Chem Biol Drug Des 2011; **77:** 206–211

Research Article

doi: 10.1111/j.1747-0285.2010.01077.x

Design, Synthesis, and Sustained-Release Property of 1,3-Cyclic Propanyl Phosphate Ester of 18β-Glycyrrhetinic Acid

Weizhi Sun, Weibing Peng, Guoqiang Li and Tao Jiang*

Key Laboratory of Marine Drugs, Ministry of Education of China, School of Pharmacy, Ocean University of China, Qingdao 266003, China

**Corresponding author: Tao Jiang,* jiangtao@ouc.edu.cn

A new class of potential prodrugs, 1,3-cyclic propanyl phosphate esters of 18β-glycyrrhetic acid, was designed and synthesized through the key reaction of 18β-glycyrrhetic acid with 1,3-cyclic propanyl phosphate ester catalysed by lithium diisopropylamide. The sustained-release properties of 1,3-cyclic propanyl phosphate esters of 18β-glycyrrhetic acid *in vivo* were also investigated. The animal experiments showed that 18β-glycyrrhetic acid was released from 1,3-cyclic propanyl phosphate esters of 18β-glycyrrhetic acid at a steady rate in rats and the plasma concentrations of 18β-glycyrrhetic acid were nearly stable. The result indicated that 1,3-cyclic propanyl phosphate esters of 18β-glycyrrhetic acid have sustained-release properties to avoid the quick metabolism of 18β-glycyrrhetic acid. These prodrugs are highlighted as a promising new strategy to improve 18β-glycyrrhetic acid metabolism.

Key words: 18β-glycyrrhetinic acid, prodrug, sustained-release properties, the mean residence time

Received 11 May 2010, revised 27 September 2010 and accepted for publication 18 December 2010

Glycyrrhiza uralensis, as one of most common herbal formulae with the functions of regulation of lung ventilation, relief of internal fever, and 'harmonizing' the other ingredients in the formula, has been clinically used in Chinese Traditional Medicine for thousands of years. Glycyrrhizin is one of the major active ingredients in licorice. Its aglycone, 18β-glycyrrhetinic acid (GA) (1,2), can be produced by hydrolysing glycyrrhizin through gastric acid or β-glucuronidase *in vivo* and has been used in the treatment of peptic ulcer and many inflammations, such as hepatitis (3). GA also has the bioactivities of anti-inflammatory, anti-allergic, anti-tumour, and anti-ulcer effects (4–6). Some of its derivatives have been used as drugs, for instance Carbenoxolone for the treatment of oesophageal ulceration and inflammation. Thus, studies on GA and its derivatives raises prospects for novel and potent drugs. However, pharmacokinetics studies showed that about half of 10 mg/kg GA after intravenous administration (i.v.) was excreted through bile in 90 min (7). After i.v. injection of 1 mg/kg GA, the half-life of GA in plasma was only 0.153 h and the mean residence time (MRT) was 1.209 h (8). The fast excretion property of GA *in vivo* limits its utilization as drugs. The MRT of GA could be longer through oral administration, but the plasma concentration was much lower than injection (9). Therefore, higher dose of GA should be used to give the same effect as injection. This can increase GA's side effects, such as hyperaldosteronism.

The structural modification of GA is an effective method to prolong GA MRT. In this work, it was found that application of 1,3-Diol cyclic phosphate prodrugs (HepDirect prodrugs) was an appropriate method to alter GA metabolism.

HepDirect prodrug is a new class of prodrugs, consisting of a 1,3-cyclic propanyl phosphate ester with a C4-aryl ring substituent. In previous research studies, these esters of phosphates and phosphonates were developed for targeting various nucleoside monophosphates (NMP) to livers (10,11). The mechanism has been confirmed as following procedure: The cyclic phosphate prodrug is primarily oxidized to produce free phosphates by liver CYP3A4 and CYP2C19. The free phosphate is then dephosphorylated by microsomal phosphatases to obtain the parent drug, as shown in Figure 1 (12). This kind of prodrugs has been considered as a potential deliver for drugs with hydroxyl groups (13–16). The mechanism also indicated that the HepDirect prodrug can deliver the original drug gradually, and delay the elimination of the original drug indirectly.

The sustained-release property of the prodrug provides a new strategy to improve GA metabolism. Two GA prodrugs, 1,3-cyclic propanyl phosphate esters of GA (PGAs), as shown in Figure 2, were designed and synthesized in this work. To release GA smoothly *in vivo* and extend GA's MRT, PGA was produced by a two-step bioconversion process with catalysts of CYP enzymes and alkaline phosphatases. Animal experiments were carried out to investigate the sustained-release properties of PGA.

Experimental

Materials

GA and glycyrrhetinic acid methyl ester (GA-Me) were purchased from Nanjing Zelang YiYao Ltd. (Nanjing, China). Male Wistar rats of 200–230 g were purchased from Qingdao Institute of Drug Control (SCXK2008010), Qingdao, China.

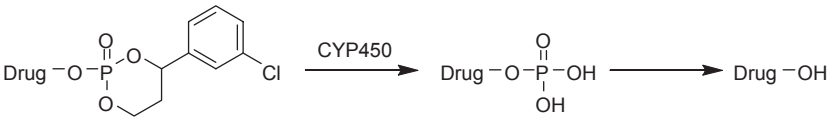


Figure 1: Proposed bioconversion of cyclic phosphate prodrugs to alcohols in hepatocytes.

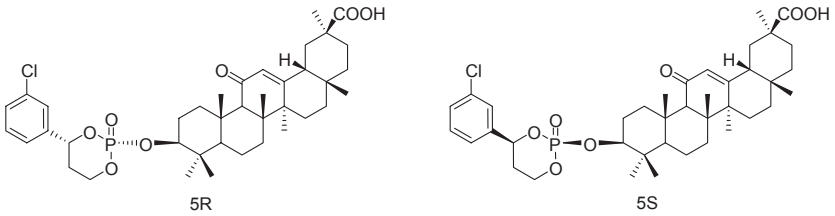


Figure 2: Structures of prodrugs of 18β-glycyrrhetinic acid (GA) (PGAs).

General methods

Melting points of the samples were determined with a Mel-temp II apparatus and were uncorrected. Column chromatography was performed using silica gel (200–300 mesh) purchased from MeiGao Ltd. (Qingdao, China). TLC was performed on a silica gel plate purchased from Merck Ltd. (Darmstadt, Germany). ^{1}H NMR and ^{13}C NMR spectra were taken on a Jeol JNM-ECP 600 spectrometer (Jeol Ltd., Tokyo, Japan) with tetramethylsilane (Me_4Si) as the internal standard, and chemical shifts were recorded as δ values. Mass spectra were recorded on a Q-TOF Global mass spectrometer. Optical rotations were recorded at 20 °C on a Jasco P-1020 polarimeter. HPLC were performed using 1100A (Agilent Ltd., Santa Clara, CA, USA) with a Kromasil ODS C_{18} column(4.6 × 250 mm, 5 μm, KR100-5C_{18}) and methanol/0.5% acetic acid aqueous solution (86:14) mixture as eluent. The flow rate was 1 mL/min with a column temperature of 25 °C. Samples eluted around 12–25 min and were monitored at 250 nm.

Synthesis

3-chlorophenyl-2-oxo-propionate (2)

A 70% sodium hydride-in-oil suspension (14.2 g, 414 mmol) was added slowly to a cold solution of 3-chlorophenylethanone (30.9 g, 200 mmol) in diethyl carbonate (300 mL) at room temperature, and then the reaction solution was stirred at 85 °C for 2 h. The mixture was poured into an ice-cold water (500 mL) solution containing acetic acid (51 mL) and extracted with three portions of ether (400 mL). The organic phase was dried and evaporated, and the residual oil was distilled at reduced pressure to give 42.0 g (92.7%) of **2**. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.86 (s, 1H), 7.76 (d, *J* 6.6 Hz, 1H), 7.50 (d, *J* 7.8 Hz, 1H), 7.37 (t, *J* 8.4 Hz, 1H), 4.16 (q, *J* 7.2 Hz, 2H), 3.92 (s, 2H), 1.21 (t, *J* 7.2 Hz, 3H). ESI-MS *m/z*: 249.0[M+Na]$^+$.

1-(3-Chlorophenyl)-1,3-propanediol (3)

3-chlorophenyl-2-oxo-propionate (**2**) (20.0 g, 88.2 mmol) was dissolved in MeOH (150 mL), and then sodium borohydride (10.0 g, 265 mmol) was added slowly at room temperature. The mixture was stirred until the reaction was completed based on TLC monitoring. After the solvent was evaporated, the residue was taken up in ethyl acetate, and washed with brine solution. The aqueous layer was extracted with ethyl acetate as many times as necessary. The organic layer was dried and concentrated. The oily residue was further purified by chromatography with petroleum ether/ethyl acetate to give 14.2 g (86%) of **3** as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.34 (s, 1H), 7.26–7.21 (m, 2H), 7.19 (d, *J* 7.2 Hz, 1H), 4.88 (q, *J* 4.2Hz, 1H), 3.80 (m, 2H), 3.28 (br s, 2H), 1.89 (m, 2H). ESI-MS *m/z*: 187.0[M+H]$^+$.

(R)-(+)-1-(3-chlorophenyl)-1,3-propanediol (3R) and (S)-(−)-1-(3-chlorophenyl)-1,3-propanediol (3S)

To a solution of diol **3** (13.5 g, 72.3 mmol), HMDS (23.4 g, 145 mmol), and THF (50 mL) were added a few drops of TMSOTf. The mixture was stirred for 1 h at room temperature. After the solvent was evaporated, the residue was taken up in ethyl acetate, washed twice with brine solution, dried and concentrated to give 21.7 g (90.4%) of 1-(3-chlorophenyl)-l,3-bis(trimethylsi1oxy) propane. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.32 (s, 1H), 7.23–7.21 (m, 1H), 7.21–7.18 (m, 2H), 7.81 (q, *J* 4.2 Hz, 1H), 3.70 (m, 1H), 3.54 (m, 1H), 1.86 (m, 1H), 1.80 (m, 1H), 0.12 (s, 9H), 0.05 (s, 9H). ESI-MS *m/z*: 331.1[M+H]$^+$.

To a solution of 1-(3-chlorophenyl)-l,3-bis(trimethylsi1oxy) propane (26.1 g, 78.9 mmol), (−)-menthone (14.6 g, 94.6 mmol) and CH_2Cl_2 (150 mL) at −40 °C, TMSOTf (1.72 mL, 9.46 mmol) was added. The mixture was stirred at −40 °C for 24 h, and then the reaction was quenched by adding pyridine. The mixture was diluted with petroleum ether and washed with aqueous $NaHCO_3$. The organic layer was dried and concentrated. The residue was purified by chromatography with petroleum ether/ethyl acetate to give 12.7 g (50%) of (2R,8R,11S)-2-(3-chlorophenyl)-8-methyl- 11-(1-methylethyl)-1,5-dioxaspiro[5.5]undecane and 12.7 g (50%) of (2S,8R,11S)-2-(3-chlorophenyl)-8-methyl-11-(1-methylethyl)-1,5-dioxaspiro[5.5]undecane.

(2R,8R,11S)-2-(3-chlorophenyl)-8-methyl-11-(1-methylethyl)-1,5-dioxaspiro[5.5]undecane: ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.36 (s, 1H), 7.30–7.22 (m, 3H), 5.03 (d, *J* 12 Hz, 1H), 4.02 (t, *J* 12 Hz, 1H), 3.86 (dd, J_1 4.2, J_2 11.4 Hz, 1H), 2.82 (d, *J* 13.2 Hz, 1H), 2.58 (m, 1H), 1.84–1.72 (m, 2H), 1.62 (d, *J* 13.2 Hz, 1H), 1.59–1.54 (m, 2H), 1.50–1.42 (m, 1H), 1.34 (d, *J* 12.6 Hz, 1H), 0.98–0.95 (t, *J* 6 Hz, 6H), 0.93 (d, *J* 6.6 Hz, 3H), 0.90 (d, *J* 9.6 Hz, 1H), 0.81 (t, *J* 13.2 Hz, 1H). ESI-MS *m/z*: 323.2[M+H]$^+$.

(2S,8R,11S)-2-(3-chlorophenyl)-8-methyl-11-(1-methylethyl)-1,5-dioxaspiro[5.5]undecane: ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.36 (s, 1H), 7.29–7.22 (m, 3H), 4.85 (d, *J* 11.4 Hz, 1H), 4.25 (t, *J* 12 Hz, 1H), 3.91 (dd, J_1 4.8, J_2 11.4 Hz, 1H), 2.83 (d, *J* 12.6 Hz, 1H), 2.50 (m, 1H), 2.05–1.90 (m, 2H), 1.85–1.81 (m, 1H), 1.73–1.67 (m, 2H), 1.55–1.53 (m, 1H), 1.52–1.49 (m, 1H), 1.31–1.27 (m, 1H), 0.98 (d, *J* 6.6 Hz, 3H), 0.95 (d, *J* 6.6 Hz, 3H), 0.91 (d, *J* 6.6 Hz, 3H), 0.80 (t, *J* 13.2 Hz, 1H). ESI-MS *m/z*: 323.1[M+H]$^+$.

A concentrated hydrochloric acid of 8 mL was added to a solution of (2R,8R,11S)-2-(3-chlorophenyl)-8-methyl-11-(1-methylethyl)-1,5-dioxaspiro[5.5]undecane or (2S,8R,11S)-2-(3-chlorophenyl)-8-methyl-11-(1-methylethyl)-1,5-dioxaspiro[5.5]undecane (10.5 g, 32.6 mmol) in MeOH (70 mL) at room temperature. The mixture was stirred for 10–20 h. Water was then added. MeOH was removed *in vacuo*. The mixture was extracted three times with ethyl acetate. The combined extracts were dried and concentrated. The residue was purified by chromatography with petroleum ether/ethyl acetate to obtain the optically active diol 5.04 g (82.8%) of **3R** or 4.78 g (69.3%) of **3S**. **3R**: $[\alpha]_D$ +54.3° (*c* 0.96, $CHCl_3$). **3S**: $[\alpha]_D$ −50.8° (*c* 0.90, $CHCl_3$).

(4R)-(+)-*trans*-2-(4-nitrophenoxy)-2-oxido-(3-chlorophenyl)-1,3,2-dioxaphosphorinane (4R) and (4S)-(−)-*trans*-2-(4-nitrophenoxy)-2-oxido-(3-chlorophenyl)-1,3,2-dioxaphosphorinane (4S)

A solution of **3R** or **3S** (3.89 g, 20.8 mmol) and Et_3N (10.7 mL, 77.5 mmol) in THF (50 mL) was added dropwise to a solution of 4-nitrophenoxyphosphorodichloridate (9.9 g, 38.7 mmol) in THF (8 mL) at 0 °C. The starting diol was consumed in 2 h. Additional Et_3N (10.7 mL, 77.5 mmol) and 4-nitrophenol (9.9 g, 38.7 mmol) were added. The mixture was stirred overnight. After the solvent was evaporated, the residue was taken up in ethyl acetate, washed with water, 0.4 M NaOH, and brine. It was dried and concentrated. The residue was purified by chromatography with petroleum ether/ethyl acetate to give 4.8 g (62.3%) of **4R** or 5.4 g (78.3%) of **4S**.

4R: mp 113–116 °C; $[\alpha]_D$ +90.1° (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 8.26 (d, *J* 8.4 Hz, 2H), 7.45 (d, *J* 8.4 Hz, 2H), 7.41 (s, 1H), 7.37–7.34 (m, 2H), 7.30–7.28 (m, 1H), 5.57 (dd, J_1 1.8, J_2 12 Hz, 1H), 4.67–4.56 (m, 2H), 2.45–2.37 (m, 1H), 2.13–2.09 (m, 1H). ESI-MS *m/z*: 370.1[M+H]$^+$.

4S: mp 112–115 °C; $[\alpha]_D$ −90.8° (*c* 1.30, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 8.25 (d, *J* 9 Hz, 2H), 7.45 (d, *J* 9.6 Hz, 2H), 7.41 (s, 1H), 7.37–7.35 (m, 2H), 7.29–7.26 (m, 1H), 5.57 (dd, J_1 2.4, J_2 12 Hz, 1H), 4.67–4.55 (m, 2H), 2.45–2.36 (m, 1H), 2.13–2.09 (m, 1H). ESI-MS *m/z*: 370.1[M+H]$^+$.

cis-3-*O*-[4-(R)-(3-Chlorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]-18β-glycyrrhetinic acid (5R) and *cis*-3-*O*-[4-(S)-(3-Chlorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]-18β-glycyrrhetinic acid (5S)

A solution of 18β-glycyrrhetinic acid (1.26 g, 2.68 mmol) in THF (30 mL) was treated with a THF solution of 2 M lithium diisopropylamide (LDA) (6.7 mL, 13.4 mmol) and stirred at room temperature. After 2 h, **4R** or **4S** (1.5 g, 4.06 mmol) was added in one portion. The mixture was stirred at room temperature for 48 h and quenched with saturated NH_4Cl. Then THF was removed *in vacuo*. 1 M HCl was dropped to the mixture at 0 °C until pH <3. The mixture was extracted with CH_2Cl_2. The organic layer was dried and concentrated. The residue was purified by chromatography to obtain 0.89 g (47.3%) of **5R** or 0.88 g (46.8%) of **5S**.

5R: mp>200 °C; $[\alpha]_D$ +102.4° (*c* 2.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.41–7.25 (m, 4H, Ar-H), 5.69 (s, 1H, 12-H), 5.38 (dd, 1H, J_1 1.2, J_2 11.4 Hz, 4′-H), 4.53–4.40 (m, 2H, 6′-H), 4.26–4.21 (m, 1H, 3-H), 2.29–2.23 (m, 2H, 5′-H), 1.36 (s, 3H, 27-H), 1.21 (s, 3H, 29-H), 1.16 (s, 3H, 25-H), 1.12 (s, 3H, 23-H), 1.08 (s, 3H, 26-H), 0.92 (s, 3H, 24-H), 0.82 (s, 3H, 28-H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 200.1 (11-C), 181.2 (30-C), 169.7 (13-C), 140.9 (1″-C), 134.8 (3″-C), 130.1 (5″-C), 128.8 (2″-C), 128.4 (12-C), 125.7 (6″-C), 123.6 (4″-C), 86.7 (3-C), 79.8 (4′-C), 67.6 (6′-C), 61.6 (9-C), 54.9 (5-C), 48.2 (18-C), 45.4 (14-C), 43.8 (20-C), 43.2 (8-C), 40.9 (5′-C), 39.1 (4-C), 39.1 (1-C), 38.6 (19-C), 37.7 (22-C), 36.7 (10-C), 34.0 (2-C), 32.7 (17-C), 31.9 (7-C), 30.9 (16-C), 28.5 (21-C), 28.4 (29-C), 28.1 (23-C), 26.5 (15-C), 26.4 (27-C), 25.2 (24-C), 23.4 (28-C), 18.7 (25-C), 17.5 (6-C), 16.4 (26-C). ^{31}P NMR ($CDCl_3$, 243MHz) δ: −7.40. ESI-MS *m/z*: 701.5[M+H]$^+$; HRMS (ESI): calcd for $C_{39}H_{54}ClO_7PNa$: 723.3228 found 723.3193.

5S: mp>200 °C; $[\alpha]_D$ +55.3° (*c* 2.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.41–7.26 (m, 4H, Ar-H), 5.71 (s, 1H, 12-H), 5.42 (dd, 1H, J_1 1.8, J_2 11.4 Hz, 4′-H), 4.51–4.38 (m, 2H, 6′-H), 4.27–4.22 (m, 1H, 3-H), 2.29–2.18 (m, 2H, 5′-H), 1.36 (s, 3H, 27-H), 1.23 (s, 3H, 29-H), 1.18 (s, 3H, 25-H), 1.13 (s, 3H, 23-H), 1.05 (s, 3H, 26-H), 0.90 (s, 3H, 24-H), 0.83(s, 3H, 28-H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 200.2 (11-C), 181.1 (30-C), 169.7 (13-C), 141.2 (1″-C), 134.7 (3″-C), 130.0 (5″-C), 128.7 (2″-C), 128.4 (12-C), 125.7 (6″-C), 123.5 (4″-C), 86.6 (3-C), 80.1 (4′-C), 67.2 (6′-C), 61.6 (9-C), 54.9 (5-C), 48.2 (18-C), 45.4 (14-C), 43.8 (20-C), 43.2 (8-C), 40.9 (5′-C), 39.1 (4-C), 39.0 (1-C), 38.7 (19-C), 37.7 (22-C), 36.7 (10-C), 34.1 (2-C), 32.7 (17-C), 31.9 (7-C), 30.9 (16-C), 28.5 (21-C), 28.4 (29-C), 28.1 (23-C), 26.5 (15-C), 26.4 (27-C), 25.2 (24-C), 23.3 (28-C), 18.7 (25-C), 17.5 (6-C), 16.4 (26-C). ^{31}P NMR ($CDCl_3$, 243 MHz) δ: −7.16. ESI-MS *m/z*: 701.4[M+H]$^+$; HRMS (ESI): calcd for $C_{39}H_{54}ClO_7PNa$: 723.3212 found 723.3193.

Biology

Aqueous solubility

To increase the aqueous solubility of **5R** and **5S**, PGAs were converted to the relevant sodium salts (PGAs-Na) by the reactions with sodium hydroxide in ethanol. Solubility of PGAs-Na was measured by dissolving prodrug of 5 mg/mL in PBS at pH 7.4. Samples were then sonicated for 5 min in a sonication bath and stayed at room temperature for 10 min. After filtration with 0.45 μm micro-pore filter membrane, the samples were diluted 1: 100 in DMSO and anal-

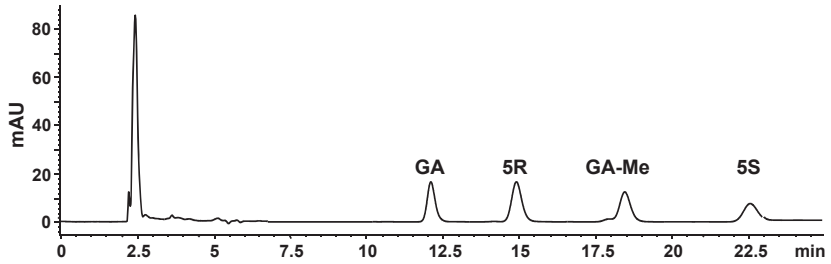


Figure 3: The retention times of 18β-glycyrrhetinic acid (GA), **5R**, **5S**, and glycyrrhetinic acid methyl ester (GA-Me).

ysed by HPLC. The aqueous solubility of PGAs-Na was more than 5 mg/mL.

Stability assay

The measurement of prodrug stability was performed using the sodium salts of **5R** or **5S** with concentrations of 100 μM in 10 mM phosphate buffer of pH 7.4 at 37 °C. Intact prodrug was quantified by HPLC technique. The t_{90} (time to 90% of starting amount) was longer than 3 days.

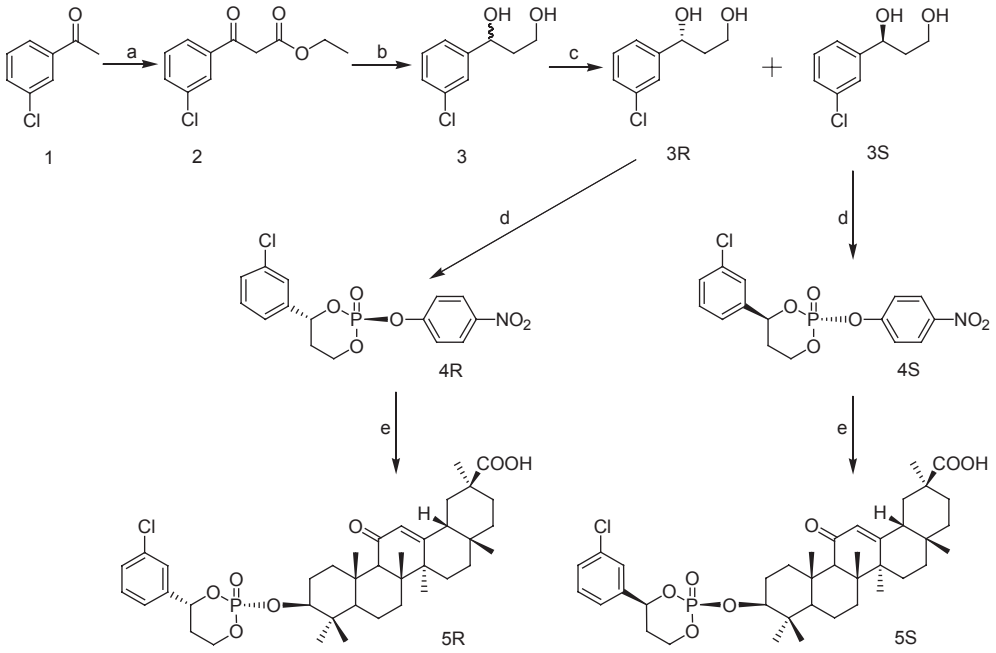
Dosing and sample collection

After intraperitoneal injection (i.p.) of the aqueous solutions of 44 mg/kg sodium salts of **5R** and **5S** to male Wistar rats, rat blood sample was collected at 0.5, 1, 2, 4, 8, 12, 24, 36, and 48 h *via* the post-orbital venous plexus veins and centrifuged at 1 000 g to isolate plasma. The supernatants were treated by methyl cyanides to remove protein and then clarified by volution and centrifugation at 16 000 g. After drying under flowing nitrogen gas at 40 °C, the supernatants were dissolved by methanol. GA-Me was added as the internal standard. The plasma concentrations of **5R**, **5S**, and GA were determined by HPLC as previously described. The retention times of compound were tested as shown in Figure 3. In this condition, the determination was not disturbed by endogenous substances in plasma. The retention times of GA, **5R**, **5S**, and GA-Me were 12.2, 14.9, 22.6 and 18.4 min, respectively.

Results and Discussion

Chemistry

PGAs (**5R**, **5S**) consist of two parts. One part is GA, which can be purchased and replaced by other drugs with hydroxyl groups. The other part is 1,3-cyclic propanyl phosphate groups. It can be replaced by cyclic intermediate with different groups. PGAs were synthesized by reacting GA with 1,3-cyclic propanyl phosphate ester through an ester exchange reaction.



Scheme 1: Synthesis process of cyclic phosphates. Reagents and conditions: (a) (i) diethyl carbonate, NaH, 80 °C; (ii) AcOH; (b) $NaBH_4$, MeOH, room temperature; (c) (i) HMDS, TMSOTf, room temperature; (ii) (−)-menthone, TMSOTf, −40 °C; (iii) HC1, MeOH; (d) 4-nitrophenyloxy dichlorophosphate, Et_3N, 4-nitrophenol, room temperature; (e) 18β-glycyrrhetinic acid (GA), lithium diisopropylamide, room temperature.

Scheme 1 shows the synthetic process of PGAs. The first step is to synthesize the corresponding 1,3-cyclic propanyl phosphate ester. 3-chlorophenylethanone was reacted with diethyl carbonate in the presence of sodium hydride at 80 °C and then was treated with AcOH to obtain 3-chlorophenyl-2-oxo-propionate (**2**) (16). This β-keto ester was reduced by $NaBH_4$ at room temperature to obtain two racemic diols (**3**) (17). The racemic diols first reacted with HMDS in the presence of catalyst of TMSOTf and then reacted with (−)-menthone at −40 °C to form diastereomeric (−)-menthone ketals. The two ketals were separated by column chromatography of silica gel. After the removal of (−)-menthone, two enantiomeric 1,3-propanediols (**3R**, **3S**) with high optical purity were obtained (18). **3R** and **3S** reacted with 4-nitrophenyl dichlorophosphate in the presence of 4 equivalents of 4-nitrophenol and Et_3N to produce the relevant enantiomeric cyclic phosphate intermediate **4R** and **4S**, respectively (>95% *trans*-isomer based on ^1H NMR) (11).

The final and most important step was ester exchange reaction. Several bases, such as *tert*-BuMgCl, *n*-BuLi, *t*-BuOK, and LDA, were used in the reaction. It was found that only LDA was feasible. This result illustrates that the alkalinity and nucleophilicity of LDA were just suitable for this reaction. The cyclic phosphate intermediates reacted with GA in the presence of LDA at room temperature to yield 1,3-cyclic propanyl phosphate ester (**5R**, **5S**) with high optical purities. To increase the yields and the conversion rates, excess LDA of 5 times was used in the process.

Biology

To investigate the sustained-release properties of PGA, the animal experiments were carried out in this work. The plasma levels of both diastereomeric *cis*-prodrugs of **5R** and **5S**, as well as GA, were investigated *in vivo* rats. After the i.p. administration of the aqueous solutions of PGAs-Na (44 mg/kg), rat blood sample was collected at different times. After the post-treatments, the plasma concentration was determined by HPLC technique.

Plasma concentration-time profiles of **5R** and **5S**, and GA in rats were shown in Figures 4 and 5, respectively. The plasma concentration of **5R** was at least twice that of **5S** in 24 h after administration, as shown in Figure 4. In the first 2 h, the plasma concentration of GA released by **5R** was obvious higher than **5S**. However, no significant different plasma concentration was observed for GA after 2 h, as shown in Figure 5. This suggested that the metabolism of **5R** was slower than **5S**.

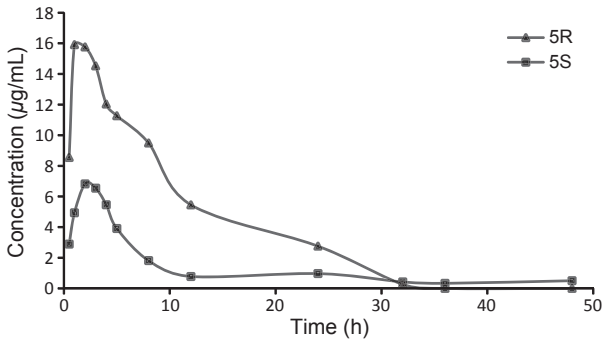


Figure 4: Plasma concentration–time profiles of **5R** and **5S** after i.p. 44 mg/kg the sodium salts of 1,3-cyclic propanyl phosphate esters of GA (PGAs-Na) in rats.

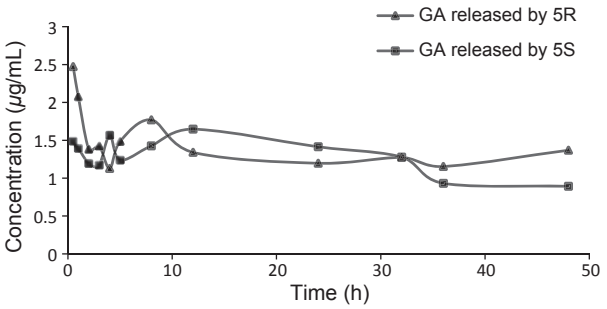


Figure 5: Plasma concentration–time profiles of released 18β-glycyrrhetinic acid (GA) by **5R** and **5S** after i.p. 44 mg/kg the sodium salts of 1,3-cyclic propanyl phosphate esters of GA (PGAs-Na) in rats.

After i.p. administrations of PGAs, the plasma concentrations of GA were relatively stable in 48 h. This result showed that GA retention time was remarkably extended.

Conclusions

Cyclic 1-(aryl)-1,3-propanyl prodrugs of nucleotide and nucleoside analogues were used to increase NTP levels in livers (19). A new strategy was found to study the mechanism of this kind of prodrugs in this work. A novel type of prodrugs of PGAs was designed to change GA metabolism by the sustained-release properties of 1,3-cyclic propanyl phosphate esters. The key reaction in the synthesis process is the coupling of GA and 1,3-cyclic propanyl phosphate ester through an ester exchange reaction. LDA was the most suitable base for this reaction. The results of animal experiments showed that the use of PGAs can obtain nearly stable plasma levels of GA in rats after i.p. administration. It successfully slowed down the GA metabolism. It is worth further studying PGAs properties in the future. This class of prodrugs provides a potential new strategy to improve GA metabolism.

Acknowledgments

This research was supported by Key International S&T Cooperation Projects (2008DFA31040) of Ministry of Science and Technology of the People's Republic of China.

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