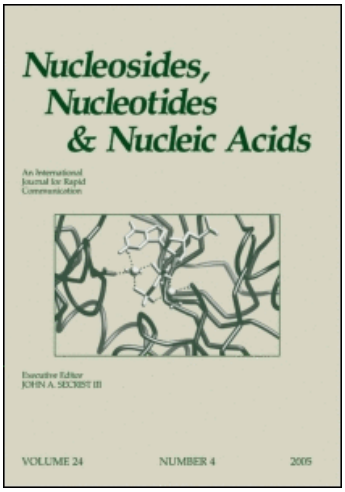

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ADDUCTS OF MANNOSE 6-PHOSPHATE WITH 5-IODO-2'-DEOXYURIDINE AND 2-5A AS POTENTIAL ANTIVIRAL AGENTS

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Abstract - To examine the possibility that the mannose 6-phosphate receptor system might be capitalized upon to facilitate uptake of nucleotides or oligonucleotides into the cell, adducts of mannose 6-phosphate with 5-iodo-2'-deoxyuridine 5'-monophosphate and with adenosine 5'-monophosphate, p5'A2'p5'A and p5'A2'p5'A2'p5'A were prepared and evaluated for their antiviral activities. The adducts with 2',5'-oligoadenylates possessed no significant antiviral activity. The adduct with 5-iodo-2'-deoxyuridine 5'-monophosphate showed activity that could be fully explained by extracellular cleavage to free 5-iodo-2'-deoxyuridine.

Many nucleosides which posses antiviral and/or antitumor properties owe their activity to anabolism to their corresponding 5'-phosphates.[1-3] Such 5'-monophosphates themselves may be inhibitory, as in the case of 5-fluoro-2'-deoxyuridine,[2] or they may be converted further to the 5'-di- or 5'-triphosphate, which then acts as the inhibitory metabolite, as in the case of acyclovir {9-[(2-hydroxyethoxy)-methyl]guanine}.[3] Nucleoside triphosphates also may be incorporated into nucleic acids resulting in fragmentation or faulty function [*e.g.*, (E)-5-(2-bromovinyl)-2'-deoxyuridine, BVDU].[4] Indeed many nucleosides can be converted to the nucleotide and may act at more than one locus (*e.g.* BVDU, acyclovir).[1-4] A common form of development of resistance to nucleoside drugs is based upon this key conversion of the nucleoside to nucleotide.[5] For example, when thymidine kinase activity is substantially reduced or lost altogether, certain nucleoside drugs become inactive. Since nucleotides cannot penetrate the cell, except perhaps with a low efficiency,[6] a considerable expenditure of effort has been invested in an attempt to develop prodrugs of nucleotides which may be readily taken up by the cell and then cleaved to active nucleotides. Quite a large variety of structural motifs have been explored in this search.[7-11]

However, so far no unequivocal proof exists that any such nucleotide derivative possesses *in vivo* activity against tumor cells resistant to the parent nucleoside.The problems and limitations of this prodrug approach have been reviewed by Bennett *et al.*[8] Nonetheless, a variety of applications for an appropriate latentiated nucleotide remain even if application to the problem of drug resistance should turn out not to be feasible.

One potential approach to transport of a nucleotide prodrug across a cell membrane is suggested from studies of I-cell disease.[12] I-cell disease is a lysosome disorder in which cells from patients genetically homozygous for this disease have virtually no hydrolases in their lysosomes. Nonetheless, in extracellular fluids very high concentrations of these enzymes are found. Such cells cannot internalize hydrolases under condition wherein normal cells can.[13] The hydrolase recognition marker has been determined to be mannose 6-phosphate, and its receptor, the mannose 6-phosphate insulin-like growth factor II receptor, is a widely occurring protein, which is essential for the transport of lysosomal enzymes from the Golgi apparatus and the cell surface to the lysosomes.[14,15] This mannose 6-phosphate recognition/receptor system has been capitalized upon to facilitate the uptake of a number of macromolecules.[16-18] In this report, we relate attempts to apply this concept to nucleotides and oligonucleotides.

EXPERIMENTAL

Adenosine 5'-monophosphoric acid, 5-iodo-2'-deoxyuridine 5'-monophosphate dilithium salt, and D-mannose 6-phosphate disodium salt were purchased from Sigma. Triphenylphosphine and 2,2'-dipyridyldisulfide were from Aldrich.

^{1}H NMR spectra were recorded with a Varian HR 220 operating at 220 MHz or with a Nicolet 360 MHz instruments. ^{13}C NMR spectra were recorded with a Jeol FX 100, at 25 MHz. The solvent was D_2O with dioxane (δ = 3.75 ppm from TMS) as an internal standard. ^{31}P NMR spectra were obtained with a Varian instrument operating at 109 MHz in D_2O as a solvent and 0.15% H_3PO_4 as an external reference.

Cell-free protein synthesis[19] and radiobinding[20] assays were performed as described earlier. The radioactive probe ppp5'A2'p5'A2'p5'A2'p5'A3'[^{32}P]p5'Cp (specific activity 3000 Ci/mmol) was purchased from Amersham.

Adenosine 5'-phosphoroimidazolidate was prepared as described by Mukaiyama and Hashimoto[21], and 5-iodo-2'-deoxyuridine 5'-phosphoromorpholidate was prepared using the method of Moffatt and Khorana.[22]

P^1-(5-Iodo-2'-deoxyuridine)-5'-P^2-D-mannose-6-pyrophosphate (ManppIdU)

4-morpholine N,N'-dicyclohexylcarboxamidinium salt (160 mg, 0.2 mmol) was dried by coevaporations with dry DMF (2 x2 ml). It was then dissolved in fresh DMF (1.5 ml) and a solution of D-mannose 6-phosphate tri-*n*-octylammonium salt (246 mg, 0.4 mmol) in DMF (2 ml) and molecular sieves (4Å, 0.5 g) were added. The mixture was kept for 3 days at room temperature. The sieves were filtered off, 2 ml of water was added to the filtrate and the mixture was applied on DEAE-Sephadex A-25 (Cl^- form) column (1.6 x 25 cm) and eluted with 1L of 0 to 0.1 M LiCl in 0.003 M HCl. Fractions absorbing at 260 nm and showing a positive test with o-aminobiphenyl reagent[24] were pooled, the pH was adjusted to 6.5 and the solution was evaporated to dryness. The residue was dissolved in methanol (5 ml) and the product was precipitated with acetone (50 ml), centrifuged, reprecipitated again and dried under vacuum. After final purification on a Sephadex G-10 gel filtration column, 55.5 mg (40.2%) of P^1-(5-iodo-2'-deoxyuridine)-5',P^2-D-mannose-6-pyrophosphate, (dilithium salt) was obtained.

When digested with snake venom phosphodiesterase the above compound gave but two detectable products as ascertained by TLC. These were mannose 6-phosphate and 5-iodo-2'-deoxyuridine 5'-monophosphate. When digested sequentially first with snake venom phosphodiesterase and then with bacterial alkaline phosphatase, the sole products were mannose and 5-iodo-2'-deoxyuridine.

^{1}H NMR (360 MHz) : δ 8.16 (s, 1, H6), 6.18 (t, 1, deoxyribose H1'), 5.09 (s, 0.7 , α-mannose H1'), 4.80 (s, 0.3, β-mannose H1'). ^{13}C NMR (25 MHz) : δ 65.5 (ribose C5'), 71.2 (α- mannose C5, $^3J_{P-C}$ 6.1 Hz), 74.8 (β-mannose C5, $^3J_{P-C}$ 8.6 Hz), 65.0 (α- and β-mannose C6, $^2J_{P-C}$ < 5 Hz).

P^1-Adenosine-5', P^2-mannose-6-pyrophosphate (ManppA)

This compound was prepared in the same way as its 5-iodo-2'-deoxyuridine analogue starting from 0.1 mmol (42 mg) of adenosine 5'-phosphoroimidazolidate[21] and 0.2 mmol (123 mg) of D-mannose 6-phosphate tri-*n*-octylammonium salt. The yield was 39 mg (66%).

This adduct of mannose 6-phosphate and AMP was digested with snake venom phosphodiesterase to give adenosine 5'-monophosphate and mannose 6-phosphate as the sole products. Sequential treatment with snake venom phosphodiesterase and bacterial alkaline phosphatase gave only adenosine and mannose. ^{1}H NMR (360 MHz) : δ 8.45 (s, 1, adenine H8), 8.20 (s, 1, adenine H2), 6.05 (d, 1, $^3J_{H1'-H2'}$ 6 Hz, ribose H1'), 5.08 (s, 0.7, α-mannose H1), 4.7 (β-mannose H1). ^{13}C NMR (25MHz) : δ 83.9 (ribose C4'),65.1 (ribose C5'), 71.1 (α-mannose C5), 74.3 (β-mannose C5), 65.1 (α- and β-mannose C6).

Adenylyl(2'-5')adenosine 5'-phosphoroimidazolidate (Imp5'pA2'p5'A).

Adenylyl (2'-5')adenosine 5'-monophosphate, triethylammonium salt (0.011mmol, 300 OD_{260} units) was dried by coevaporation with DMF (2 x 2 ml) and the residue was dissolved in fresh DMF (1 ml). To this solution triethylamine (50 µl), imidazole (0.5 mmol, 34 mg), triphenylphosphine (0.2 mmol, 52 mg) and 2,2'-dipyridyldisulfide (0.2 mmol, 44 mg) were added. The reaction progress was followed by TLC (PEI-cellulose F, 0.1 M ammonium bicarbonate, R_f: imidazolidate - 0.76, adenylate dimer - 0.51). After the reaction was completed, the reaction mixture was added to 1% solution of sodium iodide in acetone (20 ml) and pure imidazolidate precipitated as a sodium salt.

Adenylyl(2'-5')adenylyl(2'-5')adenosine 5'-phosphoroimidazolidate (Imp5'pA2'p5'A2'p5'A).

This compound was obtained in the same way as its dimer analogue, starting from 0.032 mmol (1140 OD_{260} units) of adenylate trimer 5'-monophosphate, triethylammonium salt. TLC : PEI-cellulose F, 0.1 M ammonium bicarbonate, R_f: imidazolidate - 0.51, adenylate trimer - 0.22.

P^1-Adenylyl(2'-5')adenosine-5', P^2-D-mannose-6-pyrophosphate, (ManppApA).

Imp5'A2'p5'A (0.05 mmol) and D-mannose 6-phosphate, tri -*n*-octylammnium salt (0.1 mmol, 62 mg) were dried separately by coevaporations with DMF (2 x 1 ml). Fresh DMF (0.5 ml) was added to each reagent , the solutions were mixed together (not everything dissolved) and the mixture was kept for two days at room temperature. After that time, DMSO (1 ml) was added in order to dissolve some DMF insoluble material and the mixture was kept one more day in a refrigerator. DMF was evaporated under vacuum, water (3 ml) was added and the mixture was applied on a DEAE Sephadex A-25 (Cl^- form) column (1.6 x 20 cm) and eluted with 500 ml of 0 to 0.2 M LiCl in 0.003 M HCl. Fractions absorbing at 260 nm and giving a positive test with o-aminobiphenyl reagent[24] were pooled, the pH was adjusted to 6.5 and the solution evaporated to dryness. Lithium chloride was removed by washing the residue with methanol (30 ml), acetone (3 x 30 ml) and the product was repurified on a Sephadex G-10 column, eluted with water. Yield: 440 OD_{258} units (34%).

Hydrolysis of the above product with 0.5 M KOH (overnight) gave adenosine and a mixture of p5'A2'p and p5'A3'p as determined by TLC. Under these conditions free mannose was not detected. Digestion with nucleotide pyrophosphatase produced adenosine 5'-monophosphate and mannose 6-phosphate as sole products. ^{1}H NMR (220 MHz) : δ 8.40 (s, 1, adenine H8), 8.21 (s, 2, adenine H2 and H8), 8.10 (s, 1, adenine H2), 6.14 (d, 1, $^3J_{H1'-H2'}$ 3.8 Hz, ribose H1'), 5.88 (s, 1, ribose H1'), 5.07 (s, α-mannose H1), 4.83 (s, β-mannose H1).

P^1-Adenylyl(2'-5')adenylyl(2'-5')adenosine-5', P^2-D-mannose-6-pyrophosphate (ManppApApA).

Imp5'A2'p5'A2'p5'A (0.032 mmol) and D-mannose 6-phosphate, tri-*n*-octylammonium salt (0.075 mmol, 46 mg) were dried separately by coevaporations with DMF (2 x 0.5 ml). The reagents were mixed together in DMF (3 ml) and DMSO (2 ml) and the mixture was kept for two days at room temperature. Then 5 ml of water was added and the mixture was applied to a DEAE Sephadex A-25 (Cl^- form) column (1.6 x 25 cm) and eluted with 1 L of 0 to 0.2 M LiCl in 0.003 M HCl. Fractions absorbing at 260 nm and giving a positive test with o-aminobiphenyl reagent[24] were pooled, the pH was adjusted to 6.5 and the solution was evaporated to dryness. Lithium chloride was removed by washing with acetone (3 x 20 ml) and acetone insoluble product was purified on Sephadex G-15 gel filtration column, eluted with water. Yield: 400 OD_{258} units (35%)

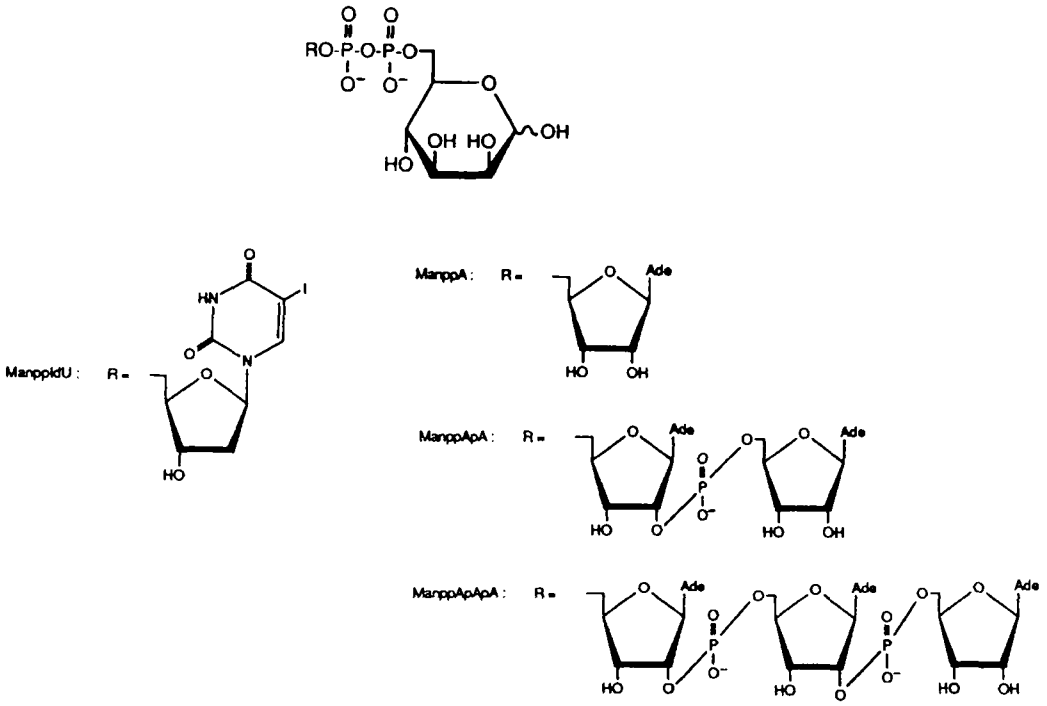
When the above product was digested with 0.5 M KOH (overnight) the products were adenosine, p5'A2'p, p5'A3'p and A2'(3')p. Under these conditions free mannose was not detected. ManppApApA also could be digested with nucleotide pyrophosphatase to give adenosine 5'-monophosphate and mannose 6-phosphate.

RESULTS and DISCUSSION

Two compounds were chosen for linkage to mannose 6-phosphate to check the possibility that the mannose 6-phosphate receptor might be utilized to ferry nucleotides into the cell. These were 5-iodo-2'-deoxyuridine 5'-monophosphate and 2-5A (ppp5'A2'p5'A2'p5'A). The former nucleotide represents a prototype for those antivirals to which drug resistance develops due to an inactivation of thymidine kinase in the resistant virus strain. The latter is an oligonucleotide involved in some aspects of interferon's antiviral action, but which is unable to penetrate cells unless rather drastic techniques are employed.[25] The mode of linkage chosen was simply to form a pyrophosphate by coupling mannose 6-phosphate either to 5-iodo-2'-deoxyuridine 5'-monophosphate or to the 5'-monophosphate of 2-5A core. Apparently, at least one form of the mannose 6-phosphate receptor recognizes methylphosphomannose residues,[26] so presumably a pyrophosphate modification might also be acceptable. *A priori*, it is not known how cleavage of such unsymmetrical pyrophosphates might occur in the cell, but on statistical basis generation of a biologically relevant product (*e.g.* 5-iodo-2'-deoxyuridine 5'-mono- or 5'-diphosphate) would be favored.

The synthetic approach to these adducts was straightforward and involved the now classical approach used by Roseman *at al.*[23] for the preparation of adducts of nucleotides with sugar 1-phosphates. Specifically mannose 6-phosphate (anomeric mixture with the biologically relevant α-anomer predominating) was reacted with an appropriately activated nucleotide 5'-phosphate

SCHEME I



either as the morpholidate or imidazolidate. The desired products were formed in fair to good yields and could be characterized by a combination of enzymatic and chemical digestion techniques as well as proton and ^{13}C NMR. Synthesized using this approach were P^1-(5-iodo-2'-deoxyuridine)-5', P^2-D-mannose-6-pyrophosphate (ManppIdU), P^1-Adenosine-5', P^2-mannose-6-pyrophosphate (ManppA), P^1-Adenylyl(2'-5')adenosine-5', P^2-D-mannose-6-pyrophosphate (ManppApA) and P^1-Adenylyl(2'-5')adenylyl(2'-5')adenosine-5', P^2-mannose-6-pyrophosphate (ManppApApA).

The adducts of mannose 6-phosphate with adenosine 5'-monophosphate and 2',5'-oligoadenylates were first examined for their ability to interact with the 2-5A dependent endonuclease.[27,28] The ability of such materials to bind to the enzyme could be ascertained with a radiobinding assay or an antagonism assay. By both criteria (Table 1), ManppA and ManppApA both were bound poorly to the endonuclease. On the other hand, ManppApApA was bound to the endonuclease virtually as well as 2-5A itself. As a consequence of their lack of efficient binding ability, ManppA and ManppApA could not activate the endonuclease as judged by their protein

Table 1

Interaction of Mannose 6-Phosphate Adducts and 2',5'-Oligoadenylates with the 2-5A Dependent Endonuclease

Compound	Binding[a]	Antagonism[b]	Translational Inhibition[c]
ManppA	N.D.[d]	>>$2x10^{-4}$ M	>>$2x10^{-4}$ M
ManppApA	N.D.	$1.5x10^{-4}$ M	>>$2x10^{-4}$ M
ManppApApA	$1.5x10^{-9}$ M	$6x10^{-7}$ M	>>$2x10^{-4}$ M
pppApApA	$7x10^{-10}$ M	N.D.	$2.5x10^{-9}$ M
pApApA	N.D.	$7x10^{-7}$ M	N.D.

a. Concentration required to displace 50% of radiolabeled probe from the endonuclease nitrocellulose complex.[20]
b. Concentration needed to give 50% prevention of 2-5A action as an inhibitor of protein synthesis.[27]
c. As assayed by inhibition of encephalomyocarditis virus mRNA translation in a L cell-free system. Results are expressed as concentration of compound required to effect a 50% inhibition of protein synthesis.[19]
d. N.D. Not determined.

Table 2

Antiviral Activity of ManppA, ManppApA and ManppApApA in E_6SM and PRK Cell Cultures

Compound	Minimum Inhibitory Concentration (μg/ml)[a]			
	E_6SM Cells		PRK Cells	
	HSV-1	Vaccinia	HSV-1	Vaccinia
ManppA	30	40	15	15
ManppApA	>200	150	>200	>200
ManppApApA	70	70	200	100
Man-6-p	>400	>400	>400	>400
5'-AMP	>400	300	>400	300
pppApApA	>200	70	>400	150
IdU	0.2	0.2	0.1	0.07

a. Concentration required to reduce virus cytopathogenicity by 50%. None of the compounds caused a microscopically detectable alteration of normal cell morphology even at concentrations >400 μg/ml.

Table 3

Antiviral Activity of ManppIdU, IdUMP and IdU in E_6SM and PRK Cell Cultures

Virus	Minimum Inhibitory Concentration (μg/ml)[a]		
	ManppIdU	IdUMP	IdU
In PRK Cells			
HSV-1 (KOS)	0.4	0.2	0.2
HSV-1 (F)	0.4	0.07	0.1
HSV-1 (McIntyre)	0.4	0.2	0.1
HSV-2 (Lyons)	0.4	0.1	0.7
HSV-2 (G)	2	2	1
HSV-2 (196)	2	1	1
Vaccinia	0.4	0.07	0.1
VSV	>400	>400	>400
In E_6SM Cells			
HSV-1 (KOS)	0.4	0.2	0.1
Vaccinia	0.4	0.2	0.1
VSV	>400	>400	>400
TK$^-$HSV-1 (B2006)	200	40	10

a. Concentration to reduce virus-induced cytopathogenicity by 50%.

synthesis inhibitory capacity. Moreover, ManppApApA, like the previously reported AppApApA,[28] could not activate the endonuclease either. Thus either ManppApApA cannot activate or, if it is degraded, it is not cleaved to yield ppApApA which is an established activator.[25]

ManppA, ManppApA and ManppApApA were also evaluated for their antiviral activity in a variety of *in vitro* systems. These tests included: measles reovirus-1, parainfluenza virus-3, sindbis virus in Vero cells, coxsackie virus B4 and poliovirus-1 in HeLa cells, herpes simplex virus-1 (HSV-1, strain KOS), vaccinia virus and vesicular stomatitis virus (VSV) in human embryonic skin-muscle (E_6SM) fibroblast cells and primary rabbit kidney (PRK) cells (Table 2). Only one compound, ManppA showed a marked antiviral activity, but it did so only against HSV-1 and vaccinia virus in PRK and E_6SM cells.

The adduct of mannose 6-phosphate with 5-iodo-2'-deoxyuridine was examined for antiviral activity in both PRK and E_6SM cultures against vaccinia virus, vesicular stomatitis virus and a number of herpes simplex virus strains (Table 3). ManppIdU turned out to be nearly as effective as IdUMP and IdU against vaccinia virus and all strains of herpes simplex virus save one, a thymidine kinase-deficient (TK$^-$) mutant. The fact that this TK$^-$ strain was not susceptible to

inhibition by ManppIdU strongly suggested that it first must be cleaved to yield the free nucleoside, which is then taken up by the cell and phosphorylated.

The degradation of ManppIdU by a component present in fetal calf serum could be demonstrated readily. In such experiments, analysis was done by means of HPLC using a μBondapak column (4.6 x250 mm) with a 0-50% 30 minute gradient of solvent B [MeOH/H_2O (1:1)] in solvent A (ammonium phosphate, 50 mM, pH 4.5). Peaks containing IdU or its derivatives could be monitored at 300 nm without interference from the UV absorption of serum components. ManppIdU, when incubated at a concentration of 2×10^{-4} M with 10% fetal calf serum in RPMI-1640 medium, was degraded in one hour to 5% IdU and 35% IdUMP with 60% ManppIdU intact. After 20 hrs, however, no ManppIdU remained. Conversion to IdU was complete. As the concentration of fetal calf serum was decreased, so was the degradation of ManppIdU such that at a concentration of 1% fetal calf serum, after one hour, 94% of the ManppIdU was intact with 6% converted to IdUMP and none to IdU. After 20 hrs in 1% fetal calf serum in RPMI-1640, 38% of the ManppIdU remained with 47% converted to IdUMP and 15% converted to IdU. In RPMI-1640 alone (no fetal calf serum), no degradation occurred.

Based upon the above results, another series of experiments was carried out in which the antiviral activities of ManppIdU and IdU were evaluated in serum-free media using E_6SM cells. In this instance, each compound was tested at 100, 10 and 1 μg/ml in triplicate against HSV-1 (KOS) or TK$^-$HSV-1 (B2006). Aliquots of the culture fluids were removed 24 hrs after infection and application of the nucleoside analog. Virus titers were determined for each sample, and the aliquots from cell cultures which had been exposed to 100 μg of compound per ml were analyzed by HPLC for the relative concentration of ManppIdU and/or IdU as appropriate (Table 4).

From these experiments, two points became clear: a) anti-HSV-1(KOS) activity of ManppIdU was accompanied by significant breakdown of ManppIdU to IdU even under such serum free conditions. Apparently, the cells must exude phosphodiesterase or pyrophosphatase activities. Thus the antiviral activity of ManppIdU under these conditions can be explained by its breakdown to IdU. b)From the experiments with the TK$^-$ strain of HSV under serum-free conditions, it was apparent that not only was the ManppIdU adduct no more active than IdU against the mutant strain , but that even though degradation of ManppIdU to IdU did occur, a considerable amount of ManppIdU remained intact. Thus, under these conditions in which the intactness of the ManppIdU adduct could be assured, the adduct provided no increase in antiviral activity over that witnessed with IdU alone.

In the above experiments, we attempted to facilitate the entry of nucleotides into cells by conjugating several nucleotidic molecules to mannose 6-phosphate, a recognition signal for the cellular mannose 6-phosphate receptor. An adduct of mannose 6-phosphate with 2-5A failed to show evidence of cellular uptake as judged by the lack of antiviral activity , and an adduct of mannose 6-phosphate with 5-iodo-2'-deoxyuridine 5'-monophosphate also failed to show

Table 4

Antiviral Activity and Catabolism of ManppIdU and IdU in Serum-Free Cultures of E_6SM Cells

Virus	Nucleoside/tide	Concentration[a]	Virus Yield[b]	Catabolites[c] in Supernatant Media	
				IdU	ManppIdU
HSV-1 (KOS)	IdU	100	1.3	71	0
		10	3.5	-	-
		1	4.7	-	-
HSV-1 (KOS)	ManppIdU	100	1.6	37	45
		10	3.3	-	-
		1	3.2	-	-
HSV-1 (KOS)	none (control)	-	5.1	-	-
TK$^-$HSV-1 (B2006)	IdU	100	2.9	75	0
		10	5.8	-	-
		1	5.3	-	-
TK$^-$HSV-1 (B2006)	ManppIdU	100	3.4	30	36
		10	6.0	-	-
		1	5.8	-	-
TK$^-$HSV-1 (B2006)	none (control)	-	6.0	-	-

a. in μg/ml
b. in $\log_{10}$ [PFU/ml]
c. Presented as percentage of total A_{300} resident in given peak. The total OD applied to the HPLC column was constant (±20%) as was the total OD/ml of supernatant. In the case of IdU a peak at 12.8 min accounted for approximately 25% of the OD for ManppIdU, the remaining OD was distributed among 4-5 other small peaks.

evidence of uptake under conditions wherein cleavage to free 5-iodo-2'-deoxyuridine was incomplete. One outstanding problem with the approach used here was the cleavage of the pyrophosphate moiety by enzymes present in serum or elaborated by cells. Facile cleavage of such pyrophosphate linkages has been demonstrated in cell sap[28] but we had not anticipated such potent activity in the external milieu of the cell. In addition there are several ambiguities concerning a) the exact structural alterations that may permit recognition by the mannose 6-phosphate receptor and b) structural elements which also would allow internalization of the ligand-receptor complex. Thus, even though methylphosphomannose residues can be recognized by the mannose 6-phosphate receptor,[26] other phosphodiester derivatives, such as diesters based on N-acetylglucosamine, are not.[29] Pyrophosphates have not been examined for their binding affinity. Another problem may be that internalization, as opposed to binding, may require a polyvalent mannose 6-phosphate ligand. Thus phosphomannan fragments with multiple phosphomonoester groups could be effectively internalized whereas pentamannosyl monophosphate is not internalized.[30,31]

A further definition of the structure-activity requirements for the mannose 6-phosphate receptor coupled with the related appropriate degradation-resistant linkage for nucleotide adducts may yet allow application of this cellular mechanism for introduction of nucleotides and/or oligonucleotides into the cell.

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