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Synthesis and Biological activities of Oseltamivir phosphate (Tamiflu), Oseltamivir and Its Derivatives

Muhammad Niyomdecha[1*2#] and Yongsak Sritana-anant[1*]

1. *Organic Synthesis Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
2. #Chemistry Department, Silpakorn University, Nakorn Pathom 73000, Thailand

* E-mail of the corresponding author: momoniyom@yahoo.com

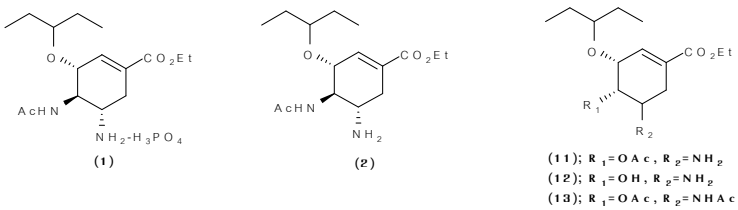
Abstract

Oseltamivir phosphate or Tamiflu™ **1**, oseltamivir **2** and three new oseltamivir derivatives **11-13** were synthesized starting from (-)-shikimic acid **3**. The title compounds were evaluated for their anti-Herpes simplex virus type-1, anti-alzheimer**,** anti-tyrosinase activity and cytotoxicity. Compound **12** exhibited inhibitory acitivity of HSV-1, AChE, tyrosinase and revealed cytotoxicity against small-cell lung cancer (NCI-H187) cells and Vero cells (African green monkey kidney).

Keywords: shikimic acid, epoxide ring opening, influenza, oseltamivir, oseltamivir phosphate, Tamiflu, anti-Herpes simplex virus type-1, anti-alzheimer**,** anti-tyrosinase activity and cytotoxicity.

1. Introduction

Oseltamivir phosphate or Tamiflu™ **1** is a potent and selective inhibitor of neuraminidase enzyme of influenza type A and B virus [1-5]. To the present date many syntheses of oseltamivir phosphate **1** have been documented [6-8, 10-16, 21-23]. While working for development for better synthetic route for oseltamivir phosphate, we also interested in evaluation of other biological activity of oseltamivir phosphate and its derivatives. Due to availability of a simple, rapid, and sensitive screening methods, oseltamivir phosphate **1** and its derivatives **2**, **11-13** (**scheme 1**) were evaluated for their anti-Herpes simplex virus type-1, anti-alzheimer, anti-tyrosinase activity and cytotoxicity[9].



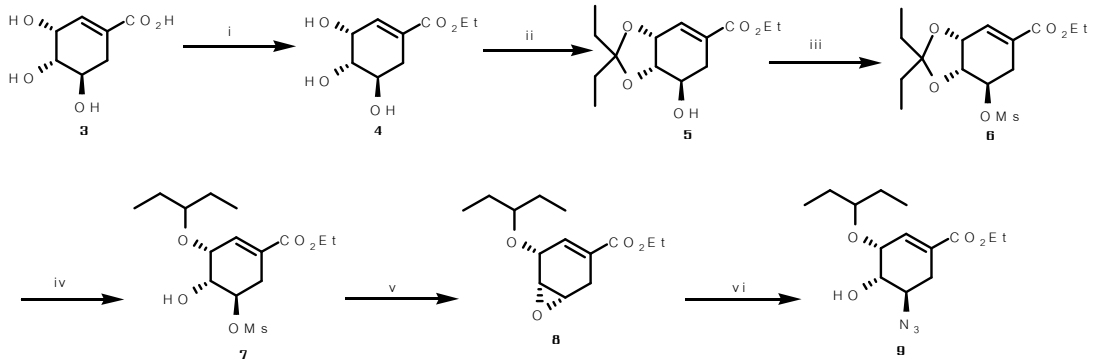
Scheme 1. Oseltamivir phosphate **1** oseltamivir **2** and oseltamivir derivatives **11-13**.

2. Materials and methods

The following analytical methods were employed unless otherwise indicated. Melting points were determined on a Stuart Scientific Melting Point apparatus (Bibby Sterlin Ltd., UK) and are uncorrected. FT-IR spectra were recorded on a Perkin-Elmer FT-IR Spectrum RXI spectrometer (Perkin Elmer Instruments LLC., USA). Solutions of samples in dichloromethane or ethyl acetate were dropped onto a potassium bromide crystal cell. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Varian Mercury NMR spectrometer operated at 400.00 MHz for ^{1}H and 100.00 MHz for ^{13}C (Varian Company, USA) using samples dissolved in $CDCl_3$ or D_2O. Mass spectra were recorded on a Waters Micromass Quatto micro API ESCi (Waters, USA).

3. Results and discussions

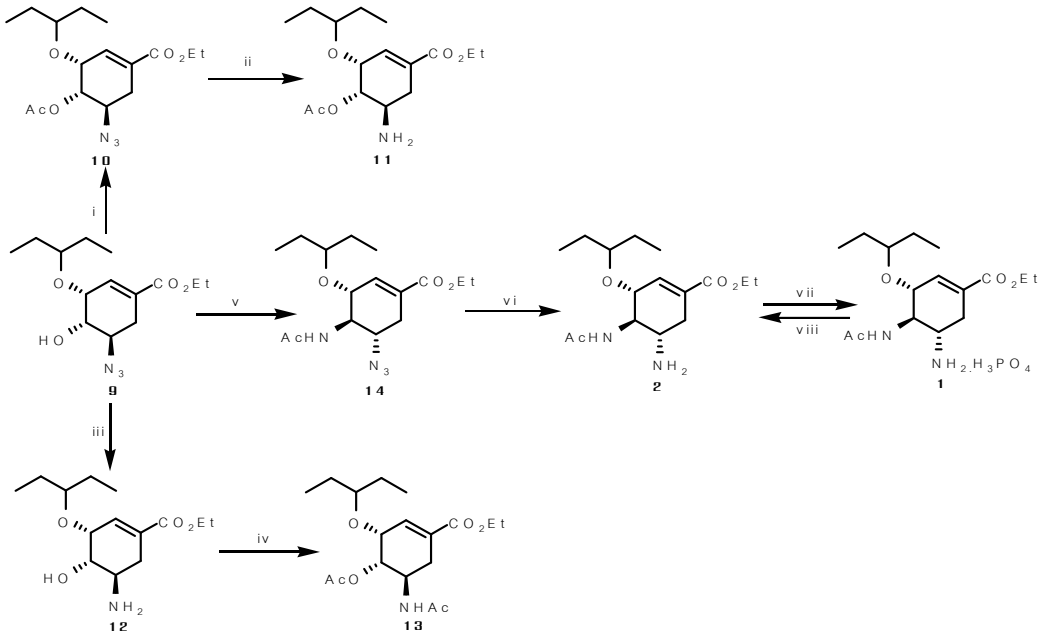
The key intermediate 5-azido-4-hydroxy-3-pentyloxy **9**, required for synthesis of oseltamivir phosphate **1**, its derivatives **2** and **11-13**, was synthesized from commercially available (-) shikimic acid **3** by modification of known methods [6-8] as shown in **Scheme 2**.



Reagents: i:$SOCl_2$, EtOH, heated to reflux, 3.0 h, ii: 3-pentanone, TfOH, rt, 3.0 h, iii: MsCl, Et_3N, EtOAc, rt, 6.0 h, iv: Et_3SiH, $AlCl_3$, CH_2Cl_2, 0 ^oC, 5.0 h, v: aq.$NaHCO_3$, EtOH-H_2O, 60 ^oC, 3.0 h, vi: NaN_3, NH_4Cl, EtOH, 70-75 ^oC, 18.0 h.
Scheme 2. Synthesis of intermediate azide **9**.

Esterification of (-)-shikimic acid **3** with ethanol and thionyl chloride gave ethyl shikimate **4** in quantitative yield. Protection of *cis*-dihydroxyl group with 3-pentanone in the presence of trifluoromethansulfonic acid afforded pentylidene ketal **5** in 83% yield. The hydroxyl group in **5** was protected using methanesulfonyl chloride and triethylamine to provide the ketal **6** in 89% yield. The resulting ketal was regioselectively reduced to give the hydroxyl ether **7** in 75% yield by modification of known method [Et_3SiH and $TiCl_4$ in CH_2Cl_2 at -32 ^oC to 0 ^oC]. Much cheaper and more easily handled $AlCl_3$ was used in place of $TiCl_4$ and the reaction was carreid out at 0 ^oC. The epoxide **8** was obtained in quantitative yield from reaction of **7** with aq. $NaHCO_3$ in EtOH/H_2O, at 60 ^oC. Ring opening of the epoxide **8** with NaN_3, NH_4Cl in EtOH-H_2O at 70-75 ^oC gave the key intermediate hydroxyl azide **9** in 55 % yield.

Acetylation of the hydroxyl azide **9** with acetyl chloride in pyridine, followed by Ph_3P reduction of the acetylated azide provided new oseltamivir derivative **11** in 70 yield after purification by column chromatography. The oseltamivir derivative **12** was obtained in 82% yield after purified by column chromatography from Ph_3P reduction of the azido group of **9**. The new diacetylated compound **13** was obtained in 91% yield from acetylations of **12** with AcCl and pyridine. To obtain the acethylated azide **14**, the azide **9** was reduced with Ph_3P followed by ring opening of *in situ* formed aziridine with NaN_3-NH_4Cl. The resulting amino azide was then acetylated with Ac_2O and Et_3N to furnish the azide **14** in 36 %yield after column chromatography. Reduction of azide **14** with Ph_3P in CH_3CN-H_2O followed by acidification with H_3PO_4 gave oseltamivir phosphate **1** 55% yield (2 steps). Oseltamivir phosphate **1** was neutralized by shaking with saturated $NaHCO_3$ provide the free base of oseltamivir **2** in quantitative yield. (**Scheme 3)**. The structures of oseltamivir and its derivatives were confirmed by their spectroscopic and analytical data.



Reagents: i: AcCl, pyridine, CH_2Cl_2, reflux, 3.0 h, ii: Ph_3P, CH_3CN-H_2O, rt, 3.0 h, iii: Ph_3P, CH_3CN-H_2O, rt, 3.0 h, iv: AcCl, pyridine, CH_2Cl_2, reflux, 3.0 h, v: a. Ph_3P, DMF, rt, 6.0 h, b. NaN_3, NH_4Cl, DMF, 70-75 ^oC, 18-20 h, c. Ac_2O, Et_3N, CH_2Cl_2, rt, 2.0 h, vi: Ph_3P, CH_3CN-H_2O, rt, 3.0 h, vii: H_3PO_4, EtOH, rt, viii: sat.$NaHCO_3$, CH_2Cl_2, 5 min.
Scheme 3. Conversion of the azide **9** to oseltamivir phosphate **1**, oseltamivir **2** and its derivatives **11-13**.

4. Biological Activity Screening

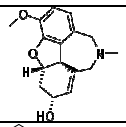
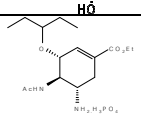
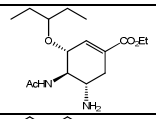
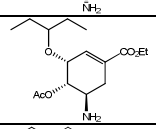
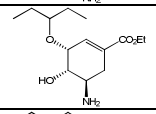
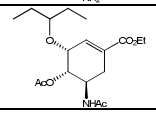
4.1 *Anti-Herpes simplex virus type 1 (anti-HSV-1)*

Antiviral activity was determined by using the green fluorescent protein (GFP)-based assay [24]. Herpes simplex virus type 1 (HSV-1) was maintained in a Vero cell line (kidney fibroblasts of an African green monkey), which was cultured in Eagle's minimum essential medium (MEM) with the addition of 10% heat inactivated fetal bovine serum (FBS) and antibiotics. Acyclovir was used as the reference compound. None of Oseltamivir and its derivatives showed HSV-1inhibition.

4.2 *Anti-alzheimer activity*

Oseltamivir and its derivatives were tested for their acetylcholinesterase inhibitory activity. Acetylcholinesterase inhibition was determined by modifying the method of Ellman [25] using acetylthiocholine as substrate. Galanthamine was used as a reference standard. Every experiment was done in triplicate. Among the test compounds, it is interesting to note that compound **12** exhibited potent anti-alzheimer activity (IC_{50} value of 91.14±2.36 µM) with 56.99 % when compare to Galantamine 100.98 %).The results shown in **table 1**.

Table 1: Acetylcholinesterase Inhibitory Activity of oseltamivir phosphate **1**, oseltamivir **2** and its derivatives **11-13**.

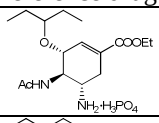
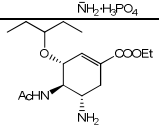
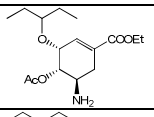
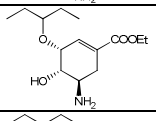
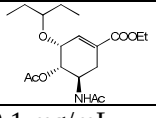
Compounds	Structure	Anti-Acetylcholinesterase Activity	
		%Inhibition	IC_{50} (μM)
Galantamine[a]		**100.98**	**1.45±0.04**
1		20.59	Inactive[b]
2		14.43	Inactive[b]
11		8.49	Inactive[b]
12		**56.99**	**91.14±2.36**
13		11.92	Inactive[b]

[a] Reference Drug; [b] Inactive at 0.1 mg/mL.

4.3 *Anti-tyrosinase Activity*

Tyrosinase-inhibition activity was determined by the modified dopachrome method using L-DOPA as a substrate [26-27]. The IC_{50} defined as the concentration of inhibitors required to inhibit tyrosinase activity by 50%, was determined. Kojic acid was used as a reference standard. Every experiment was done in triplicate. Inbitory activity evaluation of oseltamivir phosphate **1**, oseltamivir **2** and its derivatives **11-13** indicated that compound **12** is more potent than compound **11**, however the degree of inhibition is not as good as the reference compound. The results are illustrated in **table 2**.

Table 2: Anti-tyrosinase Activity of oseltamivir phosphate **1**, oseltamivir **2** and its derivatives **11-13**.

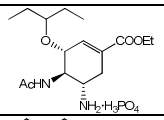
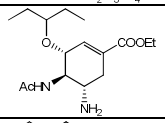
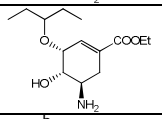
Compounds	Structure	Anti-tyrosinase Activity	
		% Inhibition	IC_{50} (μM)
Kojic acid[a]	reference drug	84.06	**80.30±0.56**
1		0.66	Inactive[b]
2		3.29	Inactive[b]
11		**58.55**	**297.25±4.79**
12		**67.09**	**145.22±1.35**
13		1.32	Inactive[b]

[a] Reference Drug; [b] Inactive at 0.1 mg/mL.

4.4 *Cytotoxicity*

Oseltamivir phosphate **1**, oseltamivir **2** and compound **12** were tested for cytotoxicity. The cytotoxicity assays against small-cell lung cancer (NCI-H187) cells, were performed using the resazurin microplate assay, which was modified for mammalian cell cytotoxicity [28] and the cytotoxicity assays against Vero cells (African green monkey kidney), were performed using the green fluorescent protein (GFP)-based assay [24]. Ellipticine was included as the reference drugs. Compound **12** showed cytotoxicity (IC_{50} value of 25.81 and 33.65 µg/mL respectively) the results showed in **table 3**.

Table 3: Cytotoxicity of oseltamivir phosphate **1**, oseltamivir **2** and its derivatives **12**.

Compounds	Structure	Cytotoxicity IC_{50} (µg/mL)	
		Vero cells[a]	NCI-H187[b]
Ellipticine[e]	reference drug	0.558	0.714
1		Non-toxic[d]	Inactive[c]
2		Non-toxic[d]	Inactive[c]
12		**25.81**	**33.65**

[a] African green monkey kidney cells; [b] Small cell lung cancer cell;

[c] Inactive at 50 µg/mL; [d] Non-cytotoxic at 50 µg/mL; [e] Reference Drugs.

5. Conclusion

Oseltamivir phosphate **1**, oseltamivir **2** and three new oseltamivir derivatives **11-13** were synthesized in this work, all in high yield. The synthezised compounds were evaluated for potential inhibitors of HSV-1, AChE and tyrosinase. The cytotoxicity assays against small-cell lung cancer (NCI-H187) cells and Vero cells (African green monkey kidney) of compounds **1**, **2** and **12** were also performed. Among the test compounds, the derivative **12** shows AChE and tyrosinase inhibition and also cytotoxicity. It could be noted that the 4-hydroxyl group on the core structure of oseltamivir is important for the bioactivities.

References and Notes

[1] Kim, C. U.; Lew, W.; Williams, M. A.; Liu, H.; Zhang, L.; Swaminathan, S.; Bischofberger, N.; Chen, M. S.; Mendel, D. B.; Tai, C. Y.; Laver, W. G.; Stevens, R. C. *J. Am. Chem. Soc.* 1997, *119*, 681.
[2] Kim, C. U.; Lew, W.; Williams, M. A.; Wu, H.; Zhang, L.; Chen, X.; Escarpe, P. A.; Mendel, D. B.; Laver, W. G.; Stevens, R. C. *J. Med. Chem.* 1998, *41*, 2451.
[3] Neumann, G.; Noda, T.; Kawaoka, Y. *Nature* 2009, *459*, 931.
[4] Hurt, A. C.; Selleck, P.; Komadina, N.; Shaw, R.; Brown, L.; Barr, I. G. *Antiviral Res.* 2007, *73*, 228.
[5] Bloom, J. D.; Gong, L. I.; Baltimore, D. *Science* 2010, *328*, 1272.
[6] Rohloff, J. C.; Kent, K. M.; Postich, M. J.; Becker, M. W.; Chapman, H. H.; Kelly, D. E.; Lew, W.; Louie, M. S.; McGee, L. R.; Prisbe, E. J.; Schultze, L. M.; Yu, R. H.; Zhang, L. *J. Org. Chem.* 1998, *63*, 4545.
[7] Kent, K. M.; Kim, C. U.; McGee, L. R.; Munger, J. D., Jr.; Prisbe, E. J.; Postich, M. J.; Rohloff, J. C.; St. John, D. E.; Williams, M. A.; Zhang, L. US Patent 5859284, January 12, 1999.
[8] Andraos, J. *Org. Process Res. Dev*. 2009, *13*, 161.
[9] Reddy, D. *J. Antimicrob. Chemother*. 2010, *65*, ii35.
[10] Magano, J. *Chem. Rev.* 2009, *109*, 4398.

[11] Magano, J. *Tetrahedron* 2011, *67*, 7875.
[12] Shibasaki, M.; Kanai, M.; Yamatsugu, K. *Isr. J. Chem.* 2011, *51*, 316.
[13] Karpf, M.; Trussardi, R. *J. Org. Chem.* 2001, *66*, 2044.
[14] Harrington, P. J.; Brown, J. D.; Foderado, T.; Hughes, R. C. *Org. Process Res. Dev*. 2004, *8*, 86.
[15] Nie, L.-D.; Shi, X.-X.; Quan, N.; Wang, F.-F.; Lu, X. *Tetrahedron: Asymmetry* 2011, *22*, 1692.
[16] Kim, H.-W.; Park, K.-J. J. *Tetrahedron Lett.* 2012, *53*, 1561.
[17] Gabel, R. A.; Groaning, M. D.; Johnston, D. A. WIPO patent WO 2007/074091, 5 July, 2007.
[18] Abrecht, S.; Karpf, M.; Trussardi, R.; Wirz, B. US Patent 6518048, February 11, 2003.
[19] Federspiel, M.; Fischer, R.; Henning, M. *Org. Process Res. Dev.* 1999, *3*, 266.
[20] Other oseltamivir syntheses used multi-steps procedures for the introduction of diamino groups, including a patent specifically describing the process: Karpf, M.; Trussardi, R. US Patent 6939986 B2, September 6, 2005.
[21] Ko, J. S.; Keum, J. E.; Ko, S. Y. *J. Org. Chem.* 2010, *75*, 7006.
[22] Nie, L.-D.; Shi, X.-X. *Tetrahedron: Asymmetry* 2009, *20*, 124.
[23] Nie, L.-D.; Shi, X.-X.; Ko, K. H.; Lu, W.-D. *J. Org. Chem.* 2009, *74*, 3970.
[24] Hunt, L., Jordan, M. Jesus, M. D. and Wurm, F. M. *Biotechnol. Bioeng.* 1999, 65, 201-205.
[25] Ellman G.L.; Courtney, D.; Andres, Featherstone, R.M. *Biochem. Pharmacol.* 1961, *7*, 88-95.
[26] Pomerants, S. H. *J. Biol. Chem.* 1963, 238, 2351-2357.
[27] Piao, L. Z., Park, H. R., Park, Y. K., Lee, S. K., Park, J. H. and Park, M. K. 2002, *Chem. Pharm. Bull.* 50, 309-311.
[28] Brien, J. O., Wilson, I., Orton, T. and Pognan, F. 2000, *Eur. J. Biochem.* 267, 5421-5426.

Experimental Section

Synthesis of ethyl (3*R*,4*S*,5*R*)-3,4,5-trihydroxy-1-cyclohexene-1-carboxylate (ethyl shikimate) 4

Thionyl chloride (0.42 g, 5.75 mmol) was added dropwise over to the stirring and ice-cooled solution of (-)-shikimic acid **3** (2.00 g, 11.50 mmol) in ethanol (10 mL) for 15 min. The reaction was refluxed for 3.0 h, then cooled to room temperature and concentrated in vacuo to give the brown oil of ethyl shikimate **4** (3.50 g, quantitative yield), R_f on TLC chromatogram = 0.125 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 1.20 (t, *J*=6.2 Hz, 3H, -C$\underline{H}_3$), 2.11(m, 1H, -C$\underline{H}_2$-), 2.75 (m, 1H, -C$\underline{H}_2$-), 3.61 (t, *J*=6.3 Hz, 1H, -C$\underline{H}$-OH), 3.96 (br-s, 1H, -C$\underline{H}$-OH), 4.10 (m, 2H, -C$\underline{H}_2$-CH_3), 4.35 (br-s, 1H, -C$\underline{H}$-OH), 5.48 (br-s, -O$\underline{H}$), 6.73 (m, 1H, -C$\underline{H}$=C-); ^{13}C NMR ($CDCl_3$) (δ, ppm): 14.1 (-$CH_2\underline{C}H_3$), 18.1 (-$\underline{C}H_2$-), 31.9 (-$\underline{C}$H-OH), 61.2 (-$\underline{C}H_2CH_3$), 66.1 (-$\underline{C}$H-OH), 66.7 (-$\underline{C}$H-OH), 130.6 (-$\underline{C}$H=C-), 136.0 (-CH=$\underline{C}$-), 166.7 (-$\underline{C}$=O); IR (neat, cm^{-1}): 3360 (-OH), 2910 (-C=C-H), 1701 (C=O), 1380, 1253 (C=C), 1081(C-O).

Synthesis of ethyl (3a*R*,7*R*,7a*S*)-2,2-diethyl-7-hydroxy-3a,6,7,7a-tetrahydrobenzo[1,3]dioxole-5-carboxylate (ethyl 3,4-*O*-isopentylidene-5-hydroxy shikimate) 5

Trifluoromethane sulfonic acid (0.70 mL, 0.74 mmol) was added dropwise with syringe to the stirring and ice-cooled solution of (-)-ethyl shikimate **4** (3.00 g, 14.85 mmol) in 3-pentanone (50 mL). After stirring for 3.0 h at room temperature, unreacted 3-pentanone was distilled off with hexane as azeotropic 2:1 mixture to give the brown oil, which was redissolved in CH_2Cl_2 (25 mL), washed with water (2x25 mL), saturated $NaHCO_3$ solution (25 mL), and dried over anhydrous Na_2SO_4. The filtered solution was then concentrated in vacuo to provide the brown oil **5** (2.24 g, 83 %), R_f on TLC chromatogram = 0.50 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.88 (t, *J*=7.0 Hz, 3H, -C($CH_2C\underline{H}_3$)$_2$), 0.92 (t, *J*=7.8 Hz, 3H, -C($CH_2C\underline{H}_3$)$_2$), 1.30 (t, *J*=7.0 Hz, 3H, -$CH_2C\underline{H}_3$), 1.74 (m, 4H, -C($C\underline{H}_2CH_3$)$_2$), 2.24 (dd, J_1=8.6 Hz, J_2=17.2 Hz, 1H, -C$\underline{H}_2$-), 2.78 (dd, J_1=4.9 Hz, 1H, -C$\underline{H}_2$-), 3.91(m, 1H, -C$\underline{H}$-OH), 4.11 (t, *J*=7.0 Hz, 1H, -C$\underline{H}$-O-), 4.22 (q, *J*=14.9, 2H, -C$\underline{H}_2CH_3$), 4.76 (m, 1H, -C$\underline{H}$-O-), 6.93 (m, 1H, -C$\underline{H}$=C-); ^{13}C NMR ($CDCl_3$) (δ, ppm): 7.8 (-C($CH_2\underline{C}H_3$)$_2$, 8.5 (-C($CH_2\underline{C}H_3$)$_2$, 14.1 (-$CH_2\underline{C}H_3$), 29.0 (-$\underline{C}H_2$-), 29.2 (-C($\underline{C}H_2CH_3$)$_2$, 29.6 (-C($\underline{C}H_2CH_3$)$_2$, 61.0 (-$\underline{C}H_2CH_3$), 68.6 (-$\underline{C}$H-OH), 72.2 (-$\underline{C}$H-O-), 77.6 (-$\underline{C}$H-O-), 113.5 (-$\underline{C}$(CH_2CH_3)$_2$, 130.2 (-CH=$\underline{C}$-), 134.1 (-$\underline{C}$H=C-), 166.2 (-$\underline{C}$=O); IR (neat, cm^{-1}): 3468 (-OH), 2976, 2932 (-C=C-H), 1712, 1650 (C=O), 1460, 1246 (C=C), 1067 (C-O).

Synthesis of ethyl (3a*R*,7*R*,7a*R*)-2,2-diethyl-7-methanesulphonyl-3a,6,7,7a-tetrahydrobenzo[1,3]dioxole-5-carboxylate (ethyl 3,4-*O*-isopentylidene-5-methanesulphonyl-shikimate) 6

Methanesulfonylchloride (0.86 mL, 11.11 mmol) was added dropwise to the stirring solution of **5** (2.00 g. 7.40 mmol) in EtOAc (10 mL). The reaction was stirred for 15 minutes and then added Et_3N (2.00 mL, 14.8 mmol). After stirring at room temperature for 6.0 hours, the solution was filtered and washed with H_2O (2x10 mL), with 1 M $NaHCO_3$ (2x10 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to give the brown oil **6** (2.30 g, 89% yield), R_f on TLC chromatogram = 0.65 (50% ethyl acetate:hexane). ^{1}H NMR

($CDCl_3$) (δ, ppm): 0.83 (t, *J*=5.5 Hz, 3H, (-C(CH_2CH_3)$_2$), 0.85 (t, *J*=7.0 Hz, 3H, (-C(CH_2CH_3)$_2$), 1.24 (t, *J*=7.0 Hz, 3H, (-CH_2CH_3)), 1.62 (m, 4H, (-C(C$H_2$$CH_3$)$_2$), 2.43 (dd, J_1=8.6 Hz, J_2=17.2 Hz, 1H, -CH_2-), 2.91 (dd, J_1=4.7 Hz, J_2=17.2, 1H, -CH_2-), 3.05(s, 3H, -OMs), 4.16 (q, *J*=7.0 Hz, 2H, (-C$H_2$$CH_3$)), 4.25 (t, *J*=7.0, 1H, (-CH-O-)), 4.75 (m, 2H, (-CH-O-), -CH-OMs), 6.90 (m, 1H, -CH=C-); ^{13}C NMR ($CDCl_3$) (δ, ppm): 7.8 (-C(CH_2CH_3)$_2$, 8.6 (-C(CH_2CH_3)$_2$, 14.2 (-CH_2CH_3), 27.9 (-CH_2-), 28.9 (-C(C$H_2$$CH_3$)$_2$, 29.6 (-C(C$H_2$$CH_3$)$_2$, 38.7 (-O-$SO_2$-O-C$H_3$), 61.2 (-CH-OH), 72.3 (-CH-O-), 75.0 (-CH-O-), 79.1 (-CH-O-), 114.4 (-C($CH_2$$CH_3$)$_2$, 129.3 (-CH=C-), 134.0 (-CH=C-), 165.3 (-C=O).

Synthesis of ethyl (3*R*,4*R*,5*R*)-3-(1-ethyl-propoxy)-4-hydroxy-5-methanesulfonyloxy-1-cyclohexene-1-carboxylate 7

Compound **6** (2.00 g, 5.75 mmol) in CH_2Cl_2 (5 mL) was added to the stirring, ice-cooled mixture of $AlCl_3$ (0.92 g, 6.90 mmol) in CH_2Cl_2 (30 mL) followed by an addition of Et_3SiH (1.37 mL, 8.62 mmol). The reaction was left at 0 ^oC for 5.0 h and then quenched by pouring into iced water. The organic layer was separated and washed with aqueous $NaHCO_3$, dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The obtained brown oil was purified by silica gel column chromatography, eluting with 10% ethyl acetate–hexane to provide the ethyl 4-hydroxy-5-methansulfonyl-3-pentylideneketal-1-cyclohexene-1-carboxylate **7** (1.50 g, 75%), R_f on TLC chromatogram = 0.60 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.86 (t, *J*=7.0 Hz, 3H, (-C(CH_2CH_3)$_2$), 0.90 (t, *J*=7.8 Hz, 3H, (-C(CH_2CH_3)$_2$), 1.27 (t, *J*=7.0 Hz, 3H, (-CH_2CH_3)), 1.52 (m, 4H, (-C(C$H_2$$CH_3$)$_2$), 2.49 (dd, J_1=6.2 Hz, J_2=18.3 Hz, 1H, -CH_2-), 2.97 (dd, J_1=5.5 Hz, J_2=17.9 Hz, 1H, -CH_2-), 3.08(s, 3H, -OMs), 3.41(quint, *J*=5.5 Hz, 1H, (-CH($CH_2$$CH_3$)$_2$), 3.91(m, 1H, -CH-O-), 4.19 (m, 2H, (-C$H_2$$CH_3$)), 4.94 (m, 2H, -CH-OMs, -CH-OH), 6.82 (m, 1H, -CH=C-); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.4 (-C(CH_2CH_3)$_2$, 9.6 (-C(CH_2CH_3)$_2$, 14.2 (-CH_2CH_3), 26.1 (-C(C$H_2$$CH_3$)$_2$, 26.4 (-C(C$H_2$$CH_3$)$_2$, 29.3 (-C$H_2$-), 38.7 (-O-$SO_2$-O-C$H_3$), 61.2 (-CH-OH), 68.6 (-CH-OH), 70.0 (-CH-O-), 71.2 (-CH-OMs), 82.1 (-CH($CH_2$$CH_3$)$_2$, 129.2 (-CH=C-), 135.0 (-CH=C-), 165.7 (-C=O).

Synthesis of ethyl (3*R*,4*R*,5*S*)-4,5-epoxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 8

A mixture of the brown oil of ethyl 5-mesyl-4-hydroxy-5-pentylidene ketal compound **7** (1.00 g, 2.86 mmol), EtOH (20 mL) and 7.5% $NaHCO_3$ solution, was heated at 60 ^oC for 3.0 hours. The reaction mixture was extracted with n-hexane (4x20 mL), washed with water (20 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to light yellow oil. The residue was purified by recrystalization with hexane at 0 ^oC to give the white crystalline solid **8** (0.70 g, 96.4%), R_f on TLC chromatogram = 0.63 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.94 (t, *J*=7.8 Hz, 3H, (-C(CH_2CH_3)$_2$), 0.96 (t, *J*=7.8 Hz, 3H, (-C(CH_2CH_3)$_2$), 1.26 (t, *J*=7.0 Hz, 3H, (-CH_2CH_3)), 1.57 (m, 4H, (-C(C$H_2$$CH_3$)$_2$)), 2.40 (dd, J_1=6.2 Hz, J_2=19.5 Hz, 1H, -CH_2-), 3.04 (d, *J*=19.5 Hz, 1H, -CH_2-), 3.46 (m, 3H, 2-CH-O-, (-CH($CH_2$$CH_3$)$_2$), 4.17 (m, 2H, (-C$H_2$$CH_3$)), 4.36 (m, 1H, -CH-O-), 6.69 (m, 1H, (-CH=C-)); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.6 (2x-C(CH_2CH_3)$_2$), 14.2 (-CH_2CH_3), 24.5 (-CH_2-), 26.5 (2x-C(C$H_2$$CH_3$)$_2$, 50.7 (-CH-O-), 53.4 (-CH-O-), 60.8 (-C$H_2$$CH_3$), 71.3 (-CH-O-), 81.6 (-CH($CH_2$$CH_3$)$_2$, 126.9 (-CH=C-), 135.5 (-CH=C-), 166.1 (-C=O).

Synthesis of ethyl (3*R*,4*S*,5*R*)-5-azido-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 9

A solution of epoxide **8** (0.78 g, 3.07 mmol) in EtOH (3 mL) was added dropwise to the mixture of sodium azide (0.40 g, 6.14 mmol), ammonium chloride (0.329 g, 6.14 mmol), water (2 mL) and EtOH (10 mL). The reaction mixture was heated at 70 ^0C for 18 hours. The residue was extracted with EtOAc (20 mL), washed with sodium bicarbonate (10 mL), water (2x10 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The resulting brown oil was purified by column chromatography on silica gel, eluting with 10% ethyl acetate–hexane to provide the ethyl 5-azido-4-hydroxy-3-pentylidene ketal compound **9** (0.84 g, 92.72 %), R_f on TLC chromatogram = 0.75 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.88 (t, *J*=7.8 Hz, 3H, (-C(CH_2CH_3)$_2$), 0.92 (t, *J*=7.8 Hz, 3H, (-C(CH_2CH_3)$_2$)), 1.28 (t, *J*=7.0 Hz, 3H, (-CH_2CH_3)), 1.54 (m, 4H, (-C(C$H_2$$CH_3$)$_2$), 2.24 (dd, J_1=7.0 Hz, J_2=18.7 Hz, 1H, -CH_2-), 2.74 (br-s, 1H, -OH), 2.87 (dd, J_1=5.5 Hz, J_2=17.9 Hz, 1H, -CH_2-), 3.42 (quint, *J*=5.5 Hz, 1H, (-CH($CH_2$$CH_3$)$_2$)), 3.74 (m, 1H, -CH-$N_3$), 3.85 (q, *J*=7.0, 1H, -CH-OH), 4.11 (m, 1H, -CH-O-), 4.20 (q, *J*=7.0 Hz, 2H, (-C$H_2$$CH_3$)), 6.82 (m, 1H, (-CH=C-)); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.6 (2x-C(CH_2CH_3)$_2$), 14.2 (-CH_2CH_3), 26.0 (-C(C$H_2$$CH_3$)$_2$), 26.5 (-C(C$H_2$$CH_3$)$_2$), 28.2 (-C$H_2$-), 58.8 (-CH-$N_3$), 61.0 (-C$H_2$$CH_3$), 70.3 (-CH-OH), 71.0 (-CH-O-), 81.8 (-CH($CH_2$$CH_3$)$_2$)), 130.3 (-CH=C-), 135.0 (-CH=C-), 165.9 (-C=O).

Synthesis of ethyl (3*R*,4*S*,5*R*)-5-azido-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 10

A mixture of compound **9** (0.20 g, 0.67 mmol), acetyl chloride (2 mL) and pyridine (0.5 mL) was refluxed for 3.0 hours. The reaction mixture was extracted into CH_2Cl_2 (5 mL) and dried the organic layer over anhydrous sodium sulfate, filtered and concentrated in vacuo to give the brown oil of **10** (0.25 g, quantitative yield), R_f on

TLC chromatogram = 0.71 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.81 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 0.87 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 1.23 (t, *J*=7.0 Hz, 3H , (-$CH_2C\underline{H}_3$)), 1.44 (m, 4H, (-C($C\underline{H}_2CH_3)_2$)), 2.09 (s, 3H, -C(O)C$\underline{H}_3$), 2.18 (dd, J_1=4.4 Hz, J_2=20.0 Hz, 1H, -C$\underline{H}_2$-), 2.82 (dd, J_1=5.5 Hz, J_2=18.7 Hz, 1H, -C$\underline{H}_2$-), 3.22 (quint, *J*=6.2 Hz, 1H, (-C$\underline{H}$($CH_2CH_3)_2$)), 4.00 (q, *J*=9.3 Hz, 1H, -C$\underline{H}$-N_3), 4.15 (q, *J*=7.0 Hz, 2H, (-$C\underline{H}_2CH_3$)), 4.20 (m, 1H, C$\underline{H}$-O-), 4.84 (dd, J_1=4.0 Hz, J_2=9.4 Hz, 1H, -C$\underline{H}$-OAc), 6.78 (m, 1H, -C$\underline{H}$=C-); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.1 (-C($CH_2\underline{C}H_3)_2$), 9.8 (-C($CH_2\underline{C}H_3)_2$), 14.2 (-$CH_2\underline{C}H_3$), 21.1 (-(CO)-$\underline{C}H_3$), 24.2 (2x-C($CH_2\underline{C}H_3)_2$), 29.6 (-C$\underline{H}_2$-), 55.6 (-$\underline{C}$H-N_3), 61.0 (-$\underline{C}H_2CH_3$), 69.3 (-$\underline{C}$H-O-), 73.2 (-$\underline{C}$H-O(C=O)-CH_3), 83.0 (-$\underline{C}$H($CH_2CH_3)_2$)), 129.6 (-CH=$\underline{C}$-), 135.2 (-$\underline{C}$H=C-), 165.6 (-CH-O($\underline{C}$=O)-CH_3), 170.5 (-$\underline{C}$=O).

Synthesis of ethyl (3*R*,4*S*,5*R*)-5-amino-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 11

Compound **10** (0.20 g, 0.588 mmol) in CH_3CN (1 mL) was added dropwise over to the stirring and ice–cooled solution of triphenyl phosphine (0.30 g, 0.882 mmol) in CH_3CN-H_2O (5:1) (6 mL) and stirred the mixture for 15 minutes, and then at room temperature for 3.0 hours. The reaction mixture was evaporated to CH_3CN and then added EtOAc (10 mL). The mixture was washed with water and saturated sodium chloride solution, dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by column chromatography through siliga gel using MeOH–EtOAc (1:9) as eluent to give a yellow oil compound **11** (0.14 g, 70%), R_f on TLC chromatogram = 0.10 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.87 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 0.94 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 1.28 (t, *J*=7.0 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.47-1.60 (m, 4H, (-C($C\underline{H}_2CH_3)_2$)), 2.02 (s, 3H, -C(O)C$\underline{H}_3$), 2.06 (d, *J*=8.58 Hz, 1H, (-C$\underline{H}_2$-), 3.04 (dd, J_1=4.7 Hz, J_2=18.0 Hz, 1H, -C$\underline{H}_2$-), 3.44 (quint, *J*=5.5 Hz, 1H, (-C$\underline{H}$($CH_2CH_3)_2$), 3.63 (m, 1H, -C$\underline{H}$-NH_2), 4.06 (m, 1H, (-C$\underline{H}$-O(C=O)-CH_3)), 4.17-4.24 (m, 3H, (-$C\underline{H}_2CH_3$), -C$\underline{H}$-OAc), 5.80 (m, 1H, (-N$\underline{H}$-(C=O)-CH_3)), 6.88 (m, 1H, -C$\underline{H}$=C-); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.1 (-C($CH_2\underline{C}H_3)_2$), 9.8 (-C($CH_2\underline{C}H_3)_2$), 14.2 (-$CH_2\underline{C}H_3$), 21.1 (-(CO)-$\underline{C}H_3$), 24.2 (2x-C($CH_2\underline{C}H_3)_2$), 29.6 (-C$\underline{H}_2$-), 55.6 (-$\underline{C}$H-N_3), 61.0 (-$\underline{C}H_2CH_3$), 69.3 (-$\underline{C}$H-O-), 73.2 (-$\underline{C}$H-O(C=O)-CH_3), 83.0 (-$\underline{C}$H($CH_2CH_3)_2$)), 129.6 (-CH=$\underline{C}$-), 135.2 (-$\underline{C}$H=C-), 165.6 (-CH-O($\underline{C}$=O)-CH_3), 170.5 (-$\underline{C}$=O).

Synthesis of ethyl (3*R*,4*S*,5*R*)-5-amino-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 12

Compound **9** (0.10 g, 0.337mmol) in CH_3CN (1 mL) was added dropwise over to the stirring and ice–cooled solution of triphenyl phosphine (0.11g, 0.404 mmol) in CH_3CN-H_2O (3:1) (4 mL) and stirred the mixture for 15 minutes, and then at room temperature for 3 hours, the reaction mixture was remove CH_3CN to give the aqueous solution and then EtOAc (5 mL) was added, the mixture was washed with water and saturated sodium chloride solution, dried over anhydrous sodium sulfate. Filtered and concentrated in vacuo and the residue was purified by column chromatography through siliga gel using MeOH–EtOAc (1:9) as eluent to give a yellow oil compound **12** (0.75 g, 82%), R_f on TLC chromatogram = 0.28 (9:1 ethyl acetate:MeOH). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.78 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 0.82 (t, *J*=7.8 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 1.20 (t, *J*=7.0 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.44 (m, 4H, (-C($C\underline{H}_2CH_3)_2$), 2.37 (m, 1H, (-C$\underline{H}_2$-)), 3.01 (dd, J_1=4.7 Hz, J_2=17.9 Hz, 1H, (-C$\underline{H}_2$-)), 3.37 (quint, *J*=4.5 Hz, 1H, (-C$\underline{H}$($CH_2CH_3)_2$), 3.46 (m, 1H, (-C$\underline{H}$-NH_2)), 3.87 (m, 1H, (-C$\underline{H}$-OH)), 4.05 (m, 1H, (-C$\underline{H}$-O-)), 4.12 (q, *J*=7.0 Hz, 2H, (-$C\underline{H}_2CH_3$)), 6.82 (m, 1H, (-C$\underline{H}$=C-)); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.6 (2x-C($CH_2\underline{C}H_3)_2$), 14.2 (-$CH_2\underline{C}H_3$), 26.0 (2x-C($\underline{C}H_2CH_3)_2$), 26.5 (-$\underline{C}H_2$-), 48.5 (-$\underline{C}$H-NH_2), 61.2 (-$\underline{C}H_2CH_3$), 65.0 (-$\underline{C}$H-OH), 66.0 (-$\underline{C}$H-OH), 82.0 (-$\underline{C}$H($CH_2CH_3)_2$)), 131.0 (-$\underline{C}$H=C-), 135.0 (-$\underline{C}$H=C-), 166.0 (-$\underline{C}$=O), MS (EI) $[M+H]^+$ = 272.367.

Synthesis of ethyl (3*R*,4*S*,5*R*)-5-acetamido-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 13

A mixture of compound **12** (0.05 g, 0.184 mmol), acytyl chloride (2 mL) and pyridine (0.5 mL) was refluxed for 3.0 hours. The reaction mixture was extracted into CH_2Cl_2 (5 mL), and dried the organic layer over anhydrous sodium sulfate, filtered and concentrated in vacuo to give the brown oil **13** (0.60 g, 91%), R_f on TLC chromatogram = 0.70 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.87 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 0.93 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 1.27 (t, *J*=7.8 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.50 (quint, *J*=7.0 Hz, 4H, (-C($C\underline{H}_2CH_3)_2$), 1.94 (s, 3H, (-C(O)C$\underline{H}_3$)), 2.10 (s, 3H, (-C(O)C$\underline{H}_3$)), 2.10 (m, 1H, (-C$\underline{H}_2$-)), 3.00 (dd, J_1=5.5 Hz, J_2=18.3 Hz, 1H, (-C$\underline{H}_2$-)), 3.33 (quint, *J*=5.5 Hz, 1H, (-C$\underline{H}$($CH_2CH_3)_2$), 4.13 (m, 1H, (-C$\underline{H}$-O-)), 4.18(q, *J*=6.2 Hz, 2H, (-$C\underline{H}_2CH_3$)), 4.57 (quint, *J*=7.0 Hz, 1H, (-C$\underline{H}$-NHAc)), 4.95 (dd, J_1=3.1 Hz, J_2=11.3 Hz, 1H, (-C$\underline{H}$-OAc)), 5.68(d, *J*=9.4 Hz, 1H, (-N$\underline{H}$-(C=O)-CH_3)), 6.83 (m, 1H, (-C$\underline{H}$=C-)); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.3 (-C($CH_2\underline{C}H_3)_2$), 10.0 (-C($CH_2\underline{C}H_3)_2$), 14.2 (-$CH_2\underline{C}H_3$), 21.3 (-O-(CO)-$\underline{C}H_3$), 23.5 (-NH-(CO)-$\underline{C}H_3$), 26.5 (2x-C($CH_2\underline{C}H_3)_2$), 31.4 (-C$\underline{H}_2$-), 44.9 (-$\underline{C}$H-O-), 61.0 (-$\underline{C}H_2CH_3$), 70.0 (-$\underline{C}$H-O-(C=O)-CH_3), 72.5 (-$\underline{C}$H-NH-(C=O)-CH_3), 83.0 (-$\underline{C}$H($CH_2CH_3)_2$), 130.8 (-CH=$\underline{C}$-), 135.0 (-$\underline{C}$H=C-), 165.9 (-$\underline{C}$=O), 169.8 (-CH-O-($\underline{C}$=O)-CH_3), 171.7 (-CH-NH-($\underline{C}$=O)-CH_3), MS (EI) $[M+H+Na]^+$ = 378.50.

Synthesis of ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-azido-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 14

A solution of ethyl-5-azido-4-hydroxy-3-pentylidene ketal compound **9** (1.70 g. 5.70 mol) in DMF (5 mL) was added dropwise to the triphenylphosphin (1.70 g. 5.70 mol) in CH_3CN (2 mL). The mixture was heated at reflux for 6.0 hours, then concentrated in vacuo to dark brown oil and then the solution of crude in DMF (2 mL) was added dropwise to the mixture of sodium azide (1.70 g. 5.70 mol), ammonium chloride (1.70 g. 5.70 mol) in DMF (2 mL), the reaction mixture was heated at 80 ^{0}C for 18.0 hours, acetic anhydride (2 mL) and triethylamine (2 mL) in CH_2Cl_2 (5 mL) were added to the reaction and then refluxed for 3.0 hours. The reaction mixture was extracted with CH_2Cl_2 (10 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to give the brown oil, and purified by column chromatography on silica gel, eluting with 10% ethyl acetate–hexane to provide the acetamido azide compound **14** (0.691 g, 36%), R_f on TLC chromatogram = 0.70 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.92 (t, *J*=7.3 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 0.93 (t, *J*=7.3 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 1.32 (t, *J*=7.1 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.47-1.59 (m, 4H, (-C($C\underline{H}_2CH_3)_2$), 2.06 (s, 3H, (-C(O)$C\underline{H}_3$)), 2.10-2.31 (m, 1H, (-$C\underline{H}_2$-)), 2.88 (dd, J_1=5.7 Hz, J_2=17.1 Hz, 1H, (-$C\underline{H}_2$-)), 3.40 (m, 2H, (-$C\underline{H}(CH_2CH_3)_2$, (-$C\underline{H}$-O-)), 4.23 (q, *J*=7.1 Hz, 2H, (-$C\underline{H}_2CH_3$)), 4.27-4.34 (m, 1H, (-$C\underline{H}$-N_3)), 4.57-4.60 (m, 1H, (-$C\underline{H}$-NHAc)), 6.01 (d, *J*=7.4 Hz, 1H, (-N$\underline{H}$-(C=O)-CH_3)), 6.81 (dd, J_1=2.2 Hz, J_2=2.3 Hz, 1H, (-$C\underline{H}$=C-)).

Synthesis of ethyl (3*R*,4*R*,5*S*)-4-*N*-acetamido-5-amino-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate phosphate (oseltamivir phosphate) 1

A solution of azido compound **14** (0.69 g. 2.04 mmol) in CH_3CN (5 mL) was added dropwise to the triphenylphosphine (0.532 g, 2.04 mmol) in CH_3CN-H_2O (3:1) (12 mL) and stirring was continued for 15 minutes. After the reaction mixture was stirred at the room temperature for 3.0 hours, the reaction mixture was evaporated to CH_3CN and then added EtOAc (10 mL). The mixture was washed with water and saturated sodium chloride solution, dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by column chromatography through siliga gel using MeOH–EtOAc (1:9) as eluent to give a yellow oil compound **2** and dissolve in abs.EtOH (10 mL) and added 85% H_3PO_4 (1 mL). Crystallization commenced immediately and after cooling to 0 ^{o}C for 12 hours the precipitate was collected by filtration to afford tamiflu **1** (0.462 g, 55%) [ ], R_f on TLC chromatogram = 0.15 (9:1 ethyl acetate:MeOH). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.65 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 0.69 (t, *J*=7.8 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 1.10 (t, *J*=7.0 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.24-1.43 (m, 4H, (-C($C\underline{H}_2CH_3)_2$), 1.89 (s, 3H, (-NH-C(O)$C\underline{H}_3$)), 2.32 (m, 1H, (-$C\underline{H}_2$-)), 2.77 (dd, J_1=6.2 Hz, J_2=16.8 Hz, 1H, (-$C\underline{H}_2$-)), 3.35 (m, 2H, (-$C\underline{H}(CH_2CH_3)_2$), (-$C\underline{H}$-$NH_2.H_3PO_4$)), 3.86 (t, *J*=10.1 Hz, 1H, (-$C\underline{H}$-NHAc)), 4.06 (q, *J*=6.2 Hz, 2H, (-$C\underline{H}_2CH_3$)), 4.14 (d, *J*=9.4 Hz, 1H, (-$C\underline{H}$-O-)), 6.83 (m, 1H, (-$C\underline{H}$=C-)). ^{13}C NMR ($CDCl_3$) (δ, ppm): 8.4 (-C($CH_2\underline{C}H_3)_2$), 8.5 (-C($CH_2\underline{C}H_3)_2$), 13.3 (-$CH_2\underline{C}H_3$), 22.4 (-O-(CO)-$\underline{C}H_3$), 25.0 (-C($CH_2\underline{C}H_3)_2$), 25.4 (-C($CH_2\underline{C}H_3)_2$), 28.1 (-$C\underline{H}_2$-), 49.1 (-$\underline{C}$H-$NH_2.H_3PO_4$), 52.6 (-$\underline{C}$H-O-), 62.4 (-$\underline{C}H_2CH_3$), 75.1 (-$\underline{C}$H-NH-(C=O)-CH_3), 84.3 (-$\underline{C}H(CH_2CH_3)_2$), 127.6 (-CH=$\underline{C}$-), 137.9 (-$\underline{C}$H=C-), 165.0 (-$\underline{C}$=O), 176.0 (-CH-NH-($\underline{C}$=O)-$CH_3$), MS (EI) M^+ = 313.397.

Synthesis of ethyl (3*R*,4*R*,5*S*)-5-amino-4-acetamido-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate (oseltamivir) 2

Oseltamivir phosphate **1** (0.010 g, 0.238 mmol) dissolved in CH_2Cl_2 (1 mL) was neutralized by shaking with saturated $NaHCO_3$ (3 mL) for 5 min. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to give the free base of oseltamivir **2** in quantitative yield. R_f on TLC chromatogram = 0.12 (1:4 ethyl acetate:MeOH). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.88 (t, *J*=7.8 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 0.89 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 1.28 (t, *J*=7.0 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.46-1.54 (m, 4H, (-C($C\underline{H}_2CH_3)_2$)), 2.03 (s, 3H, (-NH-C(O)$C\underline{H}_3$)), 2.11-2.18 (m, 1H, (-$C\underline{H}_2$-)), 2.74 (dd, J_1=5.5 Hz, J_2=17.6 Hz, 1H, (-$C\underline{H}_2$-)), 3.22 (m, 1H, (-$C\underline{H}$-NH_2)), 3.33 (quint, *J*=5.5 Hz, 1H, (-$C\underline{H}(CH_2CH_3)_2$), 3.53 (q, *J*=9.36 Hz, 1H, (-$C\underline{H}$-NHAc)), 4.19 (q, *J*=7.0 Hz, 2H, (-$C\underline{H}_2CH_3$)), 5.78 (d, *J*=7.8 Hz, 1H, (-N$\underline{H}$-(C=O)-CH_3)), 6.77 (s, 1H, (-$C\underline{H}$=C-)), FTIR, cm^{-1}: 3282 (-NH_2), 2954 (C=C-H), 1716 (C=O), 1259, 1095 (C-O), MS (EI) $[M+H]^+$ = 313.397.

Anti-acetylcholinesterase activity

Materials. Electric-eel AChE (EC 3.1.1.7), acetylthiocholine iodide (ATCI), 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB) and galanthamine were purchased from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.). All chemicals and solvents used were purchased from E. Merck, Fluka, and Sigma & Aldrich Co., unless stated otherwise.

Acetylcholinesterase inhibition was determined spectrophotometrically using acetylthiocholine as substrate by modifying the method of Ellman[24]. Briefly, in the 96-well plates, 140 µl of 10 mM sodium phosphate buffer (pH 8.0), 20 µl of a solution of AChE (0.2 U/mL in 10 mM sodium phosphate buffer, pH 8.0) and 20 µl of test compound solution dissolved in 80% methanol (a final concentration of 0.1 mg/mL) were mixed and incubated at room temperature for 15 min. The reaction was stated by adding 20 µl of mixture solution of 5 mM DTNB in 10 mM sodium phosphate buffer (pH 8.0), containing 0.1% bovine serum albumin (BSA) and 5 mM ATCI in 10

mM sodium phosphate buffer, pH 8.0 (5:1). The hydrolysis of acetylthiocholine was determined by monitoring the formation of the yellow 5-thio-2-nitrobenzoate anion as result of reaction with DTNB and thiocholines, catalyzed by enzymes at a wavelength of 405 nm on a Microtiter plate reader (Sunrise, Tecan) and the absorbance was measured after 2 minutes of incubation at room temperature. Percentage of inhibition was calculated by comparing the rate of enzymatic hydrolysis of ATCI for the sample to that of the blank (80% methanol in buffer). Galanthamine was used as a reference standard. Every experiment was done in triplicate.

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