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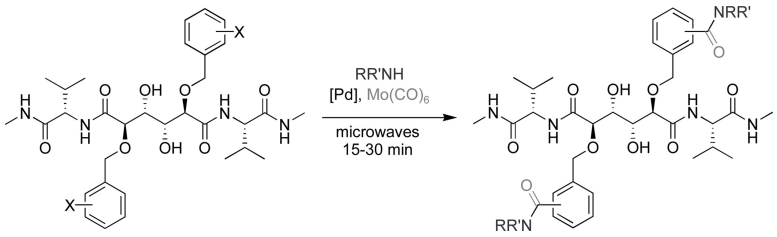

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High-Speed Synthesis of Potent C_2-Symmetric HIV-1 Protease Inhibitors by In-Situ Aminocarbonylations

Johan Wannberg,[†] Nils-Fredrik K. Kaiser,[†] Lotta Vrang,[‡] Bertil Samuelsson,[‡] Mats Larhed,[†] and Anders Hallberg*,[†]

Organic Pharmaceutical Chemistry, Department of Medicinal Chemistry, Uppsala University, BMC, Box 574, SE-751 23 Uppsala, Sweden, and Medivir AB, Lunastigen 7, SE-141 44 Huddinge, Sweden

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Two novel series of C_2-symmetric HIV-1 protease inhibitors were synthesized by microwave-promoted, palladium-catalyzed aminocarbonylations of the *o*-iodo- and *m*-bromobenzyloxy P1/P1′ substituted core structures. Molybdenum hexacarbonyl was used as a convenient solid source of carbon monoxide in these transformations. After the initial high-speed library generation, biological testing identified highly active HIV-1 protease inhibitors. Selected ortho- and meta-decorated inhibitors were subsequently resynthesized on a larger scale and retested for their affinity toward HIV-1 protease, showing micromolar to low nanomolar inhibition. The discovery of highly active inhibitors containing large phenyl amide ortho substituents in the P1/P1′ positions indicates that larger groups than previously believed are tolerated in this part of the S1/S1′ pocket.

Introduction

The era of HAART (highly active antiretroviral therapy) has led to a reevaluation of HIV infection from deadly to chronic but manageable in the developed world.[1,2] Drug-resistant viral strains,[3] severe adverse effects,[4] or both can, however, quickly reverse this for individual patients. Importantly, although progress has been made, HIV infected patients in low- and middle-income countries still have limited access to modern treatment. This is mainly due to the high cost of antiretroviral drugs and the general limitations of healthcare systems.[5] Thus, there is a true demand for both more cost-effective drugs and for novel pharmacotherapies with resistance profiles different from those in current use.

In 1998, a short and stereo-controlled method for the synthesis of C_2-symmetric HIV-1 protease inhibitors from L-mannonic-γ-lactone was reported.[6] In these inhibitors, a dihydroxy ethylene unit served as a transition state mimicking fragment and benzyloxy groups as P1/P1′ side chains. The core structures of these carbohydrate-based inhibitors were subsequently used to explore the impact of elongation of the P1/P1′ side chains by means of palladium-catalyzed coupling reactions.[7] This operation yielded several HIV-1 protease inhibitors with high potencies in enzyme and cell assays. Furthermore, compounds exhibiting high activity against ritonavir-resistant viral strains were identified. Several X-ray structures of enzyme−inhibitor complexes revealed that the para-substituted benzyloxy side chains occupied both the S1/S1′and S3/S3′ subsites of the native C_2-symmetric protease in an extended geometry. The X-ray structures also indicated that the substituents reached the surrounding water at the boundary of the active site.[7] Subsequent modeling suggested that it may also be feasible to extend the meta position of the P1/P1′ subunits to afford potent compounds. However, analyses of the available crystal structures suggested that an extension of the ortho position with large groups might be less likely to furnish good inhibitors. To our knowledge, inhibitors with aromatic ring systems in the P1 or P1′ positions with large ortho substituents have never been disclosed.

We have previously used solid $Mo(CO)_6$ as a convenient source of carbon monoxide[8] in fast aminocarbonylation reactions[9,10] promoted by efficient microwave heating[11] and palladium catalysis.[12,13] Encouraged by the smoothness and chemoselectivity of this protocol, we initially decided to investigate more complex medicinal chemistry-related substrates,[14] and subsequently employed aminocarbonylations to achieve bis-functionalization of the benzyloxy P1/P1′ side chains of our C_2-symmetric dihydroxyethylene-based inhibitor scaffold. Thus, we hoped that this high-speed methodology would efficiently allow a rapid exploration of the impact of large *ortho*- and *meta*-amide substituents on the HIV-1 protease-inhibiting capacity. Although two new amide/peptide bonds were introduced and, consequently, the reported inhibitors become less drug-like, this study provides information useful for the future design of potent HIV-1 protease inhibitors with unique characteristics.

Results and Discussion

The previously reported synthetic route[6,7] was used to prepare key intermediates **4** and **5** in a straightforward way (Scheme 1). Although these two halogen-functionalized benzyl ethers were potent inhibitors as such (compound **4**, $K_i = 4.3$ nM; compound **5**, $K_i = 0.1$ nM), our intention was to use them as aryl-palladium precursors in carbonylative

* Corresponding author. E-mail: anders.hallberg@orgfarm.uu.se.
[†] Uppsala University.
[‡] Medivir AB.

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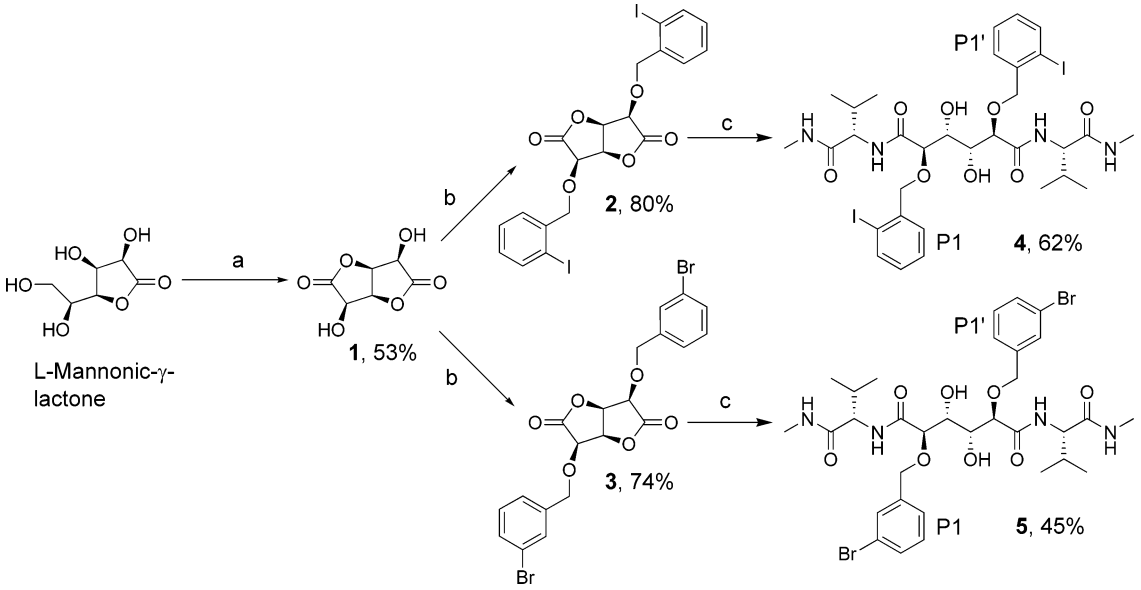
Scheme 1. Synthesis of Aryl Halide-Containing Inhibitors **4** and **5**[a]



[a] Reagents and conditions: (a) HNO_3, 90 °C, 5 h; (b) *o*-iodobenzyl-2,2,2-trichloroacetimidate or *m*-bromobenzyl-2,2,2-trichloroacetimidate, $BF_3 \times Et_2O$, rt, 18 h; (c) L-valine methylamide, dichloroethane, 50 °C, 18 h.

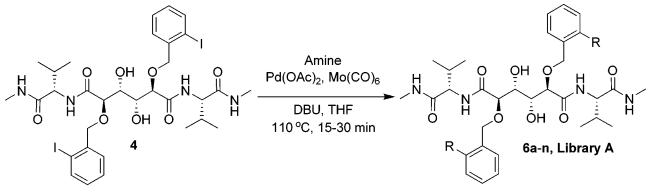
couplings to map the available size and binding features of the S1/S1′ subsites.

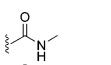
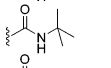
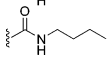
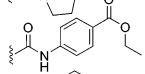
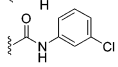
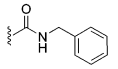
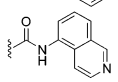
The substitutions of the halogen groups were performed in sealed vessels under noninert palladium-catalyzed aminocarbonylative conditions. The previously reported microwave protocol, in which carbon monoxide gas is released in situ from solid molybdenum hexacarbonyl[9] when heated in the reaction cocktail, was employed.[10,15] The preparative approach was (1) to conduct a large number of fast small-scale carbonylation reactions to generate libraries A and B, (2) to purify the individual library products by reversed-phase preparative LC/MS with UV-triggered fraction collection, and (3) to test the resulting purified inhibitors for their affinity for the HIV-1 protease. The most active or otherwise interesting library members identified from this first screening should then be selected for resynthesis in larger scale with thorough chemical characterization to facilitate an unambiguous determination of the inhibitory efficiency.

The iodo-functionalized core structure **4** was used as precursor for library A. The aminocarbonylations were conducted under ligandless conditions at 0.015-mmol scale with $Pd(OAc)_2$ as precatalyst and with 14 different primary and secondary amines (Table 1). In all cases, 15 or 30 min of microwave heating at 110 °C furnished complete conversion of starting material **4**. After a quick filtration and subsequent LC/MS purification, all but one of the 14 amide products were isolated in more than 95% purity.

Library B was synthesized analogously using meta-substituted aryl bromide **5**, but at a higher reaction temperature and employing a more advanced catalytic system (Table 2). The conditions previously developed for aryl bromides (Herrman's palladacycle **8**,[16] 150 °C, 15 min)[10] furnished the desired amide product but in complex reaction mixtures. Apparently product and starting material decomposed slowly at this high temperature. An addition of catalytic amounts of Fu's salt **9**[17] ($BF_4HP(t\text{-}Bu)_3$) gave a more active catalytic system,[15] enabling a reduction of reaction temperature to

Table 1. Synthesis of Library A and Results of First Screening of HIV-1 Protease Inhibition[a]



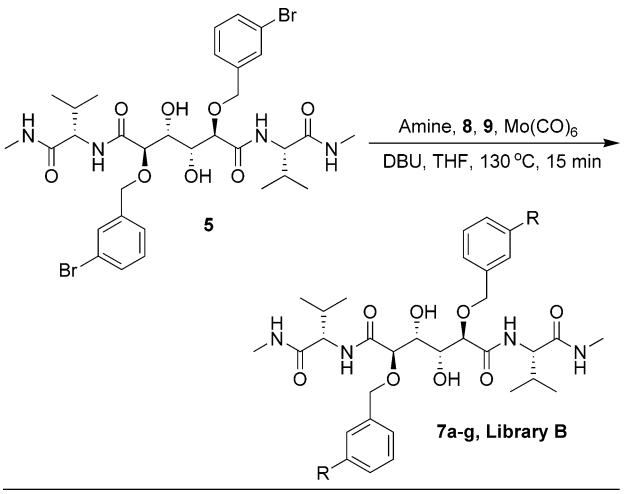
Inhibitor	R	K_i (nM)	Inhibitor	R	K_i (nM)
6a		>5000	**6h**		7
6b		700	**6i**		70
6c		600	**6j**		800
6d		400	**6k**		200
6e		>5000	**6l**		6
6f		200	**6m**		2000
6g		700	**6n**		800

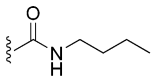
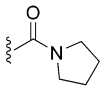
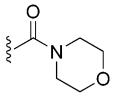
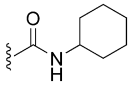
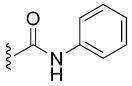
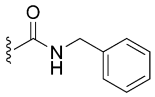
[a] The isolated yields of pure **6a**−**n** varied between 15 and 53%.

130 °C, and provided a cleaner product mixture while still allowing full conversion of **5** within 15 min (Table 2).

The inhibitors and their affinity for HIV-1 are listed in Tables 1 (library A) and 2 (library B). As expected, the meta-substituted compounds in library B were all highly active (K_i, 2−300 nM); the secondary amides proved to be particularly potent inhibitors (K_i, 2−20 nM). In contrast, the ortho-modified derivatives in library A, **6a**−**n**, displayed a wider range of activity with no clear structure−activity trend. The most surprising finding was the high activity of *ortho*-anilides **6h** and **6l**, which seems to indicate that these inhibitors are accommodated in the protease in a binding

Table 2. Synthesis of Library B and Results of First Screening of HIV-1 Protease Inhibition[a]



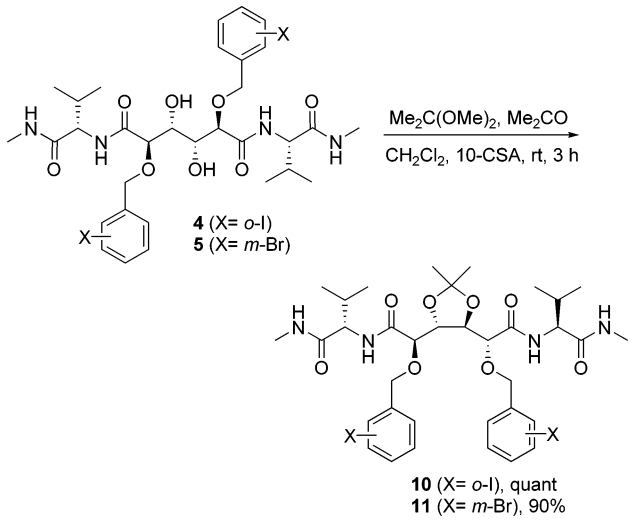
Inhibitor	R	K_i (nM)
7a		20
7b		3
7c		200
7d		300
7e		20
7f		7
7g		2

[a] The isolated yields of pure **7a**−**g** varied between 18 and 45%.

mode that has not been previously observed. To establish absolute clarity regarding the structure−activity relationship of the inhibitors from library A in particular, seven compounds (**6f**, **6h**, **6i**, **6l**, **6m**, **7b**, and **7g)** were selected for a second preparation in 0.050-mmol scale.

During the small-scale production of libraries A and B, a number of impurities were detected in minor amounts by ESI-MS.[18] Fortunately, the LC/MS retention times of these possibly active side products were sufficiently different from the target inhibitors as to not contaminate them. One of the byproducts was tentatively identified as a monolactone formed by competing intramolecular alkoxycarbonylation of **4**. Trace amounts of the corresponding carboxylic acids, formed by hydrolysis of the lactone or by direct hydroxycarbonylation of **4** and **5**, were also detected by LC/MS. Thus, to simplify the purification and to increase the yields,

Scheme 2. Dioxolane Protection of the Vicinal Diols



the central vicinal diol was acetal-protected before carrying out subsequent resynthesis of the chosen inhibitors. Resynthesis of structures **6f**, **6h**, **6i**, **6l**, **6m**, **7b**, and **7g** was conducted from the 1,3-dioxolanes **10** and **11**, smoothly generated by acid-catalyzed acetalization of parent compounds **4** and **5**, respectively (Scheme 2).

Palladium(0)-catalyzed amide-extensions of **10** and **11** were thereafter performed with controlled microwave heating under air and after deprotection with HCl/ether in MeOH delivered much improved isolated yields of free dihydroxy inhibitors (Table 3).[19] To evaluate the enzyme inhibitory effects of the resynthesized inhibitors, an assay identical to that in Tables 1 and 2 was used. As demonstrated by the results obtained, a good correlation between the first and second enzyme testing was achieved, proving a consistently high activity of ortho-substituted **6h** and **6l**.[20] The results of computational and structural chemistry investigations for rationalization of improved activity and binding mode determination of these inhibitors will be reported elsewhere.

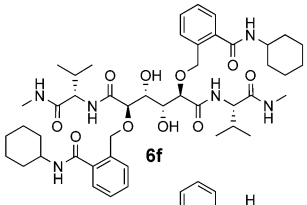
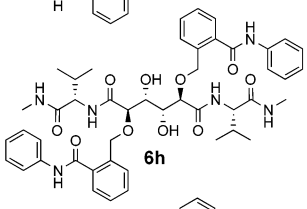
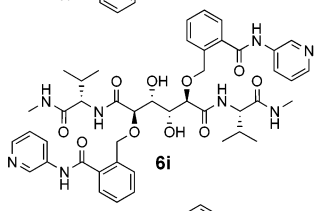
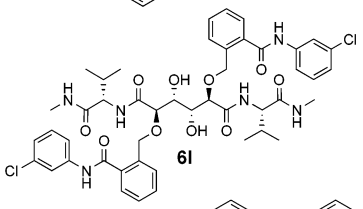
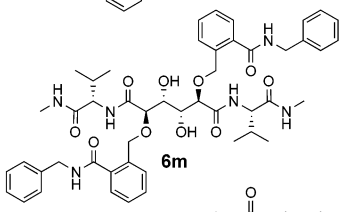
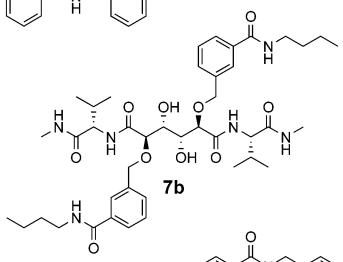
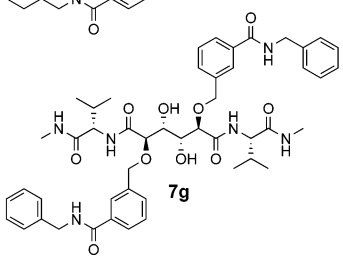
Conclusions

Reported herein are the results of a series of high-speed attempts to modify a class of C_2-symmetric 1,2-dihydroxyethylene-based HIV-1 protease inhibitors by previously unexplored amide expansions of the P1/P1′ side chains. The carbonylative couplings were performed with sequential microwave heating using 14 different nucleophilic amines and in situ release of carbon monoxide and afforded 21 novel inhibitors. From the information presented, we suggest that ortho substitution of P1 or P1′ benzyl side chains may provide a promising new approach in the search for unique HIV-1 protease inhibitors. Further studies on the efficiency of this class of inhibitors toward drug resistant virus strains are currently in progress.

Experimental Section

General Information. Microwave-assisted synthesis was carried out in a Smith/Emrys synthesizer single-mode microwave cavity producing controlled irradiation at 2450 MHz (Biotage AB, Uppsala, Sweden). Thin-layer chromatography was preformed on precoated silica gel F-254

Table 3. Isolated Yields and Inhibitory Activity of Resynthesized Compounds

Starting material	Inhibitor	Isolated Yield (%)[a]	K_i (nM)
10	 **6f**	69	196
10	 **6h**	67	20
10	 **6i**	32	118
10	 **6l**	24	8.5
10	 **6m**	69	1332
11	 **7b**	40	6.5
11	 **7g**	39	2.9

[a] Two-step yield after aminocarbonylation and deprotection; >95% pure by ^{1}H NMR.

plates, 0.25-mm plates (E. Merck) and visualized with UV light and ninhydrin. Flash column chromatography was performed using Merck Silica gel 60 (0.040−0.063 mm). THF was freshly distilled over Na/benzophenone. RP-LC/MS analysis of reaction mixtures and pure products were performed using a Gilson HPLC system with a Chromolith SpeedROD RP-18e column (50 × 4.6 mm) and a Finnigan AQA quadrupole mass spectrometer using a 4 mL/min CH_3CN/H_2O gradient (0.05% HCOOH) and detection by UV (DAD, 190−350 nm) and MS (ESI^+). Preparative RP-LC/MS purifications were performed by UV-triggered fraction collection with a similar Gilson-Finnigan AQA system and a Zorbax SB C8 column (150 × 21.2 mm) using a 15 mL/min CH_3CN/H_2O gradient (0.05% HCOOH) with UV (214, 255 nm) and MS (ESI^+) detection. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury-400 spectrometer or a JEOL JNM-EX400 spectrometer at 400 and 100.5 MHz, respectively.

HIV Protease Inhibition. The HIV-1 protease was cloned and heterologously expressed in *Esherichia coli* and purified as described elsewhere.[21] The K_i values for the synthesized compounds were determined by a fluorometric assay.[22]

2,5-*O*-Bis-(2-iodobenzyl)-L-mannaro-1,4:3,6-di-γ-lactone (2). ^{1}H NMR (400 MHz, $(CD_3)_2CO$): δ 7.90 (dd, J = 7.9, 1.3 Hz, 2H), 7.60 (dd, J = 7.7, 1.8 Hz, 2H), 7.46 (ddd, J = 7.9, 7.7, 1.3 Hz, 2H), 7.10 (ddd, J = 7.9, 7.7, 1.8 Hz, 2H), 5.56 (AA′ part of AA′XX′, 2H), 5.01 (XX′ part of AA′XX′, 2H), 4.97 (d, J = 12.7 Hz, 2H), 4.85 (d, J = 12.7 Hz, 2H). ^{13}C NMR (100.5 MHz, $(CD_3)_2CO$): δ 171.9, 140.5, 140.2, 130.7, 129.9, 129.4, 97.9, 77.2, 76.5, 75.4. Anal. Calcd (%) for $C_{20}H_{16}I_2O_6$: C, 39.63; H, 2.66. Found: C, 39.54; H, 2.76.

2,5-*O*-Bis-(3-bromobenzyl)-L-mannaro-1,4:3,6-di-γ-lactone (3). ^{1}H NMR (400 MHz, $(CD_3)_2CO$): δ 7.64 (t, J = 1.9 Hz, 2H), 7.51 (ddd, J = 7.9, 2.0, 1.2 Hz, 2H), 7.47−7.43 (m, 2H), 7.35 (t, J = 7.8 Hz, 2H), 5.45 (AA′ part of AA′XX′, 2H), 4.94 (d, J = 12.2 Hz, 2H), 4.90 (XX′ part of AA′XX′, 2H), 4.86 (d, J = 12.2 Hz, 2H). ^{13}C NMR (100.5 MHz, $(CD_3)_2CO$): δ 172.0, 140.9, 131.8, 131.5, 131.3, 127.5, 122.8, 76.0, 75.3. 72.4. Anal. Calcd (%) for $C_{20}H_{16}Br_2O_6$: C, 46.90; H, 3.15. Found: C, 47.00; H, 3.10.

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-iodobenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (4).** ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 7.88 (q, J = 4.7 Hz, 2H), 7.84 (dd, J = 7.8, 1.3 Hz, 2H), 7.75 (d, J = 9.1 Hz, 2H), 7.50 (dd, J = 7.6, 1.8 Hz, 2H), 7.41 (ddd, J = 7.4, 7.6, 1.3 Hz, 2H), 7.06 (ddd, J = 7.8, 7.4, 1.8 Hz, 2H), 4.94 (d, J = 7.3 Hz, 2H), 4.46 (m, 4H), 4.18 (dd, J = 9.1, 6.6 Hz, 2H), 4.11 (d, J = 7.3 Hz, 2H), 3.98 (m, 2H), 2.59 (d, J = 4.7 Hz, 6H), 1.98 (m, 2H), 0.84 (d, J = 6.8 Hz, 6H), 0.81 (d, J = 6.8 Hz, 6H). ^{13}C NMR (100.5 MHz, $(CD_3)_2SO$): δ 171.0, 170.1, 140.0, 138.7, 129.4, 128.6, 128.2, 97.4, 79.6, 74.8, 69.9, 57.6, 30.5, 25.5, 19.3, 18.1. Anal. Calcd (%) for $C_{32}H_{44}I_2N_4O_8$: C, 44.36; H, 5.12; N, 6.47. Found: C, 44.18; H, 4.98; N, 6.33.

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(3-bromobenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (5).** ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 7.88 (q, J = 4.5 Hz, 2H), 7.77 (d, J = 8.9 Hz, 2H), 7.54 (t, J = 1.8 Hz, 2H), 7.47 (dt, J = 7.5, 1.8 Hz, 2H), 7.35−7.26 (m, 4H), 4.86 (d, J = 7.5 Hz, 2H), 4.48−4.42 (m, 4H), 4.16 (dd, J = 8.9, 6.5 Hz, 2H), 4.02 (d, J = 7.4 Hz, 2H), 3.85 (m, 2H), 2.59 (d, J = 4.5 Hz, 6H), 2.02−1.92 (m, 2H), 0.84 (m, 12H). ^{13}C NMR (100.5 MHz, $(CD_3)_2SO$): δ 171.1, 170.3, 140.9, 130.4, 130.3, 130.1, 126.4, 121.6, 79.4, 70.1, 57.7, 30.5, 25.4, 19.3, 18.1. Anal. Calcd (%) for $C_{32}H_{44}$-

$Br_2N_4O_8$: C, 49.75; H, 5.74; N, 7.25. Found: C, 49.61; H, 5.74; N, 7.11.

General Procedure for the Synthesis of Library A. A 2-mL microwave vial was charged with **4** (13.0 mg, 0.015 mmol), $Pd(OAc)_2$ (1.0 mg, 0.0045 mmol), $Mo(CO)_6$ (26.4 mg, 0.10 mmol), amine (1.0 mmol), and dry THF (1.5 mL). Subsequent addition of DBU (45 μL, 0.30 mmol) was followed by the immediate capping of the vial with a Teflon septum, and the mixture was irradiated with microwaves to 110 °C for 15 or 30 min (30 min for **6b**, **6g**, **6j**, and **6l**). After cooling, the reaction mixture was loaded onto a plug of Celite and silica (2 + 2 cm) and eluted with 10% MeOH in $CHCl_3$ (only Celite filtration for **6i** and **6n**). Volatiles were removed under reduced pressure, and the residue was redissolved in MeCN and purified by preparative RP-LC/MS to give library members **6a**−**n** in the purities indicated below.

Purity Data for Library A. RP-LC/MS (10−70% MeCN in H_2O, 4 mL/min, 8 min, purity determined at 214 nM): **6a**, t_R = 2.46 min, >98%, *m*/*z* 729.3 $(M + H)^+$. **6b**, t_R = 3.97 min, >98%, *m*/*z* 813.4 $(M + H)^+$. **6c**, t_R = 4.13 min, >98%, *m*/*z* 813.4 $(M + H)^+$. **6d**, t_R = 3.40 min, >98%, *m*/*z* 809.4 $(M + H)^+$. **6e**, t_R = 2.88 min, >98%, *m*/*z* 841.3 $(M + H)^+$. **6f**, t_R = 4.66 min, >98%, *m*/*z* 865.4 $(M + H)^+$. **6g**, t_R = 4.12 min, 88%, *m*/*z* 867.3 $(M + H)^+$. **6h**, t_R = 4.54 min, >98%, *m*/*z* 853.3 $(M + H)^+$. **6i**, t_R = 1.95 min, >98%, *m*/*z* 855.3 $(M + H)^+$. **6j**, t_R = 5.23 min, 97%, *m*/*z* 905.3 $(M + H)^+$. **6k**, t_R = 5.21 min, 95%, *m*/*z* 997.3 $(M + H)^+$. **6l**, t_R = 5.52 min, >98%, *m*/*z* 921.2 $(M + H)^+$. **6m**, t_R = 4.55 min, >98%, *m*/*z* 881.3 $(M + H)^+$. **6n**, t_R = 2.01 min, >98%, *m*/*z* 955.3 $(M + H)^+$.

General Procedure for the Synthesis of Library B. A 2-mL microwave vial was charged with **5** (11.6 mg, 0.015 mmol), Herrman's palladacycle **8** ($Pd_2(OAc)_2(P(o\text{-}tol)_3)_2$, 1.9 mg, 0.0020 mmol), Fu's salt **9** ($[(t\text{-}Bu)_3PH]BF_4$, 2.3 mg, 0.0080 mmol), $Mo(CO)_6$ (26.4 mg, 0.10 mmol), amine (1.0 mmol), and dry THF (1.5 mL). Subsequent addition of DBU (45 μL, 0.30 mmol) was followed by the immediate capping of the vial with a Teflon septum, and the mixture was irradiated with microwaves to 130 °C for 15 min. After cooling, the reaction mixture was loaded onto a plug of Celite and silica (2 + 2 cm) and eluted with 10% MeOH in $CHCl_3$. Volatiles were removed under reduced pressure, and the residue was redissolved in MeCN and purified by preparative RP-LC/MS to give library members **7a**−**g** in the purities indicated below.

Purity Data for Library B. RP-LC/MS (10−70% MeCN in H_2O, 4 mL/min, 8 min, purity determined at 214 nM): **7a**, t_R = 2.37 min, >98%, *m*/*z* 729.3 $(M + H)^+$. **7b**, t_R = 4.06 min, 98%, *m*/*z* 813.4 $(M + H)^+$. **7c**, t_R = 3.20 min, >98%, *m*/*z* 809.4 $(M + H)^+$. **7d**, t_R = 2.60 min, >98%, *m*/*z* 841.3 $(M + H)^+$. **7e**, t_R = 4.66 min, >98%, *m*/*z* 865.4 $(M + H)^+$. **7f**, t_R = 4.32 min, 96%, *m*/*z* 853.3 $(M + H)^+$. **7g**, t_R = 4.45 min, >98%, *m*/*z* 881.3 $(M + H)^+$.

Procedure for the Protection of 4 and 5 To Produce 10 and 11. To a solution of **4** (347 mg, 0.400 mmol) or **5** (155 mg, 0.201 mmol) in 100 mL of dichloromethane/acetone (4:1) was added 20 mL of 2,2-dimethoxypropane and a catalytic amount (10 mg) of (±)-camphor-10-sulfonic acid. The reaction mixture was stirred at room temperature for 3 h and was thereafter washed with aq $NaHCO_3$ (sat.) (2 × 30 mL) and dried with $MgSO_4$. The volatiles were evaporated, and the residue was purified by silica flash chromatography (5% MeOH in $CHCl_3$) to give 365 mg of **10** (quantitative) or 147 mg of **11** (90%).

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-iodobenzyloxy)-3,4-dihydroxy-3,4-*O*-isopropylidenehexane-1,6-diamide (10).** ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (dd, J = 7.9, 1.4 Hz, 2H), 7.45 (dd, J = 7.6, 1.8 Hz, 2H), 7.35 (ddd, J = 7.9, 7.6, 1.4 Hz, 2H), 7.05 (d, J = 9.3 Hz, 2H), 7.01 (ddd, J = 7.9, 7.6, 1.8 Hz, 2H), 6.40 (br q, J = 4.8 Hz, 2H), 4.77 (t, J = 1.2 Hz, 2H), 4.75 (d, J = 12.3 Hz, 2H), 4.68 (d, J = 12.3 Hz, 2H), 4.25−4.20 (m, 4H), 2.75 (d, J = 4.8 Hz, 6H), 2.39−2.29 (m, 2H), 1.39 (s, 6H), 0.88 (d, J = 6.8 Hz, 6H), 0.77 (d, J = 6.8 Hz, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 171.1, 169.1, 139.7, 139.1, 130.2, 129.8, 128.7, 110.2, 98.6, 79.0, 77.7, 58.2, 29.9, 27.1, 26.4, 19.7, 17.6. Anal. Calcd (%) for $C_{35}H_{48}I_2N_4O_8$: C, 46.37; H, 5.34; N, 6.18. Found: C, 46.31; H, 5.28; N, 6.08.

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(3-bromobenzyloxy)-3,4-dihydroxy-3,4-*O*-isopropylidenehexane-1,6-diamide (11).** ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (s, 2H), 7.44 (d, J = 7.8, 2H), 7.30−7.19 (m, 4H), 7.00 (d, J = 9.3 Hz, 2H), 6.40 (br q, J = 4.8 Hz, 2H), 4.71−4.55 (m, 6H), 4.20 (dd, J = 9.3, 5.5 Hz, 2H), 4.08 (t, J = 1.3 Hz, 2H), 2.73 (d, J = 4.8 Hz, 6H), 2.35−2.26 (m, 2H), 0.87 (d, J = 6.8 Hz, 6H), 0.80 (d, J = 6.8 Hz, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 171.1, 169.1, 138.9, 131.6, 131.3, 130.4, 126.9, 122.9, 79.1, 77.7, 73.3, 58.1, 30.0, 27.1, 26.3, 19.5, 17.5. Anal. Calcd (%) for $C_{35}H_{48}Br_2N_4O_8$: C, 51.73; H, 5.95; N, 6.89. Found: C, 51.67; H, 5.86; N, 6.84.

General Procedure for the Resynthesis of Inhibitors 6f, 6h, 6i, 6l, and 6m. A 2-mL microwave vial was charged with **10** (45.3 mg, 0.050 mmol), $Pd(OAc)_2$ (1.7 mg, 0.0075 mmol), $Mo(CO)_6$ (26.4 mg, 0.10 mmol), amine (1.0 mmol), and dry THF (1.5 mL). Subsequent addition of DBU (75 μL, 0.50 mmol) was followed by the immediate capping of the vial with a Teflon septum under air, and the mixture was irradiated with microwaves to 110 °C for 15 min. After cooling, the reaction mixture was filtered, and volatiles were removed under reduced pressure. The residue was purified by silica gel flash chromatography using MeOH: $CHCl_3$/*i*-hexane mixtures. The isolated protected inhibitor was then dissolved in 15 mL of MeOH, followed by addition of 5 mL of HCl/ether and stirring at room temperature for 4 h. Evaporation of volatiles was followed by purification on a short silica column (MeOH/$CHCl_3$) to give the desired pure inhibitors **6** in 24−69% yield (>95% by ^{1}H NMR).

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-cyclohexylcarbamoylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (6f).** ^{1}H NMR (400 MHz, $CDCl_3$, CD_3OD, 4:1): δ 7.46−7.34 (m, 8H), 4.87 (d, J = 11.7 Hz, 2H), 4.62 (d, J = 11.7 Hz, 2H), 4.23 (d, J = 5.4 Hz, 2H), 4.00−3.95 (m, 4H), 3.90−3.80 (m, 2H), 2.72 (s, 6H), 2.36−2.23 (m, 2H), 2.02−1.92 (m, 4H), 1.81−1.71 (m, 4H), 1.44−1.12 (m, 10H), 0.94 (d, J = 6.8 Hz, 6H),

0.86 (d, $J = 6.8$ Hz, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3$, CD_3OD, 4:1): δ 172.1, 171.9, 169.8, 136.8, 134.6, 130.5, 130.3, 128.7, 127.9, 79.8, 72.0, 71.0, 58.7, 49.3, 32.9, 32.8, 30.0, 26.0, 25.6, 25.24, 25.22, 19.5, 17.5. Anal. Calcd (%) for $C_{46}H_{68}N_6O_{10}$: C, 63.87; H, 7.92; N, 9.71. Found: C, 63.77; H, 7.88; N, 9.67

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-phenylcarbamoylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (6h).** ^{1}H NMR (400 MHz, $CDCl_3$, CD_3OD, 4:1): δ 7.65−7.57 (m, 6H), 7.47−7.37 (m, 6H), 7.31 (t, $J = 7.8$ Hz, 4H), 7.12 (t, $J = 7.4$ Hz, 4H), 4.91 (d, $J = 11.9$ Hz, 2H), 4.65 (d, $J = 11.9$ Hz, 2H), 4.16 (d, $J = 5.6$ Hz, 2H), 3.95−3.90 (m, 4H), 2.64 (s, 6H), 2.27−2.17 (m, 2H), 0.90 (d, $J = 6.9$ Hz, 6H), 0.82 (d, $J = 6.9$ Hz, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3$, CD_3OD, 4:1): δ 172.1, 171.8, 169.0, 138.4, 136.8, 134.8, 130.6, 130.5, 129.1, 128.8, 128.2, 124.8, 120.7, 79.9, 72.0, 71.0, 58.7, 30.0, 26.0, 19.4, 17.5. Anal. Calcd (%) for $C_{46}H_{56}N_6O_{10}$: C, 64.77; H, 6.62; N, 9.85. Found: C, 64.59; H, 6.56; N, 9.70.

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis[2-(pyridin-3-ylcarbamoyl)benzyloxy]-3,4-dihydroxyhexane-1,6-diamide (6i).** ^{1}H NMR (400 MHz, CD_3OD): δ 8.85 (d, $J = 2.4$ Hz, 2H), 8.29−8.20 (m, 4H), 7.62 (dd, $J = 7.3$, 1.8 Hz, 2H), 7.57 (dd, $J = 7.4$, 1.5 Hz, 2H), 7.52−7.36 (m, 6H), 4.83 (d, $J = 12.0$ Hz, 2H), 4.74 (d, $J = 12.0$ Hz, 2H), 4.11 (d, $J = 6.7$ Hz, 2H), 4.07 (AA′ part of AA′BB′ system, 2H), 4.00 (BB′ part of AA′BB′ system, 2H), 2.68 (s, 6H), 2.12−2.02 (m, 2H), 0.89−0.84 (m, 12H). ^{13}C NMR (100.5 MHz, CD_3OD): δ 173.7, 173.5, 170.5, 145.5, 142.5, 137.1, 136.7, 131.8, 131.3, 129.6, 129.4, 128.9, 125.3, 81.2, 72.1, 71.4, 60.0, 31.7, 26.2, 19.7, 18.6.

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis[2-(3-chlorophenylcarbamoyl)benzyloxy]-3,4-dihydroxyhexane-1,6-diamide (6l).** ^{1}H NMR (400 MHz, $CDCl_3$, CD_3OD, 4:1): δ 7.84 (t, $J = 2.2$ Hz, 2H), 7.60−7.56 (m, 2H), 7.48−7.39 (m, 8H), 7.23 (t, $J = 8.0$ Hz, 2H), 7.08 (dd, $J = 8.0$, 2.6 Hz, 2H), 4.88 (d, $J = 11.9$ Hz, 2H), 4.68 (d, $J = 11.9$ Hz, 2H), 4.19−4.08 (m, 2H), 3.96 (AA′ part of AA′BB′ system, 2H), 3.92 (BB′ part of AA′BB′ system, 2H), 2.65 (s, 6H), 2.24−2.14 (m, 2H), 0.89 (d, $J = 6.8$ Hz, 6H), 0.82 (d, $J = 6.8$ Hz, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3$, CD_3OD, 4:1): δ 172.5, 172.3, 169.4, 140.1, 136.7, 135.3, 134.8, 131.1, 130.8, 130.4, 129.0, 128.4, 124.9, 120.9, 118.9, 80.3, 72.1, 71.2, 59.1, 30.5, 26.1, 19.6, 17.8.

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-benzylcarbamoylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (6m).** ^{1}H NMR (400 MHz, $CDCl_3$, CD_3OD, 4:1): δ 7.50 (d, $J = 7.4$ Hz, 2H), 7.44−7.22 (m, 16H), 4.82 (d, $J = 11.4$ Hz, 2H), 4.72 (d, $J = 11.4$ Hz, 2H), 4.57 (d, $J = 14.8$ Hz, 2H), 4.50 (d, $J = 14.8$ Hz, 2H), 4.19 (d, $J = 5.9$ Hz, 2H), 4.07 (AA′ part of AA′BB′ system, 2H), 3.99 (BB′ part of AA′BB′ system, 2H), 2.67 (s, 6H), 2.27−2.17 (m, 2H), 0.91 (d, $J = 6.9$ Hz, 6H), 0.85 (d, $J = 6.9$ Hz, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3$, CD_3OD, 4:1): δ 172.2, 172.1, 17.5, 138.4, 136.2, 134.9, 130.64, 130.57, 128.9, 128.8, 128.0, 127.8, 127.6, 80.2, 71.6, 71.3, 71.3, 58.8, 30.2, 26.0, 19.4, 17.7. Anal. Calcd (%) for $C_{48}H_{60}N_6O_{10}$: C, 65.44; H, 6.86; N, 9.54. Found: C, 65.17; H, 6.75; N, 9.32

General Procedure for the Resynthesis of Inhibitors 7b and 7 g. A 2-mL microwave vial was charged with **11** (40.6 mg, 0.050 mmol), Herrman's palladacycle **8** ($Pd_2(OAc)_2(P(o\text{-}tol)_3)_2$, 3.4 mg, 0.0038 mmol), Fu's salt **9** ($[(t\text{-}Bu)_3PH]BF_4$, 4.4 mg, 0.015 mmol), $Mo(CO)_6$ (26.4 mg, 0.10 mmol), amine (1.0 mmol) and dry THF (1.5 mL). Subsequent addition of DBU (75 μL, 0.50 mmol) was followed by the immediate capping of the vial with a Teflon septum under air, and the mixture was irradiated with microwaves to 130 °C for 15 min. After cooling, the reaction mixture was filtered, and volatiles were removed under reduced pressure. The residue was purified by silica gel flash chromatography using MeOH/$CHCl_3$/*i*-Hexane mixtures. The isolated protected inhibitor was then dissolved in 15 mL of MeOH, followed by addition of 5 mL of HCl/ether and stirring at room temperature for 4 h. Evaporation of volatiles was followed by purification on a short silica column (MeOH/$CHCl_3$) to give the desired pure inhibitors **7b** in 40% and **7g** in 39% yield (>95% by ^{1}H NMR).

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-*n*-butylcarbamoylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (7b).** ^{1}H NMR (400 MHz, $CDCl_3$, CD_3OD, 4:1): δ 7.83 (s, 2H), 7.79 (d, $J = 7.3$, 2H), 7.49−7.39 (m, 4H), 4.69 (s, 4H), 4.21 (d, $J = 5.9$ Hz, 2H), 4.14 (AA′ part of AA′BB′ system, 2H), 3.09 (BB′ part of AA′BB′ system, 2H), 3.41 (t, $J = 7.2$ Hz, 4H), 2.74 (s, 6H), 2.24−2.14 (m, 2H), 1.67−1.57 (m, 4H), 1.47−1.36 (m, 4H), 0.99−0.90 (m, 12H), 0.85 (d, $J = 6.9$ Hz, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3$, CD_3OD, 4:1): δ 172.1, 171.9, 168.4, 137.4, 135.3, 130.9, 129.0, 127.6, 126.4, 80.2, 72.6, 71.5, 58.6, 40.1, 31.8, 30.5, 26.0, 20.4, 19.4, 17.7, 13.9. Anal. Calcd (%) for $C_{42}H_{64}N_6O_{10} + H_2O$: C, 60.70; H, 8.01; N, 10.11. Found: C, 60.79; H, 7.97; N, 9.95.

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(3-benzylcarbamoylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (7 g).** ^{1}H NMR (400 MHz, $CDCl_3$, CD_3OD, 4:1): δ 7.88 (s, 2H), 7.84 (d, $J = 7.1$, 2H), 7.47−7.43 (m, 4H), 7.38−7.23 (m, 10H), 4.68 (s, 4H), 4.64 (d, $J = 14.7$ Hz, 2H), 4.60 (d, $J = 14.7$ Hz, 2H), 4.16−4.06 (m, 4H), 2.67 (s, 6H), 2.20−2.09 (m, 2H), 0.88 (d, $J = 6.8$ Hz, 6 H), 0.82 (d, $J = 6.8$ Hz, 6 H). ^{13}C NMR (100.5 MHz, $CDCl_3$, CD_3OD, 4:1): δ 172.1, 171.9, 168.3, 138.8, 137.4, 134.9, 131.0, 129.1, 128.8, 127.8, 127.7, 127.5, 126.4, 80.3, 72.5, 71.4, 58.6, 43.9, 30.4, 26.0, 19.4, 17.7.

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Supporting Information Available. RP-LC/MS chromatograms and ^{1}H NMR spectra of compounds **6f**, **6h**, **6i**, **6l**, **6m**, **7b**, and **7g**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Employing the *o*-iodo starting material **4**, competing mono dehalogenation resulted in small amounts (<10%) of nonsymmetric monoamidation products.

(19) Full conversion of **10** was not achieved with 3-chloroaniline as the nucleophile, which explains the relatively low isolated yield of **6l** (24%).

(20) No traces of inhibitor **4** or the corresponding dehalogenated arene was detected by LC/MS or NMR of the purified compounds.

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