SYNTHESIS OF A NOVEL 18β-GLYCYRRHETINIC ACID DERIVATIVE

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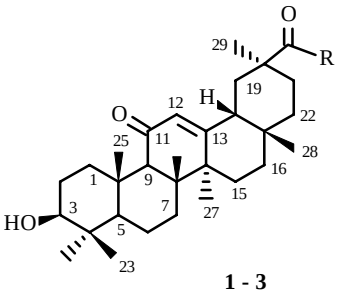
*A novel compound, biotinylated 18*β*-glycyrrhetinic acid (BGA), was synthesized. It is a compound of 18*β*-glycyrrhetinic acid linked with biotin.*

Key words: 18β-glycyrrhetinic acid, biotinylated 18β-glycyrrhetinic acid, biotin.

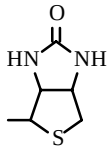
18β-Glycyrrhetinic acid (GA, **1**) is the aglycone of glycyrrhizin, a member of the triterpenoids. Recent study has shown that GA exhibits many pharmacological activities, such as anti-inflammatory (cortisol-like), anti-hepatotoxic, anti-bacterial, and anti-viral effects [1–5]. Biotin has a strong avidity for avidin and streptavidin. The biotin-(strept)avidin system has found widespread use in many analytical technology such as immunohistochemistry, enzyme-linked immunosorbent assay [6], and bioanalytical systems [7] and exhibits superiority to the conventional methods. Especially, in drug-immobilizing research, biotinylated taxol, FK506, and doxorubicin have been synthesized and immobilized to the wells of plates in order to screen their drug targets via phage display technology [8–10].

In this study, 29-carboxyl of GA was activated and then linked to 1,6-hexanediamine, after which the product **2** was attached to a molecule of biotin and a novel compound, biotinylated 18β-glycyrrhetinic acid (BGA, **3**), was synthesized for the first time. Because biotin has a carboxyl group too, BGA can also be obtained in another way, in which biotin is linked to **1**, 6-hexanediamine first.



1 - 3

1: R = OH
2: R = $NH(CH_2)_6NH_2$
3: R = $NH(CH_2)_6NHCO(CH_2)_4$



N,*N*′-Dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (NHS) were used to activate the carboxyl group of GA, making the carboxyl react with the amine of 1,6-hexanediamine easily. Then the white deposit was removed by filtration. The activated GA was added to **1**, 6-hexanediamine, and the presence of an amine in **2** can be revealed by the lower R_f (0.1) value in a ninhydrin staining experiment. Upon the addition of active biotin, the former ninhydrin spot disappeared and a new spot with a slightly higher R_f (0.15) appeared, which is **3** (BGA). The crude mixture was subjected to column chromatography, which provided BGA in 38% yield. The purity of BGA is about 94.64% by HPLC analysis. In the biotin-initiated process, the

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efficiency of biotin activation is 56%, but the yield of product e is very low after active biotin was combined with 1,6-hexanediamine because of the outgrowth of biotin-NH-C_6-NH-biotin in the side reaction. The product of reaction e to c was detected by TLC (thin-layer chromatography).

Because the imidazolyl group of biotin is a part of the linkage to avidin or streptavidin, the quantization of imidazolyl of BGA is necessary for the continuous experiment. It was carried out by addition of *p*-dimethylaminobenzaldehyde to BGA at 60°C and the color of yellow would be developed as biotin did if free imidazolyl on BGA existed. The number of free imidazolyl groups on BGA is positively correlated with the OD410 value of the reaction. This suggested that BGA could be immobilized by avidin and streptavidin and could be used as a screening target for the phage-displayed library.

In the chemical synthesis process, we chose **1**, 6-hexanediamine, as a linker between GA and biotin, because it: 1) offers a reactive amino group to GA, resulting in an effective linkage to biotin; 2) introduces a long carbon chain, which can reduce the steric hindrance between the two molecules, and maintains the extended conformation of GA; 3) may reduce the negative influence on the biological activities of GA. However, as 1,6-hexanediamine has two amino groups, a side-compound GA-NH-C_6-NH-GA or biotin-NH-C_6-NH-biotin, can be produced in the process of GA or biotin linking with 1,6-hexanediamine, respectively, and such side reactions decrease the efficiency of formation of BGA.

In conclusion, we have synthesized a novel compound, BGA for the first time. Our study provides a feasibility example for studying the natural products or compounds using the biotin-(strept)avidin system, and the addition of biotin to these molecules will not alter their pharmacological activities. In the future, we will investigate more details of GA using an intermediate compound, -BGA, on the molecular device of GA and synthesize more biotinylated drugs to study their molecular mechanism.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Bruker AVANCE 500 instrument at a working frequency of 500 MHz, in CD_3OD with TMS internal standard.

TLC was performed using petroleum ether–ethylacetate–methanol (3:10:8). Spots were developed by ninhydrin in water with subsequent heating at 110°C for 5 min, for the GA-hexanediamine-NH_2 and biotin-hexanediamine-NH_2.

High-performance liquid chromatography (HPLC) was performed using a XDB-C18 column of 4.6 × 250 mm, 5 μm (Agilent Technology Co., USA) connected in a series circuit of a dual pump system (LC-ATVP, Shimadzu, Japan) followed by development with the mobile phase of acetonitrile–water (55:45) at a flow rate of 1.00 mL/min. BGA was detected at 250 nm by UV detector [11].

DMF was dried by molecular sieve. 1,6-Hexanediamine and CH_2Cl_2 were distilled over KOH. DCC and NHS were of analytical grade. The 18β-GA was purchased from Across Organics (USA). Biotin was purchased from Bioasia.

Synthesis of BGA. To a stirred solution of GA (300 mg, 0.638 mmol), DCC (131.7 mg, 0.638 mmol) and NHS (73.5 mg, 0.638 mmol) in dry CH_2Cl_2–dimethyl formanide (DMF) (5:1, 12 mL) were added; the reaction mass was stirred for 24 h at room temperature (RT) and then filtered to remove the white deposit. The solution was added slowly dropwise to a solution of 1,6-hexanediamine (70 mg, 0.603 mmol) in CH_2Cl_2 and stirred overnight. After washing with water (3 × 20 mL), the organic layers were dried over Na_2SO_4. Finally, the solution of biotin (97.6 mg, 0.4 mmol), DCC (82.5 mg, 0.4 mmol), and NHS (46 mg, 0.4 mmol), which had reacted for 24 h, was added to the mixture and the whole stirred for 4 h at room temperature and then concentrated in vacuum, gained crude. The crude mixture was subjected to silica gel column chromatography (from petroleum ether–ethylacetate–methanol, 1:3:0.2 to 1:3:2). The quantization of imidazolyl of BGA was carried out by adding *p*-dimethylaminobenzaldehyde reagent (DR) into the solution at 60°C and incubating it for 30 min, with detection at 410 nm in a plate reader [12]. The DR solution contained a mixture of 0.1 (w/v) *p*-dimethylaminobenzaldehyde and 1% (v/v) sulfuric acid in ethanol.

BGA yield 38% (an amorphous powder), R_f 0.15 (petroleum ether–ethylacetate–methanol, 3:10:8).
PMR spectrum (500MHz, CD_3OD, δ, ppm): 0.70 (3H, s, CH_3-24); 0.89 (3H, s, CH_3-28); 1.01 (3H, s, CH_3-25); 1.04 (3H, s, CH_3-26); 1.06 (1H, m, H-16); 1.08 (1H, m, H-1); 1.19 (4H, m, CH_3-29, H-1); 1.27 (3H, s, CH_3-29); 1.31 (1H, m, H-15); 1.33 (3H, s, CH_3-23); 1.35–1.37 (4H, m, H-22, $SCHCH_2CH_2CH_2$); 1.40 (1H, m, H-7); 1.40–1.46 (6H, m, $CONHCH_2CH_2(CH_2)_3$); 1.52–1.56 (5H, m, 2H, H-2;1H, H-6; 2H, $CONHCH_2CH_2$);1.58–1.66 (4H, q, $SCHCH_2CH_2$); 1.74–1.82 (2H, m, H-5, H-7); 1.82–1.90 (2H, m, H-15, H-19); 2.04–2.11 (4H, m, H-16, H-18, H-19, H-21); 2.36 (1H, s, H-9); 2.59–2.64 (2H, t,

$SCH(CH_2)_3CH_2$); 2.81–2.84 (1H, q, H-1); 3.06–3.11 (6H, all s, $CONHCH_2$, $CONH(CH_2)_5CH_2$, SCH_2); 3.15 (1H, q, SCH); 3.46–3.47 (1H, t, SCH_2CH); 3.50–3.51 (1H, dd, H-3); 3.57–3.58 (1H, t, SCHCH); 4.19–4.21 (1H, q, SCHCHNH); 4.38–4.40 (1H, q, SCH_2CHNH); 5.53 (1H, s, H-12); 7.57–7.60 (1H, t, CONH); 7.86 (1H, s, CH_2NH). Mass spectrum: *m/z* 817.5 ($[M+Na]^+$, 100); HREIMS *m/z* 795.13 (calcd for $C_{46}H_{74}N_4O_5S$ 795.17).

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