CGC)Rh$(CO)_2^{2-}$. The rhodium loading was about 3 Rh atoms per 1 Ni atom as determined by Neutron Activation Analysis. The ability of the **O**-Ni$(CGC)^{2-}$ ligand to strongly secure the Rh moiety to the solid support implies potential for such metallodithiolato ligands to be utilized in catalysis and for modeling of enzyme active sites.

4. Experimental

The Pike MIRacle™ attachment from Pike Technologies was used for attenuated total reflectance infrared spectra for solid state samples. Electron spray ionization mass spectrometry data were obtained at the Laboratory for Biological Mass Spectrometry, Texas A&M University, College Station, Texas, using MDS Series Quasar Pulsar with a spray voltage of 5 eV. Solvents were purified according to standard procedures and were freshly distilled under N_2 prior to use or purified and degassed via a Bruker solvent system [13]. All reagents were used as received without any further purification.

Standard Fmoc Merrifield techniques were followed for all peptide syntheses. The solid supports, TentaGel S-RAM Fmoc resin beads purchased from Advanced Chem Tech averaged 90 μm in diameter, are composed of polystyrene with cross-linking via divinylbenzene and grafted with polyethyleneglycol bearing a Rink linker as the free amine termini (0.23 mmol/g loading). All synthesis were carried out in plastic fritted syringes, 10 mL, to facilitate the multiple additions and removal of reagents and wash solvents. Mixing of beads and reagents was accomplished by an automated shaker. Synthesis of **O**-Ni$(CGC)^{2-}$ and **O**-Ni(CGC)Rh$(CO)_2^{1-}$ followed previously reported procedures and was followed by washes with 3×5 ml aliquots of DMF, methanol, dichloromethane, and ether [8]. The ether wash is vital in that it removes any extraneous dichloromethane that will interfere with the Neutron Activation Analysis studies.

Metal loading was determined via Neutron Activation Analysis at the Texas A&M Center for Chemical Characterization and Analysis. Nickel and rhodium were present either separately or together in the organic matrices at concentrations from a few tenths of percent to several percent. Due to differences in half-lives of the neutron induced reaction products, the elements were determined in separate experiments. A crystalline sample of Ni(bmedaco) was used as a standard for determining the loading of Ni on the resin beads [14]. That the Rh analysis is accurate while found in the presence of Ni was confirmed by a bona-fide non-crystalline sample of [Ni(bmedaco)]Rh(CO)PPh_3^+ [15]. Irradiations were made in a pneumatic facility at the Texas A&M University Nuclear Science Center's 1 MW TRIGA reactor with at a nominal neutron flux of 3×12 cm^{-2} s^{-1}. Gamma spectroscopy was performed using a high resolution germanium detector from Ametek Ortec with relative efficiency of about 50% and resolution of 1.80 keV FWHM at the 1332 keV line of ^{60}Co. Samples, standards and quality control materials were weighed into pre-cleaned polyethylene vials, irradiated and counted sequentially for time periods which depended on the element of interest. Gamma spectra were accumulated using a Canberra Industries GeniePC based acquisition system and transferred via internet to our VMS-based Alpha system for data reduction. Canberra's ESP Genie Neutron Activation Analysis software was used for spectral analysis and for NAA computations. Greater detail in Ni and Rh sample preparation and analysis may be found in the Supplemental material.

4.1. Synthesis of resin-bound *O*-[Ni(CGC)]$^{2-}$

Standard Fmoc techniques were used to construct the N-acylated CysGlyCys peptide on the TentaGel resin beads. Cysteine deprotection via removal of the Mmt (4-methoxytrityl) group was accomplished with minimal cleavage of the tripeptide from the resin with a 1:94:5 mixture of trifluoroacetic acid:dichloromethane:triisopropyl silane deployed in 5 mL aliquots. This was done in 5 min intervals until the deprotection mixture obtained from the peptide-bound resins was no longer yellow, indicating removal of the cysteinyl Mmt thiol-protecting group. Extensive washes (3×5 mL each) with pure CH_2Cl_2 and MeOH solvents followed. A basic solution of green nickel acetylacetonate (25 mg, 0.1 mmol + 22 mg, 0.4 mmol KOH) in 5 mL of dichloromethane was introduced to the resin bed. The light-yellow color of the beads changed to a bright orange within 30 s. After 30 min, the solution containing the nickel source was removed and the beads were again washed with CH_2Cl_2 solvent until the residual washes were completely colorless. The beads were dried *in vacuo* and stored in a vacuum desiccator.

4.2. Synthesis of resin-bound *O*-Ni(CGC)Rh$(CO)_2^{1-}$

A portion of the orange **O**-Ni$(CGC)^{2-}$, was placed in a syringe and allowed to swell in dichloromethane. To this a yellow solution of $[Rh(CO)_2Cl]_2$ in CH_2Cl_2 was added by drawing up with a needle, and the syringe was then capped. The mixture was agitated for 10 min on an automated shaker. The color of the beads quickly changed from orange to purple/brown but allowed to react a total of 45 min. The beads were washed (6×10 mL $CH_2Cl_2$and ether) and dried under vacuum. ν(CO): 2067s, 1990s cm^{-1}.

4.2.1. Stability experiments

In a typical experiment a large batch of **O**-*Ni(CGC)*-Rh$(CO)_2^{1-}$ was synthesized using standard procedures described previously and identified via its signature ν(CO) bands. The batch was divided into four separate samples for the analysis shown in Table 2. Sample 1 was stored in a vacuum dessicator and not exposed to any further physical stress. Sample 2 was suspended in benzene and shaken vigorously using a lab-shaker for 2 days before being

washed with ether and dried *in vacuo*. As the **O**-Ni(CGC)-$Rh(CO)_2^{1-}$ shows a marked air stability not observed for its solution analogue, $Ni(ema)Rh(CO)_2^{1-}$, its air stability was further explored. Thus, Sample 3 was loaded into a round bottom flask, filled with benzene, and bubbled with atmospheric air continuously for 2 days. Finally, the beads were tested for their ability to retain the Ni(CGC)Rh-$(CO)_2^{1-}$ moiety through the physical stress of repeated swelling (in CH_2Cl_2) and placed in a fritted syringe with benzene and shaken for 2 h followed by ether wash and drying repeatedly for 2 days.

Acknowledgements

We sincerely thank Professor Patrick H. Toy at The University of Hong Kong and Professor Kevin Burgess at Texas A&M University for their helpful advice. We acknowledge financial support from the National Science Foundation (CHE 01-11629), the Robert A. Welch Foundation, and the National Institutes of Health (Chemistry–Biology Interface Training Grant to K. Green, T32 GM008523).

Appendix A. Supplementary material

Full detail of Neutron Activation Analysis Methods for the determination of Rh and Ni are discussed. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2006.10.051.

References

[1] (a) J. Carey, S. Ma, T. Pfister, D. Garner, H. Kim, J. Abramite, Z. Wang, Y. Lu, J. Am. Chem. Soc. 126 (2004) 10812;
(b) C. Thomas, T. Ward, Chem. Soc. Rev. 34 (2005) 337;
(c) V. Nandam, M. Rosenblatt, A. Osyczka, H. Kono, Z. Getahun, L. Dutton, J. Saven, W. Degrado, J. Am. Chem. Soc. 127 (2005) 5804.
[2] M. Skander, N. Hubert, J. Collot, J. Graninaru, A. Klein, J. Sauser, A. Zocchi, F. Gilardoni, T. Ward, J. Am. Chem. Soc. 126 (2004) 14411.
[3] (a) O. Kornyushyna, A. Stemmler, D. Graybosch, I. Bergenthal, C. Burrows, Bioconjugate Chem. 16 (2005) 178;
(b) D.A. Rockcliffe, A. Cammers, A. Murali, W.K. Russell, V.J. DeRose, Inorg. Chem. 45 (2006) 472.
[4] R. Krishnan, C.G. Riordan, J. Am. Chem. Soc. 126 (2004) 4484.
[5] (a) C. Darnault, A. Volbeda, E.J. Kim, P. Legrand, X. Vernède, P.A. Lindahl, J.C. Fontecilla-Camps, Nat. Struct. Biol. 10 (2003) 271;
(b) T.I. Doukov, T.M. Iverson, J. Seravalli, S.W. Ragsdale, C.L. Drennan, Science 298 (2002) 567–572.
[6] C.E. Webster, M.Y. Darensbourg, P.A. Lindahl, M.B. Hall, J. Am. Chem. Soc. 126 (2004) 3410.
[7] M.V. Rampersad, S.P. Jeffery, M.L. Golden, J. Lee, J.H. Reibenspies, D.J. Darensbourg, M.Y. Darensbourg, J. Am. Chem. Soc. 127 (2005) 17323.
[8] K.N. Green, S.P. Jeffery, J.H. Reibenspies, M.Y. Darensbourg, J. Am. Chem. Soc. 128 (2006) 6493.
[9] (a) D.C. Sherrington, Chem. Commun. (1998) 275;
(b) E. Bayer, Angew. Chem., Int. Ed. Engl. 30 (1991) 113;
(c) P. Hodge, Chem. Soc. Rev. 26 (1997) 417.
[10] (a) J. Vágner, G. Barany, K. Lam, V. Krchňák, N. Sepetov, J. Ostrem, P. Strop, M. Lebl, Proc. Natl. Acad. Sci. 93 (1996) 8194;
(b) U. Grewther, H. Waldmann, Chem. Eur. J. 7 (2001) 959;
(c) J. Kress, R. Zanaletti, A. Amour, A. Ladlow, M. Ladlow, J. Frey, M. Bradley, Chem. Eur. J. 8 (2002) 3769.
[11] W.C. Chan, P.C. White, Fmoc Solid Phase Peptide Synthesis: A Practical Approach, Oxford University Press, New York, NY, 2000, pp. 62–64.
[12] J. Milton HarrisPolyethylene Glycol Chemistry: Biotechnical & Biomedical Applications, vol. 10, Plenum Press, NY, 1992, pp. 13–15.
[13] A.J. Gordon, R.A. Ford, The Chemist's Companion, Wiley and Sons, New York, 1972, pp. 429–436.
[14] D. Mills, J.H. Reibenspies, M.Y. Darensbourg, Inorg. Chem. 29 (1990) 4366.
[15] S.P. Jeffery, K.N. Green, M.V. Rampersad, J.H. Reibenspies, M.Y. Darensbourg, Dalton Trans. 35 (2006) 4244.