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Fused-Ring Systems

Synthesis of Nature-Inspired Medium-Sized Fused Heterocycles from Amino Acids

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Abstract: Herein, we describe the synthesis of molecular scaffolds consisting of medium-sized fused heterocycles using amino acids, which are some of the most useful building blocks used by nature as well as chemists to create structural diversity. The acyclic precursors were assembled by using traditional Merrifield solid-phase peptide synthesis, and cyclization was carried out through acid-mediated tandem endocyclic *N*-acyliminium ion formation, followed by nucleophilic addition with internal nucleophiles. The synthesis of molecular scaffolds consisting of seven-, eight-, and nine-membered rings proceeded with full stereocontrol of the newly generated stereogenic center in most cases.

Introduction

Nature is the ultimate inspiration for pharmacologically relevant and structurally diverse compounds that exhibit all kinds of biological activities.[1] Therefore, not surprisingly, the structure of many current drugs originated from natural products[2,3] and nature-inspired structures represent the ultimate source of structural diversity for drug discovery. Searching for novel biologically active structures that mimic natural products has become an integral part of drug discovery efforts.[4,5] However, recent structural analysis of drugs and compound libraries used in high-throughput screening has revealed significant differences in the chemical space covered by individual groups of compounds.[6] Existing compound collections typically displayed a low frequency of sp^3 hybridized carbon atoms and chiral centers when compared with drugs and natural products.[7] It has been suggested[7–9] that the incorporation of a greater proportion of chiral compounds with higher degrees of saturation may improve clinical outcomes. Lovering[7] introduced a fractional sp^3 character (Fsp^3) as a ratio of the number of sp^3-hybridized carbon atoms and total carbon count. Marketed drugs tend to have a higher Fsp^3 when compared with discovery compounds.

Natural products comprising complex fused and bridged carbocycles represent particularly intriguing molecular scaffolds with 3D architecture. Structures encompass a variety of fused eight-membered ring-containing compounds, including [5+8] (dactylol, precapnelladiene, asteriscane), [6+8] (neolemnane), [6+8+6] (taxane), [5+8+5] (basmane, fusicoccane, ophiobolane), and [5+8+6] (variecolin) rings (Figure 1 a).[10] The [6+8] fused-ring system is particularly attractive because it is contained in the taxane diterpene skeleton. Taxanes, such as Paclitaxel (Taxol®), interfere with microtubules (i.e., cellular structures that help the movement of chromosomes during mitosis) and block cell growth by stopping mitosis (treatment of cancer)[11,12] (Figure 1 b). The decahydro-1,7-methanonaphthalene ring [6+6+5] system is contained in aconitine, the "queen of poisons", which is highly cardiotoxic and neurotoxic (Figure 1 a).[13]

Examples of natural products with fused seven-membered rings include [5+7+5] (alkaloid cephalotaxine,[14] thapsigargin[15]) and [5+7+6] (grayanotoxin[16]) skeletons. These are only a few illustrative structures from a large pool of natural products comprising five-, six-, seven-, and eight-membered ring systems (Figure 1 b).

Chemical synthesis of this kind of molecular scaffold has been accomplished numerous times, however, it represents a very challenging task.[17] Extensive SAR studies are typically restricted to peripheral substituents.[18] Here, we outline an alternative approach based on modular solid-phase synthesis of fused and bridged heterocycles mimicking complex molecular carbocyclic scaffolds. Modular assembly enables straightforward diversification in almost any position at the molecular scaffold and, importantly changing ring sizes.

One of the most useful synthetic strategies for the formation of a fused-ring system in one reaction is through acid-mediated cyclic iminium ion formation and subsequent second ring closure by nucleophilic addition.[19–21] Although a century of developments in the use of *N*-acyl-substituted carbocations

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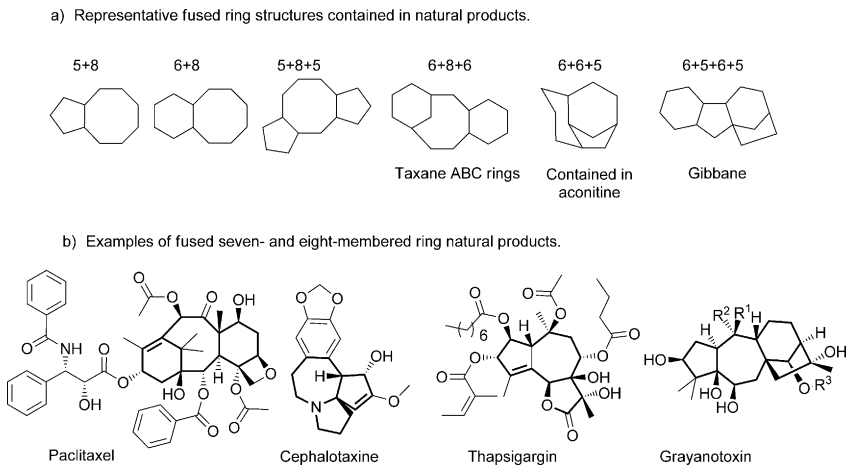


Figure 1. Fused structures and examples of natural products containing fused medium-sized rings.

has yielded a plethora of synthetic routes for accessing diverse nitrogenous heterocycles, relatively few examples of stereoselective cyclization for the formation of medium-sized rings have been reported, and the formation of these medium-sized rings has not been studied systematically.[22–24] One example is the synthesis of bicyclic lactam developed by Speckamp's group.[25] In addition, bicyclic lactams have been prepared from 2-propynyl- and allylsilanes,[26,27] but the formation of large rings typically occurs in conjunction with the formation of smaller cycles.[21] Occasionally, *N*-acyliminium cyclization has produced a new eight-membered ring with high yield (92%) under mild conditions (23 °C for 1 h),[28] probably due to high reactivity of the C-nucleophile (pyrrole nucleus) and additional constraints in the acyclic substrate.

One example of the synthetic opportunities in this area is the carbocyclic [8+5] decahydro-1*H*-cyclopenta[8]annulene fused-ring system, which is contained in a diverse range of natural products (Figure 1). However, heterocycles comprising this molecular scaffold have not been studied as potential drug-like molecules, and only a very limited number of reports have addressed this class of nitrogenous heterocycles (Figure 2).

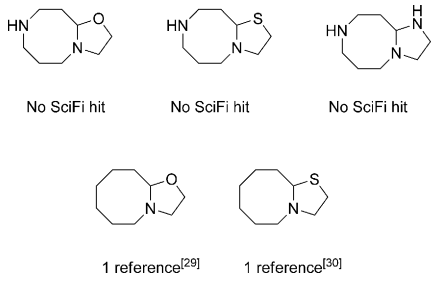


Figure 2. SciFinder search of heteroanalogues of carbocyclic [8+5] decahydro-1*H*-cyclopenta[8]annulene fused ring system.[29,30]

Our ongoing research is focused on the development of synthetic routes for pharmacologically relevant molecules possessing features that have been largely missing in traditional compounds decks (asymmetric carbons, sp^3 carbons). We recently reported the regio- and stereoselective solid-phase synthesis of medium-sized bridged heterocycles by using tandem *N*-acyliminium ion cyclization–nucleophilic addition.[31] In this communication, we report an analogous approach for the synthesis of molecular scaffolds composed of medium-sized fused rings. Synthesis of biomimetic medium rings has recently attracted attention, although using different chemical routes.[32,33]

The general concept of modular solid-phase synthesis of an acyclic precursor followed by acid-mediated cyclic iminium ion formation and subsequent second ring closure by nucleophilic addition is illustrated in Figure 3. This strategy allows the independent assembly of acyclic precursors for both the first and the second ring. A straightforward solid-phase synthesis of acyclic precursors was carried out by using protocols reported in our recent publications.[34;35]

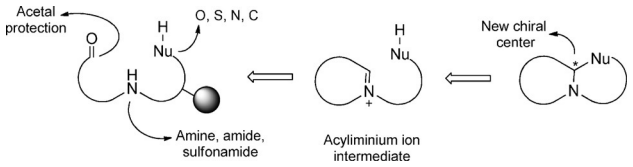
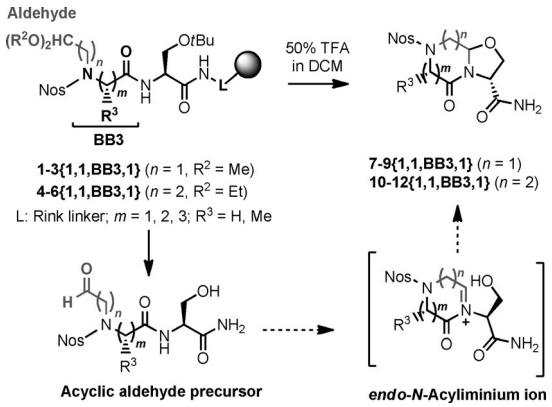


Figure 3. Tandem iminium ion cyclization–nucleophilic addition strategy on solid support.

Results and Discussion

Modular synthesis of acyclic precursors

We developed a method for the stepwise assembly of acyclic intermediates from amino acids on solid phase followed by one-step conversion of the resin-bound acyclic precursors to create a diverse range of molecular scaffolds. We previously reported the polymer-supported synthesis of [6+5] fused tetrahydro-2*H*-oxazolo[3,2-*a*]pyrazin-5(3*H*)-ones **7{1,1,1,1}** through eastbound *N*-acyliminium ion cyclization–nucleophilic addition from linear resin-bound precursors **1{1,1,1,1}** (Scheme 1).[34] To form the six-membered iminium ring, acyclic resin-bound intermediates **1{1,1,1,1}** contained α-amino acids ($m=1$) and a protected aldehyde group that was attached through a methylene group ($n=1$; Figure 5, Scheme 1). Acidic treatment of the polymer-supported acyclic compound **1{1,1,1,1}** triggered several reactions in one pot: release from the resin, removal of the protecting groups (masked aldehyde and protected internal nucleophile), and formation of the six-membered endocyclic *N*-acyliminiun ion followed by internal nucleophilic attack, providing the target molecular scaffolds **7{1,1,1,1}**.[34]



Scheme 1. Formation of medium-sized fused heterocycles via medium-sized *N*-acyliminium-ion intermediates using the oxygen of L-Ser as an internal nucleophile.

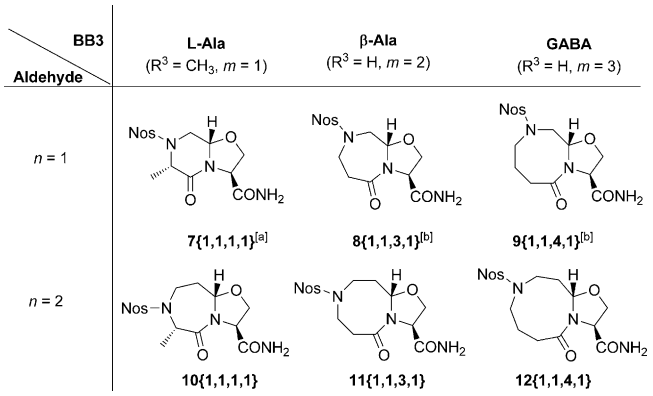


Figure 4. Medium-sized six-, seven-, eight-, and nine-membered fused rings from acyclic precursors **1**–**6{1,1,BB3,1}**. [a] Compound **7{1,1,1,1}** has been previously reported;[34] [b] Fused bicycles **8{1,1,3,1}** and **9{1,1,4,1}** were not formed.

To evaluate the potential to form medium-sized *endo*-*N*-acyliminium-ion intermediates (from seven- to nine-membered rings), a set of resin-bound precursors **2**–**6{1,1,BB3,1}** was synthesized. These model precursors contained L-Ser to provide the internal O-nucleophile that can react with the *endo*-*N*-acyliminium-ion intermediates, which can be formed by different combinations of amino acids (**BB3**: L-Ala, β-Ala, and GABA; $m=1$, 2, 3, respectively) and acetal-protected aldehydes with two- or three-carbon spacers ($n=1$, 2; Scheme 1). The solid-phase synthesis of these acyclic precursors was performed by using standard solid-phase chemistry protocols and commercially available building blocks according to our previously reported procedures.[34,36–39]

Briefly, Rink amide[40] was acylated with Fmoc-Ser(*t*Bu)-OH, followed by Fmoc removal and acylation with the second amino acid (Fmoc-L-Ala-OH, $m=1$, Fmoc-β-Ala-OH, $m=2$, and Fmoc-GABA-OH, $m=3$). The Fmoc protecting group was removed, and subsequent activation with the 4-nitrobenzenesulfonyl (4-Nos) group led to resin-bound sulfonamides, which were subjected to Mitsunobu reaction using glycolaldehyde dimethyl acetal ($n=1$, for linear precursors **2**–**3**) or 3,3-diethoxy-1-propanol ($n=2$, for linear precursors **4**–**6**) as the masked aldehyde to obtain the desired linear precursors **2**–**6{1,1,BB3,1}**.

First, acyclic precursors **2{1,1,3,1}** and **3{1,1,4,1}**, containing protected aldehyde on a two-carbon spacer ($n=1$) and β-Ala ($m=2$) and GABA ($m=3$), were treated with trifluoroacetic acid (TFA) for 2 h in an attempt to synthesize compounds **8{1,1,3,1}** and **9{1,1,4,1}** (Figure 4). However, the target fused heterocycles were not formed; both linear supported models yielded a complex mixture containing acyclic deprotected aldehydes as the major component, which was identified from the ^{1}H NMR spectra of the crude reaction mixture. We then evaluated model compounds (**BB3**: (**1**) Fmoc-L-Ala ($m=1$); (**3**) Fmoc-β-Ala ($m=2$); (**4**) GABA ($m=3$)) that were prepared by using 3,3-diethoxy-1-propanol ($n=2$; Figure 4). TFA treatment of these resin-bound precursors derived from a three-carbon spacer aldehyde led to the formation of the desired medium-sized fused heterocycles **10{1,1,1,1}**, **11{1,1,3,1}**, and **12{1,1,4,1}**. These results provided experimental evidence that

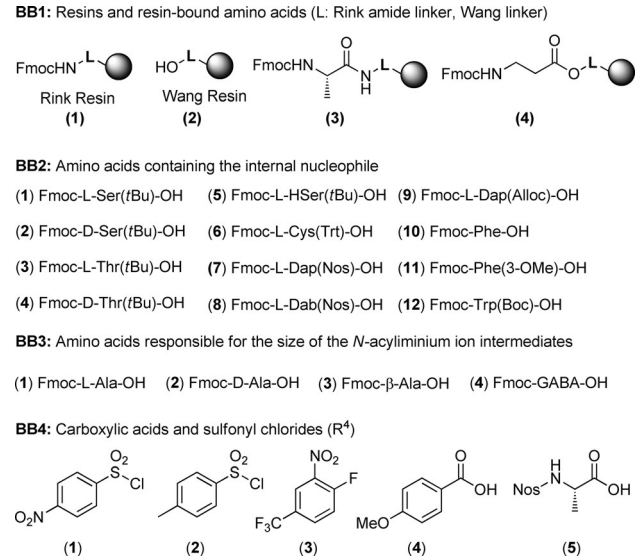


Figure 5. Building blocks used for the synthesis of resin-bound precursors of fused medium-sized bicycles **10**, **11**, and **12**.

the formation of the medium-sized *endo*-*N*-acyliminium-ion intermediates was strongly dependent on the length of the aldehyde chain. The aldehyde that contained a three-carbon spacer facilitated the closure of the iminium ion ring, and the specific ring size was controlled by the corresponding second amino acid (**BB3**; Figure 5).

Encouraged by these preliminary results, we focused on the scope and limitations of the reaction with respect to acyclic precursors containing three-carbon spacer aldehydes ($n=2$) to form medium-sized fused bicycles through *N*-acyliminium ion cyclization–nucleophilic addition. We designed and synthesized acyclic resin-bound precursors **4**, **5**, and **6**, containing different internal nucleophiles (heteroatoms and nucleophilic carbon) in the first amino acid (**BB2**) involved in the nucleophilic addition to the cyclic *N*-acyliminium-ion intermediates. The ring size was determined by the second amino acid (**BB3**). In addition, we evaluated the effect of substituents not directly involved in

the tandem reaction, such as the building blocks attached to the resin (**BB1**) and to the N-terminal amino group of the second amino acid (R^4, **BB4**). The individual building blocks used in the synthesis are shown in Figure 5.

C–Heteroatom bond formation

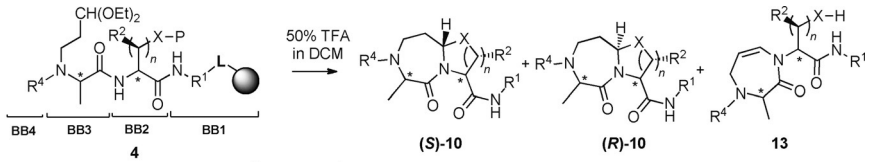
Seven-membered fused bicycles

To study the formation of seven-membered fused rings, resin-bound acyclic precursors **4** were synthesized by using α-amino acids (**BB3** = L-Ala (**1**) and D-Ala (**2**)). The treatment of acyclic precursors **4** with 50% TFA in CH_2Cl_2 triggered the formation of seven-membered *endo*-*N*-acyliminium ions, and subsequent addition of internal N, O, and S nucleophiles to form the [7+5]- and [7+6]-fused bicycles **10**. Two diastereoisomers were formed, depending on the direction of nucleophilic attack (i.e., (*S*)-**10** and (*R*)-**10**). In addition to the target fused product, the formation of enamide **13** was also observed in a few instances (Scheme 2 and Table 1).

NMR spectroscopic analysis of the stereoselectivity indicated that the configuration of the new stereogenic center was primarily dependent on the stereochemistry of the first amino acid (**BB2**: Ser, HSer, Thr, Cys, 2,3-diaminopropionic acid (Dap) and 2,4-diaminobutyric acid (Dab)). Whereas L-amino acids directed the new stereocenter to the (*S*)-configuration, D-amino acids promoted the opposite (*R*)-configuration (Table 1, entries 1 and 4). In addition, for L-Thr ($R^2 = CH_3$), the fused bicycles were formed with full stereocontrol and in good yields (entry 5), indicating a synergic effect of the nature of the R^2 group in the stereochemical outcome of the reaction. As expected from our previous studies,[34] the stereocontrol was independent of the substitution at the R^1 position (**BB1**: H and L-Ala, entries 1 and 3, respectively). The configuration of the second amino acid involved in the iminium-ion intermediate did not exhibit a significant effect because of the marginal influence on the stabilization of the conformation of the *N*-acyliminium-ion intermediate (**BB3**: L-Ala or D-Ala, entries 1 and 6, respectively). Notably, the stereochemistry of the new chiral center was not affected by the increase in the size of the second ring (from a five-membered ring (entry 1, $n = 1$) to a six-membered ring (entry 7, $n = 2$)). However, the regioselectivity of the tandem reactions decreased, allowing for enamide formation.

When compared with O-nucleophiles, N-nucleophiles exhibited a decreased tendency for the second cyclization, based on the presence of as much as 24% of enamide **13** (Table 1, entries 9 and 10). The isolation of these specific bicycles was not successful because of the formation of a complex mixture of isomers ((*S*)-**10**, (*R*)-**10**, and **13**) in addition to the dipeptide produced by acrolein elimination.[31,41] In addition, by changing the N-substituent of the internal nucleophile from sulfonamide (Nos) to carbamate (Alloc), only enamide **13{1,9,1,1}** was formed due to decreased nucleophilicity (entry 11).

The assignment of the absolute configuration of the new stereogenic centers of the fused-ring systems (*S*)-**10{1,1,1,1}** and (*R*)-**10{1,2,1,1}** was based on NOE effects observed in their ^{1}H NOESY 1D spectra (Figure 6). The NOE correlation between the CH_3 signal of the L- and D-Ala (**BB3**) with 9a-H was decisive. The C3 chiral carbon was introduced by the L- and D-amino acids containing the nucleophile (**BB2**: L- and D-Ser), which meant that its configuration was known. The observed



Scheme 2. Synthesis of fused seven-membered rings **10** from solid-supported linear precursors **4**.

Table 1. Effect of structure on product distribution for seven-membered fused heterocycles.

	Compound	R^1	BB2	BB3	R^4	(*R*)-**10**/(*S*)-**10**/**13**[a]	Purity [%][b]	Yield [%][c]
1	(*S*)-**10{1,1,1,1}**	H	Ser[d]	Ala	Nos	5:95:0	82	58
2	(*S*)-**10{1,1,1,2}**	H	Ser[d]	Ala	Tos	8:92:0	65	23
3	(*S*)-**10{3,1,1,1}**	Ala	Ser[d]	Ala	Nos	6:94:0	41	42
4	(*R*)-**10{1,2,1,1}**	H	D-Ser[d]	Ala	Nos	84:16:0	65	47
5	(*S*)-**10{1,3,1,1}**	H	Thr[d]	Ala	Nos	1:99:0	76	45
6	(*S*)-**10{1,1,2,1}**	H	Ser[d]	D-Ala	Nos	12:88:0	74	20
7	(*S*)-**10{1,5,1,1}**	H	HSer[d]	Ala	Nos	13:81:6	80	20
8	(*S*)-**10{1,6,1,1}**	H	Cys[e]	Ala	Nos	9:91:0	88	42
9	(*S*)-**10{1,7,1,1}**	H	Dap(Nos)	Ala	Nos	4:82:14	57	17[f]
10	(*R*)-**10{1,8,1,1}**	H	Dab(Nos)	Ala	Nos	3:73:24	48	45[f]
11	**13{1,9,1,1}**	H	Dap(Alloc)	Ala	Nos	0:0:100	54	25

[a] Ratio calculated by integration of the diagnostic signals in the ^{1}H NMR spectra; [b] Analysis of crude products by LC, $\lambda = 210–500$ nm; [c] Isolated yield after HPLC purification calculated from the ^{1}H NMR spectra; [d] Protecting group: *t*Bu; [e] Protecting group: Trt; [f] Not isolated, yield calculated from ^{1}H NMR spectra of the mixture of purified products including diastereoisomers **10**, enamide **13**, and the corresponding dipeptide

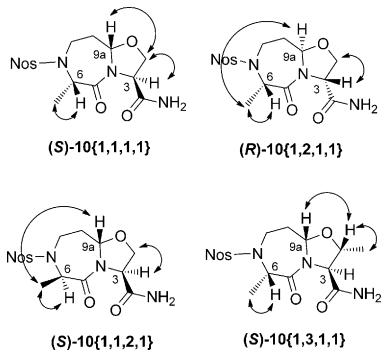
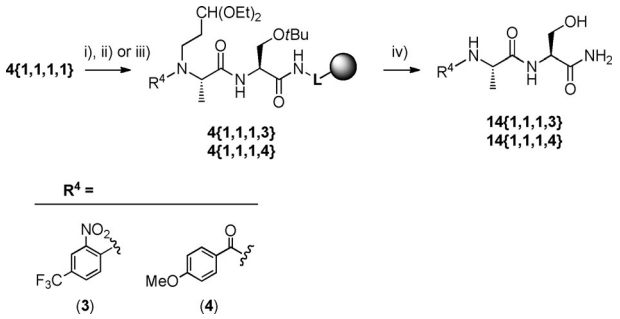


Figure 6. Configuration assignment of hexahydrooxazolo[3,2-*d*][1,4]diazepin-5(6*H*)-ones [**7+5**].

NOE correlations indicated the same relative disposition for 3-H and 9a-H in both pairs of diastereoisomers.

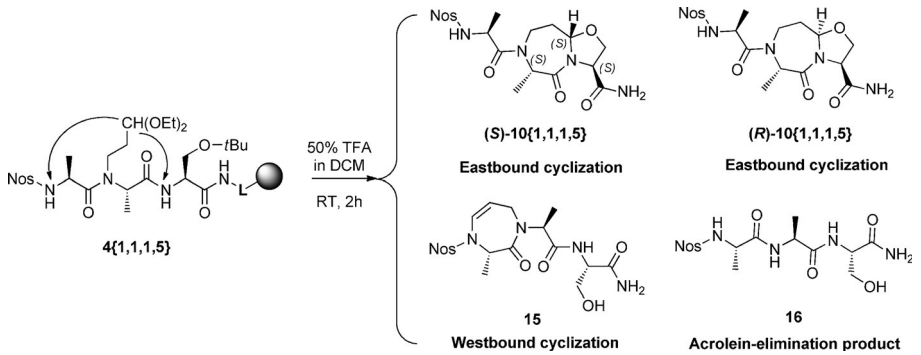
Comparison of the ^{1}H NMR data for each pair of epimers of the seven-membered fused bicycles (**10**) indicated that 9a-H appeared at a higher chemical shift (0.1–0.32 ppm) in the (*R*)-epimer compared with that for the (*S*)-epimer when L-Ala was involved in the larger cycle (**BB3**: **1**). On the other hand, C9a appeared at a higher field (0.5–1.9 ppm) in the (*R*)-epimers than in the (*S*)-epimers in the ^{13}C NMR spectra. For the model with D-Ala as the second amino acid, this trend changed the signals of the diagnostic proton and carbon at position 9a, which appeared at a higher chemical shift in the (*S*)-epimer compared with that for the (*R*)-epimer. This result was explained by considering the enantiomeric compounds that were produced (compare (*S*)-**10{1,1,2,1}** and (*R*)-**10{1,2,1,1}**). These differences between the epimers were used to tentatively assign the configuration of the remaining compounds in the series. The NMR spectral data of the diagnostic proton and carbon signals are tabulated in the Supporting Information.

In an attempt to increase the diversity of the products, the 4-Nos group in the resin-bound intermediates were cleaved (2-mercaptoethanol and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in *N*,*N*-dimethylformamide (DMF) for 5 min) and the *N*-aryl and *N*-acyl derivatives were prepared by reactions with 1-fluoro-2-nitro-4-(trifluoromethyl)benzene and 4-methoxybenzoic acid, respectively (**4{1,1,1,3}** and **4{1,1,1,4}**). However, instead of target compound **10**, N-arylated and N-acylated dipeptides **14** were formed; their structures were confirmed by LC/MS spectrometry and by NMR spectroscopy (Scheme 3). As described in our previous paper, the presence of a base in the reaction induced the elimination of acrolein from the aldehyde, providing N-substituted dipeptide **14**.[31]



Scheme 3. Modifications at the R^4 position with aromatic amine and amide functionalities. Reagents and conditions: i) mercaptoethanol, DBU, DMF, RT, 5 min; ii) 1-fluoro-2-nitro-4-(trifluoromethyl)benzene (for $R^4=3$), DIEA, DMSO, RT, overnight; iii) 4-methoxybenzoic acid (for $R^4=4$), HOBt, DIC, DMF/CH_2Cl_2, RT, overnight; iv) 50% TFA in CH_2Cl_2, RT, 2 h.

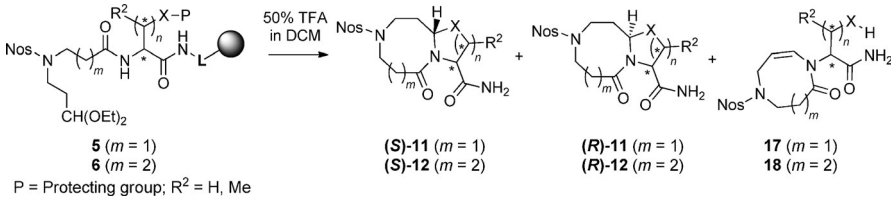
Finally, to evaluate the effect of incorporation of the seven-membered fused bicycles as peptide backbone constraints, two simple model acyclic precursors were prepared in which the fused cycle mimicked two amino acids of the peptide backbone. The first model (i.e., tripeptide(Nos-Fused bicycle-Ala-NH_2) **10{3,1,1,1}**) was obtained from acyclic intermediate **4{3,1,1,1}** in high crude purity (Table 1, entry 3). However, attempts to extend the peptide chain towards the amino terminus were not successful (Scheme 4). This second model compound was prepared from 4-Nos deprotected resin **4{1,1,1,1}** by acylation with Fmoc-Ala-OH followed by Fmoc removal and subsequent reaction with 4-Nos-Cl to afford **4{1,1,1,5}**. This linear substrate can form the cyclic iminium ion in two directions, either in the eastbound direction (i.e., towards the carboxy terminal amino acid) to produce the *N*-acyliminium ion, or in the westbound direction to produce the *N*-sulfonyliminium ion, both of which are seven-membered rings. After treatment with 50% TFA in CH_2Cl_2, a complex mixture was detected based on LC/MS analysis and this was found to contain two possible east-cyclized epimers **10{1,1,1,5}** along with the corresponding west-enamide **15** and nosylated tripeptide **16**. This complex crude mixture was not purified or characterized further. To overcome the low regioselectivity of the cyclization, we are evaluating the on-resin cyclization of Nos-intermediate **4{1,1,1,1}** followed by Nos cleavage and extension of the peptide chain.



Scheme 4. Attempts to incorporate target seven-membered ring-fused bicycle **10** into the peptide backbone.

Eight- and nine-membered fused rings

Resin-bound compounds **5** and **6**, with Fmoc-β-Ala-OH or Fmoc-GABA-OH instead of α-amino acids as **BB3**, were the precursors of the eight- and nine-membered *endo*-*N*-acyliminium-ion intermediates, respectively (Scheme 5). As in the previous study, treatment of the resin-bound acyclic precursors **5** and **6** with TFA promoted the formation of the *N*-acyliminium ion, which can undergo either nucleophilic addition to form the target fused rings **11** and **12**, respectively, or elimination to produce enamides **17** and **18**, respectively. The reaction exhibited the same regio- and stereocontrol as that observed for seven-membered fused bicycles **10**. However, as expected, the purity and yield of the target compounds were lower than



Scheme 5. Synthesis of fused eight- and nine-membered rings **11** and **12** from solid-supported linear precursors **5** and **6**.

Table 2. Effect of structure on product distribution for eight- and nine-membered fused heterocycles **11** and **12**, respectively.

	Compound	BB2	BB3	(*R*)-**11**/(*S*)-**11**/**17**[a]	Purity [%][b]	Yield [%][c]
1	(*R*)-**11{1,1,3,1}**	Ser[d]	β-Ala	5:95:0	65	4
	(*S*)-**11{1,1,3,1}**					26
2	(*R*)-**11{1,2,3,1}**	D-Ser[d]	β-Ala	99:1:0	67	26
3	(*S*)-**11{1,3,3,1}**	Thr[d]	β-Ala	1:99:0	72	30
4	(*R*)-**11{1,4,3,1}**	D-Thr[d]	β-Ala	99:1:0	81	29
5	(*R*)-**11{1,6,3,1}**	Cys[e]	β-Ala	37:63:0	73	41
6	(*S*)-**11{1,1,5,1}**	Ser	acpc[f]	1:99:0	78	33
	Compound	BB2	BB3	(*R*)-**12**/(*S*)-**12**/**18**[a]	Purity [%][b]	Yield [%][c]
7	(*S*)-**12{1,1,4,1}**	Ser[d]	GABA	5:95:0	41	4
8	(*R*)-**12{1,2,4,1}**	D-Ser[d]	GABA	99:1:0	47	13
9	(*S*)-**12{1,3,4,1}**	Thr[d]	GABA	1:99:0	53	12
10	(*R*)-**12{1,4,4,1}**	D-Thr[d]	GABA	99:1:0	70	10
11	(*R*)-**12{1,6,4,1}**	Cys[e]	GABA	36:64:0	61	5
	(*S*)-**12{1,6,4,1}**					13
12	(*R*)-**12{1,5,4,1}**	HSer[d]	GABA	–	–	NI

[a] Ratio calculated by integration of the diagnostic signals in the ^{1}H NMR spectra; [b] Analysis of the crude products by LC, $\lambda = 210–500$ nm; [c] Isolated yield after HPLC purification calculated from the ^{1}H NMR spectra; [d] Protecting group: *t*Bu; [e] Protecting group: Trt; [f] acpc: Fmoc-*cis*-2-aminocyclopentane carboxylic acid; NI: not isolated, a complex mixture of unidentified products.

those of **10** (Table 2). Interestingly, the formation of enamides **17** and **18** was not observed.

Therefore, for the [8+5]-fused bicycles, the use of L-Ser as **BB2** generated the (*S*)-configuration of the new chiral carbon (Table 2, entry 1), and the use of D-Ser induced the opposite (*R*)-configuration (entry 2). Analogously, L-Thr exhibited the previously observed synergic effect (i.e., improving the stereoselectivity; entries 1 and 3). In particular, L-Cys afforded higher yields, likely due to the higher nucleophilicity of sulfur, but lower stereoselectivity (entry 5). Unfortunately, the combination of Fmoc-β-Ala-OH and HSer generated a complex mixture of components that were neither isolated nor characterized.

To evaluate the effect of conformational constraints present on **BB3**, we synthesized a model compound using (+/-)-Fmoc-*cis*-2-aminocyclopentane carboxylic acid (acpc). The cyclization proceeded cleanly with this compound, providing the expected fused ring (Table 2, entry 6).

The more challenging nine-membered cycles included in the [9+5]-fused bicyclic systems **12** were produced in acceptable overall yields, although the yields were lower than those in the previous series, as expected (Table 2, entries 7–11). In analogy to the previously described [8+5]-fused bicyclic system, the stereochemistry of the new chiral carbon was primarily controlled by the absolute configuration of the first amino acid (**BB2**) of the acyclic precursor. Similar to the previous models, the incorporation of HSer did not allow the desired product to be isolated with sufficient purity (entry 12).

It is important to stress that the [8+5] and [9+5] fused rings were prepared from linear building blocks without any conformation restriction, which could enhance the ring formation, already observed in analogous ring closures.[28]

The assignment of the configuration of the new stereogenic center in eight- and nine-membered fused bicycles **11** and **12** was based on the NOE effect observed in the NOESY 1D and ROESY 2D spectra of L- and D-Thr derivatives **11{1,3,3,1}** compared with **11{1,4,3,1}**, and **12{1,3,4,1}** compared with **12{1,4,4,1}** (Figure 7). In all of the analyzed compounds, the

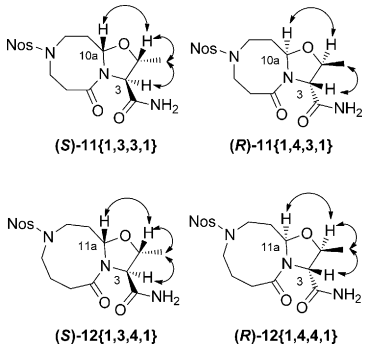


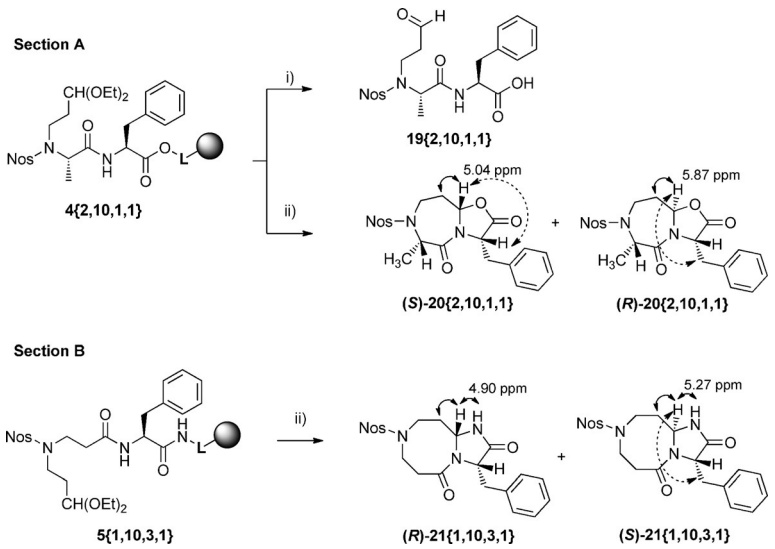
Figure 7. Configuration assignment of 5-oxooctahydro-5*H*-oxazolo[3,2-*a*][1,5]diazocine **11** and oxodecahydrooxazolo[3,2-*a*][1,5]diazonine **12**.

NOE correlation between the 2-H signal of L- and D-Thr with the corresponding 10a-H signal in **11** and 11a-H in **12** were decisive for determining the correct assignment. In addition, these correlations indicated the same *trans* relative disposition for 3-H and 10a-H or 11a-H in the eight- or nine-membered fused bicycles, respectively. Comparison of the chemical shift in the ^{1}H and ^{13}C NMR spectra of each pair of epimers indicated the same tendency observed for the series of seven-membered ring systems **10**. These differences between the epimers were used for the tentative configuration assignment of the rest of the compounds in the series. NMR data of the diagnostic proton and carbon signals are provided in the Supporting Information.

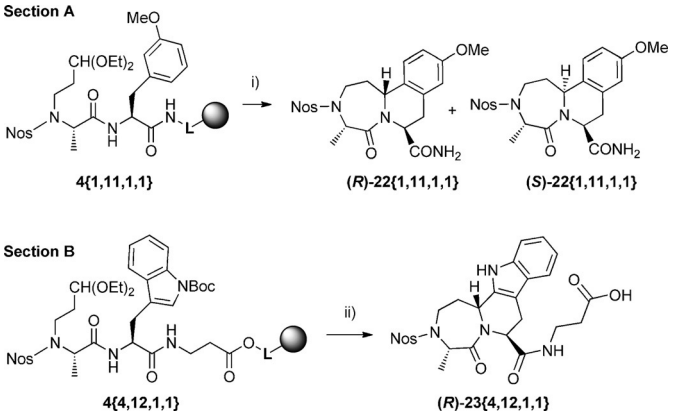
C–C bond formation

Finally, encouraged by the success of the synthesis of medium-sized bridged heterocycles and inspired by the *N*-acyliminium

Pictet–Spengler reaction,[42–44] Phe- and Trp-derived linear intermediates were synthesized (**BB2**: Phe, 3-(OMe)-Phe, Trp) to evaluate the formation of a new C–C bond during the formation of the second ring (Scheme 6 and Scheme 7). The L-Phe-derived linear precursor **4{2,10,1,1}** was treated with TFA for 16 h to afford only the corresponding aldehyde **19{2,10,1,1}**, which was identified based on LC/MS and on ^{1}H NMR spectroscopic analysis (Scheme 6 A). Exposure of the Wang-resin-derived precursor **4{2,10,1,1}** to formic acid at 60 °C for 16 h yielded an unexpected compound (i.e., tetrahydrooxazolo[3,2-*d*][1,4]diazepine-2,5(3*H*,6*H*)-dione **20{2,10,1,1}**) as a mixture of diastereoisomers (*R*)-**20** and (*S*)-**20** in a ratio of 1.1:1. In this case, the *N*-acyliminium ion was formed, and subsequent nucleophilic attack of the carboxylic acid afforded unexpected compound **20{2,10,1,1}**, the structure and stereochemistry of which was confirmed by NMR spectroscopic analysis. The analogous formation of lactones from seven-membered *N*-acyliminium ion intermediates has been reported.[24] Encouraged by this result and to confirm the structure, the Rink-resin-derived precursor for eight-membered ring intermediates **5{1,10,3,1}** was treated with formic acid at 60 °C for 16 h to yield the corresponding epimeric mixture of lactams (*R*)-**21{1,10,3,1}** and (*S*)-**21{1,10,3,1}** (ratio *S*/*R* = 3:1), the structure and stereochemistry of which were confirmed by NMR spectroscopic analysis (Scheme 6 B).



Scheme 6. Formation of lactones **20{2,10,1,1}** and lactams **21{1,10,3,1}** from linear precursors **4{2,10,1,1}** and **5{1,10,3,1}**. Reagents and conditions: i) 50% TFA in CH_2Cl_2, rt, overnight; ii) neat TFA, 60 °C, overnight. Arrows indicate COSY correlation and dotted arrows indicate NOESY correlation. The chemical shift of the fused proton 10a-H is also indicated.



Scheme 7. Formation of C–C bond by nucleophilic addition. Reagents and conditions: i) 50% TFA in CH_2Cl_2, RT, 16 h; ii) 50% TFA in CH_2Cl_2, RT, 2 d.

Phe, with an electron-donating OMe group in the *meta*-position [linear precursor **4{1,11,1,1}**], enhanced the nucleophilicity of the phenyl ring and promoted closure of the second ring to afford the corresponding mixture of epimers (*R*/*S*)-**22{1,11,1,1}** [ratio *R*/*S* = 10:1] after 16 h of treatment with TFA, with 25% overall yield (Scheme 7 A). In this case, even though two non-equivalent carbon atoms were activated, only one regioisomer was obtained as a mixture of the epimers generated during the nucleophilic attack. As expected, the major isomer **22** contained the OMe group in the less sterically hindered position. Similarly, the more nucleophilic aromatic indolyl group of acyclic precursor **4{4,12,1,1}** yielded the fused bicyclic products (*R*)-**23{4,12,1,1}** under treatment with TFA for two days (Scheme 7 B).

Conclusion

The synthesis of diverse medium-sized fused molecular scaffolds through acid-mediated tandem reactions involving seven-, eight-, and nine-membered endocyclic *N*-acyliminium ions as the key intermediates was described. The complex heterocyclic systems were synthesized from simple starting materials with low to good total yields and with high to full stereocontrol of the new chiral carbon. The molecular scaffolds covered a variety of ring sizes including [7+5], [7+6], [8+5], and [9+5] fused rings. This synthetic strategy enabled the use of a variety of heteroatoms contained in the bicycles, including N, O, and S. In addition, C-nucleophiles contained in electron-rich aromatic rings provided complex tri- and tetracycles through C–C bond formation. Further work exploring the potential applicability of this strategy to larger peptide molecules is in progress.

Experimental Section

Solid-phase syntheses were performed in plastic reaction vessels (i.e., syringes, each equipped with a porous disk) using a manually operated synthesizer. 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 prior to changing the solvent. Commercially available Rink resin (100–200 mesh, 0.66 mmol g^{-1}) and Wang resin (100–200 mesh, 1.0 mmol g^{-1}) were used. The yields of the crude products were calculated with respect to the loading of the first building block. The reaction conditions for the individual steps of the synthesis have been reported in previous communications.[34,36–39]

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Keywords: cyclization · drug discovery · fused-ring systems · regioselectivity · solid-phase synthesis

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