Asymmetric Catalysis

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Reconciling the Stereochemical Course of Nucleopalladation with the Development of Enantioselective Wacker-Type Cyclizations**

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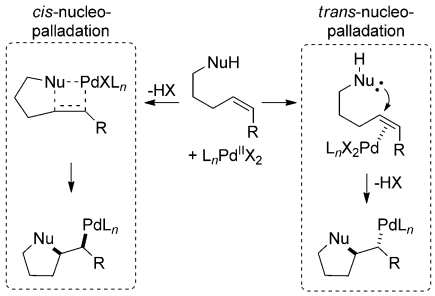
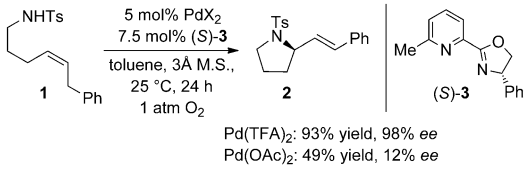
Palladium(II)-catalyzed oxidative functionalization of alkenes has been the focus of intense interest for decades, and Wacker-type cyclizations,[1] which enable synthesis of diverse heterocycles, are a prominent class of these reactions.[2] Substantial effort has been directed toward enantioselective applications, but successful examples (e.g., $\geq 90\%$ *ee*) remain rare and often exhibit limited substrate scope.[3,4] A key challenge associated with these reactions is the possibility of *cis*- or *trans*-nucleopalladation (NP) of the alkene, because the formation of diastereomeric intermediates from these pathways could have significant consequences for the development of enantioselective transformations (Scheme 1).[3] Examples of both *cis*- and *trans*-NP pathways



Scheme 1. Stereochemical pathways for alkene nucleopalladation.

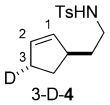
in catalytic reactions have been documented,[5,6] but only three enantioselective variants of these reactions have been characterized with respect to the stereochemical course of NP. All three examples exhibited a preference for *cis*-NP.[4f,k,n] The possible impact of the stereochemical course of NP on the enantioselectivity of a given asymmetric Wacker-type reaction has not been established. Herein, we present a mechanistic investigation of the factors that affect the stereochemical course of NP in the context of a recently discovered catalyst system for the enantioselective cyclization of γ-alkenyl tosylamides. Implementation of a novel stereochemical probe demonstrates that both the chiral neutral-donor ligand and the anionic ligands on the palladium center are capable of controlling the stereochemical pathway for amidopalladation (AP), but only the *trans*-AP pathway exhibits high enantioselectivity. These data provide the first direct correlation between NP stereoselectivity and the enantioselectivity of the transformation in question. Such insights highlight valuable considerations for the development of enantioselective reactions that involve nucleopalladation of an alkene.

Recently, we showed that a palladium(II) catalyst with a chiral pyridine oxazoline (pyrox) ligand enables preparation of pyrrolidines (e.g., **2** from **1**) in excellent yield and enantioselectivity (Scheme 2).[4p,7] Based on several closely related precedents, we predicted that the Pd^{II}/pyrox catalyst



Scheme 2. Enantioselective cyclization of γ-alkenyl tosylamides. M.S. = molecular sieves, TFA = trifluoroacetate, Ts = 4-toluenesulfonyl.

system would favor a *cis*-AP mechanism.[8–11] For example, the isotopically labeled substrate 3-D-**4** has been used to assess the mechanism of several different palladium(II) catalyst systems for the aerobic, oxidative amidation of alkenes,[5d] and Pd(OAc)$_2$/pyridine and Pd(TFA)$_2$/(−)-sparteine were among the catalyst systems shown to afford products exclusively arising from *cis*-AP of the alkene.[12] In the enantioselective cyclization of γ-alkenyl tosylamides, the identity of the anionic ligand was found to have a significant impact on the reaction outcome. Replacing [Pd(pyrox)(TFA)$_2$] with [Pd(pyrox)(OAc)$_2$] gave a significantly diminished yield and enantioselectivity under otherwise identical reaction conditions (Scheme 2). The disparity of these results raised the possibility that the reactions with these catalysts might involve different AP pathways.



Our initial attempt to probe the AP pathway for the enantioselective reaction involved the use of the substrate 3-

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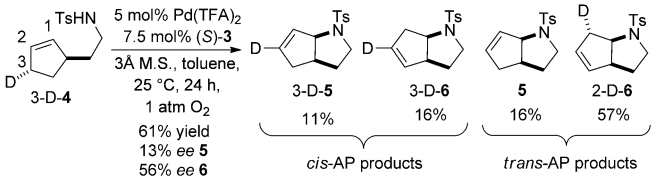
D-**4** under the previously optimized reaction conditions. However, a mixture of all four of the possible bicyclic pyrrolidines was obtained in 61% yield, favoring the *trans*-AP products in approximately a 3:1 ratio relative to *cis*-AP products (Scheme 3). This result was unexpected for two

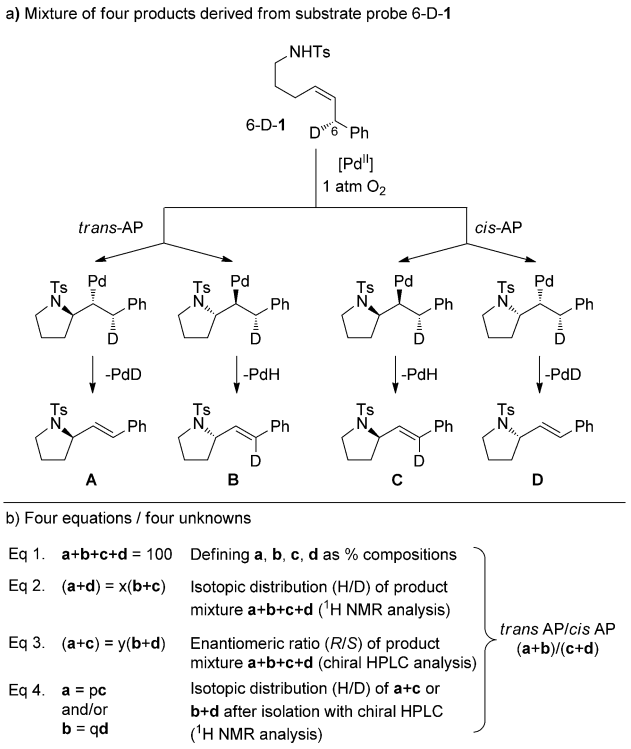


Scheme 3. Cyclization of the substrate 3-D-**4** with chiral catalyst.

reasons: first, the *cis*-AP pathway was anticipated to be dominant for these reaction conditions, and second, it seemed unlikely that a highly enantioselective reaction would involve simultaneous operation of both *cis*- and *trans*-AP pathways. Analysis of the product mixture by HPLC using a chiral stationary phase revealed poor kinetic resolution. The products 3-D-**5** and **5** were formed in 13% *ee*, and the products 3-D-**6** and 2-D-**6** were formed in 56% *ee*. The relevance of these results was not entirely clear, in part, because the cyclic alkene in 3-D-**4** could influence the stereochemical course of the AP step and may not be a good model for acyclic alkenes which undergo highly enantioselective cyclization.[4p]

To circumvent the complications associated with the use of 3-D-**4** as a mechanistic probe, we prepared a novel acyclic deuterated substrate probe, 6-D-**1**, which is a chiral analogue of substrate **1** (Scheme 4).[13] Analysis of the products formed by oxidative cyclization of 6-D-**1** is more involved than the analysis of products derived from the substrate 3-D-**4** because both the absolute configuration of the product and the loss or retention of the deuterium atom must be accounted for (the four products **A**–**D** differ only in the absolute configuration of the stereogenic center and/or the presence or absence of the styrenyl deuterium atom at C6, Scheme 4a). Reliable results with 6-D-**1** are possible because *trans*-styrenyl products are obtained with high selectivity over the *cis* isomers, and very little deuterium scrambling ($\leq 5\%$) occurs.[14]

Three independent analytical measurements were used to establish the yield of the products **A**–**D** from the reaction of 6-D-**1** under various reaction conditions. First, the H/D ratio at C6 in the four styrenyl products was obtained by ^{1}H NMR spectroscopy. This quantity established the relationship $(\mathbf{a}+\mathbf{d}) = x(\mathbf{b}+\mathbf{c})$, where **a**, **b**, **c**, and **d** represent the percent composition of the species **A**–**D**, and x = H/D at C6 (Scheme 4b). Second, the enantiomeric ratio of the products was obtained by HPLC analysis. This quantity established the relationship $(\mathbf{a}+\mathbf{c}) = y(\mathbf{b}+\mathbf{d})$, where y = [*R* products/*S* products]. Third, the two sets of enantiomeric products were separated by HPLC using a chiral stationary phase, and the H/D ratio of the enantiomerically pure products was obtained by ^{1}H NMR spectroscopy.[15] This quantity established the **a**/**c** and **b**/**d** ratios. With these data in hand and accounting for full mass balance $(\mathbf{a}+\mathbf{b}+\mathbf{c}+\mathbf{d} = 100)$, it was possible to solve

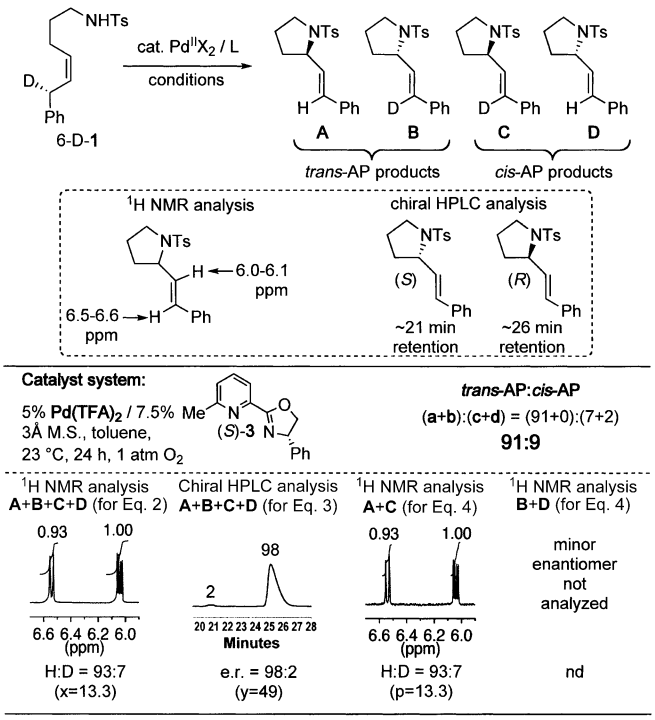


Scheme 4. a) Mechanistic pathways for the reaction of 6-D-**1** and b) mathematical relationships used to determine the yields of products **A**–**D**.

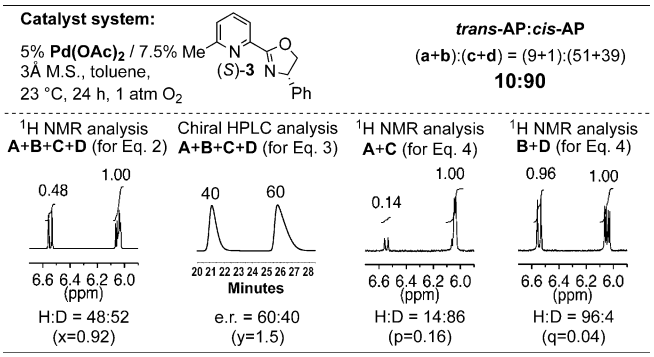
a system of four equations and four unknowns to determine the quantities **a**, **b**, **c**, and **d**, from which the *trans*-AP/*cis*-AP selectivity was obtained from the ratio $(\mathbf{a}+\mathbf{b})/(\mathbf{c}+\mathbf{d})$.[16]

The substrate 6-D-**1** was subjected to the optimized reaction condition using the chiral catalyst, and the reaction proceeded in excellent yield and enantioselectivity (90% yield and 96% *ee*), which is consistent with the reactivity of **1** reported previously (Scheme 2).[4p] ^{1}H NMR spectroscopic analysis of the initial product mixture revealed a 93:7 preference for the protio products (Scheme 5). Because we had previously determined that the *S* configuration of the pyrox ligand **3** favors formation of the *R* configuration of the pyrrolidine, the initial ^{1}H NMR and HPLC analyses were enough to conclude that product **A** was the major species and the *trans*-AP pathway was heavily favored over the *cis* pathway. The product ratio was established more definitively with ^{1}H NMR analysis of the purified major enantiomer species **A** and **C**. The three measurements show that these reactions exhibit a very high selectivity for a *trans*-AP pathway (*trans*-AP/*cis*-AP = 91:9). The correlation between the high enantioselectivity and high *trans*/*cis*-AP selectivity obtained from the substrate 6-D-**1** may be contrasted to the poor enantioselectivity and poor *trans*-AP/*cis*-AP selectivity observed with the substrate 3-D-**4** (see Scheme 3).[17,18]

These results established the utility of the substrate probe 6-D-**1** and the protocol for product analysis to correlate the enantioselectivity with the AP pathway of the oxidative cyclization reaction. We then turned our attention to the [Pd(pyrox)(OAc)$_2$]-catalyzed reaction, which proceeds with



Scheme 5. Experimental data and *trans*-AP/*cis*-AP selectivity obtained from oxidative cyclization of substrate 6-D-**1** with the optimized reaction conditions with a chiral catalyst.



Scheme 6. Experimental data and *trans*-AP/*cis*-AP selectivity obtained from oxidative cyclization of substrate 6-D-**1** with a [Pd(pyrox)(OAc)$_2$] catalyst system (refer to Scheme 5 for depiction of the reaction).

Table 1: Summary of amidopalladation studies with pyrox-ligated and ligand-free catalysts.

Entry	Pd^{II}[a]	Ligand[b]	Yield [%][c]	*ee* [%][d]	*trans*-AP/*cis*-AP[e]
1	Pd(TFA)$_2$	(*S*)-**3**	90	96	>9:1
2	Pd(OAc)$_2$	(*S*)-**3**	48	20	1:9
3	Pd(TFA)$_2$	none	55	0	1:6
4	Pd(OAc)$_2$	none	15	0	<1:9

[a] Used 5 mol%. [b] Used 7.5 mol%. [c] Determined by ^{1}H NMR analysis of the crude reaction mixture. [d] Determined by HPLC analysis of the purified products. [e] See the Supporting Information for full disclosure of the raw data.

much lower enantioselectivity under reaction conditions identical to the [Pd(pyrox)(TFA)$_2$]-catalyzed reaction. The reactivity of 6-D-**1** with [Pd[(*S*)-**3**](OAc)$_2$] as the catalyst was tested and, consistent with our prior results, the reaction proceeded in only 48% yield and 20% *ee*. ^{1}H NMR analysis of the initial product mixture revealed a 48:52 H/D ratio. After separation of the two enantiomeric products by HPLC, analysis of the *R*-configured products revealed a 14:86 H/D ratio, while the purified *S*-configured products displayed a 96:4 H/D ratio (Scheme 6). Incorporation of the data from either of these two measurements into the system of four equations led to similar product ratios for species **A**, **B**, **C**, and **D**, and the results show that the reaction strongly favored a *cis*-AP pathway (*trans*-AP/*cis*-AP = 10:90), with a 9:1:51:39 ratio for **A**/**B**/**C**/**D**. While the overall reaction exhibited low enantioselectivity, consideration of the minor products **A** and **B**, which arose from *trans*-AP of the alkene, revealed that the *trans*-AP pathway was quite enantioselective (e.r. = 9:1). Thus, with this substrate and pyrox/Pd^{II} catalyst system, the *trans*-AP pathway proceeds with high enantioselectivity while the *cis*-AP pathway exhibits low enantioselectivity. These observations represent the first direct assessment of the enantioselectivity of two different NP pathways for otherwise identical reactions.

The results of the reactions with the chiral ligands are summarized in entries 1 and 2 of Table 1. In an effort to separate the influence of the neutral-donor and anionic ligands on the stereochemical course of the AP step, we investigated the oxidative cyclization of 6-D-**1** with Pd(OAc)$_2$ and Pd(TFA)$_2$ in the absence of an ancillary neutral-donor ligand. The results show that both palladium(II) sources favor *cis*-AP of the alkene (Table 1, entries 3 and 4; see also Schemes S7 and S8 in the Supporting Information). The selectivity is considerably higher with Pd(OAc)$_2$; only trace quantities of the *trans*-AP-derived product are detected by NMR/HPLC analysis. With Pd(TFA)$_2$, the *trans*-AP/*cis*-AP selectivity is 1:6, thus suggesting that while the TFA ligand is intrinsically more compatible with the *trans*-AP mechanism, it still favors *cis*-AP. Taken together, the data in Table 1 demonstrate that the pyrox ligand plays an important role in enforcing the *trans*-AP pathway with Pd(TFA)$_2$ as the palladium(II) source. Previous efforts to understand the factors that influence *trans*- versus *cis*-AP selectivity have implicated the carboxylate ligand as a Brønsted base to mediate palladium—amidate bond formation in the *cis*-AP pathway.[5d,19] The present findings reveal that only with the combined presence of a trifluoroacetate anionic ligand and the pyrox neutral-donor ligand is a *trans*-AP pathway, initiated by substitution of TFA by the substrate alkene, favored over the *cis*-AP pathway involving formation of a Pd-amidate.

In summary, the design, synthesis and implementation of a novel chiral substrate probe (6-D-**1**) has enabled key insights into the relationship between the NP pathway and the enantioselectivity of a catalytic transformation. The ability of an ancillary neutral-donor ligand to alter the stereochemical course of NP only when a suitable anionic ligand is present highlights the challenges associated with the discovery of efficient catalysts for the asymmetric Wacker-type oxidation of alkenes. Ideally, the factors that affect the NP

stereochemistry should be considered in conjunction with the exploration of chiral ancillary ligands in the future development of enantioselective reactions.

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[1] P. M. Henry in *Palladium Catalyzed Oxidation of Hydrocarbons*, *Vol. 2*, D. Reidel Publishing Co., Dordrecht, The Netherlands, **1980**.

[2] a) G. Zeni, R. C. Larock, *Chem. Rev.* **2004**, *104*, 2285 – 2309; b) E. M. Beccalli, G. Broggini, M. Martinelli, S. Sottocornola, *Chem. Rev.* **2007**, *107*, 5318 – 5365; c) A. Minatti, K. Muñiz, *Chem. Soc. Rev.* **2007**, *36*, 1142 – 1152; d) V. Kotov, C. C. Scarborough, S. S. Stahl, *Inorg. Chem.* **2007**, *46*, 1910 – 1923; e) K. H. Jensen, M. S. Sigman, *Org. Biomol. Chem.* **2008**, *6*, 4083 – 4088.

[3] R. I. McDonald, G. Liu, S. S. Stahl, *Chem. Rev.* **2011**, *111*, 2981 – 3019.

[4] For leading primary references, see: a) T. Hosokawa, T. Uno, S. Inui, S.-I. Murahashi, *J. Am. Chem. Soc.* **1981**, *103*, 2318 – 2323; b) Y. Uozumi, K. Kato, T. Hayashi, *J. Am. Chem. Soc.* **1997**, *119*, 5063 – 5064; c) Y. Uozumi, K. Kato, T. Hayashi, *J. Org. Chem.* **1998**, *63*, 5071 – 5075; d) M. A. Arai, M. Kuraishi, T. Arai, H. Sasai, *J. Am. Chem. Soc.* **2001**, *123*, 2907 – 2908; e) R. M. Trend, Y. K. Ramtohul, E. M. Ferreira, B. M. Stoltz, *Angew. Chem.* **2003**, *115*, 2998 – 3001; *Angew. Chem. Int. Ed.* **2003**, *42*, 2892 – 2895; f) T. Hayashi, K. Yamasaki, M. Mimura, Y. Uozumi, *J. Am. Chem. Soc.* **2004**, *126*, 3036 – 3037; g) R. M. Trend, Y. K. Ramtohul, B. M. Stoltz, *J. Am. Chem. Soc.* **2005**, *127*, 17778 – 17788; h) K.-T. Yip, M. Yang, K.-L. Law, N.-Y. Zhu, D. Yang, *J. Am. Chem. Soc.* **2006**, *128*, 3130 – 3131; i) Y. J. Zhang, F. Wang, W. Zhang, *J. Org. Chem.* **2007**, *72*, 9208 – 9213; j) T. Tsujihara, T. Shinohara, K. Takenaka, S. Takizawa, K. Onitsuka, M. Hatanaka, H. Sasai, *J. Org. Chem.* **2009**, *74*, 9274 – 9279; k) W. He, K.-T. Yip, N.-Y. Zhu, D. Yang, *Org. Lett.* **2009**, *11*, 5626 – 5628; l) C. C. Scarborough, A. Bergant, G. T. Sazama, I. A. Guzei, L. C. Spencer, S. S. Stahl, *Tetrahedron* **2009**, *65*, 5084 – 5092; m) F. Jiang, Z. Wu, W. Zhang, *Tetrahedron Lett.* **2010**, *51*, 5124 – 5126; n) D. N. Mai, J. P. Wolfe, *J. Am. Chem. Soc.* **2010**, *132*, 12157 – 12159; o) K. H. Jensen, J. D. Webb, M. S. Sigman, *J. Am. Chem. Soc.* **2010**, *132*, 17471 – 17482; p) R. I. McDonald, P. B. White, A. B. Weinstein, C. P. Tam, S. S. Stahl, *Org. Lett.* **2011**, *13*, 2830 – 2833; q) G. Yang, C. Shen, W. Zhang, *Angew. Chem.* **2012**, *124*, 9275 – 9279; *Angew. Chem. Int. Ed.* **2012**, *51*, 9141 – 9145.

[5] For examples of *cis*-nucleopalladation, see the following leading references: [4f,g,k,n] and a) J. E. Ney, J. P. Wolfe, *Angew. Chem.* **2004**, *116*, 3689 – 3692; *Angew. Chem. Int. Ed.* **2004**, *43*, 3605 – 3608; b) M. B. Hay, J. P. Wolfe, *J. Am. Chem. Soc.* **2005**, *127*, 16468 – 16476; c) J. S. Nakhla, J. W. Kampf, J. P. Wolfe, *J. Am. Chem. Soc.* **2006**, *128*, 2893 – 2901; d) G. Liu, S. S. Stahl, *J. Am. Chem. Soc.* **2007**, *129*, 6328 – 6335; e) K. Muñiz, C. H. Hovelmann, J. Streuff, *J. Am. Chem. Soc.* **2008**, *130*, 763 – 773; f) B. W. Michel, L. D. Steffens, M. S. Sigman, *J. Am. Chem. Soc.* **2011**, *133*, 8317 – 8325.

[6] For examples of *trans*-nucleopalladation, see the following leading references: Refs. [4g,o, 5c,d] and a) M. P. Watson, L. E. Overman, R. G. Bergman, *J. Am. Chem. Soc.* **2007**, *129*, 5031 – 5044; b) B. M. Cochran, F. E. Michael, *J. Am. Chem. Soc.* **2008**, *130*, 2786 – 2792; c) P. A. Sibbald, C. F. Rosewall, R. D. Swartz, F. E. Michael, *J. Am. Chem. Soc.* **2009**, *131*, 15945 – 15951.

[7] Quinox and pyrox ligands have emerged as a versatile class of chiral ligands for palladium(II)-catalyzed reactions with alkenes. For examples other than those described in Refs [4k,m,o,p] and [11], see: a) N. S. Perch, R. A. Widenhoefer, *J. Am. Chem. Soc.* **1999**, *121*, 6960 – 6961; b) N. S. Perch, T. Pei, R. A. Widenhoefer, *J. Org. Chem.* **2000**, *65*, 3836 – 3845; c) Y. Zhang, M. S. Sigman, *J. Am. Chem. Soc.* **2007**, *129*, 3076 – 3077; d) T. P. Pathak, K. M. Gligorich, B. E. Welm, M. S. Sigman, *J. Am. Chem. Soc.* **2010**, *132*, 7870 – 7871; e) R. Jana, T. P. Pathak, K. H. Jensen, M. S. Sigman, *Org. Lett.* **2012**, *14*, 4074 – 4077.

[8] See Ref. [5d] and: X. Ye, G. Liu, B. V. Popp, S. S. Stahl, *J. Org. Chem.* **2011**, *76*, 1031 – 1044.

[9] Investigation of a well-defined (bipyridine)Pd^{II}-tosylamidate complex provided fundamental insights into alkene insertion into a Pd–N(R)Ts bond (i.e., the *cis*-AP step). See a) P. B. White, S. S. Stahl, *J. Am. Chem. Soc.* **2011**, *133*, 18594 – 18597. For other studies of alkene insertion into Pd–N bonds, see the following leading references: b) P. S. Hanley, J. F. Hartwig, *J. Am. Chem. Soc.* **2011**, *133*, 15661 – 15673; c) J. D. Neukom, N. S. Perch, J. P. Wolfe, *Organometallics* **2011**, *30*, 1269 – 1277.

[10] Yang and co-workers recently reported a Pd^{II}/quinoline-oxazoline (quinox)catalyst system for enantioselective oxidative cyclization of anilides bearing tethered alkenes, and stereochemical analysis of the products revealed that the reaction involves *cis*-AP of the alkene (see Ref. [4k]).

[11] Further circumstantial evidence supporting a *cis*-AP mechanism comes from the use of quinox and pyrox ligands in other palladium(II)-catalyzed reactions, such as oxidative Heck reactions, which involve *cis* addition of palladium(II) and an aryl group across an alkene C=C bond. For *cis*-NP reactions employing pyrox/quinox ligands, see Refs [4k, 5f], and: K. S. Yoo, C. P. Park, C. H. Yoon, S. Sakaguchi, J. O'Neil, K. W. Jung, *Org. Lett.* **2007**, *9*, 3933 – 3935.

[12] See Ref. [5d]. (−)-Sparteine is a chiral diamine (bidentate) ligand.

[13] The substrate 6-D-**1** was synthesized from (*S*)-mandelic acid in ten steps, with the first five steps having direct precedent in the literature: M. N. Alberti, G. Vassilikogiannakis, M. Orfanopoulos, *Org. Lett.* **2008**, *10*, 3997 – 4000. Derivatization and analysis of an advanced intermediate established the stereochemical purity of the substrate probe to be very high (> 20:1, see the Supporting Information for details).

[14] A detailed consideration of how alkene isomerization and deuterium scrambling could complicate our results is provided in the Supporting Information (Scheme S1). However, under all catalyst conditions tested in this study, the *trans*-styrenyl products are obtained in > 20:1 selectivity, and it was possible to remove trace quantities of the *cis*-styrenyl products by chromatography prior to further analysis. The extent of deuterium scrambling was characterized by ^{2}H NMR analysis of the mixture of the four products **A**–**D** (Scheme S3–S8), and only trace quantities (≤ 5%) of deuterium at the C5 position were observed in our experiments.

[15] This quantity was corroborated by ESI/MS analysis of the enantiomerically pure products (see Schemes S3–S8 in the Supporting Information).

[16] The validity of this protocol was tested by subjecting 6-D-**1** to the previously reported $Pd(OAc)_2$/pyridine oxidative cyclization conditions. The data arising from this experiment show that the reaction proceeds with very high selectivity for *cis*-AP of the alkene (*trans*/*cis* = 8:92; see Scheme S3 in the Supporting Information), thus supporting the previously reported conclusions derived from the use of substrate 3-D-**4** as the mechanistic probe (see Ref. [5d]).

[17] Cyclization of 6-D-**1** with the opposite antipode of the ligand, (*R*)-**3**, resulted in 93% yield, 96% *ee*, and a 96:4 *trans*/*cis*-AP ratio (see Scheme S5 in the Supporting Information). The similar

trans/*cis*-AP ratios obtained with ligands (*S*)-**3** and (*R*)-**3** in the reactions of 6-D-**1** show that any kinetic isotope effect associated with β-hydride elimination from the C6 position has minimal impact on the AP pathway.

[18] We previously reported DFT calculations that implicated high enantioselectivity for a *cis*-AP pathway (see Ref. [4p]). The present results suggest that at least one other *cis*-AP pathway exists that is lower in energy than the one presented in Ref. [4p]. DFT calculations to explore this issue and to analyze the *trans*-AP mechanism are currently under investigation.

[19] For other discussion of factors contributing to *cis*- versus *trans*-nucleopalladation, see Refs [3, 4g, 5c] and the following: J. A. Keith, P. M. Henry, *Angew. Chem.* **2009**, *121*, 9200 – 9212; *Angew. Chem. Int. Ed.* **2009**, *48*, 9038 – 9049.

