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Structure-based design of 5'-substituted 1,2,3-triazolyated oseltamivir derivatives as potent influenza neuraminidase inhibitors†

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Resistant viruses containing mutant neuraminidases (NAs) with diminished drug affinity continue to emerge, and new anti-influenza agents are urgently required. Several potent inhibitors targeting the hydrophobic 150-cavity of viral NAs have been developed by modifying the antiviral drugs, oseltamivir carboxylate (OSC) and zanamivir, with hydrophobic groups. Here, we describe a different strategy for exploring novel and efficient NA inhibitors by targeting the charged amino acid residues around the entrance to the 150-cavity. We synthesized a C5-substituted OSC derivative (**1e**) with a 4'-phenyl-1,2,3-triazolyl group capable of entering the 150-cavity, and solved the crystal structure of **1e** in complex with influenza A virus N5 NA. Using the resulting structural information, we next designed and synthesized two series of OSC derivatives carrying various polar substituents at the triazolyl group of **1e** and **2e**, with **2e** being a 5'-phenyl-1,2,3-triazole regioisomer of **1e**. The NA inhibition assays demonstrated that the 2 series (**2e–n**) generally had superior activity compared with the 1 series (**1e–n**). Compound **2j**, bearing a 3-phenylamino group on the triazole ring, was the most potent inhibitor of all tested NAs including an N2 NA containing the E119V OSC-resistant mutation. Moreover, **2j** potently inhibited viral replication *in vitro*, and molecular docking studies revealed that its phenylamino group can form an additional strong hydrogen bond with residue D151 near the entrance of the 150-cavity. The design method described in this study provides useful insights into the development of novel NA inhibitors. Compound **2j** warrants further structural optimization to obtain a candidate for clinical use.

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Introduction

Despite the availability of vaccines and antiviral drugs, influenza viruses continue to pose a major threat to public health because of their ability to rapidly mutate.¹ Over the past 20 years, the emergence of human-infective H5N1 and H7N9 avian influenza viruses has received extensive public attention.^{2,3} Influenza neuraminidase (NA) is an enzyme on the viral envelope that hydrolyzes sialic acids in glycan receptors on the infected host cells to enable the release and spread of progeny virus from these cells.⁴ Several neuraminidase inhibitors (NAIs),

including oseltamivir,⁵ zanamivir,^{6,7} laninamivir octanoate,⁸ and peramivir,⁹ have been successfully developed and approved for clinical use. However, the rapid emergence of viruses resistant to these drugs significantly increased the urgent need for the next generation of NAIs.^{10–13}

On the basis of phylogenetic and X-ray crystallographic studies of various NAs, this viral enzyme is classified into two subtypes.¹⁴ In group 1 NAs (N1, N4, N5, and N8), there is a 150-cavity adjacent to the enzyme active site (*i.e.*, drug-binding site) with an entrance loop (known as the 150-loop, amino acid

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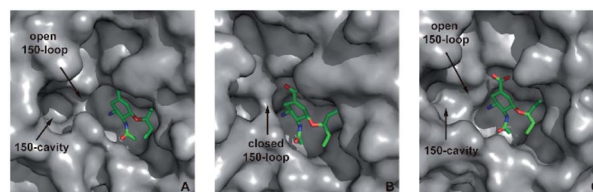


Fig. 1 Comparison of the crystal structures of representative group 1 and group 2 neuraminidases. (A) N1 virus with an open 150-loop (PDB code: 6HP0);²⁵ (B) N2 virus with a closed 150-loop (PDB code: 4GZP);²⁶ (C) N2 virus with an open 150-loop induced by oseltamivir carboxylate (PDB code: 4K1K).¹⁸



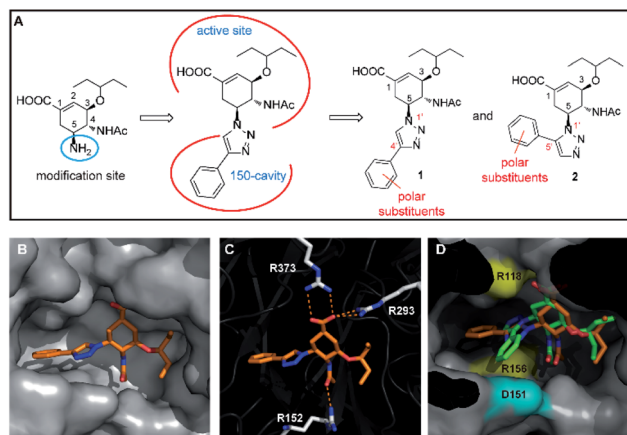


Fig. 2 Design approach and structural basis of the target neuraminidase (NA) inhibitors. (A) Design of the 1,2,3-triazolylated oseltamivir carboxylate (OSC) derivatives. (B) The X-ray crystal structure of compound **1e** in complex with the N5 NA. The 4'-phenyl-1,2,3-triazolyl group can enter the 150-cavity. (C) Key interactions (hydrogen bond interactions) of **1e** in complex with the N5 NA. The main structure (OSC scaffold) interacts with the active site in a manner similar to that of the binding pattern of OSC but without the additional strong interaction involving the phenyl group. Polar interactions are represented by dashed lines. Other interactions are not indicated. The residues involved in direct hydrogen bonds with the ligand are represented with sticks. (D) Comparison of the conformation of **1e** (orange) and **2e** (green) within the N5 NA crystal structure based on molecular docking experiments. Compound **2e** could maintain a conformation similar to that of **1e** and its phenyl group was closer to the acidic residues (cyan) and basic residues (yellow) near the entrance of the 150-cavity. The oxygen and nitrogen atoms of the compounds are colored red and blue, respectively.

residues 147–152) that usually adopts an open conformation (Fig. 1). In group 2 NAs (N2, N3, N6, N7, and N9), the 150-loop is normally closed.^{15–17} However, when the enzyme is bound to appropriate inhibitors, this flexible loop becomes open and the formerly covered 150-cavity is exposed.^{18–22} Novel NAIs that target both the active site and the 150-cavity can potentially have more extensive interactions and higher affinities toward both group 1 and 2 NAs compared with conventional inhibitors.^{23–36}

Chemical derivatization of conventional NAIs *via* a group substitution oriented toward the 150-cavity could generate such potent inhibitors. The NAIs reported thus far were mainly designed based on the hydrophobic properties of the 150-cavity and were generally obtained by attaching a hydrophobic group to the structure of oseltamivir carboxylate (OSC) or zanamivir (ZNV).^{24–36} Herein, we report a different strategy for exploring novel and efficient NA inhibitors by targeting the charged amino acid residues around the entrance to the 150-cavity. We envisioned that the designed compounds, which could simultaneously bind the active site of NA and interact with the charged residues of the 150-cavity, possess high inhibitory potency toward various NA subtypes. Therefore, two series of C5-substituted OSC derivatives carrying various polar substituents at the triazolyl group were synthesized and evaluated for their biological activity *in vitro*.

Results and discussion

Design of target NA inhibitors

Among the approved NA inhibitors, oseltamivir (OSV), with its superior pharmacokinetics and bioavailability, is the only orally available drug. Therefore, it was chosen as the template molecule for this study. Moreover, the C5 amino group of OSV is well exposed toward the open 150-cavity and can serve as a potential modification site. It has been reported that for some OSC analogs featuring a 1,2,3-triazole at the C5 position, the triazole-extended portion can enter the 150-cavity.^{24,25} Thus, our strategy involved using a triazole ring with various functional groups as the linker between the OSC scaffold and the group entering the 150-cavity. Furthermore, polar substituents were introduced into the triazole-extended portion to explore the potential for strong interactions (hydrogen bonds or salt bridges) with charged residues around the entrance to the 150-cavity (Fig. 2A).

Given these points, we first designed five C5-substituted OSC derivatives (**1a–e**) with a 4'-aryl-1,2,3-triazolyl group as these derivatives were easy for modification, and expected to be capable of entering the 150-cavity. These compounds were synthesized by copper-catalyzed azide–alkyne cycloaddition (CuAAC),³⁷ and preliminary enzymatic assays of their inhibition of N2 (H3N2) and N5 (H12N5) were performed (Table 1). Although **1c–e** exhibited moderate inhibition potency at a similar level, compound **1e** bearing a phenyl group on the triazole ring was preferred as the most promising parent molecule because of its simple structure and capacity for easy chemical modification. Subsequently, the X-ray crystal structure of compound **1e** in complex with the N5 NA were determined and can be guided for more effective modifications (Fig. 2B and C). Crystals of the complex exhibited that **1e** was indeed occupying both the catalytic site and the 150-cavity simultaneously, and its main structure (OSC scaffold) interacted with the active site in a manner similar to that of the binding pattern of OSC.³⁸ However, no additional interactions of the phenyl group were observed, except for some hydrophobic interactions, resulting in a substantial decrease in inhibitory potency. It appeared that a rational modification of **1e** would include the introduction of polar substituents such as amino, carboxyl, or alkyl-substituted N-containing groups into the benzene ring, so as to explore additional interactions with charged amino acids near the entrance of the 150-cavity (R118, E119, D151, and R156). However, with the substituent at the 4' position of the triazole, the phenyl group faced toward the base of the cavity rather than toward the entrance. Therefore, a better approach was to force the orientation of the substituents on the triazole ring toward the charged residues near the entrance of the 150-cavity, using ruthenium-catalyzed 1,3-dipolar cycloaddition (RuAAC).³⁹ This afforded the 5'-substituted isomer (**2e**) instead of a 4'-substituted triazole. Encouragingly, molecular docking studies showed that **2e** maintained a conformation similar to that of **1e** in the crystal structure of the N5 NA, allowing it to serve as the precursor molecule for the next round of inhibitor design (Fig. 2D).

Table 1 IC₅₀ values (μM) of the target compounds based on the neuraminidase inhibition assay

Compound	R	IC ₅₀ ^a (μM)		
		N5 (H12N5) ^b	N2 (H3N2) ^c	mutN2(H3N2 E119V) ^d
1a		>100	>100	— ^e
1b		>100	>100	—
1c		1.66 ± 0.25	3.80 ± 0.65	—
1d		2.85 ± 1.28	4.41 ± 1.11	—
1e		12.93 ± 1.57	11.55 ± 3.19	20.63 ± 2.97
2e		5.34 ± 0.45	3.38 ± 0.58	15.96 ± 1.95
1f		8.14 ± 1.12	2.83 ± 0.73	17.67 ± 7.05
2f		3.92 ± 0.69	1.27 ± 0.12	6.53 ± 1.31
1g		29.51 ± 8.00	11.76 ± 2.04	>100
2g		2.04 ± 0.99	0.58 ± 0.14	25.62 ± 4.41
1h		61.15 ± 23.70	21.17 ± 6.45	>100
2h		0.57 ± 0.13	0.30 ± 0.063	>100
1i		>100	>100	>100
2i		0.25 ± 0.044	0.63 ± 0.13	23.28 ± 4.38
1j		3.60 ± 0.82	1.54 ± 0.23	1.52 ± 0.26
2j		0.045 ± 0.016	0.019 ± 0.0040	0.79 ± 0.074
1k		0.52 ± 0.13	0.67 ± 0.12	4.06 ± 0.75
2k		0.41 ± 0.047	0.56 ± 0.16	3.26 ± 0.45
1l		0.74 ± 0.18	>100	>100
2l		0.14 ± 0.016	0.29 ± 0.047	9.26 ± 0.91
1m		>100	>100	>100
2m		>100	>100	>100
1n		6.95 ± 0.47	1.98 ± 0.33	>100
2n		2.43 ± 0.33	0.30 ± 0.088	26.68 ± 9.16
OSC	n/a	0.00094 ± 0.0003	0.00050 ± 0.00009	0.012 ± 0.004

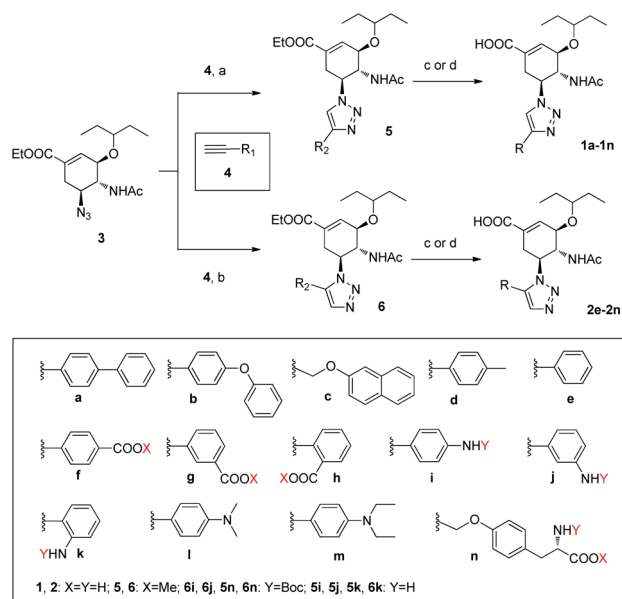
^a Concentration required to reduce neuraminidase activity to 50% of the control activity (IC₅₀). Values are shown as the mean ± SD of three experiments. ^b N5 from H12N5 A/Duck/Alberta/60/1976. ^c H3N2 (A/Moscow/10/99). ^d H3N2 (A/Moscow/10/99) with E119V mutation. ^e —: not tested.

Thus, in this study, a series of 5'-substituted 1,2,3-triazolylated OSC derivatives (2f–n) were synthesized and evaluated for their inhibitory potency toward NAs *in vitro*. Meanwhile, all the corresponding 4'-isomers (1f–n) were also tested to verify the validity of using 5'-substituted triazoles in our strategy.

Chemistry

As shown in Scheme 1, the 4'- and 5'-substituted 1,2,3-triazolylated OSC derivatives were prepared *via* coupling with azido oseltamivir (3), which can be obtained by the azidation of commercially available oseltamivir phosphate with imidazole-1-sulfonyl azide hydrochloride,²⁵ and the corresponding alkynes

(4) using either CuAAC³⁷ or RuAAC,³⁹ followed by hydrolysis or deprotection. Remarkably, although some alkynylamines require *N*-Boc protection prior to the RuAAC reaction to improve the reaction yield, no protection is required in the CuAAC reaction. All resulting triazolylated OSC derivatives were fully characterized by high-performance liquid chromatography (purity > 95%), nuclear magnetic resonance (NMR), and mass spectroscopy (MS). In most cases, the ¹H chemical shift of the triazole H for 4'-substituted 1,2,3-triazolylated OSC derivatives was over 0.4 ppm larger than the corresponding 5'-isomers, and could be served as an identifying feature of the two series.



Scheme 1 Synthesis of target compounds. Reagents and conditions: (a) sodium ascorbate, aq CuSO_4 , THF/ H_2O (3 : 1), 45 °C, 6–12 h, 68–98%; (b) $\text{Cp}^*\text{RuCl}(\text{PPh}_3)_2$, THF, 60 °C, 12–24 h, 29–89%; (c) aq NaOH, MeOH, rt, 6–12 h, 55–81%; (d) (i) aq NaOH, MeOH, rt, 6–12 h; (ii) TFA/DCM (1 : 1), rt, 3–4 h, 72–79%.

NA inhibition assay

All the 5'-substituted 1,2,3-triazolylated **OSC** derivatives, along with their corresponding 4'-isomers, were evaluated for their NA inhibition of N5 (H12N5) from group 1 and N2 (H3N2) from group 2, using 4-methylumbelliferyl-*N*-acetylneuraminic acid (MUNANA) as a fluorescent substrate.⁴⁰ To verify their inhibitory activity toward drug-resistant influenza viruses, an N2 (H3N2) NA with the E119V mutation, which confers resistance to OSV,⁴¹ was also employed in the assays. **OSC** was run in parallel as a control.

As shown in Table 1, all the 5'-substituted 1,2,3-triazolylated **OSC** derivatives exhibited higher IC_{50} values than their corresponding 4'-isomers and the parent compound (**2e**), suggesting that the 5'-substituted triazole-extended portions were well exposed toward the residues near the entrance of the 150-cavity, making it easier for these derivatives to gain additional interactions. However, all the tested compounds displayed weaker NA inhibitory activities than that of **OSC**, demonstrating that the new interactions could not compensate for the hydrogen bonds that were compromised by the insertion of the less basic triazole ring in place of the amino group at C5 of **OSC**. Generally, the tested compounds exhibited equal inhibition toward both of the NA subtypes and did not display a marked degree of selectivity. Furthermore, the decreased inhibitory activities toward mutN2 compared with wild N2 for most of the compounds suggested that interactions between the E119 residue and these compounds remained.

Structure–activity relationship analysis indicated that the substituent positions on the phenyl ring played an important role in the potency of the compounds. The compound with an

electropositive substituent at the *meta*-position of the phenyl group (**2j**) showed considerably increased potency compared with its *para*- and *ortho*-substituted isomers, whereas the compound with an electronegative substituent at the *ortho*-position (**2h**) exhibited higher inhibition activity. This phenomenon is likely largely dependent on the hydrogen-bonding strengths between polar substituents on the benzene ring of the inhibitors and the charged residues near the entrance of the 150-cavity. Furthermore, although compound **2l**, with a dimethyl-substituted derivative on the amino group of **2i**, showed slightly increased inhibition, **2m** had almost no activity. One possibility is that the conformation of **2m**, which bears a large substituent on the amino group, may be distorted within the NAs, resulting in unfavorable interactions with the binding pockets of these enzymes.

Remarkably, compound **2j** exhibited significantly higher binding potency toward N2 and N5 NA than that of other compounds, with IC_{50} values of 19 nM and 45 nM, respectively. Importantly, **2j** also showed moderate inhibition toward mutN2 in the sub-micromolar range.

Anti-influenza virus activity and cytotoxicity of **2j** and **OSC** in MDCK cells

Next, the anti-influenza virus potency of the most promising compound (**2j**) was evaluated in Madin–Darby canine kidney (MDCK) cells, and **OSC** was selected as a reference compound for testing in parallel. Three viruses, comprising H1N1 (A/Puerto Rico/8/34), H3N2 (A/Moscow/10/99), and an OSV-resistant strain of the latter containing the E119V mutation, were rescued with reverse genetics^{41,42} and used for the assays (Table 2). In agreement with its IC_{50} against the purified enzymes, compound **2j** showed lower activity than **OSC** against H1N1 and H3N2 ($\text{EC}_{50} = 11.53 \mu\text{M}$ and $7.65 \mu\text{M}$, respectively). As for the OSV-resistant influenza A virus (H3N2 E119V), both **2j** and **OSC** exhibited an EC_{50} value larger than $100 \mu\text{M}$, and at this concentration, the inhibition rates were 24.6% and 44.1%, respectively. Notably, **2j** exhibited no appreciable cytotoxicity at the highest tested concentrations ($\text{CC}_{50} > 500 \mu\text{M}$). The cell-based assay demonstrated that compound **2j**, with moderate

Table 2 Anti-influenza virus activity and cytotoxicity of **2j** and oseltamivir carboxylate (**OSC**)

Compounds	EC_{50} (μM) ^a			CC_{50} ^b
	H1N1 ^c	H3N2 ^c	H3N2 E119V ^c	
2j	11.53 ± 2.69	7.65 ± 0.70	$>100^d$	>500
OSC	0.25 ± 0.081	0.43 ± 0.080	$>100^d$	>500

^a EC_{50} refers to the concentration required to achieve 50% protection against virus-induced cytopathic effect. All the assays were performed in quadruplicate and the results are reported as the mean \pm SD.

^b CC_{50} refers to the concentration of 50% cellular toxicity. ^c A/Puerto Rico/8/1934; H3N2, A/Moscow/10/99; H3N2 E119V, and A/Moscow/10/99H3N2 background containing an E119V mutation in the neuraminidase gene. ^d At $100 \mu\text{M}$, the inhibition rates were 24.6% for **2j** and 44.1% for **OSC**, respectively.

inhibition toward influenza A viruses and low cytotoxicity *in vitro*, deserves further optimization.

Molecular docking study

Unfortunately, the crystal structure of NA in complex with **2j** could not be obtained to present the mechanism of action more intuitively. To further investigate the discrepancy in activity between the 5'-substituted 1,2,3-triazolylated **OSC** derivatives and their corresponding 4'-isomers within the 150-cavity of the NA structure, molecular docking studies of two representative compounds (**2h** and **2j**) and their 4'-isomers (**1h** and **1j**) with the crystal structure of N5 NA extracted from the complex with **1e** was conducted with GOLD software (Genetic Optimization of Ligand Docking),⁴³ and PyMOL⁴⁴ was used to visualize the results.

As shown in Fig. 3A, the triazole-extended portions of all four compounds occupied the 150-cavity while the other structural regions of the compounds interacted with the active site in a manner similar to that of the binding pattern of **OSC**. The difference was that the **2** series (**2h** and **2j**) exhibited several key interactions within the 150-cavity that were not observed for the **1** series (**1h** and **1j**). As we had speculated, hydrogen-bonding interactions were clearly observed between the amino group of **2j** and the anionic residue D151, whereas the carboxyl group of **2h** interacted with the basic amino acids R152 and R156 (Fig. 3B). The main reason for this is that the 5'-substituted triazole-extended portion formed by RuAAC was closer to the charged residues near the entrance of the 150 cavity, which resulted in improved enzyme inhibition.

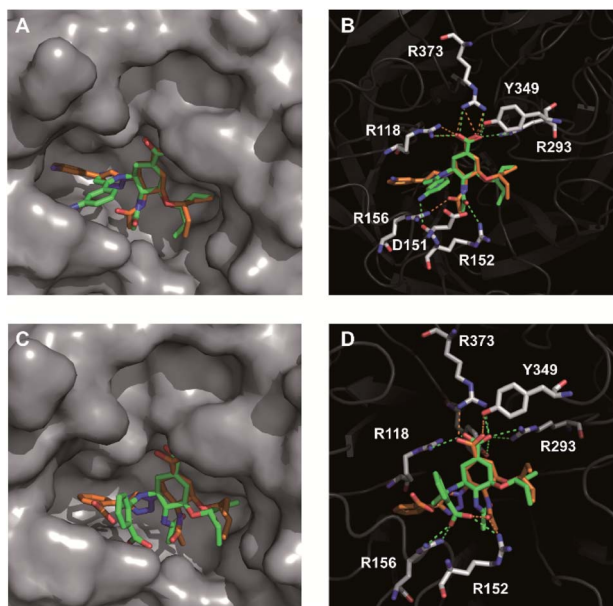


Fig. 3 Comparison of the binding modes and interactions of 5'-substituted 1,2,3-triazolylated **OSC** derivatives (green) and their 4'-isomers (orange) with N5 neuraminidase. (A and B) Overlay of N5-**2j**; (C and D) Overlay of N5-**1h** and N5-**2h**. Polar interactions are represented by dashed lines (green for the **2** series and orange for the **1** series). Other interactions are not shown. The residues involved in direct hydrogen bonds with the ligand are represented with sticks. The oxygen and nitrogen atoms of the compounds are colored red and blue, respectively.

Taken together, the molecular docking experiments were in agreement with the results of the NA inhibition assays, especially for the **OSC** analog **2j**, which bears a 3-aminophenyl group on the 5'-substituted triazole ring that can simultaneously bind the active site of NA and interact with the residues in the 150-cavity, allowing high inhibitory potency toward both NA subtypes. These findings explain the mechanism behind the remarkable ability of compound **2j** to bind and inhibit NA enzymes.

Conclusions

Since the first report of the 150-cavity in 2006, tremendous progress has been made in the development of NA inhibitors specifically targeted toward this hydrophobic cavity. In this study, we described a different strategy that designing two series of 1,2,3-triazolylated **OSC** derivatives by targeting the charged amino acid residues around the entrance to the 150-cavity. The NA inhibition assays demonstrated that the **2** series (**2e-n**) generally had better activity than the **1** series (**1e-n**), whereas the compound **2j**, bearing a 3-phenylamino group on the triazole ring, was the most potent inhibitor of all the tested NAs, including an N2 NA that contains an E119V **OSC**-resistant mutation. Moreover, **2j** exhibited acceptable potency and low cytotoxicity in cellular assays. Molecular docking studies revealed that the phenylamino group of **2j** can form an additional hydrogen bond with the D151 residue near the entrance of the NA 150-cavity. The design method described in this study provides useful insights into the development of novel NA inhibitors. The compound **2j** warrants further structural optimization to obtain a candidate for clinical use.

Experimental

General chemistry information

All chemical reagents and solvents, including **4** and **OSC**, were obtained from commercial source and used as purchased. Compound **3** was synthesized as described previously.²⁵ Silica gel (100–200 mesh) for column chromatography was purchased from Qingdao Haiyang Chemical Co., Ltd (China). Sephadex G-10 was purchased from GE Healthcare (China). IR data were recorded using a Nicolet IS5 FTIR spectrophotometer (Waltham, MA, USA). ¹H NMR (500 MHz) and ¹³C NMR (125 MHz) spectra were measured on a Bruker Advance spectrometer. ESIMS and HR-ESIMS spectra were recorded on a Bruker Apex IV FTMS spectrometer. The purity of final compounds is ≥95%, which was determined by an HPLC system (Agilent Technologies 1200) equipped with a UV detector using conditions as follows: Agilent Eclipse XDB-C18 columns (250 × 9.4 mm, 5 μm), 95 → 20% deionized water/acetonitrile gradient at 2 mL min⁻¹ over 30 min, UV detection at 254 nm wavelength.

NA inhibition assay

The N5 (H12N5, A/Duck/Alberta/60/1976), N2 (H3N2, A/Moscow/10/1999) and its **OSV**-resistant virus with E119V mutation were obtained from Sino Biological Inc. NA enzyme

activity and competitive inhibition by inhibitor (**OSC**, **1**, or **2**) were tested using MUNANA (4-methylumbelliferyl-*N*-acetylneuraminic acid) as a fluorescent substrate, according to published protocols.⁴⁰ In brief, NA activity was predetermined in an appropriate range (normally in 10–50 nM, in PBS) for fluorescence detection in the presence of MUNANA (167 μ M, in PBS). For inhibition assays, the enzyme (10 μ L) was mixed with serial 3-fold dilutions of inhibitor (10 μ L, initial concentration at 1 mM) in PBS in a 96-well microplate and incubated at 37 °C for 30 min. MUNANA (30 μ L, 167 μ M, in PBS) was then added to the mixture and fluorescence was measured on a SpectraMax M5 microplate reader (molecular devices), at excitation and emission wavelengths of 355 and 460 nm, respectively. Each assay was performed in no less than triplicates. IC₅₀ values were determined with GraphPad Prism 8.

X-ray crystallography

The N5 enzyme for X-ray crystallography was cloned, expressed, and purified as previously described.⁴⁵ The N5-**1e** complex was prepared by soaking N5 crystals in crystallization solution (0.1 M HEPES, pH 7.5, containing 12% v/v polyethylene glycol 3350) supplemented with **1e** (20 mM) at 18 °C for 3 h. The crystals were then flash-cooled at 100 K with cryoprotection. Diffraction data were collected on the beamline BL17U at Shanghai Synchrotron Radiation Facility. The data were processed and scaled with HKL2000.⁴⁶ The structure of N5 in complex with **1e** was determined by molecular replacement in Phaser using the CCP4 program suite,⁴⁷ with the known N5 structure (PDB code 3SAN)⁴⁵ as a search model. Model building and refinement were performed as described previously⁴⁵ using COOT,⁴⁸ REFMAC5,⁴⁹ and the PHENIX package.⁵⁰ The stereochemical quality of the final models was assessed with the program PROCHECK.⁵¹ All structural figures were generated using PyMOL software (<http://www.pymol.org>). Statistical support for the structures obtained is presented in ESI Table S1.† Atomic coordinates and experimental structure factors have been deposited in the PDB database under identification code 7E6Q.

Anti-influenza virus activity and cytotoxicity of **2j** and **OSC** in MDCK cells

The anti-influenza activities of compounds were measured by the EC₅₀ values, which were the concentrations of tested molecules for 50% protection of the influenza virus infection-mediated cytopathic effects (CPE).²⁹ Serial 3-fold dilutions of inhibitor (initial concentration at 200 μ M) in DMEM containing 2 mg mL⁻¹ TPCK-trypsin and 2% bovine serum albumin (BSA) were prepared and mixed with an equal volume of virus (200 TCID₅₀) in the same medium. The mixtures (100 μ L) were used to infect MDCK cells, which were grown on 96-well microplates (10⁴ cells per well). After 72 h of incubation at 37 °C in a humidified 5% CO₂ incubator, 50 μ L per well of Cell Counting Kit-8 (CCK-8, Dojindo Laboratories) reagent solution (5 μ L CCK-8 and 45 μ L media) was added. After incubation at 37 °C for additional 2 h, the absorbance was measured at 450 nm using a SpectraMax M5 fluorescence spectrophotometer (molecular

devices). The EC₅₀ values were calculated by nonlinear regressions using GraphPad Prism 8. The 50% cytotoxic concentration (CC₅₀) values of compounds to MDCK cells were determined by the procedures analogous to the EC₅₀ determination but without virus infection.

Molecular docking study

GOLD (version 5.8.1, Cambridge Crystallographic Data Centre, Cambridge, UK) is a powerful docking software which docks compounds in the active region to generate a good bonding pose depending on different scoring function and template.^{52–54} Protein N2 (PDB code: 4GZP) and N5 (extracted from N5-**1e** complex) structure was prepared using GOLD program and all hydrogens were added. The structure of native ligand present in NA proteins was removed, and the 3D structures of the studied compounds were generated using ChemBio3D Ultra 14.0 software and energy minimization using molecular mechanics (MM2). Binding site of the enzyme for docking was defined automatically by setting 6 Å around the coordinates of the native ligand. All available scoring functions in GOLD, namely CHEMPLP, ChemScore, ASP, and GoldScore were tried to redock compounds inside the NA protein and then RMSD measure was calculated to validate and find the most appropriate scoring function for docking analyses. For each compound, ten docked solutions were generated with their corresponding GOLD fitness scores. The selection of best ligand pose was done based on their interactions with amino acid residues of the target protein showing least clashes and having highest fitness score. The images were generated using PyMOL (<http://www.pymol.org>).

Synthetic protocols

General procedure for the synthesis of 5a–5n via CuAAC. To the mixture of **3** (135 mg, 0.4 mmol, 1.0 eq.) and alkyne **4** (0.5 mmol, 1.25 eq.) in 16 mL of THF/H₂O (3 : 1) was added freshly prepared 1 M solution of CuSO₄·5H₂O (24 μ L, 0.06 equiv.) in water, followed by the addition of sodium ascorbate (8 mg, 0.04 mmol, 0.1 eq.). The heterogeneous reaction mixture was stirred at room temperature for 6–12 h and monitored by TLC. After the completion, the reaction was evaporated to dryness. The crude product was purified by column chromatography, eluted with 25–75% EtOAc in petroleum ether, yielded the 4'-substituted-1,2,3-triazolylated **OSC** derivatives **5**.

General procedure for the synthesis of 6e–6n via RuAAC. To the mixture of **3** (135 mg, 0.4 mmol, 1.0 eq.) and alkyne **4** (0.6 mmol, 1.5 eq.) in 10 mL of dry THF was added Cp*RuCl(PPh₃)₂ (16 mg, 0.02 mmol, 0.05 eq.) and stirred at 60 °C for 12–24 h under a nitrogen atmosphere. The reaction mixture was monitored by TLC, and after the completion, evaporated to dryness. The crude product was purified by column chromatography, eluted with 25–75% EtOAc in petroleum ether, yielded the 5'-substituted-1,2,3-triazolylated **OSC** derivatives **6**.

General procedure A for the synthesis of 1 or 2 (reaction condition c in Scheme 1). To a solution of compound **5** or **6** (0.2 mmol) in MeOH (10 mL) was added an aqueous solution of

NaOH (1 M, 5 mL). The mixture was stirred at rt for 6–12 h, neutralized with Dowex 50 (H^+), filtered, and concentrated. The residue was dissolved in deionized water (0.5 mL) and filtered (Millipore Express 0.22 μ M filter). Purification with Sephadex G-10 (eluent: 0.1% TFA in H_2O) yielded **1** or **2** as a white foam.

General procedure B for the synthesis of 1 or 2 (reaction condition d in Scheme 1). To a solution of compound **5** or **6** (0.2 mmol) in MeOH (10 mL) was added an aqueous solution of NaOH (1 M, 5 mL). The mixture was stirred at rt for 6–12 h, neutralized with Dowex 50 (H^+), filtered, and concentrated. The residue was dissolved in CH_2Cl_2 /TFA (10 mL, 1 : 1, v/v). The mixture was stirred at rt for 3–4 h and concentrated. The resulting residue was dissolved in deionized water (0.5 mL) and filtered (Millipore Express 0.22 μ M filter). Purification with Sephadex G-10 (eluent: 0.1% TFA in H_2O) yielded **1** or **2** as a white foam.

Ethyl (3R,4R,5S)-4-acetamido-5-[4-(1,1'-biphenyl-4-yl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (5a). Using the general procedure for CuAAC, compound **5a** was afforded from **3** (135 mg, 0.4 mmol, 1.0 eq.) and 4-biphenylacetylene (89 mg, 0.5 mmol, 1.25 eq.) as a white foam (188 mg, 91%). 1H NMR (500 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.91–7.35 (m, 9H), 6.92 (s, 1H), 6.16 (s, 1H), 5.50 (dd, $J = 17.4, 11.1$ Hz, 1H), 4.77 (d, $J = 8.6$ Hz, 1H), 4.25–4.19 (m, 2H), 4.09–4.07 (m, 1H), 3.43–3.39 (m, 1H), 3.13–3.03 (m, 2H), 1.79 (s, 3H), 1.60–1.50 (m, 4H), 1.29 (t, $J = 7.1$ Hz, 3H), 0.96 (t, $J = 7.4$ Hz, 3H), 0.90 (t, $J = 7.4$ Hz, 3H). ESIMS calcd for $C_{30}H_{37}N_4O_4$ ($M + H$) $^+$: 517.3. Found: 517.1.

Ethyl (3R,4R,5S)-4-acetamido-3-(pentan-3-yloxy)-5-[4-(4-phenoxyphenyl)-1H-1,2,3-triazol-1-yl]cyclohex-1-ene-1-carboxylate (5b). Using the general procedure for CuAAC, compound **5b** was afforded from **3** (135 mg, 0.4 mmol, 1.0 eq.) and 1-(4-ethynylphenoxy)benzene (97 mg, 0.5 mmol, 1.25 eq.) as a white foam (183 mg, 86%). 1H NMR (500 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.77 (d, $J = 8.7$ Hz, 1H), 7.36 (dd, $J = 8.5, 7.5$ Hz, 2H), 7.13 (t, $J = 7.4$ Hz, 3H), 7.06–7.04 (m, 3H), 6.91 (s, 1H), 6.07 (s, 1H), 5.45 (td, $J = 11.1, 6.1$ Hz, 1H), 4.74 (d, $J = 8.8$ Hz, 1H), 4.23 (q, $J = 7.1$ Hz, 2H), 3.42–3.37 (m, 1H), 3.11–3.00 (m, 2H), 1.77 (s, 3H), 1.58–1.49 (m, 4H), 1.30 (t, $J = 7.1$ Hz, 3H), 0.94 (t, $J = 7.4$ Hz, 3H), 0.89 (t, $J = 7.4$ Hz, 3H). ESIMS calcd for $C_{30}H_{37}N_4O_5$ ($M + H$) $^+$: 533.3. Found: 533.2.

Ethyl (3R,4R,5S)-4-acetamido-5-[4-(2-naphthoxy)methyl-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (5c). Using the general procedure for CuAAC, compound **5c** was afforded from **3** (135 mg, 0.4 mmol, 1.0 eq.) and naphth-2-yloxymethylacetylene (92 mg, 0.5 mmol, 1.25 eq.) as a white foam (183 mg, 88%). 1H NMR (500 MHz, $CDCl_3$) δ 7.79 (s, 1H), 7.76–7.72 (m, 2H), 7.71 (d, $J = 3.3$ Hz, 1H), 7.43 (t, $J = 7.2$ Hz, 1H), 7.33 (t, $J = 7.2$ Hz, 1H), 7.23 (d, $J = 1.8$ Hz, 1H), 7.14 (dd, $J = 8.9, 2.3$ Hz, 1H), 6.86 (s, 1H), 6.49 (d, $J = 6.7$ Hz, 1H), 5.41 (dd, $J = 17.3, 10.9$ Hz, 1H), 5.32–5.22 (m, 2H), 4.70 (d, $J = 8.4$ Hz, 1H), 4.19 (q, $J = 7.1$ Hz, 2H), 3.99 (dd, $J = 19.5, 8.7$ Hz, 1H), 3.35 (p, $J = 5.6$ Hz, 1H), 3.09–2.94 (m, 2H), 1.63 (s, 3H), 1.54–1.45 (m, 4H), 1.27 (t, $J = 7.1$ Hz, 3H), 0.90 (t, $J = 7.4$ Hz, 3H), 0.85 (t, $J = 7.4$ Hz, 3H). HRESIMS calcd for $C_{29}H_{37}N_4O_5$ ($M + H$) $^+$: 521.2759. Found: 521.2770.

Ethyl (3R,4R,5S)-4-acetamido-5-[4-(4-methylphenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (5d). Using the general procedure for CuAAC, compound **5d** was afforded from **3** (135 mg, 0.4 mmol, 1.0 eq.) and 4-ethynyltoluene (58 mg, 0.5 mmol, 1.25 eq.) as a white foam (169 mg, 93%). 1H NMR (500 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.64 (d, $J = 7.7$ Hz, 2H), 7.16 (d, $J = 7.6$ Hz, 2H), 7.02 (d, $J = 6.5$ Hz, 1H), 6.89 (s, 1H), 5.34 (t, $J = 13.5$ Hz, 1H), 4.65 (d, $J = 7.6$ Hz, 1H), 4.24–4.19 (m, 3H), 3.46–3.36 (m, 1H), 3.10–2.96 (m, 2H), 2.34 (s, 3H), 1.66 (s, 3H), 1.57–1.51 (m, 4H), 1.28 (t, $J = 7.1$ Hz, 3H), 0.93 (t, $J = 7.3$ Hz, 3H), 0.88 (t, $J = 7.3$ Hz, 3H). HRESIMS calcd for $C_{25}H_{35}N_4O_4$ ($M + H$) $^+$: 455.2653. Found: 455.2651.

Ethyl (3R,4R,5S)-4-acetamido-3-(pentan-3-yloxy)-5-(4-phenyl-1H-1,2,3-triazol-1-yl)cyclohex-1-ene-1-carboxylate (5e). Using the general procedure for CuAAC, compound **5e** was afforded from **3** (135 mg, 0.4 mmol, 1.0 eq.) and phenylacetylene (52 mg, 0.5 mmol, 1.25 eq.) as a white foam (173 mg, 98%). 1H NMR (500 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.78 (d, $J = 7.4$ Hz), 7.38 (t, $J = 7.5$ Hz, 2H), 7.31 (t, $J = 7.3$ Hz, 1H), 6.90 (s, 1H), 6.72 (d, $J = 7.1$ Hz, 1H), 5.42–5.33 (m, 1H), 4.68 (d, $J = 8.4$ Hz, 1H), 4.22 (dd, $J = 14.2, 7.1$ Hz, 2H), 4.19–4.11 (m, 1H), 3.41 (p, $J = 5.5$ Hz, 1H), 3.11–2.97 (m, 2H), 1.69 (s, 3H), 1.56–1.52 (m, 4H), 1.29 (t, $J = 7.1$ Hz, 3H), 0.94 (d, $J = 7.4$ Hz, 3H), 0.88 (d, $J = 7.4$ Hz, 3H). HRESIMS calcd for $C_{24}H_{33}N_4O_4$ ($M + H$) $^+$: 441.2496. Found: 441.2493.

Ethyl (3R,4R,5S)-4-acetamido-5-[4-(4-methoxycarbonylphenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (5f). Using the general procedure for CuAAC, compound **5f** was afforded from **3** (135 mg, 0.4 mmol, 1.0 eq.) and 4-ethynylbenzoic acid methyl ester (80 mg, 0.5 mmol, 1.25 eq.) as a white foam (171 mg, 86%). 1H NMR (500 MHz, $CDCl_3$) δ 8.06 (d, $J = 8.1$ Hz, 2H), 8.02 (s, 1H), 7.86 (d, $J = 8.2$ Hz, 2H), 6.91 (s, 1H), 6.35 (d, $J = 4.5$ Hz, 1H), 5.45 (dd, $J = 16.9, 11.1$ Hz, 1H), 4.71 (d, $J = 8.3$ Hz, 1H), 4.23 (q, $J = 7.1$ Hz, 2H), 4.13–4.07 (m, 1H), 3.92 (s, 3H), 3.43–3.37 (m, 1H), 3.12–2.99 (m, 2H), 1.73 (s, 3H), 1.55–1.51 (m, 4H), 1.29 (t, $J = 7.1$ Hz, 3H), 0.93 (t, $J = 7.4$ Hz, 3H), 0.88 (t, $J = 7.3$ Hz, 3H). HRESIMS calcd for $C_{26}H_{35}N_4O_6$ ($M + H$) $^+$: 499.2551. Found: 499.2544.

Ethyl (3R,4R,5S)-4-acetamido-5-[4-(3-methoxycarbonylphenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (5g). Using the general procedure for CuAAC, compound **5g** was afforded from **3** (135 mg, 0.4 mmol, 1.0 eq.) and 3-ethynylbenzoic acid methyl ester (80 mg, 0.5 mmol, 1.25 eq.) as a white foam (157 mg, 79%). 1H NMR (500 MHz, $CDCl_3$) δ 8.38 (s, 1H), 8.11 (s, 1H), 7.96 (t, $J = 7.7$ Hz, 2H), 7.41 (t, $J = 7.8$ Hz, 1H), 6.96 (d, $J = 8.0$ Hz, 1H), 6.89 (s, 1H), 5.39 (td, $J = 11.1, 6.1$ Hz, 1H), 4.65 (d, $J = 8.3$ Hz, 1H), 4.29–4.17 (m, 3H), 3.86 (s, 3H), 3.41 (t, $J = 8.4$ Hz, 1H), 3.14–2.97 (m, 2H), 1.69 (s, 3H), 1.56–1.48 (m, 4H), 1.27 (t, $J = 7.1$ Hz, 3H), 0.90 (t, $J = 7.4$ Hz, 3H), 0.85 (t, $J = 7.4$ Hz, 3H). HRESIMS calcd for $C_{26}H_{35}N_4O_6$ ($M + H$) $^+$: 499.2551. Found: 499.2551.

Ethyl (3R,4R,5S)-4-acetamido-5-[4-(2-methoxycarbonylphenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (5h). Using the general procedure for CuAAC, compound **5h** was afforded from **3** (135 mg, 0.4 mmol, 1.0 eq.) and 2-ethynylbenzoic acid methyl ester (80 mg, 0.5 mmol, 1.25 eq.) as

compound **6g** was afforded from **3** (135 mg, 0.4 mmol, 1.0 eq.) and 3-ethynylbenzoic acid methyl ester (96 mg, 0.6 mmol, 1.5 eq.) as a white foam (76 mg, 38%). $^1\text{H NMR}$ (500 MHz, CDCl_3) δ 8.14 (t, $J = 4.4$ Hz, 1H), 8.01 (s, 1H), 7.72 (s, 1H), 7.60 (d, $J = 4.9$ Hz, 2H), 6.84 (s, 1H), 5.89 (d, $J = 6.0$ Hz, 1H), 5.52 (dt, $J = 17.3, 8.5$ Hz, 1H), 4.96 (d, $J = 8.1$ Hz, 1H), 4.19 (q, $J = 6.5$ Hz, 2H), 4.08 (d, $J = 6.7$ Hz, 1H), 3.94 (s, 3H), 3.29 (p, $J = 5.7$ Hz, 1H), 2.96–2.85 (m, 2H), 1.74 (s, 3H), 1.52–1.42 (m, 4H), 1.27 (t, $J = 7.1$ Hz, 3H), 0.90 (t, $J = 7.4$ Hz, 3H), 0.85 (t, $J = 7.4$ Hz, 3H). HRESIMS calcd for $\text{C}_{26}\text{H}_{35}\text{N}_4\text{O}_6$ ($\text{M} + \text{H}$) $^+$: 499.2551. Found: 499.2557.

Ethyl (3R,4R,5S)-4-acetamido-5-[5-(2-methoxycarbonylphenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (6h). Using the general procedure for RuAAC, compound **6h** was afforded from **3** (135 mg, 0.4 mmol, 1.0 eq.) and 2-ethynylbenzoic acid methyl ester (96 mg, 0.6 mmol, 1.5 eq.) as a white foam (58 mg, 29%). $^1\text{H NMR}$ (500 MHz, CDCl_3) δ 7.79–7.77 (m, 2H), 7.44 (t, $J = 7.4$ Hz, 1H), 7.11 (d, $J = 8.4$ Hz, 1H), 7.00 (t, $J = 7.4$ Hz, 1H), 6.88 (s, 1H), 5.67 (d, $J = 5.0$ Hz, 1H), 5.47 (dd, $J = 18.2, 9.0$ Hz, 1H), 4.80 (d, $J = 8.4$ Hz, 1H), 4.22 (q, $J = 7.2$ Hz, 2H), 3.86 (dd, $J = 18.2, 8.4$ Hz, 1H), 3.37–3.34 (m, 1H), 3.14 (d, $J = 13.5$ Hz, 2H), 1.69 (s, 3H), 1.56–1.44 (m, 4H), 1.36 (t, $J = 7.2$ Hz, 3H), 0.92 (t, $J = 7.4$ Hz, 3H), 0.86 (t, $J = 7.4$ Hz, 3H). HRESIMS calcd for $\text{C}_{26}\text{H}_{35}\text{N}_4\text{O}_6$ ($\text{M} + \text{H}$) $^+$: 499.2557. Found: 499.2555.

Ethyl (3R,4R,5S)-4-acetamido-3-(pentan-3-yloxy)-5-[5-(4-tert-butoxycarbonylamino)phenyl]-1H-1,2,3-triazol-1-yl]cyclohex-1-ene-1-carboxylate (6i). Using the general procedure for RuAAC, compound **6i** was afforded from **3** (135 mg, 0.4 mmol, 1.0 eq.) and *tert*-butyl (4-ethynylphenyl)carbamate (130 mg, 0.6 mmol, 1.5 eq.) as a white foam (136 mg, 61%). $^1\text{H NMR}$ (500 MHz, CDCl_3) δ 7.66 (s, 1H), 7.50 (d, $J = 8.4$ Hz, 2H), 7.29 (d, $J = 8.4$ Hz, 2H), 6.84 (s, 1H), 6.63 (s, 1H), 5.76 (d, $J = 6.4$ Hz, 1H), 5.53–5.49 (m, 1H), 4.99 (dd, $J = 9.2, 2.2$ Hz, 1H), 4.25–4.16 (m, 2H), 3.98–3.95 (m, 1H), 3.31 (dt, $J = 11.5, 5.7$ Hz, 1H), 3.02–2.98 (m, 2H), 1.73 (s, 3H), 1.49 (s, 9H), 1.53–1.43 (m, 13H), 1.27 (t, $J = 7.1$ Hz, 3H), 0.91 (d, $J = 7.4$ Hz, 3H), 0.86 (t, $J = 7.4$ Hz, 3H). ESIMS calcd for $\text{C}_{29}\text{H}_{42}\text{N}_5\text{O}_6$ ($\text{M} + \text{H}$) $^+$: 556.3. Found: 556.2.

Ethyl (3R,4R,5S)-4-acetamido-3-(pentan-3-yloxy)-5-[5-(3-tert-butoxycarbonylamino)phenyl]-1H-1,2,3-triazol-1-yl]cyclohex-1-ene-1-carboxylate (6j). Using the general procedure for RuAAC, compound **6j** was afforded from **3** (135 mg, 0.4 mmol, 1.0 eq.) and *tert*-butyl (3-ethynylphenyl)carbamate (130 mg, 0.6 mmol, 1.5 eq.) as a white foam (73 mg, 33%). $^1\text{H NMR}$ (500 MHz, CDCl_3) δ 7.64 (s, 1H), 7.58 (s, 1H), 7.37 (d, $J = 4.9$ Hz, 2H), 7.02 (d, $J = 4.1$ Hz, 2H), 6.83 (s, 1H), 6.39 (d, $J = 3.7$ Hz, 1H), 5.36 (td, $J = 11.0, 5.8$ Hz, 1H), 4.83 (d, $J = 8.1$ Hz, 1H), 4.18–4.15 (m, 3H), 3.31 (t, $J = 5.6$ Hz, 1H), 2.88 (td, $J = 17.5, 8.1$ Hz, 2H), 1.77 (s, 3H), 1.49 (s, 9H), 1.49–1.44 (m, 4H), 1.26–1.23 (m, 3H), 0.89 (d, $J = 7.4$ Hz, 3H), 0.85 (t, $J = 7.4$ Hz, 3H). HRESIMS calcd for $\text{C}_{29}\text{H}_{42}\text{N}_5\text{O}_6$ ($\text{M} + \text{H}$) $^+$: 556.3130. Found: 556.3140.

Ethyl (3R,4R,5S)-4-acetamido-3-(pentan-3-yloxy)-5-[5-(2-aminophenyl)-1H-1,2,3-triazol-1-yl]cyclohex-1-ene-1-carboxylate (6k). Using the general procedure for RuAAC, compound **6k** was afforded from **3** (135 mg, 0.4 mmol, 1.0 eq.) and 2-ethynylaniline (71 mg, 0.6 mmol, 1.5 eq.) as a white foam (84 mg, 46%). $^1\text{H NMR}$ (500 MHz, CDCl_3) δ 7.77 (s, 1H), 7.33 (t, $J = 8.5$ Hz, 1H), 7.06–6.96 (m, 3H), 6.82 (s, 1H), 5.21 (dd, $J = 15.3, 10.0$ Hz, 1H), 4.85 (d, $J = 6.4$ Hz, 1H), 4.19 (q, $J = 7.1$ Hz, 2H), 4.07 (s, 1H),

3.32–3.27 (m, 1H), 2.98–2.82 (m, 2H), 1.79 (s, 3H), 1.52–1.45 (m, 4H), 1.27 (t, $J = 7.1$ Hz, 3H), 0.90 (d, $J = 7.4$ Hz, 3H), 0.85 (t, $J = 7.4$ Hz, 3H). HRESIMS calcd for $\text{C}_{24}\text{H}_{34}\text{N}_5\text{O}_4$ ($\text{M} + \text{H}$) $^+$: 456.2611. Found: 456.2609.

Ethyl (3R,4R,5S)-4-acetamido-5-[5-(4-dimethylaminophenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (6l). Using the general procedure for RuAAC, compound **6l** was afforded from **3** (135 mg, 0.4 mmol, 1.0 eq.) and 4-ethynyl-*N,N*-dimethylaniline (87 mg, 0.6 mmol, 1.5 eq.) as a white foam (114 mg, 59%). $^1\text{H NMR}$ (500 MHz, CDCl_3) δ 7.61 (s, 1H), 7.24 (d, $J = 8.8$ Hz, 2H), 6.84–6.80 (m, 3H), 5.73 (d, $J = 6.4$ Hz, 1H), 5.58–5.52 (m, 1H), 5.06 (d, $J = 8.9$ Hz, 1H), 4.15–4.25 (m, 2H), 4.02–3.97 (m, 1H), 3.32–3.30 (m, 1H), 3.01 (s, 4H), 2.83–2.80 (m, 2H), 1.74 (s, 3H), 1.53–1.45 (m, 4H), 1.27 (t, $J = 7.1$ Hz, 3H), 0.91 (t, $J = 7.4$ Hz, 3H), 0.87 (t, $J = 7.4$ Hz, 3H). ESIMS calcd for $\text{C}_{26}\text{H}_{38}\text{N}_5\text{O}_4$ ($\text{M} + \text{H}$) $^+$: 484.3. Found: 484.3.

Ethyl (3R,4R,5S)-4-acetamido-5-[5-(4-diethylaminophenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (6m). Using the general procedure for RuAAC, compound **6m** was afforded from **3** (135 mg, 0.4 mmol, 1.0 eq.) and 4-ethynyl-*N,N*-diethylaniline (134 mg, 0.6 mmol, 1.5 eq.) as a white foam (135 mg, 66%). $^1\text{H NMR}$ (500 MHz, CDCl_3) δ 7.57 (s, 1H), 7.22 (d, $J = 8.4$ Hz, 2H), 6.85 (s, 1H), 6.72 (d, $J = 8.4$ Hz, 2H), 5.81 (d, $J = 6.6$ Hz, 1H), 5.58–5.54 (m, 1H), 5.10 (d, $J = 8.7$ Hz, 1H), 4.23–4.13 (m, 4H), 4.03 (ddd, $J = 11.6, 9.2, 6.7$ Hz, 1H), 3.47–3.29 (m, 5H), 2.86–2.70 (m, 2H), 1.75 (s, 3H), 1.56–1.45 (m, 4H), 1.26 (t, $J = 7.1$ Hz, 3H), 1.19 (t, $J = 7.0$ Hz, 6H), 0.93–0.87 (m, 6H). ESIMS calcd for $\text{C}_{28}\text{H}_{42}\text{N}_5\text{O}_4$ ($\text{M} + \text{H}$) $^+$: 512.3. Found: 512.2.

Ethyl (3R,4R,5S)-4-acetamido-3-(pentan-3-yloxy)-5-[5-[[4-((*S*)-2-*tert*-butoxycarbonylamino-3-methoxy-3-oxopropyl)phenoxy]methyl]-1H-1,2,3-triazol-1-yl]cyclohex-1-ene-1-carboxylate (6n). Using the general procedure for RuAAC, compound **6n** was afforded from **3** (135 mg, 0.4 mmol, 1.0 eq.) and methyl(*S*)-2-[[*tert*-butoxycarbonyl]amino]-3-[4-(prop-2-yn-1-yloxy)phenyl]propanoate (200 mg, 0.6 mmol, 1.5 eq.) as a white foam (99 mg, 37%). $^1\text{H NMR}$ (500 MHz, CDCl_3) δ 7.71 (s, 1H), 7.00 (d, $J = 8.0$ Hz, 2H), 6.86 (d, $J = 8.1$ Hz, 3H), 6.53 (d, $J = 7.3$ Hz, 1H), 5.36 (td, $J = 11.1, 6.3$ Hz, 1H), 5.14–5.07 (m, 2H), 5.01 (d, $J = 8.1$ Hz, 1H), 4.67 (d, $J = 8.3$ Hz, 1H), 4.50 (d, $J = 6.9$ Hz, 1H), 4.19 (q, $J = 7.1$ Hz, 2H), 3.98 (dd, $J = 18.5, 8.8$ Hz, 1H), 3.67 (s, 3H), 3.34 (q, $J = 5.5$ Hz, 1H), 3.05–2.93 (m, 4H), 1.67 (s, 3H), 1.52–1.44 (m, 4H), 1.39 (s, 9H), 1.26 (t, $J = 7.1$ Hz, 3H), 0.89 (t, $J = 7.4$ Hz, 3H), 0.84 (t, $J = 7.4$ Hz, 3H). HRESIMS calcd for $\text{C}_{34}\text{H}_{50}\text{N}_5\text{O}_9$ ($\text{M} + \text{H}$) $^+$: 672.3603. Found: 672.3602.

(3R,4R,5S)-4-Acetamido-5-[4-(1,1'-biphenyl-4-yl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (1a). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **5a** (165 mg, 0.32 mmol) gave **1a** as a white foam (123 mg, 79%). IR (neat) ν_{max} 3338.84, 2925.77, 2851.90, 1654.08, 1555.73, 1431.87, 1344.86, 1078.97 cm^{-1} . $^1\text{H NMR}$ (500 MHz, CD_3OD) δ 8.45 (s, 1H), 7.92–7.67 (m, 6H), 7.47 (t, $J = 7.7$ Hz, 2H), 7.39–7.36 (m, 1H), 6.89 (s, 1H), 5.10–5.07 (m, 1H), 4.45–4.36 (m, 2H), 3.49–3.44 (m, 1H), 3.11–3.08 (m, 2H), 1.75 (s, 3H), 1.60–1.53 (m, 4H), 0.98 (t, $J = 7.4$ Hz, 3H), 0.90 (t, $J = 7.4$ Hz, 3H). $^{13}\text{C NMR}$ (125 MHz, CD_3OD) δ 171.65, 142.48, 139.88, 129.20, 128.66, 127.62, 127.52, 126.68, 125.35, 119.13,

82.13, 71.18, 56.48, 55.54, 31.60, 25.85, 25.23, 21.36, 8.52, 8.09. HRESIMS calcd for $C_{28}H_{32}N_4NaO_4$ ($M + Na$)⁺: 511.2316. Found: 511.2318.

(3R,4R,5S)-4-Acetamido-3-(pentan-3-yloxy)-5-[4-(4-phenoxyphe-nyl)-1H-1,2,3-triazol-1-yl]cyclohex-1-ene-1-carboxylic acid (1b). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **5b** (139 mg, 0.26 mmol) gave **1b** as a white foam (99 mg, 75%). IR (neat) ν_{max} 3318.93, 2939.20, 1628.36, 1420.34, 1167.99, 1036.75 cm^{-1} . ¹H NMR (500 MHz, CD₃OD) δ 8.24 (s, 1H), 7.70–6.92 (m, 9H), 6.77 (s, 1H), 4.98–4.92 (m, 1H), 4.33–4.23 (m, 2H), 3.35–3.31 (m, 1H), 3.00–2.90 (m, 2H), 1.62 (s, 3H), 1.48–1.39 (m, 4H), 0.84 (t, $J = 7.4$ Hz, 3H), 0.77 (t, $J = 7.4$ Hz, 3H). ¹³C NMR (125 MHz, CD₃OD) δ 173.00, 172.34, 158.83, 154.88, 138.11, 129.45, 128.03, 122.44, 118.47, 81.93, 70.75, 52.76, 52.32, 35.02, 29.42, 28.98, 25.25, 9.46, 8.91. HRESIMS calcd for $C_{28}H_{31}N_4O_5$ ($M + H$)⁺: 503.2300. Found: 503.2296.

(3R,4R,5S)-4-Acetamido-5-[4-(2-naphthylxy)methyl-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (1c). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **5c** (183 mg, 0.35 mmol) gave **1c** as a white foam (107 mg, 62%). IR (neat) ν_{max} 3295.60, 2965.95, 2877.58, 1655.98, 1576.99, 1413.19, 1345.39, 1078.65 cm^{-1} . ¹H NMR (500 MHz, CD₃OD) δ 8.14 (s, 1H), 7.81–7.73 (m, 3H), 7.42 (t, $J = 7.5$ Hz, 1H), 7.37 (d, $J = 2.3$ Hz, 1H), 7.32 (t, $J = 7.1$ Hz, 1H), 7.17 (dd, $J = 8.9, 2.5$ Hz, 1H), 6.63 (s, 1H), 5.28 (s, 2H), 4.97 (dd, $J = 18.0, 9.1$ Hz, 1H), 4.30 (dd, $J = 18.4, 7.3$ Hz, 2H), 3.40 (dd, $J = 11.2, 5.6$ Hz, 1H), 3.01 (d, $J = 7.3$ Hz, 2H), 1.57 (s, 3H), 1.55–1.46 (m, 4H), 0.93 (t, $J = 7.4$ Hz, 3H), 0.84 (t, $J = 7.4$ Hz, 3H). ¹³C NMR (125 MHz, CD₃OD) δ 173.23, 157.60, 144.68, 136.05, 133.45, 130.68, 130.47, 128.56, 128.01, 127.37, 124.80, 124.51, 119.86, 108.25, 83.41, 77.34, 62.32, 60.95, 56.45, 34.03, 27.31, 26.62, 22.48, 9.93, 9.52. HRESIMS calcd for $C_{27}H_{32}N_4NaO_5$ ($M + Na$)⁺: 515.2265. Found: 515.2268.

(3R,4R,5S)-4-Acetamido-5-[4-(4-methylphenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (1d). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **5d** (169 mg, 0.37 mmol) gave **1d** as a white foam (122 mg, 77%). IR (neat) ν_{max} 3393.75, 2946.75, 2835.71, 1645.75, 1597.51, 1416.94, 1026.59 cm^{-1} . ¹H NMR (500 MHz, CD₃OD) δ 8.34 (s, 1H), 7.68 (d, $J = 8.1$ Hz, 2H), 7.24 (d, $J = 8.0$ Hz, 2H), 6.70 (s, 1H), 5.03–4.95 (m, 1H), 4.37 (s, 2H), 3.46–3.40 (m, 1H), 3.06–3.01 (m, 2H), 2.36 (s, 3H), 1.70 (s, 3H), 1.59–1.49 (m, 4H), 0.95 (t, $J = 7.4$ Hz, 3H), 0.86 (t, $J = 7.4$ Hz, 3H). ¹³C NMR (125 MHz, CD₃OD) δ 173.30, 148.67, 139.34, 134.49, 130.52, 128.95, 126.64, 120.87, 83.53, 77.32, 60.89, 56.34, 33.77, 27.32, 26.66, 22.59, 21.27, 9.94, 9.54. HRESIMS calcd for $C_{23}H_{31}N_4O_4$ ($M + H$)⁺: 427.2340. Found: 427.2340.

(3R,4R,5S)-4-Acetamido-3-(pentan-3-yloxy)-5-(4-phenyl-1H-1,2,3-triazol-1-yl)cyclohex-1-ene-1-carboxylic acid (1e). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **5e** (173 mg, 0.39 mmol) gave **1e** as a white foam (130 mg, 81%). IR (neat) ν_{max} 3396.19, 2947.34, 2836.81, 1646.87, 1417.34, 1025.08 cm^{-1} . ¹H NMR (500 MHz, CD₃OD) δ 8.39 (s, 1H), 7.81 (d, $J = 7.4$ Hz, 2H), 7.43 (t, $J = 7.6$ Hz, 2H), 7.34 (t, $J = 7.4$ Hz, 1H), 6.92 (s, 1H), 5.08 (dd, $J = 17.4, 10.5$ Hz, 1H), 4.44 (d, $J = 7.5$ Hz, 1H), 4.38–4.31 (m, 1H), 3.47–3.40 (m,

1H), 3.11–2.99 (m, 2H), 1.71 (s, 3H), 1.60–1.47 (m, 4H), 0.96 (t, $J = 7.4$ Hz, 3H), 0.87 (t, $J = 7.4$ Hz, 3H). ¹³C NMR (125 MHz, CD₃OD) δ 173.43, 168.80, 148.68, 139.47, 131.70, 129.97, 129.74, 129.38, 126.69, 121.34, 83.84, 76.67, 60.22, 56.16, 32.44, 27.27, 26.71, 22.59, 9.94, 9.52. HRESIMS calcd for $C_{22}H_{29}N_4O_4$ ($M + H$)⁺: 413.2183. Found: 413.2179.

(3R,4R,5S)-4-Acetamido-5-[4-(4-carboxyphenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (1f). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **5f** (171 mg, 0.34 mmol) gave **1f** as a white foam (112 mg, 72%). IR (neat) ν_{max} 3361.77, 2963.24, 2929.43, 2875.61, 1658.95, 1550.11, 1409.77, 1287.15, 1074.26 cm^{-1} . ¹H NMR (500 MHz, CD₃OD) δ 8.45 (s, 1H), 8.02 (d, $J = 8.2$ Hz, 2H), 7.82 (d, $J = 8.2$ Hz, 2H), 6.67 (s, 1H), 5.01 (dd, $J = 18.5, 8.6$ Hz, 1H), 4.35 (dd, $J = 18.7, 7.9$ Hz, 2H), 3.43 (dt, $J = 11.1, 5.4$ Hz, 1H), 3.06 (d, $J = 8.1$ Hz, 2H), 1.71 (s, 3H), 1.61–1.45 (m, 4H), 0.95 (t, $J = 7.4$ Hz, 3H), 0.86 (t, $J = 7.4$ Hz, 3H). ¹³C NMR (125 MHz, CD₃OD) δ 174.74, 174.17, 173.31, 148.19, 138.52, 135.51, 133.63, 130.98, 125.98, 121.80, 83.48, 77.34, 61.00, 56.55, 33.92, 27.34, 26.67, 22.60, 9.94, 9.55. HRESIMS calcd for $C_{23}H_{29}N_4O_6$ ($M + H$)⁺: 457.2082. Found: 457.2088. $C_{23}H_{28}N_4NaO_6$ ($M + Na$)⁺: 479.1901. Found: 479.1904.

(3R,4R,5S)-4-Acetamido-5-[4-(3-carboxyphenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (1g). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **5g** (157 mg, 0.32 mmol) gave **1g** as a white foam (99 mg, 69%). IR (neat) ν_{max} 3387.01, 1654.02, 1548.54, 1389.71, 1017.78 cm^{-1} . ¹H NMR (500 MHz, CD₃OD) δ 8.37 (d, $J = 3.6$ Hz, 2H), 7.90 (dd, $J = 17.6, 7.7$ Hz, 2H), 7.43 (t, $J = 7.7$ Hz, 1H), 6.66 (s, 1H), 5.00 (dd, $J = 17.4, 10.1$ Hz, 1H), 4.45–4.29 (m, 2H), 3.47–3.41 (m, 1H), 3.11–3.03 (m, 2H), 1.71 (s, 3H), 1.60–1.47 (m, 4H), 0.95 (t, $J = 7.4$ Hz, 3H), 0.87 (t, $J = 7.4$ Hz, 3H). ¹³C NMR (125 MHz, CD₃OD) δ 174.92, 174.28, 173.24, 148.47, 140.04, 135.75, 133.44, 131.36, 130.06, 129.35, 128.27, 127.69, 121.59, 83.48, 77.40, 60.96, 56.61, 33.98, 27.33, 26.65, 22.63, 9.95, 9.54. HRESIMS calcd for $C_{23}H_{29}N_4O_6$ ($M + H$)⁺: 457.2082. Found: 457.2092.

(3R,4R,5S)-4-Acetamido-5-[4-(2-carboxyphenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (1h). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **5h** (163 mg, 0.3 mmol) gave **1h** as a white foam (104 mg, 70%). IR (neat) ν_{max} 3295.18, 2964.19, 2933.58, 1649.54, 1397.34, 1076.56 cm^{-1} . ¹H NMR (500 MHz, CD₃OD) δ 8.16 (s, 1H), 7.80 (dd, $J = 7.2, 0.8$ Hz, 1H), 7.63–7.58 (m, 2H), 7.50–7.46 (m, 1H), 6.74 (s, 1H), 5.00–5.06 (m, 1H), 4.45–4.25 (m, 2H), 3.45–3.41 (m, 1H), 3.11–3.03 (m, 2H), 1.77 (s, 3H), 1.58–1.48 (m, 4H), 0.95 (t, $J = 7.4$ Hz, 3H), 0.87 (t, $J = 7.4$ Hz, 3H). ¹³C NMR (125 MHz, CD₃OD) δ 172.03, 171.10, 168.67, 145.58, 134.04, 131.16, 130.77, 130.19, 129.91, 129.34, 128.06, 122.04, 82.16, 75.80, 59.30, 54.90, 32.32, 25.89, 25.26, 21.34, 8.54, 8.12. HRESIMS calcd for $C_{23}H_{29}N_4O_6$ ($M + H$)⁺: 457.2082. Found: 457.2083.

(3R,4R,5S)-4-Acetamido-5-[4-(4-aminophenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (1i). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **5i** (178 mg, 0.39 mmol) gave **1i** as a white foam (92 mg, 55%). IR (neat) ν_{max} 3374.09, 2947.67,

2835.94, 1656.64, 14449.41, 1410.50, 1025.83 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 8.05 (s, 1H), 7.43 (d, $J = 8.4$ Hz, 1H), 6.70 (s, 1H), 6.66 (d, $J = 8.4$ Hz, 1H), 4.92–4.87 (m, 1H), 4.31–4.18 (m, 2H), 3.35–3.30 (m, 1H), 2.99–2.87 (m, 2H), 1.62 (s, 3H), 1.46–1.38 (m, 4H), 0.84 (t, $J = 7.4$ Hz, 3H), 0.77 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 171.88, 171.13, 147.65, 146.67, 129.45, 126.39, 119.67, 118.26, 114.75, 82.15, 75.59, 59.01, 54.85, 32.47, 26.75, 25.87, 21.19, 8.55, 8.14. HRESIMS calcd for $\text{C}_{22}\text{H}_{30}\text{N}_5\text{O}_4$ ($\text{M} + \text{H}$) $^+$: 428.2292. Found: 428.2292.

(3R,4R,5S)-4-Acetamido-5-[4-(3-aminophenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (1j). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **5j** (124 mg, 0.27 mmol) gave **1j** as a white foam (67 mg, 58%). IR (neat) ν_{max} 3278.09, 2965.28, 2877.64, 1658.25, 1556.55, 1374.81, 1079.39 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 8.27 (s, 1H), 7.16 (s, 1H), 7.14 (d, $J = 7.7$ Hz, 1H), 7.11 (s, 1H), 6.70 (d, $J = 7.6$ Hz, 1H), 6.66 (s, 1H), 5.01–4.93 (m, 1H), 4.40–4.32 (m, 2H), 3.43 (p, $J = 5.5$ Hz, 1H), 3.06–2.98 (m, 2H), 1.71 (s, 3H), 1.57–1.47 (m, 4H), 0.95 (t, $J = 7.4$ Hz, 3H), 0.86 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 174.26, 173.29, 149.40, 148.98, 135.69, 133.47, 132.33, 130.62, 121.12, 116.51, 116.45, 113.40, 83.51, 77.45, 60.96, 56.39, 34.06, 27.31, 26.64, 22.62, 9.93, 9.55. HRESIMS calcd for $\text{C}_{22}\text{H}_{30}\text{N}_5\text{O}_4$ ($\text{M} + \text{H}$) $^+$: 428.2292. Found: 428.2295.

(3R,4R,5S)-4-Acetamido-5-[4-(2-aminophenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (1k). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **5k** (101 mg, 0.22 mmol) gave **1k** as a white foam (58 mg, 61%). IR (neat) ν_{max} 3397.63, 2948.65, 2937.92, 1678.89, 1449.47, 1203.89, 1020.50 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 8.29 (s, 1H), 7.42 (d, $J = 7.7$, 1.3 Hz, 1H), 7.10–7.07 (m, 1H), 6.91 (s, 1H), 6.82 (d, $J = 8.0$ Hz, 1H), 6.71 (t, $J = 7.3$ Hz, 1H), 5.09–5.03 (m, 1H), 4.43–4.36 (m, 2H), 3.43 (p, $J = 5.6$ Hz, 1H), 3.07–3.04 (m, 2H), 1.71 (s, 3H), 1.58–1.48 (m, 4H), 0.95 (t, $J = 7.4$ Hz, 3H), 0.87 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 172.07, 167.48, 147.19, 144.95, 138.02, 128.67, 128.46, 127.87, 120.26, 117.41, 116.53, 114.45, 82.45, 75.35, 58.79, 54.76, 31.12, 25.88, 25.33, 21.25, 8.54, 8.14. HRESIMS calcd for $\text{C}_{22}\text{H}_{30}\text{N}_5\text{O}_4$ ($\text{M} + \text{H}$) $^+$: 428.2298. Found: 428.2291.

(3R,4R,5S)-4-Acetamido-5-[4-(4-dimethylaminophenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (1l). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **5l** (150 mg, 0.31 mmol) gave **1l** as a white foam (95 mg, 67%). IR (neat) ν_{max} 3361.70, 2963.53, 2841.42, 1645.74, 1423.30, 1018.09 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 8.16 (s, 1H), 7.63 (d, $J = 8.6$ Hz, 1H), 6.84 (s, 1H), 6.81 (d, $J = 8.6$ Hz, 1H), 4.43–4.30 (m, 2H), 3.44–4.42 (m, 1H), 3.04–2.97 (m, 8H), 1.72 (s, 3H), 1.60–1.49 (m, 4H), 0.95 (t, $J = 7.4$ Hz, 3H), 0.87 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 171.88, 150.92, 147.87, 129.45, 126.15, 118.27, 112.45, 109.92, 82.38, 72.32, 58.90, 55.96, 39.05, 31.62, 29.40, 28.88, 21.16, 8.53, 8.10. HRESIMS calcd for $\text{C}_{22}\text{H}_{30}\text{N}_5\text{O}_4$ ($\text{M} + \text{H}$) $^+$: 456.2611. Found: 456.2608.

(3R,4R,5S)-4-Acetamido-5-[4-(4-diethylaminophenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (1m). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **5m** (113 mg, 0.22 mmol) gave

1m as a white foam (67 mg, 63%). IR (neat) ν_{max} 3339.48, 2968.29, 2875.82, 1657.91, 1613.80, 1505.16, 1269.25, 1077.65 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 8.05 (s, 1H), 7.50 (d, $J = 8.9$ Hz, 1H), 6.73 (s, 1H), 6.64 (d, $J = 8.9$ Hz, 1H), 4.94–4.88 (m, 1H), 4.31–4.22 (m, 2H), 3.35–3.29 (m, 5H), 2.99–2.88 (m, 2H), 1.62 (s, 3H), 1.47–1.40 (m, 4H), 1.07 (t, $J = 7.0$ Hz, 6H), 0.85 (t, $J = 7.4$ Hz, 3H), 0.77 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 172.06, 150.73, 147.80, 129.40, 126.20, 118.40, 118.21, 112.38, 82.37, 75.50, 55.56, 54.93, 39.30, 29.44, 25.88, 25.30, 21.23, 11.63, 8.52, 8.13. HRESIMS calcd for $\text{C}_{22}\text{H}_{30}\text{N}_5\text{O}_4$ ($\text{M} + \text{H}$) $^+$: 484.2924. Found: 484.2922.

(3R,4R,5S)-4-Acetamido-5-{4-[(S)-2-amino-2-carboxyethyl]phenoxy[methyl]-1H-1,2,3-triazol-1-yl}-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (1n). Using the general procedure B for the synthesis of **1** or **2**, deprotection of compound **5n** (168 mg, 0.25 mmol) gave **1n** as a white foam (104 mg, 79%). IR (neat) ν_{max} 3287.59, 2927.55, 1659.25, 1512.02, 1435.81, 1202.45 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 8.08 (s, 1H), 7.22 (d, $J = 8.6$ Hz, 2H), 7.00 (d, $J = 8.6$ Hz, 2H), 6.90 (d, $J = 1.5$ Hz, 1H), 5.17 (d, $J = 0.7$ Hz, 2H), 5.05–5.00 (m, 1H), 4.38 (d, $J = 7.3$ Hz, 1H), 4.35–4.26 (m, 1H), 4.19 (dd, $J = 7.5$, 5.4 Hz, 1H), 3.45–3.39 (m, 1H), 3.25 (dd, $J = 14.6$, 5.3 Hz, 1H), 3.11 (dd, $J = 14.6$, 7.6 Hz, 1H), 3.02 (d, $J = 8.3$ Hz, 2H), 1.66 (s, 3H), 1.57–1.46 (m, 4H), 0.94 (t, $J = 7.4$ Hz, 3H), 0.85 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 173.32, 171.46, 168.86, 159.27, 144.66, 139.51, 131.70, 129.64, 128.10, 124.81, 116.56, 83.89, 76.79, 62.26, 60.18, 55.97, 55.36, 36.49, 32.27, 27.20, 26.66, 22.58, 9.91, 9.49. HRESIMS calcd for $\text{C}_{26}\text{H}_{36}\text{N}_5\text{O}_7$ ($\text{M} + \text{H}$) $^+$: 530.2609. Found: 530.2607.

(3R,4R,5S)-4-Acetamido-3-(pentan-3-yloxy)-5-(5-phenyl-1H-1,2,3-triazol-1-yl)cyclohex-1-ene-1-carboxylic acid (2e). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **6e** (157 mg, 0.36 mmol) gave **2e** as a white foam (115 mg, 78%). IR (neat) ν_{max} 3424.69, 2842.65, 1643.99, 1427.59, 1256.33, 1017.10 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 7.75 (s, 1H), 7.56–7.44 (m, 5H), 6.86 (d, $J = 1.3$ Hz, 1H), 5.16–5.14 (m, 1H), 5.54–5.52 (m, 1H), 4.22–4.18 (m, 1H), 3.35–3.33 (m, 1H), 3.07–3.04 (m, 2H), 1.68 (s, 3H), 1.52–1.44 (m, 4H), 0.91 (t, $J = 7.4$ Hz, 3H), 0.84 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 173.01, 168.81, 140.91, 139.57, 133.26, 130.86, 130.41, 130.15, 129.42, 127.88, 83.47, 75.15, 62.65, 57.76, 32.85, 27.22, 26.61, 22.74, 9.94, 9.48. HRESIMS calcd for $\text{C}_{22}\text{H}_{29}\text{N}_4\text{O}_4$ ($\text{M} + \text{H}$) $^+$: 413.2183. Found: 413.2180.

(3R,4R,5S)-4-Acetamido-5-[5-(4-carboxyphenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (2f). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **6f** (106 mg, 0.21 mmol) gave **2f** as a white foam (64 mg, 66%). IR (neat) ν_{max} 3383.81, 2950.17, 2839.14, 1649.36, 1393.10, 1020.19 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 8.45 (s, 1H), 8.02 (d, $J = 8.3$ Hz, 2H), 7.83 (d, $J = 8.3$ Hz, 2H), 6.67 (s, 1H), 5.04–4.97 (m, 1H), 4.41–4.33 (m, 2H), 3.44–3.41 (m, 1H), 3.07–3.04 (m, 2H), 1.71 (s, 3H), 1.57–1.47 (m, 4H), 0.95 (t, $J = 7.4$ Hz, 3H), 0.86 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 174.01, 173.31, 148.17, 135.44, 133.72, 132.35, 130.98, 129.87, 126.00, 121.82, 83.47, 77.33, 60.99, 56.55, 33.90, 27.34, 26.67, 22.60, 9.94, 9.55. HRESIMS calcd for $\text{C}_{23}\text{H}_{28}\text{N}_4\text{NaO}_6$ ($\text{M} + \text{Na}$) $^+$: 479.1901. Found: 479.1904.

(3R,4R,5S)-4-Acetamido-5-[5-(3-carboxyphenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (2g). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **6g** (76 mg, 0.15 mmol) gave **2g** as a white foam (40 mg, 58%). IR (neat) ν_{\max} 3377.18, 2966.02, 2935.50, 1657.54, 1656.63, 1397.48, 1077.71 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 8.12 (d, $J = 7.1$ Hz, 1H), 7.99 (s, 1H), 7.78 (s, 1H), 7.62–7.54 (m, 2H), 6.77 (s, 1H), 4.98–4.93 (m, 1H), 4.35–4.33 (m, 2H), 3.36–3.33 (m, 1H), 3.24–3.15 (m, 1H), 3.11 (dd, $J = 17.6$, 5.7 Hz, 1H), 1.69 (s, 3H), 1.52–1.43 (m, 4H), 0.91 (t, $J = 7.4$ Hz, 3H), 0.82 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 172.79, 172.23, 140.61, 137.50, 132.87, 131.70, 130.97, 130.28, 127.86, 111.40, 83.55, 79.61, 64.41, 57.73, 33.25, 27.24, 26.63, 22.74, 9.87, 9.52. HRESIMS calcd for $\text{C}_{23}\text{H}_{29}\text{N}_4\text{O}_6$ ($\text{M} + \text{H}$) $^+$: 457.2082. Found: 457.2090.

(3R,4R,5S)-4-Acetamido-5-[5-(2-carboxyphenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (2h). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **6h** (63 mg, 0.12 mmol) gave **2h** as a white foam (32 mg, 56%). IR (neat) ν_{\max} 3334.80, 2836.90, 2525.99, 1678.81, 1449.44, 1203.80, 1143.36, 1024.27 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 8.09 (s, 1H), 7.58 (d, $J = 7.4$ Hz, 1H), 7.36 (d, $J = 7.0$ Hz, 1H), 7.13 (d, $J = 8.4$ Hz, 1H), 6.97 (t, $J = 7.2$ Hz, 1H), 6.73 (s, 1H), 5.06 (d, $J = 6.7$ Hz, 1H), 4.37–4.24 (m, 2H), 3.41–3.37 (m, 1H), 3.05–2.97 (m, 2H), 1.63 (s, 3H), 1.53–1.47 (m, 4H), 0.91 (t, $J = 7.4$ Hz, 3H), 0.83 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 172.77, 171.63, 169.65, 139.28, 138.67, 135.84, 132.79, 130.82, 130.74, 128.49, 127.51, 121.34, 82.76, 74.47, 60.08, 55.97, 30.24, 24.14, 23.77, 20.31, 7.08, 6.99. HRESIMS calcd for $\text{C}_{23}\text{H}_{28}\text{N}_4\text{NaO}_6$ ($\text{M} + \text{Na}$) $^+$: 479.1901. Found: 479.1905.

(3R,4R,5S)-4-Acetamido-5-[5-(4-aminophenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (2i). Using the general procedure B for the synthesis of **1** or **2**, deprotection of compound **6i** (83 mg, 0.15 mmol) gave **2i** as a white foam (46 mg, 72%). IR (neat) ν_{\max} 3359.53, 2967.00, 2937.43, 2878.38, 1660.22, 1557.67, 1499.89, 1200.77 1077.20 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 7.63 (s, 1H), 7.20 (d, $J = 8.5$ Hz, 1H), 6.89 (s, 1H), 6.86 (d, $J = 8.5$ Hz, 1H), 5.17–5.11 (m, 1H), 4.56–4.54 (m, 1H), 4.25–4.21 (m, 1H), 3.39–3.35 (m, 1H), 3.10–2.98 (m, 2H), 1.70 (s, 3H), 1.55–1.48 (m, 4H), 0.94 (t, $J = 7.4$ Hz, 3H), 0.86 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 171.62, 167.56, 148.25, 140.03, 138.16, 132.39, 131.28, 129.72, 128.15, 115.56, 82.16, 74.03, 56.14, 55.47, 31.45, 25.83, 25.24, 21.34, 8.53, 8.09. HRESIMS calcd for $\text{C}_{22}\text{H}_{30}\text{N}_5\text{O}_4$ ($\text{M} + \text{H}$) $^+$: 428.2292. Found: 428.2293.

(3R,4R,5S)-4-Acetamido-5-[5-(3-aminophenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (2j). Using the general procedure B for the synthesis of **1** or **2**, deprotection of compound **6j** (74 mg, 0.13 mmol) gave **2j** as a white foam (41 mg, 73%). IR (neat) ν_{\max} 3392.69, 2970.90, 2749.20, 1678.74, 1435.55, 1204.21, 1143.03, 1019.03 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 7.84 (s, 1H), 7.70 (t, $J = 7.9$ Hz, 1H), 7.58–7.53 (m, 3H), 6.87 (s, 1H), 5.19–5.18 (m, 1H), 4.55–4.54 (m, 1H), 4.16 (t, $J = 9.6$ Hz, 1H), 3.35–3.31 (m, 1H), 3.10–3.01 (m, 2H), 1.71 (s, 3H), 1.51–1.43 (m, 4H), 0.90 (t, $J = 7.4$ Hz, 3H), 0.83

(t, $J = 7.4$ Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 173.37, 168.89, 139.62, 132.22, 129.97, 129.41, 125.00, 124.24, 117.14, 114.87, 83.61, 75.12, 57.86, 54.45, 32.84, 27.20, 26.59, 22.78, 9.91, 9.46. HRESIMS calcd for $\text{C}_{22}\text{H}_{30}\text{N}_5\text{O}_4$ ($\text{M} + \text{H}$) $^+$: 428.2292. Found: 428.2293.

(3R,4R,5S)-4-Acetamido-5-[5-(2-aminophenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (2k). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **6k** (108 mg, 0.24 mmol) gave **2k** as a white foam (70 mg, 69%). IR (neat) ν_{\max} 3391.31, 2950.09, 2838.83, 1645.74, 1417.23, 1203.74, 1021.06 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 7.69 (s, 1H), 7.24–7.21 (m, 1H), 7.03 (d, $J = 7.5$ Hz, 1H), 6.85 (d, $J = 8.0$ Hz, 1H), 6.78 (t, $J = 7.4$ Hz, 1H), 6.61 (s, 1H), 4.65–4.64 (m, 1H), 4.38–4.30 (m, 2H), 3.45–3.38 (m, 1H), 3.17–3.03 (m, 2H), 1.73 (s, 3H), 1.51–1.42 (m, 4H), 0.91 (t, $J = 7.4$ Hz, 3H), 0.82 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 171.34, 165.61, 146.57, 136.58, 132.89, 130.77, 129.48, 118.06, 117.46, 115.80, 112.58, 110.66, 82.05, 75.35, 57.07, 55.81, 32.28, 25.88, 25.21, 21.56, 8.48, 8.15. HRESIMS calcd for $\text{C}_{22}\text{H}_{30}\text{N}_5\text{O}_4$ ($\text{M} + \text{H}$) $^+$: 428.2298. Found: 428.2297.

(3R,4R,5S)-4-Acetamido-5-[5-(4-dimethylaminophenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (2l). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **6l** (98 mg, 0.20 mmol) gave **2l** as a white foam (62 mg, 67%). IR (neat) ν_{\max} 3271.17, 2963.35, 2933.31, 1657.42, 1506.99, 1362.62, 1227.19, 1021.24 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 7.53 (s, 1H), 7.18 (d, $J = 8.6$ Hz, 1H), 6.78 (d, $J = 8.6$ Hz, 1H), 6.72 (s, 1H), 5.11–4.92 (m, 1H), 4.45–4.36 (m, 1H), 4.23–4.11 (m, 1H), 3.27 (t, $J = 5.5$ Hz, 1H), 3.00–2.89 (m, 8H), 1.60 (s, 3H), 1.44–1.35 (m, 4H), 0.83 (t, $J = 7.4$ Hz, 3H), 0.75 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 171.66, 151.38, 140.19, 137.57, 131.32, 129.60, 129.43, 113.18, 112.25, 82.12, 73.96, 56.32, 55.37, 39.01, 31.57, 25.85, 25.24, 21.33, 8.52, 8.09. HRESIMS calcd for $\text{C}_{22}\text{H}_{30}\text{N}_5\text{O}_4$ ($\text{M} + \text{H}$) $^+$: 456.2611. Found: 456.2612.

(3R,4R,5S)-4-Acetamido-5-[5-(4-diethylaminophenyl)-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (2m). Using the general procedure A for the synthesis of **1** or **2**, deprotection of compound **6m** (113 mg, 0.22 mmol) gave **2m** as a white foam (64 mg, 60%). IR (neat) ν_{\max} 3362.32, 2966.94, 2932.70, 1657.04, 1613.50, 1504.52, 1268.94, 1076.93 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 7.62 (s, 1H), 7.25 (d, $J = 8.9$ Hz, 1H), 6.85–6.82 (m, 3H), 5.14–5.09 (m, 1H), 4.56–4.51 (m, 1H), 4.30–4.24 (m, 1H), 3.46 (q, $J = 7.1$ Hz, 4H), 3.37–3.35 (m, 1H), 3.11–3.01 (m, 2H), 1.70 (s, 3H), 1.54–1.46 (m, 4H), 1.20 (t, $J = 7.0$ Hz, 6H), 0.93 (t, $J = 7.4$ Hz, 3H), 0.86 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 171.54, 168.72, 148.53, 140.24, 137.49, 131.03, 129.68, 129.07, 112.04, 111.61, 81.20, 74.36, 56.05, 55.66, 43.97, 31.69, 25.85, 25.25, 21.36, 11.44, 8.53, 8.13. HRESIMS calcd for $\text{C}_{22}\text{H}_{30}\text{N}_5\text{O}_4$ ($\text{M} + \text{H}$) $^+$: 484.2924. Found: 484.2923.

(3R,4R,5S)-4-Acetamido-5-[5-[[4-((S)-2-amino-2-carboxyethyl)phenoxy]methyl]-1H-1,2,3-triazol-1-yl]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (2n). Using the general procedure B for the synthesis of **1** or **2**, deprotection of compound **6n** (99 mg, 0.15 mmol) gave **2n** as a white foam (61 mg, 78%). IR (neat) ν_{\max} 3433.92, 3013.45, 2929.50, 1686.89, 1512.86, 1436.01,

1296.51, 1205.47, 1137.42, 1025.52 cm⁻¹. ¹H NMR (500 MHz, CD₃OD) δ 8.08 (s, 1H), 7.22 (d, *J* = 8.6 Hz, 2H), 7.01 (d, *J* = 8.6 Hz, 2H), 6.90 (s, 1H), 5.17 (d, *J* = 1.8 Hz, 2H), 5.05–4.99 (m, 1H), 4.38–4.19 (m, 3H), 3.44–3.39 (m, 1H), 3.26 (dd, *J* = 14.7, 5.2 Hz, 1H), 3.13–3.07 (m, 1H), 3.04–2.95 (m, 2H), 1.65 (s, 3H), 1.56–1.48 (m, 4H), 0.94 (t, *J* = 7.4 Hz, 3H), 0.86 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (125 MHz, CD₃OD) δ 173.38, 171.27, 168.80, 159.35, 144.81, 139.47, 131.68, 129.66, 127.95, 124.53, 116.65, 83.87, 76.77, 62.27, 60.22, 55.97, 55.21, 36.48, 32.38, 27.22, 26.67, 22.55, 9.92, 9.49. HRESIMS calcd for C₂₆H₃₆N₅O₇ (M + H)⁺: 530.2609. Found: 530.2606.

Conflicts of interest

The authors declare no conflict of interest.

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Notes and references

- 1 M. P. Girard, J. S. Tam, O. M. Assossou and M. P. Kieny, *Vaccine*, 2010, **28**, 4895–4902.
- 2 T. M. Uyeki and N. J. Cox, *N. Engl. J. Med.*, 2013, **368**, 1862–1864.
- 3 J. Stevens, O. Blixt, L. M. Chen, R. O. Donis, J. C. Paulson and I. A. Wilson, *J. Mol. Biol.*, 2008, **381**, 1382–1394.
- 4 C. Liu, M. C. Eichelberger, R. W. Compans and G. M. Air, *J. Virol.*, 1995, **69**, 1099–1106.
- 5 K. McClellan and C. M. Perry, *Drugs*, 2001, **61**, 263–283.
- 6 M. von Itzstein, W. Y. Wu, G. B. Kok, M. S. Pegg, J. C. Dyason, B. Jin, T. Van Phan, M. L. Smythe, H. F. White, S. W. Oliver, P. M. Colman, J. N. Varghese, D. M. Ryan, J. M. Woods, R. C. Bethell, V. J. Hotham, J. M. Cameron and C. R. Penn, *Nature*, 1993, **363**, 418–423.
- 7 C. J. Dunn and K. L. Goa, *Drugs*, 1999, **58**, 761–784.
- 8 S. Kubo, T. Tomozawa, M. Kakuta, A. Tokumitsu and M. Yamashita, *Antimicrob. Agents Chemother.*, 2010, **54**, 1256–1264.
- 9 P. Chand, P. L. Kotian, A. Dehghani, Y. El-Kattan, T. H. Lin, T. L. Hutchison, Y. S. Babu, S. Bantia, A. J. Elliott and J. A. Montgomery, *J. Med. Chem.*, 2001, **44**, 4379–4392.
- 10 M. Kiso, K. Mitamura, Y. Sakai-Tagawa, K. Shiraiishi, C. Kawakami, K. Kimura, F. G. Hayden, N. Sugaya and Y. Kawaoka, *Lancet*, 2004, **364**, 759–765.
- 11 Q. M. Le, M. Kiso, K. Someya, Y. T. Sakai, T. H. Nguyen, K. H. L. Nguyen, N. D. Pham, H. H. Ngyen, S. Yamada, Y. Muramoto, T. Horimoto, A. Takada, H. Goto, T. Suzuki, Y. Suzuki and Y. Kawaoka, *Nature*, 2005, **437**, 1108.
- 12 Y. Hu, S. Lu, Z. Song, W. Wang, P. Hao, J. Li, X. Zhang, H. L. Yen, B. Shi, T. Li, W. Guan, L. Xu, Y. Liu, S. Wang, X. Zhang, D. Tian, Z. Zhu, J. He, K. Huang, H. Chen, L. Zheng, X. Li, J. Ping, B. Kang, X. Xi, L. Zha, Y. Li, Z. Zhang, M. Peiris and Z. Yuan, *Lancet*, 2013, **381**, 2273–2279.
- 13 J. D. Bloom, L. I. Gong and D. Baltimore, *Science*, 2010, **328**, 1272–1275.
- 14 J. D. Thompson, D. G. Higgins and T. J. Gibson, *Comput. Appl. Biosci.*, 1994, **10**, 19–29.
- 15 R. J. Russell, L. F. Haire, D. J. Stevens, P. J. Collins, Y. P. Lin, G. M. Blackburn, A. J. Hay, S. J. Gamblin and J. J. Skehel, *Nature*, 2006, **443**, 45–49.
- 16 X. Xu, X. Zhu, R. A. Dwek, J. Stevens and I. A. Wilson, *J. Virol.*, 2008, **82**, 10493–10501.
- 17 G. M. Air, *Influenza Other Respir. Viruses*, 2012, **6**, 245–256.
- 18 Y. Wu, G. Qin, F. Gao, Y. Liu, C. J. Vavricka, J. Qi, H. Jiang, K. Yu and G. F. Gao, *Sci. Rep.*, 2013, **3**, 1551.
- 19 R. E. Amaro, R. V. Swift, L. Votapka, W. W. Li, R. C. Walker and R. M. Bush, *Nat. Commun.*, 2011, **2**, 388.
- 20 R. E. Amaro, D. D. L. Minh, L. S. Cheng, W. M. Lindstrom, A. J. Olson, J. H. Lin, W. W. Li and J. A. McCammon, *J. Am. Chem. Soc.*, 2007, **129**, 7764–7765.
- 21 R. E. Amaro, X. Cheng, I. Ivanov, D. Xu and J. A. McCammon, *J. Am. Chem. Soc.*, 2009, **131**, 4702–4709.
- 22 N. Han and Y. Mu, *PLoS One*, 2013, **8**, e60995.
- 23 S. Mohan and B. M. Pinto, *Can. J. Chem.*, 2018, **96**, 91–101.
- 24 P. S. Kerry, S. Mohan, R. J. M. Russell, N. Bance, M. Niikura and B. M. Pinto, *Sci. Rep.*, 2013, **3**, 2871.
- 25 V. Zima, C. B. Albiñana, K. Rojíková, J. Pokorná, P. Páchl, P. Řezáčová, J. Hudlický, V. Navrátil, P. Majer, J. Kinvalinka, M. Kožíšek and A. Machara, *Bioorgan. Med. Chem.*, 2019, **27**, 2935–2947.
- 26 X. Zhu, R. McBride, C. M. Nycholat, W. Yu, J. C. Paulson and I. A. Wilson, *J. Virol.*, 2012, **86**, 13371–13383.
- 27 P. C. Wang, D. C. Chiu, J. T. Jan, W. I. Huang, Y. C. Tseng, T. T. Li, T. J. Cheng, K. C. Tsai and J. M. Fang, *Eur. J. Med. Chem.*, 2018, **145**, 224–234.
- 28 P. H. Hsu, D. C. Chiu, K. L. Wu, P. S. Lee, J. T. Jan, Y. S. E. Cheng, K. C. Tsai, T. J. Cheng and J. M. Fang, *Eur. J. Med. Chem.*, 2018, **154**, 314–323.
- 29 J. Zhang, V. Poongavanam, D. Kang, C. Bertagnin, H. Lu, X. Kong, H. Ju, X. Lu, P. Gao, Y. Tian, H. Jia, S. Desta, X. Ding, L. Sun, Z. Fang, B. Huang, X. Liang, R. Jia, X. Ma, W. Xu, N. A. Murugan, A. Loregian, B. Huang, P. Zhan and X. Liu, *J. Med. Chem.*, 2018, **61**, 6379–6397.
- 30 J. Zhang, N. A. Murugan, Y. Tian, C. Bertagnin, Z. Fang, D. Kang, X. Kong, H. Jia, Z. Sun, R. Jia, P. Gao, V. Poongavanam, A. Loregian, W. Xu, X. Ma, X. Ding, B. Huang, P. Zhan and X. Liu, *J. Med. Chem.*, 2018, **61**, 9976–9999.
- 31 H. Ju, J. Zhang, Z. Sun, Z. Huang, W. Qi, B. Huang, P. Zhan and X. Liu, *Eur. J. Med. Chem.*, 2018, **146**, 220–231.
- 32 R. Jia, J. Zhang, W. Ai, X. Ding, S. Desta, L. Sun, Z. Sun, X. Ma, Z. Li, D. Wang, B. Huang, P. Zhan and X. Liu, *Eur. J. Med. Chem.*, 2019, **178**, 64–80.
- 33 A. S. Chintakrindi, D. J. Gohil, A. S. Chowdhary and M. A. Kanyalkar, *Bioorgan. Med. Chem.*, 2020, **28**, 115191.
- 34 J. Ye, X. Yang, M. Xu, P. K. Chan and C. Ma, *Eur. J. Med. Chem.*, 2019, **182**, 111635.

- 35 W. Ai, J. Zhang, W. A. Zalloum, R. Jia, S. Cherukupalli, X. Ding, Z. Sun, L. Sun, X. Jiang, X. Ma, Z. Li, D. Wang, B. Huang, P. Zhan and X. Liu, *Eur. J. Med. Chem.*, 2020, **191**, 112147.
- 36 A. Mahal, M. Duan, D. S. Zinad, R. K. Mohapatra, A. J. Obaidullah, X. Wei, M. K. Pradhan, D. Das, V. Kandi, H. S. Zinad and Q. Zhu, *RSC Adv.*, 2021, **11**, 1804–1840.
- 37 V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596–2599.
- 38 W. Lew, X. Chen and C. U. Kim, *Curr. Med. Chem.*, 2000, **7**, 663–672.
- 39 L. Zhang, X. Chen, P. Xue, H. H. Y. Sun, I. D. Williams, K. B. Sharpless, V. V. Fokin and G. Jia, *J. Am. Chem. Soc.*, 2005, **127**, 15998–15999.
- 40 M. Potier, L. Mameli, M. Belisle, L. Dallaire and S. B. Melancon, *Anal. Biochem.*, 1979, **94**, 287–296.
- 41 M. Richard, O. Ferraris, A. Erny, M. Barthélémy, A. Traversier, M. Sabatier, A. Hay, Y. P. Lin, R. J. Russell and B. Lina, *Antimicrob. Agents Chemother.*, 2011, **55**, 2942–2952.
- 42 L. Zheng, J. Wei, X. Lv, Y. Bi, P. Wu, Z. Zhang, P. Wang, R. Liu, J. Jiang, H. Cong, J. Liang, W. Chen, H. Cao, W. Liu, G. F. Gao, Y. Du, X. Jiang and X. Li, *Biosens. Bioelectron.*, 2017, **91**, 46–52.
- 43 G. Jones, P. Willett, R. C. Glen, A. R. Leach and R. Taylor, *J. Mol. Biol.*, 1997, **267**, 727–748.
- 44 W. L. DeLano, *Proteins: Struct., Funct., Bioinf.*, 2002, **30**, 442–454.
- 45 M. Wang, J. Qi, Y. Liu, C. J. Vavricka, Y. Wu, Q. Li and G. F. Gao, *J. Virol.*, 2011, **85**, 8431–8435.
- 46 Z. Otwinowski and W. Minor, *Methods Enzymol.*, 1997, **276**, 307–326.
- 47 R. J. Read, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 2001, **57**, 1373–1382.
- 48 P. Emsley and K. Cowtan, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 2004, **60**, 2126–2132.
- 49 G. N. Murshudov, A. A. Vagin and E. J. Dodson, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 1997, **53**, 240–255.
- 50 P. D. Adams, P. V. Afonine, G. Bunkóczi, V. B. Chen, I. W. Davis, N. Echols, J. J. Headd, L. W. Hung, G. J. Kapral, R. W. Grosse-Kunstleve, A. J. McCoy, N. W. Moriarty, R. Oeffner, R. J. Read, D. C. Richardson, J. S. Richardson, T. C. Terwilliger and P. H. Zwart, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 2010, **66**, 213–221.
- 51 R. A. Laskowski, M. W. MacArthur, D. S. Moss and J. M. Thornton, *J. Appl. Crystallogr.*, 1993, **26**, 283–291.
- 52 K. E. Hevener, W. Zhao, D. M. Ball, K. Babaoglu, J. Qi, S. W. White and R. E. Lee, *J. Chem. Inf. Model.*, 2009, **49**, 444–460.
- 53 C. Elam, M. Lape and S. Paula, *Biophys. Chem.*, 2010, **150**, 88–97.
- 54 H. Hirota, K. Satou and K. Onodera, *J. Chem. Inf. Model.*, 2007, **47**, 1609–1618.