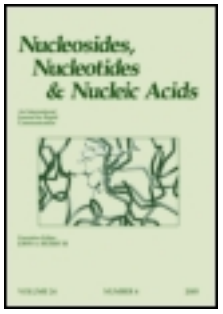

Nucleosides, Nucleotides and Nucleic Acids

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BICYCLIC NUCLEOSIDE SYNTHESIS—A PHOTOCHEMICAL APPROACH

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□ *The synthesis of a series of bicyclic nucleosides using photolytic ring-expansion of cyclobutanones is reported. The cyclobutanone precursors were prepared by [2+2] cycloaddition of a series of cyclic alkenes with chlorinated ketenes, derived from dichloro- and trichloroacetyl chloride. The synthesis of the nucleosides was achieved through photolysis of cyclobutanone precursors with 6-chloropurine by UV irradiation. The generality of this method was investigated and the absolute stereochemistry was assigned by NMR spectroscopy. The photoproducts demonstrated a marked preference for the 2′-exo conformation.*

Keywords Bicyclic nucleosides; synthesis; photolysis; ring-expansion

INTRODUCTION

It has been found that structurally modified DNA and RNA analogues have a wide range of biological activity, including inhibition of protein synthesis.[1] Such modified nucleosides were found to be effective as antiviral agents and have been utilized in the development of drugs used to combat cancer.[2]

One form of modification of natural nucleosides involves restricting its conformational flexibility. Called "bicyclic," "rigid," or "locked" nucleosides, these compounds have become some of the most promising in the antisense approach to gene therapy.[3] Natural ribonucleosides and deoxyribonucleosides exist in two distinct puckered conformations—"North" and "South." Each of these conformations can also be called 3′-*endo* and 2′-*endo*, respectively (Figure 1).[4] The overall conformational flexibility of the nucleosides is of great importance in the complementary binding of nucleotides and their interactions with other molecules. A conformational study demonstrated that the protein Reverse Transcriptase cannot

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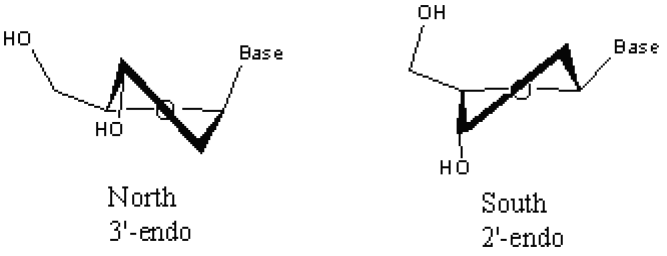


FIGURE 1 Conformations of ribonucleosides.

distinguish between the North (3′-*endo*) and South (2′-*endo*) type of DNA, but can distinguish between their complementary locked nucleosides, thus affecting this protein's function.

Certain rigid nucleosides can be frozen in one of two conformations. Due to the presence of the pseudoaxially arranged base at carbon-1′ and the steric interactions associated with it and the substituents at carbon-3′, it is expected that the 'North' (3′-*endo*) conformation would be less favorable than the "South" (2′-*endo*) conformation in DNA. Calculations performed on natural deoxyribonucleosides have confirmed this prediction.[3,6]

Different approaches to the synthesis of locked nucleosides have included fusion of a nitrogen-containing ring to the sugar[7] and fusion of rings to different sides of the sugar moiety.[8–10] Typically, the procedures for these syntheses begin with a glycone, followed by the formation of the fused ring through ring-closing metathesis of sugar substituents.[1,10] Our method starts with the fused ring to which a cyclobutanone is annealed. Cyclobutanones undergo photochemical ring expansion isomerization to an oxacarbene which can insert into purines at the N(9)-H bond, to give a photoadduct.[11] These ketones serve as glycosyl donors under relatively mild conditions.

The synthesis of each of these locked nucleosides involved two key reactions. The first is the [2+2] cycloaddition, generating substituted cyclobutanones, while the second is the photochemical isomerization of the ketone to give a furanose. This methodology has been previously developed[11] and forms the basis for the locked nucleoside preparation.

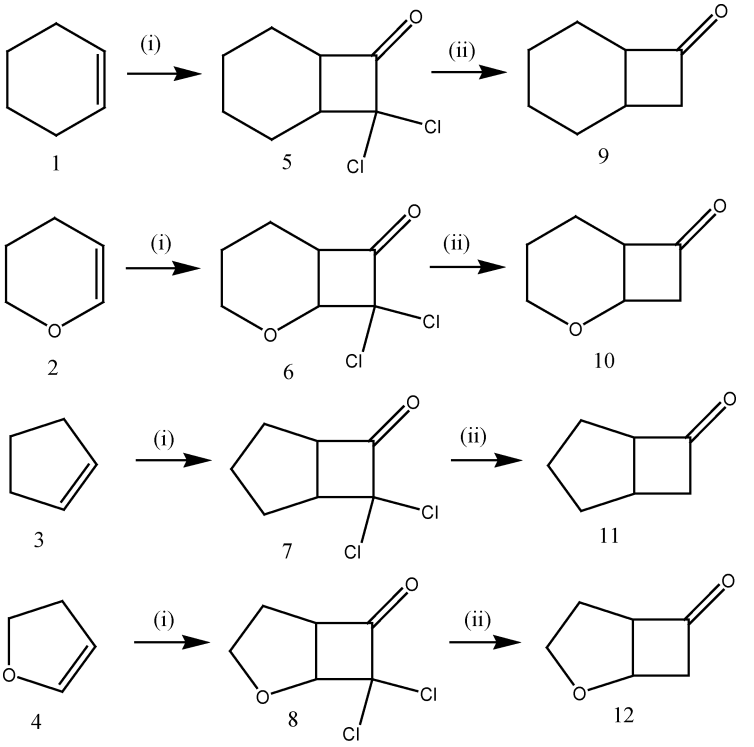
There are several advantages to using this photochemical ring-expansion in the synthesis of locked nucleosides. First, it is a simple single-step reaction involving mild conditions. In addition, the method shown can be generalized to give consistent yields of ring-expanded products. This generalization allows for a wide variety of locked nucleosides to be synthesized with minor alterations of the cyclobutanone substituents.

RESULTS AND DISCUSSION

The cycloaddition was achieved through the reaction of ketene and a suitable alkene. This reaction has long been known to generate [2 +

2] cycloaddition products by way of an antarafacial transition state which minimizes steric repulsion of the ketene substituents.[12] Parent ketene is conveniently generated by pyrolysis of acetone and cycloadds to alkene to generate cyclobutanones. However, the relative unreactive nature of ketene towards [2+2] cycloaddition with non-activated alkenes prompted us to consider the use of halogenated ketenes. Dichloroketene can be generated from dehydrochlorination of dichloroacetyl chloride or dechlorination of trichloroacetyl chloride. Cyclic alkenes **1–4** were reacted with dichloroketene generated in situ to give the dichlorinated ketones **5–8**, respectively (Scheme 1). The NMR spectra of the crude mixtures confirmed the presence of the bridgehead hydrogens of **5** and **7** at δ 3.4 and 3.6 ppm and for dichlorinated ketones **6** and **8** at δ 3.6 and 4.2 ppm. Degradation of these compounds prevented purification and the crude products carried forward to the next step. Dechlorination with zinc and ammonium chloride to give **9–12** was confirmed through IR spectroscopy (carbonyl peak shift from 1800–1810cm^{-1} to 1780–1790cm^{-1}).

Ketones **9–12** underwent photolytic insertion with 6-chloropurine by UV excitation (10–14 hours irradiation) in acetonitrile solution, to give

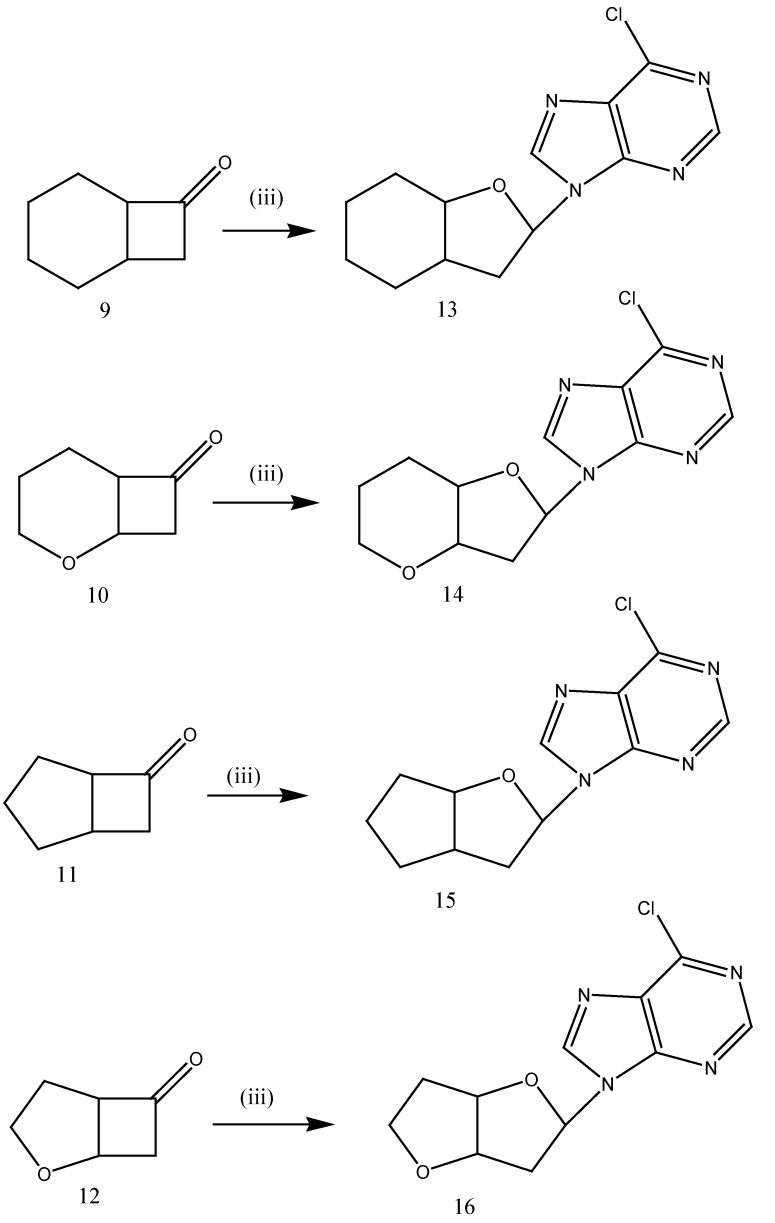


i. DCAC, TEA/Hexane; ii. Zn, NH_3Cl/MeOH

SCHEME 1 [2+2] cycloaddition.

the rigid nucleoside analogues **13–16** in yields of 19–24% (Scheme 2). The nucleosides were isolated and characterized by spectral data and high-resolution mass spectrometry.

Furanosides **13–15** were present as single stereoisomers. Compound **16** was formed as two stereoisomers in an approximate 2:1 ratio, which were not



iii. 6-Chloropurine/CH_3CN

SCHEME 2 Photolytic ring expansion.

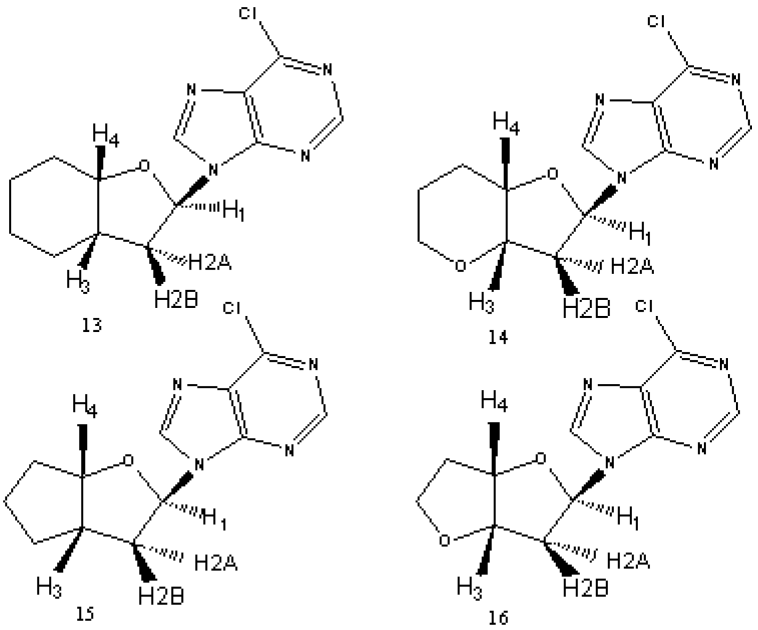


FIGURE 2 Relative stereochemistry of nucleosides **13–16**.

separable. The reason for the presence of a single isomer for furanosides **13–15**, compared to two isomers of compound **16**, is unknown.

Stereochemical assignments were based on 1-D ^{1}H NMR as well as NOESY. The structures of the four synthesized nucleosides are shown below, with specific hydrogen atoms labeled accordingly (Figure 2).

The NMR data show the configuration of the synthesized nucleosides. The NOESY analysis of the two isomers of **16** established a correlation between H-1 and H-4 in the minor product ('isomer B′) as seen in Figure 3.

This indicated that the two hydrogen atoms of this isomer ("isomer B") are closer in proximity than the H-1 and H-4 atoms of the major product ("isomer A"), suggesting that they are on the same side of the ring. In contrast, H-1 and H-4 of isomer A of **16** are facing in opposite directions (Figure 4). Thus, stereochemistry of the major isomer is assigned the *exo* configuration.

Similar results are found for the single stereoisomers of **13–15**. 1D slices from 2D NOESY for compounds **13–15** indicated that H-1 and H-4 are not correlated with the same hydrogen at C-2′ (Figure 5). Instead, each of H-1 and H-4 is correlated to a different hydrogen at C-2′. This indicates that H-1 and H-4 are in a *trans* configuration. To correlate these structures to the 3′-*endo* and 2′-*endo* conformations, we can see that in the case of the 3′-*endo* conformation, H-1 and H-4 would be closer in proximity to show a correlation in the NOESY data. Whereas direct comparison of the stereochemistry between the nucleoside analogues prepared in this study

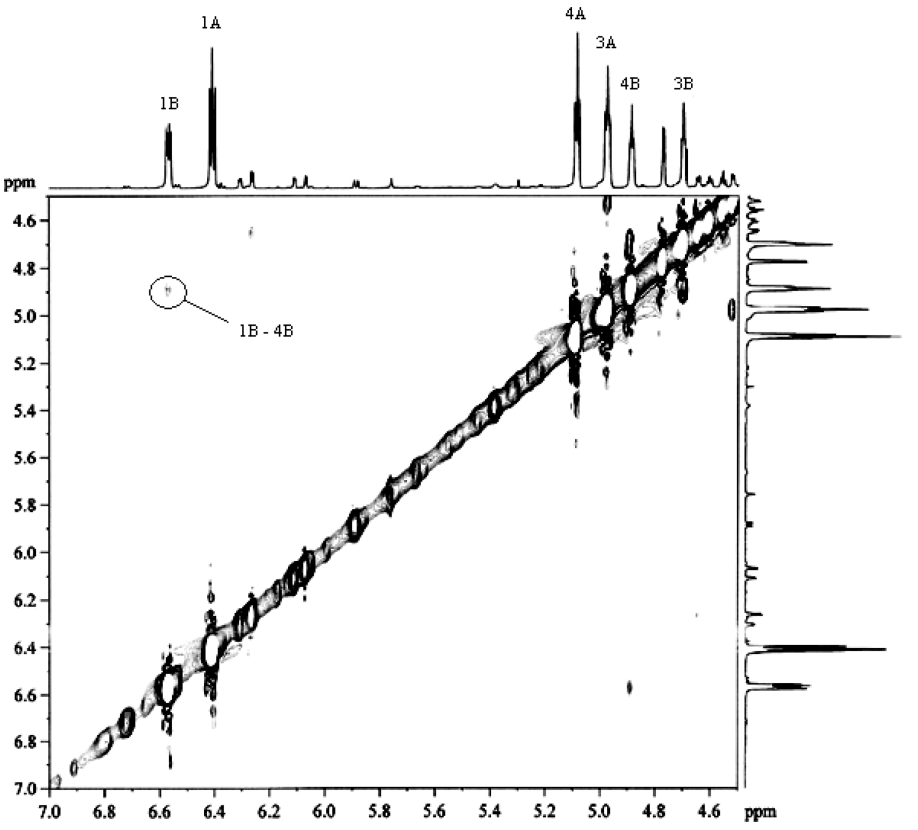


FIGURE 3 NOESY of isomers A and B of nucleoside 16.

and the natural nucleosides cannot be made due to the *cis*-ring fusion of the bicyclic ring system, it is only in the 2′-*endo* conformation where the two hydrogen atoms H-1 and H-4 are further apart in proximity compared to the 2′-*endo* conformer. Thus, these analogues serve as stereochemical models for the 3′-*endo* conformers of natural nucleosides.

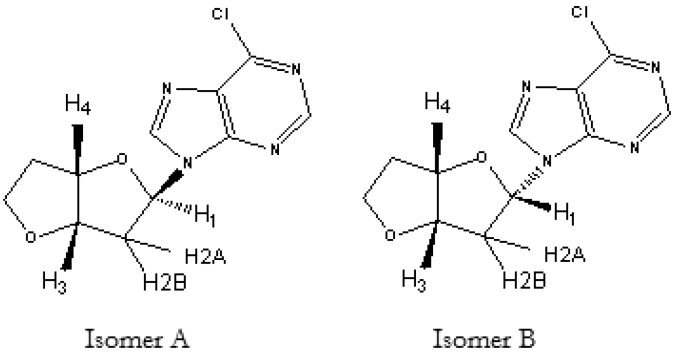


FIGURE 4 Two isolated isomers of nucleoside 16.

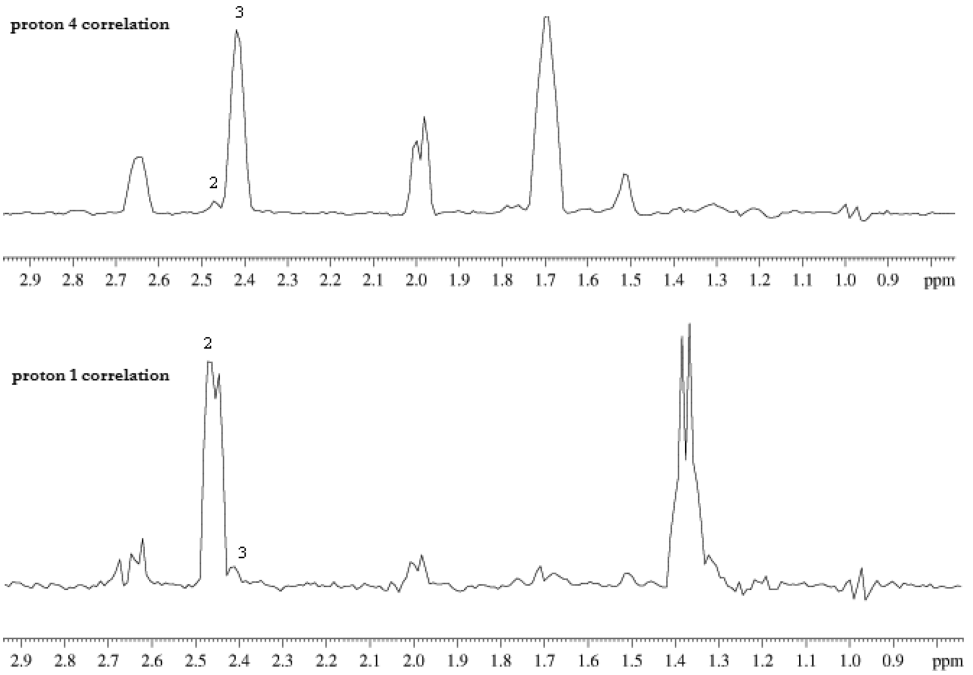


FIGURE 5 1D slice of 2D NOESY of nucleoside **13**

In summary, bicyclic cyclobutanones can be structurally elaborated to conformationally rigid nucleoside analogs by photochemical isomerization to an oxacarbene and insertion to a purine. This novel approach represents a simple and general scheme for the preparation of these derivatives.

EXPERIMENTAL

All NMR spectra were recorded on Bruker AV300 and AV400 spectrometers. NMR solvents used are as indicated. NOESY spectra were recorded at 600 MHz at 10 °C in $CDCl_3$. All IR spectra were recorded on a Genesis II Mattson 3000 FT-IR spectrometer. All thin layer chromatography (TLC) was done on silica gel 60F 254 alumina sheets. Preparative TLC was done on Silicycle silica gel 60F 254 precoated glass plates. Mass spectra were run in electron ionization at made 70 eV on a Micromass/Waters GCT Time-of-flight mass spectrometer at the McMaster Centre for Mass Spectrometry. UV spectra were recorded on an Ultrospec 4300 *pro* UV Spectrophotometer. All photolysis reactions were carried out using a Hanovia 450 W medium-pressure mercury arc lamp in a quartz immersion well. All solvents were distilled and purged with argon for 30 minutes.

8,8-Dichlorobicyclo[4.2.0] octan-7-one (5)

Cyclohexene (0.500 g, 6.10 mmol) was stirred with dichloroacetyl chloride (1.35 g, 9.16 mmol) in dry hexane (20 mL). The solution was refluxed for 20 minutes, followed by slow addition of TEA (0.930 g, 9.20 mmol) in hexane (10 mL). The mixture was allowed to reflux overnight. The solid material was removed by filtration and the solvent washed with 2 $\times$ 20 mL of saturated $NaHCO_3$. After drying over $MgSO_4$, the hexane was removed under vacuum to give 0.934 g of 5 as a colorless liquid. Formation of the dichlorocyclobutanone product was confirmed by IR spectroscopy ($\tilde{\nu}$ = 1810 cm^{-1}) and immediately carried forward to the next step.

8,8-Dichloro-2-oxabicyclo[4.2.0]octan-7-one (6)

3,4-Dihydropyran (**2**) (0.500 g, 5.95 mmol) was stirred with DCAC (1.20 g, 8.14 mmol) in dry hexane (20 mL). The solution was refluxed for 20 minutes, followed by the slow addition of TEA (0.85 g, 8.40 mmol) in hexane (10 mL). The mixture was refluxed for 15 minutes. The solution was washed with 2 $\times$ 20 mL of saturated $NaHCO_3$, dried over $MgSO_4$, and the solvent was subsequently removed under vacuum to give 0.820 g of a colorless liquid. Infrared spectroscopy ($\tilde{\nu}$ = 1808 cm^{-1}) confirmed the formation of the dichlorocyclobutanone product, which was immediately carried forward to the next step.

7,7-Dichlorobicyclo[3.2.0]heptan-6-one (7)

Cyclopentene (**3**) (0.500 g, 7.35 mmol) was dissolved in hexane (30 mL) and stirred at room temperature. DCAC (1.30 g, 8.82 mmol) was added and the mixture refluxed for 10 minutes. TEA (0.90 g, 8.89 mmol) in hexane (10 mL) was added and the mixture refluxed for 15 minutes before quenching with H_2O (20 mL). The solvent was washed with saturated $NaHCO_3$ (10 mL), dried over $MgSO_4$, and the solvent removed under vacuum to give **7**. Infrared spectroscopy ($\tilde{\nu}$ = 1811 cm^{-1}) confirmed the presence of **7**, which was immediately carried forward to the next step.

7,7-Dichloro-2-oxa-bicyclo[3.2.0]heptan-6-one (8)

2,3-Dihydrofuran (**4**) (0.500 g, 7.14 mmol) was reacted using the same method as for cyclopentene, described above. Infrared spectroscopy ($\tilde{\nu}$ = 1807 cm^{-1}) confirmed the presence of ketone **8**, which was immediately carried forward to the next step.

Bicyclo[4.2.0]octan-7-one (9)

Ketone **5** (0.400 g, 2.07 mmol) was stirred in MeOH (20 mL) at 0°C. To this solution was added zinc powder (2.00 g, 30.6 mmol) and ammonium chloride (0.90 g, 16.8 mmol). The mixture was allowed to stir and warm to room temperature overnight. The zinc was then filtered and the solvent removed under vacuum. The residue was washed with 3 × 15 mL of ether and the organic layer was washed with 2 × 10 mL saturated $NaHCO_3$, dried over $MgSO_4$, the solvent removed under vacuum, and the residue purified by column chromatography (20% ethyl acetate in hexane) to provide **9** (0.21 g, 80%). ^{1}H NMR ($CDCl_3$) δ 3.30–3.49 (m, 2H), 2.51–2.60 (m, 2H), 1.37–2.05 (m, 8H). IR (liquid film) 1779 cm^{-1}. The physical data were consistent with those previously reported (13).

2-Oxabicyclo[4.2.0]octan-7-one (10)

Ketone **6** (0.300 g, 1.54 mmol) was reacted in the same manner as compound **5**, described above. The dechlorinated product **10** was isolated (0.150 g, 79%) after purification by column chromatography (20% ethyl acetate in hexane). ^{1}H NMR ($CDCl_3$) δ 4.50 (m, 1H), 3.95 (m, 2H), 2.83–3.33 (m, 3H), 1.41–2.03 (m, 4H). IR (liquid film) 1780 cm^{-1}. The physical data were consistent with those previously reported.[14]

Bicyclo[3.2.0]heptan-6-one (11)

Dichloroketone **7** (0.500 g, 2.79 mmol) was stirred in MeOH (20 mL) at 0°C. To this solution was added zinc powder (2.0 g, 30.58 mmol) and ammonium chloride (1.10 g, 20.56 mmol). The mixture was allowed to stir and warm to room temperature overnight. The zinc was then filtered and the solvent removed under vacuum. The residue was washed with 3 × 15 mL of ether and the organic layer was washed with 2 × 10 mL saturated $NaHCO_3$, dried over $MgSO_4$, and the solvent removed under vacuum which was purified by column chromatography (20% ethyl acetate in hexane) to provide **11** (0.20 g, 66%). ^{1}H NMR ($CDCl_3$) δ 3.04–3.35 (m, 3H), 2.49–2.60 (m, 1H), 1.37–1.85 (m, 6H). IR (liquid film) 1785 cm^{-1}. The physical data were consistent with those previously reported.[15]

2-Oxa-bicyclo[3.2.0]heptan-6-one (12)

Ketone **8** (0.250 g, 1.38 mmol) was reacted using the same method as for compound **7**, described above. Purification by column chromatography (20% ethyl acetate in hexane) provided **12** (1.11 g, 72% yield). ^{1}H NMR ($CDCl_3$) δ 4.92 (m, 1H), 3.72–3.95 (m, 3H), 3.01–3.28 (m, 2H), 2.01–2.22

(m, 2H). IR (liquid film) 1782 cm^{-1}. The physical data were consistent with those previously reported.[14]

6-Chloro-9-(octahydrobenzofuran-2-yl)-9H-purine (13)

6-Chloropurine (0.300 g, 1.94 mmol) was dissolved in argon-purged CH_3CN (350 mL) and placed in a pyrex glass immersion well. To this was added **9** (0.200 g, 1.61 mmol) and the solution was placed in ice-water bath and irradiated for 10 h. The solvent was removed under vacuum and the residue washed with CH_2Cl_2 (3 × 50 mL). The product was purified by column chromatography (65% ethyl acetate in hexane) to give **13** (0.90 g, 20%) as a clear oil. ^1H NMR ($CDCl_3$) δ 8.75 (s, 1H), 8.27 (s, 1H), 6.45 (t, 1H, $J = 6.31$), 4.58 (m, 1H), 2.45 (m, 2H), 1.21–2.10 (m, 9H). MS 280 (1), 278 (M+), 181 (20), 154 (20), 125 (40), 81 (100), 71 (75), 49 (60). HRMS (EI) calc. for $C_{13}H_{15}N_4OCl$ 278.0934, found 278.0924. UV ($\lambda_{max} = 268.7$ nm) $\varepsilon = 1.75 \times 10^3$ L/mol cm.

6-Chloro-9-(hexahydro-2H-furo[3.2-b]pyran-2-yl)-9H-purine (14)

Ketone **10** (0.200 g, 1.59 mmol) was reacted in the same way as **9**, described above. The solvent was removed under vacuum and the residue washed with CH_2Cl_2 (3 × 50 mL). The product was purified by column chromatography (70% ethyl acetate in hexane) to give **14** (0.98g, 22%) as a clear oil. ^1H NMR ($CDCl_3$) δ 8.75 (s, 1H), 8.27 (s, 1H), 6.81 (m, 1H), 4.21 (m, 1H), 3.70–3.98 (m, 3H), 2.51–2.62 (m, 2H), 1.41–1.91 (m, 4H). MS 282 (1), 280 (M+), 238 (8), 210 (10), 154 (100), 119 (90), 85 (100), 67 (25), 55 (30), 41 (50). HRMS (EI) calc. for $C_{12}H_{13}N_4O_2Cl$ 280.0727, found 280.0717. UV ($\lambda_{max} = 268.8$ nm) $\varepsilon = 3.16 \times 10^3$ L/mol cm.

6-Chloro-9-(hexahydro-cyclopenta[b]furan-2-yl)-9H-purine (15)

6-Chloropurine (0.300 g, 1.94 mmol) was dissolved in argon-purged CH_3CN (350 mL) and placed in a pyrex glass immersion well. To this was added **11** (0.200 g, 1.82 mmol) and the solution was placed in ice-water bath during irradiation (10 hours). The solvent was removed under vacuum and the residue washed with CH_2Cl_2 (3 × 50 mL). The product was purified by column chromatography (65% ethyl acetate in hexane) to give **15** (0.11 g, 23%) as a clear oil. ^1H NMR ($CDCl_3$) δ 8.79 (s, 1H), 8.27 (s, 1H), 6.41 (d, 1H, $J = 5.81$), 4.82 (m, 1H), 2.25–2.40 (m, 2H), 1.21–2.10 (m, 7H). MS 266 (1), 264 (M+), 181 (13), 154 (70), 119 (45), 111 (100), 81 (80), 67 (100), 53 (25), 43 (70). HRMS (EI) calc. for $C_{12}H_{13}N_4OCl$ 264.0778, found 264.0765. UV ($\lambda_{max} = 271.2$ nm) $\varepsilon = 8.73 \times 10^3$ L/mol cm.

6-Chloro-9-(hexahydro-furo[3,2-b]furan-2-yl)-9H-purine (16)

Ketone **12** (0.200 g, 1.78 mmol) was reacted using the same method as for compound **11**, described above. Product **16** (0.090 g, 19%) was isolated as a mixture of two isomers. ^{1}H NMR ($CDCl_3$) δ 8.78 (s, 1H), 8.24 (s, 1H), 6.52 (m, 1H) 6.43 (m, 1H), 5.10 (m, 1H), 4.98 (m, 1H), 3.90–4.10 (m, 4H), 2.70–3.02 (m, 2H), 0.90–2.2 (m, 6H). MS 268 (1), 266 (M+), 244 (10), 223 (30), 188 (70), 154 (90), 140 (90), 119 (100), 113 (100), 95 (55), 81 (40), 69 (80). HRMS (EI) calc. for $C_{11}H_{11}N_4O_2Cl$ 266.0571, found 266.0577. UV ($\lambda_{max} = 268.6$ nm) $\varepsilon = 3.12 \times 10^3$ L/mol cm.

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