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Novel Lipase-Catalysed Enantioselective Deacetylation of (±)-5-Acetoxy-3-(4-fluorophenyl)-2-phenylisoxazolidine

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Abstract—(±)-5-Acetoxy-3-(4-fluorophenyl)-2-phenylisoxazolidine has been synthesised by a highly diastereoselective [3+2] cycloaddition reaction between α-(4-fluorophenyl)-*N*-phenylnitrone and vinyl acetate in good yield. *Candida rugosa* lipase catalyses the deacetylation of this (±)-5-acetoxyisoxazolidine in a highly enantioselective fashion in diisopropyl ether containing *n*-butanol affording (−)-5-acetoxy-3-(4-fluorophenyl)-2-phenylisoxazolidine in 43% yield and >99% *ee*.

Introduction

One of the important challenges facing organic chemists today is the synthesis of compounds in a highly selective and cost effective manner, utilising renewable raw materials through environmentally benign processes. The organic chemists have responded to these challenges in a befitting manner and one of the recent advances in this direction is the application of biocatalysts using pure or crude enzymes or even whole cells in their native forms in aqueous/organic media.[1,2] The most frequently used enzymes are lipases which carry out hydrolytic reactions;[3,4] hydrolytic reactions have almost 50% share of total enzyme-catalysed reactions used in organic synthesis.[2] Recently, we have demonstrated that lipases from various species can be used for regio- and stereoselective acylation/deacylation of different classes of polyphenolic compounds[5–8] and sugar derivatives.[9,10]

Isoxazolidines are masked amino acids and under appropriate conditions give rise to hydroxyamino acids and β-amino alcohols which are important components in the synthesis of a wide range of biologically active compounds, such as neopolyoxins,[11] theonellamide F[12] and WS 47083.[13] Isoxazolidines themselves possess a variety of biological activities, for example antiviral,[14] antifungal,[15] anti-inflammatory,[16] etc.

In view of the importance of isoxazolidines in medicinal chemistry, we envisaged to synthesise 5-oxygenated 2,3-diarylisoxazolidines by [3+2] cycloaddition reaction of α,*N*-diarylnitrones with vinyl and allyl acetates to provide handles for resolution. The racemic isoxazolidine **2** was synthesised by this reaction between α-(4-fluorophenyl)-*N*-phenylnitrone (**1**) and vinyl acetate in 75% yield (Scheme 1); it was found to be diastereomerically pure as only one diastereomer with the C-3 aryl and the C-5 acetoxy groups *cis* to each other was detected. The formation of a single diastereomer of isoxazolidine **2** is supported by the presence of one set of signals for C-3H, C-4Hα, C-4Hβ and C-5H at δ 4.40 (dd), 2.47 (ddd), 3.15 (ddd) and 6.58 (dd), respectively, in the ^{1}H NMR spectrum of the compound. The respective couplings of the ^{1}H NMR spectral signals due to C-3H, C-4Hα, C-4Hβ and C-5H is clearly observed in the ^{1}H–^{1}H COSY NMR spectrum of (±)-isoxazolidine **2**. The formation of a single diastereomer in this reaction was earlier confirmed by us by X-ray analysis of a similar isoxazolidine derivative.[17] A sample of (±)-**2** was found to exhibit 78% inhibition of *Mycobacterium tuberculosis* at MIC value of 6.25 μg/mL. This result prompted us to resolve the two enantiomers of (±)-isoxazolidine **2** for comparative study of the inhibitory potential of the two stereoisomers.

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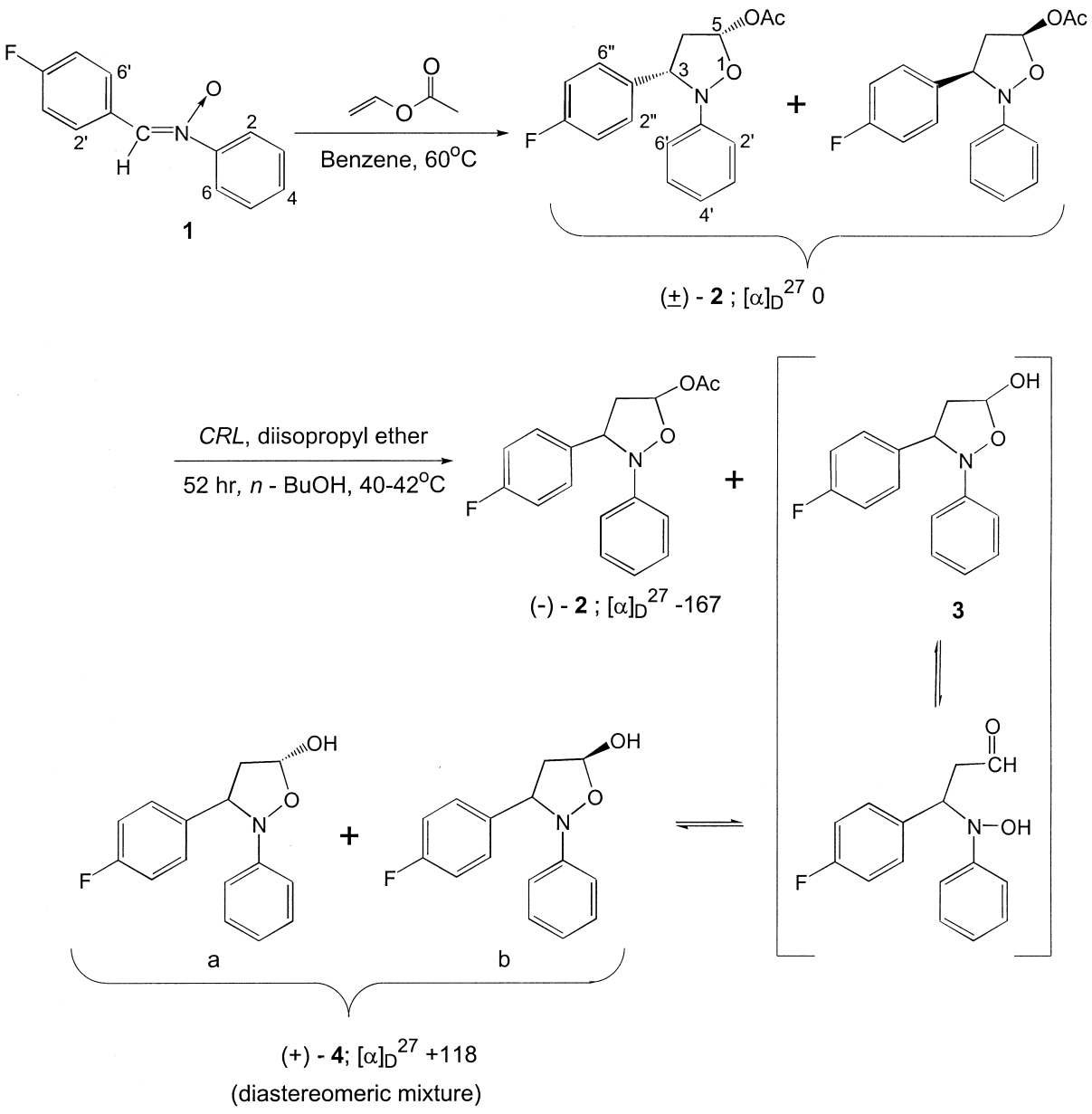

Scheme 1. Preparation and enantioselective deacetylation of 5-acetoxy-3-(4-fluorophenyl)-2-phenylisoxazolidine (**2**).

Results and Discussion

Based on our earlier studies on the use of lipases in regio- and stereocontrol of transacylation reactions on different types of compounds,[5–10] we attempted enzymatic resolution of (±)-2 with *Candida rugosa* lipase (CRL) and porcine pancreatic lipase (PPL) in different organic solvents, that is, toluene, diisopropyl ether, tetrahydrofuran, dioxane and acetonitrile. While no deacetylation of (±)-**2** was observed with PPL in any solvent, CRL in diisopropyl ether carried out a highly enantioselective biotransformation involving the deacetylation of (±)-isoxazolidine **2** (Scheme 1). In a typical reaction, (±)-5-acetoxyisoxazolidine **2** was dissolved in dry diisopropyl ether containing *n*-butanol and CRL was added to it. The suspension was stirred at 40–42 °C and the progress of the reaction was monitored by HPLC. The reaction was stopped at about 50% conversion (52 h) of the starting acetate to the product by filtering off the enzyme and worked up to afford (−)-5-acetoxy-3-(4-fluorophenyl)-2-phenylisoxazolidine (**2**) and (+)-3-(4-fluorophenyl)-5-hydroxy-2-phenylisoxazolidine (**4**) as white crystalline solids in 43 and 42% yields, respectively.[18] The spectral data (^{1}H-, ^{13}C NMR, IR, UV and mass spectra) of (−)-**2** was found to be identical with those of (±)-acetoxyisoxazolidine **2**; hydroxyisoxazolidine **4** was unambiguously identified from its spectral data. The ^{1}H and ^{13}C NMR spectra of hydroxyisoxazolidine **4** exhibited two sets of closely related peaks for all the 15 carbons and 14 protons in the molecule (cf. experimental) which revealed that it is a mixture of *cis*- and *trans*-isoxazolidines with respect to

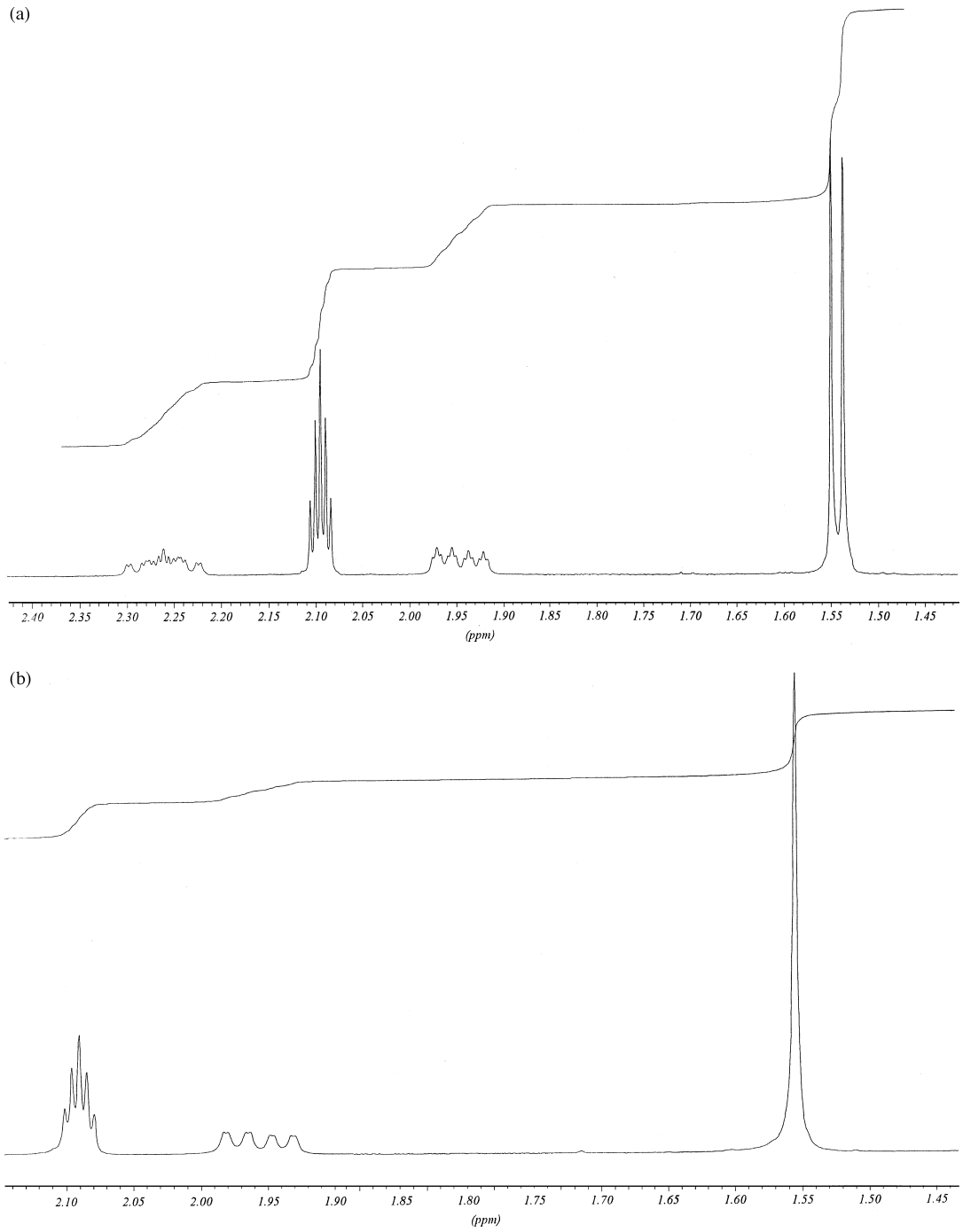

Figure 1. (a) ^{1}H NMR spectra of (±)-5-acetoxy-3-(4-fluorophenyl)-2-phenylisoxazolidine (**2**) recorded in the presence of (+)-TFAE; (b) ^{1}H NMR spectra of (−)-5-acetoxy-3-(4-fluorophenyl)-2-phenylisoxazolidine (**2**) recorded in the presence of (+)-TFAE.

the C-3 aryl and the C-5 hydroxy groups present in it. The epimerisation of initially formed *cis*-(+)-3-(4-fluorophenyl)-5-hydroxy-2-phenylisoxazolidine (**3**) to a mixture of *cis*- and *trans*- isoxazolidines **4** is very much feasible because the acetoxy group in compound **2** is at an anomeric position; isoxazolidine ring opening after deacetylation and further cyclisation leads to the formation of a diastereomeric mixture of hydroxyisoxazolidines (Scheme 1).

The enantiomeric excess (*ee*) of (−)-acetoxyisoxazolidine **2** was determined by chiral shift NMR technique using (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol [(+)-TFAE] shift reagent in toluene-d_8 at 223 K. As shown in Figure 1a, a clean separation of the acetoxy peaks of the two enantiomers of (±)-acetoxyisoxazolidine **2** has been achieved in its ^{1}H NMR spectrum recorded in the presence of (+)-TFAE. The ^{1}H NMR spectrum of (−)-**2** with (+)-TFAE exhibited the

absence of acetoxy peak of one enantiomer (Fig. 1b). This indicates that one of two enantiomers, that is, (+)-acetoxyisoxazolidine has been deacetylated selectively by CRL to afford *cis*-hydroxyisoxazolidine **3** which further isomerises to a diastereomeric mixture of hydroxyisoxazolidine **4**. The enantiomeric excess (*ee*) of (−)-isoxazolidine **2** was found to be greater than 99%, calculated on the basis of integration of acetoxy peaks in the ^{1}H NMR spectra of starting (±)-acetoxyisoxazolidine **2** and the unreacted (−)-acetoxyisoxazolidine **2** recorded in the presence of (+)-TFAE.

It is interesting to note that though the bulkiness of the aromatic moieties at the N-2 and C-3 positions of the (±)-isoxazolidine **2** seems to be very similar, CRL showed a very high enantioslectivity (>99% *ee*) in the biotransformation being reported by us. It may also be mentioned that this perhaps is the first report of an efficient enzymatic resolution of a heavily substituted isoxazolidine. There is only one report in the literature thus far on the synthesis of a remotely different type of isoxazolidines, wherein a coupling of enzymatic resolution and cycloaddition reaction with nitrone to give a bicyclo isoxazolidine in 88% *ee* is mentioned.[19]

To conclude, the present study has revealed highly selective capability of CRL for enantioselective deacetylation of (±)-isoxazolidine **2** in diisopropyl ether. The hydroxyisoxazolidine **3** formed during deacetylation of (±)-acetoxyisoxazolidine **2** epimerises to a diastereomeric mixture of hydroxyisoxazolidine **4**, which can be acetylated and put to enzymatic reaction again giving rise to higher yields of optically pure acetoxyisoxazolidine as compared to classical enzymatic resolution wherein a maximum of 50% yield of the desired stereoisomer can be achieved. As it is difficult to synthesise such compounds in enantiomerically pure form by purely chemical methods, the biocatalytic approach reported here should find utility in the synthesis of new optically enriched isoxazolidines.

Further work on generalisation of this methodology to get a series of optically pure isoxazolidines, and the evaluation of their absolute configuration and the inhibitory activities of their stereoisomers against *M. tuberculosis* is in progress.

Experimental

General

Melting points were determined on a Mettler FP62 instrument and are uncorrected. The IR and UV spectra were recorded on a Perkin–Elmer model RXI FT-IR spectrophotometer and a Cary 100 Biospectrophotometer, respectively. The optical rotations were measured on a Bellingham Stanley AD 220 polarimeter. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance-300 spectrometer at 300 and at 75 MHz, respectively, using TMS as internal standard. The chemical shift values are on the δ scale and the coupling constants (*J*) are in hertz. The EI-MS and HR-MS were recorded on a Jeol AX 505 W instrument at 70 eV. The enzymes porcine pancreatic lipase (PPL, Type II) and *C. rugosa* lipase (CRL, Type VII) were purchased from Sigma Chemical Co. (USA) and used after storing in vacuo over P_2O_5 for 24 h. The organic solvents used were distilled over ignited molecular sieves (4 Å), while *n*-butanol was dried and distilled over ignited potassium carbonate. Analytical TLCs were performed on precoated Merck silica gel $60F_{254}$ plates; the spots were detected either under UV light or by charring with 4% alcoholic H_2SO_4. Enzymatic deacetylation reaction was monitored at λ_{254} nm on a Shimadzu LC-10AS HPLC instrument with SPD-10A UV–vis detector and Shimpack CLC-ODS (4.6×150 mm) reverse phase column; solvent system used was methanol/water (3:2) at the flow rate of 0.50 mL/min. The chiral ^{1}H NMR spectral shift reagent (+)-TFAE was purchased from Aldrich Chemical Co. (USA) and used as such.

(±)-5-Acetoxy-3-(4-fluorophenyl)-2-phenylisoxazolidine (2). To a solution of α-(4-fluorophenyl)-*N*-phenylnitrone (**1**, 2.6 g, 12 mmol) in anhydrous benzene (20 mL), vinyl acetate (1.33 mL, 12 mmol) was added and the reaction mixture was stirred at 60 °C. The progress of the reaction was monitored by TLC and on completion, solvent was removed under reduced pressure to afford the crude product as an off-white solid, which was purified by column chromatography over silica gel using petroleum ether/ethyl acetate (4:1) as eluent to afford the pure compound (±)-**2** as a white crystalline solid (2.7 g) in 75% yield, mp 90–91 °C, $[\alpha]_D^{27}$ 0 (*c* 0.85, chloroform), R_f 0.41 (petroleum ether/ethyl acetate, 4:1). HR-MS: $[M]^+$ 301.1076, calcd for $C_{17}H_{16}FNO_3$ 301.1114; IR (KBr): 2362, 1736, 1601, 1510, 1490, 1361, 1233, 1196, 1078, 1058, 986, 959, 856 and 768 cm^{-1}; UV (MeOH): 204 and 240 nm; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.10 (s, 3H, $COCH_3$), 2.47 (ddd, J=13.5, 6.3 and 1.5 Hz, 1H, C-4Hα), 3.15 (ddd, J=13.5, 9.3 and 6.3 Hz, 1H, C-4Hβ), 4.40 (dd, J=9.3 and 6.3 Hz, 1H, C-3H), 6.58 (dd, J=6.3 and 1.5 Hz, 1H, C-5H), 6.93–7.12 (m, 5H, C-2′H, C-4′H, C-6′H, C-3″H and C-5″H), 7.16–7.22 (m, 2H, C-3′H and C-5′H) and 7.45 (dd, J=9.0 and 5.4 Hz, 2H, C-2″H and C-6″H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 21.26 ($COCH_3$), 45.96 (C-4), 68.72 (C-3), 94.57 (C-5), 115.69 and 117.25 (C-2′, C-6′, C-3″ and C-5″), 123.66 (C-4′), 128.75 (C-2″ and C-6″), 129.02 (C-3′ and C-5′), 136.19 (C-1″), 149.50 (C-1′), 164.13 (C-4″) and 170.36 (CO); EI-MS, *m*/*z* (% rel. int.): 301 ($[M]^+$, 3), 241 (3), 223 (3), 200 (9), 167 (3), 149 (18), 123 (9), 97 (3) and 58 (100).

***Candida rugosa* lipase-catalysed deacetylation of (±)-5-acetoxy-3-(4-fluorophenyl)-2-phenylisoxazolidine (2).** To a solution of (±)-acetoxyisoxazolidine **2** (482 mg, 1.6 mmol) in anhydrous diisopropyl ether (20 mL) containing *n*-butanol (3 mol equiv), *Candida rugosa* lipase (200 mg) was added. The suspension was stirred at 40–42 °C and progress of the reaction was monitored by HPLC. When 50% conversion of the starting material to the product was reached (in 52 h), the enzyme was filtered off and the solvent evaporated under reduced pressure to afford the crude product; column chromatography of the crude mixture over silica gel using a

gradient mixture of petroleum ether and ethyl acetate afforded the optically enriched unreacted (−)-5-acetoxy-3-(4-fluorophenyl)-2-phenylisoxazolidine (**2**) and deacetylated (+)-3-(4-fluorophenyl)-5-hydroxy-2-phenylisoxazolidine (**4**).

(−)-5-Acetoxy-3-(4-fluorophenyl)-2-phenylisoxazolidine (2). Was eluted from the column using petroleum ether/ethyl acetate (4:1) as a white crystalline solid (207 mg) in 43% yield,[18] mp 90–91 °C, $[\alpha]_D^{27}$ −167 (*c* 0.63, chloroform), R_f: 0.41 (petroleum ether/ethyl acetate, 4:1). All the spectral data of (−)-isoxazolidine **2** were found to be identical with the data of (±)-isoxazolidine (**2**).

(+)-3-(4-Fluorophenyl)-5-hydroxy-2-phenylisoxazolidine (4) (diastereomeric mixture of *cis* and *trans* isoxazolidines with respect to the orientation of the C-3 aryl and the C-5 hydroxy groups). Was eluted from the column using petroleum ether/ethyl acetate (3:2) as a white crystalline solid (173 mg) in 42% yield,[18] mp 118–20 °C, $[\alpha]_D^{27}$ +118 (*c* 1.01, chloroform), R_f: 0.35 (petroleum ether/ethyl acetate, 3:2). IR (Nujol): 3391, 1598, 1509, 1487, 1460, 1376, 1228, 1157, 837, 758 and 726 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.37–2.46 (m, 2H, C-4$H_{\alpha,cis}$ and C-4$H_{\alpha,trans}$), 2.76–2.82 (m, 2H, C-4$H_{\beta,cis}$ and C-4$H_{\beta,trans}$), 3.06 (m, 2H, C-5O_{Hcis} and C-5O_{Htrans}), 4.38 (dd, J=7.1 and 6.0 Hz, 1H, C-3$_{Hcis/trans}$), 4.82 (*dd*, J=7.1 and 5.9 Hz, 1H, C-3$_{Htrans/cis}$), 5.76 (m, 2H, C-5$_{Hcis}$ and C-5$_{Htrans}$), 6.83–6.99 (m, 6H, C-2′$_{Hcis}$, C-2′$_{Htrans}$, C-4′$_{Hcis}$, C-4′$_{Htrans}$, C-6′$_{Hcis}$ and C-6′$_{Htrans}$), 7.02–7.25 (m, 8H, C-3′$_{Hcis}$, C-3′$_{Htrans}$, C-5′$_{Hcis}$, C-5′$_{Htrans}$, C-3″$_{Hcis}$, C-3″$_{Htrans}$, C-5″$_{Hcis}$ and C-5″$_{Htrans}$) and 7.42–7.60 (m, 4H, C-2″$_{Hcis}$, C-2″$_{Htrans}$, C-6″$_{Hcis}$ and C-6″$_{Htrans}$); ^{13}C NMR ($CDCl_3$) δ 47.19 and 47.77 (C-4$_{cis}$ and C-4$_{trans}$), 66.90 and 68.98 (C-3$_{cis}$ and C-3$_{trans}$), 96.04 and 97.22 (C-5$_{cis}$ and C-5$_{trans}$), 114.47, 115.07, 115.95 and 116.93 (C-2′$_{cis}$, C-2′$_{trans}$, C-6′$_{cis}$, C-6′$_{trans}$, C-3″$_{cis}$, C-3″$_{trans}$, C-5″$_{cis}$ and C-5″$_{trans}$), 121.49 and 121.57 (C-4′$_{cis}$ and C-4′$_{trans}$), 128.17, 128.64, 129.20 and 129.30 (C-3′$_{cis}$, C-3′$_{trans}$, C-5′$_{cis}$, C-5′$_{trans}$, C-2″$_{cis}$, C-2″$_{trans}$, C-6″$_{cis}$ and C-6″$_{trans}$), 136.57 and 139.05 (C-1″$_{cis}$ and C-1″$_{trans}$), 150.02 and 153.10 (C-1″$_{cis}$ and C-1′$_{trans}$) and 160.61 and 163.86 (C-4″$_{cis}$ and C-4′$_{trans}$); EI-MS, m/z (% rel. int.): 259 ($[M]^+$, 4), 241 (63), 224 (31), 212 (7), 198 (5), 146 (36), 133 (46), 123 (47), 118 (18), 109 (14), 104 (19), 95 (21), 91 (100), 77 (85), 64 (15), 58 (23), 51 (39) and 43 (87).

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