CHIMIA 41 (1987) Nr. 9 (September)

Synthesis and NMR Spectral Data of some Esters Related to the Side Chains in Pluramycin Type Antibiotics**

Heinz Nadig and Urs Séquin*

Abstract: Simple methyl esters can serve as suitable model compounds in the structural elucidation of the side chains in pluramycin type antibiotics. The ^{1}H-NMR spectra of the model esters are in good agreement with those of the side chains of the corresponding antibiotics with respect to both, chemical shifts and coupling patterns, the former being consistently at lower fields for the natural products.

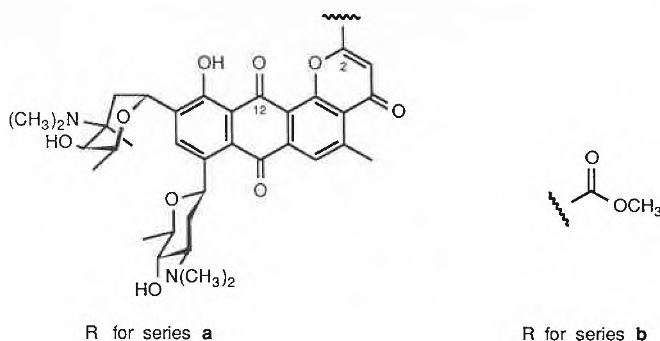
Antibiotics of the pluramycin type, such as kidamycin (**1a**), hedamycin (**6a**), and the like, are highly substituted 4*H*-anthra[1,2-*b*]pyran-4,7,12-triones. They differ primarily in the constitution of the side chain at C(2)[1]. Quite often, only very small amounts (less than 1 mg) of these substances can be isolated. Thus, ^{13}C-NMR spectra can normally not be taken, nor is chemical degradation or derivatization possible. Therefore, their constitutions are mostly determined by extensive ^{1}H-NMR spectroscopy. Modern NMR techniques such as the Fourier transform difference spectra method[2,3] or two-dimensional spectroscopy have helped a great deal in the detection and assignment of the signals of the side chain protons[4,5], which are quite often buried under other resonances.

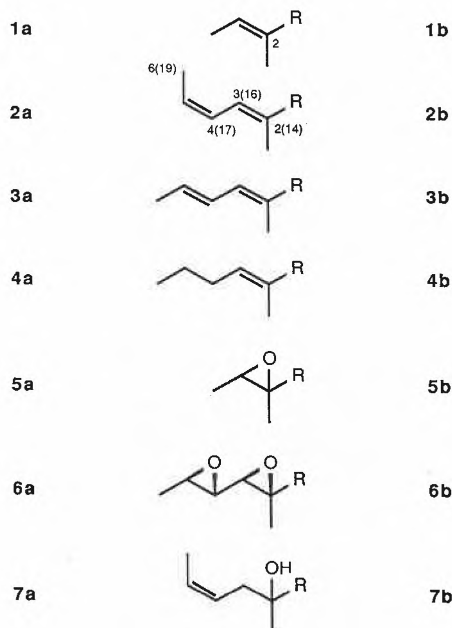
Earlier studies from our laboratory showed that simple methyl esters related to the side chains of the pluramycin antibiotics can serve as valuable model compounds helping the structural elucidation of new pluramycin-like natural products[6]. The diepoxy ester **6b**, e.g., was used for the assignment of the relative configurations in the side chain of hedamycin (**6a**)[7]. We now wish to present a compilation and discussion of the spectral differences between the pluramycin antibiotics **1a**–**7a** and the corresponding methyl esters **1b**–**7b**. The conclusions from this compilation should facilitate the structural elucidation of any new pluramycin antibiotics.

All of the model esters are known compounds with the exception of **7b**; however, not all of them were also characterized with an NMR spectrum. The models **3b**, **5b**, and **6b** have been synthesized earlier in our laboratory (cf. experimental part). Ester **2b** had been prepared by *Kim*[8] some years ago in a palladium-catalyzed reaction. We made the compound in a more conventional way starting with dibromoester **8** (Scheme 1). Treatment with KOH and subsequent reesterification with methanol and sulfuric acid gave the acetylenic ester **10**, which was then hydrogenated over Lindlar catalyst to give **2b**. Whereas *Cason* and *Kalm*[9] used a bromination-dehydrobromination process to get **4b**, we prepared this ester in analogy to **3b**[7] in a simple Reformatsky condensation with subsequent dehydration, which however resulted in the formation of some of the isomeric ester having the double bond in the 3-position. Compound **7b** was made starting with 1-bromo-2-butyne (**11**),

*Correspondence: Privatdozent Dr. U. Séquin
Institut für Organische Chemie
Universität Basel
St. Johanns-Ring 19, CH-4056 Basel

**Acknowledgement: Financial support of this work was provided by the Swiss National Science Foundation (grant No 2.629-0.82).





which was condensed with the anion of THP-protected methyl lactate (**12**, THP = tetrahydropyranyl). The resulting acetylenic ester **13** was deprotected with pyridinium *p*-toluenesulfonate (PPTS) and finally hydrogenated over Lindlar catalyst (Scheme 2).

The spectra of the esters **2b**–**4b** and of **7b** closely resemble those of the corresponding rubiflavins C-1 (**2a**), -C-2 (**3a**), -D (**4a**), and -E (**7a**) with respect to the relative positions of the resonances and to the coupling patterns (see Table 1). This is clearly a corroboration of the constitutions derived recently for the side chains of these antibiotics[3]. The data compiled in Table 1 show furthermore, that quite constant chemical shift differences are found between the resonances of the antibiotics and of the corresponding model esters. The resonances of the antibiotics are always at lower fields than those of the esters, which must be due to the magnetic anisotropy effect of the 4*H*-anthra[1,2-*b*]pyran-4,7,12-trione system. The downfield shift is more prominent in pairs of compounds which have a double bond conjugated to the pyrone ring of the antibiotics or to the carbonyl group of the esters, respectively. NOE experiments have shown that the side chain in anthrapyran derivatives of this type tends to be oriented towards the C(12) carbonyl group[3] and thus lies in the deshielding cone of this group. The deshielding is largest for H–C(16), where the chemical shift difference between antibiotic and ester is ca. 0.7–0.9 ppm. In compounds without this α,β-double bond the deshielding is reduced to ca. 0.2–0.4 ppm. In accord with the conformation mentioned for the compounds with the α,β-double bond, the deshielding of the methyl group at C(14) is smaller than in the compounds lacking the double bond. Small but rather constant chemical shift differences are observed for the resonances of the remaining protons (ca. 0.1–0.35 ppm in α,β-unsaturated and ca. 0 to 0.2 ppm in α,β-saturated compounds).

The fact, that the spectral differences between the antibiotic side chains and the model esters are either quite small or rather constant, makes that simple esters are indeed good models for the side chains in pluramycin antibiotics and can help structural determinations in this class of natural products.

Experimental Part

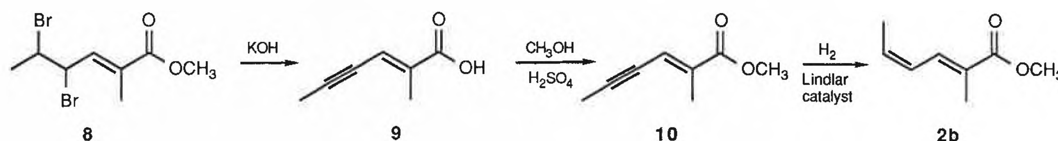
General remarks: Elemental analyses were carried out in the analytical laboratory of the Institut für Organische Chemie (*E. Thommen*), Universität Basel; NMR spectra were measured on a Bruker WH 90 at 90 MHz for ^{1}H and at 22.63 MHz for ^{13}C (*K. Aegerter*). Mass spectra (EI, 70 eV) were recorded on a AEI MS 30 (Dr. *J.-P. Stadelmann*, Institut für Physikalische Chemie der Universität Basel) or on a VG 70–250 in our institute. UV spectra were measured on a Beckmann Mod. 25, IR spectra on a Perkin-Elmer 1310. HPLC equipment: Spectra Physics pump and gradient mixer SP 8700, detector SP 8400, Knauer column LiChrosorb RP-18 (7 µm, 8 × 250 mm).

*Methyl (*E*)-2-methyl-2-butenoate* (**1b**) was obtained by esterification of tiglic acid (FLUKA) with methanol and sulfuric acid.

*(2*E*)-2-Methylhex-2-en-4-ynoic acid* (**9**): The dibromoester **8**[7] (5 g, 17 mmol) and KOH (6.5 g, 116 mmol) were suspended in water (70 mL) and refluxed for 5 h. After cooling, the reaction mixture was acidified with conc. HCl and extracted with ether (3 × 100 mL); the ether was removed in vacuo to yield **9** as a yellow solid (1.13 g, 58%). The raw-product was purified by sublimation to give **9** as a white solid (*m.p.* 120–123 °C after crystallization from hexane). – UV (EtOH): 255 (16270). – IR (KBr): 3400–2500, 2220, 1690, 1625, 1420, 1280, 1180, 1160, 990, 670. – ^{1}H-NMR ($CDCl_3$): 12.40 (s, 1H, COOH), 6.72 (m, 1H, H–C(3)), 2.07 (d, J = 2.5 Hz, 3H, H_3–C(6)), 2.03 (d, J = 0.7 Hz, 3H, H_3–C(7)). – ^{13}C-NMR ($CDCl_3$): 173.1 (s, C(1)), 137.1 (s, C(2)), 122.9 (d, C(3)), 100.7 and 76.8 (2s, C(4) and C(5)), 14.6 (q, C(7)), 4.8 (q, C(6)). – MS: 124 (M^+, 100), 107 (6.0), 96 (35), 95 (40), 81 (17), 79 (25), 78 (29), 77 (82), 53 (34), 51 (47), 43 (28), 39 (35).

*Methyl (2*E*)-2-methylhex-2-en-4-ynoate* (**10**): The acid **9** (3.8 g, 31 mmol), methanol (5 mL, 125 mmol) and a few drops of conc. H_2SO_4 were refluxed in CCl_4 (15 mL) for 8 h. After cooling, water (50 mL) was added and the solution extracted with CH_2Cl_2 (2 × 50 mL). The organic layers were washed with 10% aq. $NaHCO_3$ (2 × 100 mL) and sat. aq. NaCl (100 mL),

Scheme 1



Scheme 2

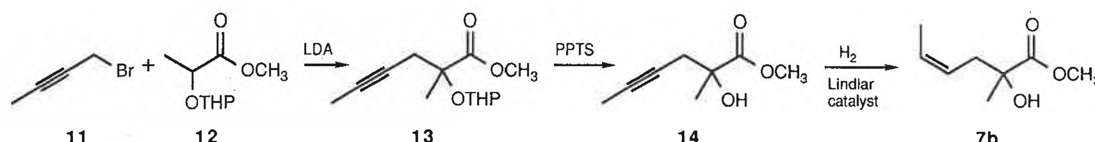


Table 1. Comparison of the ^{1}H-NMR Spectra of some Pluramycin Type Antibiotics with those of the Corresponding Esters[a].

Antibiotics: Methyl esters:	CH_3–C(14) CH_3–C(2)	H–C(16) H–C(3)	H–C(17) H–C(4)	H–C(18) H–C(5)	H–C(19) H–C(6)	CH_3O	Ref.
Kidamycin (**1a**)	2.00 s	7.49 br q (7)	2.04 d (7)				[14]
1b	1.82 s	6.82 br q (7.5)	1.76 d (7)			3.72 s	
$\Delta\delta$[b]	+0.18	+0.67	+0.28				
Rubiflavin C-1 (**2a**)	2.08 s	8.39 br d (11.5)	6.54 tq (11/2)	6.10 dq (10.5/7)	2.19		[3]
2b	1.92 br s	7.53 dq (11.6/1.3)	6.32 ddq (11.5/10.8/1.4)	5.90 dq (10.6/7)	1.88 br d (8)	3.76 s	
$\Delta\delta$	+0.16	+0.86	+0.22	+0.20	+0.31		
Rubiflavin C-2 (**3a**)	2.09 s	7.94 br d (11.5)	6.59 m (11.5/15)	6.39 m (15/7)	2.00 br d (7)		[3]
3b	1.89 s	7.11 dq (10.9/1.5)	6.37 dd (10.9/16)	6.03 dq (16/6.2)	1.86 d (6.2)	3.69 s	[7]
$\Delta\delta$	+0.20	+0.83	+0.22	+0.36	+0.14		
Rubiflavin D (**4a**)	2.01 s	7.46 br t (7.5)	2.38 m (7.5)	1.65 m (7.5)	1.08 t (7.5)		[3]
4b	1.87 d (1.5)	6.77 tq (7.3/1.8)	2.20 q (7.2)	1.50 m (7)	0.97 t (7.3)	3.75 s	
$\Delta\delta$	+0.14	+0.69	+0.18	+0.15	+0.11		
Epoxykidamycin (**5a**)	1.83 s	3.46 q	1.54 d				[5]
5b	1.46 s	3.26 q (5.5)	1.32 d (5.6)			3.69 s	
$\Delta\delta$	+0.37	+0.20	+0.22				
Hedamycin (**6a**)	1.96 s	3.32 d (6)	2.89 dd (4.7/2.1)	3.11 dq (2.2/5)	1.44 d (5.3)		[14]
6b	1.65 s	3.10 d (5.3)	2.69 dd (5.3/2.3)	3.01 dq (2.3/5.3)	1.37 d (5.3)	3.75 s	[7]
$\Delta\delta$	+0.31	+0.22	+0.20	+0.10	+0.07		
Rubiflavin E (**7a**)	1.70 s	2.84 m (14)	5.38 m (10.8/14)	5.74 m (10.8/6.8)	1.65 d (6.8)		[3]
7b	1.42 s	2.47 m	5.37 dtq (11/6.8/1.5)[c]	5.67 dtq (11/6/1.1)[c]	1.62 br d (6)	3.76 s	
$\Delta\delta$	+0.28	+0.37	+0.01	+0.07	+0.03		

[a] Chemical shifts in ppm downfield from TMS. In parentheses: coupling constants in Hz. Solvent: $CDCl_3$.
[b] $\Delta\delta = \delta_{Antibiotic} - \delta_{Ester}$.
[c] Coupling constants determined from decoupling experiments.

then dried (Na_2SO_4). After removal of the solvent, the raw-product was distilled in vacuo to give 1.77 g of **10** (41%, *b.p.* 80–83°C/16 mbar). – UV (EtOH): 258 (14600). – IR (Film): 2950, 2920, 2210, 1710, 1610, 1430, 1355, 1260, 1120, 740. – ^{1}H-NMR ($CDCl_3$): 6.57 (m, 1H, H–C(3)), 3.77 (s, 3H, OCH_3), 2.07 (s and d, 6H, H_3–C(6) and H_3–C(7)). – ^{13}C-NMR ($CDCl_3$): 167.8 (s, C(1)), 137.7 (s, C(2)), 120.6 (d, C(3)), 99.2 and 76.8 (2s, C(4) and C(5)), 51.9 (q, OCH_3), 15.0 (q, C(7)), 4.7 (q, C(6)). – MS: 138 (M^+, 82), 123 (18), 110 (29), 107 (55), 95 (16), 79 (60), 77 (100), 74 (24), 67 (15), 59 (38), 51 (28), 44 (45).

*Methyl (2*E*, 4*Z*)-2-methyl-2,4-hexadienoate* (**2b**): The ester **10** (300 mg, 2.2 mmol) was dissolved in hexane (10 mL), Lindlar catalyst (50 mg) was added, and the solution shaken at room-temperature under an atmosphere of H_2. After 3 h, the addition of H_2 was complete; the catalyst was filtered off and the solvent was removed in vacuo to give 293 mg of **2b** (96%, *b.p.* 65–70°C/16 mbar). – UV (EtOH): 266 (22000). – IR (Film): 2950, 1710, 1440, 1270, 1250, 1100, 720. – ^{1}H-NMR ($CDCl_3$): see Table 1. – ^{13}C-NMR ($CDCl_3$): 169.1 (s, C(1)), 126.8 (s, C(2)), 133.9 and 132.8 and 124.9 (3s, C(3) and C(4) and C(5)), 51.7 (q, OCH_3), 13.8 (q, C(6)), 12.4 (q, C(7)). – MS: 140 (M^+, 53), 125 (100), 109 (41), 97 (25), 95 (26), 91 (25), 81 (76), 79 (69).

*Methyl (2*E*, 4*E*)-2-methyl-2,4-hexadienoate* (**3b**) was prepared as described[7].

*Methyl (2*E*)-2-methyl-2-hexenoate* (**4b**): Zinc (50 g, 0.76 mol) was activated with 2N HCl for 5 min, then washed with water, acetone and toluene. It was then suspended in dry toluene (250 mL). A mixture of butanal (46 g, 0.64 mol) and methyl 2-bromopropionate (96 g, 0.57 mol) was added dropwise. After addition of ca. ¼ of the mixture and gentle heating, the reaction started. Addition was carried on at a rate to maintain boiling. The reaction mixture was refluxed for additional 30 min and then cooled with ice. After addition of 2N H_2SO_4 (250 mL) the mixture was stirred for 30 min. The two phases were separated and the organic layer was washed with 2N H_2SO_4 (200 mL), 10% aq. $KHCO_3$ (200 mL), and water (2 × 200 mL), then dried (Na_2SO_4). The solvent was removed in vacuo to give 60.5 g (66%) of crude methyl 3-hydroxy-2-methylhexanoate as a yellow oil.

19 g (0.12 mol) of the raw product and 20 g of P_2O_5 (adsorbed on an inert support, Fluka Nr. 79610) in toluene (125 mL) were refluxed for 90 min. After cooling, the pH was adjusted to 8 with 2N NaOH. The two phases were separated, the organic layer washed with 10% aq. $KHCO_3$ (100 mL), sat. aq. NaCl (100 mL), and water (100 mL), then dried (Na_2SO_4). The solvent was removed in vacuo and the raw-product distilled to give 3 g of a 1:2-mixture (according to GC) of **4b** and methyl (3*E*)-2-methyl-3-hexenoate (18%, *b.p.* 70–76°C/15 mbar).

A sample of this mixture was separated by HPLC on a Knauer RP-18-column with CH_3OH/water (8:2) as solvent. The products were isolated from the eluates by addition of aq. NaCl and extraction with CH_2Cl_2. In this way, 120 mg of **4b** and 86 mg of its isomer could be isolated. Data of **4b**: ^{1}H-NMR ($CDCl_3$): see Table 1. – ^{13}C-NMR ($CDCl_3$): 168.7 (C(1)), 142.4 (C(3)), 127.8 (C(2)), 51.6 (OCH_3), 30.8 (C(4)), 22.0 (C(5)), 13.8 (C(6)), 12.3 (C(7)). – MS: 142 (M^+, 17), 127 (11), 111 (17), 101 (19), 55 (87), 43 (100).

Data of *methyl (3*E*)-2-methyl-3-hexenoate* (cf.[10]): ^{1}H-NMR ($CDCl_3$): 5.45 (m, 2H, H–C(4) and H–C(5)), 3.70 (s, OCH_3), 3.12 (m, 1H, H–C(2)), 2.03 (m, 2H, H_2–C(5)), 1.28 (d, J = 6.9 Hz, 3H, H_3–C(7)), 1.02 (t, J = 7.2 Hz, 3H, H_3–C(6)). – ^{13}C-NMR ($CDCl_3$): 175.5 (C(1)), 133.8 (C(3)), 128.0 (C(4)), 51.7 (OCH_3), 42.8 (C(2)), 25.5 (C(5)), 17.5 (C(7)), 13.5 (C(6)). – MS: 142 (M^+, 4.0), 127 (3.7), 113 (1.8), 111 (2.2), 88 (22), 83 (36), 82 (15), 67 (19), 55 (100), 41 (59).

*Methyl (2*RS*, 3*SR*)-2,3-epoxy-2-methylbutanoate* (**5b**) was prepared as described[11].

*Methyl (2*RS*, 3*SR*, 4*RS*, 5*RS*)-2,3:4,5-diepoxy-2-methylhexanoate* (**6b**) was prepared as described[7].

Methyl 2-hydroxy-2-methyl-4-hexynoate (**14**): To a solution of diisopropylamine (2.8 mL, 20 mmol) in tetrahydrofuran (THF, 5 mL), BuLi (8.2 mL of a 1.6 M solution in hexane) was added at 0°C. After stirring for 10 min, the solution was cooled to −70°C. A solution of THP-protected methyl lactate (**12**, 2.39 g, 12.7 mmol, prepared in analogy to[12]) in 10 mL of hexane/THF (1:1) was added dropwise and the resulting mixture stirred for 30 min at −70°C and for another 30 min at 0°C. Then the reaction mixture was cooled again to −70°C and 1-bromo-2-butyne (**11**, 1.7 g, 12.7 mmol, prepared according to[13] from 2-butyne-1-ol obtained from EGA-Chemie) in 10 mL of hexane/THF (1:1) was added dropwise. The solution was stirred for 3 h, during which the temperature slowly rose to room temperature. Then the reaction was quenched with 0.5 N HCl (50 mL) and the product extracted with ether (2 × 50 mL). The combined etheral layers were washed with 10% aq. $KHCO_3$ (50 mL) and sat. aq. NaCl (2 × 50 mL), dried (Na_2SO_4), and evaporated to give the THP-protected ester **13** (2.84 g, 93%) as a yellow oil. The raw product was purified by column chromatography (silica gel, CH_2Cl_2/0–10% CH_3OH), yielding 1.7 g of nearly pure product.

Removal of the protecting group was effected by dissolving **13** (830 mg, 3.5 mmol) and PPTS (80 mg, 0.3 mmol) in EtOH (20 mL) and stirring for 6 h. Then 10% aq. $KHCO_3$ (20 mL) was added and the product extracted with ether (3 × 20 mL). The etheral layers were washed with sat. aq. NaCl (2 × 20 mL), dried (Na_2SO_4), and evaporated. Purification on a silica gel column with CH_2Cl_2 as eluent gave pure **14** (120 mg). – UV (EtOH): 229 (87). – IR (Film): 3500, 3000, 2960, 2920, 2230, 1740, 1450, 1370, 1280, 1220, 1120. – ^{1}H-NMR ($CDCl_3$): 3.80 (s, 3H, OCH_3), 3.43 (br. s, 1H, OH), 2.56 (q, J = 2.6 Hz, 2H, H_2–C(3)), 1.78 (t, J = 2.6 Hz, 3H, H_3–C(6)), 1.45 (s, 3H, H_3–C(7)). – ^{13}C-NMR ($CDCl_3$): 175.9 (s, C(1)), 79.0 (s, C(4)*), 74.1 (s, C(2)), 73.6 (s, C(5)*), 52.6 (q, OCH_3), 31.5 (t, C(3)), 28.4 (q, C(7)), 3.3 (q, C(6)). – MS: 156 (M^+, 25), 139 (5), 124 (8), 103 (35), 97 (35), 85 (6), 75 (5), 69 (4), 59 (4), 54 (14), 43 (100).

*Methyl (4*Z*)-2-hydroxy-2-methyl-4-hexenoate* (**7b**): The ester **14** (120 mg) was dissolved in ethyl acetate (10 mL), and Lindlar catalyst (100 mg) was added. The solution was shaken at room temperature under an atmosphere of H_2 for 90 min. Then the catalyst was removed by filtration and the solvent evaporated to give **7b** (100 mg, 82%). – UV (EtOH): 227 (115). – IR (Film): 3500, 3030, 2980, 2960, 1740, 1450, 1380, 1260, 1220, 1160, 1140, 1080, 990, 760. – ^{1}H-NMR ($CDCl_3$): see Table 1. – ^{13}C-NMR ($CDCl_3$): 177.2 (s, C(1)), 127.9 (d, C(4)*), 123.9 (d, C(5)*), 74.9 (s, C(2)), 52.5 (q, OCH_3), 37.9 (t, C(3)), 25.5 (q, C(7)), 12.9 (q, C(6)). – MS: 158 (M^+), 141, 140, 125, 115, 109, 104, 103, 101, 100, 99, 89, 85, 83, 81, 75, 57, 43, 41, 39.

Received: June 30, 1987 [FC 114]

[1] U. Séquin, *Prog. Chem. Org. Nat. Prod. 50* (1986) 57.
[2] G. Massiot, S.K. Kan, P. Gonord, C. Duret *J. Am. Chem. Soc. 97* (1975) 3277.
[3] H. Nadig, U. Séquin, *Helv. Chim. Acta 70* (1987) 1217.
[4] S.K. Gonda, K.M. Byrne, P.K. Herber, Y. Tondeur, D. Liberato, B.D. Hilton, *J. Antibiot. 37* (1984) 1344.
[5] K.M. Byrne, S.K. Gonda, B.D. Hilton, *J. Antibiot. 38* (1985) 1040.
[6] U. Séquin, M. Ceroni, *Helv. Chim. Acta 61* (1978) 2241.
[7] M. Ceroni, U. Séquin, *Helv. Chim. Acta 65* (1982) 302.
[8] J.I. Kim, *Taehan Hwahakoe Chi 27* (1983) 441 [cf. *Chem. Abstr. 100* (1984) 156199r].
[9] J. Cason, M.J. Kalm, *J. Org. Chem. 19* (1954) 1947.
[10] P.E. Pfeffer, L.S. Silbert, *J. Org. Chem. 36* (1971) 3290.
[11] U. Séquin, *Tetrahedron Lett. 20* (1979) 1833.
[12] M. Miyashita, A. Yoshikoshi, P.A. Grieco, *J. Org. Chem. 42* (1977) 3772.
[13] P.J. Ashworth, G.H. Whitham, M.C. Whiting, *J. Chem. Soc.* (1957) 4633.
[14] U. Séquin, *Tetrahedron 34* (1978) 761.