able oxindole and isatin, the total syntheses of *meso*- (**1**) and (+)-chimonanthine (**2**) were accomplished in 39 and 21% overall yield respectively. The alkylation of the dilithium dienolate of **6** in 10% DMPU/THF is notable in producing one of the two possible C_2 products with high selectivity (20:1). This alkylation reaction is a rare example of high diastereoselectivity arising from the combination a prostereogenic enolate with a chiral electrophile containing an sp^3 carbon center.[20]

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[1] Recent reviews of asymmetric synthesis of quaternary centers: a) E. J. Corey, A. Guzman-Perez, *Angew. Chem.* **1998**, *110*, 402–415; *Angew. Chem. Int. Ed.* **1998**, *37*, 388–401; b) K. Fuji, *Chem. Rev.* **1993**, *93*, 2037–2066.

[2] For an excellent recent strategy, see R. M. Lemieux, A. I. Meyers, *J. Am. Chem. Soc.* **1998**, *120*, 5453–5457.

[3] Y. Adjibadé, B. Weniger, J. C. Quirion, B. Kuballa, P. Cabalion, R. Anton, *Phytochemistry* **1992**, *31*, 317–319.

[4] H. F. Hodson, B. Robinson, G. F. Smith, *Proc. Chem. Soc. London* **1961**, 465–466.

[5] T. Tokuyama, J. W. Daly, *Tetrahedron* **1983**, *39*, 41–47.

[6] L. Verotta, T. Pilati, M. Tatò, E. Elisabetsky, T. A. Amador, D. S. Nunes, *J. Nat. Prod.* **1998**, *61*, 392–396.

[7] R. K. Duke, R. D. Allan, G. A. R. Johnston, K. N. Mewett, A. D. Mitrovic, C. C. Duke, T. W. Hambley, *J. Nat. Prod.* **1995**, *58*, 1200–1208.

[8] F. Guéritte-Voegelein, T. Sévenet, J. Pusset, M.-T. Adeline, B. Gillet, J.-C. Beloeil, D. Guénard, P. Potier, R. Rasolonjanahary, C. Kordon, *J. Nat. Prod.* **1992**, *55*, 923–930.

[9] E. S. Hall, F. McCapra, A. I. Scott, *Tetrahedron* **1967**, *23*, 4131–4141.

[10] a) R. Göschke, J. B. Hendrickson, R. Rees, *Tetrahedron* **1964**, *20*, 565–579; b) C. L. Fang, S. Horne, N. Taylor, R. Rodrigo, *J. Am. Chem. Soc.* **1994**, *116*, 9480–9486.

[11] T. Hino, S. Yamada, *Tetrahedron Lett.* **1963**, 1757–1760.

[12] J. T. Link, L. E. Overman, *J. Am. Chem. Soc.* **1996**, *118*, 8166–8167.

[13] L. E. Overman, D. V. Paone, B. A. Stearns, *J. Am. Chem. Soc.* **1999**, *121*, 7702–7703.

[14] Ditriflate **7** is available on a large scale by reaction of (+)-2,3-*O*-isopropylidene-L-threitol with triflic anhydride and iPr$_2$NEt in Et$_2$O at 0°C. Both enantiomers of this intermediate are available from (+)- and (−)-tartaric acid: E. A. Mash, K. A. Nelson, S. Van Deusen, S. B. Hemperly in *Organic Syntheses, Vol. 68* (Ed.: J. D. White), Wiley, New York, **1990**, pp. 92–103.

[15] Diol **10** had previously been converted into *meso*-chimonanthine.[12] The efficiency of this conversion is increased to 65% by utilizing the conditions we recently developed to convert a related chiral diol into (−)-chimonanthine.[13]

[16] Consistent with this interpretation, stereoselection was eroded in the presence of a sodium-selective cryptand (Kryptofix 221).

[17] R. M. Williams, E. Kwast, *Tetrahedron Lett.* **1989**, *30*, 451–454.

[18] Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-134670. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

[19] Literature value:[6] $[\alpha]_D^{23} = +264.5$ ($c = 1.0$ in EtOH).

[20] a) A recent survey of stereoselective C–C bond formation cites no examples in sections dealing with alkylation reactions with chiral electrophiles; see D. S. Matteson, *Methoden Org. Chem. (Houben-Weyl) 4th ed. 1996*, Vol. E21/2, pp. 1077–1118; b) Interconversion of atropisomers of the dienolates derived from **6** is likely to be slow under the alkylation conditions. Whether the 20:1 selectivity arises solely in the initial alkylation step or is enhanced by selective partitioning of the minor diastereomer in the cyclization step has not yet been determined.

How Strong Is the Coordination Bond between a Histidine Tag and Ni–Nitrilotriacetate? An Experiment of Mechanochemistry on Single Molecules**

Matteo Conti, Giuseppe Falini, and Bruno Samorì*

A number of extensively used methods of increasing importance in molecular biology are based on the ability of the nickel(II) ion, when chelated by nitrilotriacetate (nta), to selectively bind proteins containing stretches of consecutive histidine residues (His). Hochuli et al. established this field of applications when they discovered that proteins containing isolated histidines lead to complexes that are less stable than those arising from proteins having two consecutive His tags at one terminus (2 × His-tag).[1] Indeed, a stretch of six consecutive His tags (6 × His-tag) is now commonly appended to the primary sequence of recombinant proteins, making it possible to isolate them selectively from a flow of crude cell lysate by means of a Ni–nta-functionalized chromatographic matrix[2a,b] and to immobilize them on biosensors for biomolecular interaction analysis.[3] The stability of the anchoring of the His-tagged proteins is challenged in both cases by the frictional force exerted on them by the flow. Such interplay between external forces and chemical processes can now be transferred to the single-molecule level thanks to recent developments in nanoscale manipulation techniques.[4, 5] Single-molecule experiments can give crucial insights into binding processes that cannot be accessed by solution methods. In the single-molecule world, bond making and breaking becomes a digital "yes-or-no" process. Determination of binding constants by traditional means provides only information that is averaged on the entire ensemble of the molecules and on the overall time scale of the experiment.

We simulated the process involved in the Ni–nta protein separation techniques by reproducing the approach, binding, stretching, and unbinding under external forces between individual His tags and individual Ni–nta chelating groups in a single-molecule experiment with the scanning force microscope (SFM, see Figure 1).[6] This experiment revealed that: 1) the probability that the encounters between a 6 × His-tag and a Ni–nta group develop in a stable bond is much higher than that for a 2 × His-tag; 2) both tags can make either of two types of complex with markedly different stabilities and with different "energy landscapes" along their force-driven disso-

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ciation pathway; and 3) the probability that the 6 × His-tag forms the more stable complex is much higher than that for the 2 × His-tag.

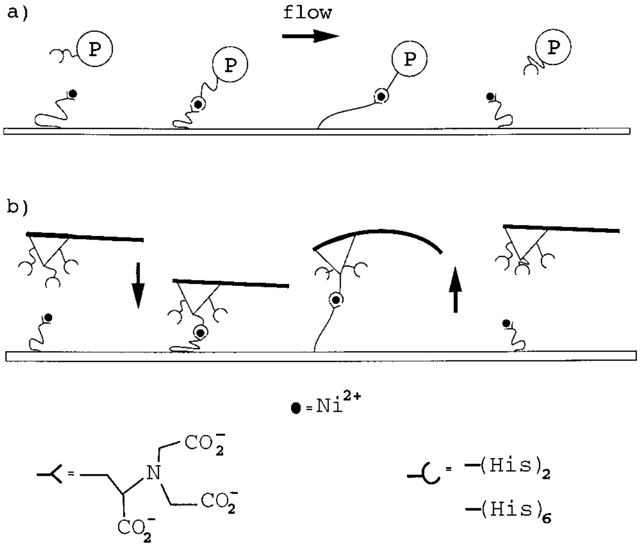

Figure 1. Sketch of the approach, binding, stretching, and unbinding of a) a His-tagged protein (p) under the flow inside an affinity chromatography column or on a biosensor surface and b) a His-tagged peptide covalently bound to an SFM tip in an approach and retraction cycle. (This figure was inspired by Figure 1 in reference[4c]).

A 2 × His-tagged peptide, HCys-$(Gly)_6$-$(His)_2$-OH, was covalently attached on its Cys end to a gold-coated SFM tip. The tip was brought into proximity to a substrate having exposed Ni–nta groups covalently bound through carboxymethylated dextran linkers, which were attached to a gold surface by an alkane C_{18} chain (sensor chip NTA, Biacore, Sweden). Whenever the 2 × His-tag of the peptide formed a chelate with a Ni–nta group, a molecular bridge was established between the tip and the substrate. The tip was subsequently retracted at a constant pulling velocity until the bridge broke (see Figure 1b). The force acting on the molecular bridge was monitored from the cantilever deflection, and force versus extension (displacement of the piezo driver) curves were recorded. At a pulling velocity of 1 $\mu m\,s^{-1}$ only 28% of the approaches led to binding events. The successful events were recognized by unbinding traces with single or multiple sawtooth patterns like those depicted in Figure 2a–c. Each tooth consists of a part with rising deflection followed by a sudden drop. The rising part corresponds to the stretching of a molecular bridge; the drop reflects its breaking and provides an estimate of the rupture force. The distribution of all the rupture forces recorded displays two maxima with different heights: ruptures at about 350 pN occurred more than twice as frequently as those at about 500 pN (Figure 3a). Figure 3b reports the distribution observed when the same experiment was performed with the homologous peptide HCys-$(Gly)_6$-$(His)_6$-OH which has a 6 × His-tag. The probability of binding events increased from the 28% of the 2 × His-tag to 42%. Most of the unbinding traces exhibited a single rupture: the distribution of the forces was very broad and peaked at about 500 pN, the same value as the lower maximum in Figure 2b.

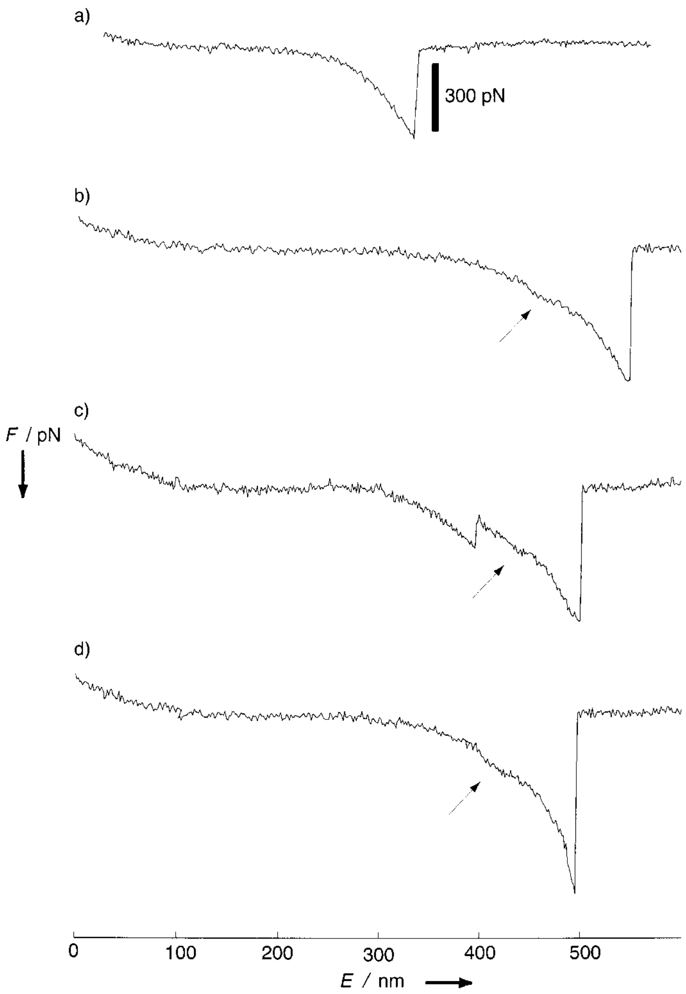

Figure 2. Characteristic shape of the force (*F*) vs extension (*E*) unbinding traces associated with the rupture of the coordination bond between His tags and nickel(II)–nta (a–c) and iron(III)–nta (d). Single rupture events (a, b, d) and sequential two-bond ruptures at about 280 and 600 pN (c) are shown. When a conformational transition of the carboxymethylated dextran chain also took place, it was revealed by the plateau at about 300 pN, which is marked by an arrows in b–d.

The first critical issue with this type of measurement is to verify that exactly one single polymer chain is picked up by the SFM tip and that only one single binding/unbinding event is measured. We followed both strategies that had been introduced previously to verify this point. 1) When the measured curves are normalized according to the length of each individual force-extension trace (which relates to the length of the single polymer chain upon rupture), their features should overlap perfectly.[7, 8] In an attempt to verify this for our case we have plotted a set of representative curves in Figure 4. 2) Polysaccharide molecules have a large enthalpic component in their elasticity. They undergo typical conformational transitions that take place at different applied forces.[4b, 7, 8, 9] Rief et al. characterized this type of transition in single molecules of carboxymethylated dextran and found that it occurs at about 300 pN.[7] The same authors utilized the corresponding plateau in the force–extension curve as an internal strain gauge to calibrate the force acting on the system.[7] We followed the same approach: the traces in which this plateau was clearly evident (e.g. Figure 2b–d) were utilized to check the direct measurements of the spring constant of different cantilevers, a determination with quite low precision (see Experimental Section). On the basis of these results we could infer that only single binding/unbinding events had been considered. This conclusion was supported by one further observation: the distribution of the rupture forces (see Figure 3) did not peak on integer multiples of an elementary force value,[10] as would be expected if simultaneous ruptures of more than one bond took place. On the other hand, it must be pointed out that none of these individual observations were as clear-cut as they in principle could be. In fact, 1) the plateau was not observed in all traces, and its normalized length was not constant; 2) the superimposition of the normalized curves was not perfect for all traces (see Figure 4); and 3) the data distributions of rupture forces were rather broad. These three observations most likely originate from a nonhomogeneous carboxymethylation of the dextran linkers and from their being not directly anchored to the substrate but to a molecular network. Our experiment should thus be improved by the use of a better defined polymeric linker. Our current data should be used just for comparing the different behaviors of the 2 × His- and 6 × His-tags rather than for assigning the absolute values of the single-bond strengths.

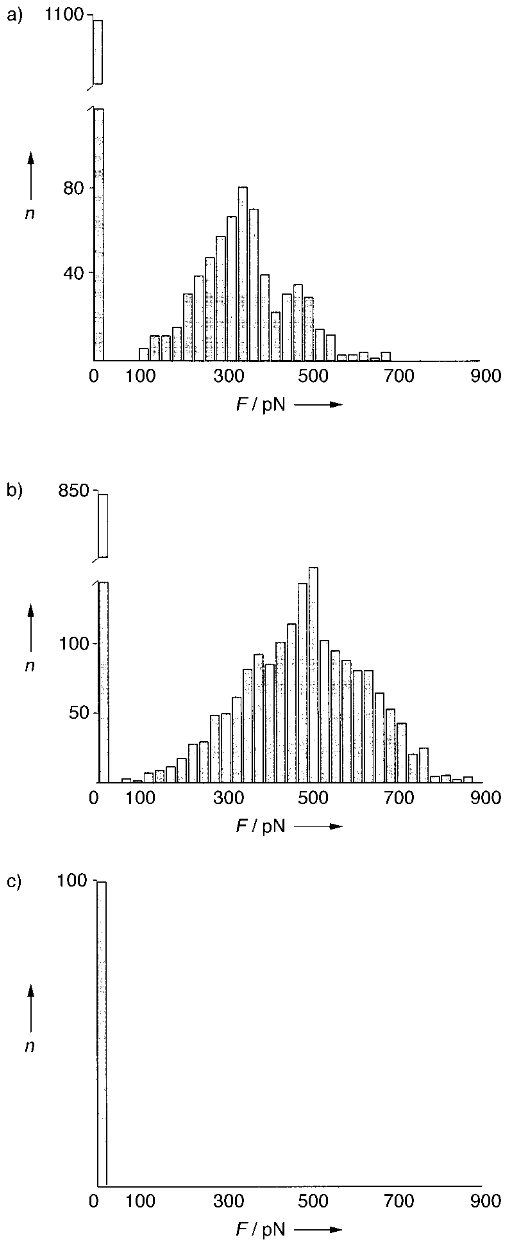

Figure 3. Histogram of the distribution (n, number of events) of all the unbinding forces (F) recorded with the Ni–nta-functionalized substrate and a) the 2xHis-tag and b) the 6xHis-tag. No binding event was recorded when the substrate was depleted of nickel(II) ions (c).

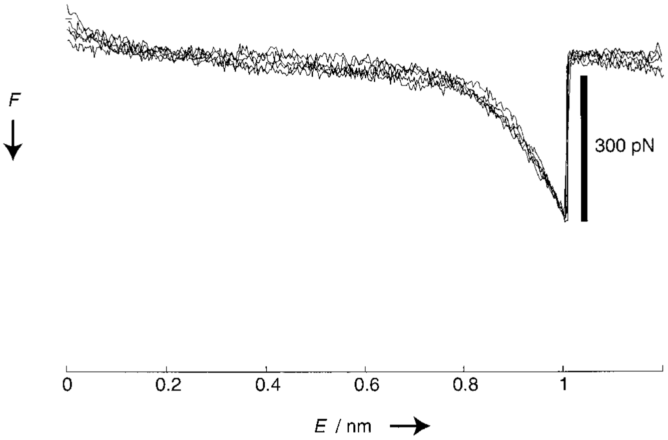

Figure 4. Superimposition of a sample of single-rupture force-extension traces normalized as described in the text.

The second critical issue with this type of measurements is to pinpoint which bond is rupturing. The bond rupture events we observed could be assigned to the Ni–His junction between the polysaccharide and the peptide sides of the system. In fact, the forces for all other bonds along the chains (C–C, C–O, C–S, including the S–Au anchor to the substrate) were recently estimated by Grandbois et al.:[4b] the weakest is the S–Au bond which, under conditions identical to those in our experiment, was found to be able to withstand forces of up to 1400 pN, far greater than the forces we applied. Our assignment was confirmed by two control experiments. 1) After the substrate was depleted of nickel(II) ions we were operating in a force regime markedly repulsive, and no adhesion between the tip and the substrate was ever observed (Figure 3c). 2) When nta was complexed to a smaller and more charged ion like iron(III), larger rupture forces (up to about 750 pN) were recorded, as expected (see Figure 2d).

We can therefore infer that the two peaks in Figure 3a and b are associated with two different complexes, or even with two classes of complexes. Therefore, when a 2 × His-tag is brought into contact with a Ni–nta group, the less stable complex (or complexes) is formed more frequently. In the case of a 6 × His-tag the more stable one (or ones) is formed more frequently instead. These two coordination bonds differ not only in stability, but also in the "energy landscape"[11] of their transition states, as revealed by the study we carried out on the dependence of their rupture forces on the force loading rate (to be published). This parameter, first introduced by Evans and Ritchie,[12] is equal to the product of the pulling velocity and the "effective" spring constant (the combination in series of the spring constant of the cantilever and of the molecular bridge).

This experiment, although not yet optimized, is casting a new light on our understanding of a bond that forms the basis for more and more important tools for molecular biology. These results make it possible not only to foresee the tremendous possibilities but also the possible problems and pitfalls of this very new and still developing SFM method for studying binding processes.

Experimental Section

The peptide HCys-(Gly)$_6$-(His)$_2$-OH was kindly provided by Prof. Peggion (Padova), and HCys-(Gly)$_6$-(His)$_6$-OH was synthesized by Tecnogen (Naples, Italy). Both were synthesized by solid-phase methods, and their purity was controlled by HPLC and MALDI-TOF MS. The Ni–nta Biacore chip was depleted of nickel(II) ions by incubation in 0.35 M ethylenediaminetetraacetic acid solution for 2 h; iron(III) or nickel(II) ions were reintroduced by treating the chip with a 100 mM solution of FeCl$_3$ or NiCl$_2$ and then rinsing with water. Si$_3$N$_4$ AFM cantilevers (DI, Santa Barbara, CA) were metalized by resistive deposition of Ti and then Au 99.99% (thicknesses of 10–15 nm and 30–40 nm, respectively) at 10^{-6} Torr. The tips were then immediately immersed in a 1 mg mL^{-1} solution of the peptide in phosphate-buffered saline (PBS) at pH 7.4 for 30 min.

The spring constants of the cantilevers were determined from their thermal excitation[13] with a precision not higher than 30%. Four cantilevers were used for the measurements. The values of their spring constants obtained by this method were in the range of 60 to 120 pN nm^{-1}. Force measurements were carried out in PBS with a Nanoscope III Multimode AFM (DI, Santa Barbara, CA).

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[1] E. Hochuli, H. Döbeli, A. Schacher, *J. Chromatogr.* **1987**, *411*, 177–184.

[2] a) L. Nieba, A. Krebber, A. Plückthun, *Anal. Biochem.* **1996**, *234*, 155–165, and references therein; b) A. M. Schmidt, H. N. Müller, A. Skerra, *Chem. Biol.* **1996**, *3*, 645–653.

[3] J. Crowe, H. Döbeli, R. Gentz, E. Hochuli, D. Stüber, *Methods in Molecular Biology*, *Vol. 31*, Humana Press, Totowa, NJ, **1994**, pp. 371–387, and references therein.

[4] a) V. T. Moy, E. L. Florin, H. E. Gaub, *Science* **1994**, *266*, 257–259; b) M. Grandbois, M. Beyer, M. Rief, H. Clausen-Schaumann, H. E. Gaub, *Science* **1999**, *283*, 1727–1730; c) J. Fritz, A. G. Katopodis, F. Kolbinger, D. Anselmetti, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 12283–12288; d) C. Bustamante, C. Rivetti, D. J. Keller, *Curr. Opin. Struct. Biol.* **1997**, *7*, 709–716; e) S. Izrailev, S. Stepaniants, M. Balsera, Y. Oono, K. Schulten, *Biophys. J.* **1997**, *72*, 1568–1581.

[5] B. Samorì, *Angew. Chem.* **1998**, *110*, 2318–2321; *Angew. Chem. Int. Ed.* **1998**, *37*, 2198–2200.

[6] In this experiment, the unbinding takes place under a rising force and in a time much shorter than those of protein separation processes. These forces are kinetic in nature.[4d,e, 11] Therefore, in these processes the bond ruptures may occur at lower forces than those we measured.

[7] M. Rief, F. Oesterhelt, B. Heymann, H. E. Gaub, *Science* **1997**, *275*, 1295–1297.

[8] H. Li, M. Rief, F. Oesterhelt, H. E. Gaub, *Adv. Mater.* **1998**, *3*, 316–319.

[9] P. E. Marszalek, A. F. Oberhauser, Y. P. Pang, J. M. Fernandez, *Nature* **1998**, *396*, 661–664.

[10] E. L. Florin, V. T. Moy, H. E. Gaub, *Science* **1994**, *264*, 415–417.

[11] R. Merkel, P. Nassoy, A. Leung, K. Ritchie, E. Evans, *Nature* **1999**, *397*, 50–53, and references therein.

[12] E. Evans, K. Ritchie, *Biophys. J.* **1997**, *72*, 1541–1555.

[13] E. L. Florin, M. Rief, H. Lehmann, M. Ludwig, C. Dornmair, V. T. Moy, H. E. Gaub, *Biosensors Bioelectronics* **1995**, *10*, 895–901.

Denticity Changes of Hydrotris(pyrazolyl)borate Ligands in Rh^{I} and Rh^{III} Compounds: From κ^3- to Ionic "κ^0"-Tp′**

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Dedicated to Professor Swiatoslaw Trofimenko

The formally tridentate cyclopentadienyl and hydrotris(pyrazolyl)borate ligands are important auxiliary ligands in organometallic chemistry.[1, 2] Most often they act as spectators, and chemical transformation occurs at the metal center or at other coordinated ligands. However, in some instances they may have a noninnocent involvement by means of hapticity changes.

Variations in coordination mode are well documented for the cyclopentadienyl system (Cp′).[3, 4] Casey and co-workers demonstrated some years ago the interconversion of η^5-C$_5$H$_5$, η^1-C$_5$H$_5$, and ionic "η^0"-C$_5$H$_5{}^-$ compounds in the reaction of $[(\eta^5\text{-C}_5\text{H}_5)\text{Re(NO)(CH}_3\text{)(PMe}_3)]$ with PMe$_3$[5] and proposed an η^3-C$_5$H$_5$ intermediate for the $\eta^5 \rightleftharpoons \eta^1$ exchange. In contrast,

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