Organic LETTERS

Letter

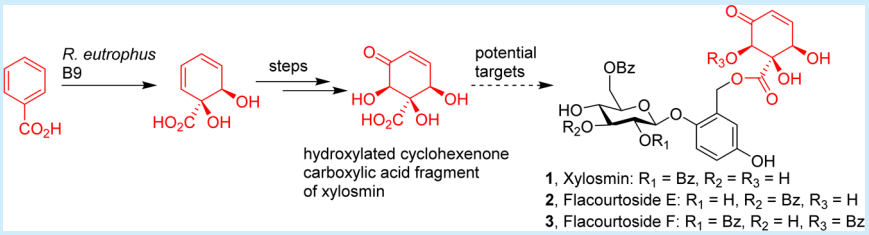
pubs.acs.org/OrgLett

A Formal Approach to Xylosmin and Flacourtosides E and F: Chemoenzymatic Total Synthesis of the Hydroxylated Cyclohexenone Carboxylic Acid Moiety of Xylosmin

Mukund Ghavre, Jordan Froese, Brennan Murphy, Razvan Simionescu, and Tomas Hudlicky*

Department of Chemistry and Centre for Biotechnology, Brock University, St. Catharines, ON L2S 3A1, Canada

Supporting Information



ABSTRACT: The hydroxylated cyclohexenone carboxylic acid moiety of xylosmin was synthesized in eight steps from benzoic acid. The key steps in the synthesis involved the enzymatic dihydroxylation of benzoic acid by the whole cell fermentation with *Ralstonia eutrophus* B9, and Henbest epoxidation. Early attempts led to the synthesis of a C6 epimer of the methyl ester of the hydroxylated cyclohexenone carboxylic acid moiety. The absolute stereochemistry of an advanced intermediate was confirmed by X-ray crystallography. Complete characterization of the previously reported but not fully characterized hydroxylated cyclohexenone carboxylic acid is provided.

Chikungunya and dengue viruses infect roughly 500 million people per year, predominantly in the tropical zone of the eastern hemisphere. Chikungunya and dengue fevers are generally characterized by symptoms such as headaches, fever, fatigue, vomiting, nausea, skin rashes, and blood-thinning and can be fatal.[1−3]

No effective vaccines are available, but broad-spectrum antivirals have had some effect in tackling the overarching virus, although the greatest levels of success have been observed in addressing the associated symptoms, usually through either retroactive or preventative measures. Phenolic glycosides, found in the *flacourtia ramontchi* plant of Madagascar, have shown replicative inhibition against these arboviral diseases. Inhibitory plant extracts containing glycosylated derivatives of 2-hydroxymethylhydroquinone as a common motif have been shown to be effective against these viral diseases (Figure 1).[4−7]

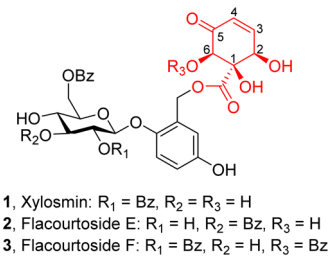


Figure 1. Naturally occurring phenolglycosides found in the *flacourtia* plant genus.[7]

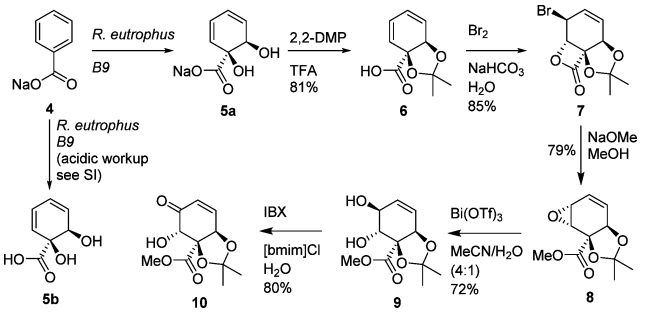
In 2013, the cyclohexenone carboxylate portion of xylosmin was reported as the product of base-catalyzed hydrolysis of a compound related to xylosmin **1** (having a free hydroxymethyl group on the monosaccharide residue, Figure 1).[5] No physical or spectral data, other than the optical rotation (reported as +13.2 (*c* 0.07, MeOH) and +12.6 (*c* 0.06, MeOH)) were provided for the free carboxylic acid. In this paper, we report the chemoenzymatic synthesis, starting from benzoic acid, of the hydroxylated cyclohexenone carboxylic acid fragment common to xylosmin (**1**),[6] flacourtoside E (**2**), and flacourtoside F (**3**) (Figure 1).[7]

Our initial approach to the target cyclohexenone fragment employed the enzymatic dihydroxylation of benzoic acid as shown in Scheme 1. The use of arene dioxygenases for desymmetrization of common aromatic compounds has gained prominent utility in the synthesis of natural products. Toluene and other arene dioxygenases that are overexpressed in *E. coli* vectors are frequently employed in chemoenzymatic syntheses, and this topic has been reviewed on several occasions.[8] Less common metabolites are the *ipso*-diols of type **5a** and **5b**[9] produced by dihydroxylation of benzoic acids with benzoate dioxygenase through the whole cell fermentation with *Ralstonia eutrophus* B9.[10] The *ipso*-diol **5a**,[10,11] derived from sodium benzoate **4**, was protected as its corresponding acetonide **6** and carefully treated with bromine to provide the *β*-lactone **7**, as

Received: January 19, 2017

DOI: 10.1021/acs.orglett.7b00194
Org. Lett. XXXX, XXX, XXX−XXX

Scheme 1. Synthesis of Enone 10 from Sodium Benzoate



shown in Scheme 1. A similar protocol was reported by Banwell in a formal approach to the C-ring of vinblastine.[12]

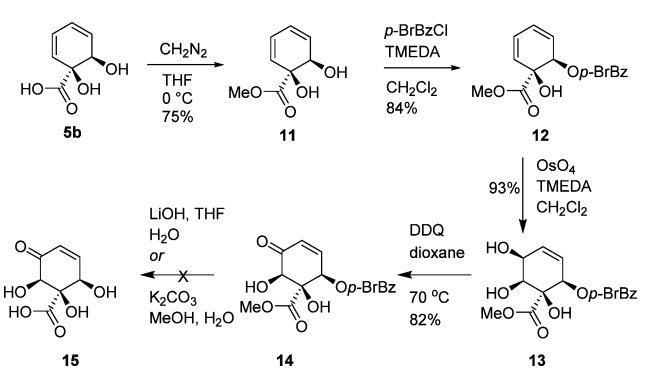
Treatment of **7** with sodium methoxide in methanol generated methyl ester **8** from lactone **7** and provided, through the intramolecular displacement of the bromide by the intermediate alkoxide, epoxide **8**. Cyclohexenyl epoxide **8** was considered as a putative precursor to the conduritol carboxylate unit found in compounds **1**−**3**.

A mild epoxide opening was achieved with catalytic $Bi(OTf)_3$ in acetonitrile and water,[13,14] leading to opening of the cyclohexenyl epoxide at the allylic position without the loss of either the ester or acetonide protecting groups. The selective oxidation of the allylic alcohol in diol **9** proved to be more difficult than initially expected. Surprisingly, oxidation with manganese dioxide always led to the 1,2-diketone product and not to the desired hydroxyenone **10**. Other oxidants (DDQ, IBX, PIFA) either proved ineffective or led to complex mixtures. Ultimately, when IBX was dissolved in the ionic liquid [bmim]Cl[15] and water, the selective oxidation of the allylic alcohol to the desired enone **10** was achieved. The expected epimerization of the C6 hydroxyl failed under both Mitsunobu conditions and base catalysis. The reported conditions for hydrolysis of the phenolglycosides ($Ba(OH)_2$, 80 °C)[5] failed as well and led only to extensive decomposition.

To approach the synthesis of the natural stereoisomer a new strategy was devised that utilized *ipso*-diol **5b**, which was converted to methyl ester **11**.[11,16] Protection of the distal alcohol (with reference to the carboxylate) in **11** as a *p*-bromobenzoate was followed by the hydroxyl-directed Donohoe osmylation[17] to furnish triol **13** in a stereo- and regioselective fashion, Scheme 2.

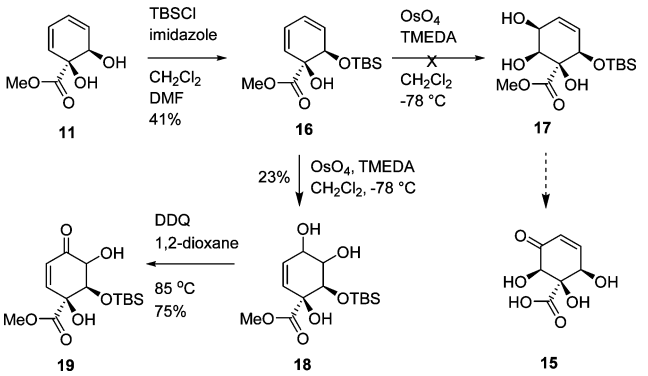
Oxidation of **13** with IBX in ionic liquid failed, in contrast to the successful oxidation of **9**; however, the allylic alcohol in **13** was cleanly oxidized to the desired enone **14** with DDQ.

Scheme 2. Synthesis of Diester 14 from Diol 5b



Unfortunately, the original plan for the double deprotection of **14** in the final step failed and the treatment of **14** under the reported conditions [$Ba(OH)_2$, or NaOMe/MeOH] resulted only in decomposition to an aromatic product. Because of this turn of events, the same sequence was executed again with a silyl protecting group instead of the benzoate at C2, as shown in Scheme 3.

Scheme 3. Failed Attempts toward the Synthesis of Enone 15



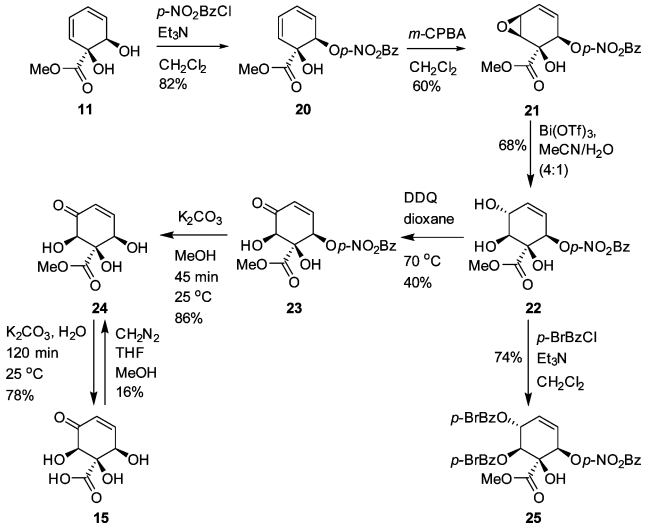
The secondary silyl ether was synthesized by a previously published sequence[18] and subjected to Donohoe dihydroxylation. Unfortunately, this dihydroxylation afforded the trihydroxy regioisomer **18**, as opposed to the regioisomer of type **17** as was previously observed. In order to confirm the assignment of structure **18**, the allylic alcohol was oxidized to afford enone **19**. The ^1H NMR spectrum of **19** did not show the expected ABX pattern of the olefinic signals and the proton at C2, but rather the presence of two clean doublets (δ 6.90 and 6.19, J = 9.9 Hz) corresponding to the olefinic hydrogens of the enone and therefore confirming the assignment of regiochemistry as that shown in **19**. The relative stereochemistry of the alcohol moieties of compounds **18** and **19** was not further investigated, but it may be assumed that these would be anti- to the TBS group, with the oxidation proceeding through a classic Upjohn mechanism, instead of the hydrogen bond-controlled Donohoe pathway.

In light of these observations, we decided to return to an approach based on the use of benzoyl protecting groups. To this end, the *p*-nitrobenzoyl ester **20** was produced from ester **11**, as shown in Scheme 4. Benzoyl ester **20** was then subjected to a Henbest epoxidation to afford epoxide **21**,[19] which was treated with $Bi(OTf)_3$ as previously described to effect the epoxide opening.

In order to confirm the absolute stereochemistry of triol **22**, this compound was subjected to benzoylation with *p*-bromobenzoyl chloride to afford the tetraester **25**. This compound was analyzed by X-ray crystallography. The structure (Figure 2) confirmed the absolute stereochemistry of **22** as shown in Scheme 4. Triol **22** was then subjected to selective oxidation with DDQ to furnish enone **23**. In contrast to previous observations involving the failure to hydrolyze *p*-bromobenzoate **14**, the *p*-nitrobenzoate ester in enone **23** was smoothly hydrolyzed with potassium carbonate (0.5 equiv) in methanol over 45 min to afford **24**, the methyl ester of the hydroxylated cyclohexenone carboxylic acid. Further hydrolysis (K_2CO_3, 0.5 equiv, H_2O, rt, 2 h) provided the desired hydroxylated cyclohexenone carboxylic acid **15** as a solid [mp = 135−137 °C (methanol/pentane, 1:3); $[\alpha]_D^{22}$ −49.4 (c = 0.5,

DOI: 10.1021/acs.orglett.7b00194
Org. Lett. XXXX, XXX, XXX−XXX

Scheme 4. Synthesis of 15, the Hydroxylated Cyclohexenone Carboxylic Acid Moiety of Xylosmin



directed epoxidation of *p*-nitrobenzoate ester **20** and subsequent manipulations that included mild conditions for the hydrolysis of the ester moieties. Carboxylic acid **15** is not a very stable compound (degradation occurs at room temperature with notable decomposition within 12 h or so; degradation proceeds at a slower rate even at −80 °C). In the original report,[5] no data were provided for the free carboxylic acid except for the optical rotation of +13.2 (*c* 0.07, MeOH) and +12.6 (*c* 0.06, MeOH). The hydrolysis of compound **26** (Figure 3), closely related to xylosmin and

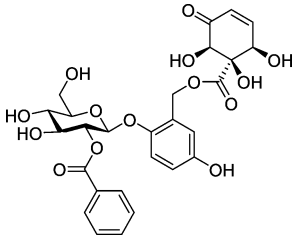


Figure 3. Free hydroxyl derivative of xylosmin **26**.[5]

methanol)]. This compound was not fully characterized in the original report,[5] and therefore this work represents the first synthesis of the hydroxylated cyclohexenone carboxylic acid moiety of xylosmin.

The previous attempts at the base-catalyzed isomerization of the C6 stereogenic center in enone **10** proved unsuccessful. Nevertheless, in order to ascertain that the stereochemical integrity of the C6 stereogenic center remained unchanged during the base hydrolysis of **24**, the freshly prepared free acid **15** was converted back to the methyl ester **24** with diazomethane. This ester was found to be identical (*vide* NMR and optical rotation) with the material used in the final hydrolysis.

Acid **15**, the hydroxylated cyclohexenone carboxylic acid fragment of xylosmin, was synthesized, and the absolute stereochemistry of ester **25** was determined to be as reported for the natural product (the structure of xylosmin was confirmed by X-ray crystallography[6]). Several approaches were investigated to prepare the hydroxylated cyclohexenone carboxylic acid **15**, and the successful one was based on the having a levorotatory optical rotation, under acidic conditions was reported[5] to give D-glucose as confirmed by the optical rotation value ($[\alpha]_D^{25}$ +50.5 (*c* 0.10, H_2O) of the sugar obtained.

The basic hydrolysis of **26**, as reported in the literature,[5] provided material, assumed to be the hydroxylated cyclohexenone carboxylic acid fragment, **15**, with a dextrorotatory optical rotation. Our experience with the hydrolysis of esters **14**, **23**, and **24** raises some questions whether the hydroxylated cyclohexenone carboxylic acid fragment was ever obtained under the harsh basic hydrolysis conditions reported. In addition, as the sign of the optical rotation of the carboxylic acid **15** obtained by synthesis is opposite to that reported for the material obtained by isolation, we may conclude that the material reported[5] from the basic hydrolysis of **26** was simply contaminated with the free sugar. The authors of the original report confirmed by correspondence that they obtained no other analytical or spectral data for the free carboxylic acid.

Based on the X-ray confirmation of structure **25** as shown, and given the fact that epimerization of the hydroxyl group at the α-position in ketones of type **14**, **23**, and **24** was not

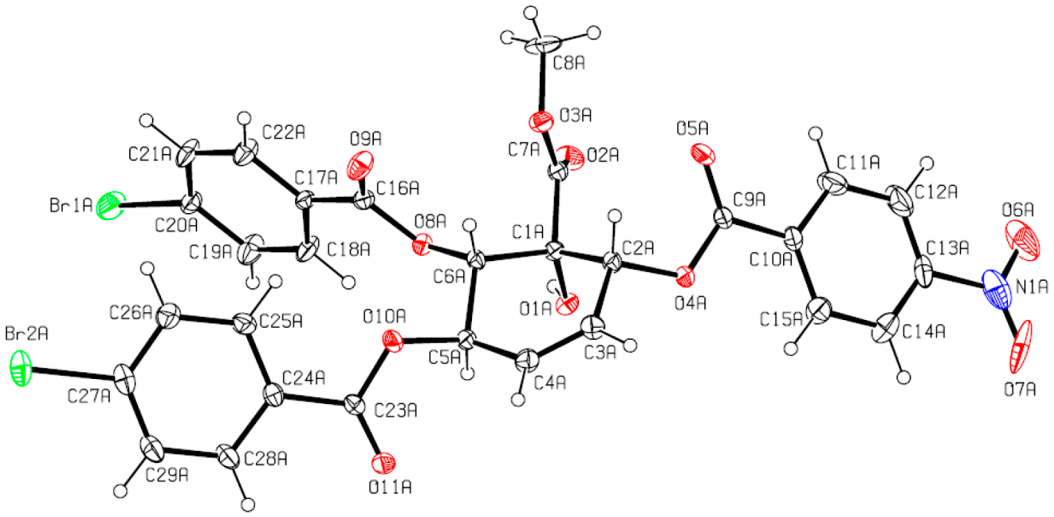


Figure 2. Crystal structure of ester **25**.

DOI: 10.1021/acs.orglett.7b00194
Org. Lett. XXXX, XXX, XXX−XXX

detected, we are reasonably certain that **15** was attained with the correct absolute stereochemistry, as that determined for the natural product.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b00194.

Experimental and spectral data are provided for the compounds **5b**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, **13**, **14**, **15**, **16**, **18**, **19**, **20**, **21**, **22**, **23**, and **24** (PDF)

X-ray data is provided for **25** (CIF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: thudlicky@brocku.ca.

ORCID

Tomas Hudlicky: 0000-0003-3239-6815

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors are grateful to the following agencies for financial support of this work: Natural Sciences and Engineering Research Council of Canada (NSERC) (Idea to Innovation and Discovery Grants), Canada Research Chair Program, Canada Foundation for Innovation (CFI), TDC Research, Inc., TDC Research Foundation, the Ontario Partnership for Innovation and Commercialization (OPIC), and The Advanced Biomanufacturing Centre (Brock University). The skillful assistance of Helen Endoma (Brock University) in the fermentations is gratefully appreciated. Hesham Shamshoum's (Brock University) help in the preparation of starting materials is appreciated. We also thank Professor Marko Mihovilovic (Vienna University of Technology) for providing a fresh culture of *Ralstonia eutrophus* B9.

■ REFERENCES

(1) Bhatt, S.; Gething, P. W.; Brady, O. J.; Messina, J. P.; Farlow, A. W.; Moyes, C. L.; Drake, J. M.; Brownstein, J. S.; Hoen, A. G.; Sankoh, O.; Myers, M. F.; George, D. B.; Jaenisch, T.; Wint, G. R.; Simmons, C. P.; Scott, T. W.; Farrar, J. J.; Hay, S. I. *Nature* **2013**, *496*, 504.

(2) Kaur, P.; Chu, J. *Drug Discovery Today* **2013**, *18*, 969.

(3) Parashar, D.; Cherian, S. *BioMed Res. Int.* **2014**, *2014*, 1.

(4) Kaou, A. M.; Mahiou-Leddet, V.; Canlet, C.; Debrauwer, L.; Hutter, S.; Laget, M.; Faure, R.; Azas, N.; Ollivier, E. J. *J. Ethnopharmacol.* **2010**, *130*, 272.

(5) Sashidhara, K. V.; Singh, S. P.; Singh, S. V.; Srivastava, R. K.; Srivastava, K.; Saxena, J. K.; Puri, S. K. *Eur. J. Med. Chem.* **2013**, *60*, 497.

(6) Gibbons, S. G.; Gray, A. I.; Waterman, P. G.; Hockless, D. C. R.; Skelton, B. W.; White, A. H. *J. Nat. Prod.* **1995**, *58*, 554.

(7) Bourjot, M.; Leyssen, P.; Eydoux, C.; Guillemot, J. C.; Canard, B.; Rasoanaivo, P.; Gueritte, F.; Litaudon, M. *J. Nat. Prod.* **2012**, *75*, 752.

(8) (a) Hudlicky, T.; Gonzalez, D.; Gibson, D. T. *Aldrichimica Acta* **1999**, *32*, 35. (b) Banwell, M. G.; Edwards, A. J.; Harfoot, G. J.; Jolliffe, K. A.; McLeod, M. D.; McRae, K. J.; Stewart, S. G.; Vogtle, M. *Pure Appl. Chem.* **2003**, *75*, 223. (c) Johnson, R. A. *Org. React.* **2004**, *63*, 117. (d) Rinner, U. In *Comprehensive Chirality*, Vol. 2; Carreira, E. M., Yamamoto, H., Eds.; Elsevier: Amsterdam, 2012; p 240. (e) Hudlicky, T.; Reed, J. W. *Synlett* **2009**, *2009*, 685. (f) Hudlicky, T.; Reed, J. W. *Chem. Soc. Rev.* **2009**, *38*, 3117. (j) Banwell, M. G.; Bolte, B.; Buckler, J. N.; Chang, E. L.; Lan, P.; Taher, E. S.; White, L. V.; Willis, A. C. *J. Proc. R. Soc. New South Wales* **2017**, *149*, 34.

(9) (a) Lewis, S. E. In *Arene Chemistry: Reaction Mechanisms and Methods for Aromatic Compounds*; Mortier, J., Ed.; Wiley-VCH: 2015; p 915. (b) Griffen, J. A.; Kenwright, S. J.; Abou-Shehada, S.; Wharry, S.; Moody, T. S.; Lewis, S. E. *Org. Chem. Front.* **2014**, *1*, 79. (c) Lewis, S. E. *Chem. Commun.* **2014**, *50*, 2821. (d) For recent use of the *ipso*-diols **5a** and **5b** in the synthesis of pleiogenone A see: Froese, J.; Overbeeke, C.; Hudlicky, T. *Chem. - Eur. J.* **2016**, *22*, 6180. [The authors are grateful to Jason Hudlicky (Vanderbilt University) for suggesting this project.]

(10) Reiner, A. M.; Hegeman, G. D. *Biochemistry* **1971**, *10*, 2530.

(11) Jenkins, G. N.; Ribbons, D. W.; Widdowson, D. A.; Slawin, A. M. Z.; Williams, D. J. J. *J. Chem. Soc., Perkin Trans. 1* **1995**, 2647.

(12) Banwell, M. G.; Edwards, A. J.; Lupton, D. W.; Whited, G. *Aust. J. Chem.* **2005**, *58*, 14.

(13) Ross, A. M.; Pohl, T. M.; Piazza, K.; Thomas, M.; Fox, B.; Whalen, D. L. *J. Am. Chem. Soc.* **1982**, *104*, 1658.

(14) Narsaiah, A. V.; Reddy, B. V. S.; Premalatha, K.; Reddy, S. S.; Yadav, J. S. *Catal. Lett.* **2009**, *131*, 480.

(15) Liu, Z.; Chen, Z.-C.; Zheng, Q.-G. *Org. Lett.* **2003**, *5*, 3321.

(16) Myers, A. G.; Siegel, D. R.; Buzard, D. J.; Charest, M. G. *Org. Lett.* **2001**, *3*, 2923.

(17) Donohoe, T. J.; Blades, K.; Moore, P. R.; Waring, M. J.; Winter, J. J. G.; Helliwell, M.; Newcombe, M. J.; Stemp, G. *J. Org. Chem.* **2002**, *67*, 7946.

(18) Palframan, M. J.; Kociok-Kohn, G.; Lewis, S. E. *Org. Lett.* **2011**, *13*, 3150.

(19) (a) Henbest, H. B.; Wilson, R. A. L. *J. Chem. Soc.* **1957**, 1958. (b) Prileschajew, N. *Ber. Dtsch. Chem. Ges.* **1909**, *42*, 4811.

DOI: 10.1021/acs.orglett.7b00194
Org. Lett. XXXX, XXX, XXX−XXX