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Structure-Activity Relationships for Unsaturated Dialdehydes 12.[1] The Reactivity of Unsaturated Dialdehydes Towards Triacetic Acid Lactone

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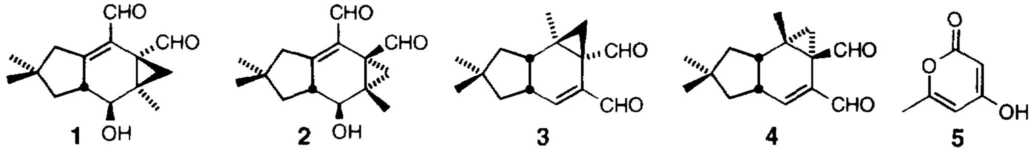
***Abstract*:.** The fungal antibiotics merulidial (**1**) and isovelleral (**3**) react stereoselectively with the natural triketide triacetic acid lactone (**5**), forming pentacyclic pyranone adducts. In buffered water solutions, the reaction rates of merulidial (**1**) and its less active isomer **2** are strongly correlated with the antibiotic activity of the two, suggesting that their electrophilicity as Michael acceptors is important for this activity. The adduct **7**, formed between merulidial and triacetic acid lactone, is identical to a compound that previously was isolated from the culture filtrate of the fungus *Merulius tremellosus*.

Naturally occurring terpenoids with an unsaturated dialdehyde functionality, for example merulidial (**1**) and isovelleral (**3**), have been isolated from various organisms such as higher plants,[2,3] fungi[4,5] and molluscs.[6] Most of the unsaturated dialdehydes possess potent bioactivities, they are for instance antibiotic[7,8] mutagenic[9,10] and hot tasting,[11,12] and some have been suggested to be natural defence compounds.[5,6]

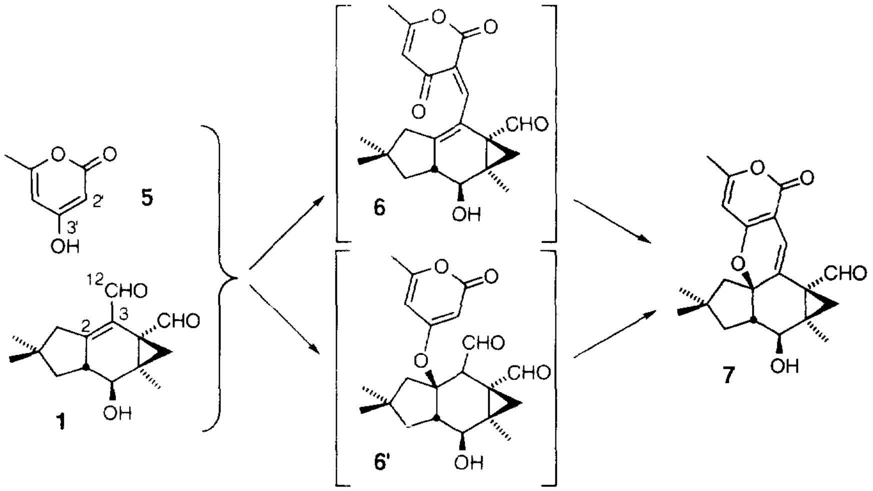


1 **2** **3** **4** **5**

Figure 1.

Several studies have demonstrated that their biological activities are linked to the unsaturated dialdehyde functionality,[9,13] the α,β-unsaturated aldehyde has been suggested to react as a Michael acceptor with for instance thiol groups of proteins[11] while the 1,4-dialdehyde moiety may form pyrrole derivatives with primary amino groups.[14] However, small structural changes may modulate the biological activities considerably, and the absolute stereochemistry of the unsaturated dialdehydes as well as their ability to form bioactive autoxidation products during a bioassay has been suggested to be of importance.[15] Recently, we isolated the pentacyclic aldehyde **7** together with merulidial (**1**) from extracts of the culture filtrate of the fungus *Merulius tremellosus*.[16] Theoretically, compound **7** might be formed by the reaction between merulidial (**1**) and the triketide triacetic acid lactone (**5**), which is a natural product produced by *Penicillium* and *Pseudomonas* strains.[17-19] In order to investigate this, and to relate the reactivity of unsaturated dialdehydes with the potency of their biological activities, we have compared the reactivity of four apparently similar unsaturated dialdehydes as Michael acceptors towards the triketide **5** under biomimetic conditions.

When merulidial (1) was stirred at room temperature with compound 5 in a buffered water solution at pH 5.6, in order to simulate the conditions during the fermentation,[16,20] the pyranone adduct 7 was formed slowly. In refluxing EtOAc, compound 7 was formed quantitatively in 24 hours, and as the previously isolated material is identical in all respects with the adduct formed in EtOAc its absolute configuration is (5aR,6S,7S,8R,9R) (as shown in Scheme 1, see Figure 2 for numbering). NMR and TLC analyses of daily samples of on-going fermentations of *Merulius tremellosus* showed that triacetic acid lactone (5) is in fact formed by the fungus, and the conclusion is that the adduct 7 isolated from the fungus[16] is formed chemically during the fermentations. Previously, compound 5 has only been indicated in minor amounts in *Penicillium patulum*,[18] during ethionine inhibition of the tropolone biosynthesis in *Penicillium stipitatum*[17] and when an organism, thought to be a *Pseudomonas*, was allowed to metabolize dehydroacetic acid.[19] The fungus *Merulius tremellosus* is therefore the first reported organism to produce significant amounts of triacetic acid lactone (5) under normal fermentation conditions.



Scheme 1. Possible intermediates in the formation of adduct **7** from **1** and **5**.

The reaction between merulidial (**1**) and lactone **5** may proceed by two principal routes, as shown in Scheme 1. Attack by C-2' on C-12 would, after loss of water, give the hypothetical intermediate **6** which can be transformed to **7** by an electrocyclic ring-closure. Alternatively, Michael addition of 3'-OH on C-2 would give the hypothetical intermediate **6'**, which also is a reasonable intermediate to **7**. Triacetic acid lactone (**5**) has previously been reported to react with aliphatic α,β-unsaturated aldehydes,[21] although the major product is formed by Michael addition of C-2' to the β-carbon and the product corresponding to **7** was only obtained as a minor product when **5** was reacted with crotonaldehyde.

The dialdehydes **2**, **3** and **4** also reacted with lactone **5** in buffered water solution at pH 5.6, although the reaction rates were considerably lower compared to merulidial (**1**), and the adducts **8**, **9** and **10** were formed as the only products. Preparative amounts were obtained in refluxing EtOAc, the structures were determinated by spectroscopy (see Table 1 for NMR data) and it was found that the lactone **5** reacts in the same way with all four dialdehydes. The relative stereochemistry of the terpenoid portion of compound **8**, **9** and **10** was determined by NOESY correlations: For compound **8**, NOESY correlations were observed between 18-H_α and 16-H_α, and between 6-H and 12-H_β as well as 16-H_β, indicating that 12-H_β and 16-H_β are on the same side as 6-H. The ^1H NMR shift difference between 12-H_β (δ 2.29) and 12-H_α (δ 1.41) suggests that 5a-O is β-oriented. This was confirmed by the NOESY correlations observed between 4-H and 6-H, 15-H_3 and 12-H_β. For compound **9**, NOESY correlations were observed between 18-H_β and 7-H, and between 7-H and 15-H_3, establishing that 15-H_3 is β and 14-H_3 α. NOESY correlations were also observed between 5a-H and 19-H, 14-H_3, 12-H_α as well as 16-H_α but not 18-H_β or 15-H_3 or 12-H_β, establishing that 5a-H has α-orientation. For compound **10**, NOESY correlations were observed between 5a-H and 18-H_α, 12-H_α, 16-H_α but not 19-H or 12-H_β or 16-H_β or 7-H, establishing that 5a-H has α-orientation.

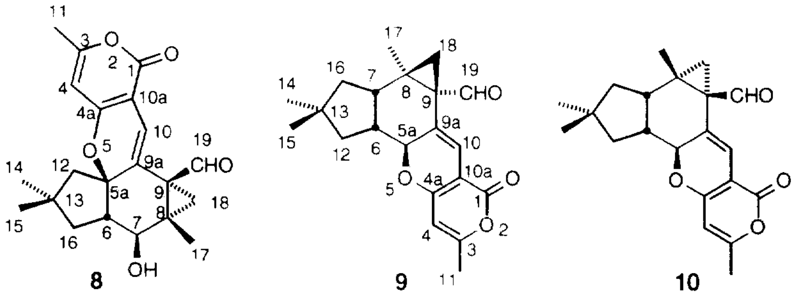


Figure 2

Table 1. ^{1}H NMR spectral data for compound **8**, **9** and **10**. The spectra were recorded in $CDCl_3$ at 500 MHz, and the coupling constants *J* are given in Hz. See Figure 2 for atom numbers.

H		**8**			**9**			**10**		
		δ		*J*	δ		*J*	δ		*J*
4	H	5.83	(*bs*)		5.82	(*bs*)		5.72	(*bs*)	
5a	H_α				4.77	(*dd*)	$J_{5a\alpha\text{-}10}=2.6$ $J_{5a\alpha\text{-}6\beta}=11.3$	4.76	(*dd*)	$J_{5a\alpha\text{-}10}=1.8$ $J_{5a\alpha\text{-}6\beta}=10.3$
6	H_β	2.99	(*m*)		2.17	(*m*)		2.59	(*m*)	
7	H_α	4.03	(*d*)	$J_{6\beta-7\alpha}=2.1$						
7	H_β				2.63	(*m*)		2.77	(*m*)	
10	H	6.68	(*s*)		6.32	(*d*)	$J_{5a\alpha\text{-}10}=2.6$	6.60	(*d*)	$J_{5a\alpha\text{-}10}=1.8$
11	H_3	2.23	(*bs*)		2.21	(*bs*)		2.20	(*bs*)	
12	H_α	1.41	(*d*)	$J_{12\alpha-12\beta}=15.3$	1.85	(*dm*)		1.35	(*m*)	
12	H_β	2.29	(*bd*)		1.68	(*dd*)	$J_{6\beta\text{-}12\beta}=7.0$ $J_{12\alpha\text{-}12\beta}=14.2$	1.91	(*ddd*)	$J_{6\beta\text{-}12\beta}=7.9$ $J_{12\alpha\text{-}12\beta}=13.1$ $J_{12\beta\text{-}16\beta}=1.8$
14	H_3	1.04	(*s*)		1.14	(*s*)		1.11	(*s*)	
15	H_3	1.16	(*s*)		1.07	(*s*)		0.99	(*s*)	
16	H_α	1.54	(*dd*)		1.36	(*m*)		1.29	(*m*)	
16	H_β	1.92	(*ddd*)	$J_{16\alpha-16\beta}=13.3$ $J_{6\beta-16\beta}=9.1$ $J_{12\beta-16\beta}=2.0$	1.73	(*dd*)	$J_{16\alpha\text{-}16\beta}=12.7$ $J_{7\beta\text{-}16\beta}=6.5$	1.83	(*ddd*)	$J_{12\beta\text{-}16\beta}=1.8$ $J_{7\beta\text{-}16\beta}=7.0$ $J_{16\alpha\text{-}16\beta}=12.3$
17	H_3	1.24	(*s*)		1.10	(*s*)		1.14	(*bs*)	
18	H_α	1.46	(*d*)	$J_{18\alpha-18\beta}=5.9$	1.82	(*d*)		1.26	(*d*)	$J_{18\alpha\text{-}18\beta}=5.6$
	H_β	1.82	(*d*)	$J_{18\alpha-18\beta}=5.9$	1.16	(*d*)	$J_{18\alpha\text{-}18\beta}=4.3$	1.55	(*dd*)	$J_{18\alpha\text{-}18\beta}=5.6$ $J_{17\text{-}18\beta}=0.8$
19	H	10.05	(*s*)		9.72	(*s*)		10.02	(*s*)	

The pyranone adducts **7-10** are formed as single stereoisomers, and compounds **7** and **8** as well as compounds **9** and **10** are diastereomers, with the same stereochemistry at C-5a but with an inverted cyclopropane ring. It is reasonable to believe that steric hindrance on the α-side of the dialdehydes makes 3'-OH of lactone **5** attack the other side of the double bond. As the biological activity of a given pair of stereoisomers of an unsaturated dialdehyde often differs considerably, the stereoselective control of 1,4-additions of nucleophiles may influence their chemical reactivity. We therefore decided to use the reaction between triacetic acid lactone (**5**) and some unsaturated dialdehydes as a model system, in order to investigate how small changes in structure might influence the chemical reactivity of these substances. The choice of the nucleophile is further motivated by the fact that it is a bi-functional nucleophile that to some extent resembles for instance cytosine.

The reactivity of **5** towards the two fungal metabolites merulidial (**1**) and isovelleral (**3**), both exhibiting antibiotic and mutagenic activities,[9,10] was therefore compared with its reactivity towards the less active isomers **2** and **4**. The reactions were carried out under biomimetic conditions at 37°C, in buffered water solutions at pH 4.0, 5.6, 7.4, and 9.0, with 5 % acetonitrile as cosolvent for the dialdehydes. Large excess (20 equivalents) triacetic acid lactone (**5**) was used to obtain pseudo-first order kinetics. In order to obtain data from the spontaneous degradation of the dialdehydes by autoxidation, which at neutral and basic pH is significant,[15] test runs without the lactone **5** were also run. Samples were taken at intervals, and the remaining amounts of dialdehyde were determined by HPLC. The disappearance of each dialdehyde due to the presence of compound **5** was obtained by subtraction of the disappearance in the negative control, and the half-lifes in hours of the dialdehydes are presented in Table 2.

Table 2. The half-lifes in hours of the dialdehydes **1**, **2**, **3** and **4** due to their reaction with compound **5**.

Dialdehyde	pH 4.0	pH 5.6	pH 7.4
1	30	140	> 4000
2	660	3300	> 4000
3	630	1100	> 4000
4	1200	1700	> 4000

Acidic conditions catalyze the reaction. This was also observed during our attempts to prepare the pyranone **7**, but preparatively, at increased temperature, it led to problems with by-products. At pH 7.4 and 9.0, the dialdehydes are actually autoxidised more rapidly than they react with compound **5**, although the autoxidation seems to be somewhat inhibited in presence of compound **5** at pH 9.0. The half-life at pH 9.0 has therefore not been calculated, and for practical reasons the reactivity of the dialdehydes can only been compared at pH 4.0 and pH 5.6. The difference between merulidial (**1**) and its isomer **2** is dramatic, the former being more than 20 times more reactive. On the other hand, isovelleral (**3**) is only slightly more reactive (50 - 100 %) then its less bioactive isomer **4**.

Interestingly, these results are in one way in accordance with the known bioactivities of the compounds, as merulidial (**1**) is much more active (antimicrobial, cytotoxic, and mutagenic activity) compared to its isomer **2** while isovelleral (**3**) is only slightly more active than its isomer **4**.[7,9,10,15] On the other hand, if we compare merulidial (**1**) with isovelleral (**3**), the former is more reactive (approximately 10 times) in this investigation while the latter possesses higher (at least 10 times) biological activities. The reactivity of the potent isovelleral (**3**) as a Michael acceptor towards lactone **5** is comparable to that of the merulidial derivative **2**, possessing quite weak bioactivities, indicating that the bioactivities of isovelleral (**3**) mainly depend on other chemical interactions. It is possible that the reactivity studied here is more important for the biological activity of certain types of dialdehydes, for instance isolactaranes like merulidial (**1**), but it is evident that other reactions of the unsaturated dialdehyde functionality, for instance autoxidations generating new bioactive compounds,[15] also may be important.

EXPERIMENTAL

General Procedures: TLC analyses were made on "Merck DC-Alufolien Kieselgel 60 F_{254}" SiO_2 plates, visualised by spraying with anisaldehyde/sulphuric acid and warming to 120°C. The EIMS spectrum (direct inlet, 70 eV) was recorded with a JEOL SX102 spectrometer, and the NMR spectra (in $CDCl_3$) with a Bruker ARX 500 spectrometer at 500 MHz (^1H, data given in Table 1) and 125 MHz (^{13}C, data given below). The chemical shifts are reported in ppm with the solvent signals (δ_H=7.26 and δ_C=77.0) as reference. The IR spectrum was recorded with a Perkin-Elmer 298 spectrometer, and the UV spectrum with a Varian Cary 219 spectrometer. The melting points (uncorrected) were determined with a Reichert microscope, and the optical rotations were measured with a Perkin-Elmer 141 polarimeter at 22°C. HPLC analyses were performed using an Altex 110A pump, a Merck 50943 LiChroCART 125-4 LiChrospher 100 RP-18 (particle size, 5μm) column, a variable wavelength absorbance detector LDC SpectroMonitor III, a Hewlett Packard HP 3396A integrator, and MeOH-H_2O mixtures (40-70 %) as elution solvent with a flow rate of 1 mL/min. The sesquiterpene dialdehydes were detected at 254 nm. Merulidial (**1**) was isolated from submerged cultures of the fungus *Merulius tremellosus* [20] and isovelleral (**3**) from fruit bodies of *Lactarius vellereus*,[5] while the isomers **2**[15] and **4**[22] were prepared as described previously. Triacetic acid lactone (**5**) was prepared from triacetic acid, according to Collie.[23]

(5aR,6S,7S,8R,9R)-9-Formyl-7-hydroxy-3,8-dimethyl-1-oxo-5a,6-(2,2-dimethyl-propano)-8,9-methano-5a,6,7,8,9-pentahydro-2,5-dioxaanthracene (7). Merulidial (**1**) (24.2 mg, 0.098 mmol) and triacetic acid lactone (**5**) (23.9 mg, 0.190 mmol) was refluxed in ethyl acetate (5 mL). The reaction was complete in 24 hours (according to TLC analysis), whereafter the solvent was removed under reduced pressure. The adduct was purified by silica gel chromatography in two steps (EtOAc followed by CH_2Cl_2-EtOAc), yielding **7** (30.4 mg, 89%) as colourless crystals (heptane-EtOAc). Its spectral data was identical in all respects to those previous published.[16]

(5aR,6S,7S,8S,9S)-9-Formyl-7-hydroxy-3,8-dimethyl-1-oxo-5a,6-(2,2-dimethyl-propano)-8,9-methano-5a,6,7,8,9-pentahydro-2,5-dioxaanthracene (8). Compound **8** was prepared by refluxing the merulidial isomer **2** (32 mg, 0.129 mmol) and triacetic acid lactone (**5**) (32 mg, 0.258 mmol) in ethyl acetate (6 mL). The substrate **2** was not completely consumed after 92 hours (according to TLC analysis), when the reaction was stopped. The solvent was removed under reduced pressure, and the adduct was purified by silica gel chromatography in three steps (EtOAc followed by CH_2Cl_2-EtOAc and Toluene-MTBE), recovering **2** (9 mg) and yielding **8** (yellowish solid, 12 mg, 26 %, but 36% in respect to consumed **2**). $[\alpha]_D$ -35° (*c* 0.51, $CHCl_3$). MS [*m/z* (% rel. int.)]: 356.1611 (M^+, 100 %, calculated for $C_{21}H_{24}O_5$ 356.1624), 340 (36), 327 (33), 311 (26), 272 (31), 254 (29), 217 (39), 149 (26), 139 (33), 84 (52), 69 (39). UV (EtOH) λ_{max} (ε): 350 nm (5400), 252 nm (6700). IR ($CHCl_3$): 3430, 2950, 2920, 2860, 1720, 1705, 1640, 1615, 1565, 1445, 1410, 1365, 1275, 1150 and 1000 cm^{-1}. ^1H NMR: see Table 1. ^{13}C NMR: 197.3, C-19; 163.1, C-1; 162.2, C-3; 162.0, C-4a; 127.9, C-9a; 112.8, C-10; 102.1, C-10a; 100.0, C-4; 87.2, C-5a; 71.6, C-7; 53.5, C-6; 52.1, C-12; 45.0, C-16; 39.5, C-9; 38.3, C-13; 36.5, C-8; 29.7, C-14; 27.9, C-15; 23.4, C-18; 20.2, C-11; 18.2, C-17.

(5aS,6R,7R,8R,9S)-9-Formyl-3,8-dimethyl-1-oxo-6,7-(2,2-dimethyl-propano)-8,9-methano-5a,6,7,8,9-pentahydro-2,5-dioxaanthracene (9). Compound **9** was prepared by refluxing isovelleral (**3**) (54 mg, 0.233 mmol) and triacetic acid lactone (**5**) (58 mg, 0.460 mmol) in ethyl acetate (10 mL). The reaction was stopped after 115 hours although isovelleral (**3**) was not completely consumed. The solvent was removed under reduced pressure, and the adduct was purified by silica gel chromatography in three steps (EtOAc followed by CH_2Cl_2-EtOAc and Toluene-MeOH), recovering **3** (7 mg) and yielding **9** (26 mg, 33 %, but 35 % in respect to consumed **3**). Yellowish crystals m.p. 183-184 °C (heptane); $[\alpha]_D$ +116° (*c* 1.00, $CHCl_3$). MS [*m/z* (% rel. int.)]: 340.1672 (M^+, 94 %, calculated for $C_{21}H_{24}O_4$ 340.1674), 311 (55), 256 (30), 244 (14), 238 (17), 217 (100), 189 (14), 173 (16), 139 (20), 86 (20), 84 (30). UV (EtOH) λ_{max} (ε): 351 nm (7400), 250 nm (9600). IR ($CHCl_3$): 3010, 2950, 2920, 2860, 1715, 1705, 1640, 1630, 1560, 1450, 1420, 1365, 1260, 1150, 1030, 1020 and 1000 cm^{-1}. ^1H NMR: see Table 1. ^{13}C NMR: 197.8, C-19; 164.4, C-4a; 162.4, C-3; 161.9, C-1; 127.6, C-9a; 116.9, C-10; 100.3, C-10a; 99.6, C-4; 76.8, C-5a; 44.6 and 44.5, C-7 and C-16; 43.4, C-12; 42.0, C-6; 40.8, C-9; 36.9, C-13; 34.6, C-8; 32.6, C-14; 32.6, C-15; 28.3, C-18; 20.2, C-11; 18.3, C-17.

(5aS,6R,7R,8S,9R)-9-Formyl-3,8-dimethyl-1-oxo-6,7-(2,2-dimethyl-propano)-8,9-methano-5a,6,7,8,9-pentahydro-2,5-dioxaanthracene (10). Compound **10** was prepared by refluxing the isovelleral isomer (**4**) (63 mg, 0.272 mmol) and triacetic acid lactone (**5**) (68 mg, 0.540 mmol) in ethyl acetate

(5 mL). The reaction was stopped after 139 hours, when large amounts of the isovelleral isomer (**4**) remained according to TLC analysis. The solvent was removed under reduced pressure, and the adduct was purified by silica gel chromatography in three steps (EtOAc followed by CH_2Cl_2-EtOAc and Toluene-MeOH), yielding **10** (yellow solid, 6 mg, 7 %). $[\alpha]_D$ -26° (*c* 0.15, $CHCl_3$). MS [*m/z* (% rel. int.)]: 340.1656 (M^+, 100 %, calculated for $C_{21}H_{24}O_4$ 340.1674), 311 (63), 256 (33), 238 (21), 217 (98), 193 (17), 173 (17), 139 (25), 91 (17), 84 (25). UV (EtOH) λ_{max} (ε): 352 nm (4800), 250 nm (7100). IR ($CHCl_3$): 2950, 2920, 2860, 1710, 1630, 1570, 1450, 1420, 1365, 1260, 1150, 1030 and 990 cm^{-1}. ^1H NMR: see Table 1. ^{13}C NMR: 197.3, C-19; 162.4, 162.1 and 162.0, C-1, C-3 and C-4a; 127.3, C-9a; 110.4, C-10; 99.6, C-4; 98.4, C-10a; 81.1, C-5a; 47.1, C-12; 46.4, C-16; 44.1, C-6; 44.1, C-7; 43.4, C-9; 38.4, C-8; 38.0, C-13; 29.6, C-15; 27.8, C-14; 21.6, C-18; 21.0, C-17; 20.1, C-11.

Degradation of the dialdehydes in buffer with triacetic acid lactone (5). Four mmol of the dialdehydes were dissolved in CH_3CN (1.00 mL) and added to the buffer (20 mL). The following buffers were used: 0.2 M phosphate / 0.1 M citric acid buffer (pH 4.0), 0.1 M phosphate buffer (pH 5.6), 0.1 M phosphate buffer (pH 7.4) and 0.025 M borax buffer (pH 9.0). The solution was divided into two equal samples, and triacetic acid lactone (**5**) (40 mmol) was added to one of the samples. The mixtures were then stirred at 37°C for a number of weeks, and samples (0.1 mL) of the reaction mixture were taken at intervals, mixed with 0.1 mL MeOH and analysed by reversed-phase HPLC.

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