Synthesis of Bicyclonucleosides Having a C−C Bridge†

Jacques Lebreton,*,‡ Jean-Marc Escudier,⊥ Laurence Arzel,‡ and Christophe Len§

Université de Nantes, CNRS, Laboratoire CEISAM-UMR-CNRS 6230, Faculté des Sciences et des Techniques, 2 rue de la Houssinière, BP 92208, 44322 Nantes Cedex 3, France, Université Paul Sabatier Laboratoire de Synthèse et Physicochimie des Molécules d'Intérêt Biologique - UMR-CNRS 5068, 31062 Toulouse Cedex, France, and Université de Technologie de Compiègne, Ecole Supérieure de Chimie Organique Minérale, EA 4297, Transformations Intégrées de la Matière Renouvelable, 1 allée du Réseau Jean-Marie Buckmaster, F-60200 Compiègne, France

Received September 5, 2008

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1. Introduction

Typical nucleosides are initially obtained from the nucleic acids, DNA and RNA, by enzymatic hydrolysis, and they are of great biological importance in metabolic pathways.[1,2] Furthermore, many natural antibiotics (e.g., bredinin)[3] belong to the nucleoside family. Their common structural characteristic is the presence of two molecular fragments: D-ribo- or D-2′-deoxyribopentofuranose as the sugar moiety and purine or pyrimidine aglycone. These two moieties are covalently bonded from N1 of pyrimidine (uracil, thymine, and cytosine) or N9 of purine (adenine and guanine) to C1′ of the glycone (the carbohydrate portion of the nucleoside) in a β-D configuration (Figure 1).

Conventional nucleosides adopt a number of conformations that can be conveniently described by three principal structural parameters (Figure 2):[4] (i) the glycosyl torsion angle χ (O4′–C1′–N9–C4 for purine nucleosides and O4′−C1′−N1−C2 for pyrimidine nucleosides), which determines the *syn* or *anti* disposition of the nucleobase relative to the sugar moiety (*syn* when the C2 carbonyl of pyrimidines or the N3 of purines lies over the sugar ring and *anti* for the opposite direction); (ii) the torsion angle γ (O5′−C5′−C4′−C3′), which determines the position of the 5′-OH relative to the C3′ carbon atom (+*sc*, *ap*, and −*sc* rotamers); (iii) the phase angle of pseudorotation P (0−360°), and the maximum out-of-plane pucker υ_{max} (0−50°), which determines the puckering of the furanose ring and its deviation from planarity, respectively.[5,6] The value of P depends on the five endocyclic sugar torsion angles (υ_0−υ_4) and on the puckering of the furanose ring. The conformation of the furanose ring around the pseudorotational cycle alternates every 18° between the envelope (E) and twist (T) form. In solution, the sugar puckering fluctuates rapidly between the North conformation with $-18° < P < 18°$ and the South conformation with $162° < P < 198°$. The transition between North and South antipodes corresponds to an inversion of ring puckering that

† This review is dedicated to Dr. Alain De Mesmaeker, an outstanding scientist who made a great contribution to the antisense field.
* To whom correspondence should be addressed. Phone: +33 2 51 12 54 03. Fax: +33 2 51 12 55 62. E-mail: jacques.lebreton@univ-nantes.fr.
‡ Université de Nantes.
⊥ Université Paul Sabatier.
§ Université de Technologie de Compiègne, Ecole Supérieure de Chimie Organique et Minérale.

10.1021/cr800465j
Published on Web 03/17/2010

Jacques Lebreton was born in Guérande (France) in 1960. He received his Ph.D. degree (1986) from the University of Paris XI-Orsay under the supervision of Professor Eric Brown (Le Mans). His thesis work included the total synthesis of C-nor D-homosteroids. In 1986, he began his first postdoctoral fellowship with Professor James A. Marshall at the University of South Carolina working on the [2,3]-Wittig rearrangement and its application in total synthesis. Following a second postdoctoral fellowship with Professor Robert E. Ireland at the University of Virginia working on the total synthesis of monensine, he joined the laboratories of CIBA-GEIGY (Novartis) in Basle in 1990, where he worked in Dr. Alain De Mesmaeker's group in the field of antisense. In 1994, he joined the CNRS and spent a few years in the group of Dr. Jean Villiéras (UMR-CNRS 6513, Nantes) concerned with organometallic chemistry. In 1998, he was promoted to Professor at the University of Nantes. His major research interest is medicinal chemistry. Most of his recent work has focused on the synthesis of bioactive molecules, such as steroids, nucleosides, alkaloids, azasugars, and marine natural products for biological evaluation purposes in the fields of HIV, central nervous system diseases, cancer, and drug delivery through academic and industrial collaborations. His research efforts also include the synthesis of labeled molecules to study biological processes.

Jean-Marc Escudier was born in Decazeville (France) in 1964. He graduated in Biochemistry and received his Ph.D. from the University Paul Sabatier in Toulouse (France) in 1992 in the field of total synthesis of optically active polyhydroxylated compounds. After a postdoctoral position in Prof. U. K. Pandit's group at the University of Amsterdam (Netherlands) on Sesbanamide A alkaloid analogue synthesis, he joined the CNRS in 1993 in the "Laboratoire de Synthèse et Physico-Chimie de Molécules d'Intérêt Biologique" at the University Paul Sabatier, Toulouse. His major research interest focuses on the development of new conformationally constrained nucleosides for the stabilization of secondary structural elements of nucleic acids.

Laurence Arzel was born in Nîmes (France) in 1974. During her undergraduate studies, she worked with Professor Jean Martinez (University of Montpellier) on short peptide cyclization for two years. In 1998, she received her M.Sc. Degree in Biomolecular Chemistry from the University of Montpellier for her work on the synthesis and characterization of the pentasaccharide developed by Sanofi and commercialized as an anticoagulant. After several months in Research and Development at Expansia (a pharmaceutical company), she joined the CNRS (1999) in Professor Marco A. Ciufolini's group at the University of Lyon. She worked on the identification and characterization of natural and synthetic compounds by mass spectrometry. In 2006, she joined Professor Jacques Lebreton's group at the University of Nantes. Her research interests are focused on the synthesis of modified nucleosides.

Christophe Len was born in L'Isle Adam (France) in 1966. He received his Ph.D. from the University of Picardie-Jules Verne (UPJV) in Amiens (France) under the supervision of Professor P. Villa in the field of carbohydrate chemistry. In 1996, he joined Dr. G. Mackenzie's group at the University of Hull (U.K.) as a postdoctoral fellow to work on the synthesis of nucleoside analogues. In 1997, he became Maître de Conférences at UPJV and worked on the chemistry of antiviral nucleoside analogues, specializing in those with novel glycone systems. In 2003, he received his habilitation and was promoted to full Professor in 2004 at the University of Poitiers (France). During 2008, he moved to the University of Technology of Compiègne (France) to develop green chemistry. His current main research interests are in the total synthesis of natural products and bioactive molecules, which include carbohydrates and green chemistry.

is accompanied by changes in the axial and equatorial disposition of all the substituents on the sugar ring. The conformations of nucleosides described by these three-state models (χ, γ, P) are in interdependent equilibria determined by steric and stereoelectronic effects (e.g., anomeric and gauche effects),[7] and the energy barrier between the preferred conformational states is usually low (Figure 2).

The conformational behavior of natural as well as modified nucleosides is of great importance for their biological activities. Only one form is present at the active site when a nucleoside or nucleotide binds to its target enzyme, such as the activating kinases and DNA polymerase. In order to investigate these enzymes for their conformational prefer-

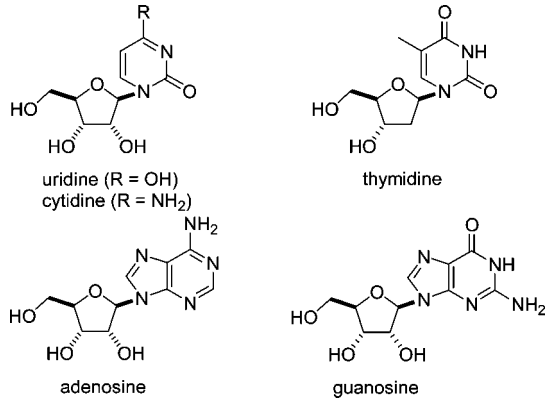


Figure 1. Classical natural nucleosides.

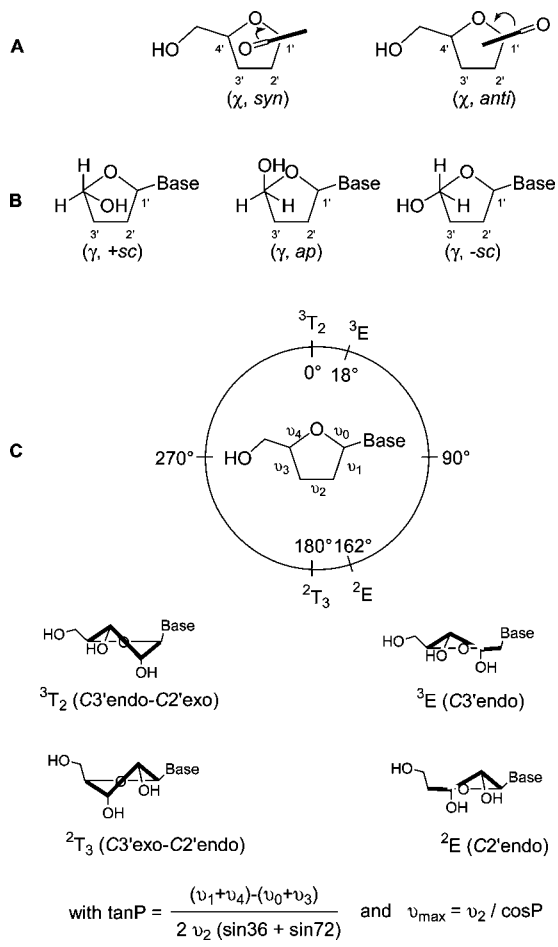


Figure 2. (A) Definition of *anti* and *syn* conformations for a pyrimidine nucleoside. (B) Definition of the torsion angle range about the C4′−C5′ bond. (C) Pseudorotational cycle of the furanose ring in a nucleoside (E = envelope and T = twist).

ence, the limitation of conformation of a nucleoside or nucleotide is widely used.[8] Conformationally constrained nucleosides can also be used to drive the molecular recognition in an oligonucleotide chain (RNA/DNA).[9] This particular conformation can be predetermined by limiting the conformational equilibrium (*syn* or *anti*, North or South, +*sc*, *ap*, or −*sc*) by the preparation of restricted polycyclic structures. Nucleosides with restricted conformations can be classified into three families: **bicyclonucleosides**[10] obtained by bonding two atoms of the furanose moiety via an alkylene unit or analogues; **cyclic phosphoesters**[10c] obtained by forming an

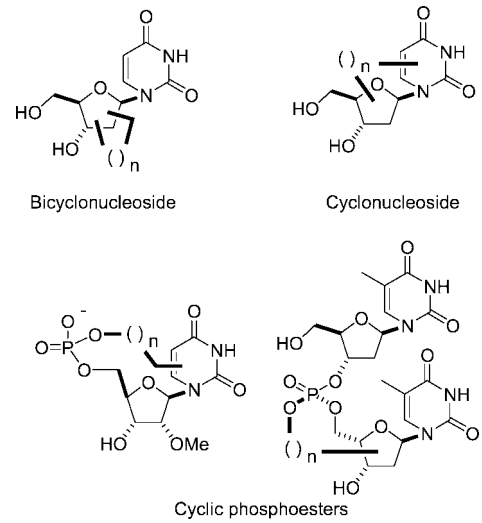


Figure 3. Different families of nucleoside analogues having a restricted conformation.

alkylene bridge or analogue between the phosphorus atom and the nucleobase or the furanose moiety; **cyclonucleosides** obtained by bonding one atom of the furanose moiety and one atom of the nucleobase via an alkylene unit or analogues (Figure 3).

For the sake of clarity, this review focuses on the synthesis of bicyclonucleosides having an alkylene group between two carbon atoms of the glycone moiety.

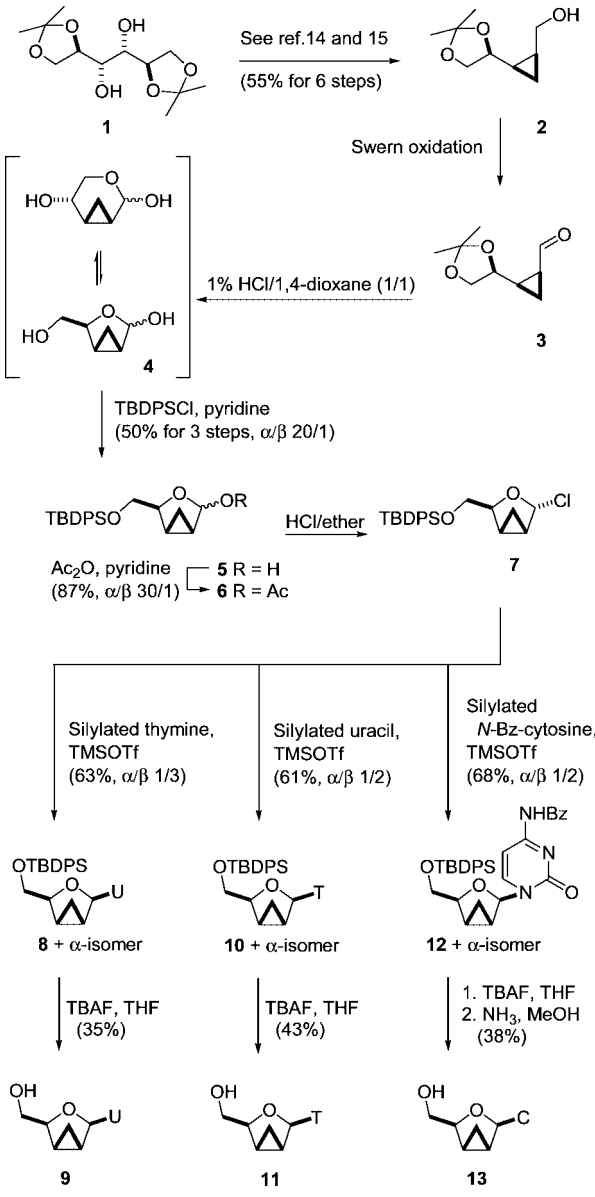
2. Synthesis of Bicyclonucleosides Having a Cyclopropane Core

Furanose ring fused nucleosides with cyclopropane, namely methano-nucleosides, have recently attracted much attention due to their potential biological activities and their use as building blocks for oligonucleotides with promising therapeutic and diagnostic applications.[11] This section presents the syntheses of methano-nucleosides by the methodologies used for the formation of the cyclopropyl core.

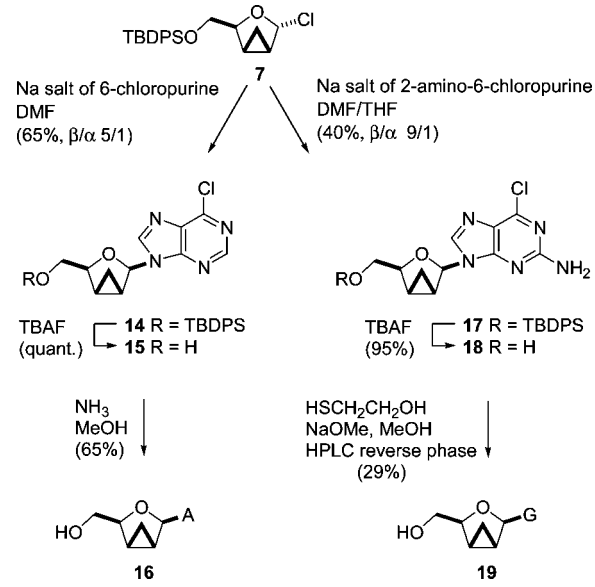
2.1. Formation via Simmons−Smith and Related Reactions

In 1998, Chu et al.[12,13] published a synthesis of sterically hindered *endo*-oriented methano-nucleoside analogues as depicted in Scheme 1. From the 1,2:5,6-di-*O*-isopropylidene-D-mannitol (**1**), the known chiral cyclopropyl allylic alcohol **2** was prepared in 55% overall yield using a six-step sequence including a Simmons−Smith reaction.[14,15] The latter alcohol **2** was oxidized using Swern oxidation and the resulting aldehyde **3** was cyclized under acidic conditions into the lactol **4**, which was in equilibrium between a furanose and pyranose form. At this stage, the lactol **4** was silylated to protect the primary alcohol function with the sterically demanding TBDPS group in order to favor the furanose intermediate **5** (50% yield for the three steps). Acetylation of lactol **5** gave rise to the corresponding acetate **6** as an α/β-anomer mixture in a 30 to 1 ratio. Due to the steric hindrance on the β face of the sugar with the methylene bridge, it was not surprising that, under the Vorbrüggen conditions, the condensation of the latter acetate mixture **6** with thymine and *N*4-benzoyl cytosine provided mainly the α-anomers (for **8** α/β 3:1 ratio, for **12** α/β 6:1 ratio). As an alternative to the conversion of the key intermediate **6** into

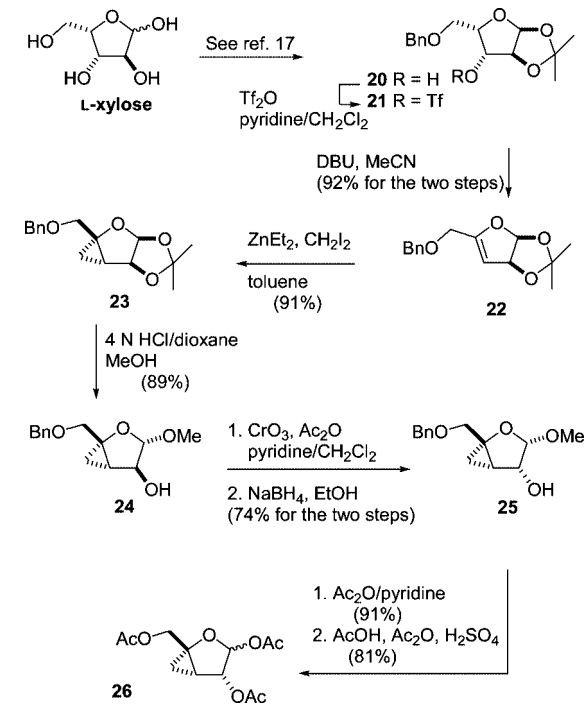
Scheme 1



Scheme 2



Scheme 3



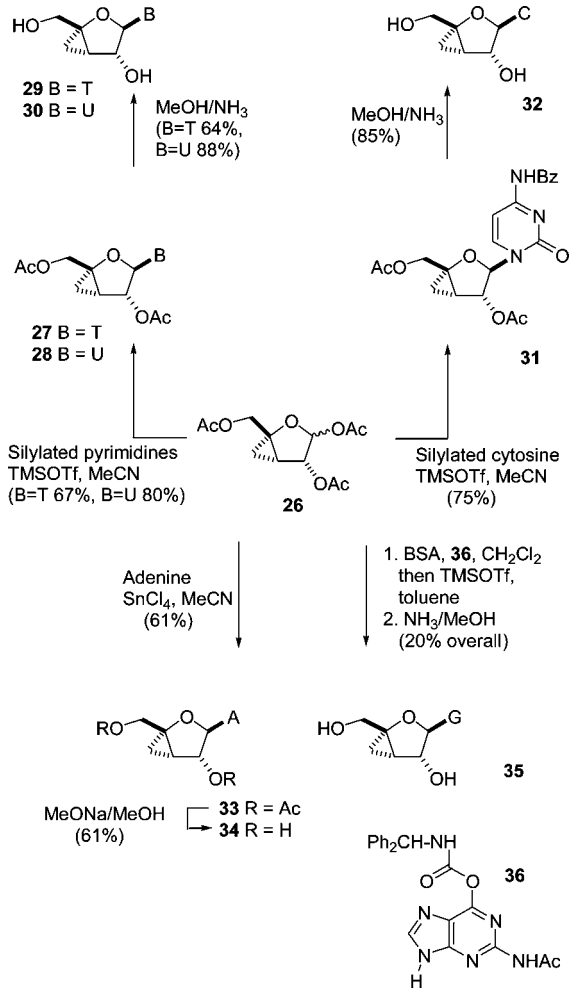
β-nucleosides, the chloride **7** emerged as the best solution. Accordingly, treatment of the acetate **6** with HCl resulted in the exclusive formation of the α isomer **7**, which, by S_N2-type condensation with the sodium salt of purines or silylated pyrimidines, led to the desired β-anomers as the major compounds. It should be pointed out that the chloride derivative **7** could be directly obtained from the lactol **5** by treatment with PPh_3 and CCl_4. In this context, uridine **8**, thymidine **10**, and cytidine **12** analogues were obtained in 63%, 61%, and 68% yields, respectively, and in α/β-anomer mixtures of ratios 1:3, 1:2, and 1:2, respectively. From these latter anomeric mixtures, removal of the protecting groups in classical conditions followed by purification by reverse-phase HPLC or silica gel column chromatography led to the desired uridine **9**, thymidine **11**, and cytidine **13** target bicyclic nucleosides in 35%, 43%, and 38% overall yields, respectively.

Concerning purine derivatives, treatment of **7** with the sodium salt of 6-chloropurine and 2-amino-6-chloropurine afforded the corresponding glycosylated compounds **14** and **17** in 65% (β/α 5:1 ratio) and 40% (β/α 9:1 ratio) yields, respectively, as outlined in Scheme 2. From these latter intermediates **14** and **17**, cleavage of the silylether followed by treatment with ammonia in refluxing MeOH or mercaptoethanol and MeONa led to the adenosine and guanosine derivatives **16** and **19**, respectively.

To highlight this work, it should be mentioned that enantiomerically pure carbocyclic cyclopropyl L-nucleosides were synthesized using the same pathway but starting from L-gulonic-γ-lactone.[13]

In the field of methano-nucleosides, Mathé et al.[16] developed an elegant approach from a chiral pool involving a diastereoselective addition of carbene to the electron-rich 3,4-unsaturated 3-deoxy sugar nucleoside **22** as the key step, followed by a classical glycosylation step (Scheme 3). The dihydrofurane intermediate **22** was prepared from **20**[17] in 92% overall yield by DBU-induced β-elimination of TfOH onto activated triflate intermediate **21**. A subsequent cyclopropanation reaction using Furukawa's procedure followed

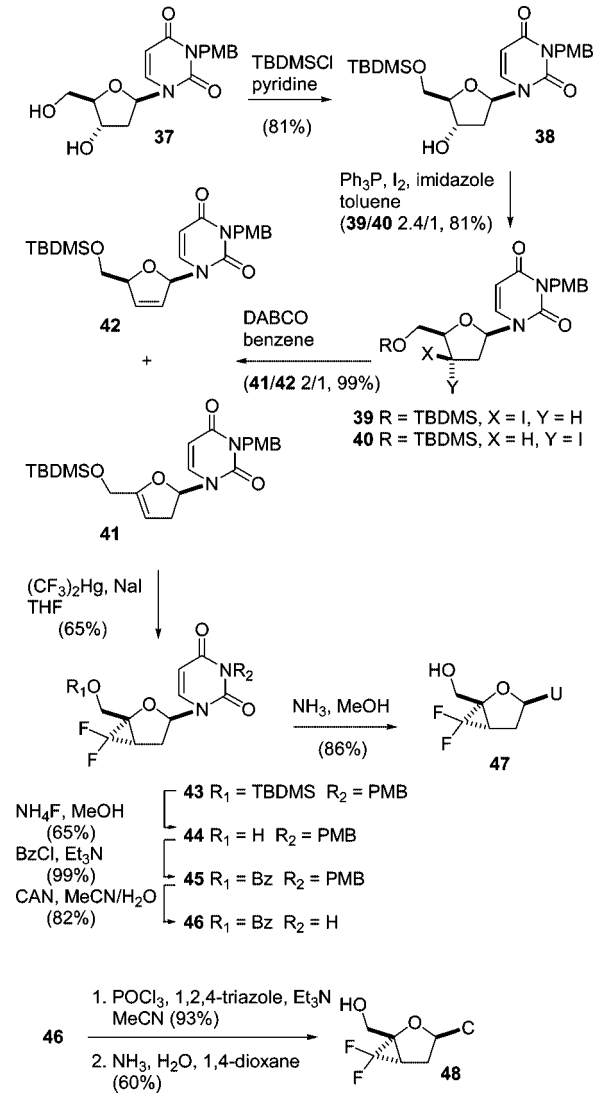
Scheme 4



by silica gel chromatography purification afforded the required pure cyclopropanic adduct **23** in 91% yield (only 1% of the other diastereoisomer was isolated). It is reasonable to assume that steric hindrance provided by the large isopropylidene group could explain this excellent diastereoselectivity. It is worth noting that from L-xylose these simple transformations provided a good synthetic access to new useful D-sugar precursors. Treatment of **23** with HCl in a dioxane−MeOH mixture afforded the α-anomer of the methyl furanoside **24** as a single isomer in 89% yield. To invert the configuration of alcohol at C4, oxidation of **24** provided the corresponding ketone which, without purification, was reduced with $NaBH_4$ to deliver the epimeric alcohol **25** as the sole diastereoisomer in 74% yield over the two steps. Protection of alcohol **25** as acetate, followed by H_2SO_4-mediated acetolysis in Ac_2O led to the cleavage of anomeric methyl and *O*-benzyl groups to furnish the peracetylated sugar building block **26** as an anomeric mixture (α/β 9:91 ratio) in 74% overall yield.

Glycosidation condensation of **26** was effected cleanly under Vorbrüggen conditions with thymine, uracil, or *N*4-benzoyl cytosine to afford the corresponding β-anomers **27**, **28**, and **31**, via a C2 acetyloxonium, in 67%, 80% and 75% yields, respectively (Scheme 4). Treatment of these latter compounds with methanolic ammonia led to the target molecules **29**, **30**, and **32**. The coupling of adenine with a sugar partner **26** in the presence of $SnCl_4$ as catalyst resulted in the isolation of the β-anomer **33**, which was then submitted to NaOH in MeOH to give the desired adenosine analogue

Scheme 5

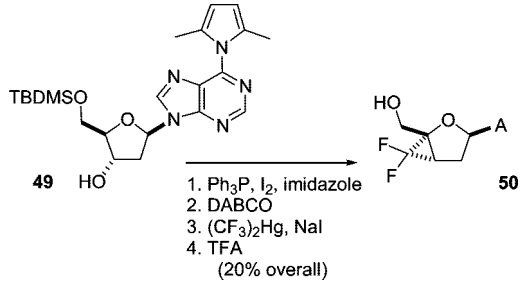


34 in 36% yield for the two steps. To complete this work, guanosine analogue **35** was synthesized as a single β-anomer in 20% overall yield by condensation of *N*2-acetyl-*O*6-(diphenylcarbamoyl)guanine (**36**) with **26** in the presence of TMSOTf as catalyst followed by methanolic ammonia treatment of the crude material.

The strategy described provides straightforward access in an efficient fashion to the four natural nucleoside analogues in a methano-nucleoside series.

Very recently, a synthesis of uridine, cytidine, and adenosine 2′-deoxy analogues with a C3′−C4′ difluoromethylene bridge was published by Robins et al.[18] employing a similar strategy with difluorocarbene, as depicted in Scheme 5. In this work, the *N*3-PMB-protected 2′-deoxyuridine **37** was prepared from uridine itself following a four-step sequence in 35% overall yield. Selective protection of the primary alcohol of **37** gave compound **38**, and then treatment with I_2 in the presence of Ph_3P and imidazole led to the isolation of the corresponding iodo derivatives as a mixture of epimers **39** and **40** in a 2.4:1 ratio and in 81% yield. The doubly inverted isomer **40** could be formed by participation of the O2 on the uracil ring via a 2,3′-*O*-anhydro intermediate. The latter mixture **39**/**40** was submitted to DABCO in refluxing benzene to promote elimination of hydrogen iodide leading to the isolation of an inseparable 2:1 mixture of the

Scheme 6

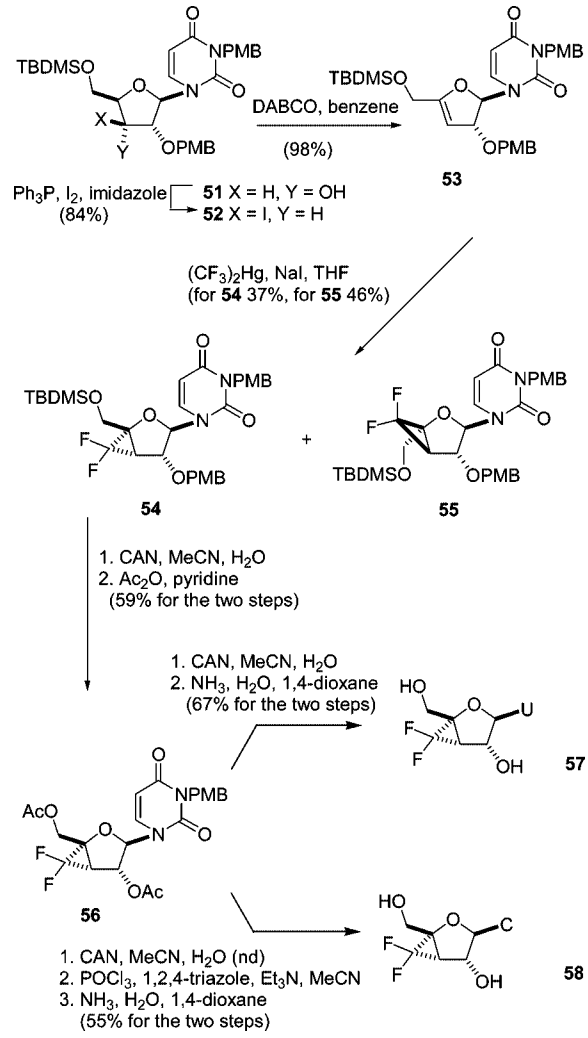


3′−4′-unsaturated key intermediate **41** and its isomer **42** in 99% yield. The iodide isomer **40** was recovered unchanged from the reaction mixture due to the *cis*-stereochemistry between the H4′ and iodo atoms. Treatment of 3′−4′-unsaturated key intermediate **41** and its isomer **42** with difluorocarbene, generated *in situ* from (trifluoromethyl)mercury and NaI, gave the unreacted less electron-rich 2′−3′-alkene **42** and the desired fluorinated adduct **43**, which was desilylated to furnish the intermediate **44** in 65% overall yield. Benzoylation of **44**, followed by sequential removal of *N*3-PMB and then benzoyl groups, led to the 2′-deoxyuridine analogue **47** in 70% overall yield. Finally, treatment of the benzoate intermediate **46** with $POCl_3$ and 1,2,4-triazole, followed by aminolysis led to the 2′-deoxycytidine analogue **48** in 56% overall yield.

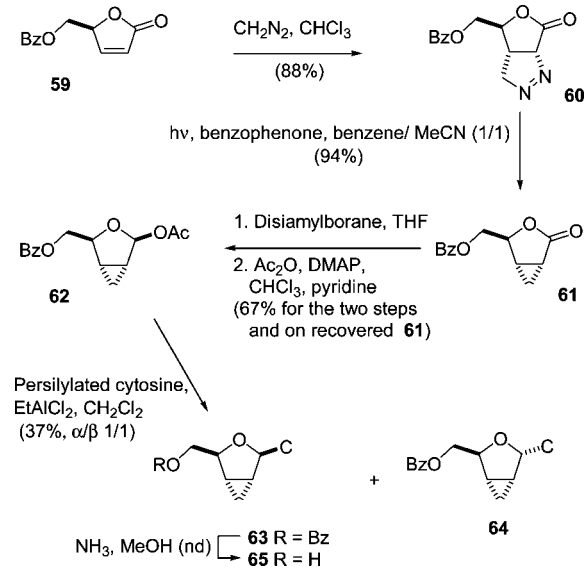
A similar sequence[18] was applied to the base-protected adenosine derivative **49** to give the corresponding 2′-deoxy analogue **50** with a difluorocyclopropane, as briefly presented in Scheme 6. It is noteworthy that the protection of the 6-amino function of adenosine as 2,5-dimethylpyrrole was necessary during the cyclopropanation step.

Having successfully developed a new entry into C3′−C4′ difluoromethylene bridged 2′-deoxynucleosides, Robins et al. extended the scope of this chemistry to the preparation of uridine and cytidine analogues as outlined in Scheme 7.[19] The protected nucleoside **51**, prepared from uridine in four steps, was treated as previously described for **38** (Scheme 5) with I_2 in the presence of Ph_3P and imidazole to afford, via a 2,3′-*O*-anhydro intermediate, the iodo derivative **52** as a unique diastereoisomer in 84% yield. Also, in contrast to previous observations in the 2′-deoxy series as presented in Scheme 5, the treatment of **52** with DABCO in hot benzene resulted in the formation of only the desired enol ether **53** in 96% yield. Addition of the difluorocarbene to intermediate **53**, about 20-fold less reactive than its 2′-deoxy congener **41**, exhibited a low diastereoselectivity due to the presence of the 2′-*O*-PMB group on the α face leading, after chromatography, to the isolation of the diastereoisomers **54** and **55** in 37% and 46% yields, respectively. From the β-D-ribo isomer **54**, the targeted uridine derivative **57** was obtained after protecting group manipulations. The cytidine analogue **58** was also synthesized from the diacetate **56** via the classical formation of the 4-triazolo intermediate followed by aminolysis.

Scheme 7



Scheme 8



2.2. Formation via 1,3-Dipolar Cycloaddition Followed by Nitrogen Extrusion

In an alternative approach, Beard et al.[20] described the synthesis of the cytidine analogue **65** using a 1,3-dipolar cycloaddition of diazomethane on the chiral butenolide **59** as a key step to afford, in diastereoselective fashion, the pyrazoline **60** intermediate. This provided, by photoirradiation in the presence of benzophenone as photosensitizer, the cyclopropane compound **61** in 83% overall yield (Scheme 8). It should be noted that this protocol is an alternative to diazomethane cyclopropanation catalyzed by metal salts[21] (e.g., Ni, Cu, or Pd). Reduction of the lactone **61** with disiamylborane followed by acetylation of the resulting lactol

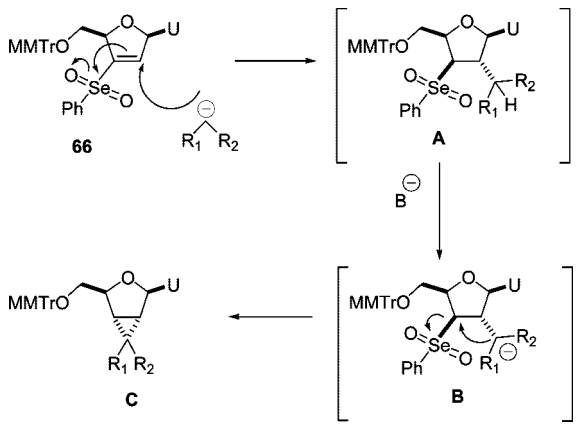
furnished the β-acetate **62** as a unique diastereoisomer. Subsequent classical glycosidation of **62** with silylated cytosine with ethylaluminium dichloride as catalyst led to an inseparable 1:1 mixture of both anomers **63** and **64** in 37% yield. The desired β-cytidine analogue **65** was obtained from the latter mixture after cleavage of the benzoate and a fastidious purification.

2.3. Formation via Michael Initiated Induced Ring Closure Reactions

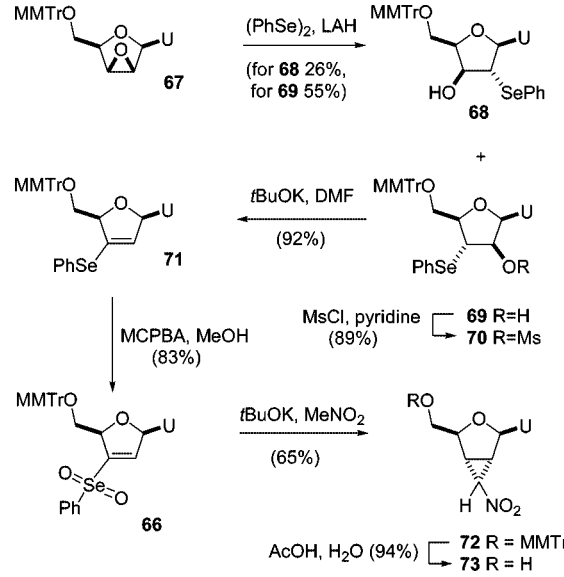
Michael induced ring closure (MIRC) reactions constitute a powerful methodology for the preparation of functionalized cyclopropanes. In this field, Chattopadhyaya et al.[22] reported an original access to [3.1.0]-bicyclic cyclopropano analogues of 2′,3′-dideoxyuridine from 3′ and 2′-phenylselenone **66** and **83** (Schemes 10, 11, and 13) combining the strong electron-withdrawing effect and the leaving group character of the selenonyl group. Addition of nucleophiles, such as anions of nitromethane ($R_1 = NO_2$, $R_2 = H$), dimethylmalonate ($R_1 = R_2 = CO_2Me$), isobutylcyanoacetate ($R_1 = CN$, $R_2 = CO_2iBu$), bis(phenylsulfonyl)methane ($R_1 = R_2 = SO_2Ph$), and bis(diethylphosphonate)methane ($R_1 = R_2 = PO(OEt)_2$), to the Michael acceptor **66**, for example, occurred by the less hindered α face to afford, after subsequent protonation by the same face, the corresponding adduct **A.** This underwent a neighboring S_N2-type nucleophilic ring closure reaction at C3′, helped by the presence of excess base, to generate a second anion **B**, as illustrated in Scheme 9.[23]

In order to illustrate the synthetic potential of this methodology, the preparation of nitrocyclopropane derivative **73** is presented in Scheme 10. The 3′-eneselenone uridine **66** was prepared from the 2′−3′-*O*-anhydro uridine **67**, which is available in five steps[24] starting from uridine. Ring opening of the epoxide **67** with phenylselenide anion led to the formation of the two isomers **68** and **69** in 26% and 55% yields, respectively, after separation by silica gel chromatography. The major isomer **69** was converted to the corresponding mesylate **70**, which, upon treatment with *t*BuOK followed by oxidation with MCPBA, yielded the key vinyl selenone uridine **66** in 68% overall yield. This latter intermediate was treated with nitromethane in the presence of excess *t*BuOK to give the nitrocyclopropane derivative **72** as a single isomer in 65% yield. Classical deprotection of the primary hydroxyl group of **72** furnished the target nucleoside **73** in 94% yield.

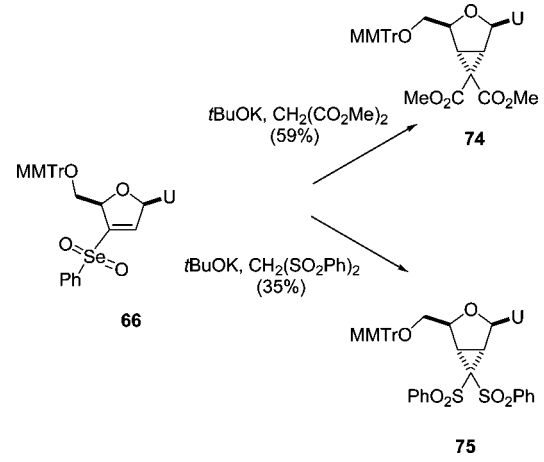
Scheme 9. Formation of the [3.1.0]-Bicyclic Cyclopropano Analogues C of 2′,3′-Dideoxyuridine from the Michael Acceptor 66



Scheme 10



Scheme 11

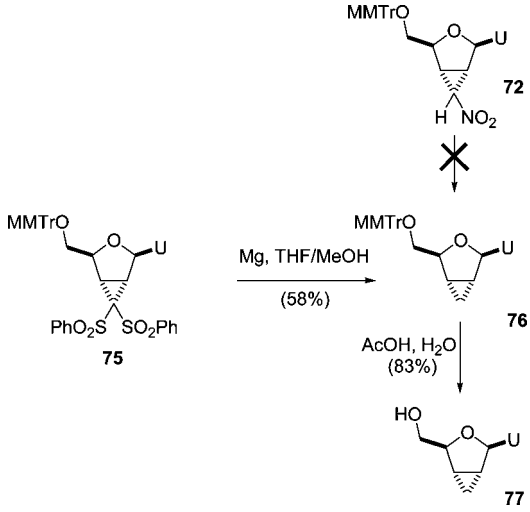


As shown in Scheme 11, the cyclopropane derivatives **74** and **75** were obtained from **66** using the same experimental conditions with the correct nucleophiles with two acidic protons.

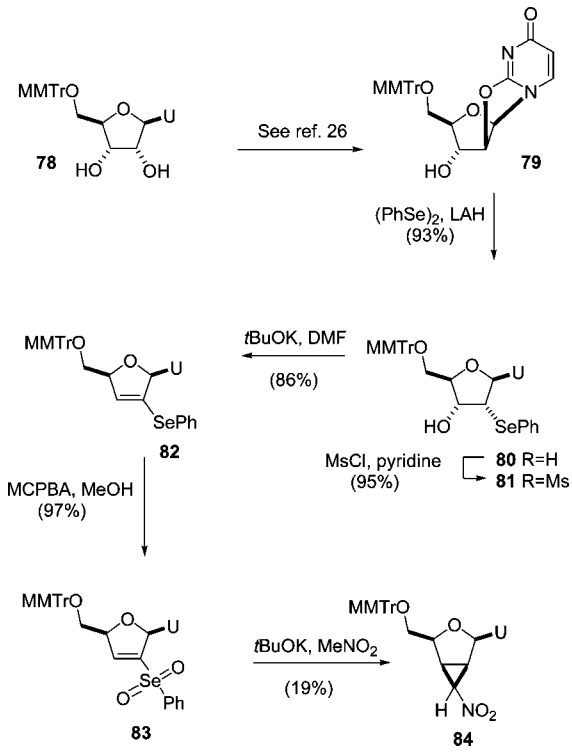
Furthermore, conversion of the derivatives **72** and **75** to the 2′−3′-α-methylene uridine **76** proved to be a challenging task. Reductive desulfonation of **75** afforded the desired compound **76** in 58% yield (Scheme 12). However, the nitro derivative **72** was inert to a radical-promoted reductive cleavage reaction. Hydrolysis of the protecting group of **76** furnished the cyclopropane derivative of 2′,3′-dideoxyuridine **77** in good yield.

To complete this work, the same authors described a stereoselective preparation of 2′-eneselenone isomer **83** from protected uridine **78** (Scheme 13).[25] Starting from 5′-*O*-protected uridine **78**, the 2,2′-*O*-anhydro uridine **79** was prepared in 85% yield following the two-step procedure of Ruyle,[26] and treatment with the phenylselenide anion provided the corresponding phenylseleno derivative **80**, which was engaged in the same set of transformations as described for isomer **69** (Scheme 10) to furnish the 2′-eneselenone isomer **83** in 74% overall yield. The reaction of several nucleophiles containing two acidic protons with the 2′-eneselenone **83** was examined under similar conditions to those previously described for the C3′ isomer **66**. While all

Scheme 12



Scheme 13

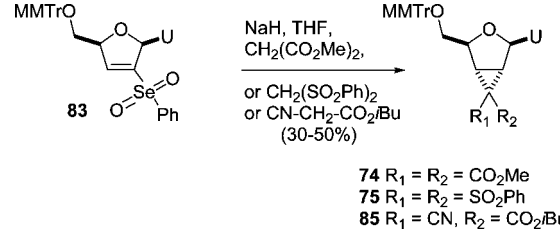


of the nucleophiles afforded the substituted cyclopropane-β-D-*ribo*-furanosyl skeleton as expected, nitromethane surprisingly gave only the nitrocyclopropane-β-D-*lyxo*-furanosyl derivative **84** in 19% yield. No argumentation was proposed by the authors concerning the diastereoselectivity of this reaction with nitromethane. The NMR data are in agreement with the proposed structures **72** and **84**, in particular the coupling constant $H_{1'}-H_{2'}$ ($J_{1'-2'} = 0$ in **72** and $J_{1'-2'} = 2.7$ Hz in **84**). It should also be noted that the absolute configuration of the carbon bearing the nitro group was later assigned by different authors (*vide infra*).

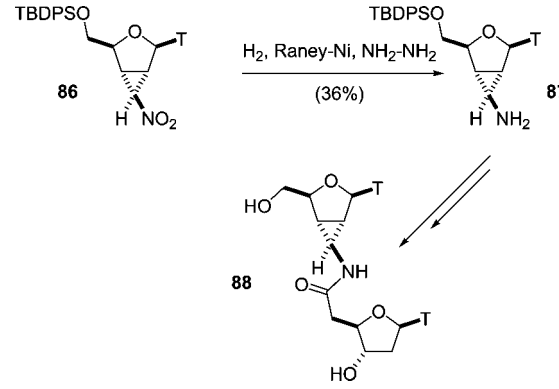
As illustrated in Scheme 14, addition of anions of dimethylmalonate or bis(phenylsulfonyl)methane to **83** led to the same adducts **74** and **75** as were prepared from the 3′-phenylselenonyl uridine **66** (Scheme 11) and the adduct **85** when using isobutylcyanoacetate.

However, this methodology has found an application in the field of antisense. The synthesis of nitrocyclopropane

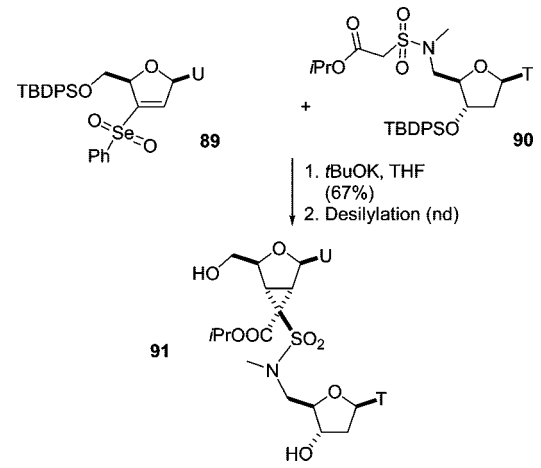
Scheme 14



Scheme 15



Scheme 16



derivative **72** was successfully reproduced by Sanghvi et al.[27] to prepare the nitrocyclopropane thymidine derivative **86**, which was reduced to the corresponding amine **87**, as outlined in Scheme 15. This key building block **87** was used for the synthesis of a new conformationally constrained thymidine−thymidine dimer **88** with a cyclopropyl amide backbone. By two-dimensional NOESY analysis, a strong correlation between the C6 proton of the thymine base and the cyclopropyl proton α to the nitrogen was observed, thereby confirming the stereochemistry assigned to the dimer **88**.

Just et al.[28] used a similar strategy with a nucleophile bearing the lower nucleoside of the dimer **91** to introduce, in an efficient manner, a rigid backbone containing cyclopropyl and a sulfonamide function as depicted in Scheme 16.

The same authors also extended the Chattopadhyaya strategy to the preparation of the dimers **92** and **93** with the cyclopropyl amide backbone isostere of **88** as presented in Figure 4.

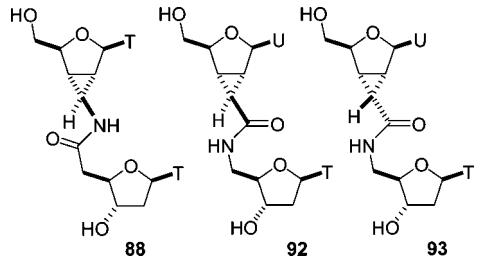


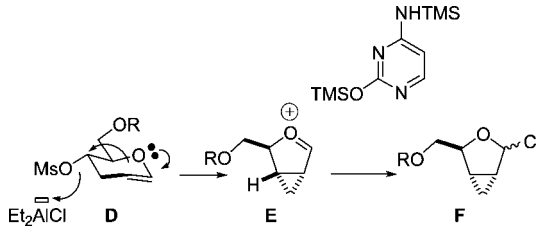
Figure 4. Dimers **92** and **93** with the cyclopropyl amide backbone isostere of **88**.

2.4. Formation via Intramolecular Rearrangement of D-Glucal

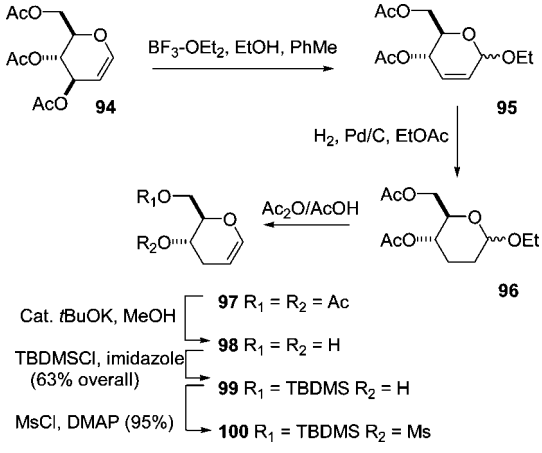
In the early work in this field, Okabe and Sun[29] disclosed an elegant route to [3.1.0] bicyclic nucleosides from the mesylate **100** (Scheme 18). This challenging strategy[30] was based on the activation of the leaving group of the D-glucal derivative **D** by a Lewis acid in order to generate, by a diastereoselective S_N2-like intramolecular displacement, an oxonium intermediate **E**, which was then trapped by the silylated nucleobase to provide the target nucleoside analogue **F** as outlined in Scheme 17.

The key compound **100** was prepared in a six-step sequence starting from commercially available tri-*O*-acetyl-D-glucal (**94**) in 60% overall yield as shown in Scheme 18. The mediated Lewis acid Ferrier rearrangement was carried out on **94** to afford the unsaturated intermediate **95**, which was hydrogenated and finally refluxed in an Ac_2O/AcOH mixture to provide the pure deoxyglucal **97**. After a classical deprotection of the hydroxyl group leading to **98** and selective protection of the primary hydroxyl group, the secondary alcohol of **99** was activated as mesylate to give the desired compound **100**.

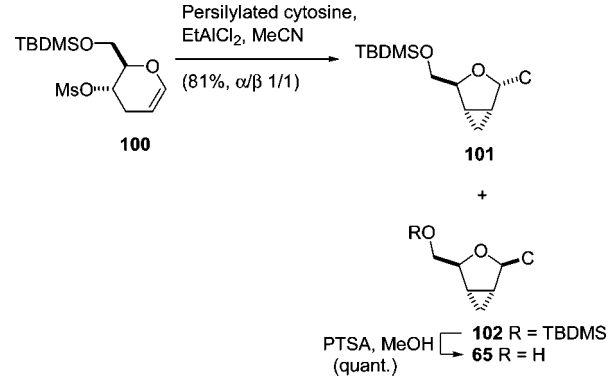
Scheme 17. Formation of the Nucleoside F via Oxonium Intermediate E



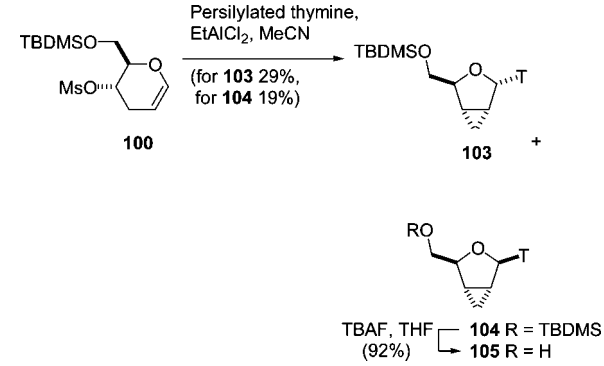
Scheme 18



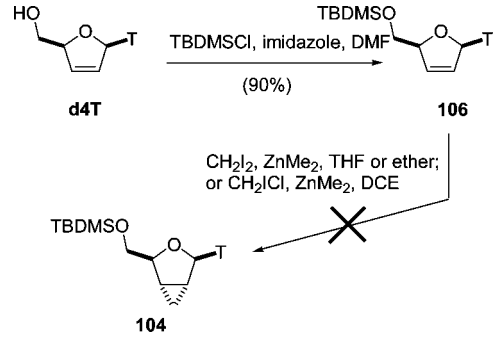
Scheme 19



Scheme 20



Scheme 21



At this point, the latter compound **100** was treated with silylated cytosine in refluxing MeCN in the presence of two equivalents of Lewis acid to afford, after 48 h, an equimolar mixture of the two α- and *β*-anomers, **101** and **102**, in 81% yield after purification (Scheme 19). The *β*-anomer **102** was isolated by recrystallization; then cleavage of the silylether by treatment with PTSA in MeOH led to the cyclopropano derivative of ddC **65** in quantitative yield.

A few years later, Sard[31] reported the synthesis of the thymidine analogue **105** using the same strategy as depicted in Scheme 20. However, it is important to note that with the silylated thymine, the reaction was completed in one hour and the corresponding α- and *β*-anomers **103** and **104** were isolated in 29% and 19% yields, respectively after chromatographic separation. Removal of the silyl protecting group of **104** provided the bicyclic nucleoside **105** in good yield.

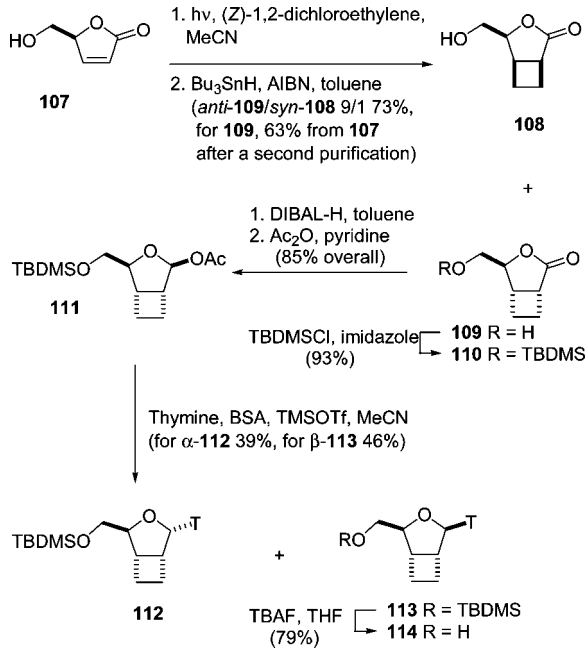
To complete this section, attempts at the cyclopropanation reaction on protected d4T **106** according to Furukawa's procedure failed as shown in Scheme 21.

3. Synthesis of Bicyclonucleosides Having a Cyclobutane Core

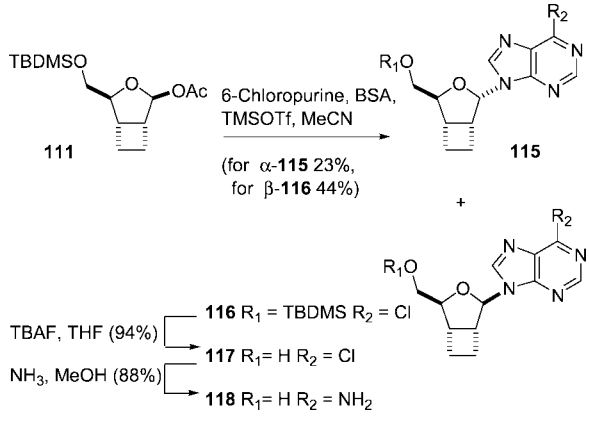
3.1. Formation via Photochemical [2 + 2] Cycloaddition

In 2006, Alibés et al.[32] published the first synthesis of 3-oxabicyclo[3.2.0]heptane nucleosides **114** and **118** as conformational analogues of d4T and d4A (Schemes 22 and 23). The required bicyclic furane derivative **109** was prepared from the commercially available (*S*)-5-hydroxymethyl-2(5*H*)-furanone (**107**) using a photochemical [2 + 2] cycloaddition reaction.[33] Direct attempts to treat **107** with ethylene under light irradiation using a high pressure 125 W mercury lamp afforded the corresponding cycloadducts **108** and **109** in modest diastereoselectivity and yield (on 1 mmol scale, *anti*-**109**/*syn*-**108** 66:44 ratio, 66% yield). On the other hand, a better outcome was obtained when using (*Z*)-1,2-dichloroethene instead of ethylene, followed by free radical dihydrodehalogenation of the crude mixture of dichlorocyclobutane derivatives to give the corresponding cyclobutanes in 73% overall yield for the two steps and in an *anti*-**109**/*syn*-**108** 9:1 ratio. The desired *anti*-intermediate **109** was isolated in pure form in 63% yield after a second purification by column chromatography. Next, **109** was converted into the

Scheme 22



Scheme 23



β-anomeric acetate **111** in 79% yield over three steps, involving protection of the primary alcohol as the silylether, reduction of the lactone with DIBAL-H, and acetylation of the resulting lactol. Under modified Vorbrüggen conditions, the condensation of the β-anomeric acetate **111** with the *in situ* persilylated thymine yielded an anomeric mixture, which was purified by column chromatography to give the α- and β-anomers **112** and **113** in 39% and 46% yields, respectively. Cleavage of the silylether in **113** furnished the target molecule **114** in good yield.

In the context of coupling procedures that involve the use of purine bases (e.g., *N*6-benzoyladenine), the regioselectivity of N9 versus N7 of this reaction is a key feature. However, from **111** the use of 6-chloropurine resulted in the exclusive formation of the desired N9-nucleoside in 67% yield as a mixture of α-**115** and β-**116** anomers in 35:65 ratio (Scheme 23). Both anomers **115** and **116** were obtained pure, after separation by column chromatography on silica gel, in 23% and 44% yields, respectively. Finally, on the β-anomer **116**, removal of the silylether afforded **117** and aminolysis provided the target molecule **118** in 83% overall yield for the two steps.

4. Synthesis of Bicyclonucleosides Having a Cyclopentane Core

4.1. Formation via a Ring Closing Metathesis Reaction

In the past decade, the metathesis reaction has been established as one of the most powerful organometallic transformations for carbon−carbon bond formation.[34,35] Robust, functionality-tolerant, highly reactive, and commercially available ruthenium-based catalysts (**I** and **II** in Figure 5) have been developed, thus extending the scope of this transformation to a wide range of alkene substrates including nucleosides.[36]

In this context, the RCM reaction is a straightforward and powerful methodology, which has stimulated the development of a variety of synthetic routes leading to bicyclic nucleosides. Also, the required alkenes have been prepared by well-established chemistry starting from nucleosides or sugar precursors.

In a series of nice papers, Nielsen et al. described the preparation of various bicyclic nucleosides, as presented below.

The synthesis of their first bicyclic nucleosides, **129**, **130**, and **131**, is reported in Schemes 24 and 25. Aldehyde **120**, prepared from diacetone-D-glucose **119** using known chemistry,[37] was reacted with vinyl magnesium bromide to afford, in a 1:4 ratio, the epimeric allylic alcohols **121** and **122** in 19% and 69% yields, respectively, after separation by silica gel chromatography.[38] Although the diastereoselectivity was

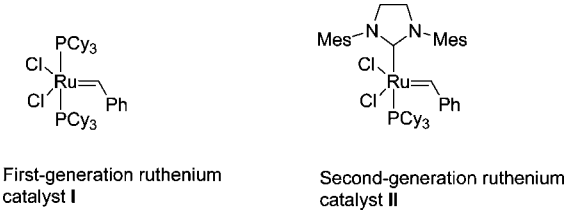
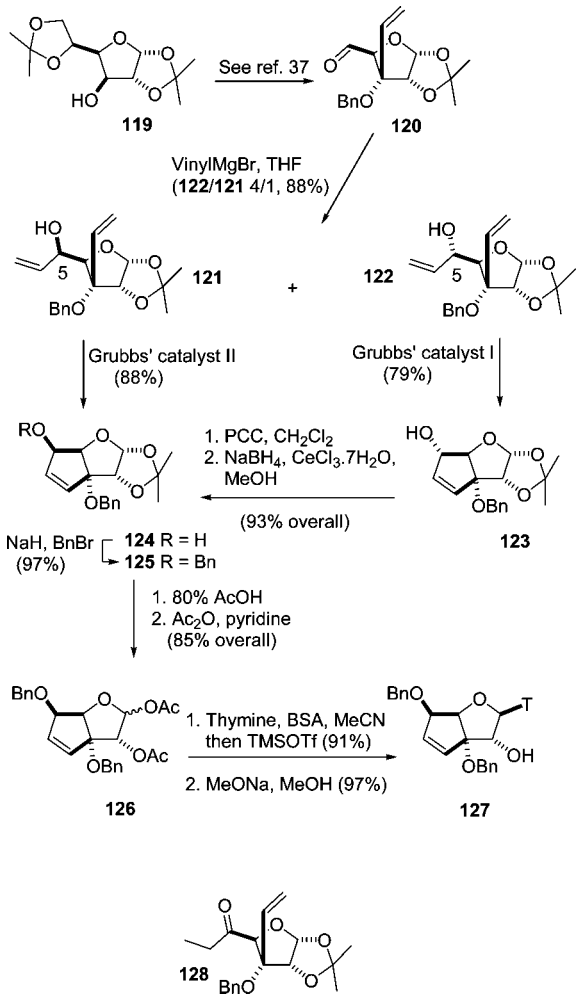
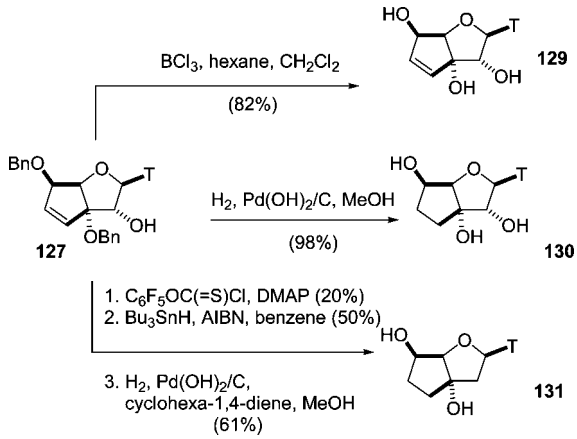


Figure 5. Ruthenium-based catalysts developed by Grubbs and used in this part.

Scheme 24

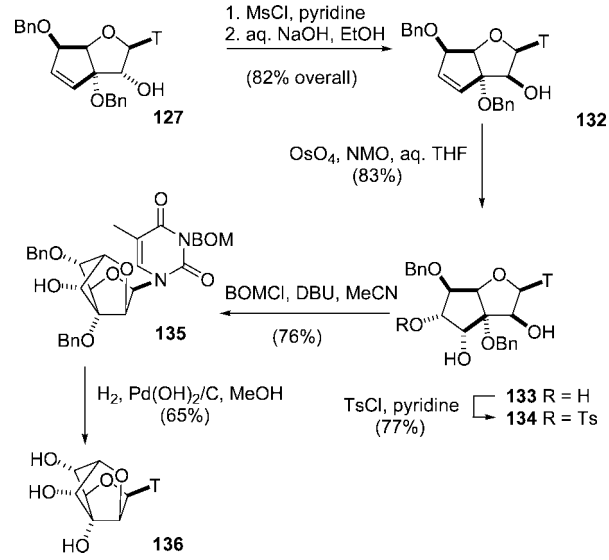


Scheme 25



not satisfactory and the undesired isomer **122** was obtained as a major product, this compound could be efficiently converted later into the required allylic alcohol **124** by inversion of the configuration at C5. It should be noted that the desired epimeric alcohol at C5 was preferentially obtained in a 1:3 ratio but in lower yield (57%) using **120** with a free tertiary alcohol. At this point, both allylic alcohols **121** and **122** were subjected to RCM reaction. For substrate **122**, twice the amount of 10 mol % Grubbs' catalyst **I** was necessary to promote the complete ring closure. Furthermore, for diene **121** in the same conditions, the desired product **124** was isolated in low yield accompanied by the saturated ketone

Scheme 26

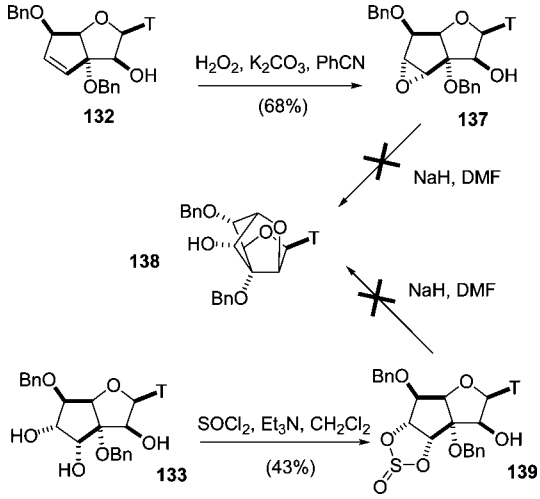


128 formed by a known ruthenium-catalyzed isomerization[39] of the allylic alcohol. Nevertheless, this problem was overcome by using Grubbs' catalyst **II** to furnish the desired nucleoside **124** in 88% yield. To afford the right C5 configuration, compound **123** was oxidized with PCC as its corresponding enone, which was subsequently reduced following Luche's procedure to yield the desired pure epimeric alcohol **124** in 93% yield: hydride was delivered by the convex α-face of the molecule with excellent diastereoselectivity. After benzylation of **124** to provide **125**, successive hydrolysis of the acetonide group and acetylation afforded the key bicyclic intermediate **126** as an anomeric mixture in good overall yield. This latter mixture was successfully subjected to a Vorbrüggen-type coupling with thymine leading to the corresponding β-nucleoside **127** in 88% yield as the sole product after deacetylation. It is clear that the acetate group on C2′ participated in neighboring group stabilization through the acetyloxonium ion intermediate to favor the formation of the desired β-nucleoside **127**.

To transform the key product **127** into the final target molecules, the BCl_3-mediated cleavage of the benzyl ethers gave the unsaturated analogue **129** (Scheme 25). On the other hand, hydrogenation of the double bond of **127** and cleavage of the benzyl ether smoothly proceeded in the presence of Pearlman's catalyst to afford **130** in 98% yield. Finally, 2′-deoxygenation of **127** was performed using the Barton−McCombie reaction. The reaction of **127** with pentafluorophenylthiochloroformate gave the thionocarbonate ester intermediate in low yield, which was then submitted to standard radical-mediated deoxygenation using Bu_3SnH and AIBN as a radical initiator to afford, after debenzylation, the target molecule **131** in 6% overall yield for the three steps.

To complete this previous work, the same group[40] published the synthesis of tricyclic nucleoside **136** from **127** as presented in Scheme 26. Conversion of the nucleoside **127** into the C2′-epimerized D-*arabino*-furanosyl intermediate **132** was efficiently accomplished via the 2,2′-*O*-anhydro nucleoside formation by subsequent mesylation followed by NaOH treatment. Next, the dihydroxylation of **132** afforded **133** in a totally diastereoselective manner due to the approach of OsO_4 from the sterically less-hindered convex α-face of the bicyclic system. Then, treatment of the diol **133** with a large excess (7 equiv) of TsCl furnished the desired monotosylated compound **134** as the sole product in 77% yield. Initially,

Scheme 27



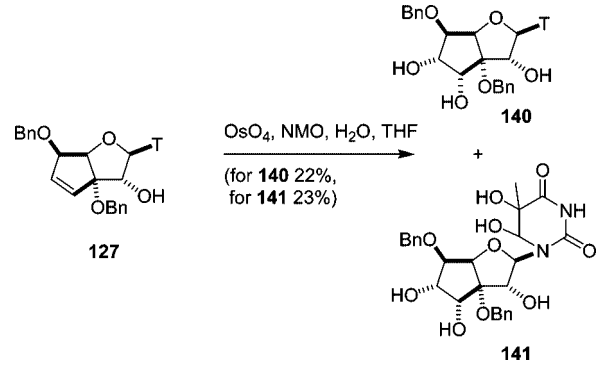
the cyclization step was attempted by treatment of **134** with NaH, but in these conditions only the 2,6′-*O*-anhydro compound was detected in the crude mixture. In order to avoid this side reaction, the N3 protection of the base with a BOM group was planned. Surprisingly, according to classical procedure, compound **134** was treated with BOMCl in the presence of excess DBU to afford the protected tricyclic product **135** as the sole product in 76% yield. Taken together, these results clearly suggested that the substrate **134** was first protected as BOM-ether *in situ*, which facilitated the cyclization step. Finally, debenzylation of **135** took place smoothly to give the fully deprotected tricyclic nucleoside **136** in 65% yield.

Other approaches to the tricyclic target nucleoside **138** from intermediates **132** and **133** were attempted without success as depicted in Scheme 27.[40] Diastereoselective epoxidation of **132** using Payne's protocol yielded epoxide **137** in 68% yield. It is worth noting that epoxidation with MCPBA was ineffective on this substrate. Unfortunately, treatment of **137** under standard NaH conditions provided only a slow decomposition of the starting material. On the other hand, cyclic sulfite **139** prepared from diol **133** was submitted to basic treatment, but no cyclized product was detected.

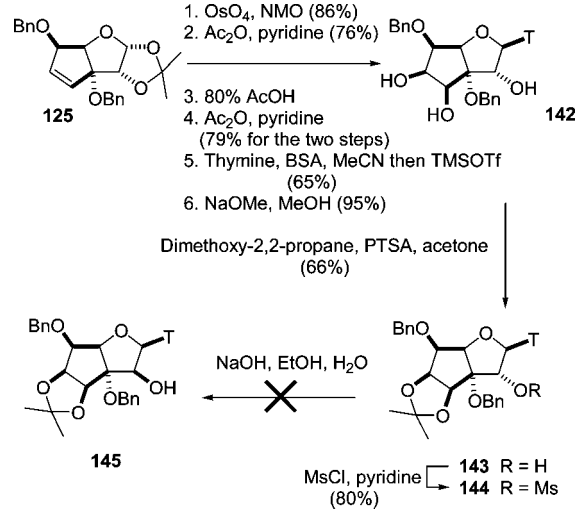
Nielsen et al.[41] used the previous intermediate **127** to prepare other polyhydroxylated bicyclic nucleosides. In order to synthesize the compound **140**, the C2′ epimer of **133**, the nucleoside **127** was dihydroxylated as previously described for **132** (Scheme 26). In contrast to this result, in the same conditions (50 °C, 5 days) the desired adduct **140** was isolated in 22% yield accompanied by the bis-dihydroxylated product **141** in 23% yield as outlined in Scheme 28. No better results were obtained when the reaction was performed at room temperature over 9 days: **140** and **141** were isolated in 33% and 22% yields, respectively, as well as 10% of starting material **127**.

An alternative route to **140** was attempted, relying on a hydroxylation step prior to the introduction of the thymine as depicted in Scheme 29. The bicyclic nucleoside **142** was prepared in 25% overall yield from compound **125** (Scheme 24) in six steps including an efficient diastereoselective dihydroxylation and a β-stereoselective Vorbrüggen condensation. Attempts to invert the hydroxyl group at C2′ of intermediate **143**, using similar conditions as applied to **127** (Scheme 26), failed: treatment of the mesylate **144** under

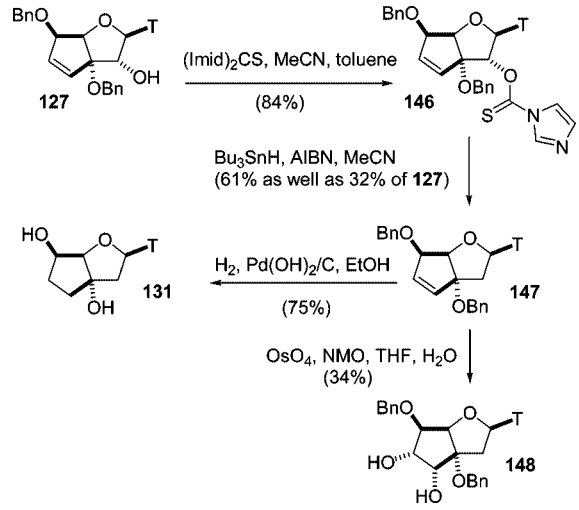
Scheme 28



Scheme 29



Scheme 30



basic conditions provided a complex mixture in which **143** was the only identified compound. No attention was paid to removing the protecting groups of **143** to afford the fully deprotected molecule. It should be pointed out that the diastereoselective dihydroxylation of **125** occurred by the concave β face; the presence of the isopropylidene and benzyl protecting groups on the α face justified the outcome of this process.

Starting from intermediate **127**, preparation of other analogues was attempted as outlined in Scheme 30.[41] The previously described analogue **131** (Scheme 25) was obtained

using this time a radical deoxygenation via the thiocarbonylimidazole **146**, which was subjected to Bu_3SnH in refluxing MeCN to afford **147**. In this latter step, the desired adduct **147** was isolated in 61% yield, as well as 32% of the alcohol **127**, which could be formed via the hemiacetal opening, a route for reversion to the starting material.[42] Catalytic hydrogenation of **147** afforded the nucleoside **131** (compared with the sequence mentioned in Scheme 25, the overall yield was increased from 6% to 38%). Further dihydroxylation of the double bond of **147** afforded diastereoselectively the diol **148** in 34% yield along with 17% of unreacted starting material. Debenzylation of **148** was not attempted.

4.2. Formation via a Radical Cyclization Reaction

Using an efficient radical cyclization reaction, the group of Chattopadhyaya has developed an elegant approach to provide an original and straightforward route to *cis*-fused bicyclic five- and six-membered 2′,4′-carbocyclic thymidine analogues, as discussed here and in section 5.3. The first series of these conformationally restricted nucleosides should be regarded as carba-analogues of 2′-*O*-,4′-*C*-methylene-bridged nucleic acids (named locked nucleic acids, or LNA,[43] now commercially available), while the second are carba-analogues of ENA[44] (2′-*O*-,4′-*C*-ethylene-bridged nucleic acids), see Figure 6.

It is worth pointing out that LNA are conformationally restricted RNA mimics (*N*-type sugar pucker that is present exclusively in A-form duplexes). LNA display a markedly increased duplex stability with complementary RNA and a greater nuclease resistance compared with native DNA. Only modified oligonucleotides with a central core of DNA flanked by LNA at both ends (so-called LNA−DNA gapmers) are capable of exhibiting significant RNase H activation.[43]

In this field, the first work from Chattopadhyaya et al.[45] is presented in Scheme 31. Starting from 4′-*C*-hydroxyethyl pentofuranose **150**[46] (prepared from the known sugar precursor **149**[47]), the corresponding allyl derivative **151** was obtained in 70% overall yield using a classical Swern oxidation/Wittig olefination sequence. From this latter intermediate **151**, acetolysis and then acetylation followed by N-glycosidation with *in situ* silylated thymine in the presence of TMSOTf led to the isolation of the β-anomer **152** in 80% yield for the two steps. Next, the acetate group of **152** was removed using methanolic ammonia, and the resulting liberated alcohol **153** was converted into the corresponding phenyl thionocarbonate derivative **154** in 72% overall yield. In order to prevent formation of uncyclized reduced product, the key intermediate **154** was treated in refluxing toluene in the presence of AIBN under highly diluted conditions by slow addition of Bu_3SnH to afford, via a 5-hexenyl type *exo* mode cyclization, the 2′,4′-*cis*-fused bicyclic nucleoside **155** in 73% yield as an inseparable mixture of diastereoisomers at C7′ in 7:3 ratio in favor of the 7′-*R* stereoisomer. Finally, cleavage of the benzyl protecting groups of **155** was carried out using Pearlman's catalyst, with ammonium formate as hydrogen-transfer agent, in MeOH to afford the desired nucleoside analogue **156** as a mixture of diastereoisomers.

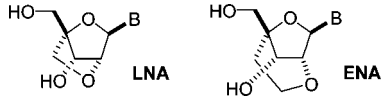
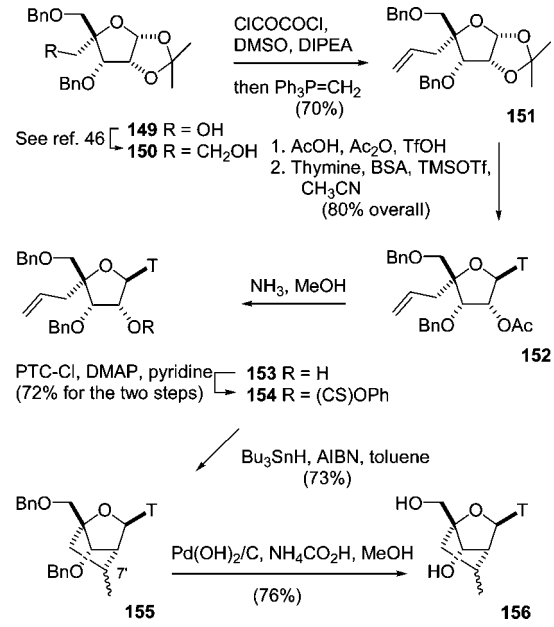


Figure 6. Structure of LNA and ENA.

Scheme 31



It should be noted that removal of the 3′- and 5′-*O*-benzyl ether is always performed using the previous conditions, with ammonium formate or cyclohexene as hydrogen-transfer agents, and this step concluded the synthesis of all the analogues presented in this section. In addition, all the modified nucleosides were incorporated into oligonucleotides after protection of the primary hydroxyl as 4,4′-dimethoxytrityl ether and activation on the secondary hydroxyl as phosphoroamidites.

This elegant strategy using a free-radical cyclization as a key step has been successfully extended by Chattopadhyaya et al.[48] using close chemistry to gain access to the various carba-LNA analogues **157**−**163** with C6′-OH, C6′-Me, or both as summarized in Figure 7. It is worth mentioning that, for incorporation into DNA sequences, the secondary alcohol function at C6′ was protected as its *p*-toluoyl derivative, while for the tertiary one no protection was necessary due to its steric hindrance and poor reactivity.

From the previous 4′-*C*-hydroxymethyl pentofuranose derivative **149**, Swern oxidation followed by a highly diastereoselective reaction with the vinyl Grignard reagent afforded the pivotal allylic alcohol **164** (Scheme 32). By

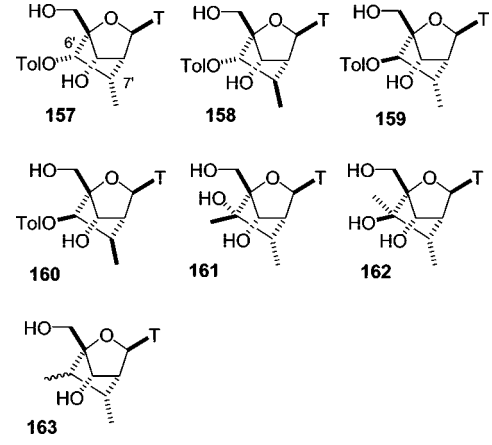
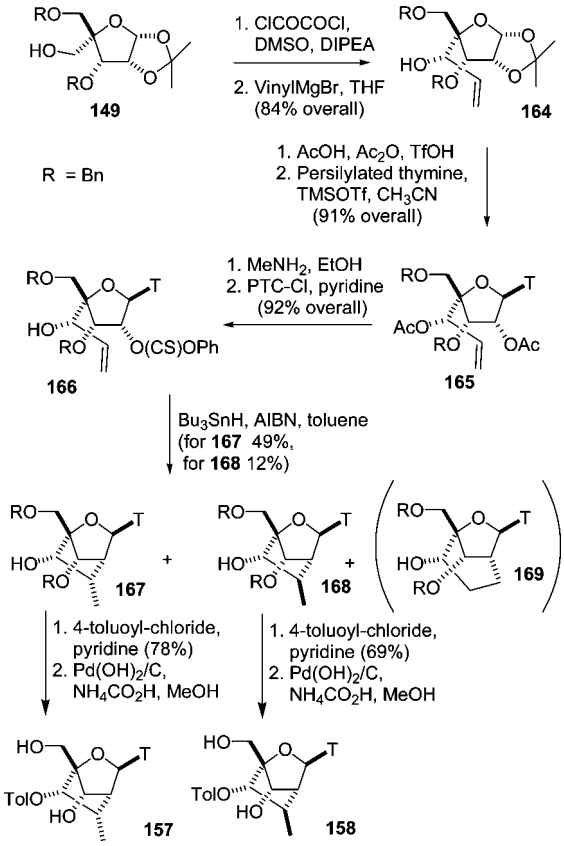


Figure 7. Carba-LNA analogues **157**−**163** with C6′-OH, C6′-Me, or both.

Scheme 32

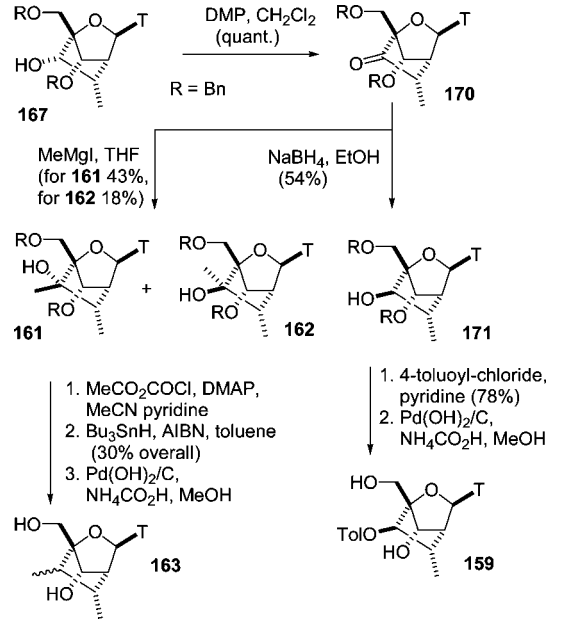


employing the same convenient route as that shown in Scheme 31, the allylic alcohol **164** was converted into the radical precursor **166** in four steps (50% overall yield). The 5-hexenyl radical cyclization on **166** was first reported to proceed exclusively by following a 5-*exo* pathway to give the bicyclonucleoside (6′-*S*,7′-*S*)-**167** as a major diastereoisomer, as well as the other (6′-*S*, 7′-*R*)-**168** diastereoisomer, in 49% and 12% isolated yields, respectively. However, at a later date, the same authors[49] reported under the previous conditions the formation of the 6-*endo* cyclized compound **169**, which was isolated together with **168** in 12% yield as an inseparable mixture in an 11:9 ratio, respectively. In contrast to the previous result with **154** (Scheme 31), the introduction of an alcohol function at C6′ in **166** has a significant impact on the stereoselectivity of the 5-*exo* cyclization pathway: the 7′-*S* stereoisomer was isolated as the major cyclized compound. Moreover, the hydroxy group seemed to enhance the 6-*endo* hexenyl cyclization mode leading to the formation of **169**.

The two other possible diastereoisomers of compounds **157** and **158** were then prepared by an oxidation/reduction sequence, as presented in Scheme 33 (only the sequence on diastereoisomer **167** is shown for the sake of clarity). The ketone intermediate **170** was used as the precursor of the analogues **161** and **162** with the tertiary alcohol function at C6′. It should be noted that the dimethyl-6′,7′-bicyclonucleoside **163**, prepared by Barton−McCombie deoxygenation of **161** via the methyl oxalate, was incorporated into DNA sequences as a mixture of diastereoisomers at C6′.

In this work, a very interesting development occurred when the terminal double bond in the side-chain of **154** (Scheme 31) was replaced by an *O*-benzyl oxime ether as an efficient radical trap. This brilliant strategy offered Chattopadhyaya et al.[50] a nice way to functionalize the C7′ leading to the

Scheme 33



preparation of the corresponding amino and aminoalcohol analogues **172**−**175**, as well as the nonsubstituted parent carba-LNA **176** (see Figure 8). Concerning these analogues, prior to dimethoxytritylation and phosphorylation, the amino function was protected as its trifluoroacetamide, and the secondary alcohol function was acetylated.

By analogy with the chemistry described above from the protected sugar **149**, the nitrile group precursor of the aldehyde function was introduced by a classical sequence to furnish **177** in 56% yield for the two steps (see Scheme 34). Then, acetolysis of **177** followed by glycosidation with thymine provided **178** in 75% overall yield. Next, the cyanonucleoside **178** was reacted with DIBAL-H to give, after an acidic aqueous workup, the corresponding aldehyde, which was subsequently treated with *O*-benzylhydroxylamine and then with PTC-Cl to release the key radical precursor **179** in 42% overall yield. The tin-mediated radical cyclization on the intermediate **179** proceeded exclusively in a 5-*exo* mode with a good level of diastereoselectivity to afford the C7′-*R* **180** and C7′-*S* **181** adducts in 60% and 4% isolated yields, respectively. The diastereoisomers **180** and **181** were subjected separately to cleavage of the N−O bond, protection of the liberated amino function as trifluoroacetamide, and finally hydrogenolysis of the benzyl ethers to afford **172** and **173**. It should be noted that the SmI_2-mediated reductive cleavage of the N−O bond failed, but this transformation

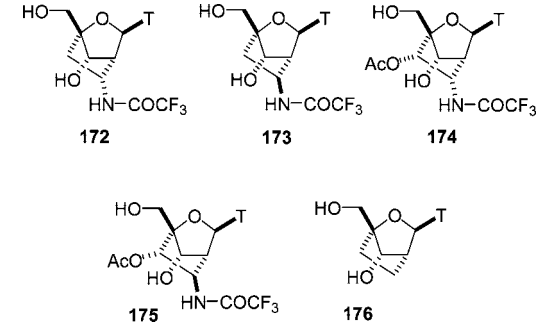
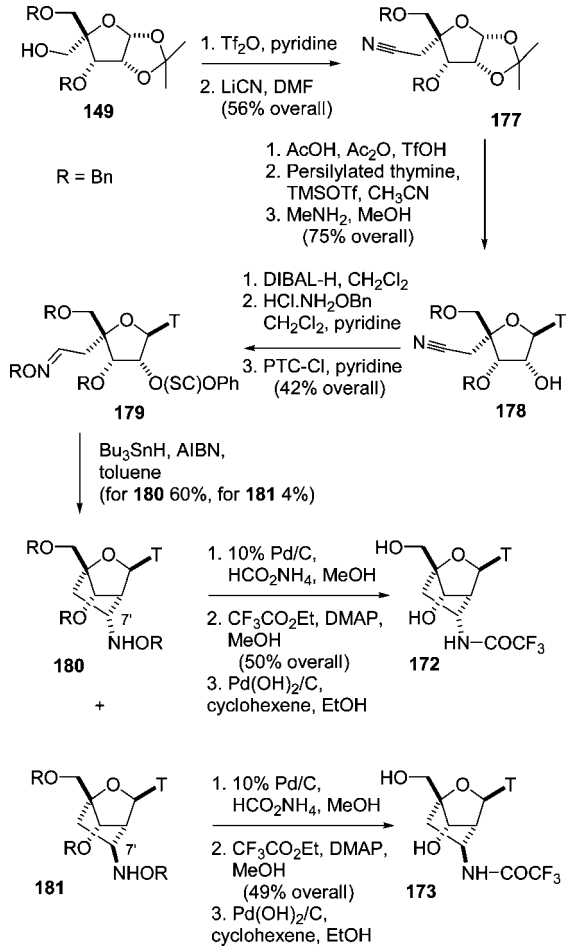
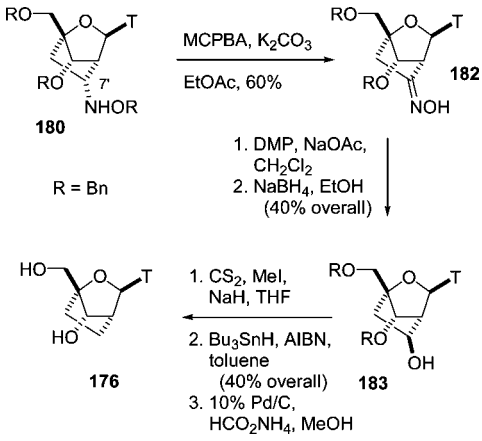


Figure 8. Carba-LNA amino and aminoalcohol analogues **172**−**175** and the nonsubstituted parent carba-LNA **176**.

Scheme 34



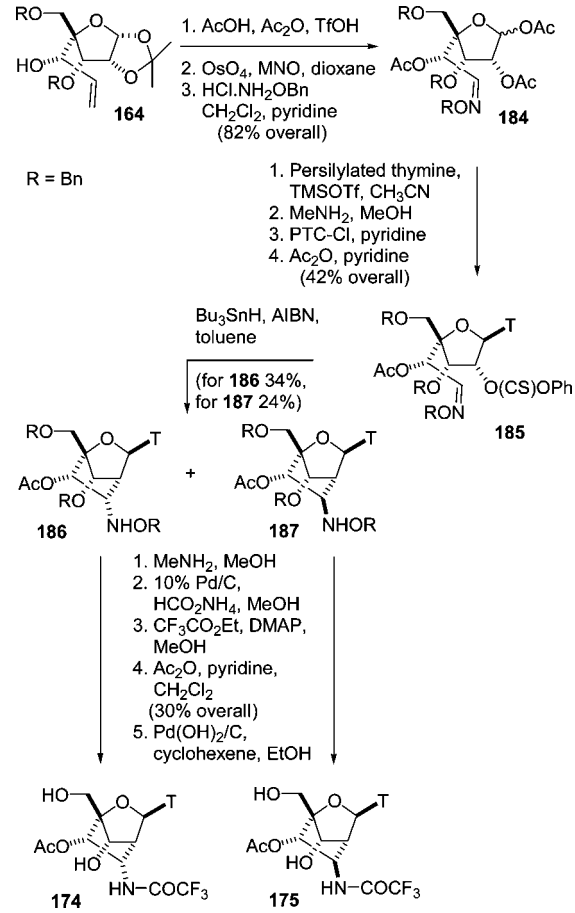
Scheme 35



was efficiently accomplished with Pd on C in the presence of a hydrogen donor, the use of Pearlman's catalyst leading to the unwanted removal of the benzyl ethers.

The nonsubstituted parent carba-LNA **176** was prepared as described in Scheme 35 from the intermediate **180**. Oxidation of the *O*-benzyl oxime ether **180** with MCPBA afforded the oxime **182**, which was treated with DMP reagent to regenerate the carbonyl group followed by reduction to furnish the alcohol **183** as the sole diastereoisomer in 24% yield for the three steps. Due to the steric hindrance of the secondary alcohol function of **183**, all attempts to introduce the phenyl thionocarbonate met with failure, so the deoxygenation was performed on the C7′-*O*-(methylthio)thiocarbonate, which was more accessible.

Scheme 36



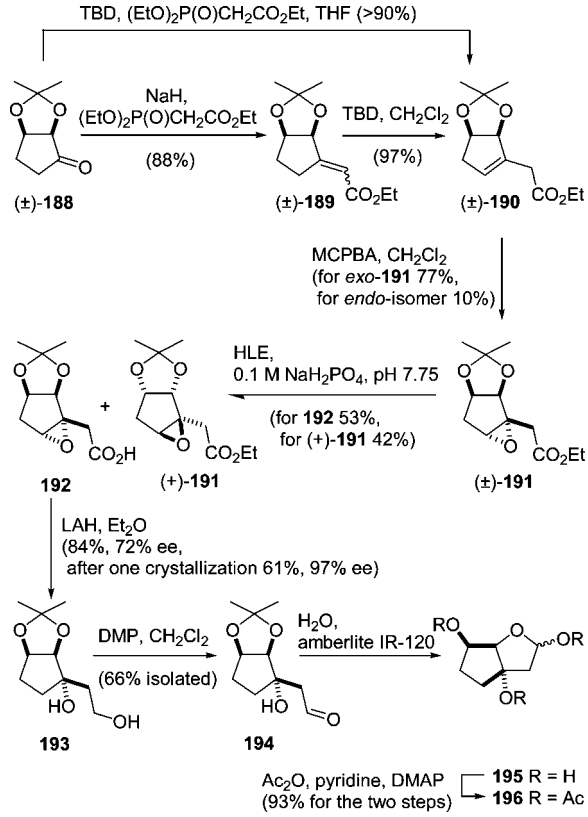
Finally, this work on radical cyclization of *O*-benzyl oxime ether was extended to the preparation of C6′-*S* hydroxyl derivatives, as outlined in Scheme 36, following the chemistry displayed in Scheme 34. It should be noted that, with *O*-benzyl oxime ethers, no products from an *endo* mode cyclization were isolated. Moreover, with the intermediate **185**, the radical cyclization afforded both potential stereoisomers **186** and **187** in close yields, 34% and 24%, respectively, after separation on a silica gel column. These two diastereoisomers **186** and **187** were subjected separately to protecting group manipulation to give the intermediates **174** and **175**, which were incorporated into DNA sequences.

From a common, readily accessible, carbohydrate-based building block **149**, available in a few steps from 1,2-*O*-isopropylidene-α-D-allofuranose,[51] the group of Chattopadhyaya prepared, through gram-scale sequences (e.g., in the glycosidation step, see Scheme 32, around 14 g of thymidine derivative **165** was isolated!), more than 12 carba-LNA analogues. The work presented in this section is an excellent illustration of what free-radical chemistry could contribute to the area of Medicinal Chemistry for C−C bond formation, especially with highly functionalized substrates.

4.3. Formation of Glycone Moiety via *de Novo* Synthesis

Leumann et al. reported the synthesis of bicyclonucleosides with an ethylene bridge between the C3′and C5′ and their

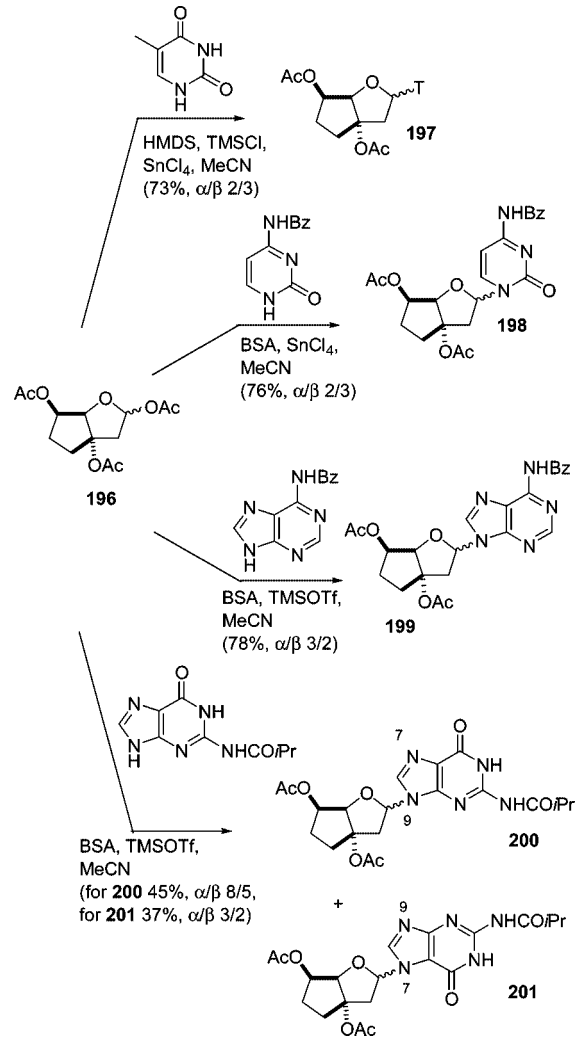
Scheme 37



hybridization properties in RNA and DNA strands, as well as their nuclease stability and their ability to induce RNase H activity.[52,9e] These chiral bicyclonucleosides were synthesized from the racemic ketone **188** (Scheme 37), and the four different bases were introduced by classical *N*-glycosylation as presented in this paragraph.

The synthesis of the first set of bicyclonucleosides **131** and **202**−**204** is described in Scheme 39.[53] The commercially available racemic ketone **188** was subjected to a Wittig−Horner reaction to give the corresponding α,*β*-unsaturated ester **189** as a mixture of *E*/*Z* isomers, which was treated with the strong organic base TBD to furnish the more stable deconjugated *β*,*γ*-unsaturated ester **190** (Scheme 37). On the other hand, this latter compound **190** could be obtained in a one-pot two-step process using 2 equiv of TBD instead of 1 equiv of NaH in the Wittig−Horner reaction. Epoxidation of the substituted cyclopentene **190** with MCPBA occurred by the convex side of the bicyclic structure to deliver, after chromatography separation, the required *exo*-epoxide **191** as the major isomer in 77% yield, as well as the *endo*-epoxide in 10% yield. At this stage, this racemic ethylester **191** was resolved by hog-liver esterase on multigram scale to afford the desired chiral acid **192** in 53% yield and in 72% ee.[54] Treatment of this crude product **192** with LAH resulted in the reduction of the carboxylic acid and the regioselective ring opening of the epoxide to afford, after purification on silica gel, the diol **193** in 84% yield. Subsequent recrystallization of this latter material afforded the enantiomerically pure (97% ee) diol **193** in 61% yield from **192**. The absolute configuration (1*S*,5*S*,6*R*) of (+)-**193** was determined by X-ray structural analysis of its (*S*)-camphanic acid derivative. Oxidation of the primary hydroxyl group of the diol **193** was best performed with DMP reagent to afford in 91% yield the unstable aldol **194**, which was used without purification in the next step. Exposure of the crude aldol **194** to strong

Scheme 38



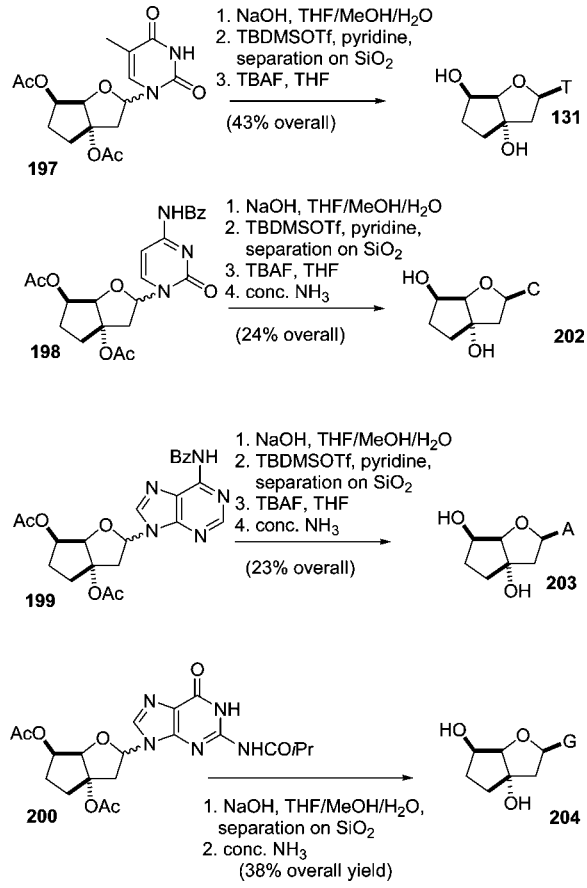
acidic ion-exchange resin in water resulted in sequential hydrolysis of the acetonide and then spontaneous intramolecular cyclization to afford the bicyclic carbohydrate framework **195** which was treated with Ac_2O to give the key glycosyl donor **196** in 93% yield as an anomeric mixture in a 1:1 ratio.

Having secured a route to the requisite chiral synthon **196**, the next task was the introduction of the four natural nucleobases (Scheme 38). Lewis acid-mediated condensation of **196** with the *in situ* persilylated nucleobases led to the corresponding nucleoside analogues **197**−**200** as α/*β*-anomeric mixtures in correct yields (yields and anomeric selectivities are summarized in Scheme 38).

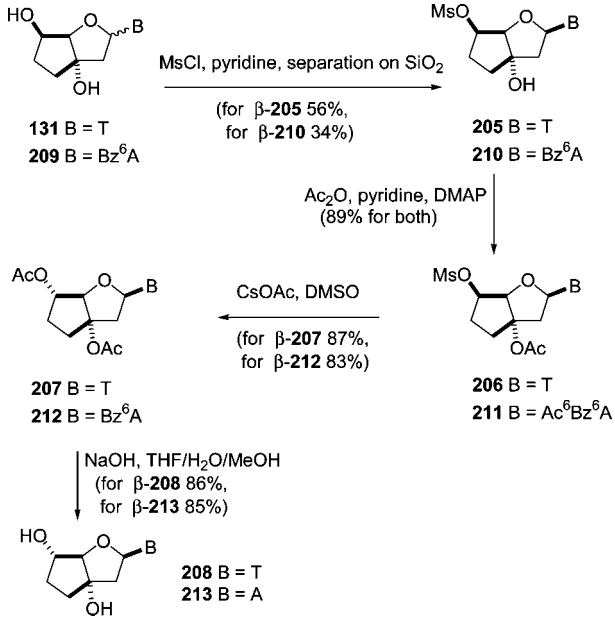
The *β*-anomerically pure nucleosides **131**, **202**, and **203** were obtained after deacetylation, silylation of the primary alcohol, and anomeric separation on silica gel column, followed by desilylation and finally treatment with ammonia for the adenosine and cytidine derivatives (overall yields are displayed in Scheme 39). From the anomeric mixture of **200**, the pure *β*-**204** was isolated after cleavage of the acetate and purification on silica gel column followed by treatment with concentrated ammonia.

Inspired by their first success, Leumann et al. focused their attention on the preparation of thymidine and adenosine C5′ epi-analogues **208** and **213** as outlined in Scheme 40.[55] In initial experiments, in order to invert the configuration of the alcohol function at C5′ of **131**, a Mitsunobu reaction was

Scheme 39

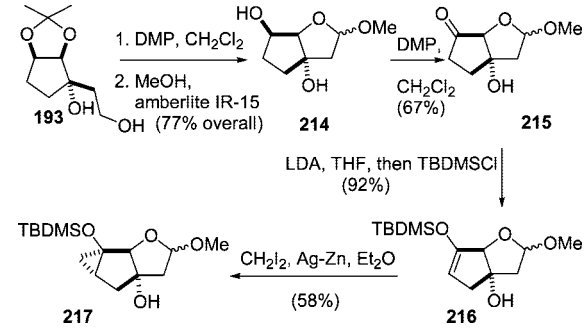


Scheme 40



attempted with various carboxylic acid partners without success. Therefore, more conventionally, selective activation of the secondary alcohol of **131** as a mesylate and isolation of pure β-anomer **205**, followed by acetylation of the tertiary alcohol to afford **206** and finally displacement of the leaving group with CsOAc yielded the corresponding diacetate **207** in 36% overall yield. Removal of the acetate groups gave the target bicyclic nucleoside **208**. The same sequence was applied to **209** to afford compound **213**. However, it is

Scheme 41



noteworthy that, during the acetylation step, the *N*6-acylated derivative was formed and its removal occurred in the last step of the sequence. Also, to complete this part, it should be mentioned that the protection of the tertiary alcohol function of **205** and **210** significantly increased the yield of the mesylate displacement with CsOAc (from 40% to 87% for **205**).

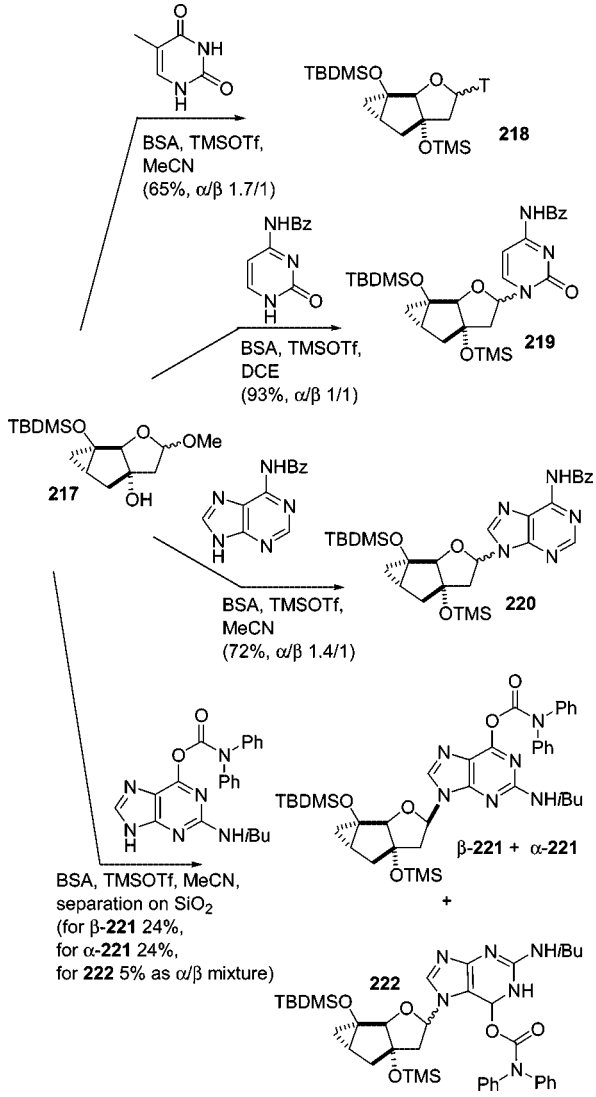
Leumann et al. also prepared tricyclodeoxynucleoside analogues **223**−**226** as shown in Scheme 43.[56] Oxidation of **193** with DMP reagent to the corresponding aldehyde followed by acid-catalyzed hydrolysis of the acetonide in MeOH gave the acetal **214** in 77% overall yield as an α/β-anomer mixture in 2:1 ratio (Scheme 41). Conversion of **214** to the silyl enol ether **216** was done in 60% overall yield by oxidation with DMP reagent and treatment of the resulting ketone **215** by LDA to form the kinetic enolate, which was then trapped by TBDMSCl. Improved Simmons−Smith cyclopropanation with Ag−Zn couple afforded, in a stereospecific manner, the adduct **217** in 58% overall yield as an α/β-anomer mixture in 2:1 ratio along with 23% of unreacted starting material. The stereoselectivity of the cyclopropanation could be rationalized by both the approach from the less hindered concave face of **216** and the directing effect of the alcohol function at C3.

At this stage, from the key glycosyl donor **217**, the four natural nucleobases were introduced under Vorbrüggen-type coupling with BSA as a silylating agent and TMSOTf as a Lewis acid (Scheme 42). In all these cases, TMSOTf was found to be more efficient than $SnCl_4$. Under these conditions, the corresponding nucleoside analogues **218**−**221** were isolated in 48−93% yields as α/β-anomeric mixtures in 1:1 to 1.7:1 ratios.

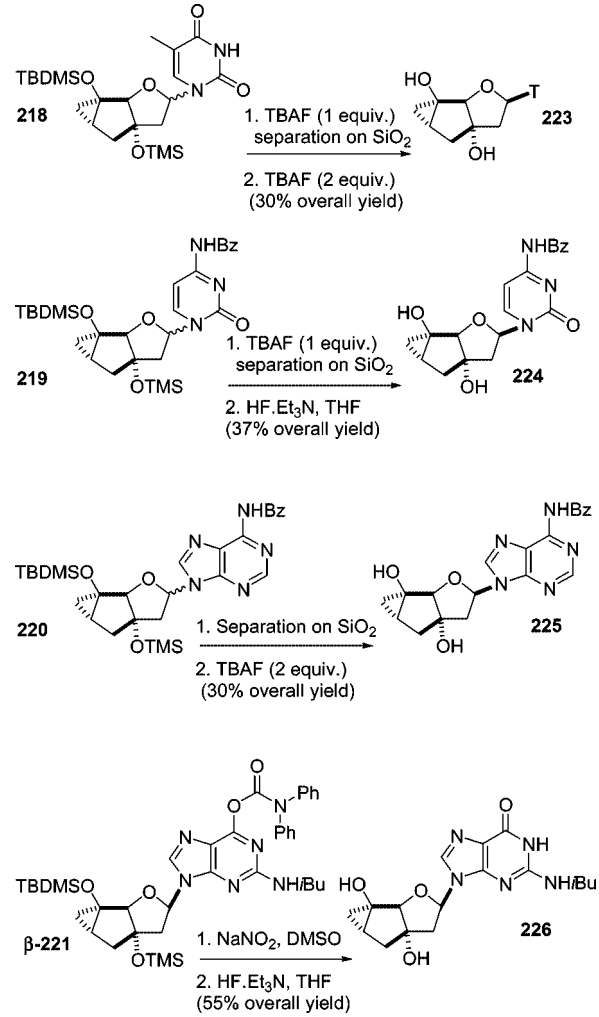
For the nucleosides **218**−**220**, the selective cleavage of the TMS group at C3′ using only one equivalent of TBAF, followed by separation on silica gel column and finally removal of the TBDMS group with an excess of TBAF, furnished the pure β-tricyclodeoxynucleoside analogues **223**−**225** (overall yields are displayed in Scheme 43). The β-nucleoside **226** was obtained from β-**221** by treatment with $NaNO_2$ to cleave the *O*6-diphenylcarbamoyl protecting group (the TMS group did not survive in these conditions) and finally desilylation.

In addition to the above-mentioned syntheses, Leumann et al. reported the preparation of the amino-functionalized bicyclo-thymidine **235** from the silyl enol ether **216** separately in both β- and α-anomer series as shown in Scheme 44.[57] Hydroboration of α-**216** gave, after separation by chromatography, the corresponding diastereomeric alcohols **227** and **228** in 73% and 21% yields, respectively. At this point, installation of an amino group with a Mitsunobu reaction was attempted without success. So, an alternative

Scheme 42



Scheme 43



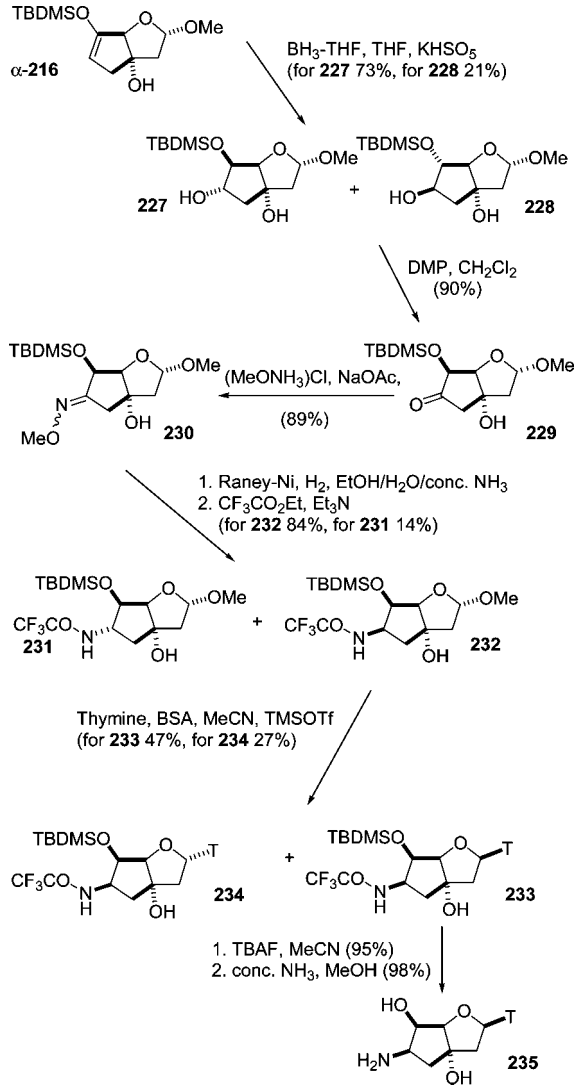
three-step sequence was performed; Dess−Martin oxidation of the latter alcohol mixture afforded the corresponding ketone **229**, which was converted to the oxime intermediate **230** followed by subsequent hydrogenolysis with Raney-Ni under hydrogen to afford a mixture of amines, isolated as their corresponding trifluoroacetates **231** and **232** in 14% and 84% yields after separation. Condensation of the glycosyl donor **232** with thymine under a modified Vorbrüggen procedure furnished the corresponding bicyclonucleosides **233** and **234** in 74% yield as an anomeric mixture in 1.7:1 ratio. After separation and removal of the protecting groups, compound **233** gave the desired amino-functionalized bicyclothymidine **235** in 93% yield.

It should be noted that, starting from β-**216**, the synthesis of **235** using the previous sequence proved to be rather difficult particularly for the hydrogenolysis and N-glycosidation steps. Only hydroboration afforded the corresponding alcohols with better diastereoselectivity (10:1 ratio) due to the steric hindrance of the methoxy on the β-face.

An application of this strategy was described by Leumann et al.[58] to afford the benzyloxyimino nucleoside analogues **241** and **246** as outlined in Scheme 45. The ketone **236**, prepared from the alcohol **214** (see Scheme 41) following a known sequence,[57] was reacted with *O*-benzylhydroxyamine in buffered EtOH/H_2O to furnish the corresponding oxime **237** in 77% yield. Classical N-glycosidation of **237** using the Vorbrüggen strategy did not afford the target nucleoside analogue. Thus, an alternative approach for the N-glycosidation using a glycal analogue was reported. After treatment of the alcohol **237** with TMSOTf, NIS-mediated addition of the *in situ* silylated thymine and the silylated glycal **238** afforded stereoselectively the nucleoside analogue **239** in 54% yield (two steps). Removal of the iodine atom of compound **239** via radical reaction followed by deprotection of the hydroxyl group of compound **240** afforded the target nucleoside analogue **241** in 69% yield (two steps). Starting from glycal **238**, application of the above strategy was reported using *N*4-benzoyl cytosine. In this case, partial deprotection of the hydroxyl group was observed, which furnished the *N*4-benzoyl cytidine analogues **242** and **244** in 15% and 26% yields, respectively. Treatment of compounds **242** and **244** with Bu_3SnH and AIBN afforded the *N*4-benzoyl cytidine derivatives **243** and **245** in 53% and 47% yields, respectively. Classical removal of the silyl group of a mixture of **243** and **245** furnished the target nucleoside analogue **246** in 46% yield.

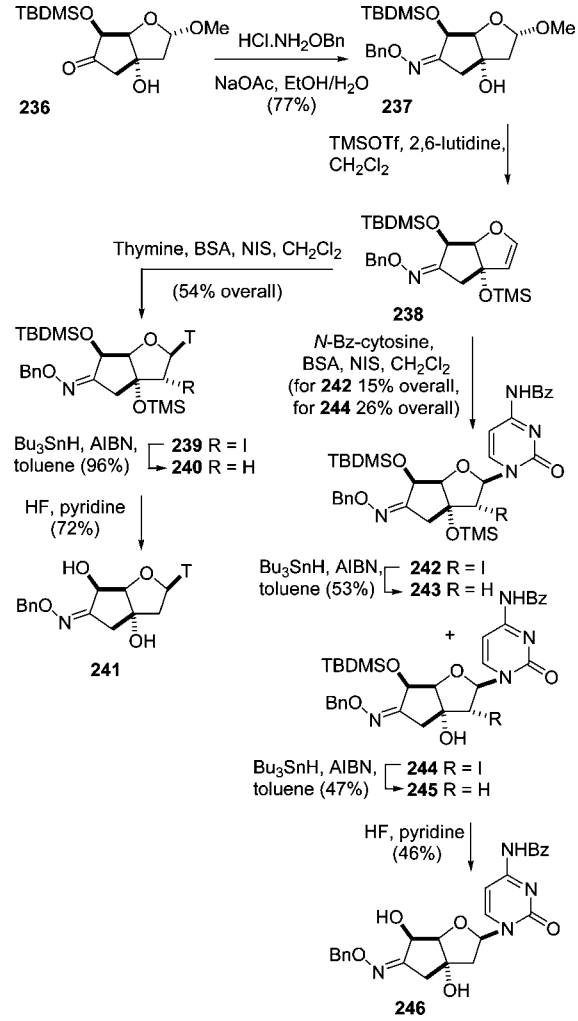
Using the same strategy, Leumann et al. described the synthesis of tricyclo-nucleoside analogues **247** and **248** in 63% and 58% overall yields, respectively, starting from the glycone derivative **217** (see Scheme 42) as presented in Scheme 46.[59] This methodology was not suitable for the preparation of N9 purine analogues since only the kinetically favored N7 isomer could be isolated.

Scheme 44

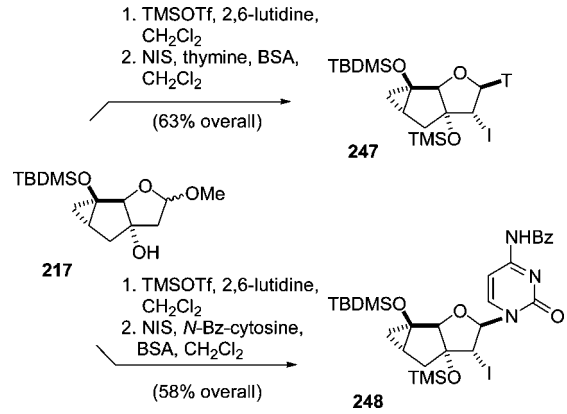


Samuelsson et al.[60] designed a short and efficient synthesis of racemic pyrimidic nucleosides **255**, **256**, and **259** as outlined in Scheme 47. In this approach, the bicyclic sugar backbone was synthesized first, and the bases were introduced at a later stage. The α-allylcyclopentanone **249** was treated with *i*PrOSiMe$_2$CH$_2$MgCl to provide the corresponding alcohol as a 4:1 *cis*/*trans* mixture, which was then subjected to Tamao−Flemming oxidation to furnish the diol **250** as a diastereomeric mixture. At this stage, benzoylation of the latter mixture and removal of the *trans* isomer by chromatography led to the desired monobenzoate **251** in 73% yield. The required methyl furanoside **252** was obtained from **251** as an anomeric mixture in 72% yield over three steps, through dihydroxylation with OsO_4 followed by periodate cleavage and finally treatment in acidic conditions. Condensation of **252** with silylated uridine in the presence of Lewis acid yielded an inseparable mixture of β/α nucleosides **253** in 1.2:1 ratio in 96% yield. It is worth noting that in the thymidine series the β/α ratio of the condensed products **254** was higher (2.8:1). At this stage, the β-anomers **255** and **256** were efficiently obtained from the previous mixture via the formation of the 2,5′-*O*-anhydro intermediates **257** and **258**. In both cases, the mixture of α/β nucleosides was debenzoylated, and the resulting primary alcohols were activated as the tosylates and treated with DBU to afford the corresponding 2,5′-*O*-anhydro intermediates **257** and **258** in

Scheme 45



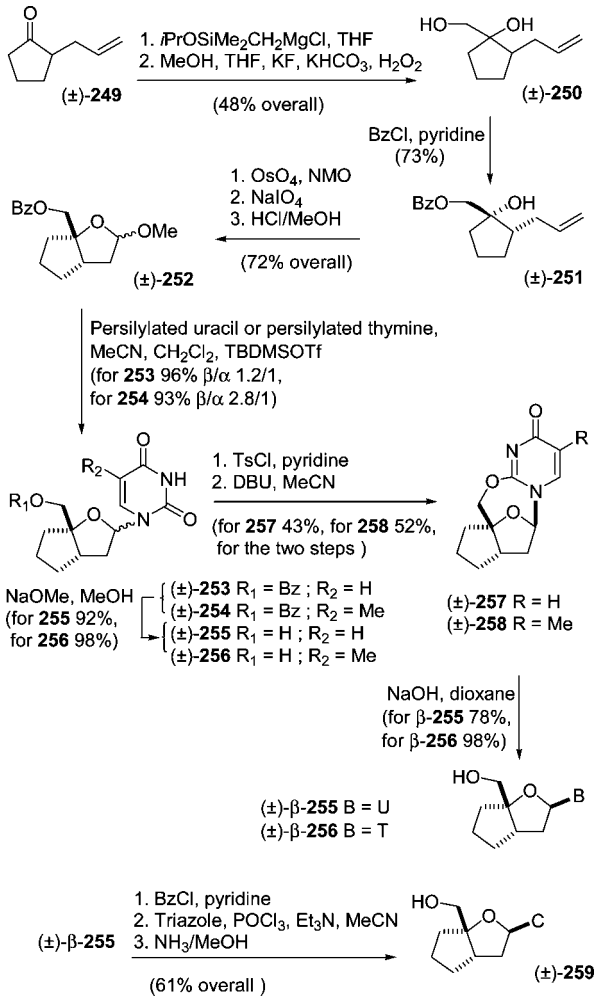
Scheme 46



43% and 52% overall yields, respectively, after purification on silica gel column. The latter compounds were submitted to NaOH to give the expected racemic targets **255** and **256** in 78% and 98% yields, respectively. To complete this work, the uridine **255** was converted to the cytidine nucleoside analogue **259** in 61% overall yield, by sequential benzoylation, formation of the 4-triazolo intermediate followed by aminolysis.

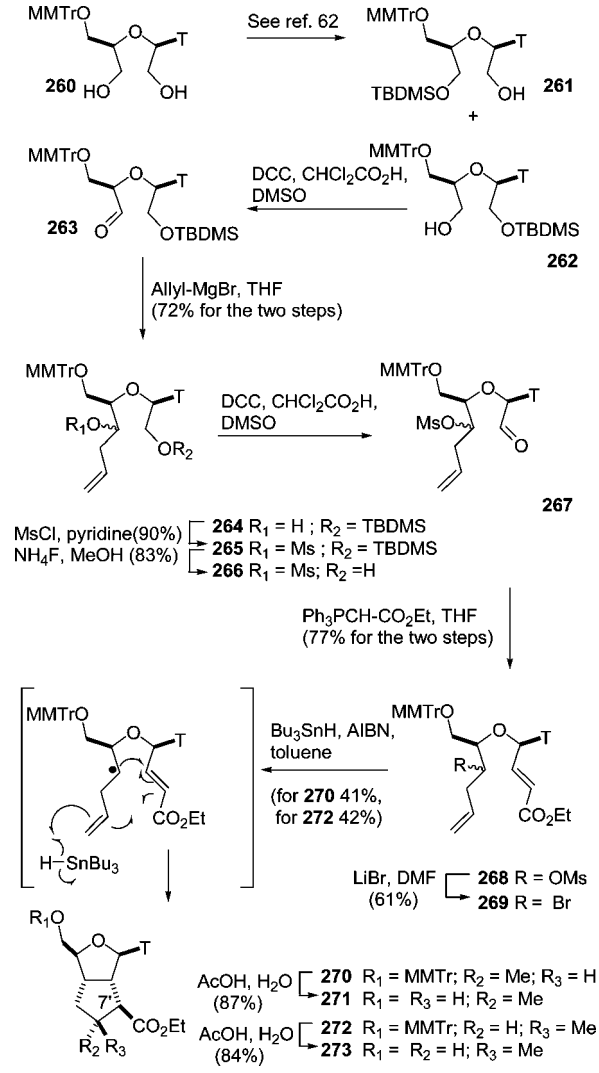
Chattopadhyaya et al. reported an original access to 2′,3′-*cis*-α-fused cyclopentane nucleosides **271** and **273** from 2′-*O*-silylether 2′,3′-*seco*-ribothymidine **262** as outlined in Scheme 48.[61] This precursor **262** was prepared from

Scheme 47



5′-*O*-MMtr-ribothymidine following a three-step sequence including: periodate oxidation of the diol, reduction of the bis-aldehyde, and monosilylation of the resulting diol **260**.[62] This latter step afforded a separable mixture of the isomeric 2′- and 3′-*O*-silylether **262** and **261** in 31% and 26% yield, respectively, as well as the corresponding bis-2′,3′-*O*-silylether in 32% yield. Pfitzner−Moffat oxidation of **262** followed by treatment of the resulting aldehyde **263** with allyl Grignard reagent afforded the corresponding homoallylic alcohol **264** as an inseparable mixture of diastereoisomers in good overall yield. Next, the alcohol function of **264** was activated as mesylate **265** and the silylether was cleaved with NH_4F in MeOH. The liberated alcohol **266** was oxidized as previously reported for **262** to furnish the aldehyde **267**, which was then treated without purification with the stabilized phosphorane reagent $Ph_3P{=}CH{-}CO_2Et$ leading to the α,β-unsaturated ester **268** having (*E*) configuration in 58% overall yield for the four steps. After displacement of mesylate by bromide using LiBr, the corresponding bromo intermediate **269** was submitted to pivotal free-radical cyclization to give a mixture of two epimeric isomers at C7′, **270** and **272**, in 41% and 42% yields, respectively, after separation by chromatography. Free-radical reduction of bromide **269** with Bu_3SnH yielded a secondary carbon-centered radical, which, by intramolecular Michael addition onto the activated double bond, led concomitantly to reconstruction of the furanose ring with an excellent stereochemical control and to the formation of an α-acyloxyalkyl radical,

Scheme 48



which, by a favored 5-*endo*-trig radical cyclization, furnished the fused cyclopentane ring. Finally, removal of the protecting group in acidic conditions afforded the two targets **271** and **273** in good yields.

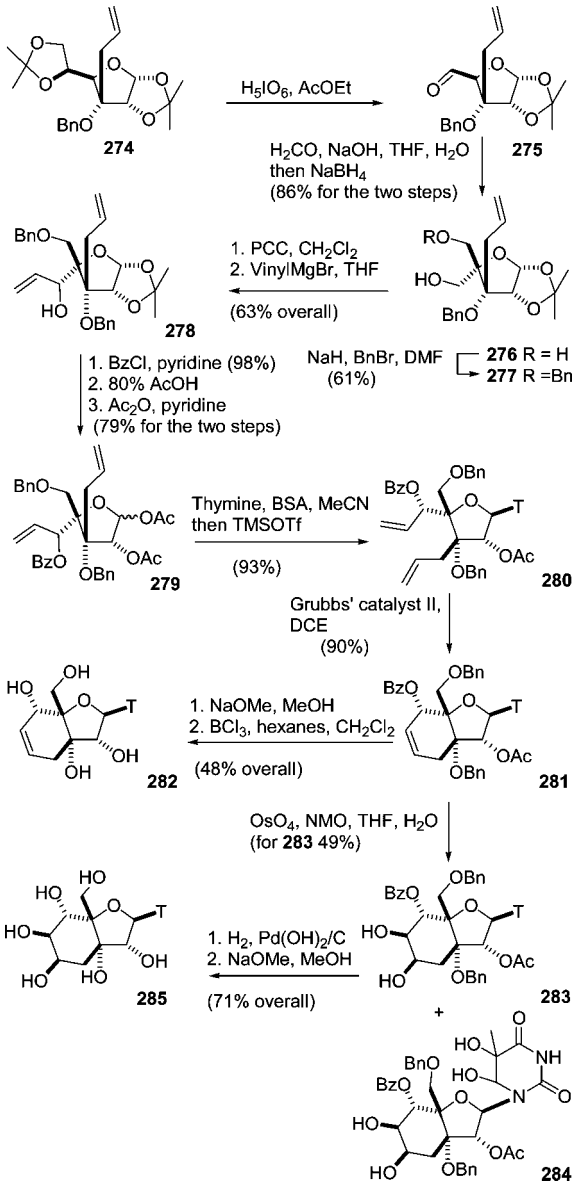
5. Synthesis of Bicyclonucleosides Having a Cyclohexane Core

5.1. Formation via a Ring Closing Metathesis Reaction

In this field, the efforts of Nielsen et al. culminated in the synthesis of three-carbon 3′,4′-linked bicyclic nucleosides **282** and **285** (see Scheme 49)[63] and 2′,4′-linked bicyclic nucleosides **293**, **294**, and **297** (see Scheme 50),[64,65] and **300**, **301**, and **304** (see Scheme 51).[65]

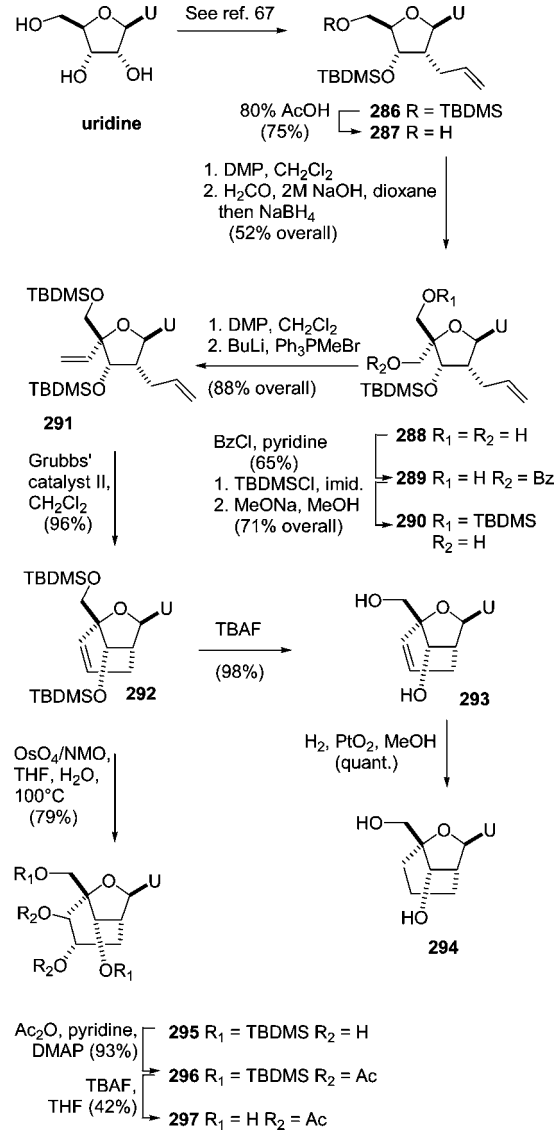
The D-allofuranose precursor **274** was readily prepared from D-glucose following a known four-step sequence.[66] Treatment of derivative **274** with periodic acid afforded the aldehyde **275**, which was condensed with formaldehyde followed by reduction of the resulting aldol with $NaBH_4$ to give the corresponding diol **276** in 86% overall yield. The diol **276** was benzylated to afford, after purification on silica gel, the desired β C5 benzylic ether **277** in 61% yield along with 22% of the other α C5-epimer. Oxidation of **277** with PCC followed by condensation with vinyl magnesium

Scheme 49



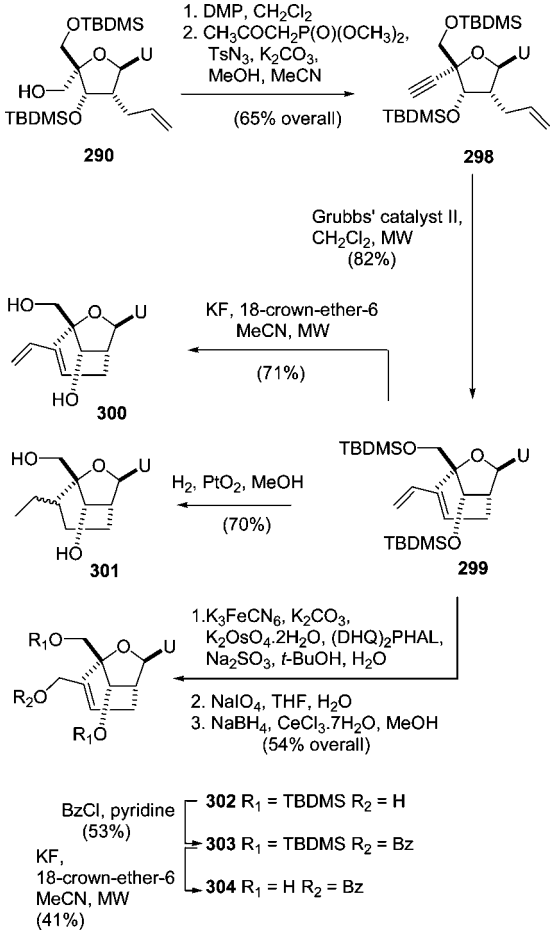
bromide gave an approximate 4:1 ratio of diastereoisomers **278** and its C6-epimer, which were separated by flash chromatography: the pure allylic alcohol was isolated in 63% yield for the two steps. At this point, the alcohol **278** was first protected as benzoate; then subsequent hydrolysis of the acetonide group followed by acetylation yielded the diacetate **279**. Vorbrüggen coupling was then carried out on the RCM reaction precursor **279** to provide, in high yield, the β-nucleoside **280** through neighboring acetate participation. Next, compound **280** was subjected to RCM reaction to give the key bicyclic intermediate **281** in 90% yield. Cleavage of the esters under basic conditions followed by removal of the benzyl ether groups with BCl_3 furnished the fully deprotected bicyclic nucleoside **282** in 48% yield for the two steps. Moreover, this synthetic approach offered the opportunity for double bond functionalization. Thus, dihydroxylation of **281** according to Upjohn's procedure gave a major diol **283** in 49% yield along with 33% of recovered starting material, as well as around 10% of the bishydroxylated compound **284**. Pd-mediated hydrogenolysis followed by de-esterification afforded the target nucleoside **285** in 71% yield (two steps).

Scheme 50



Nielsen et al.[64] also reported the synthesis of three-carbon 2′−4′-linked bicyclic nucleosides **293** and **294** as depicted in Scheme 50. In this context, free-radical allylation has proven to be extremely useful in the synthesis of many nucleoside analogues.[67] Not surprisingly, two groups identified the protected 2′-*C*-allyl uridines **286** and **305** (see Scheme 52) as valuable starting materials for the synthesis of bicyclic nucleoside analogues using an RCM reaction. These key intermediates **286** and **305** were prepared in around 30% overall yield from uridine through well-established chemistry including a diastereoselective free-radical allylation.[67] Nevertheless, it should be pointed out that, in this present work, treatment of **286** with 80% acetic acid over 3 days led to smooth and selective cleavage of the 5′-*O*-silylether in 75% yield. The primary alcohol **287** was oxidized with the DMP reagent to the corresponding aldehyde, which was engaged in an aldol condensation/reduction sequence to furnish the diol **288** in 52% overall yield. Over the next three steps, the diol **288** was converted to the monoprotected intermediate **290** in correct overall yield using standard protecting group manipulations. Treatment of **288** with excess BzCl provided the monobenzoate **289** in 65% yield along with 28% of starting material. Subsequent silylation of the remaining alcohol function

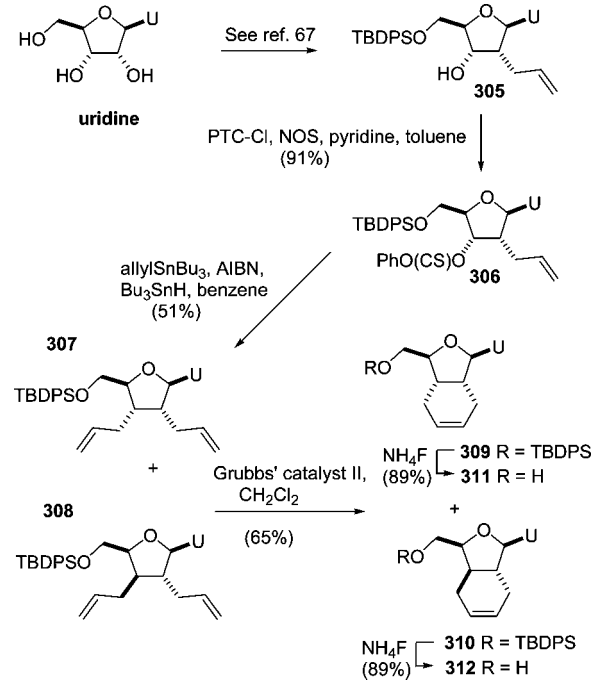
Scheme 51



followed by removal of the benzoate under basic conditions furnished the compound **290**. The latter was subjected to sequential oxidation of the primary alcohol with DMP reagent to the corresponding aldehyde followed by standard Wittig methylenation leading to the key diene **291** in 88% yield for the two steps. RCM reaction employing Grubbs' catalyst **II** gave the intended bicyclic nucleoside **292** in 96% yield. Furthermore, desilylation of **292** afforded the first target **293**, which was hydrogenated using Adams' catalyst to lead to the second target **294** in quantitative yield. Starting from the nucleoside analogue **292**, Nielsen et al.[65] reported the synthesis of the diol analogue **297** (Scheme 50). Dihydroxylation of **292** using Upjohn's procedure afforded the 6′*S*,7′*S* dihydroxy derivative **295** in 79% yield as a single diastereoisomer. After acetylation of the hydroxyl groups, the silyl groups of compound **296** were removed with TBAF to give the deprotected compound **297** in 39% yield (two steps).

Starting from the nucleoside analogue **290**, oxidation of the primary hydroxyl group using the DMP reagent and then homologation using the Bestmann−Ohira reagent afforded the enyne **298** in 65% yield (two steps) as reported in Scheme 51.[65] The enyne derivative **298** was reacted with Grubbs' second-generation catalyst, using microwave heating, which provided the diene **299** in 82% yield. Deprotection of the hydroxyl groups was carried out using KF and 18-crown-ether-6 at 100 °C under microwave irradiation, which gave the unprotected nucleoside analogue **300** in 71% yield. Complete hydrogenation of the diene **299** was achieved by the use of Adams' catalyst to give a mixture of diastereoisomers **301** (6′*R*/6′*S*, 8:1) in

Scheme 52

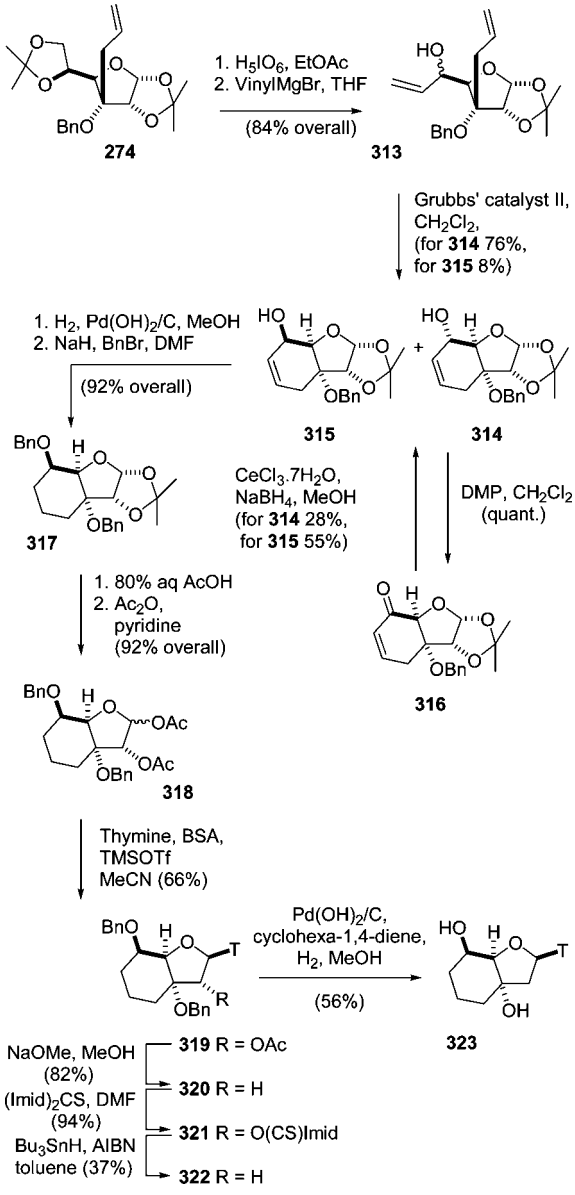


70% yield. In order to increase the hydrophilicity of the compounds **300** and **301**, selective dihydroxylation of the terminal double bond of **299**, followed by oxidative cleavage with $NaIO_4$ and selective reduction of the formyl group using Luche conditions, was achieved to give the hydroxymethyl derivative **302** in 54% yield (three steps). The primary hydroxyl group of **302** was protected as its benzoate ester **303**, and the silyl protecting groups were removed to give compound **304** in 22% yield (two steps).

Starting from the allyl derivative **305**, our group[68] published the synthesis of 2′−3′-cyclohexene bicyclic uridine analogues **311** and **312** as outlined in Scheme 52. The protected allyl derivative **305** was converted to the C3′ radical precursor **306** in 91% overall yield by the formation of the corresponding C3′ thiocarbonate under the Barton conditions, which are required in the case of hindered alcohols. Treatment of **306** under various conditions with allyltributyltin and AIBN in benzene led to only starting material or to decomposition over prolonged reaction times. Fortunately, under the above experimental conditions, addition of a small amount of Bu_3SnH was found to be crucial as a source of tributyltin radical to react on the thiocarbonate function leading to efficient fragmentation and then formation of the C3′ center radical. Following this protocol, the allylated compound was isolated in around 50% yield as a 3:1 diastereomeric mixture in favor of the *cis*-compound **307** along with a small amount of reduced product. The latter inseparable mixture of *cis*-**307** and *trans*-**308** was submitted to RCM reaction in the presence of Grubbs' catalyst **II** giving a mixture of *cis*-**309** and *trans*-**310** followed by removal of the silylether using the Robins' procedure[69] to afford the two target bicyclic nucleosides **311** and **312** in 58% overall yield. These were then separated by preparative HPLC.

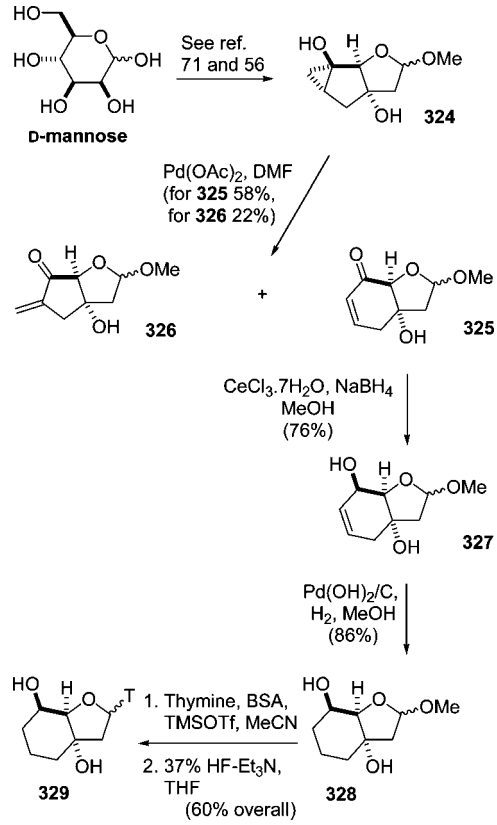
The [4.3.0]-bicyclothymidine **323** was prepared by Leumann et al. as outlined in Scheme 53.[70] After oxidative and selective cleavage of compound **274** (prepared from D-glucose following a known four-step sequence)[66] by treatment with periodic acid in ethyl acetate, the Grignard

Scheme 53



reaction with vinylmagnesium bromide afforded the vinyl derivative **313** in 84% yield as a mixture of two stereoisomers. The mixture of isomers **313** was treated with Grubbs' catalyst **II** in CH_2Cl_2 to give [4.3.0]-bicyclo derivatives **314** and **315** in 76% and 8% yields, respectively. It was notable that oxidization of the undesired 5′*S* isomer **314** and subsequent Luche reduction of the enone **316** gave the mixture of stereoisomers **314** and **315** in a ratio of 1:2. Catalytic hydrogenation of pure enol **315** followed by benzylation of the hydroxyl group afforded the protected compound **317** in 92% yield (two steps). Acidic cleavage of the dioxolane ring of **317** and *in situ* acetylation gave the sugar building block **318** in 92% yield (two steps). *N*-Glycosidation of the glycone derivative **318** with *in situ* persilylated thymine under Vorbrüggen conditions led to the nucleoside analogue **319** in 66% yield with high selectivity for the β-anomer (ratio α/β, 1:10). After classical deprotection of the 2′ hydroxyl group, treatment of the alcohol **320** with 1,1′-thiocarbonyldiimidazol followed by deoxygenation under Barton−McCombie conditions furnished the deoxy nucleoside analogue **322** in 28% yield (three steps). Deprotection of compound **322** by

Scheme 54



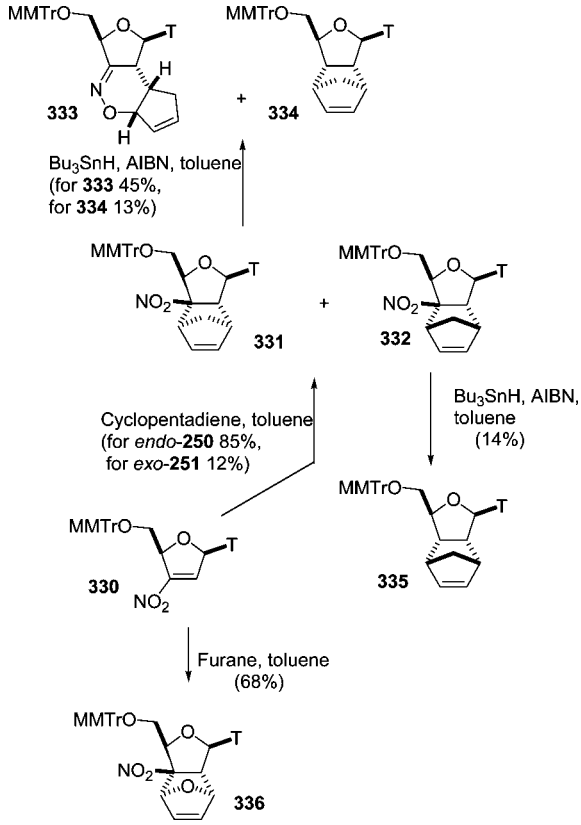
catalytic hydrogenation gave the target nucleoside analogue **323** in 56% yield.

It was notable that, starting from the alcohol **324** obtained in 21 steps from D-mannose,[71,56] its direct conversion to the analogue **325** was performed using Pd(II)-mediated ring expansion (see Scheme 54).[72] Treatment of the tricyclo compound **324** with $Pd(OAc)_2$ in DMF led to both enones **325** and **326** in 58% and 22% yields, respectively. Reduction of the enone **325** via Luche conditions yielded the allylic alcohol **327** in 76% yield. After catalytic hydrogenation, the [4.3.0]-bicyclo derivative **328** was submitted to *in situ* silylated thymine by Vorbrüggen chemistry via transient silyl protection of the hydroxyl groups of the glycone part. After removal of the silylated protecting groups, an inseparable mixture of thymidine anomers **329** (ratio α/β, 2:1) was obtained in 60% yield.

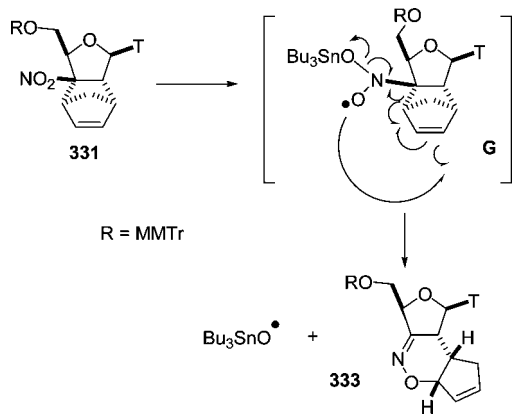
5.2. Formation via Diels−Alder Reaction

Diels−Alder reactions on the nitrothymidine **330** as the electron-deficient alkene were used by Chattopadhyaya et al. to prepare α-fused cyclohexane bicyclic nucleosides with only moderate success due to a subsequent problematic free-radical denitration as depicted in Scheme 55.[73] The condensation of the nitroolefin **330**[74] with cyclopentadiene gave, after separation on silica gel, the *endo*-**331** and *exo*-adducts **332** in 85% and 12% yields, respectively. The *endo*-**331** was treated with Bu_3SnH in the presence of AIBN in refluxing toluene to afford the sugar-fused tricyclic (4*H*-5,6-dihydro-1,2-oxazine)-nucleoside **333** in 45% yield as well as the expected 3′-denitrated product **334** in only 13% yield. The formation of **333** could be explained by the addition of the nitroxide radical intermediate **G** to the double bond, which is accessible in this isomer, followed by concomitant rearrangement and elimination of the Bu_3SnO radical (Scheme

Scheme 55



Scheme 56. Formation of the Tricyclic (4*H*-5,6-Dihydro-1,2-oxazine)-nucleoside 333

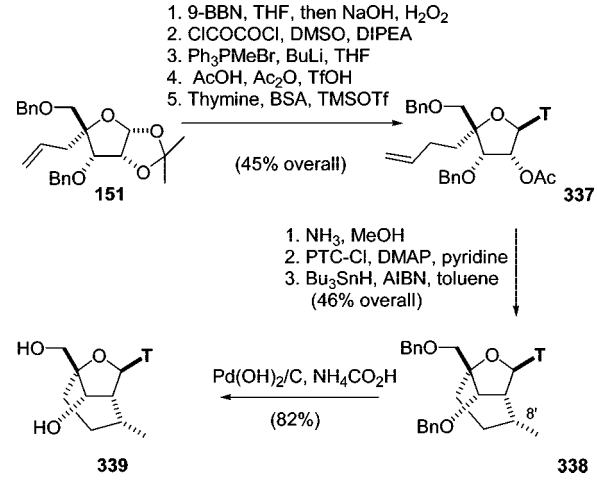


56). In the same conditions, it is not surprising that the *exo*-**332** led only to the denitrated nucleoside analogue **335** in low yield. This work was also carried out with furane (Scheme 55) and anthracene as diene.

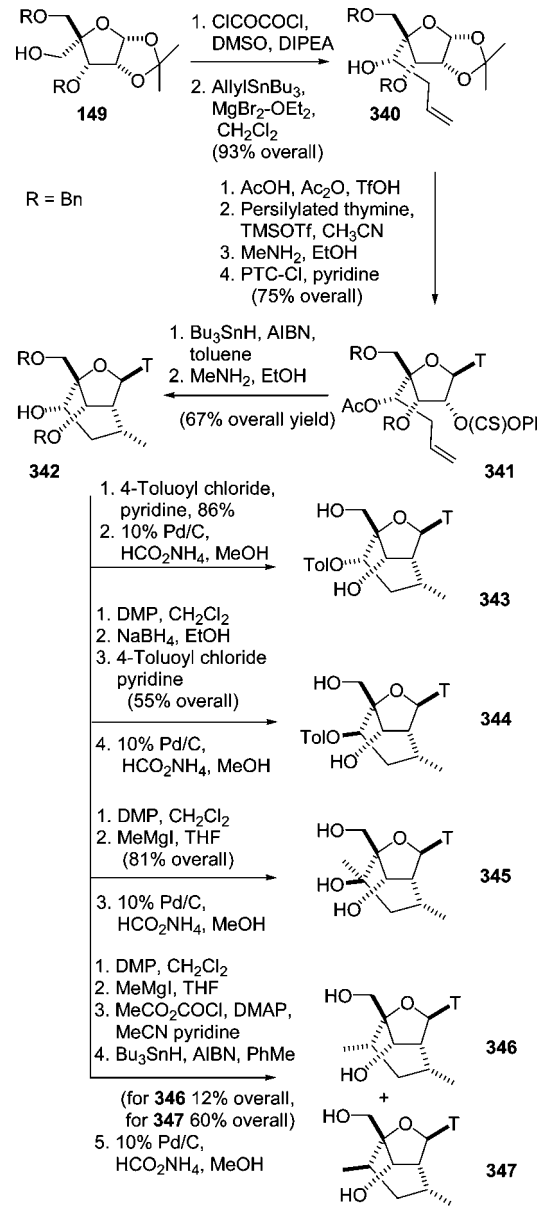
5.3. Formation via a Radical Cyclization Reaction

In order to further demonstrate the scope of radical cyclization to form a C−C bond in the field of carbobicyclic nucleosides, Chattopadhyaya et al.[45] extended their strategy described in Scheme 31 (see section 4.2) to the 4′-*C*-homoallylic derivative **337** to provide the bicyclic carba-ENA nucleoside analogue **339** (Scheme 57). The required key intermediate **337** was prepared as previously presented in Scheme 31 including at the first stage a hydroboration/oxidation on **151** followed by a Swern oxidation/Wittig olefination sequence. The 6-*exo*-heptenyl radical cyclization on **337** proceeded in an efficient manner to give the desired 8′-*R* bicyclic nucleoside **338** as the sole isomer in 76% yield.

Scheme 57



Scheme 58



Using their well-established chemistry, the Chattopadhyaya group[48] also investigated the preparation of C6′-OH and C6′-Me ENA analogues, as described in Scheme 58. The common chiral building block **149** was oxidized to the aldehyde and allylated under chelation-controlled conditions with $MgBr_2-OEt_2$ and allyltributylstannane to provide the 6′-*S* homoallylic alcohol **340** as a single diastereoisomer in 93% overall yield. This latter compound **340** was exploited, following an identical set of transformations as already discussed in section 4.2 (see Schemes 32 and 33), to afford the carba-ENA analogues **343**−**347**.

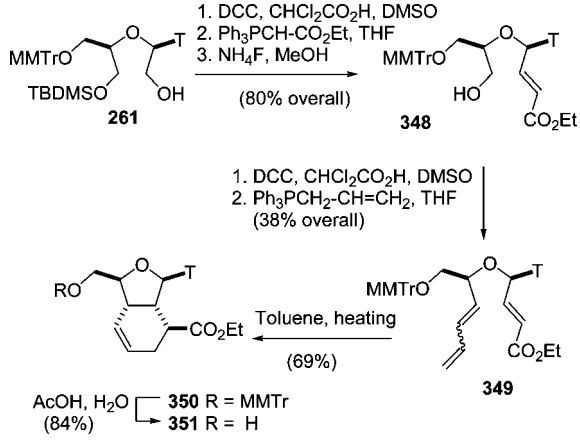
5.4. Formation of Glycone Moiety via *de Novo* Synthesis

In parallel with their work on cyclopentane bicyclic nucleosides presented in Scheme 48, Chattopadhyaya et al.[61] achieved the synthesis of a cyclohexane nucleoside **351** from 3′-*O*-silylether 2′,3′-*seco*-ribo-thymidine **261** as outlined in Scheme 59. Following a similar sequence to that described in Scheme 48 on **262**, the alcohol **348** was prepared in three steps from **261**. As previously, oxidation of **348** furnished the aldehyde, which was submitted to a Wittig reaction with $Ph_3P{=}CH{-}CH{=}CH_2$ to give the diene **349** as a mixture of *Z*/*E* isomers in 3:2 ratio (38% yield). The resulting diene **349** was subjected to thermal intramolecular Diels−Alder reaction to yield the adduct **350** as the sole diastereoisomer in 69% yield. Removal of the protecting group of **350** furnished the bicyclic nucleoside analogue **351** in 84% yield.

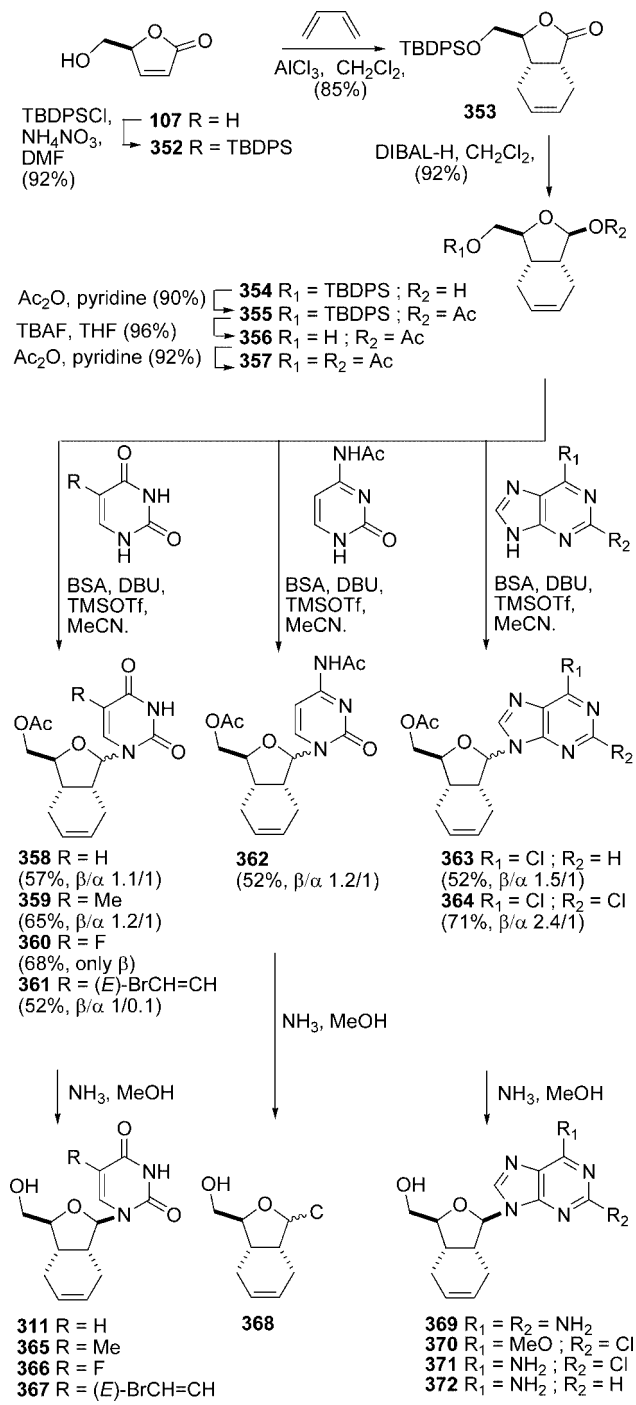
Gotor and Theodorakis et al.[75] published the synthesis of a set of various 2′−3′-cyclohexene bicyclic nucleoside analogues **311** and **365**−**372** as depicted in Scheme 60. Starting from the chiral silyl-*O*-protected butenolide **352**, prepared from D-mannitol in five steps and in 30% overall yield ((*S*)-5-hydroxymethyl-2(5*H*)-furanone **107** is commercially available), the Diels−Alder cycloaddition with butadiene[76] afforded the compound **353** as the sole diastereoisomer in 85% yield; the diene was reacted by the less hindered face. Next, the adduct **353** was converted via a four-step sequence to the corresponding diacetate **357** in 75% overall yield. Then, a variety of nucleobases were installed on **357** under Hilbert−Johnson conditions, by sequential treatment with the appropriate nucleobase in the presence of BSA, followed by addition of DBU and finally TMSOTf to provide a mixture of α- and *β*- nucleosides **358**−**364** in 52−71% yields. It should be pointed out that attempts to carry out the glycosidation step on the 5′-*O*-silylated derivative **355** failed presumably due to the steric hindrance of the protecting group. Finally, subsequent treatment with ammonia gave the desired nucleosides **311** and **365**−**372**. In some cases, the separation of the α- and *β*-anomers was not possible.

To end this work, an inosine analogue **373** was efficiently prepared using an enzymatic deamination on intermediate **372** as depicted in Scheme 61.

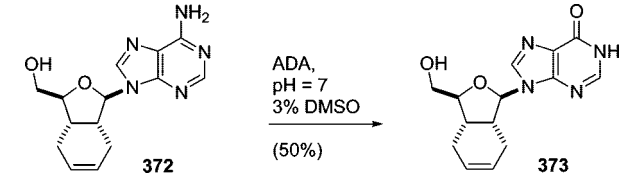
Scheme 59



Scheme 60



Scheme 61

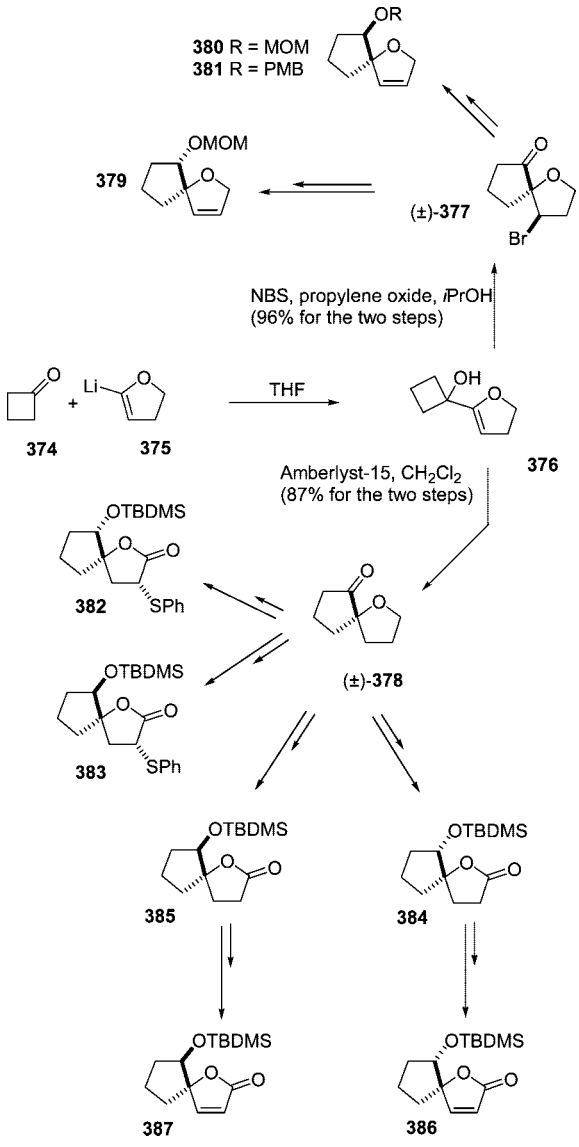


6. Synthesis of Nucleosides Having a Spirocyclic Core

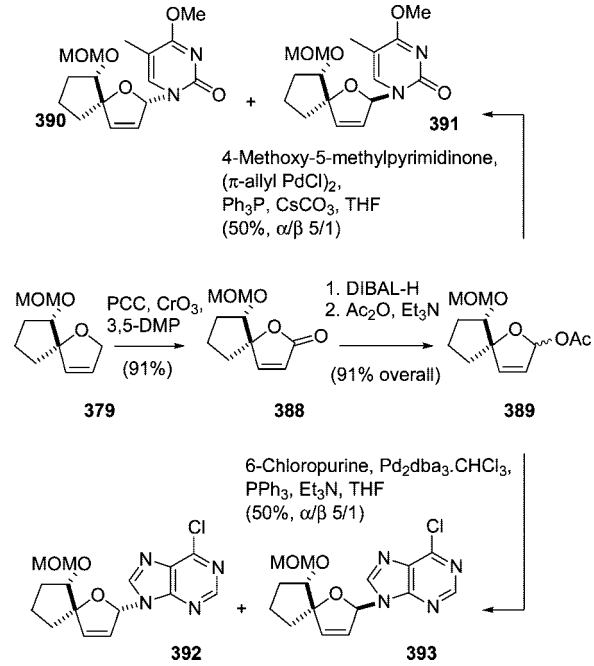
6.1. Formation via an Oxonium Ion-Initiated Pinacolic Ring Expansion Reaction

Paquette et al. reported the synthesis of a unique class of conformationally restricted spirocyclic nucleosides in the D-enantiomeric series, as discussed in this paragraph. All this work is based on acid- or bromonium ion-induced rearrangement of carbinol for the preparation of the spirocyclic moiety.[77] The efficient preparation of the racemic bromo ketone intermediate **377** relied upon a highly diastereoselective oxonium ion-promoted rearrangement of sensitive carbinol **376** with NBS in the presence of propylene oxide as an acid scavenger (Scheme 62). On the other hand, exposure of the pivotal carbinol **376** to an acidic ion-exchange resin in CH_2Cl_2 gave rise to the spiro ketone **378** in high yield. This latter racemic material was resolved, in multigram quantities, via its corresponding Johnson sulfoximine derivative for **377** or by ketalization with (*R*)-mandelic acid for **378**. Subsequent diastereoselective reduction of the carbonyl at different stages of the synthesis with suitable reducing agents and functional group manipulations provided a route toward the chiral spiro cores **379**−**387** of the target nucleosides.

Scheme 62



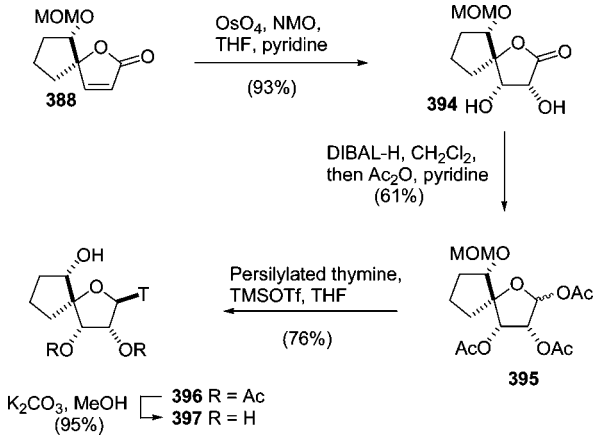
Scheme 63



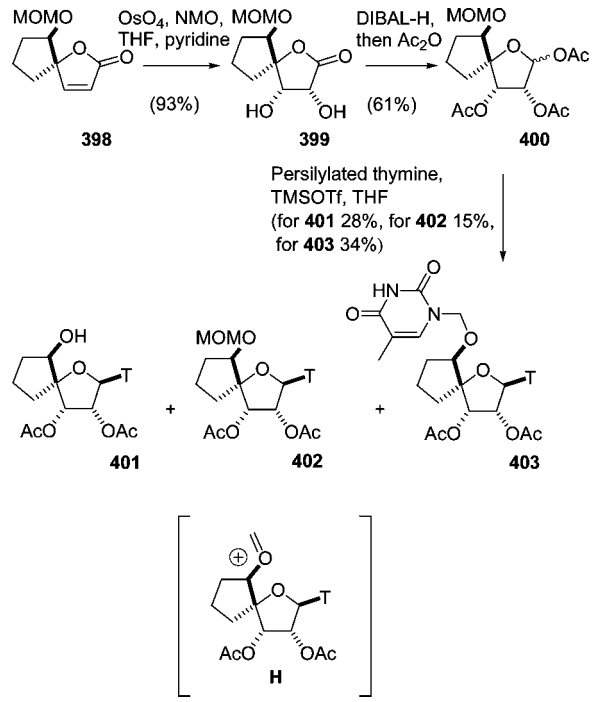
The first spirocyclic nucleosides bearing either α- or *β*-configured hydroxyl groups at C5′ were successfully prepared as outlined in the following schemes. As shown in Scheme 63, allylic oxidation of **379** with a large excess of CrO_3, PCC, and 3,5-DMP afforded the lactone **388**, which was subsequently reduced with DIBAL-H to the corresponding lactol as a 5:1 mixture of anomers followed by acetylation to furnish the glycosyl donor **389** in 82% overall yield.[78] At this point, the authors were unable to attribute the absolute configuration of each *O*-acetyl derivative. Nevertheless, it could be assumed that the reduction occurred from the less hindered α face, opposite the MOM group, to deliver the *β*-anomer as the major diastereoisomer. The answer came from the next step. From the allyl acetate anomers **389**, the Pd(0)-catalyzed introduction of the purine and pyrimidine bases with retention, as previously reported by Trost,[79] gave predominantly, in both cases, the corresponding α-nucleosides **390** and **392** in a 5:1 ratio and in 50% yields. This result proved that the reduction of **388** gave mainly the α-anomer.

So, this approach was abandoned, and the stereocontrolled introduction of the thymine was carried out, as depicted in Scheme 64, based on the assistance of the neighboring C2′ acetate group. The butenolide **388** was diastereoselectively dihydroxylated, and the resulting diol **394** was reduced with DIBAL-H to afford the corresponding lactol aluminate, which was directly treated with Ac_2O in the presence of pyridine leading to the triacetate **395** in 57% overall yield. The presence of H5′ and H3′ on the same side of compound **394** was confirmed by a NOE correlation so, in contrast with previous observations with DIBAL-H, the dihydroxylation occurred from the less hindered α-face. Persilylated thymine was glycosylated by reaction with **395** in the presence of TMSOTf to afford selectively the *β*-anomer **396** in 76% yield; the removal of the MOM protecting group occurred when an excess of Lewis acid was used. Finally, methanolysis of **396** released the first C5′-α-hydroxy spirocyclic ribosylthymine derivative **397**.

Scheme 64

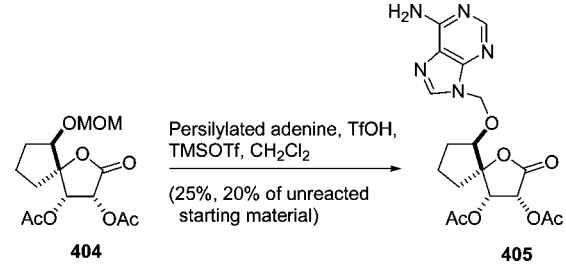


Scheme 65

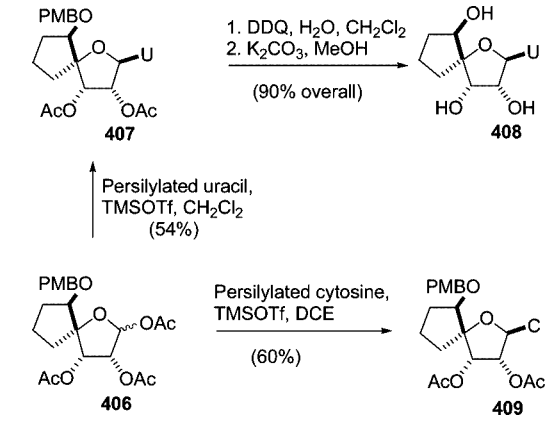


In order to synthesize the C5′-β hydroxy spirocyclic thymidine diastereoisomer of **397**, Paquette et al.[80] applied the same strategy from the spirobutenolide building block **398** (prepared from **380** by allylic oxidation) as illustrated in Scheme 65. In this way, the pivotal triacetate **400** was obtained in a 57% overall yield from **398** following the same procedures as described for **388** (Scheme 64). In contrast with the previous results in the α-oriented MOM series, the treatment of **400** with an excess of persilylated thymine and TMSOTf resulted in the formation of the desired nucleoside **401** and its MOM-protected derivative **402** in relatively low yields (28% and 15%, respectively) along with an unexpected nucleoside **403** isolated in 34% yield. Formation of **403** appeared to be due to the reaction between the persilylated thymine and the oxonium intermediate **H**. At this point, the authors suspected the presence of TfOH in TMSOTf, produced upon prolonged storage, to be responsible for the formation of **403**. Surprisingly, in the α-oriented MOM series, the formation of this side product is not observed. It is worth noting that glycosidations from MOM ethers have been reported in a few examples only for acyclic nucleoside synthesis.[81]

Scheme 66



Scheme 67

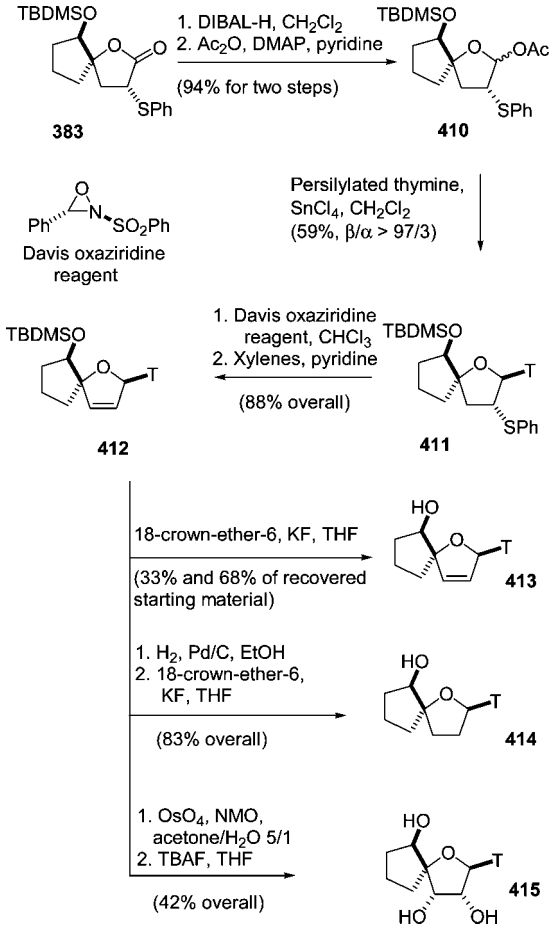


Supporting evidence for this pathway is illustrated in Scheme 66. The lactone **404** was reacted with persilylated adenine in the presence of TMSOTf and TfOH to furnish the compound **405** in 25% yield, together with 20% of unreacted starting material.

In the light of this, to avoid the formation of the oxonium intermediate **H** during the glycosidation step, the PMB protecting group was used and the desired glycosyl donor **406** was conveniently obtained from **381** as previously described in the MOM series.[80] TMSOTf-catalyzed condensation of **406** with persilylated uracil gave only the desired nucleoside **407** in 54% yield (Scheme 67). In contrast, the glycosidation step with persilylated cytosine was attempted without success under the same conditions. This reaction was then investigated in DCE leading to the protected cytidine **409**, which was isolated in an unoptimized 60% yield. From intermediate **407**, removal of PMB with CAN failed; however treatment with DDQ followed by methanolysis led to the isolation of the C5′-β-spirocyclo uridine **408** in 90% overall yield. No attempt to remove the protecting groups on **409** was mentioned.

A second approach to this family of spironucleosides was disclosed by the same authors[82] from the 2-α-phenylthiolactones **382** and **383** as shown in Schemes 68 and 69. In principle, the α-C2-phenylthiosubstituent should allow control of the β-glycosidation via an episulfenium intermediate and enable the implementation of an unsaturation.[83] The 2-α-phenylthiolactone **383**, prepared in five steps in 40% overall yield from the chiral spiro ketone **378**, was treated with DIBAL-H followed by acetylation of the resulting lactol to afford the glycosyl donor **410** (Scheme 68). Treatment of **410** with persilylated thymine and $SnCl_4$ as Lewis acid furnished the β stereoisomer **411** in 59% yield. The phenylthio group was removed by oxidative elimination using the Davis oxaziridine followed by heating in xylene-containing pyridine to give the key intermediate **412** in high overall yield. Cleavage of the silyl protecting group on 2,5-

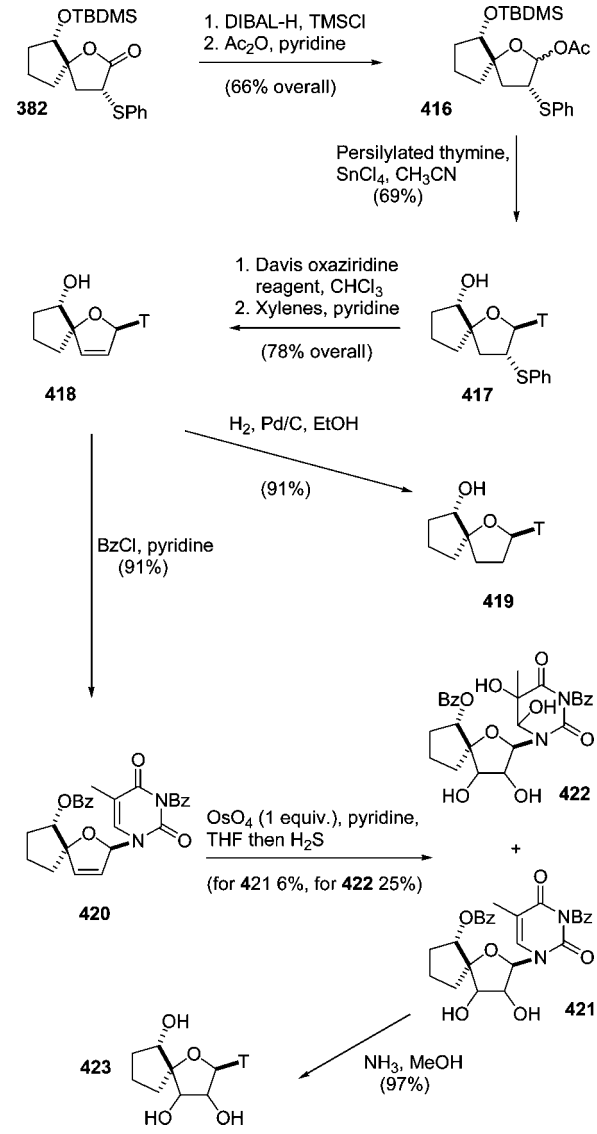
Scheme 68



dihydrofuran **412** proved to be rather tricky and at best was conducted with KF in the presence of 18-crown-ether-6 to provide the d4T analogue **413** in 33% yield along with 68% of unreacted starting material. Epimerization was observed with classical TBAF desilylation. From intermediate **412**, hydrogenation and desilylation afforded the dihydro compounds **414**. It is also noteworthy that an alternative shorter approach involving desulfurization of **411** with Raney-Ni failed. Finally, the compound **412** was treated with a catalytic amount of OsO_4 in the presence of NMO to furnish the corresponding diol as a single diastereoisomer, which was then treated with TBAF leading to the spironucleoside **415** in 42% overall yield. The uridine analogues were prepared analogously to their corresponding thymidine derivatives.

Compared with the previous series, Paquette et al. faced various difficulties with the α-C5′ epimers. First, treatment of the lactone **382** with DIBAL-H resulted in the formation of over-reduced product (up to 39%) (Scheme 69). After some experimentation, it was found that the addition of TMSCl in the reaction mixture to trap the lactol as *O*-TMS acetal, followed by DIBAL-H, minimized the formation of the side product to less than 5%. To increase the yield of the glycosidation step, the reaction was carried out in CH_3CN, instead of CH_2Cl_2 as described with **410**, providing the *β*-isomer **417** unexpectedly desilylated in 69% yield. From **417**, classical formation of the sulfoxide and thermal extrusion of phenylsulfenic acid yielded intermediate **418** in 78% yield for the whole process. Then, this material was hydrogenated under pressure to furnish the analogue **419**. On the other hand, the intermediate **418** was protected as dibenzoyl derivative **420** to facilitate further manipulations.

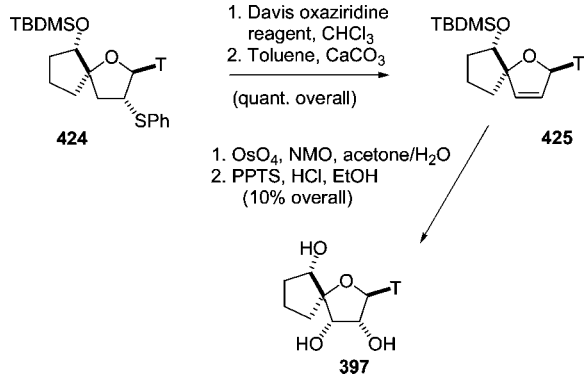
Scheme 69



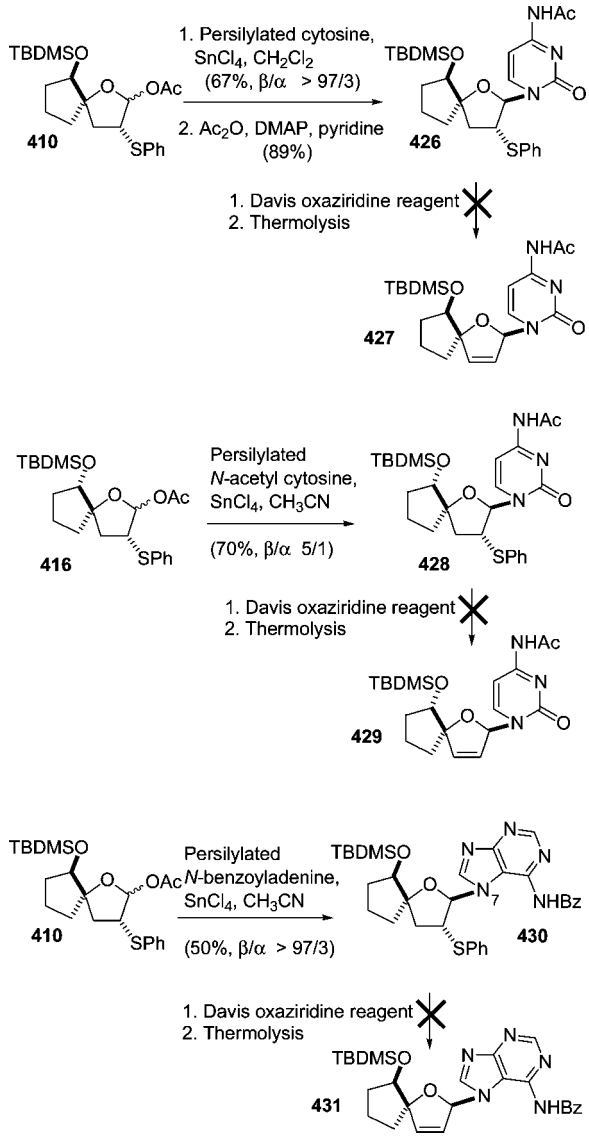
To install the vicinal diol moiety, the osmium-mediated dihydroxylation proved to be rather difficult compared with the result observed with the N3 unprotected thymidine **412**. A variety of conditions were examined; the use of an equimolar amount of OsO_4 in a mixture of THF/pyridine followed by treatment with hydrogen sulfide led to the isolation of diol **421** in only 6% yield as well as the tetraol **422** in 25% yield. In the uridine series, this ratio was inverted due to the absence of the electron-donating effect of the methyl group. Owing to the bulkiness of the protected thymine (or uracil), the dihydroxylation may occur from the less hindered face of the double bond providing preferentially the desired α,α-diastereoisomer. However, at this stage, the stereochemistry was not clearly assigned (*vide infra*). After separation, the removal of the benzoate protecting groups gave the analogue **423**. In a similar fashion, this set of analogues was also prepared in the uridine series.

In parallel, the same sequence was carried out on the silylated derivative **424** without significant difficulty (Scheme 70). The osmylation step was run in the classical catalytic version, and the diol was isolated in 36% yield. Although not mentioned by the authors, by comparison of the ^1H and ^{13}C NMR data of the deprotected dihydroxylated adduct of **425** and compound **397** prepared from another route, as

Scheme 70



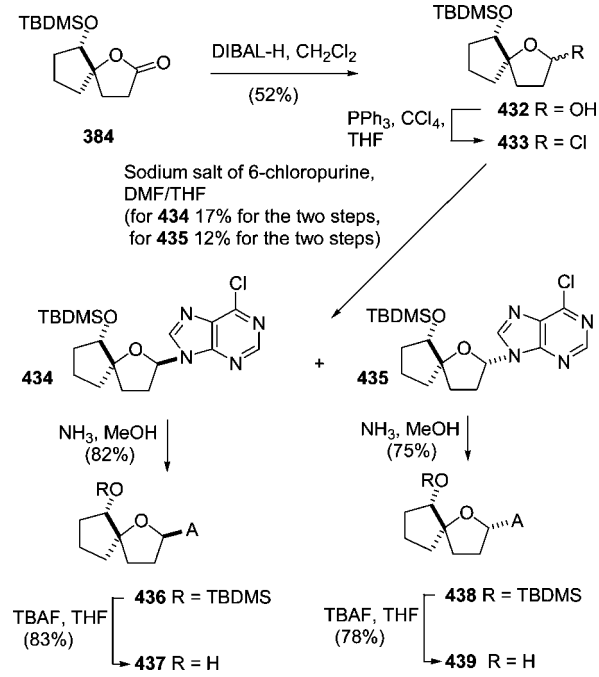
Scheme 71



outlined in Scheme 64, it appeared clearly that the NMR data were identical. This suggests that the dihydroxylated adduct of **425** has the desired α,α configuration for the vicinal diol system.

Having demonstrated the feasibility of this strategy, Paquette et al. turned their attention to the preparation of other analogues with cytosine and adenine bases from **410** and **416** as shown in Scheme 71. The coupling of glycosyl donor **410** with the persilylated cytosine in CH_2Cl_2 under

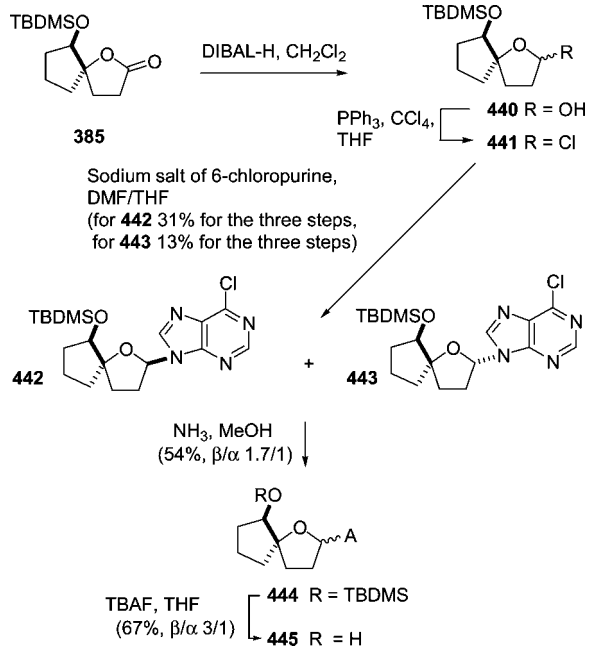
Scheme 72



catalysis with $SnCl_4$ gave, with excellent diastereoselectivity ($\beta/\alpha > 97:3$), the corresponding nucleoside **426** in 67% yield. With the C5′ diastereoisomer **416**, the *N*-glycosidation proceeded with slightly lower selectivity, even with bis-TMS-*N*-acetylcytosine,[84] which is known to improve significantly the formation of the β-isomer. Thus, the desired β-spironucleoside **428** was isolated in 70% yield as a β/α mixture in 5:1 ratio. In the adenosine series, as previously noted, the glycosidation step was very sensitive to the solvent; in CH_3CN, only the undesired N7 adduct **430** was isolated with an excellent β selectivity, while in ether or CH_2Cl_2 the formation of both N7 and N9 adducts occurred. These different intermediates **426**, **428**, and **430** were then submitted to the oxidative elimination sequence. Although the corresponding epimeric sulfoxide mixtures were obtained, unfortunately the thermolysis step led to decomposition.

To bypass the drawback of this sulfoxide pyrolytic extrusion step, Paquette et al. explored a new route to introduce purine bases from the lactones **384** and **385** via their corresponding 1-chloro intermediates using as the key step a sodium glycosidation procedure as reported in Schemes 72−75.[85] Chiral lactone **384** was reduced with DIBAL-H followed by treatment of the resulting lactol **432** with PPh_3 and CCl_4 to afford the corresponding 1-chloro spiranofuranose **433** as a mixture of anomers. This latter crude material **433** was treated with the sodium salt of the 6-chloropurine leading, after chromatography, to the isolation of the pure β- and α-anomers **434** and **435** in 17% and 12% overall yields, respectively, from lactol **432**. As already pointed out by Hildebrand and Wright,[86] this S_N2-type of condensation is not completely stereospecific and epimerization of the 1-α-chlorofurane derivative occurred during the reaction. So the use of a mixture of anomeric chloro derivatives is not a crucial issue. It should also be mentioned that the heating of the pure α-anomer **435** at 120 °C in DMF resulted in the formation of a 1:1 mixture of anomers. Under the same conditions, the β-anomer **434** appeared to be stable. In both series, aminolysis followed by desilylation gave the pure β and α adenosines **437** and **439** in good overall yields.

Scheme 73

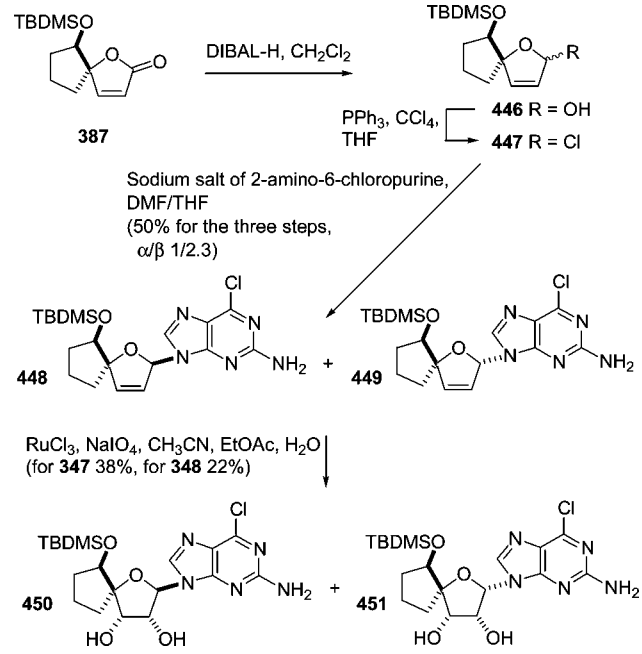


Starting from the epimeric lactone **385**, the same sequence was applied to afford the corresponding adenosine **445** as an inseparable α/*β* mixture of anomers (Scheme 73).[85] The standard reaction of the chlorosugar **441** as a mixture of α/*β*-anomers with the sodium salt of the 6-chloropurine gave after separation the pure *β*- and α-anomers **442** and **443** in 31% and 13% overall yields, respectively, from lactone **385**. However, in contrast with the previous results, at room temperature or in acidic medium an anomerization reaction occurred leading to thermodynamic equilibrium favoring the formation of the thermodynamically more stable *β*-anomer (1.7:1 ratio). Also, treatment of both anomers **442** and **443** (separately or as a mixture) with methanolic ammonia furnished the corresponding anomeric adenosine derivatives **444** in *β*/α 1.7:1 ratio and in 54% yield. Finally, removal of the silyl protecting group furnished the desired adenosine **445** as an inseparable *β*/α-anomeric mixture in 3:1 ratio.

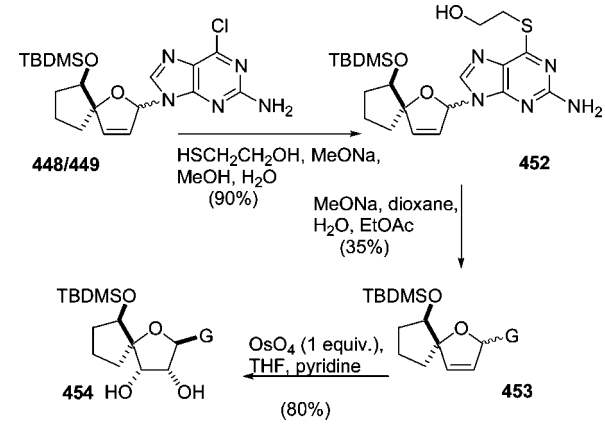
From this latter work, the same authors displayed an approach to a spiro guanosine from the chiral butenolide **387** as depicted in Scheme 74. Glycosidation of **447** with the sodium salt of the 2-amino-6-chloropurine gave an anomeric mixture of **448** and **449** in 2.3:1 ratio in 50% yield. After partial separation by careful chromatography on silica gel, each anomer was able to convert to the other at rt to reach thermodynamic equilibrium. Dihydroxylation of the latter mixture catalyzed by RuO_4, generated *in situ* from $RuCl_3$ with $NaIO_4$ as a co-oxidant, was found to be the most efficient procedure, and the diastereoisomers **450** and **451** were isolated after separation on silica gel in 38% and 22% yields, respectively. At this stage, no attempt to convert compound **450** into the corresponding guanosine analogue was mentioned.

A second approach from the mixture of **448** and **449** was also attempted starting first with the conversion of the 2-amino-6-chloropurine moiety to guanine as presented in Scheme 75. In contrast to previous results from the literature,[13,87] this latter transformation was achieved in a two-step sequence; the sulfide intermediate **452** as a 2.3:1 anomeric mixture was formed by treatment of **448/449** with 2-mercaptoethanol under basic conditions in refluxing MeOH

Scheme 74



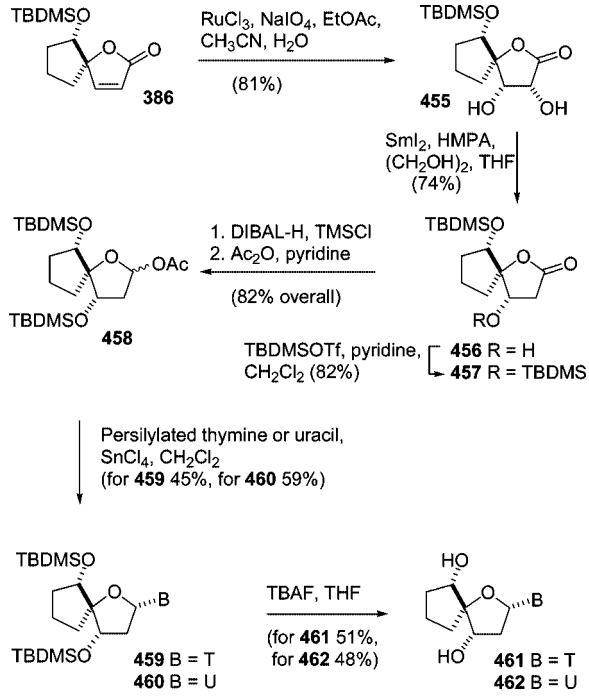
Scheme 75



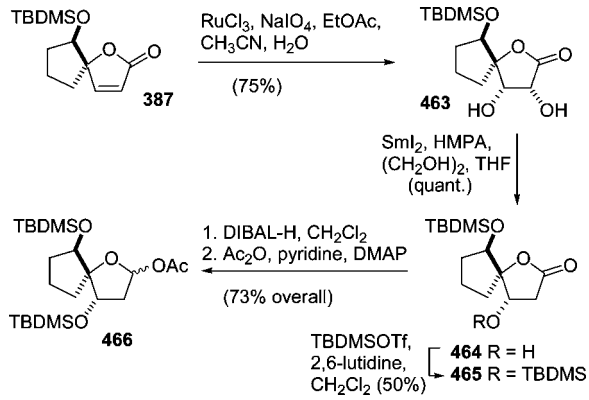
and, after purification, was refluxed in dioxane under the same basic conditions to yield **453** as a 1.6:1 anomeric mixture. Finally, dihydroxylation of the latter material in the presence of a stoichiometric amount of OsO_4 gave the desired spironucleoside **454** as the major diastereoisomer. Cleavage of the silyl group on **454**, as well as on the other guanine intermediates, with different fluoride sources met with failure.

In continuing their exploration of the potential applications of chiral spirocyclic butenolides, such as compound **386**, in the nucleoside field, Paquette et al.[88] investigated the preparation of the corresponding 2′-deoxyribonucleoside, as presented in Scheme 76. Stereoselective RuO_4-mediated dihydroxylation of **386** led to the formation of the desired diol **455** in 81% yield. Then, selective deoxygenation at the 2-position of **455** was carried out with SmI_2 in HMPA/THF in the presence of ethylene glycol as a chelating agent and proton source to afford **456** in 74% yield. Sequential silylation of the remaining C3 alcohol function of **456** and reduction of the lactone function with DIBAL-H in the presence of TMSCl, followed by acetylation, provided the glycosyl donor **458** in 70% overall yield for the three steps. With **458** in hand, the glycosidation was examined with persilylated thymine and uracil. In the case of thymine, only 12% of the *β*-anomer was formed, as indicated by proton

Scheme 76



Scheme 77



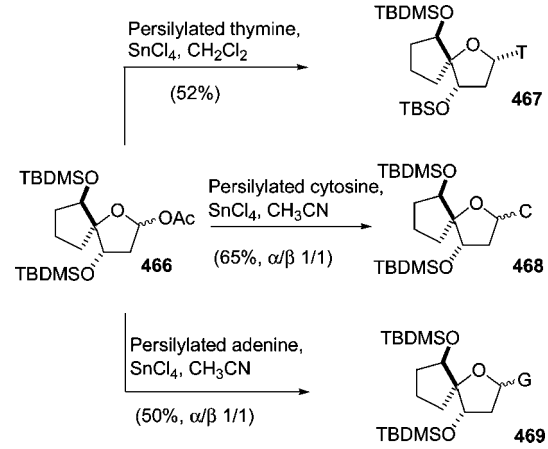
NMR, but purification led to the sole isolation of the undesired α-anomer **459** in 45% yield, while with uracil only the α-anomer **460** was formed in 59% yield. Classical removal of the protecting groups gave the nucleosides **461** and **462**.

To evaluate the influence of the configuration of the silylated alcohol at C5 on the diastereoselectivity of this glycosidation step, the epimeric derivative **466** was prepared following the same previous sequence from **387** as outlined in Scheme 77.

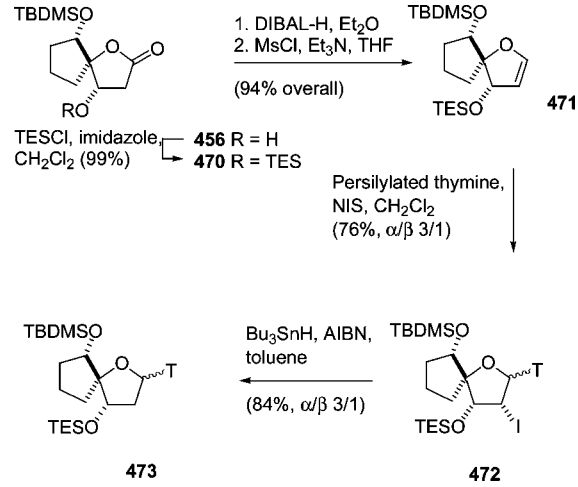
Under the same glycosidation conditions as previously described for **458**, treatment of **466** with persilylated thymine led exclusively to the formation of the α-anomer **467** in 52% yield (Scheme 78). Further experiments in CH_3CN with persilylated cytosine and adenine resulted in the formation of an inseparable 1:1 mixture of anomers of the corresponding nucleosides **468** and **469** in 65% and 50% yields, respectively. As a conclusion, the configuration at C5′ had no influence on the diastereoselectivity of the glycosidation.

To complete this work, another strategy was developed by Paquette and Dong as outlined in Scheme 79.[89] Starting from the lactone **456**, subsequent silylation of the alcohol function, reduction with DIBAL-H to the corresponding

Scheme 78



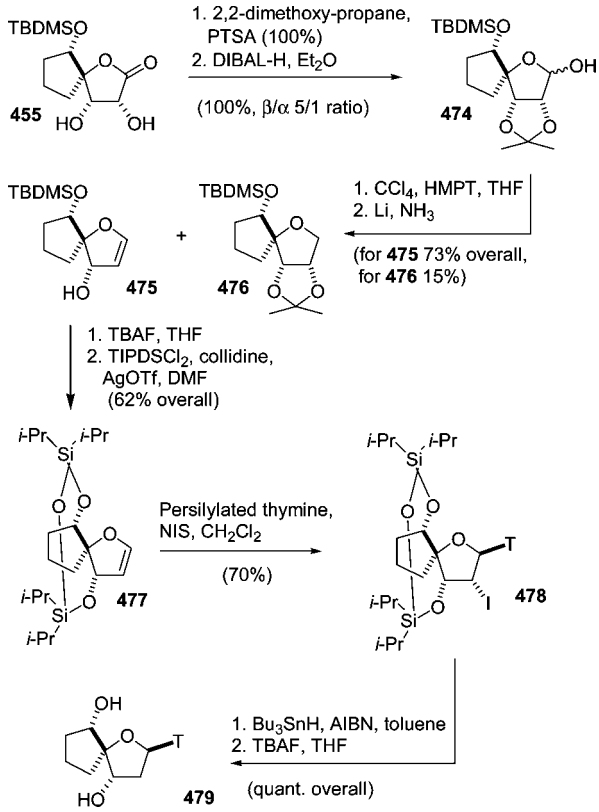
Scheme 79



lactol followed by mesylation, and *in situ* elimination afforded the glycal **471** in 94% overall yield. Glycosylation of glycal **471** with persilylated thymine using NIS as electrophile led to the isolation of the 2′-iodo-4′-spirothymidine **472** in 76% yield as an inseparable mixture of α/β-anomers in 3:1 ratio. Finally, free-radical deiodination afforded the undesired α-anomer **473** as the major compound.

As the conclusion of their contribution to the field of original nucleoside analogue synthesis, Paquette and Dong proposed an elegant solution to solve the problematic stereoselective β-anomeric glycosidation in this series.[89] Following previous reports,[90] concerning the use of the 3,5-*O*-TIPDS protecting group on 4-thiaglycals to increase the β-selectivity on electrophilic glycosidation, the TIPDS-protected glycal **477** was prepared as outlined in Scheme 80. It should be pointed out that the previous strategy to obtain the glycal **471** (Scheme 79) failed due to the ring opening of the lactol intermediate into the corresponding aldehyde induced by the strain of the disiloxane ring. To overcome this problem, the dihydroxylactone **455** was protected as acetonide and reduced to the corresponding lactol **474**. This was then engaged in Ireland's procedure leading first to the formation of the ribofuranosyl chloride followed by subsequent reductive fragmentation to afford, after separation, the glycal **475** and the dehalogenated compound **476** in 73% and 15% yield, respectively. Desilylation of **475** followed by treatment with $TIPDSCl_2$ gave the desired glycal **477**, which, when treated with persilylated thymine in the presence of NIS, led exclusively to the

Scheme 80

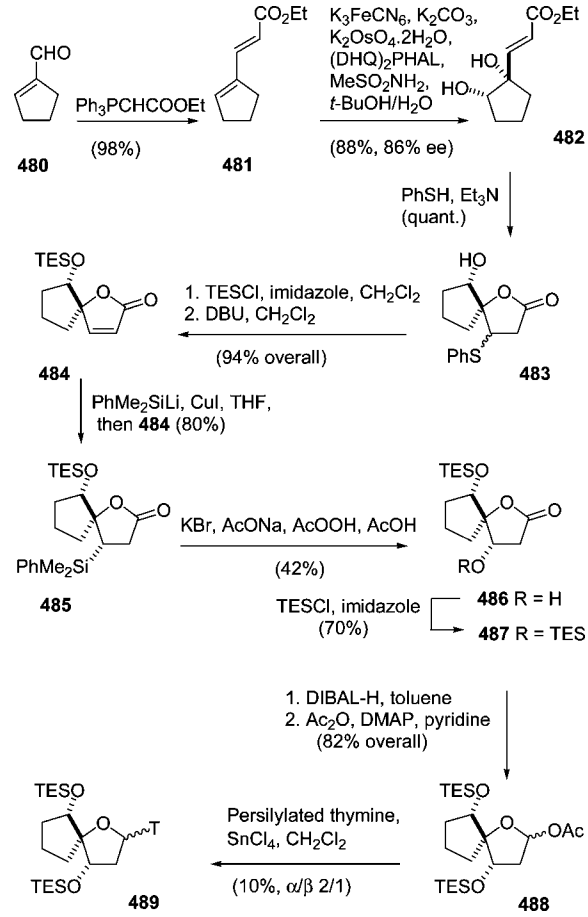


β-anomer **478** in 70% yield. It would be reasonable to assume that the stereochemical outcome of this transformation is due to the steric hindrance of the 3,5-*O*-TIPS protecting group on the top β-face favoring the formation of the α-iodonium key intermediate. Deiodination and cleavage of the TIPDS group furnished the spirothymidine **479** in high yield.

Although this strategy was not extended to the β-5′-isomer series, it should be pointed out that the same authors successfully prepared the 4′-thiaspironucleoside analogues with an α- or β-hydroxyl substituent at C5′ following the same sequence from the corresponding thiaglycals.[91]

In parallel with Paquette's work, an alternative and interesting strategy to prepare spirocyclic nucleosides was explored by Wendeborn et al.[92] at Syngenta Ltd., as highlighted in Scheme 81. The readily available α,β-unsaturated aldehyde **480** was submitted to a Wittig reaction to give the corresponding 2,4-dieonate **481**. This was subjected to Sharpless asymmetric dihydroxylation with commercially available AD-mix-α reagent under standard conditions in the presence of methanesulfonamide to provide, in excellent regioselectivity,[93] the chiral diol **482** in 86% overall yield and in 86% ee. Treatment of **482** with PhSH in the presence of Et_3N quantitatively furnished the Michael adduct, which underwent *in situ* lactonization to give the spiro lactone **483** as a 1:2 mixture of diastereoisomers at C3. To avoid isomerization via probably a retro-aldol/aldol reaction, the alcohol function of **483** was protected as a silylether prior to DBU-induced elimination of PhSH to give the chiral butenolide **484** in excellent overall yield. Attempts to install the requisite alcohol function with appropriate stereochemistry at C3, by sequential epoxidation of **484** followed by regioselective ring opening, failed. The required epoxide was obtained in only low yields and its treatment with $NaBH_4$ led to the reduction of the lactone to the corresponding diol without affecting the epoxide. Finally,

Scheme 81

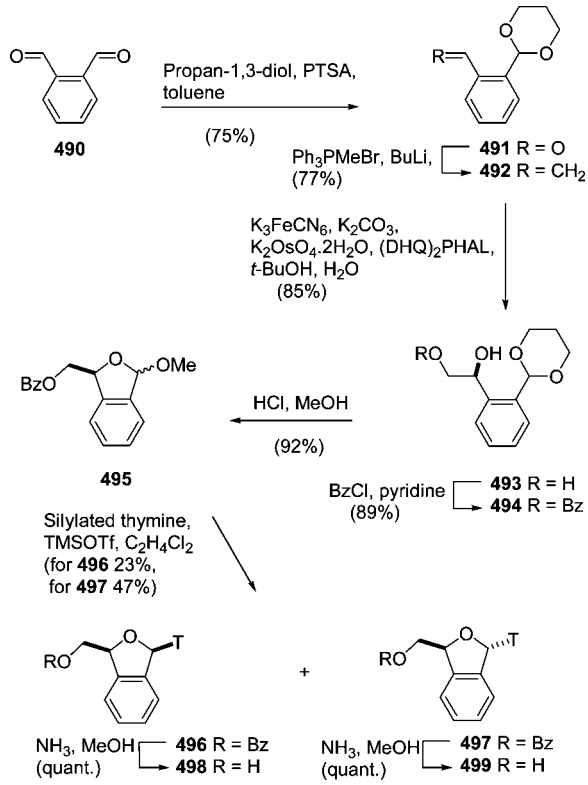


the use of the $PhMe_2Si$ group as a masked hydroxyl group was the best alternative to reach the target molecule **486**. Diastereoselective addition of the silyl cuprate reagent to the butenolide **484** yielded the corresponding α-silyl derivative **485**, which was oxidized upon treatment with AcOOH to afford the intermediate **486** in 35% overall yield. Then, silylation of alcohol **486** set up the substrate for successive reduction with DIBAL-H, immediately followed by acetylation to furnish the glycosyl donor **488** in around 60% overall yield as a mixture of anomers in 5:4 ratio. The introduction of the thymine on the anomeric position of **488** was carried out by the classical Vorbrüggen procedure leading to a mixture of α/β spirocyclic 2′-deoxyribonucleoside anomers **489** in 2:1 ratio in only 10% yield. Although this route to spirocyclic 2′-deoxyribonucleosides is quite original, the low yield and the lack of stereoselectivity in the glycosidation step limit its synthetic applications. Nevertheless, it is important to mention, as shown in Scheme 76, that no better results for this coupling step have been obtained by Paquette on the similar substrate **458** with TBDMS-silyl protecting groups: only the α-anomer was isolated in modest yield.

7. Synthesis of Nucleosides Having a Benzo[c]furan Core

Analogues of d4T having a benzo[*c*]furan core were described by Ewing et al. The target nucleosides were first obtained as a racemic mixture[94] and, in subsequent papers, as enantiomerically pure forms.[95] For simplicity, only the asymmetric synthesis of benzo[*c*]furan analogues is described here. Starting from phthalaldehyde **490**, selective protection

Scheme 82



of one of the formyl groups was achieved by acetal formation to give **491** (Scheme 82). This was followed by Wittig homologation of the remaining formyl group to give the corresponding styrene **492** in 58% yield. The ethene functional group was converted into the corresponding dihydro derivatives **493** in 85% yield (ee > 99%) using the commercial Sharpless reagent, AD-mix α. After selective benzoylation of the primary hydroxyl group of **493**, the corresponding ester **494** was cyclized and methylated to afford the corresponding 1,3-dihydrobenzo[*c*]furan derivative **495** in 82% yield (two steps), analogous to an anomeric mixture of 2′,3′-didehydro-2′,3′-dideoxyfuranosides. Both the thymidine derivatives, **496** and **497**, were obtained by standard Vorbrüggen chemistry on **495**, due to the lack of neighboring group participation to direct stereoselectivity. After removal of the benzoyl protection and subsequent silica gel chromatography, the target nucleosides **498** and **499** were obtained enantiomerically pure in 9% and 19% overall yield, respectively.

The nucleosides **498** and **499** are analogous to 2′,3′-didehydro-2′,3′-dideoxynucleosides in the D-series. The related enantiomers analogous to L-nucleosides were synthesized using the same strategy but employing AD-mix β. This work was further extended to provide the full set of related isomers **500**−**504**, having uracil and cytosine as heterocyclic bases accordingly (Figure 9). In each case, the use of the appropriate AD-mix afforded an enantiomerically pure nucleoside.[95a]

8. Conformation Analysis and Properties of C−C Bridged Bicyclonucleosides

The large number of nucleoside analogues synthesized so far have been designed for two main purposes: finding new drugs against viral infections[8] and finding new building blocks to form stable duplexes for antisense[9,96] or RNA interference approaches.[97] In both cases, knowledge of the nucleoside conformational structure is crucial, whether to understand the enzymatic inhibition behavior or the ability to form duplexes with RNA or DNA counterparts. It has been pointed out that the nucleoside metabolic pathways are very sensitive to sugar puckering in terms of binding affinity. There is also an entropic benefit in duplex formation for conformationally restricted nucleotide-containing DNA or RNA strands.[9e]

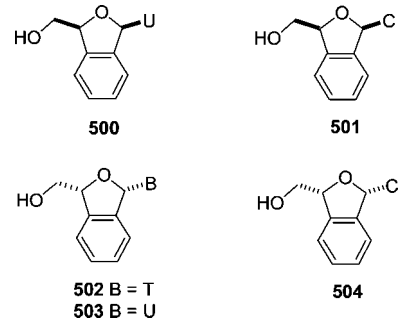


Figure 9. 2′-*C*- and 3′-*C*-dibranched nucleosides **500**−**504** having a benzo[*c*]furan core.

Most of the work on nucleosides with restricted sugar puckering has focused on the preparation of analogues with a bridge containing at least one heteroatom. The prominent recent examples of such analogues that lead to a complex with an increased thermal stability are locked nucleic acids (LNA/BNA) in which the ribofuranose subunit is locked (via a covalent bridge between the 2′-oxygen and 4′-carbon) into the C3′-*endo* conformation (North type).[43]

Figure 10 locates the various carbocyclic nucleoside analogues presented in this review around the pseudorotational cycle of sugar puckering conformations; the detailed parameters, where available, are listed in the sections below. For a better understanding, reviewed bicyclonucleosides have been classified into two families, that is, the 3′-deoxy-bicyclonucleosides with antiviral purposes and the 3′-hydroxy-bicyclonucleosides devoted to the antisense technology. As a general feature, it can be seen that the latter populate the North and the South part of the pseudorotational clock with Chattopadhyaya's carba-LNA and -ENA analogues and Leumann's bc- and tc-DNA analogues, respectively, whereas the former are relatively widespread with a higher density around the East side. Unfortunately, structural parameters are available for only 60% of the reviewed 3′-deoxy-bicyclonucleosides, which is insufficient to look for a relationship between structure and biological activity. On the other hand, structural parameters of 3′-hydroxy-bicyclonucleosides can be nicely correlated with their duplex formation ability.

8.1. Conformation and Biological Properties of 3′-Deoxy-bicyclonucleosides

8.1.1. Conformation Analysis

Table 1 contains, where available from X-ray, NMR, or *in silico* calculation data, structural parameters of 3′-deoxy-bicyclonucleosides with the torsional angles χ (indicating the base orientation) and γ (related to the C4′−C5′ bond conformation), υ_{max} (maximal deviation from planarity), and *P* (describing the conformation of the furanose ring).

There is no direct relationship between the nature, position, and absolute stereochemistry of the furanose substituents and

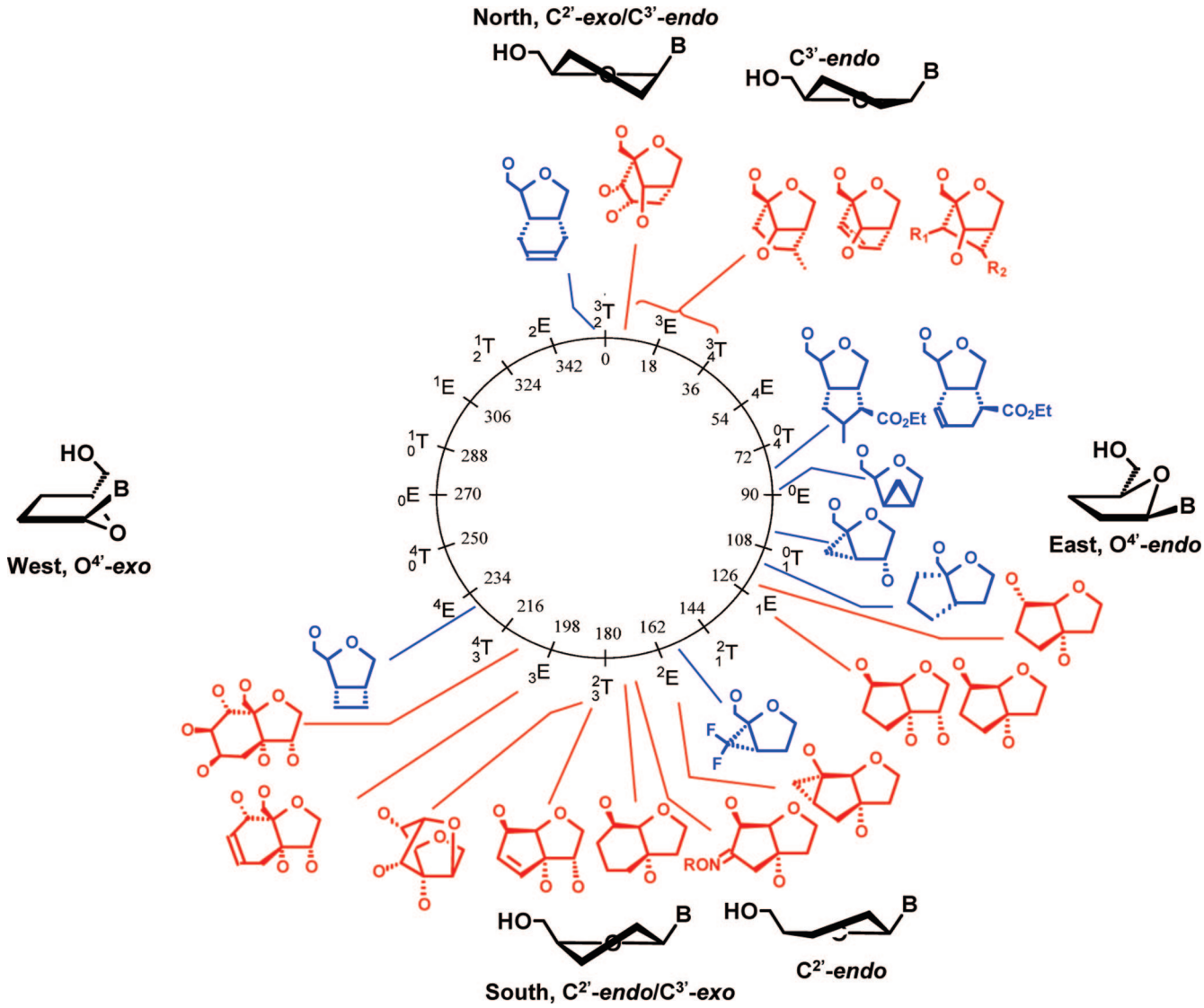


Figure 10. Position of C−C bridged bicyclonucleosides around the pseudorotational cycle with the characteristic North, East, South, and West sugar puckering conformations. Colored in blue are the 3′-deoxynucleosides and in red those suitable for incorporation within oligonucleotides. *P* values are given in degrees. Envelope (E) and twist (T) forms alternate every 18°.

the sugar puckering. For instance, cyclopropane substituents can drive the sugar puckering from the East form to the South if substituted with fluorine (Table 1, entries 1−4).[13,16,18] In the same way, fused six-membered cycles with the furanose ring can provide access to sugar either in the North conformation (entry 9)[68] or in the East form (entry 10)[61] as can fused five-membered cycles (entries 6−8).[60,61] As an exception, cyclobutane fused ring in the 2′,3′ position induces a preferential conformation between West and South (entry 7).[32]

Unfortunately, there are many analogues with no reported data about their structure (Figure 11). This is particularly unfortunate for bicyclonucleosides **413**, **414**, **418**, **419**, and **437**, which represent a very interesting new class of γ-constrained nucleosides never previously described.

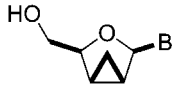
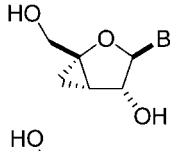
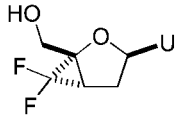
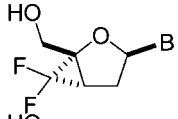
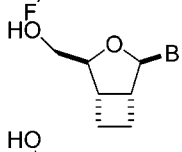
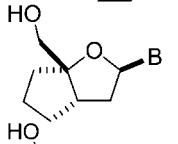
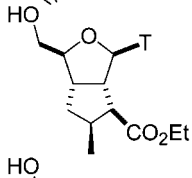
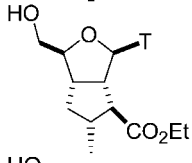
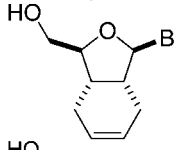
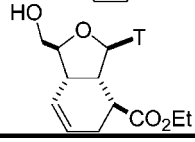
8.1.2. Biological Properties

Only a few of these 3′-deoxy-bicyclonucleoside analogues have been evaluated as antiviral agents with special attention paid to anti-HIV activity. However, none of the nucleoside analogues **9**, **11**, **13**, **16**, and **19** showed any significant activity up to 100 μM in peripheral blood mononuclear cells, while only weak activity against HIV has been reported for the cytidine analogue **65** constrained by a cyclopropane ring in the 2′,3′ position, whereas its thymidine counterpart **105** did not display activity against any of the virus strains tested but was not toxic up to 200 nM. The North configured 2′,3′-cyclohexene fused analogues **311**, **365**, **372**, and **373** were also evaluated in a similar assay, and it turned out that only the ddI analogue **373** exhibited a moderate activity with an EC_{50} of 12.3 μM, and no significant cytotoxicity was detected. A cyclopentane fused ring in the 4′,3′ position lets analogues **255**, **256**, and **259** adopt an East conformation that may not be favorable because they were found to be inactive in an *in vitro* assay for HIV-1 RT inhibition and in an XTT assay for anti-HIV-1 and cytopathic effects.

Finally, compounds **311** and **312** were evaluated against herpes simplex virus type 1 in cell cultures, but neither of them showed any antiviral activity.

Unfortunately, the small number of analogues evaluated is hindering the progress of a conformational−activity study to identify the conformational preferences of the targeted enzymes.

Table 1. Structural Parameters of 3′-Deoxy-bicyclonucleosides

Entry	Structure	Numbering [a]	Nucleoside conformation parameters				
			χ	γ	ν_{max}	*P*	Puckering
1[b]		**9** (U), **11** (T), **13** (C), **16** (A), **19** (G)	-73°	*trans*	34.5°	91.7°	$O^{4'}$-*endo*, ^{0}E (East)
2[c]		**29** (T), **30** (U), **32** (C), **34** (A), **35** (G)	nd	nd	17.2°	107.2°	$C^{1'}$-*exo*, $^{0}_{1}$T (East)
3[b]		**47**	-148.1°	nd	10.1°	159.8°	$C^{2'}$-*endo*, ^{2}E (South)
4[b]		**48** (C) **50** (A)	-130.7°	nd	25.3°	135.6°	$C^{2'}$-*endo*, $_{1}$E (South)
5[b]		**114** (T) **118** (A)	-157.1°	*trans*	22.9°	228.1°	$C^{4'}$-*endo*, ^{4}E
6[c,d]		**255** (U) **256** (T) **259** (C)	nd	nd	39.0°	112.0°	$C^{1'}$-*endo*, $^{0}_{1}$T (East)
7[b]		**271**	-154°	59°	27°	86°	$O^{4'}$-*endo*, ^{0}E (East)
8[b]		**273**	-141°	60°	39°	89°	$O^{4'}$-*endo*, ^{0}E (East)
9		**311** (U), **365** (T) **372** (A) **373** (I)	*anti*	nd	nd	nd	$C^{2'}$-*exo* $C^{3'}$-*endo*
10[c]		**351**	-141°	57°	27°	79°	$O^{4'}$-*endo* ^{0}E (East)

[a] Underlined numbers correspond to the analyzed compound. [b] From X-ray data analysis. [c] From *ab initio* calculation or energy minimization. [d] From NMR data analysis.

8.2. Conformation and Biological Properties of 3′-Hydroxy-bicyclonucleosides

8.2.1. Conformation Analysis

There are two main classes of 3′-hydroxy-bicyclonucleosides whose structural parameters are collected in Tables 2 and 3, respectively. This distinction is based on the nature of the sugar/phosphate backbone torsional angles γ/δ or γ alone involved in the bicyclonucleoside structures. These analogues have been designed to reduce the conformational states of nucleosides in order to increase their duplex formation ability via an entropic benefit, when included in oligonucleotides.

In Table 2 are summarized the data obtained mainly from the pioneering work of Leumann in the early 1990s to adapt the preorganization concept to the world of nucleic acid chemistry. These γ,δ-constrained nucleoside analogues (bc- and tc-DNA) were designed with the special aim of reducing the sugar puckering in the B-type DNA conformation (South, C2′-*endo*) and of restraining the flexible C4′−C5′ bond, which should highly preorganize the single-stranded nucleic acid and enhance the duplex formation ability.[9e]

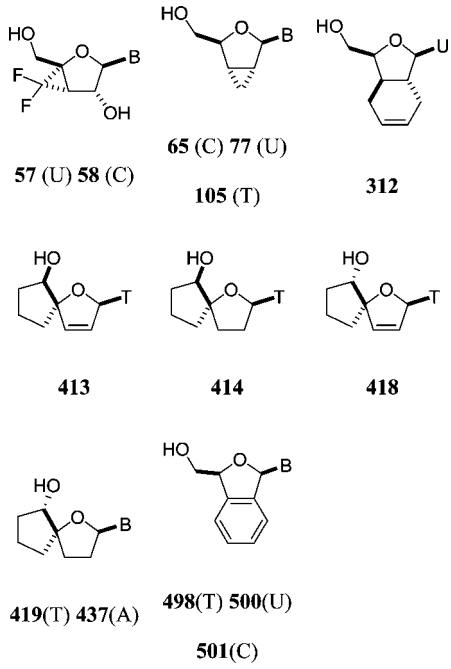


Figure 11. 3′-Deoxy-bicyclonucleosides without reported conformational parameters.

More recently, Poul Nielsen revisited the synthetic approach of bc-DNA leading to the preparation of bicyclonucleosides in the *ribo* series with a highly pronounced South conformation with a *P* of 180° (Table 2, entry 1) and a γ torsion angle of 90°, close to the canonical *gauche*(+) value.[38] Removal of the double bond in the fused cyclopentane ring made the γ torsion angle switch to a nearly *trans* conformation as observed for Leumann's bc-DNA (150°, Table 1, entries 2 and 3).[53] The latter are in a C1′-*exo* conformation, halfway between an East and South sugar conformation ($P = 128°$) but with a γ torsion angle that does not fit properly with the geometry observed in A- or B-type DNA duplexes. Surprisingly, the introduction of an amino substituent in the *R* configuration on the carbocyclic ring of bc-DNA seemed to locate γ in the *gauche*(+) range, but this was deduced from an analysis of a precursor and not from the nucleoside itself (entry 4).[57] However, the sugar puckering was fixed in the South conformation with the introduction of a benzyloxime in the same position, and the γ torsion angle was corrected in the *gauche*(+) range (entries 5 and 6).[58] Configuration inversion at C5′ drew back γ to around the *gauche*(−) range, while the sugar puckering was recovered in C1′-*exo* as observed for its 5′ epimer (entries 7 and 8 vs 3).[55] To try to circumvent the problem arising from the inadequate γ torsion angle in bc-DNA, a cyclopropane ring was introduced to obtain tc-DNA in which the South conformation of the furanose ring was more pronounced and the value of γ was slightly corrected (entry 10).[56] Very recently, bc^{4,3}-DNA, a ring-enlarged analogue of bc-DNA, was developed.[70] In this structure, the γ angle presented a *gauche* (+) conformation and the furanose moiety showed a South-type sugar pucker (entry 9). Finally in this class of compound, Poul Nielsen also proposed a tricycle system to restrain the nucleoside perfectly in the C2′-*endo*/C3′-*exo* conformation but with γ in a *trans* configuration (entry 11).[40] To summarize the results of this approach, consisting of looking for analogues fitting the geometry of nucleotides in DNA duplexes, so far none of them exhibit all the required structural parameters in the same structure.

On the other hand, the conformational parameters of carbo bicyclonucleoside analogues of LNA and ENA are collected in Table 3, together with those of two pure δ-constrained nucleosides (LNA and ENA are nucleosides in which both the δ torsional angle and the ν_2 of the furanose ring are constrained). These compounds were developed essentially by Chattopadhyaya's group[45,48,50] and to a lesser extent by Nielsen's group[65] with the aim of providing conformationally restrained nucleosides in the North conformation with the special feature of a high level of substitution on the carbocycle in order to fine tune the electrostatics and hydration around the internucleotidic phosphate linkage.

As summarized in Figure 10, and with careful attention to the *P* values reported in Table 3, it can be seen that carba-LNA and -ENA nucleosides analogues (furanose C2′ connected to C4′ via an ethylene or propylene bridge, respectively) are all locked in the North conformation with an average *P* value around 20°. When determined, the relative position of the base with respect to the furanose ring is *anti* and the torsional angle γ populates the *gauche*(+) range as classically observed in natural nucleosides. Therefore, carbocycle substitutions with hydrophilic functions (Table 3, entries 4−7) or hydrophobic functions (Table 3, entries 1−3, 12, and 13) have no influence on either the sugar pucker or its amplitude maximum. The size of the carbocycle (from five to six carbons) does not change these values either (Table 3, entries 2 vs 18 and 3 vs 21). The two exceptions in this family of δ-constrained nucleosides are the three-carbon 3′,4′-linked bicyclic nucleosides **282** and **285**, which pre-

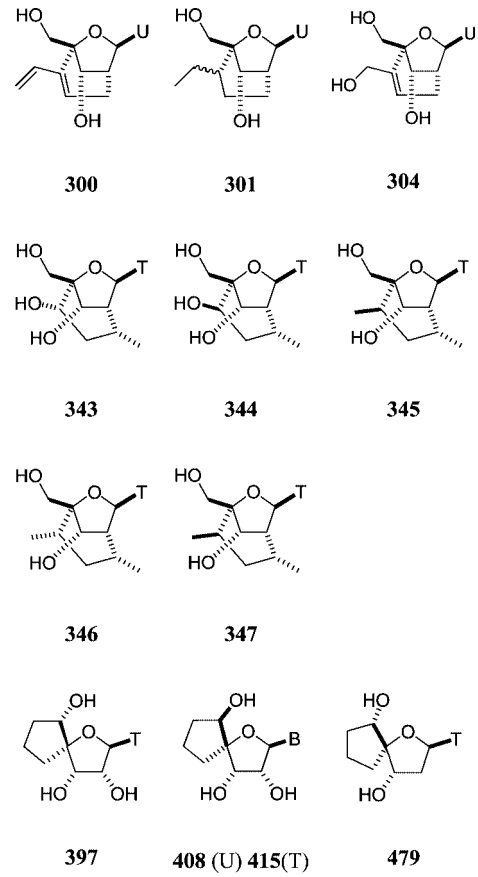
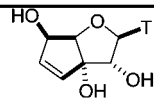
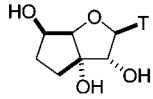
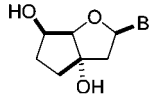
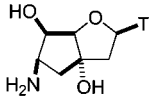
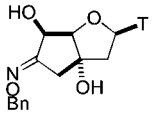
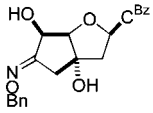
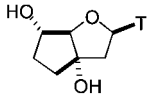
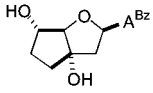
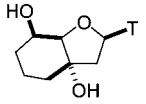
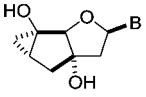
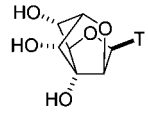


Figure 12. 3′-Hydroxy-bicyclonucleosides with no reported conformational parameters. Nucleosides are represented in their fully deprotected form.

Table 2. Structural Parameters of γ,δ-Constrained 3′-Hydroxy-bicyclonucleosides

Entry	Structure	Numbering[a]	Nucleoside conformation parameters				
			χ	γ	ν_{max}	*P*	Puckering
1[c]		**129**	nd	90°	nd	180°	$C^{2'}$-*endo*, $^{2}_{3}$T(South)
2[d]		**130**	nd	150°	nd	nd	$C^{1'}$-*exo*, $_{1}$E
3[b]		**131** (T) **202** (C) **203** (A) **204** (G)	*anti*	149°	nd	128.0°	$C^{1'}$-*exo*, $_{1}$E
4		**235**	nd	g^{+}	nd	nd	nd
5[b]		**241**	-118.1°	88°	nd	163.0	$C^{2'}$-*endo*, ^{2}E
6[b]		**246**	-167.7°	86°	nd	180	$C^{2'}$-*endo*, $^{2}_{3}$T(South)
7[c,d]		**208**	*anti*	g^{-}	nd	115.0°	$C^{1'}$-*exo*, $_{1}$E
8[c,d]		**213**	*anti*	g^{-}	nd	113.0°	$C^{1'}$-*exo*, $_{1}$E
9[b]		**323**	-105°	70°	nd	174°	$C^{2'}$-*endo*, ^{2}E
10[b]		**223** (T) **224** (C^{Bz}) **225** (A^{Bz}) **226** (G^{iBu})	-140°	130°	nd	154°	$C^{2'}$-*endo*, ^{2}E (South)
11[b]		**136**	-151.2°	*trans*	42.8°	182.2°	$C^{2'}$-*endo*, $^{2}_{3}$T(South)

[a] Underlined numbers correspond to the analyzed compound. [b] From X-ray data analysis. [c] From *ab initio* or energy minimization calculation. [d] From NMR data analysis.

sented a sugar puckering conformation close to South (Table 3, entries 16 and 17) showing that the furanose conformation was governed by the fused cyclohexane ring even in the *ribo* series.

There are a few 3′-hydroxy-bicyclonucleosides that have not been studied at the structural level (Figure 12). It is reasonable to speculate that the carba-ENA analogues **300**, **301**, **304**, and **343**−**347** should exhibit the same conformational characteristic as **339** and therefore be restrained in the North conformation, but this is still to be demonstrated. There are no conformational or duplex formation ability data available for the spiro-nucleosides **397**, **408**, **415**, and **479**. Once again, this is unfortunate because they are γ-constrained structures that could provide a very interesting new insight to understand the relative importance of γ with respect to the other angles, and they represent the missing link between the γ,δ-constrained and δ-constrained nucleosides.

Table 3. Structural Parameters of δ-Constrained 3′-Hydroxy-bicyclonucleosides[c]

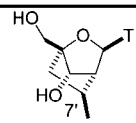
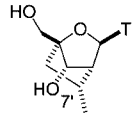
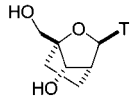
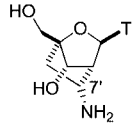
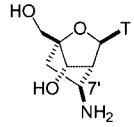
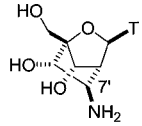
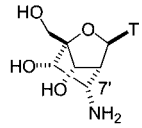
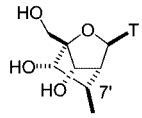
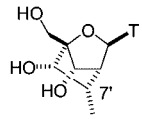
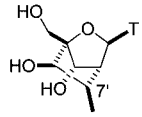
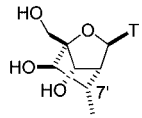
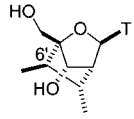
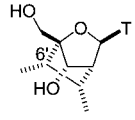
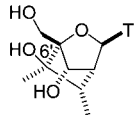
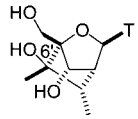
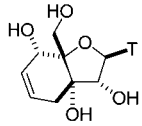
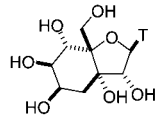
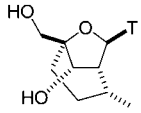
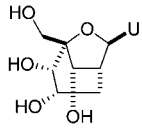
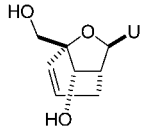
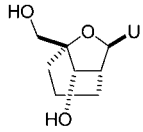
			Nucleoside conformation parameters				
Entry	**Structure**	**Numbering**	**χ**	**γ**	**ν_{max}**	***P***	**Puckering**
1[a,b]		(*7′S*)-**156**	-164.3°	g^+	47.5°	14.2°	$C^{3'}$-*endo*, ^3E
2[a,b]		(*7′R*)-**156**	-161.3°	g^+	57.8°	15.8°	$C^{3'}$-*endo*, ^3E
3[a]		**176**	nd	nd	54.9°	16.3°	$C^{3'}$-*endo*, ^3E
4[a]		**172** (*7′R*)	nd	nd	56.0°	26.1°	$C^{3'}$-*endo*, ^3E
5[a]		**173** (*7′S*)	nd	nd	55.9°	17.1°	$C^{3'}$-*endo*, ^3E
6[a]		**175** (*7′R*)	nd	nd	54.0°	15.0°	$C^{3'}$-*endo*, ^3E
7[a]		**174**(*7′S*)	nd	nd	54.5°	24.6°	$C^{3'}$-*endo*, ^3E
8[a]		**158** (*7′R*)	-163.0	g^+	55.7°	16.6°	$C^{3'}$-*endo*, ^3E
9[b]		**157** (*7′S*)	-167.7°	g^+	55.5°	18.5°	$C^{3'}$-*endo*, ^3E
10[b]		**160** (*7′R*)	-163.0°	g^+	55.7°	16.6°	$C^{3'}$-*endo*, ^3E
11[a]		**159** (*7′S*)	-167.7°	g^+	55.5°	18.5°	$C^{3'}$-*endo*, ^3E
12[a]		**163** (*6′R*)	-161.7°	g^+	56.6°	19.5°	$C^{3'}$-*endo*, ^3E
13[a]		**163′** (*6′S*)	-161.3°	g^+	56.6°	18.8°	$C^{3'}$-*endo*, ^3E

Table 3. Continued

Entry	Structure	Numbering	Nucleoside conformation parameters				
			χ	γ	ν_{max}	*P*	Puckering
14[a]		**162** (*6′R*)	-161.9°	g$^+$	56.9°	20.3°	C$^{3'}$-*endo*, ^{3}E
15[a]		**161** (*6′S*)	-163.0°	g$^+$	56.8°	22.0°	C$^{3'}$-*endo*, ^{3}E
16		**282**	nd	nd	nd	nd	C$^{3'}$-*exo*, $_3$E
17[a]		**285**	*anti*	g$^+$	46.7°	207.0°	C$^{3'}$-*exo*, $_3$E
18[a]		**339**	-150.0°	g$^+$	47.1°	19.4°	C$^{3'}$-*endo*, ^{3}E
19[a]		**297**	nd	nd	46°	7°	C$^{3'}$-*endo*, $^3_2$T(North)
20		**293**	nd	nd	49°	27°	C$^{3'}$-*endo*, ^{3}E
21		**294**	nd	nd	46°	15°	C$^{3'}$-*endo*, ^{3}E

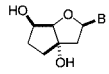
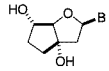
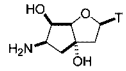
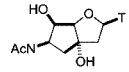
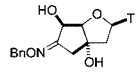
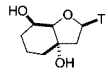
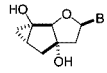
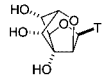
[a] From *ab initio* or energy minimization calculations. [b] From NMR data analysis. [c] Nucleosides are represented in their fully deprotected form.

8.2.2. Duplex Formation Ability

In this section, Tables 4 and 5 summarize the results in terms of thermal stability for γ,δ- and δ-constrained bicyclonucleosides within duplexes with cDNA or RNA strands. These properties have been studied in an antisense approach together with *exo*-nuclease resistance, RNase H activity induction, and miscellaneous properties by Leumann for the γ,δ-constrained nucleosides (bc-and tc-DNA) and by Chattopadhyaya/Poul Nielsen for the δ-constrained bicyclonucleoside analogues of LNA and ENA.

In the early 1990s, Leumann started to investigate the effect of structural preorganization of DNA single strands by incorporation of bicyclo-2′-deoxynucleosides **131** and **203**[98] (bc-DNA), and the study was later completed with the cytidine and guanidine derivatives **202** and **204**, respectively (Table 5, entry 1). As a first observation, the homopyrimidine bc-DNA strand made of **131** formed less stable duplexes with complementary poly(A) or dA_{10} than the homopurine bc-DNA strand made of **203** with complementary poly(U) or dT_{10} compared with the natural counterpart. In purine-rich sequences, bc-DNA induced a decrease in duplex stability, whereas it did not in pyrimidine-rich sequences. In mix-mer sequences, bc-DNA **131** induced a slight stabilization toward DNA complementary strands, while a decrease in T_m was observed with RNA counterparts. It is noteworthy that selectivity in base pairing is maintained with these analogues. Interestingly, it has been shown that bc-DNA has a strong preference for the Hoogsteen and reversed-Hoogsteen pairing mode over the Watson−Crick pairing mode originating from the 100° deviation of the γ torsional angle from the canonical value. The hybridization study on triple-helix formation with 15-mixed-sequence oligonucleotides containing bicyclothymidine **131** and bicyclodeoxycytidine **202** displayed increased thermal stability. Moreover, sequences including bc-

Table 4. Sequences and Variations in Melting Temperatures of γ,δ-Constrained Bicyclonucleoside-Containing Duplexes[g]

Entry	Structure and Numbering	Sequences (5'→3')[a]	ΔTm °C/mod /DNA	ΔTm °C/mod /RNA
		TTTTTTTTTT	-1.4/-2.0	-0.4
		AAAAAAAAAA	+0.1	+1.3
		CCCCCC	+0.3	nr
		GGGGGG	-2.4	-2.1
1[b,c]		**TTTTTTTTT**	-0.4/-0.6	nr
		GGATGGGAG	-1.7	-0.8
	131(T), **202** (C), **203** (A), **204** (G) (bc-DNA)	**CTCCCATCC**	-0.4	+0.3
		GGATG**TT**CTCGA	+0.6	-0.6
		GGATGTTC**T**CGA	+1.5	-0.5
		GGA**T**GTTC**T**CGA	+0.2	-0.7
		TTTTT**T**TTTT	-4.9	-4.9
		TTTT**TT**TTTT	-6.5	-6.6
		TTT**T**T**T**TTTT	-2.7	-5.1
2[e]		**TTTTTTTTTC**	Nd	Nd
	208 (T), **213** (A)	AAAA**A**AAAAA	-1.6	-2.7
		AAAA**AA**AAAA	-2.7	-2.3
		AAAA**A**A**A**AAA	-1.2	-1.5
		TTTTT**T**TTTT	-0.8	nr
3[f]		TTTT**TT**TTTT	-2.0	nr
	235	**T**T**T**T**T**T**T**T	+0.9	nr
		TTTTT**T**TTTT	-3.5	nr
4[f]		**T**T**T**T**T**T**T**T	-1.9	nr
	235 Ac			
		TT**TTTTT**TTT	-3.3	-0.3
5[b]		GGATG**TT**CTCGA	-2.8	-1.5
		GGATGTTC**T**CGA	-4.5	-5.5
	241 (bc[ox]-DNA)	GGA**T**GTTC**T**CGA	-4.5	-5.0
		GGA**T**GTTCTCGA	-1.4	-2.3
6[b]		GGATGTTC**T**CGA	-0.2	-0.8
		GGATG**TT**CTCGA	-0.3	+0.7
	323 (bc[4,3]-DNA)	AACTG**T**CACG	-3.0	-3.7
		p**T**T**T**T**T**T**T**	-2.5	-3.0
		p**TTTTTTTT**	-0.5(+1)[e]	+1.4(+1.4)[e]
		p**TTTTTTTT**	-0.45(+0.8)[e]	+1.7(+1.3)[e]
		p**AAAAAAAA**	+2.5(+3.2)[e]	+1.5(+1.7)[e]
		p**AAAAAAAAA**	+2.0(+2.6)[e]	+1.9(+2.2)[e]
7[b]		AACTGT**C**ACG	+2.4	+3.2
		AACTG**T**CACG	+0.9	+0.5
	223 (T), **224** (C), **225** (A), **226** (G) (tc-DNA)	AACTGTC**A**CG	-1.0	-0.4
		AACT**G**TCACG	+0.3	+1.0
		p**CGTGACAGTT**	+1.2	+2.4
		p**AACTGTCACG**	+1.1	+1.9
		CTGA**T**ATGC	-9.0	-10.0
8[d]		C**T**GA**T**A**T**GC	Nd	Nd
	136			

[a] Bold and underlined characters within sequences denote modified nucleotide. [b] T_m values measured as the maximum of the first derivative of the melting curve ($A_{260\ nm}$ vs temperature) in medium salt buffer 10 mM NaH_2PO_4, 150 mM NaCl, pH 7.0, using 2 μM concentrations of the two complementary strands. [c] Buffer 10 mM Tris, 150 mM NaCl, pH 7.0. [d] Buffer 10 mM NaH_2PO_4, 100 mM NaCl, 0.1 mM EDTA, pH 7.0, using 1.5 μM concentrations of the two complementary strands. [e] Buffer 10 mM NaH_2PO_4, 1 M NaCl, pH 7.0. [f] Buffer 10 mM sodium cacodylate, 150 mM NaCl, pH 7.0, using 4.0 μM concentrations of the two complementary strands. [g] Nd = no duplex observed; nr = not reported.

Table 5. Sequences and Variations in Melting Temperatures of δ-Constrained Bicyclonucleoside-Containing Duplexes[c]

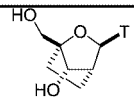
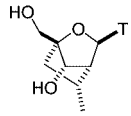
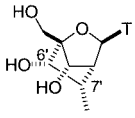
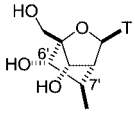
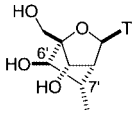
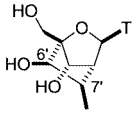
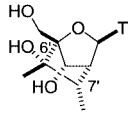
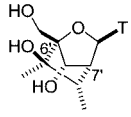
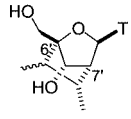
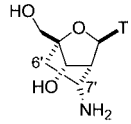
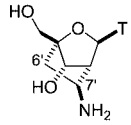
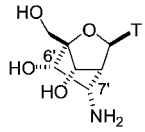
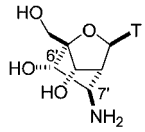
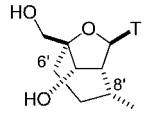
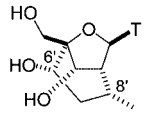
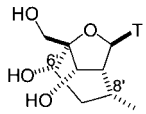
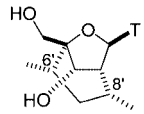
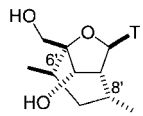
Entry	Structure and numbering	Sequences (5'→3') ΔTm °C/mod *vs.* DNA (*vs.* RNA)[a]			
		CTTCATTTTTTC**T**TC	CTTCATTTT**T**TCTTC	CTTCATT**T**TTTCTTC	CTTCA**T**TTTTTCTTC
1	 **176**	+0.6 (+3.2)	-0.8 (+3.8)	-1.9 (+3.8)	-2.1 (+2.7)
2	 **156**	0.0 (+3.5)	-1.0 (+5.0)	-1.0 (+4.0)	-2.0 (+3.5)
3	 **157**	+1.2 (+3.0)	+1.5 (+4.0)	+0.5 (+4.1)	-1.7 (+4.0)
4	 **158**	-0.7 (+1.0)	-2.1 (+1.5)	-3.1 (+1.5)	-2.6 (+2.0)
5	 **159**	+1.5 (+3.0)	+0.5 (+4.5)	0.0 (+4.5)	-1.5 (+4.0)
6	 **160**	-1.0 (+1.5)	-2.5 (+2.5)	-3.0 (+2.5)	-3.5 (+2.5)
7	 **161**	+2.0 (+2.5)	+1.0 (+3.5)	0.0 (+3.5)	-0.5 (+3.5)
8	 **162**	nr	+0.0 (+4.5)	-1.0 (+4.5)	-0.5 (+3.5)
9	 **163**	+0.5 (+2.5)	-1.5 (+3.5)	-2.0 (+3.5)	-3.0 (+3.5)
10	 **172**	+1.7 (+2.6)	+1.4 (+2.8)	0.0 (+2.6)	0.0 (+2.7)
11	 **173**	-0.6 (+0.2)	-1.6 (+0.5)	-2.5 (+0.9)	-2.9 (+1.0)

Table 5. Continued

Entry	Structure and numbering	Sequences (5'→3') ΔTm °C/mod *vs.* DNA (*vs.* RNA)[a]			
		CTTCATTTTTTC**T**TC	CTTCATTTT**T**TCTTC	CTTCATT**T**TTTCTTC	CTTCA**T**TTTTTCTTC
12	 **174**	+1.5 (+3.5)	+1.0 (+3.5)	-0.5 (+3.0)	-0.5 (+3.5)
13	 **175**	-0.5 (-1.0)	-1.5 (+1.5)	-2.0 (+1.5)	-2.0 (+2.0)
14	 **339**	-1.5 (+1.0)	-5.5 (+0.5)	-5.0 (+0.5)	-5.5 (+1.0)
15	 **343**	-1.5 (0.0)	-6.0 (-0.5)	-7.5 (-0.5)	-7.0 (+1.0)
16	 **344**	-2.0 (+1.0)	-3.3 (+1.0)	-3.5 (+1.0)	-4.5 (+1.5)
17	 **346**	-4.5 (0.0)	-3.0 (-1.0)	-8.5 (-1.0)	-8.5 (+0.5)
18	 **347**	-2.0 (+1.0)	-4.5 (+0.5)	-4.5 (+0.5)	-5.0 (+1.0)

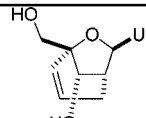
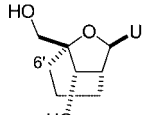
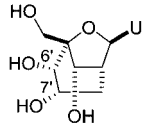
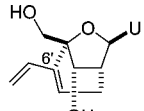
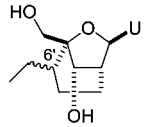
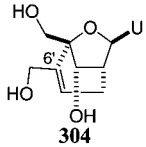
Entry	Structure and numbering	Sequences (5'→3') ΔTm °C/mod *vs.* DNA (*vs.* RNA)[b]	
		GTGA**U**ATGC	G**U**GA**U**A**U**GC
19	 **293**	-1.0 (+4.5)	-1.0 (+3.5)
20	 **294**	-1.0 (+4.0)	-2.3 (+2.3)
21	 **296**	-0.5 (+5.0)	-1.1 (+4.7)

Table 5. Continued

Entry	Structure and numbering	Sequences (5'→3') ΔTm °C/mod *vs.* DNA (*vs.* RNA)[b]	
		GTGA<u>U</u>ATGC	G<u>U</u>GA<u>U</u>A<u>U</u>GC
22	 **300**	-2.8 (+3.2)	-3.7 (+2.1)
23	 **301**	-2.8 (+3.8)	-2.7 (+2.8)
24	 **304**	-2.0 (+4.9)	-2.4 (+3.0)

[a] T_m values measured as the maximum of the first derivative of the melting curve ($A_{260\ nm}$ vs temperature) in medium salt buffer (60 mM Tris-HCl at pH 7.5, 60 mM KCl, 0.8 mM $MgCl_2$) using 1 μM concentrations of the two complementary strands. [b] Buffer 5 mM NaH_2PO_4, 100 mM NaCl, 0.1 mM EDTA, pH 7.0, using 1.5 μM concentrations of the two complementary strands. [c] **<u>T</u>** or **<u>U</u>** denotes the modified nucleotide; nr = not reported.

DNA showed an increase in resistance by a factor of 10−20 toward phosphodiesterase and were 8 times more stable than DNA in fetal calf serum (5′-exonuclease).

With a more deviating γ torsional angle (g^- compared with g^+ in naturally occurring DNA), they observed a negative effect on the duplex formation ability of bc-DNA **208**- and **213**-containing homothymidine or homoadenine, respectively (Table 4, entry 2).[55] The decrease in thermal stability is comparable with DNA and RNA complementary strands. Interestingly, although no duplex formation was observed with an undecamer with ten bc-DNA **208**, it turned out that it was 1.4 times more resistant toward snake-venom phosphodiesterase than a decamer containing its bc-DNA **131** epimer.

The 6′-amino-functionalized bc-DNA **235** was incorporated once, twice, and five times in a deca-thymidylate (Table 4, entry 3).[57] With one and two modifications, a destabilization of the duplex with the cDNA at neutral pH was observed (ΔT_m/mod. = −0.8 and −2.0 °C, respectively) whereas with the alternated five modifications, the thermal stability of the DNA−DNA duplex was increased (ΔT_m/mod. = +0.9 °C). When pH increased, this positive effect decreased because of the amino group protonation. This was not observed with **235Ac** in which the amino group was acetylated (Table 4, entry 4), but a strong destabilization was reported (up to −3.5 °C/mod) most probably due to steric hindrance between the 6′-acetylated amine group and the phosphate backbone bond.

More recently, Leumann's group has introduced bc^{ox}-DNA **241**[58] in which a lipophilic benzyloxime substituent is added on the carbocycle at the 6′-position of a bc-DNA thymidine. It was incorporated once, twice in mixed sequences, and five times in a decathymidylate (Table 4, entry 5). With cDNA, strong destabilization was noticed for one and two modifications when not consecutive. This loss in stability was reduced when the bc^{ox}-DNA **241** were positioned consecutively on the strand, maybe because of the favorable hydrophobic interaction of the oxime substituents. This phenomenon was amplified with complementary RNA with a gradually reduced destabilization from −5.5 to −0.3 °C per modification. Finally, this modification helped transfection of oligonucleotides with lipofectamine compared with unmodified DNA.

Finally, bc^{4,3}-DNA **323**, a ring-enlarged analogue of bc-DNA, has very recently been described.[70] Only a slight reduction in thermal stability was induced by bc^{4,3}-DNA **323** within oligonucleotides against DNA or RNA complementary strands (Table 4, entry 6). Consecutive modifications led to a lower destabilization against DNA and to a moderate stabilizing effect against RNA. While there was only a minor variation in terms of duplex stability relative to bc-DNA, the high discrimination level observed with bc-DNA was reduced with bc^{4,3}-DNA **323**.

The second generation of conformationally constrained cyclonucleosides (tc-DNA **223**−**226**, the most structurally complex DNA analogues synthesized so far) developed by Leumann's group arose from the intention of correcting the imperfect γ torsional angle of bc-DNA.[56] Homobasic thymine and adenine containing **223** and **225** nona- and octamers, respectively, led to the formation of very stable duplexes with cDNA and RNA polymers in high salt concentration (values in brackets), whereas at more physiological ionic strength, this stabilization was slightly lowered for tc-DNA **225** and a moderate destabilization was noticed for tc-DNA **222** only against DNA complement (Table 4, entry 7). Interestingly, in a duplex formation study with the four modified DNA decamers containing a single tc-DNA modification (**223**, **224**, **225**, or **226**) and cDNA and RNA, a pronounced increase in the stability of both duplexes with tc-DNA **223** and **224** (ΔT_m/mod. = +0.4 to +3.2 °C) was reported compared with tc-DNA **225** and **226** (ΔT_m/mod. = −1.0 to +1.0 °C). The fact that the weaker intrastrand base stacking of pyrimidine bases was more efficiently compensated by the rigid backbone of the sugar ring could explain these observations. As a general picture, it emerges that pyrimidine tc-DNA nucleotides stabilized duplex formation with a complementary strand more than purine tc-DNA nucleotides did and that tc-DNA within an oligonucleotide

favored duplex formation to a greater extent with RNA complement than with DNA complement. This point was evidenced by the high thermal stability measured for two complementary fully decamer modified tc-DNA containing all four bases in a random distribution (Table 4, entry 7). Moreover, in mismatch discrimination experiments, it appeared that tc-DNA recognized complementary RNA with increased selectivity compared with natural DNA and RNA. Therefore, tc-DNAs represent A-type DNA mimics. An explanation can be found in the compensatory effect of the cyclopropane ring on torsional angles β and γ and in the particular structural features of tc-DNA (sugar pucker, glycosidic angle) for their significantly enhanced duplex formation ability with RNA complementary strands.[99]

As for its precursor dc-DNA, tc-DNA **223** within 15-mer-sequence oligonucleotides has been evaluated for triple-helix formation. An effect of sequence composition on triplex stabilization was observed, which may originate from differences in conformation of the target duplex or third strand. Finally, tc-DNAs were investigated as siRNA modifiers. It was found that modifications close to the 3′-end, as well as modifications at three to four additional positions either in the 3′- or 5′-end of the sequence were tolerated and, in some cases, gave higher siRNA activity than wild-type RNA.[59]

It should also be noted that incorporation of these tricyclodeoxynucleotides into DNA provided complete resistance toward 3′- and 5′-exonucleases. The presence of steric hindrance due to the cyclopropane ring in close vicinity of the phosphodiester bond to be cleaved could be responsible for the greater nuclease resistance exhibited by tc-DNA compared with bc-DNA. Unfortunately, modified oligomers were not able to induce RNaseH cleavages, which make them poor candidates for antisense purposes, but it has been show that aberrant splicing can be corrected with tc-DNA-containing oligomers, which were 100-fold better than 2′-OMe phosphorothioate oligoribonucleotides.

The last member of the γ,δ-constrained nucleosides, tricyclic nucleoside **136** prepared by Poul Nielsen's group, was incorporated once and three times into 9-mer DNA sequences (Table 4, entry 8).[40] Large decreases in thermal stability with cDNA and RNA were observed for a single monomer **136** incorporation ($\Delta T_m = -9$ and -10 °C, respectively), whereas no duplex formation could be detected when three monomers were included. This tricyclic nucleoside **136** adopts a perfect *S*-type conformation and therefore should at least favor the B-form found in DNA−DNA duplexes. However, the strong destabilization observed with the DNA strand could be explained by the highly locked structure of **136** in combination with an imperfect angle γ and a more restricted torsion angle χ leading to unfavorable base pairing. The additional ring and the presence of a free hydroxyl at C-7′ could induce bc-DNA steric hindrance and also have an impact on the duplex hydration.

Data about the thermal denaturation of native carba-LNA and -ENA analogue-modified oligonucleotides with their DNA or RNA complements are presented in Table 5. Eighteen analogues (thirteen carba-LNA and five carba-ENA) have been evaluated by single incorporation in four different positions within the same sequence by Chattopadhyaya's group (Table 5, entries 1−18),[45,48,50] whereas Poul Nielsen's group has modified once and three times the same sequence to determine the behavior of their six recently synthesized carba-ENA analogues (Table 5, entries 19−24).[65]

As a general picture, carba-LNA and -ENA emerged as analogues with high to very high duplex formation ability with RNA complements depending on the carbocycle substitution (ΔT_m/mod. up to +5 °C). On the other hand, the duplexes formed with DNA complements exhibited only a slight thermal stability improvement in a few cases (average ΔT_m/mod. = +0.8 °C, Table 5, entry 10) but more often a moderate to large destabilization (average ΔT_m/mod. up to −2.1 and −6.1 °C, for carba-LNA and carba-ENA, Table 5, entries 4 and 17, respectively). With a big set of carba-LNA analogues, an interesting study of the various impacts of hydrophilic and lipophilic ring substitutions became possible, giving new insight into the modulation of antisense activity.

The direct carba-LNA analogue **176** of LNA did not have the same potency to stabilize a DNA/RNA hybrid as its 2′-oxygenated counterpart but still had a reasonable effect (Table 5, entry 1, ΔT_m for LNA is 1 °C higher).[50] This result proved that the absence of a hydrophilic substituent at C-2′ has limited influence on the thermal stability of the duplex with complementary RNA. Affinity for the target RNA was favored by hydrophobic substituents positioned at C7′ and directed toward the minor groove on carba-LNA analogues (Table 5, entry 2 vs 1, entries 3 vs 4 and 5 vs 6). When the hydrophobic substituent is pointing at the vicinal phosphate 3′, it can impair the duplex stability by perturbation of the hydration network in the minor groove, more especially with RNA.[48,50]

Replacing the hydrophobic methyl group with a hydrophilic amino group led to a destabilization of the corresponding duplexes for the C7′ *S* configured analogues whereas their C7′ *R* epimers only induced a slight negative effect (Table 5, entries 2 vs 10 and 11 and 3 vs 12 and 13).

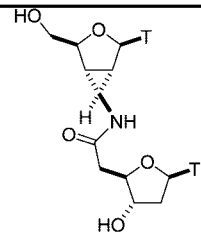
Substitution at C6′ with a hydrophobic methyl group had approximately no effect (entry 2 vs 9), whereas the presence of a hydrophilic function such as hydroxyl increased the duplex stability with both DNA and RNA complementary strands (Table 5, entries 3 and 5 vs 2 and 8 vs 9).

Therefore, the carba-LNA analogues **157** and **161** within oligomers were found to be more effective in enhancing duplex formation ability with RNA and, to a lesser extent, with DNA complements.

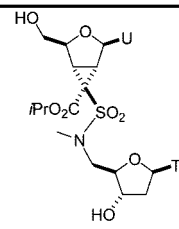
Carba-ENA analogues evaluated by Chattopadhyaya's group were all found to destabilize duplex formation with DNA (Table 5, entries 14−18) and to have a moderate positive impact toward RNA complements. They all bear a methyl substituent at C8′ oriented toward the minor groove of the duplex. In contrast to carba-LNA analogues, this substitution seemed to disfavor duplex formation when compared with unsubstituted carba-ENA (Table 5, entry 14 vs 19 and 20). These latter analogues exhibited promising properties with ΔT_m around +4 °C per modification when incorporated once and lowered to around +3 °C for three modifications when hybridized with RNA complements. The introduction of an extra hydrophilic or hydrophobic group in C6′ had a negative contribution when it was oriented toward the minor groove and associated with a methyl substituent at C8′ (Table 5, entries 15 and 17 vs 16 and 18). However, when these groups were directed to the vicinal phosphate, no change was observed (Table 5, entries 16 and 18 vs 14).

When examining the properties described for Poul Nielsen's carba-ENA analogues (Table 5, entries 19−24), one can see that the best result was obtained with a double electro-

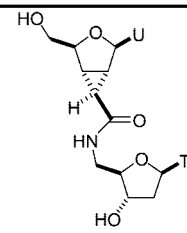
Table 6. Sequences and Variations in Melting Temperatures of Duplexes Containing Bicyclonucleosides with Amide Internucleotidic Linkage



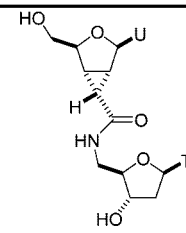
88



91



92 (*S* epimer)



93 (*R* epimer)

Sequences (5'→3')	ΔTm °C /mod *vs.* RNA[a]			
GCGTTTT**UT**TTTGCG	nr	-3.2	-6.2	-6.0
GCGTT**UTUT**TT**UT**GCG	nr	-4.0	-9.3	nr

[a] T_m values measured as the maximum of the first derivative of the melting curve ($A_{260\ nm}$ vs temperature) in medium salt buffer 10 mM NaH_2PO_4, 150 mM NaCl, pH 7.0, using 2 μM concentrations of the two complementary strands; nr = not reported.

philic substitution at C6′ and C7′ with the higher reported ΔT_m of +5 °C per modification (Table 5, entry 21), while a single hydroxymethyl substituent retained the initial stabilizing effect toward RNA but increased the destabilization observed with a DNA complement (Table 5, entry 24 vs 19). There was no destabilization effect of a lipophilic substitution at C6′ toward RNA complements, whereas the negative effect was more pronounced with DNA (Table 5, entries 23 and 22 vs 20).

Therefore, it is clear that the substitution of the extra carbocycle on carba-LNA and -ENA did not lead to the same behavior toward duplex formation ability. It turned out that C6′−C7′ substituted carba-LNA **157** and **161** together with unsubstituted carba-ENA **293**−**294** or C6′ substituted carba-ENA **296**, **300**, **301**, and **304** were worth further investigations and applications.

To have a good overview of their potential as antisense or siRNA therapeutic agents, 3′-exonuclease stability and the ability to elicit RNase H activity were investigated for carba-LNA **156**−**163** and **172**−**175** and carba-ENA **343**−**347**.[48] Analysis of the digestion patterns with snake venom phosphodiesterase showed that (i) all analogues bearing a methyl substituent at C6′ had an improved nuclease resistance by preventing the presence of a water molecule needed by the 3′-exonucleases to cleave the phosphodiester, (ii) the presence of a hydroxyl function at C6′ directed toward the vicinal scissile phosphate (carba-LNA **159**, **160**, and **162**) increased nuclease activity, and (iii) when the C6′ hydroxyl function pointed to the minor groove, improved nuclease resistance of the upstream 5-phosphate was observed.

Finally, it appeared that the RNase H activity of carba-LNA and -ENA modified oligonucleotides with the complementary RNA strand was very similar to that of the native counterpart and that the cleavage pattern of the hybrids was not affected by the nature of different substituents on the carbocyclic moiety but depended upon the site modification.

Taken together, this nice and complete study clearly highlighted the antisense potency of both 2′,4′-cis-fused bicyclic nucleosides carba-LNA and carba-ENA, even though the synthetic accessibility of these modified nucleosides is a major hindrance to further development.

8.3. Miscellaneous

In 1997, the incorporation of the conformationally rigid U−T dimers **91**, **92**, and **93** was reported. These contain cyclopropyl-amide and -sulfonamide functions in two 16-mer DNA sequences incorporating one or three dimers.[27] A strong decrease in thermal affinity toward complementary RNA was observed (see Table 6). Replacement of the P−O bonds of the phosphodiester internucleotidic bridge by the cyclopropane−amide bridge with shorter and much less flexible bonds could explain this loss in duplex formation ability. Due to these disappointing results, an analysis of the RNase H activity and nuclease stability of these modified oligonucleotides was not performed.

9. Conclusions

Since the synthesis of C2′−C3′ methano-cytidine in 1989, being the first bicyclonucleoside with an alkylene group between two carbon atoms of the glycone moiety, significant progress has been accomplished in this field, as presented in this review. The syntheses of various types of bicyclonucleosides using many different strategies have been published. Nevertheless, as clearly illustrated here, many of these present not only a restricted access to bicyclonucleosides but also a lack of stereocontrol, particularly in the *N*-glycosidation step, which still remains a crucial issue. Despite the structural diversity of the nucleoside analogues described in this review, none of the compounds evaluated for their antiviral and antitumor cell properties exhibited significant activity. Moreover, in many syntheses, the total number of steps is a weak point in the preparation of these potentially biologically active molecules in gram quantities for evaluation in the field of antisense therapy, as well as in Medicinal Chemistry.

In the future, the synthesis of new modified bicyclonucleosides, especially with a bridge at C1′, will be a challenge for synthetic organic chemists and an opportunity for biological investigations.

10. Abbreviations

Ac	acetyl
ADA	adenosine deaminase
AD-mix	asymmetric dihydroxylation mixture
All	allyl
AIBN	2,2′-azobisisobutyronitrile
aq.	aqueous
bc-DNA	bicyclo[3.3.0]-deoxyribonucleoside
$bc^{4,3}$-DNA	bicyclo[4.3.0]-deoxyribonucleoside
bc^{ox}-DNA	benzyloxime-bicyclo[3.3.0]-deoxyribonucleoside
9-BBN	9-borabicyclo[3.3.1]nonane
Bn	benzyl
BNA	bridged nucleic acid
BOM	benzyloxymethyl
BSA	*N*,*O*-bis(trimethylsilyl)acetamide
Bu	butyl
Bz	benzoyl
CAN	ceric ammonium nitrate
carba-LNA	C2′−C4′-methylene locked nucleic acid
carba-ENA	C2′−C4′-ethylene bridged nucleic acid
cat.	catalytic
conc.	concentrated
DABCO	1,4-diazabicyclo[2.2.2]octane
dba	dibenzylidene-acetonyl
DBU	1,8-diazabicyclo[5.4.0]undecen-7-ene
DCC	dicyclohexylcarbodiimide
DCE	1,2-dichloroethane
ddC	2′,3′-dideoxycytidine
ddI	2′,3′-dideoxyinosine
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
$(DHQ)_2PHAL$	1,4-bis(dihydroquininyl)phthalazine
DIBAL-H	diisobutylaluminium hydride
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMP	Dess−Martin periodinane
3,5-DMP	3,5-dimethylpyrazole
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
ΔT_m/mod.	[T_m (modified oligonucleotide) − T_m (wild-type)]/(number of modifications)
d4A	2′,3′-didehydro-2′,3′-dideoxyadenosine
d4T	2′,3′-didehydro-2′,3′-dideoxythymidine
EC_{50}	half-maximal effective concentration
ee	enantiomeric excess
ENA	O2′−C4′-ethylene bridged nucleic acid
equiv	equivalent
Et	ethyl
HIV	human immunodeficiency virus
HLE	hog liver esterase
HMDS	1,1,1,3,3,3-hexamethyldisilazane
HMPA	hexamethylphosphoramide
HMPT	hexamethylphosphorous triamide
HPLC	high-pressure liquid chromatography
Imid	imidazol-1-yl
iPr	isopropyl
LAH	lithium aluminum hydride
LDA	lithium diisopropylamide
LNA	locked nucleic acid
MCPBA	*m*-chloroperoxybenzoic acid
Me	methyl
MMTr	monomethoxytrityl
MOM	methoxymethyl
Ms	mesyl
MW	microwave
NBS	*N*-bromosuccinimide
nd	not determined
NIS	*N*-iodosuccinimide
NMO	*N*-methylmorpholine-*N*-oxide
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
NOS	*N*-hydroxysuccinimide
PMB	*p*-methoxybenzyl
PCC	pyridinium chlorochromate
Ph	phenyl
PTC-Cl	phenoxythiocarbonylchloride
PTSA	*p*-toluenesulfonic acid
quant.	quantitative
RCM	ring-closure metathesis
RNA	ribonucleic acid
RNase H	ribonuclease H
RT	reverse transcriptase
Siamyl	*sec*-2-methyl-butyl
siRNA	silencer ribonucleic acid
TBAF	tetrabutylammonium fluoride
TBD	1,5,7-triazabicyclo[4.4.0]dec-5-ene
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
TES	triethylsilyl
*t*Bu	*tert*-butyl
tc-DNA	tricyclo-deoxynucleoside
Tf	trifluoromethanesulfonate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPDS	tetraisopropyldisiloxan-1,3-diyl
T_m	melting temperature of the duplex formed with their RNA or DNA complements
TMS	trimethylsilyl
TMSOTf	trimethylsilyl trifluoromethanesulfonate
Ts	tosyl
XTT	sodium 3,3-{1-[(phenylamino)carbonyl]-3,4-tetrazolium}-bis(4-methoxy-6-nitro)benzene sulfonic acid hydrate

11. Acknowledgments

The authors are deeply indebted to the "Ministère de l'Enseignement Supérieur et de la Recherche", the "Centre National de la Recherche Scientifique" (CNRS), the "Université de Nantes", and the "Université de Toulouse" for their constant support. We also wish to thank Carol Wrigglesworth (Scientific English, Nantes, France) for linguistic assistance. One of us (J.L.) thanks his colleague Dr. André Guingant (CEISAM-UMR-CNRS 6320) for helpful discussions and encouragement.

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CR800465J