Studies on the Biosynthesis of the Antibiotic Moenomycin A

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Dedicated with Best Wishes to our Friend and Colleague Professor Horst Kunz on the Occasion of his 60th Birthday

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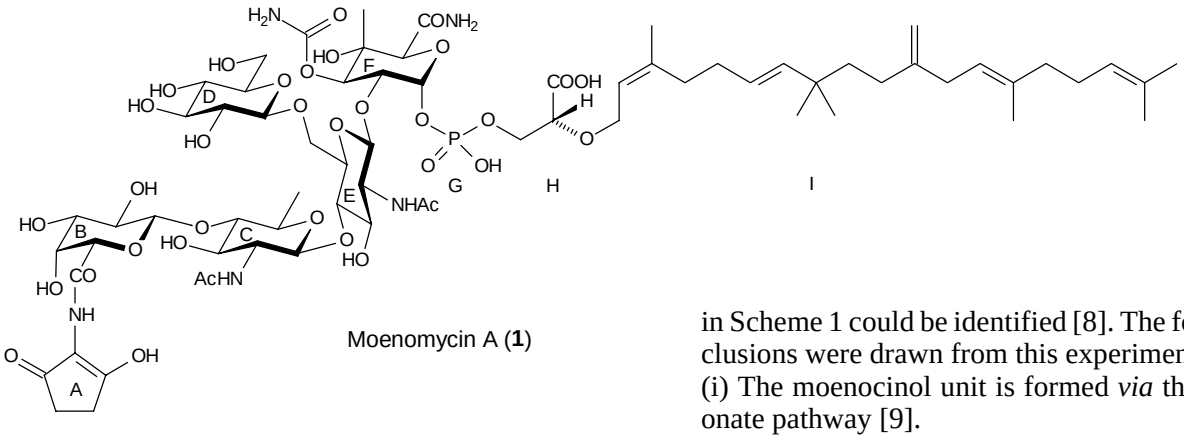
Abstract. Feeding experiments (^{13}C and ^{15}N-labeled precursors) shed light on the biosynthetic origin of the chromophore (unit A of **1**), the *N*-acetyl groups, the 4-*C*-methyl group of the moenuronamide unit (part F of **1**), the sugar units, and the lipid part (unit I of **1**) of the antibiotic moenomycin A (**1**). The lipid part is completely isoprenoid and is constructed *via* the non-mevalonate pathway. The central C_{10} part originates from a precursor like geranyl or linalyl diphosphate and is formed by a route involving ring formation between C-2 and C-6 of the monoterpene unit, two successive rearrangements to give a 7-membered ring intermediate and cleavage of the ring between C-5 and C-11 (moenocinol numbering).

The transglycosylation reaction in peptidoglycan biosynthesis is a highly promising target for new antibiotics. Some glycopeptide antibiotics and the moenomycins (see moenomycin A, **1**) interfere with this biosynthetic step. Most probably, their modes of action are different. Whereas the moenomycins have been shown to interact with the enzyme(s) [1] the glycopeptides interfere with the substrates of the transglycosylation reaction [2]. For the moenomycins (**1**), it has been demonstrated that units C-E-F-G-H-I (i.e. trisaccharide analogues, see formula **1**) are sufficient to elicit transglycosylase inhibiting and antibiotic activities [1]. A mechanism for the mode of action has been proposed [3]. It is assumed that the moenomycins are anchored to the cytoplasmic membrane *via* the lipid part and bind then highly selectively to the active site of the enzyme *via* the F-E-C trisaccharide.



Moenomycin A (**1**)

The lipid part I of the moenomycin-type antibiotics [4] is derived from a C_{25} alcohol (moenocinol) with an unusual structure. Three isoprenoid C_5 units are easily discernible whereas the central C_{10} part (C-5 through C-11) does not obey the isoprene rule in an obvious way (see formula 4). It has been speculated a long time ago [5] that this C_{10} unit could be formed by anti Markovnikov cyclization of a geranyl-type diphosphate (see 5) to give a structure of type 2 and opening of the bond between C-5 and C-11 (moenocinol numbering). A total synthesis of moenocinol has been executed based on this speculation [6].

Recently, we performed preliminary [1-^{13}C]acetate feeding experiments with cultures of *Streptomyces ghanaensis* H2 (semi-producing strain from the BC Biochemie GmbH collection [7]). The usual mixture of moenomycins [4] (containing moenomycin A as the major component) was isolated and, although the ^{13}C enrichments were very low, the labeling pattern shown in Scheme 1 could be identified [8]. The following conclusions were drawn from this experiment:

(i) The moenocinol unit is formed *via* the non-mevalonate pathway [9].

(ii) The central part results from cyclization of linalyl diphosphate in the Markovnikov sense (**5** → **6**), opposite to the previous assumption. Then exclusively the

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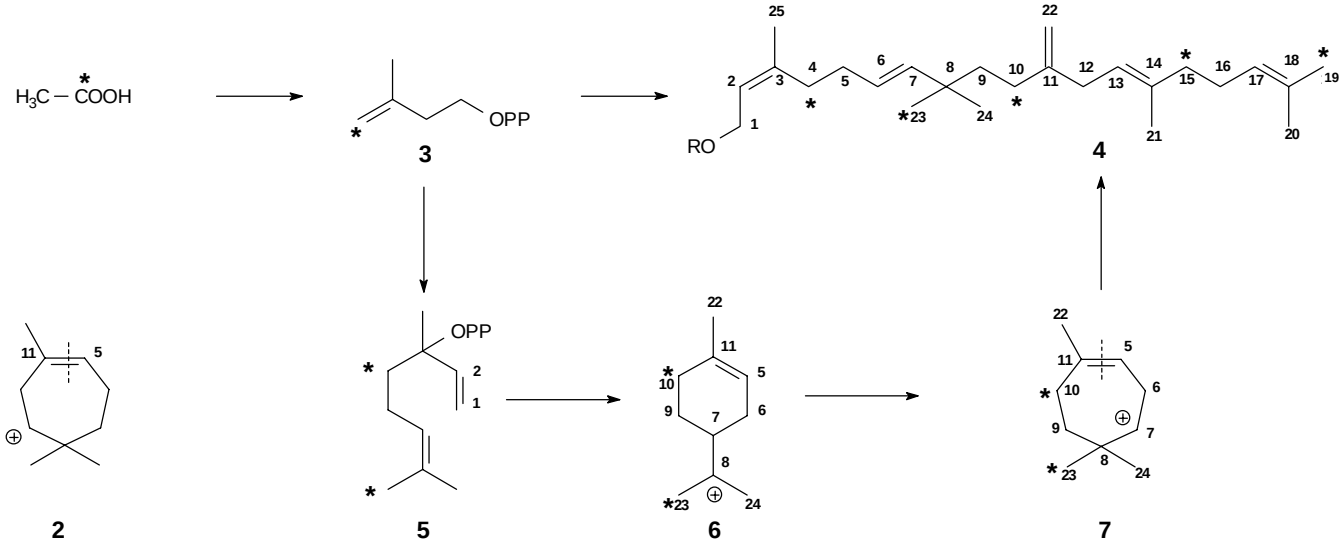


Scheme 1 [1-^{13}C]acetate feeding experiment

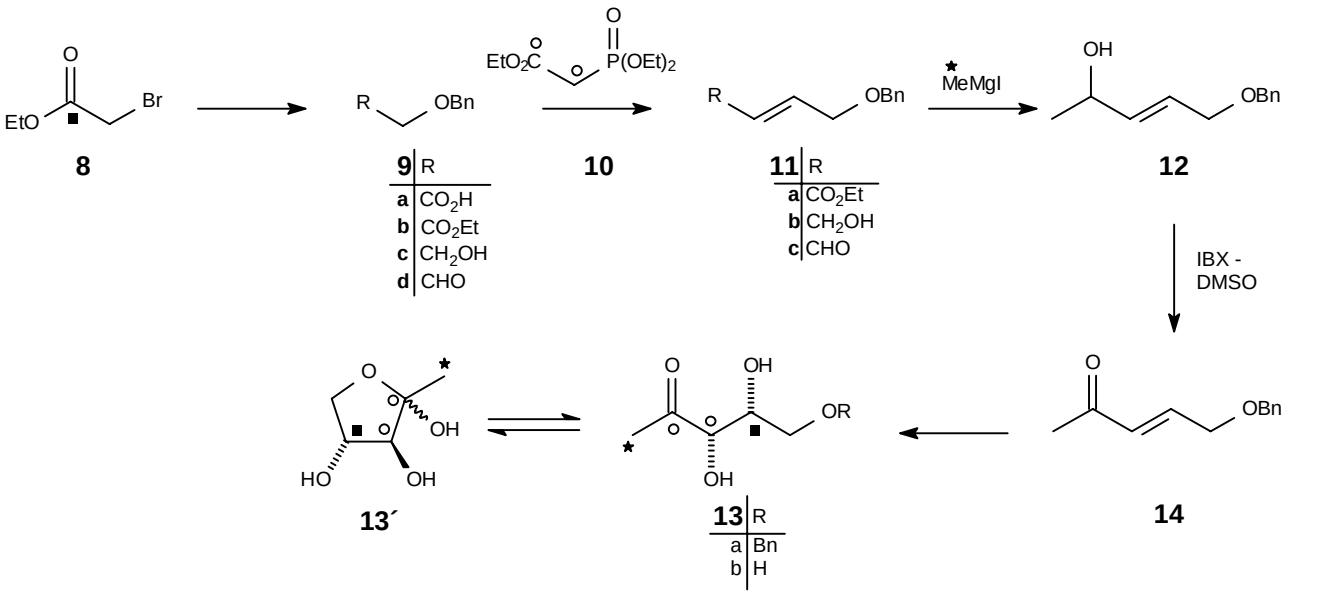
bond between C-7 and C-9 (moenomycin numbering) migrates to give a 7-membered intermediate of type **7**. Finally, cleavage of the bond between C-5 and C-11 of **7** (see Scheme 1) leads to the central C_{10} part of moenocinol.

Here, we wish to describe feeding experiments that allow a much closer look on the origin of the moenomycin lipid part [10]. Furthermore, ^{13}C and ^{15}N labeling of moenomycin A in positions which might be interesting in the context of studying the interaction of the antibiotic with the *enzyme penicillin binding protein* **1b** by NMR techniques is described.

Synthesis of Labeled Precursors

1-Deoxy-D-xylulose (**13b**) has been demonstrated to be a central intermediate in the non-mevalonate pathway of terpenoid biosynthesis. In addition, it has been found to be incorporated into terpenoid products with high efficiency [9]. We decided, therefore, to use ^{13}C-labelled **13b** to explore the origin of the moenomycin C_{25} lipid.

Known routes have been adapted to introduce ^{13}C labels into various positions of **13b** [11, 12]. Thus, bromoacetic acid (**8**) was converted to *O*-benzylglycolic acid (**9a**) from which *O*-benzylglycolaldehyde (**9d**) was



Scheme 2 Synthesis of labeled 1-deoxy-D-xylulose

obtained in three steps. Condensation of **9d** with ethyl diethylphosphonoacetate (**10**) provided ethyl *(E)*-4-benzyloxybut-2-enoate (**11a**) which in turn was converted into aldehyde **11c**. Subsequent reaction with methyl Grignard reagent followed by iodoxybenzoic acid [13] oxidation in DMSO [14] provided **14**. Enone **14** was submitted to an asymmetric Sharpless dihydroxylation [15] (AD mix β) to furnish **13a**. Finally, hydrogenolysis gave 1-deoxy-D-xylulose (**13b**). According to NMR spectroscopy **13b** mainly exists in the hemiacetal form **13'** (two anomers). The ^{13}C NMR spectrum corresponds to those reported by Spenser [11] and by Boland [16]. In the ^{13}C NMR spectra of some ^{13}C-labeled 1-deoxy-D-xyluloses the signals of the open-chain form **13b** could also be identified (see Experimental).

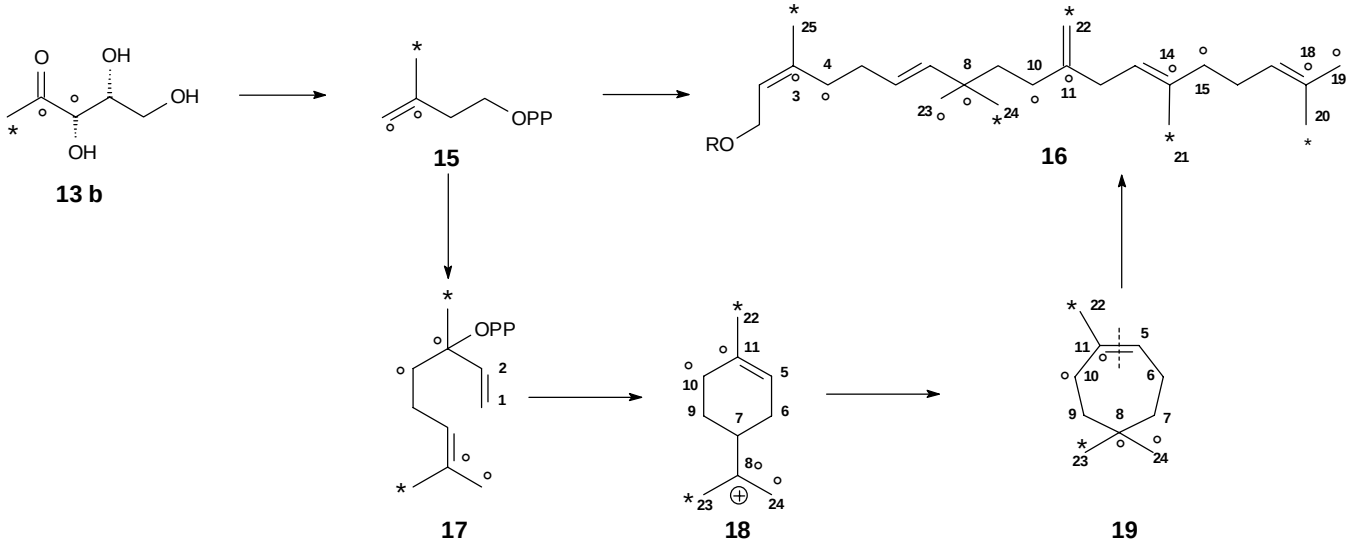
With [1-^{13}C]bromoacetic acid as starting material **13b** labeled in the 4-positon (■) was prepared. From **9d** and ethyl [1,2-$^{13}C_2$]diethylphosphonoacetate 2,3-labeled **13b** (positons *o*) was obtained, and use of ^{13}C-labeled methylmagnesium iodide in step **11c** → **12** allowed the synthesis of **13b** with the ^{13}C-label in the 1-position (indicated by *).

Incorporation of Labeled Precursors into the Lipid Part of Moenomycin

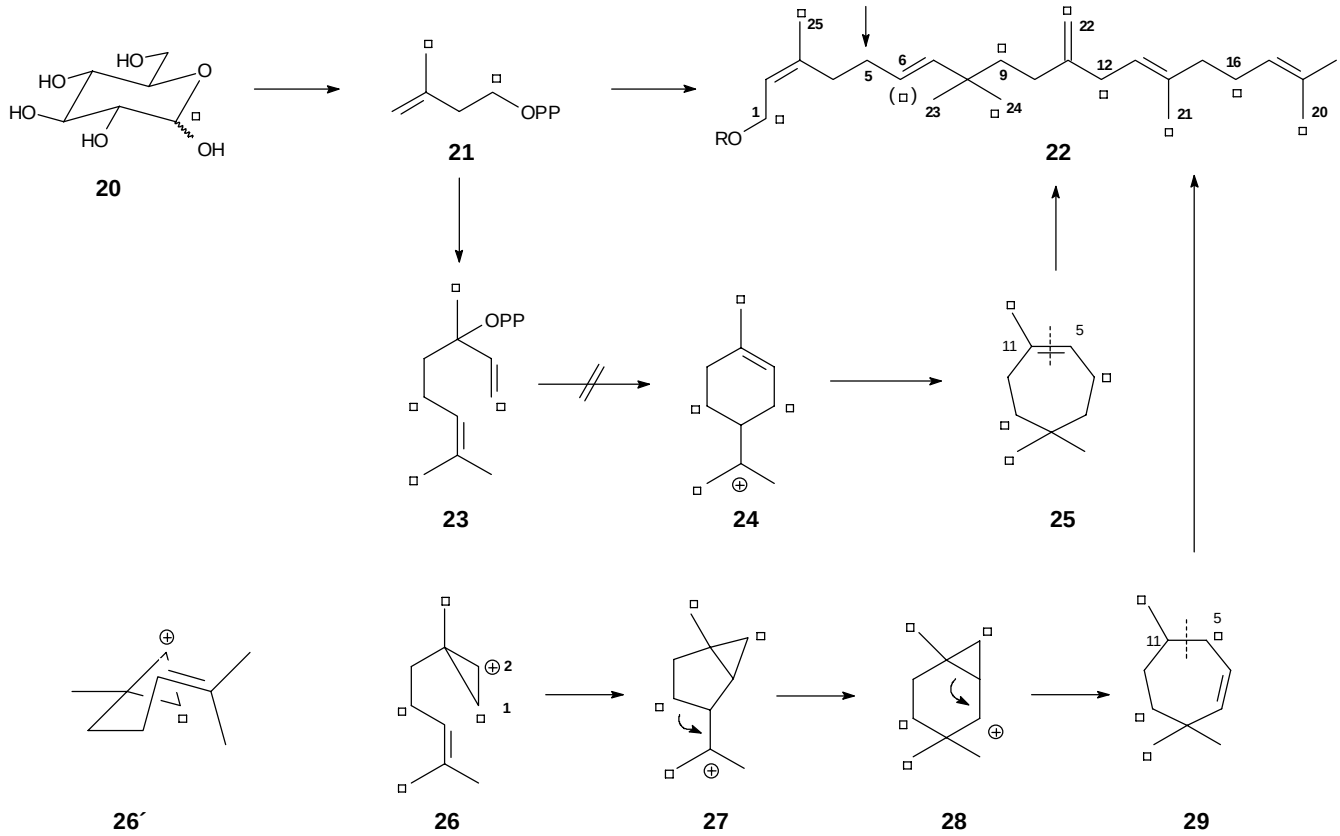
In a first set of experiments cultures of *Streptomyces ghanaensis* H2 were grown in Erlenmeyer flasks (medium 1, see Experimental). After 48 h a single dose of [1-^{13}C]-1-deoxy-D-xylulose (**13b***) was administered. The fermentation was stopped after 240 h and the mixture of the moenomycins was isolated. ^{13}C NMR spectra were recorded in 10:1 methanol-water. Well-resolved spectra were obtained. All signals of the lipid part could be assigned by comparison with previous results [17]. For a quantitative analysis the inverse gated decoupling ^{13}C NMR spectrum of the unlabeled moenomycin mixture was recorded under the same conditions. The enrichments were calculated comparing the corresponding signals (referenced to C-2 of moenomycin unit A [8], see formula 1) of labeled and unlabeled moenomycin samples using a known procedure [18]. The * positions in **16**, (Scheme 3) were enriched: C-20 (2.0%), C-21 [19] (1.3%), C-23/C-24 [20] (3.6%), C-22 (3.1%), C-25 (3.1%). This result proves the previous assumption, that all units of the moenocinol part are isoprenoid and formed *via* the non-mevalonate pathway.

In the second feeding experiment under identical conditions [2,3-$^{13}C_2$]-1-deoxy-D-xylulose (**13b** o) was administered. The ^{13}C NMR spectrum of the isolated moenomycin mixture displayed the labeling pattern shown in Scheme 3 (*o* positions: C-19 (1.1%, $^1J_{19,18}$ = 43.3 Hz), C-23/C-24 (not separated, 0.6%, $^1J_{23/24,8}$ = 35.4 Hz), C-10 (1.7% , $^1J_{10,11}$ = 41.5 Hz), C-4 (1.1%, $^1J_{4,3}$ = 41.5 Hz), C-8 (0.9%, $^1J_{8,23/24}$ = 35.4 Hz), C-15 (1.3%, $^1J_{15,14}$ = 42.4 Hz), C-18 (0.4%, $^1J_{18,19}$ = 43.3 Hz), C-14 (0.8%, $^1J_{14,15}$ = 42.4 Hz), C-3 (0.7%, broad non-resolved signal), C-11 (1.0%, $^1J_{11,10}$ = 41.5 Hz). The result is clearly in agreement with the conclusions taken from the feeding experiments discussed above.

In a third feeding experiment [1-^{13}C]-D-glucose (**20**) was administered using a somewhat different protocol (medium 2). From the ^{13}C NMR spectra the labeling pattern summarized in Scheme 4 was obtained: C-21 (0.6%), C-20 (0.4%), C-25 (0.6%), C-16 (0.5%), C-23/C-24 (0.2%), C-5 (0.5%), C-12 (0.3%), C-9 (0.4%), C-**22** (0.2%) [21]. With one exception the labels were found in the expected positions. However, contrary to the expectations C-5 was labeled in **21** rather than C-6. This



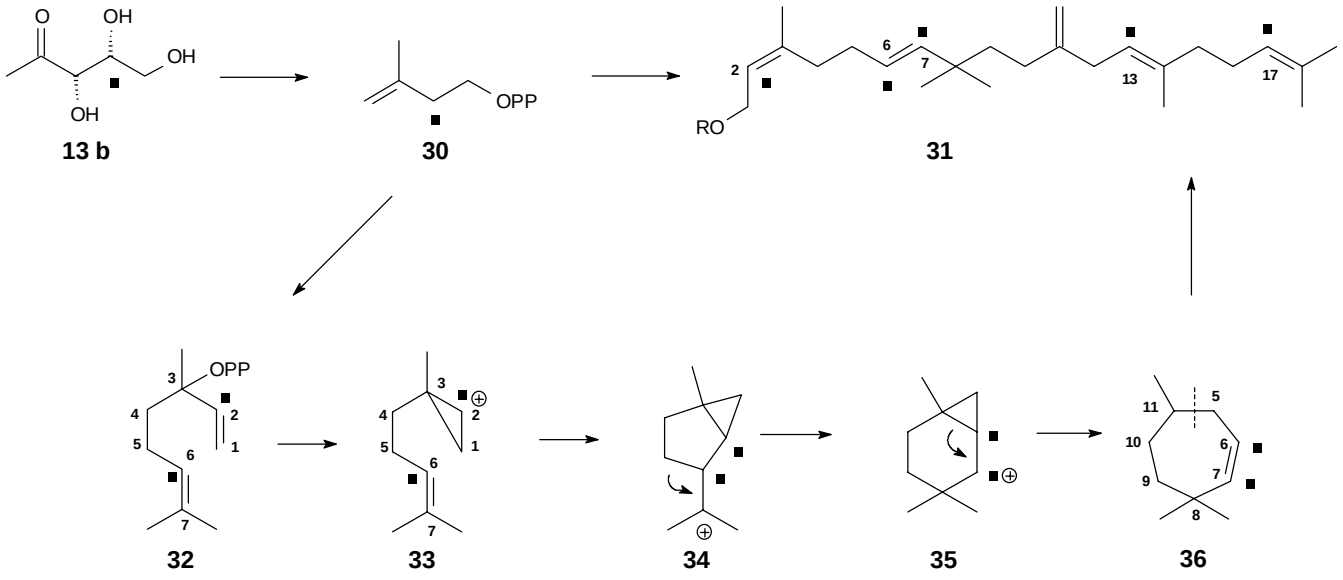
Scheme 3 Feeding of 1-deoxy-D-xylulose ^{13}C-labeled in positions **1** and **2**, **3**



Scheme 4 Feeding of [1-^{13}C]-D-glucose

result means that in the course of the formation of the 7-membered intermediate from linalyl or geranyl diphosphate, carbons 1 and 2 (geraniol numbering) must exchange their positions. A three-membered ring intermediate (see **26** and **26'** in Scheme 4) appears to offer a reasonable explanation.

With the aim of substantiating the mechanistic rationale summarized in Scheme 4, [4-^{13}C]-1-deoxy-D-xylulose (**13b** ■) was administered under the conditions of experiments 2 and 3 (*vide supra*). Using the usual procedure, in the moenocinol part the labeling pattern summarized in Scheme 5 was found: C-2 (1.7%), C-13



Scheme 5 Feeding of 1-deoxy-D-xylulose ^{13}C-labeled in position **4**

(2.3%), C-17 (1.8%), C-6 (1.3%, $^1J_{6,7}$ = 72.2 Hz), C-7 (1.2 %, $^1J_{7,6}$ = 72.2 Hz). This result nicely proves that carbons 2 and 6 (geraniol numbering) of the geraniol-/linalool-derived intermediate **33** are joined in the course of the formation of moenocinol and become moenocinol carbons 6 and 7 (see **31** and **36**), respectively.

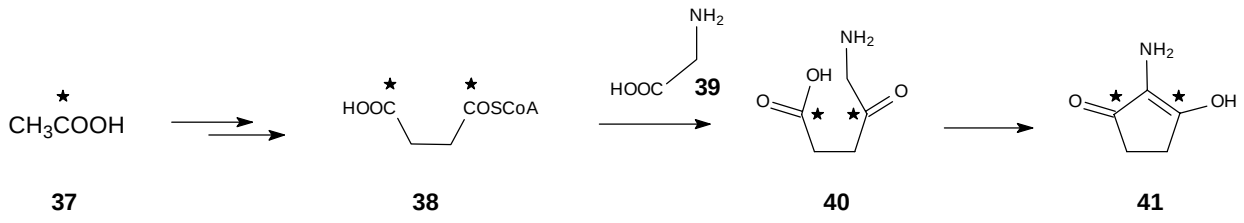
In conclusion, we have shown, that the lipid part of moenomycins A is completely isoprenoid and is constructed *via* the non-mevalonate pathway. The formation of the central C_{10} part originates from a precursor like geranyl or linalyl diphosphate and proceeds by a pathway as shown in Scheme 5 involving (i) ring formation between C-2 and C-6 (geraniol numbering), (ii) two successive rearrangements to give a 7-membered ring intermediate and (iii) cleavage of the bond between C-5 and C-11 (moenocinol numbering).

Labeling of the *N*-acetyl Groups and the Chromophore Part

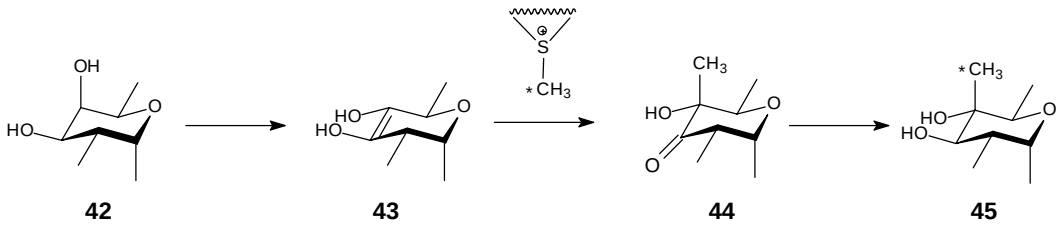
For the following experiments a somewhat different isolation procedure for the moenomycins was used including an ultrafiltration step. Sodium [1-^{13}C]acetate was administered using a pulse feeding protocol (the first dose at the end of the logarithmic phase, 45 h, medium 5). After altogether 165 h the moenomycins were isolated using this isolation procedure. ^{13}C NMR spectra were recorded in 25:1 methanol-water. Well-resolved spectra were obtained. For a quantitative analysis the inverse gated decoupling ^{13}C NMR spectrum of the unlabeled moenomycin mixture was recorded in a 0.05 mol/l $Cr(acac)_3$ solution in 25:1 methanol-water [22]. Under these conditions suppressing NOE enhancements and relaxation effects all signals gave practically the same integral. For the labeled sample, it was found that (i) acetate was incorporated into the sugar *N*-acetyl groups (4.7%), (ii) the chromophore part was labeled at positions 1 and 3 (1.7%) in agreement with the labeling pattern that could be expected according to the biosynthetic pathway elucidated by Floss and coworkers (Scheme 6) [23], (iii) enrichment of the carbamoyl-C was also observed (2.1%) indicating metabolic cleavage of acetic acid.

Feeding of Methionine

In these experiments, medium 4 was employed. The formation of moenomycin was studied in the presence of methionine. Consumption of methionine and moenomycin production were followed by HPLC. After considerable experimentation methionine was administered in a single dose after 48 h (1 g/l). HPLC indicated that it was consumed after 120 h. The moenomycin concentration in the liquid medium started to increase after all methionine had been consumed reaching finally twice the value of cultures without added methionine. When (*S*)-[$^{13}CH_3$]methionine was administered the isolated moenomycin showed one enriched carbon signal, that of the 4-methyl group in unit F (20%). We conclude from this experiment that the branching methyl group in unit F of moenomycin is introduced *via* a sequence consisting of (i) oxidation, (ii) dienolate methylation, and (iii) reduction (**42** → **43** → **44** → **45**, Scheme 7). Such a sequence was postulated for related cases many years ago [24].



Scheme 6 Origin of the chromophore unit of moenomycin A



Scheme 7 Origin of the 4-C-methyl group of moenomycin A unit F

Feeding of ^{15}N-labeled Ammonium Sulfate

The production of moenomycins by *Streptomyces ghanaesis* requires a rather complex fermentation medium. For the ^{15}N-labeling only ammonium sulfate could be replaced by [^{15}N]ammonium sulfate, whereas the soybean meal (also containing nitrogen) could be not omitted. All nitrogens of moenomycin A were labeled. The incorporation rate was about 15%. Figure 1 shows the ^{1}H NMR spectrum. The ^{15}N chemical shifts were obtained from ^{1}H ^{15}N HMQC spectra.

Further Information from the [1-^{13}C]-D-Glucose Feeding Experiment

After feeding of [1-^{13}C]-D-glucose as described above labeling of the 1-positions of all sugar units was observed with an incorporation rate of 2–3%. Labeling of the 6-positions of units B, C and F (0.6–0.8%) was also found, wheras the ^{13}C signals of C-6^{D} and C-6^{E} were hidden by other sugar signals [25]. The latter observations reflect the glycolytic/glucogenetic pathway [26]. Some incorporation into C-4 and C-5 of the chromophore part and into the methyl groups of the *N*-acetyl amino substituents was observed, too.

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Experimental

NMR: Gemini 200 and Gemini 2000 (Varian, ^{1}H NMR 200 MHz, ^{13}C NMR 50.3 MHz), Gemini 300 (Varian, ^{1}H NMR 300 MHz, ^{13}C NMR 75.5 MHz), DRX 400 (Bruker, ^{1}H NMR 400.1 MHz, ^{13}C NMR 100.6 MHz), DRX 600 (Bruker, ^{1}H NMR 600.1 MHz, ^{13}C NMR 150.9 MHz), chemical shifts are given in δ values; Mass Spectrometry: EI MS: VG-12-250 (Vacuum Generators, 70 eV), FAB MS: ZAB-HSQ (MassLab Manchester, Xenon, 8 kV), ESI MS: API 100 LC/MS-System (Perkin Elmer Applied Biosystems, Methanol, 10 mM ammonium acetate); optical rotation (sodium D-line, 0.5 dm cell) Polartronic Fa. Carl Zeiss Jena; preparative medium pressure chromatographie was performed using self made columns (65 g RP $_{18}$ material, 40-63 µm, LiChroprep®, Merck or Macherey Nagel Polygoprep 60–50 C 18). Fermentation experiments were performed in a gyrotary shaker

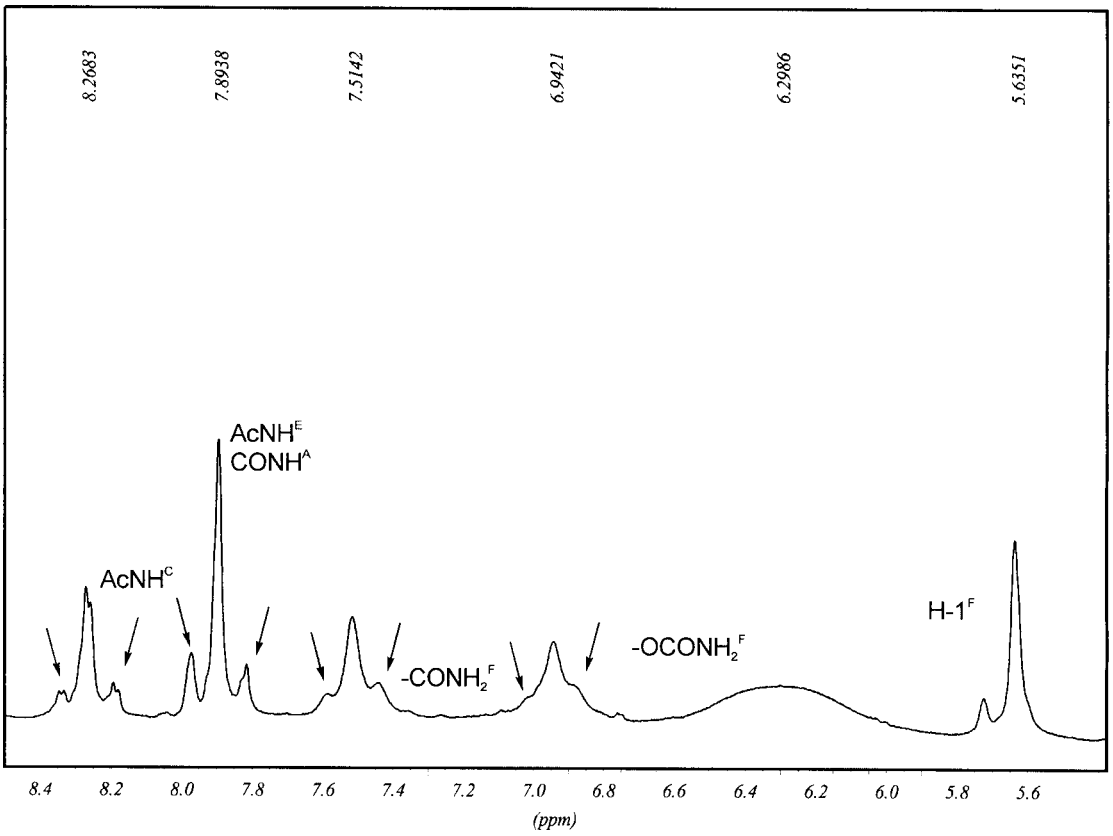

Fig. 1 NH proton signals of ^{15}N labeled moenomycin (15% incorporation rate). Arrows indicate ^{1}H ^{15}N couplings

(Thermoshaker; Fa. Gerhardt) at 37 °C and 160 r/min. Sterile works were performed in a cleanbench (Intermed. Nunc).

[1-^{13}C]-Benzyloxyacetic acid (**9a**)

Sodium (331 mg, 14.4 mmol) was dissolved in benzylalcohol (20 mL) at 60 °C. After the completion of alkoxide formation, [1-^{13}C]-bromoacetic acid (1,00 g, 7,19 mmol), dissolved in a small amount of benzylalcohol, was added. The mixture was stirred at 160 °C for 4 h. Excess benzylalcohol was removed at reduced pressure and the residue was taken up with water. The aqueous solution was extracted four times with diethylether. Hydrochloric acid was added and the aqueous phase was extracted again four times with 20 mL portions of diethylether. The combined organic extracts were dried over sodium sulfate. After solvent evaporation [1-^{13}C]-benzyloxyacetic acid (1.17 g, 97%) was obtained as a colourless oil. – ^{1}H NMR ($CDCl_3$, 200 MHz): δ/ppm = 10.96 (1H, sb, -COO<u>H</u>), 7.51–7.23 (5 H, mb, $C_6\underline{H}_5$), 4.65 (2H, s, Ph–C<u>H</u>$_2$), 4.16 (2H, d, C<u>H</u>$_2$-2, $^2J_{2\text{-}C1}$ = 4.6 Hz). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 176.35 (C-1), 137.14, 129.15, 128.80, 128.69, 73.94 (Ph–<u>C</u>H$_2$, d, $^3J_{C3\text{-}C1}$ = 2.7 Hz), 67.00 (C-2, d, $^1J_{C2\text{-}C1}$ = 60.6 Hz). – $C_8{}^{13}CH_{10}O_3$ (167.17, 167.07), EI MS: *m/z* = 166.9 $[M]^{+\cdot}$.

[1-^{13}C]-Ethyl benzyloxyacetate (**9b**)

[1-^{13}*C*]-Benzyloxyacetic acid (1.17 g, 6.99 mmol) was dissolved in ethanol (10 mL). Ten drops of thionyl chloride were added carefully at 20 °C. The solution was stirred at 20 °C for 30 min. Water (20 mL) was added and the mixture was extracted several times with dichloromethane. The combined organic extracts were dried over sodium sulfate. After solvent evaporation [1-^{13}*C*]-ethyl benzyloxyacetate (1.36 g, 99%) was obtained as a colourless liquid. – ^{1}H NMR ($CDCl_3$, 200 MHz): δ/ppm = 7.46–7.23 (5H, mb, $C_6\underline{H}_5$), 4.64 (2H, s, Ph–C<u>H</u>$_2$), 4.23 (2H, qd, C<u>H</u>$_2$–CH_3, $^3J_{4\text{-}5}$ = 7.1 Hz, $^2J_{4\text{-}C1}$ = 2.9 Hz), 4.09 (2H, d, C<u>H</u>$_2$-2, $^2J_{2\text{-}C1}$ = 4.8 Hz), 1.29 (3H, t, CH_2–C<u>H</u>$_3$, $^3J_{5\text{-}4}$ = 7.1 Hz). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 172.39 (C-1), 139.19, 130.52, 130.10, 130.03, 75.37 (Ph–<u>C</u>H$_2$, d, $^3J_{C3\text{-}C1}$ = 2.7 Hz), 69.28 (C-2, d, $^1J_{C2\text{-}C1}$ = 63.1 Hz), 62.91 (-<u>C</u>H$_2$–CH_3, $^2J_{C4\text{-}C1}$ = 2.7 Hz), 16.27 (-CH_2–<u>C</u>H$_3$, $^2J_{C5\text{-}C1}$ = 2.7 Hz). – $C_{10}{}^{13}CH_{14}O_3$ (195.22, 195.10). – EI MS: *m/z* = 195.1 $[M]^{+\cdot}$.

[1-^{13}C]-Benzyloxyethanol (**9c**)

[1-^{13}*C*]-Ethyl benzyloxyacetate (1.36 g, 6.94 mmol) was dissolved in dichloromethane (20 mL) and the solution was cooled to 0 °C. Diisobutylaluminium hydride solution (1.5 M in toluene, 14.0 mmol, 9.3 mL) was added slowly. The mixture was stirred for 2 h at 0 °C. Progress of the reaction was controlled by TLC (PE - EE 2:1) and diisobutylaluminium hydride solution was added in 1 mL portions, until all educt was consumed. Diluted hydrochloric acid was added to the mixture until the precipitate was completely redissolved. The mixture was extracted three times with dichloromethane. The combined organic extracts were dried over sodium sulfate. Evaporation provided [1-^{13}*C*]-benzyloxyethanol (1.05 g, 98%,) as a colourless oil. – ^{1}H NMR ($CDCl_3$, 200 MHz): δ/ppm = 7.46–7.21 (5H, mb, $C_6\underline{H}_5$), 4.56 (2H, s, Ph–C<u>H</u>$_2$), 3.74 (2H, dt, C<u>H</u>$_2$-1, $^1J_{1\text{-}C1}$ = 142.7 Hz, $^3J_{1\text{-}2}$ = 4.6 Hz), 3.59 (2H, td, CH_2-2, $^3J_{2\text{-}1}$ = 4.6 Hz, $^2J_{2\text{-}C1}$ = 2.7 Hz), 2.51 (1H, sb, -O<u>H</u>). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 138.55, 129.00, 128.35, 128.33, 73.75 (Ph–<u>C</u>H$_2$, d, $^3J_{C3\text{-}C1}$ = 4.2 Hz), 71.93 (C-2, d, $^3J_{C2\text{-}C1}$ = 40.4 Hz), 62.28 (C-1). – Impurity signals at: 201.12, 97.42. – $C_8{}^{13}CH_{12}O_2$ (153.18, 153.09), EI MS: *m/z* = 152.9 $[M]^{+\cdot}$.

[1-^{13}C]-Benzyloxyacetaldehyde (**9d**)

[1-^{13}*C*]-Benzyloxyethanol (1.05 g, 6.83 mmol) was added to a solution of iodoxybenzoic acid (2.83 g, 10.2 mmol) in DMSO (20 mL). The mixture was stirred for 4 h at 20 °C. Water was added and the mixture was extracted three times with dichloromethane. The combined organic phases were extracted three times with water to remove DMSO. The organic solution was dried over sodium sulfate and the solvent was removed *in vacuo*. The crude product was purified by FC (silicagel, PE - EE 2:1) to give benzyloxyacetaldehyde (800 mg, 77%) as a colourless oil. – ^{1}H NMR ($CDCl_3$, 200 MHz): δ/ppm = 9.71 (1H, d, -C<u>H</u>O, $^1J_{1\text{-}C1}$ = 175.8 Hz), 7.46–7.27 (5H, mb, $C_6\underline{H}_5$), 4.62 (2H, s, Ph–C<u>H</u>$_2$), 4.09 (2H, d, C<u>H</u>$_2$-2, $^2J_{2\text{-}C1}$ = 4.6 Hz). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 201.07 (C-1), 137.37, 129.14, 128.75, 128.58, 75.74 (C-2, d, $^1J_{C2\text{-}C1}$ = 43.5 Hz). 74.13 (Ph–<u>C</u>H$_2$, d, $^3J_{C3\text{-}C1}$ = 3.4 Hz). – Impurity signals at: 173.43, 64.34, 64.62, 40.78. – $C_8{}^{13}CH_{10}O_2$ (151.17, 151.07), FAB MS: *m/z* = 152 $[M+H]^+$.

Ethyl (E)-4-benzyloxy-2-butenoate (11a, General Procedure)

Sodium hydride (126 mg, 5.29 mmol) was suspended in benzene (10 mL) and ethyl diethylphosphonoacetate (1.18 g, 5.29 mmol) was slowly added within 10 min at 20 °C. The solution was stirred for 1.5 h at 20 °C. Benzyloxyacetaldehyde (800 mg, 5.29 mmol) was added dropwise during 5 min. A gelatinous precipitate was formed. The reaction mixture was stirred for 3 h at 20 °C. Water was added and the phases were seperated. The aqueous phase was extracted three times with diethylether. The combined organic extracts were dried over sodium sulfate and the solvent was removed *in vacuo*. FC (Silicagel, PE - Et_2O 19 : 1) furnished ethyl (*E*)-4-benzyloxybut-2-enoate (724 mg, 61%). – ^{1}H NMR (200 MHz, $CDCl_3$): δ/ppm = 7.47–7.23 (5H, mb, $C_6\underline{H}_5$), 6.99 (1H, dt, 3-H, $^3J_{3\text{-}2}$ = 15.7 Hz, $^3J_{3\text{-}4}$ = 4.3 Hz), 6.14 (1H, dt, 2-H, $^3J_{2\text{-}3}$ = 15.7 Hz, $^4J_{2\text{-}4}$ = 1.7 Hz), 4.57 (2H, s, Ph–C<u>H</u>$_2$), 4.21 (2H, q, -C<u>H</u>$_2$–CH_3, $^3J_{6\text{-}7}$ = 7.1 Hz), 4.18 (2H, dd, 4-H, $^3J_{4\text{-}3}$ = 4.3 Hz, $^4J_{4\text{-}2}$ = 1.7 Hz), 1.30 (3H, t, -CH_2–C<u>H</u>$_3$, $^3J_{7\text{-}6}$ = 7.1 Hz). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 166.85 (C-1), 144.74 (C-3), 138.27, 128.98, 128.32, 128.15, 121.94 (C-2), 73.21 (Ph–<u>C</u>H$_2$), 69.08 (C-4), 60.80 (-<u>C</u>H$_2$–CH_3), 14.62 (-CH_2–<u>C</u>H$_3$).

[1,2-$^{13}C_2$] Ethyl (E)-4-benzyloxy-2-butenoate

Prepared as described above, using [1,2-$^{13}C_2$] ethyl diethylphosphonoacetate. – ^{1}H NMR (200 MHz, $CDCl_3$): δ/ppm = 7.43–7.26 (5H, mb, $C_6\underline{H}_5$), 7.00 (1H, dtdd, 3-H, $^3J_{3\text{-}2}$ = 15.6 Hz, $^3J_{3\text{-}4}$ = 5.5 Hz, $^2J_{3\text{-}C2}$ = 4.4 Hz $^3J_{3\text{-}C1}$ = 2.4 Hz), 6.15 (1H, dddt, 2-H, $^1J_{2\text{-}C2}$ = 164.0 Hz, $^3J_{2\text{-}3}$ = 15.6 Hz, $^2J_{2\text{-}C1}$ = 3.6 Hz, $^4J_{2\text{-}4}$ = 1.8 Hz), 4.57 (2H, s, Ph–C<u>H</u>$_2$), 4.27–4.14 (4H, m, -C<u>H</u>$_2$–CH_3, 4-H, $^3J_{6\text{-}7}$ = 7.1 Hz, $^3J_{6\text{-}C1}$ = 3.0 Hz), 1.30 (3H, t, -CH_2–C<u>H</u>$_3$, $^3J_{7\text{-}6}$ = 7.1 Hz). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 166.80 (C-1, d, $^1J_{C1\text{-}C2}$ = 75.0 Hz), 144.73 (C-3, d, $^2J_{C3\text{-}C2}$ = 70.4 Hz), 138.28, 128.98, 128.32, 128.14, 121.94 (C-2, d, $^1J_{C2\text{-}C1}$ = 75.0 Hz), 73.27 (Ph–<u>C</u>H$_2$), 69.16 (C-4, d,

$^3J_{C2-C4}$ = 6.4 Hz), 60.90 (-$\underline{C}H_2$–CH_3, not seperated m), 14.77 (-CH_2–$\underline{C}H_3$, d, $^3J_{C7-C1}$ = 6.4 Hz). – $C_{11}{}^{13}C_2H_{16}O_3$ (222,25, 222,12). – EI MS: *m/z* = 222,1 $[M]^{+\cdot}$.

[3-^{13}C]-Ethyl (E)-4-benzyloxy-2-butenoate

Prepared from [1-^{13}*C*]-benzyloxyacetaldehyde and ethyl diethylphosphonoacetate. – ^{1}H NMR ($CDCl_3$, 200 MHz): δ/ppm = 7.40–7.25 (5H, mb, $C_6\underline{H}_5$), 7.00 (1 H, ddt, 3-H, $^1J_{3-C3}$ = 157.3 Hz, $^3J_{3-2}$ = 15.7 Hz, $^3J_{3-4}$ = 4.2 Hz), 6.15 (1H, ddt, 2-H, $^3J_{2-3}$ = 15.7 Hz, $^2J_{2-C3}$ = 2 Hz, $^4J_{2-4}$ = 1 Hz), 4.56 (2H, s, Ph–$C\underline{H}_2$), 4.21 (2 H, q, -$C\underline{H}_2$–CH_3, $^3J_{6-7}$ = 7.1 Hz), 4.21–4.14 (2H, mb, 4-H), 1.36 (3H, t, -CH_2–$C\underline{H}_3$, $^3J_{7-6}$ = 7.1 Hz). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 166.82 (C-1), 144.80 (C-3), 138.31, 129.00, 128.35, 128.17, 121.86 (C-2, d, $^1J_{C2-C3}$ = 71.4 Hz), 73.30 (Ph–$\underline{C}H_2$, d, $^3J_{C5-C3}$ = 3.7 Hz), 69.17 (C-4, d, $^1J_{C4-C3}$ = 45.8 Hz), 60.93 (-$\underline{C}H_2$–CH_3), 14.83 (-CH_2–$\underline{C}H_3$). – Impurity signal at: 170.86. – $C_{12}{}^{13}CH_{16}O_3$ (221.26, 221.11), EI MS: *m/z* = 221.1 $[M]^{+\cdot}$.

(E)-4-Benzyloxy-2-buten-1-ol (11b, General Procedure)

Ethyl (*E*)-4-benzyloxy-2-butenoate (724 mg, 3.25 mmol) was dissolved in dichloromethane (20 mL) and the solution was cooled to 0 °C. DIBAL-H (1.5 M in toluene, 6 ml, 9 mmol) was added slowly. The mixture was stirred at 0 °C for 6 h then an excess of methanol was added. The mixture was filtered through Celite® to remove solids. Solvent evaporation furnished *(E)*-4-benzyloxy-2-buten-1-ol (564 mg, 96%). – ^{1}H NMR (200 MHz, $CDCl_3$): δ/ppm = 7.44–7.17 (5 H, mb, $C_6\underline{H}_5$), 5.99–5.68 (2H, m, 2-H, 3-H), 4.53 (2H, s, Ph–$C\underline{H}_2$), 4.13 (2H, d, 1-H, $^3J_{1-2}$ = 3.6 Hz), 4.04 (2H, d, 4-H, $^3J_{4-3}$ = 4.0 Hz), 1.92 (1 H, sb, -O$\underline{H}$). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 138.71, 132.82 (C-2), 128.92, 128.28, 128.18 (C-3), 72.76 (Ph–$\underline{C}H_2$), 70.54 (C-4), 63.36 (C-1).

(E)-[1,2-$^{13}C_2$]-4-Benzyloxy-2-buten-1-ol

Prepared from [1,2-$^{13}C_2$]-ethyl *(E)*-4-benzyloxy-2-butenoate. – ^{1}H NMR (200 MHz, $CDCl_3$): δ/ppm = 7.48–7.22 (5H, mb, $C_6\underline{H}_5$), 5.93 (1H, m, 3-H), 5.88 (1H, dddt, 2-H, $^1J_{2-C2}$ = 154.9 Hz, $^3J_{2-3}$ = 15.5 Hz, $^2J_{2-C1}$ = 5.1 Hz, $^3J_{2-1}$ = 5.1 Hz), 4.52 (2H, s, Ph–$C\underline{H}_2$), 4.11 (2H, dddd, 1-H, $^1J_{1-C1}$ = 142.1 Hz, $^3J_{1-2}$ = 5.1 Hz, $^2J_{1-C2}$ = 4.5 Hz, $^4J_{1-3}$ = 1 Hz), 4.03 (2H, dd, 4-H, $^3J_{4-3}$ = 5.6 Hz, $^3J_{4-C2}$ = 5.6 Hz), 2.53 (1H, sb, -O$\underline{H}$). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 138.69, 133.05 (C-2, d, $^1J_{C2-C1}$ = 46.0 Hz), 128.96, 128.33, 128.23, 127.89 (C-3, dd, $^1J_{C3-C2}$ = 72.1 Hz, $^2J_{C3-C1}$ = 2.3 Hz), 72.74 (Ph–$\underline{C}H_2$), 70.61 (C-4, dd, $^2J_{C4-C2}$ = 6.3 Hz, $^3J_{C4-C1}$ = 1.7 Hz), 63.16 (C-1, d, $^1J_{C1-C2}$ = 46.0 Hz). – Impurity signals at: 41.72, 40.95, 30.70, 29.96.

(E)-[3-^{13}C]-4-Benzyloxy-2-buten-1-ol

Prepared from [3-^{13}*C*]-ethyl (*E*)-4-benzyloxy-2-butenoate. – ^{1}H NMR ($CDCl_3$, 200 MHz): δ/ppm = 7.47–7.21 (5H, mb, $C_6\underline{H}_5$), 6.00–5.80 (1H, m, 2-H), 5.83 (1H, ddt, 3-H, $^1J_{3-C3}$ = 155.8 Hz, $^3J_{3-2}$ = 15.5 Hz, $^3J_{3-4}$ = 5.8 Hz), 4.53 (2H, s, Ph–$C\underline{H}_2$), 4.22–3.96 (4H, m, 1-H, 4-H), 2.54 (1H, sb, -O$\underline{H}$). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 138.70, 133.13 (C-2, d, $^1J_{C2-C3}$ = 71.4 Hz), 128.98, 128.37, 128.25, 128.03 (C-3), 72.84 (Ph–$\underline{C}H_2$, d, $^3J_{C5-C3}$ = 3.7 Hz), 70.70 (C-4, d, $^1J_{C4-C3}$ = 48,5 Hz), 63.28 (C-1). – Impurity signals at: 62.36, 54.91, 34.78, 28.98, 27.16, 27.09, 23.14. – $C_{10}{}^{13}CH_{14}O_2$ (179.22, 179.10), EI MS: *m/z* = 179.1 $[M]^{+\cdot}$.

(E)-4-Benzyloxy-3-butenal (11c, General Procedure)

(*E*)-4-Benzyloxy-3-buten-1-ol (2 g, 11.2 mmol) was dissolved in chloroform (35 mL). Activated manganese dioxide (14 g) was added carefully in small portions. The mixture was stirred for 2 h at 20 °C and subsequently filtered through Celite®. Solvent removal furnished *(E)*-4-benzyloxy-3-butenal quantitatively. – ^{1}H NMR (200 MHz, $CDCl_3$): δ/ppm = 9.59 (1H, d, -C$\underline{H}$O, $^3J_{1-2}$ = 7.8 Hz), 7.50–7.17 (5H, mb, $C_6\underline{H}_5$), 6.85 (1H, m, 3-H, $^3J_{3-2}$ = 15.7 Hz, $^3J_{3-4}$ = 4.0 Hz), 6.41 (1H, ddt, 2-H, $^3J_{2-3}$ = 15.7 Hz, $^3J_{2-1}$ = 7.8 Hz, $^4J_{2-4}$ = 1.8 Hz), 4.60 (2H, s, Ph–$C\underline{H}_2$), 4.29 (2H, dd, 4-H, $^3J_{4-3}$ = 4.0 Hz, $^4J_{4-2}$ = 1.8 Hz). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 193.78 (C-1), 153.55 (C-3), 137.97, 132.35 (C-2), 129.07, 128.49, 128.22, 73.53 (Ph-$C\underline{H}_2$), 69.09 (C-4).

(E)-[1,2-$^{13}C_2$]-4-Benzyloxy-3-butenal

Prepared from *(E)*-[1,2-$^{13}C_2$]-4-benzyloxy-3-buten-1-ol. – ^{1}H NMR (200 MHz, $CDCl_3$): δ/ppm = 9.58 (1H, ddd, -C$\underline{H}$O, $^1J_{1-C1}$ = 172.4 Hz, $^2J_{1-C2}$ = 25.7 Hz $^3J_{1-2}$ = 7.8 Hz), 7.40–7.29 (5H, mb, $C_6\underline{H}_5$), 6.88–6.78 (1H, m, 3-H), 6.41 (1H, ddddt, 2-H, $^1J_{2-C2}$ ≈ 170 Hz $^3J_{2-3}$ = 15.8 Hz, $^3J_{2-1}$ = 7.8 Hz, $^2J_{2-C1}$ = 1.9 Hz, $^4J_{2-4}$ = 1.9 Hz), 4.60 (2H, s, Ph–$C\underline{H}_2$), 4.33–4.24 (2H, m, 4-H). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 193.78 (C-1, d, $^1J_{C1-C2}$ = 53.5 Hz), 153.57 (C-3, dd, $^1J_{C3-C2}$ = 68.3 Hz, $^2J_{C3-C1}$ = 4.6 Hz), 138.00, 132.31 (C-2, d, $^1J_{C2-C1}$ = 53.5 Hz), 129.07, 128.49, 128.21, 73.55 (Ph–$C\underline{H}_2$), 69.11 (C-4, d, $^2J_{C4-C2}$ = 7.3 Hz). – Impurity signals at: 63.58, 62.83, 31.00, 30.26. – $C_9{}^{13}C_2H_{14}O_2$ (178.20, 178.09), EI MS: *m/z* = 178.1 $[M]^{+\cdot}$.

(E)-[3-^{13}C]-4-Benzyloxy-2-butenal

Prepared from *(E)*-[3-^{13}*C*]-4-benzyloxy-3-buten-1-ol. – ^{1}H NMR ($CDCl_3$, 200 MHz): δ/ppm = 9.57 (1H, d, -C$\underline{H}$O, $^3J_{1-2}$ = 8.0 Hz), 7.43–7.28 (5H, mb, $C_6\underline{H}_5$), 6.84 (1H, m, 3-H, $^1J_{3-C3}$≈150 Hz, $^3J_{3-2}$ = 15.8 Hz, $^3J_{3-4}$ = 4.1 Hz), 6.40 (1H, m, 2-H, $^3J_{2-3}$≈ 15 Hz, $^2J_{2-1}$ = 8.0 Hz), 4.58 (2H, s, Ph–$C\underline{H}_2$), 4.33–4.21 (2H, m, 4-H). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 193.89 (C-1, d, $^1J_{C1-C3}$ = 4.2 Hz), 153.66 (C-3), 138.01, 132.26 (C-2, $^1J_{C2-C3}$ = 69.0 Hz), 129.08, 128.50, 128.22, 73.47 (Ph–$C\underline{H}_2$, $^3J_{C5-C3}$ = 3.4 Hz), 69.03 (C-4, $^1J_{C4-C3}$ = 46.2 Hz). – Impurity signals at: 148.09, 62.34, 34.66, 27.08, 22.97. – $C_{10}{}^{13}CH_{12}O_2$ (177.21, 177.09), EI MS: *m/z* = 176.8 $[M]^{+\cdot}$.

(E)-5-Benzyloxy-3-penten-2-ol (12, General Procedure)

Methyl Grignard reagent was prepared in the normal way from magnesium turnings (188 mg, 7 mmol) and methyl iodide (1.00 g, 7 mmol). Under an atmosphere of argon methylmagnesium iodide was added to a solution of *(E)*-4-benzyloxy-3-butenal (414 mg, 2.35 mmol) in anhydrous THF (10 ml). The mixture was stirred for 3 h at 20 °C, then poured into saturated aqueous ammonium chloride solution. After saturation with potassium carbonate the mixture was extracted three times with dichloromethane. The combined organic extracts were dried over sodium sulfate and the solvent was removed *in vacuo*. (*E*-5-Benzyloxy-3-penten-2-ol (426 mg, 94%) thus obtained was used without further purification. – ^{1}H NMR (200 MHz, $CDCl_3$): δ/ppm = 7.48–7.19 (5H, mb, $C_6\underline{H}_5$),

5.96–5.62 (2H, mb, 3-H, 4-H), 4.53 (2H, s, Ph–C$\underline{H}_2$), 4.44–4.23 (1H, mb, 2-H), 4.03 (2H, d, 5-H, $^3J_{5\text{-}4}$ = 3.7 Hz), 2.28 (1H, sb, -O$\underline{H}$), 1.28 (3H, d, C$\underline{H}_3$-1, $^3J_{1\text{-}2}$ = 6.2 Hz). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 138.67, 137.66 (C-3), 128.88, 128.27, 128.15, 126.68 (C-4), 72.80 (Ph–$\underline{C}H_2$), 70.60 (C-5), 68.69 (C-2), 23.68 ($\underline{C}H_3$-1).

(E)-[1-^{13}C]-5-Benzyloxy-3-penten-2-ol

Prepared from (*E*)-4-benzyloxy-3-butenal and [^{13}C]-methyl iodide. – ^{1}H NMR (200 MHz, $CDCl_3$): δ/ppm = 7.43–7.26 (5H, mb, $C_6\underline{H}_5$), 5.91–5.75 (2H, mb, 3-H,4-H), 4.52 (2H, s, Ph–C$\underline{H}_2$), 4.39–4.25 (1H, mb, 2-H), 4.02 (2H, d, 5-H, $^3J_{5\text{-}4}$ = 3.6 Hz), 2.07 (1H, sb, -O$\underline{H}$), 1.27 (3H, dd, C$\underline{H}_3$-1, $^1J_{1\text{-}C1}$ = 126.4 Hz, $^3J_{1\text{-}2}$ = 6.1 Hz). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 138.76, 137.82 (C-3), 129.01, 128.32, 128.20, 126.63 (C-4, $^3J_{C4\text{-}C1}$ = 3 Hz), 72.75 (Ph–$\underline{C}H_2$), 70.59 (C-5), 68.57 (C-2, $^2J_{C1\text{-}C2}$ = 38.5 Hz), 23.59 ($\underline{C}H_3$-1). – Impurity signals at: 32.34, 32.29, 31.28, 31.24, 30.03, 25.62, 17.49, 16.25.

(E)-[2,3-$^{13}C_2$]-5-Benzyloxy-3-penten-2-ol

Prepared from (*E*)-[1,2-$^{13}C_2$]-4-benzyloxy-3-butenal. – ^{1}H NMR (200 MHz, $CDCl_3$): δ/ppm = 7.52–7.13 (5H, mb, $C_6\underline{H}_5$), 5.80 (1H, ddddt, 3-H, $^1J_{3\text{-}C3}$ = 154.3 Hz, $^3J_{3\text{-}4}$ = 15.4 Hz, $^3J_{3\text{-}2}$ = 4.7 Hz, $^2J_{3\text{-}C2}$ = 4.7 Hz, $^4J_{3\text{-}5}$ = 1.0 Hz), 5.88–5.67 (1H, m, 4-H), 4.52 (2H, s, Ph–C$\underline{H}_2$), 4.31 (1H, dm, 2-H, $^1J_{2\text{-}C2}$ = 141 Hz), 4.06–3.97 (2H, m, 5-H), 1.98 (1H, sb, -O$\underline{H}$), 1.27 (3H, dd, C$\underline{H}_3$-1, $^3J_{1\text{-}2}$ = 6.4 Hz, $^2J_{1\text{-}C2}$ = 4.4 Hz). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 138.75, 137.81 (C-3, d, $^1J_{C3\text{-}C2}$ = 46.9 Hz), 128.94, 128.32, 128.19, 126.56 (C-4, dd, $^1J_{C4\text{-}C3}$ = 71.9 Hz, $^2J_{C4\text{-}C2}$ = 1 Hz), 72.76 (Ph–$\underline{C}H_2$), 70.59 (C-5, dd, $^2J_{C5\text{-}C3}$ = 6.1 Hz, $^3J_{C5\text{-}C2}$ = 1.5 Hz), 68.57 (C-2, d, $^1J_{C2\text{-}C3}$ = 46.9 Hz), 23.59 ($\underline{C}H_3$-1, d, $^1J_{C1\text{-}C2}$ = 38.5 Hz). – Impurity signals at: 64.77, 63.86, 63.42, 62.69, 30.82, 30.09, 23.98, 23.21.

(E)-[4-^{13}C]-5-Benzyloxy-3-penten-2-ol

Prepared from (*E*)-[3-^{13}C]-4-benzyloxy-3-butenal. – ^{1}H NMR ($CDCl_3$, 200 MHz): δ/ppm = 7.46–7.20 (5H, mb, $C_6\underline{H}_5$), 5.81 (1H, dddt, 3-H, $^3J_{3\text{-}4}$ = 15.6 Hz, $^3J_{3\text{-}2}$ = 5.9 Hz, $^2J_{3\text{-}C4}$ = 2.4 Hz, $^4J_{3\text{-}5}$ = 1 Hz), 5,78 (ddtd, 4-H, $^1J_{4\text{-}C4}$ = 155.5 Hz, $^3J_{4\text{-}3}$ = 15.6 Hz, $^3J_{4\text{-}5}$ = 5.6 Hz, $^4J_{4\text{-}2}$ = 1 Hz), 4.52 (2H, s, Ph–C$\underline{H}_2$), 4.31 (1H, m, 2-H, $^3J_{2\text{-}1}$ = 6 Hz), 4.02 (2H, dd, 5-H, $^3J_{5\text{-}4}$ = 5.6 Hz, $^2J_{5\text{-}C4}$ = 4.8 Hz), 2.13 (1H, sb, -O$\underline{H}$), 1.28 (3H, d, C$\underline{H}_3$-1, $^3J_{1\text{-}2}$ = 6 Hz). – ^{13}C NMR (50,3 MHz, $CDCl_3$): δ/ppm = 140.22, 139.38 (C-3, d, $^1J_{C3\text{-}C4}$ = 72.3 Hz), 129.83, 128.72, 128.56, 128.09 (C-4), 74.33 (Ph–$\underline{C}H_2$, d, $^3J_{C6\text{-}C4}$ = 3.6 Hz), 72.17 (C-5, d, $^1J_{C5\text{-}C4}$ = 48.5 Hz), 70.14 (C-2), 25.26 ($\underline{C}H_3$-1). – Impurity signals at: 67.21, 63.85, 44.00 an between 43 to 28. – $C_{11}{}^{13}CH_{16}O_2$ (193.25, 193.12). – EI-MS: *m/z* = 174.1 $[M–H_2O]^{+\cdot}$.

(E)-5-Benzyloxy-3-penten-2-one (14, General Procedure)

Iodoxybenzoic acid (932 mg, 3.33 mmol) was dissolved in DMSO (10 mL) and the (*E*)-5-benzyloxypent-3-en-2-on (426 mg, 2.22 mmol) dissolved in a small amount of DMSO was added. The solution was stirred until the educt was consumed. The reaction progress was monitored by TLC (PE - EE 1:1). The mixture was then diluted with water and extracted three times with dichloromethane. DMSO was removed from the combined organic phase by extraction with water. Drying over sodium sulfate and solvent evaporation yielded (*E*)-5-benzyloxypent-3-en-2-one (397 mg, 94%) which was used without further purification. – ^{1}H NMR (200 MHz, $CDCl_3$): δ/ppm = 7.48–7.22 (5H, mb, $C_6\underline{H}_5$), 6.81 (1H, dt, 4-H, $^3J_{4\text{-}3}$ = 16.1 Hz, $^3J_{4\text{-}5}$ = 4.4 Hz), 6.35 (1H, dt, 3-H, $^3J_{3\text{-}4}$ = 16.1 Hz, $^4J_{3\text{-}5}$ = 1.8 Hz), 4.57 (2H, s, Ph–C$\underline{H}_2$), 4.20 (2H, dd, 5-H, $^3J_{5\text{-}4}$ = 4.4 Hz, $^4J_{5\text{-}3}$ = 1.8 Hz), 2.26 (3H, d, C$\underline{H}_3$-1). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 198.84 (C-2), 143.66 (C-4), 138.14, 130.85 (C-3), 129.03, 128.41, 128.24, 73.44 (Ph–$\underline{C}H_2$), 69.31 (C-5), 27.78 ($\underline{C}H_3$-1). – Impurity signals at: 141.97, 133.22, 131.91, 119.99, 40.95.

(E)-[1-^{13}C]-5-Benzyloxy-3-penten-2-one

Prepared from (*E*)-[1-^{13}C]-5-benzyloxy-3-penten-2-ol. – ^{1}H NMR (200 MHz, $CDCl_3$): δ/ppm = 7.41–7.27 (5H, mb, $C_6\underline{H}_5$), 6.80 (1H, dt, 4-H, $^3J_{4\text{-}3}$ = 16.1 Hz, $^3J_{4\text{-}5}$ = 4.4 Hz), 6.34 (1H, ddt, 3-H, $^3J_{3\text{-}4}$ = 16.1 Hz, $^4J_{3\text{-}5}$ = 1.8 Hz, $^3J_{3\text{-}C1}$ = 1.8 Hz), 4.56 (2H, s, Ph–C$\underline{H}_2$), 4.21 (2H, dd, 5-H, $^3J_{5\text{-}4}$ = 4.4 Hz, $^4J_{5\text{-}3}$ = 1.8 Hz), 2.28 (3H, d, C$\underline{H}_3$-1, $^1J_{1\text{-}C1}$ = 127.3 Hz). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 198.80 (C-2, $^1J_{C2\text{-}C1}$ = 42.3 Hz), 143.59 (C-4), 138.17, 130.87 (C-3, $^2J_{C3\text{-}C1}$ = 15.3 Hz), 129.04, 128.44, 128.23, 73.39 (Ph–$\underline{C}H_2$), 69.26 (C 5), 27.68 ($\underline{C}H_3$-1). – Impurity signal at: 31.46.

(E)-[2,3-$^{13}C_2$]-5-Benzyloxy-3-penten-2-one

Prepared from (*E*)-[2,3-$^{13}C_2$]-5-benzyloxy-3-penten-2-ol. – ^{1}H NMR (200 MHz, $CDCl_3$): δ/ppm = 7.38–7.15 (5H, mb, $C_6\underline{H}_5$), 6.85–6.65 (1H, m, 4-H), 6.32 (1H, dddt, 3-H, $^1J_{3\text{-}C3} \approx 170$ Hz, $^3J_{3\text{-}4}$ = 16.2 Hz, $^2J_{3\text{-}C2}$ = 2.1 Hz, $^4J_{3\text{-}5}$ = 2.1 Hz), 4.51 (2H, s, Ph–C$\underline{H}_2$), 4.08–4.20 (2H, m, 5-H), 2.21 (3H, dd, C$\underline{H}_3$-1, $^2J_{1\text{-}C2}$ = 5.8 Hz, $^3J_{1\text{-}C3}$ = 1 Hz). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 198.82 (C-2, d, $^1J_{C2\text{-}C3}$ = 53.0 Hz), 143.66 (C-4, dd, $^1J_{C4\text{-}C3}$ = 69.4 Hz, $^2J_{C4\text{-}C2}$ = 2 Hz), 138.14, 130.80, (C-3, d, $^1J_{C3\text{-}C2}$ = 53.0 Hz), 129.00, 128.39, 128.20, 73.34 (Ph–$\underline{C}H_2$), 69.22 (C-5, d, $^2J_{C5\text{-}C2}$ = 6.1 Hz), 27.60 ($\underline{C}H_3$-1, dd, $^1J_{C1\text{-}C2}$ = 42.3 Hz, $^2J_{C1\text{-}C3}$ = 14.9 Hz). – Impurity signals at: 48 to 38.

(E)-[4-^{13}C]-5-Benzyloxy-3-penten-2-one

Prepared from (*E*)-[4-^{13}C]-5-benzyloxy-3-penten-2-ol. – ^{1}H NMR ($CDCl_3$, 200 MHz): δ/ppm = 7.40–7.06 (5.5H, mb, $C_6\underline{H}_5$, 4-H), 6.23–6.04 (1.5H, m, 3-H, 4-H), 4.54 (2H, s, Ph–C$\underline{H}_2$), 4.22–4.09 (2H, m, 5-H), 2.24 (3H, s, C$\underline{H}_3$-1). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 200.19 (C-2, d, $^2J_{C2\text{-}C4}$ = 2 Hz), 145.12 (C-4), 139.66, 132.28 (C-3, d, $^1J_{C3\text{-}C4}$ = 69.5 Hz), 130.51, 129.89, 128.71, 74.93 (Ph–$\underline{C}H_2$, d, $^3J_{C6\text{-}C3}$ = 3.7 Hz), 70.82 (C-5, d, $^1J_{C4\text{-}C5}$ = 48.5 Hz), 24.60 ($\underline{C}H_3$-1). – Impurity signals at: 43 to 30.

5-Benzyloxy-1-deoxy-d-xylulose (13a, General Procedure)

The asymmetric Sharpless dihydroxylation was carried out by a general procedure for α,β-unsaturated ketones [15]. – 1.87 g of commercial AD mix β (the amount of OsO_4 was increased to 1 mol% by addition of potassium osmate), methanesulfonamide (127 mg, 1.33 mmol) and $NaHCO_3$ (337 mg, 4.01 mmol) were stirred in 1:1 water - *t*-butanol (30 ml) at 20 °C. The clear solution was cooled to 0 °C, whereupon a precipitate was formed. The ketone (258 mg, 1.34 mmol) was added in one portion. The mixture was stirred for 24 h at 0 °C. The reaction progress was monitored by TLC (PE - EE 1:1; R_f ketone 0.41; R_f diol 0.28). The reaction was stopped by addition of sodium thiosulfate (3.8 g), the mixture was

warmed to room temperature and stirred for 1 h. The mixture was extracted several times with dichloromethane. The combined organic extracts were dried over sodium sulfate and the solvent was evaporated. FC (silicagel, PE : EE 1:1) furnished 5-benzyloxy-1-deoxy-D-xylulose (178.7 mg, 60%) as a colourless sirup. – $[\alpha]_D^{20} = +60.0$ (c 13.0, CH_2Cl_2). – ^{1}H NMR (200 MHz, CD_3OD): δ/ppm = 7.41–7.19 (5H, mb, $C_6\underline{H}_5$), 4.85 (2H, s, Ph–C$\underline{H}_2$), 4.24–4.09 (2H, m, 3-H,4-H), 3.72–3.42 (2H, m, 5-H), 2.23 (3H, d, C$\underline{H}_3$-1). – ^{13}C NMR (50.3 MHz, CD_3OD): δ/ppm = 212.32 (C-2), 139.90, 129.66, 129.19, 128.99, 79.09 (C-3), 74.69 (Ph–$\underline{C}H_2$), 72.27 (C-5), 70.19 (C-4), 26.91 ($\underline{C}H_3$-1).

[1-^{13}C]-5-Benzyloxy-1-deoxy-D-xylulose

Prepared from (*E*)-[1-^{13}*C*]-5-benzyloxy-3-penten-2-one. – $[\alpha]_D^{27} = +53.2$ (c 22.5, CH_2Cl_2). – ^{1}H NMR (200 MHz, $CDCl_3$): δ/ppm = 7.44–7.19 (5H, mb, $C_6\underline{H}_5$), 4.57 (2H, s, Ph–C$\underline{H}_2$), 4.28–4.13 (2H, m, 3-H,4-H), 3.71 (1H, sb, -OH), 3.62 (2H, d, 5-H, $^3J_{5\text{-}4}$ = 6.0 Hz), 2.39 (1H, sb, -OH), 2.27 (3H, d, C$\underline{H}_3$-1, $^1J_{1\text{-}C1}$ = 128.4 Hz). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 208.70 (C-2, $^1J_{C2\text{-}C1}$ = 42.3 Hz), 138.14, 129.42, 128.48, 128,38, 77.51 (C-3), 74.10 (Ph–$\underline{C}H_2$), 71.47 (C-5), 70.92 (C-4), 25.96 ($\underline{C}H_3$-1). – Impurity signal at: 27.48.

[2,3-$^{13}C_2$]-5-Benzyloxy-1-deoxy-D-xylulose

Prepared from (*E*)-[2,3-$^{13}C_2$]-5-benzyloxy-3-penten-2-one. – $[\alpha]_D^{27} = +60.0$ (c 17.9, CH_2Cl_2). – ^{1}H NMR (200 MHz, $CDCl_3$): δ/ppm = 7.53–7.12 (5H, mb, $C_6\underline{H}_5$), 4.54 (2H, s, Ph–C$\underline{H}_2$), ca. 4.20 (1H, sb, 3-H, $^1J_{3\text{-}C3} \approx 135$ Hz), 4.18 (1H, mb, 4-H), 3.87 (1H, sb, -OH), 3.66–3.53 (2H, m, 5-H), 3.01 (1H, sb, -OH), 2.21 (3H, dd, C$\underline{H}_3$-1, $^2J_{1\text{-}C2}$ = 5.8 Hz, $^3J_{1\text{-}C3}$ = 1 Hz). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 209.19 (C-2, d, $^1J_{C2\text{-}C3}$ = 39.3 Hz), 138.26, 129.04, 128.46, 128.44, 77.80 (C-3, d, $^1J_{C3\text{-}C2}$ = 39.3 Hz), 74.04 (Ph–$\underline{C}H_2$), 71.50 (C-5, d, $^2J_{C5\text{-}C2}$ = 3.1 Hz), 70.88 (C-4, d, $^1J_{C4\text{-}C3}$ = 39.3 Hz), 26.06 ($\underline{C}H_3$-1, dd, $^1J_{C1\text{-}C2}$ = 42.2 Hz, $^2J_{C1\text{-}C3}$ = 13.1 Hz). – Impurity signals at: 47.53, 46.74. – $C_{10}{}^{13}C_2H_{16}O_4$ (226.24, 226.11), EI MS: *m/z* = 226.0 $[M]^{+\cdot}$.

[4-^{13}C]-5-Benzyloxy-1-deoxy-D-xylulose

Prepared from (*E*)-[4-^{13}*C*]-5-benzyloxy-3-penten-2-one. – $[\alpha]_D^{22} = +41.7$ (c 1,14, CH_2Cl_2). – ^{1}H NMR (200 MHz, $CDCl_3$): δ/ppm = 7.50–7.16 (5H, mb, $C_6\underline{H}_5$), 4.55 (2H, s, Ph–C$\underline{H}_2$), 4.21 (1H, mb, 3-H), 4.19 (1H, mb, 4-H, $^1J_{4\text{-}C1} \approx$ 135 Hz), 3.84 (1H, sb, -OH), 3.66–3.47 (2H, m, 5-H), 2.92 (1H, sb, -OH), 2.23 (3H, s, C$\underline{H}_3$-1). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ/ppm = 210.52 (C-2), 139.73, 130.55, 130.15, 129.50, 79.38 (C-5, d, $^2J_{C5\text{-}C4}$ = 39.3 Hz), 78.89 (C-3, d, $^1J_{C3\text{-}C4}$ = 32.0 Hz), 75.63 (Ph–$\underline{C}H_2$, d, $^3J_{C6\text{-}C4}$ = 4.6 Hz), 72.49 (C-4), 27.73 ($\underline{C}H_3$-1). – Impurity signals at: 79.84, 79.21 78.57, 65.20, 45.20, 44.58, 31.60. – $C_{11}{}^{13}CH_{16}O_4$ (225.25, 225.11), EI-MS: *m/z* = 224.8 $[M]^{+\cdot}$.

1-Deoxy-d-xylulose (13b, General Procedure)

5-Benzyloxy-1-deoxy-D-xylulose (150 mg, 0.6 mmol) was dissolved in methanol (20 mL). A small portion of Pd–C catalyst (5 per cent) was added. This mixture was stirred overnight under an atmosphere of hydrogen. The catalyst was filtered off and the solvent was removed to provide 1-deoxy-D-xylulose in quantitative yield. – $[\alpha]_D^{21} = +32.1$ (c 5.1, H_2O). – ^{1}H NMR (200 MHz, CD_3OD): δ/ppm = 4.30–3.16 (7H, 2-H, 3-H, 4-H, CH_2-5, 2x -OH), 1.36, 1.32 (3H, 2 s, C$\underline{H}_3$-1 α,β). – ^{13}C NMR (50.3 MHz, CD_3OD, APT): δ/ppm = 110.74 (C-2) (+), 106.20 (C-2) (+), 85.40 (C-3) (–), 84.15 (C-3) (–), 79.52 (C-4) (–), 77.57 (C-4) (–), 73.12 (C-5) (+), 71.96 (C-5) (+), 19.41 ($\underline{C}H_3$-1) (–), 17.21 ($\underline{C}H_3$-1) (–).

[1-^{13}C]-1-Deoxy-D-xylulose

Prepared from [1-^{13}*C*]-5-benzyloxy-1-deoxy-D-xylulose. – ^{1}H NMR (200 MHz, CD_3OD): δ/ppm = 4.36–3.12 (7H, 2-H, 3-H, 4-H, CH_2-5, 2x -OH), 1.35–1.26 (3H, dm, C$\underline{H}_3$-1{$CH_3{}^{13}$C-OH}, $^1J_{1\text{-}C1}$ = 127 Hz). – ^{13}C NMR (50.3 MHz, CD_3OD): δ/ppm = 109.95 (C-2), 104.43 (C-2), 84.12 (C-3), 82.89 (C-3), 78.27 (C-4), 76.93 (C-4), 71.83 (C-5), 70.71 (C-5), 18.10 ($\underline{C}H_3$-1), 15.87 ($\underline{C}H_3$-1). – Due to ^1J couplings it was complicated to detect the signal of C-2. – Impurity signals at: 76.33, 64.31, 23.91, 23.17, 20.59. – $C_4{}^{13}CH_{10}O_3$ (135.12, 135.06), ESI MS (neg. mode): *m/z* = 134 $[M–H]^-$.

[2,3-$^{13}C_2$]-1-Deoxy-D-xylulose

Prepared from [2,3-$^{13}C_2$]-5-benzyloxy-1-deoxy-D-xylulose $[\alpha]_D^{21} = +30.7$ (c 5.0, H_2O). – ^{1}H NMR (200 MHz, CD_3OD): δ/ppm = 4.36–3.25 (5H, 2-H, 3-H, 4-H, CH_2-5), 3.24–3.10 (2H, m, 2 -OH), 2.24 (3H, d, C$\underline{H}_3$-1{$CH_3{}^{13}$CO}, $^2J_{1\text{-}C2}$ = 4.6 Hz), 1.47–1.10 (3H, m, C$\underline{H}_3$-1{$CH_3{}^{13}$C-OH}), (C$\underline{H}_3$-1{$CH_3{}^{13}$CO} : C$\underline{H}_3$-1{$CH_3{}^{13}$C-OH} = 1:4.1). – ^{13}C NMR (50.3 MHz, CD_3OD): δ/ppm = 209.19 (C-2, d, $^1J_{C2\text{-}C3}$ = 41.2 Hz), 111.5–108.4 (C-2, m), 106.3–102.4 (C-2, m), 85.0–81.3 (C-3, C-4, C-5, m).

[4-^{13}C]-1-Deoxy-D-xylulose

Prepared from [4-^{13}*C*]-5-benzyloxy-1-deoxy-D-xylulose. – $[\alpha]_D^{21} = +33.2$ (c 5.7, H_2O). – ^{1}H NMR (200 MHz, CD_3OD): δ/ppm = 4.66–3.17 (5H, 2-H, 3-H, 4-H, CH_2-5), 3.13–2.88 (2H, m, 2 -OH), 2.25 (3H, d, C$\underline{H}_3$-1{$CH_3{}^{13}$CO}), 1.53–1.03 (3H, m, C$\underline{H}_3$-1{$CH_3{}^{13}$C–OH}), (C$\underline{H}_3$-1{$CH_3{}^{13}$CO} : C$\underline{H}_3$-1{$CH_3{}^{13}$C-OH} = 1:30). – ^{13}C NMR (50.3 MHz, CD_3OD): δ/ppm = 109.52 (C-2), 105.00 (C-2), 84.18 (C-3, d, $^1J_{C5\text{-}C4}$ = 41.2 Hz), 82.93 (C-3, d, $^1J_{C5\text{-}C4}$ = 41.2 Hz), 78.29 (C-4), 76.35 (C-4), 71.85 (C-5, d, $^1J_{C3\text{-}C4}$ = 37.0 Hz), 70.68 (C-3, d, $^1J_{C3\text{-}C4}$ = 38.1 Hz), 18.12 ($\underline{C}H_3$-1), 15.91 ($\underline{C}H_3$-1). – Impurity signals at: 105.83, 74 to 63, 42.35, 41.60, 34.43, 32.31. – $C_4{}^{13}CH_{10}O_3$ (135.12, 135.06), EI MS: *m/z* = 134.9 $[M]^{+\cdot}$.

Nutrient Solutions

Preculture medium: cornsteep liquor (0.4 g), soybean meal (3.0 g), $CaCO_3$ (0.45 g), soybean oil (0.4 g), D-glucose (5.2 g), KH_2PO_4 (0.1 g), deion. water (0.1 l), pH 7 prior to sterilisation.

Medium 1 (feeding of xylulose): cornsteep liquor (3.3 g), soybean meal (6.4 g), $CaCO_3$ (1.65 g), soybean oil (11.7 g), $(NH_4)_2SO_4$ (1.5 g), glucose (7.5g), amylase bac. (0.5 mg), $CoSO_4$ (0.7 mg), KH_2PO_4 (50 mg), Genapol®(20 per cent in water, 12.5 g), deion. water (0.5 L), pH 7 prior to sterilisation. Additional glucose was administered after 12 h (15 g/L), 24 h (7.5 g/L) and 30 h (7.5g/L).

Medium 2 (feeding of glucose): cornsteep liquor (3.3 g), soybean meal (6.4 g), $CaCO_3$ (1.65 g), soybean oil (11.7 g), $(NH_4)_2SO_4$ (1.5 g), glucose (7.5g), amylase bac. (0.5 mg), $CoSO_4$ (0.7 mg), KH_2PO_4 (50 mg), Genapol®(20 per cent in

water, 12.5 g), deion. water (0.5 L), pH 7 prior to sterilisation. Additional glucose was administered after 12 h (15 g/L), 24 h (7.5 g/L including 2 g of [1-^{13}C]-D-glucose) and 30 h (7.5 g/L including 2 g of [1-^{13}C]-D-glucose).

Medium 3 (feeding of ^{15}N-ammonium sulfate): cornsteep liquor (5.0 g), soybean meal (19.2 g), $CaCO_3$ (2.5 g), soybean oil (11.7 g), $(^{15}NH_4)_2SO_4$ (2.3 g), corn starch (19.7 g), amylase bac. (0.5 mg), $CoSO_4$ (0.7 mg), KH_2PO_4 (100 mg), Genapol®(20 per cent in water, 18.8 g), deion. water (0.75 L), pH 7 prior to sterilisation.

Medium 4 (feeding of methionine): Identical with medium **3**, $(^{15}NH_4)_2SO_4$ was replaced by $(NH_4)_2SO_4$.

Medium 5 (feeding of acetate): cornsteep liquor (28.5 g), soybean meal (34.1 g), $CaCO_3$ (4.3 g), $(NH_4)_2SO_4$ (3.0 g), starch (32.9 g), amylase bac. (1.5 mg), $MgSO_4$ (0.23 g), $FeSO_4$ (0.2 mg), $ZnSO_4$ (0.8 mg), $CuSO_4$ (2.9 mg), $CoSO_4$ (1.0 mg), glucose (6.3 g), KH_2PO_4 (0.12 g), Genapol®(20 per cent in H_2O, 25.0 g), deion. H_2O (1.0 L), pH 6.5 prior to sterilisation. Sodium[1-^{13}C]acetate was added in total of 15 additions in intervals of 8 h. The first dose was administered after 45 h. In test experiments consumption of acetate was followed with the test kit of Boehringer (Mannheim).

Fermentation

Streptomyces ghanaensis H2 was maintained as frozen culture at −80 °C. 2 mL of a frozen culture were used to inoculate 100 ml of the preculture medium in a 500 mL Erlenmeyer flask. The cultures were incubated on a rotary shaker (160 rpm) at 37 °C for 20 h. 1 mL of this culture was used to inoculate 50 mL of the medium which was used for incorporation experiments, depending on the used precursor (medium 1 to 4). The cultures were incubated on a rotary shaker (160 rpm) at 37 °C for 240 h.

Incorporation Experiments

Feeding experiments were carried out under standard fermentation conditions. The labelled precursors were added in aqueous solutions. 1-Deoxy-D-xylulose was dissolved in deionized water (15 mL), sterilised by filtration (Sartorius Minisartâ, 0.2 µm) and was administered after 48 h. Methionine was dissolved in deionized water (15 mL), sterilised at 121 °C for 10 min and administered in a single dose after 48 h (1 g/l). Sodium [1-^{13}C]acetate (2.25 g/l) was administered using a pulse feeding protocol (the first dose at the end of the logarithmic phase, 45 h).

Isolation of the Moenomycins

Method 1: Cells were separated from the medium by centrifugation. The supernatant after solvent evaporation was stirred with 80:20 methanol-water. Cell disintegration was achieved by sonication in methanol. The filtrates from both fractions were combined and after solvent evaporation the residue was partitioned between butanol and water. The aqueous phase was purified by ultrafiltration (Amicon YM 3000 (cutoff 3000 Da)). RP_{18}-Medium-pressure LC (puffer - acetonitrile 63:37, puffer: K_2HPO_4*3 H_2O (13.1 g), KH_2PO_4 (0.3 g) and water, final volume: 1 L) followed by desalting (ultrafiltration) yielded the mixture of moenomycins.

Method 2: Cells were separated from the medium by filtration. The filtered solution was concentrated at 40 °C (rotatory evaporator) and the residue was stirred with an ice-cold 8:2 methanol-water mixture for 2 h and then filtered. Cell disintegration was achieved by sonication in ice-cold 8:2 methanol-water. After filtration the filtrates were combined and methanol was evaporated. After setting the pH 7.5 the aqueous solution was extracted with three portions of 1-butanol. Solvent evaporation from the aqueous phase, taking up the residue in 4:6 acetonitrile-buffer (K_2HPO_4*3 H_2O (13.1 g), KH_2PO_4 (0.3 g) and water, final volume: 1 L, adjusting the pH to 7.5), and medium pressure LC (RP_{18}, solvent: acetonitrile-buffer as described above) gave a fraction that was desalted by solid phase extraction (RP18, first water, then 1:1 acetonitrile-water). Acetonitrile removal by distillation and subsequent lyophilization provided the pure mixture of the moenomycins.

Table 1 Results from the [1-^{13}C]-D-glucose feeding experiment

Chemical shift in ppm	Assignment	Incorporation rate
199.84	C-1,3^{A}	–
176.83	CO_2H	–
173.99	CONH ^{E}	–
173.61	CONH ^{C}	–
173.31	C-6^{F}	0.80
169.67	C-6^{B}	0.55
158.66	$OCONH_2$	–
150.48	C-11^{I}	–
141.49	C-3^{I}	–
140.87	C-7^{I}	–
136.75	C-14^{I}	–
131.73	C-18^{I}	–
126.21	C-6^{I}	–
124.67	C-17^{I}	–
122.85	C-13^{I}	–
122.35	C-2^{I}	–
110.44	C-2^{A}	–
108.70	C-22^{I}	0.16
104.26	C-1^{D}	1.83
103.97	C-1^{B}	2.66
103.68	C-1^{E}	1.91
102.51	C-1^{C}	2.51
95.49	C-1^{F}	2.07
42.17	C-9^{I}	0.38
40.18	C-15^{I}	–
35.80	C-8^{I}	–
35.30	C-12^{I}	0.26
32.78	C-4^{I}	–
32.06	C-5^{I}	0.49
31.62	C-10^{I}	–
31.02	C-4,5^{A}	0.45
27.30	C-23,24^{I}	0.24
26.98	C-16^{I}	0.49
25.37	C-19^{I}	–
23.47	C-25^{I}	0.57
22.97	$\underline{C}H_3CONH$ ^{C,E}	0.79
22.75	$\underline{C}H_3CONH$ ^{C,E}	0.40
17.47	C-6^{C}	0.74
17.23	C-20^{I}	0.40
15.36	C-21^{I}	0.63
15.25	CH_3^{F}	–

NMR Experiments

a)The pure desalted moenomycin mixture (unlabeled and labelled samples) was dissolved in CD_3OD-D_2O 10:1 (0.7 mL) and filtered into a 5 mm NMR tube. The spectra were acquired using an inverse gated decoupling experiment. The number of scans was 9000 in case the of 1-deoxy-D-xylulose incorporation experiments and 30 000 in case of the glucose incorporation experiments. Incorporations were calculated according to Scott *et al*. [18].

b) In the [1-^{13}C]acetate feeding experiment a somewhat different procedure was used. For a quantitative analysis the inverse gated decoupling ^{13}C NMR spectrum of the unlabelled moenomycin mixture was recorded in a 0.05 mol/l $Cr(acac)_3$ solution in 25:1 methanol-water. Under these conditions suppressing NOE enhancements and relaxation effects all signals gave practically the same integral. The labeled moenomycin was recorded under identical conditions. Incorporations were calculated according to Scott *et al*. [18].

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