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Use of a Chiral Praseodymium Shift Reagent in Predicting the Complete Stereostructure of Glisoprenin A

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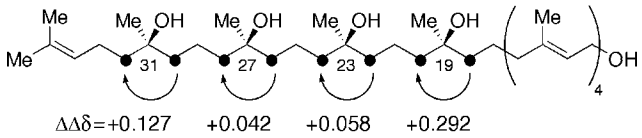
ABSTRACT



Glisoprenin A

$$\Delta\Delta\delta = \Delta\delta_{R\text{-}4}\,(\delta CX_{R\text{-}4} - \delta CY_{R\text{-}4}) - \Delta\delta_{S\text{-}4}\,(\delta CX_{S\text{-}4} - \delta CY_{S\text{-}4})$$

The complete stereostructure of glisoprenin A has been predicted via analysis of the ^{13}C NMR behaviors in the presence of (*R*)- and (*S*)-Pr(tfc)$_3$.

The polyisoprenepolyol class of natural products first came to the attention of the chemical community in 1983 when Nozoe and co-workers reported the isolation of the gymnoprenols and gymnoplin from the hallucinogenic mushroom *Gymnopilus spectablis*.[1] In 1992, while screening for acyl-CoA:cholesterol acyl transferase (ACAT) inhibitors, Omura and co-workers reported the isolation of glisoprenin A (**1**) and glisoprenin B from the fermentation broth of *Gliocladium*.[2] At least 19 members are now known in the polyisoprenepolyol family.[3] Their gross structures were elucidated through a combined use of degradation and spectroscopic studies. However, the complete stereostructure has not yet been established for any member of this class of natural products.[4] In this Letter, we report the stereochemical assignment of glisoprenin A (**1**) by employing the chiral lanthanide shift reagent approach disclosed in the preceding Letter.

Glisoprenin A (**1**) contains four asymmetric tertiary alcohols at C-19, C-23, C-27, and C-31 that are separated by a three-carbon bridge. Thus, by invoking the concept of a self-contained box, we can analyze each of the four stereogenic centers *independently*.[5] In this respect, the chiral NMR solvent approach recently developed in this laboratory (Figure 1) seems particularly well suited to the case at hand.[6]

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(1) (a) Nozoe, S.; Koike, Y.; Tsuji, E.; Kusano, G.; Seto, H. *Tetrahedron Lett*. **1983**, *24*, 1731. (b) Nozoe, S.; Koike, Y.; Kusano, G.; Seto, H. *Tetrahedron Lett*. **1983**, *24*, 1735. (c) Aoyagi, F.; Maeno, S.; Okuno, T.; Matasumoto, H.; Ikura, M.; Hikichi, K.; Matsumoto, T. *Tetrahedron Lett*. **1983**, *24*, 1991. (d) Nozoe, S.; Koike, Y.; Ito, N.; Kusano, G. *Chem. Lett*. **1984**, 1001.

(2) (a) Tomoda, H.; Huang, X. H.; Nishida, H.; Masuma, R.; Kim, Y. K.; Omura, S. *J. Antibiot*. **1992**, *45*, 1202. (b) Nishida, H.; Huang, X. H.; Tomoda, H.; Omura, S. *J. Antibiot*. **1992**, *45*, 1669.

(3) (a) Hegde, V. R.; Dai, P.; Chu, M.; Patel, M.; Bryant, R.; Terracciano, J.; Das, P. R.; Puar, M. S. *J. Antibiot*. **1997**, *50*, 983. (b) Thines, E.; Eilbert, F.; Anke, H.; Sterner, O. *J. Antibiot*. **1998**, *51*, 117. (c) Sterner, O.; Thines E.; Eilbert, F.; Anke, H. *J. Antibiot*. **1998**, *51*, 228. (d) Joshi, B. K.; Gloer, J. B.; Wicklow, D. T. *J. Nat. Prod*. **1999**, *62*, 730. (e) Sawabe, A.; Morita, M.; Kiso, T.; Kishine, H.; Ohtsubo, Y.; Ouchi, S.; Okamoto, T. *J. Agric. Food Chem*. **1999**, *47*, 588.

(4) On the basis of the CD study of a degradation product, Nozoe and co-workers predicted the absolute configuration of the *ω-tert*-hydroxyl of gymnopilin (corresponding to the C-31 hydroxyl of glisoprenin A) to be *S*: Ohta, T.; Aizawa, K.; Kamo, S.; Tabei, N.; Oshima, Y.; Nozoe, S. *38th Tennen Yuki Kagobutsu Toronkai Koen Yoshishu*, **1996**, 307. In addition, the absolute configuration of the C-2/C-3 α-glycol in gymnoprenols is known; see: (a) Nozoe, S.; Koike, Y.; Kusano, G. *Tetrahedron Lett*. **1984**, *25*, 1371. (b) Hanson, R. M. *Tetrahedron Lett*. **1984**, *25*, 3783. (c) Nozoe, S.; Ohta, T.; Koike, Y.; Kusano, G. *Tetrahedron Lett*. **1984**, *25*, 4023.

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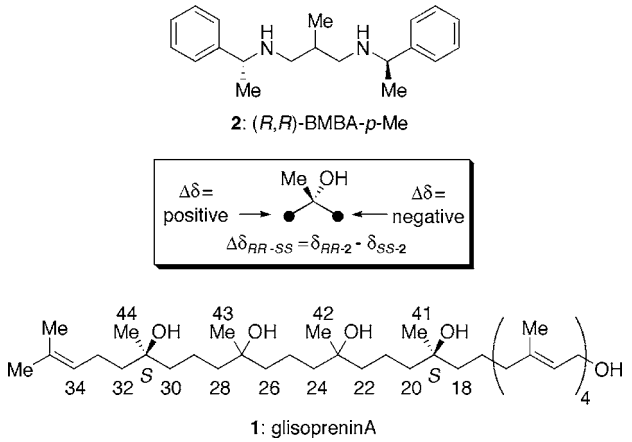

Figure 1. Prediction of the C-19 and C-31 absolute configurations of glisoprenin A (**1**) from the chiral NMR solvents (*R*,*R*)- and (*S*,*S*)-BMBA-*p*-Me (**2**). The $\Delta\delta$ values observed for the C-18 and C-32 carbons were +0.023 and −0.053, respectively.

However, there are two requirements in order to conduct stereochemical analyses by this approach. First, at least one, preferably both, of the carbons adjacent to the alcoholic center (referred to as the α-carbons) must be observed as a distinct resonance in the chiral bidendate solvent. Second, the chemical shifts of the α-carbons must be assigned unambiguously in the chiral medium. Unfortunately, when the ^{13}C NMR spectrum of glisoprenin A (**1**) was acquired in (*R*,*R*)- or (*S*,*S*)-BMBA-*p*-Me/$CDCl_3$, only two (C-18 and C-32) out of the eight α-carbons were discernible (Figure 2). Nonetheless, the $\Delta\delta$ behaviors observed for the C-18 and C-30 carbons allowed us to predict the absolute configuration of both the C-19 and C-31 alcohols to be *S*.[4] However, we were unable to treat the remaining C-23 and C-27 stereogenic

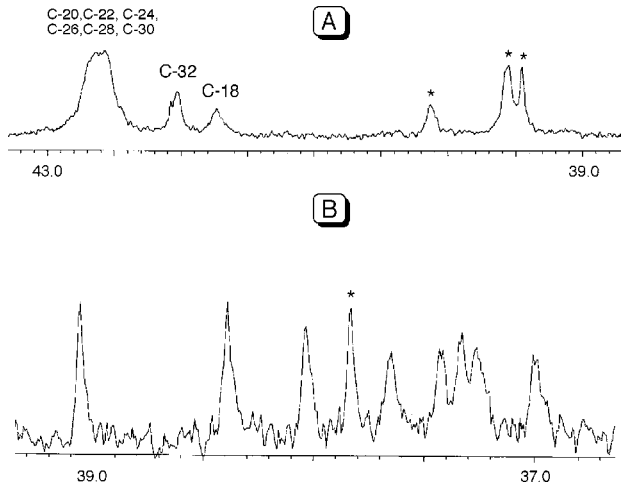

Figure 2. Comparison of the α-carbon region of the ^{13}C NMR spectrum of glisoprenin A (**1**) in (*S*,*S*)-BMBA-*p*-Me (panel A) and (*S*)-Pr(tfc)$_3$ (panel B). Asterisks (*) indicate resonance of the allylic carbon at C-4.

centers in a similar manner, because the α-carbons associated with these stereocenters overlapped to give a single broad resonance. In principle, this problem can be circumvented by either (1) acquiring the spectrum on a higher magnetic field NMR spectrometer or (2) spreading the spectrum with the use of lanthanide shift reagents.[7] In the preceding Letter, we illustrated the exciting and appealing potential the latter approach offers.[8] Our hope was that, in the presence of Pr(tfc)$_3$ (**3**), the C-18, C-20, C-22, C-24, C-26, C-28, C-30, and C-32 resonances would spread out, thereby permitting the assignment of absolute configuration of glisoprenin A (**1**).

Initially, we attempted the chiral shift reagent experiment on glisoprenin A (**1**) under the conditions optimized for the model compounds, i.e., 15 mol % per OH of (*R*)- and (*S*)-**3**.[8] However, this attempt failed to spread the chemical shifts of the α-carbon resonances to our satisfaction. Increasing the concentration of Pr(tfc)$_3$ (**3**) to 25 mol % per OH resulted in a spectrum in which all eight α-carbons were observed as separate resonances (Figure 2).

With a resolved ^{13}C NMR in hand, the next step was unambiguously to establish the chemical shifts of the eight α-carbons. We envisioned this being accomplished by following the stepwise procedures depicted in Figure 3. The

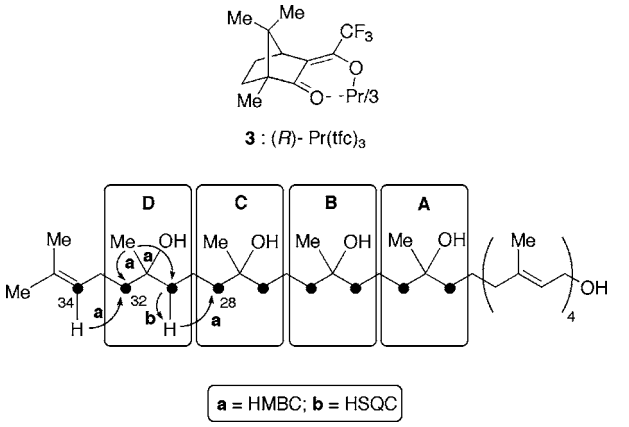

Figure 3. Strategy to assign the chemical shifts of the α-carbons of glisoprenin A (**1**) in the presence of Pr(tfc)$_3$ (**3**).

first step is to correlate through detection of long-range ^{1}H−^{13}C coupling the proton resonance (singlet) of each quaternary methyl group to the carbon resonances of two

(5) (a) Boyle, C. D.; Harmange, J. C-.; Kishi, Y. *J. Am. Chem. Soc.* **1994**, *116*, 4995. (b) Zheng, W.; DeMattei, J. A.; Wu, J.-P.; Duan, J. J.-W.; Cook, L. R.; Oinuma, H.; Kishi, Y. *J. Am. Chem. Soc.* **1996**, *118*, 7946. (c) Kobayashi, Y.; Tan, C.-H.; Kishi, Y. *Helv. Chim. Acta* **2000**, *83*, 2562. (d) Kobayashi, Y.; Tan, C.-H.; Kishi, Y. *J. Am. Chem. Soc.* **2001,** 123, 2076. (e) Higashibayashi, S.; Czechtizky, W.; Kobayashi, Y.; Kishi, Y. *J. Am. Chem. Soc.* **2003**, *125*, 14379 and references therein.
(6) (a) Kobayashi, Y.; Hayashi, N.; Kishi, Y. *Org. Lett.* **2002**, *4*, 411. (b) Kobayashi, Y.; Hayashi, N.; Kishi, Y. *Tetrahedron. Lett.* **2003**, *44*, 7489.
(7) (a) Hinckley, C. C. *J. Am. Chem. Soc.* **1969**, *91*, 5160. (b) Sanders, J. K. M.; Williams, D. H. *J. Chem. Soc., Chem. Commun.* **1970**, 422.
(8) Ghosh, I.; Zeng, H.; Kishi, Y. *Org. Lett.* **2004**, *6*, 4715. It was found that glisoprenin A was sparingly soluble in pure C_6D_6 but soluble in a 1:4 (v/v) mixture of CD_2Cl_2/C_6D_6.

α-carbons. This correlation should allow the grouping of the C18−C32 portion of glisoprenin A into four compartments **A**−**D**. The second task is to establish the connectivity of the four compartments, which begins with correlating, again through detection of long-range $^{1}H-^{13}C$ coupling, the unique C-34 vinyl proton to one of the eight α-carbons. This would permit assignment of the chemical shift of the C-32 carbon and, consequently, the C-30 carbon, as both carbons C-32 and C-30 belong to the same stereocompartment. The third step is to correlate the C-30 carbon to one of the remaining six α-carbons through a connectivity-study of carbon (C-30)→protons (C-30)→carbon (C-28). This correlation should establish the C-28 chemical shift and, consequently, the chemical shift of C-26. Repeating this procedure twice, i.e., C-26→C-24/C-22 and C-22→C-20/C-18, should enable us to assign the chemical shifts of all eight α-carbons unambiguously. It should be noted that this correlation task can equally be initiated from the right-side of the glisoprenin A backbone.

A combination of various two-dimensional NMR techniques, including HMQC/HSQC (single-bond $^{1}H-^{13}C$ correlation), COSY ($^{1}H-^{1}H$ correlation), and HMBC (multiple-bond $^{1}H-^{13}C$ correlation) experiments, are routinely employed to accomplish a task like the one outlined above.[9] In the present case, we expected that a combined use of HSQC and HMBC experiments should be sufficient to complete the required chemical shift assignments (Figure 3). However, of potential concern was the effect of the paramagnetic shift reagent on the outcome of the two-dimensional NMR experiments.

The initial two-dimensional NMR experiments were performed on a 0.05 M sample of **1** containing 20 mol % per OH of (*S*)-Pr(tfc)$_3$ (*S*-**3**).[10] The HSQC experiment provided most of the key single-bond $^{1}H-^{13}C$ correlations, but no cross-peaks associated with the C-22 and C-24 carbons were observed.[11] On the other hand, the HMBC spectrum detected *none* of the crucial multiple-bond correlations. Repeating the HMBC experiment with different parameters ($^{n}J_{CH}$ = 8−20 Hz) resulted in no marked improvement.[12,14] Diluting the sample concentration from 0.05 to 0.02 M seemed to have no beneficial effect on the outcome of the HMBC experiment. However, the corresponding HSQC spectrum showed an improved S/N ratio; indeed, at this concentration, single-bond $^{1}H-^{13}C$ correlations were observed for carbons C-22 and C-24, which were previously undetectable at higher concentration. Despite this, only the chemical shift of the C-32 carbon could be determined in the presence of (*S*)-Pr(tfc)$_3$, whereas the other α-carbons still remained unassigned.

Faced with the technical difficulties encountered with the Pr-based shift reagent, we also tested Sm- and Eu-based shift reagents but with limited success.[13] Through these studies, however, it became evident that the outcome of the HMBC experiment depends significantly on the nature of the lanthanide metal.[14] Thus, it may prove beneficial to investigate a two-dimensional NMR experiment that could provide the same correlations as the HMBC experiment but is based on the HSQC pulse sequence rather than the HMQC sequence.[15,16]

On searching the literature, we noticed a few examples where the HSQC sequence (referred to as HSQMBC) was successfully tuned to detect long-range heteronuclear ($^{1}H-^{13}C$ and $^{1}H-^{15}N$) couplings.[17,18] In our case, with a 0.02 M sample of glisoprenin A (**1**) containing 20 mol % per OH of (*S*)-Pr(tfc)$_3$, the HSQMBC experiment indeed gave cross-peaks correlating the α-carbons to the protons of the corresponding tertiary methyl group (Figure 4).[19,20] Through

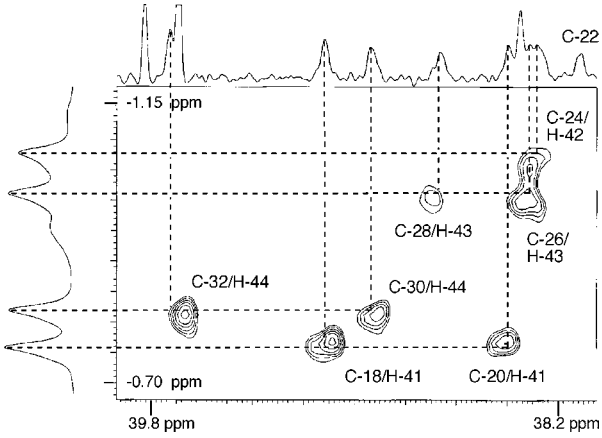

Figure 4. HSQMBC spectrum of a 0.02 M sample of glisoprenin A (**1**) in the presence of 20 mol % per OH of (*S*)-Pr(tfc)$_3$ (**3**). This spectrum correlated the ^{1}H resonance (singlet, *y*-axis) of each quaternary methyl to the ^{13}C resonances (*x*-axis) of the two relevant α-carbons, except that only one cross-peak, i.e., the C-42 methyl to the C-24 α-carbon, was detected for the C-22/C-24 compartment.

this experiment, we were finally able to establish the four compartments. It should be noted that, in principle, the

(9) For a thorough explanation of the described two-dimensional NMR experiments, including pulse sequences, see: Claridge, T. D. W. *High-Resolution NMR techiques in Organic Chemistry*; Elsevier: Oxford, 1999; Tetrahedron Organic Chemistry Series, Vol. 19.

(10) We felt that reducing the amount of shift reagent to 20 mol % per OH may improve the detectability of cross-peaks in the two-dimensional NMR experiments.

(11) The acquisition parameters for the HSQC experiment were based on: Gelis, I.; Katsaros, N.; Luchinat, C.; Piccioli, M.; Poggi, L. *Eur. J. Biochem*. **2003**, *270*, 600. Also see: Sattler, M.; Fesik, S. W. *J. Am. Chem. Soc*. **1997**, *119*, 7885. For detailed acquisition parameters, see Supporting Information.

(12) There are cases where HMBC experiments are successful in the presence of a paramagnetic metal. For an example, see: Skidmore, K.; Simonis, U. *Inorg. Chem*. **1996**, *35*, 7470.

(13) *R*)-Sm(hfc)$_3$ discriminated the enantiotopic carbons of *meso* secondary and tertiary alcohols. However, a higher concentration of (*R*)-Sm(hfc)$_3$ (ca. 25 mol %/OH) was required to observe discrimination in the case of the *meso* tertiary alcohol. The HMBC spectrum of a model 1,5-tetraol system in the presence of (*R*)-Sm(hfc)$_3$ (25 mol %/OH) showed several of the desired cross-peaks. It was found that Sm(hfc)$_3$ was less effective than Pr(tfc)$_3$ in spreading the signals. As a result, both the carbon and proton spectra in the presence of Sm(hfc)$_3$ were significantly more congested. In contrast, with (*R*)-Eu(tfc)$_3$ the HMBC spectrum was similar to (*S*)-Pr(tfc)$_3$.

(14) To detect the small long-range $^{13}C-H$ couplings ($^{n}J_{CH}$ = 8−10 Hz), the HMQC-sequence-based HMBC experiment uses rather long interpulse delays (50−60 ms), which result in its poor detectability of rapidly relaxing protons. Presumably, as a result of this, we are unable to observe any of the desired long-range $^{1}H-^{13}C$ correlations in the presence of (*S*)-Pr(tfc)$_3$ and (*R*)-Eutfc$_3$.

HSQMBC experiment should have also provided the connectivity of the C-34 vinyl proton to one of the eight α-carbons, but no relevant cross-peaks could be detected in order to make such a correlation.

Encouraged by the success of the HSQMBC experiment, we recognized that the required connectivity of the four compartments could be established from the series of two-dimensional NMR experiments depicted in Figure 5. Thus,

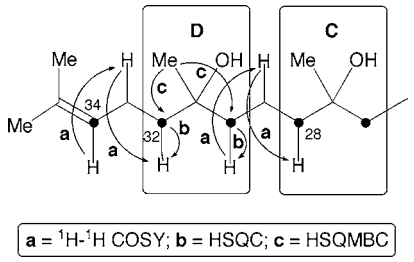

Figure 5. Modified strategy for assigning the chemical shifts of the α-carbons of glisoprenin A (**1**) in the presence of Pr(tfc)$_3$ (**3**).

a combination of ^{1}H−^{1}H COSY and HSQC experiments was performed to correlate the C-34 vinyl proton to the C-32 carbon and, consequently, to the C-30 carbon. Using ^{1}H−^{1}H COSY and HSQC experiments again, we were able to establish the connectivity of the C-28 carbon and, consequently, the C-26 carbon. Repeating this operation allowed us to establish the chemical shifts for all eight α-carbons. With the same two-dimensional NMR experiments, we then assigned the chemical shifts for all eight α-carbons in the presence of 20 mol % per OH of (*R*)-Pr(tfc)$_3$.

With the chemical shift assignments in hand, we were able to determine the $\Delta\Delta\delta$ behavior for each of the four tertiary hydroxyls *independently*. The four positive $\Delta\Delta\delta$ values thus obtained led us to conclude that the four tertiary hydroxyls possess the *S* configuration, thereby predicting the complete stereostructure of glisoprenin as **1** (Figure 6). In the following

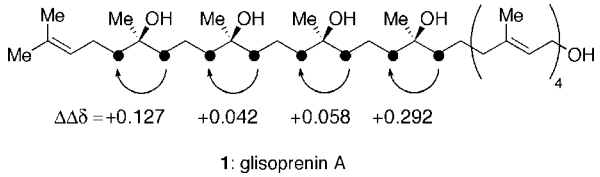

Figure 6. Complete stereostructure of glisoprenin A (**1**) predicted from the four $\Delta\Delta\delta$ values obtained in the presence of (*R*)- and (*S*)-Pr(tfc)$_3$ (**3**).

Letter,[21] we confirm this prediction and the validity of the chiral lanthanide shift approach via the total synthesis of the newly assigned stereostructure.

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Supporting Information Available: Sample preparation, detailed acquisition parameters for the one- and two-dimensional NMR experiments performed, and selected one- and two-dimensional NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) On the other hand, inverse detection two-dimensional NMR experiments such as HSQC and HMQC use relatively short interpulse delays that are set by relatively large value of $^1J_{CH}$ (140−160 Hz). These short interpulse delays assist in the detection of fast-relaxing, paramagnetically affected signals. Hence, the condition $T_2^{-1} < {}^1J_{CH}$, which is typically satisfied for resonances arising in the presence of paramagnetic metals, has resulted in the successful application of the HSQC and the HMQC experiments to such systems. For an example, see ref 11.

(16) An inspection of the HSQC and HMQC pulse sequences suggest that for a particular value of $^1J_{CH}$, the interpulse delays in an HSQC experiment are substantially shorter than those in the corresponding HMQC experiment. The shorter interpulse delays of the HSQC experiment may facilitate the detection of the fast-relaxing, paramagnetically affected signals and therefore show some of the key long-range ^{1}H−^{13}C correlations that are essential to mapping the backbone of glisoprenin A.

(17) This was achieved by setting the interpulse delays between 31 and 25 ms to allow sufficient time for the small long-range ^{1}H−^{13}C couplings to evolve.

(18) Also referred to as GSQMBC. (a) Marek, R.; Králík, L.; Sklenár, V. *Tetrahedron Lett.* **1997**, *38*, 665 and references therein. (b) Williamson, R. T.; Márquez, B. L.; Gerwick, W. H.; Köver, K. E. *Magn. Reson. Chem.* **2000**, *38*, 265.

(19) With Eu(tfc)$_3$ (C = 0.02 M, 20 mol % per OH of Eu(tfc)$_3$), we were able to obtain HSQMBC spectra comparable to or better than those obtained with Pr(tfc)$_3$. We have also formulated an empirical rule to predict the absolute configuration of an unknown compound in the presence of Eu(tfc)$_3$. However, some exceptions for the Eu-based empirical rule have been found. See ref 22 of the preceding Letter.

(20) For detailed acquistion parameters, see Supporting Information.

(21) Adams, C. M.; Ghosh, I.; Kishi, Y. *Org. Lett.* **2004**, *6*, 4723.