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Graphic Abstract

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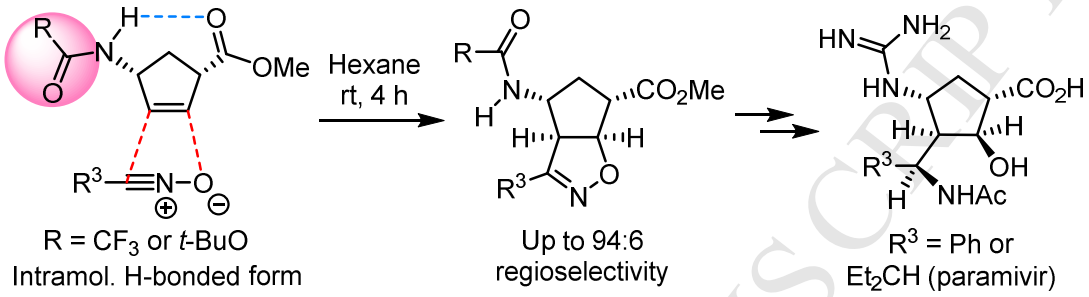

Substituent and solvent effects in the 1,3-dipolar cycloadditions for synthesis of anti-influenza agent peramivir and its analog

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ABSTRACT

Influenza remains a health problem to humans. Peramivir is a FDA approved anti-influenza drug targeting the virus neuraminidase. The (3+2) cycloaddition reaction of 2-ethylbutanenitrile oxide with the cyclopentene dipolarophile derived from Vince lactam is a key step in the conventional synthesis of peramivir. Our study showed that conducting the (3+2) cycloaddition reactions with either aliphatic or aromatic nitrile oxide in hexane solution provided high percentage of the desired regioisomer, and the *N*-substituent having electron-withdrawing property is also beneficial to the regioselectivity. This study also demonstrated an alternative synthetic pathway of (−)-peramivir and the analog having a phenyl group in place of the 3-pentyl moiety.

Keywords:

Peramivir; Nitrile oxide; Cycloaddition; Influenza; Organic synthesis.

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† C.-L.C and T.-W.C contributed equally to this work.

1. Introduction

Seasonal influenza is a major cause of mortality and morbidity among patients with acute upper respiratory diseases [1,2]. Influenza virus belongs to the family Orthomyxoviridae and has a single-stranded segmented RNA genome, which comprises eight negative-sense single-stranded RNA segments to produce at least 11 proteins [3,4]. Hemagglutinin (HA) and neuraminidase (NA) are glycoproteins anchored on the cell surface of influenza virus. Genetic diversity of influenza viruses is due to high mutation rates and frequent genetic reassortments which lead to high variable HA and NA antigens.

NA is responsible for cleaving the linkage between influenza virus and the sialyl receptor of host cell. Inhibition of the viral NA will suppress the spread of viruses to other host cells [5]. There are 11 subtypes of influenza NAs bearing conserved active sites. Therefore, an effective NA inhibitor may act as a universal anti-influenza drug to all subtypes [6].In addition to zanamivir (Relenza®) [7] and oseltamivir (Tamiflu®) [8], peramivir (Repiacta®, Fig. 1) [9−12] is a potent NA inhibitor and approved for treating influenza infection by intravenous administration [13].

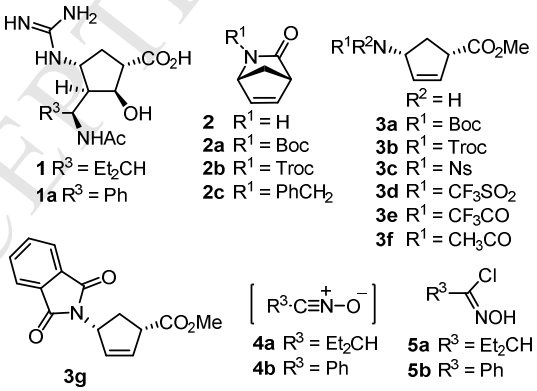
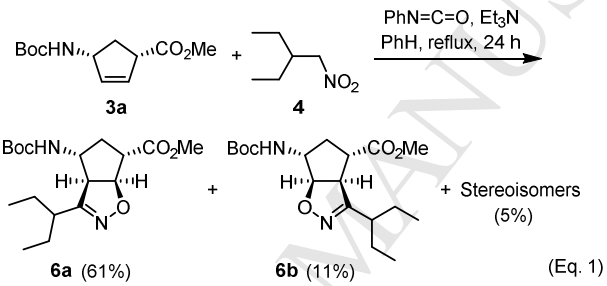


Fig. 1. Chemical structures of peramivir (**1**), analogue (**1a**), and the synthetic precursors.

Peramivir was first synthesized by Babu and coworkers via a key step of (3+2) cycloaddition reaction of 2-ethylbutanenitrile oxide (**4a**) and cyclopentene **3a**, which was

readily prepared from (−)-2-azabicyclo[2.2.1]hept-5-en-3-one (Vince lactam) [9]. As shown in Eq. 1, the nitrile oxide **4a** was generated in situ from 2-ethyl-1-nitrobutane (**4**) using phenyl isocyanate as the dehydrating reagent, and the (3+2) cycloaddition with cyclopentene **3a** in benzene (reflux, 24 h) was promoted by Et_3N to give the major product **6a** (61% isolated yield), along with the regioisomer **6b** (11%) and their stereoisomers (5%) [14]. Alternatively, the research teams of Miller and Chen treated 2-ethylbutanal oxime with NaOCl (bleach) to give the corresponding chlorination compound **5a**, which produced nitrile oxide **4a** in the presence of Et_3N and underwent the 1,3-dipolar cycloaddition with alkene **3a** in CH_2Cl_2 solution (reflux or room temperature, 20 h) [15,16].



The (3+2) cycloaddition between nitrile oxide and alkene is an efficient way to prepare isoxazoline, which can be reduced to provide the functionality of 3-amino alcohol [17−19]. The synthesis of peramivir benefits from the strategy that employs the (3+2) cycloaddition of 2-ethylbutanenitrile oxide for simultaneous formation of two covalent bonds and implantation of latent amino and hydroxyl groups. In this study, we further investigated various factors, such as the dipolarophile substrates of mono- and bicyclic alkenes, nitrile oxide 1,3-dipoles, solvents and reaction conditions, which may influence the regio- and stereochemistry of the (3+2) cycloadditions for the synthesis of peramivir and its analogs [20].

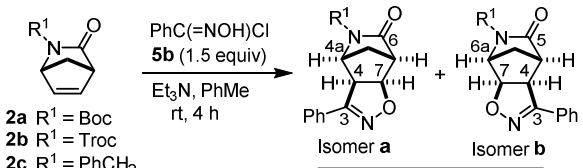
2. Results and discussion

2.1. Method for generation of nitrile oxide

In the synthesis of peramivir, the required 2-ethylbutanenitrile oxide was either prepared by dehydration of 2-ethyl-1-nitrobutane [14] or by dehydrochlorination of 2-ethyl-*N*-hydroxybutanimidoyl chloride [15, 16]. In general, nitrile oxide is an active species and easily undergoes self-dimerization to give 1,2,5-oxadiazole 2-oxide [17−20]. In addition to NaOCl, some other halogenation reagents, such as chloramine-T, *N*-bromosuccinimide and (diacetoxyiodo)benzene have been reported for conversion of oximes to hydroximoyl chlorides [21−23]. In our study, 2-ethylbutanal oxime and benzaldehyde oxime was treated with *N*-chlorosuccinimide (NCS) to give relatively stable chlorination compounds of hydroximoyl chlorides **5a** and **5b**, which were isolated and prepared as dilute solution in appropriate solvent for slow injection to a solution containing Et_3N to generate the corresponding nitrile oxides **4a** or **4b** for the in situ (3+2) cycloaddition reactions with the alkene substrates.

2.2. Cycloaddition reactions of Vince lactam

We speculated that Vince lactam having a [2.2.1] bicyclic skeleton might render better stereochemical control and facilitate the (3+2) cycloaddition by releasing the ring strain. We first tested the (3+2) cycloaddition by adding hydroximoyl chloride **5a** to a toluene solution containing lactam (±)-**2** and Et_3N. However, the reaction at 60 ^oC for 8 h only resulted in a complicated mixture containing less than 20% of cycloaddition products.



R^1 =	Ratio of isomers
Boc	**7a/7b** = 57:43
Troc	**8a/8b** = 63:37
$PhCH_2$	**9a/9b** = 37:63

(Eq. 2)

We then investigated the (3+2) cycloaddition reactions of *N*-protected Vince lactam (**2a**−**2c**) with benzonitrile oxide (**4b**). Thus, *N*-hydroxybenzimidoyl chloride (**5b**) was dissolved in toluene solution and slowly injected via syringe pump into a toluene solution containing *N*-Boc lactam (±)-**2a** and Et_3N (Eq. 2). The desired (3+2) cycloaddition proceeded smoothly at room temperature to give compounds (±)-**7a** and (±)-**7b**. The possible dimerization of reactive nitrile oxide was significantly suppressed by slow addition of hydroximoyl chloride to Et_3N for controlled generation of nitrile oxide, which was readily consumed by excess amount of alkene. Though the cycloaddition was much facilitated and no stereoisomers were formed, the regioselectivity of products was poor (**7a**/**7b** = 57:43). The isomers **7a** and **7b** were separated by silica gel chromatography, and the desired isomer **7a** (as racemic mixture) was further recrystallized from CH_2Cl_2–hexane for X-ray diffraction analysis (supplementary Fig. S1), thus confirming the regio- and stereochemistry.

On using *N*-Troc lactam (±)-**2b** as the dipolarophile, the regioselectivity of cycloaddition products (±)-**8a** and (±)-**8b** was slightly increased to 63:37. In contrast, the (3+2) cycloaddition of lactam (±)-**2c** bearing an electron-donating benzyl substituent favored the product of undesired regioisomer (±)-**9b**. Nonetheless, the minor isomer **9a** (as racemic mixture) was isolated and recrystallized from CH_2Cl_2–hexane to verify the regio- and stereochemistry by X-ray crystallography (supplementary Fig. S2).

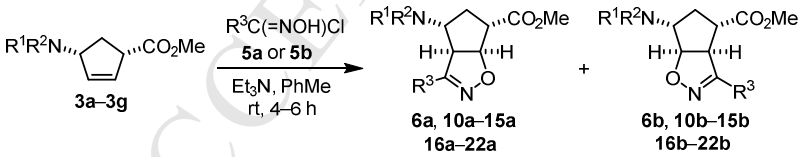
On the basis of photoelectron spectroscopic study [24], Novak and Kovač concluded that no transannular interaction exists between the π-moiety and amide group of Vince lactam, though the electronic effects are prominent in norbornadiene system. However, certain inductive effect might involve in the (3+2) cycloaddition reactions of *N*-substituted Vince lactam because the regioselectivity varies with different electronic properties of the substituents (e.g. Boc, Troc and benzyl groups in Eq. 2).

2.3. Effects of N-substituents in (3+2) cycloaddition reactions of cyclopentene dipolarophiles

Without success on using bicyclic alkenes **2a**−**2c** to establish the desired regiochemistry in the (3+2) cycloadditions with nitrile oxides, we re-examined the possible effects of *N*-substituents in the cyclopentene dipolarophiles **3a**−**3g**. The substituents include electron-withdrawing groups, such as *tert*-butoxycarbonyl (Boc), 2,2,2-trichloroethoxycarbonyl (Troc), (2-nitrophenyl)sulfonyl (Nosyl), (trifluoromethyl)sulfonyl (Tf), trifluoroacetyl, acetyl and phthalyl (Phth). The (3+2) cycloaddition reactions were similarly carried out with either benzonitrile oxide or 2-ethylbutanenitrile oxide, which were in situ generated from the corresponding hydroximoyl chlorides (Table 1). In comparison with the bicyclic dipolarophile (e.g. **2a**), the monocyclic dipolarophile (e.g. **3a**) was less reactive. The dimerization of nitrile oxides, once generated from hydroximoyl chlorides, could compete with the desired (3+2) cycloaddition reactions. Thus, more amount of hydroximoyl chloride was applied to ensure good conversion of cyclopentene dipolarophiles (Table 1).

Table 1

Substituent effect in the regioselectivity of (3+2) cycloaddition reactions[a]



entry	alkene (R^1, R^2)	$R^3C(=NOH)Cl$ (equiv)	conv. (%)[b]	ratio of isomers[c]
1	**3a** (Boc, H)	**5a** (2)	95[d]	**6a**/**6b** = 85:15[d]
2	**3b** (Troc, H)	**5a** (6)	75	**10a**/**10b** = 76:24
3	**3c** (Nosyl, H)	**5a** (8)	95	**11a**/**11b** = 68:32
4	**3d** (CF_3SO_2, H)	**5a** (8)	95	**12a**/**12b** = 68:32
5	**3e** (CF_3CO, H)	**5a** (7)	95	**13a**/**13b** = 80:20
6	**3f** (CH_3CO, H)	**5a** (8)	95	**14a**/**14b** = 80:20
7	**3g** (Phth)	**5a** (16)	45	**15a**/**15b** = 64:36
8	**3a** (Boc, H)	**5b** (5)	75	**16a**/**16b** = 67:33
9	**3b** (Troc, H)	**5b** (4)	90	**17a**/**17b** = 74:26

10	**3c** (Nosyl, H)	**5b** (6)	75	**18a**/**18b** = 74:26
11	**3d** (CF_3SO_2, H)	**5b** (4)	75	**19a**/**19b** = 66:34
12	**3e** (CF_3CO, H)	**5b** (4)	90	**20a**/**20b** = 87:13
13	**3f** (CH_3CO, H)	**5b** (4)	95	**21a**/**21b** = 76:24
14	**3g** (Phth)	**5b** (8)	60	**22a**/**22b** = 56:44

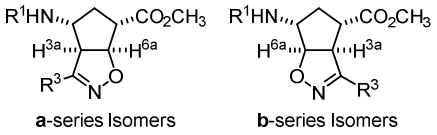
[a] The reactions of alkenes **3a**−**3g** with the nitrile oxides generated in situ from hydroximoyl chlorides **5a** and **5b** at room temperature.
[b] The conversion was estimated by ^{1}H NMR spectral analysis based on the remaining alkene (**3a**−**3g**) in the crude product mixture.
[c] The ratio of regioisomers was calculated based on the characteristic proton signals, such as H-3a, H-6a and CO_2CH_3, in the crude product mixture (Table 2).
[d] The reaction was conducted at 60 ^{o}C for 24 h.

For the 1,3-dipolar cycloaddition of **3a** bearing an *N*-Boc group in this study (Table 1, entry 1), the nitrile oxide **4a** was produced by adding hydroximoyl chloride **5a** to a toluene solution containing Et_3N, different from the previously reported method using 2-ethyl-1-nitrobutane as the precursor of **4a** [9,14]. Nonetheless, both methods gave the cycloaddition products **6a** and **6b** at 60 ^{o}C in a ratio of 85:15. We further found that the related (3+2) cycloaddition reaction of alkene (**3a**−**3g**) occurred readily at room temperature by slow injection of hydroximoyl chloride (**5a** or **5b**) to the toluene solution containing Et_3N (Table 1). The conversion of alkenes to cycloadducts was generally good (75−95%) as estimated by ^{1}H NMR spectral analysis, except for the reactions of alkene **3g** carrying phthalimido substituent (≤ 60% conversion).

The crude product mixture was subject to ^{1}H NMR spectral analysis to determine the ratio of regioisomers based on their characteristic protons, such as H-3a, H-6a and CO_2CH_3 (Table 2). In general speaking, most **a**-series isomers exhibited the H-6a at a lower field than that of **b**-series isomers, presumably because the H-6a in **a**-series isomers was deshielded by the adjacent carboxylate group. The similar deshielding effect was observed for the H-3a of **b**-series isomers. Some isomers could be separated by repeated silica gel chromatography, and 2D NMR spectra were applied to establish the chemical structures and assignment of protons.

Table 2

Comparison of chemical shifts of characteristic protons in **a**- and **b**-series regioisomers.[a]



compound	isomer **a**			isomer **b**		
	H-3a	H-6a	CO_2CH_3	H-3a	H-6a	CO_2CH_3
6a/6b	3.58	5.20	3.76	3.89	4.83	3.79
10a/10b	3.64	5.20	3.75	3.88	NA[b]	3.70
11a/11b	3.72	5.19	3.77	3.90	4.80	3.79
12a/12b	NA[b]	5.15	3.78	NA[b]	4.98	3.79
13a/13b	3.60	5.14	3.75	3.69	4.85	3.76
14a/14b	3.60	5.15	3.75	3.69	4.83	3.76
15a/15b	NA[b]	NA[b]	3.74	NA[b]	NA[b]	3.73
16a/16b	4.20	5.44	3.78	4.48	5.04	3.81
17a/17b	4.28	5.43	3.80	4.46	5.12	3.84
18a/18b	4.36	5.44	3.81	4.51	5.08	3.87
19a/19b	4.37	5.38	3.82	4.31	5.21	3.88
20a/20b	4.26	5.39	3.82	4.37	5.08	3.86
21a/21b	4.27	5.39	3.80	NA[b]	4.87	3.67
22a/22b	NA[b]	NA[b]	3.76	NA[b]	NA[b]	3.71

[a] Adapted from the ^{1}H NMR spectra (400 MHz) measured in $CDCl_3$ solution.
[b] NA is not available, due to complication by other signals.

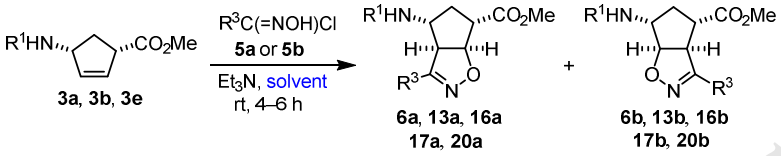
2.4. Effects of solvent, reaction temperature and catalyst

The regiochemistry in 1,3-dipolar cycloaddition reactions of nitrile oxides can be influenced by several factors such as solvent, reaction temperature, metal catalyst and Lewis acid [17−19,25,26]. In our present study, the solvent effect is most prominent (Table 3). The cycloaddition reaction appeared to proceed well in nonpolar solvent, such as hexane and toluene, to attain high regioselectivity. For example, the 1,3-dipolar cycloaddition of alkene **3b** (R^1 = Troc) with benzonitrile oxide in toluene or a mixed toluene−hexane system (Table 3, entries 7 and 8) showed higher regioselectivity for the desired product **17a** than that conducted in more polar solvent systems (Table 3, entries 9−12). In agreement with this trend, the cycloaddition reactions of alkenes **3a** (R^1 = Boc) and **3e** (R^1 = CF_3CO) in hexane solution also provided higher percentage of the desired **a**-series regioisomers than that were

performed in toluene solution. Though a previous report has demonstrated that the 1,3-dipolar cycloadditions of nitrile oxides with certain alkenes can proceed in aqueous solution [25], we did not test the possibility of using aqueous solvent in our case.

Table 3

Solvent effect in the regioselectivity of (3+2) cycloaddition reactions[a]



entry	alkene (R^1)	$R^3C(=NOH)Cl$ (equiv)	solvent	conv. (%)[b]	ratio of isomers[c]
1	**3a** (Boc)	**5a** (2)	PhMe	95[d]	**6a**/**6b** = 85:15[d]
2	**3a** (Boc)	**5a** (4)	hexane	97	**6a**/**6b** = 91:9
3	**3e** (CF_3CO)	**5a** (7)	PhMe	95	**13a**/**13b** = 80:20
4	**3e** (CF_3CO)	**5a** (7)	hexane	95	**13a**/**13b** = 90:10
5	**3a** (Boc)	**5b** (5)	PhMe	75	**16a**/**16b** = 67:33
6	**3a** (Boc)	**5b** (4)	hexane	95	**16a**/**16b** = 86:14
7	**3b** (Troc)	**5b** (4)	PhMe	90	**17a**/**17b** = 74:26
8	**3b** (Troc)	**5b** (4)	PhMe/hex (1:1)	90	**17a**/**17b** = 84:16
9	**3b** (Troc)	**5b** (4)	DMF	20	**17a**/**17b** = 62:38
10	**3b** (Troc)	**5b** (4)	CH_3CN	60	**17a**/**17b** = 63:37
11	**3b** (Troc)	**5b** (4)	THF	90	**17a**/**17b** = 68:32
12	**3b** (Troc)	**5b** (4)	CH_2Cl_2	90	**17a**/**17b** = 68:32
13	**3e** (CF_3CO)	**5b** (4)	PhMe	90	**20a**/**20b** = 87:13
14	**3e** (CF_3CO)	**5b** (4)	PhMe/hex (1:2)	95	**20a**/**20b** = 91:9
15	**3e** (CF_3CO)	**5b** (4)	hexane	95	**20a**/**20b** = 94:6

[a]–[d] Referring to the footnotes of Table 1.

The reaction temperature was not crucial to regioselectivity of the 1,3-dipolar cycloadditions in the present study. For example, the cycloaddition between **3b** and benzonitrile oxide in toluene solution (Table 3, entry 7) gave the products **17a**/**17b** in a ratio of 74:26, which was not appreciably affected by performing the reaction at −78, 0, 25 or 95 ^oC (supplementary Table S3).

It is well known that 1,3-dipolar cycloadditions of nitrile oxides with allylic alcohols or electron-deficient alkenes (e.g. α,β-unsaturated ester) can be facilitated by incorporation of

additives, such as metal-catalyst and Lewis acid, to gain good regio- and stereoselectivities [18,21,27]. Hydroximoyl chlorides **5a** and **5b** were treated with base Et_3N to generate the corresponding nitrile oxides for in situ cycloaddition with non-activated alkenes **3a**−**3g**, therefore, Lewis acid catalysis would not be compatible to the basic system in the present study. Indeed, none of additives $MgBr_2$, $Ti(O^iPr)_4$, $Zn(OAc)_2$ or $Zn(acac)_2$ could enhance the yield of the desired regioisomer **17a** (supplementary Table S3).

As an approach to peramivir and its analogs, the key (3+2) cycloadditions of **3a** (R^1 = Boc) were realized in hexane solution to give the desired products **6a** and **16a** in high regioselectivity (Table 3, entries 2 and 6). In comparison with the previously reported (3+2) cycloadditions of **3a** with nitrile oxide **4a** in toluene (or benzene) solution [14−16], our current method provided the desired isomers **6a** with somewhat better regioselectivity by conducting the cycloadditions in hexane solution. To our satisfaction, the (3+2) cycloadditions of alkene **3e** (R^1 = CF_3CO) with nitrile oxides **4a** and **4b** in hexane solution afforded the desired products **13a** and **20a** in optimized regioselectivity (Table 3, entries 4 and 15). This synthetic procedure could be performed in gram scale to provide **13a** and **20a** in ~80% isolated yield after purification by silica gel chromatography.

2.5 Rationale for regioselectivity

The 1,3-dipolar cycloadditions of nitrile oxides to disubstituted alkenes generally lead to mixtures of regioisomers, unlike the more regioselective reactions with monosubstituted alkenes and conjugated dipolarophiles, which have more distinct steric and electronic effects [28]. Although the regioselectivity has been interpreted by the HOMO, LUMO or combined HOMO–LUMO controlled modes [28], it is still difficult to predict the regioselectivity in the (3+2) cycloaddition of disubstituted alkene by frontier orbital theory.

In comparison, the (3+2) cycloaddition reactions of alkene **3e** bearing trifluoroacetamido substituent tended to attain high percentage of **a**-series isomers (**13a** and **20a**), whereas the (3+2) cycloaddition reactions of alkene **3g** carrying phthalimido substituent resulted in low regioselectivity (Table 1, entries 7 and 14). We speculate that the high regioselectivity in (3+2) cycloaddition of **3e** might be attributable to the intramolecular hydrogen bonded form, which is devoid in **3g**. Our preliminary density functional theory (DFT) calculation supports the preferable structure of **3e** (Fig. 2) having the trifluoroacetamide and ester moieties in proximity to form intramolecular hydrogen bond (N-H•••OCO). The intramolecular hydrogen bonded form also explains why the regioselectivity increased when (3+2) cycloaddition reaction was conducted in nonpolar solvent. As the conformation of cyclopentene (e.g. **3e**) is rigidified by intramolecular bonding, the influence of electron-withdrawing substituent (e.g. trifluoroacetyl), either through bonds or through space, on the C=C double bond would become substantial [24].

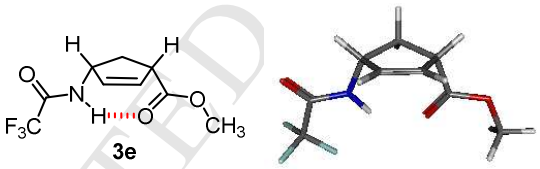
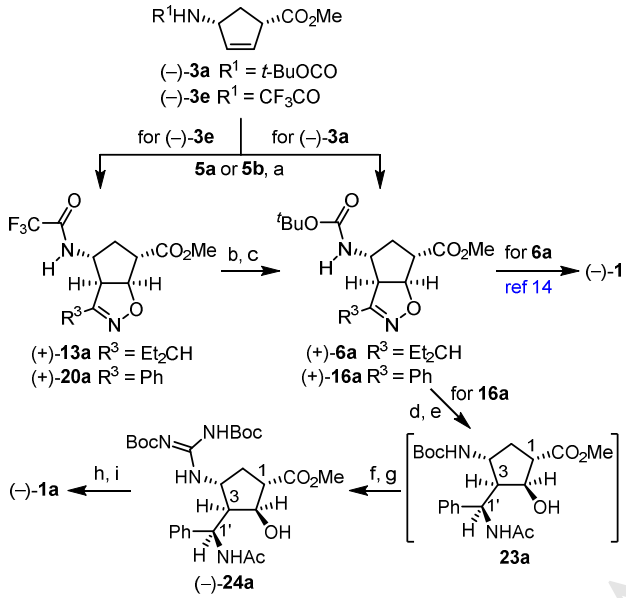


Fig. 2. DFT calculation for the optimized geometry of compound **3e**. The functional CAM-B3LYP and the basis set 6-31G(d) were used in the calculation. The number of imaginary frequencies = 0. Gibbs free energy = –928.430500 Hartree.

*2.6. Alternative approach to peramivir (−)-**1** and analog (−)-**1a***

In the conventional synthesis of (−)-peramivir [14], the key intermediate (+)-**6a** is prepared in 61% yield by the (3+2) cycloaddition of alkene (−)-**3a** with nitrile oxide **4a** in refluxing toluene (or benzene) solution for 24 h (Eq. 1). In this study (Scheme 1), we found

that the cycloaddition could be performed in hexane solution at room temperature for 4 h to provide a better yield (66%) of (+)-**6a** because of the improved regioselectivity (Table 3, entry 2).



Scheme 1. Synthesis of peramivir (−)-**1** and analog (−)-**1a** bearing a phenyl group at C-1′ position. *Reagents and conditions*: (a) Et_3N, hexane, rt, 4 h; isolated yield: (+)-**6a**, 66%; (+)-**13a**, 80%; (+)-**16a**, 52%; (+)-**20a**, 83%; (b) HCl in MeOH, reflux, 23 h; (c) Boc_2O, Et_3N, CH_2Cl_2, 0 $^oC \rightarrow$ rt, 1.5 h; isolated yield: (+)-**6a**, 74%; (+)-**16a**, 73%; (d) Raney nickel, H_2, THF, rt, 30 min; (e) Ac_2O, Et_3N, MeOH, 0 $^oC \rightarrow$ rt, 12 h; (f) CF_3CO_2H, CH_2Cl_2, 0 $^oC \rightarrow$ rt, 20 min; (g) 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea, $HgCl_2$, Et_3N, CH_2Cl_2, 14 h; 32% yield from (+)-**16a**; (h) NaOH, 30 min; (i) H_2O, reflux, 1.5 h; 99% yield.

As shown in Table 3, the (3+2) cycloadditions of alkene **3e** ($R^1 = CF_3CO$) with nitrile oxides were conducted in hexane solution to afford the desired products **13a** and **20a** in optimized regioselectivity. We thus explored the possibility in utilizing the isoxazolines **13a** and **20a** for syntheses of peramivir and its analog **1a** bearing a phenyl group at C-1′ position.

Accordingly [14], optically active Vince lactam was subjected to solvolysis in methanolic HCl, and the resulting amino ester was treated with trifluoroacetic anhydride in

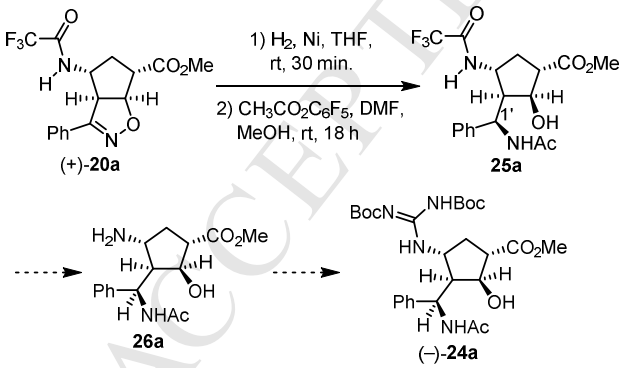
the presence of Et_3N to give (−)-**3e** bearing a trifluoroacetamido substituent. The (3+2) cycloaddition reaction was carried out by slow injection of a hexane solution of hydroximoyl chloride **5a** via syringe pump into a mixture of alkene (−)-**3e** and Et_3N in hexane to afford isoxazoline isomers **13a** and **13b** (90:10). The desired product (+)-**13a** was isolated in 80% yield by silica gel column chromatography. Compound (+)-**13a** was heated in methanolic HCl to release the amino group, followed by treatment with di-*tert*-butyl dicarbonate to give 74% yield of the *N*-Boc derivative (+)-**6a**. The physical and spectral properties of as-synthesized (+)-**6a** were in agreement with that prepared by the conventional procedure [14]. Thus, the formal synthesis of peramivir (−)-**1** was realized, though no better yield of isoxazoline (+)-**6a** was obtained by the two-step preparation via intermediacy of (+)-**13a**.

For the synthesis of peramivir analog **1a**, benzonitrile oxide was generated from hydroximoyl chloride **5b** in the presence of Et_3N and reacted in situ with (−)-**3a** to form isoxazoline (+)-**16a** in 52% yield. Alternatively, isoxazoline (+)-**20a** was prepared in high yield by the (3+2) cycloaddition of alkene (−)-**3e** with benzonitrile oxide. The trifluoroacetyl group of (+)-**20a** was removed by acid-catalyzed solvolysis, and subsequently converted to *N*-Boc group to afford (+)-**16a**. The two-step sequence gave a 61% overall yield of (+)-**16a** from (−)-**3e** ($R^1 = CF_3CO$), better than that derived from a direct (3+2) cycloaddition of (−)-**3a** (R = Boc).

The isoxazoline ring in (+)-**16a** was subjected to hydrogenolysis over Raney nickel to furnish *N*–*O* cleavage and C=N reduction [14−16,20]. On treatment with acetic anhydride, the amino group of intermediate product was converted to acetamide, giving a mixture of diastereomers **23a** and **23b** (85:15) differing at the C-1′ center. In ^{1}H NMR spectra, the major isomer **23a** with (1′*R*)-configuration exhibited its H-1′ signal at δ 5.47, whereas isomer **23b** displayed the H-1′ at a higher field (δ 5.24).

The Boc group in **23a**/**23b** was subsequently removed on treatment with trifluoroacetic acid. The resulting aminium salt was treated with a thiopseudourea reagent, *N*,*N*′-bis-(*tert*-butoxycarbonyl)-*S*-methylisothiourea, to afford the bis-Boc-guanidine compound **24a**/**24b**. The desired product (−)-**24a** was obtained by column chromatography in 32% overall yield starting from (+)-**16a**. After saponification of (−)-**24a**, the Boc protecting groups were successfully removed by heating in water [20,29] to yield the peramivir analog (−)-**1a** bearing a phenyl group at the C-1′ position.

In an attempt to obtain (−)-**24a** via a shorter pathway (Scheme 2), (+)-**20a** was reduced by Raney nickel under an atmosphere of hydrogen. The resulting amino alcohol was treated with pentafluorophenyl acetate to afford compound **25a** and its (1′*S*)-diastereomer (85:15). However, the selective hydrolysis of trifluoroaceamide in the presence of acetamide and methyl ester posed a problem [30]. So far, we have tried several reaction conditions for the hydrolysis of **25a** in either acidic or basic conditions, including refluxing in 1 M HCl aqueous solution, refluxing in 35% NH_3 methanolic solution, refluxing or stirring with K_2CO_3 in anhydrous or moist MeOH, without success to obtain the desired product **26a**.



Scheme 2. An attempted conversion of (+)-**20a** to (−)-**24a** via a short pathway.

3. Conclusion

We demonstrate in this study that chlorination of oximes with NCS gave the relatively stable hydroximoyl chlorides **5a** and **5b** as convenient precursors of nitrile oxides **4a** and **4b** for in situ (3+2) cycloadditions with alkenes. Because our initial plan of using Vince lactam as a reactive 1,3-dipolarphile could not afford good regioselectivity, we turned to investigate the (3+2) cycloaddition reactions of the cyclopentene dipolarophiles **3a**−**3g** equipped with various *N*-substituents. A suitable electron withdrawing substituent (e.g. Boc, Troc and trifluoroacetyl) on the dipolarophiles was an important factor to induce the regioselectivity. Both aliphatic nitrile oxide (**4a**) and aromatic nitrile oxide (**4b**) showed a similar trend of regioselectivity in the (3+2) cycloaddition reactions. The cycloaddition reactions proceeded smoothly in nonpolar solvent (e.g. hexane and toluene) to give high percentage of the desired **a**-series regioisomers. Up to 94:6 regioselectivity of the (3+2) cycloaddition between **3e** (R^1 = CF_3CO) and benzonitrile oxide was obtained by stirring in hexane solution at room temperature for 4 h (Table 3, entry 15).

The key intermediate (+)-**6a** for the synthesis of (−)-peramivir was obtained from the cycloaddition of (−)-**3a** (R^1 = Boc) in hexane solution, showing better regioselectivity (91:9) than that performed in toluene solution (compared entries 1 and 2 in Table 3). We also carried out the 1,3-dipolar cycloadditions of (−)-**3e** in high regioselectivity to give isoxazolines (+)-**13a** and (+)-**20a**, and demonstrated how to convert them into the *N*-Boc analogs (+)-**6a** and (+)-**16a** as an alternative approach to the syntheses of (−)-peramivir and the analog (−)-**1a** having phenyl group in place of the 3-pentyl moiety.

4. Experimental section

4.1. General

All the reagents and solvents were reagent grade and used without further purification unless otherwise specified. Dichloromethane (CH_2Cl_2) was distilled from CaH_2. All air or moisture sensitive experiments were performed under an atmosphere of argon. Analytic thin-layer chromatography (TLC) was performed on 0.25 mm E. Merck silica gel 60 F_{254} glass plates. TLC plate visualization was performed with UV light, potassium permanganate, ninhydrin or phosphomolybdic acid (PMA). E. Merck silica gel 60 (0.040–0.063 mm particle sizes) was used for column chromatography. High-performance liquid chromatography (HPLC) was conducted on a Schambeck SFD GmbH SDS2100 instrument equipped with Schambeck SFD GmbH S-3210 UV/Vis detector set at absorption wavelength of 365 nm. Dikma column (250 × 10.0 mm) packed with Inspire^{TM} silica (10 μm) was used for HPLC, and an eluent of hexane/EtOAc (86:14) at a flow rate of 1.5 mL/min was applied.

Melting points were recorded on a Yanaco micro apparatus and are uncorrected. Optical rotations were measured on digital polarimeter of Japan JASCO Co. P-2000. $[\alpha]_D$ values are given in the unit of 10^{-1} deg cm^2 g^{-1}. Infrared (IR) spectra was recorded on Nicolet Manga 550-II. Nuclear magnetic resonance (NMR) spectra were obtained on Varian Unity Plus-400 (400 MHz) or Bruker A VIII (500 MHz) NMR. Chemical shifts (δ) are given in parts per million (ppm) relative to residual $CDCl_3$ solvent: δ_H 7.24 and δ_C 77.0 (the central line of triplet). Coupling constants (*J*) are given in hertz (Hz) and the splitting patterns are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). The electrospray ionization high resolution mass spectra (ESI–HRMS) experiments were conducted on a Bruker Daltonics BioTOF III high-resolution mass spectrometer.

4.2. Representative procedure A: 1,3-dipolar cycloaddition of N-protected Vince lactam with benzonitrile oxide generated in situ from hydroximoyl chloride

A solution of *N*-Boc lactam (±)-**2a** (418 mg, 2.0 mmol) and triethylamine (0.83 mL, 6.0 mmol) in toluene (2.0 mL) was stirred at room temperature under an atmosphere of argon. A solution of hydroximoyl chloride **5b** (467 mg, 3.0 mmol) in toluene (12 mL) was injected via syringe pump at a rate of 3.0 mL/h. After completion of injection, the mixture was extracted with EtOAc and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. ^{1}H NMR spectral analysis indicated that the crude product mixture contained cycloaddition products (±)-**7a** and (±)-**7b** in a ratio of 57:43. Compounds **7a** and **7b** were separated by silica gel chromatography (CH_2Cl_2), and **7a** was further recrystallized from CH_2Cl_2–hexane for X-ray diffraction analysis.

4.3. Representative procedure B: 1,3-dipolar cycloaddition of N-protected 4-aminocyclopent-2-ene-1-carboxylate with nitrile oxide generated in situ from hydroximoyl chloride

A solution of alkene (±)-**3e** (24 mg, 0.1 mmol) and triethylamine (110 μL, 0.8 mmol) in hexane (1.0 mL) was stirred at room temperature under an atmosphere of argon. A solution of 2-ethyl-*N*-hydroxybutanimidoyl chloride (**5a**, 104 mg, 0.7 mmol) in hexane (8.0 mL) was injected via syringe pump at a rate of 2.0 mL/h. After completion of injection, the mixture was extracted with EtOAc and brine. After completion of injection, the mixture was extracted with EtOAc and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. ^{1}H NMR spectral analysis indicated the crude product mixture contained cycloaddition products (±)-**13a** and (±)-**13b** in a ratio of 90:10.

4.4. Representative procedure C: conversion of N-trifluoroacetyl protected cycloaddition product to N-Boc protected analog

Trifluoroacetamide (+)-**20a** (1.02 g, 3.1 mmol) was dissolved in 4 M HCl methanolic solution and heated at boiling for 23 h. The solution was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2, added Et_3N (1.3 mL, 9.3 mmol) and Boc_2O (0.93 mL, 4.0 mmol) at 0 ^oC. The mixture was stirred at room temperature for 1.5 h, and then concentrated under reduced pressure. The residue was re-dissolved in CH_2Cl_2, washed with H_2O, dried over $MgSO_4$, and filtered. The filtrate was concentrated by rotatory evaporation under reduced pressure. The residue was purified by silica gel column chromatography (hexane/EtOAc = 10:1) to afford carbamate (+)-**16a** (819 mg) in 73% overall yield from (+)-**20a**.

4.5. Synthetic procedure and compound characterization

*4.5.1. Tert-Butyl 3-oxo-2-azabicyclo[2.2.1]hept-5-ene-2-carboxylate (**2a**)*

To a solution of lactam (±)-**2** (307 mg, 3.0 mmol) and DMAP (183 mg, 1.5 mmol) in acetonitrile (3 mL) was added Boc_2O (687 mg, 3.15 mmol). The mixture was stirred at room temperature for 3 h, concentrated under reduced pressure, and purified by silica gel chromatography (hexane/EtOAc = 5:1) to afford the Boc derivative (±)-**2a** (531 mg, 85% yield). $C_{11}H_{15}NO_3$; white solid; mp 53–54 ^oC; TLC (hexane/EtOAc = 5:1) R_f = 0.46; IR ν_{max} (neat) 3384, 2980, 2939, 2880, 1790, 1710, 1369, 1335, 1152, 991, 935 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.76 (1 H, ddd, *J* = 5.6, 2.4, 0.4 Hz), 6.53 (1 H, ddd, *J* = 5.2, 3.2, 1.6 Hz), 4.85–4.79 (1 H, m), 3.27–3.22 (1 H, m), 2.21 (1 H, dt, *J* = 8.4, 1.6 Hz), 2.02 (1 H, dt, *J* = 8.8, 1.6 Hz), 1.36 (9 H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.1, 150.2, 139.8, 138.0, 82.4, 62.3, 54.8, 54.2, 27.9 (3 ×); ESI−HRMS calcd for $C_{11}H_{15}NO_3Na$: 232.0948, found *m/z* 232.0944 $[M + Na]^+$.

*4.5.2. 2,2,2-Trichloroethyl 3-oxo-2-azabicyclo[2.2.1]hept-5-ene-2-carboxylate (**2b**)*

To a solution of lactam (±)-**2** (109 mg, 1.0 mmol) and triethylamine (0.50 mL, 3.6 mmol) in CH_2Cl_2 (5 mL) was added 2,2,2-trichloroethyl chloroformate (0.46 mL, 3.3 mmol) at 0 ^oC under an atmosphere of argon. The mixture was stirred at room temperature for 12 h, and extracted with EtOAc and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (EtOAc/hexane = 1:10) to afford the Troc derivative (±)-**2b** (68.0 mg, 24% yield). $C_9H_8Cl_3NO_3$; white solid; mp 99–100 ^oC; TLC (hexane/EtOAc = 5:1) R_f = 0.38; IR ν_{max} (neat) 3007, 2956, 2876, 1803, 1380, 1338, 1276, 1182, 1121, 999, 882, 756, 714 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.88 (1 H, dd, *J* = 5.2, 2.4 Hz), 6.69–6.61 (1 H, m), 5.10–5.04 (1 H, m), 4.79 (1 H, d, *J* = 12.0 Hz), 4.73 (1 H, d, *J* = 12.0 Hz), 3.44–3.39 (1 H, m), 2.38 (1 H, dt, *J* = 8.4, 1.6 Hz), 2.21 (1 H, dt, *J* = 8.8, 1.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.5, 148.4, 139.9, 138.2, 94.5, 74.7, 62.8, 54.5, 53.7; ESI−HRMS calcd for $C_9H_8Cl_3NO_3$: 283.9639, found *m/z* 283.9643 $[M + H]^+$.

*4.5.3. 2-Benzyl-2-azabicyclo[2.2.1]hept-5-en-3-one (**2c**)*

Under an atmosphere of argon, sodium hydride (80 mg of a 60% suspension in mineral oil, 2.0 mmol) was placed in a flask, washed with hexane, and added anhydrous THF (4 mL). A solution of lactam (±)-**2** (109 mg, 1.0 mmol) in anhydrous THF (2 mL) was added dropwise. The mixture was stirred at room temperature for 30 min, and benzyl bromide (0.24 mL, 2.0 mmol) was added dropwise. After stirring for 8 h, the mixture was extracted with EtOAc and H_2O. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc = 5:1) to afford the benzyl derivative (±)-**2c** (144 mg, 72% yield). $C_{13}H_{13}NO$; brown oil; TLC (hexane/EtOAc = 5:1) R_f = 0.22; IR ν_{max} (neat) 3065, 3027, 3005, 2948, 2868, 1719, 1558,

1496, 1455, 1391, 1356, 1232, 1075, 930, 836, 757, 699 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30–7.19 (3 H, m), 7.17–7.13 (2 H, m), 6.62–6.44 (2 H, m), 4.40 (1 H, d, *J* = 14.8 Hz), 4.01–3.97 (1 H, m), 3.93 (1 H, d, *J* = 14.8 Hz), 3.37–3.32 (1 H, m), 2.25 (1 H, dt, *J* = 7.6, 1.6 Hz), 2.03 (1 H, dt, *J* = 7.6, 1.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.9, 139.4, 137.1, 136.3, 128.4 (2 ×), 128.2 (2 ×), 127.4, 62.5, 58.2, 53.6, 47.8; ESI−HRMS calcd for $C_{13}H_{14}NO$: 200.1072, found *m/z* 200.1075 $[M + H]^+$.

*4.5.4. Methyl 4-((tert-butoxycarbonyl)amino)cyclopent-2-ene-1-carboxylate (**3a**)*

Lactam (±)-**2** (109 mg, 1.0 mmol) was placed in a flask under an atmosphere of argon, and hydrochloride in methanol (1.25 M, 3.0 mL) was added. The mixture was heated under reflux for 12 h, and then concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (5 mL), and treated successively with triethylamine (170 μL, 1.2 mmol) and Boc_2O (0.28 mL, 1.2 mmol). The mixture was stirred at room temperature for 1 h, and extracted with CH_2Cl_2, 1 M $HCl_{(aq.)}$ and water. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (EtOAc/hexane = 1:20) to afford the Boc derivative (±)-**3a** (190.0 mg, 79% yield). $C_{12}H_{19}NO_4$; colorless oil; TLC (hexane/EtOAc = 5:1) R_f = 0.32; IR ν_{max} (neat) 3374, 3067, 2978, 2928, 1737, 1521, 1436, 1330, 1157, 1061, 860 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.95–5.74 (2 H, m), 4.87 (1 H, br), 4.76 (1 H, br), 3.69 (3 H, s), 3.49–3.41 (1 H, m), 2.47 (1 H, dt, *J* = 14.0, 8.8 Hz), 1.84 (1 H, dt, *J* = 14.0, 4.0 Hz), 1.42 (9 H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.6, 154.9, 134.6, 130.8, 79.0, 55.6, 51.9, 48.9, 34.3, 28.2 (3 ×); ESI−HRMS calcd for $C_{12}H_{19}NO_4Na$: 264.1212, found *m/z* 264.1216 $[M + Na]^+$.

*4.5.5. Methyl 4-(((2,2,2-trichloroethoxy)carbonyl)amino)cyclopent-2-ene-1-carboxylate (**3b**)*

By a procedure similar to that for **3a**, lactam (±)-**2** (109 mg, 1.0 mmol) was heated with hydrochloride in methanol (1.25 M) under reflux for 12 h, and the intermediate product was treated with 2,2,2-trichloroethyl chloroformate (0.17 mL, 1.2 mmol) in CH_2Cl_2 solution in the presence of Et_3N to give the Troc derivative (±)-**3b** (200 mg, 63% yield). $C_{10}H_{12}Cl_3NO_4$; white solid; mp 66–67 °C; TLC (hexane/EtOAc = 8:1) R_f = 0.18; IR ν_{max} (neat) 3345, 3074, 2953, 2844, 1732, 1520, 1436, 1328, 1093, 1052, 942, 819, 727, 569 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.80–5.91 (2 H, m), 5.51 (1 H, d, *J* = 9.2 Hz), 4.79 (1 H, td, *J* = 8.8, 3.2 Hz), 4.68 (1 H, d, *J* = 12.0 Hz), 4.64 (1 H, d, *J* = 12.0 Hz), 3.66 (3 H, s), 3.46 (1 H, ddd, *J* = 8.4, 4.0, 0.8 Hz), 2.47 (1 H, dt, *J* = 14.0, 8.4 Hz), 1.90 (1 H, dt, *J* = 14.0, 4.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 153.7, 133.9, 131.9, 95.5, 74.3, 56.4, 52.2, 49.1, 34.3; ESI−HRMS calcd for $C_{10}H_{12}Cl_3NO_4Na$: 337.9706, found *m/z* 337.9724 $[M + Na]^+$.

*4.5.6. Methyl 4-((2-nitrophenyl)sulfonamido)cyclopent-2-ene-1-carboxylate (**3c**)*

By a procedure similar to that for **3a**, lactam (±)-**2** (218 mg, 2.0 mmol) was heated with hydrochloride in methanol (1.25 M) under reflux for 12 h, and the intermediate product was treated with 4-nitrobenzenesulfonyl chloride (485 mg, 2.2 mmol) in CH_2Cl_2 solution in the presence of Et_3N (0.34 mL, 2.4 mmol) and DMPA (24 mg, 0.2 mmol) to give the Nosyl derivative (±)-**3c** (239 mg, 47% yield). $C_{13}H_{14}N_2O_6S$; white solid; mp 117–118 °C; TLC (EtOAc/hexane = 1:3) R_f = 0.15; IR ν_{max} (neat) 3648, 3308, 3098, 2955, 2849, 1732, 1693, 1538, 1442, 1360, 1166, 1061, 1005, 914, 786, 731, 656, 598, 562 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.15–8.08 (1 H, m), 7.84–7.77 (1 H, m), 7.75–7.68 (2 H, m), 5.89–5.80 (2 H, m), 5.66 (1 H, dt, *J* = 5.6, 2.4 Hz,), 4.65–4.55 (1 H, m), 3.65 (3 H, s), 3.42–3.34 (1 H, m), 2.28 (1 H, dt, *J* = 14.4, 8.4 Hz), 1.83 (1 H, dt, *J* = 14.0, 3.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.0, 147.7, 134.7, 133.6, 133.1, 132.9, 132.9, 130.4, 125.2, 59.4, 52.3, 49.0, 34.0; ESI−HRMS calcd for $C_{13}H_{14}N_2O_6SNa$: 349.0460, found *m/z* 349.0465 $[M + Na]^+$.

*4.5.7. Methyl 4-((trifluoromethyl)sulfonamido)cyclopent-2-ene-1-carboxylate (**3d**)*

By a procedure similar to that for **3a**, lactam (±)-**2** (218 mg, 2.0 mmol) was heated with hydrochloride in methanol (1.25 M) under reflux for 12 h, and the intermediate product was treated with trifluoromethanesulfonic anhydride (0.19 mL, 1.1 mmol) in CH_2Cl_2 solution in the presence of Et_3N to give the triflate derivative (±)-**3d** (164 mg, 60% yield). $C_8H_{10}F_3NO_4S$; colorless liquid; TLC (EtOAc /hexane = 1:8) R_f = 0.20; IR ν_{max} (neat) 3650, 3296, 3233, 3959, 2900, 2851, 1721, 1452, 1384, 1235, 1065, 1009, 928, 788, 741, 613, 572 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.02–5.95 (2 H, m), 5.91 (1 H, d, *J* = 8.4 Hz), 4.64 (1 H, t, *J* = 8.8 Hz), 3.72 (3 H, s), 3.55–3.48 (1H, m), 2.41 (1 H, dt, *J* = 14.4, 8.0 Hz), 2.09 (1 H, dt, *J* = 14.4, 1.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.5, 133.5, 133.2, 119.6 ($^1J_{CF}$ = 319 Hz), 60.1, 52.7, 49.0, 34.9; ^{19}F NMR (376 MHz, $CDCl_3$) δ −78.3 (3 ×); ESI−HRMS calcd for $C_8H_9F_3NO_4S$: 272.0204, found *m/z* 272.0200 $[M - H]^-$.

*4.5.8. Methyl 4-(2,2,2-trifluoroacetamido)cyclopent-2-ene-1-carboxylate (**3e**)*

Under an atmosphere of argon, a mixture of lactam (±)-**2** (1.09 g, 10.0 mmol) and hydrochloride in methanol (1.25 M, 20 mL) was heated under reflux for 12 h. The mixture was then concentrated under reduced pressure. To the residue were added CH_2Cl_2 (10 mL) and triethylamine (4.66 mL, 33.5 mmol). Trifluoroacetic anhydride (3.34 mL, 24.0 mmol) was then added dropwise at 0 ^oC. The mixture was stirred at room temperature for 18 h to complete the acylation reaction. The mixture was extracted with CH_2Cl_2 and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc = 10:1) to afford (±)-**3e**, 1.76 g, 75% yield). By a similar procedure, optically active lactam (−)-**2** underwent solvolysis in 4 M HCl methanolic solution for 2.5 h, followed by acylation with

trifluoroacetic anhydride, to give (−)-**3e**. $C_9H_{10}F_3NO_3$; colorless oil; $[\alpha]^{20}_D$ = −47.4 (*c* = 2.0, $CHCl_3$); TLC (EtOAc/hexane = 1:8) R_f = 0.15; IR ν_{max} (neat) 3315, 3081, 2958, 2918, 2849, 1720, 1551, 1439, 1349, 1215, 1161 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.26 (1 H, br), 5.99 (1 H, dd, *J* = 5.6, 2.8 Hz), 5.94 (1 H, dt, *J* = 5.4, 2.0 Hz), 5.06 (1 H, t, *J* = 4.4 Hz), 3.72 (3 H, s), 3.55 (1 H, dq, *J* = 8.0, 2.0 Hz), 2.42 (1 H, dt, *J* = 14.4, 8.4 Hz), 1.99 (1 H, dt, *J* = 14.0, 2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 156.0 ($^2J_{CF}$ = 37 Hz) , 133.4, 132.9, 115.8 ($^1J_{CF}$ = 286 Hz), 54.5, 52.5, 49.2, 33.9; ^{19}F NMR (376 MHz, $CDCl_3$) δ −76.6 (3 ×); ESI−HRMS calcd for $C_9H_{11}F_3NO_3$: 238.0691, found *m/z* 238.0696 $[M + H]^+$.

*4.5.9. Methyl 4-acetamidocyclopent-2-ene-1-carboxylate (**3f**)*

By a procedure similar to that for **3a**, lactam (±)-**2** (218 mg, 2.0 mmol) was heated with hydrochloride in methanol (1.25 M) under reflux for 12 h, and the intermediate product was treated with acetic anhydride (0.28 mL, 3.0 mmol) in CH_2Cl_2 solution in the presence of Et_3N to give the acetamide derivative (±)-**3f** (234 mg, 64% yield). $C_9H_{13}NO_3$, white solid; mp 59–60 °C; TLC (EtOAc/hexane = 2:1) R_f = 0.29; IR ν_{max} (neat) 3587, 3378, 3273, 3065, 2953, 2847, 1738, 1652, 1541, 1437, 1375, 1174, 1065, 1009, 927, 741, 603 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.01 (1 H, br), 5.91–5.80 (2 H, m), 5.09–5.01 (1 H, m), 3.69 (3 H, s), 3.53–3.44 (1 H, m), 2.43 (1 H, dt, *J* = 14.0, 8.4 Hz), 1.93 (3 H, s), 1.85 (1 H, dt, *J* = 14.0, 3.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.4, 169.1, 134.6, 131.6, 54.2, 52.3, 49.2, 34.3, 23.4; ESI−HRMS calcd for $C_9H_{13}NO_3Na$: 206.0793, found *m/z* 206.0796 $[M + Na]^+$.

*4.5.10. Methyl 4-(1,3-dioxoisoindolin-2-yl)cyclopent-2-ene-1-carboxylate (**3g**)*

Under an atmosphere of argon, lactam (±)-**2** (218 mg, 2.0 mmol) was placed in a flask, and hydrochloride in methanol (1.25 M, 2.5 mL) was added. The mixture was heated under reflux for 12 h, and then concentrated under reduced pressure. The residue was dissolved in

1,2-dichloroethane (5 mL), and phthalic anhydride (163 mg, 1.1 mmol) and DMAP (146 mg, 1.2 mmol) were successively added. The mixture was stirred at room temperature for 17 h, and extracted with CH_2Cl_2 and 1 M $HCl_{(aq.)}$ and water. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc = 10:1) to afford the phthalimide derivative (±)-**3g** (117 mg, 43% yield). $C_{15}H_{13}NO_4$; white solid; mp 72–73 °C; TLC (EtOAc/hexane = 1:3) R_f = 0.37; IR ν_{max} (neat) 2953, 1771, 1710, 1467, 1392, 1370, 1203, 1172, 1113, 933, 891, 718, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.82–7.76 (2 H, m), 7.71–7.65 (2 H, m), 6.14 (1 H, dt, *J* = 5.6, 2.4 Hz), 5.78 (1 H, dt, *J* = 5.6, 2.4 Hz), 5.38–5.29 (1 H, m), 3.77 (3 H, s), 3.68–3.59 (1 H, m), 2.66 (1 H, dt, *J* = 14.0, 9.2 Hz), 2.50 (1 H, dt, *J* = 13.6, 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 167.8 (2 ×), 133.8 (2 ×), 132.0, 131.8, 130.6 (2 ×), 123.1 (2 ×), 54.7, 52.0, 49.5, 31.0; ESI−HRMS calcd for $C_{15}H_{14}NO_4$: 272.0923, found *m/z* 272.0907 $[M + H]^+$.

*4.5.11. 2-Ethyl-N-hydroxybutanimidoyl chloride (**5a**)*

To a solution of 2-ethylbutanal oxime (575 mg, 5.0 mmol) in DMF (5.0 mL) was added *N*-chlorosuccinimide (800.0 mg, 6.0 mmol) at room temperature. The mixture was stirred for 4 h, and concentrated under reduced pressure. The residue was extracted with EtOAc and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford hydroximoyl chloride **5a** (566 mg, 76% yield). $C_6H_{12}ClNO$; colorless liquid; TLC (EtOAc/hexane = 1:2) R_f = 0.17; IR ν_{max} (neat) 3440, 3062, 3028, 2981, 2891, 2753, 2602, 1955, 1882, 1811, 1633, 1578, 1493, 1445, 1304, 1289, 1210, 1074, 967, 869, 756, 692 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.66 (1 H, br), 2.37 (1 H, tt, *J* = 9.2, 5.2 Hz), 1.66–1.44 (4 H, m), 0.85 (6 H, t, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.98, 49.51, 25.26 (2 ×), 11.47 (2 ×).

*4.5.12. N-Hydroxybenzimidoyl chloride (**5b**)*

To a solution of benzaldehyde oxime (605 mg, 5.0 mmol) in DMF (5 mL) was added *N*-chlorosuccinimide (730 mg, 5.5 mmol) at room temperature. The mixture was stirred for 4 h, and concentrated under reduced pressure. The residue was extracted with EtOAc and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford hydroximoyl chloride **5b** as a mixture of *E*- and *Z*-isomers (713 mg, 92% yield). C_7H_6ClNO; oily solid; TLC (EtOAc/hexane = 1:5) R_f = 0.57 and 0.61; IR ν_{max} (neat) 3405, 3063, 2972, 2934, 2877, 1766, 1635, 1606, 1461, 1383, 1279, 1172, 1146, 1089, 1059, 971, 923, 816 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, containing *E*-and *Z*-isomers) δ 8.14 (1 H, s), 7.60–7.53/7.96–7.93 (2 H, m), 7.41–7.35/7.45–7.39 (3 H, m); ^{13}C NMR (100 MHz, $CDCl_3$, containing *E*-and *Z*-isomers) δ 140.6, 132.2, 130.7, 128.4 (2 ×), 127.1(2 ×).

*4.5.13. Tert-Butyl 6-oxo-3-phenyl-3a,6,7,7a-tetrahydro-4,7-methanoisoxazolo[4,5-c]pyridine-5(4H)-carboxylate (**7a**) and tert-butyl 5-oxo-3-phenyl-4,5,7,7a-tetrahydro-4,7-methanoisoxazolo[5,4-c]pyridine-6(3aH)-carboxylate (**7b**)*

According to the representative procedure A, the cycloaddition of (±)-**2a** (418 mg, 2.0 mmol) with benzonitrile oxide, which was generated in situ from hydroximoyl chloride **5b** (467 mg, 3.0 mmol), was carried out to give a crude product containing (±)-**7a** and (±)-**7b** in a ratio of 57:43 as shown by the ^1H NMR analysis. Some **7a** (121 mg, 18% yield) and **7b** (54 mg, 8% yield) were isolated by repeated silica gel chromatography (CH_2Cl_2). Compound (±)-**7a** was further recrystallized from CH_2Cl_2–hexane for X-ray diffraction analysis.

Compound **7a**: $C_{18}H_{20}N_2O_4$; white solid; mp 174–176 ^oC; TLC (CH_2Cl_2) R_f = 0.35; IR ν_{max} (neat) 2975, 2924, 2852, 1793, 1702, 1358, 1298, 1146, 887, 773 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.83–7.75 (2 H, m), 7.45–7.40 (3 H, m), 5.23 (1 H, dt, *J* = 8.4, 1.6 Hz), 4.79–4.74 (1 H, m), 4.24 (1 H, d, *J* = 8.0 Hz), 3.32–3.27 (1 H, m), 2.00 (1 H, dt, *J* = 11.2, 1.6 Hz),

1.87 (1 H, dt, *J* = 11.2, 1.6 Hz), 1.58 (9 H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 154.7, 149.0, 130.7, 129.0 (2 ×), 127.8, 126.7 (2 ×), 84.1, 83.9, 61.1, 58.8, 54.8, 31.8, 28.1 (3 ×); ESI−HRMS calcd for $C_{18}H_{20}N_2O_4Na$: 351.1321, found *m/z* 351.1337 $[M + Na]^+$

Compound **7b**: $C_{18}H_{20}N_2O_4$; white solid; mp 200–204 ^oC; TLC (CH_2Cl_2) R_f = 0.57; IR ν_{max} (neat) 3063, 2979, 2931, 1793, 1715, 1349, 1294, 1149, 962, 765 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72–7.67 (2 H, m), 7.44–7.38 (3 H, m), 5.13 (1 H, dt, *J* = 8.0, 1.6 Hz), 4.74–4.69 (1 H, m), 4.23 (1 H, d, *J* = 8.0 Hz), 3.06–3.01 (1 H, m), 2.01 (1 H, dt, *J* = 11.6, 1.6 Hz), 1.77 (1 H, dt, *J* = 11.6, 1.6 Hz), 1.52 (s, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 155.0, 148.5, 130.5, 128.9 (2 ×), 127.3, 126.7 (2 ×), 85.4, 83.7, 62.6, 53.1, 49.1, 31.7, 27.9 (3 ×); ESI−HRMS calcd for $C_{18}H_{20}N_2O_4Na$: 351.1321, found *m/z* 351.1332 $[M + Na]^+$.

*4.5.14. 2,2,2-Trichloroethyl 6-oxo-3-phenyl-3a,6,7,7a-tetrahydro-4,7-methanoisoxazolo[4,5-c]pyridine-5(4H)-carboxylate (**8a**) and 2,2,2-trichloroethyl 5-oxo-3-phenyl-4,5,7,7a-tetrahydro-4,7-methanoisoxazolo[5,4-c]pyridine-6(3aH)-carboxylate (**8b**)*

According to the representative procedure A, the cycloaddition of (±)-**2b** (87 mg, 0.31 mmol) with benzonitrile oxide, which was generated in situ from hydroximoyl chloride **5b** (115 mg, 0.74 mmol), was carried out to give a crude product containing (±)-**8a** and (±)-**8b** in a ratio of 63:37 as shown by the ^{1}H NMR analysis (partial, 400 MHz, $CDCl_3$) δ 5.25 (0.63 H, d, *J* = 8.0 Hz, H-7)/ 5.21 (0.37 H, d, *J* = 8.0 Hz, H-7), 4.35 (0.63 H, d, *J* = 8.0 Hz, H-4)/ 4.27 (0.37 H, d, *J* = 8.0 Hz, H-4).

*4.5.15. 5-Benzyl-3-phenyl-4,5,7,7a-tetrahydro-4,7-methanoisoxazolo[4,5-c]pyridin-6(3aH)-one (**9a**) and 6-benzyl-3-phenyl-3a,6,7,7a-tetrahydro-4,7-methanoisoxazolo[5,4-c]pyridin-5(4H)-one (**9b**).*

According to the representative procedure A, the cycloaddition of (±)-**2c** (62 mg, 0.31 mmol) with benzonitrile oxide, which was generated in situ from hydroximoyl chloride **5b** (102 mg, 0.66 mmol), was carried out to give a crude product containing (±)-**9a** and (±)-**9b** in a ratio of 37:63 as shown by the ^{1}H NMR analysis. Some **9a** (9 mg, 7% yield) and **9b** (43 mg, 31% yield) were isolated by repeated silica gel chromatography (CH_2Cl_2/MeOH = 100:1). Compound (±)-**9a** was further recrystallized from CH_2Cl_2–hexane for X-ray diffraction analysis.

Compound **9a**: $C_{20}H_{18}N_2O_2$; white solid; mp 136–138 ^{o}C; TLC (hexane/EtOAc = 2:1) R_f = 0.25; IR ν_{max} (neat) 2947, 2925, 2853, 1707, 1496, 1456, 1410, 1355, 1219, 980, 884, 766, 693 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.32 (10 H, m), 5.12 (1 H, dt, *J* = 8.0, 1.6 Hz), 4.52 (1 H, d, *J* = 14.8 Hz), 4.35 (1 H, d, *J* = 14.8 Hz), 3.88–3.95 (1 H, m), 3.73–3.68 (1 H, m), 3.32–3.27 (1 H, m), 1.95 (1 H, dt, *J* = 10.4, 1.6 Hz), 1.79 (1 H, dt, *J* = 10.4, 1.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 155.0, 135.8, 130.3, 129.2 (2 ×), 128.9 (2 ×), 128.7 (2 ×), 128.4, 128.0, 126.4 (2 ×), 85.2, 61.4, 58.4, 54.1, 45.8, 34.7; ESI−HRMS calcd for $C_{20}H_{19}N_2O_2$: 319.1447, found *m/z* 319.1441 $[M + H]^+$.

Compound **9b**: $C_{20}H_{18}N_2O_2$; white solid; mp 160–161 ^{o}C; TLC (hexane/EtOAc = 2:1) R_f = 0.31; IR ν_{max} (neat) 2971, 2924, 2853, 1705, 1496, 1456, 1409, 1356, 1216, 967, 884, 767, 694 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76–7.67 (2 H, m), 7.43–7.21 (8 H, m), 4.78 (1 H, dt, *J* = 8.4, 1.6 Hz), 4.51 (1 H, d, *J* = 14.8 Hz), 4.20 (1 H, d, *J* = 14.8 Hz), 4.10 (1 H, d, *J* = 8.4 Hz), 3.95–3.89 (1 H, m), 3.32–3.27 (1 H, m), 1.91 (1 H, dt, *J* = 10.8, 1.6 Hz), 1.68 (1 H, dt, *J* = 10.8, 1.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 155.5, 136.2, 130.4, 128.9 (2 ×), 128.9 (2 ×), 128.1 (2 ×), 128.0, 127.6, 126.8 (2 ×), 85.8, 63.0, 54.4, 48.3, 45.3, 34.3; ESI−HRMS calcd for $C_{20}H_{19}N_2O_2$: 319.1447, found *m/z* 319.1450 $[M + H]^+$. The structural assignment of **9b** was confirmed by ^{1}H−^{1}H COSY analysis.

*4.5.16. Methyl 4-tert-butoxycarbonylamino-3-(1-ethylpropyl)-4,5,6,6a-tetrahydro-3aH-cyclopenta[d]isoxazole-6-carboxylate (**6a**) and methyl 6-((tert-butoxycarbonyl)amino)-3-(pentan-3-yl)-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-4-carboxylate (**6b**) [14].*

According to the representative procedure B, the cycloaddition of (±)-**3a** (750 mg, 3.1 mmol) with 2-ethylbutanenitrile oxide, which was generated in situ from hydroximoyl chloride **5a** (2.77 g, 18.6 mmol), was performed in hexane to give a crude product containing (±)-**6a** and (±)-**6b** in a ratio of 91:9 as shown by the ^{1}H NMR analysis (partial, 400 MHz, $CDCl_3$) δ 5.20 (0.83 H, d, *J* = 8.4 Hz, H-6a)/ 4.83 (0.17 H, d, *J* = 8.6 Hz, H-6a), 3.58 (0.83 H, d, *J* = 9.2 Hz, H-3a)/ 3.89 (0.17 H, m, H-3a), 3.76 (2.49 H, s, CO_2Me)/ 3.79 (0.51 H, s, CO_2Me). Compound (±)-**6a** (727 mg, 66% yield) was isolated by silica gel chromatography (EtOAc/hexane 1:9 to 1:6 gradients).

Alternatively, optically active (+)-**6a** was prepared from trifluoroacetamide (+)-**13a** according to the representative procedure C. Therefore, trifluoroacetamide (+)-**13a** (94 mg, 0.27 mmol) was dissolved in 4.6 M HCl methanolic solution and heated at boiling for 24 h. The solution was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (1 mL), added Et_3N (0.12 mL, 0.84 mmol) and Boc_2O (0.08 mL, 0.36 mmol) at 0 ^oC. The mixture was stirred at room temperature for 4 h, and then concentrated under reduced pressure. The residue was re-dissolved in CH_2Cl_2, washed with H_2O, dried over $MgSO_4$, and filtered. The filtrate was concentrated by rotatory evaporation under reduced pressure. The residue was purified by silica gel column chromatography (hexane/EtOAc = 10:1) to afford carbamate (+)-**6a** (70 mg, 74% yield).

Compound **6a**: $C_{18}H_{30}N_2O_5$; pale yellow oil; $[\alpha]^{20}_D$ = +58.3 (*c* = 2.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.57 (1 H, d, *J* = 7.2 Hz), 5.16 (1 H, d, *J* = 8.4 Hz), 4.17 (1 H, br), 3.71 (3 H, s), 3.54 (1 H, d, *J* = 9.2 Hz), 3.14 (1 H, d, *J* = 7.2 Hz), 2.46–2.45 (1 H, m), 2.10–2.03 (1 H, m), 2.00–1.96 (1 H, m), 1.71–1.53 (4 H, m), 1.40 (9 H, s), 0.90–0.83 (6 H, m); ^{13}C

NMR (100 MHz, $CDCl_3$) δ 174.3,160.4, 154.3, 86.9, 79.4, 63.5, 55.7, 52.6, 52.2, 40.7, 33.6, 28.7 (3 ×), 25.9, 24.3, 12.5, 11.2; ESI–HRMS calcd. for $C_{18}H_{31}N_2O_5$: 355.2233, found: *m/z* 355.2233 $[M + H]^+$.

*4.5.17. Methyl 3-(pentan-3-yl)-4-(((2,2,2-trichloroethoxy)carbonyl)amino)-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-6-carboxylate (**10a**) and methyl 3-(pentan-3-yl)-6-(((2,2,2-trichloroethoxy)carbonyl)amino)-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-4-carboxylate (**10b**)*

According to the representative procedure B, the cycloaddition of (±)-**3b** (16 mg, 0.05 mmol) with 2-ethylbutanenitrile oxide, which was generated in situ from hydroximoyl chloride **5a** (44 mg, 0.3 mmol), was carried out to give a crude product containing (±)-**10a** and (±)-**10b** in a ratio of 76:24 as shown by the ^{1}H NMR analysis (partial, 400 MHz, $CDCl_3$) δ 5.20 (0.76 H, d, *J* = 12 Hz, H-6a), 3.64 (0.76 H, d, *J* = 8 Hz, H-3a)/ 3.88 (0.24 H, d, *J* = 8 Hz, H-3a), 3.75 (2.28 H, s, CO_2Me)/ 3.70 (0.72 H, s, CO_2Me). Attempt to isolate **10a** and **10b** failed by silica gel chromatography.

*4.5.18. Methyl 4-((2-nitrophenyl)sulfonamido)-3-(pentan-3-yl)-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-6-carboxylate (**11a**) and methyl 6-((2-nitrophenyl)sulfonamido)-3-(pentan-3-yl)-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-4-carboxylate (**11b**)*

By analogy to the representative procedure B, the cycloaddition of (±)-**3c** (33 mg, 0.1 mmol) with 2-ethylbutanenitrile oxide, which was generated in situ from hydroximoyl chloride **5a** (119 mg, 0.8 mmol), was performed in toluene to give a crude product containing (±)-**11a** and (±)-**11b** in a ratio of 68:32 as shown by the ^{1}H NMR analysis (partial, 400 MHz, $CDCl_3$) δ 5.19 (0.68 H, d, *J* = 8 Hz, H-6a)/ 4.80 (0.32 H, d, *J* = 8 Hz), 3.72 (0.68 H, d, *J* = 8 Hz, H-3a)/ 3.90 (0.32 H, m, H-3a), 3.77 (2.04 H, s, CO_2Me)/ 3.79 (0.96 H, s, CO_2Me). The

samples of (±)-**11a** (10 mg, 22% yield) and (±)-**11b** (3 mg, 8% yield) were isolated by silica gel chromatography (EtOAc/hexane = 1:3) for characterization of their physical and spectroscopic properties.

Compound **11a**: $C_{19}H_{25}N_3O_7S$; colorless oil; TLC (EtOAc/hexane = 1:3) R_f = 0.15; IR ν_{max} (neat) 3313, 3097, 2964, 2927, 2876, 1732, 1542, 1441, 1363, 1166, 1061, 864, 785, 742, 655, 592 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.16–8.08 (1 H, m), 7.90–7.82 (1 H, m), 7.79–7.70 (2 H, m), 6.68 (1 H, d, *J* = 8.0 Hz), 5.19 (1 H, d, *J* = 8.8 Hz), 4.05 (1 H, t, *J* = 7.6 Hz), 3.77 (s, 3 H), 3.72 (1 H d, *J* = 9.2 Hz), 3.22 (1 H, d, *J* = 7.2 Hz), 2.33 (1 H, dq, *J* = 7.6, 6.0 Hz), 2.09–1.91 (2 H, m), 1.73–1.48 (4 H, m), 0.87 (6 H, dt, *J* = 17.2, 7.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.8, 160.4, 147.9, 134.5, 133.7, 132.8, 130.4, 125.4, 89.2, 63.6, 58.7, 53.0, 52.0, 40.8, 33.7, 25.7, 24.0, 12.0, 10.8; ESI−HRMS (negative mode) calcd for $C_{19}H_{24}N_3O_7S$: 438.1335, found *m/z* 438.1343 $[M - H]^-$. The structural assignment of **11a** was confirmed by $^1H-^1H$ COSY analysis.

Compound **11b**: $C_{19}H_{25}N_3O_7S$; colorless oil; TLC (EtOAc/hexane = 1:3) R_f = 0.19; IR ν_{max} (neat) 3317, 3098, 2963, 2932, 2875, 1731, 1641, 1440, 1362, 1257, 1168, 854, 742, 655, 592 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.23–8.15 (1 H, m), 7.88–7.82 (1 H, m), 7.79–7.69 (2 H, m), 6.23 (1 H, d, *J* = 7.2 Hz), 4.80 (1 H, dd, *J* = 9.0, 1.8 Hz), 4.07–3.97 (1 H, m), 3.90 (1 H, dd, *J* = 9.2, 3.2 Hz), 3.79 (3 H, s), 2.98–2.88 (1 H, m), 2.25 (1 H, dq, *J* = 8.0, 5.6 Hz), 2.19–2.06 (2 H, m), 1.68–1.48 (4 H, m), 0.86 (6 H, td, *J* = 7.2, 4.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.0, 161.4, 147.9, 133.8, 133.7, 133.0, 131.4, 125.4, 90.1, 61.3, 56.8, 53.1, 47.1, 40.8, 33.7, 25.6, 24.0, 12.0, 10.9; ESI−HRMS (negative mode) calcd for $C_{19}H_{24}N_3O_7S$: 438.1335, found *m/z* 438.1340 $[M - H]^-$.

4.5.19. Methyl 3-(pentan-3-yl)-4-((trifluoromethyl)sulfonamido)-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-6-carboxylate ***(12a)*** *and methyl 3-(pentan-3-yl)-6-*

*((trifluoromethyl)sulfonamido)-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-4-carboxylate (**12b**)*

By analogy to the representative procedure B, the cycloaddition of (±)-**3d** (27 mg, 0.1 mmol) with 2-ethylbutanenitrile oxide, which was generated in situ from hydroximoyl chloride **5a** (119 mg, 0.8 mmol), was performed in toluene to give a crude product containing (±)-**12a** and (±)-**12b** in a ratio of 68:32 as shown by the ^{1}H NMR analysis (partial, 400 MHz, $CDCl_3$) δ 5.15 (0.68 H, d, *J* = 12 Hz, H-6a)/ 4.98 (0.32 H, d, *J* = 12 Hz, H-6a), 3.78 (2.04 H, s, CO_2Me)/ 3.79 (0.96 H, s, CO_2Me). Attempt to separate **12a** and **12b** failed by silica gel chromatography.

*4.5.20. Methyl 3-(pentan-3-yl)-4-(2,2,2-trifluoroacetamido)-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-6-carboxylate (**13a**) and methyl 3-(pentan-3-yl)-6-(2,2,2-trifluoroacetamido)-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-4-carboxylate (**13b**)*

According to the representative procedure B, the cycloaddition of (±)-**3e** (24 mg, 0.1 mmol) with 2-ethylbutanenitrile oxide, which was generated in situ from hydroximoyl chloride **5a** (104 mg, 0.7 mmol), was performed in hexane to give a crude product containing **13a** and **13b** in a ratio of 90:10 as shown by the ^{1}H NMR analysis (partial, 400 MHz, $CDCl_3$) δ 5.14 (0.80 H, d, *J* = 8 Hz, H-6a)/ 4.85 (0.20 H, d, *J* = 8 Hz, H-6a), 3.60 (0.80 H, d, *J* = 12 Hz, H-3a)/ 3.69 (0.20 H, d, *J* = 8 Hz, H-3a), 3.75 (2.40 H, s, CO_2Me)/ 3.76 (0.60 H, s, CO_2Me).

In another experiment, the cycloaddition of (−)-**3e** (99 mg, 0.42 mmol) with **5a** (440 mg, 2.94 mmol) was performed in hexane solution to give optically active compounds **13a** and **13b** in a ratio of 90:10 as shown by the ^{1}H NMR analysis (partial, 400 MHz, $CDCl_3$) δ 5.15 (0.9 H, d, *J* = 8.7 Hz, H-6a)/ 4.86 (0.1 H, d, *J* = 8.8 Hz, H-6a), 3.77 (2.7 H, s, CO_2Me)/ 3.78 (0.3 H, s, CO_2Me). A sample of (+)-**13a** (110 mg, 80% yield) was isolated by silica gel

chromatography (EtOAc/hexane = 1:12 to 1:10 gradients) for characterization of their physical and spectroscopic properties.

Compound **13a**: $C_{15}H_{21}F_3N_2O_4$; light yellow oil; TLC (EtOAc/hexane = 1:6) R_f = 0.20; $[\alpha]^{20}_D$ = +94.0 (*c* = 2.0, $CHCl_3$); IR ν_{max} (neat) 3319, 2965, 2934, 2877, 2358, 1720, 1558, 1460, 1439, 1374, 1200, 1176, 885, 809 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.39 (1 H, br d, *J* = 6.0 Hz), 5,15 (1 H, d, *J* = 9.0 Hz), 4.49–4.46 (1 H, m), 3.77 (3 H, s), 3.60 (1 H, d, *J* = 8.8 Hz), 3.39–3.38 (1 H, m), 2.49 (1 H, dq, *J* = 8.6, 6.1 Hz), 2.13–2.11 (2 H, m), 1.76–1.59 (4 H, m), 0.91 (3 H, t, *J* = 7.4 Hz), 0.87 (3 H, t, *J* = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.1, 160.3, 156.6 (q, $^2J_{CF}$ = 37.0 Hz), 115.7 (q, $^1J_{CF}$ = 285.9 Hz), 87.6, 63.0, 54.9, 53.1, 52.9, 40.8, 31.7, 25.6, 23.9, 12.1, 10.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ −76.3 (3 ×); ESI−HRMS calcd for $C_{15}H_{22}F_3N_2O_4$: 351.1526, found *m/z* 351.1522 $[M - H]^+$.

*4.5.21. Methyl 4-acetamido-3-(pentan-3-yl)-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-6-carboxylate (**14a**) and methyl 6-acetamido-3-(pentan-3-yl)-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-4-carboxylate (**14b**)*

By analogy to the representative procedure B, the cycloaddition of (±)-**3f** (18 mg, 0.1 mmol) with 2-ethylbutanenitrile oxide, which was generated in situ from hydroximoyl chloride **5a** (56 mg, 0.8 mmol), was performed in toluene to give a crude product containing (±)-**14a** and (±)-**14b** in a ratio of 80:20 as shown by the ^1H NMR analysis (partial, 400 MHz, $CDCl_3$) δ 5.15 (0.8 H, d, *J* = 8 Hz, H-6a)/ 4.83 (0.2 H, d, *J* = 8 Hz, H-6a), 3.60 (0.8 H, d, *J* = 12 Hz, H-3a)/ 3.69 (0.2 H, d, *J* = 8 Hz, H-3a), 3.75 (2.4 H, s, CO_2Me)/ 3.76 (0.6 H, s, CO_2Me). Attempt to separate **14a** and **14b** failed by silica gel chromatography.

*4.5.22. Methyl 4-(1,3-dioxoisoindolin-2-yl)-3-(pentan-3-yl)-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-6-carboxylate (**15a**) and methyl 6-(1,3-dioxoisoindolin-2-yl)-3-(pentan-3-yl)-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-4-carboxylate (**15b**)*

By analogy to the representative procedure B, the cycloaddition of (±)-**3g** (27 mg, 0.1 mmol) with 2-ethylbutanenitrile oxide, which was generated in situ from hydroximoyl chloride **5a** (238 mg, 1.6 mmol), was performed in toluene to give a crude product containing (±)-**15a** and (±)-**15b** in a ratio of 64:36 as shown by the ^{1}H NMR analysis (partial, 400 MHz, $CDCl_3$) δ 3.74 (1.92 H, s, CO_2Me)/ 3.73 (1.08 H, s, CO_2Me). Attempt to separate **15a** and **15b** failed by silica gel chromatography.

*4.5.23. Methyl 4-((tert-butoxycarbonyl)amino)-3-phenyl-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-6-carboxylate (**16a**) and Methyl 6-((tert-butoxycarbonyl)amino)-3-phenyl-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-4-carboxylate (**16b**)*

According to the representative procedure B, the cycloaddition of (±)-**3a** (241 mg, 1.0 mmol) with benzonitrile oxide, which was generated in situ from hydroximoyl chloride **5b** (720 mg, 5.0 mmol), was performed in hexane to give a crude product containing (±)-**16a** and (±)-**16b** in a ratio of 86:14 as shown by the ^{1}H NMR analysis (partial, 400 MHz, $CDCl_3$) δ 5.44 (0.85 H, d, *J* = 9.2 Hz, H-6a)/ 5.04 (0.15 H, m, H-6a), 4.20 (0.85 H, d, *J* = 9.2 Hz, H-3a)/ 4.48 (0.15 H, d, *J* = 8.4 Hz, H-3a)/, 3.78 (2.7 H, s, CO_2Me)/ 3.81 (0.3 H, s, CO_2Me). A sample of (±)-**16a** (189 mg, 52% yield) was isolated by silica gel chromatography (hexane/EtOAc = 6:1) for characterization of its physical and spectroscopic properties.

Alternatively optically active (+)-**16a** was prepared in 73% isolated yield from optically active trifluoroacetamide (+)-**20a** as described in the representative procedure C.

Compound **16a**: $C_{19}H_{24}N_2O_5$; white solid; mp 172–173 ^oC; TLC (hexane/EtOAc = 4:1) R_f = 0.35; $[\alpha]^{20}_D$ = +80.7 ($CHCl_3$. *c* = 1.0); IR ν_{max} (neat) 3358, 2979, 2955, 2923, 1727, 1683,

1525, 1438, 1367, 1290, 1168, 976, 894, 764, 692 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.98–7.82 (2 H, m), 7.46–7.20 (3 H, m), 5.62 (1 H, br), 5.44 (1 H, dd, *J* = 9.2, 1.6 Hz), 4.39–4.27 (1 H, m), 4.20 (1 H, d, *J* = 9.2 Hz), 3.78 (3 H, s), 3.28 (1 H, t, *J* = 5.2 Hz), 2.02–2.13 (2 H, m), 1.44 (9 H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 156.9, 155.0, 130.2, 128.8 (2 ×), 128.1, 127.4 (2 ×), 88.5, 79.6, 60.9, 56.5, 52.6, 33.2, 28.4 (3 ×); ESI−HRMS calcd for $C_{19}H_{25}N_2O_5$ 361.1763, found *m/z* 361.1763 $[M + H]^+$. The structural assignment of **16a** was confirmed by $^1H-^1H$ COSY analysis.

*4.5.24. Methyl 3-phenyl-4-(((2,2,2-trichloroethoxy)carbonyl)amino)-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-6-carboxylate (**17a**) and methyl 3-phenyl-6-(((2,2,2-trichloroethoxy)carbonyl)amino)-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-4-carboxylate (**17b**)*

By analogy to the representative procedure B, the cycloaddition of (±)-**3b** (32 mg, 0.1 mmol) with benzonitrile oxide, which was generated in situ from hydroximoyl chloride **5b** (62 mg, 0.4 mmol), was performed in toluene to give a crude product containing (±)-**17a** and (±)-**17b** in a ratio of 74:26 as shown by the ^1H NMR analysis (partial, 400 MHz, $CDCl_3$) δ 5.43 (0.74 H, d, *J* = 12 Hz, H-6a)/5.12 (0.26 H, d, *J* = 8 Hz, H-6a), 4.28 (0.74 H, d, *J* = 12 Hz, H-3a)/ 4.46 (0.26 H, d, *J* = 8 Hz, H-3a), 3.80 (2.22 H, s, CO_2Me)/ 3.84 (0.78 H, s, CO_2Me).

The crude products obtained from several experiments were combined and subjected to silica gel chromatography (hexane/EtOAc = 1:6) and HPLC [250 × 10 mm Inspire^{TM} silica column (10 μm particle size) using an eluent of hexane/EtOAc (86:14) at a flow rate of 1.5 mL/min] to give (±)-**17a** (20 mg) and (±)-**17b** (4 mg) for characterization of their physical and spectroscopic.

Compound **17a**: $C_{17}H_{17}Cl_3N_2O_5$; white solid; mp 139–140 ^oC; TLC (hexane/EtOAc = 4:1) R_f = 0.31; HPLC t_R = 41.8 min; IR ν_{max} (neat) 3355, 2954, 1736, 1519, 1438, 1285, 1219,

1133, 1089, 1044, 891, 818, 765, 693, 570 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.96–7.86 (2 H, m), 7.45–7.38 (3 H, m), 6.38 (1 H, d, *J* = 7.2 Hz), 5.43 (1 H, dd, *J* = 9.2, 0.4 Hz), 4.77 (1 H, d, *J* = 12.0 Hz), 4.72 (1 H, d, *J* = 12.0 Hz), 4.56–4.39 (1 H, m), 4.28 (1 H, d, *J* = 9.2 Hz), 3.79 (3 H, s), 3.36 (1 H, t, *J* = 5.2 Hz), 2.18–2.10 (2 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.3, 156.4, 153.9, 130.4, 128.9 (2 ×), 127.9, 127.3 (2 ×), 95.5, 88.6, 74.4, 60.7, 57.2, 52.9, 52.8, 32.8; ESI−HRMS calcd for $C_{17}H_{18}Cl_3N_2O_5$: 435.0281, found *m/z* 435.0291 $[M + H]^+$.

Compound **17b**: $C_{17}H_{17}Cl_3N_2O_5$; white solid; mp 97–98 °C; TLC (hexane/EtOAc = 4:1) R_f = 0.28; HPLC t_R = 43.0 min; IR ν_{max} (neat) 3351, 2954, 2918, 2849, 1731, 1518, 1438, 1246, 1094, 910, 877, 814, 767, 693 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.81–7.68 (2 H, m), 7.44–7.38 (3 H, m), 5.99 (1 H, d, *J* = 7.5 Hz), 5.12 (1 H, d, *J* = 9.0 Hz), 4.76 (1 H, d, *J* = 12.0 Hz), 4.69 (1 H, d, *J* = 12.0 Hz), 4.46 (1 H, dd, *J* = 9.0, 1.5 Hz), 4.44–4.38 (1 H, m), 3.84 (3 H, s), 3.17–3.09 (1 H, m), 2.27–2.16 (2 H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.8, 157.4, 153.8, 130.4, 129.0 (2 ×), 128.0, 127.0 (2 ×), 95.5, 91.4, 74.5, 59.2, 55.2, 53.2, 48.5, 32.4; ESI−HRMS calcd for $C_{17}H_{18}Cl_3N_2O_5$: 435.0281, found *m/z* 435.0291 $[M + H]^+$. The structural assignment of **17b** was confirmed by HSQC and HMBC spectral analyses.

*4.5.25. Methyl 4-((2-nitrophenyl)sulfonamido)-3-phenyl-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-6-carboxylate (**18a**) and methyl 6-((2-nitrophenyl)sulfonamido)-3-phenyl-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-4-carboxylate (**18b**)*

By analogy to the representative procedure B, the cycloaddition of (±)-**3c** (33 mg, 0.1 mmol) with benzonitrile oxide, which was generated in situ from hydroximoyl chloride **5b** (93 mg, 0.6 mmol), was performed in toluene to give a crude product containing (±)-**18a** and (±)-**18b** in a ratio of 74:26 as shown by the ^1H NMR analysis (partial, 400 MHz, $CDCl_3$) δ 5.44 (0.74 H, d, *J* = 8 Hz, H-6a)/5.08 (0.26 H, d, *J* = 8 Hz, H-6a), 4.36 (0.74 H, d, *J* = 12 Hz,

H-3a)/ 4.51 (0.26 H, d, *J* = 8 Hz, H-3a), 3.81 (2.22 H, s, CO_2Me)/ 3.87 (0.78 H, s, CO_2Me). Attempt to isolate **18a** and **18b** failed by silica gel chromatography.

*4.5.26. Methyl 3-phenyl-4-((trifluoromethyl)sulfonamido)-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-6-carboxylate (**19a**) and methyl 3-phenyl-6-((trifluoromethyl)sulfonamido)-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-4-carboxylate (**19b**)*

By analogy to the representative procedure B, the cycloaddition of (±)-**3d** (41 mg, 0.15 mmol) with benzonitrile oxide, which was generated in situ from hydroximoyl chloride **5b** (93 mg, 0.6 mmol), was performed in toluene to give a crude product containing (±)-**19a** and (±)-**19b** in a ratio of 66:34 as shown by the ^{1}H NMR analysis (partial, 400 MHz, $CDCl_3$) δ 5.38 (0.66 H, d, *J* = 8 Hz, H-6a)/5.21 (0.34 H, d, *J* = 12 Hz, H-6a), 4.37 (0.66 H, d, *J* = 8 Hz, H-3a)/ 4.31 (0.34 H, d, *J* = 8 Hz, H-3a), 3.82 (1.98 H, s, CO_2Me)/ 3.88 (1.02 H, s, CO_2Me). A sample of (±)-**19a** (6.0 mg, 10% yield) was isolated by silica gel chromatography (hexane/CH_2Cl_2 = 1:3) for characterization of its physical and spectroscopic properties.

Compound **19a**: $C_{15}H_{15}F_3N_2O_5S$; white solid; mp 137–139 ^oC; TLC (hexane/CH_2Cl_2 = 1:3) R_f = 0.28; IR ν_{max} (neat) 3219, 2957, 2923, 2851, 1784, 1715, 1446, 1383, 1230, 1195, 1147, 1080, 1016, 919, 765, 694, 604 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80–7.22 (2 H, m), 7.47–7.41 (3 H, m), 7.41 (1 H, br), 5.38 (1 H, d, *J* = 8.8 Hz), 4.36 (1 H, d, *J* = 8.8 Hz), 4.32 (1 H, t, *J* = 7.2 Hz), 3.82 (3 H, s), 3.45 (1 H, d, *J* = 8.0 Hz), 2.27–2.07 (2 H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.7, 156.0, 130.8, 129.1, 127.3, 127.2, 119.6 ($^1J_{CF}$ = 320 Hz), 88.8, 77.2, 62.6, 60.5, 53.4, 53.0, 33.8, 29.7; ^{19}F NMR (376 MHz, $CDCl_3$) δ −77.9 (3 ×). The structural assignment of **19a** was confirmed by ^{1}H−^{1}H COSY analysis.

*4.5.27. Methyl 3-phenyl-4-(2,2,2-trifluoroacetamido)-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-6-carboxylate (**20a**) and methyl 3-phenyl-6-(2,2,2-trifluoroacetamido)-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-4-carboxylate (**20b**)*

By analogy to the representative procedure B, the cycloaddition of (±)-**3e** (120 mg, 0.5 mmol) with benzonitrile oxide, which was generated in situ from hydroximoyl chloride **5b** (329 mg, 2 mmol), was performed in toluene to give a crude product containing (±)-**20a** and (±)-**20b** in a ratio of 87:13 as shown by the ^{1}H NMR analysis (partial, 400 MHz, $CDCl_3$) δ 5.39 (0.87 H, d, *J* = 8 Hz, H-6a)/5.08 (0.13 H, d, *J* = 8 Hz, H-6a), 4.26 (0.87 H, d, *J* = 8 Hz, H-3a)/ 4.37 (0.13 H, d, *J* = 12 Hz, H-3a), 3.82 (2.61 H, s, CO_2Me)/ 3.86 (0.39 H, s, CO_2Me). When the (3+2) cycloaddition of (±)-**3e** (120 mg, 0.5 mmol) and benzonitrile oxide was performed in hexane solution, the desired product (±)-**20a** (139 mg) was isolated in 78% yield by silica gel chromatography (hexane/CH_2Cl_2 = 1:1).

In another experiment, the cycloaddition reaction of (−)-**3e** (1.00 g, 2.11 mmol) with **5b** (2.62 g, 8.43 mmol) was performed in hexane/toluene (100:1) solution to give optically active compound (+)-**20a** (1.26 g, 83% yield) after purification by silica gel chromatography (CH_2Cl_2/hexane gradients, 0:1 → 1:2 → 1:1).

Compound **20a**: $C_{16}H_{15}F_3N_2O_4$; white solid; mp 167–169 °C; $[\alpha]^{20}_D$ = +244.7 (*c* = 1.0, $CHCl_3$); TLC (EtOAc/hexane = 1:4) R_f = 0.29; IR ν_{max} (neat) 3315, 3102, 2957, 2919, 1731, 1702, 1554, 1440, 1384, 1286, 1177, 980, 900, 768, 694 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (1 H, d, *J* = 5.0 Hz), 7.99–7.91 (2 H, m), 7.48–7.40 (3 H, m), 5.39 (1 H, d, *J* = 9.0 Hz), 4.69–4.63 (1 H, m), 4.26 (1 H, d, *J* = 9.0 Hz), 3.82 (3 H, s), 3.51–3.42 (1 H, m), 2.17–2.12 (2 H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.2, 156.9 ($^2J_{CF}$ = 37.5 Hz), 155.8, 130.6, 129.0 (2 ×), 127.5, 127.4 (2 ×), 115.8 ($^1J_{CF}$ = 286.3 Hz), 88.8, 60.8, 55.9, 53.6, 53.3, 31.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ −76.5 (3 ×); ESI−HRMS calcd for $C_{16}H_{16}F_3N_2O_4$: 357.1062, found *m/z* 357.1073 $[M + H]^+$.

*4.5.28. Methyl 4-acetamido-3-phenyl-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-6-carboxylate (**21a**) and methyl 6-acetamido-3-phenyl-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-4-carboxylate (**21b**)*

By analogy to the representative procedure B, the cycloaddition of (±)-**3f** (92 mg, 0.5 mmol) with benzonitrile oxide, which was generated in situ from hydroximoyl chloride **5b** (311 mg, 2 mmol), was performed in toluene to give a crude product containing (±)-**21a** and (±)-**21b** in a ratio of 76:24 as shown by the ^{1}H NMR analysis (partial, 400 MHz, $CDCl_3$) δ 5.39 (0.76 H, d, *J* = 8 Hz, H-6a)/ 4.87 (0.34 H, d, *J* = 8 Hz, H-6a),, 4.27 (0.76 H, d, *J* = 8 Hz, H-3a), 3.80 (2.28 H, s, CO_2Me)/ 3.67 (0.72 H, s, CO_2Me). A sample of (±)-**21a** (70 mg, 46% yield) was isolated by silica gel chromatography (hexane/EtOAc = 1:1) for characterization of its physical and spectroscopic properties.

Compound **21a**: $C_{16}H_{18}N_2O_4$, white solid; mp 176–177 ^oC; TLC (EtOAc /hexane = 2:1) R_f = 0.40; IR ν_{max} (neat) 3619, 3298, 3073, 2956, 1728, 1654, 1541, 1436, 1376, 1306, 1199, 974, 895, 766, 693 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02–7.93 (2 H, m), 7.40–7.31 (3 H, m), 7.06 (1 H, d, *J* = 6.8 Hz), 5.34 (1 H, dd, *J* = 9.2, 0.8 Hz), 4.57–4.49 (1 H, m), 4.21 (1 H, d, *J* = 9.2 Hz), 3.73 (3 H, s), 3.30 (1 H, t, *J* = 4.8 Hz), 2.05–1.97 (2 H, m), 1.94 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6, 169.6, 156.4, 130.1, 128.6 (2 ×), 127.9, 127.3 (2 ×), 88.6, 60.7, 55.5, 53.2, 52.7, 32.0, 23.2; ESI−HRMS calcd for $C_{16}H_{19}N_2O_4$: 303.1345, found *m/z* 303.1343 $[M + H]^+$. The structural assignment of **21a** was confirmed by ^{1}H−^{1}H COSY analysis.

*4.5.29. Methyl 4-(1,3-dioxoisoindolin-2-yl)-3-phenyl-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-6-carboxylate (**22a**) and methyl 6-(1,3-dioxoisoindolin-2-yl)-3-phenyl-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazole-4-carboxylate (**22b**)*

By analogy to the representative procedure B, the cycloaddition of (±)-**3g** (41 mg, 0.15 mmol) with benzonitrile oxide, which was generated in situ from hydroximoyl chloride **5b** (186 mg, 1.2 mmol), was performed in toluene to give a crude product containing (±)-**22a** and (±)-**22b** in a ratio of 56:44 as shown by the ^{1}H NMR analysis (partial, 400 MHz, $CDCl_3$) δ 3.76 (1.68 H, s, CO_2Me)/ 3.71 (1.32 H, s, CO_2Me). A sample of (±)-**22a** (6 mg, 21% yield) was isolated by silica gel chromatography (hexane/EtOAc = 1:4) for characterization of its physical and spectroscopic properties.

Compound **22a**: $C_{22}H_{18}N_2O_5$; colorless oil; TLC (EtOAc/hexane = 1:2) R_f = 0.50; IR ν_{max} (neat) 2952, 2916, 2848, 1773, 1712, 1376, 1197, 887, 721 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86–7.83 (2 H, m), 7.77–7.73 (2 H, m), 7.50–7.48 (2 H, m), 7.33 (1 H, tt, *J* = 7.5, 1.5 Hz), 7.28–7.24 (2 H, m), 5.81 (1 H, dd, *J* = 10.0, 5.0 Hz), 4.81 (1 H, dt, *J* = 7.5, 5.5 Hz), 4.75 (1 H, dd, *J* = 10.0, 5.0 Hz), 3.76 (3 H, s), 3.14 (1 H, ddd, *J* = 10.0, 8.0, 5.0 Hz), 2.60–2.52 (1 H, m), 1.16 (1 H, dt, *J* = 15.0, 5.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 207.0, 172.3, 167.7 (2 ×), 157.4, 134.5 (2 ×), 131.5, 130.3, 128.9 (2 ×), 128.0, 126.9 (2 ×), 123.6 (2 ×), 88.5, 55.9, 53.9, 52.4, 51.6, 33.0; ESI−HRMS calcd for $C_{22}H_{19}N_2O_5$: 391.1294, found *m/z* 391.1308 $[M + H]^+$.

*4.5.30. Methyl (1S,2S,3R,4R)-3-[(1R)-1-acetamido-1-phenylmethyl]-4-((tert-butoxycarbonyl)amino)-2-hydroxycyclopentane-1-carboxylate (**23a**) and the isomer **23b** with (1'S)-configuration*

Slurry of 50% Raney nickel in water (4 mL) was vigorously stirred and then kept standing still until precipitation of black powder. The supernatant was removed, and the residue was washed with anhydrous THF (freshly distilled over sodium) for five times (always soaking Raney nickel in solvent!). Under an atmosphere of argon, optically active isoxazoline (+)-**16a** (300 mg, 0.83 mmol) was added to the above-prepared suspension of Raney nickel in THF (15 mL). Hydrogen gas was bubbled into the mixture for 30 min at

room temperature to furnish the reduction reaction of isoxazoline (+)-**16a**. Upon completion of reaction, the mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure to give an intermediate product of amino alcohol.

To a methanolic solution (4 mL) of the above-prepared amino alcohol was added Et_3N (464 μL, 3.33 mmol) and acetic anhydride (197 μL, 2.08 mmol) at 0 ^oC. The mixture was stirred at room temperature for 16 h, and then quenched by addition of ammonium hydroxide (25% aqueous solution, 100 μL, 1.5 mmol). The mixture was stirred for 2 h at room temperature, and concentrated by rotatory evaporation under reduced pressure. The residue was dissolved in EtOAc, and washed successively with saturated $NaHCO_3$ and AcOH (30% aqueous solution). The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a crude product of acetamido alcohol containing optically active **23a** with (1′*R*)-configuration and the (1′*S*)-diastereomer in a ratio of 85:15 as shown by the ^1H NMR analysis. ^1H NMR (400 MHz, CD_3COCD_3, **23a**/**23b** (85:15)) δ 7.79 (0.85 H, d, *J* = 8.2 Hz/ 7.85 (0.15 H, d, *J* = 8.6 Hz), 7.45–7.39 (2 H, m), 7.29–7.26 (2 H, m), 7.21–7.17 (1 H, m), 6.04 (0.85 H, d, *J* = 7.6 Hz)/ 5.63 (0.15 H, d, *J* = 8.3 Hz), 5.47 (0.85 H, t, *J* = 9.3 Hz/ 5.24 (0.15 H, t, *J* = 7.4 Hz), 4.30–4.26 (0.85 H, m/ 4.23–4.22 (0.15 H, m), 4.01 (1 H, br), 3.58 (3 H, s), 2.79–2.76 (1 H, m), 2.58–2.51 (1 H, m), 2.46–2.40 (1 H, m), 1.91 (3 H, s), 1.79–1.73 (1 H, m), 1.42 (9 H, s).

*4.5.31. Methyl (1S,2S,3R,4R)-3-[(1R)-1-acetamido-1-phenylmethyl]-4-[2,3-bis(tert-butoxycarbonyl)guanidine]-2-hydroxycyclopentane-1-carboxylate (**24a**)*

To a solution of the above-prepared acetamido alcohol **23a**/**23b** (85:15) (39 mg, 0.1 mmol) in CH_2Cl_2 (4 mL) was added trifluoroacetic acid (1 mL, 5.9 mmol) dropwise at 0 ^oC. The mixture was stirred at room temperature for 30 min, and then concentrated by rotatory evaporation under reduced pressure. The residue was dissolved in CH_2Cl_2 (5 mL), and added

Et_3N (54 μL, 0.4 mmol), 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea (33 mg, 0.12 mmol) and $HgCl_2$ (31 mg, 0.12 mmol). The mixture was stirred for 12 h at room temperature, and then filtered through a pad of Celite. The filtrate was concentrated under reduced pressure, and the desired product of bis-Boc guanidine (−)-**24a** (28.7 mg) was obtained by column chromatography (silica gel, hexane/EtOAc gradients, 3:1 → 2:1). The overall yield of (−)-**24a** starting from (+)-**16a** was 32%. $C_{27}H_{40}N_4O_8$; white wax; TLC (CH_2Cl_2/MeOH= 25:1) R_f = 0.45; $[\alpha]^{20}_D$ = −24.6 (*c* = 2.0, acetone); IR ν_{max} (neat) 3314, 3277, 2978, 2929, 1725, 1647, 1615, 1417, 1368, 1155, 1140, 1057 cm^{-1}; ^1H NMR (400 MHz, CD_3COCD_3) δ 11.43 (1 H, s), 8.38–8.34 (2 H, m), 7.37–7.35 (2 H, m), 7.25–7.22 (2 H, m), 7.18–7.14 (1 H, m), 5.52–5.48 (1 H, dd, *J* = 9.6, 5.6 Hz), 4.70–4.65 (1 H, m), 4.32 (1 H, s), 4.22 (1 H, d, *J* = 4.0 Hz), 3.65 (3 H, s), 2.83–2.81 (1H, m), 2.61–2.56 (2 H, m), 1.98 (3 H, s), 1.89–1.87 (1 H, m), 1.49 (9 H, s), 1.47 (9 H, s); ^{13}C NMR (100 MHz, CD_3COCD_3) δ 175.5, 170.8, 164.5, 156.8, 154.0, 144.2, 129.3 (2 ×), 127.7 (2 ×), 84.3, 80.1, 77.3, 56.3, 52.8, 52.7, 51.6, 51.2, 34.9, 29.0 (3 ×), 28.6 (3 ×), 24.0, 14.8; ESI–HRMS calcd for $C_{27}H_{41}N_4O_8$: 549.2919, found: *m*/*z* 549.2929 $[M + H]^+$.

*4.5.32. (1S,2S,3R,4R)-3-[(1R)-1-Acetamido-1-phenylmethyl]-4-guanidino-2-hydroxycyclopentane-1-carboxylic acid (**1a**)*

To a solution of ester (−)-**24a** (13.3 mg, 0.024 mmol) in THF (1 mL) and EtOH (1 mL) was added NaOH (1 M aqueous solution, 0.5 mL). The mixture was stirred for 40 min at room temperature, and then acidified by addition of Dowax 50W×8 to pH ≈ 4. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in H_2O (3 mL) and heated at boiling for 1.5 h. The solution was concentrated under reduced pressure. The residue was purified on a reversed-phase RP-18 column (MeOH/H_2O = 1:4) to afford the desired peramivir analog (−)-**1a** (8 mg, 99% yield). The purity of product

(−)-**1a** was 95.7% as shown by HPLC on an HC-C18 column (Merck, 4.6 × 100 mm, 2 μm particle size), t_R = 7.5 min (gradient of 0–20% aqueous MeOH in 20 min) at a flow rate of 1 mL/min). $C_{16}H_{22}N_4O_4$; pale yellow solid; mp = 261 °C (decomposed); TLC (CH_2Cl_2/MeOH= 3:1) R_f = 0.13; $[\alpha]^{20}_D$ = –21.7 (*c* = 0.5, H_2O); IR ν_{max} (film) 3385, 2921, 2850, 1637, 1559, 1397, 1303, 1273, 1113, 1085, 1033, 967, 950 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 7.46–7.44 (4 H, m), 7.40–7.37 (1 H, m), 5.21 (1 H, d, *J* = 8.9 Hz), 4.10–4.05 (1 H, m), 3.94 (1 H, d, *J* = 3.7), 2.68–2.58 (3 H, m), 1.93 (3 H, s), 1.92–1.87 (1 H, m); ^{13}C NMR (100 MHz, D_2O) δ 172.7, 155.7, 140.3, 128.9 (2 ×), 128.0, 127.2 (2 ×), 78.5, 75.4, 55.0, 53.7, 53.4, 52.6, 34.3, 22.0; ESI–HRMS calcd for $C_{16}H_{23}N_4O_4$: 335.1714, found: *m*/*z* 335.1708 $[M + H]^+$.

*4.5.32. Methyl (1S,2S,3R,4R)-3-[(1R)-1-acetamido(phenyl)methyl)-2-hydroxy-4-(2,2,2-trifluoroacetamido)cyclopentane-1-carboxylate (**25a**) and the isomer **25b** with (1'S)-configuration*

Slurry of 50% Raney nickel in water (0.6 mL) was vigorously stirred and then kept standing still until precipitation of black powder. The supernatant was removed, and the residue was washed with anhydrous THF (freshly distilled over sodium) for five times (always soaking Raney nickel in solvent!). Under an atmosphere of argon, optically active isoxazoline (+)-**20a** (32 mg, 0.09 mmol) was added to the above-prepared suspension of Raney nickel in THF (5 mL). Hydrogen gas was bubbled into the mixture for 30 min at room temperature to furnish the reduction reaction. Upon completion of reaction, the mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure to give an intermediate product of amino alcohol.

To a DMF solution (3 mL) of the above-prepared amino alcohol was added a solution of pentafluorophenyl acetate (81 mg, 0.36 mmol) in MeOH (20 μL, 0.49 mmol). The mixture was stirred at room temperature for 18 h, concentrated, and then extracted with EtOAc and

saturated aqueous $NaHCO_3$ solution. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a crude product of **25a** and its (1′*S*)-diastereomer **25b** (85:15) in about 75% yield. Two diastereomers were separated in small quantity by column chromatography (silica gel, CH_2Cl_2/MeOH gradients, 1:0 → 50:1 → 40:1) for ^{1}H NMR analysis. In agreement with **23a**/**23b**, the major isomer **25a** exhibited its H-1′ signal at δ 5.45, whereas isomer **25b** displayed the H-1′ at a higher field (δ 5.12).

25a: ^{1}H NMR (400 MHz, acetone-d_6) δ 8.28 (1 H, d, *J* = 6.9 Hz), 7.44 (1 H, br), 7.42–7.37 (2 H, m), 7.32–7.25 (2 H, m), 7.23–7.19 (1 H, m), 5.45 (1 H, t, *J* = 10 Hz), 4.61–4.53 (1 H, m), 4.43–4.42 (1 H, d, *J* = 4.9 Hz), 3.96–3.94 (1 H, m), 3.59 (3 H, s), 2.72–2.62 (2 H, m), 1.89–1.83 (1 H, m), 1.80 (3 H, s). **25b**: ^{1}H NMR (400 MHz, acetone-d_6) δ 7.81 (1 H, d, *J* = 8.0 Hz), 7.71 (1 H, d, *J* = 8.6 Hz), 7.39–7.36 (2 H, m), 7.29–7.21 (3 H, m), 5.24 (1 H, br), 5.12 (1 H, t, *J* = 8.9 Hz), 4.48–4.39 (1 H, m), 4.30 (1 H, d, *J* = 3.8 Hz), 3.61 (3 H, s), 2.59–2.49 (2 H, m), 1.93 (3 H, s), 1.87–1.80 (1 H, m).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at

References

[1] M. Imai, Y. Kawaoka, Curr. Opinion Virol. 2 (2012) 160–167.

[2] J.K. Yin, M.Y.K. Chow, G. Khandaker, C. King, P. Richmond, L. Heron, R. Booy, Vaccine 30 (2012) 3209–3222.

[3] K. Das, J.M. Aramini, L.C. Ma, R.M. Krug, E. Arnold, Nat. Struct. Mol. Biol. 17 (2010) 530−538.

[4] A.C. Lowen, P. Palese, Infect. Disord. Drug Targets 7 (2007) 318−328.

[5] A. Moscona, N. Engl. J. Med. 353 (2005) 1363−1373.

[6] R.G. Webster, P.A. Reay, W.G. Laver, Virology 164 (1988) 230−237.

[7] M. von Itzstein, W.-Y. Wu, G.B. Kok, M.S. Pegg, J.C. Dyason, B. Jin, T.V. 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, C.R. Penn, Nature 363 (1993) 418−423.

[8] C.U. Kim, W. Lew, M.A. Williams, H. Williams, L. Zhang, X. Chen, P.A. Escarpe, D.B. Mendel, W.G. Laver, R.C. Stevens, J. Med. Chem. 41 (1998) 2451−2460.

[9] Y.S. Babu, P. Chand, S. Bantia, P. Kotian, A. Dehghani, Y. El-Kattan, T.-H. Lin, T.L. Hutchison, A.J. Elliott, C.D. Parker, S.L. Ananth, L.L. Horn, G. W. Laver, J.A. Montgomery, J. Med. Chem. 43 (2000) 3482–3486.

[10] P. Chand, S. Bantia, P. Kotian, Y. El-Kattan, T.-H. Lin, Y.S. Babu, Bioorg. Med. Chem. 13 (2005) 4071–4077.

[11] E.A. Govorkova, I.A. Leneva, O.G. Goloubeva, K. Bush, R.G. Webster, Antimicrob. Agents Chemother. 45 (2001) 2723–2732.

[12] S. Bantia, C.D. Parker, S.L. Ananth, L.L. Horn, K. Andries, Antimicrob. Agent Chemother. 45 (2001) 1162–1167.

[13] P. Prince, R.K. Sharma, A. Roy, J. Pharm. Res. 3 (2010) 1602–1606.

[14] P. Chand, P.L. Kotian, A. Dehghani, Y. El-Kattan, T.-H. Lin, T.L. Hutchison, Y.S. Babu, S. Bantia, A.J. Elliott, J.A. Montgomery, J. Med. Chem. 44 (2001) 4379–4392.

[15] T. Mineno, M.J. Miller, J. Org. Chem. 68 (2003) 6591–6596.

[16] F. Jia, J. Hong, P.-H. Sun, J.-X. Chen, W.-M. Chen, Synth. Commun. 43 (2013) 2641–2647.

[17] H. Feuer, (Ed.) Nitrile oxides, nitrones, and nitronates in organic synthesis: novel strategies in synthesis. 2nd ed. Hoboken, NJ: Wiley-VCH; 2007.

[18] T. Hashimoto, Maruoka, Chem. Rev. 115 (2015) 5366−5412.

[19] S. Roscales, J. Plumet, Org. Biomol. Chem. 16 (2018) 8446−8461.

[20] D.-C. Chiu, T.-C. Lin, W.-I Huang, T.-J. Cheng, K.-C. Tsai, J.-M. Fang, Org. Biomol. Chem. 15 (2017) 9910−9922.

[21] L. Kiss, M. Nonn, F. Fülöp, Synthesis 44 (2012) 1951–1963.

[22] T.K.M. Shing, W.F. Wong, H. M. Cheng, W. S. Kwok, K. H. So, Org. Lett. 9 (2007) 753−756.

[23] B.A. Mendelsohn, S. Lee, S. Kim, F. Teyssier, V.S. Aulakh, M.A. Ciufolini, Org. Lett. 11 (2009) 1539−1542.

[24] I. Novaka, B. Kovač, Spectrochim. Acta Part A, 61 (2005) 1007–1009.

[25] C. Kesornpun, T. Aree, C. Mahidol, S. Ruchirawat, P. Kittakoop, Angew. Chem. Int. Ed. 55 (2016) 3997–4001.

[26] D. Singh, N. Devi, V. Kumar, C.C. Malakar, S. Mehra, R.K. Rawal, B.S. Kaitha, V. Singh, RSC Adv. 6 (2016) 88066−88076.

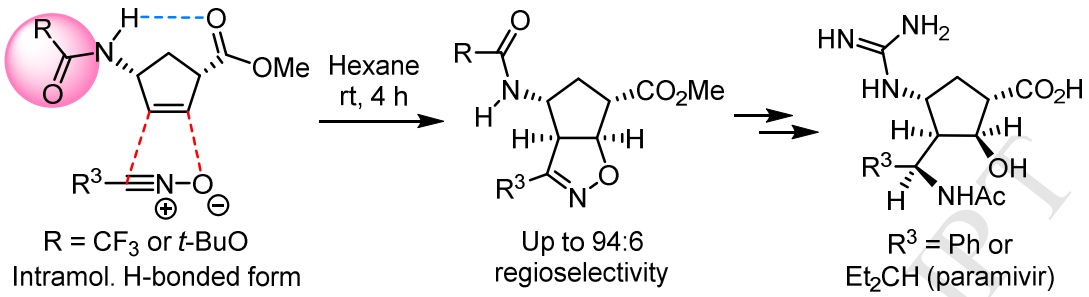
[27] A. Ros, E. Alvarez, H. Dietrich, R. Fernández, J.M. Lassaletta, Synlett (2005) 2899–2904.

[28] M. Kissane, A.R. Maguire, Asymmetric 1,3-dipolar cycloadditions of acrylamides Chem. Soc. Rev. 39 (2010) 845–883.

[29] J. Wang, Y.-L. Liang, J. Qu, Chem. Commun. (2009) 5144−5146.

[30] D.M. Andrews, S.J. Carey, H. Chaignot, B.A. Coomber, N.M. Gray, S.L. Hind, P.S. Jones, G. Mills, J.E. Robinson, M.J. Slater, Org. Lett. 4 (2002) 4475-4478.

Graphic Abstract



Legends of Tables, Figures and Schemes

Substituent and solvent effects in the 1,3-dipolar cycloadditions for synthesis of anti-influenza agent peramivir and its analog

Research highlights

• Peramivir is an anti-influenza drug targeting the virus neuraminidase.

• The synthesis involves (3+2) cycloaddition of nitrile oxide with alkene derived from Vince lactam.

• The regioselectivity of cycloaddition is improved by conducting in hexane solution.

• The cycloaddition with *N*-CF_3CO-cyclopentene derivative gives 94% desired regioisomer.

• Alternative syntheses of (−)-peramivir and the phenyl analog are demonstrated.