Chinese Journal of Chemistry, **2009**, *27*, 2296—2299 **Note**

Triflic Acid-Promoted Formylation of Ceramide in Dimethylformamide

CHIANG, Liwu(姜豊武) PAN, Sider(潘賜德) LO, Jemmau(羅建苗)
YU, Chungshan*(俞鐘山)

Department of Biomedical Engineering and Environmental Sciences, National Tsing-Hua University, Hsinchu 30043, Taiwan, China
Institute of Nuclear Engineering and Science, National Tsing-Hua University, Hsinchu 30043, Taiwan, China

The protected ceramide: *N*-((2*S*,3*S*,4*R*)-3,4-bis(benzyloxy)-1-hydroxyoctadecan-2-yl)tetracosanamide, was attempted to introduce a triflate as a leaving group followed by a nucleophilic substitution with azido group in one-pot manner. Unexpectedly, the oxazole ring formed via a thermodynamically favored intramolecular cyclization was opened to generate the original ceramide by triflic acid. In addition, the residual acid promoted a formylation of the primary hydroxy group in DMF.

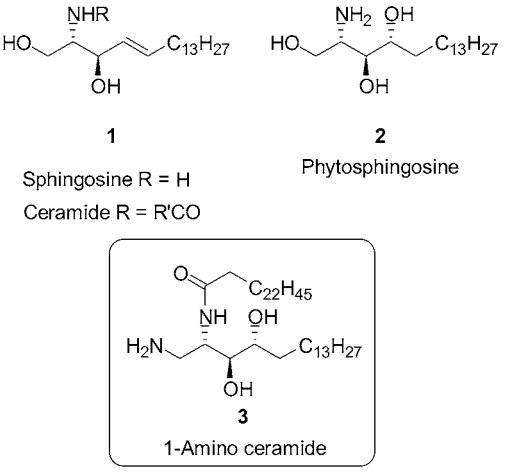
Keywords ceramide, rearrangement, oxazole

Introduction

Sphingosines and ceramide analogs have been intensively studied due to their potential inhibition effect against numerous cancer cells through apoptosis as well as immune system.[1] The bioactivity of sphingosine is mainly associated with the carbon length profile and partly or even rarely with the chirality. For example, the sum of carbon chain length of 18 carbon atoms exerted the optimal bioactivity. By contrast, only subtle differences among the four stereoisomers of *D*-erythro, *D*-threo, *L*-erythro and *L*-threo were observed. As a member of sphingosine, ceramide bearing a phytosphingosine moiety with characteristic configuration (2*S*,3*S*,4*R*) regulates the inhibition or promotion of tumors via an even complex mechanism mediated by natural killer T cells. A representative glycoceramide such as KRN 7000 has been intensively studied due to its versatile functions.[2] To accelerate the discovery of potential compounds, a combinatorial approach via constructing a library of 528 ceramide analogs has been reported and the most active compounds showed IC_{50} values in the low micromolar range.[3]

Considering the advantage of combination of easy preparation and fast assay, the recently developed *in-situ* assay of amide-forming libraries emerges as a rational approach for discovering potential compounds.[4] We are particularly interested in constructing such a core compound **3** with a scaffold of ceramide bearing a primary amino group (Scheme 1).

Scheme 1 Structures of sphingosine analogs



Experimental

N-((2*S*,3*S*,4*R*)-3,4-Bis(benzyloxy)-1-hydroxyoctadec-2-yl)tetracosanamide (4)

To a mixture of (2*S*,3*S*,4*R*)-2-amino-3,4-bis(benzyloxy)octadecan-1-ol (818 mg, 1.6 mmol) in CH_2Cl_2 (10 mL) were added $(i\text{-Pr})_2NEt$ (0.6 mL, 3.3 mmol), tetracosanoic acid (0.64 g, 1.73 mmol) and HBTU (0.67 g, 1.73 mmol) sequentially. Upon stirring at r.t. for 1 h, the slightly soluble tetracosanoic acid and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium (HBTU) turned the opaque solution to pale yellow. 3 mL of EtOAc and 1 mL of HCl (1 mol/L) were added sequentially. The resulting organic layer was collected and washed with

* E-mail: csyu@mx.nthu.edu.tw; Tel.: 00886-3-5715131(ext)35582; Fax: 00886-3-5718649
Received August 11, 2008; revised December 22, 2008; accepted July 26, 2009.
Project supported by the National Science Council of China at Taipei and CGMH_NTHU Joint Research (Nos. NSC-96-2113-M-007-028-MY2 and CGTH96N2342E1).

saturated $NaHCO_3$ (10 mL×2) and brine (10 mL). The aqueous layer was then extracted with CH_2Cl_2 (5 mL×3). The organic phase collected was treated with Na_2SO_4 and filtered through celite. After concentration under reduced pressure, the residue was chromatographed with eluent of EtOAc/*n*-hexane (1∶3, *V*∶*V*) to provide the white solid **4** in 83% yield (1.2 g). m.p. 52—53 ℃. Anal. $C_{56}H_{97}NO_4$, M_r=848.37, ESI+Q-TOF: calcd *M* 847.74 (100%); found M 847.9 (*m*/*z*), $[M+H]^+$=848.9 (*m*/*z*); ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 0.86 (t, *J*=7.0 Hz, 6H, CH_3), 1.23 (brs, 62H, 31CH_2), 1.30—1.72 (m, 6H, 3CH_2), 1.98—2.00 (m, 2H, H-2'), 2.15 (brs, 1H, OH), 3.59 (dd, J_{gem}=11.5, $J_{1a,2}$=4.5 Hz, 1H, H-1a), 3.66—3.70 (m, 2H, H-3, H-4), 3.97 (dd, J_{gem}=11.5, $J_{1b,2}$=3.5 Hz, 1H, H-1b), 4.11—4.14 (m, 1H, H-2), 4.43 (d, J_{gem}=12.0 Hz, 1H, OC**H**HPh), 4.59 (d, J_{gem}=11.5 Hz, 1H, OCH**H**Ph), 4.64 (d, J_{gem}=11.5 Hz, 1H, OC**H**HPh), 4.69 (d, J_{gem}=12.0 Hz, 1H, OCH**H**Ph), 6.03 (d, $J_{NH,2}$=8.0 Hz, 1H, NH), 7.24—7.37 (m, 10H, 2×Ph); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 14.10 (CH_3), 22.68 (CH_2), 25.64 (CH_2), 26.00 (CH_2), 29.30 (CH_2), 29.36 (CH_2), 29.52 (CH_2), 29.59 (CH_2), 29.69 (CH_2), 30.80 (CH_2), 31.90 (CH_2), 31.91 (CH_2), 36.69 (CH_2), 50.62 (CH, C-2), 62.95 (CH_2, C-1), 72.88 (CH_2, $CH_2C_6H_5$), 73.04 (CH_2, $CH_2C_6H_5$), 79.05 (CH, C-4), 82.23 (CH, C-3), 127.80 (CH, CH_2Ph), 127.95 (2CH, CH_2Ph), 128.09 (2CH, CH_2Ph), 128.16 (CH, CH_2Ph), 128.45 (2CH, CH_2Ph), 128.70 (2CH, CH_2Ph), 137.80 (C, CH_2Ph), 138.13 (C, CH_2Ph), 172.93 (CONH); ^{13}C NMR (125 MHz, C_6D_6) *δ*: 14.34 (CH_3), 23.09 (CH_2), 25.99 (CH_2), 26.55 (CH_2), 29.73 (CH_2), 29.86 (CH_2), 30.05 (CH_2), 30.10 (CH_2), 30.13 (CH_2), 30.16 (CH_2), 30.18 (CH_2), 30.21 (CH_2), 31.00 (CH_2), 32.31 (CH_2), 32.32 (CH_2), 36.59 (CH_2), 51.66 (CH, C-2), 63.07 (CH_2, C-1), 72.67 (CH_2, $CH_2C_6H_5$), 73.35 (CH_2, $CH_2C_6H_5$), 80.27 (CH, C-4), 81.41 (CH, C-3), 128.30 (CH, CH_2Ph), 128.64 (CH, CH_2Ph), 128.76 (CH, CH_2Ph), 138.88 (C, CH_2Ph), 139.05 (C, CH_2Ph), 172.23 (CONH).

4-((1*S*,2*R*)-1,2-Bis(benzyloxy)hexadecyl)-4,5-dihydro-2-tricosyloxazole (5)

A mixture of compound **4** (20 mg, 24 μmol) and pyridine (23 μL, 282 μmol) in CH_2Cl_2 (0.5 mL) was stirred at ambient temperature for 3 min. Upon cooling down to −78 ℃ during 10 min, the mixture turned white cloudy. An amount of 120 μL (equivalent to 71 μmol) was drawn from the solution of Tf_2O in CH_2Cl_2 (0.1 mL/0.9 mL, *V*/*V*) to add to the above mixture. After being stirred for 15 min, the mixture was concentrated under reduced pressure. The residue obtained was chromatographed using eluent [*V*(EtOAc)∶*V*(*n*-hexane)=1∶9] to provide the white soft solids **5** in 52% yield (12 mg). Further coevaporation with toluene afforded an oil. Anal. $C_{56}H_{95}NO_3$, M_r=830.36, ESI+Q-TOF: calcd *M*=829.73 (100%); found *M*=829.8 (*m*/*z*), $[M+H]^+$=830.8 (*m*/*z*); ^{1}H NMR (500 MHz, C_6D_6) *δ*: 0.91 (t, *J*=7.0 Hz, 6H, 2×CH_3), 1.32 (brs, 64H, 32×CH_2), 1.52—1.78 (m, 4H, 2×CH_2), 2.28 (t, *J*=7.0 Hz, 2H, H-1'), 3.72—3.75 (m, 1H, H-4), 3.87—3.91 (m, 1H, H-5a), 4.15 (dd, *J*=7.0, 8.5 Hz, 1H, H-5b), 4.38—4.46 (m, 2H, H-1", H-2"), 4.46 (d, J_{gem}=12.0 Hz, 1H, OC**H**HPh), 4.53 (d, J_{gem}=12.0 Hz, 1H, OCH**H**Ph), 4.72 (d, J_{gem}=12.0 Hz, 1H, OC**H**HPh), 4.79 (d, J_{gem}=12.0 Hz, 1H, OCH**H**Ph), 7.08—7.36 (m, 10H, 2×Ph); ^{13}C NMR (125 MHz, C_6D_6) *δ*: 14.33 (CH_3), 23.08 (CH_2), 26.21 (CH_2), 26.46 (CH_2), 28.41 (CH_2), 29.61 (CH_2), 29.76 (CH_2), 29.79 (CH_2), 29.95 (CH_2), 30.92 (CH_2), 30.12 (CH_2), 30.18 (CH_2), 30.26 (CH_2), 31.05 (CH_2), 32.31 (CH_2), 68.02 (CH, C-2), 69.41 (CH_2, C-1), 72.59 (CH_2, $CH_2C_6H_5$), 73.68 (CH_2, $CH_2C_6H_5$), 80.48 (CH, C-4), 82.68 (CH, C-3), 127.63 (CH, CH_2Ph), 127.81 (CH, CH_2Ph), 128.00 (CH, CH_2Ph), 128.19 (CH, CH_2Ph), 128.29 (CH, CH_2Ph), 128.51 (CH, CH_2Ph), 139.34 (C, CH_2Ph), 139.57 (C, CH_2Ph), 168.34 (C=N).

(2*S*,3*S*,4*R*)-3,4-Bis(benzyloxy)-2-(tetracosanamido)octadecyl formate (6)

To compound **4** (12 mg, 14 μmol) in CH_2Cl_2 (0.6 mL) was added pyridine (7 μL, 85 μmol) at r.t. Upon cooling down to −70 ℃ during 10 min, the mixture turned white cloudy. Tf_2O (8 μL, 42 μmol) was then added. After the stirring for 15 min, DMF (1 mL) and NaN_3 (3 mg, 42 μmol) were added sequentially. After concentration of the mixture under reduced pressure, the residue was chromatographed using eluent [*V*(EtOAc)∶*V*(*n*-hexane)=1∶9] to provide the white solids **6** in 36% yield (5 mg). m.p. 63—66 ℃. Anal. $C_{57}H_{97}NO_5$, M_r=876.38, ESI+Q-TOF: calcd. *M*=875.74 (100%), found *M*=875.8 (*m*/*z*), $[M+H]^+$=876.8 (*m*/*z*), $[M+Na]^+$=898.8, $[M+K]^+$=914.8 (*m*/*z*); ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 0.86 (t, *J*=7.0 Hz, 6H, 2×CH_3), 1.23 (brs, 64H, 32×CH_2), 1.45—1.68 (m, 4H, 2×CH_2), 1.92 (dt, *J*=2.5, 7.5 Hz, 2H, H-2'), 3.56—3.59 (m, 2H, H-3, H-4), 4.32—4.35 (m, 1H, H-2), 4.41—4.47 (m, 2H, H-1a, H-1b), 4.46 (d, J_{gem}=11.5 Hz, 1H, OC**H**HPh), 4.55 (d, J_{gem}=11.5 Hz, 1H, OCH**H**Ph), 4.59 (d, J_{gem}=11.5 Hz, 1H, OC**H**HPh), 4.71 (d, J_{gem}=11.5 Hz, 1H, OCH**H**Ph), 5.50 (d, $J_{NH,2}$=8 Hz, 1H, NH), 7.27—7.35 (m, 10H, 2×Ph), 7.97 (s, 1H, HCO).

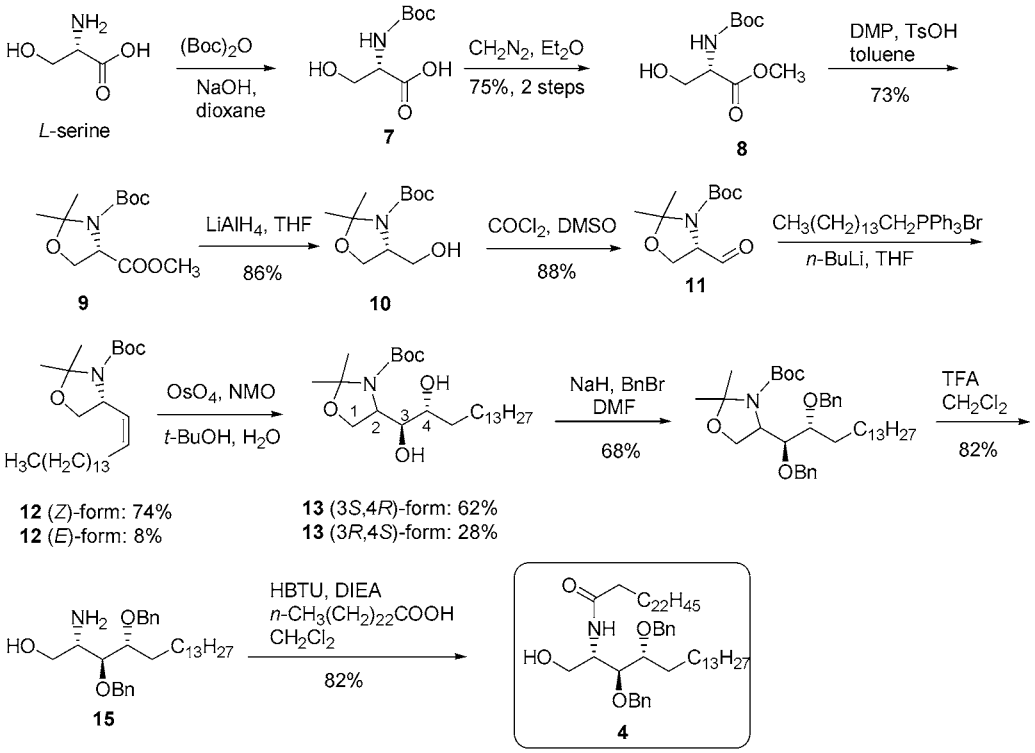
Results and discussion

Synthesis

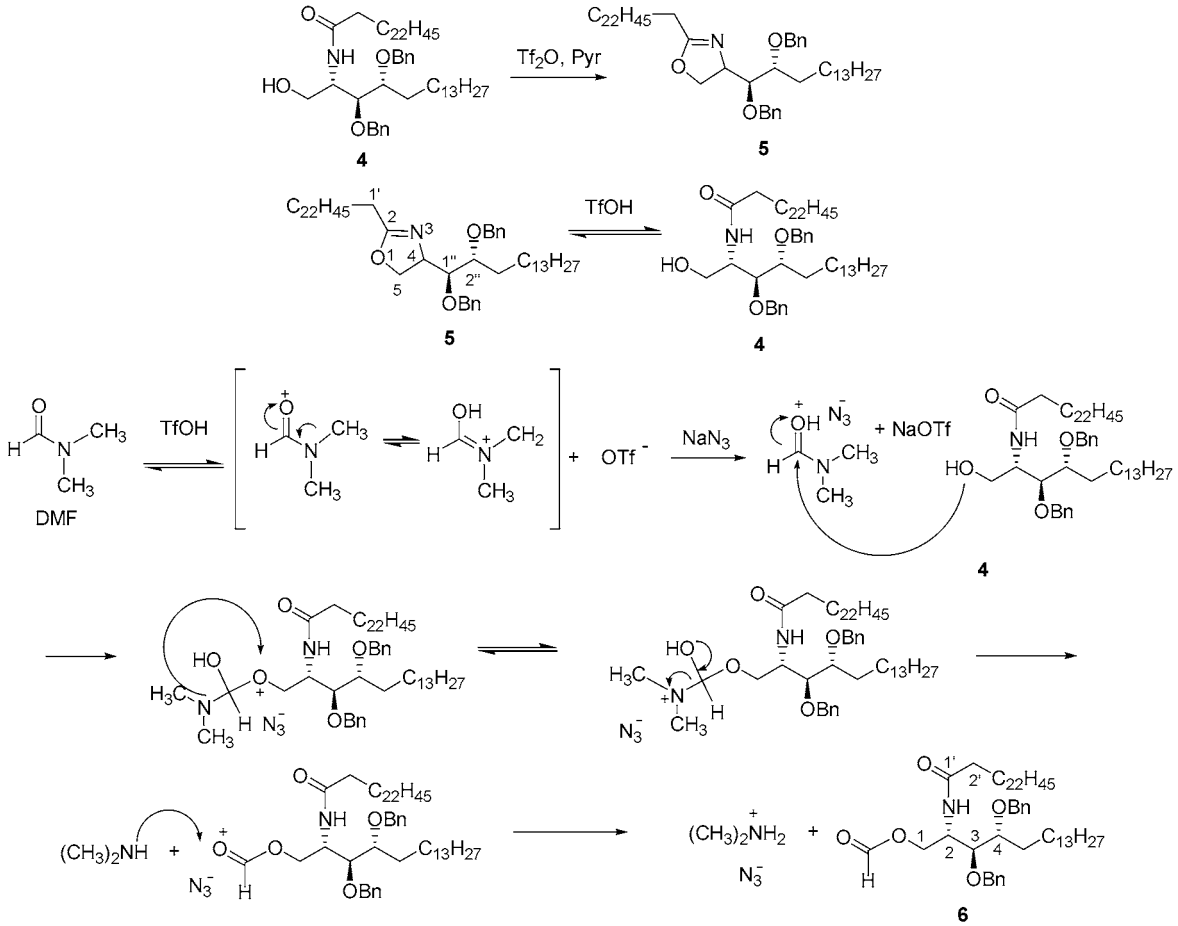
Whereas the preparation of an analog of **3**, devoid of 4-OH, starting from *γ*-butyrolacton via continuous Hofmann rearrangements and Crutius rearrangement has been reported,[6] a more straightforward pathway based on **4** would be favorable for our purpose (Scheme 2).[7]

Compound **4** was prepared from *L*-serine via Garner's aldehyde in a ten-step synthesis.[8] The triflic group[9] was introduced without encountering difficulty except the instability arising during column chromatography, even after a pretreatment with 5% triethylamine. Thus, the labile triflate needed to couple with azide through a one-pot approach (Scheme 3). As expected,

Scheme 2 Synthesis of the well-protected ceramide **4** from commercial *L*-serine



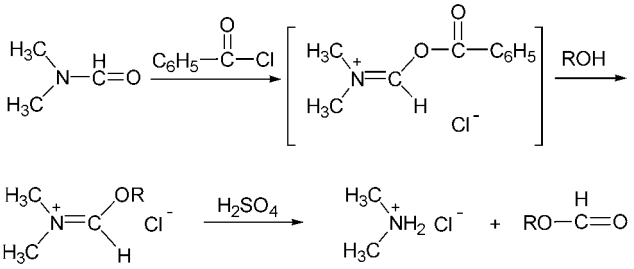
Scheme 3 Triflic-assisted formylation via rearrangement



the triflate formed was readily attacked by the imido group to provide the oxazole analog **5**. However, in a case of using 6 equiv. of pyridine, the by-product triflic acid was not completely quenched by the base. Protonation of **5** by the residual acid shifted the equilibrium from oxazole toward the starting ceramide **4**. In addition, the protonation also extended to the solvent DMF. The released triflic anion further combined with a sodium cation upon the addition of NaN_3. Hence, formation of the metastable salt of protonated DMF and azide was driven toward completion. Protonated oxo group made the carbonyl grop more susceptible to be attacked by the primary alcohol of compound **4**. After removal of the proton on the acidic carboxy group by the released secondary amine, the formyl-protected ceramide **6** was generated.

A similarity between the above findings and the work reported by Barluenga and coworkers is addressed,[10] in which the benzoyl chloride-dimethylformamide was generated for subsequent attack by alcohol (Scheme 4). It is interesting that both the halide Cl^- and pseudohalide N_3^- acted as the counter anions for stabilizing the ester-conjugated DMF and protonated DMF, respectively. The subsequent hydrolysis was required to afford the formyl group in their work. By contrast, the nucleophilic attack by our alcohol **4** onto the protonated DMF followed by a series of rearrangements seems straightforward. Whereas the rearrangement could be affected in the ceramide, a similar condition used for the formylation of benzyl alcohol could produce only marginal product observed by TLC. The neighboring amide group of the ceramide might participate in the rearrangement. The present findings need a further study for future application to the introduction of formyl group in other natural products.

Scheme 4 The reported formylation via an *in-situ* formation of benzoyl chloride-dimethylformamide



References

1 (a) Padron, J. M. *Curr. Med. Chem.* **2006**, *13*, 755.
(b) Segui, B.; Andrieu-Abadie, N.; Jaffrezou, J. P.; Benoist, H.; Levade, T. *BBA-Biomembranes* **2006**, *1758*, 2104.
(c) Gomez-Munoz, A. *FEBS Lett.* **2004**, *562*, 5.
(d) Crul, M.; Mathot, R. A. A.; Giaccone, G.; Punt, C. J. A.; Rosing, H.; Hillebrand, M. J. X.; Ando, Y.; Nishi, N.; Tanaka, H.; Schellens, J. H. M.; Beijnen, J. H. *Cancer Chem. Pharm.* **2002**, *49*, 287.
(e) Chi, L. M.; Hsieh, C. H.; Wu, W. G. *J. Chin. Chem. Soc.* **1992**, *35*, 39.
(f) Yeh, C. H.; Pan, S. D.; Chen, S. W.; Fu, Z. W.; Chiang, L. W.; Yu, C. S. *J. Chin. Chem. Soc.* **2007**, *54*, 1375.
(g) Ju, D. D.; Li, D. T.; Wei, G. J.; Her, G. R. *J. Chin. Chem. Soc.* **1994**, *41*, 323.

2 (a) Prigozy, T. I.; Naidenko, O.; Qasba, P.; Elewaut, D.; Brossay, L.; Khurana, A.; Natori, T.; Koezuka, Y.; Kulkarni, A.; Kronenberg, M. *Science* **2001**, *291*, 664.
(b) Kawano, T.; Cui, J. Q.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Motoki, K.; Ueno, H.; Nakagawa, R.; Sato, H.; Kondo, E.; Koseki, H.; Taniguchi, M. *Science* **1997**, *278*, 1626.

3 Chang, Y. T.; Choi, J.; Ding, S.; Prieschl, E. E.; Baumruker, T.; Lee, J. M.; Chung, S. K.; Schultz, P. G. *J. Am. Chem. Soc.* **2003**, *124*, 1856.

4 (a) Brik, A.; Wu, C. Y.; Wong, C. H. *Org. Biomol. Chem.* **2006**, *4*, 1446.
(b) Wu, C. Y.; Chang, C. F; Chen, J. S. Y.; Lee, S. T.; Wong, C. H.; Lin, C. H. *Angew. Chem., Int. Ed.* **2003**, *42*, 4661.
(c) Lee, L. V.; Mitchell, M. L.; Huang, S. J.; Fokin, V. V.; Sharpless, K. B.; Wong, C. H. *J. Am. Chem. Soc.* **2003**, *125*, 9588.
(d) Brik, A.; Lin, Y. C.; Elder, J.; Wong, C. H. *Chem. Biol.* **2002**, *9*, 891.
(e) Lee, S. G.; Chmielewski, J. *Chem. Biol.* **2006**, *13*, 421.
(f) Lo, J. M.; Chiang, L. W.; Chen, S. W.; Fu, Z. W.; Pei, K.; Yu, C. S. *Chim. Oggi-Chem. Today* **2007**, *25*, 46.
(g) Chiang, L. W.; Pei, K.; Chen, S. W.; Huang, H. L.; Lin, K. J.; Yen, T. C.; Yu, C. S. *Chem. Pharm. Bull.* **2009**, *57*, 714.

5 (a) Hakogi, T.; Monden, Y.; Taichi, M.; Iwama, S.; Fujii, S.; Ikeda, K.; Katsumura, S. *J. Org. Chem.* **2002**, *67*, 4839.
(b) Hakogi, T.; Fuji, S.; Morita, M.; Ikeda, K.; Katsumura, S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2141.

6 (a) Hakogi, T.; Monden, Y.; Iwama, S.; Katsumura, S. *Org. Lett.* **2000**, *2*, 2627.
(b) Hakogi, T.; Taichi, M.; Katsumura, S. *Org. Lett.* **2003**, *5*, 2801.

7 Yeh, C. H.; Pan, S. D.; Chen, S. W.; Fu, Z. W.; Chiang, L. W.; Yu, C. S. *J. Chin. Chem. Soc.* **2007**, *54*, 1375.

8 (a) Xia, C. F.; Yao, Q. J.; Schumann, J.; Rossy, E.; Chen, W. L.; Zhu, L. Z.; Zhang, W. P.; De-Libero, G.; Wang, P. G. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2195.
(b) Azuma, H.; Tamagaki, S.; Ogino, K. *J. Org. Chem.* **2000**, *65*, 3538.

9 Paquette, L. A. *Encyclopedia of Reagents for Organic Synthesis*, Wiley, New York, **1995**, pp. 5146—5152.

10 Barluenga, J.; Campos, P. J.; Gonzalez-NuÑez, E.; Asensio, G. *Synthesis* **1985**, 426.

(E0808111 Zhao, X.)