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Synthesis of Immunostimulatory α-*C*-Galactosylceramide Glycolipids via Sonogashira Coupling, Asymmetric Epoxidation, and Trichloroacetimidate-Mediated Epoxide Opening

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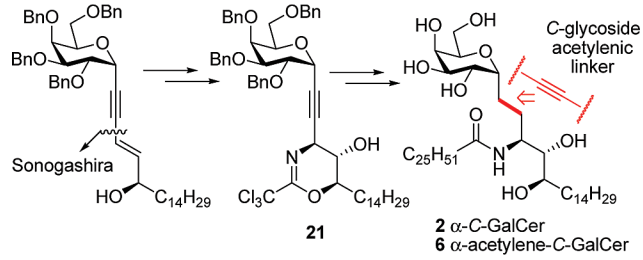
ABSTRACT



Stereocontrolled syntheses of α-*C*-GalCer (2) and its α-*C*-acetylenic analogue 6 were accomplished in high efficiency by a convergent construction strategy from 1-hexadecene and D-galactose. The key transformations include Sonogashira coupling, Sharpless asymmetric epoxidation, and Et_2AlCl-catalyzed cyclization of an epoxytrichloroacetimidate to generate protected dihydrooxazine 21.

KRN7000 [(2*S*,3*S*,4*R*)-1-*O*-(α-D-galactopyranosyl)-2-(*N*-hexacosanoylamino)-1,3,4-octadecanetriol, α-GalCer, **1**, Figure 1] is a synthetic analogue of a glycolipid extracted from the marine sponge[1] *Agelas mauritianus* during a screen of natural products possessing antitumor properties in mice by Kirin Pharmaceuticals.[2] α-GalCer forms a complex with a glycoprotein in antigen-presenting cells known as CD1d.[3] Glycolipid presentation in CD1d−ligand complexes to the T cell receptor of invariant natural killer T (iNKT) cells gives a high-affinity ternary complex[4] that activates iNKT cells in mice and humans to secrete a complex mixture of cytokines. The production of pro-inflammatory T helper (Th1) type cytokines such as interferon γ (IFN-γ) is correlated with antitumor, antiviral/antibacterial, and adjuvant activities, whereas anti-inflammatory Th2 type cytokines (such as interleukins 4, 5, 10, and 13) are regulators of some autoimmune and inflammatory diseases.[5] However, the simultaneous production of both types of conflicting cytokine activities comprises the therapeutic potential of activated

(1) Since mammalian and plant glycosphingolipids generally are β-anomers, it is surprising that sponges would produce α-glycosphingolipids. It is likely that **1** was actually derived from *Novosphingobium* bacteria that colonized *A. mauritianus*.

(2) (a) Natori, A.; Koezuka, Y.; Higa, T. *Tetrahedron Lett.* **1993**, *34*, 5591. (b) Kobayshi, E.; Motoki, K.; Uchida, T.; Fukushima, H.; Koezuka, Y. *Oncol. Res.* **1995**, *7*, 529.

(3) CD1d molecules, which are expressed by professional antigen-presenting cells such as dendritic cells, macrophages, and B cells, recognize various lipid and glycolipid antigens. Bendelac, A.; Savage, P. B.; Teyton, L. *Annu. Rev. Immunol.* **2007**, *35*, 297.

(4) Invariant NKT cells are a subset of T lymphocytes that recognize glycolipid antigens and are dependent on CD1d as the antigen-presenting molecule to their T cell receptor (TCR). In the glycolipid−CD1d complex, the two long hydrocarbon chains of α-GalCer are buried in the two long apolar channels of CD1d, and the carbohydrate headgroup protrudes above the surface toward the TCR of iNKT cells.[7]

(5) Savage, P. B.; Teyton, L.; Bendelac, A. *Chem. Soc. Rev.* **2006**, *35*, 771.

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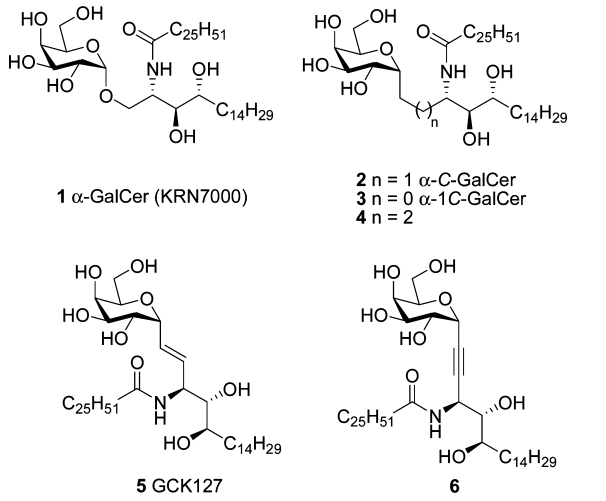


Figure 1. Structures of glycolipids **1**−**6**.

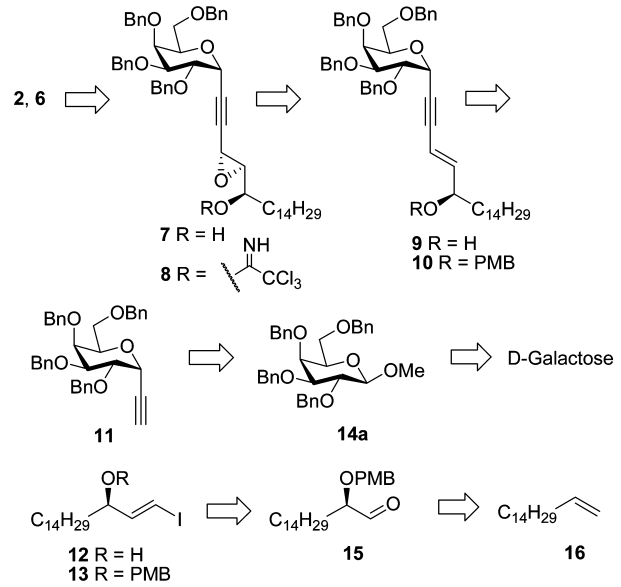
iNKT cells and interferes with a concerted biological outcome.[6] The potential clinical utility of **1** is also limited by the long-term unresponsiveness (anergy) of iNKT cells when multiple doses of **1** are administered.

α-*C*-Glycoside analogues of α-GalCer are expected to be long-lived because they are resistant to α-glycosidase activity. Moreover, replacing the glycosidic oxygen atom with a methylene group removes a hydrogen-bonding acceptor site.[7] Thus, α-*C*-GalCer analogues may bind less tightly to CD1d, which may be a factor in determining the type of cytokine release. An isosteric *C*-glycoside analogue (**2**, Figure 1) was found to be active in mice in vivo, with a biased induction of Th1 responses compared to **1**.[8] In addition, **2** produced a long-term production of IFN-γ in mice, suggesting that the α-*C*-GalCer/CD1d complex is more stable in antigen-presenting cells in vivo than the KRN7000/CD1d complex.[8e,f] We found that nonisosteric α-*C*-GalCer analogue **3**, in which the glycosidic oxygen was deleted, induced an even higher Th1-type cytokine response than **1** and **2** in human iNKT cells in vitro.[9] Interestingly, other α-*C*-glycoside homologues that contain a three-carbon linker (**4**) were inactive.[8b] GCK127 (**5**), an analogue with an *E*-alkene linker, not only exhibited activity in mice but also induced a potent stimulatory activity against human iNKT cells, which was ascribed to the preservation of an ∼170° dihedral angle in the linker region between the galactose and the ceramide (Gal-C1-O1-phytosphingosine C1′-phytosphingosine C2′).[10] These studies indicate that α-*C*-GalCer analogues are useful for presentation by CD1d to iNKT cells and have potential immunotherapeutical applications compared with **1**, the most commonly used ligand.

In order to make larger quantities of **2** available to the immunology community,[11] diverse synthetic approaches toward this important synthetic target have been developed.[8,12] However, there remains a need for efficient stereoselective methods for the preparation of **2**. We report a concise convergent synthesis of **2** from readily available, inexpensive starting materials. In addition, the synthetic route to **2** reported here permits modification of the linker region, which appears to be critical for Th1 vs Th2 selectivity. We also report the synthesis of **6**, which contains an acetylenic moiety and also preserves an ∼170° dihedral angle in the linker.

As shown in the retrosynthetic analysis (Scheme 1), we envisioned that the three contiguous stereogenic centers in

Scheme 1. Retrosynthetic Plan



the phytosphingosine moiety can be accessed from epoxy alcohol **7** after reaction with trichloroacetonitrile to give **8**, followed by a Lewis acid catalyzed epoxide opening at the propargylic carbon. The requisite epoxide **7** could be

(6) (a) Miyamoto, K.; Miyake, S.; Yamamura, T. *Nature* **2001**, *413*, 531. (b) Van Kaer, L. *Nat. Rev. Immunol.* **2005**, *5*, 31.

(7) (a) Borg, N. A.; Wun, K. S.; Kjer-Nielsen, L.; Wilce, M. C. J.; Pellicci, D. G.; Koh, R.; Besra, G. S.; Bharadwaj, M.; Godfrey, D. I.; McCluskey, J.; Rossjohn, J. *Nature* **2007**, *448*, 44. (b) Koch, M.; Stronge, V. S.; Shepherd, D.; Gadola, S. D.; Mathew, B.; Ritter, G.; Fersht, A. R.; Besra, G. S.; Schmidt, R. R.; Jones, E. Y.; Cerundolo, V. *Nat. Immunol.* **2005**, *6*, 819. (c) Schiefner, A.; Fujio, M.; Wu, D.; Wong, C.-H.; Wilson, I. A. *J. Mol. Biol.* **2009**, *394*, 71.

(8) (a) Yang, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Angew. Chem., Int. Ed.* **2004**, *43*, 3818. (b) Chen, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Org. Lett.* **2004**, *6*, 4077. (c) Pu, J.; Franck, R. W. *Tetrahedron* **2008**, *64*, 8618. (d) Franck, R. W.; Tsuji, M. *Acc. Chem. Res.* **2006**, *39*, 692. (e) Schmieg, J.; Yang, G.; Franck, R. W.; Tsuji, M. *J. Biomed. Biotechnol.* **2010**, *2010*, 283612. (f) Sullivan, B. A.; Nagarajan, N. A.; Wingender, G.; Wang, J.; Scott, I.; Tsuji, M.; Franck, R. W.; Porcelli, S. A.; Zajonc, D. M.; Kronenberg, M. *J. Immunol.* **2010**, *184*, 141.

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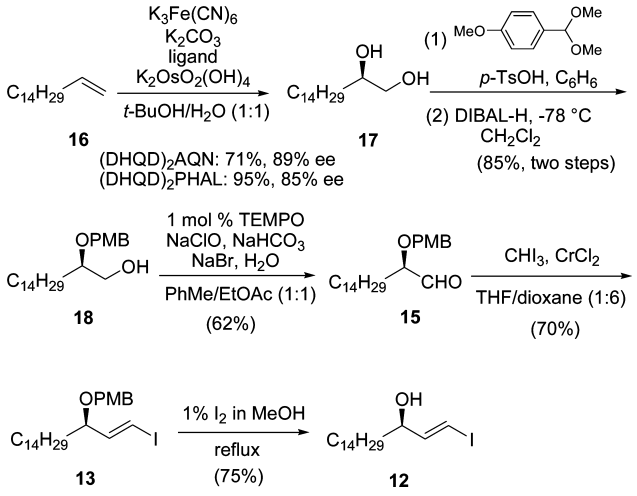
(10) (a) Chen, G.; Chien, M.; Tsuji, M.; Franck, R. W. *ChemBioChem* **2006**, *7*, 1017. (b) Li, X.; Chen, G.; Garcia-Navarro, R.; Franck, R. W.; Tsuji, M. *Immunology* **2009**, *127*, 216. (c) Li, X.; Shiratsuchi, T.; Chen, G.; Dellabona, P.; Casorati, G.; Franck, R. W.; Tsuji, M. *J. Immunol.* **2009**, *183*, 4415.

(11) The Tetramer Core Facility funded by the NIH provides reagents for NKT cell activation, including CD1d ligands, to approved investigators.

(12) Wipf, P.; Pierce, J. G. *Org. Lett.* **2006**, *8*, 3375.

furnished by Sharpless asymmetric epoxidation (SAE)[13] of **9**, which in turn could be obtained from **10** via Sonogashira cross-coupling[14] between two building blocks, **11** and **12** (or **13**). Compound **11** can be assembled via α-*C*-ethynylation of **14a** (accessible from D-galactose; see the Supporting Information), and **12** can be prepared via Takai olefination[15] of aldehyde **15**, which can be synthesized from 1-hexadecene (**16**).

Scheme 2. Synthesis of Vinyl Iodide **12**



As shown in Scheme 2, Sharpless asymmetric dihydroxylation of **16** with AD-mix-β provided the desired diol **17** (85% ee) in almost quantitative yield.[16] Alternatively, the use of the ligand $(DHQD)_2AQN$, which was reported to have a higher enantioselectivity than the PHAL-based ligand in an aliphatic system,[17] delivered **17** in a lower yield (71%) and slightly higher ee value (89% ee). Diol **17** was converted to its *p*-methoxybenzylidene (PMB) acetal, which was reduced with DIBAL-H to give alcohol **18** (85%, two steps).[18] The use of a protocol with sodium hypochlorite catalyzed by TEMPO[19] gave the desired aldehyde **15** in 62% yield without any erosion of the ee value.[20] Since an (*E*)-l-iodoalkene was required, we used the Takai reaction,[15] which is known to be highly *E* stereoselective. Condensation of aldehyde **15** with iodoform in the presence of chromium(II) chloride yielded the expected (*E*)-vinyl iodide **13** in 70% yield when the Evans modification[21] was used. Deprotection of the PMB group using I_2 in MeOH[22] afforded vinyl iodide **12** (75%).

Initially, α-*C*-ethynylgalactoside **11** was prepared by reaction of 1-acetoxy-2,3,4,6-tetra-*O*-benzyl-D-galactopyranoside (**14b**,**c**) with ethynyl precursor **19** in the presence of TMSOTf followed by desilylation of **20**.[23] However, we subsequently found that methyl β-D-galactosylpyranoside (**14a**), which is crystalline, can also react with **19** under the same conditions (Scheme 3). This reaction proceeded with very high α-stereoselectivity; no corresponding β-anomer was found by ^1H NMR. Its efficiency in the preparation of **11** is comparable to that of acetate **14c**. The two-step yield of **11** from **14c** was 54%.[23a,c] Furthermore, **14c** must be prepared from **14a** in two additional steps (~67% overall yield).[24] Thus our two-step yield of **11** from **14a** (37%) is not only comparable to that from **14c** but also offers the advantage that **14a** can be prepared from the very inexpensive D-galactose as reported in the Supporting Information.

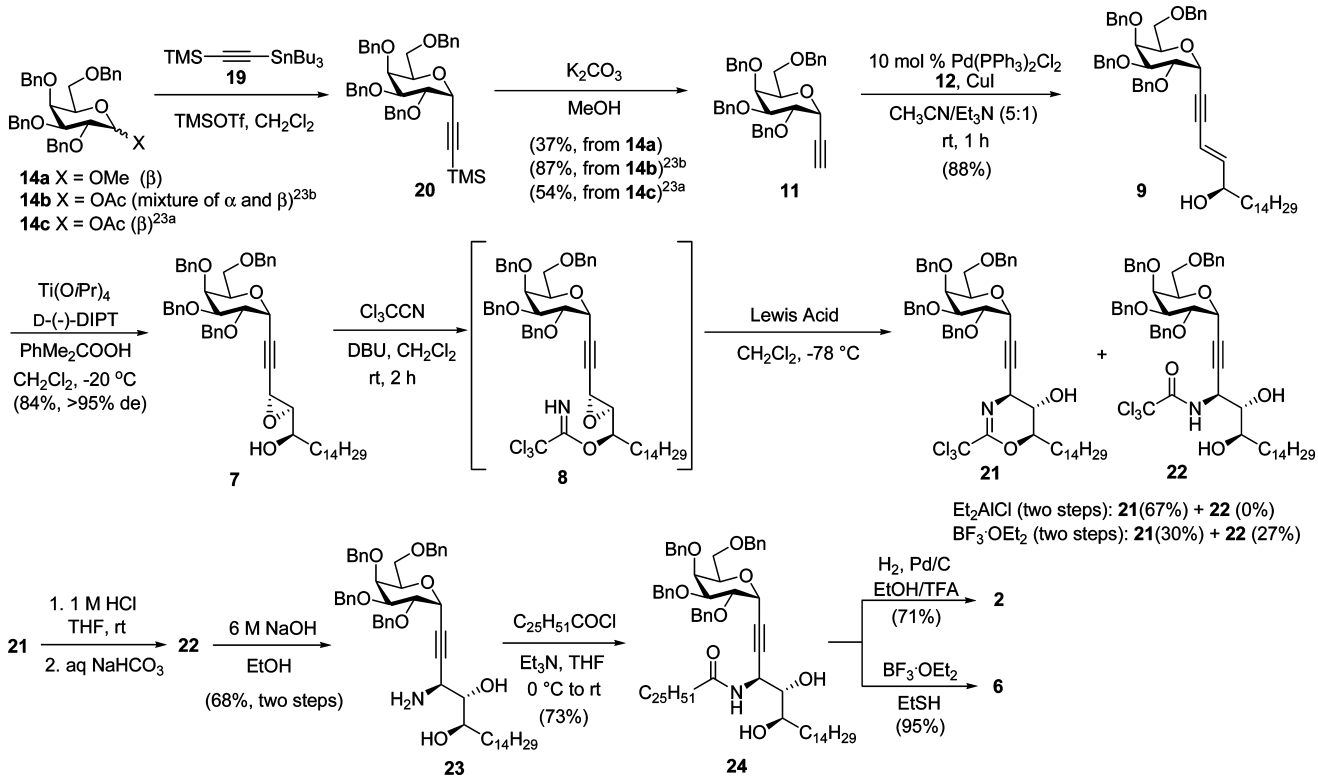
With an efficient synthesis of the two building blocks **11** and **12** established, we directed our efforts toward Sonogashira coupling (Scheme 3).[14] An initial trial of cross-coupling [$Pd(PPh_3)_4$, CuI (0.5 equiv), *i*-Pr_2NEt (6 equiv), THF] between PMB-protected alcohol **13** and alkyne **11** in THF provided enyne **10** in 56% yield. During deprotection of the PMB group of **10** with DDQ, the hydroxy group was oxidized to the corresponding ketone. However, when free alcohol **12** was coupled with **10** in the presence of $Pd(Ph_3P)_4$ and CuI in CH_3CN/Et_3N (5:1) the yield of enynol **9** improved to 88%. Use of $Pd(PPh_3)_2Cl_2$ as a precatalyst [$Pd(PPh_3)_2Cl_2$, CuI, CH_3CN/Et_3N (5:1)] afforded **9** in about the same yield as obtained with $Pd(Ph_3P)_4$.

Catalytic or substoichometric SAE of **9** was ineffective, producing little conversion after 20 h at −20 °C. The reaction using 4 equiv of cumene hydroperoxide as the epoxidizing agent catalyzed by 2.5 equiv of Ti(O-*i*-Pr)$_4$ and 2.6 equiv of D-(−)-DIPT provided propargylic epoxy alcohol **7** in high yield (84%) and excellent diastereoselectivity (>95%).[25] Chelation-controlled opening of 2,3-epoxy alcohol **7** with NaN_3 and in the presence of NH_4Cl in aqueous MeOH under reflux failed to provide the desired azido diol, delivering instead a complex mixture.[26] Et_2AlCl-catalyzed cyclization[27] of trichloroacetimidate **8**, prepared by reaction of **7** with 6.0 equiv of trichloroacetonitrile in the presence of 3.5 equiv of DBU,[27d] gave dihydrooxazine **21** in a two-step yield of 67%. It is noteworthy that $BF_3{\cdot}Et_2O$ also catalyzed cyclization of **8** to **21**; however, we obtained a mixture of **21** and its hydrolysis product **22** in a ratio of 1:1 (30% vs 27%, respectively).

Acid hydrolysis of **21** provided trichloroacetimide **22**, which was treated with ethanolic NaOH to deliver amine **23** in 68% overall yield. Reaction of amine **23** with *n*-hexacosanoyl chloride[12] gave amide **24** in 73% yield. Catalytic hydrogenation (Pd/C, H_2, EtOH/TFA)[10a] of the linking triple bonds, together with global removal of the benzyl protecting groups, afforded the target α-*C*-glycoside **2**.[28] However, attempted reduction using Pearlman's catalyst

(13) For a review of SAE, see: Katsuki, T.; Martin, V. S. *Org. React.* **1996**, *48*, 1.
(14) For a review, see: Sonogashira, K. *J. Organomet. Chem.* **2002**, *653*, 46.
(15) Takai, K.; Nitta, K.; Utimoto, K. *J. Am. Chem. Soc.* **1986**, *108*, 7408.
(16) The ee of **17** was enriched to >95% by recrystallization from EtOAc, and this highly enantiopure diol was used in the following reaction. See: He, L.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2000**, *65*, 7618.
(17) Eecker, H.; Sharpless, K. B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 448.
(18) Synthesis of primary alcohol **18** was readily achieved by following a reported synthesis of a shorter chain analogue from 1-heptene. Smith, A. B.; Chen, S. S. Y.; Nelson, F. C.; Reichert, J. M.; Salvatore, B. A. *J. Am. Chem. Soc.* **1997**, *119*, 10935.
(19) Jurczak, J.; Gryko, D.; Kobrzycka, E.; Gruza, H.; Prokopowiczit, P. *Tetrahedron* **1998**, *54*, 6051.
(20) Compound **15** was reduced back to **18** by $NaBH_4$ and subsequently converted to the (*S*)-MTPA ester using (*R*)-MTPA chloride. The ^1H NMR spectrum of the MTPA ester exhibited no signals of the other isomer; see the Supporting Information.

Scheme 3. Synthesis of **2** and **6**



[H_2, $Pd(OH)_2$, CH_2Cl_2/MeOH] resulted in incomplete saturation of the acetylenic group. The preparation of **6** from **24** was achieved by BF_3•OEt_2/EtSH deprotection of the benzyl groups,[29] leaving the acetylenic moiety intact, in almost quantitative yield.

In conclusion, a convergent and stereoselective synthetic route to α-*C*-GalCer (**2**) and its analogue **6** containing an acetylenic linker was accomplished. Notable features include the concise formation of three contiguous stereogenic centers in the phytosphingosine moiety by Sonogashira cross-coupling followed by Sharpless asymmetric epoxidation and Et_2AlCl-catalyzed cyclization of an epoxytrichloroacetimidate intermediate. This convergent construction from simple starting materials (10 steps from **14a** with 6.5% overall yield) permits the preparation of analogues with variations in the linker area.

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Supporting Information Available: Experimental procedures as well as ^1H and ^{13}C NMR spectra for all new compounds and synthetic **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) Evans, D. A.; Ng, H. P. *Tetrahedron Lett.* **1993**, *34*, 2229.
(22) Vaino, A. R.; Szarek, W. A. *Synlett* **1995**, 1157.
(23) (a) Nishikawa, T.; Koide, Y.; Kajii, S.; Wada, K.; Ishikawa, M.; Isobe, M. *Org. Biomol. Chem.* **2005**, *3*, 687. (b) Dondoni, A.; Moriotti, G.; Marra, A. *J. Org. Chem.* **2002**, *67*, 4475. (c) The stereoselectivity of the *C*-glycosidation was found to be independent of the anomeric configuration of the acetate.[23a,b]
(24) Kulkarni, S. S.; Gervay-Hague, J. *Org. Lett.* **2006**, *8*, 5765.
(25) Compound **7** was converted to the (*S*)-MTPA ester using using (*R*)-MTPA chloride. The de value was determined by analyzing the ^1H NMR spectrum of the (*S*)-MTPA ester of **7**.
(26) Two mechanisms may be responsible for the failure: (1) intramolecular 1,3-dipolar cycloaddition of the propargyl azide followed by MeOH attack on the resulting strained bicyclic triazole or (2) a triaza-Cope rearrangement followed by cyclization of the resulting allenyl azide to triazafulvene and reaction with MeOH. See: (a) Banert, K. *Chem. Ber.* **1989**, *122*, 911. (b) Banert, K. *Chem. Ber.* **1989**, *122*, 1963.
(27) For Lewis acid catalyzed cyclization of 2,3-epoxytrichloroacetimidates, see: (a) Schmidt, U.; Respondek, M.; Lieberknecht, A.; Werner, J.; Fisher, P. *Synthesis* **1989**, 256. (b) Hatakeyama, S.; Matsumoto, H.; Fukuyama, H.; Mukugi, Y.; Irie, H. *J. Org. Chem.* **1997**, *62*, 2275. (c) Lu, X.; Sun, C.; Valentine, W. J.; E, S.; Liu, J.; Tigyi, G.; Bittman, R. *J. Org. Chem.* **2009**, *74*, 3192. (d) The preparation of **8** in the presence of a catalytic amount of DBU[27b] was ineffective.
(28) The spectral data of **2** matched well with the previously reported material in 500 MHz ^1H and 125 MHz ^{13}C NMR spectra and optical rotation (see Table S1, Supporting Information).[8,12] .
(29) Xu, J.; Egger, A.; Bernet, B.; Vasella, A. *Helv. Chim. Acta* **1996**, *79*, 2004.