ORGANIC LETTERS

2012
Vol. 14, No. 9
2402–2405

Total Synthesis of Mycocyclosin

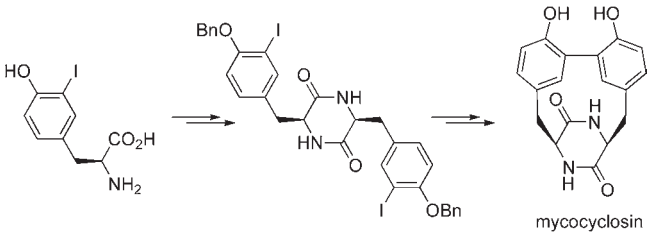
James R. Cochrane, Jonathan M. White, Uta Wille, and Craig A. Hutton*

School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, VIC 3010, Australia

chutton@unimelb.edu.au

Received April 2, 2012

ABSTRACT



The first total synthesis of mycocyclosin, a diketopiperazine natural product isolated from *M. tuberculosis*, is described. While direct oxidative coupling of tyrosine phenolic groups was unsuccessful, construction of the highly strained bicyclic framework was successfully accomplished through an intramolecular Miyaura–Suzuki cross-coupling to generate the biaryl linkage.

Mycocyclosin **1** (Figure 1) is a diketopiperazine secondary metabolite produced by *Mycobacterium tuberculosis*, discovered in 2009 by Belin and co-workers.[1] The biosynthesis of mycocyclosin proceeds through initial condensation of two molecules of tyrosyl-tRNA **2** to generate cyclo(L-Tyr–L-Tyr) **3** (Scheme 1). Formation of the diketopiperazine **3** is catalyzed by the enzyme cyclodityrosine synthetase. This enzyme is the first characterized member of a recently discovered family of enzymes known as cyclodipeptide synthetases (CDPS) that structurally resemble the catalytic domain of class Ic tRNA synthetases.[2−5] Subsequent oxidative coupling of the tyrosine aromatic rings of **3** is catalyzed by the cytochrome P450 enzyme CYP121, which generates a 3,3′-dityrosine-type biaryl cross-link to complete the bicyclic framework of **1**.[1−3] Recently, the gene encoding the CYP121 P450 enzyme, *rv2276*, was shown to be essential for *M. tuberculosis* viability,[6] leading to speculation that mycocyclosin has a vital but as yet undetermined cellular role in *M. tuberculosis* survival.

Furthermore, the *M. tuberculosis* CYP121 P450 enzyme appears to be a target for the azole antifungal/antimycobacterial drugs,[6] highlighting the potential of this enzyme as a novel *M. tuberculosis* drug target. The use of azole-type therapeutics often leads to serious side effects due to their nonselective inhibition of host P450 enzymes, and thus the design of selective inhibitors of the CYP121 P450 enzyme, based on structural analogy to mycocyclosin, could ultimately provide new therapeutics for tuberculosis with fewer side effects.

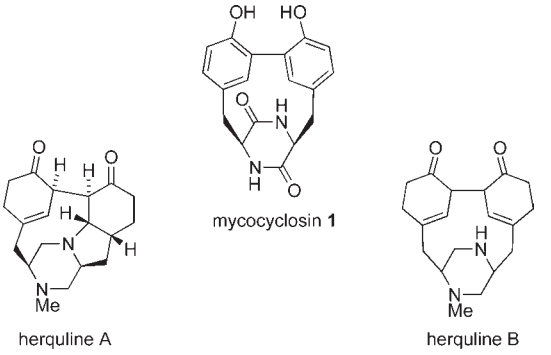


Figure 1. Structures of mycocyclosin **1** and related natural products herqulines A and B.

(1) Belin, P.; Le Du, M. H.; Fielding, A.; Lequin, O.; Jacquet, M.; Charbonnier, J.-B.; Lecoq, A.; Thai, R.; Courçon, M.; Masson, C.; Dugave, C.; Genet, R.; Pernodet, J.-L.; Gondry, M. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 7426–7431.

(2) Gondry, M.; Sauguet, L.; Belin, P.; Thai, R.; Amouroux, R.; Tellier, C.; Tuphile, K.; Jacquet, M.; Braud, S.; Courçon, M.; Masson, C.; Dubois, S.; Lautru, S.; Lecoq, A.; Hashimoto, S.-I.; Genet, R.; Pernodet, J.-L. *Nat. Chem. Biol.* **2009**, *5*, 414–420.

(3) Vetting, M. W.; Hegde, S. S.; Blanchard, J. S. *Nat. Chem. Biol.* **2010**, *6*, 797–799.

(4) Sauguet, L.; Moutiez, M.; Li, Y.; Belin, P.; Seguin, J.; Le Du, M. H.; Thai, R.; Masson, C.; Fonvielle, M.; Pernodet, J. L.; Charbonnier, J. B.; Gondry, M. *Nucleic Acids Res.* **2011**, *39*, 4475–4489.

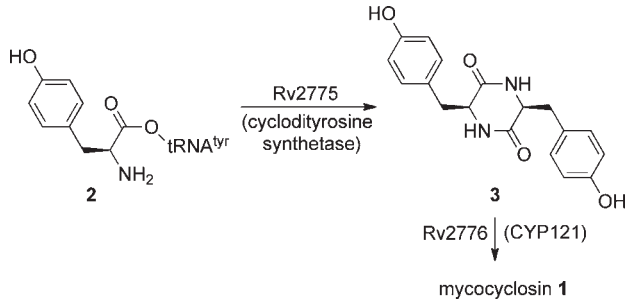
(5) Bonnefond, L.; Arai, T.; Sakaguchi, Y.; Suzuki, T.; Ishitani, R.; Nureki, O. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 3912–3917.

10.1021/ol300831t
Published on Web 04/20/2012

Construction of a molecular model of mycocyclosin **1** suggests the bicyclic compound is significantly strained. In this regard, mycocyclosin is reminiscent of strained cyclophane-type natural products containing biaryl linkages, such as haouamine and rhazinal, which have attracted significant recent interest as synthetic targets.[7−13] Mycocyclosin also bears close resemblance to the natural products herquline A and B (Figure 1), isolated by Omura and co-workers from *Penicillium herquei*,[14−16] and potentially could be a biosynthetic precursor of such compounds.

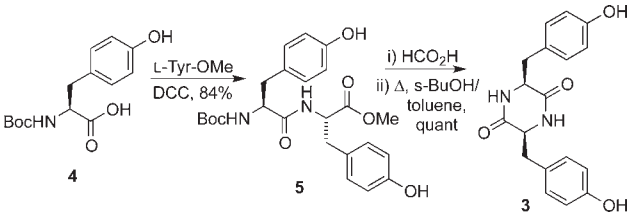
The unusual structure of mycocyclosin, together with its intriguing role in *M. tuberculosis* biology, prompted us to investigate a route toward its total synthesis.

Scheme 1. Biosynthesis of Mycocyclosin **1**



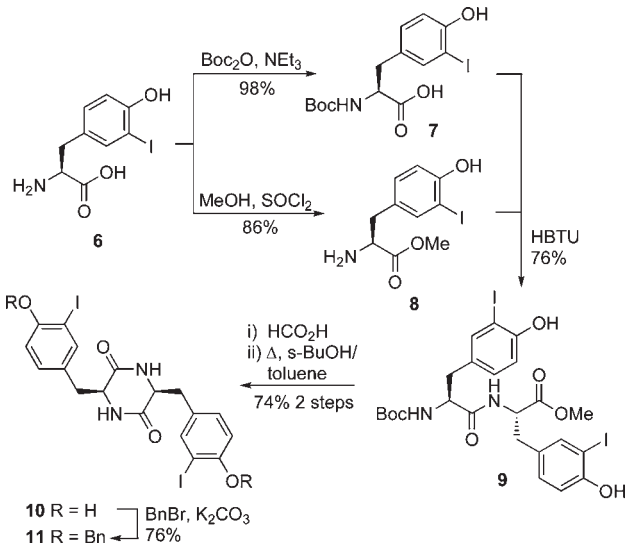
In pursuit of a synthetic route to mycocyclosin **1**, we envisaged a biomimetic approach through initial diketopiperazine synthesis followed by oxidative phenolic coupling. Cyclodityrosine has been prepared by condensation of L-tyrosine in ethylene glycol at high temperature;[17] however, this method has been shown to cause epimerization, resulting in mixtures of stereoisomers.[18] Accordingly, cyclo(L-Tyr−L-Tyr) **3** was prepared by a stepwise method.[19] Tyrosine was converted to the protected dipeptide **5** through standard amino acid protecting group and peptide coupling techniques (Scheme 2). Removal of the Boc-group and cyclization generated the diketopiperazine **3** in good yield as a single stereoisomer.

Scheme 2. Synthesis of L,L-Cyclodityrosine **3**



Direct oxidative coupling of **3** was attempted using the vanadium oxyfluoride-promoted process developed by Evans and co-workers.[20] However, under these conditions only dimers and trimers of the diketopiperazine **3** were detected, with no evidence of intramolecular phenolic coupling to generate mycocyclosin **1**. In retrospect, direct oxidative coupling to generate strained biaryl linkages has met with only limited success.[10,13]

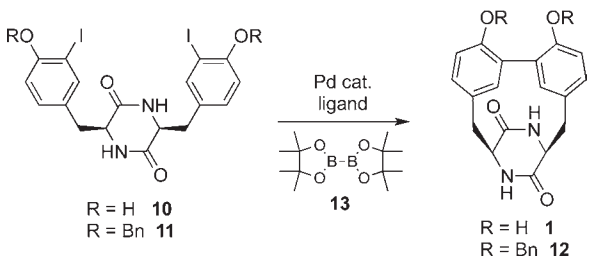
Scheme 3. Synthesis of Diketopiperazine **11**



Attention was therefore turned to a metal-catalyzed cross-coupling approach, which required the corresponding iodotyrosine-containing diketopiperazine **10**. Accordingly, cyclo(L-Tyr−L-Tyr) **3** was treated with iodine in the presence of silver sulfate; however, to drive the iodination of both tyrosine residues to completion, small amounts of the corresponding tri-iodinated product were produced, which was difficult to remove. A more efficient route to iodinated **10** was therefore pursued from L-iodotyrosine **6**. Following an analogous procedure to that used for the

(6) McLean, K. J.; Carroll, P.; Lewis, D. G.; Dunford, A. J.; Seward, H. E.; Neeli, R.; Cheesman, M. R.; Marsollier, L.; Douglas, P.; Smith, W. E.; Rosenkrands, I.; Cole, S. T.; Leys, D.; Parish, T.; Munro, A. W. *J. Biol. Chem.* **2008**, *283*, 33406–33416.
(7) Garrido, L.; Zubía, E.; Ortega, M. J.; Salvá, J. *J. Org. Chem.* **2003**, *68*, 293–299.
(8) Fürstner, A.; Ackerstaff, J. *Chem. Commun.* **2008**, 2870.
(9) Burns, N. Z.; Krylova, I. N.; Hannoush, R. N.; Baran, P. S. *J. Am. Chem. Soc.* **2009**, *131*, 9172–9173.
(10) Wipf, P.; Furegati, M. *Org. Lett.* **2006**, *8*, 1901–1904.
(11) Grundl, M. A.; Trauner, D. *Org. Lett.* **2006**, *8*, 23–25.
(12) Baran, P. S.; Burns, N. Z. *J. Am. Chem. Soc.* **2006**, *128*, 3908–3909.
(13) Bowie, A. L.; Trauner, D. *J. Org. Chem.* **2009**, *74*, 1581–1586.
(14) Omura, S.; Hirano, A.; Iwai, Y.; Masuma, R. *J. Antibiot.* **1979**, *32*, 786–790.
(15) Furusaki, A.; Matsumoto, T.; Ogura, H.; Takayanagi, H.; Hirano, A.; Omura, S. *J. Chem. Soc., Chem. Commun.* **1980**, 698.
(16) Enomoto, Y.; Shiomi, K.; Hayashi, M.; Masuma, R.; Kawakubo, T.; Tomosawa, K.; Iwai, Y.; Omura, S. *J. Antibiot.* **1996**, *49*, 50–53.
(17) Xia, H.; Suo, Z. Y.; Qiang, G. J.; Chang, C. J. *J. Appl. Polym. Sci.* **2008**, *110*, 2168–2178.
(18) Cook, B.; Hill, R. R.; Jeffs, G. E. *J. Chem. Soc., Perkin Trans. 1* **1992**, 1199.
(19) Jung, M. E.; Rohloff, J. C. *J. Org. Chem.* **1985**, *50*, 4909–4913.
(20) (a) Evans, D. A.; Dinsmore, C. J.; Evrard, D. A.; DeVries, K. M. *J. Am. Chem. Soc.* **1993**, *115*, 6426–6421. For application to dityrosine, see: (b) Reetz, M.; Merk, C.; Mehler, G. *Chem. Commun.* **1998**, 2075–2076.

Table 1. Optimization of Biaryl Coupling/Cyclization[a]



entry	R	13 (equiv)	catalyst	yield (%)
1	Bn	–	$NiCl_2(PCy_3)_2$, Zn, PPh_3[b]	0
2	Bn	–	$Ni(PPh_3)_4$[c]	0
3	H	1.2	$Pd(dppf)\cdot CH_2Cl_2$	0
4	Bn	1.2	$Pd(dppf)\cdot CH_2Cl_2$	33
5[d]	Bn	1.2	$Pd(dppf)\cdot CH_2Cl_2$	20
6	Bn	1.2	$Pd(OAc)_2$, Sphos[e]	19
7[f]	Bn	1.2	$Pd(dppf)\cdot CH_2Cl_2$	5
8[g]	Bn	1.2	$Pd(dppf)\cdot CH_2Cl_2$	26
9	**Bn**	**1.0**	**$Pd(dppf)\cdot CH_2Cl_2$**	**42**
10	Bn	0.6	$Pd(dppf)\cdot CH_2Cl_2$	27

[a] Conditions: **10** or **11**, 0.005 M in DMSO, **13**, 6 equiv K_2CO_3, catalyst (20 mol %), 90 °C. [b] DMF solvent. [c] DMF solvent, 1.5 equiv Ni(0). [d] 80 °C. [e] ligand/catalyst 3:1. [f] 1,4-Dioxane solvent. [g] Microwave irradiation, 90 °C, 2 h.

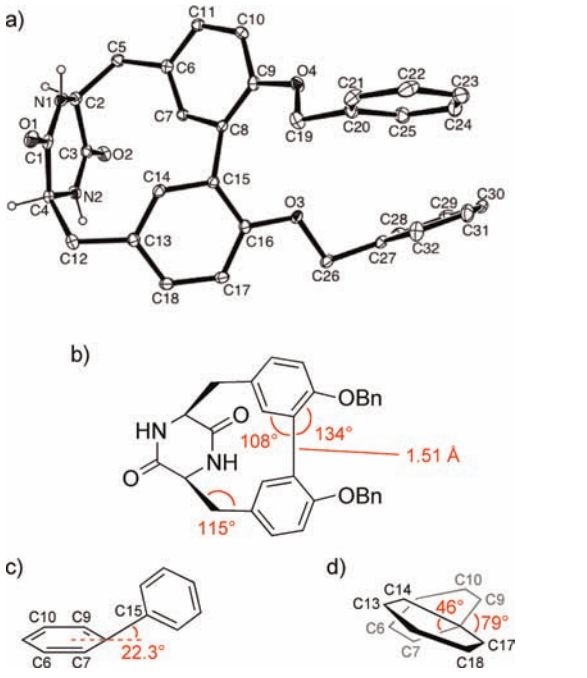


Figure 2. (a) ORTEP diagram of bis(*O*-benzyl)mycocyclosin **12**. (b) Representation of crystal structure with perturbed bond lengths and angles highlighted. (c) Representation of out-of-plane bend of biaryl bond of **12**. (d) View down biaryl bond (C15−C8) highlighting twist of aromatic rings and out-of-plane bend of biaryl bond with respect to both aromatic rings.

preparation of L,L-cyclodityrosine **3**, the L,L-cyclodi(iodotyrosine) **10** was generated from **6** in three steps and 55% yield (Scheme 3). Benzylation of **10** gave the protected compound **11** in good yield.

Attempted Ni-catalyzed Ullmann-type reductive coupling of bis-iodide **11** did not proceed (Table 1, entry 1),[21] returning a mixture of starting material and protodeiodinated material. Accordingly, we switched to a one-pot Pd-catalyzed Miyaura borylation−Suzuki coupling method we have previously employed for the preparation of dityrosine derivatives.[22] Zhu et al. also employed such an approach in an intramolecular manner to generate the biaryl linkage in a biphenomycin analogue.[23] Treatment of L,L-cyclodi(iodotyrosine) **10** with bispinacolatodiboron **13** in the presence of $Pd(dppf)Cl_2$ gave none of the cross-coupled product, with proto-deiodinated material recovered (entry 3). In contrast, treatment of the benzyl-protected L,L-cyclodi(iodotyrosine) **11** under the same conditions gave the cross-coupled adduct **12** in moderate yield (entry 4). Optimization of the cross-coupling reaction was attempted by varying temperature (entry 5), ligand (entry 6), solvent (entry 7), use of microwave irradiation (entry 8), and stoichiometry (entries 9 and 10). All conditions provided the protected mycocyclosin derivative **12** in low to moderate yield, with an optimum 42% yield obtained.

Crystals of **12** suitable for X-ray crystallographic analysis were grown from DMSO. The crystal structure of **12** confirms that the [8.2.2] bicyclic framework is highly strained (see Figure 2). The geometry of the dityrosine biaryl linkage is significantly distorted: the biaryl bond is longer than normal (1.51 Å; cf. 1.49 Å for an average biaryl bond),[24] and the associated bond angles deviate greatly from the idealized value of 120° (e.g., C7−C8−C15 108.1°, C9−C8−C15 134.4°). Notably, the C8−C15 biaryl bond is bent considerably out-of-plane with respect to the aromatic rings, displaying α-angles of 22.3° and 16.3°. These distortions are among the greatest deviations known for strained cyclophane natural products and derivatives.[7,10,25,26]

The high degree of strain present in the bicyclic compound **12** suggests it is remarkable that the cross-coupling proceeds in even moderate yield.[27] We speculate that the intermediate biaryl-palladium species represents a

(21) For use of Ni(0)-promoted reductive cyclization in the synthesis of alnusone, see: (a) Semmelhack, M. F.; Ryono, L. S. *J. Am. Chem. Soc.* **1975**, *97*, 3873–3875. For attempts at intramolecular Ni- and Pd-catalyzed couplings toward the biaryl-containing ring in vancomycin, see: (b) Boger, D. L.; Miyazaki, S.; Kim, S. H.; Wu, J. H.; Castle, S. L.; Loiseleur, O.; Jin, Q. *J. Am. Chem. Soc.* **1999**, *121*, 10004–10011. (c) Nicolaou, K. C.; Li, H.; Boddy, C. N. C.; Ramanjulu, J. M.; Yue, T.-Y.; Natarajan, S.; Chu, X.-J.; Bräse, S.; Rübsam, F. *Chem.—Eur. J.* **1999**, *5*, 2584–2601.

(22) (a) Hutton, C. A.; Skaff, O. *Tetrahedron Lett.* **2003**, *44*, 4895–4898. (b) Skaff, O.; Jolliffe, K. A.; Hutton, C. A. *J. Org. Chem.* **2005**, *70*, 7353–7363.

(23) Carbonnelle, A.-C.; Zhu, J. *Org. Lett.* **2000**, *2*, 3477–3480.

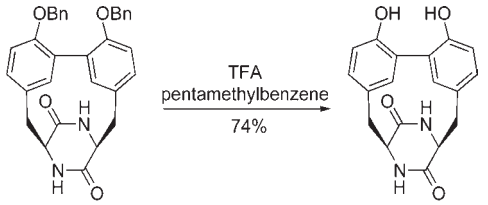
(24) Allen, F. H.; Watson, D. G.; Brammer, L.; Orpen, A. G.; Taylor, R. In *International Tables for Crystallography*, Vol. C; Prince, E., Ed.; Wiley-VCH: 2006; Chapter 9.5, pp 790−811.

(25) Cram, D.; Cram, J. M. *Acc. Chem. Res.* **1971**, *4*, 204–213.

(26) Lee, T. J.; Rice, J. E.; Allen, W. D.; Remington, R. B.; Schaefer, H. F., III. *Chem. Phys.* **1988**, *123*, 1–25.

'tethered', low-strain system in which the tyrosine C3 positions are held in close proximity, with reductive elimination then proceeding to yield the cross-linked product **12**. Such an approach proved successful in Trauner's synthesis of rhazinal in which Pd-catalyzed cross-coupling effected formation of a strained biaryl system, whereas oxidative direct coupling failed.[13]

Scheme 4. Synthesis of Mycocyclosin **1**



With bis(*O*-benzyl)-protected mycocyclosin **12** in hand, standard hydrogenolysis conditions were investigated to effect deprotection. However, under such conditions, complete decomposition of the strained bicyclic compound was observed. Fortunately, treatment of **12** with trifluoroacetic acid (TFA) in the presence of pentamethylbenzene as a cation scavenger effected clean benzyl group cleavage to generate mycocyclosin **1** in good yield (Scheme 4). The ^{1}H and ^{13}C NMR spectra of synthetic mycocyclosin closely matched that reported for the natural product.[1]

Considering the strain exhibited in the benzyl-protected mycocyclosin **12**, the natural product would be expected to possess similar strain. Though the structure of mycocyclosin **1** has been determined by mass spectrometry and ^{1}H and ^{13}C NMR spectroscopy, no confirmation of the three-dimensional structure, including analysis of the configuration about the biaryl chirality axis, has been reported. We considered that the contraction of the angles about the biaryl linkage could increase the barrier to rotation such that atropisomers are possible. Accordingly, we calculated the ground-state structure of mycocyclosin **1** and the barrier to rotation about the biaryl axis.[28]

The calculated ground-state structure of mycocyclosin **1** is in close agreement with the crystal structure of the benzyl-protected derivative **12**; the bond angles around the biaryl linkage are similarly distorted from ideal geometry, and the biaryl bond is significantly bent out-of-plane with respect to the aromatic rings (20.7° in **1**; cf. 22.3° in **12**). The barrier to rotation between atropisomers was calculated to be 39.6 kJ·mol^{-1} (Figure 3), which is in the range expected for a 1,1′-disubstituted biaryl.[29] The transition state to atropisomerization is stabilized by a strong

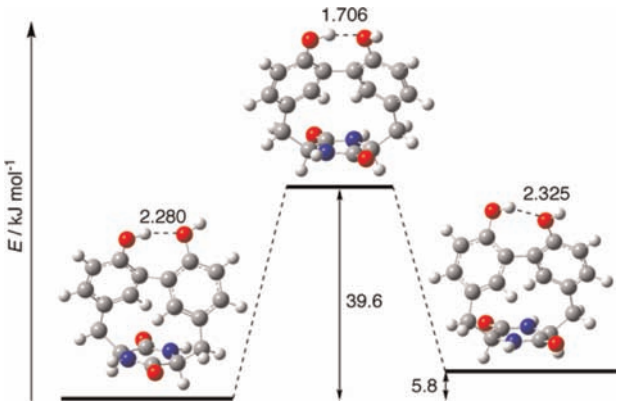


Figure 3. Calculated potential energy surface for the atropisomerization of mycocyclosin.

H-bond between the phenolic OH groups; without this stabilization the barrier to rotation would be higher. The barrier to rotation is sufficiently low that rapid interconversion of atropisomers would occur at rt. The lowest energy conformation of mycocyclosin **1** is 5.8 kJ·mol^{-1} more stable than its atropisomer and matches the configuration of the atropisomer observed in the solid state of **12**. Low temperature NMR studies of mycocyclosin are in agreement with the calculations, showing that interconversion of the atropisomers occurs rapidly at rt, with a coalescence temperature < 190 K (see Supporting Information).

In conclusion, an efficient synthesis of the highly strained meta-cyclophane-type diketopiperazine natural product, mycocyclosin **1**, has been developed employing an intramolecular, one-pot borylation–Suzuki cross-coupling to generate the biaryl linkage. A synthetic route to **1** should allow investigations into the biological role of mycocyclosin in *Mycobacterium tuberculosis* viability and also provides a synthetic route suitable for elaboration to the preparation of analogues of mycocyclosin in the search for potential antimycobacterial compounds.

Acknowledgment. Financial support from the Australian Research Council (DP110100112) is acknowledged. This work was supported by the National Computing Infrastructure (NCI) facility. Dr. Ian Luck (University of Sydney) is thanked for assistance with VT NMR experiments. Assoc. Prof. Spencer Williams (University of Melbourne) is thanked for bringing this compound to our attention.

Supporting Information Available. Full experimental details, characterization data, and ^{1}H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org. The authors declare no competing financial interest.

(27) A yield of 45% was obtained for a related intramolecular cross-coupling with much less strain; see ref 23.

(28) Calculations were performed using the BHandHLYP hybrid density functional in combination with the 6-31+G* basis set.

(29) Leroux, F. *ChemBioChem* **2004**, *5*, 644–649.

The authors declare no competing financial interest.