Cafestol to Tricalysiolide B and Oxidized Analogues: Biosynthetic and Derivatization Studies Using Non-heme Iron Catalyst Fe(PDP)

Marinus A. Bigi,[a] Peng Liu,[b] Lufeng Zou,[b] K. N. Houk,*[b] M. Christina White*[a]

[a] Roger Adams Laboratory, Department of Chemistry, University of Illinois, Urbana, IL 61801, USA
Fax +1(217)2445943; E-mail: white@scs.uiuc.edu

[b] Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095, USA
Fax +(310)2061843; E-mail: houk@chem.ucla.edu

Received: 19.10.2012; Accepted after revision: 09.11.2012

Abstract: The tricalysiolides are a recently isolated class of diterpene natural products featuring the carbon backbone of the well-known coffee extract, cafestol. Herein we validate the use of our non-heme iron complex, Fe(PDP), as an oxidative tailoring enzyme mimic to test the proposal that this class of natural products derives from cafestol via cytochrome P-450-mediated furan oxidation. Thereafter, as predicted by computational analysis, C–H oxidation derivatization studies provided a novel 2° alcohol product as a single diastereomer.

Key words: C–H oxidation, functionalization, aliphatic, non-heme iron, natural product diversification, biosynthesis, cafestol

Oxygen-activating heme[1] and non-heme iron[2] tailoring enzymes are routinely used in nature to oxidize small molecules for the purposes of biosynthesis. Using biomimetic small-molecule catalysts[3] for similar purposes would significantly streamline the synthesis and derivatization of natural products, particularly in cases where their hydrocarbon cores are readily available from isolation or fermentation. In addition to providing a synthetic route for generating large quantities of rare natural products and their derivatives for biological testing, these studies may also shed light on the biosynthetic pathways by which these molecules arise in nature.

We recently described a non-heme small-molecule catalyst, Fe(PDP) (**1**), that is able to utilize H_2O_2 to oxidize 2° and 3° C–H bonds in preparatively useful yields (ca. 50%, 1 equiv substrate) and with predictable selectivities based on electronic, steric, and steroelectronic factors within the molecule.[4,5] In analogy to nature's oxidation enzymes (e.g., cytochrome P-450s, α-ketoglutarate-dependent oxygenases) Fe(PDP) (**1**) performs both C–H hydroxylations and olefin epoxidations[6] through the intermediacy of a high-valent iron oxo.[7] Following the 'heme paradigm', **1** hydroxylates alkanes through a two-step process of radical hydrogen abstraction and rapid iron-hydroxyl rebound.[8] Because of these mechanistic similarities, and the high plasticity found within some P-450 active sites,[9] we hypothesized that **1** could facilitate a range of useful biomimetic oxidation reactions. In support of this, we recently reported that **1** promotes biomimetic mixed desaturase/oxygenase activity on aliphatic substrates containing carboxylic acids.[8] We also showed that Fe(PDP) oxidation at the C1 position of the taxane skeleton generated a short-lived radical intermediate that rearranged to the nortaxane skeleton, stimulating a hypothesis that these compounds (first found alongside taxol in the pacific yew tree) arise during the P-450-mediated oxidase phase of taxol biosynthesis.[8] Herein we report that cafestol, a compound readily isolated in multigram quantities from coffee grounds,[10] can be oxidized using Fe(PDP) to furnish tricalysiolide B, a diterpene recently isolated from the tree *tricalysia dubia* in milligram quantities (Figure 1).[11] Given that cafestol was also isolated from *T. dubia*,[12] this finding supports a biosynthetic hypothesis that the characteristic furan ring of cafestol has been oxidized within the organism to provide the tricalysiolides.

Based on the involvement of a highly electrophilic iron oxo intermediate, hydroxylation with Fe(PDP) is selective

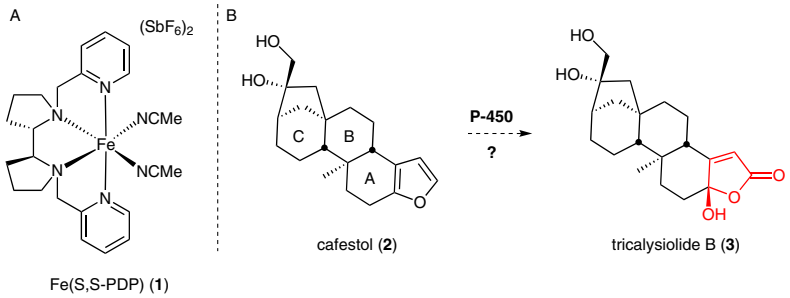


Figure 1 (A) Structure of Fe(*S*,*S*-PDP) (**1**). (B) Well-known diterpene found in coffee, cafestol, and the recently isolated tricalysiolide B

SYNLETT 2012, 23, 2768–2772
Advanced online publication: 14.11.2012
DOI: 10.1055/s-0032-1317708; Art ID: ST-2012-Y0906-C

for electron-rich C–H bonds (Figure 2). Additionally, the bulky nature of the catalyst renders it sensitive to the steric environment of different C–H bonds, and accordingly, it is observed that sterically accessible C–H bonds can be selectively oxidized. For example, we have found in cyclic systems that less sterically hindered equatorial C–H bonds are oxidized preferentially to axial C–H bonds. Stereoelectronic effects (e.g., hyperconjugative activation, relief of torsional strain, and 1,3-diaxial interactions) have been shown to play an important role in the selectivity profile, especially in cases where electronic and steric effects do not exert strong influence.[4,5] Finally, carboxylic acid directing groups can often override all of these substrate-biasing elements, enabling orthogonal site selectivities to those observed for intermolecular reactions.[13] Significantly, the 'selectivity rules' delineated above with Fe(PDP) for aliphatic C–H oxidations are proving themselves to be general,[14] particularly among other electrophilic, bulky catalysts that perform mechanistically related C–H oxidations, even with other bond types.[15]

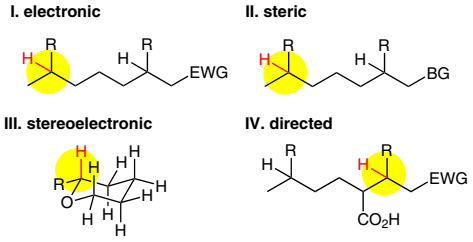
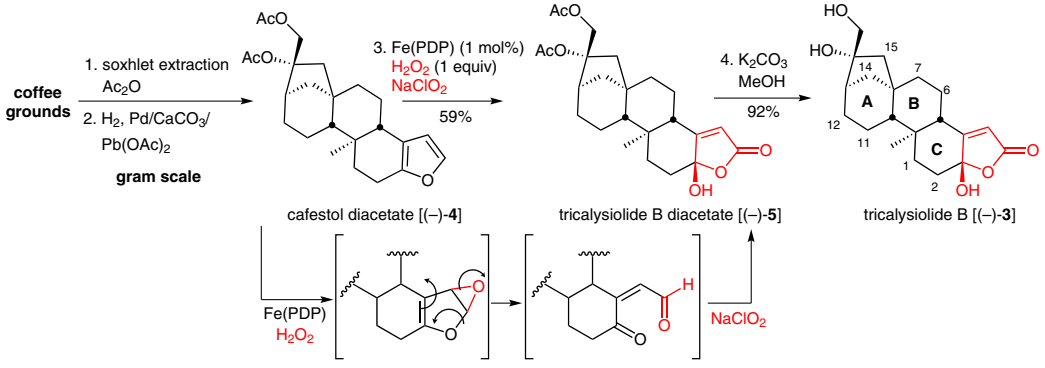


Figure 2 Fe(PDP)-derived C–H oxidation 'rules' governing site selectivity (R = H or Me)

Despite numerous sites of aliphatic C–H oxidation on tricalysiolide B being viable for the Fe(PDP) oxidant, straightforward computational analysis evaluating electronic, steric, and stereoelectronic parameters (lowest-energy conformer combined with evaluation of the NPA charges) enabled an accurate prediction of the major site of oxidation. Significantly, oxidation of tricalysiolide B triacetate furnished 6β-hydroxytricalysiolide B triacetate as a single diastereomer in useful yields. This represents a rare example of a highly diastereoselective methylene C–H hydroxylation reaction.

Cafestol (**2**), a bioactive pentacyclic diterpene belonging to the *ent*-kaurene family of natural products, is one of the major constituents of coffee (ca. 1% of whole coffee bean) (Figure 1).[16] Besides its intriguing structure, cafestol has received interest because it exhibits anticarcinogenic activity in rats[17] and has been implicated in inhibition of the progression of Parkinson's disease.[18] Recently, the tricalysiolides, a new class of bioactive natural products containing the *ent*-kaurene framework, were isolated from the wood of *Tricalysia dubia* in milligram quantities.[11] The finding that cafestol could also be isolated from *T. dubia* prompted us to hypothesize that a cytochrome P-450-mediated oxidation of its furan ring accounted for the biosynthesis of the tricalysiolides.[19] We sought to apply Fe(PDP)-mediated oxidation of cafestol as a means of rapidly accessing this class of natural products in large quantities to aid biological and derivatization studies and to provide evidence that the proposed P-450-mediated oxidation was a feasible biosynthetic transformation.

Following a patented process,[20] we isolated 2.7 grams of a mixture of cafestol and an unsaturated isomer, kahweol, from 1 kg of coffee grounds via soxhlet extraction (Scheme 1). To protect the unstable diol motif of these diterpenes, acetylation with acetic anhydride furnished diacetylated analogues that were easily purified by flash chromatography. After isolation of the inseparable mixture of cafestol diacetate and kahweol diacetate, hydrogenation with a poisoned palladium(0) catalyst [$Pd/CaCO_3/Pb(OAc)_4$] and one atmosphere of hydrogen provided pure cafestol diacetate (**4**) as a white powder. When cafestol diacetate (**4**) was exposed to mild Fe(PDP) (**1**) oxidation conditions [catalyst (1 mol%), H_2O_2 (1.0 equiv), r.t., open to the air,[21] wet solvent), epoxidation of the electron-rich furan ring followed by rearrangement furnished the α,β-unsaturated aldehyde product. The aldehyde was filtered through silica and exposed to standard Pinnick oxidation conditions,[22] chemoselective for aldehyde oxidation, to furnish the lactone **5**. Gratifyingly, this procedure provided **5** as a single diastereomer in 59% yield from cafestol diacetate (**4**), demonstrating that a



Scheme 1 Extraction of cafestol from coffee grounds and conversion into tricalysiolide B using Fe(PDP)-catalyzed furan oxidation

P-450-mediated oxidation could form the basis of the biosynthesis of the tricalysiolides. Interestingly, these results are also consistent with findings from metabolic studies on cafestol which suggest that epoxidation of the furan ring followed by formation of glutathione conjugates leads to its bioactivation.[23] Attempts to further oxidize the α,β-unsaturated aldehyde to the requisite carboxylic acid intermediate with Fe(PDP) suffered problems with competing olefin oxidation. In P-450 oxidation systems this chemoselectivity issue may be circumvented by the selective positioning of the two reactive moieties within the constraints of the enzymatic active site. Finally, hydrolysis of diacetate **5** with potassium carbonate in methanol provided tricalysiolide B (**3**) in two steps and 54% overall yield from cafestol diacetate (**4**). Notably, our route from coffee grounds can efficiently provide gram quantities of tricalysiolide B for bioassays and derivatization studies.

A key finding of our studies of Fe(PDP)-catalyzed aliphatic C–H oxidation reactions was that they were both preparative and predictably selective.[4,5] Before we began our C–H oxidation derivatization studies, we qualitatively analyzed tricalysiolide B triacetate (**6**) using our previously delineated selectivity rules based on electronics, sterics, and stereoelectronics (Figure 2). Initial analysis of triacetoxy tricalysiolide B suggested that the methylene sites within rings A, B, and C would be too similar electronically to afford useful oxidation selectivity: two methylene sites are γ and four are β to electron-withdrawing groups (Figure 3, A). We therefore turned to a 'selectivity-rules-guided' computational analysis to more accurately predict potential sites of oxidation of triacetate **6**. A conformational search of **6** was performed with MacroModel,[24] followed by DFT geometry optimizations of the lowest energy conformers at the B3LYP/6-31G(d) level using Gaussian 09[25] to locate the global energy minimum and single-point NPA charge calculations at the B3LYP/6-311++G(d,p) level. The geometry of the most stable conformer and the partial atomic charges allowed us to extract quantitative information about the relative electronic, steric, and stereoelectronic parameters of the equatorial C–H bonds. As expected, those methylene C–H bonds α to electron-withdrawing furanone and acetoxy ester subunits are the most electron-poor C–H bonds (calculated NPA charges[26] for equatorial C–H bonds: H2, 0.230; H15, 0.240), and therefore the least susceptible to oxidation, while those sites most susceptible to oxidation (H1, H6, H7, H11, H12, and H14) lie β or γ from these sites (Figure 3, B). Interestingly, in accord with our first-level analysis, the NPA charges of the majority of these sites suggest that their electronic character is too similar in nature to lead to useful selectivities. Analysis of the lowest-energy conformer of **6**, however, suggests that significant steric and stereoelectronic factors may be used to distinguish these electronically similar sites. For example, although H7 is electronically activated relative to the other sites, it is adjacent to a quaternary carbon, rendering it (and H14) sterically deactivated. Of the sites that are electronically closely matched, analysis of interatomic distances demonstrates that significant repulsion exists between equatorial hydrogens H11 and H1 and that the equatorial hydrogen H12 was partially blocked by the acetoxy group at C17, rendering those sites poorly accessible to the bulky Fe(PDP) catalyst (Figure 3, B).

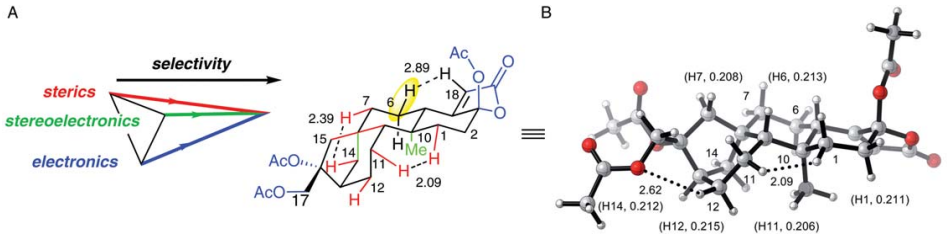
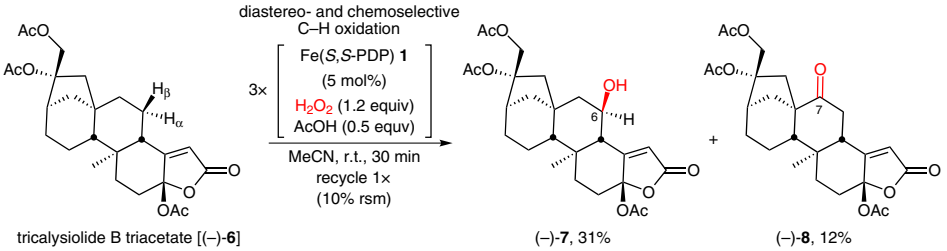


Figure 3 (A) 3D depiction of **6**, showing key electronic effects (blue), steric interactions (red, between H1 and H11; values of dotted lines represent distances in Å between indicated hydrogens for the lowest energy conformer), and stereoelectronics (green). (B) Calculated lowest-energy conformer of **6** and calculated NPA charges of equatorial H atoms using B3LYP/6-311++G(d,p)



Scheme 2 Fe(PDP)-catalyzed oxidation of tricalysiolide B derivative **6**, affording 2° alcohol **7** and ketone **8**, after recycling unreacted starting material once

Therefore, of the electronically accessible sites, H6 is predicted to be the most sterically accessible. Another factor calculated to be important for promoting C–H oxidation is alleviation of 1,3-diaxial strain.[5] Significantly, oxidation of the sterically exposed equatorial C–H bond of C6, leading to a slightly sp^2-hybridized carbon in the transition state for oxidation, should alleviate 1,3-diaxial strain between the axial C–H bond at C6 and the axial Me group at C10. Therefore, based on the experimental guidelines depicted in Figure 2 and the computational analysis of several steric and electronic parameters of **6**, we predicted that equatorial H6-derived 2° alcohol **7** would be likely to emerge as the major product due to a combination of its relative electron-richness, steric accessibility, and potential for reduction of 1,3-diaxial strain.[27]

As anticipated based on this computational analysis, C–H oxidation of tricalysiolide B diacetate **6** using Fe(*S*,*S*-PDP) led to the equatorial H6-derived 2° alcohol **7** (21%) as the major product along with the H7-derived ketone **8** in 9% yield (2.3:1 ratio) with 36% recovered starting material (Scheme 2). The poor mass balance is most likely due to unselective oxidation at numerous other electronically activated sites (Figure 3). Recycling the starting material once furnished 6β-hydroxy-tricalysiolide B triacetate (**7**) in a useful yield of 31%. Notably, this reaction constitutes a rare example of a highly diastereo- and chemoselective aliphatic C–H oxidation: the major product **7** was isolated as a single diastereomer and the electron-poor olefin found within **6** survived the highly oxidizing reaction conditions. Analysis of the energy-minimized structure of tricalysiolide B diacetate **6** reveals that the axial methyl group at C10 blocks the α-face of **6**, likely preventing the sterically encumbered catalyst from accessing the α-H at C6. This accounts for both the highly diastereoselective nature of the oxidation and the lack of significant overoxidation of **7** to the ketone oxidation state. With a new functional handle installed onto the B ring of tricalysiolide derivative **7**, one can envision rapid diversification to define structure–activity relationships and develop compounds of enhanced bioactivity. It is significant to note that using traditional methods, which rely on polar functionality for reactivity and selectivity, the incorporation of a hydroxyl moiety in a portion of a molecule comprised of isolated, unactived methylene sites would require de novo synthesis.

In summary, we report the use of Fe(PDP) as a small-molecule catalyst that enables the oxidative transformation of the readily available cafestol core to that of the tricalysiolides. In addition to enabling access to large quantities of a rare natural product from a readily available precursor, Fe(PDP) oxidation provides evidence to support the biosynthetic hypothesis that the tricalysiolides arise from P-450 enzymatic oxidation of the furan ring of cafestol. Despite the fact that the tricalysiolides have a relatively electronically unbiased core, simple 'selectivity-rules-driven' computational analysis accounting for steric, stereoelectronic (angles of van der Waals radii), as well as electronic parameters (NPA and Mulliken charges), enables an accurate prediction of the site of Fe(PDP) oxidation to furnish a novel derivative of tricalysiolide B. The Fe(PDP)-catalyzed C–H oxidation of tricalysiolide B triacetate (**6**) to furnish 6β-hydroxytricalysiolide B triacetate (**7**) illustrates a rare example of a chemo-, site-, and diastereoselective methylene oxidation with a small-molecule catalyst. Notably, hydroxylated derivatives may lead to more rapid identification of oxidized metabolites of cafestol as well as compounds with novel or enhanced biological activities.

Acknowledgment

M.C.W. and K.N.H. gratefully acknowledge the National Science Foundation under the CCI Center for Selective C–H Functionalization (Grant CHE-1205646) for financial support. K.N.H also acknowledges the NIH for financial support (NIGMS-NIH grant GM36700). Mr. P. Gormisky is thanked for providing the crystallographic data confirming the structural assignment of **7**.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

References and Notes

(1) Denisov, I. G.; Makris, T. M.; Sligar, S. G.; Schlichting, I. *Chem. Rev.* **2005**, *105*, 2253.
(2) (a) Bruijnincx, P. C. A.; van Koten, G.; Gebbink, R. J. M. K. *Chem. Soc. Rev.* **2008**, *37*, 2716. (b) Merkx, M.; Kopp, D. A.; Sazinsky, M. H.; Blazyk, J. L.; Müller, J.; Lippard, S. J. *Angew. Chem. Int. Ed.* **2001**, *40*, 2782. (c) Neumann, C. S.; Fujimori, D. G.; Walsh, C. T. *Chemistry & Biology* **2008**, *15*, 99. (d) Costas, M.; Mehn, M. P.; Jensen, M. P.; Que, L. Jr. Chem. Rev. **2004**, *104*, 939.
(3) (a) Okuno, T.; Ito, S.; Ohba, S.; Nishida, Y. J. *Chem. Soc., dalton Trans.* **1997**, 3547. (b) Kim, C.; Chen, K.; Kim, J.; Que, L. Jr *J. Am. Chem. Soc.* **1997**, *119*, 5964. (c) Wu, X.; Seo, M. S.; Davis, K. M.; Lee, Y.-M.; Chen, J.; Cho, K.-B.; Pushkar, Y. N.; Nam, W. *J. Am. Chem. Soc.* **2011**, *133*, 20088. (d) Bigi, J. P.; Harman, W. H.; Lassalle-Kaiser, B.; Robles, D. M.; Stich, T. A.; Yano, J.; Britt, R. D.; Chang, C. J. *J. Am. Chem. Soc.* **2012**, *134*, 1536. (e) Groves, J. T.; Neumann, R. *J. Org. Chem.* **1988**, *53*, 3891. (f) Breslow, R.; Huang, Y.; Zhang, X.; Yang, J. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 11156. (g) Das, S.; Incarvito, C. D.; Crabtree, R. H.; Brudvig, G. W. *Science* **2006**, *312*, 1941.
(4) Chen, M. S.; White, M. C. *Science* **2007**, *318*, 783.
(5) Chen, M. S.; White, M. C. *Science* **2010**, *327*, 566.
(6) White, M. C.; Doyle, A. G.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2001**, *123*, 7194.
(7) Chen, K.; Que, L. Jr. *J. Am. Chem. Soc.* **2001**, *123*, 6327.
(8) Bigi, M. A.; Reed, S. A.; White, M. C. *Nat. Chem.* **2011**, *3*, 218.
(9) Pochapsky, T. C.; Kazanis, S.; Dang, M. *Antioxid. Redox Signaling* **2010**, *13*, 1273.
(10) Gross, G.; Jaccaud, E.; Huggett, A. C. *Food Chem. Toxicol.* **1997**, *35*, 547.
(11) Nishimura, K.; Hitotsuyanagi, Y.; Sugeta, N.; Sakakura, K.; Fujita, K.; Fukaya, H.; Aoyagi, Y.; Hasuda, T.; Kinoshita, T.; He, D.-H.; Otsuka, H.; Takeda, Y.; Takeya, K. *Tetrahedron* **2006**, *62*, 1512.
(12) Nishimura, K.; Hitotsuyanagi, Y.; Sakakura, K.; Fujita, K.; Tachihara, S.; Fukaya, H.; Aoyagi, Y.; Hasuda, T.; Kinoshita, T.; Takeya, K. *Tetrahedron* **2007**, *63*, 4558.

(13) Bigi, M. A.; Reed, S. A.; White, M. C. *J. Am. Chem. Soc.* **2012**, *134*, 9721.
(14) White, M. C. *Science* **2012**, *335*, 807.
(15) For examples in aliphatic C–H chlorinations and fluorinations with Mn porphyrins, see: (a) Liu, W.; Groves, J. T. *J. Am. Chem. Soc.* **2010**, *132*, 12847. (b) Liu, W.; Huang, X.; Cheng, M.-J.; Nielsen, R. J.; Goddard, W. A. III.; Groves, J. T. *Science* **2012**, *337*, 1322. For an example with allylic C–H aminations using an Fe-phthalocyanine catalyst, see: (c) Paradine, S. M.; White, M. C. *J. Am. Chem. Soc.* **2012**, *134*, 2036.
(16) Kurzrock, T.; Speer, K. *Food Rev. Int.* **2001**, *17*, 433.
(17) Cavin, C.; Holzhaeuser, D.; Scharf, G.; Constable, A.; Huber, W. W.; Schilter, B. *Food Chem. Toxicol.* **2002**, *40*, 1155.
(18) Trinh, K.; Andrews, L.; Krause, J.; Hanak, T.; Lee, D.; Gelb, M.; Pallanck, L. *J. Neurosci.* **2010**, *30*, 5525.
(19) Guengerich, F. P. *Arch. Biochem. Biophys.* **2003**, *409*, 59.
(20) Bertholet, R.; US 4692534, **1987**.
(21) No differences in reactivity or selectivity have been observed running Fe(PDP) oxidations under N_2 or Ar atmosphere vs. running the reaction open to air. This along with the stereoretentive nature of oxidation suggests the reaction does not proceed via hydroxyl radicals or a long-lived carbon radical intermediate.
(22) Bal, B. S.; Childers, W. E. Jr.; Pinnick, H. W. *Tetrahedron* **1981**, *37*, 2091.
(23) van Cruchten, S. T. J.; de Haan, L. H. J.; Mulder, P. P. J.; Kunne, C.; Boekschoten, M. V.; Katan, M. B.; Aarts, J. M. M. J. G.; Witkamp, R. F. *J. Nutr. Biochem.* **2010**, *21*, 757.
(24) MacroModel, version 9.9; Schrödinger LLC: New York, **2011**.
(25) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A. Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09, Revision B.01* **2009**.
(26) NPA charges were calculated using B3LYP/6-311++G(d,p). Mulliken charges were calculated using B3LYP/6-31G(d) (see Supporting Information) and provided an analogous trend to the NPA charges. Mulliken charges are an excellent alternative when minimizing computation power and time are a consideration.
(27) These steric and electronic property analyses were all based on calculations of the reactant molecule. Density functional theory (DFT) calculations of transition-state structures and selectivities are under way in our labs to verify these selectivity rules.

