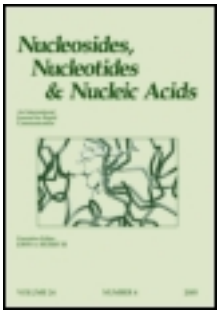

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AN EFFICIENT MULTIGRAM SYNTHESIS OF MONOMERS FOR THE PREPARATION OF NOVEL OLIGONUCLEOTIDES CONTAINING ISOSTERIC NON-PHOSPHOROUS BACKBONES

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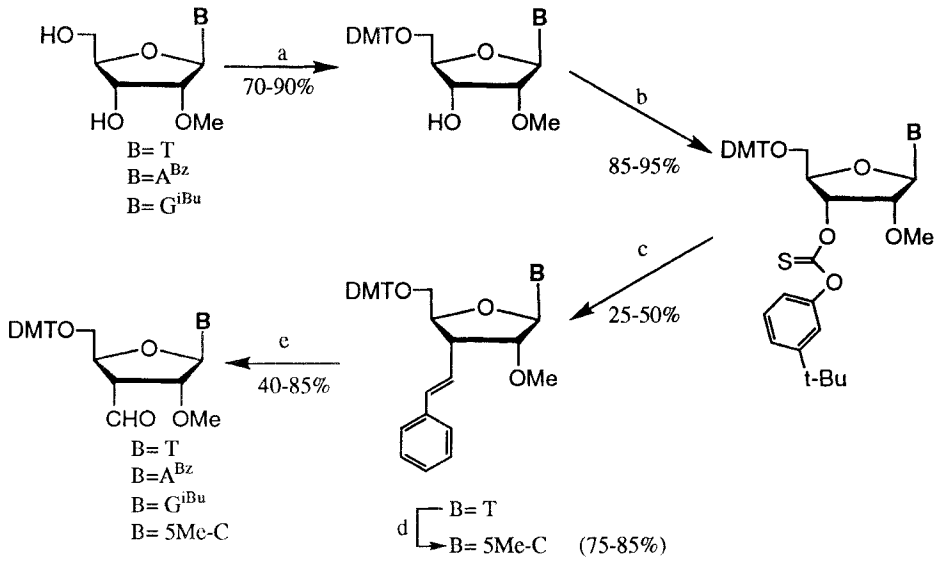
ABSTRACT: The facile preparation of two novel classes of nucleoside analogs for the inclusion as dimeric non-phosphorous containing subunits in chimeric backbones has been accomplished. The concise preparation of 3'-formylnucleosides and 5'-*O*-(N-methylhydroxylamino)-nucleosides is reported.

The introduction of achiral non-phosphorous isosteres for the natural phosphodiester backbone in oligonucleotides represents a rapidly emerging design construct within the paradigm of antisense oligonucleosides.[1] Our efforts in this area have focused on the methylene(methylimino) [MMI] unit as the primary modification. [2] With the incorporation of these nucleosidic dimers, we have seen complete nuclease resistance of the incorporated linkage, considerable protection of the neighboring phosphate residues, and an increase in binding affinity for target RNA without loss of sequence specificity. During the course of these seminal studies, we decided to further investigate the properties of 2' substituted derivatives of the initially prepared MMI constructs. [3] The introduction of the 2'-*O*-methyl group demonstrated an increase in nuclease resistance of the neighboring phosphate beyond that of the initially tested MMI and an increase in binding affinity for RNA complement with respect to the unsubstituted analog. As a result of these investigations, a robust scalable practical preparation of the monomeric subunits requisite for 2'-*O*-methyl-MMI analogs was required to supply material for more thorough investigation of these materials.

The most efficient route for preparation of 2'-*O*-methyl-MMI dimers was shown to be the condensation of a 5'-*O*-(N-methylhydroxylamino) modified nucleoside with the corresponding 3'-formyl-nucleoside to form the oxime derivative. This oxime was further subjected to *in situ* reduction to yield the desired MMI linkage directly. Intermediates from the route presented here were also utilized to investigate the possibility of using the free radical generated at 3'-*C* to capture the 5'-*O*-(formyloxime) modified lower subunit.[4]

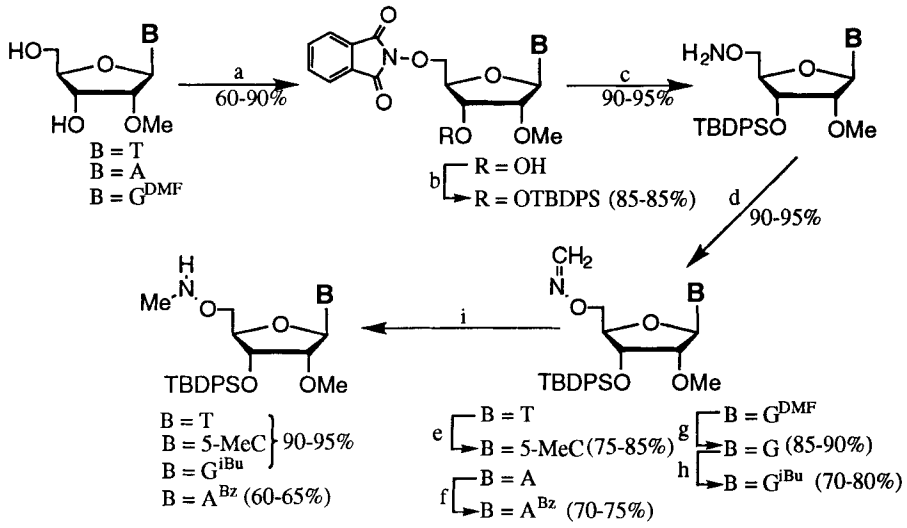
Our entry into the 3'-*C*-formylnucleosides centered around the radical mediated capture of a styryl donor reagent which could be readily oxidized to the desired aldehyde (Scheme 1). [5] The route explored focused on minimizing the number of reactions prior to and after the preparation of the critical carbon-carbon bond. The choice of 5'-*O* blocking group was dictated by our desire to utilize the dimethoxytrityl blocking group during incorporation of the resulting dimers into oligomers via automated synthesis. The dimethoxytrityl group proved to be resilient throughout the ensuing chemistry leading to final product. Preliminary base protection was also tailored toward the use of standard protocols for deprotection within the context of standard solid-phase oligomerization.



Reagents: (a) DMT-Cl, pyridine, 0°C to ambient, 18 h., (b) 3-*tert*-Butylphenyl chlorothionoformate, DMAP, DCM, 0°C to ambient, 18 h., (c) Styryltributyltin, chlorobenzene, ACN, 80-100°C, 0.2 M, 4-12 h., (d) 1)$POCl_3$, triazole, Et_3N, Acetonitrile, -5 to 5°C, 3h., 2) NH_3, Dioxane, (e) 4% OsO_4, $NaIO_4$, H_2O, dioxane.

Scheme 1

Investigations into the nature of the carbon-carbon bond forming reaction itself showed a surprising insensitivity toward the choice of aryl residue on the precursor thionocarbonate derivative. As a direct result, we focused on the use of the (3-*tert*-butylphenyl)thionochloroformate as this reagent represented a greater than 10-fold reduction in materials cost from our previously described intermediate.[4] This transformation also demonstrated a marked sensitivity to the choice of radical initiator. The substitution of 1,1'-Azobis-(cyclohexanecarbonitrile) (ACN) for the previously investigated AIBN reduced the amount of initiator required from 4-5 equivalents down to 1 equivalent or less. The inclusion of a substituent on the α-face of the nucleosidic ribose moiety led to some concern over loss of stereoselectivity at the radical capture stage. These concerns proved to be minor as all four desired nucleosidic precursors demonstrated a better than 15:1 preference for introduction of the styrene substituent in the α-configuration. The only unanticipated problem encountered during the radical process was the partial cleavage of the isobutyryl group from the N^2-position of guanosine. This can be compensated for by reintroduction of the desired isobutyryl protection after styrylation. Oxidation of the resulting 3'-styrylnucleosides proceeded readily through osmium tetroxide mediated conversion to the diol and *in situ* oxidative cleavage of the diol by sodium periodate. Although yields on the styrylation and oxidation were generally modest (e.g. 25-50%), the rapid entry into the 3'-alkylated derivatives and the ease with which these compounds were carried forward to the required aldehydes makes this route amenable to the preparation of multigram quantities of material.



Reagents: (a) PPh_3, DEAD, N-hydroxyphthalimide, THF (T) or DMF (A and G^{DMF}), 1-3 h, (b) TBDPS-Cl, imidazole, DMF, 18 h, (c) Methylhydrazine, DCM, 0°C or -10°C (G^{DMF}), 1 h, (d) 37% aq. Formaldehyde, MeOH, EtOAc, 40°C, 30 min., (e) 1)$POCl_3$, DMF, Triazole, 2) NH_3, MeOH, (f) BzCl, Pyridine, 0°C to ambient, 18 h., (g) 30% aq. NH_4OH, Dioxane, 3 h., (h) iBuCl, pyridine, 0°C to ambient, 18 h., (i) $NaBH_3CN$, AcOH, 10°C, 2 h. or $NaBH_3CN$, MeOH, HCl (pH 4) (A^{Bz}).

Scheme 2

The preparation of a 5′-*O*-(N-methylhydroxylamino)nucleosides (Scheme 2) focused on developing a route with minimal required purification of intermediates applicable to the complete set of required nucleosides. The introduction of the *O*-amino linkage was accomplished by Mitsunobu transformation of the unprotected 2′-*O*-methyl-5-methyluridine and 2′-*O*-methyladenosine. In the case of 2′-*O*-methylguanosine, the unprotected nucleoside exhibits a marked tendency to form the 5′,N^1-cycloanhydronucleoside that has been previously observed for the 2′-deoxy analog.[6] This same tendency was seen in the N^2-isobutyrylated derivative as well. In an attempt to alter the electronic nature of the guanosine base, we investigated protection of the N^2 position with the dimethylformamidine blocking group described by Caruthers et al.[7] This resulted in complete capture of the activated 5′-hydroxyl by *N*-hydroxyphthalamide in a directly analogous fashion to the adenosine and uridine derivatives. This effect appears to be general and, following further study, will be reported in due course. The only limitation introduced by dimethylformamidine protection is this group's incompatibility the reductive conditions employed later in the sequence. It should be noted that this is the only change in protecting groups required in any of the eight preparations described. Purification of the resulting *O*-phthalimide derivatives was facilitated by either performing the reaction in THF (in the case of 5-methyluridine) or removal of the DMF used as solvent for the purines and resuspension in THF. The precipitate obtained contains a small

amount of triphenylphosphine oxide as the solitary impurity. This can be removed by a second precipitation from THF but was demonstrated to be inert in subsequent transformations.

Silylation proceeded readily to block the 3'-*O* position and the products were obtained in a usable crude form by precipitation from a rapidly stirred admixture of ether and ice water. The resulting material was then thoroughly dried and treated with *N*-methylhydrazine in dichloromethane. The *N*-methylphthalhydrazide side product was removed by filtration. Any remaining unreacted *N*-methylhydrazine was then removed by extraction with dilute aqueous acetic acid. The crude filtrate from this reaction was concentrated and treated directly with formaldehyde in ethyl acetate/methanol to afford the formyloxime directly upon concentration.

The 5-methyluridine derivative was purified by column chromatography at this point and further converted to the 5-methylcytidine derivative via triazolation at C^4 followed by ammonolysis as desired. The adenosine oxime derivative afforded a crystalline solid which was protected at N^6 by benzoylation to yield another crystallizable solid. Deprotection of the crude N^2-dimethylformamidineguanosine oxime with aqueous ammonia/dioxane resulted in a crystalline precipitate which was then dried and reblocked with isobutyryl chloride to obtain the desired N^2-isobutyrylguanosine oxime by chromatographic purification.

The oximes described are extremely stable and can be stored over long periods at ambient temperature with no appreciable degradation. Generally these materials are reduced as needed by treatment with sodium cyanoborohydride in acetic acid and used crude to form dimers immediately on preparation.

The transformations accomplished in the homologous portion of the route to the lower subunits have all been optimized to proceed extremely efficiently with few if any side products. As a result, the route, although longer than the preparation reported earlier for the corresponding aldehydes, is robust, efficient, and economical enough to be considered viable for the synthesis of MMI dimers for oligomerization and testing.

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