

Conformationally Constrained Dipeptides. Obtention
of Enantiomerically Pure 6-Acetamido-5-Oxo-1,2,3,5,6,7-
Hexahydro-3-Indolizine Carboxylic Acid

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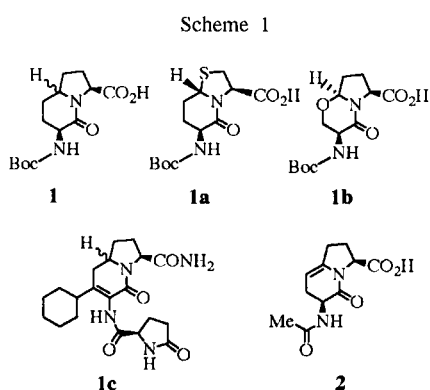
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Received March 31, 2000

The separation of a stereoisomeric mixture of esters **6**, and the synthesis of the new enantiomerically pure unsaturated 6,5-fused bicyclic lactam **2** are described. The key-step involves trimethylsilyl iodide cleavage, without racemization, of a vinylogous carbamate. The *cis* *N*-acetylamino acid **2** is a new scaffold for the synthesis of rigid β -turn mimetics.

J. Heterocyclic Chem., **37**, 1491 (2000).

Conformationally rigid analogs of natural peptides (peptidomimetics) have emerged as important tools for the study of the central role of peptides and proteins in the communication, regulation and replication of biological systems [1]. The need to replace natural amino acids in peptides in order to obtain drug-like target molecules has stimulated a great deal of innovation and has allowed a better understanding in the interactions of small molecules with proteins such as enzymes or receptors [2]. The bicyclo[4.3.0]nonane scaffold (**1**) (Scheme 1), allowing for the design of conformationally restricted moieties for Ala-Pro dipeptides, have been described [3,4] and seem to be more stable under acidic conditions than the 1-thia [5] or 8-oxa [6] analogs (compounds **1a,b**).



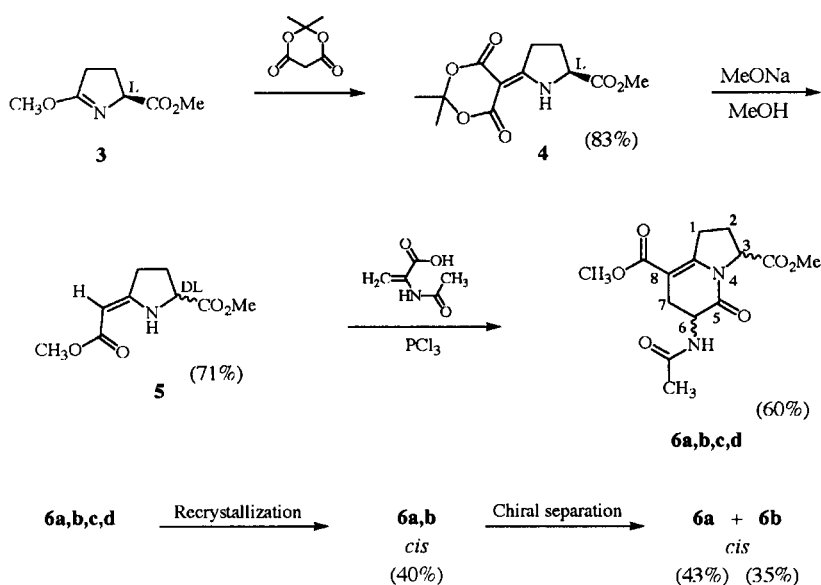
Recent reports describe the synthesis of such heterocycles [7], and continuing our research in the field of peptidomimetics of therapeutic interest [8], we describe here a flexible synthesis of indolizine **2**. To the best of our knowledge [1,7], only one example (**1c**) of unsaturated analog of **2** has been described [9]. We have also reported [10] the synthesis of racemic diester **6** and racemic acid **2**. In the present work, we studied the separation of a

stereoisomeric mixture of diesters **6** and the synthesis of the *cis* acids **2a** and **2b** starting from the *cis* compound **6**.

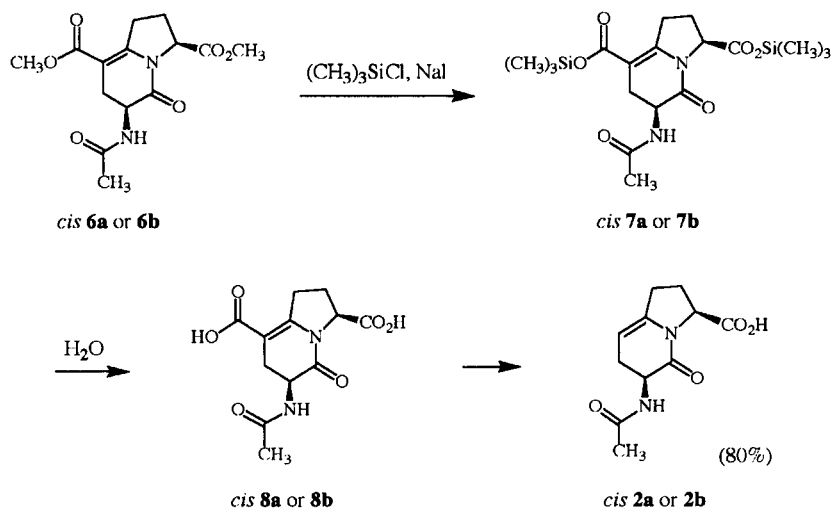
Condensation of Meldrum acid (Scheme 2) with iminoether **3** derived from L-pyroglutamic acid led to the enaminoester **4** in 83% yield [11]. Due to the acidity of its enamine function, the opening of Meldrum's ring in the ester **4** was carried out with two equivalents of sodium methoxide [12], and a complete racemization of this compound was observed [13]. After neutralization with two equivalents of hydrochloric acid, a spontaneous decarboxylation occurred, leading to 71% yield of β -enaminoester **5**. A slight modification of the published synthesis [10,12] avoided a chromatographic purification. It was checked by nmr (COSY and ROESY) that, as for other β -enaminoesters [14,15], only the *Z*-isomer was obtained. Transformation of this compound to lactam **6** was performed using a general method [16,17], by heating β -enaminoester **5** with acetamidoacrylic acid and phosphorous trichloride in a mixture of dioxane and toluene. According to Capps [16], the most likely mechanism for the ring formation is a Michael reaction followed by cyclization promoted by phosphorous trichloride.

After recrystallization from acetone/ethyl acetate (1/1), indolizine **6** was obtained in 60% yield as a 50/50 mixture of two racemics **6a,b** and **6c,d**. Generally conformationally restricted moieties have to be in the *cis* configuration [3,4] in order to act as rigid β -turn peptidomimetics. Hence, the separation of the isomers of **6** was realized. Another recrystallization of **6** in acetone yielded the pure *cis* racemic product **6a,b** [18], whose resolution in each enantiomer was performed by chiral chromatography using a Chiralpak AD column. Pure compounds *cis* **6a** and *cis* **6b** were thus obtained. The resistance of β -enaminoesters to saponification was reported [19] and special methods such as heating with boric acid have to be used for such a saponification [20]. We have previously shown

Scheme 2



Scheme 3



that saponification of β -enaminoesters derived from pyroglutamic acid with sodium hydroxide proceeds only on the 5-methoxycarbonyl group [12,21]. In the present work, it was observed that saponification of the methyl esters of **6** was effective with a sodium hydroxide solution. Nevertheless epimerization also occurred again.

Trimethylsilyl iodide [22] is a very attractive reagent for the cleavage of esters [23], carbamates [24] and vinylogous carbamates [12], frequently inducing no racemization. The sodium iodide/trimethylsilyl chloride/acetonitrile mixture generally gave the same results [25]. Here, the reaction of **6a** or **6b** with this reagent yielded

easily trimethylsilyl diesters **7a** or **7b** respectively. Water hydrolysis then afforded the corresponding diacids **8** whose enaminoacid group decarboxylates spontaneously, giving the optically pure *cis* acetylamino acids **2a** or **2b** in 80% yields.

Conclusion.

In this work, we described an efficient synthesis of new enantiomerically pure indolizines **2**. We have previously reported [10] the use of racemic derivatives of acid **2** for the synthesis of peptidomimetics and we applied these general procedures for the design of Neurokinin 1 (NK₁)

antagonists, useful in the treatment of asthma and pain [26], and for the synthesis of potential anticancer drugs, such as Ras farnesyl transferase inhibitors. We believe that this method yielding optically active products will give access to many compounds in the field of conformationally constrained peptides.

EXPERIMENTAL

Melting points are uncorrected. IR spectra were recorded on KBr pellets. ^1H nmr spectra were recorded at 300 MHz in deuteriochloroform or dimethyl- d_6 sulfoxide. Chemical shifts were recorded in ppm (δ) and coupling constants in Hertz, relative to tetramethylsilane as internal standard. The mass spectra were recorded on a quadrupolar Finnigan Mat SSQ 710 instrument in either electron impact or chemical ionization mode. Elemental analyses for C, H, N, performed by the "Service Central d'Analyses" at the CNRS, Vernaison, France, were not carried out for moisture sensitive compounds **2**. Chiral chromatography was done on a Chiralpak AD column for preparative (tris-3,5-dimethylphenylcarbamate; 250 x 50 mm i.d.; 20 μm) Daicel Chemical Industries, Chiral Technologies Europe) and analytic chromatography (tris-3,5-dimethylphenylcarbamate; 250 x 4.6 mm i.d.; 5 μm) Daicel Chemical Industries, Chiral Technologies Europe). A constant mobile phase flow was provided by a Gilson 306 pump model. Detection was performed with a Gilson 118 spectrophotometer.

Methyl (5-Methoxycarbonyl-2-pyrrolidinylidene)acetate (**5**).

A mixture of β -enaminoester **4** (**10**) (5.4 g, 20 mmoles) and sodium methoxide (30% in weight in methanol, 7.2 g, 40 mmoles) in methanol (30 ml) was refluxed for 24 hours in an inert atmosphere. Part of the mixture (20 ml) was evaporated and the residue was added to a cooled (0 $^\circ\text{C}$), well stirred, mixture of hydrochloric acid (36%, 6.1 g) and dichloromethane (200 ml). After the end of gas evolution, the organic layer was separated and the aqueous phase was extracted with dichloromethane (250 ml). The combined organic phases were washed with water saturated with sodium hydrogen carbonate and sodium chloride. After drying, the solution was evaporated, giving 71% of compound **5**. Physical data are identical to that of an authentic sample [12].

Dimethyl (3*S*,6*S*)-6-(Acetylamino)-5-oxo-1,2,3,5,6,7-hexahydroindolizine-3,8-dicarboxylate (**6a**) and Isomers.

Acetamidoacrylic acid (5 g, 38.7 mmoles) and phosphorus trichloride (3.33 ml, 38.7 mmoles) were added to a solution of β -enaminoester **5** (7 g, 35.2 mmoles) in a dry mixture of dioxane (30 ml) and toluene (15 ml). The solution was stirred and refluxed under nitrogen for 4 hours. The solvents were removed under vacuum. The residue was dissolved in chloroform and washed successively with saturated aqueous sodium hydrogen carbonate, hydrochloric acid (1 *N*), water and saturated aqueous sodium chloride. The chloroform solution was dried over magnesium sulfate, filtered and the solvent was evaporated. Recrystallization of the residue from ethyl acetate/acetone (1/1) yielded 60% in a 50/50 mixture of the two racemics (**6a,b**) and (**6c,d**), mp 181-182 $^\circ\text{C}$; ir (potassium bromide): ν 3305 (N-H), 1740 (C=O), 1690 (C=O), 1646 (C=O) cm^{-1} ; Surprisingly for a mixture, the ^1H nmr spectrum is relatively simple: ^1H nmr (deuteriochloroform): δ 1.99 (s, 3H), 2.11-2.48 (m, 2H), 2.85-3.16 (m, 2H), 3.32-3.54 (m, 2H), 3.74

(s, 3H), 3.76 (s, 3H), 4.58-4.62 (m, 1H), 4.79 (dd, 1H, $J = 7.0$ Hz, $J' = 2.9$ Hz), 6.28 (d, 1H, $J = 5.4$ Hz); ms: (chemical ionization) m/z 311 (MH^+), 279, 251, 192.

Anal. Calcd. for $\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_6$: C, 54.19; H, 5.85; N, 9.03. Found: C, 54.01; H, 5.84; N, 9.04.

Isolation of Isomers **6a**, **6b**.

Recrystallization from a minimum amount of acetone (4 ml for 100 mg of **6a,b,c,d**), yields exclusively the *cis* racemic compound **6a,b** (40%); the separation of each enantiomer (**6a**) and (**6b**) of the racemic **6a,b** was performed by chiral chromatography using a Chiralpak AD column. A constant mobile phase flow of 100 ml/minute was used and detection was realized at 280 nm. Injection volumes were 5 ml of a stock solution of 8 g/L of compound in isopropanol. Mobile phase elution was made isocratically using 80/20 hexane/isopropanol. Chromatography was performed at 40 $^\circ\text{C}$. The first eluted compound was **6a** (7.6 minutes), then **6b** (10.1 minutes); (-)-**6a** (43%), white powder, mp 190-191 $^\circ\text{C}$; $[\alpha]_{25}^D = -15.0^\circ$ (CHCl_3 , $c=1$); (+)-**6b** (35%), white powder, mp 190-191 $^\circ\text{C}$; $[\alpha]_{25}^D = +15.0^\circ$ (CHCl_3 , $c=1$). Ester **6a** or/and **6b**; ir (potassium bromide): ν 3305 (N-H), 1740 (C=O), 1690 (C=O), 1646 (C=O) cm^{-1} ; ^1H nmr (deuteriochloroform): δ 1.88 (s, 3H), 2.03-2.32 (m, 2H), 2.85-3.16 (m, 2H), 3.34-3.48 (m, 2H), 3.72 (s, 3H), 3.74 (s, 3H), 4.55-4.61 (m, 1H), 4.77 (dd, 1H, $J = 6.9$ Hz, $J' = 2.8$ Hz), 6.44 (d, 1H, $J = 5.3$ Hz); ms: (chemical ionisation) m/z 311 (MH^+), 279, 251, 192.

(3*S*,6*S*)-6-Acetylamino-5-oxo-1,2,3,5,6,7-hexahydro-3-indolizine Carboxylic Acid (**2a**) and (3*R*,6*R*) (**2b**).

Chlorotrimethylsilane (960 mg, 8.68 mmoles) and sodium iodide (1.30 g, 8.68 mmoles) were added to a solution of indolizone **6a** or **6b** (900 mg, 2.89 mmoles) in 80 ml of acetonitrile. The solution was stirred and heated at reflux under nitrogen for 48 hours. The nmr yield in **7a** or **7b** was 100%. Water (50 ml) was added and the solution was heated at 80 $^\circ\text{C}$ for 30 minutes. The solution was washed with chloroform (2 x 40 ml), the aqueous phases were evaporated under reduced pressure and boiling absolute ethanol was added to the residue. Precipitate of sodium chloride was filtered and the solvent was evaporated. The residue crystallized from absolute ethanol/methylene dichloride (1:1) yielding indolizone **2a** or **2b** in 80% yield; (-)-**2a**: white powder, mp >100 $^\circ\text{C}$ (hygroscopic); $[\alpha]_{25}^D = +23.0^\circ$ (MeOH, $c=1$); (+)-**2b**: white powder, mp >100 $^\circ\text{C}$ (hygroscopic); $[\alpha]_{25}^D = +23.0^\circ$ (MeOH, $c=1$). Acid **2a** and/or **2b**; ir (potassium bromide): ν 1730 (C=O), 1670 (C=O), 1630 (C=O) cm^{-1} ; ^1H nmr (dimethyl- d_6 sulfoxide): δ 1.85 (s, 3H), 1.90-2.60 (m, 6H), 4.10-4.25 (m, 1H), 4.40-4.45 (m, 1H), 8.20-8.30 (m, 1H); ms: (electronic impact) m/z 238 (M^+), 193 (M-COOH). Optical purity of acids **2a** and **2b** was found 100% by analytical chiral HPLC (1 ml/minutes, $t_1 = 9.2$ minutes (**2a**), $t_2 = 11.0$ minutes (**2b**), 40 $^\circ\text{C}$, hexane/isopropanol (80/20)). Acids **2a** and **2b** are hygroscopic, therefore elemental analyses was not performed.

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[26] The synthesis and biological properties of these peptidomimetics will be reported elsewhere.