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Lithium Chloride Catalyzed Asymmetric Domino Aza-Michael Addition/[3 + 2] Cycloaddition Reactions for the Synthesis of Spiro- and Bicyclic α,β,γ-Triamino Acid Derivatives

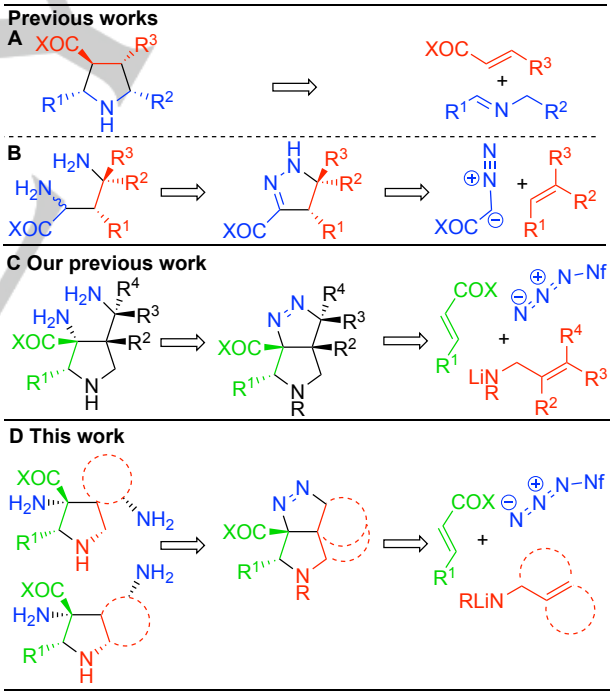
David Just,[a] Daniel Hernandez-Guerra,[a] Susanne Kritsch,[b] Radek Pohl,[a] Ivana Císařová,[c] Peter G. Jones,[b] Richard Mackman,[d] Gina Bahador,[d] and Ullrich Jahn*[a]

Abstract: Angularly and peri-fused tricyclic pyrrolidinopyrazolines are efficiently prepared by LiCl-catalyzed domino aza-Michael addition-1,3-dipolar cycloaddition reactions. The absolute stereochemistry is controlled in the aza-Michael addition step, nonaflyl azide serves as effective diazo transfer reagent to the formed enolate and the resulting diazo dipole engages in the 1,3-dipolar cycloaddition step. The resulting tricyclic pyrrolidinopyrazolines can be easily transformed to enantiomerically enriched nonproteinogenic spirocyclic α,β,γ-triamino acids, angularly or peri-fused tricyclic β-prolines or pyrimidines. The activity of the tricyclic amino acid derivatives against the hepatitis C virus was determined.

Introduction

Non-natural amino acids are an important compound class that plays a significant role in chemistry and the life sciences. They are interesting building blocks, often with atypical substitution patterns and stereochemistry. This provides ample opportunities to modulate the physical and chemical properties of products containing them. In material chemistry, non-natural amino acids have attracted considerable interest, particularly as building blocks of foldamers[1] and dendrimers.[2] They are frequently applied in medicinal chemistry as replacements for native amino acids, leading to better hydrolytic stability, biological activity and selectivity at their receptors.[3]

Peptides containing proteinogenic α-amino acids typically have low *in vivo* stability because of easy digestion by proteases, and the introduction of non-standard α-amino acids has therefore been exploited for the preparation of variously substituted chiral scaffolds that were applied in drug discovery, the preparation of bioconjugates, catalysis and biopolymers.[4] Another tool to increase the stability of conjugates are α,α-dialkyl α-amino acids, because they are usually resistant to cleavage.[5] The insertion of an additional substituted carbon atom next to the carboxylic group leads to β-amino acids, which may create new stereocenters, thus making them structurally more diverse then their α-relatives.[6] This formal insertion process can be repeated and, despite the fact that another homologation to γ-amino acids reduces the number of potential hydrogen bonds within the backbone, such peptides have been shown to adopt various stable conformations, such as helices, sheets and turns.[7] In particular, cyclic amino acids have proved to be of high importance, because of their conformational rigidity and tunable steric properties.[8]



Scheme 1. (A-C) Previous approaches to cyclic non-natural amino acids. (D) Tricyclic α,β,γ-triamino acids by tandem aza-Michael addition/1,3-dipolar cycloaddition reactions.

The prospect of combining all these amino acid classes to either homopeptides or heteropeptides (ααβ, αββ, αβγ …) opens access to an essentially unlimited structural and conformational space of peptides, with the potential to access defined secondary and tertiary structures in peptides. Therefore, the synthesis of

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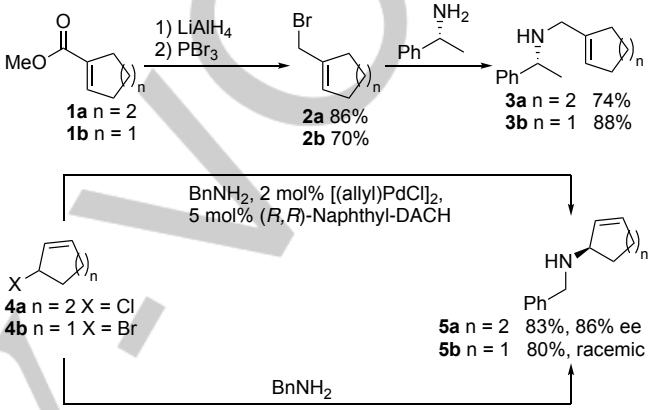
polyfunctional monomer units appears to be one of the most limiting factors because it is often a complex task and involves tedious sequences. Although a large number of methodologies for the synthesis of non-standard polyfunctional amino acids has been reported,[9] the most powerful are dipolar cycloadditions, because they generate constrained cyclic structures, are regioselective, diastereoselective and offer opportunities to apply catalytic asymmetric conditions (Scheme 1A).[10] Two strategic approaches towards amino acid synthesis by 1,3-dipolar cycloadditions have been pursued. On the one hand, the heterocycle formed by the cycloaddition is preserved as the amino acid itself; e.g. proline analogs can be approached by azomethine ylide cycloaddition reactions.[11] On the other hand the initially formed cycloadduct may be subsequently transformed to amino acids, as demonstrated for the conversion of simple pyrazolines to diamino acids (Scheme 1B).[12]

Polyfunctional nonproteinogenic amino acids have more recently become the focus of our interest.[13] Ideally, proteolytic stability, but similarity to natural amino acids should be kept and epimerization-sensitive structural features should be avoided in the design. Structural constraints to limit the number of energetically similar conformations should be an important criterion for the design. The polyfunctionality should provide branching points to three-dimensional peptides or analogous structures that nature cannot access because of the functionality pattern of proteinogenic amino acids. Previously, we reported a domino approach to bicyclic enantiopure highly substituted pyrrolidinopyrazolines by anionic aza-Michael addition followed by 1,3-dipolar cycloaddition, utilizing easily accessible starting materials such as crotonates or cinnamates and simple chiral allylic lithium amides (Scheme 1C).[14] Nonaflyl azide proved to be the reagent of choice for the generation of α-diazo derivatives from the corresponding enolates, thus introducing two sterically different nitrogen atoms, which allowed the construction of enantiopure products containing four stereocenters. These pyrrolidinopyrazolines were modified to highly substituted monocyclic amino acids, which are applicable for peptide synthesis, in which each of the amino functions can be selectively addressed. We hypothesized that these structures would provide spatially better defined motifs by introduction of additional rings, which can be further diversified to conformationally even more constrained and at the same time sterically demanding building blocks for peptides (Scheme 1D).

Here, we report an efficient synthetic strategy to approach enantiopure 5,5,5- and 5,5,6-tricyclic fused and spirocyclic pyrrolidinopyrazolines utilizing chiral cyclic allylic lithium amides bearing their chirality either in the α-methylbenzyl group or directly in the cyclic alkenylamine moiety. Their antiviral activities against the hepatitis C virus are also reported.

Results and Discussion

An efficient access to the starting cyclic allylic amines **3** and **5** is crucial for the approach to polycyclic functional amino acids (Scheme 2). Chiral amines **3a,b** with cycloalkenylmethyl units were efficiently prepared over three steps from cycloalkenecarboxylates **1a,b**. Their reduction by lithium aluminum hydride and nucleophilic exchange of the resulting alcohols provided bromides **2a,b** in good yields. Subsequent alkylation of (*R*)-1-phenylethylamine with **2a,b** furnished cyclic amines **3a,b** in 62-64% yields over three steps. (*R*)-*N*-Benzyl cyclohexenyl amine **5a** was synthesized with 86% ee by asymmetric palladium-catalyzed allylic amination of cyclohexenyl chloride **4a** and benzylamine using Trost's DACH ligand. This methodology failed for the preparation of cyclopentenylamine **5b**, which was therefore approached in racemic form by alkylation of benzylamine with cyclopentenyl bromide **4b**.



Scheme 2. Preparation of starting amines **3** and **5**.

The envisaged tandem aza-Michael/cycloaddition sequences occur spontaneously with simple allylic amines;[15] however, previous experience showed that increasing steric demand at the allylic amine unit may stall the aza-Michael addition. Therefore, the investigation commenced with the tandem reaction between enantiomerically pure cyclohexenylmethyl amine **3a** and *tert*-butyl crotonate **6a** as substrates. Deprotonation of amine **3a** by *n*-butyllithium at –78 °C and addition of **6a** based on Davies procedure,[15] followed by addition nonaflyl azide and acetic acid provided the cyclized products *trans*-**7a** and *cis*-**8a** in low (31%) yield and moderate diastereoselectivity (Table 1, entry 1). Decoupling the reaction sequence and performing the steps individually revealed that the aza-Michael addition was the inefficient step (not shown). Based on Collum's results and our own experience, the low efficiency was traced to aggregation of the lithium amide.[13b,16] Therefore, a catalytic amount of LiCl was added to the reaction mixture and the yield of tricyclic angularly fused spiranoids *trans*-**7a** and *cis*-**8a** improved to 76% with a 12:1 diastereomeric ratio. (entry 2 *vs.* 1). The optimized reaction conditions were successfully employed for domino reactions using cycloalkenylmethyl amines **3a-b** having five- or six-membered rings and α,β-unsaturated esters **6a-f** bearing alkyl or aryl substituents in β-position. It was shown that *tert*-butyl esters **6a,b** with alkyl or aryl substituents in the β-position of the Michael acceptors are equally well applicable in the tandem process, with both types of amines **3a-b** providing products **7a-d** in 68-84% yields with good diastereoselectivity (entries 2-5). Methyl cinnamate **6c** furnished tricyclic products *trans*-**7e,f** in reasonable to good yield, but with significantly reduced 4.5-6:1 **7:8**

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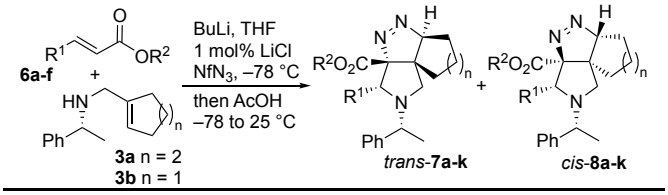
diastereomeric ratio (entries 6,7 *vs.* 4,5); however, isopropyl crotonate **6d** provided product **7g** in 78% yield with very good diastereoselectivity comparable to that observed for *tert*-butyl esters **6a,b** (entry 8). Substituted arylpyrrolidinopyrazolinecarboxylates *trans*-**7h-k** were obtained in 60-83% yields with good diastereoselectivities regardless of the electronic nature of the substituents at the aryl ring (entries 9-12).

Table 1. Scope of the domino reaction with respect to allylic amines **3a,b** and esters **6a-f**



entry	3	6	R^1	R^2	7 (%)	7:8[a]
1[b]	**a**	**a**	Me	*t*Bu	**a** 31	5:1
2	**a**	**a**	Me	*t*Bu	**a** 72	12:1
3	**b**	**a**	Me	*t*Bu	**b** 68	12:1
4	**a**	**b**	Ph	*t*Bu	**c** 68	12:1
5	**b**	**b**	Ph	*t*Bu	**d** 84	12:1
6	**a**	**c**	Ph	Me	**e** 47	4.5:1
7	**b**	**c**	Ph	Me	**f** 64	6:1
8	**a**	**d**	Me	*i*Pr	**g** 78	15:1
9	**a**	**e**	*p*-$MeOC_6H_4$	*t*Bu	**h** 60	24:1
10	**b**	**e**	*p*-$MeOC_6H_4$	*t*Bu	**i** 83	21:1
11	**a**	**f**	*p*-FC_6H_4	*t*Bu	**j** 80	8:1
12	**b**	**f**	*p*-FC_6H_4	*t*Bu	**k** 69	12:1

[a] Determined from the ^{1}H NMR spectra of the crude reaction mixtures. [b] without LiCl.

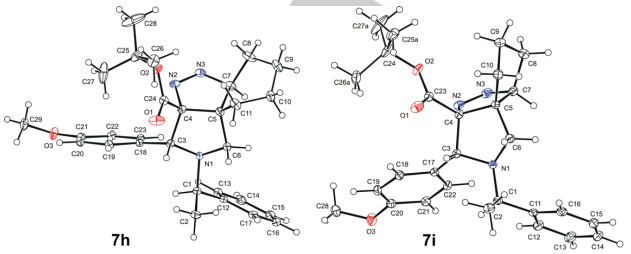


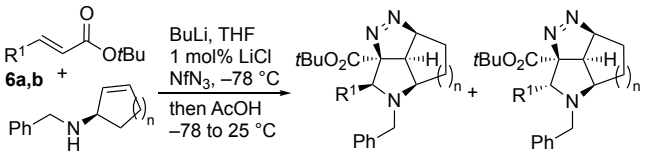
Figure 1. X-Ray crystal structure of **7h** and **7i**. The cyclohexane unit in **7h** is disordered and only one orientation of atoms C9, C10 is depicted for clarity. The *tert*-butyl ester group in **7i** is also disordered and only one orientation is depicted for clarity. The displacement ellipsoids are drawn at 30% probability level.

The structure and configuration of tricycles **7h** and **7i** were unambiguously determined by X-ray crystallography (Figure 1). The C-N bond lengths of the pyrazoline ring are 1.50 Å and 1.48 Å, respectively, whereas the N=N bond of the diazene unit is 1.25 Å long. The annulated cyclohexane ring in **7h** adopts a distorted boat conformation, whereas the cyclopentane ring in **7i** has an envelope conformation. The configuration of the other angularly annulated tricyclic pyrrolidinopyrazolines was assigned by analogy of their NMR spectral data.

Encouraged by the successful application of cyclic amines **3** in the domino reactions, the reaction of amines with the amine functions directly attached to the ring were explored. Initial attempts to employ chiral *N*-cycloalkenyl-*N*-(1-phenylethyl)amines analogous to **3** were not successful because of their too low reactivity in the aza-Michael addition step, even with LiCl catalysis. However, the conjugate addition proceeded with less bulky *N*-benzyl-*N*-((*R*)-cyclohexenyl)amine **5a** (86% ee) and crotonate **6a** (Table 2). Subsequent diazo transfer with nonaflyl azide and cycloaddition afforded peri-fused 5,5,6-tricycles *trans*-**9a** and *cis*-**10a** in good yield, but moderate 2.6:1 diastereoselectivity (entry 1). The corresponding cinnamate **6b** reacted similarly (entry 2). Products *trans*-**9a,b** and *cis*-**10a,b** crystallized easily to give enantiomerically pure compounds (See the Supporting information). Racemic *trans*-**9b** and *cis*-**10b** were also obtained in a very early experiment in moderate yields by using ethylsulfonyl azide because diazo transfer was much less efficient than with nonaflyl azide (not shown).

Employing racemic five-membered amine **5b** resulted in similar yields of 5,5,5-tricycles *trans*-**9c,d** and *cis*-**10c,d**, but the diastereoselectivity was even lower (entries 3,4). It must be noted that the aza-Michael addition step was much slower with amines **5** than with amines **3.**

Table 2. Domino reaction with allylic amines **5a-b**



entry	6	R^1	5	9+10 (%)	9:10[a]
1	**a**	Me	**a**	**a** 72	2.6:1
2	**b**	Ph	**a**	**b** 70	3.2:1
3	**a**	Me	**b**	**c** 85	1.4:1
4	**b**	Ph	**b**	**d** 72	1.7:1

[a] Determined from the ^{1}H NMR spectra of the crude reaction mixture.

The relative configurations of the major and minor peri-fused products **9b, 10b** and **9d**, **10d**, respectively, were assigned by X-

ray crystallographic analysis (Figure 2). Both the aryl and ester units are easily accommodated in the bowl-shaped structures. The bond length around the diazene unit were with 1.49-1.52 Å for the C-N bond and 1.24 Å for the N=N bonds very similar to those determined for **7h** and **7i**, indicating that steric constraints are not significant.

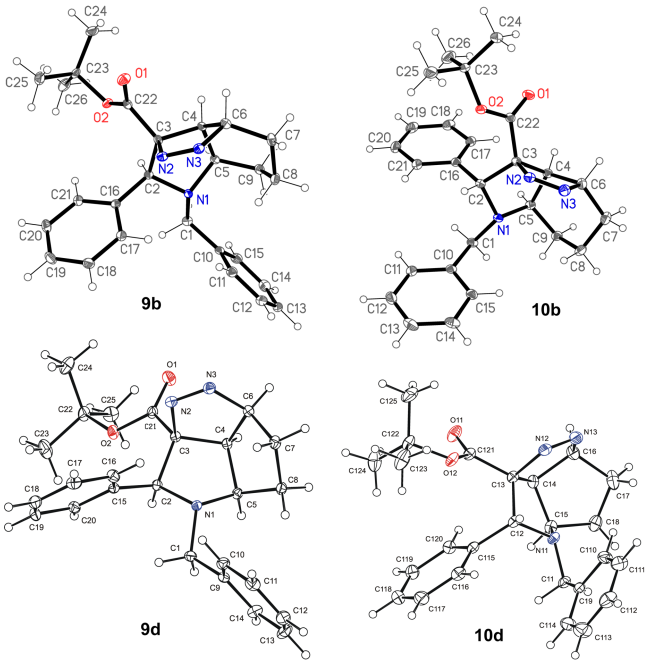
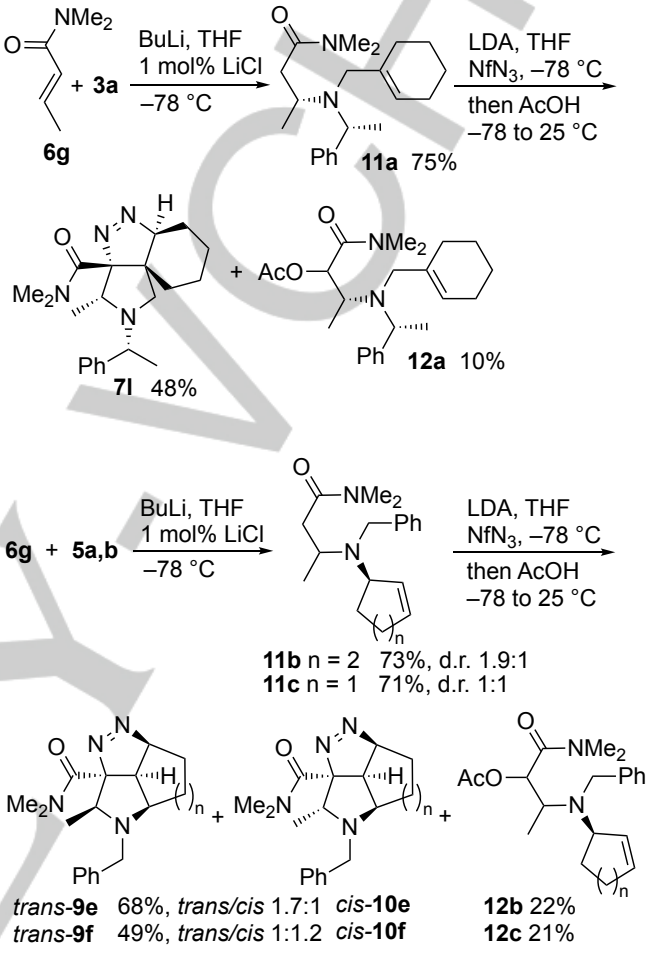


Figure 2. X-Ray crystallographic structures of diastereomeric racemic peri-annulated pyrrolidinopyrazolines *trans*-**9b**, cis-**10b** and *trans*-**9d**, *cis*-**10d**. the displacement ellipsoids are drawn at 30% probability level.

The reactivity of α,β-unsaturated amide **6g** toward lithium amides derived from amines **3a** and **5a** was also investigated and proved to be somewhat different. The tandem reactions analogous to those reported in tables 1 and 2 gave the products **7l** as well as **9e,f/10e,f** in low yields (not shown). Moreover, acetoxylation products **12a-c** formed in substantial amounts (see Scheme 3). This hinted to the fact that the aza-Michael addition proceeded satisfactorily, but that diazo transfer and cycloaddition were problematic. Therefore, a stepwise protocol was pursued (Scheme 3). The aza-Michael addition between **6g** and amines **3a** or **5a,b**, respectively, proceeded uneventfully, provided that LiCl was present, and was similarly efficient to that of the esters **6a-f**. β-Amino amides **11a-c** were isolated in 70-75% yield as a single enantiomer for **11a**, whereas **11b,c** were isolated as 1-1.9:1 diastereomeric mixtures, in line with the previous results. Deprotonation of **11a** followed by diazo transfer with nonaflyl azide and treatment with acetic acid provided tricyclic amide **7l** in 48% yield as a single diastereomer together with small amounts of α-acetoxy-β-amino amide **12a**. Amino amides **11b,c** also provided tricycles *trans*-**9e,f** and *cis*-**10e,f** in improved 49-68% yields when using the stepwise protocol. Only two diastereomers were formed from the diastereomeric mixtures of **11b,c** in similar ratios, thus indicating that the cycloaddition occurred with very high diastereoselectivity for both, *trans*-**9b,c** and *cis*-**10b,c**. β-Amino-α-(acetoxy) amides **12b-c** were, however, also isolated.

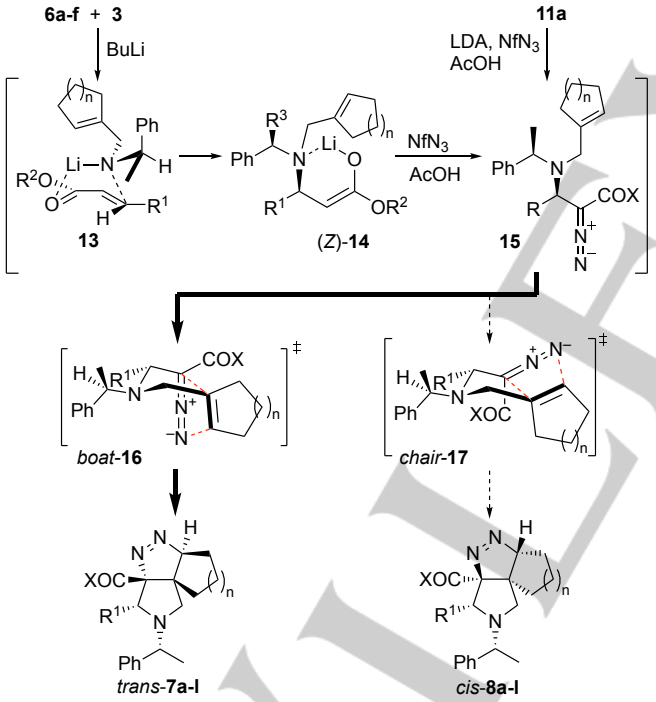


Scheme 3. Reactivity of α,β-unsaturated amide **4g**.

The good stereoselectivity observed for the formation of tricyclic angularly fused pyrrolidinopyrazolines **7** rests on several factors. The absolute stereochemistry is reliably set in the aza-Michael addition step for amines bearing cyclic allylic groups (as in amines **3a,b**) and is assumed to proceed by precoordination of the lithium amide to the Michael acceptors **6** as shown in transition state **13**.[13-15] It must be noted that in contrast to simpler unhindered *N*-allylic 1-phenylethylamines,[14] the aza-Michael addition is sensititve to the bulk of the allylic unit. The lithium amides derived from amines **3** are probably dimers in solution.[13b] In this state, the conjugate addition is apparently not efficient because of steric hindrance, raising the transition state energy. The addition of a catalytic amount of anhydrous lithium chloride accelerates the conjugate addition significantly,[16] probably by forming sterically less demanding mixed dimers of the lithium amide with LiCl, which coordinate more efficiently to **6** and add thus faster. The stereoselectivity is not compromised by LiCl

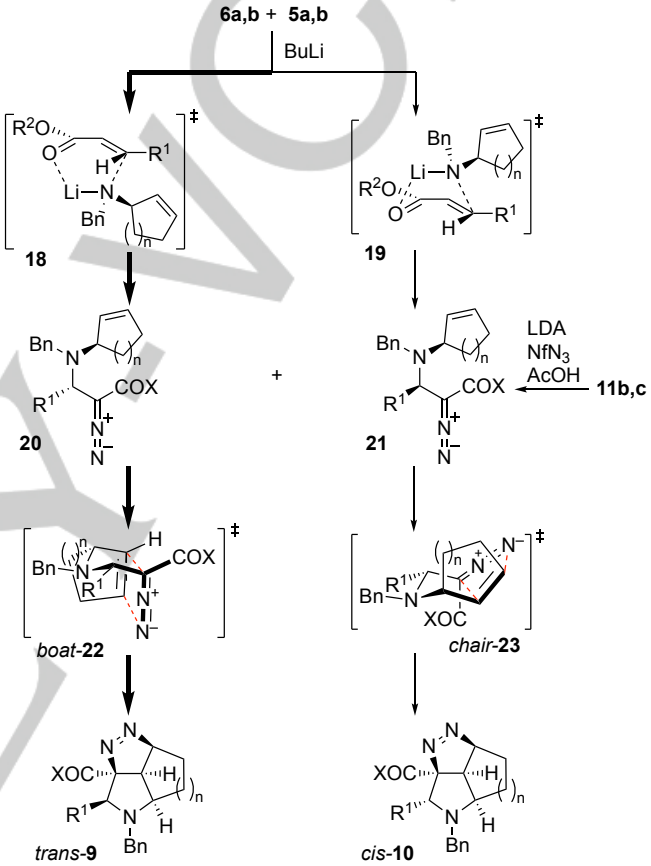
addition. The resulting (*Z*)-enolates **14** subsequently add to the terminus of the electrophilic nonaflyl azide. The triazenide thus formed is subsequently quenched by addition of acetic acid[14] and transforms to β-amino-α-diazo esters **15** (X = OR^2).

Surprisingly, amide **6g** gave only low yields of tricycle **7l** in the tandem aza-Michael addition/diazo transfer/cycloaddition reactions. The reactivity difference was traced to the diazo transfer step to enolates **14**, as the aza-Michael addition proceeded reliably with unsaturated amide **6g**. When the resulting β-amino amide **11a** was deprotonated with LDA and the resulting enolate subjected to nonaflyl azide, the diazo transfer proceeded smoothly. Reasoning that the enolate geometry may be a factor that causes this reactivity difference, both enolates, either formed by 1,4-addition or by deprotonation with LDA, were reacted with chlorotrimethylsilane (see the Supporting Information),[13b,d,15] and the resulting silyl ketene aminals were studied by ROESY NMR spectroscopy. The investigation confirmed that (*Z*)-enolates form in large excess in both cases as indicated by the contact of the vinyl proton with one of the *N*-methyl groups. This rules out enolate geometry as a factor and therefore the reason for this surprising reactivity difference based on the difference of enolate generation is still not understood.



Scheme 4. Stereochemical rationale of the domino process forming the configuration of tricyclic angularly fused spiranoids.

The final 1,3-dipolar cycloaddition step proceeded in all cases smoothly, but subtle differences in the diastereoselectivity became apparent. High diastereoselectivity for the formation of tricycles *trans*-**7** was observed with bulky *tert*-butyl or isopropyl ester groups or the dimethyl amide unit, but the selectivity decreased using a methyl ester function in the cycloaddition substrate **15**. This is consistent with a preferred transition state *boat*-**16**, in which the bulkier carbonyl and R^1 groups occupy diequatorial positions thus minimizing steric interactions. This forces the diazo unit into axial orientation and the cycloaddition proceeds in turn through a boat-like transition state. The minor isomers *cis*-**8** result from reaction via chair-like transition state **17**, which is disfavored because of the *cis* orientation of the carbonyl and R^1 groups.

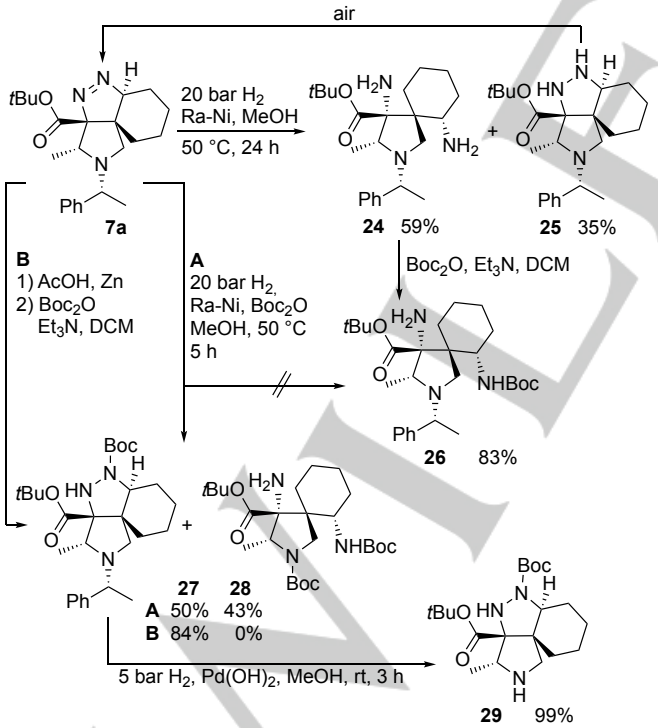


Scheme 5. Stereochemical rationale of the domino process for the formation of peri-fused tricycles **6** and **7**.

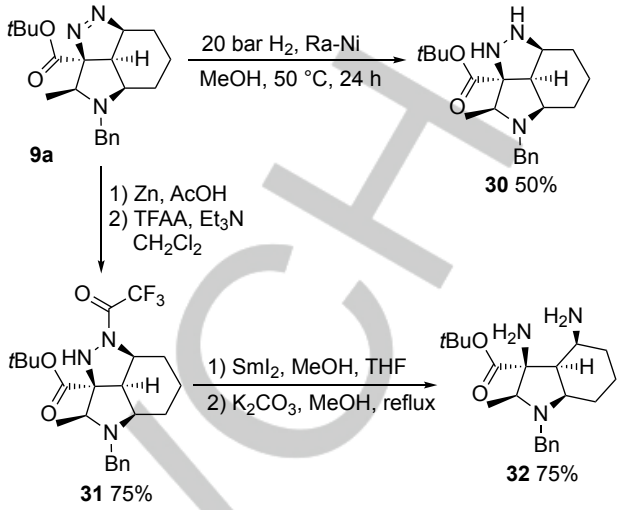
In contrast to amines **3**, where a single branching point at the amine function is present and the stereoselectivity of the overall process is strictly dictated by the stereocenter in the 1-phenylethyl unit, amines **5a,b** having the stereocenter as a part of the cycle are only little different with respect to steric demand at the stereocenter (Scheme 5). Therefore, the face selectivity of their aza-Michael addition to esters **6a,b** is not very high, lying in the range of 2:1 for the preferred attack from the bottom face via transition state **18**, where less steric interactions of the substituent R^1 with the cycloalkenyl unit are present compared to the arrangement in transition state **19**. The resulting β-amino-α-diazo carbonyl derivatives **20** and **21** undergo the cycloaddition with exclusive diastereoselectivity, since no other diastereomers than *trans*-**9** and *cis*-**10** were detected in the reaction mixtures. The

1,3-dipolar cycloaddition of **20** apparently proceeds most favorably via transition state *boat*-**22**, in which the larger substituents occupy *trans*-diequatorial positions and thus force the diazo and alkene units into axial orientations, furnishing isomers *trans*-**9**. The diastereomeric amino diazo compounds **21** can in contrast only react via transition state *chair*-**23** providing the *cis* isomers **10**.

Tricyclic spiranoid **7a** was chosen as a representative example for demonstration of scaffold diversity. Hydrogenolytic cleavage of the N=N double bond serves as a tool to approach spiro α,β,γ-triamino acid derivatives (Scheme 6). Free amine **24** was obtained in 59% yield using Raney nickel under 20 bar of dihydrogen, which was accompanied by separable pyrazolidinopyrrolidine **25**. Surprisingly, the latter was spontaneously reoxidized to starting material **7a** on exposure to air and can thus be recycled. Product **24** was selectively protected by Boc_2O at the secondary position affording orthogonally diprotected triamino ester **26**. In contrast, hydrogenation/acylation of **7a** by Raney nickel (**A**) in the presence of Boc_2O did not furnish the desired protected amine **26** but provided Boc-protected pyrazolidine **27** and the product of N-N bond cleavage and competitive hydrogenolysis of the phenylethyl unit, di-Boc-triamino ester **28**, in almost the same amounts. Pyrazolidine **27**, which also appears to be an attractive scaffold, was selectively obtained by reduction of **7a** with zinc in acetic acid and subsequent protection by Boc_2O (**B**).[17] Further hydrogenolysis of **27** selectively afforded triamino ester **29** bearing a free amino function in α- and β-position.

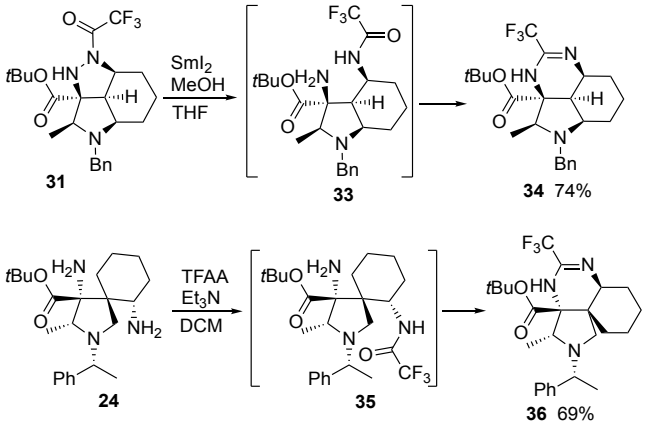


Scheme 6. Access to spiro-α,β,γ-triamino esters **24** and **26** by hydrogenolysis conditions and formation of differentially protected α-hydrazino esters **27-29**.



Scheme 7. Two-step reductive access to fused α,β,γ-triamino acids **32** under single-electron reductive conditions.

Tricycle **9a** was selected as a representative model to explore the reactivity of peri-fused ring systems. For convenience, these investigations were performed with racemic material; the results should, however, be the same with enantiomerically enriched derivatives. Hydrogenolytic ring opening of the pyrazoline ring was not possible (Scheme 7). Free N-H pyrazolidine-containing tricycle **30** was obtained in moderate yield and minor debenzylation products were competitively formed (not shown), but N-N bond cleavage did not occur. Therefore, **9a** was first transformed in good yield to trifluoroacetylpyrazolidine **31** by reduction with zinc in acetic acid, followed by trifluoroacetylation. Single electron reduction by SmI_2 followed by immediate deprotection of the trifluoroacetyl group resulted in annulated bicyclic β-protected triamino acid **32**.

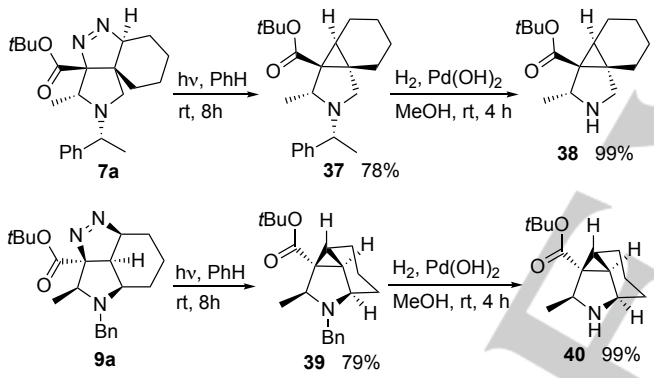


Scheme 8. Access to annulated 2-trifluoromethyltetrahydropyrimidines **34** and **36**.

On attempted isolation and spectroscopic characterization of bicyclic γ-trifluoroacetyl-protected triamino acid **33**, a slow

formation of 2-trifluoromethyltetrahydropyrimidine **34** was observed. This transformation was completed within a day upon standing in an NMR tube in $CDCl_3$ (Scheme 8). This process seems to be general, since tetrahydropyrimidine formation also occurred spontaneously from amino acid **35** when spiro triamino acid **24** was protected by trifluoroacetic anhydride. Trifluoromethylated pyridmidine **36** was isolated from **35** in 69% yield after standing in an NMR tube in $CDCl_3$ for a day.

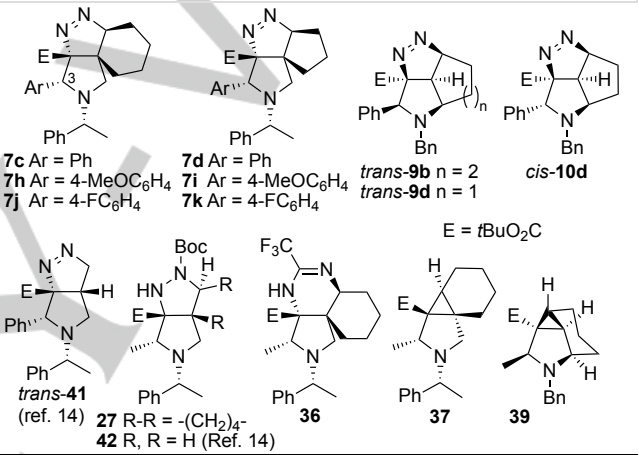
Tricyclic scaffolds **7a** and **9a** containing the 1-pyrazoline unit are not only precursors for triamino acid derivatives but are also β-amino acid surrogates by extrusion of molecular nitrogen. Irradiation of both compounds by a high pressure mercury lamp furnished angularly fused and peri-fused cyclopropanes **37** and **39**, respectively, in good yields as single diastereomers (Scheme 9).[18] Removal of the phenylethyl groups by hydrogenolysis over Pearlman's catalyst provided conformationally constrained tricyclic β-amino acids **38** and **40** reminiscent of alkaloid structures,[19] which exemplify the potential applicability of the method toward natural product synthesis and also for the preparation of β-peptide foldamers[20] or pharmaceutical analogs.[17b]



Scheme 9. Photochemical nitrogen extrusion reactions to β-prolinates **38** and **40**.

All compounds were tested against various genotypes of the hepatitis C virus in the 1B and 2A replicon assays (Table 3). 3-Aryl-substituted angular tricyclic triamino *tert*-butyl esters **7c,d,h-k** display low micromolar activity in the replicon 1B assay (entries 1,2,5-8). The corresponding methyl esters **7e,f** displayed similar activities (entries 3,4). The activity is enhanced by fluoride in *para*-position of the aryl ring (entries 7,8), while the activity was slightly decreased with electron-donating groups in *para*-position (entry 5,6). In contrast to the replicon 1B assay the EWG/EDG effect is opposite in the replicon 2A assay (entries 5-8). In all cases the substrates with five-membered carbocyclic rings **7d,f,i,k** showed higher activity than substrates with annulated six-membered carbocyclic rings **7c,e,h,j** in the replicon 1B assay (entries 2,4,6,8 vs. 1,3,5,7). A similar trend was observed for peri-fused tricycles **9**, where aryl-substituted five-membered derivative **9d** was more active than six-membered **9b**, (entry 9 *vs.* 10). Minor peri-fused product *cis*-**10d** was less active compared to *trans*-**9d** (entry 11 *vs.* 10). Interestingly, comparison with the previously synthesized bicyclic pyrrolidinopyrazolines[14] revealed that ester **41** showed activity in the 1B replicon assay, but not in the 2A replicon assay (entry 12 *vs.* 1-11). It is important to note that 3-methyl-substituted derivatives **7a,d**, **9a,c**, **10a,c** as well as all 3-methyl-substituted bicyclic analogs of **41** showed low or no activity against HCV in these assays (not shown). These trends indicate that the inhibition is probably related to a common specific binding site for compounds **7**, **9** and **41** (entries 1-10,12). Surprisingly, 3-methyl-substituted *N*-Boc-protected tricyclic pyrazolidine **27** as well as previously prepared bicyclic derivative **42**[14] proved to be active in both assays, but the additional annulation in **27** induced again higher activity compared to **42** (entries 13 *vs.* 14**)**.

Table 3. Antiviral activity of the synthesized compounds in two HCV replicon assays.[a]



entry		1B EC_{50} (μM)	2A EC_{50} (μM)	entry		1B EC_{50} (μM)	2A EC_{50} (μM)
1	**7c**	6.1	14.4	9[b]	**9b**	15.5	29.6
2	**7d**	4.9	14.8	10[b]	**9d**	7.9	11.5
3	**7e**	9.2	10.7	11[b]	**10d**	11.2	20.0
4	**7f**	6.2	12.8	12	**41**	7.2	>44
5	**7h**	12.5	15.6	13	**27**	5.8	17.2
6	**7i**	6.9	8.3	14	**42**	10.7	35.7
7	**7j**	3.5	27.6	15	**36**	11.1	27.8
8	**7k**	1.7	19.5	16	**37**	13.0	>50
				17[b]	**39**	11.6	>50

[a] Cytotoxicity values (CC_{50}) were determined in Huh-7 cells and are >50 μM for all compound except **7i** (20.7 μM), **7e** (22.0 μM). [b] racemic.

Remarkably, cyclohexane-annulated, but methyl-substituted tetrahydropyrimidine **36** and both bicyclo[4.1.0]heptane-fused β-proline derivatives **37** and **39** were active despite the fact, that they contain neither an aryl substituent at C-3 nor a five-membered carbocyclic ring (entries 15-17). The compounds were

not cytotoxic (CC_{50} >50 μM) except for **7i** (20.7 μM) and **7e** (22.0 μM).

Conclusions

Bicyclic α,β,γ-triamino acids, tricyclic pyrrolidino-tetrahydropyrimidines and tricyclic β-prolines have been efficiently accessed. Applying a one pot aza-Michael addition, diazo transfer and dipolar cycloaddition sequence led to tricyclic pyrazolo-pyrrolidines that served as precursors for polyfunctional amino acids. Enantiomerically pure products containing five stereocenters were formed using simple chiral 1-phenylethylamine as an easily removable auxiliary, which dictates the overall stereoselectivity very efficiently. All compounds were tested against the HCV virus and a number of them showed biological activity in the replicon 1B and 2A assays. It was demonstrated that these structures can be easily diversified to afford highly substituted potentially versatile amino acid derivatives that might be applied in peptide synthesis, material and medicinal chemistry in the future. Investigations along these lines are underway in these laboratories.

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Keywords: Tandem reactions • Nitrogen heterocycles • Non-natural amino acids • β-prolines • Michael addition • Diazo transfer • 1,3 dipolar cycloaddition

[1] a) T. A. Martinek, F. Fülöp, *Chem. Soc. Rev*. **2012**, *41*, 687-702; b) D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes, J. S. Moore, *Chem. Rev*. **2001**, *101*, 3893-4011; c) S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173-180; d) S. H. Yoo, H.-S. Lee, *Acc. Chem. Res.* **2017**, *50*, 832-841; e) B. A. F. Le Bailly, J. Clayden, *Chem. Commun.* **2016**, *52*, 4852-4863.

[2] a) M. Guillot-Nickowski, S. Eisler, F. Diederich, *New J. Chem.* **2007**, *31*, 1111-1127; b) M. Scholl, Z. Kadlecova, H.-A. Klok, *Prog. Polym. Sci.* **2009**, *34*, 24-61; c) K.Sadler, J. P. Tam, *Rev. Mol. Biotechnol.* **2002**, *90*, 195-229.

[3] a) M. Sánchez-Navarro, M. Teixidó, E. Giralt, *Acc. Chem. Res.* **2017**, *50*, 1847-1854; b) R. Gopalakrishan, A. I. Frolov, L. Knerr, W. J. Drury III, E. Valeur, *J. Med. Chem.* **2016**, *59*, 9599−9621; c) A. G. Jamieson, N. Boutard, D. Sabatino, W. D. Lubell, *Chem. Biol. Drug Des.* **2013**, *81*, 148-165; d) I. Kwon, S. I. Lim, *Macromol. Chem. Phys.* **2013**, *214*, 1295-1301; e) P. G. Vasudev, S. Chatterjee, N. Shamala, P. Balaram, *Chem. Rev*. **2011**, *111*, 657–687.

[4] a) D. Goyal, S. Shuaib, S. Mann, B. Goya, *ACS Comb. Sci.* **2017**, *19*, 55-80; b) J. C. Maza, H. J. Taylor, D. M. Uthappa, D. D. Young, *Synlett* **2016**, *27*, 805-813; c) T. L. Hendrickson, V. Crécy-Lagard, P. Schimmel, *Ann. Rev. Biochem.* **2004**, *73*,147-176; d) V. Lu, *Curr. Opin. Chem. Biol.* **2005**, *9*, 118-126.

[5] H. Yamaguchi, H. Kodama, S. Osada, F. Kato, M. Jelokhani-Niaraki, M. Kondo, *Biosci. Biotechnol. Biochem*. **2003**, *67*, 2269-2272.

[6] a) C. Cabrele, T. A. Martínek, O. Reiser, L. Berlicki, *J. Med. Chem*. **2014**, *57*, 9718-9739; b) T. Heck, B. Geueke, H.-P. E. Kohler, *Chem. Biodiversity* **2012**, *9*, 2388-2409; c) D. Seebach, J. Gardiner, *Acc. Chem. Res.* **2008**, *41*, 1366-1375; d) L. Kiss, I. M. Mándity, F. Fülöp, *Amino Acids* **2017**, *49*, 1441-1455; e) P. S. P. Wang, A. Schepartz, *Chem. Commun.* **2016**, *52*, 7420-7432.

[7] a) D. Seebach, A. K. Beck, D. J. Bierbaum, *Chem. Biodiversity* **2004**, *1*, 1111-1239; b) D. Seebach, D. F. Hook, A. Glättli, *Biopolymers* **2006**, *84*, 23-37; c) F. Bouillère, S. Thétiot-Laurent, C. Kouklovsky, V. Alezra; *Amino Acids* **2011**, *41*, 687-707.

[8] a) F. Fülöp, T. A. Martínek, G. K. Tóth, *Chem. Soc. Rev.* **2006**, *35*, 323-334; b) R. Singh, R. Vince, *Chem. Rev*. **2012**, *112*, 4642-4686; c) L. Kiss, F. Fülöp, *Chem. Rev*. **2014**, *114*, 1116-1169.

[9] a) O. O. Grygorenko, *Tetrahedron* **2015**, *71*, 5159-5216; b) N. Saha, B. Chatterjee, S. K. Chattopadhyay, *J. Org. Chem.* **2015**, *80*, 1896-1904; c) C. Cativiela, M. D. Díaz-de-Villegas, *Tetrahedron: Asymmetry* **2007**, *18*, 569-623; d) C. Cativiela, M. D. Díaz-de-Villegas, *Tetrahedron: Asymmetry* **2000**, *11*, 645-732.

[10] T. Hashimoto, K. Maruoka, *Chem. Rev*. **2015**, *115*, 5366-5412.

[11] a) A. S. Gothelf, K. V. Gothelf, R. G. Hazell, K. A. Jørgensen, *Angew. Chem., Int. Ed*. **2002**, *41*, 4236−4238; b) C. Chen, X. Li, S. L. Schreiber, *J. Am. Chem. Soc*. **2003**, *125*, 10174−10175; b) S. R. Vidadala, C. Golz, C. Strohmann, C.-G. Daniliuc, H. Waldmann, *Angew. Chem. Int. Ed.* **2015**, *54*, 651−655; c) X.-H. Chen, W.-Q. Zhang, L.-Z. Gong, *J. Am. Chem. Soc.* **2008**, *130*, 5652−5653.

[12] a) M. R. Mish, F. M. Guerra, E. M. Carreira, *J. Am. Chem. Soc*. **1997**, *119*, 8379-8380; b) H. Sasaki, E. M. Carreira, *Synthesis* **2000**, 135-138; c) T. Kano, T. Hashimoto, K. Maruoka, *J. Am. Chem. Soc*. **2006**, *128*, 2174-2175; d) F. M. Guerra, M. R. Mish, E. M. Carreira, *Org. Lett.* **2000**, *2*, 4265-4267; e) V. A. Gorpinchenko, D. V. Petrov, S. L. Khursan, V. A. Dokichev, Y. V. Tomilov, *Chem. Heterocycl. Comp*. **2009**, *45*, 1039-1046.

[13] a) F. Kafka, M. Holan, D. Hidasová, R. Pohl, I. Císařová, B. Klepetářová, U. Jahn, *Angew. Chem. Int. Ed.* **2014**, *53*, 9944-9948; b) F. Kafka, R. Pohl, I. Císařová, R. Mackman, G. Bahador, U. Jahn, *Eur. J. Org. Chem.* **2016**, 3862-3871; c) U. Jahn, F. Kafka, R. Pohl, P. G. Jones, *Tetrahedron* **2009**, *65*, 10917-10929; d) U. Jahn, M. Müller, S. Aussieker, *J. Am. Chem. Soc*. **2000**, *122*, 5212-5213.

[14] V. Kapras, R. Pohl, I. Císařová, U. Jahn, *Org. Lett.* **2014**, *16*, 1088-1091.

[15] a) S. G. Davies, A. D. Smith, P. D. Price, *Tetrahedron: Asymmetry* **2005**, *16*, 2833-2891; b) S. G. Davies, A. M. Fletcher, P. M. Roberts, J. E. Thomson, *Tetrahedron: Asymmetry* **2012**, *23*, 1111-1153; c) S. G. Davies, A. M. Fletcher, P. M. Roberts, J. E. Thomson, *Tetrahedron: Asymmetry* **2017**, *28*, 1842-1868

[16] Y. Ma, A. C. Hoepker, L. Gupta, M. F. Faggin, D. B. Collum, *J. Am. Chem. Soc.* **2010**, *132*, 15610-15623.

[17] a) W. Wang, D. D. Simovic, M. Di, L. Fieber, K. S. Rein, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1949-1952; b) J. K. Mukhopadhyaya, A. P. Kozikowski, E. Grajkowska, S. Pshenichkin, J. T. Wroblewski, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1919-1924; c) C.-H. Küchenthal, W. Maison, *Synthesis* **2010**, 719-740.

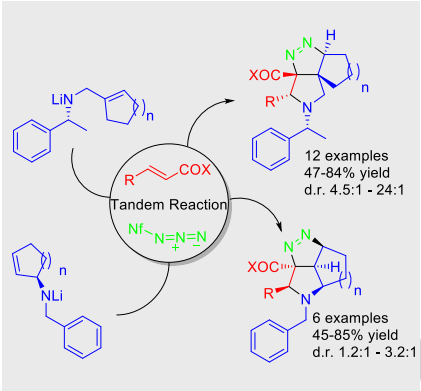
[18] a) B. S. Santos, S. C. C. Nunes, A. A. C. C. Pais, T. M. V. D. Pinho e Melo, *Tetrahedron* **2012**, *68*, 3729-3737; b) D. F. Taber, P. J. Guo, *J. Org. Chem.* **2008**, *73*, 9479-9481.

[19] a) S. R. McCabe, P. Wipf, *Angew. Chem. Int. Ed.* **2017**, *56*, 324-327; b) K. S. MacMillan, T. Nguyen, I. Hwang, D. L. Boger, *J. Am. Chem. Soc.* **2009**, *131*, 1187-1194.

[20] A. K. Medda, H.-S. Lee, *Synlett* **2009**, 921-924.

FULL PAPER

Angularly and peri-fused tricyclic pyrrolidinopyrazolines are efficiently prepared by LiCl catalyzed domino aza-Michael addition-1,3-dipolar cycloaddition. The resulting tricyclic pyrrolidinopyrazolines can be easily transformed to spirocyclic or condensed bicyclic α,β,γ-triamino acids derivatives.



Tandem Reactions

*David Just, Daniel Hernandez-Guerra, Susanne Kritsch, Radek Pohl, Ivana Císařová, Peter G. Jones, Richard Mackman, Gina Bahador, Ullrich Jahn**

Page No. – Page No.

Lithium Chloride Catalyzed Asymmetric Domino Aza-Michael Addition/[3 + 2] Cycloaddition Reactions for the Synthesis of Spiro- and Bicyclic α,β,γ-Triamino Acid Derivatives