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Spirocyclic Restriction of Nucleosides

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The concept of spirocyclic restriction as applied broadly to the field of nucleoside mimics makes possible the generation of diastereomeric pairs configured with a *syn*- or *anti*-oriented hydroxyl substituent at C5′. The development of concise synthetic routes to spiro-fused nucleosides bearing all possible natural bases and expectedly capable of enforcing a conformation favourable for duplex formation on incorporation into oligomers represents a significant new direction in the design of antisense molecules. The present overview describes the convenient approaches that have been developed in this laboratory for accessing varied members of this class, including analogues that feature sulfur and carbon at the apical position.

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Introduction

The structural modification of nucleosides continues to represent a vibrant area of synthetic organic and bioorganic chemistry because select compounds of this class are amenable to the treatment of those diseases where the normal and diseased states differ with regard to the enzymes involved in the processing of nucleic acids. Viral diseases are recognized to often fit these criteria. Furthermore, the enzymes involved appear to have strict conformational requirements with respect to the geometry adopted by the furanose ring.[1] These fascinating interdependencies have prompted several research groups to undertake the preparation of modified nucleosides that feature restrictions in conformational flexibility in order to attain a more optimal level of puckering.[2] Bicyclo[3.1.0]hexane-[3,4] and bicyclo[3.3.0]octane-based carbocyclic mimics[5] have been examined as potentially useful pseudosugars. For reasons to be detailed below, we have independently pursued a comprehensive investigation of 4′-spirocyclic nucleosides that also include the thia and carba series. The synthetic component of this major undertaking is presently nearing completion and constitutes the basis of this report.

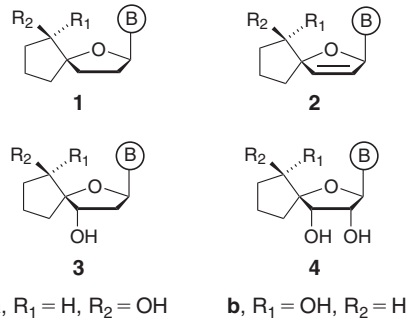

Diagram 1.

Why 4′-Spiroalkylated Nucleosides?

One may justifiably raise questions regarding the importance underlying 4′-spiroalkylated nucleosides. In fact, there are several quite relevant reasons why the unprecedented structural features resident in **1**–**4** (Diagram 1) might hold considerable potential. It will be recognized, for example, that the torsion angle about the C4′—C5′ bond of the furanose ring is necessarily fixed so that the key hydroxyl group at this site is now oriented specifically *anti* (**a** series) or *syn* (**b** series) to the furanose oxygen. Limitations are therefore placed on the magnitude of these torsion angles. Such constraints hold potential importance in modulation of the sugar–phosphate DNA backbone, and ultimately the secondary structure of DNA and base recognition.[6]

Extensive crystal structure data, most notably those for DNA and RNA fragments, reveal the existence of considerable void space (likely occupied by water molecules) in the region below C4′ of each nucleoside building block.[7] The area being referred to is sufficiently voluminous to accommodate more than the string of three methylene groups under consideration in **1**–**4**. Accordingly, there are no pertinent untoward concerns dealing with non-bonded steric superimposition.

The spirocyclic substitution pattern also precludes the possibility of free radical-induced degradation arising from hydrogen atom abstraction at C4′. The advantages offered by C4′ alkylation in warding off this destructive pathway have been responsible for the rapid rise in prominence of simpler analogues of this type.[8]

In addition to these features, the spirocyclic array resident in **1**–**4** provides an inherent atomic arrangement where some

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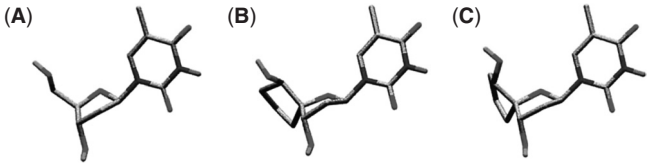
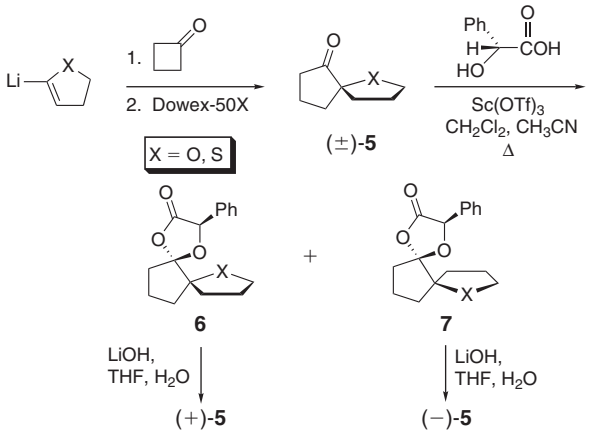


Fig. 1. The minimum-energy conformations of natural thymidine (**A**), **3a** (**B**), and **3b** (**C**).

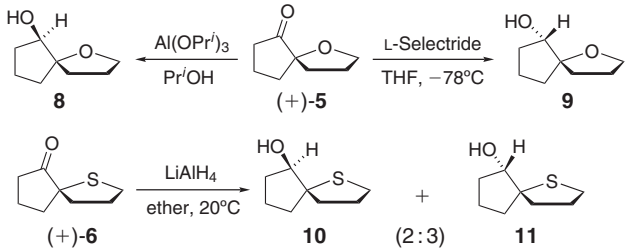
conformational biasing operates, but not to the level of structural rigidification. Fully optimized Amber calculations in the gas phase have made evident a striking similarity between the low-energy conformation adopted by natural thymidine (**A**) and those adopted by the two 1-oxaspiro[4.4]nonane isomers defined as **B** and **C** (Fig. 1).[9] The overlap of **B** on **A** is truly remarkable (root mean square 0.007). For **C**, the departure from direct superimposition is not as perfect (RMS 0.058), but nevertheless very acceptable. Note that this theoretical treatment projects the cyclopentyl hydroxyl group pseudoequatorially in both stereoisomeric series. Of additional interest is the topological change associated with epimers **B** and **C** ($\gamma = +sc$ and *ap*, respectively).

Preliminary Studies

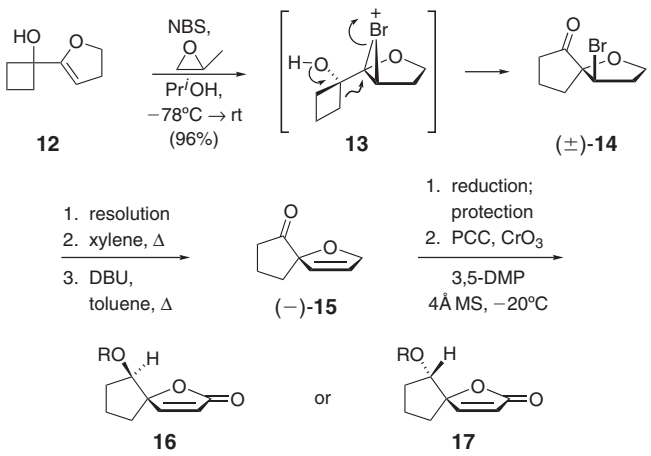
Several years ago, we reported the first examples of oxonium ion-initiated pinacolic ring expansion.[10–12] Subsequently, parallel studies revealed that thionium ions react in a parallel manner.[13] When 5-lithiated 2,3-dihydrofuran and 2,3-dihydrothiophene are reacted initially with cyclobutanone, the door is opened to a two-step entry to possible oxygen- and sulfur-containing spirocyclic precursors to **1–4** as reflected in the generation of **5**. The discovery that these ketones are particularly well suited to resolution via acetalization with (*R*)-(–)-mandelic acid under catalysis by scandium triflate[14,15] was deemed to be particularly advantageous to our goals (Scheme 1). Following the alkaline hydrolysis of **6**, it was discovered that the resulting (+)-**5** (X = O) could be reduced to **8** or **9** with essentially complete diastereoselectivity (Scheme 2).[14] The reduction of (+)-**5** (X = S) of 98% *e.e.* with lithium aluminium hydride in ether at 20°C was observed by us to proceed in a significantly less stereoselective fashion than previously reported.[16] The observed 2 : 3 partitioning of **10** and **11** was in fact welcomed by us since both diastereomeric series were accessed simultaneously, and **10** and **11** differ sufficiently in polarity to make their chromatographic separation routine.[15]



Scheme 1. Acetalization with (*R*)-(–)-mandelic acid.

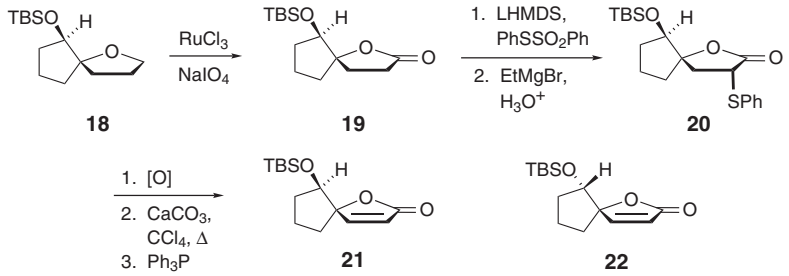


Scheme 2. Reduction stereoselectivities of spiroketones.



Scheme 3. Bromonium ion-mediated route to epimeric unsaturated lactones. NBS = *N*-bromosuccinimide, DBU = 1,8-diazabicyclo [5.4.0]undec-7-ene, PCC = pyridium chlorochromate, DMP = dimethylpyrazole.

Leo Paquette received his B.Sc. degree in chemistry magna cum laude from Holy Cross College in 1956 and his Ph.D. from MIT in 1959. After serving as a medicinal chemist at Upjohn (1959–1963), he joined the faculty at Ohio State University and was promoted to Professor in 1969. Subsequently, he held the Kimberly Professorship in chemistry (1981–1987) and now is a Distinguished University Professor there (1987–). He has held numerous Fellowships (Alfred P. Sloan Foundation 1965, Guggenheim 1976–1977, Senior Humboldt 1989) and an Awardee of the Japanese Society for the Promotion of Science (1992). On several occasions he has been honored by the American Chemical Society (Morley Medalist 1971, Columbus Section Award 1979, Award for Creative Work in Synthetic Organic Chemistry 1984, Arthur C. Cope Award 1987, Ernest Guenther Award 1992). He has also been the recipient of many international awards including, most recently, the Centenary Lectureship of the Royal Society of Chemistry and the S. T. Li Prize for Science and Technology. His current research interests focus on the total synthesis of natural and unnatural products, the development of synthetic methodology and catalytic asymmetric methods, and organometallic chemistry.



Scheme 4. Preferred route involving initial ruthenium-promoted oxidation to the lactone level. LHMDS = lithium hexamethyldisilazide, TBS = *tert*-butyl dimethylsilyl.

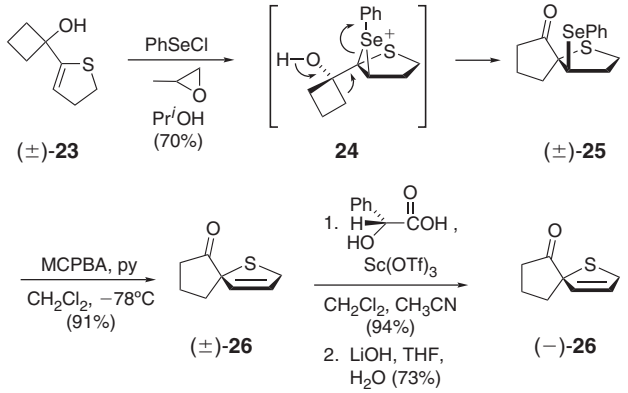
More Advanced Functionalization

The need for introducing higher levels of unsaturation into these spirocyclic intermediates was initially approached from two directions in both the oxygen and sulfur series. The first route entailed initiation of the rearrangement of **12** with NBS in isopropyl alcohol containing 10 equivalents of propylene oxide at −78°C to deter any acid buildup. The high degree of diastereoselectivity that leads to **14** can be rationalized in terms of the generation of bromonium ion **13**. Subsequent *anti* migration of the methylene unit with avoidance of unfavourable steric interactions leads to formation of the *syn* product (Scheme 3).[14] The resolution of **14** via transient coupling to Johnson's (*S*)-(+)-sulfoximine, E_2 elimination in the presence of DBU to give (–)-**15**, and regioselective oxidation of a protected form of the derived alcohols resulted in controlled formation of either **16** or **17**, both in enantiomerically pure form.[14]

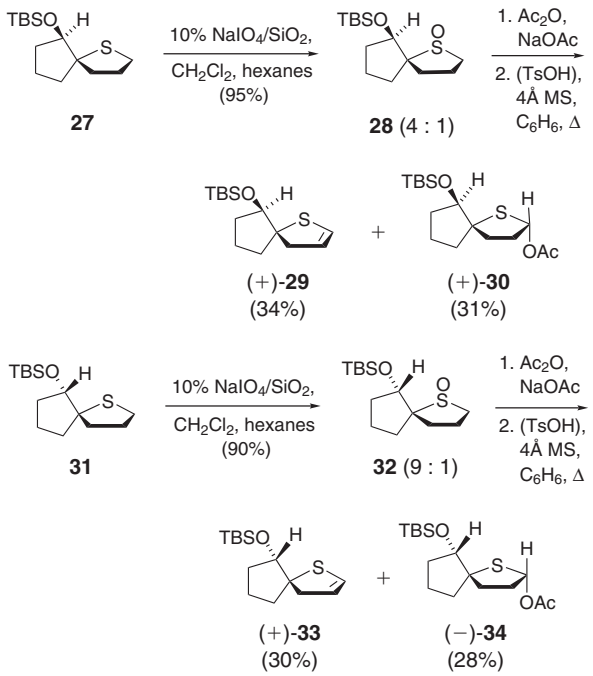
A more efficient pathway took advantage of the ease with which **18** (and its 5′-epimer) undergoes direct ruthenium tetraoxide oxidation to generate lactone **19** (Scheme 4). Ensuing two-step phenylsulfenylation[17] and sulfoxide elimination results in ready conversion into **21** (or **22**) in convenient fashion.[14,18]

An effective means for arriving at desirable intermediates in the thia series consists of phenylselenenyl chloride-initiated ring expansion/rearrangement of alcohol **23** (Scheme 5).[15] As in the preparation of (±)-**14**, it is imperative that propylene oxide be present as an acid scavenger. The conversion into **26**, best effected by chemoselective oxidation at selenium, was accomplished most effectively under basic conditions at high dilution.[19] This spiro ketone was resolved by acetalization with (*R*)-mandelic acid, purified by fractional crystallization, and subjected to alkaline saponification.

Formation of the TBS ethers of **10** and **11** provides substrates ideally suited to examination of the sulfoxidation reaction. As a direct consequence of inherent steric shielding, both **27** and **31** were found to be unreactive to an excess of MCPBA.[15] In remarkable contrast, alternative recourse to sodium periodate supported on silica gel[20,21] proved notably effective in oxidizing both tetrahydrothiophenes to **28** and **32** at the 10% level of loading (Scheme 6). Longer reaction times were required for the α-siloxy series. This kinetic retardation was accompanied by an increase in stereoselectivity from 4 : 1 to 9 : 1. The use of ammonium molybdate[22]



Scheme 5. Pathway to laevorotatory dehydro thia ketone **26**. MCPBA = *m*-chloroperbenzoic acid, THF = tetrahydrofuran.



Scheme 6. Pummerer approach to thionucleoside functionalization.

provided yet another convenient way to generate **28** and **32** without evidence of sulfone formation. With this reagent, diastereomer ratios did not exceed 3 : 2.

Pummerer reactions carried out on **28** and **32** in hot acetic anhydride containing sodium acetate[23] gave rise to complex

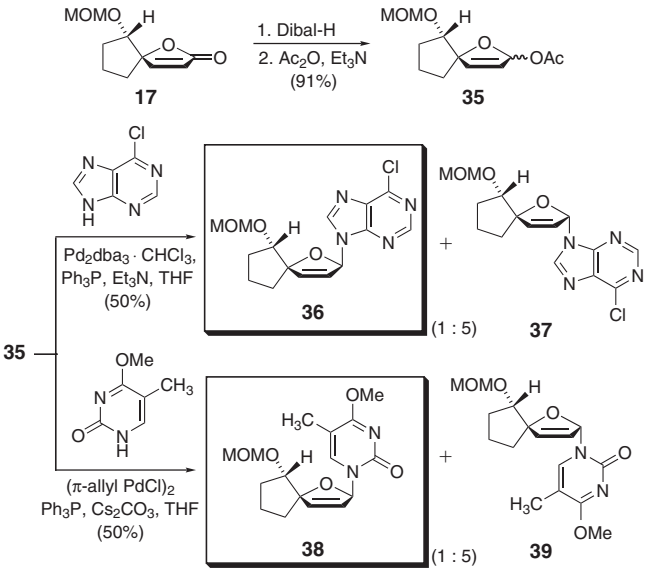
reaction mixtures, which when heated with *p*-toluenesulfonic acid in benzene containing 4 Å molecular sieves[24] converged to the elimination products **29** and **33** alongside acetates **30** and **34**. Each of the four thiospirans was formed in approximately 30% yield. The stereochemical assignments to **30** and **34** rest on the results of nuclear Overhauser effect measurements.[15]

Accessing Prototype Furanoside Mimics

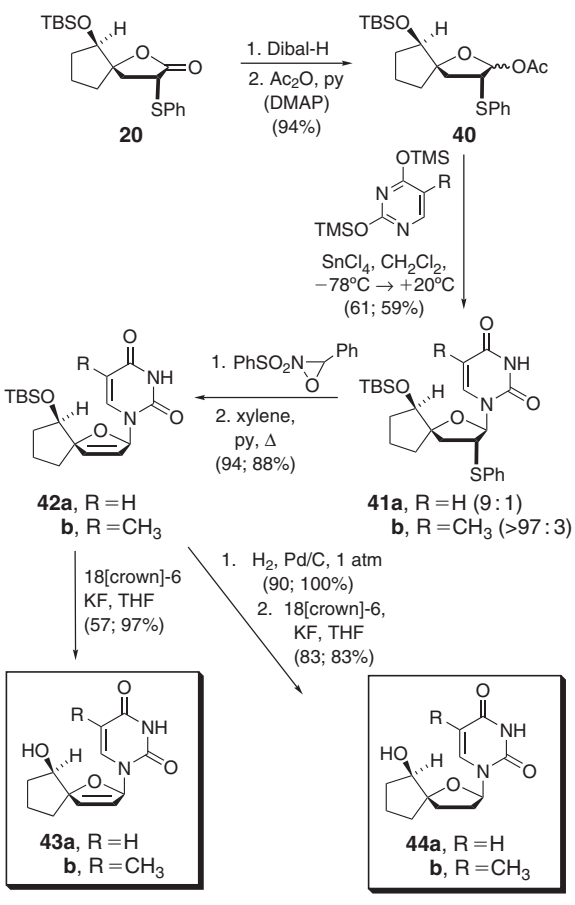
In our view, the developments defined above set a very solid foundation for arrival at nucleoside mimics endowed with oxygen or sulfur as the central heteroatom. The one remaining concern involved the possible inhibitory steric effect induced by the protected 5′-hydroxyl on proper installation of the nucleosidic bond. In an effort to gain an appreciation of operational factors, lactone **17** was reduced to its lactol and *O*-acylated to give **35** (Scheme 7). The stereodisposition of the acetate functionality in **35** could not be ascertained spectroscopically. We reasoned that if hydride delivery to **17** had occurred predominantly *anti* to the MOM group, then conversion to a π-allylpalladium species and subsequent coupling to a heterocycle should be met with overall retention.[25] In the present example, coupling of **35** to 6-chloropurine in the presence of $Pd_2(dba)_3 \cdot CHCl_3$, triphenylphosphine, and triethylamine brought about smooth conversion into a 1 : 5 mixture of **36** and **37**. When *O*-methylthymine was involved, a 1 : 5 mixture of spironucleosides was again produced, and the major anomer was confirmed to be **39** by X-ray crystallography.[26]

The implications that were drawn in the light of these findings were that spirocyclic system **35** undergoes these palladium-catalyzed reactions productively and that the DIBAL-H reduction/*O*-acetylation sequence delivers predominantly the α-acetate. The need for a directing group was obvious. To this end, recourse was made to the readily available α-phenylthio lactone **20**, submission of which to the same two-step protocol afforded an anomeric mixture of **40** (Scheme 8).[18] The expectation was that $SnCl_4$-promoted coupling reactions between **40** and persilylated uracil or thymine[27,28] would foster the intervention of an episulfonium ion intermediate[29] and/or complexation of the tin salt at the sulfur atom[30] and eventuate in selective formation of the desired 1,2-*trans* glycosyl bond.[31–34] While **41a** was formed as a 9 : 1 mixture rich in the β-anomer, the somewhat more bulky thymine reagent gave rise uniquely to **41b** in line with customary steric effects.

Following arrival at **41a** and **41b**, two avenues were pursued to remove the phenylthio substituent. The oxidative option involved conversion into the sulfoxide with the Davis oxaziridine reagent[35] and subsequent thermal extrusion of phenylsulfenic acid in the presence of pyridine. The unprotected didehydrodideoxy nucleosides **43a** and **43b** were generated without evidence of epimerization by reaction with potassium fluoride in THF.[18] As indicated in Scheme 8, saturation of the dihydrofuran double bond in these systems was performed most efficaciously while the TBS group remained installed.

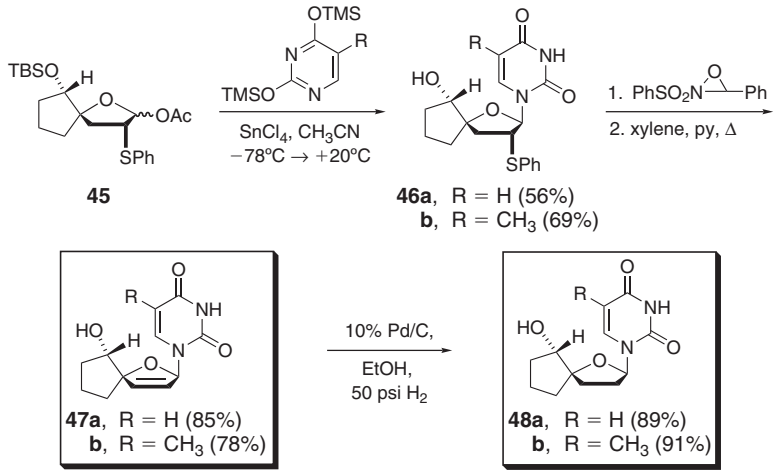


Scheme 7. Synthesis of the first *syn*-dideoxy nucleoside. MOM = methoxymethyl, dba = dibenzylideneacetone, Dibal-H = diisobutyl aluminium hydride.

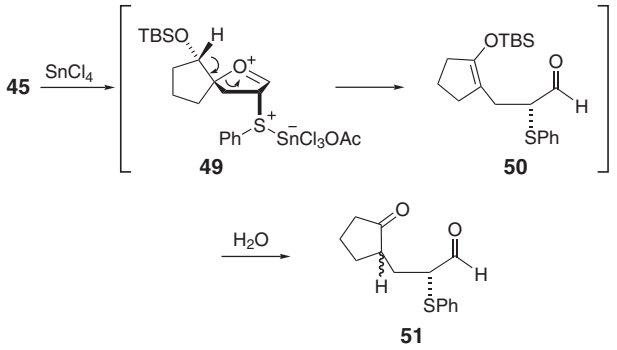


Scheme 8. Nucleoside formation involving **40**. DMAP = 4-dimethylaminopyridine.

The parallel conversion of **45** to **47** and **48** proved initially to be problematic, as reflected in the low yields (<25%) incurred during introduction of the nucleobases (Scheme 9). It was soon discovered that **45** is prone to a fragmentation



Scheme 9. Nucleoside formation involving **45**.

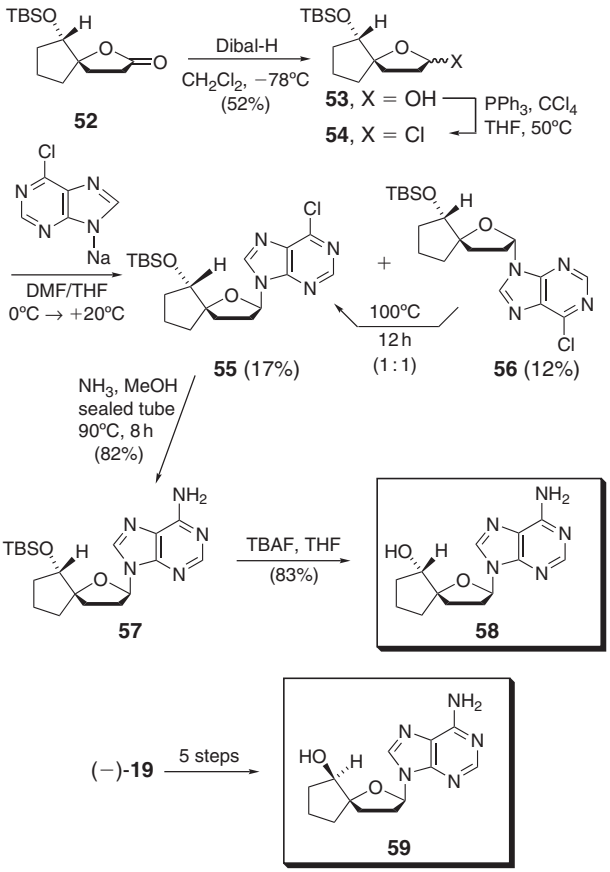


Scheme 10. Ring cleavage of **45**.

reaction not operative in **40**. Evidently, the stereoelectronic features resident in **45** are conducive to operation of an E_2 elimination (Scheme 10). Once this irreversible step operates, the resulting silyl enol ether functionality in **50** becomes subject to hydrolysis during the aqueous workup with liberation of the cyclopentanone carbonyl group in **51**.

Matters were significantly upgraded when the glycosylation step was performed instead in acetonitrile. This solvent switch was met with unexpected loss of the TBS protecting group to give **46** directly. The advantages offered by this eventuality were further reinforced by the finding that thermal elimination of the derived sulfoxides gave rise to **47** and ultimately **48** in an efficient manner.

A creditworthy attribute of the global research plan is its potential for the introduction of purine bases into target nucleosides. This is notable because, with very few exceptions, standard approaches to C4′-substituted nucleosides all derive from the Cannizzaro reaction of a thymidine-based 5′-aldehyde followed by reoxidation under somewhat forcing conditions. The vigorous nature of the chemistry employed in this sequence, using existing nucleosides as starting materials, restricts it to the more robust bases. The end result is that it is very unusual to see 4′-substituted systems with anything other than thymine as base. The strategy that we have used for the synthesis of purine mimics has involved the S_N2-type displacement of halide ion from chlorides such as **54** with the sodium salt of 6-chloropurine.[35–38] The highly reactive



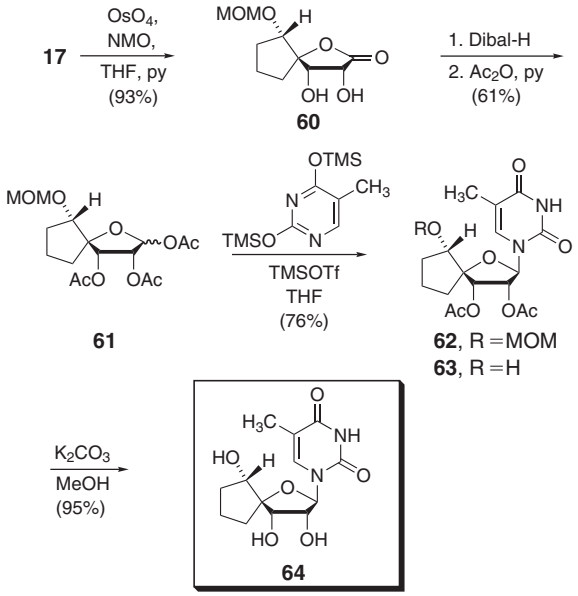
Scheme 11. Elaboration of adenine analogues. TBAF = tetrabutyl ammonium fluoride.

electrophile **54** was prepared in a non-stereoselective manner from **52** by sequential Dibal-H reduction and exposure to triphenylphosphine and carbon tetrachloride (Scheme 11). The involvement of **54** in glycosylation proceeded with the formation of **55** and **56** in comparable amounts. The generation of an epimeric mixture at this point was not an element of concern since thermal activation of **56** at 100°C induced its epimerization to the desired **55**. Substitutive amination followed by routine desilylation afforded **58**. When

recourse was made to laevorotatory lactone **19**, conversion into **59** was realised uneventfully by way of the identical five-step sequence.[39]

An equally rewarding thrust involved the osmium tetraoxide-catalyzed dihydroxylation of unsaturated spiro lactones in the presence of NMO. An early series of experiments involved the methoxymethyl derivative **17**, where introduction of the two hydroxyl groups occurred in a highly diastereoselective manner as in **60** (Scheme 12).[26] The ensuing one-pot reaction involved reduction with Dibal-H and direct acetylation. Triacetate **61** so generated made possible the implementation of the Vorbrüggen process. Specific use of bis-*O*-silylated thymine and trimethylsilyl triflate gave rise to **63** from which **64** was generated by routine deacetylation.

The reaction pathway developed for the conversion of **17** into **64** is not limited to the 5′α series, but appears to be generally applicable to the related β-epimer. Two representative examples are illustrated in Scheme 13. In the first instance, the coupling to triacetate **65** with persilylated uracil afforded **66** as the sole nucleosidic product. Its twofold deprotection proceeded with excellent chemoselectivity to make **67** available.[26] The single-phase sodium-salt glycosylation protocol was used to generate **68**. In this instance, dihydroxylation was most satisfactorily implemented by reaction with ruthenium tetraoxide in a two-phase solvent system consisting of ethyl acetate, acetonitrile, and water.[40] The rapidity of this important step (complete within 5 min), its stereoselectivity, and its overall efficiency (60%) are noteworthy.[39] Generation of the spiro guanosine **70** was achieved by reaction with 2-mercaptoethanol under basic conditions.[41–43]

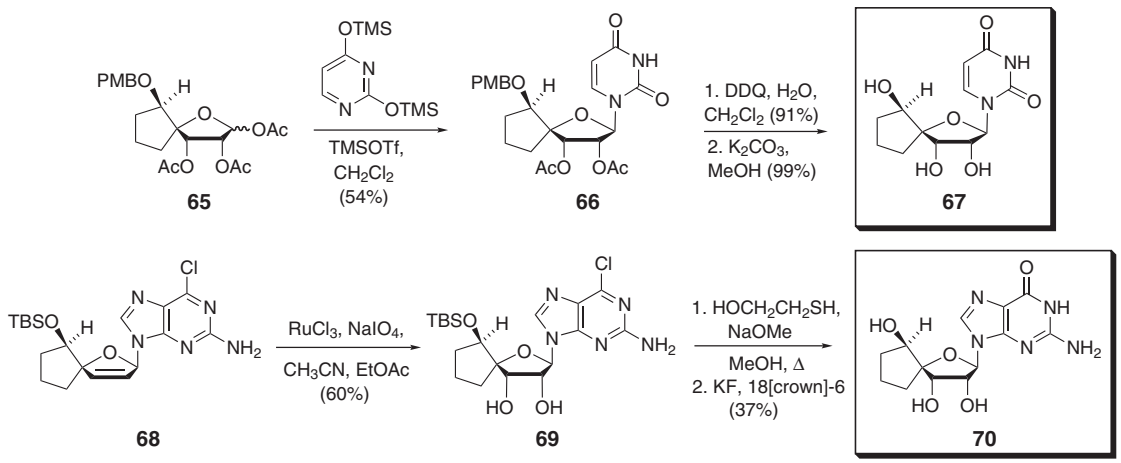


Scheme 12. Synthesis of the first fully substituted spironucleoside. NMO = *N*-methylmorpholine-*N*-oxide.

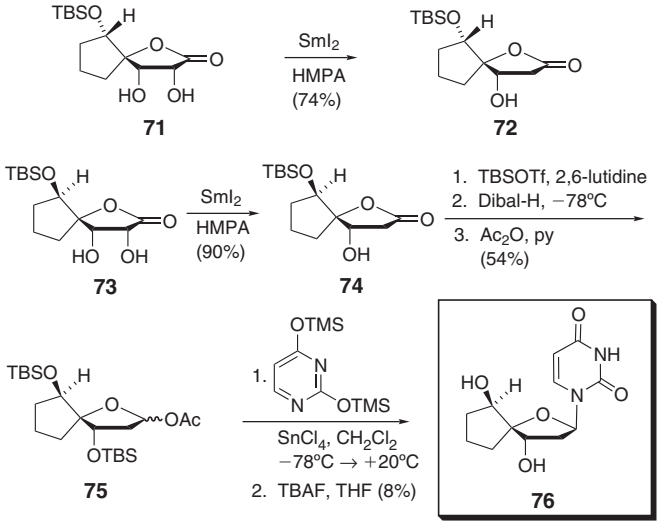
The Quest for 2′-Deoxy Spironucleosides

Since DNA is, like RNA, involved in a wide array of biological functions, 2′-deoxy congeners of the spirocyclic type loom as interesting targets for deliberate incorporation in a site-specific manner into nucleotide strands. This component of our research undertaking has more recently been accorded preliminary attention. Our expectation is that these new mimics will hopefully hold interest as useful medicinal agents in their own right in addition to imparting valuable therapeutic properties when incorporated into oligomers.

The ready availability of unsaturated lactones generically represented as **16** and **17** and the feasibility of dihydroxylating the conjugated double bond in a relatively efficient manner led to the selection of intermediates such as **71** and **73** as appropriate precursors to the key 2′-deoxy building blocks (Scheme 14).[44,45] The highly polar character and elevated water solubility of these substances prompted consideration of their direct exposure to samarium iodide. The use of HMPA as solvent resulted in reductive cleavage of the C2′ hydroxyl with generation of **72** and **74**, respectively.[46] Dihydroxy lactone **71** reacts more sluggishly than does **73** and requires a considerably greater period of time to proceed to completion. Following silylation of the OH functionality in **74**, conversion into the anomeric acetates **75** proved uneventful. These accomplishments allowed for the ultimate incorporation of uracil as in **76**. Although the efficiency of this Vorbrüggen



Scheme 13. Generation of uridine and guanosine prototypes. PMB = *p*-nitrobenzoate, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

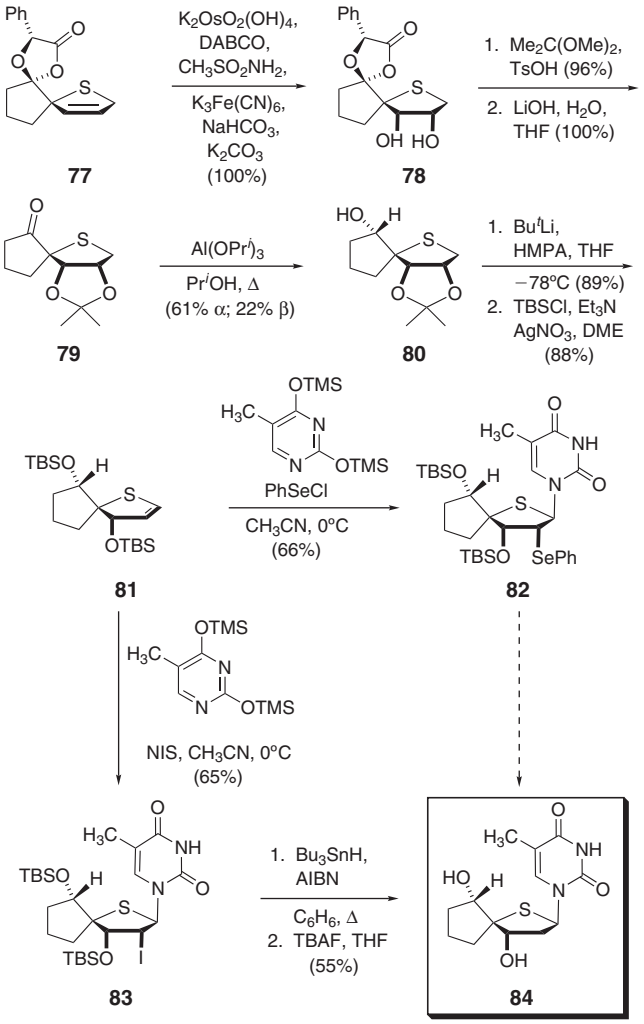


Scheme 14. Realization of a synthetic approach to 2′-deoxy spironucleosides. HMPA = hexamethyl phosphoramide.

reaction sequence is presently low, albeit in line with existing precedent,[28c] improvements continue to be sought.[44,45]

More Advances in the Spirothianucleoside Sector

Concurrently, we have addressed an expeditious means for the generation of 2′-deoxy thiaspironucleosides. As a direct consequence of the divergent chemical reactivity of tetrahydrofuran and tetrahydrothiophene ring systems, the development of an entirely different approach was needed. The most attractive pathway to surface at this time is summarized in Scheme 15.[47] The enantiomerically pure mandelic acid acetal was initially prepared from **26** as outlined previously. It proved particularly advantageous to preserve the acetal functionality during the course of the ensuing dihydroxylation. The conversion of **77** into **78** was effected in quantitative yield in the presence of potassium osmate and DABCO without evidence of competing oxidation at sulfur.[48] Also encouraging, acetonide formation and saponification proceeded in 96% overall yield to give **79**. We next explored the stereochemical course of various reductions of the carbonyl group in this ketone and settled on the use of Meerwein–Ponndorf–Verley conditions. α-Alcohol **80** was isolated in 61% yield along with 22% of the β-isomer. This reduction gave the most equitable distribution of the two key epimeric intermediates. Introduction of a 1′,2′-double bond was achieved by treating **80** with *tert*-butyllithium in THF containing HMPA.[49] Subsequent silylation resulted in masking of the two hydroxyl groups in a manner identical to that in **81**. This compound was then examined for its ability to react in the desired way with either phenylselenenyl chloride or *N*-iodosuccinimide in the presence of persilylated thymine.[50] Both reactions proved to be remarkably successful, giving rise to **82** and **83** as single diastereomers. The indicated stereochemical assignments are based on the results of NOESY experiments. To complete the conversion into **84**, we selected radical conditions for the chemoselective reductive cleavage. The nine-step conversion of **77** into this enantiopure spirothianucleoside

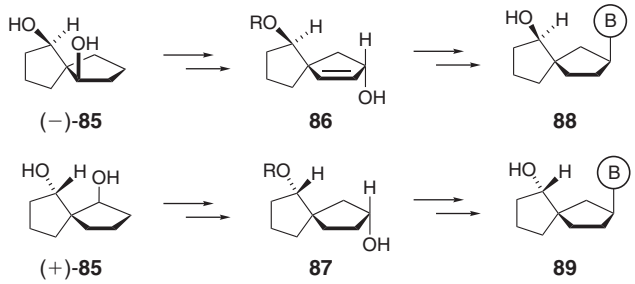


Scheme 15. An effective route to 2′-deoxy spirothianucleosides. DABCO = 1,4-diazabicyclo[2.2.2]octane, AIBN = azobis(isobutyronitrile).

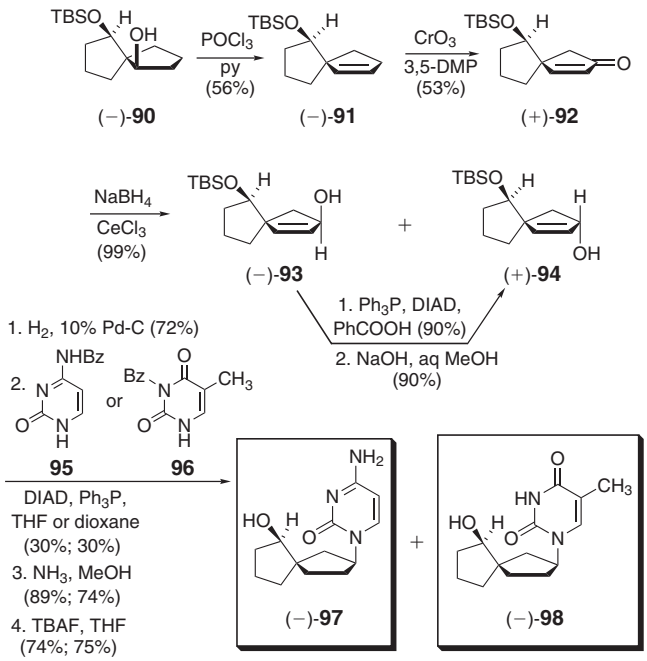
is illustrative of a convenient means for assembling these attractive compounds.

Carbaspironucleosides Having Natural C1′ Absolute Configuration

The explosive interest in carbocyclic nucleosides witnessed in the past decade is a likely reflection of the potent antiviral and antitumour properties of select members of this class[51–53] and their improved metabolic stability as a consequence of the absence of a glycosyl linkage.[54] Retrosynthetic consideration of the spiro equivalents **88** and **89** led us back to readily available (±)-spiro[4.4]nonane-1,6-dione,[55] the reduction of which with $LiBu^t(Bu^i)_2AlH$ has previously been shown to deliver the racemic *cis,cis*-diol[57] (Scheme 16). The last compound has been effectively resolved with generation of (–)-**85** and (+)-**85** via ketalization with (1*R*)-(+)-camphor.[56] According to our strategy, the laevorotatory enantiomer was to serve as a practical precursor to **88** in light of the absolute configurational features held in common by this pair of compounds. Our ready procurement of **89** on the other hand was to rest on the suitable monoprotection of



Scheme 16. Retrosynthetic construction of the epimeric spirocarbanucleosides reflecting the 'merger of chirality' concept.

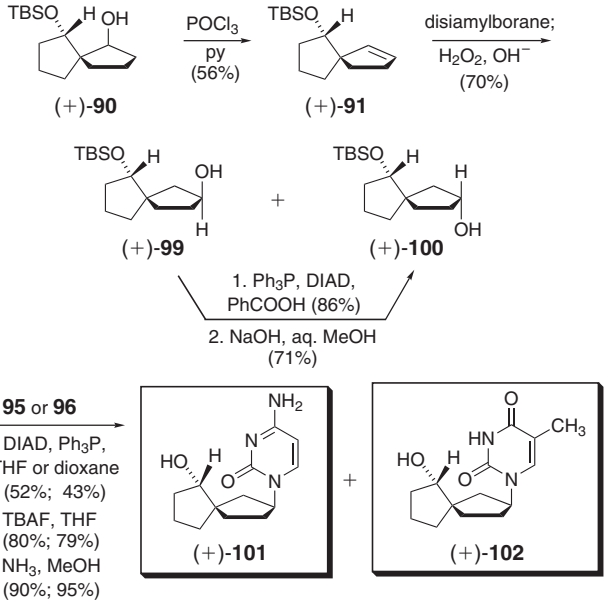


Scheme 17. Exemplary spirocarbanucleoside construction. DIAD = diisopropyl azodicarboxylate.

(+)-**85**, subsequent conversion into **87** presumably via the cyclopentene, and ultimately deployment of an S_N2 reaction to invert configuration.

Consistent with the non-bonded steric compression present in **90**, its dehydration proved to be problematic. Ultimately, phosphorous oxychloride was found to be capable of generating **91** in satisfactory yield[57] (Scheme 17). Expectedly, the major competing process was Wagner–Meerwein rearrangement. Sequential oxidation with the chromium trioxide–3,5-dimethylpyrazole complex[58,59] and reduction according to Luche[60] produced **93** and **94** in a 1 : 1 diastereomeric ratio. Following their ready chromatographic separation, **93** could be individually transformed into **94**, thereby allowing for the acquisition of appreciable amounts of the latter. This accomplished, **94** was subjected to catalytic hydrogenation and cleanly converted into both **97** and **98** by means of established processes.[61–64] The selectivity of the individual reactions was rewardingly high.

The next phase of this investigation called again for the dehydration of **90**, although now in the form of the (+)-enantiomer[58] (Scheme 18). The regiodirected hydration



Scheme 18. The conversion of (+)-**90** into spirocarbanucleosides properly configured at C1′.

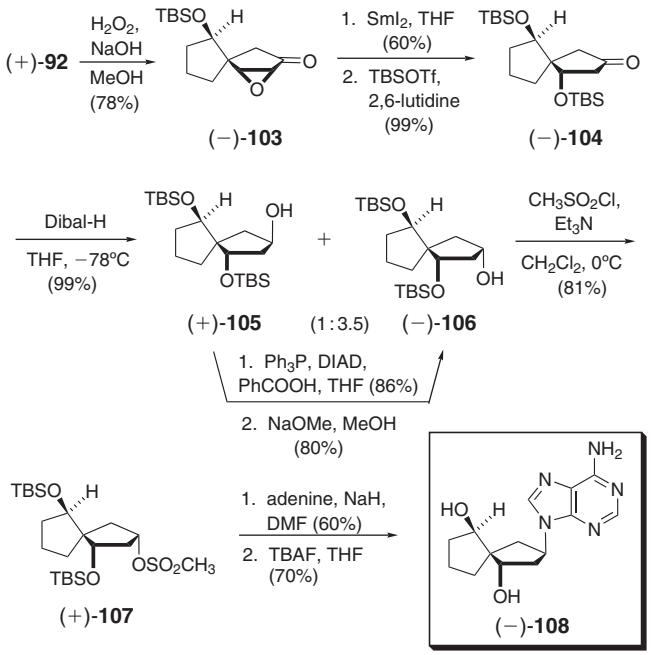
of (+)-**91** was properly directed by involving the sterically demanding disiamylborane reagent. The distribution of isolated **99** and **100** was 19 and 51%. For the purpose of maximizing the available amount of **100**, the Mitsunobu reaction was successfully applied once again. At this point, practical crossover to the diastereomeric manifold was realised and the acquisition of **101** and **102** was accomplished in the manner developed earlier.

This conversion of the enantiomers of **90** by different synthetic pathways into epimeric dideoxy spirocytidines and spirothymidines having natural absolute stereochemistry at C1′ constitutes an unprecedented 'merger-of-chirality' principle of potentially bioactive application.

Another spirocarbanucleoside theme developed more recently in this laboratory involves the conversion of spirocyclic enones such as (+)-**92** into 2′-deoxy nucleosides as exemplified by the adenosine derivative **108**[65] (Scheme 19). The successful pathway relies on the susceptibility of **92** and structural analogues thereof to undergo base-promoted conversion to the respective epoxy ketones, whose reduction with samarium iodide in THF[66] furnishes the β-hydroxycarbonyl product resulting from cleavage of the α-C—O bond. In the specific case of **92**, the conversion into **103** was highly stereoselective, thus allowing for clear definition of the configuration of C3′ in **104**. The Dibal-H reduction of **104** gave rise to a chromatographically separable mixture of **105** and **106** in a 1 : 3.5 ratio. Standard conditions for the Mitsunobu reaction allow for total conversion of **105** into **106** and full use of the material supply. The mesylation of **106** to give **107** followed by S_N2 displacement with the sodium salt of adenine[67] and desilylation affords in good yield the adenosine analogue **108**.

Future Prospects

For antisense chemotherapy to be successful, the newly developed spirocyclic nucleotides will need to be resistant to



Scheme 19. Acquisition of a deoxy spirocarbanucleoside.

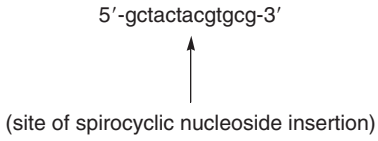


Diagram 2.

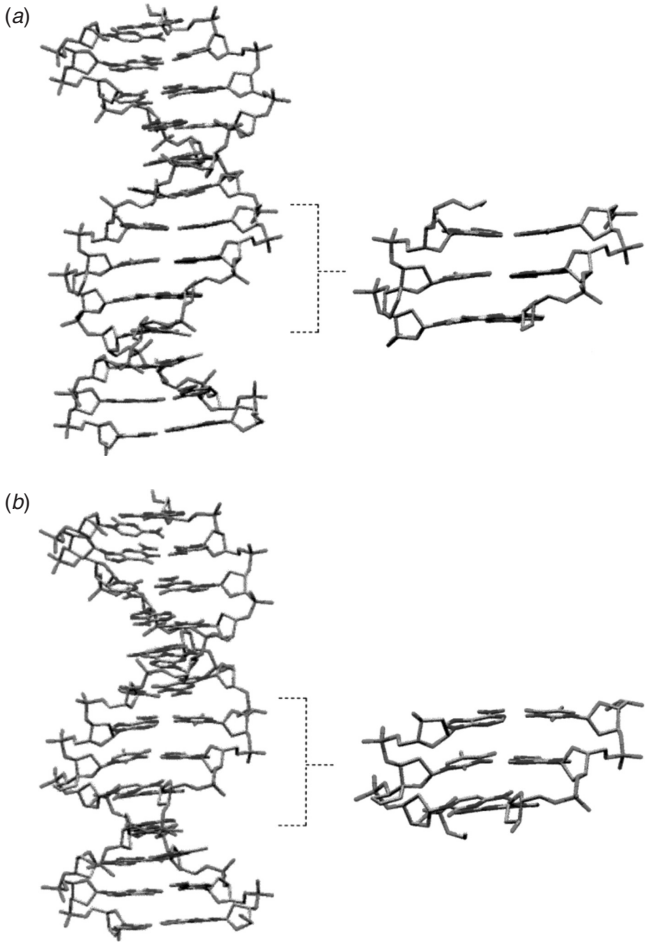


Fig. 2. Computed changes in the duplex structure of a 13-*mer* duplex as a central thymidine is exchanged for **3a** (B = Th) and **3b** (B = Th) (Amber program, Na^+ ions not shown for clarity).

nuclease activity and be capable of modulating viral gene expression.[68] As discussed at the outset, **1–4** may be resistant to enzymatic transformations and have improved bioavailability. However, will nucleosides incorporating these building blocks provide duplexes with complementary nucleic acids that are stable? Molecular modelling studies have provided the exciting prospect that spirocyclo-DNA/RNA hybrids are quite capable of realizing base-pairing interactions closely comparable to natural nucleic acids. Of course, confirmation of these predictions must await the actual determination of their properties, most particularly thermal denaturation.

These results of molecular modelling studies are illustrated here for 13-*mer* systems having the base sequence depicted in Diagram 2.

The computations made provision for the inclusion of sodium ions, which were subsequently deleted from Fig. 2 for purposes of clarity. For this model-building exercise, a centrally located thymidine unit was replaced by **3b** (B = Th) as well as by **3a** (B = Th). The fidelity of the base-pairing in all three systems is remarkably good. The magnified regions are intended to provide improved visualization of the central core of these oligonucleotides. In light of these indicators, the anticipated reduction in the entropy term upon duplex formation with both **3a** and **3b** (a consequence of structural reorganization) should materialize despite the obvious disparity in backbone and furanose ring torsional angles. The possibility of arresting DNA synthesis without sequentially altering the hydrogen-bonding geometry of template bases has been reported.[69]

An increasing variety of gene products are being discovered that are regulated by antisense RNA molecules expressed intracellularly.[70,71] It is hoped that the spirocyclic restriction approach outlined herein will ultimately prove feasible for therapeutic applications in the context of gene therapy. Increasing RNA binding affinity to synthetic oligonucleotides does not alone lead to biological efficacy. These compounds must also penetrate cells and reach cytoplasm where they must bind effectively to m-RNA and particularly the crucial initiation codon region.[72] The conservative spirocyclic modifications proposed here are apt to possess improved properties such as suitably enhanced lipophilicity, resistance to alkaline hydrolysis, as well as stability toward nuclease degradation. We end by quoting Piet Herdewijn:[2b] '… the design and synthesis of conformationally restricted oligonucleotides remains challenging research with great potential for future drug development in all fields of oligomeric structures.'

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