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Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:

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H. Rosemeyer^a; E. Feiling^a; Wolfgang Nierling^b; F. Seela^a

^a Laboratorium für Organische und Bioorganische Chemie, ^b Physikalische Chemie, Institut für Chemie, Universität Osnabrück, Osnabrück, Germany

To cite this Article Rosemeyer, H. , Feiling, E. , Nierling, Wolfgang and Seela, F.(1999) 'Glycerol-Furcated Oligonucleotides: Synthesis and Aggregation', *Nucleosides, Nucleotides and Nucleic Acids*, 18: 6, 1563 — 1564

To link to this Article: DOI: 10.1080/07328319908044784

URL: <http://dx.doi.org/10.1080/07328319908044784>

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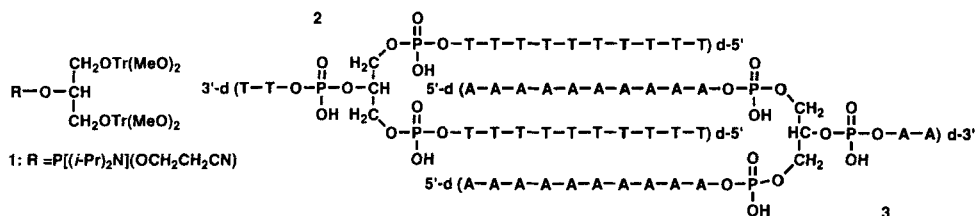
GLYCEROL-FURCATED OLIGONUCLEOTIDES: SYNTHESIS AND AGGREGATION

H. Rosemeyer¹, E. Feiling¹, Wolfgang Nierling², and F. Seela*¹

¹Laboratorium für Organische und Bioorganische Chemie, ²Physikalische Chemie, Institut für Chemie, Universität Osnabrück, Barbarastr. 7, D-49069 Osnabrück, Germany

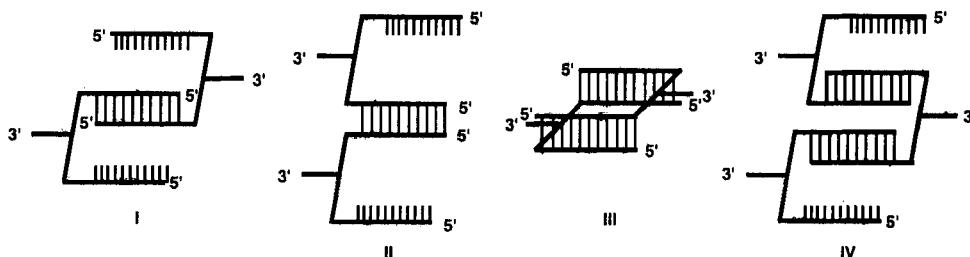
ABSTRACT: The synthesis of the bi-furcated complementary oligonucleotides **2** and **3** is described and their complex formation is studied as a function of their molar ratio and ionic strength by means of temperature-dependent UV- and CD spectroscopy as well as by dynamic light scattering. Structural proposals for the different aggregates are given.

The role of branched RNA's as intermediates of RNA splicing¹ has initiated the synthesis of branched oligonucleotides which have found application as poly-labelled DNA probes². We report on the bi-furcated oligomers **2** and **3** with glycerol as a flexible ramification point. They were prepared by solid-phase synthesis applying the phosphoramidite **1**. Their composition was confirmed by MALDI-TOF mass spectrometry.

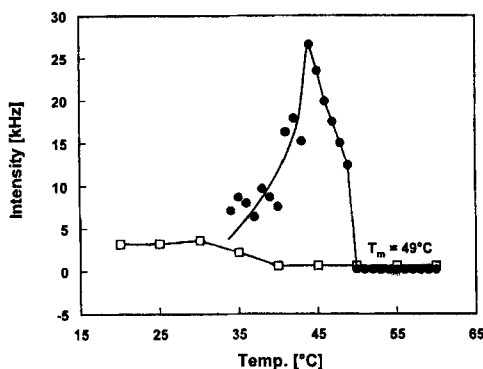


Temperature-dependent UV- and CD measurements of an equimolar mixture of **2** and **3** (3 μM , each, 10 mM Na-cacodylate, 10 mM MgCl_2 , 100 mM NaCl, pH 7) showed a duplex formation with a T_m value of 36°C and a thermal hypochromicity (H, 5–80°C) of 30 % which is similar to the data of $d(A_{10}) \bullet d(T_{10})$ ³. The CD spectra resemble those of homomeric oligo(dA)•oligo(dT). From concentration-dependent T_m measurements as well as from fitting of individual melting curves to a two-state model the thermodynam-

ics of duplex formation ($\Delta H^\circ = -78$ kcal/mol, $\Delta S^\circ = -225$ cal/K mol) were evaluated. Also these data correspond to calculated values of $d(A_{10}) \bullet d(T_{10})$ ⁴. This implies the formation of either aggregate I or II and disclosure of structure III. A 2:1 mixture (2: 4 μ M; 3: 2 μ M) exhibits a similar T_m value as a 1:1 mixture (37°C) making structure IV unlikely.



Enhancement of the $MgCl_2$ concentration to 110 mM raises the T_m value of both, the 2:1- (2: 6 μ M; 3: 3 μ M; $T_m = 49^\circ C$) and the 1:1 mixture of the oligomers (2, 3: 3 μ M, each, $T_m = 47^\circ C$). Cooling a 2:1 mixture of **2**•**3** below $\approx 40^\circ C$ results in a reversible precipitation of a fluffy oligonucleotide material which does neither happen in the case of equimolar mixtures nor at low Mg^{2+} ion concentrations. Dynamic light scattering (FIG.) on a 2:1 mixture (2: 6 μ M; 3: 3 μ M) shows that down to $49^\circ C$ an ideal solution with a diffusion coefficient, $D_{app,z}(q,c)$, of $4.5 \mu m^2/sec$ exists. Between the T_m ($49^\circ C$) and $44^\circ C$



the light scattering intensity increases drastically indicating the formation of large but still soluble particulates. Below $44^\circ C$ the precipitation of a condensed DNA phase occurs (Ψ -DNA)⁵.

FIG. Dynamic light scattering of **2** (6 μ M) + **3** (3 μ M) (●●●) as well as of an equimolar mixture (3 μ M, each, □□□).

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