Enantioselective Synthesis of a Highly Preorganised 2′-Deoxy-spiro-nucleoside

Sebastian Wendeborn,* Gregory Binot, Mafalda Nina, Tammo Winkler

Syngenta AG, CH-4002 Basel, Switzerland
Fax +41(61)3235500; E-mail: sebastian.wendeborn@syngenta.com

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Abstract: The synthesis of a novel spiro-nucleotide, in which the C(4′) and the C(5′) of a nucleotide are connected by an additional ethylene bridge, is reported. The γ-torsional angle of the resulting novel nucleotides is in the region of the one observed in natural double stranded DNA.

Keywords: DNA, asymmetric synthesis, dihydroxylations, nucleosides, spiro compounds

Recently, a number of nucleotide modifications were reported in the literature, in which the sugar pucker is locked in a certain conformation, preferentially the C3′-*endo* conformation.[1] Some of those modifications result, when incorporated in oligonucleotides, in a dramatic stabilization of duplexes formed with complementary oligoribonucleotides or oligodeoxyribonucleotides. The most prominent modification of this type is the 'locked nucleic acid' **I**, shown in Figure 1, in which the C(4′) and the C(2′)OH are linked by a methylene group, thereby locking the sugar pucker in the C3′-*endo* conformation.[1a,b,2]

Molecular model analysis of a double stranded DNA reveals that replacement of the C(4′)H and either of the C(5′)H's with a ethylene bridge results in a highly rigidified spiro nucleotide compatible with the overall geometry of DNA (Figure 1). Introduction of the spiro functionality reduces rotational freedom around the C(4′)–C(5′) bond, resulting in a γ-torsional angle of 66° for **IIα** and 44° for **IIβ** as observed in the corresponding gas phase DFT/BP DN** optimized geometries (vide infra). Those values may be compared to the average γ-torsional angles in natural A-form and B-form DNA crystal structures of 57° and 51° respectively.[3,4] Nucleotides, such as **IIα** or **IIβ** may therefore be very interesting candidates for preorganising DNA or RNA strands into which they may be incorporated in search for better antisense and antigene oligonucleotides. High fidelity DNA binding oligonucleotides are currently a prime target of research for potential applications in antisense and antigene therapies[5] and site directed mutagenesis experiments.[6]

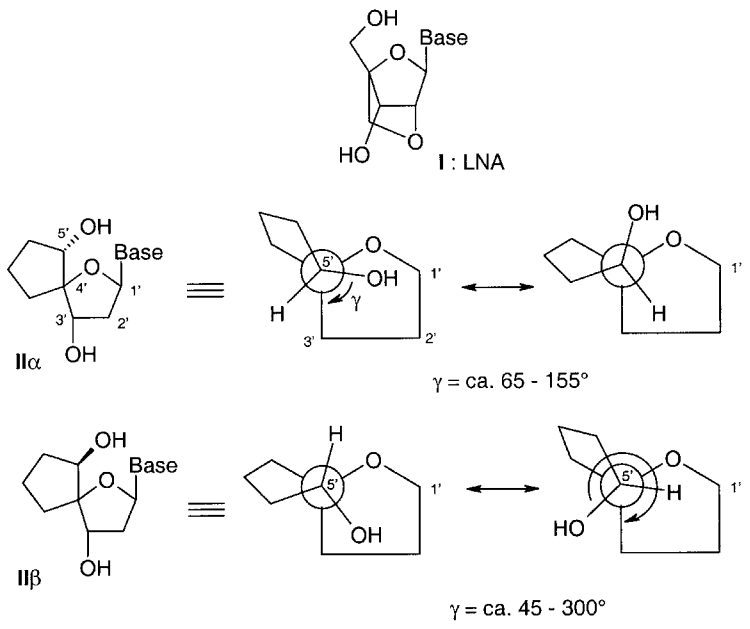

Figure 1 Conformations of spironucleosides **IIα** and **IIβ**.

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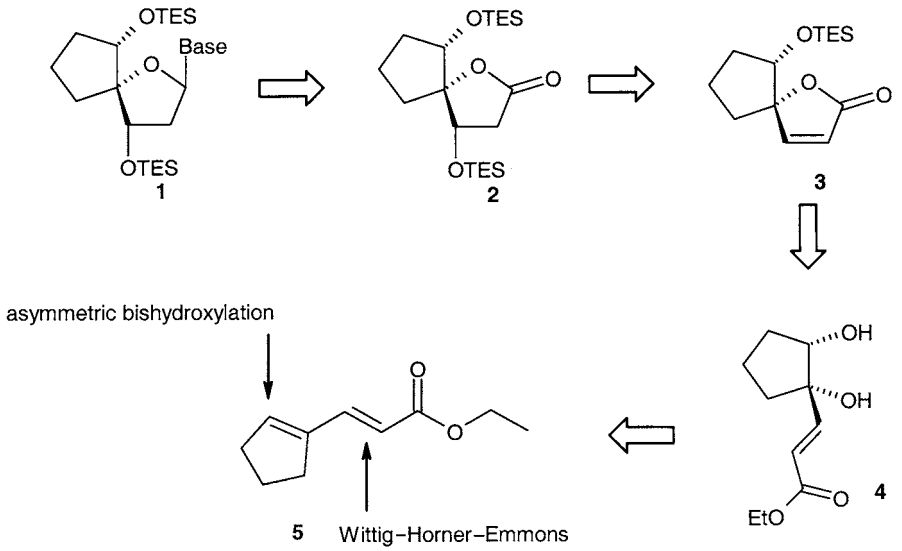
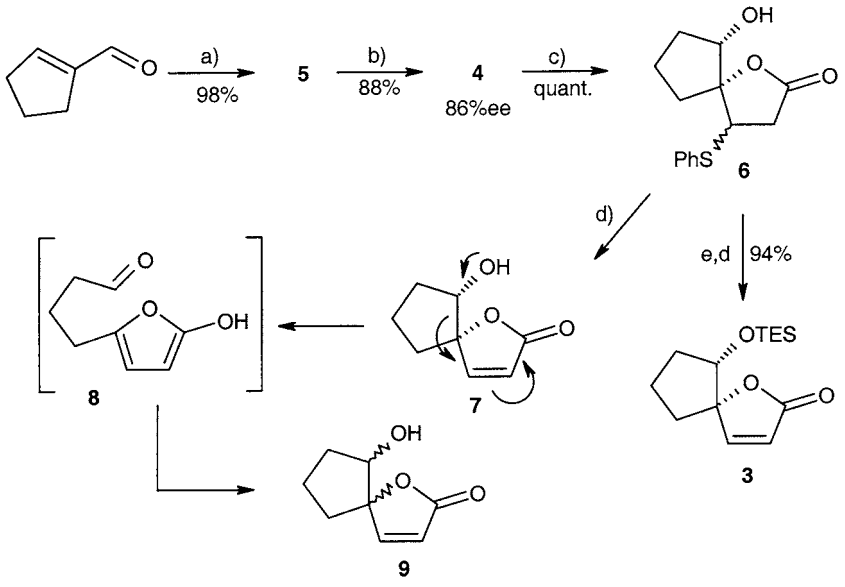


Scheme 1 Retrosynthetic analysis



Scheme 2 a) 1.3 equiv Ph_3P=CCOOEt, CH_2Cl_2, 0 °C, 72 h; b) 1.5 equiv α-AD-mix, 1 equiv $MeSO_2NH_2$, *t*-BuOH–H_2O (1:1), 4 °C, 18 h; c) 1.1 equiv PhSH, 1.1 equiv Et_3N, 70 °C, 1.5 h; d) 1.1 equiv Et_3SiCl, 3 equiv imidazole, CH_2Cl_2, r.t., 0.15 h; e) 2 equiv DBU, CH_2Cl_2, r.t., 18 h.

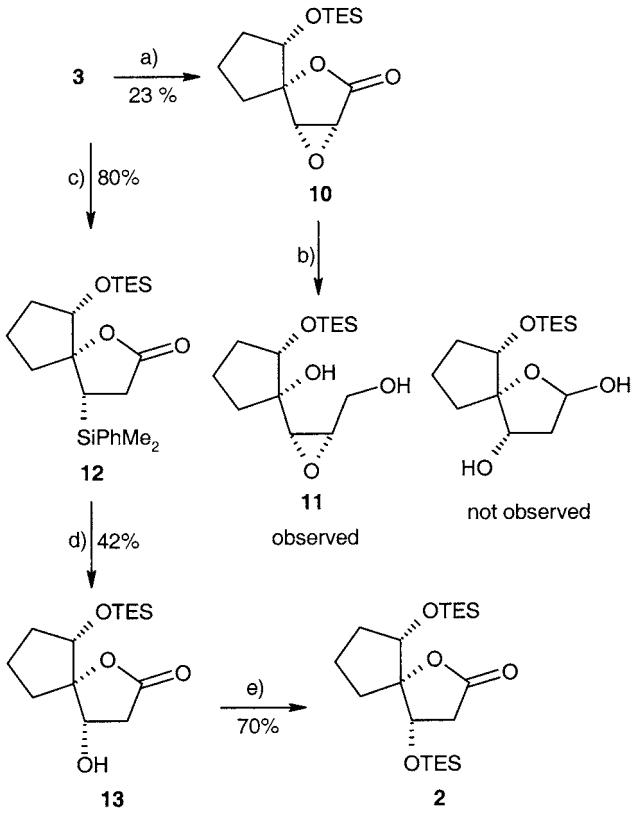
During the preparation of this manuscript the synthesis of a related spirocyclic nucleoside was reported by Paquette et al.[7] The approach that we have chosen is fundamentally different from Paquette's, even though it leads to the same common intermediate **3**. Complementary to Paquette's work, which leads to spiro ribonucleosides, our work focused on the synthesis of the corresponding 2′-deoxyribonucleosides. Here we report our studies towards the synthesis of enantiomerically pure **IIα**. The retrosynthetic analysis is outlined in Scheme 1.

We envisioned introduction of the thymine through a Vorbrüggen type nucleosidation of a lactol derivative corresponding to lactone **2**.[8] The C(3′) alcohol would be available through functionalization of α,β-unsaturated lactone **3**, and we anticipated oxygenation of C(3′) from the less hindered α-face. Compound **3** would be obtained from **4** through isomerization of the *trans* double bond and deprotection of the diol, which itself would be introduced through chemoselective and asymmetric bishydroxylation of diene **5**.

Scheme 2 summarizes the efficient and enantioselective construction of spiro lactone **3**. Wittig–Horner–Emmons reaction of known cyclopenten-1-al gave α,β-unsaturated ester **5** in excellent yield. Sharpless asymmetric bishydroxylation resulted in clean formation of diol **4** in 88% yield and 86% ee.[9] The absolute stereochemistry of **4** was assigned according to literature precedent of related compounds.[10,11] 1,4-Addition of thiophenol in the presence of triethylamine to **4** resulted in quantitative formation of spiro lactone **6** as a 1:2 mixture of diasteromers at C(3′).

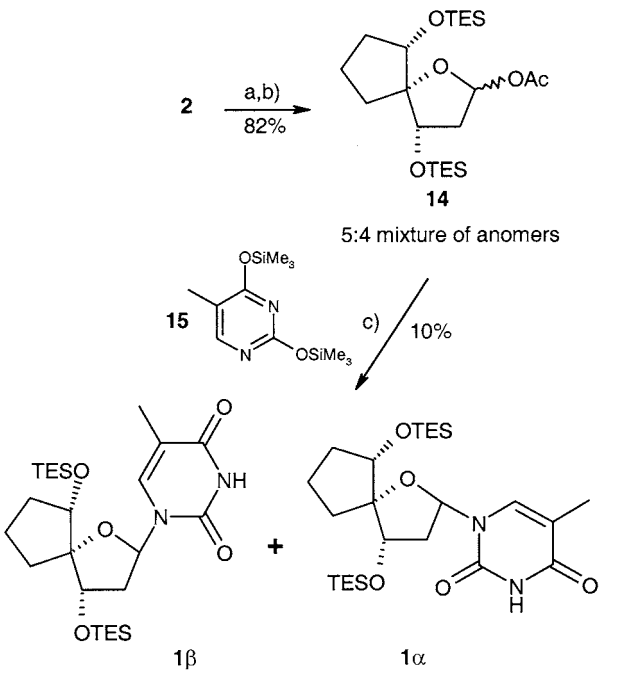
Reinstallation of the double bond through DBU-induced elimination of thiophenol could be accomplished but resulted in scrambling of relative and presumably absolute stereochemistry at C(4′) and C(5′), probably through a retro-aldol/aldol isomerisation reaction resulting in diastereomeric mixture of **9**.[12] Isomerisation could be avoided, when alcohol **6** was silyl protected prior to thiophenol elimination, yielding **3** in excellent yield.

Oxygenation of C(3′) proved to be difficult. Epoxidation proceeded in low yields to give **10**, which gave upon treatment with $NaBH_4$ the undesired diol **11** (Scheme 3).[13] Upon some experimentation we were able to add Flemings silyl cuprate to the unsaturated lactone resulting in clean 1,4-addition with complete α-selectivity.[14] The resulting Si–C bond of **12** was oxidized according to literature procedures to give **13**, which was silylated to key intermediate **2**.[15]



Scheme 3 a) 2.9 equiv NaOCl, pyridine, 0 °C → r.t., 3 h; b) $NaBH_4$, EtOH; c) 2 equiv $PhMe_2SiLi$, 1.05 equiv CuI, THF, –23 °C, 4 h, then –78 °C, **3** in THF, 0.5 h; d) 1.8 equiv KBr, 12 equiv NaOAc, 12 equiv HOOAc, HOAc, r.t., 4 h; e) 1.1 equiv Et_3SiCl, 3 equiv imidazole, CH_2Cl_2, r.t., 0.15 h.

The final steps of the synthesis are reported in Scheme 4. Reduction of lactone **2** resulted in formation of the corresponding unstable lactol, which was immediately acylated to give **14** as a mixture of anomers. Inspection of molecular models of **14** indicates that introduction of a thymine into the anomeric position may be in conflict with severe steric hindrance caused by the two triethylsilyl groups below and above the anomeric center. Nevertheless, to our



Scheme 4 a) 1.35 equiv DIBAL, PhMe, –78 °C, 1 h; b) 5 equiv Ac_2O, 0.1 equiv DMAP, pyridine, 0 °C → r.t., 1 h; c) 2 equiv **15**, 1 equiv $SnCl_4$, CH_2Cl_2, –78 °C → r.t., 1 h.

satisfaction, introduction of thymine could be accomplished under Vorbrüggen's conditions, even though in low yield and without stereoselectivity (**1α**:**1β** ≅ 2:1).[16]

Theoretical calculations. Two model compounds were used in the present work to evaluate the gas phase optimized geometries of **IIα** and **IIβ** which better mimic the the γ-torsional angles in natural double strand DNA. Quantum mechanical calculations were carried out with the Spartan program (release 5.1.2) using the density functional theory (DFT), BP exchange correlations functionals and DN** basis set.[17] Energy minimizations were performed with the default tolerances in the Spartan program. The orientation of the thymine relative to the furanose i.e. χ-dihedral angle was fixed to reproduce the *anti* orientation found in the natural DNA. The adiabatic potential energies surfaces as a function of the γ-dihedral angle in model compounds **IIα** and **IIβ** were calculated using the DFT/BP DN** level (Figure 2). Results show that the γ-dihedral angles corresponding to the global minimum energy geometries for **IIα** and **IIβ** are 120° and 310° respectively.

The intramolecular hydrogen bond between the C(5′) alcohol group and the O(4′) oxygen from the ribose accounts for the global minimum energy (γ = 120°) found for the model compound **IIα**. In the context of a DNA single strand the linkage prevents this intramolecular hydrogen bond to occur. Thus the local minimum where γ is 66° and the corresponding sugar pucker is C2′-*endo* is likely to be the more stable conformation of compound **IIα** when the alcohol group is involved in the DNA linkage (Figure 3, A). The two minima differ by 3.2 kcal/mol and

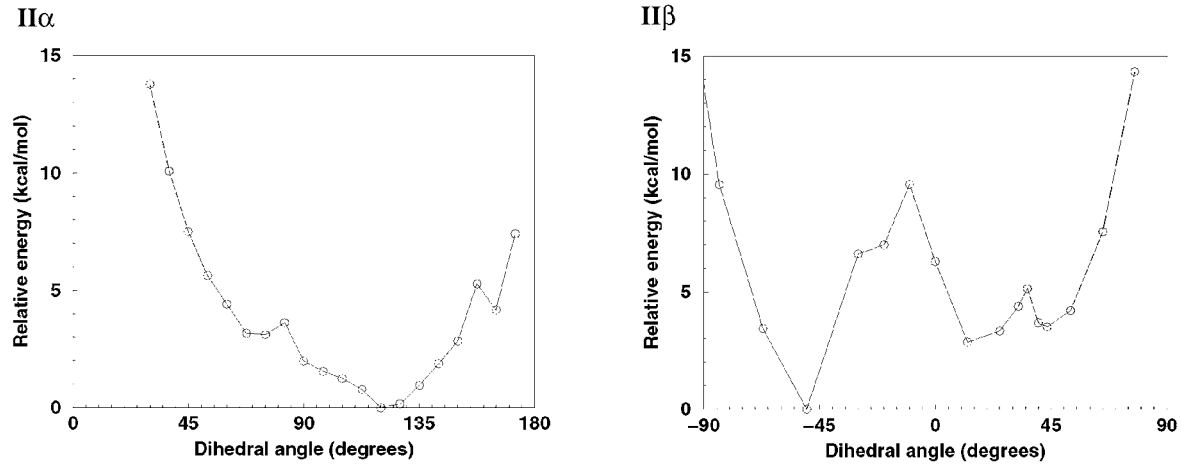


Figure 2 Potential energy surfaces as a function of γ-dihedral angle for compounds **IIα** and **IIβ**.

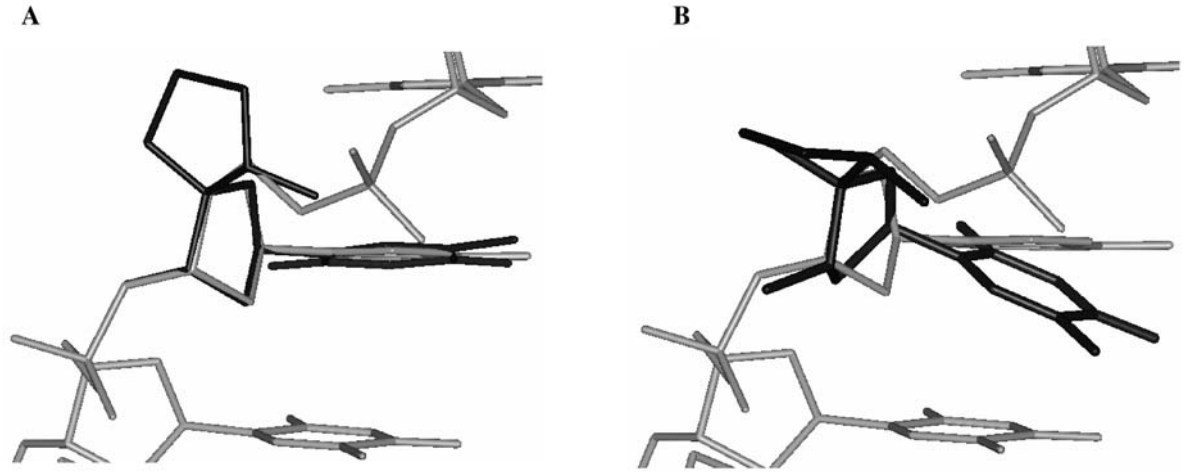


Figure 3 The crystal structure of the B-DNA CGATCGATCG at 1.5 Å resolution is shown in grey.[18] **A**. **IIα** model compound gas phase geometry optimized with DFT/BP DN** basis set. The γ-dihedral angle is 66° and the sugar pucker is C2′-*endo*. **B**. **IIβ** model compound gas phase geometry optimized with DFT/BP DN** basis set. The γ-dihedral angle is 44° and the sugar pucker is C3′-*endo* (local minimum).

the energy barrier between them is of 3.6 kcal/mol. The **IIβ** surface indicates three energy minima that occur around 12°, 44°, and 310° (global minimum). The **IIβ** local minimum, where γ is 44°, has a sugar pucker in C3′-*endo* conformation (Figure 3, B). No intramolecular hydrogen bond was observed. The two local minima differ by 0.7 kcal/mol in energy and the energy barrier between both is 2.3 kcal/mol. The energy barrier between the global minimum and two minima is 9.5 kcal/mol.

In summary, we have succeeded in the synthesis of a highly preorganized spiro-nucleotide. Further studies are required to optimize the final steps of the sequence to allow for a more efficient and selective introduction of thymine. Molecular modelling studies and DFT theoretical calculations predict, that a successful incorporation of such spiro-nucleotides into a DNA or RNA backbones may have a highly preorganizing effect on the overall structure of the oligomer, potentially resulting in increased binding affinities to DNA or RNA targets. The reported spiro nucleotide and analogs thereof may therefore find application in RNA- or DNA- targeting with implications for antisense-, antigene- and site directed mutagenesis strategies but could also have applications as anti-viral agents.

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(12) Ee of **9** was not determined.
(13) Literature precedent in a related system suggested that $NaBH_4$ would result in chemoselective reduction of the epoxide at C(2′) along with reduction of the lactone to the lactol. Inspection of the ^{13}C NMR data presented by the authors, however, indicates that their results were misinterpreted: Ortuno, R. M.; Cardellach, J.; Font, J. *J. Heterocycl. Chem.* **1987**, *24*, 79.
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(16) **1α**: ^1H NMR (500 MHz, $CDCl_3$): δ = 7.95 (b, 1 H, NH), 7.74 (q, *J* = 1.0 Hz, 1 H, H-6), 6.38 (dd, *J* = 8.3 and 1.8 Hz, 1 H, H-1′), 4.09 (d, *J* = 5.3 Hz, 1 H, H-3′), 3.78 (dd, *J* = 9.4 and 7.3 Hz, 1 H, H-5′), 2.85 (ddd, *J* = 14.4, 8.3 and 5.3 Hz, 1 H, H-2′β), 1.92 [d, *J* = 1.0 Hz, 3 H, CH_3-C(5)], 1.83 (dd, *J* = 14.4 and 1.8 Hz, 1 H, H-2'α), 1.5–2.1 (m, 6 H, H-6′, H-7′, H-8′), 0.97 (m, 18 H, CH_3CH_2Si), 0.62 (m, 12 H, CH_3CH_2Si). The configuration of C-1′ was established by NOE: Both H-1′ and H-3′ show a strong NOE with H-2′β. No NOE was observed between H-3′ and H-6. Furthermore, a strong NOE was observed between H-3′ and H-5′ proving the configuration of C-3′ and C-5′ given in Scheme 4. ^{13}C NMR (125 MHz, $CDCl_3$, as obtained from the HSQC and HMBC spectra): δ = 163.7 (C-4), 150.3 (C-2), 137.6 (C-6), 109.7 (C-5), 97.8 (C-4′), 85.5 (C-1′), 78.3 (C-5′), 75.6 (C-3′), 42.9 (C-2′), 31.9 and 29.4 (C-8′ and C-6′), 17.9 (C-7′), 12.4 (CH_3-C-5), 6.7 (CH_3CH_2Si), 4.9 (CH_3CH_2Si). ESI-MS: 511 ($M + H^+$); 509 ($M - H^+$).
1β: ^1H NMR (500 MHz, $CDCl_3$): δ = 8.01 (b, 1 H, NH), 8.00 (q, *J* = 1.0 Hz, 1 H, H-6), 6.28 (dd, *J* = 7.8 and 5.5 Hz, 1 H, H-1′), 4.23 (dd, *J* = 5.6 and 3.0 Hz, 1 H, H-3′), 3.91 (dd, *J* = 9.5 and 8.3 Hz, 1 H, H-5′), 2.25 (ddd, *J* = 12.9, 5.5 and 3.0 Hz, 1 H, H-2′α), 2.11 (ddd, *J* = 12.9, 7.8 and 5.6 Hz, 1 H, H-2′β), 1.94 [d, *J* = 1.0 Hz, 3 H, CH_3-C(5)], 1.5–2.1 (m, 6 H, H-6′, H-7′, H-8′), 0.97 (m, 18 H, CH_3CH_2Si), 0.62 (m, 12 H, CH_3CH_2Si). The configuration of C-1′ was established by NOE: H-1′ shows a strong NOE with H-2′α, and H-3′ a strong NOE with H-2′β. A weak NOE was observed between H-3′ and H-6. Furthermore, a strong NOE was observed between H-3′ and H-5′ proving the configuration of C-3′ and C-5′ given in Scheme 4. ^{13}C NMR (125 MHz, $CDCl_3$, as obtained from the HSQC and HMBC spectra): δ = 163.7 (C-4), 150.3 (C-2), 136.6 (C-6), 109.7 (C-5), 95.7 (C-4′), 84.4 (C-1′), 76.9 (C-5′), 74.4 (C-3′), 41.8 (C-2′), 31.4 and 29.2 (C-8′ and C-6′), 17.8 (C-7′), 12.4 (CH_3-C-5), 6.7 (CH_3CH_2Si), 4.9 (CH_3CH_2Si). ESI-MS: 511($M + H^+$); 509 ($M - H^+$).
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