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Regioselective Incorporation of Backbone Constraints Compatible with Traditional Solid-Phase Peptide Synthesis

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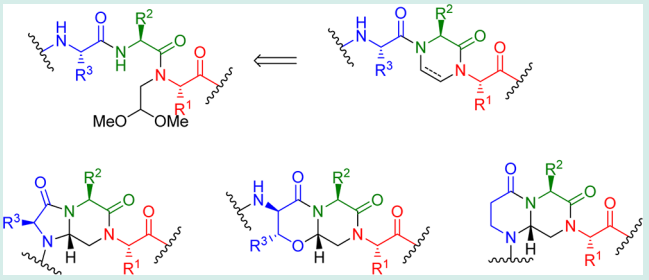
Supporting Information

ABSTRACT: A protected aldehyde was attached via a two-carbon spacer to a peptide backbone amide nitrogen during a traditional Merrifield solid-phase synthesis. Acid-mediated unmasking of the aldehyde triggered the regioselective formation of cyclic *N*-acyliminiums between the aldehyde and the neighboring peptide amide nitrogen. In the absence of an internal nucleophile, the cyclic iminiums formed dihydropyrazinones, a six-membered peptide backbone constraint between two peptide amides. In the presence of an internal nucleophile, tetrahydropyrazinopyrimidinediones or tetrahydroimidazopyrazinediones were formed via tandem *N*-acyliminium ion cyclization-nucleophilic addition. The outcome of this nucleophilic addition was dependent on the substituent on the nitrogen nucleophile.



KEYWORDS: *iminium, bisheterocycles, solid-phase synthesis, regioselectivity*

INTRODUCTION

Although peptides possess a wide range of biological activities, they suffer from serious drawbacks as therapeutic agents. Peptides are prone to rapid proteolytic degradation; they are poorly transported to the brain and are rapidly excreted through the liver and kidneys, which limits their use as drugs in clinical practice. Therefore, a significant effort has been dedicated to overcoming the aforementioned negative characteristics by designing and synthesizing peptidomimetics.[1−4] Victor Hruby describes peptidomimetics as compounds whose essential elements (pharmacophore) mimic a natural peptide or protein in 3D space but retain the ability to interact with a biological target, thus producing the same biological effect.[2] Several different approaches to peptidomimetics were followed including amino acid modifications, backbone alterations, the introduction of global restrictors such as head-to-tail and side-chain to side-chain cyclization and the synthesis of backbone scaffolds. Recent review articles have described these individual approaches in detail.[1−4]

Among the numerous potential structural variations, a straightforward method could allow for rigidifying the peptide backbone by bridging two neighboring amide nitrogens via a two-carbon spacer. The formation of a cyclic *N*-acyliminium ion from an acyclic intermediate that hosts a protected aldehyde attached to the amide nitrogen via a two-carbon spacer could provide a synthetic methodology to facilitate this bridging, offering access to expanded structural diversity (**I**, Scheme 1). Iminium chemistry has been reviewed in in the literature.[5−7] Scheme 1 depicts two possible routes for cyclic *N*-acyliminium ion formation;[8] the cyclic *N*-acyliminium **IIa** formed with the amide facing the peptide amino terminus (referred to as westbound)[3,9−12] and the iminium **IIb** formed with the amide facing the peptide carboxyl terminus (referred to as eastbound).[13] The direction is based on standard peptide/protein nomenclature with an N-terminal amino acid on the left. In the absence of a nucleophile, the intermediates **IIa** and **IIb** lead to 3,4-dihydropyrazin-2(1*H*)-ones **IIIa** and **IIIb**, respectively.[14,15] Both scenarios were documented in the literature for simple model compounds that allowed the six-membered ring to close in one direction only.[16]

These cyclic *N*-acyliminium ions can be transformed into structures that are more complex. The presence of an internal nucleophile can trigger a fused-ring formation, and a bicyclic system can be formed via tandem *N*-acylium ion cyclization−nucleophilic addition.[5−7,17,18]

Tandem *N*-acylium ion cyclization−nucleophilic addition was particularly useful for the synthesis of several peptidomimetics in the westbound direction. Baldwin and co-workers[19] reported the synthesis of fused ring piperazine derivatives as bicyclic γ-lactam dipeptide mimetics. Patek's group[9] used tandem *N*-acyliminium ion cyclization−nucleophilic addition to directly access the bi-, tri-, and tetracyclic derivatives of 1-acyl-3-oxopiperazines (**V**) (Scheme 2). Kahn and co-workers[3,10,11] took advantage of Patek's solid-phase synthesis[9] and prepared

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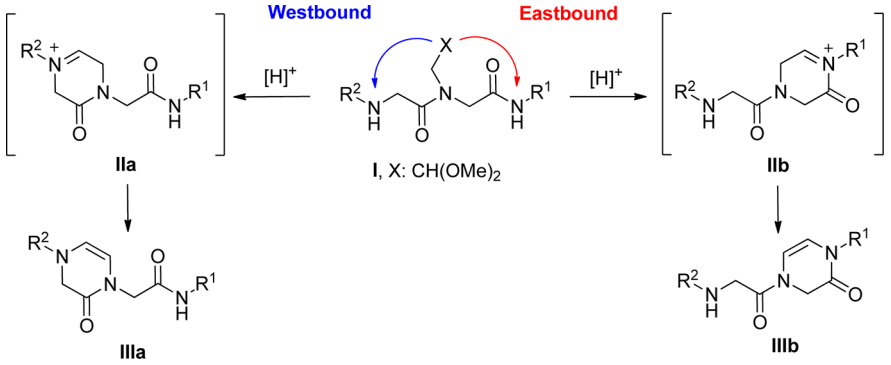
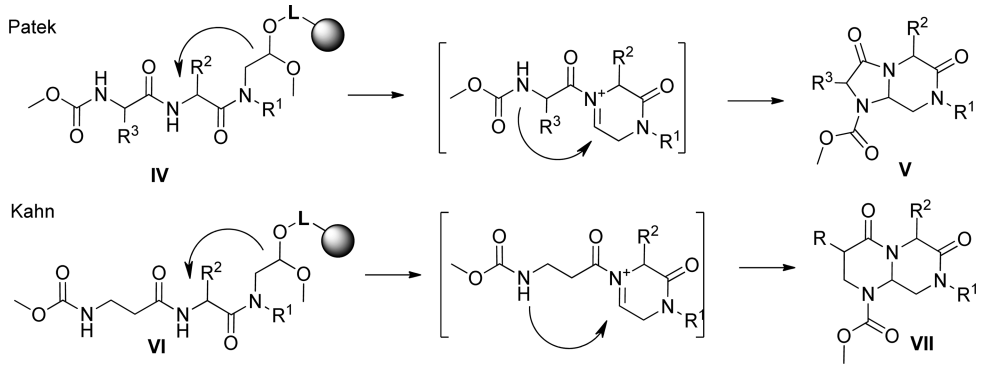
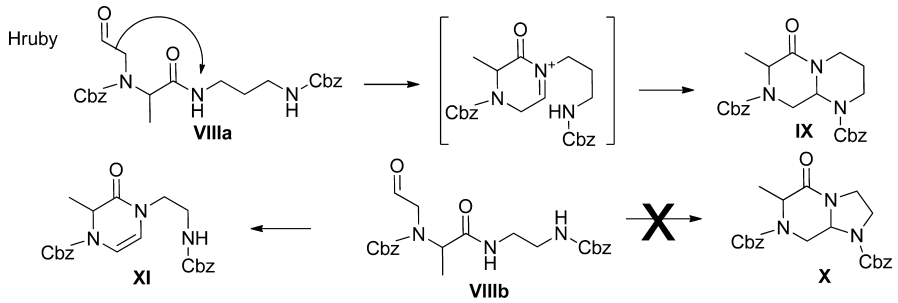
Scheme 1. Two Possible Synthetic Routes for Cyclic *N*-acyliminium



Scheme 2. Reported Iminium Ion Formation in the Westbound Direction



Scheme 3. Reported Iminium Ion Formation in the Eastbound Direction



β-turn mimetics (**VII**) from a dipeptide containing a β-amino acid and an α-amino acid (**VI**). The bicyclic β-turn peptidomimetics, 1,3,6,8-substituted tetrahydro-2*H*-pyrazino-[1,2-*a*]pyrimidine-4,7-diones, were prepared by Kahn's group from a similar linear precursor via tandem cyclization after cleavage from acetal or olefin linkers.[10,11] The aldehyde function was located on a side chain attached to the amide nitrogen of the C-terminal amino acid (**IV** and **VI** linear precursors).

Hruby[13] also developed a synthesis for β-turn mimetics via cyclic acyliminium formation in the opposite direction (the eastbound direction). In this case, the aldehyde was located on the amide of the N-terminal amino acid (**VIIIa** and **VIIIb**, Scheme 3). The fused ring **IX** was formed from the *N*-(3-aminopropyl) derivative. Interestingly, no analogous reaction with a dipeptide precursor has been reported. No ring closure to the five-membered ring (**X**) was observed with the olefinic product (**XI**) forming instead. Pyrazinopyrimidine moieties have also been included by Kohn and Zhang into a peptide as constrained β-turn mimics, which were formed spontaneously from analogous linear intermediates during the TFA cleavage of linear peptides from a solid support.[12]

The results indicated that the formation of the second fused ring strongly depended on the direction of cyclization (west or east), the fused ring size, the type of nucleophile, and the substituents.

Typically, constrained peptide mimetics have been prepared in an independent synthesis then incorporated into the peptide. We describe the synthesis of acyclic precursors during a traditional Merrifield peptide synthesis and the formation of a ring system during cleavage from the resin. The cyclic iminium can be formed in both directions, that is, toward the amino or the carboxyl terminus; therefore, we evaluated the regioselectivity of the piperazine ring formation and reported the conditions for the unequivocal synthesis of cyclic iminiums in either direction. We then addressed the factors influencing the fused-ring formation in the westbound direction. Cyclization in the eastbound direction will be reported in a subsequent communication.

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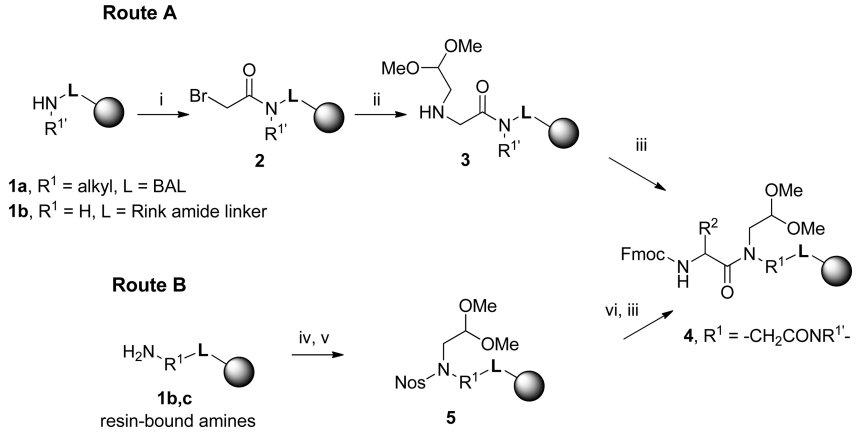
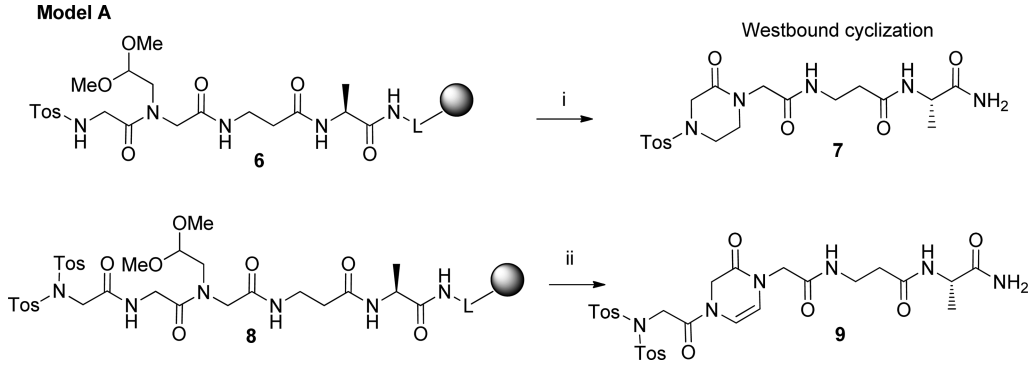
Scheme 4. Preparation of the Masked Aldehyde Precursors 4[a]



[a]Reagents and conditions: (i) bromoacetic acid, DIC, DIEA, DCM, rt, 2 h; (ii) aminoacetaldehyde dimethylacetal, DIEA, DMF, rt, 2 h; (iii) Fmoc-α-amino acid, DIC, THF, rt, 16 h or Fmoc-α-amino acid, DIC, *N*-hydroxybenzotriazole (HOBt), DMF/DCM (1:1), rt, 16 h; (iv) 4-nitrobenzenesulfonyl chloride, lutidine, DCM, rt, 16 h; (v) glycolaldehyde dimethyl acetal, DIAD, PPh_3, THF, 50 °C, 16 h; (vi) mercaptoethanol, DBU, DMF, rt, 5 min.

Scheme 5. Regioselectivity of the Iminium Ion Formation[a]



[a]Reagents and conditions: (i) 50% TFA, 10% TES in DCM, rt, 1 h; (ii) 50% TFA in DCM, rt, 1 h.

As an analogy, conformational constraints have been incorporated into peptides via silylated amino acids with the subsequent conversion into *N*-acyliminium ions.[20−22] *N*-Boc-1,3-oxazinane-masked aldehyde building blocks have been incorporated into peptides and used to synthesize structurally diverse bicyclic dipeptide mimetics.[23,24] The present work is an extension of our previous studies.[15,18]

RESULTS AND DISCUSSION

First, we describe the results of experiments focused on the regioselectivity of cyclic iminium ion formation. Then, we address the fused-ring formation via tandem *N*-acyliminium ion cyclization−nucleophilic addition with respect to the fused ring size, the type of nucleophile, and its substituents.

Regioselectivity. As mentioned previously, the *N*-(2-oxoethyl)-derivatized peptides can, in principle, form *N*-acyliminium species in two directions (Scheme 1): toward the peptide amino terminus (referred to as westbound, intermediate **IIa**) and toward the carboxyl peptide terminus (eastbound direction, intermediate **IIb**). Cyclization in either direction is viable, and both directions of the *N*-acyliminium ion formation have been reported in model compounds that allowed only one direction of cyclization.[9,12−14]

We evaluated the regioselectivity of cyclic iminium formation on model compounds that allowed both directions of cyclic iminium formation. Cyclic *N*-acyliminium intermediates were formed from an aldehyde and a nitrogen atom contained in different functionalities including the amide, sulfonamide, and aniline derivatives. Among the various methods for iminium ion synthesis, the condensation of secondary amides with aldehydes is regarded as one of the most versatile.[17,25−27] The acetal-protected aldehyde was attached to the peptide backbone amide nitrogen via a two-carbon spacer. The aldehyde was then unmasked, and the target compounds were cleaved from the resin via acid-mediated cleavage to initiate the cyclic *N*-acyliminium formation.

The synthesis of the model compounds was carried out using commercially available polystyrene-1% DVB resin with Rink-amide[28] and BAL[29] linkers (Scheme 4). The BAL resin was reacted with primary amines under reductive amination conditions to yield polymer-supported secondary amines.[30] Two different routes were used to introduce the two-carbon side-chain with protected aldehydes. The resins labeled **1** were acylated with bromoacetic acid using in situ-prepared symmetrical anhydrides. Then, the α-bromoacylamide resin **2** was reacted with aminoacetaldehyde dimethyl acetal to yield the intermediates labeled **3** (Scheme 4, route A).[12] A sample of resin **3** was then reacted with Fmoc-OSu for LC/MS analysis. In the last synthesis step of the cycle precursor, the secondary amines **3** were acylated with DIC/HOBt or the in situ-prepared

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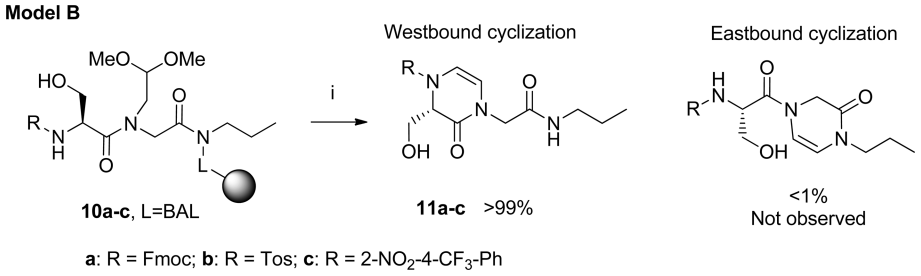
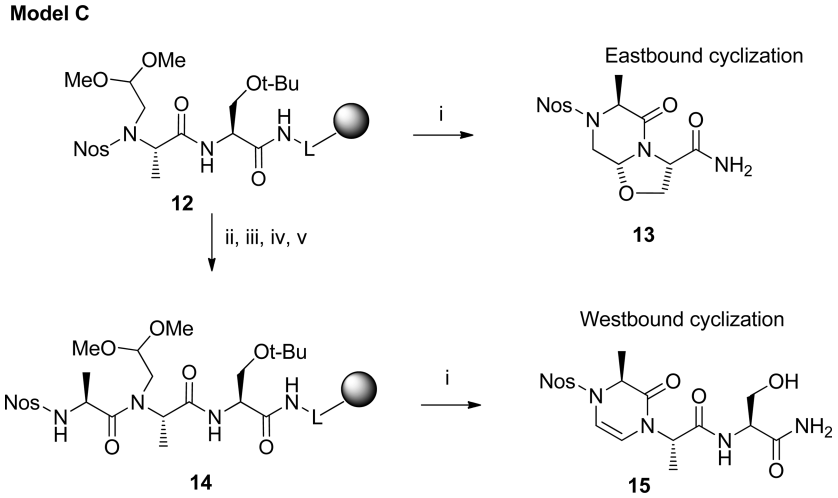
Scheme 6. Effect of the *N*-substituent on the Regioselectivity[a]



[a]Reagents and conditions: (i) 50% TFA in DCM, rt, 1 h.

Scheme 7. Regioselectivity of Iminium Formation and Demonstration of Eastbound Viability[a]



[a]Reagents and conditions: (i) 50% TFA, 10% TES in DCM, rt, 1 h; (ii) mercaptoethanol, DBU, DMF, rt, 5 min; (iii) Fmoc-α-amino acid, DIC, HOBt, DMF/DCM (1:1), rt, 16 h; (iv) 50% piperidine in DMF, rt, 20 min; (v) 4-nitrobenzenesulfonyl chloride, lutidine, DCM, rt, 4 h.

symmetrical anhydrides of different amino acids including Fmoc-Gly-OH, Fmoc-Ala-OH, Fmoc-Phe-OH, Fmoc-Val-OH, Fmoc-Ile-OH, Fmoc-Glu(O*t*-Bu)−OH, and Fmoc-Lys(Boc)−OH. A ∼10 mg sample of resin **4** was reacted with Fmoc-OSu, and the product was released from the resin to analyze the completion of the acylation reaction.

An alternative strategy to introduce the masked aldehyde was based on Fukuyama *N*-alkylation[31] (Scheme 4, route B). This route involved the sulfonylation of resin-bound amines (Rink resin **1b** or generic resin-bound amine **1c**) with 4-nitrobenzenesulfonyl (4-Nos) chloride followed by the Fukuyama variation of a Mitsunobu *N*-alkylation with glycolaldehyde dimethyl acetal to produce resin **5**. The corresponding diethyl acetal did not provide the expected *N*-alkylated species; only starting material was detected in all cases. The final Nos-group cleavage of **5** produced a secondary resin-bound amine that was then acylated with the Fmoc-protected amino acids to yield resin **4**, as previously described.

On the basis of these strategies, three different model systems (Models A−C shown in Schemes 5−7, respectively) were synthesized using traditional peptide chemistry, and the regioselectivity of the iminium formation was evaluated. The first polymer-supported tetrapeptide **6** could form both cyclic *N*-acyliminium regioisomers: toward the peptide carboxyl terminus (eastbound) or toward the peptide amino terminus (westbound cyclization) (Model A, Scheme 5). Under TFA exposure, the cyclic *N*-acyliminium was formed exclusively in the westbound direction to yield only a single regioisomer, as confirmed by ^{1}H and ^{13}C NMR analyses. Triethylsilane (TES) present in the cleavage cocktail reduced the iminium to 1,4-substituted-piperazinone (**7**).[14] The cyclic iminium was formed with sulfonamide. We extended the peptide backbone by one amino acid and synthesized pentapeptide **8** to form the iminium with amide (two Tos groups prevented the formation of a fused ring). Only one regioisomer (**9**) was detected, isolated, and characterized, demonstrating the same west directional preference. Next, we extended the peptide toward the amino terminus and synthesized three tetrapeptides on the Rink-amide resin, 4-Nos-Ala-Gly-Ala-*N*-(2-oxo-ethyl)-Ala-NH-Resin, 4-Nos-Ala-Ala-Ala-*N*-(2-oxo-ethyl)-Ala-NH-Resin, and 4-Nos-Ala-Lys-Ala-*N*-(2-oxo-ethyl)-Ala-NH-Resin, and observed only regioselective westbound formation of the piperazinone derivatives.

To further evaluate the potential effect of the amide type and *N*-substituent on the regioselectivity of the iminium ion formation, we prepared model compounds **10a−c** (Model B, Scheme 6). The *N*-propyl carboxyl-terminal amide (**10**) provided increased nucleophilicity and steric accessibility when compared with the previous model compounds. We also introduced different *N*-substituents including urethane, sulfonamide, and aryl derivatives and found that neither the type of secondary amide nor the substituents influenced the regioselectivity. We observed the exclusive formation of west regioisomers (**11a−c**) in all cases.

Finally, the third model system also documented the westbound-directed cyclization route; the linear precursor **14**,

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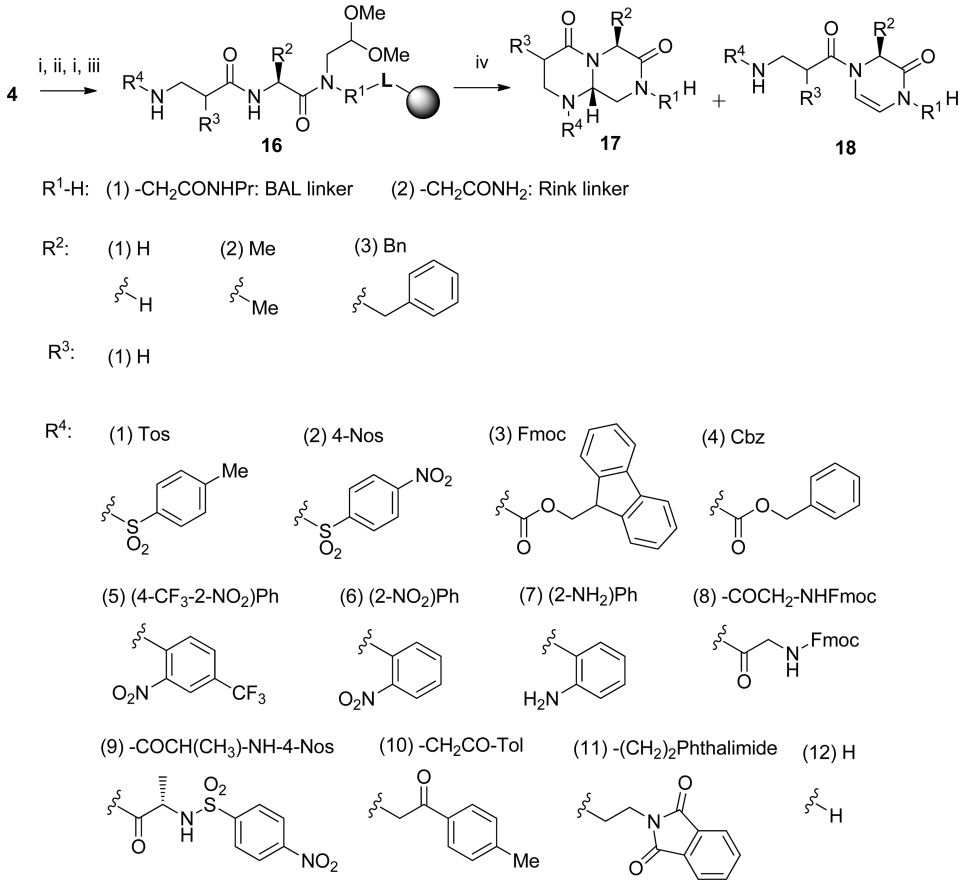
Scheme 8. Synthesis of 6 + 6 Fused Rings[a]



[a]Reagents and conditions: (i) 50% piperidine in DMF, 15 min, rt; (ii) Fmoc-*β*-Ala-OH, DIC, HOBt, DMF/DCM (1:1), rt, 16 h; (iii) for R^4 = 1,2: benzenesulfonyl chlorides, lutidine, DCM, rt, 16 h; for R^4 = 4: Cbz-Cl, DIEA, DCM, rt, 16 h; for R^4 = 5: 4-fluoro-2-nitrobenzotrifluoride, DIEA, DMSO, rt, 16 h; for R^4 = 6: 1-fluoro-2-nitrobenzenes, DMSO, 50 °C, 16 h; for R^4 = 7: 1-fluoro-2-nitrobenzenes, DMSO, 50 °C, 16 h followed by $SnCl_2$, DIEA, and DMF saturated with N_2, rt, 16 h; for R^4 = 8, 9: carboxylic acid or Fmoc-AA, DIC, HOBt, DMF/DCM (1:1), rt, 16 h; for R^4 = 10: 2-bromo-1-*p*-tolyl-ethanone, DIEA, DMF, rt, 16 h; for R^4 = 11: (*N*)-2-hydroxyethylphthalimide, DIAD, PPh_3, THF, rt, 16 h; (iv) 50% TFA in DCM, 1 h.

with the ability to cyclize in both directions, reacted via the west-iminium ion exclusively to generate the 1,4-disubstituted-3,4-dihydro-1*H*-pyrazin-2-one **15** as the sole product (Model C, Scheme 7). A similar substrate (**12**) that allowed iminium formation toward the east direction only was also examined. After treatment with TFA, resin **12** generated the double cycle **13**, proving that the formation of eastbound iminium was feasible in the absence of a west-iminium path.

In conclusion, we demonstrated that the *N*-(2-oxo-ethyl)-derivatized peptides were regioselectively converted either to 3,4-dihydropyrazin-2(1*H*)-one upon the acid-mediated deprotection of the aldehyde or to piperazin-2-one in the presence of the reducing agent TES. This conclusion was supported by our recently published synthesis of 3,4-dihydropyrazin-2(1*H*)-ones and piperazin-2-ones from polymer-supported acyclic intermediates via *N*-acyl iminiums.[15] Thus, this synthesis can incorporate a six-membered peptide backbone constraint during traditional Merrifield solid-phase peptide synthesis.

Tandem *N*-acylium Ion Cyclization–Nucleophilic Addition. The second part of this study focused on the formation of a fused ring via an internal nucleophile. The tandem *N*-acyliminium ion cyclization–nucleophilic addition was studied using two ring sizes (five and six-membered rings) and an internal *N*-arylsulfonyl, *N*-acyl, *N*-aryl, *N*-alkyl, or carbamate nitrogen nucleophile.

6 + 6 Fused Rings. To study the formation of 6 + 6 fused rings, common intermediates **4** (Scheme 4) were acylated using Fmoc-*β*-Ala-OH, followed by Fmoc group cleavage and *N*-derivatization with a wide variety of functional groups in the R^4 position to evaluate the influence of the different nitrogen nucleophiles on the acyliminium reaction (resin **16**, Scheme 8, Table 1). Exposure to TFA caused cleavage from the resin, and the deprotection of the aldehyde triggered the formation of iminium ions, which could undergo nucleophilic addition to form the target hexahydro-pyrazino[1,2-*a*]pyrimidine-4,7-diones **17** or enamides **18**.[13] The crude products were analyzed using LC/MS and/or ^{1}H NMR spectroscopy. To assess the effect of the cleavage cocktail, several analytical samples were also exposed to 5% water in TFA and neat formic acid. These cleavage cocktails yielded the same major products observed with 50% TFA in DCM.

N-arylsulfonyl Derivatives. Both the Tos (**16**(1,1,1,1), **16**(2,1,1,1), **16**(2,2,1,1)) and 4-Nos derivatives (**16**(2,1,1,2), **16**(2,2,1,2)) provided clean conversions to the fused-ring systems **17** with no detectable formation of enamides. The purity of the crude compounds was high, >90% as determined by LC/MS traces at 240 nm. The NMR spectra indicated that

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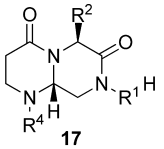
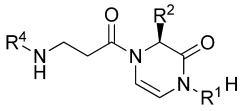
Table 1. Evaluation of the Internal Nitrogen Nucleophile during 6 + 6 Ring Formation[e]


17


18

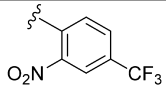
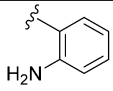
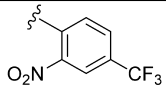
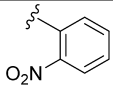
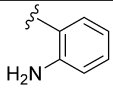
Entry	R^1H	R^2	R^4	Ratio[a] 17:18	Compound Yield[b]
			Sulfonamides		
16(1,1,1,1)	-$CH_2CONHPr$	H	Tos	>99:1	**17**(1,1,1,1) 49%
16(2,1,1,1)	-CH_2CONH_2	H	Tos	>99:1	**17**(2,1,1,1) 76%
16(2,2,1,1)	-CH_2CONH_2	Me	Tos	>99:1	**17**(2,2,1,1) 78%
16(2,1,1,2)	-CH_2CONH_2	H	4-Nos	>99:1	**17**(2,1,1,2) 65%
16(2,2,1,2)	-CH_2CONH_2	Me	4-Nos	>99:1	**17**(2,2,1,2) 63%
			Urethanes		
16(2,1,1,4)	-CH_2CONH_2	H	Cbz	30:70	**17**(2,1,1,4) + **18**(2,1,1,4) 44%[d]
16(2,2,1,3)	-CH_2CONH_2	Me	Fmoc	75:25[c]	NI
16(2,2,1,4)	-CH_2CONH_2	Me	Cbz	40:60	**17**(2,2,1,4) + **18**(2,2,1,4) 50%[d]
			***N*-aryl**		
16(2,1,1,5)	-CH_2CONH_2	H		1:99	**18**(2,1,1,5) 46%
16(2,1,1,7)	-CH_2CONH_2	H		>99:1	**17**(2,1,1,7) 50%
16(2,2,1,5)	-CH_2CONH_2	Me		1:99	**18**(2,2,1,5) 85%
16(2,3,1,6)	-CH_2CONH_2	Bn		69:31	**17**(2,3,1,6) 56%
16(2,3,1,7)	-CH_2CONH_2	Bn		93:7	**17**(2,3,1,7) 66%

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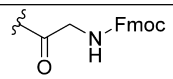
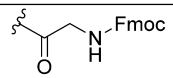
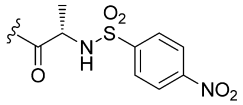
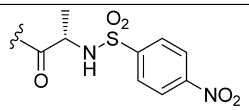
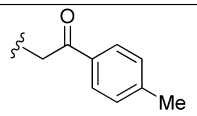
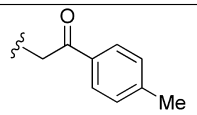
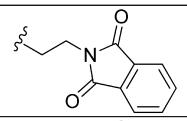
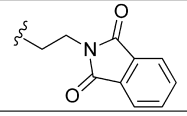
Table 1. continued

Entry	R^1H	R^2	R^4	Ratio[a] 17:18	Compound Yield[b]
			Amides		
16(2,1,1,8)	$-CH_2CONH_2$	H		<1:99[c]	NI
16(2,2,1,8)	$-CH_2CONH_2$	Me		<1:99[c]	NI
16(2,1,1,9)	$-CH_2CONH_2$	H		<1:99	**18**(2,1,1,9) 92%
16(2,2,1,9)	$-CH_2CONH_2$	Me		<1:99	**18**(2,2,1,9) 45%
			***N*-alkyl**		
16(2,1,1,10)	$-CH_2CONH_2$	H		<1:99[c]	NI
16(2,2,1,10)	$-CH_2CONH_2$	Me		<1:99[c]	NI
16(2,1,1,11)	$-CH_2CONH_2$	H		<1:99[c]	NI
16(2,2,1,11)	$-CH_2CONH_2$	Me		<1:99[c]	NI
			***N*-H**		
16(2,1,1,12)	$-CH_2CONH_2$	H	H	<1:99	NI
16(2,2,1,12)	$-CH_2CONH_2$	Me	H	<1:99	**18**(2,2,1,12) 46%

[a]The relative ratio of products **17:18** was estimated from the LC traces at 240 nm and/or from the crude ^{1}H NMR spectrum. [b]The total yield after HPLC purification of the products prepared in a 6- to 10-step synthesis. [c]These compounds were unstable and decomposed during purification; the products were identified in the ^{1}H NMR of the crude preparations through their characteristic olefinic protons. [d]Inseparable mixture of isomers **17** and **18**; the ratio of **17**:**18** was calculated from the ^{1}H NMR spectra. [e]NI stands for not isolated.

the nucleophilic addition proceeded with full stereocontrol of the newly formed asymmetric carbon. The 4-Nos group was chosen because it could be easily removed.[31,32]

N-carbamates. The polymer-supported Fmoc derivatives (**16**(2,2,1,3)) and Cbz derivatives (**16**(2,1,1,4), **16**(2,2,1,4)) formed a mixture of products with the bisheterocycles **17** as the major products in the case of Fmoc derivatives. The Cbz derivatives primarily yielded the enamides **18**. Replacing Gly with Ala appeared to have a little effect.

N-aryl Derivatives. The reaction outcome with the *N*-aryl model compounds (possessing low basicity, pK_a 19−31) was dependent on the aromatic substituents. The electron-deficient aromatic rings yielded only the enamides (**18**(2,1,1,5), **18**(2,2,1,5)) or a mixture of products (**16**(2,3,1,6)), while the electron-donating substituents promoted the nucleophilic addition and generated the fused products in good yields (**16**(2,1,1,7), **17**(2,3,1,7)).

N-acyl Compounds. The presence of the nonbasic amide functionality did not lead to the fused ring system; the

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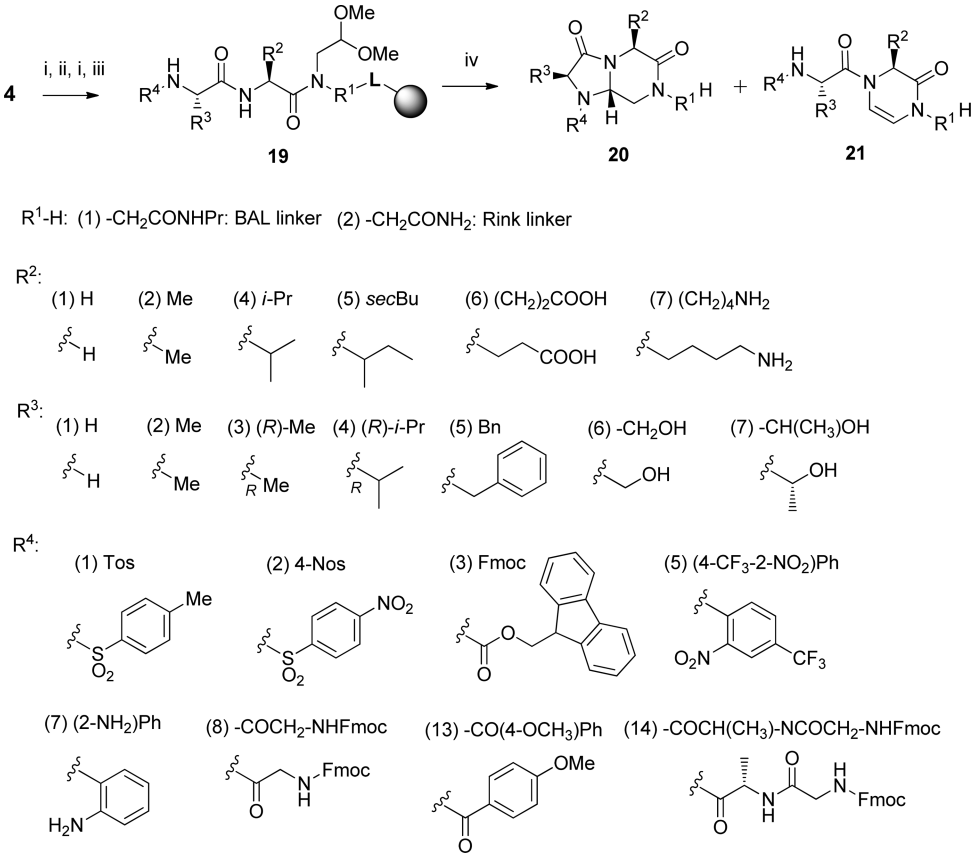
Scheme 9. Synthesis of 5 + 6 Fused Rings[a]



[a]Reagents and conditions: (i) piperidine, DMF, 15 min, rt; (ii) Fmoc-α-amino acid, DIC, HOBt, DMF/DCM (1:1), rt, o/n; (iii) for R^4 = 1, 2: benzenesulfonyl chlorides, lutidine, DCM, rt, 16 h; for R^4 = 5: 4-fluoro-2-nitrobenzotrifluoride, DIEA, DMSO, rt, 16 h; for R^4 = 7: 1-fluoro-2-nitrobenzenes, DMSO, 50 °C, 16 h, followed by $SnCl_2$, DIEA, DMF saturated with N_2, rt, 16 h; for R^4 = 8, 13, 14 (twice): carboxylic acid or Fmoc-AA, DIC, HOBt, DMF/DCM (1:1), rt, 16 h; (iv) 50% TFA in DCM, 1 h.

enamides were isolated as the only products (**18**(2,1,1,8), **18**(2,2,1,8), **18**(2,1,1,9), **18**(2,2,1,9)). Despite forcing different conditions, including high temperature, extended reaction times, and the use of trifluoroacetic anhydride or formic acid, no formation of the fused heterocycle was observed.

N-H and N-alkyl Compounds. The acyclic resin-bound substrates with strongly basic amine functionalities (pK_a higher than 40) formed the enamides exclusively (**18**(2,1,1,10), **18**(2,2,1,10), **18**(2,1,1,11), **18**(2,2,1,11), **18**(2,1,1,12), and **18**(2,2,1,12)), most likely because of the protonation of the amino group.

5 + 6 Fused Rings. To form a five-membered fused ring, resin **4** was acylated overnight with Fmoc-protected amino acids (both L- and D-) via DIC activation in the presence of HOBt at ambient temperature, although the reaction time was reduced to 1 h for unprotected Fmoc-Ser-OH (Scheme 9). The final derivatization using standard reaction conditions generated sulfonamides, urethanes, amides, and *N*-aryl derivatives **19** and enabled us to evaluate the effect of the *N*-substituent on the reaction outcome. After treating resin **19** with a TFA solution (50% in DCM), the crude products were analyzed using LC/MS and/or ^{1}H NMR spectroscopy. As in the previous study, the iminium ion could either undergo nucleophilic addition to form the target fused five-membered ring **20** or elimination to produce enamide **21**. We observed that the formation of enamide **21** strongly depended on both the *N*-R^4 substituent and the type of amino acids (R^2 and R^3).

N-arylsulfonyl Derivatives. We prepared model sulfonamides with tosyl and nosyl chlorides (sulfonamides, Table 2) and evaluated the effect of amino acids (R^2 and R^3 substituents) on the ratio of the fused-ring compounds to the enamides. Unlike the previously described six-membered ring formation, this outcome was highly influenced by the types of arylsulfonyl group and amino acid. The *N*-Tos derivatives prepared using Fmoc-Gly-OH (no Cα substituent in R^1) (**19**(1,1,2,1), **19**(2,1,2,1), and **19**(2,1,6,1)) formed both the fused heterocycles **20** and the enamides **21**. However, the Cα-substituted amino acids (Ala, (**19**(2,2,2,1), and **19**(2,2,6,1))) formed the fused ring predominantly (>99:1). Moreover, Ser and Thr with or without the *t*-butyl protected hydroxyl group formed the fused west ring through the amino group and not the hydroxyl group (potential six-membered ring formation). In contrast, the Nos derivatives produced a mixture of products in all cases examined. The presence of a Cα substituent ($R^2 \neq$ H) favored the second cyclization, and the proportion of bicyclic products increased independently of the electronic and steric characteristics of the R^2 group (compare the substrate **19**(2,1,2,2) with substrates **19**(2,2,2,2), **19**(2,2,5,2), **19**(2,4,1,2), **19**(2,4,2,2), **19**(2,5,1,2), **19**(2,5,2,2), **19**(2,6,1,2), **19**(2,7,2,2), and **19**(2,7,5,2)).

The formation of an asymmetric carbon during the tandem *N*-acylium ion cyclization−nucleophilic addition was stereoselective; we isolated only one diastereoisomer (**20**(2,2,2,1), **20**(2,2,2,2), **20**(2,2,5,2), **20**(2,4,1,2), and **20**(2,4,2,2)) when a

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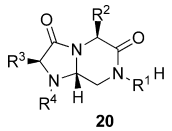
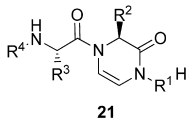
Table 2. Evaluation of Internal Nitrogen Nucleophiles in a 5 + 6 Double Ring


20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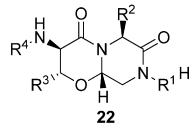

22

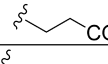
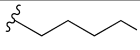
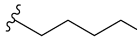
Entry	R^1	R^2	R^3	R^4	Ratio[a] 20:21	Compound Yield[b]
				Sulfonamides		
19(1,1,2,1)	-$CH_2CONHPr$	H	Me	Tos	72:28	**20**(1,1,2,1) 15%[c]
19(2,1,2,1)	-CH_2CONH_2	H	Me	Tos	81:19	**20**(2,1,2,1) 67%[c] **21**(2,1,2,1) 15%
19(2,1,6,1)	-CH_2CONH_2	H		Tos	70:30	**20**(2,1,6,1) 44%[d]
19(2,2,2,1)	-CH_2CONH_2	Me	Me	Tos	>99:1	**20**(2,2,2,1) 53%
19(2,2,6,1)	-CH_2CONH_2	Me		Tos	>99:1	**20**(2,2,6,1) 53%
19(2,1,2,2)	-CH_2CONH_2	H	Me	4-Nos	10:90	**20**(2,1,2,2) 8%[c] **21**(2,1,2,2) 65%
19(2,2,2,2)	-CH_2CONH_2	Me	Me	4-Nos	51:49	**20**(2,2,2,2) 55% **21**(2,2,2,2) 30%
19(2,2,3,2)	-CH_2CONH_2	Me	(*R*)-Me	4-Nos	15:85	**21**(2,2,3,2) 87%
19(2,2,4,2)	-CH_2CONH_2	Me	(*R*)-*i*-Pr	4-Nos	15:85	**21**(2,2,4,2) 75%
19(2,2,5,2)	-CH_2CONH_2	Me	Bn	4-Nos	56:44	**20**(2,2,5,2) 45% **21**(2,2,5,2) 15%
19(2,4,1,2)	-CH_2CONH_2	*i*Pr	H	4-Nos	NC	**20**(2,4,1,2) 58%
19(2,4,2,2)	-CH_2CONH_2	*i*Pr	Me	4-Nos	48:52	**20**(2,4,2,2) 58%
19(2,5,1,2)	-CH_2CONH_2	*sec*Bu	H	4-Nos	71:29	NI
19(2,5,2,2)	-CH_2CONH_2	*sec*Bu	Me	4-Nos	45:55	NI
19(2,6,1,2)	-CH_2CONH_2		H	4-Nos	62:38	NI
19(2,7,2,2)	-CH_2CONH_2		Me	4-Nos	60:40	NI
19(2,7,5,2)	-CH_2CONH_2		Bn	4-Nos	63:37	NI

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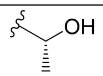
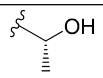
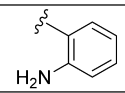
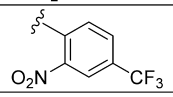
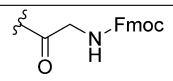
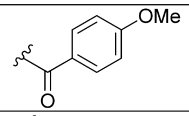
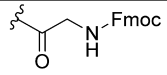
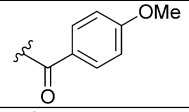
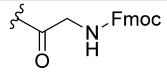
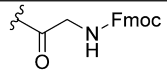
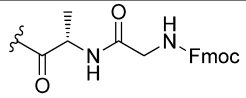
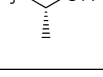
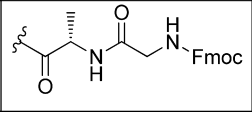
Table 2. continued

Entry	R^1	R^2	R^3	R^4	Ratio[a] 20:21	Compound Yield[b]
				Urethanes		
19(2,1,1,3)	-CH_2CONH_2	H	H	Fmoc	6:90	**21**(2,1,1,3) 14%
19(2,1,2,3)	-CH_2CONH_2	H	Me	Fmoc	<1:99[e]	NI
19(2,1,5,3)	-CH_2CONH_2	H	Bn	Fmoc	5:95[e]	NI
19(2,1,7,3)	-CH_2CONH_2	H		Fmoc	99% of **22**	**22**(2,1,7,3) 54%
19(2,2,5,3)	-CH_2CONH_2	Me	Bn	Fmoc	<1:99[e]	NI
19(2,2,7,3)	-CH_2CONH_2	Me		Fmoc	99% of **22**	**22**(2,2,7,3) 90%
				***N*-aryl**		
19(2,1,2,7)	-CH_2CONH_2	H	Me		>99:1	**20**(2,1,2,7) 16%
19(2,2,2,5)	-CH_2CONH_2	Me	Me		<1:99	**21**(2,2,2,5) 15%
				***N*-acyl**		
19(2,1,2,8)	-CH_2CONH_2	H	Me		<1:99[e]	NI
19(2,1,2,13)	-CH_2CONH_2	H	Me		<1:99[e]	NI
19(2,1,5,8)	-CH_2CONH_2	H	Bn		<1:99[e]	NI
19(2,1,5,13)	-CH_2CONH_2	H	Bn		<1:99[e]	NI
19(2,2,2,8)	-CH_2CONH_2	Me	Me		<1:99[e]	NI
19(2,2,5,8)	-CH_2CONH_2	Me	Bn		<1:99[e]	NI
19(2,2,6,14)	-CH_2CONH_2	Me			77% of **22**	**22**(2,2,6,14) 77%
19(2,2,7,14)	-CH_2CONH_2	Me			99% of **22**	**22**(2,2,7,14) 92%

[a]The relative ratio of the products **20:21** was estimated from the LC traces at 240 nm and/or from the crude ^{1}H NMR spectra. NC stands for not calculated because of overlap. NI stands for not isolated; [b]The total yield after HPLC purification of the products prepared in a 6- to 10-step synthesis. [c]Mixture of diastereoisomers. [d]This sample produced a mixture of diastereoisomers, but only one was isolated. [e]These compounds were unstable and decomposed during purification; the products were identified in the ^{1}H NMR spectra of the crude preparations through their characteristic olefinic protons.

chiral amino acid was present in the first ring of the target structure ($R^2 \neq H$). This was independent of the nature of the second amino acid (the R^3 influence). The spatial arrangement of the iminium salt enabled the formation of a fused ring from

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only one side without any dependence on the east ring, and we obtained the optically pure compounds **20**(2,2,2,1), **20**(2,2,2,2), **20**(2,2,5,2), **20**(2,4,1,2), and **20**(2,4,2,2). The configuration of the new chiral carbon was determined using the Karplus equation[33] with coupling constants from the ^{1}H NMR spectra or the 2D NOE experiments to verify that all structures were the *S* isomers. However, when the chiral amino acid was present only in the second cycle (e.g., **19**(2,1,2,1) with Fmoc-Gly-OH as the first amino acid), a mixture of diastereoisomers was formed as indicated by NMR (see substrates **19**(1,1,2,1), **19**(2,1,2,1), and **19**(2,1,2,2)). The ratio of diastereoisomers was 65:35 in favor of isomer **19**.

Surprisingly, the presence of the opposite stereochemistry (*R*) in the R^3 position obtained through the introduction of D-Ala or D-Val as the second amino acid strongly hindered the second cyclization, leading to the enamides **21** as the major products (**21**(2,2,3,2) and **21**(2,2,4,2)) likely because of steric effects.

N-carbamates. Resin-bound Fmoc derivatives provided only minor quantities of the fused ring compounds (substrates **19**(2,1,1,3), **19**(2,1,2,3), **19**(2,1,5,3), and **19**(2,2,5,3)) except for the Ser- and Thr-substituted compounds in the R^2 position (**19**(2,1,7,3) and **19**(2,2,7,3)). However, in this case the presence of an alcohol group allowed the closure of the second ring through the oxygen nucleophile to form the 6 + 6 tetrahydropyrazino[2,1-*b*][1,3]oxazine-4,7(6*H*,8*H*)-diones **22**(2,1,7,3) and **22**(2,2,7,3) (Figure 1).

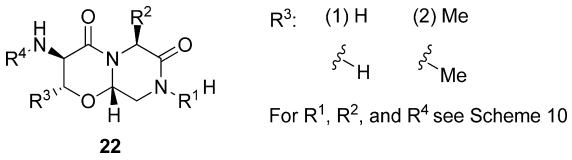

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Figure 1. Six +6-membered rings: tetrahydropyrazino[2,1-*b*][1,3]-oxazine-4,7(6*H*,8*H*)-diones.

N-aryl Compounds. The outcome of the *N*-aryl derivatives was in good agreement with the 6-membered ring formation. The *N*-aryl iminium ions formed the double-fused ring with the electron-rich aryl groups (**20**(2,1,2,7)), or lost a proton and yielded an enamide with the electron-deficient aromatic rings (**21**(2,2,2,5)).

N-acyl Compounds. Model compounds prepared with an *N*-acyl substituent provided results analogous to the previous six-membered ring formation in which only the enamides **21** were detected except for those substrates with Ser or Thr as the second amino acid (**19**(2,2,6,14) and **19**(2,2,7,14)). These exceptions produced the heterofused rings **22**(2,2,6,14) and **22**(2,2,7,14) through the participation of oxygen as an internal nucleophile. Because the fused ring **20** was not formed, the enamide **21** was incorporated as a six-membered peptide backbone constraint during the traditional Merrifield solid-phase peptide synthesis.

CONCLUSION

N-(2-Oxoethyl)-derivatized peptides formed the *N*-acyliminiums toward the peptide amino terminus, referred to here as westbound cyclization. In the absence of the westbound amide nitrogen, the *N*-acyliminium ions were formed toward the peptide carboxyl terminus (eastbound cyclization). The fused 6 + 6 and 6 + 5 bisheterocycles were formed via internal nucleophilic addition to cyclic iminium ions. The formation of the two ring systems exhibited analogous trends; however, the six-membered rings were formed more efficiently than the five-membered rings (Table 3). The observed favored six-membered ring formation followed Baldwin's rules.[34] In both cases, the nature of the R^4 group was critical to defining the final destination of the iminium salt. In both the 6 + 6 and 6 + 5 fused rings, the second cyclization was strongly promoted by the *N*-terminal sulfonamide groups. Furthermore, both the 6 + 6 and 6 + 5 bisheterocycles were produced with full stereocontrol of the newly formed asymmetric carbon when a chiral carbon was present at the R^2 position. The *N*-aryl derivatives formed the double cycle only when an electron-donating substituent was present, while the *N*-alkyl and *N*-acyl acyclic precursors did not form a second ring. The *N*-Arylsulfonyl derivatives of compounds prepared using Ser and Thr formed five-membered rings via a nitrogen nucleophile, while the *N*-Fmoc and *N*-acyl derivatives closed six-membered rings via an oxygen nucleophile, thus enabling selection between the competitive cyclizations by choosing the appropriate *N*-substituent.

Table 3. Bicycle and Enamide Formation As a Function of *N*-substituent

derivative	6 + 6 bicycle	enamide	5 + 6 bicycle	enamide
N-arylsulfonyl	>99%	<1%	15−70%	85−30%
N-carbamates	30−75%	70−25%	1−6%	99−94%
N-aryl derivatives	1−99%	99−1%	1−99%	99−1%
N-acyl compounds	<1%	>99%	<1%	>99%
N-H compounds	<1%	>99%	<1%	>99%

EXPERIMENTAL PROCEDURES

Acylation with Bromoacetic Acid (Resin 2). Resin **1a** was a commercial Rink resin (loading = 0.6 mmol/g), and the Fmoc protecting group was cleaved by a 20-min exposure to 50% piperidine in DMF. Resin **1b** was prepared from a BAL resin according to the reported procedure.[30] A fritted polypropylene reaction vessel charged with resin **1a** or **1b** (1 g) was washed 3 times with DCM. A solution of bromoacetic acid (700 mg, 5 mmol) was made in 10 mL of DCM in a fritted syringe, and DIC (386 μL, 2.5 mmol) was added. The precipitated *N,N*′-di-*i*-propylurea (DIU) was filtered after 10 min shaking, the DIEA (436 μL, 2.5 mmol) was added, and the solution was placed in the syringe with resin **1a** or **1b**. The resin slurry was shaken at ambient temperature for 4 h and then washed 5 times with DCM. The completion of the acylation to obtain resin **2** followed using the bromophenol blue test.

Reaction with Aminoacetaldehyde Dimethylacetal (Resin 3). A fritted polypropylene reaction vessel charged with resin **2** (1 g) was washed 3 times with DCM and 3 times with DMF. An aminoacetaldehyde dimethylacetal (1.09 mL, 10 mmol) and DIEA (1.74 mL, 10 mmol) solution in 10 mL of DMF was added. The resin slurry was shaken at ambient temperature for 2 h and then washed 3 times with DMF and 3 times with DCM.

Quantification of Resin Loading. A ∼10 mg resin sample was reacted with Fmoc-OSu (169 mg, 0.5 mmol) in 1 mL of DCM for 30 min at ambient temperature. The resin was washed 5 times with DCM, dried, precisely weighed, and the product was cleaved from the resin with TFA:TES:DCM (5:1:4) for 30 min. The cleavage cocktail was evaporated by a stream of nitrogen, and the cleaved compounds extracted into 1

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mL of MeOH. These samples of Fmoc derivatives of **3** were analyzed using LC/MS, and the analyses were compared with that of the standard (Fmoc-Ala-OH; concentration 1 mg/mL). The loading of the resin was determined using the external standard method.

Acylation with Fmoc-Amino Acid (Resin 4). A fritted polypropylene reaction vessel charged with resin **4a** or **4b** (250 mg) was washed 3 times with DCM and 3 times with DMF. The Fmoc protecting group was cleaved with 50% piperidine in DMF as previously described. The resin was washed 3 times with DMF and 3 times with DCM. The Fmoc-amino acid (0.4 mmol) was dissolved in 1 mL of DMF and 1 mL of DCM. Then, HOBt (61.2 mg, 0.4 mmol) and DIC (63 μL, 0.4 mmol) were added, and the solution was added to the resin. The reaction slurry was shaken at ambient temperature for 1 h (Fmoc-Ser-OH) or overnight (for other amino acids), and then the resin was washed 5 times with DCM. A sample of the resin was reacted with Fmoc-OSu, followed by product cleavage with 50% TFA and analysis using LC/MS as previously described.

Sulfonylation with 4-Methyl-benzenesulfonyl Chloride (Resins 5, 13), or 2- or 4-Nitrobenzenesulfonyl Chloride (Resins 5, 15, 16, 19). A fritted polypropylene reaction vessel charged with resin **1b**, **1c**, or **4** (250 mg) was washed 3 times with DCM and 3 times with DMF. The Fmoc group was cleaved with 20% piperidine in DMF as previously described above, and the resin was washed 3 times with DMF and 3 times with DCM. A solution of 4-methylbenzenesulfonyl chloride (190 mg; 1 mmol) or 2-nitrobenzenesulfonyl chloride (222 mg; 1 mmol) and DIEA (174 μL; 1 mmol) in 2 mL of DCM was added to the resin, and the reaction slurry was shaken at ambient temperature overnight. The resin was then washed 5 times with DCM. A sample of resin was reacted with Fmoc-OSu, the product cleaved with 50% TFA and analyzed by LCMS as previously described.

Sulfonylation with 2- or 4-Nitrobenzenesulfonyl Chloride Followed by the Fukuyama Variation of the Mitsunobu *N*-alkylation with Glycolaldehyde Dimethyl Acetal (Resins 5, 12). Resins **1b, c** (330 mg) were sulfonylated as previously described and washed 3 times with anhydrous THF. A solution of Ph_3P (260 mg, 1 mmol) and glycolaldehyde dimethyl acetal (100 μL, 1 mmol) in anhydrous THF (5 mL) was added to the swollen resin which was contained in a fritted polypropylene reaction vessel. This vessel was connected to another syringe containing DIAD (200 μL, 1 mmol) in anhydrous tetrahydrofuran (THF, 4 mL), and both syringes were cooled in a freezer for 30 min. The DIAD solution was then added to the resin suspension, and the resulting mixture was shaken at room temperature for 30 min and then at 50 °C overnight. The resin was washed 3 times with THF, once with MeOH, and 3 times with DCM. A sample of resin was treated with TFA:TES:DCM (5:1:4), and the liberated products were analyzed using LCMS as previously described.

Reaction with Benzyloxycarbonyl (Cbz) Chloride (Resin 16). A fritted polypropylene reaction vessel charged with resin **4** (250 mg) was washed 3 times with DCM and 3 times with DMF. The Fmoc group was cleaved with 20% piperidine in DMF as previously described, and the resin was washed 3 times with DMF and 3 times with DCM. A solution of carbobenzoxy chloride (60 μL, 0.42 mmol) in DCM (2 mL) was added to the swollen resin contained in a fritted polypropylene reaction vessel. This vessel was connected to another syringe containing a DIEA (75 μL, 0.42 mmol) solution in DCM (1.5 mL), and both syringes were cooled in a freezer for 30 min. The DIEA solution was then added to the resin suspension, and the resulting mixture was shaken overnight at room temperature. The resin was next washed 5 times with DCM, and the completion of the reaction was confirmed using the bromophenol blue test.

Arylation with 4-Fluoro-2-nitrobenzotrifluoride (10c, 16, 19) or with 1-Fluoro-2-nitrobenzene (16, 19). A fritted polypropylene reaction vessel charged with acylated resin **4** (250 mg) was washed 3 times with DCM and 3 times DMF. The Fmoc protecting group was cleaved with 20% piperidine in DMF as previously described, and the resin was washed 3 times with DMF and 3 times with DCM. A solution of 4-fluoro-2-nitrobenzotrifluoride (700 μL, 5 mmol) or 1-fluoro-2-nitrobenzotrifluoride (530 μL, 5 mmol) and DIEA (870 μL, 5 mmol) in 5 mL of DMSO was added to the resin, and the reaction slurry was shaken overnight at ambient temperature (for 4-fluoro-2-nitrobenzotrifluoride) or at 80 °C (for 1-fluoro-2-nitrobenzotrifluoride). The resin was next washed 3 times with DMSO and 3 times with DCM. The completion of the reaction was confirmed using the bromophenol blue test.

Reduction with Tin(II) Chloride Dihydrate (Resins 16, 19). Tin(II) chloride dihydrate (912 mg, 4 mmol) was dissolved in 3 mL of DMF saturated with N_2, and DIEA (700 μL, 4 mmol) was added. The solution was added to resin **10** (250 mg), and the reaction slurry was shaken overnight at ambient temperature for 2 h, or 2 times overnight (**16**, **19**). Finally, the resin was washed 3 times with DMF and 3 times with DCM.

Alkylation with 2-Bromo-1-*p*-tolyl-ethanone (Resin 16). A fritted polypropylene reaction vessel charged with acylated resin **4** (250 mg) was washed 3 times with DCM and 3 times with DMF. The Fmoc protecting group was cleaved with 20% piperidine in DMF as previously described, and the resin was washed 3 times with DMF and 3 times with DCM. The resultant resin was sulfonylated as previously described above and then washed 3 times with DMF. A solution of 2-bromo-1-*p*-tolyl-ethanone (320 mg, 1.5 mmol) and DIEA (525 μL, 3 mmol) in 3 mL of DMF was added. The resin slurry was shaken overnight at ambient temperature, then washed 3 times with DMF and 3 times with DCM. The completion of the reaction was confirmed using the bromophenol blue test. Finally, the nosyl group was removed by treating the resin with a solution of mercaptoethanol (105 μL, 1.5 mmol) and DBU (75 μL, 0.5 mmol) in DMF (2.5 mL) at ambient temperature for 10 min. The resin was then washed 3 times with DMF and 3 times with DCM.

Alkylation with (*N*)-2-hydroxyethylphthalimide (Resin 16). Acylated resin **4** (250 mg) was Fmoc deprotected and sulfonylated as previously described. The resin was then washed 3 times with anhydrous THF. A solution of Ph_3P (230 mg, 0.9 mmol) and (*N*)-2-hydroxyethylphthalimide (169 mg, 0.9 mmol) in anhydrous THF (5 mL) was next added to the swollen resin contained in a fritted polypropylene reaction vessel. This vessel was connected to another syringe containing DIAD (180 μL, 0.9 mmol) in anhydrous THF (3 mL), and both syringes were cooled in a freezer for 30 min. The DIAD solution was then added to the resin suspension, and the resulting mixture was shaken overnight at room temperature. Finally, the resin was washed 3 times with THF, once with MeOH, and 3 times with DCM.

Acylation with *p*-Methoxybenzoic Acid (Resin 19). A fritted polypropylene reaction vessel charged with acylated

resin **4** (250 mg) was washed 3 times with DCM and 3 times with DMF. The Fmoc protecting group was cleaved with 20% piperidine in DMF as described above, and the resin was washed 3 times with DMF and 3 times with DCM. A solution of *p*-methoxybenzoic acid (93 mg, 0.6 mmol) was made in 3 mL of DCM in a fritted syringe, and DIC (94 μL, 0.6 mmol) was added. The precipitated *N,N′*-di-*i*-propylurea (DIU) was filtered after 10 min of shaking, and the solution was added to the syringe with swollen resin. The resin slurry was shaken overnight at ambient temperature and then washed 5 times with DCM. The completion of the reaction was confirmed using the bromophenol blue test.

Cleavage from Resin (6, 8, 10, 12, 14, 16, 19). The resin-bound compounds (250 mg) in a fritted polypropylene reaction vessel were treated with 5 mL of 50% TFA in DCM for 1 h, except the Ser- and Thr-containing resins, which had reaction times of 2 h. The TFA solution was then collected, and the resin was washed 3 times with 50% TFA. The washes were collected, and the combined extracts were evaporated by a stream of nitrogen. The crude product was purified using HPLC, and the collected fractions were concentrated under a stream of nitrogen or under reduced pressure, frozen, and lyophilized. The yield was calculated with respect to the resin loading after incorporation of the first building block (typical loading = 0.35−0.50 mmol/g).

■ ASSOCIATED CONTENT

Supporting Information

Analytical data of individual compounds and copies of NMR spectra associated with this article. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ REFERENCES

(1) Grauer, A.; König, B. Peptidomimetics - A Versatile Route to Biologically Active Compounds. *Eur. J. Org. Chem.* **2009**, 5099−5111.

(2) Vagner, J.; Qu, H.; Hruby, V. J. Peptidomimetics, a synthetic tool of drug discovery. *Curr. Opin. Chem. Biol.* **2008**, *12*, 292−296.

(3) Eguchi, E.; Kahn, M. Design, synthesis, and application of peptide secondary structure mimetics. *Mini-Rev. Med. Chem.* **2002**, *2*, 447−462.

(4) Burgess, K. Solid-phase syntheses of beta-turn analogues to mimic or disrupt protein-protein interactions. *Acc. Chem. Res.* **2001**, *34*, 826−835.

(5) Yazici, A.; Pyne, S. G. Intermolecular Addition Reactions of N-Acyliminium Ions (Part 1). *Synthesis* **2009**, 339−368.

(6) Yazici, A.; Pyne, S. G. Intermolecular Addition Reactions of N-Acyliminium Ions (Part 2). *Synthesis* **2009**, 513−541.

(7) Maryanoff, B. E.; Zhang, H. C.; Cohen, J. H.; Turchi, I. J.; Maryanoff, C. A. Cyclizations of N-Acyliminium Ions. *Chem. Rev.* **2004**, *104*, 1431−1628.

(8) Lewis, J. G.; Bartlett, P. A. Amino acid-derived heterocycles as combinatorial library targets: Bicyclic aminal lactones. *J. Comb. Chem.* **2003**, *5*, 278−284.

(9) Vojkovsky, T.; Weichsel, A.; Patek, M. Solid-Phase Synthesis of Heterocycles Containing an 1-Acyl-3-oxopiperazine Skeleton. *J. Org. Chem.* **1998**, *63*, 3162−3163.

(10) Eguchi, M.; Lee, M. S.; Nakanishi, H.; Stasiak, M.; Lovell, S.; Kahn, M. Solid-Phase Synthesis and Structural Analysis of Bicyclic beta-Turn Mimetics Incorporating Functionality at the i to i + 3 Position. *J. Am. Chem. Soc.* **1999**, *121*, 12204−12205.

(11) Eguchi, M.; Lee, M. S.; Stasiak, M.; Kahn, M. Solid-phase synthesis and solution structure of bicyclic ß-turn peptidomimetics: diversity at the I position. *Tetrahedron Lett.* **2001**, *42*, 1237−1239.

(12) Kohn, W. D.; Zhang, L. Solid-phase synthesis of peptide-heterocycle hybrids containing a tripeptide-derived 6,6-fused bicyclic subunit. *Tetrahedron Lett.* **2001**, *42*, 4453−4457.

(13) Min, B. J.; Gu, X.; Yamamoto, T.; Petrov, R. R.; Qu, H.; Lee, Y. S.; Hruby, V. J. Synthesis of a novel benzyl-octahydropyrazino[1,2-a]pyrimidin-6-one derivative as a convenient internal bicyclic peptidomimetic. *Tetrahedron Lett.* **2008**, *49*, 2316−2319.

(14) Lee, S. C.; Park, S. B. Practical Solid-Phase Parallel Synthesis of +ö5−2-Oxopiperazines via N-Acyliminium Ion Cyclization. *J. Comb. Chem.* **2007**, *9*, 828−835.

(15) Vankova, B.; Brulikova, L.; Wu, B.; Krchnak, V. Synthesis of Piperazinones, Piperazines, Tetrahydropyrazines and Dihydropyrazinones from Polymer-Supported Acyclic Intermediates via N-Alkyl and N-Acyl Iminiums. *Eur. J. Org. Chem.* **2012**, 5075−5084.

(16) Kim, J. H.; Lee, Y. S.; Kim, C. S. Synthesis of Praziquantel via N-Acylimium Ion Cyclization of Amido Acid. *Heterocycles* **1998**, *48*, 2279−2285.

(17) Royer, J.; Bonin, M.; Micouin, L. Chiral heterocycles by Iminium Ion Cyclization. *Chem. Rev.* **2004**, *104*, 2311−2352.

(18) Cankarova, N.; Krchnak, V. Polymer-Supported Stereoselective Synthesis of Benzimidazolinopiperazinones. *J. Org. Chem.* **2012**, *77*, 5687−5695.

(19) Baldwin, J. E.; Hulme, C.; Edwards, J. A.; Schofield, C. J. Synthesis of a bicyclic gama-laclam dipeptide analogue. *Tetrahedron Lett.* **1993**, *34*, 1665−1668.

(20) Sun, H.; Moeller, K. D. Silyl-Substituted Amino Acids:New Routes to the Construction of Selectively Functionalized Peptidomimetics. *Org. Lett.* **2002**, *4*, 1547−1550.

(21) Sun, H.; Moeller, K. D. Building Functionalized Peptidomimetics:New Electroauxiliaries and the Use of a Chemical Oxidant for Introducing N-Acyliminium Ions into Peptides. *Org. Lett.* **2003**, *5*, 3189−3192.

(22) Sun, H.; Martin, C.; Kesselring, D.; Keller, R.; Moeller, K. D. Building Functionalized Peptidomimetics:Use of Electroauxiliaries for Introducing N-Acyliminium Ions into Peptides. *J. Am. Chem. Soc.* **2006**, *128*, 13761−13771.

(23) Nielsen, T. E.; Le Quement, S.; Meldal, M. Solid-Phase Synthesis of Bicyclic Dipeptide Mimetics by Intramolecular Cyclization of Alcohols, Thiols, Amines, and Amides with N-Acyliminium Intermediates. *Org. Lett.* **2005**, *7*, 3601−3604.

(24) Nielsen, T. E.; Meldal, M. Solid-phase intramolecular N-acyliminium Pictet-Spengler reactions as crossroads to scaffold diversity. *J. Org. Chem.* **2004**, *69*, 3765−3773.

(25) Remuson, R.; Gelas-Mialhe, Y. A convenient access to the piperidine ring by cyclization of allylsilyl substituted N-acyliminium and iminium ions: Application to the synthesis of piperidine alkaloids. *Mini-Rev. Org. Chem.* **2008**, *5*, 193−208.

(26) Speckamp, W. N.; Moolenaar, M. J. New developments in the chemistry of N-acyliminium ions and related intermediates. *Tetrahedron* **2000**, *56*, 3817−3856.

(27) Overman, L. E. Charge as a key component in reaction design. The invention of cationic cyclization reactions of importance in synthesis. *Acc. Chem. Res.* **1992**, *25*, 352−359.

(28) Rink, H. Solid-phase synthesis of protected peptide fragments using a trialkoxydiphenyl-methylester resin. *Tetrahedron Lett.* **1987**, *28*, 3787−3790.
(29) Jensen, K. J.; Alsina, J.; Songster, M. F.; Vagner, J.; Albericio, F.; Barany, G. Backbone Amide Linker (BAL) Strategy for Solid-Phase Synthesis of C-terminal Modified and Cyclic Petides. *J. Am. Chem. Soc.* **1998**, *120*, 5441−5452.
(30) Krchnak, V.; Smith, J.; Vagner, J. Solid-phase traceless synthesis of selected nitrogen-containing heterocyclic compounds. The Encore technique for directed sorting of modular solid support. *Collect. Czech. Chem. Commun.* **2001**, *66*, 1078−1106.
(31) Fukuyama, T.; Jow, C. K.; Cheung, M. 2- and 4-Nitrobenzenesulfonamides: Exceptionally versatile means for preparation of secondary amines and protection of amines. *Tetrahedron Lett.* **1995**, *36*, 6377−6374.
(32) Miller, S. C.; Scanlan, T. S. Site-Selective N-Methylation of Peptides on Solid Support. *J. Am. Chem. Soc.* **1997**, *119*, 2301−2302.
(33) Karplus, M. Vicinal proton coupling in nuclear magnetic resonance. *J. Am. Chem. Soc.* **1963**, *85*, 2870−2871.
(34) Baldwin, J. E. Rules for ring closure. *J. Chem. Soc., Chem. Commun.* **1976**, 734−736.