Selected Biological Evaluation of Synthesized Iridoid

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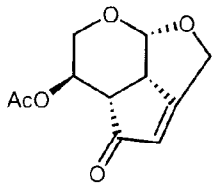
Summary: A general synthetic strategy has been used for the synthesis of an important class of natural product, the iridoids, which were then biologically evaluated. All the compounds were characterized by ^{1}H NMR, EIMS, IR, UV techniques and analyzed by CHN analyzer.

Introduction

Iridoid is a class of naturally occurring compounds which have diverse biological activities including antiviral [1], anti-allergic, anti-anaphylactic, analgesic, [2] antioxidant, [3,4] antimicrobial, antirheumatic, cholagogue, choleretic, hepatoprotective, laxative, hypotensive, sedative, antitumor, [1,5] immunostimulant activities, [6,7] antimicrobial [8,9] and hepatotoxic [10] Some of the iridoids showed radical scavenging activity against DPPH or antioxidant activity against β-carotene. Some iridoid showed significant inhibition of UVB-induced Activator Protein-1 (AP-1) activity in cell cultures [11].

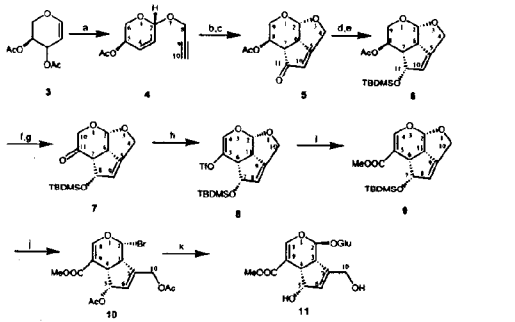
There are several reports available in the literature on the synthesis of iridoids [12]. In view of diverse biological activities associated with this class of compounds and difficulties in preparation of iridoid skeleton, we planned to synthesize iridoid skeleton from L-arabinose according to synthetic scheme 1. In our synthetic strategy, we used Pauson-Khand reaction for the preparation of cyclopentaannulated adducts type A, which was a key intermediate of this synthetic scheme.



Cyclopentaannulated Intermediate Type A

The structures of all newly synthesized compounds were determined by ^{1}HNMR and mass spectroscopy and purity was confirmed by CHN analysis. All newly synthesized compounds were screened for their random biological activities including phytotoxic, antifungal and antioxidant activities.

Synthetic pathway to synthesize iridoid **11** is shown in scheme 1. Compound **4** was synthesized by treating L-arabinal (**3**) with propargyl alcohol using modified Ferrier's protocol [13]. It was then converted to compound **5** using Pauson-Khand reaction [14]. However, attempt to prepare compound **6** from compound **5** faced a lot of problems. According to scheme, when we treated the compound **5** with sodium borohydride/cerium trichloride in methanol at 0°C we got a complex mixture of products, which was very difficult to purify. At this stage we altered our proposed strategy and used sodium borohydride/ cerium trichloride hexahydrated in methanol followed by basic hydrolysis surprisingly we isolated fully reduced molecule **12** in 22% yields. The compound **12** was easily converted into compound **13** in 90% yields by treating with acetic anhydride and pyridine (Scheme 2).



a) Propargyl Alcohol, I_2; b) $Co_2(CO)_8$, CH_2Cl_2; c) NMO, 0°C; d) $NaBH_4$, $CeCl_3$; e) TBDMSCl, Imidazole. f) MeOH:H_2O:Et_3N (5:4:1); g) PCC, CH_2Cl_2; h) LDA, THF, -78°C, Triflic anhydride; i) Pd, PPh_3, MeOH, NEt_3, LiCl, THF, CO, reflux; j) 30% HBr in AcOH; k) 2,3,4,6-tetra-*O*-acetyl-β-D-sodium gluconate, Deacetylation

Scheme 1

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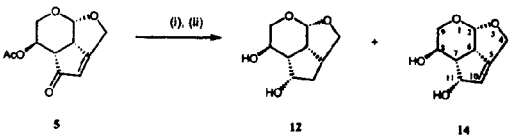
5 12 13

(i) $NaBH_4/CeCl_3.6H_2O$, MeOH, 0°C: (ii) MeOH, H_2O, Et_3N: (iii) Ac_2O/Pyridine

Scheme 2

In another attempt using sodium borohydride /anhydrous cerium trichloride at -78°C followed by usual basic hydrolysis compound **5** afforded a mixture of compounds **12** and **14** in 3 to 1 ratio (overall 60% yields), respectively, (Scheme 3).

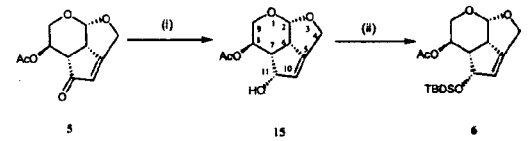


5 12 14

(i) $NaBH_4/CeCl_3$, MeOH, -78°C: (ii) MeOH, H_2O, Et_3N

Scheme 3

After various modifications in reaction conditions it was revealed that when compound **5** was treated with sodium borohydride/anhydrous cerium trichloride but at -10°C, it afforded compound **15** in 28% yields. The compound **15** was reacted with *t*-butyldimethyl silyl chloride in the presence of imidazole at 0°C and then at room temperature to afford compound **6**.



5 15 6

(i) $NaBH_4/CeCl_3$, MeOH, -10°C: (ii) TBDMSCl, Imidazole

(Scheme 4)

The compound **6** was treated with a mixture of methanol, water and triethyl amine (5:4:1) to accomplish deacetylation. The product so obtained was *in situ* treated with pyridinium chlorochromate to oxidize the alcoholic moiety present on tetrahydropyran ring to afford compound **7**. Compound **7** was treated with LDA at -78°C to produce enolate of ketone moiety present on tetrahydropyran ring, which was *in situ* trapped using triflouromethanesulfonic acid anhydride to afford triflate **8** in 90% yields. Compound **8** produced ester **9** in good yields, when it was reacted with $Pd(PPh_3)_2(Cl)_2$, triphenylphosphine, methanol and carbon monoxide. The ester **9** afforded brominated product **10** after cleavage of tetrahyrofuran ring on reacting with 30% hydrobromic acid in acetic acid. The desired iridoid skeleton was prepared by reacting compound **10**, with 2,3,4,6-tetra-*O*-acetyl-β-D-sodium gluconate followed by deacetylation in good yields.

Biological Studies

In order to evaluate selected biological effects of our newly synthesized compounds, compounds **4-15** were tested for their phytotoxicity [15], antioxidant and antifungal activities [16-20].

Phytotoxic Bioassay

Phytotoxicity of all the synthesized compounds **4-15** was tested by using the modified protocol of McLaughlin [15]. These experiments were done at three different concentrations *i.e.*, 500 μg/ml, 50 μg/ml and 5 μg/ml. The results of phytotoxic bioassay are shown in Table-1.

Table-1: Results of *Lemna Welv.* Phytotoxic Bioassay

S.No.	Compound	500 μg/ml	50 μg/ml	5 μg/ml
1.	**4**	54	51	48
2.	**5**	00	17	23
3.	**6**	100	100	100
4.	**7**	100	100	19
5.	**8**	100	0	23
6.	**9**	86	51	59
7.	**10**	40	8	00
8.	**11**	00	17	23
9.	**12**	60	00	00
10.	**13**	100	21	19
11.	**14**	100	17	23
12.	**15**	30	00	00

Compound 6, which contains *t*-butyldimethylsilyl functionality found highly active compound in this series having 100% plant growth inhibition at all the three levels of concentrations i.e. 500 μg/ml, 50 μg/ml and 5 μg/ml. Compound 7 appeared to be the second most active compound having 100% plant growth inhibition at 500 μg/ml, 50 μg/ml, while almost inactive at 5 μg/ml level. The compounds 4 and 9 exhibited moderate activity at all three levels of concentration. The compounds 8, 13 and 14 showed 100% plant growth inhibition at 500 μg/ml, however, found almost inactive on other concentration.

Antifungal Bioassay

All the synthesized compound were tested for their fungicidal activity against *Tricophyton longifusus, Candida albicans, Aspergillus flavus,*

Table 2: Results of Antifungal Bioassay

Compound Number	4	5	6	7	8	9	11	12	13	14	15
T. longifusus	27	32	00	00	65	84	00	00	00	65	00
C. albicans	00	00	00	00	00	100	00	00	00	00	00
A. flavus	00	00	60	00	00	00	00	00	00	00	00
M. canis	00	00	00	00	35	00	00	00	45	37	00
F. solani var. lycopersici	00	00	00	00	00	00	00	00	00	00	00
F. moniliformis	00	00	00	00	00	00	00	00	00	00	00

Microsporum canis, Fusarium solani var. lycopersici and *Fusarium moniliformis* fungal strains. The results of antifungal assays are collected in Table-2.

The results indicated that these compounds are not very active against the tested fungal strains except in few cases they exhibited variant degree of activity. The compounds **8, 9** and **14** showed significant activity against *T. longifusus*, whereas compounds **4** and **5** showed weak activities against the same fungus. Compound **6** showed some activities against *A. flavus,* while compound **8, 13** and **14** found to be weakly active against *M. canis*.

Compound **9** demonstrated strong activities against *C. Albicans*, indicating that compound **9** can be considered as lead compound for design of drug against candidiasis.

Antioxidant Bioassay

Reactive oxygen species (ROS) are generated when oxygen is supplied in access and its reduction is insufficient. The best explore ROS are super oxide anion radical (O^2). Its protonated from perhydroxyle radical, hydrogen peroxide and hydroxyl radical (HO) [21].

ROS are harmful for cell and are the cause of many diseases, e.g., ischemic/reperfusion" States", inflammatory diseases cancer, drug toxicity, degenerative processes associated with aging and neurogenerative diseases such as Parkinson's disease, Alzeihmer dementia and multiplic sclerosis [22-25].

All the synthesized compounds were screened for their free radical scavenging activity. The results of free radical scavenging activity are depicted in Table-3.

Compound **11** exhibited maximum free radical scavenging activity in this series of compounds, which was even higher than standard (propyl gallate), whereas compound **5** and compound **14** also demonstrated very high degree of free radical scavenging activity.

Table-3: Results of Antioxidant Bioassay.

S.No.	Compound No.	Free radical scavenging activity (%)
1.	**4**	–
2.	**5**	72.70
3.	**6**	-
4.	**7**	-
5.	**8**	-
6.	**9**	-
7.	**10**	-
8.	**11**	93.28
9.	**12**	-
10.	**13**	-
11.	**14**	88
12.	**15**	52
Standard propyl gallate		91

These all compounds scavenging at 1 m. molar concentration against 1,1-diphenyl-2-pierylhydrazy radical (DPPH)

General Experimental Procedures

All reactions were performed by taking necessary precautionary measures. The reactions with moisture sensitive reagents were performed under oxygen free conditions under positive pressure of nitrogen or argon and by using anhydrous solvents. Anhydrous ethers were prepared by using sodium and benzophenone ketyl. Alcohols were dried by using iodine and magnesium turnings. Anhydrous chloroform was prepared by refluxing with P_2O_5 followed by distillation. All anhydrous solvents were stored over molecular sieves of proper mesh size. Since compounds were prepared for the purpose of pharmacological screening yields of the reactions were not optimized. The column chromatography was performed on silica gel 60 70-230 mesh size. The solvent used for work up and column chromatography was distilled before use. The thin layer chromatography was done on silica gel PF 254 precoated 0.25 mm plates that were visualized by iodine vapour or by ceric sulphate followed by heating. Mass spectra were recorded on MAT-312 or JEOL JMS-HX 110 instruments. ^{1}H NMR spectra were recorded on Brücker AM-300, AM-400 or

AMX500 spectrophotometer using tetramethylsilane as an internal standard. Chemical shifts are recorded in ppm.

3,4-Di-O-acetyl L-Arabinal (3).

2,3,4-Tetra-*O*-acetyl-α-L-bromoarabinopyranoside (5 g, 15 mmol) was dissolved in glacial acetic acid (35 ml) at 30-35°C and added during 10 min to a mechanically stirred mixture of zinc dust (10 g) and 50% acetic acid (54 ml) containing a few drops of a solution of chloroplatinic acid, kept at -10° by a freezing mixture. At intervals of 30 minutes a few drops of a solution of chloroplatinic acid were added to maintain a vigorous reaction. The stirring was continued for 3 h, and the mixture was kept overnight at 0°C. After filtration of the excess zinc dust, the filtrate was extracted with chloroform. The chloroform extracts were washed with saturated aqueous sodium hydrogen carbonate solution and then with water and finally dried (Na_2SO_4). Removal of the solvent by evaporation at 40°C afforded syrup. Fractional distillation gave 3,4-di-*O*-acetyl-L-arabinal, which was recrystallized from ethanol; m. p. 95-97°C, $[\alpha]_D^{20}$ + 35.2° (*c*, 0.90 in chloroform) in agreement with literature values. The structure of product was further confirmed by mass and NMR spectroscopy.

2-(Prop-2-ynyl)-5-O-Acetyl-3,4-Dideoxy-α-D-Erythro-Hex-3-Enopyranoside (4).

3,4-Di-*O*-acetyl-L-arabinal (**3**) (2.5 g, 12.5 mmol), propargyl alcohol (0.8 ml, 13.5 mmol) and iodine (0.5 g, 3.9 mmol) were added under argon to a 250 ml round-bottomed flask containing 50 ml of THF. The solution was stirred under argon at room temperature for 1 h, upon which time it was diluted with 75 ml of ether. The resulting dark-red colored mixture was washed with 50 ml of 10% aqueous $Na_2S_2O_3$, turning the solution completely colorless. The aqueous layer was extracted with ether (3 x 100 ml). The combined organic layers dried over Na_2SO_4 and the solvent was removed by rotary evporator. The resulting crude solid was purified by silica gel column chromatography using 5:1 hexane: ethyl acetate providing 2.62 g (96%) of 2-(prop-2-ynyl)-5-*O*-acetyl-2,3-dideoxy-α-D-erythro-hex-2-enopyranoside (**4**) as an oil, $[\alpha]_D^{25}$ -133 (c 0.5, $CHCl_3$). The structure of product was confirmed by mass and NMR spectroscopy. ^{1}HNMR (400 MHz, $CDCl_3$): δ 5.98 (dd, 1H, *J* = 10.4, 2.6 Hz, H-3), 5.61 (dd, 1H, *J* = 10.4, 2.1 Hz, H-4), 4.74 (ddd, 1H, *J* = 4.4, 4.1, 2.1 Hz, H-5), 4.63 (d, 1H, *J* = 2.6 Hz, H-2), 4.21 (d, 1H, *J* = 16.1 Hz, H-8), 4.17 (d, 1H, *J* = 16.1 Hz, H-8′), 3.96 (dd, 1H, *J* = 13.4, 4.4 Hz, H-6), 3.67 (dd, 1H, *J* =13.4, 4.1 Hz, H-6′), 2.3 (s, 1H, H-10), 2.0 (s, 3H, CH_3). FDMS 196 (M^+,H); Anal Calc. for $C_{10}H_{12}O_4$ (196.200)[Found: C (60.98), H (6.12) requires C(61.2), H(6.16)]

Preparation of Compound (5).

To a solution of compound **4** (1.34 g, 5 mmol) in dry dichloromethane, under argon at room temperature, $CO_2(CO)_8$ (1.88 g, 35 mmol) was added and the reaction mixture was stirred until no starting material left (TLC analysis). The reaction was cooled at 0°C and *N*-methyl morpholine oxide (NMO) (3.51 g) was added in one portion. The reaction mixture was stirred for 3 h at room temperature; the crude mixture was filtered through Celite and thoroughly washed with dichloromethane, and the solvent was evaporated under reduced pressure on a rotary evaporator. The crude mixture was loaded on a column and eluted with a 7:3 mixture of hexane and ethyl acetate to afford pure compound **5** in 90% yields as viscous liquid, $[\alpha]_D^{25}$ -122 (c 0.32, $CHCl_3$). The structure of product was confirmed by mass and NMR spectroscopy. ^{1}HNMR (400 MHz, $CDCl_3$): δ 6.54, (br s, 1H, H-10), 5.21 (m, 1H, H-8), 5.01 (d, 1H, *J* =3.0 Hz, H-2), 4.24 (d, 1H, *J* = 13.0 Hz, H-4), 4.19 (d, 1H, *J* = 13.0 Hz, H-4′), 4.02 (dd,1H, *J* = 13.3, 4.4 Hz, H-9), 3.69 (dd, 1H, *J* = 13.3, 4.2 Hz, H-9′), 3.11 (dd, 1H, *J* = 3.0, 3.8 Hz, H-6), 2.83 (dd, 1H, *J* = 9.2, 3.8, Hz, H-7), 2.13 (s, 3H,CH_3). Anal. Calcd. for $C_{11}H_{12}O_5$:(224.210) [Found: C (59.01), H (5.41) requires C(58.98), H(5.39)].

Preparation of Compound (6).

Compound **5** (2.5 g 11 mmol) was dissolved in methanol and a (2.5 g, 11 mmol) of anhydrous cesium chloride in methanol was cooled to 10°C and treated with equimolar amount of sodium borohydride. The reaction was completed in 1.5 h (TLC analysis) and the reaction was quenched with saturated solution of ammonium chloride. The solvent was evaporated by stream of nitrogen and the crude material was dissolved in anhydrous DMF and imidazole (1.1 eq) in anhydrous DMF was added at 0°C and stirred for 15 min. A solution of *t*-butyldimethylsilyl chloride (1.1 eq) in anhydrous DMF was added to it with constant stirring. Reaction mixture was stirred for additional 12 h at room temperature,

after completion of reaction (TLC analysis), it was diluted with dichloromethane, washed with saturated aqueous solution ammonium chloride. The aqueous phase was again washed with dichloromethane and combined organic phase was washed with water and dried over anhydrous sodium sulfate. It was filtered and solvent was evaporated on a rotary evaporator under reduced pressure. The residue was loaded on a column and eluted with a 8:2 mixture of hexane and ethyl acetate to afford compound **6** in 28% yield as yellowish liquid, $[\alpha]^{25}_D$ - 94 (c 0.25, $CHCl_3$). The structure of compound was confirmed by mass and NMR spectroscopy. ^{1}HNMR (400 MHz,$CDCl_3$): δ 6.3 (br s, 1H, H-10), 5.88 (m, 1H, H-8), 5.1 (d, 1H, *J* = 3.0 Hz, H-2), 4.66 (m, 1H, H-11), 4.31 (d, 1H, *J* = 13.2 Hz, H-4), 4.26 (d, 1H, *J* = 13.2 Hz, H-4′), 4.18 (dd, 1H, *J* = 13.5, 4.5 Hz, H-9), 3.94 (dd, 1H, *J* = 13.5, 4.5 Hz, H-9′), 3.32 (dd, *J* = 3.0, 5.1 Hz, H-6), 3.14 (ddd, 1H, *J* = 9.3, 10.2, 5.18 Hz, H-7), 2.14 (s, 3H, CH_3), 0.88 (s, 9H, $SiC(CH_3)_3$), 0.08 (s, 6H, $Si(CH_3)_2$); FDMS 340 (M^+), Anal. Calcd. for $C_{17}H_{28}O_5Si$: (340.487) [Found: C (59.92), H (8.25) requires C (59.97), H (8.29)].

Preparation of Compound (7).

Compound **6** in methanol was slowly added to a mixture of methanol, water and triethyl amine (5:4:1) at 0° C with constant stirring. Stirring was continued for 2 h at room temperature and after completion of reaction (TLC analysis), the solvent of reaction mixture was evaporated on a two stage pump. The resulting solid was dissolved in anhydrous methylene chloride and it was added in one portion to a stirred suspension of pyridinium chlorochromate 3.50 g (16 mmol) in 20 mL in anhydrous methylene chloride. Stirring was continued for 2 h at room temperature. The completion of reaction was monitored by TLC, when no staring material was left; the solvent of reaction mixture was evaporated under reduced pressure. The residue was suspended in water and extracted with ether (3 x 50 ml). The combined extracts were washed with water and saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, filtered and solvent was evaporated under reduced pressure. The residue was chromatographed on a silica gel column using 8:2 hexane/ethyl acetate as eluent to afford compound **7**, 2.46 g (84%) as an oil, $[\alpha]^{25}_D$ - 167 (c 0.65, $CHCl_3$). ^{1}HNMR (400 MHz,$CDCl_3$): δ 6.01 (d, 1H, *J* = 2.1, H-9), 5.06 (dd, 1H *J* = 3.2 Hz, H-2), 5.01 (dd, 1H, *J* = 2.1, 4.6, Hz, H-8), 4.29 (d, 1H, *J* = 13.6 Hz, H-4), 4.22 (d, 1H, *J* = 13.6 Hz, H-4′), 4.11 (d, 1H, *J* = 13.4 Hz, H-10′), 3.92 (d, 1H, *J* = 13.4 Hz, H-10′), 3.29 (dd, 1H, *J* = 3.2, 5.8 Hz, H-6), 3.11 (dd, 1H, *J* = 5.8, 4.6 Hz, H-7), 0.90 (s, 9H, $SiC(CH_3)_3$), 0.09 (s, 6H, $Si(CH_3)_2$); FDMS: 296 (M^+); Anal. Calcd. for $C_{15}H_{24}O_4Si$: (296.434) [Found: C (60.76), H (8.19) requires C (60.78), H (8.16)].

Preparation of Compound (8).

To a 14 molar solution lithiumdiisopropylamide in anhydrous THF (prepared from diisopropylamine and n-butyl lithium) a solution of compound **7**, 2 g (6.76 mmol) in 25 mL anhydrous THF was added drop wise under argon *via* a syringe with constant stirring at -78°C. The reaction was monitored by TLC, when all starting material was consumed (about 1 h), trifluoromethanesulfonic acid anhydride (5.8 g, 6.8 mmol) was added *via* a syringe drop wise under argon at -78 °C. The stirring was continued for four more hours at room temperature, after completion of reaction (TLC analysis), it was quenched with drop wise addition moist ether. The solvent of reaction mixture was evaporated under reduced pressure; the residue was suspended in water and extracted with ether (3 x 50 ml). The combined extracts were washed with water and saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, filtered and solvent was evaporated under reduced pressure. The residue was loaded on a silica gel column and eluted with 8:2 hexane/ethyl acetate to afford pure compound **8**; Yield was 3 g (91%), as an oil, $[\alpha]^{25}_D$ - 45 (c 0.22, $CHCl_3$). ^{1}HNMR (400 MHz,$CDCl_3$): δ 6.69 (d, 1H, *J* = 1.91 Hz, H-4), 5.99 (d, 1H, *J* = 2.2, H-8), 5.2 (d, 1H, *J* = 2.9 Hz, H-2), 4.89 (dd, 1H, *J* = 2.2, 9.6 Hz, H-7), 4.39 (d, 1H, *J* = 12.2 Hz, H-10), 4.08 (d, 1H, *J* = 12.2 Hz, H-10′), 3.33 (dd, 1H, *J* = 2.9, 4.5 Hz, H-11), 3.09 (ddd, 1H, *J* = 1.9, 4.5, 9.68 Hz, H-6), 1.02 (s, 9H, $SiC(CH_3)_3$), 0.07 (s, 6H, $Si(CH_3)_2$); FDMS = 428 (M^-). Anal. Calcd. for $C_{16}H_{23}FO_6SSi$: (428.497) [Found: C (44.78), H (5.41) requires C (44.85), H (5.41)].

Preparation of Compound (9).

A 200 mL glass pressure bottle equipped with two valves and a pressure gauge was charged with 8 mg of $Pd(PPh_3)_2(Cl)_2$ (prepared from Pd, PPh_3 and LiCl), 40 mg of triphenylphosphine and 2 g of compound **8**. The flask was then filled with argon and 0.5 ml of triethyl amine and 0.5 mL of methanol was added. The apparatus was then pressurized with 3 atm of carbon monoxide, sealed and heated for 18 h at 120-125°C and then allowed to cool. 10% Hydrochloric acid (2.4 mL) was added to the viscous

orange-brown reaction mixture in 2 mL portions with constant agitation, water was added to the reaction mixture. It was extracted with dicholoromethane (3x50 ml), the combined extracts were washed with saturated aqueous sodium bicarbonate solution then with water, dried over anhydrous sodium sulfate, filtered and solvent was evaporated under reduced pressure. The residue was loaded on a silica gel column and eluted with 7;3 hexane/ethyl acetate to afford pure compound **9**, 1.23 g (74 %), as viscous liquid, $[\alpha]^{25}_{D}$ - 72 (c 0.7, $CHCl_3$). ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.62 (d, 1H, *J* = 1.61, H-4), 6.24 (d, 1H, *J* = 2.2 Hz, H-8), 5.39 (d, 1H, *J* = 2.7 Hz, H-2), 4.97 (dd, 1H, *J* = 2.2, 4.6 Hz, H-7), 4.43 (d, 1H, *J* = 12.3 Hz, H-10), 4.04 (d, 1H, *J* = 12.3 Hz, H-10′), 3.59 (s, 3H, OCH_3), 3.61 (ddd, 1H, *J* = 1.6, 4.4, 4.67 Hz, H-6), 3.42 (dd, 1H, *J* = 2.7, 4.4 Hz, H-11), 0.99 (s, 9H, $3CH_3$), 0.09 (s, 6H, $2CH_3$); FDMS 338 (M^+). Anal. Calcd. for $C_{17}H_{26}O_5Si$: (338.471) [Found: C (60.21), H (7.69) requires C (60.32), H (7.74)].

Preparation of Compound (10).

Hydrogen bromide-acetic acid (30%) 0.91 mL (15.9 mmol) was added drop wise to 1.8 g (5.3 mmol) of compound **9** with constant stirring on an ice salt bath during a period of 10 min and then stirring was continued at room temperature for 30 min. Water was then added and then the mixture was neutralized with sodium carbonate. The neutral solution was then extracted with ether, the combined extracts were washed with water and then with saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, filtered and solvent was evaporated under reduced pressure to afford crude compound **10**, 3.07 g (94 %) as amorphous solid, $[\alpha]^{25}_{D}$ - 75 (c 0.43, $CHCl_3$).. A small portion of this unstable compound was recrystallized from a mixture of ether and hexane for spectroscopy and elemental analysis. ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.11 (d, 1H, *J* = 1.8, H-8), 6.78 (d, 1H, *J* = 7.0 Hz, H-2), 6.66 (d, 1H, *J* = 2.3 Hz, H-6), 5.71 (dd, 1H, *J* = 2.3, 10.0, Hz, H-5), 4.92 (d, 1H, *J* = 13.0 Hz, H-10), 4.87 (d, 1H, *J* = 13.0 Hz, H-10′), 3.80 (dd, 1H, *J* = 7.0, 9.2 Hz, H-3) 3.63 (ddd, 1H, *J* = 1.8, 9.2, 10.0 Hz, H-4), 3.71 (s, 3H, OCH_3), 2.02 (s, 3H, CH_3), 1.97 (s, 3H, CH_3); FDMS 389 (M^+). Anal. Calcd. for $C_{15}H_{17}BrO_7$: (389.195) [Found: C (46.31), H (4.35) requires C (46.29), H (4.40)].

Preparation of Compound (11).

To a stirred solution of 1 g (3 mmol) of 2,3,4, 6-tetra-*O*-acetyl-*β*–D-sodium gluconate in nitromethane, 1.20 g (3 mmol) of crude compound **10** and 0.71 g (3 mmol) of mercuric cyanide were then added. The reaction mixture was stirred for 6 h, the progress of reaction was monitored by TLC, after completion of reaction, the mixture was then concentrated to a yellowish syrup by distillation under reduced pressure on a water bath. The syrup was dissolved in 120 ml of hot benzene and then allowed to stand for 12 h at 0°C. The mercury salts was filtered off on a sintered glass funnel and washed the solid residue with several small portion of benzene. The benzene was evaporated and the residue was treated with a mixture of methanol, water and triethyl amine (5:4:1), after completion of reaction (TLC analysis), the solvent was evaporated on a two stage pump. The solid so obtained was pure compound **11** in 60% yields, $[\alpha]^{25}_{D}$ + 203 (c 0.52, H_2O), having satisfactory spectroscopic and elemental analysis. ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.13 (d, 1H, *J* = 1.8, H-8), 6.26 (d, 1H, *J* = 4.5 Hz, H-6), 5.36 (d, 1H, *J* = 5.2 Hz, H-2), 4.71 (dd, 1H, *J* = 4.5, 5.1 Hz, H-5), 4.92 (d, 1H, *J* = 13.1 Hz, H-10), 4.87 (d, 1H, *J* = 13.1 Hz, H-10′), 3.72 (s, 3H, CH_3), 3.96-3.85 (m, 2H, H-6a″/6b″), 3.67 (dd, 1H, *J* = 1.8, 9.73, 5.17 Hz, H-4), 3.52 (dd, 1H, *J* = 9.7, 5.2 Hz, H-3), 3.3-3.18 (m, 2H, H-3″/H-5″), 3.11-3.03 (m, 2H, H-4″/2″), 3.0-2.92 (m, 1H, H-1″); FDMS = 488 (M^+). Anal. Calcd. for $C_{21}H_{28}O_{13}$: (488.439) [Found: C (51.58), H (5.69) requires C (51.64), H (5.78)].

Preparation of Compound (12).

Compound **5** (1.25 g 5.5 mmol) was dissolved in methanol and a 5.5 molar solution cesium chloride hexahydrate in methanol was cooled to 0°C and treated with equimolar amount of sodium borohydride. The reaction was completed in 1.5 h (TLC analysis) and the reaction was quenched with saturated solution of ammonium chloride. It was extracted with dichrolomethane and the solvent was evaporated by stream of nitrogen and the crude was treated with a mixture of methanol, water and triethyl amine (5:4:1), after completion of reaction (TLC analysis), the solvent was evaporated on a two stage pump. The residue was loaded on a column and eluted with a 7:3 mixture of hexane and ethyl acetate to afford pure compound **12** in 22% overall yields, as viscous liquid, $[\alpha]^{25}_{D}$ - 33 (c 0.32, CH_3OH). The structure of compound was confirmed by mass and NMR spectroscopy. ^{1}HNMR (400 MHz, $CDCl_3$): δ 5.61 (d, 1H, *J* = 2.61 Hz, H-2), 4.28 (dd, 1H, *J* = 2.58, 7.1 Hz, H-11), 4.11 (dd, 1H, *J* = 9.7, 10.2 Hz H-

8), 3.66 (2H, m, H-9/9′), 3.66 (m, 1H, H-4/4′), 2.49 (m, 1H, H-5), 2.18 (m, 1H, H-6), 2.26 (m, 1H, H-7), 1.88 (m, 2H, H-10/10′). FDMS = 186 (M^+). Anal. Calcd. for $C_9H_{14}O_4$: (186.205) [Found: C (58.00), H (7.60) requires C (58.05), H (7.58)].

Preparation of Compound (13).

To a solution of compound **12** in anhydrous pyridine (5 ml) was added 2.5 equivalent of acetic anhydride at 0°C with constant stirring. The stirring was continued for further 24 h at room temperature till completion of reaction (TLC analysis). The reaction mixture was poured on ice water mixture and extracted with dichloromethane (3 x 50 ml), the combined organic phase was washed with aqueous saturated sodium bicarbonate solution, again with water and dried over anhydrous sodium sulphate. The solid was filtered and the solvent was evaporated under reduced pressure. The residue was flashed chromatographed using 7:2 haxane/ethyl acetate as eluent to afford pure compound **13** in 90% yields as amorphous solid, $[\alpha]^{25}_D$ - 68 (c 0.57, $CHCl_3$). ^{1}HNMR (400 MHz,$CDCl_3$): δ 5.52 (d, 1H, J = 2.7 Hz, H-2), 5.01 (dd, 1H, J = 2.6, 7.2 Hz, H-11), 4.81 (dd, 1H, J = 10.0, 10.2 Hz, H-8), 4.0-4.32 (m, 2H, H-9/9′), 3.64 (dd, 1H, J = 5.2, 12.2 Hz, H-4), 3.55 (dd, 1H, J = 5.2, 12.2 Hz, H-4′), 2.57-2.65 (m, 1H, H-5), 2.46-2.51 (m, 1H, H-7), 2.31-2.43 (m, 1H, H-6), 2.12-2.22 (m, 2H, H-10/10′), 2.03 (s, 3H, CH_3), 2.00 (s, 3H, CH_3). FDMS = 270 (M^+). Anal. Calcd. for $C_{13}H_{18}O_6$: (270.278) [Found: C (57.67), H (6.66) requires C (57.77), H (6.71)].

Preparation of Compound (14).

Compound **5** (2.5 g 11 mmol) was dissolved in methanol and a (2.5 g, 11 mmol) of anhydrous cesium chloride in methanol was cooled to -78°C and treated with equimolar amount of sodium borohydride. The reaction was completed in 2.5 h (TLC analysis) and after usual work up, it was treated with a mixture of methanol, water and triethyl amine (5:4:1), after completion of reaction (TLC analysis), the solvent was evaporated on a two stage pump. The residue was loaded on a silica gel column and eluted with 8:2 hexane/ethyl acetate to afford compounds **12** and **14** in 3:1 ratio (overall 40% yield). The compound **12** showed satisfactory NMR and mass spectroscopic data as we found in the previous experiment. The compound **4** was found to be a viscous liquid, $[\alpha]^{25}_D$ - 97 (c 0.39, $CHCl_3$), having satisfactory spectroscopic and elemental analysis data. ^{1}HNMR (400 MHz,$CDCl_3$): δ 5.25 (d, 1H, J = 2.6 Hz, H-2), 4.86 (dd, 1H, J = 2.7, 7.2 Hz, H-11), 4.81 (dd, 1H, J = 10.1, 10.3 Hz, H-8), 4.0-4.32 (m, 2H, H-9/9′), 3.64 (dd, 1H, J = 5.8, 12.6 Hz, H-4), 3.55 (dd, 1H, J = 5.8, 12.6 Hz, H-4′), 2.57-2.65 (m, 1H, H-5), 2.46-2.51 (m, 1H, H-7), 2.31-2.43 (m, 1H, H-6), 2.12-2.22 (m, 2H, H-10/10′). FDMS = 186 (M^+). Anal. Calcd. for $C_9H_{14}O_4$: (186.205) [Found: C (58.00), H (7.49) requires C (58.05), H (7.58)].

Preapration of Compound (15).

Compound **5** (2.5 g 11 mmol) was dissolved in methanol and a (2.5 g, 11 mmol) of anhydrous cesium chloride in methanol was cooled to -10°C and treated with equimolar amount of sodium borohydride. The reaction was completed in 2.5 h (TLC analysis) and after usual work up, the residue was loaded on a silica gel column and eluted with 7:3 hexane/ethyl acetate to afford pure compound **15** as viscous liquid, $[\alpha]^{25}_D$ - 87 (c 0.59, $CHCl_3$). ^{1}HNMR (400 MHz,$CDCl_3$): δ 5.32 (d, 1H, J = 2.6 Hz, H-2), 4.81 (dd, 1H, J = 10.0, 10.2 Hz, H-8), 4.41 (dd, 1H, J = 2.7, 7.2 Hz, H-11), 4.02-4.15 (m, 2H, H-9/9′), 3.62 (dd, 1H, J = 5.7, 12.2 Hz, H-4), 3.50 (dd, 1H, J = 5.7, 12.2 Hz, H-4′), 2.62-2.69 (m, 1H, H-5), 2.42-2.51 (m, 1H, H-7), 2.21-2.28 (m, 1H, H-6), 1.98-2.11 (m, 2H, H-10/10′), 2.03 (s, 3H, CH_3). FDMS = 186 (M^+). Anal. Calcd. for $C_{11}H_{16}O_5$: (228.242) [Found: C (57.81), H (7.04) requires C (57.88), H (7.07)].

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