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Abstract: Based on classical drug design theory, a novel series of gentiopicroside derivatives was designed and synthesized. All synthesized compounds were then biologically evaluated for their inhibition of influenza virus and anti-HCV activity *in vitro*. Some of the gentiopicroside derivatives, such as **11a**, **13d** and **16** showed interesting anti-influenza virus activity with IC_{50} at 39.5 μM, 45.2 μM and 44.0 μM, respectively. However, no significant anti-HCV activity was found for all of gentiopicroside derivatives. The preliminary results indicate that modification of the sugar moiety on gentiopicroside was helpful for enhancing the anti-influenza activities. Our works demonstrate the importance of secoiridoid natural products as new leads in the development of potential antiviral inhibitors.

Keywords: Natural product, Secoiridoid, Gentiopicroside derivatives, Antiviral agents, Anti-influenza virus.

1. Introduction

Viral infections pose a threat to virtually every organism in every domain of life. Some are of great public health importance worldwide, such as influenza virus and Hepatitis C virus (HCV). Influenza virus is a major human pathogen that can cause annual epidemics and occasional pandemics. It was estimated that influenza epidemics cause 250 000 to 500 000 deaths every year worldwide [1]. Currently, two classes of anti-influenza drugs, M2 ion channel inhibitors and neuraminidase

inhibitors, are approved by the FDA for the treatment of influenza virus infection. However, resistance to individual antiviral drugs is probably to appear [2]. On the other hand, HCV is a major cause of chronic liver diseases which can lead to permanent liver damage, hepatocellular carcinoma and death [3]. The World Health Organization estimates that 130-170 million individuals have detectable antibodies to HCV worldwide, corresponding to 3% of the world's population. Prior to 2011, HCV infections were treated with a combination of pegylated interferon A and ribavirin [4]. In 2011 the protease inhibitors boceprevir and telaprevir became available to treat HCV infection with genotype 1 in combination with ribavirin and pegylated interferon [5, 6]. However, the SVR (Sustained Virologic Response) with current treatment is not optimal, and significant side effects (depression, fatigue, irritability, worsening of mania, insomnia) exist for these drugs. Therefore, there is still a grand challenge for the development of new antiviral inhibitors with unique scaffolds for higher efficacy and improved tolerability.

Natural products play a crucial role in the development of drugs for the treatment of human diseases [7], and to this very day numerous marketed drugs are of natural origin, either as original compounds or after modification [8]. It was found that 10% of the drugs on the market are unaltered natural products, 29% are their derivatives (semi-synthetics) and the rest (61%) have a synthetic origin [9]. The modification of natural products in an effort to alter their biochemical capacity is a common technique utilized by synthetic and medicinal chemists. Moreover, the structural modification of biologically active natural products aims at increasing potency and selectivity, improving physico-chemical, biochemical and pharmacokinetic properties, and eliminating or reducing side effects. More recently, the analogues of natural products are increasingly reported as antiviral inhibitors [10].

Gentiopicroside (GPS), a secoiridoid compound isolated from *Gentiana lutea* which is called *Qin Jiao* in Chinese (**Fig.1**), is one of the most common herbal medicines used in China. Animal experiments have revealed choleretic, anti-hepatotoxic, adaptogenic, and anti-inflammatory activities [11]. It has been investigated for its possible effects on the central nervous system, such as

antidepressant, anticonvulsant, and analgesic activities in mice [12]. Recently, Khuraman Mustafayeva *et al.* evaluated the possible genotoxic, mutagenic, and clastogenic effects of gentiopicroside [13]. Very recently, L. Yang *et al.* reported the hepatoprotective effect of gentiopicroside on anit-induced cholestatic liver injury in mice [14]. However, the main drawback of the gentiopicroside currently being evaluated in clinical trials is its relatively poor lipophilicity and suboptimal pharmacokinetic properties.

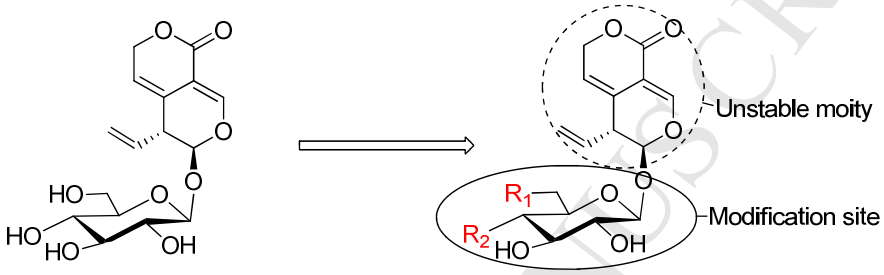


Fig. 1. Structure of gentiopicroside and the designed derivatives.

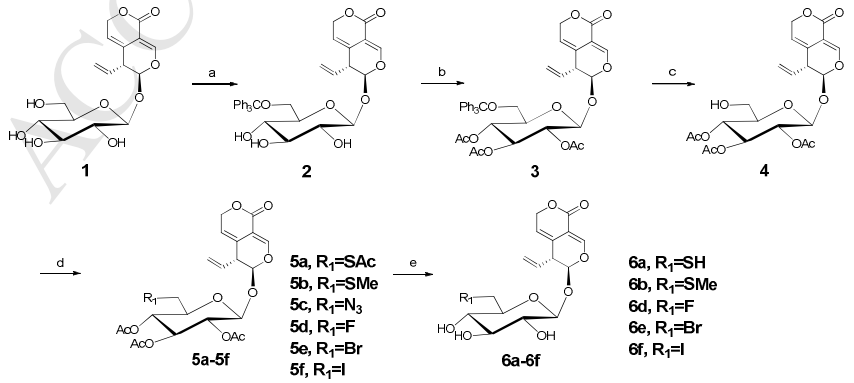
As part of our ongoing program in the study of gentiopicroside [15,16], in the current work, we describe the design, synthesis and pharmacological evaluation of a series of gentiopicroside derivatives as potential antiviral inhibitors.

2. Results and discussion

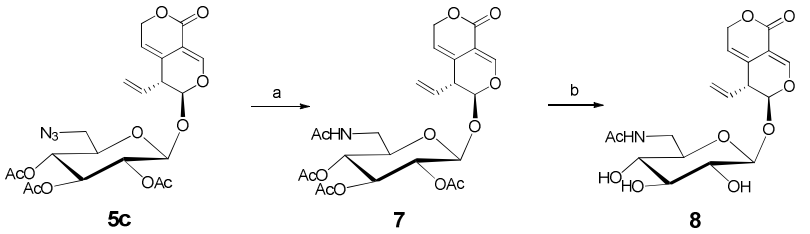
2.1. Chemistry

Structurally, gentiopicroside possesses a complex skeleton featuring fused dipyran glycoside. The compact structure of **1** containing two stereocenters, conjugation system and hemiketal, is further exacerbated by its extreme acid and base sensitivity. So we need a mild and efficient protocol for the regioselective preparation of gentiopicroside derivatives. The expeditious and straightforward synthetic route is depicted in **Scheme 1**. Our synthetic studies commenced with the gentiopicroside that was isolated from the *Gentiana lutea*. Regioselective protection of the primary hydroxyl group was accomplished by the reaction of **1** with triphenylmethyl chloride. Subsequent acetylation of the remaining hydroxyl groups with acetic anhydride gave

the triacetyl derivative **3**. Detriphenylmethylation by treatment with $FeCl_3$ produced the desired intermediates with a free hydroxyl group at primary position [17]. With the alcohol **4** in hands, some common atom or functional group in drug design, such as halogen [18], sulfur [19], and amino group [20] and so on, could be introduced at primary position. The alcohol **4** was smoothly converted to the corresponding iodide **5f** and bromide **5e** in good yield using Br_2/Ph_3P [21] and I_2/Ph_3P [22] system. Furthermore, the introduction of a triflate at C-6' in alcohol **4** afforded a unstable intermediate which was followed by displacement with thioacetate [23], thiomethoxide, azide [24], fluor [25] to yield the desired compounds **5a-5d** in good yield at two steps. In addition, the azide compound **5c** was reduced into amino compound by Staudinger reaction [26] as shown in **scheme 2**. Unfortunately, two compounds, which could not be separated by silica gel chromatography, were obtained on account of acetyl migration [27]. After acetylation, one pure compound **7** was obtained in excellent yield. Final deacetylation could afford the target compound **8**. However, attempt to deprotection of the acetyl groups of **5** using various reagents such as (i) NaOMe/MeOH [28], (ii) K_2CO_3/MeOH [29], (iii) $Et_3N/MeOH/H_2O$ [30], (iv) NH_4OH/ MeOH [31] failed to give the desired compounds; all of compounds underwent decomposition or no reaction under such conditions. Nevertheless, the deprotections were achieved *via* treating the compounds in methanol with dibutyltin oxide [32] under reflux condition, furnishing the target compounds **6a-6f** in excellent yield.



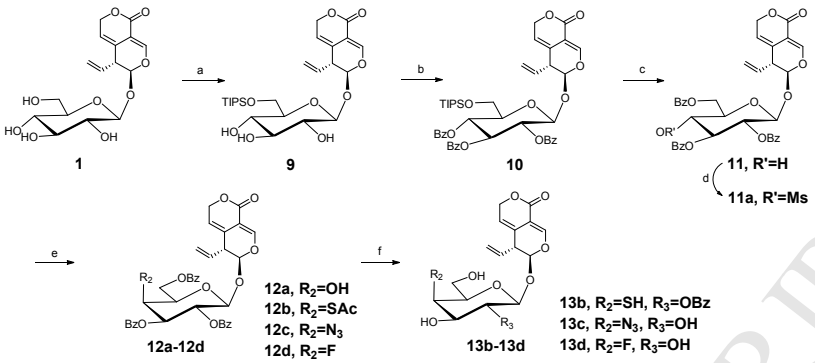
Scheme 1. Reagents and conditions: (a) Ph_3CCl, pyridine, 80 °C, 3 h, 85%; (b) Ac_2O, DMAP, pyridine, RT, overnight, 90%; (c) $FeCl_3$, DCM, RT, 1 h, 82%; (d) i) Tf_2O, pyridine, DCM, 0 °C, 15 min, then KSAc, DMF, RT, 2 h, 91%; ii) Tf_2O, pyridine, DCM, 0 °C, 15 min, then NaSMe, DMF, RT, 3 h, 40%; iii) Tf_2O, pyridine, DCM, 0 °C, 15 min, then NaN_3, DMF, RT, 2 h, 89%; iv) Tf_2O, pyridine, DCM, 0 °C, 15 min, then TBAF, THF, 0 °C, 1 h, 69%; v) Imazole, Ph_3P, I_2, Toluene, 70 °C, 30 min, 53%; vi) Ph_3P, Br_2, pyridine, 0 °C, overnight, 62%; (e) Bu_2SnO, MeOH, reflux, 12 h, 74% for 6a, 60% for 6b, 62% for 6d, 67% for 6e, 51% for 6f.



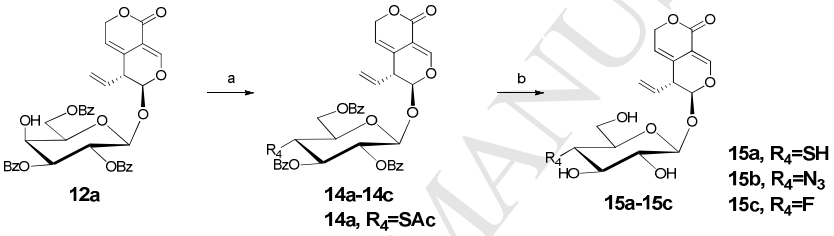
Scheme 2. Reagents and conditions: (a) i) Ph_3P, H_2O, THF, 50 °C, 24 h; ii) Ac_2O, DMAP, pyridine, RT, overnight, 90% in two steps; (b) Bu_2SnO, MeOH, reflux, 12 h, 46%.

On the other hand, the sugar moiety of gentiopicroside was selectively and quantitatively converted into the corresponding 6'-TIPS derivative **9** by treatment with TIPSCl in DMF at room temperature for overnight in the presence of imidazole. Subsequent benzoylation reaction generated the compound **10** in high yields. Treatment of **10** with TBAF cleaved the TIPS protecting group to give rise an intramolecular migration of the benzoyl group at C-4' to the less crowded C-6' position [33]. The structures of **11** was confirmed by its conversion into the corresponding mesyl derivatives **11a** as its ^{1}H NMR for H-4' of the glucose moiety displayed deshielded signals at δ 5.18 (dd, $J_{4,5}$ = 12.2 Hz, $J_{3,4}$ = 9.7 Hz), indicating the position of the formed free hydroxyl in the derivative **11** to be at 4' position in sugar moiety. The alcohol **11** was then converted into the corresponding triflate derivative, followed by nucleophilic substitution with KNO_2, KSAc, NaN_3, and TBAF to provide the derivatives **12a-12d** in excellent yields. Finally the desired compounds were obtained in moderate yields after debenzoylation under the aforementioned conditions. At the same time, the alcohol **12a** was converted into the corresponding equatorial SH, N_3, F substituents by the same two-step process. The final deprotection of **12b-12d** using dibutyltin oxide proceeded successfully to afford the target molecules **13b-13d** in moderate yields (**Scheme 3.**). It is worth to note that the C-4' triflate was reacted with TBAF starting from the suitably protected **12a** to unsuccessfully give the desired 4'-fluoro compound **14c**, the elimination product **16** (see supporting information) was observed as described in the literature [34]. DAST reagent was used to introduce an equatorial fluorine at C-4' of sugar moiety in gentiopicroside. Final deprotection of **14a-14c** by using dibutyltin oxide proceeded to afford the target molecules **15a-15c** in moderate yields (**Scheme 4.**). Unfortunately, **15b** and **15c** were obtained as mixture of

compounds.



Scheme 3. Reagents and conditions: (a) TIPSCl, Imazole, DMAP, DMF, RT, overnight, 91%; (b) BzCl, DMAP, pyridine, RT, overnight, 85%; (c) TBAF, THF, 0 °C, 1 h, 78%; (d) MsCl, Et_3N, DCM, 0 °C, 1 h; 92%; (e) i) Tf_2O, pyridine, DCM, 0 °C, 15 min, then KNO_2, DMF, 50 °C, 2 h, 85%; ii) Tf_2O, pyridine, DCM, 0 °C, 15 min, then KSAc, DMF, RT, 2 h, 93%; iii) Tf_2O, pyridine, DCM, 0 °C, 15 min, then NaN_3, DMF, RT, 2 h, 91%; iv) Tf_2O, pyridine, DCM, 0 °C, 15 min, then TBAF, THF, 0 °C, 1 h, 51%. (f) Bu_2SnO, MeOH, reflux, 20 h, 65% for 13b, 40% for 13c, 42% for 13d.



Scheme 4. Reagents and conditions: (a) i) Tf_2O, pyridine, DCM, 0 °C, 15 min, then KSAc, DMF, RT, 2 h, 76%; ii) Tf_2O, pyridine, DCM, 0 °C, 15 min, then NaN_3, DMF, RT, 1 h, 78%; iii) DAST, pyridine, DCM, -25 °C, 3 h, 80%. (b) Bu_2SnO, MeOH, reflux, 12 h, 47% for 15a, 31% for 15b, 46% for 15c.

The synthesized compounds were fully characterized by physicochemical and spectral means. The MS and ^1H NMR, ^{13}C NMR spectral data were found in agreement with the assigned molecular structures.

2.2. Biological evaluations

2.2.1 Inhibition of influenza virus infectivity

To explore the novel gentiopicroside derivatives as anti-influenza agents, we first determined their cytotoxicity in MDCK cells by the CellTiter-Glo® assay. As shown in **Fig 2A**, except compound **2**, all the other derivatives of gentiopicorside showed no cytotoxicity to uninfected MDCK cells at concentration of 50 μM. Compound **2** exhibited strong cell toxicity at the same concentration indicating that the triphenylmethyl group could be a toxic group, which agreed well with previous work by Sirion *et al.* They first reported that the introduction of triphenylmethyl ester group into C-19 of andrographolide led to increase in toxicity against a series of cancer cell

lines [35].

Except compound **2**, gentiopicroside and some of its derivatives were selected for evaluation against the influenza A/WSN/33 (H1N1) virus that was propagated in MDCK cells by the cytopathic effect (CPE) reduction assay. Oseltamivir (OSV), an inhibitor of influenza neuraminidase, was used as a positive control. The screening results are shown in **Fig. 2B.** We found that: 1) compounds **11a**, **13d**, and **16** significantly reduced the viral CPE, but the other compounds had less activity; 2) the hydrophobicity of gentiopicroside may be helpful for the binding with its target since two gentiopicroside derivatives **11a** and **16** are two multi-benzoyl substituted gentiopicroside derivatives with good anti-influenza activity; 3) 4'-substituted group has important effect on the anti-influenza activity. Chang of the conformation of 4'-fluoro from 4'-axial (**13d**) to 4'-equatorial (**15c**) or shift of the fluoro substituent from 4'-axial (**13d**) to the adjacent 6' (**6d**) significantly decreased or even eliminated the activity.

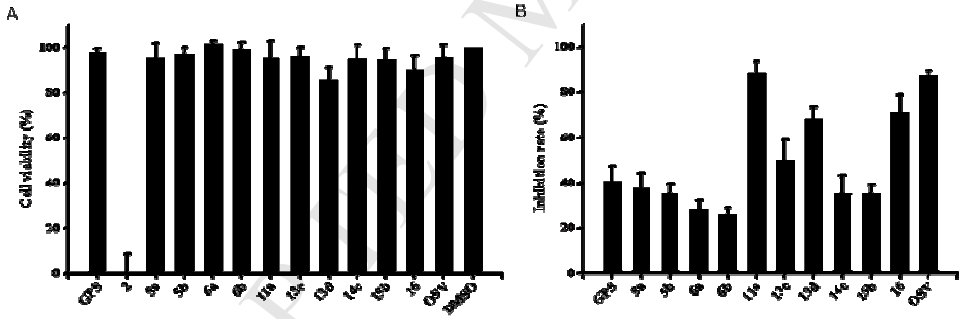


Fig. 2. Inhibitory effects of gentiopicorside derivatives (50 μM) against anti-influenza virus. (A) The cytotoxic effects of gentiopicorside derivatives (50 μM) using CellTiter-Glo® Assay. DMSO was used as a negative control. (B) The anti-influenza virus activity of gentiopicorside derivatives (50 μM) and positive control, respectively. Error bars indicate standard deviations of triplicate experiments.

Three compounds **11a**, **13d** and **16** as well as OSV identified with high inhibition rates in the initial screening were selected for the dose response assays. The concentrations required to inhibit viral replication by 50% (IC_{50}) are summarized in Table 1. Though compounds **13d** and **16** showed a little weaker activity than OSV (IC_{50}: 45.2±2.9 μM vs 44.0±4.1 μM), they can be used as a new lead compound of anti-influenza inhibitor for further structure modification.

Table 1. In vitro anti-influenza virus activity of the lead compounds

Compound	IC_{50} (μM)
11a	39.5 ± 3.2
13d	45.2 ± 2.9
16	44.0 ± 4.1
OSV	33.6 ± 2.2

The anti-influenza activity of **11a**, the most representative compound in this study, was further confirmed by direct microscopic observation. Far less CPE was observed when treated with **11a** at 50 μM than DMSO (Fig. **3**).

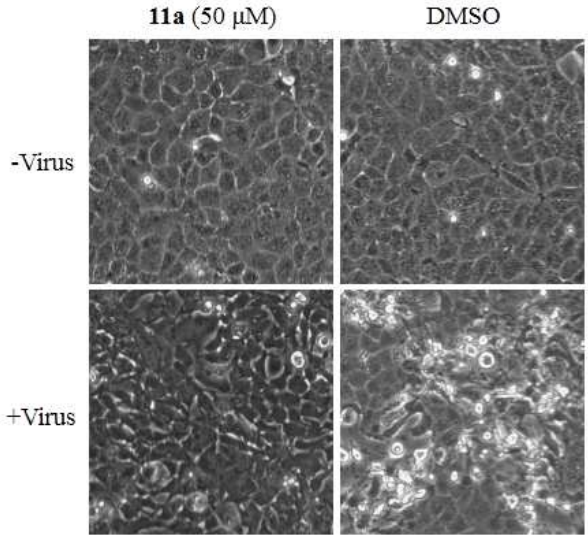


Fig. 3. Direct microscopic observation of the CPE inhibition by compound **11a**.

2.2.2 Inhibition of HCV infectivity

Concerning the fact that gentiopicroside has the hepatoprotective effect on anit-induced cholestatic liver injury in mice [14], the synthesized compounds (**2~16**) were first evaluated for their anti-HCVpp entry activity by using a previous method [36]. Two concentrations, 1 μM and 5 μM, were tested for each compound. DMSO is a negative control for monitoring the maximum HCVpp entry in the presence of 1% DMSO. Echinocystic acid (EA) is a positive control to reflect the blocking of HCVpp entry. As compared to their parent compound gentiopicroside, compound **11a** showed better anti-HCVpp entry activity at the concentrations of 1 μM and 5 μM, three other compounds **14a**, **15c** and **16** also showed better inhibition in a significant

dose-dependence manner in the primary assay. However, they all showed much weaker inhibition of HCVpp infection than the positive control (see Table **S1.**). Those results indicated that gentiopicorside is not a good lead compound for HCV entry.

3. Conclusion

In summary, a novel type of gentiopicroside derivatives have been designed and synthesized efficiently, and their antiviral activities against influenza virus and HCV were evaluated *in vitro*. No significant anti-HCV activity was found for those new gentiopicroside derivatives. However, some of the gentiopicroside derivatives, such as **11a**, **13d** and **16**, exhibited potent anti-influenza activities with IC_{50} at 39.5 μM, 45.2 μM and 44.0 μM, respectively. The preliminary results indicate that modification of the sugar moiety on gentiopicroside is essential for enhancing biological activities.

Further optimization of new gentiopicroside derivatives based on the lead compound **13d** aiming at improving inhibition activities against anti-influenza virus is currently ongoing in our groups and will be reported in due course.

4. Experimental section

4.1. Chemistry-general

All reactions were performed under nitrogen atmosphere. Reagents were all analytically or chemically pure and used without further purification unless specified. Solvents were reagent grade and, when necessary, were purified and dried by standard methods. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 spectrometer using $CDCl_3$ as solvent and tetramethylsilane as internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). High resolution mass spectral (HRMS) analyses were taken on a Bruker micrOTOF mass spectrometer using electron spray ionization. Optical rotations were determined with a JASCO P-2000 polarimeter. TLC was performed on Silica Gel GF254 for TLC (Merck) and spots were visualized by CAM containing Cerium Sulfate (1.0 g) and ammonium molybdate (VI) tetrahydrate (25.0 g) in 10% H_2SO_4 (500 mL) followed by heating or by irradiation with UV light (254 nm).

Flash column chromatography was performed on column packed with Silica Gel 60 (200-300 mesh, Merck).

4.2. Synthesis

4.2.1. *6'-O-Triphenylmethyl gentiopicroside (**2**)*

A solution of gentiopicroside (502 mg, 1.41 mmol, 1.0 equiv.) and TrCl (708 mg, 2.54 mmol, 1.8 equiv.) in pyridine (5 mL) was stirred an 80 ℃ for 3 h. The mixture was concentrated in vacuum and co-concentrated with toluene (5 mL). The residue was dissolved in CH_2Cl_2 (2×20 mL), washed with water saturated sodium hydrogen carbonate solution (20 mL) and brine (20 mL). The organic phase was dried by anhydrous magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (Ethyl Acetate/methanol/Cyclohexane, 5:1:2) to give compound **2** (718.8 mg, 85%) as a yellow foam. R_f = 0.35 (Ethyl Acetate/methanol, 5:1). $[\alpha]_D^{20}$ = -90.2 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, Acetone-d_6): δ 7.38 (dd, J = 8.5, 2.0 Hz, 5H, H arom), 7.28 (d, J = 1.2 Hz, 1H, H-3), 7.25-7.03 (m, 10H, H arom), 5.79-5.60 (m, 1H, H-8), 5.55 (d, J = 3.2 Hz, 2H, H-1, H-6), 5.24-5.04 (m, 2H, H-10a, H-10b), 5.01-4.77 (m, 2H, H-7a, H-7b), 4.64 (d, J = 7.8 Hz, 1H, H-1'), 3.52-3.39 (m, 1H, H-5'), 3.39-3.23 (m, 4H, H-3', H-4', H-9, H-6'a), 3.24-3.06 (m, 2H, H-6'b, H-2'). ^{13}C NMR (101 MHz, Acetone-d_6): δ 163.36 (C-11), 149.39 (C-3), 145.27 (C arom quat), 135.01 (C-8), 129.61, 128.58, 127.82 (C arom tert), 126.76 (C-5), 118.51 (C-10), 117.13 (C-6), 104.90 (C-4), 100.39 (C-1'), 98.29 (C-1), 87.02 ($\underline{C}Ph_3$), 78.09 (C-5'), 76.73 (C-3'), 74.33 (C-2'), 71.42 (C-4'), 69.93 (C-7), 64.41 (C-6'),46.43 (C-9). HRMS (ESI): *m/z* Calcd. for $C_{35}H_{34}O_9Na$ $[M+Na]^+$ 621.2095, found 621.2141 (-4.6 ppm).

4.2.2. *2',3',4'-Tri-O-acetyl-6'-O-Triphenylmethyl gentiopicroside (**3**)*

Acetic anhydride (0.6 mL, 6.0 mmol, 5.0 equiv.) was added to a solution of **2** (718.2 mg, 1.2 mmol, 1.0 equiv.), DMAP (14.6 mg, 0.12 mmol, 0.1 equiv.) in anhydrous pyridine (20 mL) and then stirred at room temperature under nitrogen for 8 h. The solution was evaporated in vacuum, then dissolved in CH_2Cl_2 (30 mL), washed with water (20 mL), saturated sodium hydrogen carbonate solution (20 mL) and brine (20 mL). The organic phase was dried anhydrous magnesium sulfate, filtered and

concentrated. The residue was purified by flash column chromatography (Ethyl Acetate/Cyclohexane, 1:3) to give compound **3** (779.5 mg, 90%) as a yellow foam. R_f = 0.33 (Ethyl Acetate/Cyclohexane, 2:3). $[\alpha]_D^{20}$ = -65.1 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41-7.17 (m, 16H, 15×H arom, H-3), 5.67 (ddd, *J* = 17.3, 10.2, 7.2 Hz, 1H, H-8), 5.57 (ddt, *J* = 4.4, 2.5, 1.1 Hz, 1H, H-6), 5.47 (d, *J* = 2.6 Hz, 1H, H-1), 5.25 (dt, *J* = 9.1, 1.2 Hz, 1H, H-10a), 5.23-5.20 (m, 1H, H-10b), 5.20-5.16 (m, 1H, H-4'), 5.15-5.07 (m, 1H, H-3'), 5.06-4.89 (m, 3H, H-7a, H-7b, H-2'), 4.83 (d, *J* = 8.0 Hz, 1H, H-1'), 3.56 (ddd, *J* = 9.5, 4.4, 2.4 Hz, 1H, H-5'), 3.38-3.25 (m, 2H, H-9, H-6'a), 3.07 (dd, *J* = 10.7, 4.4 Hz, 1H, H-6'b), 1.95 (s, 3H, C<u>H</u>$_3$CO), 1.93 (s, 3H, C<u>H</u>$_3$CO), 1.72 (s, 3H, C<u>H</u>$_3$CO). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.26 (CH_3<u>C</u>O), 169.24 (CH_3<u>C</u>O), 169.00 (CH_3<u>C</u>O), 163.26 (C-11), 148.14 (C-3), 143.60 (C arom), 132.90 (C-8), 128.76-127.20 (C arom), 125.44 (C-5), 118.85 (C-10), 116.75 (C-6), 104.35 (C-4), 96.72 (C-1), 96.38 (C-1'), 86.75 (<u>C</u>Ph$_3$), 73.73 (C-5'), 72.75 (C-3'), 70.83 (C-2'), 69.46 (C-7), 68.58 (C-4'), 61.85 (C-6'), 45.39 (C-9), 20.69 (<u>C</u>H$_3$CO), 20.59 (<u>C</u>H$_3$CO), 20.48 (<u>C</u>H$_3$CO). HRMS (ESI): *m/z* Calcd. for $C_{41}H_{40}O_{12}Na$ $[M+Na]^+$ 747.2413, found 747.2412 (-0.1 ppm).

4.2.3. *2',3',4'-Tri-O-acetyl gentiopicroside* ***(4)***

To a solution of tritylated **3** (42 mg, 0.058 mmol, 1.0 equiv.) in CH_2Cl_2 (3 mL) was added solid $FeCl_3$ (19 mg, 0.12 mmol, 2.0 equiv.). The mixture was stirred at room temperature for 40 min, after which time the reaction was complete as indicated by TLC. Water (5 mL) was added and the mixture was diluted with Ethyl Acetate (2×5 mL). The organic layers were combined, dried over anhydrous magnesium sulfate, The residue was purified by flash column chromatography (Ethyl Acetate/Cyclohexane, 1:1) to give compound **4** (23 mg, 82%) as a white foam. R_f = 0.29 (Ethyl Acetate/Cyclohexane, 2:1). $[\alpha]_D^{20}$ = -159.6 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36 (q, *J* = 1.3 Hz, 1H, H-3), 5.62 (ddd, *J* = 17.3, 10.3, 7.1 Hz, 1H, H-8), 5.54 (dd, *J* = 3.6, 2.6 Hz, 1H, H-6), 5.44 (d, *J* = 2.3 Hz, 1H, H-1), 5.28-5.21 (m, 1H, H-3'), 5.21-5.19 (m, 1H, H-10a), 5.19-5.15 (m, 1H, H-10b), 5.10-4.97 (m, 2H, H-7a, H-4'), 4.97-4.90 (m, 1H, H-7b), 4.90-4.84 (m, 2H, H-2', H-1'), 3.83-3.67 (m,

1H, H-6'a), 3.64-3.48 (m, 2H, H-6'b, H-5'), 3.26 (ddd, *J* = 6.9, 2.5, 1.2 Hz, 1H, H-9), 2.03 (s, 3H, $C\underline{H}_3CO$), 1.97 (s, 3H, $C\underline{H}_3CO$), 1.91 (s, 3H, $C\underline{H}_3CO$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.41 ($CH_3\underline{C}O$), 170.08 ($CH_3\underline{C}O$), 169.13 ($CH_3\underline{C}O$), 163.27 (C-11), 147.87 (C-3), 132.78 (C-8), 125.20 (C-5), 118.70 (C-10), 116.85 (C-6), 104.36 (C-4), 96.59 (C-1), 96.08 (C-1'), 74.56 (C-5'), 72.22 (C-2'), 70.72 (C-4'), 69.44 (C-7), 68.50 (C-3'), 60.95 (C-6'), 45.11 (C-9), 20.70 ($\underline{C}H_3CO$), 20.64 ($\underline{C}H_3CO$), 20.52 ($\underline{C}H_3CO$). HRMS (ESI): *m/z* Calcd. for $C_{22}H_{26}O_{12}Na$ $[M+Na]^+$ 505.1316, found 505.1316 (0 ppm).

4.2.4. *2',3',4'-Tri-O-acetyl-6'-(1,1,1-trifluoromethanesulfonyl) gentiopicroside (**5**)*

Trifluoromethanesulfonic anhydride (41 µL, 0.24 mmol, 2.0 equiv.) was added dropwise at 0 ℃ to a stirred solution of **4** (56 mg, 0.12 mmol, 1.0 equiv.) in CH_2Cl_2 (3 mL). After 2 min, pyridine (100 µL) was added to this stirred solution at the same temperature. After 15 min, the reaction mixture was diluted with CH_2Cl_2 (5 mL) and washed successively with 1 M HCl (2 mL), saturated aqueous sodium hydrogen carbonate (5 mL) and water (5 mL). The separated organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuum at low temperature. The residue was used directly in the next step without further purification. $\mathbf{R_f}$ = 0.38 (Ethyl Acetate/Cyclohexane, 1:1).

4.2.5. *2',3',4'-Tri-O-acetyl-6'-S-acetyl-6'-thio-6'-deoxy gentiopicroside (**5a**)*

Potassium thioacetate (10.0 mg, 0.082 mmol, 2.0 equiv.) was added to a solution of the protected triflate residue **5** (25.2 mg, 0.041 mmol, 1.0 equiv.) in anhydrous DMF (2 mL). After stirring at room temperature for 2 h, the mixture was diluted with Ethyl Acetate (2×5 mL) and washed with brine (5 mL). The organic phase was dried with anhydrous magnesium sulfate and concentrated in vacuum. Purification of the residue by flash column chromatography (Ethyl Acetate/Cyclohexane, 1:1) afforded the thiolacetate derivative **5a** (22 mg, 98% in two steps). R_f = 0.35 (Ethyl Acetate/Cyclohexane, 2:1). $[\alpha]_D^{20}$ = -70.4 (c 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.41-7.32 (m, 1H, H-3), 5.72-5.59 (m, 1H, H-8), 5.54 (dd, *J* = 3.5, 2.8, 1.6 Hz, 1H, H-6), 5.42-5.34 (m, *J* = 3.4, 1H, H-1), 5.21 (dd, *J* = 7.9, 1.2 Hz, 1H, H-10a), 5.18 (t, *J*

= 1.2 Hz, 1H, H-10b), 5.14 (t, *J* = 9.5 Hz, 1H, H-3'), 5.05 (ddt, *J* = 17.7, 2.5, 1.2 Hz, 1H, H-7a), 4.95 (d, *J* = 3.2 Hz, 1H, H-7b), 4.94-4.90 (m, 1H, H-4'), 4.90-4.84 (m, 1H, H-2'), 4.82-4.74 (m, *J* = 7.8, 1H, H-1'), 3.64 (ddd, *J* = 9.7, 6.6, 3.0 Hz, 1H, H-5'), 3.32-3.16 (m, 2H, H-9, H-6'a), 3.06 (dd, *J* = 14.4, 6.6 Hz, 1H, H-6'b), 2.32 (s, 3H, SAc), 2.04 (d, *J* = 7.2 Hz, 3H, C$\underline{H}_3$CO), 1.95 (s, 3H, C$\underline{H}_3$CO), 1.90 (d, *J* = 5.5 Hz, 3H, C$\underline{H}_3$CO). ^{13}C NMR (101 MHz, $CDCl_3$): δ 194.41 (SAc), 170.04 (CH$_3\underline{C}$O), 169.76 (CH$_3\underline{C}$O), 169.08 (CH$_3\underline{C}$O), 163.17 (C-11), 147.90 (C-3), 132.74 (C-8), 125.29 (C-5), 118.78 (C-10), 116.81 (C-6), 104.37 (C-4), 96.64 (C-1), 96.00 (C-1'), 73.47 (C-5'), 72.33 (C-3'), 70.59 (C-4'), 70.36 (C-2'), 69.42 (C-7), 45.13 (C-9), 30.48 (SCO$\underline{C}$H$_3$), 29.99 (C-6'), 20.73 ($\underline{C}$H$_3$CO), 20.60 ($\underline{C}$H$_3$CO), 20.51 ($\underline{C}$H$_3$CO). HRMS (ESI): *m/z* Calcd. for $C_{24}H_{28}O_{11}SNa$ $[M+Na]^+$ 563.1306, found 563.1302 (0.7 ppm).

4.2.6. *2',3',4'-Tri-O-acetyl-6'-S-methyl-6'-thio-6'-deoxy gentiopicroside (**5b**)*

Sodium thiomethoxide (31.0 mg, 0.44 mmol, 1.3 equiv.) was added to a solution of the protected triflate residue **5** (163 mg, 0.43 mmol, 1.0 equiv.) in anhydrous DMF (2 mL). After stirring at room temperature for 2 h, the mixture was diluted with Ethyl Acetate (2×5 mL) and washed with brine (5 mL). The organic phase was dried with anhydrous magnesium sulfate and concentrated in vacuum. Purification of the residue by flash column chromatography (Ethyl Acetate/Cyclohexane, 1:1) afforded the thiolacetate derivative **5b** (71.0 mg, 98% in two steps). R_f = 0.42 (Ethyl Acetate/Cyclohexane, 1:1). $[\alpha]_D^{20}$ = -54.0 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41 (d, *J* = 1.6 Hz, 1H, H-3), 5.65 (ddd, *J* = 19.6, 10.1, 7.2 Hz, 1H, H-8), 5.55 (ddd, *J* = 5.5, 3.7, 2.2 Hz, 1H, H-6), 5.47-5.38 (m, 1H, H-1), 5.23 (dt, *J* = 4.9, 1.2 Hz, 1H, H-10a), 5.22-5.19 (m, 1H, H-10b), 5.18 (d, *J* = 9.6 Hz, 1H, H-3'), 5.12-5.04 (m, 1H, H-7a), 5.00 (d, *J* = 9.6 Hz, 1H, H-4'), 4.99-4.94 (m, 1H, H-2'), 4.94-4.90 (m, 1H, H-7b), 4.85 (d, *J* = 8.1 Hz, 1H, H-1'), 3.70 (ddd, *J* = 10.3, 7.2, 3.5 Hz, 1H, H-5'), 3.30-3.20 (m, 1H, H-9), 2.75-2.57 (m, 2H, H-6'a, H-6'b), 2.22-2.15 (m, 3H, SC$\underline{H}_3$), 2.04 (d, *J* = 2.5 Hz, 3H, C$\underline{H}_3$CO), 2.00 (d, *J* = 9.4 Hz, 3H, C$\underline{H}_3$CO), 1.95 (d, *J* = 3.5 Hz, 3H, C$\underline{H}_3$CO). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.22 (CH$_3\underline{C}$O), 169.72 (CH$_3\underline{C}$O), 169.24 (CH$_3\underline{C}$O), 163.29 (C-11), 148.09 (C-3), 132.79 (C-8), 125.42 (C-5), 118.92

(C-10), 116.82 (C-6), 104.42 (C-4), 96.87 (C-1), 96.33 (C-1'), 74.94 (C-5'), 72.49 (C-3'), 71.41 (C-4'), 70.75 (C-2'), 69.50 (C-7), 45.32 (C-9), 35.73 (C-6'), 20.84 ($\underline{C}H_3CO$), 20.72 ($\underline{C}H_3CO$), 20.63 ($\underline{C}H_3CO$), 17.34 ($S\underline{C}H_3$). HRMS (ESI): *m/z* Calcd. for $C_{23}H_{28}SO_{11}Na$ $[M+Na]^+$ 535.1239, found 535.1245 (1.0 ppm).

4.2.7. *2',3',4'-Tri-O-acetyl-6'-azido-6'-deoxy gentiopicroside (**5c**)*

NaN_3 (8.1 mg, 0.124 mmol, 2.0 equiv.) was added to a solution of the protected triflate residue **5** (38.0 mg, 0.062 mmol, 1.0 equiv.) in anhydrous DMF (2 mL). This reaction mixture was stirred at room temperature for 1 h, and then the reaction mixture was diluted with Ethyl Acetate (5 mL) and washed with water (5 mL). The separated aqueous layer was washed with Ethyl Acetate (2×5 mL). The combined organic layers were dried over anhydrous magnesium sulfate and concentrated in vacuum. The crude product was purified by column chromatography to give compound **5c** (26.0 mg, 83%) as a white foam. R_f = 0.46 (Ethyl Acetate/Cyclohexane, 1:1). $[\alpha]_D^{20}$ = -110.0 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41 (d, *J* = 1.7 Hz, 1H, H-3), 5.63 (ddd, *J* = 17.2, 10.1, 7.1 Hz, 1H, H-8), 5.56 (dt, *J* = 3.7, 1.9 Hz, 1H, H-6), 5.43 (d, *J* = 2.6 Hz, 1H, H-1), 5.23 (dd, *J* = 2.3, 1.1 Hz, 1H, H-2'), 5.22-5.20 (m, 1H, H-10a), 5.19 (d, *J* = 3.9 Hz, 1H, H-10b), 5.12-5.03 (m, 1H, H-3'), 4.99 (t, *J* = 2.0 Hz, 1H, H-7a), 4.97 (d, *J* = 3.8 Hz, 1H, H-4'), 4.95-4.92 (m, 1H, H-7b), 4.89 (d, *J* = 8.1 Hz, 1H, H-1'), 3.75 (ddd, *J* = 9.8, 7.2, 2.5 Hz, 1H, H-5'), 3.42 (dd, *J* = 13.4, 7.3 Hz, 1H, H-6'a), 3.33-3.13 (m, 2H, H-9, H-6'b), 2.03 (s, 3H, $C\underline{H}_3CO$), 1.99 (s, 3H, $C\underline{H}_3CO$), 1.94 (d, *J* = 3.0 Hz, 3H, $C\underline{H}_3CO$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.12 ($CH_3\underline{C}O$), 169.62 ($CH_3\underline{C}O$), 169.15 ($CH_3\underline{C}O$), 163.24 (C-11), 148.01 (C-3), 132.65 (C-8), 125.33 (C-5), 119.01 (C-10), 116.90 (C-6), 104.48 (C-4), 96.91 (C-1), 96.23 (C-1'), 74.19 (C-5'), 72.23 (C-2'), 70.56 (C-4'), 69.52 (C-3'), 69.51 (C-7), 51.07 (C-6'), 45.24 (C-9), 20.71 ($\underline{C}H_3CO$), 20.67 ($\underline{C}H_3CO$), 20.58 ($\underline{C}H_3CO$). HRMS (ESI): *m/z* Calcd. for $C_{22}H_{25}NO_{11}Na$ $[M+Na]^+$ 530.1381, found 530.1366 (3.0 ppm).

4.2.8. *2',3',4'-Tri-O-acetyl-6'-fluoro-6'-deoxy gentiopicroside (**5d**)*

Tetrabutylammonium fluoride (1M in THF) (205 µL, 0.20 mmol, 5.0 equiv.) was slowly added to a solution of the protected triflate residue **5** (25 mg, 0.04 mmol, 1.0

equiv.) in dry THF (2 mL) at 0 ℃. The reaction mixture was then stirred at room temperature for 2 h, diluted with Ethyl Acetate (3 mL) and washed with 1M HCl (3 mL). The combined aqueous phases were extracted once with Ethyl Acetate (2×5 mL), and the combined organic phases washed with saturated sodium hydrogen carbonate (5 mL), water (5 mL), dried over anhydrous magnesium sulfate and concentrated under vacuum. The residue was purified by column chromatography (Ethyl Acetate/Cyclohexane, 2:1) to yield as a white foam **5d** (12 mg, 60%). R_f = 0.2 (Ethyl Acetate/Cyclohexane, 2:3) $[\alpha]_D^{20}$ = -117.8 (c 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39 (q, *J* = 1.4 Hz, 1H, H-3), 5.65 (ddd, *J* = 17.3, 10.3, 7.1 Hz, 1H, H-8), 5.59-5.54 (m, 1H, H-6), 5.45 (d, *J* = 2.4 Hz, 1H, H-1), 5.27-5.23 (m, 1H, H-3'), 5.23-5.21 (m, 1H, H-10a), 5.19 (d, *J* = 1.2 Hz, 1H, H-10b), 5.08 (dd, *J* = 17.5, 1.1 Hz, 1H, H-7a), 5.02 (dd, *J* = 10.2, 9.4 Hz, 1H, H-4'), 4.99-4.95 (m, 1H, H-2'), 4.95-4.90 (m, 1H, H-7b), 4.88 (d, *J* = 8.0 Hz, 1H, H-1'), 4.58-4.48 (m, 1H, H-6'a), 4.46-4.36 (m, 1H, H-6'b), 3.77 (m, *J* = 20.9, 10.2, 4.6, 2.7 Hz, 1H, H-5'), 3.28 (dd, *J* = 7.0, 1.3 Hz, 1H, H-9), 2.04 (s, 3H, C$\underline{H}_3$CO), 1.99 (s, 3H, C$\underline{H}_3$CO), 1.93 (s, 3H, C$\underline{H}_3$CO). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.23 ($CH_3\underline{C}O$), 169.57 ($CH_3\underline{C}O$), 169.17 ($CH_3\underline{C}O$), 163.31 (C-11), 147.95 (C-3), 132.81 (C-8), 125.35 (C-5), 118.92 (C-10), 117.01 (C-6), 104.55 (C-4), 96.72 (C-1), 96.13 (C-1'), 82.13 (C-6'), 80.38 (C-6'), 73.26 (C-5'), 73.07 (C-5'), 72.46 (C-3'), 70.61 (C-2'), 69.56 (C-7), 68.04 (C-4'), 67.97 (C-4'), 45.26 (C-9), 20.75 ($\underline{C}H_3CO$), 20.74 ($\underline{C}H_3CO$), 20.64 ($\underline{C}H_3CO$). ^{19}F NMR (376 MHz, $CDCl_3$): δ -74.52. HRMS (ESI): *m/z* Calcd. for $C_{22}H_{25}O_{11}FNa$ $[M+Na]^+$ 507.1279, found 507.1273 (-5.0 ppm).

4.2.9. *2',3',4'-Tri-O-acetyl-6'-bromo-6'-deoxy gentiopicroside (**5e**)*

Bromine (7 µL, 0.14 mmol, 2.0 equiv.) was added dropwise to a stirred solution of the **4** (33.0 mg, 0.068 mmol, 1.0 equiv.) and triphenylphosphine (36.0 mg, 0.14 mmol, 2.0 equiv.) in anhydrous pyridine (3 mL) at 0 ℃. The mixture was allowed to warm to room temperature overnight. The solution was diluted with Ethyl Acetate (10 mL) and washed with saturated saturated sodium hydrogen carbonate solution (5 mL) and water (5 mL). The combined organic layers were dried over anhydrous magnesium

sulfate and concentrated in vacuum. The crude product was purified by column chromatography to give compound **5e** (23 mg, 62%) as a white foam. R_f = 0.41 (Ethyl Acetate/Cyclohexane, 1:1). $[\alpha]_D^{20}$ = -102.9 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45-7.35 (m, 1H, H-3), 5.66 (ddd, *J* = 17.3, 10.3, 7.1 Hz, 1H, H-8), 5.60-5.52 (m, 1H, H-6), 5.45 (d, *J* = 2.5 Hz, 1H, H-1), 5.26-5.23 (m, 1H, H-10a), 5.21 (s, 1H, H-10b), 5.19 (d, *J* = 7.8 Hz, 1H, H-3'), 5.12-5.03 (m, 1H, H-7a), 4.99 (d, *J* = 4.1 Hz, 1H, H-4'), 4.98-4.95 (m, 4H, H-7b), 4.95-4.92 (m, 1H, H-2'), 4.88 (d, *J* = 8.1 Hz, 1H, H-1'), 3.75 (ddd, *J* = 9.7, 7.0, 2.6 Hz, 1H, H-5'), 3.48 (dd, *J* = 11.4, 2.6 Hz, 1H, H-6'a), 3.38 (dd, *J* = 11.4, 7.0 Hz, 1H, H-6'b), 3.30 (d, *J* = 6.4 Hz, 1H, H-9), 2.05 (s, 3H, C<u>H</u>$_3$CO), 2.00 (s, 3H, C<u>H</u>$_3$CO), 1.95-1.92 (m, 3H, C<u>H</u>$_3$CO). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.20 (CH_3<u>C</u>O), 169.62 (CH_3<u>C</u>O), 169.20 (CH_3<u>C</u>O), 163.32 (C-11), 148.01 (C-3), 132.81 (C-8), 125.42 (C-5), 118.97 (C-10), 116.96 (C-6), 104.54 (C-4), 96.86 (C-1), 96.13 (C-1'), 73.71 (C-5'), 72.36 (C-3'), 70.96 (C-2'), 70.71 (C-4'), 69.57 (C-7), 45.25 (C-9), 30.62 (C-6), 20.84 (<u>C</u>H$_3$CO), 20.74 (<u>C</u>H$_3$CO), 20.65 (<u>C</u>H$_3$CO). HRMS (ESI): *m/z* Calcd. for $C_{22}H_{25}BrO_{11}Na$ $[M+Na]^+$ 567.0487, found 567.0472 (-2.6 ppm).

4.2.10. *2',3',4'-Tri-O-acetyl-6'-iodo-6'-deoxy gentiopicroside (**5f**)*

Compound **4** (32.3 mg, 0.09 mmol, 1.0 equiv.) was dissolved in anhydrous toluene (5 mL), imdazole (2 mg, 0.014 mmol, 0.15 equiv.), triphenylphosphere and iodine were sequence added after dissolution of each reagent. The mixture was heated under reflux at 70 ℃ for 15 min, the reaction mixture was diluted with Ethyl Acetate (10 mL) and washed with $Na_2S_2O_3$ (5 mL). The combined organic layers were washed with brine (5 mL), dried over anhydrous magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (Ethyl Acetate/Cyclohexane, 1:1) to give compound **5f** (82.0 mg, 53%) as a white foam. R_f = 0.39 (Ethyl Acetate/Cyclohexane, 1:1). $[\alpha]_D^{20}$ = -67.5 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47-7.36 (m, 1H, H-3), 5.67 (ddd, *J* = 17.3, 10.3, 7.1 Hz, 1H, H-8), 5.61-5.54 (m, 1H, H-6), 5.47 (d, *J* = 2.6 Hz, 1H, H-1), 5.31-5.24 (m, 1H, H-10a), 5.24-5.21 (m, 1H, H-10b), 5.19 (d, *J* = 9.4 Hz, 1H, H-3'), 5.12-5.04 (m, 1H, H-7a),

5.01-4.96 (m, 1H, H-7b), 4.96-4.92 (m, 1H, H-2'), 4.90 (d, *J* = 6.2 Hz, 1H, H-1'), 4.88-4.85 (m, 1H, H-4'), 3.62-3.49 (m, 1H, H-5'), 3.31 (d, *J* = 2.6 Hz, 1H, H-9), 3.29 (d, *J* = 2.7 Hz, 1H, H-6'a), 3.15 (dd, *J* = 11.1, 8.2 Hz, 1H, H-6'b), 2.05 (s, 3H, C$\underline{H}_3$CO), 1.99 (s, 3H, C$\underline{H}_3$CO), 1.94 (s, 3H, C$\underline{H}_3$CO). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.20 ($CH_3\underline{C}O$), 169.67 ($CH_3\underline{C}O$), 169.25 ($CH_3\underline{C}O$), 163.35 (C-11), 148.11 (C-3), 132.85 (C-8), 125.48 (C-5), 119.02 (C-10), 116.92 (C-6), 104.53 (C-4), 97.02 (C-1), 96.18 (C-1'), 73.86 (C-5'), 72.21(C-3'), 72.16 (C-4'), 70.86 (C-2'), 69.59 (C-7), 45.28 (C-9), 20.88 ($\underline{C}H_3CO$), 20.75 ($\underline{C}H_3CO$), 20.67 ($\underline{C}H_3CO$), 2.85 (C-6'). HRMS (ESI): *m/z* Calcd. for $C_{22}H_{25}IO_{11}Na$ $[M+Na]^+$ 615.0333, found 615.0334 (0.2 ppm).

4.2.11. *6'-thio-6'-deoxy gentiopicroside (**6a**)*

Dibutyltin oxide (35.0 mg, 0.14 mmol, 1.0 equiv.) was added to a solution of the globe protected compound **5a** (72.0 mg, 0.14 mmol, 1.0 equiv.) in anhydrous methanol (10 mL). The mixture was heated under reflux at 70 ℃. The progress of the reaction was monitored with thin layer chromatography. Upon completion of the reaction, the mixture was evaporated to give a residue. Purification of the residue by flash column chromatography (CH_2Cl_2/MeOH, 95:5) afforded the thio derivative **6a** (36.7 mg, 74%) as white foam. R_f = 0.42 (Ethyl Acetate/Methanol, 5:1). $[\alpha]_D^{20}$ = -91.3 (c 1.0, MeOH). ^{1}H NMR (400 MHz, D_2O): δ 7.50 (d, *J* = 1.3 Hz, 1H, H-3), 5.88-5.73 (m, 1H, H-8), 5.67 (ddt, *J* = 4.4, 2.7, 1.5 Hz, 1H, H-6), 5.58 (d, *J* = 3.2 Hz, 1H, H-1), 5.35-5.22 (m, 2H, H-10a, H-10b), 5.20-4.97 (m, 2H, H-7a, H-7b), 4.77-4.65 (m, *J* = 7.9 Hz, 1H, H-1'), 3.43-3.31 (m, 4H, H-9, H-3', H-4', H-2'), 3.28-3.16 (m, 1H, H-5'), 3.09-2.95 (m, 1H, H-6'a), 2.71 (dd, *J* = 14.0, 6.6 Hz, 1H, H-6'b). ^{13}C NMR (101 MHz, D_2O): δ 167.77 (C-11), 150.16 (C-3), 133.73 (C-8), 124.95 (C-5), 119.31 (C-10), 117.78 (C-6), 104.34 (C-4), 99.50 (C-1'), 98.65 (C-1), 76.91 (C-5'), 76.04 (C-2'), 73.26 (C-3'), 72.29 (C-4'), 71.21 (C-7), 45.28 (C-9), 25.77 (C-6'). HRMS (ESI): *m/z* Calcd. for $C_{16}H_{20}O_8Na$ $[M+Na]^+$ 395.0777, found 395.0775 (0.5 ppm).

4.2.12. *6'-S-methyl-6'-thio -6'-deoxy gentiopicroside (**6b**)*

Dibutyltin oxide (39.0 mg, 0.15 mmol, 1.0 equiv.) was added to a solution of the globe protected compound **5b** (79.0 mg, 0.15 mmol, 1.0 equiv.) in anhydrous

methanol (10 mL). The mixture was heated under reflux at 70 °C. The progress of the reaction was monitored with thin layer chromatography. Upon completion of the reaction, the mixture was evaporated to give a residue. Purification of the residue by flash column chromatography (CH_2Cl_2/MeOH, 95:5) afforded the thio derivative **6b** (36.0 mg, 60%) as white foam. R_f = 0.50 (Ethyl Acetate / MeOH, 5:1). $[\alpha]_D^{20}$ = -101.8 (c 1.0, MeOH). ^{1}H NMR (400 MHz, MeOD): δ 7.51 (s, 1H, H-3), 5.87-5.72 (m, 1H, H-8), 5.67 (M, *J* = 6.1, 4.4, 2.5 Hz, 1H, H-6), 5.56 (d, *J* =3.2 Hz, 1H, H-1), 5.37-5.21 (m, 2H, H-10a, H-10b), 5.17-4.98 (m, 2H, H-7a, H-7b), 4.71 (d, *J* = 7.9 Hz, 1H, H-1'), 3.62-3.46 (m, 1H, H-5'), 3.42-3.29 (m, 3H, H-3', H-4', H-9), 3.22 (dd, *J* = 9.0, 7.9 Hz, 1H, H-2'), 3.01 (dd, *J* = 14.2, 2.4 Hz, 1H, H-6'a), 2.78-2.64 (m, 1H, H-6'b), 2.20 (s, 3H, C$\underline{H}_3$). ^{13}C NMR (101 MHz, MeOD): δ 166.18 (C-11), 150.69 (C-3), 134.85 (C-8), 127.00 (C-5), 118.81 (C-10), 117.29 (C-6), 104.95 (C-4), 100.45 (C-1'), 98.88 (C-1), 78.01 (C-5'), 76.97 (C-3'), 74.53 (C-2'), 73.97 (C-4'), 70.89 (C-7), 46.70 (C-9), 36.76 (C-6'), 17.01 ($\underline{C}H_3$). HRMS (ESI): Calcd. for $C_{17}H_{22}O_8SNa$ $[2M+Na]^+$ 409.0933, found 409.0929 (0.4 ppm).

4.2.14. *6'-fluoro-6'-deoxy gentiopicroside (**6d**)*

Dibutyltin oxide (36.0 mg, 0.15 mmol, 1.0 equiv.) was added to a solution of the globe protected compound **5d** (70.0 mg, 0.15 mmol, 1.0 equiv.) in anhydrous methanol (10 mL). The mixture was heated under reflux at 70 ℃. The progress of the reaction was monitored with thin layer chromatography. Upon completion of the reaction, the mixture was evaporated to give a residue. Purification of the residue by flash column chromatography (CH_2Cl_2/MeOH, 90:10) afforded the fluoro derivative **6d** (32.0 mg, 62%) as white foam. R_f = 0.47 (Ethyl Acetate/MeOH, 5:1). $[\alpha]_D^{20}$ = -159.4 (c 1.0, MeOH). ^{1}H NMR (400 MHz, MeOD): δ 7.49 (d, *J* = 1.3 Hz, 1H, H-3), 5.78 (ddd, *J* = 17.3, 10.3, 7.0 Hz, 1H, H-8), 5.67 (dd, *J* = 3.7, 2.7, 1.5 Hz, 1H, H-6), 5.57 (d, *J* = 3.0 Hz, 1H, H-1), 5.32-5.27 (m, 1H, H-10a), 5.27-5.23 (m, 1H, H-10b), 5.12 (ddt, *J* = 17.6, 2.5, 1.2 Hz, 1H, H-7a), 5.07-4.97 (m, 1H, H-7b), 4.71 (d, *J* = 4.0 Hz, 1H, H-1'), 4.62 (dd, *J* = 10.0, 8.2 Hz, 1H, H-6'a), 4.60-4.55 (m, 1H, H-6'b), 3.58-3.47 (m, 1H, H-5'), 3.46-3.42 (m, 1H, H-3'), 3.41-3.39 (m, 1H, H-4'), 3.39 (d, *J*

= 7.0 Hz, 1H, H-9), 3.20 (t, *J* = 8.5 Hz, 1H, H-2'). ^{13}C NMR (101 MHz, MeOD): δ 166.36 (C-11), 150.65 (C-3), 135.00 (C-8), 127.03 (C-5), 118.80 (C-10), 117.44 (C-6), 105.11 (C-4), 100.42 (C-1'), 98.82 (C-1), 84.08 (C-6'), 82.38 (C-6'), 77.91 (C-3'), 76.88 (C-5'), 76.70 (C-5'), 74.49 (C-2'), 71.04 (C-7), 70.28 (C-4'), 70.21 (C-4'), 46.74 (C-9). ^{19}F NMR (376 MHz, MeOD): δ -80.04. HRMS (ESI): *m/z* Calcd. for $C_{16}H_{19}FO_8Na$ $[M+Na]^+$ 381.0962, found 381.0959 (0.3 ppm).

4.2.15. *6'-bromo-6'-deoxy gentiopicroside (**6e**)*

Dibutyltin oxide (36.0 mg, 0.14 mmol, 1.0 equiv.) was added to a solution of the globe protected compound **5e** (78.0 mg, 0.14 mmol, 1.0 equiv.) in anhydrous methanol (10 mL). The mixture was heated under reflux at 70 ℃. The progress of the reaction was monitored with thin layer chromatography. Upon completion of the reaction, the mixture was evaporated to give a residue. Purification of the residue by flash column chromatography (CH_2Cl_2/MeOH, 95:5) afforded the bromo derivative **6e** (40.0 mg, 67%) as white foam. R_f = 0.29 (DCM/MeOH, 9:1). $[\alpha]_D^{20}$ = -109.0 (c 1.0, MeOH). ^{1}H NMR (400 MHz, MeOD): δ 7.51 (d, *J* = 1.2 Hz, 1H, H-3), 5.79 (ddd, *J* = 17.3, 10.3, 7.1 Hz, 1H, H-8), 5.73-5.65 (m, 1H, H-6), 5.58 (d, *J* = 3.1 Hz, 1H, H-1), 5.34-5.28 (m, 1H, H-10a), 5.28-5.24 (m, 1H, H-10b), 5.17-5.09 (m, 1H, H-7a), 5.08-5.00 (m, 1H, H-7b), 4.74 (d, *J* = 7.9 Hz, 1H, H-1'), 3.83 (dd, *J* = 11.0, 2.2 Hz, 1H, H-6'a), 3.64-3.58 (m, 1H, H-6'b), 3.50 (ddd, *J* = 8.9, 6.5, 2.2 Hz, 1H, H-5'), 3.41 (q, *J* = 8.2, 7.5 Hz, 3H, H-3', H-9, H-4'), 3.22 (dd, *J* = 9.1, 8.0 Hz, 1H, H-2'). ^{13}C NMR (101 MHz, MeOD): δ 166.35 (C-11), 150.69 (C-3), 135.01 (C-8), 127.09 (C-5), 118.83 (C-10), 117.43 (C-6), 105.10 (C-4), 100.37 (C-1'), 98.97 (C-1), 77.73 (C-3'), 76.77 (C-5'), 74.60 (C-2'), 73.50 (C-4'), 71.04 (C-7), 46.77 (C-9), 33.76 (C-6'). HRMS (ESI): *m/z* Calcd. for $C_{32}H_{38}O_{16}BrNa$ $[2M+Na]^+$ 859.0429, found 859.0419 (-4.5 ppm).

4.2.16. *6'-iodo-6'-deoxy gentiopicroside (**6f**)*

Dibutyltin oxide (28.0 mg, 0.11 mmol, 1.0 equiv.) was added to a solution of the globe protected compound **5f** (67.0 mg, 0.11 mmol, 1.0 equiv.) in anhydrous methanol (10 mL). The mixture was heated under reflux at 70 ℃. The progress of the reaction

was monitored with thin layer chromatography. Upon completion of the reaction, the mixture was evaporated to give a residue. Purification of the residue by flash column chromatography (CH_2Cl_2/MeOH, 95:5) afforded the iodo derivative **6f** (27.0 mg, 51%) as white foam. R_f = 0.38 (Ethyl Acetate/MeOH, 5:1). $[\alpha]_D^{20}$ = -71.1(c 1.0, MeOH). ^{1}H NMR (400 MHz, MeOD): δ 7.51 (d, *J* = 1.3 Hz, 1H, H-3), 5.79 (ddd, *J* = 17.3, 10.3, 7.2 Hz, 1H, H-8), 5.68 (ddt, *J* = 4.4, 2.8, 1.5 Hz, 1H, H-6), 5.61 (d, *J* = 3.3 Hz, 1H, H-1), 5.35-5.29 (m, 1H, H-10a), 5.29-5.25 (m, 1H, H-10b), 5.19-4.99 (m, 2H, H-7a, H-7b), 4.73 (d, *J* = 8.0 Hz, 1H, H-1'), 3.66 (dd, *J* = 10.7, 1.8 Hz, 1H, H-6'a), 3.45-3.37 (m, 3H, H-9, H-5', H-6'b), 3.30-3.16 (m, 3H, H-3', H-2', H-4'). ^{13}C NMR (101 MHz, MeOD): δ 166.30 (C-11), 150.77 (C-3), 135.05 (C-8), 127.19 (C-5), 118.89 (C-10), 117.38 (C-6), 105.10 (C-4), 100.40 (C-1'), 99.10 (C-1), 77.56 (C-5'), 76.76 (C-2'), 75.26 (C-3'), 74.74 (C-4'), 71.04 (C-7), 46.83 (C-9), 6.63 (C-6'). HRMS (ESI): *m/z* Calcd. for $C_{32}H_{38}O_{16}INa$ $[2M+Na]^+$ 955.0153, found 955.0141 (-1.2 ppm).

4.2.17. *2',3',4'-Tri-O-acetyl-6'-acetamido -6'-deoxy gentiopicroside (**7**)*

To a solution of **5c** (280 mg, 0.58 mmol, 1.0 equiv.) in THF (10 mL) and water (0.2 mL) was added PPh_3 (305mg, 1.16 mmol, 2.0 equiv.). The reaction mixture was reacted at 50 ℃ for 7 h, the mixture was evaporated under diminished pressure, and the residue was used directly in the next step without further purification. R_f = 0.37 (DCM/MeOH, 95:5).

Acetia anhydride (0.17 mL, 1.74 mmol, 3.0 equiv.) was added to a solution of the residue in anhydrous pyridine (5 mL) and then stirred at room temperature under nitrogen for overnight. The solution was evaporated in vacuum, then dissolved in CH_2Cl_2 (30 mL), washed with water (20 mL), saturated sodium hydrogen carbonate solution (20 mL) and brine (20 mL). The organic phase was dried anhydrous magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (DCM/MeOH, 97:3) to give compound **7** (273 mg, 90% in two steps) as a yellow foam. R_f = 0.13 (DCM/MeOH, 97:3). $[\alpha]_D^{20}$ = -110.4 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36 (s, *J* = 1.3 Hz, 1H, H-3), 5.85 (t, *J* = 6.1

Hz, 1H, NHAc), 5.63 (ddd, *J* = 17.3, 10.3, 7.1 Hz, 1H, H-8), 5.55 (m, *J* = 4.4, 2.6, 2.1, 1.2 Hz, 1H, H-6), 5.39 (d, *J* = 2.4 Hz, 1H, H-1), 5.22 (dt, *J* = 7.1, 1.2 Hz, 1H, H-10a), 5.20-5.18 (m, 1H, H-10b), 5.16 (d, *J* = 9.5 Hz, 1H, H-3'), 5.05 (dd, *J* = 17.6, 2.4 Hz, 1H, H-7a), 4.98-4.92 (m, 1H, H-7b), 4.92-4.89 (m, 1H, H-4'), 4.89-4.85 (m, 1H, H-2'), 4.83 (d, *J* = 8.1 Hz, 1H, H-1'), 3.62 (ddd, *J* = 10.0, 4.4, 3.2 Hz, 1H, H-5'), 3.50 (ddd, *J* = 6.3, 3.8, 2.1 Hz, 2H, H-6a', H-6b'), 3.24 (ddd, *J* = 6.2, 2.5, 1.2 Hz, 1H, H-9), 2.02 (s, 3H, NHC$\underline{H}_3$CO), 1.96 (d, *J* = 4.1 Hz, 6H, C$\underline{H}_3$CO), 1.90 (s, 3H, C$\underline{H}_3$CO). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.28-169.77 (3×C$H_3\underline{C}$O), 169.15 (NHC$H_3\underline{C}$O), 163.14 (C-11), 147.83 (C-3), 132.70 (C-8), 125.22 (C-5), 118.83 (C-10), 116.93 (C-6), 104.42 (C-4), 96.70 (C-1), 96.24 (C-1'), 73.03 (C-5'), 72.26 (C-3'), 70.58 (C-4'), 69.41 (C-7), 68.55 (C-2'), 45.18 (C-9), 38.86 (C-6'), 23.29 ($\underline{C}H_3$CO), 20.81-20.42 (3×$\underline{C}H_3$CO). HRMS (ESI): *m/z* Calcd. for $C_{24}H_{29}NO_{12}Na$ $[M+Na]^+$ 546.1587, found 546.1585 (0.3 ppm).

4.2.18. *6'-acetamido-6'-deoxy gentiopicroside (**8**)*

Dibutyltin oxide (153 mg, 0.62 mmol, 1.5 equiv.) was added to a solution of the globe protected compound **7** (214 mg, 0.41 mmol, 1.0 equiv.) in anhydrous methanol (10 mL). The mixture was heated under reflux at 70 ℃. The progress of the reaction was monitored with thin layer chromatography. Upon completion of the reaction, the mixture was evaporated to give a residue. Purification of the residue by flash column chromatography (CH_2Cl_2/MeOH, 83:17) afforded the compound **8** (74 mg, 46%) as white foam. $\mathbf{R_f}$ = 0.26 (DCM/Methanol, 9:1). $[\alpha]_D^{20}$ = -95.8 (c 1.0, MeOH). ^{1}H NMR (400 MHz, MeOD): δ 7.52 (s, 1H, H-3), 5.82 (ddd, *J* = 17.3, 10.3, 7.0 Hz, 1H, H-8), 5.74-5.68 (m, 1H, H-6), 5.67 (d, *J* = 3.1 Hz, 1H, H-1), 5.35-5.26 (m, 2H, H-7a, H-7b), 5.15 (dd, *J* = 17.7, 2.7, 1.3 Hz, 1H, H-10a), 5.06 (dd, *J* = 17.7, 3.5, 1.2 Hz, 1H, H-10b), 4.77-4.67 (d, *J* = 8.9 Hz, 1H, H-1'), 3.73-3.64 (m, 1H, H-6'a), 3.60-3.45 (m, 2H, H-6'b, H-3'), 3.40-3.35 (m, 1H, H-5'), 3.29-3.18 (m, 2H, H-4', H-2'), 2.04 (s, 3H, C$\underline{H}_3$). ^{13}C NMR (101 MHz, MeOD): δ 173.37 (C$H_3\underline{C}$O), 166.17 (C-11), 150.57 (C-3), 134.93 (C-8), 127.00 (C-5), 118.61 (C-10), 117.20 (C-6), 104.94 (C-4), 100.22 (C-1), 98.53 (C-1'), 77.39 (C-3'), 76.33 (C-5), 74.63 (C-4'), 72.64 (C-2'), 70.87 (C-7), 46.65

(C-9), 41.61 (C-6'), 22.47 (<u>C</u>H_3). HRMS (ESI): *m/z* Calcd. for $C_{16}H_{20}O_8Na$ $[M+Na]^+$ 395.0777, found 395.0771 (0.5 ppm).

4.2.19. *6'-O-Triisopropylsilyl gentiopicroside (**9**)*

Gentiopicrin (32.3 mg, 0.09 mmol, 1.0 equiv.) and Imidazole (18.4 mg, 0.27 mmol, 3.0 equiv.) were dissolved in anhydrous DMF (2 mL) and then DMAP (2 mg, 0.014 mmol, 0.15 equiv.) was added. The reaction mixture cooled to 0 °C. TIPSCl (41 µL, 0.19 mmol, 2.1 equiv.) was slowly added and then the reaction mixture was allowed to warm to room temperature. After 16h the reaction mixture was washed with water and the aqueous layer extracted with Ethyl Acetate (2×5 mL). The combined organic layers were washed with brine (5 mL), dried over anhydrous magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (Ethyl Acetate/Cyclohexane/MeOH, 5:5:1) to give compound **9** (42.6 mg, 92%) as a white foam. $\mathbf{R_f}$ = 0.35 (Ethyl Acetate/Cyclohexane/MeOH, 5:5:1). $[\alpha]_D^{20}$ = -123.1 (c 1.0, MeOH). ^{1}H NMR (400 MHz, Acetone-d_6): δ 7.36 (s, J = 1.4 Hz, 1H, H-3), 5.77-5.67 (m, 1H, H-8), 5.65 (ddd, J = 3.2, 2.5, 1.3 Hz, 1H, H-6), 5.54 (d, J = 2.9 Hz, 1H, H-1), 5.30-5.16 (m, 2H, H-10a, H-10b), 5.08-4.90 (m, 2H, H-7a, H-7b), 4.67 (d, J = 7.9 Hz, 1H, H-1'), 4.11 (dd, J = 10.9, 1.6 Hz, 1H, H-6'a), 3.97-3.82 (m, 1H, H-6'b), 3.47-3.34 (m, 3H, H-5', H-3', H-4'), 3.26 (dd, J = 7.3, 2.8, 1.4 Hz, 1H, H-9), 3.19 (t, J = 8.3 Hz, 1H, H-2'), 1.20-1.01 (m, 21H, 3×(C<u>H</u>$_3$)$_2$C<u>H</u>). ^{13}C NMR (101 MHz, Acetone-d_6): δ 163.36 (C-11), 149.22 (C-3), 134.89 (C-8), 126.51 (C-5), 118.21 (C-10), 117.03 (C-6), 104.77 (C-4), 99.99 (C-1'), 97.87 (C-1), 78.18 (C-5'), 77.86 (C-3'), 74.22 (C-2'), 70.90 (C-4'), 69.85 (C-7), 64.15 (C-6'), 46.27 (C-9), 18.37 (<u>C</u>H_3), 12.72 (<u>C</u>H). HRMS (ESI): *m/z* Calcd. for $C_{25}H_{40}O_9SiNa$ $[M+Na]^+$ 535.2342, found 535.2334 (-1.6 ppm).

4.2.20.*2',3',4'-Tri-O-benzoyl-6'-O-Triisopropylsilyl gentiopicroside (**10**)*

Benzoyl chloride (0.33 mL, 2.8 mmol, 5.0 equiv.) was added dropwise to a stirring mixture of **9** (288.5 mg, 0.56 mmol, 1.0 equiv.) in anhydrous pyridine (10 mL) under an argon atmosphere at room temperature, and then DMAP (6.8 mg, 0.056 mmol, 0.1 equiv.) was added, the reaction mixture was stirred for 20 h at room temperature. The mixture was quenched with saturated sodium hydrogen carbonate solution (5 mL) and

the aqueous phase was extracted with Ethyl Acetate (2×10 mL). The combined organic layers were washed with brine (5 mL), dried over anhydrous magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (Ethyl Acetate/Cyclohexane, 1:4) to give compound **10** (431.0 mg, 93%) as a white foam. R_f = 0.40 (Ethyl Acetate/Cyclohexane, 1:2). $[\alpha]_D^{20}$ = -96.7 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94-7.27 (m, 15H, H arom), 7.22 (d, *J* = 1.5 Hz, 1H, H-3), 5.86 (t, *J* = 9.7 Hz, 1H, H-3'), 5.65-5.57 (m, 1H, H-8), 5.56-5.51 (m, 2H, H-4', H-1), 5.48-5.40 (m, 2H, H-2',H-6), 5.19 (dt, *J* = 3.7, 1.2 Hz, 1H, H-10a), 5.17-5.11 (m, 2H, H-10b, H-1'), 4.71 (ddt, *J* = 17.4, 3.9, 1.0 Hz, 1H, H-7a), 4.47 (ddt, *J* = 17.4, 2.3, 1.0 Hz, 1H, H-7b), 3.98-3.89 (m, 3H, H-6'a, H-6'b, H-5'), 3.28-3.14 (m, 1H, H-9), 1.13-1.03 (m, 18H, C$\underline{H}_3$), 1.03-0.96 (m, 3H, C$\underline{H}$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 165.91 (Ph$\underline{C}$O), 165.31 (Ph$\underline{C}$O), 165.20 (Ph$\underline{C}$O), 162.82 (C-11), 147.69 (C-3), 133.82-128.47 (C arom), 133.00 (C-8), 125.57 (C-5), 118.65 (C-10), 116.75 (C-6), 104.29 (C-4), 96.35 (C-1), 96.13 (C-1'), 76.12 (C-5'), 73.02 (C-3'), 71.52 (C-2'), 69.60 (C-4'), 68.98 (C-7), 62.91 (C-6'), 45.18 (C-9), 18.08 (3×$\underline{C}H_3$), 12.16 (3×$\underline{C}$H). HRMS (ESI): *m/z* Calcd. for $C_{46}H_{52}O_{12}SiNa$ $[M+Na]^+$ 847.3126, found 847.3105 (2.0 ppm).

4.2.21. *2',3',6'-Tri-O-benzoyl gentiopicroside (**11**)*

Compound **10** (275.7 mg, 0.34 mmol, 1.0 equiv.) was stirred with TBAF 1.0 M (0.25 mL, 0.25 mmol, 0.7 equiv.) at 0℃ under argon. After 15min, 0.25 ml of TBAF 1.0 M was added again and the mixture was stirred for an additional 30 min at 0 °C. Then quenched with the addition of saturated aqueous sodium hydrogen carbonate (5 mL) and extracted with Ethyl Acetate (2×5 mL). The combined organic layers were washed with saturated aqueous brine (5 mL), dried over anhydrous magnesium sulfate, and concentrated in vacuum. The residue was purified by flash column chromatography (Ethyl Acetate/Cyclohexane, 1:3) to give compound **11** (154.5 mg, 70%) as a white foam. R_f = 0.29 (Ethyl Acetate/Cyclohexane, 2:3). $[\alpha]_D^{20}$ = -87.5 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24-7.32 (m, 15H, H arom), 7.20 (s, 1H, H-3), 5.58 (ddd, *J* = 17.2, 10.1, 7.0 Hz, 1H, H-8), 5.52-5.45 (m, 2H, H-1, H-3'), 5.41

(t, *J* = 8.9 Hz, 2H, H-6, H-2'), 5.16 (d, *J* = 5.5 Hz, 1H, H-10a), 5.14-5.03 (m, 2H, H-10b, H-1'), 4.78 (dd, *J* = 12.2, 4.0 Hz, 1H, H-6a), 4.73-4.59 (m, 2H, H-6b, H-7a), 4.45-4.30 (m, 1H, H-7b), 4.00-3.92 (m, 1H, H-4'), 3.88 (ddd, *J* = 9.8, 4.0, 2.2 Hz, 1H, H-5'), 3.67 (d, *J* = 4.2 Hz, 1H, OH), 3.21 (d, *J* = 6.7 Hz, 1H, H-9). ^{13}C NMR (101 MHz, $CDCl_3$): δ 167.39 (PhCO), 167.05 (PhCO), 165.32 (PhCO), 162.80 (C-11), 147.55 (C-3), 133.92-128.64 (C arom), 132.83 (C-8), 125.46 (C-5), 118.70 (C-10), 116.75 (C-6), 104.28 (C-4), 96.13 (C-1), 95.92 (C-1'), 76.25 (C-3'), 75.15 (C-5'), 70.85 (C-2'), 69.66 (C-4'), 68.92 (C-7), 63.28 (C-6'), 44.96 (C-9). HRMS (ESI): *m/z* Calcd. for $C_{37}H_{32}O_{12}Na$ $[M+Na]^+$ 691.1768, found 691.1786 (2.5 ppm).

4.2.22. *2',3',6'-Tri-O-benzoyl-4'- O-methylsulfonyl gentiopicroside (**11a**)*

Methanesulfonyl chloride (6 µL, 0.078 mmol, 2.0 equiv.) was added dropwise at 0 °C to a stirred solution of **11** (26 mg, 0.039 mmol, 1.0 equiv.) in CH_2Cl_2 (3 mL). After 2 min, pyridine (100 µL) was added to this stirred solution at the same temperature. After 40 min, the reaction mixture was diluted with CH_2Cl_2 (5 mL) and washed successively with 1 M HCl (2 mL), saturated aqueous $NaHCO_3$ (5 mL) and water (5 mL). The separated organic layer was dried over $MgSO_4$ and concentrated in vacuum. The residue was purified by flash column chromatography (Ethyl Acetate/Cyclohexane, 1:3) to give compound **11a** (26.5 mg, 92%) as a yellow foam. R_f = 0.38 (Ethyl Acetate/Cyclohexane, 1:1). $[\alpha]_D^{20}$ = -57.89 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18-7.25 (m, 15H, H arom), 7.23-7.16 (s, 1H, H-3), 5.81 (t, *J* = 9.6 Hz, 1H, H-3'), 5.64-5.50 (m, 1H, H-8), 5.50-5.46 (d, 1H, H-1), 5.46-5.39 (m, 2H, H-2', H-6), 5.23-5.06 (m, 3H, H-4', H-10, H-1'), 4.84 (dd, *J* = 12.5, 2.2 Hz, 1H, H-6'a), 4.73-4.63 (m, 1H, H-7a), 4.63-4.53 (m, 1H, H-6'b), 4.40 (d, *J* = 17.5 Hz, 1H, H-7b), 4.07 (ddd, *J* = 9.9, 4.0, 2.2 Hz, 1H, H-5'), 3.20 (d, *J* = 6.9 Hz, 1H, H-9), 2.88 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.19 (PhCO), 165.63 (PhCO), 164.96 (PhCO), 162.52 (C-11), 147.25 (C-3), 133.88-128.33(C arom), 132.59 (C-8), 125.27 (C-5), 118.79 (C-10), 116.88 (C-6), 104.36 (C-4), 96.14 (C-1), 95.79 (C-1'), 74.17 (C-4'), 72.77 (C-5'), 72.05 (C-3'), 71.01 (C-2'), 68.88 (C-7), 61.99 (C-6'), 44.83 (C-9), 38.94 (CH_3). HRMS (ESI): *m/z* Calcd. for $C_{37}H_{32}O_{12}Na$ $[M+Na]^+$ 769.1529,

found 769.1561 (4.2 ppm).

4.2.23. *2',3',6'-Tri-O-benzyl gentiopicroside (**12a**)*

Potassium nitrite (11.2 mg, 0.11 mmol, 5.0 equiv.) was added to a solution of the protected triflate residue **12** in dry DMF (3 mL). After stirring at 50 °C for 2 h, the mixture was diluted with Ethyl Acetate (2×5 mL) and washed with brine (5 mL). The organic phase was dried with anhydrous magnesium sulfate and concentrated in vacuum. Purification of the residue by flash column chromatography (Ethyl Acetate/Cyclohexane, 1:3) afforded the inversion products **12a** (22 mg, 81% in two steps). R_f = 0.29 (Ethyl Acetate/Cyclohexane, 2:3). $[\alpha]_D^{20}$ = -97.2 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06-7.29 (m, 15H, H arom), 7.18 (d, *J* = 1.5 Hz, 1H, H-3), 5.79 (dd, *J* = 10.4, 8.1 Hz, 1H, H-2'), 5.62-5.55 (m, 1H, H-8), 5.55-5.52 (m, 1H, H-1), 5.40 (m, *J* = 3.8, 2.2 Hz, 1H, H-6), 5.35 (dd, *J* = 10.4, 3.2 Hz, 1H, H-3'), 5.15 (dt, *J* = 4.4, 1.2 Hz, 1H, H-10a), 5.11 (dt, *J* = 2.7, 1.2 Hz, 1H, H-10b), 5.06 (d, *J* = 8.1 Hz, 1H, H-1'), 4.70 (dd, *J* = 11.5, 6.3 Hz, 1H, H-6'a), 4.66-4.63 (m, 1H, H-6'b), 4.63-4.56 (m, 1H, H-7a), 4.47-4.39 (m, 1H, H-4'), 4.35 (ddt, *J* = 17.4, 2.3, 0.9 Hz, 1H, H-7b), 4.19-4.10 (m, *J* = 6.3 Hz, 1H, H-5'), 3.27-3.18 (m, 1H, H-9), 3.14 (d, *J* = 4.8 Hz, 1H, O<u>H</u>). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.51 (Ph<u>C</u>O), 165.81 (Ph<u>C</u>O), 165.30 (Ph<u>C</u>O), 162.80 (C-11), 147.40 (C-3), 133.69-128.49 (C arom), 132.87 (C-8), 125.29 (C-5), 118.44 (C-10), 116.68 (C-6), 104.16 (C-4), 96.06 (C-1'), 95.87 (C-1), 73.82 (C-3'), 73.06 (C-5'), 68.84 (C-7), 68.82 (C-2'), 67.09 (C-4'), 62.81 (C-6'), 44.85 (C-9). HRMS (ESI): *m/z* Calcd. for $C_{37}H_{32}O_{12}Na$ $[M+Na]^+$ 691.1791, found 691.1811 (-4.5 ppm).

4.2.24. *2',3',6'-Tri-O-benzoyl-4'-S-acetyl-4'-thio-4'-deoxy gentiopicroside (**12b**)*

Potassium thioacetate (6.5 mg, 0.057 mmol, 3.0 equiv.) was added to a solution of the protected triflate residue **12** (15 mg, 0.02 mmol, 1.0 equiv.) in anhydrous DMF (3 mL). After stirring at room temperature for 2 h, the mixture was diluted with Ethyl Acetate (2×5 mL), washed with brine (5 mL). The organic phase was dried over anhydrous magnesium sulfate and concentrated in vacuum. Purification of the residue by flash column chromatography (Ethyl Acetate/cyclohexane, 1:1) afforded the

thiolacetate derivative **12b** (24 mg, 88% in two steps). R_f = 0.46 (Ethyl Acetate/Cyclohexane, 1:1). $[\alpha]_D^{20}$ = -109.9 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11-7.33(m, 15H, H arom), 7.24-7.16 (m, 1H, H-3), 5.67 (dd, *J* = 10.3, 4.6 Hz, 1H, H-3'), 5.59 (ddd, *J* = 17.2, 10.2, 7.0 Hz, 1H, H-8), 5.50 (d, *J* = 2.1 Hz, 1H, H-1), 5.47 (d, *J* = 7.9 Hz, 1H, H-6), 5.45 (d, *J* = 8.0 Hz, 1H, H-2'), 5.18 (dt, *J* = 5.1, 1.1 Hz, 1H, H-10a), 5.17-5.12 (m, 1H, H-10b), 5.09 (d, *J* = 7.9 Hz, 1H, H-1'), 4.74 (d, *J* = 1.3 Hz, 1H, H-7a), 4.73-4.70 (m, 1H, H-7b), 4.68 (d, *J* = 4.1 Hz, 1H, H-4'), 4.48 (d, *J* = 5.7 Hz, 1H, H-7b), 4.46 (d, *J* = 4.9 Hz, 1H, H-5'), 4.43 (t, *J* = 5.2 Hz, 1H, H-6'b), 3.26 (d, *J* = 6.7 Hz, 1H, H-9), 2.30 (s, 3H, SAc). ^{13}C NMR (101 MHz, $CDCl_3$): δ 192.74 (SAc), 166.20 ($CH_3\underline{C}O$), 165.41 ($CH_3\underline{C}O$), 165.16 ($CH_3\underline{C}O$), 162.63 (C-11), 147.31 (C-3), 133.88-128.57 (C arom), 132.83 (C-8), 125.39 (C-5), 118.64 (C-10), 116.90 (C-6), 104.35 (C-4), 96.59 (C-1), 96.04 (C-1'), 72.42 (C-5'), 71.45 (C-3'), 70.17 (C-2'), 68.91 (C-7), 63.62 (C-6'), 46.53 (C-4'), 44.91 (C-9), 30.78 (SAc). HRMS (ESI): *m/z* Calcd. for $C_{39}H_{34}O_{12}SNa$ $[M+Na]^+$ 749.1669, found 749.1689 (-3.4 ppm).

4.2.25. *2',3',6'-Tri-O-benzoyl-4'-azido-4'-deoxy gentiopicroside (**12c**)*

The protected triflate residue **12** (39 mg, 0.05 mmol, 1.0 equiv.) was dissolved in anhydrous DMF (3 mL), and then sodium azide (6.4 mg, 0.1 mmol, 2.0 equiv.) was added. This reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with Ethyl Acetate (5 mL), washed with water (5 mL). The separated aqueous layer was washed with Ethyl Acetate (2×5 mL). The combined organic layers were dried over anhydrous magnesium sulfate and concentrated in vacuum. The crude product was purified by column chromatography (Ethyl Acetate / Cyclohexane, 1:2) to give compound **12c** (28mg, 83%) as a white foam. R_f = 0.39 (Ethyl Acetate/Cyclohexane, 1:1). $[\alpha]_D^{20}$ = -180.9 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13-7.34 (m, 15H, H arom), 7.22-7.14 (m, 1H, H-3), 5.72 (dd, *J* = 10.4, 8.0 Hz, 1H, H-2'), 5.65-5.57 (m, 1H, H-8), 5.57-5.53 (m, 1H, H-3'), 5.51 (d, *J* = 2.1 Hz, 1H, H-1), 5.48-5.42 (m, 1H, H-6), 5.19-5.15 (m, 1H, H-10a), 5.14 (d, *J* = 1.2 Hz, 1H, H-10b), 5.03 (d, *J* = 8.0 Hz, 1H, H-1'), 4.71-4.68 (m, 1H, H-7a), 4.68-4.63 (m,

1H, H-6'a), 4.54 (dd, *J* = 11.4, 6.7 Hz, 1H, H-6'b), 4.40 (d, *J* = 17.4 Hz, 1H, H-7b), 4.35 (dd, *J* = 3.6, 1.2 Hz, 1H, H-4'), 4.23-4.06 (m, 1H, H-5'), 3.25 (d, *J* = 6.8 Hz, 1H, H-9). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.22 (PhCO), 165.81 (PhCO), 165.15 (PhCO), 162.72 (C-11), 147.27 (C-3), 134.05-128.34 (C arom), 132.87 (C-8), 125.34 (C-5), 118.68 (C-10), 117.00 (C-6), 104.43 (C-4), 96.17 (C-1'), 96.00 (C-1), 73.21 (C-3'), 71.60 (C-5'), 68.95 (C-7), 68.85 (C-2'), 62.87 (C-6'), 60.49 (C-4'), 44.96 (C-9). HRMS (ESI): *m/z* Calcd. for $C_{37}H_{31}N_3O_{11}Na$ $[M+Na]^+$ 716.1856, found 716.1853 (0.2 ppm).

4.2.26. *2',3',6'-Tri-O-benzoyl-4'-fluoro-4'-deoxy gentiopicroside (**12d**)*

The tetrabutylammonium fluoride (1M in THF) (0.6 mL, 0.60 mmol, 10.0 equiv.) was slowly added to a solution of the protected triflate residue (48 mg, 0.06mmol, 1.0 equiv.) in anhydrous THF (3 mL) at 0 ℃. The reaction mixture was then stirred at room temperature for 2 h, diluted with CH_2Cl_2 (5 mL), and washed with 1M HCl (3 mL). The combined aqueous phases were extracted once with CH_2Cl_2 (2×5 mL), and the combined organic phases washed with saturated sodium hydrogen carbonate (5 mL), water (5 mL), dried over anhydrous magnesium sulfate and concentrated under vacuum. The residue was purified by flash column chromatography (Ethyl Acetate/Cyclohexane, 1:3) to give **12d** (16 mg, 40% in two steps) as white foam. R_f = 0.41 (Ethyl Acetate/Cyclohexane, 2:3). $[\alpha]_D^{20}$ = -88.0 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17-7.31 (m, 15H, H arom), 7.21 (s, 1H, H-3), 5.76 (dd, *J* = 10.2, 8.4 Hz, 1H, H-3'), 5.60 (ddd, *J* = 17.4, 10.3, 7.1 Hz, 1H, H-8), 5.54 (d, *J* = 1.6 Hz, 1H, H-1), 5.45 (s, 1H, H-6), 5.44-5.33 (m, 1H, H-2'), 5.26-5.18 (m, 1H, H-10a), 5.17 (s, 1H, H-4'), 5.14-5.07 (m, 1H, H-10b), 4.77-4.69 (m, 1H, H-1'), 4.70-4.63 (m, 2H, H-6'a, H-7a), 4.58 (dd, *J* = 11.3, 6.8 Hz, 1H, H-7b), 4.39 (d, *J* = 17.2 Hz, 1H, H-7b), 4.28-4.10 (m, 1H, H-5'), 3.26 (d, *J* = 6.8 Hz, 1H, H-9). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.19-165.11 (3×PhCO), 162.68 (C-11), 147.29 (C-3), 133.88-128.68 (C arom), 132.85 (C-8), 125.34 (C-5), 118.68 (C-10), 116.97 (C-6), 104.41 (C-4), 96.03 (C-1'), 95.96 (C-1), 87.08 (C-4'), 85.21 (C-4'), 71.98 (C-5'), 71.96 (C-5'), 71.80 (C-2'), 68.92 (C-7), 68.54 (C-3'), 61.85 (C-6'), 61.80 (C-6'), 44.95 (C-9). ^{19}F NMR (376

MHz, $CDCl_3$): δ -74.36. HRMS (ESI): *m/z* Calcd. for $C_{37}H_{31}FO_{11}Na$ $[M+Na]^+$ 693.1748, found 693.1761 (-2.6 ppm).

4.2.27. *2'-O-benzoyl-4'-thio-4'-deoxy gentiopicroside (**13b**)*

Dibutyltin oxide (110.0 mg, 0.44 mmol, 2.0 equiv.) was added to a solution of the globe protected compound **12b** (158.6 mg, 0.22 mmol, 1.0 equiv.) in anhydrous methanol (15 mL). The mixture was heated under reflux at 70 ℃. The progress of the reaction was monitored with thin layer chromatography. Upon completion of the reaction, the mixture was evaporated to give a residue. Purification of the residue by flash column chromatography (CH_2Cl_2/MeOH, 90:10) afforded the iodole derivative **13b** (67.7 mg, 65%) as white foam. R_f = 0.39 (DCM/MeOH, 9:1). $[\alpha]_D^{20}$ = -155.6 (c 1.0, MeOH). ^{1}H NMR (400 MHz, MeOD): *δ* 8.10-7.47 (m, 5H, H arom), 7.21-7.11 (s, 1H, H-3), 5.84-5.69 (m, 2H, H-1, H-8), 5.63 (m, *J* = 2.4 Hz, 1H, H-6), 5.35 (dd, *J* = 9.8, 8.0 Hz, 1H, H-2'), 5.28-5.17 (m, 2H, H-10a, H-10b), 5.01 (d, *J* = 8.0 Hz, 1H, H-1'), 4.82 (dd, *J* = 4.0, 0.9 Hz, 1H, H-7a), 4.69-4.57 (m, 1H, H-7b), 4.19 (dd, *J* = 9.8, 4.5 Hz, 1H, H-3'), 4.10-4.02 (m, 1H, H-5'), 3.95 (dd, *J* = 11.4, 7.1 Hz, 1H, H-6'a), 3.85 (dd, *J* = 11.4, 4.8 Hz, 1H, H-6'b), 3.58 (dd, *J* = 4.6, 1.7 Hz, 1H, H-4'), 3.37-3.32 (m, 1H, H-9). ^{13}C NMR (101 MHz, MeOD): *δ* 167.11 (C arom), 165.36 (C-11), 149.07 (C-3), 134.59 (C-8), 130.87-129.73 (C arom), 126.46 (C-5), 118.25 (C-10), 118.08 (C-6), 105.01 (C-4), 98.22 (C-1'), 97.59 (C-1), 76.72 (C-5'), 73.40 (C-2'), 71.74 (C-3'), 70.32 (C-7), 63.53 (C-6'), 46.05 (C-9), 45.75 (C-4'). HRMS (ESI): *m/z* Calcd. for $C_{16}H_{20}SO_8Na$ $[M+Na]^+$ 499.1039, found 499.1035 (0.4 ppm).

4.2.28. *4'-azido-4'-deoxy gentiopicroside (**13c**)*

Dibutyltin oxide (80.0 mg, 0.32 mmol, 2.0 equiv.) was added to a solution of the globe protected compound **12c** (109.4 mg, 0.16 mmol, 1.0 equiv.) in anhydrous methanol (15 mL). The mixture was heated under reflux at 70 °C. The progress of the reaction was monitored with thin layer chromatography. Upon completion of the reaction, the mixture was evaporated to give a residue. Purification of the residue by flash column chromatography (CH_2Cl_2/MeOH, 90:10) afforded the iodole derivative **13c** (67.7 mg, 83%) as white foam. R_f = 0.39 (DCM/MeOH, 9:1). $[\alpha]_D^{20}$ = -155.6 (c

1.0, MeOH). ^{1}H NMR (400 MHz, MeOD): δ 7.50 (t, J = 1.4 Hz, 1H, H-3), 5.80 (ddd, J = 17.2, 10.3, 6.9 Hz, 1H, H-8), 5.69 (ddd, J = 3.7, 2.7, 1.3 Hz, 1H, H-6), 5.65 (d, J = 2.8 Hz, 1H, H-1), 5.37-5.28 (m, 1H, H-10a), 5.28-5.24 (m, 1H, H-10b), 5.14 (ddt, J = 17.6, 2.6, 1.3 Hz, 1H, H-7a), 5.05 (ddt, J = 17.6, 3.6, 1.2 Hz, 1H, H-7b), 4.65 (d, J = 7.8 Hz, 1H, H-1'), 3.99-3.90 (m, 1H, H-4'), 3.83 (dd, J = 9.6, 3.9 Hz, 1H, H-3'), 3.79-3.75 (m, 1H, H-6'a), 3.74 (d, J = 5.3 Hz, 1H, H-6'b), 3.72-3.68 (m, 1H, h-5'), 3.57-3.47 (m, 1H, H-2'), 3.40-3.35 (m, 1H, H-9). ^{13}C NMR (101 MHz, MeOD): δ 166.23 (C-11), 150.47 (C-3), 134.95 (C-8), 126.92 (C-5), 118.48 (C-10), 117.26 (C-6), 104.97 (C-4), 100.72 (C-1'), 98.52 (C-1), 75.35 (C-3'), 75.13 (C-5'), 71.94 (C-2'), 70.88 (C-7), 63.99 (C-4'), 62.33 (C-6'), 46.58 (C-9). HRMS (ESI): *m/z* Calcd. for $C_{16}H_{19}N_3O_8Na$ $[M+Na]^+$ 404.1070, found 404.1073 (-0.3 ppm).

4.2.30. *4'-fluoro-4'-deoxy gentiopicroside (**13d**)*

Dibutyltin oxide (69.7 mg, 0.28 mmol, 2.0 equiv.) was added to a solution of the globe protected compound **12d** (94.0 mg, 0.14 mmol, 1.0 equiv.) in anhydrous methanol (15 mL). The mixture was heated under reflux at 70 ℃. The progress of the reaction was monitored with thin layer chromatography. Upon completion of the reaction, the mixture was evaporated to give a residue. Purification of the residue by flash column chromatography (CH_2Cl_2/MeOH, 90:10) afforded the iodole derivative **13d** (21.0 mg, 42%) as white foam. R_f = 0.51 (Ethyl Acetate/MeOH, 5:1). $[\alpha]_D^{20}$ = -151.5 (c 1.0, MeOH). ^{1}H NMR (400 MHz, MeOD): δ 7.50 (d, J = 1.3 Hz, 1H, H-3), 5.80 (ddd, J = 17.2, 10.3, 6.9 Hz, 1H, H-8), 5.68 (s, 1H, H-6), 5.68 (s, 1H, H-1), 5.34-5.28 (m, 1H, H-10a), 5.28-5.23 (m, 1H, H-10b), 5.13 (ddt, J = 17.6, 2.5, 1.2 Hz, 1H, H-7a), 5.09-4.99 (m, 1H, H-7b), 4.77 (dd, J = 35.4, 1.9 Hz, 1H, H-4'), 4.70 (dd, J = 7.2, 1.8 Hz, 1H, H-1'), 3.83-3.78 (m, 1H, H-6'a), 3.77 (s, 1H, H-6'b), 3.71-3.66 (m, 1H, H-5'), 3.66-3.55 (m, 1H, H-3'), 3.55-3.46 (m, 1H, H-2'), 3.43-3.37 (m, 1H, H-9). ^{13}C NMR (101 MHz, MeOD): δ 166.38 (C-11), 150.65 (C-3), 135.09 (C-8), 127.10 (C-5), 118.67 (C-10), 117.41 (C-6), 105.13 (C-4), 100.61 (C-1'), 98.72 (C-1), 91.24 (C-4'), 89.45 (C-4'), 75.89 (C-5'), 75.71 (C-5'), 73.70 (C-3'), 73.52 (C-3'), 72.07 (C-2'), 71.03 (C-7), 61.46 (C-6'), 61.41 (C-6'), 46.76 (C-9). HRMS (ESI): *m/z* Calcd.

for $C_{16}H_{19}FO_8Na$ $[M+Na]^+$ 381.0962, found 381.0960 (0.2 ppm).

4.2.31. *2',3',6'-Tri-O-benzoyl-4'-S-acetyl-4'-thio-4'-deoxy gentiopicroside (**14a**)*

Potassium thioacetate (17.5 mg, 0.15 mmol, 3.0 equiv.) was added to a solution of the protected triflate residue (41.0 mg, 0.05 mmol, 1.0 equiv.) in anhydrous DMF (3 mL). After stirring at room temperature for 2 h, the mixture was diluted with Ethyl Acetate (2×5 mL), washed with brine (5 mL). The organic phase was dried over anhydrous magnesium sulfate and concentrated in vacuum. Purification of the residue by flash column chromatography (Ethyl Acetate/cyclohexane, 1:2) afforded the thiolacetate derivative **14a** (28 mg, 76% in two steps). R_f = 0.29 (Ethyl Acetate/Cyclohexane, 1:2). $[\alpha]_D^{20}$ = -40.9 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18-7.32 (m, 15H, H arom), 7.19 (s, 1H, H-3), 5.73 (dd, *J* = 10.8, 9.7 Hz, 1H, H-3'), 5.56 (ddd, *J* = 17.2, 10.1, 7.0 Hz, 1H, H-8), 5.48 (d, *J* = 2.2 Hz, 1H, H-1), 5.42 (d, *J* = 8.1 Hz, 1H, H-2'), 5.40 (d, *J* = 8.2 Hz, 1H, H-6), 5.20-5.14 (m, 1H, H-10a), 5.13 (d, *J* = 10.4 Hz, 1H, H-10b), 5.10 (d, *J* = 8.2 Hz, 1H, H-1'), 4.75-4.68 (m, 1H, H-6'a), 4.68-4.63 (m, 1H, H-7a), 4.60 (dd, *J* = 12.2, 4.7 Hz, 1H, H-6'b), 4.45-4.33 (m, 1H, H-7b), 4.23-4.14 (m, 1H, H-5'), 4.14-4.02 (m, *J* = 11.2, 1H, H-4'), 3.21 (d, *J* = 6.9 Hz, 1H, H-9), 2.20 (s, 3H, SAc). ^{13}C NMR (101 MHz, $CDCl_3$): δ 192.71 (SAc), 166.37 (PhCO), 165.77 (PhCO), 165.16 (PhCO), 162.69 (C-11), 147.45 (C-3), 133.84-128.60 (C arom), 132.84 (C-8), 125.44 (C-5), 118.68 (C-10), 116.80 (C-6), 104.34 (C-4), 96.09 (C-1'), 95.87 (C-1), 73.61 (C-5'), 72.37 (C-2'), 71.52 (C-3'), 68.92 (C-7), 63.73 (C-6'), 44.94 (C-9), 44.69 (C-4'), 30.92 (SAc). HRMS (ESI): *m/z* Calcd. for $C_{39}H_{34}O_{12}SNa$ $[M+Na]^+$ 749.1669，found 749.1663 (-0.5 ppm).

4.2.32. *2',3',6'-Tri-O-benzoyl-4'-azido-4'-deoxy gentiopicroside (**14b**)*

The protected triflate residue (38.6 mg, 0.048 mmol, 1.0 equiv.) was dissolved in anhydrous DMF (3 mL), and then sodium azide (6.3 mg, 0.1 mmol, 2.0 equiv.) was added. This reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with Ethyl Acetate (5 mL), washed with water (5 mL). The separated aqueous layer was washed with Ethyl Acetate (2×5 mL). The combined organic layers were dried over anhydrous magnesium sulfate and concentrated in

vacuum. The crude product was purified by column chromatography (Ethyl Acetate/Cyclohexane, 1:2) to give compound **14b** (31.5 mg, 78% in two steps) as a white foam. R_f = 0.38 (Ethyl Acetate/Cyclohexane, 1:1). $[\alpha]_D^{20}$ = -53.0 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19-7.33 (m, 15H, H arom), 7.23-7.14 (m, 1H, H-3), 5.69 (t, *J* = 9.8 Hz, 1H, H-3'), 5.57 (ddd, *J* = 17.2, 10.2, 7.0 Hz, 1H, H-8), 5.47 (d, *J* = 2.3 Hz, 1H, H-1), 5.45-5.41 (m, 1H, H-6), 5.38 (dd, *J* = 9.9, 8.1 Hz, 1H, H-2'), 5.15 (dd, *J* = 4.1, 1.2 Hz, 1H, H-10a), 5.13-5.05 (m, 2H, H-10b, H-1'), 4.77 (dd, *J* = 12.3, 2.3 Hz, 1H, H-6'a), 4.72-4.61 (m, 2H, H-6'b, H-7a), 4.41 (d, *J* = 17.4 Hz, 1H, H-7b), 3.94 (t, *J* = 9.9 Hz, 1H, H-4'), 3.83 (ddd, *J* = 10.2, 4.1, 2.3 Hz, 1H, H-5'), 3.20 (d, *J* = 6.6 Hz, 1H, H-9). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.21 (Ph<u>C</u>O), 165.61 (Ph<u>C</u>O), 165.22 (Ph<u>C</u>O), 162.57 (C-11), 147.38 (C-3), 133.95-128.58 (C arom), 132.73 (C-8), 125.39 (C-5), 118.75 (C-10), 116.84 (C-6), 104.36 (C-4), 96.16 (C-1), 95.92 (C-1'), 73.39 (C-3'), 73.25 (C-5'), 71.14 (C-2'), 68.90 (C-7), 63.15 (C-6'), 60.97 (C-4'), 44.91 (C-9). HRMS (ESI): *m/z* Calcd. for $C_{37}H_{31}N_3O_{11}Na$ $[M+Na]^+$ 716.1856, found 716.1850 (0.5 ppm).

4.2.33. *2',3',6'-Tri-O-benzoyl-4'-fluoro-4'-deoxy gentiopicroside (**14c**)*

To a solution of **12a** (208.5 mg, 0.31 mmol, 1.0 equiv.) in anhydrous DCM (5 mL) at -30 ℃ was added diethylaminosulfur trifluoride (122 μL, 0.93 mmol, 3.0 equiv.) and pyridine (100 μL). The reaction mixture was stirred for 3 h at the room temperature, and quenched by addition with MeOH (several drops) at 0 ℃. The residue was diluted with Ethyl Acetate (20 mL), and washed with waterl (10 mL), washed with saturated $NaHCO_3$ (10 mL), brine (10 mL), dried over anhydrous magnesium sulfate and concentrated under vacuum. The residue was purified by flash column chromatography (Ethyl Acetate/Cyclohexane, 1:4) to give **14c** (166.8 mg, 80%) as white foam. R_f = 0.47 (Ethyl Acetate/Cyclohexane, 1:2). $[\alpha]_D^{20}$ = -52.9 (c 2.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14-7.34 (m, 15H, H arom), 7.25-7.14 (s, 1H, H-3), 5.84 (ddd, *J* = 13.8, 10.0, 8.9 Hz, 1H, H-3'), 5.69-5.52 (m, 1H, H-8), 5.48 (d, *J* = 2.3 Hz, 1H, H-1), 5.45-5.35 (m, 2H, H-6, H-2'), 5.19-5.09 (m, 3H, H-1', H-10a, H-10b), 4.93-4.78 (m, 1/2H, H-4'), 4.76 (d, *J* = 1.9 Hz, 1.5H, H-6'a, H-4'), 4.73-4.65

(m, 1H, H-7a), 4.62 (ddd, *J* = 12.3, 4.5, 1.5 Hz, 1H, H-6'b), 4.42 (dd, *J* = 17.5, 2.4, 1.1 Hz, 1H, H-7b), 4.18-4.01 (m, 1H, H-5'), 3.24-3.07 (d, 1H, H-9). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.16 (Ph<u>C</u>O), 165.50 (Ph<u>C</u>O), 165.07 (Ph<u>C</u>O), 162.50 (C-11), 147.30 (C-3), 133.94-128.38 (C arom), 132.63 (C-8), 125.33 (C-5), 118.76 (C-10), 116.83 (C-6), 104.34 (C-4), 96.19 (C-1), 95.88 (C-1'), 88.22 (C-4'), 86.34 (C-4'), 72.50 (C-3'), 72.07 (C-5'), 70.61 (C-2'), 68.85 (C-7), 62.33 (C-6'), 44.88 (C-9). HRMS (ESI): *m/z* Calcd. for $C_{37}H_{31}FO_{11}Na$ $[M+Na]^+$ 693.1748, found 693.1744 (0.4 ppm).

4.2.34. *4'-thio-4'-deoxy gentiopicroside (**15a**)*

Dibutyltin oxide (127.0 mg, 0.51 mmol, 3.0 equiv.) was added to a solution of the globe protected compound **14a** (120.3 mg, 0.17 mmol, 1.0 equiv.) in anhydrous methanol (15 mL). The mixture was heated under reflux at 70 °C. The progress of the reaction was monitored with thin layer chromatography. Upon completion of the reaction, the mixture was evaporated to give a residue. Purification of the residue by flash column chromatography (CH_2Cl_2/MeOH, 90:10) afforded the iodole derivative **15a** (21.9 mg, 47%) as white foam. R_f = 0.45 (EtOAc/MeOH, 5:1). $[\alpha]_D^{20}$ = -145.3 (c 1.0, MeOH). ^{1}H NMR (400 MHz, MeOD): δ 7.52-7.49 (m, 1H, H-3), 5.94-5.74 (m, 1H, H-8), 5.76-5.62 (m, 2H, H-1, H-6), 5.38-5.22 (m, 2H, H-10a, H-10b), 5.19-5.01 (m, 2H, H-7a, H-7b), 4.74 (d, *J* = 7.9 Hz, 1H, H-1'), 4.09-3.95 (m, 1H, H-6'a), 3.89-3.78 (m, 1H, H-6'b), 3.48 (m, *J* = 12.9, 7.7, 4.4, 2.6 Hz, 1H, H-5'), 3.38-3.36 (m, 1H, H-9),3.36-3.30 (m, 1H, H-3'), 3.27-3.16 (t, 1H, H-2'), 2.83-2.71 (t, 1H, H-4'). ^{13}C NMR (101 MHz, MeOD): δ 166.22 (C-11), 150.58 (C-3), 134.97 (C-8), 127.00 (C-5), 118.50 (C-10), 117.17 (C-6), 104.93 (C-4), 100.12 (C-1'), 98.45 (C-1), 79.78 (C-5'), 78.91 (C-3'), 75.54 (C-2'), 70.86 (C-7), 63.22 (C-6'), 46.60 (C-9), 43.26 (C-4'). HRMS (ESI): *m/z* Calcd. for $C_{16}H_{20}SO_8Na$ $[M+Na]^+$ 395.0777, found 395.0774 (0.3 ppm).

4.2.37. *2',3',6'-Tri-O-benzoyl-4',5'-olefin gentiopicroside **(16)***

The tetrabutylammonium fluoride (TBAF, 1M in THF) (0.37 mL, 0.37 mmol, 5.0 equiv.) was slowly added to a solution of the protected triflate residue (59.0 mg, 0.074

mmol, 1.0 equiv.) in anhydrous THF(3 mL) at 0 ℃. The reaction mixture was then stirred at room temperature for 2 h, diluted with Ethyl Acetate (5 mL), and washed with 1M HCl (3 mL). The combined aqueous phases were extracted once with Ethyl Acetate (2×5 mL), and the combined organic phases washed with saturated sodium hydrogen carbonate (5 mL), water (5 mL), dried over anhydrous magnesium sulfate and concentrated under vacuum. The residue was purified by flash column chromatography (Ethyl Acetate/Cyclohexane, 1:2) to give **16** (19 mg, 40% in two steps) as a white foam. R_f = 0.28 (Ethyl Acetate/Cyclohexane, 1:2). $[\alpha]_D^{20}$ = -54.3 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14-7.42 (m, 15H, H arom), 7.40 (d, *J* = 7.5 Hz, 1H, H-3), 5.70 (dd, *J* = 3.6, 0.9 Hz, 1H, H-1'), 5.56 (dd, *J* = 3.3, 2.2 Hz, 1H, H-2'), 5.54 (d, *J* = 3.8 Hz, 1H, H-3'), 5.53-5.50 (m, 1H, H-8), 5.49 (d, *J* = 3.3 Hz, 1H, H-4'), 5.46 (d, *J* = 3.7 Hz, 1H, H-1), 5.36 (d, *J* = 3.0 Hz, 1H, H-6), 5.13 (dd, *J* = 2.0, 1.2 Hz, 1H, H-10a), 5.09 (dt, *J* = 9.7, 1.1 Hz, 1H, H-10b), 4.96-4.87 (m, 1H, H-6' a), 4.87-4.76 (m, 2H, H -6'b, H-7a), 4.69-4.59 (m, 1H, H-7b), 3.23-3.14 (m, 1H, H-9). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.02 (Ph<u>C</u>O), 165.77 (Ph<u>C</u>O), 165.05 (Ph<u>C</u>O), 163.08 (C-11), 149.08 (C-5'), 148.93(C-3), 133.94-128.50 (C arom), 132.48 (C-8), 126.02 (C-5), 119.35 (C-10), 115.96 (C-6), 104.10 (C-4), 99.18 (C-4'), 96.39 (C-1), 92.62 (C-1'), 69.16 (C-7), 68.44 (C-2'), 65.56 (C-3'), 63.24 (C-6'), 45.28 (C-9). HRMS (ESI): *m/z* Calcd. for $C_{37}H_{30}O_{11}Na$ [M+Na]: 673.1679, found 673.1680 (0.2 ppm).

4.3. Cytopathic effect (CPE) reduction assay

The assay was performed as described by Noah et al. with some modifications [37]. MDCK cells were seeded into 96-well plates, incubated overnight and infected with influenza virus (MOI ¼ 0.1) suspended in DMEM supplemented with 1% FBS, containing test compound and 2 mg/mL TPCK-treated trypsin, with a final DMSO concentration of 1% in each well. After 40 h of incubation, CellTiterGlo reagent (Promega Corp., Madison, WI, USA) was added and the plates were read using a plate reader (Tecan Infinite M2000 PRO™; Tecan Group Ltd., Mannedorf, Switzerland).

4.4. Cytotoxicity test

Cells grown in 96-well plates overnight were cultured in 1% FBS with increasing amounts of the test compounds for 40 h. Cytotoxicity was assessed with the CellTiter-Glo assay described as above.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://

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Design, synthesis and biological evaluation of gentiopicroside derivatives as potential antiviral inhibitors

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Highlights:

- A novel series of gentiopicroside derivatives was designed and synthesized.
- All the newly synthesized compounds were evaluated for the inhibition of influenza virus and anti-HCV activity *in vitro*.
- Compound **11a**, **13d** and **16** displayed interesting anti-influenza virus activity.
- Compound **13d** could be as new lead compound in the development of potential antiviral inhibitors.