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[^{15}N]-Isotopic labeling: a suitable tool to study the reactivity of bis lactams

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Abstract

The unexpected reactivity of 2,5-diketopiperazines under basic conditions, thanks to *N*-Boc activation, allows access to valuable pharmacological scaffolds, such as original statine derivatives. Toward this transannular rearrangement of activated lactams (TRAL), we report here the study of bis lactam reactivity using [^{15}N]-isotopic labeling.

Keywords: Diketopiperazine; [^{15}N]-Isotopic labeling; Lactam activation; Stereoselective synthesis; TRAL

^{15}N NMR spectroscopy has become a pertinent method for the characterization of amino acid derivatives, including peptides and proteins.[1] Nitrogen-15 enrichment can be used to overcome the problem of low sensitivity in ^{15}N NMR spectroscopy, which is partly due to the low natural abundance of ^{15}N nuclei (0.37%). Using selective ^{15}N labeled compounds, ^{15}N NMR spectroscopy allows characterization of minor modifications in the structure of nitrogen-containing molecules[2] due to this favorable spectral characteristic.

The transannular rearrangement of activated lactams (TRAL) is a new reaction of broad interest for organic or medicinal chemists in the field of five-membered heterocycles.[3] Starting from suitable activated diketopiperazines, the TRAL allows a total regioselective ring contraction leading to the synthesis of substituted pyrrolidine-2,4-diones. Actually, the reactivity under basic conditions of *N*-Boc activated 2,5-diketopiperazines provides a highly stereoselective pathway to original pyrrolidine-2,4-diones, which could be subsequently diastereoselectively reduced and opened into valuable statines analogues.[4]

First, regarding the pharmacological interest of the synthesized compounds and secondly, to study more thoroughly the reactivity of bis lactams towards the TRAL, we have considered mono-isotopic labeling with nitrogen-15 as an appropriate tool. For this purpose, we decided to examine the TRAL with di-protected *cyclo*-[Gly-Val] as starting material, labeling the glycine nitrogen to visualize the center of the TRAL. This atom of nitrogen-15 will be a suitable probe to check structural changes at the molecular level. Furthermore, the nitrogen-15, which is a no radioactive isotope, could also be seen as an interesting tool for in vivo imagery by magnetic resonance of [^{15}N]-labeled compounds of pharmacological interest (see Scheme 1).[5]

We choose to synthesize the starting material of the TRAL, (3*S*)-1,4-di-*tert*-butoxycarbonyl-3-isopropyl- piperazine-2,5-dione **8**, using the commercially available [^{15}N]-glycine (labeled at 98%), as shown in Scheme 2.

Several papers describe the synthesis of diketopiperazines by thermal heating of the corresponding dipeptide in solvents of high boiling points[6] such as DMF or 1,2-dichlorobenzene. However, the major drawback of this method is the partial racemization of the amino acid residues. Some procedures using solvent-free conditions[7]

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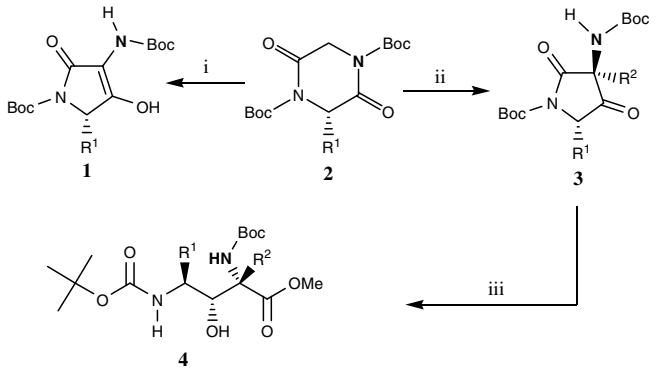
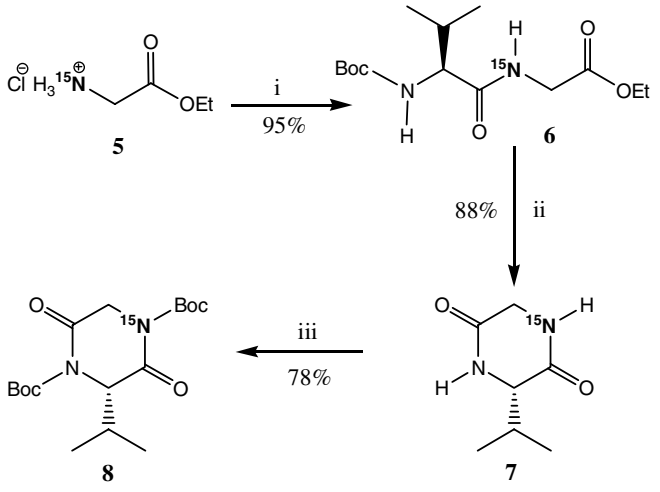


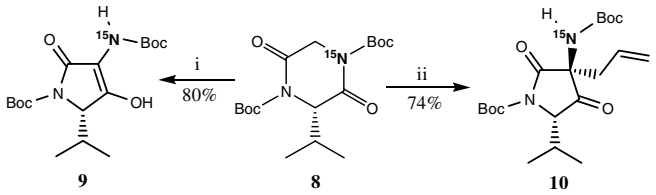
Scheme 1. TRAL: five-membered heterocycles and statine analogues synthesis from activated lactams. Reagents and conditions: (i) *t*BuOK, THF, rt, 3 h; (ii) R^2–Br, NaH, THF, 0 °C → rt, 8 h; (iii) $NaBH_4$, MeOH, 0 °C, 30 min.



Scheme 2. Reagents and conditions: (i) Boc-Val-OH, BOP, Et_3N, DMF, rt, overnight; (ii) H_2O, DMF, MW, 150 °C, 2 h 30 min; (iii) Boc_2O, DMAP, DMF, rt, 1 h.

and/or microwave irradiations[8] are also reported. Nevertheless, when a dipeptide methyl ester is used, yields decrease with degradation, and a lack of reproducibility is observed. We then decided to develop a more efficient procedure[9] for the synthesis of enantiopure diketopiperazines. Opting for the higher stability of the ethyl ester to decrease degradation, and a mixture of water and DMF (H_2O–DMF 11:1) as solvent for the critical step of cyclization, we were able to improve the yield to 88%. Subsequent Boc substitutions were then carried out under conventional conditions,[10] to obtain **8** in good yield.

We then examined the TRAL using the labeled activated diketopiperazine **8**. As shown in Scheme 3, we were able to reproduce the TRAL[3] and its alkylating variant in good yields, and with excellent ee and de. Compounds **9** and **10** were obtained, respectively, in one step starting from the labeled bis lactam **8**. We observed a total regioselective ring contraction allowing the exclusion from the ring system of the ^{15}N of the activated bis lactam. A total retention



Scheme 3. Reagents and conditions: (i) *t*BuOK, THF, rt, 3 h; (ii) allyl bromide, NaH, THF, 0 °C → rt, 8 h.

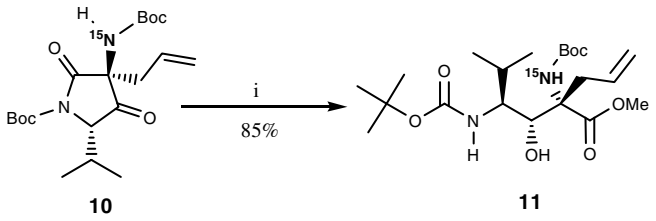
of configuration of the valine residue and in the case of tandem TRAL rearrangement/alkylation, a highly stereoselective alkylation is detected, as shown by chiral HPLC analyses.

By applying subsequently the one-step reductive-opening strategy,[4] the α,γ-diamino-β-hydroxyacid ester **11** could be obtained from the corresponding 3-aminopyrrolidine-2-one **10** with excellent stereoselectivity in 85% yield (Scheme 4).[4] It is interesting here to notice a structural analogy of these molecules with sub-units occurring in biologically active compounds, such as statines and bleomycin, that make them really attractive. Further works are currently devoted to incorporate labeled statine analogues into peptidic sequences.

All the original compounds **5**–**11** were analyzed and studied by ^1H, ^{13}C, and ^{15}N NMR spectroscopies.[11] We revealed that all synthesized molecules spread out on a wide range of chemical shifts, from 32.2 to 117.2 ppm, and that numerous couplings with the nitrogen-15 are observed (Table 1).

By crossing the data collected with those described in the literature, it was possible to highlight relationships between the structures and the measured heteronuclear coupling constants. The nitrogens-15 of the different synthesized compounds are each one implicated in different chemical bonds (amine, amide, lactam, *N*-carboxyl-lactam, *N*-carboxyl-enamine, and linear *N*-carboxyl-amine) and we observed here that chemical shifts of the ^{15}N isotope are clearly related to the electronic effects underwent by the nitrogen. In addition, we also detected the strong effect of the electron withdrawing groups moving the signal to the low fields. Thanks to the ^{15}N NMR spectroscopy, we were firstly able to unambiguously attribute each signal. This point is crucial for imagery if we need to localize and identify those potentially bio-active compounds in vivo.[5]

We then analyze the coupling constants $^1J(^{15}N–^1H)$. All possess very close values (91.0–93.1 Hz) that could be



Scheme 4. Reagents and conditions: (i) $NaBH_4$, MeOH, 0 °C, 30 min.

Table 1
Selected NMR data for compounds **5**–**11**

Entry	Product	δ^{c} ^{15}N (ppm)	$^{n}J(^{1}H–^{15}N)$ (Hz)	$^{n}J(^{13}C–^{15}N)$ (Hz)	
1	**5**[a]	32.2	—	$^{1}J = 7.6$	(CH_2 Gly)
2	**6**[b]	86.5	$^{1}J = 92.3$	$^{1}J = 13.1$	(CH_2 Gly)
				$^{1}J = 14.7$	(CO amide)
				$^{2}J = 7.2$	(C^{*})
3	**7**[a]	107.9	$^{1}J = 91.0$	$^{1}J = 8.4$	(CH_2 Gly)
				$^{1}J = 14.8$	(CO lactam)
				$^{2}J = 6.4$	(C^{*})
4	**8**[b]	117.2	$^{2}J = 0.8$ (CH_2 Gly)	$^{1}J = 8.7$	(CH_2 Gly)
			$^{2}J = 0.9$ (CH_2 Gly)	$^{1}J = 10.5$	(CO lactam)
			$^{3}J = 1.7$ (CH^{*})	$^{2}J = 6.9$	(C^{*})
5	**9**[b]	93.6	$^{1}J = 93.1$	—	
6	**10**[b]	76.5	$^{1}J = 93.1$	$^{1}J = 14.4$	($C_{quat}{}^{*}$)
				$^{1}J = 24.4$	(CO Boc)
7	**11**[b]	69.7	$^{1}J = 91.1$	$^{1}J = 12.0$	($C_{quat}{}^{*}$)

[a] Solvent: DMSO-d_6.
[b] Solvent: $CDCl_3$.
[c] Reference: ammonia (experimental reference: CH_3NO_2).

initially seen as irrelevant. However, these values inform us about the orientation of the amide bond. More exactly, it translates the fact that this one is 'cisoïd' or 'transoïd' (i.e., if ω angle value is 180° or 0°, respectively), by following the general rule[12] below, only valid when the nitrogen-15 is involved in an amide bond:

$$\text{If } 92.15\text{ Hz} < {}^{1}J({}^{15}\text{N}–{}^{1}\text{H}) < 94.45\text{ Hz} \rightarrow (\omega = 0^{\circ})$$

$$\text{If } 89.30\text{ Hz} < {}^{1}J({}^{15}\text{N}–{}^{1}\text{H}) < 91.13\text{ Hz} \rightarrow (\omega = 180^{\circ})$$

To study the conformation of (3*S*)-1,4-di-*tert*-butoxycarbonyl-3-isopropylpiperazine-2,5-dione **8**, we chose to apply the Karplus et al. equations.[13]

$$^{3}J(^{15}\text{N}–^{1}\text{H})(\theta) = -1.514 \times a^{2}(^{3}J(^{1}\text{H}–^{1}\text{H})(\theta)) \quad (1)$$

$$^{3}J(^{1}\text{H} - {}^{1}\text{H}) = 7.0 - 1.0\cos(\theta) + 5.0\cos(2\theta) \quad (2)$$

The crystallographic data of **8** by X-ray diffraction[14] (Fig. 1) allowed us to measure the required value of the dihedral angle ^{15}N(2)–C(3)–C(4)–H ($\theta = +155.4°$). Applying then Eq. 2, we calculated the value of the resulting $^{3}J(^{1}H–^{1}H)$ as 11.18 Hz. Considering the fact that '*a*' in Eq. 1 is the partial s character of nitrogen, and that the nitrogen hybridization is sp^2, its value is 0.33. We were then able to calculate a theoretical value of 1.8 Hz for $^{3}J(^{15}N–^{1}H)$, while a real value of 1.7 Hz was measured on the 200 MHz NMR spectra (solvent: $CDCl_3$). According to these results, we can postulate that the conformation of the activated bis lactam **8** in solution is very close to its crystalline state. The proposed mechanism of the TRAL could then be validated,[3] the locked conformation of the activated diketopiperazine being critical to obtain a total diastereoselectivity. Besides, the well-determined orientations by X-ray of the two *tert*-butoxycarbonyl groups (Boc) corroborate the kinetic formation of the first enolate on the α-carbon of the glycine residue, which is less bulky than the α-carbon of the valine residue (see Scheme 5).

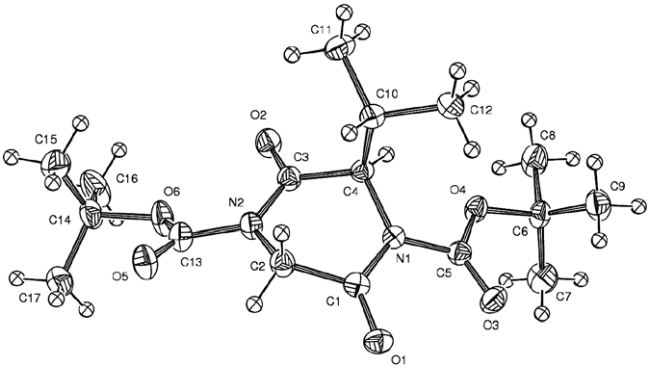
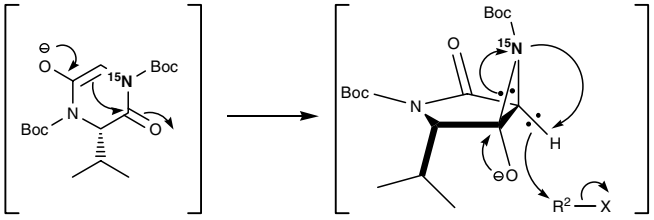


Fig. 1. ORTEP representation of (*S*)-**8**.



Scheme 5. Proposed mechanism to explain the observed stereoselectivity of the TRAL.

In conclusion, after the development of a new and efficient method to prepare (*S*)-3-isopropyl-piperazine-2,5-dione with excellent optical purity, we have initiated a [^{15}N]-labeled adaptation of the TRAL chemistry already developed in our group, to comfort a postulated mechanism. Original [^{15}N]-labeled optically pure compounds of biological interest such as diketopiperazine, pyrroline-2-one, pyrrolidine-2,4-dione, and statine analogues were prepared in excellent yields. Further works are actually devoted for their applications in medical imagery using ^{15}N magnetic resonance (in vitro protocols).

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