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Development of Oleanane-Type Triterpenes as a New Class of HCV Entry Inhibitors

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ABSTRACT

Development of hepatitis C virus (HCV) entry inhibitors represents an emerging approach that satisfies a tandem mechanism for use with other inhibitors in a multifaceted cocktail. By screening Chinese herbal extracts, oleanolic acid (**OA**) was found displaying weak potency to inhibit HCV entry with IC_{50} at 10 μM. Chemical exploration of this triterpene compound revealed its pharmacophore requirement for blocking HCV entry - ring A, B and E are conserved while ring D is tolerant to some modifications. Hydroxylation at C-16 significantly enhanced its potency for inhibiting HCV entry with IC_{50} at 1.4 μM. Further modification by conjugation of this new lead with a disaccharide at 28-COOH removed the undesired hemolytic effect, and more importantly increased its potency by ~5-fold (**54a**, IC_{50} 0.3 μM). Formation of a triterpene dimer via a linker bearing triazole (**70**) dramatically increased its potency with IC_{50} at ~10 nM. Mechanistically, such functional triterpenes interrupt the interaction between HCV envelope protein E2 and its receptor CD81 via binding to E2, thus blocking virus and host cell recognition. This study establishes the importance of triterpene natural products as new leads for the development of potential HCV entry inhibitors.

Keywords:

Triterpene; Oleanolic acid; Echinocystic acid; HCV entry inhibitor; SAR

INTRODUCTION

Hepatitis C virus (HCV) is the leading cause of liver fibrosis and cirrhosis that eventually lead to liver carcinomas.[1] Treatment of HCV infection with ribavirin/interferon has been used for almost 30 years. The recently approved Telaprevir and Boceprevir targeting HCV replication represent the beginning of a new era in the control of HCV infection.[2] However, resistance to individual antiviral drug is likely to appear and a combination of drugs targeting different stages of HCV life cycle is required. Inhibition of virus entry into HCV-permissive cells represents an emerging field for the prevention and reduction of infection. 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.

Pentacyclic triterpenes are secondary plant metabolites found in different plant organs, with a few species containing up to 30% of their dry weight.[3] These triterpenes have been proposed possessing defense activities due to their capability to prevent host from various pathogen and herbivore infections.[4] Betulinic acid, a lupane-type triterpene, has been confirmed by many studies displaying significant inhibiting activity against HIV entry and virus mature/release. One derivative, bevirimat (PA-457), is already in clinical trial.[5] Moronic acid and maslinic acid, two oleanane-type triterpenes, also display anti-HIV activity *in vitro*.[6] Other diverse and promising biological activities of triterpenes, including anti-inflammatory, hepatoprotective, analgesic, antimicrobial, antimycotic, virostatic, immunomodulatory and tonic effects, warrant further pharmaceutical development and even clinical investigation.[7]

Recently, a variety of compounds have been shown to exhibit anti-HCV entry activity with their IC_{50} at μM to even sub-μM level, depending on the sensitivity of

the assay in different laboratories.[8] Here, we report oleanolic acid (**OA**) and echinocystic acid (**EA**), two naturally occurring oleanane-type triterpenes (**Figure 1**), and their derivatives displaying substantial activity to inhibit HCV entry with one derivative (**70**) showing IC_{50} at ~10 nM. Further studies showed that these compounds represented a group of specific anti-HCVpp agents targeting viral entry process. The putative mechanism underlying the blocking of HCV entry by **EA** is its strong binding to E2, one of the envelope proteins of HCV, which blocks the interaction between E2 and CD81, a well-identified receptor for HCV entry. This study establishes the importance of triterpene natural products as leads for the development of potential HCV entry inhibitors.

RESULTS AND DISCUSSION

Discovery of Oleanolic Acid (OA) as a HCV-entry inhibitor. A library of Chinese herbal extracts was screened using a HCV pseudo particle (HCVpp) entry assay.[8b, 9] Vesicular stomtatis virus G protein pseudo particle (VSVpp) was also tested in parallel to determine the specificity and toxicity as previously reported.[8b, 9] The n-butanol/H_2O crude extract of *Dipsacus asperoides*, a traditional Chinese herbal medicine, displayed specific anti-HCV entry activity with 50% of viral entry being inhibited at the concentration of 50 μg/mL (**S Figure 1**). Subsequent bioactivity-guided phytochemical studies indicated the major active constituents in the extract were saponin due to their strong polarity and weak UV absorption at 254 nm (**S Figure 2**). One major aglycone isolated from the saponin was **OA** (**Figure 1**), a relatively non-toxic triterpene natural product exhibiting attractive hepatoprotective, antitumor and antiviral properties.[10] Biological assays indicated that **OA** displayed substantial activity on blocking HCV entry, with IC_{50} at ~10 μM (**Figure 1**), but was far less potent than their parental saponin mixture, which was more potent following

increase of their polarity (**S Figure 2**).

Identification of the Pharmacophore of OA. To assess the anti-HCV potential of structurally related triterpene compounds and determine the structure-activity relationship, a series of commercially available triterpenes were acquired from ChromaDex and Sigma. In addition, a variety of chemical modifications were carried out at the 3-OH, 28-COOH positions of **OA** to identify the structural requirements. As shown in **Figure 1**, initial exploration of **OA** indicated that 3-OH, the chemically modifiable group at ring A, was functionally important since its oxidation (**1**), acetylation (**2**) and glucosylation (**3**) all significantly decreased or even eliminated the activity. Further exploration at ring A indicated that not only 3-OH but also other positions were also less tolerant to some modifications: hydroxylation at C-2 (**4**), C-23 (**5**) or both (**6**) reduced the activity greatly. The potency of **5** was totally lost upon further oxidation of the 23-CH_2OH group into 23-CHO (**7**) or 23-COOH (**8**), or glucosidation at 3-OH (**9**). In addition to ring A, we found ring B was unmodifiable since hydroxylation at C-6 (**10**) completely abolished the potency of **OA**. These data suggested the left part of **OA**, including ring A and B, was conserved and any modification might significantly decrease its activity.

We subsequently explored the structure-activity relationship of ring C, in particular the allyl skeleton, and found that a substituent hydroxyl group at either the methylene (**11**) or vinyl skeleton (**12**) had almost no or just marginal effect on the potency of **OA**. In addition, introduction of a keto at the allyl skeleton (**13**) also had no obvious effect, suggesting ring C is a modifiable site. Continued exploration of the ring D of **OA** indicated that hydroxylation at C-16 (**EA**) remarkably enhanced the anti-HCV activity and the resulting **EA** showed an increase of IC_{50} by almost 8-fold (1.4 μM). This result suggested ring D was a modifiable part and more potent leads

might be prepared by modifying ring D. In addition, we synthesized a series of **OA** derivatives and found ester or amide substitutions at 28-COOH (**14**, **15**) maintained or even enhanced the potency, suggesting carboxyl acid is not absolutely required and can be modified.

Shift of C-29-methyl group from C-20 to adjacent C-19 (**16**) at ring E decreased the anti-HCV activity by more than 50%. Switch of 28-COOH with 29-methyl group plus oxidation at C-11 (**17**), a rather tolerant site, also decreased the anti-HCV potency. However, replacement of ring E, a 6-member ring, with a 5-member ring by switching the linker C-21-C-20 to C-21-C-19 (betulinic acid, **18**) significantly decreased the report readings in both HCVpp and VSVpp infected cells. This observation might reflect the cellular toxicity rather than potency enhancement by the replacement of oleanolic skeleton with betulinic acid, a relatively cytotoxic triterpene being developed as an anti-cancer drug.[8] In this study, the VSVpp entry assay, performed in parallel with HCVpp entry, was originally used to monitor the inhibitory specificity towards different viruses. Later, we found VSVpp assay is also useful to monitor chemical toxicity since the VSVpp, unlike HCVpp which infects only liver cell (Huh7), has broad host range and infects almost all cell lines. When a chemical is cytotoxic, the expression of luciferase reporter, delivered by VSVpp, in host cells will be inhibited. We found the VSVpp assay is even more sensitive to chemical toxicity than alamarBlue assay. Overall, **OA** SAR data provide positional requirements and their pharmacophore contributions on the antiviral activity, suggesting part of **OA** right side could be modified. Further modification at this region, just like **EA**, might enhance its anti-HCV potency.

Pharmacophore confirmation via chemical modification of EA. **EA** is an analog of **OA**, with a hydroxyl group at C-16 (**Figure 1**). Such a small variation significantly

differentiated **EA** from **OA** with almost 10-fold potency gain for **EA**. Therefore, **EA** was chosen as a new lead for SAR exploration and the same conclusion was obtained. As shown in **Table 1**, modifications of 3-OH at ring A, including sulfonation (**19**), amination (**20**), benzylation (**21**), oxidation (**22**), methylation (**23**), elimination (**24**) and other modifications (**25, 26**), made these compounds less potent than **EA**. Hydroxylation at other sites of ring A, including at C-1 (**27**) or C-23 (**28**), also significantly decreased potency. Further oxidation of 23-CH_2OH into 23-CHO (**29**) and then 23-COOH (**30**) completely abolished the potency of **EA**. Similarly, modification of ring B, including hydroxylation at C-6 (**31**) or C-7 (**32**) via biotransformation approach,[11] abolished the antiviral activity of **EA**. Therefore, the left side of **EA**, including ring A and B, is functionally conserved and some modification at this side might remarkably attenuate its potency.

Modification of ring C indicated that a keto (**33**) or a hydroxyl (**34**) substitute at the vinyl skeleton had almost no effect or only marginal effect on the potency of **EA**, similar to the case of **OA** that ring C is tolerant for chemical modifications. Explorations of ring D (**35-45**) indicated that the potency gained by the introduction of a hydroxyl group at C-16 was lost upon acetylation or elimination of 16-OH. However, oxidation of 16-OH seemed to bring less effect on the potency. Furthermore, **46**, a glycoside conjugate at both 3-OH and 28-COOH, also displayed remarkable potency with >90% HCV entry being inhibited at 10 μM (**Table 1**). The significant inhibition of HCV entry by glucose substitution at the conserved 3-OH (**47**) implied that the potency of **EA** can be further enhanced if the glycoside was kept at 28-COOH, consistent with the initial observation that the more polar the saponin extract the more potent the virus entry blocking activity. An independent biotransformation experiment indicated that the ring E of **EA** was not a potential modifiable site since hydroxylation

at C-21 and C-29 (**48**, **49**) all significantly decreased its potency on blocking HCV entry. [11] Apparently, intact rings A, B and E were common structural features that were required for the anti-HCV entry activity.

Development of more potent HCV entry inhibitors. Pentacyclic triterpenes, such as **EA** and **OA**, are hydrophobic, which may affect their potential pharmacokinetic properties. To increase their solubility, a variety of carbohydrate moieties, including mono-, di- and even tri-saccharides, were conjugated to **EA** via 28-COOH, a modifiable site within ring D. As outlined in **Scheme 1**, direct esterification of **EA** with 2,3,4,6-tetra-*O*-acetyl-*α*-D-glucosyl bromide afforded **50a**, which was then hydrolyzed in the presence of CH_3ONa to give **50b**.[12] In the same fashion, conjugations of other saccharide with **EA** (**51a-55b**, **Table 1**) were carried out.

The HCV entry assay indicated that the **EA**-oligosaccharide conjugates, either carrying protective acetyl groups or not, exhibited more potent effects than **EA** (**Table 1**). Among them, acetylated galactose displayed the highest potency followed by acetyl protected glucose and ribose. For disaccharide conjugates, the terminally acetylated galactose (**54a**) showed the most promising inhibitory activity with IC_{50} at 0.3 μM, 5-fold more potent than **EA** (**Figure 2**). However, deacetylation of **EA** glycoconjudates reduced the potency, even though the solubility was significantly increased, especially in the case of **54a** versus **54b**. This result might be due to the reduction of hydrophobicity, which decreased the affinity of **EA**-conjugates to the protein targets that is usually via hydrophobic interactions.

A parallel experiment was carried out by conjugating **EA** with the same carbohydrate moieties through a triazole linker rather than an ester bond. Such alteration might theoretically enhance the stability of **EA** glycoconjugates (**58a-68b**, **Table 1**). The synthesis was based on a Cu(I) azide-alkyne cycloaddition reaction

(CuAAC) that linked acetyl-glucosyl azide and **57**, generating 1,4-disubstituted-1,2,3-triazole glucoconjugates in high yields (**Scheme 2**).[13] Biological evaluations indicated that almost all such conjugates with acetyl groups in the carbohydrate moieties exhibited comparable potency as their counterparts (**Table 1**). Deprotection of **EA** conjugates remarkably decreased their activities, independent of a mono-, di- or trisaccharide conjugate, just like the cases in the ester bond conjugates (**50a**-**55b**). In addition, we found that **EA** glycoconjugates with mono-, di- or trisaccharide display almost the identical activity, suggesting introduction of a monosaccharide is sufficient to enhance its biological activity.

Alternatively, combination of two pharmacophores together is one potential way to further increase the activity of **EA**. Here, two types of **EA** dimer, **69** and **70**, were synthesized as shown in **Scheme 3.** We found that **69**, the **EA** dimer linked by 1,3-propanediamine, displayed almost the same activity as **EA**. Unexpectedly, **70**, the **EA** dimer bearing a triazole linker, exhibited significantly higher potency with IC_{50} at 10.3 nM (**Figure 2**), almost two orders of magnitudes more potent than **EA** (1.4 μM). To our knowledge, this is the most potent compound based on HCVpp entry assay. Further explorations of this new lead are ongoing to obtain promising HCV entry inhibitors.

Mechanistic investigation of EA-mediated HCV entry inhibition. It is clear that intact ring A, B and E are common structural features among **EA** and their active derivatives and analogs, which leads us to hypothesize that they are the pharmacophore for blocking HCV entry, either via interacting with HCVpp, host Huh7 cells or both. To clarify this hypothesis, five different assay conditions were set up as previously reported.[14] In the first condition, the co-treatment assay, **EA** was present in the culture medium during the entire viral entry process. Briefly, cells were

infected with HCVpp or VSVpp in the presence of 10 μM **EA** and incubated for 72 h at 37 °C to allow virus entry. In the second condition, the pretreatment assay, cells were first pretreated with 10 μM of **EA** at 37 ^{o}C for 3 h, washed to remove unbound compound and then exposed to viruses at 37 ^{o}C for 72 h. In the third condition, the prebinding assay, cells were exposed to viruses in the presence of **EA** at 4 ^{o}C for 3 h, washed to remove unbound viruses and compound and then cultured at 37 ^{o}C for 72 h. In the fourth condition, the post-binding and pre-entry assay, cells were first incubated with viruses at 4 ^{o}C for 3 h, washed to remove unbound virus and then treated with **EA** at 37 ^{o}C for 72 h. In the fifth condition, the post-entry assay, cells were first treated with viruses at 37 ^{o}C for 6 h to allow the virus entry into cells. After washed unbound viruses, infected cells were treated with the compound at 37 ^{o}C for 72 h.

Under all five conditions, CD81 antibody was utilized as a positive control because it blocks HCV virus entry via binding to CD81 receptors. IM2865 was an unrelated compounds used as another control and 0.5% DMSO (final concentration) was used for normalization in each condition.[14] As compared to the co-treatment assay, a short pretreatment of the cells with the compound prior to virus infection (pretreatment) or co-treatment of cells with viruses and the compound at 4 ^{o}C (prebinding) resulted in very weak, if any, activity (**Figure 3**), suggesting that **EA** exerts its inhibitory activity post virus binding. This is remarkably different from CD81 antibody which interferes with virus attachment to the target cells by interacting with the surface receptors. In the post-binding or pre-entry condition, a high activity of **EA** was noted, suggesting that **EA** interferes with the subsequent step following virus attachment to target cells, presumably the step of viral envelope-cell membrane fusion. Under the post-entry condition, antiviral activity was not observed at all, indicating that **EA** did not interfere with the multiple processes after viral entry.

In conclusion, these data suggested that **EA** exerts its inhibitory activity at the stage of post-virus binding but before virus entry, presumably interfering with virus envelope-host cell membrane fusion.

EA-mediated disruption of CD81 and E2 interaction. In order to determine which target **EA** potentially binds to and thereby blocks HCV entry, a biotin-labeled **EA** conjugate (**73**) was synthesized (**Scheme 4**)[15] and then utilized as a probe to pull-down the targets in HCVpp packaging cell lysate. Conjugation of the biotin molecule at its 28-COOH through CuAAC had no detectable effect on the potency of **EA** (**Table 1**). As shown in **Figure 4**, HCV envelope protein E2 was selectively pulled down by streptavidin (SA) immobilized agrose. In contrast, no protein was captured in a parallel pull-down experiment wherein free biotin or **EA** was utilized, replacing **73** as the probe. Furthermore, competitive experiments indicated that no E2 was pull down when extra free **EA** was added into the cell lysate, and such competition was verified by an alternative assay - surface plasmon resonance (SPR) experiments wherein the binding of E2 to biotin-labeling **EA** chip was decreased by free **EA** in a significant dose-dependence manner (**S Figure 3**). Therefore, both supported the direct binding of **EA** to E2 protein. To confirm the binding is indeed due to the interaction between **EA** and E2, we then selected another set of experiments where SA-biotin interaction was used for the immobilization of E2 on the SA sensor tip in BIAcore assay. The sensor was then treated with samples of different concentrations of CD81 and CD81 together with **EA**. The sensor immobilized E2 on superstreptavidin (SSA) was subjected to treatment with sample **EA**.[16] A series of BIAcore assays indicated that the K_D between E2 and **EA** is around 24 μM, about 1000-fold lower than E2 and CD81 (K_D = 21 nM), and no binding was detected at all for **EA** to CD81 (**Figure 5**). Characterization of E2 and CD81 binding in the presence

of 10 μM **EA** indicated that their K_D decreased to 100 nM, ~5-fold lower than that without **EA**. Based on data from the biological and affinity assays, we proposed a tentative mechanism for **EA**-mediated blocking of HCV entry (**Figure 6**): **EA** binds to HCV envelope protein E2 and thus disrupts its interaction with CD81 receptor; such disruption does not block virus-host cell attachment but the followed fusion of the virus envelope and host cell membrane.

Hemolytic and cytotoxic studies of EA and its derivatives. A series of studies have demonstrated that the aglycon of triterpenoids possess crucial influence on the hemolytic properties, one of the well-known characteristics for saponins.[17] Compared with **OA** which possesses mild hemolytic activity, **EA** gains substantial hemolytic side effect with CC_{50} at 15 μM. Such hemolytic property may restrict these **EA** derivatives used as potential anti-HCV entry inhibitors. Here, we found that almost all the **EA** derivatives depleted the hemolytic activity upon modification of C-28 carboxyl group (**S Figure 4**). In addition, the cytotoxicity of the most representative compounds in this study, **EA**, **54a** and **70**, towards HepG2 (a liver cancer cell), Hela (a cervical cancer cell line) and 293T (a derived kidney normal cell) has been tested based on alamarBlue assay and no toxicity was detected even at concentration high as 50 μM (**S Figure 5**). Furthermore, data from the VSVpp entry, a useful assay reflecting the cytotoxicity of screen chemicals, also support that most **EA** derivatives display far less cytotoxicity (**Table 1**).

CONCLUSION

We identified oleanane-type triterpenes, oleanolic acid (**OA**) and echinocystic acid (**EA**), as new scaffolds for blocking HCV entry. Guided by SAR studies, a new class of HCV inhibitors with IC_{50} from μM to nM was prepared. Mechanistic studies indicated that such functional triterpenes bind to HCV envelope protein E2 and thus

interrupt the interaction between E2 and CD81 receptor. Such disruption does not block virus and host cell attachment but the followed virus-host fusion (**Figure 6**). This study establishes the importance of triterpene natural products as new leads for the development of potential HCV entry inhibitors.

EXPERIMENTAL SECTION

Chemistry. High Resolution Mass Spectra (HRMS) 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 ambient temperature. ^{1}H NMR chemical shifts were referenced to the internal standard TMS (δ_H = 0.00) or the solvent signal (δ_H = 3.31 for the central line of MeOD). ^{13}C NMR chemical shifts are referenced to the solvent signal (δ_C = 77.00 for the central line of $CDCl_3$, δ_C = 49.00 for the central line of MeOD). Reactions were monitored by thin-layer chromatography (TLC) on a pre-coated silica gel 60 F_{254} plate (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 method employed in determining the purity of synthesized compounds was HPLC using a Waters e2695 instrument and Waters 2998 photodiode array detector, with chromatography performed on a Diamonsil 250 × 4.6 mm, 5 μm C18(2) column, and eluted with CH_3OH-H_2O (80:20 to 100:0) at a flow rate of 1.0 mL/min.

General procedure A for the synthesis of EA glycoconjugates (50a-55a). To the glycosyl bromide (0.8 mmol) and **EA** (189 mg, 0.4 mmol) stirring in CH_2Cl_2/H_2O (10:1, 22 mL) was added K_2CO_3 (138 mg, 1 mmol) and Bu_4NBr (64 mg, 0.2 mmol). The mixture was refluxed under nitrogen atmosphere. After completion (TLC) the

reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with water (10 mL × 3), brine and dried over Na_2SO_4. The crude product was purified by column chromatography.

General procedure B for the *O*-deacetylation of EA glycoconjugates (50b-55b, 58b-68b). To the *O*-pentacetylated compound stirring in methanol was added sodium methoxide (cat.). The mixture was stirred at rt. After completion (TLC) the reaction mixture was neutralized with HCl (1 M). Water was added and the resulting suspension was filtered. Crude product was purified by column chromatography.

General procedure C for the CuAAC "click" reaction (58a-68a). To a solution of alkyne (0.16 mmol) and azide (0.19 mmol) in CH_2Cl_2 (3 mL) and H_2O (3 mL), was added $CuSO_4$ (30 mg, 0.19 mmol) and Na-L-ascorbate (75 mg, 0.38 mmol). The resulting solution was stirred vigorously for 12 h at rt. The reaction mixture was diluted with H_2O (10 mL), then extracted with CH_2Cl_2 (10 mL × 3). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography.

Compound 50a. Prepared from **EA** (189 mg, 0.4 mmol) and acetyl-glucosyl bromide (329 mg, 0.8 mmol) according to General procedure A. The residue was purified by column chromatography (petroleum ether/AcOEt, 2/1 v/v) to give **50a** as a white solid (252 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.70, 0.75, 0.88, 0.89, 0.92, 0.96, 1.32 (7 × CH_3), 1.99, 2.00, 2.00, 2.05 (4 × CH_3CO), 0.72-2.16 (m, other aliphatic ring protons), 2.97 (dd, 1H, J = 4.0, 14.3 Hz), 3.19 (dd, 1H, J = 4.2, 10.6 Hz), 3.74-3.78 (m, 1H), 4.02 (dd, 1H, J = 2.1, 12.4 Hz), 4.25 (dd, 1H, J = 4.4, 12.4 Hz), 4.39 (brs, 1H), 5.08-5.24 (m, 3H), 5.39 (t, 1H, J = 3.2 Hz), 5.54 (d, 1H, J = 8.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.4, 15.6, 17.0, 18.2, 20.5 (3C), 20.6, 23.2, 24.5, 26.8, 27.1, 28.0, 30.2 (2C), 32.6, 33.0, 35.1, 35.5, 36.9, 38.5, 38.7, 39.5, 40.4, 41.3, 46.0, 46.6, 48.8,

55.2, 61.4, 67.9, 69.9, 72.4, 72.7, 74.2, 78.8, 91.6, 123.2, 141.9, 169.1, 169.4, 170.0, 170.5, 174.7. ESI-HRMS (*m/z*): $[M+Na]^+$calcd for $C_{44}H_{66}NaO_{13}$, 825.4396, found 825.4387.

Compound 50b. Prepared from **50a** (50 mg, 0.06 mmol) according to General procedure B. The residue was purified by column chromatography (CH_2Cl_2/MeOH, 10/1 v/v) to afford **50b** as a white solid (12.4 mg, 31%). ^{1}H NMR (400 MHz, MeOD): δ 0.77, 0.79, 0.89, 0.96, 0.97, 1.37 (7 × CH_3), 0.74-1.96 (m, other aliphatic ring protons), 2.29 (t, 1H, J = 13.3 Hz), 2.99 (dd, 1H, J = 4.0, 14.2 Hz), 3.15 (dd, 1H, J = 5.0, 11.4 Hz), 3.27-3.34 (m, 4H), 3.67 (dd, 1H, J = 4.3, 12.0 Hz), 3.82 (d, 1H, J = 11.1 Hz), 4.53 (brs, 1H), 5.32 (t, 1H, J = 3.4 Hz), 5.35 (d, 1H, J = 8.1 Hz). ^{13}C NMR (100 MHz, MeOD): δ 16.1, 16.3, 17.8, 19.5, 24.5, 25.0, 27.3, 27.9, 28.7, 31.3, 31.7, 33.3, 34.2, 36.3, 36.4, 38.2, 39.8, 40.0, 40.8, 42.1, 42.7, 47.8, 48.2, 50.0, 56.9, 62.4, 71.1, 74.0, 74.9, 78.3, 78.7, 79.7, 95.7, 123.6, 144.6, 177.2. ESI-HRMS (*m/z*) $[M+Na]^+$calcd for $C_{36}H_{58}NaO_9$, 657.3973, found 657.3987.

Compound 51a. Prepared from **EA** (189 mg, 0.4 mmol) and acetyl-xylosyl bromide (270 mg, 0.8 mmol) according to General procedure A. The residue was purified by column chromatography (petroleum ether/AcOEt, 2/1 v/v) to give **51a** as a white solid (202 mg, 67%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.69, 0.74, 0.86, 0.88, 0.91, 0.94, 1.31 (7 × CH_3), 2.01, 2.01, 2.02 (3 × CH_3CO), 0.79-2.15 (m, other aliphatic ring protons), 2.97 (dd, 1H, J = 4.1, 14.1 Hz), 3.17 (dd, 1H, J = 4.2, 10.4 Hz), 3.45 (dd, 1H, J = 8.5, 12.0 Hz), 4.05-4.10 (m, 1H), 4.38 (brs, 1H), 4.89-5.00(m, 2H), 5.16 (t, 1H, J = 8.2 Hz), 5.37 (t, 1H, J = 3.3 Hz), 5.56 (d, 1H, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.5, 15.6, 17.0, 18.2, 20.6, 20.6, 23.3, 24.6, 26.8, 28.0, 30.2, 32.6, 33.0, 35.1, 35.4, 36.9, 38.5, 38.7, 39.5, 40.4, 41.3, 46.1, 46.6, 48.9, 55.2, 62.5, 68.2, 69.4, 70.9, 74.1,

76.7, 77.0, 77.4, 78.8, 91.9, 123.1, 142.1, 169.2, 169.7, 169.8, 174.7. ESI-HRMS (*m/z*) $[M+Na]^+$calcd for $C_{41}H_{62}NaO_{11}$, 753.4184, found 753.4199.

Compound 51b. Prepared from **51a** (90 mg, 0.12 mmol) according to General procedure B. The residue was purified by column chromatography (CH_2Cl_2/MeOH, 10/1 v/v) to afford **51b** as a white solid (58.6 mg, 78%). ^{1}H NMR (400 MHz, MeOD): δ 0.78, 0.89, 0.95, 0.96, 0.97, 1.37 (7 × CH_3), 0.74-1.93 (m, other aliphatic ring protons), 2.28 (t, 1H, J = 13.3 Hz), 3.02 (dd, 1H, J = 4.0, 14.2 Hz), 3.15 (dd, 1H, J = 5.0, 11.4 Hz), 3.25-3.31 (m, 2H), 3.38 (t, 1H, J = 8.4 Hz), 3.47-3.53 (m, 1H), 3.88 (dd, 1H, J = 5.0, 11.5 Hz), 4.51 (brs, 1H), 5.31-5.33 (m, 2H). ^{13}C NMR (100 MHz, MeOD): δ 16.1, 16.3, 17.8, 19.5, 24.5, 25.1, 27.3, 27.9, 28.8, 31.3, 31.9, 33.4, 34.3, 36.3, 36.4, 38.2, 39.8, 40.0, 40.8, 42.1, 42.7, 47.7, 48.2, 50.1, 56.9, 67.2, 70.8, 73.5, 74.9, 77.3, 79.7, 96.2, 123.7, 144.6, 177.3. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{35}H_{56}NaO_8$, 627.3867, found 627.3881.

Compound 52a. Prepared from **EA** (189 mg, 0.4 mmol) and acetyl-arabinosyl bromide (270 mg, 0.8 mmol) according to General procedure A. The residue was purified by column chromatography (petroleum ether/AcOEt, 2/1 v/v) to give **52a** as a white solid (341 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.72, 0.75, 0.87, 0.89, 0.92, 0.96, 1.31 (10 × CH_3), 2.02, 2.04, 2.11 (3 × CH_3CO), 0.69-2.14 (m, other aliphatic ring protons), 3.00 (dd, 1H, J = 4.0, 14.3 Hz), 3.19 (dd, 1H, J = 4.2, 10.6 Hz), 3.70 (dd, 1H, J = 1.8, 12.9 Hz), 3.97 (dd, 1H, J = 3.8, 13.0 Hz), 4.38 (t, 1H, J = 3.4 Hz), 5.10 (dd, 1H, J = 3.5, 8.9 Hz), 5.19-5.26 (m, 2H), 5.41 (t, 1H, J = 3.2 Hz), 5.51 (d, 1H, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.5 (2C), 17.0, 18.2, 20.6, 20.7, 20.8, 23.2, 24.8, 26.8, 27.1, 28.0, 29.8, 30.2, 32.6, 33.0, 35.0, 35.5, 36.9, 38.5, 38.7, 39.5, 40.5, 41.4, 46.1, 46.6, 49.0, 55.2, 63.5, 67.0, 68.0, 69.6, 73.9, 78.8, 91.9, 123.1, 141.9, 169.3, 169.8,

170.1, 174.6. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{41}H_{62}NaO_{11}$, 753.4184, found 753.4204.

Compound 52b. Prepared from **52a** (50 mg, 0.07 mmol) according to General procedure B. The residue was purified by column chromatography (CH_2Cl_2/MeOH, 10/1 v/v) to afford **52b** as a white solid (38.6 mg, 93%). ^{1}H NMR (400 MHz, MeOD): δ 0.76, 0.77, 0.88, 0.94, 0.96, 1.36 (7 × CH_3), 0.73-1.93 (m, other aliphatic ring protons), 2.27 (t, 1H, J = 13.3 Hz), 3.05 (dd, 1H, J = 4.1, 14.3 Hz), 3.14 (dd, 1H, J = 5.0, 11.4 Hz), 3.54 (dd, 1H, J = 9.7 Hz), 3.64-3.65 (m, 2H), 3.86-3.90 (m, 2H), 4.51 (brs 1H), 5.33 (t, 1H, J = 3.2 Hz), 5.37-5.41 (m, 1H). ^{13}C NMR (100 MHz, MeOD): δ 16.1, 16.3, 17.8, 19.5, 24.5, 25.2, 27.3, 27.9, 28.7, 31.3, 31.8, 33.4, 34.2, 36.2, 36.3, 38.1, 39.8, 39.9, 40.8, 42.0, 42.6, 47.6, 48.2, 50.2, 56.8, 66.1, 68.2, 71.2, 73.5, 74.8, 79.7, 95.7, 123.7, 144.6, 177.2. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{35}H_{56}NaO_8$, 627.3867, found 627.3880.

Compound 53a. Prepared from **EA** (189 mg, 0.4 mmol) and acetyl-galactosyl bromide (329 mg, 0.8 mmol) according to General procedure A. The residue was purified by column chromatography (petroleum ether/AcOEt, 2/1 v/v) to give **53a** as a white solid (98.5 mg, 31%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.75, 0.78, 0.91, 0.92, 0.95, 0.99, 1.34 (7 × CH_3), 1.99, 2.02, 2.04, 2.17 (4 × CH_3CO), 0.73-2.14 (m, other aliphatic ring protons), 2.99-3.01 (brs, 1H, J = 10.6 Hz), 3.22 (dd, 1H, J = 4.1, 10.4 Hz), 4.00 (t, 1H, J = 6.7 Hz), 4.10-4.15 (m, 2H), 4.39 (brs, 1H), 5.07 (dd, 1H, J = 3.4, 10.4 Hz), 5.31 (t, 1H, J = 10.3 Hz), 5.40-5.44 (m, 2H), 5.54 (d, 1H, J = 8.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.5, 15.6, 17.1, 18.2, 20.4, 20.6 (2C), 20.6, 23.3, 24.7, 26.8, 27.1, 28.0, 29.8, 30.2, 32.6, 33.0, 35.1, 35.5, 37.0, 38.5, 38.7, 39.5, 40.5, 41.4, 46.1, 46.6, 49.0, 55.2, 60.7, 66.7, 67.6, 70.7, 71.4, 74.1, 78.8, 92.0, 123.1, 141.9, 169.3, 169.8, 170.1, 170.2,

174.6 ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{44}H_{66}NaO_{13}$, 825.4396, found 825.4387; $[M+NH_4]^+$ calcd for $C_{44}H_{70}NO_{13}$, 820.4842, found 820.4839.

Compound 53b. Prepared from **53a** (50 mg, 0.06 mmol) according to General procedure B. The residue was purified by column chromatography (CH_2Cl_2/MeOH, 10/1 v/v) to afford **53b** as a white solid (47 mg, 99%). ^{1}H NMR (400 MHz, MeOD): δ 0.77, 0.78, 0.89, 0.96, 0.97, 1.37 (7 × CH_3), 0.74-1.96 (m, other aliphatic ring protons), 2.29 (t, 1H, J = 13.3 Hz), 3.00 (dd, 1H, J = 3.8, 14.2 Hz), 3.15 (dd, 1H, J = 4.9, 11.4 Hz), 3.50 (dd, 1H, J = 3.2, 9.7 Hz), 3.58-3.65 (m, 2H), 3.69-3.71 (m, 2H), 3.88 (d, 1H, J = 3.0 Hz), 4.55 (brs, 1H), 5.31-5.33 (m, 2H). ^{13}C NMR (100 MHz, MeOD): δ 16.1, 16.3, 17.8, 19.5, 24.5, 25.0, 27.3, 27.9, 28.7, 31.3, 31.8, 33.4, 34.2, 36.3, 36.4, 38.2, 39.8, 40.0, 40.8, 42.1, 42.6, 47.8, 48.2, 50.0, 56.9, 62.0, 70.0, 71.3, 74.9, 75.2, 77.4, 79.7, 96.2, 123.6, 144.6, 177.3. ESI-HRMS (*m/z*) $[M+NH_4]^+$calcd for $C_{36}H_{62}NO_9$, 652.4419, found 652.4415.

Compound 54a. Prepared from **EA** (189 mg, 0.4 mmol) and acetyl-lactosyl bromide (559 mg, 0.8 mmol) according to General procedure A. The residue was purified by column chromatography (petroleum ether/AcOEt, 2/1 v/v) to give **54a** as a white solid (234 mg, 59%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.71, 0.78, 0.90, 0.92, 0.94, 0.98, 1.34 (7 × CH_3), 1.96, 2.02, 2.03, 2.05, 2.07, 2.10, 2.15 (7 × CH_3CO), 0.74-2.17 (m, other aliphatic ring protons), 2.98 (dd, 1H, J = 3.9, 14.4 Hz), 3.21 (dd, 1H, J = 3.8, 10.6 Hz), 3.69-3.72 (m, 1H), 3.83-3.88 (m, 2H), 4.05-4.15 (m, 3H), 4.36-4.39 (m, 2H), 4.47 (d, 1H, J = 7.9 Hz), 4.95 (dd, 1H, J = 3.4, 10.4 Hz), 5.05-5.12 (m, 2H), 5.23 (t, 1H, J = 9.2 Hz), 5.35 (d, 1H, J = 3.2 Hz), 5.39 (brs, 1H), 5.53 (d, 1H, J = 8.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.4, 15.5, 17.0, 18.2, 20.4, 20.5 (2C), 20.6 (2C), 20.7, 20.7, 23.2, 24.5, 26.8, 27.1, 28.0, 30.0, 30.2, 32.6, 33.0, 35.1, 35.4, 36.9, 38.5, 38.7, 39.5, 40.4, 41.3, 46.0, 46.6, 48.8, 55.2, 60.8, 61.6, 66.6, 69.0, 70.3, 70.7, 70.9, 72.6, 73.3, 74.2,

75.7, 78.8, 91.4, 100.9, 123.2, 141.9, 168.9, 169.4, 169.5, 170.0, 170.1, 170.2, 170.3, 174.6. ESI-HRMS (*m/z*) $[M+Na]^+$calcd for $C_{56}H_{82}NaO_{21}$, 1113.5241, found 1113.5238; $[M+NH_4]^+$ calcd for $C_{56}H_{86}NO_{21}$, 1108.5687, found 1108.5698.

Compound 54b. Prepared from **54a** (140 mg, 0.13) according to General procedure B. The residue was purified by column chromatography (CH_2Cl_2/MeOH, 7/1 v/v) to afford **54b** as a white solid (92.6 mg, 91%). ^{1}H NMR (400 MHz, Pyridine-d_5): δ 0.99, 1.03, 1.06, 1.08, 1.17, 1.23, 1.85 (7 × CH_3), 0.90-2.61 (m, other aliphatic ring protons), 2.83 (t, 1H, J = 13.4 Hz), 3.46-3.56 (m, 2H), 3.98-4.00 (m, 1H), 4.12-4.17 (m, 3H), 4.29-4.57 (m, 7H), 5.12 (d, 1H, J = 7.8 Hz), 5.31 (brs, 1H), 5.65 (brs 1H), 6.27 (d, 1H, J = 8.2 Hz), 6.43 (d, 1H, J = 3.5 Hz). ^{13}C NMR (100 MHz, Pyridine-d_5): δ 16.3, 17.1, 18.1, 19.4, 24.4, 25.1, 27.8, 28.7, 29.3, 31.4, 32.7, 33.7, 34.1, 36.5, 36.6, 38.0, 39.6, 39.9, 40.7, 41.9, 42.6, 47.7, 47.8, 49.7, 56.5, 62.2, 62.5, 70.6, 73.0, 74.2, 74.9, 75.7, 77.5, 77.7, 77.8, 78.7, 82.0, 95.9, 106.3, 123.3, 144.9, 176.4. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{42}H_{68}NaO_{14}$, 819.4501, found 819.4492.

Compound 55a. Prepared from **EA** (189 mg, 0.4 mmol) and acetyl-maltosyl bromide (559 mg, 0.8 mmol) according to General procedure A. The residue was purified by column chromatography (petroleum ether/AcOEt, 2/1 v/v) to give **55a** as a white solid (83 mg, 19%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.72, 0.78, 0.90, 0.92, 0.94, 0.98, 1.34 (7 × CH_3), 2.00, 2.02, 2.02, 2.05, 2.10, 2.12 (7 × CH_3CO), 0.74-2.17 (m, other aliphatic ring protons), 2.98 (dd, 1H, J = 3.9, 14.2 Hz), 3.21(dd, 1H, J = 3.9, 10.5 Hz), 3.76-3.80 (m, 1H), 3.91-3.94 (m, 1H), 4.00-4.05 (m, 2H), 4.20-4.26 (m, 2H), 4.36-4.40 (m, 2H), 4.84 (dd, 1H, J = 4.0, 10.5 Hz), 4.98-5.07 (m, 2H), 5.26-5.40 (m, 4H), 5.59 (d, 1H, J = 8.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.4, 15.5, 17.0, 18.2, 20.5, 20.5 (3C), 20.6, 20.7, 20.8, 23.2, 24.5, 26.8, 27.1, 28.0, 30.1, 30.2, 32.6, 33.0, 35.2, 35.4, 36.9, 38.5, 38.7, 39.5, 40.4, 41.3, 46.1, 46.6, 48.8, 55.2, 61.4, 62.5, 68.0,

68.5, 69.2, 70.0, 70.7, 72.6, 72.8, 74.3, 75.2, 78.8, 91.2, 95.6, 123.2, 141.9, 169.4 (2C), 169.8, 170.0, 170.3, 170.4, 170.5, 174.5. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{56}H_{82}NaO_{21}$, 1113.5241, found 1113.5247.

Compound 55b. Prepared from **55a** (41 mg, 0.04 mmol) according to General procedure B. The residue was purified by column chromatography (CH_2Cl_2/MeOH, 7/1 v/v) to afford **55b** as a white solid (25 mg, 83%). ^{1}H NMR (400 MHz, MeOD): δ 0.77, 0.79, 0.89, 0.95, 0.97, 1.37 (7 × CH_3), 0.74-1.96 (m, other aliphatic ring protons), 2.28 (t, 1H, J = 13.4 Hz), 3.00 (dd, 1H, J = 3.9, 14.2 Hz), 3.15 (dd, 1H, J = 5.0, 11.3 Hz), 3.23-3.28 (m, 1H), 3.34-3.38 (m, 1H), 3.43-3.46 (m, 2H), 3.58-3.72 (m, 5H), 3.82-3.84 (m, 3H), 4.53 (brs, 1H), 5.18 (d, 1H, J = 3.8 Hz), 5.32(brs, 1H), 5.37 (d, 1H, J = 8.2 Hz). ^{13}C NMR (100 MHz, MeOD): δ 16.1, 16.3, 17.8, 19.5, 24.5, 25.1, 27.3, 27.9, 28.7, 31.2, 31.7, 33.3, 34.2, 36.4 (2C), 38.2, 39.8, 40.0, 40.8, 42.1, 42.7, 47.8, 48.2, 50.1, 56.9, 61.8, 62.8, 71.6, 73.6, 74.1, 74.8, 74.9, 75.1, 77.4, 78.1, 79.7, 80.5, 95.6, 102.8, 123.7, 144.6, 177.2. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{42}H_{68}NaO_{14}$, 819.4501, found 819.4491.

Compound 56. To **EA** (944 mg, 2 mmol) and TBTU (963 mg, 3 mmol) stirring in 20 mL THF was added DIEA (0.5 mL, 3 mmol). The mixture was stirred at room temperature overnight. After completion (TLC) the reaction mixture was filtered, and the filtration was concentrated. The crude was purified by column chromatography (petroleum ether/AcOEt, 4/1 v/v) to give **56** as a white solid (1.01 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.79, 0.83, 0.92, 0.98, 1.01, 1.04, 1.45 (7 × CH_3), 0.75-2.35 (m, other aliphatic ring protons), 3.16 (dd, 1H, J = 4.2, 14.2 Hz), 3.23 (dd, 1H, J = 3.8, 10.7 Hz), 4.85 (brs, 1H), 5.49 (t, 1H, J = 3.2 Hz), 7.34 (d, 1H, J = 8.3 Hz), 7.39-7.55 (m, 2H), 8.05 (d, 1H, J = 8.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.5, 15.6, 17.3, 18.2, 23.3, 24.4, 26.9, 27.1, 28.1, 30.4, 31.1, 32.6, 33.0, 35.1, 36.0, 37.0, 38.5, 38.7,

39.6, 41.0, 41.5, 46.2, 46.5, 49.3, 55.2, 74.2, 78.9, 108.0, 120.5, 124.2, 124.7, 128.6, 128.6, 141.4, 143.4, 172.7. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{36}H_{52}N_3O_4$, 590.3952, found 590.3949.

Compound 57. To **56** (589 mg, 1 mmol) and Na_2CO_3 (106 mg, 1 mmol) stirring in 8 mL DMF was added 2-propynylamine (0.1 mL, 1.5 mmol). The mixture was stirred at room temperature for 20 min. After completion (TLC) the solvent was removed under reduced pressure. The mixture was resolved in AcOEt and washed with water and brine twice. The organic layer was dried over $MgSO_4$, then filtered and concentrated. The crude was purified by column chromatography (petroleum ether/AcOEt, 2/1 v/v) to give **57** as a white solid (473 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.79, 0.81, 0.91, 0.93, 0.95, 1.00, 1.38 (7 × CH_3), 0.73-2.30 (m, other aliphatic ring protons), 2.21 (t, 1H, J = 2.6 Hz), 2.70 (dd, 1H, J = 3.8, 13.7 Hz), 3.22 (dd, 1H, J = 3.7, 10.8 Hz), 3.86-4.00 (m, 2H), 4.40 (brs, 1H), 5.54 (t, 1H, J = 3.4 Hz), 6.42 (t, 1H, J = 4.8 Hz). ^{13}C NMR (100 MHz, CDCl3): δ 15.6 (2C), 17.1, 18.2, 23.4, 24.9, 26.7, 27.1, 28.0, 29.4, 29.6, 30.2, 32.5, 32.5, 35.1, 35.2, 36.9, 38.6, 38.7, 39.6, 41.6, 41.8, 46.6, 46.9, 49.0, 55.1, 71.6, 75.3, 78.8, 79.4, 123.4, 144.0, 177.5. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{33}H_{52}NO_3$, 510.3942, found 510.3941.

Compound 58a. Prepared from **57** (82 mg, 0.16 mmol), acetyl-glucosyl azide (71 mg, 0.19 mmol) according to General procedure C. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **58a** as a white solid (117 mg, 83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.71, 0.78, 0.90, 0.91, 0.99, 1.36 (7 × CH_3), 1.87, 2.03, 2.07, 2.09 (4 × CH_3CO), 0.76-2.38 (m, other aliphatic ring protons), 2.75-2.76 (m, 1H, J = 10.9 Hz), 3.22 (dd, 1H, J = 2.9, 10.1 Hz) 4.00-4.03 (m, 1H), 4.15 (d, 1H, J = 12.6 Hz), 4.26-4.32 (m, 3H), 4.37 (brs, 1H), 4.55 (dd, 1H, J = 5.8, 15.2 Hz), 5.23-5.27 (m, 1H), 5.40-5.47 (m, 2H), 5.55 (brs, 1H), 5.86 (d, 1H, J = 8.6Hz), 6.92 (t,

1H, J = 4.6 Hz), 7.79 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.5, 15.6, 16.8, 18.2, 20.1, 20.5 (2C), 20.6, 23.3, 25.2, 26.8, 27.1, 28.0, 29.0, 30.1, 32.4, 32.5, 35.0, 35.1 (2C), 36.9, 38.5, 38.7, 39.6, 41.6, 41.7, 46.7, 46.8, 49.1, 55.1, 61.5, 67.6, 70.3, 72.6, 75.0, 75.1, 78.8, 85.6, 120.9, 123.5, 143.5, 145.2, 168.7, 169.3, 169.9, 170.5, 178.0. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{47}H_{71}N_4O_{12}$, 883.5063, found 883.5053.

Compound 58b. Prepared from **58a** (80 mg, 0.09 mmol) according to General procedure B. The crude product was washed with water three times without further purification to afford **58b** as a white solid (61 mg, 95%). ^{1}H NMR (400 MHz, MeOD): δ 0.66, 0.78, 0.89, 0.94, 0.96, 0.97, 1.37 (7 × CH_3), 0.76-2.00 (m, other aliphatic ring protons), 2.35 (t, 1H, J = 13.2 Hz), 2.87 (dd, 1H, J = 3.2, 13.6 Hz), 3.15 (dd, 1H, J = 4.9, 11.4 Hz), 3.47-3.58 (m, 3H), 3.71 (dd, 1H, J = 5.3, 12.1 Hz), 3.83-3.89 (m, 2H), 4.31 (d, 1H, J = 15.2 Hz), 4.35 (brs, 1H), 4.43 (d, 1H, J = 15.2 Hz), 5.47 (brs, 1H), 5.57 (d, 1H, J = 9.2 Hz), 7.98 (s, 1H). ^{13}C NMR (100 MHz, MeOD): δ 16.2, 16.3, 17.7, 19.5, 24.5, 25.4, 27.3, 27.9, 28.7, 31.2, 31.7, 33.3, 33.9, 36.0 (2C), 36.3, 38.1, 39.8, 39.9, 40.8, 42.3, 42.8, 48.0, 48.2, 50.0, 56.8, 62.4, 70.9, 74.0, 75.6, 78.4, 79.7, 81.1, 89.5, 123.5, 124.3, 144.9, 145.9, 180.2. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{39}H_{63}N_4O_8$, 715.4640, found 715.4647.

Compound 59a. Prepared from **57** (89 mg, 0.17 mmol), acetyl-xylosyl azide (57 mg, 0.19 mmol) according to General procedure C. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **59a** as a white solid (106 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.67, 0.78, 0.90, 0.91, 0.91, 0.99, 1.36 (7 × CH_3), 1.88, 2.05, 2.08 (3 × CH_3CO), 0.63-2.25 (m, other aliphatic ring protons), 2.74 (dd, 1H, J = 3.3, 13.5 Hz), 3.22 (dd, 1H, J = 3.5, 10.4 Hz), 3.60 (t, 1H, J = 10.8 Hz), 4.26-4.31 (m, 2H), 4.38 (brs, 1H), 4.54 (dd, 1H, J = 5.7, 15.2 Hz), 5.14-5.18 (m, 1H), 5.40-5.43 (m, 2H), 5.53 (brs, 1H), 5.76 (d, 1H, J = 8.6 Hz), 6.90 (t, 1H, J = 5.1 Hz), 7.77

(s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.5 (2C), 16.7, 18.1, 20.1, 20.5 (2C), 23.3, 25.1, 26.7, 27.0, 27.9, 29.2, 30.1, 32.4, 32.5, 35.0 (2C), 35.1, 36.8, 38.5, 38.6, 39.5, 41.5, 41.7, 46.6, 46.7, 48.9, 55.1, 65.3, 68.3, 70.3, 71.9, 75.0, 78.7, 86.1, 120.9, 123.3, 143.5, 145.1, 168.7, 169.6, 169.8, 177.9. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{44}H_{67}N_4O_{10}$, 811.4852, found 811.4858.

Compound 59b. Prepared from **59a** (55 mg, 0.07 mmol) according to General procedure B. The crude product was washed with water three times without further purification to afford **59b** as a white solid (45 mg, 97%). ^{1}H NMR (400 MHz, MeOD): δ 0.52, 0.76, 0.88, 0.91, 1.34, 0.95 (7 × CH_3), 0.70-2.01 (m, other aliphatic ring protons), 2.32 (t, 1H, J = 13.2 Hz), 2.85-2.88 (m, 1H,), 3.15 (dd, 1H, J = 4.8, 11.0 Hz), 3.45-3.55 (m, 2H), 3.66-3.72 (m, 1H), 3.88 (t, 1H, J = 9.0), 4.00 (dd, 1H, J = 5.3, 11.2 Hz), 4.35 (brs, 1H), 4.39 (brs, 2H), 5.45 (brs, 1H), 5.50 (d, 1H, J = 9.1 Hz), 7.97 (s, 1H). ^{13}C NMR (100 MHz, MeOD): δ 16.1, 16.3, 17.6, 19.3, 24.4, 25.3, 27.3, 27.7, 28.7, 31.1, 31.7, 33.2, 33.8, 35.7, 35.8, 36.3, 38.0, 39.7, 39.9, 40.6, 42.2, 42.6, 48.0 (2C), 49.9, 56.7, 69.6, 70.6, 73.7, 75.5, 78.4, 79.7, 89.9, 123.7, 124.2, 144.8, 145.8, 180.1. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{38}H_{61}N_4O_7$, 685.4535, found 685.4526.

Compound 60a. Prepared from **57** (79 mg, 0.15 mmol), acetyl-arabinosyl azide (60 mg, 0.2 mmol) according to General procedure C. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **60a** as a white solid (111 mg, 92%). ^{1}H NMR (400 MHz,$CDCl_3$): δ 0.69, 0.78, 0.91, 0.99, 1.36 (7 × CH_3), 1.89, 2.04, 2.23 (3 × CH_3CO), 0.72-2.30 (m, other aliphatic ring protons), 2.75 (dd, 1H, J = 2.5, 13.0 Hz), 3.22 (dd, 1H, J = 2.0, 10.0 Hz), 3.95 (d, 1H, J = 13.3 Hz), 4.18 (d, 1H, J = 13.4 Hz), 4.27 (dd, 1H, J = 4.4, 15.0 Hz), 4.36 (brs, 1H), 4.56 (dd, 1H, J = 5.8, 15.1 Hz), 5.25 (dd, 1H, J = 3.3, 10.0 Hz), 5.43 (brs, 1H), 5.54-5.60 (m, 2H), 5.72 (d, 1H, J = 9.1 Hz), 6.90 (t, 1H, J = 4.7 Hz), 7.82 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.5 (2C),

16.8, 18.2, 20.2, 20.5, 20.9, 23.3, 25.2, 26.8, 27.1, 28.0, 29.0, 30.1, 32.4, 32.5, 35.0, 35.1 (2C), 36.9, 38.5, 38.7, 39.5, 41.5, 41.7, 46.7, 46.8, 49.0, 55.1, 67.1, 67.6, 68.1, 70.4, 75.1, 78.8, 86.6, 120.9, 123.4, 143.5, 145.0, 168.9, 169.8, 170.1, 177.9. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{44}H_{67}N_4O_{10}$, 811.4852, found 811.4859.

Compound 60b. Prepared from **60a** (77 mg, 0.9 mmol) according to General procedure B. The crude product was washed with water three times without further purification to afford **60b** as a white solid (64 mg, 99%). ^{1}H NMR (400 MHz, MeOD): δ 0.59, 0.77, 0.89, 0.92, 0.96, 0.97, 1.36 (7 × CH_3), 0.72-1.99 (m, other aliphatic ring protons), 2.36 (t, 1H, J = 13.2 Hz), 2.87 (dd, 1H, J = 3.2, 13.5 Hz), 3.15 (dd, 1H, J = 4.8, 11.2 Hz), 3.71 (dd, 1H, J = 3.3, 9.4 Hz), 3.85 (d, 1H, J = 12.4 Hz), 3.95 (brs, 1H), 4.01 (dd, 1H, J = 1.6, 12.7 Hz), 4.14 (t, 1H, J = 9.3 Hz), 4.32-4.45 (m, 3H),5.46 (brs, 1H), 5.46 (d, 1H, J = 9.0 Hz), 7.59 (t, 1H, J = 5.3 Hz), 8.00 (s, 1H). ^{13}C NMR (100 MHz, MeOD): δ 16.1, 16.4, 17.6, 19.4, 24.4, 25.4, 27.3, 27.9, 28.7, 31.2, 31.9, 33.3, 33.9, 35.9, 36.1, 36.3, 38.0, 39.8, 39.9, 40.7, 42.3, 42.7, 48.0, 48.1, 49.9, 56.7, 70.2, 70.7, 71.3, 74.8, 75.6, 79.6, 90.4, 123.0, 124.3, 144.9, 146.0, 180. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{38}H_{61}N_4O_7$, 685.4535, found 685.4540.

Compound 61a. Prepared from **57** (80 mg, 0.16 mmol), acetyl-mannosyl azide (71 mg, 0.19 mmol) according to General procedure C. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **61a** as a white solid (112 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.51, 0.79, 0.90, 0.90, 0.92, 0.98, 1.35 (7 × CH_3), 2.00, 2.10, 2.10, 2.15 (4 × CH_3CO), 0.70-2.27 (m, other aliphatic ring protons), 2.72 (dd, 1H, J = 3.2, 13.4 Hz), 3.21 (dd, 1H, J = 3.5, 10.3 Hz), 3.93-3.97 (m, 1H), 4.20 (dd, 1H, J = 1.8, 12.4 Hz), 4.28-4.46 (m, 4H), 5.25-5.39 (m, 2H), 5.54 (brs, 1H), 5.66 (d, 1H, J = 2.2 Hz), 6.14 (brs, 1H), 6.90 (t, 1H, J = 5.3 Hz), 7.76 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.5, 15.6, 16.6, 18.1, 20.4, 20.6 (2C), 20.7, 23.3, 24.9,

26.8, 27.1, 28.0, 29.7, 30.2, 32.4, 32.5, 35.0, 35.1, 35.3, 36.9, 38.5, 38.7, 39.5, 41.5, 41.7, 46.6, 46.9, 48.9, 55.2, 62.1, 64.8, 68.7, 70.8, 75.5, 75.6, 78.9, 84.6, 121.5, 123.7, 143.5, 144.6, 169.2, 169.5, 169.8, 170.5, 178.0. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{47}H_{71}N_4O_{12}$, 883.5063, found 883.5065.

Compound 61b. Prepared from **61a** (70 mg, 0.08 mmol) according to General procedure B. The crude product was washed with water three times without further purification to afford **61b** as a white solid (53 mg, 93%). ^{1}H NMR (400 MHz, MeOD): δ 0.62, 0.78, 0.89, 0.94, 0.96, 0.97, 1.37 (7 × CH_3), 0.73-1.97 (m, other aliphatic ring protons), 2.35 (t, 1H, J = 13.1 Hz), 2.87 (dd, 1H, J = 3.0, 13.5 Hz), 3.15 (dd, 1H, J = 4.9, 11.3 Hz), 3.52-3.54 (m, 1H), 3.73-3.80 (m, 3H), 3.92 (dd, 1H, J = 1.8, 12.1 Hz), 4.09 (brs, 1H), 4.31 (d, 1H, J = 14.6 Hz), 4.35 (brs, 1H), 4.42 (d, 1H, J = 15.1 Hz), 5.47 (brs, 1H), 5.98 (brs, 1H), 8.10 (s, 1H). ^{13}C NMR (100 MHz, MeOD): δ 16.2, 16.3, 17.6, 19.4, 24.5, 25.4, 27.3, 27.9, 28.7, 31.2, 31.8, 33.3, 33.9, 36.0 (2C), 36.3, 38.1, 39.8, 39.9, 40.7, 42.3, 42.8, 48.0, 48.2, 49.9, 56.8, 62.6, 67.8, 72.3, 75.0, 75.6, 79.6, 81.5, 88.2, 124.1, 124.3, 144.9, 145.3, 180.1. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{39}H_{63}N_4O_8$, 715. 4640, found 715.4637.

Compound 62a. Prepared from **57** (95 mg, 0.19 mmol), acetyl-galactosyl azide (82 mg, 0.22 mmol) according to General procedure C. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **62a** as a white solid (160 mg, 98%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.72, 0.78, 0.90, 0.92, 0.99, 1.36 (7 × CH_3), 1.88, 2.01, 2.05, 2.24 (4 × CH_3CO), 0.76-2.21 (m, other aliphatic ring protons), 2.74 (dd, 1H, J = 3.5, 13.6 Hz) 3.22-3.24 (m, 1H), 4.11-4.30 (m, 4H), 4.36 (brs, 1H), 4.56 (dd, 1H, J = 6.0, 15.2 Hz), 5.25 (dd, 1H, J = 3.3, 10.2 Hz), 5.51-5.56 (m, 3H), 5.80 (d, 1H, J = 9.3 Hz), 6.90 (t, 1H, J = 5.2 Hz), 7.83 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.5, 15.6, 16.8, 18.2, 20.2, 20.4, 20.6 (2C), 23.3, 25.2, 26.9, 27.1, 28.0, 28.9,

30.1, 32.4, 32.5, 35.0, 35.1 (2C), 36.9, 38.5, 38.7, 39.6, 41.7, 41.8, 46.7, 46.8, 49.1, 55.1, 61.1, 66.7, 67.9, 70.7, 73.9, 75.2, 78.8, 86.2, 121.0, 123.5, 143.5, 145.1, 168.9, 169.8, 170.0, 170.3, 177.9. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{47}H_{71}N_4O_{12}$, 883.5063, found 883.5062.

Compound 62b. Prepared from **62a** (70 mg, 0.08 mmol) according to General procedure B. The crude product was washed with water three times without further purification to afford **62b** as a white solid (53 mg, 93%). ^{1}H NMR (400 MHz, MeOD): δ 0.65, 0.78, 0.89, 0.94, 0.96, 0.97, 1.37 (7 × CH_3), 0.73-1.98 (m, other aliphatic ring protons), 2.36 (t, 1H, J = 13.2 Hz), 2.87 (dd, 1H, J = 3.3, 13.4 Hz), 3.15 (dd, 1H, J = 4.9, 11.4 Hz), 3.69 (dd, 1H, J = 3.2, 9.5 Hz), 3.75 (d, 2H, J = 6.0 Hz), 3.81-3.84 (m, 1H), 3.99 (d, 1H, J = 2.9 Hz), 4.14 (t, 1H, J = 9.4 Hz), 4.32 (dd, 1H, J = 5.2, 15.6 Hz), 4.36 (brs, 1H), 4.44 (dd, 1H, J = 5.3, 15.2 Hz), 5.48 (brs, 1H), 5.54 (d, 1H, J = 9.2 Hz), 7.60 (t, 1H, J = 5.2 Hz), 8.02 (s, 1H). ^{13}C NMR (100 MHz, MeOD): δ 16.2, 16.3, 17.6, 19.4, 24.5, 25.4, 27.3, 27.9, 28.7, 31.2, 31.8, 33.3, 33.9, 36.0, 36.1, 36.3, 38.1, 39.8, 39.9, 40.7, 42.3, 42.8, 48.0, 48.2, 50.0, 56.8, 62.2, 70.2, 71.3, 75.3, 75.6, 79.7, 79.8, 90.1, 123.1, 124.3, 144.9, 146.0, 180.3. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{39}H_{63}N_4O_8$, 715.4640, found 715.4646.

Compound 63a. Prepared from **57** (77 mg, 0.15 mmol), acetyl-rhamnosyl azide (60 mg, 0.19 mmol) according to General procedure C. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **63a** as a white solid (109 mg, 89%). ^{1}H NMR (400 MHz, CDCl3): δ 0.57, 0.78, 0.89, 0.91, 0.99, 1.36 (7 × CH_3), 1.99, 2.10, 2.12 (3 × CH_3CO), 1.35 (d, 3H, J = 6.2 Hz), 0.71-2.46 (m, other aliphatic ring protons), 2.70 (dd, 1H, J = 3.3, 13.5 Hz), 3.21 (dd, 1H, J = 3.6, 10.2 Hz), 3.79-3.86 (m, 1H), 4.34 (dd, 1H, J = 5.4, 15.0 Hz), 4.42-4.47 (m, 2H), 5.15-5.20 (m, 1H), 5.24 (dd, 1H, J = 3.1, 10.2 Hz), 5.51 (brs, 1H), 5.66 (dd, 1H, J = 1.0, 2.9 Hz), 6.12

(d, 1H, J = 0.8 Hz), 6.90 (t, 1H, J = 10.5 Hz), 7.74 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.4, 15.5, 16.6, 17.4, 18.1, 20.4, 20.5, 20.6, 23.2, 24.8, 26.7, 27.0, 28.0, 29.8, 30.1, 32.5 (2C), 34.9, 35.0, 35.2, 36.8, 38.4, 38.6, 39.4, 41.4, 41.6, 46.6, 46.8, 48.7, 55.1, 69.0, 69.5, 70.7, 73.8, 75.2, 78.7, 84.6, 121.4, 123.5, 143.4, 144.4, 169.1, 169.7, 169.8, 177.9. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{45}H_{69}N_4O_{10}$, 825.5008, found 825.5018.

Compound 63b. Prepared from **63a** (70 mg, 0.08 mmol) according to General procedure B. The crude product was washed with water three times without further purification to afford **63b** as a white solid (56 mg, 95%). ^{1}H NMR (400 MHz, MeOD): δ 0.51, 0.77, 0.89, 0.91, 0.96 (6 × CH_3), 1.36-1.38 (2 × CH_3), 0.72-1.99 (m, other aliphatic ring protons), 2.37 (t, J = 13.1 Hz), 2.84 (dd, 1H, J = 3.0, 13.3 Hz), 3.14 (dd, 1H, J = 4.5, 11.0 Hz), 3.51 (brs, 2H), 3.67 (brs, 1H), 4.06 (brs, 1H), 4.31-4.44 (m, 3H), 5.45 (brs, 1H), 5.93 (brs, 1H), 8.01 (s, 1H). ^{13}C NMR (100 MHz, MeOD): δ 16.2, 16.4, 17.6, 18.2, 19.5, 24.4, 25.3, 27.3, 27.9, 28.7, 31.3, 32.0, 33.3, 33.9, 35.9, 36.0, 36.4, 38.1, 39.8, 39.9, 40.7, 42.3, 42.8, 48.1, 48.1, 49.9, 56.7, 72.3, 73.1, 74.7, 75.6, 76.9, 79.6, 88.2, 124.0, 124.3, 144.9, 145.3, 180.1. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{39}H_{63}N_4O_7$, 699.4691, found 699.4697.

Compound 64a. Prepared from **57** (88 mg, 0.17 mmol), acetyl-lyxosyl azide (57 mg, 0.19 mmol) according to General procedure C. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **64a** as a white solid (87 mg, 64%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.52, 0.79, 0.90, 0.92, 0.99, 1.35 (7 × CH_3), 2.02, 2.09, 2.13 (3 × CH_3), 0.70-2.27 (m, other aliphatic ring protons), 2.71 (dd, 1H, J = 3.6, 13.6 Hz), 3.21 (dd, 1H, J = 3.9, 10.4 Hz), 3.54 (dd, 1H, J = 10.0, 11.1 Hz), 4.29-4.46 (m, 4H), 5.25-5.36 (m, 2H), 5.53 (brs, 1H), 5.67 (dd, 1H, J = 1.1, 2.8 Hz), 6.05 (d, 1H, J = 0.84 Hz), 6.88 (t, 1H, J = 5.4 Hz), 7.72 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.5 (2C), 16.6, 18.1, 20.5 (2C), 20.7, 23.3, 24.9, 26.8, 27.1, 28.0, 29.8, 30.2,

32.4, 32.5, 35.0, 35.1, 35.3, 36.9, 38.5, 38.7, 39.5, 41.5, 41.7, 46.6, 46.9, 48.9, 55.2, 65.4, 65.8, 68.8, 70.3, 75.4, 78.9, 85.1, 121.4, 123.6, 143.5, 144.5, 169.1, 169.8, 169.9, 177.9. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{44}H_{67}N_4O_{10}$, 811.4852, found 811.4862.

Compound 64b. Prepared from **64a** (40 mg, 0.05 mmol) according to General procedure B. The crude product was washed with water three times without further purification to afford **64b** as a white solid (32 mg, 95%). ^{1}H NMR (400 MHz, MeOD): δ 0.56, 0.77, 0.88, 0.93, 0.96, 0.97, 1.36 (7 × CH_3), 0.72-1.97 (m, other aliphatic ring protons), 2.35 (t, 1H, J = 13.2 Hz), 2.88 (dd, 1H, J = 3.4, 13.6 Hz), 3.15 (dd, 1H, J = 4.9, 11.4 Hz), 3.39 (t, 1H, J = 10.7 Hz), 3.66 (dd, 1H, J = 2.9, 9.2 Hz), 3.92-3.98 (m, 1H), 4.03-4.07 (m, 2H), 4.32-4.43 (m, 3H), 5.45 (brs, 1H), 5.89 (brs, 1H), 8.03 (s, 1H). ^{13}C NMR (100 MHz, MeOD): δ 16.1, 16.3, 17.6, 19.4, 24.4, 25.4, 27.3, 27.9, 28.7, 31.2, 31.9, 33.3, 33.9, 36.0 (2C), 36.3, 38.1, 39.8, 39.9, 40.7, 42.3, 42.8, 48.0, 48.2, 49.9, 56.8, 67.3, 69.8, 72.0, 75.0, 75.6, 79.7, 88.8, 124.0, 124.2, 144.9, 145.5, 180.0. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{38}H_{61}N_4O_7$, 685.4535, found 685.4527.

Compound 65a. Prepared from **57** (60 mg, 0.12 mmol), acetyl-lactosyl azide (126 mg, 0.19 mmol) according to General procedure C. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **65a** as a white solid (120 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.69, 0.78, 0.91, 0.99, 1.36 (7 × CH_3), 1.86, 2.00, 2.02, 2.05, 2.11, 2.12, (7 × CH_3CO), 0.72-2.38 (m, other aliphatic ring protons), 2.71-2.74 (m, 1H), 3.21-3.23 (m, 1H), 3.70-3.71 (m, 1H), 3.93-3.97 (m, 2H), 4.06-4.15 (m, 2H), 4.27 (dd, 1H, J = 4.6, 15.2 Hz), 4.35 (brs, 1H), 4.40 (dd, 1H, J = 4.2, 12.5 Hz), 4,50-4.58 (m, 3H), 4.93-4.97 (m, 1H), 5.06-5.20 (m, 2H), 5.38-5.40 (m, 2H), 5.54 (brs, 1H), 5.75-5.82 (m, 1H), 6.93 (t, 1H, J = 5.1 Hz), 7.73 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.5 (2C), 16.7, 18.1, 20.1, 20.3, 20.4 (3C), 20.6, 20.7, 23.3, 25.1, 26.8, 27.0, 27.9, 28.9, 30.1, 32.3, 32.5, 35.0, 35.1 (2C), 36.8, 38.5, 38.6, 39.5, 41.6, 41.7, 46.6,

46.7, 49.0, 55.0, 61.4, 61.5, 67.6, 70.4, 71.4, 72.0, 72.2, 72.7, 75.1, 75.7 (2C), 78.7, 85.4, 100.7, 121.0, 123.4, 143.5, 145.0, 168.9, 169.0, 169.2, 169.5, 170.0, 170.1, 170.4, 178.0. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{59}H_{87}N_4O_{20}$, 1171.5908, found 1171.5877.

Compound 65b. Prepared from **65a** (80 mg, 0.07 mmol) according to General procedure B. The crude product was washed with water three times without further purification to afford **65b** as a white solid (52 mg, 91%). ^{1}H NMR (400 MHz, MeOD): δ 0.62, 0.77, 0.88, 0.93, 0.96, 0.97, 1.36 (7 × CH_3), 0.72-1.97 (m, other aliphatic ring protons), 2.35 (t, 1H, J = 13.3 Hz), 2.89 (dd, 1H, J = 3.3, 13.4 Hz), 3.15 (dd, 1H, J = 5.0, 11.2 Hz), 3.52-3.96 (m, 12H), 4.36-4.47 (m, 4H), 5.46 (brs, 1H), 5.70 (d, 1H, J = 9.2 Hz), 8.19 (s, 1H). ^{13}C NMR (100 MHz, MeOD): δ 16.2, 16.4, 17.7, 19.4, 24.4, 25.4, 27.3, 27.8, 28.7, 31.2, 31.8, 33.3, 33.9, 35.5, 36.0, 36.3, 38.1, 39.8, 39.9, 40.7, 42.1, 42.7, 48.0, 48.1, 49.9, 56.7, 61.4, 62.5, 70.3, 72.4, 73.7, 74.7, 75.5, 76.5, 77.1, 79.6 (2C), 89.7, 105.0, 124.2, 124.6, 144.8, 145.2, 180.3. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{45}H_{73}N_4O_{13}$, 877.5169, found 877.5169.

Compound 66a. Prepared from **57** (75 mg, 0.15 mmol), acetyl-maltosyl azide (126 mg, 0.19 mmol) according to General procedure C. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **66a** as a white solid (163 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.69, 0.78, 0.91, 0.99, 1.36 (7 × CH_3), 1.85, 2.02, 2.04, 2.07, 2.11, 2.14, (7 × CH_3CO), 0.72-2.25 (m, other aliphatic ring protons), 2.71-2.74 (m, 1H), 3.21-3.23 (m, 1H), 3.98 (d, 2H, J = 9.6 Hz), 4.07 (d, 1H, J = 11.8 Hz), 4.14 (t, 1H, J = 9.2 Hz), 4.24-4.28 (m, 3H), 4.36 (brs, 1H), 4.48-4.56 (m, 2H), 4.88 (dd, 1H, J = 3.8, 10.5 Hz), 5.08 (t, 1H, J = 9.8 Hz), 5.30-5.55 (m, 4H), 5.54 (brs, 1H), 5.85 (d, 1H, J = 9.2 Hz), 6.90 (t, 1H, J = 4.8 Hz), 7.71 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.5, 15.6, 16.8, 18.2, 20.1, 20.5 (3C), 20.6, 20.7, 20.8, 23.3, 25.1, 26.8, 27.1, 28.0, 29.0, 30.1, 32.4, 32.5, 35.0, 35.1 (2C), 36.8, 38.5, 38.7, 39.5, 41.6,

41.7, 46.7, 46.8, 49.0, 55.1, 61.4, 62.4, 67.8, 68.7, 69.1, 69.9, 70.9, 72.3, 75.0, 75.2, 75.2, 78.7, 85.2, 95.8, 120.9, 123.5, 143.5, 145.1, 169.0, 169.3, 169.9 (2C), 170.2, 170.4, 170.5, 178.0. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{59}H_{87}N_4O_{20}$, 1171.5908, found 1171.5910.

Compound 66b. Prepared from **66a** (100 mg, 0.09 mmol) according to General procedure B. The crude product was washed with water three times without further purification to afford **66b** as a white solid (64 mg, 86%). ^{1}H NMR (400 MHz, MeOD): δ 0.63, 0.77, 0.88, 0.94, 0.96, 0.97, 1.36 (7 × CH_3), 0.72-1.97 (m, other aliphatic ring protons), 2.34 (t, 1H, J = 13.1 Hz), 2.88-2.91 (m, 1H), 3.15 (dd, 1H, J = 4.6, 10.8 Hz), 3.26-3.29 (m, 1H), 3.50 (dd, 1H, J = 3.6, 9.7 Hz), 3.63-3.94 (m, 10H), 4.37-4.48 (m, 3H), 5.26 (d, J = 3.6 Hz), 5.46 (brs, 1H), 5.70 (d, 1H, J = 8.4 Hz), 8.24 (s, 1H). ^{13}C NMR (100 MHz, MeOD): δ 16.2, 16.4, 17.7, 19.4, 24.5, 25.4, 27.3, 27.8, 28.7, 31.2, 31.8, 33.3, 33.9, 35.4, 36.0, 36.3, 38.1, 39.8, 39.9, 40.7, 42.1, 42.7, 48.0, 48.1, 50.0, 56.8, 61.6, 62.6, 71.5, 73.7, 74.1, 74.8, 75.0, 75.5, 77.9, 79.6, 80.1, 89.9, 102.9, 124.2, 144.8, 180.3. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{45}H_{73}N_4O_{13}$, 877. 5169, found 877.5174.

Compound 67a. Prepared from **57** (82 mg, 0.16 mmol), acetyl-cellobiosyl azide (126 mg, 0.19 mmol) according to General procedure C. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **67a** as a white solid (163 mg, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.69, 0.78, 0.91, 0.99, 1.36 (7 × CH_3), 1.87, 1.98, 2.06, 2.07, 2.09, 2.11, 2.17 (7 × CH_3CO), 0.72-2.25 (m, other aliphatic ring protons), 2.71 (dd, 1H, J = 3.0, 13.4 Hz), 3.21-3.23 (m, 1H), 3.92-3.96 (m, 3H), 4.08-4.18 (m, 3H), 4.27 (dd, 1H, J = 4.7, 15.2 Hz), 4.35 (brs, 1H), 4.47-4.54 (m, 3H), 4.98 (dd, 1H, J = 3.4, 10.4 Hz), 5.13 (dd, 1H, J = 8.0, 10.3 Hz), 5.36-5.41 (m, 3H), 5.55(brs, 1H), 5.79 (dd, 1H, J = 8.7 Hz), 6.90 (t, 1H, J = 5.1 Hz), 7.72 (s, 1H). ^{13}C NMR

(100 MHz, $CDCl_3$): δ 15.5, 15.6, 16.8, 18.2, 20.2, 20.4, 20.6 (3C), 20.7 (2C), 23.3, 25.2, 26.8, 27.1, 28.0, 28.9, 30.1, 32.4, 32.5, 35.0, 35.1 (2C), 36.9, 38.5, 38.7, 39.5, 41.7 (2C), 46.7, 46.8, 49.1, 55.1, 60.8, 61.6, 66.5, 69.0, 70.5, 70.8 (2C), 72.5, 75.2, 75.5, 75.8, 78.8, 85.5, 101.0, 121.0, 123.6, 143.5, 145.1, 169.0 (2C), 169.5, 170.0, 170.1 (2C), 170.3, 178.0. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{59}H_{87}N_4O_{20}$, 1171.5908, found 1171.5901.

Compound 67b. Prepared from **67a** (100 mg, 0.09 mmol) according to General procedure B. The crude product was washed with water three times without further purification to afford **67b** as a white solid (69 mg, 92%). ^{1}H NMR (400 MHz, MeOD): δ 0.62, 0.78, 0.88, 0.94, 0.96, 0.97, 1.36 (7 × CH_3), 0.72-1.97 (m, other aliphatic ring protons), 2.34 (t, 1H, *J* = 13.2 Hz), 2.89 (dd, 1H, *J* = 2.7, 10.4 Hz), 3.15 (dd, 1H, *J* = 4.7, 11.0 Hz), 3.26-3.44 (m, 2H), 3.52-3.95 (m, 10H), 4.35-4.50 (m, 4H), 5.46 (brs, 1H), 5.69 (d, 1H, *J* = 8.4 Hz), 5.70 (d, 1H, *J* = 8.4 Hz), 8.24 (s, 1H). ^{13}C NMR (100 MHz, MeOD): δ 16.2, 16.4, 17.6, 19.4, 24.4, 25.4, 27.3, 27.8, 28.7, 31.2, 31.8, 33.3, 33.9, 35.5, 36.0, 36.3, 38.0, 39.8, 39.9, 40.7, 42.1, 42.7, 48.0, 48.1, 49.9, 56.7, 61.3, 62.3, 71.3, 73.7, 74.8, 75.5, 76.5, 77.7, 78.1, 79.5, 79.6, 79.6, 89.6, 104.5, 124.2, 124.5, 144.8, 145.4, 180.3. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{45}H_{73}N_4O_{13}$, 877.5169, found 877.5170.

Compound 68a. Prepared from **57** (68 mg, 0.13 mmol), acetyl-maltotriosyl azide (180 mg, 0.19 mmol) according to General procedure C. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **68a** as a white solid (163 mg, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.69, 0.78, 0.91, 0.99, 1.36 (7 × CH_3), 1.85, 2.00, 2.04, 2.04, 2.06, 2.10, 2.02, 2.17 (10 × CH_3CO), 0.72-2.25 (m, other aliphatic ring protons), 2.72 (dd, 1H, *J* = 3.2, 13.5 Hz), 3.21 (dd, 1H, *J* = 3.2, 10.3 Hz), 3.93-4.35 (m, 12H), 4.47-4.56 (m, 3H), 4.77 (dd, 1H, *J* = 4.0, 10.3 Hz), 4.86 (dd, 1H, *J* = 4.0, 10.5 Hz), 5.08 (t, 1H, *J* = 9.9 Hz), 5.29-5.49 (m, 5H), 5.54 (brs, 1H), 5.85 (d, 1H,

J = 9.1 Hz), 6.88 (t, 1H, J = 5.1 Hz), 7.70 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.5, 15.6, 16.8, 18.2, 20.1, 20.5 (4C), 20.6, 20.7 (3C), 20.8, 23.3, 25.1, 26.8, 27.1, 28.0, 29.1, 30.1, 32.5, 35.1, 35.2 (2C), 36.9, 38.5, 38.7, 39.6, 41.7, 41.8, 46.7, 46.8, 49.1, 53.4, 55.1, 61.3, 62.2, 62.6, 67.9, 68.5, 69.2, 69.3, 70.0, 70.4, 71.0, 71.5, 72.5, 73.4, 74.9, 75.2, 75.3, 78.8, 85.2, 95.6, 96.1, 120.9, 123.6, 143.5, 145.1, 169.1, 169.4, 169.6, 169.8 (2C), 170.3 (2C), 170.5 (3C), 178.0. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{71}H_{103}N_4O_{28}$, 1459.6753, found 1459.6784.

Compound 68b. Prepared from **68a** (110 mg, 0.07 mmol) according to General procedure B. The crude product was washed with water three times without further purification to afford **68b** as a white solid (54 mg, 73%). ^{1}H NMR (400 MHz, MeOD): δ 0.65, 0.78, 0.89, 0.94, 0.95, 0.97, 1.37 (7 × CH_3), 0.72-1.98 (m, other aliphatic ring protons), 2.35 (t, 1H, J = 7.9 Hz), 2.88 (dd, 1H, J = 3.0, 13.4 Hz), 3.15 (dd, 1H, J = 4.9, 11.3 Hz), 3.26-3.29 (m, 1H), 3.45-3.95 (m, 16H), 4.32 (d, 1H, J = 15.4 Hz), 4.35 (brs, 1H), 4.43 (d, 1H, J = 15.2 Hz), 5.17 (d, 1H, J = 3.6 Hz), 5.26 (d, 1H, J = 3.6 Hz), 5.47 (brs, 1H), 5.62 (d, 1H, J = 8.9 Hz), 7.99 (s, 1H). ^{13}C NMR (100 MHz, MeOD): δ 16.2, 16.4, 17.7, 19.5, 24.5, 25.4, 27.3, 27.9, 28.7, 31.2, 31.7, 33.3, 33.9, 36.0, 36.0, 36.3, 38.1, 39.8, 39.9, 40.8, 42.2, 42.8, 48.0, 48.1, 50.0, 56.8, 61.8, 62.1, 62.7, 71.5, 73.4, 73.6, 73.7, 74.2, 74.7, 74.9, 75.0, 75.6, 78.1, 79.5, 79.6, 80.3, 81.3, 89.3, 102.7, 102.9, 123.5, 124.2, 144.9, 146.0, 180.2. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{51}H_{83}N_4O_{18}$, 1039.5697, found 1039.5707.

Compound 69. To **56** (75 mg, 0.13 mmol) and K_2CO_3 (68 mg, 1 mmol) stirring in 3 mL DMF was added 1,3-propanediamine (5.5 μL, 0.06 mmol). The mixture was stirred at room temperature overnight. After completion (TLC) the solvent was removed under reduced pressure. The mixture was resolved in CH_2Cl_2 and washed with water and brine twice. The organic layer was dried over $MgSO_4$, then filtered

and concentrated. The crude was purified by column chromatography (CH_2Cl_2 CH_3OH, 30/1 v/v) to give product as a white solid (35 mg, 55%). ^{1}H NMR (400 MHz, MeOD): δ 0.76, 0.88, 0.92, 0.95, 0.96, 1.35 (14 × CH_3), 0.71-2.01 (m, other aliphatic ring protons), 2.27 (t, 2H, J = 13.3 Hz), 2.86-2.89 (m, 2H), 3.03-3.17 (m, 6H), 4.30 (brs, 2H), 5.51 (brs, 2H), 7.09 (brs, 2H). ^{13}C NMR (100 MHz, MeOD): δ 16.1, 17.7, 19.0, 24.1, 25.6, 27.1, 27.3, 28.5, 30.0, 30.8, 31.0, 33.0, 33.4, 35.6, 35.8, 37.3, 37.6, 39.4, 39.4, 40.3, 41.8, 42.4, 47.5, 47.5, 56.1, 75.0, 79.2, 123.6, 144.4, 179.8. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{63}H_{103}N_2O_6$, 983.7811, found 983.7804.

Compound 70. Prepared from **57** (112 mg, 0.22 mmol), 1,3-diazidopropane (12.6 mg, 0.1 mmol) according to General procedure C. The residue was purified by column chromatography (CH_2Cl_2/CH_3OH, 25/1 v/v) to give product as a white solid (100 mg, 87%). ^{1}H NMR (400 MHz, MeOD): δ 0.54, 0.71, 0.76, 0.88, 0.96, 0.96, 0.96, 1.37(s, 14 × CH_3), 0.71-2.01 (m, other aliphatic ring protons), 2.35 (t, 2H, J = 13.2 Hz), 2.46-2.53 (m, 2H), 2.87 (dd, 2H, J = 3.1, 9.9 Hz), 3.14 (dd, 2H, J = 5.0, 10.9 Hz), 4.36-4.44 (m, 10H), 5.47 (brs, 2H), 7.82 (s, 2H). ^{13}C NMR (100 MHz, MeOD): δ 16.2, 16.4, 17.7, 19.4, 24.5, 25.4, 27.3, 27.9, 28.7, 31.3, 31.8, 32.0, 33.3, 33.9, 36.0, 36.0, 36.4, 38.1, 39.8, 39.9, 40.7, 42.3, 42.8, 48.1, 48.2, 50.0, 56.7, 75.6, 79.6, 124.2, 124.7, 145.0, 146.1, 180.1. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{69}H_{109}N_8O_6$, 1145.8465, found 1145.8438.

Compound 71. To D-biotin (488 mg, 2 mmol) stirring in 8 mL DMF, N-hydroxysuccinimide (344 mg, 3 mmol) and EDC (576 mg, 3 mmol) were added. The mixture was stirred at rt overnight. The reaction mixture was then poured into ice (400 mL) and the precipitate was filtered. The precipitate was washed twice and dried under reduced pressure to afford a white solid (580 mg, 85%). The analytical data was in accordance with the reported characterization data.[18]

Compound 72. To **71** (96 mg, 0.28 mmol) stirring in 3 mL DMF, T**EA** (80 μL, 0.70 mmol) and 2-azidoethylamine (48 mg, 0.56 mmol) were added. The reaction was stirred for 12 h at rt. The contents were evaporated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2/CH_3OH/AcOH, 100/10/1 v/v/v) to give **72** as a white solid (46.4 mg, 53%). The analytical data was in accordance with the reported characterization data.[18]

Compound 73. To a solution of **57** (51 mg, 0.1 mmol) and **72** (37.4 mg, 0.12 mmol) in THF (3 mL) and H_2O (1 mL), $CuSO_4$ (19.2mg, 0.12 mmol) and Na-L-ascorbate (47.5 mg, 0.24 mmol). The resulting solution was stirred for 12 h at 40 ^oC. After completion (TLC), the contents were evaporated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2/CH_3OH, 10/1 v/v) to give **73** as a white solid (58.4 mg, 71%). ^1H NMR (400 MHz, Pyridine-d_5): δ 0.88, 0.97, 1.11, 1.04, 1.07, 1.23, 1.75 (7 × CH_3), 0.90-2.10 (m, other aliphatic ring protons), 2.29-2.37 (m, 3H), 2.44-2.48 (m, 1H), 2.78 (t, 1H, J = 13.1 Hz), 2.88 (d, 1H, J = 12.4 Hz), 2.96 (dd, 1H, J = 4.9, 12.6 Hz), 3.21-3.26 (m, 1H), 3.38 (dd, 1H, J = 3.3, 13.5 Hz), 3.44-3.48 (m, 1H), 3.87-3.92 (m, 2H), 4.38-4.41 (m, 1H), 4.54-4.57 (m, 1H), 4.67-4.75 (m, 3H), 4.85 (dd, 1H, J = 5.5, 14.9 Hz), 5.10 (brs, 1H), 5.66 (brs, 1H), 7.47 (brs, 1H), 8.12 (s, 1H), 8.19 (t, 1H, J = 5.2 Hz), 8.83 (t, 1H, J = 5.7 Hz). ^{13}C NMR (100 MHz, Pyridine-d_5): δ 16.2, 16.9, 17.6, 19.1, 24.2, 25.6, 26.3 (2C), 27.6, 28.5, 29.1, 29.3 (2C), 31.1, 32.1, 33.5, 33.6, 36.2, 36.4 (2C), 37.7, 39.5, 39.7, 40.4, 40.4, 41.4, 42.0, 42.6, 47.6, 47.8, 49.6, 50.1, 56.2, 56.6, 61.0, 62.7, 75.2, 78.4, 123.4, 123.9, 145.0, 146.2, 164.9, 173.9, 178.3. ESI-HRMS (*m/z*) $[M+H]^+$ calcd for $C_{45}H_{72}N_7O_5S$, 822.5310, found 822.5318.

HCV and VSV pseudovirus entry assay. All compounds were tested using the HCVpp and VSVpp entry assay as described previously. Briefly, pseudotyped viruses

were produced by cotransfecting 293T cells with plasmid encoding HCV E1, E2 or vesicular stromatis G protein (VSVG) and the envelope and Vpr deficient HIV vector carrying a luciferase reporter gene inserted into the Nef position 72 h after transfection, HCVpp or VSVpp was harvested from the supernatant of the transfected cells. For compound library screening, infections were performed in 96-well plates by adding diluted HCVpp or VSVpp into 5×10^3 Huh-7 cells/well in the presence or absence of test compounds, followed by incubation for 72 h at 37 ^{o}C. Luciferase activity, reflecting the degree of the pseudoparticles into host cells, was measured 3 days after infection using the Bright-Glo Reagent (Promega). Test compounds were serially diluted to give a final concentration of 1 μM and 5 μM in 1% dimethyl sulfoxide (DMSO). Maximum activity (100% of control) and background were derived from control wells containing DMSO alone or from uninfected wells, respectively. 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 inhibition values were then calculated by subtracting this value from 100. The specificity of the compounds for inhibiting HCV was determined by evaluating inhibition of VSVpp infection in parallel. Each sample was done in duplicate, and experiments were repeated for at least three times.

Hemolytic Assay. Hemolytic activity was measured as following:[19] 2% rabbit red blood cells in erythrocyte buffer (130 mM NaCl, 20 mM Tris-HCl, pH 7.4) were incubated with serial diluted compounds. After incubating for 60 minutes at 37°C, hemolysis was monitored by measuring absorption at 540 nm with a microplate reader. Percentage of hemolysis was then calculated as the routine method.

ASSOCIATED CONTENT

Supporting Information

Further chemical and biological experimental details, including HPLC data and NMR spectra are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

OA, oleanolic acid; EA, echinocystic acid; DMSO, dimethyl sulfoxide; DMF, dimethylformamide; DMAP, 4-(*N,N*-dimethylamino)pyridine; TBTU, *O*-benzotriazol-1-yl-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate; DIEA, *N,N*-diisopropylethylamine; THF, tetrahydrofuran; TEA, triethylamine; EDC, *N*-(3-Dimethylaminopropyl)-*N* ′ -ethylcarbodiimide hydrochloride; TBAB, tetrabutylammonium bromide.

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Figure legends

Figure 1. Structures and anti-HCV entry activities of **OA**, its derivatives (**1-15**) and analogs (**16-18**).

Figure 2. The inhibition curves for compound **EA**, **54a** and **70**. The concentration of **EA** were 1 nM, 10 nM, 100 nM, 1 μM, 10 μM and 100 μM, and the concentration of **54a** and **70** were 0.5 nM, 5 nM, 50 nM, 0.5 μM, 5 μM and 50 μM. Each concentration was tested triplicate and results were expressed as mean and standard deviation from triplicate assays.

Figure 3. Mechanistic studies of **EA**-mediated blocking of HCVpp entry by five different assay conditions. CD81 antibody, an entry inhibitor targeting host cell membrane, was utilized as a positive control and IM2865, a non-relevant compound, was used as negative control. 0.5% DMSO (final concentration) was used for normalization in each condition. The final concentration of all chemicals in the assay was 10 μM and the concentration of CD81 antibody was 5 nM.

Figure 4. Identification of **EA**-targeting proteins by pull-down assay. A biotin-labeling **EA** was utilized as a probe that selectively pulled down HCV envelope protein E2 from HCVpp packaging cell lysate by streptavidin immobilized on agrose.

Figure 5. Kinetic assays by Biacore for valuation of E2, CD81 and chemical EA interactions. Blue curves were the experimental trace obtained from biolayer interferometry experiments, and red curves were the best global fits to the data used to calculate the K_D. (**A**) Binding curves for E2 and CD81 interaction. E2 were loaded onto SA coated biosensors and incubated with varying concentrations of CD81 from 40.9 nM to 654.5 nM, $K_D = 2.1 \times 10^{-8}$ M ($R^2 = 0.99$); (**B**) Binding curves for E2 and **EA** interaction. E2 were loaded onto SSA coated biosensors and incubated with varying concentrations of **EA** from 3.125 μM to 100 μM, $K_D = 2.4 \times 10^{-5}$ M (R^2 =

0.94); (**C**) Binding curves for E2 and CD81 interaction in the presence of **EA**. E2 were loaded onto SA coated biosensors and incubated with varying concentrations of CD81 from 40.9 nM to 654.5 nM and then 100 μM **EA** was added, $K_D = 1.5 \times 10^{-6}$ M ($R^2 = 0.97$).

Figure 6. A proposed mechanism for EA-mediated blocking of HCV entry, where in ring A, B and E of EA are conserved while ring D is modifiable.

Scheme 1[a]. [a] Reagents and conditions: (a) Ac_2O, DMAP, pyridine, rt, 12 h; (b) HBr·AcOH, CH_2Cl_2, 0 ^oC to rt, 12 h; (c) acetyl-glucosyl bromide, TBAB, K_2CO_3, CH_2Cl_2, N_2, reflux, overnight; (d) MeONa, MeOH, rt, 1 h.

Scheme 2[a]. [a] Reagents and conditions: (a) Ac_2O, DMAP, pyridine, rt, 12 h; (b) HBr·AcOH, CH_2Cl_2, 0 ^oC to rt, 12 h; (c) NaN_3, DMF, rt, overnight; (d) TBTU, DIEA, THF, rt, overnight; (e) 2-propynylamine, Na_2CO_3, DMF, rt, 20 min; (f) $CuSO_4$, Na-L-ascorbate, azidosacchrides, CH_2Cl_2/H_2O, rt, overnight; (g) MeONa, MeOH, rt, 1h.

Scheme 3[a]. [a] Reagents and conditions: (a) 1,3-propanediamine, Na_2CO_3, DMF, rt, overnight; (b) $CuSO_4$, Na-L-ascorbate, 1,3-diazidopropane, CH_2Cl_2/H_2O, rt, overnight.

Scheme 4[a]. [a] Reagents and conditions: (a) N-hydroxysuccinimide, EDC, DMF, rt, overnight; (b) 2-azidoethylamine, TEA, DMF, rt, 12 h; (c) $CuSO_4$, Na-L-ascorbate, Compound **57**, THF/H_2O, 40 ^oC, overnight.

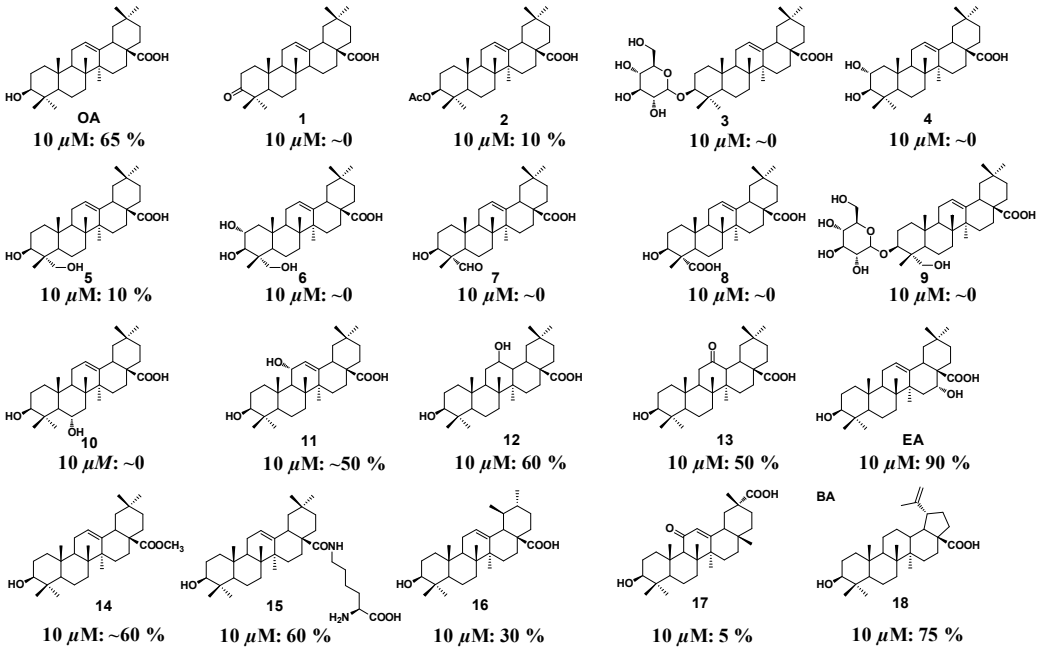


Figure 1

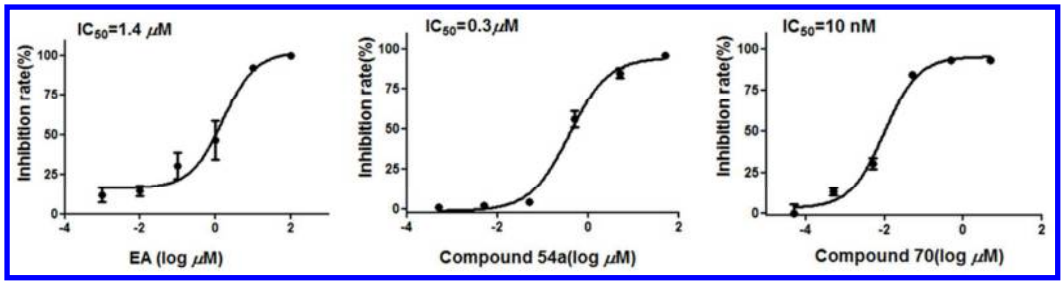


Figure 2

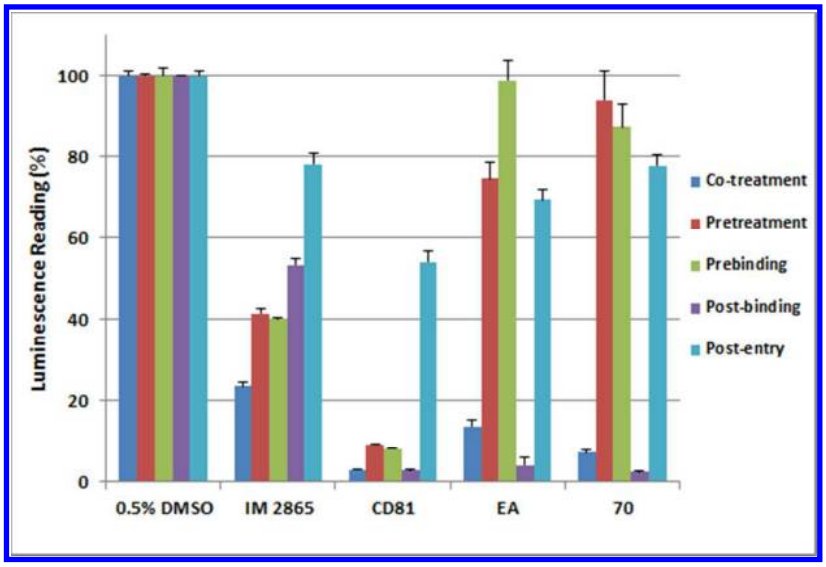


Figure 3

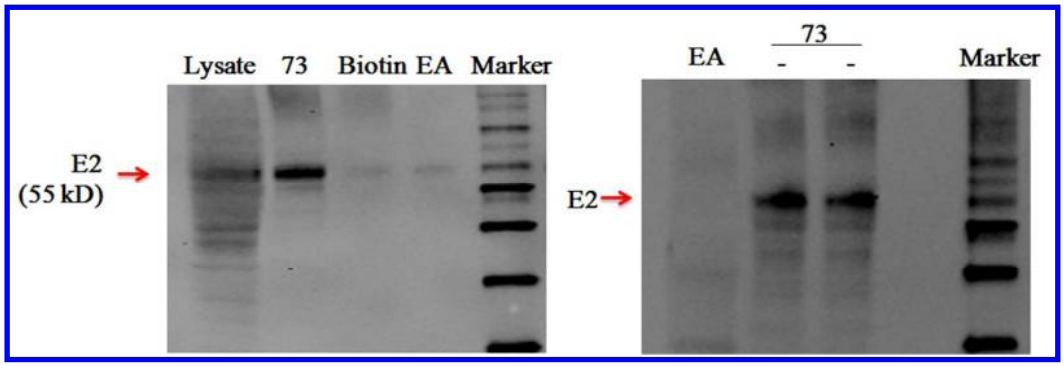


Figure 4

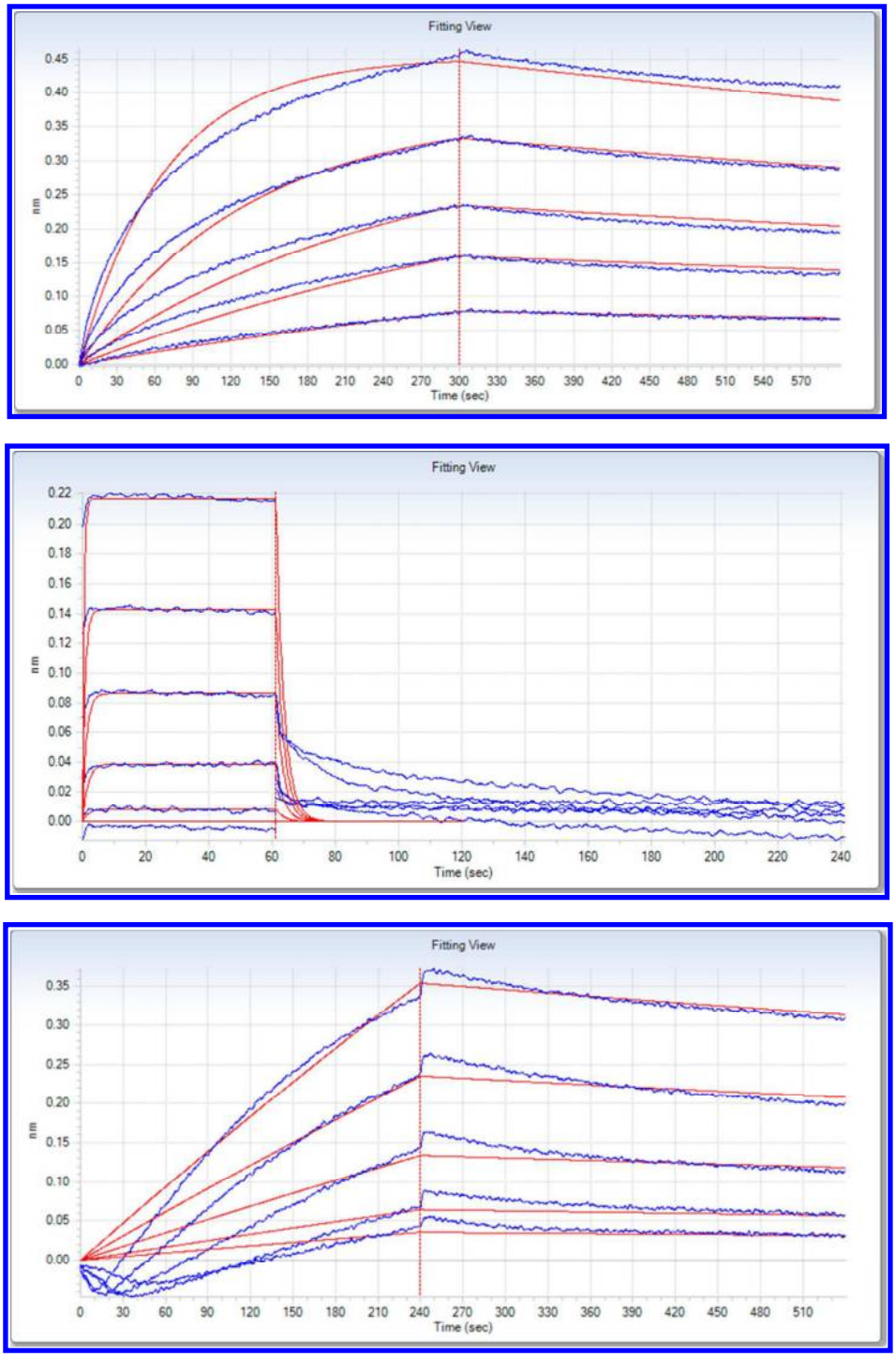

Fitting View
nm
Time (sec)
Fitting View
nm
Time (sec)
Fitting View
nm
Time (sec)

Figure 5

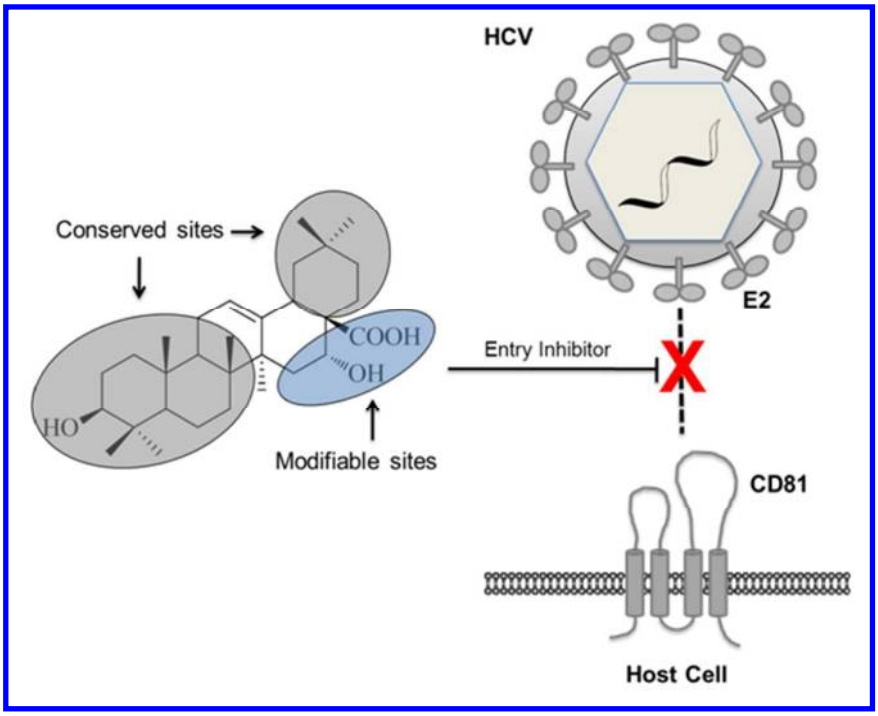
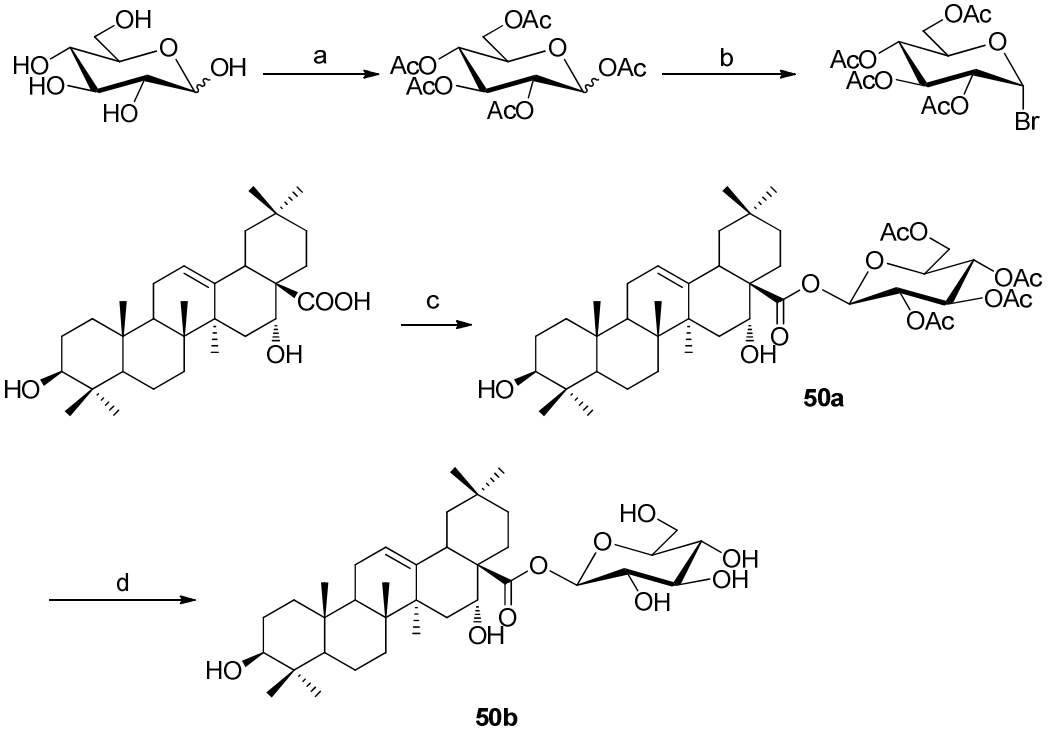
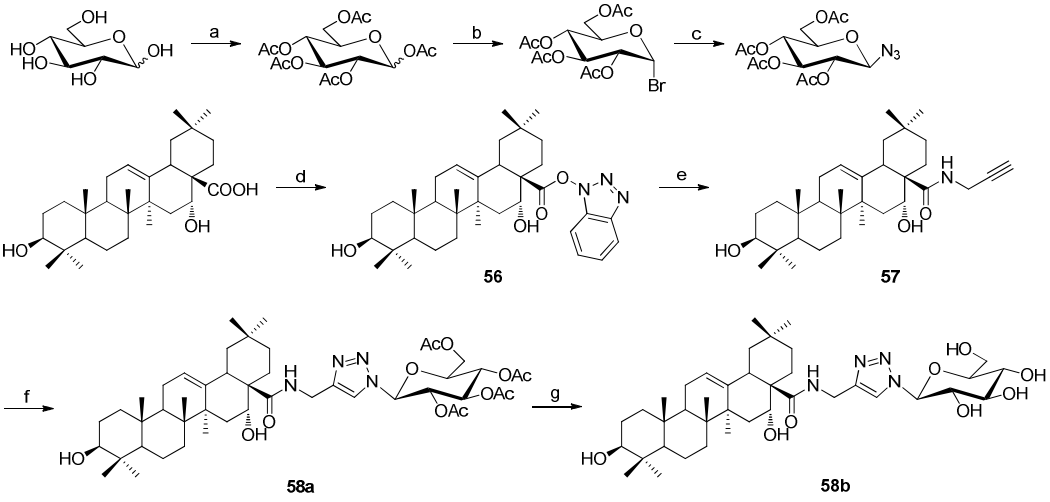

HCV
E2
Conserved sites
COOH
OH
HO
Modifiable sites
Entry Inhibitor
CD81
Host Cell

Figure 6

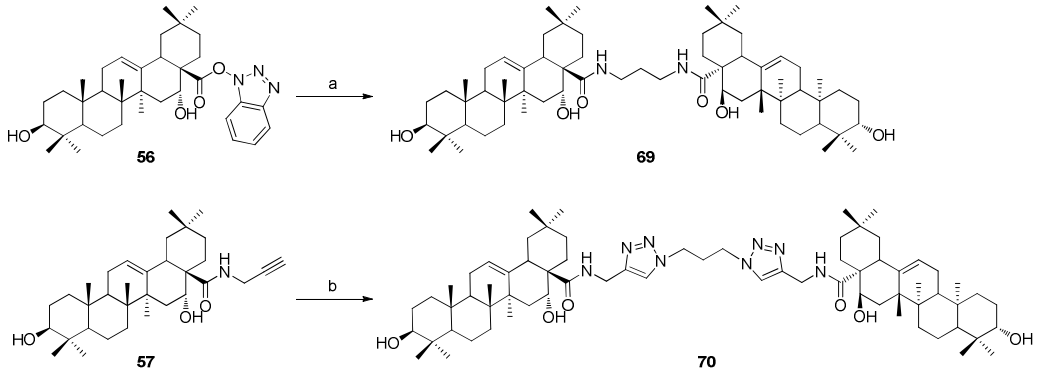
Scheme 1[a]



Scheme 2[a]



Scheme 3[a]



Scheme 4[a]

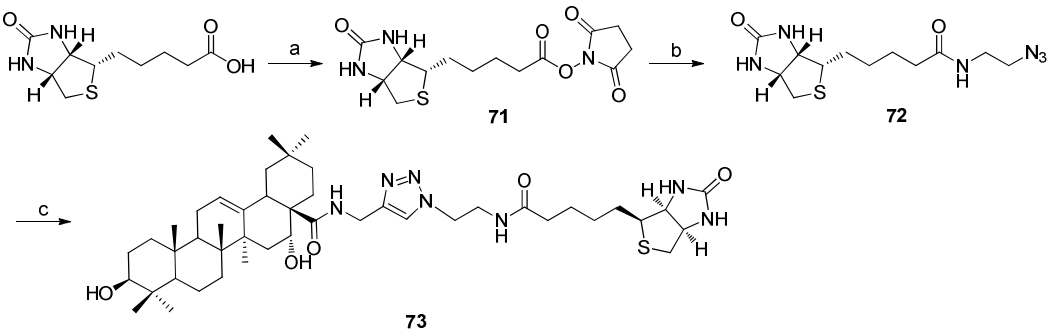
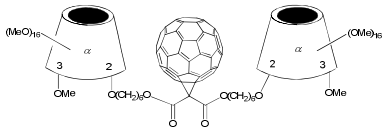
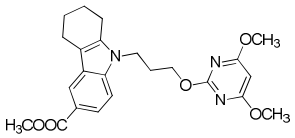
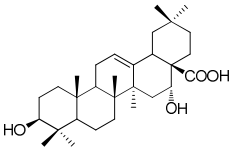
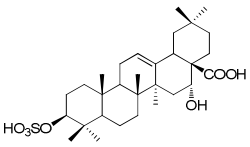
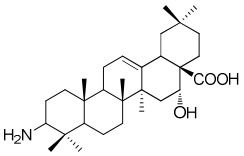
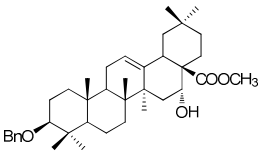
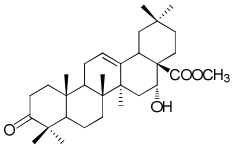
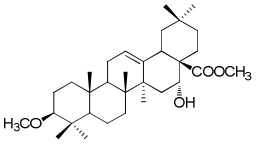
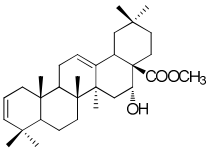
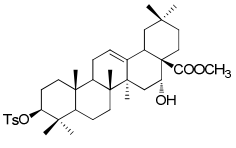
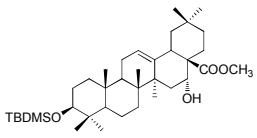
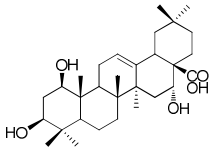
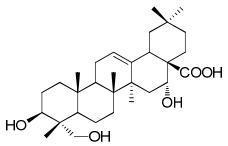
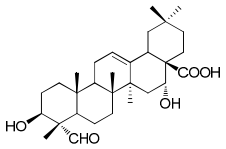
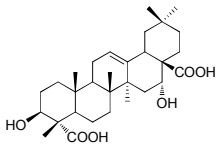
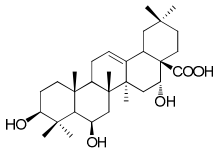
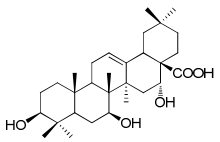
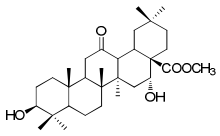
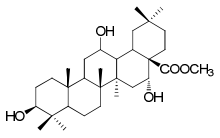
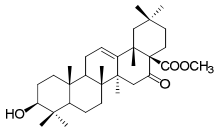
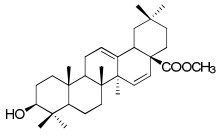
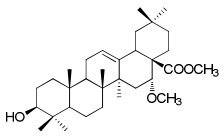
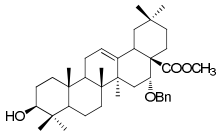
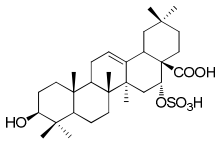
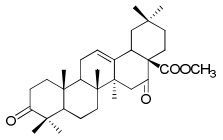
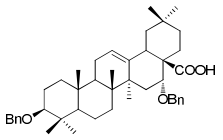
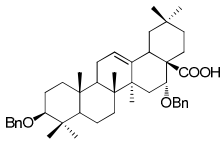
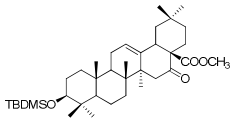
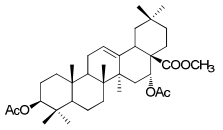
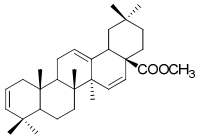
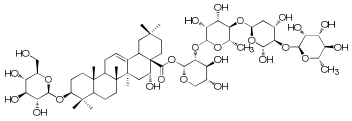
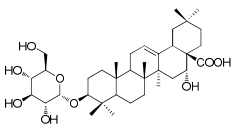
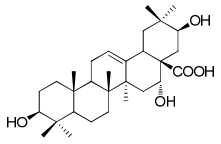
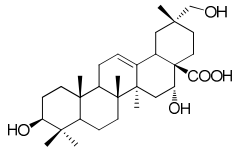
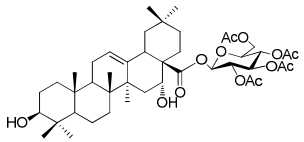
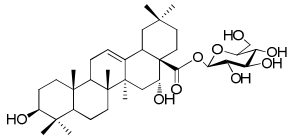
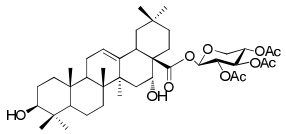
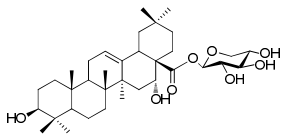
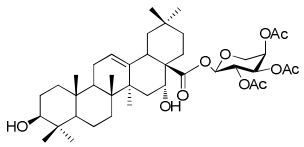
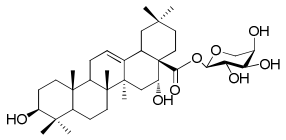
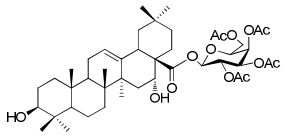
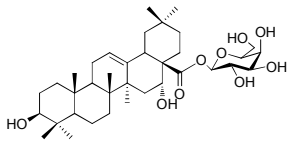
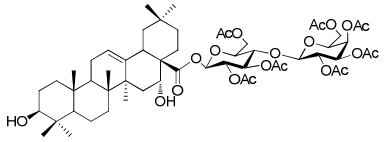


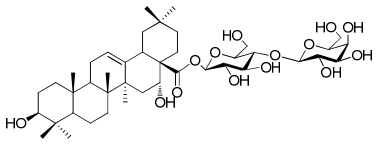
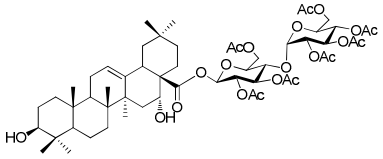
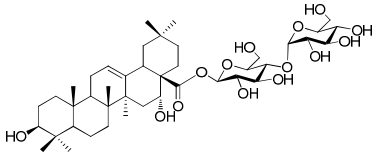
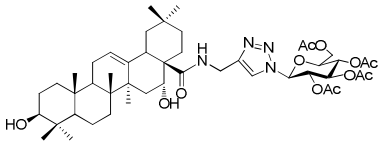
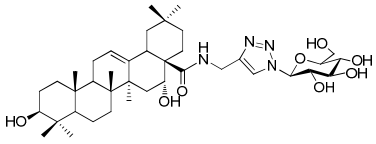
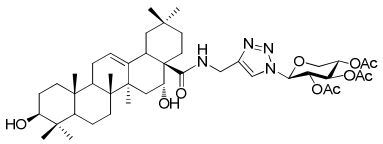
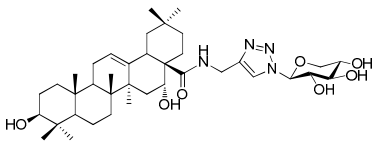
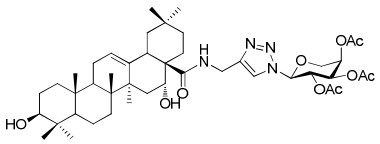
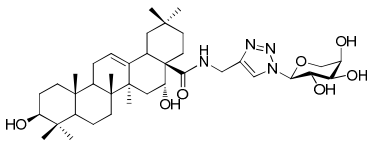
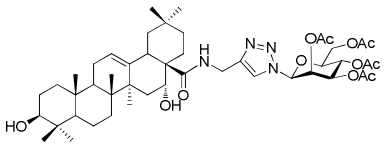
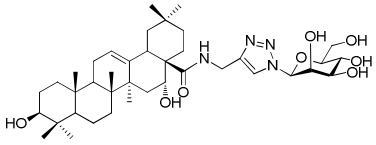
Table 1. Structures and anti-HCVpp entry activities of EA, EA derivatives and analogs

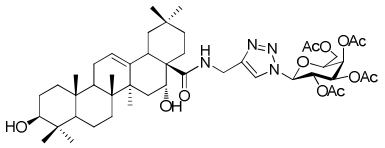
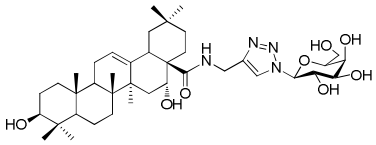
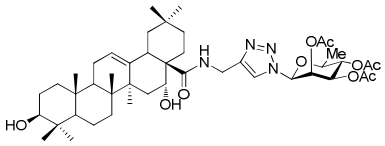
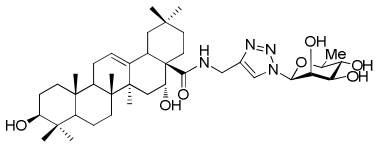
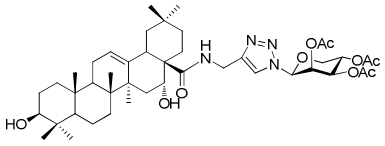
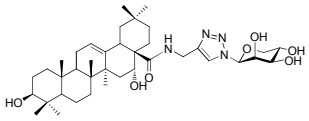
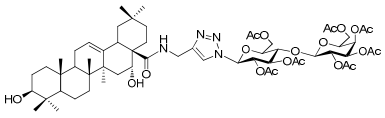
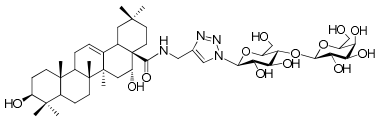
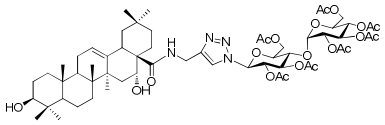
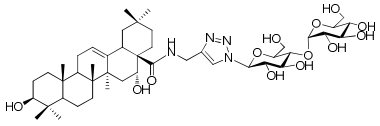
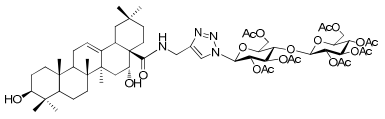
Compound		Inhibition of HCVpp entry (%)[a]		Inhibition of VSVpp entry (%)[a]	
		1 μM	5 μM	1 μM	5 μM
Positive control 1[b]		75.3 ±1.6	94.4 ±1.4	7.3±0.7	7.5±0.9
Positive control 2[b]		21.2±1.1	34.0±0.9	11.7±2.2	12.8±1.8
EA		27.5 ± 2.1	77.7 ± 3.3	7.2 ± 1.7	9.0 ± 0.8
19		33.4 ± 1.4	55.2 ± 1.8	39.15 ± 2.3	40.35 ± 1.3
20		12.1 ± 0.7	10.5 ± 0.1	23.6 ± 0.7	28.4 ± 0.1
21		10.5 ± 0.7	5.2 ± 0.4	12.6 ± 1.1	25.0 ± 1.5
22		3.5 ± 0.6	64.7 ± 2.9	21.5 ± 1.8	55.4 ± 2.9
23		-14.0 ± 0.9	-53.7 ± 2.6	9.9 ± 0.3	30.1 ± 1.2
24		-3.3 ± 0.2	-8.9 ± 0.7	7.6 ± 0.4	9.7 ± 0.7
25		12.3 ± 1.1	16.4 ± 0.7	38.3 ± 2.4	39.3 ± 3.6

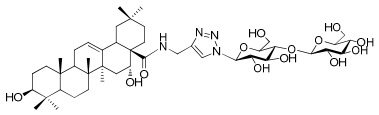
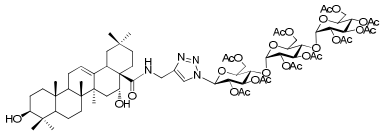
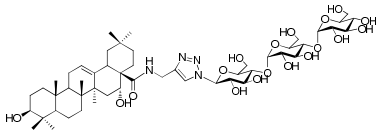
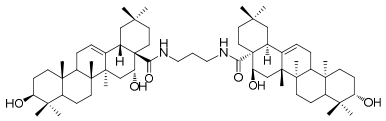
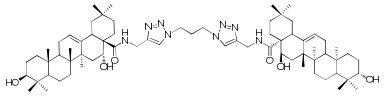
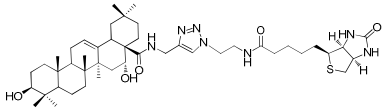
26		44.3 ± 2.3	27.8 ± 2.0	27.4 ± 1.6	37.4 ± 3.0
27		14.3 ± 1.1	35.2 ± 2.9	7.9 ± 0.7	11.8 ± 0.9
28		NT	~20 (10 μM)	NT	NT
29		NT	~0 (10 μM)	NT	NT
30		NT	~0 (10 μM)	NT	NT
31		NT	~5 (10 μM)	NT	NT
32		30.4 ± 2.6	53.5 ± 3.4	1.6 ± 0.1	5.6 ± 0.6
33		4.9 ± 0.3	-8.7 ± 0.7	42.7 ± 3.5	46.1 ± 4.1
34		22.6 ± 1.9	51.0 ± 0.2	35.8 ± 1.5	43.5 ± 0.1
35		34.7 ± 1.8	87.2 ± 0.7	20.3 ± 1.7	21.5 ± 2.1
36		12.3 ± 0.8	27.9 ± 2.7	15.2 ± 0.2	18.8 ± 1.4

37		17.5 ± 2.8	33.7 ± 3.9	7.3 ± 1.4	9.9 ± 3.5
38		4.6 ± 0.2	5.8 ± 1.3	1.6 ± 0.1	-3.9 ± 0.2
39		1.0 ± 0.2	-2.7 ± 0.1	38.5 ± 2.3	34.7 ± 1.1
40		48.2 ± 2.4	70.1 ±0.3	-10.0 ± 0.7	-2.8 ± 0.2
41		5.5 ± 0.6	28.2 ± 1.8	28.8 ± 1.2	30.6 ± 2.7
42		1.7 ± 0.1	7.9 ± 0.2	8.5 ± 0.7	14.2 ± 1.0
43		11.5 ± 1.1	-3.8 ± 0.3	45.3 ± 1.8	40.2 ± 2.0
44		13.5 ± 0.5	20.1 ± 1.2	15.8 ± 0.2	24.2 ± 1.8
45		35.8 ± 2.5	14.0 ± 1.0	20.9 ± 1.5	33.9 ± 2.8
46		81.7 ± 5.2	94.4 ± 7.1	20.7 ± 1.8	33.2 ± 2.7
47		16.5 ± 0.5	36.5 ± 3.2	-2.5 ± 0.4	3.6 ± 1.3

48		NT	~15 (10 μM)	NT	NT
49		NT	~90 (10 μM)	NT	NT
50a		35.7 ± 0.6	78.3 ± 2.2	3.7 ± 0.3	13.9 ± 1.6
50b		30.6 ± 2.3	67.5 ± 2.0	12.9 ± 0.2	5.6 ± 0.1
51a		17.5 ± 1.3	70.7 ± 3.6	20.1 ± 0.1	21.7 ± 2.1
51b		33.8 ± 2.5	77.7 ± 3.2	36.5 ± 0.6	13.0 ± 1.1
52a		19.3 ± 1.2	60.6 ± 2.6	17.1 ± 1.0	20.1 ± 0.3
52b		34.7 ± 2.6	54.6 ± 0.5	15.6 ± 1.1	5.8 ± 0.1
53a		43.4 ± 2.7	81.6 ± 2.7	29.3 ± 0.5	30.7 ± 1.9
53b		40.9 ± 3.2	61.6 ± 3.6	8.1 ± .5	14.6 ± 1.2
54a		80.4 ± 2.8	88.6 ± 0.4	11.2 ± 0.7	14.9 ± 0.3

54b		32.7 ± 1.1	40.1 ±2.6	22.6 ± 0.5	16.7 ± 1.1
55a		36.5 ± 0.1	50.0 ± 3.2	20.9 ± 1.2	23.4 ± 2.1
55b		36.4 ± 1.9	43.7 ± 2.0	21.6 ± 1.1	21.9 ± 1.9
58a		20.3 ± 0.2	89.7 ± 2.0	-9.1 ± 0.7	12.5 ± 0.3
58b		12.4 ± 0.6	33.1 ± 1.9	-15.2 ± 0.1	19.1 ± 1.6
59a		35.9 ± 2.2	87.3 ± 0.5	39.8 ± 1.6	47.7 ± 0.2
59b		14.8 ± 0.7	27.8 ± 1.4	34.4 ± 0.7	27.0 ± 1.7
60a		26.3 ± 0.7	72.6 ± 3.6	-6.6 ± 0.1	20.9 ± 2.1
60b		18.7 ± 1.8	32.2 ± 2.3	8.4 ± 0.4	25.1 ± 1.4
61a		21.8 ± 0.7	81.5 ± 3.0	-5.3 ± 0.1	14.7 ± 0.8
61b		17.5 ± 0.3	25.0 ± 1.3	17.9 ± 1.5	19.5 ± 1.5

62a		20.4 ± 0.2	66.5 ± 2.6	24.3 ± 2.1	30.6 ± 2.3
62b		16.6 ± 0.3	36.3 ± 0.2	11.5 ± 0.3	25.3 ± 1.5
63a		17.0 ± 1.5	71.6 ± 2.1	32.6 ± 1.5	-10.7 ± 0.8
63b		32.0 ± 2.8	67.0 ± 1.5	7.7 ± 0.8	13.9 ± 0.6
64a		21.8 ± 1.2	65.8 ± 3.0	29.3 ± 1.1	32.2 ± 3.5
64b		20.3 ± 1.5	43.2 ± 3.7	7.0 ± 0.8	35.2 ± 1.7
65a		24.6 ± 1.3	71.8 ± 4.5	39.0 ± 2.8	32.1 ± 0.9
65b		23.1 ± 2.4	14.6 ± 1.5	47.0 ± 2.5	47.3 ± 3.2
66a		56.4 ± 4.6	79.7 ± 1.5	13.2 ± 1.0	28.9 ± 2.3
66b		9.3 ± 0.3	29.7 ± 7.0	25.8 ± 2.3	48.1 ± 1.8
67a		11.9 ± 0.4	64.8 ± 3.4	36.8 ± 3.2	38.8 ± 2.9

67b		15.2 ± 1.2	11.8 ± 0.5	26.6 ± 0.9	50.5 ± 3.5
68a		45.4 ± 1.1	62.9 ± 2.5	17.7 ± 0.2	14.0 ± 0.3
68b		12.8 ± 0.7	39.0 ± 0.3	19.7 ± 1.1	27.4 ± 1.7
69		44.6 ± 7.4	58.2 ± 2.8	18.7 ± 0.9	27.6 ± 1.6
70		86.3 ± 1.8	89.0 ± 0.7	16.5 ± 1.3	11.9 ± 0.2
73		34.2 ± 2.5	76.7 ± 4.6	10.3 ± 1.2	12.0 ± 0.8

[a] Inhibition (%) = 100% − DMSO negative control (%) of each tested compound; NT, not tested in this assay.

[b] Positive control compounds from reference 14(b) and 8(a), respectively.

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