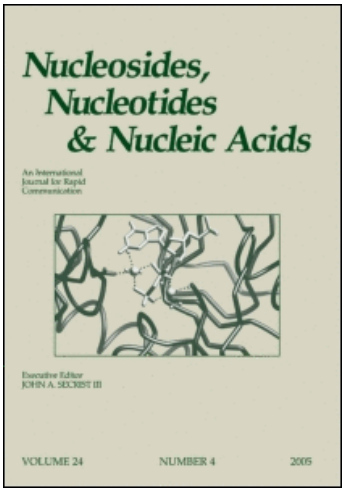
This article was downloaded by:
On: *26 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis of 3',5'-Dithymidylyl-α-hydroxyphosphonate Dimer Building Blocks for Oligonucleotide Synthesis—A New Pro-oliguncleotide

Ralf P. Mauritz[a]; Chris Meier[a]; Eugen Uhlmann[b]
[a] Institute of Organic Chemistry, Johann Wolfgang Goethe-University, Frankfurt am Main, Germany [b] Hoechst AG, General Pharma Research G838, Frankfurt am Main, Germany

To cite this Article Mauritz, Ralf P. , Meier, Chris and Uhlmann, Eugen(1997) 'Synthesis of 3',5'-Dithymidylyl-α-hydroxyphosphonate Dimer Building Blocks for Oligonucleotide Synthesis—A New Pro-oliguncleotide', Nucleosides, Nucleotides and Nucleic Acids, 16: 7, 1209 — 1212
To link to this Article: DOI: 10.1080/07328319708006159
URL: http://dx.doi.org/10.1080/07328319708006159

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.

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 1209-1212 (1997)

SYNTHESIS OF 3',5'-DITHYMIDYLYL-α-HYDROXYPHOSPHONATE DIMER BUILDING BLOCKS FOR OLIGONUCLEOTIDE SYNTHESIS

- A NEW PRO-OLIGONUCLEOTIDE APPROACH -

Ralf P. Mauritz[a], Chris Meier[a]*, and Eugen Uhlmann[b]

a) Institute of Organic Chemistry, Johann Wolfgang Goethe-University, Marie-Curie-Str.11, D-60439 Frankfurt am Main, Germany
b) Hoechst AG, General Pharma Research G838, D-65926 Frankfurt am Main, Germany

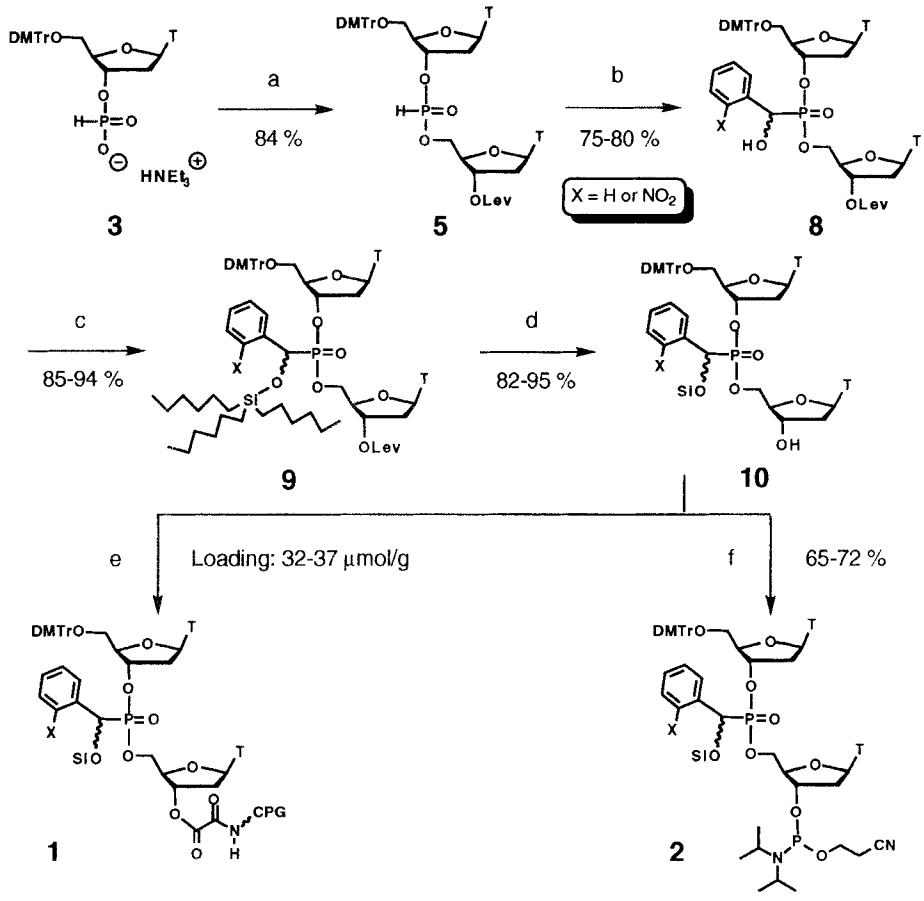
ABSTRACT: The synthesis of the dimer building blocks **1** and **2** and their introduction into $(T)_{15}$-oligonucleotides is described. The stability against 3'-exonuclease digestion (SVP) as well as the hybridization properties (T_m values) were examined.

Downloaded At: 15:49 26 January 2011

DNA or RNA antisense oligonucleotides are an important possibility to treat viral diseases. The mode of action is the hybridization of an antisense oligonucleotide with a complementary sequence of the sense-RNA target strand[1]. Unfortunately natural oligonucleotides showed no biological activity because of low penetration through biomembranes and of high instability against cellular endo- and exonuclease activity. In order to circumvent these limitations different chemically modified oligonucleotides have been synthesized: methylphosphonates, phosphorothioates, phosphorodithioates and phosphotriesters as backbone modifications were introduced[2]. All these modifications are more lipophilic than the natural phosphodiester oligonucleotide and much more stable against exonucleases: Most of them are not degradable which is not always desired because of toxic side effects. The consequence for this purpose is the use of ***Pro-oligonucleotides***[3].

In this work we present the α-hydroxybenzylphosphonate moiety as a new lipophilic phosphodiester-backbone modification, which could also act as a ***Pro-oligonucleotide-concept***[4]. It was shown before, that α-hydroxybenzylphosphonates bearing strong electron-withdrawing substituents in the aromatic ring rearrange to yield benzylphosphotriesters[5]. On the other hand, introduction of an electron-donating substituent leads to the direct cleavage reaction[5]. Therefore two different derivatives of the dimer building blocks were synthesized: one dimer contains a α-hydroxy-2-nitrobenzyl residue (predominantly rearrangement) whereas the second is the unsubstituted α-hydroxybenzyl moiety (exclusively direct cleavage). Both dimer building blocks were prepared as 3'-oxalyl-linked on CPG-support **1** and as 3'-phosphoamidites **2**. These dimers should allow the incorporation of the new backbone modi-

Copyright © 1997 by Marcel Dekker, Inc.



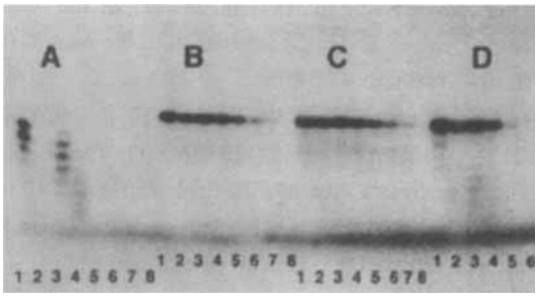
a) 3'-(OLev)T **4**, pivaloylchloride, pyridine, rt, 8 min.; b) benzaldehyde **6** (or 2-nitrobenzaldehyde **7**), CH_2Cl_2, NEt_3 (cat.), rt, 8 h; c) (n-Hex)$_3$SiCl, pyridine, rt, 7 h; d) hydrazine hydrate, pyridine/HOAc (3:2), pyridine, rt, 3 min.; e) oxalylchloride, 1,2,4-triazole, CH_3CN, pyridine, rt, 1 h, then AP-CPG-support (550 Å), rt, 16 h; f) bis(diisopropylamino)-β-cyanoethylphosphine, CH_2Cl_2/CH_3CN (1:1), 1H-tetrazole, rt, 3 h

SCHEME 1: Synthesis of the dimer building blocks **1** and **2**

fication into an oligonucleotide following the phosphoamidite chemistry at different positions: at the 3'-, at the 5'-terminus as well as mixed modified oligonucleotides containing internal and terminal modifications. Here we concentrate on the synthesis of 3'-modified oligo's.

The syntheses of **1** and **2** are summarized in scheme 1. The syntheses, which uses H-phosphonate chemistry, starts from thymidyl-3'-H-phosphonate **3** which was coupled with 3'-levulinylthymidine **4** [3'-(OLev)T] to yield the H-phosphonate diester **5**[6]. Diester **5** was reacted with benzaldehydes (X=H **6** or NO_2 **7**) to give the corresponding α-hydroxybenzyl-phosphonates **8**[7]. The α-hydroxyl groups of **8** were protected using the trihexylsilyl group to give **9**. Compound **10** is the key intermediate for **1** and **2**, which could be isolated after deprotection of the levulinyl group from **9** with hydrazine hydrate. **1** could be prepared using

Downloaded At: 15:49 26 January 2011



A: 5'-$(T)_{13}$TpT-3' **B:** 5'-$(T)_{13}$TpT-3' **C:** 5'-$(T)_{13}$TpT-3' **D:** 5'-$(T)_{13}$TpT-3'

Probes 1-8: **1**: 0 min., **2**: 2 min., **3**: 5 min., **4**: 10 min., **5**: 30 min., **6**: 60 min., **7**: 120 min., **8**: 300 min.; Test: 1 OD of each oligonucleotide

FIGURE 1: PAGE-Gel (15% acrylamide, 10 M urea) of the digestion of the $(T)_{15}$-oligonucleotides with 0.009 U of SVP in 32 mM TRIS buffer with 15 mM $MgCl_2$ at 37 °C

TABLE 1: Melting temperatures (T_m values) of the different modified $(T)_{15}$-oligonucleotides on a 1.0 μM-scale in 10 mM HEPES buffer with 140 mM NaCl (pH 6.8)

Oligonucleotide	5'-$(T)_{13}$TpT-3'	5'-$(T)_{13}$TpT-3'	5'-$(T)_{13}$TpT-3'	5'-$(T)_{13}$TpT-3'
T_m value against $(dA)_{15}$ [°C]	38.1	38.4	37.0	38.2
T_m value against $(rA)_{15}$ [°C]	33.6	33.9	33.1	33.0

the procedure of Letsinger[8] with an average loading of 32-37 μmol/g. The phosphoamidites **2** were obtained from **10** in 65-72% yield after reaction with bis(di*iso*propylamino)-β-cyanoethylphosphine in the presence of 1H-tetrazole.

3'-modified $(T)_{15}$-oligonucleotides were synthesized with the dimer building blocks **1** on a 1.0 μM-scale. After deprotection of the β-cyanoethyl groups with di*iso*propylamine at room temperature for 14 h, the oligomers were liberated from the solid support within 5 min. with a cold saturated solution of ammonia in dry methanol. It is necessary to avoid any moisture in this step. Then the cleavage was stopped by dropwise addition of 50% HOAc until pH≤7. After desalting on a Bond Elut C18-column the purity of the oligonucleotides were examined by HPLC and electrospray (ESI) mass spectrometry.

The stability of the synthesized 3'-modified $(T)_{15}$-oligonucleotides was tested against snake venom phosphodiesterase (SVP) in a 32 mM TRIS buffer containing 15 mM $MgCl_2$

Downloaded At: 15:49 26 January 2011

(pH 6.8) at 37 °C (as shown in Figure 1). The stability of the modified oligonucleotides against 3'-exonucleases (lane B, C and D) in contrast to the natural $(T)_{15}$-mer (lane A) was six fold enhanced from ≈ 8 min. to ≈ 60 min.

The hybridization properties of the 3'-modified $(T)_{15}$-oligonucleotides were measured against $(dA)_{15}$ and $(rA)_{15}$ as references (see table 1). The melting temperatures were determined in a 1.0 μM-scale in a 10 mM HEPES buffer with 140 mM NaCl (pH 6.8) in a range from 10 °C to 80 °C. The observed T_m values of the unmodified and the modified oligonucleotides were in a range of 0.5 °C in all cases.

In summary, the use of the dimer building blocks **1** and **2** allows efficient incorporation of the α-hydroxybenzylphosphonate moiety into oligonucleotide conjugates. These should act as potential ***pro-oligonucleotide***, releasing the unmodified oligonucleotide after rearrangement and hydrolytic cleavage of the resulting phosphotriester or after direct cleavage. The stability of the modified $(T)_{15}$-oligonucleotides against exonucleases showed a significant enhancement in contrast to the natural $(T)_{15}$-oligonucleotide. The melting temperatures (T_m values) of the modified oligonucleotides against $(dA)_{15}$ and $(rA)_{15}$ were in the same range as the unmodified oligonucleotide.

Downloaded At: 15:49 26 January 2011

REFERENCES

1. Uhlmann, E.; Peyman, A.; *Chem. Rev.* **1990**, *90*, 543-584
2. Amarnath, V.; Broom, A.D.; *Chem. Rev.* **1977**, *77*, 183-217
3. a) Iyer, R.P.; Yu, D.; Devlin, T.; Ho, N.; Agrawal, S.; *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1917-1922; b) Tosquellas, G.; Barber, I.; Morvan, F.; Rayner, B.; Imbach, J.-L.; *Bioorg. Med. Chem. Lett.* **1996**, *6*, 457-462
4. Meier, C.; Mauritz, R.P.; *Nucleosides & Nucleotides* **1995**, *14*, 803-804
5. a) Meier, C.; Habel, L.; Laux, W.H.G.; de Clercq, E.; Balzarini, J.; *Nucleosides & Nucleotides* **1995**, *14*, 759-762; b) Meier, C.; *Liebigs Ann.* **1995**, 2195-2202
6. a) Garegg, P.J.; Regberg, T.; Stawinski, J.; Strömberg, R.; *Chem. Scripta* **1985**, *25*, 280-282; b) Garegg, P.J.; Stawinski, J.; Strömberg, R.; *ibid* **1986**, *26*, 59-62
7. a) Meier, C.; *Angew. Chem.* **1993**, *105*, 1854-1856; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1704-1706; a) Kondo, K.; Ohnishi, N.; Takemoto, K.; Yoshida, H.; *J. Org. Chem.* **1992**, *57*, 1622-1625
8. Alul, R.H.; Singman, C.N.; Zhang, G.; Letsinger, R.L.; *Nucl. Acids Res.* **1991**, *19*, 1527-1532