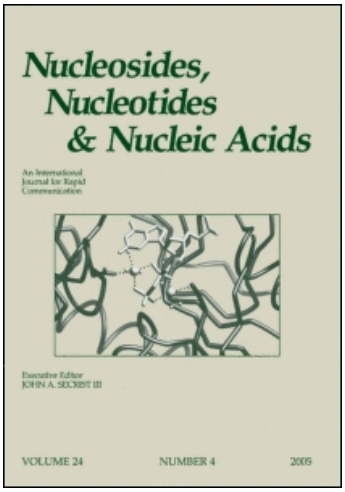

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Synthesis Of The Bicyclo-[4.3.0]-Thymidyl-Nucleoside Via Pd(Ii)-Mediated Ring Expansion Chemistry

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SYNTHESIS OF THE BICYCLO-[4.3.0]-THYMIDYL-NUCLEOSIDE VIA Pd(II)-MEDIATED RING EXPANSION CHEMISTRY

Andrea Stauffiger and Christian J. Leumann □ *Department of Chemistry and Biochemistry, University of Bern, Bern, Switzerland*

□ *A variety of modified nucleosides to improve antisense oligodeoxynucleotide properties such as target affinity, nuclease resistance, and pharmacokinetics were developed in the last two decades. In the context of conformational restriction we present here the synthesis of the [4.3.0]-bicyclo-DNA thymine monomer via Pd(II)-mediated ring expansion of an intermediate of the tricyclo-DNA synthesis.*

Keywords Antisense oligodeoxynucleotide properties; target affinity; nuclease resistance; pharmacokinetics

INTRODUCTION

A wide variety of different DNA analogs containing conformationally constrained nucleosides were synthesized in recent years with the aim of stabilizing duplexes via single strand preorganization.[1−3] In our laboratory we concentrated on the synthesis and properties of bicyclo- (bc-DNA) and tricyclo-DNA (tc-DNA). In both analogs the backbone torsion angles γ and δ were restricted by incorporation into an additional ring.[4,5] However, bc- and tc-DNA both show non-optimal (anticlinal) arrangements of torsion angle γ as a direct consequence of the newly introduced ring. In order to match the geometry of an artificial DNA duplex with that of the naturally occurring A- and B-DNA duplexes, a correction of angle γ to the gauche range is needed. A novel nucleoside that could potentially fulfill these requirements is [4.3.0]-bicyclo-DNA, in which torsion angle γ is predicted to be 85° according to modeling studies. It structurally differs from bc-DNA only by the replacement of the additional five-membered ring by a six-membered ring. Herein we describe the synthesis of the thymidyl-nucleoside of [4.3.0]-bc-DNA via a Pd(II)-mediated ring expansion, starting from a known

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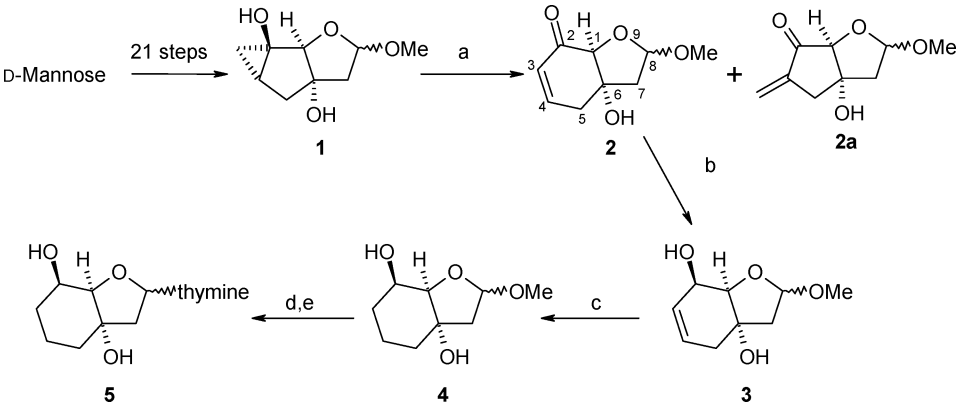
tricyclic building block. The monomer was obtained in four steps as a mixture of anomers.

RESULTS AND DISCUSSION

The synthesis of the unprotected [4.3.0]-bicyclo-DNA thymine monomer **5** is outlined in Scheme 1. The tricyclic intermediate **1** served as starting material and was obtained in 21 steps from D-mannose according to known procedures for tricyclo-DNA synthesis.[6] The direct conversion of **1** to **2** was attempted via Pd(II)-mediated ring expansion.[7] Treatment of **1** with $Pd(OAc)_2$ in DMF at rt lead to **2** together with **2a,** resulting from alternative ring opening**,** in approximately equal amounts and a total yield of 80%. Reduction of the α,β-unsaturated ketone **2** was performed with $NaBH_4$ under *Luche* conditions,[8,9] and yielded the allylic alcohol **3** as a single diastereoisomer in 76% yield. Catalytic hydrogenation then gave the free sugar **4** (86%) which was coupled to *in situ* silylated thymine by *Vorbrüggen* chemistry via transient TMS protection of the hydroxy functions of the sugar. The desired thymine monomer **5** was obtained as a (inseparable) mixture of anomers (α:β 2:1, determined by NMR integration) in 60% yield.

This synthetic approach to [4.3.0]-bicyclo-DNA is based on intermediate **1,** which is available in gram quantities in our laboratory. However, the unselective ring expansion and the anomeric mixture of nucleosides after *Vorbrüggen* coupling are serious disadvantages that need to be addressed in a refined synthetic strategy.



SCHEME 1 (a) $Pd(OAc)_2$, DMF, rt, 24 h, (80%); (b) $CeCl_3 \bullet 7H_2O$, $NaBH_4$, MeOH, 0°C, 30 min, (76%); (c) 20% $Pd(OH)_2/C$, H_2, MeOH, rt, 3 h, (86%); (d) Thymine, BSA, TMS-OTf, CH_3CN, 50°C, 4 h, (60%, 2:1 α:β); (e) 37% HF/Et_3N, THF, rt, 15 min, (quant.).

EXPERIMENTAL PART

General

Solvents were distilled prior to use. All moisture sensitive reactions were performed in ovendried glassware under Ar. External bath temperatures were used to record all reaction temperatures. Spectral data (NMR, MS) were measured with standard equipment and are indicated in standard format. Flash chromatography (FC) was performed using silica gel with an average particle size of 40 μm. Thin-layer chromatography (TLC) was performed on silica gel plates (0.25 mm).

(1S,6S,8R/S)-6-hydroxy-8-methoxy-9-oxabicyclo[4.3.0]non-3-en-2-one (2). Compound **1** (146.7 mg, 0.79 mmol) was dissolved in dry DMF (1.5 ml) and $Pd(OAc)_2$ (32.2 mg, 0.16 mmol) was added. The orange-black mixture was stirred at room temperature in the presence of 4Å molecular sieves and a $CaCl_2$-tube for 24 h before another portion of $Pd(OAc)_2$ (32.2 mg, 0.16 mmol) was added. The reaction was stopped after 24 h and the catalyst and the molecular sieve were filtered off over a bed of celite/silice and washed with MeOH. The solvents were evaporated under reduced pressure and the crude product was purified by FC (100% TBME) to yield ketone **2** (84.1 mg, 0.46 mmol, 58%) as a clear, colorless oil. ^{1}H-NMR (400MHz, $CDCl_3$): 6.87 (*ddd*, 1H, J = 7.7, 4.1, 1.9, H-C(4)), 6.10 (*ddd*, 1H, J = 7.7, 2.1, 1.0, H-C(3)), 5.14 (*d*, 1H, J = 3.5, H-C(8)), 4.42 (*s*, 1H, H-C(1)), 4.13 (s, 1H, OH), 3.48 (*s*, 3H, OMe), 2.94 (*ddd*, 1H, J = 15.0, 3.8, 0.9, H-C(5)), 2.83 (*dt*, 1H, J = 15.0, 4.3, 2.2, H-C(5)), 2.08 (*m*, 2H, H-7). ^{13}C-NMR (75 MHz, $CDCl_3$): 196.6 (C(2)), 147.1 (C(4)), 127.8 (C(3)), 104.0 (C(8)), 87.1 (C(1)), 78.3 (C(6)), 55.7 (-O-CH_3), 43.4 (C(7)), 33.9 (C(5)). HR-MS (EI^+, $[M + H]^+$): 184.0736 (calc.184.0736).

(1R,2R,6S,8RS)-2,6-dihydroxy-8-methoxy-9-oxabicyclo[4.3.0]non-3-en (3). Compound **2** (217.8 mg, 1.18 mmol) was dissolved in dry MeOH (15 ml) and cooled to 0°C. $CeCl_3 \cdot 7H_2O$ (881.0 mg, 2.36 mmol) was added followed by the slow and portionwise addition of $NaBH_4$ (89.3 mg, 2.36 mmol). The white solution was stirred at 0°C for 30 min then dissolved with EtOAc and quenched with a solution of aq. sat. NH_4Cl. The aqueous phase was extracted with EtOAc until no more product was detectable (TLC control). The organic phase was washed with a solution of aq. sat. NH_4Cl (1 × 20 ml), then dried ($MgSO_4$), filtered, and the solvent evaporated *in vacuo*. The crude product was purified by FC (98:2 to 95:5 CH_2Cl_2/MeOH) to yield product **3** (167.9 mg, 0.90 mmol, 76%) as a colorless oil. ^{1}H-NMR (300MHz, $CDCl_3$): 5.89 (*td*, 1H, J = 9.8, 3.0, H-C(3)), 5.81 (*m*, 1H, J = 9.6, 8.5, 3.0, H-C(4)), 5.09 (*dd*, 1H, J = 2.8, 2.1, H-C(8)), 4.32 (*m*, 1H, H-C(2)), 4.27 (*d*, 1H, J = 5.5, H-C(1)), 3.42 (*s*, 3H, OMe), 3.31 (*s*, 1H, OH), 2.53

(*dd*, 1H, J = 16.9, 5.4, H-C(5)), 2.26 (*m*, 1H, J = 16.8, H-C(5)), 2.18 (*s*, 2H, H-C(7)). ^{13}C-NMR (101MHz, $CDCl_3$): 129.9 (C(3)), 126.8 (C(4)), 104.8 (C(8)), 86.7 (C(1)), 66.2 (C(2)), 54.9 (-O-CH_3), 46.8 (C(5)), 33.5 (C(7)). HR-MS (ESI-MS$^+$, $[M + Na]^+$): 209.0785 (calc. 209.0789).

(1R,2R,6S,8RS)-2,6-dihydroxy-8-methoxy-9-oxabicyclo[4.3.0]nonane (4). Compound **3** (160 mg, 0.86 mmol) was dissolved in dry MeOH (16 ml) and 20% $Pd(OH)_2/C$ (153 mg) was added. The black solution was degassed with argon then flushed with H_2. It was then stirred at room temperature for 3 h. Pd was filtered off over Celite. The solvent was evaporated and the crude product purified by FC (98:2 CH_2Cl_2/MeOH) to yield product **4** (mixture of anomers, 139.5 mg, 0.74 mmol, 86%) as white crystals. ^{1}H-NMR (300MHz, D_2O): 5.11(*dd*, 1H, J = 6.0, 4.5, H-C(8)), 3.98 (*d*, 1H, J = 3.6, H-C(1)), 3.90 (*m*, 1H, H-C(2)), 3.42 (*s*, 3H, OMe), 2.42 (*dd*, 1H, J = 13.2, 6.0, H-C(7)), 1.83 (*dd*, 1H, J = 13.5, 4.5, H-C(7)), 1.63-1.47 (*m*, 6H, H-C(3), H-C(4), H-C(5)). ^{13}C-NMR (101MHz, $CDCl_3$): 105.2 (C(8)), 86.4 (C(1)), 67.0 (C(2)), 55.2 (-O-CH_3), 44.4, 32.6 , 28.4, 16.4 (C(3), C(4), C(5), C(7)). HR-MS (ESI-MS$^+$, $[M + Na]^+$): 211.0940 (calc. 211.0946).

(1′R,2′R,6′S,8′RS)-2′,6′-dihydroxy-8′-(thymin-1-yl)-9′-oxabicyclo[4.3.0] nonane (5). The sugar **4** (41.0 mg, 0.22 mmol) was dissolved in dry acetonitrile (3.5 ml). Thymine (54.9 mg, 0.44 mmol) and N,O-bis-(trimethylsilyl)acetamide (0.32 ml, 1.30 mmol) were added and the suspension was stirred at 50°C. After 90 min the clear solution was cooled to 0°C and trimethylsilyl-trifloromethansulfonate (0.08 ml, 0.44 mmol) was added dropwise. The solution was stirred at 50°C for 3 h. It was then poured onto an ice-cold solution of aq. sat. $NaHCO_3$ and extracted with CH_2Cl_2 (3 ml). The organic phase was dried ($MgSO_4$), filtered, and evaporated *in vacuo* to yield the crude product. This was dissolved in dry THF (1 ml), and 37% HF/Et_3N (0.21 ml, 0.43 mmol) was added. The solution was stirred for 30 min, then diluted with EtOAc and quenched by the addition of silica (1 g). Purification by FC (94:6 CH_2Cl_2/MeOH) yielded product **5** (mixture of anomers, 36.9 mg, 0.13 mmol, 60%, 2:1 α:β) as a white foam. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.67 (*d*, 1H, J = 1.11, H-C(6_β)), 7.41 (*d*, 2H, J = 1.14, H-C(6_α 6.22 (*dd*, 1H, J = 7.52, 3.89, H-C($8'_\beta$)), 6.04 (*dd*, 2H, J = 7.53, 3.90, H-C($8'_\alpha$)), 4.46 (*d*, 2H, J = 5.28, H-C($1'_\alpha$)), 4.16 (*m*, 3H, H-C($1'_\beta$, H-C($2'_\alpha$)), 4.05 (*m*, 1H, H-C($2'_\beta$)), 2.70 (*dd*, 2H, J = 13.68, 7.47, H-C($7'_\beta$)), 2.59 (*dd*, 4H, J = 8.96, 5.74, H-C($7'_\alpha$)), 2.11-1.61 (*m*, 18H, H-C($3'_\alpha$, H-C($4'_\alpha$, H-C($5'_\alpha$, H-C($3'_\beta$, H-C($4'_\beta$, H-C($5'_\beta$)), 1.94 (*d*, 6H, J = 1.11, 5-$CH_{3\alpha}$), 1.92 (*d*, 3H, J = 1.14, 5-$CH^3_{3\beta}$C-NMR (101MHz, MeOD): 163.4, 151.1 (C($Thymine_{quart}$)), 139.0 (C(6)), 110.6 (C(5)), 86.3 (C(1′)), 86.0 (C(8′)), 78.5 (C(6)), 68.4 (C(2′)), 44.8 (C(7′)), 36.0, 30.8, 18.3 (C(3′),

C(4′), C(5′)), 12.5 (C(C5-CH3)). HR-MS (ESI-MS$^+$, $[M + Na]^+$): 305.1121 (calc. 305.1113).

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