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Selective photochemical synthesis of 3,3′-dimethoxy-4,2′-dihydroxybiphenyl

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Abstract

3,3′-Dimethoxy-4,2′-dihydroxybiphenyl (4,2′-DHBP) can be synthesized with high selectivity from 2-methoxyphenol (guaiacol) by photochemical excitation. Alternate methods to synthesize guaiacol dimers, e.g. peroxidase-catalyzed or electrochemically initiated dimerizations, generate a very low yield of 4,2′-DHBP in comparison. Product yield of the photochemical synthesis of 4,2′-DHBP can be enhanced by the addition of benzophenone as an activator without measurable loss in selectivity. Analytical GC–MS as well as a preparative HPLC method for the isolation of 4,2′-DHBP are described. The enzymatic and photochemical approach reveal similar selectivities of about 90%, however the peroxidase reaction leads to 3,3′-dimethoxy-4,4′-dihydroxybiphenyl (4,4′-DHBP) whereas the photochemical reaction affords 3,3′-dimethoxy-4,2′-dihydroxybiphenyl (4,2′-DHBP).

Keywords: Guaiacol; 2-Methoxyphenol; Dimerization; 3,3′-Dimethoxy-4,2′-dihydroxybiphenyl; Photochemical synthesis

1. Introduction

2-Methoxyphenol (guaiacol) is a commonly used reagent in biochemistry for the quantitative analysis of the enzymatic activity of peroxidases [1]. In addition, it is a structural unit of lignin and is frequently used as a model compound in this context [2]. In both cases, the formation of dimers from guaiacol (**I**) is an essential step (Fig. 1). In the enzymatic reaction, dimerization causes the coloration of the solution which serves as an indicator. In lignin, the network comprising guaiacol subunits is related to fiber strength. Five different dimers (**II**–**VI**) can be obtained through theses processes.

The determination of peroxidase activity with guaiacol (**I**) as an indicator is widely used. Peroxidases cause the oxidation of **I** in a single electron reaction which leads to the formation of phenoxyl radicals (**I•**). A complex mixture of products is observed which comprises five dimers and two trimers of guaiacol [1]. The main product obtained is 3,3′-dimethoxy-4,4′-dihydroxybiphenyl (**V**) which is further oxidized by peroxidase to 3,3′-dimethoxy-4,4′-biphenoquinone (**VII**) (Fig. 2). The later compound is responsible for the absorption of the solution with a maximum at 470 nm [3]. Other dimers, in particular 3,3′-dimethoxy-4,2′-dihydroxybiphenyl (4,2′-DHBP), occur as minor side products only. A ratio of 96:4 for 4,4′-DHBP:4,2′-DHBP is reported [1].

We analyzed whether photochemical or electrochemical instead of enzymatic generation of guaiacol radicals affects the dimer distribution. We found that with high selectivity the 4,2′-DHBP (**VI**) dimer is obtained upon UV-excitation of undiluted guaiacol. Similar results are obtained in solutions of guaiacol in nonprotic solvents where only small amounts of the 4,4′-DHBP (**V**) were observed as a side product. With increasing dilution and increasing protic character of the solvent 4,4′-DHBP becomes the main product, the same dimer which is obtained in the enzymatic reaction. The dimers can

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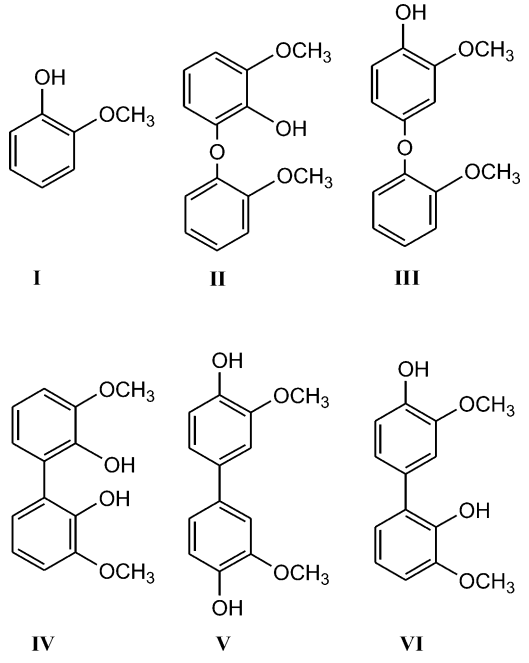

Fig. 1. Structures of guaiacol (**I**) and its five dimers (**II–VI**).

be isolated effectively by preparative column chromatography. This approach allows the selective preparation of any dimer of interest.

2. Experimental

Guaiacol purum (≥98%, GC), benzophenone, potassium nitrosodisulfonate and horse radish peroxidase were obtained from Sigma-Aldrich, Taufkirchen, and used as received.

For screening purposes, light exposure of guaiacol was undertaken in sealed quartz cuvettes for 18 h in a Suntest XLS+ (Atlas) using a Xenon arc lamp without a glass filter. The power at the sample was 765 W/m^2. Preparative reactions were undertaken in sealed quartz cuvettes in a UV reactor equipped with a 150-W mercury high-pressure lamp (Heraeus TQ150).

Peroxidase reactions were undertaken in Eppendorf vials. Nine hundred and sixty microliters of 100 mM phosphate buffer, pH 7.0, was mixed with 170 μl of 18 mM aqueous guaiacol solution. Upon addition of 70 μl of peroxidase solution having an activity of 25 U/ml and 150 μl 8 mM aqueous H_2O_2 solution, the color changed to brownish under gentle agitation at room temperature within a minute. The aqueous phase was extracted with 500 μl of chloroform. The chloroform phase was immediately subjected to GC–MS analysis.

Electrochemical synthesis and cyclovoltammetric analysis was undertaken in a three-electrode system comprising a platinum net anode, a glassy carbon cathode and a calomel reference electrode (SCE). A model 273A potentiostat controlled by the model 270 electrochemical analysis system software (both from EG & G) was used. Guaiacol (10 ml) was dissolved in a mixture of 0.1 M acetate buffer (20 ml) pH 4.7 and acetonitrile (20 ml) and the mixture was electrolyzed at 1 V versus SCE for 2 h. A total charge flow of 63.5 C was observed.

GC–MS analysis of the reaction products was undertaken using a Shimadzu GCMS-QP5050A equipped with a 30-m column type FS-SE-54-CB-0.25 (Chromatographie Service, Langerwehe). The mass detector spans the range from 50 to 700 *m*/*z*. An analytical method for the GC–MS analysis of the reaction products was developed. Helium 5.0 gas was used as a mobile phase at a flow rate of 0.6 ml/min (split ratio 9). The injector temperature was 300 °C and the interface was thermostated to 230 °C. A temperature gradient of 3 °C/min starting form 160 °C and ending at 280 °C appeared to be useful. The final value was kept constant for 40 min. Mass detection started at 12 min run time in order to cut-off the large peak resulting from remaining guaiacol. Electron ionization (EI) at 70 eV was applied.

Analytical HPLC analysis was done on a Agilent model 1050 system equipped with a diode array detector. The col-

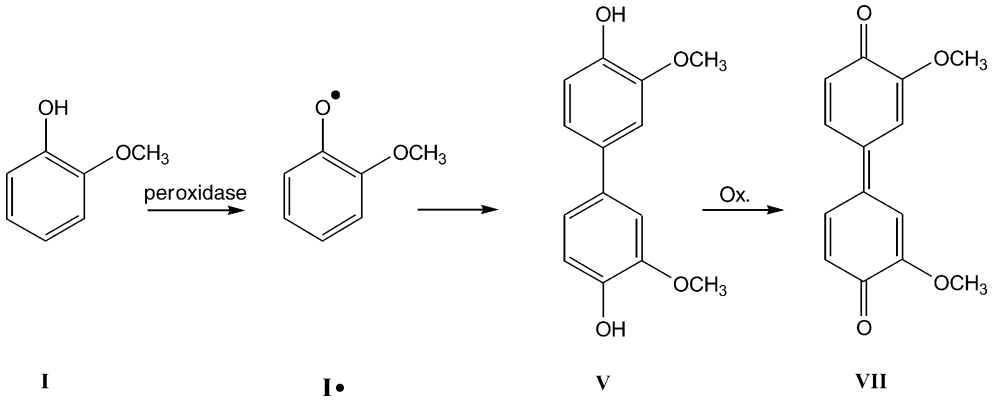

Fig. 2. Selective formation of 3,3′-dimethoxy-4,4′-biphenyl (**V**) catalyzed by peroxidase in aqueous solution. **V** is further oxidized by peroxidase to 3,3′-dimethoxy-4,4′-biphenoquinone (**VII**).

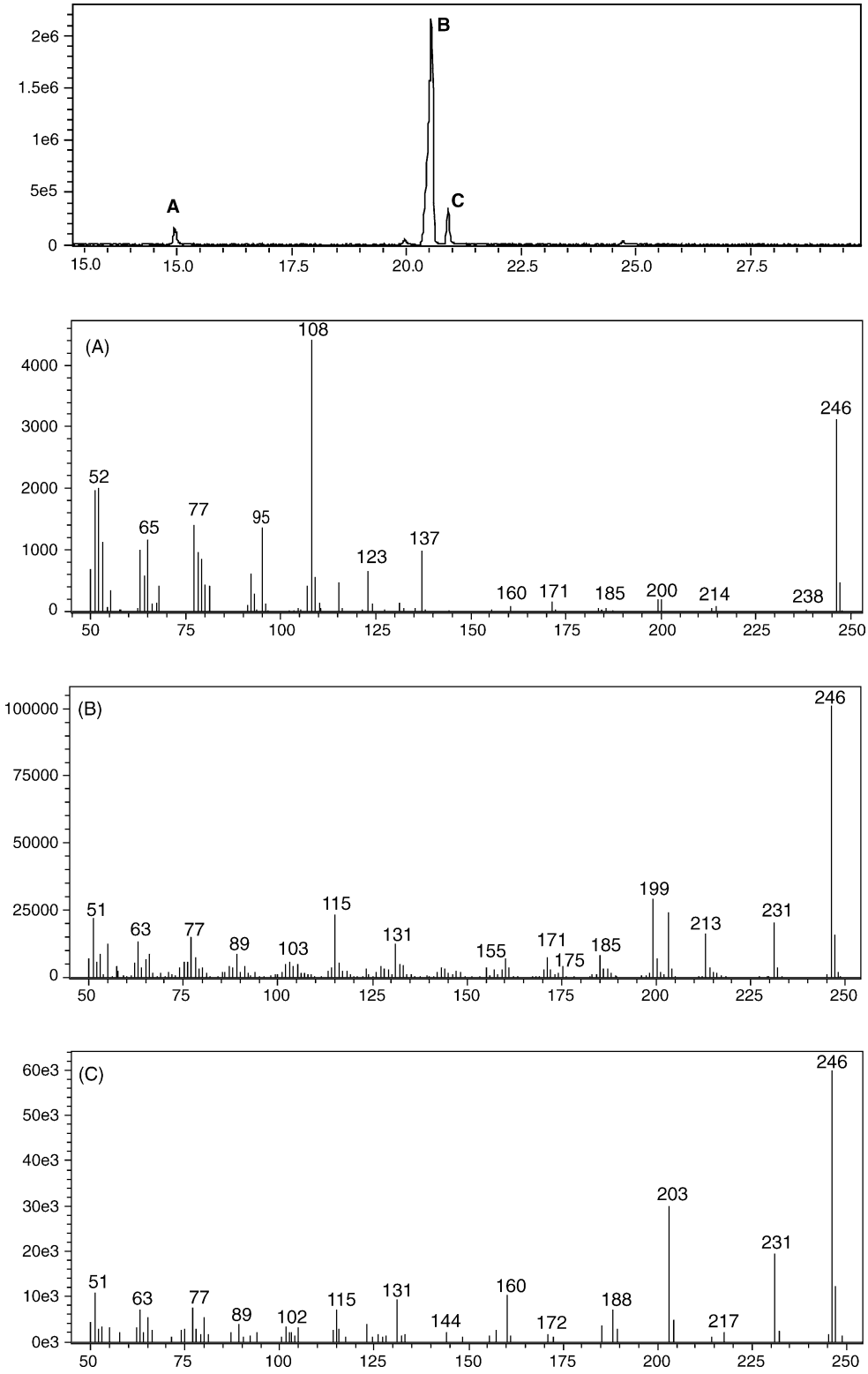

Fig. 3. GC–MS analysis of guaiacol dimers obtained from photochemical-induced guaiacol dimerization. (Top) Total ion count (TIC) of guaiacol products after UV exposure. (Below) Mass spectra of the peaks A to C.

umn used was a 250 × 4.0 mm RP 18 loaded with 3 μm material (Nucleosil RP 18, Bischoff, Leonberg). Separations were done in an isocratic run of 27-min duration with a mixture of 35% acetonitrile and 65% water (containing 3% H_3PO_4) at a flow rate of 1 mL/min at a column temperature of 40 °C.

Preparative separation of the photo-products was done using a preparative HPLC system (Knauer) equipped with a 32 × 250 mm sized RP18 column (EnCaPharm 100RP18, 10 μm).

NMR spectra were recorded on a 500 MHz spectrometer (Avance 500, Bruker) in $CDCl_3$ solution at 300 K.

3. Results and discussion

3.1. Photochemically induced guaiacol dimers

Upon exposure of guaiacol with UV light, a dark reddish brown colored liquid is obtained. Using the developed GC–MS protocol (see Section 2) three products were identified which have different retention times but an identical mass of 246 *m/z* (Fig. 3). This corresponds with the formation of three of the possible dimers of guaiacol (MW = 124 g/mol) as detailed in Table 1. The main product peak B represents about 90% of the dimer fraction. The fragmentation pattern of peak C identifies it as the 4,4′-DHBP dimer which is the main product of the peroxidase reaction [4]. Whereas peak A can probably be assigned to either dimer **II** or **III**, from its MS fragmentation pattern peak B is the desired 4,2′-DHBP.

3.2. Isolation and identification of 3,3′-dimethoxy-4,2′-dihydroxybiphenyl

In order to identify the composition of peak B, a method was developed to isolate this isomer by preparative HPLC. Isocratic separation of the isomer mixture (35/65 acetonitrile–water mixture on a RP18 column) was achieved on an analytical HPLC system, and transposed to preparative HPLC using a flow rate of 10 mL/min acetonitrile/water (3:7) (50 min run). Subsequent gradient increases of acetonitrile (10% per 5 min) allowed isolation of pure material.

^{1}H,[1] ^{13}C[2] and COSY NMR analyses identified the structure as 4,2′-DHBP. The fragmentation pattern of 4,2′-DHBP resulting from electron ionization[3] was consistent (see Fig. 3), suggesting the photochemical route allows selective synthesis of 4,2′-DHBP in one simple step.

Table 1
Isomer composition of photochemically dimerized guaiacol

Product	Retention time (Rt) (min)	Product distribution (%)[a]
A	14.9	3
B	20.9	90
C	21.5	7

[a] Area % of peaks in total ion count (TIC).

From analytical HPLC runs, the absorption spectra of the two dimers 4,2′-DHBP and 4,4′-DHBP were obtained (Fig. 4). Both compounds have no absorption in the visible region and therefore appear colorless. Intense color appears upon oxidation of the isolated 4,2′-DHBP dimer, e.g., with Fremy's salt [6]. A single product is found in GC–MS having a mass of 262 *m/z*. This indicates that a oxygen was introduced into the molecule and a hydroquinone/quinone system was generated and absorption in the visible region appears (Fig. 4).

3.3. Dimer composition obtained by electrochemical, enzymatic and photochemical syntheses

The dimer composition obtained by three different synthetic routes has been compared (Fig. 5). In addition to the above photochemical activation, enzymatic synthesis catalyzed by peroxidase [7] and electrochemical synthesis have been examined (Table 2). The peroxidase reaction leads with high selectivity to 4,4′-DHBP as previously reported. Electrochemical radical generation in a 1:1 mixture of acetonitrile and 0.1 M acetate buffer pH 4.7 leads to a distribution of about 2:1 of 4,4′-DHBP and 4,2′-DHBP but in addition significant amounts of other unidentified dimers appear. These results suggest the photochemical protocol is the method of choice as 4,2′-DHBP is obtained in high yield [8].

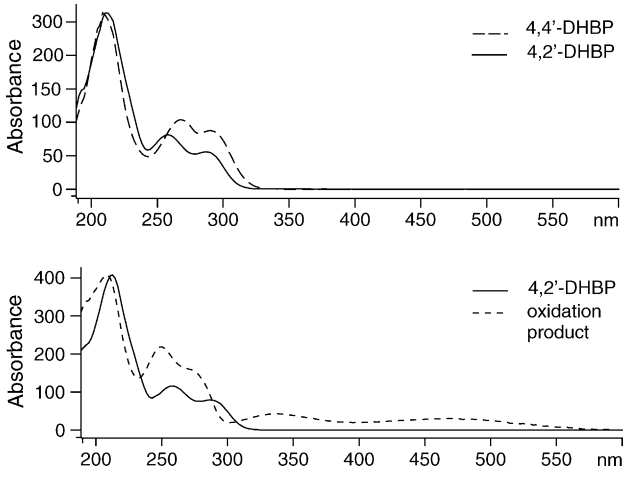

Fig. 4. (Top) UV–vis absorption spectra of 3,3′-dimethoxy-4,4′-dihydroxybiphenyl (**V**) and 3,3′-dimethoxy-4,2′-dihydroxybiphenyl (**VI**). (Bottom) UV–vis absorption spectra of **VI** and its oxidation product obtained from the reaction with Fremy's salt.

[1] ^{1}H NMR: δ_H 2.20 (6H, s), 5.69 (1H, s), 5.89 (1H, s), 6.88 (1H, dd, J = 7.9 Hz, J = 1.7 Hz), 6.93 (1H, t, J = 7.9 Hz), 6.98 (1H, dd, J = 7.9 Hz, J = 1.7 Hz), 7.01 (1H, d, J = 8.1 Hz), 7.13 (1H, dd, J = 8.1 Hz, J = 1.9 Hz), 7.20 (1H, d, J = 1.9 Hz) ppm.

[2] ^{13}C NMR: δ_c 55.9 (OCH_3), 56.2 (OCH_3), 109.4 (C_{ar}—H), 112.0 (C_{ar}—H), 114.2 (C_{ar}—H), 119.6 (C_{ar}—H), 122.2 (C_{ar}—H), 122.5 (C_{ar}—H), 127.6 (C_{ar}—C_{ar}), 129.8 (C_{ar}—C_{ar}), 142.7 (C_{ar}—O), 144.9 (C_{ar}—O), 146.2 (C_{ar}—O), 146.9 (C_{ar}—O) ppm.

[3] *m/z* (relative intensity): 246 [M^+, 100], 231 (20), 213 (16), 203 (24), 199 (29), 185 (8), 171 (8), 131 (12), 115 (23), 89 (8), 77 (15), 63 (13), 51 (22).

Table 2
Isomer distribution in dependence on activation method

		4,2′-DHBP (%)[a]	4,4′-DHBP (%)[a]	Other (%)[a]
Photochemical	Guaiacol liqu.	90	7	3
Electrochemical	In acetate buffer/acetonitrile (1:1)	15	33	52
Peroxidase	In phosphate buffer	5	91	4

[a] Area % of peaks in total ion count (TIC).

3.4. Benzophenone-enhanced photochemical dimerization

Benzophenone is widely used as an activator in photosynthesis [5], and in this case the yield of 4,2′-DHBP can be increased with no effect on selectivity or dimer distribution. For example, addition of 29.4% (w/w) benzophenone [9], i.e. 0.2 equiv., to the guaiacol increased the yield of dimers by a factor of about 3.

4. Conclusion

Photochemical dimerization and preparative HPLC purification enable the synthesis and isolation of suitable amounts of 4,2′-DHBP. In comparison of the three methods (photochemical, enzymatic and electrochemical), only the photochemical route displays high selectivity for 4,2′-DHBP. Both the photochemical and the enzymatic synthesis show excellent selectivities of about 90% for single but different

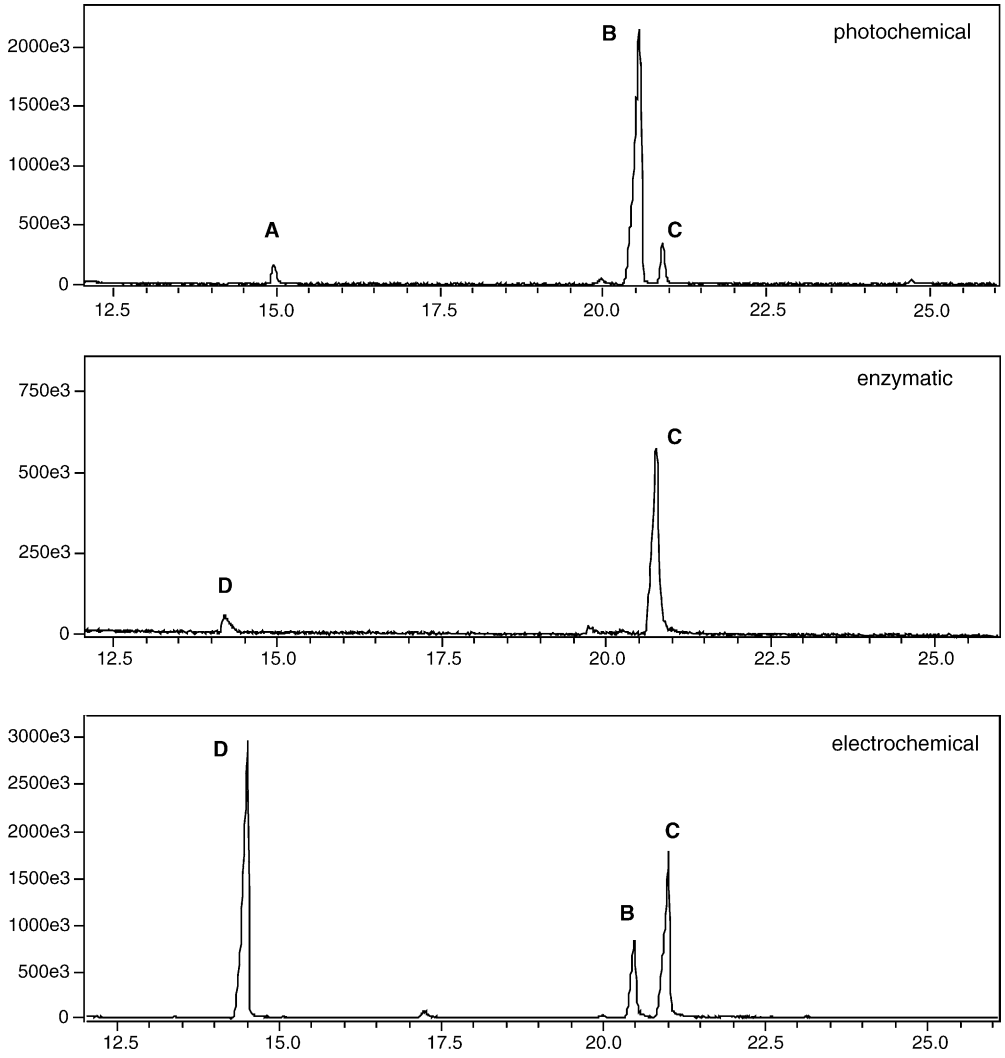

Fig. 5. Dimer composition in dependence on the synthetic route. The dimer composition obtained through (top) photosynthesis, (middle) peroxidase and (bottom) electrosynthesis is quite different. The photochemical and the enzymatic route show similar selectivities of about 90% but for different dimers (B = 4,2′-DHBP, C = 4,4′-DHBP).

dimers (see Fig. 5). The enzymatic approach is the method of choice for the generation of 4,4′-DHBP and the photochemical method for 4,2′-DHBP synthesis.

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