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Preparation and Biological Evaluation of γ-Fluoromethyl-α-methylene-γ-butyrolactone and γ-butyrolactam

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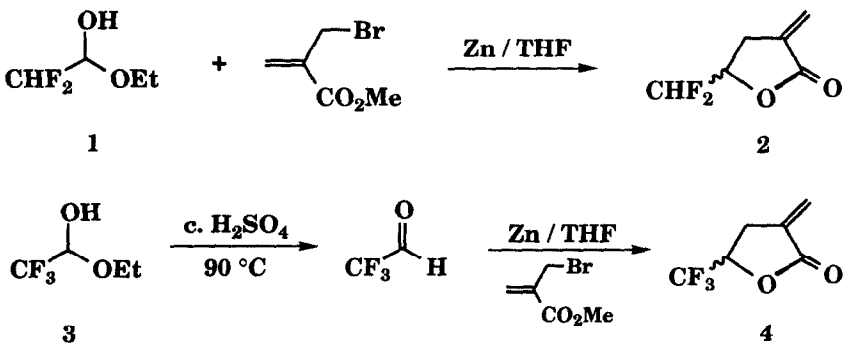
Abstract: γ-Difluoromethyl-α-methylene-γ-butyrolactone **2** was readily prepared from the Reformatsky-type reaction of difluoroacetaldehyde ethyl hemiacetal **1** and methyl 2-(bromomethyl)acrylate in Zn-THF system, and γ-difluoromethyl-α-methylene-γ-butyrolactams **6** were also prepared from difluoroethanimine. γ-Difluoromethyl-α-methylene-γ-butyrolactone **2** was found to possess comparable or even stronger *in vitro* antitumoral activity than the currently used 5-fluorouracil.

Since the discovery of the antitumoral activity of uracil with a fluorine,[1] researches for the modification of molecules with fluorine(s), which produced profound and unexpected results on biological activity,[2] have been extensive in recent years. Particularly, the difluoro-methyl group is preferred due to its ability to act as a hydrogen donor, allowing the possibility to interact with solvents and biological molecules.[3] Recently, we have reported the biological activity of 4,4-difluorothreonine and its versatility as chiral building units with three easily differentiated functional groups.[4] Herein, we report the synthesis of γ-difluoromethyl-α-methylene-γ-butyrolactone **2** and lactam **6** and their antitumoral activities.

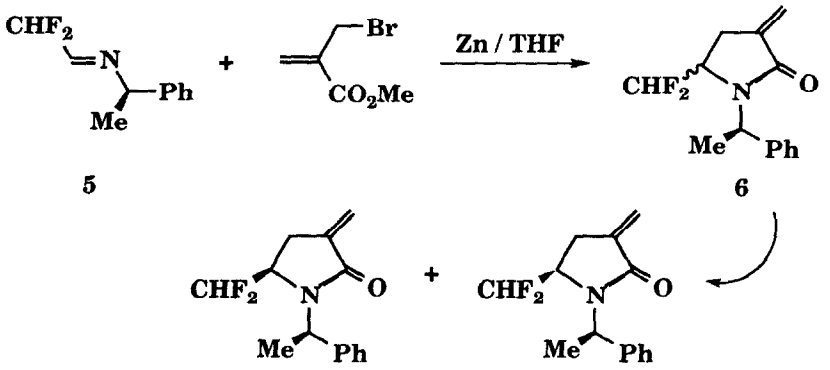
Difluoroacetaldehyde ethyl hemiacetal **1** acts as a difluoroacetaldehyde equivalent in Reformatsky-type reaction[5] using the system of methyl 2-(bromomethyl)acrylate and Zinc in THF to form γ-difluoromethyl-α-methylene-γ-butyrolactone. After a survey of conditions, the reaction of hemiacetal **1** (1 equiv) with this Reformatsky-type reagent (2 equiv) in THF was found to afford lactone **2** in 58% yield.[6] More than 2 equiv of the nucleophiles as well as, in some cases, 1 equiv of the Lewis acid per mole of the hemiacetal **1** were required. Obviously, 1 equiv of nucleophile or Lewis acid was consumed by the hydroxy group in difluoroacetaldehyde ethyl hemiacetal **1**, which generates the difluoroacetaldehyde *in situ*.

Scheme 1



In contrast, trifluoroacetaldehyde ethyl hemiacetal **3** failed to react with this Reformatsky-type reagent. The preparation of lactone **4** was achieved by the reaction of trifluoroacetaldehyde generated from hemiacetal **3** with this Reformatsky-type reagent.[7]

The synthesis of γ-difluoromethyl-α-methylene-γ-butyrolactams **6**,[8] which have been receiving increasing attention because of their properties as suicide inhibitors,[1,2] has been explored *via* the reaction of difluoroethanimine **5** with methyl 2-(bromomethyl)acrylate in the presence of Zinc. Diastereomeric mixture was separated by column chromatography on silica gel. However, trifluoroethanimine did not undergo the Reformatsky-type reaction.



For each of those lactones and lactam, the *in vitro* growth inhibitory action towards tumor cell lines was determined. The reported IC_{50} values represent the concentration of inhibitor producing 50 percent inhibition of cell growth. Table I gathers the results for *racemic γ-difluoromethyl-α-methylene-γ-butyrolactone* **2** with the established antimetabolite, 5-fluorouracil (5-FU), as a reference. A comparison of IC_{50} values demonstrated the potential of this difluorinated lactone as an antitumoral substance, which interestingly

showed more effective nature than 5-FU in inhibition of cell growth of human cancer or melanoma cells. However, γ-trifluoromethyl-α-methylene-γ-butyrolactone **4** and γ-difluoromethyl-α-methylene-γ-butyrolactam **6** retained low to zero activities.

Table I Effect of Racemic γ-lactone (2) on the Growth of Various Tumor Cell *in vitro* [a]

Cell Line	IC_{50} (μg/mL)	
	γ-lactone (2)	5-FU
L1210/c[b]	3	0.2
CCRF CEM[c]	4	4
T24[d]	0.5	6
P36[e]	2	11
SW480[f]	4	6

a) Each tumor cell line ($1x10^4$ cells/well) was incubated in the presence or absence of compound for 72 h. Then, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) was added for $OD^{578-588}$ measurements. IC_{50} (μg/mL) was given as the concentration at 50% inhibition of cell growth. % Inhibition = {1-($OD^{578-588}$ of sample well) / ($OD^{578-588}$ of control well)} x 100.
b) Mouse leukemia. c) Human leukemia. d) Human cancer.
e) Human melanoma. f) Human cancer.

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6) Preparation of γ-difluoromethyl-α-methylene-γ-butyrolactone **2** [5-difluoromethyl-4,5-dihydro-3-methylene-2(*3H*)furanone]. To a suspension of Zn powder (4.15 g) in freshly dried tetrahydrofuran (60 ml), the mixture of difluoroacetaldehyde ethyl hemiacetal **1** (1.74 g, 13.5

mmol) and methyl 2-(bromomethyl)acrylate (4.96 g, 27 mmol) in THF at 0 °C under an atmosphere of nitrogen. After 4 hrs of stirring at room temperature, the mixture was quenched with 1N HCl (120 ml) and then oily materials were extracted with diethyl ether (30 ml x 3). The extracts were dried over anhydrous magnesium sulfate. Removal of the solvent and flash chromatography using the mixture of n-hexane-ethyl acetate (3 ; 1) gave **2** in 58% yield: Physical properties of 2 : Rf 0.25 (n-hexane : AcOEt = 2:1), ^{1}H NMR ($CDCl_3$) δ 3.05 (1 H, dt, $J_{H,H}$ = 2.7, 5.5 Hz), 3.07 (1 H, t, $J_{H,H}$ = 2.9 Hz), 4.67 (1 H, m), 5.76 (1 H, t, $J_{H,H}$ = 2.6 Hz), 5.85 (1 H, ddd, $J_{H,H}$ = 2.7 Hz, $J_{H,F}$ = 54.0, 56.1 Hz), 6.32 (1 H, t,$J_{H,H}$ = 2.9 Hz). ^{13}C NMR ($CDCl_3$) δ 26.00 (dd, J = 2.7, 4.7 Hz), 73.18 (dd, J = 25.7, 30.0 Hz), 110.8 (dd, J = 243, 246 Hz), 123.9, 131.6, 168,9. ^{19}F NMR ($CDCl_3$) δ 33.82 (ddd, $J_{F,H}$ = 16.8, 56.5 Hz, $J_{F,F}$ = 296 Hz), 39.55 (ddd, $J_{F,H}$ = 5.7, 53.8 Hz, $J_{F,F}$ = 296 Hz). IR (neat) γ 1780 cm^{-1}; high-resolution mass calcd for $C_6H_6O_2F_2$ $(MH)^+$ 149.0414 , found 149.0421.

7) Physical properties of γ-Trifluoromethyl-α-methylene-γ-butyrolactone **4** : Rf 0.23 (n-hexane:AcOEt = 3:1), yield 70%; ^{1}H NMR ($CDCl_3$) δ 3.04 (1 H, ddt, $J_{H,H}$ = 4.7, 18.0, 2.7 Hz), 3.22 (1 H, ddt, $J_{H,H}$ = 8.8, 18.0, 2.9 Hz), 4.82 (1 H, ddq, $J_{H,H}$ = 4.7, 8.8, 6.2 Hz), 5.83 (1 H, t, $J_{H,H}$ = 2.6 Hz), 6.38 (1 H, t, $J_{H,H}$ = 2.9 Hz). ^{13}C NMR δ 3.05 ^{19}F NMR ($CDCl_3$) δ 81.80 (d, $J_{F,H}$ = 6.1 Hz). IR (neat) γ 1780 cm^{-1}; high-resolution mass calcd for $C_6H_5O_2F_3$ $(M)^+$ 166.0242 , found 166.0234.

8) To a suspension of Zn (0.23 g) in tetrahydrofuran (5 ml), the mixture of (α*R*)-N-(2,2-difluoroethylidene)methylbenzylamine **5** (0.37 g, 2 mmol) and methyl 2-(bromomethyl)acrylate (0.36 g, 2 mmol) in THF at 0 °C under an atmosphere of nitrogen. After 4 hrs of refluxing, worked up similarly, giving **6a** and **6b** in 84% yield: Physical properties of **6a** : Rf 0.19 (n-hexane:AcOEt = 3:1). $[\alpha]_D^{21}$ -106.6 (c 1.04, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.66 (3 H, d, $J_{H,H}$ = 7.2 Hz), 2.68 (1 H, ddt, $J_{H,H}$ = 8.5, 17.5, 2.9 Hz), 2.84 (1 H, ddt, $J_{H,H}$ = 2.4, 17.2, 2.9 Hz), 3.48 (1 H, dddt, $J_{H,H}$ = 5.9, 8.6, 18.7, 2.7 Hz), 5.42 (1 H, ddd, $J_{H,H}$ = 0.6, 2.0, 2.8 Hz), 5.57 (1 H, q, $J_{H,H}$ = 7.3 Hz), 5.74 (1 H, ddd, $J_{H,H}$ = 2.6 Hz, $J_{H,F}$ = 54.7, 56.7 Hz), 6.09 (1 H, ddd, $J_{H,H}$ = 0.7, 2.6, 3.3 Hz), 7.28-7.45 (5 H, m). ^{13}C NMR δ 17.72 (t, J = 1.7 Hz), 24.92 (dd, J = 3.6, 5.2 Hz), 51.75 (s), 54.89 (dd, J = 23.4, 28.0 Hz), 114.18 (dd, J = 244, 248 Hz), 116.63 (s), 127.62 (s), 128.09 (s), 128.91 (s), 137.50 (s), 138.35 (s), 168.61 (s). ^{19}F NMR ($CDCl_3$) δ 30.10 (ddd, $J_{F,H}$ = 18.3, 56.5 Hz, $J_{F,F}$ = 287 Hz), 36.76 (ddd, $J_{F,H}$ = 5.3, 54.2 Hz, $J_{F,F}$ = 287 Hz). IR (neat) γ 1780 cm^{-1}; high-resolution mass calcd for $C_{14}H_{16}NOF_2$ $(MH)^+$ 252.1200 , found 252.1208: Physical properties of **6b** : Rf 0.14 (n-hexane:AcOEt = 3:1). $[\alpha]_D^{20}$ -156.7 (c 0.75, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.70 (3 H, d, $J_{H,H}$ = 7.2 Hz), 2.79 (1 H, ddddd, $J_{H,H}$ = 0.5, 2.8, 3.3, 8.9, 17.5 Hz), 2.90 (1 H, ddt, $J_{H,H}$ = 2.1, 17.5, 2.3 Hz), 3.93 (1 H, dddt, $J_{H,H}$ = 3.9, 8.8, 20.9, 2.2 Hz), 4.91 (1 H, ddd, $J_{H,H}$ = 2.2 Hz, $J_{H,F}$ = 55.1, 56.3 Hz), 5.43 (1 H, ddd, $J_{H,H}$ = 0.6, 2.0, 2.8 Hz), 5.60 (1 H, q, $J_{H,H}$ = 7.2 Hz), 6.08 (1 H, ddd, $J_{H,H}$ = 0.6, 2.3, 3.3 Hz), 7.30-7.40 (5 H, m). ^{13}C NMR δ 15.96 (s), 24.63 (dd, J = 3.2, 5.6 Hz), 50.59 (s), 54.60 (dd, J = 23.5, 29.8 Hz), 113.26 (dd, J = 243, 249 Hz), 116.56 (s), 127.21 (s), 128.05 (s), 128.76 (s), 137.66 (s), 140.37 (s), 168.60 (d, J = 1.5 Hz). ^{19}F NMR ($CDCl_3$) δ 30.10 (ddd, $J_{F,H}$ = 18.3, 56.5 Hz, $J_{F,F}$ = 287 Hz), 36.76 (ddd, $J_{F,H}$ = 5.3, 54.2 Hz, $J_{F,F}$ = 287 Hz).