

## SYNTHESIS OF THYMIDINE-THYMIDINE-DINUCLEOTIDES WITH AMINO INTERNUCLEOTIDE LINKAGE

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### ABSTRACT

*$\beta$ -Thymidine was protected with 4,4'-dimethoxytrityl group at 5'-OH, then treated with 2-chloro-N-methylamine hydrochloride to give 1. The nucleoside 1 was coupled with the pentofuranuronic acid 2 in presence of diphenylphosphorylazide and triethylamine to afford the dinucleotide 4a.*

*Coupling of 1 with 5'-iodo- $\beta$ -thymidine (3) in presence of triethylamine gave the dinucleotide 4b.*

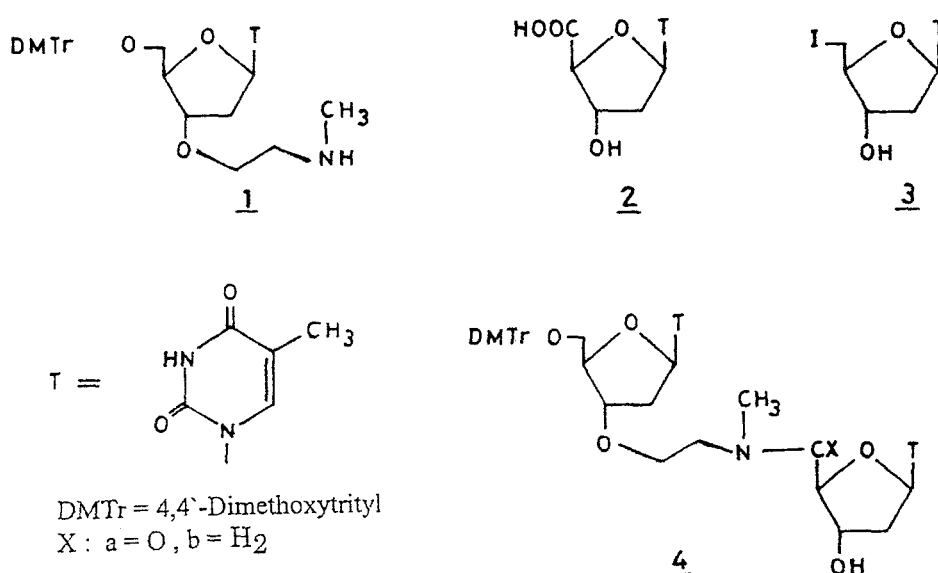
### INTRODUCTION

Phosphate-modified oligonucleotide analogues such as phosphorothioates<sup>1,2</sup> and methylphosphonates<sup>3</sup>, initially chosen for their ease of synthesis and resistance to nuclease degradation, have demonstrated the effectiveness of antisense oligonucleotides in vitro and in vivo. However, both phosphorothioate and methylphosphonate modification suffer from some drawbacks, e.g. introduction of diastereomers causing variable capability to hybridize with target sequences. With this and some desirable oligonucleotide traits in mind such water solubility, resistance to nuclease degradation, ability to penetrate the cell membrane, and low cost preparation methods, researcher are now replacing some or all the anionic phosphate linkers with neutral non-phosphate linkers<sup>4</sup>, two of such are described in this paper

## RESULTS AND DISCUSSION

The new dinucleotides (T\*T) described here contain 5-atom N-methylcarboxamide or N-methylamine linker connecting the two pentofuranosyl moieties, since we believe a shorter linker would be too rigid compared to the natural 4-atom phosphate linker. Further, this linkers were chosen for its non-ionic character, a desirable property for antisense applications

The amin5'-O-(4,4'-dimethoxytrityl)-3'-O-(2-N-methylethyl) thymidine (1) was prepared from 5'-O-(4,4'-dimethoxytrityl) thymidine<sup>5</sup> and 2-chloro-N-methylethylamine hydrochlorid<sup>6</sup> in the presence of potassium hydroxide in large excess by the method described by Griffen and Todd<sup>7</sup>. The basic properties of the amino group was good help in the purification phase since it did seem to reduce the lability of the 5'-O-(4,4'-dimethoxytrityl) group when exposed to acid.



Oxidation of thymidine in the 5'-position as described by Moss *et al.*<sup>8</sup> is a very specific reaction, and the carboxylic acid **2** was sufficiently pure after precipitation from reaction mixture. The carboxamide T\*T dimer **4a** was prepared from **1** and **2** under mild, neutral conditions using diphenylphosphorylazide (PhO)<sub>2</sub> P(O) N<sub>3</sub><sup>9,10</sup> as the condensing agent and the product was obtained in 30% yield after purification on silica gel. The amine T\*T dimer **4b** was prepared from **1** and 5'-iodothymidine (**3**) when heated for 40 h in presence of triethylamine and the product was obtained in 12% yield after purification on silica gel. . The structure of **1** and **4** were verified by use of <sup>1</sup>H- and <sup>13</sup>C-NMR spectroscopy beside the elemental analysis.

#### EXPERIMENTAL

<sup>1</sup>H- and <sup>13</sup>C- NMR spectra were recorded on a Bruker AC 250 spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane in CDCl<sub>3</sub>. Elemental analyses were performed in the microanalytical centre at Cairo University

#### **Preparation of 2-chloro-N-methylethylamine hydrochloride **6**:**

N-Methylethanolamine (19 g) were neutralized with conc. HCl using a pH paper. Water was completely removed under reduced pressure. The residue was suspended in 250 ml dry benzene and cooled to 0°C. To the cold solution was added very quickly 30 ml of thionyl chloride. The reaction mixture was left to warm to room temperature and then refluxed for 2 h. The solvent and the excess of thionyl chloride were evaporated under reduced pressure to give a white solid. The solid was washed with ether. m.p 89-90°C; yield 80%.

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**Synthesis of 3'-O-(2-N-methylaminoethyl)-5'-O-(4,4'-dimethoxytrityl) thymidine 1:**

A suspension of 5'-O-(4,4'-dimethoxytrityl) thymidine<sup>5</sup> (5.4 g, 10 m mole) and KOH (15 g, 270 m mole) in 30 ml benzene and 10 ml dioxane was refluxed and stirred for 1 h. To this suspension was added 2-chloro-N-methylethylamine hydrochloride (8.12 g, 70 m mole). The reaction mixture was stirred in the refluxing solvent for 12 h. The solvent was evaporated under reduced pressure. The residue was mixed with 200 ml of water and then extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, evaporated, then chromatographed on silica gel (30 g) by using CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub> (2-7%) as eluent. m.p 95-97°C; yield 37%. <sup>1</sup>H-NMR δ 1.44 (s, 3H, CH<sub>3</sub>); 2.20 (m, 2H, 2'-H); 2.51 (s, 3H, CH<sub>3</sub>N); 2.90 (m, 2H, CH<sub>2</sub>N); 3.46 (m, 4H, CH<sub>2</sub>O and 5'-H); 3.78 (s, 6H, CH<sub>3</sub>O); 4.10 (m, 2H, 4',3'-H); 6.84 (t, J=7.7 Hz, 1H, 1'-H); 7.25-7.39 (m, 13 H, arom.) 7.60 (s, 1H, 6-H).

<sup>13</sup>C-NMR δ 11.68 (CH<sub>3</sub>); 35.42 (CH<sub>3</sub>N); 40.42 (C-2'); 50.32 (CH<sub>3</sub>N); 55.11 (CH<sub>3</sub>O); 63.73 (C-5'); 67.32 (CH<sub>2</sub>O); 80.26 (C-3'); 83.81 (C-3'); 84.62 (C-Ar); 86.81 (C-4'); 111.18 (C-5); 113.14, 127.02, 128.00, 129.95 (arom.); 135.22 (C-6); 144.17 (arom.); 150.51 (C-2); 158.6 (arom.); 163.87 (C-4). Found (C,63.3;H, 6.6; N, 6.6%, C<sub>34</sub>H<sub>39</sub>N<sub>3</sub>O<sub>7</sub> · 5/2 H<sub>2</sub>O requires C, 63.14; H, 6.86; N, 6.50%.

**Synthesis of 1',2'-Dideoxy-(N-[5'-O-(4,4'-dimethoxytrityl) thymidin-3'-yloxyethyl]-1'-thyminy)β-D-erythro-N-methyl pentofuranuronamide (T\*14a):**

To a solution of 1 (983 mg, 1.64 m mole) in 14 ml dry DMF was

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added **2**<sup>8</sup> (349 mg, 1.36 m mole) at -20°C. Diphenylphosphorylazide (450 mg, 1.64 m mole) and triethylamine (165 mg, 1.64 m mole) were added at the same temperature. The reaction mixture was left to warm to room temperature and stirred for 10 h. The solvent was evaporated under reduced pressure (1 Torr). The residue was chromatographed on silica gel (50 g) by using acetonitrile as eluent. The product obtained as foam, yield 30% <sup>1</sup>H-NMR δ 1.46, 1.48 (2x s, 6H, 2x CH<sub>3</sub>); 2.10 (s, 3H, CH<sub>3</sub>N); 2.12-2.55 (m, 4H, 2',2''-H); 2.96 (t, J=6.0 HZ, 2H, CH<sub>2</sub>N); 3.12-3.55 (m, 4H, CH<sub>2</sub>O, 5'-H); 3.78 (s, 6H, CH<sub>3</sub>O); 4.10 (m, 2H, 4',3'-H); 4.55-4.70 (m, 2H, 4'',3''-H); 5.12 (s, 1H, OH); 6.28 (t, J=6.7 Hz, 1H, 1'-H); 6.35 (t, J=7.0 Hz, 1H, 1''-H); 7.0-7.50 (m, 13H, arom.); 7.60, 7.66 (2x s, 2H, 6'-H); 10.20 (s, 2H, 2x NH). <sup>13</sup>C-NMR δ 11.81, 12.48 (2x CH<sub>3</sub>); 33.26 (CH<sub>3</sub>N); 37.24, 39.31 (C-2', 2''); 49.51 (CH<sub>2</sub>N); 55.15 (CH<sub>3</sub>O); 64.06 (C-5'); 77.42 (CH<sub>2</sub>O); 81.21, 84.44 (C-3', 3''), 83.88, 84.40 (C-1', 1''); 84.81 (C-Ar); 86.95, 87.21 (C-4', 4''); 111.04, 111.21 (C-5, 5'); 113.22, 127.10, 128.01, 129.95 (arom.); 135.21, 133.31 (C-6, 6'); 144.15 (arom.); 151.11, 151.62 (C-2, 2'); 158.60 (arom.); 163.88; 164.55 (C-4, 4'); 171.23 (CO). Found: C, 60.2; H, 6.3; N, 7.0 %. C<sub>44</sub>H<sub>49</sub>N<sub>5</sub>O<sub>12</sub> · 2H<sub>2</sub>O requires C, 60.33; H, 6.10; N, 7.10%.

**Synthesis of 1',2'-Dideoxy-(N[5'-O-(4,4'-dimethoxytrityl) thymidin-3'-yloxyethyl]-N-methyl) 5'-amino-1'- thyminyll β-D-erythro-pentofuranose (T\*T**4b**):**

To a solution of **1** (651 mg, 1 m mole) in 5 ml abs.EtOH was added 5'-iodothymidine (**3**)(352mg, 1m mole) and triethylamine (101 mg, 1 m mole). The reaction mixture was refluxed for 40 h. The solvent was evaporated under reduced pressure and the residue was

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chromatographed on silica gel (40 g) by using CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub> (2-5%) as eluent. The product obtained as foam. Yield 12%.

<sup>1</sup>H-NMR δ 1.40, 1.42 (2x s, 6H, 2x CH<sub>3</sub>); 2.05 (s, 3H, CH<sub>3</sub>N); 2.05-2.50 (m, 4H, 2', 2''-H); 2.93 (t, J=5.8 Hz, CH<sub>2</sub>N); 3.10-3.38 (m, 4H, CH<sub>2</sub>O, 5'-H); 3.40 (s, 6H, CH<sub>3</sub>O); 4.10-4.20 (m, 2H, 4', 3''-H); 4.52-4.60 (m, 2H, 4'', 3''-H); 5.22 (s, 1H, OH); 6.20 (t, J=6.7 Hz, 1H, 1'-H); 6.35 (t, J=7.0 Hz, 1H, 1''-H); 7.20-7.50 (m, 13 H, arom.); 7.61-7.63 (2x s, 2H, 6,6'-H); 9.20 (s, 2H, 2x NH). <sup>13</sup>C-NMR δ 11.01, 11.50 (2x CH<sub>3</sub>); 31.20 (CH<sub>3</sub>N); 38.24, 40.86 (C-2', 2''); 48.50 (CH<sub>2</sub>N); 56.00 (CH<sub>3</sub>O); 63.02, 65.57 (C-5', 5''); 76.23 (CH<sub>2</sub>O); 81.00, 81.12 (C-3', 3''); 82.90, 83.20 (C-1', 1''); 84.80 (C-Ar); 86.16, 86.22 (C-4, 4'); 111.30, 111.35 (C-5, 5'); 112.25, 127.10, 128.81, 129.84 (arom.); 135.80, 136.02 (C-6, 6'); 145.00 (arom.); 150.09 (C-2, 2'); 158.17 (arom.); 163.2, 163.5 (C-4, 4'). Found: C, 61.7; H, 6.5; N, 8.0%. C<sub>44</sub>H<sub>51</sub>N<sub>5</sub>O<sub>11</sub> · 3/2 H<sub>2</sub>O requires C, 61.96; H, 6.38; N, 8.21.

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### الملخص العربى

تخليق ثنائى نيكليوتيدات الثيميدين - ثيميدين ذات رابطته نيكليوتيديه  
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