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Synthesis of Propane-2,3-diol Combinatorial Monomers

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Base catalyzed reaction of heterocyclic bases with R-(+)-glycidol gives heteroaryl-propane-2,3-diols which are functionalized to the 3-O-dimethoxytrityl-2-O-phosphoramidites. A dimethoxytrityl-glycidol is reacted with alkyl or aryl Grignard reagents and then phosphitylated to yield 1-O-dimethoxytrityl-2-phosphoramidites. These compounds can be used for combinatorial oligomer synthesis.

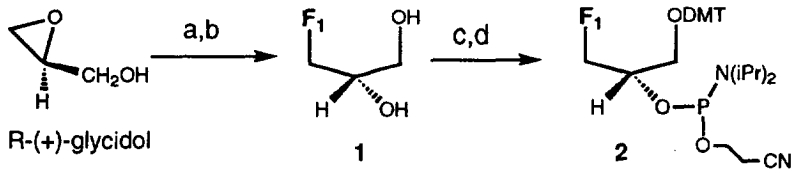
The synthesis and screening of oligonucleotide combinatorial libraries has become a major tool in the search for novel lead sequences against a variety of cellular and viral targets.[1,2] However, oligonucleotide libraries which contain only the four natural nucleobases are limited in the chemical and topological diversity that they incorporate. This limitation is dictated by the stringency of enzymatic methods[3,4] commonly used to amplify the sequence of an active oligomer in a pool found to be active in a screening assay.[5] Our laboratory has previously used a combinatorial method SURF[6] that relies on whole cell antiviral or antiproliferative assays to determine that an oligonucleotide phosphorothioate d($T_2G_4T_2$) is a potent inhibitor of the HIV virus.[7] SURF methodology uses the iterative synthesis and screening of increasingly simplified subsets of oligomer libraries and does not rely on enzymatic amplification at any stage. These findings have prompted our efforts to incorporate more diverse functionalities on simple, regularly repeating propane-phosphodiester oligomers prepared by the oligomerization of propane-2,3-diol phosphoramidite monomers. The synthesis of these monomers is described herein.

The design of propane-2,3-diol monomers, each bearing a unique functionality, meet several criteria: first, functionalities in three chemical classes were identified: a) Watson-Crick bases b) long chain alkyl or aromatic c) heteroaromatic rings. Second, the chemistry developed to incorporate functional groups on a propane-2,3-diol was chosen to be general and simple, in order to quickly synthesize a number of monomers representative of each class. Third, the functionalities in these classes were chosen for their compatibility with the automated phosphoramidite coupling chemistries used in DNA synthesis. Finally, the chirality of the backbone was fixed by the use of a R-(+)-glycidol as starting material. Nucleophilic ring opening of this material at C-1 yielded S-propane-2,3-diols, thus insuring that deconvolution of a library would yield a unique isomer sequence with a defined stereochemistry of the backbone. Here we report on the facile synthesis of propane-2,3-diol monomers representing these classes and their functionalization to phosphoramidites suitable for automated synthesis on solid support.

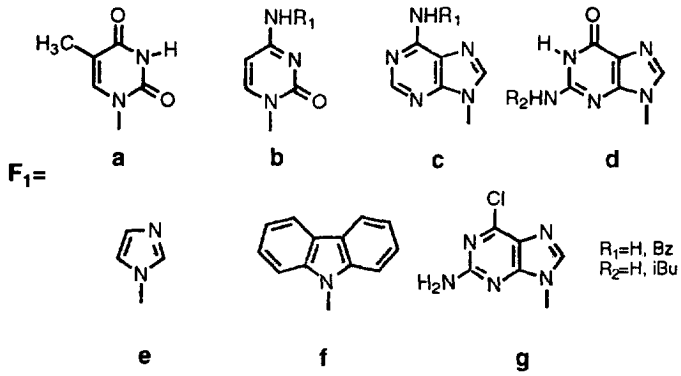
In Scheme 1, carbazole and imidazole were reacted with R-(+)-glycidol in DMF containing potassium carbonate to give the 1-substituted propane-2,3-diols **1e,f**.[8,9] easily purifed by chomatography and crystallization from methanol. This reaction was extended to the synthesis of the

alkylated nucleobases T, C and A (**1a-c**, R_1=H)) and of the guanine precursor, 2-amino-6-chloropurine (**1g**).[10,11] The alkylation of adenine occurred predominantly at N-9 and was separated

Scheme 1



Reagents and conditions: a) F_1H/potassium carbonate/DMF; b) flash chromatography or trituration with hot methanol; c) dimethoxytrityl chloride-pyridine; d) 2-cyanoethyl N,N-diisopropylchlorophosphoramidite, diisopropyl ethylamine in anh. acetonitrile

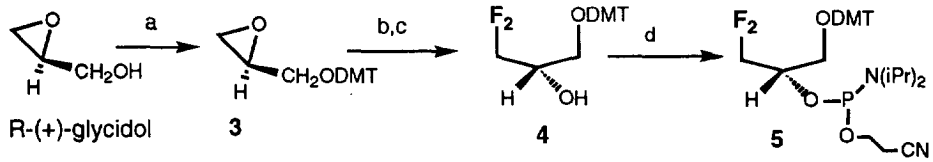


from the N-7 isomer by recrystallization from methanol. Likewise, the 2-amino-6-chloropurine was alkylated at N-9 without significant reaction at N-7. This material was converted to the guanine (**1d**, R_2=H) by a treatment in hot 1 N HCl. Cytosine was alkylated in high yield at N-1 without a product resulting from alkylation at N-3. However, considerable manipulation of the reaction conditions was required to bias the product ratio towards alkylation of thymine at N-1.[12] Thus, a slow introduction of 0.67 equivalents of R-(+)-glycidol into DMF containing thymine and catalytic potassium carbonate at 85 °C yielded the 1-(thymin-1-yl)-propane-2,3-diol (**1a**), free of the product of alkylation at N-3.

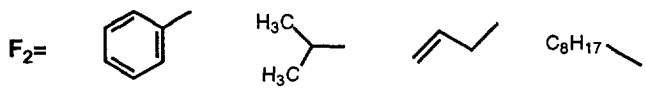
Protection of the amine groups of the adenine- and cytosine-propane-2,3-diols was performed by the transient protection method of Jones.[13] We found that three equivalents of trimethylsilyl chloride followed by treatment with benzoyl chloride provided the adenine N(6)- and cytosine N(4)-benzoylated compounds. The guanine **1d** was likewise transiently silylated and then reacted with isobutyryl chloride to give the N(2)-isobutyryl derivative **1d**, R_2=iBu. In all cases, we were able to selectively react the primary hydroxyl group of the propane-2,3-diols under the same conditions of anhydrous pyridine/DMT chloride used for tritylation of the 5'-hydroxyl groups of deoxyribose nucleosides. The products were characterized by their ^{1}H nmr spectra, which exhibited a doublet (exchangeable with D_2O) for a secondary hydroxyl hydrogen. The tritylated compounds were

routinely phosphitylated using 2-cyanoethyl N,N-diisopropylchlorophosphoramidite in THF. In coupling with a T-derivatized CPG resin, the phosphoramidite monomers **2a-f** exhibited coupling efficiencies greater than 90 %, based on trityl cation yields of the NpT dimers as measured at 498 nm.

Scheme 2.



Reagents and conditions: a) Dimethoxytrityl chloride, triethylamine in dichloromethane; b) F_2MgX, Li_2CuCl_4 in THF; c) aq. NH_4Cl; filter, flash chromatography; d) N,N,N',N'-tetraisopropyl-cyanoethylphosphoramidite, diisopropyl tetrazolide in THF.



Alternatively, reaction of R-(+)-glycidol with DMT chloride in dichloromethane gave the tritylated intermediate **3**.[14] This material was reacted with benzylmagnesium bromide, isopropylmagnesium chloride, allylmagnesium bromide or octylmagnesium chloride to obtain moderate yields of tritylated products **4h-k**.[15] These products also exhibited a signal consistent with a secondary hydroxyl hydrogen in their ^1H nmr, indicative of exclusive nucleophilic attack of the Grignard reagents at the primary carbon of epoxide **3**. Compounds **5h-k** were obtained from routine phosphitylation of **4h-k** using N,N,N',N'-tetraisopropyl-2-cyanoethyl-phosphoramidite in THF.

Alkylated diols **1a-f** were tested for their stability during oligomer synthesis. Thus, the propane-2,3-diols were reacted with standard solutions of dichloroacetic acid in dichloromethane or iodine in THF for periods corresponding to twenty automated coupling cycles. In all cases, the diols remained stable under these conditions. The dimethoxytrityl groups were removed from tritylated compounds **4h-k** using trichloroacetic acid and the products were found stable to the conditions of automated DNA synthesis as descibed above. Also, it was found that acyl protecting groups on C, A and G derivatives **1b-d** (R_1=Bz, R_2=iBu) were easily removed under the conditions of ammonium hydroxide/55 °C/18 hr, thus ensuring unmasking of Watson-Crick hydrogen bonding functionalities following oligomerization.

We believe that the propane-2,3-diol monomers described above will be useful in oligomerizing functionalities in addition to the A, G, T and C heterocyclic bases present in oligonucleotide combinatorial libraries. Novel heterocyclic, aromatic and alkyl groups will provide additional interactions with biological targets. The conformational flexibility of the propane-phosphodiester backbone relative to a deoxyribose backbone will be a factor in determining the

tertiary structure or shape of our combinatorial oligomers . It has been proposed that unique molecular shapes are responsible for the biological activity of novel pharmacophores derived from comibnatorial libraries. [16,17] The synthesis of libraries and the screening of these against a variety of targets will be reported elsewhere.

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9. **1-Imidazolyl-propane-2,3-diol:** A solution of R-(+)-glycidol (12.0 g, .16 mol) in acetonitrile was heated to 50 °C and then treated with imidazole (13.8 g, 0.20 mol). The mixture was heated for an hour to yield a biphasic solution. The lower phase was collected and concentrated to an oil which was flash chromatographed using ethyl acetate-methanol (19:1 and then 9:1). Yield of crystalline solid is 10.2 g (39 %). ^{1}H nmr (dmso-d_6): δ, 3.3 (m, 2, $\underline{CH_2}OH$); 3.8 (bs, 1, CH); 3.9 (dd, 1, CH_2); 4.1 (dd, 1, CH_2); 4.9 (bs, 1, OH, exchanges with D_2O); 5.1, (bs, 1, OH, xchanges with D_2O); 6.9, 7.1, and 7.6 (3 s, 3, imidazole).
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15. **1-O-Dimethoxytrityl-2,3-undecanediol:** Dilithium tetrachlorocuprate (0.1 M solution in THF, 1.9 mL, 0.5 eq.) in THF was cooled to -68 ° and then treated with octyl magnesium chloride (2.0 M in THF, 9.4 mL, 5 eq.) Dimethoxytrityl glycidol (1.4 g, 3.7 mmol) in 20 mL THF was added dropwise and the solution warmed to room temperature for 2 hr, followed by carful addition of saturated aqueous ammonium chloride. The mixture was partitioned between water and ethyl acetate and the organic layer was washed with water, dried and concentrated. Flash chromatography using hexanes-ethyl acetate (19:1) yielded 1.1 g of a sirup (58 %). ^{1}H nmr (dmso-d_6): δ, 0.9 (t, 3, CH_3); 1.2-1.4 (m, 16, aliphatic); 2.35 (d, 1, OH, exch. with D_2O); 2.9-3.2 (m, 2, CH_2O); 3.7-3.8 (m, 7, dimethoxy and methine); 6.9-7.5 (2 m, 13, aryl).
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