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Synthesis and Anti-HCV Entry Activity Studies of β-Cyclodextrin–Pentacyclic Triterpene Conjugates

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In our previous studies, oleanolic acid (OA) and echinocystic acid (EA), isolated from *Dipsacus asperoides*, were found to have anti-HCV entry properties. The major issue for members of this type of triterpene is their low water solubility. In this study, a series of new water-soluble triazole-bridged β-cyclodextrin (CD)–pentacyclic triterpene conjugates were synthesized via click chemistry. Thanks to the attached β-CD moiety, all synthesized conjugates showed lower hydrophobicity (Alog *P*) than their parent compounds. Several conjugates exhibited moderate anti-HCV entry activity. With the exception of per-O-methylated β-CD–pentacyclic triterpene conjugates, all other conjugates showed no cytotoxicity based on an alamarBlue assay carried out with HeLa, HepG2, MDCK, and 293T cells. More interestingly, the hemolytic activity of these conjugates disappeared upon the introduction of β-CDs. Easy access to such conjugates that combine the properties of β-CD and pentacyclic triterpenes may provide a way to obtain a new class of anti-HCV entry inhibitors.

Introduction

Hepatitis C virus (HCV) is the leading cause of chronic hepatitis, cirrhosis, and eventually leads to liver carcinomas.[1] In 2002 there were ~170 million individuals infected worldwide, comprising ~3% of the global population.[2] Treatment of HCV infection with peginterferon/ribavirin has been used for almost 30 years. In 2011, the US Food and Drug Administration (FDA) approved the HCV NS3 protease inhibitors telaprevir (Incivek) and boceprevir (Victrelis) for targeting HCV replication, representing the beginning of a new era in the control of HCV infection.[3] However, resistance to individual antiviral drugs is likely to appear,[4] and a combination of drugs that target different stages of the HCV life cycle is required. The recently approved olysio (Simeprevir) is also an HCV protease inhibitor; however, a decrease in efficacy was observed in patients infected with HCV genotype 1a with an NS3 protease Q80K polymorphism, a strain of HCV commonly found in the United States.[5] Inhibition of virus entry into HCV-permissive cells is an emerging approach for the prevention and reduction of infection.[6] Development of HCV entry inhibitors could satisfy the tandem use with other inhibitors of viral replication, leading to a multifaceted approach to control HCV infection more effectively.

Natural products play a significant role in the drug discovery and development process.[7] It has been estimated that over 40% of medicines have their origins in active natural products.[8] Pentacyclic triterpenes are secondary plant metabolites, widespread in fruit peels, leaves, and stem bark,[9] with a few species containing triterpenes up to 30% of their dry weight.[10] It has been estimated that there are more than 20000 triterpenoids in nature.[11]

It is generally believed that the physiological function of triterpenoids is defense (a plant's acquired resistance) against plant pathogens.[12] Plant-derived pentacyclic triterpenoids of lupine and oleanane families provide a versatile structural platform for the discovery of new biologically active compounds (Figure 1). Betulinic acid (BA), a lupane-type triterpene, has been confirmed by many studies to display significant inhibiting activity against HIV entry and virus maturation/release.[13] One derivative, bevirimat (PA-457), is already in clinical trials.[14] Ursolic acid (UA) and moronic acid (MA), two oleanane-type triterpenes, also display anti-HIV activity in vitro.[15] In our previous studies,[16] we found that oleanolic acid (OA) and echinocystic acid (EA), two naturally occurring oleanane-type triterpenes, and their derivatives display substantial activity to inhibit HCV entry. The putative mechanism behind EA blocking of HCV entry is its strong binding to HCV envelope glycoprotein E2. Other diverse and promising biological activities of triterpenes, including anti-inflammatory, hepatoprotective, analgesic, antimicrobial, antimycotic, virostatic, immunomodulatory, and tonic effects, warrant further pharmaceutical develop-

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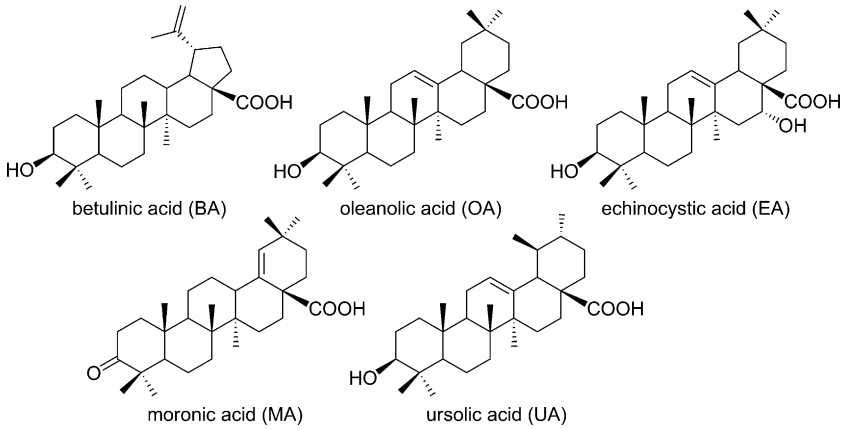


Figure 1. Structures of pentacyclic triterpenes.

ment and even clinical investigation.[17] In particular, the lupane and oleanane triterpenes display no prominent cytotoxicity, which has been accepted through their long-term use in complementary medicinal history. However, the low solubility of triterpenes in biological environments and high hydrophobicity (log *P*) is a drawback for further broad applications, in particular, for development into a therapeutic medicine. For example, the solubilities of BA and OA in water are ~0.02 and 0.05 μg mL^{-1}, respectively.[18]

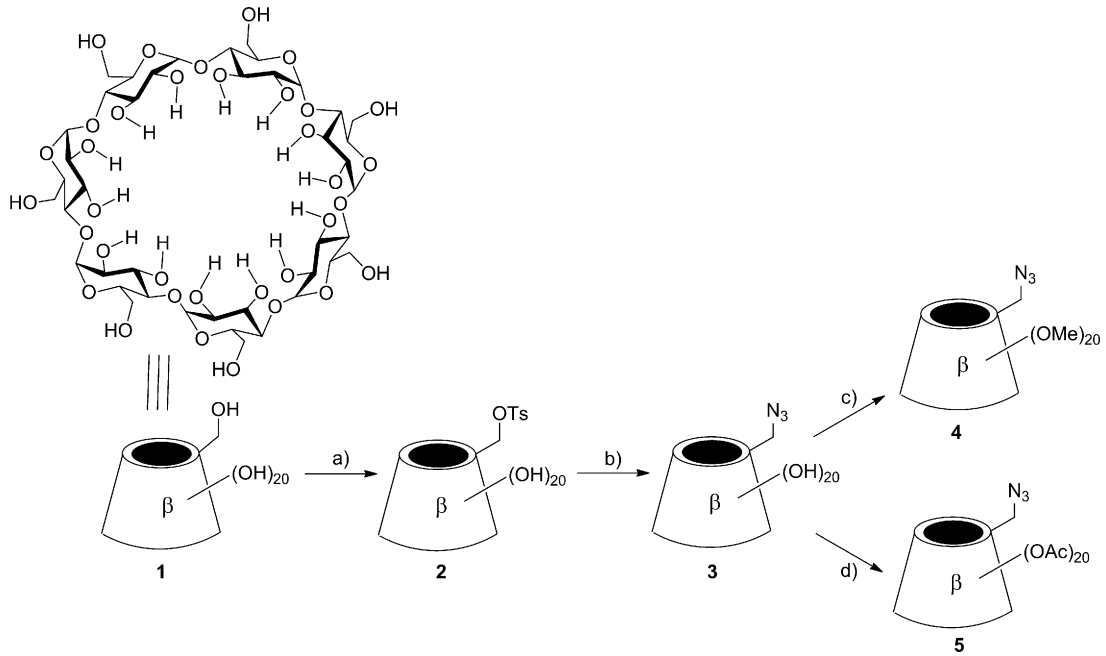
Cyclodextrins (CDs) are cyclic (α-1,4-)-linked water-soluble oligosaccharides of α-glucopyranose containing a relatively hydrophobic central cavity and hydrophilic outer surface. The most common cyclodextrins are α-CD, β-CD, and γ-CD, which consist of six, seven, and eight glucopyranose units, respectively. Given their hydrophilic external surface and hydrophobic inner surface structure, CDs and their derivatives have proven to be very efficient host molecules for the binding of a large number of organic and inorganic guest molecules.[19] Thus, CDs have frequently been applied in pharmacy,[19a–d] analytical chemistry,[19b] enzyme mimics,[19f] as well as agriculture and environmental engineering,[19h] etc. For instance, in the pharmaceutical field,[19a,e,20] CDs are versatile excipients used to enhance the solubility, stability, safety, and bioavailability of drugs. In addition, chemically modified CD derivatives have also been widely used in the design of artificial enzymes and for enantiomeric separation.[19b,f,g]

As a continuation of our ongoing studies,[16] we describe herein a simple and reliable synthetic protocol involving a copper-catalyzed azide–alkyl cycloaddition (CuAAC, 'click chemistry') for the preparation of β-CD–pentacyclic triterpene conjugates as a new class of anti-HCV entry inhibitors.

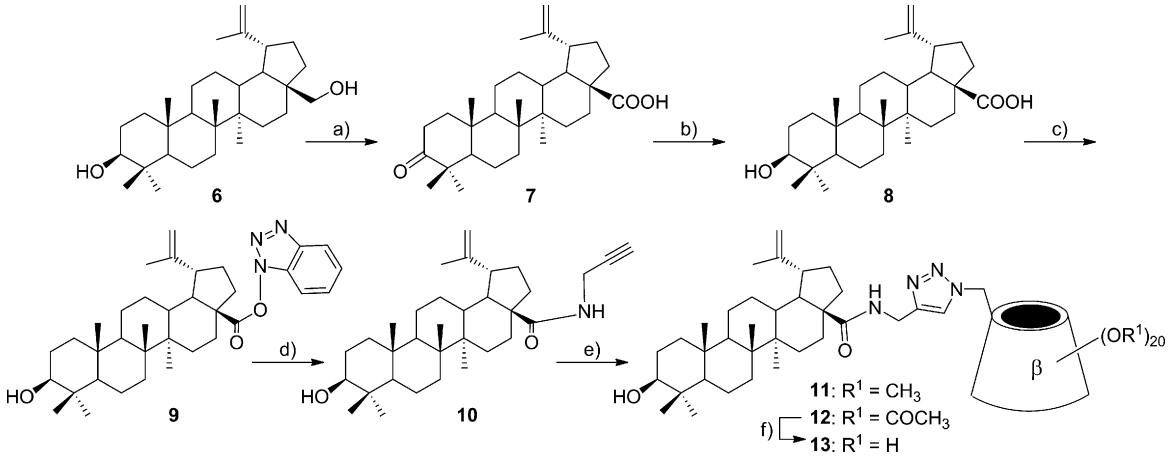
Results and Discussion

Chemistry

The synthetic routes for 6^A-azide-6^A-deoxyper-O-alkylated β-CDs **4** and **5** are depicted in Scheme 1. Briefly, selective monotosylation of β-CD **1**, according to the procedure described by Thatcher and colleagues.[21] with minor modifications, afforded compound **2**. This was followed by nucleophilic substitution with sodium azide in *N*,*N*-dimethylformamide (DMF) to provide the key intermediate 6^A-azide-6^A-deoxy-β-CD **3** in quantitative



Scheme 1. *Reagents and conditions*: a) H_2O, NaOH, TsCl; b) DMF, NaN_3, 80 °C, 18 h; c) DMF, NaH, CH_3I; d) pyridine, DMAP, Ac_2O.



Scheme 2. *Reagents and conditions*: a) H_2CrO_4, acetone, 0 °C→RT, 18 h; b) $NaBH_4$, THF, RT, 2.5 h; c) TBTU, DIPEA, THF; d) propargylamine, K_2CO_3, DMF, 1 h; e) THF/H_2O (1:1, *v*/*v*), $CuSO_4$, sodium L-ascorbate, **4** or **5**; f) CH_3ONa/CH_3OH, RT, 8–10 h.

yield, which was used without further purification in the next step. Methylation of crude **3** with methyl iodide in the presence of sodium hydride afforded the 6^A-azide-6^A-deoxyper-O-methylated β-CD **4** in 86% yield. 6^A-Azide-6^A-deoxyper-O-acetylated β-CD **5** was obtained in 92% yield by treating crude **3** with acetic anhydride in the presence of 4-dimethylaminopyridine (DMAP) and pyridine.

The synthesis of β-CD–BA conjugates **11**–**13** is illustrated in Scheme 2. At first, BA was synthesized by a modified method of Kim et al.[22] Oxidation of betulin **6** with Jones reagent in acetone, followed by reduction with sodium borohydride in tetrahydrofuran gave betulinic acid **8** in 42% yield (two steps).[23] Activation of the carboxyl group of **8** with 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) gave the stable intermediate **9** in excellent yield and purity, which was treated with propargylamine under basic conditions to afford **10** in 1 h in 92% yield. Compound **10** then underwent a click reaction with **4** or **5** in THF/H_2O (1:1) in the presence of a catalytic amount of copper sulfate and sodium ascorbate as reducing agent to yield **11** and **12** in 72 and 79% yields, respectively. The acetyl groups of **12** were removed by Zemplén reaction to afford **13**.

The structures of conjugates **11**–**13** were identified by 1D and 2D NMR spectroscopy as well as ESI high-resolution mass spectrometry (ESI-HRMS). In the aromatic region (Figure 2), the signal at $\delta = 7.58$–7.83 ppm referring to 1H, according to its ^1H–^1H and ^1H–^{13}C correlation spectra, could be assigned to triazole-CH. An additional signal was observed for the amide proton at 6.31–6.47 ppm for conjugates **11**–**12** (in $CDCl_3$) and at $\delta = 8.16$ ppm for conjugate **13** (in CD_3OD), indicating that these conjugates are indeed connected by a triazole linker. As mono-functionalization of β-CD itself causes loss of C_7 symmetry, spectral overlap in the 1D and 2D COSY spectra make assignment of the ^1H and ^{13}C signals of the glucose moieties extremely complex. The lower-field regions of these conjugates were relatively well resolved and fully assigned with the aid of ^1H–^1H COSY and ^1H–^{13}C HSQC spectra (Figure 3), which could be useful for assignment of the other signals by 2D COSY and HSQC. Similarly, the CuAAC reaction for the synthesis of other triterpene–β-CD conjugates **23**–**31** were carried out from commercially available pentacyclic triterpenes OA, EA, and UA in good yields (61–91%; Scheme 3). The aromatic region signals (triazole-CH and CONH) of conjugates **23**–**25** are similar to those of conjugate **11** (Figure 2). Figure 4 presents the characteristic parts ($\delta = 4.2$–5.6 ppm) of the ^1H NMR spectra (in $CDCl_3$) of conjugates **11** and **23**–**25**. The signals have been fully assigned with the aid of ^1H–^1H COSY and ^1H–^{13}C HSQC spectra.

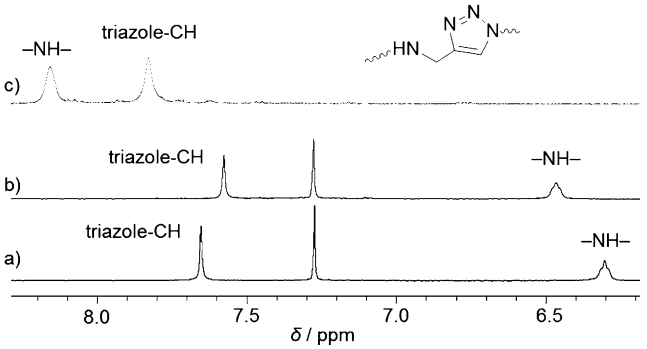


Figure 2. Aromatic region of the 400 MHz ^1H NMR spectra of conjugates a) **11** ($CDCl_3$), b) **12** ($CDCl_3$), and c) **13** (CD_3OD) recorded at 25 °C.

Calculated ALog*P*

The logarithm of the 1-octanol/water partition coefficient (log*P*) is a well-known measure of molecular hydrophobicity (or lipophilicity).[24] Lipophilicity governs the interaction of a given molecule with the intestinal membrane, yet such a compound must also possess hydrophobic character for passive transport across intestinal epithelia. In our study, the calculated Alog*P* values were determined using Pipeline Pilot software version 7.5 (Accelrys Corp., San Diego, CA, USA).[25] All the conjugates **11**–**13** and **23**–**31** showed increased hydrophilicity relative to their parent compound (Table 1). For example, a de-

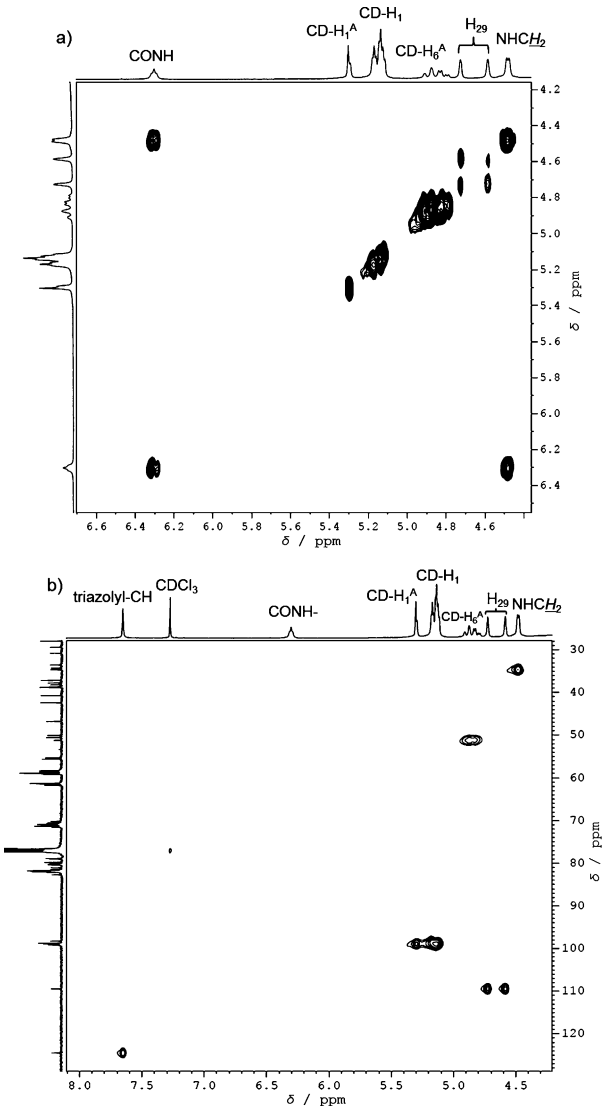


Figure 3. Characteristic portions of the 400 MHz a) 2D-COSY and b) HSQC spectra of conjugate **11** recorded in $CDCl_3$ at 25 °C.

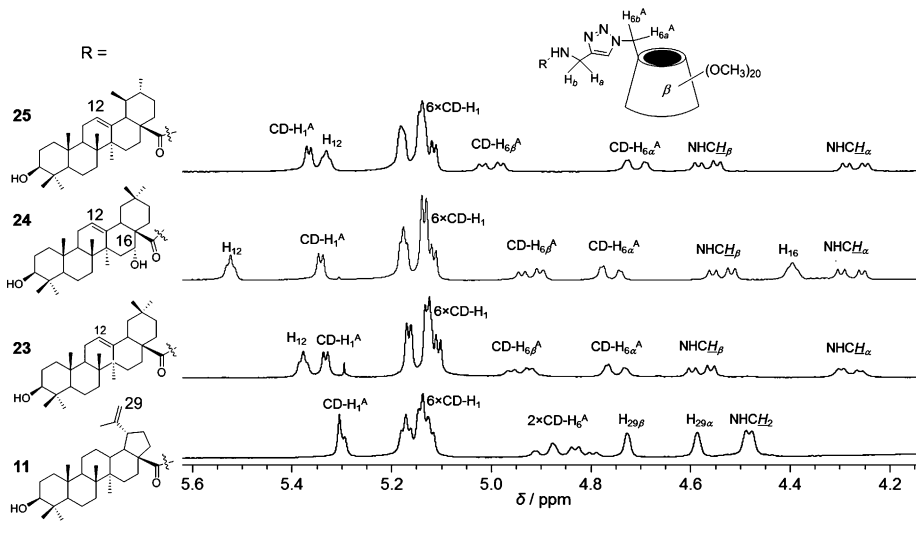


Figure 4. Characteristic portions of the 400 MHz ^1H NMR spectra of conjugates **11**, **23**, **24**, and **25** recorded in $CDCl_3$ at 25 °C

Table 1. Calculated Alog*P* values of β-CD–pentacyclic triterpene conjugates.[a]

Compd	Alog*P*	Compd	Alog*P*	Compd	Alog*P*
BA	6.546	**13**	−5.765	**28**	0.616
OA	6.447	**23**	2.306	**29**	−6.966
EA	5.345	**24**	1.204	**30**	1.764
UA	6.492	**25**	2.352	**31**	−5.819
11	2.405	**26**	1.718		
12	1.817	**27**	−5.864		

[a] Alog*P* values (Ghose and Crippen octanol–water partition coefficient at 25 °C) were calculated using Pipeline Pilot software, version 7.5 (Accelrys Corp., San Diego, CA, USA).

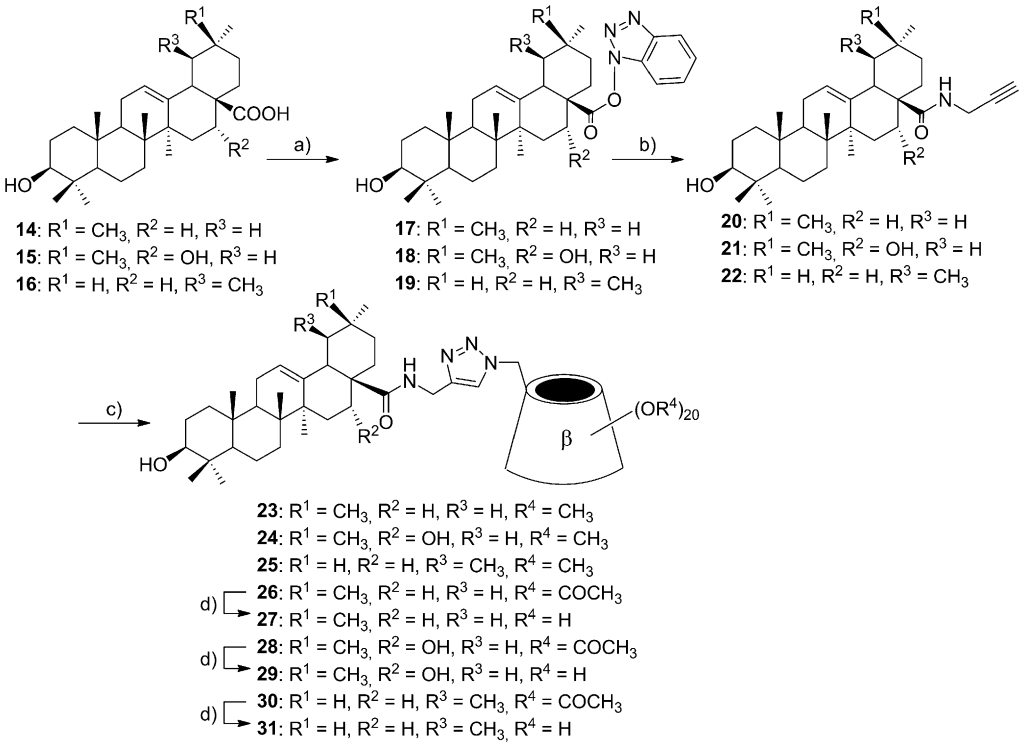
crease in Alog*P* value in the order BA > **11** > **12** > **13** was observed for BA and its conjugates. Similar results were also observed for OA, EA, and UA. Benefiting from the hydrophilic β-CD moiety, conjugates **13**, **27**, **29**, and **31** showed the lowest Alog*P* values.

Anti-HCV entry activity

β-CD has had well-known use in the improvement of certain properties of drugs such as bioavailability.[19] Our previous studies suggested that pentacyclic triterpenes OA and EA display substantial activity in blocking HCV entry.[16] Could β-CD improve the bioavailability of EA and OA, thereby increasing their anti-HCV entry activity? With this in mind, a series of β-CD–triterpene conjugates were designed and synthesized. As listed in Table 2, we found that: 1) the anti-HCV entry activities of the four pentacyclic triterpenes increased in the order: EA > BA ⩾ UA > OA, and the potencies of EA and BA are greater than those of the highly potent compound reported by Mittapalli et al.;[26] 2) except for BA, the introduction of β-CD on the triterpene resulted in modest (**27** vs. OA and **31** vs. UA) or the largest (**29** vs. EA) increases in potency, whereas no inhibition was observed in for anti-VSV (vesicular stomatitis virus) entry activity; and 3) all the per-*O*-methylated β-CD–triterpene conjugates (conjugates **11**, **23**, **24**, and **25**) showed more potent anti-VSV entry activities than their parent compound, especially for conjugates **11** and **23**, indicating that methylation of β-CD may impart some degree of cytotoxicity.

In vitro cytotoxic activity

The in vitro cytotoxic activity was evaluated for all of the synthesized conjugates **11**–**13** and **23**–**31** against HeLa (cervical cancer), HepG2 (liver cancer), MDCK (Madin–Darby canine kidney), and 293T (human



Scheme 3. *Reagents and conditions*: a) TBTU, DIPEA, THF; b) propargylamine, K_2CO_3, DMF, 1 h; c) THF/H_2O (1:1, *v/v*), $CuSO_4$, sodium L-ascorbate; d) CH_3ONa/CH_3OH, RT, 10 h.

Table 2. Anti-HCV and VSV pseudo-particle (pp) entry activities of β-CD–pentacyclic triterpene conjugates.

Compd	Inhibition [%][a]				CC_{50}/EC_{50}[b]
	HCVpp Entry		VSVpp Entry		
	1 μM	5 μM	1 μM	5 μM	
Ctrl[c]	21.2±1.1	34.0±0.9	11.7±2.2	12.8±1.8	–
EA	27.5±2.1	77.7±3.3	7.2±1.7	9.0±0.8	–
OA	22.4±1.4	26.4±0.9	10.6±2.0	23.6±2.2	–
UA	13.8±3.2	35.1±3.7	2.8±0.7	3.9±0.4	–
BA	4.2±0.8	65.2±3.2	−2.0±4.3	1.9±2.1	–
11	88.2±3.6	98.2±2.5	4.5±0.8	98.6±0.3	~1
12	70.3±5.6	81.7±4.7	8.7±1.5	10.3±3.1	–
13	37.1±2.3	42.7±1.9	−1.7±3.5	3.8±1.6	–
23	82.7±3.8	98.2±5.1	24.9±1.7	99.5±0.8	~1
24	59.5±3.5	74.5±2.1	11.5±1.1	49.8±0.9	–
25	10.1±1.0	37.7±2.6	21.6±1.3	24.9±1.7	–
26	56.7±3.5	89.7±4.3	−9.2±3.6	7.6±2.0	–
27	25.2±0.8	67.1±3.9	−1.4±4.8	18.5±3.2	–
28	−4.0±2.0	37.3±0.7	5.6±0.8	19.6±1.4	–
29	86.5±4.4	95.9±0.7	3.4±1.2	9.3±0.3	–
30	62.6±4.6	80.7±3.5	−1.8±2.3	−2.0±3.7	–
31	13.6±1.8	48.9±2.7	−2.8±2.6	−2.4±3.1	–

[a] Percent inhibition = (100%) − (DMSO negative control%) for each tested compound; data represent the mean ± SEM of three independent experiments performed in triplicate. [b] Except for compounds **11** and **23**, no significant toxicity was observed at 100 μM. [c] Positive control: methyl 9-(3-(4,6-dimethoxypyrimidin-2-yloxy)propyl)-2,3,4,9-tetrahydro-1*H*-carbazole-6-carboxylate.[26]

kidney) cell lines, by using an alamarBlue assay. The results showed that BA, UA, and four per-*O*-methylated β-CD–triterpene conjugates showed significant activity against HeLa cells relative to positive control (paclitaxel, PTX), whereas almost no cytotoxic activity was observed for EA, OA, and other β-CD conjugates (Figure 5). Similar results were also observed for the HepG2, 293T, and MDCK cell lines, except for BA, which showed no cytotoxic activity toward MDCK cells. In all experiments, four per-*O*-methylated β-CD–triterpene conjugates showed some cytotoxicity at 5 μM and strong cytotoxicity at 50 μM, indicating per-*O*-methylated β-CD may have some cytotoxicity in vitro.

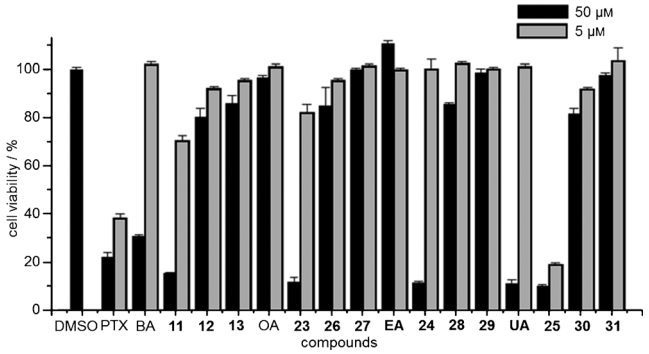


Figure 5. In vitro activity of conjugates **11**–**13** and **23**–**31** against HeLa cells. The cells were seeded at a density of 1×10^4 cells per well in 96-well plates. The plates were left for 16 h to allow the cells to adhere to the well surface. Conjugates were added at 5 or 50 μM in triplicate, and the cells were left for another 48 h. Control wells contained the equivalent volume of culture medium with 1% DMSO. An alamarBlue assay was performed to determine the level of anti-proliferation. Error bars represent standard deviations.

Hemolytic assays

A series of studies have demonstrated that the aglycons of triterpenoids exert a significant influence on hemolytic properties, one of the well-known characteristics of saponins.[27] In our previous studies, the substantial hemolytic property of triterpenes disappeared upon biotransformation or modification at the C28 carboxyl group.[16] In this study we also found that all the β-CD–pentacyclic triterpene conjugates were depleted of hemolytic activity upon the introduction of β-CD or its derivatives at the C28 carboxyl group (Figure 6).

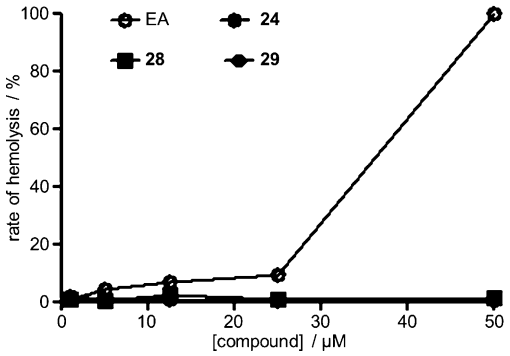


Figure 6. Hemolysis assay of EA and its β-CD conjugates.

Conclusions

We have shown the synthesis, anti-HCV entry activity, and cytotoxicity, including hemolytic exploration of new β-CD–pentacyclic triterpene conjugates. As expected, all the new conjugates showed lower hydrophobicity (Alog*P*) than their respective parent compounds. Several conjugates exhibited moderate anti-HCV entry activity in an HCV pseudo-particle (HCVpp) entry assay. Except these per-O-methylated β-CD–pentacyclic triterpene conjugates, other conjugates showed no apparent cytotoxicity in an alamarBlue assay using four cell lines. In addition, the hemolytic toxicity of these new conjugates disappeared upon coupling with β-CD. Easy access to such conjugates that combine the properties of β-CD and pentacyclic triterpenes may provide a way to obtain a new class of anti-HCV entry inhibitors.

Experimental Section

Chemistry

The syntheses of **2**–**5**, **7**–**8**, **18**, and **21** were performed as previously reported (Supporting Information).[16b,21,22] IR spectra were recorded on a Nicolet Nexus 470 spectrometer (American Thermo Nicolet Co.) in the range 400–4000 cm^{-1} using KBr pellets. ESI-HRMS data were obtained with an APEX IV FT_MS (7.0 T) spectrometer (Bruker) in positive ESI mode. NMR spectra were recorded on a Bruker DRX 400 spectrometer at room temperature. ^{1}H NMR chemical shifts are referenced to the internal standard TMS (δ_H = 0.00 ppm) or the solvent signal (δ_H = 3.31 ppm for the central line of MeOD). ^{13}C NMR chemical shifts are referenced to the solvent signal (δ_C = 77.00 ppm for the central line of $CDCl_3$, δ_C = 49.00 ppm for the central line of MeOD). Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel 60 F_{254} plates (layer thickness: 0.2 mm, E. Merck, Darmstadt, Germany) and detected by staining with a yellow solution containing $Ce(NH_4)_2(NO_3)_6$ (0.5 g) and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (24.0 g) in 6% H_2SO_4 (500 mL) followed by heating. Flash column chromatography was performed on silica gel 60 (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd.). The calculated Alog*P* and water solubility values were determined using Pipeline Pilot software v. 7.5 (Accelrys Corp., San Diego, CA, USA).

General procedure A for the synthesis of 1-benzotriazolyl triterpene ester: To a solution of triterpene BA, OA, EA, or UA (0.22 mmol) in THF (10 mL) was added TBTU (106 mg, 0.33 mmol) and DIPEA (0.12 mL, 0.66 mmol) at room temperature. The reaction mixture was stirred for 5 h under nitrogen. The solvent was removed in vacuo. The residue was dissolved with CH_2Cl_2 (15 mL), washed with brine (3×5 mL), dried with Na_2SO_4, filtered, and the solvent was removed in vacuo. The crude product was purified by flash chromatography.

General procedure B for the synthesis of *N*-propargyl triterpene amide: To a solution of 1-benzotriazolyl triterpene ester (0.74 mmol) in DMF (10 mL) was added K_2CO_3 (153 mg, 1.11 mmol) and 2-propargylamine (33 μL, 0.89 mmol) at room temperature. The reaction mixture was stirred for 1 h under nitrogen. The solvent was removed in vacuo. The residue was dissolved with 25 mL CH_2Cl_2, washed with brine (3×5 mL), dried with Na_2SO_4, filtered, and the solvent was removed in vacuo. The crude product was purified by flash chromatography.

General procedure C for the click reaction: To a solution of alkyne (0.09 mmol) and azide (0.06 mmol) in THF/H_2O (1:1 *v/v*, 5 mL) was added $CuSO_4$ (9.5 mg, 0.06 mmol) and sodium ascorbate (24 mg, 0.12 mmol). The resulting solution was stirred vigorously for 12 h at room temperature. The reaction mixture was extracted with CH_2Cl_2 (3×10 mL). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography.

General procedure D for the deacetylation reaction: The per-*O*-acetylated β-CD–triterpene conjugate was dissolved in dry CH_3OH (~5 mL per 100 mg compound) and a solution of $NaOCH_3$ (30% in CH_3OH, 0.1 equiv [mol acetate]$^{-1}$) was added. The solution was stirred at room temperature for 4–6 h. After completion (TLC) the reaction mixture was neutralized with Amberlite IR-120 (H^+) ion-exchange resin, then filtered and concentrated. The crude product was purified by RP column chromatography (eluted by CH_3OH).

1-Benzotriazolyl 3β-hydroxylup-20(29)-en-28-oate (9): According to general procedure A, the residue was purified by flash chromatography (eluent: petroleum ether (PE)/EtOAc = 4:1) to afford **9** as a white solid in 93% yield. R_f = 0.43 (PE/EtOAc = 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.08 (d, 1H, *J* = 8.4 Hz), 7.55 (t, 1H, *J* = 7.4 Hz), 7.42 (t, 1H, *J* = 7.6 Hz), 7.37 (d, 1H, *J* = 8.3 Hz), 4.73 (s, 1H), 4.64 (s, 1H), 3.19 (dd, 1H, *J* = 4.9, 11.1 Hz), 2.95 (td, 1H, *J* = 4.9, 11.1 Hz), 2.64 (m, 1H), 2.41 (dd, 1H, *J* = 7.8, 13.0 Hz), 2.22 (td, 1H, *J* = 3.6, 12.4 Hz), 2.04–2.15 (m, 1H), 0.78–1.82 (m, other aliphatic ring protons), 1.71, 1.05, 0.99, 0.98, 0.81, 0.76 (s, each 3H), 0.70 ppm (d, 1H, *J* = 10.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.80, 149.20, 143.59, 128.87, 128.61, 124.65, 120.59, 107.90, 110.34, 78.90, 57.00, 55.37, 50.55, 49.95, 46.55, 42.46, 40.78, 38.84, 38.73, 38.49, 37.18, 36.75, 34.36, 31.39, 30.28, 30.10, 27.97, 27.36, 25.41, 20.79, 19.37, 18.26, 16.12, 16.07, 15.33, 14.81 ppm; IR (KBr): $\tilde{\nu}$ = 3434, 2944, 2871, 1808,

1643, 1452, 1020, 741 cm^{-1}; ESI-HRMS calcd for $C_{36}H_{52}N_3O_3$ $[M+H]^+$: 574.4003, found: 574.4003.

***N*-propargyl 3β-hydroxylup-20(29)-en-28-amide (10)**: According to general procedure B, the residue was purified by flash chromatography (eluent: PE/EtOAc = 4:1) to afford **10** as a white solid in 90% yield. R_f = 0.43 (PE/EtOAc = 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.81 (t, 1H, J = 4.8 Hz), 3.94–4.10 (m, 2H), 3.10–3.20 (m, 2H), 2.42 (td, 1H, J = 3.3, 12.7 Hz), 2.21 (t, 1H, J = 2.4 Hz), 0.74–1.96 (m, other aliphatic ring protons), 1.68, 0.97, 0.96, 0.94, 0.82, 0.75 (s, each 3H), 0.67 ppm (d, 1H, J = 8.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.83, 150.77, 109.36, 80.14, 78.91, 71.06, 55.62, 55.35, 50.59, 50.13, 46.66, 42.41, 40.75, 38.81, 38.69, 38.06, 37.71, 37.16, 34.35, 33.55, 30.76, 29.35, 28.94, 27.95, 27.37, 25.56, 22.62, 20.86, 19.45, 18.26, 16.10, 15.32, 14.61 ppm; IR (KBr): $\tilde{\nu}$ = 3368, 3312, 2946, 2869, 1643, 1453, 626 cm^{-1}; ESI-HRMS calcd for $C_{33}H_{52}NO_2$ $[M+H]^+$: 494.3993, found: 494.3991.

***N*-[1-(6^A-deoxyper-*O*-methyl-β-CD-6-yl)-1*H*-1,2,3-triazol-4-yl]-methyl 3β-hydroxylup-20(29)-en-28-amide (11)**: Prepared from **4** and **10** according to general procedure C, the residue was purified by flash chromatography (eluent: PE/EtOAc/acetone = 1:1:1) to afford **11** as a white solid in 62% yield. R_f = 0.30 (CH_2Cl_2/CH_3OH = 15:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.65 (s, 1H), 6.31 (t, 1H), 5.30 (d, 1H, $J_{1,2}$ = 3.2 Hz, overlap with CH_2Cl_2), 5.16–5.18 (m, 3H), 5.11–5.14 (m, 4H), 4.78–4.91 (m, 2H), 4.72 (br s, 1H), 4.58 (br s, 1H), 4.48 (d, 2H, J = 4.8 Hz), 4.04–4.08 (m, 1H), 3.32–3.99 (m, 92H), 3.16–3.26 (m, 7H), 3.07–3.14 (m, 1H), 3.02 (dd, 1H, J = 3.4, 9.8 Hz), 2.37–2.44 (m, 1H), 0.88–2.04 (m, other aliphatic ring protons), 1.67, 0.96, 0.95, 0.84, 0.81, 0.75 (6×CH_3), 0.67 ppm (d, 1H, J = 8.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 176.24, 150.79, 144.33, 124.45, 109.43, 99.19, 98.93, 98.90, 98.85, 98.75, 98.23, 82.70, 82.14, 82.02, 82.01, 81.96, 81.88, 81.78, 81.76, 81.51, 81.08, 80.39, 80.27, 80.18, 79.79, 79.02, 78.90, 71.57, 71.32, 71.25, 71.04, 70.96, 70.92, 70.76, 70.56, 70.20, 61.77, 61.48, 61.45, 61.34, 61.32, 61.28, 59.20, 59.06, 58.99, 58.93, 58.70, 58.63, 58.58, 58.43, 58.32, 58.29, 55.62, 55.33, 51.21, 50.56, 50.08, 46.76, 42.43, 40.73, 38.83, 38.67, 38.20, 37.76, 37.18, 34.65, 34.29, 33.54, 30.82, 29.40, 27.96, 27.38, 25.56, 20.88, 19.37, 18.28, 16.11, 15.95, 15.35, 14.61 ppm; IR (KBr): $\tilde{\nu}$ = 3436, 2930, 1660, 1460, 1038, 554 cm^{-1}; ESI-HRMS calcd for $C_{95}H_{161}N_4O_{36}$ $[M+H]^+$: 1934.0885, found: 1934.0836.

***N*-[1-(6^A-deoxyper-*O*-acetyl-β-CD-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl 3β-hydroxylup-20(29)-en-28-amide (12)**: Prepared from **5** and **10** according to general procedure C, the residue was purified by flash chromatography (eluent: PE/EtOAc/acetone = 1:1:1) to afford **12** as a white solid in 73% yield. R_f = 0.34 (CH_2Cl_2/CH_3OH = 15:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.58 (s, 1H), 6.47 (t, 1H), 5.54 (d, 1H, $J_{1,2}$ = 3.2 Hz), 5.25–5.38 (m, 6H), 5.13–5.19 (m, 2H), 5.00–5.13 (m, 6H), 5.05 (m, 1H), 4.94 (dd, 1H, J = 4.0, 8.3 Hz), 4.66–4.87 (m, 7H), 4.73 (m, 2H), 4.69 (m, 1H), 4.52–4.61 (m, 5H), 4.58 (m, 1H), 4.01–4.39 (m, 13H), 4.35(m, 1H), 3.69–3.77 (m, 5H), 3.62 (t, 1H, J = 8.3 Hz), 3.54 (t, 1H, J = 8.9 Hz), 3.18 (dd, 1H, J = 4.6, 11.2 Hz), 3.09–3.13 (m, 1H), 2.48 (t, 1H, J = 9.7 Hz), 2.03–2.18 (m, 60H), 0.86–1.90 (m, other aliphatic ring protons), 1.67, 1.26, 0.96, 0.91, 0.82, 0.75 (6×CH_3), 0.67 ppm (d, 1H, J = 8.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 176.29, 170.82, 170.72, 170.67, 170.57, 170.51, 170.46, 170.36, 170.25, 169.51, 169.39, 169.34, 169.29, 169.13, 150.93, 144.89, 124.73, 109.34, 97.06, 96.92, 96.84, 96.80, 96.71, 96.60, 96.41, 78.95, 77.87, 77.20, 77.02, 76.84, 76.69, 76.55, 75.95, 71.50, 71.34, 71.28, 71.12, 70.98, 70.82, 70.46, 70.39, 70.18, 70.04, 69.98, 69.87, 69.66, 69.54, 69.49, 69.37, 69.29, 62.77, 62.69, 62.59, 62.46, 62.25, 55.63, 55.36, 50.62, 50.08, 49.55, 46.66, 42.45, 40.72, 38.83, 38.70, 38.16, 37.66, 37.40, 37.18, 34.71, 34.36, 33.49, 30.84, 29.44, 29.24, 27.95, 27.40, 25.60, 20.87, 20.77, 20.72, 20.63, 19.40, 18.29, 16.10, 16.07, 15.33, 14.59 ppm; IR (KBr): IR (KBr): $\tilde{\nu}$ = 3468, 2949, 1751, 1658, 1373, 1238, 1045, 603 cm^{-1}; ESI-HRMS (*m/z*) calcd for $C_{115}H_{161}N_4NaO_{56}$ $[M+Na+H]/2^+$: 1258.4875, found: 1258.4836; $C_{115}H_{161}KN_4O_{56}$ $[M+K+H]/2^+$: 1266.4744, found: 1266.4754.

***N*-[1-(6^A-deoxy-β-CD-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl 3β-hydroxylup-20(29)-en-28-amide (13)**: Prepared from **12** according to general procedure D, the residue was purified by RP flash chromatography (eluent: CH_3OH) to afford **13** as a white solid in 91% yield. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.16 (br s, 1H), 7.83 (br s, 1H), 5.13 (d, 1H, $J_{1,2}$ = 2.4 Hz), 4.97–5.02 (m, 7H, overlap with H_2O), 4.72 (s, 1H), 4.58 (s, 1H), 4.33–4.55 (m, 3H), 4.06 (t, 1H), 3.68–3.96 (m, 22H), 3.42–3.60 (m, 12H), 3.57 (m, 1H), 3.35–3.40 (m, 2H, overlap with CH_3OH), 3.05–3.16 (m, 2H), 2.55 (t, 1H, J = 10.5 Hz), 2.15 (d, 1H, J = 11.6 Hz), 1.82–1.92 (m, 2H), 0.85–1.75 (m, other aliphatic ring protons), 1.69, 1.01, 0.95, 0.93, 0.87, 0.76 (6×CH_3), 0.71 ppm (d, 1H, J = 8.6 Hz); ^{13}C NMR (100 MHz, CD_3OD): δ = 179.40, 152.27, 147.21, 126.25, 110.10, 103.99, 103.90 (2C), 103.87, 103.83, 103.79, 103.55, 84.85, 83.11, 83.06, 83.01, 82.94, 82.82, 79.65, 74.79, 74.70, 74.59, 74.42, 74.21, 74.12, 74.07, 73.88, 73.66, 73.21, 72.11, 70.59, 61.83, 61.34, 57.01, 56.92, 52.29, 52.12, 49.85, 48.04, 43.51, 42.04, 40.07, 39.96, 39.32, 39.01, 38.33, 35.92, 35.63, 34.00, 31.99, 30.63, 28.69, 28.06, 27.03, 22.21, 19.71, 19.56, 16.91, 16.25, 15.10 ppm; IR (KBr): 3400, 2926, 1641, 1453, 1155, 1031, 581 cm^{-1}; ESI-HRMS calcd for $C_{75}H_{121}N_4O_{36}$ $[M+H]^+$: 1653.7755, found: 1653.7821; $C_{75}H_{120}N_4NaO_{36}$ $[M+Na]^+$: 1675.7574, found: 1675.7494.

1-Benzotriazolyl 3β-hydroxyolean-12-en-28-oate (17): According to general procedure A, the residue was purified by flash chromatography (eluent: PE/EtOAc = 4:1) to afford **17** as a white solid in 93% yield. R_f = 0.42 (PE/EtOAc = 2:1); δ = 8.05 (d, 1H, J = 8.4 Hz), 7.52 (t, 1H, J = 7.4 Hz), 7.39 (t, 1H, J = 8.0 Hz), 7.37 (d, 1H, J = 8.2 Hz), 5.38 (t, 1H, J = 3.4 Hz), 3.22 (dd, 1H, J = 4.2, 11.4 Hz), 2.98 (dd, 1H, J = 4.1, 13.6 Hz), 2.26 (td, 1H, J = 5.4, 14.9 Hz), 2.14 (td, 1H, J = 4.6, 13.5 Hz), 0.78–2.05 (m, other aliphatic ring protons), 1.22, 1.00, 0.97, 0.91, 0.85, 0.77 (6×CH_3), 0.75 ppm (d, 1H, J = 11.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.57, 143.51, 142.06, 128.73, 128.40, 124.56, 123.87, 120.46, 108.11, 78.91, 55.21, 47.57, 47.53, 45.44, 41.87, 41.56, 39.44, 38.92, 38.72, 38.48, 36.98, 34.07, 33.64, 32.88, 32.81, 32.39, 30.60, 29.44, 28.09, 28.02, 27.14, 25.70, 23.48, 23.43, 23.09, 22.91, 22.62, 20.11, 19.13, 18.28, 17.25, 15.56, 15.33, 14.37, 11.35 ppm; IR (KBr): $\tilde{\nu}$ = 3433, 2945, 1810, 1632, 1381, 1048, 743 cm^{-1}; ESI-HRMS calcd for $C_{36}H_{52}N_3O_3$ $[M+H]^+$: 574.4033, found: 574.4014.

1-Benzotriazolyl 3β-dihydroxyurs-12-en-28-oate (19): According to general procedure A, the residue was purified by flash chromatography (eluent: PE/EtOAc = 4:1) to afford **19** as a white solid in 93% yield. R_f = 0.42 (PE/EtOAc = 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04 (d, 1H, J = 8.4 Hz), 7.51 (t, 1H, J = 7.4 Hz), 7.39 (t, 1H, J = 7.5 Hz), 7.35 (d, 1H, J = 8.3 Hz), 5.38 (t, 1H, J = 3.5 Hz), 3.23 (m, 1H), 2.39 (d, 1H, J = 1.2 Hz), 2.29 (td, 1H, J = 4.0, 13.4 Hz), 1.93–2.21 (m, 6H), 0.85–1.71 (m, other aliphatic ring protons), 1.12, 1.01, 1.00, 0.94, 0.92, 0.91, 0.80 (6×CH_3), 0.75 ppm (d, 1H, J = 11.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.25, 143.45, 136.76, 128.68, 128.30, 127.04, 124.49, 120.37, 108.12, 78.84, 55.21, 52.94, 49.04, 47.47, 42.22, 39.63, 38.96, 38.69, 38.64, 36.90, 36.62, 33.14, 30.42, 28.31, 28.10, 27.14, 24.28, 23.29, 20.95, 18.23, 17.45, 16.81, 15.59, 15.46 ppm; IR (KBr): $\tilde{\nu}$ = 3433, 2927, 1806, 1620, 1458, 1047, 742 cm^{-1}; ESI-HRMS calcd for $C_{36}H_{52}N_3O_3$ $[M+H]^+$: 574.4003, found: 574.4005.

***N*-propargyl 3β-hydroxyolean-12-en-28-amide (20)**: According to general procedure B, the residue was purified by flash chromatography (eluent: PE/EtOAc = 4:1–3:1) to afford **20** as a white solid in

95% yield. R_f = 0.40 (PE/EtOAc = 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.12 (t, 1H, J = 4.5 Hz), 5.42 (t, 1H, J = 3.1 Hz), 3.87–4.09 (m, 2H), 3.22 (dd, 1H, J = 4.3, 11.1 Hz), 2.54 (dd, 1H, J = 3.3, 12.6 Hz), 2.21 (t, 1H, J = 2.4 Hz), 0.81–2.03 (m, other aliphatic ring protons), 1.17, 0.99, 0.91, 0.80, 0.79 (7×CH_3), 0.73 ppm (d, 1H, J = 11.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.95, 144.62, 123.10, 79.58, 78.77, 71.52, 55.02, 47.44, 46.56, 46.20, 42.02, 41.92, 39.30, 38.67, 38.43, 36.85, 33.98, 32.90, 32.24, 32.14, 30.62, 29.25, 28.01, 27.16, 27.05, 25.67, 23.78, 23.50, 23.44, 18.19, 16.87, 15.50, 15.29 ppm; IR (KBr): $\tilde{\nu}$ = 3436, 2942, 2876, 1658, 1509, 1045 cm^{-1}; ESI-HRMS calcd for $C_{33}H_{52}NO_3$ $[M+NH_4]^+$: 494.3993, found: 494.3991.

***N*-propargyl 3β-dihydroxyurs-12-en-28-amide (22)**: According to general procedure B, the residue was purified by flash chromatography (eluent: PE/EtOAc = 4:1) to afford **22** as a white solid in 93% yield. R_f = 0.41 (PE/EtOAc = 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.12 (br s, 1H), 5.36 (br s, 1H), 3.87–4.06 (m, 2H), 3.21 (dd, 1H, J = 3.6, 11.2 Hz), 2.22 (br t, 1H), 0.87–2.02 (m, other aliphatic ring protons), 1.10, 0.99, 0.95, 0.92, 0.87, 0.80, 0.78 (7×CH_3), 0.72 ppm (d, 1H, J = 11.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.80, 139.60, 125.97, 79.63, 78.77, 71.51, 55.04, 53.59, 47.63, 47.43, 42.33, 39.60, 39.45, 38.93, 38.65, 38.57, 36.81, 36.76, 32.60, 30.73, 29.26, 28.04, 27.71, 27.08, 24.83, 23.29, 23.18, 21.12, 18.16, 17.13, 16.82, 15.54, 15.42 ppm; IR (KBr): $\tilde{\nu}$ = 3421, 2936, 2870, 1649, 1516, 1456, 1045, 723 cm^{-1}; ESI-HRMS calcd for $C_{33}H_{52}NO_3$ $[M+NH_4]^+$: 494.3993, found: 494.3989.

***N*-[1-(6^A-deoxyper-*O*-methyl-β-CD-6-yl)-1*H*-1,2,3-triazol-4-yl]-methyl 3β-hydroxyolean-12-en-28-amide (23)**: Prepared from **4** and **20** according to general procedure C, the residue was purified by flash chromatography (eluent: PE/EtOAc/acetone = 1:2:1) to afford **23** as a white solid in 73% yield. R_f = 0.53 (PE/EtOAc/acetone = 1:2:3); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.64 (s, 1H), 6.59 (br s, 1H), 5.38 (t, 1H, J = 4.0 Hz), 5.33 (d, 1H, $J_{1,2}$ = 3.5 Hz), 5.16 (d, 2H, $J_{1,2}$ = 3.3 Hz), 5.12–5.13 (m, 3H), 5.10 (d, 1H, $J_{1,2}$ = 3.6 Hz), 4.94 (dd, 1H, J = 5.0, 14.0 Hz), 4.75 (br d, 1H, J = 12.4 Hz), 4.58 (dd, 1H, J = 5.7, 15.0 Hz), 4.28 (dd, 1H, J = 4.4, 15.2 Hz), 4.03–4.05 (m, 1H), 3.47–3.96 (m, 72H), 3.26–4.43 (m, 18H), 3.38 (m, 1H), 3.29 (m, 1H), 3.24 (dd, 1H, J = 3.5, 9.44 Hz), 3.16–3.22 (m, 6H), 2.98 (dd, 1H, J = 3.4, 9.8 Hz), 2.53 (br d, 1H, J = 9.2 Hz), 0.85–2.01 (m, other aliphatic ring protons), 1.14, 0.97, 0.88, 0.87, 0.86, 0.77 (6×CH_3), 0.70–0.72 (m, 1H), 0.61 ppm (CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.21, 144.30, 144.17, 124.79, 123.17, 99.22, 98.97, 98.86, 98.79, 98.60, 98.20, 82.27, 82.13, 82.03, 81.99, 81.89, 81.80, 81.76, 81.70, 81.42, 81.11, 80.47, 80.40, 80.27, 79.76, 79.70, 78.88, 78.78, 71.67, 71.32, 71.22, 71.03, 70.98, 70.89, 70.78, 70.60, 70.15, 69.47, 61.74, 61.51, 61.49, 61.33, 61.29, 59.24, 59.07, 58.99, 58.94, 58.69, 58.64, 58.42, 58.31, 58.26, 55.06, 51.07, 47.52, 46.57, 46.21, 42.00, 41.90, 39.31, 38.72, 38.41, 36.93, 34.82, 34.05, 32.96, 32.46, 32.31, 30.68, 28.05, 27.28, 27.13, 25.77, 23.76, 23.62, 23.43, 18.25, 16.60, 15.54, 15.32 ppm; IR (KBr): $\tilde{\nu}$ = 3429, 2929, 1659, 1461, 1367, 1040, 972, 556 cm^{-1}; ESI-HRMS calcd for $C_{95}H_{160}N_4NaO_{36}$ $[M+Na]^+$: 1956.0704, found: 1956.0713.

[1-(6^A-deoxyper-*O*-methyl-β-CD-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl 3β,16α-dihydroxyolean-12-en-28-amide (24): Prepared from **4** and **21** according to general procedure C, the residue was purified by flash chromatography (eluent: PE/EtOAc/acetone = 1:2:1) to afford **24** as a white solid in 65% yield. R_f = 0.38 (PE/EtOAc/acetone = 1:2:3); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.60 (br s, 1H), 6.86 (t, 1H, J = 5.1 Hz), 5.52 (br s, 1H), 5.34 (d, 1H, J = 3.6 Hz), 5.17–5.18 (m, 2H), 5.11–5.14 (m, 4H), 4.92 (dd, 1H, J = 5.3, 14.4 Hz), 4.76 (dd, 1H, J = 2.2, 14.3 Hz), 4.54 (dd, 1H, J = 5.4, 15.1 Hz), 4.39 (br s, 1H), 4.27 (dd, 1H, J = 4.7, 15.0 Hz), 4.02–4.06 (m, 1H), 3.17–3.96 (m, 39H), 3.65 (s, 9H), 3.63 (s, 12H), 3.52, 3.51, 3.50, 3.48 (4×s, 21H), 3.40 (s, 3H), 3.39 (2×s, 6H), 3.38 (s, 3H), 3.34 (s, 3H), 3.33 (s, 3H), 3.00 (dd, 1H, J = 2.4, 10.8 Hz), 2.74 (dd, 1H, J = 3.3, 13.4 Hz), 2.22 (t, 1H, J = 13.3 Hz), 0.86–2.02 (m, other aliphatic ring protons), 1.35, 0.99, 0.91, 0.90, 0.78, 0.65 ppm (7×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.70, 144.13, 143.38, 124.42, 123.55, 99.16, 98.92, 98.81, 98.73, 98.59, 98.18, 82.16, 82.12, 81.98, 81.96, 81.84, 81.74, 81.70, 81.39, 81.13, 80.39, 80.30, 80.24, 79.78, 79.64, 78.82, 78.70, 75.37, 71.57, 71.32, 71.22, 71.03, 70.96, 70.94, 70.88, 70.79, 70.58, 70.14, 61.71, 61.48, 61.44, 61.33, 61.29, 59.21, 59.06, 58.98, 58.92, 58.65, 58.63, 58.61, 58.44, 58.34, 58.30, 55.14, 50.97, 48.94, 46.77, 46.68, 41.74, 41.50, 39.56, 38.72, 38.50, 36.90, 35.24, 35.22, 35.11, 32.54, 32.48, 30.53, 30.19, 28.01, 27.13, 26.85, 25.12, 23.33, 18.18, 16.81, 15.56 ppm; IR (KBr): $\tilde{\nu}$ = 3434, 2929, 1730, 1658, 1462, 1368, 1038, 971, 554 cm^{-1}; ESI-HRMS (*m/z*) calcd for $C_{95}H_{160}N_4O_{37}Na$ $[M+Na]^+$: 1972.0654, found: 1972.0618.

[1-(6^A-deoxyper-*O*-methyl-β-CD-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl 3β-dihydroxyurs-12-en-28-amide (25): Prepared from **4** and **22** according to general procedure C, the residue was purified by flash chromatography (eluent: PE/EtOAc/acetone = 1:2:1) to afford **25** as a white solid in 73% yield. R_f = 0.53 (PE/EtOAc/acetone = 1:2:3); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.62 (s, 1H), 6.57 (br s, 1H), 5.36 (d, 1H, $J_{1,2}$ = 3.2 Hz), 5.33 (br s, 1H), 5.14–5.18 (m, 5H), 5.11 (d, 1H, $J_{1,2}$ = 3.3 Hz), 5.08 (dd, 1H, J = 4.8, 14.4 Hz), 4.70 (d, 1H, J = 12.9 Hz), 4.56 (dd, 1H, J = 5.6, 14.9 Hz), 4.27 (dd, 1H, J = 4.9, 14.7 Hz), 4.01–4.05 (m, 1H), 3.15–3.94 (m, 40H), 3.65 (s, 12H), 3.63 (s, 9H), 3.52 (s, 6H), 3.51 (s, 6H), 3.50 (2×s, 6H), 3.48 (s, 3H), 3.39 (2×s, 12H), 3.35 (s, 3H), 3.34 (s, 3H), 2.98 (dd, 1H, J = 3.1, 9.8 Hz), 0.85–2.02 (m, other aliphatic ring protons), 2.02 (m, 1H), 1.08, 0.98, 0.93, 0.89, 0.85, 0.86, 0.77 (6×CH_3), 0.71 (d, 1H, J = 11.4 Hz), 0.64 ppm (CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.07, 144.11, 139.21, 126.08, 124.71, 99.20, 98.95, 98.85, 98.77, 98.52, 98.16, 82.14, 82.10, 82.01, 81.98, 81.89, 81.88, 81.77, 81.71, 81.40, 81.11, 80.48, 80.36, 80.29, 79.76, 79.68, 78.90, 78.67, 71.69, 71.33, 71.24, 71.08, 70.99, 70.89, 70.80, 70.60, 70.15, 61.76, 61.51, 61.47, 61.33, 61.29, 61.27, 61.20, 59.26, 59.07, 58.99, 58.94, 58.68, 58.63, 58.42, 58.30, 58.28, 55.08, 53.57, 50.97, 47.64, 47.51, 42.33, 39.68, 39.49, 38.98, 38.72, 38.56, 37.01, 36.91, 34.78, 32.67, 30.81, 28.08, 27.82, 27.17, 24.85, 23.29, 21.16, 18.23, 17.15, 16.62, 15.59, 15.44 ppm; IR (KBr): $\tilde{\nu}$ = 3436, 2929, 1659, 1459, 1369, 1040, 972, 554 cm^{-1}; ESI-HRMS calcd for $C_{95}H_{161}N_4O_{36}$ $[M+H]^+$: 1934.0885, found: 1934.0854.

[1-(6^A-deoxyper-*O*-acetyl-β-CD-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl 3β-hydroxyolean-12-en-28-amide (26): Prepared from **5** and **20** according to general procedure C, the residue was purified by flash chromatography (eluent: PE/EtOAc/acetone = 1:1:1) to afford **26** as a white solid in 61% yield. R_f = 0.38 (CH_2Cl_2/CH_3OH = 15:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.58 (s, 1H), 6.63 (t, 1H, J = 5.3 Hz), 5.62 (d, 1H, $J_{1,2}$ = 3.8 Hz), 5.40 (br s, 1H), 5.26–5.36 (m, 6H, overlap with CH_2Cl_2), 5.16–5.20 (m, 3H), 5.08–5.13 (m, 3H), 5.07 (d, 1H, J = 3.7 Hz), 5.03 (d, 1H, J = 3.6), 4.95 (dd, 1H, J = 3.9, 8.6 Hz), 4.74–4.88 (m, 6H), 4.12–4.70 (m, 22H), 3.69–3.77 (m, 5H), 3.65 (t, 1H, J = 8.2 Hz), 3.51 (t, 1H, J = 9.5 Hz), 3.20 (dd, 1H, J = 4.2, 11.2 Hz), 2.60–2.62 (m, 1H), 2.02–2.18 (m, 60H), 0.75–1.90 (m, other aliphatic ring protons), 1.26, 1.15, 0.98, 0.89, 0.88, 0.77, 0.59 (7×CH_3), 0.73 ppm (t, 1H, J = 11.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.13, 170.78, 170.74, 170.71, 170.66, 170.58, 170.54, 170.48, 170.42, 170.35, 170.33, 170.28, 170.22, 169.54, 169.40, 169.37, 169.36, 169.28, 169.15, 144.36, 144.22, 125.14, 123.05, 97.08, 96.98, 96.85, 96.66, 96.61, 96.54, 96.37, 78.89, 77.36, 77.20, 76.92, 76.85, 76.66, 75.91, 71.63, 71.23, 71.17, 70.89, 70.79, 70.68, 70.41, 70.34, 70.12, 69.97, 69.82, 69.73, 69.66, 69.60, 69.51, 69.47, 69.42, 69.29, 62.80, 62.57, 62.42, 62.35, 62.22, 55.07, 49.17, 47.51, 46.53, 46.17, 41.92, 41.87,

39.28, 38.71, 38.42, 36.89, 35.01, 34.03, 32.97, 32.48, 32.34, 30.66, 28.03, 27.27, 27.13, 25.74, 23.72, 23.57, 23.41, 20.84, 20.81, 20.76, 20.74, 20.71, 20.68, 20.65, 20.62, 20.57, 18.23, 16.58, 15.51, 15.28 ppm; IR (KBr): $\tilde{\nu}$ = 3433, 1932, 1750, 1651, 1372, 1238, 1045, 603 cm^{-1}; ESI-HRMS (*m/z*) calcd for $C_{115}H_{160}N_4Na_2O_{56}$ $[M+2Na]^{2+}$:1269.4796, found: 1269.4846.

[1-(6^A-deoxy-β-CD-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl 3β-hydroxyolean-12-en-28-amide (27): Prepared from **26** according to general procedure D, the residue was purified by RP flash chromatography (eluent: CH_3OH) to afford **27** as a white solid in 92% yield. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 5:1): δ = 7.79 (s, 1H), 5.36 (brs, 1H), 5.07 (d, 1H, $J_{1,2}$ = 3.4 Hz), 4.93–4.96 (m, 7H, overlap with H_2O), 4.56 (dd, 1H, *J* = 7.4, 14.3 Hz), 4.48 (d, 1H, *J* = 15.2 Hz), 4.34 (d, 1H, *J* = 15.2 Hz), 4.05–4.09 (m, 1H), 3.70–3.91 (m, 23H), 3.37–3.57 (m, 14H), 3.25 (t, 1H, *J* = 9.4 Hz), 3.14–3.15 (m, 2H), 2.76–2.80 (m, 1H), 2.03–2.10 (m, 1H), 1.90–1.92 (m, 1H), 1.77 (t, 1H, *J* = 13.4 Hz), 0.90–1.62 (m, other aliphatic ring protons), 1.16, 0.97, 0.93, 0.91, 0.77 (6× CH_3), 0.75 (d, 1H, *J* = 12.0 Hz), 0.66 ppm (CH_3); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ 5:1): δ = 180.25, 146.12, 144.87, 126.14, 124.03, 103.92, 103.78, 103.73, 103.32, 84.69, 82.87, 82.80, 82.67, 79.50, 78.94, 74.51, 74.30, 74.22, 74.03, 73.86, 73.67, 73.39, 73.01, 71.80, 61.59, 61.07, 56.44, 51.94, 49.85, 47.39, 47.33, 42.72, 42.42, 40.43, 39.67, 39.62, 37.95, 35.84, 34.93, 33.97, 33.60, 33.52, 31.49, 28.65, 28.31, 27.64, 26.45, 24.38, 24.06, 23.95, 19.30, 17.61, 16.27, 15.91 ppm; IR (KBr): $\tilde{\nu}$ = 3435, 2946, 1750, 1651, 1372, 1238, 1045, 603 cm^{-1}; ESI-HRMS (*m/z*) calcd for $C_{75}H_{121}N_4O_{36}$ $[M+H]^+$:1653.7755, found: 1653.7673, calcd for $C_{75}H_{120}N_4NaO_{36}$ $[M+Na]^+$:1675.7574, found: 1675.7566.

[1-(6^A-deoxyper-*O*-acetyl-β-CD-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl 3β,16α-dihydroxyolean-12-en-28-amide (28): Prepared from **5** and **21** according to general procedure D, the residue was purified by flash chromatography (eluent: PE/EtOAc/acetone = 1:1:1) to afford **28** as a white solid in 68% yield. R_f = 0.25 (eluent: PE/EtOAc/acetone = 1:2:3); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.52 (brs, 1H), 6.90 (brs, 1H), 5.58 (d, 1H, $J_{1,2}$ = 3.3 Hz), 5.52 (brs, 1H), 5.24–5.37 (m, 6H), 5.19 (t, 1H, *J* = 8.4 Hz), 5.17 (d, 1H, $J_{1,2}$ = 3.7 Hz), 5.15 (m, 1H), 5.10 (d, 1H, $J_{1,2}$ = 4.0 Hz), 5.08 (d, 1H, $J_{1,2}$ = 4.0 Hz), 5.06 (d, 1H, $J_{1,2}$ = 3.7 Hz), 5.04 (d, 1H, $J_{1,2}$ = 3.7 Hz), 4.99 (d, 1H, $J_{1,2}$ = 3.6 Hz), 4.92 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 8.7 Hz), 4.78–4.84 (m, 3H), 4.73–4.76 (m, 2H), 4.09–4.68 (m, 23H), 3.67–377 (m, 5H), 3.62 (m, 1H, *J* = 8.3 Hz), 348 (m, 1H, *J* = 9.0 Hz), 3.18 (dd, 1H, *J* = 4.6, 10.7 Hz), 3.18 (dd, 1H, *J* = 2.5, 13.5 Hz), 2.01–2.13 (m, 60H), 1.32, 0.97, 0.91, 0.88, 0.87, 0.75, 0.62 ppm (7× CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.64, 170.77, 170.70, 170.68, 170.60, 170.54, 170.47, 170.44, 170.34, 170.32, 170.27, 170.22, 169.52, 169.38, 169.36, 169.34, 169.27, 169.14, 144.41, 143.19, 124.82 (triazole-CH_2), 123.42, 97.03 (2C), 96.81, 96.65, 96.60, 96.56, 96.41, 78.84, 77.31, 77.20, 76.97, 76.75, 76.67, 76.56, 75.88, 75.15, 71.57, 71.27, 71.22, 70.91, 70.57, 70.41, 70.29, 70.17, 70.06, 69.98, 69.83, 69.67, 69.63, 69.53, 69.47, 69.32, 62.78, 62.62, 62.58, 62.47, 62.41, 62.24, 55.21, 49.27, 49.07, 46.77, 46.68, 41.78, 41.46, 39.63, 38.73, 38.56, 36.90, 35.32, 35.21, 35.07, 32.55, 30.13, 29.03, 28.01, 27.17, 26.89, 25.37, 23.33, 20.82, 20.78, 20.76, 20.73, 20.70, 20.69, 20.65, 20.61, 20.57, 18.21, 16.85, 15.54 ppm; IR (KBr): $\tilde{\nu}$ = 3435, 2960, 1750, 1656, 1372, 1237, 1045, 603 cm^{-1}; ESI-HRMS calcd for $C_{115}H_{160}N_4NaO_{57}$ $[M+NH_4]^+$: 2531.9637, found: 2531.9640.

[1-(6^A-deoxy-β-CD-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl 3β,16α-dihydroxyolean-12-en-28-amide (29): Prepared from **28** according to general procedure D, the residue was purified by RP flash chromatography (eluent: CH_3OH) to afford **29** as a white solid in 89% yield. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 5:1): δ = 7.78 (s, 1H), 5.48 (brs, 1H), 5.06 (d, 1H, $J_{1,2}$ = 3.6 Hz), 4.93–4.97 (m, 6H), 4.91 (brs, 1H), 4.57 (dd, 1H, *J* = 6.9, 14.3 Hz), 4.49 (d, 1H, *J* = 15.3 Hz), 4.27–4.33 (m, 2H), 4.03–4.09 (m, 1H), 3.71–3.93 (m, 23H), 3.41–3.59 (m, 13H), 3.38 (brs, 1H), 3.23 (t, 1H, *J* = 9.4 Hz), 3.16 (t, 1H, *J* = 5.0 Hz), 3.10–3.15 (m, 1H), 2.88–2.91 (m, 1H), 2.28 (t, 1H, *J* = 13.3 Hz), 1.92–1.96 (m, 3H), 1.80–1.85 (m, 1H), 0.70–1.67 (m, other aliphatic ring protons), 1.35, 0.97, 0.94, 0.89, 0.77, 0.71 ppm (7× CH_3); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ 5:1): δ = 180.04, 145.79, 144.43, 126.01, 124.03, 103.84, 103.71, 103.70, 103.63, 103.26, 84.63, 82.86, 82.81, 82.67, 79.43, 75.11, 74.45, 74.41, 74.38, 74.23, 74.13, 73.96, 73.79, 73.61, 73.36, 73.31, 72.92, 71.70, 61.55, 61.05, 56.48, 52.01, 49.99, 47.90, 47.59, 42.67, 42.13, 40.61, 39.71, 39.62, 37.87, 35.88, 33.66, 33.12, 30.95, 30.79, 28.59, 27.62, 27.32, 25.75, 24.27, 19.21, 17.72, 16.23, 16.16 ppm; IR (KBr): $\tilde{\nu}$ = 3399, 2929, 1639, 1155, 1081, 1032, 581 cm^{-1}; ESI-HRMS (*m/z*) calcd for $C_{75}H_{121}N_4O_{37}$ $[M+H]^+$: 1669.7704, found: 1669.7677; calcd for $C_{75}H_{120}N_4NaO_{37}$ $[M+Na]^+$: 1691.7524, found: 1691.7489.

[1-(6^A-deoxyper-*O*-acetyl-β-CD-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl 3β-dihydroxyurs-12-en-28-amide (30): Prepared from **5** and **22** according to general procedure D, the residue was purified by flash chromatography (eluent: PE/EtOAc/acetone = 1:1:1) to afford **30** as a white solid in 61% yield. R_f = 0.38 (CH_2Cl_2/CH_3OH = 15:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.57 (s, 1H), 6.63 (t, 1H, *J* = 5.0 Hz), 5.62 (d, 1H, $J_{1,2}$ = 3.8 Hz), 5.26–5.38 (m, 6H), 5.35 (m, 1H), 5.16–5.23 (m, 3H), 5.12 (d, 1H, *J* = 4.0 Hz), 5.08–5.11 (m, 2H), 5.07 (d, 1H, *J* = 3.6 Hz), 5.01 (d, 1H, *J* = 3.7 Hz), 4.95 (dd, 1H, *J* = 3.8, 8.7 Hz), 4.75–4.88 (m, 6H), 4.69 (d, 1H, *J* = 11.4 Hz), 4.63 (m, 1H), 4.08–4.60 (m, 27H), 3.69–3.78 (m, 5H), 3.65 (t, 1H, *J* = 8.4 Hz), 3.52 (t, 1H, *J* = 9.1 Hz), 3.20 (dd, 1H, *J* = 4.0, 11.4 Hz), 2.02–2.18 (m, 60H), 0.90–2.10 (m, other aliphatic ring protons), 1.26, 1.09, 0.98, 0.89, 0.86, 0.77, 0.62 (7× CH_3), 0.71 ppm (d, 1H, *J* = 11.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.04, 170.79, 170.74, 170.72, 170.67, 170.63, 170.57, 170.55, 170.50, 170.43, 170.35, 170.29, 170.24, 169.56, 169.41, 169.39, 169.37, 169.35, 169.29, 169.16, 144.25, 139.04, 126.03, 125.16, 97.10, 97.00, 96.85, 96.67, 96.61, 96.59, 96.38, 78.93, 77.36, 76.94, 76.89, 76.69, 76.62, 75.90, 71.62, 71.25, 71.22, 70.91, 70.79, 70.65, 70.40, 70.35, 70.12, 70.05, 70.02, 69.96, 69.75, 69.66, 69.61, 69.52, 69.46, 69.28, 62.80, 62.61, 62.57, 62.43, 62.38, 62.22, 55.10, 53.47, 49.18, 47.60, 47.51, 42.28, 39.64, 39.48, 38.88, 38.71, 38.58, 36.99, 36.87, 34.92, 32.71, 30.81, 29.22, 28.07, 27.81, 27.17, 24.77, 23.29, 21.17, 20.86, 20.83, 20.77, 20.74, 20.69, 20.66, 20.63, 20.58, 18.22, 17.12, 16.61, 15.56, 15.40 ppm; IR (KBr): $\tilde{\nu}$ = 3419, 2930, 1640, 1155, 1081, 1033, 582 cm^{-1}; HRMS calcd for $C_{115}H_{160}N_4NaO_{56}$ $[M+Na]^+$: 2515.9687, found: 2515.9626.

[1-(6^A-deoxy-β-CD-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl 3β-dihydroxyurs-12-en-28-amide (31): Prepared from **30** according to general procedure D, the residue was purified by RP flash chromatography (eluent: CH_3OH) to afford **31** as a white solid in 91% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.89 (brs, 1H), 5.11 (d, 1H, $J_{1,2}$ = 3.7 Hz), 4.95–4.99 (m, 6H, 5× CD-H_1), 4.93 (m, 1H, overlap with H_2O), 4.65 (dd, 1H, *J* = 7.6, 14.4 Hz), 4.43 (d, 1H, *J* = 15.3 Hz), 4.37 (d, 1H, *J* = 15.3 Hz), 4.06–4.10 (m, 1H), 3.42–3.93 (m, 36H), 3.37 (m, 1H), 3.31 (m, 1H, overlap with CH_3OH), 3.12–3.17 (m, 2H), 2.15 (d, 1H, *J* = 10.8 Hz), 2.05–2.13 (m, 1H), 0.80–1.95 (m, other aliphatic ring protons), 1.13, 0.98, 0.91, 0.93, 0.79, 0.67 (7× CH_3), 0.75 ppm (d, 1H, *J* = 11.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 180.35, 145.96, 139.83, 127.31, 126.73, 104.01, 103.99, 103.88, 103.79, 103.48, 84.78, 83.08, 83.05, 83.02, 82.95, 82.86, 79.67, 74.80, 74.73, 74.59, 74.40, 74.23, 74.20, 74.08, 73.97, 73.87, 73.68, 73.30, 71.89, 61.92, 61.84, 61.35, 56.68, 54.14, 52.43, 49.85, 48.92, 43.26, 40.84, 40.27, 39.93, 39.84, 38.59, 38.09, 35.72, 34.14, 31.88, 29.54, 28.93, 28.78, 27.89, 25.22, 24.38, 24.09, 21.62, 19.46, 17.79, 17.71, 16.45, 16.13 ppm; IR (KBr):

$\tilde{\nu}$ = 3422, 2927, 1635, 1155, 1081, 1033, 581 cm^{-1}; ESI-HRMS calcd for $C_{75}H_{121}N_4O_{36}$ $[M+H]^+$: 1653.7755, found: 1653.7767.

Bioassays

HCV and VSV pseudovirus entry assays: All compounds were tested using the HCV and VSV pseudo-particle (HCVpp and VSVpp) entry assay as described previously.[6a,16] Pseudotyped viruses were produced by co-transfecting plasmid expressing HCV E1E2 or vesicular stomatis G protein (VSVG) with pNL4–3 HIV proviral DNA (AIDS Reagent Program, NIH, Bethesda, MD, USA), the envelope- and Vpr-deficient HIV vector carrying a luciferase reporter gene inserted into the Nef position in 293T producer cells. For compound library screening, Huh-7 cells (5×10^3 cells per well) were seeded into 96-well plates for 24 h, and then were infected with HCVpp or VSVGpp in the presence or absence of compounds, followed by incubation at 37 °C. Test compounds were diluted to a final concentration of 1 and 5 μM in 1% DMSO. Luciferase activity, reflecting the amount of pseudovirus entering host cells, was measured three days after infection using the Bright-Glo Reagent (Promega). Maximum activity (100% of control) and background were derived from control wells containing DMSO alone or from uninfected wells, respectively. CD81 and IM2281 were used as two positive controls, as reported previously.[6a] The individual signals in each of the compound test wells were then divided by the averaged control values (wells lacking inhibitor) after background subtraction, and multiplied by 100 to determine percent activity. The corresponding percent inhibition values were then calculated by subtracting this value from 100. The specificity of compounds for inhibiting HCV was determined by evaluating inhibition of VSVGpp infection in parallel. Each sample was performed in duplicate, and experiments were repeated at least three times.

Cytotoxicity assays: All of the reported conjugates were evaluated for cytotoxicity in HeLa (cervical cancer), HepG2 (liver cancer), 293T (human kidney), and MDCK (Madin–Darby canine kidney) cells. The cells (1×10^4 per well) were seeded in 96-well tissue culture plates and incubated for 16 h at 37 °C in an atmosphere of 5% CO_2 to allow the cells to adhere to the surface of the wells. The culture medium was then replaced with fresh medium containing the compounds at 5 and 50 μM in triplicate, and control wells contained the equivalent volume of medium with 1% DMSO, after which they were incubated for 48 h at 37 °C under an atmosphere of 5% CO_2. Following incubation, alamarBlue (10 μL per well) was added aseptically to the wells, and cultures were returned to the incubator; 2 h later, the fluorescence level was measured with an excitation wavelength of 530 nm, and emission wavelength of 590 nm. The percent cell viability (CV) was calculated using the equation: $CV(\%) = (F/F_0)\times100$, in which F is the fluorescence of the compound and F_0 is the fluorescence of DMSO.

Hemolysis assays: Hemolytic activity was determined as previously reported.[16,28] Briefly, 2% rabbit red blood cells (5×10^8 RBC mL^{-1}) in PBS (pH 7.4) were incubated with serially diluted compounds. After incubating at 37 °C for 60 min, hemolysis was monitored by measuring absorption at λ 541 nm with a microplate reader. Percentage of hemolysis was then calculated using the equation: $\%\text{hemolysis} = (OD_{541\,sample} - OD_{541\,PBS})/(OD_{541\,TX100} - OD_{541\,PBS})$, in which TX100 is 1% *v/v* Triton X-100. The observed hemolysis of RBC in PBS solutions and in 1% *v/v* Triton X-100 solution were used as negative and positive controls, respectively. All hemolysis experiments were carried out in triplicate.

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Keywords: antiviral agents · click chemistry · echinocystic acid · HCV entry inhibitors · pentacyclic triterpenes · cyclodextrins

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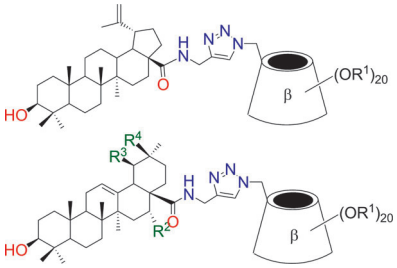
Synthesis and Anti-HCV Entry Activity Studies of β-Cyclodextrin–Pentacyclic Triterpene Conjugates



An awesome CD collection: A series of water-soluble triazole-bridged β-cyclodextrin (CD)–pentacyclic triterpene conjugates were synthesized, and their hydrophobicity, anti-HCV entry activities, and toxicity were studied. Easy access to such conjugates may provide a way to obtain a new class of anti-HCV entry inhibitors.