Synthesis of Acyclo-*C*-nucleosides in the Imidazo[1,2-*a*]pyridine and Pyrimidine Series as Antiviral Agents

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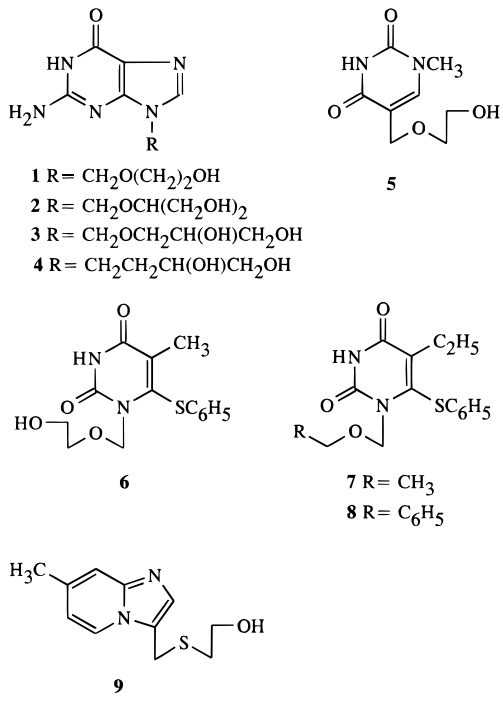
The synthesis and the antiviral activities of C-3 acyclic nucleoside analogues of imidazo[1,2-*a*]pyridine and pyrimidine are reported. From these compounds, **20**, **21**, **22**, **23**, **28**, and **34** showed a specific activity against cytomegalovirus and/or varicella-zoster virus.

Among the antiviral agents, acyclic nucleosides have received much attention. Acyclovir (ACV) (**1**), ganciclovir (GVC) (**2**), iNDG (**3**), and buciclovir (**4**) (Chart 1) are active against herpes simplex virus (HSV), varicella-zoster virus (VZV), and/or cytomegalovirus (CMV).[1] From the reported acyclo-*C*-nucleosides,[2] the 1-methylpseudouridine (**5**) showed activity against HSV-1. Recently, Tanaka et al.[3a] have reported synthesis and antiviral activity of an acyclo-*N*-nucleoside derivative of pyrimidine, bearing a phenylthio group at the position 6, called HEPT (**6**). This compound showed a marked anti-HIV-1 activity and was 20-fold less toxic for the bone marrow than azidothymidine (AZT). In addition, they showed that ethoxymethyl (E-EPU) or benzyloxymethyl (E-BPU) derivatives (**7**, **8**) were active against AZT-resistant HIV-1 strains.[3b,c]

In continuation of our studies on nitrogen bridgehead heterocycles,[4] we were interested in the synthesis and antiviral activity of 3-substituted imidazo[1,2-*a*]azine and -diazine. In previous studies, we have described the preparation[5] and activity of 3-[[(hydroxyethyl)thio]-methyl]-7-methylimidazo[1,2-*a*]pyridine (**9**) against TK$^-$ varicella-zoster virus.[6]

In contrast, some derivatives in the imidazo[1,2-*a*]-pyrimidine series were devoid of antiviral activity.[7] In this work, we report the synthesis and the antiviral activity of side-chain-modified imidazo[1,2-*a*]pyridinic and -pyrimidinic derivatives and the influence of the antiviral activity of the nature and position of various substituents in the imidazo[1,2-*a*]pyridinic ring system.

Chart 1



Chemistry

Substituted imidazo[1,2-*a*]pyridine (**11a**−**f**) were obtained by condensation of suitable 2-aminopyridine (**10**) with bromoacetaldehyde or ethyl bromopyruvate according to modified Roe's procedure.[8] The Vilsmeier−Haack formylation on **11** gave the corresponding aldehyde **12**, which was reduced to the corresponding alcohol **13** using sodium borohydride in methanol. In the case of **12h** the reduction was complicated by transesterification to give the methyl ester derivative **13i**. Proof of the structure was done by ^{1}H-NMR spectroscopy with a methyl absorption at δ 3.95 and at δ 52.2 in ^{13}C-NMR spectroscopy. Further confirmation was given by mass spectroscopy with a *m*/*z* at 206. From the difficulty of extraction and purification of the aldehydes, direct hydroxymethylation was assayed according to the procedure of Teulade,[9] modified as follows. The imidazopyridine derivative **11**, formaldehyde, acetic acid, and sodium acetate were heated at 90 °C in a bomb apparatus for 2 h. The yields and physical properties of the original compounds are reported in Table 1. Preparation of the nucleosides analogs was achieved according

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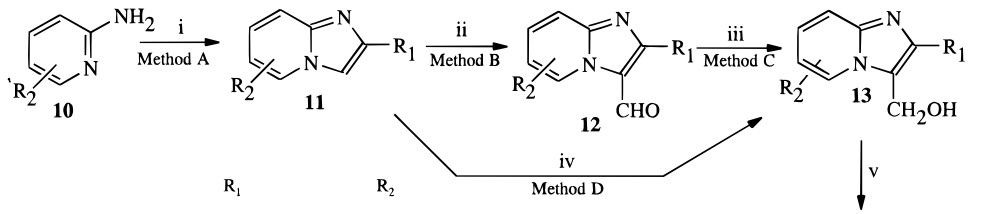
Scheme 1[a]



	R_1	R_2
a	H	H
b	H	7-CH_3
c	H	5-CH_3
d	H	8-CH_3
e	H	6,8-Br_2-7-CH_3
f	H	6,8-Br_2-5,7-$(CH_3)_2$
g	H	6-CF_3-8-Cl
h	$CO_2C_2H_5$	H
i	CO_2CH_3	H

[a] Reagents and conditions: (i) $BrCH_2CHO$ or $BrCH_2COCO_2Et$, EtOH, reflux; (ii) DMF, $POCl_3$; (iii) $NaBH_4$, MeOH; (iv) HCHO, AcONa, AcOH, bomb apparatus; (v) (a) $SOCl_2$, (b) $HSCH_2R_3$, CH_3CN, pyridine, reflux.

Table 1. Preparation Method, Yield, and Melting Points for Original Intermediates

compd	method	yield,[a] %	mp, °C
11e	A	70	103−104
11f	A	55	144−145
11g	A	75	104−105
12a	B	30	111−114
12b	B	59	82
12c	B	15	130
12d	B	50	98−100
12h	B	53	112−114
13b	C	91	131−132
13c	C	98	173−174
13d	C	98	142−143
13e	D	25	195−196
13f	D	35	216−217
13g	D	30	125−126
13i	C	72	180−184

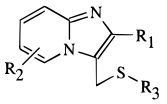
[a] After chromatography.

to our previously described procedure.[5−7] Thus the alcohol **13** was converted to the corresponding halide in refluxing thionyl chloride. After evaporation to dryness, the residue was dissolved in acetonitrile and refluxed in the presence of the suitable thiol and pyridine to give the attempted derivatives (**14**−**33**) (Scheme 1). The structures, yields, and physical properties of these compounds are reported in Table 2.

Biological Assays

Antiviral activity was assayed against a broad range of RNA and DNA viruses. Some compounds showed a marked activity against CMV and VZV (Table 3). The most potent was **22**, but this compound also showed marked cytotoxicity. Substantial antiviral activity against CMV was also noted for **20**, **21**, **23**, **28**, and **34**,while compounds **20**, **21**, and **22** proved to be active against VZV replication. For **28** the cytotoxic concen-

Table 2. Structure and Physical Properties of Synthesized Thioethers



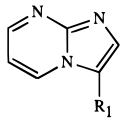
compd	R_1	R_2	R_3	yield,[a] %	mp, °C
14[6]	H	H	CH_2CH_2OH		
15	CO_2Me	H	CH_2CH_2OH	62	147−149
16	H	7-Me	CH_2CH_3	40	oil
17	H	7-Me	$CH_2C_6H_5$	60	117−118
18	H	7-Me	$CH_2CH(OH)CH_3$	45	107−108
19	H	7-Me	$CH_2CH(OH)CH_2OH$	40	118
20	H	7-Me-6,8-Br_2	$CH_2C_6H_5$	30	oil
21	H	8-Me	CH_2CH_3	40	oil
22	H	8-Me	$CH_2C_6H_5$	50	81−82
23	H	8-Me	CH_2CH_2OH	45	106−107
24	H	5-Me	$CH_2C_6H_5$	38	86−87
25	H	5-Me	CH_2CH_2OH	50	109−110
26	H	5-Me	$CH_2CH(OH)CH_3$	35	104−105
27	H	5-Me	$CH_2CH(OH)CH_2OH$	25	113−115
28	H	5,7-Me_2-6,8-Br_2	$CH_2C_6H_5$	55	90−91
29	H	5,7-Me_2-6,8-Br_2	CH_2CH_2OH	50	154−155
30	H	7-Me	CH_2CO_2Et	60	104−106
31	H	7-Me-6,8-Br_2	CH_2CH_2OH	40	oil
32	H	6-CF_3-8-Cl	$CH_2CH_2CH_2OH$	50	oil
33	H	7-Me	$CH_2CH_2CH_2OH$	20	oil

[a] After chromatography.

Table 3. Anti-CMV and -VZV Activities and Cytotoxic Properties in Human Embryonic Lung (HEL) Cells

	minimum inhibitory concentration[a] (μg/mL)						
	CMV		VZV				
compd	AD169 strain	Davis strain	OKA strain TK^+	YS strain TK^+	07/1 strain TK^-	YS/R strain TK^-	minimum cytotoxic concentration[b] (μg/mL)
14	40	35	25	25	ND	25	>100
15	>50	>50	>25	>25	ND	>25	>100
16	>10	10	>10	>10	>10	>10	35
17	3.5	3.5	15	17	ND	10	45
18	>50	>50	>100	>100	ND	100	>100
19	>50	>50	100	>100	ND	50	>100
20	>1	2	2.7	1.8	2.3	2	30
21	2	2	10	5	6.8	>10	35
22	0.2	0.2	0.9	0.3	0.4	>1	8
23	7	6	25	10	15	>10	85
25	>10	>10	>10	>10	>10	>10	40
26	>10	>10	30	20	24	24	90
27	>10	25	>40	>40	>40	>40	75
28	1.7	4	>40	>40	40	40	>100
29	>10	>10	>40	>40	>40	>10	70
30	25	23	>40	>40	>40	>40	80
31	20	30	35	>40	22	20	50
32	>40	>40	>40	>40	>40	>40	>100
33	30	30	>40	>40	>40	>40	>100
34	2	2	8.8	2.4	5	>10	40
35	>40	>40	>40	>40	>40	>40	>100
cidofovir	0.05	0.05	0.04	0.033	ND	0.004	>100
ganciclovir	0.5	0.5					>100
acyclovir			0.82	0.95	25	40	>100
brivudin			0.0009	0.002	>50	>20	>50

[a] Required to reduce virus-induced pathogenicity by 50%. [b] Required to reduce cell proliferation by 50%. ND: not determined.



Compd	R_1
34[7]	$CH_2SCH_2C_6H_5$
35[7]	$CH_2SCH_2CH(OH)CH_3$

tration was higher than for the other active compounds. The compounds did not prove active against HIV-1 and HIV-2 (in CEM cells), HSV-1 (strain KOS), HSV-2 (strain G), vaccinia virus, vesicular stomatitis virus, TK- HSV-1 (strain B2006), TK^-/TK^+ HSV-1 (strain VMW 1837) (in E_6SM cells), parainfluenza-3 virus, reo-1 virus, Sindbis virus, Coxsackie B4 virus, Semliki forest virus (in Vero cells), and vesicular stomatitis virus, coxsackie B4 virus, and polio-1 virus (in HeLa cells) at a concentration up to cytotoxic concentration (Table 4).

Antiviral Assays

Viruses, cells, antiviral assay and methods, and abbreviations are as described previously.[10]

Structure−Activity Relationship

From these results and our previous results some interesting conclusions could be drawn. All the hydroxylated side chain derivatives gave compounds that had little, if any, antiviral efficacy. The best results were obtained with the benzylthiomethyl or ethylthiomethyl substituent. The nature and the position of the substituent on the pyridinic moiety appeared to be more important. Thus, a methyl group at the 7- or 8-position seemed necessary for the antiviral activity. At this point, it seems that derivatives that are unsubstituted

Table 4. Cytotoxicity of Evaluated Compounds against Cells in Culture

	CC_{50} (μg/mL)			
compd	CEM cells[a]	E_6SM cells[b]	Vero cells[b]	HeLa cells[b]
14	29 ± 19	400	400	200
15	87 ± 18	>400	>400	>200
16	11 ± 0.85	40	200	100
17	3.1 ± 0.35	100	40	40
18	60 ± 18	>400	>400	400
19	>100	>400	>400	400
20	5 ± 1.4	40	40	40
21	4.1 ± 0.64	200	400	10
22	0.5 ± 0.02	40	100	4
23	9.5 ± 1.2	400	400	40
24	9.9 ± 2.0	10	40	40
25	22 ± 4.8	200	200	100
26	27 ± 0.49	400	>400	100
27	45 ± 3.2	400	400	>100
28	35 ± 18	>40	>40	>100
29	17 ± 4.1	100	>200	>200
30	37 ± 1.3	200	>200	>100
31	37 ± 6.9	>200	>40	>100
32	27 ± 3.1	100	>100	100
33	17 ± 1.8	>100	>40	40
34	4.2 ± 0.64	100	200	40
35	>100	>400	>400	400
zidovudine	>125			
didanosine	>100			
ganciclovir		>100		
acyclovir		>400		
brivudin		>400	>400	>400
ribavirin		>400	>400	>200
(*S*)-DHPA			>400	>400
C-c3Ado			>400	>400

[a] 50% cytotoxic concentration, or concentration required to reduce CEM cell viability by 50%. [b] 50% cytotoxic concentration, or concentration required to cause a microscopically detectable alteration of normal cell morphology.

at position 2 are the most potent compounds. The activity of imidazo[1,2-*a*]pyrimidine **34** requires the synthesis of further compounds in this series in order to make a structure−activity relationship analysis. Finally, from a comparison of **20** with **28** it appears that the methyl group at position 5 diminishes the cytotoxicity.

Conclusions

The thioether derivatives reported here appear to be original antiviral agents. The fact that they show selective activity against CMV and VZV suggests that they are preferentially metabolized by the virus-infected cells and/or show a particullary affinity for virus-specific enzymes. Rationalization of these results using molecular modeling is currently under investigation, while the synthesis of new compounds is in progress.

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Supporting Information Available: General experimental procedures, including ^{1}H and ^{13}C NMR spectral data for all new compounds (5 pages). Ordering information is given on any current masthead page.

References

(1) De Clercq, E. Recent advances in the search for selective antiviral agents. *Adv. Drug Res.* **1988**, *17*, 1−59.

(2) Chu, C. K.; Butler, S. J. Chemistry and antiviral activities of acyclonucleotides. *J. Heterocycl. Chem.* **1986**, *23*, 289−319.

(3) (a) Miyasaka, T.; Tanaka, B.; Baba, M.; Hayakawa, H.; Walker, R. T.; Balzarini, J.; De Clercq, E. A novel lead for specific anti-HIV-1 agents: 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine. *J. Med. Chem.* **1989**, *32* (12), 2507−2509. (b) Tanaka, H.; Baba, M.; Ubasawa, M.; Takashima, H.; Sekiya, K.; Nitta, I.; Shigeta, S.; Walker, R. T.; De Clercq, E.; Miyasakia, T. Synthesis and anti-HIV-1 activity of 2-, 3- and 4-substituted analogues of 1-[-(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT). *J. Med. Chem.* **1991**, *34* (4), 1394−1399. (c) Tanaka, H.; Baba, M.; Saito, S.; Miyasaka, T.; Takashima, H.; Sekika, K.; Ubasawa, M.; Nitta, I.; Walker, R. T.; Nakashima, H.; De Clercq, E. Specific anti-HIV-1 "acyclonucleosides" which cannot be phosphorylated: synthesis of some deoxy analogues of 1-[-(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine. *J. Med. Chem.* **1991**, *34* (4), 1508−1511.

(4) (a) Gueiffier, A.; Milhavet, J. C.; Blache, Y.; Chavignon, O.; Teulade, J. C.; Madesclaire, M.; Viols, H.; Dauphin, G.; Chapat, J. P. Synthesis of 1*H*-imidazo[1,2-*a*]pyrazolo[3,4-*c*]pyridines. *Chem. Pharm. Bull.* **1990**, *38* (9), 2352−2356. (b) Gueiffier, A.; Blache, Y.; Viols, H.; Chapat, J. P.; Chavignon, O.; Teulade, J. C.; Dauphin, G.; Debouzy, J. C.; Chabart, J. C. Carbon-nitrogen bond formation in cyclisation by deoxygenation, thermolysis or photolysis of phenylimidazo[1,2-*a*][1,8]naphthyridine. *J. Heterocycl. Chem.* **1992**, *29*, 283−287.

(5) Elhakmaoui, A.; Gueiffier, A.; Debouzy, J. C.; Blache, Y.; Chavignon, O.; Teulade, J. C.; Milhavet, J. C.; Dauphin, G.; Chapat, J. P. Synthesis of acyclo-C-nucleosides of imidazo[1,2-*a*]pyridine as potential antiviral agents; Studies of model membrane passage. *Boll. Chim. Farm.* **1995**, submitted.

(6) Elhakmaoui, A.; Gueiffier, A.; Milhavet, J. C.; Blache, Y.; Chapat, J. P.; Chavignon, O.; Teulade, J. C.; Snoeck, R.; Andrei, G.; De Clercq, E. Synthesis and antiviral activity of 3-substituted imidazo[1,2-*a*]pyridine. *BioOrg. Med. Chem. Lett.* **1994**, *4* (16), 1937−1940.

(7) Gueiffier, A.; Blache, Y.; Chapat, J. P.; Alhakmaoui, A.; Essassi, E. M.; Andrei, G.; Snoeck, R.; De Clercq, E.; Chavignon, O.; Teulade, J. C.; Fauvelle, F. Synthesis and antiviral activity of 2 and 3-substituted imidazo[1,2-*a*]pyrimidines. *Nucleosides Nucleotides* **1995**, *14* (3−5), 551−554.

(8) Roe, A. M. The thermal condensation of imidazoles with carbonyl compounds. *J. Chem. Soc.* **1963**, 2195−2200.

(9) Teulade, J. C.; Bonnet, P. A.; Rieu, J. N.; Viols, H.; Chapat, J. P.; Grassy, G.; Carpy, A. C-3 hydroxylation of some imidazo-[1,2-*a*]azines, *J. Chem. Res.* (*M*) **1986**, 1842−1874.

(10) (a) De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R. T.; Jones, A. S.; Torrence, P. F.; Shugar, D. Comparative efficacy of antiherpes drugs against different strains of herpes simplex virus. *J. Infect. Dis.* **1980**, *141* (5), 563−574. (b) De Clercq, E. Antiviral and antimetabolic activities of neplanocins. *Antimicrob. Agents Chemother.* **1985**, *28* (1), 84−89. (c) De Clercq, E.; Holy, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. A novel selective broad-spectrum anti-DNA virus agents. *Nature* **1986**, *323* (6087), 464−467. (d) Pauwels, R.; Balarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. *J. Virol. Methods* **1988**, *20*, 309−321. (e) Schols, D.; De Clercq, E.; Balzarini, J.; Baba, M.; Witvrouw, M.; Hosoya, M.; Andrei, G.; Snoeck, R.; Neyts, J.; Pauwels, R.; Nagy, M.; Györgyi-Edelényi, J.; Machovich, R.; Horvath, I.; Löw, M.; Görög, S. Sujphated polymers are potent and selective inhibitors of various enveloped viruses, including herpes simplex virus, cytomegalovirus, vesicular stomatitis virus, respiratory synctial virus and toga-, arena- and retroviruses. *Antiviral Chem. Chemother.* **1990**, *1* (4), 233−240. (f) De Clercq, E. in vitro and ex-viro test systems to rationalize drug design and delivery. In *Minutes collection*; Crommelin, D., Couvreur, P., Duchêne, D., Eds.; 1994; pp 108−125.

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