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Robust synthesis of enantiopure cyclohexenyl analogues of 2/3-deoxyribose sugars as carbocyclic nucleoside precursors

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ABSTRACT

An expedient synthesis of 2-deoxy (**10**) and 3-deoxy (**11**) cyclohexenyl analogues of 2-deoxy and 3-deoxy-D-ribose sugar from commercially available starting materials is reported. Highly efficient enzymatic resolution of the key compound **10** is described using lipase under hydrolytic conditions. The robust methodology applied here will be useful to synthesize cyclohexenyl nucleosides, which possess potent antiviral activity and are capable of gene silencing via RNAi or antisense applications.

1. Introduction

Investigations on synthetic oligonucleotides that can mimic the properties of oligoribonucleotides have been much focused on the synthesis of conformationally restricted *N*-type nucleoside analogues.[1] The LNA (Locked Nucleic Acids) are the best examples that show very high DNA/RNA duplex stability due to its locked *N*-type sugar conformation.[2] In six-membered ring analogues, the cyclohexene nucleosides are structurally considered as one of the best mimics of natural furanose nucleosides (Fig. 1),[3] as the conformational equilibrium and the values of the thermodynamic parameters are very similar between a cyclohexenyl nucleoside (ΔG 1.8 kJ/mol between $^2H_3$ or *S*-type and $^3H_2$ or *N*-type and equilibrium occurs via the eastern hemisphere with a barrier of 10.9 kJ/mol) and in natural ribose nucleosides (difference between *N*-type and *S*-type is 2 kJ/mol, and equilibrium occurs via the eastern hemisphere with a barrier of 4–20 kJ/mol).[4] Also, the $\pi-\sigma^*$ interaction mimics the anomeric effect in furanose nucleoside.[5] At the nucleoside level the CeNA (Cyclohexenyl Nucleic Acids) exists in both *N*-type and *S*-type sugar conformations due to highly flexible cyclohexene ring with low energy barrier between the two forms. The cyclohexenyl nucleosides have exhibited potent antiviral activity and the CeNA oligomers have been shown to mimic the function of RNA with increased enzymatic and chemical stability.[6] Additionally, the conformational flexibility allowed CeNA oligomers to have tremendous application in antisense therapeutics because of the consequential ability to induce RNase-H activity[7] as compared to any other modified nucleic acid oligomers except for the FANAs.[8] They have also been shown to be useful in siRNA applications.[9] The main limitation that has stymied the scope of this highly valuable discovery is the lack of a robust and scalable synthetic strategy that would ensure access to the useful 2′-deoxyribose sugar analogue **14** as an intermediate in enantiomerically pure form. There are two synthetic methodologies that have been applied so far. In the first case, *R*-(−)-carvone[10] was used as starting material in a multistep synthesis to obtain both D and L isomers but the overall yield of this synthesis was low (2–3%), which is not

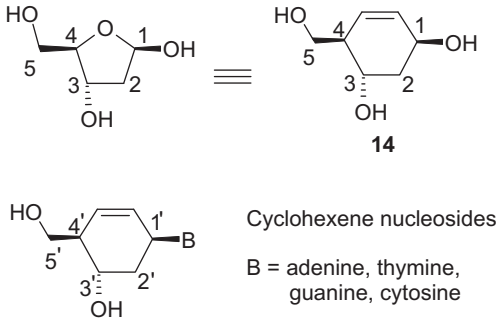

Fig. 1. Cyclohexene mimics of 2-deoxyribose and cyclohexene nucleosides.

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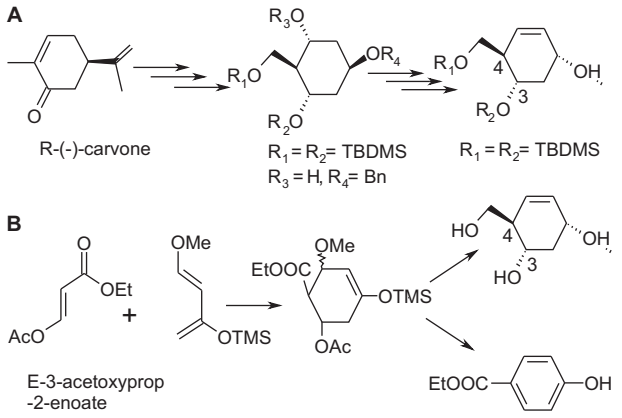
practical for oligonucleotide work (Scheme 1, A). Another improved method involved a synthetic approach[11] to the sugar precursor in the racemic form starting from ethyl (2*E*)-3-acetoxyprop-2-enoate as dienophile[12] and Danishefsky's diene[13] in a Diels–Alder reaction to construct the six-membered ring skeleton (Scheme 1, B).



Scheme 1. Reported synthetic methods.

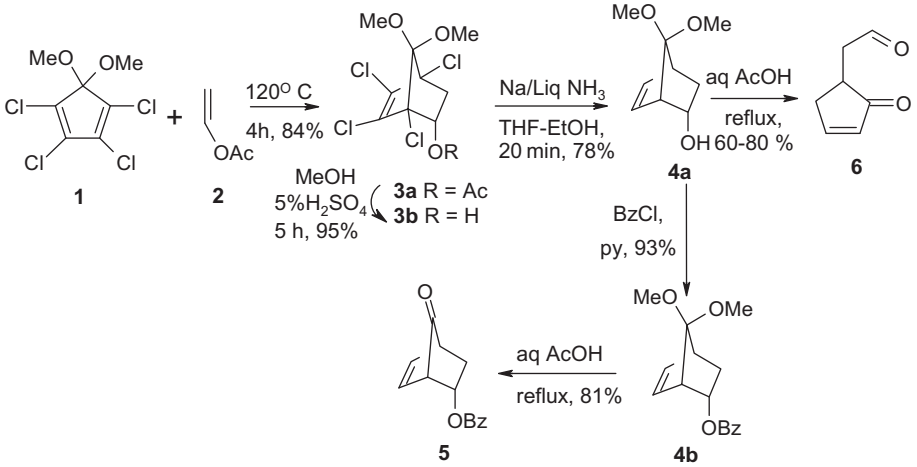
We have been recently involved in developing synthetic approaches towards conformationally restricted analogues of 2′-5′ *iso*DNA for their applications in antisense research.[14] During a careful search of the literature for the synthesis of isomeric carbocyclic mimic of 3-deoxyribose and the analogues nucleoside for its incorporation in 2′-5′ *iso*DNA, we came across a very interesting report[15] about the synthesis of carbasugars and confused carbasugars from commercially available starting materials. The required 2-deoxy and 3-deoxy cyclohexenyl analogues of 2-deoxy and 3-deoxyribose sugars in racemic form were found to be the intermediate compounds in this synthesis. We report in this article the optimized synthetic protocols to achieve the synthesis of cyclohexene containing mimic of 2-deoxy-(D,L)-ribose sugar, cyclohexene containing mimic of 3-deoxy-(D,L)-ribose and an important cyclohexene analogue of 2-deoxy-L-ribose sugar with inverted configuration at C4 centre. We also report the enzymatic resolution of the Diels–Alder adducts as well as that of the benzylidene protected intermediate to obtain enantiomerically pure carbocyclic sugars.

2. Results and discussion

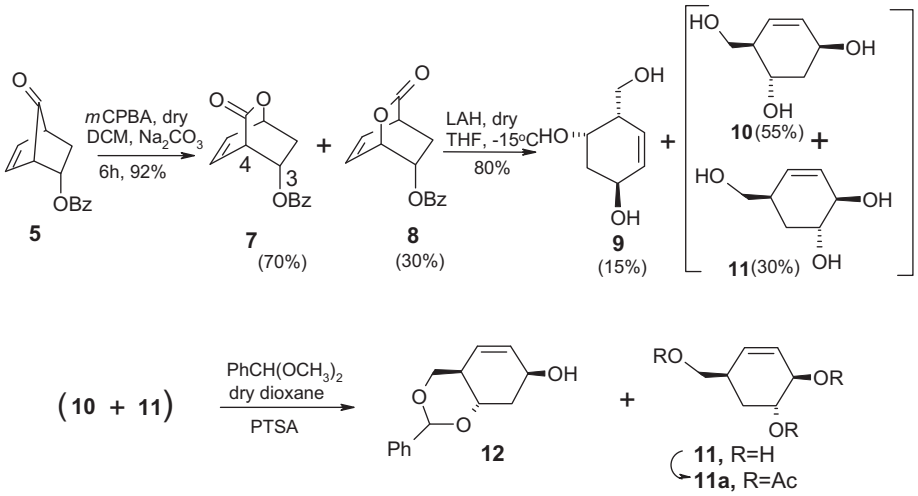
Diels–Alder reaction between commercially available 5,5-dimethoxy-1,2,3,4-tetrachlorocyclopentadiene **1** and vinyl acetate **2** at 120 °C gave exclusively the *endo* adduct (±)-**3a**.[15] The ester group in (±)-**3a** was hydrolyzed under acidic conditions to get the free alcohol (±)-**3b**. Compound (±)-**3b** was then subjected to reductive dehalogenation under Birch conditions to get (±)-**4a**. The purpose of the acetate hydrolysis prior to dehalogenation was to improve the yields of the dehalogenation reaction (Scheme 2).[16] The bicyclic system and the formation of *endo* adduct set the desired trans-relationship of the substituents corresponding to the position 3 and 4 in ribose sugar. The ketal hydrolysis of compound (±)-**4a** to get the free ketone (±)-**5** proved to be difficult as we found that the formation of compound (±)-**6** was the major product under variety of ketal hydrolysis conditions. We employed different protecting groups for the secondary hydroxyl group in (±)-**4a**, such as TBDMS, benzyl or acetate[15] but each time compound (±)-**6** was formed as a major product in acidic hydrolysis. The silyl ether, benzyl ether and the acetate protecting group probably underwent hydrolytic cleavage, concomitant with the ketal hydrolysis and as a consequence, (±)-**6** is obtained as the major fragmentation product. A report was found in the literature where a similar retro aldol type of rearrangement was observed and a similar major reaction product was obtained.[17] Then we used benzoyl protection as suggested by Sgarbi and Clive[18] and found that the aqueous acetic acid reflux conditions gave a clean ketal hydrolyzed product (±)-**5** in 81% yield. The benzoyl protection was found to be stable under these conditions and the rearranged product (±)-**6** was not observed. As expected, the Baeyer–Villiger's oxidation of ketone (±)-**5** gave two regioisomeric inseparable mixture of lactones (±)-**7** and (±)-**8** in 7:3 proportion, respectively, in high yield (Scheme 3). It was confirmed by 2D COSY NMR spectroscopy (Supplementary data).

The mixture of lactones was reduced with $LiAlH_4$ to result (±)-**9** (the epimerized product of (±)-**10**) and inseparable mixture of trihydroxy substituted cyclohexene derivatives (±)-**10** as 2′-deoxyribosugar analogue and (±)-**11** as 3′-deoxyribosugar analogue.

Epimeric triol (±)-**9** could be separated by column chromatography and was characterized by NMR and mass spectroscopy. The mixture of regioisomers (**10** and **11**) obtained was subjected to chemoselective 1,3-diol protection and only compound (±)**10** was protected to furnish (±)**12** quantitatively. The unreacted triol (±)-**11** could be then easily separated chromatographically from (±)-**12**. After purification, the structure of triol (±)-**11** was

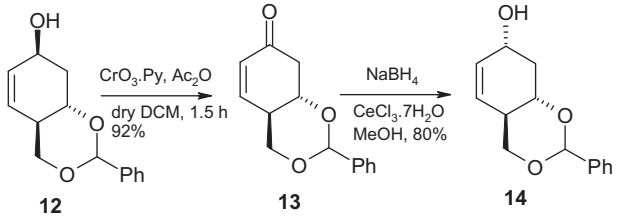


Scheme 2. Synthesis of desired dehalogenated *endo* adduct **5**.



Scheme 3. Synthesis and separation of the triols **9**, **11** and protected **12**.

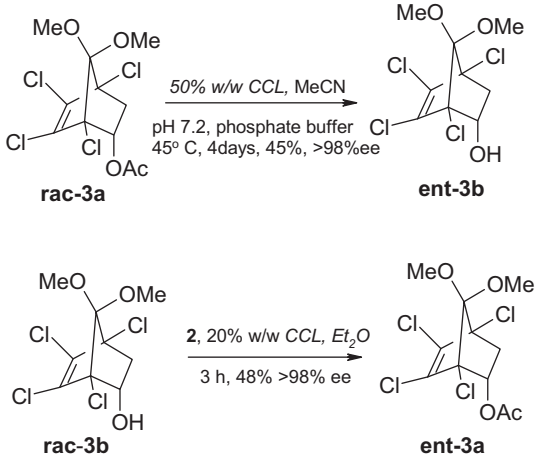
confirmed by converting it to the known triacetate (±)-**11a**.[15] All the three isomers were easily purified in very good yield. The separation protocol employed here avoids the difficult chromatographic separation[15] of the two regioisomers **7** and **8**, which had to be further individually processed to get **9**, **10** and **11**. With this we completed the racemic synthesis of both the 3-deoxy-cyclohexenyl sugar analogues (±)-**11** and benzylidene protected 2-deoxy-cyclohexenyl sugar analogues (±)-**12** in high yields. Compound (±)-**12** was then oxidized using CrO_3/pyridine in less than 3 h to get the enone (±)-**13**. The reported MnO_2 oxidation reported earlier[11] takes very long time and depends mainly on the quality of MnO_2 used in the reaction. The use of CrO_3 reduces the reaction time and is highly reproducible. Compound (±)-**13** was then subjected to Luche reduction to get epimeric alcohol (±)-**14** using $NaBH_4$ in the presence of $CeCl_3 \cdot 7H_2O$ (Scheme 4).[11] Compound (±)-**14** can then be converted to the cyclohexenyl nucleosides units using reported procedures.[19]



Scheme 4. Epimerization at C-1.

After achieving the synthesis of racemic **9**, **11**, **12** and **14**, we undertook the enantiopure synthesis of these sugar analogues. Enzymatic resolution of compound (±)-**3b** is known in the literature using *Candida cylindracea* lipase (CCL) and vinyl acetate as an acyl donor.[20]

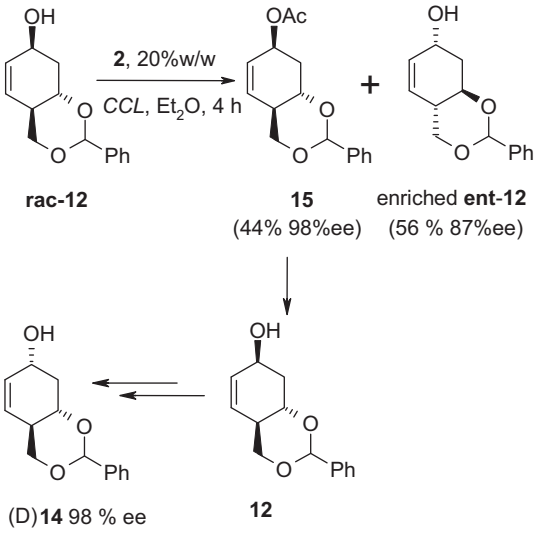
In our laboratory this reaction was very sluggish, took almost 7 days to complete and could not be scaled up. As the acylation was sluggish but enantiospecific, we thought of doing enzymatic hydrolysis of (±)-**3a** using the same lipase enzyme. Use of acetonitrile as co-solvent along with phosphate buffer (pH 7.2) gave very good enantioselective hydrolysis in 4 days (Scheme 5, yield 45%, ee 98%). Continuing the hydrolysis of the remaining enantiomerically enriched acetate for 24 h, the other isomer as unreacted acetate **3a** was obtained in good yield and high ee (yield 42%, ee 98%). Alternatively, the improvement of the resolution time could 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 3 h (Scheme 5). The enantiomeric identity of the resolved acetate **3a** and *ent*-**3b** was established after comparing with the known compound and the enantiomeric purity was established by chiral HPLC analysis and ^{1}H NMR using chiral shift reagent (Supplementary data). Following the same reactions as in Schemes 2–4, compounds **9**, **11**, **12** and **14**, can now be synthesized in enantiomerically pure form from enantiomerically pure Diels–Alder adducts **3a**/**b**.



Scheme 5. Enzymatic resolution of **3a**/**3b**.

As the enzymatic resolution at such an early stage to get the different sugar analogues, such as **14** and enantiopure compound **12**, *ent*-**12** would be time consuming, we embarked to resolve the two enantiomers of (±)-**12** using enzymatic acylation. Compound (±)-**12** was subjected to acylation using vinyl acetate as donor and *C. cylindracea* lipase (CCL) to get **15** in excellent yield and high enantiomeric purity (Scheme 6). The high enantiomeric purity was established by ^{1}H NMR using chiral shift reagent (Supplementary data). The enantiomeric identity of the resolved acetate **15** and *ent*-**12** was established after converting the acetate **15** (hydrolysis of the acetate followed by oxidation and reduction as described in Scheme 4) to the known compound, i.e., enantiomerically pure D-isomer **14** and comparing the reported[19] HPLC retention time on chiral HPLC column (Supplementary data). The enriched *ent*-**12** was

further treated under same enzymatic conditions and *ent*-**12** could be isolated by silica gel chromatography. Synthesis of pure 2′deoxy-D-sugar analogue *ent*-**12** was not possible by the earlier reported route.[11]



Scheme 6. Synthesis of **14** via enzymatic resolution and epimerization of **12**.

3. Conclusions

In conclusion, this paper outlines a robust method to synthesize important cyclohexenyl-2-deoxyribose sugar and 3-deoxyribose sugar analogues in excellent yields from commercially available starting materials. The synthesis of both the sugars in enantiopure form can be achieved from the resolved DA adducts. The enzymatic resolution of cyclohexene analogue of 2-deoxyribose sugar was achieved in high yields and enantiomeric purity. The easy access to these sugars as outlined in this paper would allow further exploitation of the cyclohexenyl as well as cyclohexane nucleic acid analogues. 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.[21]

4. Experimental section

4.1. General

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 GF $_{254}$ 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 *C. cylindracea* lipase was purchased from Ltd. Aldrich Inc.

*4.1.1. (±)(1S,2S,4R)-1,4,5,6-Tetrachloro-7,7-dimethoxybicyclo[2.2.1]hept-5-en-2-yl acetate (**3a**).* 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 5 h. 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 (±)-**3a** (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.

*4.1.2. (±) (1S,2S,4R)-1,4,5,6-Tetrachloro-7,7-dimethoxybicyclo[2.2.1]hept-5-en-2-ol (**3b**).* To a solution of (±)-**3a** (20 g, 57.14 mmol) in methanol (150 mL) was added 5% aqueous H_2SO_4 (40 mL) and stirred at 65 °C for 5 h. Methanol was removed on rotavapor in vacuo. The residue was diluted with EtOAC and water, saturated aqueous $NaHCO_3$ and brine wash were given. Organic layer was dried over Na_2SO_4, filtered, concentrated in vacuo and purified by silica gel chromatography (pet. ether/EtOAc=92:8) to result **3b** (16.8 g) in 95% yield as a white solid, along with the recovered **3a** (400 mg, 2% yield).

^{1}H NMR (200 MHz, $CDCl_3$): 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, CH_2), 2.12 (d, 1H, J=4.8 Hz), 1.79 (dd, 1H, J=12.3, 2.4 Hz, CH_2); ^{13}C NMR (200 MHz, $CDCl_3$): δ 130.8, 127.3, 112.0, 79.8, 76.4, 74.2, 52.5, 51.6, 44.2.

*4.1.3. (±)(1S,2S,4S)-7,7-Dimethoxybicyclo[2.2.1]hept-5-en-2-ol (**4a**).* To a suspension of liquid ammonia (700 mL) and sodium (4.78 g, 207.8 mmol) at −78 °C, solution of (±)-**3b** (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 NH_4Cl solution and kept at rt 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 Na_2SO_4, filtered, concentrated in vacuo and purified by silica gel chromatography (pet. ether/EtOAc=85:15) to result (±)-**4a** (3.4 g) in 78% yield as a pale yellow thick liquid.

^{1}H NMR (200 MHz, $CDCl_3$): 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, CH_2), 0.85 (dd, 1H, J=12.3, 2.2 Hz, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ 137.9, 128.3, 119.2, 76.7, 70.2, 51.6, 50.7, 49.6, 45.6, 36.2.

*4.1.4. (±) (1S,2S,4S)-7,7-Dimethoxybicyclo[2.2.1]hept-5-en-2-yl benzoate (**4b**).* To a solution of (±)-**4a** (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 4 h. 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_2SO_4, concentrated in vacuo. The residue was purified by silica gel chromatography (pet. ether/EtOAc=89:11) to afford (±)-**4b** (13.5 g) in 93% yield.

^{1}H NMR (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, CHOBz), 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 $C_{16}H_{18}O_4Na$ $(M+Na)^+$ 297.1102, observed 297.1113; IR ($CHCl_3$): ν_{max} 2950, 1720, 1602 cm^{-1}

*4.1.5. (±) (1S,2S,4S)-7-Oxobicyclo[2.2.1]hept-5-en-2-yl benzoate (**5**).* Solution of (±)-**4b** (4.5 g, 16.4 mmol) in 180 mL of 6/1 mixture of acetic acid/water was refluxed for 4 h. 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_2SO_4, concentrated in vacuo. ^{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, CHOBz), 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, IR ($CHCl_3$): ν_{max} 3064, 2947, 1790, 1720, 1600 cm^{-1}.

*4.1.6. (±) (1S,4S,8S,)-3-Oxo-2-oxabicyclo[2.2.2]oct-5-en-8-yl benzoate (**7**).* To a solution of (±)-**5** (5 g, 21.1 mmol) in 100 mL of dry DCM, was added Na_2CO_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 6 h at rt. The reaction mixture was quenched with 10% aqueous solution of $Na_2S_2O_5$ (30 mL). Organic layer was separated and aqueous layer was extracted with DCM. The combined organic layer was washed with saturated aqueous $NaHCO_3$ followed by brine, and dried over anhydrous Na_2SO_4. The organic layer was concentrated in vacuo followed by silica gel chromatography (pet. ether/EtOAc=90:10) to give a mixture of **7** and **8** (92%) in 70:30 ratio along with the recovered **5** (4%).

^1H NMR (200 MHz, $CDCl_3$): δ 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), 2.85 (m, 1H, major), 2.60 (m, 1H, minor), 1.75 (d, *J*=6.0 Hz, 1H, major), 1.59 (m, 1H, minor); ^{13}C NMR (50 MHz, $CDCl_3$): δ 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 $(M+H)^+$ 245.0812; IR ($CHCl_3$): ν_{max} 1759,1716 cm^{-1}

*4.1.7. (±) (1S,3S,6R)-6-(Hydroxymethyl) cyclohex-4-ene-1,3-diol (**10**).* To a solution of mixture **7**,**8** (3.3 g, 13.5 mmol) in dry THF (400 mL) at −15 °C, LAH (1.5 g, 40.5 mmol) was added and the resulting mixture was stirred at the same temperature for 2 h. The reaction mixture was then quenched cautiously with ethyl acetate (50 mL) followed by saturated aqueous solution of Na_2S0_4, to precipitate out aluminium salts. After the filtration, filtrate was concentrated in vacuo, to result epimeric mixture of **9**, **10** and the other regioisomer **11**. The mixture was purified by silica gel chromatography (MeOH/EtOAc=1:99) to give pure **9** (15%) and a mixture of **10** and **11** in 85% yield, which was used further without separation.

^1H NMR (200 MHz, $CDCl_3$) of compound **9**: δ 5.86–5.72(m, 2H, Alkene CH), 4.38–4.32 (m, 1H, allylic CH–O), 4.14–4.08 (m, 1H, CH_2O), 3.87–3.79 (m, 1H, CH_2O), 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, $CDCl_3$): δ 130.5, 127.9, 66.1, 65.4, 61.7, 41.5, 35.3. LCMS: mass calculated for $C_7H_{12}O_3Na$ $(M+Na)^+$ 167.06, observed $(M+Na)^+$ 167.17.

*4.1.8. (±) (4aR,7S,8aS)-2-Phenyl-4a,7,8,8a-tetrahydro-4H-benzo[d][1,3]dioxin-7-ol (**12**).* Mixture of compounds **10** and **11** (3g, 21 mmol) was dissolved in dry dioxane, and benzaldehyde dimethylacetal (6 mL, 27 mmol), PTSA (200 mg, 1.05 mmol) was added to it slowly and the reaction was stirred at rt for 24 h. 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 Na_2SO_4 and concentrated in vacuo, followed by silica gel chromatography (pet. ether/EtOAc=88:12) to afford (±)-**12** in 70% yield.

^1H NMR (200 MHz, $CDCl_3$): 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, CHOH), 4.34 (dd, 1H, *J*=10.7, 4.5 Hz, CH_2O), 3.89–3.83 (m, 1H, CH–O), 3.70 (t, 1H, *J*=11.4 Hz, CH_2O), 2.48–2.46 (m, 1H, OH), 2.25–2.16 (m, 1H, CH), 2.03–1.87 (m, 2H, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ 138.1, 130.2, 128.9, 128.3, 126.8, 126.1, 102.3, 75.3, 70.6, 65.2, 40.4, 37.2; HRMS: mass calculated for $C_{14}H_{17}O_3$ $(M+H)^+$ 233.1177, observed $(M+H)^+$ 233.1173.

*4.1.9. (±) (4aR,8aS)-2-Phenyl-8,8a-dihydro-4H-benzo[d][1,3]dioxin-7(4aH)-one (**13**).* To a solution of CrO_3 (90 mg, 0.9 mmol), Ac_2O (0.085 mL, 0.9 mmol), pyridine (0.14 mL, 1.81 mmol) in dry DCM (15 mL), was added 5 mL solution of 12 (200 mg, 0.9 mmol) in DCM stirred for 1.5 h at rt. Reaction mixture was filtered on Celite and purified by silica gel chromatography (pet. ether/EtOAc=91:9) to afford **13** (185 mg) in 92% yield.

^1H NMR (200 MHz, $CDCl_3$): 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 Hz, CH_2O), 4.07 (m, 1H, CH–O), 3.81 (t, 1H, *J*=11.3 Hz, CH_2O), 3.02–2.86 (m, 2H, CH_2CH), 2.68 (dd, 1H, *J*=16.4, 12.8 Hz, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ 196.8, 145.0, 137.5, 132.1, 129.2, 128.4, 126.1, 101.7, 76.5, 69.3, 44.5, 40.0; IR ($CHCl_3$) : ν_{max} 1720, 1610 cm^{-1}.

*4.1.10. (±) (4aR,7R,8aS)-2-Phenyl-4a,7,8,8a-tetrahydro-4H-benzo[d][1,3]dioxin-7-ol (**14**).* To a solution of **13** (165 mg, 0.717 mmol) in 10 mL dry MeOH, was added $CeCl_3{\cdot}7H_2O$ (400 mg, 1.07 mmol) stirred for 1 h at rt. $NaBH_4$ (33 mg, 0.86 mmol) was added in portions, stirred for 2 h at rt. Reaction was quenched with crushed ice and stirred for 30 min. Reaction mixture concentrated in vacuo, residue was dissolved in EtOAc and washed with water, brine. EtOAc layer was dried over Na_2SO_4 concentrated in vacuo and purified by silica gel chromatography (pet. ether/EtOAc=90:10) to afford **14** (135 mg) in 82% yield.

^1H NMR (200 MHz, $CDCl_3$): δ 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, CHOH), 4.32 (dd, 1H, *J*=10.8, 4.4 Hz, CH_2O), 3.76–3.57 (m, 2H, CH–O, CH_2O), 2.59–2.49 (m, 2H, CH_2, CH), 1.89–1.73 (m, 1H, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ 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 $C_{14}H_{16}O_3Na$ $(M+Na)^+$ 255.1, observed $(M+Na)^+$ 255.3.

4.2. Enzymatic resolution reactions

*4.2.1. Enzymatic hydrolysis of racemic **3a**.* To a solution of racemic compound **3a** (500 mg, 1.43 mmol) in 10 mL acetonitrile, was added 5 mL of 1 M phosphate buffer with pH 7.2 and 20% (w/w) of *C. cylindracea* lipase (CCL) stirred for 4 days at 45 °C. Enzyme was filtered, filtrate was concentrated in vacuo and subjected for column chromatography (pet. ether/EtOAc=91:9) to yield enantiopure **3b** (45%, 98% ee) $[\alpha]_D^{20}$ −26.1° (*c* 1.05g/100 mL in $CHCl_3$).

*4.2.2. Enzymatic acylation of racemic **3b**.* To a solution of racemic compound **3b** (500 mg, 1.64 mmol) in 15 mL diethyl ether, was added vinyl acetate (0.15 mL, 1.64 mmol) and 20% (w/w) of *C. cylindracea* lipase (CCL) stirred for 3 h at rt. Enzyme was filtered, filtrate was concentrated in vacuo and subjected for column chromatography (pet. ether/EtOAc=91:9) to yield enantiopure **3a** (48%, 98% ee). $[\alpha]_D^{20}$ −8.0° (*c* 1.01g/100 mL in $CHCl_3$).

*4.2.3. (+)(4aR,7S,8aS) 7-O-acetyl-2-phenyl-4a,7,8,8a-tetrahydro-4H-benzo[d][1,3]dioxin-7-ol (**15**).* To a solution of compound (±)-**12** (500 mg, 2.15 mmol) in 15 mL diethyl ether, was added vinyl acetate (0.2 mL, 2.15 mmol) and 20% (w/w) of CCL stirred for 3 h at rt. Enzyme was filtered, filtrate was concentrated in vacuo and subjected to column chromatography (pet. ether/EtOAc=94:6) to yield **15** (44%, >98% ee) and enriched *ent*-**12** (56%, 87% ee). ^1H NMR (200 MHz, $CDCl_3$): δ 7.55(m, 2H, Aromatic), 7.39 (m, 3H, Aromatic), 5.89–5.81 (m, 1H, Alkene CH), 5.66 (s, 1H, CHPh), 5.46 (m, 1H, Alkene CH), 4.33 (dd, 1H, *J*=10.8, 4.5 Hz), 3.94–3.81 (m, 1H), 3.73 (t, 1H, *J*=11.2 Hz), 2.56–2.45 (m, 1H), 2.26–2.18 (m, 1H), 2.09 (s, 3H) 2.07–1.94(m, 1H); $[\alpha]_D^{20}$ −150.4° (*c* 1.1g/100 mL in $CHCl_3$) LCMS:

mass calculated for $C_{16}H_{18}O_4Na$ $(M+Na)^+$ 297.1103, observed $(M+Na)^+$ 297.1081.

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Supplementary data

^1H, ^{13}C, LCMS, HRMS analysis of relevant compounds, chiral HPLC and ^1H NMR with chiral shift reagents. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.06.003.

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