Polyether Mimics of Naturally Occurring Cytotoxic Annonaceous Acetogenins

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On the basis of the ionophore model, polyether analogues **4** and **6** were designed and synthesized to mimic the naturally occurring annonaceous acetogenins corossolin (**2**) and bullatin (**5**), which were discovered as members of a large family of novel polyketides with cytotoxicity, antitumoral, and other biological activities since 1982. The preliminary screening shows that they have compatible cytotoxicity with the corresponding natural annonaceous acetogenins. These results open a potential way to find more active antitumor agents with simplified structures based on natural annonaceous acetogenins.

Introduction

Annonaceous acetogenins, a relatively new class of natural products known since 1982, have been attracting worldwide attention in recent years because of their potent biological activities, especially as growth inhibitors of certain tumor cells.[1−5] To this date, the study of action mechanism found the acetogenins inhibited the complex I of the mitochondrial respiration chain.[6] Their structures are characterized by a butenolide part, one to three tetrahydrofuran (THF) rings, and an alkyl chain between and another chain on the other side. These novel features and multiple chiral centers, especially more than four chiral centers in the THF parts, make them challenging synthetic targets. Also due to the scant natural resources and the requirement of substantial amounts of enantiomerically pure samples for further biological and clinical studies, a number of total syntheses of annonaceous acetogenins have been reported in the literature[7−9] since the 1990s.

In a project dealing with the synthesis of bioactive natural products from sugar we synthesized the mono-THF annonaceous acetogenin corossolone (**1**) and corossolin (**2**) and its stereoisomers.[10−12] At that time, the ionophoric ability in the THF part of acetogenins was considered to be responsible, at least partly, for their cytotoxicity. Later Sasaki et al. also postulated their ionophoric function and reported the observation of their Ca^{2+} complex by NMR.[13−15] From the viewpoint of ionophore we proposed that if the THF part was substituted by simple diethylene or triethylene glycol ethers, the mimetic acetogenin would conserve their ionophoric ability and hence bioactivity. Thus, corossolone (**1**)[16] or corossolin (**2**)[16] would be simplified to an easily reached compound, **3** or **4**, and the corresponding bis-THF acetogenin, bullatin (**5**),[17] to triethylene glycol **6** (Scheme 1). On the basis of this consideration, we have synthesized a series of such acetogenin mimics.[18] Herewith, we would like to report the first synthesis and cytotoxicity testing results of the two simplest mimics, **4** and **6**.

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Chemical Synthesis

During our synthesis of corossolone and (10*RS*)-corossolin, the butenolide segment **7** was synthesized from ethyl L-lactate and methyl undecenate.[4] With this segment in hand, the mimetic acetogenins **4** and **6** could be similarly obtained by coupling with a long chain polyethelene ether acetylene, followed by hydrogenation and elimination of the MOM group.

Starting from diethylene glycol or triethylene glycol, two-directional *O*-alkylation with propargyl bromide gave compound **8a** or **8b** in high yields. Monoalkylation of **8** with *n*-octyl bromide or *n*-heptyl bromide respectively afforded **9a** or **9b** along with some bis-alkylation products. Regioselective ring open[19] of the epoxide **7** with the alkynyllithium of **9** in the presence of boron−trifluoride etherate gave the coupling products **10** in excellent yield. Catalytic hydrogenation of **10** in the presence of PtO_2 gave the saturated products in moderate yield. However, when palladium on charcoal was used as the catalyst, even lower yield was obtained due to the hydrogenolysis of the ether linkage. β-Elimination of the MOM protecting group with DBU yielded the target molecule **4** and **6** as a mixture of diastereoisomers at C-10 (Scheme 2).

Biological Evaluation

The synthesized mimics **4** and **6** have been submitted to other institutes for testing of their cytotoxicity. Both compounds showed activity compatible with the corresponding natural annonaceous acetogenins.

The preliminary cytotocxicity screening results from Shanghai Institute of Materia Medica are shown in Table 1. For comparison, we also list some results from known samples, such as our synthesized corossolone (**1**) and (10*RS*)-corossolin (**2**) and natural solamin (**12**) bullatacin (**13**). These results also showed that both of our synthetic mimics have moderate activities. It is somewhat confusing that the most active anonnaceous acetogenin so far reported, bullatacin showed even lower activity than these mimics.

Summary

We have designed and synthesized the mimics of annonaceous acetogenins. The preliminary screening

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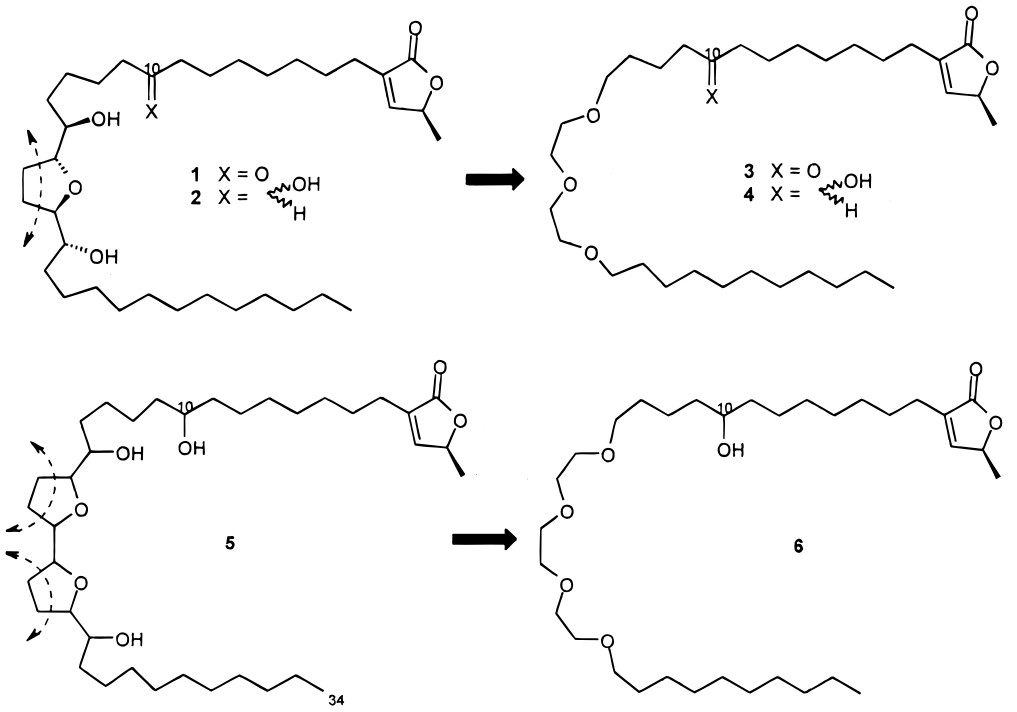
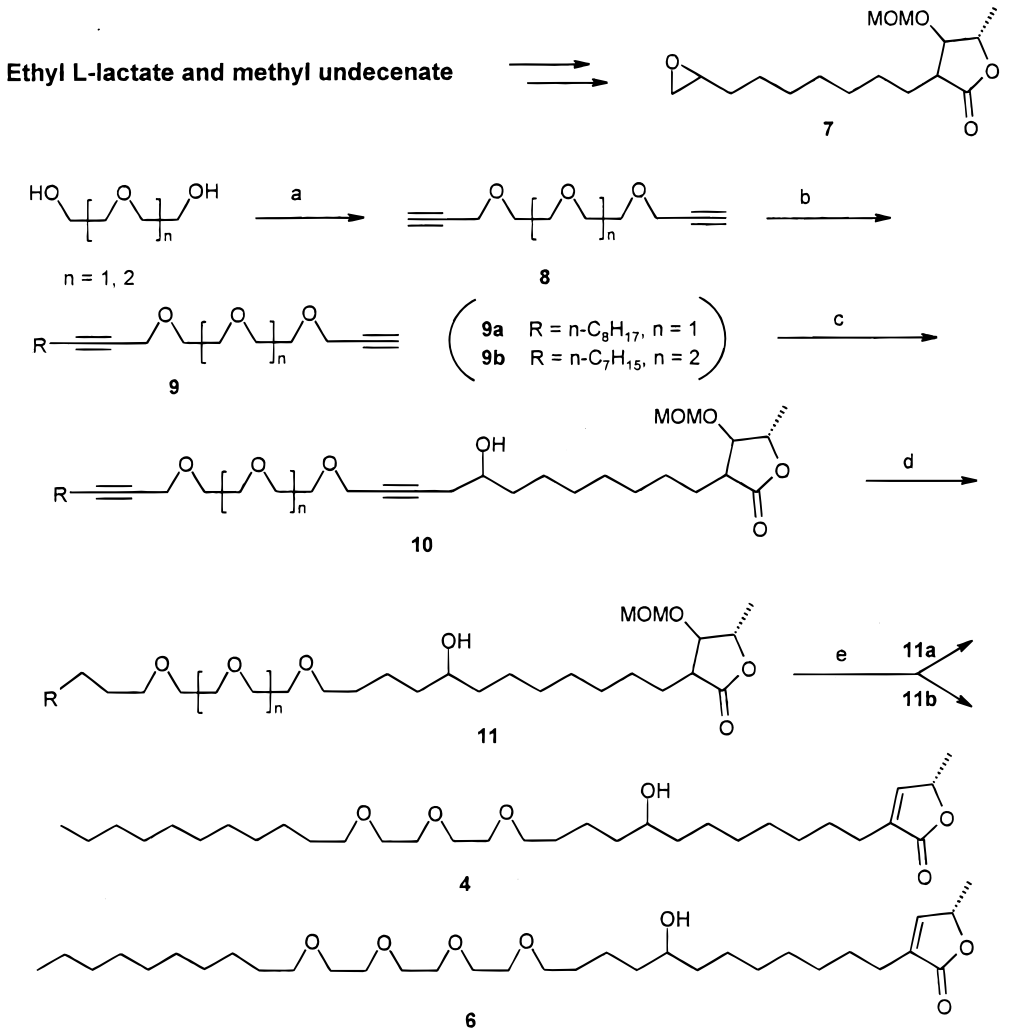
Scheme 1

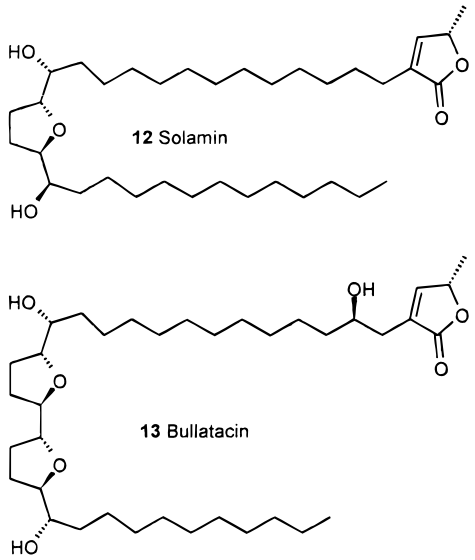


Scheme 2[a]



[a] Reagents and conditions: (a) NaH, $BrCH_2C{\equiv}CH$, THF, 0 °C to rt; (b) *n*-BuLi, *n*-$C_8H_{17}Br$ (or *n*-$C_7H_{15}Br$), THF−HMPA, −78 °C; (c) *n*-BuLi,BF_3·OEt, **7**, THF, −78 °C; (d) H_2, PtO_2, rt; (e) DBU, THF.

Table 1. In Vitro Testing against the HL-60 and K562 Cell Lines



	IG %					
	for HL-60			for K562		
concn (μM)	100	10	1	100	10	1
compound **4**	100	50	0	31	18	0
compound **6**	100	65	21	55	25	22
corossolone (**1**)	68	29	0	53	16	2
(10*RS*)-corossolin (**2**)	63	56	5	10	2	0
solamin (**12**)	24	8	0	59	39	29
bullatacin (**13**)	73	7	0	53	39	27

shows that they have compatible cytotoxicity with the corresponding natural annonaceous acetogenins. These results open a potential way to find more active antitumor agents with simplified structures based on natural annonaceous acetogenins. More synthetic mimics designed according to this strategy will be reported in due time.

Experimental Section

The melting points were uncorrected. Optical rotations were measured on a Perkin-Elmer 241 MC autopol polarimeter. IR spectra were obtained on an IR-440 or a Perkin-Elmer 983 spectrophotometer. ^{1}H NMR spectra were taken on a Varian EM-390, AMX-300, or AMX-600 spectrometer. Mass spectra were measured on a HP 5989A spectrometer. Microanalyses were carried out in the microanalytic laboratory of this institute. Flash column chromatography was performed on silica gel H (10−40 μm).

α,ω-Bis(*O*-propargyl)diethylene Glycol (8a). To a solution of diethylene glycol (4.24 g) in 100 mL of anhydrous THF was added NaH (80% w/w, 2.88 g) at 0 °C. After hydrogen was entirely emitted, a catalytic amount of tetrabutylammonium iodide and propargyl bromide (8.6 mL) was added, respectively. The mixture was then warmed to room temperature and stirred for an additional 3 h. After removal of the solvent, the residue was purified by column chromatography on silica gel, giving a clear pale yellow liquid, **8a** (5.194 g, 71.3%). IR (neat) ν_{max}: 3310 (s), 2820 (s), 2120 (m), 1465 (s), 1450 (s), 1350 (s), 1285 (s), 1245 (s), 1100 (s) cm^{-1}. ^{1}H NMR (60 MHz, CCl_4) δ_H: 4.01 (4H, d, J = 2.7 Hz), 3.53 (8H, s), 2.20 (2H, t, J = 2.7 Hz) ppm. EIMS (*m*/*z*, %): 182 (M^+, 1.4), 127 (0.5), 99 (0.9), 83 (6.7), 45 (100).

α-(*O*-Undecan-2-ynyl)-ω-(*O*-propargyl)diethylene Glycol (9a). To a solution of **8a** (5.132 g) in 80 mL of THF was injected *n*-butyllithium in hexane (2.5 M, 11.75 mL) slowly at −78 °C under N_2 atmosphere, and the reaction was stirred for an hour. A solution of 1-bromooctane (7.62 g) in HMPA (16 mL) was then added via syringe. After being stirred for 3.5 h, the reaction was warmed to room temperature for an additional 3 h. The mixture was treated with saturated NH_4Cl (50 mL) and extracted with ether (100 mL). The organic layer was washed with brine and dried (Na_2SO_4). The crude product was purified by column chromatography on silica gel, yielding monosubstituted product **9a** (3.460 g, 41.7%). IR (neat) ν_{max}: 3300 (s), 2920 (s), 2860 (s), 2280 (w), 2200 (w), 2100 (w), 1470 (s), 1440 (s), 1380 (m), 1350 (s), 1280 (m), 1240 (m), 1090−1140 (brs) cm^{-1}. ^{1}H NMR (90 MHz, CCl_4) δ_H: 4.06 (4H, m), 3.58 (8H, m), 2.26 (1H, m), 2.14 (2H, m), 1.30 (12H, m), 0.89 (3H, m) ppm. EIMS (*m*/*z*, %): 167 (0.8), 137 (9), 83 (26), 81 (100), 55 (98).

Compound 9b. The procedure was the same as for **9a**, yielding 42%. IR (neat) ν_{max}: 3250 (s), 2920 (s), 2850 (s), 2200 (w), 1460 (s), 1350 (m), 1130 (s), 1100 (s) cm^{-1}. ^{1}H NMR (60 MHz, CCl_4) δ_H: 4.17 (4H, m), 3.63 (12H, m), 2.33 (1H, m), 2.26 (2H, m), 1.39 (10H, m), 0.94 (3H, m) ppm. EIMS (*m*/*z*, %): 225 (0.6), 211 (4), 171 (5), 127 (32), 99 (13), 81 (93), 43 (100).

Compound 10a. To a solution of **9a** (588 mg) in 15 mL of anhydrous THF, were added *n*-butyllithium (2.4 M in hexane, 0.86 mL) and $BF_3{\cdot}Et_2O$ (0.268 mL), respectively at −78 °C, and the mixture was stirred for 15 min. The epoxide **7** (300 mg) in THF (10 mL) was added via syringe. The reaction was stirred for 2 h, quenched with saturated NH_4Cl (10 mL), and then allowed to warm to room temperature. The mixture was extracted with ether and dried (Na_2SO_4). The crude product was purified by column chromatography on silica gel, giving a light yellow oil, **10a** (556 mg, 94%). IR (neat) ν_{max}: 3450 (m), 2940 (s), 2860 (s), 2200 (w), 1780 (s), 1470 (m), 1380 (m), 1240 (m), 1100 (brs) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 4.69 (2H, s), 4.35 (1H, dq, J = 6.2 Hz, 6.2 Hz), 4.21 (2H, t, J = 2.1 Hz), 4.19 (2H, t, J = 2.1 Hz), 4.13 (1H, dd, J = 7.1 Hz), 3.67−3.77 (9H, m), 3.40 (3H, s), 2.65 (1H, dt, J = 6.7 Hz, 6.7 Hz), 2.48 (1H, ddt, J = 16.7, 4.6, 2.2 Hz), 2.34 (1H, ddt, J = 16.6, 6.9, 2.1 Hz), 2.21 (2H, tt, J = 7.0, 2.1 Hz), 1.8−2.0 (2H, m), 1.6−1.7 (1H, m), 1.2−1.6 (27H, m), 0.89 (3H, s) ppm. EIMS (*m*/*z*, %): 580 ([M − CH_3]$^+$, 1), 550 (2), 351 (1), 287 (5), 257 (7), 255 (15), 225 (22), 45 (100).

Compound 10b. The procedure was the same as that for **10a**, yielding 85%. IR (neat) ν_{max}: 3460 (m), 2940 (s), 2870 (s), 2200 (w), 1780 (s), 1460 (s), 1355 (s), 1100 (s) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 4.67 (3H, m), 4.20 (4H, m), 4.03 & 4.08 (1H, m), 3.66 (13H, m), 3.39 (3H, s), 2.60 (1H, m), 2.42 (1H, m), 2.37 (1H, m), 2.19 (2H, m), 1.26−1.95 (27H, m), 0.88 (3H, s) ppm. EIMS (*m*/*z*, %): 625 ([MH]$^+$, 0.9), 607 ([M − H_2O]$^+$, 0.5), 593 (0.1), 339 (14), 255 (20), 225 (65), 45 (100).

Compound 11a. A mixture of **10a** (145 mg), $PtO_2{\cdot}H_2O$ (12 mg), and EtOAc (3 mL) was stirred at room temperature for 24 h under hydrogen. After filtration and removal of the solvent, the residue was purified by column chromatography on silica gel, affording a colorless liquid, **11a** (89 mg, 61%). IR (neat) ν_{max}: 3450 (s), 2920 (s), 2860 (s), 1780 (s), 1460 (s), 1380 (m), 1350 (m), 1240 (m), 1180 (s), 1100 (s), 1050 (s) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 4.68 (2H, s), 4.34 (1H, dq, J = 6.2 Hz, 6.2 Hz), 3.74 (1H, dd, J = 7.1 Hz, 5.7 Hz), 3.56−3.67 (8H+1H, m), 3.46 (4H, t+t), 3.39 (3H, s), 2.65 (1H, dt, J = 6.7 Hz, 6.7 Hz), 1.2−1.9 (41H, m), 0.88 (3H, t, J = 6.7 Hz) ppm. EIMS (*m*/*z*, %): 604([MH]$^+$, 2), 586 (6), 343 (8), 341 (26), 325 (17), 297 (19), 243 (22), 85 (100), 45 (87).

Compound 11b. The procedure was the same as that for **11a**, except PtO_2 was replaced with 10% Pd−C (wet, 50%) as the catalyst, yielding 24%. IR (neat) ν_{max}: 3460 (m), 2920 (s), 2860 (s), 1790 (s), 1470 (s), 1380 (m), 1355 (m), 1240 (m), 1100 (brm) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 4.65 (3H, m), 4.10 (1H, m), 3.65 (13H, m), 3.46 (4H, m), 3.39 (3H, s), 2.62 (1H, m), 1.15−2.05 (39H, m), 0.88 (3H, t, J = 6.6 Hz) ppm. EIMS (*m*/*z*, %): 634 ([MH]$^+$, 4), 616 (7), 341 (24), 325 (23), 311 (11), 229 (16), 85 (58), 45 (100).

2-(8′-Hydroxyl-13′,16′,19′-trioxotriacontyl)-4-methylbutenic 2-Lactone (4). To the solution of **11a** (126 mg) in anhydrous THF (3 mL) under nitrogen was added DBU (98%, 71 μL). The reaction completed after refluxing for 2 h. The

mixture was cooled, several drops of acetic acid were added, and the mixture was evaporated to dryness. Column chromatography on silica gel of the residue gave a white solid, **4**, with low melting point (103 mg, 91%). Mp: 49.2−50.6 °C. $[\alpha]_D^{20} = -4.46°$ (0.11, $CHCl_3$). IR (neat) ν_{max}: 3450 (br, w−m), 2920 (s), 2860 (s), 1760 (s), 1460 (m), 1380 (m), 1240 (m), 1120 (s), 1030 (s) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ_H: 6.98 (1H, d, J = 1.5 Hz), 4.99 (1H, dq, J = 6.9 Hz, 1.6 Hz), 3.57−3.66 (8H+1H, m), 3.46 (4H,tt), 2.27 (2H, t, J = 7.7 Hz), 1.26−1.68 (27H, m), 0.88 (3H, t, J = 6.7 Hz) ppm. EIMS (*m*/*z*, %): 524 ([M^+ − OH], 2), 342 (7), 281 (29), 279 (55), 263 (39), 243 (16), 181 (23), 85 (100). Anal. Calcd for $C_{32}H_{60}O_6$: C, 71.07; H, 11.18. Found: C, 70.74; H, 11.12.

2-(8-Hydroxyl-13′,16′,19′,22′-tetraoxadotriacontyl)-4-methylbutenic 2-Lactone (6). The procedure was the same as that of **4**; compound **6** was finally obtained as a white, low-melting solid (91%). Mp: 39−41 °C. $[\alpha]_D^{20}$ −17.7° (*c* 0.11, $CHCl_3$). IR (neat) ν_{max}: 3460 (m), 2940 (s), 2860 (s), 1760 (s), 1470 (m), 1350 (m), 1320 (m), 1100 (br) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ_H: 6.99 (1H, d, J = 1.4 Hz), 4.99 (1H, dq, J = 1.5, 6.7 Hz), 3.61 (12H+1H, m), 3.46 (4H, m), 2.26 (2H, t, J = 6.7 Hz), 1.2−1.7 (37H, m), 0.87 (3H, t, J = 6.7 Hz) ppm. EIMS (*m*/*z*, %): 431 (1), 229 (6), 185 (12), 139 (9), 97 (45), 43 (100). Anal. Calcd for $C_{33}H_{62}O_7$: C, 69.43; H, 10.95. Found: C, 69.30; H, 10.72.

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