Pergamon

Tetrahedron Letters, Vol. 37, No. 48, pp. 8739-8742, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)02016-3

Synthesis of Acylphosphates of Purine Ribonucleosides

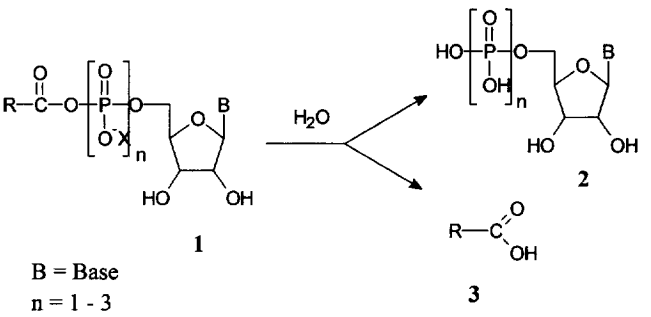
Annett Kreimeyer, Joël Ughetto-Monfrin, Abdelkader Namane, Tam Huynh-Dinh*

Unité de Chimie Organique, associé au CNRS 487

Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France

Abstract: Nucleotides do not penetrate cells at a sufficient rate to realize their therapeutic potential. To overcome this limitation we have envisaged acyl nucleodi(tri)phosphates (ND(T)Ps) as suitable membrane permeable prodrugs because a) preliminary experiences have shown that these compounds are preferably cleaved at their mixed carboxylic phosphoric bond to generate the corresponding carboxylic group (**3**) and nucleotides (**2**)[1], and b) the potential modification of the acyl group R allows to vary the lipophilicity of the acyl nucleotide derivative. Copyright © 1996 Published by Elsevier Science Ltd

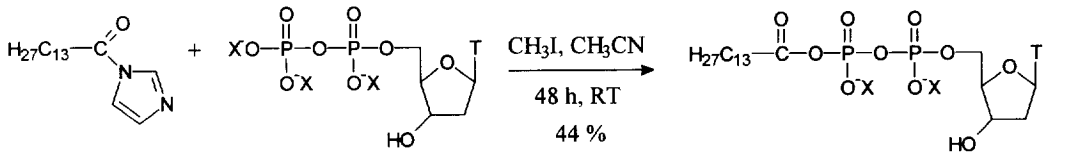
In a previous study acylphosphates of azidothymidine, dideoxythymidine and 3'-O-acetyl thymidine were synthesized using DCC as activator.[2, 3] Next, we were interested in designing analogous potential membrane permeable derivatives of ribonucleotides bearing a purine base. Thus, synthetic problems associated with the presence of several potential acylation sites present in ribofuranosyl purine nucleotides (OH groups of the sugar moiety, NH_2 group of the base) had to be considered. However, because of the low stability of the mixed anhydride moiety under typical deprotection conditions, protecting groups were of limited interest. Therefore, two new methods allowing the synthesis of acyl phosphates of thymidine, guanosine and adenosine nucleosides *via* selective acylation of the terminal phosphate group of free nucleotides were designed.



In a first attempt the procedure recently developed in our laboratory[2, 3] was applied to the synthesis of the myristic acid derivative of adenosine 5′-diphosphate (ADP). But reaction of myristoyl diphosphate with adenosine in the presence of DCC led to the desired compound in a very low yield (0.6 %), obviously due to the possible acylation at the 2′-OH, 3′-OH and NH_2 groups of adenosine. Indeed, among the by-products, a derivative additionally acylated at the 3′-OH group of the sugar moiety could be isolated. Thus, a new synthetic route to acylphosphates of ribonucleotides was developed.

Activation of carboxylic acids by conversion into their corresponding imidazolides is well-known.[4] Besides, when Wang and Chen studied the reactivity of ribonucleotides towards N-acylimidazoles, they found that the addition of an organic base to the reaction system accelerates the acylation of the hydroxyl groups of the ribose moiety as well as that of the amino group of the heterocyclic ring.[5, 6] Thus, to minimize any possible synthetic problems associated with the free NH_2 group, thymidine was first selected as a model

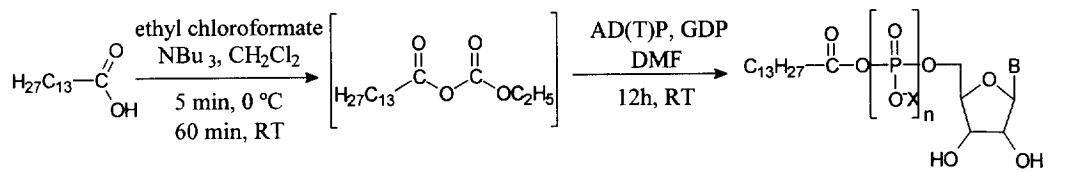
compound: thymidine 5'-diphosphate (dTDP) was treated with myristoylimidazole (**4**) under neutral conditions. However, no reaction was observed even when modifying the solvent system. Based on the assumption that methylation at the N-1 of the imidazolide (**4**) promotes a more active reagent for the acylation on the β-phosphate group of dTDP (**5**), methyliodide was added to the reaction mixture.



4 **5:** X = $NHBu_3^+$ **6:** X = $NHBu_3^+$

Indeed, the target compound (**6**) was isolated in a yield of 44 %, significantly improved according to previous experiments using DCC.[1, 3] With this method, acylation at the 3'-OH group of the sugar moiety occured in about 3 - 4 %. Encouraged by these results, we attempted to apply this new synthetic procedure to ADP. However, upon addition of methyliodide to the reaction mixture a precipitate was formed, probably due to the methylation of position N-7 of adenosine, yielding a charged and therefore insoluble derivative. No further reaction was observed.

Finally, acylphosphates of purine ribonucleotides were obtained using ethyl chloroformate to activate the carboxylic acid. This route was based on the synthesis of aminoacyl nucleoside 5'-monophosphates performed by Michelson and Letters.[7] Treatment of myristic acid (**7**) in dioxane with ethyl chloroformate provided the mixed anhydride **8**, not isolated, which then reacted with the nucleotide, dissolved in CH_2Cl_2 / DMF (4 : 1), to yield the acyl ND(T)P (**9, 10, 11**).



7 **8** **9, 10, 11:** X = $NHBu_3^+$; n = 2, 3; B = adenine, guanine

It should be noted that when DMF was omitted the reaction course was very slow (> 7 days) and could not be pushed to completion. It is known that the solvent has a marked influence on acylation performed with mixed anhydrides;[4] besides, in peptide synthesis the use of DMF as solvent is recommended to avoid any side reactions of the amine compound.[9, 10] Herein, both a solvent effect as well as a catalytic function of DMF should be considered. All products were isolated as their tributylammonium salts. By this method the acylphosphates of AD(T)P and GTP (**9, 10, 11**) were obtained with overall yields ranging from 44 % to 52 %.

Table 1: Synthesis of acyl-nucleotides (6, 9, 12, 13)

Compound	N	Activating conditions[a]	Yield (%)
9	adenine	B	48
6	thymine	A	44
10	adenine	B	52
11	guanine	B	44

[a] Activating conditions: A) R-imidazole, CH_3I; B) ethyl chloroformate

Taking advantage of our preceding NMR studies on acylphosphates,[3] we could conclude that the acyl group was specifically linked to the terminal phosphorus of the nucleotide. The most significant data on the ^{31}P NMR spectra of acyl ND(T)Ps is the chemical shift of the terminal phosphorus (P_β, P_γ), which resonates 9 to 10 ppm higher field than that of a non acylated ND(T)P-$P_{\beta(\gamma)}$. This fact was due to the shielding effect of the carboxylic substituent.

In summary, with the presented synthetic procedures acyl nucleotides were now easily obtained in high yields and with a short reaction time. Further modifications on the acyl group allow a fine turning of their lipophilic balance and hydrolytic stability.

Experimental

In order to obtain a CH_2Cl_2-soluble form of the nucleotides, their commercial available sodium salts were changed into their tributylammonium form ($NHBu_3^+$) according to the method of van Wijk et al. [11] Nucleoside 5´-triphosphates degraded during the course of this procedure. Thus, the countercation exchange was performed by passing them twice through a Dowex AG50 WX8 200 $NHBu_3^+$ form column at 4 °C. Eluats were lyophilized. Dioxane was distilled from sodium / benzophenone. CH_2Cl_2 was distilled from phosphorus pentoxide. Dry acetonitrile was purchased from Merck. Standard benchtop conditions were used for working under anhydrous conditions. TLC were performed on Merck silica gel 60 F_{254} precoated plates. The spots were visualized with UV light as well as anisaldehyde-sulfuric acid spray for the nucleotides and phosphomolybdic acid spray (4 % in methanol) for the imidazole derivative. Column chromatography was conducted on reversed phase C-18 silica gel at 4 °C using a 5-45 % water-acetonitrile gradient. NMR spectra were taken on a Bruker AC-300 spectrometer (^1H: 300 MHz). Chemical shifts are reported in units of δ relative to TMS as external standard in D_2O and $CDCl_3$. ^{31}P NMR were recorded at 121 MHz using 85 % H_3PO_4 as external standard.[12] Mass spectra were determined by fast atom bombardment (FAB, electrospray ionisation). Because of the inherent instability of the compounds (**6**, **9**, **10**, **11**) towards hydrolysis of the mixed anhydride bond, their purity was checked by reversed phase HPLC separation (column: 5C18) instead of elemental analysis. The elution was performed within 20 min gradient of 5-70 % acetonitrile in 0.01 M triethylammonium acetate (pH7.0) and a flow rate of 1mL/min. Detection was performed at 265 nm. Retention times are indicated below.

N-tetradecanoyl-imidazole (**4**). The imidazolide **4** was prepared as described by Staab, Lüking and Dürr.[13] After recristallization in ethyl acetate, **4** was isolated as a white solid in 64% yield: mp. 77.9 °C; ^1H-NMR (D_2O) δ: 8.17 (s, 1H), 7.48 (s, 1H), 7.10 (s, 1H), 2.86 (t, 2H, J = 7 Hz), 1.80 (quintet, 2H, J = 7 Hz), 1.26 (m, br, 20H), 0.88 (t, 3H, J = 7 Hz); MS FAB$^+$ m/z 279.3 $(M+H)^+$; Anal. Calcd for $C_{17}H_{30}N_2O$: C, 73.33; H, 10.86; N, 10.06. Found: C, 73.45; H, 10.96; N, 10.04.

Tetradecanoyl-(5'-thymidinyl)-diphosphate (**6**). To a stirred solution of the imidazolide **4** (56 mg, 0.2 mmol) and dTDP (48 mg, 0.05mmol) in dry CH_3CN (7 mL) CH_3I (0.013 mL, 0.2 mmol) was added. After 48 hours at 25 °C under argon atmosphere the mixture was diluted with 5 mL of toluene. CH_2Cl_2 was removed by evaporation. The product was extracted into water (3 x 5 mL) at 0 °C using a centrifuge for separating the phases. The aqueous phases were collected and lyophilized. The resulting white solid was purified by flash chromatography on reversed phased silica gel to give the pure compound **6** in 44% yield: ^1H-NMR (D_2O) δ: 7.79 (s, 1H), 6.31 (t, 1H, J = 7 Hz), 4.59 (s, br, 1H), 4.15 (d, br, 3H, J = 15 Hz), 3.11 (t, 6H, J = 8 Hz), 2.41 (m, 2H), 2.31 (m, 2H), 1.92 (s, 3H), 1.65 (m, 6H), 1.55 (m, 2H), 1.36 (m, 6H), 1.23 (s, br, 20H), 0.92 (t, 9H, J = 7 Hz), 0.85 (t, 3H, J = 6 Hz); ^{31}P NMR (D_2O, proton decoupled) δ: -9.70 (d, 1P, Jp_p = 24 Hz), -17.90 (d, 1P, Jp_p = 24 Hz); MS FAB$^-$ of $C_{24}H_{40}N_2O_{12}P_2$ m/z 611.3 $(M-H)^-$; HPLC: 14.03 min (92.5 %).

General procedure for the synthesis of the acyl ribonucleotides (**9**, **10**, **11**). Myristic acid (**7**) (137 mg, 0.6 mmol) was dissolved in dry dioxane (4 mL). Tributylamine (NBu_3) (0.22 mL, 0.9 mmol) was added, followed by ethyl chloroformate (0.06 mL, 0.6 mmol) to the ice cold solution. After 5 min at 0 °C, the mixture was kept for 60 min at rt under argon atmosphere and then the nucleotide (0.2 mmol), dissolved in 4 mL CH_2Cl_2 and 1 mL DMF, was added. The reaction was kept under anhydrous conditions for 12 hours.

As in former case, the work-up was an extraction between toluene and water following a purification of the lyophilized product by flash chromatography.

Tetradecanoyl-(5'-adenosinyl)-diphosphate (**9**). prepared in 48 % yield as described above: ^{1}H NMR (D_2O) δ: 8.62 (s, 1H), 8.31 (s, 1H), 6.12 (d, 1H, J = 5 Hz), 4.65 (t, 1H, J = 5 Hz), 4.53 (t, 1H, J = 4 Hz), 4.36 (m, br, 1H), 4.28 (m, br, 2H), 3.11 (t, 6H, J = 8 Hz), 2.33 (t, 2H, J = 7 Hz), 1.66 (m, 6H), 1.36 (m, 6H), 1.06 (d, br, 22H, J = 22 Hz), 0.91 (t, 9H, J = 7 Hz); 0.73 (t, 3H, J = 6 Hz); ^{31}P NMR (D_2O, proton decoupled) δ: -10.96 (d, 1P, J_{P_P} = 21 Hz), -19.32 (d, 1P, J_{P_P} = 21 Hz); MS FAB$^-$ of $C_{24}H_{41}N_5O_{11}P_2$ m/z 636.1 $(M-H)^-$; HPLC: 13.72 min (96.5 %).

Tetradecanoyl-(5'-adenosinyl)-triphosphate (**10**). prepared in 52 % as described above: ^{1}H NMR (D_2O) δ: 8.65 (s, 1H), 8.35 (s, 1H), 6.10 (d, 1H, J = 5 Hz), 4.69 (t, 1H, J = 5 Hz), 4.55 (t, 1H, J = 3 Hz), 4.37 (m, br, 1H), 4.29 (m, br, 2H), 3.12 (t, 6H, J = 8 Hz), 2.36 (t, 2H, J = 7 Hz), 1.66 (m, 6H), 1.37 (m, 6H), 1.15 (d, br, J = 25 Hz, 22H), 0.92 (t, 9H, J = 7 Hz), 0.82 (t, 3H, J = 7 Hz); ^{31}P NMR (D_2O, proton decoupled) δ: -10.67 (d, 1P, J_{P_P} = 20 Hz), -19.46 (d, 1P, J_{P_P} = 17 Hz), -22,44 (t, 1P, J_{P_P} = 20 Hz); MS FAB$^-$ of $C_{24}H_{42}N_5O_{14}P_3$ m/z 717.2 $(M-H)^-$; HPLC: 11.92 min (98.7 %).

Tetradecanoyl-(5'-guanosinyl)-diphosphate (**11**). prepared in a 44.2% yield as described above: ^{1}H NMR (D_2O) δ: 8.15 (s, 1H, H8), 5.88 (d, 1H, J = 5 Hz), 4.64 (t, 1H, J = 5 Hz), 4.47 (t, br, 1H, J = 4 Hz), 4.27 (m, br, 1H), 4.23 (m, br, 1H), 3.09 (t, 6H, J = 8 Hz), 2.34 (t, 2H, J = 7 Hz), 1.63 (m, 6H), 1.46 (m, 2H), 1.33 (m, 6H), 1.13 (br, m, 20H), 0.89 (t, 9H, J = 7 Hz), 0.76 (t, 3H, J = 7 Hz); ^{31}P NMR (D_2O, proton decoupled) δ: -11.03 (d, 1P, J_{P_P} = 20 Hz), -19.42 (d, 1P, J_{P_P} = 20 Hz); MS FAB$^-$ for $C_{24}H_{39}N_5O_{12}P_2$ m/z 652.1 $(M-H)^-$; HPLC: 14.13 min (100%).

Acknowledgement. We are grateful to the *Deutsche Forschungsgemeinschaft*, Bonn, Germany, for financial support of this research.

References and notes

1. Bonnaffé, D.; Dupraz, B.; Ughetto-Monfrin, J.; Namane, A.; Huynh-Dinh, T. *Nucleosides and Nucleotides*, **1995**, *14*, 783 -787.
2. Bonnaffé, D.; Dupraz, B.; Ughetto-Monfrin, J.; Namane, A.; Huynh-Dinh, T. *Tetrahedron Lett.* **1995**, *36*, 531 - 534.
3. Bonnaffé, D.; Dupraz, B.; Ughetto-Monfrin, J.; Namane, A.; Huynh-Dinh, T. *J. Org. Chem.* **1996**, *61*, 895 - 902.
4. Staab, H. A. *Angew. Chem.* **1962**, *74*, 407 - 423.
5 Wang, Y.; Chen, Y- Q, *Heterocycles*, **1989**, *28*, 593 -601.
6. Wang, Y.; Liu, X-Y.; Yang, Z-W.; Wang, Q-W.; Xu, Y-Z.; Wang, Q-Z.; Xu, J-F. *Nucleic Acid Res.* **1987**, *15*, 4291 - 4305.
7. Michelson, A. M.; Letters, R. *Biochim. Biophys. Acta.* **1964**, *80*, 242 - 246.
8. Chen, F. M. F.; Benoiton, N. L. *Can. J. Chem.* **1987**, *65*, 619 - 625.
9. Benoiton, N. L.; Chen, F. M. F. *FEBS Lett.* **1981**, *125*, 104 - 106.
10. Meienhofer, J. In *The Peptides*. Vol 1. E. Gross and J. Meienhofer (Eds). Academic Press: New York. **1979**. p. 263.
11. van Wijk, G.M.T., Hostetler, K.Y., Kroneman, E., Richman, D.D., Sridhar, C.N., Kumar, R., van de Bosch, H. *Chem. Phys. Lipids.* **1994**, *70*, 213 - 222.
12 Phosphorus chemical shift greatly depends on the solvent and the pH. To compare ^{31}P chemical shifts, acylated and unacylated nucleotides were mixed and ^{31}P NMR spectra of this mixture were recorded in D_2O.
13 Staab, H. A.; Lüking, M. ; Dürr, F. H. *Chem. Ber.* **1962**, *95*, 1275 - 1283.

(*Received in France* 12 *August* 1996; *accepted* 9 *October* 1996)