# A Synthesis of Sugar-modified *S*-Adenosyl-L-homocysteine (AdoHcy) Analogues as Inhibitors of AdoHcy Hydrolase

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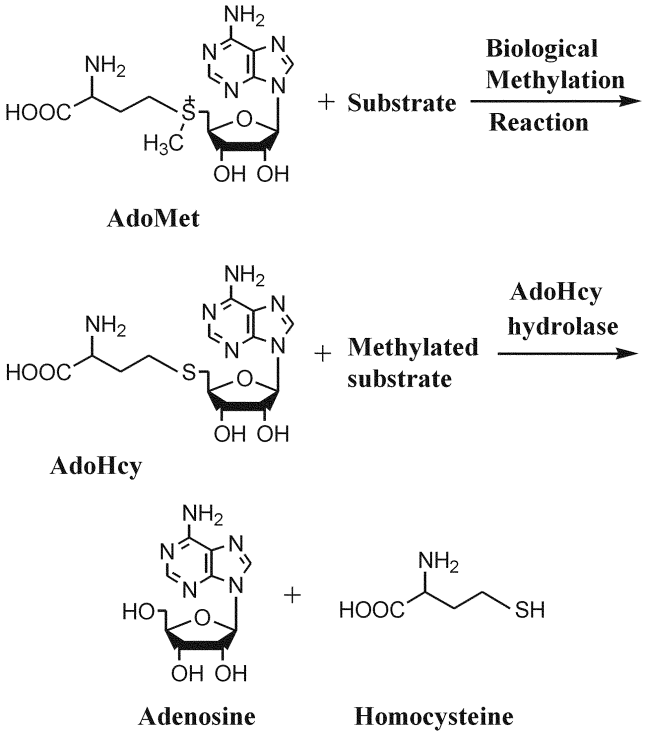
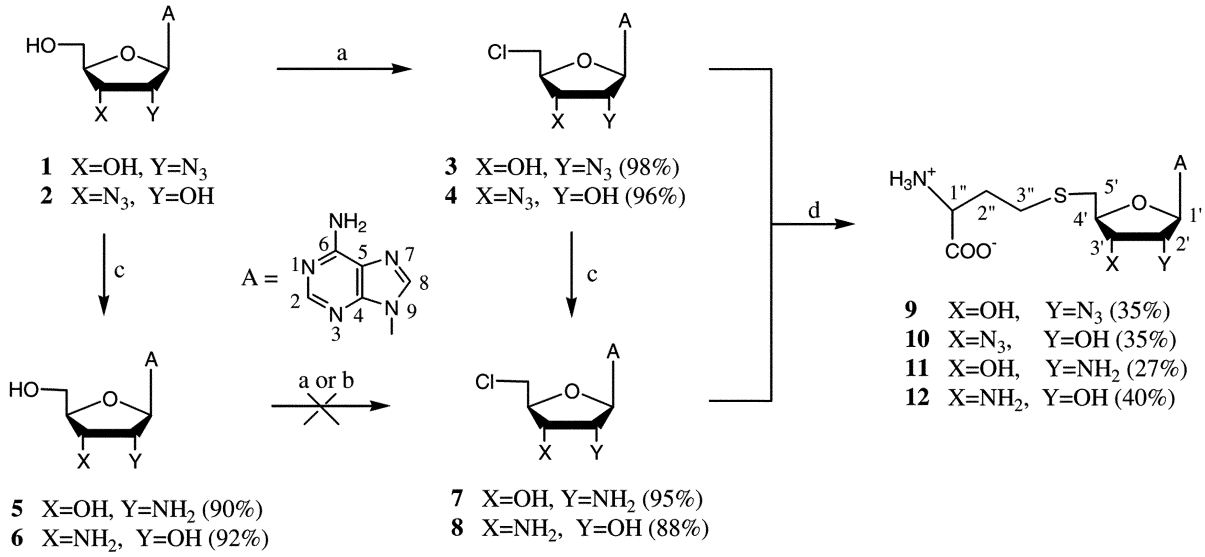
*S*-Adenosyl-L-methionine (AdoMet) serves as a methyl donor in a variety of biomethylation reactions, and the byproduct, *S*-adenosyl-L-homocysteine (AdoHcy), is a potent feedback inhibitor of AdoMet-dependent methyltransferase enzymes.[1] The AdoHcy is hydrolyzed to adenosine amd L-homocysteine by AdoHcy hydrolase in the cytoplasm[2] (Scheme 1). It is known that inhibition of AdoHcy hydrolase results in a rapid accumulation of intracellular AdoHcy and the consequent inhibition of transmethylation processes which are essential for viral multiplication[3] and cell division.[4] Therefore AdoHcy hydrolase has become an attractive target for the molecular design of broad-spectrum antiviral and anticancer agents.[5]

Based on the proposal by Palmer and Abeles,[6] major efforts to synthesize the inhibitors of AdoHcy hydrolase have focused on the mechanism-based nucleosides, mainly Neplanocine A or aristeromycin-derived nucleosides.[7] However, continuous attention has not been given to studies on the class of AdoHcy analogues and the evaluation of their activities, mainly due to the difficulty in their preparation. After X-ray crystallographic structure of the rat liver and human AdoHcy hydrolase was recently determined,[8] a few AdoHcy derivatives have been synthesized with the intention to prove the interactions of AdoHcy with residues at the binding site of AdoHcy hydrolase.[9] These synthetic efforts would thereby improve our understanding of this enzyme, which can consequently could provide important clues for the design of novel inhibitors.

Among the reported compounds structurally related to AdoHcy, the modification in AdoHcy molecules is mainly focused on the amino acid and/or base moieties.[10] However, there have been very few studies on the AdoHcy derivatives with variations in the sugar portion of AdoHcy.[11] So, it was interesting to us to synthesize sugar-modified AdoHcy analogues and to investigate the role of sugar portion of AdoHcy in the interaction with the enzyme and the consequent effect of functionalities in sugar moiety on the inhibitory activity. With the above point in mind, as one of several approaches to prepare AdoHcy derivatives having diverse functionality at 2' or 3'-position of sugar moiety, we designed azidosugar (and aminosugar) AdoHcy derivatives (**9**-**12**, Scheme 2) in which 2'- or 3'-hydroxy group in ribose moiety is replaced with a azido group and amino group. Since amino group is similar to hydroxy group in size and in hydrogen-bonding character, and azido group shows interesting biological activities when it is introduced to sugar moiety as shown in the example of AZT,[12] it is envisioned to be interesting to investigate the interaction of these functionalities with the active site of AdoHcy Hydrolase. The synthetic precursors, 2' (and 3')- azido (and amino)-2' (and 3')-deoxyadenosine derivatives (**1**, **2**, **5**, and **6**, Scheme 2) were recently reported to be prepared from adenosine by Hwang *et al*.[13] The compounds **1**, **2**, **5**, and **6** were converted to 5'-chloroadenosine analogues, **3**, **4**, **7**, and **8** by employing similar methodologies reported previously.[14,15] With those chlorinated compounds in hand, the AdoHcy analogues, **9**, **10**, **11**, and **12** were finally prepared *via* coupling with L-homocysteine sodium salt (Scheme 2). The synthetic details and the activity test against AdoHcy hydrolase will be discussed in this note.




**Scheme 1**




**Scheme 2**. (a) $SOCl_2$, HMPA, rt; (b) $SOCl_2$, MeCN/Py (1 : 1), rt; (c) Pd/C, $H_2$, MeOH, rt; (d) Homocysteine sodium salt, KI, $H_2O$, 100 °C.

## Experimental Section

Melting points were recorded on Electrothermal melting point apparatus and are uncorrected. Mass and NMR spectra were recorded on JEOL JMS-DX 303 (3 KV) and Jeol 400 MHz spectrometer, respectively. Thin layer chromatography was performed on pre-coated silica gel 60 F-254 (Merck). The L-homocyteine sodium salt was prepared via Birch reduction of L-methionine according to reported methodology.[16]

**Typical procedure for the preparation of chlorinated compounds (3, 4, 7, and 8).**[14] To a pre-cooled solution of thionyl chloride (0.33 mL, 4.51 mmol) in HMPA (4 mL) was added compound **1** (0.22 g, 0.75 mmol). The reaction mixture was stirred at ambient temperature for 1.5 h under Ar atmosphere, then quenched with water (3 mL). The mixture was applied to ion exchange column chromatography (Dowex 50W × 4-200, $H^+$ form). The column was washed with water and eluted with 2 N aqueous $NH_4OH$ (30 mL) followed by 2 N aqueous $NH_4OH$-MeOH (1 : 1, v/v). The chromatographically identical fractions were collected and extracted with EtOAc (100 mL × 2). The combined organic layer was dried over $MgSO_4$ and evaporated to give **3** (0.23 g, 98%) as a white solid. The compounds **7** and **8** were obtained through hydrogenation of compounds **3** and **4** ($H_2$ // Pd/C in MeOH). **3**: 98%; mp: 48-50 °C (soften); IR (KBr) $cm^{-1}$ 2119 (strong, -$N_3$); $^1$H-NMR (DMSO-d6) $\delta$8.34 (s, 1H, H8), 8.16 (s, 1H, H2), 7.37 (br, 2H, $NH_2$), 6.26 (d, 1H, *J* = 5.2 Hz, -OH, exch), 6.04 (d, 1H, *J* = 5.2 Hz, H1'), 4.90 (t, 1H, *J* = 5.2 Hz, H2'), 4.67 (dd, 1H, *J* = 5.2, 10.4 Hz, H3'), 4.15 (dd, 1H, *J* = 4.8, 10.4, H4'), 3.94 (dd, 1H, *J* = 4.8, 11.6 Hz, H5'a), 3.84 (dd, 1H, *J* = 6.4, 11.6, H5'b); $^{13}$C-NMR $\delta$ 156.16, 152.88, 149.13, 139.71, 119.13, 85.27, 83.83, 71.84, 63.46, 59.77; MS (FAB) $[M+H]^+$ 311. **4**: 96%; mp: 155-157 °C; IR (KBr) $cm^{-1}$ 2112 (strong, -$N_3$); $^1$H-NMR (DMSO-d6) $\delta$8.34 (s, 1H, H8), 8.16 (s, 1H, H2), 7.32 (br, 2H, $NH_2$), 6.36 (d, 1H, *J* = 6.0 Hz, -OH, exch), 5.94 (d, 1H, *J* = 6.0 Hz, H1'), 5.17 (q, 1H, *J* = 6.0 Hz, H2'), 4.39 (t, 1H, *J* = 4.8 Hz, H3'), 4.15 (q, 1H, *J* = 4.8, H4'), 3.97 (dd, 1H, *J* = 4.8, 11.6 Hz, H5'a), 3.92 (dd, 1H, *J* = 6.0, 11.6, H5'b); $^{13}$C-NMR $\delta$ 156.11, 152.75, 149.32, 139.77, 119.11, 87.46, 80.77, 73.07, 62.43, 44.50; MS (FAB) $[M+H]^+$ 311. **7**: 95%; mp: 221 °C (decomposed); $^1$H-NMR, DMSO-d6 $\delta$ 8.33 (s, 1H, H8), 8.14 (s, 1H, H2), 7.29 (br, 2H, $NH_2$), 5.73 (d, 1H, *J* = 7.6 Hz, H1'), 4.10 (m, 3H, H2',3',4'), 3.93 (dd, 1H, *J* = 6.4, 11.2 Hz, H5'a), 3.83 (dd, 1H, *J* = 6.4, 11.2 Hz, H4'); $^{13}$C-NMR $\delta$ 156.09, 152.63, 149.72, 140.01, 119.27, 88.28, 84.91, 72.07, 56.16, 44.65; MS (FAB) $[M+H]^+$ 285. **8**: 89%; mp: 187-190 °C (decomposed); $^1$H-NMR (DMSO-d6) $\delta$ 8.27 (s, 1H, H8), 8.15 (s, 1H, H2), 7.35 (b, 1H, -OH, exch), 7.30 (br, 2H, $NH_2$), 5.94 (d, 1H, *J* = 2.4 Hz, H1'), 4.41 (dd, 1H, *J* = 2.4, 5.6 Hz, H2'), 3.98 (dd, 1H, *J* = 2.4, 11.2 Hz, H5'a), 3.89 (m, 1H, H4'), 3.84 (dd, 1H, *J* = 6.0, 11.2 Hz, H5'b), 3.55 (dd, 1H, *J* = 5.6, 7.2, H4'); $^{13}$C-NMR $\delta$156.07, 152.69, 149.06, 139.32, 118.99, 88.97, 83.67, 74.18, 54.73, 48.59; MS (FAB) $[M+H]^+$ 285.

**Typical procedure for the synthesis of AdoHcy analogues (9-12).** AdoHcy analogues (**9**, **10**, **11**, and **12**) were prepared according to the modified method of Ramalingam and Woodard.[16a] Thus, L-Homocysteine sodium salt (104 mg, 0.58 mmol) and KI (30 mg) was added to a suspension of **3** (120 mg, 0.42 mmol) in a deoxygenated water (3 mL, boiled for 1 hour and immediately cooled), and the reaction temperature increased slowly to 100 °C. A clear solution was resulted at 80 °C. The mixture was stirred for 5 h under Ar atmosphere. The reaction was immediately cooled in ice bath and adjusted to pH 3.5 with 1 N HCl (aq). The whole mixture was applied to column (1 × 20 cm) of Dowex 50W × 4-200 ($NH_4^+$ form), and the column was washed with water thoroughly, eluted with 1N aqueous $NH_4OH$ at the flow rate of ~2.5 mL/10 min. The chromatographically identical fractions (BuOH-AcOH-$H_2O$ = 60 : 15 : 25, $R_f$ =

0.33) were collected, and then evaporated to give **9** (54 mg, 35%) as a pale yellow solid. **9**: 35%; $^1$H-NMR ($D_2O$) $\delta$8.25 (s, 1H, H8), 8.13 (s, 1H, H2), 5.94 (d, 1H, *J* = 5.2 Hz, H1'), 4.74 (m, 1H, H2'), 4.54 (m, 1H, H3'), 4.14 (m, 1H, H4'), 3.60 (m, 1H, H1''), 2.92 (dd, 1H, *J* = 4.8, 14.0 Hz, H5'a), 2.84 (dd, 1H, *J* = 6.4, 14.0 Hz, H5'b), 2.58 (m, 2H, H3''), 2.01, 1.92 (m, 2H, H2''); $^{13}$C-NMR $\delta$174.67, 156.00, 153.39, 149.02, 140.33, 119.22, 86.64, 85.33, 73.47, 65.65, 54.17, 33.73, 31.01, 28.41; MS (FAB) $[M+H]^+$ 410. **10**: 35%; $R_f$ = 0.38; $^1$H-NMR (DMSO-d6) $\delta$8.38 (s, 1H, H8), 8.15 (s, 1H, H2), 7.33 (br, 2H, -$NH_2$), 5.89 (d, 1H, *J* = 5.6 Hz, H1'), 5.18 (m, 1H, H2'), 4.30 (m, 1H, H3'), 4.03 (m, 1H, H4'), 3.41 (m, 1H, H1'', overlapped with water peak), 2.90 (m, 2H, H5'a, H5'b). 2.60 (m, 2H, H3''), 1.93, 1.73 (m, 2H, H2''); $^{13}$C-NMR $\delta$ 170.18, 156.10, 152.79, 149.40, 139.95, 119.12, 87.31, 80.92, 73.11, 63.88, 53.04, 33.85, 31.50, 28.19; MS (FAB) $[M+H]^+$ 410. **11**: 27%; $R_f$ = 0.09; $^1$H-NMR ($D_2O$) $\delta$8.14 (s, 1H, H8), 7.97 (s, 1H, H2), 5.72 (d, 1H, *J* = 7.6 Hz, H1'), 4.14 (m, 2H, H3',4'), 3.93 (m, 1H, H2'), 3.63 (t, 1H, *J* = 6.0 Hz, H1''), 2.81 (m, 2H, H5'), 2.51 (t, 2H, *J* = 7.6 Hz, H3''), 1.95 (m, 2H, H2''); $^{13}$C-NMR $\delta$174.87, 156.08, 153.41, 149.71, 140.66, 119.30, 88.02, 85.32, 73.85, 57.30, 54.36, 34.48, 31.12, 28.25; MS (FAB) $[M+H]^+$ 384. **12**: 40%; $R_f$ = 0.07; $^1$H-NMR ($D_2O$) $\delta$8.09 (s, 1H, H8), 7.99 (s, 1H, H2), 5.87 (d, 1H, *J* = 1.2 Hz, H1'), 4.65 (m, 1H, H2'), 4.02 (m, 1H, H3'), 3.59 (t, 1H, *J* = 6.0 Hz, H1''), 3.45 (m, 1H, H4'), 2.93 (dd, 1H, *J* = 3.6, 14.8 Hz, H5'a), 2.76 (dd, 1H, *J* = 6.4, 14.8 Hz, H5'b). 2.47 (m, 2H, H3''), 1.91 (m, 2H, H2''); $^{13}$C-NMR $\delta$ 175.03, 156.08, 153.37, 149.07, 140.29, 119.21, 89.50, 83.85, 7626, 55.74, 54.41, 34.18, 31.15, 28.57; MS (FAB) $[M+H]^+$ 384.

**Assay of AdoHcy hydrolase for synthetic direction.**[17] Recombinant human placental AdoHcy hydrolase purified to homogeneity was used in this study. The compounds were preincubated with the enzyme at various concentrations from 0.1 to 100 $\mu$M in 500 $\mu$L of 50 mM potassium phosphate buffer (pH 7.2) containing 1 mM EDTA (buffer A) at 37 °C for 5 min. To this mixture, 0.2 mM adenosine (5 $\mu$L) and 5 mM L-homocysteine (50 $\mu$L) were added, and the mixture was incubated for 5 min at the same temperature. The reaction was terminated by the addition of 25 $\mu$L of 5 N $HClO_4$. The terminated reaction mixture was kept in ice for 5 min and microcentrifuged. The supernatant was analyzed for AdoHcy by HPLC equipped C-18 reversed-phase column (Econosphere C18, 5 $\mu$m, 250 × 4.6 mm, Alltech, Deerfield, IL). The elution was carried out at a flow rate of 1 mL/min in two sequential linear gradients: 6-15% A over 15 min, 15-50% A over later 5 min, where mobile phase A was acetonitrile and B was 50 mM sodium phosphate buffer ($NaH_2PO_4/H_3PO_4$), pH 3.2. The peak of AdoHcy was monitored at 258 nm. The concentration was determined by the peak area of AdoHcy.

## Results and Discussion

The starting materials **1**, **2**, **5** and **6** were prepared according to the reported procedures developed by Hwang *et al*.[13] Now although the conversion of **1**, **2**, **5**, and **6** to their chlorinated compounds **3**, **4**, **7** and **8** were made eventually by employing the previously reported procedure,[14,15] some restriction about this reaction deserve some mention. Thus, treatment of compounds **1** and **2** with $SOCl_2$ in HMPA as solvent at ambient temperature for 1h provided the chlorinated compounds, **3** and **4** in over 96% yield. On the other hand, the chlorination attempts of **5** and **6** to prepare **7** and **8** under the same reaction condition for the preparation of **3** and **4** or the alternative reaction ($SOCl_2$/pyridine/$CH_3CN$),[15] were not successful, but only starting materials were recovered. This is presumably due to the base character of amino group in sugar moiety, which could scavenge chlorine source. However, the compounds **7** and **8** were obtained by adopting a different route, namely, through hydrogenation of compounds **3** and **4** ($H_2$ // Pd/C in MeOH) in over 90% yield. Finally, AdoHcy analogues (**9**, **10**, **11**, and **12**) were prepared in moderate yields *via* the coupling 5'-chloroadenosine analogues with L-homocysteine sodium salt according to the modified method of Ramalingam and Woodard.[16a] The optimal molar ratio of 5'-chloro derivatives and L-homocysteine sodium salt was 1 : 1.5, and the reaction mixture was refluxed in deoxygenated water for 4-5 hours. The product was isolated in moderate yield (27-40%) by a cation-exchange resin chromatography eluted with 1 N aqueous $NH_4OH$ at the very slow flow rate (~2.5 mL/10 min). The moderate yields seem to be caused by the unstable nature of the products, which decompose fairly rapid at over 90 °C. The reduction of reaction time showed no improvement in yield. This decomposition phenomenon is consistent with the results observed in the preparation of similar compound, *S*-adenosyl-L-methionine (AdoMet).[16] Now, those final AdoHcy derivatives **9**, **10**, **11**, and **12** were tested toward the residual activities of human recombinant AdoHcy Hydrolase using adenosine and L-homocysteine and their results are shown in Table 1. The preliminary assay of these AdoHcy derivatives (**9**-**12**) against AdoHcy hydrolase showed, however, no inhibitory effects at 100 mM, while the nucleosides (**1**, **2**, **5** and **6**) interestingly showed 5-15% of inhibitory activity at this concentration. Among the nucleosides compound **5** showed the potential inhibitory activity and so the further modifications of **5** in sugar or base are in progress.

In conclusion, we could synthesize the sugar-modified AdoHcy derivatives as a possible AdoHcy hydrolase inhibitor in moderate yields *via* coupling 5'-chloroadenosine

**Table 1**. Inhibitory activities of compounds against human AdoHcy hydrolase (% enzyme activity at 100 mM)

| Nucleosides | Inhibition | AdoHcy Analogues | Inhibition |
|---|---|---|---|
| **1** | 5 | **9** | ND[a] |
| **2** | 5 | **10** | ND[a] |
| **5** | 15 | **11** | ND[a] |
| **6** | ND[a] | **12** | ND[a] |

[a]No inhibitory activity showed at 100 mM.

analogues with L-homocysteine sodium salt. However, their inhibitory activity against AdoHcy hydrolase were not shown at the concentration of 100 mM.

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## References

1. (a) Narayan, P.; Rottman, F. M. In *Advances in Enzymology*; Meister, A., Ed.; John Wiley Inc.: New York, 1994; pp 255-285. (b) Barber, J. R.; Clarke, S. *J. Biol. Chem.* **1984**, *259*, 7115. (c) Schatz, R. A.; Wilens, T. E.; Sellinger, O. Z. *Biochem. Biophys. Res. Commun.* **1981**, *98*, 1097.
2. De la Haba, G.; Cantoni, G. L. *J. Biol. Chem.* **1959**, *234*, 603.
3. (a) Hasobe, M.; McKee, J. G.; Borchardt, R. T. *Antimicrob. Agents Chemother.* **1989**, *33*, 828. (b) Ransohoff, R. M.; Narayan, P.; Ayers, D. F.; Rottman, F. M.; Nilsen, T. W. *Antiviral Res.* **1987**, *7*, 317. (c) Bray, M.; Driscoll, J.; Huggins, J. W. *Antiviral Res.* **2000**, *45*, 135.
4. (a) Wnuk, S. F.; Yuan, C.-S.; Borchardt, R. T.; Balzarini, J.; De Clercq, E.; Robins M. J. *J. Med. Chem.* **1997**, *40*, 1608. (b) De Clercq, E. *Nucleosides Nucleotides* **1998**, *17*, 625.
5. (a) Yuan, C.-S.; Liu, S.; Wnuk, S. F.; Robins, M. J.; Borchardt, R. T. *Design and Synthesis of S-Adenosylhomocysteine Hydrolase, Inhibitors as Broad-Spectrum Antiviral Agents* in *Advances in Antiviral Drug Design*; De Clercq, E., Ed.; JAI Press: Geenwich, C.T., 1996; Vol. 2, pp 41-88. (b) Liu, S.; Wolfe, M. S.; Borchardt, R. T. *Antiviral Res.* **1992**, *19*, 247. (c) Wolfe, M. S.; Borchardt, R. T. *J. Med. Chem.* **1991**, *34*, 1521.
6. Palmer, J. L.; Abeles, R. H. *J. Biol. Chem.* **1979**, *254*, 1217.
7. Chiang, P. K. *Pharmacol. Ther.* **1998**, *77*, 115 and references therein.
8. (a) Turner, M. A.; Yuan, C.-S.; Borchardt, R. T.; Herhfield, M. S.; Smith, G. D.; Howell, P. L. *Nature Struct. Biol.* **1998**, *5*, 369. (b) Hu, Y.; Komoto, J.; Huang, Y.; Gomi, T.; Ogawa, H.; Takata, Y.; Fujioka, M.; Takusagawa, F. *Biochemistry* **1999**, *38*, 8323.
9. (a) Miles, R. W.; Nielsen, L. P. C.; Ewing, G. J.; Yin, D.; Borchardt, R. T.; Robins, M. J. *J. Org. Chem.* **2002**, *67*, 8258. (b) Steere, J. A.; Sampson, P. B.; Honek, J. F. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 457.
10. (a) Borchardt, R. T.; Huber, J. A.; Wu, Y. S. *J. Org. Chem.* **1976**, *41*, 565. (b) Ramalingam, K.; Woodard, R. W. *J. Org. Chem.* **1984**, *49*, 1291. (c) Chiang, P. K.; Cantoni, G. L.; Bader, J. P.; Shannon, W. M.; Tomas, J.; Montgomery, J. A. *Biochem. Biophys. Res. Commun.* **1978**, *82*, 417.
11. (a) Chiang, P. K.; Richards, H. H.; Cantoni, G. L. *Mol. Pharmacol.* **1977**, *13*, 939. (b) Johnson, C. R.; Esker, J. L.; Van Zandt, M. C. *J. Org. Chem.* **1994**, *59*, 5854.
12. Chen, B.-C.; Quinlan, S. L.; Reid, J. G. *Tetrahedron Lett.* **1995**, *36*, 7961.
13. Kim, B. T.; Kim, S. K.; Lee, S. J.; Hwang, K. J. *Bull. Korean Chem. Soc.* **2004**, *25*, 243.
14. Kikugawa, K.; Ichino, M. *Terahedron Lett.* **1971**, 87.
15. Robins, M. J.; Hansske, F.; Wnuk, S. F.; Kanai, T. *Can. J. Chem.* **1991**, *69*, 1468.
16. (a) Ramalingam, K.; Woodard, R. W. *Terahedron Lett.* **1985**, *26*, 1135. (b) Matos, J. R.; Raushel, F. M.; Wong, C.-H. *Biotechnol. Appl. Biochem.* **1987**, *9*, 39.
17. Jeong, L. S.; Yoo, S. J.; Lee, K. M.; Koo, M. J.; Choi, W. J.; Kim, H. O.; Moon, H. R.; Lee, M. Y.; Park, J. G.; Lee, S. K.; Chun, M. W. *J. Med. Chem.* **2003**, *46*, 201.