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Direct and Facile Syntheses of Heterocyclic Vinyl-*C*-Nucleosides for Recognition of Inverted Base Pairs by DNA Triple Helix Formation: First Report by Direct Wittig Route

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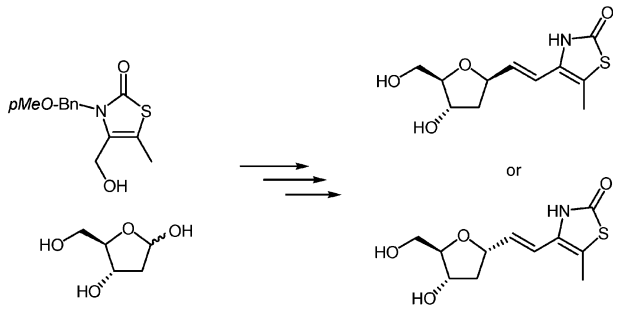

The ability to recognize specific gene sequences canonically would allow precise means for genetic intervention. However, specific recognition of two of the four possible base pairs by triplex-forming oligonucleotides (TFO) as X·T−A and Y·C−G within a triplex currently remains elusive. A series of C1-vinyl nucleosides have been proposed, and their stability and specificity have been evaluated extensively by molecular dynamics simulation. Because most *C*-nucleoside syntheses extend through direct substitution at the C1-position, a more convenient strategy for their syntheses via a direct Wittig coupling is presented here.

The growing knowledge of gene sequences has made DNA a suitable drug target. This demands the development of a method for sequence-specific recognition of DNA duplex. Because genes are directly responsible for function and dysfunction that cause chronic maladies and disease, the ability to suppress, delete, or modify them directly will lead toward more direct avenues for therapy. The first step toward developing synthetic bioorganic devices that operate upon gene sequences is to have the means for their recognition.

The formation of intermolecular DNA triple helices offers the possibility of designing compounds with extensive sequence recognition properties that would be useful as anti-gene agents or tools in molecular biology. One remaining major limitation of this approach is that with natural nucleosides these triplex structures are generally restricted to homopurine−homopyrimidine target sites (Figure 1). Triplex-forming oligonucleotides (TFO) strands in parallel orientation H-bonds the polypurine duplex strand of A−T and G−C in the major groove (Hoogsteen bonding) as T·A−T and C^+·G−C, respectively (Figure 2). The current inability to form stable triplexes by recognizing inverted base pairs, T−A and C−G, directly by TFO is the major deterrent in the development of anti-gene methodologies

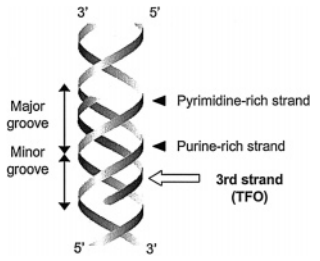

FIGURE 1. DNA triplex; TFO strand parallel or antiparallel.

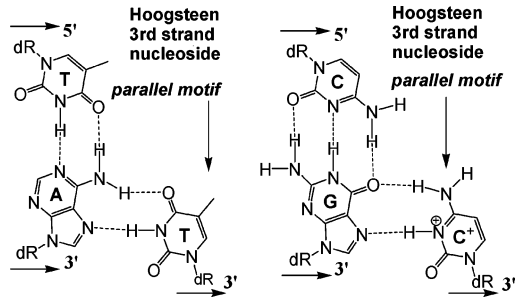

FIGURE 2. T·A−T triplet and C^+·G−C triplet.

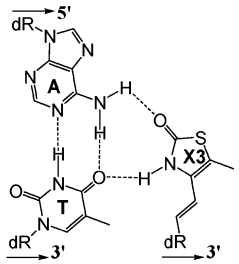

FIGURE 3. X3·T−A triplet.

Proposed here are the syntheses of a prototype 2′-deoxy-*C*-vinyl β-D-ribonucleoside analogue, X3 (Figure 3). Because TFOs that incorporate interspersed α-anomer nucleoside analogues have shown augmented binding stability,[1,2] synthesis of the nucleoside α-anomer is also of interest. The design of these 2′-deoxy-*C*-vinyl β-D-ribonucleosides is based upon extensive molecular dynamics evaluations[3,4] that display significantly favorable major groove Hoogsteen H-bonding of the T−A base pair orientation. Specificity is also preserved, which shows unfavorable interactions toward other unintended base pair combinations.[5]

C-Nucleoside syntheses in the literature have been, most commonly, for aryl nucleobases, where the synthetic strategies generally employ an activated deoxyribose C1-position. One of the more versatile procedures involves treatment of 3,5-di-

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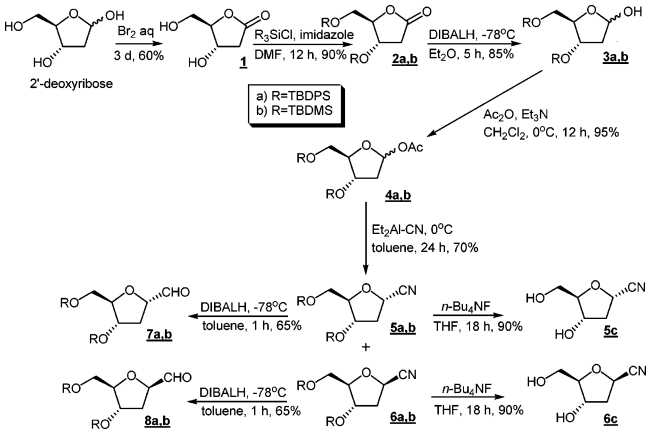
O-silyl protected 2-deoxyribonolactone with an aryllithium, whereby the resulting hemiacetal is reduced with Et_3SiH.[6] Other popular methods to produce aryl C-nucleosides include treatment of Hoffer's α-chlorosugar (3,5-di-*O*-toluoyl-1-chloro-2-deoxy-α-D-ribofuranose) with diaryl cadmium[7,8] and Heck coupling of aryl triflates or iodides to ribofuranoid glycols.[9−11]

Regarding *C*-vinyl deoxyribose derivatives, a few synthetic strategies have been reported. The strategy of Takase et al.[12] commences with the addition of alkynyllithium reagents to 3,5-di-*O*-benzyl-2-deoxyribofuranose to afford diastereomeric mixtures of the corresponding ring-opened alkynyldiols. A cobalt-mediated cyclization (intramolecular Nicholas reaction) follows to give *C*-alkynyl-3,5-di-*O*-benzyl-2-deoxy-ribofuranosides with some *β*-selectivity. These may be further modified to *C*-vinyl derivatives. In another example, a one-pot transformation of unprotected monosaccharides to give styrenyl *C*-glycosides, via a Horner−Wadsworth−Emmons ring-closure and a tandem halogenation/Ramberg−Bäcklund sequence, proceeds in reasonable yield of the *C*-vinyl deoxyribose with equal α- and *β*-anomeric preference. Nonetheless, the availability of the nucleobase as a sulfonylphosphonate is a requirement.[13] A six-step intramolecular cyclization strategy that allows *C*-derivatization via Wittig addition at the C1 of a protected 5-iodo glucofuranose followed by its recyclization to a *C*-vinyl 2-deoxy-*β*-ribofuranoside is also available.[14] Ring reclosure appears to be quite sterically hindered and also requires relatively high temperatures for cyclization; however, yields are high for the *E*-methacrylate example.

These examples are clever and imaginative strategies; however, some either may not be the most suitable for the creation of vinyl *C*-nucleosides with reactively vulnerable base moieties or may require protective groups with incompatible deprotection conditions. Silyl ether protection offers significant advantages with regard to minimal unreactiveness toward exposure to ylide and to orthogonal deprotection conditions following attachment of the nucleobase. Additionally, most of these procedures do not have effective stereocontrol of nucleobase addition at the C1 center. This leads to squandering nucleobase, especially if no corrective epimerization pathway is available.

Examples of Wittig coupling of 2-deoxyribofuranosyl carbaldehydes with even simple α-ester phosphoranes, let alone any heterocyclic phosphoranes, are surprisingly limited. One notable case is the Wittig coupling of a methoxycarbonylmethylene phosphorane to a 3-*O*-TBDMS-5-*O*-benzyl-2-deoxy-ribofuranosyl carbaldehyde.[15] For this particular synthesis and others, preparation of C1-carbaldehydes[14] has been commonly achieved through Hoffer's α-chlorosugar via substitution with NaCN to form 2-deoxy-*β*-D-ribofuranosyl cyanide. This is followed by reduction with sodium hypophosphate/Raney nickel

SCHEME 1



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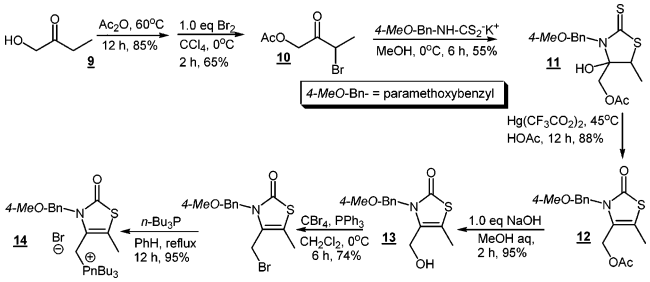

to form the 2-deoxy-*β*-D-ribofuranosyl carbaldehyde that is then captured as the *N*,*N*′-diphenylethyleneimine. Treatment with TsOH liberates the carbaldehyde.[16] Many aspects of this strategy are incompatible with the necessity of 3,5-*O*-disubstituted silyl ether protection. A more viable and effective strategy is illustrated below.

The strategy shown here allows a direct accession of these derivatives where the vinyl nucleoside base adds to a 3,5-di-*O*-silyl protected 2-deoxy-D-ribofuranosyl carbaldehyde via Wittig coupling at the final phase. The 2-deoxyribofuranosyl carbaldehydes **7** and **8** are obtained from the nitriles, **5** and **6**, respectively, according to Scheme 1. The phosphonium salt **14** was synthesized according to Scheme 2.

Synthesis of the 2-deoxy-D-ribofuranosyl carbaldehyde commences with 3,5-bis-*O*-silyl protection of 2-deoxy-D-ribono-1,4-lactone (**1**)[17] that is available via aqueous bromine oxidation of 2-deoxy-D-ribose.[18] Reduction of the lactones **2a**,**b** with DIBALH provides the 3,5-bis-*O*-silyl protected 2-deoxy-D-ribose **3a,b** in high yield.[17] This is then converted to a mixture of *O*-acetyl-2-deoxy-D-ribose anomers **4a,b** to allow for subsequent transformation to the nitriles **5a,b** and **6a,b** (Scheme 2). The α- and *β*-anomers, **5a,b** and **6a,b**, were reduced with DIBALH to their corresponding aldehydes, **7** and **8**, respectively. The **7** and **8** series aldehydes were stable for months when stored at −78 °C (Scheme 1).

The predominance of the α-anomers **5a,b** would be consistent with the model proposed by Woerpel et al.[19] that suggests a

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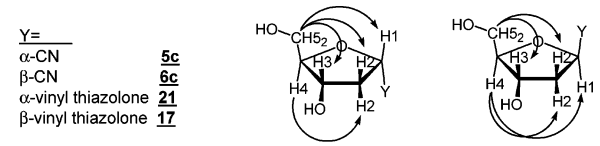

FIGURE 4. Observed NOEs for the α- and *β*-anomers, **5c** and **21** and **6c** and **17**, respectively; assignments are based upon ^{1}H-^{1}H COSY data.

nucleophilic "inside"-attack of the five-membered oxocarbenium ion intermediate. In this particular case, a lower energy pseudoaxial configuration of the 3,5-bis-*O*-silyl substituted oxocarbenium would favor the 1,3 syn addition. Consequently, treatment of **10** with trimethylsilyl cyanide (TMSCN) and BF_3·OEt_2 at −40 °C yielded a 2:1 α/*β* anomeric ratio. Treatment with Et_2Al−CN at 0 °C produced a better yield of the *β*-anomer, but the α-anomer was still preferred at a 4:3 ratio.

Treatment of Hoffer's α-chlorosugar with either NaCN[14] or TMSCN/BF_3·OEt_2[20] predominantly yields the *β*-anomer as a 1:3 α/*β* anomeric ratio. Moreover, with Et_2Al−CN, the predominant preparation of *β*-C1-nitrile is achieved from a 1:1[21] to a 1:5[22] α/*β* anomeric ratio. Additionally, treatment of 1,3,5-*O*-benzoyl-2-deoxy-D-ribofuranose with TMSCN and BF_3·OEt_2 also mainly yields the *β*-C1-nitrile[23] in a 3:7 ratio. To elucidate the mechanisms of these anomeric preferences Narasaka and co-workers[24] have studied the influence of 3-substitution upon the anomeric product ratio in the *C*-glycosidation of 1-*O*-acetyl-5-*O*-benzyl-2-deoxy-D-ribofuranose. In accord with Woerpel's studies, 3-*O*-benzyl derivatization favors the α-anomer 82:18 (α/*β*), while 3-*O*-CH_2(SO)Me derivatization deviates to favor the *β*-anomer 32:68 (α/*β*). The cause of this deviation is postulated to be due to the sulfoxide oxygen shielding the α-face, likely through lone pair association with the oxocarbenium cation. Similarly, the difference in stereoselectivity between the ether and the ester 3,5-disubstituted 2-deoxyribose at C1 may likely be due to a more prominent configurational association of the more proximal 3-ester carbonyl lone pair toward the α-face of the oxocarbenium cation intermediate than that of the more distal 5-ester toward the *β*-face.

The chromatographically separated α-and *β*-nitrile anomer pairs, **5a**/**6a** and **5b**/**6b**, are identified by the presence of unique NOE between H4′ and H1′ protons for the *β*-anomer and for the α-anomer by the presence of unique NOE between H1′ and H5′ protons[14,25] (Figure 4). In addition to the NOE results, the nonequivalence in chemical shift of the H2-methylene protons of the nitrile *β*-anomer **6** being generally greater than that of the α-anomer **5** (Figure 5) is also consistent with what is typically observed for C1 substituted 2-deoxyribose.[26] The same NOE pattern and H2-methylene chemical shift nonequivalence correlation pattern (Figure 6) is observed for the α and *β C*-vinyl thiazolone derivative anomers, **17** and **21**, respectively.

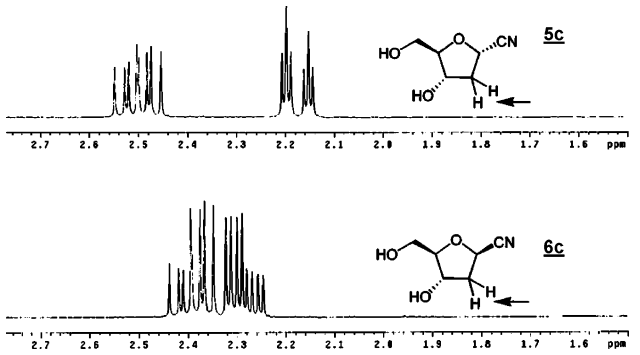

FIGURE 5. ^{1}H NMR of H2′ methylenes for α and *β* nitriles, **5c** and **6c**, respectively.

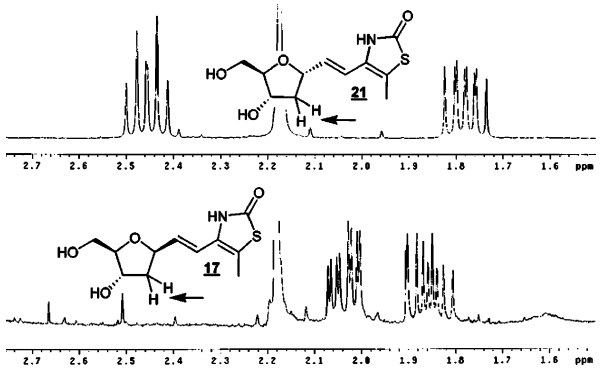

FIGURE 6. ^{1}H NMR of H2′ methylenes for α- and *β*-vinyl thiazolones, **21** and **17**, respectively.

The initial 2-mercapto-4-methyl-5-hydroxy-5-acetoxymethyl thiazole core (**11**) of the vinyl nucleoside X3 is synthesized by the addition of 4-methoxybenzyl dithiocarbamate (created in situ via CS_2 and 4-methoxybenzylamine) to the bromoketone **10**, which was obtained by acetylation and subsequent bromination[27] of 1-hydroxy-butan-2-one (**9**). Desulfurization and dehydration of **11** is achieved in one step via mercuric trifluoroacetate to yield *N*-(4-methoxybenzyl)-4-acetoxymethyl-5-methyl-thiazolone, **12**. Hydrolysis of its ester moiety to the alcohol **13** was facile, and subsequent halogenation of the alcohol via CBr_4 was achieved by the careful addition of PPh_3. Quaternization of the 4-bromomethyl thiazolone to the phosphonium salt **14** with *n*-Bu_3P is achieved in the usual manner (Scheme 2).

The Wittig coupling employing the α-anomer **13a** yields predominantly the *Z*-alkene, **18**, despite employment of the Schlosser modification[28] or salt and solvent alteration. However, following deprotection of the thiazolone moiety to yield **19**, olefin photoisomerization toward the more thermodynamically stable *E*-alkene, **20**, was easily achieved with catalytic I_2. Apparently, attempts at photoisomerization of the 4-methoxybenzyl protected thiazolone, **18**, were not successful. This may likely be due to stabilizing electronic effects available through *π*-overlap of the 4-methoxybenzyl moiety with the TBDPS ether moiety. Of the TBDPS-protected 2-deoxy-D-ribofuranosyl carbaldehyde derivatives, **13a** and **14a**, only the *C*-nucleoside α-anomer **13a** was capable of the Wittig coupling. No *β*-anomer

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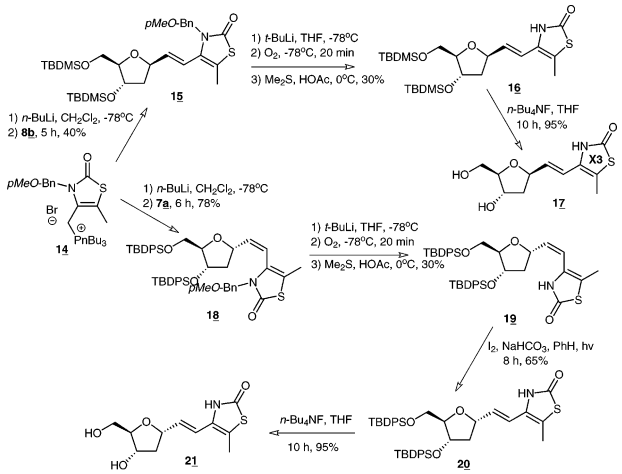
SCHEME 3



product from **14a** was detected when employing reaction conditions of up to 60 °C. However, employment of the less sterically encumbered TBDMS-protected 2-deoxy-β-D-ribofuranosyl carbaldehyde **14b** allowed the generation of the vinyl *C*-nucleoside β-anomer, **15**, through the Wittig procedure in almost entirely the *E*-configuration. The *E* and *Z* configurations of these nucleosides were identified by their vinyllic ^{1}H NMR coupling constants, 15 and 12 Hz, respectively. Noticeable epimerization was not detected for either anomer at the C1 position under Wittig conditions. The preservation of stereochemistry avoids squandering the nucleobase component on the undesired anomer. Furthermore, attempts at epimerization of the C1 center via TFA or TsOH[29] of the 3,5-di-*O*-acetyl protected vinyl thiazolone *C*-nucleoside merely resulted in the decomposition of the nucleobase.

Deprotection of the 4-methoxybenzylamides, **15** and **18**, was effected by formation of the benzyl anion with *tert*-butyllithium at −78 °C, with subsequent oxidation by O_2 where the resulting hemi-aminal sustains loss of 4-methoxybenzaldehyde.[30] Deprotection of their silyl ethers by tetra-*n*-butylammonium fluoride generates the thiazolone nucleoside α- and β-anomers, **17** and **21**, respectively (Scheme 3). This strategy offers a shorter, more modular direct approach toward the assembly of *C*-vinyl ribonucleosides with respect to those available as described in the introduction. The phosphonium salt components are easily generated and available; epimerization of the carbaldehyde is negligible under the proposed Wittig conditions. Moreover, further control of olefin stereochemistry is available through a photoisomerization route. With regard to some of the more elaborate strategies, this direct, one-stage assembly and deprotection strategy, which utilizes the option of mild Wittig conditions and silyl ether deprotection conditions, also allows for the use of a wide range of nucleobases.

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Experimental Procedures

4-[1-(3,5-Bis-*O*-(*t*-butyldiphenylsilyl)-2-deoxy-α-D-ribofuranosyl)-2-*Z*-vinyl]-*N*-(4-methoxybenzyl)-5-methyl-thiazol-2-one.[18] Compound **6** (787 mg, 1.47 mmol) was added to a flame-dried flask. The flask was then purged with and placed under an atmosphere of argon. Twenty milliliters of dry dichloromethane was added to the flask to dissolve the starting material. The solution was chilled to −78 °C, and *n*-butyllithium (1.6 M hexanes; 1.00 mL, 1.62 mmol) was added over 5 min to the stirring solution. After the mixture was stirred for 15 min, **13a** (918 mg, 1.47 mmol) that was dissolved in 5 mL of dry dichloromethane was added over 15 min. The solution was stirred for 2 h, warmed to 0 °C, and stirred for an additional 5 min. The reaction was quenched/washed with 20 mL of saturated aqueous ammonium chloride, and the organic phase was separated, dried over magnesium sulfate, and evaporated by rotary evaporator to yield a clear yellow oil. The residue was purified by flash chromatography ($R_f = 0.50$) with 3:1 hexanes/ethyl acetate (the sample was loaded with a small, yet sufficient, amount of dichloromethane to improve solubility) to yield 972 mg (78% from **6**) as a clear yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66−7.26 (20H, m), 7.15 (d, 2H, $J = 8.7$ Hz), 6.82 (d, 2H, $J = 8.7$ Hz), 6.25 (dd, 1H, $J = 9.6$ Hz, 10.8 Hz), 5.75 (d, 1H, $J = 10.8$ Hz), 4.92 (d, 1H, $J = 15.3$ Hz), 4.60 (d, 1H, $J = 15.3$ Hz), 4.58 (m, 1H), 4.46 (m, 1H), 4.09 (m, 1H), 3.76, 3.75 (2s, 3H), ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5, 159.1, 141.7, 136.0, 135.9, 135.7, 135.6, 133.7, 133.6, 133.3, 133.2, 130, 129.9, 129.0, 128.8, 128.0, 127.9, 127.8, 127.5, 117.5, 114.3, 110.4, 87.5, 76.1, 75.6, 64.8, 55.6, 46.9, 41.1, 27.3, 27.1, 19.5, 13.7. (doubling of some signals due to the presence of TBDPS rotamers). High-resolution FAB MS MH^+ [Found: (MH^+), 853.3665; calculated for $C_{51}H_{59}O_5NSi_2S$, (MH^+), 853.3653].

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Supporting Information Available: Detailed experimental procedures for all compounds and spectroscopic data are available free of charge via the Internet at http://pubs.acs.org.

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