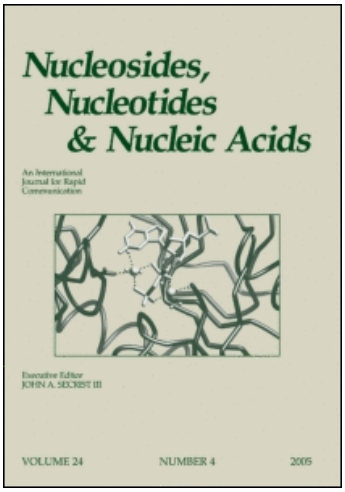


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Novel Synthesis of a New Class of Polynuclear Pyridinethione Nucleosides

Galal E. H. Elgemeie[a]; Adel M. E. Attia[b]; Nahed M. Fathy[c]
[a] Chemistry Department, Faculty of Science, Helwan University, Cairo, Egypt [b] Chemistry Department, Faculty of Education, Tanta University, Egypt [c] Photochemistry Department, National Research Centre, Cairo, Egypt

To cite this Article Elgemeie, Galal E. H. , Attia, Adel M. E. and Fathy, Nahed M.(1997) 'Novel Synthesis of a New Class of Polynuclear Pyridinethione Nucleosides', Nucleosides, Nucleotides and Nucleic Acids, 16: 4, 485 — 493
To link to this Article: DOI: 10.1080/07328319708001365
URL: http://dx.doi.org/10.1080/07328319708001365

NUCLEOSIDES & NUCLEOTIDES, 16(4), 485-493 (1997)

NOVEL SYNTHESIS OF A NEW CLASS OF POLYNUCLEAR PYRIDINETHIONE NUCLEOSIDES

Galal E. H. Elgemeie*[a], Adel M. E. Attia[b] and Nahed M. Fathy[c]

a Chemistry Department, Faculty of Science, Helwan University, Helwan, Cairo, Egypt

b Chemistry Department, Faculty of Education, Tanta University, Egypt

c Photochemistry Department, National Research Centre, Cairo, Egypt

Abstract: A novel synthesis of a new class of polynuclear pyridinethione glycosides utilizing the reactions of phenanthridine analogues and ∝-bromoglucose or ∝-bromogalactose tetraacetate as starting components is described.

As a part of our program directed towards the development of new, simple and efficient procedures for the synthesis of antimetabolites[1-3], we have recently reported different successful approaches for the synthesis of mercaptopurine, 3-deazapyrimidine nucleosides and folic acid analogues[4,5]. An important series of antimetabolites bearing a structural resemblance to the naturally occurring pyrimidine nucleosides uridine, cytidine and thymidine consists of their 3-deaza analogues[6]. Although a number of N-glycosylpyridines have been prepared, no pyridinethione nucleosides have been synthesized or biologically evaluated. A new class of anti HIV agents has been identified recently[7]. These compounds, like 1-[(2-hydroxyethoxy)-methyl]-6-(phenylthio)thymine (HEPT) and 1-(benzoyloxymethyl)-5-ethyl-6-(phenylthio)uracil (EBPU), show a high selectivity for the HIV-1 reverse transcriptase. The present paper deals with the novel synthesis of a new class of polynuclear pyridinethione nucleosides related to HEPT and EBPU. As far as we know this is the first coupling reaction of this type to be reported for pyridine-2(1*H*)thiones and their condensed derivatives.

Thus it has been found that 1- tetralylidenecyanothioacetamide **1** reacted with β-(2-furanyl)- and β-(2-thienyl)methylenemalononitriles **2** in refluxing ethanol containing catalytic amounts of piperidine for 3h to give the unexpected phenanthridine analogues **3** (Chart 1). The formation of **3** from **1** and **2** is assumed to proceed via addition of the active methylene group of **1** to the double bond of **2** to give Michael intermediates, which then cyclize via malononitrile elimination and oxidation under the reaction conditions to yield **3**, a support for the mechanism for the formation of **3** has been previously reported by us[8-10]. The structure of compounds **3** could be established and confirmed for the reaction products on the basis of their elemental analysis and spectral data (MS , IR, ^{1}H-NMR). Thus, the mass spectrum of **3a** was compatible with the molecular formula $C_{18}H_{12}N_2SO$ (m/z 304), and ^{1}H-NMR spectrum had signals at δ 2.64 (s, CH_2), 2.88 (s, CH_2), 7.16-7.48 (m, Ar-H) and 14.1 (br, NH).

Compounds **3** can be coupled with different classes of sugar halides to give a novel ring system of glycosides, for example **3** reacted with 2,3,4,6,-tetra-O-acetyl-α-D-gluco- and galactopyranosyl bromides in the presence of aqueous potassium hydroxide to give the corresponding polynuclear pyridinethione glycosides **4a-d**. Although the coupling of **3** with the glycosyl bromides could also give the corresponding thioglycosides, the formation of **4a-d** was proven chemically . Reaction of **3** with hexamethyldisilazane in the presence of ammonium sulfate gave the corresponding 3-trimethylsilylthioisoquinolines **5**, which were subsequently treated with peracetylated sugar in the presence of redistilled $SnCl_4$ to afford the corresponding N-glycosyl compounds. All previous literature reports that the Lewis acid- induced coupling reactions of S-silylated heterocyclic bases with peracetylated sugars gave the corresponding N-nucleosides as the sole product [11-13]. The structures of the reaction products **4** were established and confirmed by their elemental analyses and spectral data (MS, IR, UV, ^{1}H-NMR, ^{13}C-NMR). Thus, the analytical data for **4a** revealed a molecular formula $C_{32}H_{30}N_2SO_{10}$ (m/z 634). The ^{1}H - NMR spectrum showed the anomeric proton as a doublet at δ 6.26 with a spin-spin coupling constant of 10.68 Hz corresponding to a diaxial orientation of H-1' and H-2' protons indicating the β-configuration, while the other six glucose protons resonated at δ 4.01-5.76. The four acetyl groups appeared as four singlets at δ1.92-2.02. The ^{13}C-NMR spectra were characterized by a signal at δ 80.0 corresponding to the C-1 atom of the β -D-glucopyranose. The four signals appearing at δ169.2-169.6 are due to the four

acetoxy carbonyl carbon atoms, while the four signals at δ19.8-20.3 are attributed to the acetate methyl carbons. Another five signals at δ 61.8, 68.2, 68.9, 72.9 and 75.0 were assigned to C-6', C-4', C-2', C-3' and C-5', respectively .The UV spectrum of **4a** proved that the reaction had led selectively to the formation of N- glucosyl derivatives and excluded substitution at the sulfur atom. Thus, whereas the S-methyl derivative of **3a** showed three maxima at 241, 282 and 373 nm, its N-glucosyl derivative exhibited two maximum absorption bands at 275 and 298 nm. Removal of the acetyl groups with ammonia in methanol gave the free glycosides **6a-d** after chromatographic purification. The structures of compounds **6** were further confirmed by elemental analyses and spectral data .The ^{13}C-NMR spectrum of **6a** contained a signal at δ 83.9 corresponding to the C-1' atom of β-D-glucopyranose. Another five signals at δ 60.7, 69.6, 71.7, 78.5 and 81.7 were assigned to C-6', C-4', C-2', C-3', and C-5' of the glucose moiety, respectively. The glycosides obtained by these syntheses seem promising for further carbohydrate transformations.

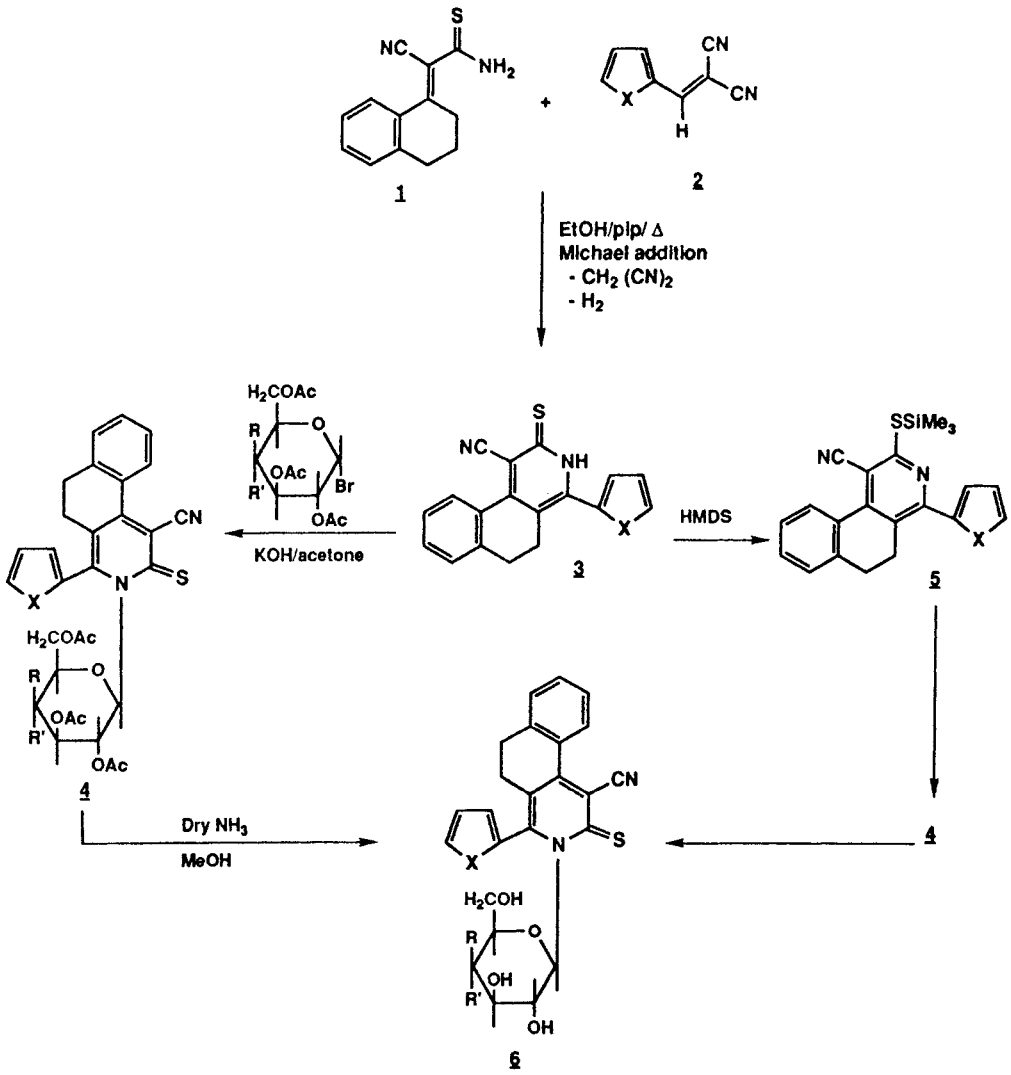
The anti-HIV activity and cytotoxicity of 7, 8-dihydrobenzo[f]-isoquinoline-3-thione glycosides and galactosides are shown in Table **1**. Among these types of glycosides the 2-(2,3,4,6,-tetra-O-acetyl-D-galacto-pyranosyl)-7,8-dihydrobenzo[f]-isoquinoline-3-thione **4b** turned out to be the most selective anti-HIV agent while the other glycosides devoid any therapeutic index at the indicated concentration.

Experimental

All evaporation were carried out under reduced pressure at 40 °C. Melting points were uncorrected. TLC aluminium silica gel sheets 60 F_{254} (Merck) were used for thin layer chromatography. Detection was effected by viewing under a short-wavelength UV lamp. IR spectra were obtained (KBr disc) on a Pye Unicam Spectra-1000. ^{1}H-NMR and ^{13}C-NMR spectra were measured on a Wilmad 270 MHz or on Varian 400 MHz spectrometer for solutions in (DMSO-d_6) using $SiMe_4$ as internal standard. Mass spectra were recorded on a Varian MAT 112 Mass spectrometer using EI mode. Analytical data were obtained from the Microanalytical data Center at Cairo University. Compounds **3** were prepared following the literature procedures[14] .

7,8-Dihydrobenzo[f]isoquinoline-3(2*H*)-thiones 3

To a mixture of **1** and **2a,b** (0.01mol) in ethanol (50 ml), piperidine (0.3 ml) was added. The reaction mixture was heated under reflux for 3hr, and then set aside



key for 4

	X	R	R'
a	O	H	OAc
b	O	OAc	H
c	S	H	OAc
d	S	OAc	H

key for 6

	X	R	R'
a	O	H	OH
b	O	OH	H
c	S	H	OH
d	S	OH	H

Table 1: Comparative potency and selectivity of 7,8-dihydrobenzo[f]isoquinoline-3-thioneglycosides and -galactosides as inhibitors of HIV replication in MT-4 cells.

Compd	EC_{50}[a] μM	IC_{50}[b] μM	TI[c] (ratio IC_{50}/EC_{50})
4a		>23.2	
4b	98	195	>1.98
4c		>9.1	
4d		>16.6	
6a		>11.5	
6b		>60.2	
6c		>33.3	
6d		>40.8	

a. Approximate values for 50% effective concentration of MT-4 cells against the cytopathic effect of HIV (EC_{50}).
b. Inhibitory concentration for 50% (IC_{50}).
c. Therapeutic index TI (IC_{50}/EC_{50}).

overnight. The resultant precipitate was filtered off and crystallized from dioxane to yield yellow crystals.

3a: MP. 200 °C, yield 24%. IR (KBr) 3280 (NH), 2216 (CN); ^{1}H-NMR (DMSO-d_6) δ 2.51 (s, 2H, $_2$), 2.82 (s, 2H, CH_2), 6.82 (d, 1H, furan 4-H), 7.34 (m, 4H, Ar-H), 7.94 (d, 1H, furan 3-H), 8.06 (s, 1H, furan 5-H) 13.92 (s, br, 1H, NH); m/z 304 (Found: C, 71.2; H, 4.1; N, 9.0. $C_{18}H_{12}N_2SO$ calculated C, 71.1; H, 3.9; N, 9.2%).

3b: MP. 276°C, yield 20%. IR (KBr) 3300 (NH), 2215 (CN); m/z 320 (Found: C, 67.2; H, 4.0; N, 9.1. $C_{18}H_{12}N_2S_2$ calculated C, 67.5; H, 3.8; N, 8.8%).

2-(2,3,4,6-Tetra-O-acetyl-D-gluco- and -galactopyranosyl)-7,8-dihydrobenzo[f] isoquinoline-3thiones 4a-d

General coupling procedures:

Method A. To a solution of 7,8-Dihydrobenzo[f]isoquinoline-3(2*H*)-thiones **3** (0.01mol) in aqueous potassium hydroxide [0.56 g (0.01 mol) in 6ml of distilled

water], a solution of 2,3,4,6-tetra-O-acetyl-α-D-gluco- or galactopyranosyl bromide (4.521g, 0.011 mol) in acetone (30 ml) was added. The mixture was stirred at room temperature until the reaction was judged complete by TLC (30 min to 20h). The mixture was evaporated under reduced pressure at 40°C and the residue was washed with distilled water to remove KBr. The product was dried and crystallized from EtOH to afford pale yellow crystals.

Method B. The 7,8-dihydrobenzo[f]isoquinoline-3(2*H*)-thiones **3** (0.01 mol) were boiled under reflux, , under anhydrous conditions for 48 hours with hexamethyldisilazane (25ml) and $(NH_4)_2SO_4$ (0.02g). The excess hexamethyldisilazane was removed under diminished pressure to provid the silylated bases **5** as a colourless oils. To a solution of the silylated base in dry MeCN (30 ml), a solution of α-D-glucose- or α-D-galactose pentaacetate (0.011 mol) in dry MeCN (20 ml) was added followed by the addition of $SnCl_4$ (1.6 ml). The reaction mixture was stirred at room temperture until the reaction was completed as indicated by TLC (3 to 6 h). The solution was poured into a saturated $NaHCO_3$ solution and extracted with $CHCl_3$. The organic layers were dried over $MgSO_4$, filtered and concentrated to give the crude nucleosides which were purified by recrystallization from EtOH to afford pale yellow crystals.

4a: MP. 172°C, yield 70%. IR (KBr) 2214 (CN), 1746 (CO); ^{1}H-NMR (DMSO-d_6) δ 1.92-2.02 (4s, 12H, $4CH_3CO$), 2.82 (t, 2H, CH_2), 2.98 (t, 2H, CH_2), 4.05 (m, 2H, H-6', 6"), 4.36 (m, 1H, H-5'), 5.02 (t, 1H, H-4') 5.26 (t, J 8.7 Hz, 1H, H-2'), 5.76 (t, 1H, H-3'), 6.26 (d, $J_{1'-2'}$=10.68 Hz, 1H, H-1'), 6.84 (m,1H, furan 4-H), 7.38 (m, 4H, C_6H_4), 8.05 (m, 1H, furan 3-H), 8.46 (dd, 1H, furan 5-H); ^{13}C-NMR (DMSO-d_6) δ 19.8-24.4 ($4CH_3$), 26.1, 26.4 ($2CH_2$), 61.8 (C-6'), 68.2 (C-4'), 68.9 (C-2'), 72.9 (C-3'), 75.0 (C-5'), 103.2 (C-3), 115.5 (CN), 125.6-156.8 (Ar-C), 169.2-169.6 (4CO); m/z 634 (Found: C, 60.3; H, 4.6; N, 4.1. $C_{32}H_{30}N_2SO_{10}$ calculated C, 60.6; H, 4.7; N,4.4%).

4b: MP. 143°C, yield 71%. IR (KBr) 2212 (CN), 1755 (CO); m/z 634 (Found: C,60.6; H, 5.0 N; 4.2. $C_{32}H_{30}N_2SO_{10}$ calculated C, 60.6; H, 4.7; N, 4.4%).

4c: MP. 200°C, yield 73%. IR (KBr) 2208 (CN), 1752 (CO); ^{1}H-NMR (DMSO-d_6)δ 1.94-2.15 (4s, 12H, $4CH_3CO$), 2.64 (t, 2H, CH_2), 2.78 (t, 2H, CH_2), 4.05 (m, 2H, H-6', 6"), 4.74 (m, 1H, H-5'), 5.04 (t, 1H, H-4'), 5.24 (t, J 8.4 Hz, 1H, H-2'), 5.74 (t, 1H, H-3'), 6.30 (d, J_{1-2} = 10.51 Hz, 1H, H-1'), 7.28 (m, 1H, thiophen 4-H), 7.38 (m, 4H, C_6H_4), 7.86 (m, 1H, thiophen 3-H), 8.18 (m, 1H, thiophen 5-H); m/z 650 (Found: C, 58.9; H, 4.8; N, 4.1. $C_{32}H_{30}N_2S_2O_9$ calculated C, 59.1; H, 4.6; N, 4.3%).

4d: MP. 161°C, yield 74%. IR (KBr) 2208 (CN), 1752 (CO); ^{1}H-NMR (DMSO-d_6) δ

1.93-2.16 (4s, 12H, $4CH_3CO$), 2.66 (t, 2H, CH_2), 2.83 (t, 2H, CH_2), 4.04 (m, 3H, H-6', 6" and H-5'), 4.48 (t, 1H, H-4'), 4.68 (t, J 10.8 Hz, 1H, H-2'), 5.44 (t, 1H, H-3'), 6.28 (d, J_{1-2} = 10.38 Hz, 1H, H-1'), 6.84 (s, 1H, thiophen 4-H), 7.39 (m, 4H, C_6H_4), 7.81 (m, 1H, thiophen 3-H), 8.17 (dd, 1H, thiophen 5-H); ^{13}C-NMR (DMSO-d_6) δ 19.8-20.3 ($4CH_3$), 24.0, 26.4 ($2CH_2$), 61.8 (C-6'), 66.3 (C-4'), 68.2 (C-2'), 70.8 (C-3'), 74.4 (C-5'), 89.3 (C-1'), 105.4 (C-3), 116.5 (CN), 125.6-158.1 (Ar-C), 169.4-169.9 (4CO); m/z 650 (Found: C, 58.8; H, 4.5; N, 4.0. $C_{32}H_{30}N_2S_2O_9$ calculated C, 59.1; H, 4.6; N, 4.3%).

2-(-β-D-Gluco- and -galactopyranosyl)-7,8-dihydrobenzo[f]isoquinoline-3-thiones 6-a-d

General procedure for nucleoside deacylation:

Dry gaseous NH_3 was passed through a solution of protected nucleosides **4a-d** (0.5 g) in dry MeOH (25 ml) at O°C for about 0.5 hour, then the reaction mixture was stirred until judged complete by TLC (4 to 18 h). The resulting reaction mixture was evaporated under reduced pressure at 40°C giving a solid residue which was crystallized from MeOH to afford colourless crystals.

6a: MP. 217°C, yield 88%. IR (KBr) 3610-3190 (OH), 2204 (CN), UV max 279 and 302 nm; ^{1}H-NMR (DMSO-d_6) δ 2.80 (s, 2H, CH_2), 2.94 (t, 2H, CH_2), 3.28-3.76 (m, 6H, H-6', 6", H-5', H-4', H-3' and H-2'), 4.44 (s, 2'-OH), 5.20 (d ,2H, 3'- OH and 4'-OH), 5.62 (s, 1H, 6'-OH), 5.86 (d, $J_{1'-2'}$= 8.72 Hz, 1H, H-1'), 6.78 (d, 1H, furan 4-H), 7.42 (m, 4H, C_6H_4), 8.01 (d, 1H, furan 3-H), 8.22 (dd, 1H, furan 5-H); ^{13}C - NMR (DMSO-d_6) δ 24.0 (CH_2), 26.4 (CH_2), 60.7 (C-6'), 69.6 (C-4'),71.7 (C-2'), 78.5 (C-3'), 81.7 (C-5'), 83.9 (C-1'), 102.8 (C-3), 113.6 (CN), 125.6-159.2 (Ar-C); m/z 466 (Found: C, 61.5; H, 5.0; N, 5.8. $C_{24}H_{22}N_2SO_6$ calculated C, 61.8; H, 4.7; N, 6.0%).

6b: MP. 204 °C, yield 85%. IR (KBr) 3640-3180 (OH), 2212 (CN); m/z 466 (Found: C, 61.6; H, 4.8; N, 6.2. $C_{24}H_{22}N_2SO_6$ calculated C, 61.8; H, 4.7; N, 6.0%).

6c: MP. 212°C, yield 86%. IR (KBr) 3650-3200 (OH), 2210 (CN); m/z 482 (Found: C, 59.5; H, 4.6; N, 6.0. $C_{24}H_{22}N_2S_2O_5$ calculated C, 59.6; H, 4.6; N, 5.8%).

6d: MP. 198°C, yield 87%. IR (KBr) 3600-3200 (OH), 2208 (CN), ^{1}H-NMR (DMSO-d_6) δ 2.66 (t, 2H, CH_2), 2.78 (t, 2H, CH_2), 3.32-3.68 (m, 6H, H-6', 6", H-5', H-4', H-3' and H-2'), 4.58 (m, 2H, 2'-OH and 3'-OH), 5.14 (d, 1H, 4'-OH), 5.52 (d, 1H, 6'-OH), 5.78 (d, $J_{1'-2'}$= 8.64 Hz, 1H, H-1'), 6.85 (s, 1H, thiophen 4-H), 7.38 (m, 4H, C_6H_4), 7.84 (d, 1H, thiophen 3-H), 8.18 (d, 1H, thiophen 5-H); ^{13}C-NMR (DMSO-d_6) δ 24.0

(CH_2), 27.2 (CH_2), 60.2 (C-6'), 68.4 (C-4'), 70.6 (C-2'), 74.8 (C-3'), 79.6 (C-5'), 89.3 (C-1'), 105.3 (C-3), 116.6 (CN), 125.6-158.9 (Ar-C); m/z 482 (Found:C, 60.0; H, 4.8; N, 6.0. $C_{24}H_{22}N_2S_2O_5$ calculated C, 59.8; H, 4.6; N, 5.8%).

Biological Procedure

The compounds **4a-d** and **6a-d** were dissolved in dimethyl sulfoxide then diluted 1:100 in cell culture medium before preparing serial half-Log_{10} dilutions. T_4 Lymphocytes were added and after a brief interval HIV-1 was added, resulting in 1:200 final dilution of the compound. Uninfected cells with the compound served as a toxicity control, and infected and uninfected cells without the compound served as basic controls. Cultures were incubated at 37 °C in a 5% carbon dioxide atmosphere for 6 days. The tetrazolium salt, XTT, was added to all wells, and cultures were incubated to allow formazan color development by viable cells. Individual wells were analyzed spectrophotometrically to quantitate formazan production, and in addition were viewed microscopically for detection of viable cells and confirmation of protective activity. Drug-treated virus-infected cells were compared with drug-treated noninfected cells and with other appropriate controls on the same plate. Data were reviewed in comparison with other tests done at the same time and a determination about activity was made.

Acknowledgements

The authors are greatly indebted to Dr. V. L. Narayanan, Drug Synthesis and Chemistry Branch, Department of Health and Human Services, National Cancer Institute, Maryland USA, for supplying in vitro anti-HIV and antitumor activity data for some of our samples.

References

1- G. E. H. Elgemeie, S. E. Elezbawy, H. A. Ali and A. K. Mansour, Bull. Chem. Soc. Jpn., 1994, **67**, 738.

2- G. E. H. Elgemeie, A. M. E. Attia, D. S. Farag and S. M. Sherif, J. Chem. Soc., Perkin Trans, 1, 1994, 1285.

3- G. E. H. Elgemeie and B. A. Hussain, Tetrahedron, 1994, **50**, 199.

4- G. E. H. Elgemeie, A. M. E. Attia, A. M. Elzanate and A. K. Mansour, Bull. Chem. Soc. Jpn., 1994, **67**, 1627.

5- G. E. H. Elgemeie, N. M. Fathy, L. M. Faddah, M. K. Elsaid and M.Y. Ebeid, Arch. Pharm. (Weinheim), 1991, **324**, 149.

6- R. R. Copp and V. E. Marquez. J. Med. Chem, (1991), **34**, 208.

7- T. Miyasaka, H. Tanaka, M. Baba, H. Hayakawa, R. T. Walker, J. Balzarini and E. A. De Clereq, J. Med. Chem., **32**, 2507 (1989).

8- G. E. H. Elgemeie, H. A. Regaila and N. Shehata, J. Chem. Soc. Perkin Trans. 1, 1267 (1990).

9- G. E. H. Elgemeie, A. M. Elzanate and A. K. Mansour, J. Chem. Soc. Perkin Trans. 1, 1073 (1992).

10- G. E. H. Elgemeie, N. M. Fathy and A. M. Attia, J. Chem. Research (S), 292 (1995).

11- U. Niedballa and H. Vorbrüggen, J. Org. Chem., 1974, **39**, 3654.

12- H. Vorbrüggen and K. K, Angew. Chem. Int. Ed. Engl., 1975, **14**, 255.

13- F. .Lichtenthaler, P. Voss and A. Heerd, Tetrahedron Lett., 1974, 2141.

14- G. E. H. Elgemeie, N. M. Fathy , Tetrahedron , 1995 , **51** , 3345 .

Received August 15, 1996
Accepted March 10, 1997