PREPARATION OF 2′(3′)-O-PHOSPHONYLMETHYL DERIVATIVES OF RIBONUCLEOSIDES

Ivan ROSENBERG and Antonín HOLÝ

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

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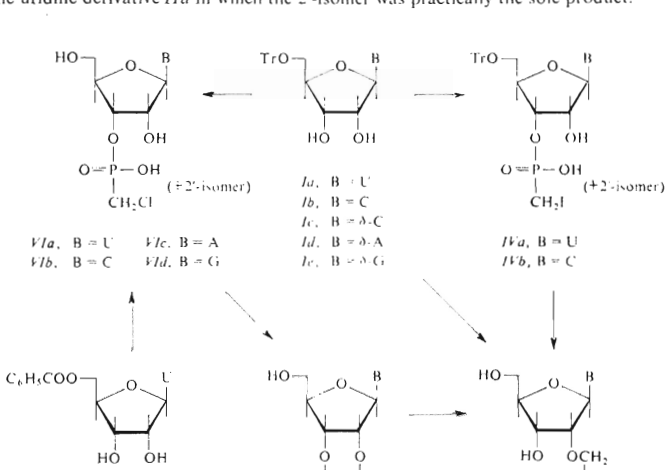
Reaction of 5′-O-tritylribonucleosides I with dimethyl *p*-toluenesulfonyloxymethanephosphonate (*III*) followed by hydrolysis afforded 2′(3′)-O-phosphonylmethyluridine (*IIa*), -cytidine (*IIb*) and -adenosine (*IIc*). With 2′,5′-di-O-trityluridine (*IX*), this procedure led to the 3′-isomer of *IIa*, with 3′,5′-di-O-trityluridine (*X*) to the 2′-isomer. 5′-O-Trityluridine (*Ia*) and -cytidine (*Ib*) were converted by reaction with iodomethanephosphonic acid and N,N′-dicyclohexylcarbodiimide into the 2′(3′)-O-iodomethanephosphonyl derivatives *IVa, b* which on reaction with sodium hydride and subsequent hydrolysis gave the compounds *IIa* and *IIb*. Reaction of compounds *I* or 5′-O-benzoyluridine (*VIII*) with chloromethanephosphonyl dichloride (*V*) and removal of the protecting groups afforded 2′(3′)-O-chloromethanephosphonylribonucleosides *VI* which on reaction with sodium hydride, or better with aqueous alkali, gave 2′(3′)-O-phosphonylmethyl derivatives of uridine (*IIa*), cytidine (*IIb*), adenosine (*IIc*) and guanosine (*IId*). 2′,3′-O-Isopropylideneribonucleosides *XI* reacted with the compound *V* to give, after hydrolysis, 5′-O-chloromethanephosphonyluridine (*XIIa*) and -cytidine (*XIIb*). These compounds were not affected by an alkali metal hydride or hydroxide.

In our preceding communication[1] we described the synthesis of 5′-ribonucleotide analogues in which the nucleoside 5′-hydroxyl, instead of being esterified by phosphoric acid moiety, was etherified by methanephosphonic acid. The O—C—P arrangement in these analogues resists phosphomonoester hydrolases. Several previous communications also describe non-cleavable 3′-nucleotide analogues: in addition to phosphothioates[2] which fulfil this requirement only partially, attention was aimed mainly at 3′-deoxy-3′-phosphonylalkylnucleoside derivatives[3,4]. These compounds, however, lack the important oxygen atom in the position 3′ of the sugar component.

We investigated the preparation and properties of 2′(3′)-O-phosphonylmethylribonucleosides (*II*) – a new type of ribonucleoside 2′- and 3′-phosphate analogues. The direct nucleophilic substitution with chloromethanephosphonic acid which can be realized in small yields with 5′-hydroxy groups in nucleosides[1], fails completely with alkoxides of secondary hydroxy groups in 5′-O-tritylribonucleosides *I*. We used the more reactive dialkyl *p*-toluenesulfonyloxymethanephosphonate *III* (ref.[1]) which on reaction with compounds *I* in the presence of sodium hydride, followed by acid hydrolysis of the protecting group and alkaline saponification, afforded directly the

desired 2′(3′)-O-phosphonylmethylnucleosides *II* (Scheme 1). One can assume that participation of the neighbouring 2′ or 3′-hydroxyl in the intermediary acyclic monoester of the compound *II* leads to the cyclic monoester which then is hydrolyzed to the salt of the acid *II*. The reaction affords a mixture of two isomeric derivatives with the 2′-isomer preponderating. This shift in favour of the 2′-isomer, due obviously to higher nucleophilicity of the 2′-alkoxide, was particularly marked in the case of the uridine derivative *IIa* in which the 2′-isomer was practically the sole product.



Ia, B = U
Ib, B = C
Ic, B = δ-C
Id, B = δ-A
Ie, B = δ-G

VIa, B = U
VIb, B = C
VIc, B = A
VId, B = G

IVa, B = U
IVb, B = C

VIII

VII
VIIa, B = U

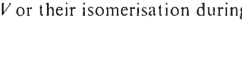
IIa, B = U
IIb, B = C
IIc, B = A
IId, B = G

In formulae *I*—*VIII*, U = uracil-1-yl, C = cytosin-1-yl, A = adenin-9-yl, G = guanin-9-yl, δ = N-dimethylaminomethylene, Tr = trityl residue

SCHEME 1

However, the yields of this reaction were not very high; the exceptionally facile participation of the hydroxyl and the tendency to formation of cyclic intermediates led us to a principally new synthetic approach to compounds *II*. The 5′-O-trityl derivatives *I* were converted by reaction with iodomethanephosphonic acid in the presence

of N,N'-dicyclohexylcarbodiimide into the 2'(3')-O-iodomethanephosphonyl derivatives *IV*. On treatment with sodium hydride these compounds underwent an intramolecular substitution reaction with alkoxide of the neighbouring 2'- or 3'-hydroxyl. The resulting cyclic intermediate with the O—C—P bond on acid hydrolysis of the trityl group afforded the known 2'(3')-O-phosphonylmethyl derivative *II*. Identity of this reaction product with an authentic compound whose O—C—P bond was formed by a direct reaction of compound *III* was confirmed by ^{31}P NMR and ^{1}H NMR spectra as well as by HPLC analysis. Also in this case a mixture of 2'- and 3'-isomer was formed; since compounds *II*, unlike nucleotides, cannot isomerize, the population of the isomeric products in compound *II*, prepared by intramolecular substitution of iodomethanephosphonates *IV*, reflects the population of isomeric compounds *IV* or their isomerisation during the reaction.



III

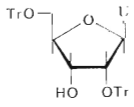
V

For identification of compounds *II*, the pure isomers of the uridine series (*IIa*) were prepared by reaction of both the 2',5'-(*IX*) and 3',5'-(*X*) ditrityl derivatives with the *p*-toluenesulfonate *III*. According to the NMR and HPLC data, the obtained products were isomerically pure and corresponded to the compounds, formed from the trityl derivative *Ia* by both methods. The ditrityl derivatives *IX* and *X* cannot naturally be used for preparation of pure isomers *IIa* by the intramolecular substitution method, since under the conditions of removal of 2'- or 3'-O-trityl function the intermediate of the type *IVa* is necessarily isomerized.

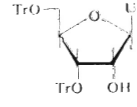
The same principle of intramolecular substitution of 2'(3')-halogenomethanephosphonyl derivatives was used also in the third variant of preparation of compounds *II*. The corresponding ribonucleoside, protected in position 5' with an acid-labile (type *I*) or alkali-labile (type *VIII*) group, reacted smoothly with chloromethanephosphonyl dichloride (*V*) in the presence of a slight excess of a weak tertiary base in an inert solvent. This almost quantitative reaction, followed by removal of the protecting groups, afforded the 2'(3')-chloromethanephosphonates *VI*. Similarly to their iodomethanephosphonate analogues *IV*, these compounds were smoothly converted into the compounds *II* by reaction with sodium hydride. It was not necessary to isolate the intermediary 2'(3')-chloromethanephosphonates *VI* from the reaction mixture of *I* (*VIII*) or *V*; they could be used directly in the next step. They were completely stable in 0·5M-H_2SO_4, methanolic or aqueous ammonia and in methanolic triethylamine. This observation determines also the choice of the suitable protecting groups for the starting nucleoside derivatives.

At the same time, we observed that the compounds *VI* underwent rapid intramolecular substitution not only with a hydride in an aprotic medium but also with aqueous alkali metal hydroxides. In neither case analysis of this mixture revealed any 2′(3′)--hydroxymethanephosphonate[5]. Half-time of the reaction of compound *VI* with 0·5M alkali metal hydroxide is 5 min; the reaction proceeds *via* the 2′,3′- (or 3′,2′--cyclic intermediate *VII* which undergoes a slower hydrolysis to give the compound *II*. After 15 h at room temperature, the compound *VI* was converted quantitatively into the compound *II*. Besides the direct identification by HPLC comparison with authentic material[6], formation of the cyclic derivative *VII* as the primary intramolecular cyclization product follows unequivocally from the reaction of compound *VI* with sodium methoxide or hydroxide in methanol; this reaction afforded a mixture of 2′(3′)-isomeric methyl esters *XIII* as a secondary product of reaction of compounds *VII* with the methoxide ion. Their alkaline hydrolysis led again to the compounds *II*. The exceptionally facile conversion of chloromethanephosphonates *VI* into compounds *VII* by intramolecular cyclization is noteworthy: its rate is incomparably higher than that of usual nucleophilic substitutions in chloromethanephosphonates. The unequivocal course of this reaction and its high rate indicate a thermodynamic control, *i.e.* formation of a bicyclic system, arising by annelation of the five-membered tetrahydrofuran and the six-membered phosphonate rings.

In accord with the previous finding, this reaction afforded exclusively a mixture of the 2′- and 3′-isomers of the compound *II*; the 5′-isomer which could arise by a concurrent reaction of the 3′-chloromethanephosphonate *VI* with the free 5′-hydroxyl was not found among the products.



IX

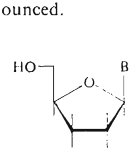


X

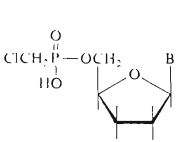
U = uracil-1-yl, Tr = trityl residue

Such reaction would require the formation of a seven-membered ring which makes it much less probable than the formation of a six-membered cycle. We tried to enforce such reaction by a reverse procedure: 2′,3′-O-isopropylidene derivatives *XI* on reaction with chloromethanephosphonyl dichloride (*V*) followed by acid hydrolysis were converted into the 5′-O-chloromethanephosphonyl derivatives *XII*. However, these compounds, containing free 3′-hydroxyl, were completely stable towards 1M-KOH or sodium hydride in dimethyl sulfoxide (as evidenced by HPLC). The

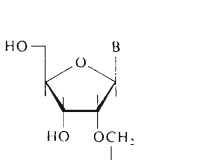
accellerating effect in intramolecular substitution of compounds *VI* in a situation which enables formation of the six-membered phosphonate ring is thus even more pronounced.



XIIa, B = U
XIIb, B = BzC



XIIIa, B = U
XIIIb, B = C



XIIIa, B = U
XIIIb, B = A

In formulae *XI*—*XIII*, U = uracil-1-yl, C = cytosin-1-yl, A = adenin-9-yl, BzC = N^4-benzoylcytosin-1-yl residue

The last variant, *i.e.* reaction of suitably protected ribonucleosides with chloromethanephosphonyl dichloride (*V*), followed by hydrolysis with aqueous alkali, is thus the method of choice. Analogously to nucleotides, under certain circumstances the isomeric compounds *II* can be separated by chromatography on an ion-exchange resin; this holds particularly for the cytidine and adenosine derivatives. The arising compounds *II* were chemically stable, did not isomerize in alkaline or acid medium and were not cleaved with phosphomonoester hydrolases. The reaction was performed with all the four natural ribonucleosides and the obtained derivatives were characterized by the usual chromatographic and electrophoretic criteria. Their UV spectra in acid and alkaline media did not differ from those of the corresponding nucleotides. Compounds *II* represent an important starting material for preparation of other nucleotide analogues which will be described elsewhere.

EXPERIMENTAL

Unless stated otherwise, the solutions were evaporated at 40°C/2 kPa and the compounds were dried at 13 Pa over phosphorus pentoxide. Paper chromatography was performed on a Whatman paper No 1 in the system S1, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2), thin-layer chromatography on Silufol UV 254 plates in the system S2, chloroform–methanol (7 : 3). Paper electrophoresis was carried out on a paper Whatman No 3 MM in the system E1, 0·1M triethylammonium hydrogen carbonate, pH 7·5. The HPLC analyses were performed on a 3·3 × 150 mm column of Separon SI C18 (5 μ) (Laboratorní přístroje, Prague) in 0·1M triethylammonium hydrogen carbonate, pH 7·5, containing the following amount of methanol (wt/wt): S3 — 0%, S4 — 5%, S5 — 10%, S6 — 20%; flow rate 0·4 ml/min at room temperature, detection at 260 nm. The UV absorption spectra were measured in aqueous solutions on a Specord UV-VIS (Carl Zeiss,

Jena) instrument; for quantitative determinations the tabulated[7] molar extinction coefficients at pH 2 were used.

Solvents and Chemicals

Dimethylformamide, dimethyl sulfoxide and 1,2-dichloroethane were distilled from phosphorus pentoxide; pyridine was dried over calcium hydride and distilled. Triethylamine was distilled from sodium borohydride. Dimethyl *p*-toluenesulfonyloxymethanephosphonate (*III*) was prepared according to ref.[1], chloromethanephosphonyl dichloride (*V*) according to ref.[8]. 5′-O-Trityl-ribonucleosides were prepared by the described procedures: *Ia* (ref.[9]), *Ib* (ref.[10]), *IX* and *X* (ref.[11]). 5′-O-Benzoyluridine (*VIII*) was synthesized according to ref.[12], 2′,3′-O-isopropylidene derivatives *XIa* and *XIb* according to ref.[13] and ref.[14], respectively.

N-Dimethylaminomethylene-5′-O-tritylribonucleosides *Ic*—*Ie* (*cf.* ref.[15])

Dimethylformamide dimethylacetal (20 ml) was added to a solution of 5′-O-tritylcytidine[10] (*Ib*; 10 mmol), 5′-O-trityladenosine[16] or 5′-O-tritylguanosine[17] in dimethylformamide (50 ml). After standing overnight at room temperature the mixture was taken down at 40°C/13 Pa, mixed with 50% aqueous pyridine and with dry ice (about 1 g) and set aside for 1 h with intermittant stirring. The mixture was taken down at 40°C/13 Pa and the residue was codistilled under the same conditions with pyridine (3 × 25 ml) and toluene (3 × 25 ml). The residue was dissolved in chloroform (20—40 ml) and the solution was added dropwise under stirring into light petroleum (500 ml). The separated solid was filtered, washed with light petroleum and dried *in vacuo*. The products obtained in this way were used for further reactions.

2′(3′)-O-Phosphonylmethyluridine (*IIa*) from *IVa* (Method *A*)

A mixture of 5′-O-trityluridine (*Ia*; 4.86 g; 10 mmol), iodomethanephosphonic acid[18] (3·33 g; 15 mmol) and pyridine (50 ml) was stirred under exclusion of moisture. N,N′-Dicyclohexylcarbodiimide (6·2 g; 30 mmol) was added and the mixture was stirred in a stoppered flask at room temperature for 10 days. Water (10 ml) was added, followed after 1 h with chloroform (200 ml). After filtration, the solution was diluted with the same volume of 1-butanol and water was added till the phases separated. The lower layer was again extracted with butanol, which had been saturated with water (100 ml), and taken down *in vacuo*. The residue was dissolved in methanol, the solution was made alkaline with triethylamine and taken down. After precipitation from chloroform (25 ml) with ether (300 ml) under stirring, the solid was collected on filter, washed with light petroleum and dried *in vacuo*, affording 7·7 g (95% for the mono-triethylammonium salt) of the chromatographically pure product *IVa*: R_F 0·80 in S1, 0·10 in S2). A solution of this compound (9·5 mmol) in dimethyl sulfoxide (65 ml) was stirred with sodium hydride (2·28 g; 95 mmol) for 3 h (calcium chloride tube) and set aside in a stoppered flask for 2 days. Acetic acid (6 ml; 100 mmol) was added and the mixture was taken down at 60°C/13 Pa. The residue was refluxed with 80% acetic acid (250 ml) for 1 h. After evaporation, the residue was mixed with water (250 ml) and extracted with ether (3 × 50 ml). The aqueous phase was evaporated *in vacuo*, the residue was taken up in water (300 ml), made acid to pH 3·5 with hydrochloric acid, and treated with charcoal (until the mixture had $A_{260} \sim 1$). The mixture was centrifuged, the solid was washed with water (3 × 100 ml), stirred with 2·5% ammonia in 30% aqueous methanol (200 ml) and filtered through Celite which was then washed with the same solvent mixture (100 ml). The filtrate was taken down *in vacuo* and the residue was dissolved in water (50 ml) and applied on an 80 × × 4 cm column of DEAE cellulose (HCO_3^-, Cellex D). After washing with water till the UV

absorption dropped, the product was eluted with water and 0—0·3M triethylammonium hydrogen carbonate, pH 7·5 (linear gradient, 2 l each; 3 ml/min, fractions 10 min). The product fractions were taken down *in vacuo*, the residue was codistilled with water (3 × 20 ml) and applied on a column of Dowex 50X8 (25 ml; Li^+). The column was washed with water, the UV-absorbing eluate was evaporated *in vacuo*, the residue was codistilled with ethanol (3 × 25 ml) and precipitated with ether (200 ml) from methanol (5 ml). The product was collected on filter, washed with ether and dried *in vacuo*. Yield 0·77 g (22%) of dilithium salt of compound *IIa*; chromatographically (S1, HPLC) and electrophoretically homogeneous. According to ^1H NMR spectrum (the H_6 doublet ratio) the ratio of the 2′ and 3′-isomers was 55 : 45. For $C_{10}H_{13}Li_2N_2O_9P$ (350·1) calculated: 8·00% N, 8·86% P; found: 7·82% N, 8·94% P. The physical constants are given in Table II.

2′(3′)-O-Phosphonylmethylcytidine (*IIb*) from *IVb* (Method *A*)

The reaction was carried out with 10 mmol of 5′-O-tritylcytidine in the same manner as described for compound *IIa*. Precipitation with light petroleum from chloroform afforded 6·4 g (7·9 mmol, *i.e.* 79% for the mono-triethylammonium salt) of compound *IVb* (R_F 0·80 in S1). This product was stirred with dimethyl sulfoxide (70 ml) and sodium hydride (3 g; 124 mmol) till the mixture became homogeneous. After standing for 2 days at room temperature, acetic acid (7·5 ml) was added. The mixture was taken down *in vacuo* and the residue was worked up with acetic acid as described for *IIa*. The aqueous solution was extracted with ether, taken down and the residue was aplied on a column of Dowex 50X8 (H^+, 300 ml). After washing with water (3 ml/min) to drop of UV-absorption, the product was eluted with 2·5% ammonia. The ammonia eluate was taken down *in vacuo* and the residue was applied on a column (100 ml) of Dowex 1X2 (acetate). The column was washed with water until the UV-absorption ceased and then with a linear gradient of water and acetic acid (0—1M, 2 l each). The product was eluted with 0·7—0·9M acid; the combined fractions were taken down *in vacuo*, the residue was codistilled with water (3 × 25 ml) and ethanol (2 × 25 ml), and the product was precipitated with ether from methanol; yield 0·30 g (11% based on *IVb*) of the free acid *IIb*, homogeneous according to S1, E1 and HPLC. For $C_{10}H_{16}N_3O_8P$ (337·3) calculated: 12·46% N, 9·20% P; found: 11·81% N, 9·07% P. The physical constants are given in Table II.

2′(3′)-O-Chloromethanephosphonylribonucleosides *VI*

Pyridine (40 mmol), followed by chloromethanephosphonyl chloride (*V*; 20 mmol), was added to a stirred solution or suspension of compound *I*, *IX* or *X* (10 mmol) in 1,2-dichloroethane (100 ml). After stirring for 24 h at room temperature, 5% aqueous pyridine (100 ml) was added and the stirring was continued for 2 h. The mixture was taken down *in vacuo* and the residue was warmed with 1M-H_2SO_4 in 50% dioxane (100 ml) to 40°C for 15 h. Water (200 ml) was added, the dioxane was evaporated *in vacuo* under simultaneous addition of water and the suspension (about 200 ml) was filtered. The filtrate was extracted with ether (3 × 50 ml), the aqueous phase was neutralized with barium hydroxide and centrifuged. The sediment was washed with water (2 × 50 ml) and the solution was taken down *in vacuo*. In the case of *VIa* and *VId*, the residue was applied on a column (2·5 × 30 cm) of Dowex 1X2 (formate). After washing with water to disappearance of UV-absorption, the product was eluted with 0—2M aqueous formic acid (linear gradient, 2 l each). The product fraction was taken down, the residue was codistilled with water (4 × 50 ml) and ethanol (2 × 25 ml) and precipitated with ether from methanol. The product was filtered, washed with ether and dried *in vacuo*. Compounds *VIb, c* were chromatographed on a column (2·5 × 30 cm) of Dowex 50X8 (H^+) in water. The product-containing fractions (second

TABLE I

Ribonucleoside chloromethanephosphonates *VI*, *XII*

Compound	Starting compound (mmol)	Yield ,% (free acid)	R_F (S1) E_{Up} [a]	k [b]	Isomer ratio	Formula (mol. w.)	Cl : N : P found/ /(calc.)
VIa	*VIII* (1·00)	29	0·49 0·58	1·16[c]	33	$C_{10}H_{14}ClN_2O_8P$ (356·8)	0·96 : 2·13 : 1 (1 : 2 : 1)
	X (10·00)	77		1·71	67		
VIb	*Ic* (15·00)	62	0·51 0·53	1·85[d] 2·99	42 58	$C_{10}H_{15}ClN_3O_7P$ (355·8)	1·13 : 3·05 : 1 (1 : 3 : 1)
VIc	*Id* (6·75)	47	0·54 0·42	1·48[e] 2·90	50 50	$C_{11}H_{15}ClN_5O_6P$ (379·7)	1·04 : 4·82 : 1 (1 : 5 : 1)
VId	*Ie* (10·00)	6	0·35 0·50	—	—	$C_{11}H_{15}ClN_5O_7P$ (395·7)	—
XIIa	*XIa* (1·00)	50[f]	0·40 0·63	1·65[c]	—	$C_{10}H_{13}ClLiN_2O_8P$ (362·6)	1·11 : 1·95 : 1 (1 : 2 : 1)
XIIb	*XIb* (2·00)	71[f]	0·42 0·56	1·33[c]	—	$C_{10}H_{14}ClLiN_3O_7P$ (361·6)	1·04 : 3·05 : 1 (1 : 3 : 1)

[a] Electrophoretic mobility in E1 (referred to uridine 3'-phosphate); [b] Retention (capacity) factor (HPLC), $k = (t_R - t_M)/t_M$ (t_R retention time, t_M hold-up time) in the system [c] S5, [d] S4, [e] S6; [f] lithium salt.

absorbing peak) were combined and worked up as described for *VIa*, *d*. The yields and properties of the thus-obtained compounds *VI* are given in Table I.

2′(3′)-O-Phosphonylmethylribonucleosides *II*

Method B. Sodium hydride (15 mmol) was added to a solution of the nucleoside *I*, *IX* or *X* (5 mmol) in dimethylformamide (50 ml). After stirring with exclusion of moisture for 30 min, compound *III* (5 mmol, see ref.[1]) was added and the mixture was stirred at room temperature for 60 h. Acetic acid (15 mmol) was added, the mixture was taken down at 40°C/3 Pa, the residue was warmed with 50 ml of 1M-H_2SO_4 in 50% aqueous dioxane to 40°C for 15 h and diluted with water (200 ml). Dioxane was evaporated *in vacuo* with simultaneous addition of water. The suspension (about 200 ml) was filtered, the filtrate was extracted with ether (3 × 50 ml), the aqueous phase was neutralized with barium hydroxide and centrifuged. The sediment was washed with water (3 × 50 ml) and the combined supernatants were taken down *in vacuo*. The residue was heated for 15 h to 50°C with 1M-NaOH (50 ml), neutralized with Dowex 50X8 (H^+) and filtered. The Dowex was washed with 1% aqueous ammonia (100 ml) and the filtrate was taken down *in vacuo*. In the preparation of compound *IIa* the above neutral residue was chromatographed on a column (1·5 × 20 cm) of Dowex 1X2 (Cl^-); elution first with 0·01M-HCl and then with

TABLE II

2′(3′)-O-Phosphonylmethylribonucleosides *II*

Compound	Starting compound (mmol)	Method	Yield %	Isomer ratio	k^a	N : P found (calc.)
IIa[b]	*Ia* (10·0)	*A*	22	55 : 45	3·00; 3·51[f]	1·92 (2)
	Ia (11·0)	*B*	44	95 : 5	3·00; 3·51[f]	2·05 (2)
	VIa (0·1)	*C*	74	55 : 45	3·00; 3·51[f]	—
	VIa (1·0)	*D*	96	53 : 47	3·00; 3·51[f]	2·10 (2)
	IX (1·4)	*B*	4	—	3·51[f]	—
	X (5·0)	*B*	37	—	3·00[f]	1·95 (2)
IIb[c]	*Ib* (10·0)	*A*	9	57 : 43	2·18; 3·10[f]	2·88 (3)
	Ib (5·0)	*B*	5	80 : 20	2·18; 3·10[f]	—
	Ic (2·0)	*B*	22	82 : 18	2·18; 3·10[f]	—
	VIb (5·0)	*C*	33	59 : 41	2·18; 3·10[f]	—
	VIb (5·0)	*D*	46	51 : 49	2·18; 3·10[f]	—
IIc[d]	*Id* (2·0)	*B*	9	75 : 25	1·93; 3·04[g]	5·05 (5)
	VIc (3·0)	*C*	49	48 : 52	1·93; 3·04[g]	—
IId[e]	*VId* (0·6)	*D*	85	33 : 67	1·52; 2·35[h]	4·90 (5)

[a] Retention (capacity) factor (HPLC); [b] lithium salt $C_{10}H_{13}Li_2N_2O_9P$ (350·1): E_{Up} 0·94(E1), R_F 0·20 (S1); [c] $C_{10}H_{16}N_3O_8P$ (337·2): E_{Up} 0·83 (E1), R_F 0·18 (S1); [d] $C_{11}H_{16}N_5O_7P$ (361·3): E_{Up} 0·80 (E1), R_F 0·18 (S1); [e] $C_{11}H_{16}N_5O_8P$ (377·3): E_{Up} 0·77 (E1), R_F 0·12 (S1); [f] in S3; [g] in S5; [h] in S4.

0—0·2M-LiCl in 0·01M-HCl (linear gradient, 2 l each). The product fraction was neutralized with lithium hydroxide, taken down and the residue was codistilled with ethanol (2 × 100 ml) and stirred with ethanol–acetone (1 : 1; 100 ml) for 1 h. After centrifugation, the sediment was washed with the same mixture (2 × 150 ml), with ether (50 ml), and dried *in vacuo*. The yield and properties of lithium salt of compound *IIa* are given in Table II. In preparation of compounds *IIb* and *IIc* the above neutral residue was chromatographed on a column (1·5 × 20 cm) od Dowex 50X8 (H^+); the column was washed with water until the eluate was transparent in the UV, and then with 2·5% aqueous ammonia. The ammonia eluate was taken down and the residue was chromatographed on a column of Dowex 1X2 (formate; 1·5 × 20 cm). After washing with water to drop in UV-absorption, the product was eluted with linear gradient of formic acid (end concentration 0·01M for *IIb*, 0·5M for *IIc*). The product-containing fractions were taken down, the residue was codistilled with water (4 × 25 ml), with ethanol (2 × 25 ml), and precipitated with ether from methanol. Yields and properties of the compounds (free acids) are given in Table II.

Method C. Sodium hydride (25 mmol) was added to a suspension of compound *VIb* or *VIc* (5 mmol) (free acid) in dimethyl sulfoxide (50 ml). The mixture was stirred overnight in a stoppered flask, set aside for 24 h at 40°C, diluted with water (500 ml), adjusted to pH 3 with Dowex 50X8 (H^+), made alkaline with ammonia and filtered. The filtrate was taken down and the product *IIb* or *IIc* was isolated as described for method *B*. Yields and properties of the products are given in Table II.

Method D. A solution of compound *VI* (free acid) in 1M-KOH or 1M-LiOH (10 ml) was set aside overnight at 40°C, neutralized with Dowex 50X8 (H^+), made alkaline with ammonia, filtered, and the filtrate was taken down *in vacuo*. Compound *IIa* was isolated by chromatography on a column (20 × 1·5 cm) of DEAE-Sephadex A-25 (formate): the column was washed with water to drop in UV-absorption and then with water and formic acid (linear gradient 0—2M, 2 l each). Compound *IIa* was eluted with 2M formic acid; the eluate was taken down and the free acid *IIa* was processed as described for *IIb*—*IId*. Compounds *IIb* and *IId* were isolated by chromatography on Dowex 1X2 (formate; 20 × 1·5 cm), elution with water and formic acid (linear gradient; 2 l each; final concentration 0·01M for *IIb* and 1 M for *II d*). The products (free acids) were isolated as described under *B*. Yields and properties of the products are given in Table II.

2′(3′)-O-Phosphonylmethyluridine (*IIa*) from 5′-O-Benzoyluridine (*VIII*)

The reaction was performed with compound *VIII* (1 mmol) according to the procedure for preparation of compounds *VI*. The crude reaction mixture was taken down, the residue was codistilled with ethanol, set aside overnight with methanol (20 ml) and adjusted to alkaline reaction (moist pH-paper) with 1M sodium methoxide. The mixture was neutralized with Dowex 50X8 (H^+), filtered, taken down *in vacuo* and the residue was chromatographed on 2 sheets of paper Whatman No 3 MM in the system S1. The product *VIa* was eluted with 0·5% ammonia, and the eluate was taken down *in vacuo*. The residue (*VIa*; ammonium salt, chromatographically homogeneous) was dried at 13 Pa and subsequently stirred with sodium hydride (12 mg) in dimethyl sulfoxide (1 ml) till the mixture was homogeneous. After standing overnight at 40°C, water (2 ml) was added, the mixture was neutralized with Dowex 50X8 (H^+) and filtered. The filtrate was taken down and the residue was chromatographed on 1 sheet of paper Whatman No 3 MM in S1. Compound *IIa* was eluted with 0·5% ammonia, the eluate was taken down, the residue was codistilled with ethanol and precipitated with ether from methanol. The yield and properties of the ammonium salt of *IIa* are given in Table II.

5'-O-Chloromethanephosphonyluridine (*XIIa*) (*cf.*[19])

Pyridine (0·35 ml; 4 mmol), followed by compound *V* (335 mg; 2 mmol), was added to a stirred suspension of compound *XIa* (1 mmol) in 1,2-dichloroethane (10 ml). After stirring for 15 h in a stoppered flask, 5% aqueous pyridine (10 ml) was added and after stirring for additional 2 h the mixture was taken down. The residue was warmed with 1M-H_2SO_4 (20 ml) to 40°C for 15 h, neutralized with barium hydroxide and filtered through Celite. The filtrate was taken down *in vacuo* and the product was isolated by chromatography on a column (1·5 × 20 cm) of Dowex 1X2 (formate): after washing with water to drop in UV absorption the product was eluted with 0–2M formic acid (linear gradient, 2 l each). The product fraction (2M formic acid) was taken down, the residue was codistilled with water (3 × 25 ml) and chromatographed on a column (10 × 1·5 cm) of Dowex 50X8 (Li^+) in water. The UV absorbing eluate was taken down, the residue was codistilled with ethanol and precipitated with ether from methanol. The yield and properties of lithium salt of *XIIa* are given in Table I.

5'-O-Chloromethanephosphonylcytidine (*XIIb*)

The reaction was carried out with 2 mmol of compound *Xlb* as described for preparation of *XIIa*. After evaporation, the mixture was dissolved in methanol (20 ml), made alkaline (moist pH-paper) with 1M sodium methoxide, set aside for 6 h at 40°C and neutralized with Dowex 50X8 (H^+). The resin was filtered, washed with 1% triethylamine in 50% methanol and the filtrate was taken down. Further work-up with sulfuric acid was carried out as described for compound *XIIa*. The residue after evaporation of the crude barium salt of *XIIb* was purified on a column (1·5 × 20 cm) of Dowex 50X8 (H^+). The product was eluted with water (the second UV-absorbing peak) and converted into the lithium salt in the same manner as the compound *XIIa*. Yield and properties of the lithium salt of *XIIb* are given in Table I.

Methyl Ester of 2'(3')-O-Phosphonylmethyluridine (*XIII*)

A solution of compound *VIa* (0·5 mmol) in 1M methanolic sodium methoxide (20 ml) was set aside for 15 h at 40°C and then neutralized with dry Dowex 50X8 (H^+). After filtration, the filtrate was taken down and the residue was converted into the lithium salt as described for *XIIa*. This product was chromatographed on a column (1·5 × 50 cm) of Separon SI C18 (20 μ) in 0·05M triethylammonium hydrogen carbonate, pH 7·5 (4 ml/min), the product-containing fractions were combined and stirred with Dowex 50X8 (pyridinium form; 80 ml) for 30 min. The Dowex was removed by filtration, washed with 5% aqueous pyridine and the filtrate was taken down at 30°C/2 kPa. The residue was converted into the lithium salt as described for compound *XIIa*, affording 0·36 mmol (72%) of compound *XIII*, $E_{Up} = 0·53$, $R_F = 0·42$ (S1), $k = 1·57$ (30%), 1·76 (70%) (S4). On standing in 1M-NaOH (KOH, LiOH) for 15 h at 40°C it gave quantitatively the compound *IIa*.

Stability of Compounds *VI* and *XII*

The stability was studied in 3 mM solution of compounds *VIa* and *VIc* in (*a*) 5% aqueous ammonia, (*b*) 30% ammonia in methanol, (*c*) 10% triethylamine in methanol, (*d*) 1M sodium methoxide in methanol, (*e*) 0·5M-KOH, (*f*) 0·5M-KOH in methanol, and (*g*) 0·5M-H_2SO_4. The samples were analyzed by HPLC. In the systems (*a*), (*b*), (*c*) and (*g*) both compounds did not react after 15 h; in systems (*d*) and (*f*) quantitative formation of *XIII* was observed after 15 h, in (*e*) 50% of *II* was formed after 5 min; after 15 h the conversion into *II* was quantitative.

The solutions (3 mM) of compounds *XIIa*, *b* in (*a*) 1M-KOH, (*b*) in dimethyl sulfoxide, containing 5 equivalents of sodium hydride, were analyzed by HPLC after standing for 15 h at 40°C. No reaction products were detected.

REFERENCES

1. Holý A., Rosenberg I.: This Journal *47*, 3447 (1982).
2. Scheit K. H.: *Nucleotide Analogs*. Wiley, New York 1980.
3. Albrecht H. P., Jones G. H., Moffatt J. G.: J. Amer. Chem. Soc. *92*, 5511 (1970).
4. Hampton A., Parini F., Harper P. J.: Biochem. *12*, 1730 (1973).
5. Holý A., Hong Ng. D.: This Journal *37*, 2066 (1972).
6. Holý A., Rosenberg I.: This Journal, in press.
7. *Physical Properties of Nucleic Acid Derivatives*. Calbiochem, Los Angeles 1964.
8. Bannard R. A. B., Gilpin J. R., Vavasour G. R., McKay A. F.: Can. J. Chem. *31*, 976 (1953).
9. Yung N. C., Fox J. J.: J. Amer. Chem. Soc. *83*, 3060 (1961).
10. Kanai T., Ichino M.: Chem. Pharm. Bull. *16*, 1848 (1968).
11. Blank H. U., Pfleiderer W.: Ann. N. Y. Acad. Sci. *742*, 1 (1970).
12. Holý A., Souček M.: Tetrahedron Lett. *1971*, 185.
13. Thomas J. in the book: *Nucleic Acid Chemistry* (L. B. Townsend, R. S. Tipson, Eds), p. 765. Wiley, New York 1978.
14. Holý A., Pischel H.: This Journal *39*, 3863 (1974).
15. Žemlička J., Chládek S., Holý A., Smrt J.: This Journal *31*, 3198 (1966).
16. Blank H. U., Frahne D., Myles A., Pfleiderer W.: Ann. N. Y. Acad. Sci. *742*, 34 (1970).
17. Žemlička J., Chládek S., Haladová Z., Rychlík I.: This Journal *34*, 3755 (1969).
18. Pitre D., Grabit Z. E. B.: J. Prakt. Chem. *32*, 317 (1966).
19. Khropov J. B., Gulyaev N. N., Severin E. S.: Biokhimiya *42*, 1742 (1977).

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