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Privileged Structures as Peptide Backbone Constraints: Polymer-Supported Stereoselective Synthesis of Benzimidazolinopiperazinone Peptides

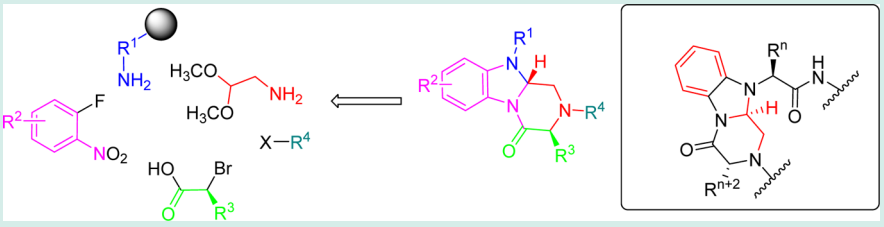
Pilar Ventosa-Andrés,[†] Ludmila Hradilová,[‡] and Viktor Krchňák*[,†,§]

[†]Department of Organic Chemistry, Institute of Molecular and Translational Medicine, Faculty of Science, Palacký University, 17 Listopadu 12, 771 46 Olomouc, Czech Republic

[‡]Farmak, Na Vlčinci 16/3, Klášterní Hradisko, 779 00 Olomouc, Czech Republic

[§]Department of Chemistry and Biochemistry, University of Notre Dame, 251 Nieuwland Science Center, Notre Dame, Indiana 46556, United States

Supporting Information



ABSTRACT: A molecular scaffold comprising a privileged structure was designed and synthesized to serve as a peptide backbone conformational constraint. The synthesis of highly functionalized 2,3,10,10*a*-tetrahydrobenzo[4,5]imidazo[1,2-*a*]pyrazin-4(1*H*)-ones on a solid-phase support was performed via a tandem *N*-acyl-*N*-aryliminium ion cyclization−nucleophilic addition reaction. The synthesis proceeded with full stereocontrol of the newly formed stereogenic center. Conventional and microwave-assisted syntheses were compared with respect to efficiency and the optical integrity of the target compounds. Significant epimerization was observed during acylation with (*S*)- and (*R*)-2-bromopropionic acids under microwave conditions.

KEYWORDS: *solid-phase synthesis, iminiums, privileged structure, peptidomimetics*

INTRODUCTION

The three-dimensional structures of peptides and proteins are substantially different. Whereas proteins form defined secondary and tertiary structures, peptides tend to be conformationally flexible, and their 3D architecture is highly dependent on their sequence and environment. The determination of receptor-bound conformations of peptide ligands not only contributes to the understanding of receptor−ligand interactions but also allows for the design and synthesis of conformationally constrained analogues of peptides with "frozen," biologically active conformations.[1−4] Moreover, a large number of cellular processes involve peptide−protein and protein−protein interactions (PPIs), such as antigen−antibody interactions, intercellular communication, and programmed cell death.[5−7] Therefore, these interactions have become valuable targets of investigation in the search for new therapeutic agents.[8] A promising approach in the search for modulators of protein−protein interactions is the design and synthesis of molecules that are able to mimic the electronic and conformational properties of the interaction surfaces, such as the mimetics of peptide secondary structure elements. Of the several types of reverse turns that have been identified,[9] β-turns have been postulated to be essential for the interaction of linear peptides with receptors, enzymes and antibodies.[10] Consequently, the preparation of fused heterocycles, which could be considered peptidomimetics of conformationally constrained turns,[2] and their incorporation into traditional Merrifield solid-phase synthesis is considered to be one of the most successful approaches.[4]

The traditional approach to the design and synthesis of constrained peptides involves the synthesis of a scaffold that provides the desired conformational restrictions without considering the pharmacological relevance of the scaffold. The aim of our present work was to design and synthesize a privileged-structure-containing scaffold that could serve as a peptide backbone constraint.[11] A privileged structure, a term introduced by Ben Evans,[12] is a molecular scaffold that possesses the ability to bind to multiple targets (i.e., proteins). Binding to individual targets can be tuned through the decoration of the scaffold (i.e., modification of functional groups).[13] The fused ring system of benzo[4,5]imidazo[1,2-*a*]pyrazin-4(1*H*)-one (**I**) is considered a hybrid of benzimidazoline and hexahydroimidazo-[1,2-*a*]pyrazin-5(1*H*)-one; both scaffolds are present in bioactive compounds and natural products. The benzimidazole ring is a privileged scaffold[12,13] present in numerous pharmacologically

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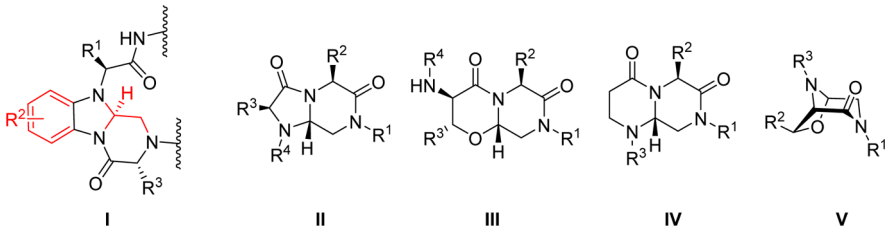
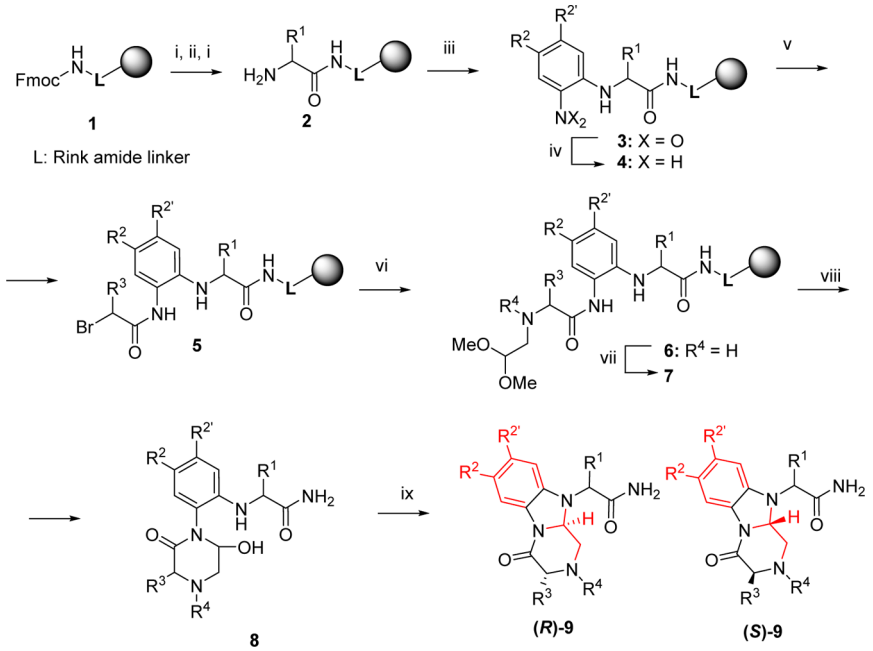


Figure 1. Fused and bridged heterocycles synthesized via iminium chemistry.

Scheme 1. Synthesis of Benzo[4,5]imidazo[1,2-*a*]pyrazin-4(1*H*)-ones 9 on a Solid-Phase Support[a]



[a]Reagents and conditions: (i) 50% piperidine in DMF (v/v), rt, 15 min; (ii) Fmoc-amino acid (5 equiv), HOBt (5 equiv), DIC (5 equiv), NMP; (iii) 4,5-disubstituted *o*-fluoronitrobenzenes (10 equiv), DIEA (10 equiv), DMSO; for R^2 = piperidin-1-yl: piperidine (10 equiv), DMSO; (iv) $Na_2S_2O_4$ (10 equiv), K_2CO_3 (14 equiv), TBAHS (1 equiv), DCM/H_2O (1:1); (v) α-bromocarboxylic acids (2 equiv), DIC (1 equiv), DCM; (vi) aminoacetaldehyde dimethyl acetal (5 equiv), DIEA (5 equiv), DMF; (vii) 4-nitrobenzenesulfonyl chloride (4-Nos-Cl) or 4-methylbenzenesulfonyl chloride (Tos-Cl), 2,6-lutidine, DCM, or acid chloride (2 equiv), DIEA (2 equiv), THF; (viii) 50% TFA in DCM, rt, 30 min; (ix) 50% TFA in DCM, rt, 16 h.

relevant molecules, such as antagonists for the angiotensin II receptor,[14] inhibitors of trypsin-like serine protease (factor Xa),[15] antibacterial agents,[16] anticancer agents,[17,18] anthelmintic agents,[19] and antiulcer agents.[20,21] The hexahydroimidazo[1,2-*a*]pyrazin-5(1*H*)-one ring is present in inhibitors of HIV-1 integrase[22] and antiviral drugs.[23]

Herein, we describe the synthesis of the pharmacologically relevant nitrogenous scaffold **I**, which is designed to serve as a conformational constraint for the peptide backbone (the backbone constraint is shown in red). This scaffold, consisting of fused heterocycles, was prepared via tandem *N*-acyliminium ion cyclization−nucleophilic addition, a very powerful strategy that has been successfully applied to the synthesis of numerous diverse heterocycles.[24−27] We have previously reported the synthesis of several fused heterocycles (Figure 1, structures **II**−**V**)[28−31] as peptide backbone conformational constraints.

RESULTS AND DISCUSSION

Synthesis. To evaluate both the scope and limitations of the proposed synthetic routes, with a particular focus on stereochemistry, a set of model compounds was prepared as shown in Scheme 1 (the constraint for the peptide backbone is shown in red). The reported dramatic increases in the reaction rates, yields, and purities of diverse products prepared under nonconventional and energy-efficient heating in a microwave cavity[32−34] prompted us to compare the synthesis under traditional reaction conditions (room temperature or conventional heating) with microwave-assisted synthesis and to evaluate the potential benefits of synthesis under microwave conditions.

We applied the conventional reaction conditions reported in our previous communications[28−31] for the individual steps of the synthetic route outlined in Scheme 1. The microwave-assisted synthesis was performed under the following reaction conditions. Rink amide resin[35] **1** was acylated with Fmoc-α-amino acids (BB1; for structures and numbering of building blocks, refer to Figure 2) activated by *N*,*N*′-diisopropylcarbodiimide (DIC) in the presence of HOBt in 1-methyl-2-pyrrolidone (NMP) at 86 °C for 10 min using the microwave heating conditions developed for peptide synthesis.[36] The Fmoc protecting group was removed with piperidine at room temperature, and resin-bound amine **2** was reacted with three different 1-fluoro-2-nitrobenzenes to yield resin-bound nitroaniline **3**. Whereas substitution of fluorine in the activated

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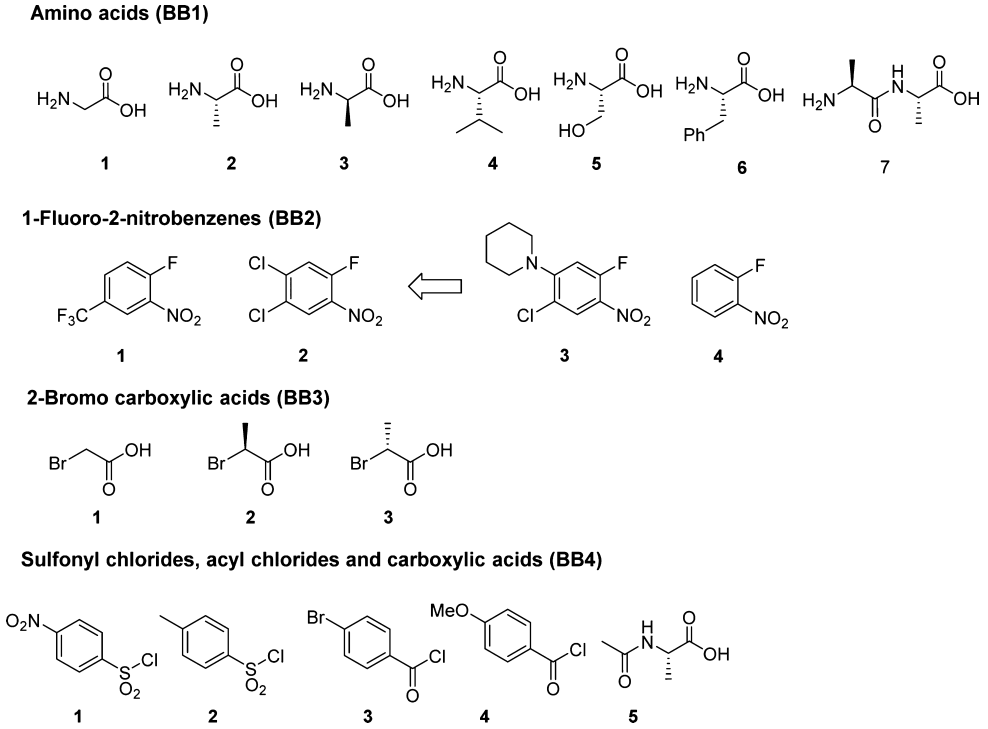

Figure 2. Building blocks used for the synthesis of **9**.

Table 1. Comparison of Conventional and Microwave-Assisted Syntheses

		conventional conditions			microwave conditions		
step	building blocks	time (h)	T (°C)	conversion (%)[a]	time (min)	T (°C)[b]	conversion (%)[a]
coupling with Fmoc-amino acids	BB1 (2−7)	2−16	rt	99	10	86	99
substitution with 2-fluoronitrobenzenes	BB2 (1)	16	rt	99	5	80	99
	BB2 (2)	16	rt	99	5	80	99
	BB2 (4)	16	50	99	60	100	99
acylation with bromocarboxylic acids	BB3 (1)	16	rt	99	1	50	99
	BB3 (2)	72	rt	99	10	86	99
	BB3 (3)	72	rt	99	10	86	99
nucleophilic substitution	aminoacetaldehyde dimethyl acetal	2	rt	99	1	90	99
nosylation	BB4 (1)	4	rt	95	15	150	86
tosylation	BB4 (2)	4	rt	90	15	150	80
acylation with Fmoc-amino acids	BB4 (5)	16	rt	90	10	86	90

[a]Conversion calculated via LC/MS. [b]Maximum power 100 W.

1-fluoro-2-nitrobenzenes BB2(1) and BB2(2) proceeded at 80 °C in DMSO within 5 min, the reaction with 1-fluoro-2-nitrobenzene required a temperature of 100 °C for 1 h. To increase the diversity of the final compound, the 4,5-dichloro-2-nitrobenzene derivative **3**($\mathbf{R^1}$,**2**) was treated with piperidine in DMF to yield the piperidine-substituted intermediate **3**($\mathbf{R^1}$,**3**). Reduction of the nitro group was accomplished using sodium dithionite ($Na_2S_2O_4$) in the presence of K_2CO_3; tetrabutylammonium hydrogen sulfate (TBAHS) was used as a phase-transfer catalyst in DCM:H_2O at room temperature[37] and afforded polymer-supported primary amine **4**.

The acylation of resin **4** using bromoacetic acid and DIC in the presence of DIEA did not afford intermediate **5**; LC/MS analysis revealed the presence of the primary amine. Microwave-assisted reaction conditions without DIEA as the base provided the desired intermediate **5** within 1 min at 50 °C. When these reaction conditions were applied to the acylation with (*R*)- and (*S*)-2-bromopropionic acids, only the primary amine **4** was detected via LC/MS. Complete acylation was observed when the reaction was conducted at 86 °C for 10 min. Subsequent nucleophilic displacement of the bromine with aminoacetaldehyde dimethyl acetal was carried out under microwave conditions at 90 °C in DMF for 1 min. Reaction of **6** with 4-Nos-Cl or Tos-Cl at 150 °C for 15 min afforded sulfonamide **7**. Acylation with either Fmoc-Ala-OH via the symmetric anhydride or benzoyl chlorides (4-methoxy, 4-bromo) was utilized to provide derivatives of the amino group.

The comparison of the reaction conditions for the individual synthetic steps is summarized in Table 1. The completion of all steps of this solid-phase synthesis of acyclic precursor **7** using traditional reaction conditions (room temperature or conventional heating) required 3 to 6 days, depending on the reactivity of the individual building blocks. Microwave-assisted synthesis allowed a substantial reduction in the reaction times, and the LC/MS analysis indicated excellent conversion for each step. However, the LC/MS analysis did not provide any information

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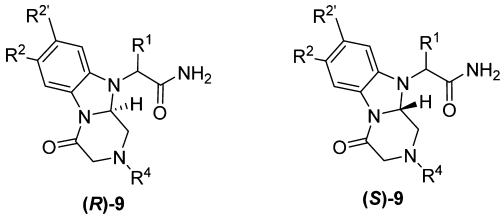
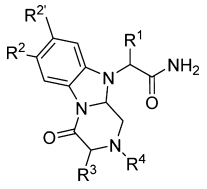
Table 2. Effects of R^1 and R^2 on Diastereomer Formation



entry	cmpd	R^1	R^2	$R^{2'}$	R^4	isolation	R/S^a	% *de*	purity[b]	yield[c]
1	**9(1,1,1,1)**	H	H	CF_3	Nos	HPLC			46	22
2	**9(2,1,1,1)**	(*S*)-CH_3	H	CF_3	Nos	HPLC	60:40	20	64	58
3	**9(2,1,1,2)**	(*S*)-CH_3	H	CF_3	Tos	HPLC	77:23	54	77	45
4	**9(2,2,1,1)**	(*S*)-CH_3	Cl	Cl	Nos	Precip	65:35	30	76	52
5	**9(2,3,1,2)**	(*S*)-CH_3	Cl	piperidin-1-yl	Tos	HPLC	66:34	32	56	32
6	**9(3,1,1,1)**	(*R*)-CH_3	H	CF_3	Nos	precip	57:43	14	88	55
7	**9(4,1,1,1)**	(*S*)-*i*Pr	H	CF_3	Nos	HPLC	65:35	30	77	86
8	**9(5,1,1,1)**	(*S*)-CH_2OH	H	CF_3	Nos	precip	47:53	6	73	27
9	**9(6,1,1,1)**	(*S*)-Bn	H	CF_3	Nos	HPLC	64:36	28	60	34

[a]R/S ratio calculated via ^{1}H NMR of the crude product. [b]Purity of the crude product. [c]Total yield after purification via HPLC or precipitation in MeOH of products prepared using a 9- to 10-step synthesis.

Table 3. Synthesized Derivatives of Benzimidazolinopiperazinones 9



entry	cmpd	R^1	R^2	$R^{2'}$	R^{3a}	R^4	method[b]	isol	R/S^c	*d*[e]	purity[d]	yield[e]
1	**9(1,1,2,1)**	H	H	CF_3	(*S*)-CH_3	Nos	B	HPLC			75	19
2	**9(1,1,3,1)**	H	H	CF_3	(*R*)-CH_3	Nos	B	HPLC			77	21
3	**9(2,1,3,2)**	(*S*)-CH_3	H	CF_3	(*R*)-CH_3	Tos	A	HPLC	58:42	16	68	27
4	**9(2,1,3,4)**	(*S*)-CH_3	H	CF_3	(*R*)-CH_3	CO-Ph-OMe	A	HPLC	55:45	10	45	37
5	**9(2,3,3,1)**	(*S*)-CH_3	Cl	piperidin-1-yl	(*R*)-CH_3	Nos	A	HPLC	53:47	6	54	58
6	**9(3,1,2,1)**	(*R*)-CH_3	H	CF_3	(*S*)-CH_3	Nos	A	HPLC	37:62	25	79	57
7	**9(3,1,3,1)**	(*R*)-CH_3	H	CF_3	(*R*)-CH_3	Nos	A	HPLC	52:48	5	68	42
8	**9(2,1,2,1)**	(*S*)-CH_3	H	CF_3	(*S*)-CH_3	Nos	B	PREC	11:89	78	77	56
9	**9(2,1,3,1)**	(*S*)-CH_3	H	CF_3	(*R*)-CH_3	Nos	B	PREC	77:33	44	78	39
10	**9(2,1,3,3)**	(*S*)-CH_3	H	CF_3	(*R*)-CH_3	CO-Ph-Br	B	PREC	73:27	46	70	39
11	**9(2,2,3,1)**	(*S*)-CH_3	Cl	Cl	(*R*)-CH_3	Nos	B	HPLC	55:45	20	62	37
12	**9(6,1,2,1)**	(*S*)-Bn	H	CF_3	(*S*)−CH_3	Nos	B	PREC	16:84	68	78	23
12	**9(6,1,3,1)**	(*S*)-Bn	H	CF_3	(*R*)−CH_3	Nos	B	PREC	76:24	52	69	24

[a]Configuration of building block used in the synthesis. [b]Acylation with 2-bromopropionic acid. Method A: Microwave conditions (86 °C, 100 W, 10 min). Method B: Conventional protocol (rt, 72 h). [c]R/S ratio calculated from ^{1}H NMR of a crude sample. [d]Purity of the crude product. [e]Total yield after purification via HPLC or precipitation in MeOH of products prepared using a 9- to 10-step synthesis.

regarding the optical integrity of the chiral intermediates and the final products.

After acid-mediated unmasking of the aldehyde and cleavage of the acyclic intermediates from the resin, cyclic *N*-acyl-*N*-aryliminium ions were formed. Although *N*-acyl-*N*-alkyliminium chemistry has been widely studied and utilized in numerous syntheses, *N*-acyl-*N*-aryliminium ion chemistry has been investigated much less frequently.[38,39] After 30 min and cleavage with 50% TFA in DCM, LC/MS analysis showed the formation of hydroxy intermediate **8** (not isolated or characterized). The presence of the aromatic amine as an internal nucleophile facilitated formation of the fused ring, and benzo[4,5]imidazo-[1,2-*a*]pyrazin-4(1*H*)-one (**9**) was obtained after 16 h of treatment with the cleavage cocktail. The substitution patterns of the synthesized benzo[4,5]imidazo[1,2-*a*]pyrazin-4(1*H*)-ones **9**, the purity of the crude products and the yields after purification are summarized in Tables 2 and 3.

All selected building blocks yielded the expected final products. However, the yield of the compounds prepared using 1-fluoro-2-nitrobenzene (BB2 #4) was very low because of the premature release of dihydroquinaxolinone **10** from resin **4** via a cyclative cleavage mechanism (Scheme 2). Therefore, we did not utilize this building block in further syntheses. Analogous release from an amide linkage has previously been reported.[40,41]

Stereoselectivity. The synthesis of target compound **9** via *N*-acyl-*N*-aryliminium ion cyclization−nucleophilic addition generated a new chiral center. The cyclization of an achiral

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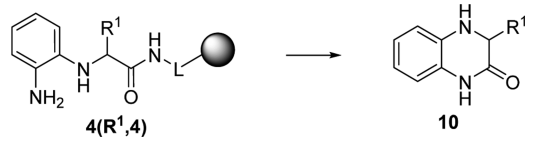
Scheme 2. Release of Dihydroquinoxalinone via Cyclative Cleavage



precursor yielded a mixture of enantiomers (Table 2, entry 1). To stereoselectively obtain target compound **9**, we evaluated the effects of chiral R^1 and R^3 substituents on the stereochemical outcome of the fused ring formation.

First, we describe the effect of a stereogenic center located on the R^1 substituent, that is, outside of the fused ring. The stereochemistry of the fused ring was investigated using a set of model compounds with different α-amino acids (Table 2). (*S*)-Amino acids in the R^1 position favored an (*R*)-configuration for the new stereogenic center with a *de* of 54% (Table 2, entry 3). The chirality of the amino acid and the substituents on the aromatic ring (Table 2, entries 4−6) did not markedly affect the reaction. This poor stereocontrol was expected and is dependent on the steric hindrance of the *N*-acyliminium ion intermediate. Interestingly, a 6% excess of the (*S*)-epimer was obtained with Ser (Table 2, entry 8). This change in stereoselectivity could be attributed to H-bond formation between the amide of Ser and the carbonyl group and between the hydroxy group of the lateral chain and the sulfonyl of 4-Nos in the cyclic intermediate, thus favoring nucleophilic attack on the opposite face.

The next set of compounds was synthesized using (*S*)- and (*R*)-2-bromopropionic acids. We observed complete stereocontrol of the newly formed chiral center. The compounds with (*R*)- and (*S*)-configurations in the R^3 position of the acyclic precursor **7** exclusively induced the (*R*)- and (*S*)-configurations of the new stereogenic center, respectively (Table 3, entries 1 and 2). The stereocontrol was dependent on the chirality of the substituent in the R^3 position, and it was not influenced by the R^1 substituent. Our results agree with those of previous studies; there is precedent in the literature for complete stereoselectivity in syntheses via *N*-acyl-*N*-aryliminium ion cyclization−nucleophilic addition reactions.[28−30,42−44]

We have described the effects of *N*-acyl-*N*-aryliminium ion cyclization−nucleophilic addition reactions on the stereochemistry of the newly formed stereogenic center. However, these results do not address the stereochemical integrity of resin-bound intermediates, particularly the potential epimerization of (*S*)- and (*R*)-2-bromopropionic acids during the acylation reaction. Because the fused ring formation is stereoselective, the epimerization of (*S*)- and (*R*)-2-bromopropionic acids would yield a mixture of (*S*,S) and (*R*,*R*) enantiomers with identical NMR spectra. Thus, to address epimerization during synthesis, we prepared a set of model precursors with two stereogenic centers in both the R^1 (a chiral amino acid) and R^3 positions. The results revealed significant epimerization of the R^3 center when acylation of the (*S*)- and (*R*)-2-bromopropionic acids was performed at elevated temperatures in a microwave cavity (Table 3, entries 3−7). The epimerization was not influenced by the chirality of the amino acid, substitution on the aromatic ring or the R^4 substituent, indicating that the epimerization occurred during the activation step. Acylation at room temperature required long reaction times and showed less epimerization; however, we still observed the formation of diastereomers (entries 8−13). To address the epimerization of (*S*)- and (*R*)-2-bromopropionic acids during acylation on a simple model compound, we acylated Ala attached to the Rink resin with both (*S*)- and (*R*)-2-bromopropionic acids under standard conditions (acid, DIC, HOBt (1:1:1), 0 °C, and acid, DIC (2:1)). The NMR of the crude products indicated 5% epimerization (*de* 90%, manufacturer specification *de* > 85%).

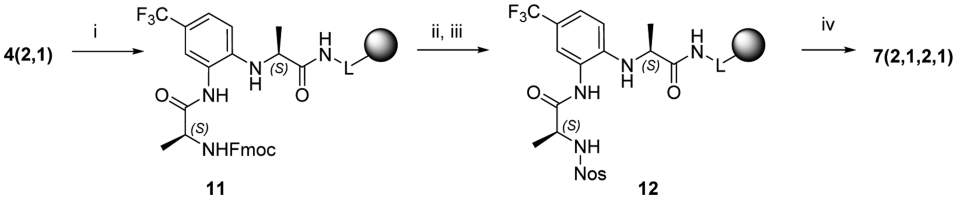
The epimerization prompted us to explore an alternative synthetic route: the acyclic resin-bound precursor **7(2,1,2,1)** was prepared via acylation of the resin-bound amine **4(2,1)** with Fmoc-Ala-OH, followed by incorporation of the protected aldehyde via the Mitsunobu reaction. Reaction with Fmoc-Ala-OH via the symmetric anhydride method in THF yielded acylated compound **11** (Scheme 3). After removal of the Fmoc protective group, the resin-bound primary amine was activated with 4-Nos-Cl in DCM in the presence of 2,6-lutidine to yield sulfonamide **12**. Subsequent Mitsunobu alkylation with glycolaldehyde dimethyl acetal afforded the acyclic intermediate **7(2,1,2,1)**. Treatment with 50% TFA in DCM for 16 h afforded product **9(2,1,2,1)** without epimerization.

Assignment of Configuration. The 10a-H protons of compounds **9** were observed as a doublet of doublets at 5.37−6.12 ppm in the ^{1}H NMR spectra, which is a diagnostic signal for a fused ring system. Accordingly, the ^{13}C NMR spectra revealed the corresponding fusion carbon C_{10a} at 73.6−77.0 ppm.

The assignment of the absolute configuration of the new stereogenic center in the Ala derivatives of **9** was based on the NOE effect observed in their ^{1}H NOESY 1D spectra. The C_3 chiral carbon was introduced by the (*S*)- or (*R*)-2-bromopropionic acid; thus, its configuration was known. The observed NOE correlations displayed the same relative configuration for 3-CH_3 and 10a-H in both pairs of diastereoisomers.

Comparing the ^{1}H NMR data of each pair of epimers of benzo[4,5]imidazo[1,2-*a*]pyrazin-4(1*H*)-one **9**, 10a-H appeared at a higher chemical shift (0.1−0.44 ppm) in the (*R*)-epimer than in the (*S*)-epimer. Furthermore, the coupling constants $J_{10a\text{-}1}$ were 9−9.5 and 3.5−3.7 Hz in the (*R*)-epimer, whereas the

Scheme 3. Alternate Synthetic Strategy[a]



[a]Reagents and conditions: (i) Fmoc-Ala-OH (2 equiv), DIC (1 equiv), THF, 40 °C, 16 h; (ii) 50% piperidine in DMF (v/v), rt, 15 min; (iii) 4-Nos-Cl, 2,6-lutidine, DCM, rt, 16 h; (iv) 0.1 M glycolaldehyde dimethyl acetal, 0.1 M PPh_3, anhydrous THF, −20 °C, then 0.1 M DIAD in anhydrous THF, 40 °C, 16 h.

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coupling constants in the (*S*)-epimer were 7.2−8.6 and 4.0−4.8 Hz. On the other hand, C_{10a} and C_3 appeared at a higher field (0.8−1.4 ppm) in the (*R*)-epimers than in the (*S*)-epimers in the ^{13}C NMR spectra. These tendencies changed when R^1 was Bn **9**(**6**,**1**,**3**,**1**), in which case the signals of the diagnostic proton and carbon in position 10a appeared at a higher chemical shift (0.51 ppm in ^1H NMR and 0.98 ppm in ^{13}C NMR) in the (*S*)-epimer than in the (*R*)-epimer (Figure 3). These differences

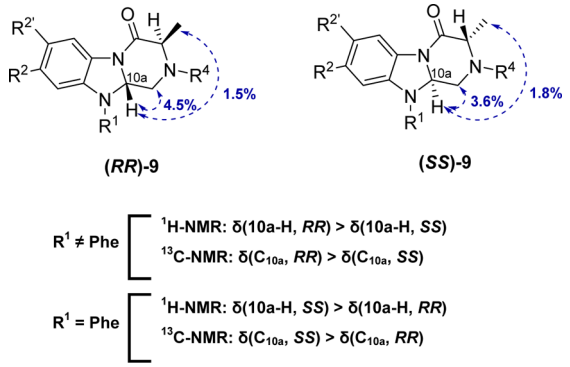


Figure 3. NMR NOEs of **9**(R^1,R^2,**2**,R^4) derivative diastereomers.

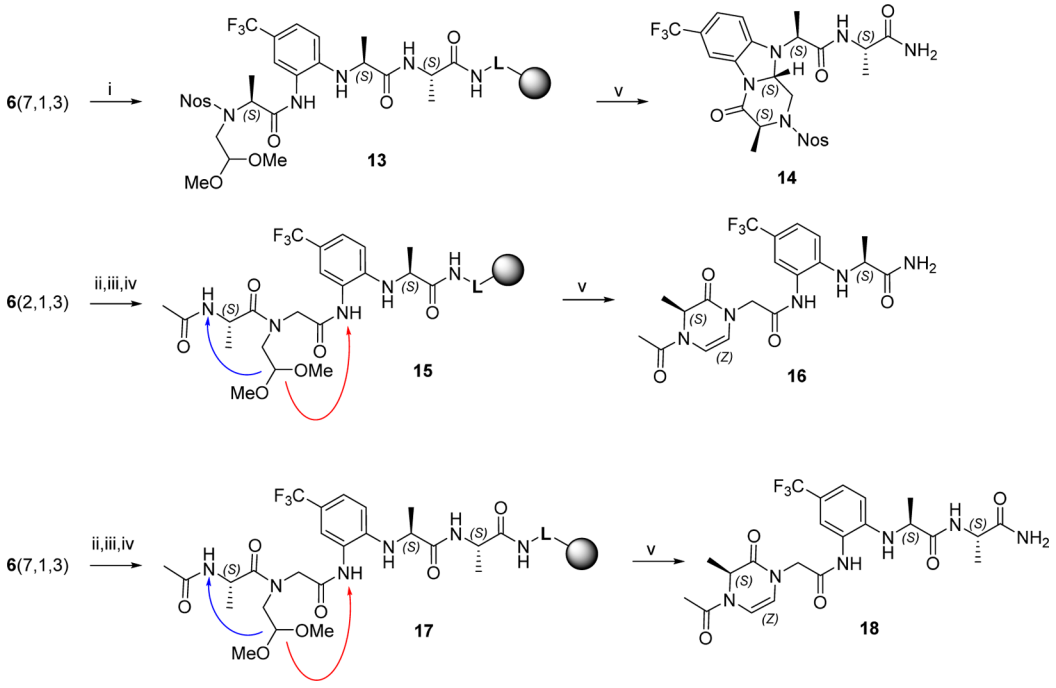
between epimers were used to tentatively assign the configuration of the remainder of the synthesized compounds. The NMR spectral data of the diagnostic protons and carbons are tabulated in the Supporting Information.

Extension of Peptide Backbone. To evaluate the incorporation of nitrogenous fused benzimidazolinopiperazinones **9** as peptide backbone constraints (β-turns) during traditional peptide synthesis, the peptide backbone was extended toward the amino and carboxy termini. We prepared model systems of tetra-(Nos-Fused bicycle-Ala-Ala-NH_2), penta-(Ac-Gly-Fused bicycle-Ala-NH_2), and hexapeptides (Ac-Gly-Fused bicycle-Ala-Ala-NH_2) in which our fused cycle **9** mimicked two amino acids of the sequence. The tetrapeptide model **14** was obtained from acyclic intermediate **13** without any changes in the outcome of the reaction, as expected. The extension of the peptide chain toward the amino terminus by one amino acid yielded resin-bound intermediates **15** and **17**. The penta- and hexapeptide precursors were prepared from the resins **6**(**2**,**1**,**1**) and **6**(**7**,**1**,**1**), respectively, via coupling with Fmoc-Ala-OH, followed by removal of the Fmoc protecting group and further acylation with Ac_2O (Scheme 4). In these cases, the formation of cyclic iminium is possible in two directions, that is, either *N*-aryliminium (red arrow) or *N*-acyliminium (blue arrow). After treatment of the penta- and hexapeptide precursors with 50% TFA in DCM, cyclic *N*-acyliminium was formed exclusively and led to only one regioisomer, **16** or **18**, respectively, which was isolated and then purified via HPLC. The structures of the regioisomers were confirmed via NMR. In conclusion, the *N*-acyliminium intermediate was exclusively formed in the west-bound direction, in accordance with our previous observations of analogous intermediates.[28,29] We are currently evaluating extension of the peptide at the N-terminus after the formation of the fused ring system **9** on a resin support.

CONCLUSION

We describe the synthesis of a peptide backbone conformational constraint that contains a privileged structure. Stereoselective solid-phase synthesis of benzimidazolinopiperazinones was performed via *N*-acyl-*N*-aryliminium ion cyclization−nucleophilic addition using commercially available building blocks. The synthesis proceeded under full stereocontrol of the newly formed stereogenic center. The effects of the individual building blocks and reaction conditions on stereochemical integrity were evaluated. Microwave-assisted synthesis resulted

Scheme 4. Incorporation of Bicyclic Constraints 9 during Peptide Synthesis[a]



[a]Reagents and conditions: (i) 4-nitrobenzenesulfonyl chloride (4-Nos-Cl), 2,6-lutidine, DCM, rt, 16 h; (ii) Fmoc-Ala-OH (3 equiv), DIC (1.5 equiv), THF, rt, 16 h; (iii) 50% piperidine in DMF (v/v), rt, 15 min; (iv) Ac_2O, rt, 1 h; (v) 50% TFA in DCM, rt, 16 h.

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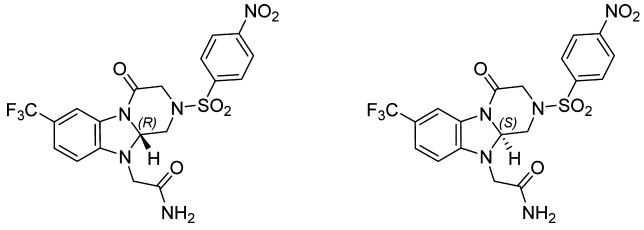
in a substantial reduction in reaction times; however, we observed significant epimerization during microwave-assisted acylation with (*S*)- and (*R*)-2-bromopropionic acids.

EXPERIMENTAL PROCEDURES

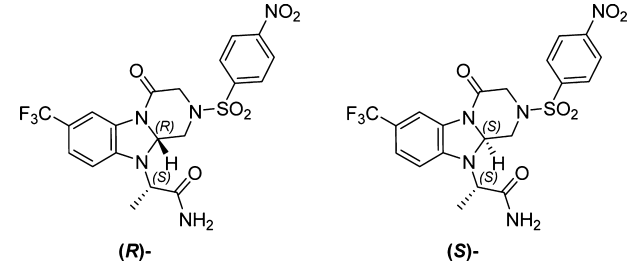
The solid-phase syntheses were performed in plastic reaction vessels (syringes, each equipped with a porous disc) using a manually operated synthesizer.[45] The volume of the wash solvent was 10 mL per 1 g of resin. For washing, the resin slurry was shaken with fresh solvent for at least 1 min before changing the solvent. Commercially available Rink resin (100−200 mesh, 0.66 mmol/g) and Wang resin (100−200 mesh, 1.0 mmol/g) were used. The yields of the crude products were calculated with respect to the loading of the first building block. The conventional reaction conditions for the individual steps of the synthesis have been reported in previous communications.[28−31] The microwave-assisted synthesis was performed in a CEM Focused Microwave Synthesis System, Model Discover, at 100 W; the reaction temperatures and times are summarized in Table 1.

Analytical Data of Representative Compounds. *(10aRS)-2-(2-((4-Nitrophenyl)sulfonyl)-4-oxo-7-(trifluoromethyl)-1,3,4,10a-tetrahydrobenzo[4,5]imidazo[1,2-a]piprazin-10(2H)-yl)acetamide* ***9(1,1,1,1)***.



Yield: 4.9 mg (22%) of amorphous solid. Purified by semipreparative HPLC. HPLC: RT = 1.86 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) δ 8.41 (d, J = 9.0 Hz, 2H, 4-Nos), 8.15 (d, J = 9.0 Hz, 2H, 4-Nos), 7.69 (d, J = 1.9 Hz, 1H, 6-H), 7.60 (bs, 1H, NH), 7.29 (d, J = 8.1 Hz, 1H, 8-H), 7.28 (s,1H, NH), 6.65 (d, J = 8.2 Hz, 1H, 9-H), 5.55 (dd, J = 9.4, 3.7 Hz, 1H, 10a-H), 4.46 (dd, J = 12.1, 3.7 Hz, 1H, 1-H), 4.30 (d, J = 16.9 Hz, 1H, 3-H), 3.98 (q, J = 17.4 Hz, 2H, Gly), 3.72 (d, J = 16.9 Hz, 1H, 3-H), 3.22 (dd, J = 12.1, 9.5 Hz, 1H, 1-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 169.7 (C, $CONH_2$-Ala), 161.2 (C, C_4), 150.2 (C, 4-Nos), 145.7 (C, C_{9a}), 141.6 (C, 4-Nos), 130.9 (C, C_{6a}), 129.1 (2CH, 4-Nos), 124.8 (2CH, 4-Nos), 123.7 (m, CH, C_8), 110.7 (m, CH, C_6), 106.2 (CH, C_9), 77.0 (CH, C_{10a}), 47.9 (CH_2, C_α-Gly), 46.6 (CH_2, C_3), 45.9 (CH_2, C_1). HRMS (ESI-TOF) m/z calcd for $C_{19}H_{16}F_3N_5NaO_6S$ $[M + Na]^+$: 522.0666, found 522.0662.

(S)-2-((RS)-2-((4-Nitrophenyl)sulfonyl)-4-oxo-7-(trifluoromethyl)-1,3,4,10a-tetrahydrobenzo[4,5]imidazo[1,2-a]pyrazin-10(2H)-yl)propanamide ***9(2,1,1,1)***.



(*R*)- (*S*)-

Yield: 59 mg (58%) of amorphous solid. Purified by semipreparative HPLC. (*R*)/(*S*) ratio of the crude = 60:40. (*R*)/(*S*) ratio of the purified product = 60:40. HPLC: RT = 2.06 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) *R*-epimer (60%) 8.42 (d, J = 8.9 Hz, 1H, 4-Nos), 8.18 (d, J = 8.9 Hz, 1H, 4-Nos), 7.73 (d, J = 1.8 Hz, 1H, 6-H), 7.57 (bs, 1H, NH), 7.33−7.26 (m, 2H, 8-H and NH), 6.65 (d, J = 8.2 Hz, 1H, 9-H), 5.70 (dd, J = 9.4, 3.6 Hz, 1H, 10a-H), 4.46 (dd, J = 12.0, 3.6 Hz, 1H, 1-H), 4.32 (d, J = 16.8 Hz, 1H, 3-H), 4.27 (q, J = 7.1 Hz, 1H, α-H-Ala), 3.73 (d, J = 17.0 Hz, 1H, 3-H), 3.43−3.28 (m, 1H, 1-H), 1.34 (d, J = 7.4 Hz, 3H, CH_3-Ala); *S*-epimer (40%) 8.39 (d, J = 8.9 Hz, 2H, 4-Nos), 8.14 (d, J = 8.9 Hz, 1H, 4-Nos), 7.71 (d, J = 1.9 Hz, 1H, 6-H), 7.66 (bs, 1H, NH), 7.36 (bs, 1H, NH), 7.32−7.26 (m, 1H, 8-H), 6.73 (d, J = 8.3 Hz, 1H, 9-H), 5.59 (dd, J = 9.2, 3.6 Hz, 1H, 10a-H), 4.36 (dd, J = 9.4, 3.6 Hz, 1H, 1-H), 4.33 (d, J = 7.4 Hz, 1H, α-H-Ala), 4.29 (d, J = 17.0 Hz, 1H, 3-H), 3.80 (d, J = 17.0 Hz, 1H, 3-H), 3.41−3.30 (m, 1H, 1-H), 1.32 (d, J = 7.5 Hz, 3H, CH_3-Ala). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) *R*-epimer 172.1 (C, $CONH_2$-Ala), 161.3 (C, C_4), 150.2 (C, 4-Nos), 143.6 (C, C_{9a}), 141.7 (C, 4-Nos), 131.8 (C, C_{6a}), 129.1 (2CH, 4-Nos), 124.8 (2CH, 4-Nos), 124.6 (q, J = 270.8 Hz, C, CF_3), 122.9 (q, J = 4.2 Hz, CH, C_8), 118.9 (q, J = 32.0 Hz, C, C_7), 110.7 (q, J = 3.6 Hz, CH, C_6), 108.4 (CH, C_9), 75.9 (CH, C_{10a}), 53.1 (CH, C_α-Ala), 47.8 (CH_2, C_3), 47.0 (CH_2, C_1), 12.8 (CH_3, CH_3−Ala); *S*-epimer 172.2 (C, $CONH_2$-Ala), 161.4 (C, C_4), 150.1 (C, 4-Nos), 144.4 (C, C_{9a}), 141.9 (C, 4-Nos), 130.9 (C, C_{6a}), 129.0 (2CH, 4-Nos), 124.8 (2CH, 4-Nos), 124.6 (q, J = 270.5 Hz, C, CF_3), 122.9 (q, J = 4.4 Hz, CH, C_8), 118.5 (q, J = 32.0 Hz, C, C_7), 110.5 (q, J = 3.8 Hz, CH, C_6), 107.0 (CH, C_9), 74.8 (CH, C_{10a}), 53.7 (CH, C_α-Ala), 47.8 (CH_2, C_3), 46.9 (CH_2, C_1), 13.3 (CH_3, CH_3-Ala). HRMS (ESI-TOF) m/z calcd for $C_{20}H_{19}F_3N_5O_6S$ $[M + H]+$ 514.1003, found 514.1008.

ASSOCIATED CONTENT

Supporting Information

Copies of NMR spectra associated with this article. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: vkrchnak@nd.edu.

Notes

The authors declare no competing financial interest.

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