Journal of Medicinal Chemistry Article

J. Med. Chem. **2010,** *53,* 7377–7391 **7377**
DOI: 10.1021/jm100822f

Carbocycles Related to Oseltamivir as Influenza Virus Group-1-Specific Neuraminidase Inhibitors. Binding to N1 Enzymes in the Context of Virus-like Particles

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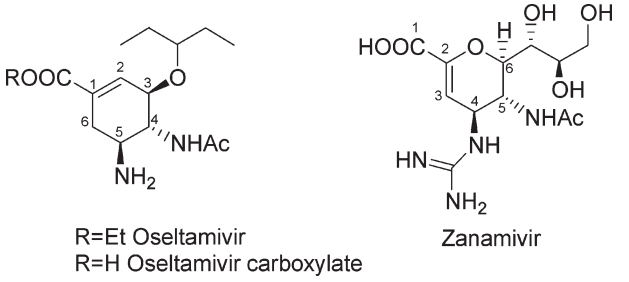
Received July 2, 2010

We report here the exploitation of the 150-cavity in the active sites of group-1 neuraminidases for the design of new triazole-containing carbocycles related to oseltamivir. Inhibition studies with virus-like particles (VLPs) containing the influenza virus neuraminidase-1 (N1) activity indicate that several candidates are inhibitors, with K_i values in the $10^{-5}-10^{-8}$ M range. In contrast, a known candidate that preserves the free amino group and a new candidate containing a guanidine function are better inhibitors, with K_i values of 1.5×10^{-9} and 4.6×10^{-10} M, respectively. The most active inhibitor of the N1 enzyme in the triazole series was selective for the N1 class and showed significantly less inhibition (K_i = 2.6 μM vs 0.07 μM) of the free influenza virus neuraminidase-2 (N2). In addition, saturation transfer difference (STD) NMR spectroscopic studies with this compound and the VLPs show that the entire molecule forms contacts with residues in the active site. These data taken together support our proposed binding mode in which the active site and the adjoining 150-cavity are both occupied.

Introduction

Influenza A and B viral infections continue to be one of the serious health problems facing the human population worldwide. In addition to the seasonal reappearance of the previously circulated viral strains, the appearance of new viral strains, such as the swine flu virus (H1N1) now circulating worldwide, through antigenic variation has resulted in three major pandemics during the past 100 years. The worst scenario in the history of influenza outbreaks was the so-called "Spanish flu" (1918) which killed nearly 1% of the world's population. Inhibition of a viral surface glycoprotein called neuraminidase, which catalyzes the release of newly formed virions from the infected cells, has proved to be a suitable approach in the design of antiviral drugs.[1−4] This approach has been successfully demonstrated by the two neuraminidase inhibitors zanamivir[3] and oseltamivir,[4] which are currently in clinical use for the treatment of influenza viral infections (Chart 1). The worldwide stockpiling of these two antiviral drugs as part of pandemic preparedness highlights the overall importance of neuraminidase inhibitors. The alarming threat of a potential influenza pandemic posed by the avian influenza virus H5N1[5,6] and the recent isolations of oseltamivir-resistant H5N1 underscore the increased demand for the development of new antiviral drugs.[7]

Chart 1



R=Et Oseltamivir
R=H Oseltamivir carboxylate

Zanamivir

The phylogenetic tree divides all of the nine known neuraminidase (NA[a]) subtypes from influenza A into two groups: group 1 contains N1, N4, N5, and N8 subtypes, whereas group 2 contains N2, N3, N6, N7, and N9.[8] Prior to 2006, crystal structures of only two subtypes N2 and N9 from group-2 enzymes were available. In 2006, Russell et al.[9] reported the crystal structures of three subtypes, N1, N4, and N8, from group-1 enzymes. They found that the three-dimensional shapes of their active sites are virtually identical but, interestingly, are very different when compared to those of group-2 enzymes N2 and N9. In the cases of N1, N4, and N8 subtypes, a loop of amino acids consisting of residues 147−152 (also known as 150-loop), including the active site residues Asp151 and Glu119, was found to adopt an unusual open conformation compared to the N2 and N9 subtypes in which the 150-loop was found to have a closed conformation. As a result of this open-loop conformation, a cavity near the active site (also called 150-cavity) becomes accessible in the case of N1, N4, and N8 subtypes. The observation of two different active-site conformations between certain subtypes

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[a]Abbreviations: VLPs, virus-like particles; NA, influenza neuraminidase; STD NMR, saturation transfer difference nuclear magnetic resonance; CuAAC, copper-catalyzed azide−alkyne cycloaddition; TLC, thin layer chromatography; PDC, pyridinium dichromate; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofuran; DCE, 1,2-dichloroethane; TFA, trifluoroacetic acid; HEPES, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid; Neu5Ac2en, 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid; COSY, correlation spectroscopy; HSQC, heteronuclear single quantum coherence; MUN, 4-methylumbelliferyl *N*-acetyl-α-D-neuraminide.

Published on Web 09/28/2010

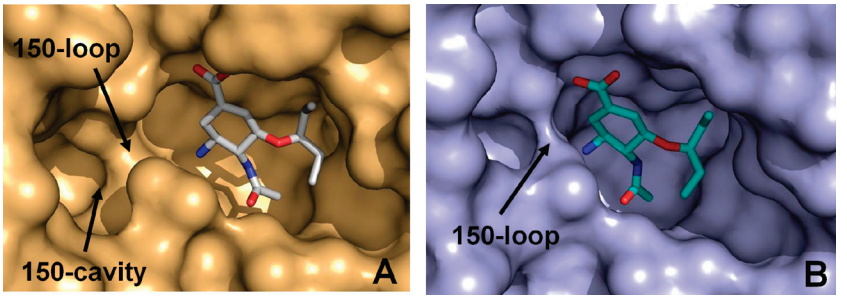


Figure 1. Active site comparison of group-1 and group-2 enzymes: oseltamivir carboxylate bound in the active site of (A) the N1 subtype and (B) the N9 subtype.

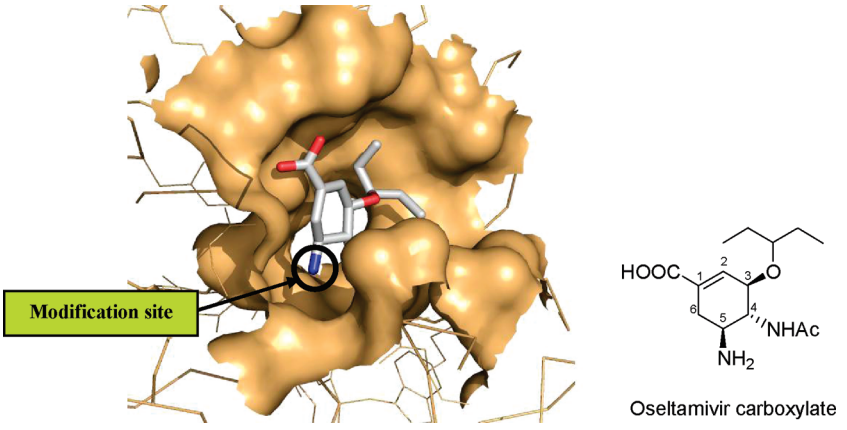


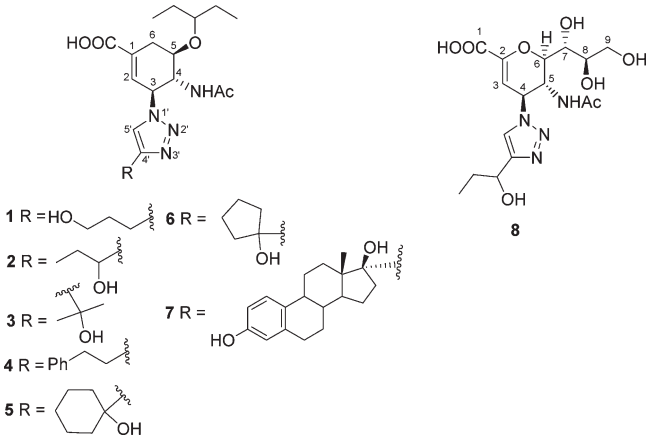
Figure 2. Active site complex of the N1 subtype with oseltamivir carboxylate showing the C-5 amino group as a potential modification site.

of group-1 and group-2 enzymes suggested that these two groups are not only genetically distinct but also structurally distinct.[9] Oseltamivir carboxylate (active metabolite of oseltamivir, Chart 1) when bound to the N1 subtype showed similar binding interactions for the ligand as seen in the group-2 enzymes except that the 150-loop adopted the open conformation as seen in the apo forms of group-1 enzymes (Figure 1); however, under certain crystallization conditions, the 150-loop adopted the closed conformation as seen in apo and holo forms of group-2 enzymes, suggesting a slow loop closure upon inhibitor binding.[9] Recent computational studies of the N1 subtype also suggest that the 150-loop has remarkable mobility and may even open to a greater extent than observed in the crystal structures.[10,11]

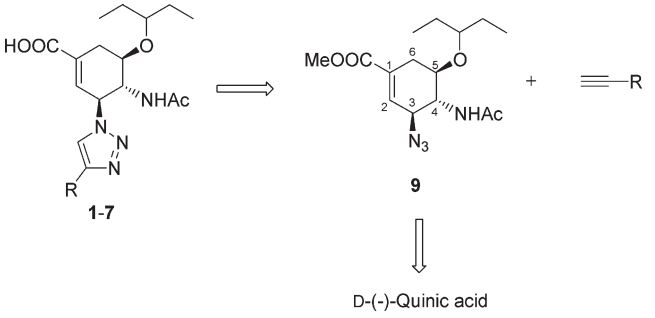
The flexibility of 150-loop and also the opening of 150-cavity near the active sites of group-1 enzymes have been considered as important starting points for the design of group-1 specific neuraminidase inhibitors.[9−11] Although, a universal drug candidate against all nine subtypes is highly desirable, group-1-specific inhibitors that exploit the newly found 150-cavity would be of use in combating the potential pandemic threat posed by avian influenza virus H5N1 and also in the treatment of currently circulating swine flu virus H1N1.

One proposed strategy to achieve group-1 specific inhibitors is to extend the structures of existing inhibitors by attaching additional groups of suitable shape, size, and hydrophobicity to fill the newly found cavity.[9] This action would presumably also keep the mobile loop in the energetically favorable open conformation.[10,11] Close examination of the crystal structure of oseltamivir carboxylate bound in the N1 subtype revealed that the C-5 amino group of oseltamivir carboxylate is well exposed toward the newly discovered cavity and could serve as the potential modification site in the design of group-1 specific inhibitors (Figure 2). In this work, we describe the synthesis of triazole containing carbocycles (**1**−**7**, Chart 2) related to oseltamivir as potential group-1 specific neuraminidase inhibitors. The basis of the study was to investigate the design of selective inhibitors, taking advantage of the novel structural features of influenza virus group-1 neuraminidases. We envisioned that in these compounds (**1**−**7**) the C-4′ substituent of the triazole ring could serve as the cavity filling group and the rigid triazole ring could serve as the linker between the cavity filling group and oseltamivir-like scaffold. It has been noted in previous studies that the C-5 amino group of oseltamivir carboxylate makes key interactions with Asp151 and Glu119 residues in group-2 enzymes (closed 150-loop conformation).[4] However, in the crystal structures of group-1 enzymes with an open 150-loop conformation, the C-5 amino group of oseltamivir is located further from these residues, and as a result, the C-5 amino group completely loses its hydrogen bonding interaction with Asp151.[9] Recent computational studies also support this observation.[10] Since the target compounds (**1**−**7**) are intended to exploit the 150-cavity, which indeed requires an open loop conformation, it was neither necessary nor appropriate to maintain a basic moiety at the C-5 position and hence could be substituted with the less basic triazole linker. We anticipated that while the C-5 amino group interactions observed with the closed 150-loop would be lost, new interactions in the open loop region and 150-cavity could be introduced from the triazole moiety and its alkyl substituent that should be compensatory. In addition, the triazole linker provides rapid access to different target compounds with a variety of 150-cavity binders essentially from one key intermediate. It is noteworthy that similar modification of the zanamivir scaffold has resulted in the discovery of an inhibitor (**8**, Chart 2) with a

Chart 2



Scheme 1



comparable EC_{50} value (6.4 μM) against avian influenza virus H5N1 to that of the parent compound, zanamivir (2.8 μM).[12] Ensemble-based virtual screening studies indicated that some of the highest affinity, potential 150-cavity binders interact primarily through hydrophobic interactions in the 150-cavity.[11] Hence, we have chosen variety of lipophilic alkyl groups, from simple alkyl chains to a complex steroidal group, as 150-cavity binders in the target compounds (**1**−**7**). Included in this list is also the 1-hydroxypropyl chain of the most potent compound (**8**) from the triazole-modified zanamivir series.[12]

Results and Discussion

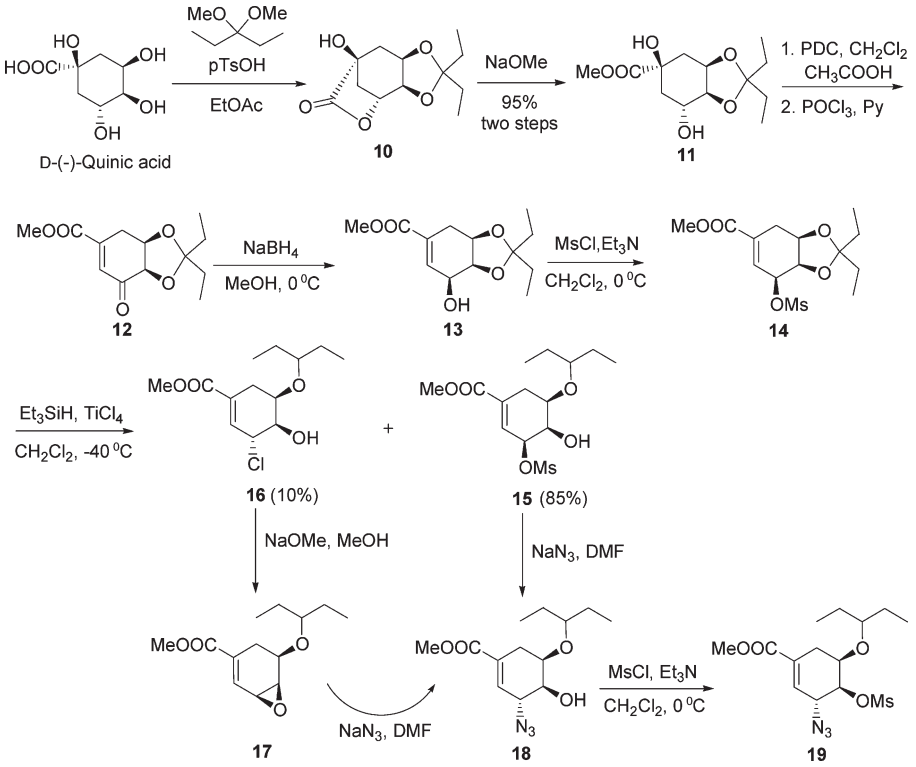
Retrosynthetic analysis indicated that the target carbocycles (**1**−**7**) could be conveniently synthesized from the azido intermediate **9** and various terminal alkynes via copper-catalyzed azide−alkyne cycloaddition (CuAAC)[13] as shown in Scheme 1. For the synthesis of the intermediate **9**, we followed conceptually similar synthetic strategies that were used in the synthesis of oseltamivir.[4,14] For example, we also employed reductive ring-opening of a pentylidineketal protecting group to install the C-5-pentyloxy side chain (step 6 in Scheme 2) and opening of a *N*-acetylaziridine ring using sodium azide to install the *trans*-3,4-diamino functionality in a regio- and stereospecific manner (step 3 in Scheme 3).

The preparation of the enone intermediate **12** was achieved using a modified literature procedure starting from D-(−)-quinic acid as shown in Scheme 2.[15] According to the reported procedure,[15] lactone **10** was obtained by heating D-(−)-quinic acid in boiling 3-pentanone in the presence of a catalytic amount of phosphoric acid with azeotropic removal of water for 12 h. However, in our hands, this reaction was found to be low yielding (25% conversion by TLC) and failed to produce the reported yield of lactone **10** (94%). Hence, we synthesized compound **10** according to the method reported for a different acetal protecting group.[14] Thus, D-(−)-quinic acid was treated with 3,3-dimethoxypentane[16] in the presence of a catalytic amount of *p*-toluenesulfonic acid (pTsOH) in ethyl acetate. Continuous removal of ethyl acetate by distillation from the reaction vessel along with the byproduct methanol led to complete consumption of quinic acid in 1 h (as indicated by TLC) and gave the desired lactone **10** in quantitative yield. The remaining conversion of lactone **10** to the required enone **12** was patterned after the reported procedure. Thus, the lactone **10** was first converted into the methyl ester **11** using catalytic NaOMe,[14] and pyridinium dichromate (PDC) oxidation followed by elimination gave the desired enone **12** (Scheme 2).[15] Subsequent regioselective reduction of the enone **12** was achieved using $NaBH_4$ at 0 °C to give the allylic alcohol **13** in 80% yield. The intermediate **13** was then transformed into the mesylate **14** using MsCl and triethylamine. The mesylate **14**, upon reaction with Et_3SiH and $TiCl_4$ at −40 °C, underwent regioselective, reductive ring-opening to give the desired secondary alcohol **15** in 85% yield. An interesting side product was also formed in this reaction which was isolated (10% yield) and characterized as the chloride intermediate **16**.[17] We predicted the stereochemistry at C-3 in the chloride **16** as *R* and the anti relationship between the C-3 chloro substituent and the C-4 hydroxyl group based on the assumption that the chloride might have formed via S_N2 displacement of the mesyl group in **15**. The proposed stereochemistry at C-3 was confirmed later by the successful conversion of the chloride intermediate **16** into an epoxide (**17**) by reaction with NaOMe. The mesylate **15** was transformed into the azido alcohol **18** using sodium azide. Of note, the side product **16** in the previous step was also transformed into the desired azido intermediate **18** via a two-step process, namely, reaction with NaOMe followed by reaction with sodium azide, as shown in Scheme 2. The azido alcohol **18** was then reacted with MsCl and triethylamine to give the mesylate **19** in 85% yield.

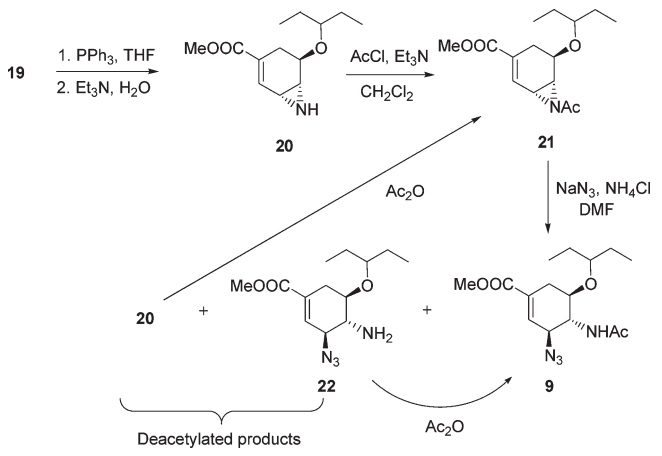
Reduction of the azido group in **19** using PPh_3 and triethylamine/water mixture resulted in the formation of the aziridine compound **20**, as shown in Scheme 3. The intermediate **20**, upon reaction with acetyl chloride and triethylamine at 0 °C, gave the *N*-acetylaziridine **21**. Reaction of **21** with sodium azide and NH_4Cl gave a mixture of three products, as indicated by TLC (Scheme 3). The major product was isolated (60% yield) and characterized as the desired azido intermediate **9**. The other two side products could not be separated by column chromatography. However, we anticipated that these two side products could have been formed as a result of the deacetylation of both starting material **21** and product **9**. Hence, the reaction was repeated and when the starting material was fully consumed (as indicated by TLC), the reaction was stopped, and after processing, the crude product was treated with acetic anhydride at room temperature for 2 h. TLC of the reaction mixture indicated only two spots corresponding to starting material **21** and product **9**, thereby confirming that the two side products observed in the previous step were indeed the deacetylated products **20** and **22**. In this case, the desired azido intermediate **9** was isolated in 68% yield and the recovered starting material **21** (20%) was then recycled.

With the key intermediate **9** in hand, we then performed a copper-catalyzed azide−alkyne cycloaddition[13] using various terminal alkynes following the standard protocol,[18] as shown in Scheme 4.

Scheme 2



Scheme 3



Initially, the hydrolysis of the methyl ester **25** was performed using 1 M NaOH and MeOH as solvent (Scheme 5). Surprisingly, the ^{1}H NMR spectrum of the crude product indicated three different products. Fortunately, the desired compound **3** was conveniently precipitated (40% yield, 98% purity) by the addition of ethyl acetate to the crude product. From the ethyl acetate soluble fraction, we were able to isolate one of the side products (41% yield, 96% purity) by crystallization, and it was then characterized as being **32** by 1D and 2D NMR analyses. From the remaining mother liquor, the other side product was isolated (13% yield) as a mixture containing 28% of **32** and characterized as being **36**. Similar results were obtained for the hydrolysis of the other esters **23**, **24**, and **26**.[19]

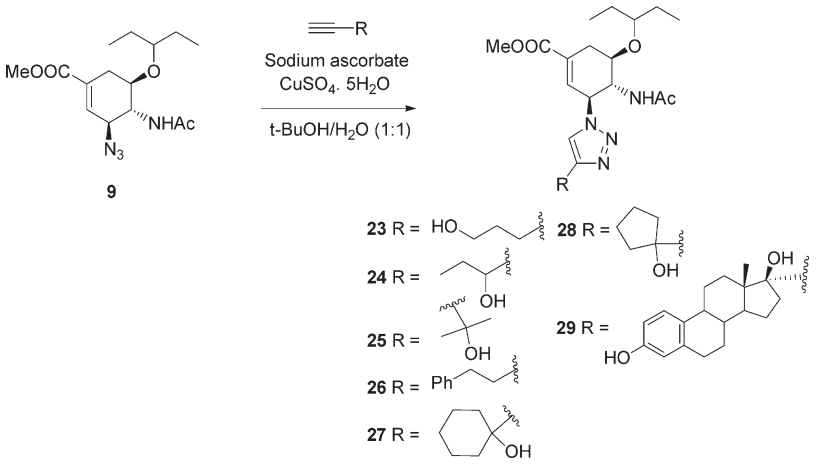
We attribute the formation of **30**−**33** and **34**−**37** to the base-catalyzed double bond migration and epimerization at the C-3 stereocenter, respectively, as shown for compound **25** in Scheme 6. In an attempt to provide further evidence for the proposed mechanism, we have performed the hydrolysis of compound **25** using deuterated sodium hydroxide in deuterated methanol. As expected, deuterium was incorporated in the isolated products **3**(**D**), **32**(**D**), and **36**(**D**) at the C-3, C-1, and C-3 positions, respectively, as indicated by ^{1}H NMR analyses; the spectrum of compound **32**(**D**) lacked the H-1 signal (Figure 3b) when compared to **32** (Figure 3a). Similarly, the spectra of compounds **3**(**D**) and **36**(**D**) lacked the H-3 signals, thus indicating that deuterium incorporation had occurred at C-3 in both of these compounds (data not shown).

In addition, the splitting patterns of the adjacent protons indicated the incorporation of deuterium. For example, the ^{1}H NMR spectrum of **32** showed a doublet for H-2 at 6.73 ppm and a ddd for both H-6a and H-6b at 2.25 and 1.96 ppm (Figure 3a), respectively, whereas in the case of **32**(**D**), the spectrum showed a singlet for H-2 and a dd for both the H-6a and H-6b protons (Figure 3b). Similarly, the dd corresponding to the H-4 proton observed in the ^{1}H NMR spectrum of **3** (δ 4.20 ppm) and **36** (δ 4.65 ppm) was changed into a doublet in the spectra of the respective deuterated products **3**(**D**) and **36**(**D**) (data not shown). In a reaction performed in an NMR tube, the ratio of the three products did not change even after 3 days, indicating that the double bond migration and epimerization at the C-3 stereocenter must be occurring prior to the hydrolysis of the ester group.

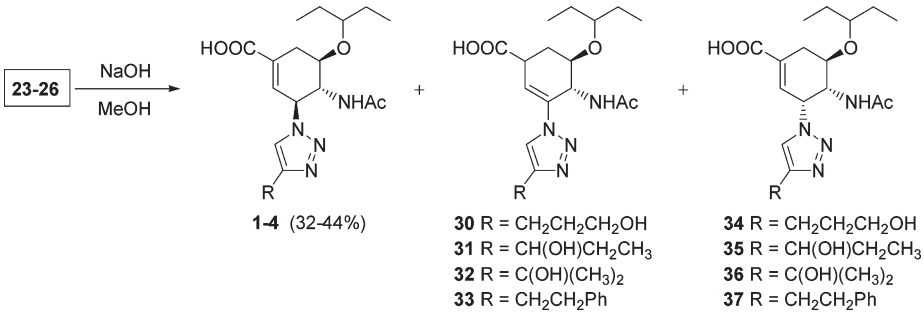
To overcome the difficulty of epimerization at C-3 and double bond migration, we employed trimethyltin hydroxide[20] to hydrolyze the methyl esters **27**−**29**. The reactions proceeded smoothly, and the products were obtained in higher yields, as shown in Scheme 7.

Finally, the key intermediate **9** was also converted into the double bond isomer of oseltamivir **38** and its corresponding guanidine derivative **39**, as shown in Scheme 8. We note that compound **38** is a known 30 nM inhibitor of neuraminidase.[21]

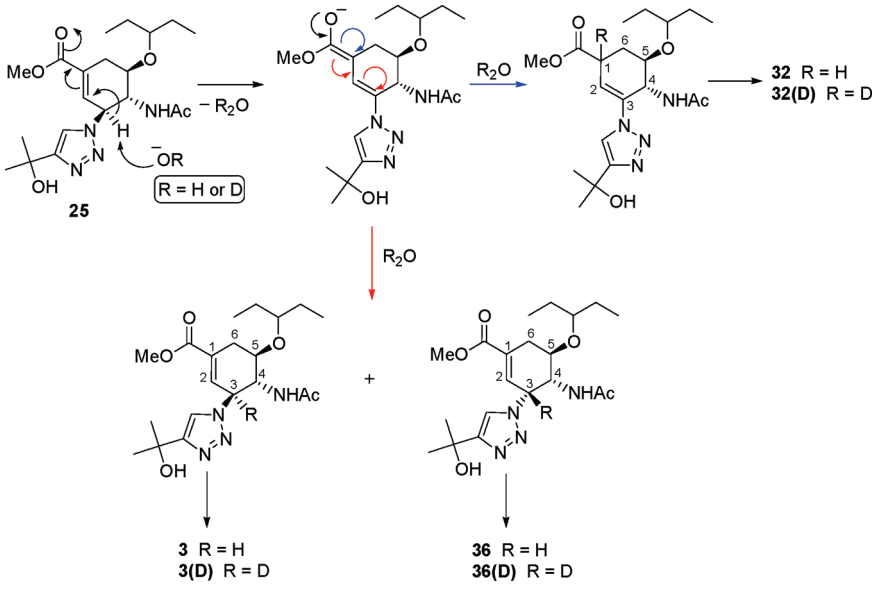
Scheme 4



Scheme 5



Scheme 6



Thus, intermediate **9**, upon treatment with trimethyltin hydroxide, gave the intermediate **40** which was then reduced to the amine **38** by bubbling hydrogen gas into the heterogeneous mixture of **40** and Lindlar's catalyst in ethanol. On the other hand, bubbling hydrogen gas into the heterogeneous mixture of the intermediate **9** and Lindlar's catalyst gave compound **41**, which was then converted into the Boc-protected guanidine derivative **42** using Boc-protected thiourea and $HgCl_2$, as shown in Scheme 8. The methyl ester **42** was first hydrolyzed using 1 M KOH, and then the Boc protecting groups were removed using a 1:1 mixture of trifluoroacetic acid (TFA) and CH_2Cl_2 to yield compound **39**.

We have tested the inhibitory activities of the target compounds **1**−**7**, **38**, and **39** against virus-like particles (VLPs) that contain an influenza A virus N1 activity.[22] Because of the insoluble nature of compounds **4** and **7** in water, the corresponding L-arginine salts **43** and **44** (Chart 3), respectively, were made and tested. In order to study the importance of the

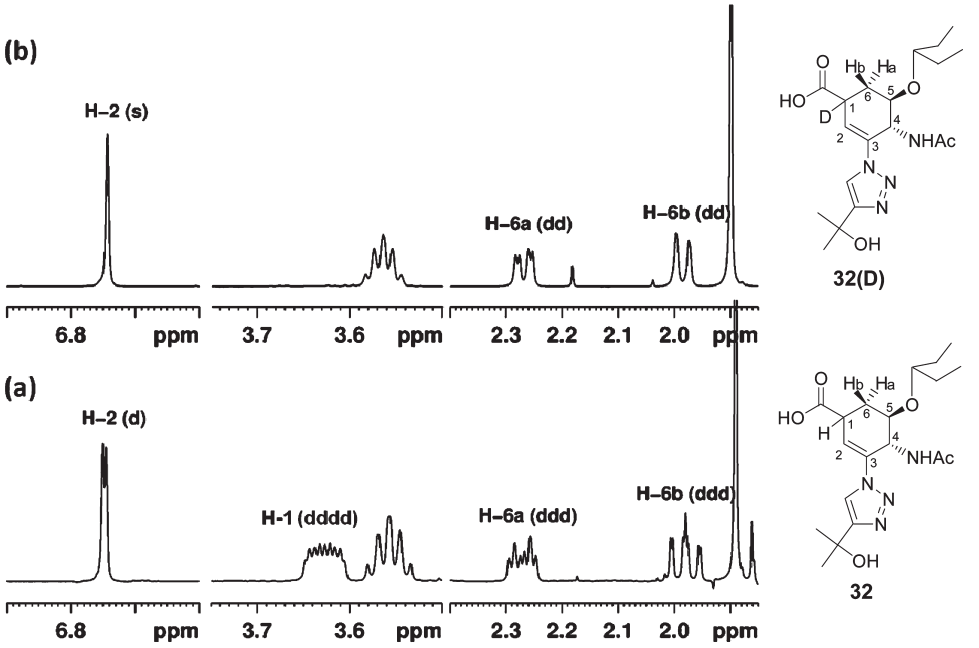
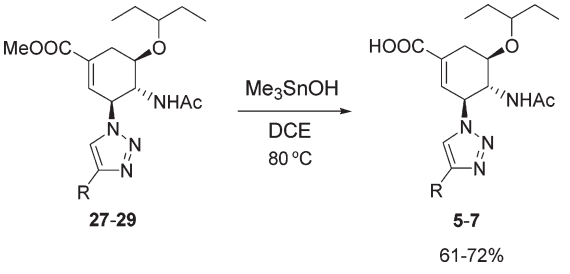


Figure 3. 1D traces of ^{1}H NMR spectra of compounds **32** and **32(D)**.

Scheme 7



regiochemistry of the double bond, we have also tested the inhibitory activities of compounds **32** and **33** as their L-arginine salts **45** and **46** (Chart 3), respectively. The results are summarized in Table 1.

Our results indicate that all of the triazole modified compounds **1**−**3**, **5**, **6**, **43**, and **44** were inhibitors, with K_i values ranging from 0.07 to 11 μM (Table 1), but less active than the parent compound **38**, its guanidine derivative **39**, or zanamivir. The parent compound **38** has a K_i value of 1.5 nM against the virus-like particles containing an influenza A/N1 activity. However, its guanidine derivative **39** was found to be a more potent inhibitor with a K_i value of 0.46 nM. In comparison, zanamivir has a K_i of 0.16 nM under these assay conditions. Comparison of the inhibitory activities of **3** and **43** with those of the corresponding double bond isomers **45** and **46**, respectively, leads us to conclude that the presence of a double bond between C-1 and C-2 is essential for proper orientation of the triazole ring and its alkyl substituent toward the 150-cavity.

In order to confirm whether the triazole-extended carbocycles (**1**−**3**, **5**, **6**, **43**, and **44**) make use of the 150-cavity that is specific to the group-1 subtypes for binding, a representative compound **2**, the most active compound in the triazole series, was screened against soluble influenza A virus N2, a group-2 neuraminidase in which the 150-cavity is absent. As expected, compound **2** was found to be significantly less active against the N2 subtype (K_i = 2.6 μM) compared to the N1 subtype (K_i = 0.07 μM). The observed 37-fold selectivity between N1 and N2 subtypes suggests that these triazole-extended carbocycles indeed take advantage of the 150-cavity found in the N1 subtype for binding.

As a final point of interest, we have used STD NMR spectroscopy to investigate the ligand interactions with N1 influenza VLPs. Once again, the most active compound in the triazole series, **2**, was chosen as a representative candidate for this study. Figure 4a shows the 1D ^{1}H NMR spectrum of a mixture of compound **2** and VLPs containing the N1 subtype in deuterated HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid) buffer. Figure 4b shows the corresponding STD NMR spectrum. Strong STD signals for the protons corresponding to the oseltamivir-like portion and the triazole extended portion of the molecule were detected. This clearly indicates that the entire molecule **2** makes contacts with the residues in the active site of N1-VLPs. Specifically, the STD signals corresponding to the H-5′, H-d, H-e, H-f protons (STD signals from the triazole-extended portion marked by the red arrows in Figure 4b) confirm that this portion of the molecule does indeed make contacts with the receptor molecule (N1), suggesting that the 150-cavity is also occupied.

Our first series performed as we expected, providing an excellent leadlike structure that then could be further modified to increase the potency. Our aim was to demonstrate that we could access this 150-cavity through the replacement of the C5-amino group with functionalized triazole substituents on the oseltamivir-like template and derive selectivity between group-1 and group-2 neuraminidases and maintain high potency. This has clearly been demonstrated. A lead compound, **2** with a K_i value of 72 nM and 37-fold group-1 selectivity, has been identified for further optimization. We also note that the selective inhibition of group-1 neuraminidases, shown here, has not been demonstrated previously for the oseltamivir-like template.

In summary, we have synthesized a series of triazole-containing carbocycles using copper catalyzed azide−alkyne cycloaddition as a key reaction. One of these compounds, **2**, was shown to be an effective group-1 neuraminidase inhibitor with a K_i value of 72 nM against VLPs containing an influenza

Scheme 8

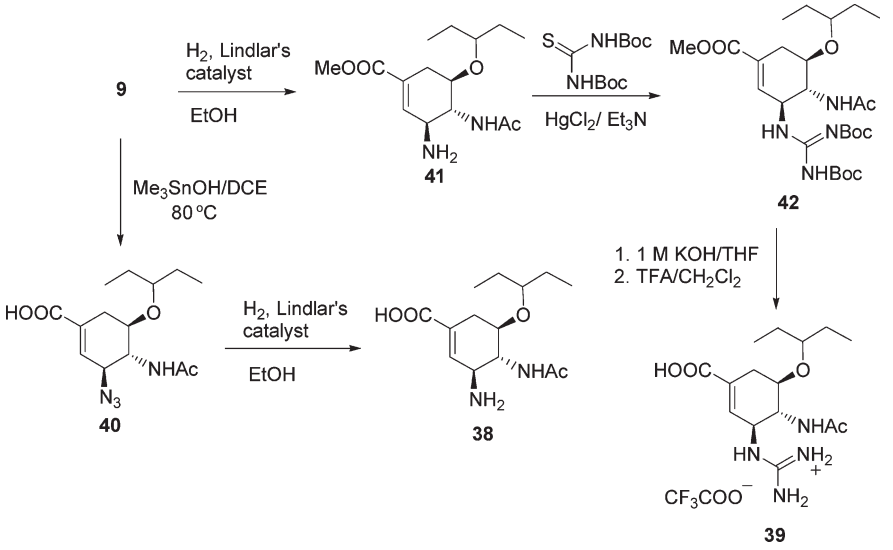


Chart 3

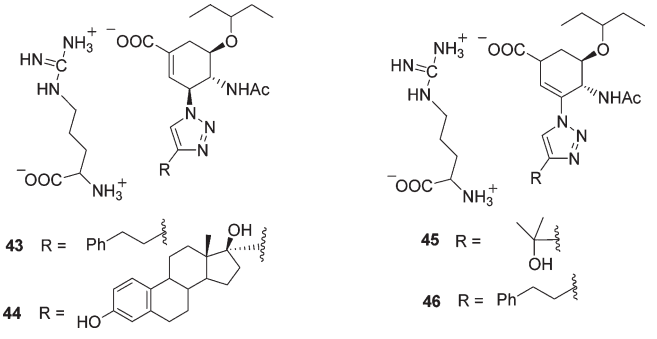


Table 1. Inhibitory Activities of Compounds **1**−**3**, **5**, **6**, **38**, **39**, and **43**−**46** against Virus-like Particles That Contain an Influenza A Virus N1 Activity

compd	K_i (M)
1	4.6×10^{-7}
2	7.2×10^{-8}
3	1.3×10^{-7}
5	4.8×10^{-6} [a]
6	1.1×10^{-5} [a]
38	1.5×10^{-9}
39	4.6×10^{-10}
43	1.2×10^{-6}
44	5.8×10^{-6}
45	1.9×10^{-5}
46	6.0×10^{-5}
Neu5Ac2en	5.9×10^{-6}
4-deoxy-4-guanidino-Neu5Ac2en (zanamivir)	1.6×10^{-10}

[a] IC_{50} value.

A virus N1 activity. On the basis of the observation of selective inhibition of the N1 subtype by compound **2** and of the ligand binding studies using STD NMR spectroscopy, it is likely that the triazole series of compounds (**1**−**3**, **5**, **6**, **43**, and **44**) make use of the 150-cavity found in the group-1 subtypes, thus providing lead structures for further optimization. In addition, we have also discovered a new candidate **39** (K_i = 0.46 nM) which has inhibitory activity comparable to that of zanamivir (K_i = 0.16 nM). Unlike the triazole series, compound **39** is not expected to discriminate between the two groups of influenza virus neuraminidases on the basis of the structural comparison of **39** with that of zanamivir.

Experimental Section

General Methods. ^1H and ^{13}C NMR spectra were recorded at 600 or 500 and 150 or 125 MHz, respectively. All assignments were confirmed with the aid of two-dimensional $^1H,^1H$ (COSY) and/or $^1H,^{13}C$ (HSQC) experiments using standard pulse programs. Processing of the spectra was performed with MestRec and/or MestReNova software. Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with silica gel 60F-254 as the adsorbent. The developed plates were air-dried, exposed to UV light and/or sprayed with a solution containing 1% $Ce(SO_4)_2$ and 1.5% molybdic acid in 10% aqueous H_2SO_4, and heated. Column chromatography was performed with silica gel 60 (230−400 mesh). High resolution mass spectra were obtained by the electrospray ionization method, using an Agilent 6210 TOF LC/MS high resolution magnetic sector mass spectrometer. The purity of all compounds was determined to be ≥98% from ^1H and ^{13}C NMR spectra (see Supporting Information).

Influenza Virus Sialidase Activity Assay. In a standard 96-well plate format, by use of previously described virus-like particles (VLPs) that contain an influenza virus N1 activity[22] (kindly provided by Dr. Jean-Michel Garcia and Jimmy Lai, Hong Kong University-Pasteur Research Centre, Hong Kong) or native influenza virus N2 sialidase (kindly provided by Prof. John Skehel, Mill Hill, U.K.), the synthesized compounds were assayed for their capacity to inhibit influenza virus sialidase by a modification[23] of the fluorometric method of Potier et al.[24] using the fluorogenic substrate 4-methylumbelliferyl *N*-acetyl-α-D-neuraminide (MUN). Specifically, 7 μL of 50 mM sodium acetate−6 mM $CaCl_2$ buffer (pH 5.5) was added to each well of a 96-well solid black plate on ice, followed by 1 μL of inhibitor, 1 μL of N1-containing VLP or native N2 sialidase, and finally 1 μL of the substrate MUN. The plate was then briefly centrifuged up to 1000 rpm for approximately 10 s to combine all components, and the mixture was incubated at 37 °C with 900 rpm shaking for 20 min. To stop the reaction, 250 μL of 0.25 M glycine, pH 10, was added to each well, and the fluorescence was read (1 s per well) at an excitation of 355 nm and emission of 460 nm. All inhibition assays were done in triplicate over four inhibitor concentrations and at two concentrations of the substrate MUN (0.05 and 0.1 mM). Inhibitor concentrations were

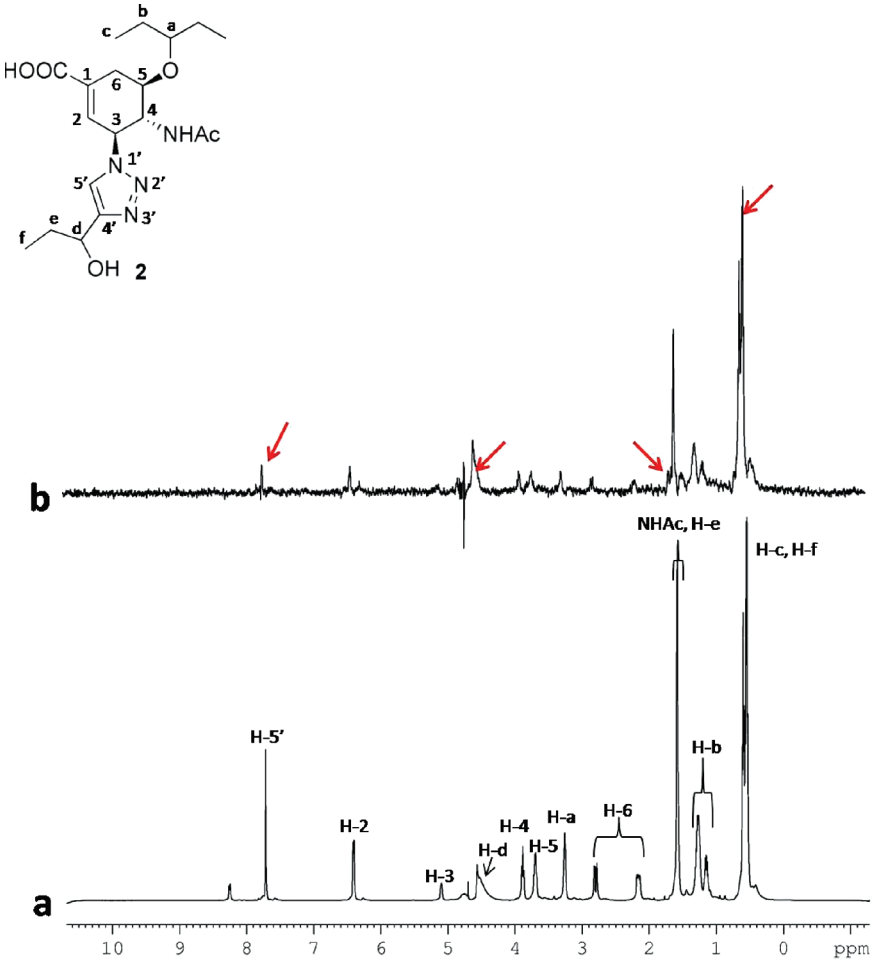


Figure 4. (a) 1D ^{1}H NMR spectrum of compound **2** in the presence of H5(N1) influenza VLPs. (b) The corresponding STD NMR spectrum.

selected to give a percentage inhibition of sialidase activity between 5% and 95%, and data analysis was carried out using SigmaPlot Enzyme Kinetics Module.[25]

STD NMR Analysis. The 1D ^{1}H NMR spectrum and STD NMR spectrum of compound **2** (960 μg) in the presence of H5(N1) influenza VLPs were acquired at 600 MHz and 278 K. The on-resonance frequency was set to −1.00 ppm and the off-resonance frequency to 300 ppm. The protein was saturated with 40 Gaussian soft pulses with a duration of 50 ms, each resulting in a total saturation time of 2.0 s. The residual water signal was reduced by applying the WATERGATE sequence.

Methyl 4,5-*O*-Isopentylidene-3-dehydro-4-*epi*-shikimate (12). To a stirred solution of D-(−)-quinic acid (10 g, 52.1 mmol) in ethyl acetate (250 mL) was added 3,3-dimethoxypentane[16] (20.7 g, 156.3 mmol) and a catalytic amount of *p*-toluenesulfonic acid (100 mg). The reaction mixture was subjected to slow distillation. Continuous removal of ethyl acetate (~125 mL) from the reaction vessel along with the byproduct methanol led to complete consumption of starting material in 1 h (as indicated by TLC). The reaction mixture was then concentrated under reduced pressure to give the lactone **10** in quantitative yield. To the crude lactone **10** was added a solution of sodium methoxide (280 mg, 5.2 mmol) in 200 mL of methanol. After stirring at room temperature for 2 h, the reaction mixture was stored at −20 °C for 16 h. The mixture was then neutralized with glacial acetic acid (0.5 mL) at −20 °C. All of the volatile components were removed under reduced pressure and the resulting crude mixture was purified by column chromatography (EtOAc/hexanes 4:1) to give **11** as a pale yellow oil (13.5 g, 94%). This material was identical in all respects to that reported previously.[15] The methyl ester **11** was then transformed into the enone **12** using a reported procedure.[15]

Methyl (3*S*,4*R*,5*R*)-4,5-*O*-Isopentylidene-3,4,5-trihydroxycyclohexene-1-carboxylate (13). Sodium borohydride (4.5 g, 118.1 mmol) was added slowly (portionwise) to a stirred solution of compound **12** (6 g, 23.6 mmol) in dry methanol (150 mL) at 0 °C, and the stirring was continued for 1 h at 0 °C. The reaction was quenched by the addition of NH_4Cl and followed by extraction with CH_2Cl_2 (200 mL × 2). The organic layers were combined and washed with water (100 mL), brine, dried over anhydrous Na_2SO_4, and concentrated. Chromatographic purification (EtOAc/hexanes, 1:2) of the crude compound afforded compound **13** as a colorless oil (4.9 g, 80%) which solidifies up on storing. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 6.91 (1H, br dddd-like, H-2), 4.61 (1H, ddd, $J_{5,6a}$ = 2.4, $J_{5,6b}$ = 3.6, $J_{5,4}$ = 7.8 Hz, H-5), 4.54 (1H, ddd, $J_{4,3}$ = 4.8, $J_{4,2}$ = 1.5 Hz, H-4), 4.06 (1H, dddd, $J_{3,6b}$ = $J_{3,2}$ = 2.4, $J_{3,-OH}$ = 10.2 Hz, H-3), 3.74 (3H, s, -COOCH_3), 3.07 (1H, ddd, $J_{6a,6b}$ = 16.8, $J_{6a,2}$ = 0.6 Hz, H-6a), 2.72 (1H, d, -OH), 1.91 (1H, dddd, $J_{6b,2}$ = 2.4 Hz, H-6b), 1.61−1.53 (4H, m, (CH_3-CH_2)$_2$-), 0.86 and 0.73 (3H, t, J = 7.8 Hz, (CH_3CH$_2$)$_2$-). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 166.3 (-COOCH$_3$), 143.1 (C-2), 128.6 (C-1), 113.0 ((R)$_2$-C-(OR)$_2$), 76.4 (C-4), 72.6 (C-5), 68.5 (C-3), 52.2 (COOCH$_3$), 29.1 and 28.3 (CH_3CH_2)$_2$-), 26.7 (C-6), 8.9 and 7.8 (CH$_3$CH$_2$)$_2$-). HRMS Calcd for $C_{13}H_{21}O_5$ (M + H): 257.1389. Found: 257.1393.

Methyl (3*S*,4*R*,5*R*)-4,5-*O*-Isopentylidene-3-*O*-(methanesulfonyl)-3,4,5-trihydroxycyclohex-1-ene-1-carboxylate (14). A solution of methanesulfonyl chloride (3.82 mL, 49.2 mmol) in 10 mL of CH_2Cl_2 was added dropwise to a stirred solution of compound **13** (6.0 g, 23.4 mmol) and Et_3N (8.48 mL, 60.9 mmol) in CH_2Cl_2 (160 mL) at 0 °C. After the addition, the reaction mixture was stirred at room temperature for 5 h. The reaction mixture was then poured into ice cold water and extracted with

CH_2Cl_2 (100 mL × 2). Combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. Chromatographic purification (EtOAc/hexanes, 1:4) of the crude compound afforded compound **14** as a pale yellow oil (7.4 g, 94%). However, pure mesylate **14** was found to be unstable (as indicated by ^1H and ^{13}C NMR), and hence, it was taken to the next step immediately. ^1H NMR ($CDCl_3$, 500 MHz): δ 6.92 (1H, br dddd-like, H-2), 5.03 (1H, ddd, $J_{3,6b}$ = 2.4, $J_{3,4}$ = 4.0, $J_{3,2}$ = 2.0 Hz, H-3), 4.73 (1H, ddd, $J_{4,5}$ = 7.5, $J_{4,2}$ = 2.0 Hz, H-4), 4.63 (1H, ddd, $J_{5,6a}$ = 2.4, $J_{5,6b}$ = 4.0 Hz, H-5), 3.76 (3H, s, -COOCH_3), 3.20 (3H, s, -OMs), 3.10 (1H, br ddd-like, $J_{6a,6b}$ = 17.0 Hz, H-6a), 1.93 (1H, br dddd-like, $J_{6b,2}$ = 3.0 Hz, H-6b), 1.65−1.53 (4H, m, (CH_3CH_2)$_2$-), 0.86 and 0.73 (3H, t, J = 7.8 Hz, (CH_3CH_2)$_2$-). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 165.5 (-COO-CH_3), 136.7 (C-2), 130.8 (C-1), 113.7 ((R)$_2$-C-(OR)$_2$), 76.3 (C-3), 75.5 (C-4), 72.7 (C-5), 52.4 (-COOCH_3), 39.3 (-OMs), 29.0 and 28.1 (CH_3CH_2)$_2$-), 27.4 (C-6), 8.9 and 7.6 (CH_3CH_2)$_2$-).

Methyl (3*S*,4*R*,5*R*)-5-(1-Ethylpropoxy)-4-hydroxy-3-(methanesulfonyloxy)cyclohex-1-ene-1-carboxylate (15). To a stirred solution of compound **14** (27 g, 80.8 mmol) in CH_2Cl_2 (600 mL) at −40 °C, triethylsilane (15.5 mL, 97.0 mmol) was added slowly followed by the dropwise addition of $TiCl_4$ (9.8 mL, 89.7 mmol) dissolved in 150 mL of CH_2Cl_2. The reaction temperature was maintained at −40 °C throughout the addition of $TiCl_4$ which was completed in 1.5 h. The reaction mixture was then poured into ice cold water (500 mL) and stirred for 10 min. The aqueous layer from the resulting biphasic layer was removed. The organic layer was washed with aqueous $NaHCO_3$ (200 mL × 2), dried over anhydrous Na_2SO_4, and all of the volatile components were removed under reduced pressure. The crude compound was purified by column chromatography (EtOAc/hexanes 1:3) to give the desired compound **15** as a pale yellow oil (23.3 g, 85%) and also the side product, chloride **16**, as a colorless oil (2.7 g, 10%). However, chloride **16** was found to be unstable, and hence, only ^1H NMR and HRMS data were obtained. Data for **15**: ^1H NMR ($CDCl_3$, 600 MHz) δ 6.67 (1H, br dddd-like, H-2), 5.33 (1H, br dddd-like, H-3), 4.30 (1H, ddd, $J_{4,3}$ = 4.2, $J_{4,5}$ = $J_{4,2}$ = 1.8 Hz, H-4), 3.74 (3H, s, -COOCH_3), 3.62 (1H, ddd, $J_{5,6a}$ = 6.0, $J_{5,6b}$ = 9.0 Hz, H-5), 3.26 (1H, m, (CH_3CH_2)$_2$-CH-O-), 3.13 (3H, s, -OMs), 2.62 (1H, br dddd-like, $J_{6a,6b}$ = 18.0, $J_{6a,3}$ = $J_{6a,2}$ = 1.2 Hz, H-6a), 2.47 (1H, dddd, $J_{6b,3}$ = $J_{6b,2}$ = 3.0 Hz, H-6b), 1.54−1.42 (4H, m, (CH_3CH_2)$_2$CH-O-), 0.89 and 0.87 (3H, t, J = 7.8 Hz, (CH_3CH_2)$_2$CH-O-). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 166.1 (-COOCH$_3$), 132.4 (C-2), 132.3 (C-1), 81.0 (CH_3-CH_2)$_2$$C$H-O-), 76.4 (C-3), 73.1 (C-5), 68.3 (C-4), 52.4 (-COO-CH_3), 39.3 (-OMs), 27.0 (C-6), 26.5 and 26.4 (CH_3CH_2)$_2$CH-O-), 9.9 and 9.7 (CH_3CH_2)$_2$CH-O-). Anal. Calcd for $C_{14}H_{24}O_7S$: C, 49.98; H, 7.19. Found: C, 49.96; H, 7.30.

Methyl (3*R*,4*R*,5*R*)-3-Chloro-5-(1-ethylpropoxy)-4-hydroxycyclohex-1-ene-1-carboxylate (16). ^1H NMR ($CDCl_3$, 500 MHz): δ 6.81 (1H, ddd, $J_{2,3}$ = 4.0, $J_{2,6a}$ = $J_{2,6b}$ = 2.0 Hz, H-2), 4.62 (1H, dddd, $J_{3,4}$ = 5.5, $J_{3,6a}$ = $J_{3,6b}$ = 2.0 Hz, H-3), 4.0 (1H, dd, $J_{4,5}$ = 2.5 Hz, H-4), 3.91 (1H, ddd, $J_{5,6a}$ = 5.5, $J_{5,6b}$ = 6.5 Hz, H-5), 3.75 (3H, s, -COOCH_3), 3.30 (1H, m, (CH_3CH_2)$_2$CH-O-), 2.58 (1H, dddd, $J_{6a,6b}$ = 18.5 Hz, H-6a), 2.52 (1H, dddd, H-6b), 2.40 (1H, br s, -OH), 1.57−1.38 (4H, m, (CH_3CH_2)$_2$CH-O-), 0.89 and 0.84 (3H, t, J = 7.8 Hz, (CH_3CH_2)$_2$CH-O-). HRMS Calcd for $C_{13}H_{22}ClO_4$ (M + H): 277.1207. Found: 277.1233.

Methyl (3*S*,4*R*,5*R*)-3,4-Epoxy-5-(1-ethylpropoxy)cyclohex-1-ene-1-carboxylate (17). To a stirred solution of chloride **16** (560 mg, 2.03 mmol) in dry methanol (25 mL) at room temperature, sodium methoxide (219.2 mg, 4.06 mmol) was added, and the stirring was continued for 18 h. The mixture was then diluted with CH_2Cl_2 (75 mL) and washed with water (50 mL × 2). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated to dryness. The crude material was purified by column chromatography (EtOAc/hexanes 1:4) to give the epoxide **17** (299 mg, 61%) as a colorless oil. ^1H NMR ($CDCl_3$, 600 MHz): δ 6.96 (1H, dd, $J_{2,3}$ = $J_{2,6b}$ = 3.6 Hz, H-2), 3.80 (1H, ddd, $J_{5,4}$ = 1.2, $J_{5,6a}$ = 6.6, $J_{5,6b}$ = 10.8 Hz, H-5), 3.71 (3H, s, -COOCH_3), 3.58 (1H, ddd, $J_{4,3}$ = 4.2, $J_{4,6a}$ = 1.8 Hz, H-4), 3.42 (1H, dd, H-3), 3.31 (1H, m, (CH_3CH_2)$_2$CH-O-), 2.80 (1H, ddd, $J_{6a,6b}$ = 16.8 Hz, H-6a), 2.14 (1H, ddd, H-6b), 1.55−1.46 (4H, m, (CH_3CH_2)$_2$-), 0.92 and 0.90 (3H, t, J = 7.8 Hz, (CH_3CH_2)$_2$-). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 166.4 (-COOCH$_3$), 134.0 (C-1), 133.8 (C-2), 81.6 ((CH_3CH_2)$_2$$C$H-O-), 73.1 (C-5), 57.5 (C-4), 52.3 (COOCH_3), 48.6 (C-3), 27.3 (C-6), 26.85 and 26.77 ((CH_3-CH_2)$_2$CH-O-), 10.0 and 9.8 ((CH_3CH_2)$_2$CH-O-). HRMS Calcd for $C_{13}H_{21}O_4$ (M+H): 241.1440. Found: 241.1444.

Methyl (3*R*,4*S*,5*R*)-3-Azido-5-(1-ethylpropoxy)-4-hydroxycyclohex-1-ene-1-carboxylate (18). To a stirred solution of compound **15** (22.5 g, 66.9 mmol) in dry DMF (600 mL) was added sodium azide (19.5 g, 301 mmol), and the resulting mixture was stirred at room temperature for 16 h. *Note: The following workup was done in four small batches, and combined crude material was taken for purification. Heating of the material at above 30 °C was avoided at anytime during the workup and purification process as a safety precaution.* The reaction mixture was diluted with ethyl acetate (200 mL) and washed with saturated NH_4Cl, water, dried over Na_2SO_4, and solvents were removed at 30 °C under reduced pressure. The combined crude material was purified by column chromatography (EtOAc/hexanes 1:3) to give compound **18** (16.9 g, 89%) as a colorless oil. ^1H NMR ($CDCl_3$, 600 MHz): δ 6.70 (1H, ddd, $J_{2,3}$ = 3.0, $J_{2,6a}$ = 1.2, $J_{2,6b}$ = 2.4 Hz, H-2), 4.21 (1H, dddd, $J_{3,4}$ = 7.2, $J_{3,6a}$ = 1.8, $J_{3,6b}$ = 2.4 Hz, H-3), 3.84 (1H, ddd, $J_{5,6a}$ = $J_{5,6b}$ = 4.2, $J_{5,4}$ = 3.0 Hz, H-5), 3.74 (3H, s, -COOCH_3), 3.72 (1H, ddd, $J_{4,-OH}$ = 7.8 Hz, H-4), 3.27 (1H, m, (CH_3CH_2)$_2$CH-O-), 2.62 (1H, dddd, $J_{6a,6b}$ = 18.6 Hz, H-6a), 2.43 (1H, dddd, H-6b), 2.36 (1H, d, -OH), 1.58−1.34 (4H, m, (CH_3CH_2)$_2$CH-O-), 0.88 and 0.80 (3H, t, J = 7.8 Hz, (CH_3CH_2)$_2$CH-O-). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 166.6 (-COOCH$_3$), 133.8 (C-2), 130.3 (C-1), 80.3 (CH_3CH_2)$_2$$C$H-O-), 73.0 (C-5), 72.4 (C-4), 61.6 (C-3), 52.3 (-COOCH_3), 28.6 (C-6), 26.7 and 26.3 (CH_3CH_2)$_2$CH-O-), 9.9 and 9.7 (CH_3CH_2)$_2$CH-O-). HRMS Calcd for $C_{13}H_{22}N_3O_4$ (M + H): 284.1610. Found: 284.1602.

Methyl (3*R*,4*S*,5*R*)-3-Azido-5-(1-ethylpropoxy)-4-(methanesulfonyloxy)cyclohex-1-ene-1-carboxylate (19). A solution of methanesulfonyl chloride (11.2 mL, 144.1 mmol) in 50 mL of CH_2Cl_2 was added dropwise to a stirred solution of compound **18** (19.4 g, 68.6 mmol) and Et_3N (24.8 mL, 178.4 mmol) in CH_2Cl_2 (550 mL) at 0 °C. After the addition, the reaction mixture was stirred at 0 °C for 1 h. *Note: The following workup was done in four small batches, and combined crude material was taken for purification. Heating of the material at above 30 °C was avoided at anytime during the workup and purification process as a safety precaution.* The reaction mixture was poured into ice cold water and extracted with CH_2Cl_2 (100 mL × 2). Combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated at 25 °C under reduced pressure. Chromatographic purification (EtOAc/hexanes, 1:3) of the combined crude material afforded compound **19** (21.1 g, 85%) as a pale yellow oil. ^1H NMR ($CDCl_3$, 600 MHz): δ 6.73 (1H, ddd, $J_{2,3}$ = 2.4, $J_{2,6a}$ = 1.2, $J_{2,6b}$ = 3.0 Hz, H-2), 4.58 (1H, dd, $J_{4,3}$ = 7.2, $J_{4,5}$ = 2.4 Hz, H-4), 4.52 (1H, dddd, $J_{3,6a}$ = 1.8, $J_{3,6b}$ = 3.0 Hz, H-3), 4.01 (1H, ddd, $J_{5,6a}$ = $J_{5,6b}$ = 4.2 Hz, H-5), 3.75 (3H, s, -COOCH_3), 3.32 (1H, m, (CH_3CH_2)$_2$CH-O-), 3.11 (3H, s, -OMs), 2.62 (1H, dddd, $J_{6a,6b}$ = 18.6 Hz, H-6a), 2.52 (1H, dddd, H-6b), 1.54−1.36 (4H, m, (CH_3CH_2)$_2$CH-O-), 0.88 and 0.80 (3H, t, J = 7.8 Hz, (CH_3CH_2)$_2$CH-O-). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 166.1 (-COOCH$_3$), 132.4 (C-2), 131.1 (C-1), 81.8 (CH_3-CH_2)$_2$$C$H-O-), 81.1 (C-4), 71.9 (C-5), 59.0 (C-3), 52.4 (-COO-CH_3), 38.6 (-OMs), 29.8 (C-6), 26.4 and 26.1 (CH_3CH_2)$_2$CH-O-), 9.8 and 9.5 (CH_3CH_2)$_2$CH-O-). HRMS Calcd for $C_{14}H_{24}N_3O_6S$ (M + H): 362.1386. Found: 362.1363. $C_{14}H_{23}N_3NaO_6S$ (M + Na): 384.1205. Found: 384.1200. $C_{14}H_{23}KN_3O_6S$ (M + K): 400.0945. Found: 400.0944.

Methyl (3*R*,4*R*,5*R*)-3,4-(Acetylepimino)-5-(1-ethyl-propoxy)-cyclohex-1-ene-1-carboxylate (21). A solution of compound **19** (22.1 g, 61.2 mmol) and triphenylphosphine (17.6 g, 67.3 mmol) in THF (1 L) was stirred at room temperature. After 2 h of

stirring, water (100 mL) was added followed by the addition of Et_3N (25.3 mL, 183.6 mmol). Stirring was continued for 14 h. Solvent was evaporated, and the residue was dissolved in CH_2Cl_2 (300 mL) and washed with water (150 mL × 2). The organic layer was dried over Na_2SO_4, filtered, and concentrated. Attempts to remove the triphenylphosphine oxide byproduct by column chromatography were unsuccessful. Hence, the crude compound was suspended in ether (400 mL) and stirred for 2 h. The separated triphenylphosphine oxide was removed by filtration. The filtrate was then concentrated and resuspended in 50 mL of ether and cooled to −10 °C for 2 h. Once again, the separated solid was removed by filtration and the filtrate was concentrated to give a 9:1 (14.3 g) mixture of aziridine compound **20** and triphenylphosphine oxide. This mixture was dissolved in CH_2Cl_2 (650 mL) and stirred at 0 °C. Et_3N (48.8 mL, 351.0 mmol) was then added followed by the dropwise addition of freshly distilled acetyl chloride (12.5 mL, 175.7 mmol). The reaction temperature was raised to room temperature, and stirring was continued for 0.5 h. The reaction mixture was diluted with ethyl acetate and washed with water (200 mL × 2). The organic layer was dried over anhydrous Na_2SO_4, concentrated, and column-purified (EtOAc/hexanes 1:4) to give compound **21** (10.2 g, 59% for two steps) as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.17 (1H, ddd, $J_{2,3}$ = 3.0, $J_{2,6a}$ = 1.8, $J_{2,6b}$ = 3.6 Hz, H-2), 4.20 (1H, ddd, $J_{5,4} = J_{5,6a}$ = 2.4, $J_{5,6b}$ = 3.0 Hz, H-5), 3.73 (3H, s, -COOCH_3), 3.23 (1H, m, $(CH_3CH_2)_2$CH-O-), 3.12-3.09 (2H, m, H-3, H-4), 2.90 (1H, ddd, $J_{6a,6b}$ = 16.8 Hz, H-6a), 2.12 (3H, s, -NHCOCH_3), 2.06 (1H, ddd, H-6b), 1.52−1.32 (4H, m, $(CH_3CH_2)_2$CH-O-), 0.88 and 0.78 (3H, t, J = 7.8 Hz, $(CH_3CH_2)_2$CH-O-). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 181.8 (-NH*C*OCH$_3$), 166.8 (-*C*OOCH$_3$), 133.4 (C-2), 130.5 (C-1), 81.3 ($CH_3CH_2)_2$*C*H-O-), 68.1 (C-5), 52.2 (-COO*C*H$_3$), 41.2 (C-3)*, 33.1 (C-4)*, 26.9 and 26.7 ($CH_3CH_2)_2$CH-O-), 26.4 (C-6), 23.5 (-NHCO*C*H$_3$), 10.1 and 9.7 (*C*H$_3$CH$_2$)$_2$CH-O-), where ∗ indicates interchangeable. HRMS Calcd for $C_{15}H_{24}NO_4$ (M + H): 282.1705. Found: 282.1704.

Methyl (3*S*,4*R*,5*R*)-4-Acetamido-3-azido-5-(1-ethylpropoxy)-cyclohex-1-ene-1-carboxylate (9). To a stirred solution of compound **21** (10.0 g, 35.6 mmol) in dry DMF (350 mL) was added sodium azide (11.6 g, 177.9 mmol) and NH_4Cl (7.5 g, 142.4 mmol). The resulting mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with water, dried over Na_2SO_4, and all of the volatile components were removed at 30 °C under reduced pressure. The crude compound was dissolved in acetic anhydride (75 mL) and stirred for 2 h. Acetic anhydride was removed carefully under reduced pressure. The resulting crude material was purified by column chromatography (EtOAc/hexanes 1:2) to give compound **9** (7.8 g, 68%) as a yellow oil which solidifies up on storing. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.69 (1H, br dd, $J_{2,3}$ = 2.5, $J_{2,6b}$ = 3.0, H-2), 5.64 (1H, d, $J_{-NH,4}$ = 7.5 Hz, -N*H*Ac), 4.77 (1H, dddd-like, $J_{3,4}$ = 8.5 Hz, H-3), 4.03 (1H, ddd, $J_{5,4}$ = 10.0, $J_{5,6a}$ = 5.5, $J_{5,6b}$ = 9.0 Hz, H-5), 3.75 (3H, s, -COOCH_3), 3.39 (1H, ddd, H-4), 3.27 (1H, m, $(CH_3CH_2)_2$C*H*-O-), 2.86 (1H, br dddd-like, $J_{6a,6b}$ = 18.0 Hz, H-6a), 2.19 (1H, dddd, $J_{6b,3}$ = 3.5, $J_{6b,2}$ = 3.0 Hz, H-6b), 2.00 (3H, s, -NHCOCH_3), 1.53−1.38 (4H, m, $(CH_3CH_2)_2$CH-O-), 0.86 and 0.85 (3H, t, J = 7.5 Hz, $(CH_3CH_2)_2$CH-O-). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 171.2 (-NH*C*OCH$_3$), 166.4 (-*C*OOH), 134.6 (C-2), 130.7 (C-1), 81.2 ($CH_3CH_2)_2$*C*H-O-), 71.0 (C-5), 59.7 (C-3), 57.8 (C-4), 52.4 (-COO*C*H$_3$), 31.5 (C-6), 26.4 and 25.9 ($CH_3CH_2)_2$CH-O-), 24.0 (-NHCO*C*H$_3$), 9.8 and 9.4 (*C*H$_3$CH$_2$)$_2$CH-O-). HRMS Calcd for $C_{15}H_{25}N_4O_4$ (M + H): 325.1876. Found: 325.1878.

Methyl (3*S*,4*R*,5*R*)-4-Acetamido-5-(1-ethylpropoxy)-3-[4-(3-hydroxypropyl)[1,2,3]triazol-1-yl]cyclohex-1-ene-1-carboxylate (23). To a stirred solution of compound **9** (100 mg, 0.31 mmol) and pent-4-yn-1-ol (37.5 μL, 0.40 mmol) in a 1:1 mixture (2 mL) of water and *tert*-butanol, copper(II) sulfate pentahydrate (5.4 mg) was added followed by the addition of freshly prepared 1 M solution (0.11 mL) of sodium ascorbate in water. The reaction went to completion after vigorous stirring at room temperature for 2 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with 10% NH_4OH (50 mL × 2) and water (50 mL × 2). The organic layer was dried over anhydrous Na_2SO_4, concentrated to dryness, and column-purified (MeOH/$CHCl_3$ 1:9) to give compound **23** (116 mg, 92%) as a yellow foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.40 (1H, s, H-5′), 6.73 (1H, br dd, $J_{2,3}$ = 2.5, $J_{2,6b}$ = 3.0 Hz, H-2), 5.93 (1H, br dddd, $J_{3,4}$ = 10.0, $J_{3,6a}$ = 2.0, $J_{3,6b}$ = 4.0 Hz, H-3), 5.92 (1H, d, $J_{-NH,4}$ = 8.0 Hz, -N*H*Ac), 4.08 (1H, ddd, $J_{5,4}$ = 10.0, $J_{5,6a}$ = 5.5, $J_{5,6b}$ = 9.5 Hz, H-5), 3.94 (1H, ddd, H-4), 3.75 (3H, s, -COOCH_3), 3.64 (2H, m, HOCH_2CH$_2$CH$_2$-), 3.31 (1H, m, $(CH_3CH_2)_2$CH-O-), 3.01 (1H, br ddd, $J_{6a,6b}$ = 18.0 Hz, H-6a), 2.80 (2H, dd, J = 7.8 Hz, HOCH$_2$CH$_2$CH_2-), 2.36 (1H, dddd, H-6b), 2.0 (br s, -O*H*), 1.90 (2H, m, HOCH$_2$CH_2CH$_2$-), 1.86 (3H, s, -NHCOCH_3), 1.54−1.40 (4H, m, $(CH_3CH_2)_2$CH-O-), 0.87 and 0.83 (3H, t, J = 7.8 Hz, $(CH_3CH_2)_2$CH-O-). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.3 (-NH*C*OCH$_3$), 166.0 (-*C*OOCH$_3$), 147.7 (C-4′), 134.0 (C-2), 131.2 (C-1), 121.4 (C-5′), 81.6 ($CH_3CH_2)_2$*C*H-O-), 72.6 (C-5), 61.0 (HO*C*H$_2$CH$_2$CH$_2$-), 60.8 (C-3), 56.0 (C-4), 52.3 (-COO*C*H$_3$), 32.1 (C-6), 31.9 (HOCH$_2$*C*H$_2$CH$_2$-), 26.2 and 25.7 ($CH_3CH_2)_2$CH-O-), 23.1 (-NHCO*C*H$_3$), 21.9 (HOCH$_2$-CH$_2$*C*H$_2$-), 9.5 and 9.2 (*C*H$_3$-CH$_2$)$_2$-CH-O-). HRMS Calcd for $C_{20}H_{33}N_4O_5$ (M+H): 409.2451. Found: 409.2452. $C_{20}H_{32}$-N_4NaO_5 (M + Na): 431.2270. Found: 431.2266.

Methyl (3*S*,4*R*,5*R*)-4-Acetamido-5-(1-ethylpropoxy)-3-[4-(1-hydroxypropyl)[1,2,3]triazol-1-yl]cyclohex-1-ene-1-carboxylate (24). Compound **24** (pale yellow foam, 114 mg, 90%) was obtained as a 1:1 mixture of diastereomers from compound **9** (100 mg, 0.31 mmol) and pent-1-yn-3-ol (34.7 μM, 0.40 mmol) using the procedure as that described to obtain **23**. Data for the mixture of diastereomers: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.53 (2H, s, H-5′), 6.74 and 6.73 (1H, br dd, $J_{2,3}$ = 2.5, $J_{2,6b}$ = 3.0 Hz, H-2), 6.0 (2H, br d, $J_{3,4}$ = 10.0 Hz, H-3), 5.96 (2H, d, $J_{-NH,4}$ = 7.5 Hz, -N*H*Ac), 4.77 (2H, dd, CH$_3$CH$_2$C*H*(OH)-), 4.15 and 4.14 (1H, ddd, $J_{5,4}$ = 10.0, $J_{5,6a}$ = 5.5, $J_{5,6b}$ = 10.0 Hz, H-5), 3.87 and 3.86 (1H, ddd, H-4), 3.75 (6H, s, -COOCH_3), 3.31 (2H, m, $(CH_3CH_2)_2$CH-O-), 3.0 (2H, br dddd-like, $J_{6a,6b}$ = 18.0 Hz, H-6a), 2.36 (2H, dddd, $J_{6b,2} = J_{6b,3}$ = 3.0 Hz, H-6b), 2.10 (br s, -O*H*), 1.92−1.80 (4H, m, CH$_3$CH_2CH(OH)-), 1.86 and 1.85 (3H, s, -NHCOCH_3), 1.54−1.40 (8H, m, $(CH_3CH_2)_2$CH-O-), 0.95 (6H, t, J = 7.5 Hz, CH_3CH$_2$CH(OH)-), 0.88 and 0.83 (6H, t, J = 7.5 Hz, $(CH_3CH_2)_2$CH-O-). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.5 and 171.4 (-NH*C*OCH$_3$), 166.13 and 166.12 (-*C*OOCH$_3$), 151.9 and 151.8 (C-4′), 134.1 and 134.0 (C-2), 131.2 and 131.1 (C-1), 122.0 and 121.8 (C-5′), 81.6 ($CH_3CH_2)_2$*C*H-O-), 72.5 (C-5), 68.2 and 68.1 (CH$_3$CH$_2$*C*H(OH)-), 60.9 and 60.8 (C-3), 56.4 (C-4), 52.38 and 52.36 (-COO*C*H$_3$), 32.2 (C-6), 30.5 and 30.4 (CH$_3$-*C*H$_2$CH(OH)-), 26.3 and 25.8 ($CH_3CH_2)_2$CH-O-), 23.3 and 23.2 (-NHCO*C*H$_3$), 10.0 and 9.9 (*C*H$_3$CH$_2$CH(OH)-), 9.57, 9.56, 9.30, and 9.29 (*C*H$_3$-CH$_2$)$_2$-CH-O-). HRMS Calcd for $C_{20}H_{33}$-N_4O_5 (M + H): 409.2451. Found: 409.2452. $C_{20}H_{32}N_4NaO_5$ (M + Na): 431.2270. Found: 431.2266.

Methyl (3*S*,4*R*,5*R*)-4-Acetamido-5-(1-ethylpropoxy)-3-[4-(1-hydroxy-1-methylethyl)[1,2,3]triazol-1-yl]cyclohex-1-ene-1-carboxylate (25). Compound **25** (385 mg, 88%) was obtained as a colorless foam from compound **9** (350 mg, 1.08 mmol) and 2-methylbut-3-yn-2-ol (0.14 mL, 1.40 mmol) using the procedure as that described to obtain **23**. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.49 (1H, s, H-5′), 6.75 (1H, br dd, $J_{2,3} = J_{2,6b}$ = 2.5 Hz, H-2), 6.01 (1H, dddd, $J_{3,4}$ = 9.5, $J_{3,6a}$ = 1.5, $J_{3,6b}$ = 3.0 Hz, H-3), 5.69 (1H, d, $J_{-NH,4}$ = 7.5 Hz, -N*H*Ac), 4.16 (1H, ddd, $J_{5,4}$ = 9.5, $J_{5,6a}$ = 5.5, $J_{5,6b}$ = 10.0 Hz, H-5), 3.86 (1H, ddd, H-4), 3.75 (3H, s, -COOCH_3), 3.31 (1H, m, $(CH_3CH_2)_2$CH-O-), 2.99 (1H, br ddd, $J_{6a,6b}$ = 18.0 Hz, H-6a), 2.37 (1H, dddd, H-6b), 1.87 (3H, s, -NHCOCH_3), 1.60 (6H, s, $(CH_3)_2$C(OH)-), 1.53-1.40 (4H, m, $(CH_3CH_2)_2$CH-O-), 0.88 and 0.82 (3H, t, J = 7.8 Hz, $(CH_3CH_2)_2$CH-O-). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.3 (-NH*C*OCH$_3$), 166.1 (-*C*OOCH$_3$), 156.2 (C-4′), 134.1 (C-2), 131.1 (C-1), 121.1 (C-5′), 81.5 ($CH_3CH_2)_2$*C*H-O-), 72.7 (C-5),

68.4 ($(CH_3)_2C$(OH)-), 60.9 (C-3), 56.2 (C-4), 52.3 (-COOCH_3), 32.2 (C-6), 30.7 and 30.4 ($(CH_3)_2$C(OH)-), 26.2 and 25.8 (CH_3CH_2)$_2$CH-O-), 23.2 (-NHCOCH_3), 9.6 and 9.2 (CH_3CH_2)$_2$-CH-O-). HRMS Calcd for $C_{20}H_{33}N_4O_5$ (M + H): 409.2451. Found: 409.2453. $C_{20}H_{32}N_4NaO_5$ (M + Na): 431.2270. Found: 431.2268.

Methyl (3*S*,4*R*,5*R*)-4-Acetamido-5-(1-ethylpropoxy)-3-(4-phenethyl[1,2,3]triazol-1-yl)cyclohex-1-ene-1-carboxylate (26). Compound **26** (370 mg, 76%) was obtained as a pale yellow foam from compound **9** (350 mg, 1.08 mmol) and 1-phenyl-but-3-yne (0.2 mL, 1.40 mmol) using the procedure as that described to obtain **23**. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.29 (2H, dd, J = 7.5 Hz, Ar), 7.21 (1H, dd, J = 7.5 Hz, Ar), 7.19 (2H, dd, J = 7.5 Hz, Ar), 7.24 (1H, s, H-5′), 6.74 (1H, br dd, $J_{2,3}$ = $J_{2,6b}$ = 2.5 Hz, H-2), 6.02 (1H, dddd, $J_{3,4}$ = 10.0, $J_{3,6a}$ = 1.5, $J_{3,6b}$ = 3.0 Hz, H-3), 5.81 (1H, d, $J_{-NH,4}$ = 7.5 Hz, -N*H*Ac), 4.18 (1H, ddd, $J_{5,4}$ = 10.0, $J_{5,6a}$ = 5.5, $J_{5,6b}$ = 10.0 Hz, H-5), 3.88 (1H, ddd, H-4), 3.79 (3H, s, -COOCH_3), 3.35 (1H, m, (CH_3CH_2)$_2$C*H*-O-), 3.08−2.98 (5H, m, H-6a, PhCH_2CH_2-), 2.38 (1H, dddd, $J_{6b,6a}$ = 18.0 Hz, H-6b), 1.87 (3H, s, -NHCOCH_3), 1.56−1.45 (4H, m, (CH_3CH_2)$_2$CH-O-), 0.91 and 0.87 (3H, t, J = 7.8 Hz, (CH_3CH_2)$_2$CH-O-). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.8 (-NH*C*OCH$_3$), 165.9 (-*C*OO-CH$_3$), 147.3 (C-4′), 141.0, 128.32, 128.30, and 126.0 (6C, Ar), 134.1 (C-2), 131.0 (C-1), 121.0 (C-5′), 81.4 (CH_3CH_2)$_2$*C*H-O-), 72.5 (C-5), 60.4 (C-3), 55.6 (C-4), 52.1 (-COO*C*H$_3$), 35.5 (PhCH_2CH_2-), 31.8 (C-6), 27.6 (PhCH_2CH_2-), 26.0 and 25.6 (CH_3CH_2)$_2$CH-O-), 23.1 (-NHCO*C*H$_3$), 9.4 and 9.2 (CH_3CH_2)$_2$-CH-O-). HRMS Calcd for $C_{25}H_{35}N_4O_4$ (M + H): 455.2658. Found: 455.2659. $C_{25}H_{34}N_4NaO_4$ (M + Na): 477.2478. Found: 477.2473.

Methyl (3*S*,4*R*,5*R*)-4-Acetamido-5-(1-ethylpropoxy)-3-[4-(1-hydroxycyclohexyl)[1,2,3]triazol-1-yl]cyclohex-1-ene-1-carboxylate (27). Compound **27** (295 mg, 85%) was obtained as a colorless foam from compound **9** (250 mg, 0.77 mmol) and 1-ethynylcyclohexanol (191.4 mg, 0.92 mmol) using the procedure as that described to obtain **23**. ^{1}H NMR (CD_3OD, 600 MHz): δ 7.84 (1H, s, H-5′), 6.75 (1H, br dd, $J_{2,3}$ = 2.5, $J_{2,6b}$ = 3.0 Hz, H-2), 5.54 (1H, dddd, $J_{3,4}$ = 9.5, $J_{3,6a}$ = 1.8, $J_{3,6b}$ = 3.5 Hz, H-3), 4.21 (1H, dd, $J_{4,5}$ = 10.0 Hz, H-4), 3.91 (1H, ddd, $J_{5,6a}$ = 5.5, $J_{5,6b}$ = 9.0 Hz, H-5), 3.77 (3H, s, -COOCH_3), 3.40 (1H, m, (CH_3CH_2)$_2$C*H*-O-), 3.01 (1H, ddd, $J_{6a,6b}$ = 18.0 Hz, H-6a), 2.42 (1H, dddd, H-6b), 2.04−1.98 (2H, m), 1.84 (3H, s, -NHCO-CH_3), 1.83−1.72 (4H, m), 1.62−1.56 (7H, m, (CH_3CH_2)$_2$CH-O-), 1.42−1.35 (1H, m), 0.92 and 0.86 (3H, t, J = 7.8 Hz, (CH_3CH_2)$_2$CH-O-). ^{13}C NMR (CD_3OD, 150 MHz): δ 173.5 (-NH*C*OCH$_3$), 167.6 (-*C*OO*C*H$_3$), 157.2 (C-4′), 134.6 (C-2), 132.9 (C-1), 121.9 (C-5′), 83.0 (CH_3CH_2)$_2$*C*H-O-), 74.1 (C-5), 70.4 (1C), 62.8 (C-3), 57.0 (C-4), 52.9 (-COO*C*H$_3$), 39.02 and 39.0 (2C), 33.1 (C-6), 27.3 and 27.0 (CH_3CH_2)$_2$CH-O-), 26.7 (1C), 23.3 and 23.2 (2C), 23.0 (-NHCO*C*H$_3$), 9.9 and 9.8 (CH_3CH_2)$_2$CH-O-). HRMS Calcd for $C_{23}H_{37}N_4O_5$ (M + H): 449.2764. Found: 449.2763. $C_{23}H_{36}N_4NaO_5$ (M + Na): 471.2583. Found: 471.2583.

Methyl (3*S*,4*R*,5*R*)-4-Acetamido-5-(1-ethylpropoxy)-3-[4-(1-hydroxycyclopentyl)[1,2,3]triazol-1-yl]cyclohex-1-ene-1-carboxylate (28). Compound **28** (360 mg, 90%) was obtained as a colorless foam from compound **9** (300 mg, 0.93 mmol) and 1-ethynylcyclopentanol (0.13 mL, 1.12 mmol) using the procedure as that described to obtain **23**. ^{1}H NMR (CD_3OD, 600 MHz): δ 7.83 (1H, s, H-5′), 6.75 (1H, br dd, $J_{2,3}$ = $J_{2,6b}$ = 2.5 Hz, H-2), 5.55 (1H, dddd, $J_{3,4}$ = 9.6, $J_{3,6a}$ = 1.8, $J_{3,6b}$ = 3.6 Hz, H-3), 4.20 (1H, dd, $J_{4,5}$ = 9.6 Hz, H-4), 3.92 (1H, ddd, $J_{5,6a}$ = 5.5 Hz, $J_{5,6b}$ = 9.6 Hz, H-5), 3.78 (3H, s, -COOCH_3), 3.39 (1H, m, (CH_3CH_2)$_2$C*H*-O-), 3.01 (1H, br ddd, $J_{6a,6b}$ = 18.0 Hz, H-6a), 2.42 (1H, dddd, H-6b), 2.11−2.04 (2H, m), 1.99−1.89 (4H, m), 1.84 (3H, s, -NHCOCH_3), 1.83−1.76 (2H, m), 1.57−1.44 (4H, m, (CH_3CH_2)$_2$CH-O-), 0.92 and 0.86 (3H, t, J = 7.8 Hz, (CH_3-CH_2)$_2$CH-O-). ^{13}C NMR (CD_3OD, 150 MHz): δ 173.6 (-NH-*C*OCH$_3$), 167.6 (-*C*OOCH$_3$), 156.1 (C-4′), 134.5 (C-2), 133.0 (C-1), 121.9 (C-5′), 83.0 (CH_3CH_2)$_2$*C*H-O-), 79.7 (1C), 74.8 (C-5), 62.7 (C-3), 57.0 (C-4), 52.9 (-COO*C*H$_3$), 41.95 and 41.92 (2C), 33.0 (C-6), 27.3 and 27.0 (CH_3CH_2)$_2$CH-O-), 24.62 and 24.61 (2C), 23.0 (-NHCO*C*H$_3$), 9.9 and 9.8 (CH_3CH_2)$_2$CH-O-). HRMS Calcd for $C_{22}H_{35}N_4O_5$ (M + H): 435.2607. Found: 435.2607. $C_{22}H_{34}N_4NaO_5$ (M + Na): 457.2427. Found: 457.2423.

Methyl (3*S*,4*R*,5*R*)-4-Acetamido-3-[4-((17α)-estra-1,3,5(10)-triene-3,17-dihydroxy-17-yl)[1,2,3]triazol-1-yl]-5-(1-ethylpropoxy)-cyclohex-1-ene-1-carboxylate (29). Compound **29** (507 mg, 76%) was obtained as a pale yellow foam from compound **9** (350 mg, 1.08 mmol) and 17α-ethynylestradiol (383.6 mL, 1.30 mmol) using the procedure as that described to obtain **23**. ^{1}H NMR (CD_3OD, 600 MHz): δ 7.77 (1H, s, H-5′), 6.99 (1H, d, J = 8.5 Hz, Ar), 6.78 (1H, br dd, $J_{2,3}$ = 2.5, $J_{2,6b}$ = 3.0 Hz, H-2), 6.50 (1H, dd, J = 8.5, 3.0 Hz, Ar), 6.46 (1H, d, J = 3.0 Hz, Ar), 5.53 (1H, dddd, $J_{3,4}$ = 9.5, $J_{3,6a}$ = 2.5, $J_{3,6b}$ = 3.0 Hz, H-3), 4.24 (1H, dd, $J_{4,5}$ = 10.0 Hz, H-4), 3.88 (1H, ddd, $J_{5,6a}$ = 5.5, $J_{5,6b}$ = 9.5 Hz, H-5), 3.78 (3H, s, -COOCH_3), 3.38 (1H, m, (CH_3CH_2)$_2$C*H*-O-), 3.02 (1H, br ddd, $J_{6a,6b}$ = 18.0 Hz, H-6a), 2.81−2.71 (2H, m), 2.46−2.39 (1H, m), 2.40 (1H, dddd, H-6b), 2.17−2.12 (1H, m), 2.10−2.05 (1H, m), 2.0−1.88 (3H, m), 1.84 (3H, s, -NHCOCH_3), 1.64−1.59 (2H, m), 1.56−1.45 (5H, m, (CH_3CH_2)$_2$CH-O-), 1.44-1.27 (3H, m), 1.03 (3H, s, -CH_3), 0.91 and 0.86 (3H, t, J = 7.8 Hz, (CH_3-CH_2)$_2$CH-O-), 0.66 (1H, ddd). ^{13}C NMR (CD_3OD, 150 MHz): δ 173.4 (-NH*C*OCH$_3$), 167.6 (-*C*OOCH$_3$), 156.0 (1C, Ar), 155.6 (C-4′), 139.0 (1C, Ar), 134.8 (C-2), 132.74 (1C, Ar), 132.73 (C-1), 127.3 (1C, Ar), 123.8 (C-5′), 116.2 and 113.8 (2C, Ar), 83.3 (1C), 83.0 (CH_3CH_2)$_2$*C*H-O-), 74.3 (C-5), 62.8 (C-3), 57.2 (C-4), 52.9 (-COO*C*H$_3$), 49.5, 48.5, 44.8, 41.2, 38.7, and 34.4 (6C), 33.3 (C-6), 30.9, 28.8, and 27.7 (3C), 27.3 and 27.0 (CH_3CH_2)$_2$CH-O-), 24.8 (1C), 23.1 (-NHCO*C*H$_3$), 15.0 (-*C*H$_3$), 9.9 and 9.8 (CH_3CH_2)$_2$-CH-O-). HRMS Calcd for $C_{35}H_{49}N_4O_6$ (M + H): 621.3652. Found: 621.3650.

(3*S*,4*R*,5*R*)-4-Acetamido-5-(1-ethylpropoxy)-3-[4-(3-hydroxypropyl)[1,2,3]triazol-1-yl]cyclohex-1-ene-1-carboxylic Acid (1). NaOH (32 mg, 0.8 mmol) was added to a solution of the methyl ester **23** (65 mg, 0.16 mmol) in a 1:1 mixture of MeOH and water (3 mL), and the reaction mixture was stirred at room temperature for 3−4 h. The pH of the reaction mixture was adjusted to 6 using 0.1 N HCl, and solvents were evaporated. The crude compound was dissolved in MeOH and passed through a pad of silica gel, and the filtrate was concentrated to give a pale yellow gum. Addition of ethyl acetate precipitated the desired compound **1** as a colorless powder (28 mg, 44%). ^{1}H NMR (CD_3OD, 600 MHz): δ 7.76 (1H, s, H-5′), 6.70 (1H, br t, H-2), 5.51 (1H, br d, $J_{3,4}$ = 9.0 Hz, H-3), 4.19 (1H, dd, $J_{4,5}$ = 9.6 Hz, H-4), 3.89 (1H, dt, $J_{5,6a}$ = 5.4, $J_{5,6b}$ = 9.0 Hz, H-5), 3.57 (2H, t, J = 6.6 Hz, HOC$H_2CH_2CH_2$-), 3.40 (1H, m, J = 6.0 Hz, (CH_3CH_2)$_2$C*H*-O-), 3.01 (1H, br dd, $J_{6a,6b}$ = 18 Hz, H-6a), 2.76 (2H, t, J = 7.8 Hz, HOCH$_2$CH$_2$CH_2-), 2.39 (1H, ddt, $J_{6b,2}$ = $J_{6b,3}$ = 3.0 Hz, H-6b), 1.87 (2H, m, J = 7.2 Hz, HOCH$_2$-CH_2CH$_2$-), 1.84 (3H, s, -NHCOCH_3), 1.56−1.45 (4H, m, (CH_3-CH_2)$_2$CH-O-), 0.92 and 0.86 (3H, t, J = 7.8 Hz, (CH_3CH_2)$_2$-CH-O-). ^{13}C NMR (CD_3OD, 150 MHz): δ 173.5 (-NH*C*OCH$_3$), 169.3 (-*C*OOH), 149.2 (C-4′), 134.2 (C-1), 133.7 (C-2), 122.6 (C-5′), 83.0 (CH_3CH_2)$_2$*C*H-O-), 74.3 (C-5), 63.0 (C-3), 62.0 (HO*C*H$_2$CH$_2$CH$_2$-), 57.1 (C-4), 33.3 (HOCH$_2$*C*H$_2$CH$_2$-), 33.3 (C-6), 27.3 and 27.0 (CH_3CH_2)$_2$CH-O-), 23.0 (-NHCO*C*H$_3$), 22.9 (HOCH$_2$CH$_2$*C*H$_2$-), 9.9 and 9.8 (*C*H$_3$-CH$_2$)$_2$-CH-O-). HRMS Calcd for $C_{19}H_{31}N_4O_5$ (M + H): 395.2294. Found: 395.2282.

(3*S*,4*R*,5*R*)-4-Acetamido-5-(1-ethyl-propoxy)-3-[4-(1-hydroxypropyl)[1,2,3]triazol-1-yl]cyclohex-1-ene-1-carboxylic Acid (2). Compound **2** (41 mg, colorless powder, 42% yield) was obtained as a 1:1 mixture of diastereomers from compound **24** (100 mg, 0.25 mmol) using the same procedure as described to obtain **1**. Data for the mixture of diastereomers. ^{1}H NMR (CD_3OD, 500 MHz): δ 7.89 and 7.87 (1H, s, H-5′), 6.76 and 6.75 (1H, br t, H-2), 5.50 (2H, br d, $J_{3,4}$ = 9.0 Hz, H-3), 4.73 and 4.72 (1H, dd, J = 7.0 Hz, CH_3CH_2C*H*(OH)-), 4.24 and 4.22 (1H, dd, $J_{4,5}$ = 10.0 Hz, H-4), 3.91 (2H, dt, $J_{5,6a}$ = 5.5, $J_{5,6b}$ = 9.5 Hz, H-5),

3.41 (2H, m, J = 5.5 Hz, $(CH_3CH_2)_2CH$-O-), 3.0 (2H, br dd, $J_{6a,6b}$ = 18 Hz, H-6a), 2.42 (2H, ddt, $J_{6b,2} = J_{6b,3}$ = 3.5 Hz, H-6b), 1.93−1.81 (4H, m, CH_3CH_2CH(OH)-), 1.86 and 1.85 (3H, s, -NHCOCH_3), 1.59−1.47 (8H, m, $(CH_3CH_2)_2$CH-O-), 0.96 and 0.95 (3H, t, J = 7.5 Hz, CH_3CH_2CH(OH)-), 0.94 and 0.88 (6H, t, J = 7.5 Hz, $(CH_3CH_2)_2$CH-O-). ^{13}C NMR (CD_3OD, 125 MHz): δ 172.2 and 172.1 (-NHCOCH$_3$), 167.5 (-COOH), 151.9 and 151.8 (C-4′), 132.9 (C-2), 132.4 and 132.3 (C-1), 121.3 and 121.2 (C-5′), 81.7 $(CH_3CH_2)_2CH$-O-), 73.0 (C-5), 67.9 and 67.8 $(CH_3CH_2CH$(OH)-), 61.8 and 61.7 (C-3), 55.8 and 55.7 (C-4), 31.9 and 31.8 (C-6), 30.2 and 30.1 $(CH_3CH_2CH$(OH)-), 26.0 and 25.7 $(CH_3CH_2)_2$CH-O-), 21.7 (-NHCOCH$_3$), 9.0 and 8.9 (CH$_3$CH$_2$CH(OH)-), 8.7, 8.6, and 8.5 (CH$_3$-CH$_2$)$_2$-CH-O-). HRMS Calcd for $C_{19}H_{31}N_4O_5$ (M + H): 395.2294. Found: 395.2284.

(3*S*,4*R*,5*R*)-4-Acetamido-5-(1-ethylpropoxy)-3-[4-(1-hydroxy-1-methylethyl)[1,2,3]triazol-1-yl]cyclohex-1-ene-1-carboxylic Acid (3). Compound **3** (78 mg, colorless powder, 40% yield) was obtained from compound **25** (200 mg, 0.49 mmol) using the same procedure as described to obtain **1**. From the ethyl acetate soluble layer, compound **32** (80 mg, colorless powder, 41% yield) was obtained by crystallization. Chromatographic purification of the mother liquor yielded compound **36** as a mixture containing 28% of **32** (25 mg, 13% yield). Similarly, the corresponding deuterated compounds **3**(**D**), **32**(**D**), and **36**(**D**) were obtained from **25** using deuterated sodium hydroxide and deuterated methanol.

Data for 3. ^{1}H NMR (CD_3OD, 600 MHz): δ 7.83 (1H, s, H-5′), 6.74 (1H, br t, H-2), 5.53 (1H, br d, $J_{3,4}$ = 8.4 Hz, H-3), 4.20 (1H, dd, $J_{4,5}$ = 9.6 Hz, H-4), 3.90 (1H, dt, $J_{5,6a}$ = 5.4, $J_{5,6b}$ = 9.0 Hz, H-5), 3.39 (1H, m, J = 5.4 Hz, $(CH_3CH_2)_2CH$-O-), 3.0 (1H, br dd, $J_{6a,6b}$ = 18 Hz, H-6a), 2.41 (1H, ddt, $J_{6b,2} = J_{6b,3}$ = 3.0 Hz, H-6b), 1.84 (3H, s, -NHCOCH_3), 1.57 (6H, s, $(CH_3)_2$-C(OH)-), 1.60-1.46 (4H, m, $(CH_3CH_2)_2$CH-O-), 0.92 and 0.86 (3H, t, J = 7.8 Hz, $(CH_3CH_2)_2$CH-O-). ^{13}C NMR (CD_3OD, 150 MHz): δ 173.6 (-NHCOCH$_3$), 168.9 (-COOH), 157.5 (C-4′), 134.1 (C-2), 133.7 (C-1), 121.2 (C-5′), 83.0 $(CH_3CH_2)_2CH$-O-), 74.2 (C-5), 69.2 $((CH_3)_2C$(OH)-), 62.9 (C-3), 57.1 (C-4), 33.0 (C-6), 30.7 $((CH_3)_2$C(OH)-), 27.3 and 27.0 $(CH_3CH_2)_2$CH-O-), 23.0 (-NHCOCH$_3$), 9.9 and 9.8 (CH$_3$CH$_2$)$_2$CH-O-). HRMS Calcd for $C_{19}H_{31}N_4O_5$ (M + H): 395.2294. Found: 395.2304.

Data for 3(D). ^{1}H NMR (CD_3OD, 600 MHz): δ 7.83 (1H, s, H-5′), 6.36 (1H, d, $J_{2,6b}$ = 2.5 Hz, H-2), 4.17 (1H, d, $J_{4,5}$ = 9.6 Hz, H-4), 3.83 (1H, ddd, $J_{5,6a}$ = 5.5 Hz, $J_{5,6b}$ = 9.6 Hz, H-5), 3.39 (1H, m, J = 6.0 Hz, $(CH_3CH_2)_2CH$-O-), 3.04 (1H, dd, $J_{6a,6b}$ = 18.0 Hz, H-6a), 2.41 (1H, ddd, H-6b), 1.84 (3H, s, -NHCOCH_3), 1.56 (6H, s, $(CH_3)_2$C(OH)-), 1.58-1.45 (4H, m, $(CH_3CH_2)_2$CH-O-), 0.92 and 0.86 (3H, t, J = 7.8 Hz, $(CH_3CH_2)_2$CH-O-). HRMS Calcd for $C_{19}H_{30}DN_4O_5$ (M + H): 396.2356. Found: 396.2356. $C_{19}H_{29}DN_4NaO_5$ (M + Na): 418.2176. Found: 418.2176. $C_{19}H_{29}DKN_4O_5$ (M + K): 434.1915. Found: 434.1923.

Data for 32. ^{1}H NMR (CD_3OD, 500 MHz): δ 7.96 (1H, s, H-5′), 6.73 (1H, d, $J_{2,1}$ = 2.5 Hz, H-2), 5.13 (1H, br dd-like, H-4), 3.86 (1H, ddd, $J_{5,4}$ = 2.5, $J_{5,6a}$ = 5.0, $J_{5,6b}$ = 2.0 Hz, H-5), 3.61 (1H, dddd, $J_{1,6a}$ = 5.0, $J_{1,6b}$ = 11.0, $J_{1,4}$ = 2.5 Hz, H-1), 3.54 (1H, m, J = 6.0 Hz, $(CH_3CH_2)_2CH$-O-), 2.25 (1H, ddd, $J_{6a,6b}$ = 14.0 Hz, H-6a), 1.96 (1H, ddd, H-6b), 1.87 (3H, s, -NHCOCH_3), 1.59 (6H, s, $(CH_3)_2$C(OH)-), 1.60-1.44 (4H, m, $(CH_3CH_2)_2$CH-O-), 0.94 and 0.89 (3H, t, J = 7.8 Hz, $(CH_3CH_2)_2$CH-O-). ^{13}C NMR (CD_3OD, 125 MHz): δ 176.4 (-COOH), 173.0 (-NHCOCH$_3$), 157.2 (C-4′), 134.1 (C-3), 122.3 (C-2), 119.7 (C-5′), 82.0 $(CH_3CH_2)_2CH$-O-), 74.8 (C-5), 69.1 $((CH_3)_2C$(OH)-), 49.0 (C-4), 38.8 (C-1), 30.72 and 30.70 $((CH_3)_2$C(OH)-), 27.8 and 27.7 $(CH_3CH_2)_2$CH-O-), 27.4 (C-6), 22.5 (-NHCOCH$_3$), 10.3 and 10.2 (CH$_3$-CH$_2$)$_2$CH-O-). HRMS Calcd for $C_{19}H_{31}N_4O_5$ (M + H): 395.2294. Found: 395.2295. $C_{19}H_{30}N_4NaO_5$ (M + Na): 417.2114. Found: 417.2119. $C_{19}H_{30}KN_4O_5$ (M + K): 433.1853. Found: 433.1855.

Data for 32(D). ^{1}H NMR (CD_3OD, 600 MHz): δ 7.97 (1H, s, H-5′), 6.72 (1H, s, H-2), 5.13 (1H, d, $J_{4,5}$ = 2.5 Hz, H-4), 3.86 (1H, ddd, $J_{5,6a}$ = 4.5, $J_{5,6b}$ = 2.0 Hz, H-5), 3.54 (1H, m, J = 6.0 Hz, $(CH_3CH_2)_2CH$-O-), 2.24 (1H, dd, $J_{6a,6b}$ = 14.0 Hz, H-6a), 1.96 (1H, ddd, H-6b), 1.87 (3H, s, -NHCOCH_3), 1.59 (6H, s, $(CH_3)_2$C(OH)-), 1.60-1.45 (4H, m, $(CH_3CH_2)_2$CH-O-), 0.93 and 0.89 (3H, t, J = 7.8 Hz, $(CH_3CH_2)_2$CH-O-). HRMS Calcd for $C_{19}H_{30}DN_4O_5$ (M + H): 396.2356. Found: 396.2356. $C_{19}H_{29}DN_4NaO_5$ (M + Na): 418.2176. Found: 418.2176. $C_{19}H_{29}DKN_4O_5$ (M + K): 434.1915. Found: 434.1912.

Data for 36 (from a Mixture Containing 28% of 32). ^{1}H NMR (CD_3OD, 600 MHz): δ 7.80 (1H, s, H-5′), 6.98 (1H, br s, H-2), 5.80 (1H, ddd, $J_{3,4}$ = 5.5, $J_{3,2}$ = 2.5, $J_{3,6a}$ = 3.0 Hz, H-3), 4.65 (1H, dd, $J_{4,5}$ = 5.5 Hz, H-4), 3.92 (1H, ddd, $J_{5,6a}$ = 3.0, $J_{5,6b}$ = 3.5 Hz, H-5), 3.40 (1H, m, J = 6.0 Hz, $(CH_3CH_2)_2CH$-O-), 2.69 (1H, dddd, $J_{6a,6b}$ = 19.0, $J_{6a,2}$ = 2.5 Hz, H-6a), 2.60 (1H, br ddd-like, H-6b), 1.79 (3H, s, -NHCOCH_3), 1.58 (6H, s, $(CH_3)_2$-C(OH)-), 1.61-1.45 (4H, m, $(CH_3CH_2)_2$CH-O-), 0.98 and 0.90 (3H, t, J = 7.8 Hz, $(CH_3CH_2)_2$CH-O-). ^{13}C NMR (CD_3OD, 150 MHz): δ 172.1 (-NHCOCH$_3$), 167.9 (-COOH), 155.6 (C-4′), 132.3 (C-1), 131.3 (C-2), 120.6 (C-5′), 80.8 $(CH_3CH_2)_2CH$-O-), 71.5 (C-5), 67.7 $((CH_3)_2C$(OH)-), 56.8 (C-3), 49.9 (C-4), 29.3 and 29.2 $((CH_3)_2$C(OH)-), 26.8 (C-6), 26.1 and 26.0 $(CH_3CH_2)_2$CH-O-), 20.9 (-NHCOCH$_3$), 8.7 and 8.5 (CH$_3$CH$_2$)$_2$CH-O-).

Data for 36(D) (from a Mixture Containing 33% of 32(D)). ^{1}H NMR (CD_3OD, 600 MHz): δ 7.81 (1H, s, H-5′), 6.99 (1H, br s, H-2), 4.64 (1H, d, $J_{4,5}$ = 5.5 Hz, H-4), 3.93 (1H, ddd-like, $J_{5,6a}$ = 3.5, $J_{5,6b}$ = 3.0 Hz, H-5), 3.44 (1H, m, J = 6.0 Hz, $(CH_3CH_2)_2$-CH-O-), 2.69 (1H, ddd, $J_{6a,6b}$ = 19.2, $J_{6a,2}$ = 2.5 Hz, H-6a), 2.60 (1H, br d, H-6b), 1.79 (3H, s, -NHCOCH_3), 1.56 (6H, s, $(CH_3)_2$C(OH)-), 1.61-1.45 (4H, m, $(CH_3CH_2)_2$CH-O-), 0.98 and 0.90 (3H, t, J = 7.8 Hz, $(CH_3CH_2)_2$CH-O-).

(3*S*,4*R*,5*R*)-4-Acetamido-5-(1-ethylpropoxy)-3-(4-phenethyl-[1,2,3]triazol-1-yl)cyclohex-1-ene-1-carboxylic Acid (4). Compound **4** (46 mg, colorless powder, 32% yield) was obtained from compound **26** (150 mg, 0.37 mmol) using the same procedure as described to obtain **1**. From the ethyl acetate soluble layer, compound **33** (44 mg, colorless powder, 30% yield) was obtained by crystallization.

Data for 4. ^{1}H NMR (CD_3OD, 600 MHz): δ 7.62 (1H, s, H-5′), 7.29−7.14 (5H, m, Ar), 6.66 (1H, br t, H-2), 5.50 (1H, br d, $J_{3,4}$ = 9.6 Hz, H-3), 4.18 (1H, dd, $J_{4,5}$ = 9.6 Hz, H-4), 3.87 (1H, dt, $J_{5,6a}$ = 5.4, $J_{5,6b}$ = 9.6 Hz, H-5), 3.38 (1H, m, J = 6.0 Hz, $(CH_3CH_2)_2CH$-O-), 3.03-2.89 (5H, m, H-6a, PhCH_2CH_2-), 2.38 (1H, ddt, $J_{6b,6a}$ = 18.0, $J_{6b,2} = J_{6b,3}$ = 3.0 Hz, H-6b), 1.83 (3H, s, -NHCOCH_3), 1.55−1.45 (4H, m, $(CH_3CH_2)_2$CH-O-), 0.91 and 0.86 (3H, t, J = 7.8 Hz, $(CH_3CH_2)_2$CH-O-). ^{13}C NMR (CD_3OD, 150 MHz): δ 173.5 (-NHCOCH$_3$), 168.8 (-COOH), 148.9 (C-4′), 142.50, 129.7, 129.6, and 127.3 (Ar), 134.3 (C-2), 133.6 (C-1), 122.7 (C-5′), 83.0 $(CH_3CH_2)_2CH$-O-), 74.3 (C-5), 62.8 (C-3), 57.0 (C-4), 36.7 (PhCH_2CH_2-), 33.1 (C-6), 28.6 (PhCH$_2$CH$_2$-), 27.3 and 27.0 $(CH_3CH_2)_2$CH-O-), 23.0 (-NHCOCH$_3$), 9.9 and 9.8 (CH$_3$CH$_2$)$_2$CH-O-). HRMS Calcd for $C_{24}H_{33}N_4O_4$ (M + H): 441.2502. Found: 441.2511.

Data for 33. ^{1}H NMR (CD_3OD, 500 MHz): δ 7.73 (1H, s, H-5′), 7.26−7.14 (5H, m, Ar), 6.68 (1H, br d, $J_{2,1}$ = 2.5 Hz, H-2), 5.07 (1H, br dd-like, H-4), 3.83 (1H, ddd, $J_{5,4}$ = 2.5, $J_{5,6a}$ = 4.5, $J_{5,6b}$ = 2.0 Hz, H-5), 3.59 (1H, dddd, $J_{1,6a}$ = 5.0, $J_{1,6b}$ = 11.0, $J_{1,4}$ = 3.0 Hz, H-1), 3.52 (1H, m, J = 6.0 Hz, $(CH_3CH_2)_2CH$-O-), 3.04-2.96 (4H, m, Ph-CH_2-CH_2-), 2.24 (1H, ddd, $J_{6a,6b}$ = 13.5 Hz, H-6a), 1.94 (1H, ddd, H-6b), 1.85 (3H, s, -NHCOCH_3), 1.60−1.43 (4H, m, $(CH_3CH_2)_2$CH-O-), 0.93 and 0.87 (3H, t, J = 7.8 Hz, $(CH_3CH_2)_2$CH-O-). ^{13}C NMR (CD_3OD, 125 MHz): δ 176.4 (-COOH), 173.0 (-NHCOCH$_3$), 148.5 (C-4′), 142.3, 129.6, 127.3 (Ar), 133.9 (C-3), 122.1 (C-2), 121.3 (C-5′), 82.1 $(CH_3CH_2)_2CH$-O-), 74.8 (C-5), 48.8 (C-4), 38.8 (C-1), 36.5 (PhCH_2-CH$_2$-), 28.3 (PhCH$_2CH_2$-), 27.8 and 27.7 $(CH_3CH_2)_2$CH-O-), 27.4 (C-6), 22.5 (-NHCOCH$_3$), 10.3 and 10.2 (CH$_3$CH$_2$)$_2$CH-O-). HRMS Calcd for $C_{24}H_{33}N_4O_4$ (M + H): 441.2502. Found: 441.2499. $C_{24}H_{32}N_4NaO_4$ (M + Na): 463.2321. Found: 463.2318.

(3*S*,4*R*,5*R*)-4-Acetamido-5-(1-ethylpropoxy)-3-[4-(1-hydroxycyclohexyl)[1,2,3]triazol-1-yl]cyclohex-1-ene-1-carboxylic Acid (5). A mixture of compound **27** (137 mg, 0.31 mmol) and trimethyltin

hydroxide (442 mg, 2.45 mmol) in 8 mL of dichloroethane (DCE) was heated at 80 °C for 5 h. Solvents were removed under reduced pressure, and the crude mass was purified by column chromatography (EtOAc/MeOH, 7:3 (v/v)). Compound **5** was obtained as a colorless foam (80 mg, 61%). ^{1}H NMR (CD_3OD, 600 MHz): δ 7.85 (1H, s, H-5′), 6.71 (1H, br t, H-2), 5.52 (1H, br d, $J_{3,4}$ = 9.0 Hz, H-3), 4.22 (1H, dd, $J_{4,5}$ = 9.6 Hz, H-4), 3.89 (1H, dt, $J_{5,6a}$ = 6.0, $J_{5,6b}$ = 9.6 Hz, H-5), 3.40 (1H, m, J = 5.4 Hz, $(CH_3CH_2)_2CH$-O-), 3.01 (1H, br dd, $J_{6a,6b}$ = 18 Hz, H-6a), 2.41 (1H, ddt, $J_{6b,2}$ = $J_{6b,3}$ = 3.5 Hz, H-6b), 2.04−1.98 (2H, m), 1.84 (3H, s, -NHCOCH_3), 1.83−1.72 (4H, m), 1.61−1.56 (7H, m, $(CH_3CH_2)_2$CH-O-), 1.42-1.36 (1H, m), 0.92 and 0.86 (3H, t, J = 7.8 Hz, $(CH_3CH_2)_2$CH-O-). ^{13}C NMR (CD_3OD, 150 MHz): δ 173.5 (-NH*C*OCH$_3$), 169.4 (-*C*OOH), 157.1 (C-4′), 134.4 (C-1), 133.5 (C-2), 121.8 (C-5′), 83.0 ($CH_3CH_2)_2$*C*H-O-), 74.3 (C-5), 70.4 (1C), 63.0 (C-3), 57.1 (C-4), 39.0 and 38.9 (2C), 33.2 (C-6), 27.3 and 27.0 ($CH_3CH_2)_2$CH-O-), 26.7 (1C), 23.3 and 23.2 (2C), 23.0 (-NHCO*C*H$_3$), 9.9 and 9.8 (*C*H$_3$CH$_2$)$_2$CH-O-). HRMS Calcd for $C_{22}H_{35}N_4O_5$ (M + H): 435.2607. Found: 435.2611.

(3*S*,4*R*,5*R*)-4-Acetamido-5-(1-ethylpropoxy)-3-[4-(1-hydroxycyclopentyl)[1,2,3]triazol-1-yl]cyclohex-1-ene-1-carboxylic Acid (6). Compound **6** (63 mg, 62%, colorless foam) was obtained from compound **28** (106 mg, 0.24 mmol) using the same procedure as described to obtain **5**. ^{1}H NMR (CD_3OD, 600 MHz): δ 7.84 (1H, s, H-5′), 6.74 (1H, br t, H-2), 5.54 (1H, br d, $J_{3,4}$ = 9.0 Hz, H-3), 4.20 (1H, dd, $J_{4,5}$ = 9.6 Hz, H-4), 3.90 (1H, dt, $J_{5,6a}$ = 5.4, $J_{5,6b}$ = 9.6 Hz, H-5), 3.39 (1H, m, J = 5.4 Hz, $(CH_3CH_2)_2$C*H*-O-), 2.99 (1H, br dd, $J_{6a,6b}$ = 18 Hz, H-6a), 2.41 (1H, ddt, $J_{6b,2}$ = $J_{6b,3}$ = 3.5 Hz, H-6b), 2.12−2.04 (2H, m), 1.98−1.89 (4H, m), 1.84 (3H, s, -NHCOCH_3), 1.82−1.77 (2H, m), 1.57−1.45 (4H, m, $(CH_3CH_2)_2$CH-O-), 0.92 and 0.86 (3H, t, J = 7.8 Hz, $(CH_3CH_2)_2$CH-O-). ^{13}C NMR (CD_3OD, 150 MHz): δ 173.5 (-NH*C*OCH$_3$), 172.9 (-*C*OOH), 156.0 (C-4′), 138.6 (C-1), 129.4 (C-2), 121.5 (C-5′), 82.9 ($CH_3CH_2)_2$*C*H-O-), 79.6 (1C), 74.7 (C-5), 63.1 (C-3), 57.3 (C-4), 41.9 and 41.8 (2C), 34.0 (C-6), 27.4 and 27.0 ($CH_3CH_2)_2$CH-O-), 24.6 (2C), 23.0 (-NHCO*C*H$_3$), 10.0 and 9.8 (*C*H$_3$CH$_2$)$_2$CH-O-). HRMS Calcd for $C_{21}H_{33}N_4O_5$ (M + H): 421.2451. Found: 421.2454.

(3*S*,4*R*,5*R*)-4-Acetamido-3-[4-((17α)-estra-1,3,5(10)-triene-3,17-dihydroxy-17-yl)[1,2,3]triazol-1-yl]-5-(1-ethylpropoxy)cyclohex-1-ene-1-carboxylic Acid (7). Compound **7** (79 mg, 72%, yellow foam) was obtained from compound **29** (115 mg, 0.18 mmol) using the same procedure as described to obtain **5**. ^{1}H NMR (CD_3OD, 600 MHz): δ 7.78 (1H, s, H-5′), 6.99 (1H, d, J = 8.4 Hz, Ar), 6.78 (1H, br t, H-2), 6.50 (1H,br dd, J = 8.4 Hz, Ar), 6.45 (1H, d, J = 2.4 Hz, Ar), 5.53 (1H, br d, $J_{3,4}$ = 9.6 Hz, H-3), 4.26 (1H, dd, $J_{4,5}$ = 10.0 Hz, H-4), 3.87 (1H, dt, $J_{5,6a}$ = 5.4, $J_{5,6b}$ = 9.6 Hz, H-5), 3.38 (1H, m, J = 6.0 Hz, $(CH_3CH_2)_2$C*H*-O-), 3.01 (1H, br dd, $J_{6a,6b}$ = 18.0 Hz, H-6a), 2.80−2.70 (2H, m), 2.46−2.41 (1H, m), 2.40 (1H, ddt, $J_{6b,2}$ = $J_{6b,3}$ = 3.5 Hz, H-6b), 2.16−1.88 (5H, m), 1.85 (3H, s, -NHCOCH_3), 1.64−1.59 (2H, m), 1.56−1.45 (5H, m, $(CH_3CH_2)_2$CH-O-), 1.44-1.27 (3H, m), 1.03 (3H, s), 0.91 and 0.86 (3H, t, J = 7.8 Hz, $(CH_3CH_2)_2$CH-O-), 0.66 (1H, m). ^{13}C NMR (CD_3OD, 150 MHz): δ 173.3 (-NH-*C*OCH$_3$), 168.8 (-*C*OOH), 156.0 (1C, Ar), 155.6 (C-4′), 139.0 (1C, Ar), 134.6 (C-2), 133.3 (C-1), 132.7 and 127.3 (2C, Ar), 123.9 (C-5′), 116.2 and113.8 (2C, Ar), 83.3 ($CH_3CH_2)_2$*C*H-O-), 83.0 (1C), 74.4 (C-5), 62.9 (C-3), 57.2 (C-4), 49.5, 48.5, 44.8, 41.2, 38.6, and 34.4 (6C), 33.4 (C-6), 30.9, 28.8, and 27.7 (3C), 27.3 and 27.0 ($CH_3CH_2)_2$CH-O), 24.8 (1C), 23.2 (-NHCO*C*H$_3$), 15.0 (1C), 9.9 and 9.8 (*C*H$_3$CH$_2$)$_2$CH-O-). HRMS Calcd for $C_{34}H_{47}N_4O_6$ (M + H): 607.3496. Found: 607.3516.

(3*S*,4*R*,5*R*)-4-Acetamido-3-azido-5-(1-ethylpropoxy)cyclohex-1-ene-1-carboxylic Acid (40). A mixture of compound **9** (100 mg, 0.31 mmol) and trimethyltin hydroxide (280 mg, 1.54 mmol) in 3 mL of dichloroethane (DCE) was heated at 80 °C for 3.5 h. Solvents were removed under reduced pressure, and the crude mass was purified by column chromatography (EtOAc/MeOH, 7:3 (v/v)). Compound **40** was obtained as a colorless foam (56 mg, 58%). ^{1}H NMR (CD_3OD, 600 MHz): δ 6.67 (1H, ddd, $J_{2,3}$ = $J_{2,6b}$ = 3.0, $J_{2,6a}$ = 0.6 Hz, H-2), 4.21 (1H, dddd, $J_{3,4}$ = 9.0, $J_{3,6a}$ = 1.8, $J_{3,6b}$ = 3.6 Hz, H-3), 3.93 (1H, dd, $J_{4,5}$ = 9.6 Hz, H-4), 3.68 (1H, ddd, $J_{5,6a}$ = 5.4, $J_{5,6b}$ = 9.0 Hz, H-5), 3.34 (1H, m, $(CH_3CH_2)_2$C*H*-O-), 2.85 (1H, dddd, $J_{6a,6b}$ = 18.0 Hz, H-6a), 2.23 (1H, dddd, H-6b), 1.99 (3H, s, -NHCOCH_3), 1.53−1.44 (4H, m, $(CH_3CH_2)_2$CH-O-), 0.90 and 0.89 (3H, t, J = 7.8 Hz, $(CH_3CH_2)_2$CH-O-). ^{13}C NMR (CD_3OD, 150 MHz): δ 173.9 (-NH*C*OCH$_3$), 169.0 (-*C*OOH), 135.3 (C-2), 132.6 (C-1), 83.0 ($CH_3CH_2)_2$*C*H-O-), 74.1 (C-5), 62.8 (C-3), 56.4 (C-4), 32.7 (C-6), 27.3 and 27.0 ($CH_3CH_2)_2$CH-O-), 23.3 (-NHCO*C*H$_3$), 10.0 and 9.8 (*C*H$_3$CH$_2$)$_2$CH-O-). HRMS Calcd for $C_{14}H_{23}N_4O_4$ (M + H): 311.1719. Found: 311.1716. $C_{14}H_{22}N_4NaO_4$ (M + Na): 333.1539. Found: 333.1535. $C_{14}H_{22}KN_4O_4$ (M + K): 349.1278. Found: 349.1288.

(3*S*,4*R*,5*R*)-4-Acetamido-3-amino-5-(1-ethylpropoxy)cyclohex-1-ene-1-carboxylic Acid (38). Hydrogen gas was bubbled through a mixture of compound **40** (20 mg, 0.06 mmol) and Lindlar's catalyst (8 mg) in 4 mL of ethanol at room temperature for 2.5 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated and purified by column chromatography (EtOAc/MeOH/H_2O, 2:3:0.1 (v/v)). Compound **38** was obtained as yellow foam (14 mg, 82% yield). ^{1}H NMR (D_2O, 600 MHz): δ 6.29 (1H, br t, H-2), 4.07 (1H, dd, $J_{4,3}$ = $J_{4,5}$ = 9.6 Hz, H-4), 4.0 (1H, br d, H-3), 3.80 (1H, dt, $J_{5,6a}$ = 5.4, $J_{5,6b}$ = 9.6 Hz, H-5), 3.48 (1H, m, J = 5.4 Hz, $(CH_3CH_2)_2$C*H*-O-), 2.89 (1H, br dd, $J_{6a,6b}$ = 17.4 Hz, H-6a), 2.33 (1H, ddt, $J_{6b,2}$ = $J_{6b,3}$ = 3.0 Hz, H-6b), 2.06 (3H, s, -NHCOCH_3), 1.59−1.44 (4H, m, $(CH_3CH_2)_2$CH-O-), 0.86 (6H, t, J = 7.8 Hz, $(CH_3CH_2)_2$CH-O-). ^{13}C NMR (D_2O, 150 MHz): δ 174.9 (-NH*C*OCH$_3$), 173.8 (-*C*OOH), 138.7 (C-1), 125.0 (C-2), 83.3 ($CH_3CH_2)_2$*C*H-O-), 73.4 (C-5), 52.7 (C-4), 52.6 (C-3), 32.3 (C-6), 25.3 ($CH_3CH_2)_2$-CH-O-), 22.3 (-NHCO*C*H$_3$), 8.7 and 8.4 (*C*H$_3$CH$_2$)$_2$CH-O-). HRMS Calcd for $C_{14}H_{25}N_2O_4$ (M + H): 285.1814. Found: 285.1813.

Methyl (3*S*,4*R*,5*R*)-4-Acetamido-3-amino-5-(1-ethylpropoxy)-cyclohex-1-ene-1-carboxylate (41). Hydrogen gas was bubbled through a stirred mixture of compound **9** (250 mg, 0.77 mmol) and Lindlar's catalyst (80 mg) in 5 mL of EtOH at room temperature for 3 h. The reaction mixture was then filtered through a pad of Celite, and the filtrate was concentrated to dryness. The crude material was purified by column chromatography (MeOH/EtOAc 1:2) to give compound **41** (140 mg, 61%) as a pale yellow oil. ^{1}H NMR (CD_3OD, 600 MHz): δ 6.78 (1H, br ddd-like, H-2), 3.82 (1H, dd, $J_{4,3}$ = 7.8, $J_{4,5}$ = 9.0 Hz, H-4), 3.75 (3H, s, -COOCH_3), 3.67 (1H, ddd, $J_{5,6a}$ = 5.4, $J_{5,6b}$ = 7.8 Hz, H-5), 3.41 (1H, br ddd-like, H-3), 3.33 (1H, m, $(CH_3CH_2)_2$-C*H*-O-), 2.73 (1H, dddd, $J_{6a,6b}$ = 18.0, $J_{6a,3}$ = 1.8, $J_{6a,2}$ = 1.2 Hz, H-6a), 2.31 (1H, dddd, $J_{6b,3}$ = $J_{6b,2}$ = 3.0 Hz, H-6b), 2.0 (3H, s, -NHCOCH_3), 1.54−1.41 (4H, m, $(CH_3CH_2)_2$CH-O-), 0.90 and 0.88 (3H, t, J = 7.8 Hz, $(CH_3CH_2)_2$CH-O-). ^{13}C NMR (CD_3OD, 150 MHz): δ 174.1 (-NH*C*OCH$_3$), 168.6 (-*C*OOCH$_3$), 140.7 (C-2), 129.3 (C-1), 82.7 ($CH_3CH_2)_2$*C*H-O-), 74.5 (C-5), 57.6 (C-4), 53.5 (C-3), 52.6 (-COO*C*H$_3$), 31.5 (C-6), 27.4 and 27.1 (CH_3-*C*H$_2$)$_2$CH-O-), 23.2 (-NHCO*C*H$_3$), 10.0 and 9.8 (*C*H$_3$CH$_2$)$_2$-CH-O-). HRMS Calcd for $C_{15}H_{27}N_2O_4$ (M + H): 299.1965. Found: 299.1963.

Methyl (3*S*,4*R*,5*R*)-4-Acetamido-3-[2,3-bis(*tert*-butoxycarbonyl)guanidino]-5-(1-ethylpropoxy)cyclohex-1-ene-1-carboxylate (42). To a stirred solution of compound **41** (100 mg, 0.34 mmol), *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea (110 mg, 0.41 mmol), and Et_3N (15 mL) in dry DMF (5 mL) at 0 °C was added $HgCl_2$ (100 mg, 0.36 mmol). After the addition, the reaction temperature was maintained at 0 °C for 1 h and then continued at room temperature for 30 min. The reaction mixture was then diluted with ethyl acetate and filtered. The filtrate was concentrated to dryness and purified by column chromatography (MeOH/$CHCl_3$ 1:9) to give compound **42** (102 mg, 56%) as a colorless foam. ^{1}H NMR (CD_3OD, 600 MHz): δ 6.81 (1H, ddd, $J_{2,3}$ = 4.2, $J_{2,6a}$ = $J_{2,6b}$ = 1.8 Hz, H-2), 4.82 (1H, dddd, $J_{3,4}$ = 4.2,

$J_{3,6a} = J_{3,6b}$ = 1.8 Hz, H-3), 4.12 (1H, dd, $J_{4,5}$ = 6.0 Hz, H-4), 3.88 (1H, ddd, $J_{5,6a} = J_{5,6b}$ = 4.2 Hz, H-5), 3.77 (3H, s, -COOCH_3), 3.29 (1H, m, $(CH_3CH_2)_2CH$-O-), 2.58 (1H, dddd, $J_{6a,6b}$ = 18.6 Hz, H-6a), 2.48 (1H, dddd, H-6b), 1.94 (3H, s, -NHCOCH_3), 1.62−1.43 (4H, m, $(CH_3CH_2)_2$CH-O-), 1.51 and 1.48 (9H, s, -$(Boc)_2$), 0.91 and 0.83 (3H, t, J = 7.8 Hz, $(CH_3CH_2)_2$CH-O-). ^{13}C NMR (CD_3OD, 150 MHz): δ 173.3 (-NH*C*OCH$_3$), 168.6 (-*C*OOCH$_3$), 164.6 and 153.7 ([-*C*OOC-$(CH_3)_3]_2$), 156.8 ((-NH)(NHBoc)*C*=NBoc), 135.7 (C-2), 130.4 (C-1), 84.5 and 80.6 ([-COO*C*$(CH_3)_3]_2$), 83.1 $(CH_3CH_2)_2$*C*H-O-), 72.8 (C-5), 52.68 (-COO*C*H$_3$)*, 52.67 (C-4)*, 49.8 (C-3), 28.7 and 28.4 ([-COOC(*C*$H_3)_3]_2$), 27.9 (C-6), 27.48 and 27.46 (CH_3-*C*$H_2)_2$CH-O-), 22.8 (-NHCO*C*H$_3$), 10.7 and 9.8 ($(CH_3CH_2)_2$-CH-O-), where ∗ indicates interchangeable. HRMS Calcd for $C_{26}H_{45}N_4O_8$ (M + H): 541.3237. Found: 541.3225.

(3*S*,4*R*,5*R*)-4-Acetamido-5-(1-ethylpropoxy)-3-guanidinocyclohex-1-ene-1-carboxylic Acid Trifluoroacetate Salt (39). 1 N KOH (2.1 mL) was added to a solution of compound **42** (70 mg, 0.13 mmol) in 7 mL of THF, and the reaction mixture was stirred at room temperature for 28 h. The pH of the reaction mixture was adjusted to 7 by bubbling CO_2, and the solvents were evaporated. The crude mass was purified by column chromatography (EtOAc/MeOH, 9:1 (v/v)), and the resulting carboxylic acid (53 mg) was then dissolved in 1:1 mixture of TFA and dichloromethane (2 mL) and stirred at room temperature for 2 h. Solvents were evaporated, and the crude mass was then triturated with dichloromethane (2 × 2 mL) to yield compound **39** as a colorless foam (29 mg, 51% yield). ^{1}H NMR (D_2O, 600 MHz): δ 6.70 (1H, dd, $J_{2,3} = J_{2,6a}$ = 2.4 Hz, H-2), 4.38 (1H, br d, $J_{3,4}$ = 9.0 Hz, H-3), 4.07 (1H, dd, $J_{4,5}$ = 9.0 Hz, H-4), 3.85 (1H, dt, $J_{5,6a}$ = 5.4, $J_{5,6b}$ = 9.0 Hz, H-5), 3.50 (1H, m, J = 5.4 Hz, $(CH_3CH_2)_2$C*H*-O-), 2.95 (1H, br dd, $J_{6a,6b}$ = 18 Hz, H-6a), 2.33 (1H, ddt, $J_{6b,2} = J_{6b,3}$ = 3.5 Hz, H-6b), 2.03 (3H, s, -NHCO-CH_3), 1.60−1.43 (4H, m, $(CH_3CH_2)_2$CH-O-), 0.87 and 0.86 (3H, t, J = 7.8 Hz, $(CH_3CH_2)_2$CH-O-). ^{13}C NMR (D_2O, 150 MHz): δ 174.6 (-NH*C*OCH$_3$), 169.3 (-*C*OOH), 162.9 (q, $J_{C,F}$ = 35.3 Hz, CF_3COO^-), 156.8 (NH_2C(NH)-NH-), 135.2 (C-2), 130.5 (C-1), 116.3 (q, $J_{C,F}$ = 290.0 Hz, *C*F_3COO^-), 83.4 (CH_3-$CH_2)_2$*C*H-O-), 73.1 (C-5), 54.4 (C-4), 53.6 (C-3), 31.2 (C-6), 25.4 and 25.3 $(CH_3CH_2)_2$CH-O-), 22.1 (-NHCO*C*H$_3$), 8.7 and 8.2 ($(CH_3CH_2)_2$CH-O-). HRMS Calcd for $C_{17}H_{27}F_3N_4O_6$ (M − CF_3COO^-): 327.2032. Found: 327.2031.

(3*S*,4*R*,5*R*)-4-Acetamido-5-(1-ethylpropoxy)-3-(4-phenethyl-[1,2,3]triazol-1-yl)cyclohex-1-ene-1-carboxylic Acid L-Arginine Salt (43). A mixture of L-arginine (4.15 mg, 0.02 mmol) and compound **4** (10.5 mg, 0.02 mmol) was stirred in dry MeOH at room temperature for 4 h. The solvents were removed, and the resulting salt **43** (14 mg, hygroscopic colorless solid) was dried under vacuum.

(3*S*,4*R*,5*R*)-4-Acetamido-3-[4-((17α)-estra-1,3,5(10)-triene-3,17-dihydroxy-17-yl)[1,2,3]triazol-1-yl]-5-(1-ethylpropoxy)cyclohex-1-ene-1-carboxylic Acid L-Arginine Salt (44). A mixture of L-arginine (2.95 mg, 0.02 mmol) and compound **7** (9.8 mg, 0.02 mmol) was stirred in dry MeOH at room temperature for 4 h. The solvents were removed, and the resulting salt **44** (11.4 mg, hygroscopic brown solid) was dried under vacuum.

(1*R*/*S*,4*R*,5*R*)-4-Acetamido-5-(1-ethylpropoxy)-3-[4-(1-hydroxy-1-methylethyl)[1,2,3]triazol-1-yl]cyclohex-2-ene-1-carboxylic Acid L-Arginine Salt (45). A mixture of L-arginine (3.5 mg, 0.02 mmol) and compound **32** (8.0 mg, 0.02 mmol) was stirred in dry MeOH at room temperature for 4 h. The solvents were removed, and the resulting salt **45** (10.1 mg, hygroscopic colorless solid) was dried under vacuum.

(1*R*/*S*,4*R*,5*R*)-4-Acetamido-5-(1-ethylpropoxy)-3-(4-phenethyl-[1,2,3]triazol-1-yl)cyclohex-2-ene-1-carboxylic Acid L-Arginine Salt (46). A mixture of L-arginine (3.6 mg, 0.02 mmol) and compound **33** (9.1 mg, 0.02 mmol) was stirred in dry MeOH at room temperature for 4 h. The solvents were removed, and the resulting salt **46** (11.2 mg, hygroscopic colorless solid) was dried under vacuum.

Acknowledgment. We are grateful to Simon Fraser University for financial support in the form of a Prototype Development Grant and M.v.I. gratefully acknowledges the financial support of the Honda Foundation, Australia.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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