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Achiral Internucleoside Linkages 2: O-CH$_2$-CH$_2$ Linkage

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Abstract: **The synthesis of an O-CH$_2$-CH$_2$ internucleoside linkage has been achieved. The crucial carbon to oxygen bond was formed by trapping a π-allyl palladium complex with 3' alcohol 6. An antisense oligonucleoside containing this dimer modification hybridized to the sense sequence.**

Recently nucleoside analogs possessing three atom replacements of the natural phosphodiester internucleotide linkage of DNA have been attracting attention as potential antisense therapeutics.[1] The strategy behind the replacement of the phosphodiester is to increase nuclease stability and enhance cellular uptake of the antisense strand while maintaining the ability to hybridize to the targeted RNA. For example, the phosphodiester (O-P(O)$_2^-$-O) linkage of **1** has been replaced by the formacetal (O-CH$_2$-O) of **2**[2], the hydroxylamine (CH$_2$-NH-O) of **3**[3] and the sulfamide (NH-SO$_2$-CH$_2$) of **4**[4]. Herein we report the synthesis of an O-CH$_2$-CH$_2$ internucleoside linkage which is hydrolytically stable, formed under mild conditions and amenable to the formation of higher oligomers.

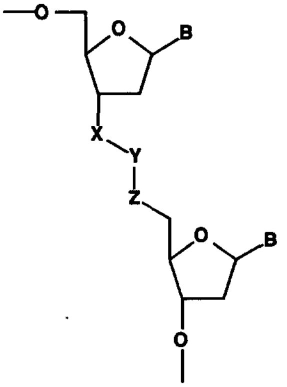


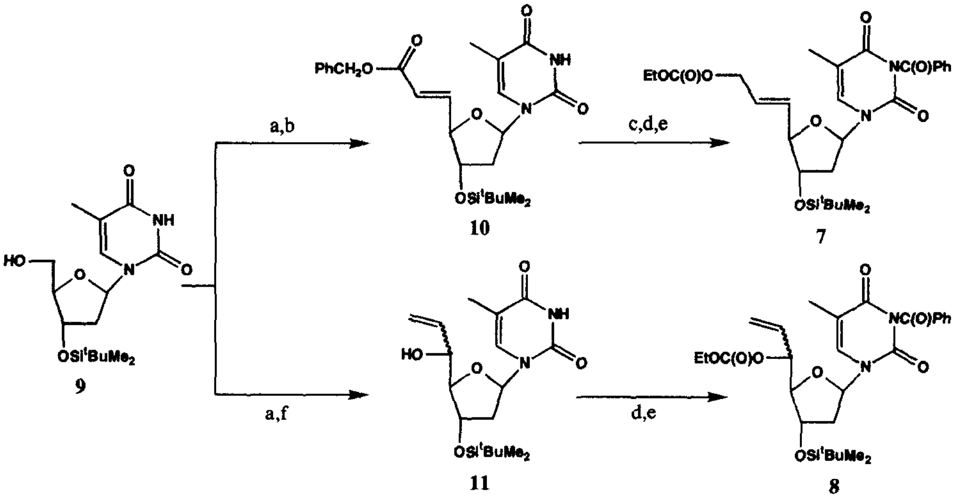
Table 1: Internucleoside Linkages

	1	2	3	4	5
X	O	O	CH$_2$	NH	O
Y	P(O)$_2^-$	CH$_2$	NH	SO$_2$	CH$_2$
Z	O	O	O	CH$_2$	CH$_2$

In order to form the desired ether internucleoside linkage found in dimer **5** without undue protecting group exchange reactions, a method for forming a carbon to oxygen bond under conditions which are tolerant of the protecting groups common to oligonucleotide synthesis is needed.[5] In principle trapping a π-allyl palladium complex with an alcohol would permit the formation of the desired carbon to oxygen bond under mild conditions.[6] Therefore it was anticipated that the O-CH$_2$-CH$_2$ internucleoside linkage could be formed by the palladium(0) catalyzed coupling of 3' alcohol **6** with either ethyl allylcarbonate **7** or **8**.

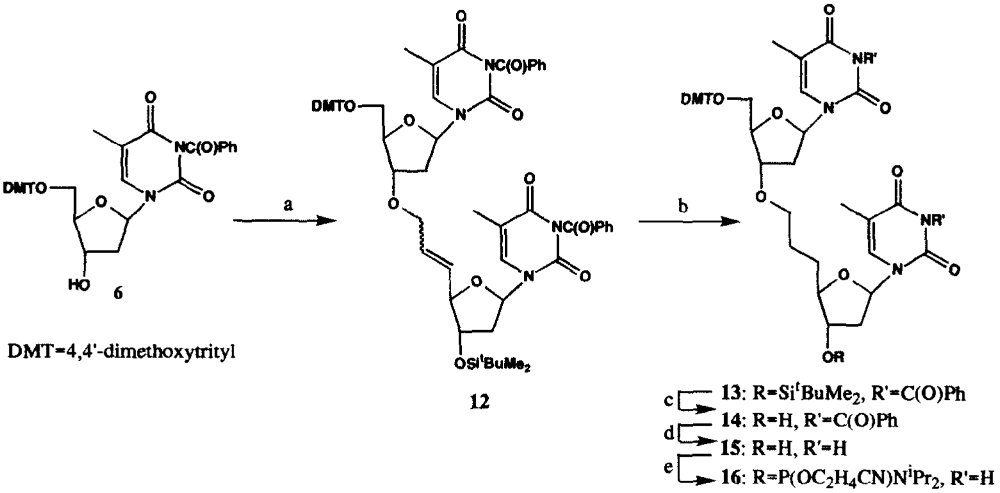
Primary ethyl allylcarbonate **7** was synthesized as depicted in **Scheme 1**. 3'-O-*tert*-butyldimethylsilyl-

Scheme 1[a]: *Synthesis of Ethyl allylcarbonates 7 and 8*



[a]Reagents and conditions: (a) 1.1 equiv of Dess-Martin Periodane, CH_2Cl_2, 1 h; (b) 1.1 equiv of $Ph_3PCHCO_2CH_2Ph$; (c) 3.2 equiv of DiBAl-H, -78°C, 3 h, (d) 10 equiv of ethyl chloroformate, 10 equiv of pyr., CH_2Cl_2, 0°C, 2 h; (e) 10 equiv of benzoyl chloride, 10 equiv of EtN^iPr_2, 1 equiv of DMAP, pyr., 0→25°C, 2 h; (f) excess vinylmagnesium bromide, THF, -78°C.

Scheme 2[a]: Coupling and Synthesis of the Dimer



[a] Reagents and conditions: (a) 4 mol% of tris(dibenzylideneacetone)dipalladium(0), 12 mol% 1,4-bis-(diphenylphosphino)butane, THF, 53°C, 15 min, then 2 equiv of 7 or 8, 53°C; (b) Pd/C, H_2, EtOAc, 2 h; (c) 1.1 equiv of nBu_4NF ,-40→0°C, 16 h, (d) NH_3 in CH_3OH, 16 h; (e) 1.3 equiv of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite, 1.5 equiv of EtN^iPr_2, CH_2Cl_2, 0°C, 2 h.

deoxythymidine (**9**)[7] was treated with Dess-Martin periodane reagent[8] followed by benzyloxycarbonylmethylenetriphenylphosphorane[9] to provide the E-α,β-unsaturated ester (**10**) in 94% yield from a one-pot procedure. The benzyl ester was reduced by treatment with DiBAl-H to give an allylic alcohol which was transformed to the ethyl allylcarbonate in the usual manner. At this point the pyrimidine nitrogen was blocked with a benzoyl group to give the desired synthon **7** in 64% yield from **10**. Protection of the nucleophillic pyrimidine nitrogen proved necessary to avoid preferential N-alkylation.[10]

As depicted in **Scheme 1**, secondary ethyl allylcarbonate **8** was synthesized by Dess-Martin oxidation of **9** followed by treatment of the crude aldehyde with vinylmagnesium bromide to give allyl alcohol **11** as an inseparable mixture of diastereoisomers in 40% yield. Treatment of **11** with ethyl chloroformate followed by blocking of the pyrimidine nitrogen with benzoyl chloride gave synthon **8** as a mixture of diastereoisomers.

With the desired ethyl allylcarbonates (**7** and **8**) in hand, their π-allyl palladium complexes were trapped with alcohol **6** (**Scheme 2**). The critical coupling was accomplished by the slow addition of a THF solution of **7** or **8** to a flask containing tris(dibenzylideneacetone)dipalladium(0), 1,4-bis-(diphenylphosphino)butane[11] and **6** at 53°C thus producing olefin **12** as an inseparable mixture of **E** and **Z** isomers. It is interesting to note that both ethyl allylcarbonates displayed the same regioselectivity in the palladium catalyzed coupling reaction to produce **12**. Coupling of **8** to **6** as described above was achieved with a 63% isolated yield of **12**. Although the coupling of primary ethyl allylcarbonate **7** was not as efficient (45%), no other regioisomers of coupled product were detected by ^1H NMR analysis (300 MHz). Although the reaction of **7** with alcohol **6** was not optimized, experiments indicated that the primary ethyl allylcarbonate had limited stability under the reaction coditions relative to secondary ethyl allylcarbonate **8**.

The synthesis of the required phosphoramidite for oligonucleotide synthesis was completed as shown in **Scheme 2**. Reduction of the internucleoside double bond of **12** produced **13**[12] which upon exposure to nBu_4NF gave alcohol **14** in 83% yield from **12**. The benzoyl blocking groups of the pyrimidine nitrogens were removed upon exposure to methanolic ammonia to produce in 95% yield 5'-dimethoxytritylated dimer **15**.[12] Treatment of **15** with 2-cyanoethyl N,N-diisopropylchlorophosphoramidite gave phosphoramidite **16**.

Phosphoramidite **16** was incorporated into a 17-mer mixed base sequence **19**. The coupling yield with **16** was in the range of 96-99% in automated DNA synthesis as determined by DMT assay. The oligo sequence was analyzed by negative FAB-MS spectra and the expected deprotonated molecular ion $(MW-1)^-$ was observed.

Thermal melting studies with strand **19** and the complementary sequence **17** showed a characteristic sigmoidal transition with a T_m of 64°C. This result is consistent with the formation of a duplex structure.[13] The magnitude of ΔT_m with the incorporation of O-CH_2-CH_2 linkage is in line with that of other achiral linkages such as CH_2-CH_2-NH, NH-CH_2-CH_2[14] and O-CH_2-O[15] when hybridized with complementary ss-DNA.

Table 2: Melting Temperature (T_m)[a]	T_m(°C)	ΔT_m(°C)
17: d-CpCpCpGpCpTpApApCpApCpApCpApCpCpC		
18: d-GpGpGpTpGpTpGpTpGpTpTpApGpCpGpGpG	68	
19: d-GpGpGpTpGpTpGpTpGp**T-O-CH_2-CH_2-T**pApGpCpGpGpG	64	-4.0

(a) Melting Temperatures (T_m) measured at 5.0 μM oligomer concentration containing 100 mM NaCl, 10 mM Na_2HPO_4 (pH=7.0) and 0.1 mM EDTA. Melting curves were recorded in steps of 0.5°C/min.

In conclusion, an efficient synthesis of a novel hydrolytically stable internucleoside linkage has been demonstrated. The synthetic strategy allows for the synthesis of dimers and higher oligomers under mild conditions which are tolerant of common nucleotide protecting groups. Thermal melting studies indicated formation of a duplex.

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12. Data for selected compounds. **13**: white foam; R_f 0.57 (silica, 1:1 EtOAc:hexane); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.87-7.83 (m, 4 H), 7.70 (s, 1 H), 7.60-7.18 (m, 15 H), 7.13 (s, 1 H), 6.80 (d, J=8.3 Hz, 4 H), 6.28 (m, 1 H), 6.10 (t, J=6.8 Hz, 1 H), 4.12-3.97 (m, 3 H), 3.78-3.71 (m, 1 H), 3.73 (s, 6 H), 3.47-3.36 (m, 3 H), 3.27 (m, 1 H), 2.47-2.39 (m, 1 H), 2.25-2.17 (m, 2 H), 2.01-1.93 (m, 2 H), 1.85 (s, 3 H), 1.68-1.40 (m, 3 H), 1.41 (s, 3 H), 0.78 (s, 9 H), -0.03 (s, 6 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 158.8, 135.3, 134.9, 130.4, 130.0, 129.0, 128.2, 128.1, 128.0, 127.2, 113.3, 86.4, 85.2, 85.1, 84.4, 79.9, 74.9, 68.7, 63.8, 55.2, 40.6, 38.0, 30.2, 26.3, 25.6, 12.7, 11.9; FAB/MS: M/z = 1119 $(M+H)^+$. **15**: white foam; R_f 0.62 (silica, 1:9 CH_3OH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.72 (s,1 H), 7.50-7.33 (m, 9 H), 7.23 (s, 1 H), 6.93 (d, J=8.9 Hz, 4 H), 6.40 (dd, J=5.1, 8.8 Hz, 1 H); 6.34 (t, J=6.5 Hz, 1 H), 4.30-4.20 (m, 3 H), 3.99-3.95 (m, 1 H), 3.89 (s, 6 H), 3.63-3.52 (m, 3 H), 3.40 (dd, J=2.4, 10.6 Hz,1 H), 2.60 (m, 1 H), 2.53-2.45 (m, 1 H), 2.27-2.17 (m, 2 H), 2.01 (s, 3 H), 1.98-1.80 (m, 4 H), 1.58 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 158.8, 144.4, 135.4, 135.3, 130.1, 128.3, 128.1, 128.0, 127.2, 113.3, 111.6, 111.2, 85.5, 85.0, 84.7, 84.4, 80.2, 74.6, 67.6, 64.0, 55.3, 40.1, 37.4, 30.0, 25.9, 12.7, 11.9; FAB/MS: M/z = 796 $(M)^+$.
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