Vol. 1, No.4
Summer, 1990

J.Sci.I.R.Iran

THE SYNTHESES OF 1- [[2- AMINO -1 - (HYDROXYMETHYL) ETHOXY] METHYL] URACIL AND 1, 3-BIS (2- HALOETHOX-YMETHYL) PYRIMIDINE

G. H. Hakimelahi* and A. Khalafi-Nezhad

Department of Chemistry, Faculty of Science, University of Shiraz, Shiraz, Islamic Republic of Iran

Abstract

The syntheses of the title compounds are described. 1- [[2- Amino -1 - (hydroxymethyl) ethoxy] methyl] uracil exhibits little activity against herpesviruses (HSV) *in vivo*.

Introduction

Nucleoside analogues have gained increasing importance through their biological activity, particularly as antiviral and anticancer compounds [1]. Recently, a novel guanine acyclic nucleoside analogue, 9- [[2-hydroxy -1 (hydroxymethyl) ethoxy] methyl] guanine (BIOLF-62, DHPG, 2'-NDG), was reported by several independent groups of investigators [2-7] to have an antiviral activity compatible to that of 9- [(2-hydroxyethoxy) methyl] guanine (acyclovir, zovirax) [8] [9] which was approved by the Federal Drug Administration (FDA) for the topical and intravenous treatment of primary genital herpes and for cutaneous herpes simplex infections in immunocompromised patients. It was also claimed that BIOLF-62 has activity *in vitro* against some HSV-1 strains which are resistant to acyclovir [6]. In this report we wish to describe the synthesis of new uracil acyclic nucleoside analogues of BIOLF-62.

A number of pyrimidine bases have conferred activity to nucleoside analogues. Halogens present at the 5- position of uracil have induced antitumor and antiviral activity to nucleoside analogues [10-14]. 5-Methyluracil arabinosides have been reported to selectively inhibit HSV-1 and HSV-2 [15]. 5-Propyluracil nucleosides have been reported to possess antiviral activity [16, 17]. 3- Deazauracil nucleosides inhibit replication in bacteria and RNA viruses [1]. 5- Nitrouracil nucleosides show potent cytotoxicity [18]. The (E) - 5 - (2 - bromovinyl) uracil nucleosides have significant inhibitory activity against HSV-1.

We have prepared 9 - [[2 - amino - 1 - (hydroxymethyl) ethoxy] methyl] uracil (8) and tested it for activity against herpes viruses.

Key words: Pyrimidine Derivatives, Acyclonucleosides

Results and Discussion

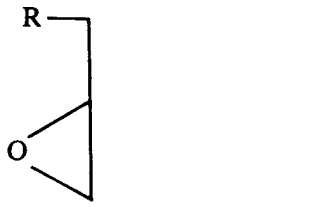
The general procedure for the synthesis of compound **8** is outlined in **Scheme 1.** Epichlorohydrin (1) was chosen as the starting material. Reaction of **1** with potassium phthalimide in the presence of phthalimide in DMF at 25° afforded after 15 h, **3** (70%). The above reaction in the absence of phthalimide gave, after 10 h, **2**(90%).Treatment of compound **2** with benzoic acid in the presence of $NaHCO_3$ in DMF at 25° afforded, after 15 h, compound **4** in 30% yield only. However, when the reaction was carried out with sodium benzoate / benzoic acid in DMF at reflux temperature compound **4** was obtained in excellent yield after 3 h. The structure of **4** was based on the fact that the H_2-C (1) exhibits a lowering of the ^{1}H-NMR chemical shift to 4.48 ppm, relative to that of the H-C (2) which appears at 4.09 ppm (cf. data of glycerol; 4.00 and 4.06 ppm, resp.). It should be noted that compound **3** was also transformed to **4** (50%) in refluxing DMF after 6 h.

The chloromethyl ether **5** was prepared, in good yield, from 1 - benzoyloxy - 3 - phtalimido - 2 - hydroxypropane (4) and 1, 3, 5 - trioxane in the presence of HCl gas after 8 h. Compound **6** was then synthesized by condensing the persilylated uracil with 1 - benzoyloxy - 3 - phthalimido - 2 - chloromethoxypropane (5). Both mercuric cyanide and tetra - n - butylammonium iodide (Bu_4NI) were used as catalysts in the coupling reaction. While both are effective catalysts, Bu_4NI has the advantage of lower toxicity, is required in smaller quantities, and the reactions are generally easier to manipulate during work-up. Treatment of **6** with NH_3/MeOH resulted in the uracil compound **7** (98%). The removal of the phthalimido group from **7** to give the unprotected compound **8** (70%) was achieved in pyridine / AcOH 3:2 containing 0.5 M phenylhydrazine. This compound was tested for activity against HSV-1 and herpes - Zoster *in vivo* and showed no significant activity.

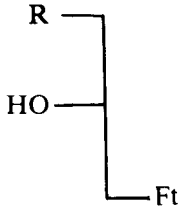
However, compound **8** is a valuable intermediate for the synthesis of a novel cyclophosphamide derivative possessing structure **9** as anticancer agent.

Next, it was decided to synthesize 1, 3 - bis (2 - haloethoxymethyl) uracil and 1, 3 - bis (2 - haloethoxymethyl) thymine. Bifunctional alkylating agents of proper structure form covalent derivatives with DNA. The covalent modification of DNA prevents both replication and transcription [19]. This could account for the pronounced cytotoxicity and therefore anticancer properties of certain bifunctional alkylating agents [20]. Since the model studies with CPK - atomic models indicate that the distance between the CH_2X functions in the best conformation of compounds **12-15** is such that they might undergo nucleophilic attack by the N (7) guanine residues of the gene to form cross links between adjacent segments of the DNA, it was decided to find a procedure for the exclusive preparation of the aforementioned compounds. This could possibly result in preparing excellent anticancer agents.

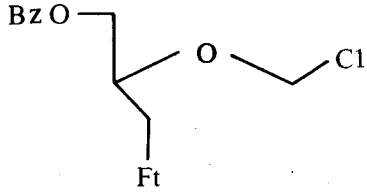
Scheme 1



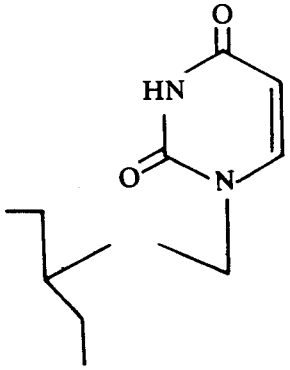
1 R = Cl
2 R = Ft



3 R=Cl
4 R=OBz

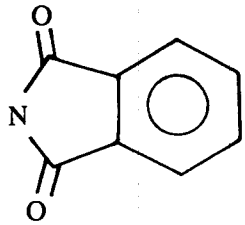


5

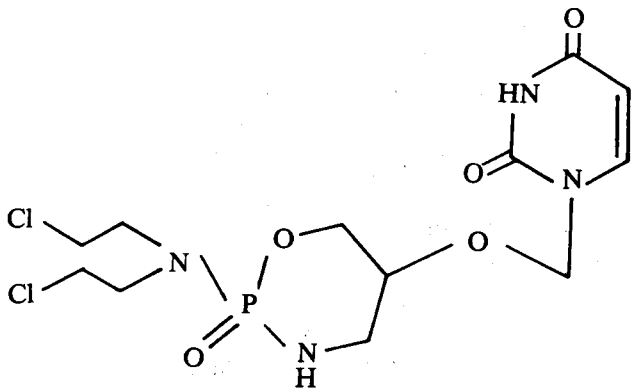


6 R=Oz , R¹=Ft
7 R=OH, R¹=Ft
8 R=OH, R¹=NH2

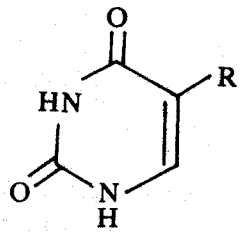
Ft=



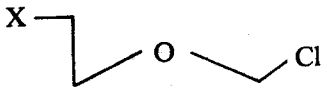
Bz=COPh



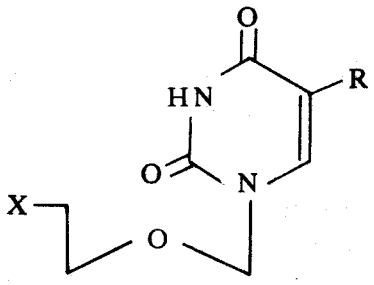
9



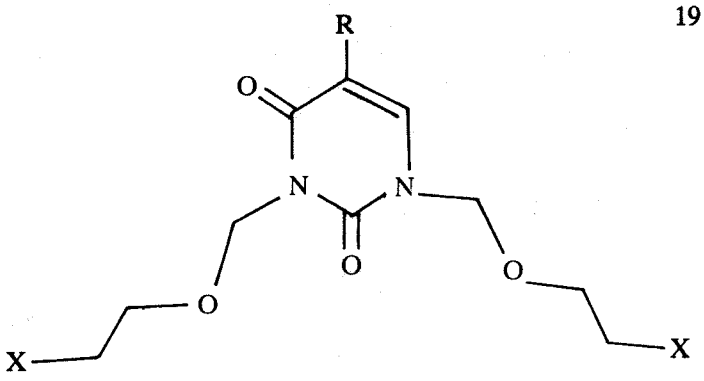
10 a R=H
10 b R=Me



11 a X=Cl
11b X = Br



16 R = H, X = Cl
17 R = H, X = Br
18 R = Me, X = Cl
19 R = Me, X = Br



12 R = H, X = Cl
13 R = H, X = Br
14 R = Me, X = Cl
15 R = Me, X = Br

20 R = Me, X = $PO(OMe)_2$
21 R = Me, X = $PO(OH)_2$

Compounds **12-15** were prepared in 95% yield (Scheme 2) by condensing the persilylated uracil (10a) or thymine (10b) with the corresponding chloromethyl ethers **11a-b** in refluxing dichloroethane. BU_4NI was used as catalyst in the coupling reactions. It should be mentioned that the above reactions in refluxing dichloromethane result in the preparation of a mixture of compounds **12-15** and **16-19** [21]. However, the exclusive preparation of compounds **16-19** could be achieved by condensation of **10a-b** with the respective chloromethyl ethers **11a-b** in THF using BU_4NF as catalyst [7, 8].

At this point, we became interested in preparing the phosphonate derivative **21**. The probable participation of **21** in gene synthesis of the tumor cells might result in interesting biochemical consequences. Therefore, compound **15** was reacted with trimethyl phosphite to afford **20** (40%). All attempts to hydrolyse the phosphonate esters to produce **21** failed and resulted in the destruction or recovery of the starting material.

Experimental Section

General Remarks. See [22].

Epiphthalimidohydrin (2). Epichlorohydrin (0.01 mol) and potassium phthalimide (0.01 mol) were suspended in dry DMF (150 ml). The reaction mixture was stirred at 20° for 10 h. Then it was partitioned between AcOEt (250 ml) and H_2O (250 ml). The organic layer was washed with H_2O (5 × 100 ml), dried (Na_2SO_4), filtered and evaporated to give a syrup.

Chromatography on silica gel and elution with CH_2Cl_2 afforded **2** (90%), m.p. 100°. R_f (ether) 0.72. IR (nujol): 1715 (amide), 1125 (epoxide) cm^{-1}. ^1H-NMR ($CDCl_3$): 2.80 (m, 2H, CH_2Ft); 3.29 (br. m, 1H, CH); 3.91 (m, 2H, CH_2); 7.80 (m, 4H, Ph).

1-Chloro- 3-phthalimido- 2-hydroxypropane (3). Epichlorohydrin (0.01 mol), potassium phthalimide (0.01 mol), and phthalimide (0.01 mol) were dissolved in dry DMF (150 ml). The reaction mixture was stirred at 25° for 15 h. The solution was extracted with AcOEt and H_2O. The AcOEt layer was dried (Na_2SO_4), filtered, and evaporated. The residue was purified on silica gel using CH_2Cl_2 as eluent to afford **3** (70%), m.p. 223°. R_f (ether) 0.56. IR (nujol): 3380 (OH), 1725 (amide) cm^{-1}. ^1H-NMR ($CDCl_3$): 3.40 - 3.80 (m, 4H, CH_2Ft and CH_2Cl; 3.81 - 4.20 (m, 1H, CH); 4.41 (br., 1H, OH, exchanged with D_2O); 7.55 (s, 4H, Ph).

1-Benzoyloxy- 3-phthalimido- 2-hydroxypropane (4). Epiphthalimidohydrin (**2**, 0.01 mol), sodium benzoate (0.01 mol), and benzoic acid (0.01 mol) were dissolved in DMF (150 ml). The reaction mixture was refluxed for 3 h. AcOEt (300 ml) was added to the reaction mixture and the solution was washed with H_2O (5 × 150 ml). The organic layer was dried (Na_2SO_4), filtered, and evaporated to leave a residue. Chromatography on silica gel and elution with $CHCl_3$ gave **4** (85%) m.p. 195°. R_f (ether) 0.38. IR (nujol): 3430 (OH), 1720 (amide), 1770 (ester). ^1H-NMR ($CDCl_3$): 3.68 - 4.05 (m, 2H, CH_2Ft); 4.09 (m, 1H, CH); 4.48 (br. s, 2H, CH_2O); 4.78 (br., 1H, OH, exchanged with D_2O); 7.15 - 8.08 (m, 9H, Ph).

Compound **4** was also prepared from **3** and sodium benzoate, in the absence of benzoic acid, in refluxing DMF after 6 h in 50% yield.

1-Benzoyloxy- 3-Phthalimido- 2 - Chloromethoxypropane (5), 1-Chloro - (2- Chloromethoxy) ethane (11a), and 1-Bromo - (2- chloro-methoxy) ethane (11b). Compound **4** (0.1 mol) and 1, 3, 5 - trioxane (0.17 mol) were added to CH_2Cl_2 (300 ml) The mixture was cooled in an ice - water bath and dry HCl was bubbled through the stirred mixture of 8 h. Anhydrous $CaCl_2$ was added and after 30 min. the solution was collected by filtration. The solution was concentrated at reduced pressure (bath temperature 50°) to a syrup. An ^1H-NMR spectrum of this material indicated that it contained 50% of **5** and 50% unreacted **4** which does not interfere in subsequent condensation. ^1H-NMR (CCl_4): 5.70 - 4.01 (m, 2H, CH_2Ft); 4.12 (m, 1H, CH); 4.50 (m, 2H, CH_2O), 5.67 (s, 2H, OCH_2Cl); 7.01 - 8.12 (m, 9H, Ph).

Compounds **11a** and **11b** were similarly prepared (~ 95 - 98%) and purified by distillation at 95° / 5-6 Toor. Spectroscopic data similar to that of the **5** except for variations due to substitutions [7].

1 - [[2 - Phthalimido - 1 - (benzoyloxymethyl) ethoxy] methyl] uracil (6). Uracil (10a, 0.01 mol) and $(NH_4)_2SO_4$ (0.5 g) were added to hexamethyldisilazene (50ml) The mixture was heated at reflux until the solution became clear (1.5 h). The solvent was removed at reduced pressure and the residue was dried under vacuum. The residue was dissolved in benzene (50 ml) and Hg $(CH)_2$ (5 g, 0.02 mol) was added. The mixture was heated below reflux temperature and chloromethyl ether **5** (0.015 mol) was added. The mixture was heated at reflux under a nitrogen atmosphere for 3 h. The solution was collected by filtration and concentrated to a gum at reduced pressure. CH_2Cl_2 (300 ml) was added and the solution was extracted first with 30% KI solution followed by H_2O. The solution was dried (Na_2SO_4), filtered and evaporated to leave 7 g of material. This material was purified by chromatography on silica gel using AcOEt to give **6** (55%), foam. R_f (ether / MeOH 9:1) 0.75. UV (EtOH): 260 nm. IR (CH_2Cl_2): 1670, 1720 (amide), 1760 (ester), 1110 (ether). ^1H-NMR ($CDCl_3$): 5.50 - 5.86 (m, 2H, CH_2Ft); 4.10 (m, 1H, CH); 4.48 (m, 2H, CH_2O); 5.56 (s, 2H, OCH_2N); 5.80 (d, 1H, H-C(5), J = 8 Hz); 7.21 (d, 1H, H-C(6), J = 8 Hz); 7.40 - 8.00 (m, 9H, Ph); 9.80 (br., 1H, NH).

Compound **6** was also prepared in a similar fashion except that 0.1 mmol of Bu_4NI was used in place of Hg $(CN)_2$ as catalyst. The coupling reaction was carried out in CH_2Cl_2 instead of benzene. After heating at reflux for 1.5 h, the solution was cooled to 25° and diluted with H_2O (15 ml) and MeOH (40 ml). After evaporation at reduced pressure, the residue was partitioned between AcOEt and H_2O. The organic layer was dried (Na_2SO_4), filtered, and evaporated to afford, after purification like above, **6** (80%).

General Procedure for the Preparation of Acyclonucleo - sides **12 -15** *and* **16 -19**. All compounds were prepared in high yield in a similar fashion except that **12-15** was synthesized in $ClCH_2CH_2Cl$ while a mixture of **12-15** and **16-19** was prepared in CH_2Cl_2, see [2]. Their ^1H-NMR and IR spectra were similar except for variations due to substitutions: Representative Procedure.

Uracil (**10a**, 40 mmol) and $(NH_4)_2SO_4$ (1 g) were

dissolved in hexamethyldisilazane (100 ml). The mixture was heated for 2 h. The solvent was evaporated at reduced pressure to leave a residue. The residue was dissolved in $ClCH_2CH_2Cl$ (200 ml) and Bu_4NI (0.10 mmol) was added. The mixture was refluxed and chloromethyl ether **11a** (85 mmol) was added. After 2 h, the solution was washed successively with $NaHSO_3$ solution (100 ml) and water (300 ml). The organic layer was dried (Na_2SO_4), filtered, and evaporated to leave a syrup. Chromatography on silica gel and elution with CH_2Cl_2 gave **12** (95%) as an oil. R_f (AcOEt / ether 1:1) 0.80. UV (EtOH): 260 nm. ^{1}H-NMR ($CDCl_3$): 3.60 (m, 4H, $2CH_2Cl$); 3.89 (m, 4H, $2CH_2O$); 5.21, 5.49 (2s, 4H, $2OCH_2N$); 5.82 (d, 1H, H-C(5), J = 8 Hz); 7.35 (d, 1H, H-C(6), J = 8 Hz). Representative ^{1}H-NMR spectrum of **15** ($CDCl_3$); 1.92 (s, 3H, CH_3); 3.22 - 3.58 (m, 4H, $2CH_2Br$); 3.68 - 4.01 (m, 4H, $2CH_2O$) 5.21, 5.48 (2s, 4H, $2OCH_2N$); 7.30 (s, 1H, H-C(6)). UV (EtOH): 264 nm. R_f (ether) 0.58.

Representative ^{1}H-NMR spectrum of **18** ($CDCl_3$): 1.90 (s, 3H, CH_3); 3.50 - 4.11 (m, 4H, $ClCH_2CH_2O$); 5.22 (s, 2H, OCH_2N); 7.21 (s, 1H, H-C(6)); 9.99 (br. s, 1H, NH). UV (EtOH): 264 nm.

IR (CH_2Cl_2): 3400 - 3500 (NH), 2980 (CH_3, CH_2), 1680, 1715 (amide), 1111 (ether). R_f (AcOEt / ether 1:1) 0.53.

*General Method for the Exclusive Preparation of compounds **16-19**.* This method was already reported. See [7,8].

1, 3- bis (2- Dimethylphosphonoethoxymethyl) thymine (20). Compound **15** (5 mmol) and trimethyl phosphite (30 mmol) were heated together at 160° for 30 h. After cooling, it was poured into ether (500 ml). The resulting oil was separated and applied to a silica gel column. Elution with $CHCl_3$ gave **20** (40%), as a foam. R_f (ether) 0.08. UV (EtOH): 264. IR (neat): 1680, 1720 (amide), 1300 - 1460 (phosphonate), 1120 (ether). ^{1}H-NMR ($CDCl_3$): 1.21 - 2.38 (m, 4H, $2CH_2P$); 1.90 (s, 3H, CH_3); 3.60, 3.80 (d, 12H, $4CH_3OP$, J = 12 Hz); 4.15 (m, 4H, $2CH_2O$); 5.16, 5.47 (2s, 4H, $2OCH_2N$); 7.13 (s, 1H, CH).

1- [[2- Phthalimido- 1- (hydroxymethyl) ethoxy] methyl] uracil (7). To a solution of **6** (0.01 mol) in MeOH (20 ml), 80 ml of saturated NH_3/MeOH was added. The solution was sealed and maintained at 25° for 24 h. The mixture was concentrated to 30 and left overnight to afford **7** (98%), m.p. 188°. R_f (ether / MeOH 9:1) 0.50. UV (EtOH): 260 mn. IR (nujol): 3400 - 3500 (OH, NH), 1680, 1720 (amide), 1120 (ether). ^{1}H-NMR (DMSO-d6): 5.48 - 4.21 (m, 6H, $HOCH_2CHCH_2Ft$); 5.50 (s, 2H, OCH_2N); 5.70 (d, 1H, H-C(5), J = 8 Hz); 7.30 (d, 1H, H-C(6), J = 8 Hz); 7.8 (m, 4H, Ph); 9.90 (br., 1H, NH).

1- [[2- Amino- 1- (hydroxymethyl) ethoxy] methyl] uracil (8). Compound **7** (1 mmol) was dissolved in Pyridine (1 ml), and 10 equiv. of 0.5 M Phenylhydrazine in pyridine / AcOH 3:2 were added. After 2 h, pentane – 2, 4 – dione (10 equiv.) was added with cooling. Solvents were removed, and the residue was suspended in $CHCl_3$ to dissolve impurities. The precipitate was filtered and washed with $CHCl_3$ to afford **8** (80%), m.p. 220°. R_f (ether / MeOH 9:1) 0.28. UV (EtOH): 260 nm. IR (nujol): 3120 - 3510 (NH, NH_2, OH), 1680, 1720 (2C = O), 1110 (ether). ^{1}H-NMR (DMSO-d6): 3.30 (br., 2H, NH_2, exchanged with D_2O); 5.39 - 5.60 (m, 2H, CH_2N); 5.71 - 4.22 (m, 4H, $HOCH_2CH$); 5.21 (s, 2H, OCH_2N); 5.40 (d, 1H, H-C(5), J = 8 Hz); 7.40 (d, 1H, H-C(6), J = 8 Hz); 10.10 (br., 1H, NH, exchanged with D_2O).

References

1. R. J. Walker, E. de Clercq, and F. Eckstein (ed.), «*Nucleo - side Analogues: Chemistry, Biology, and Medical Applications*», Plenum Press, N. Y. 1979.
2. K. O. Smith, K. S. Galloway, W. L. Kennell, K. K. Ogilvie, and B. K. Radatus, *Antimicrob. Agents Chemother.* **22**, 55 (1982).
3. W. T. Ashton, J. D. Karkas, A. K. Field, and R. L. Tolman, *Biochem. Biophys, Res. Commun.* **108**, 1716 (1982).
4. J. C. Martin, C. A. Dvorak, D. F. Smee, T. R. Matthews, and J. P. H. Verheyden, *J. Med. Chem.* **26**, 759 (1983).
5. D. F. Smee, J. C. Martin, J. P. H. Verheyden, and T. R, Matthews, *Antimicrob. Agents Chemother.* **23**, 679 (1983).
6. H. J. Schaeffer in «*Nucleosides, Nucleotides, and their Biological Applications*», J. L. Rideout, D. W. Henry and L. M. Beascham III (eds.), Academic Press, N. Y., 1983, pp. 1-17.
7. G. H. Hakimelahi and Ali Khalafi-Nezhad, *Helv. Chim. Acta* (1989); to appear.
8. G. H. Hakimelahi, F. Mohanazadeh, Ali Khalafi-Nezhad, and M. Zakerinia, *Med. J. I. R. Iran*, **4**, (1989); to appear.
9. H. J. Schaeffer, L. Beauchamp, P. de Miranda, and G. B. Elion, *Nature*, **272**, 583 (1978).
10. A. F. Cook, M. J. Holman, M. J. Kramer, and P. W. Trown, *J. Chem.* **22**, 1330 (1979).
11. A. Rosowsky, S. H. Kim, D. Trites, and M. Wick, *J. Med. Chem.* **25**, 1034 (1982).
12. C. Heidelberger, *Cancer. Res.* **30**, 1549 (1970).
13. P. H. Fischer, M. S. Chen, and W. H. Prusoff, *Biochem. Biophys.*

Acta, **606**, 236 (1980).
14. B. Goz, *Pharmacol. Rev.* **29**, 249 (1978).
15. W. E. G. Maller, R. K. Zahn, A. Maidhof, R. Beyer, and J. Arendes, *Chem. Biol. Interact.* **23**, 141 (1978).
16. E. de Clercq, J. Deschamps, and D. Shugar, *Antimicrob. Agents Chemother.* **13**, 545 (1978).
17. J. L. Ruth and Y. C. Cheng, *J. Biol. Chem.* **257**, 10261 (1982).
18. W. L. Washtien and D. V. Santi, *J. Med. Chem.* **25**, 1252 (1982).
19. C. O. Wilson, O. Gisvold, and R. F. Doerge, «*Textbook of Organic Medicinal and Pharmaceutical Chemistry*», J. B. Lippincott Co., Toronto, 1972, pp. 170 - 171.
20. *Ibid*, 172 - 175.
21. G. H. Hakimelahi, F. Mohanazadeh, and G. R. M. A. Afshari, *J. Sci. I. R. Iran* **1**, (1989).
22. G. H. Hakimelahi, M. Zarrinehzad, A. A. Jarrahpour, and H. Sharghi, *Helv. Chim. Acta*, **70**, 219 (1987).