Synthesis, lipase catalyzed kinetic resolution, and determination of the absolute configuration of enantiomers of the Morita-Baylis-Hillman adduct 3-hydroxy-2-methylenebutanenitrile

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Dedicated to Prof. Jacek Młochowski on the occasion of his 80th birthday

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Abstract

3(*RS*)-Hydroxy-2-methylenebutanenitrile was synthesized from acetaldehyde and acrylonitrile with DABCO as a catalyst. Optimization of the reaction conditions was conducted because some literature procedures were not reproducible. Asymmetric transesterification of the Morita-Baylis-Hillman adduct was carried out using ten lipases. The hydroxyl-nitrile was also esterified chemically and subjected to enzymatic hydrolysis with the same set of enzymes. The best results were obtained with lipase from *Candida antarctica* and Novozyme 435. The absolute configuration of an optically pure hydroxyl-nitrile was determined by Mosher's method.

Keywords: Morita-Baylis-Hillman reaction, transesterification, hydrolysis, lipases, biocatalysis, stereochemistry

Introduction

The Morita-Baylis-Hillman (MBH) reaction is a useful tool for the synthesis of multifunctionalized compounds from electrophiles and activated alkenes (Scheme 1).

Scheme 1. General scheme of the MBH reaction.

Since the end of the 1960s many modifications of the Morita-Baylis-Hillman reaction have been developed including asymmetric versions with application of organocatalysts¹ such as chiral phosphoroorganic compounds,²⁻⁴ chiral Brønsted acids⁵ or thiourea derivatives.^{3,6-8} Biocatalysts⁹⁻¹¹ have also been employed as chiral auxiliaries in MBH reactions, but in general they are less effective in terms of both substrate conversion and stereoselectivity. Our previous research focused on the synthesis and evaluation of biological activities of novel terpenoid derivatives.¹²⁻¹⁵ We have focused recently on the possibility of using MBH adducts in the synthesis of novel δ -lactones with potential olfactory properties. We chose 3-(*RS*)-hydroxy-2-methylenebutanenitrile **1(RS)** as a model substrate for further studies. The use of simple aldehydes and activated alkenes provides a good model for the optimization of MBH reactions in various environments.¹⁶⁻¹⁸ Most studies of this sort have been carried out using ring-substituted benzaldehydes.

β -Hydroxy-nitriles are less commonly known to possess interesting aroma profiles but there is some evidence of their usefulness as fragrance agents.¹⁹ The synthesis of Morita-Baylis-Hillman adducts in the absence of chiral auxiliaries results in stereomeric mixtures of products. Kinetic resolution can be employed to obtain optically pure or enriched molecules therefrom.²⁰⁻²² The use of enzymes for the kinetic resolution of secondary carbinols is well documented,^{23,24} and has proved to be economically and environmentally advantageous. Previous studies regarding biocatalytic resolution of enantiomers of 3(*RS*)-hydroxy-2-methylenebutanenitrile **1(RS)** were focused on the optimization of transesterification reaction conditions using lipase from *Pseudomonas cepacia*.²⁵ The optimized reaction conditions resulted in a moderate conversion of MBH adduct (27%) with excellent *ee* (98%). A major disadvantage of this procedure is the very long reaction time (474 h), which makes it unpractical. Rotation of the plane of polarized light of the acetylated product was described as levorotary but the specific rotatory power was not determined. Adduct **1(RS)** was also used to study nitrile hydrolase activity of *Rhodococcus erythropolis* SET1.²⁶ The yield of recovered nitrile and reaction time were satisfactory (42% and 48 h respectively); *ee* was moderate (76.5%). The configuration of the stereogenic secondary carbinol center was assigned only tentatively (*R*), and the specific rotatory power also was not determined.

The aim of studies presented herein was to optimize the synthesis and biocatalytic kinetic resolution of racemic aliphatic MBH adduct to give optically pure model β -hydroxy-nitriles and their acetates. In addition, we aimed to determine the absolute configuration of the 3-hydroxy-2-methylenebutanenitrile.

Results and Discussion

3(*RS*)-Hydroxy-2-methylenebutanenitrile **1(RS)** was prepared by the Morita-Baylis-Hillman reaction of acetaldehyde and acrylonitrile using 1,4-diazabicyclo[2.2.2]octane (DABCO) as a catalyst (Scheme 2).

Scheme 2. Synthesis of the model racemic MBH adduct.

Optimization of the reaction conditions was carried out in a solvent-free environment (Table 1), and in THF, 1,4-dioxane, and their water mixtures (Table 2). An accelerated rate and increased yields in the Morita-Baylis-Hillman reaction of acetaldehyde and acrylonitrile was observed, as in previous reports regarding aromatic aldehydes and activated alkenes.^{18,27} In general, a stoichiometric amount of a base is usually used for MBH transformations. In this report we show that an increase in the molar ratio of DABCO results in lower product yields (entries 1-4). Increasing the molar ratio of acetaldehyde is more favorable than of acrylonitrile, and results in higher yields and fewer side products (entries 5 and 6).

Table 1. Optimization of MBH reaction of acetaldehyde and acrylonitrile under solvent-free conditions

Entry	Acetaldehyde ^a	Acrylonitrile ^a	DABCO ^a	Yield ^b (%)
1	1	1	0.2	97
2	1	1	0.5	93
3	1	1	0.75	90
4	1	1	1	77
5	1	1.5	1	70
6	1.5	1	1	84

^a Molar ratio. ^b Isolated yield.

Optimization of the reaction conditions was also carried out in THF, 1,4-dioxane and their water mixtures using equimolar amounts of both substrates, and 20 mol% DABCO. Yields of MBH transformations in pure solvents were moderate to poor (entries 1 and 4), but in their water mixtures reactions yields were excellent. Similar outcomes were obtained in the coupling of acetaldehyde and methyl acrylate in organic solvent-water systems.²⁸

Table 2. Optimization of MBH reaction of acetaldehyde and acrylonitrile carried out in THF, 1,4-dioxane, and their mixtures with water

Entry	Acetaldehyde ^a	Acrylonitrile ^a	DABCO ^a	THF ^b	H ₂ O ^b	1,4-Dioxane ^b	Yield ^c (%)
1	1	1	0.2	1	-	-	40
2	1	1	0.2	1	1	-	77
3	1	1	0.2	0.7	0.3	-	94
4	1	1	0.2	-	-	1	19
5	1	1	0.2	-	1	1	93
6	1	1	0.2	-	0.3	0.7	91

^a Molar ratio. ^b Volume ratio. ^c Isolated yield.

The first attempt at kinetic resolution of **1(RS)** was based on enzymatic transesterification (Scheme 3) under anhydrous conditions with vinyl acetate as an acyl donor. We chose ten lipases for the initial screening (Table 3). Entry numbers in Table 3 identify the enzymes used in Tables 4 and 5.

Scheme 3. Enzymatic transesterification of 3(RS)-hydroxy-2-methylenebutanenitrile.

Table 3. Biocatalysts selected for the screening procedure

Entry	Lipase
1	Novozyme 435
2	PS Amano lipase from <i>Pseudomonas cepacia</i>
3	Immobilized lipozyme from <i>Mucor miehei</i>
4	Lipase from <i>Rhizopus oryzae</i>
5	G Amano lipase from <i>Penicillium camemberti</i>
6	AYS Amano lipase
7	AS Amano lipase
8	Lipase A from <i>Candida antarctica</i>
9	RMIM lipozyme
10	M Amano lipase from <i>Mucor javanicus</i>

Table 4. Results of biocatalytic transesterification of 3(RS)-hydroxy-2-methylenebutanenitrile

Entry	1h				24h				48h			
	Alc. ^a ee%	Acetate ^b ee%	Conv. ^c %	E ^d	Alc. ^a ee%	Acetate ^b ee%	Conv. ^c %	E ^d	Alc. ^a ee%	Acetate ^b ee%	Conv. ^c %	E ^d
1	33	97	25	91	63	82	44	19	29	60	32	5
2	9	100	9	-	19	96	17	59	48	96	33	79
3	5	100	4	-	54	95	36	67	81	94	46	81
4	0	0	-	-	0	0	-	-	0	0	-	-
5	0	0	-	-	0	0	-	-	0	0	-	-
6	0	0	-	-	1	100	1	-	3	63	4	5
7	0	0	-	-	1	100	1	-	2	86	3	14
8	61	95	39	73	87	44	67	7	73	29	71	4
9	1	100	1	-	18	96	15	58	26	96	21	63
10	0	0	-	-	0	0	-	-	1	100	<1	-

^a (R)-acetate. ^b (S)-alcohol. ^c Based on GC, calculated according to the formula $\text{conv.} = ee_s / (ee_s + ee_p)$. ^d E-value calculated according to the formula $E = \{\ln[(1-ee_s)/(1+ee_s/ee_p)]\} / \{\ln[(1+ee_s)/(1+ee_s/ee_p)]\}$

As a result of these biotransformations we were able to obtain acetate **2(S)** in excellent enantiomeric excess, and moderate to very good yield (Table 4, entries 1-3 and 8). Novozyme 435 and lipase A from *Candida antarctica* transformed *S*-alcohol most rapidly (Table 4 entries 1 and 8; 25% and 39% of substrate conversion after 1 h). PS Amano lipase from *Pseudomonas cepacia* and immobilized lipozyme from *Mucor miehei* required more time to achieve higher conversion, and in these cases there was little or no effect on enantioselectivity.

The second strategy for obtaining pure enantiomers of 3-hydroxy-2-methylenebutanenitrile was enzymatic hydrolysis of 3(*RS*)-acetoxy-2-methylenebutanenitrile **2(RS)**, which was synthesized from **1(RS)** using acetyl chloride and pyridine in anhydrous dichloromethane (Scheme 4). Both compounds were found to be not useful as fragrances: alcohol **1(RS)** is odorless, and acetate **2(RS)** causes irritation to the nasal mucosa. Biotransformations were carried out using the same set of enzymes at room temperature with shaking (150 rpm) in a biphasic system consisting of a phosphate buffer (pH 7) and *i*-Pr₂O (Scheme 4).

Scheme 4. Synthesis and enzymatic hydrolysis of 3(*RS*)-acetoxy-2-methylenebutanenitrile.

The three tested enzymes gave acceptable results (Table 5, entries 1,2 and 8). It can be seen that enzymatic hydrolysis results in higher conversions than transesterification reactions, but at the expense of enantioselectivity. The (*S*)-acetate is consumed considerably faster than the *R*-isomer, but the hydrolysis in a biphasic system is a slower process, and in this case it requires 24 h to obtain excellent ees of an acetate (>99%). Conversions above 50% indicate that both isomers of acetate **2** are being consumed. Careful monitoring of the hydrolysis progress is required to obtain optically pure **1(S)**.

Table 5. Results of biocatalytic hydrolysis of 3(*RS*)-acetoxy-2-methylenebutanenitrile

Entry	1h				24h				48h			
	Acetate ^a ee%	Alc. ^b ee%	Conv. ^c %	E ^d	Acetate ^a ee%	Alc. ^b ee%	Conv. ^c %	E ^d	Acetate ^a ee%	Alc. ^b ee%	Conv. ^c %	E ^d
1	66	100	40	>200	100	71	58	30	100	54	65	16
2	23	100	19	>200	100	84	54	59	100	78	56	41
3	0	0	-	-	0	0	-	-	3	100	3	-
4	0	0	-	-	0	0	-	-	1	11	8	-
5	0	0	-	-	0	0	-	-	0	0	-	-
6	1	100	1	-	39	54	42	5	60	47	56	5
7	3	27	10	2	86	5	95	2	100	2	98	3
8	87	100	47	>200	100	49	67	14	100	33	75	9
9	0	0	-	-	0	0	-	-	0	0	-	-
10	0	0	-	-	0	0	-	-	0	0	-	-

^a (*R*)-acetate. ^b (*S*)-alcohol. ^c Based on GC, calculated according to the formula $\text{conv.} = ee_s / (ee_s + ee_p)$ ^dE-value calculated according to the formula $E = \{\ln[(1-ee_s)/(1+ee_s/ee_p)]\} / \{\ln[(1+ee_s)/(1+ee_s/ee_p)]\}$

The last phase of this study was determination of the absolute configuration of enantiomers of the Morita-Baylis-Hillman adduct **1**. We chose the Mosher ester method.²⁹ Optically pure acetate obtained via enzymatic hydrolysis (intact substrate, Table 5 entry 2, 24 h) was hydrolyzed chemically in a biphasic system. Subsequent esterification with both enantiomers of Mosher's acid chloride in anhydrous conditions gave two esters **3(R,R)** and **3(S,R)** (Scheme 5).

Scheme 5. Synthesis of Mosher's esters from the enzymatic hydrolysis product.

All relevant chemical shifts of Mosher's esters are shown in Table 6.

Table 6. Chemical shifts of **3(R,R)** and **3(S,R)**

Group	$\delta_{(S)\text{-ester}}$ [ppm]	$\delta_{(R)\text{-ester}}$ [ppm]
Methylene H	5.96	6.03
Methylene H	5.94	6.01
Methanetriyl H	5.57	5.56
Methyl H	1.50	1.43

Methyl and methanetriyl hydrogens of the (S)-ester lie downfield with respect to peaks of the (R)-ester, and methylene hydrogens the opposite. This shielding/deshielding pattern (Figure 1) indicates that initial alcohol has the *R* configuration.

Figure 1. Shielding/deshielding pattern for Mosher's esters of **1(R)**

Conclusion

We report above the strategy of the synthesis of the multifunctional building block 3-hydroxy-2-methylene-butanenitrile **1**, its kinetic resolution into optically pure isomers, and determination of the absolute configuration of the isomers of the title compound. Solvent-free Morita-Baylis-Hillman reaction has proven to give the best results (Table 1 entry 1). Two strategies of kinetic resolution allowed us to obtain the (*S*)-acetate with excellent *ee* (97% enzymatic transesterification – Table 4 entry 1), and optically pure (*S*)-alcohol (>99% – Table 5 entry 1) and (*R*)-acetate (>99% – Table 5 entries 1,2 and 8). The absolute configuration of the enzymatic hydrolysis product (Scheme 5) was determined by Mosher's method; the shielding/deshielding pattern indicates that the initial alcohol has the *R* configuration.

Experimental Section

General. All reagents obtained from commercial suppliers (Sigma-Aldrich, Poznań, Poland; Merck, Warszawa, Poland; Avantor, Gliwice, Poland) were of analytical grade and were used without further purification. The course of all reactions, composition of products, and their purities were monitored by gas chromatography (GC). Analytical GC was performed on Agilent 7890A apparatus with flame ionization detector with nitrogen as make up gas. Analyses were carried out on Thermo TR-5 column [(5% phenyl)-methylpolysiloxane] 30 m×0.32 mm ID×1.0 μm film using helium as carrier gas at a flow rate of 3.8462 mL/min in a split ratio of 1:100 and the following programme: (a) 40 °C for 5 min; (b) rate of 10.0 °C/min from 40 to 190 °C and hold for 5 min. Chiral gas chromatography was carried out with Varian CP Chirasil-Dex CB column, 25 m x 0.25 mm ID x 0.25 μm film. Analyses were carried out using helium as carrier gas, flow 0.5 mL/min, split ratio 35:1 with programme: (a) 120 °C for 10 min. Injector and detector were held at 250 °C. High resolution mass spectra (HRMS) were recorded using electron spray ionization (ESI) technique on Waters LCT Premier XE mass spectrometer. The middle-infrared spectra (4000–400 cm⁻¹) were collected on a Fourier transform, Bruker VERTEX 70 V vacuum spectrometer equipped with an air-cooled DTGS detector (Ettlingen, Germany). The liquid samples were placed on the diamond crystal of the ATR accessory. The spectral data

were recorded at a resolution of 2 cm^{-1} with 64 scans collection and further elaborated using Bruker OPUS software. ^1H and ^{13}C NMR spectra were recorded in CDCl_3 with TMS as an internal standard on Bruker Avance TM DRX 600 instrument. Chemical shifts (δ) are reported in ppm and coupling constants (J) are given in Hz. Optical rotation measurements were carried out on a Krüss P3000 automatic polarimeter.

3(*RS*)-Hydroxy-2-methylenebutanenitrile (**1**(*RS*))

To a mixture of acetaldehyde (5 g, 6.35 mL, 0.11 mol) and acrylonitrile (5.8 g, 7.2 mL, 0.11 mol) was added DABCO (2.5 g, 22 mmol). After stirring for 96 h at rt, the reaction mixture was diluted with 100 mL of EtOAc. The organic layer was washed three times with 5% water solution of HCl, once with water, once with 10% water solution of NaHCO_3 , and then with brine, dried over Na_2SO_4 and filtered. Evaporation of the solvents gave a yellow liquid, which was purified through flash chromatography on silica gel (hexane/EtOAc from 0 to 20% EtOAc) to give 3(*RS*)-hydroxy-2-methylenebutanenitrile **1**(*RS*) as a colorless, odorless liquid (10.4 g, 97%). IR (ATR, ν_{max} , cm^{-1}): 3465 (br), 2235 (w). ^1H NMR (600 MHz, CDCl_3): δ_{H} 1.47 (3H, d, $^3J_{\text{HH}}$ 6.6 Hz, $-\text{CH}_3$), 2.08 (1H, s, $-\text{OH}$), 4.47 (1H, q, $^3J_{\text{HH}}$ 6.6 Hz, $-\text{CH}$), 5.98 and 6.04 (2H, 2x d, $^4J_{\text{HH}}$ 0.6 Hz, $\text{CH} \times 2$ olefinic) ^{13}C NMR (CDCl_3 , 150 MHz): δ_{C} 22.3 (CH_3), 68.3 (CH_3CH), 117.1 (CHCCH_2), 127.8 (CHCCH_2), 129.1 (CHCCN), HRMS (TOFMS EI+) m/z calculated [$\text{C}_5\text{H}_7\text{NO} + \text{H}^+$] 98.0600, found 98.0615

General procedure for enzymatic transesterification of **1**(*RS*)

Vinyl acetate (42 mg, 45 μL , 0.49 mmol) and MBH adduct **1**(*RS*) (25 mg, 0.26 mmol) were placed in a 5 mL glass vial and dissolved in 2 mL of anhydrous *i*-Pr $_2$ O. A lipase (20 mg) was added, and reaction was stirred for 46 h. 200 μL of reaction mixture was collected after 1 h, 24 h and 48 h, and subjected to chiral gas chromatographic analysis.

3(*RS*)-Acetoxy-2-methylenebutanenitrile (**2**(*RS*)). Acetyl chloride (4.04 g, 3.67 mL, 51.5 mmol) was added dropwise to a mixture of 3(*RS*)-hydroxy-2-methylenebutanenitrile **1**(*RS*) (5 g, 51.5 mmol) and pyridine (4.1 g, 4.2 mL, 51.5 mmol) in 100 mL of DCM at 0 °C. The reaction was stirred for additional 2 h at rt. The reaction mixture was diluted with 50 mL of DCM. The organic layer was washed three times with 5% water solution of HCl, once with water, once with 10% water solution of NaHCO_3 , and then with brine, dried over Na_2SO_4 and filtered. Evaporation of the solvents gave a yellow liquid, which was purified through flash chromatography on silica gel (hexane/EtOAc from 0 to 10% EtOAc) to give 3(*RS*)-acetoxy-2-methylenebutanenitrile **2**(*RS*) as a colorless liquid, irritating to nasal mucosa (6.2 g, 86%). IR (ATR, ν_{max} , cm^{-1}): 2227 (w), 1743 (s). ^1H NMR (600 MHz, CDCl_3): δ_{H} 1.49 (3H, d, $^3J_{\text{HH}}$ 6.6 Hz, CHCH_3), 2.13 (3H, s, COCH_3), 5.44 (1H, q, $^3J_{\text{HH}}$ 6.6 Hz, CH), 6.03 and 6.05 (2H, 2x d, $^4J_{\text{HH}}$ 0.6 Hz, $\text{CH} \times 2$ olefinic) ^{13}C NMR (CDCl_3 , 150 MHz): δ_{C} 19.2 (CH_3CO), 21.0 (CHCH_3), 69.5 (CH_3CH), 116.2 (CHCCH_2), 123.8 (CHCCH_2), 131.7 (CHCCN), 169.7 (CH_3CO) HRMS (TOFMS EI+) m/z calculated [$\text{C}_7\text{H}_9\text{NO}_2 + \text{H}^+$] 140.0706, found 140.0720

General procedure for enzymatic hydrolysis of **2(*RS*).** Acetate **2**(*RS*) (25 mg, 0.18 mmol) was placed in a 10 mL glass vial and dissolved in 2 mL of *i*-Pr $_2$ O. A lipase (20 mg) and 2 mL of distilled water were added, and reaction was stirred for 46 h. 200 μL of reaction mixture was collected after 1 h, 24 h and 48 h, and subjected to chiral gas chromatographic analysis.

(-)-3(*R*)-Acetoxy-2-methylenebutanenitrile (**2**(*R*)). Acetate **2**(*RS*) (800 mg, 5.76 mmol) was placed in a 20 mL glass vial and dissolved in 5 mL of *i*-Pr $_2$ O. PS Amano lipase from *Pseudomonas cepacia* (500 mg) and 5 mL of distilled water were added, and reaction was stirred for 24 h. Lipase was filtered off, phases were separated, and the water phase was extracted twice with 2 mL of *i*-Pr $_2$ O. The combined organic phases were dried over Na_2SO_4 and filtered. Evaporation of the solvents gave a colorless liquid, which was purified through flash

chromatography on silica gel (hexane/EtOAc from 0 to 10% EtOAc) to give (2*R*)-3-(*R*)-acetoxy-2-methylenebutanenitrile **2(R)** as a colorless liquid, irritating to nasal mucosa (100 mg, 25%). $[\alpha]^{24} = -90$ [1.0, CHCl₃], NMR spectra correspond with the spectra of the racemic form.

Monitoring the progress of kinetic resolution

Biocatalytic transesterifications and hydrolysis were monitored via chiral gas chromatography under conditions described in the experimental "general" section. Determination of absolute configuration of the enzymatic hydrolysis product provided the possibility to identify respective isomers: $R_t = 5.39$ min ((*R*)-acetate); $R_t = 5.72$ min ((*S*)-acetate); $R_t = 8.41$ min ((*S*)-alcohol); $R_t = 8.74$ min ((*R*)-alcohol).

(-)-3(*R*)-Hydroxy-2-methylenebutanenitrile (1(*R*)). Acetate **2(R)** (100 mg, 0.72 mmol) was placed in a 10 mL glass vial and dissolved in 3 mL of *i*-Pr₂O. A solution of NaOH (~60 mg, ~1.44 mmol) in 3 mL of distilled water was added, and reaction was stirred for 6 h. After this time phases were separated, and the water phase was extracted twice with 2 mL of *i*-Pr₂O. The combined organic phases were dried over Na₂SO₄ and filtered. Evaporation of the solvents gave a pure (2*R*)-3-(*R*)-hydroxy-2-methylenebutanenitrile **2(R)** as a colorless and odorless liquid (65 mg, 92%). $[\alpha]^{24} = -10$ [0.65, CHCl₃], NMR spectra correspond with the spectra of the racemic form.

General procedure for Mosher's esters preparation²⁹. Mosher's acid chloride (3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid chloride, 3 μ L, 15.6 μ mol) was added to a mixture of 3(*R*)-hydroxy-2-methylenebutanenitrile **1(R)** (1 mg, 10.3 μ mol) and dry pyridine (2 μ L, 25 μ mol) in 300 μ L of CDCl₃. The reaction mixture was stirred until completion (~2 h, TLC: hexane/EtOAc 4:1) then diluted with 300 μ L of CDCl₃.

2(R)-3-Cyanobut-3-en-2-yl 2(*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (3(*S,R*)). ¹H NMR (600 MHz, CDCl₃): δ_H 1.43 (3H, d, ³ J_{HH} 6.6 Hz, -CH₃), 3.35 (3H, s, -OCH₃), 5.56 (1H, q, ³ J_{HH} 6.6 Hz, -CH), 6.01 and 6.03 (2H, 2x d, ⁴ J_{HH} 0.6 Hz, CH x 2 olefinic), 7.28-7.36 (5H, m, aromatic)

2(R)-3-Cyanobut-3-en-2-yl 2(*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (3(*R,R*)). ¹H NMR (600 MHz, CDCl₃): δ_H 1.50 (3H, d, ³ J_{HH} 6.6 Hz, -CH₃), 3.35 (3H, s, -OCH₃), 5.57 (1H, q, ³ J_{HH} 6.6 Hz, -CH), 5.94 and 5.96 (2H, 2x d, ⁴ J_{HH} 0.6 Hz, CH x 2 olefinic), 7.28-7.40 (5H, m, aromatic)

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