CHAPTER 2

Design and synthesis of cyclohexenyl and α-L-cyclohexenyl nucleosides
2. Design and synthesis of cyclohexenyl and α-L-cyclohexenyl nucleosides

2.1 Introduction

Antisense oligonucleotides have attracted a lot of attention for drug designing over the last two decades. These are specifically designed to target mRNA. The key role of antisense oligonucleotides in targeting mRNA, siRNA, miRNA, splice correction, etc. has further highlighted the need for the development of modified oligonucleotides. Natural oligonucleotides cannot be used as therapeutic agents due to poor stability against nucleases. Therefore, modifications have been introduced to enhance the therapeutic efficiency like increasing the binding affinity, water solubility, degradation of RNA by RNase-H enzyme, cellular uptake, etc.

Six-membered cyclic analogs are one of the several other prominent modifications for antisense therapy. The furanose ring of natural nucleoside was replaced by a six-membered ring which conferred suitable rigidity to antisense constructs. Therefore, upon formation of a double-stranded complex loss of entropy was less. The 1,5-anhydrohexitol nucleic acid (HNA) modification exhibited selective and strong RNA recognition, yielding the desired A-type double helix but prohibiting RNase H recognition. The double bond was introduced to increase flexibility allowing adaptation and enzymatic recognition, hopefully preserving the increased affinity for complementary targets.

![Figure 1 Natural nucleic acids and modified analogues.](image)

As per structural considerations, the cyclohexene nucleosides are one of the best mimics of natural furanose nucleosides in six-membered ring analogues (Figure 1). The thermodynamic parameters of cyclohexenyl nucleoside (ΔG 1.8 kJ/mol) between S-type (H3) and N-type (H3) and equilibrium occurs via the eastern hemisphere with a barrier of 10.9 kJ/mol are very similar to natural ribose.
nucleosides ($\Delta G$ 2 kJ/mol between N-type and S-type, and equilibrium occurs \textit{via} the eastern hemisphere with a barrier of 4–20 kJ/mol). The $\Pi$-$\sigma^*$ interaction of cyclohexenyl nucleoside mimics the anomeric effect in furanose nucleoside. CeNA (cyclohexenyl nucleic acids) exists in both N-type and S-type sugar conformations at the nucleoside level (Figure 2) due to highly flexible cyclohexene ring with low energy barrier between the two forms.

![Figure 2: Comparison of the conformational equilibrium](image)

The cyclohexenyl nucleosides have demonstrated potent antiviral activity and the CeNA oligomers have been shown to mimic the function of RNA with increased enzymatic and chemical stability. The presence of the double bond allows enough flexibility within a CeNA-RNA double helix, to be recognized by RNase H. CeNA also been shown to be useful in siRNA applications.

### 2.2 Synthesis of Cyclohexenyl nucleosides

Although cyclohexenyl nucleic acids have tremendous applications, the main limitation that has stymied the scope of this highly valuable discovery is the lack of a robust and scalable synthetic strategy of the key intermediate in enantiomerically pure form. Two synthetic routes were reported in the literature for enantiopure synthesis of cyclohexenyl nucleic acids.

First synthetic route was reported by Herdewijn, P \textit{et al.} (scheme 1) Synthesis started from R-(-)-carvone, which converted into 2 by reported procedures within 5 steps. Epoxide ring in 2 was opened by using LiTMP, Et$_2$AlCl followed by hydroboration of the double bond using 9BBN to get 4. $\alpha$-isomer at 4-carbon also was observed as a side product in addition to the required compound 4. Then TBDMS protection of primary hydroxyl group then mesylation of secondary hydroxyl compound and benzyl group deprotection yields 7. Compound 7 was further taken for
oxidation of hydroxyl group to get 8 followed by elimination to get 9. Compound 9 was reduced to alcohol 10 which was subjected to Mitsunobu reaction conditions to obtain the required cyclohexenyl adenine nucleoside. The main drawback of this synthesis is the overall yield was low (2–3%), which is not practical for oligonucleotide synthesis.

**Scheme 1** Synthesis of cyclohexenyl adenine nucleoside from R-(-)-carvone

**Reagents and conditions:** (a) LiTMP, Et₂AlCl; (b) 9BBN, THF; (c) TBDMSCl, imidazole, DMF, rt, 70%; (d) MsCl, EtN, DCM, 0 °C, 92%; (e) Pd-C (10%), HCOONH₄, MeOH, reflux, 76%; (f) MnO₂, CH₂Cl₂, rt, 48% and 47% recovery of 7; (g) NaBH₄, CeCl₃.7H₂O, MeOH, 0 °C to rt, 91%; (h) Adenine, DEAD, PPh₃; (i) TFA/H₂O(3:1).

Second synthetic route was also reported by Herdewijn, P et al. They started the synthesis with Diels–Alder reaction of ethyl (2E)-3-acetoxyprop-2-enoate as dienophile and Danishefsky’s diene to construct the six-membered ring skeleton 1. The major drawback in this was compound 1 could easily undergo aromatization to give 3 which is not useful for further synthesis (scheme 2).
2.3 Our design and rationale

We were interested in developing an easy and straightforward synthetic route for the cyclohexenyl nucleosides. The enzymatic resolution also would give rise to the other stereoisomer corresponding to α-L-cyclohexenyl nucleoside which is not known in the literature (Figure 3).

The unprecedented thermal stability of duplexes involving LNA has inspired to investigate the properties of the stereoisomers of LNA and stereo isomeric analog termed as α-L-LNA. The main difference between LNA and its isomer was LNA is in N-type conformation whereas α-L-LNA was in S-type (DNA-like) sugar geometry which is capable to show RNase H activity.

Figure 3: structures of cyclohexenyl nucleoside and α-L-cyclohexenyl nucleoside
Chapter 2

We designed the stereo isomer of cyclohexene nucleoside named as α-L-cyclohexenyl nucleoside. The major advantage of α-L-cyclohexenyl nucleoside is DNA like (S-type) sugar geometry, which is capable of eliciting the RNase-H activity as well as advantages of both α-L-LNA and CeNA. We proposed a most efficient common synthetic route for synthesis of both cyclohexenyl and α-L-cyclohexenyl nucleosides (Figure 4).

![Figure 4: Design of α-L-cyclohexenyl nucleoside](image)

2.4 Methodology, Results and Discussion

Diels-Alder reaction between commercially available 5,5-dimethoxy-1,2,3,4-tetrachlorocyclopentadiene 1 and vinyl acetate at 120 °C gave exclusively endo adduct (±)-2.\(^{17}\) The acetate group in (±)-2 was hydrolysed under acidic conditions to give the free alcohol (±)-3. Compound (±)-3 was then subjected to reductive dehalogenation under Birch conditions to get (±)-4. The purpose of the acetate hydrolysis was to improve the yields of the dehalogenation reaction (Scheme 3).\(^{18}\)

**Scheme 3** Synthesis of dehalogenated bicyclic intermediate 4

![Scheme 3](image)

**Reagents and conditions:** (i) vinyl acetate, 120 °C, 5h, 84% (ii) H₂SO₄, MeOH, 85 °C, 8-10 h, 95% (iii) Na, Liq NH₃, THF: EtOH 0.5h, 74%.

Next aim was to hydrolyze the ketal group of 4 to get free ketone (±) 6 proved to be difficult as we found that the formation of compound (±)-5 was a major product under variety of ketal hydrolysis conditions. We employed different protecting groups for the secondary hydroxy group in (±)-4 such as TBS, benzyl or acetate protection\(^{18}\) but each time compound (±)-5 was formed as a major product in the acidic
Chapter 2

hydrolysis. The silyl ether, benzyl ether and the acetate protecting groups probably undergo hydrolytic cleavage (scheme 4). A report was found in the literature where a similar retro aldol type of rearrangement was observed and a similar major product was obtained. Then we tried benzoyle protection as suggested by Sgarbi et al. 20

Scheme 4 Different conditions tried for ketal hydrolysis and mechanism for rearranged product.

This was found to be stable under these reaction conditions and the rearranged product (±)-5 was not formed. We observed that the aqueous acetic acid reflux conditions of (±)-7 gave a clean ketal hydrolysis product (±)-8 in 81% yield (scheme 5). The Bayer-Villager’s oxidation of ketone (±)-8 gave two regioisomeric inseparable mixture of lactones (±)-9 and (±)-10 in 7:3 proportion respectively, in high yield (Scheme 5). The products were confirmed by 2D COSY NMR spectroscopy. The mixture of lactones (9+10) was reduced with LiAlH4 and gave the tri hydroxy substituted cyclohexene derivatives (±)-11 and (±)-12 as an inseparable mixture (scheme 6). The cis geometry of C1&C4 and trans placement of substituent at C3 was fixed at this point in compound 11 and 12. The mixture of regioisomers
Chapter 2

obtained was subjected to chemo selective 1,3-diol protection and only compound (±)-11 was protected to furnish (±)-13 quantitatively.

Scheme 5 Synthesis of the mixture of lactones 9 and 10

Reagents and conditions: (i) BzCl, pyridine, 3h, 93% (ii) CH₃COOH : H₂O (6:1), 120 °C, 4 h, 81% (iii) mCPBA, DCM, 0 °C, 5h, 92%, 9:10 (70:30).

Then the unreacted triol (±)-12 could be easily separated chromatographically. Successfully we have synthesized key intermediate (±)-13 with good yield. The compound (±)-12 obtained as byproduct may be used further to achieve 2'-5'linkages of 3'-deoxy cyclohexenyl nucleosides which may be useful in therapeutic studies. Compound (±)-13 subjected for oxidation using CrO₃/pyridine in 3h to get the enone (±)-14 (scheme 6). The reported MnO₂ oxidation takes very long time and depends on the quality of MnO₂ used in the reaction.

Scheme 6 Synthesis of 1,3 diol protected key intermediate 13

Reagents and conditions: (i) LiAlH₄, dry THF, -15 °C, 2h, 72 % (ii) PhCH(OMe)₂, PTSA, dry dioxane, rt, 24 h, 81% (iii) CrO₃, dry pyridine, Ac₂O, dry DCM, 2 h, 92% (iv) NaBH₄, CeCl₃·7H₂O, MeOH, 3h, 80%.
Chapter 2

The use of CrO3 reduces the reaction time and is highly reproducible. Compound (±)-14 was then subjected to Luche reduction to get epimeric alcohol (±)-15 using NaBH4 in the presence of CeCl3.7H2O (Scheme 6) and it can be converted to cyclohexenyl nucleosides units using reported procedures.

2.5 Enzymatic resolution

In the field of organic synthesis the use of enzymes has become an interesting area. Since many enzymes have been demonstrated to possess activity and widely used to carry out synthetic transformations. Hydrolases are the most frequently used enzymes due to their considerable stability and broad substrate spectrum. Enzymes are commercially available and they work under mild reaction conditions. Enzyme catalyzed kinetic resolution of racemic alcohols through trans esterification is an attractive route among the numerous synthetic methods for asymmetric synthesis.

Enzymatic resolution of (±)-3 is known in the literature using Candida cylindracea lipase (CCL) and vinyl acetate as an acyl donor. We followed the reported reaction conditions but reaction was very sluggish, it took almost 7 days to complete and could not be scaled up (scheme 7.1). The acylation was sluggish but enantio specificly was good, then we tried enzymatic hydrolysis of (±)-2 using the same lipase in phosphate buffer (pH 7.2). The acetate compound was less soluble in buffer due to highly non polar nature of the compound. When acetonitrile was used as co-solvent along with phosphate buffer (pH 7.2), good enantioselective hydrolysis was observed in 4 days (Scheme 7.2, yield 45%, ee 98%). Continuing the hydrolysis of the remaining enantiomerically enriched acetate for 24 h, the other enantiomer as unreacted acetate 2 was obtained in good yield and high ee (yield 42%, ee 98 %). Alternatively, optimization of the resolution time was also be achieved by using CCL for esterification using vinyl acetate as acyl donor in diethyl ether solvent. The acylation was enantioselective, giving the acetate (48% yield and >98% ee) in 3h (Scheme 7.3).
Chapter 2

**Scheme 7** Enzymatic resolution of racemic compounds 2 and 3

The enantiomeric identity of the resolved acetate 2 was established by \(^1\)H NMR using chiral shift reagent and chiral HPLC analysis.

### 2.6 Enantiopurity confirmation by chiral shift reagent NMR of rac-2 and ent-2

In principle, the enantiomeric molecules do not differ in physical properties unless they are placed in a chiral environment. We used Tris[3-(heptafluoropropyl-hydroxymethylene)-d-camphoratol] europium(III) derivative as chemical shift reagent which contains chiral ligand and the resulting complexes with chiral molecules are diastereotopic. Protons attached to chiral centers will give rise to separate signals only if the molecule is placed in a diastereotopic environment.

We demonstrated the splitting patterns of the protons of rac-2 and ent-2 in chiral shift reagent NMR (Table 1). The splitting pattern of H\(^1\) and H\(^2\) protons in enantiopure compound was dd whereas in racemic compound it was multiplet. The methyl protons (H\(^3\)) of acetate group showing one singlet in ent-2 and two singlets in rac-2 (Figure 5 & Table 1).
Table 1 Chemical shift values of rac-2 and ent-2 with NMR shift reagent

<table>
<thead>
<tr>
<th>Structure</th>
<th>Proton</th>
<th>δ-value</th>
<th>Splitting pattern</th>
<th>δ-value</th>
<th>Splitting pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H¹</td>
<td>6.03</td>
<td>multiplet</td>
<td>5.97</td>
<td>dd</td>
</tr>
<tr>
<td></td>
<td>H²</td>
<td>3.04</td>
<td>multiplet</td>
<td>3.02</td>
<td>dd</td>
</tr>
<tr>
<td></td>
<td>CH₃³</td>
<td>2.43</td>
<td>2 singlets</td>
<td>2.37</td>
<td>singlet</td>
</tr>
</tbody>
</table>

rac-2

ent-2

Figure 5: 'H NMR splitting patterns of rac-2 and ent-2 with chiral shift reagent
The enantiopurity was confirmed by chiral HPLC. The rac-2 compound showed two peaks, whereas only one peak observed in HPLC chromatogram for ent-2 (Figure 6).

**Figure 6:** Chiral HPLC chromatograms of rac-2 and ent-2 compounds

Successfully we have introduced an efficient method for enzymatic resolution with good yields as well as good enantiomeric excess. The enzymatic resolution at such an early stage to get the different sugar analogues would be time consuming, we
Chapter 2

decided to resolve the two enantiomers of (+)-13 using enzymatic acylation. Compound (+)-13 was subjected to acylation using vinyl acetate as donor and Candida cylindracea lipase (CCL) to get 16 in excellent yield and high enantiomeric purity (Scheme 8).

Scheme 8 Enzymatic resolution of racemic compounds 13

\[
\text{OH} \quad \xrightarrow{\text{Candida cylindracea}} \quad \text{OAc} \\
\text{13} \quad \text{4h} \quad \text{16} \quad \text{17} \\
\text{HOAc, Et}_2\text{O} \\
\text{(45%, 98% ee) (55%, 87% ee)}
\]

cyclohexenyl nucleoside \quad \alpha\text{-l-cyclohexenyl nucleoside}

The high enantiomeric purity was established by \(^1\text{H}\) NMR using chiral shift reagent. The enantiomeric identity of the resolved acetate 16 and 17 was established after converting the acetate 16 (hydrolysis of the acetate followed by oxidation and reduction as described in Scheme 6) to the known compound i.e. enantiomerically pure D-isomer 15 and comparing with the reported HPLC retention time on chiral HPLC column.

Table 2 Chemical shift values of rac-15 and ent-15 with NMR shift reagent

<table>
<thead>
<tr>
<th>structure</th>
<th>Proton</th>
<th>rac-16</th>
<th></th>
<th>ent-16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(\delta)-value</td>
<td>Splitting pattern</td>
<td>(\delta)-value</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>(\text{H}^1)</td>
<td>3.07</td>
<td>multiplet</td>
<td>3.11</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>(\text{CH}_3^2)</td>
<td>3.37</td>
<td>2 singlets</td>
<td>3.50</td>
</tr>
</tbody>
</table>
Chapter 2

In case rac-16 and ent-16 also we noticed the difference in splitting patterns of the protons of in chiral shift reagent NMR (Table 2). The splitting pattern of H¹ proton in enantiopure compound was dd, whereas in racemic compound it was multiplet. The methyl protons (H²) of acetate group showing one singlet in ent-16 and two singlets in rac-16 (Figure 7) and chiral HPLC chromatogram showed in Figure 8.

Figure 7: ¹H NMR splitting patterns of rac-16 and ent-16 with chiral shift reagent
Figure 8: Chiral HPLC chromatograms of rac-16 and ent-16 compounds
The enriched enantiomer 17 was further subjected for same enzymatic acylation conditions and pure enantiomer 17 was isolated by silica gel chromatography, which was not possible to synthesize by earlier reported synthetic route.\textsuperscript{13}

### 2.7 Synthesis of cyclohexenyl thymine nucleoside

After successful synthesis of enantiopure intermediates ent-15 and 17, ent-15 was used for the synthesis of cyclohexenyl nucleoside. Compound ent-15 was subjected for Mitsunobu reaction conditions to introduce thymine base moiety onto the cyclohexenyl ring (scheme 9).

**Scheme 9** Synthesis of cyclohexenyl thymine nucleoside

We observed O-alkylated product 19 instead of required N-alkylated product 18. In literature also we found that in case of thymine there could be a competition between N-nucleophile and O-nucleophile. Initially we tried the reaction with N\textsuperscript{3}-benzoyl protected thymine as nucleophile and diisopropyl azodicarboxylate as base in THF at room temperature, we observed the O-alkylated product 19 (scheme 9a). Then we
used the same reaction conditions with low temperature at -10 °C, was also yielded the undesired product (scheme 9b). We also used the known literature procedure where sodium benzoate used in Mitsunobu reaction was also ended with O-alkylated product (scheme 9c). Diethyl azodicarboxylate was used instead of diisopropyl azodicarboxylate and solvent changed to dioxane which also gave 19 (scheme 9d). Finally we obtained the desired N-alkylated product 18 with 30% yield by changing the reaction conditions to unprotected thymine as nucleophile and reflux at 100 °C in dioxane (scheme 9e). To improve the yield, we changed the reaction conditions, which again ended with O-alkylated product (scheme 9f,9g,9h). Compounds 18 and 19 were confirmed by chemical shift value of the allylic proton in ¹H NMR. The chemical shift value of the proton was 5.3 ppm for N-alkylated product, whereas in case of O-alkylated the value shifted to around 5.6 ppm.

2.8 Synthesis of Enantiopure α-L-Cyclohexenyl Adenine, Guanine Nucleosides

Synthesis of α-L-cyclohexenyl adenine nucleoside was obtained from compound 17. Enantiopure compound 17 was subjected for Mitsunobu reaction in presence of adenine, DEAD, triphenylphosphine and dioxane was used as a solvent to yield 20 which was further treated with 80% aq acetic acid for deprotection of benzylidene group to give the α-L-cyclohexenyl adenine 21 in 32% yield (scheme 10).

Scheme 10 Synthesis of α-L-cyclohexenyl adenine nucleoside

Reagents and conditions: (i) Adenine, DEAD, PPh₃, dry THF, rt, overnight, 38 % (ii) 80% aq CH₃COOH, 60 °C, 8 h, 65%.

To complete the synthesis of α-L-cyclohexenyl guanine nucleoside initially we protected the amine group of 2-amino-6-chloropurine with isobutyryl group yielded 23 to avoid the formation of unwanted product with N-7 isomer in further nucleophilic substitution. Compound 17 was subjected with protected 2-amino-6-
chloropurine 23 under Mitsunobu reaction condition to get 24 with 34% yield, which was again treated with aq acetic acid to yield the final α-L-cyclohexenyl guanine nucleoside 25 (scheme 11).

**Scheme 11** Synthesis of α-L-cyclohexenyl adenine nucleoside

![Scheme 11](image)

**Reagents and conditions:** (i) Isobutyric anhydride, dry N,N dimethyl acetamide, 150 °C, 2h, 72 % (ii) 23, DIAD, PPh3, dry THF, overnight, 34% (iii) 80% aq CH3COOH, 60 °C, 8 h, 68%.
Chapter 2

2.9 Conclusions:

- Introduced a robust method for the synthesis of important cyclohexenyl-2-deoxyribose sugar analogues in excellent yields from commercially available starting materials.

- Both cyclohexenyl and \(\alpha\)-L-cyclohexenyl nucleosides was achieved by using our designed synthetic route which was not possible with previous reports.

- An excellent enzymatic resolution method was accomplished for non polar compounds which can produce high yields and enantiomeric purity.

- Enantiopurity was established by using chiral shift reagent NMR as well as chiral HPLC.

- The easy access to these sugars as outlined in this chapter would allow further exploitation of the cyclohexenyl as well as cyclohexane nucleic acid analogs. These synthons will have applications not only in the synthesis of nucleoside/oligonucleotide analogues but also in carbohydrate chemistry where modified carbasugars as sugar mimics have potential applications.
2.10 Experimental Section

All the non-aqueous reactions were carried out under the inert atmosphere of Nitrogen/Argon and the chemicals used were of laboratory or analytical grade. All solvents used were dried and distilled according to standard protocols. TLCs were carried out on pre-coated silica gel GF254 sheets (Merck 5554). Column chromatographic separations were performed using silica gel 60-120 mesh (Merck) or 200-400 mesh (Merck) and using the solvent systems EtOAc/Pet ether and MeOH/DCM. IR spectra were recorded on an infrared Fourier Transform spectrophotometer using chloroform or neat. $^1$H and $^{13}$C spectra were obtained using Bruker AC-200, AC-400 and AC-500 NMR spectrometers. The chemical shifts are reported in delta (δ) values and referred to internal standard TMS for $^1$H. Enzyme Candida cylindracea lipase was purchased from Ltd. Aldrich Inc.

(±)(1S,2S,4R)-1,4,5,6-tetrachloro-7,7-dimethoxy-bicyclo[2.2.1]hept-5-en-2-yl acetate (2):

To 5,5-dimethoxy-1,2,3,4-tetrachlorocyclopentadiene (12 mL, 45.45 mmol) was added vinyl acetate (7.82 mL, 90.9 mmol) and allowed to stir at 120°C for 5h. The excess vinyl acetate was removed in vacuo and the residue was purified by silica gel chromatography (pet ether:EtOAc = 97:3) to afford exclusively the endo adduct (±)-3 (13.36 g) in 84% yield.

$^1$H NMR (200 MHz, CDCl$_3$): δ 5.51 (dd, 1H, J = 7.7, 2.3 Hz, CHOAc), 3.60 (s, 3H, OMe), 3.56 (s, 3H, OMe), 2.83 (dd, 1H, J = 12.6, 7.8 Hz, CH$_2$), 2.07 (s, 3H, OAc), 1.76 (dd, 1H, J = 12.8, 2.6 Hz, CH$_2$); $^{13}$C NMR (50 MHz, CDCl$_3$): δ 170.2, 131.0, 127.8, 111.8, 76.4, 73.9, 52.6, 51.7, 43.8, 20.6; LCMS: mass calculated for C$_{11}$H$_{12}$Cl$_4$O$_4$K (M+ K$^+$) 386.91, observed 385.19.

(±) (1S,2S,4R)-1,4,5,6-tetrachloro-7,7-dimethoxybicyclo[2.2.1]hept-5-en-2-ol (3):

To a solution of (±)-2 (20 g, 57.14 mmol) in methanol (150 mL) was added 5% aqueous H$_2$SO$_4$ (40 mL) and stirred at 65°C for 5h. Methanol was removed on rotavapor in vacuo. The residue was diluted with EtOAC and water, saturated aqueous NaHCO$_3$
Chapter 2

and brine wash were given. Organic layer was dried over Na2SO4, filtered, concentrated in vacuo and purified by silica gel chromatography (pet ether:EtOAc = 92:8) to result 3 (16.8 g) in 95% yield as a white solid,

1H NMR (200 MHz, CDCl3): δ 4.66 (m, 1H, CHOH), 3.58 (s, 3H, OMe), 3.55 (s, 3H, OMe), 2.67 (dd, 1H, J = 12.4, 8.0 Hz, CH2), 2.12 (d, 1H, J = 4.8 Hz), 1.79 (dd, 1H, J = 12.3, 2.4 Hz, CH2); 13C NMR (50 MHz, CDCl3): δ 130.8, 127.3, 112.0, 79.8, 76.4, 74.2, 52.5, 51.6, 44.2.

(±)(1S,2S,4S)-7,7-dimethoxybicyclo[2.2.1]hept-5-en-2-ol (4):

To a suspension of liquid ammonia (700 mL) and sodium (4.78 g, 207.8 mmol) at -78 °C, solution of (±)-3 (8 g, 25.97 mmol) in 10/1 mixture of THF (100 mL)/EtOH (10 mL) was added dropwise. After completion of addition, stirring was continued for 15 min, reaction mixture was quenched with saturated aqueous NH4Cl solution and kept at room temperature overnight to allow liquid ammonia to evaporate. THF/EtOH was removed on rotavapor in vacuo, residue was diluted with DCM. Water wash and brine wash were given to the organic layer, dried over Na2SO4, filtered, concentrated in vacuo and purified by silica gel chromatography (pet ether:EtOAc, 85:15) to result (±)-4 (3.4 g) in 78% yield as a pale yellow thick liquid.

1H NMR (200 MHz, CDCl3): δ 6.49 (m, 1H, Alkene CH), 6.10 (m, 1H,Alkene CH), 4.85 (s, 1H,OH), 4.56 (m, 1H,CHOH), 3.26 (merged, 1H,CH), 3.18 (s, 3H,OMe), 3.15 (s, 3H,OMe), 2.87 (m, 1H,CH), 2.42 (m, 1H,CH2), 0.85 (dd, 1H, J = 12.3, 2.2 Hz, CH2) 13C NMR (50 MHz, CDCl3): δ 137.9, 128.3, 119.2, 76.7, 70.2, 51.6, 50.7, 49.6, 45.6, 36.2.

(±) (1S,2S,4S)-7,7-dimethoxybicyclo[2.2.1]hept-5-en-2-yl benzoate (7):

To a solution of (±)-4 (9 g, 17.6 mmol) in pyridine (45 mL) was added benzoyl chloride (8.1 mL, 58.23 mmol) and the reaction mixture was stirred at rt for 4h. Pyridine was removed in vacuo and the residue was diluted with EtOAc. Water wash and brine...
wash were given to the organic layer, dried over Na$_2$SO$_4$, concentrated in vacuo. The residue was purified by silica gel chromatography (pet ether:EtoAc, 89:11) to afford (±)-7 (13.5 g) in 93% yield.

$^1$HNMR (200 MHz, CDCl$_3$): δ 7.99-7.94 (m, 2H, Aromatic), 7.55-7.27 (m, 3H, Aromatic), 6.43 (m, 1H, Alkene), 6.11 (m, 1H, Alkene), 5.61 (m, 1H, CHOBOz), 3.41 (m, 1H, CH), 3.26 (s, 3H, OMe), 3.19 (s, 3H, OMe), 2.97 (m, 1H, CH), 2.58-2.48 (m, 1H, CH$_2$), 1.18 (dd, 1H, $J = 12.5$, 2.4 Hz, CH$_2$); $^{13}$C NMR (50 MHz, CDCl$_3$): δ 166.4, 136.0, 133.6, 132.8, 130.1, 129.5, 128.3, 119.0, 73.8, 51.9, 49.9, 48.7, 45.2, 33.4; HRMS: mass calculated for C$_{16}$H$_{18}$O$_4$Na (M+Na$^+$) 297.1102, observed 297.1113; IR (CHCl$_3$): $\nu_{max}$ 2950, 1720, 1602 cm$^{-1}$

(±) (1S,2S,4S)-7-oxobicyclo[2.2.1]hept-5-en-2-yl benzoate (8):

Solution of (±)-7 (4.5 g, 16.4 mmol) in 180 mL of 6/1 mixture of acetic acid/water was refluxed for 4h. Solvent was removed in vacuo, residue diluted with the EtOAc and water wash, saturated aqueous NaHCO$_3$ wash and finally brine wash were given. The organic layer was dried over Na$_2$SO$_4$, concentrated in vacuo and purified by silica gel chromatography (pet ether:DCM, 50:50) to give a mixture of 8 (81%).

$^1$H NMR (200 MHz, CDCl$_3$): δ 8.06 - 7.94 (m, 2H, Aromatic), 7.58-7.39 (m, 3H, Aromatic), 6.79 (m, 1H, Alkene CH), 6.50 (m, 1H, Alkene CH), 5.68-5.60 (m, 1H, CHOBOz), 3.57 (m, 1H, CH), 3.09 (m, 1H, CH), 2.66-2.53 (m, 1H, CH$_2$), 1.49 (dd, 1H, $J = 13.5$, 3.0 Hz, CH$_2$); $^{13}$C NMR (50 MHz, CDCl$_3$): δ 201.0, 166.1, 134.4, 133.3, 133.2, 129.7, 129.5, 128.9, 128.4, 69.0, 51.8, 47.4 32.5; LCMS: mass calculated for C$_{14}$H$_{12}$O$_3$Na (M+ Na$^+$) 251.0786, observed 251.4641; IR (CHCl$_3$): $\nu_{max}$ 3064, 2947, 1790, 1720, 1600 cm$^{-1}$.

(±) (1S,4S)-3-oxo-2-oxabicyclo[2.2.2]oct-5-en-8-yl benzoate (9 + 10): To a solution of (±)-8 (5 g, 21.1 mmol) in 100 mL of dry DCM, was added Na$_2$CO$_3$ (2.23 g, 21.1 mmol), stirred and cooled to 0°C. m-CPBA (5.2 g, 21.1 mmol) was added to this suspension and stirred it for 6h at rt. The
Chapun-2

reaction mixture was quenched with 10% aq. solution of Na$_2$S$_2$O$_5$ (30 mL). Organic layer was separated and aqueous layer was extracted with DCM. The combined organic layer was washed with saturated aq NaHCO$_3$ followed by brine, and dried over anhydrous Na$_2$SO$_4$. The organic layer was concentrated in vacuo followed by silica gel chromatography (pet ether:EtOAc, 90:10) to give a mixture of 9 and 10 (92%) in 70:30 ratio.

$^1$H NMR (200 MHz, CDCl$_3$): $\delta$ 7.98 (m, 2H, Aromatic), 7.56-7.41 (m, 3H, Aromatic), 6.78 (m, 1H, minor), 6.72 (m, 1H, major) 6.54 (m, 1H), 5.60 (m, 1H, minor), 5.48 (m, 1H, major), 5.42 (m, 1H, minor), 5.32 (m, 1H, major), 4.05 (m, 1H, major), 3.57 (m, 1H, minor), 2.60 (m, 1H, minor), 1.75 (d, $J = 6.0$ Hz, 1H, major), 1.59 (m, 1H, minor); $^{13}$C NMR (50 MHz, CDCl$_3$): $\delta$ 172.2, 170.7, 165.5, 134.3, 133.4, 133.3, 132.5, 129.5, 129.3, 129.0, 128.9 128.4, 128.2, 76.6, 73.5, 73.0, 68.8, 65.5, 46.2, 40.3, 35.0, 29.3; HRMS: mass calculated for C$_{14}$H$_{13}$O$_4$ (M+H$^+$) 245.0813, observed 245.0812; IR (CHCl$_3$): $\nu$$_{max}$ 1759, 1716 cm$^{-1}$.

(±)(1S,3S,6R)-6-(hydroxymethyl)cyclohex-4-ene-1,3-diol (11+12):

To a solution of mixture 9+10 (3.3g, 13.5mmol) in dry THF (400mL) at −15°C, LAH (1.5g, 40.5 mmol) was added and the resulting mixture was stirred at the same temperature for 2h. The reaction mixture was then quenched cautiously with ethyl acetate (50 mL) followed by aqueous saturated solution of Na$_2$SO$_4$, to precipitate out aluminium salts. After the filtration, filtrate was concentrated in vacuo, to result inseparable epimeric mixture of 11 and 12 in 85% yield which was used further without separation.

$^1$H NMR (200 MHz, CDCl$_3$) of compound (11+12): $\delta$ 5.86-5.72(m, 2H, Alkene CH), 4.38-4.32 (m, 1H, allylic CH-O), 4.14-4.08 (m, 1H, CH$_2$O), 3.87-3.79 (m, 1H, CH$_2$O), 3.68-3.60 (m, 1H, CH-O), 2.50 (m, 1H, Allylic CH), 2.11-2.02 (m, 1H,CH$_2$), 1.88-1.73 (m, 1H, CH$_2$); $^{13}$C NMR (50 MHz,CDCl$_3$): $\delta$ 130.5, 127.9, 66.1,65.4,61.7, 41.5, 35.3. LCMS: mass calculated for C$_7$H$_{12}$O$_3$Na (M+ Na$^+$) 167.06, observed 167.17.
Chapter 2

(±)-(4aR,7S,8aS)-2-phenyl-4a,7,8,8a-tetrahydro-4H-benzo[d][1,3]dioxin-7-ol (13):

Mixture of compounds 11 and 12 (3g, 21mmol) was dissolved in dry dioxane, and benzaldehyde dimethylacetal (6 mL, 27 mmol), PTSA (200mg, 1.05mmol) was added to it slowly and the reaction was stirred at rt for 24h. Reaction mixture was quenched with ice and stirred for 30 min. Extracted with EtOAc three times, combined organic layer was washed with water and brine solution, dried over Na2SO4 and concentrated in vacuo, followed by silica gel chromatography (pet ether:EtOAc 88:12) to afford (±)-13 in 70% yield.

1H NMR (200 MHz, CDCl3): δ 7.55-7.50 (m, 2H, Aromatic), 7.41-7.36 (m, 3H, Aromatic), 5.89-5.83 (m, 1H, Alkene CH), 5.65 (s, 1H, CHPh), 5.53 (dd, 1H, J = 9.7, 1.5 Hz, Alkene CH), 4.41 (m, 1H, CHO), 4.34 (dd, 1H, J = 10.7, 4.5 Hz, CH2O), 3.89-3.83 (m, 1H, CH-O), 3.70 (t, 1H, J = 11.4 Hz, CH2O), 2.48-2.46 (m, 1H, OH), 2.25-2.16 (m, 1H, CH), 2.03-1.87 (m, 2H, CH2); 13C NMR (50 MHz, CDCl3): δ 138.1, 130.2, 128.6, 128.3, 126.8, 126.1, 102.3, 75.3, 70.6, 65.2, 40.4, 37.2; HRMS: mass calculated for C14H17O3 (M+ H+) 233.1177, observed 233.1173.

(±)-(4aR,8aS)-2-phenyl-8,8a-dihydro-4H-benzo[d][1,3]dioxin-7-one (14):

To a solution of CrO3 (90 mg, 0.9 mmol), Ac2O (0.085 mL, 0.9 mmol), Pyridine (0.14 mL, 1.81 mmol) in dry DCM (15 mL), was added 5mL solution of 13 (200 mg, 0.9 mmol) in DCM stirred for 1.5h at rt. Reaction mixture was filtered on celite and purified by silica gel chromatography (pet ether:EtOAc = 91:9) to afford 14 (185 mg) in 92% yield.

1H NMR (200 MHz, CDCl3): δ 7.55 (m, 2H, Aromatic), 7.43-7.38 (m, 3H, Aromatic), 6.63 (dd, 1H, J = 9.8, 1.8 Hz, Alkene CH), 6.18 (m, 1H, Alkene CH), 5.64 (s, 1H, CHPh), 4.49 (dd, 1H, J = 10.9, 4.5, CH2O), 4.07 (m, 1H, CH-O), 3.81 (t, 1H, J = 11.3 Hz, CH2O), 3.02-2.86 (m, 2H, CH2CH), 2.68 (dd, 1H, J = 16.4, 12.8Hz, CH2); 13C NMR (50MHz,CDCl3): δ 196.8, 145.0, 137.5, 132.1, 129.2, 128.4, 126.1, 101.7,
Chapter 7

76.5, 69.3, 44.5, 40.0; LCMS: mass calculated for C\textsubscript{14}H\textsubscript{14}O\textsubscript{3} (M+ K\textsuperscript{+}) 269.0943, observed 269.1373; IR (CHCl\textsubscript{3}): \textit{v} max 1720, 1610 cm\textsuperscript{-1}.

\((\pm)(4aR,7R,8aS)-2\text{-phenyl}-4a,7,8,8a\text{-tetrahydro-4H-benzo[d][1,3]dioxin-7-ol}(15)\):

To a solution of 14 (165 mg, 0.717 mmol) in 10 mL dry MeOH, was added CeCl\textsubscript{3}.7H\textsubscript{2}O (400 mg, 1.07 mmol) stirred for 1h at rt. NaBH\textsubscript{4} (33 mg, 0.86 mmol) was added in portions, stirred for 2h at rt. Reaction was quenched with crushed ice and stirred for 30 min. Reaction mixture concentrated \textit{in vacuo}, residue was dissolved in EtOAc and washed with water, brine. EtOAc layer was dried over Na\textsubscript{2}SO\textsubscript{4} concentrated \textit{in vacuo} and purified by silica gel chromatography (pet ether:EtOAc = 91:9) to afford 15 (135 mg) in 82% yield.

\textbf{1H NMR (200 MHz, CDC\textsubscript{3}):} \(\delta\) 7.52 (m, 2H, Aromatic), 7.39 (m, 3H, Aromatic), 5.79 (m, 1H, Alkene CH), 5.61 (s, 1H, CHPh), 5.47 (m, 1H, Alkene CH), 4.55 (m, 1H, CHO\textsubscript{H}), 4.32 (dd, 1H, \(J = 10.8, 4.4\) Hz, CH\textsubscript{2}O), 3.76-3.57 (m, 2H, CH-0, CH\textsubscript{2}O), 2.59-2.49 (m, 2H, CH\textsubscript{2},CH), 1.89-1.73 (m, 1H, CH\textsubscript{2}); \textbf{13C NMR (50 MHz, CDC\textsubscript{3}):} \(\delta\) 138.0, 132.8, 129.0, 128.4, 126.2, 124.8, 102.17, 76.5, 70.7, 67.7, 40.0, 38.3; LCMS: mass calculated for C\textsubscript{14}H\textsubscript{16}O\textsubscript{3}Na (M+ Na\textsuperscript{+}) 255.15, observed 255.37.

\textbf{5-methyl-1-((4aR,7S,8aS)-2-phenyl-4a,7,8,8a-tetrahydro-4H-benzo[d][1,3]dioxin-7-yl)pyrimidine-2,4(1H,3H)-dione (18) :}

To a solution of pure ent-15 (300 mg, 1.28 mmol) in 10mL dry dioxane, was added thymine (320 mg, 2.2 mmol) and triphenylphosphine (660 mg, 2.2 mmol), stirred for 15 min at rt. Reaction mixture cooled to ice bath temperature and DIAD added slowly then ice bath removed and reflux the reaction mixture at 100 °C for 3h. Reaction mixture concentrated under reduced pressure and residue was dissolved in EtOAc and washed with water, brine. EtOAc layer was dried over Na\textsubscript{2}SO\textsubscript{4} concentrated \textit{in vacuo} and purified by silica gel chromatography (pet ether:EtOAc = 70:30) to afford 18 (138 mg) in 30% yield.
**Chapter 2**

\( ^1H \text{ NMR of } 17 \) (400 MHz, CDCl\(_3\)) \( \delta \) 1.97 (s, 3 H), 2.19 - 2.26 (m, 2 H), 2.52 - 2.62 (m, 1 H), 3.64 (s, 1 H), 3.79 (t, \( J = 11.17 \) Hz, 1 H), 4.38 - 4.44 (m, 1 H), 5.31 (br. s., 1 H), 5.63 (s, 1 H), 5.66 - 5.72 (m, 1 H), 5.94 (d, \( J = 9.54 \) Hz, 1 H), 7.18 (s, 1 H), 7.33 - 7.41 (m, 3 H), 7.44 - 7.59 (m, 9 H), 7.69 (d, \( J = 7.28 \) Hz, 3 H), 7.67 (d, \( J = 8.03 \) Hz, 2 H), 8.31 (br. s., 1 H) ppm; \( ^13C \text{ NMR (125 MHz, CDCl}_3) \) \( \delta \) 12, 14.2, 34.8, 39.7, 53.0, 70.2, 102.1, 111.8, 126.1, 127.7, 128.3, 129.1, 130.1, 130.4, 131.6, 135.0, 136.1, 137.8, 149.8, 162.7, 168.9 ppm; LCMS: mass calculated for C\(_{19}\)H\(_{20}\)N\(_2\)O\(_4\)Na (M+ Na\(^+\)) 363.1423, observed 363.08.

\( ^1H \text{ NMR 19 (500 MHz, CDCl}_3) \) \( \delta \) 1.82 - 1.90 (m, 1 H), 1.99 (s, 3 H), 2.59 - 2.66 (m, 1 H), 2.69 - 2.77 (m, 1 H), 3.71 (t, \( J = 11.14 \) Hz, 1 H), 3.85 (ddd, \( J = 12.13 \), 9.08, 3.20 Hz, 1 H), 4.35 (dd, \( J = 10.99 \), 4.58 Hz, 1 H), 5.51 - 5.57 (m, 1 H), 5.64 (s, 2 H), 5.80 (d, \( J = 9.77 \) Hz, 1 H), 7.10 (d, \( J = 1.22 \) Hz, 1 H), 7.35 - 7.41 (m, 3 H), 7.47 - 7.54 (m, 5 H), 7.63 - 7.68 (m, 1 H), 7.93 (dd, \( J = 8.39 \), 1.37 Hz, 2 H) ppm; LCMS: mass calculated for C\(_{19}\)H\(_{20}\)N\(_2\)O\(_4\)K (M+ K\(^+\)) 379.1423, observed 379.06.

9-((4aS,7S,8aR)-2-phenyl-4a,7,8,8a-tetrahydro-4H-benzo[d][1,3]dioxin-7-yl)-9H-purin-6-amine (20):

To a solution of pure enantiomer 17 (150 mg, 0.64 mmol) in 10mL dry dioxane, was added adenine (175 mg, 1.28 mmol) and triphenylphosphine (340 mg, 1.28 mmol), stirred for 15 min at rt. Reaction mixture cooled to ice bath temperature and DEAD (0.02 mL, 1.28 mmol) added slowly the removed the ice bath and stirred the reaction mixture at rt for overnight. Reaction mixture concentrated under reduced pressure and residue was dissolved in EtOAc and washed with water, brine. EtOAc layer was dried over Na\(_2\)SO\(_4\) concentrated in vacuo and purified by silica gel chromatography (methanol:DCM = 5:95) to afford 20 (86 mg) in 38% yield.

\( ^1H \text{ NMR (500 MHz, CDCl}_3) \) \( \delta \) 2.04 (d, \( J = 7.63 \) Hz, 1 H), 2.16 (q, \( J = 11.90 \) Hz, 1 H), 2.74 - 2.86 (m, 2 H), 3.75 (t, \( J = 11.29 \) Hz, 1 H), 3.95 (ddd, \( J = 12.21 \), 9.16, 3.05 Hz, 1
Chapter 2

H), 4.37 (dd, J=10.83, 4.43 Hz, 1 H), 5.59 (tdd, J=8.74, 8.74, 4.20, 2.14 Hz, 1 H), 5.67 (s, 1 H), 5.74 - 5.83 (m, 2 H), 7.33 - 7.41 (m, 3 H), 7.51 (dd, J=7.78, 1.68 Hz, 2 H), 7.87 (s, 1 H) ppm; $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 36.3, 39.8, 51.5, 70.4, 102.2, 114.1, 119.6, 126.1, 127.7, 128.4, 128.6, 129.1, 137.8, 138.4, 139.3, 149.7, 153.0, 155.6 ppm; LCMS: mass calculated for C$_{19}$H$_{20}$N$_5$O$_2$ (M+ H$^+$) 350.16, observed 350.12.

(1R,2S,5S)-5-(6-amino-9H-purin-9-yl)-2-(hydroxymethyl)cyclohex-3-en-1-ol (21):

Compound 20 (100 mg, 0.27 mmol) was dissolved in 80% aq CH$_3$COOH solution and stirred reaction mixture for 8 h at 60 °C. Reaction mixture concentrated under reduced pressure and residue was dissolved in EtOAc and washed with water, brine. EtOAc layer was dried over Na$_2$SO$_4$ concentrated in vacuo and purified by silica gel chromatography (methanol:DCM = 10:90) to afford 21 (47 mg) in 65% yield.

$^1$H NMR (200 MHz, DMSO-$d_6$) $\delta$ 1.95 - 2.08 (m, 2 H), 2.23 (br. s., 2 H), 3.61 - 3.82 (m, 3 H), 4.67 (br. s., 1 H), 4.95 (br. s., 1 H), 5.21 (br. s., 1 H), 5.69 (d, J=8.46 Hz, 1 H), 5.92 (d, J=8.72 Hz, 1 H), 7.25 (br. s., 2 H), 8.13 (br. s., 2 H) ppm; LCMS: mass calculated for C$_{12}$H$_{15}$N$_5$O$_2$K (M+ K$^+$) 300.13, observed 300.98.

N-(6-chloro-9H-purin-2-yl)isobutyramide (23):

To a solution of commercially available 2-amino-6-chloropurine (1g, 6 mmol) in N,N-dimethylacetel 30 mL, was added isobutyric anhydride (2.7 mL, 15 mmol) and reflux the reaction mixture for 2 h at 150 °C, cooled to room temperature and evaporated under reduced pressure to 1/10 of its volume. The precipitated crude product was collector and crystallized from boiling of ethanol/water (1:1, 200 mL) to yield 23 (710 mg).

$^1$H NMR (200 MHz, CD$_3$OD) $\delta$ 1.23 (d, J=6.95 Hz, 6 H), 2.71 - 2.88 (m, 1 H), 8.40 (s, 1 H) ppm; LCMS: mass calculated for C$_9$H$_{10}$ClN$_5$ONa (M+ Na$^+$) 262.04, observed 261.84.
Chapter 2

N-(6-chloro-9-((4aS,7S,8aR)-2-phenyl-4a,7,8,8a-tetrahydro-4Hbenzo[d][1,3]dioxin-7-yl)-9H-purin-2-yl)isobutyramide (24):

To a solution of 17 (100 mg, 0.43 mmol) in 10mL dry THF, was added 23 (205 mg, 0.86 mmol) and triphenylphosphine (225 mg, 0.86 mmol), stirred for 15 min at rt. Reaction mixture cooled to ice bath temperature and DIAD added slowly the removed the ice bath and reflux the reaction mixture stirred at room temperature for overnight. Reaction mixture concentrated under reduced pressure and residue was dissolved in EtOAc and washed with water, brine. EtOAc layer was dried over Na2SO4 concentrated in vacuo and purified by silica gel chromatography (pet ether:EtOAc = 70:30) to afford 24 (67 mg) in 35% yield.

1H NMR (200 MHz, CDCl3) δ 1.28 (s, 3 H), 1.32 (s, 3 H), 2.71 - 3.01 (m, 3 H), 3.75 (t, J=11.18 Hz, 1 H), 3.96 (ddd, J=12.22, 9.19, 3.09 Hz, 1 H), 4.38 (dd, J=10.80, 4.48 Hz, 1 H), 5.57 - 5.71 (m, 2 H), 5.79 (s, 2 H), 8.07 (s, 1 H), 8.20 (s, 1 H) ppm; 13C NMR (50 MHz, CDCl3) δ 19.3, 36.2, 39.7, 52.0, 70.3, 76.4, 102.2, 126.1, 127.0, 128.4, 128.6, 129.1, 129.3, 131.5, 132.0, 132.2, 133.6, 137.7, 142.6, 152.0, 152.4, 160.3, 175.5 ppm; LCMS: mass calculated for C23H26ClN5O2Na (M+ Na+) 462.94.13, observed 463.25.

N-(9-((1S, 4S, 5R)-5-hydroxy-4-(hydroxymethyl)cyclohex-2-en-1-yl)-6-oxo-6,9 dihydro-1H-purin-2-yl)isobutyramide (25):

Compound 24 (100 mg, 0.22 mmol) was dissolved in 80% aq CH3COOH solution and stirred reaction mixture for 8h at 60 °C. Reaction mixture concentrated under reduced pressure and residue was purified by silica gel chromatography (methanol:DCM = 10:90) to afford 25 (52 mg) in 68% yield.

1H NMR (200 MHz, CDCl3) δ 1.28 (s, 7 H), 2.28 - 2.53 (m, 2 H), 2.53 - 2.83 (m, 2 H), 3.60 - 3.96 (m, 3 H), 5.18 - 5.35 (m, 1 H), 5.81 (d, J=10.23 Hz, 1 H), 6.01 (dt, J=10.11, 2.27 Hz, 1 H), 8.02 (br. s., 1 H) ppm; LCMS: mass calculated for C16H21N3O4Na (M+ Na+) 370.37, observed 370.11.

### Compounds - Spectral data

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (^1)H NMR &amp; (^{13})C NMR</td>
<td>57</td>
</tr>
<tr>
<td>2 DEPT NMR &amp; LC MS</td>
<td>58</td>
</tr>
<tr>
<td>3 (^1)H NMR &amp; (^{13})C NMR</td>
<td>59</td>
</tr>
<tr>
<td>3 DEPT NMR &amp; 4 (^1)H NMR</td>
<td>60</td>
</tr>
<tr>
<td>4 (^{13})C NMR &amp; DEPT NMR</td>
<td>61</td>
</tr>
<tr>
<td>7 (^1)H NMR &amp; (^{13})C NMR</td>
<td>62</td>
</tr>
<tr>
<td>7 DEPT NMR &amp; LC MS</td>
<td>63</td>
</tr>
<tr>
<td>8 (^1)H NMR &amp; (^{13})C NMR</td>
<td>64</td>
</tr>
<tr>
<td>8 DEPT NMR &amp; LC MS</td>
<td>65</td>
</tr>
<tr>
<td>9 + 10 (^1)H NMR &amp; (^{13})C NMR</td>
<td>66</td>
</tr>
<tr>
<td>9 + 10 COSY</td>
<td>67</td>
</tr>
<tr>
<td>9 + 10 LC MS</td>
<td>68</td>
</tr>
<tr>
<td>12 (^1)H NMR &amp; (^{13})C NMR</td>
<td>69</td>
</tr>
<tr>
<td>12 DEPT NMR &amp; LC MS</td>
<td>70</td>
</tr>
<tr>
<td>13 (^1)H NMR &amp; (^{13})C LC MS MR</td>
<td>71</td>
</tr>
<tr>
<td>13 DEPT NMR &amp; LC MS</td>
<td>72</td>
</tr>
<tr>
<td>14 (^1)H NMR &amp; (^{13})C NMR</td>
<td>73</td>
</tr>
<tr>
<td>14 DEPT NMR &amp; LC MS</td>
<td>74</td>
</tr>
<tr>
<td>15 (^1)H NMR &amp; (^{13})C NMR</td>
<td>75</td>
</tr>
<tr>
<td>15 DEPT NMR &amp; LC MS</td>
<td>76</td>
</tr>
<tr>
<td>18 (^1)H NMR &amp; (^{13})C NMR</td>
<td>77</td>
</tr>
<tr>
<td>18 DEPT NMR &amp; LC MS</td>
<td>78</td>
</tr>
<tr>
<td>19 (^1)H NMR &amp; LC MS</td>
<td>79</td>
</tr>
<tr>
<td>20 (^1)H NMR &amp; (^{13})C NMR</td>
<td>80</td>
</tr>
<tr>
<td>20 DEPT NMR &amp; 21 (^1)H NMR</td>
<td>81</td>
</tr>
<tr>
<td>21 LC MS &amp; 23 (^1)H NMR</td>
<td>82</td>
</tr>
<tr>
<td>23 LC MS &amp; 24 (^1)H NMR</td>
<td>83</td>
</tr>
<tr>
<td>24 (^{13})C NMR &amp; DEPT NMR</td>
<td>84</td>
</tr>
<tr>
<td>24 LC MS &amp; 25 (^1)H NMR</td>
<td>85</td>
</tr>
<tr>
<td>25 (^{13})C NMR &amp; DEPT NMR</td>
<td>86</td>
</tr>
<tr>
<td>25 LC MS</td>
<td>87</td>
</tr>
</tbody>
</table>
Chapter 2
Chapter 2

![NMR Spectra](image)

**Chemical Shift (ppm)**

- Chloroform-d
- [Diagram showing chemical shifts and normalized intensity]

![1H NMR Spectrum](image)

**Chemical Shift (ppm)**

- [Diagram showing chemical shifts and normalized intensity]
Chapter 2

Elemental composition calculator

Target m/z: 297.1113 amu
Tolerance: +5.0000 ppm
Result type: Elemental
Max num of results: 100
Min DBE: -0.5000 Max DBE: +50.0000
Electron state: OddAndEven
Num of charges: 0
Add water: N/A
Add proton: N/A
File Name: 10MAR2011.wiff

<table>
<thead>
<tr>
<th>Elements</th>
<th>Min Number</th>
<th>Max Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>H</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Na</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>O</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

Formulas | Calculated m/z (amu) | mDa Error | PPM Error | DBE |
-------|----------------------|-----------|-----------|-----|
1      | C16 H18 O4 Na        | 297.1102  | 1.0210    | 3.4364 | 7.5 |
### Elemental Composition Calculator

| Target m/z: | +245.0812 amu |
| Tolerance: | +5.0000 ppm |
| Result type: | Elemental |
| Max num of results: | 100 |
| Min DBE: | -0.5000 |
| Max DBE: | +50.0000 |
| Electron state: | OddAndEven |
| Num of charges: | 0 |
| Add water: | N/A |
| Add proton: | N/A |
| File Name: | 10MAR2011.wiff |

<table>
<thead>
<tr>
<th>Elements</th>
<th>Min Number</th>
<th>Max Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>N</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Na</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>O</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Formula</th>
<th>Calculated m/z (amu)</th>
<th>mDa Error</th>
<th>PPM Error</th>
<th>DBE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14 H13 O4</td>
<td>245.0813</td>
<td>-0.1840</td>
<td>-0.7510</td>
<td>8.5</td>
</tr>
</tbody>
</table>
Chapter 2

![Chemical Shift Diagram](image1)

![Chemical Shift Diagram](image2)
### Elemental Composition Calculator

- **Target m/z:** +233.1173 amu
- **Tolerance:** +5.0000 ppm
- **Result type:** Elemental
- **Max num of results:** 100
- **Min DBE:** -0.5000  **Max DBE:** +50.0000
- **Electron state:** OddAndEven
- **Num of charges:** 0
- **Add water:** N/A
- **Add proton:** N/A
- **File Name:** 10MAR2011.wiff

<table>
<thead>
<tr>
<th>Elements</th>
<th>Min Number</th>
<th>Max Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>H</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Na</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>O</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Formula</th>
<th>Calculated m/z (amu)</th>
<th>mDa Error</th>
<th>PPM Error</th>
<th>DBE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14 H17 O3</td>
<td>233.1177</td>
<td>-0.4696</td>
<td>-2.0144</td>
<td>6.5</td>
</tr>
</tbody>
</table>

---

Chapter 2

---

Chapter 2

**Chemical Shift (ppm)**

[Image: Spectroscopy data for compound ent-15 in Chloroform-d]

- Chemical Shift (ppm): 7.54, 7.32, 7.50, 7.39, 7.46, 7.36, 7.27
- Normalized Intensity: 1.00, 0.75, 0.50, 0.25, 0.10

**ent-15**

- Structure: Hexagonal ring with OH and Ph groups

---

Chapter 2

Chemical Shift (ppm)

Normalized Intensity

m/z

Relative Abundance

Chapter 2

[Diagrams of NMR spectra showing chemical shifts and normalized intensities for compounds 20 and another compound with NH2 functional group.

The first diagram shows normalized intensity on the y-axis and chemical shift (ppm) on the x-axis. The second diagram is similar but shows chemical shift (ppm) on a broader scale.]

Chapter 2

[Chemical Shift Diagrams]

Chapter 2

![Chemical Structure Diagram]

- Molecular formula: 
- Relative Abundance:
- m/z values: 0, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100
- Mass values: 130.24, 152.04, 222.03, 244.01, 249.03, 284.21, 319.13, 348.13, 402.09, 442.14, 489.18, 553.41, 580.19

Chapter 2

2.12 References


Chapter 2


