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BRIDGED NUCLEIC ACIDS: DEVELOPMENT, SYNTHESIS AND PROPERTIES

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Abstract – Restricting the sugar moiety of a nucleic acid to a single conformation can be accomplished by forming a bridge in the sugar. A large number of bridged nucleic acids with variable bridged structures and conformations have been developed. The synthesis and properties of these artificial nucleic acid analogues are discussed. Such conformational restriction of the sugar moiety significantly improved the properties of the nucleic acid.

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1. INTRODUCTION

Recently, much attention has been focused on the development of nucleic acid based technologies and therapies. Research interest in nucleic acid based technologies such as antisense,[1] antigenes,[2] ribozymes[3] and RNA interference[4] increases daily. Chemically modified oligonucleotides are highly applicable to these technologies. Oligonucleotides are chemically modified in order to improve target binding affinity, nuclease resistance, target specificity, cellular uptake and biodistribution. Several nucleic acids, modified either in the sugar, the base or the backbone have been developed during the past few decades:[5-7] Some of these modified nucleic acids exhibited increased hybridizing ability while other characteristics remained unchanged or diminished. Indeed, most early attempts at modifying nucleic acids resulted in the improvement of only a single property, rather then the simultaneous improvement of all these properties mentioned above. For example, phosphorthioate modification improved the nuclease resistance of oligonucleotides, at the expense of target binding affinity.[8] Despite a slight decrease in hybridizing ability, the first antisense drug containing a phosphorthioate oligonucleotide was approved by the FDA, and appeared on the market in 1998 for the treatment of cytomegalovirus-induced retinitis.[9] Since then, tremendous effort has been made to develop novel nucleic acid derivatives with improved properties.

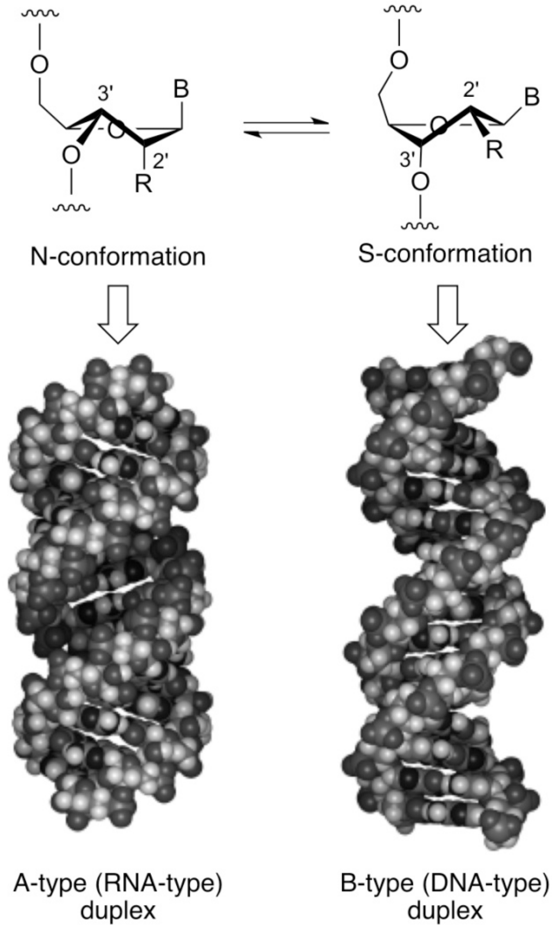


Figure 1 Conformation of nucleoside sugar moiety and structures of A- and B-type duplexes.

Our strategy for developing novel modified nucleic acid analogues was based on restricting the conformation of the sugar in the nucleotides. The sugar moiety of nucleic acids possesses high conformational flexibility and is in equilibrium between several conformations, such as N-type (North type) and S-type (South type) (Figure 1). When nucleic acids hybridize with their complementary strand, this conformational freedom is drastically restricted. The entropy loss associated with conformational restriction during the hybridization process is an important consideration when developing a nucleic acid analogue with high binding affinity to its complementary strand. It is well known that the sugar conformations in A-form RNA predominantly exist in the N-type conformation, and that B-form DNA possesses an S-type sugar pucker. Therefore, modified oligonucleotides in which the sugar conformation is preorganized into the N-type conformation are expected to form stable duplexes with complementary single-stranded RNA (ssRNA), and that preorganized S-type oligonucleotides will form stable duplexes with single-stranded DNA (ssDNA). Preorganization of nucleic acids in a suitable form, for example by adding an extra bridge in the sugar moiety, would be a promising strategy for preventing loss of entropy.

Based on these concepts, we developed a novel class of nucleic acid analogues with fixed conformations by bridging the sugar moiety of the nucleotide; these derivative are called bridged nucleic acids (BNAs).[10] Of the various BNA analogues synthesized to date, 2'-*O*,4'-*C*-methylene bridged nucleic acid (2',4'-BNA),[11,12] with N-type sugar conformation has found wide application in nucleic acid base technologies. This nucleic acid analogue is locked in the N-type sugar conformation. Nucleic acid derivatives with the N-type sugar pucker have been found to be very useful in antisense and antigene technologies.[2,7] The enhanced affinity of the N-type sugar pucker for complementary ssRNA or dsDNA is attributed to improved stacking to the nearest neighbours and the quenching of local backbone motion, thereby reducing the entropic penalty in the free energy of stabilization of duplex and triplex structures.[13] Hence, the development of N-type sugar puckered nucleic acids is essential for nucleic acid base technologies. 2',4'-BNA, the most promising N-type nucleic acid analogue, was first synthesized in our laboratory in 1997.[11] Soon after this publication, Wengel et al. independently synthesized the same nucleic acid analogue in 1998 and named it locked nucleic acid, or LNA.[14,15] This nucleic acid analogue 2',4'-BNA or LNA showed unprecedented target binding affinity and has emerged as the most promising nucleic acid analogue used in molecular biology.[16-18] Because of its interesting properties, 2',4'-BNA is commercially available and 2',4'-BNA-modified antisense oligonucleotides have entered human clinical trials.[19] The outstanding properties of 2',4'-BNA have encouraged continued research in this field.

Apart from the development of 2',4'-BNA/LNA, a number of other BNAs with N-type sugar conformation have been developed by us and others. Wengel *et al*. reported 2',4'-BNA/LNA analogues such as 2'-amino-LNA[20,21] and several derivatives[22] and 2'-thio-LNA[20] in which the 2'-oxygen atom of 2',4'-BNA/LNA was replaced by 2'-nitrogen and 2'-sulfur atom, respectively. We have synthesized the amino congeners of 2',4'-BNA analogues, namely, 3'-amino-2',4'-BNA[23,24] and 5'-amino-2',4'-BNA,[25] by dual modifications, i.e, modifications of both the sugar moiety and the backbone. 2',4'-BNA with unnatural bases was also developed by us[26-31] and other groups.[32-34] Carbocyclic five-membered bridged BNA analogues A, B ware recently reported.[35,36] BNA analogue C with a six-membered bridge structure with a 2'-*C*,4'-*C* architecture was first developed by Wang *et al*.[37,38] Ethylene bridged nucleic acid (ENA) with a six-membered bridge structure bearing a 2'-*O* architecture was introduced by Koizumi *et al*.[39,40] Chattopadhyaya *et al*. reported a 2'-*N*-containing ENA, *i.e*., Aza-ENA[41] and carbocyclic ENA anlogues.[35]

Several carbocyclic six-membered 2’,4’-linked BNAs D, E were developed by Nielsen *et al.*[42] We have developed several derivatives of a highly potent BNA analogue, 2’-aminomethylene BNA (2’,4’-BNA^{NC}), bearing a six-membered bridge structure with a functionalizable nitrogen atom on the bridge.[43-46]

N-type

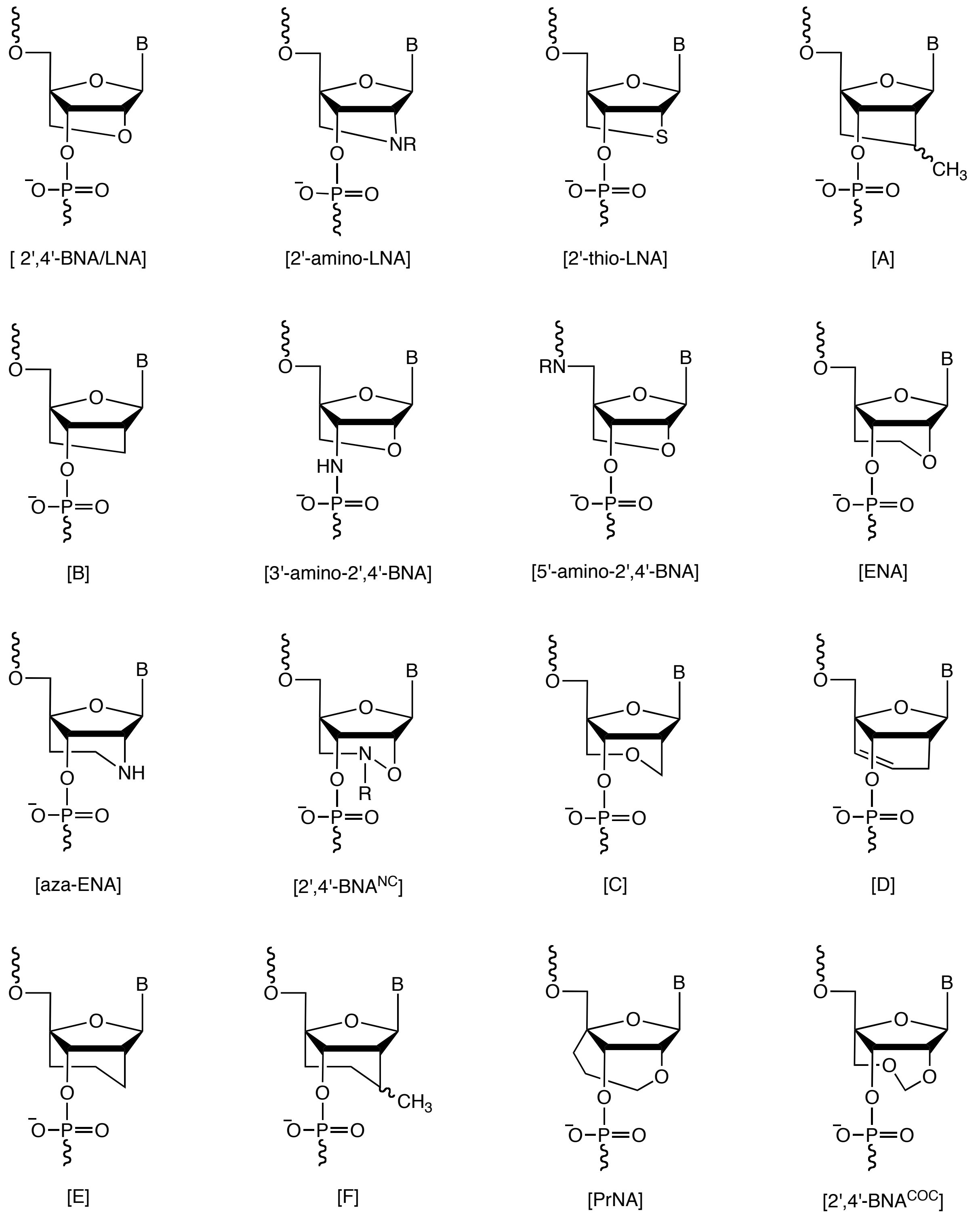


Figure 2 Bridged Nucleic Acids with N-type sugar conformation.

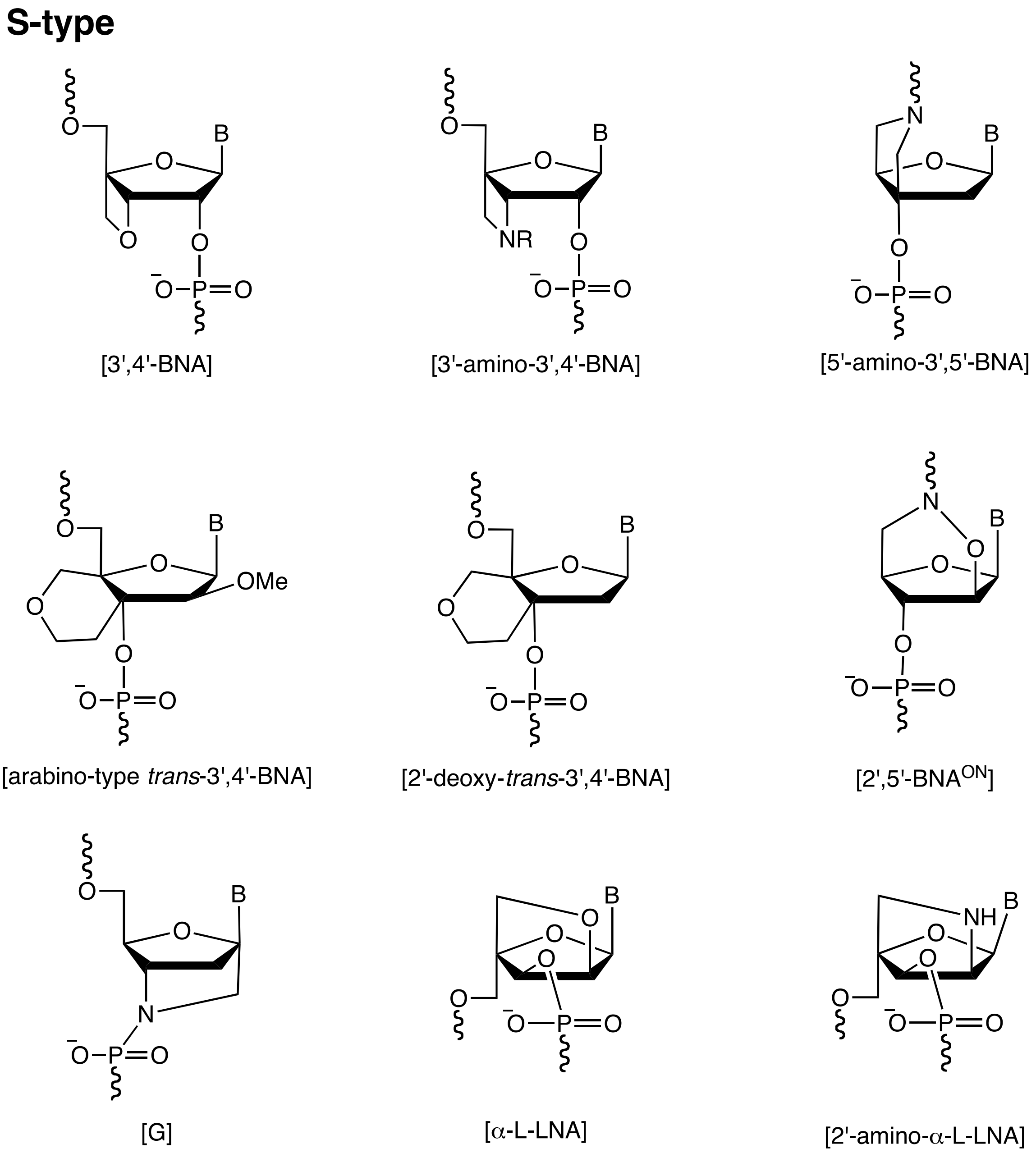


Figure 3. Bridged Nucleic Acids with S-type sugar conformation.

BNA analogues with a seven-membered bridged moiety, such as propylene bridged nucleic acid (PrNA),[40] was developed by Koizumi *et al.*, and 2’-*O*,4’-*C*-methylene oxymethylene BNA (2’,4’-BNA^{COC})[47,48] was developed by us. A light-responsive 2’,4’-BNA^{COC} derivative was synthesized by us very recently.[49]

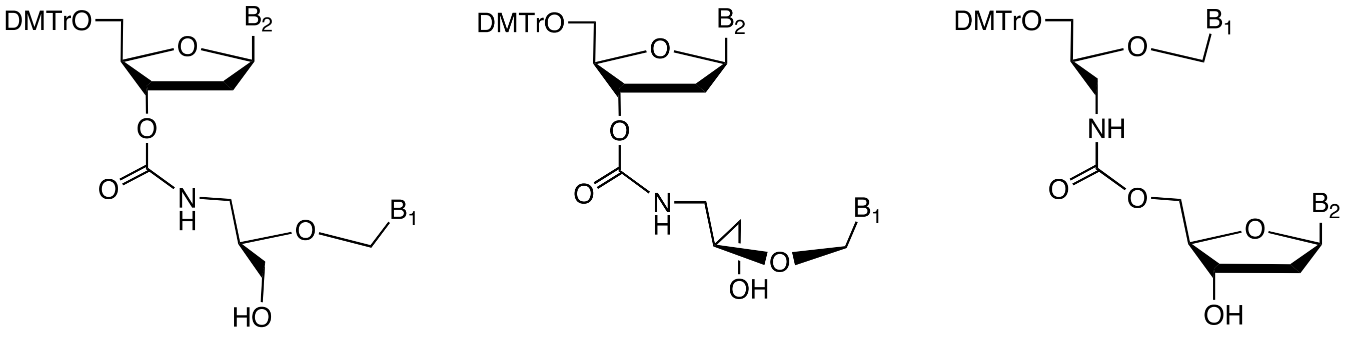
A number of BNA analogues with a fixed S-type sugar pucker were also synthesized by us. Oligonucleotides consisting of an S-type sugar puckered nucleoside are expected to form stable B-form duplexes with complementary ssDNA. Therefore, this type of oligonucleotides might find applications in DNA microarrays[50] or decoy strategies.[51] The various S-type BNAs developed in our laboratory include 3’,4’-BNA,[52-55] 3’-amino-3’,4’-BNA,[56] 5’-amino-3’,5’-BNA,[57,58] and various *trans*-3’,4’-BNA analogues,[59-65] 2’,5’-BNA^{ON}.[66] Wengel synthesized a 3’,1’-aminomethylene linked DNA analogue[67] G, and an α-configured arabino-type BNA, α-L-LNA[68] and 2’-amino-α-L-LNA[69] which possessed the S-type sugar conformation.

This review will give an account of the development and synthesis of these various BNA analogues and their properties.

2. BEFORE BRIDGED NUCLEIC ACIDS –OUR ACYCLIC NUCLEOSIDES–

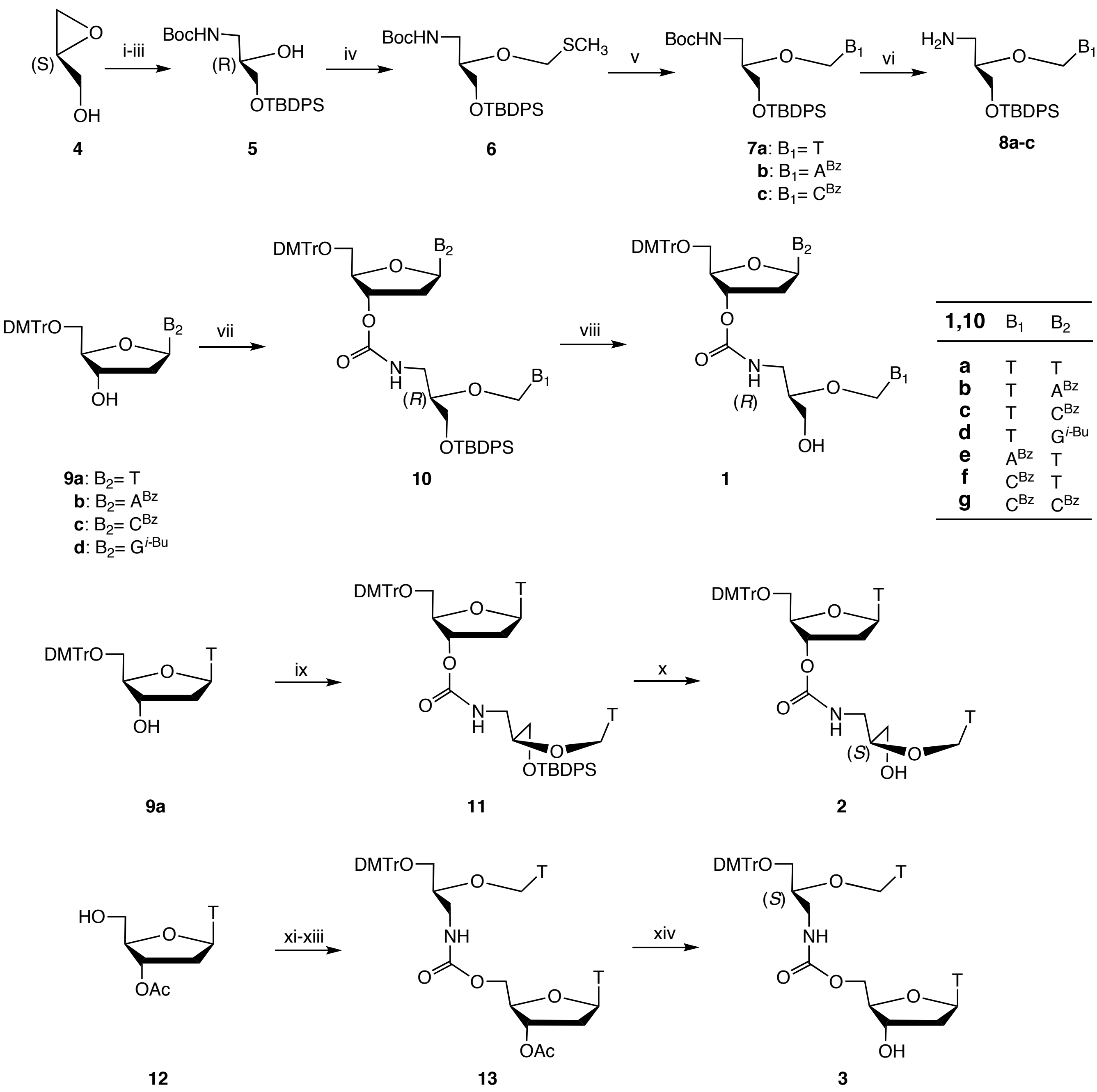
In 1995, we reported the synthesis of novel nucleoside dimer analogues **1**-**3** which contain an acyclic nucleoside and a carbamate linkage (Fig. 4).[70] These formed the foundation of our development of novel artificial nucleic acids. The carbamate-linked oligonucleotides had been synthesized before; however, their ability to hybridize with DNA or RNA complements varied depending on the sequence of the oligonucleotides.[71] The carbamate linkage is a little shorter than the phosphodiester linkage in natural nucleic acids. Therefore, we employed an acyclic nucleoside in combination with a rigid carbamate linkage in order to relax the strain caused in the hybridization step.

The synthesis of nucleoside dimers **1**-**3** was accomplished as shown in Scheme 1. (*S*)-Glycidol (compound **4**) was treated with aqueous ammonia, and the amino and primary hydroxyl groups of the obtained compound were protected with Boc and TBDPS groups, respectively, to afford **5**. Alcohol **5** was converted into methylthiomethyl ether **6**, which was then treated with nucleobases in the presence of NBS to give the acyclic nucleoside analogues **7a**-**7c** (B_1 = thymin-1-yl (T), N^6-benzoyladenin-9-yl (A^{Bz}), or N^4-benzoylcytosin-9-yl (C^{Bz})). After deprotection of the Boc group, **8** was coupled with natural 2’-deoxyribonucleoside **9** activated by *N*,*N*’-carbonyldiimidazole (CDI) to afford **1** *via* **10**. The other isomer, **2**, was readily synthesized in a similar manner using (*S*)-**8a** derived from (*R*)-glycidol. In addition, (*S*)-**8a** was also coupled with **12** to give the corresponding dimer **13**, which was transformed into **3**.



1 **2** **3**

Figure 4. Structures of our first nucleoside analogues.



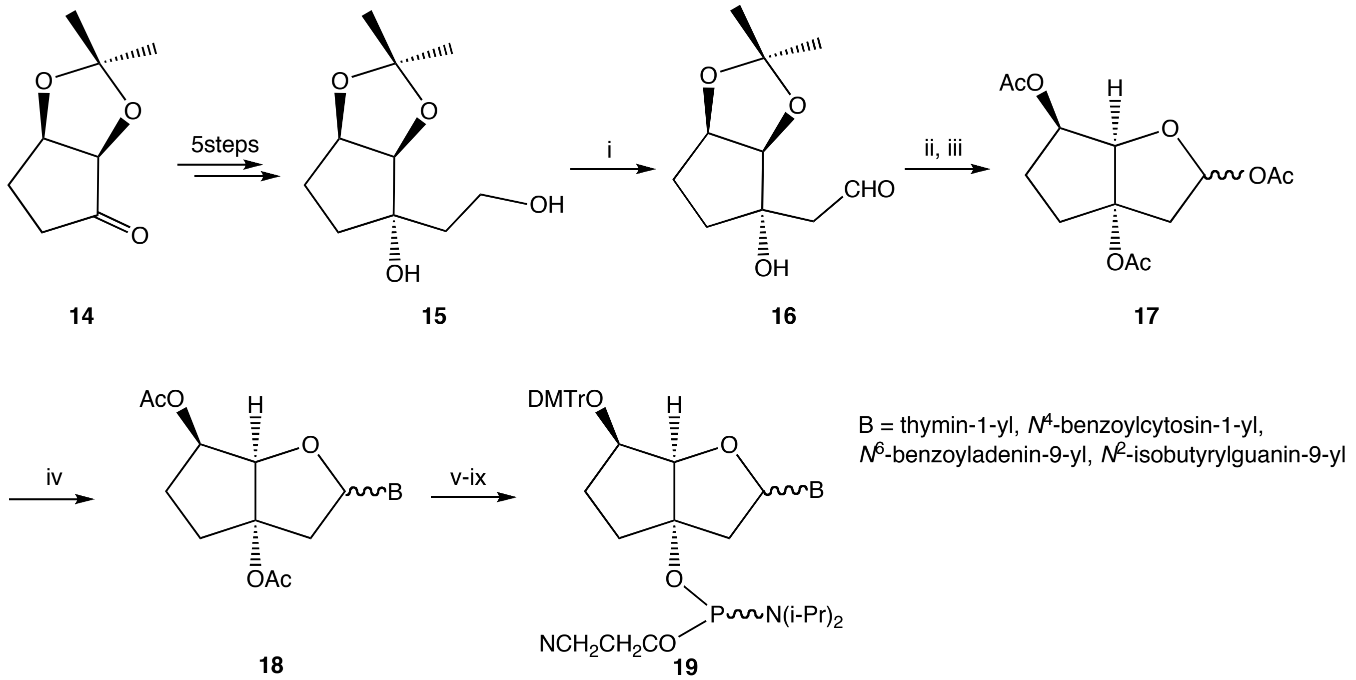
1,10	B_1	B_2
a	T	T
b	T	A^{Bz}
c	T	C^{Bz}
d	T	$G^{i\text{-}Bu}$
e	A^{Bz}	T
f	C^{Bz}	T
g	C^{Bz}	C^{Bz}

Scheme 1. *Reagents and conditions*: i, NH_3 aq.; ii, di-*tert*-butyl dicarbonate, Et_3N, DMF; iii, *tert*-butyldiphenylsilyl chloride, Et_3N, DMAP, CH_2Cl_2; iv, DMSO, Ac_2O, AcOH; v, silylated base, molecular sieves(4A), CH_2Cl_2 then NBS; vi, TFA, CH_2Cl_2; vii, 1,1'-carbonyldiimidazole, pyridine, then (*R*)-**8**, DMAP, pyridine (for **10a-f**) or bis(4-nitrophenyl)carbonate, Et_3N, DMF, then (*R*)-**8c**, Et_3N, DMF (for **10g**); viii, tetra-*n*-butylammonium fluoride, THF; ix, 1,1'-carbonyldiimidazole, pyridine, then (*S*)-**8a**, DMAP, pyridine; x, tetra-*n*-butylammonium fluoride, THF; xi, 1,1'-carbonyldiimidazole, pyridine, then (*S*)-**8a**, DMAP, pyridine; xii, tetra-*n*-butylammonium fluoride, THF; xiii, 4,4'-dimethoxytrityl chloride, pyridine; xiv, NaOH aq., EtOH/pyridine. Boc= *tert*-butoxycarbonyl, TBDPS= *tert*-butyldiphenylsilyl, T= thymin-1-yl, A^{Bz}= N^6-benzoyladenin-9-yl, C^{Bz}= N^4-benzoylcytosin-1-yl, $G^{i\text{-}Bu}$= N^2-isobutyrylguanin-9-yl, DMTr= 4,4'-dimethoxytrityl.

Nucleoside dimers **1** and **3** were converted into their phosphoramidite derivatives and incorporated into oligonucleotides using an automated DNA synthesizer. Unfortunately, UV melting experiments showed that the oligonucleotides containing these nucleoside dimers did not exhibit increased hybridization with their complementary strands, although these oligonucleotide derivatives were highly resistant to enzymatic degradation.[72]

3. SEVERAL BRIDGED NUCLEIC ACIDS –BEFORE 2’,4’-BNA/LNA–

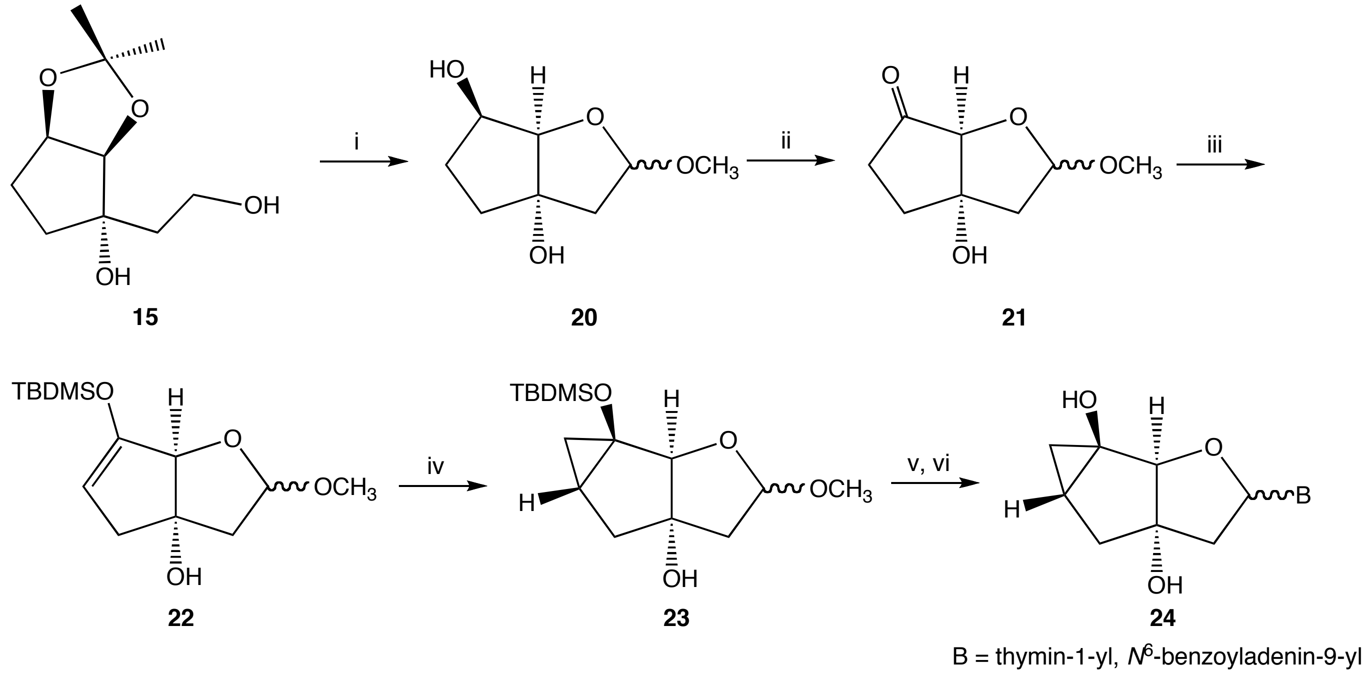
The above results showed that conformationally flexible acyclic nucleosides decreased hybridization when incorporated into oligonucleotides. This finding inspired us to produce novel nucleic acid analogues bearing a conformationally restricted or locked sugar moiety. The advantages of a conformationally restricted sugar moiety were described above. To our knowledge, the first example of this new class of nucleic acids, bicyclonucleosides with an additional 3’,5’-ethylene bridge, was reported by Leumann *et al.* in 1993.[73] This is a pioneering work had considerable impact on subsequent related research. The synthesis of the bicyclonucleosides is outlined in Scheme 2. The key intermediate **15** was prepared from starting material **14** in a 5-step sequence.[73] The primary alcohol of **15** was oxidized to give aldehyde **16**, which was converted into bicyclic compound **17**. Coupling reaction of **17** with nucleobases was carried out under Vorbrüggen conditions[74,75] to give anomeric mixtures of the bicyclo-DNA monomer **18**. After deprotection and separation of each anomer, the bicyclo-DNA monomer was converted into phosphoramidite derivative **19**, which was successfully incorporated into oligonucleotides. Introduction of bicyclo-DNA into oligonucleotides stabilized the duplexes and triplexes formed with RNA and DNA.[76] Later, the same laboratory reported several derivatives of bicyclo-DNA, including tricyclo-DNA, a tricyclic congener of bicyclo-DNA, which has a cyclopropane ring to further restrict the flexibility of the nucleic acid backbone.[77] As shown in Scheme 3, tricyclo-DNA was prepared from diol **15**, which was



B = thymin-1-yl, N^4-benzoylcytosin-1-yl, N^6-benzoyladenin-9-yl, N^2-isobutyrylguanin-9-yl

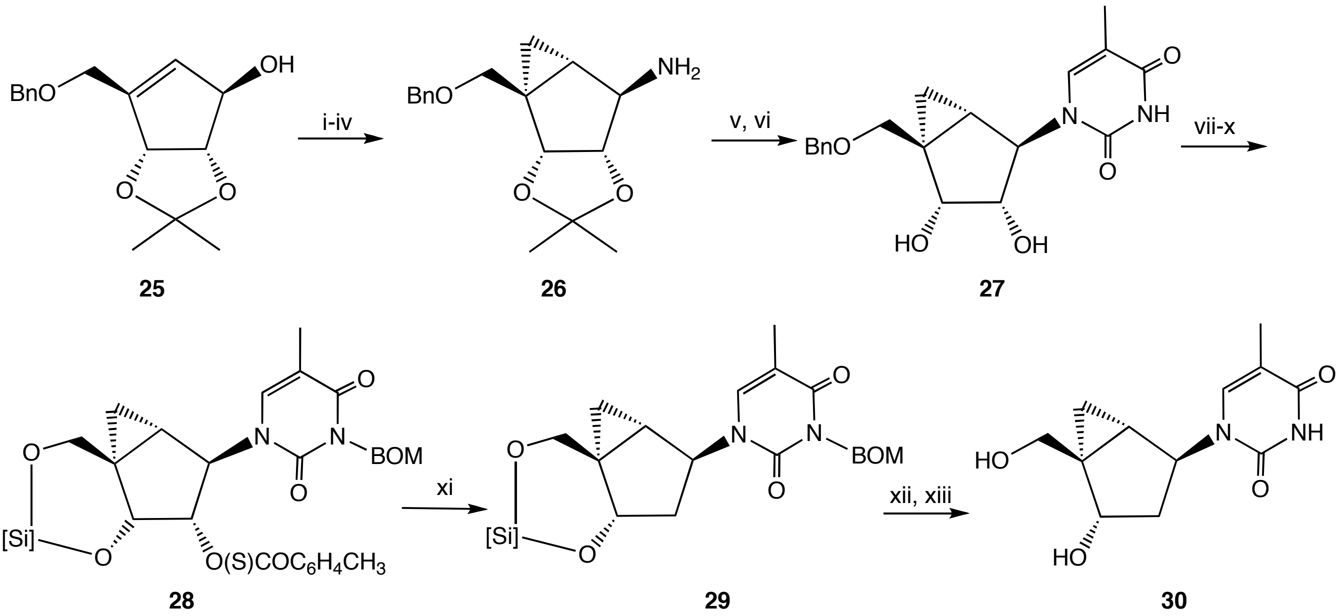
Scheme 2. *Reagents and conditions*: i, *Dess-Martin* reagent, CH_2Cl_2; ii, *IR-120* (H^+), H_2O; iii, DMAP, pyridine/Ac_2O; iv, silylated base, $SnCl_4$ or TMSOTf, MeCN; v, NaOH, THF/MeOH/H_2O; vi, TMSOTf, pyridine, vii, tetra-*n*-butylammonium fluoride; viii, 4,4'-dimethoxytrityl triflate, 2, 6-lutidine or pyridine; ix, $(NCCH_2CH_2O)[(i\text{-}Pr)_2N]PCl$, $(i\text{-}Pr)_2EtN$, THF or MeCN. DMTr= 4,4'-dimethoxytrityl.

used in the synthesis of bicyclo-DNA. Compound **15** was converted to olefin **22** *via* a series of reactions including Dess-Martin periodinate oxidation, deketalization/acetylation, deprotonation and silylation. The cyclopropane ring was introduced into olefin derivative **22** by the Simmons-Smith reaction to give **23**, and a subsequent coupling reaction with nucleobases and desilylation gave the tricyclo-DNA monomer **24**.[77]

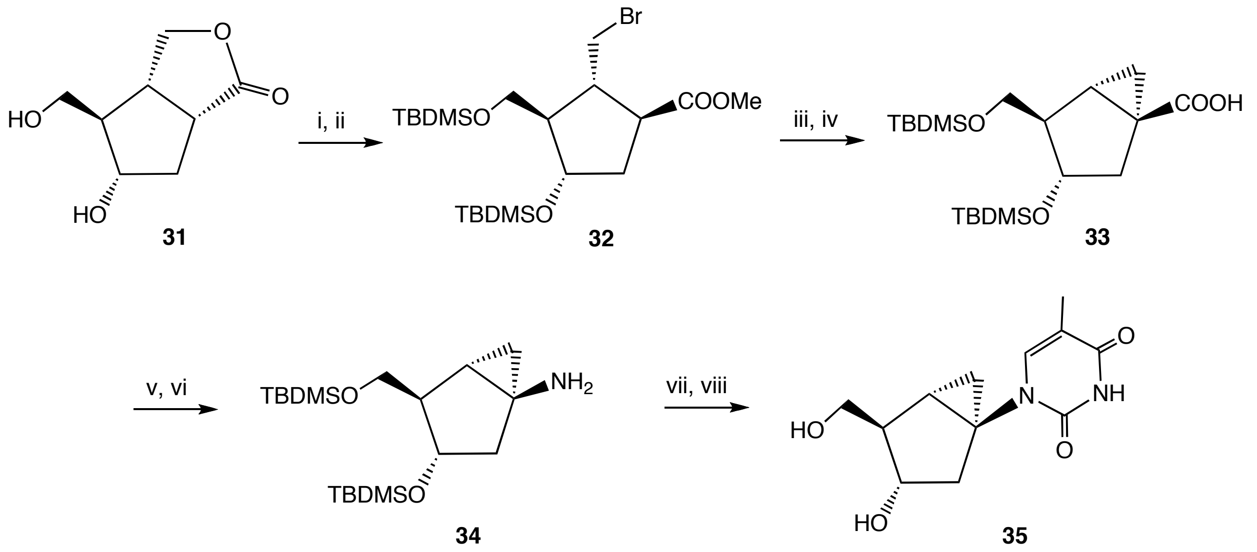


Scheme 3. *Reagents and conditions*: i, 1. *Dess-Martin* reagent, CH_2Cl_2, 2. *Amberlyst 15*, MeOH; ii, *Dess-Martin* reagent, CH_2Cl_2; iii, 1. LDA, THF, 2. TBDMSCl, THF; iv, CH_2I_2/Ag-Zn, Et_2O; v, silylated base, TMSOTf, MeCN or 1,2-dichloroethane; vi, tetra-*n*-butylammonium fluoride, THF.

Another example is the methano carbocyclic nucleosides reported by Altman *et al.* in 1994.[78] They prepared 4',6'- and 1',6'-methano carbocyclic thymidines **30** and **35** as shown in Schemes 4 and 5, respectively. Cyclopentenol **25** was stereoselectively converted to **26** by Simmons-Smith cyclopropanation, tosylation, azidation and reduction. Thymine nucleobase was constructed by reaction with the acylisocyanate derivative and subsequent acid-mediated cyclization. Debenzylation of **27** by Pd-catalyzed hydrogenolysis, thioacylation of 2'-OH, silyl protection of the 3'- and 5'-OH groups, and benzyloxymethyl (BOM) protection of the thymine nucleobase produced **28** which was subjected to radical reduction to give **29**. Deprotection of the silyl and BOM groups afforded the desired 4',6'-methano carbocyclic thymidine **30**.

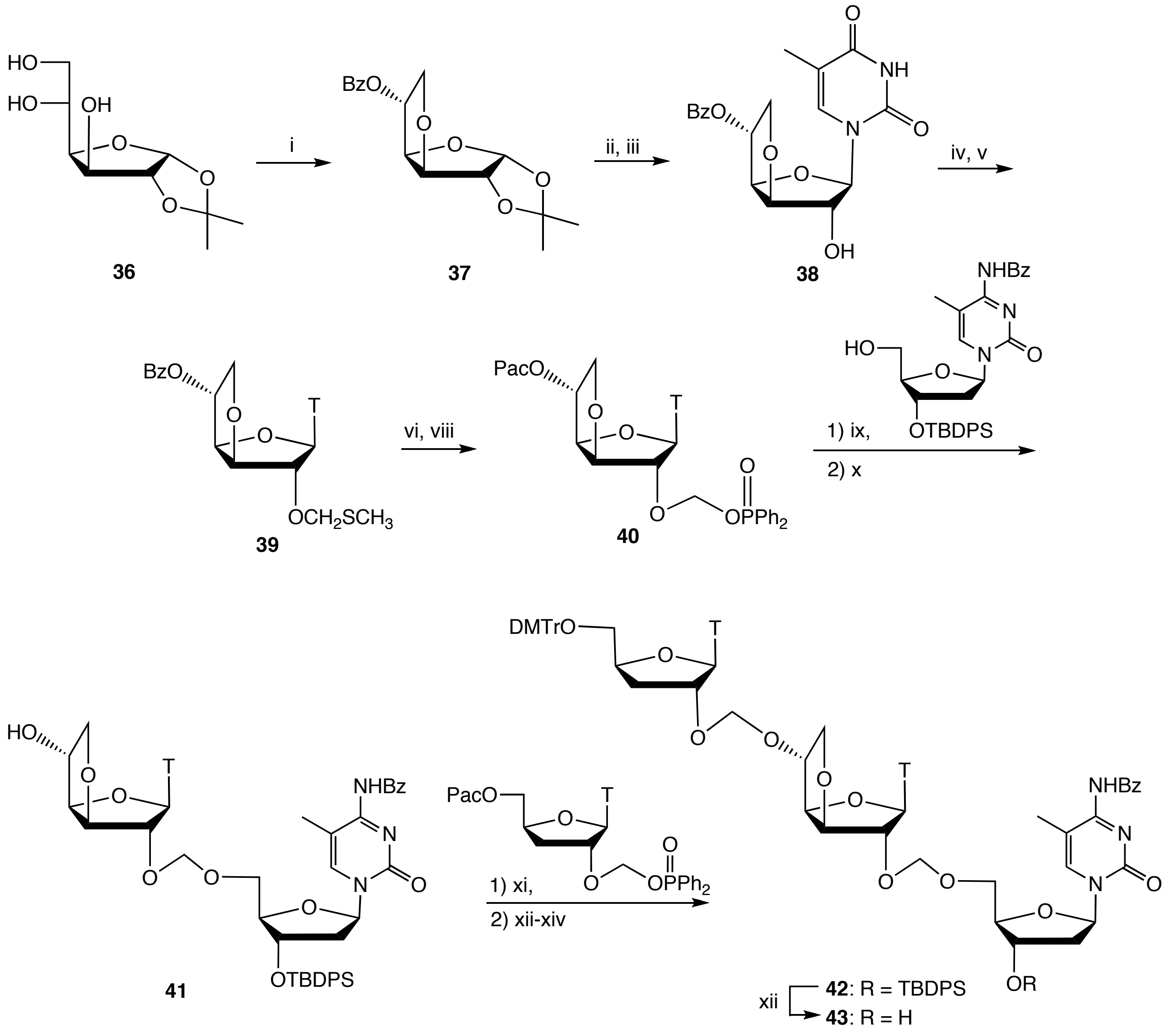


Scheme 4. *Reagents and conditions*: i, Zn/Cu, CH_2Cl_2, Et_2O; ii, TsCl, DMAP, Et_3N, CH_2Cl_2; iii, NaN_3, DMF; iv, H_2, Lindlar's catalyst; v, $CH_3OCH{=}C(CH_3)CONCO$, CH_2Cl_2; vi, HCl, $EtOH/H_2O$; vii, H_2, Pd-C, AcOEt/MeOH; viii,1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, imidazole, DMF; ix, benzyl chloromethyl ether, DBU, MeCN; x, $CH_3C_6H_4OC(S)Cl$, DMAP, Et_3N, CH_2Cl_2; xi, Bu_3SnH, AIBN, 1,2-dimethoxyethane; xii, tetra-*n*-butylammonium fluoride, THF; xiii, H_2, Pd-C, then NaOMe. [Si]= 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl, BOM= benzyloxymethyl.



Scheme 5. *Reagents and conditions*: i, TMSBr, MeOH, $ZnBr_2$; ii, *N*-TBDMS *N*-methyl acetamide, DMF; iii, KO*t*-Bu, *t*-BuOH; iv, KOH, EtOH; v, diphenylphosphoryl azide, Et_3N, toluene; vi, H_2, Pd-C, toluene; vii, $CH_3OCH{=}C(CH_3)C(O)NCO$, CH_2Cl_2; viii, HCl, $EtOH/H_2O$.

On the other hand, the synthesis of 1',6'-methano carbocyclic thymidine **35** started with bicyclic lactone **31**, which was converted to **32** by opening of the lacone and silylation.[79] Cyclopropane ring formation under basic conditions and saponification gave bicyclic acid **33**. Formation of the carbocyclic acid azide, *in situ* Curtius rearrangement,[80] and quenching by benzyl alcohol and subsequent reduction afforded **34**. Introduction of a thymine nucleobase followed by desilylation gave 4',6'-methano carbocyclic thymidine **35**.

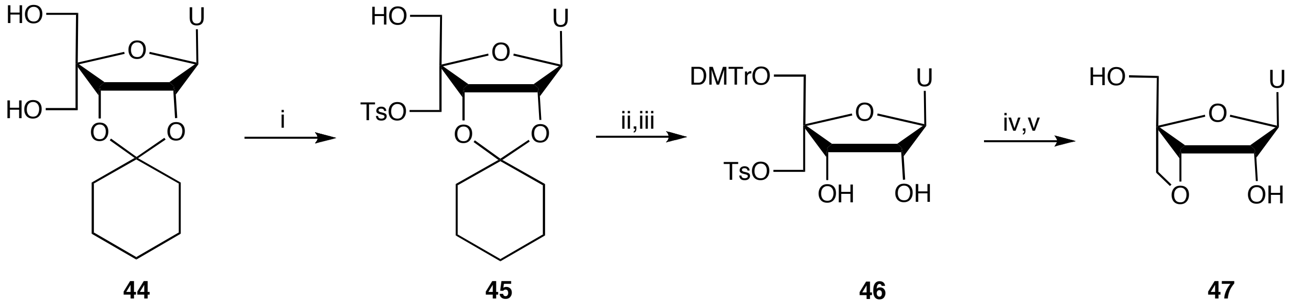


Scheme 6. *Reagents and conditions*: i, $PhC(OMe)_3$, TsOH, CH_2Cl_2; ii, AcOH, Ac_2O, H_2SO_4; iii, silylated thymine, TMSOTf, MeCN; iv, NaOMe, THF; v, Me_2S, Bz_2O_2, MeCN; vi, NaOMe, MeOH; vii, phenoxyacetyl chloride, pyridine, CH_2Cl_2; viii, *N*- iodosuccinimide, Ph_2PO_2H, 1,2-dichloroethane; ix, TMSOTf, MeCN/dichloroethane; x, NH_3, MeOH; xi, TMSOTf, MeCN/1,2-dichloroethane; xii, NH_3, MeOH; xiii, 4,4'-dimethoxytrityl chloride, pyridine, CH_2Cl_2; xiv, tetra-*n*-butylammonium fluoride, THF. T= thymin-1-yl, Pac= phenoxyacetyl, TBDPS= *tert*-butyldiphenylsilyl, DMTr= 4,4'-dimethoxytrityl.

Matteucci *et al.* developed a nucleoside trimer containing a 3',6'-anhydroglucose derivative and 2',5' formacetal internucleoside linkages.[81] As shown in Scheme 6, starting material **36** was treated with $PhC(OMe)_3$/TsOH to give the 3,6-anhydroglucose derivative **37** *via* a glucose ortho ester intermediate. Acetolysis and subsequent introduction of a thymine nucleobase gave the bicyclic nucleoside **38**, which was then converted to the methylthiomethyl ether **39**. After removal of the 5'-*O*-benzoyl group and replacement with the phenoxyacetyl (Pac) group, the 2'-*O*-methylthiomethyl derivative **39** was converted to the methyl diphenylphosphinate **40**. Condensation with the cytidine derivative and deprotection at the

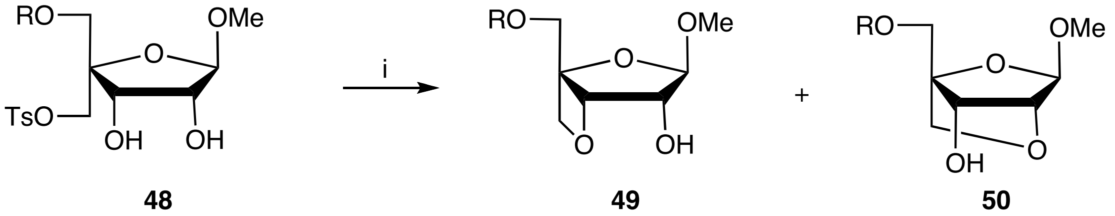
5’-position gave dimer **41**, which was then coupled with the diphenylphosphinate derivative of thymidine to give **43** *via* **42**.

In 1997, we reported the synthesis of 3’-*O*,4’-*C*-methyleneribonucleosides (3’,4’-BNAs), which have a 2,6-dioxabicyclo[3.2.0]heptane ring system.[52] The first synthesis of 3’,4’-BNA monomer was accomplished from a known 4’-(hydroxymethyl)uridine derivative **44** (Scheme 7).



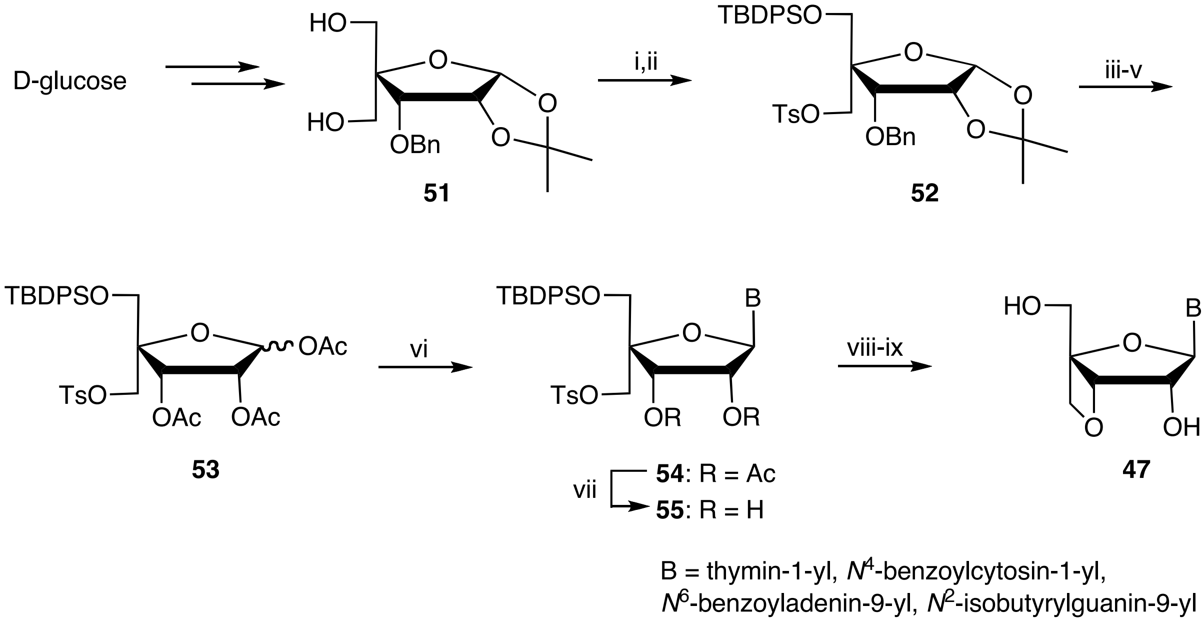
Scheme 7. *Reagents and conditions*: i, TsCl; ii, CF_3CO_2H, H_2O; iii, 4,4'-dimethoxytrityl chloride, DMAP, pyridine; iv, sodium hexamethyldisilazide, THF; v, CF_3COOH, H_2O/CH_2Cl_2. DMTr= 4,4'-dimethoxytrityl. U= uracil-1-yl.

Selective tosylation of the diastereotopic hydroxymethyl groups in **44** gave **45**. Deprotection of the cyclohexylidene group of **45** and subsequent protection of the primary hydroxyl group afforded **46**. Oxetane ring formation from **46** was achieved under basic conditions to give the desired nucleoside monomer **47**.



Scheme 8. *Reagents and Conditions*: i, sodium hexamethyldisilazide, THF.

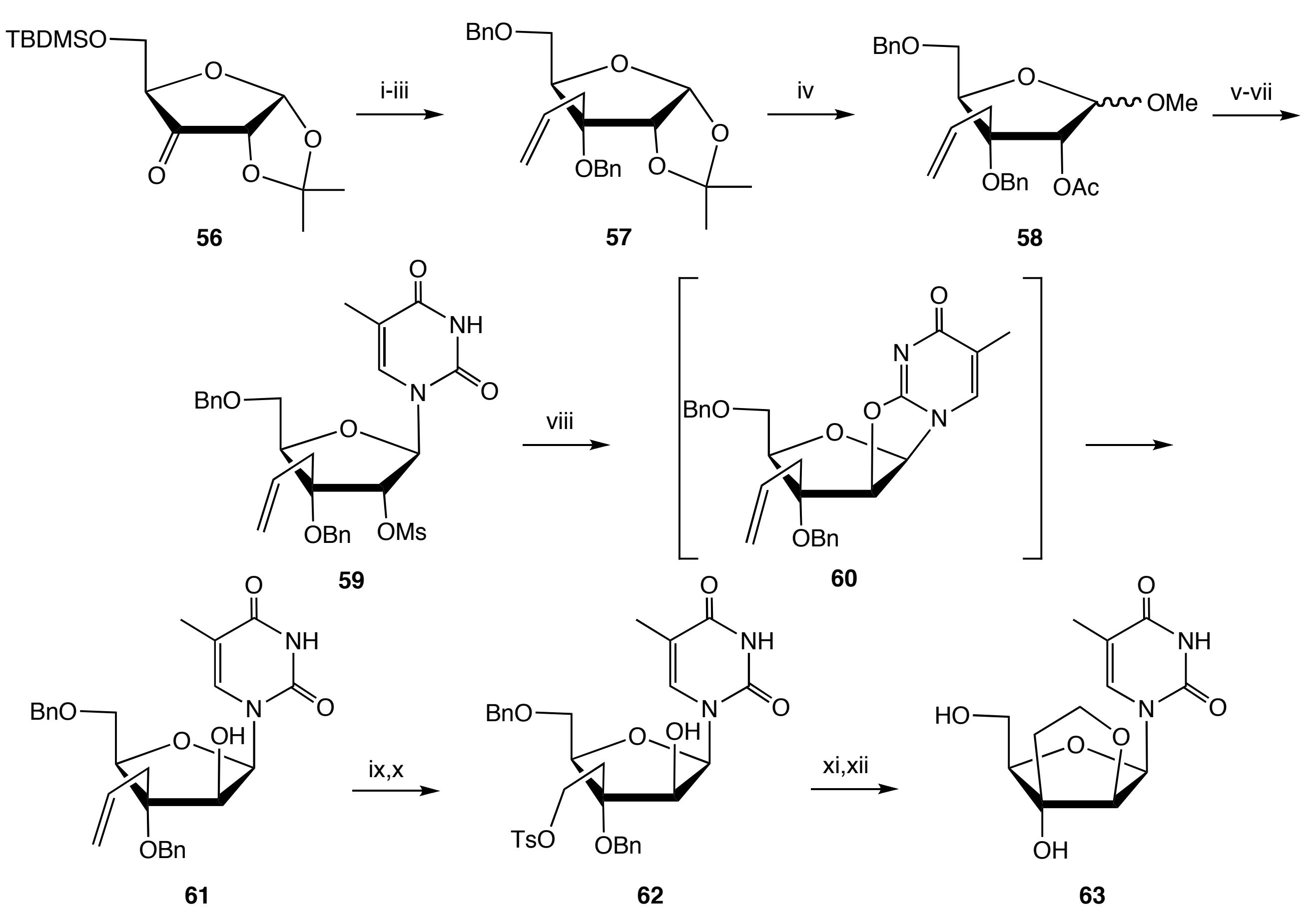
We expected the formation of the 2’,4’-BNA monomer by a ring closure reaction between the 2’-OH group and the 4’-tosyloxymethyl group, since we had evidence that 1-methoxy congener **48** yielded 2’,4’-cyclized product **50** along with 3’,4’-BNA precursor **49** under the same reaction conditions (Scheme 8). However, we observed **47** as the sole isomer, probably due to a difference in the sugar conformation between **46** and **48**. The 2’-OH group of **46** might be distant from the 4’-tosyloxymethyl group. We observed that the 3’,4’-BNA monomers bearing pyrimidine nucleobases were effectively synthesized by this route. Next, we attempted to develop an alternate route to access the purine congener of 3’,4’-BNA by introducing nucleobases into a 1-*O*-acetylribofuranose derivative and then forming an oxetane ring under basic conditions (Scheme 9).[54,55] The known ribofuranose derivative **51** was silylated stereoselectively, and the obtained alcohol was tosylated to give **52**, which was then converted into diacetate **53**. After debenzylation and acetylation at the 3-hydroxy group, all four nucleobases (thymine, *N*^{4}-benzoylcytidine, *N*^{6}-benzoyladenine, and *N*^{2}-isobutyrylguanine) were successfully introduced. Deacetylation, oxetane ring formation, and deprotection at the 5'-position proceeded smoothly to afford the desired 3',4'-BNA monomer **55** having all four nucleobases. This nucleic acid analogue showed similar or slightly increased RNA affinity and decreased DNA affinity.[53,82] The adenine analogue of 3',4'-BNA was also used as a potent RNase L agonist.[83]



B = thymin-1-yl, *N*^{4}-benzoylcytosin-1-yl, *N*^{6}-benzoyladenin-9-yl, *N*^{2}-isobutyrylguanin-9-yl

Scheme 9. *Reagents and conditions*: i, *tert*-butyldiphenylsilyl chloride, Et_3N, CH_2Cl_2; ii, TsCl, Et_3N, DMAP, CH_2Cl_2; iii, AcOH, Ac_2O, H_2SO_4; iv, H_2, Pd-C, AcOEt/$CHCl_3$; v, Ac_2O, pyridine; vi, silylated base, TMSOTf, 1,2-dichloroethane; vii, K_2CO_3, MeOH; viii, sodium hexamethyldisilazide, THF; ix, tetra-*n*-butylammonium fluoride, THF. TBDPS= *tert*-butyldiphenylsilyl.

Wengel *et al.* reported the synthesis of a nucleoside analogue bearing an extra bridge between the 2'- and 3'-positions.[84] As shown in Scheme 10, the known ulose **56** was used as a starting material. Stereoselective Grignard addition of an allyl group, removal of the TBDMS group, and subsequent benzylation gave **57**. Thymine nucleobase was introduced after deprotection and acetylation (compound **58**), and the configuration at the 2'-position of **59** was inverted *via* a 2-*O*,2'-*O*-anhydro intermediate **60**. Oxidative cleavage of the allyl group and concomitant reduction gave the hydroxyethyl derivative **62**. After tosylation of the primary hydroxyl group, cyclization was carried out under basic conditions to give a bicyclic nucleoside which was debenzylated to provide 2',3'-BNA analogue **63**.

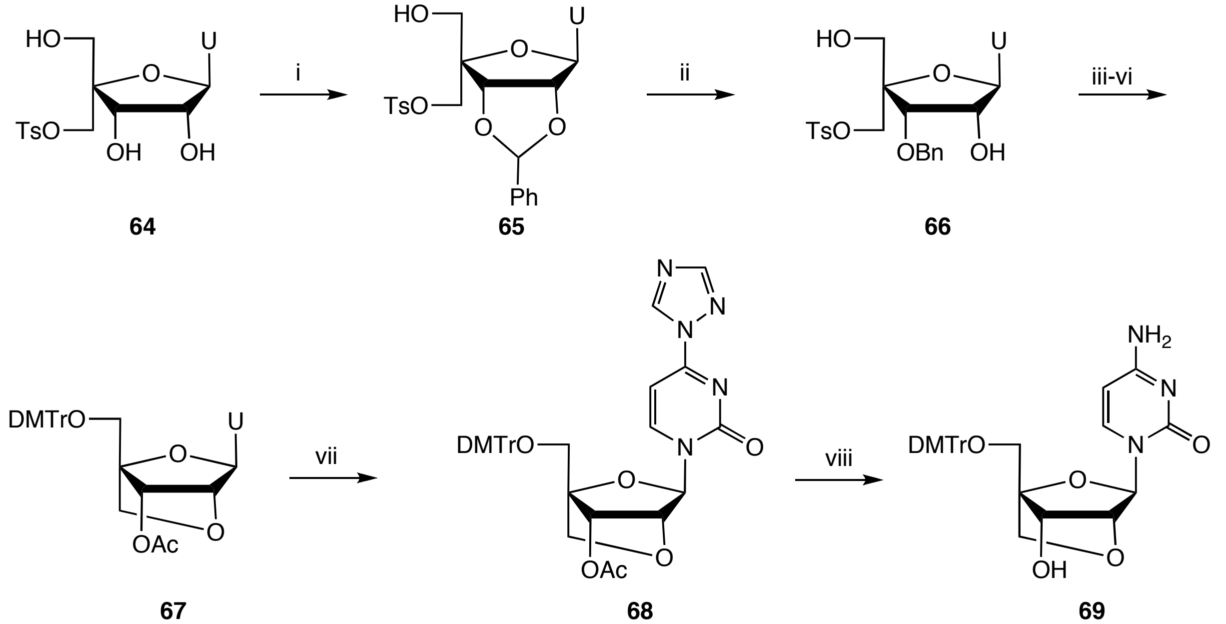


Scheme 10. *Reagents and conditions*: i, allylMgBr, Et_2O, THF; ii,tetra-*n*-butylammonium fluoride, THF; iii, BnBr, NaH, DMF; iv, AcOH, then Ac_2O, pyridine; v, silylated thymine, TMSOTf, MeCN; vi, MeONa, MeOH; vii, MsCl, pyridine; viii, NaOH, $EtOH/H_2O$; ix, OsO_4, $NaIO_4$, THF/H_2O, then $NaBH_4$, THF/H_2O; x, TsCl, pyridine; xi, NaH, DMF; xii, H_2, $Pd(OH)_2$-C, EtOH.

4. 2’,4’-BNA/LNA AND ITS DERIVATIVES –BRIDGED NUCLEIC ACIDS WITH N-TYPE SUGAR CONFORMATION–

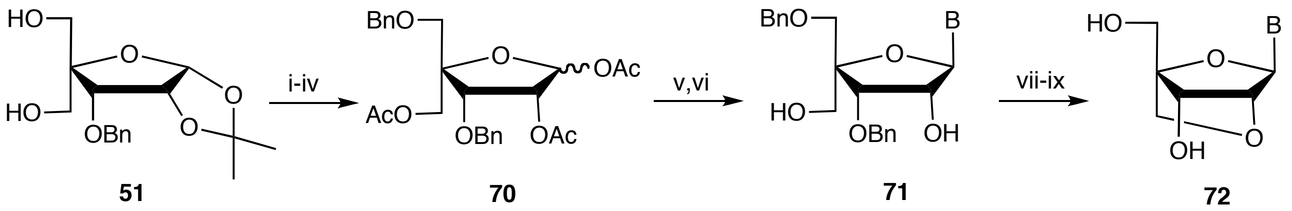
In 1997, we achieved the first synthesis of 2’,4’-BNA monomers bearing uracil and cytosine nucleobases,[11] where the sugar puckering was exclusively N-type (C3’-endo conformation) due to the methylene bridge between the O2’ and C4’ atoms. On the other hand, Wengel *et al*. independently accomplished the synthesis of the same nucleic acid analogue in 1998[14] and called it Locked Nucleic Acid (LNA). Oligonucleotides containing the 2’,4’-BNA/LNA monomers showed unprecedented hybridization ability to their RNA complements,[12] superior triplex-forming ability,[85-90] and enzymatic stability.[76] These properties of 2’,4’-BNA/LNA make them suitable for many kinds of genome technologies, e.g., oligonucleotide-based therapy and as probes for DNA detection. 2’,4’-BNA/LNA is now commercially available and is widely used in many research fields, as described above. The synthesis of 2’,4’-BNA/LNA has been summarized in previous reviews;[10,82,91] therefore, only a brief account is presented here. As shown in Scheme 11, our first synthesis of the 2’,4’-BNA monomers started from compound **64**, was an intermediate in the synthesis of 3’,4’-BNA (the precursor of compound **46** in

Scheme 7).[11,52] Compound **64** was converted to the 2,3-*O*-benzylidene derivative **65**, which was subjected to regioselective reductive cleavage to afford **66** with the 3'-*O*-benzyl group. Ring closure and subsequent deprotection gave the desired 2',4'-BNA monomer **67**. The uracil nucleobase of **67** was converted to cytosine (compound **69**) by a conventional method.



Scheme 11. *Reagents and conditions*: i, PhCHO, $ZnCl_2$; ii, $NaBH_3CN$, $TiCl_4$, MeCN; iii, sodium hexamethyldisilazide, THF; iv, H_2 Pd-C, MeOH; v, 4,4'-dimethoxytrityl chloride, DMAP, pyridine; vi, Ac_2O, pyridine; vii, 4-chlorophenyl phosphorodichloridate, 1,2,4-triazole, pyridine; viii, NH_4OH, dioxane. U= uracil-1-yl.

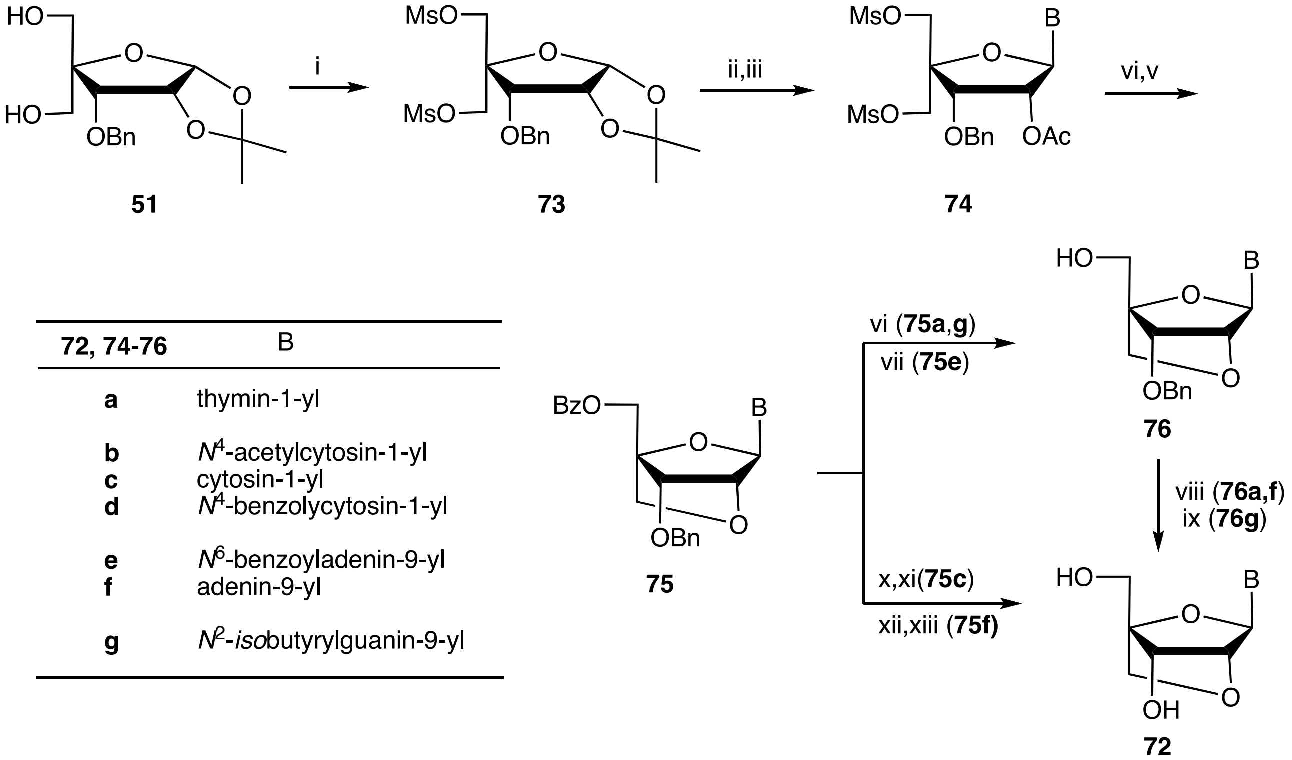
On the other hand, the synthesis of 2',4'-BNA/LNA by Wengel *et al.* started from 4'-*C*-hydroxymethyl pentofuranose derivative **51** (Scheme 12).[14,92] Regioselective 5-*O*-benzylation, acetylation, acetolysis, and a second acetylation gave **70**. Incorporation of nucleobases and deacetylation afforded **71**. Tosylation followed by ring closure under basic conditions and debenzylation gave the desired 2',4'-BNA/LNA monomer **72**.



B = thymin-1-yl, uracil-1-yl, N^2-isobutyrylguanin-9-yl, N^4-benzoylcytosin-1-yl, N^6-benzoyladenin-9-yl, cytosin-1-yl, adenin-9-yl

Scheme 12. *Reagents and conditions*: i, NaH, BnBr, DMF; ii, Ac_2O, pyridine; iii, AcOH; iv, Ac_2O, pyridine; v, silylated nucleobase, TMSOTf, MeCN (or dichloroethane); vi, NaOMe, MeOH; vii, TsCl, pyridine; viii, NaH, DMF; ix, H_2, $Pd(OH)_2$-C, EtOH, (or 1,4-cyclohexadiene, $Pd(OH)_2$-C, MeOH or BCl_3, CH_2Cl_2/hexane).

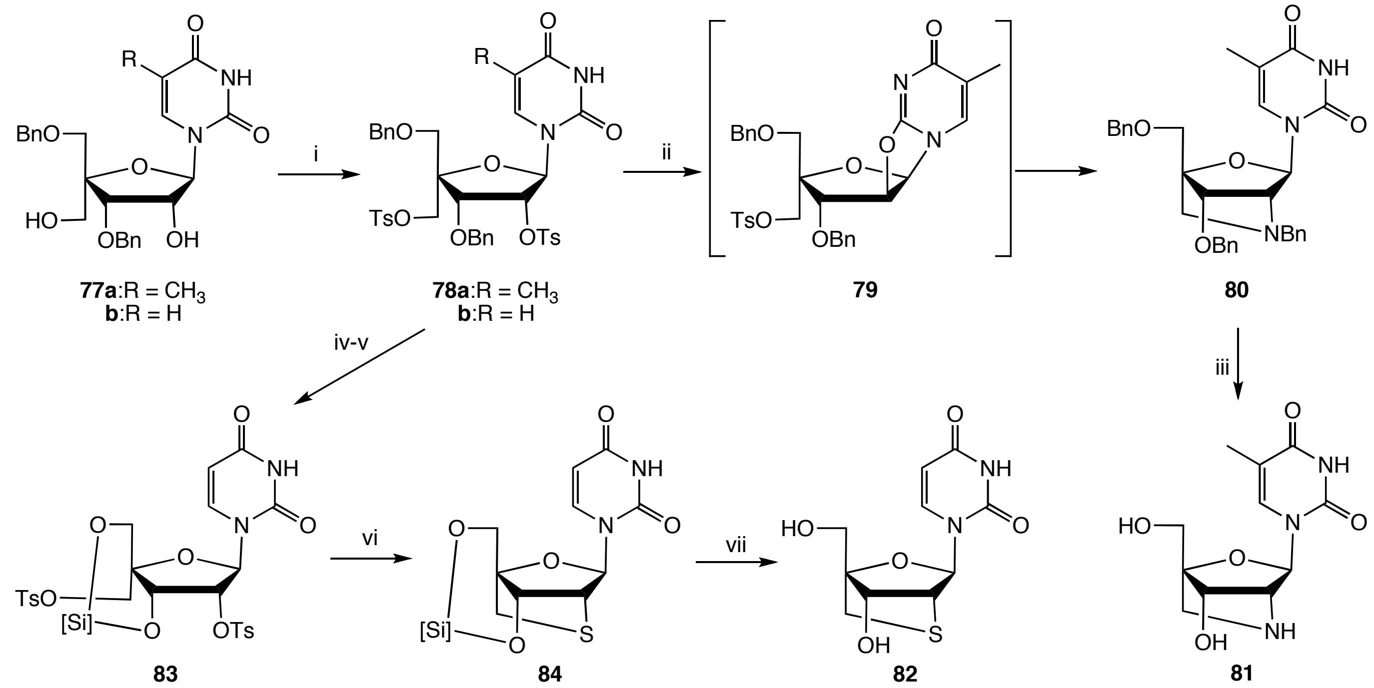
Later, Koshkin *et al*. reported a more efficient route to the 2',4'-BNA/LNA monomers (Scheme 13).[93] Their synthesis also started from pentofuranose derivative **51**, which was converted to di-*O*-mesylated compound **73**. Acetolysis and the incorporation of nucleobases afforded **74**, which was converted to bicyclic nucleoside **75** by one-pot deacetylation, ring closure, then nucleophilic displacement of the 5'-*O*-mesyl group by sodium benzoate. Subsequent saponification of the benzoyl group and removal of the 3'-*O*-benzyl group by hydrogenolysis gave the 2',4'-BNA/LNA monomer **76**.



72, 74-76	B
a	thymin-1-yl
b	N^4-acetylcytosin-1-yl
c	cytosin-1-yl
d	N^4-benzolycytosin-1-yl
e	N^6-benzoyladenin-9-yl
f	adenin-9-yl
g	N^2-*iso*butyrylguanin-9-yl

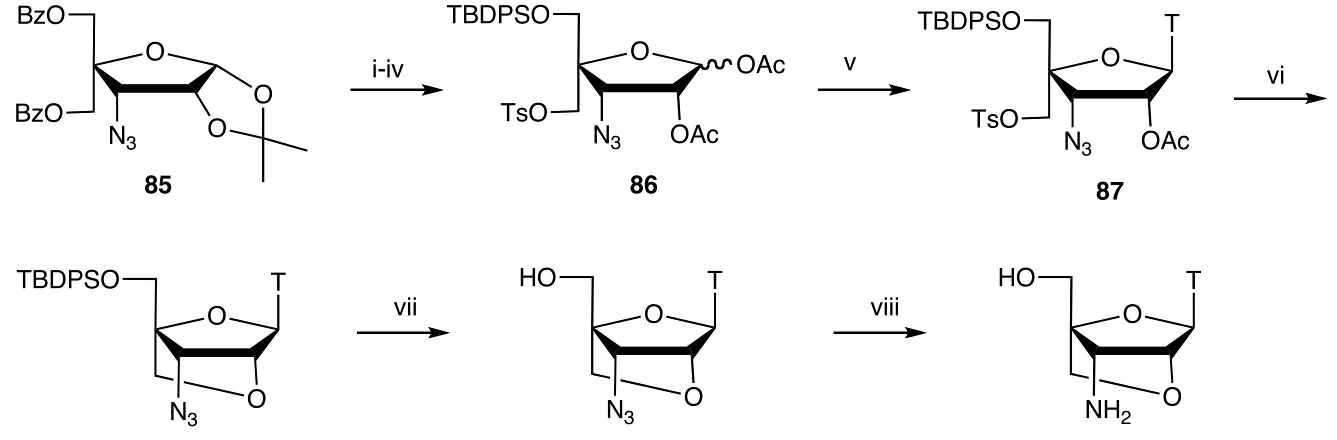
Scheme 13. *Reagents and conditions*: i, MsCl, pyridine; ii, TFA, then Ac_2O, pyridine or AcOH, Ac_2O, H_2SO_4; iii, silylated nucleobase, TMSOTf, MeCN or 1,2-dichloroethane; iv, NaOH or LiOH, THF or dioxane (+NH_4OH for **74f**); v, NaOBz, DMF; vi, NaOH, THF or EtOH/pyridine; vii, NH_4OH, $MeNH_2$, MeOH; viii, $Pd(OH)_2$-C, ammonium formate, MeOH; ix, Pd-C, HCO_2H, MeOH; x, $Pd(OH)_2$-C, ammonium formate, MeOH; xi, NH_4OH; xii, $Pd(OH)_2$-C, ammonium formate, MeOH; xiii, BzCl, pyridine, then NaOH, EtOH/pyridine.

As simple and interesting derivatives of 2',4'-BNA/LNA, the oxygen atoms in the 2',4'-BNA/LNA structure were replaced with other hetero atoms. For example, 2'-amino and 2'-thio congeners were developed by Wengel *et al*. (Scheme 14).[21,22] The 4'-*C*-hydroxymethylnucleoside derivatives **77a** and **77b** was converted into di-*O*-tosylates **78a** and **78b**, which were treated with benzylamine to give **80** *via* 2,2'-anhydro intermediate **79**. Debenzylation of **80** with ammonium formate and 10% $Pd(OH)_2/C$ gave the desired 2'-amino-LNA monomer **81**. Alternatively, 2'-thio-LNA monomer **82** was obtained from **78b** as shown in Scheme 14. The benzyl groups of **78b** were removed and reprotected with TIPDS groups to afford **83**. Treatment with potassium thioacetate followed by desilylation gave **82** *via* **84**.



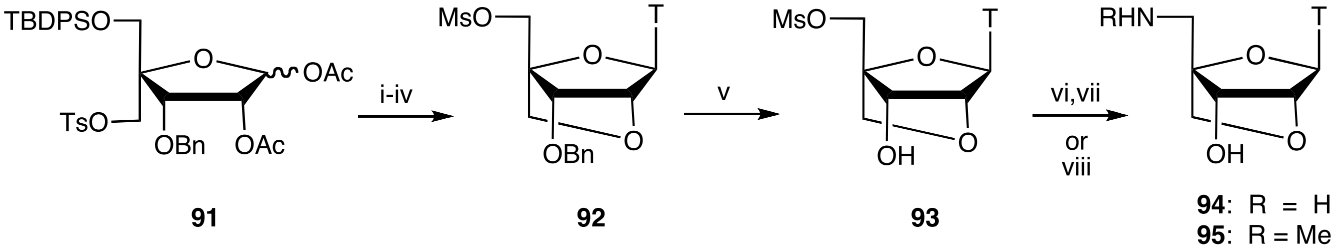
Scheme 14. *Reagents and conditions*: i, TsCl, DMAP, CH_2Cl_2; ii, $BnNH_2$; iii, H_2, $Pd(OH)_2$-C, EtOH, then Pd-C, ammonium formate, MeOH; iv, H_2, $Pd(OH)_2$-C, EtOH; v, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, pyridine; vi, KSAc, DMF; vii, tetra-*n*-butylammonium fluoride, THF. [Si]= 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl.

3'-Amino and 5'-amino congeners of 2',4'-BNA were developed by our group.[23-25] As shown in Scheme 15, 3'-amino-2',4'-BNA monomer was synthesized starting from 3-azidofuranose derivative **85**. Saponification, regioselective silylation, tosylation and acetolysis gave **86**. Incorporation of thymine nucleobase and subsequent base-mediated deacetylation and ring closure smoothly proceeded to give **88**. The desired 3'-amino-2',4'-BNA monomer **90** was obtained by Pd-mediated reduction of the corresponding 3'-azido congener **89**, which is an analogue of AZT with N-type sugar conformation.[94]



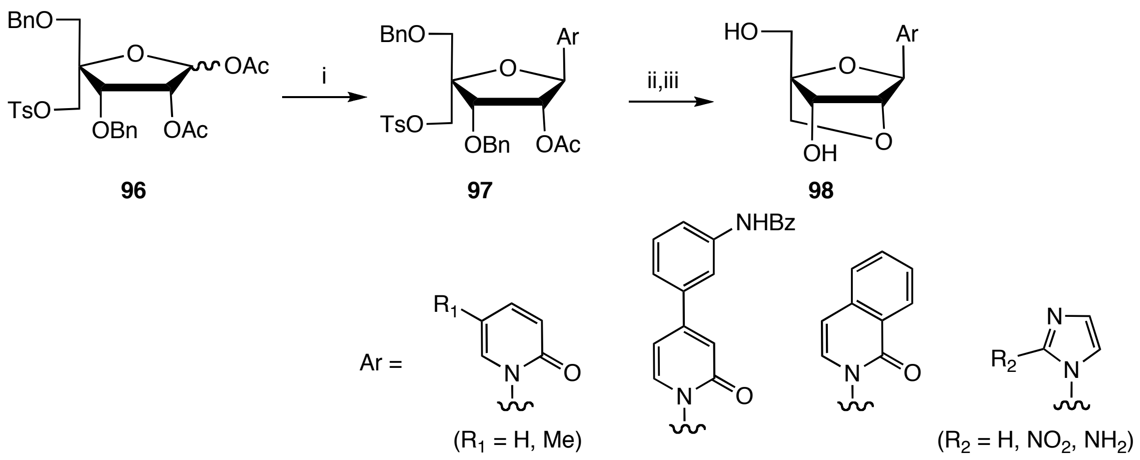
Scheme 15. *Reagents and conditions*: i, K_2CO_3 aq., MeOH; ii, *tert*-butyldiphenylsilyl chloride, Et_3N, CH_2Cl_2; iii, TsCl, Et_3N, DMAP, CH_2Cl_2; iv, Ac_2O, AcOH, H_2SO_4; v, silylated thymine, $SnCl_4$, 1,2-dichloroethane; vi, K_2CO_3, MeOH; vii, tetra-*n*-butylammonium fluoride, THF; viii, H_2, Pd-C, EtOH. TBDPS= *tert*-butyldiphenylsilyl, T= thymin-1-yl.

On the other hand, 5'-amino-2',4'-BNA monomer **94**[25] and its *N*-methyl derivative **95**[95] were prepared from **91** (Scheme 16). Compound **92** was obtained from **91** *via* sequential nucleosidation, cyclization, desilylation and mesylation. Debenzylation produced **93**, the 5'-mesyloxy group of which was replaced by an azido group and finally converted to 5'-amino-2',4'-BNA monomer **94** by reduction under Staudinger conditions. Alternatively, **93** was treated with methylamine in a sealed tube to afford 5'-methylamino derivative **95**.



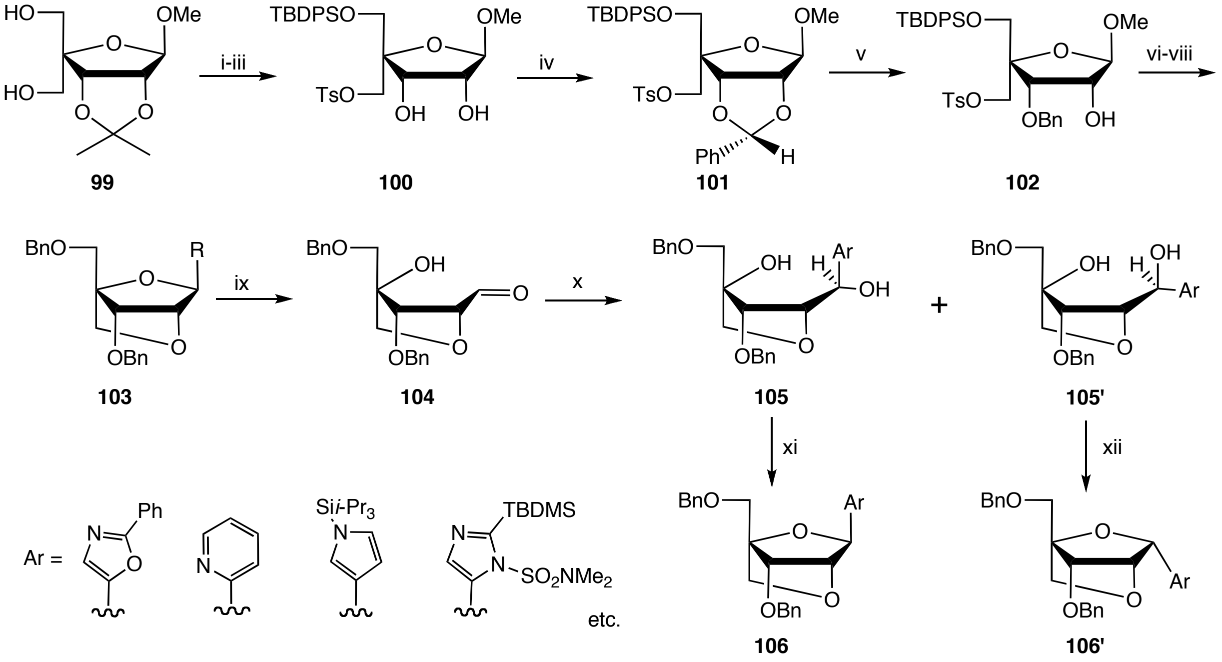
Scheme 16. *Reagents and conditions*: i, silylated thymine, TMSOTf, 1,2-dichloroethane; ii, K_2CO_3, MeOH; iii,tetra-*n*-butylammonium fluoride, THF; iv, MsCl, pyridine; v, $Pd(OH)_2$-C, cyclohexene, EtOH; vi, NaN_3, DMF; vii, Ph_3P, pyridine, then NH_4OH aq.; viii, $MeNH_2$, T= thymin-1-yl.

A variety of unnatural nucleobases were also introduced into the 2',4'-BNA/LNA skeleton for development of efficient triple helix-forming oligonucleotides or for other purposes.[26-31,96,97] The structures of nucleosides with unnatural nucleobases are categorized into two groups: N-nucleoside and C-nucleoside. The former contains a linkage between C1' in the furanose ring and a nitrogen atom in the heteroaromatics as unnatural nucleobases, and the latter involves a linkage between C1' and a carbon atom of the unnatural nucleobase. For the construction of several N-nucleoside-type 2',4'-BNA monomers, we chose 2-pyridone,[27] 5-methyl-2-pyridone,[28] 4-(3-benzamidophenyl)-2-pyridone,[31] 1-isoquinolone,[29] imidazole and 2-aminoimidazole[30] as unnatural nucleobases. These heteroaromatics were designed for recognition of C•G or T•A interuption sites in a homopurine•homopyrimidine dsDNA. The synthesis of the 2',4'-BNA monomers bearing unnatural nucleobase **98** was accomplished by coupling these heteroaromatics and furanose derivative **96**, 2'-*O*-deacetylation and subsequent ring closure, and 3'-*O*- and 5'-*O*-debenzylation (Scheme 17). Thus, this approach, based on Wengel's synthetic route to 2',4'-BNA/LNA (Scheme 12), is a good route for the construction of N-nucleoside-type 2',4'-BNA/LNA bearing unnatural nucleobases. Koshkin also reported the N-nucleoside-type 2',4'-BNA/LNA bearing other heteroaromatics.[34]

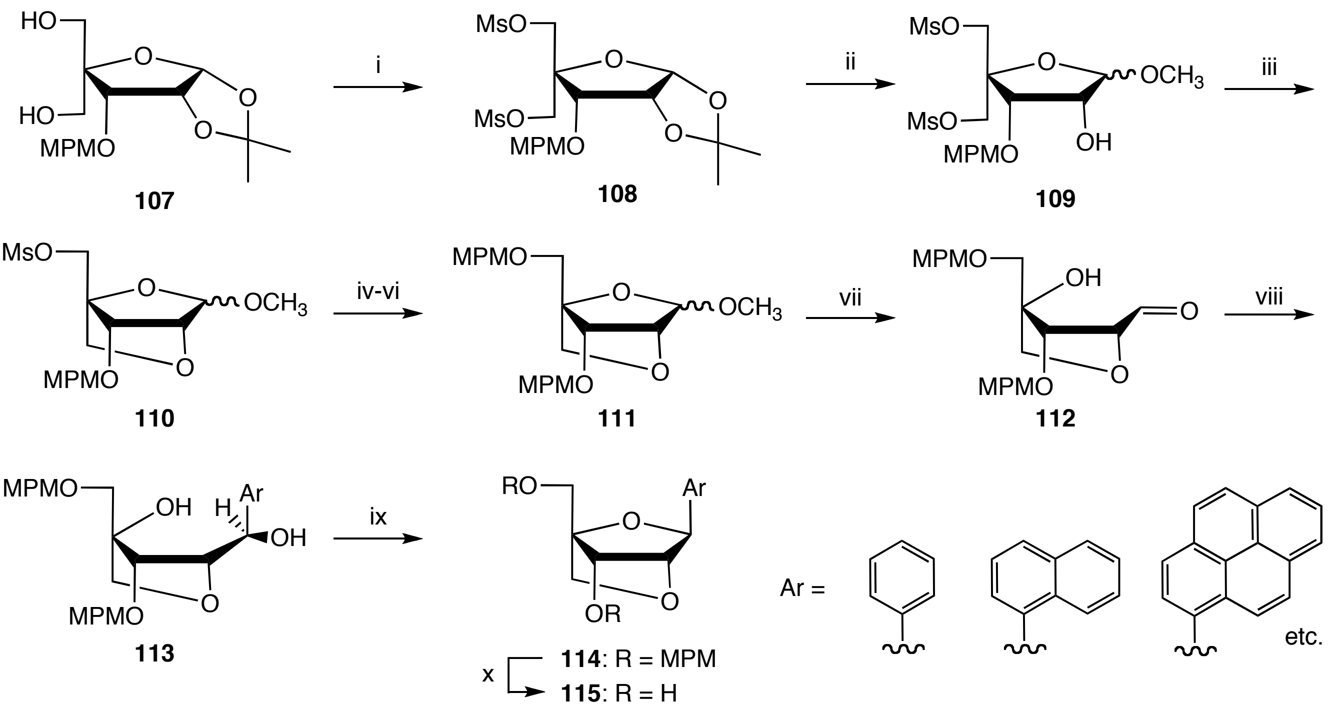


Scheme 17. *Reagents and conditions*: i, silylated heteroaromatics, TMSOTf, dichloroethane; ii, K_2CO_3, MeOH; iii, $Pd(OH)_2$-C, cyclohexene, EtOH.

Compared to N-nucleosides, C-nucleosides can possess a great variety of heteroaromatics as unnatural nucleobases; therefore, we developed a synthetic route for C-nucleoside-type 2',4'-BNA/LNA monomers.[31,98,99] As shown in Scheme 18, aldehyde **104** as a key intermediate was prepared straightforwardly from methyl 4-hydroxymethylribofuranoside derivative **99**. Aldehyde **104** was coupled with the lithium derivative of several heteroaromatics to afford diols **105** and **105'** as diastereomeric mixtures. On the other hand, the corresponding magnesium derivative of the heteroaromatics gave the desired diol **105** stereoselectively. Each stereoisomer of diols **105** and **105'** was separated and converted into ring closure products **106** and **106'** under Mitsunobu conditions. Subsequent debenzylation of desired β-isomer **106** afforded the C-nucleoside-type 2',4'-BNA/LNA monomers.[98,99]



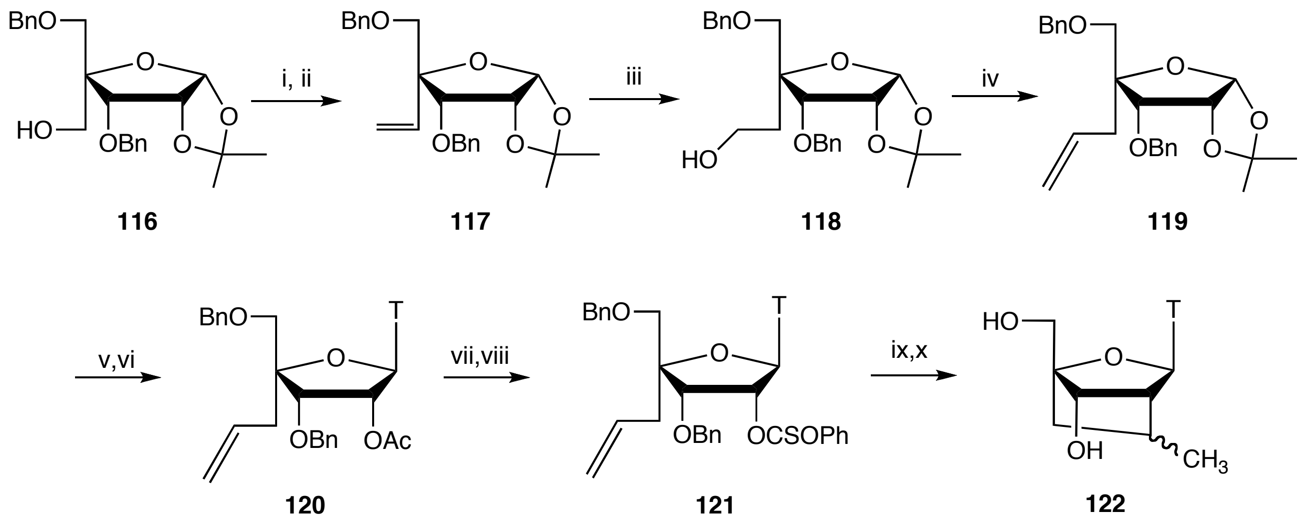
Scheme 18. *Reagents and conditions*: i, *tert*-butyldiphenylsilyl chloride, Et_3N, CH_2Cl_2; ii, TsCl, Et_3N, DMAP, CH_2Cl_2; iii,TFA, THF /H_2O; iv, PhCHO, $ZnCl_2$; v, $NaBH_3CN$, $TiCl_4$, MeCN; vi, sodium hexamethyldisilazide, THF; vii, tetra-*n*-butylammonium fluoride, THF; viii, BnBr, NaH, DMF; ix, HCl, THF; x, ArMgBr, or ArLi, THF; xi, diethylazodicarboxylate, PPh_3, THF or *N,N,N',N'*-tetramethylazodicarboxamide, *n*-Bu_3P, benzene; xii, diethylazodicarboxylate, Ph_3P, THF. TBDPS= *tert*-butyldiphenylsilyl.



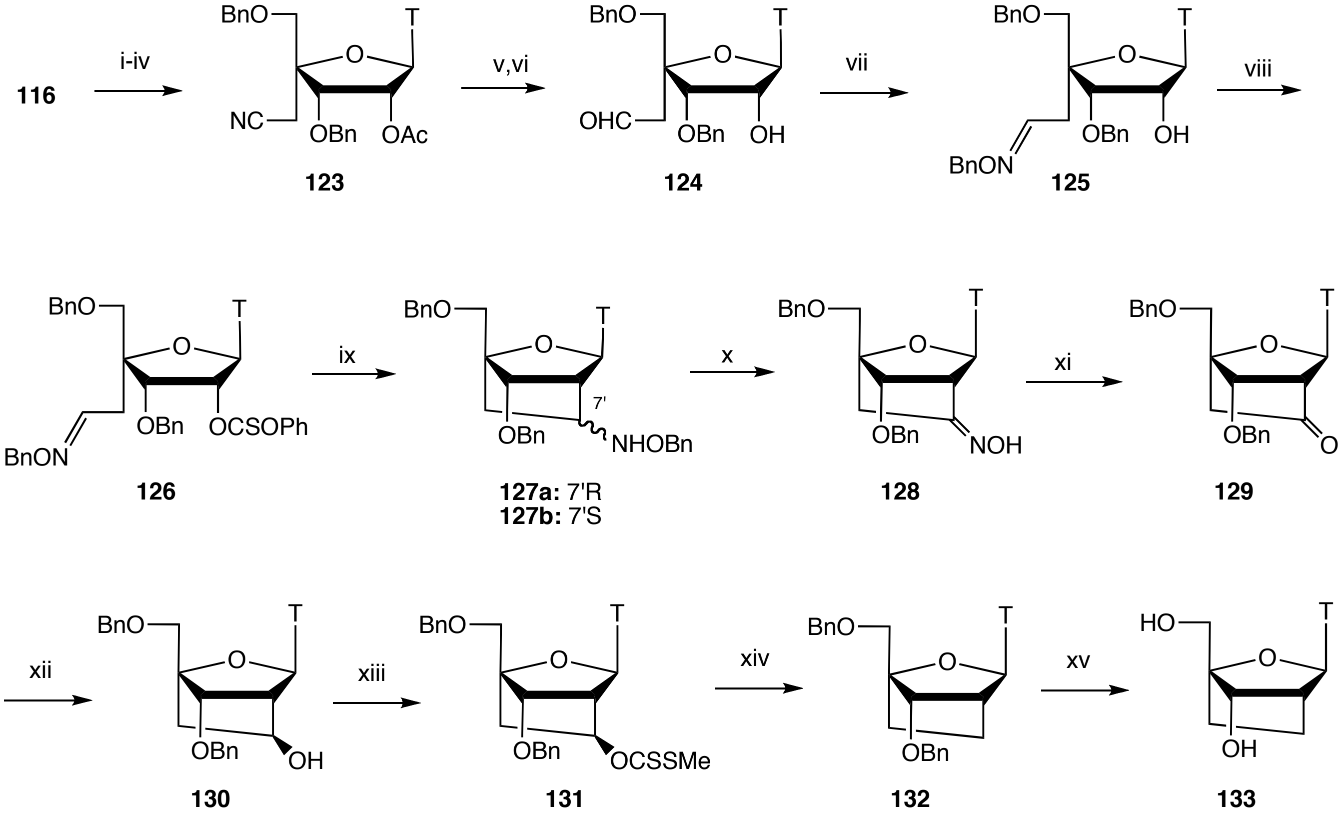
Scheme 19. *Reagents and conditions*: i, MsCl, pyridine; ii, HCl H_2O/CH_3OH; iii, NaH, DMF; iv, KOAc, dioxane, 18-crown-6; v, NH_3, MeOH; vi, *p*-methoxybenzyl chloride, NaH, THF; vii, AcOH; viii, ArMgBr, THF; ix, *N,N,N',N'*-tetramethylazodicarboxamide, *n*-Bu_3P, benzene; x, DDQ, CH_2Cl_2, H_2O. MPM= *p*-methoxybenzyl.

Wengel *et al*. also prepared 2’,4’-BNA/LNA derivatives bearing benzene, naphthalene, and pyrene as nucleobases (Scheme 19).[32,33] Starting material **107**, where the *p*-methoybenzyl group was used to protect the 3-OH group, was converted into di-*O*-mesylate **108**. Methanolysis and base-induced cyclization afforded methyl furanoside **110** as an anomeric mixture. Replacement of the mesyloxy group by the acetoxy group, followed by deacetylation and *p*-methoxybenzylation, gave **111**, which was then treated with acetic acid to yield aldehyde **112**. Likewise to our results (Scheme 18), coupling of aldehyde **112** with several aryl Grignard reagents, followed by cyclization under Mitsunobu conditions, proceeded smoothly to afford **114**. Oxidative removal of the *p*-methoxybenzyl group was achieved using DDQ to give the desired diol **115**.

A carbocyclic 2’,4’-BNA derivative was synthesized from the known dibenzyl derivative **116** by Chattopadhyaya *et al*. (Scheme 20).[35] Oxidation of the primary alcohol of **116** followed by Wittig reaction gave **117**, which was subjected to hydroboration-oxidation reaction to give alcohol **118**. Swern oxidation and further Wittig reaction provided **119** into which a thymine nucleobase was incorporated. Deacetylation and esterification with phenyl chlorothioformate afforded **121**. Cyclization by a radical mediated procedure followed by debenzylation provided the carbocyclic BNA monomer **122**.



Scheme 20. *Reagents and conditions:* i, $(COCl)_2$, DMSO, diisopropylethylamine, CH_2Cl_2; ii, $Ph_3P{=}CH_2$, THF; iii, 9-BBN, NaOH, H_2O_2, THF; iv) 1) $(COCl)_2$, DMSO, diisopropylethylamine, CH_2Cl_2, 2) $Ph_3P{=}CH_2$, THF; v, AcOH, Ac_2O, TfOH; vi, silylated thymine, TMSOTf, MeCN; vii, NH_3, MeOH; viii, phenylchlorothionoformate, DMAP, pyridine; ix, Bu_3SnH, toluene, AIBN; x, $Pd(OH)_2$-C, ammonium formate, MeOH. T= thymin-1-yl.



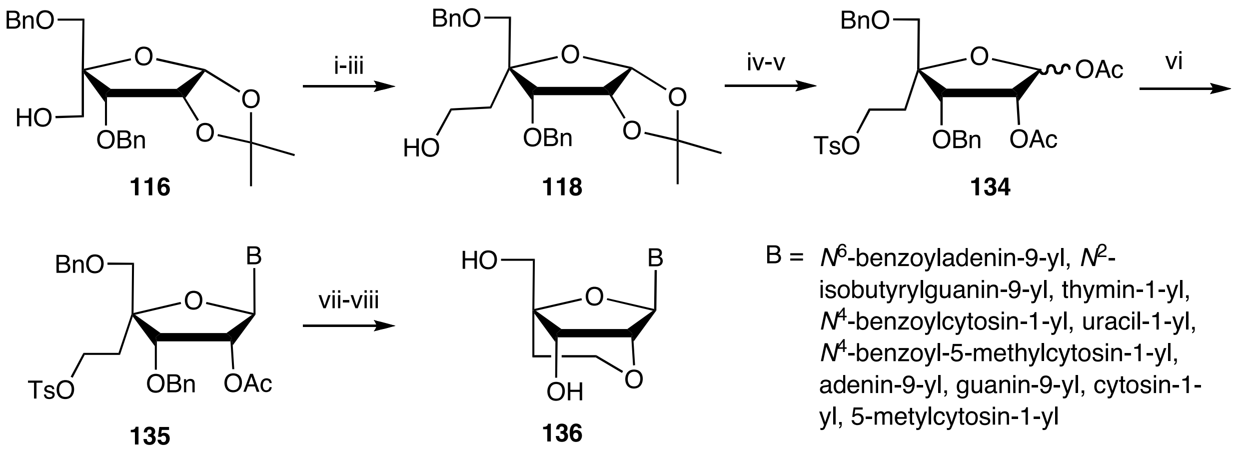
Scheme 21. *Reagents and conditions:* i, Tf_2O, pyridine, CH_2Cl_2; ii, LiCN, DMF; iii, AcOH, TFA, Ac_2O; iv, silylated thymine, TMSOTf, MeCN; v, $MeNH_2$, MeOH; vi, DIBAL-H, CH_2Cl_2; vii, $HCl{\bullet}NH_2OBn$, pyridine, CH_2Cl_2; viii, phenyl chlorothioformate, pyridine; ix, Bu_3SnH, toluene, AIBN; x, m-CPBA, AcOEt, K_2CO_3; xi, Dess-Martin reagent, NaOAc, CH_2Cl_2; xii, $NaBH_4$,EtOH; xiii, CS_2, MeI, NaH, THF; xiv, Bu_3SnH, AIBN, toluene; xv, $Pd(OH)_2$-C, ammonium formate, MeOH. T= thymin-1-yl.

Another carbocyclic five-membered BNA analogue, **133** (Scheme 21), was synthesized very recently using the same starting material **116**.[36] Triflation, replacement with cyanide, acetolysis, and introduction of a thymine nucleobase afforded **123**. Deacetylation of **123** and subsequent DIBAL reduction gave aldehyde **124**, which was treated with *O*-benzylhydroxylamine to produce oxime **125**. Esterification with phenyl chlorthioformate (compound **126**) followed by radical cyclization afforded a mixture of two diastereomers, **127a** and **127b**, with **127a** (7’R) as the major product. Oxidation of the benzyloxyamine of **127a** with mCPBA provided oxime **128**. Dess-Martin periodinate oxidation gave ketone **129** which was reduced stereoselectively to alcohol **130**. Esterification with sterically less bulky (methylthio)thiocarbonate (comapred to the phenyl thiocarbonate group in **126**) afforded **131**, which was transformed to benzyl-protected carba-2’,4’-BNA analogue **132** *via* radical deoxygenation. Debenzylation provided the carbocyclic 2’,4’-BNA monomer **133**.

5. 2’,4’-BNA/LNA DERIVATIVES WITH EXPANDED RING SIZE

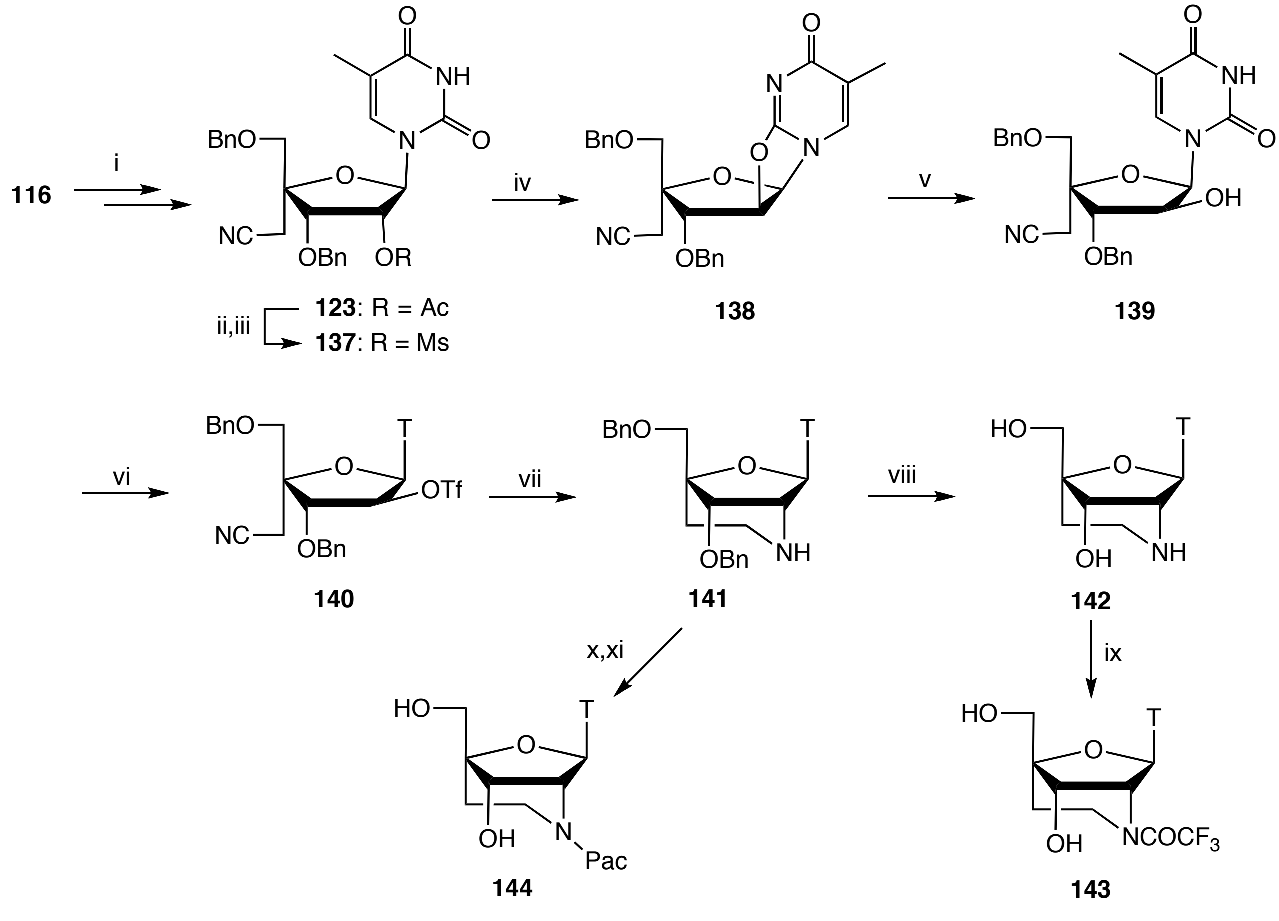
2’,4’-BNA/LNA and its analogues have an additional methylene linkage between the 2’-oxygen/amino and 4’-carbon atoms to form a bicyclo[2.2.1]heptane skeleton. The sugar conformation is exclusively N-type, locked by the five-membered bridged structure. Various BNA analogues with expanded ring size having different bridge moiety architectures were also developed, some with a 2’-*O* linked bridge, some with a 2’-*N* bridge, and some with a 2’-*C* linked or carbocyclic BNA structure.

Ethylene-bridged nucleic acid (ENA) is one example of a six-membered BNA analogue.[39,40] The synthesis of ENA was accomplished from the known dibenzyl derivative **116** as shown in Scheme 22. Compound **116** with a hydroxymethyl group was converted to compound **118** with an additional methylene group by Swern oxidation, Wittig reaction and hydroboration, followed by oxidation. After tosylation and acetolysis, nucleobases were introduced to give the 4’-(hydroxyethyl)ribonucleoside derivative **135** *via* **134**. Base-induced one-pot deacetylation and ring closure followed by Pd-mediated hydrogenolysis afforded the desired ENA monomer **136**. ENA monomers with a variety of bases have been synthesized.[40]



B = N^6-benzoyladenin-9-yl, N^2-isobutyrylguanin-9-yl, thymin-1-yl, N^4-benzoylcytosin-1-yl, uracil-1-yl, N^4-benzoyl-5-methylcytosin-1-yl, adenin-9-yl, guanin-9-yl, cytosin-1-yl, 5-metylcytosin-1-yl

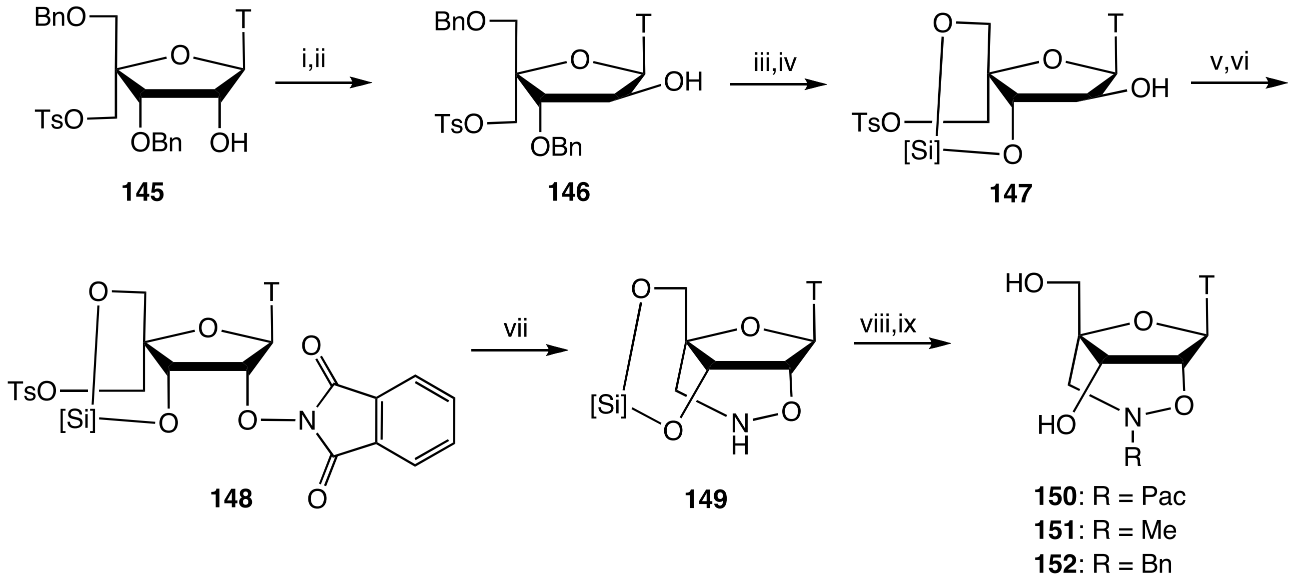
Scheme 22. *Reagents and conditions*: i, $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2; ii, $Ph_3P=CH_2$, DMSO; iii, 9-BBN, THF then H_2O_2, NaOH; iv, TsCl, Et_3N, CH_2Cl_2; v, Ac_2O, H_2SO_4, AcOH; vi, silylated nucleobase, TMSOTf, 1,2-dichloroethane; vii, NaOH, pyridine/H_2O; viii, H_2, $Pd(OH)_2$-C, MeOH.



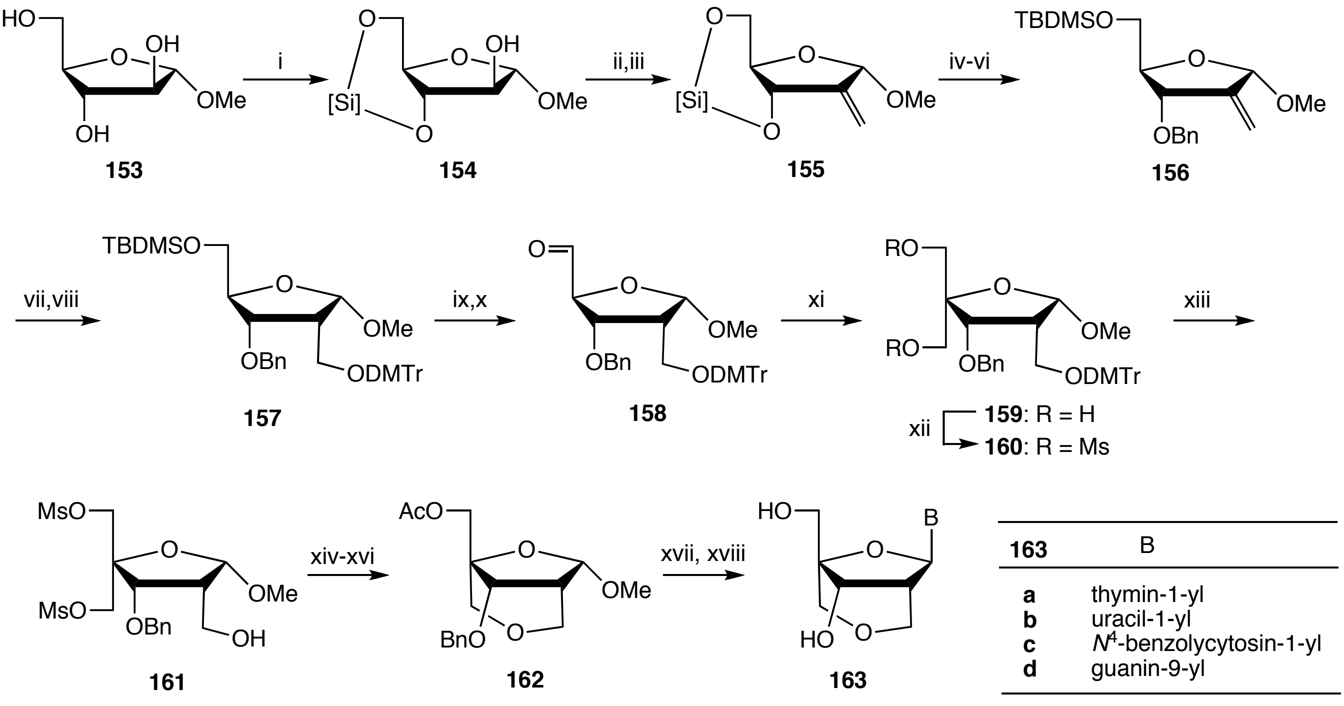
Scheme 23. *Reagents and conditions:* i, See Scheme 21; ii, NaOMe, MeOH; iii, MsCl, pyridine; iv, DBU, MeCN; v, H_2SO_4, acetone; vi, Tf_2O, pyridine, DMAP, CH_2Cl_2; vii, $NaBH_4$, TFA, THF; viii, $Pd(OH)_2$-C, ammonium formate, MeOH, then BCl_3, CH_2Cl_2; ix, ethyl trifluoroacetate, DMAP, MeOH; x, phenoxyacetyl chloride, pyridine; xi, $Pd(OH)_2$-C, ammonium formate, MeOH, then BCl_3, CH_2Cl_2. Pac= phenoxyacetyl, T= thymin-1-yl.

A thymine derivative of a 2'-aza analogue of ENA was reported by Chattopadhyaya *et al.*[41] The synthesis of aza-ENA is shown in Scheme 23. Known dibenzyl derivative **116** was converted to **123** as described in Scheme 21. Deacetylation of **123** with NaOMe afforded alcohol **124**, which was directly mesylated to give **137**. Compound **137** was treated with DBU to produce 2,2'-anhydronucleoside **138**, which was refluxed with 0.1 M H_2SO_4 in acetone to provide the arabino-type product **139** quantitatively. Compound **139** was converted to triflate **140** and the cyano group was reduced to an amino group using sodium tris(trifluoroacetoxy)borohydride (prepared *in situ* from $NaBH_4$ and TFA) which spontaneously underwent intramolecular cyclization to give **141**. Debenzylation with $Pd(OH)_2$-C/ammonium formate followed by BCl_3 provided the aza-ENA monomer **142**. For efficient incorporation into oligonucleotides, *N*-protected aza-ENA analogues were synthesized. *N*-Trifluoroacetyl and *N*-phenoxyacetyl aza-ENA derivatives **143** and **144** were synthesized from **142** and **141**, respectively. Compound **142** was reacted with excess ethyl trifluoroacetate in methanol to give *N*-trifluoroacetyl aza-ENA analogue **143**. On the other hand, **141** was treated with Pac-Cl in pyridine and the resultant phenoxyacetyl derivative was subjected to debenzylation to produce the phenoxyacetyl derivative of aza-ENA **144**. Both these derivatives were incorporated into oligonucleotides successfully.

We also developed 2',4'-BNA^{NC} which contains a unique O-N linkage in the six-membered bridge structure (Scheme 24).[43-46] The key step in the construction of 2',4'-BNA^{NC} is the O-N bond formation. First, we attempted direct amination of the 2'-OH group of ribonucleoside derivative **145**, but none of the desired product was obtained. Therefore, the O-N linkage was introduced by the following multi-step sequence. Ribonucleoside derivative **145** was converted into arabinonucleoside **146** by 2'-*O*-mesylation and concomitant hydrolysis. After debenzylation of the 3'- and 5'-OH groups, the resultant triol was selectively silylated with the tetraisopropyldisiloxy group to give **147**. Triflation of the 2'-OH group, and subsequent S_N2 reaction with *N*-hydroxyphthalimide afforded **148**. Then, compound **148** was treated with hydrazine in the presence of DABCO and pyridine, a one-pot deprotection and cyclization reaction, to give **149** with the desired six-membered bridged moiety containing an O-N linkage. Protection or substitution on the nitrogen of the additional bridge followed by desilylation of the 3'- and 5'-OH groups gave the 2',4'-BNA^{NC} monomer **150**.



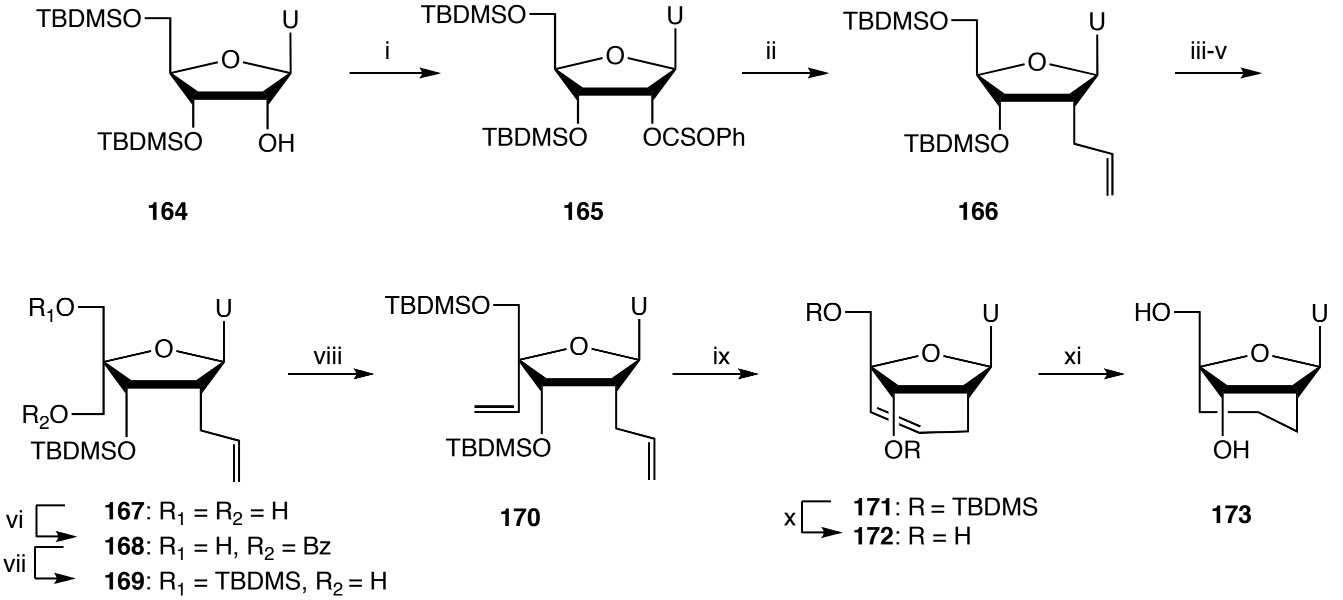
Scheme 24. *Reagents and conditions*: i, MsCl, pyridine; ii, NaOH, EtOH; iii, $Pd(OH)_2$-C, cyclohexene, EtOH; iv, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, imidazole, DMF; v, Tf_2O, DMAP, pyridine; vi, *N*-hydroxyphthalimide, DBU, MeCN; vii, H_2NNH_2, DABCO, pyridine; viii, phenoxyacetyl chloride, Et_3N, CH_2Cl_2 (for **150**), HCHO aq., $NaBH_3CN$, PPTS, MeOH (for **151**) or BnBr, Et_3N, CH_2Cl_2 (for **152**); ix, tetra-*n*-butylammonium fluoride, THF. [Si]= 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl, T= thymin-1-yl, Pac= phenoxyacetyl.



163	B
a	thymin-1-yl
b	uracil-1-yl
c	N^4-benzolycytosin-1-yl
d	guanin-9-yl

Scheme 25. *Reagents and conditions*: i, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, pyridine; ii, DMSO, DCC, TFA, pyridine; iii, $Ph_3P{=}CH_2$, ether; iv, TBAF, THF; v, TBDMSCl, pyridine; vi, BnBr, NaH, THF; vii, 9-BBN, THF, then $NaBO_3$, H_2O; viii, DMTrCl, pyridine; ix, tetra-*n*-butylammonium fluoride, THF; x, DMSO, DCC, TFA, pyridine; xi, HCHO, NaOH, dioxane/H_2O; xii, MsCl, pyridine; xiii, AcOH, H_2O; xiv, NaH, THF; xv, NaOH aq.; xvi, Ac_2O, pyridine; xvii, silylated nucleobase, $SnCl_4$, 1,2-dichloroethane; xviii, BCl_3, CH_2Cl_2 (for **163a-c**), NH_4OH, dioxane, then H_2, $Pd(OH)_2$-C (for **163d**). [Si]= 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl.

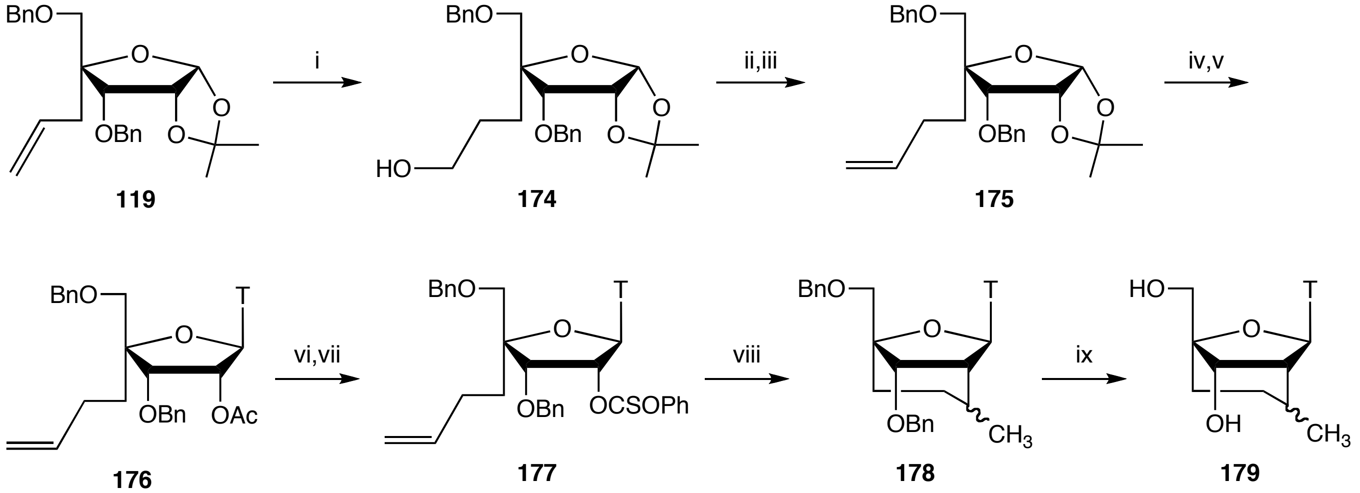
Several other six-membered 2'-*C*,4'-*C*-BNA analogues have been synthesized. In 1999, Wang *et al.* described the synthesis of 2'-*C*,4'-*C* bridged bicyclonucleotide **163** (Scheme 25).[37] The hydroxyl groups at the 3- and 5-positions of 1-α-methyl arabinoside (compound **153**) were protected with a silyl group to give **154**. Oxidation of **154** followed by Wittig reaction afforded **155**. Removal of the silyl group at the 3- and 5-positions, and further protection of the primary alcohol with a TBDMS group and the secondary alcohol with a benzyl group, afforded **156**. Hydroboration of the olefin and protection with a dimethoxytrityl (DMTr) group gave **157**. Desilylation followed by oxidation of the resulting alcohol furnished the aldehyde **158**. Aldol condensation, Cannizzaro reaction and subsequent mesylation afforded **160**. After removal of the DMTr group of **160**, the obtained alcohol **161** was cyclized under basic conditions and then converted to **162**. Reaction of **162** with a silylated nucleobase in the presence of $SnCl_4$ and concomitant deprotection gave the nucleoside derivative **163**.



Scheme 26. *Reagents and conditions:* i, PhOC(S)Cl, DMAP, MeCN; ii, allyltributyltin, AIBN toluene, iii, AcOH; iv, Dess-Martin reagent, CH_2Cl_2; v, HCHO, NaOH, dioxane, then $NaBH_4$; vi, BzCl, pyridine MeCN; vii, TBDMSCl, imidazole, DMF, then NaOMe, MeOH; viii, Dess-Martin reagent, CH_2Cl_2, then $Ph_3P{=}CH_2$, THF; ix, Grubbs second-generation catalyst, CH_2Cl_2; x, tetra-*n*-butylammonium fluoride, THF; xi, H_2, PtO_2, MeOH. U= uracil-1-yl.

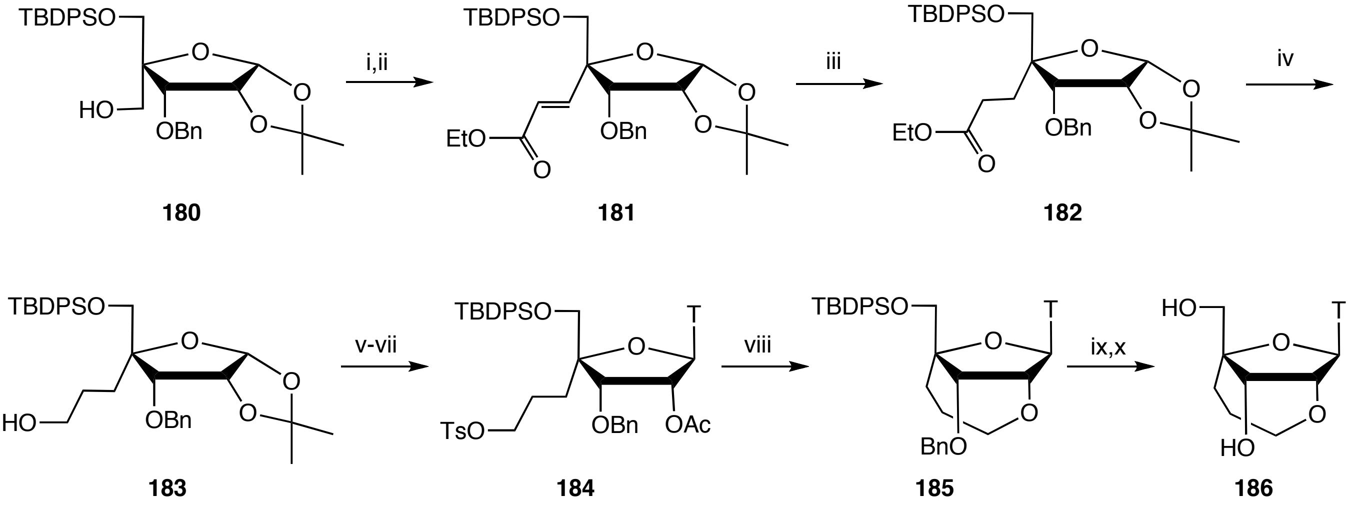
Two BNA analogues with 3 carbons at the 2',4'-linkage were synthesized by Nielsen *et al.* using a ring closing metathesis.[42] The synthesis used 3',5'di-*O*-protected uridine **164** as the starting material as shown in scheme 26. Compound **164** was converted to 2'-*C*-allyl derivative **166** by radical-mediated alkylation *via* **165**. Selective removal of the 5'-*O*-TBDMS group with 80% acetic acid provided an alcohol, which was oxidized to the ketone and ultimately converted to diol **167** by aldol condensation and reduction. The more reactive 4'-*C*-hydroxymethyl group of **167** was protected with a benzoyl group, the remaining 5'-OH group was then protected with TBDMSCl, then the 4'-hydroxymethyl group was released *via* deprotection with sodium methoxide to deliver **169**. Compound **169** was converted to diene **170** efficiently by Dess-Martin periodinate followed by Wittig reaction. Treatment with Grubbs catalyst caused the two terminal alkynes of **170** to react by ring closing metathesis to furnish the bicyclic nucleoside **171**. The silyl protecting group was removed to give monomer **172** with an unsaturated bridged moiety. Hydrogenolysis using Adam's catalyst provided the saturated derivative **173**. Both **172** and **173** were incorporated into oligonucleotides *via* a phosphoramide protocol.

Synthesis of a six-membered carbocyclic BNA analogue was recently reported by Chattopadhyaya *et al.* (Scheme 27).[35] Hydroboration-oxidation of **119** gave alcohol **174**, which was subjected to Swern oxidation and Wittig reaction to afford olefin **175**. Introduction of the thymine base by conventional methods gave **176**. Deacetylation followed by esterification with phenyl chlorothioformate yielded ester **177**. Radical-mediated cyclization produced *exo* product **178**, which was converted to the desired nucleoside analogue **179**.



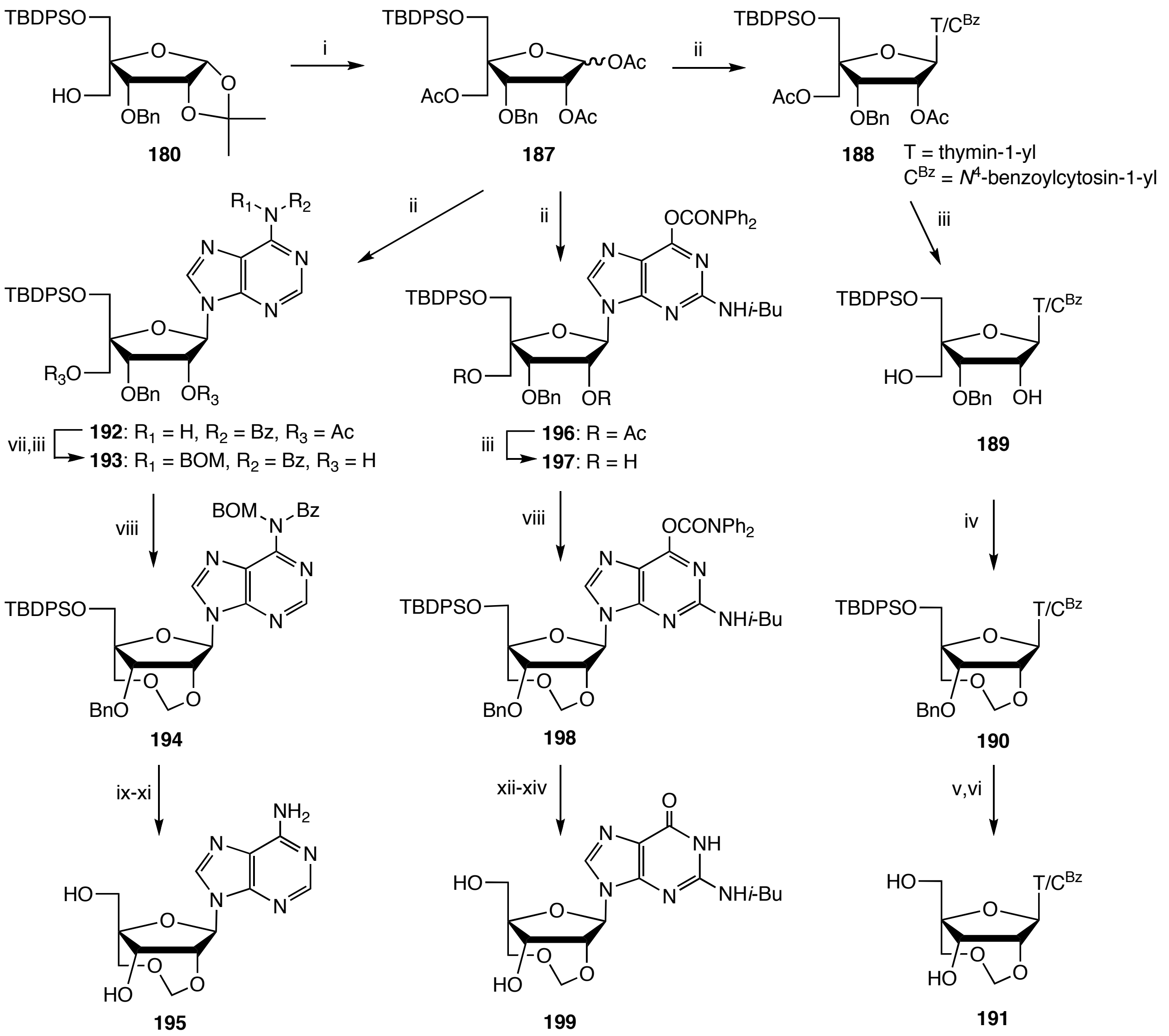
Scheme 27. *Reagents and conditions:* i, 9-BBN, THF, NaOH, H_2O_2; ii, $(COCl)_2$, DMSO, diisopropylethylamine, CH_2Cl_2; iii, $Ph_3P{=}CH_2$, THF; iv, AcOH, Ac_2O, TfOH; v, silylated thymine, TMSOTf, MeCN; vi, NH_3, MeOH; vii, phenyl chlorothionoformate, DMAP, pyridine; viii, Bu_3SnH, AIBN, toluene; ix, $Pd(OH)_2$-C, ammonium formate, MeOH. T= thymin-1-yl.

2',4'-BNA/LNA analogues with a seven-membered bridged structure such as PrNA[40] and 2',4'-BNA^{COC} were also developed.[47,48] The synthesis strategy for the PrNA monomer is depicted in Scheme 28. Swern oxidation and carbon chain elongation by Horner-Wadsworth-Emmons olefination of the pentofuranose derivative **180** gave the (*E*)-enolate **181**. Selective reduction of the olefine using $Pd(OH)_2$ afforded **182**, which was then treated with L-selectride to give 4-(hydroxypropyl)furanose derivative **183**. Tosylation, acetolysis and introduction of thymine nucleobase afforded **184**. Deacetylation and subsequent ring closure proceeded smoothly under alkaline conditions to give **185**. Deprotection of the 5'- and 3'-OH groups afforded the PrNA monomer **186**.



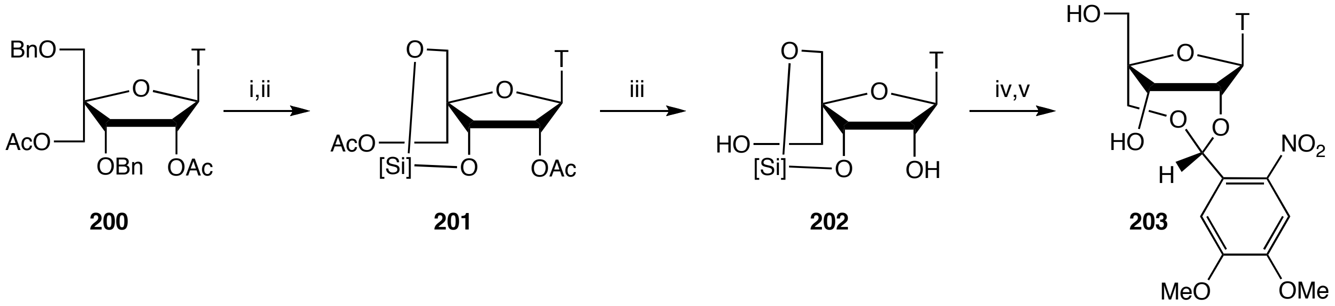
Scheme 28. *Reagents and conditions*: i, $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2; ii, $(EtO)_2P(O)CH_2COOEt$, NaH, THF; iii, H_2, $Pd(OH)_2$-C, MeOH; iv, L-selectride, THF; v, TsCl, Et_3N, CH_2Cl_2; vi, Ac_2O, H_2SO_4, AcOH; vii, silylated thymine, TMSOTf, 1,2-dichloroethane; viii, NaOH, pyridine/H_2O; ix, tetra-*n*-butylammonium fluoride, THF; x, H_2, $Pd(OH)_2$-C, MeOH. TBDPS= *tert*-butyldiphenylsilyl, T= thymin-1-yl.

The synthesis of 2’,4’-BNA^{COC} bearing various nucleobases is shown in Scheme 29. Pentofuranose derivative **180** was converted to triacetate **187**.[47,48] Pyrimidine nucleobase (thymine or *N*^{4}-benzoylcytosine) was introduced using silylated base to give **188**. Deacetylation of **188** gave diol **189**, which was cyclized by treatment with paraformaldehyde under acidic conditions to obtain **190** bearing a seven-membered bridged structure. Desilylation and debenzylation gave 2’,4’-BNA^{COC} monomer **191** with a pyrimidine nucleobase. Synthesis of a 2’,4’-BNA^{COC} analogue with a purine nucleobase was reported recently by our group using compound **187**. To incorporate adenine nucleobase, triacetate **187** was coupled with *N*^{6}-benzoyladenine to afford compound **192**. Synthesis of the 2’,4’-BNA^{COC} structure (methylene acetal formation) from this nucleoside analogue using a procedure similar to that applied for the pyrimidine base was troublesome due to depurination under acidic conditions. After several attempts, we found methyleneacetal formation proceeded using **193**, where the *N*^{6}-amide nitrogen is protected by a BOM group, upon treatment with NBS and DMSO. Sequential deprotection of the benzoyl, silyl, benzyl and BOM groups yielded 2’,4’-BNA^{COC}-A monomer **195**. 2’,4’-BNA^{COC} with a guanine nucleobase was also synthesized from the same triacetate derivative **187**.[48] Introduction of the guanine nucleobase was carried out using *O*^{6}-(diphenylcarbamoyl)-*N*^{2}-isobutyrylguanine to give **196**, which was deacetylated and then cyclized to form a seven-membered ring with a methylene acetal moiety (compound **198**) using the same conditions used for the adenine derivative. Removal of the diphenylcarbamoyl group, desilylation and debenzylation furnished 2’,4’-BNA^{COC}-G monomer **199**.

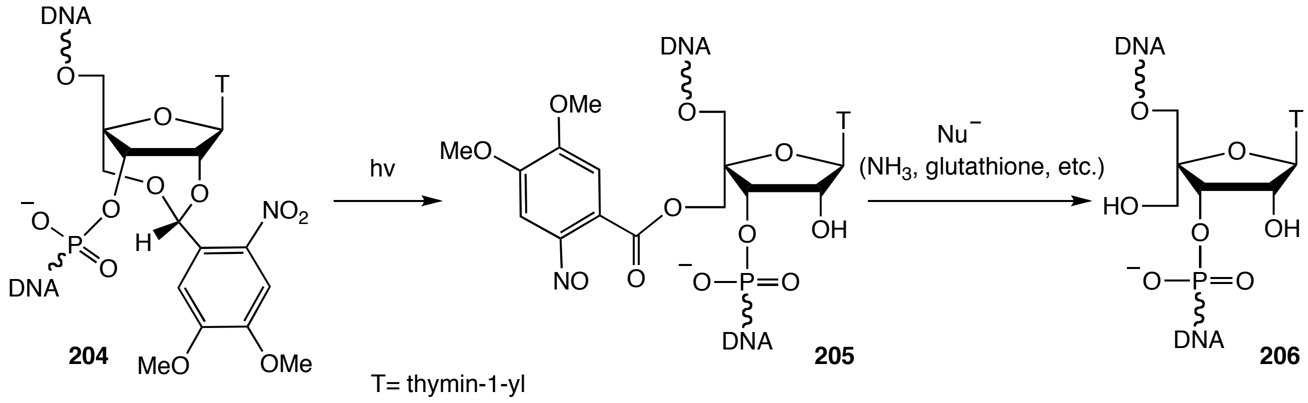


Scheme 29. *Reagents and conditions:* i, Ac_2O,AcOH, H_2SO_4; ii, silylated nucleobase, TMSOTf, MeCN; iii, K_2CO_3, MeOH for T derivative or LiOH, THF/H_2O for C derivative; iv, paraformaldehyde, TsOH, 1,2-dichloroethane; v, tetra-*n*-butylammonium fluoride, THF; vi, $Pd(OH)_2$-C, cyclohexene, MeOH; vii, benzyloxymethyl chloride, DBU, DMF; viii, NBS, DMSO; ix, NH_3 aq., THF; x, tetra-*n*-butylammonium fluoride, THF; xi, $Pd(OH)_2$-C, ammonium formate, EtOH/AcOH; xii, $NaNO_2$, DMSO; xiii, tetra-*n*-butylammonium fluoride, THF; xiv, H_2, $Pd(OH)_2$-C, MeOH. BOM= benzyloxymethyl, *i*-Bu= *iso*-butyl.

Synthesis of light-responsive 2',4'-BNA^{COC} derivatives **203** (nucleoside monomer, Scheme 30) and **204** (oligonucleotide-containing **203**, Scheme 31) was also accomplished in our laboratory recently.[49] As shown in Scheme 30, two *O*-benzyl groups of **200** were removed by catalytic hydrogenolysis and the resulting diol was reprotected with a silyl group to give **201**. Deacetylation of **201** with aqueous methylamine produced diol **202**, which was treated with 6-nitroveratraldehyde in the presence of zinc chloride to give a bicyclic nucleoside, which was desilylated to afford the desired light-responsive 2',4'-BNA^{COC} monomer **203**. This monomer was then transformed into the phosphoramidite derivative and incorporated into oligonucleotides *via* a conventional phosphoramidite protocol. Photoirradiation converted the oligonucleotide containing this BNA residue (structure **204**, Scheme 31) into the unbridged nucleic acid derivative **205** with reduced target binding affinity. This derivative reacted with several nucleophiles such as ammonia or glutathione to produce 4'-*C*-hydroxymethyl RNA analogue **206**, which possessed high binding affinity to ssRNA.



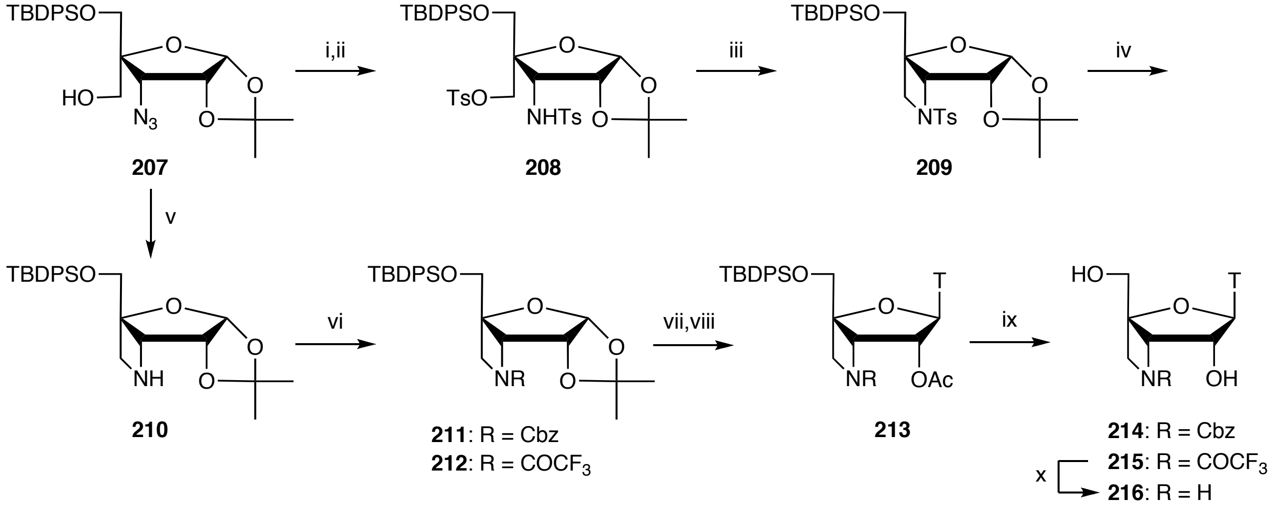
Scheme 30. *Reagents and conditions:* i, H_2, $Pd(OH)_2$-C, AcOEt; ii, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, imidazole, DMF; iii, $MeNH_2$ aq., THF; iv, 6-nitroveratraldehyde, $ZnCl_2$, hexafluoroisopropanol; v, tetra-*n*-butylammonium fluoride, THF. [Si]= 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl, T= thymin-1-yl.



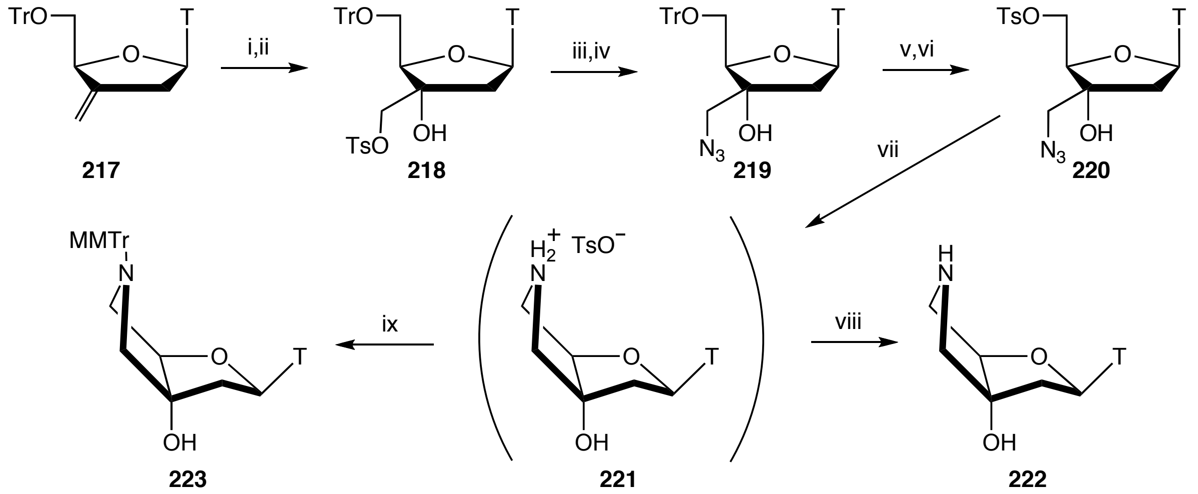
Scheme 31

6. BRIDGED NUCLEIC ACIDS WITH S-TYPE SUGAR CONFORMATION

Apart from the N-type BNAs described above, we also synthesized several BNAs with S-type sugar conformation. S-type sugar puckered BNAs are interesting candidates for application in post-genome technologies such as DNA microarrays and decoy nucleic acids. The first S-type BNA synthesized by us was 3',4'-BNA[52-55] which was synthesized prior to 2',4'-BNA and is described in the section 3. A slight modification of this BNA analogue, *i.e.*, replacing 3'-oxygen atom by a nitrogen, resulted in a sugar-fused azetidine ring containing the nucleic acid derivative, 3'-amino-3',4'-BNA.[56] The sugar conformation of this compound is thought to be S-type, due to constraints on the azetidine ring. The synthesis of 3'-amino-3',4'-BNA is shown in Scheme 32. The 3'-azido derivative **207** prepared from **85** was reduced by conventional hydrogenolysis and the resulting amino alcohol was treated with *p*-toluenesulfonyl chloride to give compound **208**. Ring-closure and detosylation of the cyclized product **209** produced **210**, which was also obtained from **207** in a single step using Staudinger's conditions.[100] After benzyloxycarbonylation or trifluoroacetylation (compounds **211** and **212**, respectively), thymine nucleobase was introduced (compound **213**) using standard procedures. Finally, one-pot deacetylation, followed by desilylation, furnished azetidine nucleosides **214** and **215**. Unsubstituted azetidine derivative **216** was prepared by removing the trifluoroacetyl group with conc. ammonia.

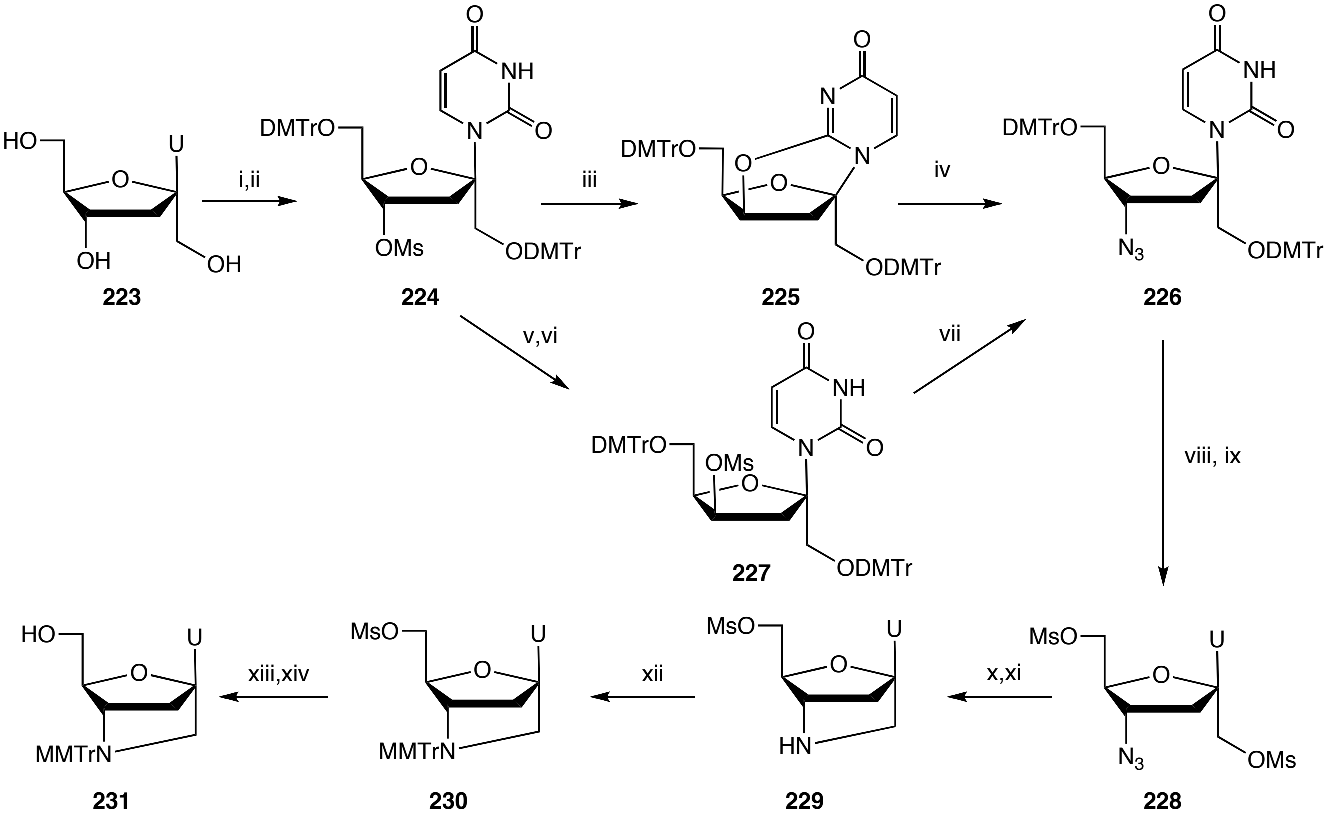


Scheme 32. *Reagents and conditions*: i, H_2, Pd-C, AcOEt; ii, TsCl, DMAP, Et_3N, CH_2Cl_2; iii, NaH, THF; iv, Na, amyl alcohol; v, Ph_3P, *o*-xylene; vi, benzyl chloroformate (for **211**) or $(CF_3CO)_2O$ (for **212**), DMAP, CH_2Cl_2; vii, Ac_2O, AcOH, H_2SO_4; viii, silylated thymine, TMSOTf, dichloroethane; ix, K_2CO_3, MeOH, then tetra-*n*-butylammonium fluoride, THF; x, NH_3 aq., dioxane. TBDPS= *tert*-butyldiphenylsilyl, T= thymin-1-yl, Cbz= benzyloxycarbonyl.



Scheme 33. *Reagents and conditions:* i, OsO_4, *N*-methylmorpholine *N*-oxide, pyridine, H_2O, *t*-BuOH, ii, TsCl, *n*-Bu_2SnO, Et_3N, CH_2Cl_2; iii, K_2CO_3, MeOH; iv, NaN_3, DMF; v, camphor sulfonic acid, CH_2Cl_2/MeOH; vi, TsCl, pyridine; vii, H_2, Pd-C, MeOH; viii, Et_3N, MeOH; ix, 4-monomethoxytrityl chloride, pyridine. T= thymin-1-yl, MMTr= 4-monomethoxytrityl.

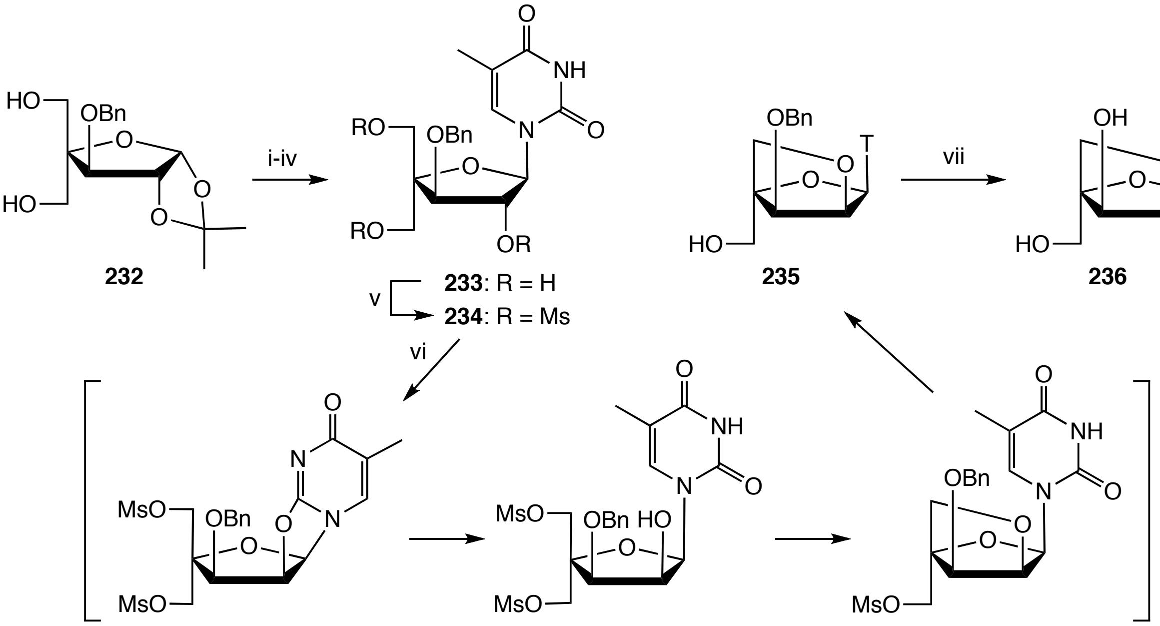
Another nitrogen containing BNA analogue with the S-type sugar conformation, 5'-amino-3',5'-BNA, was synthesized in our laboratory from **217** as depicted in Scheme 33.[57,58] Osmium tetraoxide-mediated stereoselective dihydroxylation followed by tosylation of the primary alcohol afforded **218**. Epoxidation of **218** and subsequent azidation provided **219**, whose C5' trityl (Tr) group was replaced with a tosyl group to give **220**. Reduction of the azido group by palladium on carbon under a H_2 atmosphere resulted in pyrrolidine ring formation to afford bicyclic nucleoside **221** as a salt, from which 5'-amino-3',5'-BNA monomer **222** was obtained by treating with Et_3N. Compound **221** can be converted without purification to the monomethoxytrityl (MMTr) derivative **223** for oligomerization.



Scheme 34. *Reagents and conditions:* i, 4,4'-dimethoxytrityl chloride, pyridine, CH_2Cl_2; ii, MsCl, pyridine; iii, NaH, DMF; iv, NaN_3, DMF; v, NaOH, dioxane; vi, MsCl, pyridine; vii, NaN_3, DMF; viii, AcOH; ix, MsCl, pyridine; x, H_2, Pd-C, MeOH; xi, DMF; xii, 4-monomethoxytrityl chloride, pyridine; xiii, NaOBz, DMF; xiv, NaOMe, MeOH. DMTr= 4,4'-dimethoxytrityl, MMTr= 4-monomethoxytrityl, U= uracil-1-yl.

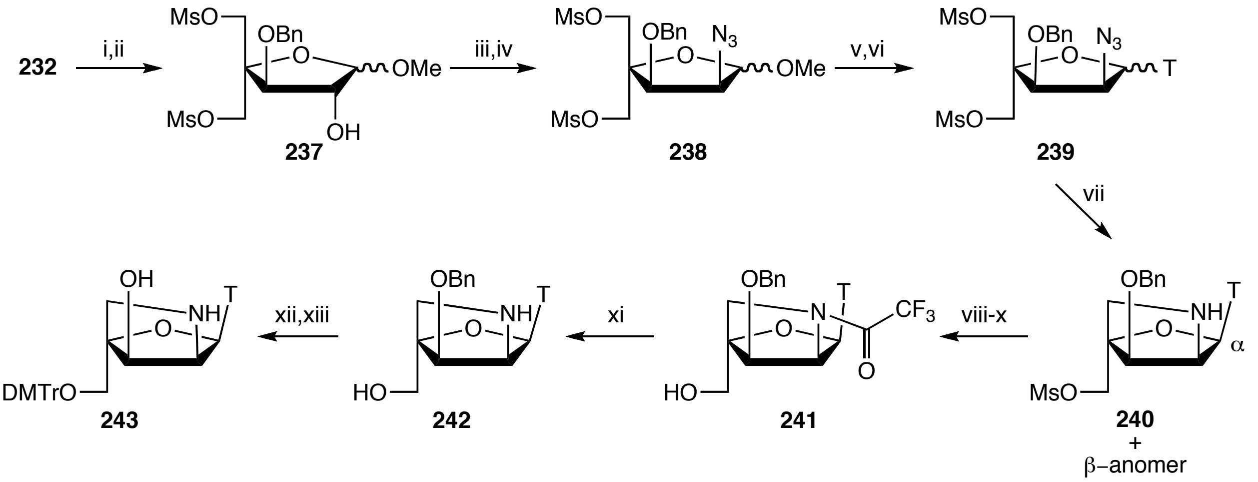
Wengel *et al*. reported a 3'-amino-1',3'-BNA analogue with S-type sugar pucker.[67] The synthesis strategy is shown in Scheme 34. Known nucleoside analogue **223** was transformed into **224** *via* tritylation and mesylation. Compound **224** was converted to the azido derivative **226** by two different procedures. Intramolecular nucleophilic attack to form 2,3'-anhydro product **225** followed by nucleophilic opening (conditions iii and iv, respectively) resulted in **226** in relatively poor yield. Alternatively, inversion of the C3' configuration (compound **227**) followed by nucleophilic substitution provided **226** in higher yield. Removal of the dimethoxytrityl (DMTr) groups and subsequent mesylation gave dimesylate **228**. Reduction of the azido group followed by cyclization afforded bicyclonucleoside **229,** which was tritylated and demesylated to furnish **231** *via* **230**.

The synthesis of α-L-ribo-configured BNA or LNA, α-L-LNA, was described by Wengel *et al*. (Scheme 35).[68] Thymine nucleoside derivative **233** was synthesized from the β-L-*threo*-pentofuranose **232** straightforwardly as described in another report by Wengel *et al*.[101] Mesylation of the hydroxyl groups and cyclization afforded 2'-*O*,5'-*C*-methylene linked bicyclofuranose derivative **235**. This conversion from **234** to **235** is a reaction cascade involving anhydro intermediate formation, hydrolysis to afford C2' inversion, intramolecular nucleophilic attack on the 5'-mesyloxy group, and removal of the remaining mesyloxy group. Debenzylation of **235** provided α-L-LNA monomer **236**.



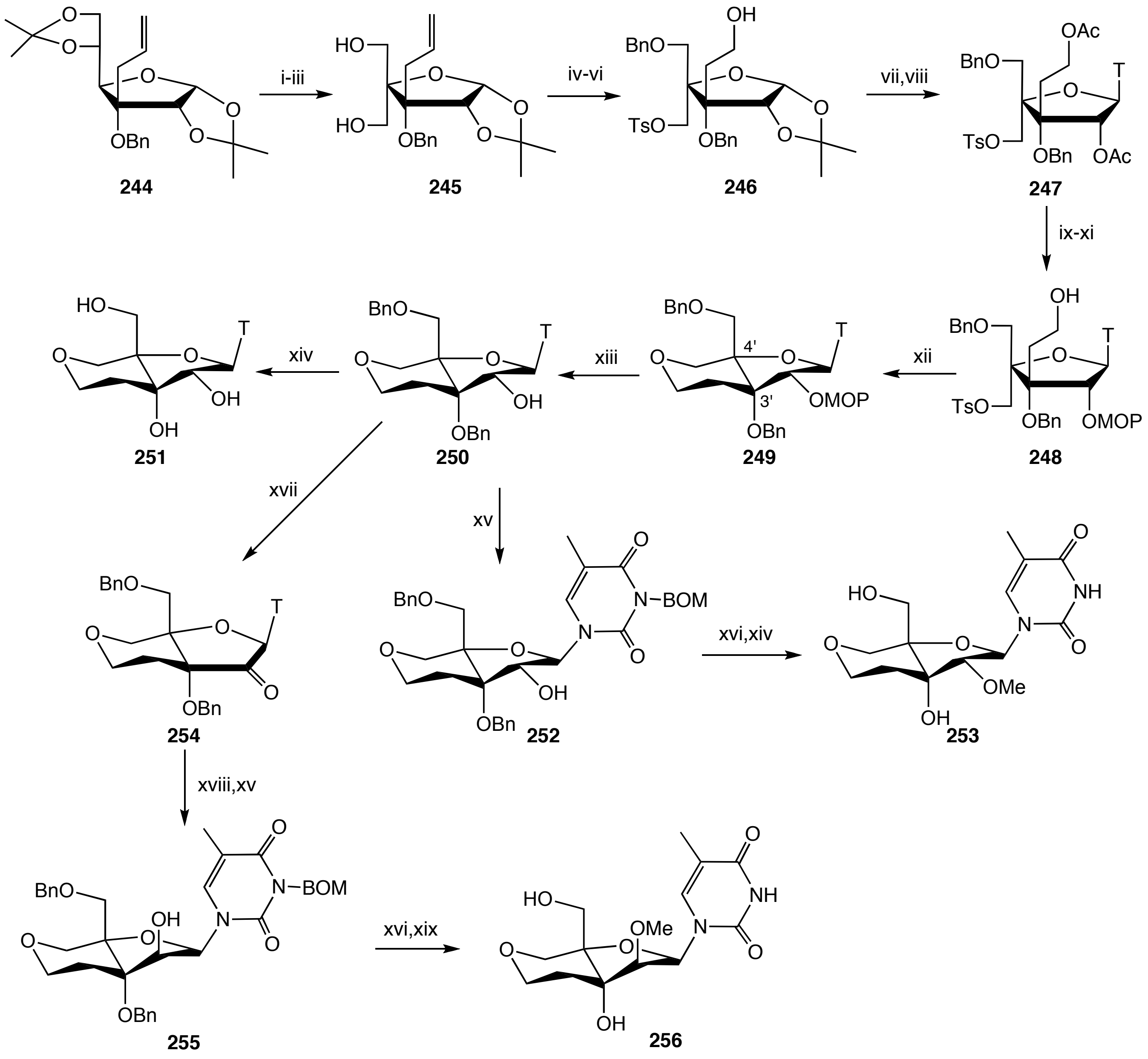
Scheme 35. *Reagents and conditions:* i, BzCl, pyridine; ii, AcOH, then Ac_2O, pyridine; iii, silylated thymine, TMSOTf, MeCN; iv, NaOMe, MeOH; v, MsCl, pyridine; vi, NaOH, $EtOH/H_2O$; vii) H_2, $Pd(OH)_2$-C, EtOH. T= thymin-1-yl.

2'-Amino-α-L-LNA, a 2'-amino congener of α-L-LNA, was also synthesized from the same starting material **232** as shown in Scheme 36.[69] Mesylation of **232** followed by treatment with acetyl chloride in methanol resulted in formation of an anomeric mixture of methyl furanoside **237**. Azidation of **237** was accomplished *via* activation of the 2'-OH group to 2'-triflate to give **238**. Introduction of thymine nucleobase was performed *via* acetolysis of **238** followed by treatment with silylated thymine in the presence of trimethylsilyl triflate. The anomeric mixture of azido nucleoside **239** was treated under Staudinger's conditions[100] to afford an easily separable bicyclic nucleoside **240**. The α-anomer of **240** was transformed to **241** *via* protection of the secondary amine with a trifluoroacetyl group and subsequent demesylation by nucleophilic substitution with potassium acetate followed by deacetylation. The trifluoroacetyl group of **241** was removed to produce amino alcohol **242**. Synthesis of **242** from **240** directly by demesylation was not achieved satisfactorily. Finally, tritylation and debenzylation afforded 2'-amino-α-L-LNA monomer **243**.



Scheme 36. *Reagents and conditions:* i, MsCl, pyridine; ii, AcCl, MeOH; iii, Tf_2O, pyridine, CH_2Cl_2; iv, NaN_3, 15-crown-5, DMF; v, Ac_2O, AcOH, H_2SO_4; vi, silylated thymine, TMSOTf, dichloroethane; vii, Me_3P, NaOH aq., THF; viii, $(CF_3CO)_2O$, pyridine, CH_2Cl_2; ix, KOAc, 18-crown-6, dioxane; x, NH_3, MeOH; xi, $NaBH_4$, EtOH; xii, 4,4'-dimethoxytrityl chloride, DMAP, pyridine; xiii, ammonium formate, $Pd(OH)_2$-C, AcOEt. T= thymin-1-yl, DMTr= 4,4'-dimethoxytrityl.

We also synthesized a number of *trans*-3',4'-BNA monomers[59,60] (compounds **251**, **253** and **256** in Scheme 37) from the known allyl derivative **244**.[102] Preparation of the hydroxymethyl derivative **245** was accomplished by a conventional three-step procedure using the aldol-Cannizzaro reaction. Selective benzylation and tosylation of the primary alcohols and oxidative cleavage of the olefin followed by reduction produced **246**. Compound **246** was also accessible from a corresponding vinyl derivative *via* hydroboration albeit in lower yield.[60] Incorporation of a thymine nucleobase into **246** according to Vorbruggen's conditions afforded nucleoside derivative **247**. Selective deacetylation of **247** followed by reprotection with a 2-methoxy-2-propyl (MOP) group and subsequent deprotection of the primary alcohol at the 3'-position furnished **248**, which was cyclized to form **249** with a *trans*-3',4'-BNA skeleton. Deprotection of the MOP protected hydroxyl group and debenzylation of the resulting **250** provided *trans*-fused 4,7-dioxabicyclo[4.3.0]nonane structure **251** or *trans*-3',4'-BNA monomer. For effective incorporation of this monomer into oligonucleotides, the 2'-OH group must be removed or suitably protected. Removal of the 2'-OH was found to be difficult; therefore, a small protecting group (methyl group) was introduced to synthesize a 2-methoxy-*trans*-3',4'-BNA analogue **253**. To accomplish this, thymine nucleobase was protected with a benzyloxymethyl (BOM) group (compound **252**), the 2'-OH group was methylated using a conventional procedure, and finally the BOM and benzyl groups were removed simultaneously to give **253**. ^{1}H-NMR and X-ray analysis revealed that **253** has a typical S-type sugar conformation. However, the *trans*-3,4-BNA analogue **253** could not be oligomerized since the corresponding phosphoramidite could not be synthesized.[60] This is attributed to the steric hindrance of the 2'-OMe group. Next, the arabino-type 2'-methoxy-*trans*-3,4'-BNA monomer **256** was synthesized by inverting the stereochemical configuration at the C2'-position. Oxidation of the 2'-OH to a keto group (compound **254**) and subsequent stereoselective reduction (compound **255**), followed by introduction of a

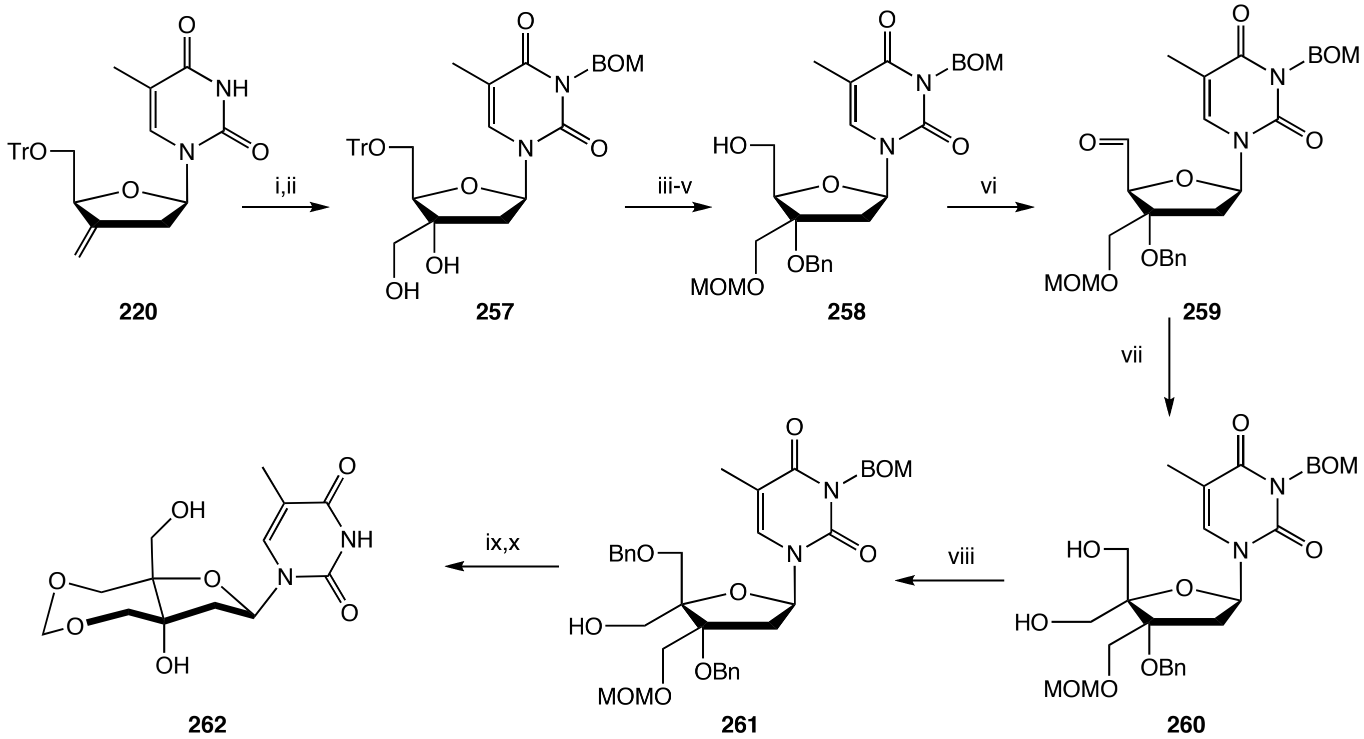


Scheme 37. *Reagents and conditions*: i, AcOH aq.; ii, $NaIO_4$,THF/H_2O; iii, HCHO aq., NaOH, THF/H_2O; iv, BnBr, NaH, DMF; v, TsCl, Et_3N, DMAP, CH_2Cl_2; vi, OsO_4, $NaIO_4$, then $NaBH_4$, THF/H_2O; vii, Ac_2O, AcOH, H_2SO_4; viii, silylated thymine, TMSOTf, 1,2-dichloroethane; ix, $MeNH_2$ aq. THF; x, 2-methoxypropane, TsOH, CH_2Cl_2; xi, NaOH aq. MeOH/THF; xii, sodium hexamethyldisilazide, THF; xiii, TsOH, MeOH/THF; xiv, $Pd(OH)_2$-C, cyclohexene, EtOH; xv, benzyl chloromethyl ether, DBU, DMF; xvi, MeI, NaH, DMF; xvii, Dess-Martin reagent, CH_2Cl_2; xviii, DIBAL-H, THF; xix, $Pd(OH)_2$-C, ammonium chloroformate, MeOH. T= thymin-1-yl, MOP= 2-methoxy-2-propyl, BOM= benzyloxymethyl.

methyl group and debenzylation, provided arabino-type *trans*-3’,4’-BNA analogue **256** which was successfully incorporated into oligonucleotides.

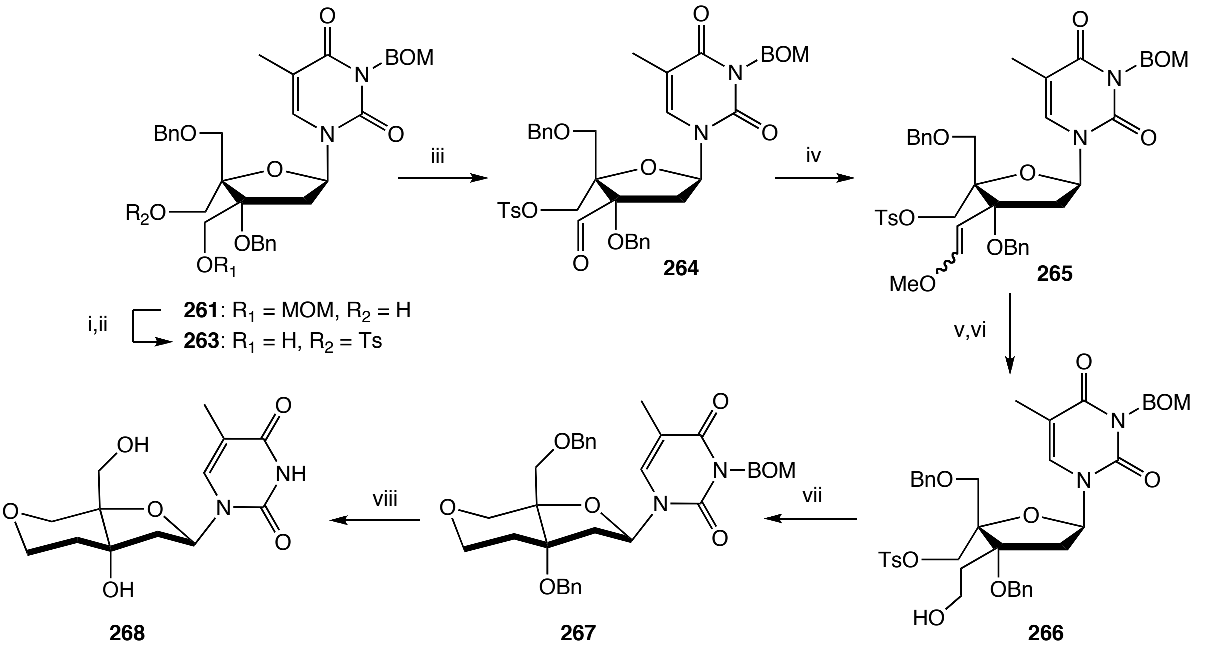
Trans-fused 3’,4’-BNA with a 2’-substituent has low reactivity for both phosphoramidite formation and oligomerization reactions. Moreover, incorporation of arabino-type 2’-methoxy-*trans*-3’,4’-BNA **256** into oligonucleotides was found to destabilize DNA and RNA duplexes. Therefore, 2’-deoxy-*trans*-3’,4’-BNAs were synthesized as shown in Schemes 38 and 39.[61,62,65] The first deoxy-type *trans*-3’,4’-BNA monomer synthesized (compound **262**) has a seven-membered ring with a methylene acetal fused at the

3'- and 4'-positions of the furanose ring (3,5,8-trioxabicyclo[5.3.0]decane structure).[61,62] As shown in Scheme 38, stereoselective oxidation of the olefin of **220** and BOM protection of the thymine produced **257**, which was transformed to **258** by protection-deprotection sequences. Oxidation of the primary hydroxyl group followed by aldol-Cannizzaro reaction gave **260** *via* **259**. Selective benzylation provided **260** which was cyclized under acidic conditions and finally debenzylated to furnish 2'-deoxy-*trans*-3',4'-BNA monomer **262**.



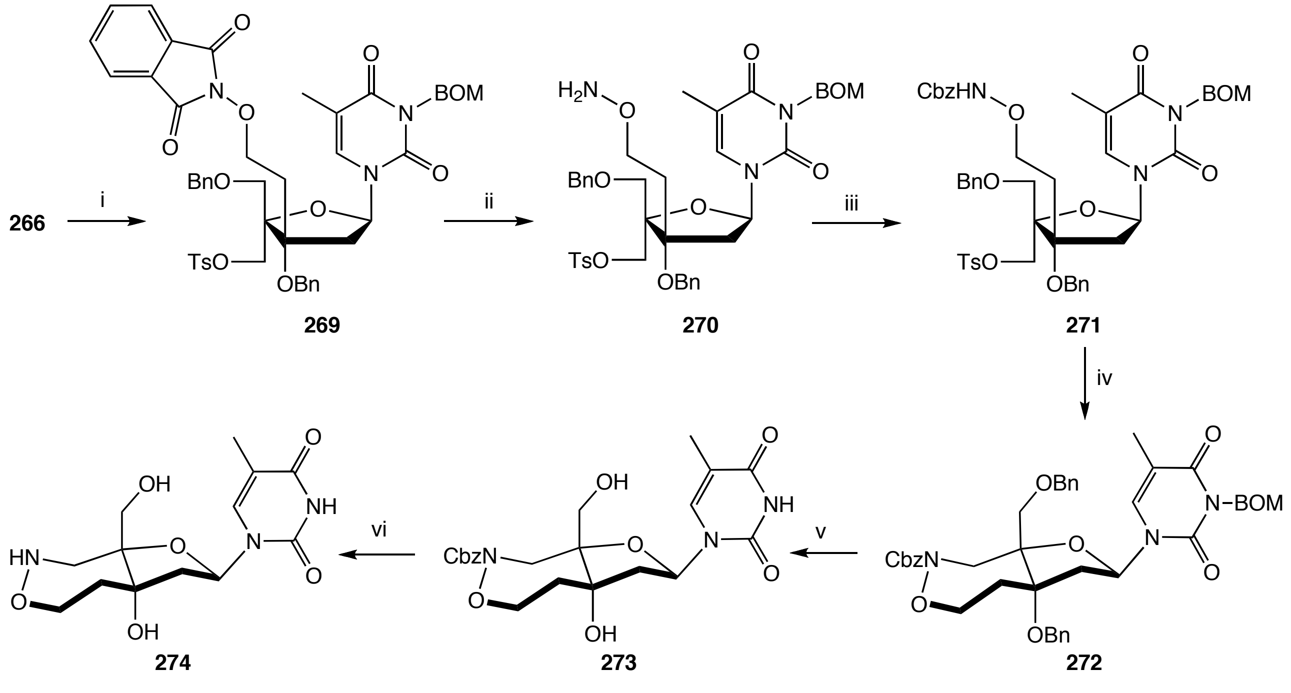
Scheme 38. *Reagents and conditions:* i, OsO_4, *N*-methylmorpholine *N*-oxide, pyridine, H_2O, *t*-BuOH; ii, benzyl chloromethyl ether, DBU, DMF; iii, chloromethyl methyl ether, diisopropylethylamine, pyridine/dichloroethane; iv, NaH, *n*-Bu_4NI, DMF; v, (+)-10-camphorsulfonic acid, CH_2Cl_2/MeOH; vi, Dess-Martin reagent, CH_2Cl_2; vii, HCHO aq., NaOH aq., THF then $NaBH_4$, THF; viii, BnBr, NaH, DMF; ix, TsOH, paraformaldehyde, 1,2-dichloroethane; x, ammonium formate, $Pd(OH)_2$-C, EtOH. BOM= benzyloxymethyl, MOM= methoxymethyl.

Another deoxy-type *trans*-3',4'-BNA with a six-membered fused ring having the 4,7-dioxabicyclo[4.3.0]nonane structure was also synthesized (Scheme 39).[62,65] Selective benzylation of **260** gave **261**, which was converted to **263** *via* tosylation and MOM deprotection. Oxidation of **263** followed by Wittig reaction of the resulting aldehyde **264** afforded enol ether **265**. Compound **265** was transformed to **266** by removal of the methyl group followed by reduction. Ring-closure and debenzylation provided the 2'-deoxy-*trans*-3',4'-BNA analogue **268** *via* **267**. The phosphoramidites of this deoxy analogue were recently synthesized and incorporated into oligonucleotides. This analogue showed pronounced increased thermal stability of the duplex, in contrast to the arabino-type 2'-methoxy-*trans*-3',4'-BNA analogue.[63]



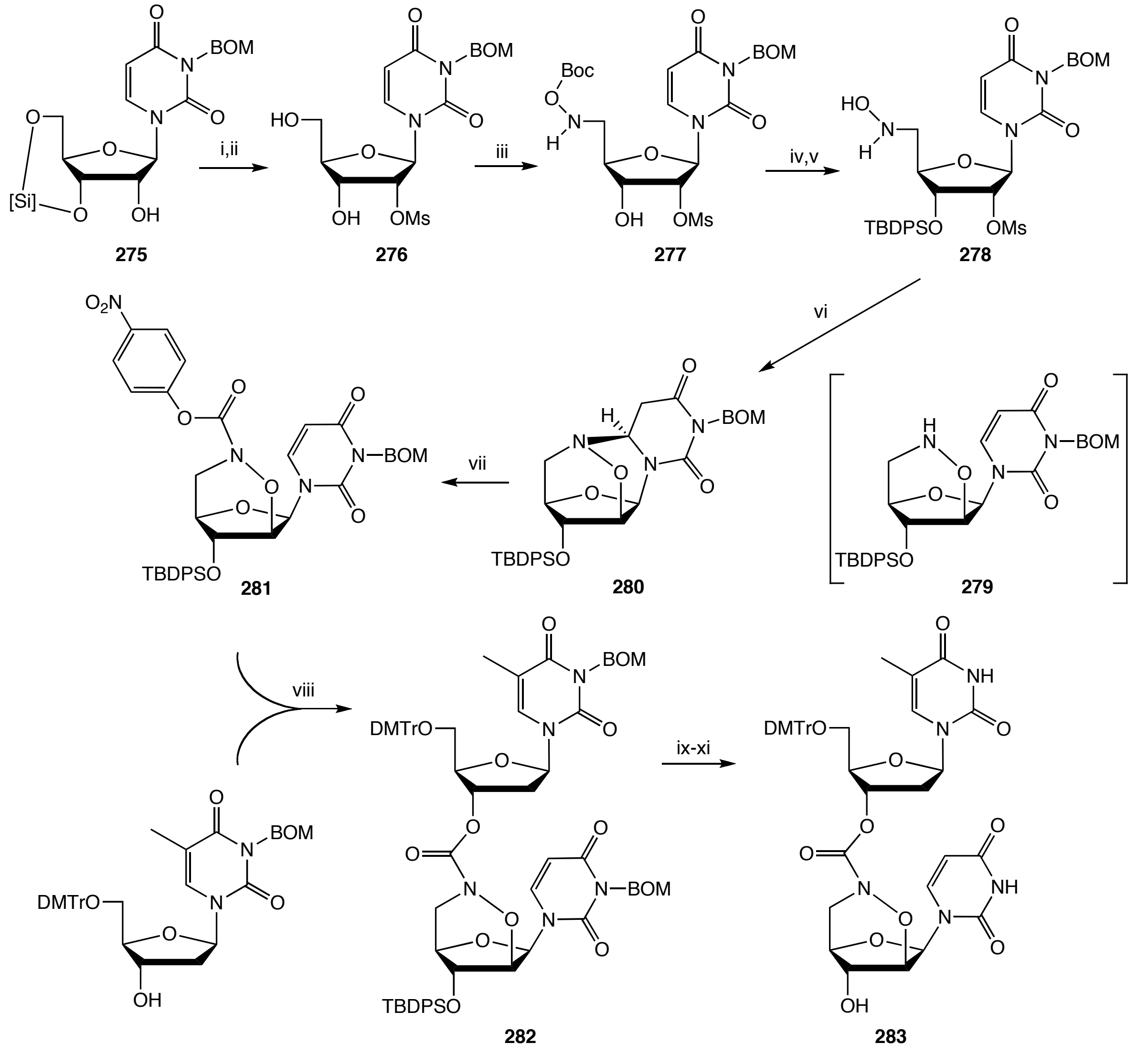
Scheme 39. *Reagents and conditions:* i, TsCl, Et_3N, DMAP, CH_2Cl_2; ii, TMSBr, CH_2Cl_2; iii, Dess-Martin reagent, CH_2Cl_2; iv, Ph_3P=CHOMe, THF; v, $Hg(OAc)_2$, *n*-Bu_4NI, THF/H_2O; vi, $NaBH_4$, THF/H_2O; vii, NaHMDS, THF; viii, ammonium formate, $Pd(OH)_2$-C, EtOH. MOM= methoxymethyl, BOM= benzyloxymethyl.

Recently, another 2'-deoxy-*trans*-3',4'-BNA monomer having a 4,8-dioxa-5-azabicyclo[5.3.0]decane skeleton was synthesized (Scheme 40).[64] Mitsunobu reaction with **266** using *N*-hydroxyphthalimide gave **269**, which was treated with hydrazine to deliver the aminoxy derivative **270**. Carbamation of the amino group of **270** followed by cyclization afforded bicycle nucleoside **272** *via* **271**. Removal of BOM produced **273**, debenzylation of which gave the *trans*-3',4'-BNA analogue **274**.



Scheme 40. *Reagents and conditions:* i, *N*-hydroxyphthalimide, Ph_3P, diethyl azodicarboxylate, THF; ii, hydrazine hydrate, CH_2Cl_2; iii, benzyl chloroformate, pyridine; iv, K_2CO_3, DMF; v, DDQ, CH_2Cl_2; vi, BCl_3, CH_2Cl_2. BOM= benzyloxymethyl, Cbz= benzyloxycarbonyl.

A uridine analogue of 2'-*O*,5'-*N* bridged nucleic acid (2',5'-BNA^{ON}) having S-type sugar pucker was synthesized using the known uridine derivative **275**.[66] Desilylation and mesylation of **275** produced mesylated diol **276**, whose primary hydroxyl group was substituted by *N*,*O*-di-Boc-hydroxylamine under Mitsunobu conditions to give **277**. Silyl protection and removal of the Boc groups afforded **278**. Cyclization of **278** to form the 2',5'-bridged structure failed to produce the desired product **279**, but rather resulted in the formation of tetracyclic structure **280**. Trying several experimental approaches resulted in the generation of 2',5'-BNA^{ON} structure **281** from this tetracyclic compound **280** was achieved by *N*-acylative β-elimination using *p*-nitrophenyl chloroformate. Compound **281** was coupled with 3'-*N*-benzyloxymethyl-5'-*O*-(4,4-dimethoxytrityl)thymidine to give the fully protected dimer **282**, which was transformed to dimer **283** containing the 2',5'-BNA^{ON} residue. This dimer after phosphitylation was incorporated into oligonucleotide.



Scheme 41. *Reagents and conditions:* i, MsCl, Et_3N, CH_2Cl_2; ii, tetra-*n*-butylammonium fluoride, THF; iii,Cbz-NH-O-Boc, Ph_3P, dibenzyl azodicarboxylate, THF; iv, *tert*-butyldiphenylsilyl chloride, imidazole, DMF; v, TFA, CH_2Cl_2; vi) potassium hexamethyldisilazide, THF; vii, *p*-nitrophenyl chloroformate, *n*-Bu_4NI, 2,6-di-*tert*-butyl-4-methylpyridine, *o*-xylene; viii, NaH, THF; ix, BCl_3, CH_2Cl_2; x, 4,4'-dimethoxytrityl chloride, pyridine; xi, tetra-*n*-butylammonium fluoride, THF. [Si]= 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl. Cbz= benzyloxycarbonyl, BOM= benzyloxymethyl, Boc= *tert*-butoxycarbonyl, TBDPS= *tert*-butyldiphenylsilyl, DMTr= 4,4'-dimethoxytrityl.

7. PROPERTIES OF BRIDGED NUCLEIC ACIDS

Restricting the conformation of a nucleic acid *via* bridging dramatically improves the properties of the nucleic acid in most cases. Hybridizing ability, sequence selectivity and nuclease resistance towards exo and endonucleases has been improved greatly by introducing BNA analogues. Hybridizing ability was monitored by UV melting temperature (T_m). Unprecedented increases in the T_m of oligonucleotides against complementary ssRNA, ssDNA and dsDNA was noticed by 2',4'-BNA modification which has N-type sugar conformation. Typical increases in T_m per modification (ΔT_m/modification) were found to range between +4 to +8 °C for complementary ssRNA and +2 to +5 °C for ssDNA.[82,92] The enzymatic stability of 2',4'-BNA modified oligonucleotides against snake venom phosphodiesterase (SVPDE) was markedly superior to that of natural oligonucleotides, as described previously.[25,82] Resistance to endonucleases in serum was also found to be superior.[103-105] The 3'-amino-2',4'-BNA analogue showed almost the same hybridizing ability as the 2',4'-BNA analogue towards complementary ssRNA and dsDNA, and affinity for ssDNA is also only slightly lower compare to that of 2',4'-BNA, indicating that it has slightly better RNA selectivity.[23,24] In addition, the nuclease resistance of 3'-amino-2',4'-BNA is much higher than that of 2',4'-BNA.[24] The mismatch discrimination of both nucleic acid derivatives is similar and generally superior to that of natural oligonucleotides. The 5'-amino-2',4'-BNA analogue also has a very high hybridizing profile to form duplexes and triplexes, and has remarkably high nuclease resistance.[25] This BNA analogue is cleavable under mild acidic conditions, allowing it to be utilized as a probe for sequence-specific dsDNA sensing.[95,106] The 2',4'-BNA analogue with an unnatural base such as 2-pyridone was found to form a stable triplex with a C•G or T•A base pair, where natural nucleobases were less effective.[27,28,31] Unnatural C-nucleosides possess universal recognition properties.[32,33] Although extensive hybridization studies with five-membered carbocyclic 2',4'-BNA analogues **122** and **133** developed by Chattopadhyaya *et al*. have not been done, a comparison with 2',4'-BNA reveals that the hybridizing ability to ssRNA is lower than that of 2',4'-BNA.[35] However, the affinity for DNA complements was similar or decreased compared to natural oligonucleotides.[35,36] Resistance to nucleolytic digestion was enhanced greatly by employing carbocyclic analogues.[36]

Among the six-membered BNA analogues, ENA showed similar or slightly lower duplex forming ability compared to 2',4'-BNA,[39] however, triplex formation was enhanced, especially with the fully modified TFO.[107] The nuclease resistance of this analogue was found to be superior to that of the 2',4'-BNA analogue.[40] The duplex-forming ability of aza-ENA was considerably higher than that of natural oligonucleotides,[41] but slightly lower than that of 2',4'-BNA.[35] Triplex formation was not investigated. Aza-ENA modified oligonucleotides showed improved nuclease resistance in denatured PAGE experiments, and were also reported to have superior serum stability compared to 2',4'-BNA.[41] The six-membered BNA analogue 2',4'-BNA^{NC}, developed by us, showed extraordinarily high duplex- and triplex-forming ability.[43-46] The duplex-forming ability of 2',4'-BNA^{NC} is slightly higher than that of 2',4'-BNA, and its triplex-forming ability is enhanced compared to 2',4'-BNA.[43] The sterically hindered 2',4'-BNA^{NC}[N-Me] analogue showed high binding affinity comparable to 2',4'-BNA and with better RNA-selectivity.[44] Fully modified TFO, or TFO composed of consecutive 2',4'-BNA^{NC} analogues, showed remarkable enhancement of target affinity compared to 2',4'-BNA.[44,46] Resistance to nucleolytic degradation was also much higher than that of 2',4'-BNA. The BNA analogue **163** developed by Wang *et al*. showed moderate increase in T_m value.[38] Carbocyclic BNA analogues **172** and **173** developed by Nielsen showed an increase in T_m from +2.3 to +4.5°C per modification against ssRNA.[42] This increase in T_m was lower than that of the 2',4'-BNA analogue using the same sequence. The T_{m} value towards ssDNA was decreased, implying that the carbocyclic analogue is RNA selective.[35,36,42]

PrNA with a seven-membered bridge structure showed decreased hybridizing ability to both ssRNA and ssDNA,[40] although nuclease resistance was very high. On the other hand, 2',4'-BNA^{COC} developed by us exhibited excellent RNA-selective hybridizing ability.[47,48] Fully modified 2',4'-BNA^{COC} duplex was found to possess extraordinarily high thermal stability.[48] The properties of N-type BNAs developed by our group are summarized in Table 1.

Table 1. Hybridizing ability and enzymatic stability of N-type BNAs developed by our group

BNA analogue	hybridizing ability			enzymatic stability	reference
	ssDNA	ssRNA	dsDNA		
DNA	±	±	±	±	–
2',4'-BNA	+++	+++	+++	+	12,82
3'-amino-2',4'-BNA	++	+++	+++	+++	23,31
5'-amino-2',4'-BNA	+++	++	++	++	25
ENA	±	++	+++	++	39,107
PrNA	–	±	ND*	+++	40
2',4'-BNA^{COC}	–	+	+	+++	47,48
2',4'-BNA^{NC}	+	+++	+++	+++	43-46

* ND denotes 'not determined'.

5-Triphosphate analogues of 2',4'-BNA and its derivatives, such as 2',4'-BNA^{NC} and 2',4'-BNA^{COC}, were prepared and enzymatically incorporated into nucleic acids or oligonucleotides.[18,108-110] Although further evaluation is still needed, these results would be highly useful for development of a variety of biological tools, such as aptamer.[108]

Among the S-type BNAs, 3',4'-BNA showed slightly decreased affinity for ssRNA and a considerable decrease in T_{m} against DNA.[82] The 1',3'-BNA analogue reported by Wengel showed a large decrease in duplex stability[67] whereas α-L-LNA improved the thermal stability of duplexes significantly.[68]

5’-Amino-3’,5’-BNA possessed excellent binding affinity towards ssRNA and ssDNA targets and showed high nuclease resistance profiles. While 5’-amino-DNA had greatly decreased target binding affinity, 5’-amino-3’,5’-BNA showed significantly increased target affinity.[57] The increased target affinity of the 5’-amino-3’,5’-BNA analogue is attributed to optimization of the γ dihedral angle *via* bridging. The *trans*-3’,4’-BNA analogue with a 2’-*O*-methyl substituent displayed lower binding affinity towards ssRNA and ssDNA complements, possibly due to steric repulsion exerted by the methyl group.[60] On the other hand, 2’-deoxy-type *trans*-3’,4’-BNA analogues showed increased binding affinity towards DNA and RNA complements.[63] Lastly, 2’,5’-BNA^{ON} was found to significantly decrease the thermal stability of duplexes.[66]

8. ACKNOWLEDGEMENTS

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