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Synthesis of N-Substituted Hydroxyprolinol Phosphoramidites for the Preparation of Combinatorial Libraries.

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Abstract: *A series of N-substituted DMT-hydroxymethylpyrrolidinol phosphoramidites has been prepared from trans-4-hydroxyproline. These can be coupled in high yield and purity using automated synthesis techniques, allowing a wide range of functionalities to be introduced into phosphodiester oligomers.*

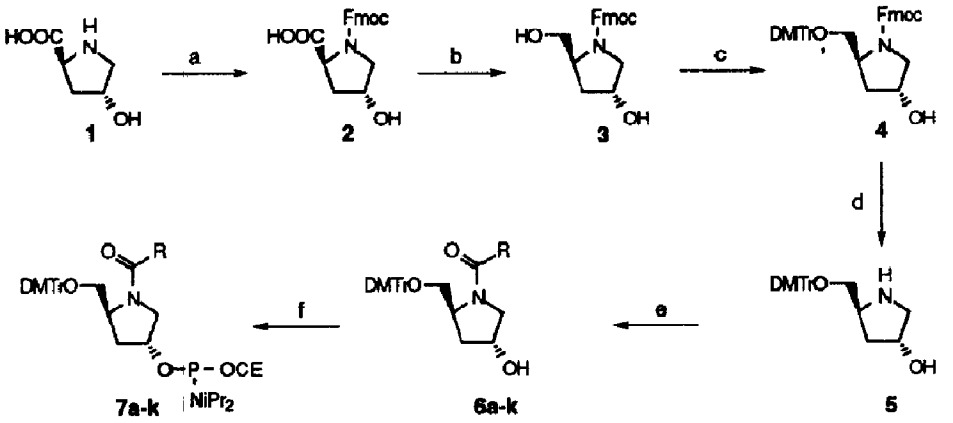
Considerable interest has recently emerged in the use of combinatorial libraries as a strategy for the discovery of novel pharmaceutical leads and diagnostic reagents.[1] This is a powerful technique for discovering novel pharmacophores. It relies on the assumption that oligomeric compounds have activities derived from cooperative interactions that their monomeric components are incapable of producing. The library of oligomers is screened either free in solution or attached to a solid support and the sequence with the highest activity is identified.

We have previously reported the result of our efforts in the area of combinatorial drug discovery which have led to the identification of a new phosphorothioate oligonucleotide with anti-HIV activity.[2] Because of the similarity of functional groups present in the monomers, oligonucleotides can form a limited number of interactions with their environment.[3] In order to extend the range of functional groups available in phosphodiester oligomers, we have prepared a novel series of phosphoramidite monomers. These permit the rapid and facile incorporation of a wide range of functionalities into combinatorial libraries allowing hydrogen bonding, hydrophobic and aromatic stacking, dipolar interactions, and ion pairing. Given the nature of these substituents, it is expected that additional binding mechanisms will be available to the phosphodiester oligomers which are not possible for oligonucleotides.

The design of phosphoramidite monomers suitable for the preparation of combinatorial libraries requires a convenient chemical handle for the attachment of the diverse functionalities, as well as the means to differentiate the termini of the oligomers. Ideally, the synthesis of the monomers should be convergent and begin with a readily available optically pure starting material. Here we describe such a methodology based on the use of *trans*-4-hydroxy -L-proline, which fulfills all these requirements (Scheme 1).[4] The free amino acid **1** was protected in quantitative yield using Fmoc-Cl in aqueous dioxane buffered with $NaHCO_3$.[5] The carboxylate function of **2** was then reduced using BH_3-Me_2S complex in refluxing THF.[6] Treatment of diol **3** with a slight excess of DMT-Cl in pyridine gave a 65% yield of the primary DMT ether **4** after crystallization from methanol.[7] Unreacted starting material and ditritylated product accounted for the remaining mass. The Fmoc group was then removed by treatment with 3-5 equivalents of piperidine in anhydrous DMF. The free amine was isolated as an oil and could be precipitated from hexanes to give a yellow powder of sufficient purity to be used directly. The DMT-hydroxyprolinol **5** was conveniently prepared on 200 g scale in this way, and served as the starting material for the synthesis of the phosphoramidite monomers.[8]

Attention was then focused on introducing chemically diverse functional groups onto the hydroxyprolinol backbone. The secondary alcohol was transiently protected as the TMS ether in initial experiments, but this was found to be unnecessary due to the greater reactivity of the amino function of **5** over the alcohol. Amine **5** was acylated with a wide variety of carboxylic acids using EDC and HOBT as dehydrating agent and catalyst.[9] The yield of amides **6a-k** (Table 1) ranged from 55 to 95% depending on the reactivity and solubility of the carboxylic acids in the reaction solvent. Reactions performed in CH_2Cl_2 gave consistently better yields than those run in DMF. The sulfonamide derivative **6l** was also readily prepared by treatment of **5** with methanesulfonyl chloride. Reactive substituents (R) on the carboxylic acids were protected with base labile blocking groups to allow deprotection with NH_4OH at the end of oligomer synthesis. The carboxylic acid function in **6b** was protected as the fluorenylmethyl ester. Fmoc protection was successful for the pyrrolidine nitrogen in **6c** while primary amine **6k** was more effectively blocked as the trifluoroacetamide. Although Fmoc-Glycine could be coupled to **5**, the Fmoc group was found to be too base-labile to allow the preparation and purification of the phosphoramidite (vide infra).[10] The phenolic and amino function of **6g** and **6h** were protected using benzoyl groups. The alcohol function of **6a-k** was then phosphitylated[11] using cyanoethyl tetraisopropylphosphorodiamidite with diisopropylammonium tetrazolide as catalyst in acetonitrile to give phosphoramidites **7a-k** in 70-90% yield after chromatography (EtOAc/Hexanes + 0.5% Et_3N).[12] The more reactive cyanoethyl diisopropylamino chlorophosphite was unsuitable due to the decomposition of the products under the reaction conditions, and the difficulty in removing the H-phosphonate hydrolysis products which invariably contaminated the desired materials.

Scheme 1: Synthesis of Amidites

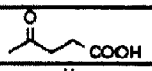
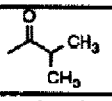
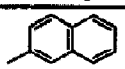
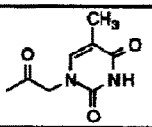
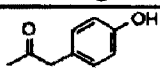
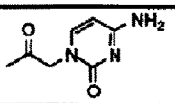
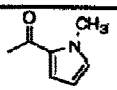
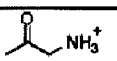


Reagents and conditions: a) Fmoc-Cl, $NaHCO_3$, H_2O/Dioxane; b) BH_3-Me_2S, THF, 65 °C; c) DMT-Cl (1.05 eq), DMAP, Et_3N, Pyridine; d) Piperidine, DMF; e) RCOOH, EDC, HOBT, CH_2Cl_2 or DMF; f) $(iPr_2N)_2P$-OEtCN, DIAT, CH_3CN.

The suitability of monomers **7a-k** for the synthesis of oligomers was established by coupling tests. Each phosphoramidite **7a-k** (0.1 M in CH_3CN) was coupled to deoxythymidine (dT) derivatized CPG using tetrazole activation followed by oxidation using I_2/H_2O/Lutidine/THF. The dimers were then cleaved from the solid support using concentrated NH_4OH at room temperature for one hour. The ammonia solution was

then incubated at 55 °C overnight. The terminal DMT was removed from each dimer using 20% aqueous acetic acid at room temperature for 15 minutes. The coupling yield and purity of the products were measured by reverse phase HPLC. It was established that the DMT protecting group on the terminal monomer should be removed after ammonia cleavage to avoid deacylation of the pseudo 5' residue through anchimeric participation of the hydroxymethyl group. This side reaction was confirmed by comparison with the N-unsubstituted dimer prepared using amidite **7c**. The purity of the dimers was unaffected by heating in ammonia overnight as long as the DMT was still present. Also, complete conversion of the fluorenylmethyl ester to the carboxylic acid in dimers prepared from **7b** was only possible by piperidine treatment prior to ammonia cleavage from the support, due to competing ammonolysis of the ester. The other phosphoramidites were incorporated into dimers without incident. **Table 1** summarizes the results of the coupling tests for each amidite. Each of the amidites **7a-k** were also oligomerized with the same efficiency (results not shown).

Table 1: Coupling efficiency of Hydroxyprolinol Phosphoramidites

Amidite	R	Protecting group	Coupling Yield	Purity[13]
7a		-	99%	98%
7b		FM	99%	99% [a]
7c		FMOC	99%	92%
7d		-	97%	92%
7e		-	97%	97%
7f		-	98%	95%
7g		Bz	98%	98%
7h		Bz	90%	99%
7i		-	97%	99%
7j		-	98%	95%
7k		$COCF_3$	94%	95%

Notes: HPLC conditions: Waters 625 LC system, with a 991 Diode Array detector (260nm). Column: Waters Deltapak C18. Buffer A: 0.1M NH_4OAc, pH 7.0, buffer B: CH_3CN. Gradient: 0 to 70% B in 50 min @ 1.0 ml/min. [a] requires treatment with 5% Piperidine in DMF prior to NH_4OH cleavage.

In conclusion, we have devised a rapid method for introducing new functionalities into phosphodiester oligomers. The technique involves the attachment of a chemical functionality to a pyrrolidine backbone, and elaboration to a phosphoramidite. A wide range of suitably protected phosphoramidites have been prepared and evaluated. The monomers **7a-k** are fully compatible with automated oligonucleotide synthesis, and give high levels of incorporation and purity. The pyrrolidine phosphoramidites are currently being incorporated into combinatorial libraries for screening against a variety of targets. The advantages of the method include the rapid preparation of new monomers and the efficient incorporation of functionalities for the facile generation of chemically diverse oligomer libraries.

References and Notes.

1. Gordon, E.M.; Barrett, R.W.; Dower, W.J.; Fodor, S.P.A.; Gallop, M.A. *J. Med. Chem.* **1994**, *37*, 1385-1401.
2. a) Ecker, D.J.; Vickers, T.A.; Hanecak, R.; Driver, V.; Anderson, K. *Nucleic Acids Res.* **1993**, *21*, 1853-56. b) Wyatt, J.R.; Vickers, T.A.; Roberson, J.L.; Buckheit, R.W. Jr.; Klimkait, T.; DeBaets, L.E.; Davis, P.W.; Rayner, B.; Imbach, J.-L.; Ecker, D.J. *Proc. Natl. Acad. Sci.* **1994**, *91*, 1356.
3. Saenger, W. *Principles of Nucleic Acid Structure;* Springer-Verlag: New York. **1984**; pp. 385- 431
4. A similar strategy has been used to prepare 3'-modified oligonucleotides: a) Gamper, H.B.; Reed, M.W.; Cox, T.; Virosco, J.S.; Adams, A.D.; Gall, A.A.; Scholler, J.K.; Meyer, R.B.Jr. *Nucleic Acids Res.* **1993**, *21*, 145-150. b) Reed, M.W.; Adams, A.D.; Nelson, J.S.; Meyer, R.B.Jr. *Bioconjugate Chem.* **1991**, *2*, 217-225.
5. Greene, T.W.; Wuts, P.G.M. *Protective Groups in Organic Synthesis- 2nd edition.* Wiley: New York. **1991**; p 318.
6. a)Jordis, U.; Sauter, F.; Siddiqi, S.M.; Küenburg, B.; Bhattacharya, K. *Synthesis*, **1990**, 925-930. b) Baker, G.L.; Fritschel, S.J.; Stille, J.R.; Stille, J.K. *J. Org. Chem.* **1981**, *46*, 2954-2960.
7. Jones, R.A. *Oligonucleotide Synthesis: a Practical Approach;* Gait, M.J. Ed.; Oxford University Press: New York. **1984**, pp. 23-34.
8. **5**: ^{1}H NMR: (200 MHz, $CDCl_3$) δ 7.42-6.80 (13 H, ArH), 4.35 (1H, m, H3), 3.77 (6H, s, 2 OCH_3), 3.62 (1H, m, H5), 3.13-2.88 (4H, m, 2 H6, 2 H2), 1.87 (1H, q, H4a), 1.65 (1H, m, H4b); ^{13}C NMR: (50.3 MHz, $CDCl_3$) δ 158.74, 145.02, 136.22, 130.09, 128.20, 127.84, 127.71, 126.79, 113.14, 85.97, 72.25, 65.85, 56.68, 55.25, 54.66, 38.45.
9. Typical procedure: EDC (15 mmol) was added to the carboxylic acid (12 mmol) and HOBT (12 mmol) in CH_2Cl_2 (40 ml) When the carboxylic acid was insoluble in CH_2Cl_2 , DMF was used instead. After 30 min amino alcohol **5** (10 mmol) was added. At completion, the reaction was quenched with sat $NaHCO_3$, extracted with ethyl acetate and purified by flash chromatography. ^{1}H NMR spectra were consistent with the presence of two rotamers in $CDCl_3$ or DMSO-d6 solution.
10. The Fmoc group was cleaved by triethylamine used in the chromatography solvent and by diisopropylamine liberated from the phosphitylating reagent.
11. Bannwarth, W.; Trzeciak, A. *Helv. Chim. Acta*, **1987**, *70*, 175-186.
12. Typical procedure: The alcohol **6** was dissolved in dry CH_3CN (0.1M). Cyanoethyl tetraisopropylphosphorodiamidite added (1.5 eq), followed by diisopropylammonium tetrazolide (0.5 eq). Once the reaction was complete (2-16 h) the solvent was removed and the oil applied to a silica gel column and eluted with a mixture of ethyl acetate/hexanes containing 0.5% triethylamine.
13. The purity of the dimers is measured from the peak areas on the HPLC chromatograms. Unreacted dT and protecting group hydrolysis products are not considered impurities.

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