REVIEW

Bicyclic nucleosides and conformational restriction of oligonucleotides

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1 Introduction

Synthesis of modified nucleosides and oligonucleotides has received much attention in the last decade in part stimulated by the prospects of developing therapeutically active analogues of the natural nucleosides and nucleic acids. Modified oligonucleotides have been synthesised especially with the aim of creating analogues able to bind complementary single stranded RNA with very high affinity and specificity, thereby offering the possibility of inhibiting the biosynthesis of a disease related protein in the so-called antisense strategy (therapeutic intervention based on duplex formation between an antisense oligonucleotide and the messenger RNA target).[1,2]

It is well established that conformational restriction may lead to favourable complex formation due to an entropic advantage. This concept has been investigated quite intensively in nucleoside and especially oligonucleotide chemistry in recent years. A number of reviews focusing on the properties of oligonucleotides preorganized for RNA-binding have been published.[3–6] In these reviews, the structural considerations behind preorganization of nucleosides and oligonucleotides are outlined and they will therefore only be briefly mentioned herein. This review is focused on *synthetic routes* towards various classes of conformationally restricted nucleosides and oligonucleotide analogues composed thereof. Only bicyclic nucleosides based on a furanose or a cyclopentane moiety are included, and the treatment of modified oligonucleotides is confined to those containing conformationally restricted bicyclic monomeric units linked together as phosphate diesters, and to those with the nucleobase attached at the anomeric or pseudoanomeric position (the latter for cyclopentane derivatives).

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The following three parameters are among those useful for describing the conformation of a pentofuranose nucleoside, i) the glycosyl torsion angle, ii) the torsion angle determining the orientation of the 5′-hydroxy group relative to the C3′ atom, and iii) the conformation of the furanose ring, *i.e.* its position on the pseudorotational cycle as determined by the phase angle of pseudorotation, *P*. The concept of pseudorotation was originally introduced in relation to the cyclopentane conformation[7] and it has been adopted for description of the furanose conformations of nucleosides.[8] By convention, a phase angle *P* of 0° corresponds to a symmetrical $^{3}T_{2}$ conformation (absolute north conformation), and a phase angle *P* of 180° to the corresponding $^{2}T_{3}$ conformation (absolute south conformation).[8–10] The puckering changes every 18° around the circle between the envelope (*E*) and twist (*T*) conformations of the furanose ring (Fig. 1). The conformation of the furanose ring tends to cluster into two domains, a north-domain centered around a C3′-*endo* (^{3}E) type conformation ($P = 0$ to 36°) and a south-domain centered around a C2′-*endo* (^{2}E) type conformation ($P = 144$ to 180°).[8,9] The energy barrier between the different conformations is low and the natural nucleosides exist in a rapidly changing equilibrium between the north and south type conformers.

The exact position of the equilibrium between different conformers for a given nucleoside depends on various steric and stereoelectronic effects, *e.g.* anomeric and *gauche* effects. One way of restricting the conformation of the furanose ring is to chemically manipulate these effects. A convincing example is the use of 3′-*N*-phosphoramidates (prepared by oligomerization of 3′-amino-3′-deoxynucleosides) as RNA mimics.[11,12] The exchange of the 3′-oxygen atom with the less electronegative nitrogen atom weakens the *gauche* effect along the C3′–C4′ bond thereby inducing an increased population of the north type conformer for these derivatives compared to the natural nucleosides. As the conformation of the furanose ring of the nucleotide monomers of oligonucleotides that bind strongly to RNA (anticipating the formation of an A-type duplex) generally is of the north type, the conformational tuning of the 3′-*N*-phosphoramidates leads to preorganization which offers one explanation for the formation of duplexes of increased stability compared to the reference duplexes entirely composed of the natural RNA and/or DNA nucleotide monomers. The reader is referred to recent reviews for more information on the relation between furanose conformation and nucleic acid duplex structure and stability.[4–6,13]

Another approach towards restricting the conformation of the furanose ring of nucleosides is to convert this into a bicyclic system. At least in some cases this has proven efficient for

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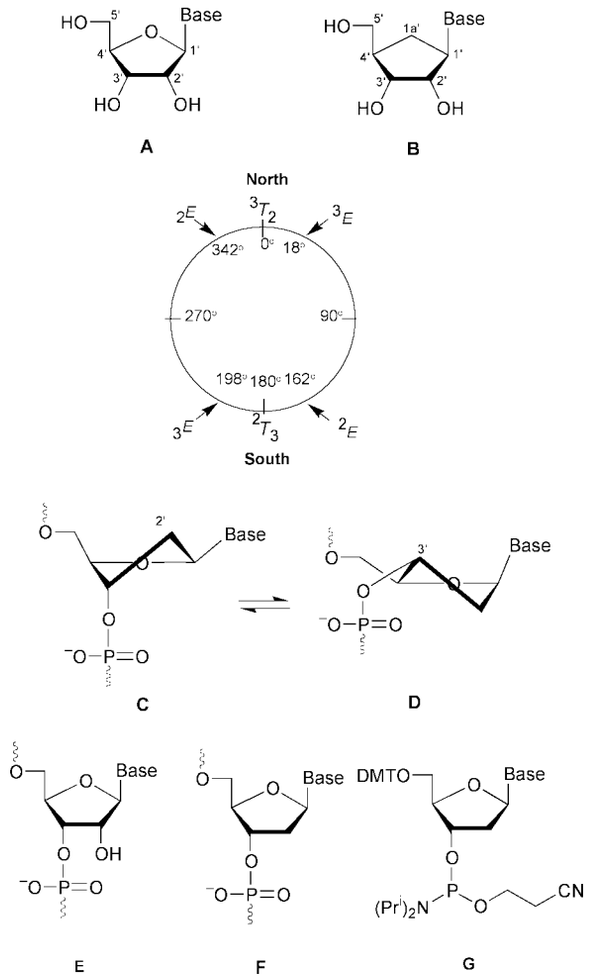


Fig. 1 The general structure of a natural pentofuranose-based ribonucleoside **A** and a cyclopentane-based ribonucleoside analogue **B** are shown with the numbering systems used throughout this review (also for bicyclic nucleosides). The pseudorotation cycle describing the conformation of the furanose ring is shown together with the equilibrium between a south (C2′-*endo*/C3′-*exo*, $^2T_3$) type conformation **C** and a north (C3′-*endo*/C2′-*exo*, $^3T_2$) type conformation **D** for a 2′-deoxyribonucleoside. Likewise shown is the structure of a ribonucleotide RNA monomer **E** (adopting a north type conformation in RNA:RNA A-type duplexes) and the structure of a 2′-deoxyribonucleotide DNA monomer **F** (adopting a south type conformation in DNA:DNA B-type duplexes). Base = adenin-9-yl, cytosin-1-yl, guanin-9-yl, thymin-1-yl and uracil-1-yl for the natural nucleosides and nucleotides. The symbols A, C, G, T and U are used for the five natural nucleobases in a number of the Schemes of this review. For more details about the conformation of nucleosides, see refs. 8–10. The 5′-*O*-dimethoxytrityl (DMT) protected 3′-*O*-phosphoramidite derivative **G** allows the incorporation of the natural DNA monomers (to give monomer **F**) into oligonucleotides by the use of an automated DNA-synthesiser. Analogously, incorporation of a modified monomer is possible using the same automated chemistry giving the opportunity to evaluate the effect of a given chemically modified monomer on the stability of duplexes.

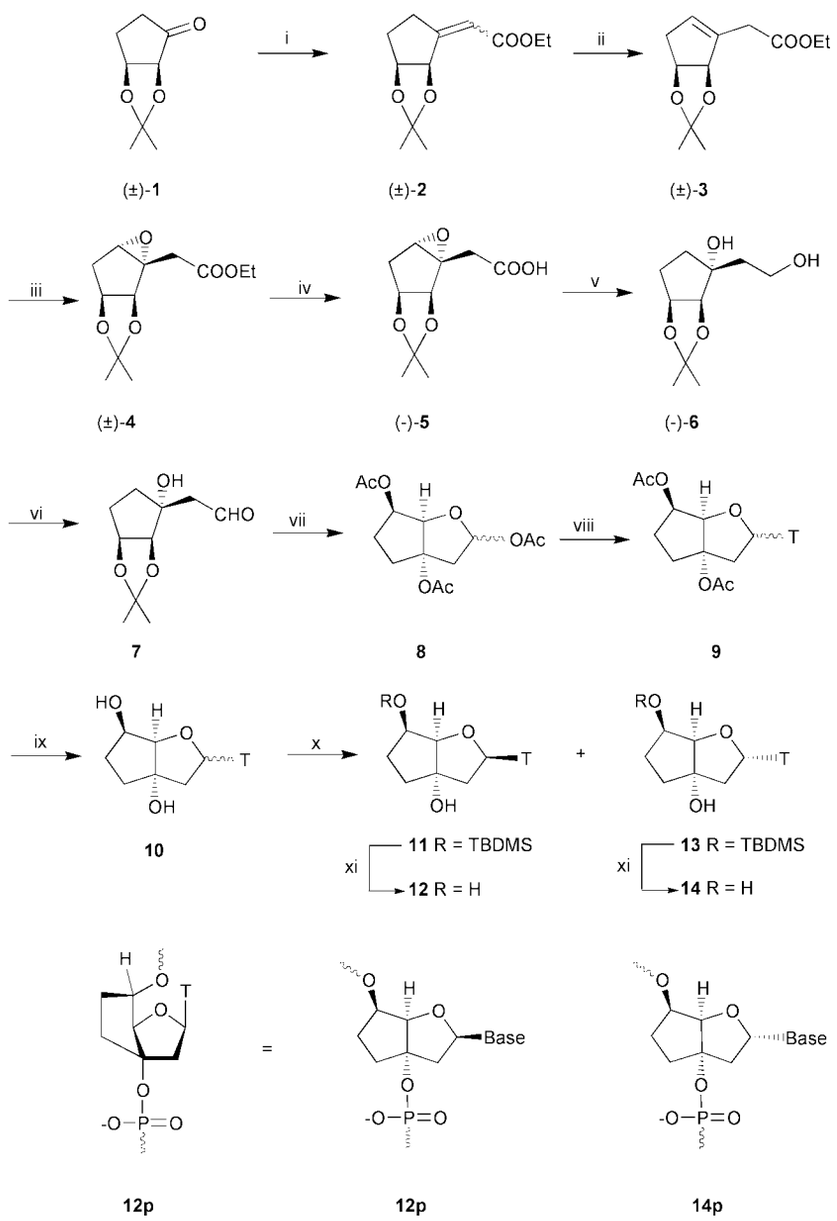
restricting or even locking the furanose conformation in a predictable way. This covalently based approach will be described in this review with the focus on the synthetic routes applied and on the effects obtained in relation to the binding properties of the modified oligonucleotides. These effects are conveniently estimated by comparing the melting temperature (T_m value) of a duplex involving a partly or fully modified oligonucleotide and an unmodified complementary target sequence, with the T_m value of the corresponding reference duplex involving two unmodified oligonucleotide sequences. In this review, the change in melting temperature per modification incorporated into the strand (ΔT_m value) is given when possible. Care, however, should be taken when comparing the ΔT_m values for the different modifications as these values are somewhat dependent on the exact sequence and hybridization conditions. In the series of furanose-based bicyclic nucleosides and oligonucleotides the exact position of the two bridge-head atoms, as well as the chemical nature of the additional linker, has been subject to variation, and a substantial number of structural variations have been studied.

2 Synthesis of 3′-*C*,5′-*C*-linked bicyclic nucleosides. Bicyclo-DNA and derivatives

Stimulated by the work of Eschenmoser and co-workers on "homo-DNA"[14,15] and the opportunity of entropically favorable duplex formation using conformationally restricted monomers, Leumann and co-workers introduced the concept of "bicyclic oligonucleotides," and in a series of papers the synthesis and properties of 3′,5′-ethano-linked bicyclic nucleosides (bicyclo[3.3.0]octane scaffold) and the corresponding "bicyclo-DNA" derivatives have been thoroughly studied. The synthesis of the nucleosides is shown in Scheme 1.[16] Horner–Wittig reaction of the racemic ketone (±)-**1** gave the α,β-unsaturated ester (±)-**2**. The *E*:*Z*-ratio varied depending on the base applied but both isomers were applicable in the subsequent isomerisation of the double bond. Thus, using a catalytic amount of strong base the thermodynamically more stable deconjugated cyclopentene (±)-**3** was obtained. The two steps could also be performed in a one-pot reaction using two equivalents of strong base in the Horner–Wittig reaction which led to (±)-**3** in a combined yield of 90%. Introduction of the tertiary alcohol functionality was accomplished by an epoxidation–reduction strategy *via* epoxide (±)-**4** which was formed stereoselectively. Separation of the two enantiomers was efficiently performed at this stage by partial ester hydrolysis using hog-liver esterase. The acid (−)-**5** could be separated from the remaining ester by extraction giving (+)-**4** in 42% yield with an enantiomeric excess (ee) of 96%. The yield of the acid (−)-**5** was 53%, and the ee was determined after reduction with $LiAlH_4$ to be 72%. Recrystallisation, however, increased the ee of the alcohol (−)-**6** to 97%. Dess–Martin oxidation of the primary alcohol to the unstable aldehyde **7** followed by acid-catalysed removal of the isopropylidene protecting group with concomitant ring closure and peracetylation afforded the key bicyclic intermediate **8** as an anomeric mixture. Mixture **8** was successfully applied in Vorbrüggen-type coupling reactions with different nucleobases, *e.g.* thymine as shown in Scheme 1, affording the anomeric mixture **9**. Neither this mixture nor the corresponding deacetylated mixture **10** could be separated by column chromatography. However, silylation of the secondary hydroxy group allowed isolation of the two anomers **11** and **13** which after desilylation afforded the parent 3′,5′-ethano-linked bicyclic nucleosides **12** and **14**, respectively (Scheme 1). After coupling with 4-*N*-benzoylcytosine and 6-*N*-benzoyladenine, separation of the two anomers likewise required a silylation–desilylation procedure. In the case of the isobutyryl-protected guanine derivatives, the desired N9-isomers were separated from the N7-isomers, and the anomeric mixture separated after deacetylation. By use of these routes, the 3′,5′-ethano-linked bicyclic nucleoside derivatives of all the four natural DNA nucleobases were obtained.[16]

Conformational analysis of the β-configured monomers, both in the solid state and in solution, revealed them to exist in a south (C1′-*exo*, $_1E$) type conformation with a pseudorotational angle *P* of 128°.[16]

Protection at the secondary hydroxy group by conversion into the DMT (4,4′-dimethoxytrityl) ether (the thymine derivatives shown as examples) and phosphitylation at the tertiary hydroxy group of the bicyclic nucleosides **12** and **14** resulted in building blocks suitable for incorporation of monomers **12p** and **14p** into β-[17–21] as well as α-oligonucleotides,[22] respectively, on a DNA synthesiser. The incorporation of the β-nucleoside derivatives (monomer **12p**) into oligonucleotides (bicyclo-

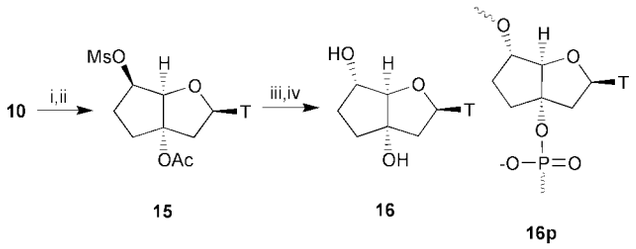


Scheme 1[16] *Reagents and conditions*: i) $(EtO)_2P(O)CH_2COOEt$, NaH, THF, 0 °C, then rt (88%); ii) DBU, CH_2Cl_2 (97%); iii) MCPBA, CH_2Cl_2, 0 °C to rt (77%); iv) hog-liver esterase, 0.1 M NaH_2PO_4, pH 7.75 (53%); v) $LiAlH_4$, Et_2O, −30 °C then reflux (61%, 97% ee); vi) 1,1,1-triacetyl-1,1-dihydro-1λ^5,2-benziodoxol-3(1*H*)-one, CH_2Cl_2, rt (91%); vii) a. H_2O/IR-120(H^+), 50 °C, b. Ac_2O, pyridine, DMAP, 0 °C to rt (93%); viii) thymine, HMDS, TMSCl, $SnCl_4$, MeCN, 0 to 55 °C (73%); ix) NaOH, THF, MeOH, H_2O, 0 °C (94%); x) TBDMSOTf, pyridine, 0 °C (28% α, 62% β); xi) $Bu_4NF{\cdot}3H_2O$ (**12**: 69%, **14**: 77%). T = thymin-1-yl.

DNA) resulted in relatively minor changes in thermal stability ($\Delta T_m = -2.4$ to $+0.3$ °C towards complementary DNA/RNA for mixed base sequences[20]) compared to the corresponding reference duplexes.[17,18,20] Homopyrimidine bicyclo-DNA is very prone to form triplexes, which however are of an unusual structure as the duplex is held together by Hoogsteen hydrogen bonds and the third bicyclo-DNA strand is bound to the duplex by Watson–Crick hydrogen bonds.[19] Hybridization between two complementary bicyclo-DNA strands involves the Hoogsteen and reversed Hoogsteen binding modes. Hybridization with bicyclo-DNA is generally entropically favoured but enthalpically disfavoured as compared to the unmodified duplexes.[18]

The anomeric α-bicyclo-DNA oligomers containing monomer **14p** form parallel duplexes with DNA complements involving Watson–Crick hydrogen bonds. The thermal stability of duplexes towards DNA or RNA complements is comparable to, or slightly lower than, those of the corresponding natural reference duplexes.[22]

As a consequence of the introduction of the 3′,5′-ethano linker, the 5′-hydroxy group of, *e.g.*, nucleoside **12** is fixed into a unnatural orientation. Thus, the torsional angle C3′–C4′–C5′–O5′ is restricted to the anticlinical range (+*ac*). In order to possibly mimic the synclinical (+*sc*) range found in natural A- or B-type duplexes, the 5′-epimeric bicyclo-DNA adenine and thymine nucleosides were synthesised as shown in Scheme 2 for the thymine derivative.[23,24] Initially it was attempted without success to invert the configuration at C5′ using Mitsunobu conditions on the anomeric mixture **10**. Instead, mesylation of the secondary hydroxy group followed by treatment with caesium acetate not only effected the desired nucleophilic substitution reaction with inversion but also provided a substantial amount (40%) of an elimination product. Acetylation of the tertiary hydroxy group to give **15** suppressed this side reaction and the 3′-*O*,5′-*O*-diacetylated intermediate was subsequently obtained from **15** in satisfactory 87% yield. Deacetylation afforded the parent nucleoside **16** which was DMT-protected at the secondary hydroxy group and phosphitylated at the tertiary hydroxy

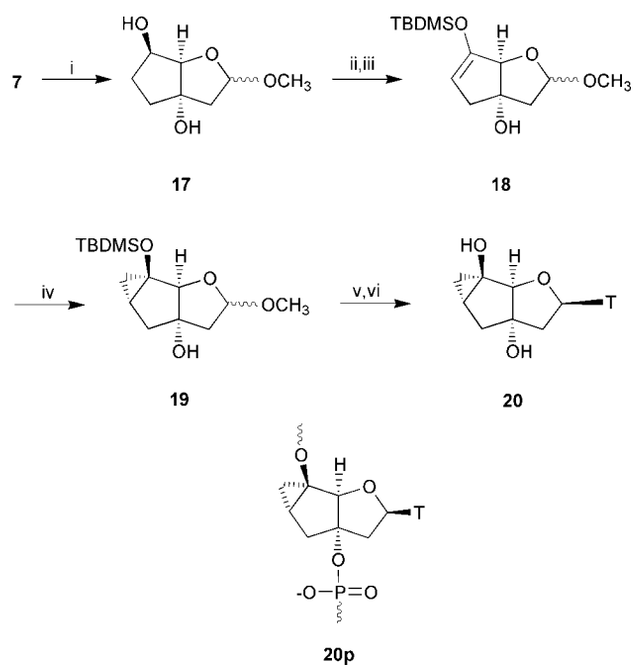


Scheme 2[23,24] *Reagents and conditions*: i) MsCl, pyridine, 0–55 °C (93%); ii) Ac_2O, DMAP, pyridine, 0 °C to rt (89%); iii) CsOAc, DMSO, 70–90 °C (87%); iv) NaOH, THF, MeOH, H_2O, 0 °C (86%). T = thymin-1-yl.

group to give the desired phosphoramidite building block for automated incorporation of monomer **16p** (Scheme 2).[23,24]

Conformational analysis showed that whereas the C5′ inversion had no significant effect on the conformation of the furanose moiety, the orientation of the 5′-hydroxy group was changed from a pseudoequatorial to a pseudoaxial position and that the relevant torsional angel was changed into the $-sc$ range. This is a conformation not found in natural duplexes and hence *epi*-bicyclo-DNA displayed pronounced decreases in thermal stability (ΔT_m values of −2 to −9 °C).[24]

In order to further investigate the effect of increased conformational restriction and the C3′–C4′–C5′–O5′ torsional angle on the structure and stability of duplexes, a tricyclic analogue of bicyclo-DNA ("tricyclo-DNA") was synthesised (Scheme 3).[25–27] The aldehyde **7** was used as starting material and after treatment with methanol under acidic conditions was converted into the anomeric mixture of the methyl glycoside **17**. Oxidation, regioselective deprotonation and silylation of the enolate afforded glycoside **18** which by a Simmons–Smith cyclopropanation reaction afforded the tricyclic glycoside **19**. This intermediate was successfully used in Vorbrüggen coupling reactions with thymine or adenine nucleobases, resulting in anomeric mixtures of the nucleosides. The nucleoside derivatives were obtained as pure anomers after selective deprotection of the trimethylsilyl group (introduced during the coupling reactions), whereupon removal of the *tert*-butyldimethylsilyl protecting group afforded the parent tricyclo-DNA nucleosides, *e.g.* the thymine derivative **20**. Standard DMT protection and phosphitylation afforded the building blocks for automated synthesis of tricyclo-DNA containing the thymine monomer **20p** or the corresponding adenine monomer.[25–27]

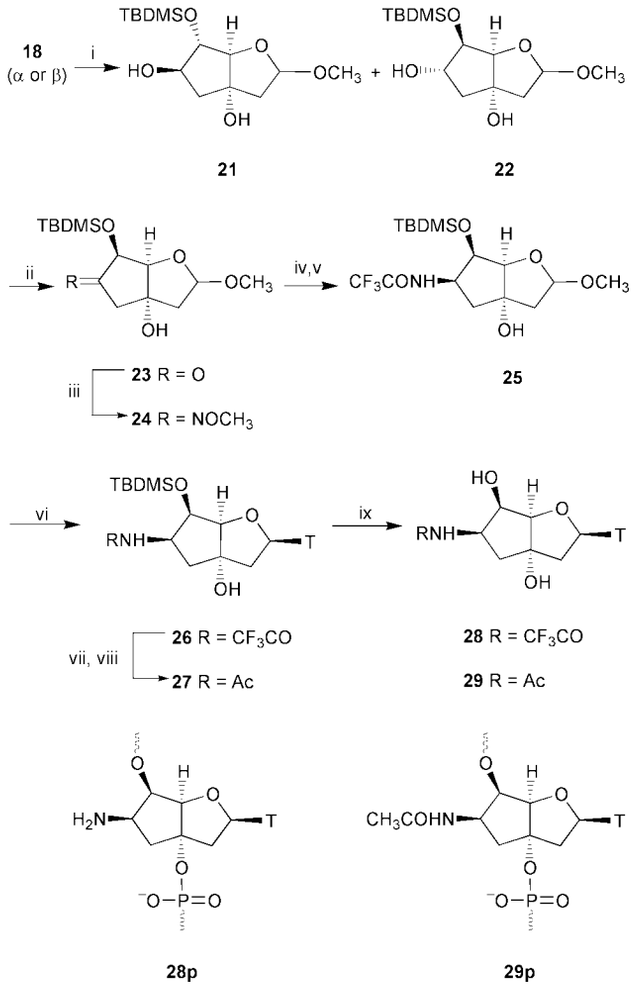


Scheme 3[25] *Reagents and conditions*: i) Amberlyst 15, MeOH (77%); ii) 1,1,1-triacetyl-1,1-dihydro-1λ^5,2-benziodoxol-3(1*H*)-one, CH_2Cl_2 (67%); iii) a. LDA, THF, −74 °C, b. TBDMSCl, THF, −74 °C (92%); iv) CH_2I_2, Ag/Zn, Et_2O, 34 °C (58%); v) a. thymine, *N*,*O*-bis(trimethylsilyl)acetamide, TMS-triflate, MeCN, rt, then 81 °C (65%, α : β = 1.7 : 1), b. TBAF (1 equiv.), THF (87%); vi) TBAF, THF (93%). T = thymin-1-yl.

Conformational analysis of the tricyclo-DNA nucleosides disclosed that they adopt a C2′-*endo* (^2E) conformation with the C3′–C4′–C5′–O5′ torsional angle in the $+ac$ (120°) range. The thermal stability of tricyclo-DNA was measured for homopurine and homopyrimidine sequences with varying degrees of modification. Generally, an increase in thermal stability (ΔT_m = +1 to +2 °C) towards DNA complements was seen, and the possibility of formation of stable triplexes was confirmed. Complexes formed between two complementary tricyclo-DNA strands were shown to be very stable, and the base-pairing selectivity obtained with tricyclo-DNA was, in general, very good.[25–27]

A derivative of bicyclo-DNA containing an amino substituent at the ethano linker has been synthesised in order to make zwitterionic bicyclo-DNA (Scheme 4).[28] Hydroboration of the vinylic silyl ether **18** (as its isolated α- or β-anomer) gave a mixture of **21** and **22**, the latter predominating regardless of the anomeric configuration of starting material **18**, which correlates well with attack by borane from the sterically less-hindered convex face of **18**. To introduce the amino functionality a Mitsunobu reaction was attempted without success. As an alternative, a redox procedure was used. Thus, Dess–Martin oxidation of compound **22** to give ketone **23** followed by reaction with methoxyammonium chloride afforded the oxime **24** in high yield. Reduction using H_2/Ra-Ni followed by trifluoroacetylation gave compound **25**, which was used in a Vorbrüggen coupling reaction to give the β-D-nucleoside **26** together with the corresponding α-anomer. At low temperature, the formation of the β-anomer was favoured whereas high temperatures induced preferential formation of the α-anomer indicating that the β-anomer is the kinetic product and the α-anomer the thermodynamic product. Nucleoside derivative **26** was either desilylated to give the trifluoroacetylated nucleoside **28** or converted into the *N*-acetyl derivative **27** which was subsequently desilylated to give nucleoside **29**. The *N*-acetyl group is stable during deprotection (by use of concentrated aqueous ammonia) of oligonucleotides after completion of the desired sequence, and therefore bicyclo-DNA containing an amino group (monomer **28p**) or an acetamido group (monomer **29p**) could be obtained after DMT protection, phosphitylation, and automated oligonucleotide synthesis.[28]

The thermal stability of duplexes between modified oligonucleotides containing one or more monomers of structures **28p** or **29p** and complementary DNA was evaluated at different pH values.[28] At pH 7, introduction of the amino-containing monomer **28p** induced a weak destabilization compared to the corresponding DNA : DNA duplex (ΔT_m = −0.8 to −2 °C). However, the presence of five monomers of **28p** induced a ΔT_m value of +1 °C. As expected, duplexes between a modified oligonucleotide involving the amino-containing monomer **28p** and complementary DNA exhibited lower thermal stability at higher pH values, probably because of reduced protonation of the amino group (and thus reduced zwitterionic character). A similar trend was not observed for the corresponding oligonucleotides containing the acetamido-substituted monomer **29p**. In fact, incorporation of **29p** induced the formation of less stable duplexes between the modified oligonucleotides and complementary DNA (ΔT_m = −1.9 to −3.5 °C) compared to the DNA–DNA reference, and no pH dependence was observed. Besides the possible reduction of the repulsive electrostatic force between the two complementary strands, the positive effect of the amino group *relative to* the acetamido group on the thermal stability of duplexes could also be caused



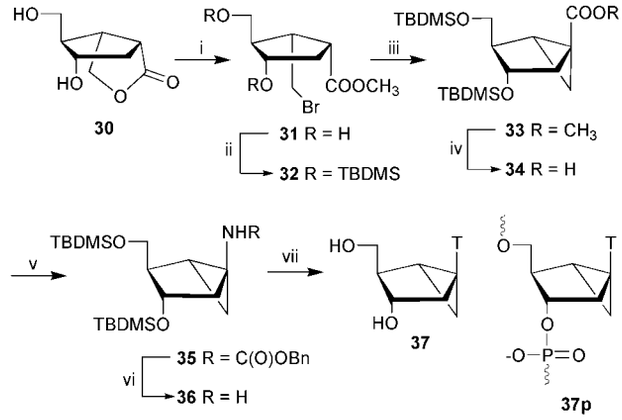
Scheme 4[28] *Reagents and conditions*: i) BH_3·THF, THF, −78 °C, then rt; ii) 1,1,1-triacetyl-1,1-dihydro-1λ^5,2-benziodoxol-3(1*H*)-one, CH_2Cl_2; iii) $(CH_3O)NH_3Cl$, NaOAc, EtOH; iv) H_2 (10 bar), Ra-Ni, EtOH, H_2O, conc. NH_3; v) CF_3COOEt, Et_3N, vi) thymine, *N*,*O*-bis(trimethylsilyl)acetamide, TMS-triflate, MeCN; vii) conc. NH_3, MeOH; viii) Ac_2O, pyridine, 0 °C; ix) TBAF, MeCN, AcOH (**28**) or Et_3N·HF, pyridine (**29**). T = thymin-1-yl.

by repulsive non-bonding interactions between the acetamido substituent and the adjacent O5′–P ester bond.[28]

3 Synthesis of methanocarbocyclic nucleosides and their oligonucleotides

Substitution of the oxygen atom in the furanose ring with a methylene group yields nucleoside analogues based on a cyclopentane moiety. This is a rather drastic modification since the anomeric effect and a number of other stereoelectronic effects are lost, which leads to stabilization towards cleavage of the glycosylic bond and to a change in structural properties of the nucleosides. Therefore, the predominant conformations of the cyclopentane-based nucleosides are different from those of the natural nucleosides.[29] In order to restrict the conformation of cyclopentane-based nucleotide monomers for modified oligonucleotides, two different methano-linked analogues have been synthesised.

1′,1′a-Methanocarbocyclic nucleosides (bicyclo[3.1.0]hexane scaffold) have been synthesised with all five natural nucleobases. Two different strategies have been employed for introduction of the cyclopentane ring. The first published route involved the homochiral bicyclic lactone **30** as starting material for the synthesis of the thymine derivative (Scheme 5)[30] *via* the unstable γ-bromo ester **31** which was silylated immediately after its purification. The best silylation agent proved to be *N*-*tert*-butyldimethylsilyl-*N*-methylacetamide, which, in DMF, afforded compound **32**. Efficient ring closure under basic conditions



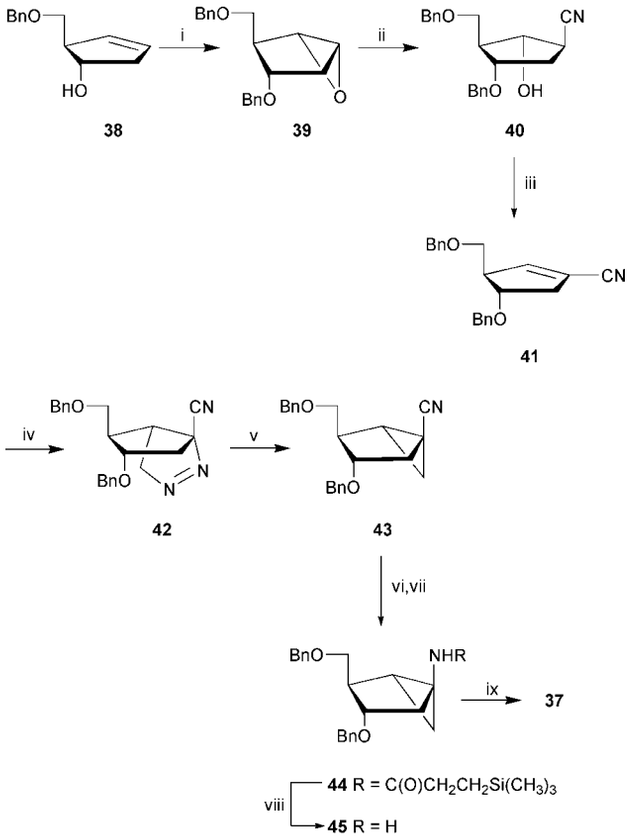
Scheme 5[30] *Reagents and conditions*: i) TMSBr, MeOH, $ZnBr_2$ (cat.), 0 °C (70%); ii) *N*-*tert*-butyldimethylsilyl-*N*-methylacetamide, DMF, 0 °C to rt (64%); iii) KOBu^t, Bu^{t}OH, rt (76%); iv) KOH, EtOH, 80 °C (78%); v) a. DPPA, Et_3N, toluene, 0 °C to rt, then 80 °C, b. BnOH, 80 °C, then 100 °C (85%); vi), H_2, 10% Pd-C, toluene (84%); vii) $CH_3OCH{=}C(CH_3)C(O)NCO$, CH_2Cl_2, 0 °C to rt (85%); viii) HCl–EtOH, reflux (80%). T = thymin-1-yl.

afforded compound **33** as the sole isomer. Hydrolysis of the ester followed by a three-step, one-pot procedure using an *in situ* Curtius rearrangement, and subsequent hydrogenolysis, afforded the bicyclic amine **36** *via* derivatives **34** and **35**. The nucleobase was subsequently constructed by a two-step procedure involving reaction with β-methoxy-α-methacryloyl isocyanate followed by acid-catalysed ring closure with simultaneous removal of the silyl protecting groups to give the bicyclic nucleoside **37**.[30]

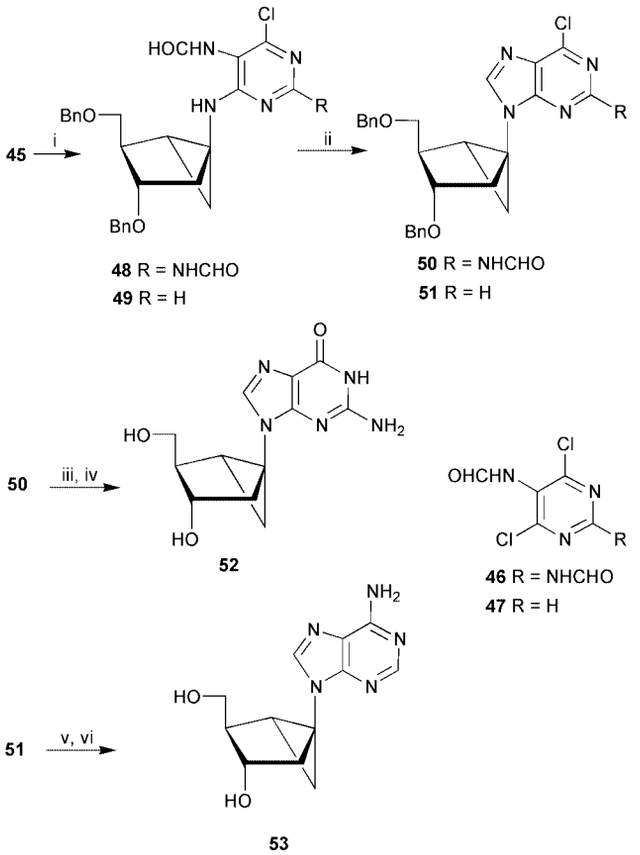
An alternative route for synthesis of the thymine nucleoside **37** starting from the structurally simple homochiral cyclopentene **38** has been developed (Scheme 6).[31,32] The cyclopentene **38** was epoxidized using a Sharpless epoxidation procedure and subsequently benzylated to give compound **39**. Nucleophilic opening of the epoxide using potassium cyanide afforded the *anti*-addition product **40**, which, by a *syn*-β-elimination of an intermediary thiocarbonylimidazolide, was converted into the α,β-unsaturated nitrile **41**. The use of azide instead of cyanide for these transformations would significantly ease the synthesis, but the azido-substituted compound did not undergo the subsequent elimination, even under forcing conditions.[31] Regio- and stereoselective 1,3-dipolar cycloaddition of diazomethane to **41** followed by photolytic rearrangement afforded the bicyclic cyanide **43** (*via* **42**), which *via* **44** was converted into the amine **45** by a Curtius rearrangement and subsequent deprotection. From this amine, all the natural nucleobases were introduced, *e.g.* the thymine moiety using the same principles as shown in Scheme 5. The uracil derivative was converted into the corresponding cytosine derivative by treatment first with $POCl_3$–1,2,4-triazole and then with aqueous ammonia.[32]

The synthesis of the purine nucleosides is shown in Scheme 7.[32] The bicyclic amine **45** was reacted with the *N*-formylated heterocycles **46** and **47** and subsequent cyclisations afforded the purines **50** and **51**, respectively. Treatment of **50** with formic acid followed by ammonia afforded the benzylated guanine analogue, which was debenzylated to give the parent bicyclic carbonucleoside **52**. For the synthesis of the adenine derivative **53**, compound **51** was treated with ammonia and subsequently debenzylated using BCl_3. Treatment of the bicyclic amine **45** with either 5-amino-4,6-dichloropyrimidine or 2,5-diamino-4,6-dichloropyrimidine resulted in little or no reaction.[32]

X-Ray crystallography showed the carbonucleoside derivative **37** to adopt a conformation in which the pseudosugar moiety is puckered in a south type (C3′-*exo*, $_3E$) conformation.[30] In the crystal, the nucleobase displayed a *syn* orientation with its 2-oxygen atom involved in intramolecular



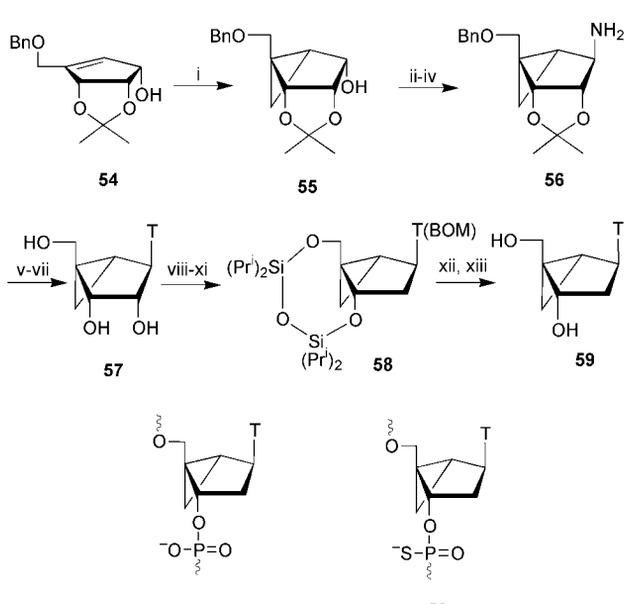
Scheme 6[31,32] *Reagents and conditions*: i) ref. 33; ii) KCN, $LiClO_4$, MeCN, 70 °C (75%); iii) 1,1′-thiocarbonyldiimidazole, DMAP, DMF, rt then 80 °C (84%); iv) CH_2N_2, Et_2O, 0 °C to rt (94%); v) *hv* (250–400 nm), benzophenone, benzene–MeCN (1 : 1) (79%); vi) NaOH, EtOH, reflux (62%); vii) diphenylphosphoryl azide, Et_3N, toluene, rt, then $Me_3SiCH_2CH_2OH$, 80 °C (56%); viii) TBAF, THF, 70 °C (~100%); ix) a. $CH_3OCH{=}C(Me)C(O)NCO$, toluene, rt (55%), b. HCl–EtOH, reflux (84%), c. BCl_3, CH_2Cl_2, −78 °C (72%).



Scheme 7[32] *Reagents and conditions*: i) **46** or **47**, Pr^i_2EtN or Et_3N, 1,4-dioxane; ii) $AcOCH(OEt)_2$ or $HC(OEt)_3$–HCl (**50**: 53%, **51**: 47%, two steps); iii) a. HCOOH, reflux, b. NH_4OH, MeOH, 40 °C (94%); iv) HCOOH, Pd-C, MeOH (86%); v) NH_3, MeOH, 80 °C (97%); vi) BCl_3, CH_2Cl_2, −78 °C (93%).

hydrogen bonding with the 5′-hydroxy group.[30] ^{1}H-NMR studies revealed the conformation of the cyclopentane ring to be the same in solution as shown by the $^3J_{HH}$ coupling constants. Several of the dihedral angles in the carbonucleoside approach 90° leading to coupling constants of ~0 Hz in the ^{1}H-NMR spectrum.[30,31] Selective introduction of a 5′-*O*-DMT protecting group followed by phosphitylation of the 3′-hydroxy group of **37** allowed the synthesis of modified oligonucleotides containing the 1′,1′a-methanocarbonucleotide thymine monomer **37p** (Scheme 5). In general, substitution of thymidine with monomer **37p** resulted in a destabilization of the resulting duplex between the modified oligonucleotide and complementary DNA/RNA sequences (compared with the duplexes containing the corresponding unmodified oligonucleotide reference sequences). Based on evaluation of partly or fully modified homopyrimidine sequences, this destabilization is more pronounced for DNA than RNA targets (ΔT_m values of −2 to −4 °C (DNA) and −1 to −2 °C (RNA)),[30] which, keeping the DNA-like conformation of the carbonucleoside **37** in mind, appears surprising.

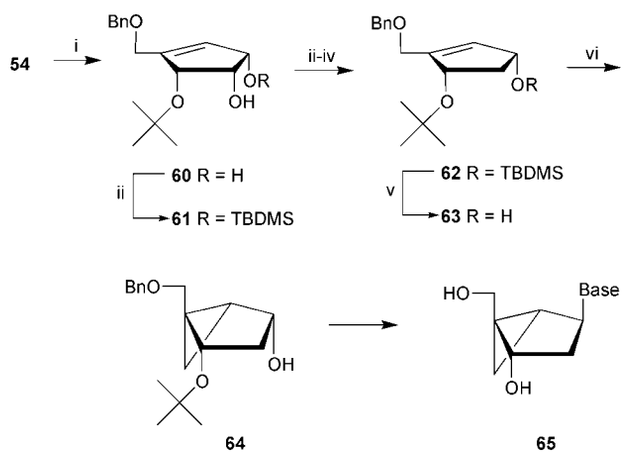
Several strategies have been applied for synthesis of 1′a,4′-methanocarbocyclic nucleosides (bicyclo[3.1.0]hexane scaffold), a class of bicyclic nucleoside analogues conformationally restricted into a north type conformation. Thus, with regard to conformational restriction, the 1′,1′a-methanocarbocyclic nucleosides described earlier and the 1′a,4′-methanocarbocyclic nucleosides constitute an interesting pair. In the first published synthetic strategy, the cyclopentene derivative **54** (obtained with an optical purity of ~50% from D-ribonolactone in seven steps) was used as starting material (Scheme 8).[33–35] Simmons–Smith cyclopropanation of **54** afforded the rigid tri-



Scheme 8[34] *Reagents and conditions*: i) Zn/Cu, CH_2I_2, Et_2O, reflux (73%); ii) TsCl, Et_3N, CH_2Cl_2, DMAP, rt, (77%); iii) NaN_3, DMF, 70 °C (88%); iv) H_2, Lindlar's catalyst (~100%); v) $H_3COCH{=}C(CH_3)$-C(O)NCO, CH_2Cl_2, −78 °C to rt (95%); vi) HCl, EtOH, H_2O, reflux (80%); vii) H_2, 10% Pd-C, EtOAc–MeOH; viii) $TIPDSCl_2$, imidazole, DMF (67%, two steps); ix) BOMCl, DBU, MeCN, rt (85%); x) $CH_3C_6H_4OC(S)Cl$, DMAP, Et_3N, CH_2Cl_2, rt, then 40 °C (90%); xi) Bu_3SnH, AIBN, DME, 80 °C (preparative chiral HPLC, 65%, 100% ee); xii) TBAF, THF, rt (99%); xiii) a. H_2, 10% Pd-C, rt, b. NaOMe, rt (88%). T = thymin-1-yl.

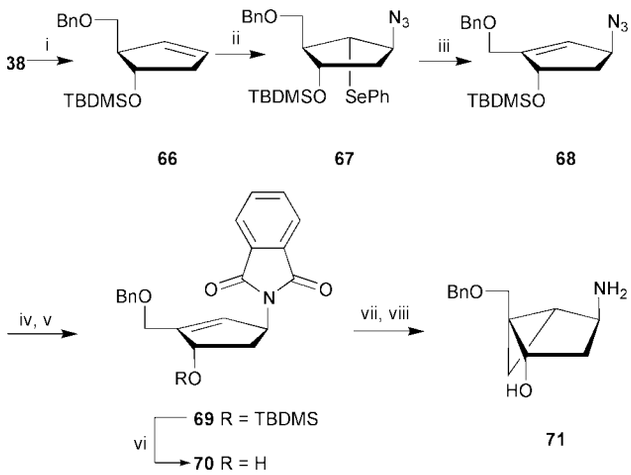
cyclic pseudosugar **55** in a diastereoselective manner due to the directing effect of the allylic alcohol. Tosylation followed by nucleophilic substitution using sodium azide and mild reduction afforded the amine **56**. The thymine nucleobase was constructed on this amine and the nucleoside was subsequently deprotected to give nucleoside **57**. Protection of the 3′- and 5′-hydroxy groups was achieved with the bidentate protecting group 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPDS). For deoxygenation at the C2′ position, the 2′-hydroxy functionality of the TIPDS-protected intermediate nucleoside was converted into the corresponding thiocarbonate. Surprisingly and in contrast to the similar ribonucleosides, substantial 3-*N*-thiocarbonylation occurred in addition to the desired 2′-*O*-thiocarbonylation wherefore N3 protection was effected by reaction with benzyl chloromethyl ether (BOMCl) in the presence of the strong base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Following radical reduction of the thiocarbonate, purification using chiral HPLC yielded derivative **58** in optically pure form which subsequently was converted into the parent bicyclic carbonucleoside **59**.[34]

A drawback of the strategy depicted in Scheme 8 is the final purification on HPLC, and a more convenient strategy has been developed (Scheme 9).[35,36] The isopropylidene protecting group of compound **54** was regioselectively opened to give the glycol **60** which was then mono-protected as its *tert*-butyldimethylsilyl ether at the sterically less hindered hydroxy group to give **61**. Barton deoxygenation and desilylation afforded the alcohol **63** (*via* **62**), which was subjected to a cyclopropanation reaction. Removal of the silyl protecting group of **62** is important since the free hydroxy group in **63** directs the Simmons–Smith cyclopropanation to give the desired bicyclo[3.1.0]hexane derivative **64** as the only product. The alcohol **64** was useful as a precursor for synthesis of all the derivatives **65** of the natural nucleobases in low to medium yields *via* a Mitsunobu reaction using the proper heterocycle followed by deprotection.[35,36]



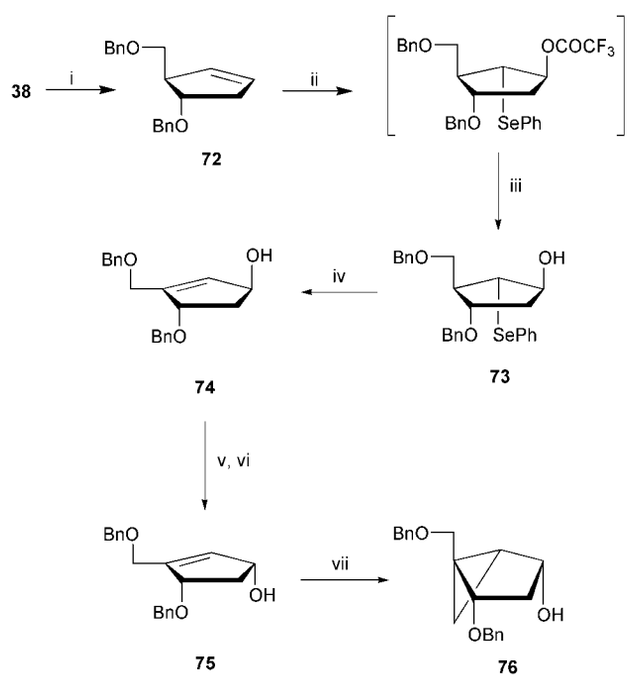
Scheme 9[35,36] *Reagents and conditions*: i) $AlMe_3$, CH_2Cl_2, −78 °C (54%); ii) TBDMSCl, imidazole, DMF (87%); iii) CS_2, NaH, MeI, THF (82%); iv) Bu_3SnH, AIBN, toluene, reflux (77%); v) TBAF, THF (92%); vi) Sm, $HgCl_2$, $ClCH_2I$, THF (96%), Base = A, C, G, T and U.

Yet another method for synthesis of the 1′a,4′-methanocarbocyclic nucleosides has been developed in order to avoid the two-step deoxygenation procedures of the two previous methods (Schemes 8 and 9). Silylation of the cyclopentene **38** afforded derivative **66** (Scheme 10)[37] which was subjected to azidation–phenylselenylation which occurred with complete stereochemical control furnishing derivative **67** after regioselective azide attack away from the benzyloxymethyl substituent. The protecting group at the secondary alcohol was either benzyl or *tert*-butyldimethylsilyl, but the latter was preferred due to higher step-wise yields and its easy selective removal. After oxidation to an intermediate selenoxide, regioselective elimination produced the allylic azide **68**. This intermediate was reduced to the corresponding amine which was protected as the phthalamide derivative **69**, a necessary step in order to allow the unmasked secondary hydroxy group of intermediate **70** to direct the Simmons–Smith cyclopropanation in a stereoselective way. Thus, the amine intermediate **71** was obtained which allowed the synthesis of the partly protected thymine derivative **59** in a step-wise manner.[37]

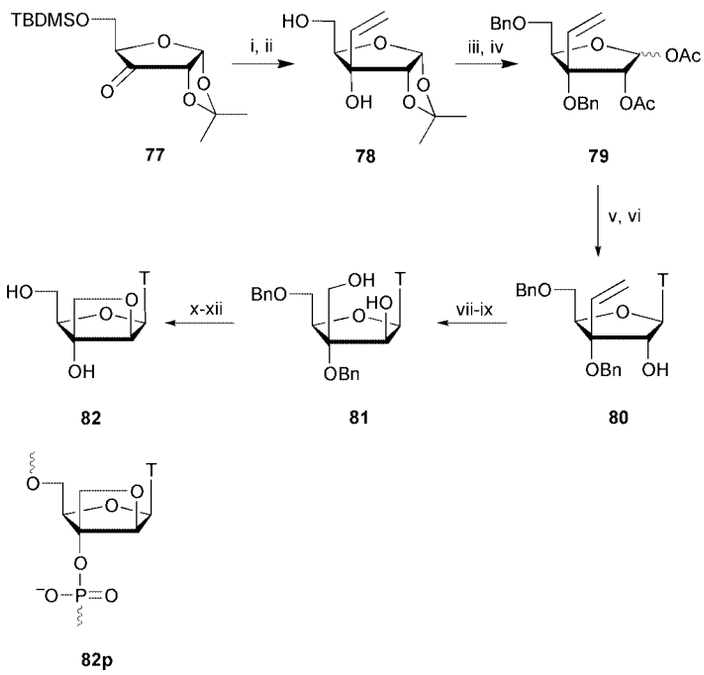


Scheme 10[37] *Reagents and conditions*: i) TBDMSCl, imidazole, DMF (76%); ii) PhSeCl, NaN_3, DMSO (87%); iii) $NaIO_4$, MeOH, H_2O (76%); iv) Ph_3P, THF, H_2O, reflux (90%); v) phthalic anhydride, pyridine, 90 °C (77%); vi) $Et_3N{\cdot}3HF$, MeCN, reflux (90%); vii) Et_2Zn, CH_2I_2, CH_2Cl_2 (96%); viii) N_2H_4, MeOH, 50 °C (~100%).

A very short synthesis of the bicyclic cyclopentane derivative **76** has recently been reported (Scheme 11).[38] The cyclopentene **38** was benzylated to give cyclopentene **72** which was treated with PhSeCl and a nucleophile, in this case trifluoroacetate, to give an intermediate, which, without isolation, was directly deacylated to give the alcohol **73**. Subsequent oxidation followed by regioselective elimination afforded the allylic alcohol **74** in satisfactory 74% yield. Inversion of configuration using a Mitsunobu reaction followed by debenzoylation afforded the alcohol **75** which by a hydroxy-directed stereoselective



Scheme 11[38] *Reagents and conditions*: i) BnBr, NaH, THF (35%); ii) CF_3COOAg, PhSeCl, DMSO; iii) KOH, EtOH (74%); iv) $NaIO_4$, MeOH, H_2O (73%); v) Ph_3P, DEAD, $PhCO_2H$, benzene (85%); vi) K_2CO_3, MeOH (80%); vii) Et_2Zn, CH_2I_2, CH_2Cl_2 (80%).



Scheme 12[39] *Reagents and conditions*: i) vinyl MgBr, Et_2O, THF; ii) TBAF, THF (75%, two steps); iii) BnBr, NaH, DMF (91%); iv) a. 80% acetic acid, 90 °C, b. Ac_2O, pyridine (87%); v) thymine, *N*,*O*-bis(trimethylsilyl)acetamide, MeCN, TMS-triflate (83%); vi) NaOMe, MeOH (97%); vii) MsCl, pyridine (84%); viii) NaOH, EtOH, H_2O (74%); ix) $NaIO_4$, catalytic OsO_4, THF, H_2O, then $NaBH_4$, THF, H_2O (36%); x) MsCl, pyridine, xi) NaH, DMF (93%, two steps); xii) H_2, $Pd(OH)_2$-C, EtOH (86%). T = thymin-1-yl.

Simmons–Smith cyclopropanation reaction was converted into the bicyclic cyclopentane alcohol **76**, a suitable precursor for synthesis of nucleoside derivatives under Mitsunobu conditions. This precursor was synthesised in fewer steps as compared to the similar precursor **64**, and a single-step deprotection of the nucleoside analogues is possible, as has been exemplified in the synthesis of the adenine derivative (**65**, Base = A).[38]

Detailed conformational analysis has been performed on the 1′a,4′-methanocarbocyclic adenine nucleoside (**65**, Base = A) revealing that the cyclopentane pseudosugar moiety is restricted to a north (C2′-*exo*, $_2E$) type conformation. X-Ray crystallography showed the existence of two different conformers in the unit cell of the crystal, differing only in the orientation of the primary hydroxy group.[35] Likewise, in the ^{1}H-NMR spectrum the pseudoanomeric proton appears as a doublet since two of the three dihedral angles to the neighbouring protons are close to 90° resulting in $^3J_{HH} \sim 0$ Hz.[35] A similar north type conformation was found for the corresponding thymine nucleoside **59**.[34]

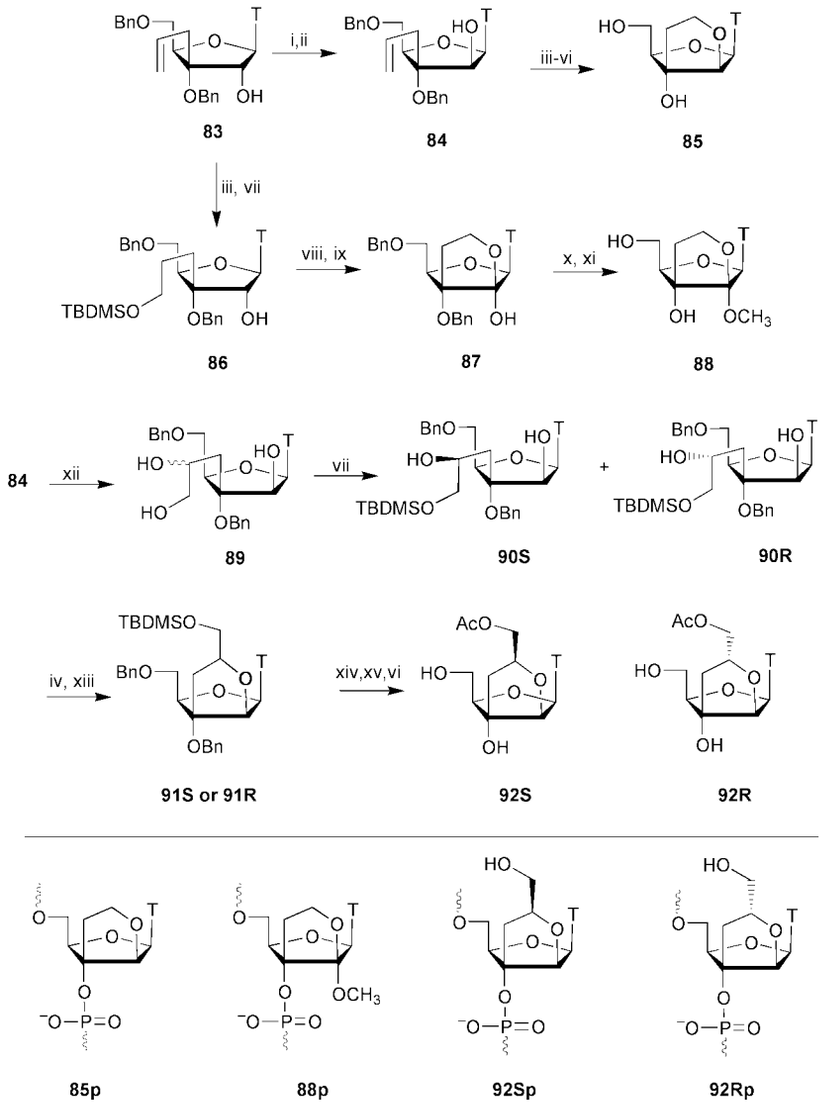
Oligonucleotides containing the thymine 1′a,4′-methanocarbocyclic nucleotide monomer **59p** (natural phosphodiester linkages; see Scheme 8) have been prepared and the thermal stability of the resulting duplexes towards an RNA target evaluated. In the phosphodiester series, a single monomer **59p** was introduced into a homopyrimidine 15-mer DNA sequence at two different positions, and both modified oligonucleotides displayed increased thermal stability (ΔT_m values of +0.8 and +2.1 °C) compared to the reference DNA–RNA duplex.[34] Similarly, a ΔT_m value of +1.3 °C was observed for a 15-mer phosphothioate DNA sequence with nucleotide monomer **59s** (phosphothioate linkages; see Scheme 8) incorporated ten times.[36] However, as the duplex formed between the modified oligonucleotide and RNA was not a substrate for RNase H, an enzyme able to cleave the RNA strand in a DNA : RNA duplex, further evaluation of this class of modified oligonucleotides was not performed.[36]

4 Synthesis of 2′-*O*,3′-*C*-linked bicyclic nucleosides and their oligonucleotides

A large group of bicyclic nucleosides based on a furanose moiety with alkyl linkers between one of the carbon atoms of the furanose ring and a heteroatom positioned as a substituent on the furanose ring has been prepared. Common to these bicyclic structures is that they have been synthesised with the aim of evaluating their effect on the binding affinity of modified oligonucleotides towards complementary DNA/RNA. A bicyclic nucleoside with a 2′-*O*,3′-*C*-methylene-linked furanose moiety (bicyclo[3.2.0]heptane scaffold) has been synthesised (Scheme 12).[39] Stereoselective addition of a vinyl substituent to the ulose **77** by a Grignard reaction afforded the furanose **78** in 75% yield. Removal of the silyl protecting group, benzylation, acetolysis and acetylation afforded the anomeric mixture **79** which was used as a glycosyl donor in a Vorbrüggen coupling reaction with silylated thymine affording the β-anomer as the sole product, probably due to the expected anchimeric assistance from the 2′-*O*-acetyl moiety. Deacetylation yielded the 3′-*C*-vinyl nucleoside **80** which *via* 2,2′-anhydro nucleoside formation was efficiently converted into the C2′-epimerised D-arabinofuranosyl nucleoside **81** after a one-pot conversion of the 3′-C-vinyl into a 3′-*C*-hydroxymethyl substituent (dihydroxylation, oxidative cleavage and reduction of the intermediary aldehyde). Selective mesylation of the primary hydroxy group was followed by very efficient ring closure and debenzylation yielding the parent bicyclic nucleoside **82** (Scheme 12). To prepare for incorporation of monomer **82p** into oligonucleotides, DMT-protection and phosphitylation were performed.[39]

Molecular modeling of nucleoside **82** suggested it to be in an east (O4′-*endo*, ^0E) conformation with a pseudorotational angle *P* of 94°. Compared to the usual furanose puckering found in nucleosides and nucleotides (north type, 0–36°, or south type, 144–180°) this corresponds to an unnatural conformation.[39] It was not possible to use NOE data to obtain further conformational information.

Modified DNA oligonucleotides with a varying number of monomer **82p** incorporated were synthesised, and the thermal stability of their duplexes formed with complementary DNA and RNA determined. Compared to the corresponding DNA reference, incorporation of few of the bicyclic monomers into a mixed sequence oligonucleotide induced only minor changes in the thermal stability towards complementary DNA/RNA.



Scheme 13[40–44] *Reagents and conditions*: i) MsCl, pyridine (89%); ii) NaOH, EtOH, H_2O, reflux (74%), iii) a. $NaIO_4$, cat. OsO_4, ^{t}BuOH, aq. THF, b. $NaBH_4$, aq. THF (49%); iv) TsCl, pyridine; v) NaH, DMF (83%, two steps); vi) H_2, 20% $Pd(OH)_2$-C, EtOH (**85**: 82%; **92S**: 95%; **92R**: 94%); vii) TBDMSCl, pyridine (**86**: 92%; **90S**: 53%; **90R**: 31%); viii) PDC, Ac_2O, 3 Å molecular sieve powder, CH_2Cl_2 (84%); ix) 0.5% HCl in MeOH (94%); x) NaH (two equiv.), CH_3I (7.4 equiv.), CH_2Cl_2, 36 °C (62%); xi) H_2, 20% $Pd(OH)_2$-C, MeOH (79%); xii) OsO_4, NMO, aq. THF (88%); xiii) K_2CO_3, 18-crown-6, DMF (**91R**: 45% from **90S**; **91S**: 52% from **90R**); xiv) TBAF, THF (**R**: 80%; **S**: 93%); xv) Ac_2O, pyridine (**R**: 96%; **S**: 92%). T = thymin-1-yl.

However, an oligonucleotide consisting of thirteen 2′-*O*,3′-*C*-methylene monomers **82p** and only one unmodified thymidine monomer displayed increased thermal stability towards complementary RNA and DNA ($\Delta T_m = +1.2$ and $+1.8$ °C, respectively).[39]

The corresponding 2′-*O*,3′-*C*-ethylene linked bicyclic nucleosides (bicyclo[3.3.0]octane scaffold) and some derivatives thereof have been synthesised from the 3′-*C*-allyl β-D-ribofuranosyl nucleoside **83** (Scheme 13)[40–43] which was obtained in a similar manner as described for nucleoside **80**. The bicyclic nucleoside **85** was synthesised in a similar way as described for **82**. Thus, C2′-inversion followed by oxidative cleavage of the allyl moiety, reduction, ring closure and debenzylation afforded **85** (*via* **84**) in overall 22% yield from **83**.[40,41] It was shown by molecular modeling that the furanose moiety of **85** adopts a C1′-*exo* ($_1E$) conformation with a pseudorotational angle *P* of 129°.[39] DMT-protection and phosphitylation yielded the corresponding phosphoramidite derivative which was used to introduce monomer **85p** into oligonucleotides.[40,41]

Incorporation of monomer **85p** into a DNA strand induced decreased thermal affinity towards complementary DNA and RNA compared to the unmodified DNA strand ($\Delta T_m \sim -3$ °C).[40,41] It was however possible to obtain strong and RNA-selective hybridization for an oligonucleotide containing monomer **85p** incorporated four or thirteen times consecutively ($\Delta T_m = +0.3$ and $+0.9$ °C, respectively).[40,41] The need for consecutive incorporations of monomers **82p** and **85p** in order to obtain large binding affinities probably originates from the unusual furanose conformations of these monomers leading to the formation duplexes of non-standard overall conformations. These promising results encouraged the synthesis of further modified nucleosides with a 2′-*O*,3′-*C*-ethylene linkage (Scheme 13).

The synthesis of the 2′-*O*,3′-*C*-ethylene-2′-*O*-methyl nucleoside **88** was accomplished in six steps from nucleoside **83** (Scheme 13).[42] Thus, oxidative cleavage of the allyl moiety followed by reduction and selective silylation afforded nucleoside **86**. Oxidation to the 2′-ulose followed by desilylation resulted in spontaneous ring closure to give the bicyclic hemiacetal **87**. In order to fix the bicyclic constitution, a chemoselective methylation with NaH and CH_3I in dichloromethane was performed followed by debenzylation which gave the 2′-*O*-methyl derivative **88**.[42] Incorporation of the corresponding nucleotide monomer **88p** into oligonucleotides demonstrated a detrimental effect of **88p** on the thermal stability of duplexes.[44]

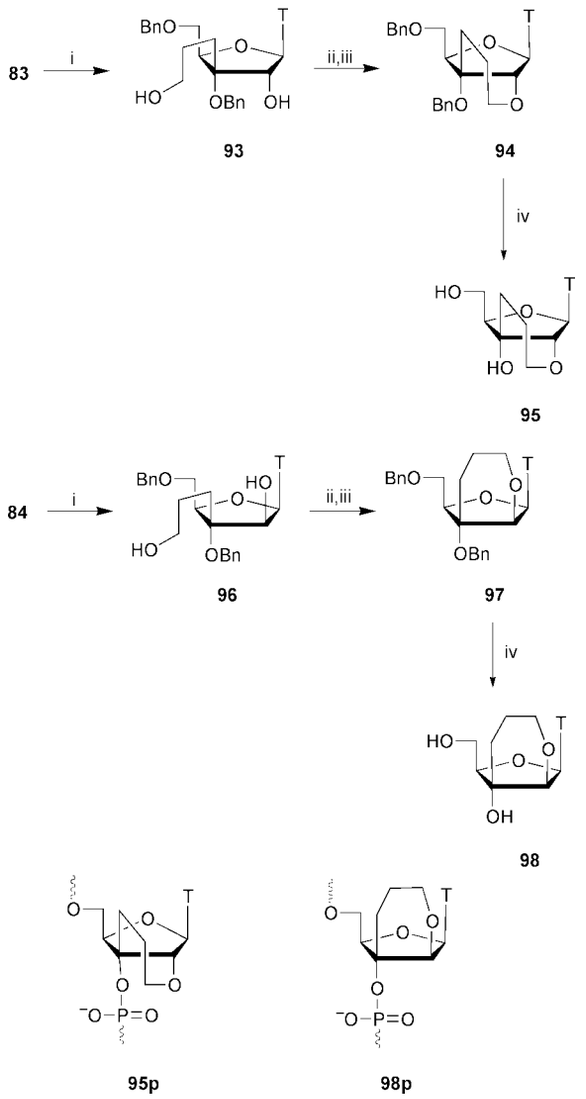
The 2′-*O*,3′-*C*-ethylene-linked nucleosides **92S** and **92R** containing an additional *C*-hydroxymethyl functionality attached at the ethylene linker (**S** and **R** designate *S*- and *R*-configur-

ations at the additional asymmetric carbon atom in the ethylene linker, respectively) were obtained in seven steps starting from nucleoside **84** (Scheme 13).[43] The inseparable diastereomeric mixture **89** was obtained by OsO_4-catalysed dihydroxylation. However, after silylation of the primary hydroxy groups, the two diastereoisomers **90S** and **90R** could be separated by column chromatography. Monotosylation expectedly afforded the derivatives with the 2′-hydroxy group free due to sterical hindrance from the thymine moiety, and base-induced ring closure furnished the bicyclic nucleosides **91R** and **91S**, respectively, by intramolecular substitution reactions with inversion. To prepare for oligonucleotide synthesis, the silyl protecting groups were exchanged with an acetyl which is removable during standard deprotection of oligonucleotides after completion of the desired sequence. Debenzylation furnished the two diastereoisomeric 2′-*O*,3′-*C*-ethylene-linked monomers **92S** and **92R** containing an *C*-acetoxymethyl functionality. Both diastereoisomers were DMT-protected and phosphitylated and subsequently incorporated into oligonucleotides by the phosphoramidite approach as monomers **92Sp** and **92Rp**, respectively (Scheme 13).[43]

Oligonucleotides containing the *S*-isomer **92Sp** displayed properties closely resembling those of the parent 2′-*O*,3′-*C*-ethylene-linked bicyclic nucleotide monomer **85p**.[41,43] Duplexes between partly modified oligonucleotides containing the *R*-isomer **92Rp**, with the additional *C*-hydroxymethyl substituent pointing towards the nucleobase, and complementary sequences were thermally less stable. However, a fully modified oligonucleotide containing monomer **92Rp** displayed self-complexation with a T_m value of 60 °C. An explanation supported by molecular modeling for this behaviour might be the existence of an intramolecular hydrogen bond between the additional *C*-hydroxymethyl group and the C2-carbonyl group of the thymine moiety, thereby orienting the nucleobase in a *syn* conformation allowing the formation of a stable homoduplex.[43]

For the synthesis of the *trans* and *cis* fused 2′-*O*,3′-*C*-propylene-linked bicyclic nucleosides **95** and **98** (bicyclo-[4.3.0]nonane structure), the strategies depicted in Scheme 14 were used.[41] With the *ribo*- and *arabino*-configured nucleosides **83** and **84** at hand as convenient starting materials, hydroboration of the double bond in the allyl substituents afforded the nucleosides **93** and **96** in 54 and 56% yield, respectively. Selective tosylation of the primary hydroxy group proved difficult in both cases as the ditosylated compounds together with starting material were obtained as by-products. However, ring closure of the monotosylated compound with sodium hydride in DMF afforded the bicyclic nucleosides **94** and **97** in yields of only 13 and 19%, respectively, for two steps. Debenzylation afforded the parent 2′-*O*,3′-*C*-propylene-linked bicyclic nucleosides **95** and **98** which were incorporated into oligonucleotides in the usual manner allowing evaluation of monomers **95p** and **98p**.[41]

It was indicated by molecular modeling that nucleoside **95** adopts a south (C2′-*endo*, $_2E$) type conformation. On the other hand, nucleoside **98** was shown to be able to adopt several different conformations despite its bicyclic nature. Both monomers **95p** and **98p** induced strongly decreased thermal affinity of homothymine sequences towards complementary DNA and RNA ($\Delta T_m = -5$ to -10 °C). An explanation for this dramatic effect on the thermal stability of duplexes could be sterical conflicts in the duplexes due to the bulkiness of the six-membered additional ring.[41]

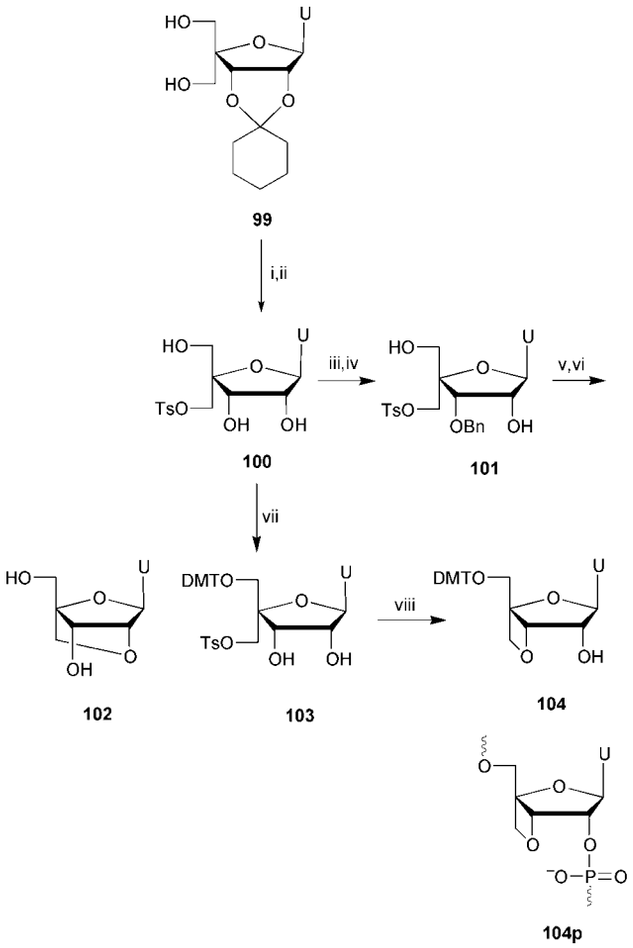


Scheme 14[41] *Reagents and conditions*: i) a. BH_3·1,4-oxathiane, THF; b. NaOH, H_2O, H_2O_2 (**93**: 54%; **96**: 56%); ii) TsCl, pyridine; iii) NaH, DMF (**94**: 13% two steps; **97**: 19%, two steps); iv) H_2, $Pd(OH)_2$-C, EtOH (73% for **95**; mixture of substrates used for **98**).

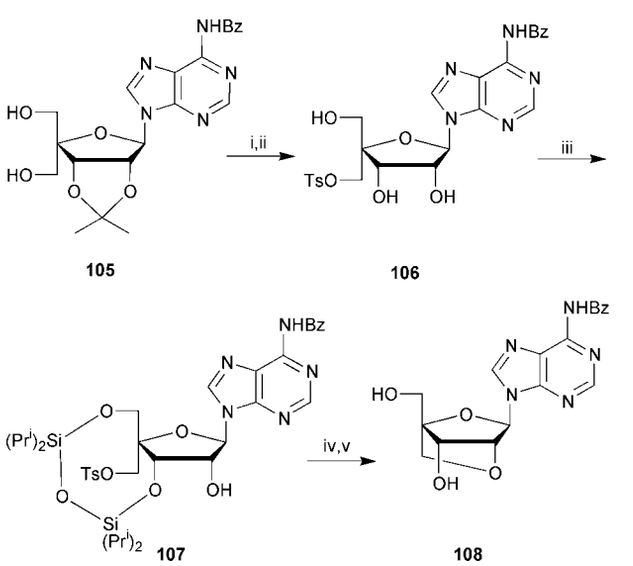
5 Synthesis of 2′-*O*,4′-*C*-linked bicyclic nucleosides. LNA (Locked Nucleic Acid) and derivatives

A very interesting class of bicyclic nucleosides for modification of oligonucleotides are 2′-*O*,4′-*C*-methylene-linked ribonucleosides (bicyclo[2.2.1]heptane scaffold). The resulting oligonucleotide analogues have been named LNA (Locked Nucleic Acid)[45,46] because of the locked conformation of their furanose moiety (*vide infra*), and the notation "LNA nucleosides" is used in the following for the 2′-*O*,4′-*C*-methylene-linked ribonucleosides. For synthesis of these bicyclic nucleosides, both linear and convergent strategies have been applied. Examples of linear strategies are given in Scheme 15[47,48] and in Scheme 16.[49]

In one strategy, the known 4′-*C*-hydroxymethyl nucleoside **99**[50] was regioselectively tosylated and the cyclohexylidene protecting group subsequently removed to give nucleoside **100**. The 2′- and 3′-hydroxy groups were protected as the benzylidene acetal, which then was regioselectively reduced to give nucleoside **101** with the benzyl protecting group attached at the 3′-*O*-position. This selectivity during the reduction was ascribed to the presence of the sterically demanding 4′-*C*-substituent. Subsequent ring closure and debenzylation gave the parent 2′-*O*,4′-*C*-methylene-linked uracil nucleoside **102**. Selective DMT-protection at the 5′-hydroxy group of compound **100** furnished nucleoside **103** which was cyclised to give the 3′-*O*,4′-*C*-methylene-linked bicyclic nucleoside **104** (Scheme 15). Analogously, attempts to directly cyclise triol **100** resulted predominantly in the formation of the 5′-hydroxy derivative of the oxetane **104**.[51] From these results, protection of the 3′-hydroxy group seems essential for synthesis of LNA nucleosides. It is therefore noteworthy that the selective opening



Scheme 15[47,48] *Reagents and conditions*: i) TsCl, 110 °C (69%); ii) aqueous TFA (94%); iii) PhCHO, $ZnCl_2$ (80%); iv) $NaCNBH_3$, $TiCl_4$, MeCN (75%); v) $(Me_3Si)_2NNa$ (3.3 equiv.), THF (61%); vi) H_2, Pd-C, MeOH (~100%); vii) DMTCl, DMAP, pyridine (56%); viii) $(Me_3Si)_2NNa$ (10 equiv.), THF (63%). U = uracil-1-yl.

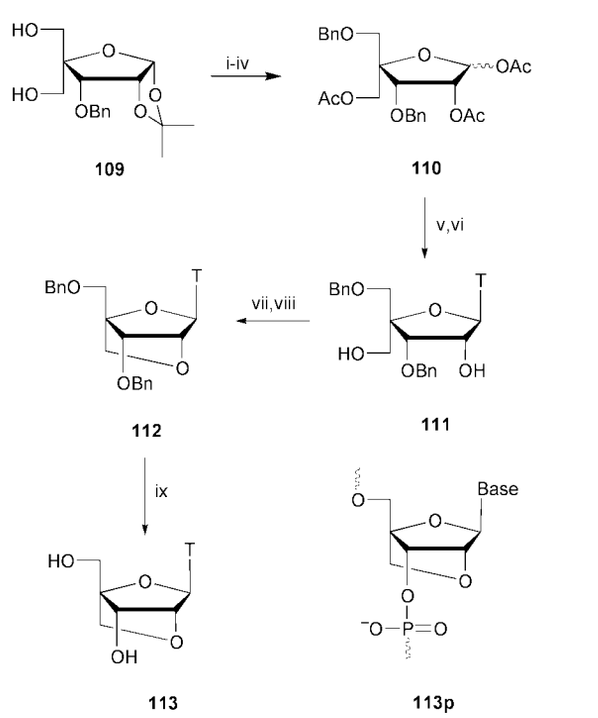


Scheme 16[49] *Reagents and conditions*: i) TsCl, pyridine (37%); ii) aqueous CF_3COOH (94%); iii) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSCl_2$), pyridine (78%); iv) NaH, anhydrous THF (84%); v) $Et_3N{\cdot}3HF$, anhydrous THF.

of the benzylidene protecting group used in the synthesis of the uridine derivative **101** has not been demonstrated with other nucleobases.

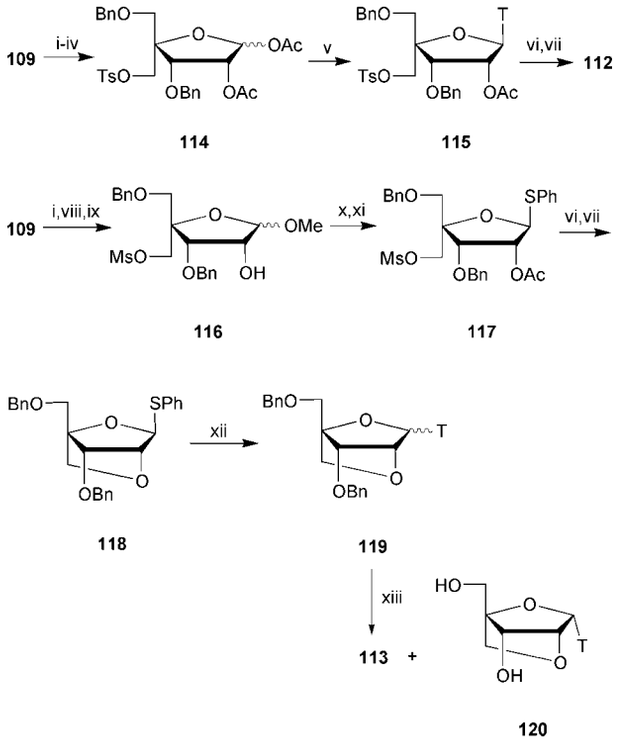
Another linear approach involving selective 3′-*O*-protection during ring closure is depicted in Scheme 16.[49] The tosylated nucleoside **106** was obtained from the known 4′-C-hydroxymethyl nucleoside **105**[50] as shown. The 3′- and 5′-hydroxy groups of **106** were protected with the bidentate TIPDS group giving derivative **107**. Subsequent cyclisation proceeded smoothly and after desilylation the target bicyclic nucleoside **108** was obtained.[49] An advantage of this TIPDS strategy compared to the benzyl strategy shown in Scheme 15 is that it involves fewer synthetic steps. In addition, the TIPDS strategy is probably more generally applicable for synthesis of LNA nucleosides containing other nucleobases. Unfortunately, the selective tosylation (or mesylation) of the 4′-*C*-hydroxymethyl group of nucleoside derivatives like **99** or **105** generally proceeds in rather limited yields. In addition, the hydroxymethylation of ribonucleosides is troublesome. Therefore, a convergent strategy for the synthesis of LNA nucleosides appears more viable.

The LNA nucleosides containing all the natural nucleobases have been synthesised using the convergent strategy shown in Scheme 17.[45,46] The key intermediate **110** was obtained from the 4-*C*-branched pentofuranose **109**[52] by selective benzylation,[53] acetylation, and acetolysis followed by another acetylation. Intermediate **110** was used as a glycosyl donor in coupling reactions with silylated nucleobases affording the thymine derivative **111** as an example after subsequent deacetylation. Selective tosylation followed by ring closure then furnished the protected derivatives (*e.g.* **112**) which upon debenzylation was converted into the target LNA nucleosides, *e.g.* the thymine derivative **113**. In the case of the cytosine and adenine derivatives, the debenzylation reactions resulted in concomitant *N*-debenzoylation of the nucleobase moieties necessitating a reprotection procedure (TMS-protection of the free hydroxy groups followed by benzoylation of the free exocyclic amine and subsequent desilylation) to obtain the protected adenine and cytosine LNA nucleosides useful for automated synthesis of LNA.[45,46]



Scheme 17[45,46] *Reagents and conditions*: i) NaH, BnBr, DMF (71%); ii) Ac_2O, pyridine (90%); iii) 80% aqueous AcOH; iv) Ac_2O, pyridine (86%, two steps); v) thymine, *N*,*O*-bis(trimethylsilyl)acetamide, TMS-triflate, MeCN, 65 °C (76%); vi) $NaOCH_3$, MeOH (97%); vii) TsCl, pyridine; viii) NaH, DMF (42%, two steps); ix) H_2, $Pd(OH)_2$-C, EtOH (98%). T = thymin-1-yl.

The selective tosylation of diol **111** is a rather low-yielding step. In order to overcome this synthetic problem the use of other glycosyl donors has been investigated (Scheme 18).[49,54]



Scheme 18[49,54] *Reagents and conditions*: i) NaH, BnBr, DMF; ii) TsCl, pyridine; iii) 80% aqueous AcOH; iv) Ac_2O, pyridine (59% from **109**); v) thymine, *N*,*O*-bis(trimethylsilyl)acetamide, TMS-triflate, MeCN (92%); vi) NH_3, MeOH (87%); vii) NaH, DMF (**112**: 92%; **118**: 95%; two steps); viii) MsCl, pyridine (99%); ix) 20% HCl in MeOH/H_2O (7 : 1, v/v) (95%); x) Ac_2O, pyridine (97%); xi) TMSSPh, TMS-triflate, CH_2Cl_2 (66%); xii) thymine, HMDS, then NBS, **118**, 4 Å molecular sieves, CH_2Cl_2 (61%, α : β = 2 : 1); xiii) H_2, 20% $Pd(OH)_2$–C, EtOH, CH_2Cl_2 (**113**: 12%; **120**: 25%). T = thymin-1-yl.

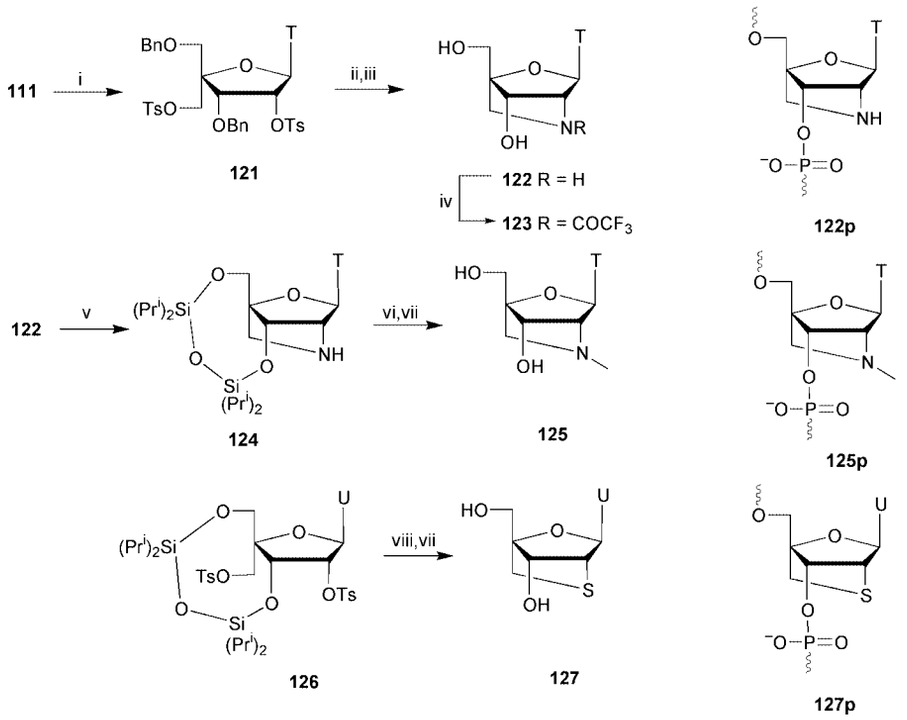
Introduction of a tosyl instead of an acetyl group at the 4-*C*-hydroxymethyl substituent after selective 5-*O*-benzylation afforded the key synthon **114** after standard exchange of the 1,2-*O*-isopropylidene group with two acetyl groups. Coupling between furanose **114** and silylated thymine using Vorbrüggen conditions afforded efficiently and stereoselectively the thymine nucleoside **115** which after mild deacetylation with methanolic ammonia and treatment with strong base was cyclised to give the desired LNA nucleoside **112**.[49] Another alternative convergent approach involved the bicyclic thioglycoside **118** as glycosyl donor.[54] Thus, benzylation of furanoside **109** followed by mesylation and methanolic acetolysis afforded the methyl furanoside **116**. Ring closure of this furanoside proved very efficient but the resulting bicyclic adduct was shown not to be suitable as a glycosyl donor in a Vorbrüggen coupling reaction towards the LNA nucleosides **119** since its strained bicyclic ring system opened in course of the coupling reaction.[54] However, acetylation and thiophenylation of **116** to give **117**, with the β-anomer as sole product, was followed by deacetylation and ring closure to give the bicyclic phenyl thiofuranoside **118** which was used in a coupling reaction with silylated thymine using NBS as thiophilic activator. Thereby, the anomeric mixture **119** (α : β = 2 : 1; 61% yield) was obtained. After debenzylation, the two anomers **113** (β-D-*ribo* configuration) and the α-LNA nucleoside **120** (α-D-*ribo* configuration) were obtained in 12 and 25% yield, respectively. Whereas the route involving the glycosyl donor **115** constitutes an improvement compared to the one involving **110**, the bicyclic glycosyl donors, *e.g.* the thioglycoside **118**, appear of only limited use for synthesis of the parent β-D-*ribo* configured LNA nucleosides. However, because of the lack of 2′-*O*-acyl substituent and thus anchimeric assistance during the coupling reaction, they might be useful for synthesis and investigations on the corresponding α-D-*ribo* configured LNA nucleosides and α-LNA.

As predicted from molecular modeling, conformational analysis of LNA nucleosides has shown their furanose moieties to adopt a north type (C3′-*endo*, ^3E) conformation with a pseudorotational angle *P* of 17°. This knowledge comes from X-ray crystallographic studies[47] and from NMR studies (*e.g.*, no coupling between H1′ and H2′ and between H2′ and H3′).[45–47]

LNA nucleoside derivatives of all the natural nucleobases have been incorporated into oligonucleotides (monomer **113p**, Scheme 17) using an automated DNA-synthesiser after conversion of the corresponding LNA nucleosides, *e.g.* the thymine derivative **113**, to phosphoramidite building blocks by 5′-*O*-DMT-protection and 3′-*O*-phosphitylation.[45,46,55] By virtue of their fixed furanose conformation, this novel class of oligonucleotides containing *one or more* monomeric LNA nucleotides was named "LNA" (Locked Nucleic Acid).[45,46] LNA displays unprecedented binding affinities towards both DNA and RNA complements as judged by the strongly increased T_m values compared to the unmodified reference duplexes.[45,46,55–57] Incorporated into a 9-mer mixed DNA sequences, ΔT_m values of +5 to + 6 °C towards complementary DNA were observed together with ΔT_m values of +7 to +8 °C towards complementary RNA.[46,57] When incorporated three times into a 9-mer mixed RNA sequence, even more pronounced increases in the thermal stability were observed with ΔT_m values of +8 to +9 °C.[56] In addition, fully modified LNA (same sequence context) was able to hybridize with target DNA and RNA complements even more tightly than the partly modified LNAs,[46] and LNA : LNA hybridization constitutes the most stable duplexes, based on natural phosphordiester internucleoside linkages, reported so far.[57] Importantly, despite the very strong binding affinities between LNA and complementary DNA/RNA, base-pairing specificity is comparable to, or even better than, that observed for the corresponding unmodified duplexes[45,58] demonstrating that LNA obeys the Watson–Crick base-pairing rules. Based on preliminary thermodynamic studies it appears that the extraordinary stability of duplexes involving partly modified LNA originates from a favorable entropic term.[55,57] This correlates well with NMR studies on LNA duplexes showing that LNA monomers in a partly modified LNA induce a conformational shift in neighbouring unmodified monomers towards an RNA-like north type conformations thereby overall preorganising the LNA for duplex formation.[59]

Several LNA derivatives have been synthesised. The syntheses leading to the introduction of 2′-amino-LNA and 2′-thio-LNA are depicted in Scheme 19.[60–62] For synthesis of the 2′-amino-LNA, the ditosylated nucleoside **121** was a key intermediate obtained by ditosylation of nucleoside **111**. Treatment of **121** with neat benzylamine at 130 °C afforded a tribenzylated 2′-amino-LNA nucleoside formed *via* the 2,2′-anhydro intermediate which explains the retention of configuration at C2′ during the reaction.[60,61] Debenzylation gave the parent 2′-amino-LNA nucleoside **122** which was protected as its *N*-trifluoroacetyl derivative **123** before DMT-protection and phosphitylation[60,61] *en route* to 2′-amino-LNA containing the monomeric nucleotide **122p**. The 2′-methylamino-LNA nucleoside **125** was obtained by selective methylation of the 3′-*O*,5′-*O*-TIPDS-protected tricyclic nucleoside **124** to give nucleoside **125** after concomitant desilylation. The TIPDS protecting group was introduced during the synthesis of **125** from **122** to ensure chemoselective *N*-methylation. Also nucleoside **125** was incorporated into oligonucleotides (as monomer **125p**, Scheme 19).[61]

The key step in the synthesis of the 2′-thio-LNA nucleoside **127** (Scheme 19) was reaction of the di-*O*-tosylated TIPDS-protected nucleoside **126** with potassium thioacetate in DMF. After desilylation, nucleoside **127** was obtained and subsequently incorporated into oligonucleotides as the 2′-thio-LNA monomer **127p**.[61,62] As was the case in the synthesis of the 2′-amino-LNA nucleoside **122**, the likely intermediacy of a 2,2′-anhydro nucleoside offers an explanation of the required



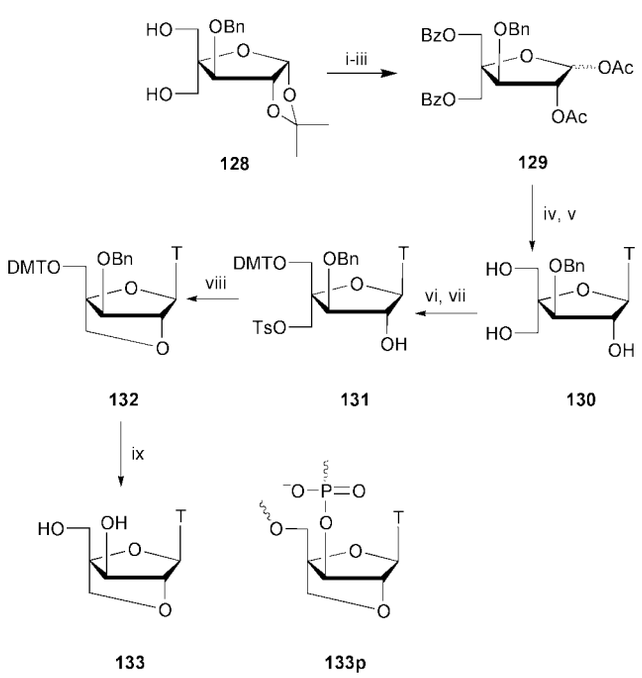
Scheme 19[60–62] *Reagents and conditions*: i) TsCl, DMAP, CH_2Cl_2 (80%); ii) $BnNH_2$, 130 °C (52%); iii) ammonium formate, 10% Pd-C, MeOH, reflux (68%); iv) ethyl trifluoroacetate, DMAP, MeOH (81%); v) $TIPDSCl_2$, pyridine; vi) MeI, DBU, THF–CH_2Cl_2 4:1, −10 to 10 °C (74%); vii) TBAF, THF; viii) KSAc, DMF (77%). T = thymin-1-yl. U = uracil-1-yl.

overall retention of the C2′ configuration. Attempts to synthesise **127** from 3′,5′-di-*O*-benzyl intermediates were unsuccessful due to failed debenzylation after introduction of the thiomethylene linker.

Structural analysis by NMR spectroscopy of all three 2′-modified LNA nucleosides **122**, **125** and **127** gave results similar to those obtained for the parent LNA nucleoside **113** (base = thymin-1-yl), *i.e.* that a locked C3′-*endo* conformation is adopted.[60,61] In the 9-mer mixed DNA sequence, 2′-amino-LNA, containing one or more 2′-amino-LNA monomer(s) **122p** or 2′-methylamino-LNA monomers **125p**, displays significantly increased thermal stability towards complementary DNA (ΔT_m = +3 °C) and complementary RNA (ΔT_m = +6 °C) when compared to the corresponding unmodified reference duplex.[61] These results indicate that the amino group in 2′-amino-LNA could serve as an attachment point for different moieties facing the rim of the minor groove. The binding affinity of 2′-thio-LNA, containing one or more 2′-thio-LNA monomer(s) **127p**, appears as good as that of LNA itself as judged from ΔT_m values of +4 to +6 °C towards complementary DNA and +8 °C towards complementary RNA.[62]

Stereoisomeric forms of LNA have been synthesised, *i.e.* *xylo*-LNA (containing the β-D-*xylo* configured monomer **133p**) and α-L-LNA (containing the α-L-*ribo* configured monomer **136p**).[63–65]

The *xylo*-LNA nucleoside **133** (Scheme 20) was synthesised from 4-*C*-hydroxymethyl-β-L-*threo*-pentofuranose **128**,[66] which by benzoylation followed by acetolysis and acetylation, was converted into the tetra-*O*-acylated glycosyl donor **129** in 83% yield. Nucleoside coupling with silylated thymine followed by deacylation gave nucleoside **130**. This nucleoside was selectively monotosylated at the 4′-*C*-hydroxymethyl substituent positioned at the opposite face of the furanose ring to thymine, but base-induced ring closure was unsuccessful for this intermediate. However, after monoprotection of the remaining hydroxy group by reaction with DMTCl to give compound **131**, cyclisation was efficiently accomplished in 96% yield affording the protected *xylo*-LNA nucleoside **132** which was deprotected in one step to give the parent *xylo*-LNA nucleoside **133**. Subsequent selective DMT-protection and phosphitylation



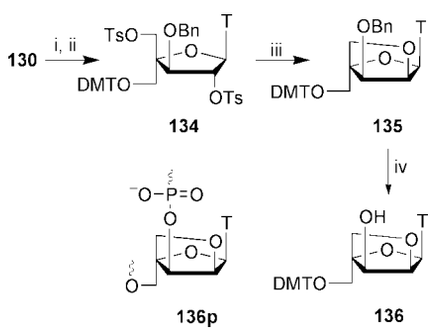
Scheme 20[63–65] *Reagents and conditions*: i) BzCl, pyridine (90%); ii) 80% aqueous AcOH; iii) Ac_2O, pyridine (92%, two steps); iv) *N*,*O*-bis(trimethylsilyl)acetamide, thymine, TMS-triflate, MeCN (85%); v) NaOMe, MeOH (89%); vi) TsCl, pyridine, CH_2Cl_2 (35%); vii) DMTCl, DMAP, pyridine (75%); viii) NaH, DMF (96%); ix) H_2, 10% Pd–C, MeOH (82%). T = thymin-1-yl.

afforded a convenient phosphoramidite building block for introduction of monomer **133p** into oligonucleotides.[63,64]

By virtue of the conformationally locked bicyclo[2.2.1]-heptane scaffold equivalent to that of LNA, the furanose ring of *xylo*-LNA nucleosides and the corresponding nucleotide monomers likewise exist in C3′-*endo* conformation.[63] Oligomerisation using the *xylo*-LNA phosphoramidite derivative of **133** on an automated synthesiser proved to be very difficult, which might be attributed to sterical hindrance at the β-face of

the furanose ring. Similar difficulties were obtained during attempted oligomerisation of the corresponding 2′-deoxy *xylo*-DNA phosphoramidite building blocks.[67] However, exchange of the standard activator 1*H*-tetrazole with pyridinium chloride increased the stepwise coupling yields to >99%.[64] Incorporation of a single *xylo*-LNA monomer **133p** in a homothymine 10-mer strongly reduced the binding affinity towards both DNA and RNA complements,[64] but for the corresponding fully modified *xylo*-LNA very efficient hybridization was observed (ΔT_m = +3.1 °C towards DNA and +4.3 °C towards RNA.[65]

The last steps in the synthesis of the α-L-LNA thymine nucleoside derivative **136** are indicated in Scheme 21.[64,68] Ring closure of nucleoside **134**[68] by treatment of base (involving a reaction cascade with 2,2′-anhydro nucleoside formation, hydrolysis and intramolecular nucleophilic substitution) afforded the α-L-*ribo* configured α-L-LNA nucleoside **135**.[68] Debenzylation of **135** using ammonium formate on palladium black in refluxing methanol proceeded chemoselectively without affecting the DMT group yielding nucleoside **136**.[64,68] Phosphitylation of **136** followed by oligomerisation led to the introduction of α-L-LNA containing one or more α-L-LNA nucleotide monomer(s) **136p** (Scheme 21).[64]

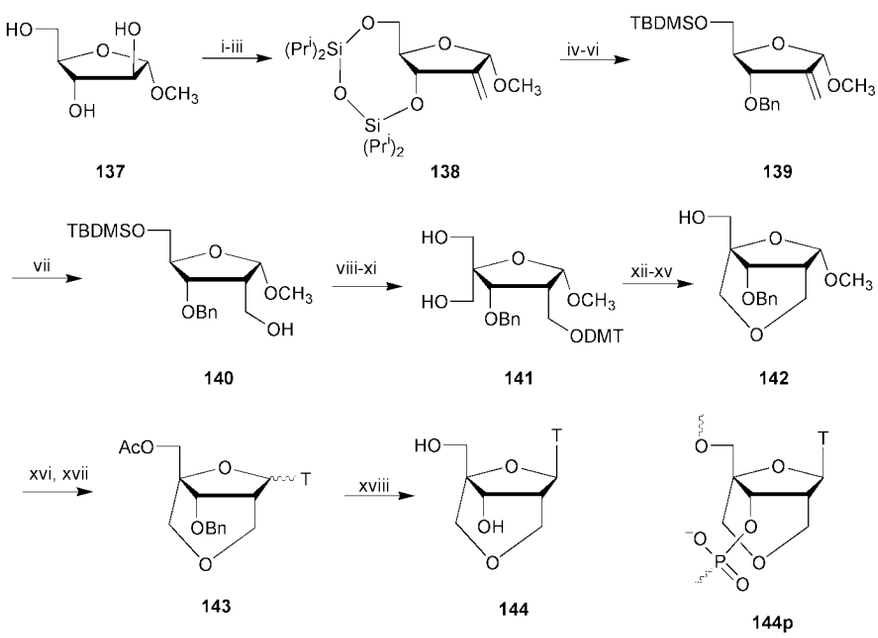


Scheme 21[64,68] *Reagents and conditions*: i) DMTCl, $AgNO_3$, THF, pyridine (31%); ii) TsCl (10 equiv), DMAP, pyridine (63%); iii) NaOH, EtOH, H_2O, reflux (81%); iv) ammonium formate, 10% Pd-C, MeOH, reflux (80%). T = thymin-1-yl.

Modeling and NMR studies revealed that the nucleoside is fixed in a south type (C3′-*exo*, $_3E$) conformation.[64,68] Interestingly, comparison of the thymine α-L-LNA nucleoside (detritylated derivative of **136**) and the thymine LNA nucleoside (**113**) indicates a strong spatial overlap between three atoms of prime importance for hybridization, namely N1, O3′ and C5′. Accordingly, the binding properties for α-L-LNA closely approach those of LNA[64,65] despite their different configurations at three out of the four stereocenters in their scaffold composed of the rigid bicyclo[2.2.1]heptane moiety.

6 Synthesis of other bicyclic nucleosides and their oligonucleotides

A close analogue of LNA based on a bicyclo[3.2.1]octane scaffold has very recently been synthesised as shown in Scheme 22.[69,70] Protection of the 3- and 5-hydroxy group of the D-arabinofuranoside **137** as its TIPDS derivative followed by oxidation and Wittig methylenation afforded the methylene furanoside **138**. Following a series of deprotection and protection steps, derivative **139** was obtained, which by a stereoselective hydroboration sequence furnished 2-*C*-hydroxymethyl derivative **140**. DMT-protection of the primary hydroxy group followed by desilylation, Swern oxidation and subsequent crossed aldol condensation with concomitant *in situ* Cannizzaro reduction afforded the 2,4-di-*C*-hydroxymethyl furanose **141**. Dimesylation followed by removal of the DMT-group, base-induced ring closure and hydrolysis of the remaining mesyl group yielded the bicyclic furanoside **142**. Following acetylation of the 5-hydroxy group, the bicyclic furanose was used as a glycosyl donor in a Vorbrüggen coupling with silylated thymine (shown as example). As expected, no anchimeric assistance occurred and an anomeric mixture of nucleosides **143** was obtained. In the case of pyrimidines, the α-anomer was obtained as the major (or only) product when using TMS-triflate as the Lewis acid. On changing to $SnCl_4$, the outcome of the coupling reaction was different and anomeric mixtures with increased content of the desired β-anomer (thymine α:β ~4:1 and 4-*N*-acetylcytosine α:β ~9:1) were obtained.[69] Deprotections afforded the parent thymine nucleoside **144** and the corresponding 4-*N*-acetylcytosine derivative, which were prepared for oligomerisation in the usual manner allowing incorporation of the thymine monomer **144p**, and the corresponding cytosine monomer, into oligonucleotides.[70]



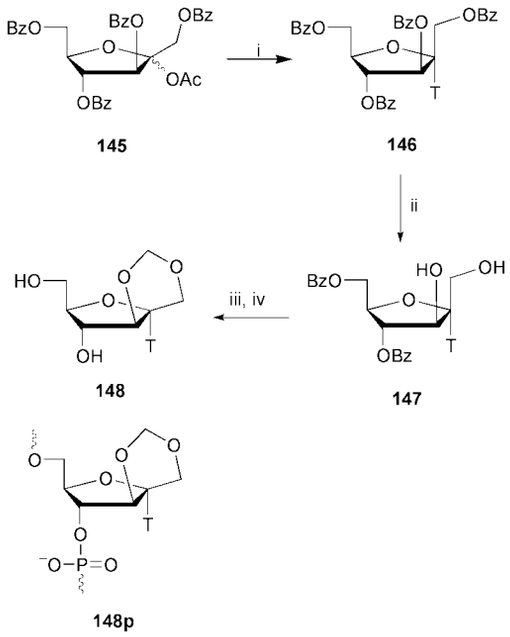
Scheme 22[69,70] *Reagents and conditions*: i) $TIPDSCl_2$, pyridine (88%); ii) DMSO, DCC, TFA, pyridine (89%); iii) Ph_3PCH_2, ether, −10 °C (91%); iv) TBAF, THF (88%); v) TBDMSCl, pyridine; vi) BnBr, NaH, THF (76%, two steps); vii) a. 9-BBN, THF, 40 °C; b. $NaBO_3$, H_2O, 50 °C (93%); viii) DMTCl, pyridine; ix) TBAF, THF (94%, two steps); x) DMSO, DCC, TFA, pyridine (90%); xi) CH_2O, NaOH, H_2O, dioxane (95%); xii) MsCl, pyridine; xiii) AcOH, H_2O (90%, two steps); xiv) NaH, THF, 55 °C; xv) NaOH, H_2O, reflux (98%, two steps); xvi) Ac_2O, pyridine (99%); xvii) silylated thymine, $SnCl_4$, CH_2Cl_2, reflux (89%); xviii) BCl_3, CH_2Cl_2 (56%). T = thymin-1-yl.

Conformational analysis of the bicyclic nucleoside **144** by X-ray crystallography and NMR showed that the furanose ring is fixed in a north type (C3′-*endo*, ^3E) conformation with a pseudorotational angle P of 16°, and that the six-membered ring adopts a chair conformation.[69] When incorporated into oligonucleotides, this modification (monomer **144p**) only induced relatively minor changes in the thermal stability of duplexes towards complementary DNA ($\Delta T_m = -0.4$ to $+1$ °C). However, towards complementary RNA the results were more encouraging ($\Delta T_m = +1.9$ to $+3.3$ °C).[70] An oligonucleotide containing a bicyclic nucleoside based on the same scaffold as **144** but linked by an all-carbon propylene linker has been mentioned in a review on modified oligonucleotides.[71] Interestingly, that specific modification induced a decrease in the thermal stability of a duplex towards complementary DNA ($\Delta T_m = -2.3$ °C) compared to the reference duplex.[71] The different effect of monomer **144p** containing an 2-oxapropylene linker and the corresponding propylene-linked monomer might be a result of different hydration of the two monomers, possibly influencing binding affinity through entropic and/or enthalpic effects.

Synthesis of the 3′-*O*,4′-*C*-methylene nucleoside **104** (bicyclo-[3.2.0]heptane scaffold) is depicted in Scheme 15. Nucleoside **104** adopts a south type (C2′-*endo*/C1′-*exo*, $^2T_1$) conformation.[47] Phosphitylation of **104** gave the building block for automated synthesis of modified DNA oligonucleotides containing monomer **104p** (linked as phosphate diesters *via* the 2′- and 5′-hydroxy groups, Scheme 15).[72] When incorporated into mixed DNA sequences, the monomer **104p** caused, dependent on the exact sequence, both decreased and increased thermal affinity towards complementary RNA ($\Delta T_m = -2$ to $+4$ °C), whereas the effect was negative or neutral towards complementary DNA ($\Delta T_m = -6$ to 0 °C).[72]

The bicyclic α-D-*arabino* configured nucleoside **148** (bicyclo-[4.3.0]nonane scaffold) was synthesised as shown in Scheme 23 and subsequently incorporated into oligonucleotides.[73] The known fructose derivative **145**[74] was condensed with silylated thymine in a Vorbrüggen coupling giving the α-anomer **146** in 75% yield as the only product due to anchimeric assistance from the benzoyloxy group neighbouring the anomeric carbon atom. Selective debenzoylation was accomplished by careful addition of sodium methoxide to a solution of **146** in THF. The resulting dihydroxy nucleoside was subsequently converted into a bicyclic nucleoside in a reaction with paraformaldehyde catalysed by acid. It was deprotected to give the parent nucleoside **148** containing an acetal functionality.[73] Modeling experiments suggested a preference of the furanose ring to adopt a south type (C3′-*exo*, $_3E$) conformation. This correlates well with the fact that a fully modified oligonucleotide containing monomer **148p** and the corresponding 5-methylcytosine monomer was unable to form complexes with complementary RNA.[73]

A few other oligonucleotide modifications exist which are based on bicyclic furanose scaffolds.[75–77] However, as the internucleotide linkages between these deviate from the natural phosphodiester type these are not included herein.



Scheme 23[73] *Reagents and conditions*: i) thymine, *N*,*O*-bis(trimethylsilyl)acetamide, $SnCl_4$, MeCN (75%); ii) NaOMe, THF (49%); iii) $(CH_2O)_n$, $MeSO_3H$, CH_2Cl_2 (90%); iv) NaOMe, MeOH (no yield was given). T = thymin-1-yl.

7 Summary and outlook

The use of monomeric nucleotides containing bicyclic furanose moieties has proven a very productive way of improving the binding affinity of oligonucleotide analogues towards complementary DNA and RNA. Based on the results described herein it can be concluded that the bicyclic monomers with furanose moieties restricted to north type conformations in general induce the most promising binding properties.

From the work on bicyclo-DNA, important knowledge on the relation between conformational restriction of nucleotide monomers and duplex structure and stability has been obtained. Among the different chemically modified oligonucleotides so far synthesised, LNA stands out in several ways. First of all, its binding affinity towards both DNA and RNA complements is extraordinary. Secondly, its affinity-enhancing effect is not restricted to a fully modified context but also embraces sequences composed of LNA- and, *e.g.*, DNA- or RNA-monomers. Thirdly, a family of LNA analogues has been demonstrated to hybridize with unprecedented binding affinity, including *e.g.* 2′-amino-LNA and α-L-LNA.

As has been clearly illustrated in this review, the synthetic accessibility of the bicyclic nucleoside monomers is somewhat restricted. Multi-step syntheses are needed and careful control of regio- as well as stereochemistry is of major concern. It is therefore clear that there is still room (and need) not only for development of improved syntheses of known interesting analogues, but also for introduction of novel modifications within this field. Besides the need for high-affinity oligonucleotide analogues for antisense therapy and diagnostic arrays, the application of oligonucleotides and analogues in areas like supramolecular chemistry and materials science is likely to expand in the near future.

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8 References

1 E. Uhlmann and A. Peyman, *Chem. Rev.*, 1990, **90**, 543.
2 A. De Mesmaeker, R. Häner, P. Martin and H. E. Moser, *Acc. Chem. Res.*, 1995, **28**, 366.
3 P. Herdewijn, *Liebigs Ann.*, 1996, 1337.
4 E. T. Kool, *Chem. Rev.*, 1997, **97**, 1473.
5 M. Egli, *Antisense Nucleic Acid Drug Dev.*, 1998, **8**, 123.
6 P. Herdewijn, *Biochem. Biophys. Acta*, 1999, 167.
7 J. E. Kilpatrick, K. S. Pitzer and R. Spitzer, *J. Am. Chem. Soc.*, 1947, **69**, 2483.
8 C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, 1972, **94**, 8205.
9 For IUPAC–IUB recommended abbreviations and symbols for the description of conformations of polynucleotide chains, see: *Eur. J. Biochem.*, 1983, **131**, 9.
10 V. E. Marquez, A. Ezzitouni, P. Russ, M. A. Siddiqui, H. Ford, Jr., R. J. Feldman, H. Mitsuya, C. George and J. J. Barchi, Jr., *J. Am. Chem. Soc.*, 1998, **120**, 2780.
11 S. Gryaznov and J.-K. Chen, *J. Am. Chem. Soc.*, 1994, **116**, 3143.

12 R. G. Schultz and S. M. Gryaznov, *Nucleic Acids Res.*, 1996, **24**, 2966.
13 J. Wengel, *Acc. Chem. Res.*, 1999, **32**, 301.
14 M. Böhringer, H.-J. Roth, J. Hunziker, M. Göbel, R. Krishnan, A. Giger, B. Schweizer, J. Schreiber, C. Leumann and A. Eschenmoser, *Helv. Chim. Acta*, 1992, **75**, 1416.
15 J. Hunziker, H.-J. Roth, M. Böhringer, A. Giger, U. Diederichsen, M. Göbel, R. Krishnan, B. Jaun, C. Leumann and A. Eschenmoser, *Helv. Chim. Acta*, 1993, **76**, 259.
16 M. Tarköy, M. Bolli, B. Schweizer and C. Leumann, *Helv. Chim. Acta*, 1993, **76**, 481.
17 M. Tarköy and C. Leumann, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 1432.
18 M. Tarköy, M. Bolli and C. Leumann, *Helv. Chim. Acta*, 1994, **77**, 716.
19 M. Bolli and C. Leumann, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 694.
20 M. Bolli, H. U. Trafelet and C. Leumann, *Nucleic Acids Res.*, 1996, **24**, 4660.
21 M. Bolli, J. C. Litten, R. Schütz and C. J. Leumann, *Chem. Biol.*, 1996, **3**, 197.
22 M. Bolli, P. Lubini and C. Leumann, *Helv. Chim. Acta*, 1995, **78**, 2077.
23 J. C. Litten, C. Epple and C. J. Leumann, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 1231.
24 J. C. Litten and C. Leumann, *Helv. Chim. Acta*, 1996, **79**, 1129.
25 R. Steffens and C. Leumann, *Helv. Chim. Acta*, 1997, **80**, 2426.
26 R. Steffens and C. J. Leumann, *J. Am. Chem. Soc.*, 1997, **119**, 11548.
27 R. Steffens and C. J. Leumann, *J. Am. Chem. Soc.*, 1999, **121**, 3249.
28 R. Meier, S. Grüschow and C. Leumann, *Helv. Chim. Acta*, 1999, **82**, 1813.
29 A. Kálmán, T. Koritsánszky, J. Béres and G. Sági, *Nucleosides Nucleotides*, 1990, **9**, 235.
30 K.-H. Altmann, R. Imwinkelried, R. Kesselring and G. Rihs, *Tetrahedron Lett.*, 1994, **35**, 7625.
31 A. Ezzitouni, J. J. Barchi, Jr. and V. E. Marquez, *J. Chem. Soc., Chem. Commun.*, 1995, 1345.
32 A. Ezzitouni and V. E. Marquez, *J. Chem. Soc., Perkin Trans. 1*, 1997, 1073.
33 K. Biggadike, A. D. Borthwick, D. Evans, A. M. Exall, B. E. Kirk, S. M. Roberts, L. Stephenson and P. Youds, *J. Chem. Soc., Perkin Trans. 1*, 1988, 549.
34 K.-H. Altmann, R. Kesselring, E. Francotte and G. Rihs, *Tetrahedron Lett.*, 1994, **35**, 2331.
35 M. A. Siddiqui, H. Ford, Jr., C. George and V. E. Marquez, *Nucleosides Nucleotides*, 1996, **15**, 235.
36 V. E. Marquez, M. A. Siddiqui, A. Ezzitouni, P. Russ, J. Wang, R. W. Wagner and M. D. Matteucci, *J. Med. Chem.*, 1996, **39**, 3739.
37 A. Ezzitouni, P. Russ and V. E. Marquez, *J. Org. Chem.*, 1997, **62**, 4870.
38 V. E. Marquez, P. Russ, R. Alonso, M. A. Siddiqui, S. Hernandez, C. George, M. C. Nicklaus, F. Dai and H. Ford, Jr., *Helv. Chim. Acta*, 1999, **82**, 2119.
39 N. K. Christensen, M. Petersen, P. Nielsen, J. P. Jacobsen, C. E. Olsen and J. Wengel, *J. Am. Chem. Soc.*, 1998, **120**, 5458.
40 P. Nielsen, H. M. Pfundheller and J. Wengel, *Chem. Commun.*, 1997, 825.
41 P. Nielsen, H. M. Pfundheller, C. E. Olsen and J. Wengel, *J. Chem. Soc., Perkin Trans. 1*, 1997, 3423.
42 A. A. Koshkin and J. Wengel, *J. Org. Chem.*, 1998, **63**, 2778.
43 M. Raunkjær, C. E. Olsen and J. Wengel, *J. Chem. Soc., Perkin Trans. 1*, 1999, 2543.
44 H. M. Pfundheller, A. A. Koshkin, C. E. Olsen and J. Wengel, *Nucleosides Nucleotides*, 1999, **18**, 2017.
45 S. K. Singh, P. Nielsen, A. A. Koshkin and J. Wengel, *Chem. Commun.*, 1998, 455.
46 A. A. Koshkin, S. K. Singh, P. Nielsen, V. K. Rajwanshi, R. Kumar, M. Meldgaard, C. E. Olsen and J. Wengel, *Tetrahedron*, 1998, **54**, 3607.
47 S. Obika, D. Nanbu, Y. Hari, K. Morio, Y. In, T. Ishida and T. Imanishi, *Tetrahedron Lett.*, 1997, **38**, 8735.
48 S. Obika, K. Morio, D. Nanbu and T. Imanishi, *Chem. Commun.*, 1997, 1643.
49 A. A. Koshkin, V. K. Rajwanshi and J. Wengel, *Tetrahedron Lett.*, 1998, **39**, 4381.
50 G. H. Jones, M. Taniguchi, D. Tegg and J. G. Moffatt, *J. Org. Chem.*, 1979, **44**, 1309.
51 M. Meldgaard and J. Wengel, unpublished results.
52 R. D. Youssefyeh, J. P. H. Verheyden and J. G. Moffatt, *J. Org. Chem.*, 1979, **44**, 1301.
53 T. Waga, T. Nishizaki, I. Miyakawa, H. Ohrui and H. Meguro, *Biosci. Biotech. Biochem.*, 1993, **57**, 1433.
54 P. Nielsen and J. Wengel, *Chem. Commun.*, 1998, 2645.
55 S. Obika, D. Nanbu, Y. Hari, J. Andoh, K. Morio, T. Doi and T. Imanishi, *Tetrahedron Lett.*, 1998, **39**, 5401.
56 S. K. Singh and J. Wengel, *Chem. Commun.*, 1998, 1247.
57 A. A. Koshkin, P. Nielsen, M. Meldgaard, V. K. Rajwanshi, S. K. Singh and J. Wengel, *J. Am. Chem. Soc.*, 1998, **120**, 13252.
58 H. Ørum, M. H. Jakobsen, T. Koch, J. Vuust and M. B. Borre, *Clin. Chem.*, 1999, **45**, 1898.
59 C. B. Nielsen, S. K. Singh, J. Wengel and J. P. Jacobsen, *J. Biomol. Struct. Dyn.*, 1999, **17**, 175.
60 S. K. Singh, R. Kumar and J. Wengel, *J. Org. Chem.*, 1998, **63**, 6078.
61 S. K. Singh, R. Kumar and J. Wengel, *J. Org. Chem.*, 1998, **63**, 10035.
62 R. Kumar, S. K. Singh, A. A. Koshkin, V. K. Rajwanshi, M. Meldgaard and J. Wengel, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 2219.
63 V. K. Rajwanshi, R. Kumar, M. Kofod-Hansen and J. Wengel, *J. Chem. Soc., Perkin Trans. 1*, 1999, 1407.
64 V. K. Rajwanshi, A. E. Håkansson, B. M. Dahl and J. Wengel, *Chem. Commun.*, 1999, 1395.
65 V. K. Rajwanshi, A. E. Håkansson, R. Kumar and J. Wengel, *Chem. Commun.*, 1999, 2073.
66 T. F. Tam and B. Fraser-Reid, *Can. J. Chem.*, 1979, **57**, 2818.
67 F. Seela, K. Wörner and H. Rosemeyer, *Helv. Chim. Acta*, 1994, **77**, 883.
68 A. E. Håkansson, A. A. Koshkin, M. D. Sørensen and J. Wengel, *J. Org. Chem.*, 2000, **65**, 5167. The C3′-*exo* conformation assigned to α-L-LNA nucleosides reflects a direct comparison with the remaining bicyclic nucleosides. Alternatively, the conformation of α-L-LNA nucleosides can be described as C3′-*endo* taking the L-configuration into account.
69 G. Wang, J.-L. Girardet and E. Gunic, *Tetrahedron*, 1999, **55**, 7707.
70 G. Wang, E. Gunic, J.-L. Girardet and V. Stoisavljevic, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 1147.
71 S. M. Freier and K.-H. Altmann, *Nucleic Acids Res.*, 1997, **25**, 4429.
72 S. Obika, K. Morio, Y. Hari and T. Imanishi, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 515.
73 R. Zou and M. D. Matteucci, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 3049.
74 A. Bouali, D. F. Ewing and G. Mackenzie, *Nucleosides Nucleotides*, 1994, **13**, 491.
75 R. J. Jones, S. Swaminathan, J. F. Milligan, S. Wadwani, B. S. Froehler and M. D. Matteucci, *J. Am. Chem. Soc.*, 1993, **115**, 9816.
76 R. Zou and M. D. Matteucci, *Tetrahedron Lett.*, 1996, **37**, 941.
77 J. Wang and M. D. Matteucci, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 229.