Desymmetrization of Pibrentasvir for Efficient Prodrug Synthesis

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ABSTRACT: A novel and practical desymmetrization tactic is described to access a new class of pibrentasvir prodrugs. The homotopic benzimidazoles of pibrentasvir (PIB) are differentiated via a one-pot di-Boc/mono-de-Boc selective N-Boc protection and formaldehyde adduct formation sequence, both enabled by crystallization-induced selectivity. The first step represents the only known application of the Horeau principle of statistical amplification for C₂-symmetric polyheterocycle regioselective functionalization. The resulting versatile intermediate is employed in the high-yielding preparation of several pibrentasvir prodrug candidates.

INTRODUCTION

Mavyret® is a pan-genotype treatment for hepatitis C virus (HCV) containing glecaprevir, an NS3/4A protease inhibitor, and pibrentasvir (1, PIB), an NS5A inhibitor (Figure 1). As the first pan-genotypic 8-week cure for people suffering from HCV, Mavyret® was approved by the U.S. Food and Drug Administration (FDA) in 2017 for the treatment of genotype 1-6 chronic HCV after a 98% 8-week cure rate was reported for treatment-naïve patients without cirrhosis or with compensated cirrhosis. Enabling formulations were required for further improving bioavailability due to challenging physicochemical properties, so a prodrug approach was pursued, resulting in the discovery of phosphates 2, 3, and 4. Pibrentasvir (1, PIB) is a large (MW 1113 g/mol) C₂-symmetric drug molecule which presents several uniquely challenging structural features when considering a solubility-enhancing prodrug approach (Figure 1). The end-cap amino acid fragments, methoxycarbonyl (Moc)-protected O-Me-L-threonines, are prone to epimerization, β-elimination, and facile Moc cleavage with nucleophiles and bases. For these reasons, preliminary attempts to attach cleavable prodrug moieties to the end-caps proved futile. The four PIB benzimidazole nitrogen atoms initially appeared too similar in reactivity to be functionalized selectively, complicated by the two tertiary aniline nitrogens and the fact that PIB exists as a mixture of tautomers and rotamers in solution. Finally, and most significantly when considering synthetic efficiency challenges, the C₂-symmetric nature of PIB renders each end homotopic. This feature facilitated efficient two-directional chain synthesis in the preparation of PIB° with stereoregular purity enhancement via the Horeau principle. However, without a readily apparent internal functionalization or steric proximity effect to avoid bis(functionalization), a statistical mixture of products was anticipated and observed in early syntheses of 2-4.
FIGURE 2. Examples of desymmetrization reactions of complex C$_2$-symmetric molecules in target-oriented syntheses

Since the seminal reviews by Schreiber$^8$ and Magnus$^9$ describing two-directional synthesis and terminus differentiation, this strategy has been used successfully in a number of additional complex molecule syntheses (Figure 2). For example, in Hoye’s synthesis of the annonaceous acetogenin (+)-parviflorin, bis(epoxide) 5 was desymmetrized via reaction with a limited quantity of a lithium acetylide, giving alcohol 6 (29%) along with recovered 5 (53%).$^{10}$ Two syntheses from the Burke group utilized this approach, including a related annonaceous acetogenin, uvaricin (not shown), using a dihydroxylation,$^11$ and the C(37)-C(54) halichondrin B subunit, using an olefination/hydroboration/oxidation sequence to convert bis(lactone) 7 to desymmetrized primary alcohol 8 in 40% yield.$^{12}$ In a remarkably brief route to (+)-roxaticin that showcases this approach, the Krische group desymmetrized diol 9 via mono-selenide 10 formation in 50% yield.$^{13}$ In each example, a maximum yield of 50% was expected and observed due to the statistical mixtures of starting material/mono/di-functionalized products (1:2:1 ratio) obtained in homotopic terminus differentiation, requiring starting material recovery and resubjection to improve material throughput. In contrast, this work describes a rare example of highly controlled monofunctionalization of a complex C$_2$-symmetric molecule via Horeau amplified di-Boc protection/crystallization-induced selective mono-Boc deprotection (de-Boc) with n-BuNH$_2$, giving mono-N-Boc-PIB 11 in 94% yield from PIB. An overall yield of ~50% from PIB was a requirement for consideration of a pibrentasvir prodrug as a development candidate due to the high value of PIB, so this desymmetrization tactic was critical.

Figure 3. Benzimidazole nitrogen desymmetrizing and regioselective functionalization strategy

RESULTS AND DISCUSSION

Beyond homotopic terminus differentiation complexity, potential strategies to selectively functionalize PIB had to simultaneously address benzimidazole regioisomer selectivity (Figure 3). Since two equivalent PIB nitrogens are para-fluoro (“down” - red) and two are meta-fluoro (“up” - blue), some electronic bias was anticipated. However, early routes to PIB prodrugs demonstrated poor alkylation selectivities under a variety of conditions with challenging isomer separations further complicating the aforementioned statistical terminus differentiation issue.$^4$ Since no direct PIB alkylation appeared to provide any useful levels of selectivity or clear opportunities for efficient end differentiation, a selective and mild Boc protection strategy was envisioned to simplify the problem to a single benzimidazole regioselective alkylation. All reaction conditions would need to be mild enough to not degrade PIB and efficient enough to enable the 50% overall yield target. While considering the potential for biocatalytic or Miller peptide-based catalyst approaches for desymmetrization,$^{14}$ approaches using simple reagents which could benefit from inherent substrate bias and/or solubility properties were prioritized.

Scheme 1. Initial attempt to mono-Boc protect PIB, 1
Not surprisingly, initial attempts to mono-Boc protect PIB with 1 molar equivalent of di-tert-butyl dicarbonate (Boc₂O) gave a mixture of six compounds (Scheme 1), reflecting a 1:2:3 statistical ratio of starting material 1, mono-Boc (11 + 12), and di-Boc (13 + 14 + 15) isomers with a 3:1 ratio of down-mono-Boc 11 (Boc para to fluoro) to up-mono-Boc 12 (Boc meta to fluoro). This ratio favoring 11 was presumably influenced by the more acidic nature of the nitrogens para to the fluorine. Following tedious chromatography, a 48% yield of mono-Boc isomers 11 and 12 was obtained. Slurring the mixture in 3:1 MTBE/EtOAc gave a crystalline solid of 11 containing < 1% 12 (3:1 12/11 in the filtrate), indicating a significant solubility difference between mono-Boc benzimidazole regiosomers. This solubility difference would prove to be important in optimizing the formation of down-mono-Boc PIB 11.

**Scheme 2.** Selective di-Boc to a mixture of 13, 14, and 15

Reasoning that selective de-Boc of a mixture of di-Boc benzimidazoles 13, 14, and 15 may facilitate selective protection, and that both 13 and 14 could undergo Boc deprotection to give mono-down-Boc isomer 11 if relative rates of Boc cleavage were favorable, a complete reaction to a mixture of di-Boc compounds 13, 14, and 15 was carried out (Scheme 2). Following initial optimization of solvent and temperature to minimize 15, treatment of PIB with Boc₂O in THF at -35 °C in the presence of catalytic DMAP gave a 69:28:3 ratio of 13, 14, and 15, respectively. Since (up,down)-di-Boc isomer 14 contains both an up-Boc and a down-Boc, this result represents an 83 (69 + 28/2):17 (28/2 + 3) total down/up-N-Boc ratio (5:1). When considering all species containing at least one down-Boc benzimidazole (13 and 14), this mixture constitutes a 97 (69 + 28):3 ratio (3:1), provided all undesired up-Boc can be selectively removed. While the first Boc protection benefits from the electronic bias imparted by the fluorine atom, the enhancement observed in the second Boc protection can be considered an example of the Horeau principle of statistical amplification.⁷

The Horeau principle is typically invoked in asymmetric synthesis to, for example, explain the upgrade in optical purity of a low ee scalemic sample through coupling to a bifunctional linker to form a C₅/meso mixture which can be more easily separated at the expense of yield. A review on this topic recently appeared.⁷ Figure 4 shows Horeau's first application of this principle in upgrading the enantiomeric purity of a 60% ee secondary alcohol to 87% ee by forming a mixture of C₅/meso carbonates, removing the meso isomer, and cleaving the carbonate.⁶ In this example, since 20% (R) isomer becomes meso and 80% (S) isomer becomes meso (statistically), 32% of the carbonate mixture is readily removed, leaving a 64:4 (16:1) ratio of (R)/(S) after carbonate cleavage (87% ee). A second cycle further enhances the mixture to 96% ee at the further expense of yield. In the present case, rather than the minor/major (or equivalent major/minor) reaction product 14 being an undesirable meso isomer, the resulting up/down di-Boc 14 still contains a desired down-Boc (Scheme 2). Therefore, down/up-N regioselectivity is enhanced from 51 to 32 via the Horeau principle of statistical amplification, with 97% of the total mixture containing at least one down-N-Boc. This constitutes the first known application of this principle for the regioselective functionalization of a C₅-symmetric poly-heterocycle.¹⁷ However, while both 13 and 14 contain at least one down-Boc benzimidazole, their simultaneous selective conversion to down-mono-Boc 11 without further de-Boc to PIB remained a significant hurdle to accomplishing PIB desymmetrization.

**Scheme 3.** De-Boc of di-Boc mixture to give 11 selectively. De-Boc conditions: n-BuNH₂ (1.5 equiv), MTBE (8 mL/g), 23 °C

Since mono-Boc regiosomers 11 and 12 showed favorable solubility differences in MTBE (vide supra), and it was
anticipated that a primary aliphatic amine may deprotect the benzimidazoles at a slow enough rate that mono-Boc \(\text{II} \) could be protected from further de-Boc to PIB by crystallization,\(^6\) the reaction solvent was switched from THF to MTBE and \(n\)-butylamine was added to the reaction mixture (Scheme 3). Much to our delight, under these conditions, up-Boc cleaved significantly faster than down-Boc, with \(\text{14} \) converting to \(\text{II} \) and \(\text{15} \) converting to \(\text{12} \) quite rapidly over the first 2.5 h while \(\text{13} \) converted more slowly to \(\text{11} \). At this point, the solution was seeded with \(\text{11} \) (1 wt%), initiating a crystallization event. After 40 h at 23 °C, all mono-\(N\)-Boc and di-\(N\)-Boc isomers besides \(\text{13} \) and \(\text{11} \) were consumed, and a ratio of 5/8/4/1 for \(\text{13}/\text{II}/\text{11} \) was observed. Filtration gave a solid consisting of > 95% \(\text{II} \).\(^9\) Since product solubility in acetonitrile (ACN) was low, a re-slurry in ACN gave \(\text{II} \) with > 98% purity in 75% isolated yield.\(^9\) Here, terminus differentiation of \(C_2\)-symmetric di-Boc isomer \(\text{13} \), the most significant component of the di-Boc mixture, was made possible through crystallization-induced protection of mono-Boc \(\text{II} \) against further de-Boc to PIB. Without crystallization of \(\text{II} \), a statistical 1:2:1 ratio of di/mo/PIB would have been generated (Figure 2). Therefore, both Horeau amplification (di-Boc stage) and crystallization-induced protection (de-Boc stage) were required to deliver simultaneous regioselective benzimidazole functionalization and desymmetrization.

![Scheme 4](image)

**Scheme 4.** One-pot di-Boc/mono-de-Boc with ACN solvent switch. Conditions: Boc(O) (1.9 equiv), DMAP (0.1 equiv), THF (5 mL/g), -40 °C, 3 h; \(n\)-BuNH\(_2\) (0.8 equiv), MTBE (10 mL/g), 23 °C, 18 h; \(n\)-BuNH\(_2\) (1 equiv), ACN (10 mL/g), 23 °C, 21 h; mother liquor re-subjection.

To further increase the yield of the desymmetrization reaction, the conditions in Scheme 4 were employed, taking advantage of the even lower solubility of Boc-PIB \(\text{II} \) in ACN compared to MTBE. Once di-Boc \(\text{14} \) was nearly consumed in MTBE where up/down Boc cleavage rate differences were optimal, a solvent switch to ACN was carried out to minimize crystallization losses and maximize protection of \(\text{II} \) from further de-Boc. Following product isolation in 84% yield, mother liquors were concentrated and resubjected to the reaction conditions to increase the yield to 94%. Overall, this di-Boc/mono-de-Boc PIB desymmetrization tactic provided a high isolated yield of a single down-mono-Boc product \(\text{11} \) out of the six species initially observed in attempted mono-Boc protection. While selective protection simplified the problem of PIB prodrug synthesis, regioselective functionalization of the remaining unprotected benzimidazole in \(\text{11} \) remained a formidable challenge (Figure 3).

![Scheme 5](image)

**Scheme 5.** One-pot hydroxymethylation/acylation of \(\text{11} \).

Direct alkylation of Boc-PIB \(\text{II} \) with chloromethyl esters was feasible and used successfully for early syntheses of PIB prodrugs.\(^4\) However, regioselectivities were poor and yields for the alkylation step variable after significant optimization efforts. Despite very limited precedent for regioselective benzimidazole formaldehyde adduct formation/acylation,\(^26\) the reversible nature of such an adduct offered potential benefits that could prove advantageous. To probe this strategy, Boc-PIB \(\text{11} \) was treated with paraformaldehyde and \(i\)-Pr\(_2\)NEt in DMF for 1 h at 70 °C (Scheme 5), then cooled to -20 °C and acylated with acid chloride \(\text{16} \).

**Table 1.** Acylation leaving group screen results

While good conversion to desired product was observed (85% overall yield), a disappointing 1:8:1 ratio of benzimidazole regioisomers \(17 \) and \(18 \) resulted, suggesting poor selectivity in the hydroxymethylation step and/or equilibration during the acylation. Undeterred by this preliminary result, Boc-PIB formaldehyde adduct \(19/20 \) mixture was isolated by precipitation from EtOAc/MTBE/hexanes for investigation of acylation leaving group identity (Table 1).\(^22\) Standard acylating conditions \((i\)-Pr\(_2\)NEt, DMAP\) were chosen as a basis for comparison between the acylating reagents, resulting in a wide range of reaction rates and product ratios. Reaction temperature was chosen for each acylating reagent to afford some conversion to product in order to measure the product isomer ratios. An increase in leaving group propensity resulted in greater conversion.
to the product isomer mixture as well as higher regioselectivity. With a relatively poor leaving group (OSu) in 21, the reaction reached 20% conversion after 18 h at 23 °C, and a similar product ratio was observed as in the 1-pot reaction (1.8:1). Employing the original highly electrophilic acid chloride 16 vastly improved rate (95% conversion after 1 h at -15°C) and surprisingly improved the isomer ratio to 20:1 in favor of the desired product. Since conditions for the acylation were nearly identical to the one-pot reaction, the improved regioselectivity clearly resulted from isolation of the solid formaldehyde adduct. Therefore, the hydroxymethylation constituted a second consecutive crystallization-induced selective reaction.²¹

While the DMF/paraformaldehyde conditions provided adequate material for preliminary studies, a more convenient formaldehyde adduct formation was desired to avoid DMF distillation on larger scale (Scheme 6). Reaction of Boc-PIB 11 with 37% aqueous formaldehyde in EtOAc for 3 h in the absence of base was found to give a high mass balance of hydroxymethylolation product (>10:1 19:11 ratio by 1H NMR in DMSO).²² Efficient isolation was achieved by partial concentration and slow addition of heptanes with product seeding to facilitate crystallization. After filtration and drying, key intermediate 19 was isolated as a white solid in 99% yield.

Scheme 6. Preparation of key intermediate 19

Having secured a robust and scalable procedure for the synthesis of compound 19, we proceeded to investigate its use in the preparation of lead prodrugs of PIB.⁴ The encouraging results observed in the model system (Table 1) provided a useful starting point for the acylation of 19 with more complex structures. We were pleased to find that treatment of a solution of 19 with dibenzyl (4-(2-chloro-2-oxoethyl)phenyl) phosphate (Scheme 7, R’COCI) in the presence of DMAP and 1-Pr₂NEt in THF at -30 °C rapidly afforded a mixture of isomers with high conversion (> 90%) and selectivity (> 20:1 isomer ratio). Nevertheless, we encountered difficulties in the removal of byproducts from these initial reactions, which led us to investigate stronger bases under cryogenic conditions.²³ Fortunately, treatment of 19 with lithium hexamethyldisilazide (LiHMDS) and the acid chloride at -65 °C rapidly afforded the desired product with a significantly improved reaction profile, albeit with slightly reduced conversion and selectivity (85% conv., 10:1 isomer ratio). After Boc-deprotection of the crude material with TFA in CH₂Cl₂, the regioisomers were efficiently separated via flash silica gel chromatography, affording the penultimate dibenzylphosphate intermediate 25 in 62% yield over 3 steps from 11.

Scheme 7. Preparation of Prodrug 2

The final hydrogenolysis step revealed some inherent solubility and isolation challenges with the phosphate prodrug 2. Initial reactions in THF or mixtures of THF and protic solvents (e.g. HOAc, MeOH, H₂O) resulted in incomplete conversion and aggregation of the product with the catalyst; however, reactions in neat HOAc as solvent with 0.7 mol% Pd/C and 50 psi H₂ were complete in < 2 h. After removal of the catalyst bed and concentration in vacuo, a final slurry of the crude material in IPA (10 mL/g) afforded compound 2 in 83% yield (Scheme 7, 51% from Boc-PIB 11).²⁴

Preliminary observations made during the synthesis of compound 2 informed the subsequent preparation of prodrug 3 (Scheme 8). Cryogenic treatment of 19 with LiHMDS in THF was followed by rapid addition of a preformed solution of the acid chloride 27 in THF to give nearly quantitative acylation in < 5 min.²⁵ Boc-deprotection of the crude material gave a mixture of products with a 29:1 regioisomer ratio. The major isomer was then efficiently separated using silica gel chromatography to give the penultimate dibenzylphosphate in 83% yield from 11. Optimization studies of the final phosphate deprotection to generate compound 2 indicated that THF instead of HOAc was the preferred solvent in this case, giving complete debenzylation in 20 h at ambient temperature with 5% Pd/C and 50 psi H₂. Crude material was purified via hot IPA crystallization and a final slurry in acetone to give 3 in 64% isolated yield.²⁴
To prepare phosphonoxyethyl prodrug 4 from key intermediate 19, a new strategy was required that would rapidly and selectively forge a P-O bond (Scheme 9). After several failed attempts to directly form this bond at the phosphate oxidation state, an efficient phosphate formation was realized by employing dibenzylphosphoramidite reagent 28 with tetrazole as base.25 Following addition of hydrogen peroxide and de-Boc of the resulting crude phosphate, dibenzylphosphonoxyethyl product 29 was obtained in good yield (65%). Finally, benzyl hydrogenolysis proceeded without incident, giving PIB prodrug 4.

![Scheme 9. Preparation of Prodrug 4](image)

**CONCLUSION**

The initial synthetic routes to prodrugs 2 - 4 employed unselective direct alkylations of PIB 1 or Boc-PIB 11 with the requisite chloromethyl esters, where tedious separation of mixtures resulted in low overall yields.4 Ultimately, the development of a scalable route to key intermediate 19 facilitated regioselective preparation of these lead prodrugs from PIB 1 and suitably positioned them for consideration as clinical candidates. The identification of high-yielding functionalization reactions of 19 and subsequent phosphate deprotection completed the syntheses of prodrugs 2 - 4, improving overall yields from 6-11% to 44-56% from PIB 1. The desymmetrization tactic employed here to differentiate the homotopic termini of PIB 1 also highlights the importance of considering classical and perhaps under-utilized strategies such as statistical amplification and exploitation of solubility differences for selectivity in even the most complex chemical settings.

**ASSOCIATED CONTENT**

**Supporting Information.** Experimental details and characterization data for all new compounds. This material is available free of charge via the internet at http://pubs.acs.org

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**ACKNOWLEDGMENT**

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**ABBREVIATIONS**

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Supporting Information for:
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General Methods and Materials

Experimental Procedures and characterization data

NMR Spectra

General Methods and Materials

Unless otherwise noted, commercial solvents and reagents were purchased and used without additional purification. Proton and carbon magnetic resonance spectra ($^1$H NMR and $^{13}$C NMR) were measured with either a Varian 400 MHz or Varian Inova 500 MHz NMR Spectrometer. $^1$H NMR chemical shifts are expressed in parts per million (δ) downfield from tetramethylsilane (CHCl$_3$ standardized at 7.26 ppm). $^{13}$C NMR chemical shifts are expressed in parts per million (δ) downfield from tetramethylsilane (central peak of CHCl$_3$ standardized at 77.16 ppm). Reactions were monitored using either LC-MS or thin-layer chromatography (TLC). Analytical LC-MS was performed on a Thermo MSQ-Plus mass spectrometer and Agilent 1100/1200 HPLC system running Xcalibur 2.0.7, Open-Access 1.4, and custom login software. The mass spectrometer was operated under positive APCI or ESI ionization conditions dependent on the system used. The HPLC system comprised an Agilent Binary pump, degasser, column compartment, autosampler and diode-array detector, with a Polymer Labs ELS-2100 evaporative light-scattering detector. The column used was a Phenomenex Kinetex C8, 2.6 μm 100 Å (2.1mm × 30mm), at a temperature of 65 °C. For LC-MS AA long method, a gradient of 3-100% acetonitrile (A) and 10 mM ammonium acetate in water (B) was used, at a flow rate of 0.5 mL/min (0-0.25 min 3% A, 0.25-5.2 min 3-100% A, 5.2-5.9 min 100% A, 5.9-6.0 min 100-3% A, 0.25 min post-run delay). Visualization of TLC was accomplished with UV light (254 or 364 nm) and/or aqueous potassium permanganate or ceric ammonium nitrate stain followed by heating. Purification of the crude reaction products, when performed, was accomplished on a Grace Revealeris X2 normal-phase chromatography system using silica gel cartridges purchased from Grace or Silicycle. Specific parameters used in the separation of individual compounds are detailed under each entry. Unless otherwise noted, reactions were carried out under an atmosphere of nitrogen. Yields refer to isolated yields of analytically pure (>95%) material unless otherwise noted.
Experimental Procedures and Characterization Data

I. Preliminary Experiments Toward mono-Boc Protection of PIB (1):

**Boc-Pibrentasvir isomer mixture:** A solution of dimethyl ((2S,2'S,3R,3'R)-(2S,2'S)-2,2'-(5,5'-(2R,5R)-1-(3,5-difluoro-4-(4-(4-fluorophenyl)piperidin-1-yl)phenyl)pyrroloidine-2,5-diyl)bis(6-fluoro-1H-benzo[d]imidazole-5,2-diyl))bis(pyrroolidine-2,1-diyl))bis(3-methoxy-1-oxobutane-2,1-diyl))dicarbamate (pibrentasvir, 10.0 g, 8.98 mmol) in THF (90 mL) was stirred at ambient temperature and di-t-butyl dicarbonate (Boc₂O, 1.96 g, 8.98 mmol) was added. A solution of 4-dimethylaminopyridine (DMAP, 0.11 g, 0.90 mmol) in THF (90 mL) was added dropwise at 25 °C over 3 h. After the addition, the reaction mixture was stirred for 16 h at 25 °C and analyzed by HPLC:

| compound  | 1 | 11 | 12 | 13 | 14 | 15 |
|-----------|---|----|----|----|----|----|
| peak area%| 25.7 | 38.6 | 12.2 | 12.3 | 7.9 | 14 |

Isolated: 48% yield, 3:1 11/12

**Method Info:** Ascentis C18 2.7 μm 4.6x150nm; 35°C; 1.5 ml/min
30:70 to 90:10 ACN :0.1% Perchloric acid

**Sample Info:** crude product mixture

![HPLC chromatogram](image)
The reaction mixture was then concentrated and purified twice by silica gel column chromatography on 300 g columns using first a 0-10% methanol and ethyl acetate gradient followed by 0-2% methanol and ethyl acetate gradient. The resulting solid was slurried in ethyl ether and hexane, filtered, and dried at 45 °C under vacuum to afford a 3:1 mixture of 11/12 (5.24 g, 48% yield). A portion of this material was purified by preparative HPLC and pure fractions were concentrated, giving major isomer tert-butyl 6-((2R,5R)-(1-(3,5-difluoro-4-(4-fluorophenyl)piperidin-1-yl)phenyl)-5-(6-fluoro-2-(S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazole-5-yl)pyrrolidin-2-yl)-5-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazole-1-carboxylate (down-Boc-PIB 11): 1H NMR (500 MHz, CDCl₃, -20 °C) (tautomeric species also present; data given for major tautomeric form): δ 11.00 (s, 1H), 7.69 (br s, 1H), 7.54 (d, J = 7.0 Hz, 1H), 7.40 (d, J = 9.8 Hz, 1H), 7.31 (d, J = 5.0 Hz, 1H), 7.20 (m, 2H), 7.00 (apparent t, J = 8.6 Hz, 2H), 6.87 (d, J = 10.3 Hz, 1H), 6.45 (br s, 1H), 6.05 (d, J = 7.6 Hz, 1H), 5.89 (dd, J = 14.3 Hz, 2H), 5.51 (d, J = 7.5 Hz, 1H), 5.40 (d, J = 7.5 Hz, 1H), 5.37 (d, J = 10 Hz, 1H), 4.70 (t, J = 10 Hz, 1H), 4.66 (br m, 1H), 4.23 (br m, 1H), 3.95 – 3.63 (m, 4H), 3.81 (s, 3H), 3.73 (s, 3H), 3.54 (br m, 1H), 3.35 (s, 3H), 3.31 (s, 3H), 3.21 – 2.89 (m, 6H), 2.59 – 2.28 (m, 6H), 2.22 – 2.08 (m, 4H), 1.90 – 1.69 (m, 5H), 1.57 (d, J = 19.9 Hz, 2H), 1.47 (s, 9H), 1.23 (d, J = 6.1 Hz, 3H), 1.12 (d, J = 6.2 Hz, 3H). 13C NMR (151 MHz, CDCl₃, 27 °C) (tautomeric species also present): δ 170.8, 169.0, 161.2 (d, J = 248.0 Hz), 160.3 (d, J = 252.6 Hz, 2C), 158.1, 157.1, 157.0, 156.7, 154.4, 148.5, 142.2, 141.9, 141.7 (d, J = 13.9 Hz), 138.3, 133.3 (d, J = 13.9 Hz), 129.0, 128.1 (d, J = 7.7 Hz, 2C), 125.5 (d, J = 17.0 Hz), 123.2 (d, J = 17.0 Hz), 117.6, 117.3, 115.0 (d, J = 21.6 Hz, 2C), 113.6, 106.2 (d, J = 24.6 Hz), 98.5 (d, J = 27.7 Hz), 97.7 (d, J = 26.0 Hz, 2C), 85.6, 77.1, 76.9, 57.8, 57.7, 57.0, 56.8, 56.7, 56.7, 56.2, 56.1, 54.9, 52.7, 52.6, 52.4, 52.3, 47.9, 47.5, 47.5, 41.6, 34.5, 34.4, 31.2, 31.0, 30.9, 28.0 (3C),
The compounds "up-Boc-PIB" 12 and "down-Boc-PIB" 11 were characterized via NMR utilizing the 1H, 13C (1H), 1H-1H COSY, 1H-1H ROESY, 1H-1H TOCSY, 1H-13C HSQC and 1H-13C HMBC NMR techniques (see NMR Spectra section). Due to the presence of tautomer peaks resulting in broad proton signals and limited resolution, the carbon and proton atoms in up-Boc-PIB 12 and down-Boc-PIB 11 were assigned in multiple solvent systems ((CD3)2SO, CDCl3 and C6D6) to identify the solvent that would allow the complete assignment of the proton and carbon atoms for both samples. While all analyses were considered, data collected in CDCl3 at 27 °C and -20 °C resulted in sufficient signals for full proton and carbon assignment for comparison of both the "up-Boc-PIB" 12 and "down-Boc-PIB" 11 samples. The compounds of interest in this study are differentiated by the substitution of the BOC group on one of the benzimidazole rings. For each compound in every solvent system, 1H-1H ROESY data reveals correlations between the BOC methyl protons and one or the other of the benzimidazole protons supporting these nonbonded protons are within 3.5 Å in space. There are two protons on the benzimidazole ring and for each product they are differentiated by their scalar coupling to the fluorine atom which could be used to assign the protons on the benzimidazole ring. The larger 1H-19F coupling is expected for the benzimidazole proton adjacent to the fluorine. In analysis of the down-Boc-PIB 11 data (temp = -20 °C), the BOC methyl protons have the ROESY correlation to the benzimidazole peak observed at 7.54 ppm (1H, d, 4JH,F= 6.99 Hz) which has been assigned H18 due to the smaller scalar coupling between H18 and F62 as compared to proton H15 (7.40 ppm, 1H, d, 3JH,F= 9.85 Hz). Proton H18 has a strong 1H-13C HMBC correlation to the pyrrolidine carbon assigned C1. This strong 1H-13C HMBC correlation is expected to be observed between proton H18 and carbon C1 as opposed to an 1H-13C HMBC correlation between proton H15 and carbon C1. For compound up-Boc-PIB 12 (temp = 27 °C), the BOC methyl protons have the ROESY correlation to the benzimidazole peak at 7.68 ppm (1H, d, 3JH,F= 10.71 Hz) which has been assigned to H15 due to its larger scalar coupling. The larger proton H18 peak is observed at 7.12 ppm and its coupling to F62 is measured to be 4JH,F = 6.82 Hz. The expected strong 1H-13C HMBC correlation between benzimidazole proton H18 and the pyrrolidine carbon C1 is observed in the "up-Boc-PIB" 12 data collected at 27 °C.
II. Development of diBoc/deBoc Protocol for Synthesis of monoBoc PIB (1)

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\text{t-Butyl } 6\text{-((2R,5R)-1-((3,5\text{-difluoro-4-((4-fluorophenyl)piperidin-1-yl)phenyl})-5-((6-fluoro-2-((S)-1-((2S,3R)-3\text{-methoxy-2-((methoxycarbonyl)amino}butanoyl)pyrrolidin-2-yl)pyrrolidin-2-yl)-5-fluoro-2-((S)-1-((2S,3R)-3\text{-methoxy-2-((methoxycarbonyl)amino}butanoyl)pyrrolidin-2-yl)-1H-benzimidazole-5-yl)pyrrolidin-2-yl)-5-fluoro-2-((S)-1-((2S,3R)-3\text{-methoxy-2-((methoxycarbonyl)amino}butanoyl)pyrrolidin-2-yl)-1H-benzimidazole-1-carboxylate: A solution of dimethyl \((2S,2'S,3R,3'R)-((2S,2'S)-2,2'-((5,5'-((2R,5R)-1-((3,5\text{-difluoro-4-((4-fluorophenyl)piperidin-1-yl)phenyl})pyrrolidin-2,5-diyl)bis(6-fluoro-1H-benzimidazole-5,2-diyl))bis(pyridine-2,1-diyl))bis(3\text{-methoxy-1-oxobutane-2,1-diyl})\) dicarbamate (pibrentasvir, 20.0 g, 18.0 mmol), THF (200 mL), and di-tert-butyl dicarbonate (Boc\(_2\)O, 8.63 g, 39.5 mmol) was cooled to \(< -35^\circ C\) and 4\text{-dimethylaminopyridine (DMAP, 0.219 g, 1.80 mmol) was added. The white slurry was held between \(-35\) and \(-40^\circ C\) (internal temperature) and followed by LC-MS (AA long method, see general methods for details), manually integrating signals at the indicated retention times at 220 nm detection wavelength, with identities established via comparison to previous non-selective Boc-protection procedure (compound structures/numbers shown there). After 3 h, all PIB 1 and mono-up-Boc isomer 12 consumed, so warmed to 15^\circ C (complete consumption of 11 to give the de-Boc profile) and concentrated on a rotovap to 33 g total mass. To remove residual THF/tBuOH, toluene (40 mL) was added and concentrated to 33 g total mass (up to 35^\circ C bath temp, starting to foam). Added MTBE (160 ml) and \(n\)-butylamine (2.67 ml, 26.9 mmol) and followed by LC-MS (AA long method).}

| time (h) | 13 (4.73 min) | 14 (4.78 min) | 15 (4.95 min) | 11 (4.23 min) | 12 (4.31 min) | 1 (3.70 min) |
|---------|---------------|---------------|---------------|---------------|---------------|---------------|
| 0       | 0             | 0             | 0             | 0             | 0             | 100           |
| 0.5     | 9.9           | 7.1           | 1             | 54.4          | 8.5           | 19.1          |
| 1       | 25.2          | 16.2          | 2.2           | 50.2          | 3.7           | 2.5           |
| 2       | 48.6          | 24.2          | 2.7           | 24            | 0.6           | 0             |
| 3       | 60.5          | 27.9          | 2.9           | 8.6           | 0             | 0             |
| 4       | 68.8          | 28.5          | 2.7           | 0             | 0             | 0             |
| 2.5     | 40.2          | 4.8           | 0             | 48.3          | 2.6           | 4.1           |
| 16      | 14.2          | 0             | 0             | 76.6          | 0             | 9.2           |
| 24      | 9.5           | 0             | 0             | 80.5          | 0             | 10            |
| 40      | 4.6           | 0             | 0             | 84.4          | 0             | 11.1          |

Di-Boc

De-Boc
After 16 h, a white slurry was observed. After 40 h, the slurry was filtered, washing with MTBE (2 x 20 mL). Dried the white solid in a vacuum oven at 50 °C, giving product with ~95% purity. Acetonitrile (146 mL) was added, the slurry was heated to 70 °C, then cooled to ambient temperature over 1 h, stirred 1 h, and filtered, washing with ACN (2 x 18 mL). Dried the white solid in a vacuum oven at 50 °C, giving tert-butyl 6-((2R,5R)-1-(3,5-difluoro-4-(4-fluorophenyl)piperidin-1-yl)phenyl)-5-(6-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benz[d]imidazol-5-yl)pyrrolidin-2-yl)-5-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benz[d]imidazole-1-carboxylate (16.4 g, 75%). LC-MS showed 97.9 pa% desired product 11 and 2.1 pa% PIB 1. Characterization data were indistinguishable from that obtained from the previous non-selective Boc-PIB 11 procedure.
tes-t-butyl 6-((2R,5R)-1-(3,5-difluoro-4-(4-(fluorophenyl)piperidin-1-yl)phenyl)-5-(6-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxy carbonylamino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-5-yl)pyrrolidin-2-yl)-5-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxy carbonylamino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-1-carboxylate (11): A solution of DMAP (0.439 g, 3.59 mmol), dimethyl (2S,2’S,3R,3’R)-(2S,2’S)-2’-(5,5’-((2R,5R)-1-((3,5-difluoro-4-(4-(fluorophenyl)piperidin-1-yl)phenyl)pyrrolidine-2,5-diyl)bis(6-fluoro-1H-benzo[d]imidazol-5,2-diyl))bis(3-methoxy-1-oxobutane-2,1-diyl))dicarbonate (40.0 g, 35.9 mmol), and THF (200 mL) was cooled to -40 °C and Boc₂O (14.9 g, 68.3 mmol) was added. Held between -40 and -45 °C (internal temperature) and followed by LC-MS AA long method. After 3 h, 1.5% down-mono-Boc 12 remained, so warmed to 23 °C, giving a 52.2/26.1/2.9/18.5/0.4/0 ratio of 13/14/15/11/12/1. Concentrated to 66.3 g total mass (tan oil/foam), then added MTBE (400 mL) and n-butylamine (2.85 mL, 28.7 mmol) and followed by LC-MS AA long method. After 18 h, a 29.2/0/0/63.7/0/6.0 ratio of 13/14/15/11/12/1 was observed, so concentrated to minimal volume, dissolved in ACN (200 mL), heated to 75 °C, and cooled the white slurry to 23 °C over 1 h. Added n-butylamine (3.56 mL, 35.9 mmol) and followed by LC-MS AA long method. After 21 h, 1.7/0/0/86.4/0/11.8 ratio of 13/14/15/11/12/1 was observed. Filtered, washed with ACN (2 x 40 mL slurry washes), then added ACN (400 mL) to the wet cake, heated to 75 °C, cooled the white slurry to 23 °C over 30 min, stirred 30 min, and filtered, washing with ACN (2 x 40 mL slurry washes). Dried the white solid in a vacuum oven at 50 °C for 14 h, giving tert-butyl 6-((2R,5R)-1-(3,5-difluoro-4-(4-(fluorophenyl)piperidin-1-yl)phenyl)-5-(6-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxy carbonylamino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-5-yl)pyrrolidin-2-yl)-5-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxy carbonylamino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-1-carboxylate (11): (36.65 g, 30.2 mmol, 84 % yield).

LC-MS:

solid 1: 3.5% PIB 1, 95.9% 11, 0.6% 13; mother liquor 1: 68.2% PIB 1, 21.7% 11, 10.1% 13

cold 2: 1.7% PIB 1, 98.3% 11; mother liquor 2: 38.8% PIB 1, 57.9% 11, 3.2% 13

The combined mother liquors were concentrated to 14.6 g total mass (including ~ 5.7 mmol mixed 1/11/13 in a 57.8/34.6/7.6 ratio + n-BuNHBOc and DMAP) and THF (30 mL) was added. The solution was cooled to -35 °C and Boc₂O (2.35 g, 10.8 mmol) was added. After 30 min between -35 and -40 °C LC-MS AA long method showed a 64/31/5 ratio of 13/14/15. Concentrated to minimal volume, added MTBE (60 mL) and n-butylamine (0.534 mL, 5.39 mmol) and LC-MS AA long method showed 37.2/0/0/54.5/0/6.9 ratio of 13/14/15/11/12/1 after 16 h. Switched to ACN (60 mL, still solution), seeded with product 11 (~10 mg), and added n-butylamine (1.68 mL, 10.8 mmol). After 5 h, a 10.9/74.7/14.3 ratio of 13/11/1 was observed by LC-MS. Filtered, washing with ACN (2 x 6 mL slurry washes), then added ACN (60 mL) to the wet cake, heated to 75 °C, cooled the white slurry to 23 °C over 30 min, stirred 30 min, and filtered, washing with ACN (2 x 6 mL slurry washes). Dried the white solid in a vacuum oven at 50 °C to constant weight, giving 11 (4.25 g, 3.50 mmol). This material was 98.5% 11 by LC-MS, so combined with first cycle material, giving tert-butyl 6-((2R,5R)-1-(3,5-difluoro-4-(4-(fluorophenyl)piperidin-1-yl)phenyl)-5-(6-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxy carbonylamino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-5-yl)pyrrolidin-2-yl)-5-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxy carbonylamino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-1-carboxylate 11 (40.9 g, 33.7 mmol, 94 % yield). Characterization data were indistinguishable from that obtained from the previous non-selective Boc-PIB 11 procedure.

III. Preparation and Acylation Studies of Hydroxymethyl Intermediate 19

Initial synthesis of formaldehyde adduct 19:
Compound 11 (200 mg, 0.17 mmol), paraformaldehyde (6.7 mg), N,N-dimethyl formamide (2 ml), and N-ethyl-N-isopropylpropan-2-amine (13 µl, 0.08 mmol) were added to a 4 mL vial. The mixture was stirred for 1 h at 70 °C, then cooled to 23 °C and concentrated to a yellow oil. The crude product was suspended in a solvent mixture of MTBE (1 mL), hexane (1 mL), and EtOAc (0.3 mL), resulting in crystallization upon sonication. Filtration of the slurry and drying of the solid under high vacuum afforded 19 (119 mg, 0.10 mmol, 60% yield). Omission of the isolation of 19 resulted in an unselective acylation with 16, giving a 1.8:1 ratio of 17 and 18 when the general acylation procedure was applied:

**Optimized synthesis of formaldehyde adduct 19:**

A solution of tert-butyl 6-((2R,5R)-1-(3,5-difluoro-4-(4-(4-fluorophenyl)piperidin-1-yl)phenyl)-5-(6-fluoro-1-(hydroxymethyl)-2-(S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzod[d]imidazol-5-yl)pyrrolidin-2-yl)-5-fluoro-2-(S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzod[d]imidazole-1-carboxylate (19): A solution of tert-butyl 6-((2R,5R)-1-(3,5-difluoro-4-(4-(4-fluorophenyl)piperidin-1-yl)phenyl)-5-(6-fluoro-2-(S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzod[d]imidazol-5-yl)pyrrolidin-2-yl)-5-fluoro-2-(S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzod[d]imidazole-1-carboxylate (18.5 g, 15.3 mmol) in EtOAc (185 ml) was stirred at 23 °C and formaldehyde (37% aqueous, 5.68 ml, 76.0 mmol) was added. After 4 h, ¹H NMR analysis (100 uL blown dry with air, dissolved in DMSO-d₆, and immediately obtained data) showed nearly complete reaction (>7:1 pdt/sm integrating 5.3/5.1 ppm signals; partial hydrolysis always observed by ¹H NMR). The mixture was concentrated to 63 g total (44 g, 49 mL EtOAc) and K₂titration showed ~ 1.1 equiv water. Then, heptanes (147 mL) were added dropwise. After 60 mL had been added over 90 min, solid material had oiled out. Product seed crystals were then added (185 mg, 1%), and the slurry stirred overnight at 23 °C.
The remaining heptanes were added over 1 h, the mixture stirred 1 h, and filtered, washing with 3:1 hep/EtOAc (20 mL). The white solid was dried in an ambient temperature vacuum oven for 2 days, giving tert-butyl 6-((2R,5R)-1-(3,5-difluoro-4-(4-fluorophenyl)piperidin-1-yl)phenyl)-5-(6-fluoro-1-(hydroxymethyl)-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)piperidin-2-yl)-1H-benzo[d]imidazol-5-yl)pyrrolidin-2-yl)-5-fluoro-2-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)piperidin-2-yl)-1H-benzo[d]imidazole-1-carboxylate (18.8 g, 15.1 mmol, 99% yield). Kf titration showed 0.73 equiv water remaining after drying, but further drying was not attempted to avoid loss of formaldehyde.

NMR check for reaction completion after 4 h with 19 at 5.3 ppm and 11 at 5.1 ppm (ratio during reaction or when characterizing product changes based on time in DMSO-d6 solution, so only qualitative; 14:1 ratio here):

2,5-dioxopyrrolidin-1-yl 2-(4-(benzoyloxy)phenyl)acetate (21): 2-(4-(benzoyloxy)phenyl)acetic acid (10 mmol, 2.42 g) was dissolved in a solution of DCC (11 mmol, 2.26 g) and N-hydroxysuccinimide (11 mmol, 1.27 g) in acetone (50 ml) and ethyl acetate (150 mL). The mixture was stirred in an ice bath at 0°C for 1 h and allowed to warm to room temperature overnight, yielding a white precipitate. The solid was filtered off, and the filtrate was concentrated to afford crude product (3.86 g). The product was purified by silica
gel flash chromatography using 50% acetone in hexanes to afford 21 (2.78 g, 80%). $^1$H NMR (400 MHz, DMSO-d$_6$) δ 7.47 – 7.42 (m, 2H), 7.42 – 7.35 (m, 2H), 7.33 (m, 1H), 7.26 (m, 2H), 7.00 (m, 2H), 5.09 (s, 2H), 4.02 (s, 2H), 2.80 (s, 4H).

MS calculated for C$_{19}$H$_{19}$NO$_6$ [M+H$_2$O]$^+$: 357.4. Found 357.0.

4-nitrophenyl 2-(4-(benzylloxy)phenyl)acetate (22): To a 250 mL round bottom flask, 2-(4-(benzylloxy)phenyl)acetic acid (1 g, 4.1 mmol), 4-nitrophenol (0.632 g, 4.54 mmol), and methylene chloride (41 ml) were added. The flask was cooled to 0°C and N,N'-methanediylidenedicyclohexanamine (0.937 g, 4.54 mmol) and N,N-dimethylpyridin-4-amine (0.050 g, 0.413 mmol) were charged. The flask was warmed to r.t. and stirred for 18 h. At reaction completion, 5 mL 3M HCl was added and the flask chilled to -20°C in a freezer for 6 h. The slurry was filtered over a Celite pad and washed with ice-cold DCM (30 mL). The resultant filtrate was washed with saturated NaHCO$_3$ (10 mL), water (10 mL), dried over sodium sulfate, filtered, and concentrated to a solid. The crude product was purified by flash chromatography (0-30% EtOAc/Heptane) to afford 22 (1.22 g, 81%). $^1$H NMR (400 MHz, CDCl$_3$) δ 8.30 (m, 2H), 7.52 – 7.26 (m, 10H), 7.07 – 7.00 (m, 2H), 5.12 (s, 2H), 3.88 (s, 2H). No mass spectral data is available due to instability of the compound under analysis conditions.

perfluorophenyl 2-(4-(benzylloxy)phenyl)acetate (24): To a 250 mL round bottom flask, 2-(4-(benzylloxy)phenyl)acetic acid (1 g, 4.1 mmol), 2,3,4,5,6-pentafluorophenol (0.836 g, 4.54 mmol), and methylene chloride (41 ml) were added. The flask was cooled to 0°C and N,N'-methanediylidenedicyclohexanamine (0.937 g, 4.54 mmol) and N,N-dimethylpyridin-4-amine (0.050 g, 0.413 mmol) were charged. The flask was warmed to ambient temperature and stirred for 18 h. At reaction completion, 3M HCl (5 mL) was added and the flask chilled to -20°C in a freezer for 6 h. The slurry was filtered over a Celite pad and washed with ice-cold DCM (30 mL). The resultant filtrate was washed with saturated aqueous NaHCO$_3$ (10 mL), water (10 mL), dried over sodium sulfate, filtered, and concentrated to a solid. The crude product was purified by silica gel flash chromatography (0-30% EtOAc/Heptane) to afford 24 (1.25 g, 74%). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.57 – 7.36 (m, 5H), 7.36 – 7.24 (m, 2H), 7.04 (m, 2H), 5.12 (s, 2H), 3.96 (s, 2H). No mass spectral data is available due to instability of the compound under analysis conditions.

General procedure for acylation of 19 with 16, 21, 22, 24:

Compound 19 (20 mg, 0.016 mmol) and tetrahydrofuran (250 µl) were added to a 4mL vial. The vial was cooled to -20°C and charged with N-ethyl-N-isopropylpropan-2-amine (7 µl, 0.06 mmol), 16, 21, 22, or 24 (0.04 mmol) in tetrahydrofuran (250 µl), and N,N-dimethylpyridin-4-amine (0.2 mg, 1.6 µmol) in tetrahydrofuran (125 µl). The mixture was stirred at the indicated temperature and analyzed by HPLC to determine conversion to product and isomer ratio.
Procedure for acylation of 19 with in situ-generated 23:

2-(4-((benzoxyl)phenyl)acetic acid (5.9 mg, 0.024 mmol) and HATU (10.7 mg, 0.028 mmol) were added to a 4 ml vial. The contents of the vial were dissolved in acetonitrile (300 µl). Then, N-ethyl-N-isopropylpropan-2-amine (9 µl, 0.05 mmol) was added to the above vial, and the contents were stirred at 23 °C for 0.5 h. The vial was cooled to 0 °C and a solution of 12 (20 mg, 0.016 mmol) in N,N-dimethyl formamide (300 µl) was added. After 1 h at 0 °C, the reaction was analyzed by HPLC.

Representative HPLC for acylation products 17 and 1:

Method information: Ascentis Express C18 15 cm x 4.6 mm, 2.7 micron. Column oven: 35°C. Flow rate: 1.5 mL/min. Mobile Phase A: 0.1% formic acid in water. Mobile Phase B: acetonitrile. Gradient: 0 min 30% B, gradient over 15 min to 95% B, hold until 20 min at 95% B, gradient over 2 min to 10% B, Stop time 22 min.
Desired product 17: 15.99 min; Undesired isomer 18: 16.11 min.

The crude product mixture was worked up by dilution with methylene chloride and sequential washing with 1M HCl (1 mL), saturated sodium bicarbonate (1 mL), and saturated brine (1 mL) solutions. The resultant product was analyzed by LCMS: calculated for C_{73}H_{81}F_{15}N_{10}O_{11} [M-C_{6}H_{5}O_{2}+3H]^{2+}: 684. Found: 684.

Deprotection of crude 17/18 to form SI-1/SI-2:
A portion of crude 17/18 was treated with trifluoroacetic acid (80 equiv) to generate a mixture of products \textit{Si}-1 and \textit{Si}-2, which were isolated by successive flash chromatography (silica, 0-5\% MeOH/CH$_2$Cl$_2$, then C-18 functionalized silica, 10-90\% MeCN/water). A fraction containing a 4:1 mixture of \textit{Si}-1 and \textit{Si}-2 was then analyzed by NMR spectroscopy for structural assignment of the product regioisomers. The isolated material was analyzed by LCMS: calculated for C$_{73}$H$_{81}$F$_{5}$N$_{10}$O$_{11}$ [M+2H]$^{2+}$: 684. Found: 684.

\textit{Regioisomer Assignment:}

Sample 15001377-604-ISOLATED_4-1 was analyzed by utilizing the following NMR techniques: $^1$H-NMR, $^1$H-$^1$H ROESY, $^1$H-$^1$C HSQC, $^1$H-$^1$C HMBC. The major compound in 15001377-604-ISOLATED_4-1 was confirmed to be regioisomer \textit{Si}-1 due to the $^1$H-$^1$H ROESY correlation observed between the connecting C73 methylene proton H73 having $^1$H-$^1$H ROESY correlations to benzimidazole proton H14 (1H NMR 7.63 ppm) which shares $^3$J$_{HF}$ coupling to F40 with a measured $^3$J$_{HF}$ = 9.66 Hz. The smaller coupling for the benzimidazole proton H17 appears to be closer to $^3$J$_{HF}$ = 7.02 Hz keeping in mind there is extensive peak overlap. The assignments of these protons were made with the consideration of compound 12 assignments generated from 2D NMR data collected in (CH$_3$)$_2$SO$_2$.

\textbf{IV. Preparation of PIB Prodrugs 2, 3, and 4 from Intermediate 19}
tert-butyl 6-(((2R,5R)-1-((3,5-difluoro-4-(4-(4-fluorophenyl)piperidin-1-yl)phenyl)-5-(6-fluoro-1-(hydroxymethyl))-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-5-yl)pyrrolidin-2-yl)-5-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-1-carboxylate (Compound 19, 48.0 g, 38.6 mmol) was dissolved in THF (600 mL) at -10 °C. The resulting solution was cooled to < -70 °C before addition of LiHMDS (1M in THF, 73.4 mL, 73.4 mmol) slowly via syringe over 5 minutes, maintaining the internal temperature below -65 °C during the addition. After the addition was complete, the mixture was stirred for 3 min before addition of dibenzyl (4-(2-chloro-2-oxoethyl)phenyl) phosphate (41.6 g, 97 mmol) dropwise as a solution in 75 mL of THF (120 mL) over 7 minutes. The internal temperature did not exceed -65 °C during this addition. The reaction was allowed to stir for 15 min at the same temperature then warmed to -50 °C by removing the flask from the acetone-dry ice bath. The reaction was subsequently quenched by addition of 2:1 saturated ammonium chloride:water mixture (150 mL). The mixture was diluted with ethyl acetate (300 mL) and stirred at ambient temperature for 30 min. The layers were separated, and the organic layer was washed with 1M HCl (3x50 mL) and brine (100 mL) then dried over sodium sulfate and concentrated in vacuo to give a crude solid (~63 g), which was used directly in the Boc-deprotection without additional purification as follows:

tert-butyl 6-(((2R,5R)-1-((2-((bis(benzyloxy)phosphoryl)oxy)phenyl)acetoxy)methyl)-6-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-5-yl)pyrrolidin-2-yl)-5-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-1-carboxylate (63.2 g, 38.6 mmol) was dissolved in 150 mL of DCM and cooled to approximately 10 °C before addition of TFA (89.0 mL, 1.15 mol) via syringe, maintaining the temperature below 25 °C. The flask was removed from the ice-water bath and stirred at RT for 90 min, at which point it was complete as indicated by LC-MS analysis. The reaction mixture was concentrated in vacuo and azeotroped with 2x200 mL of EtOAc to give a crude product that was purified via flash chromatography, eluting on a 1.5 kg silica gel column with a heptanes:acetone mobile phase (isocratic 95:5 heptanes:acetone for 5 min, 95:5 to 65:35 over 15 min, then isocratic 65:35) until complete elution of 25. This method efficiently rejected the minor isomer 26 and unreacted starting materials/byproducts to give material of > 95% HPLC purity. Note that NMR spectral data are generally complicated by the presence of rotameric and/or tautomeric forms, and spectra have been included from solvents that minimize these additional forms. Attempts at obtaining
clearer NMR spectra at elevated temperatures were unsuccessful. **Major isomer (25):** ¹H NMR (400 MHz, THF-d₈) δ 10.99 (t, J = 50.3 Hz, 1H), 7.45 (d, J = 10.3 Hz, 1H), 7.36 – 7.22 (m, 12H), 7.20 (dd, J = 8.5, 5.6 Hz, 4H), 7.16 – 7.13 (m, 1H), 7.13 – 7.09 (m, 2H), 6.95 (t, J = 8.8 Hz, 2H), 6.56 (d, J = 11.7 Hz, 1H), 6.39 (d, J = 18.9 Hz, 1H), 6.25 (d, J = 11.7 Hz, 1H), 6.12 (s, 1H), 5.86 (d, J = 12.5 Hz, 2H), 5.51 (d, J = 29.4 Hz, 2H), 5.34 (dd, J = 8.0, 4.5 Hz, 1H), 5.27 (s, 1H), 5.10 (s, 2H), 5.09 (s, 2H), 4.44 (dd, J = 8.3, 5.3 Hz, 1H), 4.28 (dd, J = 8.6, 4.3 Hz, 1H), 3.92 – 3.68 (m, 5H), 3.66 (d, J = 5.0 Hz, 2H), 3.36 (d, J = 6.2 Hz, 3H), 3.53 (s, 3H), 3.42 – 3.30 (m, 2H), 3.16 (s, 2H), 3.12 – 2.92 (m, 6H), 2.75 (s, 3H), 2.22 – 1.93 (m, 4H), 1.93 – 1.80 (m, 3H), 1.35 – 1.21 (m, 4H), 1.14 – 1.04 (m, 1H), 1.12 – 1.05 (m, 1H), 1.01 (d, J = 6.3 Hz, 3H), 0.85 (d, J = 6.2 Hz, 3H).

**¹³C NMR:**
(101 MHz, THF-d₈) δ 172.17, 171.74, 170.7, 164.4, 163.4, 163.5, 163.4, 162.0, 161.1, 161.0, 160.2, 159.0, 158.6, 158.5, 157.9, 152.0, 144.4, 144.3, 140.5, 138.1, 138.0, 135.6, 135.4, 132.3, 132.0, 130.1, 130.0, 130.0, 129.9, 129.6, 126.8, 126.5, 121.8, 121.7, 121.6, 121.5, 119.4, 119.0, 119.0, 116.6, 116.4, 99.8, 99.5, 99.2, 98.9, 78.7, 78.1, 71.1, 71.2, 71.1, 59.4, 58.7, 58.6, 57.9, 57.7, 56.5, 54.4, 54.3, 52.9, 52.8, 49.0, 48.7, 43.5, 41.3, 36.3, 32.9, 32.7, 31.4, 31.2, 30.8, 30.6, 26.9, 26.8, 24.4, 23.9, 23.9, 21.9, 20.4, 17.4, 16.8, 15.3. **¹⁹F NMR:**
(162 MHz, THF-d₈) δ -5.9; **¹⁹F NMR:**
(376 MHz, THF-d₈) δ -118.8 (td, J = 9.1, 4.5 Hz), -120.1 (s), -124.7 (s), -126.4 (s), -128.0 (s); Specific rotation: [α] D21.4 = +3.0 (c = 1.0, MeOH); ESI-LCMS: Calculated for [(M/2) + H]+: 769.78; found: 769.80.

![Chemical Structure](image)

(6-((2R,5R)-1-(3,5-difluoro-4-(4-(fluorophenyl)piperidin-1-yl)phenyl)-5-(6-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-5-yl)pyrrolidin-2-yl)-5-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-1-yl)methyl 2-(4-((bis(benzyloxy)phosphoryl)oxy)phenyl)acetate (Compound 26, minor diastereomer):

Obtained as the minor component from flash chromatographic separation on silica gel as described for compound 25. Note that this compound exists as a mixture of tautomers and/or rotamers. Attempts to obtain sharper spectral peaks at higher temperatures were unsuccessful and were generally worse in alternative solvents. **¹H NMR:**
(500 MHz, DMSO-d₆) δ 12.11 (s, 1H), 7.47 (d, J = 36.7 Hz, 2H), 7.35 (qd, J = 4.7, 2.4 Hz, 11H), 7.27 (d, J = 18.5 Hz, 12H), 6.50 – 6.25 (m, 2H), 6.01 – 5.78 (m, 2H), 5.74 – 5.43 (m, 2H), 5.31 – 5.19 (m, 1H), 5.18 (s, 1H), 5.14 (d, J = 1.2 Hz, 2H), 5.12 (d, J = 1.1 Hz, 2H), 4.36 – 4.16 (m, 2H), 3.92 (d, J = 4.1 Hz, 1H), 3.88 – 3.72 (m, 3H), 3.58 (s, 1H), 3.57 – 3.51 (m, 4H), 3.48 (d, J = 2.5 Hz, 3H), 3.27 (d, J = 2.9 Hz, 1H), 3.17 (s, 3H), 3.14 (s, 1H), 3.04 (s, 2H), 2.77 (s, 3H), 2.58 (s, 3H), 2.12 (s, 3H), 2.12 – 1.91 (m, 3H), 1.88 – 1.74 (m, 3H), 1.56 (br d, J = 110.5 Hz, 6H), 1.04 (d, J = 6.2 Hz, 3H), 1.01 (d, J = 6.2 Hz, 3H). ESI-LCMS:
Calculated for [(M/2) + H]+: 769.78; found: 769.80.
(5-((2R,5R)-1-(3,5-difluoro-4-(4-(4-fluorophenyl)piperidin-1-yl)phenyl)-5-(5-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxy carbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-6-yl)pyrrolidin-2-yl)-6-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxy carbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-1-yl)methyl 2-(4-(phosphonoxy)phenyl)acetate (compound 3): (5-((2R,5R)-1-(3,5-difluoro-4-(4-(4-fluorophenyl)piperidin-1-yl)phenyl)-5-(5-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxy carbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-6-yl)pyrrolidin-2-yl)-6-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxy carbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-1-yl)methyl 2-(4-(bis(benzoxypyrophosphoryl)oxy)phenyl)acetate (Compound 25).

**Preparation of Trimethyl Lock Acid S4**

1. Preparation 1: 25.0, 24.8, 19.5, 15.2, 15.1.

**Spectral data was consistent with those previously reported for the title compound.**

1. Spectral data was consistent with those previously reported for the title compound.  

**ESI-LCMS : Calculated for [(M/2) + H]⁺: 679.66; found: 679.50.**
The synthesis of 3-[2'-((dibenzylphosphono)oxy)-4',6'-dimethylphenyl]-3,3-dimethylpropionic acid (S4) was accomplished in 3 steps as shown in Scheme S1. The synthesis was first reported by Boorchardt et al.\textsuperscript{2} where S4 was prepared in 6 steps. Starting from the same commercially available 3,5-dimethylphenol (S1) and methyl 3,3-dimethylacrylate, the lactone S2 was prepared in methanesulfonic acid. At this point, several steps were eliminated from the synthesis, including a reduction, protection, and deprotection, by opening the lactone with potassium hydroxide in methanol to generate potassium 3-(2,4-dimethyl-6-oxidophenyl)-3-methylbutanoate (S3). The dipotassium salt S3 was phosphorylated at the phenolic hydroxylate group using tetrabenzy1 pyrophosphate to generate the trimethyl lock carboxylic acid S4.

\textbf{Scheme S1. New abbreviated synthesis of trimethyl lock acid S4}

\textbf{Compound S2.} Compound S2 was prepared according to the published procedure\textsuperscript{2}

\textbf{Compound S3:} KOH powder (34.5 g, 5.23 mmol, 2.4 eq) was dissolved in methanol (445 mL). The solution was cooled to 25 °C and Compound S2 (44.5 g, 218 mmol) was added. The reaction mixture was stirred at room temperature and monitored by NMR. After 3 h, the reaction mixture was clarified through a 0.45 micron filter and concentrated under reduced pressure. The resulting residue was chased with heptane to afford 3-(2,4-dimethyl-6-oxidophenyl)-3-methylbutanoate (S3) (78 g, 78% w/w, quant.) as a foam. The product is hygroscopic and should be stored under nitrogen. \textsuperscript{1}H NMR (400 MHz, DMSO-d6) δ 5.73 (d, J = 2.1 Hz, 1H), 5.36 (d, J = 2.2 Hz, 1H), 2.60 (s, 2H), 2.23 (s, 3H), 1.89 (s, 3H), 1.46 (s, 6H).
3-(2-((bis(benzyloxy)phosphoryl)oxy)-4,6-dimethylphenyl)-3-methylbutanoic acid (S4): To a solution of Compound S3 (1.68 g, 5.63 mmol) in DMF (24 mL) was added tetrabenzyl pyrophosphate (3.18 g, 5.91 mmol) dissolved in DMF (8 mL) at 80 C. After the addition, the reaction mixture was warmed to 25 C. After 2 h, the reaction mixture was quenched with water (100 mL) followed by extraction with MTBE (100 mL). The organic layer was washed with brine, dried with sodium sulfate, and concentrated. The crude product was purified by reverse phase chromatography to afford S4 (1.29 g, 53.5%) as a white solid. 1H NMR (400 MHz, CDCl3) δ 7.45 – 7.28 (m, 10H), 7.04 (s, 1H), 6.80 – 6.71 (m, 1H), 5.23 – 5.03 (m, 5H), 2.89 (s, 2H), 2.54 (s, 3H), 2.17 (s, 3H), 1.64 (s, 6H). MS(ESI) [M-H]: calculated 482.5; found 481.2

Reverse-Phase Chromatography: The gradient was 30-90% B in 4 min, 90-90% B in 2 min, 90-30% B in 0.01 min, and then hold at 30% B for 0.5 min (1 mL/min flow rate). Mobile phase A was 10 mM NH4HCO3, mobile phase B was HPLC grade CH3CN. The column used for the chromatography is a 2.1 x 50 mm Xbridge C18 column (5 µm particle size).

5-((2R,5R)-1-((3,5-difluoro-4-(4-fluorophenyl)piperidin-1-yl)phenyl)-5-(5-fluoro-2-(((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-6-yl)pyrrolidin-2-yl)-6-fluoro-2-(((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-1-yl)methyl-3-(2-((bis(benzyloxy)phosphoryl)oxy)-4,6-dimethylphenyl)-3-methylbutanoate (S5): A solution of 3-((2-((bis(benzyloxy)phosphoryl)oxy)-4,6-dimethylphenyl)-3-methylbutanoic acid S4 (388 mg, 0.804 mmol) in DCM (4 mL) was stirred at 23 C and DMF (0.1 M in DCM, 0.080 mL, 0.008 mmol) and oxalyl chloride (0.106 mL, 1.21 mmol) were added. After 1 h, LC-MS TFA method (aliquot MeOH quench) showed nearly complete acid chloride formation. The mixture was concentrated to minimal volume, THF (1 mL) was added, and the mixture was concentrated to minimal volume again. The resulting yellow oil acid chloride 27 was used without purification or characterization.

A solution of tert-butyl 6-((2R,5R)-1-((3,5-difluoro-4-(4-fluorophenyl)piperidin-1-yl)phenyl)-5-(5-fluoro-1-(hydroxymethyl)-2-(((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-5-yl)pyrrolidin-2-yl)-5-fluoro-2-(((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-5-yl)-1H-benzo[d]imidazol-1-carboxylate 19 (500 mg, 0.402 mmol) in THF (5 mL) was cooled to < -75 C and LHMDS (1 M in THF, 0.764 mL, 0.764 mmol) was added over 2 min at < -74 C. After 2 min, added the acid chloride prepared above in THF (0.4 + 0.3 + 0.3 mL) at < -72 C over 2 min and LC-MS AA long method showed 97% conversion (manually integrating the peaks at 4.17 min and 4.94 min). Removed the cooling bath, added water (5 mL) and EtOAc (20 mL), separated layers, and washed the organic layer with saturated aqueous NaHCO3 (5 mL) and brine (2 mL). Dried (Na2SO4), concentrated, and dissolved crude tert-butyl 6-((2R,5R)-5-1-((3-((2-((bis(benzyloxy)phosphoryl)oxy)-4,6-dimethylphenyl)-3-methylbutanoxy)methyl)-6-fluoro-2-(((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-5-yl)-1-(3,5-difluoro-4-(4-fluorophenyl)piperidin-1-yl)phenyl)pyrrolidin-2-yl)-5-fluoro-2-(((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazole-1-in DCM (4 mL).
TFA (1 mL) was added and the solution was stirred at 23 °C for 1 h, when LC-MS AA long method showed a 29:1 ratio of regioisomers (integrating peaks at 4.46 min = minor and 4.52 min = major). Added EtOAc (20 mL) and saturated aqueous NaHCO₃ (20 mL), separated layers, and washed with satd aq NaHCO₃ (2 x 10 mL) and brine (5 mL). Dried (Na₂SO₄), concentrated, and silica gel chromatography (40-75% acetone/heptanes gradient elution; good regioisomer separation at ~ 60% acetone/heptanes) gave S5 (541 mg, 0.337 mmol, 84 % yield): ¹H NMR (500 MHz, THF-d₈) δ 11.07 (d, J = 64.3 Hz, 1H), 7.44 – 7.23 (m, 12H), 7.20 (dd, J = 8.6, 5.6 Hz, 3H), 7.12 (d, J = 6.7 Hz, 1H), 7.03 (s, 1H), 6.94 (t, J = 8.8 Hz, 2H), 6.47 (s, 1H), 6.43 (d, J = 11.7 Hz, 2H), 6.11 (d, J = 8.5 Hz, 1H), 5.99 (d, J = 11.8 Hz, 1H), 5.85 (br d, J = 12.9 Hz, 2H), 5.60 – 5.46 (m, 2H), 5.27 (dd, J = 8.1, 4.7 Hz, 2H), 5.06 (s, 2H), 5.05 (s, 2H), 4.45 (dd, J = 8.3, 5.4 Hz, 1H), 4.26 (dd, J = 8.6, 4.3 Hz, 1H), 3.91 – 3.68 (m, 4H), 3.56 (s, 3H), 3.52 (s, 3H), 3.40 – 3.33 (m, 1H), 3.20-2.87 (m, 8H), 2.71 (s, 3H), 2.64-2.46 (m, 9H), 2.43-2.36 (m, 2H), 2.33 (s, 3H), 2.22-2.18 (m, 2H), 2.09 (s, 3H), 2.06 (br s, 1H), 1.96-1.79 (m, 4H), 1.79-1.75 (m, 2H), 1.70 – 1.63 (m, 2H), 1.54 (s, 3H), 1.51 (s, 3H), 1.01 (d, J = 6.2 Hz, 3H), 0.91 – 0.85 (m, 1H), 0.82 (d, J = 6.2 Hz, 3H). ¹³C NMR (101 MHz, THF-d₈) δ 170.9, 169.9, 168.9, 162.5, 161.6, 160.1, 159.2, 158.3, 157.8, 157.8, 156.8, 156.0, 154.4, 150.2, 142.5, 138.6, 137.9, 136.2, 136.0, 133.6, 131.4, 130.6, 128.4, 128.3, 128.2, 128.2, 128.1, 127.9, 127.9, 127.8, 127.8, 127.8, 127.8, 123.9, 118.8, 117.4, 114.8, 114.6, 98.0, 97.7, 97.4, 97.1, 76.8, 76.2, 69.4, 64.9, 57.6, 56.9, 56.0, 54.8, 52.5, 52.3, 51.1, 47.0, 41.6, 39.2, 34.4, 31.1, 29.6, 29.3, 19.5, 15.6, 15.1. ³¹P NMR (162 MHz, THF-d₈) δ -6.3; ¹⁹F NMR (376 MHz, THF-d₈) δ -117.7 – 118.9 (br s), -119.9 (s), -124.3 (s), -126.1 (s), -127.6 (s); Specific rotation: [α]D °20.9 = -6.2 (c = 1.0, MeOH). No molecular ion was observed for this intermediate under numerous ionization conditions.

![Chemical structure](image)

**A2**

3-(2,4-dimethyl-6-(phosphonoxy)-3-methylbutanoate (2): A solution of (5-((2R,5R)-1-(3,5-difluoro-4-(4-(4-fluorophenyl)piperidin-1-yl)phenyl)-5-(6-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxy carbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-5-
pyrrolidin-2-yl)-6-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxy carbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-5-yl)methyl)benzyl 3-(2,4-dimethyl-6-(phosphonoxy)-3-methylbutanoate S5 (1.00 g, 0.622 mmol) in THF (7 mL) was added to 5% Pd/C (wet JM#9) (0.099 g) in a 20 mL Barnstead Hastelloy stainless steel reactor and stirred for 24 h at 50 psi hydrogen pressure at 25 °C. The mixture was filtered, washing the catalyst bed with THF (2 x 5 mL). The solution was concentrated, IPA (25 mL) was added, the slurry was heated to 70 °C, and a dark solution was observed. This was cooled to ambient temperature, filtered, and the gray solid was dried in an ambient temperature vacuum oven, giving crude product (644 mg, 73%). This material was slurried in acetone (20 mL) for 10 min, filtered, and washed with acetone (2 x 5 mL). The white solid was dried in an ambient temperature vacuum oven to constant weight, giving 2 (565 mg, 0.396 mmol, 64%): ¹H NMR (500 MHz, CD₃OD) δ 7.34 (d, J = 10.3 Hz, 2H), 7.26 – 7.18 (m, 3H), 7.15 (dd, J = 10.5, 6.5 Hz, 2H), 6.96 (t, J = 8.8 Hz, 2H),
Dibenzyl (5-((2R,5R)-1-(3,5-difluoro-4-(4-(4-fluorophenyl)piperidin-1-yl)phenyl)-5-(5-fluoro-2-((R)-1-((2R,3S)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)pyrrolidin-2-yl)-6-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzod[d]imidazol-6-yl)methyl phosphate (S6): A solution of 1H-tetrazole (0.45 M in acetonitrile) (8.94 ml, 4.02 mmol) was cooled in a dry ice/acetonitrile bath, and tert-butyl 6-((2R,5R)-1-(3,5-difluoro-4-(4-(4-fluorophenyl)piperidin-1-yl)phenyl)-5-(5-fluoro-1-(hydroxymethyl)-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzod[d]imidazol-5-yl)pyrrolidin-2-yl)-5-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzod[d]imidazol-1-carboxylate (1.00 g, 0.804 mmol) was added. The cooled mixture was vigorously stirred while dibenzyl diisopropylphosphoramidite (1.55 ml, 4.02 mmol) was added dropwise over 10 min. The mixture was allowed to slowly warm to -10 °C over 2 h. The mixture was slowly warmed to 0 °C, then hydrogen peroxide (50% aq) (0.493 ml, 8.04 mmol) was added, and the resulting solution was allowed to warm to ambient temperature. Diluted with EtOAc, washed with aq Na2SO4 and aq NaHCO3, dried over Na2SO4, filtered, and concentrated. The crude product was dissolved in DCM (8 ml) and TFA (trifluoroacetic acid) (4 ml, 51.9 mmol) was added. After stirring for 30 min, concentrated to give an oil. The oil was dissolved in DCM (20 ml), washed with satd aq NaHCO3, dried over Na2SO4, filtered and concentrated. The residue was purified by column chromatography on silica gel using a solvent gradient of 30-80% acetone in heptanes, giving S6 (725 mg, 65%). A minor byprod eluted just before the desired prod, which is a bis-phosphomethyl byprod (98 mg, MW = 1694).

S6: 1H NMR (500 MHz, CD3OD) δ 7.46 (d, J = 10.0 Hz, 1H), 7.39-7.26 (m, 7H), 7.27-7.06 (s, 7H), 6.98 (t, J = 8.8 Hz, 2H), 6.42 (t, J = 11.9 Hz, 1H), 6.16 (t, J = 12.1 Hz, 1H), 5.82 (d, J = 12.9 Hz, 2H), 5.64 – 5.51 (m, 2H), 5.29 – 5.18 (m, 2H), 5.02 (dd, J = 9.0, 6.8 Hz, 4H), 4.58 (br s, 2H), 4.43 (br s, 1H), 4.33 (d, J = 4.0 Hz, 1H), 3.93 (m, 3H), 3.81 (dt, J = 9.5, 6.9 Hz, 1H), 3.66 (br s, 3H), 3.65 (s, 3H), 3.56 – 3.49 (m, 1H), 3.12-3.00 (m, 3H), 2.97-2.89 (br m, 2H), 2.81 (s, 3H), 2.62-2.49 (m, 3H), 2.44-2.34 (m, 1H), 2.35-2.18 (m, 4H), 2.15-2.06 (m, 2H), 2.09-1.86 (m, 4H), 1.86-1.73 (m, 5H), 1.09 (d, J = 6.2 Hz, 3H), 0.96 (d, J = 6.3 Hz,
\[\text{3H}. \quad ^{13}\text{C NMR (101 MHz, CD}_3\text{OD)} \delta 170.4, 170.0, 162.4, 161.5, 161.4, 160.0, 159.1, 159.0, 158.8, 158.4, 157.9, 156.8, 156.7, 156.4, 156.4, 142.3, 137.6, 135.3, 135.19, 135.21, 133.1, 133.0, 128.5, 128.5, 128.3, 128.0, 127.9, 127.8, 127.7, 127.3, 127.2, 125.0, 124.9, 119.4, 117.4, 117.2, 115.0, 114.5, 113.0, 110.2, 100.9, 98.2, 97.9, 97.9, 97.1, 76.1, 75.7, 69.9, 69.9, 69.8, 69.8, 69.2, 67.1, 57.5, 57.2, 56.1, 55.9, 55.2, 52.8, 52.4, 51.6, 41.3, 34.1, 31.2, 30.7, 25.0, 24.9, 15.3, 15.1, 14.3; ^{31}\text{P NMR (162 MHz, CD}_3\text{OD)} \delta -2.3. \quad ^{19}\text{F NMR (376 MHz, CD}_3\text{OD)} \delta -119.4 (\text{dd}, J = 14.0, 8.8, 5.3 \text{ Hz}), -120.2 (\text{s}), -120.5 (\text{s}), -124.1 - -124.7 (\text{m}), -126.3 (\text{s}). \quad \text{Specific rotation: } [\alpha]_D^{21.3} = +16.6 (c = 1.0, \text{MeOH}); \quad \text{ESI-LCMS: Calculated for } [(\text{M}/2)+\text{H}]^{+}: 702.72; \text{found: 702.58.}

(5-((2R,5R)-1-((3,5-difluoro-4-(4-(4-fluorophenyl)piperidin-1-yl)phenyl)-5-(5-fluoro-2-((2R,3S)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-6-yl)pyrrolidin-2-yl)-6-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-6-yl)methyl dihydrogen phosphate 4: A solution of dibenzyl (5-((2R,5R)-1-((3,5-difluoro-4-(4-(4-fluorophenyl)piperidin-1-yl)phenyl)-5-(5-fluoro-2-((2R,3S)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-6-yl)pyrrolidin-2-yl)-6-fluoro-2-((S)-1-((2S,3R)-3-methoxy-2-((methoxycarbonyl)amino)butanoyl)pyrrolidin-2-yl)-1H-benzo[d]imidazol-6-yl)methyl phosphate S6 (11.3 g, 8.05 mmol) in THF (110 mL) and water (110 mL) was added to 5\% Pd/C (2.26 g, 9.90 mmol) in a 600 mL 316SS reactor and stirred for 1.7 h at 50 psi hydrogen pressure without external heating. The reaction mixture was filtered and concentrated until THF distillation was complete, giving a white slurry. Sonicated the slurry, stirred 30 min, and filtered, washing with water (2 x 50 mL). The wet cake was stirred with ACN (110 mL) overnight and the white solid was filtered, washed with ACN (2 x 22 mL). After drying in a vacuum oven at 50 °C to constant weight, 4 (8.92 g, 91\%) was obtained as a white solid: \text{H NMR (600 MHz, CD}_3\text{OD) } \delta 7.60 (d, J = 10.3 \text{ Hz, 1H}), 7.33 (d, J = 10.0 \text{ Hz, 1H}), 7.25 – 7.17 (m, 3H), 7.14 (d, J = 6.6 \text{ Hz, 2H}), 6.96 (t, J = 8.8 \text{ Hz, 2H}), 6.28 – 6.15 (m, 1H), 5.97 (dd, J = 11.1, 9.2 \text{ Hz, 1H}), 5.81 (d, J = 12.6 \text{ Hz, 2H}), 5.52 (t, J = 7.6 \text{ Hz, 3H}), 5.44 (t, J = 7.1 \text{ Hz, 1H}), 5.23 (dd, J = 8.1, 5.2 \text{ Hz, 1H}), 4.41 (d, J = 4.5 \text{ Hz, 1H}), 4.34 (d, J = 4.0 \text{ Hz, 1H}), 4.01 – 3.78 (m, 4H), 3.64 (s, 3H), 3.62 (s, 3H), 3.56 (qd, J = 6.3, 3.8 \text{ Hz, 2H}), 3.34 (s, 1H), 3.16 – 2.97 (m, 5H), 2.92 (dt, J = 11.7, 3.5 \text{ Hz, 2H}), 2.85 (s, 3H), 2.60 – 2.47 (m, 5H), 2.38 (dq, J = 12.6, 7.4 \text{ Hz, 1H}), 2.35 – 2.22 (m, 3H), 2.19 (dq, J = 12.2, 6.0 \text{ Hz, 1H}), 2.09 (tt, J = 8.3, 5.2 \text{ Hz, 1H}), 1.95 (dt, J = 10.6, 5.3 \text{ Hz, 3H}), 1.72 (tdd, J = 17.3, 8.2, 3.2 \text{ Hz, 4H}), 1.14 (d, J = 6.3 \text{ Hz, 1H}), 1.07 (d, J = 6.3 \text{ Hz, 3H}), 0.98 (d, J = 6.3 \text{ Hz, 3H}). \quad \text{C NMR (101 MHz, CD}_3\text{OD) } \delta 170.3, 169.9, 162.5, 161.5, 160.1, 159.1, 159.0, 158.6, 157.9, 157.8, 157.1, 157.0, 156.9, 156.2, 142.4, 142.3, 142.3, 137.5, 133.7, 133.6, 128.0, 127.9, 124.3, 124.1, 117.2, 116.5, 116.5, 114.6, 114.4, 98.3, 98.0, 97.3, 97.0, 77.4, 76.1, 75.7, 67.9, 57.6, 57.5, 57.1, 56.1, 55.9, 55.8, 55.5, 53.4, 52.5, 52.4, 51.5, 51.4, 51.2, 41.4, 34.1, 34.1, 31.3, 31.2, 30.7, 30.6, 24.9, 24.8, 15.3, 15.0, 14.3, 14.2. \quad \text{P NMR (162 MHz, CD}_3\text{OD) } \delta -2.3. \quad \text{F NMR (376 MHz, CD}_3\text{OD) } \delta -119.4 (\text{dd}, J = 14.0, 8.8, 5.3 \text{ Hz}), -120.2 (\text{s}), -120.5 (\text{s}), -124.1 - -124.7 (\text{m}), -126.3 (\text{s}). \quad \text{Specific rotation: } [\alpha]_D^{21.6} = +16.6 (c = 1.0, \text{MeOH}); \quad \text{ESI-LCMS: Calculated for } [(\text{M}/2)+\text{H}]^{+}: 612.60; \text{found: 612.34.}
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Spectral Data for New Compounds
Compound 12, $^1$H NMR (CDCl$_3$, 600 MHz):
Compound 12, $^{13}$C ($^1$H) NMR (CDCl$_3$, 151 MHz):
Compound 12, $^{19}$F NMR (CDCl$_3$, 376 MHz):
Compound 12, $^1$H NMR (CDCl$_3$, 600 MHz):
Compound 12, $^{13}$C ($^1$H) NMR (CDCl$_3$, 151 MHz):

The NMR spectrum shows the chemical shifts for various carbon atoms in compound 12. The spectrum includes peaks at various ppm values, indicating the presence of different chemical environments.

Specifically, the spectrum displays signals at ppm values ranging from 0 to 200, with some peaks being more pronounced than others. The assignments of these peaks to specific carbon atoms are indicated in the figure.
Compound 12, $^1$H-$^1$H ROESY NMR (CDCl$_3$, 600 MHz):

[Image of a ROESY spectrum with peaks labeled from 1 to 52, and chemical shifts indicated for various peaks.]
Compound 12, $^1$H-$^1$H ROESY NMR (CDCl$_3$, 600 MHz):
Compound 12, $^1$H NMR (CDCl$_3$, 600 MHz)

Temperature = 27 C assignments

| Atom | Chemical Shift |
|------|----------------|
| 1 C  | 57.27          |
| H    | 5.41           |
| 2 C  | 30.96          |
| H'   | 1.86           |
| H''  | 2.53           |
| 3 C  | 31.04          |
| H'   | 1.87           |
| H''  | 2.51           |
| 4 C  | 57.37, 57.57   |
| H    | 5.44           |
| 5 N  | 80.90          |
| 6 C  | 141.76         |
| 7 C  | 97.52          |
| H    | 5.79           |
| 8 C  | 160.22         |
| 9 C  | 117.72         |
| 10 C | 160.22         |
| 11 C | 97.52          |
| H    | 5.79           |
| 12 C | 124.00         |
| 13 C | 125.95         |
| 14 C | 157.79         |
| 15 C | 103.09         |
| H    | 7.68           |
| 16 C | 132.36         |
| 17 C | 137.90         |
| 18 C | 117.76, 118.26 |
| H    | 7.12, 7.37     |
| 19 C | 108.79, 117.51 |
| H    | 6.91, 7.32     |

| Atom | Chemical Shift |
|------|----------------|
| 20 C | 138.66         |
| 21 C | 132.68         |
| 22 C | 97.99, 105.68  |
| H    | 7.09, 7.44     |
| 23 C | 157.40         |
| 24 C | 56.08          |
| H    | 5.95           |
| 25 C | 55.09          |
| H    | 5.44           |
| 26 C | 30.71          |
| H'   | 2.05           |
| H''  | 2.37           |
| 27 C | 24.02          |
| H'   | 2.07           |
| H''  | 2.17           |
| 28 C | 47.03          |
| H'   | 3.82           |
| H''  | 3.93           |
| 29 N | 132.81         |
| 30 N | 137.53         |
| 31 C | 47.82          |
| H'   | 3.74           |
| H''  | 3.77           |
| 32 C | 25.28          |
| H'   | 2.10           |
| H''  | 2.23           |
| 33 C | 28.02          |
| H'   | 2.25           |
| H''  | 2.87           |
| 34 C | 170.64         |
| 35 C | 168.93         |
| 36 O | 56.29          |
| H    | 4.51, 4.59     |
| 38 O | 56.57          |
| H    | 4.31, 4.40     |
| 40 N | 78.25          |
| H    | 5.58           |
| 41 N | 79.22          |
| H    | 5.68           |
| 42 C | 157.27         |
| 43 O | 56.57          |
| 44 O | 3.00           |
| 45 C | 75.93          |
| H    | 3.74           |
| 46 O | 16.56          |
| H'   | 1.16           |
| 48 C | 156.99         |
| 49 O | 76.85          |
| H    | 3.68           |
| 51 O | 52.41          |
| H3   | 3.69           |

| Atom | Chemical Shift |
|------|----------------|
| 55 C | 52.19          |
| H3   | 3.63           |
| 56 N | 56.29          |
| 57 C | 156.95         |
| 58 N | 251.44         |
| 59 N | 10.50          |
| 60 C | 154.79         |
| 61 N | 7.17           |
| 62 F | -121.74, -121.36 |
| 63 F | -126.03, -124.25 |
| 64 N | 39.15          |
| 65 C | 52.52          |
| H''  | 3.00           |
| 66 C | 3.05           |
| 67 F | 41.68          |
| 68 C | 34.40          |
| H'   | 1.75           |
| H''  | 1.78           |
| 69 C | 52.52          |
| H'   | 3.00           |
| H''  | 3.05           |
| 70 F | -119.23        |
| 71 F | -119.23        |
| 72 C | 142.40         |

| Atom | Chemical Shift |
|------|----------------|
| 73 C | 128.13         |
| H    | 7.17           |
| 74 C | 114.86         |
| 75 C | 6.95           |
| 76 C | 161.23         |
| 77 C | 114.86         |
| 78 F | 6.95           |
| 79 C | 128.13         |
| 80 C | 7.17           |
| 81 C | 148.25         |
| 82 O | 86.34          |
| 83 C | 28.00          |
| 84 C | 1.70           |
| 85 C | 28.00          |
| 86 O | 1.70           |
| 87 C | 28.00          |
| H3   | 1.70           |
Compound 11, $^1$H NMR (CDCl$_3$, 500 MHz, -20 °C)
Compound 11, $^{13}$C NMR (CDCl$_3$, 125 MHz)
Compound 11, $^{19}$F NMR (CDCl$_3$, 376 MHz)
Compound 11, $^1$H NMR (CDCl$_3$, 500 MHz, -20 ºC)
Compound 11, $^1$H-$^1$H ROESY NMR (CDCl$_3$, 500 MHz, -20 °C)
Compound 11, -20°C assignments NMR (CDCl$_3$, 500 MHz)

Temperature = -20°C assignments

| Atom | Chemical Shift | Atom | Chemical Shift | Atom | Chemical Shift | Atom | Chemical Shift |
|------|----------------|------|----------------|------|----------------|------|----------------|
| 1 C  | 58.04         | 19 C| 117.05        | 33 C| 27.25         | 92 C| 15.33         |
| H    | 5.40          | H   | 7.31          | H   | 2.80, 2.90    | H   | 1.12          |
| 2 C  | 30.92         | 20 C| 137.69        | 34 C| 171.09        | H   | 7.20          |
| H    | 1.87          | 21 C| 134.04, 134.61|     |                | H   | 7.00          |
| H$^+$| 2.43          | 22 C| 99.24, 99.36  | 35 C| 169.11        | H   | 7.20          |
| 3 C  | 51.53         | H   | 6.79, 6.87    | 36 O| 55.86         | H   | 7.00          |
| H    | 1.72          | 23 C| 156.59        | 37 C| 55.86         | H   | 7.00          |
| H$^+$| 2.52          | 24 C| 56.08         |     |                | H   | 7.00          |
| 4 C  | 57.94         | H   | 6.05          | 38 O| 4.65          | H   | 7.00          |
| H    | 5.37          | 25 C| 54.55, 55.05  |     |                |      |               |
| 5 N  | 141.81        | 26 C| 30.98         | 39 C| 56.82         | H   | 7.00          |
| 7 C  | 97.86         | H   | 2.15          | 40 N| 4.53, 4.70    | H   | 7.00          |
| H    | 5.45, 5.93    | H   | 7.00          | 41 N| 5.85          |      |               |
| 8 C  | 160.33        | 27 C| 23.78         |     |                | H   | 7.00          |
| 9 C  | 117.45        | H$^+$| 2.13         | 42 C| 57.33         | H   | 7.00          |
| 10 C | 160.33        | H$^+$| 2.33         | 43 O| 66.0          |      |               |
| 11 C | 97.86         | 28 C| 47.80         | 44 O| 65.80         | H$^+$| 3.03         |
| H    | 5.84, 5.93    | H$^+$| 3.92         | 45 C| 76.97         |      |               |
| 12 C | 122.45, 122.59| H    | 4.23          | 46 O| 66.0          |      |               |
| 13 C | 125.07        | 29 N| 7.33, 7.40    | 47 C| 16.15         | H$^+$| 3.11         |
| 14 C | 157.32        | 30 N| 47.95         | 48 S| 1.23, 1.72    |      |               |
| 15 C | 106.48        | 31 C| 7.33, 7.40    | 49 C| 157.20        | H   | 2.56          |
| 16 C | 141.46        | H$^+$| 3.83         | 50 C| 77.32         | H$^+$| 1.80         |
| 17 C | 128.66        | 32 C| 25.34         |      |                |      |               |
| 18 C | 113.51, 114.23| H    | 2.16          | 51 O| 52.80         | H$^+$| 3.03         |
| H    | 7.47, 7.54    | H$^+$| 2.37          |      |                |      |               |

Note: The table lists the chemical shifts for various atoms in Compound 11 at -20°C, measured in CDCl$_3$ using a 500 MHz NMR spectrometer.
Compound 11, $^1$H-NMR (CDCl$_3$, 600 MHz):
Compound 11, $^1$H-$^1$H ROESY NMR (CDCl$_3$, 600 MHz):
Compound 11, $^{13}$C {$^1$H} (DMSO-d6, 151 MHz):

The image contains a 13C NMR spectrum with various chemical shifts and peaks labeled with numbers and corresponding frequencies. The spectrum is used to identify the chemical structure and properties of compound 11.
Compound 11, 27 °C assignments NMR (CDCl₃, 600 MHz)

**Mol Formula:** C₉₇H₆₂F₆N₁₀O₁₀<br>
**Mono Mass:** 1212.54<br>
**Molecule Name:** 27C<br>
**Molecule Label:** 27C

| Atom | Chemical Shift |
|------|----------------|
| 1 C  | 57.65          |
| H    | 5.42           |
| 2 C  | 31.05          |
| H'   | 1.85           |
| H''  | 2.51           |
| 3 C  | 31.18          |
| H'   | 1.90           |
| H''  | 2.52           |
| 4 C  | 57.79          |
| H    | 5.40           |
| 5 N  |                |
| 6 C  | 141.81         |
| 7 C  | 97.76          |
| 8 C  | 5.86           |
| 9 C  | 117.70         |
| 10 C | 160.33         |
| 11 C | 97.76          |
| H    | 5.86           |
| 12 C |                |
| 13 C | 125.58         |
| 14 C | 157.46         |
| 15 C | 106.20         |
| H    | 7.31, 7.41     |
| 16 C | 141.69         |
| 17 C | 128.98         |
| 18 C | 113.47         |
| H    | 7.50           |

**BOTTOM Temperature = 27 °C**

| Atom | Chemical Shift |
|------|----------------|
| 19 C | 117.32         |
| H    | 6.68, 7.31     |
| 20 C |                |
| 21 C |                |
| 22 C | 98.43          |
| H    | 7.01, 7.47     |
| 23 C | 157.48         |
| 24 C | 56.24          |
| H    | 5.97           |
| 25 C | 54.98          |
| H    | 5.45           |
| 26 C | 30.86          |
| H'   | 2.11           |
| H''  | 2.42           |
| 27 C | 24.14          |
| H'   | 2.08           |
| H''  | 2.23           |
| 28 C | 47.52          |
| H'   | 3.92           |
| H''  | 4.07           |
| 29 N |                |
| 30 N |                |
| 31 C | 47.85          |
| H'   | 3.73           |
| H''  | 3.80           |
| 32 C | 25.20          |
| H'   | 2.12           |
| H''  | 2.27           |
| 33 C | 27.75          |
| H'   | 2.91           |
| H''  | 2.23           |
| 34 C | 170.82         |
| 35 C | 169.01         |
| 36 O |                |
| 37 C | 56.24          |
| H    | 4.61           |
| 38 O |                |
| 39 C | 56.62          |
| H    | 4.51, 4.56     |
| 40 N |                |
| 41 N |                |
| H    | 6.35           |
| 42 C | 157.02         |
| 43 O |                |
| 44 O |                |
| 45 C | 76.85          |
| H    | 3.75           |
| 46 O |                |
| 47 C | 16.32          |
| H3   | 1.29           |
| 48 C | 157.02         |
| 49 O |                |
| 50 C | 77.04          |
| H    | 3.64           |
| 51 O |                |
| 52 C | 15.10          |
| H3   | 1.13           |
| 53 O |                |
| 54 C | 52.37          |
| H3   | 3.70           |
| 55 C | 52.37          |
| H3   | 3.70           |
| 56 N |                |
| 57 C | 158.23         |
| 58 N |                |
| 59 N |                |
| 60 C |                |
| 61 N |                |
| H    | 10.71          |
| 62 F | -122.89        |
| 63 F | -123.93        |
| 64 N |                |
| 65 C | 52.74          |
| H'   | 3.04           |
| H''  | 3.11           |
| 66 C | 34.44          |
| H2   | 1.76           |
| 67 C | 41.62          |
| H    | 2.53           |
| 68 C | 34.44          |
| H2   | 1.76           |
| 69 C | 52.74          |
| H'   | 3.04           |
| H''  | 3.11           |
Compound 21, $^1\text{H}$ NMR (DMSO-d$_6$, 400 MHz)
Compound 22, \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz):
Compound 24 $^1$H NMR (CDCl$_3$, 400 MHz):
Compounds SI-1, (SI-2 4:1 mixture) $^1$H NMR (DMSO-d6, 600 MHz):
Compounds SI-1 (SI-2), $^1$H-$^1$H ROESY (DMSO-d6, 600 MHz):
Compounds SI-1 (SI-2), $^1$H-$^{13}$C HMBC (DMSO-d6, 600 MHz):
Compound SI-1 (SI-2), $^1$H-$^{13}$C HSQC (DMSO-d6, 600 MHz):
Compound 25, $^1$H NMR (THF-d8, 600 MHz):
Compound 25, $^{13}$C NMR (THF-d8, 151 MHz):
Compound 25, $^{19}$F NMR (THF-d8, 376 MHz)
Compound 25, $^{31}$P NMR (THF-d8, 162 MHz):
Compound 26, $^1$H NMR (DMSO-d6, 500 MHz)
Compound 3, $^1$H NMR (CD$_3$OD, 400 MHz):
Compound 3, $^{13}$C NMR (CD$_3$OD, 101 MHz):
Compound 3, $^{19}$F NMR (CD$_3$OD, 376 MHz):
Compound 3, $^{31}$P NMR (CD$_3$OD, 162 MHz):
Compound S6, $^1$H NMR (CD$_3$OD, 400 MHz):
Compound S6, $^{13}$C NMR (CD$_3$OD, 101 MHz):
Compound S6, $^{19}$F NMR (CD$_3$OD, 376 MHz):
Compound S6, $^{31}$P NMR (CD$_3$OD, 162 MHz):
Compound 4, $^1$H NMR (CD$_3$OD, 400 MHz):
Compound 4, $^{13}$C NMR (CD$_3$OD, 101 MHz):
Compound 4, $^{19}$F NMR (CD$_3$OD, 376 MHz):
Compound 4, $^{31}$P NMR (CD$_3$OD, 162 MHz):
Compound S5, $^1$H NMR (THF-d$_8$, 500 MHz):
Compound S5, $^{13}$C NMR (THF-d8, 125 MHz):
Compound S5, $^{19}$F NMR (THF-d8, 376 MHz):
Compound S5, $^{31}$P NMR (THF-d8, 162 MHz):
Compound 2, $^1$H NMR (CD$_3$OD, 400 MHz):
Compound 2, $^{13}$C NMR (CD$_3$OD, 101 MHz):
Compound 2, $^{19}$F NMR (CD$_3$OD, 376 MHz):
Compound 2, $^{31}$P NMR (CD$_3$OD, 162 MHz):
