

*Supporting information*

*Chemoselective activation of nucleoside 3'-O-methylphosphonothioates with  
1,3,5-triazinyl morpholinium salts*

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Table of Contents	S1
General Procedures	S2-S3
Spectra	S4-S11

## General procedures

Reactions were carried out under positive pressure of dry argon. Solvents and reagents were purified according to standard laboratory techniques and distilled directly into reaction vessels. Column chromatography and TLC analyses were performed on silica gel (Kiesel gel 60, 240-400 mesh, E.Merck Inc.), and silica gel HP TLC precoated F<sub>254</sub> plates (purchased from E.Merck Inc.), respectively. NMR spectra were recorded on a Bruker Advance DRX 500 spectrometer, operating at 500.13 MHz (<sup>1</sup>H), and 202.46 MHz (<sup>31</sup>P). Chemical shifts (δ) are reported relative to TMS (<sup>1</sup>H) and 80% H<sub>3</sub>PO<sub>4</sub> (<sup>31</sup>P) as external standards. Mass spectra were recorded on a Finnigan Mat 95 (NBA, Cs<sup>+</sup> gun operating at 13 keV).

### *General procedure for preparation of triethylammonium salts of nucleoside methylphosphonothioates (5)*

Into a solution of 1,2,4-triazole (5 mmol, 2.5 equiv) and triethylamine (6 mmol, 3 equiv) in dry THF (10 mL), cooled in ice bath, methyldichlorophosphine (2.2 mmol, 1.1 equiv) was added with vigorous stirring, and a reaction mixture was stirred for 20 min at this temperature. The immediate formation of white precipitate was observed. 5'-O-DMT-N-protected nucleoside (2 mmol) dissolved in THF (10 mL) was added to this mixture dropwise at 0°C, and ice bath was removed. Stirring was continued for 30 min. After this time sulfur was added, and reaction mixture was left overnight. Triethylamine and water (1:4 v/v) was added to the reaction mixture until the solution became clear, and stirring was continued for additional 0.5 hour. After this time, the reaction mixture was diluted with NaHCO<sub>3</sub> (0.1M), and extracted 3 times with chloroform. The combined organic fractions were additionally washed with NaHCO<sub>3</sub> and water, dried with MgSO<sub>4</sub> and concentrated. The obtained product (as a foam) was dissolved in small amount of CH<sub>2</sub>Cl<sub>2</sub> and precipitated to hexane or petrol ether. Obtained product (mixture of diastereomers in ca 1:1 ratio, 1.18g) was separated by a short-path column chromatography on silica-gel (40 g) using a mixture of chloroform and ethanol (19:1, v/v) containing 1% of triethylamine as an eluent. The *R*<sub>P</sub>- and *S*<sub>P</sub>-5 were separated by the column chromatography on fine silica-gel (50 g) using a mixture of chloroform and ethanol (39:1, v/v) containing up to 5% of Et<sub>3</sub>N as eluent.

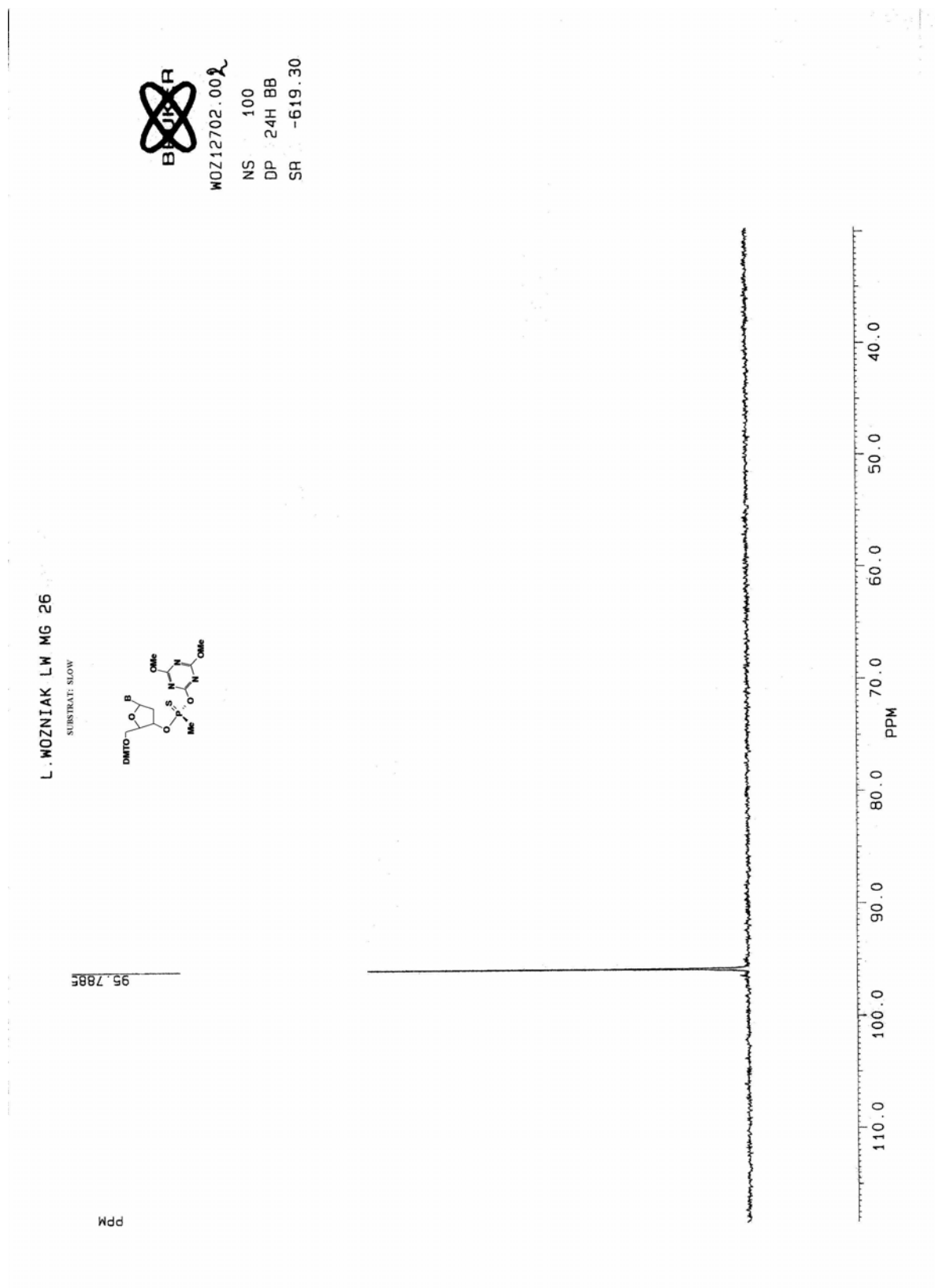
### *General procedure for preparation of 5'-O-DMT-N-protected nucleoside 3'-O-(4,6 dimethoxy-1,3,5-triazin-2-yl) methylphosphonothioate (7)*

Nucleoside methylphosphonothioates **5** (dried over molecular sieves 4Å) and *N*-methyl-*N*-[4,6-dimethoxy-1,3,5-triazin-2-yl] morpholinium chloride (**1**) (2 equiv.) were stirred at room

temp. for about 0.5 h in dry MeCN. After the reaction was complete (TLC control) the reaction mixture was concentrated, redissolved in  $\text{CHCl}_3$  and washed with saturated  $\text{NaHCO}_3$ , and water. The organic layer was dried over  $\text{MgSO}_4$ , concentrated, and applied to a silica gel column. Obtained product **7** was separated by a short-path column chromatography on silica-gel, using a mixture of chloroform and ethanol (gradient 0÷2% EtOH in chloroform, with addition of 0.05% of  $\text{Et}_3\text{N}$ ) as an eluent.

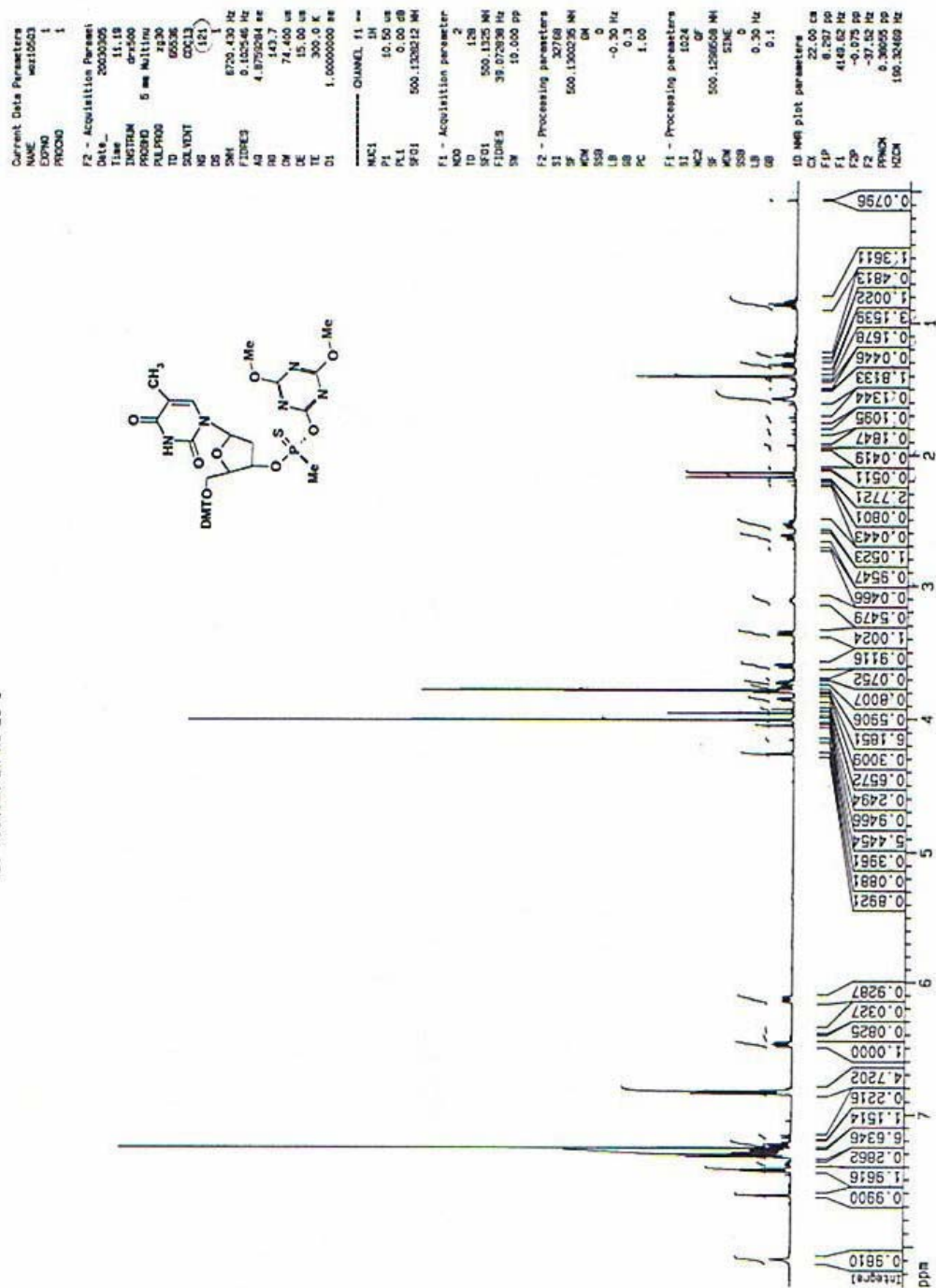
***General procedure for dinucleoside (3',5')-methylphosphonothioate (4)***

5'-O-DMT-N-protected nucleoside 3'-O-(4,6 dimethoxy-1,3,5-triazin-2-yl) methylphosphonothioates (**7**) (1 equiv) and properly protected nucleosides **9** (2 equiv) were dissolved in  $\text{CH}_2\text{Cl}_2$  and dried overnight over freshly activated molecular sieves 4 Å. To this solution DBU was added (4 equiv) and the reaction mixture was stirred with for 24 h at room temp. The reaction mixture was diluted with additional  $\text{CH}_2\text{Cl}_2$ , and washed twice with diluted citric acid (0.01 M) and water. Organic layer was dried with  $\text{MgSO}_4$ , concentrated to dryness and coevaporated twice with toluene. The residue was dissolved in small volume of dry THF and treated with  $\text{Et}_3\text{N}\cdot 3\text{HF}/\text{Et}_3\text{N}$  (3:1 v/v). After deprotection was completed (3-4 hours), the reaction mixture was diluted with chloroform, washed twice with  $\text{NaHCO}_3$  (0.1 M) concentrated, and subjected to a silica gel column chromatography (gradient 0÷6% EtOH in chloroform, with addition of 0.05% of  $\text{Et}_3\text{N}$ ).



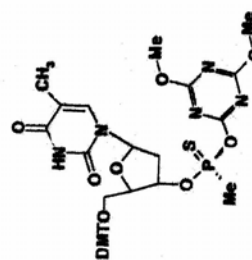
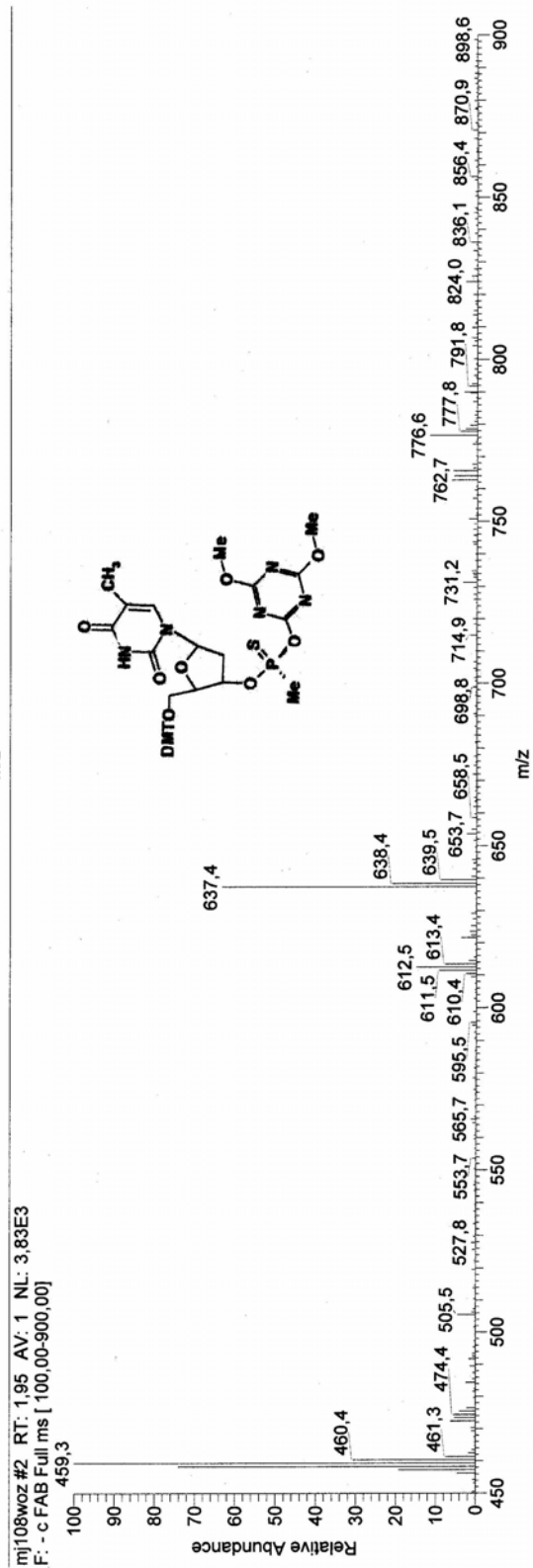
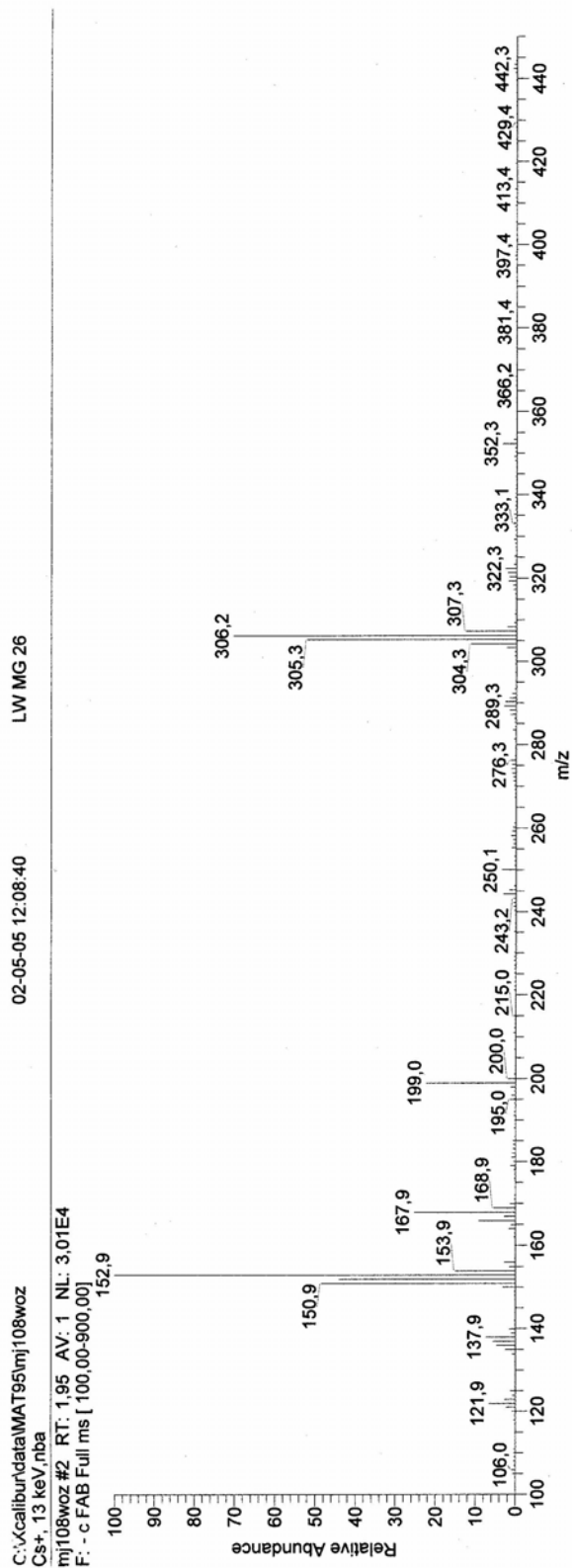
Compound  $S_{P-7}$

L. Moznioak LN MS 20-1



Compound *Sp*-7

Compound *S<sub>P</sub>*-7



Compound Rp-7

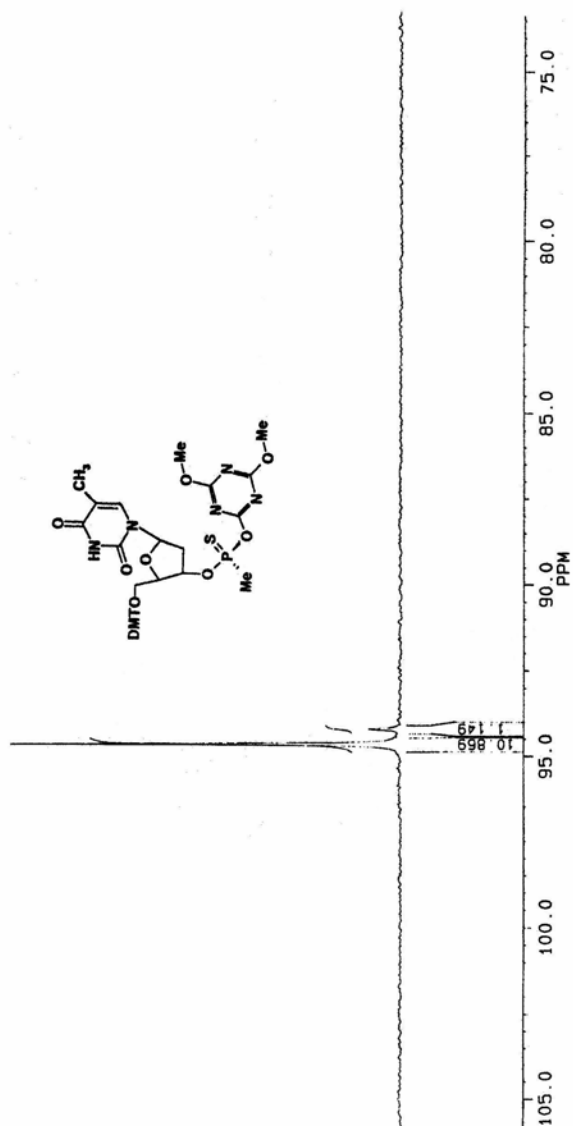
L. WOZNIAK LW MG 54

94.6551  
94.2063

Wdd

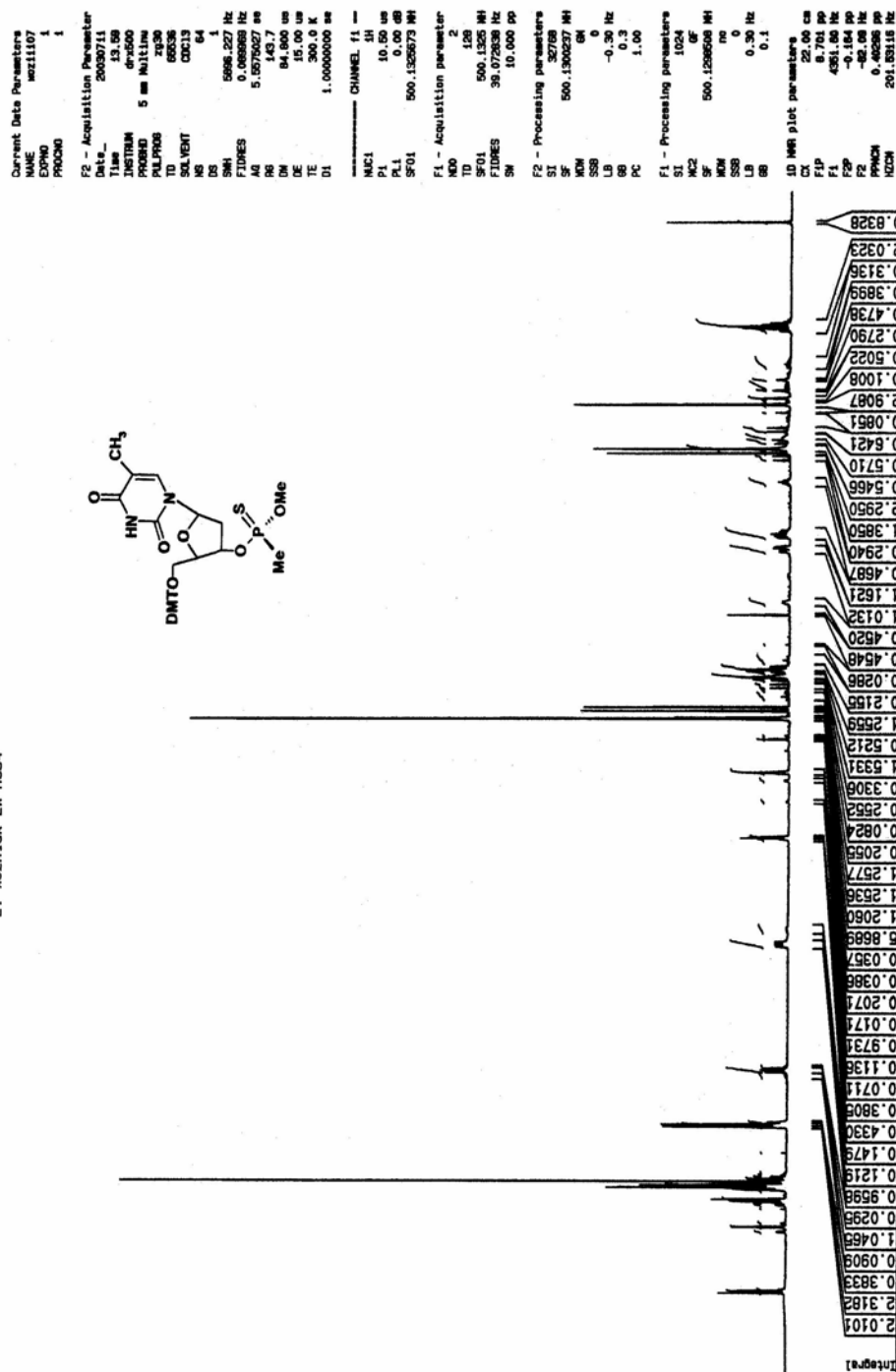
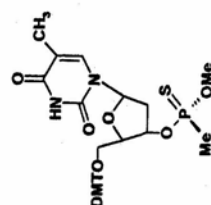


WOZ10707.001  
NS 116  
DP 24H BB  
SR -985.51



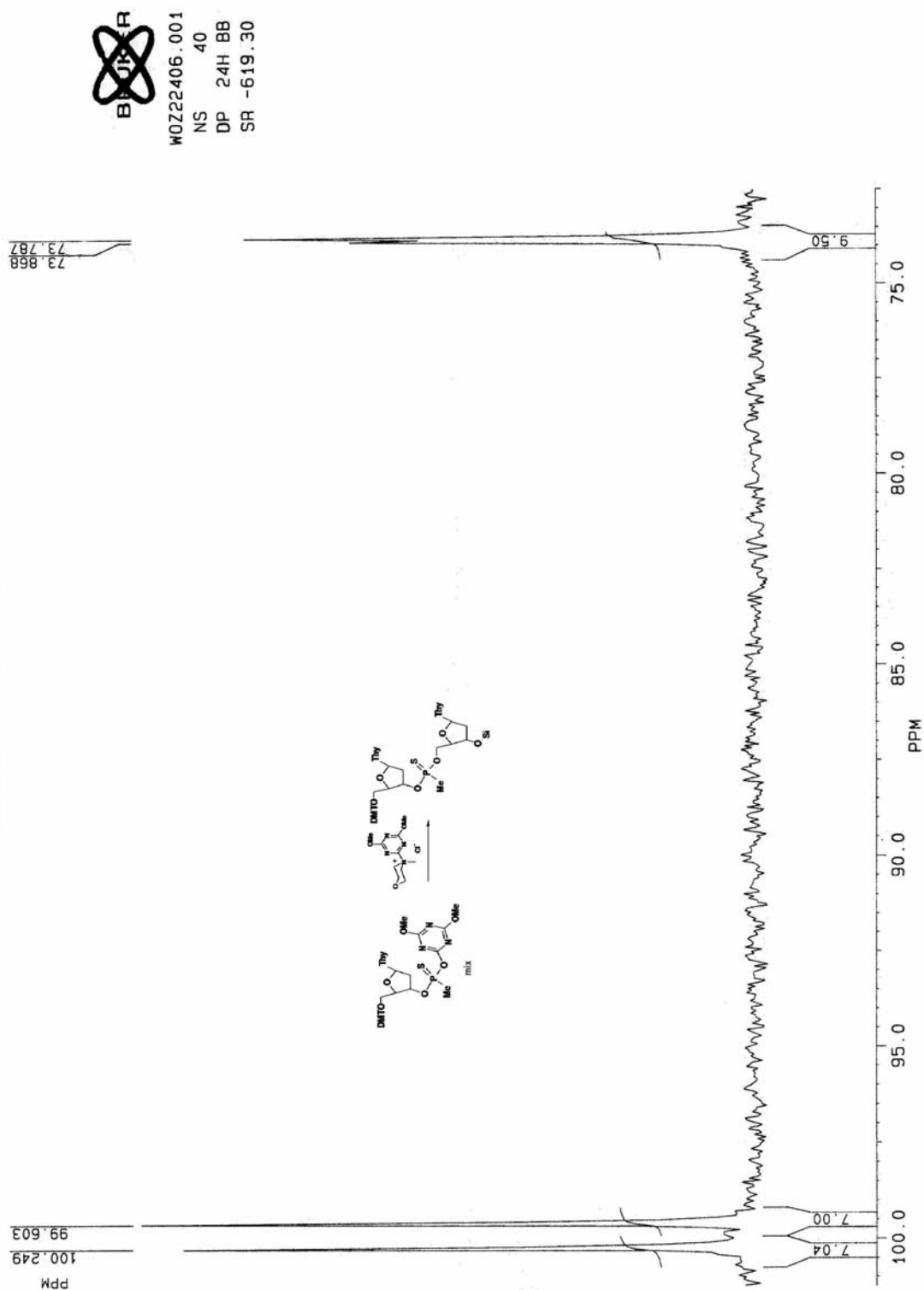
# Compound *S<sub>P</sub>*-8

L. Nozniak LW M654



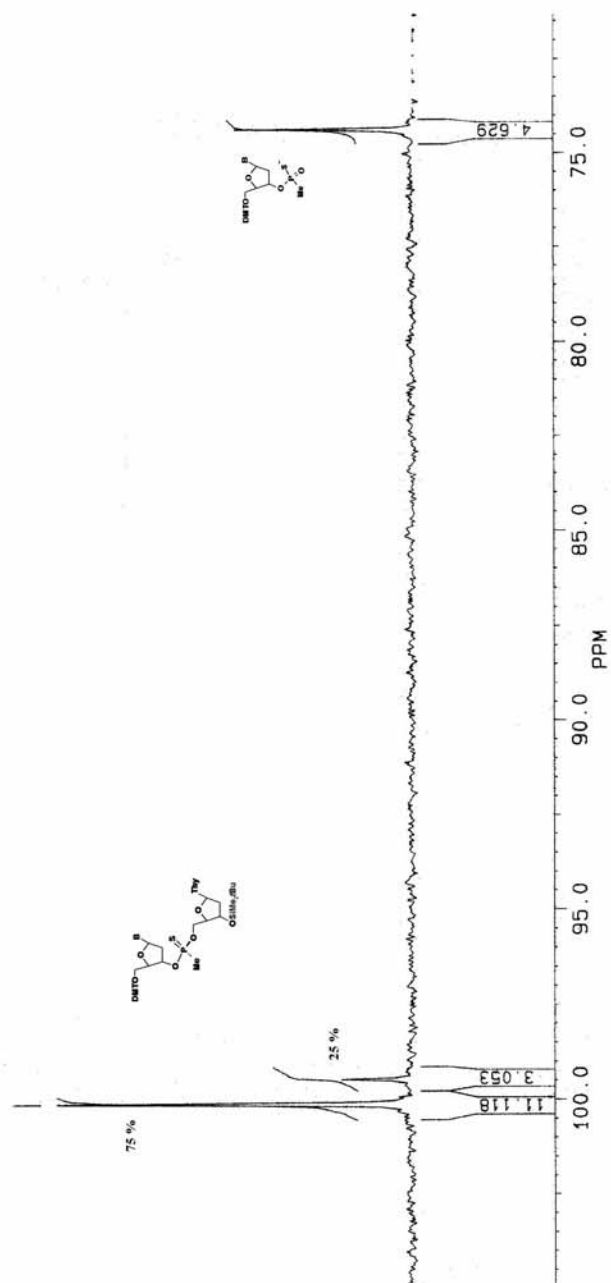
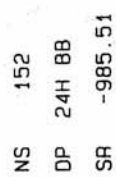


L.WOZNIAK = LW MG 49 =

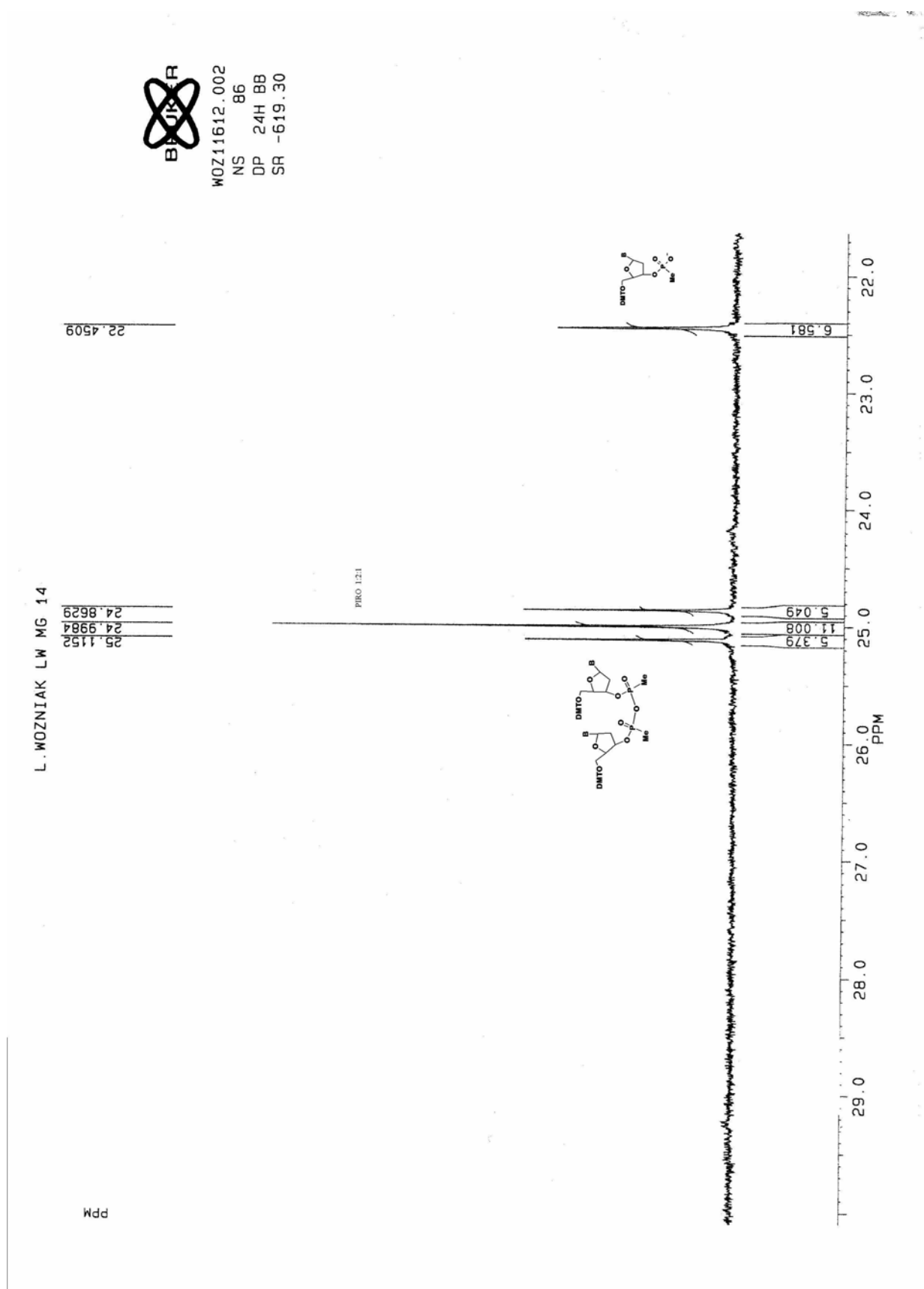


Compound Mix-4 (100.25 and 99.6 ppm) and Mix-5 (73.87 and 73.71 ppm)

Wdd



S10



Compound **12** (25.12, 24.99, 24.83 ppm) and **11** (22.45 ppm)