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Chirality-Driven Folding of Short β-Lactam Pseudopeptides

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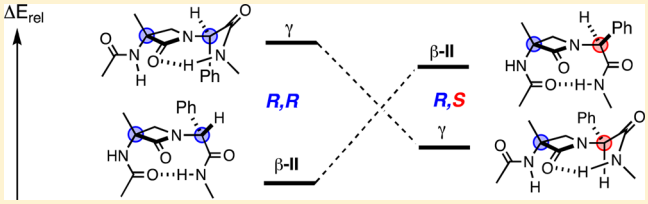
Supporting Information

ABSTRACT: Novel enantiopure pseudopeptide models containing a central -(β-lactam)-(Aa)- scaffold characterized by the combined presence of an α-alkyl-α-amino-β-lactam (*i* +1) residue and a α-substituted (*i* + 2) amino acid have been readily synthesized from α-alkyl serines. The conformational analysis of such β-lactam pseudopeptides conducted in $CDCl_3$ and DMSO-d_6 solutions using 1D- and 2D-NMR techniques revealed an equilibrium between β-II turn and γ-turn conformers, which was ultimately modulated by the relative configuration of the -(β-lactam)-(Aa)- residues. Long-range chiral effects on the α-lactam pseudopeptide conformers were also found when two (*i*) and (*i* + 3) chiral residues were attached to the termini of a central -(β-lactam)-(Aib)- segment. In such mimetics, heterochiral (*i*) and (*i* + 3) residues reinforced a β-II turn conformer, whereas homochiral corner residues stabilized an overlapped β-II/ β-I double turn motif. No β-hairpin nucleation was observed in any instance. In good agreement with the conformers found in solution, β-turned and open structures were also characterized by X-ray crystallography. Relative stabilities of the different conformers were estimated computationally at a B3LYP/6-31++G** calculation level, and finally, a conformation equilibrium model based on steric inter-residual interactions around the -(β-lactam)-(*i* + 2)- segment was proposed to account for the observed chiral effects.



INTRODUCTION

Chirality is at the origin of the regular folding patterns occurring in proteins and natural peptides. It is well-known that short "all-L" peptides may experience dramatic changes in their backbone conformation due to the replacement of a single residue by an achiral unit or a D-configuration amino acid.[1] This phenomenon was first found in natural peptides and is now widely used as a strategy to design peptidomimetics stabilizing reverse turn motifs such as β-turns[2] **1** and γ-turns[3] **2** (Figure 1, top). Indeed, changing the configuration of some backbone residues in functional short peptides may result in increased potency, altered selectivity,[4] or improved proteolytic stability. Furthermore, these effects can become amplified when conformationally constrained lactam scaffolds **3** incorporating interresidual bridges within contiguous CαH and NH positions (Freidinger lactams)[5] are embedded in the native peptide backbone (Figure 1, bottom). Formation of more complex structures, such as the doubly hydrogen-bonded β-hairpins **3** (antiparallel β-sheet nucleators), is also strongly dependent on the configurations of the terminal residues (*i*) and (*i* + 3).[6]

Unfortunately, most of the (*i* + 1)-(*i* + 2) dipeptide lactam surrogates used routinely as turn mimetics[7] possess comparatively large external fragments, and their restraint elements (cycles) and recognition groups (R^1 and R^2) are often crammed in the scaffold.[8] Alternatively, the studies carried out on proline peptides[9] were only of limited success because of their inherent ring-puckering. This makes it very difficult to interpret the stereoelectronic interactions arising from the chirality effect of residues neighboring the lactam moiety. Hence, a scaffold size reduction strategy intended to design "minimal" short peptide models becomes particularly attractive in terms of design simplicity and folding interpretation.

In our first accomplishment in this area,[10] we introduced the β-lactam peptides **4** containing a central α-alkyl-α-amino-β-lactam ring placed as the (*i+1*) residue.[11] This approach, termed "β-lactam scaffold-assisted design" (β-LSAD), involved the formal insertion of a single carbon atom (Cα-*H* + *H*-N → Cα-CH_2-N) in the native peptide to form "minimal" β-lactam pseudopeptides **4**, which displayed a set of interesting structural features. First, the linear disposition of the Cα, N and Cα′ atoms within the β-lactam ring confers to peptidomimetics **4** a high potential for β-hairpin nucleation. Second, they possess a $\psi_{(i+1)}$ dihedral angle fixed at 120° by the β-lactam ring, which prevents the formation of type-I and/or type-III β-turns (both with $\psi_{(i+1)}$ near −30°) and favors only the β-II-turn/γ-turn conformational equilibrium.[12] Indeed, a preliminary conformational study of β-LSAD pseudopeptides containing a glycine (*i*

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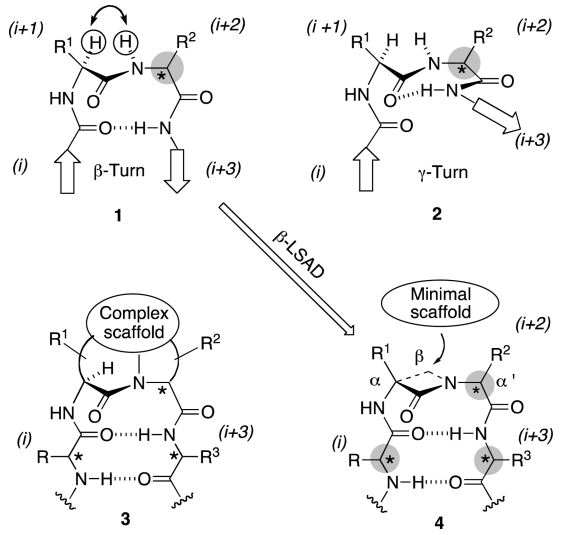

Figure 1. (Top) Peptides folded into β-turn (**1**) and γ-turn (**2**) motifs. (Bottom) Freidinger's lactam peptidomimetics (**3**) and β-LSAD concept (β-lactam scaffold-assisted design) to design minimally scaffolded β-lactam pseudopeptides **4**, differing from the parent peptides in a single carbon atom.

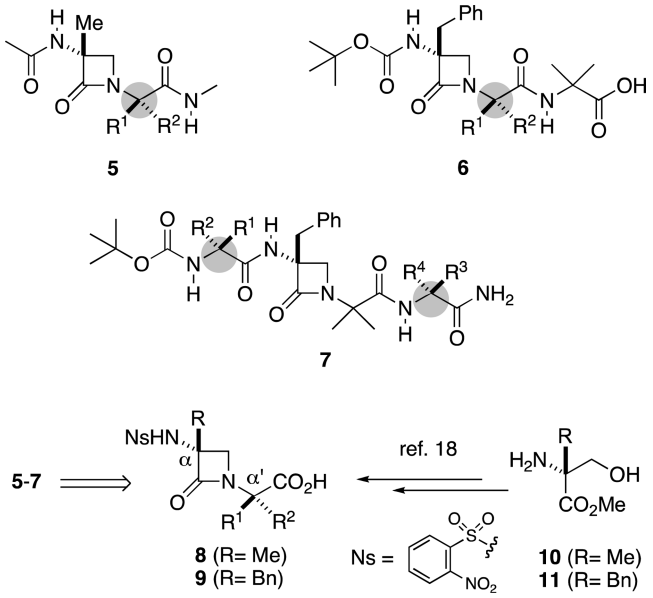

Figure 2. (Top) β-Lactam pseudopeptide models **5**−**7** selected to study the chiral effect of residues (i), (i + 2), and (i + 3) on the peptide folding. (Bottom) Synthesis of the β-lactam dipeptide scaffolds **8** and **9**.

+ 2) residue (**4**; R^2 = H) conducted in our laboratory confirmed their strong bias toward forming type-II β-turns which survive even in the presence of the highly coordinating solvent DMSO.[10a] Finally, α-alkyl-α-amino-β-lactam peptides are expected to be chemically much more stable than common α-amino-β-lactam antibiotics, which undergo easy ring-opening to β-amino acid derivatives. This transformation usually involves an Ad_N nucleophilic attack onto the C=O sp^2 carbon atom following the Bürgi−Dunitz trajectory.[13] In β-lactams **4**, such a trajectory is hindered by the α-substituents at both sides of the 2-azetidinone plane, thus making the ring-opening reaction more difficult with respect to other α-monosubstituted β-lactam analogues.[14] Recently, the surprisingly high "amidicity" of monocyclic β-lactams has also been invoked to account for their chemical stability.[15]

As mentioned above, only the solution conformation of short β-lactam pseudopeptides containing a central -(β-lactam)-(Gly)- core has been studied so far, and the possible β-turn stabilizing (or destabilizing) effect exerted by the chiral α-amino acid residues neighboring the β-lactam nucleus remains to be explored. Herein we report the synthesis and detailed conformational analysis of three families of β-lactam pseudopeptide models designed to show the chiral effects of residues (i), (i + 2) and (i + 3) on the folding of short β-lactam peptides **4** in solution and in the solid state. Finally, we further examine the ability of this motif to nucleate a β-hairpin, the shortest β-sheet structure.

RESULTS AND DISCUSSION

Design and Synthesis of β-Lactam Pseudopeptide Models. Short pseudopeptide models **5**−**7** (Figure 2) with a homogeneous (R) configuration at the Cα stereocenter of the β-lactam ring were selected for development because they are reminiscent of constrained natural L α-amino acid residues.[16] Configurationally related β-lactam peptides **5** and **6** containing a single additional stereocenter at residue (i + 2) were designed to carry terminal groups with different steric demands. Accordingly, mimetics **5** [Ac-(α-Me-β-lactam)-Aa-Me] represented the smallest capped β-lactam dipeptide structures containing three peripheral methyl groups, whereas mimetics **6** [Boc-(α-Bn-β-lactam)-Aa-Aib-OH] had a larger central benzyl group and bulkier achiral N- and C-terminal groups (Boc and Aib-OH). Finally, pseudopeptides **7** [Boc-Ala*-(α-Bn-β-lactam)-Aib-Ala*-NH_2] were designed to ascertain the chiral effect of two additional stereocenters at the (i) and (i + 3) alanine residues on the possible β-hairpin nucleation.

The enantiopure scaffolds **8** and **9** required to form the central -(i + 1)-(i + 2)- pseudopeptide segments were easily obtainable from the corresponding α-alkylserinates[17] **10** and **11** according to an operationally very simple method developed in our laboratory.[18] Tables 1 and 2 show the details of the transformation of the dipeptide scaffolds **8**, **9**, and **14** into the target β-lactam pseudopeptide models.

As shown in Table 1, the synthesis of pseudopeptides **5** and **6** required the coupling of the C-terminal carboxylic acid group in **8** and **9** with methylamine and α-aminoisobutiric benzyl ester, respectively. Formation of *N*-methylamides **12a**−**e** from nonhindered β-lactam carboxylic acid precursors was best performed using the mixed anhydride method assisted by isobutyl chloroformate and *N*-methylmorpholine, whereas couplings involving hindered Aib residues were most efficiently accomplished with EEDQ[19] reagent (products **13**, **15**, **16**, and **7a**−**d**). Denosylation of compounds **12** and **13** proceeded smoothly with thiophenol[20] in the presence of potassium carbonate to afford the intermediate α-amino-β-lactams, which were trapped in situ with acetic anhydride or di-*tert*-butyl dicarbonate to provide the desired mimetic models **5** or the analogues **6** after hydrogenolytic deprotection of the C-terminal benzyl ester. Stereochemical integrity was maintained in all transformations and no epimerization of the α' stereocenter could be detected via NMR and HPLC analysis of the reaction crudes. Following a similar protocol (Table 2), mimetics **7** were obtained in good overall yields from the α',α'-dimethyl-β-lactam **14** after consecutive coupling with N-protected alanines

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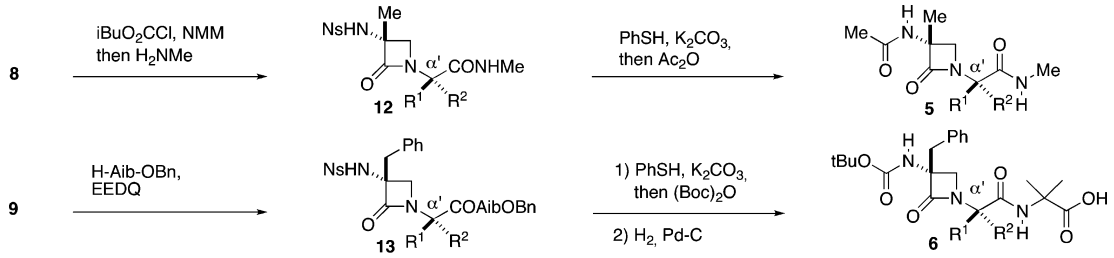
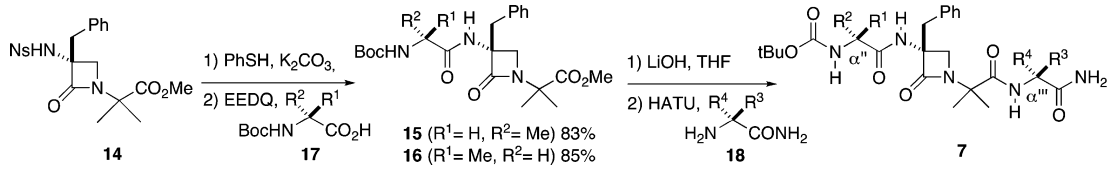
Table 1. Preparation of β-Lactam Peptidomimetics 5 and 6



R^1	R^2	config Cα′	product	yield (%)	product	yield (%)
H	H		**12a**	80	**5a**	84
Me	H	*R*	**12b**	58	**5b**	72
H	Me	*S*	**12c**	59	**5c**	81
Ph	H	*R*	**12d**	78	**5d**	89
H	Ph	*S*	**12e**	85	**5e**	94
Me	Me		**12f**	75[a]	**5f**	82
Me	H	*R*	**13b**	79	**6b**	79
H	Me	*S*	**13c**	70	**6c**	80
Ph	H	*R*	**13d**	55	**6d**	82
H	Ph	*S*	**13e**	66	**6e**	80
Me	Me				**6f**[b]	92

[a]EEDQ reagent was used as the amide formation reagent. [b]The product was prepared from acid Boc-(β-lactam)-Aib-OH, which was coupled to H-Aib-OBn and submitted to hydrogenolysis.

Table 2. Preparation of β-Lactam Peptidomimetics 7



R^1	R^2	config Cα″	R^3	R^4	config Cα‴	product	yield[a] (%)
H	Me	*R*	H	Me	*R*	**7a**	80
H	Me	*R*	Me	H	*S*	**7b**	65
Me	H	*S*	Me	H	*S*	**7c**	73
Me	H	*S*	H	Me	*R*	**7d**	77

[a]Overall yield from **15** and **16**.

17 and alaninamides **18** using, respectively, the EEDQ and HATU/HOAT[21] reagents.

Solution Conformation of β-Lactam Peptides 5 and 6 with a Chiral (*i* + 2) Residue. With the model β-lactam peptides in hand, we first addressed the NMR conformational analysis of the simplest families **5** [Ac-(α-Me-β-lactam)-Aa-Me] and **6** [Boc-(α-Bn-β-lactam)-Aa-Aib-OH] in order to characterize the presumably more populated β-II turn conformers in solution and to assess the possible stabilizing or destabilizing effects exerted by the chiral Aa residues on the conformer equilibrium. Although water was considered first as the most biologically meaningful medium for the study, finally DMSO-d_6 solvent was chosen for practical reasons to ensure solubility. In the case of peptides **5a−f**, however, the recurrent overlapping of NH amide signals in dimethylsulfoxide precluded a straightforward interpretation of the results and $CDCl_3$ was used as solvent. Both solvents (99.80% D) were used as purchased and were not subjected to any particular pretreatment.

Solutions of each peptide **5a−f** and **6a−f** (5×10^{-3} M) yielded sharp, well-resolved ^{1}H NMR spectra consisting of single sets of signals in both solvents. All protons were unambiguously assigned from DQF-COSY and HMBC spectra (Figures S1−S10, Supporting Information). Chemical shift variations upon solvent change and ^{1}H NMR thermal coefficients[22] for the exchangeable amide protons of the pseudopeptides in DMSO-d_6 solutions were determined over a temperature range of 300−325 K at 5 K intervals (Table 3). An inspection of the data recorded immediately revealed a totally different deshielding for the β-lactam N_2H amide protons and the N_7H protons of the (*i* + 3) residue, suggesting the formation of a turned conformer in most of the cases with the N_7H amide protons participating in the intramolecular hydrogen bond network of the β-lactam peptides and the N_2H protons exposed to the solvent. This was evident from the analysis of thermal coefficients in DMSO-d_6 (see Table 3, two right-hand columns) showing in the range −2.3 to −4.9 ppb/K for N_7H protons, whereas amides and carbamates N_2H gave larger absolute thermal coefficient values (up to −8.9 ppb/K). Consistently, the solvent change from nonacceptor $CDCl_3$ to acceptor DMSO-d_6 resulted in a very small chemical shift variation for N_7H amides in all peptides (0.1−1.1 ppm) but in a significantly larger downfield shift (2.3 − 2.8 ppm) in N_2H β-lactam amides. While seeking more insight into the conforma-

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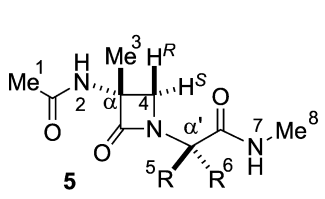
Table 3. Amide Proton ^{1}H NMR Chemical Shifts (δ) and Thermal Coefficients ($\Delta\delta/\Delta T$) for β-Lactam Peptidomimetic Models 5a–f and 6a–f



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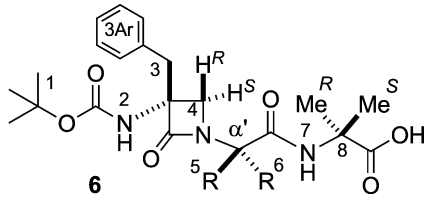

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entry	compd	R^5	R^6	config C α'	$N_2H(\delta)^a$	$N_7H(\delta)^a$	$N_2H(\Delta\delta/\Delta T)^b$	$N_7H(\Delta\delta/\Delta T)^b$
1	**5a**	H	H		5.63 (+2.77)	8.09 (+0.08)	−5.3	−3.2
2	**5b**	Me	H	*R*	5.60 (+2.72)	8.08 (+0.18)	−4.8	−3.4
3	**5c**	H	Me	*S*	5.57 (+2.66)	7.94 (+0.29)	−4.5	−3.9
4	**5d**	Ph	H	*R*	5.64 (+2.77)	8.41 (+0.16)	−5.0	−4.0
5	**5e**	H	Ph	*S*	5.62 (+2.52)	7.12 (+1.15)	−4.5	−4.9
6	**5f**	Me	Me		5.55 (+2.81)	8.19 (+0.16)	−6.5	−3.9
7	**6a**	H	H		4.89 (+2.21)	7.82 (+0.01)	−8.3	−2.3
8	**6b**	Me	H	*R*	4.76 (+2.84)	7.93 (+0.03)	−8.9	−2.8
9	**6c**	H	Me	*S*	4.90 (+2.29)	7.83 (+0.10)	−9.0	−3.0
10	**6d**	Ph	H	*R*	4.85 (+2.69)	8.18 (+0.14)	−8.3	−3.1
11	**6e**	H	Ph	*S*	4.94 (+2.32)^c	7.85 (+0.56)	−8.9	−4.2
12	**6f**	Me	Me		4.84 (+2.73)	7.76 (+0.10)	−8.6	−2.5

^{a}Chemical shifts (ppm) measured in $CDCl_3$ at 300 K. Values in parentheses represent variations of the chemical shift when the $CDCl_3$ solvent was changed to DMSO-d_6. ^{b}Thermal coefficients in ppb/K measured in DMSO-d_6 from 300 to 325 K at 5 K intervals. ^{c}A mixture of conformers was observed at room temperature in $CDCl_3$, which turned to a single set of peaks in DMSO-d_6.

tional differences between the β-lactam peptide diastereomers with (i + 2) chiral α-amino acid residues (Table 3, entries 2–5 and 8–11), we noticed that the thermal coefficients of the N_7H protons where routinely larger for (i + 2)-(*S*) diastereomers (**5c**, **5e** and **6c**, **6e**) than for (i + 2)-(*R*) ones (**5b**, **5d** and **6b**, **6d**), and furthermore, this effect was appreciably enhanced in pseudopeptides containing the bulkier phenylglycine residues (compare entries 4–5 and 10–11). This lower exposure of the (*R*) diastereomers to the DMSO-d_6 coordinating solvent was consistent with the tight intramolecular $N_7H\cdots O{=}C$ hydrogen bonding of β-turned β-lactam peptides **4** (Figure 1), whereas the weaker hydrogen bonds of the (*S*) diastereomers (entries 3, 5, 9, and 11) suggested a partial folding of the peptide backbone into γ-turned conformers or open conformers possessing more exposed amide protons. Finally, the β-lactam pseudopeptides with nonchiral Gly or Aib (i + 2) residues (entries 1, 6, 7, and 12) exhibited only very shielded N_7H protons in DMSO-d_6, consistent with their likely participation in stable β-turns.

Next, relevant noncontiguous interproton distances for peptides **5** and **6** were calculated following the ISPA (isolated spin-pair approximation) method[23] from the integration of key NOESY or ROESY cross-peak signals recorded at 300 K (500 MHz) at mixing times of 200–400 ms (Tables S1 and S2, Figures S15 and S16, Supporting Information).

Some expansions of the NOESY spectra of **5d**,**e** (in $CDCl_3$) and **6d**,**e** (in DMSO-d_6), incorporating a chiral phenylglycine (i + 2) residue, are shown in Figure 3 as an example. The spectra gave evidence for a number of NOE contacts, including the excellent diagnostic correlation peaks provided by the pro-*R* and pro-*S* diastereotopic C_4 protons placed, respectively, at the upper and lower faces of the β-lactam ring, as depicted in Figure 3. Only the $4H^R$ proton gave a characteristic strong NOE contact (cross-peak **a**) with the 3Me singlet in peptides **5** and with the benzylic moiety 3/3Ar in peptides **6**. Conversely, the $4H^S$ proton oriented to the lower face of the azetidin-2-one ring plane was used to monitor the $4H^S$–N_7H distances (cross-peak **b**) and to estimate the preferred formation of canonical β-II turns (≈ 2.5–2.8 Å), distorted γ-turns (≈ 3.8–4.0 Å) or open conformers (>4 Å). Additionally, the orientation of the R^5 and R^6 substituents of the chiral (i + 2) residue relative to the β-lactam ring could be established on the basis of the distances of some of their key protons to $4H^R$ and $4H^S$ (cross-peaks **c** and **d**). In the case of **6d**, the abnormal shielding of 5Ar *ortho* protons (6.52 ppm) arising from the diamagnetic effect of the neighboring benzylic 3Ar ring provided a further indirect sign of β-turn conformation. Finally, as none of the peptides **5** and **6** gave measurable $4H^R/N_7H$ correlation cross-peaks, a significant participation in the conformational equilibrium of inverse γ^i-turn β-lactam pseudopeptides with the (i + 2)-(i + 3) branch oriented toward the top side of the azetidinone ring was ruled out (see empty boxes in Figure 3).

From the interproton distances collected in Figure 3, a regular chiral effect trend could be observed for the β-turn/γ-turn equilibrium as the α'-(*R*) configuration of peptides **5d**/**6d** changed to α'-(*S*) in their diastereomers **5e**/**6e**. Indeed, the average $4H^S$ –N_7H distances (cross-peak **b**) were significantly shorter for **5d** and **6d** (2.6 and 3.0 Å) than for their respective isomers **5e** and **6e** (3.8 Å and >4 Å), suggesting that in the latter cases β-turns were weakened or broken in favor of γ-turns. This assumption was further supported by the close distances of the CαH^S protons of the (*S*)-phenylglycine residue to $4H^R$ and $4H^S$ in **5e**/**6e** and also by the absence of a comparable interaction for CαH^6 in **5d**/**6d**. In line with these observations, β-lactam pseudopeptides **5b**/**6b** and **5c**/**6c** derived, respectively from (*R*)-alanine and (*S*)-alanine, showed similar β-turn destabilization trends upon (i + 2) α' stereocenter configuration inversion, albeit in these cases the chiral effect was less pronounced (Figures S15 and S16, Supporting Information). Finally, all the β-lactam pseudopeptides with achiral (i + 2) residues of glycine (**5a**/**6a**) or α-aminoisobutyric acid Aib (**5f**/**6f**) populated exclusively very

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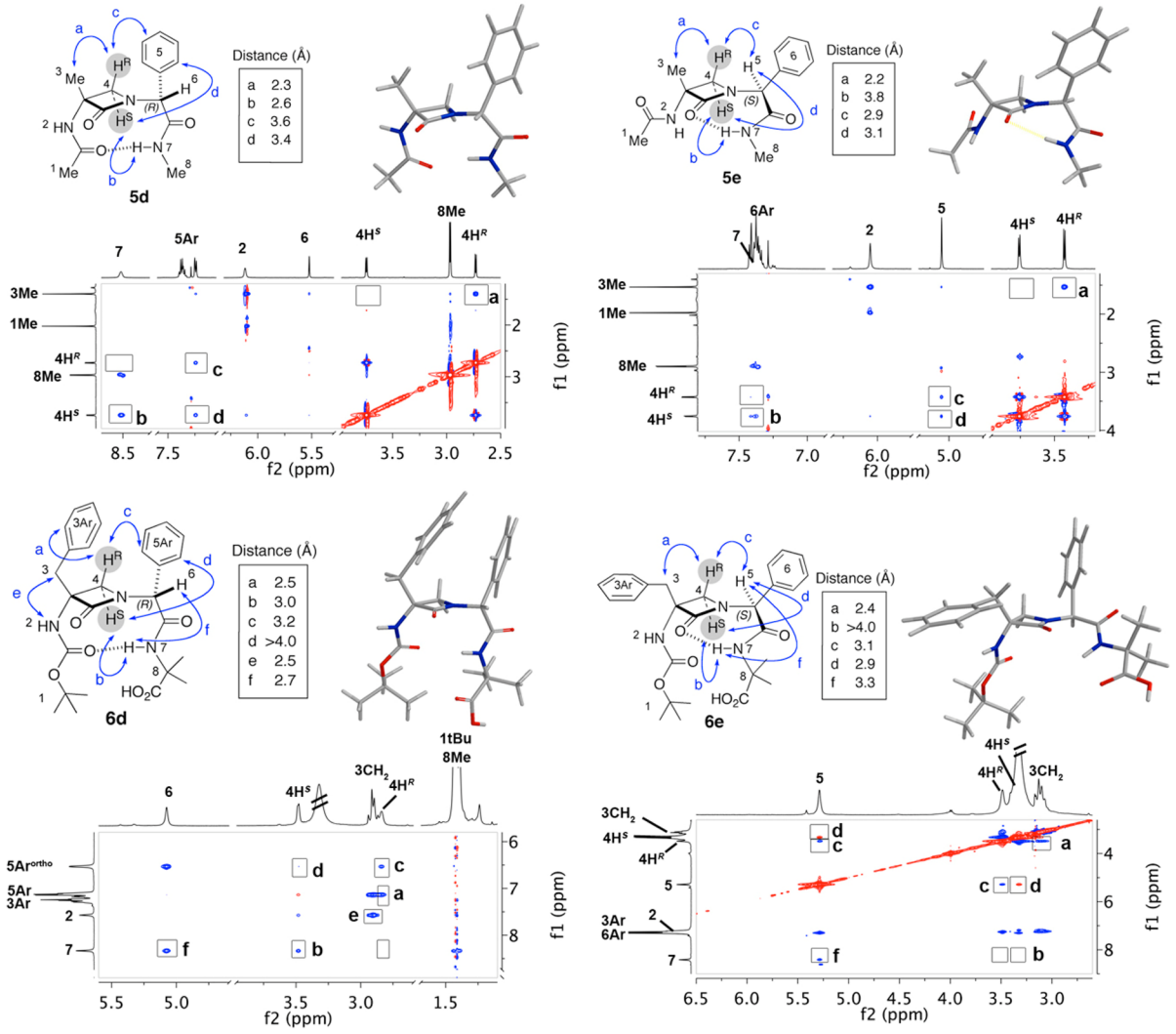
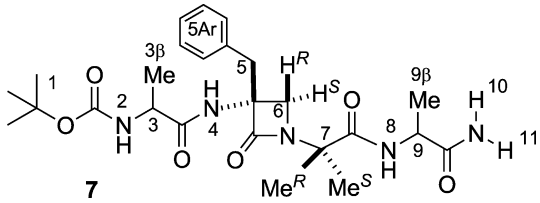


Figure 3. Expansion of the NOESY spectra of pseudopeptides **5d–e** in $CDCl_3$ (top) and **6d–e** in DMSO-d_6 (bottom) showing a preferred β-II turn conformation (left-hand) and a γ-turn conformation (right-hand). Key NOE correlations with the upper ($4H^R$) and lower ($4H^S$) sides of the β-lactam ring plane are highlighted inside boxes. Empty boxes show the expected position of alternative **b** cross-peaks with $4H^R$ protons. Model figures show energy-minimized SYBYL molecular mechanics structures restrained with NOESY interproton distances (±0.3 Å) (Tables S1 and S2, Supporting Information).

stable β-turn conformations and showed no tendency to form γ-turns.

Solution Conformation of β-Lactam Peptides 7 with Chiral (i) and (i + 3) Residues. Following the same NMR methodology disclosed above, we next studied the conformational behavior of β-lactam peptidomimetics **7a–d** in DMSO-d_6 (2×10^{-3} M) to check the chiral effect of (i) and (i + 3) alanine residues on the nucleation of β-sheets. The thermal coefficient data collected in Table 4 show that all the (i + 3) N_8H amide protons participate in strong intramolecular hydrogen bonds, likely forming stable β-II turns. Interestingly, unlike the "heterochiral" diastereomers **7b** and **7d**, "homochiral" diastereomers **7a** (*R*,*R*) and **7c** (*S*,*S*) showed solvent-shielded $N_{10}H$ terminal *trans*-amide protons which were expected to be accepted by the Boc groups to form β-hairpin type secondary structures. A more detailed examination of the interproton distances from ROESY spectra of **7a**, however, revealed that the actual acceptor of the $N_{10}H$ amide proton was the β-lactam carbonyl oxygen, thus forming two overlapped β-II/β-I turns (Figure 4). In particular, this assumption was

Table 4. Amide Proton ^{1}H NMR Thermal Coefficients ($\Delta\delta/\Delta T$)[a] for β-Lactam Peptidomimetic 7



7

entry	compd	config (C_3, C_9)	N_2H	N_4H	N_8H	$N_{10}H$	$N_{11}H$
1	**7a**	*R*,*R*	−7.4	−6.6	−2.3	−2.4	−5.2
2	**7b**	*R*,*S*	−7.3	−6.7	−2.8	−3.8	−4.1
3	**7c**	*S*,*S*	−6.3	−5.8	−3.5	−2.5	−5.2
4	**7d**	*S*,*R*	−7.0	−5.8	−2.5	−4.3	−4.5

[a]Thermal coefficients in ppb/K measured in DMSO-d_6 from 300 to 325 K at 5 K intervals.

further supported by the long-range N_8H–$N_{10}H$ NOE diagnostic cross-peak **h** observed for the homochiral peptide

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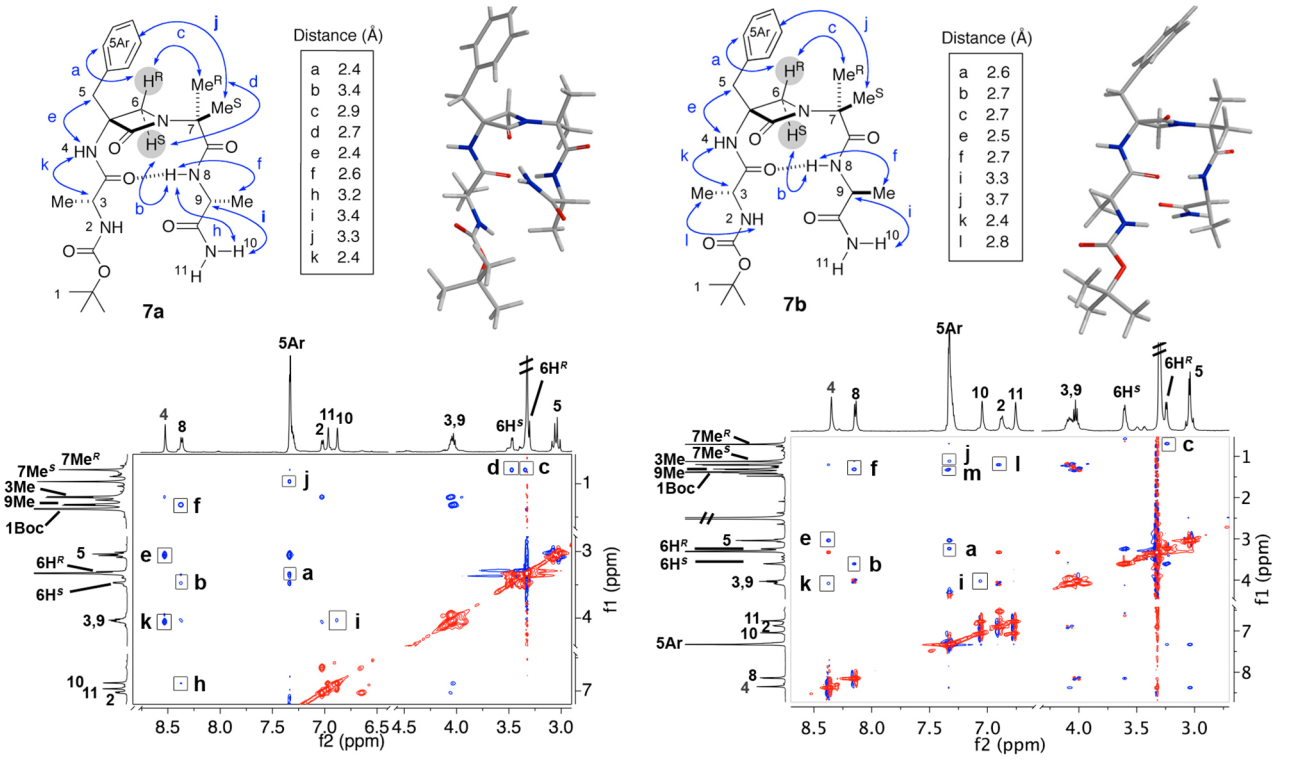

Figure 4. Expansion of the ROESY spectra of pseudopeptides **7a** and **7b** in DMSO-d_6 showing two overlapped β-turns sharing the β-lactam ring and the $N_8H{-}N_{10}H$ close distance NOE correlation (**h**). The model figure shows the energy-minimized structure restrained with ROESY interproton distances (±0.3 Å) (Table S3, Supporting Information).

7a, but not for the heterochiral peptide **7b** (for an analogous behavior for diastereomers **7c**/**7d**, see Figure S17, Supporting Information).

Solid-State Conformation of β-Lactam Peptidomimetics 5–7. Single crystals of the β-lactam pseudopeptides **5e**, **5f**, **7a**, and **7d** were grown from their solutions in MeOH and were used for X-ray crystal structure determination at 160 K (Figure 5). While **5e** and **5f** are ansolvates and there is no free space within the structures that might accommodate solvent molecules, **7a** crystallizes as a 1:1 methanol solvate and **7d** crystallizes as its monohydrate. A detailed analysis of some key structural parameters, Table 5, revealed the formation of well-defined β-II turn conformers in all instances, with the exception of the (*S*)-phenylglycine-derived mimetic **5e**, which showed an extended backbone structure with the (*i*) acetamido group and the (*i* + 3) phenylglycine *N*-methylcarbamoyl group lying on opposite sides of the plane of the β-lactam ring. Consistent with the extremely destabilized γ-turn conformation observed for this pseudopeptide in solution by NMR, no intramolecular hydrogen bond was detected in the solid state. Instead, each NH amide group acted as a donor to form intermolecular bifurcated hydrogen bonds with the C═O oxygen atom of the β-lactam ring.

Mimetic models **5f**, **7a**, and **7d** containing the (*i* + 2) Aib residue fulfilled all the structural criteria of canonical type-II β-turns in terms of $_{(i)}C{=}O{\cdots}HN_{(i+3)}$ hydrogen bond distances (1.9–2.1 Å), $_{(i)}O{\cdots}H{-}N_{(i+3)}$ angles (159–167°) and the δ pseudodihedral angle formed by the four Cα atoms (5–14°), which was very close to the ideal null value. Only a slight distortion (15–20°) caused by the coplanar arrangement of $N_{(i+1)}$, $C\alpha_{(i+1)}$, $N_{(i+2)}$ and $C\alpha_{(i+2)}$ atoms around the central β-lactam ring, was observed for the torsion angles $\phi{-}\psi$ in

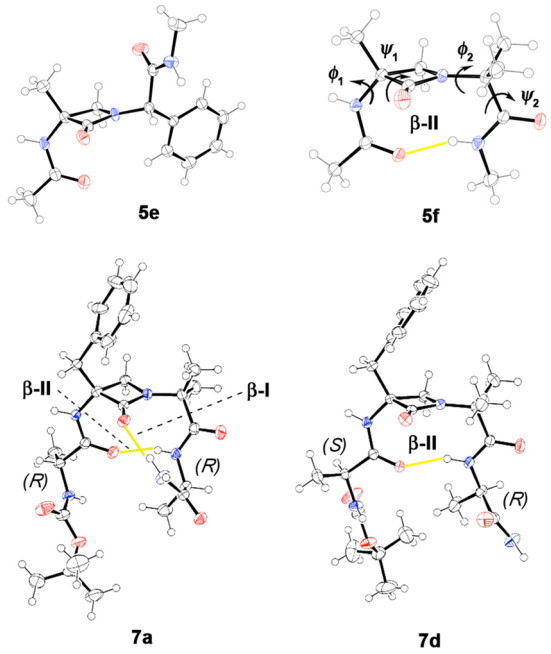

Figure 5. ORTEP plots (50% probability ellipsoids) of the molecular structures of β-lactam peptidomimetics **5e**, **5f**, **7a**, and **7d** in the solid state. Compound **7a** shows overlapping β-II/β-I turns.

residues (*i* + 1)-(*i* + 2) with respect to the ideal values for a type-II β-turn. On the other hand, none of the peptides **7a** and **7d** showed a second hydrogen bond between the terminal Boc and NH_2 groups denoting the formation of β-sheet structures. In spite of that, the NH_{trans} terminal amide proton of peptide **7a** acted as a donor to the β-lactam carbonyl oxygen atom to form

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Table 5. Selected Geometric Parameters for β-Lactam Peptidomimetic Models 5e, 5f, 7a, and 7d[a]

compd	$_{(i)}$C═O···HN$_{(i+3)}$ (Å)	$\phi_{(i+1)}$; $\psi_{(i+1)}$ (deg)	$\phi_{(i+2)}$; $\psi_{(i+2)}$ (deg)	δ^b (deg)	$_{(i)}$C═O···H−N$_{(i+3)}{}^c$ (deg)
5e	>4.0	−39.0(4); 126.6(3)	−95.2(4); 134.9(3)	−157.0	
5f	1.92(3)	−38.3(2); 125.4(2)	90.8(2); −2.8(2)	14.4	160(3)
7a (β-II)	2.07(3)	−39.7(3); 118.9(2)	47.0(4); 30.4(3)	−31.3	167(2)
7a (β-I)	1.87(4)	47.0(4); 30.4(3)	67.2(3); 24.3(3)	−77.3	168(5)
7d	1.97(3)	−40.6(3); 123.9(2)	95.4(3); −2.5(3)	15.9	159(3)
β-II (ideal)	1.8−2.5	−60; 120	80; 0	0	160
β-I (ideal)	1.8−2.5	60; 30	90; 0	0	160
γ (ideal)	1.8−2.5	−; −	50; −60	0	160

[a]Measured at 170 K. Solvates: **7a**·MeOH and **7d**·H_2O. [b]Pseudodihedral angle formed by the four Cα atoms of the peptide models. [c]O···H−N angle.

a quasi-canonical β-I turn partially overlapping the β-II turn. To the best of our knowledge, this is the first example of such a type of double motif in solid pseudopeptide structures.[24]

Finally, a rationale for the chiral effect caused by the (*i* + 2) residue on the relative stability of β-II/ γ conformers in pseudopeptides **5** was established on the basis of the steric interactions of R^1 and R^2 groups with the carbonyl and methylene groups of the β-lactam ring (*i* + 1). Accordingly, we submitted structures **5a−f** to a DFT conformational optimization[25] consisting of a Monte Carlo search (6-31G*), followed by structure minimization at the B3LYP/6-31++G** level[26] (for details, see the Supporting Information, S39). Energy values collected in Figure 6 show a general trend for the preferred stabilization (2−3 kcal/mol) of type II β-turns, over the normal γ-turns. As expected, no type-I β-turn could be characterized as a stable energy minimum. In addition, a clear chiral-effect destabilizing the β-II turn was apparent in the case of heterochiral (*R*,*S*) mimetics **5c** and **5e** with respect to the homochiral (*R*,*R*) mimetics **5b** and **5d**, and this energy-difference increased when the size of R group was increased from methyl to phenyl. In the latter case, a dramatic inversion in the stability of the γ-turn is also observed. Finally, models **5a** and **5f** bearing respectively achiral (*i* + 2) residues of Gly and Aib displayed very similar relative stability patterns favoring the type β-II conformer over the γ-turns, albeit in the latter case the energy difference was lower.

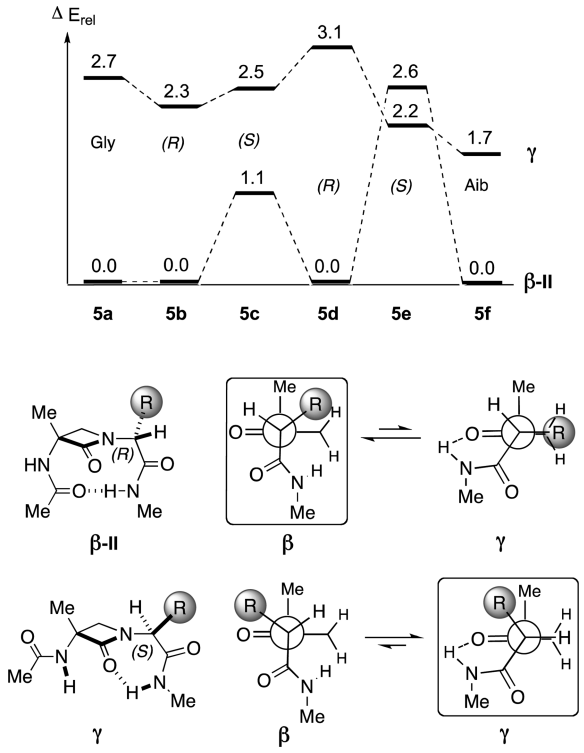

Figure 6. (Top) Relative zero energies (kcal·mol^{-1}) of β-II turn and γ-turn conformers for pseudopeptides **5a−f**. (Bottom) Steric interactions accounting for the chiral-effect and relative conformer stabilities. The energy values for the isomer pairs **5b−c** and **5d−e** are assigned to the lower energy conformer in each case.

Figure 6 also depicts the Newman projections of models **5** with the β-lactam nitrogen atom and Cα′ eclipsed. In homochiral (*R*,*R*) isomers, the group R can adopt an alternative disposition only when the β-turn-II conformation is reached, leading to the more stable conformations. The exception to this general bias only occurs when the (*i* + 2) residue is heterochiral with respect to the β-lactam (*i* + 1) stereocenter and the size-difference between R and the β-lactam α- group is large. For instance, in model **5e** with a (*S*)-phenylglycine (*i* + 2) residue, conformer β-II is destabilized by the steric repulsion of the phenyl group and the β-lactam carbonyl oxygen atom, resulting in the stabilization of the γ-turn conformer.

CONCLUSIONS

The present work illustrates the intrinsic ability of short β-lactam peptide chains to form β-II and γ-turn structures in solution. It also emphasizes that "one carbon-scaffolded" β-lactam pseudopeptides provide very precise information on the H-bonding pattern in short peptide models, which complements prior quantum chemistry and gas-phase laser-desorption experiments.[25c] 2D NMR experiments conducted in solution have provided compelling evidence that β-lactam pseudopeptides **5** and **6** containing a central homochiral -[(*R*)-β-Lactam-(*R*)-Aa]- segment behave as betagenic, whereas the heterochiral analogues -[(*R*)-β-lactam-(*S*)-Aa]- destabilize β-turn conformations and favor γ-turned peptides, especially when the (*i* + 2) Aa residue carries sterically demanding Cα backbone substituent groups. In addition, pseudopeptides **7** containing a central betagenic -[(*R*)-β-lactam-Aib]- nucleus flanked by heterochiral (*i*) and (*i* + 3) α-amino acid residues do not show a straightforward ability to stabilize β-hairpins. Instead, the homochiral counterparts show a strong tendency to form unprecedented β-II/β-I overlapped double turns around the central β-lactam core.

In the light of the current results, it can be stated that chirality-driven conformational effect may ultimately determine the preferred type of turn stabilized by β-lactam pseudopeptides. Therefore, the β-LASD design model, consisting in the incorporation of an intraresidual methylene bridge to frozen type-II β-turns, must be applied cautiously to "all-L" peptide natural segments when chiral residues neighboring the central

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β-lactam core are involved. Despite this limitation, the β-LASD design method provides a simple steric model that opens up the route for a better understanding of the steric interactions driving the shallow β-/γ- transition in larger peptides.

EXPERIMENTAL SECTION

General Experimental Procedures. All reactions were carried out under an atmosphere of nitrogen in oven- or flame-dried glassware with magnetic stirring. Solvents were distilled prior to use. Anhydrous tetrahydrofuran (THF) and dichloromethane were distilled, respectively, from sodium metal/benzophenone ketyl and from calcium hydride. Reaction products were purified by flash chromatography using silica gel 60 (230−400 mesh). Analytical TLC was performed on 0.25 mm silica gel 60-F plates and spots were visualized, either with UV light or with the spray reagent phosphomolybdic acid-ammonium cerium(IV) nitric-sulfuric acid−water, followed by heating. Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 and 125.7 MHz, respectively, and are reported as δ values (ppm) relative to residual $CDCl_3$ (δ 7.26), DMSO (δ 2.49), and $CDCl_3$ (δ 77.0), DMSO (δ 39.5) as internal standards. Mass spectra were obtained from samples ionized under EI (70 eV) or CI conditions and detected using a quadrupole or a TOC mass analyzer, after direct injection (HRMS) or GC−MS coupling (column: fused silica gel, 15 m, 0.25 mm, 0.25 nm phase SPB-5). The preparations and spectroscopic data of compounds **6a**, **8a**, **9a−f**, and **14** were reported previously.[10a,18]

General Procedure for the Preparation of 1-Carboxyalkyl-3-methyl-3-(2-nitrobenzenesulfonylamino)azetidin-2-ones 8b−f.[18] To a solution of (*R*)-methyl 2-methylserinate **10** (1.90 mmol, 0.32 g) and 2-nitrobenzenesulfonyl chloride (NsCl, 4.18 mmol, 0.935 g) in acetonitrile (30 mL) was added $KHCO_3$ (9.50 mmol, 0.951 g), and the suspension was stirred at reflux for 16 h. Then saturated aqueous $NaHCO_3$ (15 mL) was added, and the mixture was extracted with EtOAc (3 × 15 mL). The organic layer was dried ($MgSO_4$), and the solvents were evaporated at reduced pressure to give pure (2*R*)-2-methyl-2-methoxycarbonyl-1-(2-nitrobenzenesulfonyl)aziridine. A solution of the aziridine (1.51 mmol) and the corresponding β-(*tert*-butyldimethylsilyloxy)ethylamine (1.51 mmol) in dry acetonitrile (30 mL) was stirred at room temperature for 20 h. Then saturated aqueous $NaHCO_3$ (15 mL) was added, the mixture was extracted with EtOAc (3 × 15 mL), the organic fractions were dried ($MgSO_4$) and filtered, and solvents were evaporated in vacuo to give the corresponding β-azaalanine, which was purified by column chromatography (hexanes/EtOAc 4:1). To a stirred solution of the β-azaalanine (1 mmol) in dry THF (15 mL) was added 2.5 mmol of LiHMDS in THF (1 M). After being stirred for 12 h, the mixture was washed with a saturated aqueous solution of $NaHCO_3$ (3 × 10 mL), and the aqueous layer was extracted with CH_2Cl_2. The solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography (hexanes/EtOAc, 1:7). The intermediate obtained was oxidized following two different procedures.

Oxidation Method A.[27] BAIB (1.069 mmol, 0.344 g), TEMPO (0.243 mmol, 0.038 g), and the corresponding *N*-hydroxyethyl-β-lactam (0.486 mmol) were combined in the reaction vessel, and to this mixture was added 1:1 acetone−water solution (2 mL). The reaction mixture was stirred for 3 h before evaporation of the solvents under reduced pressure. The resulting product was purified by column chromatography (hexanes/EtOAc, 1:10 or CH_2Cl_2/MeOH, 10:1).

Oxidation Method B. The Jones reagent[28] (1.48 mmol) was added dropwise to the solution of the corresponding *N*-hydroxyphenylethyl-β-lactam (0.74 mmol) in acetone (10 mL) at 0 °C, and the mixture was stirred at the same temperature for 2 h. The oxidant excess was quenched with ^{i}PrOH (10 mL), and the dark solid formed was dissolved with H_2O (4 mL). The mixture was extracted with EtOAc (3 × 10 mL), and the combined organic fractions were washed with brine (5 mL). The organic solution was dried ($MgSO_4$) and evaporated under reduced pressure, and the resulting product was purified by column chromatography (MeOH/CH_2Cl_2, 1:10).

*(3R)-1-[(R)-1-Carboxyethyl]-3-methyl-3-(2-nitrobenzenesulfonylamino)azetidin-2-one (**8b**).* The general procedure was followed (oxidation method A) from *N*-[(2*R*)-2-hydroxy-1-methylethyl]-β-lactam (0.524 mmol, 0.180 g): yield 0.160 g (87%); yellowish solid; mp 55 °C; $[\alpha]^{25}_D$ +7.7 (0.45, MeOH); IR (KBr) ν_{max} 3320, 2944, 1747, 1718, 1545, 1169, 832 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.22−7.78 (m, 4H), 4.09 (q, 1H, *J* = 7.2 Hz), 3.72 (d, 1H, *J* = 5.8 Hz), 3.35 (d, 1H, *J* = 5.8 Hz), 1.52 (s, 3H), 1.38 (d, 3H, *J* = 7.3 Hz); ^{13}C NMR (75 MHz, CD_3OD) δ 177.7, 169.4, 149.4, 136.4, 135.0, 133.7, 131.9, 125.9, 66.5, 54.8, 54.3, 20.5, 16.5; MS *m*/*z* (ESI, negative polarity) MS-1 355.9; MS2(355.9) 308.8, 200.7, 185.7, 137.8. Anal. Calcd for $C_{13}H_{15}N_3O_7S$: C, 43.69; H, 4.23; N, 11.76. Found: C, 43.50; H, 4.17; N, 11.49.

*(3R)-1-[(S)-1-Carboxyethyl]-3-methyl-(2-nitrobenzenesulfonylamino)azetidin-2-one (**8c**).* The general procedure was followed (oxidation method A) from *N*-hydroxy-(2*S*)-methylethyl-β-lactam (0.350 mmol, 0.120 g): yield 0.110 g (88%); white solid; mp 58−60 °C; $[\alpha]^{25}_D$ −21.3 (0.5, MeOH); IR (KBr) ν_{max} 3260, 2901, 1742, 1728, 1543, 1170, 820 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.17−7.83 (m, 4H), 4.23 (q, 1H, *J* = 7.5 Hz), 3.63 (d, 1H, *J* = 5.5 Hz), 3.50 (d, 1H, *J* = 5.5 Hz), 1.58 (s, 3H), 1.38 (d, 3H, *J* = 7.5 Hz); ^{13}C NMR (75 MHz, CD_3OD) δ 176.1, 168.1, 147.9, 135.1, 133.6, 132.2, 130.1, 124.4, 65.0, 52.5, 51.5, 19.3, 14.9; MS *m*/*z* (ESI, negative polarity) MS-1 356.0; MS2(356.0) 200.7, 137.8; MS3(200.7) 136.8. Anal. Calcd for $C_{13}H_{15}N_3O_7S$: C, 43.69; H, 4.23; N, 11.76. Found: C, 43.54; H, 4.46; N, 11.47.

*(3R)-3-Methyl-3-(2-nitrobenzenesulfonylamino)-1-[(R)-1-phenylcarboxymethyl]azetidin-2-one (**8d**).* The general procedure was followed (oxidation method B) from *N*-hydroxy-(2*R*)-phenylethyl-β-lactam (1.43 mmol, 0.58 g): yield 0.330 g (55%); yellowish powder; mp 115 °C; $[\alpha]^{25}_D$ 56.1 (0.7, MeOH); IR (KBr) ν_{max} 3050, 2934, 1749, 1725, 1530, 1156, 802 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.24−7.32 (m, 9H), 5.37 (s, 1H), 3.97 (d, 1H, *J* = 6.0 Hz), 3.08 (d, 1H, *J* = 6.0 Hz), 1.41 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 170.9, 166.4, 146.4, 134.2, 132.2, 130.9, 129.1, 126.9, 126.5, 126.4, 123.1, 63.8, 58.4, 52.5, 17.8; MS *m*/*z* (ESI, positive polarity) MS+23: 442.0, 420.0; MS2(420.1) 392.0; 204.9; MS3(392.0) 346.0, 205.0, 158.9. Anal. Calcd for $C_{18}H_{17}N_3O_7S$: C, 51.55; H, 4.09; N, 10.02. Found: C, 51.78; H, 4.28; N, 9.76.

*(3R)-3-Methyl-3-(2-nitrobenzenesulfonylamino)-1-[(S)-1-phenylcarboxymethyl]azetidin-2-one (**8e**).* The general procedure was followed (oxidation method B) from *N*-hydroxy-(2*S*)-phenylethyl-β-lactam (0.740 mmol, 0.300 g): yield 0.180 g (58%); yellowish powder; mp 188−190 °C; $[\alpha]^{25}_D$ +66.3 (1.25, MeOH); IR (KBr) ν_{max} 3360, 2944, 1741, 1736, 1536, 1167, 826 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ_H 7.83−7.33 (m, 9H), 5.38 (s, 1H), 3.56 (d, 1H, *J* = 5.6 Hz), 3.23 (d, 1H, *J* = 5.6 Hz), 1.60 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 173.4, 168.1, 147.8, 135.6, 134.9, 133.6, 132.2, 130.0, 128.4, 128.3, 127.7, 124.5, 65.0, 59.8, 52.8, 19.7; MS *m*/*z* (ESI, positive polarity) MS+23 442.0, 420.1; MS2(420.1) 392.0; MS3(392.0) 346.0, 205.0, 159.0. Anal. Calcd for $C_{18}H_{17}N_3O_7S$: C, 51.55; H, 4.09; N, 10.02. Found: C, 51.32; H, 3.82; N, 9.82.

*(3R) 1-(1-Carboxyisopropyl)-3-methyl-3-(2-nitrobenzenesulfonylamino)azetidin-2-one (**8f**).* To the solution of *N*-(methoxycarbonyl-isopropyl)-β-lactam (0.259 mmol, 0.110 g) in THF (1 mL) was added a solution of $LiOH·H_2O$ (1.299 mmol, 0.055 g) in H_2O (1 mL). The reaction mixture was stirred at room temperature for 2 h and then was acidified with 2 M HCl and extracted with EtOAc (3 × 5 mL). The organic layer was dried over $MgSO_4$ and evaporated to give a product which was purified by column chromatography (hexanes/EtOAc, 2:1): yield 0.090 g (93%); yellow oil; $[\alpha]^{25}_D$ −33.0 (1.2, MeOH); IR (KBr) ν_{max} 3260, 2989, 1785, 1764, 1532, 1179, 816 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.16−7.78 (m, 4H), 3.68 (d, 1H, *J* = 5.0 Hz), 3.34 (d, 1H, *J* = 5.0 Hz), 1.61 (s, 3H), 1.52 (s, 3H), 1.44 (s, 3H). ^{13}C NMR (75 MHz, CD_3OD) δ 175.9, 169.1, 149.3, 136.4, 135.0, 133.6, 131.7, 125.9, 65.6, 60.2, 54.5, 24.3, 24.2, 20.9; MS *m*/*z* (ESI, negative polarity) MS-1: 383.0; MS2(383.0) 200.7, 185.7, 137.8; MS3(137.8) 117.9. Anal. Calcd for $C_{14}H_{17}N_3O_7S$: C, 45.28; H, 4.61; N, 11.32. Found: C, 44.97; H, 4.77; N, 11.53.

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General Procedure for the Preparation of 3-Methyl-1-[(*N*-methylcarbamoyl)alkyl]-3-(2-nitrobenzenesulfonylamino)-azetidin-2-ones (12a–f). To the solution of the corresponding β-lactam **8** (0.175 mmol, 0.060 g) in THF (1.5 mL) were successively added NMM (0.175 mmol, 0.020 mL) and IBCF[29] (0.175 mmol, 0.022 mL) at −20 °C. After an activation period of 3 min, 40% aqueous methylamine (0.350 mmol, 0.012 mL) was added, and the resulting solution was stirred for 45 min at −20 °C. After the solvent was evaporated under reduced pressure, the residue was directly loaded on silica gel (hexanes/EtOAc, 1:7) to give the corresponding *N*-methylcarbamoyl-β-lactam.

*(3R)-3-Methyl-1-(N-methylcarbamoylmethyl)-3-(2-nitrobenzenesulfonylamino)azetidin-2-one (**12a**).* The general procedure was followed from β-lactam (**8a**) (0.175 mmol, 0.060 g). The obtained crude product was purified by preparative TLC (hexanes/EtOAc, 1:7): yield 0.050 g (80%); white powder; mp 145–147 °C; $[\alpha]^{25}_{D}$ −87.3 (1.5, CH_2Cl_2); IR (KBr) ν_{max} 3108, 2990, 1760, 1732, 1526, 1140, 825 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 8.10–7.26 (m, 4H), 6.98 (br s, 1H), 5.95 (s, 1H), 4.35 (d, 1H, *J* = 17.0 Hz), 3.89 (d, 1H, *J* = 5.5 Hz), 3.57 (d, 1H, *J* = 17.0 Hz), 3.37 (d, 1H, *J* = 5.5 Hz), 2.90 (d, 3H, *J* = 5.5 Hz), 1.62 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 167.4, 167.1, 147.6, 134.9, 133.9, 133.2, 130.8, 125.5, 67.9, 57.4, 45.6, 26.3, 21.8; MS *m*/*z* (ESI, negative polarity) MS-1 354.9; MS2(354.9) 135.8 137.7 185.7, 193.7. Anal. Calcd for $C_{13}H_{16}N_4O_6S$: C, 43.82; H, 4.53; N, 15.72. Found: C, 43.80; H, 4.69; N, 15.47.

*(3R)-3-Methyl-1-[(1R)-1-(N-methylcarbamoyl)ethyl]-3-(2-nitrobenzenesulfonylamino)azetidin-2-one (**12b**).* The general procedure was followed from β-lactam (**8b**) (0.08 mmol, 0.030 g). The crude product was purified by preparative TLC (hexanes/EtOAc, 1:7): yield 0.018 g (58%); white solid; mp 152 °C; $[\alpha]^{25}_{D}$ −67.3 (0.75, CH_2Cl_2); IR (KBr) ν_{max} 3150, 2963, 1750, 1735, 1520, 1160, 825 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 8.09–7.74 (m, 4H), 7.08 (br s, 1H), 5.92 (s, 1H), 4.47 (q, 1H, *J* = 7.3 Hz), 3.74 (d, 1H, *J* = 5.5 Hz), 3.40 (d, 1H, *J* = 5.5 Hz), 2.88 (d, 3H, *J* = 4.7 Hz), 1.60 (s, 3H), 1.42 (d, 3H, *J* = 7.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.1, 166.9, 147.6, 135.0, 133.9, 133.2, 130.8, 125.4, 66.5, 53.9, 50.3, 26.4, 21.9, 14.5; MS *m*/*z* (ESI, negative polarity) MS-1 369.0; MS2(369.0) 185.7, 137.8, 126.8. Anal. Calcd for $C_{14}H_{18}N_4O_6S$: C, 45.40; H, 4.90; N, 15.11. Found: C, 45.41; H, 4.78; N, 15.20.

*(3R)-3-Methyl-1-[(1S)-1-(N-methylcarbamoyl)ethyl]-3-(2-nitrobenzenesulfonylamino)azetidin-2-one (**12c**).* The general procedure was followed from β-lactam (**8c**) (0.078 mmol, 0.028 g). The crude product was purified by preparative TLC (hexanes/EtOAc, 1:7): yield 0.017 g (59%); white solid; mp 163–164 °C; $[\alpha]^{25}_{D}$ −69.3 (0.67, CH_2Cl_2); IR (KBr) ν_{max} 3120, 2959, 1765, 1745, 1540, 1100, 808 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 8.12–7.72 (m, 4H), 6.56 (br s, 1H), 5.96 (s, 1H), 4.06 (q, 1H, *J* = 7.3 Hz), 3.84 (d, 1H, *J* = 5.5 Hz), 3.39 (d, 1H, *J* = 5.5 Hz), 2.84 (d, 3H, *J* = 4.8 Hz), 1.58 (s, 3H), 1.57 (d, 3H, *J* = 7.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 171.1, 167.4, 148.0, 135.8, 134.2, 133.5, 131.2, 125.8, 66.5, 55.9, 53.9, 26.8, 21.9, 15.9; MS *m*/*z* (ESI, negative polarity) MS-1 369.0; MS2(369.0) 185.7, 137.8, 126.9; MS3(137.0) 121.8, 90.0. Anal. Calcd for $C_{14}H_{18}N_4O_6S$: C, 45.40; H, 4.90; N, 15.11. Found: C, 45.04; H, 4.88; N, 15.23.

*(3R)-3-Methyl-1-[(1R)-1-(N-methylcarbamoyl)-1-phenylmethyl]-3-(2-nitrobenzenesulfonylamino)azetidin-2-one (**12d**).* To a solution of β-lactam (**8d**) (0.24 mmol, 0.100 g) and methylamine (2 M in THF; 0.357 mmol, 0.179 mL) in CH_2Cl_2 (5 mL) were added sequentially DCC (0.334 mmol, 0.069 g) and HOBt (0.286 mmol, 0.039 g) at 0 °C. The reaction mixture was allowed to warm to rt and stirred for 12 h. Then solvent was removed under reduced pressure, and the resulting crude product was purified by preparative TLC (hexanes/EtOAc, 1:5): yield 0.080 g (78%); yellowish powder; mp 145 °C; $[\alpha]^{25}_{D}$ −144.4 (0.75, CH_2Cl_2); IR (KBr) ν_{max} 3320, 2984, 1768, 1732, 1540, 1158, 828 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 8.14–7.25 (m, 10H), 5.52 (s, 1H), 3.80 (d, 1H, *J* = 5.8 Hz), 2.99 (d, 3H, *J* = 4.7 Hz), 2.94 (d, 1H, *J* = 5.8 Hz), 1.46 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 169.1, 167.2, 147.9, 135.4, 134.3, 133.7, 131.3, 129.6, 129.2, 125.9, 66.7, 60.1, 55.3, 27.0, 21.8; MS *m*/*z* (ESI, negative polarity) MS+1 431.0; MS2(431.0) 193.7, 188.8, 137.8; MS3(188.8) 137.8, 131.8. Anal. Calcd for $C_{19}H_{20}N_4O_6S$: C, 52.77; H, 4.66; N, 12.96. Found: C, 52.91; H, 4.70; N, 12.61.

*(3R)-3-Methyl-1-[(1S)-1-(N-methylcarbamoyl)-1-phenylmethyl]-3-(2-nitrobenzenesulfonylamino)azetidin-2-one (**12e**).* According to the aforementioned procedure (compound **12d**) product **12e** was prepared starting from β-lactam (**8e**) (0.31 mmol, 0.130 g) and methylamine (2 M in THF; 0.465 mmol, 0.233 mL). An epimerization took place, and the mixture of diastereisomers **12e** and **12d** (ratio 96:4) was separated by preparative TLC (hexanes/EtOAc, 1:5): yield 0.113 g (85%); yellowish powder; mp 148–150 °C; $[\alpha]^{25}_{D}$ +38.9 (0.7, CH_2Cl_2); IR (KBr) ν_{max} 3210, 2943, 1745, 1725, 1544, 1164, 821 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.92–7.35 (m, 10H), 5.99 (br s, 1H), 5.93 (br s, 1H), 5.38 (s, 1H), 3.62 (s, 2H), 2.79 (d, 3H, *J* = 4.7 Hz), 1.62 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 168.7, 167.1, 147.5, 135.3, 133.9, 133.5, 132.9, 130.7, 129.2, 128.9, 128.4, 125.3, 65.9, 59.2, 54.8, 26.4, 21.3; MS *m*/*z* (ESI, negative polarity) MS-1: 431.0; MS2(431.0) 137.8, 188.8, 185.7. Anal. Calcd for $C_{19}H_{20}N_4O_6S$: C, 52.77; H, 4.66; N, 12.96. Found: C, 53.00; H, 4.59; N, 12.58.

*(3R)-3-Methyl-1-[1-(N-methylcarbamoyl)isopropyl]-3-(2-nitrobenzenesulfonylamino)azetidin-2-one (**12f**).* The β-lactam (**8f**) (0.162 mmol, 0.160 g) was dissolved in CH_2Cl_2 (2.5 mL) and cooled to 0 °C. To this solution was added methylamine (2 M THF) (0.323 mmol, 0.162 mL), and then the reaction mixture was cooled to −12 °C (ice/NaCl) and EEDQ (0.072g, 0.292 mmol) was added as a CH_2Cl_2 solution (2.5 mL). The reaction was slowly warmed to room temperature and stirred overnight. After this time, the reaction mixture was concentrated under reduced pressure, and the crude product was purified by preparative TLC (hexanes/EtOAc, 1:7) to afford pure *N*-methylamide **12f** as a white powder: yield 0.050 g (75%); mp 135 °C; $[\alpha]^{25}_{D}$ −46.2 (1.75, CH_2Cl_2); IR (KBr) ν_{max} 3315, 2934, 1758, 1728, 1540, 1166, 798 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ_H 8.14–7.84 (m, 4H), 3.63 (d, 1H, *J* = 5.0 Hz), 3.41 (d, 1H, *J* = 5.0 Hz), 2.79 (s, 3H,), 1.57 (s, 3H), 1.54 (s, 3H), 1.45 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ_C 174.5, 168.3, 148.2, 135.4, 134.1, 132.6, 130.2, 124.8, 64.8, 60.2, 53.8, 25.8, 23.4, 23.3, 20.1; MS *m*/*z* (ESI, negative polarity) MS-1 383.0; MS2(383.0) 200.7, 185.7, 137.8; MS3(137.8) 117.9. Anal. Calcd for $C_{15}H_{20}N_4O_6S$: C, 46.87; H, 5.24; N, 14.57. Found: C, 47.02; H, 5.33; N, 14.36.

General Procedure for the Preparation 3-Acetamido-3-methyl-1-[(*N*-methylcarbamoyl)alkyl]azetidin-2-ones (5a–f). To a stirred solution of the corresponding β-lactam **12** (0.067 mmol, 0.045 g) in dry CH_3CN (5 mL) were added thiophenol (0.335 mmol, 0.034 g) and K_2CO_3 (0.268 mmol, 0.037 g). After the solution was stirred at room temperature for 1 h (monitored by TLC and ^1H NMR), acetic anhydride (0.402 mmol, 0.088 g) was added, and the reaction was stirred overnight. The solvent was evaporated under reduced pressure, and the crude was purified by preparative TLC (hexanes/EtOAc, 1:7).

*(R)-3-Acetamido-3-methyl-1-[1-(N-methylcarbamoyl)methyl]-azetidin-2-one (**5a**).* The general procedure was followed from α-(2-nosyl)-β-lactam **12a** (0.084 mmol, 0.030 g). The crude product was purified by preparative TLC (hexanes/EtOAc, 1:7): yield 0.015 g (84%); yellowish oil; $[\alpha]^{25}_{D}$ +94.8 (0.5, CH_2Cl_2); IR (KBr) ν_{max} 3210, 2965, 1740, 1728 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 8.19 (br s, 1H), 6.16 (s, 1H), 4.44 (d, 1H, *J* = 17.6 Hz), 3.89 (d, 1H, *J* = 5.0 Hz), 3.57 (d, 1H, *J* = 17.6 Hz), 3.37 (d, 1H, *J* = 5.0 Hz), 2.90 (d, 3H, *J* = 4.6 Hz), 2.06 (s, 3H), 1.58 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.7, 168.8, 168.0, 65.0, 53.5, 45.3, 26.3, 22.9, 20.5; MS *m*/*z* (ESI, negative polarity) MS-1 211.8; MS2(212.0) 181.8, 169.8, MS3(169.8) 126.8, 112.8, 100.8, 96.0, 82.9. Anal. Calcd for $C_9H_{15}N_3O_3$: C, 50.69; H, 7.09; N, 19.71. Found: C, 50.72; H, 7.35; N, 19.62.

*(3R)-3-Acetamido-3-methyl-1-[1-(1R)-(N-methylcarbamoyl)-ethyl]azetidin-2-one (**5b**).* The general procedure was followed from α-(2-nosyl)-β-lactam **12b** (0.064 mmol, 0.024 g). The crude product was purified by preparative TLC (hexanes/EtOAc, 1:7): yield 0.010 g (72%); yellowish solid; mp 159–160 °C; $[\alpha]^{25}_{D}$ +58.2 (0.5, CH_2Cl_2); IR (KBr) ν_{max} 3125, 3060, 1782, 1768 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 8.13 (br s, 1H), 5.96 (s, 1H), 4.47 (q, 1H, *J* = 7.3 Hz), 3.73 (d, 1H, *J* = 5.1 Hz), 3.23 (d, 1H, *J* = 5.1 Hz), 2.84 (d, 3H, *J* = 4.6 Hz), 2.04 (s, 3H), 1.55 (s, 3H), 1.40 (d, 3H, *J* = 7.3 Hz); ^{13}C NMR (125

MHz, $CDCl_3$) δ_C 170.6, 168.6, 63.5, 50.2, 49.8, 26.5, 23.0, 20.7, 14.4; MS *m/z* (ESI, negative polarity) MS-1 225.9; MS2(225.9) 183.8; MS3(183.8) 126.8, 96.9. Anal. Calcd for $C_{10}H_{17}N_3O_3$: C, 52.85; H, 7.54; N, 18.49. Found: C, 52.86; H, 7.60; N, 18.53.

*(3R)-3-Acetamido-3-methyl-1-[1-(1R)-(N-methylcarbamoyl)-ethyl]azetidin-2-one (**5c**).* The general procedure was followed from α-(2-nosyl)-β-lactam **12c** (0.060 mmol, 0.022 g). The crude product was purified by preparative TLC (hexanes/EtOAc, 1:7): yield 0.011 g (81%); white powder; mp 115 °C; $[\alpha]^{25}_D$ +58.6 (0.5, CH_2Cl_2); IR (KBr) ν_{max} 3310, 3140, 1785, 1758 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 8.01 (br s, 1H), 5.96 (s, 1H), 3.80 (d, 1H, *J* = 5.0 Hz), 3.75 (q, 1H, *J* = 7.4 Hz), 3.17 (d, 1H, *J* = 5.0 Hz), 2.86 (d, 3H, *J* = 4.7 Hz), 2.03 (s, 3H), 1.73 (d, 3H, *J* = 7.5 Hz), 1.55 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 171.4, 170.5, 168.6, 63.4, 56.2, 53.5, 26.4, 23.0, 20.6, 16.3; MS *m/z* (ESI, negative polarity) MS-1 225.9; MS2(225.9) 195.8, 183.8; MS3(183.8) 126.8, 96.9. Anal. Calcd for $C_{10}H_{17}N_3O_3$: C, 52.85; H, 7.54; N, 18.49. Found: C, 52.59; H, 7.68; N, 18.84.

*(3R)-3-Acetamido-3-methyl-1-[1-(1R)-(N-methylcarbamoyl)-phenylmethyl]azetidin-2-one (**5d**).* The general procedure was followed from α-(2-nosyl)-β-lactam **12d** (0.108 mmol, 0.047 g). The crude product was purified by preparative TLC (CH_2Cl_2/MeOH 10:1): yield 0.028 g (89%); white powder; mp 99 °C; $[\alpha]^{25}_D$ +21.2 (0.5, CH_2Cl_2); IR (KBr) ν_{max} 3125, 3094, 1760, 1725 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 8.44 (br s, 1H), 7.37−7.22 (m, 5H), 5.99 (br s, 1H), 5.50 (s, 1H), 3.73 (d, 1H, *J* = 5.0 Hz), 2.95 (d, 3H, *J* = 4.6 Hz), 2.72 (d, 1H, *J* = 5.0 Hz), 2.00 (s, 3H), 1.38 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.9, 169.2, 168.3, 134.3, 129.0, 128.9, 128.4, 63.4, 59.4, 51.3, 26.6, 22.9, 20.2; MS *m/z* (ESI, negative polarity) MS-1 288.1; MS2(288.0) 245.8, 230.8, 171.8, 126.8, 111.9; MS3(245.8) 126.8; MS4(126.8) 111.8, 98.9. Anal. Calcd for $C_{15}H_{19}N_3O_3$: C, 62.27; H, 6.62; N, 14.52. Found: C, 62.40; H, 6.51; N, 14.75.

*(3R)-3-Acetamido-3-methyl-1-[1-(1S)-(N-methylcarbamoyl)-phenylmethyl]azetidin-2-one (**5e**).* The general procedure was followed from α-(2-nosyl)-β-lactam **12e** (0.144 mmol, 0.062 g). The crude product was purified by preparative TLC (CH_2Cl_2/MeOH 10:1): yield 0.039 g (94%); white crystals; mp 198−199 °C; $[\alpha]^{25}_D$ +133.5 (0.75, MeOH); IR (KBr) ν_{max} 3210, 2998, 1744, 1725 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.43−7.39 (m, 5H), 7.14 (br s, 1H), 5.66 (s, 1H), 5.77 (s, 1H), 3.77 (d, 1H, *J* = 5.0 Hz), 3.46 (d, 1H, *J* = 5.0 Hz), 2.93 (d, 3H, *J* = 4.7 Hz), 2.02 (s, 3H), 1.58 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.1, 168.9, 168.3, 134.6, 128.8, 128.7, 128.5, 76.6, 63.4, 62.3, 53.9, 26.5, 23.0, 20.4; MS *m/z* (ESI, negative polarity) MS-1: 288.1; MS2(288.0) 245.8, 230.8, 188.8, 171.8, 126.8; MS3(245.8) 126.8; MS4(126.8) 111.8, 58.3. Anal. Calcd for $C_{15}H_{19}N_3O_3$: C, 62.27; H, 6.62; N, 14.52. Found: C, 62.67; H, 6.70; N, 14.30.

*(3R)-3-Acetamido-3-methyl-1-[1-(N-methylcarbamoyl)-isopropyl]azetidin-2-one (**5f**).* The general procedure was followed from α-(2-nosyl)-β-lactam **12f** (0.091 mmol, 0.035 g). The crude product was purified by preparative TLC (hexanes/EtOAc, 1:7): yield 0.018 g (82%); yellowish powder; mp 216−218 °C; $[\alpha]^{25}_D$ +57.7 (0.75, CH_2Cl_2); IR (KBr) ν_{max} 3320, 3180, 1769, 1748 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 8.28 (br s, 1H), 6.30 (s, 1H), 3.73 (d, 1H, *J* = 5.0 Hz), 3.20 (d, 1H, *J* = 5.0 Hz), 2.82 (d, 3H, *J* = 4.6 Hz), 2.02 (s, 3H), 1.71 (s, 3H), 1.52 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 173.7, 170.7, 168.3, 62.2, 60.3, 51.0, 26.6, 24.8, 24.3, 22.9, 20.7; MS *m/z* (ESI, positive polarity) MS+23 264.0, 242.0; MS2(242.0) 129.0; MS3(129.0) 101.1, 58.4. Anal. Calcd for $C_{11}H_{19}N_3O_3$: C, 54.76; H, 7.94; N, 17.42. Found: C, 54.50; H, 8.00; N, 17.09.

General Procedure for the Preparation of Ns-(β-lactam)-Aa-Aib-OBn Compounds (13b−e). To a stirred solution of the corresponding *N*-carboxymethyl β-lactam **9b−e** (1 mmol) in CH_2Cl_2 (20 mL) cooled to −0 °C was added H-Aib-OBn (1 mmol, 0.193 g) dissolved in CH_2Cl_2 (9 mL). The solution was cooled to −12 °C, EEDQ (1.2 mmol, 0.310 g) was added, and the reaction mixture was slowly warmed to room temperature and stirred overnight. The resulting solution was washed with 1 M HCl (3 × 10 mL), the aqueous layer was extracted with CH_2Cl_2, and the combined organic phases were dried ($MgSO_4$) and evaporated. The product was purified by flash column chromatography (hexanes/EtOAc, 2:1).

*Compound **13b**.* The general procedure was followed from the *N*-carboxyalkyl-β-lactam **9b** (1 mmol, 0.433 g): yield 0.486 g (79%); white solid; mp 216−218 °C; $[\alpha]^{25}_D$ +54.7 (0.42, CH_2Cl_2); IR (KBr) ν_{max} 3396, 3085, 1762, 1681, 1539, 1347, 1153 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 8.15−7.72 (m, 4H, o-Ns), 7.37−7.21 (m, 10H, Ar), 7.17 (s, 1H, N*H*CMe$_2$), 5.89 (s, 1H, N*H*-Ns), 5.21 (d, 1H, *J* = 12.3 Hz, OC*H*$_2$Ph), 5.09 (d, 1H, *J* = 12.6 Hz, OC*H*$_2$Ph), 4.32 (m, 1H, NC*H*Me), 3.55 (d, 1H, *J* = 5.7 Hz, C*H*$_2$NCO), 3.34 (d, 1H, *J* = 5.7 Hz, C*H*$_2$NCO), 3.14 (d, 1H, *J* = 13.5 Hz, NCC*H*$_2$Ph), 3.03 (d, 1H, *J* = 13.5 Hz NCC*H*$_2$Ph), 1.59 (s, 3H, *Me*$_2$C), 1.56 (s, 3H, *Me*$_2$C), 0.83 (d, 3H, *J* = 6.9 Hz, *Me*CHNH); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 173.8, 168.8, 166.1, 147.4, 134.9, 133.8, 133.2, 132.6, 130.9, 130.2, 128.9, 128.5, 128.2, 128.1, 125.3, 70.1, 67.0, 56.6, 50.5, 49.5, 40.3, 25.4, 24.7, 13.4; MS *m/z* (ESI, positive polarity) MS+23: 264.0, 242.0; MS2(242.0) 129.0; MS3(129.0) 101.1, 58.4. Anal. Calcd for $C_{30}H_{32}N_4O_8S$: C, 59.20; H, 5.30; N, 9.20. Found: C, 58.86; H, 5.64; N, 9.01.

*Compound **13c**.* The general procedure was followed from the *N*-carboxyalkyl-β-lactam **9b** (1 mmol, 0.433 g): yield 0.426 g (70%); white solid; mp 216−218 °C; $[\alpha]^{25}_D$ +106.7 (0.3, CH_2Cl_2); IR (KBr) ν_{max} 3378, 3105, 1748, 1539, 1356, 1165 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ_H 8.14−7.74 (m, 4H, o-Ns), 7.40−7.25 (m, 10H, Ar), 6.93 (s, 1H, N*H*CMe$_2$), 5.86 (s, 1H, N*H*-Ns), 5.18 (s, 2H, OC*H*$_2$Ph), 3.89 (q, 1H, *J* = 1.6 Hz, NC*H*(Me)CO), 3.72 (d, 1H, *J* = 5.7 Hz, C*H*$_2$NCO), 3.47 (d, 1H, *J* = 5.7 Hz, C*H*$_2$NCO), 3.14 (q, 2H, *J* = 1.6 Hz, CqC*H*$_2$Ph), 1.59 (s, 3H, *Me*$_2$C), 1.58 (s, 3H, *Me*$_2$C), 1.46 (d, 3H, *J* = 7.25 Hz, *Me*CHNH); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 173.9, 169.5, 166.5, 147.4, 133.6, 133.2, 133.1, 130.9, 130.0, 129.0, 128.5, 128.2, 128.1, 127.9, 125.3, 69.5, 67.2, 56.6, 53.8, 53.5, 40.6, 29.7, 24.9, 15.1; MS *m/z* (TOF MS ES+) MS[+Na] 630.9, MS[+H] 608.8, MS: 562.8, 500.9, 472.9, 394.0, 359.9, 115.9. Anal. Calcd for $C_{30}H_{32}N_4O_8S$: C, 59.20; H, 5.30; N, 9.20. Found: C, 59.14; H, 5.55; N, 8.92.

*Compound **13d**.* The general procedure was followed from the *N*-carboxyalkyl-β-lactam **9d** (1 mmol, 0.497 g): yield 0.368 g (55%); white solid; mp 72−74 °C; $[\alpha]^{25}_D$ −83.8 (0.65, CH_2Cl_2); IR (KBr) ν_{max} 3378, 3028, 1768, 1742, 1677, 1534, 1148 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 8.15−7.68 (m, 4H), 7.50−6.85 (m, 15H), 5.90 (br s, 1H), 5.32 (s, 1H), 5.20 (d, 1H, *J* = 12.5 Hz), 5.16 (d, 1H, *J* = 12.5 Hz), 3.69 (d, 1H, *J* = 6.0 Hz), 3.04 (d, 1H, *J* = 6.0 Hz), 3.00 (d, 1H, *J* = 14.0 Hz), 2.95 (d, 1H, *J* = 14.0 Hz), 1.67 (s, 3H), 1.64 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 173.8, 167.8, 165.9, 147.5, 136.0, 134.8, 134.0, 133.4, 133.3, 132.5, 131.1, 130.1, 129.1, 128.9, 128.7, 128.6, 128.5, 128.2, 128.1, 128.0, 125.5, 69.9, 67.2, 59.2, 55.9, 52.3, 40.3, 25.5, 24.9; MS *m/z* (ESI, positive polarity) MS 693.2; MS2(693.2) 671.2, 666.1, 665.2, 478.3; MS3(665.2) 479.2, 478.2, 463.1. Anal. Calcd for $C_{35}H_{34}N_4O_8S$: C, 62.67; H, 5.11; N, 8.35. Found: C, 62.60; H, 4.95; N, 8.07.

*Compound **13e**.* The general was followed from the *N*-carboxyalkyl-β-lactam **9e** (0.42 mmol, 0.2 g) and α-aminoisobutyric acid benzyl ester (0.44 mmol; 0.085 g): yield 0.023 g (66%); mp 64−65 °C; $[\alpha]^{25}_D$ +9.4 (0.65, CH_2Cl_2); IR (KBr) ν_{max} 3350, 2928, 1744, 1726, 1682, 1536, 1148 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.95−7.61 (m, 4H), 7.37−7.26 (m, 15H), 6.44 (br s, 1H), 5.81 (br s, 1H), 5.33 (s, 1H), 5.19 (d, 1H, *J* = 12.5 Hz), 5.15 (d, 1H, *J* = 12.5 Hz), 3.76 (d, 1H, *J* = 5.5 Hz), 3.52 (d, 1H, *J* = 5.5 Hz), 3.25 (dd, 2H, *J* = 11.9 Hz), 1.59 (s, 3H), 1.56 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 173.9, 167.4, 166.7, 147.3, 135.6, 135.3, 133.6, 133.5, 133.0, 131.1, 129.9, 129.2, 129.1, 129.0, 128.7, 128.5, 128.2, 127.9, 125.4, 69.3, 67.5, 59.7, 57.1, 53.6, 41.1, 25.0, 24.5; MS *m/z* (ESI, positive polarity) MS 693.3; MS2(693.2) 666.1, 665.2, 478.2, 361.2; MS3(665.2) 479.1, 478.2, 463.2. Anal. Calcd for $C_{35}H_{34}N_4O_8S$: C, 62.67; H, 5.11; N, 8.35. Found C, 62.32; H, 5.06; N, 7.98.

General Procedure for the Preparation of Boc-(β-lactam)-Aa-Aib-OH Compounds 6b−e. Thiophenol (5.00 mmol, 0.469 mL) and K_2CO_3 (4.00 mmol, 0.567 g) were added to a solution of the corresponding Ns-(β-Lactam)-Aa-Aib-OBn **13b−e** (1 mmol) in dry MeCN (15 mL), and the suspension was stirred at room temperature for 2 h (monitoried by TLC and ^1H NMR). Upon completion, the

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reaction mixture was filtered through a pad of Celite, evaporated in vacuo, and quickly purified by column chromatography (CH_2Cl_2, then MeOH). The corresponding intermediate amine H-(β-lactam)-Aa-Aib-OBn was redissolved in MeCN (15 mL), di-*tert*-butyl dicarbonate (0.402 mmol; 0.088 g) was added, and the solution was stirred overnight at room temperature. The solution was evaporated, treated with saturated aqueous $NaHCO_3$, and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic solutions were dried ($MgSO_4$), and the solvents were evaporated under reduced pressure to afford the corresponding intermediate Boc-(β-lactam)-Aa-Aib-OBn, which was purified by preparative TLC (hexanes/EtOAc, 2:1). This benzyl ester was dissolved in ethanol (15 mL) containing 10% Pd/C (0.050 g), and the suspension was stirred at room temperature under hydrogen for 16 h. After this time, the reaction mixture was filtered through a pad of Celite, washed with ethanol (2 × 10 mL), and the combined organic solution was evaporated under reduced pressure to afford the corresponding carboxyl peptide product **6b–e**.

*2-[2-[(3R)-3-Benzyl-3-(tert-butoxycarbonylamino)-2-oxoazetidin-1-yl]-(R)-propanamido]-2-methylpropanoic Acid (**6b**).* The general procedure was followed from compound **13b** (1 mmol, 0.608 g): yield 0.342 g (79%); oil; $[\alpha]^{25}_D$ −15.0 (0.2, CH_2Cl_2); IR (KBr) ν_{max} 3320, 3180, 1769, 1748 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ_H 7.31–7.29 (m, 5H, Ar), 4.0 (m, 1H, C*H*Me), 3.67 (d, 1H, *J* = 4.8 Hz, CH_2NCO), 3.35 (d, 1H, *J* = 4.8 Hz, CH_2NCO), 3.07 (d, 1H, *J* = 13.3 Hz, CH_2Ph), 2.97 (d, 1H, *J* = 13.3 Hz, CH_2Ph), 1.47 (s, 9H, *t*BuO), 1.46 (s, 6H, *Me*$_2$CH), 0.58 (d, 3H, *J* = 7.1 Hz, *Me*CH); ^{13}C NMR (125 MHz, CD_3OD) δ_C 181.2, 171.2, 171.0, 156.6, 135.6, 131.8, 129.4, 128.4, 81.0, 68.8, 59.5, 50.8, 39.1, 28.7, 26.9, 25.8, 13.9; HPLC-MS, MeOH/HCO_2H *m*/*z* (ESI, positive polarity) MS+23 264.0, 242.0; MS2(242.0) 129.0; MS3(129.0) 101.1, 58.4. Anal. Calcd for $C_{22}H_{31}N_3O_6$: C, 60.95; H, 7.21; N, 9.69. Found C, 61.33; H, 6.95; N, 9.60.

*2-[2-[(3R)-3-Benzyl-3-(tert-butoxycarbonylamino)-2-oxoazetidin-1-yl]-(S)-propanamido]-2-methylpropanoic acid (**6c**).* The general procedure was followed from compound **13c** (1 mmol, 0.608 g): overall yield 0.346 g (80%); white solid; mp 216–218 °C; $[\alpha]^{25}_D$ −28.1 (0.36, CH_2Cl_2); IR (KBr) ν_{max} 2974, 1739, 1698, 1583, 1363, 1166 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.77 (s, 1H, N*H*CMe$_2$), 7.39–7.19 (m, 5H, Ar), 4.9 (s, 1H, Boc*H*N), 4.34 (m, 1H, C*H*Me), 3.65 (d, 1H, *J* = 4.4 Hz, CH_2NCO), 3.38 (d, 1H, *J* = 5.4 Hz, CH_2NCO), 3.19 (d, 1H, *J* = 13.5 Hz, CH_2Ph), 2.94 (d, 1H, *J* = 13.5 Hz, CH_2Ph), 1.55 (s, 3H, *Me*$_2$C), 1.55 (s, 3H, *Me*$_2$C), 1.45 (s, 9H, *t*BuO), 0.85 (d, 3H, *J* = 7.25 Hz, *Me*CH); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 180.6, 170.2, 169.8, 155.5, 134.6, 130.7, 128.4, 127.4, 79.9, 67.7, 58.4, 49.8, 38.1, 29.7, 27.7, 25.8, 24.8, 12.9. HRMS (TOF CI) *m*/*z* 433.2221, $C_{22}H_{31}N_3O_6$ requires 433.2216.

*2-[[(3R)-3-Benzyl-3-(tert-butoxycarbonylamino)-2-oxoazetidin1-yl]-(R)-phenylacetamido]-2-methylpropanoic Acid (**6d**).* The general procedure was applied from β-lactam **13d** (1.00 mmol, 0.670 g): yield 0.355 g (82%); white solid; mp = 238 °C; $[\alpha]^{25}_D$ +35.8 (0.65, MeOH); IR (KBr) ν_{max} 3320, 2928, 2851, 1744, 1686, 1670, 1651 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ_H 7.32–6.64 (m, 10H), 5.03 (s, 1H), 3.58 (d, 1H, *J* = 5.5 Hz), 3.02 (d, 1H, *J* = 13.5 Hz), 2.85 (d, 1H, *J* = 13.5 Hz), 2.83 (d, 1H, *J* = 5.5 Hz), 1.61 (s, 3H), 1.57 (s, 3H), 1.50 (s, 9H); ^{13}C NMR (125 MHz, CD_3OD) δ_C 176.8, 169.7, 168.9, 155.4, 133.7, 133.6, 130.3, 128.7, 128.3, 127.8, 127.2, 79.8, 67.6, 58.6, 56.8, 48.5, 27.3, 25.4, 23.7. HPLC-MS, MeOH/HCO_2H *m*/*z* (ESI, positive polarity) 566.4; MS2(566.4) 436.1, 435.0, 434.2, 374.1, 202.1; MS3(434.2) 375.1, 374.1, 188.0, 187.0, 178.0, 173.0, 172.0, 159.0, 131.0, 130.0; MS4(374.1) 188.0, 187.0, 172.0, 170.0, 159.0, 130.9. Anal. Calcd for $C_{27}H_{33}N_3O_6$: C, 65.44; H, 6.71; N, 8.48. Found C, 65.30; H, 6.49; N, 8.13.

*2-[[(3R)-3-Benzyl-3-(tert-butoxycarbonylamino)-2-oxoazetidin1-yl](S)-phenylacetamido]-2-methylpropanoic Acid (**6e**).* The general procedure was applied from β-lactam **13e** (1.00 mmol, 0.670 g): yield 0.346 g (80%); mp 113–115 °C; $[\alpha]^{25}_D$ +44.5 (0.3, MeOH); IR (KBr) ν_{max} 3326, 2980, 2916, 2853, 1744, 1698, 1674, 1660 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ_H 8.41 (s, 1H), 7.30–7.22 (m, 10H), 5.29 (s, 1H), 3.48 (br s, 1H), 3.32 (br s, 1H), 3.17–3.10 (br s, 2H), 1.36 (br s, 15H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.8, 168.1, 167.3, 154.8, 136.3, 136.1, 130.5, 128.7, 128.5, 128.4, 128.1, 127.0, 79.2, 67.5, 58.4, 56.0, 51.4, 38.8, 28.6, 25.2, 25.0. HPLC-MS *m*/*z* (ESI, negative polarity) 494.3; MS2(494.3) 420.0; MS3(420.0) 377.0, 334.0, 333.1, 291.0, 273.0, 260.9, 249.9, 248.0, 174.8, 127.9; MS4(377.0) 333.0, 275.9, 249.9, 247.9, 207.0, 159.9, 127.9 188.0, 187.0, 172.0, 170.0, 159.0, 130.9. Anal. Calcd for $C_{27}H_{33}N_3O_6$: C, 65.44; H, 6.71; N, 8.48. Found C, 65.14; H, 6.98; N, 8.20.

Preparation of 2-[[(3*R*)-3-Benzyl-3-(*tert*-butoxycarbonylamino)2-oxoazetidin1-yl]dimethylacetamido]-2-methylpropanoic Acid (6f). *(3R)-3-Benzyl-3-tert-butoxycarbonyl-1-[1-(methoxycarbonyl)isopropyl]azetidin-2-one.* Thiophenol (5 mmol, 0.469 mL) and K_2CO_3 (4 mmol, 0.567 g) were added to a solution of (3*R*)-3-benzyl-3-(2-nitrobenzenesulfonylamino)-1-[1-(methoxycarbonyl)isopropyl]-azetidin-2-one[1] (1 mmol, 0.461 g) in MeCN (5 mL). The suspension was stirred for 2 h at rt, the solvent was evaporated in vacuo, and the residue was purified by column chromatography (EtOAc, then MeOH) to afford (3*R*)-3-benzyl-3-amino-1-[1-(methoxycarbonyl)isopropyl]azetidin-2-one in 90% yield (0.248 g). To a solution of this material in CH_2Cl_2 (5 mL) was added $(Boc)_2O$ (3 mmol, 0.654g, and the mixture was stirred at rt for 16 h. After evaporation of the solvent, the crude was purified by column chromatography hexanes/EtOAc, 5:1): overall yield 0.188 g (50%); yellow oil; $[\alpha]^{25}_D$ +10.2 (0.6, CH_2Cl_2); IR (KBr) ν_{max} 3318, 1760, 1742, 1714 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.38–7.25 (m, 5H, Ph), 4.78 (s, 1H, NH), 3.66 (s, 3H, OCH_3), 3.58 (d, 1H, *J* = 5.2 Hz, CH_2NCO), 3.38 (d, 1H, *J* = 5.2 Hz, CH_2NCO), 3.11 (s, 2H, CH CH_2Ph), 1.48 (s, 9H, *t*BuO), 1.35 (s, 3H, C*Me*$_2$), 1.21 (s, 3H, C*Me*$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) 173.2, 166.9, 153.4, 135.3, 130.3, 128.4, 127.1, 65.8, 58.8, 52.6, 49.9, 39.1, 28.2, 23.7, 23.6; MS *m*/*z* (ESI, positive polarity) +MS2 343.1, +MS4 283.0. Anal. Calcd for $C_{20}H_{28}N_2O_5$: C, 63.81; H, 7.50; N, 7.44. Found: C, 63.64; H, 7.36; N, 7.19.

*Compound **6f**.* To a solution of (3*R*)-3-benzyl-3-*tert*-butoxycarbonyl-1-[1-(methoxycarbonyl)isopropyl]azetidin-2-one (1 mmol, 0.376 g) in MeOH (20 mL) was added LiOH·.H_2O (10 mmol, 0.485 g) in 10 mL of H_2O, and the mixture was stirred at rt for 4 h. Acidification of the resulting mixture with 1 M HCl and extraction with CH_2Cl_2 (3 × 8 mL) provided an organic solution of (3*R*)-3-benzyl-3-*tert*-butoxycarbonyl-1-[1-(carboxy)isopropyl]azetidin-2-one, which was dried (Na_2SO_4) and filtered. H-Aib-OBn (1.3 mmol, 0.248 g) was added, the mixture was cooled to −12 °C, and EEDQ (1.3 mmol, 0.331 g) was added. The reaction mixture was stirred during 16 h, while rt was slowly reached. The resulting solution was washed with 1 M HCl (3 × 10 mL), the aqueous layer was extracted with CH_2Cl_2, and the combined organic phases were dried ($MgSO_4$) and evaporated. The crude was purified by flash column chromatography (hexanes/EtOAc, 5:1) to afford benzyl 2-[[(3*R*)-3-benzyl-3-(*tert*-butoxycarbonylamino)2-oxoazetidin1-yl]dimethylacetamido]-2-methylpropanoate: white solid; 0.494 g (92%). A suspension of this benzyl ester (0.92 mmol) and 20% Pd/C in EtOAc (35 mL) was stirred for 1 h at rt under H_2 atmosphere. The mixture was filtered through a Celite pad, and the filtrate was evaporated: yield 0.411 g (100%); white solid; mp 213–215 °C; $[\alpha]^{25}_D$ +65.2 (0.48, CH_2Cl_2); IR (KBr) 3346, 1736, 1702, 1685 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.87 (s, 1H, N*H*CMe$_2$), 7.38–7.27 (m, 5H, Ar), 4.84 (s, 1H, BocN*H*), 3.62 (d, 1H, *J* = 5.1 Hz, CH_2NCO), 3.35 (d, 1H, *J* = 5.1 Hz, CH_2NCO), 3.14 (d, 1H, *J* = 13.4 Hz, CH_2Ph), 2.89 (d, 1H, *J* = 13.4 Hz, CH_2Ph), 1.58 (s, 3H, *Me*$_2$C), 1.57 (s, 3H, *Me*$_2$C), 1.45 (s, 9H, *t*BuO), 1.32 (s, 3H, *Me*$_2$C), 0.95 (s, 3H, *Me*$_2$C); ^{13}C NMR (75 MHz, $CDCl_3$) 175.8, 174.5, 168.3, 154.7, 133.5, 130.8, 129.1, 128.2, 81.6, 66.4, 59.9, 57.9, 48.6, 39.5, 28.7, 25.9, 25.3, 24.4, 23.8; MS *m*/*z* (ESI, positive polarity) MS 446.2, MS2(446.2) 372.8, 372.1, MS3(372.1) 329.0, MS4(329.0) 329.1, 242.9, 201.9, 199.9, 172.0, 168.9, 153.9, 127.9, 111.0. Anal. Calcd for $C_{23}H_{33}N_3O_6$: C, 61.73; H, 7.43; N, 9.39. Found: C, 61.90; H, 7.26; N, 9.34.

General Procedure for the Preparation of Boc-Ala-(α-Bn-β-lactam)-Aib-OMe Compounds 15 and 16. Thiophenol (5 mmol, 0.469 mL) and K_2CO_3 (4 mmol, 0.567 g) were added to a stirred solution of (3*R*)-3-benzyl-1-[1-(methoxycarbonyl)isopropyl]-3-(2-nitrobenzenesulfonylamino)azetidin-2-one **14** (1 mmol, 0.461 g) in

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MeCN (5 mL). The mixture was stirred over 2 h, the solvent was evaporated in vacuo, and the crude was purified by flash column chromatography (EtOAc, then MeOH): yield 90%. A solution of the resulting amine and the corresponding Boc-Ala-OH (1.3 mmol, 0.248 g) in anhydrous CH_2Cl_2 (20 mL) was cooled to −12 °C, and EEDQ (1.3 mmol, 0.331 g) was added. The mixture was stirred during 16 h, while rt was slowly reached. The resulting solution was washed with 1 M HCl (3 × 10 mL), the aqueous layer was extracted with CH_2Cl_2, and the combined organic phases were dried ($MgSO_4$) and evaporated. The product was purified by flash column chromatography (hexanes/EtOAc, 2:1).

*Methyl 2-[[(3R)-3-Benzyl-3-[2-(tert-butoxycarbonylamino)-(2R)-propanamido]2-oxoazetidin1-yl]-2-methylpropanoate (**15**).* The general procedure was followed from compound **17** (1 mmol, 0.461 g) and Boc-D-Ala-OH (1.3 mmol, 0.248 g): yield 0.371 g (83%); white solid; mp 135−137 °C; $[\alpha]^{25}_D$ +28.5 (0.2, CH_2Cl_2); IR (KBr) ν 3309, 3000, 1763, 1752, 1704, 1656, 1160, 672 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.28−7.32 (m, 5H, Ar), 6.62 (bs, 1H, CON*H*CCH$_2$Ph), 4.9 (bs, 1H, CON*H*CH(CH$_3$)CO), 4.14 (m, 1H, NHC*H*(Me)CO), 3.71 (s, 3H, CO$_2$*Me*), 3.63 (d, 1H, J = 5.5 Hz, NCH_2CCH$_2$Ph), 3.45 (d, 1H, J = 5.4 Hz, NCH_2CCH$_2$Ph), 3.21 (d, 1H, J = 14.2 Hz, CH_2Ph), 3.18 (d, 1H, J = 14.0 Hz, CH_2Ph), 1.45 (s, 9H, *t*BuO), 1.43 (s, 3H, *Me*$_2$CCO$_2$Me), 1.36 (s, 3H, *Me*$_2$CCO$_2$Me), 1.31 (d, 3H, J = 7.1 Hz, CH(*Me*)CONH); ^{13}C NMR (75 MHz, $CDCl_3$) 173.1, 172.7, 166.8, 155.3, 134.8, 130.2, 128.4, 127.2, 80.2, 65.7, 58.9, 52.5, 49.4, 38.7, 28.3, 23.6, 18.3, 16.8; HPLC-MS, MeOH/HCO_2H m/z (ESI, positive polarity) 187.1 (35), 133.1 (99), 126.1 (62), 112.1 (41), 97.1 (41), 72.1 (85), 59.1 (100), 57.1 (48), 55.1 (53); HRMS (TOF CI) m/z 447.2372, $C_{23}H_{33}N_3O_6$ requires 447.2369.

*Methyl 2-[(3R)-3-Benzyl-3-[2-(tert-butoxycarbonylamino)(2S)-propanamido]2-oxoazetidin-1-yl]-2-methylpropanoate (**16**).* The general procedure was followed from compound **17** (1 mmol, 0.461 g) and Boc-Ala-OH (1.3 mmol, 0.248 g): yield 0.380 g (85%); yellowish solid; $[\alpha]^{25}_D$ −22.4 (0.5, CH_2Cl_2); IR (KBr) ν 3271, 2993, 1753, 1721, 1716, 1673, 1167, 740 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.32 (m, 5H, Ar), 4.08 (m, 1H, COC*H*(Me)NH), 3.65 (s, 3H, CO$_2$Me), 3.59 (d, 1H, J = 5.5 Hz, NCH_2C), 3.41 (d, 1H, J = 5.3 Hz, NCH_2C), 3.15 (dd, 2H, J = 13.7 Hz, CH$_2$Ph), 1.45 (s, 9H, *t*BuO), 1.33 (s, 3H, C*Me*$_2$CO$_2$Me), 1.29 (d, 1H, J = 6.9 Hz, CH*Me*NH), 1.23 (s, 3H, C*Me*$_2$CO$_2$Me); ^{13}C NMR (75 MHz, $CDCl_3$) 173.2, 172.9, 155.3, 134.9, 130.3, 128.4, 127.1, 79.6, 65.9, 58.9, 53.5, 52.5, 49.8, 38.5, 28.3, 23.4, 18.3; HPLC-MS, MeOH/HCO_2H m/z (ESI, positive polarity) 187.1 (32), 134.1 (28), 133.1 (100), 115.6 (27), 102.5 (21), 92.1 (33), 91.1 (41), 58 (56); HRMS (TOF CI) m/z 447.2348, $C_{23}H_{33}N_3O_6$ requires 447.2369.

General Procedure for the Preparation of Boc-Ala-(β-Lactam)-Aib-Ala-NH$_2$ Compounds 7a−d. To a solution of the corresponding methyl 2-[(3*R*)-3-benzyl-3-[2-(*tert*-butoxycarbonylamino)2*R*-propanamido]2-oxoazetidin-1-yl]-2-dimethylpropanoate **15** or **16** (1 mmol, 0.447 g) in MeOH (20 mL) was added a solution of LiOH·H_2O (10 mmol, 0.485 g) in MeOH/H_2O 10:5 mL. The mixture was stirred for 2 h and acidified with 1 M HCl (5 mL), and the aqueous phase was extracted with CH_2Cl_2 (3 × 15 mL) to afford the intermediate carboxylic acid in 85% yield. To this crude material dissolved in anhydrous DMF (15 mL) cooled to 0 °C was added successively the corresponding alaninamide (1.5 mmol, 0.131 g), HATU (1.5 mmol, 0.570 g), HOAT (1.4 mmol, 0.191 g), and $KHCO_3$ (15 mmol, 1.5 g). The mixture was stirred during 16 h while slowly reaching rt. The solvent was evaporated at low pressure, and the residue was redissolved in CH_2Cl_2 and washed with 1 M HCl (3 × 10 mL) and satd $NaHCO_3$ (3 × 10 mL). After evaporation of the organic solvent, the crude product was purified by flash column chromatography (hexanes/EtOAc, 5:1 or CH_2Cl_2/MeOH, 15:1).

*2-[2-[(3R)-Benzyl-3-[2-(tert-butoxycarbonylamino)-(2R)-propanamido]2-oxoazetidin1-yl]-2-methylpro panamido]-(2R)-propanamide (**7a**).* The general procedure was followed from methyl 2-[(3*R*)-3-benzyl-3-[2-(*tert*-butoxycarbonylamino)(2*R*)-propanamido]2-oxoazetidin1-yl]-2-dimethylpropanoate **15** (1 mmol, 0.447 g) and (*R*)-alaninamide **18** (1.5 mmol, 0.131 g): yield 0.629 g (80%); white solid; mp 190−192 °C; $[\alpha]^{25}_D$ +36.0 (0.30, CH_2Cl_2); IR (KBr) ν_{max} 3416, 3308, 2965, 1741, 1726, 1694, 1536, 691 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ_H 8.3 (s, 1H, N*H*CCH$_2$Ph), 8.16 (d, 1H, J = 6.9 Hz, N*H*CH(Me)CONH$_2$), 7.33−7.28 (m, 5H, Ph), 7.1 (bs, 1H, NH$_2$), 6.87 (d, 1H, J = 8.1 Hz, BocN*H*), 6.83 (bs, 1H, NH$_2$), 4.13 (m, 1H, BocHNC*H*(Me), 4.06 (m, 1H, C*H*(Me)CONH$_2$), 3.58 (d, 1H, J = 5.1 Hz, CH_2NCMe$_2$), 3.26 (d, 1H, J = 5.0 Hz, CH_2NCMe$_2$), 3.05 (d, 1H, J = 13.4 Hz, CH$_2$Ph), 3.02 (d, 1H, J = 13.5 Hz, CH$_2$Ph), 1.39 (s, 9H, *t*BuO), 1.29 (d, 3H, J = 7.2 Hz, CH(*Me*)CONH$_2$), 1.20 (d, 3H, J = 6.9 Hz, BocHNCH(*Me*), 1.12 (s, 3H, *Me*$_2$CCO), 0.7 (s, 3H, *Me*$_2$CCO); ^{13}C NMR (75 MHz, $CDCl_3$) 175.0, 173.6, 173.2, 166.8, 156.1, 133.1, 130.3, 128.7, 127.8, 80.9, 77.2, 65.8, 59.8, 49.6, 38.9, 28.3, 24.3, 23.6, 15.9, 16.5; HPLC-MS, MeOH/HCO_2H m/z (ESI, positive polarity) 217.2 (19), 167.1 (33), 149.0 (100), 119.1 (26), 85.9 (29); HRMS (TOF CI) m/z 503.2763, requires 503.2744. Anal. Calcd for $C_{25}H_{37}N_5O_6$: C, 59.63; H, 7.41; N, 13.91. Found: C, 59.70; H, 7.01; N, 13.91.

*2-[2-[(3R)-Benzyl-3-[2-(tert-butoxycarbonylamino)-(2R)-propanamido]2-oxoazetidin1-yl]2-methylpro panamido]-(2S)-propanamide (**7b**).* The general procedure was followed from methyl 2-[(3*R*)-3-benzyl-3-[2-(*tert*-butoxycarbonylamino)(2*R*)-propanamido]2-oxoazetidin1-yl]-2-dimethylpropanoate **15** (1 mmol, 0.447 g) and (*S*)-alaninamide **18** (1.5 mmol, 0.131 g): yield 0.327 g (65%); white solid; mp 202−204 °C; $[\alpha]^{25}_D$ −80.0 (0.50, CH_2Cl_2); IR (KBr) ν_{max} 3412, 3213, 1733, 1730, 1681, 1629, 696, 734 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) 8.41 (s, 1H, N*H*CCH$_2$Ph), 8.30 (d, 1H, J = 6.7 Hz, N*H*CH(Me)CONH$_2$), 7.33−7.28 (m, 5H, Ph), 7.10 (s, 1H, NH$_2$), 6.88 (d, 1H, J = 7.8 Hz, N*H*Boc), 6.84 (s, 1H, NH$_2$), 4.13 (m, 1H, *t*BuOCONHC*H*Me), 4.06 (m, 1H, C*H*(Me)CONH$_2$), 3.58 (d, 1H, J = 5.3 Hz, CH_2NCMe$_2$), 3.26 (d, 1H, J = 5.0 Hz, CH_2NCMe$_2$), 3.06 (d, 1H, J = 13.4 Hz, CH$_2$Ph), 3.02 (d, 1H, J = 13.6 Hz, CH$_2$Ph), 1.39 (s, 9H, *t*BuO), 1.29 (d, 3H, J = 7.1 Hz, CH(*Me*)CONH$_2$), 1.20 (d, 3H, J = 6.9 Hz, BocHNCH(*Me*), 1.09 (s, 3H, *Me*$_2$CCO), 0.75 (s, 3H, *Me*$_2$CCO); ^{13}C NMR (75 MHz, $CDCl_3$) 175.2, 175.1, 173.5, 173.3, 167.0, 156.1, 133.1, 130.3, 128.7, 127.8, 80.8, 77.2, 65.8, 59.8, 49.7, 47.5, 38.8, 28.3, 24.3, 16.6; HPLC-MS, MeOH/HCO_2H m/z (ESI, positive polarity) 119.1 (88), 97.1 (60), 91.1 (72), 71.1 (67), 69.1 (63), 57.1 (100), 55.1 (90); HRMS (TOF CI) m/z 503.2727, $C_{25}H_{37}N_5O_6$ requires 503.2744.

*2-[2-[(3R)-Benzyl-3-[2-(tert-butoxycarbonylamino)(2S)-propanamido]2-oxoazetidin1-yl]2-methylpro panamido]-(2S)-propanamide (**7c**).* The general procedure was followed from methyl 2-[(3*R*)-3-benzyl-3-[2-(*tert*-butoxycarbonylamino)(2*S*)-propanamido]2-oxoazetidin-1-yl]-2-dimethylpropanoate **16** (1 mmol, 0.447 g) and (*S*)-alaninamide **18** (1.5 mmol, 0.131 g): yield 0.326 g (73%); yellowish solid; mp 160−162 °C; $[\alpha]^{25}_D$ +66.0 (0.20, CH_2Cl_2); IR (KBr) ν_{max} 3334, 2969, 1748, 1682, 1536, 1165 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ_H 8.32 (s, 1H, N*H*CCH$_2$Ph), 8.16 (d, 1H, J = 6.5 Hz, N*H*CH(Me)CONH2), 7.33−7.28 (m, 5H, Ph), 7.06 (bs, 1H, NH$_2$), 6.91 (d, 1H, J = 7.0 Hz, N*H*Boc), 6.77 (bs, 1H, NH$_2$), 4.08 (m, 1H, BocNHC*H*Me), 4.02 (m, 1H, C*H*(Me)CONH$_2$), 3.61 (d, 1H, J = 4.7 Hz, CH_2NCMe$_2$), 3.24 (d, 1H, J = 4.8 Hz, CH_2NCMe$_2$), 3.05 (d, 1H, J = 13.4 Hz, CH$_2$Ph), 3.01 (d, 1H, J = 13.5 Hz, CH$_2$Ph), 1.38 (s, 9H, *t*BuO), 1.31 (d, 3H, J = 7.2 Hz, CH(*Me*)CONH$_2$), 1.20 (d, 3H, J = 7.2 Hz, BocNHCH*Me*), 1.12 (s, 3H, *Me*$_2$CCO), 0.7 (s, 3H, *Me*$_2$CCO); ^{13}C NMR (75 MHz, $CDCl_3$) 175.0, 173.6, 173.0, 166.7, 155.9, 133.0, 130.3, 128.7, 127.8, 80.9, 75.8, 65.9, 59.8, 49.6, 47.6, 38.9, 28.3, 24.3, 23.6, 16.5; HPLC-MS, MeOH/HCO_2H m/z (ESI, positive polarity) 187.1 (49), 133.1 (37), 91.1 (100), 70.1 (60), 57.1 (42). HRMS (TOF CI) m/z 503.2771, requires 503.2744. Anal. Calcd for $C_{25}H_{37}N_5O_6$: C, 59.63; H, 7.41; N, 13.91. Found: C, 59.85; H, 7.34; N, 13.92.

*2-[2-[(3R)-Benzyl-3-[2-(tert-butoxycarbonylamino)(2S)-propanamido]2-oxoazetidin1-yl]2-methylpro panamido]-(2R)-propanamide (**7d**).* The general procedure was followed from methyl 2-[(3*R*)-3-benzyl-3-[2-(*tert*-butoxycarbonylamino)(2*S*)-propanamido]2-oxoazetidin1-yl]-2-dimethylpropanoate **16** (1 mmol, 0.447 g) and (*R*)-alaninamide **18** (1.5 mmol, 0.131 g): yield 0.392 g (77%); yellowish solid; mp 219−220 °C; $[\alpha]^{25}_D$ −15.9 (0.20, CH_2Cl_2); IR (KBr) ν_{max} 3413, 3303, 2978, 2914, 1744, 1663, 1542 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ_H 8.53 (s, 1H, N*H*CCH$_2$Ph), 8.37 (d, 1H, J =

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7.3 Hz, N*H*CH(Me)CONH$_2$), 7.33−7.28 (m, 5H, Ar), 7.03 (d, 1H, *J* = 6.7 Hz, BocN*H*), 6.97 (s, 1H, NH$_2$), 6.88 (s, 1H, NH$_2$), 4.04 (m, 1H, BocNHC*H*Me), 4.03 (m, 1H, C*H*(Me)CONH$_2$), 3.46 (d, 1H, *J* = 4.8 Hz, C*H*$_2$NCMe$_2$), 3.34 (d, 1H, *J* = 5.0 Hz, C*H*$_2$NCMe$_2$), 3.06 (d, 1H, *J* = 13.4 Hz, CH$_2$Ph), 3.01 (d, 1H, *J* = 13.4 Hz, CH$_2$Ph), 1.38 (s, 9H, *t*BuO), 1.31 (d, 3H, *J* = 7.2 Hz, CH(*Me*)CONH$_2$), 1.19 (d, 3H, *J* = 7.0 Hz, BocHNCH*Me*), 0.97 (s, 3H, *Me*$_2$CCO), 0.79 (s, 3H, *Me*$_2$CCO); ^{13}C NMR (75 MHz, CDCl$_3$) 175.9, 173.4, 172.5, 167.7, 155.9, 132.7, 130.3, 128.8, 127.9, 80.8, 77.2, 65.6, 58.9, 49.5, 48.1, 38.8, 28.3, 23.7, 17.3; HPLC-MS, MeOH/HCO$_2$H *m*/*z* (ESI, positive polarity) 187.1 (44), 173.1 (28), 172.1 (26), 117.1 (37), 116 (25), 115 (26), 113.1 (35), 91 (100), 70.0 (47); HRMS (TOF CI) *m*/*z* 503.2742, requires 503.2744. Anal. Calcd for $C_{25}H_{37}N_5O_6$: C, 59.63; H, 7.41; N, 13.91. Found: C, 59.67; H, 7.62; N, 13.88.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR spectra of compounds **5**−**7** and **13d**−**e**. ^{13}C NMR spectra of compounds **5**−**13b,c**, **15**, and **16**. NMR/ROESY spectra and interproton distances of compounds **5**−**7**. Gaussian output data of structures **5a**−**f**. X-ray data for **5e**, **5f**, **7a**, and **7d** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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