

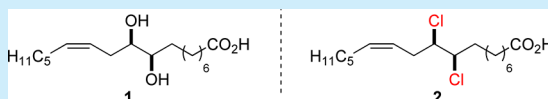
Synthesis and Biological Evaluation of Chlorinated Analogs of Leukotoxin Diol

Christian Nilewski, Camille Le Chapelain, Susanne Wolfrum, and Erick M. Carreira*

Vladimir Prelog Weg 3, Laboratory of Organic Chemistry, ETH-Zürich, CH-8093 Zurich, Switzerland

Supporting Information

ABSTRACT: This study documents that chlorinated analogs of leukotoxin diol **1**, in which the *vic*-diol has been replaced with *vic*-chlorides (**2**), induce caspase 3 activity and apoptosis on HepG2 cells in a dose-dependent manner in analogy to the parent diol. This suggests that chlorides may substitute for hydroxyls in certain lipids as bioisosteres in defined biological settings.



As part of our ongoing studies on chlorinated natural products, we became interested in investigating the effects of chlorides in lipids vis-à-vis their biological activity.¹ Chloride atoms and hydroxyl groups are regarded as classical isosteres,² and isosteric displacement of substituents is an approach widely employed to fine tune the biological, physicochemical, and pharmacokinetic properties of a bioactive molecule.³ Herein, we document that chlorinated analogs of leukotoxin diol **1**, wherein the *vic*-diol has been replaced with *vic*-chlorides to give dichloride **2**, induce caspase 3 activity and apoptosis on HepG2 cells in a dose-dependent manner in analogy to the parent diol (Figure 1).

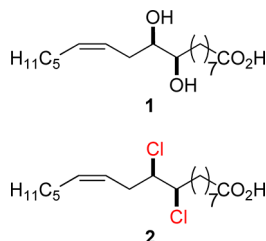


Figure 1. Structures of leukotoxin diol **1** and its chlorinated analog.

Despite their consideration as isosteres, there are prominent differences between chlorides and alcohols. First, the substitution of a hydroxyl by a chloride leads to marked increase in lipophilicity (*i*-PrOH clog *P* = 0.38 vs 2-chloropropane clog *P* = 1.61). Second, alcohols can participate in hydrogen bonding as acceptors and donors, while organochlorides, however, cannot, but instead, they can engage in halogen bonding.⁴ A noteworthy example of hydroxyl–chloride exchange has been provided by the replacement of three of the hydroxyl groups of sucrose **3** by chlorides to furnish the noncaloric artificial sweetener sucralose (**4**) (Figure 2).⁵ Sucralose is known to be considerably sweeter than sucrose, and it constitutes the major ingredient of several commercialized artificial sweeteners. This difference has been correlated with the increase of lipophilicity and enhanced permeability of chlorinated derivative **4** compared to natural sugar **3** (Figure 2).

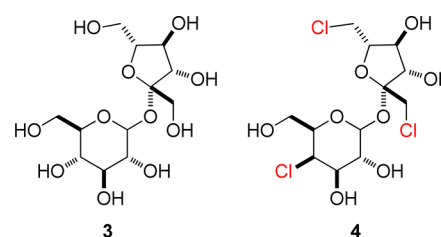
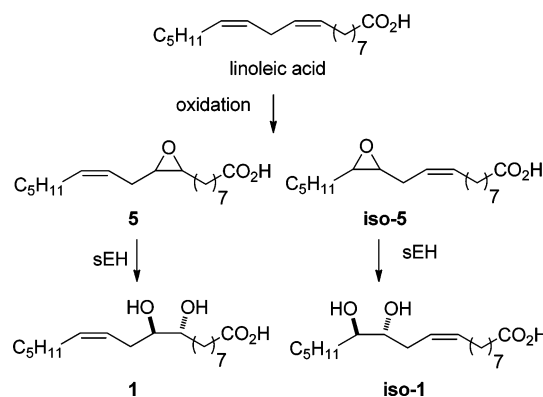


Figure 2. Structures of sucrose (**3**) and its chlorinated analog sucralose (**4**).

In analogy, we wondered whether there may be situations in which substitution of *vic*-diols in certain lipids with the corresponding *vic*-chlorides might lead to structures which reproduce the biological responses observed with the parent *vic*-diols in the naturally occurring biomolecule. As an initial approach, our attention turned to leukotoxin diol **1**, and we were eager to see whether its biological effects could be retained in the chloride analog **2**. Leukotoxin diol **1** and its regioisomer *iso*-**1** are biosynthesized from linoleic acid in mammalian neutrophils (Scheme 1).⁶ Linoleic acid is oxidized

Scheme 1. Biosynthesis of Leukotoxin Diol **1** in Mammals

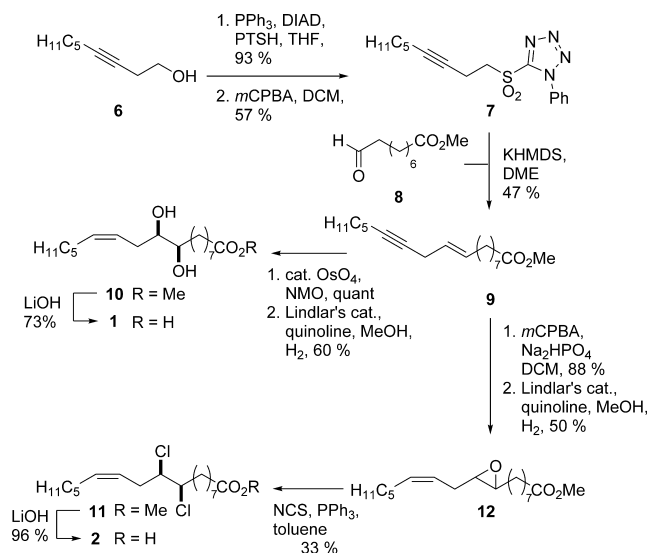
Received: September 29, 2015

to leukotoxin 5 by cytochrome P-450 dependent monooxygenase or by reactive oxygen species (ROS) such as hydroxyl radicals generated by respiratory burst oxidase.⁷ The epoxide is then converted to the *threo* diol by action of epoxide hydrolase, mostly soluble epoxide hydrolase (sEH).⁸

Shortly after its discovery, leukotoxin 5 was associated with numerous toxic effects, including pulmonary edema, vasodilatation, acute respiratory distress syndrome (ARDS), cardiovascular toxicity, mitochondrial dysfunction, nitric oxide elevation, and more recently, immunosuppression.⁹ Interestingly, in vitro studies on sEH were conducted that reported leukotoxin to act only as a protoxicant, with its hydrolyzed metabolites 1 and *iso*-1 being responsible for the observed adverse effects.¹⁰ This appeared as a surprise as hydrolysis of epoxides contributes usually to detoxification processes.¹¹ Later reports shed more critical light on this conclusion.¹² However, in vivo assays on Swiss webster mice supported the hydrolysis of the epoxide to be the trigger for toxicity.¹³ The role of leukotoxin and its diol on mitochondria has been documented in early reports;¹⁴ however, the exact mechanism of action of these compounds has not yet been elucidated.¹⁵ In order to gain insights on the effects of the Cl/OH exchange, we set out to synthesize leukotoxin diol 1, its chlorinated analog 2, as well as the corresponding *erythro* diastereomers.

Our synthetic endeavors commenced with alcohol 6 (Scheme 2), which was converted to sulfone 7 in two steps

Scheme 2. Synthesis of Diols 1 and 10 and Dichlorides 2 and 11.

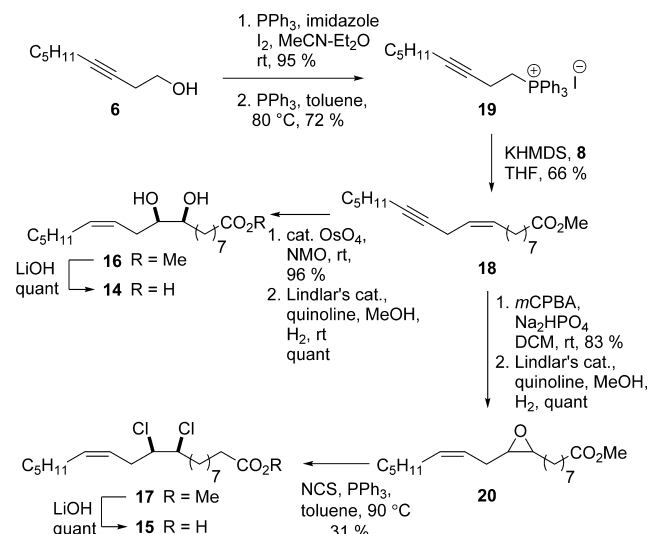


through a Mitsunobu reaction with 1-phenyl-1H-tetrazole-5-thiol and subsequent oxidation of the intermediate sulfide with $m\text{-CPBA}$.¹⁶ Julia–Kocienski coupling between aldehyde 8¹⁷ and sulfone 7 using KMDS in DME enabled the formation of (*E*)-enyne 9 as a single diastereomer.¹⁸ Upjohn dihydroxylation of the (*E*) alkene followed by Lindlar reduction of the alkyne moiety delivered ester 10,^{19,20} which underwent facile hydrolysis under standard conditions to afford leukotoxin diol (1). The corresponding dichlorides 2 and 11 could be derived from enyne 9 as well. Epoxidation of the alkene moiety in 9 with $m\text{-CPBA}$ and subsequent Lindlar reduction of the intermediate *trans*-epoxide led to the formation of epoxide 12. The epoxide moiety was then converted into a *trans* dichloride motif by implementation of a method developed by

Yoshimitsu and co-workers.^{21,22} Facile saponification of dichloroester 11 completed the synthesis of dichloride 2.

Having established a synthetic route to leukotoxin diol (1) and its parent dichloride 2, we focused on the synthesis of their *erythro*-diastereoisomeric counterparts (Scheme 3). Thus,

Scheme 3. Synthesis of Diols 14 and 16 and Dichlorides 15 and 17

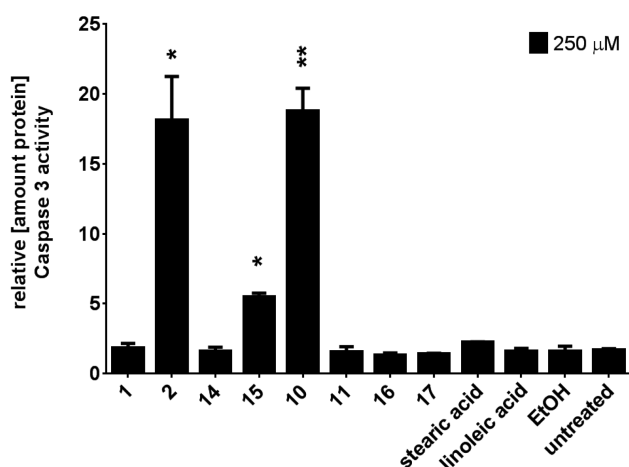


erythro diol 14 and its chlorinated analog 15, as well as their corresponding methyl esters 16 and 17, were prepared via a similar sequence of reactions starting from (*Z*)-enyne 18 (Scheme 3). Enyne 18 was obtained as a single diastereoisomer by a Wittig reaction between aldehyde 8 and triphenylphosphine iodide 19.^{23,24} Subsequent dihydroxylation followed by Lindlar reduction led to dihydroxy methyl ester 16, which was readily hydrolyzed to *erythro*-leukotoxin diol 14. Gratifyingly, enyne 18 could also be readily converted to epoxide 20 in two steps, including epoxidation with $m\text{-CPBA}$ and subsequent Lindlar reduction. Homoallylic epoxide 20 was then transformed into *erythro*-dichloride 17 by applying again Yoshimitsu's methodology. Ester hydrolysis of 17 proceeded as expected and provided *erythro*-dichloride 15.

With both diastereoisomers of leukotoxin diol (1 and 14) and their chlorinated counterparts (2 and 15) in hand, the stage was set for the biological evaluation of their cytotoxicities against different cell lines. In this regard, we measured the activation of caspase 3, the key mediator of programmed cell death (apoptosis) following exposure to the leukotoxin diol analogs.²⁵ None of the analogs triggered activation of caspase 3 in human breast and lung carcinoma cells (MCF-7/HTB-22; A-549/CCL-185). In contrast, some of the synthesized compounds induced activation of caspase 3 in human liver carcinoma cells (HepG2/HB8065) and murine hepatoma cells (Hepa 1–6/CRL-1830) (Figure 3a,b). In Hepa cells, the *threo* form (dichloride 2 and diol methyl ester 10) seemed to be more active than the *erythro* form (dichloride 15) (Figure 3a). In HepG2 cells, both leukotoxin diol 1 and its chlorinated counterpart 2 showed a strong increase in caspase 3 activity, while the corresponding methyl esters 10 and 11 showed a reduced pro-apoptotic effect compared to the free acids. *threo*-Dichloride 15 also induced moderate apoptosis (Figure 3b).

The human HepG2 cell line was chosen for a more thorough dose-dependent analysis of the synthesized leukotoxin diol

a) Hepa 1-6



b) HepG2

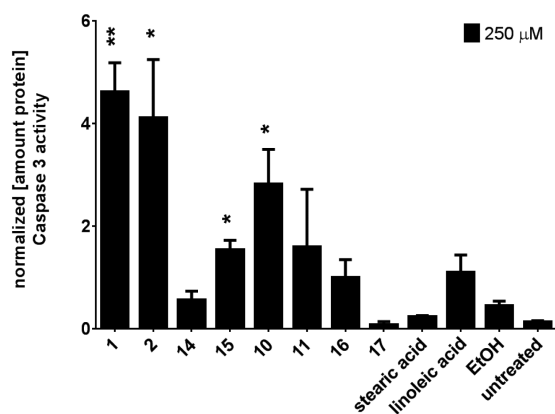


Figure 3. Relative caspase 3 activity measured on (a) Hepa 1–6 and on (b) HepG2 cells at 250 μM for each compound. The significance of each result is shown above the bar: $p < 0.05 = *$; $p < 0.01 = **$.

analogs. As shown in Figure 4, a dose-dependent decrease of caspase 3 activity was seen for all active compounds, indicating that this is a genuine and not an unspecific effect. In summary, the polar terminal function as well as the configuration of the diol motif appeared to influence the bioactivity. Interestingly,

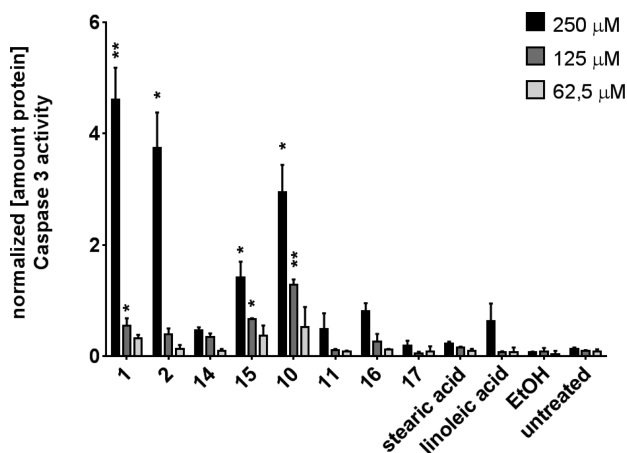


Figure 4. Dose–response for HepG2 cells.

these results correlate with a previous report in which chlorinated analogs of stearic and myristic acids were documented to exhibit cytotoxic effects toward HepG2 cells.²⁶ The relatively high concentration of compounds (250 and 125 μM) needed to induce apoptosis could be the consequence of low intracellular concentrations due to inefficient uptake of the leukotoxin diols and/or a natural mechanism that ensures that cell death is only induced in critical situations indicated by high abundance of leukotoxin diols. A plausible explanation for the noninduction of caspase 3 activity in lung and breast cancer cells could be their high levels of resistance to endogenous toxins, a key feature of cancer cells.

In summary, we have conducted a comparative study of both diastereoisomers (*threo/erythro*) of leukotoxin diol as well as their chlorinated counterparts. Cytotoxicity assays were performed on a variety of cell lines by measuring caspase 3 activity. Leukotoxin diol and its chlorinated analog, and to a lesser extent their methyl esters, were found to induce apoptosis in HepG2 cells in a dose-dependent manner. It is noteworthy that the biological effect of leukotoxin diol on HepG2 cells was retained in the parent dichloride, which indicates that it may be possible to generate analogs of leukotoxin diols with chlorides while retaining biological readout.

■ ASSOCIATED CONTENT

§ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs-orglett.5b02814.

Experimental procedures and characterization data for all reactions and products, including ^1H NMR and ^{13}C NMR spectra of new compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: carreira@org.chem.ethz.ch.

Notes

The authors declare no competing financial interest. The work is taken in part from undergraduate research work (Semesterarbeit) conducted by C.L.C. at ETH-Zürich.

■ ACKNOWLEDGMENTS

We thank the ERC for support of this work in part through grant 320666_CHLIP.

■ REFERENCES

- (1) (a) Nilewski, C.; Geisser, R. W.; Carreira, E. M. *Nature* **2009**, 457, 573–576. (b) Nilewski, C.; Geisser, R. W.; Ebert, M.-O.; Carreira, E. M. *J. Am. Chem. Soc.* **2009**, 131, 15866–15876. (c) Nilewski, C.; Deprez, N. R.; Fessard, T. C.; Li, D. B.; Geisser, R. W.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2011**, 50, 7940–7943. (d) Huwyler, N.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2012**, 51, 13066–13069. (e) Shemet, A.; Sarlah, D.; Carreira, E. M. *Org. Lett.* **2015**, 17, 1878–1881.
- (2) Burger, A. *Medicinal Chemistry*, 3rd ed.; Wiley: New York, 1970; p 127.
- (3) Naumann, K. *Pest Manag. Sci.* **2000**, 56, 3–21 and references cited therein.
- (4) For a seminal review on halogen bonding, see: Metrangolo, P.; Neukirch, H.; Pilati, T.; Resnati, G. *Acc. Chem. Res.* **2005**, 38, 386–395.
- (5) Mathlouthi, M.; Seuvre, A.-M.; Birch, G. G. *Carbohydr. Res.* **1986**, 152, 47–61.

- (6) (a) Hayakawa, M.; Sugiyama, S.; Takamura, T.; Yokoo, K.; Iwata, M.; Suzuki, K.; Taki, F.; Takahashi, S.; Ozawa, T. *Biochem. Biophys. Res. Commun.* **1986**, *137*, 424–430. (b) Ozawa, T.; Sugiyama, S.; Hayakawa, M.; Taki, F.; Hanaki, Y. *Biochem. Biophys. Res. Commun.* **1988**, *152*, 1310–1318.
- (7) (a) Hayakawa, M.; Ogawa, T.; Sugiyama, S.; Ozawa, T. *Biochem. Biophys. Res. Commun.* **1989**, *161*, 1077–1085. (b) Iwase, H.; Takatori, T.; Aono, K.; Iwade, K.; Takahashi, M.; Nakajima, M.; Nagao, M. *Biochem. Biophys. Res. Commun.* **1995**, *216*, 483–488. (c) Iwase, H.; Takatori, T.; Nijima, H.; Nagao, M.; Amano, T.; Iwade, K.; Matsuda, Y.; Nakajima, M.; Kobayashi, M. *Biochim. Biophys. Acta, Lipids Lipid Metab.* **1997**, *1345*, 27–34.
- (8) Moghaddam, M. F.; Motoba, K.; Borhan, B.; Pinot, F.; Hammock, B. *Biochim. Biophys. Acta, Gen. Subj.* **1996**, *1290*, 327–339.
- (9) (a) Fukushima, A.; Hayakawa, M.; Sugiyama, S.; Ajioka, M.; Ito, T.; Satake, T.; Ozawa, T. *Cardiovasc. Res.* **1988**, *22*, 213–218. (b) Sakai, T.; Ishizaki, T.; Ohnishi, T.; Sasaki, F.; Ameshima, S.; Nakai, T.; Miyabo, S.; Matsukawa, S.; Hayakawa, M.; Ozawa, T. *Am. J. Physiol.* **1995**, *269*, L326–331. (c) Ishizaki, T.; Shigemori, K.; Nakai, T.; Miyabo, S.; Ozawa, T.; Chang, S.-W.; Voelkel, N. F. *Am. J. Physiol.* **1995**, *269*, L65–70. (d) Ishizaki, T.; Shigemori, K.; Yamamura, Y.; Matsukawa, S.; Nakai, T.; Miyabo, S.; Hayakawa, M.; Ozawa, T.; Voelkel, N. F. *Biochem. Biophys. Res. Commun.* **1995**, *210*, 133–137. (e) Ishizaki, T.; Takahashi, H.; Ozawa, T.; Chang, S.-W.; Voelkel, N. F. *Am. J. Physiol.* **1995**, *268*, L123–128. (f) Ozawa, T.; Nishikimi, M.; Sugiyama, S.; Taki, F.; Hayakawa, M.; Shionoya, H. *Biochem. Int.* **1988**, *16*, 369–373. (g) Edwards, L. M.; Lawler, N. G.; Nikolic, S. B.; Peters, J. M.; Horne, J.; Wilson, R.; Davies, N. W.; Sharman, J. E. *J. Lipid Res.* **2012**, *53*, 1979–1986.
- (10) (a) Moghaddam, M.; Grant, D. F.; Cheek, J. M.; Greene, J. F.; Williamson, K. C.; Hammock, B. D. *Nat. Med.* **1997**, *3*, 562–566. (b) Moran, J.; Weise, R.; Schnellmann, R.; Freeman, J.; Grant, D. *Toxicol. Appl. Pharmacol.* **1997**, *146*, 53–59. (c) Greene, J.; Newman, J.; Williamson, K.; Hammock, B. *Chem. Res. Toxicol.* **2000**, *13*, 217–226.
- (11) (a) Aran, M.; Oesch, F. *Mammalian xenobiotic epoxide hydrolases. Enzyme Systems that Metabolize Drugs and Other Xenobiotics*; Wiley: New York, 2002; pp 459–483. (b) Hammock, B. D.; Storms, D. H.; Grant, D. F. *Epoxide hydrolases. Biotransformations*; Pergamon Press: Oxford, 1997; Vol. 3, pp 283–305.
- (12) (a) Moran, J. H.; Mitchell, L. A.; Bradbury, J. A.; Qu, W.; Zeldin, D. C.; Schnellmann, R. G.; Grant, D. F. *Toxicol. Appl. Pharmacol.* **2000**, *168*, 268–279. (b) Mitchell, L. A.; Moran, J. H.; Grant, D. F. *Toxicol. Lett.* **2002**, *126*, 187–196 and references cited therein.
- (13) Zheng, J.; Plopper, C.; Lakritz, J.; Storms, D.; Hammock, B. *Am. J. Respir. Cell Mol. Biol.* **2001**, *25*, 434–438.
- (14) Ozawa, T.; Hayakawa, M.; Takamura, T.; Sugiyama, S.; Suzuki, K.; Iwata, M.; Taki, F.; Tomita, T. *Biochem. Biophys. Res. Commun.* **1986**, *134*, 1071–1078.
- (15) (a) Sisemore, M. F.; Zheng, J.; Yang, J. C.; Thompson, D. A.; Plopper, C. G.; Cortopassi, G. A.; Hammock, B. D. *Arch. Biochem. Biophys.* **2001**, *392*, 32–37. (b) Moran, J. H.; Mon, T.; Hendrickson, T. L.; Mitchell, L. A.; Grant, D. F. *Chem. Res. Toxicol.* **2001**, *14*, 431–437.
- (16) Bonini, C.; Chiumminto, L.; Videtta, V. *Synlett* **2005**, 3067–3070.
- (17) Gung, B. W.; Dickson, H. *Org. Lett.* **2002**, *4*, 2517–2519.
- (18) Blakemore, P. R.; Cole, W. J.; Kocieński, P. J.; Morley, A. *Synlett* **1998**, 1998, 26–28.
- (19) VanRheenen, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, *17*, 1973–1976.
- (20) Lindlar, H. *Helv. Chim. Acta* **1952**, *35*, 446.
- (21) Yoshimitsu, T.; Fukumoto, N.; Tanaka, T. *J. Org. Chem.* **2009**, *74*, 696–702.
- (22) The relative configuration of the vicinal dichloride motif of **11** was supported by selective conversion of **11** to the corresponding diene followed by analysis of the ^1H NMR spectrum: (a) Sonnet, P. E.; Oliver, J. E. *J. Org. Chem.* **1976**, *41*, 3279–3283. (b) Frost, D. J.; Gunstone, F. D. *Chem. Phys. Lipids* **1975**, *15*, 53–85.
- (23) Skaanderup, P. R.; Poulsen, C. S.; Hyldtoft, L.; Jørgensen, M. R.; Madsen, R. *Synthesis* **2002**, 2002, 1721–1727.
- (24) For a review of the Wittig olefination, see: Maryanoff, B. E.; Reitz, A. B. *Chem. Rev.* **1989**, *89*, 863–927.
- (25) Nicholson, D. W.; Ali, A.; Thornberry, N. A.; Vaillancourt, J. P.; Ding, C. K.; Gallant, M.; Gareau, Y.; Griffin, P. R.; Labelle, M.; Lazebnik, Y. A.; Munday, N. A.; Raju, S. M.; Smulson, M. E.; Yamin, T.-T.; Yu, V. L.; Miller, D. K. *Nature* **1995**, *376*, 37–43.
- (26) Lystad, E.; Hostmark, A. T.; Jebens, E. *Pharmacol. Toxicol.* **2001**, *89*, 85–91.