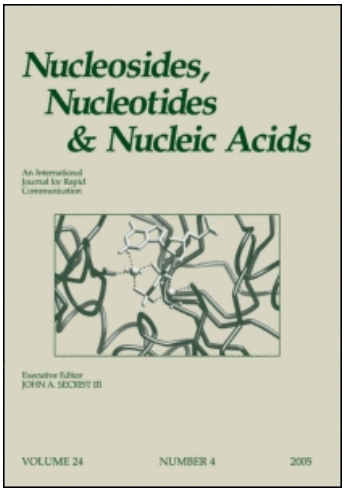

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis of a New Hydroxyamino Linked Thymidine Dimer via a Radical *C-C* Bond Formation

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To cite this Article Sanghvi, Yogesh S. and Cook, P. Dan(1995) 'Synthesis of a New Hydroxyamino Linked Thymidine Dimer via a Radical *C-C* Bond Formation', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 859 — 862
To link to this Article: DOI: 10.1080/15257779508012489
URL: http://dx.doi.org/10.1080/15257779508012489

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 859-862 (1995)

SYNTHESIS OF A NEW HYDROXYAMINO LINKED THYMIDINE DIMER VIA A RADICAL *C-C* BOND FORMATION

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ABSTRACT: An efficient synthesis of a thymidine nucleoside dimer [T-3′-β-O-N(CH_3)-CH_2-5′-T] has been accomplished *via* an intermolecular radical coupling reaction. The novel dimer contains an achiral and neutral backbone linkage which may have potential application in constructing backbone modified antisense oligonucleosides.

Structural modifications of oligonucleotides are becoming increasingly important as possible clinical applications of antisense oligonucleotides (AO) emerge.[1] A recent focus of our research has been directed toward modifications wherein phosphodiester linkages are replaced by achiral and neutral linkages.[2] These analogs are less susceptible to degradation by cellular nucleases and are likely to be transported into the cells due to their increased lipophilicity. In search for superior backbone linkages, we recently reported methylene(methylimino) (MMI) linkage as a novel linker with potential applications in AO.[3] The MMI linkage **1** was not cleaved by cellular nucleases, and MMI containing AO hybridized to their complementary RNA effectively with a high level of base pair specificity. To further explore the structure-activity relationship (SAR) of MMI linked AO and their properties, we synthesized a new hydroxy(methyliminomethylene) (HMIM) linkage as a shorter-positional isomer of MMI linkage.

We believe that a simple atom switch in the MMI linkage **1** (see Figure 1) would alter the internucleosidic distance and provide us with a better understanding of the effects (distance) and requirements of conformational changes in designing improved backbone linkages. In this communication, we describe a convenient synthesis of HMIM linked T*T nucleoside dimer **2**.

Retrosynthetic analysis of desired HMIM dimer **2** indicated that 5′-deoxy-5′-iodothymidine **4** and 1-(3′-*O*-methyleneamino-2′-deoxy-5′-*O*-*t*-butyldimethylsilyl-β-D-

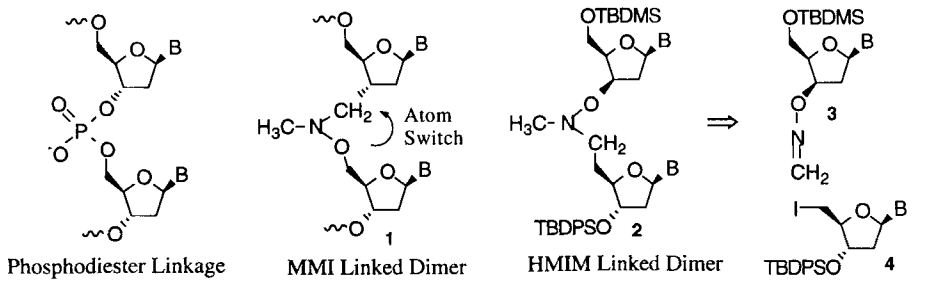


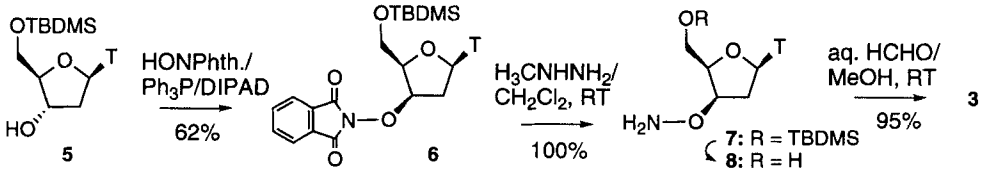
Figure 1

threo-pentofuranosyl)thymidine **3** would serve as key building-blocks for an intermolecular radical *C-C* bond formation reaction. A convenient synthesis of the radical acceptor **3** was accomplished in four-steps from thymidine, following a similar procedure reported in the literature.[4] Mitsunobu reaction of protected **5**[5] resulted in the exclusive formation of 3′-*O*-phthalimido derivative **6** in 62% yield. The 3′-β-configuration of *O*-phthalimido group in **6** was established by COSY and NOESY techniques. Hydrazinolysis of **6** with methylhydrazine gave **7** in quantitative yield. Deprotection of the silyl group of **7** with TBAF furnished 1-(3′-*O*-amino-2′-deoxy-β-D-*threo*-pentofuranosyl)thymidine (**8**), a *threo*-analog of 3′-*O*-aminothymidine, an anti-HIV compound.[4c] *N*-alkylation of **7** with one equivalent of aq. HCHO led to the formation of oxime ether **3** in excellent yield. The synthesis of iodo nucleoside **4** as a radical precursor was accomplished following standard literature procedures.[6]

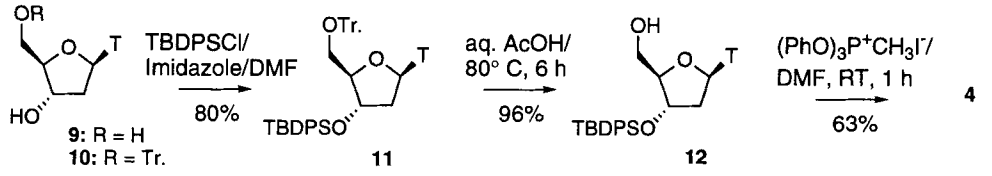
A solution of **2**, **3**, and pinacolate **13**[7] in benzene was refluxed for 16 h to afford, after purification of the reaction mixture by silica gel chromatography, a 52% yield of the protected HMIM dimer **14**. Reductive methylation[3] of **14** with aq. HCHO/$NaCNBH_3$/AcOH furnished **15** in 85% yield. Deprotection of **14** with TBAF led to **15**, which on dimethoxytritylation followed by phosphitylation[8], provided **16** in 65% overall yield. Initial attempts to incorporate **16** in an oligonucleotide *via* standard phosphoramidite chemistry by automated synthesis failed in our hands. Attempts to utilize other coupling chemistries is in progress.

In summary, a covenient synthesis of a *threo*-analog of 3′-*O*-aminothymidine, an anti-HIV nucleoside has been achieved. In addition, synthesis of a new T*T dimer containing an achiral and neutral linkage has been accomplished *via* an intermolecular radical *C-C* bond formation reaction. We believe that HMIM dimer may have applications in constructing backbone-modified antisense oligonucleotide analogs.

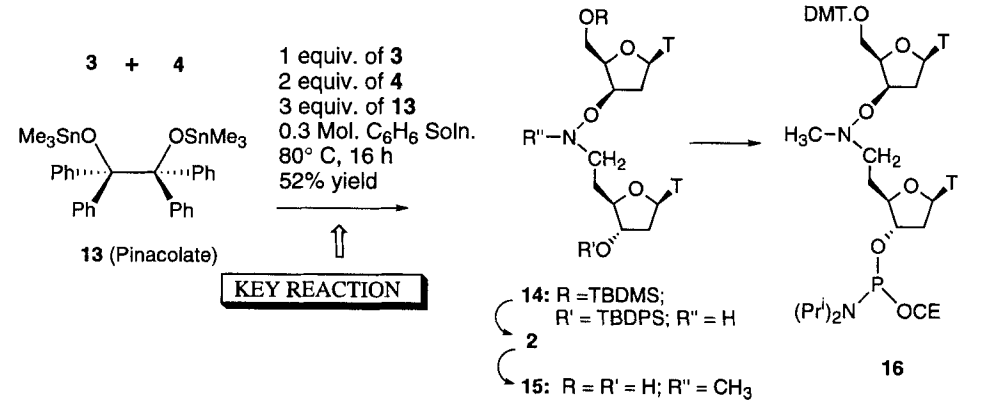
Synthesis of Radical Acceptor 3



Synthesis of Radical Precursor 4



Intermolecular Radical Coupling of 3 and 4



ACKNOWLEDGEMENTS

The poster at 11th IRT indicated α-configuration for the structures **6-8**, which has been corrected to β-configuration. We thank Professor Jean M. Tronchet for sending us a preprint of his manuscript described the synthesis of **8**. Our sample of **8** had similar properties with that reported by Tronchet *et al.* (*Nucleosides & Nucleotides* 13, **1994**, in press). Thanks are also due to Mr. Patrick Wheeler for the NMR work.

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