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Design of substituted tetrahydrofuran derivatives for HIV-1 protease inhibitors: synthesis, biological evaluation, and X-ray structural studies†‡

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Substituted tetrahydrofuran derivatives were designed and synthesized to serve as the P2 ligand for a series of potent HIV-1 protease inhibitors. Both enantiomers of the tetrahydrofuran derivatives were synthesized stereoselectivity in optically active forms using lipase-PS catalyzed enzymatic resolution as the key step. These tetrahydrofuran derivatives are designed to promote hydrogen bonding and van der Waals interactions with the backbone atoms in the S2 subsite of the HIV-1 protease active site. Several inhibitors displayed very potent HIV-1 protease inhibitory activity. A high-resolution X-ray crystal structure of an inhibitor-bound HIV-1 protease provided important insight into the ligand binding site interactions in the active site.

Introduction

The development of HIV-1 protease inhibitor drugs and their introduction into combination therapy with reverse transcriptase inhibitors marked the beginning of an important breakthrough for the treatment of patients with HIV-1 infection and AIDS.[1,2] The advent of this active antiretroviral therapy (ART) helped transform HIV-1 infection from an inevitably fatal disease into a manageable chronic ailment.[3,4] Currently there is no treatment to eradicate HIV from an infected patient. However, ART treatment regimens significantly improve quality of life, enhance HIV management, and reduce mortality and morbidity in patients with HIV-1 infection and AIDS.[5,6] Despite these advances, there are major drawbacks

associated with the current treatment, particularly peptide-like features, poor ADME properties, drug toxicity and drug side effects.[7,8] Another major alarming problem is the emergence of drug resistant HIV-1 strains, which may limit long-term treatment options.[9,10] Therefore, the development of a new generation of protease inhibitors (PIs) with improved drug properties as well as broad-spectrum activity against multidrug resistant HIV-1 variants is critically important for future HIV/AIDS management.[11,12]

Over the years, our research efforts have focused on the design and development of nonpeptide protease inhibitors incorporating stereochemically defined novel heterocycles to interact with the backbone residues in the active site of HIV-1 protease. We reported a variety of highly potent inhibitors that showed intriguing structural features and displayed broad-spectrum antiviral activity against multidrug resistant HIV-1 variants.[13–15] One of these PIs is the FDA approved drug, darunavir (**1**, Fig. 1), which became a first-line therapy for the treatment of HIV/AIDS patients.[16,17] One of the intriguing features of darunavir is the incorporation of a structure-based design nonpeptide ligand, 3(*R*),3a(*S*),6a(*R*)-bis-tetrahydrofuranylurethane (bis-THF).[18,19] The P2 bis-THF ligand is specifically designed to promote maximum interactions, particularly hydrogen bonding interactions with the backbone atoms in the S2 subsite of the HIV-1 protease active site. This "backbone binding concept" emerged from the observation that the backbone conformation of the active site of mutant proteases shows only minimal distortion.[20,21] Therefore, design strategies enhancing inhibitor protein backbone interactions may

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†This manuscript is dedicated to Professor Sukh Dev, an exemplary teacher, a scholar, and a pioneer, on the occasion of his 100th birthday.
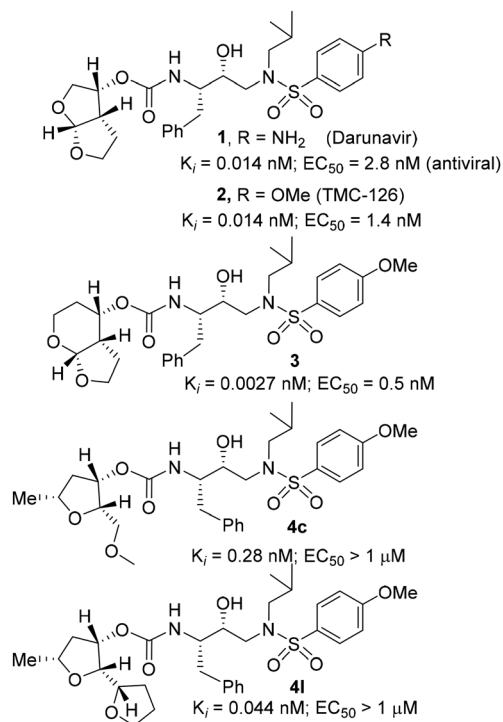‡Electronic supplementary information (ESI) available: Crystallographic data collection and refinement statistics. See DOI: https://doi.org/10.1039/d4ob00506f

7354 | *Org. Biomol. Chem.*, 2024, **22**, 7354–7372

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Fig. 1 Structures of PIs **1–3**, **4c**, and **4l**.

likely lead to inhibitors sustaining inhibitor affinity and potency.[22,23] A number of X-ray crystallographic studies of darunavir-bound HIV-1 protease revealed that both oxygen atoms of the bis-THF ligand form very strong hydrogen bonding interactions with the backbone amide NHs of Asp29 and Asp30 in the S2 subsite.[24,25] Furthermore, the bicyclic bis-THF scaffold nicely fills in the hydrophobic pocket, thereby enhancing van der Waals interactions with the enzyme active site residues. Darunavir's P2′ amino benzenesulfonamide ligand also forms hydrogen bonds with the Asp30 backbone NH as well as with the side chain carboxylic acid. These extensive interactions from the S2 to S2′ subsites of HIV-1 protease are likely to be responsible for darunavir's potency and effectiveness against multidrug-resistant HIV-1 variants.[26,27]
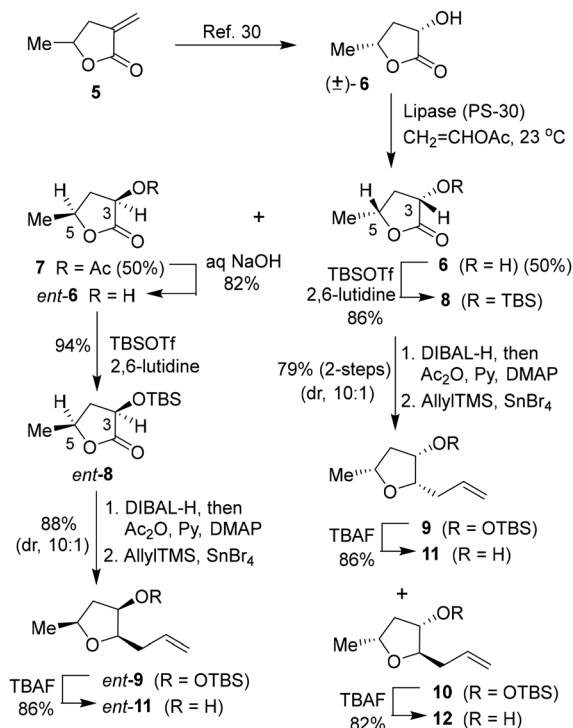
Based upon the X-ray structures of darunavir and its P2′ methoxy derivative **2**, we further designed a bicyclic acetal bearing a tetrahydropyranyl–tetrahydrofuran ring system in inhibitor **3**.[28] We speculated that a larger tetrahydropyranyl (THP) ring may improve hydrogen bonding with the active site aspartate backbone NHs due to increase in the dihedral angle of the bicyclic acetal oxygen atoms. Also, a larger THP-ring may enhance van der Waals interactions. Indeed, inhibitor **3** exhibited high enzyme affinity and antiviral activity and maintained excellent antiviral activity against a variety of multi-drug resistant clinical HIV-1 strains.[29] Our PI design promoting backbone hydrogen bonding interactions also led to the development of diverse classes of PIs that exhibited marked antiviral activity and displayed robust activity against highly multidrug-resistant HIV-1 strains.[13,20,21] Based upon the X-ray struc-

ture of darunavir bound HIV-1 protease, we have now investigated the potential of various substituted tetrahydrofuran derivatives with acyclic and cyclic functionalities to function as effective P2 ligands for the S2 subsite of the HIV-1 protease substrate binding site, exemplified in structures **4c** and **4l**. Herein, we report the design and syntheses of a variety of nonpeptide HIV-1 protease inhibitors incorporating functionalized tetrahydrofuran derivatives as the P2 ligands in combination with (R)-(hydroxyethylamine)sulfonamide isosteres. Functionalized tetrahydrofuran derivatives were synthesized stereoselectively in optically active form using lipase-PS catalyzed enzymatic resolution as the key step. All inhibitors were evaluated in HIV-1 protease inhibitory and antiviral assays. The X-ray crystal structure of an inhibitor-bound HIV-1 protease was determined to obtain molecular insights into the inhibitor protease interactions.

Results and discussion

Based on the X-ray crystal structure of darunavir and wild-type HIV-1 protease, we planned to explore new PIs containing acyclic tetrahydrofuran derivatives that can accommodate key ligand binding site interactions of the P2 bis-THF ligand of daruanvir.[24,25] Furthermore, we envisioned that such substituted tetrahydrofuran derivatives can be optimized to improve the polar interactions with both Asp29 and Asp30 active site backbone atoms. Also, we speculated that installing appropriate alkyl substituents at the C2 and C5 positions would enhance hydrophobic contact with the S2 subsite residues. Accordingly, we designed several trisubstituted tetrahydrofuran derivatives with varying stereochemistry at the C2, C3, and C5 positions. For detailed assessment of the ligand-binding site interactions, the syntheses of these substituted tetrahydrofuran derivatives were carried out in an enantioselective manner. As shown in Scheme 1, commercially available α-methylene-γ-valerolactone **5** was subjected to ozonolysis to provide the corresponding keto lactone that was hydrogenated with 10% Pd–C in ethyl acetate under a hydrogen-filled balloon to afford the racemic α-hydroxy lactone **6** in 62% yield in two steps.[30,31] Enzymatic resolution of racemic alcohol with amano-lipase PS-30 in a mixture (1:1) of vinyl acetate and THF at 23 °C for 12 h resulted in the optically active alcohol **6** and acetate derivative **7** in excellent yield. Saponification of acetate **7** with aqueous NaOH at 23 °C for 12 h furnished the optically active alcohol ent-**6** in 61% yield. Both enantiomeric alcohols were obtained with over 90% ee. Stereochemical identity of the enantiomers was previously determined by X-ray crystallographic analysis.[31] Both enantiomeric alcohols were prepared in a multigram scale for the synthesis of various enantiomeric ligands for our studies.
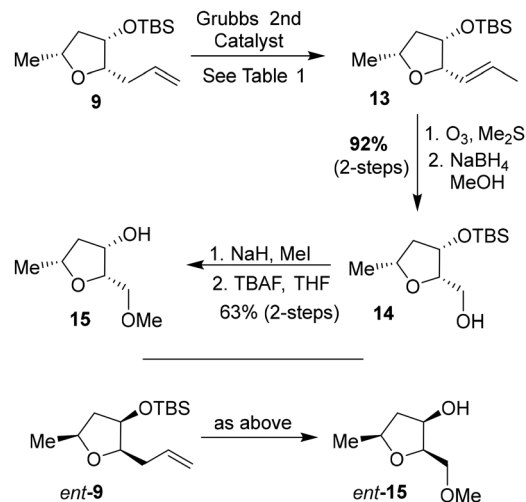
The optically active (3S,5R)-3-hydroxy-5-methyl 2-furanone **6** was reacted with TBSOTf and 2,6-lutidine in CH_2Cl_2 at 0 °C for 2 h to provide TBS-ether **8** in 96% yield. DIBAL-H reduction of lactone at −78 °C for 2 h gave the corresponding lactol that was immediately treated with acetic anhydride and triethyl-

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Org. Biomol. Chem., 2024, **22**, 7354–7372 | **7355**

Scheme 1 Synthesis of optically active (2S,3S,5R)-2-allyl-5-methyl-tetrahydrofuran-3-ol and its enantiomer.

Scheme 2 Synthesis of (2S,3S,5R)-2-(methoxymethyl)-5-methyl-tetrahydrofuranol **15** and *ent*-**15**.

Table 1 Optimization of terminal olefin isomerization[a]

Entry	Grubbs 2nd (mol%)	Reaction conditions[c]	Yield[b] (%)
1	20	MeOH, 60 °C, 4 h	67
2	5	CH_2Cl_2, 60 °C, 24 h	61
3	10	CH_2Cl_2, 110 °C, 24 h	95
4	15	CH_2Cl_2, 60 °C, 72 h	54
5	5	Toluene, 110 °C, 24 h	60
6	10	Toluene, 110 °C, 24 h	47

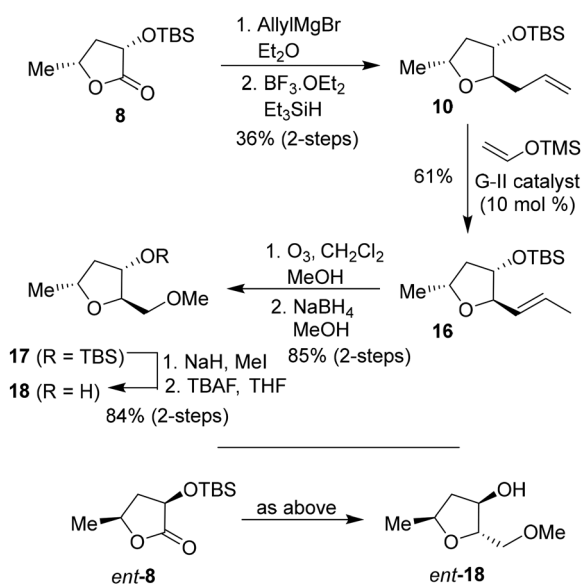[a] Reaction were carried out at the 0.1 mmol scale and in 0.012 M solution. [b] Isolated yield at chromatography. [c] Trimethyl(vinyloxy)silane (10 equiv.) was used for all experiments except those shown in entry 1.

amine in the presence of a catalytic amount of DMAP at −78 °C for 2 h to furnish the corresponding acetate in 90% yield in two steps. Exposure of the resulting acetate to allyl trimethyl silane in the presence of $SnBr_4$ in CH_2Cl_2 at −78 °C to 23 °C for 2 h resulted in the allyl derivative **9** as the major product (dr, 10 : 1 by 1 H-NMR analysis) along with a small amount of the minor diastereomer **10** in 88% yield.[31,32] The diastereomers were separated by using silica gel chromatography. Treatment of the resulting allyl derivative **9** with TBAF in THF at 0 °C to 23 °C for 3 h furnished the trisubstituted ligand alcohol **11** in 86% isolated yield. Similarly, the removal of the TBS group from diastereomer **10** provided alcohol **12** in 82% yield. The enantiomeric ligand alcohol *ent*-**11** was prepared from the optically active alcohol *ent*-**6** using the same sequence of reactions as for the synthesis of ligand **11**. The minor isomer *ent*-**12** also formed in the reaction.

We then planned to investigate the substituted tetrahydrofuran derivative containing a C2 methoxymethyl side chain. The synthesis of (2S,3S,5R)-2(methoxymethyl)-5-methyl-3-tetrahydrofuranol is shown in Scheme 2. The synthesis of the requisite aldehyde requires the introduction of a C2 allyl chain followed by olefin isomerization and cleavage of the resulting olefin. We investigated the olefin isomerization of the C2 substituted tetrahydrofuran derivative under a variety of conditions and the results are shown in Table 1. We first examined terminal alkene isomerization of **9** using 20 mol% Grubbs 2nd generation catalyst in MeOH at 60 °C for 4 h, as described by Hanessian and co-workers.[33] This condition provided the di-

substituted olefin **13** in 67% yield (entry 1). We then examined olefin isomerization using 5 mol% Grubbs 2nd generation catalyst in the presence of 10 equivalents of trimethyl(vinyloxy) silane in CH_2Cl_2 at 60 °C in a sealed tube for 24 h, as reported by Nishida and co-workers.[34] This condition provided the isomerized olefin product **13** in 61% yield (entry 2). The reaction of **9** with 10 mol% Grubbs 2nd generation catalyst and 10 equivalents of trimethyl(vinyloxy)silane in CH_2Cl_2 at 110 °C in a sealed tube for 24 h furnished the isomerization product **13** in 95% yield (entry 3). We also investigated 15 mol% catalyst at 60 °C for 72 h, which provided a lower yield of **13**. Interestingly, the isomerization in toluene resulted in a significant reduction in product yields (entries 5 and 6). For the synthesis of the ligand alcohol **14**, olefin **13** was subjected to ozonolysis in a mixture (1 : 1) of CH_2Cl_2 and MeOH at −78 °C for 2 h followed by reduction with $NaBH_4$ at −78 °C for an additional 2 h. These conditions resulted in alcohol **14** in 92% yield in two steps. The treatment of alcohol **14** with NaH in THF at 0 °C followed by the reaction with MeI at 0 °C to 23 °C

7356 | *Org. Biomol. Chem.*, 2024, **22**, 7354–7372

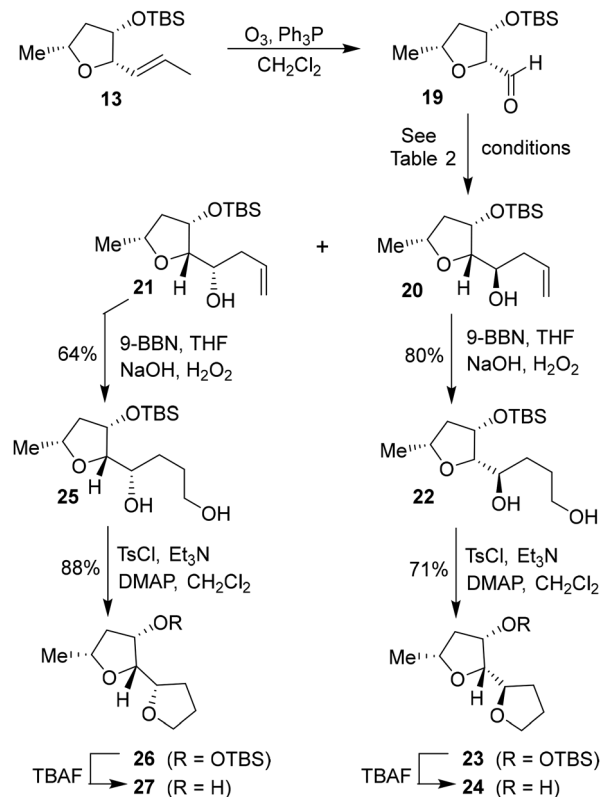This journal is © The Royal Society of Chemistry 2024

afforded the corresponding methyl ether derivative in 63% yield. The reaction of the resulting methyl ether with TBAF at 0 °C to 23 °C for 3 h furnished the ligand alcohol **15** in near-quantitative yield. The enantiomeric ligand alcohol *ent*-**15** was synthesized from *ent*-**9** following the same sequence of reaction as for methyl ether **15**.

To investigate the effect of the stereochemistry at the C2 methoxymethyl side chain, we prepared the corresponding diastereomeric tetrahydrofuran derivatives in a stereoselective manner, as shown in Scheme 3. TBS protected lactone **8** was treated with allyl magnesium bromide in ether at −78 °C for 3 h to provide the corresponding hemiketal intermediate in 63% yield. Reduction of the resulting hemiketal using triethylsilane in the presence of BF₃·OEt₂ in CH₂Cl₂ at −78 °C for 3 h furnished the allyl derivative **10** as a major product (dr, 10 : 1 by ¹H-NMR) in 58% yield. Olefin isomerization using Grubbs second generation catalyst as described above resulted in olefin **16** in 61% yield.[33,34] The ozonolysis of **16** followed by reduction with NaBH₄ afforded alcohol **17** in 85% yield over 2 steps. Alcohol **17** was converted to its methyl ether with NaH and MeI. Removal of the TBS ether using TBAF in THF furnished (2*R*,3*S*,5*R*)-2(methoxymethyl)-5-methyl tetrahydrofuran-3-ol **18** in 84% yield over 2 steps. The corresponding enantiomeric ligand alcohol *ent*-**18** was prepared following the same sequence of reactions as compound **18**.

Based upon our previous design of the bicyclic fused bis tetrahydrofuran (bis-THF) ligand, we planned to examine the feasibility of substituted 2-tetrahydrofuranyl–tetrahydrofuran derivatives as the P2 ligands. The synthesis of diastereomeric ligands utilizing isomerized olefin **13** is shown in Scheme 4. The ozonolytic cleavage of olefin **13** with Ph₃P at −78 °C for 1.5 h provided aldehyde **19** in 76% yield. Aldehyde **19** was subjected to allylation under a variety of conditions. The results
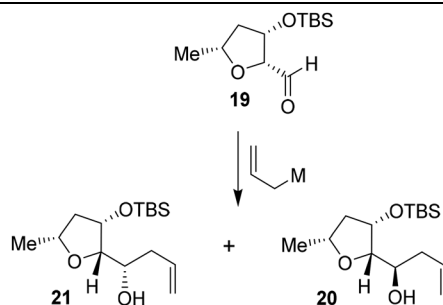

Scheme 4 Synthesis of (2*R*,2'*R*,3*S*,5*R*)-5-methyloctahydro-[2,2'-bifuran]-3-ol **24** and its diastereomer **27**.

are shown in Table 2. The reaction of **19** with allyl trimethylsilane in the presence of 3 equivalents of BF₃·OEt₂ at −78 °C for 4 h provided a mixture (82 : 18) of diastereomers **20** and **21** in 78% combined yield (entry 1). The diastereomeric ratios were determined by ¹H-NMR (500 MHz) analysis. The use of allyl tributyl stannane under the above conditions afforded a mixture (83 : 17) of alcohols **20** and **21** in 79% yield (entry 2). The use of MgBr₂·OEt₂ as the Lewis acid resulted in a marginal diastereoselective (45 : 55 ratio) and reduction in yield (entry 3). Treatment of **19** with allylMgBr in ether at −78 °C for 3 h provided diastereomers **20** and **21** as a mixture (45 : 55) in 59% yield (entry 4). The use of ZnCl₂ as the bidentate Lewis acid and allyl tributyl stannane at −78 °C to 23 °C for 18 h resulted in the reversal of diastereoselectivity and diastereomers **20** and **21** were obtained as a mixture (25 : 75) in 75% yield. These diastereomers were separated by silica gel chromatography and the major diastereomer **20** (from entry 2) was treated with 9-BBN in THF at 0 °C to 23 °C for 6 h.

Oxidation of the resulting organoborane with 3 N NaOH and 30% H₂O₂ at 23 °C for 3 h furnished diol **22** in 80% yield. Treatment of diol **22** with tosyl chloride and Et₃N in the presence of a catalytic amount of DMAP in CH₂Cl₂ at 0 °C to 23 °C for 48 h furnished the bicyclic tetrahydrofuran derivative **23** in 71% yield. Deprotection of the TBS group using TBAF in THF at 0 °C to 23 °C for 3 h afforded the ligand alcohol **24** in near-quantitative yields. The diastereomeric alcohol **21** (from
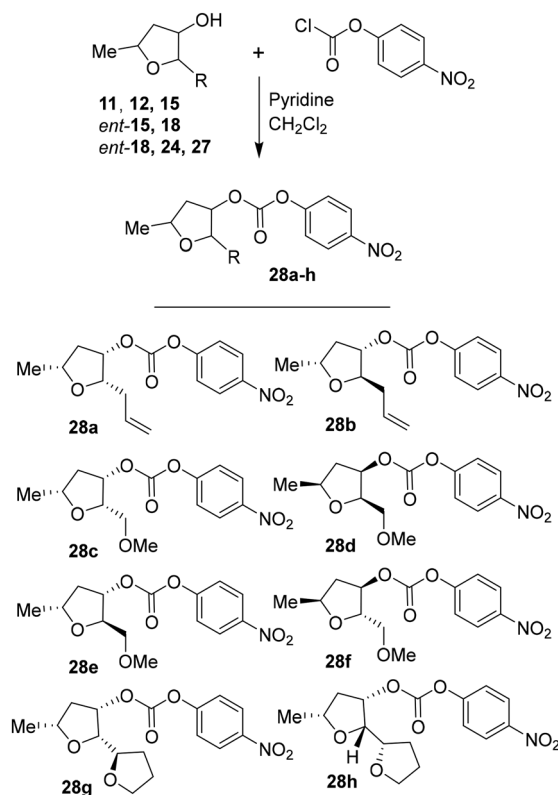

Scheme 3 Synthesis of (2*R*,3*S*,5*R*)-2-(methoxymethyl)-5-methyl-tetrahydrofuranol **18** and *ent*-**18**.

Table 2 Addition of allyl metals to aldehyde **19**[a]

Entry	M	Lewis acid and conditions	Yield[b] (%)	Ratio (**20** : **21**)[c]
1	SiMe$_3$	BF$_3$·OEt$_2$, CH$_2$Cl$_2$, −78 °C, 4 h	78	4.2 : 1
2	SnBu$_3$	BF$_3$·OEt$_2$, CH$_2$Cl$_2$, −78 °C, 18 h	79	4.3 : 1
3	SnBu$_3$	MgBr$_2$·OEt$_2$, CH$_2$Cl$_2$, 23 °C, 4 h	75	1 : 1.3
4	MgBr	ZnCl$_2$, Et$_2$O, −78 °C, 3 h	59	1 : 1.2
5	SnBu$_3$	ZnCl$_2$, CH$_2$Cl$_2$, −78 °C, 18 h	75	1 : 3
6	SiMe$_3$	ZnCl$_2$, CH$_2$Cl$_2$, −78 °C, 18 h	66	1 : 2.5

[a] Reactions were carried at the 0.1 mmol scale. [b] Isolated yield at chromatography. [c] Ratios were determined by ^{1}H-NMR analysis.

entry 5) was then converted to the diastereomeric tetrahydro-furanyl–tetrahydrofuran derivative **27** using the same sequence of reactions.

For the synthesis of inhibitors with substituted tetrahydro-furans, various ligand alcohols were converted to the corresponding mixed activated carbonate derivatives, as shown in

Scheme 5. Reactions of optically active ligand alcohols (**11, 12, 15**, *ent*-**15, 18**, *ent*-**18, 24** and **27**) were reacted with 4-nitrophenyl chloroformate and pyridine in CH$_2$Cl$_2$ at 0 °C to 23 °C for 12 h, furnishing mixed carbonates **28a–h** in good yields.[26] Syntheses of inhibitors with (*R*)-(hydroxyethylamine)sulfonamide isosteres containing 4-methoxysulfonamide and 4-aminosulfonamide as the P2′-ligands are shown in Scheme 6. Treatment of known amine derivatives **29** and **30** with mixed activated carbonates **28a–e** in the presence of diisopropylethylamine (DIPEA) in CH$_3$CN at 23 °C for 12–36 h provided inhibitors **4a–l**.[26] The full structures of these inhibitors are shown in Table 3.

The selected PIs containing tri-substituted tetrahydrofuran derivatives as the P2-ligands were designed and synthesized to assess their interactions in the S2-subsite with respect to the bis-THF ligand of darunavir.[16] The structure and activity of
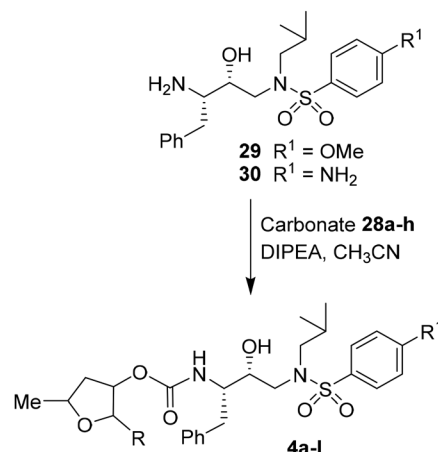

Scheme 5 Synthesis of activated carbonates **28a–h**.

Scheme 6 Synthesis of protease inhibitors **4a–l**.

7358 | *Org. Biomol. Chem.*, 2024, **22**, 7354–7372

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Table 3 Activity of PIs with substituted tetrahydrofuran ligands
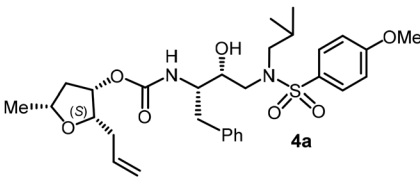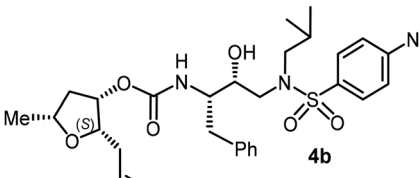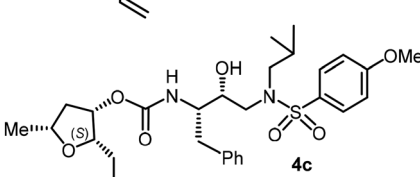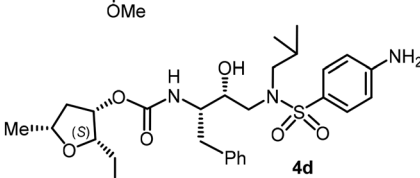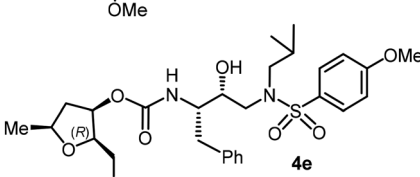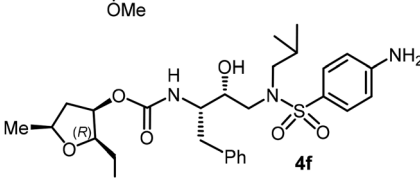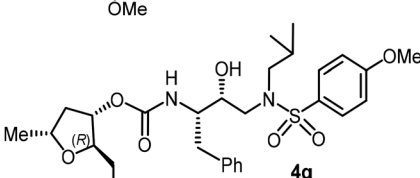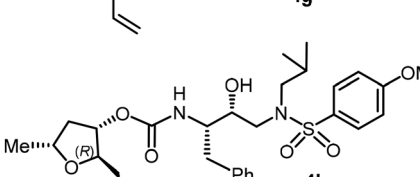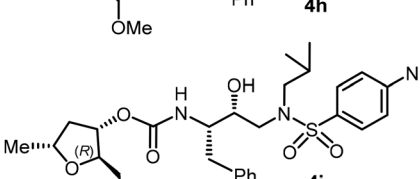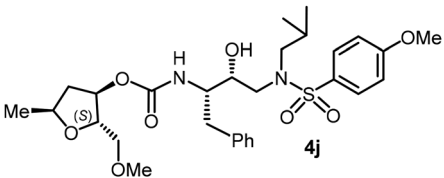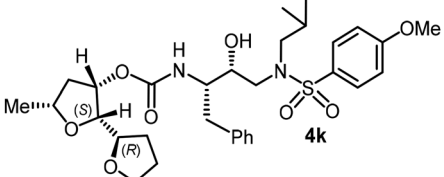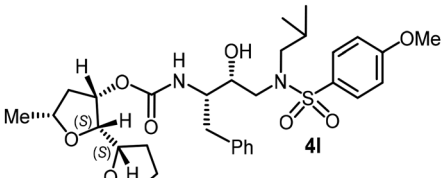
Entry	Inhibitor	$K_i{}^a$ (nM)	$EC_{50}{}^b$ (µM)
1	**4a**	0.4	>1
2	**4b**	26	>1
3	**4c**	0.28	>1
4	**4d**	88	>1
5	**4e**	1.2	>1
6	**4f**	35.3	>1
7	**4g**	0.78	>1
8	**4h**	0.049	>1
9	**4i**	9.4	>1

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Org. Biomol. Chem., 2024, **22**, 7354–7372 | **7359**

Table 3 (Contd.)

Entry	Inhibitor	$K_i{}^a$ (nM)	$EC_{50}{}^b$ (µM)
10	4j	2.6	>1
11	4k	0.31	>1
12	4l	0.044	>1

[a] K_i values represent at least 5 data points. Standard error in all cases was less than 7%. Darunavir exhibited K_i = 16 pM. [b] Values are means of at least three experiments in MT-2 cells. Darunavir exhibited EC_{50} = 1.6 nM.

these new PIs are shown in Table 3. The synthetic PIs were first evaluated using the spectrofluorometric assay described by Toth and Marshall.[35] As shown, inhibitor **4a** with C-2(S) allyl and C-5(R) methyl substituents showed a HIV-1 protease inhibitory K_i value of 0.4 nM. We then determined the antiviral activity of these PIs in MT-2 human-T-lymphoid cells exposed to HIV$_{LAI}$.[30] In this cell-based antiviral assay, inhibitor **4a** did not exhibit any appreciable antiviral activity.[36,37] Compound **4b** with 4-aminosulfonamide as the P2′-ligand displayed significant loss of HIV-1 protease inhibitory activity (entry 2). Compound **4c** with a C2(S) methoxymethyl substituent was designed to make hydrogen bonding interactions in the S2-subsite. However, compound **4c** exhibited marginal improvement of protease inhibitory activity over compound **4a** without a polar substituent at the C2 position (entry 3). It turns out that the choice of P2′-ligand is important for activity as the 4-methoxysulfonamide P2′ ligand showed enhanced potency over the 4-aminosulfonamide P2′ ligand as compound **4d** exhibited over 300-fold reduction of protease inhibitory activity over compound **4c**. Interestingly, none of these derivatives showed any appreciable antiviral activity (entries 1–4). We then examined the effect of the enantiomeric P2 ligand in compounds **4e** and **4f**. However, compound **4e** showed a nearly 4-fold reduction of enzyme activity over **4c** which contains an enantiomeric P2-ligand. In compound **4g**, we altered the C2 lipophilic allyl side chain stereochemistry. However, compound **4g** displayed HIV-1 protease inhibitory activity that was comparable to that of compound **4a** containing the C2(S)-allyl substituent (entries 1 and 7). Compound **4h** with a C2(R)-methoxymethyl substituent exhibited very potent enzyme inhibitory activity (K_i 49 pM) compared to compound **4c** with a

C2(S)-methoxymethyl substituent. The corresponding compound **4i** with a P2′-aminosulfonamide ligand showed significant reduction of enzyme K_i value (entries 8 and 9). Compound **4j** with an enantiomeric P2 ligand is significantly less potent than compound **4h**, indicating that the C2(R)-methoxymethyl stereochemistry is more suitable for polar and van der Waals interactions in the S2 subsite. We then examined the effect of a C2-tetrahydrofuranyl substituent in compounds **4k** and **4l**, differing the stereochemistry at the C2′-position. Interestingly, compound **4k** with a 2(S),2′(R) tetrahydrofuranyl–tetrahydrofuran ligand exhibited HIV-1 protease inhibitory activity (K_i 0.31 nM) that was comparable to that of compound **4c** with a C2 methoxymethyl side chain (entries 3 and 11). Compound **4l**, on the other hand, with a diastereomeric tetrahydrofuranyl–tetrahydrofuran ligand (2S,2S′ configuration) displayed very potent HIV-1 protease inhibitory activity with a K_i value of 44 pM comparable to that of darunavir (entry 12). However, both compounds **4k** and **4l** did not exhibit any appreciable antiviral activity. The reason for the lack of antiviral activity is not clear. The observed high EC_{50} values may be due to fact that these compounds do not penetrate the cell. We and others observed such differences between HIV-1 inhibitory activity and cell-based antiviral activity, previously.[38,39] The compounds in Table 3 have also shown relatively higher clog P (lipophilicity) values (**4l**, clog P 5) than darunavir (clog P 2.9). Compound **4l** showed marked HIV-1 protease affinity, possibly due to strong hydrogen bonding interactions with the backbone residues as well as van der Waals interactions in the S2 subsite of HIV-1 protease.

To obtain molecular insight into the HIV-1 protease–inhibitor interactions, we determined the X-ray crystal structure of

7360 | *Org. Biomol. Chem.*, 2024, **22**, 7354–7372

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HIV-1 protease complexed with compound **4l**. An active site interaction of **4l**-bound HIV-1 protease structure is shown in Fig. 2. The X-ray structure of wild-type HIV-1 protease co-crystallized with inhibitor **4l** (GRL-072-17A) was determined and refined to 1.32 Å resolution. Two conformations of the inhibitor with a C2-symmetry were visible in the active site of the protease dimer at relative occupancy of 0.55/0.45. The protease dimer structure is similar to that of the PR/DRV complex with a RMSD of 0.17 Å for 198 equivalent Cα atoms.[25] Most differences in Cα positions are less than 0.4 Å. The largest disparities of about 0.6 Å for Pro81′ and the adjacent Gly49 and Gly49′ are likely due to the large P2 group of (2*S*,2′*S*,5*R*)-5-methyloctahydro-2,2′-bifuran instead of the bis-tetrahydrofuran of DRV.[25]

The inhibitor retains the majority of hydrogen bonds observed between DRV and the main chain atoms of protease with the exception of the altered P2 group. An overlay of X-ray structures of darunavir-bound HIV-1 protease and compound **4l**-bound HIV-1 protease is shown in Fig. 3. The number of van

der Waals interactions for the P2 tetrahydrofuranyl–tetrahydrofuran ligand of inhibitor **4l** with the protease are reduced compared to those of protease and darunavir interactions. The new P2 group has an added methyl group and a single bond to separate the two tetrahydrofurans in the opposite direction to create a tetrahydrofuranyl–tetrahydrofuran derivative. The changes in the P2 group increase its flexibility in the binding cavity of the HIV protease. The P1–P2′ scaffold which links the P2 group with the rest of the inhibitor shifts toward the flap to create space for the additional methyl group. The atom bearing the methyl group sinks into a hydrophobic bowl-shaped depression and forms new van der Waals interactions with the hydrophobic side chains of Ala28, Val32 and Ile84, while maintaining a weak C–H···O interaction with the carboxyl oxygen of Asp30. The flexible linkage allows the outer tetrahydrofuran ring to reduce the unfavorable interaction with the carboxyl oxygen of Gly48 to 3.5 Å compared with 3.0 Å in the darunavir-bound HIV-1 protease structure.[25] The oxygen atom of the outer tetrahydrofuran in the P2 group preserves hydrogen bonds with the amide nitrogen atom and side chain oxygen of Asp29 at distances of 2.9 Å and 3.3 Å, respectively. However, the oxygen atom of the inner tetrahydrofuran has lost both hydrogen bonds to the amide nitrogen atoms of Asp29 and Asp30 (interatomic distances of 3.8 Å) unlike the bis-THF ligand of darunavir. Overall, the alteration in the P2 group decreases the inhibitor interactions with HIV protease compared to those seen in the bis-THF ligand with the HIV-1 protease active site.[24,25]
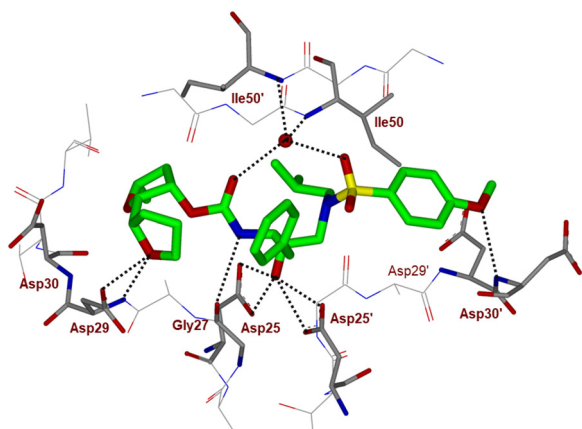
Fig. 2 X-ray structure of inhibitor **4l** (green color)-bound to the active site of wild-type HIV-1 protease (PDB code: 9B2H). All strong hydrogen bonding interactions are shown as dotted lines.

Conclusion

In summary, we investigated the use of a series of substituted tetrahydrofuran derivatives as P2 ligands for HIV-1 protease inhibition. These ligands were designed based upon the X-ray crystal structure of darunavir-bound HIV-1 protease. In particular, the substituted tetrahydrofuran derivatives containing polar and lipophilic substituents are designed to form hydrogen bonds and fill in the hydrophobic pocket similar to the bis-THF ligand of darunavir in the S2 subsite of HIV-1 protease. The syntheses of the substituted tetrahydrofuran were carried out in a stereoselective manner in an optically active form. In general, inhibitors containing these ligands exhibited very potent HIV-1 protease inhibitory activity and a number of compounds displayed protease inhibitory activity comparable to darunavir. The influence of ligand stereochemistry is clearly evident. Compound **4h** with a 2(*R*)-methoxymethyl side chain exhibited over 5-fold improvement of enzyme affinity compared to compound **4c** with a 2(*S*)-methoxymethyl side chain. Also, inhibitors **4k** and **4l** with tetrahydrofuranyl–tetrahydrofuran moieties with C2(*R*) and C2(*S*) configuration, respectively, displayed preference for the C2(*S*) configuration as compound **4l** exhibited a HIV-1 protease inhibitory K_i of 44 pM, comparable to that of darunavir (K_i 14 pM). Several inhibitors containing these stereochemically defined ligands displayed very potent HIV-1 protease inhibitory activity; however they did
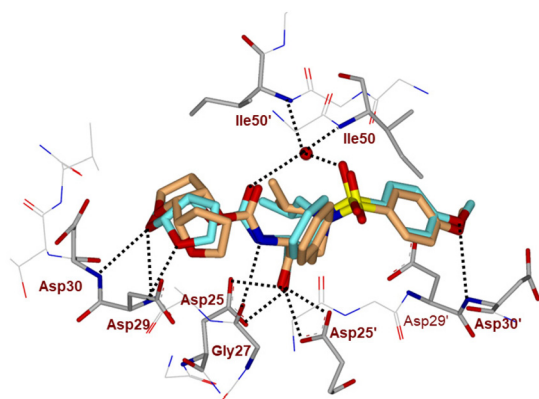

Fig. 3 Overlay of the X-ray structure of inhibitor **4l** (orange color)-HIV-1 protease (PDB code: 9B2H) and X-ray structure of TMC-126 (turquoise color)-bound HIV-1 protease (PDB code: 3I7E).

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Org. Biomol. Chem., 2024, **22**, 7354–7372 | **7361**

not exhibit any appreciable antiviral activity. Inhibitors containing the P′2-methoxysulfonamide ligand were significantly more potent than inhibitors with the P′2-aminosulfonamide ligand. We determined the high resolution X-ray structure of the inhibitor **4l**-bound HIV-1 protease. The structure revealed that C2 substituted THF oxygen forms a strong hydrogen bond with the backbone Asp29 amide NH in the S2 subsite. However, the oxygen atom of the inner THF ring does not form any hydrogen bond with the Asp29 and Asp30 backbone NH, like the bis-THF ligand of darunavir. This loss of the backbone hydrogen bonding interaction as well higher lipophilicity may be responsible for the lack of antiviral activity of these PIs. Further design and optimization of inhibitor properties are in progress in our laboratory.

Experimental section

All reactions were carried out under an inert atmosphere, either N_2 or Ar, using magnetic stirring and oven-dried glassware. All solvents were anhydrous and distilled prior to use. Dichloromethane and triethylamine were distilled from calcium hydride. Tetrahydrofuran, diethyl ether, and benzene were distilled from sodium/benzophenone. All other solvents were of HPLC grade or better. Flash column chromatography was performed using EM Science 60–200 mesh silica gel. Thin-layer chromatography was performed using 60 F-254 E. Merck silica gel plates. ^{1}H- and ^{13}C-NMR were recorded using Bruker AV-400, Avance DRX-500, Varian Mercury-Vx-300, and Gemini-2300 spectrometers and Me_4Si as an internal standard. Optical rotations were recorded on a PerkinElmer 341 polarimeter. A Thermo Finnigan LCQ Classic mass was used for MS analyses. The purity of the test compounds was determined by HRMS and HPLC analysis. All test compounds showed ≥95% purity.

3-Hydroxy-5-methyldihydrofuran-2(3H)-one ((±)-6)

To a stirred solution of α-methylene-γ-butyrolactone **5** (6.12 g, 54.6 mmol) in CH_2Cl_2 (20 mL) at −78 °C was bubbled a stream of ozone until blue color persisted. The ozone stream was then stopped and purged with a stream of oxygen to remove the excess ozone. After adding dimethyl sulfide (12 mL, 163.7 mmol), the reaction mixture was warmed to 23 °C and stirred for 4 h. The reaction mixture was concentrated under reduced pressure and the crude product (4.9 g, 79% yield) was used for the next step without any further purification.

To a stirred solution of the unsaturated lactone intermediate (3.5 g, 31 mmol) in EtOAc (20 mL) was added Pd–C (150 mg, 10 wt%). The resulting solution was stirred at 23 °C under 1 atm H_2 gas over 24 h. Upon completion of the reaction, the mixture was filtered through a plug of Celite, and the solvents were removed under reduced pressure. The crude product was purified by silica gel column chromatography (50% EtOAc in hexanes) to give racemic lactone **6** (2.87 g, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.57 (dd, J = 11.3, 8.4 Hz, 1H), 4.54–4.45 (m, 1H), 4.03 (brs, 1H), 2.70 (ddd, J = 12.6, 8.4,

5.1 Hz, 1H), 1.90–1.80 (m, 1H), 1.43 (d, J = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ, 178.2, 73.9, 69.1, 38.8, 20.9.

(3S,5R)-3-Hydroxy-5-methyldihydrofuran-2(3H)-one ((+)-6)

To a solution of racemic lactone **6** (960 mg, 8.23 mmol) in THF (15 mL) were added vinyl acetate (13.3 mL, 144.9 mmol) and lipase PS-30 (0.9 g) at 23 °C under an argon atmosphere. The reaction mixture was stirred for 2 h (50 : 50 by ^{1}H NMR). After this period, the reaction mixture was filtered through a plug of Celite and solvents were removed under reduced pressure. The crude product was purified by silica gel column chromatography (20% to 50% EtOAc in hexanes) to give alcohol **(+)-6** (485 mg, 50% yield) as a colorless oil and acetate **7** (650 mg, 50% yield). Alcohol **(+)-6**; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.58 (dd, J = 11.2, 8.4 Hz, 1H), 4.52–4.44 (m, 1H), 4.18 (brs, 1H), 2.69 (ddd, J = 12.9, 8.3, 5.1 Hz, 1H), 1.89–1.79 (m, 1H), 1.42 (d, J = 6.3 Hz, 3H); ^{13}C (100 MHz, DMSO-d$_6$) δ: 178.3, 73.9, 69.0, 38.8, 20.9; LRMS-ESI (m/z): 139.2 $(M + Na)^+$, $[\alpha]_D^{20}$ +2.8 (c 1.0, $CHCl_3$). Acetate **7**; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.45 (dd, J = 10.9, 8.6 Hz, 1H), 4.59–4.47 (m, 1H), 2.76 (ddd, J = 12.7, 8.5, 5.3 Hz, 1H), 2.10 (s, 3H), 1.83 (dt, J = 12.5, 10.6 Hz, 1H), 1.43 (d, J = 6.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ, 172.5, 169.8, 73.6, 69.0, 36.7, 21.0, 20.6; LRMS-ESI (m/z): 181.2 $(M + Na)^+$; $[\alpha]_D^{20}$ −18.45 (c 1.0, $CHCl_3$).

(3R,5S)-3-Hydroxy-5-methyldihydrofuran-2(3H)-one (ent-6)

To a stirred solution of acetate **7** (320 mg, 2.0 mmol) in MeOH (5 mL) was added aqueous NaOH (10% solution, 5 mL) and the mixture was stirred at 23 °C for 12 h. After this period, the reaction mixture was acidified with 1 N HCl solution and solvents were concentrated under reduced pressure. The aqueous layer was extracted multiple times with EtOAc. The combined organic layers were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (60% EtOAc in hexane) to give alcohol **ent-6** (144 mg, 61%) as a colorless oil. LRMS-ESI (m/z): 139.2 $(M + Na)^+$; $[\alpha]_D^{20}$ −2.3 (c 1.0, CH_3OH); for NMR data, please see lactone **(+)-6**.

(3S,5R)-3-((tert-Butyldimethylsilyl)oxy)-5-methyldihydrofuran-2(3H)-one (8)

To a stirred solution of the optically active alcohol **(+)-6** (485 mg, 4.2 mmol) in CH_2Cl_2 (10 mL) were added 2,6-lutidine (1.45 ml, 12.5 mmol) and TBSOTf (1.45 mL, 6.27 mmol) at 0 °C under an argon atmosphere. The reaction mixture was warmed to 23 °C and stirred for 1 h. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The extracts were washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (10% EtOAc in hexanes) and TBS lactone **8** (923 mg, 96%) was obtained as a white amorphous solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.46–4.34 (m, 2H), 2.56 (ddd, J = 12.6, 8.1, 5.3 Hz, 1H), 1.77 (dt, J = 12.4, 10.3 Hz, 1H), 1.37 (d, J = 6.3 Hz, 3H), 0.85 (d, J = 3.8 Hz, 9H), 0.10 (d, J = 17.0 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ, 175.8, 72.6, 69.8, 40.0,

7362 | *Org. Biomol. Chem.*, 2024, **22**, 7354–7372

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25.7, 21.1, 18.2, −4.7, −5.3; LRMS-ESI (m/z): 253.2 (M + Na)$^+$; HRMS (ESI), calcd for $C_{11}H_{23}O_3Si$: m/z 231.1416 [M + H]$^+$, found 231.1408; $[\alpha]_D^{20}$ −16.0 (c 1.0, CHCl$_3$).

(((2S,3S,5R)-2-Allyl-5-methyltetrahydrofuran-3-yl)oxy)(tert-butyl)dimethylsilane (9)

To a stirred solution of lactone **8** (415 mg, 1.80 mmol) in CH$_2$Cl$_2$ (16 mL) at −78 °C was added DIBAL-H (1 M in hexanes, 2.70 ml, 2.70 mmol) under an argon atmosphere and stirred at the same temperature for 2 h. The reaction was quenched by the addition of MeOH (3 mL) and warmed to 23 °C. Then, a saturated aqueous solution of sodium potassium tartrate was added and stirred vigorously at 23 °C for 2 h until it formed a white suspension. The white suspension was filtered through a plug of Celite and the filtrate was concentrated under reduced pressure. To crude lactol (417 mg) were added DMAP (44 mg, 0.36 mmol), Et$_3$N (1 mL, 7.19 mmol) and Ac$_2$O (0.42 mL, 4.50 mmol) at 0 °C under an argon atmosphere and stirred for 2 h. Upon completion of the reaction, the solvents were concentrated under reduced pressure and the crude residue was purified by silica gel column chromatography (15% EtOAc in hexane) to give the acetate intermediate (445 mg, 90% yield in two steps).

To a solution of the acetate intermediate (344 mg, 1.26 mmol) in CH$_2$Cl$_2$ (12.6 mL, 0.1 M) was added allyl trimethylsilane (0.8 mL, 5 mmol) at 23 °C under an argon atmosphere and then cooled to −78 °C. After the addition of SnBr$_4$ (660 mg, 1.50 mmol), the mixture was warmed to 23 °C over 2 h. Upon completion, the reaction was quenched by the addition of saturated aqueous Na$_2$HPO$_4$ and extracted with CH$_2$Cl$_2$. The combined organic layers were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. ^{1}H NMR analysis of the unpurified crude product showed a pair of diastereomers in a 10 : 1 ratio. The crude product was purified by silica gel column chromatography (70% CH$_2$Cl$_2$ in hexanes) to give the major olefin **9** (256 mg, 80%) as a colorless oil and the minor olefin **10** (25 mg, 8%) as a colorless oil. Olefin **9**: ^{1}H NMR (400 MHz, CDCl$_3$) δ: 5.85 (ddt, J = 17.1, 10.2, 6.9 Hz, 1H), 5.09 (d, J = 17.2 Hz, 1H), 5.02 (d, J = 10.2 Hz, 1H), 4.22 (dt, J = 6.4, 3.7 Hz, 1H), 3.96–3.89 (m, 1H), 3.63 (q, J = 6.2 Hz, 1H), 2.42–2.32 (m, 2H), 2.26 (dt, J = 13.4, 6.9 Hz, 1H), 1.47 (ddd, J = 13.0, 6.6, 2.9 Hz, 1H), 1.29 (d, J = 6.2 Hz, 3H), 0.89 (s, 9H), 0.04 (d, J = 4.3 Hz, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 135.9, 116.4, 83.2, 73.7, 73.5, 43.5, 34.4, 25.9, 22.2, 18.2, −4.4, −4.9; LRMS-ESI (m/z): 279.3 (M + Na)$^+$; HRMS (APCI), calcd for $C_{14}H_{29}O_2Si$: m/z 257.1936 [M + H]$^+$ found m/z 257.1935; $[\alpha]_D^{20}$ +16.8 (c 1.0, CHCl$_3$).

(2S,3S,5R)-2-Allyl-5-methyltetrahydrofuran-3-ol (11)

To a stirred solution of olefin **9** (21 mg, 0.08 mmol) in THF (2 mL) was added TBAF solution (1M in THF, 0.20 mL, 0.20 mmol) at 0 °C under an argon atmosphere. The reaction mixture was warmed to 23 °C and stirred for 3 h. After this period, the reaction was quenched with water and the aqueous layer was extracted with EtOAc. The combined organic layers were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced

pressure. The crude residue was purified by silica gel column chromatography (20% EtOAc in hexane) to give alcohol **11** (10 mg, 86%) as a white amorphous solid. ^{1}H NMR (400 MHz, CDCl$_3$) δ: 5.94–5.82 (m, 1H), 5.20–5.05 (m, 2H), 4.27–4.17 (m, 1H), 3.98–3.88 (m, 1H), 3.66–3.55 (m, 1H), 2.50–2.35 (m, 3H), 1.70 (brs, 1H), 1.54–1.44 (m, 1H), 1.33 (d, J = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 134.7, 117.0, 82.3, 73.5, 73.1, 43.1, 33.4, 21.9; LRMS-ESI (m/z): 165.1 (M + Na)$^+$; HRMS (APCI), calcd for $C_8H_{15}O_2$: m/z 143.1072 [M + H]$^+$, found m/z 143.1069; $[\alpha]_D^{20}$ +8.6 (c 0.33, CHCl$_3$).

(3R,5S)-3-((tert-Butyldimethylsilyl)oxy)-5-methyldihydrofuran-2(3H)-one (ent-8)

Lactone **ent-8** (268 mg, 94% yield) was obtained as a white amorphous solid from alcohol **ent-6** (144 mg, 1.24 mmol) by following the procedure described above for its enantiomer **8**. For NMR data, please see lactone **8**; LRMS-ESI (m/z): 253.2 (M + Na)$^+$; HRMS (ESI), calcd for $C_{11}H_{23}O_3Si$: m/z 231.1416 [M + H]$^+$, found 231.1414; $[\alpha]_D^{20}$ +15.5 (c 1.0, CHCl$_3$).

(((2R,3R,5S)-2-Allyl-5-methyltetrahydrofuran-3-yl)oxy)(tert-butyl)dimethylsilane (ent-9)

By following the procedure outlined for the preparation **9**, olefin **ent-9** was obtained as a colorless oil (210 mg, 0.77 mmol) with dr 10 : 1 after the diastereoselective allylation reaction. LRMS-ESI (m/z): 257 (M + H)$^+$; HRMS (APCI), calcd for $C_{14}H_{29}O_2Si$: m/z 257.1936 [M + H]$^+$ found m/z 257.1934; $[\alpha]_D^{20}$ −14.0 (c 1.0, CHCl$_3$). For NMR data, please see the enantiomeric compound **9**.

(2R,3R,5S)-2-Allyl-5-methyltetrahydrofuran-3-ol (ent-11)

By following the procedure outlined for the preparation of its enantiomer **11**, alcohol **ent-11** was obtained as an amorphous solid. LRMS-ESI (m/z): 165.1; HRMS (APCI), calcd for $C_8H_{15}O_2$: m/z 143.1072 [M + H]$^+$, found m/z 143.1077. Using similar deprotection to TBS ether **10** provided alcohol **12**.

tert-Butyldimethyl(((2S,3S,5R)-5-methyl-2-(prop-1-en-1-yl)tetrahydrofuran-3-yl)oxy)silane 13

To a stirred mixture of olefin **9** (144 mg, 0.56 mmol) and vinyloxytrimethylsilane (0.84 mL, 5.60 mmol) in CH$_2$Cl$_2$ (45 mL) was added the Grubb's second generation catalyst (48 mg, 0.056 mmol) at 23 °C under an argon atmosphere. The reaction mixture was refluxed at 110 °C for 24 h in a sealed tube. The mixture was cooled to 23 °C and concentrated under reduced pressure to remove solvents. The crude residue was purified by silica gel column chromatography (2% diethyl ether in hexanes) to give olefin **13** (136 mg, 95%) as a colorless oil; ^{1}H NMR (400 MHz, CDCl$_3$) δ: 5.73–5.62 (m, 2H), 4.24–4.23 (m, 1H), 4.04–3.97 (m, 2H), 2.31–2.28 (m, 1H), 1.71 (d, J = 5.2 Hz, 3H), 1.60–1.50 (m, 1H), 1.33 (d, J = 5.2 Hz, 3H), 0.88 (s, 9H), 0.02 (s, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 129.4, 128.4, 84.5, 74.9, 73.6, 43.4, 25.7, 22.0, 18.1, 17.8, −4.4, −4.7; LRMS-ESI (m/z): 279.3 (M + Na)$^+$; HRMS (APCI), calcd for $C_{14}H_{29}O_2Si$: m/z 257.1936 [M + H]$^+$ found m/z 257.1936; $[\alpha]_D^{20}$ +28.0 (c 1.0, CHCl$_3$).

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Org. Biomol. Chem., 2024, **22**, 7354–7372 | **7363**

((2S,3S,5R)-3-((*tert*-Butyldimethylsilyl)oxy)-5-methyltetrahydrofuran-2-yl)methanol 14

A solution of olefin **13** (36 mg, 0.14 mmol) in a 1 : 1 mixture of CH$_2$Cl$_2$ and MeOH (3.6 mL) was cooled down to −78 °C. Ozone was passed into the solution until the color of the solution turned pale blue. The reaction mixture was then purged with oxygen for 5 min and NaBH$_4$ (16 mg, 0.43 mmol) was added. After stirring for 2 h at −78 °C, the reaction mixture was diluted with EtOAc and quenched with saturated aqueous NH$_4$Cl. The aqueous layer was extracted with EtOAc. The combined organic layers were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (10% EtOAc in hexanes to 30% EtOAc in hexanes) to give alcohol **14** (32 mg, 92% yield in two steps) as a white amorphous solid; ^{1}H NMR (400 MHz, CDCl$_3$) δ: 4.54–4.46 (m, 1H), 3.98–3.92 (m, 1H), 3.83–3.77 (m, 3H), 2.32–2.27 (m, 1H), 1.36–1.27 (m, 1H), 1.24 (d, J = 6.2 Hz, 3H), 0.90 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 81.2, 74.5, 73.5, 62.7, 43.4, 25.6, 21.3, 17.8, −4.7, −5.3; LRMS-ESI (m/z): 269.2 (M + Na)$^+$; HRMS (APCI), calcd for C$_{12}$H$_{27}$O$_3$Si: 247.1729 m/z [M + H]$^+$, found m/z 247.1725; $[\alpha]_D^{20}$ +13.9 (c 1.0, CHCl$_3$).

(2S,3S,5R)-2-(Methoxymethyl)-5-methyltetrahydrofuran-3-ol 15

To a stirred solution of alcohol **14** (32 mg, 0.13 mmol) in THF (2.6 mL) was added NaH (60% dispersion in mineral oil, 31 mg, 0.77 mmol) at 0 °C under an argon atmosphere. After stirring for 10 min at 0 °C, MeI (88 μL, 1.42 mmol) was added and the reaction mixture was slowly warmed to 23 °C over 3 h. Upon completion, the reaction was quenched with saturated aqueous NH$_4$Cl and the aqueous layer was extracted with diethyl ether. The combined organic layers were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography gave the corresponding methyl ether **14a** (21 mg, 0.08 mmol) as a colorless oil. ^{1}H NMR (400 MHz, CDCl$_3$) δ: 4.35–4.29 (m, 1H), 3.98 (q, J = 6.8 Hz, 1H), 3.81 (q, J = 6.4 Hz, 1H), 3.58–3.52 (m, 2H), 3.38 (s, 3H), 2.32–2.25 (m, 1H), 1.54–1.44 (m, 1H), 1.32 (d, J = 6.4 Hz, 3H), 0.88 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 81.8, 74.1, 73.1, 72.1, 59.1, 43.4, 25.6, 21.8, 17.9, −4.8, −5.3; LRMS-ESI (m/z): 283.2 (M + Na)$^+$; $[\alpha]_D^{20}$ +18.7 (c 1.0, CHCl$_3$).

To a stirred solution of the above-mentioned methyl ether (16 mg, 0.06 mmol) in THF (2 mL) was added TBAF solution (1M in THF, 0.15 mL, 0.15 mmol) at 0 °C under an argon atmosphere. The reaction mixture was warmed to room temperature and stirred for 3 h. The reaction was quenched with water and the aqueous layer was extracted with CH$_2$Cl$_2$. The combined organic layers were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by flash silica gel column chromatography (40% EtOAc in hexanes) to give alcohol **15** (8 mg, quantitative) as a white amorphous solid. ^{1}H NMR (400 MHz, CDCl$_3$) δ: 4.44–4.39 (m, 1H), 3.99–3.89 (m, 1H), 3.85–3.60 (m, 3H), 3.41 (s, 3H), 2.75 (brs, 1H), 2.44–2.32 (m, 1H), 1.56–1.45 (m, 1H),

1.33 (d, J = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 80.2, 73.9, 73.8, 71.6, 59.4, 43.2, 21.4; LRMS-ESI (m/z): 169.1 (M + Na)$^+$; HRMS (APCI), calcd for C$_7$H$_{15}$O$_3$: 147.1021 m/z [M + H]$^+$, found m/z 147.1015; $[\alpha]_D^{20}$ +3.8 (c 0.33, CHCl$_3$).

(2R,3R,5S)-2-(Methoxymethyl)-5-methyltetrahydrofuran-3-ol (*ent*-15)

Alcohol *ent*-**15** (9 mg, 93% yield) was prepared as a white amorphous solid from compound *ent*-**9** by following the procedure described above for its enantiomer **9**. LRMS-ESI (m/z): 169.1 (M + Na)$^+$; HRMS (APCI), calcd for C$_7$H$_{15}$O$_3$: 147.1021 m/z [M + H]$^+$, found m/z 147.1017; $[\alpha]_D^{20}$ −4.2 (c 1.0, CHCl$_3$). For NMR data, please see compound **15**.

(((2R,3S,5R)-2-Allyl-5-methyltetrahydrofuran-3-yl)oxy)(*tert*-butyl)dimethylsilane (10)

To a stirred solution of lactone **8** (317 mg, 1.38 mmol) in CH$_2$Cl$_2$ (14 mL) was added allyl magnesium bromide solution (1.0 M in Et$_2$O, 1.65 mL, 1.65 mmol) at −78 °C under an argon atmosphere. After stirring for 3 h at the same temperature, the reaction was quenched by the addition of saturated aqueous NH$_4$Cl and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The crude product was passed through a flash silica gel column to afford the hemiketal (282 mg, 61%).

To the hemiketal (282 mg, 1.04 mmol) in CH$_2$Cl$_2$ (8.5 mL) were consecutively added Et$_3$SiH (1.03 mL, 6.47 mmol) and BF$_3$ OEt$_2$ (0.38 mL, 3.11 mmol) at −78 °C under an argon atmosphere. After stirring for 2 h at the same temperature, the reaction was quenched by the addition of saturated aqueous NaHCO$_3$ and the aqueous layer was extracted with CH$_2$Cl$_2$. The combined organic layers were washed with brine, dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (5% EtOAc in hexanes) to afford **10** (154 mg, 58%) as a yellow oil; ^{1}H NMR (400 MHz, CDCl$_3$) δ: 5.84 (ddt, J = 17.0, 9.8, 6.8 Hz, 1H), 5.14–5.01 (m, 2H), 4.20–4.11 (m, 1H), 4.04–3.97 (m, 1H), 3.85–3.79 (m, 1H), 2.35–2.27 (m, 1H), 2.28–2.16 (m, 2H), 1.57–1.50 (m, 1H), 1.28 (d, J = 6.2 Hz, 3H), 0.88 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (101 MHz, CDCl$_3$) δ: 134.8, 116.8, 83.5, 76.1, 73.6, 42.4, 37.6, 25.7, 22.1, 17.8, −4.6, −4.9; LRMS-ESI (m/z): 279.3 (M + Na)$^+$; HRMS (ESI), calcd for C$_{14}$H$_{29}$O$_2$Si: m/z 257.1936 [M + H]$^+$, found m/z 257.1930; $[\alpha]_D^{20}$ +30.8 (c 1.0, CHCl$_3$).

tert-Butyldimethyl(((2R,3S,5R)-5-methyl-2-(prop-1-en-1-yl)tetrahydrofuran-3-yl)oxy)silane 16

By following the procedure outlined for the preparation of olefin **13**, olefin **16** (40 mg, 34%; 61% brsm) was obtained as a colorless oil from compound **10** (115 mg, 0.45 mmol) after terminal olefin migration; 51 mg of the starting material **10** was recovered. ^{1}H NMR (400 MHz, CDCl$_3$) δ: 5.78–5.65 (m, 1H), 5.44–5.31 (m, 1H), 4.23–4.15 (m, 1H), 4.12–3.95 (m, 2H), 2.30–2.21 (m, 1H), 1.69 (d, J = 4.1 Hz, 3H), 1.68–1.55 (m, 1H), 1.28 (d, J = 4.1 Hz, 3H), 0.87 (s, 9H), 0.03 (s, 6H); ^{13}C NMR

7364 | *Org. Biomol. Chem.*, 2024, **22**, 7354–7372

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(100 MHz, CDCl$_3$) δ, 130.2, 128.6, 85.2, 76.6, 73.4, 42.4, 25.6, 22.2, 18.0, 17.6, −4.7, −5.3. LRMS-ESI (*m/z*): 279.3 (M + Na)$^+$; HRMS (ESI), calcd for C$_{14}$H$_{29}$O$_2$Si: *m/z* 257.1936 [M + H]$^+$, found *m/z* 257.1938.

((2*R*,3*S*,5*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-methyltetrahydrofuran-2-yl)methanol (17)

By following the procedure outlined for the preparation of alcohol **14**, alcohol **17** (16 mg, 85% yield in two steps) was obtained as a yellowish oil from olefin **16** (19 mg, 0.08 mmol) after ozonolysis and NaBH$_4$ reduction. ^{1}H NMR (400 MHz, CDCl$_3$) δ: 4.28–4.14 (m, 2H), 3.86–3.79 (m, 1H), 3.80–3.50 (m, 2H), 2.31–2.20 (m, 1H), 2.01 (brs, 1H), 1.65–1.58 (m, 1H), 1.30 (d, *J* = 5.2 Hz, 3H), 0.88 (s, 9H), 0.06 (s, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 84.6, 74.4, 72.8, 62.2, 42.9, 26.6, 21.8, 17.8, −4.7, −5.3. LRMS-ESI (*m/z*): 269.2 (M + Na)$^+$; HRMS (APCI), calcd for C$_{12}$H$_{27}$O$_3$Si: 247.1729 *m/z* [M + H]$^+$, found *m/z* 247.1724; $[\alpha]_D^{20}$ +33.0 (*c* 1.0, CHCl$_3$).

(2*R*,3*S*,5*R*)-2-(Methoxymethyl)-5-methyltetrahydrofuran-3-ol (18)

By following the procedure outlined for the preparation of methyl ether **15**, the corresponding methyl ether derivative (14 mg, 90% yield) was obtained as a yellow oil from alcohol **17** (15 mg, 0.06 mmol) after methylation. ^{1}H NMR (400 MHz, CDCl$_3$) δ: 4.44–4.15 (m, 2H), 3.90–3.85 (m, 1H), 3.51–3.47 (m, 1H), 3.40–3.38 (m, 1H), 3.38 (s, 3H), 2.28–2.20 (m, 1H), 1.60–1.52 (m, 1H), 1.29 (d, *J* = 6.2 Hz, 3H), 0.88 (s, 9H), 0.01 (s, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ: 83.3, 74.1, 73.3, 72.8, 59.2, 42.7, 25.6, 21.8, 17.9, −4.6, −5.3; LRMS-ESI (*m/z*): 283.2 (M + Na)$^+$; $[\alpha]_D^{20}$ +37.0 (*c* 0.67, CHCl$_3$).

By following the procedure outlined for the preparation of alcohol **15**, alcohol **18** (7 mg, 93% yield) was obtained as a colorless oil (14 mg, 0.05 mmol) after TBAF deprotection. ^{1}H NMR (400 MHz, CDCl$_3$) δ: 4.30–4.14 (m, 2H), 3.96–3.88 (m, 1H), 3.54–3.47 (m, 1H), 3.46–3.40 (m, 1H), 3.38 (s, 3H), 2.43–2.35 (m, 1H), 2.12 (brs, 1H), 1.63–1.57 (m, 1H), 1.30 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ: 82.6, 74.4, 74.2, 73.4, 59.3, 42.2, 21.8; LRMS-ESI (*m/z*): 169.1 (M + Na)$^+$; HRMS (APCI), calcd for C$_7$H$_{15}$O$_3$: 147.1021 *m/z* [M + H]$^+$, found *m/z* 147.1014; $[\alpha]_D^{20}$ +17.3 (*c* 0.33, CHCl$_3$).

(((2*S*,3*R*,5*S*)-2-Allyl-5-methyltetrahydrofuran-3-yl)oxy)(*tert*-butyl)dimethylsilane (*ent*-10)

The allyl derivative *ent*-**10** (43 mg, 28% in two steps) was obtained as a single isomer from lactone *ent*-**8** (144 mg, 0.63 mmol) in two steps as a colorless oil by following the procedure described above for the allyl derivative **10**. For NMR data, please see compound **10**. LRMS-ESI (*m/z*): 279.3 (M + Na)$^+$; HRMS (APCI), calcd for C$_{14}$H$_{29}$O$_2$Si: *m/z* 257.1936 [M + H]$^+$ found *m/z* 257.1937; $[\alpha]_D^{20}$ −35.2 (*c* 1.0, CHCl$_3$).

tert-Butyldimethyl(((2*S*,3*R*,5*S*)-5-methyl-2-(prop-1-en-1-yl)tetrahydrofuran-3-yl)oxy)silane (*ent*-16)

To a stirred mixture of olefin *ent*-**10** (43 mg, 0.17 mmol) and vinyloxytrimethylsilane (0.25 mL, 1.68 mmol) in CH$_2$Cl$_2$

(8.2 mL) was added the second-generation Grubb's catalyst (7 mg, 0.008 mmol) at 23 °C under an argon atmosphere. The reaction mixture was heated to 110 °C and vigorously stirred for 24 h. After this period, the mixture was cooled to 23 °C and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (2% diethyl ether in hexanes) to give olefin *ent*-**16** (20 mg, 47%) as a colorless oil. For NMR data, please see compound **16**. LRMS-ESI (*m/z*): 279.3 (M + Na)$^+$; HRMS (ESI), calcd for C$_{14}$H$_{29}$O$_2$Si: *m/z* 257.1936 [M + H]$^+$, found *m/z* 257.1933.

((2*S*,3*R*,5*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-methyltetrahydrofuran-2-yl)methanol (*ent*-17)

Alcohol *ent*-**17** (15 mg, 78%) was obtained as a yellow oil from olefin *ent*-**16** (20 mg, 0.08 mmol) by following the procedure described above for compound **17**. For NMR data, please see compound **17**. LRMS-ESI (*m/z*): 269.2 (M + Na)$^+$; HRMS (APCI), calcd for C$_{12}$H$_{27}$O$_3$Si: 247.1729 *m/z* [M + H]$^+$, found *m/z* 247.1729; $[\alpha]_D^{20}$ −32.2 (*c* 0.50, CHCl$_3$).

(2*S*,3*R*,5*S*)-2-(Methoxymethyl)-5-methyltetrahydrofuran-3-ol (*ent*-18)

Alcohol *ent*-**17** (14.5 mg, 0.06 mmol) was converted to methyl ether (9.5 mg, 63%) and obtained as a yellow oil by following the procedure described above. Compound *ent*-**18** (4.6 mg, 96%) was obtained after the removal of TBS ether (8 mg, 0.03 mmol) by following the procedure described above for compound **18**. For NMR data, please see compound **18**. LRMS-ESI (*m/z*): 169.1 (M + Na)$^+$; HRMS (APCI), calcd for C$_7$H$_{15}$O$_3$: 147.1021 *m/z* [M + H]$^+$, found *m/z* 147.1015; $[\alpha]_D^{20}$ −16.7 (*c* 0.13, CHCl$_3$).

(*R*)-1-((2*S*,3*S*,5*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-methyltetrahydrofuran-2-yl)but-3-en-1-ol (20)

Ozone gas was bubbled into a solution of isomerized olefin **13** (78 mg, 0.30 mmol) in CH$_2$Cl$_2$ (5 mL) at −78 °C for 10 min. After the solution turned pale blue, oxygen was bubbled into the solution for 10 min and triphenylphosphine (96 mg, 0.36 mmol) was added. The reaction mixture was stirred for 15 min at −78 °C and warmed to rt for 1 h. After this period, the solvent was concentrated under reduced pressure and the crude residue was passed through a flash silica gel column to afford aldehyde **19** (56 mg, 76% yield) as a colorless oil. This sensitive aldehyde was used immediately after the quick purification step with 50% EtOAc in hexanes.

To a stirred solution of aldehyde **19** (56 mg, 0.23 mmol) in distilled CH$_2$Cl$_2$ (5 mL) were consecutively added allyl tributyl stannane (0.29 mL, 0.91 mmol) and BF$_3$ OEt$_2$ (85 µL, 0.69 mmol) at −78 °C under an argon atmosphere. The reaction mixture was slowly warmed to room temperature over 6 h and stirred at room temperature for 12 h. After this period, the reaction was quenched with saturated aqueous NH$_4$Cl and the aqueous layer was extracted with CH$_2$Cl$_2$. The combined organic layers were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (5% EtOAc in hexanes to

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Org. Biomol. Chem., 2024, **22**, 7354–7372 | **7365**

10% EtOAc in hexanes) to give olefin **20** (52 mg, 79%) as a colorless oil with a dr of 4.2 : 1; ^{1}H NMR (400 MHz, CDCl$_3$) δ: 5.98–5.86 (m, 1H), 5.21–5.05 (m, 2H), 4.56 (dt, J = 6.6, 5.5 Hz, 1H), 4.02–3.84 (m, 2H), 3.56 (dd, J = 7.5, 5.4 Hz, 1H), 3.00 (brs, 1H), 2.57–2.48 (m, 1H), 2.35–2.23 (m, 2H), 1.54 (ddd, J = 12.6, 8.1, 5.5 Hz, 1H), 1.29 (d, J = 6.1 Hz, 3H), 0.91 (s, 9H), 0.11 (d, J = 2.3 Hz, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 135.2, 117.1, 82.6, 74.3, 73.4, 70.2, 42.7, 38.3, 25.7, 21.7, 17.8, −4.4, −5.3; LRMS-ESI (m/z): 309.2 (M + Na)$^+$; HRMS (APCI), calcd for C$_{15}$H$_{31}$O$_3$Si: m/z 287.2042 [M + H]$^+$ found m/z 287.2043; $[\alpha]_D^{20}$ +24.8 (c 1.0, CHCl$_3$).

(S)-1-((2S,3S,5R)-3-((tert-Butyldimethylsilyl)oxy)-5-methyltetrahydrofuran-2-yl)but-3-en-1-ol (21)

Aldehyde **19** (43 mg, 65% yield) was obtained from olefin **13** (57 mg, 0.22 mmol) as described above. To a stirred solution of aldehyde **19** (43 mg, 0.18 mmol) in distilled CH$_2$Cl$_2$ (5 mL) were consecutively added ZnCl$_2$ (72 mg, 0.53 mmol) and allyl tributyl stannane (0.27 mL, 0.88 mmol) at −78 °C under an argon atmosphere. The reaction mixture was slowly warmed to room temperature over 6 h and stirred at room temperature for 12 h. After this period, the reaction was quenched with saturated aqueous NaHCO$_3$ and the aqueous layer was extracted with CH$_2$Cl$_2$. The combined organic layers were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (5% EtOAc in hexanes to 10% EtOAc in hexanes) to afford olefin **21** (38 mg, 75%) as a major product with a dr of 3 : 1; ^{1}H NMR (400 MHz, CDCl$_3$) δ: 5.90 (ddt, J = 17.2, 10.2, 7.0 Hz, 1H), 5.18–5.04 (m, 2H), 4.42 (dt, J = 6.5, 4.7 Hz, 1H), 4.01–3.88 (m, 2H), 3.57 (t, J = 4.6 Hz, 1H), 3.14 (brs, 1H), 2.33 (dt, J = 19.5, 6.4 Hz, 3H), 1.54 (td, J = 7.8, 3.8 Hz, 1H), 1.34 (d, J = 6.1 Hz, 3H), 0.90 (s, 9H), 0.09 (s, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 135.1, 116.9, 83.2, 74.6, 73.4, 70.1, 43.7, 37.7, 25.6, 21.4, 17.8, −4.5, −5.3; LRMS-ESI (m/z): 309.2 (M + Na)$^+$; HRMS (APCI), calcd for C$_{15}$H$_{31}$O$_3$Si: m/z 287.2042 [M + H]$^+$ found m/z 287.2045; $[\alpha]_D^{20}$ +16.7 (c 0.67, CHCl$_3$).

(R)-1-((2S,3S,5R)-3-((tert-Butyldimethylsilyl)oxy)-5-methyltetrahydrofuran-2-yl)butane-1,4-diol (22)

To a stirred solution of olefin **20** (45 mg, 0.16 mmol) in distilled THF (1.6 mL) was added dropwise a solution of 9-BBN (0.5 M in THF, 0.80 mL, 0.40 mmol) at 0 °C under an argon atmosphere. The mixture was warmed to room temperature and stirred for 6 h. Upon completion of the reaction, aqueous 3 N NaOH solution (0.5 mL) and 30% H$_2$O$_2$ (1 mL) were added and the resulting mixture was stirred for 3 h at room temperature. Water (2 mL) was added and the aqueous layer was extracted with Et$_2$O (3 × 20 mL). The combined organic layers were washed with brine, dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (60% EtOAc in hexanes to 80% EtOAc in hexanes) to provide diol **22** (38 mg, 80%) as a colorless oil; ^{1}H NMR (400 MHz, CDCl$_3$) δ: 4.57 (q, J = 6.1 Hz, 1H), 3.99–3.89 (m, 1H), 3.84 (ddd, J = 10.2, 8.1, 2.6 Hz, 1H),

3.66 (dtd, J = 15.8, 11.0, 5.4 Hz, 2H), 3.55 (dd, J = 7.7, 5.7 Hz, 1H), 2.27 (dt, J = 12.8, 6.4 Hz, 1H), 1.90 (dtd, J = 13.9, 6.7, 2.6 Hz, 1H), 1.76 (dq, J = 9.1, 6.9, 6.4 Hz, 2H), 1.53 (ddd, J = 12.9, 8.5, 6.0 Hz, 2H), 1.27 (d, J = 6.1 Hz, 3H), 0.90 (s, 9H), 0.11 (s, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 82.5, 74.6, 73.3, 71.2, 63.0, 42.5, 30.8, 29.3, 25.6, 21.6, 17.8, −4.4, −5.3; LRMS-ESI (m/z): 327.2 (M + Na)$^+$; HRMS (ESI), calcd for C$_{15}$H$_{33}$O$_4$Si: m/z 305.2148 [M + H]$^+$, found m/z 305.2146; $[\alpha]_D^{20}$ +18.7 (c 1.0, CHCl$_3$).

tert-Butyldimethyl(((2S,2′R,3S,5R)-5-methyloctahydro-[2,2′-bifuran]-3-yl)oxy)silane (23)

To a stirred solution of diol **22** (35 mg, 0.11 mmol) in CH$_2$Cl$_2$ (1.8 mL) were added triethylamine (0.10 mL, 0.71 mmol) and DMAP (2.6 mg, 0.02 mmol) at 0 °C under an argon atmosphere. After stirring for 10 min, 4-toluenesulfonyl chloride (27.2 mg, 0.14 mmol) was added and the resulting mixture was stirred for 24 h at room temperature. The reaction was quenched with saturated aqueous NH$_4$Cl and the aqueous layer was extracted with CH$_2$Cl$_2$. The combined organic layers were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (20% EtOAc in hexanes) to give bicyclic ether **23** (23 mg, 71%) as a colorless oil. ^{1}H NMR (400 MHz, CDCl$_3$) δ: 4.35 (ddd, J = 6.6, 4.3, 2.7 Hz, 1H), 4.06–3.97 (m, 2H), 3.85 (td, J = 7.8, 7.3, 3.7 Hz, 1H), 3.71–3.60 (m, 2H), 2.26 (ddd, J = 13.4, 7.7, 6.0 Hz, 1H), 2.05–1.96 (m, 1H), 1.94–1.82 (m, 3H), 1.49 (ddd, J = 13.1, 6.1, 2.8 Hz, 1H), 1.30 (d, J = 6.2 Hz, 3H), 0.89 (s, 9H), 0.06 (s, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 80.1, 77.0, 73.9, 73.0, 67.7, 43.2, 28.1, 25.9, 25.7, 22.0, 18.0, −5.0, −5.2; LRMS-ESI (m/z): 309.2 (M + Na)$^+$; HRMS (APCI), calcd for C$_{15}$H$_{31}$O$_3$Si: m/z 287.2042 [M + H]$^+$ found m/z 287.2044; $[\alpha]_D^{20}$ +4.3 (c 1.0, CHCl$_3$).

(2R,2′R,3S,5R)-5-Methyloctahydro-[2,2′-bifuran]-3-ol (24)

To a stirred solution of bicyclic ether **23** (18 mg, 0.06 mmol) was added TBAF solution (1M in THF, 0.10 mL, 0.10 mmol) at 0 °C under an argon atmosphere. The mixture was stirred for 3 h at room temperature. The reaction was quenched with saturated aqueous NH$_4$Cl and the aqueous layer was extracted with CH$_2$Cl$_2$. The combined organic layers were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (80% EtOAc in hexanes) to give alcohol **24** (10 mg, 99%) as a colorless oil. ^{1}H NMR (400 MHz, CDCl$_3$) δ: 4.46 (dt, J = 7.4, 3.8 Hz, 1H), 4.11 (q, J = 7.1 Hz, 1H), 4.02–3.92 (m, 1H), 3.89 (dt, J = 8.2, 6.4 Hz, 1H), 3.75 (dt, J = 8.2, 6.9 Hz, 1H), 3.49 (dd, J = 7.8, 4.4 Hz, 1H), 2.93 (s, 1H), 2.36 (dt, J = 13.8, 7.0 Hz, 1H), 2.19–2.10 (m, 1H), 1.98–1.88 (m, 2H), 1.87–1.75 (m, 1H), 1.60–1.51 (m, 1H), 1.33 (d, J = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 84.5, 77.8, 74.6, 73.3, 68.2, 42.1, 29.7, 25.4, 21.6. LRMS-ESI (m/z): 195.1 (M + Na)$^+$; HRMS (APCI), calcd for C$_9$H$_{17}$O$_3$: m/z 173.1177 [M + H]$^+$ found m/z 173.1172; $[\alpha]_D^{20}$ +10.0 (c 0.34, CHCl$_3$).

7366 | *Org. Biomol. Chem.*, 2024, **22**, 7354–7372

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(S)-1-((2S,3S,5R)-3-((tert-Butyldimethylsilyl)oxy)-5-methyltetrahydrofuran-2-yl)butane-1,4-diol (25)

To a stirred solution of olefin **21** (33 mg, 0.12 mmol) in distilled THF (1.6 mL) was added dropwise a solution of 9-BBN (0.5 M in THF, 0.80 mL, 0.40 mmol) at 0 °C under an argon atmosphere. The mixture was warmed to room temperature and stirred for 6 h. Upon completion of the reaction, aqueous 3 N NaOH solution (0.5 mL) and 30% H_2O_2 (1 mL) were added and the resulting mixture was stirred for 3 h at room temperature. Water (2 mL) was added and the aqueous layer was extracted with Et_2O (3 × 20 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (60% EtOAc in hexanes to 80% EtOAc in hexanes) to provide diol **25** (22 mg, 64%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ: 4.47–4.38 (m, 1H), 3.94 (dt, J = 7.6, 6.3 Hz, 1H), 3.87 (dt, J = 8.0, 4.0 Hz, 1H), 3.66 (dt, J = 7.5, 5.6 Hz, 2H), 3.60–3.51 (m, 1H), 2.30 (dt, J = 13.1, 6.7 Hz, 1H), 1.79–1.70 (m, 2H), 1.67 (ddd, J = 11.9, 5.7, 2.8 Hz, 2H), 1.52 (ddd, J = 13.0, 7.7, 4.3 Hz, 1H), 1.32 (d, J = 6.2 Hz, 3H), 0.89 (s, 9H), 0.08 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ, 84.1, 74.6, 73.4, 70.8, 62.8, 43.7, 30.6, 29.8, 25.6, 21.4, 17.8, −4.5, −5.3; LRMS-ESI (m/z): 327.2 (M + Na)$^+$; HRMS (ESI), calcd for $C_{15}H_{33}O_4Si$: m/z 305.2148 [M + H]$^+$, found m/z 305.2149; $[\alpha]_D^{20}$ +20.4 (c 1.0, $CHCl_3$).

tert-Butyldimethyl(((2S,2′S,3S,5R)-5-methyloctahydro-[2,2′-bifuran]-3-yl)oxy)silane (26)

To a stirred solution of diol **25** (22 mg, 0.07 mmol) in CH_2Cl_2 (1.8 mL) were added triethylamine (0.10 mL, 0.71 mmol) and DMAP (2.6 mg, 0.02 mmol) at 0 °C under an argon atmosphere. After stirring for 10 min, 4-toluenesulfonyl chloride (27.2 mg, 0.14 mmol) was added and the resulting mixture was stirred for 24 h at room temperature. The reaction was quenched with saturated aqueous NH_4Cl and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (20% EtOAc in hexanes) to give compound **26** (18 mg, 88%). ^1H NMR (400 MHz, $CDCl_3$) δ: 4.29 (ddd, J = 6.5, 4.1, 2.7 Hz, 1H), 4.18–4.08 (m, 1H), 4.06–3.98 (m, 1H), 3.93–3.83 (m, 1H), 3.80–3.72 (m, 1H), 3.48 (dd, J = 8.4, 4.2 Hz, 1H), 2.33–2.24 (m, 1H), 2.10–1.98 (m, 1H), 1.90–1.83 (m, 2H), 1.55–1.44 (m, 2H), 1.34 (d, J = 6.1 Hz, 3H), 0.88 (s, 9H), 0.05 (d, J = 4.7 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ, 86.2, 78.1, 74.4, 73.4, 67.9, 43.5, 28.0, 25.8, 25.6, 22.0, 17.8, −4.2, −5.3; LRMS-ESI (m/z): 309.2 (M + Na)$^+$; HRMS (APCI), calcd for $C_{15}H_{31}O_3Si$: m/z 287.2042 [M + H]$^+$ found m/z 287.2041; $[\alpha]_D^{20}$ +30.9 (c 0.50, $CHCl_3$).

(2R,2′S,3S,5R)-5-Methyloctahydro-[2,2′-bifuran]-3-ol (27)

To a stirred solution of compound **26** (17 mg, 0.06 mmol) was added TBAF solution (1M in THF, 0.10 mL, 0.10 mmol) at 0 °C under an argon atmosphere. The mixture was stirred for 3 h at room temperature. The reaction was quenched with saturated

aqueous NH_4Cl and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (80% EtOAc in hexanes) to give alcohol **27** (11 mg, 99%). ^1H NMR (400 MHz, $CDCl_3$) δ: 4.36 (dt, J = 6.5, 4.5 Hz, 1H), 4.16 (dt, J = 7.0, 3.5 Hz, 1H), 4.02–3.88 (m, 2H), 3.86–3.78 (m, 1H), 3.65 (dd, J = 5.0, 3.9 Hz, 1H), 2.40–2.29 (m, 1H), 2.10–1.80 (m, 5H), 1.58–1.52 (m, 1H), 1.32 (d, J = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ, 83.2, 78.1, 74.2, 73.4, 68.9, 43.7, 28.0, 25.6, 21.7. LRMS-ESI (m/z): 195.1 (M + Na)$^+$; HRMS (APCI), calcd for $C_9H_{17}O_3$: m/z 173.1177 [M + H]$^+$ found m/z 173.1171; $[\alpha]_D^{20}$ +24.6 (c 0.33, $CHCl_3$).

General procedure for the preparation of activated carbonate

To a solution of alcohol in CH_2Cl_2 (10 mL per mmol of alcohol) was added pyridine (5 equiv.) at 23 °C under an argon atmosphere, and the reaction mixture was cooled to 0 °C followed by the addition of 4-nitrophenyl chloroformate (2.2 equiv.). The reaction mixture was warmed to 23 °C and stirred for 12 h. Upon completion of the reaction, the solvents were removed under reduced pressure and the crude product was purified by silica gel column chromatography.

Activated carbonates (28a)

Following the general procedure A, activated carbonate **28a** (5 mg, 24% yield) was prepared as a white amorphous solid from alcohol **11** (10 mg, 0.07 mmol). ^1H NMR (400 MHz, $CDCl_3$) δ: 8.28 (d, J = 9.2 Hz, 2H), 7.38 (d, J = 9.2 Hz, 2H), 5.91–5.80 (m, 1H), 5.26 (ddd, J = 6.6, 3.9, 2.3 Hz, 1H), 5.22–5.07 (m, 2H), 4.02 (ddt, J = 13.5, 7.3, 6.2 Hz, 1H), 3.83 (ddd, J = 7.3, 6.5, 3.9 Hz, 1H), 2.64–2.55 (m, 1H), 2.55–2.46 (m, 2H), 1.77–1.69 (m, 1H), 1.37 (d, J = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ, 155.4, 152.1, 145.3, 133.9, 125.2, 121.6, 117.4, 80.9, 80.7, 73.7, 40.5, 33.4, 21.3; LRMS-ESI (m/z): 330.1 (M + Na)$^+$. HRMS (APCI), calcd for $C_{15}H_{18}NO_6$: m/z 308.1134 [M + H]$^+$, found m/z 308.1129.

Activated carbonates (28b)

Following the general procedure, activated alcohol **28b** (10 mg, 30% yield) was prepared as a yellow oil from alcohol **12** (16 mg, 0.11 mmol). ^1H NMR (500 MHz, $CDCl_3$) δ: 8.28 (d, J = 9.2 Hz, 2H), 7.39 (d, J = 9.2 Hz, 2H), 5.89–5.78 (m, 1H), 5.20–5.11 (m, 2H), 5.03 (ddd, J = 7.1, 3.4, 2.7 Hz, 1H), 4.36–4.28 (m, 1H), 4.26 (td, J = 6.7, 2.7 Hz, 1H), 2.60–2.51 (m, 1H), 2.34 (tt, J = 6.8, 1.3 Hz, 2H), 1.82 (ddd, J = 13.9, 5.8, 3.5 Hz, 1H), 1.34 (d, J = 6.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ, 155.4, 152.1, 145.4, 133.4, 125.3, 121.8, 118.0, 83.3, 82.1, 73.9, 53.4, 38.6, 37.3, 21.7; LRMS-ESI (m/z): 330.1 (M + Na)$^+$; $[\alpha]_D^{20}$ +15.1 (c 0.64, $CHCl_3$). HRMS (APCI), calcd for $C_{15}H_{18}NO_6$: m/z 308.1134 [M + H]$^+$, found m/z 308.1136.

Activated carbonates (28c and 28d)

Following the general procedure, activated carbonate **28c** (17 mg, 91% yield) was prepared as a white amorphous solid from alcohol **15** (8 mg, 0.06 mmol). ^1H NMR (400 MHz, $CDCl_3$)

δ: 8.28 (d, J = 9.0 Hz, 2H), 7.38 (d, J = 9.0 Hz, 2H), 5.34 (p, J = 3.8 Hz, 1H), 4.10–3.95 (m, 2H), 3.67 (d, J = 5.7 Hz, 2H), 3.41 (s, 3H), 2.66–2.53 (m, 1H), 1.76–1.70 (m, 1H), 1.37 (d, J = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 155.4, 152.0, 145.3, 125.2, 121.6, 80.2, 79.7, 74.1, 70.6, 59.3, 40.3, 21.1; LRMS-ESI (m/z): 312 (M + H)$^+$; HRMS (APCI), calcd for C$_{14}$H$_{18}$NO$_7$: m/z 312.1083 [M + H]$^+$, found m/z 312.1082; [α]$_D^{20}$ +5.8 (c 0.33, CHCl$_3$). Following the general procedure, activated carbonate **28d** (16 mg, 70% yield) was prepared as a white amorphous solid from alcohol *ent*-**15** (9 mg, 0.07 mmol). For NMR data, please see compound **28c**; LRMS-ESI (m/z): 312 (M + H)$^+$; HRMS (APCI), calcd for C$_{14}$H$_{18}$NO$_7$: m/z 312.1083 [M + H]$^+$, found m/z 312.1091; [α]$_D^{20}$ −6.2 (c 0.33, CHCl$_3$).

Activated carbonates (28e and 28f)

Following the general procedure, activated carbonate **28e** (13 mg, 78% yield) was prepared as a white amorphous solid from alcohol **18** (7 mg, 0.05 mmol). ^{1}H NMR (400 MHz, CDCl$_3$) δ: 8.28 (d, J = 9.2 Hz, 2H), 7.38 (d, J = 9.2 Hz, 2H), 5.22 (ddd, J = 7.2, 4.1, 3.1 Hz, 1H), 4.40–4.32 (m, 1H), 4.29 (td, J = 4.5, 3.1 Hz, 1H), 3.57–3.48 (m, 2H), 3.39 (s, 3H), 2.57 (dt, J = 13.9, 7.1 Hz, 1H), 1.82 (ddd, J = 13.6, 6.2, 4.0 Hz, 1H), 1.34 (d, J = 6.3 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 155.4, 152.2, 145.5, 125.3, 121.7, 81.9, 81.7, 75.2, 73.1, 59.5, 39.2, 21.5; LRMS-ESI (m/z): 312 (M + H)$^+$; HRMS (APCI), calcd for C$_{14}$H$_{18}$NO$_7$: m/z 312.1083 [M + H]$^+$, found m/z 312.1082 [α]$_D^{20}$ +18.9 (c 0.13, CHCl$_3$).

By following the general procedure, activated alcohol **28f** (4 mg, 40% yield) was prepared as a white amorphous solid from alcohol *ent*-**18** (4.6 mg, 0.03 mmol). For NMR data, please see compound **28e**. LRMS-ESI (m/z): 312 (M + H)$^+$; HRMS (APCI), calcd for C$_{14}$H$_{18}$NO$_7$: m/z 312.1084 [M + H]$^+$, found m/z 312.1081; [α]$_D^{20}$ −19.1 (c 0.13, CHCl$_3$).

Activated carbonates (28g)

By following the general procedure, activated carbonate **28g** (17 mg, 82%) was prepared from alcohol **24** (10 mg, 0.06 mmol). ^{1}H NMR (400 MHz, CDCl$_3$) δ: 8.27 (d, J = 9.2 Hz, 2H), 7.39 (d, J = 9.2 Hz, 2H), 5.40 (ddd, J = 7.0, 4.3, 2.9 Hz, 1H), 4.18–4.10 (m, 1H), 4.07 (td, J = 7.3, 6.1 Hz, 1H), 3.91–3.84 (m, 1H), 3.76 (dt, J = 8.3, 6.8 Hz, 1H), 3.68 (dd, J = 7.9, 4.3 Hz, 1H), 2.58 (dt, J = 14.2, 7.1 Hz, 1H), 2.19–2.05 (m, 1H), 2.01–1.84 (m, 3H), 1.75 (ddd, J = 14.2, 7.1, 2.9 Hz, 1H), 1.35 (d, J = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 125.2, 121.7, 83.2, 80.0, 76.2, 74.2, 68.3 40.2, 29.2, 25.6, 21.3; LRMS-ESI (m/z): 360.2 (M + Na)$^+$; HRMS (APCI), calcd for C$_{16}$H$_{20}$NO$_7$: m/z 338.1239 [M + H]$^+$ found m/z 338.1249; [α]$_D^{20}$ +21.9 (c 0.30, CHCl$_3$).

Activated carbonates (28h)

By following the general procedure, activated carbonate **28h** (8 mg, 44%) was prepared from alcohol **27** (9 mg, 0.05 mmol). ^{1}H NMR (400 MHz, CDCl$_3$) δ: 8.28 (d, J = 9.1 Hz, 2H), 7.38 (d, J = 9.1 Hz, 2H), 5.29 (p, J = 3.3 Hz, 1H), 4.15 (q, J = 7.4 Hz, 1H), 4.12–4.04 (m, 1H), 3.97–3.88 (m, 1H), 3.88–3.78 (m, 1H), 3.72 (dd, J = 7.2, 4.3 Hz, 1H), 2.61 (dt, J = 14.2, 7.1 Hz, 1H), 2.09–2.01 (m, 1H), 1.95–1.91 (m, 1H), 1.76 (ddd, J = 14.2, 7.4, 2.9 Hz, 1H), 1.65 (dq, J = 11.8, 8.1 Hz, 2H), 1.39 (d, J = 6.1 Hz,

3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 155.3, 152.1, 145.4, 125.2, 121.6, 83.8, 80.6, 74.2, 68.4, 60.3, 40.6, 28.1, 25.7, 21.1. LRMS-ESI (m/z): 360.1 (M + Na)$^+$; HRMS (APCI), calcd for C$_{16}$H$_{20}$NO$_7$: m/z 338.1239 [M + H]$^+$ found m/z 338.1240; [α]$_D^{20}$ +9.6 (c 0.27, CHCl$_3$).

General procedure for inhibitor synthesis using amine 29 or 30

To a stirred solution of activated carbonate and amine **29** or **30** in acetonitrile (2 mL) was added DIPEA (8 equiv.) at 0 °C under an argon atmosphere. The reaction mixture was stirred at room temperature until completion of the reaction. Upon completion, the solvents were removed under reduced pressure and the crude residue was purified by silica gel column chromatography.

(2S,3S,5R)-2-Allyl-5-methyltetrahydrofuran-3-yl((2S,3R)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)carbamate (4a)

Following the general procedure, inhibitor **4a** (7 mg, 97% yield) was prepared as a white amorphous solid from amine **29** and activated carbonate **28a** (5 mg, 0.02 mmol). ^{1}H NMR (500 MHz, CDCl$_3$) δ: 7.72 (d, J = 8.9 Hz, 2H), 7.30–7.19 (m, 5H), 6.98 (d, J = 8.9 Hz, 2H), 5.70 (ddt, J = 18.8, 9.1, 6.9 Hz, 1H), 5.08–4.97 (m, 3H), 4.85 (d, J = 8.2 Hz, 1H), 3.88 (s, 3H), 3.86–3.81 (m, 3H), 3.62 (ddd, J = 7.8, 5.9, 4.0 Hz, 1H), 3.15 (dd, J = 15.1, 7.7 Hz, 1H), 3.07–2.93 (m, 3H), 2.90–2.75 (m, 2H), 2.41 (dt, J = 14.3, 7.2 Hz, 1H), 2.19 (dt, J = 14.5, 7.4 Hz, 1H), 2.14–2.06 (m, 1H), 1.89–1.77 (m, 1H), 1.45 (ddd, J = 14.0, 7.3, 2.4 Hz, 1H), 1.28 (d, J = 6.2 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) δ, 163.0, 155.8, 137.4, 134.4, 129.4, 129.3, 128.5, 126.5, 116.9, 114.3, 81.3, 75.6, 73.5, 72.7, 58.7, 55.6, 54.9, 53.7, 53.4, 40.7, 35.3, 33.2, 27.2, 21.3, 20.1, 19.8; LRMS-ESI (m/z): 575.0 (M + H)$^+$; HRMS-ESI (m/z): C$_{30}$H$_{42}$N$_2$O$_7$S; calc'd for [M + Na]$^+$: 597.2605, found 597.2611.

(2S,3S,5R)-2-Allyl-5-methyltetrahydrofuran-3-yl((2S,3R)-4-((4-amino-N-isobutylphenyl)sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)carbamate (4b)

Following the general procedure, inhibitor **4b** (7 mg, 62% yield) was prepared as a yellow amorphous solid from amine **30** and activated carbonate **28a** (7 mg, 0.02 mmol). ^{1}H NMR (400 MHz, CDCl$_3$) δ: 7.55 (d, J = 8.2 Hz, 2H), 7.34–7.14 (m, 5H), 6.69 (d, J = 8.2 Hz, 2H), 5.72–5.62 (m, 1H), 5.07–5.03 (m, 1H), 5.02–4.96 (m, 2H), 4.85 (d, J = 7.8 Hz, 1H), 3.92–3.85 (m, 1H), 3.90–3.84 (m, 2H), 3.62–3.60 (m, 1H), 3.18–2.72 (m, 6H), 2.44–2.30 (m, 1H), 2.25–2.05 (m, 2H), 1.88–1.78 (m, 1H), 1.49–1.41 (m, 1H), 1.27 (d, J = 6.2 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 155.9, 137.6, 134.5, 129.5, 129.4, 128.5, 126.6, 117.0, 114.2, 81.42, 75.7, 73.6, 72.8, 58.8, 55.0, 53.8, 40.8, 35.4, 33.3, 27.3, 21.4, 20.2, 19.9; LRMS-ESI (m/z): 560.0 (M + H)$^+$; HRMS-ESI (m/z): C$_{29}$H$_{41}$N$_3$O$_6$S; calc'd for [M + Na]$^+$: 582.2608, found 582.2615.

7368 | *Org. Biomol. Chem.*, 2024, **22**, 7354–7372

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Paper

(2S,3S,5R)-2-(Methoxymethyl)-5-methyltetrahydrofuran-3-yl ((2S,3R)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)carbamate (4c)

Following the general procedure, inhibitor **4c** (8 mg, 58% yield) was prepared as a white amorphous solid from amine **29** and activated carbonate **28c** (7 mg, 0.02 mmol). ^{1}H NMR (400 MHz, CDCl$_3$) δ: 7.71 (d, J = 8.9 Hz, 2H), 7.34–7.17 (m, 5H), 6.98 (d, J = 8.9 Hz, 2H), 5.18–5.11 (m, 1H), 4.84 (d, J = 8.3 Hz, 1H), 3.93 (q, J = 6.6 Hz, 1H), 3.87 (s, 3H), 3.86–3.77 (m, 4H), 3.38–3.31 (m, 2H), 3.30 (s, 3H), 3.14 (dd, J = 15.2, 8.3 Hz, 1H), 3.07–2.91 (m, 3H), 2.86 (dd, J = 14.1, 8.4 Hz, 1H), 2.79 (dd, J = 13.4, 6.7 Hz, 1H), 2.47–2.36 (m, 1H), 1.87–1.76 (m, 1H), 1.46 (dd, J = 14.0, 7.7 Hz, 1H), 1.29 (d, J = 6.1 Hz, 3H), 0.91 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 163.0, 148.4, 137.5, 129.7, 129.4, 129.3, 128.5, 126.5, 114.26, 80.4, 75.5, 74.0, 72.6, 70.8, 59.2, 58.7, 55.5, 54.9, 55.6, 40.6, 35.2, 27.2, 21.2, 20.1, 19.8; LRMS-ESI (m/z): 578.0 (M + H)$^+$; HRMS-ESI (m/z): C$_{29}$H$_{42}$N$_2$O$_8$S; calc'd for [M + H]$^+$: 579.2735, found 579.2740.

(2S,3S,5R)-2-(Methoxymethyl)-5-methyltetrahydrofuran-3-yl ((2S,3R)-4-((4-amino-N-isobutylphenyl)sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)carbamate (4d)

Following the general procedure, inhibitor **4d** (7 mg, 69% yield) was prepared as a yellow amorphous solid from amine **30** and activated carbonate **28c** (6 mg, 0.02 mmol). ^{1}H NMR (400 MHz, CDCl$_3$) δ: 7.55 (d, J = 8.7 Hz, 2H), 7.32–7.20 (m, 5H), 6.68 (d, J = 8.7 Hz, 2H), 5.18–5.12 (m, 1H), 4.85 (d, J = 8.4 Hz, 1H), 3.98–3.89 (m, 1H), 3.86–3.81 (m, 3H), 3.40–3.32 (m, 2H), 3.31 (s, 3H), 3.14 (dd, J = 15.1, 8.3 Hz, 1H), 3.08–2.81 (m, 5H), 2.76 (dd, J = 13.4, 6.6 Hz, 1H), 2.41 (dt, J = 14.1, 7.2 Hz, 1H), 1.88–1.74 (m, 2H), 1.52–1.42 (m, 2H), 1.30 (d, J = 6.1 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 155.7, 150.6, 137.6, 129.4, 129.3, 128.4, 128.3, 126.5, 114.0, 80.4, 77.1, 75.5, 74.0, 72.7, 70.9, 59.2, 58.8, 54.9, 53.7, 40.6, 35.3, 27.2, 21.2, 20.1, 19.8; LRMS-ESI (m/z): 564.0 (M + H)$^+$; HRMS-ESI (m/z): C$_{28}$H$_{41}$N$_3$O$_7$S; calc'd for [M + H]$^+$: 564.2738, found 564.2733.

(2R,3R,5S)-2-(Methoxymethyl)-5-methyltetrahydrofuran-3-yl ((2S,3R)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)carbamate (4e)

Following the general procedure, inhibitor **4e** (7 mg, 71% yield) was prepared as a white amorphous solid from amine **29** and activated carbonate **28d** (5 mg, 0.02 mmol). ^{1}H NMR (400 MHz, CDCl$_3$) δ: 7.71 (d, J = 8.8 Hz, 2H), 7.33–7.17 (m, 5H), 6.99 (d, J = 8.8 Hz, 2H), 5.11–5.05 (m, 1H), 4.84 (d, J = 8.6 Hz, 1H), 3.94 (h, J = 6.4 Hz, 1H), 3.88 (s, 3H), 3.86–3.77 (m, 4H), 3.52–3.49 (m, 2H), 3.34 (s, 3H), 3.14 (dd, J = 15.1, 8.4 Hz, 1H), 3.05–2.94 (m, 3H), 2.89–2.76 (m, 3H), 2.38–2.30 (m, 1H), 1.90–1.77 (m, 1H), 1.60 (brs, 1H), 1.33–1.28 (m, 2H), 0.93 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 163.0, 155.7, 137.5, 129.7, 129.4, 128.4, 126.5, 114.3, 80.1, 75.8, 74.0, 72.5, 71.1, 59.2, 58.7, 55.5, 54.9, 53.6, 40.6, 35.3, 27.2, 21.3, 20.1, 19.8; LRMS-ESI (m/z): 578.0 (M + Na)$^+$;

HRMS-ESI (m/z): C$_{29}$H$_{42}$N$_2$O$_8$S; calc'd for [M + Na]$^+$: 601.2554, found 601.2557.

(2R,3R,5S)-2-(Methoxymethyl)-5-methyltetrahydrofuran-3-yl ((2S,3R)-4-((4-amino-N-isobutylphenyl)sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)carbamate (4f)

Following the general procedure, inhibitor **4f** (16 mg, 54% yield) was prepared from amine **30** and activated carbonate **28d** (16 mg, 0.05 mmol). ^{1}H NMR (500 MHz, CDCl$_3$) δ: 7.54 (d, J = 8.7 Hz, 2H), 7.33–7.16 (m, 5H), 6.67 (d, J = 8.7 Hz, 2H), 5.06 (ddd, J = 6.8, 4.3, 2.5 Hz, 1H), 4.87 (d, J = 8.6 Hz, 1H), 4.18 (brs, 1H), 3.94 (ddt, J = 13.6, 7.4, 6.2 Hz, 1H), 3.89–3.81 (m, 4H), 3.50 (d, J = 4.7 Hz, 2H), 3.33 (s, 3H), 3.13 (dd, J = 15.1, 8.2 Hz, 1H), 3.05–2.91 (m, 3H), 2.83 (dd, J = 14.0, 8.6 Hz, 1H), 2.76 (dd, J = 13.3, 6.6 Hz, 1H), 2.33 (dt, J = 14.2, 7.1 Hz, 1H), 1.88–1.75 (m, 1H), 1.27 (d, J = 15.5 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) δ, 155.6, 150.7, 137.6, 129.4, 128.4, 126.5, 126.4, 126.0, 114.0, 80.1, 75.7, 74.1, 72.6, 71.1, 59.2, 58.8, 54.9, 53.7, 40.6, 35.3, 27.2, 21.3, 20.1, 19.8; LRMS-ESI (m/z): 564.0 (M + Na)$^+$; HRMS-ESI (m/z): C$_{28}$H$_{41}$N$_3$O$_7$S; calc'd for [M + Na]$^+$: 586.2557, found 586.2562.

(2R,3S,5R)-2-Allyl-5-methyltetrahydrofuran-3-yl((2S,3R)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)carbamate (4g)

Following the general procedure, inhibitor **4g** (8.5 mg, 68% yield) was prepared as a white amorphous solid from amine **29** and activated carbonate **28b** (6.5 mg, 0.02 mmol). ^{1}H NMR (800 MHz, CDCl$_3$) δ: 7.71 (d, J = 8.8 Hz, 2H), 7.33–7.20 (m, 5H), 6.98 (d, J = 8.5 Hz, 2H), 5.81–5.70 (m, 1H), 5.12–5.03 (m, 2H), 4.83 (d, J = 9.0 Hz, 1H), 4.81–4.78 (m, 1H), 4.22–4.15 (m, 1H), 3.88 (s, 3H), 3.87–3.77 (m, 3H), 3.12 (dd, J = 15.0, 8.5 Hz, 1H), 3.04–2.98 (m, 2H), 2.96 (dd, J = 13.2, 8.4 Hz, 1H), 2.89 (dd, J = 14.0, 8.8 Hz, 1H), 2.79 (dd, J = 13.5, 6.7 Hz, 1H), 2.40–2.33 (m, 1H), 2.25–2.16 (m, 2H), 1.86–1.78 (m, 1H), 1.65–1.53 (m, 2H), 1.24 (d, J = 6.4 Hz, 3H), 0.91 (d, J = 6.2 Hz, 3H), 0.87 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 163.1, 156.0, 137.6, 134.0, 129.8, 129.5, 129.5, 128.5, 126.6, 117.4, 114.4, 82.3, 78.9, 73.7, 72.6, 58.8, 55.6, 55.1, 53.7, 39.0, 37.3, 35.4, 27.3, 21.7, 20.1, 19.9; LRMS-ESI (m/z): 575.0 (M + H)$^+$; HRMS-ESI (m/z): C$_{30}$H$_{42}$N$_2$O$_7$S; calc'd for [M + Na]$^+$: 597.2605, found 597.2613.

(2R,3S,5R)-2-(Methoxymethyl)-5-methyltetrahydrofuran-3-yl ((2S,3R)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)carbamate (4h)

Following the general procedure, inhibitor **4h** (10 mg, 76% yield) was prepared as a white amorphous solid from amine **29** and activated carbonate **28e** (7 mg, 0.02 mmol). ^{1}H NMR (400 MHz, CDCl$_3$) δ: 7.70 (d, J = 8.9 Hz, 2H), 7.31–7.20 (m, 5H), 6.97 (d, J = 8.8 Hz, 2H), 4.96–4.91 (m, 1H), 4.88 (d, J = 8.4 Hz, 1H), 4.28–4.19 (m, 1H), 3.87 (s, 3H), 3.85–3.79 (m, 1H), 3.43–3.35 (m, 2H), 3.33 (s, 3H), 3.16–3.07 (m, 1H), 3.03–2.85 (m, 5H), 2.79 (dd, J = 13.3, 6.6 Hz, 1H), 2.41–2.29 (m, 1H), 1.87–1.75 (m, 1H), 1.63–1.51 (m, 2H), 1.29–1.26 (m, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ, 163.0, 156.0, 137.5, 129.7, 129.4, 128.4, 126.5, 114.3,

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Org. Biomol. Chem., 2024, **22**, 7354–7372 | **7369**

108.6, 82.1, 76.8, 74.6, 73.1, 72.5, 59.2, 58.7, 55.5, 55.1, 53.6, 39.3, 35.3, 27.2, 21.4, 20.1, 19.8. LRMS-ESI (*m/z*): 579.0 (M + H)$^+$; HRMS-ESI (*m/z*): C$_{29}$H$_{42}$N$_2$O$_8$S; calc'd for [M + Na]$^+$: 601.2554, found 601.2558.

(2*R*,3*S*,5*R*)-2-(Methoxymethyl)-5-methyltetrahydrofuran-3-yl ((2*S*,3*R*)-4-((4-amino-*N*-isobutylphenyl)sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)carbamate (4i)

Following the general procedure, inhibitor **4i** (6.5 mg, 60% yield) was prepared as a yellow amorphous solid from amine **30** and activated carbonate **28e** (6 mg, 0.02 mmol). ^{1}H NMR (500 MHz, CDCl$_3$) *δ*: 7.54 (d, *J* = 8.7 Hz, 2H), 7.33–7.16 (m, 5H), 6.68 (d, *J* = 8.5 Hz, 2H), 4.95–4.90 (m, 1H), 4.88 (d, *J* = 8.8 Hz, 1H), 4.29–4.18 (m, 2H), 3.94–3.87 (m, 2H), 3.87–3.75 (m, 2H), 3.41–3.36 (m, 2H), 3.34 (s, 3H), 3.11 (dd, *J* = 15.1, 8.3 Hz, 1H), 3.03–2.84 (m, 5H), 2.76 (dd, *J* = 13.3, 6.7 Hz, 1H), 2.42–2.31 (m, 1H), 1.88–1.74 (m, 1H), 1.60–1.49 (m, 1H), 1.24 (d, *J* = 4.1 Hz, 3H), 0.91 (d, *J* = 6.6 Hz, 3H), 0.86 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) *δ*, 155.9, 150.6, 137.5, 129.4, 129.4, 128.4, 126.5, 114.0, 82.1, 74.6, 73.1, 72.5, 59.2, 58.8, 55.0, 53.7, 39.3, 35.3, 27.2, 21.4, 20.1, 19.8; LRMS-ESI (*m/z*): 564.0 (M + H)$^+$; HRMS-ESI (*m/z*): C$_{28}$H$_{41}$N$_3$O$_7$S; calc'd for [M + Na]$^+$: 586.2557, found 586.2564.

(2*S*,3*R*,5*S*)-2-(Methoxymethyl)-5-methyltetrahydrofuran-3-yl ((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)carbamate (4j)

Following the general procedure, inhibitor **4j** (4.3 mg, 66% yield) was prepared as a white amorphous solid from amine **29** and activated carbonate **28f** (3.5 mg, 0.01 mmol). ^{1}H NMR (400 MHz, CDCl$_3$) *δ*: 7.70 (d, *J* = 8.7 Hz, 2H), 7.34–7.17 (m, 5H), 6.98 (d, *J* = 8.7 Hz, 2H), 4.93–4.88 (m, 1H), 4.85–4.80 (m, 1H), 4.26–4.20 (m, 1H), 4.07–4.04 (m, 1H), 3.88 (s, 3H), 3.87–3.82 (m, 2H), 3.41 (d, *J* = 4.3 Hz, 2H), 3.34 (s, 3H), 3.20–3.10 (m, 1H), 3.05–2.89 (m, 5H), 2.84–2.75 (m, 1H), 2.38–2.26 (m, 1H), 1.68–1.61 (m, 1H), 1.51–1.44 (m, 1H), 1.22 (d, *J* = 6.2 Hz, 3H), 0.91 (d, *J* = 6.4 Hz, 3H), 0.87 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) *δ*, 163.1, 156.0, 137.6, 129.8, 129.5, 129.3, 128.5, 126.6, 114.4, 82.0, 77.2, 74.8, 73.2, 72.6, 59.3, 58.8, 55.6, 55.0, 53.8, 39.4, 35.6, 27.3, 21.6, 20.1, 19.9; LRMS-ESI (*m/z*): 579.0 (M + H)$^+$; HRMS-ESI (*m/z*): C$_{29}$H$_{42}$N$_2$O$_8$S; calc'd for [M + Na]$^+$: 601.2554, found 601.2560.

(2*S*,2′*S*,3*S*,5*R*)-5-Methyloctahydro-[2,2′-bifuran]-3-yl((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)carbamate (4k)

Following the general procedure, inhibitor **4k** (16 mg, 83% yield) was prepared from amine **29** and activated carbonate **28g** (11 mg, 0.03 mmol) as a white amorphous solid. ^{1}H NMR (400 MHz, CDCl$_3$) *δ*: 7.71 (d, *J* = 8.7 Hz, 2H), 7.36–7.17 (m, 5H), 6.98 (d, *J* = 8.9 Hz, 2H), 5.25–5.20 (m, 1H), 4.82 (d, *J* = 7.9 Hz, 1H), 3.98 (q, *J* = 6.6 Hz, 1H), 3.90 (s, 1H), 3.88 (s, 3H), 3.86–3.80 (m, 3H), 3.67–3.63 (m, 2H), 3.16–2.91 (m, 6H), 2.81 (dd, *J* = 13.4, 6.8 Hz, 1H), 2.43 (dt, *J* = 14.2, 7.1 Hz, 1H), 1.89–1.78 (m, *J* = 6.5 Hz, 5H), 1.58–1.40 (m, 1H), 1.27 (d, *J* = 6.1 Hz, 3H), 0.91 (d, *J* = 6.6 Hz, 3H), 0.87 (d, *J* = 6.6 Hz, 3H);

^{13}C NMR (100 MHz, CDCl$_3$) *δ*, 162.9, 155.9, 137.5, 129.8, 129.4, 129.4, 128.4, 126.4, 114.2, 83.3, 76.3, 75.3, 73.9, 72.4, 68.0, 58.6, 55.5, 54.9, 53.5, 40.6, 35.0, 28.1, 27.1, 25.9, 21.4, 20.1, 19.8; LRMS-ESI (*m/z*): 605.03 (M + H)$^+$; HRMS-ESI (*m/z*): C$_{31}$H$_{45}$N$_2$O$_8$S; calc'd for [M + H]$^+$: 605.2896, found 605.2906.

(2*S*,2′*R*,3*S*,5*R*)-5-Methyloctahydro-[2,2′-bifuran]-3-yl((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)carbamate (4l)

Following the general procedure, inhibitor **4l** (4.8 mg, 62% yield) was prepared from amine **29** and activated carbonate **28h** (4 mg, 0.01 mmol). ^{1}H NMR (800 MHz, CDCl$_3$) *δ*: 7.72 (d, *J* = 8.8 Hz, 2H), 7.28 (t, *J* = 7.6 Hz, 2H), 7.21 (d, *J* = 7.6 Hz, 3H), 6.99 (d, *J* = 8.4 Hz, 2H), 5.14–5.05 (m, 1H), 4.84 (d, *J* = 8.4 Hz, 1H), 3.99–3.95 (m, 1H), 3.88 (s, 3H), 3.86–3.80 (m, 2H), 3.79–3.71 (m, 2H), 3.51 (dd, *J* = 7.8, 4.4 Hz, 1H), 3.35 (s, 1H), 3.16 (dd, *J* = 15.2, 8.1 Hz, 1H), 3.08 (dd, *J* = 14.3, 4.0 Hz, 1H), 3.03–2.93 (m, 2H), 2.82–2.78 (m, 2H), 2.42–2.34 (m, 1H), 1.85–1.78 (m, 2H), 1.77–1.72 (m, 1H), 1.51–1.46 (m, 1H), 1.35–1.31 (m, 1H), 1.30 (d, *J* = 6.1 Hz, 3H), 0.93 (d, *J* = 6.6 Hz, 3H), 0.88 (d, *J* = 2.5 Hz, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) *δ*, 163.1, 155.6, 137.6, 129.8, 129.5, 129.4, 128.5, 126.5, 114.4, 84.3, 77.5, 75.6, 74.3, 73.0, 68.2, 58.8, 55.6, 55.0, 53.7, 41.0, 35.6, 27.2, 25.9, 21.4, 20.2, 19.9. LRMS-ESI (*m/z*): 605.0 (M + H)$^+$; HRMS-ESI (*m/z*): C$_{31}$H$_{45}$N$_2$O$_8$S; calc'd for [M + H]$^+$: 605.2896, found 605.2899.

Methods: determination of X-ray structures of the HIV-1 protease–inhibitor complex

HIV-1 protease was expressed and purified as described.[25,40] The protease–inhibitor complex (PR/GRL-072-17A) was crystallized by hanging drop vapor diffusion method with a solution of 1.7 M NaCl and 0.1 M sodium acetate at pH 5.5 in the well. X-ray diffraction data were collected on a single crystal cooled to 90 K at SER-CAT (22-ID beamline), Advanced Photon Source, Argonne National Lab (Chicago, USA) with X-ray wavelength of 1.0 Å. X-ray data were processed by HKL-2000[41] to give an Rmerge of 8.9%. The crystal structure was solved by PHASER[42] in CCP4i Suit[43–45] using one of the previously reported isomorphous structures[46] as the initial model, and refined by SHELX-2014[47,48] and Refmac5[49] using X-ray data at 1.32 Å resolution. PRODRG-2[50] and JLigand[51] were used to construct the inhibitor and geometric restraints for refinement. COOT[52,53] was used to modify the PR–inhibitor structure. Alternative conformations were modeled, and anisotropic atomic displacement parameters (B factors) were applied for all protein atoms including solvent molecules. The final refined solvent structure comprised two Na$^+$ ions, four Cl$^-$ ions, two glycerol molecules, one formic acid, and 221 water molecules. The crystallographic statistics are listed in Table 1. The coordinates and structural factors of the protease complexes with GRL-072-17A have been deposited in the Protein Data Bank[54] with accession codes 9B2H.

7370 | *Org. Biomol. Chem.*, 2024, **22**, 7354–7372

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Author contributions

Arun K. Ghosh: writing – review & editing, writing – original draft, supervision, resources, project administration, methodology, investigation, funding acquisition, formal analysis, and conceptualization. Daniel Lee: methodology and investigation. Ashish Sharma: methodology and investigation. Megan E. Johnson: methodology and data curation. Ajay K. Ghosh: methodology and data curation. Yuan-Fang Wang: methodology and investigation. Johnson Agniswamy: methodology and investigation. Masayuki Amano: methodology and investigation. Shin-ichiro Hattori: methodology and investigation. Irene T. Weber: writing, editing, supervision, methodology, and data curation. Hiroaki Mitsuya: supervision, methodology, data curation, and funding acquisition.

Data availability

The authors confirm that the data supporting these studies are available within the article and its ESI.‡

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

References

1 R. C. Ogden and C. W. Flexner, *Protease Inhibitors in AIDS Therapy*, New York, 2011, pp. 1–300.

2 J. A. Esté and T. C. Cihlar, *Antiviral Res.*, 2010, **85**, 25–33.

3 C. W. Diffenbach and A. S. Fauci, *Ann. Intern. Med.*, 2011, **154**, 766–771.

4 M. S. Cohen, Y. Q. Chen and M. McCauley, *N. Engl. J. Med.*, 2011, **365**, 493–505.

5 J. Maenza and C. Flexner, *Am. Fam. Physician*, 1998, **57**, 2789–2798.

6 M. Vitoria, A. Rangaraj, N. Ford and M. Doherty, *Curr. Opin. HIV AIDS*, 2019, **14**, 143–149.

7 G. Barbaro, *Lancet*, 2004, **363**, 900–901.

8 A. Chawla, C. Wang, C. Patton, M. Murray, Y. Punekar, A. de Ruiter and C. Steinhart, *Infect. Dis. Ther.*, 2018, 183–195.

9 C. W. Dieffenbach and A. S. Fauci, *Ann. Intern. Med.*, 2011, **154**, 766–771.

10 K. A. Bosh, A. S. Johnson, A. L. Hernandez, J. Prejean, J. Taylor, R. Wingard, L. A. Valleroy and H. I. Hall, *Morb. Mortal. Wkly. Rep.*, 2020, **69**, 1717–1724.

11 L. Waters and M. Nelson, *Int. J. Clin. Pract.*, 2007, **61**, 983–990.

12 C. E. Reust, *Am. Fam. Physician*, 2011, **83**, 1443–1451.

13 A. K. Ghosh, I. T. Weber and H. Mitsuya, *Chem. Commun.*, 2022, **58**, 11762–11782.

14 A. K. Ghosh, H. L. Osswald and G. Prato, *J. Med. Chem.*, 2016, **59**, 5172–5208.

15 A. K. Ghosh and B. D. Chapsal, *Aspartic Acid Proteases as Therapeutic Targets*, Wiley-VCH, Weinheim, Germany, 2010, pp. 169–204.

16 A. K. Ghosh, Z. L. Dawson and H. Mitsuya, *Bioorg. Med. Chem.*, 2007, **15**, 7576–7580.

17 A. Curran and E. R. Pascuet, *Enfermedades Infecciosas y Microbiología Clínica*, 2008, vol. 10, pp. 14–22.

18 A. K. Ghosh, P. R. Sridhar, N. Kumaragurubaran, Y. Koh, I. T. Weber and H. Mitsuya, *ChemMedChem*, 2006, **1**, 939–950.

19 A. K. Ghosh, B. Chapsal and H. Mitsuya, *Aspartic Acid Proteases as Therapeutic Targets*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2010, pp. 205–243.

20 A. K. Ghosh, D. D. Anderson, I. T. Weber and H. Mitsuya, *Angew. Chem., Int. Ed.*, 2012, **51**, 1778–1802.

21 A. K. Ghosh, B. Chapsal, I. T. Weber and H. Mitsuya, *Acc. Chem. Res.*, 2008, **41**, 78–86.

22 A. K. Ghosh and B. D. Chapsal, in *From Introduction to Biological and Small Molecule Drug Research and Development*, ed. R. Jefferis, 2013, pp. 355–384.

23 J. Mallolas, *AIDS Rev.*, 2017, **19**, 105–112.

24 A. Y. Kovalevsky, F. Liu, S. Leshchenko, A. K. Ghosh, J. M. Louis, R. W. Harrison and I. T. Weber, *J. Mol. Biol.*, 2006, **363**, 161–173.

25 Y. Tie, P. I. Boross, Y.-F. Wang, L. Gaddis, A. K. Hussain, S. Leshchenko, A. K. Ghosh, J. M. Louis, R. W. Harrison and I. T. Weber, *J. Mol. Biol.*, 2004, **338**, 341–352.

26 Y. Koh, H. Nakata, K. Maeda, H. Ogata, G. Bilcer, T. Devasamudram, J. F. Kincaid, P. Boross, Y.-F. Wang,

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Org. Biomol. Chem., 2024, **22**, 7354–7372 | **7371**

Y. Tie, P. Volarath, L. Gaddis, R. W. Harrison, I. T. Weber, A. K. Ghosh and H. Mitsuya, *Antimicrob. Agents Chemother.*, 2003, **47**, 3123–3129.

27 S. De Meyer, H. Azijn, D. Surleraux, D. Jochmans, A. Tahri, R. Pauwels, P. Wigerinck and M.-P. de Béthune, *Antimicrob. Agents Chemother.*, 2005, **49**, 2314–2321.

28 A. K. Ghosh, B. D. Chapsal, A. Baldridge, M. P. Steffey, D. E. Walters, Y. Koh, M. Amano and H. Mitsuya, *J. Med. Chem.*, 2011, **54**, 622–634.

29 M. Amano, P. M. Salcedo-Gómez, R. S. Yedidi, R. Zhao, H. Hayashi, K. Hasegawa, T. Nakamura, C. D. Martyr, A. K. Ghosh and H. Mitsuya, *Antimicrob. Agents Chemother.*, 2019, **63**, e00466–e00419.

30 A. K. Ghosh and P. R. Nyalapatla, *Tetrahedron*, 2017, **73**, 1820–1830.

31 A. K. Ghosh and Y. Chen, *Tetrahedron Lett.*, 1995, **36**, 505–508.

32 A. K. Ghosh and P. R. Nyalapatla, *Org. Lett.*, 2016, **18**, 2296–2299.

33 S. Hanessian, S. Giroux and A. Larsson, *Org. Lett.*, 2006, **8**, 5481–5484.

34 M. Arisawa, Y. Terada, K. Takahashi, M. Nakagawa and A. Nishida, *J. Org. Chem.*, 2006, **71**, 4255–4261.

35 M. V. Toth and G. R. Marshall, *Int. J. Pept. Protein Res.*, 1990, **36**, 544–550.

36 Y. Koh, M. Amano, T. Towata, M. Danish, S. Leshchenko-Yashchuk, D. Das, M. Nakayama, Y. Tojo, A. K. Ghosh and H. Mitsuya, *J. Virol.*, 2010, **84**, 11961–11969.

37 K. Yoshimura, R. Kato, M. F. Kavlick, A. Nguyen, V. Maroun, K. Maeda, K. A. Hussain, A. K. Ghosh, S. V. Gulnik, J. W. Erickson and H. Mitsuya, *J. Virol.*, 2002, **76**, 1349–1358.

38 A. K. Ghosh, Z. Xia, S. Kovela, W. L. Robinson, M. E. Johnson, D. W. Kneller, Y. F. Wang, M. Aoki, Y. Takamatsu, I. T. Weber and H. Mitsuya, Potent HIV-1 Protease Inhibitors Containing Carboxylic and Boronic Acids: Effect on Enzyme Inhibition and Antiviral Activity and Protein-Ligand X-ray Structural Studies, *ChemMedChem*, 2019, **14**, 1863–1872.

39 N. M. Midde, B. J. Patters, P. S. S. Rao, T. J. Cory and S. Kumar, *Expert Opin. Invest. Drugs*, 2016, **25**, 1189–1200.

40 J. Agniswamy, D. W. Kneller, R. Brothers, Y.-F. Wang, R. W. Harrison and I. T. Weber, *ACS Omega*, 2019, **4**, 8707–8719.

41 Z. Otwinowski, W. Minor, C. W. Carter Jr. and R. M. Sweet, *Processing of X-ray Diffraction Data Collected in Oscillation Mode*, Academic Press, New York, 1997, pp. 307–326.

42 A. J. McCoy, R. W. Grosse-Kunstleve, P. D. Adams, M. D. Winn, L. C. Storoni and R. J. Read, *J. Appl. Crystallogr.*, 2007, **40**, 658–674.

43 J. Agirre, M. Atanasova, H. Bagdonas, C. B. Ballard, *et al.*, *Acta Crystallogr.*, 2023, **79**, 449–461.

44 M. D. Winn, C. C. Ballard, K. D. Cowtan, E. J. Dodson, P. Emsley, P. R. Evans, R. M. Keegan, E. B. Krissinel, A. G. W. Leslie, A. McCoy, S. J. McNicholas, G. N. Murshudov, N. S. Pannu, E. A. Potterton, H. R. Powell, R. J. Read, A. Vagin and K. S. Wilson, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 2011, **67**, 235–242.

45 Collaborative Computational Project, Number 4, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 1994, **50**, 760–763.

46 C.-H. Shen, Y.-F. Wang, A. Y. Kovalevsky, R. W. Harrison and I. T. Weber, *FEBS J.*, 2010, **277**, 3699–3714.

47 G. M. Sheldrick, *Acta Crystallogr., Sect. A: Found. Crystallogr.*, 2008, **64**, 112–122.

48 G. M. Sheldrick and T. R. Schneider, *Methods Enzymol.*, 1997, **277**, 319–343.

49 G. N. Murshudov, A. A. Vagin and E. J. Dodson, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 1997, **53**, 240–255.

50 A. W. Schuettelkopf and D. M. F. van Aalten, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 2004, **60**, 1355–1363.

51 A. A. Lebedev, P. Young, M. N. Isupov, O. V. Moroz, A. A. Vagin and G. N. Murshudov, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 2012, **68**, 431–440.

52 P. Emsley, B. Lohkamp, W. G. Scott and K. Cowtan, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 2010, **66**, 486–501.

53 P. Emsley and K. Cowtan, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 2004, **60**, 2126–2132.

54 H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov and P. E. Bourne, *Nucleic Acids Res.*, 2000, **28**, 235–242.

7372 | *Org. Biomol. Chem.*, 2024, **22**, 7354–7372

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