Tetrahedron 64 (2008) 8638–8641

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Direct conversion of polyconjugated compounds into their corresponding carboxylic acids by *Acetobacter aceti*

Elena Pini [a,*], Vittorio Bertacche [a], Francesco Molinari [b], Diego Romano [b], Raffaella Gandolfi [a]

[a] *Istituto di Chimica Organica 'A. Marchesini', Università degli Studi, Via Venezian 21, 20133 Milano, Italy*
[b] *Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi, Via Mangiagalli 25, 20133, Milano, Italy*

ARTICLE INFO

Article history:
Received 30 April 2008
Received in revised form 13 June 2008
Accepted 3 July 2008
Available online 8 July 2008

Keywords:
Polyconjugated compounds
Oxidation
Acetobacter aceti

ABSTRACT

The conversion of polyconjugated aldehydes or alcohols into their corresponding acids was carried out using *Acetobacter aceti*. The analytical results were compared with those of the acids chemically obtained using a Horner–Wittig reaction.

1. Introduction

In recent years, the interest in polyconjugated systems has increased due to the antioxidant properties of these molecules.[1–3] Polyunsaturated aldehydes and alcohols have been evaluated in a number of biochemical and pharmacological studies,[4–6] which have identified their potential role as antioxidant and antiproliferative molecules.

Polyconjugated aldehydes were obtained following Kuhn[7] and Blout[8] procedures by self-condensation of crotonaldehyde or by condensation between crotonaldehyde and acetaldehyde using piperidinium acetate as catalyst. The compounds obtained were subsequently reduced by $NaBH_4$ giving the corresponding alcohols.

The aim of this work was the direct conversion of compounds **1** and **2** into the corresponding carboxylic acids **3** (Scheme 1). In the literature it is known that the conventional methods such as permanganate[9] or Jones reagent[10] could not be applied to α,β-unsaturated compounds since double bonds are affected under these conditions. Balkrishna et al.[11] suggested the use of sodium chlorite, but this method gave double bond oxidation with these molecules as well.

Nevertheless, several methods [12–15] are available for the synthesis of α,β-unsaturated carboxylic acids, but these reactions have low yields and did not allow the direct oxidation.

To overcome these synthetic problems, microbial cells endowed with dehydrogenases or oxidases able to perform the selective oxidation of aldehydes and/or alcohols under mild conditions can be employed.[16] Acetic acid bacteria are known to be particularly suited for carrying out these oxidations also on industrial scale.[17,18]

In this work, we have investigated the oxidation of different polyunsaturated aldehydes and alcohols catalysed by *Acetobacter aceti* MIM2000/28, a microorganism already used for a number of selective bio-oxidations.[19,20]

1a-f R= CHO

3a-f

2a-f R= CH_2OH

a n=2 **b** n=2 **c** n=3
d n=5 **e** n=6 **f** n=7

Scheme 1. Direct oxidation of polyconjugated aldehydes and alcohols.

2. Results and discussion

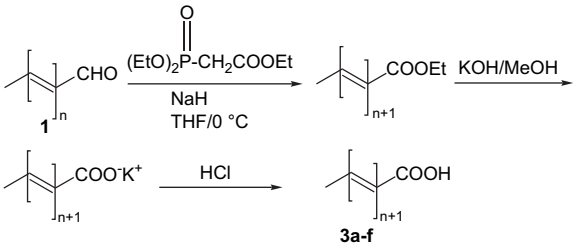
The direct chemical oxidation of polyconjugated aldehydes (**1a–f**, Scheme 1) with conventional reagents, such as potassium permanganate, Jones reagent and the Balkrishna method was unsuccessful.

The polyunsaturated carboxylic acids (**3a–f**) were obtained using an indirect method. The ethyl esters obtained by the

* Corresponding author. Tel.: +39 2 50314606; fax: +39 2 50314615.
E-mail address: elena.pini@unimi.it (E. Pini).

0040-4020/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2008.07.011

Horner–Wittig reaction carried out between the *all trans* aldehydes and the ylide from triethyl phosphonoacetate were hydrolysed with methanolic potassium hydroxide[21] (Scheme 2) leading to the carboxylic acids. In this case, the reaction product is a molecule with $n+1$ unsaturation compared with the starting aldehyde; the olefinic systems kept *all trans* configuration but the final yields were low due to the number of steps involved. All the acids obtained following this procedure were isolated and characterised by ^{1}H and ^{13}C NMR spectroscopies, two-dimensional analyses (COSY and HETCOR), infrared spectroscopy and mass spectrometry.



Scheme 2. Synthesis of polyunsaturated acids **3** by Horner–Wittig reaction.

A. aceti MIM2000/28 was used for the oxidation of the polyunsaturated aldehydes furnishing good yields compared with the ones obtained by chemical means (Table 1). The molar conversion decreased from 2,4-hexadienal **1a** to 2,4,6,8,10-dodecapentaenal **1d**; no reaction was observed with aldehydes having longer chain lengths (**1e** and **1f**). This trend can be due to the lower solubility in water of the substrates with higher chain length and/or to the increased toxicity of the substrates towards the enzymatic systems. The biocatalytic oxidation of aldehydes **1c** and **1d** reached maximum conversion after 24 and 48 h, respectively; no change of substrate/product composition was observed at prolonged times. The time-course of the oxidation of **1d** is reported in Figure 1.

The study was then extended to alcohols **2a**–**f**, obtained by reduction of the corresponding aldehydes with $NaBH_4$; alcohols are generally more soluble in water, more stable and tend to be less toxic towards the dehydrogenases involved in these reactions. The results are summarised in Table 2.

The oxidation of the different polyconjugated alcohols catalysed by *A. aceti* occurred with no detectable aldehyde accumulation, indicating that the second step of the oxidation (aldehyde into acid) is faster than the first one (alcohol into aldehyde). The occurrence of different membrane-bound dehydrogenases in acetic acid bacteria has been exhaustively studied; normally aldehyde dehydrogenases are more active than alcohol dehydrogenases so that aldehydes (potentially toxic to many enzymes) are not accumulated.[20,22]

The conversion of these substrates was higher than the ones observed starting from the aldehydes. The time-course of the oxidation of **2d** is reported in Figure 2.

Table 1
Molar conversion of aldehydes **1** into acids **3** obtained by chemical and biocatalytic methods

Acid	Chemical yield (%)	Biocatalytic yield (%)	Biocatalytic time conversion (h)
3a	10	86	2
3b	23	89	3
3c	51	60[a]	24
3d	20	65[a]	48
3e	10	—	—
3f	10	—	—

[a] The resuspended cells' concentration was six times the concentrate compared with cell cultures. In the other cases the ratio was 2:1.

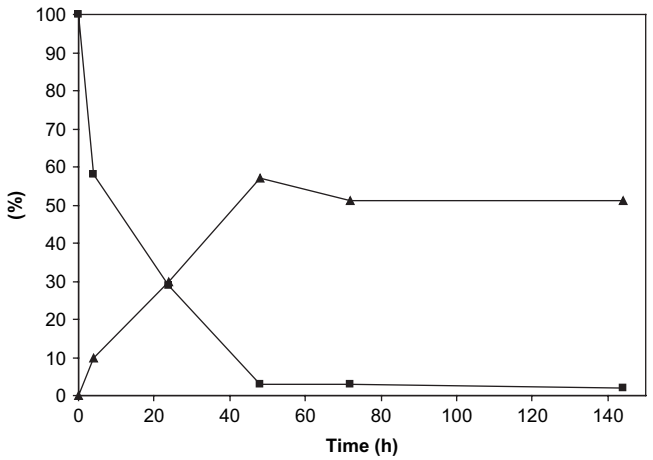


Figure 1. Time-course of the biotransformation of 2,4,6,8,10-dodecapentaenal **1d** catalysed by *A. aceti* MIM2000/28. ■, aldehyde; ▲, acid.

The acid yields lowered by increasing the chain length of the substrate, as observed when aldehydes were used as substrate.

3. Conclusion

The use of a biocatalytic approach for the direct oxidation of polyconjugated aldehydes seems to be a promising and possibly general method to furnish acids in good yields. The analytical profile of the biotransformation products is comparable to the profile of the chemically synthesised acids; moreover, the ^{1}H NMR spectroscopic analyses confirm the *all trans* configuration of double bonds.

4. Experimental section

4.1. Materials and methods

The ^{1}H and ^{13}C NMR spectra and two-dimensional analyses (COSY and HETCOR) were carried out on a Varian Gemini 200 operating at 200 MHz. Chemical shifts were expressed as parts per million (δ). Samples of about 5 mg were dissolved in 0.7 mL of DMSO.

HPLC/DAD analyses were performed using a Perkin–Elmer Series 200 DAD equipped with a manual injector Rheodyne 7125 (loop 20 μL), an online degasser and a column block heater. The column employed was a Purosphere STAR RP 18-e (Merck, MA, USA), 150×4.6 mm i.d. (5 μm particle size); mobile phase was methanol with 0.1% acetic acid (v/v) eluted at 0.8 mL/min with a temperature maintained at 30 °C. The UV–vis peaks' spectra were recorded ranging from 200 to 650 nm.

MS analyses were performed by using a Thermo Finnigan (MA, USA) LCQ Advantage system MS spectrometer with an electrospray ionisation source and an 'Ion Trap' mass analyser. The MS spectra

Table 2
Conversion of polyunsaturated alcohols **2** to corresponding acids

Substrate	Conversion time (h)	Acid yields (%)
2a	2	100
2b	2	100
2c	44	82
2d[a]	24	25

[a] The reported data concern the maximum acid formation; extending the conversion time degradation phenomena begin.

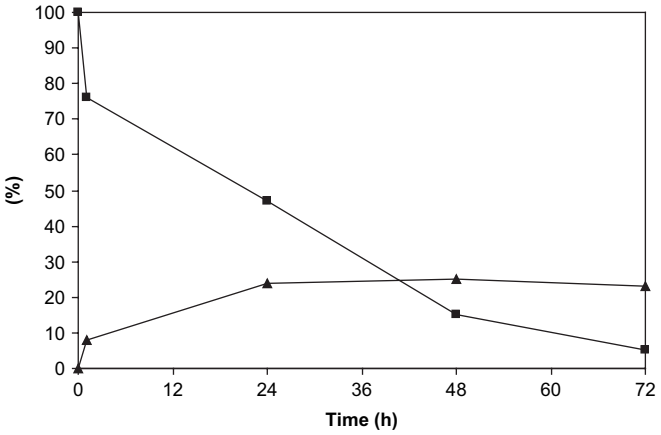


Figure 2. Time-course of the biotransformation of 2,4,6,8,10-dodecapentaenol **2d** catalysed by *A. aceti* MIM2000/28. ■, alcohol; ▲, acid.

were obtained by direct infusion of a sample solution in a mixture MeOH/H_2O/AcOH 10:89:1 under ionisation, ESI positive.

FTIR spectra were collected by using a Perkin–Elmer (MA, USA) FT-IR Spectrometer 'Spectrum One' in a spectral region between 4000 and 450 cm^{-1}. Samples were mixed in a mortar with KBr (1:100) and pressed in a hydraulic press (14 tons) to small tablets, which were then analysed by transmittance technique with 32 scansions and 4 cm^{-1} resolutions.

All reagents, solvents and spectroscopic solvent grade employed for HPLC and MS analyses and DMSO-d_6 for NMR were purchased from Aldrich Chemical.

4.2. General procedure to obtain alcohols 2a–f

To a stirred solution of aldehyde **1a–f** (2.2 mmol) in absolute ethanol (20 mL), $NaBH_4$ (3.3 mmol) in absolute ethanol (5 mL) was added under nitrogen. The reaction mixture was stirred at room temperature for 1–5 h (monitored by TLC). After distilled water (30 mL) addition, the aqueous layer was extracted with diethyl ether (3×30 mL) and the organic layers were dried under sodium sulfate, filtered and then evaporated under reduced pressure leading to a residue, which was purified by recrystallisation. Low solubility of compounds **2e** and **2f** did not allow ^{13}C analysis.

4.2.1. Compound **2a**[23]

Reaction time: 1 h. Colourless oil; yield 90%.

4.2.2. Compound **2b**[24]

Reaction time: 1.5 h. Recrystallisation from petroleum ether gave a white solid; yield 90%; mp 99–100 °C.

4.2.3. Compound **2c**[25]

Reaction time: 2 h. Recrystallisation from ethanol; white solid; yield 70%; mp 99–100 °C.

4.2.4. Compound **2d**[26]

Reaction time: 3 h. Recrystallisation from ethyl acetate; pale yellow solid; yield 80%; mp 203–204 °C; λ_{MeOH}: 310.5, 324.1, 340.5 nm; ν_{max} (KBr): 3280, 3186, 3014, 2920, 2870, 1684, 1654, 1378, 1004, 964 cm^{-1}; δ_H (200 MHz, $CDCl_3$): 6.27–6.12 (8H, m, *CH*), 5.85–5.74 (2H, m, $CHCH_2OH$, *CH*CH_3), 4.25 (2H, d, *J* 5.8 Hz, CH_2OH), 1.82 (3H, d, *J* 6.6 Hz, CH_3), 1.31 (1H, s, CH_2*OH* exch. with D_2O); δ_C (53 MHz, $CDCl_3$): 133.5, 133.1, 133.9, 131.8–130.3, 18.3; MS: MH^+, found 177. $C_{12}H_{16}O$ requires 176.

4.2.5. Compound **2e**

Reaction time: 5 h. Recrystallisation from ethyl acetate; yellow solid; yield 60%; mp dec >200 °C; λ_{MeOH}: 333.7, 350.5, 369.7 nm; ν_{max} (KBr): 3422, 2962, 2924, 2852, 1654, 1636, 1004, 964 cm^{-1}; δ_H (200 MHz, $CDCl_3$): 6.27–6.12 (10H, m, *CH*), 5.78–5.73 (2H, m, $CHCH_2OH$, *CH*CH_3), 4.03 (2H, d, *J* 7.3 Hz, CH_2OH), 1.76 (3H, d, *J* 6.6 Hz, CH_3), 1.35 (1H, s, OH exch. with D_2O); MS: MH^+, found 203. $C_{14}H_{18}O$ requires 202.

4.2.6. Compound **2f**

Reaction time: 5 h. Recrystallisation from ethyl acetate; dark yellow solid; yield 40%; mp dec >200 °C; λ_{MeOH}: 354.9, 373.3, 394.9 nm; ν_{max} (KBr): 3430, 2962, 2924, 2852, 1684, 1654, 1268, 1098, 1018, 800 cm^{-1}; δ_H (200 MHz, $CDCl_3$): 6.32–6.17 (12H, m, *CH*), 5.86–5.75 (2H, m, $CHCH_2OH$, *CH*CH_3), 4.02 (2H, d, *J* 5.2 Hz, CH_2OH), 1.76 (3H, d, *J* 6.1 Hz, CH_3), 1.31 (s, 1H, OH exch. with D_2O); MS: MH^+, found 229. $C_{16}H_{20}O$ requires 228.

4.3. General procedure to obtain acids 3a–f

Triethyl phosphonoacetate (4.52 mmol, 0.89 mL) was added under nitrogen to a stirred suspension of sodium hydride (4.82 mmol, 192.85 mg of a 1:1 w/w dispersion in mineral oil) in anhydrous THF (20 mL) at 0 °C. By maintaining this temperature the appropriate aldehyde (4.098 mmol) was added and the reaction mixture was allowed to stand overnight at 4 °C. The mixture was then poured into ice water and the reaction product was extracted with diethyl ethers or precipitated in the reaction medium, filtered and washed with water. The ethyl esters were suspended in 5% KOH in methanol (50 mL) and refluxed for about 30 min. After cooling, the precipitate was filtered and washed with cold methanol. A solution of 5% aqueous HCl was added to the potassium salt until acidic pH was reached. Acids **3a–f** were filtered and washed with a little amount of water.

4.3.1. Compound **3a**[27]

White solid; yield 23%; mp 125–126 °C.

4.3.2. Compound **3b**[27]

Pale yellow solid; yield 23%; mp 188–189 °C; λ_{MeOH}: 297 nm; ν_{max} (KBr): 3017, 2921, 1682, 1637, 1611, 1419, 1375, 1300, 1154, 1002, 967, 922 cm^{-1}; δ_H (200 MHz, DMSO): 12.1 (1H, s, CH*COOH* exch. with D_2O), 7.15 (1H, dd, *J* 15.27, 10.99, *CH*=CHCOOH), 6.7 (1H, dd, *J* 14.97, 10.69 Hz, *CH*=CHCH=CHCOOH), 6.3–6.0 (2H, m, *CH*), 5.9 (1H, dq, *J* 6.72, 13.13 Hz, *CH*CH_3), 5.8 (1H, d, *J* 15.27 Hz, =*CH*COOH), 1.7 (3H, d, *J* 6.72 Hz, CH_3); δ_C (53 MHz, DMSO): 168.3, 145.1, 141.4, 135.5, 132.0, 128.4, 121.6, 19.0; MS: MH^+, found 139. $C_8H_{10}O_2$ requires 138.

4.3.3. Compound **3c**[28]

Yellow solid; yield 51%; mp 258–260 °C; λ_{MeOH}: 329 nm; ν_{max} (KBr): 3015, 2918, 2849, 1682, 1618, 1594, 1422, 1384, 1309, 1276, 1008, 923 cm^{-1}; δ_H (200 MHz, DMSO): 12 (1H, s, CH*COOH* exch. with D_2O), 7.2 (1H, dd, *J* 15.27, 11.3 Hz, *CH*=CHCOOH), 6.7 (1H, dd, *J* 14.66, 10.69 Hz, *CH*=CHCH=CHCOOH), 6.3–6.0 (4H, m, *CH*), 5.9 (1H, dq, J_9 6.72, 13.13 Hz, *CH*CH_3), 5.8 (1H, d, *J* 15.27 Hz, =*CH*COOH), 1.8 (3H, d, *J* 6.72 Hz, CH_3); δ_C (53 MHz, DMSO): 168.4, 144.6, 141.2, 137.8, 133.4, 132.4, 130.4, 130.1, 122.2, 19.0; MS: MH^+, found 165. $C_{10}H_{12}O_2$ requires 164.

4.3.4. Compound **3d**[21]

Dark yellow solid; yield 20%; mp 260–262 °C.

4.3.5. Compound **3e**[29]

Dark orange solid; yield 10%; mp dec >200 °C; λ_{MeOH}: 379 nm; ν_{max} (KBr): 3012, 2924, 2852, 1712, 1621, 1589, 1447, 1384, 1368,

1300, 1274, 1248, 1227, 1138, 1008, 916 cm^{-1}; δ_H (200 MHz, DMSO): 12 (1H, s, CH*COOH* exch. with D_2O), 7.3 (1H, dd, *J* 15.03, 11.36, *CH*=CHCOOH), 6.6 (1H, dd, *J* 10.99, 14.66 Hz, *CH*=CHCH=CHCOOH), 6.3–6.0 (8H, m, *CH*), 5.9 (1H, d, *J* 15.03 Hz, =*CH*COOH), 5.8 (1H, dq, *J* 6.96, 14.15 Hz, *CH*CH_3), 1.8 (3H, d, *J* 6.96 Hz, *CH*$_3$); δ_C (53 MHz, DMSO): 167.8, 144.9, 141.2, 137.8, 136.2, 135.3–130.5, 129.8, 120.0, 18.7; MS: MH^+, found 217. $C_{14}H_{16}O_2$ requires 216.

4.3.6. Compound **3f**[30]

Dark orange solid; yield 10%; mp dec >200 °C; λ_{MeOH}: 397 nm; ν_{max} (KBr): 3011, 2925, 2853, 1709, 1621, 1434, 1374, 1304, 1260, 1238, 1137, 1007 cm^{-1}; δ_H (200 MHz, DMSO): 12 (1H, s, CH*COOH* exch. with D_2O), 7.3 (1H, dd, *J* 15.03, 10.99 Hz, *CH*=CHCOOH), 6.8–6.0 (11H, m, *CH*), 5.8 (1H, d, *J* 15.03 Hz, =*CH*COOH), 5.95–5.7 (1H, m, *CH*CH_3), 1.8 (3H, d, *J* 6.59 Hz, CH*CH*$_3$); MS: MH^+, found 243. $C_{16}H_{18}O_2$ requires 242.

4.4. Microorganism

A. aceti MIM2000/28 from our collection (MIM: Microbiologia Industriale Milano) was employed. Microorganism was routinely maintained on GYC solid medium (glucose 50 g/L, yeast extract 10 g/L, $CaCO_3$ 30 g/L, agar 12 g/L, pH 6.3, distilled water) at 28 °C. The strain grown on GYC slants for 24 h at 28 °C was inoculated into Erlenmeyer flask containing 50 mL of GLY medium (glycerol 25 g/L, yeast extract 10 g/L, pH 5, distilled water) on a reciprocal shaker (120 spm).

4.5. Biotransformation

According to substrate reactivity the culture rate of *A. aceti* MIM2000/28 employed was 200 mL for the biotransformation of compounds **a** and **b** and 600 mL for the other substrates.

The cultures were centrifuged (4000 rpm for 10 min) and suspended in 0.1 M phosphate buffers (100 mL) at pH 6.8. Polyunsaturated compounds dissolved in ethanol were added to the biotransformation system to give 1 mg/mL of substrate concentration and 10% of solvent.

Samples (250 μL) were taken at intervals, brought to pH 1 by addiction of 5% HCl, extracted with an equal volume of ethyl acetate and analysed by TLC (80% AcOEt/MeOH) and HPLC/DAD in the conditions as above described.

Acknowledgements

This work was supported by Fondo Interno Ricerca Scientifica e Tecnologica (FIRST), Università degli Studi di Milano.

References and notes

1. Bertelli, A.; Pini, E.; Stradi, R. Patent WO 03/95403 A1, 2003.
2. Pini, E.; Rossi, E.; Celentano, G.; Stradi, R. *Org. Prep. Proced. Int.* **2002**, *34*, 198.
3. Pini, E.; Nava, D.; Stradi, R. *Org. Prep. Proced. Int.* **2003**, *36*, 166.
4. Morelli, R.; Loscalzo, R.; Stradi, R.; Bertelli, A.; Falchi, M. *Drugs Exp. Clin. Res.* **2003**, *29*, 95.
5. Pini, E.; Bertelli, A.; Stradi, R.; Falchi, M. *Drugs Exp. Clin. Res.* **2004**, *30*, 203.
6. Tagliazucchi, D.; Ghelardoni, D.; Maltinti, S.; Ronca, G.; Conte, A.; Pini, E. *Pharmacologyonline* **2006**, *3*, 765.
7. Kuhn, R.; Grundmann, C. *Chem. Ber.* **1937**, *70*, 1318.
8. Blout, E. R.; Fields, M. *J. Am. Chem. Soc.* **1948**, *70*, 180.
9. Lee, D. G.; Brownridge, J. R. *J. Am. Chem. Soc.* **1973**, *95*, 3033.
10. Wiberg, K. B.; Deutsch, C. J. *J. Am. Chem. Soc.* **1973**, *95*, 3034.
11. Balkrishna, S. B.; Childers, W. E.; Pinnick, H. W. *Tetrahedron* **1981**, *37*, 2091.
12. Grieco, P. A.; Wang, C. L.; Burke, S. D. *J. Chem. Soc., Chem. Commun.* **1975**, 537.
13. Jones, G. *Org. React.* **1967**, *15*, 204.
14. Black, T. H.; Zhang, Y. *Synth. Commun.* **1995**, *25*, 15.
15. Arnel, B.; Maruoka, K.; Yamamoto, H. *Tetrahedron* **1995**, *51*, 4011.
16. Molinari, F.; Gandolfi, R.; Villa, R.; Urban, E.; Kiener, A. *Tetrahedron: Asymmetry* **2003**, *14*, 2041.
17. Romano, A.; Gandolfi, R.; Nitti, P.; Rollini, M.; Molinari, F. *J. Mol. Catal. B: Enzym.* **2002**, *17*, 235.
18. Mitsukura, K.; Sato, Y.; Yoshida, T.; Nagasawa, T. *Biotechnol. Lett.* **2004**, *26*, 1643.
19. Holland, H. L. *Organic Synthesis with Oxidative Enzyme*; Wiley-VCH: Weinheim, 1992.
20. Asai, T. *Acetic Acid Bacteria*; University of Tokyo: Tokyo, 1968.
21. Souto, A. A.; Acuna, A. U.; Armat-Guerri, F. *Tetrahedron Lett.* **1994**, *35*, 5907.
22. Molinari, F. *Curr. Org. Chem.* **2006**, *10*, 1247.
23. Chuitan, S.; Hartwing, J. *Angew. Chem.* **2004**, *43*, 4794.
24. Troast, D. M.; Porco, J. *Org. Lett.* **2002**, *4*, 991.
25. Steven, L. V.; Smith, S.; Woodward, P. *Tetrahedron* **2005**, *70*, 1154.
26. D'Amico, K.; Manos, C.; Christiensen, R. *J. Am. Chem. Soc.* **1980**, *102*, 1977.
27. Kuhn, R.; Grundmann, C. *Chem. Ber.* **1936**, *69*, 227.
28. Kuhn, R.; Hoffer, A. *Chem. Ber.* **1931**, *64*, 1977.
29. Kuhn, R.; Grundmann, C. *Chem. Ber.* **1937**, *70*, 1325.
30. Schmitt, J.; Obermeit, A. *Justus Liebigs Ann. Chem.* **1941**, *547*, 291.