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Solid-phase Synthesis, Antiviral Activity and Cytotoxicity of Some Functionalized Lactones

N. GOUAULT, M. DAVID, J. F. CUPIF, A. SAULEAU AND M. AMOROS

U.P.R.E.S., Pharmacochimie de Molécules de Synthèse et de Substances Naturelles, Faculté de Pharmacie, 2 avenue du Professeur Léon Bernard, 35043 Rennes Cedex, France

Abstract

Thio-substituted γ- and δ-lactones were prepared via a resin-bound epoxide by ring-opening reaction with sodium thiolates and subsequent cleavage.

The compounds were ineffective against herpes simplex virus type 1 and poliovirus. However, the cytotoxicity on murine tumour lines of 3,3-dimethyl lactone appeared to be promising.

Applications of combinatorial synthesis in medicinal chemistry promise to revolutionize new lead discovery and to optimize biological activity (Terrett et al 1995; Nefsi et al 1997). In the past few years, efforts have been directed towards the development of solid-phase chemistry as the pre-eminent method for the construction of small molecular libraries (Thompson & Ellman 1996). Excess of reagents is easily separated from support-bound products by simple filtration and reactions can be driven to completion without the difficult isolation and purification steps.

Lactones are prominent moieties in biologically active compounds (Caine et al 1992; Paquette et al 1994; Ellman & Plunkett 1995; Ogiku et al 1995) and are attractive targets for solid-phase synthesis. They afford the opportunity to combine the cyclization and resin cleavage steps in order to provide a traceless synthesis. This is exemplified herein in the preparation of thio-substituted γ- and δ-lactones. In-vitro evaluation of cytotoxicity and antiviral activity against poliovirus, an RNA virus and herpes simplex virus type 1 (HSV-1), a DNA virus is reported.

Materials and Methods

Chemistry

IR spectra were recorded on a 16PC FTIR Perkin Elmer spectrometer. Solids were examined with a diffuse reflectance accessory. For liquids, a horizontal attenuated total reflectance with a ZnSe crystal was used. ^{1}H and ^{13}C NMR were recorded using a Brucker DMX at 500 and 125 MHz, respectively. $CDCl_3$ was used as solvent and tetramethylsilane as the internal standard. Chemical shifts (δ) are in ppm. Compounds were purified by column chromatography with silica gel 60 (70–230 Mesh) purchased from Merck. DMF and CH_2Cl_2 were distilled from CaH_2 and P_2O_5, respectively, and stored under N_2. Merrifield resin was commercially available. Purity was estimated by gas chromatography (Carlo Erba 4160, column SE52) and NMR analysis after cleavage.

Correspondence: M. David, U.P.R.E.S., Pharmacochimie de Molécules de Synthèse et de Substances Naturelles, Faculté de Pharmacie, 2 avenue du Professeur Léon Bernard, 35043 Rennes Cedex, France.

*Polymeric epoxides **1a–d***

These compounds were obtained by coupling alkenoic acids to Merrifield resin (1·7 mmol Cl g^{-1}, 1% divinylbenzene) followed by epoxidation with *m*-chloroperbenzoic acid according to Le Hetet et al (1997).

Representative methods for synthesis of various compounds

*Polymeric β-hydroxythioethers **2a–d***. The products **2a–d** were prepared by ring opening with sodium thiolates in DMF at room temperature as previously described (Le Hetet et al 1997). The polymer obtained was filtered off, washed with DMF, DMF:H_2O (1:1), H_2O, H_2O:THF (1:1), THF and Et_2O and then dried at 50°C under vacuum for 4 h. IR (ν cm^{-1}): 3330 (OH), 1728 (CO).

γ-Butyrolactones **5**–***8***. Trifluoroacetic acid (50% in CH_2Cl_2, 16 mL) was added to the resin **2a**–**d** (0·6 g, 1·02 mmol) and shaken for 2 h. The resulting solution was filtered from the resin, concentrated to dryness and analysed by gas chromatography. The residue was purified by column chromatography.

Identification of ***5***. After chromatography (CH_2Cl_2), **5** was obtained in 67% yield. IR $\nu\,cm^{-1}$: 1774 (CO). ^1H NMR δ: 1·98–2·06 and 2·36–2·43 (2H, m, H_4), 2·47–2·61 (2H, m, H_3), 3·02–3·06, 3·32–3·36 and 4·59–4·64 (3H, m, ABX, H_5 and CH_2-5), 7·22–7·41 (5H, m, C_6H_5). ^{13}C NMR δ: 26·9 (C_4), 28·4 (C_3), 38·6 (CH_2-5), 78·6 (C_5), 129·2 (C_3'), 129·9 (C_4'), 130·2 (C_2'), 134·8 (C_1'), 176·5 (C_2).

Identification of ***6***. After chromatography (CH_2Cl_2) **6** (1:1 mixture of diastereoisomers) was obtained in 50% yield. IR $\nu\,cm^{-1}$: 1770 (CO). ^1H NMR δ: 1.27–1·28 (3H, d, CH_3-3), 1·60–1·66, 2·01–2·08, 2·30–2·36 and 2·50–2·68 (2H, m, H_4), 2·72–2·79 (1H, m, H_3), 2·98–3·05, 3·28–3·37, 4·42–4·50 and 4·60–4·65 (3H, m, ABX, H_5 and CH_2-5, JAB = 13·8 and 13·84, JAX = 3·94 and 5·70, JBX = 5·07 and 8·00), 7·20–7·40 (5H, m, C_6H_5). ^{13}C NMR δ: 23·4 and 24·9 (CH_3), 30·1 and 30·7 (C_4), 32·2 and 32·9 (C_3), 34·7 and 35·0 (CH_2-5), 72·7 and 72·9 (C_5), 123·4 and 123·5 (C_3'), 125·6 and 125·7 (C_4'), 126·6 and 126·7 (C_2'), 131·1 and 131·3 (C_1'), 175·3 and 175·9 (C_2).

Identification of ***7***. After chromatography (CH_2Cl_2–CH_3COOEt, 80:20) **7** was obtained in 25% yield. IR $\nu\,cm^{-1}$: 1772 (CO). ^1H NMR δ: 1·20–1·25 (6H, s, CH_3), 1·80–1·90 and 2·20–2·28 (2H, m, H_4), 3·05–3·08, 3·35–3·40 and 4·48–4·55 (3H, m, ABX, H_5 and CH_2-5), 7·10–7·40 (5H, m, C_6H_5). ^{13}C NMR δ: 24·6 and 25·0 (CH_3), 38·8 (C_4), 40·5 (C_3), 38·6 (CH_2-5), 75·0 (C_5), 126·9 (C_3'), 129·2 (C_4'), 130·0 (C_2'), 134·8 (C_1'), 181·2 (C_2).

Identification of ***8***. Crude yield 63%. IR $\nu\,cm^{-1}$: 1732 (CO). ^1H NMR δ: 1·55–1·65 and 1·78–1·88 (2H, m, H_4), 1·85–1·95 and 2·10–2·15 (2H, m, H_5), 2·40–2·50 and 2·54–2·64 (2H, m, H_3), 2·95–3·05, 3·35–3·42 and 4·40–4·50 (3H, m, ABX, H_6 and CH_2-6), 7·22–7·39 (5H, m, C_6H_5). ^{13}C NMR δ: 18·2 (C_4), 26·8 (C_5), 29·5 (C_3), 38·6 (CH_2-6), 79·2 (C_6), 125·8 (C_3'),126·5 (C_4'), 126·7 (C_2'), 135·1 (C_1'), 171·6 (C2).

Identification of ***9***. Yield 45%. IR $\nu\,cm^{-1}$: 1774 (CO). ^1H NMR δ: 1·95–2·05 and 2·38–2·45 (2H, m, H_2), 2·32 (3H, s, CH_3), 2·48–2·62 (2H, m, H_3), 2·95–3·05, 3·25–3·32 and 4·53–4·62 (3H, m, ABX, H_5 and CH_2-5), 7·10–7·40 (4H, m, C_6H_4). ^{13}C NMR δ: 21·0 (CH_3), 26·9 (C_4), 28·5 (C_3), 39·3 (CH_2-5), 78·7 (C_5), 130·0 (C_3'), 130·9 (C_4'), 131·0 (C_2'), 137·4 (C_1'), 176·5 (C_2).

Identification of ***10***. Compound **10** (1:1 mixture of diastereoisomers) was obtained in 30% yield. IR $\nu\,cm^{-1}$: 1772 (CO). ^1H NMR δ: 1·27–1·28 (3H, d, CH_3-3), 2·34 (3H, s, CH_3), 1·60–1·66, 2·01–2·08, 2·30–2·36 and 2·58–2·68 (2H, m, H_4), 2·60–2·70 (1H, m, H_3), 2·98–3·05, 3·28–3·37, 4·42–4·50 and 4·60–4·65 (3H, m, ABX, H_5 and CH_2-5), 7·20–7·40 (4H, m, C_6H_4).

Polymeric β-hydroxythioethers ***3a***–***b***, ***4a***

The products **3a**–**b** and **4a** were prepared by ring opening **1a**–**b** with thiols in the presence of $Ti(OiPr)_4$. To the resin **1a**–**b** (600 mg, 1·02 mmol) in dry DMF (6 mL) was added R_3SH (1·02 mmol) and $Ti(OiPr)_4$ (1·02 mmol). The resulting mixture was stirred at room temperature under nitrogen for 2 h. The resin (**3a**–**b**, **4a**) obtained was filtered and washed with DMF, THF, H_2O, Et_2O and dried under vacuum. IR ($\nu\,cm^{-1}$): 3330 (OH), 1728 (CO).

γ-Butyrolactones ***9***–***11***. These cyclic esters were obtained by cyclization during the opening of the epoxide ring. Sodium thiolate generated by treatment of NaH in dry DMF with an excess of thiol was added to the resin **1a**–**b** in dry DMF (8 mL) at 0°C for 18 h. The reaction mixture was filtered. After washing the polymer with DMF, the solution was extracted at pH 8 (NaOH 10%) with CH_2Cl_2 (5 × 10 mL), dried (Na_2SO_4) and evaporated. The residue was purified by chromatography on silica gel.

Antitumour activity

Cell culture. Murine L1210 leukaemia cells and 3LL Lewis Lung carcinoma cells were cultured in RPMI 1640 medium (Eurobio) containing 10% heat-inactivated foetal calf serum (Eurobio), 2 mM L-glutamine, 1% non-essential amino acids, 50 int. units mL^{-1} penicillin and 50 $\mu g\,mL^{-1}$ streptomycin in a humidified atmosphere of 95% air and 5% CO_2, at 37°C. Viability was checked with trypan blue and cell numbers were determined using a hemocytometer.

Sample preparation. Stock solutions (100 mM) were prepared in dimethylsulphoxide, distributed in 0·2-mL fractions and stored at −20°C.

In-vitro proliferation assays. All antiproliferative assays were carried out in 96-well culture plates

(Corning), seeded with 100 μL 2·5 $\times$ 10^3 L1210 cells/well at the experiment time or with 100 μL 10^3 3LL cells/well 24 h before the start of the experiments. Diluted stock solutions (10 μL) and two-fold dilutions ranging from 8 to 0·25 mM (final concentrations in wells from 0·72 to 0·023 mM) were added in triplicate to cell suspensions in the multi-plate. Six wells without drugs served as a control for cell growth. Peripheral wells containing only 100 μL medium were not considered for assays, because of variations due to liquid evaporation at plate edges, and served as blank controls. After incubation at 37°C for 48 h, 10 μL 5 mg mL^{-1} MTT in phosphate-buffered saline was added to each well and incubated for a further 4 h. The 96-well plates were then centrifuged at 1000 *g* for 5 min and supernatants were carefully removed. The blue formazan crystals produced by viable cells were dissolved in dimethylsulphoxide to allow assay using a microplate spectrophotometer (Multiscan Life-Science) at 540 nm (test) and 690 nm (background). Cell growth inhibition was calculated in comparison with control cell growth and the EC50 was calculated using the Biolise software.

Antiviral activity

Cells and viruses. African green monkey kidney cells (Vero) were grown in Eagle's minimum essential medium (MEM) supplemented with 10% foetal calf serum, 160 int. units mL^{-1} penicillin and 80 μg mL^{-1} gentamycin. The viruses used were HSV-1 strain H29S and poliovirus type 2, vaccinal strain Sabin. Virus stocks were prepared on Vero cells and virus titrations were performed with the limit dilution method (Burleson et al 1992). The virus titre was estimated from cytopathogenicity and expressed as 50% tissue cultures infectious doses per mL (TCID50).

Evaluation of cytotoxicity of lactones on Vero cells
To assess the effect of lactones on uninfected Vero cells dilutions from 25 $\times$ 10^3 to 98 nM were added to confluent one-day-old monolayers of Vero cells grown in 96-well microplates. After 96 h incubation at 37°C, cell growth and survival were measured by the tetrazolium dye procedure (Denizot & Lang 1986). The optical density of each well was read using an automated plate reader (Titertek Multiscan) and all data was immediately analysed on a microcomputer with the Biolise software. Each assay was performed in triplicate and each experiment was repeated six times. The 50% cytotoxic concentration (CC50) was defined as the concentration of compound that reduced the absorbance (OD_{540}) of the control sample by 50%.

Antiviral activity
Lactones from CC50 to CC50/4 were added to confluent Vero cells just before virus infection at a low multiplicity of infection (0·001 and 0·01 TCID50 per cell). Toxicity controls, cells controls and virus controls were run simultaneously.

Each compound was assayed in triplicate and each experiment was repeated three times. Plates were incubated at 37°C for multiple cycles of replication (48 h for poliovirus and 72 h for HSV-1). Cell survival was measured as above by the tetrazolium dye procedure. The concentration of drug that reduced the viral cell destruction by 50% was expressed as the 50% inhibitory (or cytopathic effect) concentration EC50.

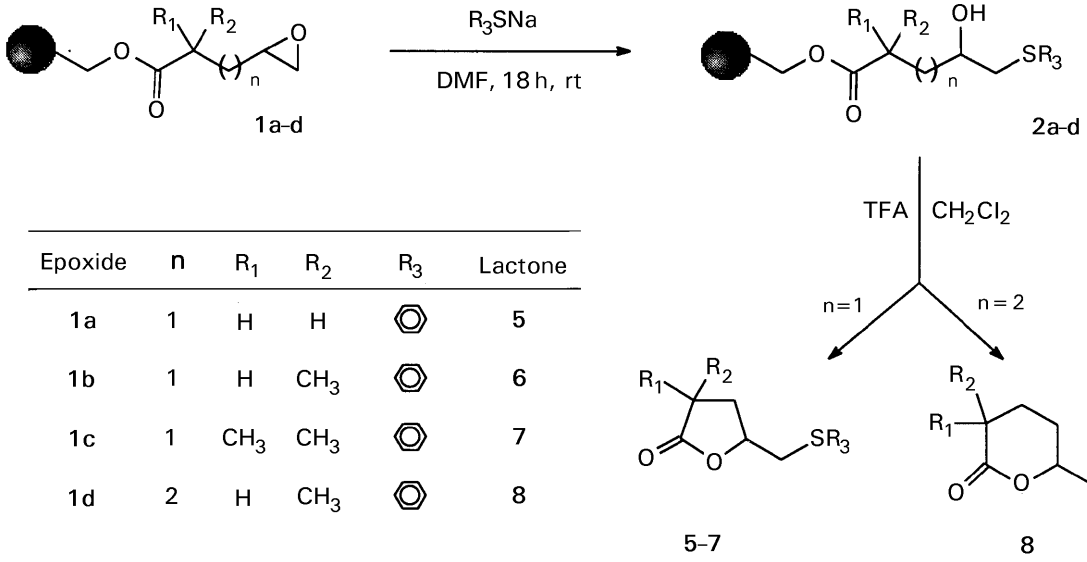
Results and Discussion

Chemistry
Our first choice for a polymer was the widely used Merrifield resin (chloromethylated styrene–divinylbenzene copolymer). The starting material for the synthesis of lactones **5**–**11** were epoxides **1a**–**d**. The latter were obtained from alkenoic acids (Le Hetet et al 1997) and were submitted to ring-opening with various thiolates R_3SNa. The reaction afforded the β-hydroxythioethers **2a**–**d** with C_6H_5SNa as indicated in Figure 1. FTIR was successfully used in reaction monitoring. A band at 3330 cm^{-1} (hydroxile group) indicated the epoxide ring-opening.

In the case of other thiolates (Figure 2), the FTIR spectrum showed the disappearance of absorption at 1730 cm^{-1} of the ester moiety (attachment to the polymer support) indicating cyclization during the opening of the epoxide. The filtration and washing of the resin delivered γ-butyrolactones **9**–**11** in the filtrate. An extraction was necessary to isolate **9**–**11** in moderate yields.

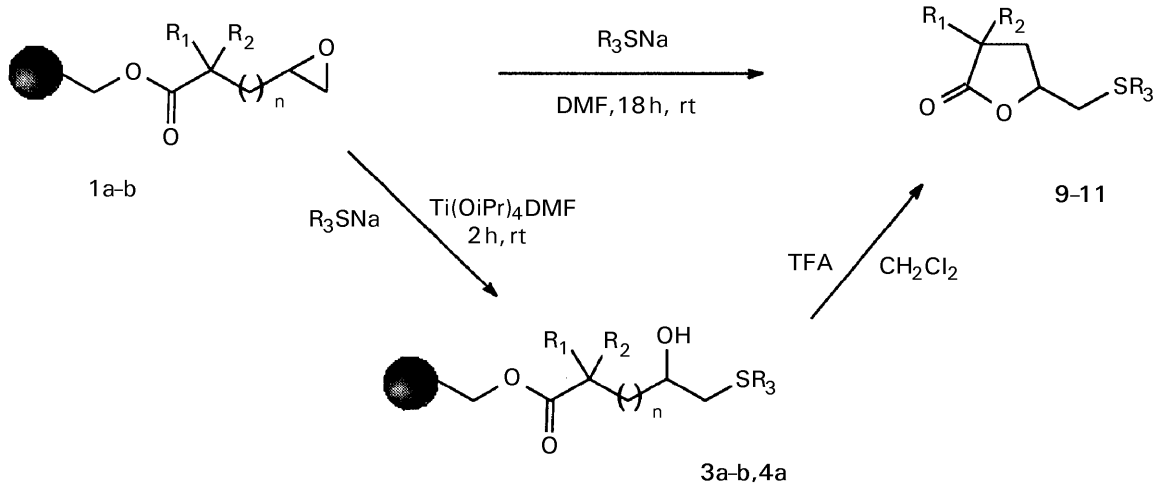
To avoid this lactonization in-situ, another strategy can be suggested for the synthesis of compounds **3a**–**b** and **4a**. Epoxides **1a**–**d** were opened by thiolates in the presence of titatium tetra-isopropoxide over 2 h and gave the expected β-hydroxythioethers (Figure 2).

Treatment of resins **2**, **3** and **4** with trifluoroacetic acid (Ho et al 1983) in methylene chloride caused spontaneous lactonization and the desired products **5**–**11** were obtained in good yields. Examination of the resin by FTIR showed no absorption at 1730 cm^{-1}, and the appearance of a band at



Epoxide	n	R_1	R_2	R_3	Lactone
1a	1	H	H		5
1b	1	H	CH_3		6
1c	1	CH_3	CH_3		7
1d	2	H	CH_3		8

Figure 1. Synthesis of lactones **5–8**.



Epoxide	n	R_1	R_2	R_3	Lactone
1a	1	H	H		9
1b	1	H	CH_3		10
1a	1	H	H		11

Figure 2. Synthesis of lactones **9–11**.

1784 cm^{-1} ($COCF_3$) indicated that cleavage was complete. All the products were identified by ^1H and ^{13}C NMR.

These results establish that supported epoxides have synthetic potential in the preparation of 3,5-disubstituted γ- and δ-butyrolactones. The strategy via hydroxyethers is currently being explored to afford sulphoxide and sulphone derivatives.

Biological results

Cytotoxicity on Vero cells and the effect of lactones **5–10** on viral cell destruction is summarized in

Table 1. Cytotoxicity on Vero cells and in-vitro antiviral effect of lactones **5**–**10**.

Compound	CC50 (μM)	HSV-1 EC50 (μM)	CC50/EC50	Poliovirus EC50 (μM)	CC50/EC50
5	625	Inactive	–	Inactive	–
6	375	250	1·5	375	2
7	187	Inactive	–	Inactive	–
8	750	Inactive	–	375	2
9	305	Inactive	–	Inactive	–
10	375	Inactive	–	250	1·5

Vero cells were infected at a multiplicity of infection of 0·001. CC50, 50% cytotoxic concentration; EC50, 50% inhibitory concentration.

Table 2. Cytotoxicity of lactones **5**–**10** on 3LL and L1210 cells.

Compound	CC50 (μM) 3LL	L1210
Doxorubicin	10×10^{-3}	2×10^{-3}
5	325	80
6	125	70
7	50	60
8	75	120
9	170	100
10	116	130

Doxorubicin served as positive control.

Table 1. Compounds **5**, **7** and **9** were completely ineffective against poliovirus and HSV-1. Lactone **6** appeared to be a weak inhibitor of both viruses, whereas lactones **8** and **10** exhibited only anti-poliovirus activity. These results were obtained at the lowest multiplicity of infection (0·001). The poor selectivity indexes indicate that the compounds did not have antiviral activity.

No significant differences in the cytotoxicity of the six compounds were noted. The cytotoxicity of lactones **5**–**10** on L1210 and 3LL murine tumour lines is given in Table 2. Doxorubicin was used as positive control. Compounds **6** and **10**, with one methyl group on the lactone ring, appeared to be slightly more cytotoxic than compound **5**. Lactone **7** with two methyl groups showed even greater cytotoxicity. Other derivatives based on the structural features of **7** should be synthesized in order to reach levels of cytotoxicity comparable with doxorubicin.

Further study of lactone **7** cytotoxicity on other tumour cell lines, especially human tumours, would be of interest and asymmetric epoxidation and ring opening with other nucleophiles to prepare additional derivatives is also required.

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