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Convergent Synthesis and Biological Evaluation of Syringolin A and Derivatives as Eukaryotic 20S Proteasome Inhibitors

Jérôme Clerc,[a] Barbara Schellenberg,[b] Michael Groll,[c] André S. Bachmann,[d] Robert Huber,[e,f,g] Robert Dudler,[b] and Markus Kaiser*[a]

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A convergent synthesis of SylA was developed and consists of the synthesis of a fully functionalized macrocycle, which is subsequently coupled with a urea moiety. For cyclization, ring-closing metathesis of a conformationally preorganized precursor was employed. The established synthetic route was then applied to the synthesis of SylA derivatives by using various peptidic side chains for decoration of the SylA macrocycle. The resulting collection of SylA analogues was tested for proteasome inhibition, revealing PEGylated SylA derivatives as the most potent proteasome inhibitors.

Introduction

The ubiquitin proteasome system represents the central degradation pathway in all eukaryotic cells.[1] Its core proteolysis system is the 20S proteasome, a chambered multicatalytic protease that features three distinct proteolytic activities.[2] The β5 subunit hosts chymotrypsin-like activity, the β2 subunit hosts trypsin-like activity, and the β1 subunit hosts caspase-like activity.[3] 20S proteasome inhibition represents a promising strategy for the chemotherapeutic treatment of certain cancers, which was pointed out by the recent FDA approval for the use of the proteasome inhibitor Bortezomib (Velcade®) for treatment of relapsed and/or refractory multiple myeloma. In addition, several clinical trials are currently ongoing to assess its efficacy in other cancer types.[4] However, Bortezomib therapy is often hampered by severe side effects and emerging drug resistances.[5] Consequently, the discovery of alternative inhibitors is still a persistent challenge and several small molecule proteasome inhibitors have lately entered advanced clinical trials[6] or have been used as chemical tools for studying proteasome function in live cells.[7]

In the last years, natural products (NPs) have been demonstrated to be an invaluable source for the identification of novel proteasome inhibitory lead structures. To date, several NPs such as salinosporamide, epoxomicin, fetullamides, TMC-95A, and argyrins have been reported as promising proteasome inhibitors.[8] In 1998, the natural product syringolin A (SylA, Figure 1) was isolated from strains of the bacterial plant pathogen *Pseudomonas syringae pv. syringae* (*Pss*),[9] in which it is biosynthesized under infection conditions by a mixed nonribosomal peptide synthetase (NRPS)/polyketide synthetase (PKS) cluster.[10] SylA exerts potent biological activities such as inhibition of proliferation of neuroblastoma and ovarian cancer cells,[11,12] resulting from potent and, under physiological conditions, irreversible proteasome inhibition.[12] Despite its irreversible binding mode, SylA shows surprising selectivity for covalent proteasome

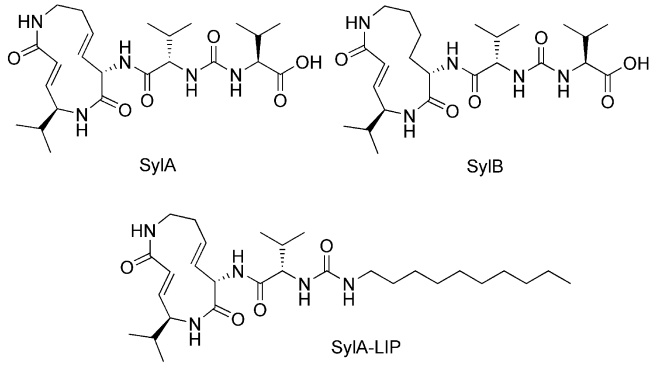

Figure 1. Chemical structures of the natural product proteasome inhibitors syringolin A (SylA), syringolin B (SylB), and synthetic derivative SylA-LIP.

[a] Chemical Genomics Centre der Max-Planck-Gesellschaft, Otto-Hahn-Str. 15, 44227 Dortmund, Germany
Fax: +49-231-9742-6479
E-mail: markus.kaiser@cgc.mpg.de
[b] Zurich-Basel Plant Science Center, Institute of Plant Biology, University of Zürich, Zollikerstr. 107, 8008 Zürich, Switzerland
[c] CIPSM, Department Chemie, TU München, Lichtenbergstr. 4, 85747 Garching, Germany
[d] Cancer Research Center of Hawaii, University of Hawaii at Manoa, 1236 Lauhala Street, Honolulu, HI 96813, USA
[e] Max-Planck-Institut für Biochemie, Am Klopferspitz 18a, 82152 Martinsried, Germany
[f] Zentrum für Medizinische Biotechnologie, Universität Duisburg-Essen, 45117 Essen, Germany
[g] Cardiff University, Cardiff CF10 3US, United Kingdom
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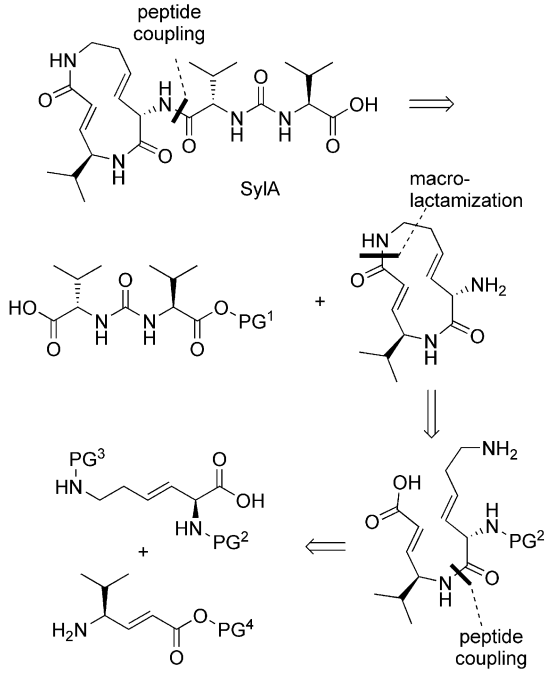
inhibition, highlighting its potential as a promising lead structure for drug discovery efforts.[13] However, even slight structural variations of the natural product SylA have a significant impact on proteasome inhibition and subsite selectivity.[12,14] In fact, only the attachment of a lipophilic side chain to the SylA core structure has resulted in the derivative SylA-LIP (Figure 1) that proved to be 100-times more potent than the parent natural product SylA. Consequently, derivatization of SylA holds promise to lead to proteasome inhibitors with enhanced inhibitory and pharmacokinetic properties. To this end, the establishment of a synthetic route that allows the facile and rapid generation of SylA derivatives would be highly desirable.

Here, we wish to report our findings on the implementation of such a synthesis route by employing a convergent assembly strategy. In addition, we present its application to the synthesis of SylA and SylA analogues and their subsequent biochemical evaluation.

Results and Discussion

In our previously published synthesis of SylA, we installed the fully functionalized ring system of SylA in the penultimate step of the synthesis. This approach however proved impractical for the synthesis of derivatives, as individual optimization of reaction conditions was required for each different side-chain residue.[14] To overcome this limitation, we envisaged a convergent assembly strategy, in which the molecule was divided into two major parts: The synthesis of a fully functionalized SylA macrocycle and the exocyclic residues (Scheme 1). The advantage of such an approach is that the SylA macrocycle can be easily decorated with alternative side chains to yield SylA analogues. For implementation of such a convergent strategy, an efficient generation of the SylA macrocycle is however required.



Scheme 1. First retrosynthetic pathway to SylA by using macrolactamization as a key step for ring closure.

As a macrolactamization approach proved to be a suitable strategy for the synthesis of SylB (Figure 1),[14] we first tested an analogous tactic for the synthesis of the SylA macrocycle. As depicted in Scheme 1, such an approach is straightforward, relying on simple, iterative peptide couplings of previously prepared amino acid building blocks by standard peptide chemistry.

We therefore initiated our studies with the synthesis of the required β,γ-dehydrolysine derivative that could be obtained by a chiral pool approach by using Garner's aldehyde **1** as the starting material.[15] The installation of the critical *trans*-β,γ double bond was envisaged by a Johnson–Claisen rearrangement (Scheme 2). Accordingly, vinylmagnesium bromide was added to (D)-serine-derived Garner aldehyde **1**.[16] Resulting alcohol **2** was obtained in good yields as a mixture of diastereomers. Both isomers were treated with triethyl orthoacetate and propionic acid, yielding **3** with the desired *trans* double bond.[17] The ester was subsequently saponified to acid **4**, which upon a modified Curtius rearrangement with the use of diphenyl phosphoryl azide and an excess amount of trichloroethanol led directly to orthogonally protected β,γ-dehydrolysinol derivative **5**.[18] First attempts to achieve formation of **7** in a one-pot approach by Jones oxidation failed, as only low yields of the desired product were obtained. Therefore, oxazolidine **5** was instead converted into **6** by a catalytic amount of *p*-toluenesulfonic acid in methanol under reflux conditions. Then, a two-step oxidation of alcohol **6** was performed. In the first step, Dess–Martin periodinane was used to provide the unstable aldehyde, which was then immediately oxidized to corresponding β,γ-dehydrolysine building block **7** by a Pinnick oxidation procedure.[19]

With **7** in hand, we continued the macrolactamization approach by attachment of an α,β-unsaturated valine residue (Scheme 3). To this end, literature-known **8**[14] was deprotected at the *N*-terminus and coupled with **7** to yield dipeptide **9**. Selective deprotection of the Troc protecting group with zinc powder in ammonium acetate buffer pH 5.0, followed by methyl ester saponification then yielded macrolactamization precursor **10** in good yield.[20] For the realization of the macrolactamization, several different cyclization protocols such as *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate/1-hydroxy-7-azabenzotriazole (HATU/HOAt) under dilution conditions were tested, but disappointingly none of them delivered the desired product. LC–MS analysis of the corresponding reaction mixtures revealed that desired product **11** was formed only in trace amounts, independently of our employed reaction conditions. Instead, mostly dimers and trimers of **11** were detected as side products, leading to the assumption that high ring strain of the 12-membered macrocycle might be responsible for the unsuccessful cyclization. As ring-closing metathesis (RCM) has proven to be a versatile ap-

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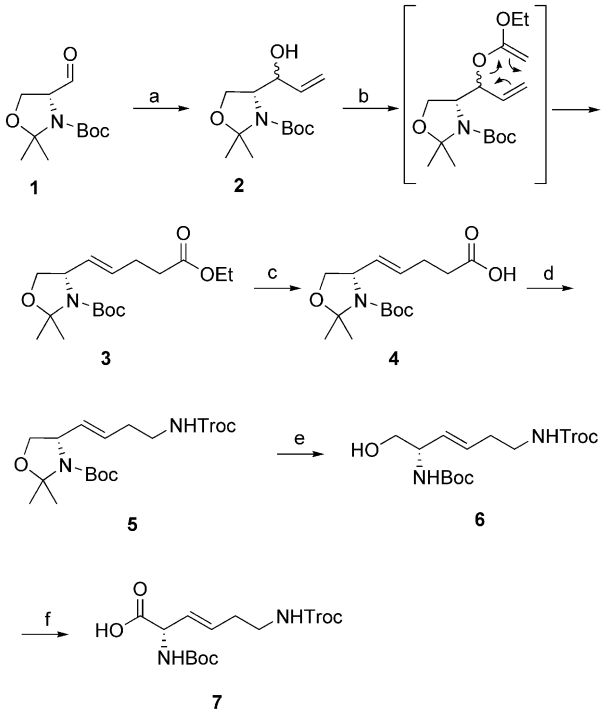
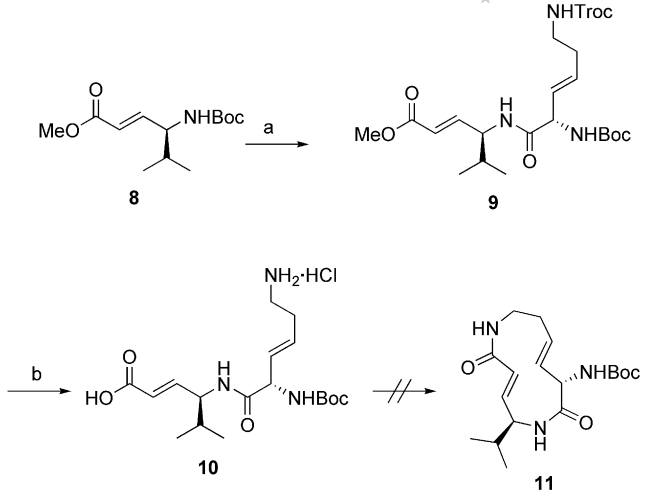
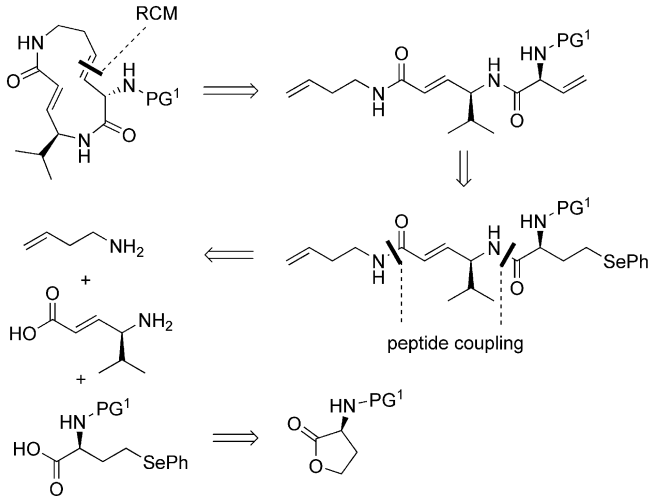


Scheme 2. Synthesis of protected β,γ-dehydrolysine building block **7**. Reagents and conditions: (a) vinylmagnesium bromide (3 equiv.), THF, 30 min at –78 °C and then 2 h at room temp., 75%, *syn*/*anti*, 1:3; (b) triethyl orthoacetate (9 equiv.), propionic acid (0.2 equiv.), xylenes, reflux, 24 h, 90%; (c) 1 M aq. LiOH (3 equiv.), MeOH/H_2O (3:1), 0 °C to room temp. over 30 min, >98%; (d) 1. diphenyl phosphoryl azide (1 equiv.), Et_3N (1.2 equiv.), toluene, 30 min at room temp. and then 4 h at reflux; 2. trichloroethanol (3 equiv.) at 50 °C and then 20 h at reflux, 93% (two steps); (e) pyridinium *p*-toluenesulfonate (PPTS, 0.2 equiv.), MeOH, overnight, reflux, >98%; (f) 1. Dess–Martin periodinane (2 equiv.), DCM, 2 h, 0 °C; 2. aq. $NaClO_2$ (7 equiv.), aq. NaH_2PO_4 (4 equiv.), *t*BuOH/2-methyl-2-butene (3:1), 0 °C to room temp. over 30 min, 69% (two steps).



Scheme 3. Attempted synthesis of macrocycle **11** using a macrolactamization approach. Reagents and conditions: (a) 1. 25% TFA/DCM, 30 min; 2. **7** (1 equiv.), PyBop (1.5 equiv.), HOAt (1.5 equiv.), DIEA (2 equiv.), DCM, 0 °C to room temp. overnight, 64% (two steps); (b) 1. Zn (150 equiv.), THF/NH_4OAc buffer solution 1 M pH 5.0 (5:1), 3 h; 2. 1 M aq. LiOH (2 equiv.), MeOH/H_2O (3:1), 0 °C to room temp. over 30 min; 3. aq. HCl, 82% (three steps).



Scheme 4. Retrosynthesis for the generation of the core macrocycle by using ring-closing metathesis (RCM) as a key step.

proachalso for the synthesis of strained macrocycles, we therefore revised our synthetic approach to include RCM as a key reaction for ring closure (Scheme 4).[21]

The corresponding retrosynthesis however leads to a RCM tripeptide precursor that features a vinylglycine residue at its *N*-terminus. As vinylglycines are known to easily undergo unwanted isomerization to the α,β-conjugated system under peptide coupling conditions, the use of a previously reported phenyl selenyl derivative that can be transformed into a vinylglycine just prior to RCM was envisaged instead.[22] Further disconnections at the peptide bonds result in three major building blocks that are all rapidly accessible by known methods.

Accordingly, (L)-homoserine was converted into protected vinylglycine derivative **12** by following the protocol of Berkowitz (Scheme 5). Saponification of **8**, coupling with commercially available 3-butenylamine, and Boc deprotection yielded amine building block **13**. Coupling of **12** and **13** by using PyBop/HOAt activation led to intermediate **14**, which upon one-pot oxidation/elimination of the phenyl selenyl group yielded RCM precursor **15** in good yields.[23]

However, as observed during the macrolactamization approach, all our attempts to cyclize **15** to macrocycle **11** failed again. Although different Grubbs catalysts and reaction conditions were screened, desired product **11** could not be isolated; instead, only dimers and trimers both in the ring-opened and ring-closed forms and various other side products were detected by LC–MS analysis. Thus, to achieve ring closure, an alteration of the conformation prior to ring closure was necessary. We imagined that this could be achieved through simple modification of the RCM precursor by replacing the central double bond by a spatially different five-membered ring system, arising from dihydrox-

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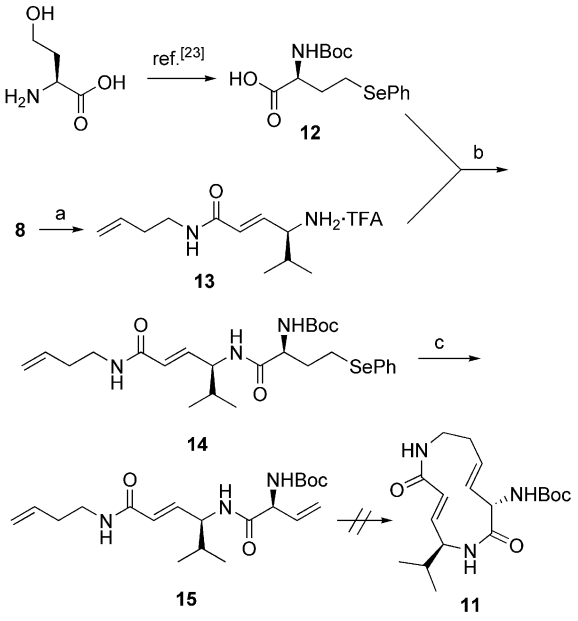
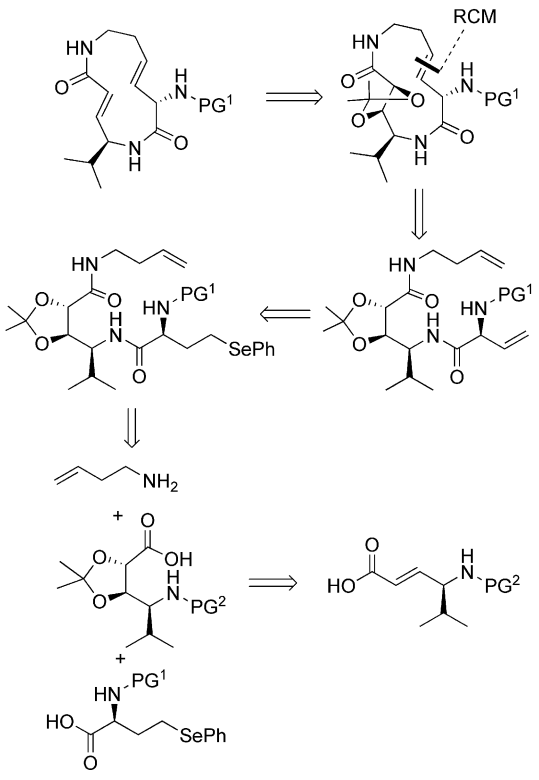


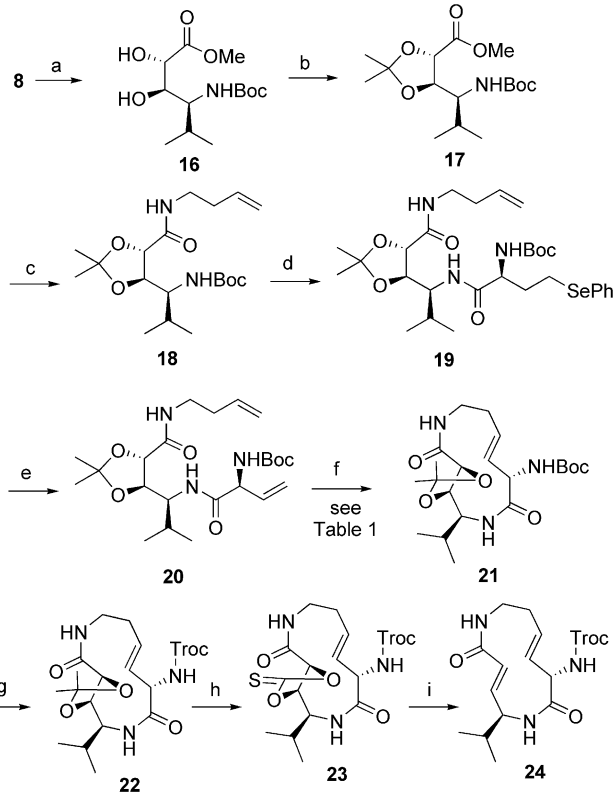
Scheme 5. Attempted synthesis of core macrocycle **11** by using a RCM approach. Reagents and conditions: (a) 1. 1 M aq. LiOH (6 equiv.), MeOH/H_2O (3:1), 0 °C to room temp. over 30 min; 2. 3-butenylamine hydrochloride (1.1 equiv.), PyBop (1.1 equiv.), DIEA (3 equiv.), DCM, 0 °C to room temp. overnight, 82% (two steps), 3. 20% TFA/DCM, 30 min, >98%; (b) **12** (1 equiv.), **13** (1 equiv.), PyBop (1.01 equiv.), HOAt (1.01 equiv.), DIEA (2.7 equiv.), DCM, from 0 °C to room temp. overnight, 93%; (c) 30% aq. H_2O_2/DIEA (1:1), DCM, 50 °C, 3 h, 67%.



Scheme 6. Retrosynthesis for the generation of the core macrocycle by using ring-closing metathesis (RCM) on a conformationally pre-oriented precursor.

ylation and subsequent acetonide formation. Reinstallment of the double bond could then be achieved by a Corey–Winter elimination (Scheme 6).

To put this into practice, α,β-unsaturated methyl ester **8** was dihydroxylated to **16** with a high diastereomeric ratio (96%*dr*) by using osmium tetroxide and 4-methyl morpholine *N*-oxide (NMO, Scheme 7).[24] Protection of diol **16** as an acetonide led to conformationally preorganized intermediate **17**. Methyl ester saponification and derivatization by peptide coupling with 3-butenylamine yielded **18**. Its conversion into **19** was achieved by chemoselective Boc deprotection with TMSOTf and 2,6-lutidine,[25] followed by coupling with phenylselenyl building block **12**. One-pot oxidation and elimination yielded the vinylglycinyl intermedi-



Scheme 7. Synthesis of the SylA macrocycle core structure by using a RCM approach with structural preorganization. Reagents and conditions: (a) OsO_4 (0.05 equiv.), NMO (1.5 equiv.), Ac_2O/H_2O (2:1), 48 h at room temp., 85%; (b) 2,2-DMP (30 equiv.), PPTS (0.05 equiv.), DCM, reflux, >98%; (c) 1. 1 M aq. LiOH (3 equiv.), MeOH/H_2O (1:1), 0 °C to room temp. over 30 min; 2. 3-butenylamine hydrochloride (1.2 equiv.), PyBop (1.5 equiv.), HOAt (1.5 equiv.), DIEA (2 equiv.), DCM, 0 °C to room temp. overnight, 80% (two steps); (d) 1. 2,6-lutidine (2 equiv.), TMSOTf (1.5 equiv.), DCM, room temp., 15 min; 2. **12** (1.3 equiv.), PyBop (1.5 equiv.), HOAt (1.5 equiv.), DIEA (2 equiv.), DCM, from 0 °C to room temp. overnight, 87% (two steps); (e) 30% aq. H_2O_2/DIEA (1:1), DCM, 3 h at 50 °C, 93%; (f) Grubbs II catalyst (0.15 equiv.), toluene, 18 h, 90 °C, 49%; (g) 1. 2,6-lutidine (2 equiv.), TMSOTf (1.5 equiv.), DCM, room temp., 15 min; 2. 2,2,2-trichloroethyl chloroformate (1.1 equiv.), $NaHCO_3$ (2 equiv.), THF, 0 °C to room temp. over 90 min, 81% (two steps); (h) 1. MW, 150 W, 140 °C, 30 min, *p*TsOH·H_2O, MeOH/H_2O/THF (2:2:1); 2. $(Im)_2CS$, DMAP, THF, 80 °C, overnight, 86% (two steps); (i) $P(OMe)_3$, 130 °C, 2.5 h, 88%.

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Table 1. Catalyst and reaction conditions screen for optimizing the RCM conversion of **20** into **21**.

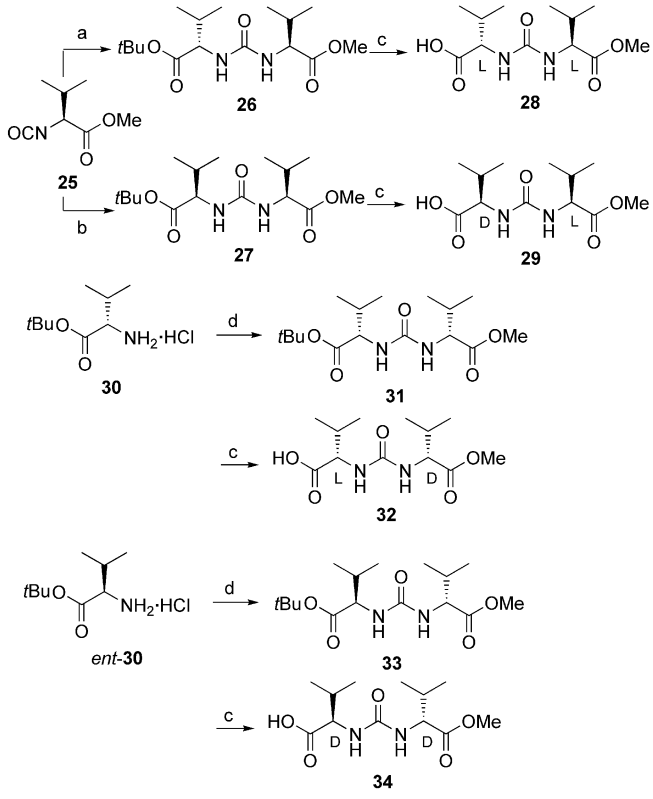
Entry	Catalyst	*E*/*Z* selectivity	Time [h]	Temperature [°C]	Solvent	Yield **21** [%][a]	Conversion [%][b]
1	Grubbs I	10:1	24	r.t.	DCM	n.i.[c]	25
2	Grubbs II	1.1:1.0	24	r.t.	DCM	42	95
3	Grubbs II	3.1:1.0	20	110	toluene	39	95
4	Grubbs II	2.6:1.0	20	90	toluene	49	95
5	Hoveyda–Grubbs I	n.d.	24	r.t.	DCM	n.i.[c]	5
6	Hoveyda–Grubbs II	1.1:1.0	48	r.t.	DCM	22	90

[a] Yield of (*E*) isomer only. [b] Determined by LC–MS (ESI). [c] n.i.: not isolated.

ate and RCM precursor **20**. For the subsequent key RCM reaction, several reaction conditions and RCM catalysts were screened (Table 1). We started our investigations with Grubbs I catalyst (Table 1, Entry 1). Whereas the Grubbs I catalyst provided **21** with the desired stereoselectivity (*E*/*Z* = 10:1), only low conversion and thus yield was observed. We then tested the Grubbs II catalyst, performing the RCM at room temperature for 24 h. To our delight, ring closure was achieved in high yield but with a poor stereoselectivity. In fact, 42% of the desired (*E*) isomer and 39% of the (*Z*) isomer could be isolated. An improvement in selectivity could be obtained when the reaction was performed at 110 °C in toluene (*E*/*Z* = 3.1:1; Table 1, Entry 3). However, under these reaction conditions, side products arose that led to serious purification problems. Adjacent optimization of reaction conditions finally allowed the generation of **21** with 2.6:1 *E*/*Z* diastereoselectivity and 49% yield of the desired (*E*) isomer if the reaction was performed for 20 h in toluene at 90 °C (Table 1, Entry 4).

The Grubbs–Hoveyda I catalyst however was even less reactive than the Grubbs I catalyst, resulting in almost no conversion to the cyclized products (Table 1, Entry 5). The Grubbs-Hoveyda II catalyst (Table 1, Entry 6) showed comparable selectivity to that of the Grubbs II catalyst (Table 1, Entry 2) under similar reaction conditions but required a much longer reaction time to achieve the same level of conversion. We therefore decided to apply the Grubbs II-catalyzed RCM at 90 °C in toluene (Table 1, Entry 4) for the conversion of **20** into **21**, performing the reaction with a similar yield on a gram scale. As the next step, selective deprotection of the acetonide group to install the required precursor for the Corey–Winter elimination was envisaged. Unfortunately, all our trials to chemoselectively deprotect the acetonide failed. As a consequence, a protecting group exchange was performed, yielding Troc-protected amine **22** (Scheme 7). With **22** in hand, microwave-assisted acidic cleavage of the acetonide to the dihydroxy intermediate and further conversion into thiocarbonate **23** with 1,1′-thiocarbonyldiimidazole and DMAP provided the necessary precursor for the Corey–Winter elimination. Elimination due to the desired (*E*)-configured double bond of SylA core macrocycle **24** was then achieved by heating **23** in trimethyl phosphite for 2.5 h at 130 °C.[26]

To complete the synthesis of SylA, the urea building block for attachment to the macrocyclic core was also required. For further biological assays and to study the influence of the side-chain stereochemistry on the overall structure of SylA, we planned to synthesize and couple all four stereoisomers of the urea dipeptide unit to the macrocycle. Thus, commercial isocyanate **25** was treated with either the (L)- or (D)-configured *tert*-butyl valine hydrochloride salt (**30** or *ent*-**30**, respectively) to generate bis-protected ureas **26** and **27**, which upon treatment with wet formic acid yielded acids **28** and **29** in excellent yields (Scheme 8). For the synthesis of the two other isomers, (L)- or (D)-configured salt **30** or *ent*-**30**, respectively, was employed again by using triphosgene followed by addition of (D)-valine methyl ester to form corresponding ureas **31** and **33**. Acidic cleavage of the *tert*-butyl ester group with wet formic acid then yielded the two alternative ureas **32** and **34**.



Scheme 8. Synthesis of all four stereoisomers of the urea building blocks. Reagents and conditions: (a) **30** (1 equiv.), DIEA (2 equiv.), DCM, room temp., overnight, 90%; (b) *ent*-**30** (1 equiv.), DIEA (2.5 equiv.), DCM, room temp., 1 h, 91%; (c) wet HCO_2H, **28**: 91%, **29**: 91%, **32**: >98%, **34**: 92%; (d) triphosgene (1.1 equiv.), DIEA (2.2 equiv.), DCM, 5 min at room temp. then (D)-valine methyl ester hydrochloride (1 equiv.), DIEA (2.2 equiv.), DCM, 10 min at room temp., **31**: 30%, **33**: 76%.

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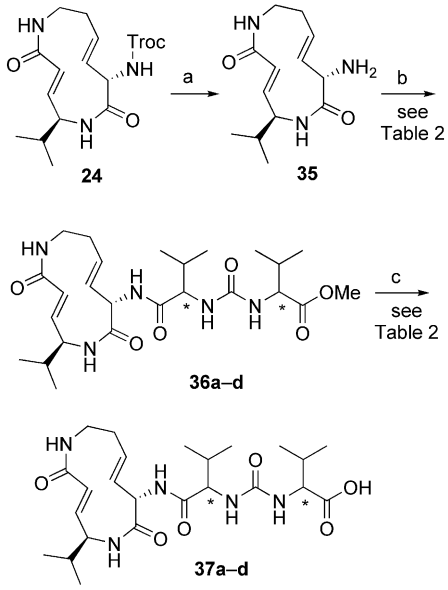
Zn-mediated cleavage of the Troc-protecting group of **24** to free amine **35**, followed by peptide coupling of the previously synthesized urea building blocks then led to the SylA derivatives **36a**–**d** (Scheme 9 and Table 2). Finally, methyl ester cleavage under mild conditions with aluminum tribromide and tetrahydrothiophene yielded the desired natural product SylA (**37a**) and three isomers thereof (i.e., **37b**–**d**).[27]



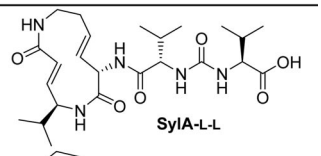
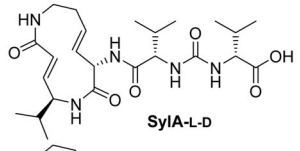
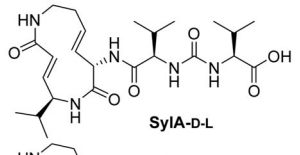
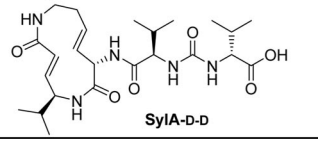
Scheme 9. Synthesis of four SylA stereoisomers. Reagents and conditions: (a) Zn (150 equiv.), THF/AcOH (1:1), 3 h, >98%; (b) urea **28**, **29**, **32**, or **34** (1.1 equiv.), PyBop (1.2 equiv.), HOAt (1.2 equiv.), DIEA (2 equiv.), DMF, 0 °C to room temp. over 40 min, for yields see Table 2; (c) $AlBr_3$ (8 equiv.), tetrahydrothiophene, room temp., 1 h, for yields see Table 2.

To gain some insight into the impact of the stereochemistry of the side chain on the structure of SylA, we investigated the NMR spectra of **37a**–**d** (Figure 2). It turned out that as expected the α-protons of the exocyclic valine residues but interestingly also the signals of the β-olefinic proton of the β,γ-unsaturated lysine were most sensitive to the stereochemical environment. All signals of the NMR spectrum of the "all (L)" isomer **37a** matched those of natural SylA, as reported previously by us. In contrast, derivatives **37c** and **37d**, which feature a (D)-valine residue adjacent to the macrocycle displayed a shift of ≈0.48 ppm at the β-olefinic proton of the β,γ-unsaturated lysine, indicating that this modification has a considerable effect on the overall arrangement of the molecule. The signals of the α-protons of the exocyclic valine residues were also unique for each isomer, resulting in an individual pattern for isomers **37a**–**d** in the NMR spectra.

With our modified convergent synthesis in hand, we then turned our attention to rationally designed derivatives of SylA. Previous studies revealed that the attachment of a lipophilic chain to the SylA core structure resulted in a derivative SylA-LIP with a significantly higher inhibition potency (Figure 1).[14] Although SylA-LIP was >100-fold more active than SylA, the attachment of this lipid chain however reduced its overall water solubility and consequently limited its pharmacokinetic suitability.

We therefore envisaged the synthesis of SylA derivatives that featured a PEG moiety instead of the lipophilic chain of SylA-LIP, thereby hopefully retaining its potent biological activity but enhancing its water solubility. Consequently, an analogous PEG derivative of SylA-LIP was generated, starting from triethylene glycol monomethyl ether

Table 2. Yields for the last two steps of the synthesis of SylA derivatives.

Entry	Urea	Coupling product, yield [%]	Deprotection product, yield [%]	Structure of the product
1	**28**	**36a**, 95	**37a**, 84	 SylA-L-L
2	**32**	**36b**, 87	**37b**, 93	 SylA-L-D
3	**29**	**36c**, 76	**37c**, 53	 SylA-D-L
4	**34**	**36d**, 95	**37d**, 68	 SylA-D-D

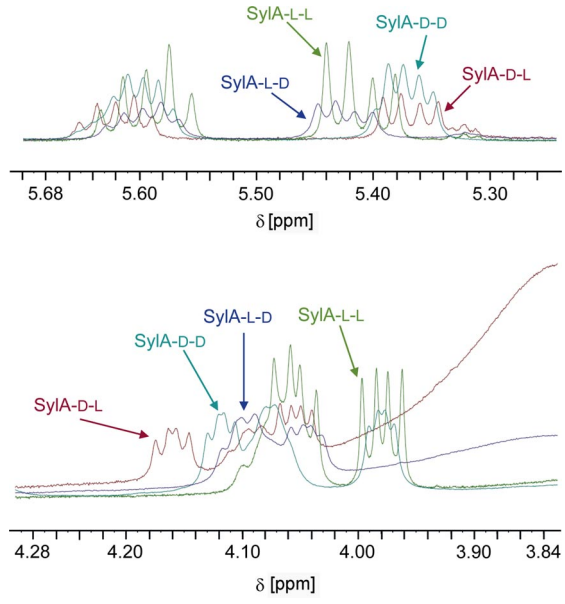


Figure 2. Overlay of the characteristic part of the ^1H NMR spectra of **37a** (SylA-L-L), **37b** (SylA-L-D), **37c** (SylA-D-L), and **37d** (SylA-D-D).

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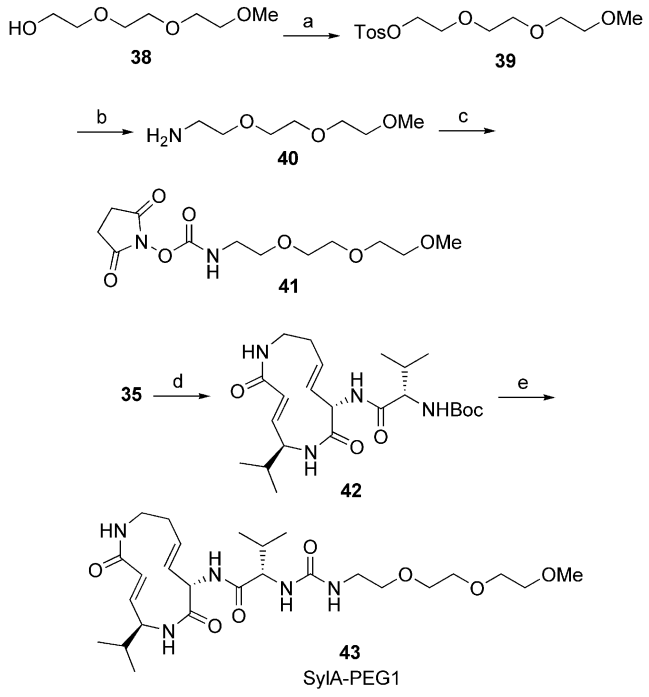
38 (Scheme 10). Transformation of **38** into tosylate **39**, followed by nucleophilic displacement with sodium azide and subsequent triphenylphosphane-mediated reduction led to amine **40**.[28] Reaction with disuccinimidyl carbonate then resulted in the PEG succinimidyl carbamate **41** as a precursor for urea formation.[29] To complete the synthesis of the PEG derivative, macrocycle **35** was then coupled with Boc-(L)-valine to yield **42**. Subsequent Boc deprotection and coupling with **41** then led to desired SylA-PEG1 derivative **43**.

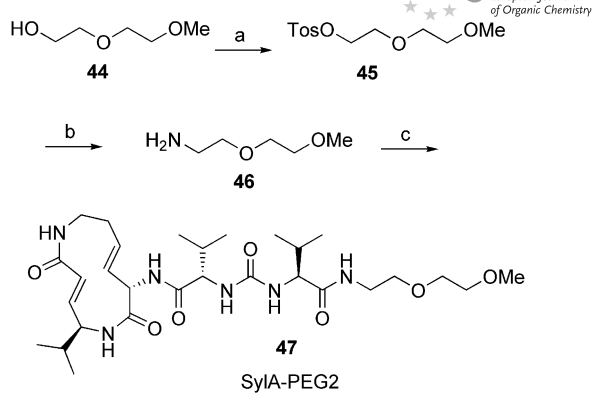


Scheme 10. Synthesis of SylA PEG derivative **43**. Reagents and conditions: (a) TosCl (1 equiv.), Et_3N (1.5 equiv.), DCM, 2 h at 0 °C then 30 min at room temp., 76%; (b) 1. NaN_3 (2.5 equiv.), DMF, 10 h at 67 °C; 2. PPh_3 (1.1 equiv.), Et_2O, 1 h at 0 °C then 90 min at room temp., 82%; (c) DSC (2 equiv.), Et_3N (2 equiv.), DMF, room temp., 90 min, >98%; (d) Boc-(L)-valine (1.2 equiv.), PyBop (1.5 equiv.), HOAt (1.5 equiv.), DIEA (2 equiv.), DMF, 0 °C to room temp. over 1 h, 95%; (e) 1. 25% TFA/DCM, 30 min; 2. **41** (8 equiv.), DIEA (3 equiv.), DMF, 0 °C to room temp. overnight, 68%.

In addition, PEG derivative **47** featuring a shorter PEG chain than **43** but with the dipeptide urea moiety of SylA was synthesized (Scheme 11). To this end, first amine **46** was synthesized in an analogous manner as **40**. Amine **46** was then coupled to SylA-L-L (**37a**) with PyBop/HOAt to yield desired SylA-PEG2 derivative **47** in good yields.

To evaluate the biological activity of the newly synthesized derivatives, biochemical activity assays with the use of human 20S proteasome were performed and compared with previously prepared SylA derivatives (Table 3).

As expected, natural SylA (**37a**) proved to be the most potent proteasome inhibitor of all four SylA isomers. Introduction of a (D)-configured valine, as in **37c** or **37d**, adjacent to the macrocycle however reduced significantly the proteasome inhibition potency. Also, SylA derivative **37b** in



Scheme 11. Synthesis of SylA-PEG2 derivative **47**. Reagents and conditions: (a) TosCl (1 equiv.), Et_3N (1.5 equiv.), DCM, 2 h at 0 °C then 30 min at room temp., 53%; (b) 1. NaN_3 (2.5 equiv.), DMF, 10 h at 67 °C; 2. PPh_3 (1.1 equiv.), Et_2O, 1 h at 0 °C then 90 min at room temp., 59%; (c) **37a** (0.66 equiv.), PyBop (0.8 equiv.), HOAt (0.8 equiv.), DIEA (2 equiv.), DMF, 0 °C to room temp. over 40 min, 79%.

Table 3. Inhibition of the chymotryptic activity of human 20S proteasome by SylA derivatives.

Entry	Urea	K_i' C-L activity [nM]
1	**37b** (SylA-L-D)	11117 ± 2222
2	**37c** (SylA-D-L)	>100000
3	**37d** (SylA-D-D)	33962 ± 11317
4	**43** (SylA-PEG1)	586 ± 69
5	**44** (SylA-PEG2)	401 ± 37
6	natural SylA (**37a**, SylA-L-L)	843 ± 8.4[a]
7	lipophilic SylA (SylA-LIP)	8.65 ± 1.33[b]

[a] Literature values from ref.[12] [b] Literature values from ref.[14]

which the terminal valine residue was converted into a (D)-isomer displayed significantly reduced activity, being roughly 10-times less potent than natural SylA. Structural analysis of the SylA/yeast 20S proteasome complex has revealed a pattern of hydrogen bonds between aspartate-144 of the proteasomal β6 subunit and the carboxyl moiety of the terminal valine residue. It is therefore tempting to speculate that the observed reduced activity of **37b** versus that of natural SylA (**37a**) is a consequence of a lack of hydrogen bonds between these two residues. Nevertheless, **37b** is still a potent proteasome inhibitor and could therefore still be used as a small molecule probe in plant biology studies. Interestingly, PEG derivatives **43** and **47** proved to be the most potent inhibitors of the small SylA collection, and there were found to be slightly more potent than the parent compound SylA but less potent than the lipophilic SylA derivative SylA-LIP.

Conclusions

In summary, a convergent synthesis of syringolin A and derivatives thereof has been developed that should enable rapid synthesis of SylA derivatives for further optimization of the proteasomal inhibition potency of SylA. To this end, a synthetic route has been built up that consists of the generation of the SylA macrocycle by RCM of a conformation-

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ally preorganized precursor. The resulting fully functionalized macrocycle can then be decorated with various peptide chains for derivatization. Using this approach, four SylA isomers were prepared and subsequently tested for inhibition, thereby verifying the previous stereochemical assignment of SylA and probing the impact of stereochemistry on its biological activity. In addition, two PEGylated derivatives were generated that proved to be more potent than natural SylA. Subsequent plant biology studies with these derivatives are currently being pursued and will be reported in due course.

Experimental Section

General Methods: Unless otherwise noted, all reagents and solvents were purchased from Acros, Fluka, Sigma–Aldrich, or Merck and used without further purification. Dry solvents were purchased as anhydrous reagents from commercial suppliers. LC–MS analyses were performed with an HPLC system from Agilent (1200 series) with an Eclipse XDB-C18, 5 μm (column dimensions: 150 × 4.60 mm) column from Agilent and a Thermo Finnigan LCQ Advantage Max ESI-Spectrometer. Two gradients were used for the analyses using H_2O with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B) at a flow rate of 1 mL min^{-1}. Gradient 1: from 0 to 1 min: 75% solvent A/25% solvent B; from 1 to 10 min: from 75% solvent A/25% solvent B to 0% solvent A/100% solvent B; from 10 to 12 min: 0% solvent A/100% solvent B; from 12 to 15 min: from 0% solvent A/100% solvent B to 90% solvent A/10% solvent B. Gradient 2: from 0 to 1 min: 90% solvent A/10% solvent B; from 1 to 10 min: from 90% solvent A/10% solvent B to 0% solvent A/100% solvent B; from 10 to 12 min: 0% solvent A/100% solvent B; from 12 to 15 min: from 0% solvent A/100% solvent B to 90% solvent A/10% solvent B. Preparative HPLC was conducted with a Varian HPLC system (Pro Star 215) with a VP 250/21 Nucleosil C18PPN-column from Macherey-Nagel. The corresponding gradients are described in the synthesis section. NMR spectra were recorded with a Varian Mercury 400 system (400 and 100 MHz for ^{1}H and ^{13}C NMR, respectively), a Bruker Avance DRX 500 system (500 and 125 MHz for ^{1}H and ^{13}C NMR, respectively), or a Varian Unity Inova 600 system (600 and 150 MHz for ^{1}H and ^{13}C NMR, respectively). ^{1}H NMR spectra are reported in the following manner: chemical shifts calculated with reference to solvent standards based on tetramethylsilane, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; sept., septuplet; m, multiplet), coupling constant(s), and number of protons. TLC analyses were performed with TLC aluminum sheets 20 × 20 cm silica gel 60 F254 from Merck. HRMS measurements were performed with a LC–HRMS (ESI-FT) machine from Thermo Electron Corporation. Microwave-assisted reactions were conducted by using a focused microwave unit (Discover® Reactor from CEM Corporation). The instrument consists of a continuous focused microwave power delivery system with operator-selectable power output from 0–300 W. In all experiments, the microwave power was held constant to ensure reproducibility. Reactions were performed in 10-mL glass vessels, which were sealed with a septum and locked into a pressure device, which controlled the pressure in the reaction vessel (maximum 10 bar). The specified reaction time corresponds to the total irradiation time. The temperature was monitored by an infrared temperature sensor positioned below the reaction vessel. The indicated temperature correlates with the maximum temperature reached during each experiment.

Methyl (4*S*)-*tert*-Butoxycarbonylamino-(2*S*,3*R*)-dihydroxy-5-methylhexanoate (16): To a solution of **8** (643 mg, 2.50 mmol, 1 equiv.) dissolved in acetone/water (2:1, 22.5 mL) in a 100-mL flask was consecutively added 4-methylmorpholine *N*-oxide (440 mg, 3.75 mmol, 1.5 equiv.) and osmium tetroxide solution (4 wt.-%/H_2O, 764 μL, 125 μmol, 0.05 equiv.). The flask was flushed with argon, and the reaction mixture was stirred for 2 d. The reaction was quenched by the addition of a saturated aqueous $NaHSO_3$ solution, and the acetone was evaporated in vacuo. Ethyl acetate and further water were added, the layers were separated in a funnel, and the organic layer was dried with Na_2SO_4, filtered through Celite, and concentrated to dryness to give a crude mixture of diastereoisomers. Product **16** (583 mg, 2.00 mmol, 80%) was obtained by recrystallization (cyclohexane) as a pure single diastereoisomer as colorless crystals. The residual mixture was then purified by flash column chromatography (70% diethyl ether in petroleum ether) to afford another portion of **16** (38 mg, 0.13 mmol, 5%) as colorless crystals (overall yield of 85%). TLC (70% diethyl ether in petroleum ether): R_f = 0.23. HPLC (gradient 2): t_R = 7.55 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.78 (d, J = 10.0 Hz, 1 H), 4.32 (br. s, 1 H), 4.01 (br. s, 1 H), 3.81–3.86 (m, 1 H), 3.81 (s, 3 H), 3.52–3.59 (m, 1 H), 2.59 (br. s, 1 H), 2.08–2.18 (m, 1 H), 1.43 (s, 9 H), 0.98 (d, J = 6.8 Hz, 3 H), 0.91 (d, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.04, 157.49, 80.36, 72.16, 71.17, 57.35, 52.73, 28.38, 27.99, 20.11, 16.67 ppm. HRMS (ESI): calcd. for $C_{13}H_{25}O_6NH^+$ $[M + H]^+$ 292.1755; found 292.1757.

Methyl (5*R*)-[(1*S*)-*tert*-Butoxycarbonylamino-2-methylpropyl]-2,2-dimethyl-[1,3]dioxolane-(4*S*)-carboxylate (17): To a solution of **16** (3.53 g, 12.12 mmol, 1 equiv.) dissolved in dichloromethane (45 mL) in a 250-mL, flame-dried flask was added 2,2-dimethoxypropane (45 mL, 364.00 mmol, 30 equiv.) and pyridinium *p*-toluenesulfonate (153 mg, 0.61 mmol, 0.05 equiv.). The flask was flushed with argon, and the solution was heated to reflux for 5 h. After evaporation to dryness, desired product **17** (3.93 g, 11.88 mmol, >98%) was obtained as a colorless solid. TLC (15% ethyl acetate in cyclohexane): R_f = 0.26. HPLC (gradient 1): t_R = 9.78 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.46 (d, J = 6.0 Hz, 1 H), 4.41–4.45 (m, 1 H), 4.11 (dd, J = 9.2, 6.4 Hz, 1 H), 3.76 (s, 3 H), 3.69–3.76 (m, 1 H), 2.09 (sept.d, J = 6.8, 3.6 Hz, 1 H), 1.44 (s, 3 H), 1.42 (s, 9 H), 1.41 (s, 3 H), 0.94 (d, J = 6.8 Hz, 3 H), 0.86 (d, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.87, 156.07, 111.88, 79.50, 77.97, 57.50, 52.47, 28.52, 28.39, 27.23, 26.02, 19.83, 15.70 ppm. HRMS (ESI): calcd. for $C_{16}H_{29}O_6NH^+$ $[M + H]^+$ 332.2068; found 332.2069.

***tert*-Butyl [(1*S*)-{(5*R*)-But-3-enylcarbamoyl-2,2-dimethyl-[1,3]dioxolan-4*S*-yl}-2-methylpropyl]carbamate (18):** To a solution of **17** (1.40 g, 4.23 mmol, 1 equiv.) dissolved in methanol/water (1:1, 20 mL) in a 50-mL flask was added a 1 M aq. lithium hydroxide solution (13 mL, 533 mg, 12.69 mmol, 3 equiv.) at 0 °C. The mixture was stirred for 30 min at room temperature. After evaporation of methanol, a 20% aq. citric acid solution was added to acidify the reaction mixture. The mixture was extracted with dichloromethane (3 × 50 mL), and the organic layer was dried with Na_2SO_4, filtered, and concentrated to yield the cleaved intermediate (1.31 g, 4.15 mmol, >98%) as a white powder. To this intermediate (1.33 g, 4.20 mmol, 1 equiv.) was then added a solution of 3-butenylamine hydrochloride (0.54 g, 5.10 mmol, 1.2 equiv.), HOAt (858 mg, 6.30 mmol, 1.5 equiv.), PyBop (3.28 g, 6.30 mmol, 1.5 equiv.) dissolved in dichloromethane (5 mL) in a 10-mL flask. *N*,*N*-Diisopropylethylamine (1.46 mL, 8.40 mmol, 2 equiv.) was added at 0 °C, and the resulting mixture was stirred overnight at room temperature. The reaction was stopped by quenching with a 20% aq. citric acid solution, and **18** was extracted from the mixture with chloro-

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form (3 × 50 mL). The combined organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by flash column chromatography (20% ethyl acetate in cyclohexane) to afford **18** (1.27 g, 3.43 mmol, 82%) as a colorless solid. TLC (30% ethyl acetate in cyclohexane): R_f = 0.49. HPLC (gradient 2): t_R = 10.79 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.63 (br. s, 1 H), 5.75 (ddt, J = 17.2, 10.4, 6.8 Hz, 1 H), 5.19 (d, J = 9.2 Hz, 1 H), 5.06–5.13 (m, 2 H), 4.30 (d, J = 6.0 Hz, 1 H), 4.06 (dd, J = 9.2, 6.0 Hz, 1 H), 3.64–3.73 (m, 1 H), 3.37–3.46 (m, 1 H), 3.24–3.33 (m, 1 H), 2.24–2.31 (m, 2 H), 2.01–2.11 (m, 1 H), 1.45 (s, 3 H), 1.43 (s, 9 H), 1.36 (s, 3 H), 0.96 (d, J = 7.2 Hz, 3 H), 0.87 (d, J = 7.2 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.40, 156.60, 135.05, 117.49, 111.36, 79.17, 78.36, 58.11, 37.99, 33.77, 29.59, 28.47, 27.28, 26.32, 19.81, 16.25 ppm. HRMS (ESI): calcd. for $C_{19}H_{34}O_5N_2H^+$ [M + H]$^+$ 371.2541; found 371.2540.

***tert*-Butyl {(1*S*)-[(1*S*)-{(5*R*)-But-3-enylcarbamoyl-2,2-dimethyl-[1,3]-dioxolan-(4*S*)-yl}-2-methylpropylcarbamoyl]-3-phenylselanylpropyl}-carbamate (19):** To a solution of **18** (710 mg, 1.92 mmol, 1 equiv.) dissolved in dichloromethane (2 mL) under an atmosphere of argon in a 10-mL flame-dried flask was added 2,6-lutidine (446 μL, 3.84 mmol, 2 equiv.) and trimethylsilyl trifluoromethanesulfonate (522 μL, 2.88 mmol, 1.5 equiv.), and the resulting mixture was stirred for 15 min. The reaction was quenched upon addition of a saturated aqueous NH_4Cl solution. The pH of the water phase was adjusted to 9 by addition of a 2 M aqueous NaOH solution and was extracted with dichloromethane. The combined organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated to yield the deprotected intermediate (508 mg, 1.88 mmol, >98%) as a white powder. This intermediate (512 mg, 1.90 mmol, 1 equiv.), **12** (878 mg, 2.45 mmol, 1.3 equiv.), PyBop (1.48 g, 2.85 mmol, 1.5 equiv.), and HOAt (388 mg, 2.85 mmol, 1.5 equiv.) were then dissolved in dichloromethane (10 mL) in a 25-mL flask. The solution was cooled to 0 °C and *N*,*N*-diisopropylethylamine (662 μL, 3.80 mmol, 2 equiv.) was added. The reaction was stirred overnight at room temperature, quenched by addition of a 20% aqueous citric acid solution, and extracted with chloroform (3 × 50 mL). The combined organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by flash column chromatography (20% ethyl acetate in cyclohexane) to afford **19** (1.03 g, 1.69 mmol, 89%) as a colorless solid. TLC (25% ethyl acetate in cyclohexane): R_f = 0.27. HPLC (gradient 2): t_R = 11.68 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42–7.46 (m, 2 H), 7.17–7.23 (m, 3 H), 6.97 (d, J = 8.4 Hz, 1 H), 6.64 (t, J = 5.6 Hz, 1 H), 5.72 (ddt, J = 17.6, 9.6, 6.8 Hz, 1 H), 5.19 (d, J = 8.0 Hz, 1 H), 5.04–5.10 (m, 2 H), 4.16–4.25 (m, 1 H), 4.10 (d, J = 6.8 Hz, 1 H), 4.03 (dd, J = 8.8, 6.8 Hz, 1 H), 3.96 (ddd, J = 8.8, 3.6, 3.6 Hz, 1 H), 3.33–3.42 (m, 1 H), 3.17–3.26 (m, 1 H), 2.83–2.90 (m, 2 H), 2.16–2.28 (m, 3 H), 2.07 (sept.d, J = 6.8, 3.6 Hz, 1 H), 1.91–2.00 (m, 1 H), 1.42 (s, 3 H), 1.40 (s, 9 H), 1.33 (s, 3 H), 0.90 (d, J = 6.8 Hz, 3 H), 0.87 (d, J = 7.2 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.58, 171.15, 155.38, 134.87, 132.51, 130.08, 129.01, 126.79, 117.49, 111.29, 79.76, 78.41, 78.24, 56.85, 54.96, 37.90, 33.64, 32.98, 29.90, 28.33, 26.95, 25.88, 23.22, 19.57, 16.40 ppm. HRMS (ESI): calcd. for $C_{29}H_{45}O_6N_3{}^{80}SeH^+$ [M + H]$^+$ 612.2546; found 612.2543. HRMS (ESI): calcd. for $C_{29}H_{45}O_6N_3{}^{78}SeH^+$ [M + H]$^+$ 610.2554; found 610.2558.

***tert*-Butyl {(1*S*)-[(1*S*)-{(5*R*)-But-3-enylcarbamoyl-2,2-dimethyl-[1,3]dioxolan-4*S*-yl}-2-methylpropylcarbamoyl]allyl}carbamate (20):** To a solution of **19** (925 mg, 2.04 mmol) dissolved in dichloromethane (85 mL) in a 250-mL flask. was added hydrogen peroxide (30% in water, 10 mL) and *N*,*N*-diisopropylethylamine (10 mL), and the resulting mixture was heated to 50 °C for 3 h. The reaction was quenched by addition of a saturated aqueous $CuSO_4$ solution. Addition of ethyl acetate (50 mL) and a 10% aqueous $KHSO_4$ solution (50 mL) generated a biphasic mixture, which was separated in a funnel. The organic phase was washed with a 5% aqueous $NaHCO_3$ solution (50 mL) and brine (50 mL), dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by flash column chromatography (20% ethyl acetate in cyclohexane) to afford **20** (861 mg, 1.90 mmol, 93%) as a colorless solid. TLC (25% ethyl acetate in cyclohexane): R_f = 0.16. HPLC (gradient 2): t_R = 10.27 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.95 (d, J = 8.8 Hz, 1 H), 6.67 (t, J = 5.6 Hz, 1 H), 5.93 (ddd, J = 17.2, 10.4, 6.4 Hz, 1 H), 5.76 (ddt, J = 17.6, 9.6, 6.8 Hz, 1 H), 5.49 (br. s, 1 H), 5.38 (ddd, J = 17.2, 1.2, 1.2 Hz, 1 H), 5.23 (ddd, J = 10.4, 1.2, 1.2 Hz, 1 H), 5.08–5.14 (m, 2 H), 4.67 (br. s, 1 H), 4.15 (d, J = 6.4 Hz, 1 H), 4.06 (dd, J = 9.2, 6.8 Hz, 1 H), 3.98 (ddd, J = 9.2, 3.6, 3.6 Hz, 1 H), 3.36–3.45 (m, 1 H), 3.25–3.34 (m, 1 H), 2.25–2.31 (m, 2 H), 2.09 (sept.d, J = 6.8, 3.6 Hz, 1 H), 1.45 (s, 3 H), 1.44 (s, 9 H), 1.36 (s, 3 H), 0.94 (d, J = 7.2 Hz, 3 H), 0.90 (d, J = 7.2 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.31, 170.21, 155.08, 134.78, 134.20, 117.41, 117.22, 111.33, 79.61, 78.45, 78.02, 57.51, 56.89, 37.90, 33.59, 30.03, 28.29, 26.90, 25.82, 19.50, 16.49 ppm. HRMS (ESI): calcd. for $C_{23}H_{39}O_6N_3H^+$ [M + H]$^+$ 454.2912; found 454.2906.

21: A solution of **20** (737 mg, 1.620 mmol, 1 equiv.) dissolved in toluene (800 mL) under an atmosphere of argon in a 1-L flame-dried flask was heated to 90 °C. A solution of Grubbs 2nd generation catalyst (207 mg, 0.243 mmol, 0.15 equiv.) in toluene (25 mL) was added over 8 h with a syringe pump to the preheated mixture. The resulting solution was stirred for 10 h at 90 °C. After concentration to dryness, the crude product was purified by flash column chromatography (50% ethyl acetate in cyclohexane) to afford **21** (335 mg, 0.787 mmol, 49%) as a light-brown solid. The product was pure enough to be used in the next step without further purification. Nevertheless, a second flash column chromatography was performed to completely eliminate the remaining trace amounts of ruthenium residues. TLC (60% ethyl acetate in cyclohexane): R_f = 0.29. HPLC (gradient 2): t_R = 8.56 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.63–5.77 (m, 2 H), 5.47–5.55 (m, 2 H), 5.12 (ddd, J = 15.2, 10.0, 0.8 Hz, 1 H), 4.46 (dd, J = 10.0, 8.4 Hz, 1 H), 4.34 (t, J = 8.0 Hz, 1 H), 3.83–3.95 (m, 2 H), 3.68 (d, J = 8.0 Hz, 1 H), 2.82–2.90 (m, 1 H), 2.36–2.44 (m, 1 H), 1.98–2.04 (m, 1 H), 1.83–1.94 (m, 1 H), 1.36 (s, 9 H), 1.34 (s, 3 H), 1.33 (s, 3 H), 0.88 (d, J = 6.8 Hz, 3 H), 0.86 (d, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.86, 169.67, 154.82, 131.54, 130.49, 111.65, 79.93, 79.81, 76.14, 58.04, 56.71, 37.07, 34.62, 30.09, 28.43, 26.90, 26.27, 19.75, 15.98 ppm. HRMS (ESI): calcd. for $C_{21}H_{35}O_6N_3H^+$ [M + H]$^+$ 426.2599; found 426.2596.

22: To a solution of **21** (295 mg, 0.69 mmol, 1 equiv.) dissolved in dichloromethane (4 mL) under an atmosphere of argon in a 10-mL flame-dried flask was added 2,6-lutidine (161 μL, 1.38 mmol, 2 equiv.) and trimethylsilyl trifluoromethanesulfonate (188 μL, 1.04 mmol, 1.5 equiv.) at room temperature, and the resulting mixture was stirred for 15 min. Addition of a saturated aqueous NH_4Cl solution quenched the reaction. The pH was adjusted to 9 by addition of a 2 M NaOH solution, and the desired product was extracted from the water phase with dichloromethane. The combined organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated to yield the deprotected intermediate (221 mg, 0.68 mmol, 98%) as a white solid. This intermediate (166 mg, 510 μmol, 1 equiv.) and sodium hydrogen carbonate (86 mg, 1.02 mmol, 2 equiv.) were then dissolved under an atmosphere of argon in tetrahydrofuran (15 mL) in a 25-mL flame-dried flask. 2,2,2-Trichloroethyl chloroformate (76 μL, 560 μmol, 1.1 equiv.) was added dropwise at 0 °C, and the resulting mixture was stirred

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for 90 min at room temperature. The solvents were removed in vacuo, and the remaining residue was partitioned between a saturated ammonium chloride solution (50 mL) and dichloromethane (50 mL). The aqueous layer was extracted three more times with dichloromethane, and the combined organic layer was finally dried with Na_2SO_4. After concentration to dryness, the crude product was purified by flash column chromatography (50% ethyl acetate in cyclohexane) to afford **22** (212 mg, 423 μmol, 83%) as a colorless solid. TLC (50% ethyl acetate in cyclohexane): R_f = 0.40. HPLC (gradient 2): t_R = 9.32 min. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.99 (d, J = 9.7 Hz, 1 H), 7.90 (d, J = 6.3 Hz, 1 H), 7.54 (d, J = 10.2 Hz, 1 H), 5.61 (ddd, J = 14.8, 11.2, 3.8 Hz, 1 H), 5.40 (dd, J = 15.1, 9.6 Hz, 1 H), 4.82 (d, J = 12.4 Hz, 1 H), 4.69 (d, J = 12.4 Hz, 1 H), 4.54 (dd, J = 9.6, 6.3 Hz, 1 H), 4.35 (dd, J = 10.5, 7.7 Hz 1 H), 3.82 (ddd, J = 10.4, 10.2, 3.4 Hz, 1 H), 3.73 (d, J = 7.6 Hz, 1 H), 3.66–3.77 (m, 1 H), 2.70–2.78 (m, 1 H), 2.22–2.30 (m, 1 H), 1.88–2.00 (m, 2 H), 1.33 (s, 3 H), 1.32 (s, 3 H), 0.86 (d, J = 6.9 Hz, 3 H), 0.79 (d, J = 6.9 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 171.17, 167.69, 153.46, 130.41, 130.23, 109.63, 96.14, 79.82, 76.71, 73.32, 57.56, 55.11, 35.57, 32.32, 29.31, 26.73, 25.94, 19.44, 15.79 ppm. HRMS (ESI): calcd. for $C_{19}H_{28}O_6N_3Cl_3H^+$ [M + H]$^+$ 500.1117; found 500.1113. HRMS (ESI): calcd. for $C_{19}H_{28}O_6N_3Cl_2{}^{37}ClH^+$ [M + H]$^+$ 502.1087; found 502.1082.

23: In a 10-mL vessel was placed **22** (20 mg, 40 μmol, 1 equiv.), *p*-toluenesulfonic acid monohydrate (7.6 mg, 40 μmol, 1 equiv.), methanol/water/tetrahydrofuran (2:2:1, 5 mL), and a magnetic stirring bar. The vessel was sealed with a septum, placed into the microwave cavity and locked with the pressure device. Constant microwave irradiation of 150 W as well as simultaneous air-cooling (300 kPa, 45 Psi) were used during the entire reaction time (30 min, 140 °C, resulting reaction pressure 12 bar). After cooling to room temperature, the solvent was removed under reduced pressure to afford the dihydroxy intermediate (18 mg, 39 μmol, >98%) as a colorless solid. The product was pure enough to be used in the next step without further purification. After performing this reaction several times, all product fractions were pooled, and the resulting residue of the dihydroxy derivative (124 mg, 269 μmol, 1 equiv.) was dissolved in tetrahydrofuran (80 mL) under an atmosphere of argon in a 250-mL flame-dried flask. To this solution was added 1,1′-thiocarbonyl diimidazole (480 mg, 2.69 mmol, 10 equiv.) and 4-(dimethylamino)pyridine (329 mg, 2.69 mmol, 10 equiv.). The resulting reaction mixture was heated to 80 °C and stirred at this temperature overnight. After cooling to room temperature, a small portion of silica gel was added, and the solvent was removed under reduced pressure. The adsorbed crude product was purified by flash column chromatography (50% ethyl acetate in cyclohexane) to yield **23** (119 mg, 237 μmol, 88%) as a colorless solid. TLC (50% ethyl acetate in cyclohexane): R_f = 0.30. HPLC (gradient 2): t_R = 9.29 min. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.52 (d, J = 9.8 Hz, 1 H), 8.07 (d, J = 6.1 Hz, 1 H), 7.82 (d, J = 10.4 Hz, 1 H), 5.62 (ddd, J = 14.8, 11.1, 3.5 Hz, 1 H), 5.48 (dd, J = 15.2, 9.5 Hz, 1 H), 4.98 (dd, J = 10.5, 9.9 Hz, 1 H), 4.84 (d, J = 12.4 Hz, 1 H), 4.74 (d, J = 9.6 Hz, 1 H), 4.70 (d, J = 12.4 Hz, 1 H), 4.51 (dd, J = 9.4, 6.2 Hz, 1 H), 4.23 (ddd, J = 10.5, 10.4, 3.8 Hz, 1 H), 3.63–3.76 (m, 1 H), 2.90 (dd, J = 12.8, 4.6 Hz, 1 H), 2.29–2.36 (m, 1 H), 1.92–2.06 (m, 2 H), 0.90 (d, J = 6.9 Hz, 3 H), 0.84 (d, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 190.08, 171.56, 163.17, 153.59, 130.49, 130.36, 96.08, 83.69, 82.58, 73.37, 57.73, 53.56, 36.27, 31.84, 29.07, 19.06, 15.65 ppm. HRMS (ESI): calcd. for $C_{17}H_{22}O_6N_3Cl_3SH^+$ [M + H]$^+$ 502.0368; found 502.0365. HRMS (ESI): calcd. for $C_{17}H_{22}O_6N_3Cl_2{}^{37}ClSH^+$ [M + H]$^+$ 504.0338; found 504.0331.

24: A solution of **23** (70 mg, 139 μmol, 1 equiv.) dissolved in trimethyl phosphite (1.0 mL) under an atmosphere of argon in a 10-mL flame-dried flask was heated at reflux in a prewarmed oil bath at 130 °C for 2.5 h. After concentration to dryness, the crude product was purified by flash column chromatography (6% methanol in dichloromethane) to yield **24** (52 mg, 122 μmol, 88%) as a colorless solid. Importantly, to assure high yields during this reaction, a minimal amount of trimethyl phosphite should be employed in the reaction, thereby facilitating precipitation of desired product **24**. TLC (6% methanol in dichloromethane): R_f = 0.30. HPLC (gradient 2): t_R = 7.79 min. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.96–8.05 (m, 2 H), 7.45 (t, J = 7.1 Hz, 1 H), 6.69 (dd, J = 15.5, 5.5 Hz, 1 H), 6.08 (d, J = 15.6 Hz, 1 H), 5.66 (dt, J = 15.4, 7.7 Hz, 1 H), 5.38 (dd, J = 15.9, 7.9 Hz, 1 H), 4.82 (d, J = 12.4 Hz, 1 H), 4.75 (d, J = 12.3 Hz, 1 H), 4.67 (dd, J = 7.9, 7.7 Hz, 1 H), 4.03–4.11 (m, 1 H), 3.08–3.22 (m, 2 H), 2.26–2.35 (m, 1 H), 1.90–2.01 (m, 1 H), 1.69–1.79 (m, 1 H), 0.95 (d, J = 6.6 Hz, 3 H), 0.91 (d, J = 6.6 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 168.97, 166.21, 153.91, 143.29, 133.53, 124.90, 121.56, 96.03, 73.48, 56.07, 55.50, 42.29, 35.07, 31.43, 19.70, 19.20 ppm. HRMS (ESI): calcd. for $C_{16}H_{22}O_4N_3Cl_3H^+$ [M + H]$^+$ 426.0749; found 426.0748. HRMS (ESI): calcd. for $C_{16}H_{22}O_4N_3Cl_2{}^{37}ClH^+$ [M + H]$^+$ 428.0719; found 428.0717.

(8*S*)-Amino-(5*S*)-isopropyl-1,6-diazacyclododeca-(3*E*,9*E*)-diene-2,7-dione (35): Compound **24** (34 mg, 81 μmol, 1 equiv.) was dissolved in tetrahydrofuran (1 mL) in a 10-mL flask. Acetic acid was added (1 mL), followed by zinc powder (798 mg, 12.2 mmol, 150 equiv.), which was added in portions over 30 min. After 3 h of vigorous stirring, the mixture was filtered through a small plug of Celite and washed with ethyl acetate. After evaporation to dryness, **35** (20 mg, 79 μmol, 98%) was obtained as a colorless solid. TLC (1% triethylamine + 5% methanol in dichloromethane): R_f = 0.31. HPLC (gradient 2): t_R = 2.06 min; NMR (400 MHz, [D$_6$]DMSO): δ = 8.42 (d, J = 8.3 Hz, 1 H), 7.48 (t, J = 6.9 Hz, 1 H), 6.73 (dd, J = 15.5, 5.2 Hz, 1 H), 6.05 (d, J = 15.6 Hz, 1 H), 5.68 (dt, J = 15.8, 7.8 Hz, 1 H), 5.50 (d, J = 15.8, 7.5 Hz, 1 H), 4.30 (d, J = 7.4 Hz, 1 H), 4.10–4.17 (m, 1 H), 3.10–3.31 (m, 2 H), 2.27–2.37 (m, 1 H), 1.94–2.05 (m, 1 H), 1.70–1.80 (m, 1 H), 0.95 (d, J = 6.6 Hz, 3 H), 0.92 (d, J = 6.8 Hz, 3 H) ppm. HRMS (ESI): calcd. for $C_{13}H_{21}O_2N_3H^+$ [M + H]$^+$ 252.1707; found 252.1708.

SylA-L-L Methyl Ester (36a): Compound **28** (14 mg, 50 μmol, 1.1 equiv.), **35** (11.6 mg, 46 μmol, 1 equiv.), PyBop (30 mg, 56 μmol, 1.2 equiv.), and HOAt (8 mg, 56 μmol, 1.2 equiv.) were dissolved in *N*,*N*-dimethylformamide (1.0 mL) in a 10-mL flask. The solution was cooled to 0 °C and *N*,*N*-diisopropylethylamine (16 μL, 92 μmol, 2 equiv.) was added. The reaction was stirred for 40 min at room temperature. After concentration to dryness, the crude product was purified by flash column chromatography (10% methanol in dichloromethane) to yield **36a** (22.2 mg, 44 μmol, 95%) as a colorless solid. TLC (8% methanol in dichloromethane): R_f = 0.19. HPLC (gradient 2): t_R = 6.84 min. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 8.04 (d, J = 7.2 Hz, 1 H), 8.00 (d, J = 8.7 Hz, 1 H), 7.44 (t, J = 7.1 Hz, 1 H), 6.68 (dd, J = 15.5, 5.5 Hz, 1 H), 6.42 (d, J = 8.8 Hz, 1 H), 6.22 (d, J = 9.1 Hz, 1 H), 6.09 (d, J = 15.5 Hz, 1 H), 5.60 (dt, J = 15.6, 7.7 Hz, 1 H), 5.41 (dd, J = 15.9, 7.7 Hz, 1 H), 4.86 (t, J = 7.4 Hz, 1 H), 4.00–4.11 (m, 3 H), 3.61 (s, 3 H), 3.10–3.24 (m, 2 H), 2.24–2.32 (m, 1 H), 1.86–2.02 (m, 3 H), 1.69–1.78 (m, 1 H), 0.95 (d, J = 6.7 Hz, 3 H), 0.90 (d, J = 6.7 Hz, 3 H), 0.82–0.87 (m, 9 H), 0.78 (d, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 190.46, 176.03, 174.32, 173.30, 172.93, 164.17, 131.91, 130.03, 83.95, 82.19, 58.59, 58.21, 56.74, 54.93, 52.26, 37.01, 32.75, 31.29, 29.21, 26.78, 19.24, 19.11, 18.95, 18.10,

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17.64, 15.41 ppm. HRMS (ESI): calcd. for $C_{25}H_{41}O_6N_5H^+$ [M + H]$^+$ 508.3130; found 508.3134.

SylA-L-D Methyl Ester (36b): Compound **32** (7 mg, 25 μmol, 1.1 equiv.), **35** (5.8 mg, 23 μmol, 1 equiv.), PyBop (15 mg, 28 μmol, 1.2 equiv.), and HOAt (4 mg, 28 μmol, 1.2 equiv.) were dissolved in *N*,*N*-dimethylformamide (1.0 mL) in a 10-mL flask. The solution was cooled to 0 °C and *N*,*N*-diisopropylethylamine (8 μL, 46 μmol, 2 equiv.) was added. The reaction was stirred for 40 min at room temperature. After concentration to dryness, the crude product was purified by flash column chromatography (10% methanol in dichloromethane) to yield **36b** (10.1 mg, 20 μmol, 87%) as a colorless solid. TLC (8% methanol in dichloromethane): R_f = 0.30. HPLC (gradient 2): t_R = 6.93 min. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 8.10 (d, J = 7.0 Hz, 1 H), 8.01 (d, J = 8.2 Hz, 1 H), 7.43 (t, J = 6.9 Hz, 1 H), 6.68 (dd, J = 15.5, 5.4 Hz, 1 H), 6.51 (d, J = 8.9 Hz, 1 H), 6.27 (d, J = 9.2 Hz, 1 H), 6.10 (d, J = 15.5 Hz, 1 H), 5.55–5.64 (m, 1 H), 5.42 (dd, J = 16.0, 7.7 Hz, 1 H), 4.86–4.92 (m, 1 H), 4.05–4.12 (m, 3 H), 3.62 (s, 3 H), 3.09–3.24 (m, 2 H), 2.24–2.32 (m, 1 H), 1.85–2.02 (m, 3 H), 1.70–1.78 (m, 1 H), 0.95 (d, J = 6.6 Hz, 3 H), 0.91 (d, J = 6.6 Hz, 3 H), 0.85 (d, J = 6.9 Hz, 3 H), 0.83 (d, J = 6.5 Hz, 3 H), 0.82 (d, J = 6.5 Hz, 3 H), 0.77 (d, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 173.04, 171.33, 168.88, 166.20, 157.24, 143.12, 133.08, 125.81, 121.45, 57.45, 57.10, 55.42, 53.55, 51.43, 42.51, 34.98, 31.41, 31.36, 30.58, 19.70, 19.14, 19.09, 18.87, 17.64, 17.47 ppm. HRMS (ESI): calcd. for $C_{25}H_{41}O_6N_5H^+$ [M + H]$^+$ 508.3130; found 508.3126.

SylA-D-L Methyl Ester (36c): Compound **29** (7 mg, 25 μmol, 1.1 equiv.), **35** (5.8 mg, 23 μmol, 1 equiv.), PyBop (15 mg, 28 μmol, 1.2 equiv.), and HOAt (4 mg, 28 μmol, 1.2 equiv.) were dissolved in *N*,*N*-dimethylformamide (1.0 mL) in a 10-mL flask. The solution was cooled to 0 °C and *N*,*N*-diisopropylethylamine (8 μL, 46 μmol, 2 equiv.) was added. The reaction was stirred for 40 min at room temperature. After concentration to dryness, the crude product was purified by flash column chromatography (10% methanol in dichloromethane) to yield **36c** (9.6 mg, 19 μmol, 76%) as a colorless solid. TLC (8% methanol in dichloromethane): R_f = 0.30. HPLC (gradient 2): t_R = 6.95 min. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 8.16 (d, J = 7.5 Hz, 1 H), 8.00–8.06 (m, 1 H), 7.43 (t, J = 6.4 Hz, 1 H), 6.68 (dd, J = 15.5, 3.2 Hz, 1 H), 6.53 (d, J = 8.9 Hz, 1 H), 6.31 (d, J = 9.1 Hz, 1 H), 6.10 (d, J = 15.4 Hz, 1 H), 5.55–5.66 (m, 1 H), 5.34–5.42 (m, 1 H), 4.87–4.94 (m, 1 H), 4.16 (dd, J = 8.9, 5.6 Hz, 1 H), 4.05–4.12 (m, 2 H), 3.62 (s, 3 H), 3.09–3.22 (m, 2 H), 2.23–2.32 (m, 1 H), 1.84–2.02 (m, 3 H), 1.69–1.77 (m, 1 H), 0.89–0.97 (m, 6 H), 0.85 (d, J = 6.8 Hz, 3 H), 0.82 (d, J = 6.8 Hz, 3 H), 0.80 (d, J = 6.7 Hz, 3 H), 0.76 (d, J = 6.7 Hz, 3 H) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 173.02, 171.26, 169.02, 166.23, 157.26, 143.18, 132.97, 126.03, 121.49, 57.48, 57.06, 55.43, 53.64, 51.44, 42.61, 34.99, 31.58, 31.34, 30.58, 19.65, 19.19, 19.06, 18.89, 17.63, 17.42 ppm. HRMS (ESI): calcd. for $C_{25}H_{41}O_6N_5H^+$ [M + H]$^+$ 508.3130; found 508.3127.

SylA-D-D Methyl Ester (36d): Compound **34** (7 mg, 25 μmol, 1.1 equiv.), **35** (5.8 mg, 23 μmol, 1 equiv.), PyBop (15 mg, 28 μmol, 1.2 equiv.), and HOAt (4 mg, 28 μmol, 1.2 equiv.) were dissolved in *N*,*N*-dimethylformamide (1.0 mL) in a 10-mL flask. The solution was cooled to 0 °C and *N*,*N*-diisopropylethylamine (8 μL, 46 μmol, 2 equiv.) was added. The reaction was stirred for 40 min at room temperature. After concentration to dryness, the crude product was purified by flash column chromatography (10% methanol in dichloromethane) to yield **36d** (12.0 mg, 24 μmol, 95%) as a colorless solid. TLC (8% methanol in dichloromethane): R_f = 0.21. HPLC (gradient 2): t_R = 6.75 min. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 8.00–8.12 (m, 2 H), 7.43 (t, J = 6.9 Hz, 1 H), 6.69 (dd, J = 15.4, 5.3 Hz, 1 H), 6.42 (d, J = 8.7 Hz, 1 H), 6.26 (d, J = 9.1 Hz, 1 H), 6.10 (d, J = 15.5 Hz, 1 H), 5.55–5.64 (m, 1 H), 5.34–5.42 (m, 1 H), 4.87–4.93 (m, 1 H), 4.12 (dd, J = 9.1, 5.6 Hz, 1 H), 4.05–4.11 (m, 1 H), 4.03 (dd, J = 8.7, 5.3 Hz, 1 H), 3.61 (s, 3 H), 3.09–3.24 (m, 2 H), 2.23–2.31 (m, 1 H), 1.84–2.01 (m, 3 H), 1.69–1.77 (m, 1 H), 0.95 (d, J = 6.6 Hz, 3 H), 0.91 (d, J = 6.6 Hz, 3 H), 0.86 (d, J = 6.8 Hz, 3 H), 0.84 (d, J = 6.9 Hz, 3 H), 0.80 (d, J = 6.7 Hz, 3 H), 0.77 (d, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 172.98, 171.24, 169.03, 166.25, 157.41, 143.20, 133.11, 126.03, 121.46, 57.82, 57.27, 55.44, 53.52, 51.37, 42.52, 34.98, 31.36, 30.18, 19.66, 19.19, 19.08, 19.02, 17.79, 17.44 ppm. HRMS (ESI): calcd. for $C_{25}H_{41}O_6N_5H^+$ [M + H]$^+$ 508.3130; found 508.3127.

SylA-L-L (37a): Compound **36a** (20.0 mg, 39.4 μmol, 1 equiv.) and aluminum bromide (84 mg, 316 μmol, 8 equiv.) were dissolved in tetrahydrothiophene (2 mL) under an atmosphere of argon in a 10-mL flame-dried flask. The resulting mixture was stirred for 1 h at room temperature. After concentration to dryness, the remaining residue was purified by preparative HPLC (using H_2O with 0.1% TFA (solvent A) and acetonitrile with 0.1% TFA (solvent B) at a flow rate of 25 mL min^{-1}. Gradient: from 0 to 10 min: 90% solvent A/10% solvent B; from 10 to 30 min: from 90% solvent A/10% solvent B to 70% solvent A/30% solvent B; from 30 to 50 min: from 70% solvent A/30% solvent B to 40% solvent A/60% solvent B; from 50 to 60 min: from 40% solvent A/60% solvent B to 0% solvent A/100% solvent B; from 60 to 80 min: 0% solvent A/100% solvent B) to yield **37a** (SylA-L-L; 16.3 mg, 33.1 μmol, 84%) as a colorless solid. TLC (2% acetic acid + 15% methanol in dichloromethane): R_f = 0.32. HPLC (gradient 2): t_R = 6.13 min. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.35 (br. s, 1 H), 8.03 (d, J = 8.4 Hz, 1 H), 7.99 (d, J = 6.7 Hz, 1 H), 7.40–7.48 (m, 1 H), 6.68 (dd, J = 15.2, 4.3 Hz, 1 H), 6.32 (d, J = 8.9 Hz, 1 H), 6.25 (d, J = 9.0 Hz, 1 H), 6.10 (d, J = 15.4 Hz, 1 H), 5.59 (dt, J = 15.5, 7.1 Hz, 1 H), 5.40 (dd, J = 15.5, 7.5 Hz, 1 H), 4.82–4.88 (m, 1 H), 4.01–4.10 (m, 2 H), 3.97 (dd, J = 8.7, 4.7 Hz, 1 H), 3.07–3.25 (m, 2 H), 2.23–2.32 (m, 1 H), 1.86–2.03 (m, 3 H), 1.69–1.78 (m, 1 H), 0.94 (d, J = 6.2 Hz, 3 H), 0.90 (d, J = 6.3 Hz, 3 H), 0.80–0.88 (m, 9 H), 0.77 (d, J = 6.5 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 173.95, 171.37, 168.83, 166.18, 157.60, 143.19, 132.97, 125.90, 121.51, 57.50, 57.35, 55.42, 53.53, 42.50, 34.96, 31.36, 31.02, 30.12, 19.71, 19.21, 19.17, 19.13, 17.58, 17.53 ppm. HRMS (ESI): calcd. for $C_{24}H_{39}O_6N_5H^+$ [M + H]$^+$ 494.2973; found 494.2978.

SylA-L-D (37b): Compound **36b** (10.0 mg, 19.7 μmol, 1 equiv.) and aluminum bromide (42 mg, 158 μmol, 8 equiv.) were dissolved in tetrahydrothiophene (1 mL) under an atmosphere of argon in a 10-mL flame-dried flask. The resulting mixture was stirred for 1 h at room temperature. After concentration to dryness, the remaining residue was purified by preparative HPLC (using H_2O with 0.1% TFA (solvent A) and acetonitrile with 0.1% TFA (solvent B) at a flow of 25 mL/min. Gradient: from 0 to 10 min: 90% solvent A/10% solvent B; from 10 to 30 min: from 90% solvent A/10% solvent B to 70% solvent A/30% solvent B; from 30 to 50 min: from 70% solvent A/30% solvent B to 40% solvent A/60% solvent B; from 50 to 60 min: from 40% solvent A/60% solvent B to 0% solvent A/100% solvent B; from 60 to 80 min: 0% solvent A/100% solvent B) to yield **37b** (SylA-L-D; 9.0 mg, 18.3 μmol, 93%) as a colorless solid. TLC (2% acetic acid + 15% methanol in dichloromethane): R_f = 0.40. HPLC (gradient 2): t_R = 6.51 min. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 12.43 (br. s, 1 H), 8.07 (d, J = 6.6 Hz, 1 H), 8.00 (d, J = 8.5 Hz, 1 H), 7.43 (t, J = 6.7 Hz, 1 H), 6.68 (dd, J = 15.4, 4.9 Hz, 1 H), 6.40 (d, J = 9.1 Hz, 1 H), 6.28 (d, J = 8.9 Hz, 1 H), 6.10 (d, J = 15.6 Hz, 1 H), 5.60 (dt, J = 15.8, 7.2 Hz, 1 H), 5.42 (dd, J = 15.8, 8.1 Hz, 1 H), 4.88 (t, J = 6.6 Hz, 1 H),

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4.05–4.12 (m, 2 H), 4.04 (dd, *J* = 8.2, 5.2 Hz, 1 H), 3.10–3.24 (m, 2 H), 2.24–2.31 (m, 1 H), 1.93–2.03 (m, 2 H), 1.85–1.93 (m, 1 H), 1.69–1.78 (m, 1 H), 0.95 (d, *J* = 6.4 Hz, 3 H), 0.90 (d, *J* = 6.4 Hz, 3 H), 0.85 (d, *J* = 6.8 Hz, 3 H), 0.83 (d, *J* = 6.4 Hz, 3 H), 0.82 (d, *J* = 6.4 Hz, 3 H), 0.77 (d, *J* = 6.5 Hz, 3 H) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 174.06, 171.52, 169.00, 166.26, 157.38, 143.26, 133.17, 125.73, 121.52, 57.15, 57.07, 55.41, 53.57, 42.52, 35.03, 31.52, 31.45, 30.57, 19.81, 19.21, 19.15, 19.11, 17.56, 17.44 ppm. HRMS (ESI): calcd. for $C_{24}H_{39}O_6N_5H^+$ [M + H]$^+$ 494.2973; found 494.2969.

SylA-D-L (37c): Compound **36c** (9.0 mg, 17.7 μmol, 1 equiv.) and aluminum bromide (38 mg, 142 μmol, 8 equiv.) were dissolved in tetrahydrothiophene (1 mL) under an atmosphere of argon in a 10-mL flame-dried flask. The resulting mixture was stirred for 1 h at room temperature. After concentration to dryness, the remaining residue was purified by preparative HPLC (using H_2O with 0.1% TFA (solvent A) and acetonitrile with 0.1% TFA (solvent B) at a flow of 25 mL/min. Gradient: from 0 to 10 min: 90% solvent A/10% solvent B; from 10 to 30 min: from 90% solvent A/10% solvent B to 70% solvent A/30% solvent B; from 30 to 50 min: from 70% solvent A/30% solvent B to 40% solvent A/60% solvent B; from 50 to 60 min: from 40% solvent A/60% solvent B to 0% solvent A/100% solvent B; from 60 to 80 min: 0% solvent A/100% solvent B) to yield **37c** (SylA-D-L; 4.6 mg, 9.3 μmol, 53%) as a colorless solid. TLC (2% acetic acid + 15% methanol in dichloromethane): R_f = 0.40. HPLC (gradient 2): t_R = 6.56 min. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 12.39 (br. s, 1 H), 8.12 (d, *J* = 7.8 Hz, 1 H), 8.05 (d, *J* = 8.7 Hz, 1 H), 7.43 (t, *J* = 6.9 Hz, 1 H), 6.69 (dd, *J* = 15.6, 5.3 Hz, 1 H), 6.42 (d, *J* = 9.0 Hz, 1 H), 6.32 (d, *J* = 9.0 Hz, 1 H), 6.10 (d, *J* = 15.5 Hz, 1 H), 5.62 (dt, *J* = 15.4, 7.6 Hz, 1 H), 5.37 (dd, *J* = 16.1, 7.7 Hz, 1 H), 4.92 (t, *J* = 7.6 Hz, 1 H), 4.16 (dd, *J* = 8.9, 5.5 Hz, 1 H), 4.06–4.12 (m, 1 H), 4.05 (dd, *J* = 8.9, 4.7 Hz, 1 H), 3.10–3.23 (m, 2 H), 2.24–2.31 (m, 1 H), 1.93–2.03 (m, 2 H), 1.84–1.92 (m, 1 H), 1.70–1.78 (m, 1 H), 0.95 (d, *J* = 6.6 Hz, 3 H), 0.91 (d, *J* = 6.6 Hz, 3 H), 0.86 (d, *J* = 6.7 Hz, 3 H), 0.82 (d, *J* = 6.8 Hz, 3 H), 0.80 (d, *J* = 6.8 Hz, 3 H), 0.76 (d, *J* = 6.8 Hz, 3 H) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 173.86, 171.34, 169.07, 166.19, 157.36, 143.21, 133.10, 125.83, 121.46, 57.20, 57.10, 55.44, 53.62, 42.50, 34.98, 31.56, 31.35, 30.53, 19.64, 19.19, 19.07, 19.03, 17.40 ppm. HRMS (ESI): calcd. for $C_{24}H_{39}O_6N_5H^+$ [M + H]$^+$ 494.2973; found 494.2967.

SylA-D-D (37d): Compound **36d** (11.8 mg, 23.2 μmol, 1 equiv.) and aluminum bromide (50 mg, 186 μmol, 8 equiv.) were dissolved in tetrahydrothiophene (1 mL) under an atmosphere of argon in a 10-mL flame-dried flask. The resulting mixture was stirred for 1 h at room temperature. After concentration to dryness, the remaining residue was purified by preparative HPLC (using H_2O with 0.1% TFA (solvent A) and acetonitrile with 0.1% TFA (solvent B) at a flow of 25 mL/min. Gradient: from 0 to 10 min: 90% solvent A/10% solvent B; from 10 to 30 min: from 90% solvent A/10% solvent B to 70% solvent A/30% solvent B; from 30 to 50 min: from 70% solvent A/30% solvent B to 40% solvent A/60% solvent B; from 50 to 60 min: from 40% solvent A/60% solvent B to 0% solvent A/100% solvent B; from 60 to 80 min: 0% solvent A/100% solvent B) to yield **37d** (SylA-D-D; 7.8 mg, 15.8 μmol, 68%) as a colorless solid. TLC (2% acetic acid + 15% methanol in dichloromethane): R_f = 0.35. HPLC (gradient 2): t_R = 6.21 min. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 8.03–8.10 (m, 2 H), 7.44 (br. s, 1 H), 6.69 (dd, *J* = 15.2, 4.3 Hz, 1 H), 6.31 (d, *J* = 8.9 Hz, 1 H), 6.27 (d, *J* = 9.0 Hz, 1 H), 6.10 (d, *J* = 15.4 Hz, 1 H), 5.60 (dt, *J* = 15.7, 5.6 Hz, 1 H), 5.37 (dd, *J* = 15.5, 7.6 Hz, 1 H), 4.90 (t, *J* = 6.3 Hz, 1 H), 4.12 (dd, *J* = 8.1, 5.9 Hz, 1 H), 4.05–4.11 (m, 1 H), 3.98 (dd, *J* = 8.3, 4.8 Hz, 1 H), 3.10–3.24 (m, 2 H), 2.24–2.32 (m, 1 H), 1.92–2.02 (m, 2 H), 1.84–1.90 (m, 1 H), 1.70–1.77 (m, 1 H), 0.95 (d, *J* = 6.4 Hz, 3 H), 0.91 (d, *J* = 6.4 Hz, 3 H), 0.86 (d, *J* = 6.6 Hz, 3 H), 0.83 (d, *J* = 6.7 Hz, 3 H), 0.80 (d, *J* = 6.8 Hz, 3 H), 0.77 (d, *J* = 6.7 Hz, 3 H) ppm. ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 174.09, 171.41, 169.08, 166.42, 157.64, 143.42, 133.15, 125.65, 121.34, 57.38, 57.21, 55.30, 53.47, 42.36, 34.80, 31.29, 31.20, 29.88, 19.93, 19.56, 18.97, 18.84, 17.78, 17.36 ppm. HRMS (ESI): calcd. for $C_{24}H_{39}O_6N_5H^+$ [M + H]$^+$ 494.2973; found 494.2966.

Human 20S Proteasome Assays: Proteasome assays were performed and evaluated as previously reported in ref.[12] by using commercially available human erythrocyte 20S proteasome from Biomol. Compounds **37b–d**, **43**, and **47** were dissolved as a stock solution in DMSO, and a dilution series in DMSO was prepared for determining the corresponding K_i' values. For each data point, three independent measurements were performed (n = 3).

Supporting Information (see footnote on the first page of this article): Synthesis of compounds **2–10**, **12–15**, **26–29**, **31–34**, and **39–47**.

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