Nucleic Acid Mimics. Synthesis of Ethylene Glycol- and Propoxy-Linked Thymidyl-Tetrahydrofuranylthymine Dimers via a Vorbrüggen-Type Glycosylation Reaction

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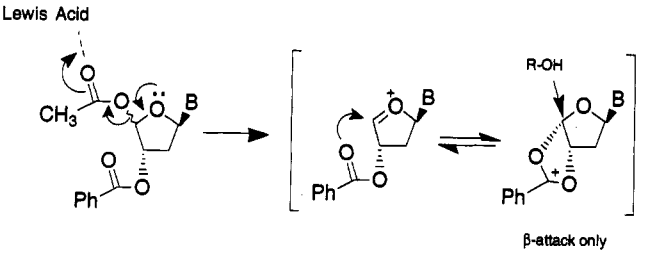
Summary: Two thymidyl-tetrahydrofuranylthymine dimers, **15** and **16**, were synthesized stereospecifically in good yield via a Vorbrüggen-type reaction between glycol ether **4** or propoxy alcohol **7** and acetate **12**.

Examination of oligonucleotide analogs as potential drugs is an active research area.[1] Modifications designed to enhance oligonucleotide pharmacokinetic and pharmacodynamic properties have been directed to all major portions of the nucleotide repeating units, including sugar, phosphodiester moiety, heterocycle, and sites of attachments between glycosyl and heterocycle moieties.[1] A recent focus has been directed toward modifying the sugar–phosphate backbone. In particular, the phosphodiester moiety linking the ribofuranosyl or deoxyribofuranosyl nucleosides has been replaced with a variety of four-atom linkages which are achiral and neutral.[2] In the interest of further simplification of the sugar–phosphate backbone of nucleic acids, we have considered the design and synthesis of oligomers consisting of tetrahydrofuranyl–heterocycles connected by four-atom linkers. These novel *oligonucleoside* types[3] should be incapable of nucleolytic degradation, have appropriate hybridization properties (binding affinities and base-pair specificity), and be amenable to large-scale synthesis.[1]

We describe a versatile Vorbrüggen-type glycosylation coupling reaction for the synthesis of tetrahydrofuranylthymine linked to thymidine by an ethylene glycol or propoxy moiety. Essential for the success of this approach is the development of a general method to stereospecifically connect tetrahydofuranyl heterocycles together such that the structure of the linker could be optimized.

We reasoned that tetrahydrofuranyl-substituted heterocycles could be stereochemically coupled by a Vorbrüggen-type glycosylation.[4] The C5′ carbon of one tetrahydrofuranyl–nucleobase bearing 4′,5′-diacyloxy groups would react with a nucleophilic linker attached to the C3′ of the second nucleosides (or C4′ of a tetrahydrofuranyl–nucleobase). The nucleophilic linker would be stereospecifically trapped by the incipient carbocation

Scheme 1



(acyloxonium ion) generated at the C5′-position by Lewis acids. Stereochemistry of the C5′-position would be controlled by the adjacent C4′-acyloxy group (Scheme 1). Key synthons for this coupling strategy are 3-((benzyloxy)methyl)-3′-*O*-(2-hydroxyethyl)-5′-*O*-(*tert*-butyldiphenylsilyl)thymidine (glycol ether **4**) and 3-((benzyloxy)methyl)-3′-deoxy-3′-(3-hydroxypropyl)-5′-*O*-(*tert*-butyldiphenylsilyl)thymidine (propoxy **7**) as nucleophilic components, and the nucleophilic acceptor is 1-(5′-acetoxy-4′-(benzoyloxy)tetrahydrofuran-2′-yl)-3-((benzyloxy)methyl)thymine (acetate **12**).

Glycol ether **4** and propoxy **7** are obtained by chemical manipulations of the 3′-oxygen atom of 3-((benzyloxy)methyl)-5′-*O*-(*tert*-butyldiphenylsilyl)thymidine (**2**) (Scheme 2). Starting from 5′-*O*-(*tert*-butyldiphenylsilyl)-thymidine (**1**),[5] the 3-position was regioselectively protected with (benzyloxy)methyl chloride in the presence of Hünig's base to provide the N-alkylated product **2**. Alkylation of **2** with ethyl bromoacetate and subsequent reduction of the ester **3** with $NaBH_4$[6] afforded the glycol ether **4**. Thiocarbonation of **2** with phenyl chlorothionoformate gave the thionocarbonate **5**, which was treated with allyl tributyltin and AIBN[7] to afford 3′-deoxy-3′-allylthymidine **6**. Hydroboration/oxidation of **6** furnished the propoxy **7**.

Acetate **12** is synthesized from 3-((benzyloxy)methyl)-3′-*O*-benzoylthymidine (**9**), which is easily accessible from **2**, by elimination of the 5′-methylene group via sequential oxidation, deesterification, and oxidative decarboxylation (Scheme 2). Benzoylation of **2** provided benzoate **8** which was desilylated to give **9**. Corey's one-step oxidation procedure[8] was utilized to transform primary alcohol **9** to the *tert*-butyl ester **10**. Treatment of **10** with CF_3COOH gave uronic acid **11**. Oxidative decarboxylation via a modified Hunsdiecker reaction[9] converted **11** to acetate

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(1) (a) Cook, P. D. *Anti-Cancer Drug Design* **1991**, *6*, 585. (b) Cook, P. D. Medicinal Chemistry Strategies for Antisense Research. In *Antisense Research & Applications*; Crooke, S. T., LeBleu, B., Eds.; CRC Press, Inc.: Boca Raton, FL, 1993, p 149. (c) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543.

(2) (a) Matteucci, M. *Tetrahedron Lett.* **1990**, *31*, 2385. (b) Veeneman, G. H.; van der Marel, G. A.; Van der Elst, H.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1990**, *109*, 449. (c) Vasseur, J.-J.; Debart, F.; Sanghvi, Y. S.; Cook, P. D. *J. Am. Chem. Soc.* **1992**, *114*, 4006. (d) Debart, F.; Vasseur, J.-J.; Sanghvi, Y. S.; Cook, P. D. *Tetrahedron Lett.* **1992**, *33*, 2645. (e) Debart, F.; Vasseur, J.-J.; Sanghvi, Y. S.; Cook, P. D. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1479. (f) Sanghvi, Y. S.; Vasseur, J.-J.; Debart, F.; Cook, P. D. *Nucleic Acids Res. Symp. Ser.* **1992**, No. 27, 133. (g) Sanghvi, Y. S.; Cook, P. D. Towards Second Generation Synthetic Backbones for Antisense Oligonucleosides. In *Nucleosides and Nucleotides as Antitumor and Antiviral Agents*; Chu, C. K., Baker, D. C., Eds.; Plenum Press: New York, 1993; p 311. (h) Sanghvi, Y. S.; Vasseur, J.-J.; Debart, F.; Cook, P. D. *Coll. Czech. Chem. Commun.* **1993**, *58*, 158.

(3) We refer to modified oligonucleotides that lack the phosphorus atom in the backbone linkage as oligonucleosides.[2c]

(4) Niedballa, U.; Vorbrüggen, H. *J. Org. Chem.* **1974**, *39*, 3654.

(5) Matulic-Adamic, J.; Watanabe, K. A. *J. Chem. Soc., Chem. Commun.* **1985**, *21*, 1535.

(6) Examples of the reduction of an α-alkoxy ester to the alcohol with boron hydride are: (a) Taniguchi, M.; Koga, K.; Yamada, S. *Tetrahedron* **1974**, *30*, 3547. (b) Adam, G.; Seebach, D. *Synthesis* **1988**, 373.

(7) (a) Chu, C. K.; Doboszewski, B.; Schmidt,W.; Ullas, V. G. *J. Org. Chem.* **1989**, *54*, 2767. (b) Fiandor, J.; Huryn, D. M.; Sluboski, B.; Tam, S. *Nuclesides & Nucleotides* **1989**, *8*, 1107. (c) Fiandor, J.; Tam, S. *Tetrahedron Lett.* **1990**, *31*, 597.

(8) Uronic acid **11** can also be produced in a single step by oxidation of **9** with pyridinium dichromate in DMF (Corey, E. J.; Samuelsson, B. *J. Org. Chem.* **1984**, *49*, 4735); however, removal of the chromium salt from the desired product was not easily achieved.

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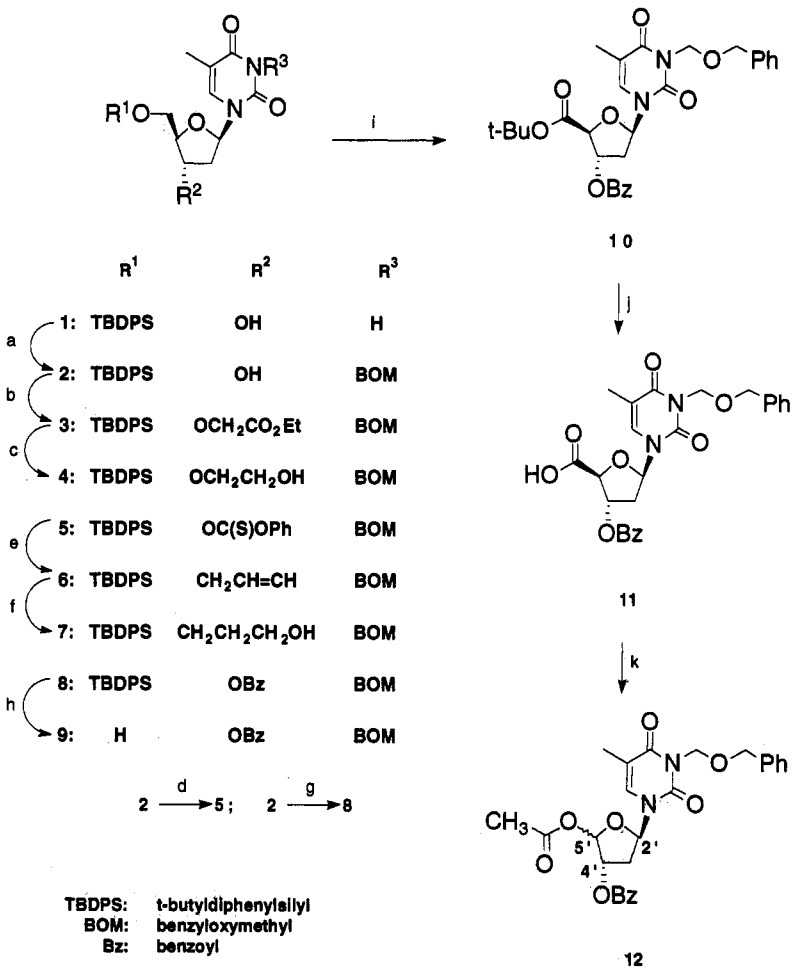
Scheme 2[a]



[a] Key: (a) BOMCl, (i-Pr)$_2$NEt, CH_2Cl_2, 23 °C (88%); (b) ethyl bromoacetate, NaH, DMF, 0–23 °C (76%); (c) $NaBH_4$, MeOH, 0–23 °C (90%); (d) phenyl chlorothionoformate, *N*-hydroxysuccinimide, pyridine, toluene, 80 °C (75%); (e) allyltributyltin, AIBN, benzene, 80 °C (83%); (f) (i) BH_3, THF, 23 °C; (ii) 30% H_2O_2, 23 °C (86%); (g) BzCl, Et_3N, CH_2Cl_2, 23 °C (87%); (h) HF–pyridine, THF, 23 °C (97%); (i) CrO_3, pyridine, Ac_2O, t-BuOH, CH_2Cl_2, DMF 0–23 °C, (65%); (j) CF_3COOH (98%); (k) $Pb(OAc)_4$, pyridine, DMF, 23 °C (60%).

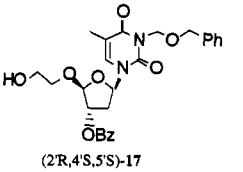
12 as a 3:7 α/β anomeric mixture at C5′. Separation of these anomers was readily achieved by silica gel column chromatography.[10] Since the stereochemical outcome of a Vorbrüggen glycosylation is controlled by the stereochemistry of the 4′-benzoyl group, separation of the anomers is unnecessary (Scheme 1).[11] Reaction of acetate **12** with glycol ether **4** or propoxy **7** in the presence of $TMSOT_f$ at −23 °C for 16 h led to the exclusive formation of the β-glycosylation adducts **13** or **14**, respectively, in 82% and 76% yields. The β-configuration of the newly created C5′ stereocenter of the tetrahydrofuranyl moiety was confirmed by a NMR NOESY experiment which shows a positive NOE between the H2′ and H5′ proton signals. Attempts to shorten the reaction time by raising the temperature to 23 °C resulted in the decomposition of acetate **12** while glycol ether **4** and propoxy **7** were recovered unchanged. Sequential removal of the protecting groups of dimers **13** and **14** by catalytic hydrogenation, base hydrolysis, and desilylation provided glycol-linked T*T dimer **15** and propoxy-linked T*T dimer **16**.[12]

In summary, we have devised a Vorbrüggen-type glycosylation reaction that is useful for the stereospecific coupling of a tetrahydrofuranyl thymine to thymidine via

(9) Chu, C. K.; Ahn, S. K.; Kim, H. O.; Beach, J. W.; Alves, A. J.; Jeong, L. S.; Islam, Q.; Van Roey, P.; Schinazi, R. F. *Tetrahedron Lett.* **1991**, *32*, 3791.

(10) For (2′*R*,4′*S*,5′*S*)-**12** (minor isomer): R_f (hexanes/AcOEt (1:1)) 0.53; ^{1}H NMR ($CDCl_3$) δ 1.95 (d, 3H, J = 1.1 Hz, 5-CH_3), 2.05 (s, 3H, acetoxy-CH_3), 2.52–2.75 (m, 2H, 3′-H), 4.70 (s, 2H, OCH_2Ph), 5.49 (s, 2H, NCH_2O), 5.60–5.65 (m, 1H, 4′-H), 6.40 (dd, 1H, J = 7.9, 3.8 Hz, 2′-H), 6.72 (d, 1H, J = 4.0 Hz, 5′-H), 7.06 (d, 1H, J = 1.1 Hz, 6-H), 7.22–7.60 (m, 8H, aromatic-H), 8.02 (d, 2H, J = 7.6 Hz, aromatic-H); ^{13}C NMR ($CDCl_3$) δ 13.04, 20.73, 33.81 (3′-C), 70.45, 71.08 (4′-C), 72.11, 85.47 (2′-C), 94.10 (5′-C), 110.90 (5-C), 127.47, 128.11, 128.45, 128.64, 129.54, 133.55, 133.92 (6-C), 137.75, 150.55 (2-C), 163.01 (4-C), 165.43 (benzoyl C=O), 169.18 (acetoxy C=O). Anal. Calcd for $C_{26}H_{26}N_2O_8$: C, 63.16; H, 5.26; N, 5.67. Found: C, 63.12; H, 5.38; N, 5.45. For (2′*R*,4′*S*,5′*R*)-**12** (major isomer): R_f (hexanes/AcOEt (1:1)) 0.47; ^{1}H NMR ($CDCl_3$) δ 1.95 (s, 3H, 5-CH_3), 2.18 (s, 3H, acetoxy-CH_3), 2.33 (ddd, 1H, J = 14.9, 8.2, 4.9 Hz, 3′-H_β), 2.75 (dd, 1H, J = 14.9, 6.2 Hz, 3′-H_α), 4.70 (s, 2H, OCH_2Ph), 5.50 (s, 2H, NCH_2O), 5.52 (d, 1H, J = 4.9 Hz, 4′-H), 6.42 (s, 1H, 5′-H), 6.73 (dd, 1H, J = 8.2, 6.2 Hz, 2′-H), 7.22–7.63 (m, 9H, 6-H and aromatic-H), 8.04 (d, 2H, J = 7.5 Hz, aromatic-H); ^{13}C NMR ($CDCl_3$) δ 13.40, 20.82, 34.95 (3′-C), 70.51, 72.07, 76.64 (4′-C), 87.15 (2′-C), 98.61 (5′-C), 111.03 (5-C), 127.45, 128.11, 128.45, 129.68, 133.02, 133.69 (6-C), 137.77, 150.91 (2-C), 162.84 (4-C), 165.12 (benzoyl C=O), 169.13 (acetoxy C=O). Anal. Calcd for $C_{26}H_{26}N_2O_8 \cdot H_2O$: C, 60.94; H, 5.47; N, 5.47. Found: C, 60.98; H, 5.18; N, 5.30.

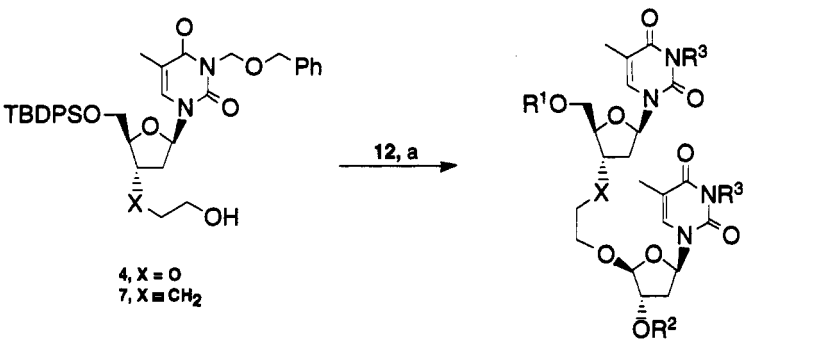
(11) Treatment of either isomer, (2′*R*,4′*S*,5′*R*)-**12** or (2′*R*,4′*S*,5′*S*)-**12**, with 1,2-bis(trimethylsiloxy)ethane in CH_2Cl_2 in the presence of $TMSOT_f$ at −23 °C afforded β-glycosidation adduct (2′*R*,4′*S*,5′*S*)-**17** as the only product in 60–70% yield.



(2′R,4′S,5′S)-17

(12) All new compounds exhibited satisfactory spectral and analytical data.

Scheme 3[a]



	X	R^1	R^2	R^3
13,	O	TBDPS	Bz	BOM
14,	CH_2	TBDPS	Bz	BOM
15,	O	H	H	H
16,	CH_2	H	H	H

13 $\xrightarrow{b}$ 15; 14 $\xrightarrow{b}$ 16

[a] Key: (a) TMSOTf, Et_3N, CH_2Cl_2, −23 °C (13: 82%; 14: 78%); (b) (i) H_2, $Pd(OH)_2$, MeOH/acetone (4:1), 23 °C; (ii) HF-pyridine, THF, 23 °C; (iii) KOH, MeOH, 23 °C (15: 89%; 16: 81%).

an ethylene glycol or a propoxy linker. The reaction provides exclusively the β-glycosylation product in high yield. The preparation of the acetoxytetrahydrofuranyl derivative of other nucleobases (C, U, A, G) and the use of this novel coupling reaction would allow the synthesis of various nucleoside-tetrahydrofuranyl nucleobase dimers with a variety of linkers. The incorporation of T*T dimers 15 and 16 into nucleic acids to determine their effects on biochemical and biophysical properties of oligonucleotides is in progress.[13]

Acknowledgment. We thank our colleagues, Dr. Ramesh Bharadwaj for scale-up synthesis of acetate 12, and Mr. Patrick Wheeler for assistance in 2D-NMR studies.

Supplementary Material Available: Synthetic procedures and spectroscopic and analytical data for compounds 2–16 (14 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(13) Dimers 15 and 16 have been converted to their 5′-(dimethoxytrityl) and 4′-phosphoramidites for automated synthesis. Studies to incorporate these dimers into antisense oligonucleotides are underway.