

This article was downloaded by:

On: 26 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597286>

Reinvestigation of 4-Thiothymidine-5'-triphosphate Synthesis

H. Bazin^a; S. Sauvaigo^b

^a CIS biointernational/DIVT/Research and New Technologies, Bagnols/Cèze Cedex, France ^b CEA Grenoble/DRFMC/SCIB/Lésions des Acides Nucléiques, Grenoble Cedex 9, France

To cite this Article Bazin, H. and Sauvaigo, S.(1999) 'Reinvestigation of 4-Thiothymidine-5'-triphosphate Synthesis', *Nucleosides, Nucleotides and Nucleic Acids*, 18: 4, 965 — 966

To link to this Article: DOI: 10.1080/15257779908041614

URL: <http://dx.doi.org/10.1080/15257779908041614>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

REINVESTIGATION OF 4-THIOTHYMIDINE-5'-TRIPHOSPHATE SYNTHESIS

Bazin, H.^{1*} and Sauvaigo, S.²

¹CIS biointernational/ DIVT/ Research and New Technologies, BP 175, F-30203 Bagnols/Cèze Cedex, France. E-mail: hbazin@compuserve.com

²CEA Grenoble/DRFMC/SCIB/Lésions des Acides Nucléiques, 17 rue des Martyrs, F-38054 Grenoble Cedex 9, France.

ABSTRACT : The 4-Thiothymidine-5'-triphosphate **1** (S⁴TTP) was known to be a substrate for polymerase, however a commercial sample of this compound failed to be incorporated into DNA. Mass spectrometry combined to alkaline phosphatase digestion and ³¹P-NMR showed that this sample was in fact 5'-chloro-5'-deoxy-4-thiothymidine-3'-triphosphate **2**. The desired S⁴TTP was synthesized by two alternate routes, was fully characterized and was shown to be incorporated in a DNA polymerase assay.

Sample analysis: The commercial dNTP (USB #77153, Amersham) gave the following MS-(ES⁻), after exchange with TEA : (M-H)⁻ 515.1 (100%) and 517.2 (40%). These data were inconsistent with a mass of 498 expected for S⁴TTP (Calc. for free acid C₁₀H₁₇N₂O₁₃P₃S). The 18 units shift and the isotopic motive suggested the replacement of a hydroxyl by chlorine. The ³¹P-NMR showed the presence of a triphosphate and the UV (λ_{max} = 335nm in H₂O) was consistent¹ with S⁴T structure. The dNTP (2.5 μl of the 10mM commercial solution) in 50μl buffer (0.1M Tris pH 9, 0.1M NaCl, 15 mM MgCl₂) was treated with 5μl Snake Venom Phosphodiesterase (Pharmacia, 5U/μl) no change was observed in HPLC² in contrast with dTTP which was hydrolysed to dTMP. The dNTP (10μl of 10mM) in 85μl buffer (as above) was treated with 5μl of alkaline phosphatase (125U), within 15mn HPLC³ showed complete hydrolysis to a new product (R_t= 20mn)³. This product was distinct from authentic 4-thiothymidine¹ (R_t= 13.1 mn)³ and the ES-MS data [(M-H)⁻ 275.1 (50%), 277.2 (15%)] was consistent with chlorine substituted 4-thiothymidine. The proton coupled ³¹P-NMR of the dNTP sample displayed a quartet at -11.2

ppm ($P\alpha$) with $J_{PH}=8.2\text{Hz}$ and $J_{PP}=19\text{Hz}$. Upon selective decoupling of H_3' (5.13ppm), the quartet collapsed to a doublet ($J=19\text{Hz}$) and upon H_5' , $5''$ decoupling (4.1ppm), the signal remained unaffected. These data indicated a triphosphate chain at 3' position, correlated with a resistance to SVP and in favour of structure **2** for commercial dNTP.

4-STTP Synthesis: To understand the origin of compound **2**, and considering the reported $S^4\text{TTP}$ synthesis⁴, one striking feature was the use of pyridine/ $(\text{EtO})_3\text{PO}$ mixture for phosphorylation (presumably to prevent glycosyl cleavage). Since the $S^4\text{TTP}$ used in ref.5 was prepared in $(\text{EtO})_3\text{PO}$ alone, we tested the action of pyridine. $S^4\text{T}^1$ (50 μmol) in pyridine (24 μl)/ $(\text{EtO})_3\text{PO}$ (150 μl) mixture (0°C) was reacted with POCl_3 (50 μl) for 5h. The main product was eluted on DEAE-sepharose with TEAB 0.18M ($R_t=19.5\text{mn}$)², the ^1H coupled ^{31}P -NMR (doublet, $J_{PH}=7.3\text{Hz}$) and MS-(ES^-) [($\text{M}-\text{H}^-$) 355.1(100%), 357.1 (35%)] were compatible with 5'-chloro-5'-deoxy- $S^4\text{T}$ -3'-phosphate structure. Similarly $S^4\text{T}$ was phosphorylated in $(\text{EtO})_3\text{PO}$ alone, the main product eluted on DEAE-sepharose with TEAB 0.17M ($R_t=7.7\text{mn}$)². The ^{31}P -NMR and MS-(ES^-) [($\text{M}-\text{H}^-$) 337.1(100%)] were compatible with 4-thiothymidine-5'-monophosphate **3** structure. The $S^4\text{TMP}$ **3** was converted to $S^4\text{TTP}$ **1** by the carbonyl-diimidazole procedure⁴, the product (8.7 μmol) was eluted on DEAE-sepharose with TEAB 0.38M ($R_t=16.3\text{mn}$)², MS-(ES^-): ($\text{M}-\text{H}^-$) 497.1. The $S^4\text{T}$ was also phosphorylated by an alternate procedure⁶ and yielded $S^4\text{TTP}$ as above.

CONCLUSIONS: Pyridine induced the formation of 5'-chlorinated nucleotide in the POCl_3 phosphorylation, and reported $S^4\text{TTP}$ data⁴ were questionable. Authentic $S^4\text{TTP}$ was synthesized and was incorporated in a polymerase assay (Pol I Klenow fragment).

REFERENCES

1. Kraszewski, A. ; Delort, A.M. and Teoule, R., *Tetrahedron Lett.*, **1986**, 27, 861-864.
2. Lichrospher^R RP18E(5 μ)125x4, Eluant A: 4% ACN in 5mM TBAP 50mM Na_2HPO_4 pH6; B : ACN. 10 to 30%B in 20min, then 30 to 50%B in 5mn. 1ml/mn.
3. Same column. Eluant A: TEAB 50mM pH7; B : ACN. 0 to 60%B in 30mn. 1ml/mn.
4. Scheit, K.H. and Faerber, P. *Nucleic Acids chemistry*. Townsend, L.B. and Tipson, R.S. (Ed), John Wiley and Sons, **1978** , Vol.2, 793-799.
5. Hofer, B and Köster H. *Nucleic Acids Res.*, **1981**, 9, 753-767 and references herein.
6. Ruth, J.L and Cheng, Y-C. *Mol. Pharmacol.*, **1981**, 20, 415-422.