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Design, Synthesis, Biological Evaluation and X-ray Structural Studies of HIV-1 Protease Inhibitors Containing Substituted Fused-Tetrahydropyranyl Tetrahydrofuran as P2-Ligands

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Abstract

Design, synthesis, biological and X-ray crystallographic studies of a series of potent HIV-1 protease inhibitors are described. Various polar functionalities have been incorporated on the tetrahydropyranyl-tetrahydrofuran-derived P2 ligand to interact with the backbone atoms in the S2 subsite. The majority of the inhibitors showed very potent enzyme inhibitory and antiviral activity. Two high-resolution X-ray structures of **30b**- and **30j**-bound HIV-1 protease provide insight into ligand-binding site interactions. In particular, the polar functionalities on the P2 ligand appear to form unique hydrogen bonds with Gly48 amide NH and amide carbonyl groups in the flap region.

Keywords

HIV-1 protease inhibitors; antiviral; darunavir; multidrug-resistant; design; synthesis; X-ray crystal structure; backbone binding

Introduction

The development of human immunodeficiency virus type 1 (HIV-1) protease inhibitors (PIs) as part of the current active antiretroviral therapy has significantly reduced the mortality of HIV/AIDS patients.[1,2] However, one of the major drawbacks of many approved protease

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inhibitors is the emergence of drug resistant HIV-1 strains which may severely limit long-term treatment options.[3,4] Therefore, design and development of protease inhibitors with broad-spectrum activity against multidrug-resistant HIV-1 variants are critically important. As part of our continuing interest to develop novel PIs, we reported a variety of novel inhibitors that are potent against HIV-1 variants resistant to currently approved PIs.[5,6] One of these PIs, darunavir (**1**, Figure 1) which contains a structure-based designed nonpeptidic P2 ligand, 3(*R*),3*a*(*S*),6*a*(*R*)-bis-tetrahydrofuranyl urethane (bis-THF), was approved as a first-line therapy for the treatment of HIV/AIDS patients.[7–10]

The bis-THF ligand in darunavir is responsible for its superb activity against multidrug resistant HIV-1 strains.[5,7] In an effort to further optimize the structural feature of bis-THF, we have designed a furopyranol or tetrahydropyranyl tetrahydorfuran (Tp-THF) ligand.[11,12] The X-ray structure of inhibitors **1** and **2** with HIV-1 protease revealed that both ring oxygens are involved in strong hydrogen bonding interactions with the backbone NHs of Asp29 and Asp30 in the S2 subsite. Also, the bicyclic ring system fills in the hydrophobic pocket in the S2 site. We have recently incorporated substituents on the bis-THF and Cp-THF rings (inhibitor **3**) to effect specific ligand-binding site interactions in the S2-subsite of HIV-1 protease.[13–16] Hohlfeld and co-workers have also reported potent HIV-1 protease inhibitors incorporating substituted bis-THF ligands.[17,18] To further optimize ligand-binding site interactions, we speculated that a larger tetrahydropyranyl ring in place of the tri-substituted THF ring may improve hydrogen bonding with the active site aspartate backbone NHs due to an increase in the dihedral angle of the bicyclic acetal bearing the tetrahydropyranyl tetrahydrofuran ring system. Furthermore, a larger THP-ring in the fused THP-THF (Tp-THF) ligand may enhance van der Waals interactions. Tp-THF ligand-derived inhibitor **4** exhibited high enzyme affinity and antiviral activity. Also, inhibitor **4** has been shown to retain high potency against a variety of multi-drug resistant clinical HIV-1 strains with EC_{50} values in low nanomolar range. In an effort to further improve backbone ligand-binding site interactions, based upon the X-ray structure of **4**-bound HIV-1 protease, we have incorporated various polar substituents at the C5-position of Tp-THF ligand. We speculated that a stereochemically defined hydroxyl group, alkoxy group or amine derivative could form a hydrogen bond with the backbone atoms of Gly48 located in the flap region of HIV-1 protease. Also, the corresponding alkyl chain may enhance additional van der Waals interactions in the active site. Herein we report our studies on the design, synthesis, biological evaluation and X-ray structural studies of C5-substituted tetrahydropyranyl tetrahydrofuran ligands. A number of inhibitors exhibited very high enzyme inhibitory and antiviral activity. For the synthesis of the tetrahydropyranyl tetrahydrofuran ring system P2-ligands in this study, we developed a highly diastereoselective tetrahydropyranyl tetrahydrofuran ring system using a [2,3]-sigmatropic rearrangement as the key reaction.

Results and discussion

Synthesis of the fused tetrahydropyranyl-tetrahydrofuran ligand is shown in Scheme 1. Methyl ester **5** is obtained in four steps from *L*-malic acid as described by Solladié and coworkers.[19] Dibal-H reduction of **5** provided the corresponding aldehyde. Wittig olefination of the resulting aldehyde in MeOH at 0 °C afforded α,β-unsaturated ester **6** in 70% yield as a mixture (8:1) of *cis*/*trans* isomers. Dibal-H reduction of **6** provided the

corresponding *cis*-alcohol. Cesium hydroxide promoted *O*-alkylation with *t*-butyl bromoacetate gave the desired alkylated product **7** in 95% yield over 2 steps. Sigmatropic rearrangement of **7** using LiHMDS at −65 °C resulted in a mixture of rearrangement products **9** and **10** in 77% yield (diastereomeric ratio 8.5:1).[16,20] The absolute stereochemical configuration of the major isomer **9** was determined through the use of 2D COSY and 1D NOE after conversion to the subsequent ligand derivatives.

The stereochemical outcome of the [2,3]-sigmatropic rearrangement can be rationalized based upon the transition state model **8** as described in the literature. [21,22] The observed selectivity is somewhat lower as compared to the isopropylidene derivative. This is possibly due to the greater flexibility of the 6-membered dioxane ring in substrate **7**. Mesylation of compound **9** followed by lithium aluminum hydride reduction gave alcohol **11** in 70 % yield over 2 steps. Ozonolysis and subsequent acid catalyzed cyclization resulted in the desired Tp-THF ligand **12** in 70 % yield. The route provided Tp-THF ligand **12** in 25% overall yield from **5**. This ligand was converted to the desired inhibitor as described previously.[14,23] The sigmatropic rearrangement product **9** is an effective intermediate for the synthesis of substituted P2-ligands. As shown in Scheme 2, the hydroxyl group was converted to methyl ether **13** using NaH and methyl iodide in THF. Reduction of **13** by $LiAlH_4$, ozonolytic cleavage of the double bond, and subsequent treatment of the resulting aldehyde with *p*-TsOH in THF provided the corresponding C3-methoxy substituted ligand **14**.

Rearrangement product **9** was subjected to Mitsunobu inversion with *p*-nitrobenzoic acid to provide benzoate **15**.[24] Selective deprotection of the *p*-nitrobenzoate in the presence of the *t*-butyl ester was accomplished in K_2CO_3 and MeOH at 0 °C over 1 h. Alkylation of the resulting alcohol using NaH and respective alkyl halide provided the corresponding alkylated product in high yield (88 – 96%). Ozonolysis of the *O*-alkylated products **16–18**, followed by acid catalyzed cyclization, gave various C3 *O*-alkylated Tp-THF ligands **19–21** in 60 – 70 % yield.

The synthesis of various C3-amine derivatives was accomplished utilizing hydroxy ester **9**. As shown in Scheme 3, reduction of **9** with LAH provided the corresponding diol. Protection of the primary alcohol with trityl chloride and Et_3N afforded the trityl derivative **22** over 92% yield in two-steps. Alcohol **22** was subjected to Mitsunobu azidation using diphenyl phosphorazidate (DPPA), triphenyl phosphine and diethyl azodicarboxylate (DEAD) to provide the corresponding azide derivative in 70% yield. Ozonolysis of the resulting olefin followed by treatment with *p*-TsOH in CH_2Cl_2 afforded bicyclic azide derivative **23** in 74% yield in two-steps. Catalytic hydrogenation of **23** with 10% Pd/C under a hydrogen-filled balloon provided the corresponding amine in near quantitative yield. The resulting amine was converted to various benzyl derivatives as follows: reductive amination with benzyldehyde in the presence of $NaBH_3CN$ in MeOH affording the corresponding benzylamine, which was then subjected to further reductive amination with formaldehyde, acetaldehyde or propionaldehyde to provide substituted amines **24 – 26** in 45–81% yield. This double alkylation was carried out in a single pot operation.[25]

For the synthesis of C3-substituted Tp-THF-derived inhibitors, various selected alcohol derivatives were converted to the corresponding mixed activated carbonate derivatives **27a–**

h. As shown in Scheme 4, optically active ligand alcohols (**14, 19–21**, **24–26**) were reacted with 4-nitrophenyl chloroformate and pyridine in CH_2Cl_2 at 0 °C to 23 °C for 12 h, furnishing mixed carbonates **27a–h** in 24–90% yields.[26] The synthesis of inhibitors with hydroxyethylsulfonamide isosteres with *p*-methoxysulfonamide and *p*-aminosulfonamide as the P2′-ligand is shown in Scheme 5. Treatment of amines **28** and **29** with mixed activated carbonates **27a–e** in CH_3CN in the presence of diisopropylethylamine (DIPEA) provided inhibitors **30a–e**, **g**. For the synthesis of inhibitor **30f**, inhibitor **30e** was subjected to catalytic hydrogenation using Pearlman's Catalyst in MeOH at 60 psi hydrogen pressure for 12 h. This has resulted in inhibitor **30f** in 38% yield over two steps. Similarly, hydrogenation of azide **30g** over 10% Pd-C in ethyl acetate under a hydrogen-filled balloon for 1 h provided inhibitor **30h** in 85% yield.

For synthesis of inhibitors **30i–l**, amines **28** and **29** were reacted with active mixed carbonate **27f–h** in the presence of DIEPA to provide corresponding urethanes. Catalytic hydrogenation of the resulting urethanes provided inhibitors **30i–l**. For the synthesis of inhibitor **30m**, methylamine derivative **30i** was subjected to reductive amination using $Na(CN)BH_3$ in MeOH in the presence of paraformaldehyde in CH_2Cl_2 to provide **30m** in 67% yield.

As stated earlier, our main objective is to investigate the effect of additional inhibitor-HIV-1 protease interactions in the S2 subsite. In particular, we planned to target the Gly48 backbone atoms in the flap region of HIV-1 protease. We first investigated the effect of the hydroxyl group and its alkyl ethers at the C4 position of the Tp-THF ligand. The structure and activity of these inhibitors are shown in Table 1. Enzyme inhibitory potency was evaluated using assay developed by Toth and Marshall.[27] Inhibitors **30a** and **30b** were designed to examine the importance of the *R/S* configuration of the C5-methoxy group. As it turned out, compound **30b** with a 5-(*R*)-configuration has shown over 300-fold better enzymatic K_i compared to compound **30a** with a 5-(*S*)-methoxy group. Antiviral activity of selected compounds were evaluated using protocol described previously.[28] Consistent with potent enzyme inhibitory activity of **30b**, it showed an antiviral IC_{50} value of 0.2 nM, which is over 85-fold better than the antiviral activity of **30a**. Based upon our preliminary model, we presume that C5-(*R*)-configuration is optimum for hydrogen bonding interactions with Gly48 backbone atoms. We subsequently investigated other C5-substituents. Compound **30c** with 4-aminosulfonamide as the P2′-ligand showed reduction of potency compared to **30b**. Compound **30d** with 5-(*R*)-ethoxy substituent showed comparable enzyme inhibitory activity. Benzyl ether derivative **30e** also showed similar antiviral potency as compound **30b**. Compound **30f** with a 5-(*R*)-hydroxy group also showed very potent enzyme inhibitory and antiviral activity, however it was 10-fold less potent than compound **30b**. Interestingly, inhibitor **30f** maintained full antiviral activity against $HIV_{DRV}{}^{R}{}_{P20}$ drug resistant strains of HIV (IC_{50} = 3.3 nM). Another objective of our investigation is to incorporate a basic amine functionality on the P2-ligand. Particularly, incorporation of an alkyl amine may serve the purpose of hydrogen bonding interaction with Gly48 backbone atoms through the amine NH. Also, a suitable alkyl group may fill in the hydrophobic pocket in the S2-subsite. Towards this goal, we have incorporated various amine and substituted amine derivatives at the C5-position of the Tp-THF ligand. Based upon previous observation of the preference

for the C5-(*R*)-configuration, we have investigated derivatives containing 5-(*R*)-configuration. The structures and activity of amine derivatives are shown in Table 2. As can be seen, compounds **30g** and **30h** with C5-azide and C5-amine functionalities are significantly less potent than the corresponding methoxy and hydroxyl derivatives **30b** and **30f** respectively. Compound **30i** with a methylamine substituted is very potent. Compound **30j** with an ethylamine functionality showed very potent antiviral activity with an IC_{50} value of 0.8 nM. The corresponding 4-aminosulfonamide derivative **30k**, however, showed significant reduction in antiviral potency. The propyl amine derivative **30l**, with a sterically demanding alkyl side chain, showed nearly 3-fold reduction in antiviral potency compared to ethylamine derivative **30j**. Compound **30m** with a dimethylamine functionality showed reduction in enzyme K_i, however it showed 3-fold improved antiviral activity over its methyl derivative **30i**.

In order to obtain molecular insight into the HIV-1 protease-inhibitor interactions, we have determined X-ray structures of HIV-1 protease complexed with compounds **30b** and **30j**. A stereoview of **30b**-bound HIV-1 protease structure is shown in Figure 2. The crystal structure of wild type HIV-1 protease with the inhibitor **30b** was determined and refined at 1.22 Å resolution. This crystal structure contains the protease dimer and the inhibitor bound in two orientations related by a 180° rotation with 55/45% relative occupancies. The overall structure is similar to the structure of HIV-1 protease with darunavir[29] with root mean square differences of 0.13 Å for Cα atoms. Inhibitor **30b** has a tetrahydropyrano-tetrahydrofuran (Tp-THF) group at P2, which bears a methoxy group. The Tp-THF ring shows a slight bend compared to the complex with the related bis-THF inhibitor bis-THF derived (pdb code: 3QAA).[30] As shown, the methoxy group on the Tp-THF ligand forms a water-mediated interaction with the amide NH of Gly 48. These interactions likely stabilize the flexible flap region of the active site cavity. The new interactions with the flap region may be responsible for its robust antiviral activity.

We have also determined the X-ray crystal structure of **30j**-bound HIV-1 protease. A stereoview of the active site is shown in Figure 3. This structure was determined and refined at 1.62Å resolution. Similar to structure for **30b**, the structure contains the protease dimer and inhibitor **30j** is bound in two orientations related to 180 ° rotation with 55/45 relative occupancies. The structure is related to **30b**-bound HIV-1 protease. The major difference is in the binding of the ethylamino substituent on the Tp-THF ring. As it turns out, the amine NH on the Tp-THF ring of the inhibitor forms a water-mediated hydrogen bonding interaction with the backbone amide NH of Gly48. In addition, the amine NH forms a direct hydrogen bond with the carbonyl oxygen of Gly48. This interaction is lacking for inhibitor **30b**. The ethylamino nitrogen atom in inhibitor **30j** shifts its position to enhance the interactions with the wild-type HIV-1 protease. These interactions are likely to stabilize the flexible flap region of the active site. This may help maintain antiviral activity of the inhibitor against multidrug-resistant HIV-1 variants.

Conclusion

In summary, we have designed and synthesized alkoxy- and amine-substituted derivatives of a tetrahydropyran-tetrahydrofuranyl ligand. These molecules are specifically designed to

make hydrogen bonding interactions with the flap region of HIV-1 protease. In addition, the alkyl substituents are expected to make hydrophobic interactions in the S2 subsite. The syntheses of the substituted ligands were carried out in a stereoselective manner using a [2,3]-sigmatropic rearrangement as the key step. A number of inhibitors with alkoxy and alkylamine substituents on the P2 ligands showed excellent enzyme K*i* and antiviral IC_{50} values. Inhibitors with a 5-(*R*)-configuration showed improved potency over inhibitors with a 5-(*S*)-configuration. Both inhibitors **30b** and **30j** with a methoxy and ethylamine functionalities, respectively displayed subnanomolar antiviral activity. Our preliminary assessment of inhibitor **30f** showed that it maintained full antiviral activity against the $HIV_{DRV}P_{20}$ resistant HIV-1 variant. We have determined high resolution X-ray crystal structures of **30b-** and **30j**-bound HIV-1 protease. The structure of **30b**-bound HIV-1 protease revealed that the methoxy oxygen forms a strong water-mediated hydrogen bond with the amide NH of Gly48 located in the flap region of the HIV-1 protease active site. The ethylamino group in inhibitor **30j** also forms a water-mediated interaction with Gly48. In addition, the amine NH forms a direct hydrogen bond with Gly48 carbonyl oxygen. The ethyl side chain also nicely packed in the hydrophobic pocket in the S2-subsite. The basic amine functionality on the inhibitor may be important for improving pharmacological properties. More detailed drug-resistance studies as well as further optimization of enzyme-inhibitor interactions are currently in progress.

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 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 MHz, Avance DRX-500, Varian Mercury-Vx-300, and Gemini-2300 spectrometers and use Me_4Si as an internal standard. Optical rotations were recorded on a Perkin-Elmer 341 polarimeter. A Thermo Finnigan LCQ Classic mass was used for MS analyses. The purity of test compounds was determined by HRMS and HPLC analysis. All test compounds showed ≥95% purity.

(*S*,*Z*)-Ethyl 3-(2,2-dimethyl-1,3-dioxan-4-yl)acrylate (6)

To a solution of (*S*)-methyl 2,2-dimethyl-1,3-dioxane-4-carboxylate (**5**) (3.0g, 17.2 mmol) in CH_2Cl_2 (100 mL) at −78 °C was added Dibal-H (1M in CH_2Cl_2, 19.0 mL). The solution was allowed to warm slowly to −50 °C over 1 h. Upon completion, methanol (100 mL) was added to the reaction followed by Ph_3PCHCO_2Et (4.20 g, 12.1 mmol) and the reaction was allowed to stir for 1 h at 0 °C. The reaction was concentrated under vacuum and the solid was washed with CH_2Cl_2 (3×20 mL) and concentrated under vacuum. The crude mixture was purified on silica gel using 5– 10% ethyl acetate/hexanes. The desired product **6** was obtained as a separable mixture of *cis/trans* (8:1) isomers (2.6 g, 70 % yield). *cis:* R_f = 0.38 (10% ethyl acetate/hexanes). $[\alpha]^{23}_{D}$ = −12.5 (*c* 1.01, $CHCl_3$). ^{1}H NMR (300 MHz,

Chloroform-d) δ 6.17 (dd, *J* = 11.4 Hz, 7.1 Hz, 1H), 5.76 (d, *J* = 11.7 Hz, 1H), 5.52 (q, *J* = 7.1 Hz, 1H), 4.24 – 4.10 (q, *J* = 7.5 Hz, 2H), 4.10 – 3.97 (m, 1H), 3.90 – 3.76 (d, *J* = 16 Hz, 1H), 1.67 – 1.61 (m, 2H), 1.50 (s, 3H), 1.39 (s, 3H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.7, 149.7, 119.2, 98.3, 66.8, 60.2, 59.5, 30.0, 29.4, 19.3, 14.2. *trans:* R_f = 0.20 (10% ethyl acetate/hexanes). ^{1}H NMR (400 MHz, Chloroform-d) δ 6.86 (dd, *J* = 15.7 Hz, 7.1 Hz, 1H), 6.04 (d, *J* = 11.8 Hz, 1H), 4.27 – 4.08 (q, *J* = 7.5 Hz, 2H), 4.01 (ddt, *J* = 14.9 Hz, 8.3 Hz, 2.7 Hz, 1H), 3.90 – 3.76 (m, 1H), 3.76 – 3.59 (m, 1H), 1.82 – 1.60 (m, 1H), 1.60 – 1.50 (m, 1H), 1.48 (s, 3H), 1.41 (s, 3H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.4, 147.0, 120.3, 108.6, 98.5, 67.8, 60.3, 59.5, 30.4, 29.7, 19.0, 14.1; LRMS-CI (m/z): 215 $(m + H)^+$.

(*S,Z*)-t-butyl-2-(3-(2,2-dimethyl-1,3-dioxan-4-yl)allyloxy)-acetate (7)

Diisobutyl aluminum hydride (1 M in CH_2Cl_2, 27.5 mL, 27.5 mmol,) was slowly added to a cold solution (−78 °C) of **6** (12.0 g, 56.0 mmol) in dichloromethane (200 mL). The solution was allowed to stir for 15 min at −78 °C. A saturated solution of Rochelle's salt (50 mL) was added and the reaction mixture was warmed to room temperature. The reaction was stirred until both layers were clear. The organic layer was separated and the aqueous layer was extracted with dichloromethane (3 × 15 mL). The organic layers were combined, washed with brine and dried over $MgSO_4$. The solid was filtered out and the organic layer was concentrated under vacuum. The crude mixture was purified on silica gel using 20% ethyl acetate/hexanes to obtain the desired allyl alcohol (9.4 g, 98% yield) as a colorless oil. R_f = 0.27 (40% ethyl acetate/hexanes). ^{1}H NMR (300 MHz, Chloroform-d) δ 5.78 – 5.74 (m, 1H), 5.50 (ddt, *J* = 11.2 Hz, 7.0 Hz, 1.3 Hz, 1H), 4.78 – 4.62 (m, 1H), 4.24 (dd, *J* = 12.9 Hz, 7.0 Hz, 1H), 4.20 – 4.08 (m, 1H), 4.00 (td, *J* = 12.2 Hz, 2.8 Hz, 1H), 3.83 (ddd, *J* = 11.8 Hz, 5.4 Hz, 1.5 Hz, 1H), 2.47 – 2.27 (m, 1H), 1.86 – 1.63 (m, 1H), 1.55 (s, 3H), 1.48 – 1.45 (m, 1H), 1.39 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 132.3, 131.5, 98.5, 65.7, 59.6, 58.8, 31.2, 29.9, 19.1

To a round bottom flask charged with activated molecular sieves (6.0 g) was added a solution of substrate, (*S,Z*)-3-(2,2-dimethyl-1,3-dioxan-4-yl)prop-2-en-1-ol (9.0 g, 52.0 mmol) in acetonitrile (150 mL), followed by *t*-butylbromoacetate (9.90 mL, 67.2 mmol), tetrabutylammonium iodide (2.0 g, 5.2 mmol) and cesium hydroxide monohydrate (13.1 g, 78.0 mmol) at room temperature. The reaction was allowed to stir for 6 h. The solid was filtered out and the solvent was concentrated under vacuum; the residue was purified by flash column chromatography (5% ethyl acetate/hexanes) to afford **7** (14.4 g, 97% yield) as a colorless oil. R_f = 0.57 (30% ethyl acetate/hexanes). $[\alpha]^{23}_D$ = + 22.9 (*c* 1.29, $CHCl_3$) ^{1}H NMR (300 MHz, Chloroform-d) δ 5.72 – 5.51 (m, 2H), 4.77 – 4.65 (m, 1H), 4.26 – 4.09 (m, 2H), 4.01 (dd, *J* = 17.3, 2.6 Hz, 1H), 3.93 (d, *J* = 3.6 Hz, 2H) 3.82 (ddd, *J* = 11.8 Hz, 5.4 Hz, 1.4 Hz, 1H), 1.79 – 1.65 (m, 1H), 1.48 (s, 3H), 1.46 (s, 10H), 1.37 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.5, 134.1, 127.7, 98.3, 81.5, 67.6, 66.8, 65.5, 59.5, 31.1, 29.9, 28.0, 19.1; LRMS-ESI (m/z): 438.4 $(m + Na)^+$.

(2*S*,3*S*)-*t*-Butyl 3-((*S*)-2,2-dimethyl-1,3-dioxan-4-yl)-2-hydroxypent-4-enoate (9)

A solution of LiHMDS (84.0 mL 1.0 M in THF, 84.0 mmol) was added to a cold solution (−60 °C) of **7** (12.0 g, 42.0 mmol) in THF (250 mL) (LiHMDS was added at a rate that did

not exceed −50 °C). The reaction mixture was allowed to warm slowly to −20 °C over 2 h. The reaction was quenched with saturated ammonium chloride (10 mL) extracted with ethyl acetate (3×20 mL) after warming to room temperature. The organic layers were combined washed with brine, dry over anhydrous $MgSO_4$ and reduce under vacuum. The residue was purified with a 5 – 10 % gradient of ethyl acetate/hexanes. The desired **9** (9.0 g, 77% yield, 8.5:1 mixture of diastereomers) was obtained as a colorless oil. R_f = 0.30 (20% ethyl acetate/hexanes). $[\alpha]^{23}_{D}$ = + 5.8 (*c* 1.02, $CHCl_3$); ^{1}H NMR (300 MHz, Chloroform-d) δ 5.86 – 5.74 (m, 1H), 5.26 – 5.01 (dd, *J* = 12.1 Hz, 2H), 4.23 (t, *J* = 4.23 Hz, 1H), 4.12 (m, 1H), 3.99 (td, *J* = 11.9 Hz, 2.8 Hz, 1H), 3.86 (dd, *J* = 11.1 Hz, 4.9 Hz, 1H), 3.15 (d, *J* = 4.2 Hz, 1H), 2.48 – 2.45 (m, 1H), 1.90 – 1.64 (m, 1H), 1.45 (d, *J* = 4.7 Hz, 13H), 1.35 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 133.1, 119.3, 98.5, 82.5, 71.0, 69.3, 59.8, 52.2, 29.8, 28.6, 28.1, 19.2; LRMS-ESI (m/z); 309.3 $(M^- + Na)^+$.

Minor isomer 10:

R_f = 0.37 (20% ethyl acetate/hexanes) $[\alpha]^{23}_{D}$ = −17.5 (*c* 1.8, $CHCl_3$) ^{1}H NMR (300 MHz, Chloroform-d) δ 5.69 – 5.51 (m, 1H), 5.20 – 5.04 (m, 2H), 4.46 (dd, *J* = 5.1 Hz, 2.2 Hz, 1H), 4.08 – 3.80 (m, 3H), 2.94 (d, *J* = 5.2 Hz, 1H), 2.40 (td, *J* = 9.8 Hz, 2.2 Hz, 1H), 1.53 – 1.34 (m, 17H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.1, 132.1, 119.9, 98.6, 82.4, 69.0, 67.0, 60.1, 53.9, 30.0, 29.9 28.1, 19.2; LRMS-ESI (m/z): 309.3 $(M^- + Na)^+$.

(*R*)-3-((*S*)-2,2-dimethyl-1,3-dioxan-4-yl)pent-4-en-1-ol (11)

To a cold (0 °C) THF (25 mL) solution of **9** (0.98 g, 3.42 mmol) was added triethyl amine (0.57 mL, 4.10 mmol) followed by MsCl (0.32 mL, 4.1 mmol). The reaction was stirred for 2 h at 0 °C. The reaction was quenched with saturated ammonium chloride (10 mL) then extracted with ethyl acetate (3×15 mL). The organic layers were combined and dried over anhydrous $MgSO_4$. The solvent was concentrated under vacuum and the crude mixture was purified using 10% ethyl acetate/hexanes to obtain the desired mesylated compound (1.2 g, 99 % yield) as a colorless oil. R_f = 0.37 (20% ethyl acetate/hexanes). ^{1}H NMR (400 MHz, Chloroform-d) δ 5.77 (dt, *J* = 17.2 Hz, 9.9 Hz, 1H), 5.20 (dd, J = 21.0 Hz, 12.9 Hz, 2H), 5.06 (d, *J* = 4.1 Hz, 1H), 4.07 – 4.01 (m, 1H), 4.01 – 3.92 (m, 1H), 3.89 – 3.81 (m, 1H), 3.15 (s, 3H), 2.66 – 2.61 (m, 1H), 1.74 (qd, *J* = 12.3 Hz, 5.5 Hz, 1H), 1.48 (s, 10H), 1.43 (s, 3H), 1.36 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 132.0, 120.4, 98.6, 83.4, 78.0, 68.1, 59.7, 51.0, 39.4, 29.7, 28.2, 28.0, 19.0.

The mesylated compound above was dissolved (1.23 g, 3.39 mmol) in THF (20 mL). At 0 °C lithium aluminum hydride (0.54 g, 14.2 mmol) was added and the reaction was stirred for 1 h at 0 °C then stirred for 6 h at room temperature. A small aliquot of the reaction was quenched and checked by NMR to determine the reaction's progress. The reaction was cooled to 0 °C and diluted with ethyl acetate. This was followed by the stepwise addition of 3N NaOH (0.5 mL) and H_2O (1 mL). The reaction was stirred until a white precipitate formed. $MgSO_4$ was added to the solution and the white solid was filtered out. The solvent was removed under vacuum and the crude was purified by flash column chromatography (gradient 10% – 20% ethyl acetate/hexanes) to give **11** (0.46 g, 70% yield, 2 steps) as a colorless oil. R_f = 0.45 (50% ethyl acetate/hexanes). $[\alpha]^{23}_{D}$ = −29.2 (*c* 1.4, $CHCl_3$); ^{1}H NMR (400 MHz, Chloroform-d) δ 5.75 (m, 1H), 5.20 – 5.00 (m, 2H), 4.01 – 3.86 (m, 2H),

3.97 – 3.87 (m, 1H), 3.85 – 3.80 (m, 1H), 3.69 – 3.57 (m, 1H), 2.36 – 2.22 (m, 1H), 2.13 (bs, 1H), 1.86 – 1.59 (m, 2H), 1.44 (s, 3H), 1.37 (s, 3H), 1.34 – 1.21 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.1, 117.0, 98.4, 71.4, 60.8, 59.87, 46.0, 33.5, 29.8, 27.5, 19.1; LRMS-CI (m/z): 201.0 (M + H)+.

(3*aS*,4*S*,7*aR*)-hexahydro-2*H*-furo[2,3-*b*]pyran-4-ol (12)

Into a cold a solution of **11** (0.45 g, 2.25 mmol) in CH_2Cl_3/MeOH (20 mL, 4:1) at −78 °C was bubbled a stream of ozone until a blue color persisted. The ozone stream was stopped and a stream of argon was bubbled through the reaction mixture to remove the excess ozone. Dimethyl sulfide (0.50 mL, 6.9 mmol) was added to the reaction and the mixture was warmed to room temperature and stirred an additional 3 h. *p*-TsOH (10.0 mg, 5 mol %) was added and the reaction mixture was stirred for 16 h. The reaction was again carefully concentrated and the residue was purified on silica gel (20% ether/hexanes to 40% ether/ hexanes) to afford compound **12**, (0.22, 70 % yield) as a white solid. R_f = 0.23 (70% ethyl acetate/hexanes). $[\alpha]^{23}_D$ = −34.2 (*c* 1.4, $CHCl_3$); ^{1}H NMR (400 MHz, Chloroform-d) δ 4.97 (d, *J* = 3.4 Hz, 1H), 4.26 – 4.09 (m, 2H), 4.01 – 3.82 (m, 2H), 3.42 – 3.20 (m, 1H), 2.49 (m, 1H), 2.20 (bs, 1H), 2.09 – 1.98 (m, 1H), 1.94 – 1.82 (m, 1H), 1.82 – 1.58 (m, 2H). ^{13}C NMR (100 MHz**,** $CDCl_3$) δ 101.3, 68.4, 67.3, 61.1, 46.3, 29.3, 21.8

(2*S*,3*R*)-*t*-Butyl 3-((*S*)-2,2-dimethyl-1,3-dioxan-4-yl)-2-methoxypent-4-enoate (13)

To a cold (0 °C) solution of **11** (0.18 g, 0.63 mmol) and methyl iodide (50 μL, 0.75 mmol) in THF (5 mL) was added sodium hydride (20 mg, 0.8 mmol). The reaction was allowed to stir for 2 h at 23 °C then quenched with saturated ammonium chloride (5 mL). The reaction mixture was extracted with ethyl acetate (3×10 mL). The organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. The solvent was reduced under vacuum and the residue was purified on silica gel to obtain **13** (0.17 g, 90% yield) as a colorless oil. R_f = 0.54 (20% ethyl acetate/hexanes. ^{1}H NMR (400 MHz, Chloroform-d) δ 5.78 (dt *J* = 17.2 Hz, 9.5 Hz, 1H), 5.21 – 5.00 (m, 2H), 4.03 – 3.88 (m, 2H), 3.84 (ddd, *J* = 11.7 Hz, 5.5 Hz, 1.7 Hz, 1H), 3.79 (d, *J* = 4.7 Hz, 1H), 3.36 (s, 3H), 2.45 (dt, *J* = 10.1 Hz, 5.3 Hz, 1H), 1.82 – 1.69 (m, 1H), 1.47 (m, 9H), 1.42 (s, 3H),1.35 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 134.0, 118.8, 98.4, 81.5, 80.4, 68.5, 59.9, 58.2, 52.0, 29.8, 28.5, 28.2, 19.13.

(2*R*,3*R*)-1-*t*-Butoxy-3-((*S*)-2,2-dimethyl-1,3-dioxan-4-yl)-1-oxopent-4-en-2-yl4-nitrobenzoate (15)

Into a cold (0 °C) solution of **9** (0.15 g, 0.52 mmol) in THF (10 mL) was added triphenylphosphine (0.55 g, 2.1 mmol) p-nitrophenylbenzoic acid (0.35 g, 2.1 mmol) and diethyl azodicarboxylate (0.95 μL, 2.1 mmol). The reaction was allowed to stir 36 h. The reaction was diluted with ethyl acetate (10 mL) and quenched with a saturated solution of sodium bicarbonate (10 mL). The reaction was extracted with ethyl acetate (3×15 ml). The organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. The solvent was concentrated under vacuum and the crude mixture was purified on silica gel using 5% ethyl acetate/hexanes to obtain **15** (0.21 g, 91%) as a pale yellow solid. R_f = 0.36 (10% ethyl acetate/hexanes). $[\alpha]^{23}_D$ = −0.27 (*c* 1.1, $CHCl_3$). ^{1}H NMR (300 MHz,

Chloroform-d) δ 8.36 – 8.21 (m, 4H), 5.86 (dt, *J* = 17.2 Hz, 10.0 Hz, 1H), 5.32 – 5.09 (m, 2H), 4.24 (dt, *J* = 11.8 Hz, 2.7 Hz, 1H), 3.97 (td, *J* = 12.0 Hz, 2.8 Hz, 1H), 3.89 – 3.73 (m, 1H), 2.65 (td, *J* = 9.5 Hz, 2.9 Hz, 1H), 1.93 – 1.74 (m, 1H), 1.44 (s, 9H), 1.34 (s, 3H), 1.27 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.3, 163.8, 150.68, 134.9, 132.3, 130.8, 123.6, 120.4, 98.4, 82.8, 73.6, 66.6, 59.7, 50.9, 29.6, 28.4, 28.0, 18.8; LRMS-ESI (m/z); 481.5 (M + Na)$^+$.

(2*R*,3*R*)-*t*-Butyl 3-((*S*)-2,2-dimethyl-1,3-dioxan-4-yl)-2-methoxypent-4-enoate (16)

To a cold (0 °C) solution of **15** (0.42 g, 0.96 mmol) in methanol was added potassium carbonate (0.16 g, 1.16 mmol). The reaction was allowed to stir for 0.5 h. The reaction was quenched with a saturated ammonium chloride (5 mL) and the methanol was removed under vacuum. The solution was extracted with ethyl acetate (3×10 mL) and the combined organic layer was combined, washed with brine and dried over anhydrous sodium sulfate. The solvent was removed under vacuum and the residue was purified on silica gel using 10% ethyl acetate/hexanes to obtain the free secondary alcohol (0.28 mg, 99% yield) as an amorphous solid. R_f = 0.38 (30% ethyl acetate/hexanes). $[\alpha]^{23}_D$ = −21.6 (*c* 1.1, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 5.92 (dt, *J* = 17.2 Hz, 10.0 Hz, 1H), 5.29 – 5.03 (m, 2H), 4.22 (dt, *J* = 11.8 Hz, 2.8 Hz, 1H), 4.11 (dd, *J* = 8.9 Hz, 6.2 Hz, 1H), 3.95 (td, *J* = 12.1 Hz, 2.8 Hz, 1H), 3.80 (ddd, *J* = 11.7, 5.4, 1.6 Hz, 1H), 3.28 (d, *J* = 9.1 Hz, 1H), 2.32 – 2.27 (m, 1H), 1.85 – 1.74 (m, 1H), 1.46 (s, 9H), 1.44 (s, 3H), 1.35 (s, 3H), 1.19 (d, *J* = 13.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 134.1, 118.8, 98.5, 82.1, 73.0, 68.6, 59.7, 52.5, 29.7, 28.4, 28.1, 18.9

To a cold (0 °C) solution of (2*R*,3*S*)-*t*-Butyl 3-((*S*)-2,2-dimethyl-1,3-dioxan-4-yl)-2-hydroxypent-4-enoate (0.12 g, 0.40 mmol) in THF (5 mL) was added sodium hydride (20.0 mg, 0.8 mmol) followed by methyl iodide (50 μL, 0.8 mmol). The reaction was allowed to stir for 2 h at 23 °C then quenched with saturated ammonium chloride (5 mL). The reaction mixture was extracted with ethyl acetate (3×10 mL). The organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. The solvent was reduced under vacuum and the residue was purified on silica gel to obtain **16** (0.12 g, 96% yield) as a colorless oil. R_f = 0.54 (20% ethyl acetate/hexanes. ^{1}H NMR (300 MHz, Chloroform-d) δ 5.74 (dt, *J* = 17.2 Hz, 10.1 Hz, 1H), 5.22 – 4.93 (m, 2H), 4.29 (d, *J* = 11.9 Hz, 1H), 3.95 (td, *J* = 12.0 Hz, 2.8 Hz, 1H), 3.83 – 3.73 (m, 2H), 3.35 (s, 3H), 2.26 (td, *J* = 9.9 Hz, 2.2 Hz, 1H), 1.79 – 1.71 (m, 1H), 1.43 (s, 9H), 1.41 (s, 3H), 1.35 (s, 3H), 1.19 – 1.07 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3, 132.9, 119.8, 98.2, 81.5, 80.5, 65.9, 60.0, 58.1, 52.3, 29.8, 28.4, 28.1, 19.1

(2*R*,3*R*)-*t*-Butyl 2-(benzyloxy)-3-((S)-2,2-dimethyl-1,3-dioxan-4-yl)pent-4-enoate (18)

Compound **18** was prepared by following the procedure outlined for o-alkylation outlined above. Compound **18** was obtained in 88% yield. R_f = 0.70 (20% ethyl acetate/hexanes). ^{1}H NMR (300 MHz, Chloroform-d) δ 7.43 – 7.27 (m, 5H), 5.76 (dt, *J* = 17.2 Hz, 10.1 Hz, 1H), 5.24 – 4.94 (m, 2H), 4.58 (d, *J* = 11.3 Hz, 1H), 4.48 – 4.28 (m, 2H), 3.99 – 3.89 (m, 2H), 3.86 – 3.70 (m, 1H), 2.37 (td, *J* = 10.0 Hz, 2.1 Hz, 1H), 1.82 −1.68 (m, 1H), 1.44 (s, 9H), 1.37 (s, 3H), 1.33 (s, 3H) 1.15 (d, *J* = 12.9, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2,

137.4, 132.8, 128.3, 127.9, 119.9, 98.2, 81.4, 78.4, 72.5, 65.8, 59.9, 52.4, 29.8, 28.3, 28.1, 19.1.

(3*S*,3*aS*,4*S*,7*aS*)-3-Methoxyhexahydro-2*H*-furo[2,3-*b*]pyran-4-ol (14)

To a cold (0 °C) solution of **13** (0.14 mg, 0.47 mmol) in THF (5 mL) was added lithium aluminum hydride (41 mg, 1.03 mmol). The reaction was allowed to stir for 1 h at 23 °C after which the reaction was cooled to 0 °C and quenched by adding excess ethyl acetate, 1 N NaOH (0.5 mL), H_2O (0.5 mL). After a white precipitate formed magnesium sulfate was added and stirred for 15 min. The reaction mixture was filtered and concentrated under vacuum to provide the corresponding diol. ^{1}H NMR (400 MHz, Chloroform-d) δ 5.87 (dt, *J* = 17.3 Hz, 9.6 Hz, 1H), 5.19 (dd, *J* = 10.3 Hz, 2.0 Hz, 1H), 5.10 (ddd, *J* = 17.2 Hz, 2.0, 0.6 Hz, 1H), 4.13 (dt, *J* = 11.9 Hz, 2.6 Hz, 1H), 3.95 (td, *J* = 12.1 Hz, 2.8 Hz, 1H), 3.79 (ddd, *J* = 11.7 Hz, 5.4 Hz, 1.6 Hz, 1H), 3.74 – 3.55 (m, 2H), 3.38 (s, 3H), 3.31 – 3.28 (m, 1H), 2.71 (bs, 1H), 2.37 – 2.32 (m, 1H), 1.80 (qd, *J* = 12.5 Hz, 5.5 Hz, 1H), 1.44 (s, 3H), 1.34 (s, 3H), 1.22 (m, *J* = 13.2 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.8, 118.8, 98.3, 82.7, 67.0, 61.0, 59.9, 57.7, 50.4, 29.7, 28.6, 19.0

The crude diol. mixture was taken up in $CHCl_3$/MeOH (4:1) and a stream of O_3 was bubble through the solution at −78 °C until a blue color persisted. Argon was bubbled through the blue solution until the solution became clear. Dimethyl sulfide (0.13 mL, 5.0 eq) was added to the reaction and the mixture was warmed to room temperature and stirred an additional 3 h. To the reaction mixture was added *p*-TsOH (10 mol %) the mixture was stirred for 18 h at room temperature. The reaction was carefully concentrated and the residue was purified on silica gel (20% ether/hexanes to 50% ether/hexanes) to afford **14**, (24 mg, 30 % yield 2 steps) as a colorless oil. R_f = 0.20 (60% ethyl acetate/hexanes). $[\alpha]^{23}_D$ = −111.7 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, Chloroform-d) δ 4.99 (d, *J* = 4.1 Hz, 1H), 4.24 – 4.17 (m, 1H), 4.17 – 4.14 (m, 2H), 4.14 – 4.02 (m, 1H), 4.01 – 3.96 (m, 1H), 3.43 – 3.37 (d, *J* = 13.6 Hz, 1H), 3.32 (s, 3H), 2.74 (d, *J* = 10.1 Hz, 1H), 2.53 – 2.48 (m, 1H), 2.00 – 1.85 (m, 1H), 1.80 – 1.74 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 101.5, 80.7, 73.3, 67.3, 60.7, 57.7, 47.5, 33.0.

(3*R*,3*aS*,4*S*,7*aS*)-3-Methoxyhexahydro-2*H*-furo[2,3-*b*]pyran-4-ol (19)

Methyl ether derivative **19** was obtained by following the two-step procedures outlined above for the formation of compound **14**. Methyl ether **19** was obtained in 45 % yield. $\mathbf{R_f}$ = 0.20 (70% ethyl acetate/hexanes). ^{1}H NMR (300 MHz, Chloroform-d) δ 5.06 (d, *J* = 3.8 Hz, 1H), 4.38 – 4.22 (m, 2H), 4.21 – 4.13 (m, 1H), 3.94 – 3.86 (m, 3H), 3.33 (s, 3H), 2.53 – 2.47 (m, 2H), 1.92 – 1.76 (m, 1H), 1.76 – 1.54 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 101.4, 78.9, 72.2, 66.3, 60.5, 57.9, 51.1, 30.2.

(3*R*,3*aS*,4*S*,7*aS*)-3-Ethoxyhexahydro-2*H*-furo[2,3-*b*]pyran-4-ol (20)

Ethyl ether derivative **20** was prepared by following the two-step procedures described for compound **19**. Ethyl ether **20** was obtained in 60 % yield. R_f = 0.20 (50% ethyl acetate/ hexanes). ^{1}H NMR (400 MHz, Chloroform-d) δ 5.05 (d, *J* = 3.8 Hz, 1H), 4.45 – 4.26 (m, 2H), 4.26 – 4.07 (m, 1H), 4.01 – 3.87 (m, 1H), 3.85 (dd, *J* = 8.6 Hz, 4.7 Hz, 1H), 3.59 – 3.41 (m, 2H), 3.32 (td, *J* = 11.9 Hz, 2.3 Hz, 1H), 2.64 – 2.41 (m, 2H), 1.91 – 1.78 (m, 1H), 1.78 –

1.64 (m, 1H), 1.19 (t, *J* = 7.0 Hz, 3H**).** ^{13}C NMR (100 MHz, $CDCl_3$) δ 101.3, 77.1, 72.6, 66.6, 66.0, 60.7, 50.8, 30.2, 15.5.

(3*R*,3*aS*,4*S*,7*aS*)-3-(Benzyloxy)hexahydro-2*H*-furo[2,3-*b*]pyran-4-ol (21)

Benzyl ether derivative **21** was prepared by following the two-step procedures as described for compound **19**. Benzyl ether **21** was obtained in 88 % yield. $[\alpha]^{23}_D$ = +45 (*c* 1.1, $CHCl_3$); R_f = 0.22 (60% ethyl acetate/hexanes). ^{1}H NMR (300 MHz, Chloroform-d) δ 7.47 – 7.18 (m, 5H), 5.05 (d, *J* = 3.7 Hz, 1H), 4.51 (d, *J* = 1.6 Hz, 2H), 4.49 – 4.36 (m, 1H), 4.26 (dd, *J* = 9.0 Hz, 6.8 Hz, 1H), 4.21 – 4.06 (m, 1H), 3.93 – 3.85 (m, 2H), 3.31 (td, *J* = 11.8 Hz, 2.4 Hz, 1H), 2.66 – 2.54 (m, 1H), 2.49 (bs, 1H), 1.93 – 1.75 (m, 1H), 1.75 – 1.47 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.4, 128.5, 128.0, 128.0, 101.3, 72.7, 72.5, 66.4, 60.6, 51.2, 30.1.

(2*S*,3*S*)-3-((*S*)-2,2-Dimethyl-1,3-dioxan-4-yl)-1-(trityloxy)pent-4-en-2-ol (22)

To a cold (0 °C) solution of **9** (1.50 g, 5.30 mmol) in THF (30 mL) was added lithium aluminum hydride (0.45 g, 11.7 mmol). The reaction was allowed to stir for 1 h at 23 °C after which the reaction was cooled to 0 °C and quenched by adding excess ethyl acetate, 1 N NaOH (0.5 mL), H_2O (0.5 mL). After a white precipitate formed magnesium sulfate was added and stirred for 15 min. The reaction mixture was filtered and concentrated under vacuum.

The crude 1,2-diol was dissolved in CH_2Cl_2 (20.0 mL). To that mixture was added triethyl amine (1.6 mL, 11.1 mmol) and triphenylmethyl chloride (1.6 g, 5.83 mmol). The reaction was allowed to stirr for 24 h. Upon completion the reaction was concentrated under vacuum and purified on silica gel to obtain the **22**. (2.20 g, 92% yield) as a white solid. R_f = 0.30 (20% ethyl acetate/hexanes). $[\alpha]^{23}_D$ = −1.86 (*c* 1.5, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.48 – 7.39 (m, 6H), 7.35 – 7.18 (m, 10H), 5.85 (dt, *J* = 17.4 Hz, 10.1 Hz, 1H), 5.16 (dd, *J* = 10.3 Hz, 2.1 Hz, 1H), 4.99 (dd, *J* = 17.3 Hz, 2.1 Hz, 1H), 4.17 – 3.98 (m, 2H), 3.93 (td, *J* = 12.1 Hz, 2.7 Hz, 1H), 3.79 (dd, *J* = 11.2 Hz, 4.8 Hz, 1H), 3.21 – 3.06 (m, 2H), 3.04 (d, *J* = 1.1 Hz, 1H), 2.30 (dt, *J* = 9.8 Hz, 3.3 Hz, 1H), 1.80 (dd, *J* = 12.5 Hz, 5.3 Hz, 1H), 1.30 (d, *J* = 5.5 Hz, 6H), 1.29 – 1.15 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.0, 128.7, 127.8, 127.0, 119.8, 98.2, 86.5, 73.0, 71.2, 64.9, 59.8, 50.9, 29.8, 28.7, 19.0; LRMS-ESI (m/z); 481.5 $(M + Na)^+$.

(3*R*,3*aS*,4*S*,7*aS*)-3-Azidohexahydro-2*H*-furo[2,3-*b*]pyran-4-ol (23)

To a solution of **22** (2.2 g, 4.80 mmol) in THF (50.0 mL) at 0 °C was added triphenyl phosphine (5.0 g, 19.2 mmol), diethylazodicarboxylate (3.3 g, 19.2 mmol), Diphenylphosphoryl azide (2.5 g, 9.6 mmol) sequentially. The reaction mixture was stirred at room temperature for 24 h after which it was concentrated under vacuum and purified on silica gel to get the desired azide (1.6 g, 70 % yield). R_f = 0.35 (10% ethyl acetate/hexanes). $[\alpha]^{23}_D$ = + 16.3 (*c* 1.6, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.56 – 7.39 (m, 6H), 7.38 – 7.13 (m, 10H), 5.48 (dt, *J* = 17.3 Hz, 10.1 Hz, 1H), 4.96 (dd, *J* = 10.3 Hz, 1.9 Hz, 1H), 4.84 (dd, *J* = 17.3 Hz, 1.8 Hz, 1H), 4.30 (dt, *J* = 11.9 Hz, 2.4 Hz, 1H), 3.98 (td, *J* = 12.1 Hz, 2.7 Hz, 1H), 3.88 – 3.71 (m, 1H), 3.69 – 3.64 (m, 1H), 3.38 (dd, *J* = 10.1 Hz, 2.6 Hz, 1H), 3.07 (dd, *J* = 10.0 Hz, 7.9 Hz, 1H), 1.98 (td, *J* = 10.2 Hz, 2.2 Hz, 1H), 1.81 – 1.59 (m,

1H), 1.49 (s, 3H), 1.35 (s, 3H), 1.20 – 1.03 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.8, 133.6, 128.6, 127.8, 127.0, 119.6, 98.4, 87.0, 67.2, 65.4, 62.3, 59.9, 50.5, 29.7, 28.4, 18.9.

(*S*)-4-((3*S*,4*R*)-4-azido-5-(trityloxy)pent-1-en-3-yl)-2,2-dimethyl-1,3-dioxane (.08 g, 0.17 mmol) was taken up in CH_2Cl_3/MeOH (20 ml, 4:1) and a stream of O_3 was bubble through the solution at −78 °C until a blue color persisted. Argon was bubbled through the blue solution until the solution became clear. Dimethyl sulfide (0.5 mL) was added to the reaction and the mixture was warmed to room temperature and stirred an additional 3 h. To the reaction mixture was added p-TsOH (10 mol %) the mixture was stirred for 18 h at room temperature. The reaction was carefully concentrated and the residue was purified on silica gel (20% ether/hexanes to 50% ether/hexanes) to afford **23**, (23 mg, 74 % yield) as a white solid. R_f = 0.33 (50% ethyl acetate/hexanes). $[\alpha]^{23}_D$ = −20.4 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 5.07 (d, *J* = 3.7 Hz, 1H), 4.42 – 4.27 (m, 2H), 4.21 (dt, *J* = 10.9 Hz, 5.7 Hz, 1H), 3.90 (ddd, *J* = 12.2 Hz, 4.3 Hz, 2.3 Hz, 1H), 3.83 – 3.71 (m, 1H), 3.32 (td, *J* = 12.0 Hz, 2.0 Hz, 1H), 2.63 – 2.45 (m, 2H), 1.79 (ddd, *J* = 13.2 Hz, 3.8 Hz, 1.6 Hz, 1H), 1.69 (td, *J* = 11.6 Hz, 4.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 101.7, 72.3, 65.5, 60.8, 59.0, 52.01, 29.8; LRMS-CI (m/z); 186.0 (M + H)+.

General Procedure for the Synthesis of N-alkylated Ligands

(3*R*,3*aS*,4*S*,7*aS*)-3-azidohexahydro-2*H*-furo[2,3-*b*]pyran-4-ol was dissolved in a solution methanol and placed under argon. 10 % Palladium on carbon (10 mol%) was added and the mixture was stirred under a hydrogen balloon for 1 h. Upon completion the reaction was filtered through a plug of silica. The solvent was removed under vacuum and the product was used without further purification.

The amino alcohol obtained from the previous step was dissolved in methanol and treated with benzaldehyde (1.0 eq) and sodium triacetoxyborohydride (1.2 eq). The reaction was allowed to stir for 18 h (or until the starting material was consumed). The desired aldehyde (1.5 eq) was added followed by additional sodium triacetoxyborohydride (1.5 eq) and the reaction was allowed to continue for an additional 12 h to give the desired dialkylated amino-Tp-THF ligand.

(3*R*,3*aS*,4*S*,7*aS*)-3-(Benzyl(methyl)amino)hexahydro-2*H*-furo[2,3-*b*]pyran-4-ol (24)

Tertiary amine **24** was prepared following the general procedure outlined above. Amine **24** was obtained in 81 % yield. R_f = 0.61 (10% MeOH/DCM). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.46 – 7.10 (m, 5H), 4.93 (d, *J* = 3.7 Hz, 1H), 4.19 (q, *J* = 11.4 Hz, 1H), 4.07 – 3.94 (m, 3H), 3.84 (ddd, *J* = 12.3 Hz, 4.5 Hz, 1.9 Hz, 1H), 3.76 (d, *J* = 12.9 Hz, 1H), 3.54 (d, *J* = 12.9 Hz, 1H), 3.25 (td, *J* = 12.5 Hz, 1.8 Hz, 1H), 2.72 – 2.65 (m, 1H) 2.32 (s, 3H), 1.81 (dd, *J* = 13.4 Hz, 5.6 Hz, 1H), 1.68 – 1.52 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 128.9, 128.6, 127.7, 100.6, 67.9, 62.8, 61.3, 60.3, 60.2, 43.5, 36.0, 30.5; LRMS-ESI (m/z): 286.3 $(M + Na)^+$.

(3*R*,3*aS*,4*S*,7*aS*)-3-(Benzyl(ethyl)amino)hexahydro-2*H*-furo[2,3-*b*]pyran-4-ol (25)

Tertiary amine **25** was obtained following the general procedure outlined above. Amine **25** was obtained in 82 % yield. R_f = 0.34 (5% MeOH/DCM). $[\alpha]^{23}_D$ = +74.6 (*c* 1.23,

$CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.48 – 7.12 (m, 5H), 4.91 (d, *J* = 3.6 Hz, 1H), 4.16 (q, *J* = 11.3 Hz, 1H), 4.09 – 4.04 (m, 2H), 4.03 – 3.86 (m, 2H), 3.85 – 3.75 (m, 1H), 3.33 (d, *J* = 13.3 Hz, 1H), 3.23 (td, *J* = 12.4 Hz, 1.6 Hz, 1H), 2.80 (dq, *J* = 14.7 Hz, 7.4 Hz, 1H), 2.66 – 2.63 (m, 1H), 2.43 (dq, *J* = 13.6 Hz, 6.9 Hz, 1H), 1.76 (dd, *J* = 13.3 Hz, 5.6 Hz, 1H), 1.57 – 1.42 (m, 1H), 1.12 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.7, 128.9, 128.6, 127.5, 100.4, 67.9, 64.1, 61.2, 57.9, 54.3, 44.1, 43.9, 30.6, 13.0

(3*R*,3*aS*,4*S*,7*aS*)-3-(Benzyl(propyl)amino)hexahydro-2*H*-furo[2,3-*b*]pyran-4-ol (26)

Tertiary amine **26** was obtained following the general procedure outlined above. Amine **26** was obtained in 45 % yield. R_f = 0.48 (5% MeOH/DCM). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.40 – 7.22 (m, 5H), 4.91 (d, *J* = 3.6 Hz, 1H), 4.16 (q, *J* = 11.5 Hz, 1H), 4.08 – 4.01 (m, 2H), 3.98 (d, *J* = 13.3 Hz, 1H), 3.94 – 3.87 (m, 1H), 3.81 (ddd, *J* = 12.2 Hz, 4.4 Hz, 1.8 Hz, 1H), 3.33 (d, *J* = 13.3 Hz, 1H), 3.23 (td, *J* = 12.5 Hz, 1.8 Hz, 1H), 2.69 – 2.62 (m, 2H), 2.41 – 2.29 (m, 1H), 1.75 (dd, *J* = 13.3 Hz, 5.6 Hz, 1H), 1.70 – 1.56 (m, 1H), 1.56 – 1.38 (m, 2H), 0.87 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.7, 129.0, 128.5, 127.5, 100.5, 67.9, 64.0, 61.2, 58.0, 55.4, 51.8, 43.9, 30.5, 21.0, 11.5; LRMS-ESI (m/z): 314.5 $(M + Na)^+$.

General Procedure for the preparation of activated carbonates from polycyclic P2-ligands

To a solution of the desired Tp-THF alcohol in dry CH_2Cl_2 was added pyridine (2.3 equivalents). The resulting mixture was cooled to 0 °C under argon and 4-nitrophenylchloroformate (2.2 equivalents) was added in one portion. The resulting mixture was stirred at 0 °C until completion. The reaction mixture was evaporated to dryness and the residue was purified by flash column chromatography on silica gel using a gradient of 20–40% ethyl acetate/hexanes to afford the desired mixed carbonate.

(3*S*,3*aR*,4*S*,7*aS*)-3-Methoxyhexahydro-2*H*-furo[2,3-*b*]pyran-4-yl 4-nitrophenyl carbonate (27a)

Following the general procedure outlined above, activated carbonate **27a** was obtained in 81 % yield. R_f = 0.55 (60% ethyl acetate/hexanes). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.29 (d, *J* = 9.2 Hz, 2H), 7.39 (d, *J* = 9.2 Hz, 2H), 5.26 (dt, *J* = 11.2 Hz, 6.3 Hz, 1H), 5.10 (d, *J* = 4.0 Hz, 1H), 4.27 (dd, *J* = 9.7 Hz, 1.6 Hz, 1H), 4.15 – 4.08 (m, 1H), 4.05 (m, 1H), 3.50 – 3.41 (m, 2H), 3.40 (s, 3H), 2.58 – 2.54 (m, 1H), 2.39 (qd, *J* = 11.9 Hz, 4.8 Hz, 1H), 1.95 – 1.86 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.4, 152.0, 145.4, 125.3, 121.7, 101.4, 80.1, 74.7, 74.6, 60.2, 58.5, 45.4, 28.2; LRMS-ESI (m/z): 362.4 $(M + Na)^+$.

(3*R*,3*aR*,4*S*,7*aS*)-3-Methoxyhexahydro-2*H*-furo[2,3-*b*]pyran-4-yl 4-nitrophenyl carbonate (27b)

Following the general procedure outlined above, activated carbonate **27a** was obtained in 90 % yield. R_f = 0.29 (40% ethyl acetate/hexanes).^{1}H NMR (300 MHz, Chloroform-d) δ 8.27 (d, *J* = 9.1 Hz, 2H), 7.38 (d, *J* = 9.3 Hz, 2H), 5.22 – 4.97 (m, 2H), 4.36 (dd, *J* = 9.0 Hz, 6.8 Hz, 1H), 4.27 – 4.22 (m, 1H), 4.07 – 3.84 (m, 2H), 3.51 – 3.34 (m, 1H), 3.34 (s, 3H), 2.96 – 2.91 (m, 1H), 2.06 – 1.79 (m, 2H**).** ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.5, 151.5,

145.4, 125.3, 121.7, 101.4, 79.2, 73.7, 72.6, 60.1, 58.0, 48.2, 26.9; LRMS-ESI (m/z): 362.4 $(M + Na)^+$.

(3*R*,3*aR*,4*S*,7*aS*)-3-Ethoxyhexahydro-2*H*-furo[2,3-*b*]pyran-4-yl 4-nitrophenyl carbonate (27c)

Following the general procedure outlined above, activated carbonate **27c** was obtained in 48 % yield. R_f = 0.17 (30% ethyl acetate/hexanes). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.28 (m, *J* = 9.2 Hz, 2H), 7.40 (m, 2H), 5.24 – 5.15 (m, 1H), 5.14 (d, *J* = 3.8 Hz, 1H), 4.42 – 4.27 (m, 2H), 4.01 (dt, *J* = 12.3 Hz, 3.7 Hz, 1H), 3.97 – 3.87 (m, 1H), 3.50 (q, *J* = 7.0 Hz, 2H), 3.45 – 3.33 (m, 1H), 2.98 – 2.95 (m, 1H), 2.03 – 1.90 (m, 2H), 1.15 (t, *J* = 7.0 Hz, 3H).

(3*R*,3*aR*,4*S*,7*aS*)-3-(Benzyloxy)hexahydro-2*H*-furo[2,3-*b*]pyran-4-yl 4-nitrophenyl carbonate (27d)

Following the general procedure outlined above, activated carbonate **27d** was obtained in 87 % yield. R_f = 0.35 (40% ethyl acetate/hexanes). ^{1}H NMR (300 MHz, Chloroform-d) δ 8.11 (d, *J* = 7.1 Hz, 2H), 7.44 – 7.16 (m, 5H), 7.04 (d, *J* = 7.1 Hz, 2H), 5.27 – 5.07 (m, 2H), 4.59 – 4.42 (m, 3H), 4.33 (dd, *J* = 9.1 Hz, 6.9 Hz, 1H), 4.03 – 3.96 (m, 2H), 3.44 – 3.36 (m, 1H), 3.09 – 3.04 (m, 1H), 2.04 – 1.91 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.2, 151.5, 145.1, 137.4, 128.3, 127.9, 125.0, 121.6, 101.3, 77.3, 73.8, 72.9, 60.2, 48.3, 26.8; LRMS-ESI (m/z): 438.4 $(M + Na)^+$.

(3*R*,3*aS*,4*S*,7*aS*)-3-Azidohexahydro-2*H*-furo[2,3-*b*]pyran-4-yl 4-nitrophenyl carbonate (27e)

Following the general procedure outlined above, activated carbonate **27e** was obtained in 80 % yield. R_f = 0.25 (30% ethyl acetate/hexanes). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.28 (d, *J* = 9.2 Hz, 2H), 7.39 (d, *J* = 9.2 Hz, 2H), 5.29 – 5.22 (m, 1H), 5.15 (d, *J* = 3.6 Hz, 1H), 4.41 (q, *J* = 8.0 Hz, 1H), 4.41 – 4.37 (m, 1H), 4.02 (dd, *J* = 12.5 Hz, 4.6Hz, 1H), 3.87 (dd, *J* = 9.0 Hz, 4.9 Hz, 1H), 3.42 (td, *J* = 12.2 Hz, 2.0 Hz, 1H), 2.88 – 2.79 (m, 1H), 2.15 – 2.01 (m, 1H), 1.95 (td, *J* = 12.1 Hz, 4.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.2, 151.6, 145.5, 125.3, 121.7, 101.7, 73.5, 72.5, 60.4, 59.3, 48.9, 26.6; LRMS-ESI (m/z): 373.2 $(M + Na)^+$.

(3*R*,3*aS*,4*S*,7*aS*)-3-(Benzyl(methyl)amino)hexahydro-2*H*-furo[2,3-*b*]pyran-4-yl 4-nitrophenyl carbonate (27f)

Following the general procedure outlined above, activated carbonate **27f** was obtained in 36 % yield. R_f = 0.4 (40% ethyl acetate/hexanes**).** ^{1}H NMR (400 MHz, Chloroform-d) δ 8.20 (d, *J* = 9.2 Hz, 2H), 7.34 – 7.09 (m, 7H), 5.25 – 5.07 (m, 2H), 4.10 (dd, *J* = 9.4 Hz, 4.4 Hz, 1H), 4.05 – 3.94 (m, 2H), 3.85 (td, *J* = 8.1 Hz, 4.4 Hz, 1H), 3.62 (d, *J* = 13.0 Hz, 1H), 3.50 (d, *J* = 13.1 Hz, 1H), 3.47 – 3.37 (m, 1H), 3.11 – 3.06 (m, 1H), 2.21 (s, 3H),1.95 (dt, *J* = 8.6, 4.5 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.4, 151.7, 145.3, 138.4, 129.2, 128.2, 127.2, 125.2, 121.8, 101.3, 74.6, 64.9, 62.0, 60.3, 59.6, 43.9, 36.4, 26.7.

(3*R*,3*aS*,4*S*,7*aS*)-3-(Benzyl(ethyl)amino)hexahydro-2*H*-furo[2,3-*b*]pyran-4-yl 4-nitrophenyl carbonate (27g)

Following the general procedure outlined above, activated carbonate **27g** was obtained in 77 % yield. R_f = 0.6 (30% ethyl acetate/hexanes). ^{1}H NMR (400 MHz, Chloroform-d) δ

8.23 (d, *J* = 9.2 Hz, 2H), 7.32 – 7.14 (m, 7H), 5.24 −5.19 (m, 1H), 5.17 (d, *J* = 3.9 Hz, 1H), 4.08 – 3.98 (m, 2H), 3.97 (t, *J* = 3.6 Hz, 1H), 3.87 (d, *J* = 4.5 Hz, 1H), 3.75 (d, *J* = 13.7 Hz, 1H), 3.49 – 3.36 (m, 2H), 3.03 – 2.98 (m, 1H), 2.62 (p, *J* = 7.2 Hz, 1H), 2.52 – 2.39 (p, *J* = 6.8 Hz, 1H), 1.98 – 1.85 (m, 2H), 1.01 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.4, 151.6, 145.3, 139.1, 131.0, 129.1, 128.1, 127.0, 125.2, 121.9, 101.1, 74.6, 66.1, 60.3, 59.1, 53.1, 45.8, 44.5, 26.9, 12.5.

(3*R*,3a*S*,4*S*,7a*S*)-3-(Benzyl(propyl)amino)hexahydro-2*H*-furo[2,3-*b*]pyran-4-yl 4-nitrophenyl carbonate (27h)

Following the general procedure outlined above, activated carbonate **27h** was obtained in 32 % yield. R_f = 0.6 (40% ethyl acetate/hexanes. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.23 (d, *J* = 9.2 Hz, 2H), 7.32 – 7.13 (m, 7H), 5.23 – 5.18 (m, 1H), 5.17 (d, *J* = 3.9 Hz, 1H), 4.03 (d, *J* = 5.6 Hz, 2H), 4.01 – 3.95, (m, 1H), 3.85 (m, 1H), 3.76 (d, *J* = 13.8 Hz, 1H), 3.48 (d, *J* = 13.8 Hz, 1H), 3.42 (td, *J* = 12.2 Hz, 11.8 Hz, 3.3 Hz, 1H), 2.98 – 2.93 (m, 1H), 2.53 – 2.46 (m, 1H), 2.41 – 2.30 (m, 1H), 1.97 – 1.82 (m, 2H), 1.65 – 1.48 (m, 1H), 1.41 – 1.23 (m, 1H), 0.82 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.5, 151.7, 145.3, 139.2, 129.0, 128.2, 127.0, 125.3, 121.8, 101.3, 74.7, 66.6, 60.4, 59.4, 54.1, 52.6, 45.7, 26.9, 20.6, 11.7.

(3*S*,3a*R*,4*S*,7a*S*)-3-Methoxyhexahydro-2*H*-furo[2,3-*b*]pyran-4-yl (2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl carbamate (30a)

To a stirred solution of amine **28** in CH_3CN at cooled to 0 °C, DIPEA (5 equivalent) followed by activated ligand **27a**. The resulting solution was stirred at 23 °C until the reaction was complete. The solution was evaporated to dryness and the crude residue purified by flash column chromatography on silica gel to yield the desired inhibitor. The titled inhibitor was synthesized using the general procedure outlined above. The desired inhibitor was obtained as a white solid (15 % yield). R_f = 0.25 (60% ethyl acetate/ hexanes). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.72 (d, *J* = 8.8 Hz, 2H), 7.35 – 7.16 (m, 5H), 6.98 (d, *J* = 8.9 Hz, 2H), 5.02 – 4.96 (m, 2H), 4.89 (d, *J* = 8.8 Hz, 1H), 4.16 (d, *J* = 10.0 Hz, 1H), 4.03 – 3.92 (m, 3H), 3.92 – 3.84 (s, 4H), 3.78 (bs, 1H), 3.46 – 3.27 (m, 3H), 3.19 (s, 3H), 3.16 – 3.08 (m, 1H), 3.10 – 2.94 (m, 3H), 2.81 (dd, *J* = 13.4 Hz, 6.7 Hz, 2H), 2.31 (d, *J* = 4.4 Hz, 1H), 2.18 (dd, *J* = 12.1 Hz, 4.4 Hz, 1H), 1.90 – 1.80 (m, 1H), 0.93 (d, *J* = 6.6 Hz, 3H), 0.87 (t, *J* = 7.6 Hz, 3H). Mass: HRMS (ESI), Calcd for $C_{30}H_{42}N_2O_9S$: *m/z* 629.2509 (M+Na), found *m/z* 629.2505 (M+Na).

(3*R*,3a*R*,4*S*,7a*S*)-3-Methoxyhexahydro-2*H*-furo[2,3-*b*]pyran-4-yl (2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-ylcarbamate (30b)

The titled inhibitor was synthesized using the general procedure outlined above. Inhibitor **30b** was obtained in 61 % yield. R_f = 0.38 (60% ethyl acetate/hexanes). ^{1}H NMR (300 MHz, Chloroform-d) δ 7.70 (d, *J* = 8.6 Hz, 2H), 7.43 – 7.13 (m, 5H), 6.97 (d, *J* = 8.7 Hz, 2H), 5.12 (d, *J* = 3.7 Hz, 1H), 5.09 – 4.96 (m, 1H), 4.93 (d, *J* = 8.5 Hz, 1H), 4.17 – 4.12 (m, 1H), 3.87 (s, 9H), 3.47 – 3.25 (m, 1H), 3.12 (s, 3H), 2.96 (dd, *J* = 13.9 Hz, 7.4 Hz, 4H), 2.78 (dd, *J* = 13.3 Hz, 6.7 Hz, 1H), 2.55 (d, *J* = 4.5 Hz, 1H), 1.94 – 1.72 (m, 2H), 1.62 (dd, *J* = 16.9 Hz, 7.9 Hz, 1H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.86 (d, *J* = 6.6 Hz, 3H). Mass: HRMS (ESI), Calcd for $C_{30}H_{42}N_2O_9S$: *m/z* 629.2509 (M+Na), found *m/z* 629.2505 (M+Na).

(3*R*,3a*R*,4*S*,7a*S*)-3-Methoxyhexahydro-2*H*-furo[2,3-*b*]pyran-4-yl (2*S*,3*R*)-4-(4-amino-*N*-isobutylphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-ylcarbamate (30c)

The titled inhibitor was synthesized from amine **29** and activated carbonate **27b** using the general procedure outlined above. Inhibitor **30c** was obtained in 24% yield. ^{1}H NMR (300 MHz, Chloroform-d) δ 7.54 (d, *J* = 8.5 Hz, 2H), 7.41 – 7.00 (m, 5H), 6.67 (d, *J* = 8.6 Hz, 2H), 5.13 (d, *J* = 3.8 Hz, 1H), 5.03 (dd, *J* = 10.2 Hz, 5.2 Hz, 1H), 4.96 (d, *J* = 8.2 Hz, 1H), 4.39 – 3.99 (m, 3H), 3.99 – 3.70 (m, 6H), 3.48 – 3.24 (m, 2H), 3.12 (s, 3H), 3.02 – 2.83 (m, 4H), 2.75 (dd, *J* = 13.3 Hz, 6.3 Hz, 1H), 2.54 (d, *J* = 4.7 Hz, 1H), 1.89 – 1.64 (m, 3H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.86 (d, *J* = 6.6 Hz, 3H). Mass: LRMS: *m/z* 592.8 (M+H). Mass: HRMS (ESI), Calcd for $C_{29}H_{41}N_3O_8S$: *m/z* 614.2513 (M+Na), found *m/z* 614.2502 (M+Na).

(3*R*,3a*S*,4*S*,7a*S*)-3-Hydroxyhexahydro-2*H*-furo[2,3-*b*]pyran-4-yl (2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenyl-sulfonamido)-1-phenylbutan-2-ylcarbamate (30f)

For the synthesis of inhibitor **30f**, compound **30e** was treated with $Pd(OH)_2$ (10 mol %) in methanol (2.0 mL) at 60 Psi for 12 h. The reaction was filtered through a plug of Celite and concentrated under vacuum. The crude product was purified by silica gel chromatography to give the desired inhibitor **30f** as an amorphous solid. (38 % yield, 2 steps). R_f = 0.22, (50 % ethyl acetate/hexanes). ^{1}H NMR (300 MHz, Chloroform-d) δ 7.72 (d, *J* = 7.1 Hz, 2H), 7.30 – 7.22 (m, 5H), 6.99 (d, *J* = 8.9 Hz, 2H), 5.17 (s, 1H), 5.04 (d, *J* = 3.7 Hz, 2H), 4.57 (s, 1H), 4.32 (t, *J* = 8.4 Hz, 1H), 3.88 (s, 7H), 3.73 (dd, *J* = 9.2 Hz, 5.0 Hz, 1H), 3.41 – 3.08 (m, 2H), 3.02 – 2.90 (m, 4H), 2.79 (dd, *J* = 13.5 Hz, 6.8 Hz, 1H), 2.47 (s, 1H), 1.93 – 1.52 (m, 3H), 0.88 (dd, *J* = 13.2 Hz, 6.4 Hz, 6H). Mass: HRMS (ESI), Calcd for $C_{29}H_{40}N_2O_9S$: *m/z* 593.2532 (M+H) and 615.2352 (M+Na), found *m/z* 593.2520 (M+H) and 615.2330 (M +Na).

(3*R*,3a*R*,4*S*,7a*S*)-3-Ethoxyhexahydro-2*H*-furo[2,3-*b*]pyran-4-yl (2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenyl-sulfonamido)-1-phenylbutan-2-ylcarbamate (30d)

The titled inhibitor **30d** was synthesized using the general procedure outlined above. Inhibitor **30d** was obtained in 47 % yield. R_f = 0.20 (40% ethyl acetate/hexanes). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.69 (d, *J* = 8.3 Hz, 2H), 7.41 – 7.12 (m, 5H), 6.97 (d, *J* = 8.9 Hz, 2H), 5.14 (s, 1H), 5.04 (bs, 1H), 4.28 – 4.05 (m, 1H), 3.87 (s, 8H), 3.34 (d, *J* = 7.0 Hz, 3H), 3.10 – 2.91 (d, 5H), 2.86 – 2.65 (m, 1H), 2.54 (bs, 1H), 1.75 – 1.63 (m, 3H), 1.14 – 1.09 (m, 3H), 0.98 – 0.85 (m, 6H). Mass: LRMS: *m/z* 621.83 (M+H). Mass: HRMS (ESI), Calcd for $C_{31}H_{44}N_2O_9S$: *m/z* 643.2665 (M+Na), found *m/z* 643.2660 (M+Na).

(3*R*,3a*R*,4*S*,7a*S*)-3-(Benzyloxy)hexahydro-2H-furo[2,3-*b*]pyran-4-yl (2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxy-phenylsulfonamido)-1-phenylbutan-2-ylcarbamate (30e)

The titled inhibitor **30e** was synthesized using the general procedure outlined above. Inhibitor **30e** was obtained in 82 % yield. R_f = 0.6, (50 % ethyl acetate/hexanes).^{1}H NMR (300 MHz, Chloroform-d) δ 7.69 (d, *J* = 8.9 Hz, 2H), 7.39 – 7.10 (m, 10H), 6.96 (d, *J* = 8.9 Hz, 2H), 5.13 (d, *J* = 3.7 Hz, 1H), 5.05 (d, *J* = 4.3 Hz, 1H), 4.82 (d, *J* = 8.4 Hz, 1H), 4.37 (s, 2H), 4.23 – 3.99 (m, 2H), 3.99 – 3.73 (m, 8H), 3.37 (t, *J* = 10.2 Hz, 1H), 3.09 (dd, *J* = 15.1 Hz, 8.4 Hz, 1H), 2.95 – 2.95 (m, 4H), 2.78 (dd, *J* = 13.5 Hz, 6.7 Hz, 1H), 2.64 – 2.59 (m, 1H), 1.79 (d, *J* = 7.4 Hz, 2H), 1.71 – 1.53 (m, 1H), 0.88 (dd, *J* = 14.5 Hz, 7.0 Hz, 6H). Mass:

LRMS: *m/z* 683.9 (M+H). Mass: HRMS (ESI), Calcd for $C_{36}H_{46}N_2O_9S$: *m/z* 705.2822 (M +Na), found *m/z* 705.2816 (M+Na).

(3*R*,3*aS*,4*S*,7*aS*)-3-Azidohexahydro-2*H*-furo[2,3-*b*]pyran-4-yl (2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenyl-sulfonamido)-1-phenylbutan-2-ylcarbamate (30g)

The titled inhibitor **30g** was synthesized using the general procedure outlined above. The desired inhibitor was obtained as an amorphous solid in 75% yield. R_f = 0.23 (40% ethyl acetate/hexanes). ^{1}H NMR (400 MHz, CD_3OD) δ 7.75 (d, *J* = 8.8 Hz, 2H), 7.24 (d, *J* = 4.4 Hz, 4H), 7.19– 7.15 (m, 1H), 7.11 – 7.01 (d, *J* = 8.8 Hz, 2H), 5.09 (d, *J* = 3.7 Hz, 1H), 5.06 – 5.01 (m, 1H), 4.26 – 4.21 (m, 1H), 4.23 – 4.14 (m, 1H), 3.86 (s, 4H), 3.81 (bs, 2H), 3.67 (dd, *J* = 8.9 Hz, 4.2 Hz, 1H), 3.39 (dd, *J* = 10.8 Hz, 4.1 Hz, 2H), 3.12 – 3.00 (m, 3H), 2.90 – 2.85 (m, 1H), 2.72 – 2.60 (m, 1H), 2.48 – 2.41 (m, 1H), 2.04 – 1.97 (m, 1H), 1.83 – 1.62 (m, 2H), 0.97 – 0.80 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0, 155.4, 137.4, 129.7, 129.4, 128.4, 126.6, 114.3, 99.3, 72.4, 72.3, 71.6, 59.4, 58.7, 55.5, 55.3, 55.0, 53.5, 44.3, 35.3, 28.2, 27.2, 20.0, 19.8. Mass: HRMS (ESI), calcd for ($C_{29}H_{39}N_5O_8S$): m/z 618.2598 (M+H), found m/z 618.2597.

(3*R*,3*aS*,4*S*,7*aS*)-3-Aminohexahydro-2*H*-furo[2,3-*b*]pyran-4-yl (2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-ylcarbamate (30h)

Inhibitor **30g** was dissolved in ethyl acetate and 10% Pd/C (10 % by weight) was added. The resulting mixture was stirred under a hydrogen-filled balloon for 1 h. The mixture was filtered through a plug of Celite and concentrated under vacuum. The crude product was purified by silica gel chromatography to obtain inhibitor **30h** as an amorphous solid in 85 % yield. R_f = 0.18 (5% (5% NH_3/MeOH)/dichloromethane). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.71 (d, *J* = 8.8 Hz, 2H), 7.37 – 7.14 (m, 5H), 6.97 (d, *J* = 8.7 Hz, 2H), 5.53 (s, 1H), 5.34 – 5.13 (m, 2H), 4.07 (dd, *J* = 9.8 Hz, 7.0 Hz, 1H), 3.98 – 3.73 (m, 8H), 3.67 (dd, *J* = 9.6 Hz, 4.9 Hz, 1H), 3.36 – 3.27 (m, 1H), 3.17 (dd, *J* = 14.9 Hz, 8.9 Hz, 1H), 3.04 – 2.68 (m, 5H), 2.66 – 2.16 (bs, 2H), 2.10 – 1.95 (m, 1H), 1.82 (dd, *J* = 14.1, 6.9 Hz, 2H), 1.46 – 1.28 (m, 1H), 0.89 (d, *J* = 6.5 Hz, 3H), 0.85 (d, *J* = 6.5 Hz, 3H). Mass: LRMS: *m/z* 592.8 (M+H). Mass: HRMS (ESI), Calcd for $C_{29}H_{41}N_3O_8S$: *m/z* 592.2693 (M+H), found *m/z* 592.2686 (M+H).

(3*R*,3*aS*,4*S*,7*aS*)-3-(Methylamino)hexahydro-2*H*-furo[2,3-*b*]pyran-4-yl (2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl carbamate (30i)

To a stirred solution of amine **28** and activated carbonate **27f** in CH_3CN, DIPEA (5 equivalent) was added and the resulting mixture was stirred for 24 h. Standard work up and purification as described for inhibitor **30a** provided the corresponding carbamate derivative. Above carbamate was dissolved in 5% ammonia in MeOH (2 mL) and 10% $Pd(OH)_2$ was added. The mixture was stirred under a hydrogen-filled balloon for 4 h. The mixture was filtered through a plug of Celite and the filtrate was concentrated under reduced pressure. The residue was chromatographed on a silica gel column to provide inhibitor **30i** in 76% yield for two-steps. R_f = 0.20 (10% methanol/DCM).^{1}H NMR (400 MHz, CD_3OD) δ 7.77 (d, *J* = 8.9 Hz, 2H), 7.32 – 7.21 (m, 4H), 7.20 – 7.17 (m, 1H), 7.07 (d, *J* = 8.7 Hz, 2H), 5.08 (d, *J* = 3.7 Hz, 1H), 5.00 – 4.91 (m, 1H), 4.21 (t, *J* = 8.4 Hz,1H), 3.87 (s, 3H), 3.86 – 3.65

(m, 4H), 3.50 – 3.34 (m, 3H), 3.15 (dd, *J* = 14.0 Hz, 3.6 Hz, 1H), 3.06 (dd, *J* = 13.7 Hz, 8.2 Hz, 1H), 2.95 (dd, *J* = 14.9 Hz, 8.1 Hz, 1H), 2.86 (dd, *J* = 13.6 Hz, 6.9 Hz, 1H), 2.60 (dd, *J* = 14.0 Hz, 10.7 Hz, 1H), 2.38 – 2.26 (m, 2H), 2.24 (s, 3H), 2.10 – 1.92 (m, 1H), 1.85 – 1.74 (m, 1H), 1.75 – 1.58 (m, 1H), 0.92 (d, *J* = 6.6 Hz, 3H), 0.87 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.5, 157.4, 140.2, 132.1, 130.6, 130.3, 129.2, 127.3, 115.4, 103.3, 74.2, 73.8, 70.5, 61.2, 59.0, 58.9, 57.1, 56.2, 53.9, 36.6, 34.5, 28.5, 28.1, 20.5. Mass: HRMS (ESI), Calcd for $C_{30}H_{43}N_3O_8S$: *m/z* 606.2849 (M+H), found *m/z* 606.2840 (M+Na).

(3*R*,3*aS*,4*S*,7*aS*)-3-(Ethylamino)hexahydro-2*H*-furo[2,3-*b*]pyran-4-yl (2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-ylcarbamate (30j)

The titled inhibitor was synthesized using the general procedure outlined above. The desired inhibitor **30j** was obtained as an amorphous solid in 50 % yield for the 2 steps. R_f = 0.25 (10% methanol/DCM). ^{1}H NMR (400 MHz, CD_3OD) δ 7.76 (d, *J* = 8.8 Hz, 2H), 7.36 – 7.13 (m, 5H), 7.13 – 7.02 (d, *J* = 9.2 Hz, 2H), 5.09 (d, *J* = 3.8 Hz, 1H), 5.01 (dq, *J* = 10.7 Hz, 5.3 Hz, 4.7 Hz, 1H), 4.25 – 4.14 (t, *J* = 8.8 Hz, 1H), 3.87 (s, 3H), 3.86 – 3.79 (m, 3H), 3.78 – 3.72 (m, 1H), 3.70 (dd, *J* = 8.7 Hz, 5.1 Hz, 1H), 3.50 (td, *J* = 7.3 Hz, 5.3 Hz, 1H), 3.46 – 3.35 (m, 2H), 3.15 (dd, *J* = 14.0 Hz, 3.7 Hz, 1H), 3.05 (dd, *J* = 13.6 Hz, 8.1 Hz, 1H), 2.96 (dd, *J* = 14.9 Hz, 8.3 Hz, 1H), 2.87 (dd, *J* = 13.6 Hz, 6.9 Hz, 1H), 2.65 – 2.51 (m, 2H), 2.50 – 2.41 (m, 1H), 2.29 (td, *J* = 6.8 Hz, 4.0 Hz, 1H), 2.07 – 1.96 (m, 1H), 1.82 – 1.60 (m, 2H), 1.10 (t, *J* = 7.1 Hz, 3H), 0.91 (d, *J* = 6.6 Hz, 3H), 0.86 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 164.5, 157.4, 140.2, 132.2, 130.6, 130.3, 129.3, 127.3, 115.4, 103.1, 74.3, 74.1, 70.4, 60.9, 58.8, 57.4, 57.2, 56.2, 53.9, 43.5, 36.7, 28.6, 28.1, 20.5, 15.0. Mass: HRMS (ESI), Calcd for $C_{31}H_{45}N_3O_8S$: *m/z* 620.3005 (M+Na), found *m/z* 620.3000 (M+Na).

(3*R*,3*aS*,4*S*,7*aS*)-3-(Ethylamino)hexahydro-2*H*-furo[2,3-*b*]pyran-4-yl (2*S*,3*R*)-4-(4-amino-*N*-isobutylphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-ylcarbamate (30k)

The titled inhibitor **30k** was synthesized following the general procedure outlined above. The desired inhibitor was obtained as an amorphous solid in 61 % yield for the 2 steps. ^{1}H NMR (400 MHz, CD_3OD) δ 7.47 (d, *J* = 8.8 Hz, 2H), 7.32 – 7.10 (m, 5H), 6.68 (d, *J* = 9.1 Hz, 2H), 5.09 (d, *J* = 3.7 Hz, 1H), 5.04 – 4.92 (m, 2H), 4.21 (t, *J* = 8.0 Hz, 1H), 3.95 – 3.79 (m, 2H), 3.90 – 3.79 (m, 2H), 3.53 – 3.48 (m,1H), 3.44 – 3.33 (m, 3H), 3.17 (dd, *J* = 14.0 Hz, 3.7 Hz, 2H), 2.99 (dd, *J* = 13.6 Hz, 8.2 Hz, 1H), 2.89 (dd, *J* = 14.8 Hz, 8.4 Hz, 1H), 2.80 (dd, *J* = 13.5 Hz, 6.8 Hz, 1H), 2.65 – 2.51 (m, 2H), 2.50 – 2.43 (m, 1H), 2.31 – 2.26 (m, 1H), 2.03 – 1.98 (m, 1H), 1.78 – 1.63 (d, 2H), 1.10 (t, *J* = 7.1 Hz, 3H), 0.92 (d, *J* = 6.5 Hz, 3H), 0.89 – 0.80 (d, *J* = 6.5 Hz, 3H). Mass: LRMS: *m/z* 605.8 (M+H). Mass: HRMS (ESI), Calcd for $C_{30}H_{44}N_4O_7S$: *m/z* 605.3009 (M+H), found *m/z* 605.3005 (M+H).

(3*R*,3*aS*,4*S*,7*aS*)-3-(Propylamino)hexahydro-2*H*-furo[2,3-*b*]pyran-4-yl (2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-ylcarbamate (30l)

The titled inhibitor **30l** was synthesized following the general procedure outlined above. Inhibitor **30l** was obtained as an amorphous solid in 60% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.76 (d, *J* = 8.9 Hz, 2H), 7.35 – 7.14 (m, 5H), 7.08 (d, *J* = 8.9 Hz, 2H), 5.10 (d, *J* = 3.8 Hz, 1H), 5.04 – 4.99 (m, 1H), 4.21 (t, *J* = 6.8 Hz, 1H), 3.88 (s, 4H), 3.86 – 3.79 (m, 1H), 3.79 – 3.68 (m, 2H), 3.53 – 3.48 (m, 1H), 3.47 – 3.35 (m, 3H), 3.26 – 3.24 (m, 1H),

3.18 – 3.11 (dd, J = 14.0 Hz, 3.6 Hz, 1H), 3.05 (dd, *J* = 13.4 Hz, 8.4 Hz, 1H), 2.96 (dd, *J* = 14.9 Hz, 8.3 Hz, 1H), 2.86 (dd, *J* = 14.9 Hz, 8.3 Hz, 1H), 2.62 (dd, *J* = 14.0 Hz, 10.7 Hz, 1H), 2.55 – 2.45 (m, 1H), 2.45 – 2.35 (m, 1H), 2.34 – 2.30 (m, 1H), 2.07 – 1.94 (m, 1H), 1.83 – 1.62 (m, 2H), 1.55 – 1.45 (m, 1H), 0.97 – 0.89 (m, 6H), 0.86 (d, *J* = 6.7 Hz, 3H). Mass: LRMS: *m/z* 634. 9 (M+H). Mass: HRMS (ESI), Calcd for $C_{32}H_{47}N_3O_8S$: *m/z* 634.3163 (M+H), found *m/z* 634.3156 (M+H).

(3*R*,3a*S*,4*S*,7a*S*)-3-(Dimethylamino)hexahydro-2*H*-furo[2,3-*b*]pyran-4-yl (2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-ylcarbamate (30m)

Compound **30h** was dissolved in 1,2-dichloroethane (2 mL). Powdered paraformaldehyde (3 equivalent) and sodium triacetoxy borohydride (3 equivalent) were added and the mixture was stirred for 24 h. The crude inhibitor was purified by silca gel chromatography to obtain the desired inhibitor **30m** as an amorphous solid in 67 % yield. R_f = 0.25 (10% methanol/ DCM). ^{1}H NMR (400 MHz, CD_3OD) δ 7.77 (d, *J* = 8.8 Hz, 2H), 7.29 – 7.18 (m, 5H), 7.08 (d, J = 8.8 Hz, 2H), 5.18 (d, *J* = 4.3 Hz, 1H), 5.01 – 4.98 (m, 1H), 4.18 – 4.01 (m, 1H), 3.96 – 3.83 (m, 5H), 3.84 – 3.76 (m, 2H), 3.50 – 3.42 (m, 3H), 3.15 (dd, *J* = 13.8 Hz, 2.5 Hz, 1H), 3.06 (dd, *J* = 13.7 Hz, 8.1 Hz, 1H), 2.96 (dd, *J* = 14.8 Hz, 7.8 Hz, 1H), 2.87 (dd, *J* = 13.6 Hz, 6.9 Hz, 1H), 2.67 – 2.50 (m, 1H), 2.23 (s, 3H), 1.97 (s, 3H), 1.81 – 1.71 (m, 1H), 1.70 – 1.61 (m, 1H), 0.92 (d, *J* = 6.6 Hz, 3H), 0.87 (d, *J* = 6.6 Hz, 3H). Mass: LRMS: *m/z* 620.8 (M+H). Mass: HRMS (ESI), Calcd for $C_{31}H_{45}N_3O_8S$: *m/z* 620.3006 (M+H), found *m/z* 620.2998 (M+H).

Methods: Determination of X-ray structures of HIV-1 protease-inhibitor complexes

The optimized HIV-1 protease was expressed and purified as described.[31] The protease-inhibitor complex was crystallized by the hanging drop vapor diffusion method with well solutions of 1.18 M NaCl, 0.1 M Sodium Acetate buffer (pH 4.6) for the complex with inhibitor **30b** and 1.3M NaCl, 0.1M Sodium Acetate buffer (pH 5.5) for the complex with inhibitor **30j** respectively. X-ray diffraction data were collected on a single crystal cooling to 90 K at SER-CAT (22-BM beamline), Advanced Photon Source, Argonne National Lab (Chicago, USA) with X-ray wavelength of 1.0 Å, and processed by HKL-2000[32] with an Rmerge of 6.8% and 5.7%, respectively, for the **30b** and **30j** complexes. Using the isomorphous structure[33], the crystal structures were solved by PHASER[34] in CCP4i Suite[35, 36] and refined by SHELX-97[37] to 1.22 Å and 1.62 Å resolution, respectively. COOT[38] was used for visual modification of the structures. PRODRG-2[39] was used to construct the inhibitor and the restraints for refinement. Alternative conformations were modeled. Anisotropic atomic displacement parameters (B factors) were applied for all atoms, including solvent molecules in the higher resolution complex of HIV protease with inhibitor **30b**. The final refined solvent structure comprised two sodium ions, three chloride ions, one acetate ion and 186 water molecules for HIV protease with inhibitor **30b** and one sodium ion, three chloride ions and 138 water molecules for HIV protease with inhibitor **30j**. The crystallographic statistics are listed in Table 2 (ESI). The coordinates and structure factors of the HIV-1 protease complexes with inhibitor **30b** and inhibitor **30j** have been deposited in the Protein Data Bank with accession codes 5DGU and 5DGW.

Supplementary Material

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Acknowledgments

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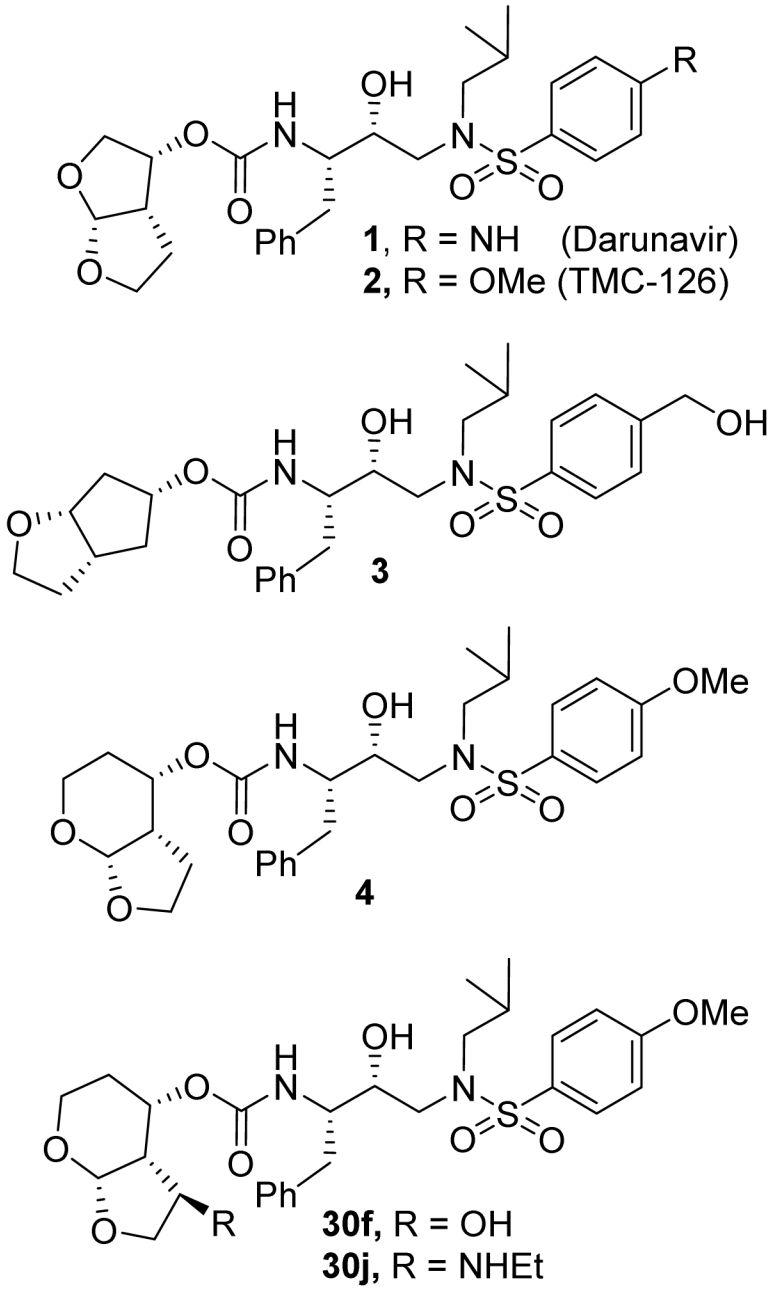


Figure 1.
Structure of PIs **1–4** and **30f,j**

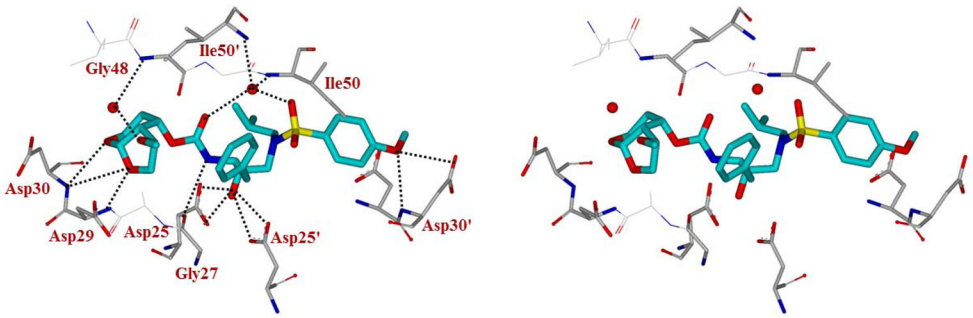


Figure 2.

Stereoview of the X-ray structure of inhibitor **30b** (turquoise color)-bound to the active site of wild-type HIV-1 protease (PDB code: 5DGU). All strong hydrogen bonding interactions are shown as dotted lines.

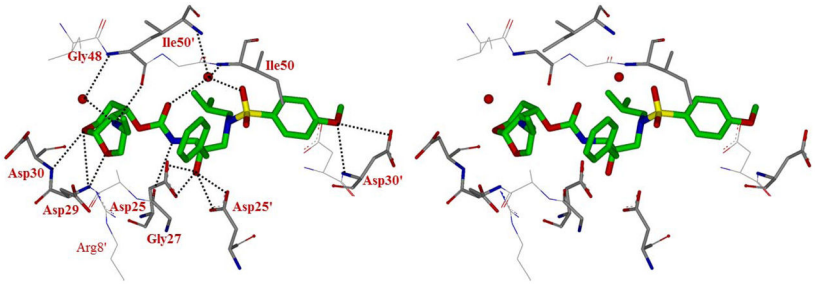
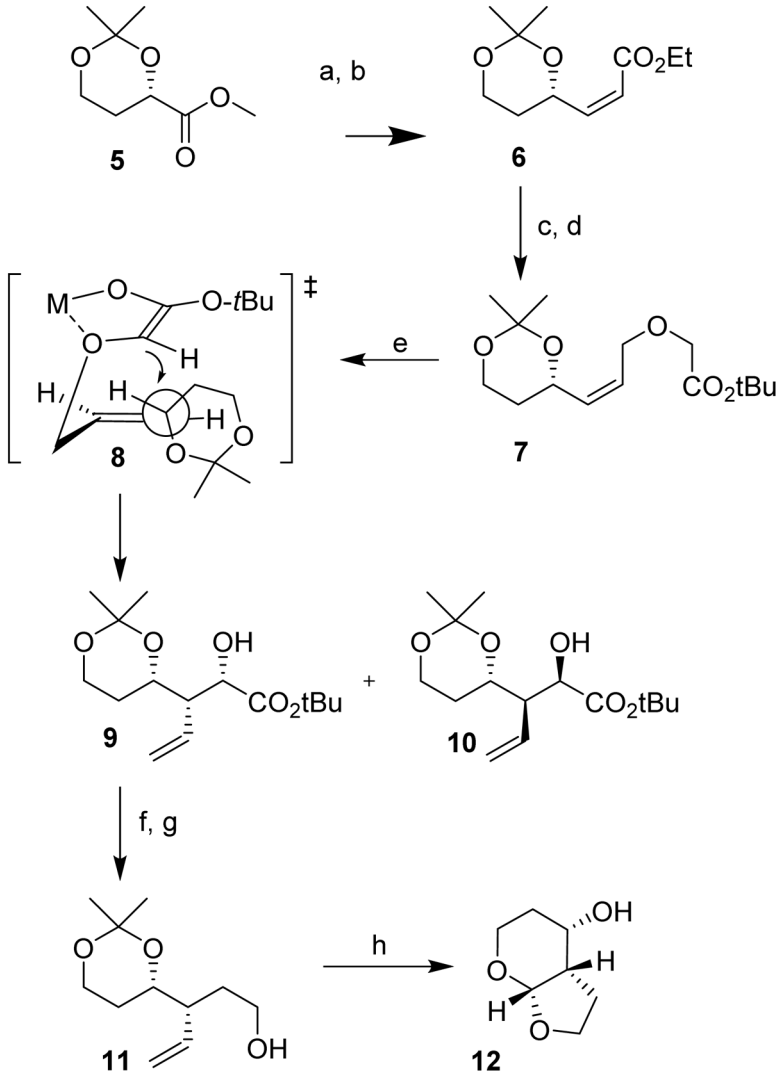


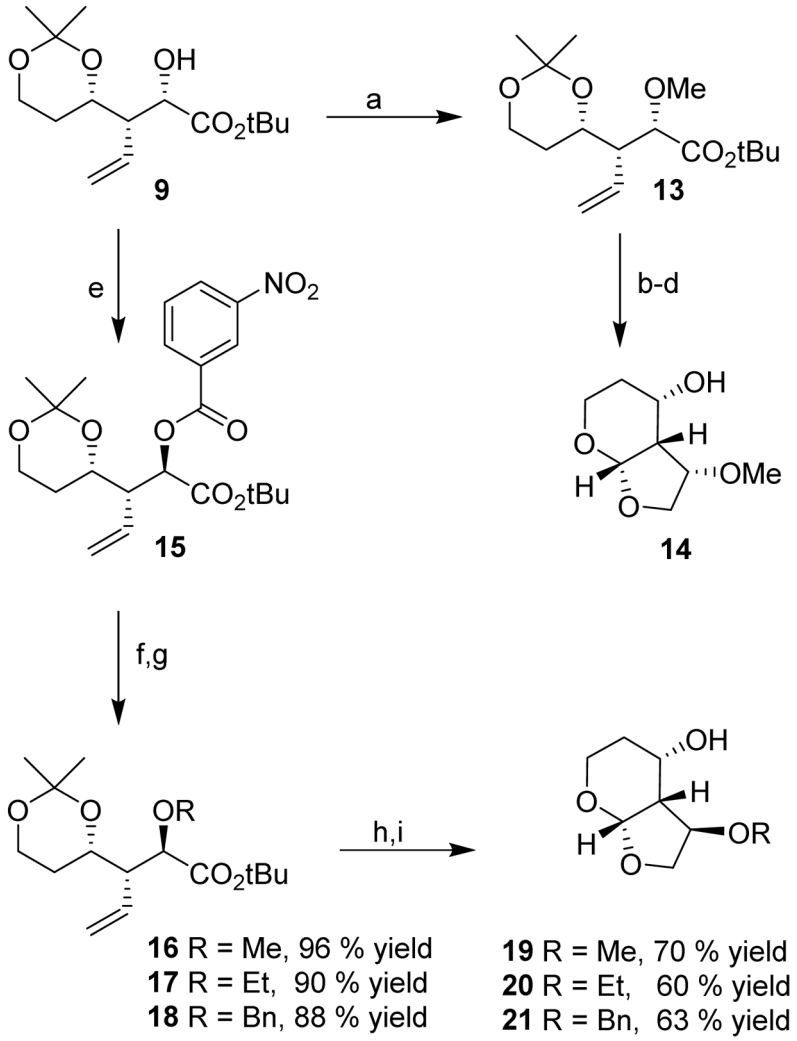
Figure 3.

Stereoview of the X-ray structure of inhibitor **30j** (green color)-bound to the active site of wild-type HIV-1 protease (PDB code: 5DGW). All strong hydrogen bonding interactions are shown as dotted lines.



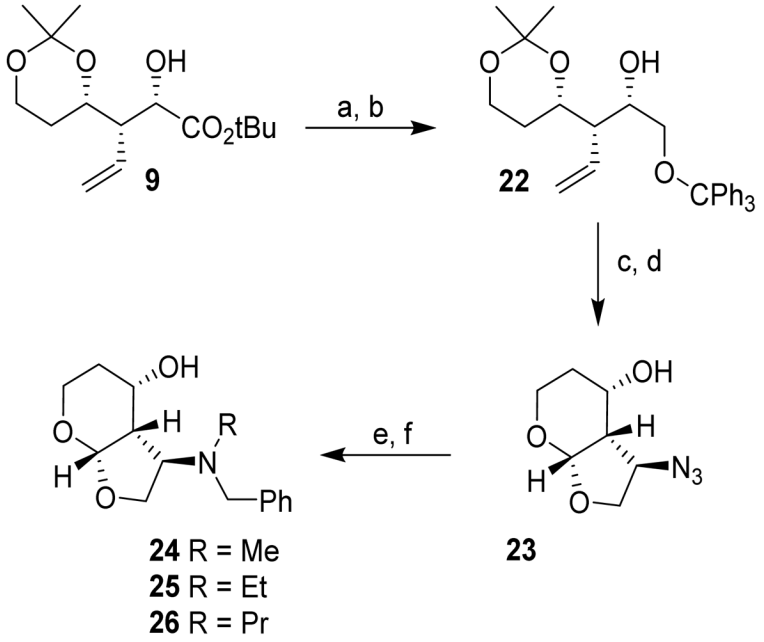
Scheme 1.

Reagents and conditions: (a) Dibal-H, CH_2Cl_2, −78 °C, 1 h; (b) MeOH, $Ph_3P{=}CHCO_2Et$, 0 °C, 70%; (c) Dibal-H, Ch_2Cl_2, −78 °C; (d) *t*-butyl bromoacetate, $CsOH{\cdot}H_2O$, TBAI, CH_3CN, 12 h, 95% 2 steps; (e) LiHMDS, THF, −65 °C −20 °C, 2 h, 77% overall, major/minor (8.5:1); (f) MsCl, Et_3N, THF, 0 °C, 1 h; ($g_)$ LAH, THF, 23 °C, 24 h, 70% 2 steps; (h) O_3, DMS, −78 °C, then *p*-TsOH, 23 °C, 18 h, 70%.



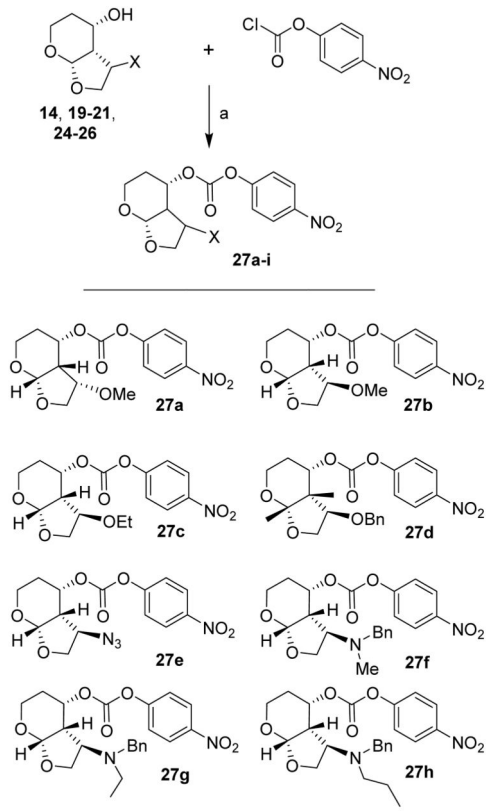
Scheme 2.

Reagents and conditions: (a) NaH, MeI, THF, 0 °C – 23 °C, 90%; (e) PPh_3, DIAD, *p*-nitrobenzoic acid, DCM, 0 °C, 18 h, 81%; (b) LAH; (c) O_3, DMS; (d), THF, *p*-TsOH, 30%, **2** steps; (f) K_2CO_3/MeOH, 0 °C, 1 h, 98%; (g) MeI, NaH, THF, 0 °C – 23 °C, or, BnBr, TBAI, NaH, THF, 0 °C to 23 °C 2 h; (h) LAH, THF, 0 °C, 1 h; (i) O_3, DMS, then, THF, *p*-TsOH.



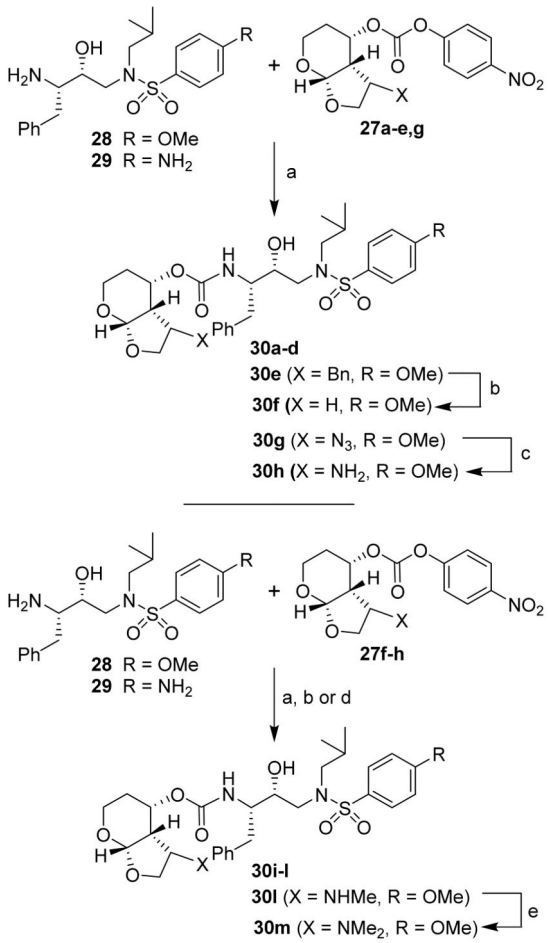
Scheme 3.

Reagents and conditions: (a) LAH, THF, 0 °C – 23 °C, 1h; (b) TrCl, Et_3N, DCM, 23 °C, 18 h, 92% 2 steps; (c) PPh_3, DPPA, DEAD, THF, 0 °C – 23 °C, 24 h, 70%; (d) O_3, DMS, −78 °C, then *p*-TsOH, 23 °C, 18h, 74%; (e) Pd/C, H_2, MeOH, 1h; (f) PhCHO, $NaBH_3CN$, MeOH, then RCHO, 24 h, 45 – 81%.



Scheme 4.

Reagents and conditions: (a) pyridine, CH_2Cl_2, 0 °C – 23 °C, 12 h (32–90%).

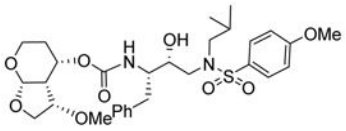
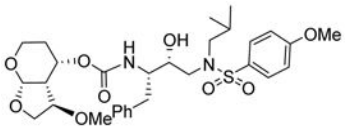
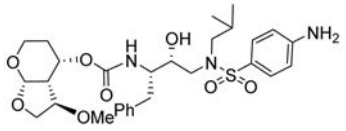
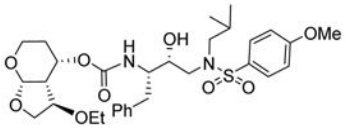
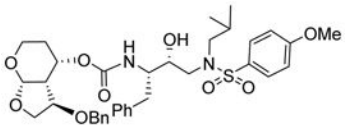
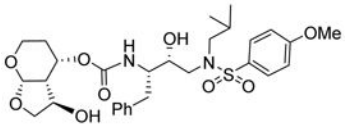


Scheme 5.

Reagents and conditions: (a) DIPEA, CH_3CN, 23 °C, 24 h (30–85%); (b) H_2, $Pd(OH)_2$, MeOH, 60 psi; (c) H_2, 10% Pd-C, EtOAc; (d) H_2, $Pd(OH)_2$, 5% NH_3 in MeOH; (e) $NaBH_3CN$, HCHO, MeOH,

Table 1

Structure and activity of *O*-substituted inhibitors

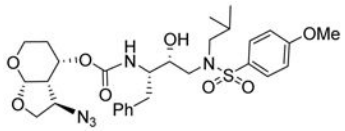
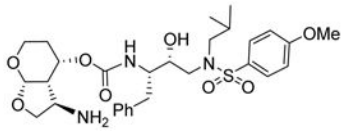
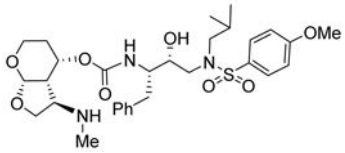
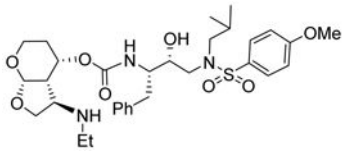
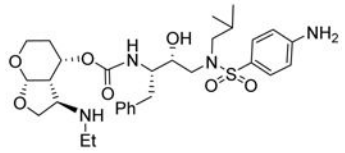
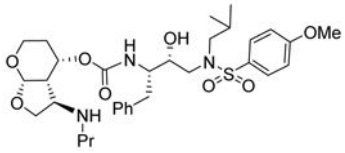
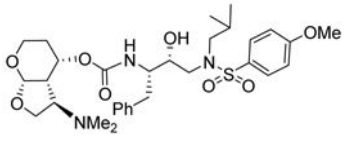
Entry	Inhibitor	K_i(nM)[a]	IC_{50}(nM)[a,b]
1.		1.38	47
2.		0.0045	0.2
3.		0.037	15
4.		0.0033	nt
5.		0.012	0.45
6.		0.06	3.15

[a] Ki values represents at least 5 data points. Standard error in all cases less than 7%. Darunavir (control) exhibited K_i = 16 pM.

[b] IC_{50} values were determined using the MTT assay. Values are means of at least three experiments. Standard error in all cases less than 5%. Darunavir exhibited IC_{50} = 1.6 nM. nt, not tested.

Table 2

Structure and activity of *N*-substituted inhibitors

Entry	Inhibitor	K_i(nM)[a]	IC_{50}(nM)[b]
1.		0.043	44
2.		21.4	520
3.		0.008	27
4.		0.009	0.8
5.		0.0058	22
6		0.0073	2.8
7.		0.023	0.8

[a] Ki values represents at least 5 data points. Standard error in all cases less than 7%. Darunavir (control) exhibited K_i = 16 pM.

[b] IC_{50} values were determined using the MTT assay. Values are means of at least three experiments. Standard error in all cases less than 5%. Darunavir exhibited IC_{50} = 1.6 nM.