The Synthesis of *N,N'-O*-Trisubstituted Hydroxylamines *via* a Mild Reductive Alkylation Procedure: An Improved Synthesis of the MMI Backbone

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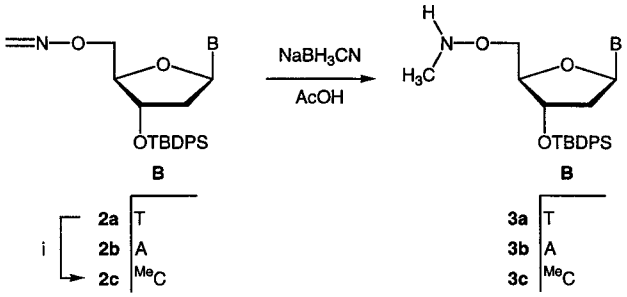
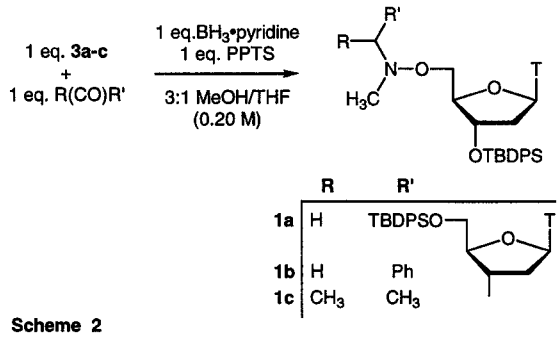
Abstract: A mild procedure for the synthesis of *N,N',O*-trisubstituted hydroxylamines *via* the reductive alkylation of *N,O*-disubstituted hydroxylamines with both aldehydes and ketones has been developed. We have applied this reaction to the synthesis of methylene(methylimino) (MMI) linked dimeric nucleosides, which are of potential use in antisense applications.

Our research toward the development of modified oligonucleotides for use in antisense[1] applications has resulted in a focus on the methylene(methylimino) (MMI) linkage as our preferred backbone modification.[2] Oligonucleotide analogs which contain alternating phosphodiester and MMI linkages are achiral, have reduced charge, are stable to nucleolytic degradation, and hybridize to their complement RNA with good affinity and base-pair specificity.[3] We have therefore concentrated our synthetic efforts to develop a general, convenient, and high yielding synthesis of nucleosidic dimers such as **1a** (Scheme 2) which contain the MMI backbone, yet retain the necessary hydroxyl groups for subsequent functionalization and use in automated DNA synthesis. Both of our previous syntheses[3,4] of MMI dimers required several steps from precursors to the requisite MMI dimers, and are intolerant of common amide base protecting groups, and also the acid labile 4,4'-dimethoxytriphenylmethyl group commonly used for automated solid phase DNA synthesis. We have therefore reduced oxime **2a**[3] to give the 5'-*O*-(*N*-methylamino) thymidine derivative **3a** (Scheme 1), and investigated methods for a *mild, single-step* reductive alkylation of **3a** with an appropriate aldehyde.



Scheme 1. (i) 1,2,4-triazole/Et_3N/$POCl_3$/MeCN then NH_3/1,4-dioxane; T=Thymine, A=adenine, MeC=5-methylcytosine

We initially attempted a coupling of 5'-*O*-*tert*-butyldiphenylsilyl-3'-deoxy-3'-*C*-formylthymidine[5] with **3a** using standard methods for the reductive alkylation of amines with carbonyl compounds, including $NaBH_3CN$,[6] $NaBH(OAc)_3$[7] and $Zn(BH_4)_2$[8] in a variety of solvents, with and without added acid catalyst (AcOH, HCl, or PPTS). Under all conditions attempted, we observed no formation of the desired dimer **1a** (Scheme 2), and obtained either no reaction, intractable mixtures of products, or preferential reduction of the aldehyde to alcohol. This last difficulty could presumably be overcome by employing excess aldehyde, however, because we intended to use valuable 3'-*C*-formyl nucleoside derivatives as our precursors, a reaction which required no excess aldehyde was imperative. We also attempted BH_3•pyridine mediated conditions[9] and observed no reaction, however, upon the addition of 1 eq. of pyridinium *para*-toluenesulfonate (PPTS),[10] the solution effervesced,[11] and both starting components were consumed to form the desired dimer **1a**. We subsequently optimized the reaction conditions to 1 eq. aldehyde, 1 eq. **3a**, 1 eq. PPTS, and 1 eq. BH_3•pyridine in 3:1 MeOH/THF (0.2 M, THF added for solubility), which provided **1a** in 81% isolated yield.[12] We were also able to alkylate **3a** with simple aldehydes (benzaldehyde) and ketones (acetone) to prepare **1b** and **1c** in 88% and 91% isolated yields, respectively, which serves to illustrate in part the generality of this method.

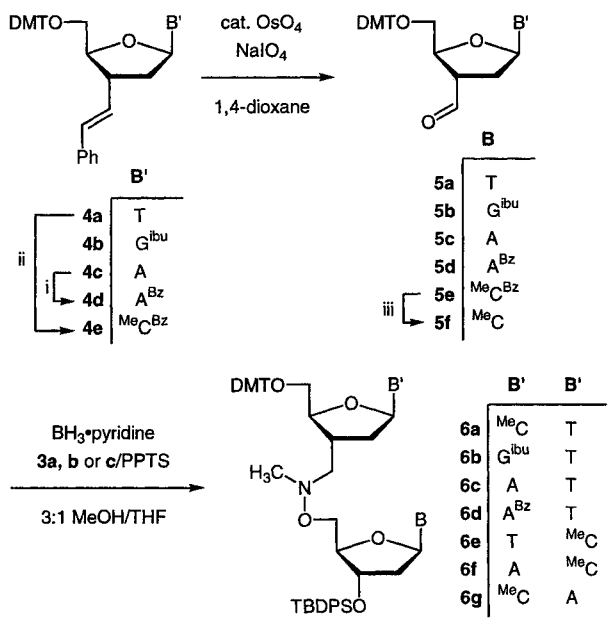


Scheme 2

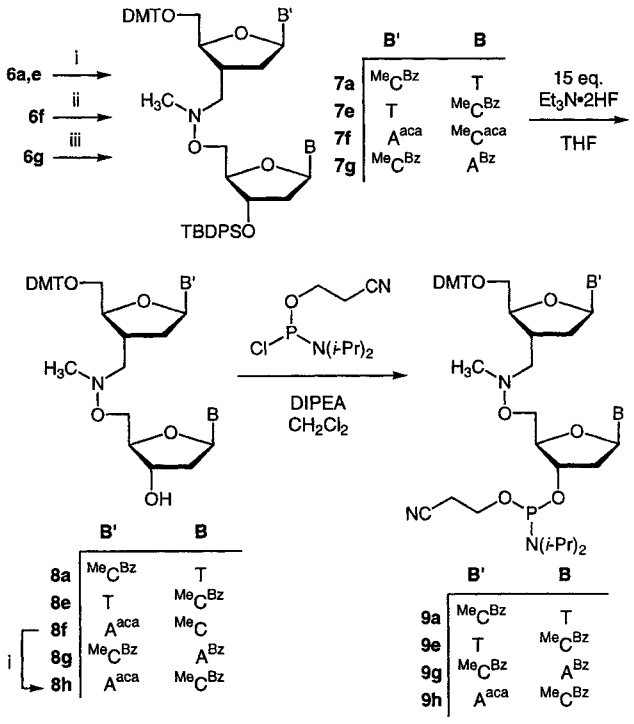
The versatility of this method for the synthesis of MMI dimers was demonstrated by the synthesis of a series of nucleosidic dimers and the corresponding phosphoramidites necessary for automated oligomerization. In order to test the compatibility of the BH_3•pyridine reaction conditions with the four natural bases necessary for the synthesis of any desired dimer, we prepared the 5'-*O*-DMT-protected styrenes **4a-c** (Scheme 3) in a manner analogous to that previously described for the 5'-*O*-TBDPS-protected derivatives.[13] Protection of **4c** in the standard way[14] gave **4d**, and the benzoyl-protected 5-methylcytosine derivative **4e** was prepared from the thymidine derivative **4a** by displacement of the corresponding triazolide with sodium benzamide in 75% yield.[15] The aldehydes **5a-e** were prepared in acceptable yields by a one-pot *cis*-dihydroxylation/oxidation sequence, and **5f** was prepared by ammonolysis of **5e**, then hydrolysis of the resulting imine.

Reductive alkylation of **3a** with **5b** and **5c** proceeded smoothly to give excellent yields of the G^{ibu}*T and A*T dimers **6b** and **6c**, however reaction with the benzoyl-protected aldehyde **5d** resulted in significant depurination, and the A^{Bz}*T dimer **6d** was isolated in only 45% yield. The attempted synthesis of the $^{Me}C^{Bz}$*T dimer **7a** directly from **3a** and **5e** resulted an intractable mixture of products; however, use of the unprotected aldehyde **5f** gave an 84% yield of **6a**, which was then benzoylated to give **7a** in 93% yield (Scheme 4). After desilylation with Et_3N•2HF[16] to provide 95% of **8a**, the appropriately protected phosphoramidite **9a** was prepared in nearly quantitative yield after precipitation of the crude product into hexane.[17] This sequence provides the phosphoramidite necessary for incorporation into oligonucleotides in 70% overall yield from the basic building blocks **5f** and **3a**, and represents a dramatic improvement over previous methods for the synthesis of these dimers.[3,4]

We also prepared several other mixed base dimers *via* the alkylation of **3b** and **3c**, which were readily prepared from the previously reported oximes **2a** and **3a**.[3] From these hydroxylamines and the aldehydes **5a**, **5c**, and **5f**, the dimers **6e-g** were prepared in 70-85% isolated yields. Benzoylation of **6e**, followed by desilylation and phosphitylation



Scheme 3. (i) BzCl/pyridine then 1 N NaOH/EtOH/dioxane (2:5:5 , 0 °C); (ii) 1,2,4-triazole/Et_3N/$POCl_3$/MeCN then NaH/$PhCONH_2$/dioxane; (iii) NH_3/MeOH/THF then EtOAc/1 N AcOH; Bz=N-benzoyl, ibu=N-isobutyryl



Scheme 4. (i) Bz_2O/DMF; (ii) dimethyl-acetamide dimethyl acetal/ MeOH; (iii) BzCl/pyridine then 1 N NaOH/EtOH/dioxane (2:5:5 , 0 °C); aca=N-[1-(dimethylamino)ethylene]

provided the phosphoramidite **9e** in similar overall yield to that obtained for **9a**. Since 6-*N*-benzoyl-2',3'-dideoxyadenosine derivatives underwent partial depurination under our mildly acidic reaction conditions, we reasoned that they would be unstable to detritylation conditions used for solid phase oligomerization.[18] We therefore opted to protect dimers containing a 2',3'-dideoxyadenosine moiety as the 'upper' unit as their dimethylacetamidine derivatives,[19] which provided the desired stability toward depurination.

To illustrate this strategy, **6f** was converted to the *bis*-dimethylacetamidine derivative **7f** in quantitative yield, however the amidine protecting group on the 5-methylcytosine unit was lost during desilylation to give **8f**. This material was selectively benzoylated in 80% yield to give **8h**, then converted to the phosphoramidite **9h**. For protection of **6g**, a simpler strategy could be used, as the adenine-containing unit is a 2'-deoxy nucleoside, and therefore the 6-*N*-benzoyl derivative is stable to detritylation conditions. As Bz_2O/DMF is insufficient to protect an adenine nucleobase, **7g** was prepared in 89% yield by per-benzoylation then partial hydrolysis. Desilylation and phosphitylation gave the phosphoramidite **9g** in excellent yield.

In conclusion, an expedient synthesis of MMI dimers has been developed, which utilizes a new method of reductive alkylation of *N,O*-disubstituted hydroxylamines as the key step. The mild conditions of this reaction allow use of the highly acid labile DMT group for protection of the 5'-hydroxyl groups throughout the sequence, which is especially convenient since it is required in standard automated synthesis of oligonucleotides. We have demonstrated that all four bases are compatible with this methodology, and elucidated base-protection strategies amenable to the synthesis and oligomerization of all sixteen possible MMI dimer phosphoramidites,[20] which should allow a rigorous evaluation of the value of the MMI backbone in antisense strategies. Additionally, the BH_3•pyridine/PPTS conditions have been shown to effect the reductive alkylation of an *N,O*-disubstituted hydroxylamine with a representative non-nucleosidic aldehyde and ketone, and the high yields may allow for use in the rapidly expanding field of combinatorial chemistry.[21] Furthermore, the near neutral conditions of this reaction permit the presence of both acid and base sensitive groups in either substrate, and the 1:1 stoicheometry should facilitate the convergent synthesis of complex molecules which contain substituted hydroxylamines.[22]

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References and Notes

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10. Commercial BH_3•pyridine complex (Aldrich) contains excess pyridine, and the resulting solution is pH~5.

11. As no gas evolution was noted upon addition of BH_3•pyridine to PPTS in MeOH, the gas evolved was presumably produced as the reaction proceeded. It is reasonable to guess that H_2 was produced as the boron-containing species produced upon donation of a hydride decomposed under the reaction conditions.

12. **General Procedure for Reductive Alkylation.** To a solution of aldehyde or ketone (1 eq), **3a** (1 eq), and pyridinium *para*-toluene sulfonate (1 eq) in 3:1 v/v MeOH/THF (5 mL/mmole) was added pyridine-borane complex (1 eq, 8 M in BH_3) dropwise. Stirring was continued until the reaction was complete as judged by TLC. The mixture was partitioned between EtOAc (20 mL/mmole) and 5% aqueous $NaHCO_3$ (20 mL/mmole), and the organic layer washed with 5% aqueous $NaHCO_3$ (10 mL/mmole) and brine, then dried ($MgSO_4$), concentrated, and purified by flash column chromatography. All new compounds were characterized by ^1H NMR, ^{13}C NMR, HRMS and/or elemental analysis.

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18. This was confirmed by treating **6d** with 3% trichloroacetic acid in CH_2Cl_2. In addition to DMT removal, the formation of 6-*N*-benzoyladenine was evident by TLC, with an estimated $t_{1/2}$ of <5 minutes. This is not surprising as sequential removal of hydroxyl groups from adenosine is known to greatly enhance the rate of depurination (Srivastava, P. C.; Robins, R. K.; Meyer, R. B. Jr. Synthesis and Properties of Purine Nucleosides and Nucleotides. In *Chemistry of Nucleosides and Nucleotides, Vol. 1;* Townsend, L. B. Ed.; Plenum Press: New York, 1988; pp. 266-267).

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20. Studies toward this end are in progress, and will be reported in due course. The phosphoramidites **9a**, **9e**, **9g**, and **9h**, have all been incorporated into antisense oligonucleotides on a Millipore Expidite automated DNA synthesizer with coupling efficiencies equal to the natural DNA monomers. The synthesis and biological evaluation of these oligomers will be reported separately.

21. Work at Isis has identified several scaffolds which contain *N,O*-disubstituted hydroxylamines (An, H.; Cook, P. D. *Tetrahedron Lett.* **1996**, *37*, 7233-7236 and Cook, P. D.; Sanghvi, Y. S.; Kung, P.-P. PCT Int. Appl. WO 95/18623, July 13, 1995.). It is straightforward to envision the functionalization of scaffolds of this type with aldehydes and ketones *via* the methods described herein, and studies directed toward this end are ongoing.

22. For an example, see: Nicolaou, K. C.; Groneberg, R. D. *J. Am. Chem. Soc.* **1990**, *112*, 4085-4086.