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Synthesis of a Novel Conformationally Locked Carbocyclic Nucleoside Ring System

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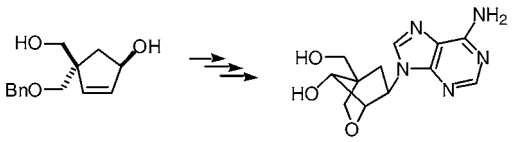
ABSTRACT



A fast and efficient synthetic route to novel Northern locked carbocyclic nucleosides (as precursors of carbocyclic locked nucleic acids or cLNAs) is described. The target nucleoside with a oxabicyclo[2.2.1]heptane ring system was prepared from a simple starting material, diethyl malonate. Ring closure by intramolecular O-alkylation provided the target ring system as the major isomer over the [3.2.0] oxetane system. The adenine moiety was introduced through a reactive triflate after inversion of the stereochemistry of the corresponding alcohol by oxidation and reduction.

In general, the ribose rings of nucleosides and nucleotides may adopt a range of conformations; this has been described as a pseudorotational cycle.[1] The Northern ((N), 2′-*exo*, 3′-*endo*) and Southern ((S), 2′-*endo*, 3′-*exo*) conformations are the most relevant to the biological activities observed for nucleosides and nucleotides in association with DNA, RNA, and various enzymes.[1−6]

In studies of the ribose ring conformation while searching for improved ligands for binding to G protein-coupled receptors, we synthesized ring-constrained carbocyclic-type analogues.[2,3] A methanocarba approach introduced by Marquez and co-workers[4,5] was used to constrain the pseudosugar (cyclopentane) ring of the nucleoside to either an (N) or (S) conformation. In this approach, a cyclopropane moiety is fused to the cyclopentane ring at one of two positions. Such analogues helped to define the role of sugar puckering in stabilizing the active receptor-bound conformation and thereby allowed identification of a favored isomer, although the possible influence of the cyclopropyl methylene group on bioactivity distinct from the conformational effect has been discussed.[2] A preference for the (N) conformation of ribose both at the adenosine A_3 receptor and at the $P2Y_1$ nucleotide receptor was defined by using methanocarba analogues.[2,3] For example, the bisphosphate derivative **1**, MRS 2279, which is locked in a (N) conformation by the bicyclo[3.1.0]hexane ring system, is the most potent known antagonist of the $P2Y_1$ receptor.[6] Subsequently, we discovered that $P2Y_2$, $P2Y_4$, and $P2Y_{11}$ receptors can recognize (N)-methanocarba nucleotide triphosphates **2** and **3** roughly as potently as the corresponding ribosides.[3] However, in the case of $P2Y_6$ receptors, the (N)-methanocarba equivalent **4**

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(1) Altona, C.; Sundaralingham, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205.
(2) Jacobson, K. A.; Ji, X.-d.; Li, A. H.; Melman, N.; Siddiqi, M. A.; Shin, K. J.; Marquez, V. E.; Ravi, R. G. *J. Med. Chem.* **2000**, *43*, 2196.
(3) Kim, H. S.; Ravi, R. G.; Marquez, V. E.; Maddileti, S.; Wihlborg, A.-K.; Erlinge, D.; Malmsjö, M.; Harden, T. K.; Boyer, J. L.; Jacobson, K. A. *J. Med. Chem.* **2002**, *45*, 208.
(4) Marquez, V. E.; Siddiqui, M. A.; Ezzitouni, A.; Russ, P.; Wang, J.; Wagner, R. W.; Matteucci, M. D. *J. Med. Chem.* **1996**, *39*, 3739.
(5) Shin, K. J.; Moon, H. R.; George C.; Marquez V. E. *J. Org. Chem.* **2000**, *65*, 2172.
(6) Boyer, J. L.; Adams, M.; Ravi, R. G.; Jacobson, K. A.; Harden, T. K. *Br. J. Pharmacol.* **2002**, *135*, 2004.

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of the natural nucleotide activator, UDP, was inactive.[3] This prompted us to search for other (N)-like ring systems, which may adopt a slightly different conformation according to the pseudorotational cycle (Figure 1).

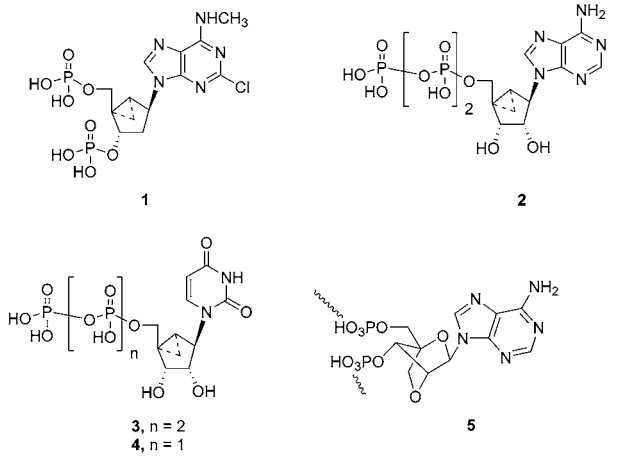


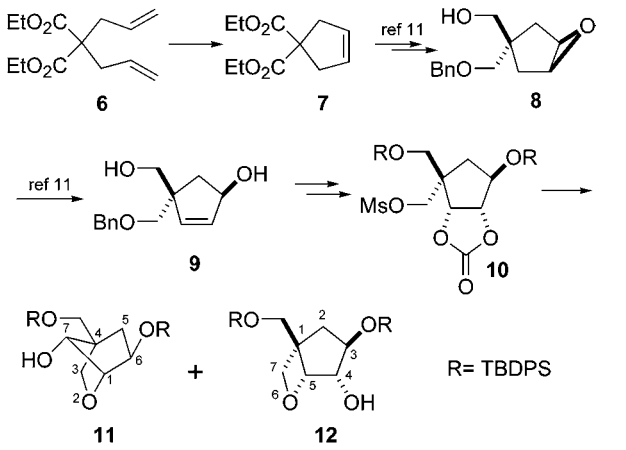
Figure 1.

Following their introduction several years ago, the locked nucleic acids **5** (LNAs)[7−9] have demonstrated excellent mismatch discrimination toward complementary RNA and have been shown to form more thermally stable complexes. The derivatization of LNAs is fully compatible with conventional DNA/RNA chemistry. Several synthetic routes to ring oxygen-containing LNAs were developed.[7−9]

Here we present the first synthetic route to nucleoside monomers of carbocyclic LNAs (cLNAs), in which the ring oxygen is replaced with a methylene group. As shown in the synthetic Scheme 2, the coupling of pseudosugar and base moieties could be accomplished at a late stage within this route. This would permit a common route to lead efficiently to the preparation of many nucleosides and nucleotides. Also, cLNAs would be more stable than oxygen LNAs because of their nonglycosidic nature, as has been discussed for methanocarba ribose in comparison with natural ribose.[2] These potential advantages encouraged us to devise a synthetic route to novel cLNA monomers of the (N) conformation. We anticipate that this synthesis will be applicable to nucleoside and nucleotide chemistry, leading to the development of new G protein-coupled receptor ligands, new antisense oligonucleotide variations, and other RNA targeting strategies.

Initially, our primary interest was the formation of the bicyclo[2.2.1]heptane system. As shown in Scheme 1, the

Scheme 1



key starting material, diol **9**, was prepared according to a ring closure reaction with Grubbs catalyst[10] and a known procedure[11] from a commercially available starting material, diethyl diallyl malonate **6**. During the process, ring opening of a *meso*-epoxide **8** by the chiral base (1*S*,2*R*)-norephedrine provided an allyl alcohol **9** containing a chiral quaternary carbon, which is usually elusive synthetically. Protection of diol **9** with the *tert*-butyldiphenylsilyl group and successive treatment with sequential dihydroxylation[12] and debenzylation gave an intermediate triol, which was converted into mesyl carbonate **10** by sequential treatment with triphosgene and mesyl chloride. Deprotection of the cyclic carbonate group and intramolecular O-alkylation could be accomplished in one pot in basic conditions (K_2CO_3 in methanol), yielding two isomers **11**and **12** in a ratio of 2:1, which were separated by column chromatography.

At the stage of isolation of the two isomers, 1D and 2D proton NMR and NOE spectra were collected for **11** and **12**, and all protons were carefully analyzed and assigned.[13] In the spectrum of isomer **12**, there was axial-axial coupling between H-3 and H-4 and between H-3 and the axial proton of two H-2s, which was consistent with isomer **12** being in

(7) (a) Obika, S.; Nanbu, D.; Fari, Y.; Morio, K.-I.; In, Y.; Ishida, T.; Imanishi, T. *Tetrahedron Lett.* **1997**, *38*, 8735. (b) Obika, S.; Hari, Yoshiyuki; Morio, K.-I.; Imanishi, T. *Tetrahedron Lett.* **2000**, *41*, 215.

(8) (a) Petersen, M.; Bondensgaard, K.; Wengel, J.; Jacobsen, J. P. *J. Am. Chem. Soc.* **2002**, *124*, 5974. (b) Petersen, M.; Wengel, J. *Trends Biotechnol.* **2003**, *21*, 74.

(9) Koshkin, A. A.; Fensholdt, J.; Pfundheller, H. M.; Lomholt, C. *J. Org. Chem.* **2001**, *66*, 8504.

(10) (a) Nguyen, S. T.; Johnson, L. K.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 3974. (b) Grubbs, R. H.; Miller, S. J. *Acc. Chem. Res.* **1995**, *28*, 446. (c) Schmalz, H.-G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1833. (d) Grubbs, R. H.; Chang, S. *Tetrahedron* **1998**, *54*, 4413.

(11) Hodgson, D. M.; Gibbs, A. R.; Drew, M. G. B. *J. Chem. Soc., Perkin Trans. 1* **1999**, *24*, 3579.

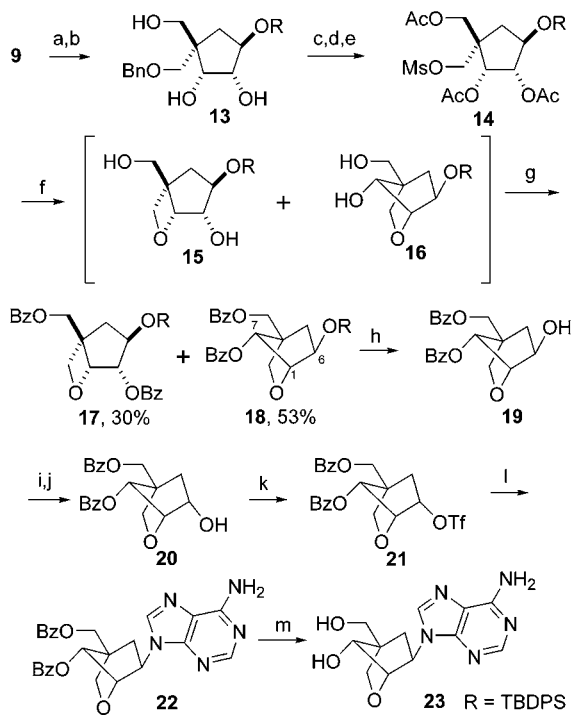
(12) (a) Alex, G.; Kruger, A. W.; Meyers, A. I. *Tetrahedron Lett.* **2001**, *42*, 4305. (b) Arrington, M. P.; Meyers, A. I. *Chem. Commun.* **1999**, *15*, 1371.

(13) **Data for 11**: ^{1}H NMR (CD_3OD) δ 7.34−7.71 (m, 20H, aromatic protons on TBDPS groups), 4.36 (s, 1H, H-7), 3.86 (d, 1H, J = 10.4 Hz, one of CH_2-OTBDPS), 3.85 (ddd, 1H, J = 1.7, 2.8, 7.7 Hz, H-6), 3.72 (d, 1H, J = 1.7 Hz, H-1), 3.61 (d, 1H, J = 10.4 Hz, one of CH_2-OTBDPS), 3.61 (dd, 1H, J = 2.8, 6.3 Hz, one of two H-3s), 3.18 (d, 1H, J = 6.3 Hz, one of two H-3s), 1.84 (ddd, 1H, J = 2.7, 2.8, 13.2 Hz, one of two H-5s), 1.66 (dd, 1H, J = 7.7, 13.2 Hz, onf of two H-5s), 1.06 (s, 18H, *tert*-butyl of TBDPS). **Data for 12**: ^{1}H NMR (CD_3OD) δ 7.26−7.83 (m, 20H, aromatic protons on TBDPS groups), 4.66 (ddd, 1H, J = 6.6, 8.0, 9.9 Hz, H-3), 4.60 (d, 1H, J = 4.1 Hz, H-5), 4.41 (dd, 1H, J = 1.1, 6.0 Hz, one of two H-7s), 3.79 (dd, 1H, J = 4.1, 8.0 Hz, H-4), 3.76 (d, 1H, J = 6.0 Hz, one of two H-7s), 3.71 (d, 1H, J = 10.4 Hz, one of CH_2-OTBDPS), 3.55 (d, 1H, J = 10.4 Hz, CH_2-OTBDPS), 1.62 (ddd, 1H, J = 1.1, 9.9, 12.9 Hz, one of two H-2s), 1.44 (dd, 1H, J = 6.9, 12.9 Hz, one of two H-2s), 1.09 (s, 9H, *t*-Bu on TBDPS), 1.05 (s, 9H, *t*-Bu on TBDPS).

the (S) conformation.[14] In contrast, in the spectrum of the desired oxabicyclo[2.2.1]heptane system **11**, H-7 (pseudoaxial) was found as singlet, and H-1 (pseudoequatorial) and H-6 (pseudoequatorial) showed two small coupling constants ($J_{1,6}$ = 1.7 Hz, $J_{5,6}$ = 2.8 Hz). These observations are consistent with the assigned (N) conformation of isomer **11**. Also in the NOESY spectra of **11** and **12**, NOE was detected between H-1 and H-6 in **11** but there was no NOE between H-3 and H-4 in **12**. This also supported a (S) conformation of **12**, in which H-3 and H-4 would be pseudoaxial. We also noted that following the acetylation of **11** and **12**, H-7 in **11** and H-4 in **12** were shifted downfield by ~1 ppm,[15] which further supported our conformational analysis and proton assignment.

To obtain the adenine adduct of the oxabicyclo[2.2.1]-heptane system, an alternate protection scheme was employed (Scheme 2). Selective protection[16] of the allylic alcohol **9** by treatment with *tert*-butyldiphenylsilyl chloride and a catalytic amount of 4-(dimethylamino)pyridine and sequential dihydroxylation with OsO_4 at 0 °C gave triol **13** as an oil. Triacetylation with acetic anhydride and debenzylation followed by mesylation of the primary alcohol afforded the triacetyl mesylate **14**, which was a desired substrate for formation of the bicyclo[2.2.1]heptane ring system. The acetyl group was selected for alcohol protection because of a low yield in the formation of the cyclic carbonate using triphosgene (i.e., formation of **10** in Scheme 1) and a side reaction upon debenzylation with cyclic carbonate. The debenzylation step was sluggish, and a side reaction of desilylation was unavoidable with Pd/C; however, Pd black in formic acid[17] at 75 °C provided the optimal yield.

Scheme 2 [a]



[a] Reaction conditions: (a) 1 equiv of TBDPSCl, TEA, cat. DMAP, CH_2Cl_2, 0 °C, overnight, 76%. (b) cat. OsO_4, NMO, acetone/water (15/1), 0 °C, overnight, 94%. (c) Ac_2O, TEA, cat. DMAP, CH_2Cl_2, 5 °C, overnight, 95%. (d) Pd Black, HCO_2H, MeOH, 75 °C, 4 h, 87%. (e) MsCl, TEA, CH_2Cl_2, 0 °C, 30 min. (f) K_2CO_3, MeOH, 0 °C, overnight, and then rt, 1 h, 89% two steps yield. (g) BzCl, TEA, CH_2Cl_2, 0 °C, overnight, and then separation of **17** and **18**. (h) TBAF, THF, 10 °C, 2 h, 83%. (i) PDC, CH_2Cl_2, reflux, 4 h. (j) $NaBH_4$, EtOH, −20 °C, 1 h and then 0 °C, 1 h, 87% two steps yield. (k) Tf_2O, DMAP, CH_2Cl_2, 0 °C, 5 min, 83%. (l) adenine, K_2CO_3, 18-Crown ether-6, DMF, 40−45 °C, 6 h, 76%. (qm) K_2CO_3, MeOH, rt, 4 h, 91%.

The oxabicyclo[2.2.1]heptane system was constructed by simultaneous deacetylation and intramolecular O-alkylation of **14**. The ring-closure step resulted in a mixture of two isomers containing the [3.2.0] oxetane ring system **15** and oxabicyclo[2.2.1]heptane **16**, similar to Scheme 1. The structural analysis of the mixture of two isomers was based on NMR chemical shifts and a unique coupling pattern observed in the spectra of **11** and **12** in Scheme 1. The ratio obtained was ~1.76:1 in favor of the desired oxabicyclo-[2.2.1]heptane system **16** and was dependent on the reaction temperature. Although a low temperature increased the fraction of the major isomer **16** over the minor oxetane **15**, the reaction did not reach completion at 0 °C. Therefore, the reaction was carried out at room temperature to achieve completion. We hope to optimize this route for exclusive formation of the major isomer, and we noted a possible lead for this improvement.[7a]

Benzoylation of a mixture of two isomers, **15** and **16**, made separation possible and gave **17** and **18**. The ratio of isolated isomers indicated in Scheme 2 was consistent with that calculated by ^{1}H NMR at the stage of a mixture of **15** and **16**. The major benzoylated isomer **18** was deprotected by treatment with TBAF in THF to yield dibenzoyl alcohol **19**.[18] Inversion of the C-6 stereochemistry was accomplished successfully in two steps from **19** (oxidation with PDC, reduction with $NaBH_4$). The resulting inverted alcohol **20** was converted to a triflate **21** by treatment with triflic anhydride and DMAP. Although mesylate was initially chosen as the leaving group instead of triflate, the reactivity of mesylate was insufficient for the substitution reaction. We surmised that this was mainly because of steric hindrance to attack by a nucleophile, due to the proximal quaternary carbon and the unique ring system. This problem was overcome by the introduction of a more reactive leaving

(14) Obike, S.; Morio, K.-I.; Nanbu, D.; Hari, Y.; Itoh, H.; Imanishi. T. *Tetrahedron* **2002**, *58*, 3039.

(15) By acetylation of **11** and **12**, H-7 in **11** was shifted downfield by 0.97 ppm while H-4 in **12** shifted downfield by 0.99 ppm. For comparison, the NMR spectra were obtained in the same deuterated solvent, CD_3OD.

(16) The secondary alcohol was selectively protected over neopentyl alcohol, which was confirmed by acetylation of the silylated compound. Chemical shift ($CDCl_3$) of methylene group was changed from 3.32 to 4.17 ppm after acetylation.

(17) Elamine, B.; Anatharomaiah, G. M.; Royer, G. P.; Means, G. E. *J. Org. Chem.* **1979**, *44*, 3442.

(18) The proton NMR spectrum for **19** after desilylation showed all protons separated and gave a better splitting pattern, which made interpretation easier than **18**. ^{1}H NMR ($CDCl_3$) δ 7.34−8.08 (m, 10H), 5.46 (s, 1H), 4.58 (one of AB quartet, 1H, J = 11.5 Hz), 4.53 (one of AB quartet, 1H, J = 11.8 Hz), 4.26 (s, 1H), 4.09−4.19 (m, 2H), 3.68 (d, 1H, J = 7.1 Hz), 2.26 (dd, 1H, J = 7.9, 13.4 Hz), 1.81 (ddd, 1H, J = 2.8, 3.0, 13.5 Hz).

group, triflate, which would be a better leaving group and might add S_N1 character to the substitution reaction. Substitution of triflate **21** by adenine proceeded smoothly at 40 °C to give dibenzoyl cLNA **22**. The benzoyl groups were then removed with potassium carbonate to give the target molecule, a novel locked carbocyclic nucleoside **23** (as precursor to cLNAs). In the spectrum of **20**, **21**, and the dibenzoyl cLNA monomer **22**, one instance of characteristic W-type long-range coupling for each compound was detected. In addition to a unique long-range coupling pattern, two coupling constants of $J_{1,6}$ and $J_{1,7}$ were zero in the spectrum of dibenzoyl cLNA monomer **22**. As mentioned before, it is thought that the relationship between H-1 and H-6 is equatorial-equatorial and that between H-1 and H-7 is equatorial-axial, which indicated an (N)-like conformation of the oxabicyclo[2.2.1] ring system.

Generally, the formation of carbocyclic nucleosides requires more synthetic steps than does the preparation of the corresponding natural nucleosides. The synthetic route we describe here would actually decrease the number of synthetic steps when applied to the synthesis of many nucleoside and nucleotide derivatives. This is primarily because the carbocyclic ribose equivalent can be coupled with the base at a late stage in the synthetic pathway. This efficiency, derived from the ability to prepare a large quantity of the triflate substrate as a common intermediate, is an important advantage in synthetic nucleoside chemistry. Development of a more efficient synthetic route is under way in our laboratory.

In conclusion, we have introduced a novel carbocyclic nucleoside ring system, which promises to have broad biological interest due to its defined conformational properties and would complement studies of LNAs.

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Supporting Information Available: Experimental procedures and spectroscopic data for compounds **13**−**23**. This material is available free of charge via the Internet at http://pubs.acs.org.

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