Tetrahedron Vol. 48, No. 44, pp. 9695-9706, 1992
Printed in Great Britain

0040-4020/92 $5.00+.00
Pergamon Press Ltd

5-Methoxy-pentono-1,4-lactones from (R)-2-Hydroxy-5-methoxy-3-pentenoic Acid Obtained by Bioreduction of the 2-Oxo Acid

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(*Received in Germany* 16 *July* 1992)

Abstract: Various 5-methoxy-pentono-1,4-lactones and derivates thereof were synthesised from propargyl alcohol or its methyl ether. The key step was the enantioselective reduction of 2-oxo-5-alkoxy-3-pentenoic acids by resting cells of *Proteus vulgaris*. The (R)-2-hydroxy-5-methoxy-3-pentenoic acid (ee. > 96%) was further functionalized via epoxidation, or hydroxylation with osmium tetroxide, or bromine addition, with modest to good diastereoselectivity, leading to 5-methoxy-pentono-1,4-lactones of the series (L)-*arabino*, (D)-*ribo*, (L)-*lyxo*, and (D)-*xylo*. A new way to obtain 2-oxo-3-enoic acids is described, using Pd mediated cross coupling between vinyl tin compounds and oxalyl chloride monoesters.

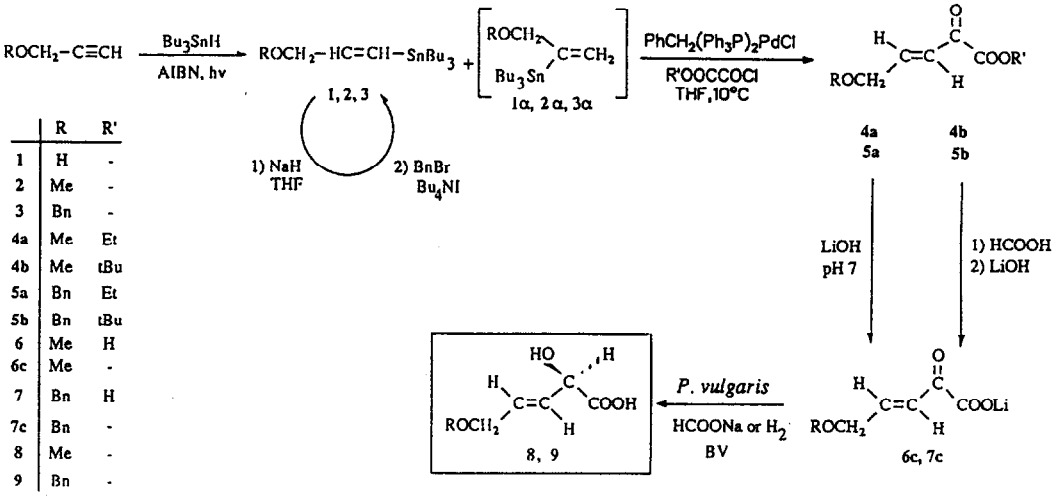
Introduction

Previous studies of this laboratory[1a-f] have shown that the bioreduction of 2-oxo carboxylates with resting cells of *P. vulgaris* leads to (R)-2-hydroxy acids, with high enantioselectivity (>97%) and high yields. From 2-oxo-3-enoic acids the valuable synthons (R)-2-hydroxy-3-enoic acids were prepared[1f] and the C-C-double bond functionalized[2].
Starting from propargyl alcohol or its methyl ether we synthezised various 2-oxo-5-alkoxy-3-pentenoic acids which were reduced by *P. vulgaris* to the corresponding (R)-2-hydroxy-5-alkoxy-3-pentenoic acids (Scheme I). Further functionalisation of the ß-γ double bond leads to pentonic acids and derivates (Scheme II), which are valuable synthons in asymmetric synthesis[3a-c]. So far only few syntheses of monosaccharides or derivatives from achiral starting materials are known[4].

Results and Discussion

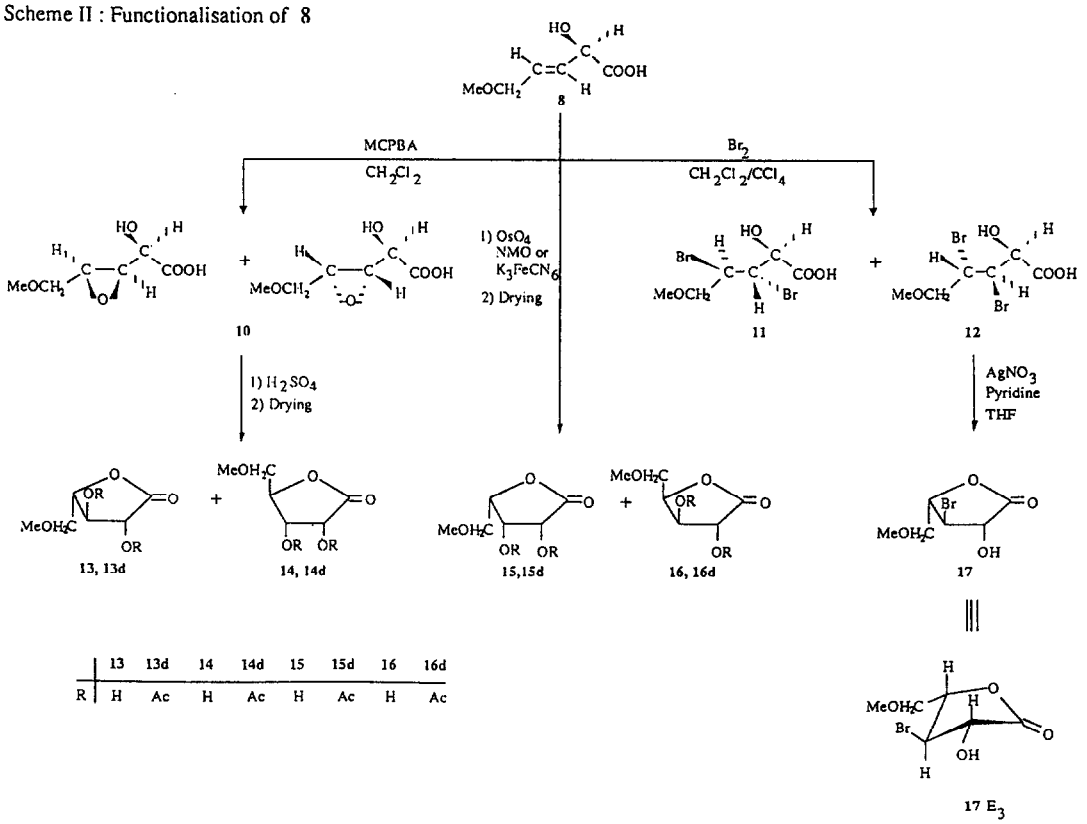
Preparation of 2-oxo-5-alkoxy-3-pentenoic acids. Whereas reacting 3-methoxy-1-bromo-1-propene with diethyl oxalate according to Rambaud et al.[5] did not lead to the expected 2-oxo ester, the Stille reaction[6a-d] was successfully applied. We used a mono ester of oxaloyl chloride as organic halide (Scheme I). To our knowledge, this is the first time that a palladium mediated cross coupling was performed with such chlorides.
The organotin compound **2** has already been prepared by Verlhac et al.[7], using a procedure of Corey and Wollenberg[8], in which methyl propargyl ether is heated with tributyltin hydride in the presence of catalytic amounts of azo-isobutyronitrile. The resulting exothermic reaction was difficult to control, leading to unreproducible yields and variations in the ratio of Bu_3Sn addition to the C-2 or C-3 of the propargyl ether (Scheme I). In-

Scheme I : Synthesis of (R)-2-hydroxy-5-alkoxy-3-pentenoic acids



	R	R'
1	H	-
2	Me	-
3	Bn	-
4a	Me	Et
4b	Me	tBu
5a	Bn	Et
5b	Bn	tBu
6	Me	H
6c	Me	-
7	Bn	H
7c	Bn	-
8	Me	-
9	Bn	-

Scheme II : Functionalisation of 8



	13	13d	14	14d	15	15d	16	16d
R	H	Ac	H	Ac	H	Ac	H	Ac

stead of heat, we used light initiation with better results. A total yield of 91% showed **E2/Z2/2α** in a ratio of 60:30:10. The benzyl ether **3**, already described[9], was prepared by hydrostannylation of propargyl alcohol using again light initiation, followed by etherification of the crude mixture[10]. The overall yield, for the two steps after distillation, was 74% (**E3/Z3/3α**=68:16:16).

During the synthesis of **4a** and **5a**, with ethyl oxaloyl chloride, **2** and **3** reacted smoothly in tetrahydrofuran at 10°C with palladium catalyst ($PhCH_2(Ph_3P)_2PdCl$). With the mono tert.-butyl ester oxaloyl chloride, the yields dropped to 40-50% for **4b** and to 50% for **5b** due to a decarbonylation leading to 5-alkoxy-2-butenoic acid esters.

Starting from the E/Z mixture of **2** and **3** the esters **4a**, **5a**, **4b** and **5b** were obtained as E form only, even when performing the reaction with the pure **Z2** prepared according to the literature[11].

Optimizing the hydrolysis of **4a** at pH 7.0 with 0.1 M LiOH resulted in 80% 2-oxo acid salt, however, always contaminated by 15-20% unidentified impurities. Ion exchange, column chromatography or multi layer coil chromatography failed to purify **6**. As the impurities did not interfere during the microbial reduction, **6c** was always used without purification. Hydrolysis of **5a** resulted in 80% of a major unidentified product. Basic hydrolysis was the only way to deprotect **4a** and **5a**. Acid hydrolysis, boron tribromide or trimethylsilyl iodide failed.

To overcome the problems arising from the basic hydrolysis, we prepared the tert.-butyl esters **4b** and **5b**. Hydrolysis of **5b** in 98-100% formic acid yielded >90% of the free acid **7**, and 85% of the isolated lithium salt. As checked by NMR and HPLC on a TEAP column, the product was pure. The neutralisation of **7** with 1 M LiOH did not lead to the degradation product obtained from the basic hydrolysis of **5a**. Unfortunately, the stability of **7** and its lithium salt **7c** was very low, even at -18°C.

Microbial reduction of 2-oxo-5-alkoxy-3-pentenoic acids. The reduction was carried out as described[1f,12].

Small scale reductions with H_2 as electron donor: For comparison of different substrates it is useful to introduce the productivity number (PN) defined as mmol product formed per kg of dry weight biocatalyst per hour. Reduction of **6c** proceeded with a PN of 80% of that observed for phenylpyruvate, which shows PN values of about 20000[1b]. At the end of the reaction about 90% of the expected amount of H_2 was consumed for **6c** and 95% for phenylpyruvate (100% is not reached as small quantities of endogenous electron donors are always present in the cells). Reductions of **7** as its lithium salt proved to be disappointing as only 45-50% conversion was reached. This is probably due to a rather fast decomposition of this substrate under the reaction conditions.

As the hydrolysis of **4a** lacked good yields and reproducibility we tried a one pot hydrolysis/reduction of this ester. The hydroxy carboxylate viologen oxidoreductase of *P. vulgaris* is not able to reduce 2-oxo esters, but, *in situ* hydrolysis, spontaneously or enzyme catalysed by lipase or esterase, generates the 2-oxo carboxylate, which should be quickly reduced to the more stable 2-hydroxy carboxylate. This method was useful for the reduction of labile 2-oxo carboxylates[1f]. With **4a**, using spontaneously or enzymically catalysed hydrolysis, only 40 to 50% conversion was reached. This was probably due to a reaction between the ester and the reduced mediator, as the latter was consumed during the reaction. Use of mediators with less negative potentials such as cobalt sepulchrate (-301 mV), carbamoylmethylviologen (-296 mV) or 2,6-antraquinone disulfonate (-184 mV) compared to BV (-360 mV), did not lead to an enhancement of the conversion of **4a**.

Preparative reduction of 2-oxo-5-alkoxy-3-pentenoic acid salts. The preparative reductions were carried out with sodium formate as electron donor as previously described[1f]. For **6c** the electron mediators carbamoylmethyl- or benzyl-viologen were tested. In both cases 60-65% yield of isolated product was obtained and the enantiomeric excess of the product was > 96%. The yield of isolated product was lower than expected from the results of the small scale experiments with H_2 as electron donor. HPLC analysis during the reaction showed the formation of a single product. The accompaning impurity of the starting material did not increase its concentration. This means a nearly quantitative reduction of the 2-oxo carboxylate was accomplished. The yield of the product was lowered by multiple purification steps required for the isolation of the hydrophilic hydroxy acid **8**.
7c was reduced on a preparative scale, giving **9** in poor isolated yield of 20%. Like **7** and **7c**, **9** also showed a quick degradation, leading to benzyl alcohol and benzaldehyde. This decay explains the low yield of **7** and **9**

It should be noted that chemical reductions with $NaBH_4/CeCl_4$[13] or 9-BBN carried out on **6**, its lithium salt or **4a**, only led to complex mixtures.

Functionalisation of the C-C double bond of 8. The carbon-carbon double bond of **8** allows further functionalisation, as previously shown for other (R)-2-hydroxy-3-enoic acids[2]. Three types of reaction were chosen: i) Trans dihydroxylation by epoxidation with meta-chloro-perbenzoic acid (MCPBA) and subsequent hydrolysis of the epoxide under acidic conditions. ii) Cis dihydroxylation using osmium tetroxide. iii) Halogenation reactions. All these reactions led to 5-methoxy-*pentono*-1,4-lactones and their derivates: i) To 5-methoxy-(L)-*arabino*- and 5-methoxy-(D)-*ribo*-1,4-lactones **13** and **14**. ii) To 5-methoxy-(L)-*lyxo* and 5-methoxy-(D)-*xylo*-1,4-lactones **15** and **16**. iii) To 5-methoxy-3-bromo-(L)-*arabino*-1,4-lactone **17** (Scheme II). Due to the chirality at C-2, diastereomeric excess were achieved in the range of 10 to 58%. The results are summarized in Table 3.

Dihydroxylation via epoxidation/hydrolysis. Epoxidation of **8** with MCPBA in methylene chloride proceeded with a de of 38% as shown by HPLC analysis. The acid hydrolysis of **10** in 0.4 M H_2SO_4 needed one week for completion at room temperature. The overall yield of pure isolated products was 68%. After lactonisation HPLC analysis showed the presence of two products in a 70:30 ratio. The major isomer **13** ((L)-*arabino*) could be partly removed by crystallization, while the remaining mixture was separated by chromatography.

Dihydroxylation with osmium tetroxide. In order to regenerate the catalytic osmium tetroxide, N-methyl-morpholine-N-oxide[14] (NMO) or potassium ferricyanide[15] were used as oxidising agents in equimolar amount. Performing the reaction with NMO and 1.25 mol% OsO_4 in acetone/water 1:1, a de of 10% in favor of the (L)-*lyxo* diastereomer **15** was achieved. This de is reversed to 8% in favor of **16** when using potassium ferricyanide in a 1:1 mixture of tert.-butanol/water, giving the (D)-*xylo* isomer **16** as major product. The de values were evaluated by NMR analysis. Spectra of the crude reaction mixture showed only two methoxy peaks and no more starting material. The de was calculated from the integration of the methoxy peaks. After purification and lactonisation[16b], HPLC analysis of the mixture showed only two products in an identical ratio as the one determined by NMR of the reaction mixture. The assignment to the (L)-*lyxo* or (D)-*xylo* series was accomplished by Nuclear Overhauser Effect (NOE). Attempts to run the dihydroxylation with achiral bulky amines to complex the osmium tetroxide, or, by

Table 1: Comparison of coupling constants of **13-16** with the corresponding free pentono-1,4-lactone[16a,b].

Compound	J (Hz)[a]	Pentonic acid γ-lactone	J (Hz)[b]
13	J_{2-3} = 8.0 J_{3-4} = 8.0 J_{4-5} = 2.0 $J_{4-5'}$ = 5.0	(D)-*arabino*	J_{2-3} = 8.4 to 8.7 J_{3-4} = 6.3 to 7.8 J_{4-5} = 2.6 $J_{4-5'}$ = 5.1 to 7.3
14	J_{2-3} = 6.0 J_{3-4} = 1.0 J_{4-5} = 3.0 $J_{4-5'}$ = 8.0	(D)-*ribo*	J_{2-3} = 5.3 to 5.7 J_{3-4} = 0.5 to 0.8 J_{4-5} = 3.2 to 3.6 $J_{4-5'}$ = 3.2 to 4.0
15	J_{2-3} = 4.0 J_{3-4} = 3.0 J_{4-5} = 4.0 $J_{4-5'}$ = 8.0	(D)-*lyxo*	J_{2-3} = 4.8 J_{3-4} = 2.8 to 3.2 J_{4-5} = 4.6 to 6.4 $J_{4-5'}$ = 5.2 to 6.7
16	J_{2-3} = 7.0 J_{3-4} = 7.0 J_{4-5} = 4.0 $J_{4-5'}$ = 3.0	(D)-*xylo*	J_{2-3} = 7.3 J_{3-4} = 6.7 to 7.3 J_{4-5} = 2.8 to 3.1 $J_{4-5'}$ = 3.0

[a] Coupling constants, registered at 360 MHZ in acetone d_6 with a drop of D_2O for **13**, **14** and **16**, and in methanol d_4 for **15**. [b] Literature data for solutions of pentonic acid γ-lactone in different solvents[16a-b], this explains the variations of coupling constants.

using the enantioselelective catalytic osmylation described by the group of Sharpless[17a-c], did not improve the de. However, the reaction led to 60% isolated **15** and **16** with both NMO and $K_3Fe(CN)_6$. Purification required repeated ion exchange chromatography, which caused some losses. After lactonisation[16b], most of the (L)-*lyxo* isomer could be removed by crystallisation and the remaining mixture was separable by chromatography.

Determination of the absolute configuration of the products of dihydroxylation 13, 14, 15 and 16. It seems that from these four diasteromers, only the 5-methoxy-(L)-*arabino*-1,4-lactone has already been synthesized[18a-b]. Based on melting point and optical rotation the (L)-*arabino* configuration was attributed to **13**, the major isomer of trans dihydroxylation. This was further confirmed by NMR NOE effect studies. The optical rotation of the isolated product was about 25% higher than reported in the literature[18b]. Aldonic acids have been extensively studied by NMR for conformational structure determinations[16a,b]. We assumed that the coupling constants of the free lactones and their 5-methoxy derivative should not be very different. As shown in Table 1 the coupling constants of **13-16** are compared with those of the literature[16a,b]. Such similarities cannot be a proof for the absolute configuration of the obtained products, but all these assignments were further confirmed by NOE experiments.
NOE experiments were performed on **13d**, **14d** and **16d**, which are the O-acetylation products of **13**, **14** and **16**, and on free **15**. The 2,3-di-O-acetyl or 2,3-isopropylidene derivatives of **15** show for H-2 and H-3 exactly the same chemical shift. Therefore, only the underivatized **15** was used for 2D-NMR

Table 2: NOE as determined by ROESY, NOESY and NOE difference spectra.

Compound	NOE effects	Configuration
13d	MeO --> H3[a] H2 --> H4[a] H3 --> H5-H5'[a]	(L)-*arabino*
14d	MeO --> H3[a] MeO --> H2[a] H3 --> H5-H5'[a][b]	(D)-*ribo*
15	H2 --> H4[a] H3 --> H4[a]	(L)-*lyxo*
16d	MeO --> H2[a][c] H2 --> H5-H5'[b] H3 --> H4[a]	(D)-*xylo*

[a] From phase sensitive NOESY. In all these spectra, there was always a cross peak between protons in an α position ($r < 5$ Å, even for trans hydrogens). For **15** and **16d** the high intensity of the H3-H4 cross peak indicates that these protons are in a cis arrangement. [b] From phase sensitive ROESY. [c] From NOE difference spectrum.

spectra. Acetylation was performed in acetic anhydride under acid catalysis by periodic acid[19]. A NOE difference spectrum was performed for **16d**, phase sensitive ROESY and phase sensitive NOESY spectra for **13d**, **14d**, **16d** and **15**. Assignments to the D or L series arise from the R configuration at C-2. Results are summarized in Table 2.

Halogenation: Iodolactonisation with I_2/KI in aqueous KOH and bromolactonisation with N-bromosuccinimide in DMF, which have proved to be very efficient and diastereoselective with other 2-(R)-hydroxy-3-enoic acids, with no oxygen on C-5, such as (R)-2-hydroxy-3-pentenoic acid[2], failed with **8**. Whereas bromination with Br_2 in CCl_4/CH_2Cl_2 at -20°C proceeded smoothly with a de of 58% and 84% yield as determined by NMR. The major isomer **12** could be isolated by crystallisation in 43% yield. It was further lactonized to **17** using Ag^+ as catalyst and pyridine as base. Configuration of **17** was deduced from coupling constants: J_{2-3} and J_{3-4} of 9 Hz, this means ax-ax couplings, which is only possible with **17** in an E_3 conformation[16a]. As no epimerisation occurred on C-3 during the lactonisation the configuration of C-3 in **12** was also R. Addition of bromine to olefins in apolar solvents is known to proceed in a trans fashion, this implies a S configuration for C-4 of **12**. Retention of configuration during lactonisation in the presence of Ag^+ is not surprising as a SN1 mechanism is possible, leading to a carbocation at C-4 and subsequent formation of the more thermodynamically favored lactone **17**; only in this case all bulky groups are trans to each other and can be in an equatorial position.

Concluding remarks. *P. vulgaris* proved, once more, to be a versatile and higly enantioselective biocatalyst for the reduction of 2-oxo carboxylates to (R)-2-hydroxy carboxylates. Its broad substrate specificity[1a-f] has been extended to 2-oxo-5-alkoxy-3-pentenoic acids. The new mild synthetic

Table 3: Summary of the different functionalisations of **8**.

Reagent	Products	Ratio	Yield
Br2	**11/12**	78/22	84%[a]
1)MCPBA/2)H^+	**13/14**	69/31	68%[b]
$OsO_4/K_3Fe(CN)_6$	**15/16**	46/54	60%[b]
OsO_4/NMO	**15/16**	55/45	60%[b]

[a] Determined by NMR as dibromide product. [b] Mixture of isolated lactones.

access to 2-oxo-5-alkoxy-3-enoic acids developed here should be useful for the synthesis of such labile compounds. Pentonic acids are known to be useful synthetic blocks in asymmetric synthesis[3a-c], the total synthesis of 5-methoxy pentonic acids presented here may also be of interest for specific isotope labelling of the 3,4 and/or 5 position of these lactones as well as the specific deuteration in the 2-position[20].

EXPERIMENTAL

Material, instruments and general procedures: The chemicals of the highest purity were purchased from Aldrich, Merck, Fluka and Sigma. Solvents were dried according to the usual procedures and distilled before use. Reactions were carried out under nitrogen in carefully dried flasks. Gas liquid chromatography was performed on a Fractovap 2400T (Carlo Erba, Italy). If not mentioned otherwise, HPLC analyses were carried out on Nucleosil RP-18 column, from Macherey & Nagel (D-5160 Düren), with methanol/water as eluent. Besides this, triethylammonium-polyol (TEAP, 0.75x25cm, SERVA, D-6900 Heidelberg) and Chiral I columns (L-hydroxy-prolin Cu^{2+} bound to silica gel) from Macherey & Nagel were used. The refractive index and UV absorption (214 or 254 nm) were simultaneously recorded. TLC analyses were performed on silica gel (Kieselgel 60 F_{254} coated plates, Merck D-6100 Darmstadt). The plates were developed with hexane/AcOEt or AcOEt/iPrOH/H_2O and visualized by UV_{254}, I_2 or induced with 10% H_2SO_4 in ethanol and subsequent heating to induce charring. Chromatography was performed on silica gel (Kieselgel 60, Merck) using hexane/AcOEt as eluent. Cells of *P. vulgaris* (DSM 30 115) were grown as described[12] and stored as wet packed cells under exclusion of oxygen at -15°C. ^1H, ^{13}C, NOE difference, ROESY, NOESY and XHCORRC spectra were recorded on Bruker AM 360, WP 200 or MX 200. Chemical shifts are given in ppm relative to internal tetramethylsilane or sodium 3-(trimethylsilyl)-propanesulfonate standards. Melting points as well as boiling points are not corrected. MS spectra (electron impact or chemical ionisation) were obtained from a Varian MAT CH5 instrument. Optical rotation were measured on an ORD Spectral Photometer J5 from Jasco (Tokyo, Japan) or on a digital polarimeter Type 71 from Roussel-Jouan (Paris, France).

Phenyl pyruvate was used as a reference substrate. For further details on bioreductions, ommission of oxygen etc, see l.c.[1b,1f,12].

(E/Z)-1-(Tri-n-butyl)stannyl-3-methoxy-1-propene (2)[7]. In a quartz flask, under nitrogen atmosphere, 5.8 g (83 mmol) freshly distilled methyl propargyl ether, 24 g (82 mmol) tri-n-butyltin hydride and 164 mg (1 mmol) (AIBN) were mixed. The mixture was cooled to 0°C and irradiated with a high pressure mercury lamp for two hours. The temperature was raised to 20-25°C, and the flask irradiated for one additional hour. The mixture was distilled using a small Vigreux column (0.1 torr, 85-93°C), leading to 27 g (91%) of a mixture **2**/**2α** in the proportions **E2:Z2:2α** = 60:30:10.

^1H-NMR for the major E isomer ($CDCl_3$) δ 6.20 (dt, J=19.0, 1.3Hz, 1H), 6.04 (dt, J=19.0, 5.0Hz, 1H), 3.95 (dd, J=5.0, 1.3Hz, 2H), 3.34 (s, 3H), 1.49 (m, 6H), 1.30 (m, 6H), 0.9 (m, 15H). ^{13}C-NMR for the major E isomer ($CDCl_3$) δ 144.3, 131.3, 76.3, 57.8, 29.2, 27.4, 13.6, 9.6. Anal. calcd. for $C_{16}H_{34}OSn$: C,53.21; H,9.49. Found: C,53.14; H,9.50.

(E/Z)-1-(Tri-n-butyl)-stannyl-3-benzyloxy-1-propene (3). The procedure for the hydrostannylation was the same as described for **2** whith the exception of the reaction temperature. After mixing the starting materials (100 mmol scale), the temperature was set to 0°C, the flask irradiated and the temperature raised in 2 hours to 25-30°C and kept at this temperature until no more propargyl alcohol could be detected by glc (PEG 4000 110°C). The resulting mixture was directly benzylated as described[9,10]. Distillation (0.1 torr, 140-160°C, lit[9]: 140-150°C) gives 32.4 g (74% for the two steps) of a mixture **E3:Z3:3α** = 68:16:16.
3 (E isomer major product): ^{1}H NMR ($CDCl_3$) δ 7.40-7.20 (m, 5H), 6.24 (dt, J=19.0, 1.5Hz, 1H), 6.09 (dt, J=19.0, 5.0Hz, 1H), 4.51 (s, 2H), 4.05 (dd, J=5.0, 1.5Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 144.5, 139.4, 131.5, 128.4, 127.7, 127.4, 74.0, 72.0, 29.14, 27.4, 13.7, 10.6.

2-Oxo-5-alkoxy-3-pentenoic acid esters (4a, 4b, 5a, 5b). For the preparation of the ethyl esters, ethyl oxaloyl chloride was used; for the tert.-butyl esters the mono tert.-butyl ester chloride was prepared as decribed[21]. The reaction of **2** with ethyl oxaloyl chloride is described as an example: In a dry two-necked flask equipped with a calcium chloride guard (oxygen must not be omitted as it catalyzes the reaction [6a-c]) and a magnetic bar, 16 mg (21 μmol, 0.01 mol%) of palladium catalyst and 46 mg (0.21 mmol, 1 mol%) of 2,6-di-tert.-butyl-p-cresol (radical scavanger) were dissolved in 20 mL dry THF. The mixture was cooled to 10°C. Ethyl oxaloyl chloride (2.4 mL, 21 mmol, 1.5 eq) was added, followed by 5.1 g (14 mmol) of **2** over a 5 min period. The progress of the reaction was registered by HPLC and usually went to completion during 2 hours at 10°C and additional 2 hours at room temperature. The THF was evaporated under vacuum and the residue chromatographed on silica gel. **4a** was obtained in 90% yield based on the content of **2** in the mixture of **2/2α**. This reaction can easily be scaled up to 50 mmol.
4a: ^{1}H NMR ($CDCl_3$) δ 7.16 (dt, J=15.9, 4.0Hz, 1H), 6.90 (dt, J=15.9, 2.2Hz, 1H), 4.35 (q, J=7.0Hz, 2H), 3.43 (s, 3H), 1.39 (t, J=7.0Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 183.1, 160.0, 149.3, 132.9, 71.3, 62.5, 58.9, 14.0. IR (film) 1630, 1675, 1700, 1730 cm^{-1}. MS 172 ($M^{+\cdot}$). Anal. calcd. for $C_8H_{12}O_4$: C,55.8; H,7.0. Found: C,55.0; H,7.0.
4b: 40-50% yield. ^{1}H NMR ($CDCl_3$) δ 7.08 (dt, J=16.0, 4.0Hz, 1H), 6.80 (dt, J=16.0, 2.0Hz, 1H), 4.17 (dd, J=4, 2.0Hz, 2H), 3.42 (s, 3H), 1.57 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 184.2, 161.6, 148.5, 124.1, 84.1, 71.3, 58.8, 27.8.
5a: 70% yield. ^{1}H NMR ($CDCl_3$) δ 7.40-7.30 (m, 5H), 7.19 (dt, J=16.0, 4.0Hz, 1H), 6.97 (dt, J=16.0, 2.0Hz, 1H), 4.59 (s, 2H), 4.35 (q, J=7.0Hz, 2H), 4.27 (dd, J=4.0, 2.0Hz, 2H), 1.37 (t, J=7.0Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 183.1, 162.0, 149.3, 137.4, 128.5, 128.3, 127.9, 127.7, 124.0, 73.0, 68.8, 62.4, 14.0. IR (film) 1630, 1675, 1700, 1730 cm^{-1}. MS 248 ($M^{+\cdot}$) or 249 ($M^{+\cdot}$+1).
5b: 50% yield. ^{1}H NMR ($CDCl_3$) δ 7.40-7.30 (m, 5H), 7.11 (dt, J=16.0, 3.5Hz, 1H), 6.87 (dt, J=16.0, 2.5Hz), 4.60 (s, 2H), 4.26 (dd, J=3.5, 2.5Hz, 2H), 1.58 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 184.2, 161.6, 148.6, 128.5, 127.9, 127.7, 124.2, 84.0, 72.9, 68.8, 27.9. IR (film) 1630, 1675, 1720 cm^{-1}. Anal. calcd. for $C_{16}H_{20}O_4$: C,69.55; H,7.29. Found: C,69.20; H,7.34.

2-Oxo-5-methoxy-3-pentenoic acid lithium salt (6c). In a three-necked flask with dropping funnel and pH electrode, 50 mL water and 30 mL acetonitrile were set at pH 7.0. **4a** 2.7 g (15,7 mmol) dissolved in 20 mL acetonitrile was slowly added during one hour, while the pH was maintained at 7.0 whith the help of a pH-Stat by adding 0.1 M LiOH. The reaction was followed by TLC (hexane/AcOEt 7:3). When the starting material was consumed, the mixture was evaporated under vacuum to remove the acetonitrile and three times extracted with 50 mL diethyl ether. After lyophilisation, the purity of the resulting salt was checked by HPLC. In this experiment 2.17 g 2-oxo acid proved to be 86% pure corresponding to a yield of 80%.
6c: ^{1}H NMR (D_2O) δ 6.05 (dt, J=16.0, 2.0Hz, 1H), 4.30 (dd, J=4.5, 2.0Hz, 2H), 3.45 (s, 3H). ^{13}C NMR (D_2O) δ 199.7, 174.5, 153.2, 128.2, 73.5, 60.9.

2-Oxo-5-benzyloxy-3-pentenoic acid (7) and lithium salt (7c). 1.1 mmol of **5b** was solubilized in 4 mL formic acid 98-100%. The reaction proceeded smoothly at room temperature and was finished in one hour (TLC: hexane/AcOEt 8:2). The formic acid was evaporated under vacuum. This hydrolysis was nearly quantitative and the free acid **7** pure by HPLC

(TEAP column MeCN 20%, phosphate buffer 20 mM 80%, H_3PO_4 until pH 3.2). Ten mL water was poured into the free acid and the pH was set at 6.5 with 1 M LiOH solution, workup described as for **6c** gave 736 mg salt (85%), which proved to be pure by HPLC. However, based on the two vinylic protons, the NMR integration of the benzyl ring protons resulted in 7 instead of 5H. After a few days storage at -18°C, a NMR spectrum revealed that this difference had increased (10 aromatic protons instead of five), while it was possible to detect benzyl alcohol and benzaldehyde by glc (PEG 4000, 160°C).
7c : ^{1}H NMR (D_2O) δ 7.5-7.3 (m, 7H), 7.01 (dt, J=17.0, 4.5Hz, 1H), 6.42 (dt, J=17.0, 2.0Hz, 1H), 4.81 (s, 2H), 4.34 (dd, J=4.5, 2.0Hz, 2H). ^{13}C NMR (D_2O) δ 199.5, 174.4, 153.2, 139.6, 131.5-130.9, 128.3, 75.5, 71.3.

(R)-2-Hydroxy-5-alkoxy-3-pentenoic acid (8,9). In a thermostated cell at 35°C, 2.0 g (29 mmol, 1.5 eq) of sodium formate was dissolved in 30 mL deoxygenated phosphate buffer 0.1M, pH 7.0 and 1.0 g (wet weight) *P. vulgaris* cells suspended in 5 mL phosphate buffer were added. Nineteen mmol (2.87g) **6c** was dissolved in 10 mL buffer. From this solution 2.5 mL were added into the reaction cell. After the first portion of **6c** was reduced, three more portions of 2.5 mL were added. The pH was maintained at 7.0 by adding 1 M HCOOH via a pH-Stat. When the reaction was finished (90 min) the pH was lowered to 2 with sulfuric acid, to precipitate the proteins, which were removed by centrifugation. The supernatant was collected, the precipitate washed twice with 10 mL 10 mM H_2SO_4. The collected supernatants were lyophilized after setting the pH at 7.0. The resulting solid material was solubilized in 20 mL water, the pH lowered to 2.0 and the aqueous phase extracted six times with 20 mL methylethyl ketone/tert.-butanol 80:20. The resulting organic layers were concentrated under vacuum. The residue was neutralized and purified on 40 mL Dowex IX8 100-200 (formate form), eluting with a gradient of formic acid 0 to 1 M. The resulting 1.76 g (63%) of **8** crystallized slowly on standing.
8: mp 45-46°C.
$[\alpha]_D$ = -53.8°(c=0.1M, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 6.20 (ddt, J=15.5, 5.4, 1.6Hz, 1H), 5.87 (ddt, J=15.6, 5.2, 1.3Hz, 1H), 4.74 (dd, J=5.2, 1.5Hz, 1H), 4.01 (d , J=5.4Hz, 2H), 3.40 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 175.6, 128.9, 128.7, 71.8, 70.3, 58.1. IR (KBr) 1735, 3400cm^{-1}. Anal. calcd. for $C_6H_{10}O_4$, 0.25 H_2O: C,47.84; H,7.03. Found: C,47.87; H,6.85.
9 was obtained with 20% yield and decomposed slowly during storage. ^{1}H NMR ($CDCl_3$) δ 7.40-7.20 (m, 5H), 6.55 (b, 2H), 6.04 (dtd, J=15.0, 5.0, 1.0Hz, 1H), 5.88 (dd, J=15.0, 5.0Hz, 1H), 4.71 (dd, J=5.0, 1.0Hz, 1H), 4.53 (s, 2H), 4.06 (d, J=5.0Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 176.0, 137.5, 129.4, 128.4, 127.9, 72.4, 70.4, 69.4.

Determination of the stereochemical purity of 8 : *P. vulgaris* exclusively forms the R enantiomer, as already shown for many 2-hydroxy acids obtained from 2-oxo carboxylates[1a-f]. Determination of the enantiomeric purity of **8** was made by ligand exchange chromatography on a chiral HPLC column (Chiral I)[22]. It was shown[1e-f] for many 2-hydroxy carboxylates that the R enantiomer has the higher retention time. This is in accordance with literature[22]. Therefore, we assume that this is also the case for 2-hydroxy acids for which no independent determination was conducted. With racemic **8** it was proved that on Chiral I the two enantiomers were separated. As chemical reduction of either **6** or **4a** was not achieved **8** obtained from microbial reduction of **6** was racemized by refluxing in a 1:1 mixture of pyridine/water[23]. Microbiologically prepared **8** gave only one peak on Chiral I ($CuSO_4$ 10 mM, 0.5 mL/min: Rt = 10.2 min), whereas racemized **8** resulted in two (Rt = 9.0 min and 10.2 min). By adding small amounts of the racemate to pure **(R)-8**, the enantiomeric excess was determined to be at least >96%.

(R)-2-Hydroxy-3,4-epoxy-pentanoic acids (10). 2.1 g (6 mmol, 1.2 eq) MCPBA containing 40-50% water was dissolved in 10 ml diethyl ether and extracted with 5 mL water. The organic layer was dried over magnesium sulfate and concentrated to 5 mL. Under an atmosphere of nitrogen, the MCPBA solution was added to a slowly stirred solution of 730 mg (5 mmol) **8** in 5 mL dry CH_2Cl_2. The reaction was followed by HPLC and was finished after 30 h at room temperature. After adding 20 mL of water this solution was extracted three times by 20 ml diethyl ether, and lyophilized leading to 771 mg (95%) of epoxide mixture **10**, which was not further purified.
10 (mixture of diastereomers): ^{13}C NMR ($CDCl_3$) δ 173.2/172.7, 72.7, 70.2/70.0, 58.9,

56.7/56.2, 54.5/54.1. IR (film) 1740 cm^{-1}. MS 162 ($M^{+\cdot}$) or 163 ($M^{+\cdot}+1$).

5-Methoxy-(L)-*arabino* and (D)-*ribo*-1,4-lactones (13,14). Epoxide mixture **10** 760 mg (4.7 mmol) was dissolved in 15 mL acetonitrile, 3 mL water and 4 mL 1 M sulphuric acid. The reaction was followed by HPLC and needed five days at room temperature to go to completion. Acetonitrile was evaporated and the pH set to 7.0. This mixture was chromatographed on 50 mL Dowex IX8 200-400 eluting with a linear gradient 0 to 0.6 M in acetic acid. The fractions containing the products were concentrated, passed through an Amberlyst 15 (H^+) column (15 mL) and evaporated under vacuum. The lactonisation was achieved in 3 days in a desiccator under vacuum and over phosphorus pentoxide[16b], leading to 520 mg (72%) of a 69/31 mixture of 5-methoxy-(L)-*arabino*-1,4-lactone **13** and 5-methoxy-(D)-*ribo*-1,4-lactone **14**. **13** could be partly crystallized (hexane/AcOEt) and the remaining mixture separated by column chromatography.

13: mp 135-136°C (lit[18a]: 135°C, lit[18b]: 137-138°C). $[\alpha]_D$ = -54.8° (19 mM, acetone), lit[18b]: -43.7° (17 mM, acetone), $[\alpha]_{546}$ = -67.5° (62 mM, acetone). ^1H NMR (acetone d_6, one drop D_2O) δ 4.37 (d, J=8.0Hz, 1H), 4.20 (m, J=8.0, 5.0, 2.0Hz, 1H), 4.17 (t, J=8.0Hz, 1H), 3.69 (dd, J=14.0, 2.0Hz, 1H), 3.58 (dd, J=14.0, 5.0, 1H), 3.34 (s, 3H). ^{13}C NMR (acetone d_6, one drop D_2O) δ 174.5, 80.6, 75.2, 74.4, 71.4, 59.2. IR (KBr) 1775 cm^{-1} (5 ring lactone). Anal. calcd. for $C_6H_{10}O_5$: C,44.45; H,6.22. Found: C,44.43; H,6.10.

14: mp 105-107°C. $[\alpha]_{546}$ = +38.5° (32 mM, acetone). ^1H NMR (acetone d_6, one drop D_2O) δ 4.49 (d, J=6.0Hz, 1H), 4.40 (dt, J=3.0, 1.0Hz, 1H), 4.28 (dd, J=6.0, 1.0Hz, 1H), 3.63 (dd, J=11.0, 3.0Hz, 1H), 3.58 (dd, J=11.0, 3.0Hz, 1H), 3.30 (s, 3H). ^{13}C NMR (acetone d_6, one drop D_2O) δ 176.3, 84.14, 72.6, 70.8, 69.7, 59.4. IR (KBr) 1750 cm^{-1} (5 ring lactone). Anal. calcd. for $C_6H_{10}O_5$: C,44.45; H,6.22. Found: C,44.46; H,6.09.

5-Methoxy-(L)-*lyxo* and (D)-*xylo*-1,4-lactones (15, 16). Dihydroxylation using $K_3Fe(CN)_6$ as oxidant: In a 25 mL flask 2.0 g (8 mmol, 4 eq) $K_3Fe(CN)_6$, 830 mg (8 mmol, 4 eq) K_2CO_3, 3 mL water, 5 mL tert.-butanol and 160 μL of a 157 mM aqueous OsO_4 solution (1.25 mol%) were mixed. Two mmol (292 mg) of **8** dissolved in 2 mL water was added. The reaction was followed by HPLC, and went to completion in three days at room temperature. Sodium hydrogensulfite (400mg) was added to reduce excess oxidant and OsO_4. This mixture was then desalted on a Dowex MR3 mixed bed resin (80 mL, 1.3 eq). The products were eluted with a linear 0 to 1 M formic acid gradient. The fractions containing the products were evaporated under vacuum and once more chromatographed as described for the trans dihydroxylation. After lactonisation, a diastereomeric mixture of 197 mg (61%) was obtained. From this mixture of 5-methoxy-(L)-*lyxo*-1,4-lactone **15** and 5-methoxy-(D)-*xylo*-1,4-lactone **16** it was possible to crystallize a part of **15** (hexane/AcOEt) and thereafter to separate the remaining mixture by column chromatography.

Dihydroxylation using NMO as reoxidant: The same conditions as above were used but with 1.3 equivalents of NMO in a 1:1 mixture of water/acetone. When the reaction was finished, excess NMO and OsO_4 were reduced with sodium hydrogensulfite and the solution purified on Dowex IX8 200-400 (acetate form).

15: mp 125-126°C. $[\alpha]_{546}$ = -28.5° (62 mM, acetone). ^1H NMR (methanol d_4) δ 4.58 (ddd, J=7.5, 4.5, 3.0Hz, 1H), 4.56 (d, J=4.5Hz, 1H), 4.41 (dd, J=4.5, 3.0Hz, 1H), 3.8 (dd, J=11.0, 4.0Hz, 1H), 3.71 (dd, J=11.0, 7.5Hz, 1H), 3.44 (s, 3H). ^{13}C NMR (acetone d_6) δ 174.5, 80.6, 75.2, 74.4, 71.4, 59.2. IR (KBr) 1770 cm^{-1} (5 ring lactone). Anal. calcd. for $C_6H_{10}O_5$: C,44.45; H,6.22. Found: C,44.45; H,6.08.

16: Oil. $[\alpha]_{546}$ = +87.0° (62 mM, acetone). ^1H NMR (acetone d_6, one drop D_2O) δ 4.57 (m, J=7.0, 4.0Hz, 1H), 4.36 (t, J=7.0Hz, 1H), 4.32 (d, J=7.0Hz, 1H), 3.67 (dd, J=11.0, 4.0Hz, 1H), 3.61 (dd, J=11.0, 3.0Hz, 1H), 3.28 (s,3H). ^{13}C NMR (methanol d_4) δ 175.7, 79.5, 75.1, 74.1, 70.9, 59.4. IR (Film) 1770 cm^{-1} (5 ring lactone). MS 163 ($M^{+\cdot}+1$). Anal. calcd. for $C_6H_{10}O_5$: C,44.45; H,6.22. Found: C,44.53; H,6.02.

Acetylation of 5-methoxy-pentono-1,4-lactones[19] : One mmol of pentono-1,4-lactone was suspended in 2 mL acetic anhydride. The temperature was lowered to 0°C and 5 μL periodic acid 60% was added. The lactone solubilized immediatly and the reaction was finished within 15 min. After usual workup, yields were ranging from quantitative to 83%.

13d: oil ^1H NMR ($CDCl_3$) δ 5.62 (d, J=7.0Hz, 1H), 5.53 (t, J=7.0Hz, 1H), 4.46 (m, J=7.0,4.0,3.0Hz, 1H), 3.72 (dd, J=11.0, 3.0Hz, 1H), 3.64 (dd, J=11.0, 4.0Hz, 1H), 3.42 (s, 3H), 2.15 (s, 3H), 2.11 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 169.8, 169.5, 168.6, 79.0, 72.5,

72.3, 70.5, 59.5, 20.5, 20.3.
14d: oil ^{1}H NMR ($CDCl_3$) δ 5.77 (d, J=6.0Hz, 1H), 4.49 (d, J=6.0Hz, 1H), 4.57 (t, J=2.0Hz, 1H), 3.72 (dd, J=10.0, 2.0Hz, 1H), 3.66 (dd, J=10.0, 2.0Hz, 1H), 3.40 (s, 3H), 2.15(s, 3H), 2.11 (s, 3H). ^{13} NMR ($CDCl_3$,) δ 171.1, 169.8, 169.2, 82.0 (C-4), 71.1 (C-5), 70.8 (C-3), 67.0 (C-2), 59.6 (OMe), 20.5, 20.2.
15d: mp 77-79°C. ^{1}H NMR ($CDCl_3$) δ 5.71 (d, J=2.0Hz, 2H), 4.73 (m, J=6.5, 5.0, 2.0Hz, 1H), 3.72 (dd, J=10.0, 6.5Hz, 1H), 3.66 (dd, J=10.0, 2.0Hz, 1H), 3.40 (s, 3H), 2.15 (s, 3H), 2.13 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 169.7, 169.4, 169.0, 77.0, 69.4 (C-5 from DEPT), 69.3, 68.0, 59.5, 20.3, 20.1.
16d: oil ^{1}H NMR ($CDCl_3$) δ 5.70 (d, J=8.0Hz, 1H), 5.51 (t, J=8.0Hz, 1H), 4.75 (dt, J=8.0, 2.5Hz, 1H), 3.60 (dd, J=11.0, 3.0Hz, 1H), 3.46 (dd, J=11.0, 2.0Hz, 1H), 3.33 (s, 3H), 2.10 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 169.9, 169.2, 168.9, 75.6, 72.6, 70.5, 68.8, 59.2, 20.2.

Bromination of 8. One mmol (146 mg) **8** was dissolved in 10 mL CH_2Cl_2/5 mL CCl_4 and the temperature lowered to -20°C. A solution of 0.5 g bromine (0.16 mL, 3 mmol) in 10 ml CH_2Cl_2/CCl_4 1:1 was slowly added until the colour of bromine did not longer disappear quickly. HPLC showed that all the starting material had reacted. After adding 100 mg sodium hydrogen sulfite in 10 mL water, the mixture was stirred for 5 min at RT, the pH set at 6.0 and the solution extracted three times with 20 mL ether. The water layer was saturated with NaCl, acidified to pH 1.0 and extracted five times with 20 ml 2-butanone. The dried organic layer was evaporated giving 257 mg (84%) of a brownish oil, which was almost pure according to NMR. Cristallisation from CH_2Cl_2/hexane gave 132 mg **12**.
12: mp 98°C (decomp.). $[\alpha]_D$ = -9.1° (14 mM, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 5.0 (d, J=1.5Hz, 1H), 4.76 (dd, J=11.0 ,1.5Hz, 1H), 4.44 (ddd, J=11.0, 4.0, 2.0Hz, 1H), 4.08 (dd, J=11.0, 4.0Hz, 1H), 3.91 (dd, J=11.0, 2.0Hz, 1H), 3.49 (s, 3H). ^{13} NMR ($CDCl_3$) δ 175.1, 74.7, 71.8, 59.4, 54.5, 50.8. IR (KBr) 1700, 1730 cm^{-1}. Anal. calcd. for $C_6H_{10}O_4Br_2$: C,23.6; H,3.4. Found: C,24.1; H,3.5.

Lactonisation of 12. This reaction was not optimized. In a light protected dry flask 47 mg **12** (0.15 mmol) were dissolved in 6 mL dry THF. To this solution 100 mg $AgNO_3$ (0.60mmol) and 24 μL dry pyridine (0.30 mmol) were added. The reaction went to completion in 24 h. Five mL silica gel 120μ were added, the mixture evaporated and chromatographed, leading to 10 mg of **17** (30%).
17: ^{1}H NMR ($CDCl_3$) δ 4.82 (d, J=9.0Hz, 1H), 4.69 (dt, J=9.0, 2.0Hz, 1H), 4.49 (t, J=9.0Hz, 1H), 3.91 (dd, J=11.0, 2.0Hz, 1H), 3.77 (dd, J=11.0, 2.0Hz, 1H), 3.39 (s, 3H), 3.08 (b, 1H). ^{13}C NMR ($CDCl_3$) δ 174.5, 73.6, 71.4, 59.3, 38.7, 30.0.

NMR spectra: In ROESY experiments 200 ms mixing time was chosen and in NOESY experiments mixing time was set to the average of the proton relaxation time (2 to 3s), zero quantum field cross peaks were strongly reduced by 1% random variation of mixing time. 1K data points was used in t_1 and t_2 dimension. A π/3 shifted squared sine bell was applied in both domains prior to Fourier transform.

Acknowledgment: D. B. thanks Elf Aquitaine for a personal grant. We are grateful to Dr. H. White and C. Schinschel for *P. vulgaris* cells, F.P. Schmidtchen for fruitful discussions, especially on the Stille reaction, P. Rauschenbach and F. Wendling for advice regarding HPLC analysis and K. Worm as well as W. Eisenreich for NMR advice.

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