Synthesis of Penicillamine- and Cysteine-containing Nucleoamino Acids as Potential Antivirals and Aminopeptidase B Inhibitors

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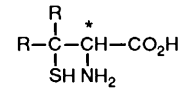
Nucleoamino acids, wherein D- and L-penicillamine and D-and L-cysteine are attached to uridine or thymidine through a carboxylic ester linkage, have been synthesized and evaluated as antivirals and aminopeptidase B (AP-B) inhibitors. The coupling of the α-amino-β-mercapto acids, *N,S*-protected as *N*-formylthiazolidines, to 2',3'-*O*-isopropylideneuridine, 3'-*O*-acetylthymidine, 5'-*O*-tritylthymidine, and thymidine was achieved *via* the mixed anhydride formed from *N,N*-bis-(2-oxooxazolidin-3-yl)phosphorodiamidic chloride and the corresponding protected amino acid in the presence of 4-(dimethylamino)pyridine. Treatment of the protected compounds with 1 mol dm^{-3} HCl in refluxing MeOH, under argon, afforded the corresponding deprotected nucleoamino acids free of racemization. Neither the compounds herein described nor D-penicillamine showed anti-HIV-1 activity in MT-4 cells or antiviral activity against some other viruses at concentrations below the cytotoxicity threshold. Penicillamine and cysteine monoesters were equipotent with the corresponding free amino acid in inhibiting AP-B with an IC_{50} in the 10^{-4} mol dm^{-3} range, while the bis-(α-amino-β-mercaptoacyl)thymidine derivatives were approximately twice as potent as the monoesters.

D-Penicillamine **1a**, a well known drug clinically used for treatment of various diseases such as rheumatoid arthritis, Wilson's disease, or cystinuria,[1] has been reported as an inhibitor of replication of HIV-1 (causative agent of AIDS) in H9 cell cultures by Chandra and Sarin.[2] Later studies from these authors[3] indicated that this antiviral activity could be due to the inhibition of transactivation of HIV-1 LTR by the transactivator protein TAT, which is a cysteine-rich protein,[4] either by forming stable interdisulphide bonds with these cysteine residues or by chelation of metal ions that are normally bound to TAT. Based on these results, clinical trials with **1a** in HIV-1-infected patients were initiated, from which it has been implied that D-penicillamine **1a** may be an effective drug for suppressing HIV-1 expression *in vivo*.[5] Taking into account that TAT is a nucleic acid binding protein specific to HIV-1, and with the aim of enhancing the anti-HIV-1 activity and selectivity of compound **1a**, we have synthesized D-penicillamine–nucleoside conjugates, as well as the analogue nucleoamino acids containing L-penicillamine **1b**, and D- and L-cysteine **2a** and **2b**, by attachment of these α-amino-β-mercapto acids to uridine and thymidine. All these compounds were evaluated against HIV-1 in MT-4 cells, and against some other viruses in cell cultures.

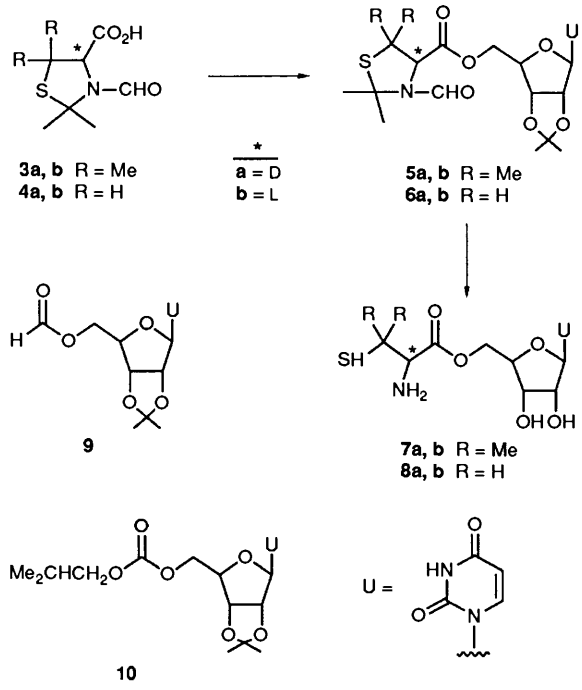


	R	*
1a	Me	D
1b	Me	L
2a	H	D
2b	H	L

The effectiveness of penicillamine **1a** in treating rheumatoid arthritis, decreasing serum immunoglobulin levels and rheumatoid factor titres,[6] along with the induction of some autoimmune diseases by compound **1a**, suggest the possibility of an immunoregulatory role for this compound.[7] Considering these facts, and the importance of cell surface-bound aminopeptidases in the modulation of the immune response,[8] we have recently studied[9] the inhibition of aminopeptidase B (AP-B) by compounds **1a** and **2b**. We now describe the inhibition of AP-B by these penicillamine- and cysteine-nucleoside conjugates.

Results and Discussion

Chemistry.—As indicated in Schemes 1 and 2, for the synthesis of the penicillamine- and cysteine-containing nucleoamino acids **7a**, **b**, **8a**, **b** and **17a–22b** we have used these α-amino-β-mercapto acids, protected as the *N*-formylthiazolidine derivatives **3a**, **b**[10] and **4a**, **b**.[11] These derivatives, on the one hand, exhibit a higher resistance to racemization compared



Scheme 1

Table 1 Physical and analytical data for the nucleoamino acids **5a, b** **22b**

Compound	Yield (%)	M.p. (°C)	$[\alpha]_D^{20}$ (°) (*c*, solvent)	t_R HPLC (min) (solvent)[a]	Formula	Found (%) (Required) C	H	N
5a	75	*b*	+25 (1.00, MeOH)		$C_{21}H_{29}N_3O_8S$	52.1 (52.2)	5.9 (6.0)	8.5 (8.7)
5b	76	*b*	+2.4 (0.99, MeOH)		$C_{21}H_{29}N_3O_8S$	52.0 (52.2)	5.8 (6.0)	8.5 (8.7)
6a	99	*b*	+72 (0.95, MeOH)		$C_{19}H_{25}N_3O_8S$	49.9 (50.1)	5.4 (5.5)	9.2 (9.2)
6b	97	*b*	−29 (1.00, MeOH)		$C_{19}H_{25}N_3O_8S$	49.9 (50.1)	5.2 (5.5)	8.9 (9.2)
7a	66	139[c]	+16 (0.99, water)	26.55 (A) 4.50 (C)	$C_{14}H_{21}N_3O_7S\cdot HCl$[d] $0.5\ Me_2CHOH\cdot H_2O$	39.6 (40.0)	5.9 (6.0)	9.3 (9.0)
7b	50	137[c]	+25 (1.00, water)	21.21 (A) 6.43 (C)	$C_{14}H_{21}N_3O_7S\cdot HCl$[d] $0.5\ Me_2CHOH\cdot H_2O$	39.8 (40.0)	5.7 (6.0)	9.3 (9.0)
8a	60	118[c]	+40 (0.88, water)	6.64 (B) 4.50 (D)	$C_{12}H_{17}N_3O_7S\cdot HCl$[d] $0.5\ Me_2CHOH\cdot H_2O$	37.3 (37.5)	5.1 (5.3)	10.0 (9.7)
8b	72	123[c]	+12 (0.96, water)	5.53 (B) 4.68 (D)	$C_{12}H_{17}N_3O_7S\cdot HCl$[d] $0.5\ Me_2CHOH\cdot H_2O$	37.3 (37.5)	5.4 (5.3)	10.0 (9.7)
11a	71	*b*	+28.8 (1.22, MeOH)		$C_{21}H_{29}N_3O_8S$	52.1 (52.2)	6.3 (6.0)	8.7 (8.7)
12b	80	*b*	−29 (1.28, MeOH)		$C_{19}H_{25}N_3O_8S$	49.9 (50.1)	5.8 (5.5)	9.5 (9.2)
13a	70	*b*	+39.3 (0.89, MeOH)		$C_{38}H_{41}N_3O_7S$	66.4 (66.8)	5.8 (6.0)	6.3 (6.2)
14b	99	*b*	−26.3 (1.18, MeOH)		$C_{36}H_{37}N_3O_7S$	65.7 (65.9)	5.7 (5.7)	6.5 (6.4)
15a	70	*b*	+51 (1.05, MeOH)		$C_{28}H_{40}N_4O_9S_2$	52.5 (52.5)	6.5 (6.3)	8.7 (8.7)
16b	99	*b*	−54 (1.02, MeOH)		$C_{24}H_{32}N_4O_9S_2$	49.5 (49.3)	5.7 (5.5)	9.5 (9.6)
17a	74	*e*	−0.4 (0.94, water)	5.19 (C) 5.46 (D)	$C_{15}H_{23}N_3O_6S\cdot HCl$[d] $0.5\ Me_2CHOH\cdot H_2O$	43.6 (43.3)	6.3 (6.6)	9.2 (9.2)
18b	65	113[c]	0 (0.96, water)	16.76 (A) 4.57 (C)	$C_{13}H_{19}N_3O_6S\cdot HCl$[d] $0.5\ Me_2CHOH\cdot H_2O$	40.7 (40.5)	5.9 (6.3)	10.0 (9.8)
19a	54	153[c]	+1.6 (0.90, water)	5.75 (C) 5.64 (D)	$C_{15}H_{23}N_3O_6S\cdot HCl$[d] $0.5\ Me_2CHOH\cdot H_2O$	43.6 (43.3)	6.3 (6.6)	9.3 (9.2)
20b	69	133[c]	−6 (0.94, water)	24.30 (A) 4.47 (C)	$C_{13}H_{19}N_3O_6S\cdot HCl$[d] $0.5\ Me_2CHOH\cdot H_2O$	40.6 (40.5)	6.0 (6.3)	9.7 (9.8)
21a	74	188[c]	+5 (0.98, water)	12.85 (C)	$C_{20}H_{32}N_4O_7S_2\cdot 2HCl$[d] $0.5\ Me_2CHOH\cdot H_2O$	41.1 (41.3)	6.5 (6.4)	9.0 (9.0)
22b	90	*e*	+1 (0.90, water)	8.91 (C)	$C_{16}H_{22}N_4O_7S_2\cdot 2HCl$[d] $Me_2CHOH\cdot 2H_2O$	37.2 (37.0)	6.10 (5.8)	8.8 (9.1)

[a] A 2.5 mmol dm^{-3} sodium octanesulphonate in water–MeOH–AcOH (75:25:0.1 v/v); B 2.5 mmol dm^{-3} sodium octanesulphonate in water–MeOH–AcOH (50:50:0.1); C water–MeOH–AcOH (90:10:0.1); D water–MeOH–AcOH (50:50:0.1). [b] Foam solids. [c] Decomp. and from Me_2CHOH. [d] Me_2CHOH was also detected by ^1H NMR spectroscopy. [e] Very hygroscopic, m.p. could not be measured.

with other *N*,*S*-protected α-amino-β-mercapto acids,[12] and on the other, are easily opened by mild acid or basic solvolysis.[11,13] By contrast, removal of other *S*-blocking groups requires harsh conditions (*i.e.*, HF, Hg^{II}, Na–NH_3), which generally give multiple side-reactions.[14]

Initial attempts to obtain the uridinyl esters **5** by the *N*,*N*′-dicyclohexylcarbodiimide (DCC)-mediated condensation of compounds with 2′,3′-*O*-isopropylideneuridine in 1,2-dimethoxyethane (DME) afforded the 5′-*O*-formyluridine derivative **9** as the only reaction product (Scheme 1). Although a similar condensation using compound **4** led to esterification of the carboxylic acid in 53% yield, 50% racemization occurred, as detected by the ^1H NMR spectra of the products **6a, b** and reverse-phase HPLC analysis (solvent system B) of the corresponding deprotected compounds **8a, b** (Tables 1 and 2). In view of these results, the following amino acid esterification methods were attempted: (a) transesterification of the active *N*-hydroxysuccinimide esters of compounds **3** and **4**, (b) activation of the carboxylic acids **3** and **4** by formation of a mixed anhydride with isobutyl chloroformate,[15] (c) direct esterification of acids **3** and **4** with 2′,3′-*O*-isopropylideneuridine in pyridine and in the presence of toluene-*p*-sulphonyl chloride,[16] and, (d) treatment of acids **3** and **4** with *N*,*N*-bis-(2-oxooxazolidin-3-yl)phosphorodiamidic chloride (BOP-Cl)[17] in pyridine, followed by *in situ* reaction[18] with 2′,3′-*O*-isopropylideneuridine. While no reaction took place following method (a), and the 5′-*O*-isobutoxycarbonyluridine derivative **10** was formed by method (b), the esters **5a, b** and **6a, b** were obtained in 36 and 43% yield, respectively, but with 100% racemization, when method (c) was used. The best results were found by application of method (d) in the presence of 4-(dimethylamino)pyridine (DMAP) (1 mol equiv.). In this manner, compounds **5a, b** and **6a, b** were obtained, free of racemization, in 75 and 99% yield, respectively. When this method was followed in the absence of DMAP, 5% racemization was detected by reverse HPLC analysis of the corresponding deprotected derivatives and, in the case of the penicillamine derivatives **3**, a mixture of compounds **5a, b** and **9** was obtained in a 1:1 ratio.

Treatment of esters **5a, b** and **6a, b** with 1 mol dm^{-3} HCl in refluxing MeOH, under argon to prevent oxidation, provided the fully deprotected nucleoamino acids **7a, b** and **8a, b** as their hydrochlorides, free of racemization.

It is important to note that the cysteine derivatives **8a, b** were unstable in solution due to their great tendency to oxidize to the

Table 2 Relevant ^{1}H NMR data (δ) of the nucleoamino acids **5a, b–22b**

		Nucleoside					α-amino-β-mercapto acid		
Compound	Solvent	1′-H	$J_{1',2'}$ (Hz)	5′-H	3′-H	3-NH	α-H	β-H	SH
5a	$CDCl_3$	5.77	2	4.65, 4.27	4.79	9.00	4.69		
5b	$CDCl_3$	5.67	2	4.53, 4.33	4.84	8.69	4.74		
6a	$CDCl_3$	5.73	2.5	4.42, 4.48	4.82	8.43	5.02	3.33, 3.28	
6b	$CDCl_3$	5.60	2	4.44	4.85	9.25	5.08	3.34, 3.27	
7a	$(CD_3)_2SO$	5.79	5	4.44, 4.39	4.04	11.39	4.21		3.49
7b	$(CD_3)_2SO$	5.79	5.5	4.43, 4.39	4.02	11.39	4.18		3.49
8a	$(CD_3)_2SO$	5.77	5	4.45, 4.38	4.04	11.40	4.45	3.01	2.91
8b	$(CD_3)_2SO$	5.76	5	4.45, 4.36	4.05	11.38	4.45	3.01	3.01
11a	$(CD_3)_2SO$	6.17	6 8	4.41, 4.32	5.15	11.39	4.67		
12b	$(CD_3)_2SO$	6.16	6.5, 7.5	4.33	5.20	11.33	4.49	3.49, 3.21	
13a	$CDCl_3$	6.56	4.5, 8.5	3.50	5.56	9.55	4.71		
14b	$(CD_3)_2SO$	6.23	6, 8	3.32, 3.25	5.36	11.40	4.92	3.45, 3.21	
15a	$CDCl_3$	6.34	5.5, 9	4.88, 4.25	5.30	8.53	4.72, 4.70		
16b	$CDCl_3$	6.23	5, 9	4.49	5.32	9.00	5.04	3.37, 3.26	
17a	$(CD_3)_2SO$	6.20	7	4.38	4.29	11.34	4.19		3.56
18b	$(CD_3)_2SO$	6.18	7	*a*	*a*	11.31	*a*	3.30	2.88
19a	$(CD_3)_2SO$	6.28	6, 8	3.65	5.38	11.36	4.16		3.68
20b	$(CD_3)_2SO$	6.27	7.5	3.64	5.37	11.34	4.38	3.04	3.04
21a	$(CD_3)_2SO$	6.27	6, 9	4.43, 4.54	5.42	11.43	4.17, 4.25		3.73, 3.78
22b	$(CD_3)_2SO$	6.26	6, 8	4.47	5.43	11.41	4.41	3.08	3.08, 3.20

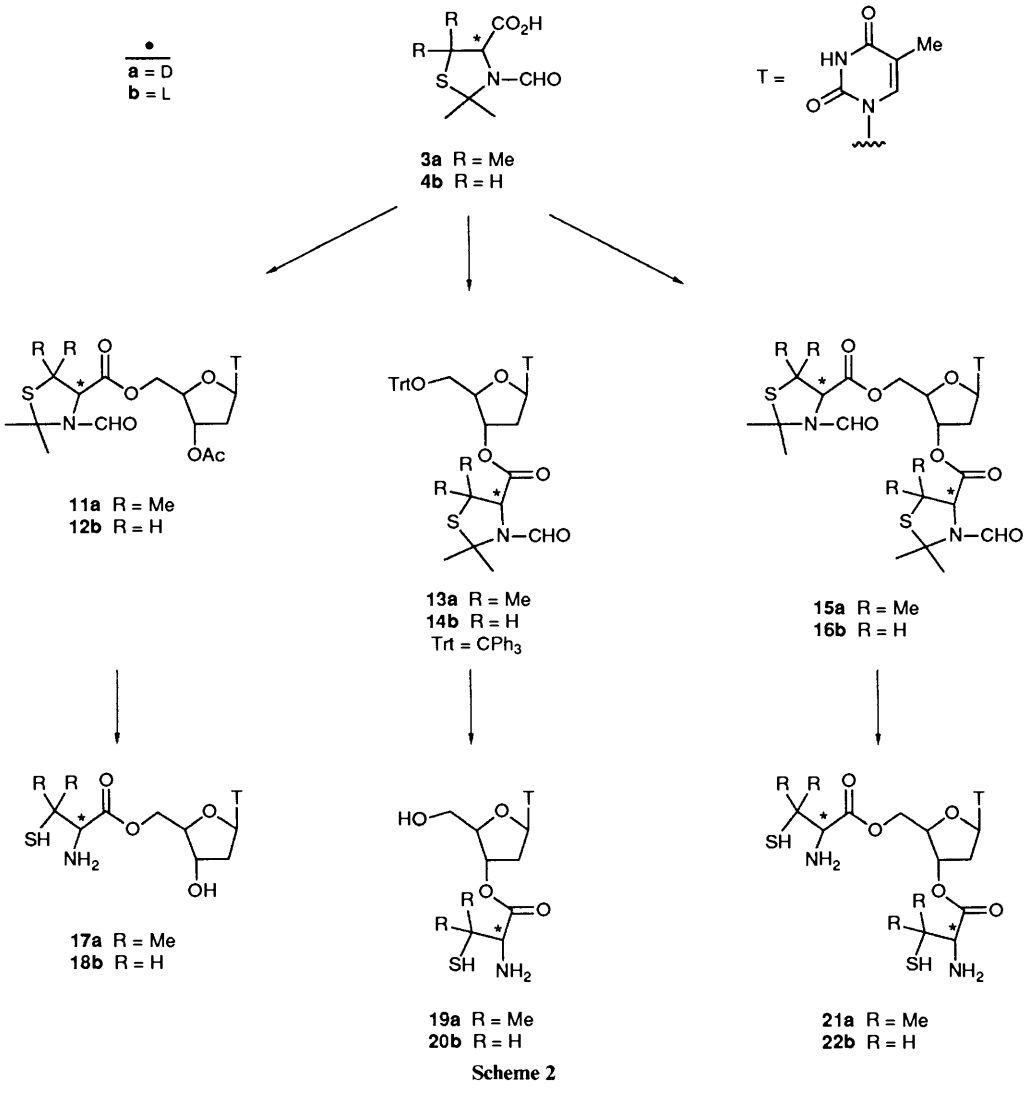
[a] Complex multiplet.

cystine analogues.[14] Thus, 50% oxidation was determined by the duplicity of signals and the decreased peaks corresponding to the SH protons in the ^{1}H NMR spectra of these compounds after 24 h in $(CD_3)_2SO$. This oxidation was also detected by the appearance (HPLC; solvent system D) of additional peaks at 2.77 min for **8a** and 2.80 min for **8b** which comprised up to 80% of the mixture after 4 days in MeOH solution. However, compounds **8a, b** remained stable after drying, and storage in a refrigerator in the absence of moisture. No oxidation was observed for the penicillamine analogues **7a, b**.

As indicated in Scheme 2, the thymidine derivatives **17a–22b** were obtained following a similar synthetic pathway to that used for the synthesis of the aforementioned uridinyl derivatives. Thus, the 5′-*O*- and 3′-*O*-acylated compounds **11a** and **12b**, and **13a** and **14b**, were prepared, respectively by acylation of 3′-*O*-acetylthymidine and 5′-*O*-tritylthymidine with the carboxylic acids **3a** or **4b**, previously activated by reaction with BOP-Cl in pyridine, and in the presence of DMAP (1 mol equiv.). The acylation of thymidine with 2 mol equiv. of the protected α-amino-β-mercapto acids **3a** and **4b** led to the 3′,5′-di-*O*-acyl analogues **15a** and **16b**, while when this condensation was carried out with 1 mol equiv. of acid **3a** and **4b**, a 3:1:2 mixture of the corresponding 5′- and 3′-*O*-acyl and 3′,5′-di-*O*-acyl derivatives was obtained. Deprotection of thymidine derivatives **11a–16b**, as indicated for the uridinyl derivatives **5a, b** and **6a, b**, afforded the fully deprotected compounds **17a–22b** as their hydrochlorides. As before, the ^{1}H NMR spectra of all these thymidine derivatives (Table 2) and HPLC analysis of the deprotected compounds (Table 1) showed the lack of racemization, and the instability of the cysteine-containing derivatives **18b**, **20b** and **22b** in solution. In this sense, compound **22b** bearing two cysteine residues was markedly unstable, since the ^{1}H NMR spectrum and chromatogram (solvent system C) of a recently dissolved sample indicated that 20% oxidation had already occurred.

The structural assignment of all these penicillamine- and cysteine-containing nucleoamino acids was based on their analytical and ^{1}H NMR spectroscopic data (Tables 1 and 2). The presence of a singlet at δ 8.5–9.5 in $CDCl_3$ or δ 11.4 in $(CD_3)_2SO$ in their ^{1}H NMR spectra, corresponding to the 3-NH pyrimidine proton, and the downfield chemical shift of the 5′-H protons in the uridinyl or thymidinyl derivatives **5a, b, 6a, b, 11a** and **12b** as compared with the same protons of the corresponding starting nucleosides, established the aminoacylation site. A similar downfield shift of the 3′- or 3′- and 5′-H protons was observed in the case of the thymidinyl derivatives **13a** and **14b** or **15a** and **16b**.

Biological Data.—Antiviral activity. All the synthesized nucleoamino acids were evaluated as anti-HIV-1 ($HTLV\text{-}III_B$) agents in MT-4 cells and as antivirals against some other viruses, *i.e.* herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), vaccinia virus (VV) and vesicular stomatitis virus (VSV) in primary rabbit kidney cell cultures;



Scheme 2

VSV, Coxsackie virus B4 and polio virus 1 in HeLa cells; parainfluenza-3 virus, reovirus, Sindbis virus, Coxsackie virus B4 and Semliki forest virus in Vero cell cultures. In all the assays, D-penicillamine **1a** was also included as a model compound. Neither **1a** nor any of the nucleoamino acids showed activity below the cytotoxicity threshold against these viruses. In view of the reports of Chandra and co-workers on the anti-HIV-1 activity of D-penicillamine in H9 cell cultures,[2,3,5] the lack of activity of this compound in our assay systems may be surprising. Perhaps this disagreement could be due to the differences in the assay systems. At this point, it is interesting to note that Bitterlich *et al.*[19] have recently found that D-penicillamine **1a**, when incubated with HIV-1-infected H9 cells for 8 days, did not exhibit appreciable anti-HIV-1 activity, while when incubated with the cells for 6 weeks it reduced the number of HIV-1-infected H9 cells.

Aminopeptidase B inhibition. The deprotected nucleoamino acids **7a**, **b**, **8a**, **b** and **17a**–**22b** were evaluated as inhibitors of AP-B activity, which is associated with the surface of murine L cells, using L-lysine β-naphthylamide (Lys-NA) as substrate.[8a] The α-amino-β-mercapto acids **1a** and **2b**, which are moderate inhibitors[9] of AP-B, were included for comparison purposes. As shown in Table 3, the penicillamine or cysteine monoesters **7a**, **b**, **17a** and **19a** or **8a**, **b**, **18b** and **20b**, regardless of the nature and configuration of the amino acid and nucleoside moiety, were equipotent with compounds **1a** and **2b**, with IC_{50} values in the 10^{-4} mol dm^{-3} range. The diesters **21a** and **22b** were approximately twice as potent as the monoesters. These observations suggest that the esters and diesters are hydrolysed to the corresponding amino acid which should be the true inhibitor of AP-B.

Table 3 Inhibitory potency of the compounds on AP-B associated with the surface of mouse L Cells

Compound	IC_{50}[a] ($\times 10^{-4}$ mol dm^{-3})	Compound	IC_{50}[a] ($\times 10^{-4}$ mol dm^{-3})
7a	6.0	**19a**	6.1
7b	5.5	**20b**	6.9
8a	6.7	**21a**	2.7
8b	6.5	**22b**	3.5
17a	5.5	**1a**	6.7
18b	6.5	**2b**	6.4

[a] Values are the mean of 4–5 experiments with 3–5 different concentrations of the inhibitor. Standard errors were less than 10% of the mean.

Experimental

Chemistry.—All reagents were of commercial quality. Solvents were dried and purified by standard methods. M.p.s were taken on a Reichert-Jung Kofler micro hot stage apparatus and are uncorrected. Elemental analyses were obtained using a

Heraeus CHN-O-RAPID instrument. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. ^{1}H NMR spectra were recorded with a Varian EM-390 (90 MHz) and a Varian XL-300 (300 MHz) spectrometer, with $SiMe_4$ as internal standard. Analytical TLC was performed on aluminium sheets coated with a 0.2 mm layer of silica gel 60 F_{254}, obtained from Merck. Silica gel 60 (230–400 mesh), also a Merck product, was used for flash chromatography. Reverse-phase HPLC analyses were performed on a μ-Bondapak C-18 stainless-steel column (3.9 × 300 mm) in a Waters Associates instrument fitted with a 254 nm wavelength UV detector, with a flow rate of 1 cm^3 min^{-1}, and as the mobile phase the following solvent systems: A: water–MeOH–AcOH (75:25:0.1 v/v) with 2.5 mmol dm^{-3} sodium octanesulphonate; B: water–MeOH–AcOH (50:50:0.1) with 2.5 mmol dm^{-3} sodium octanesulphonate; C: water–MeOH–AcOH (90:10:0.1); D: water–MeOH–AcOH (50:50:0.1).

General Procedure for the Synthesis of the Protected 5′-O-(α-*Amino*-β-*mercaptoacyl*)*uridine Derivatives* **5a**, **b** *and* **6a**, **b** *and* 5′- *and* 3′-O-(α-*Amino*-β-*mercaptoacyl*)*thymidine Derivatives* **11a**, **12b**, **13a** *and* **14b**.—BOP-Cl (3.5 mmol) was added to a solution of the appropriate 3-formyl-2,2-dimethylthiazolidine-4-carboxylic acid **3a**, **b** or **4a**, **b** (3 mmol) in dry pyridine (10 cm^3) cooled at 0 °C. After being stirred for 30 min at this temperature the mixture was treated with 2′,3′-*O*-isopropylideneneuridine, 3′-*O*-acetylthymidine, or 5′-*O*-tritylthymidine (3 mmol), along with DMAP (3 mmol). This mixture was stirred at 0 °C for 24 h, and then evaporated to dryness. The residue was taken up in $CHCl_3$ (100 cm^3), washed successively with water (20 cm^3) and brine (20 cm^3) and dried over Na_2SO_4, and after evaporation the residue was purified by flash chromatography with $CHCl_3$–MeOH mixtures as eluant. The properties and analytical and spectral data for the protected compounds thus obtained, **5a**, **b**, **6a**, **b**, **11a**, **12b**, **13a** or **14b**, are summarized in Tables 1 and 2.

General Procedure for the Preparation of the Protected 3′,5′-O-*bis*-(α-*amino*-β-*mercaptoacyl*)*thymidine Derivatives* **15a** *and* **16b**.—BOP-Cl (3.5 mmol) was added to a solution of the appropriate 3-formyl-2,2-dimethylthiazolidine-4-carboxylic acid **3a** or **4b** (3 mmol) in dry pyridine (10 cm^3) cooled at 0 °C. After the mixture had been stirred for 30 min at this temperature, thymidine (1 mmol) and DMAP (3 mmol) were added, and the mixture was stirred at 0 °C for 24 h and then worked up as above. The properties and data for compounds **15a** and **16b** are summarized in Tables 1 and 2.

General Procedure for the Preparation of Compounds **7a**, **b**, **8a**, **b** *and* **17a–22b** *by Deprotection of Compounds* **5a**, **b**, **6a**, **b** *and* **11a–16b**.—A solution of the corresponding protected α-amino-β-mercaptoacyl nucleoside **5a**, **b**, **6a**, **b** or **11a–16b** (1 mmol) in 1 mol dm^{-3} HCl in MeOH (20 cm^3) was refluxed under argon for 4 h. The solvent was evaporated off under argon, and the residue was crystallized from propan-2-ol. The analytical data of the deprotected nucleoamino acids **7a**, **b**, **8a**, **b** and **17a–22b** are summarized in Table 1, and their relevant ^{1}H NMR in Table 2.

5′-O-*Formyl*-2′,3′-O-*isopropylideneneuridine* **9**.—DCC (0.63 g, 3 mmol) was added to a solution of compound **3a** (0.65 g, 3 mmol) and 2′,3′-*O*-isopropylideneuridine (0.71 g, 2.5 mmol) in dry DME (25 cm^3). After the mixture had been stirred at room temperature for 48 h, the white precipitate was filtered off, and washed with DME and the filtrates were evaporated to dryness. The residue was purified by flash chromatography [EtOAc–hexane (1:2)] to afford *title compound* **9** as a solid (0.15 g, 65%), m.p. 146—148 °C (from propan-2-ol); (Found: C, 49.9; H, 5.1; N, 8.9. $C_{13}H_{16}N_2O_7$ requires C, 50.0; H, 5.2; N, 9.0%); $[\alpha]_D^{20}$ −4.3° (*c* 1.00, MeOH); δ_H(300 MHz; $CDCl_3$) 1.36 and 1.58 (6 H, 2 s, isopropylidene group), 4.41 (3 H, m, 4′-H and 5′-H_2), 4.87 (1 H, dd, *J* 4 and 6.5 Hz, 3′-H), 5.05 (1 H, dd, *J* 2 and 6.5 Hz, 2′-H), 5.61 (1 H, d, *J* 2 Hz, 1′-H), 5.75 (1 H, d, *J* 8 Hz, 5-H), 7.25 (1 H, d, *J* 8 Hz, 6-H), 8.09 (1 H, s, HC=O), 9.45 (br s, NH).

5′-O-*Isobutoxycarbonyl*-2′,3′-O-*isopropylideneuridine* **10**.—Isobutyl chloroformate (0.52 cm^3, 4 mmol) and triethylamine (0.56 cm^3, 4 mmol) were added to a solution of compound **3a** (0.65 g, 2 mmol) in dry CH_2Cl_2 (20 cm^3) cooled to −10 °C. After being stirred for 30 min at this temperature, the mixture was treated with 2′,3′-*O*-isopropylideneuridine (0.56 g, 2 mmol) and triethylamine (0.28 cm^3, 2 mmol) and stirred overnight. Then the reaction mixture was diluted with CH_2Cl_2 (50 cm^3), washed successively with water (15 cm^3) and brine (15 cm^3), dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography [EtOAc–hexane (1:1)] to afford *title compound* **10** as a solid (0.68 g, 92%), m.p. 134–136 °C (from propan-2-ol) (Found: C, 55.1; H, 6.7; N, 7.8. $C_{17}H_{24}N_2O_7$ requires C, 55.3; H, 6.6; N, 7.6%); $[\alpha]_D^{20}$ +3.74° (*c* 1.00, MeOH); δ_H(300 MHz; $CDCl_3$) 0.89 (6 H, d, *J* 6 Hz, $CHMe_2$), 1.33 and 1.56 (6 H, 2 s, isopropylidene group), 1.83–2.10 (1 H, m, $CHMe_2$), 3.90 (2 H, d, *J* 6 Hz, OCH_2), 4.35 (3 H, m, 4′-H and 5′-H_2), 4.80 (1 H, dd, *J* 4 and 6.5 Hz, 3′-H), 4.96 (1 H, dd, *J* 2 and 6.5 Hz, 2′-H), 5.73 (1 H, d, *J* 8 Hz, 5-H), 5.76 (1 H, d, *J* 2 Hz, 1′-H) 7.33 (1 H, d, *J* 8 Hz, 6-H) and 9.30 (1 H, br s, NH).

Biological Methods.—Antiviral activity. HIV-1-Induced cytopathogenicity assays were carried out with the HTLV-III_B strain. The virus was prepared from the culture supernatant of a persistently HTLV-III_B-infected HUT-78 cell line. The antiviral assays were based on an inhibition of HIV-1-induced cytopathogenicity in human MT-4 lymphocytes as previously described.[20]

The antiviral test procedures were based on an inhibition of virus-induced cytopathogenicity in either primary rabbit kidney, HeLa or Vero cell cultures following previously established procedures.[21] Briefly, confluent cell cultures in microtitre trays were inoculated with 100 $CCID_{50}$ of virus (HSV-1, HSV-2, VV, VSV, Coxsackie virus B4, polio virus-1, parainfluenza-3 virus, reovirus-1, Sindbis virus and Semliki forest virus), 1$CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After 1 h of virus adsorption, residual virus was removed, and the cell cultures were incubated at 37 °C in the presence of varying concentrations of the test compounds. Viral cytopathogenicity was recorded as soon as it reached completion in the control virus-infected cell cultures.

AP-B Assays. Cell surface-associated AP-B activities were determined following the Aoyagi method.[8a] The incubation mixture consisted of 2 mmol dm^{-3} L-Lys-Na (0.25 cm^3), Hank's balanced salt solution (0.65 cm^3) and distilled water (0.1 cm^3) with, or without, the inhibitor. After incubation (3 min; 37 °C) the mixture was added to monolayer cultures of murine L cells (5×10^5 cells), and the incubation was stopped after 30 min by the addition of the stabilized diazonium salt Garnet GBC (1 cm^3; 1 mg cm^{-3}) in 1 mol dm^{-3} acetic acid buffer at pH 4.2, containing 10% Tween 20. The mixture was left at room temperature for 15 min, then centrifuged and its absorbance was measured at 525 nm.

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