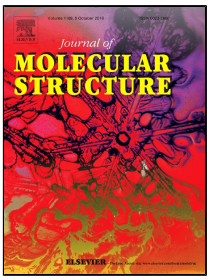
Accepted Manuscript



Synthesis and spectroscopic characterization of cyclobutyl hydantoins

Juan Caturelli, M. Florencia Martini, Lucas Fabian, Graciela Y. Moltrasio, Albertina G. Moglioni

PII:	S0022-2860(18)30708-7
DOI:	10.1016/j.molstruc.2018.06.007
Reference:	MOLSTR 25294
To appear in:	*Journal of Molecular Structure*
Received Date:	13 March 2018
Accepted Date:	01 June 2018

Please cite this article as: Juan Caturelli, M. Florencia Martini, Lucas Fabian, Graciela Y. Moltrasio, Albertina G. Moglioni, Synthesis and spectroscopic characterization of cyclobutyl hydantoins, *Journal of Molecular Structure* (2018), doi: 10.1016/j.molstruc.2018.06.007

Synthesis and spectroscopic characterization of cyclobutyl hydantoins

Juan Caturelli[a], M. Florencia Martini[a,b], Lucas Fabian[a,b], Graciela Y. Moltrasio[c] and Albertina G. Moglioni[a,b,*]

[a] *Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Cátedra de Química Medicinal, Junín 956 PP (1113), Buenos Aires, Argentina.*
[b] *CONICET-Universidad de Buenos Aires. Instituto de la Química y Metabolismo del Fármaco (IQUIMEFA), Junín 956 SS (1113) Buenos Aires, Argentina.*
[c] *Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Química Orgánica, Junín 956 3er floor, Buenos Aires, Argentina.*
** Corresponding author: Departamento de Farmacología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Instituto de la Química y Metabolismo del Fármaco (IQUIMEFA), Junín 956, (1113) Buenos Aires, Argentina.*
E-mail addresses: amoglio@ffyb.uba.ar

Abstract

The hydantoin moiety has proved to be an important pharmacophore that confers a wide range of biological properties to different derivatives. Thus, synthetic methods have been developed to obtain such molecules. Herein, we describe the heterocyclization process to obtain imidazolidine-2,4-diones (hydantoin compounds) from methylcyclobutyl ketones and cyclobutanones derived from (-)-(1*S*)-α-pinene and (-)-(1*S*)-verbenone through the Bucherer-Berg reaction. The methylcyclobutyl hydantoins and the spirohydantoin obtained were fully characterized, determining their absolute stereochemistry by nuclear magnetic resonance experiments and theoretical calculations.

Keywords: methylcyclobutyl ketones, cyclobutanones, Bucherer-Berg reaction, hydantoins, nuclear magnetic resonance

Introduction

Hydantoins and their derivatives constitute a group of privileged structures [1] displaying a wide range of biological activities [2]. These properties have encouraged their use in fragment-based drug design processes [3]. These procedures have led to the discovery of novel entities with therapeutic utility (Figure 1) [4].

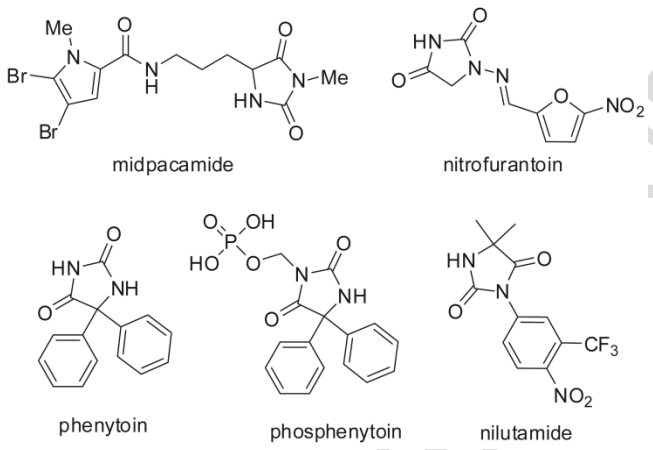


Figure 1. Some therapeutically useful hydantoins

The hydantoin core is present in some natural products, for example (+)-hydantocidin [2], which is a spironucleoside structure with herbicidal activity. This compound can be isolated from *Streptomyces hygroscopicus*, growing in Japan. The hydantoin moiety is also present in many alkaloids present in sponges and corals, such as aplysinopsins [2], which are known to have cytotoxic properties (Figure 2).

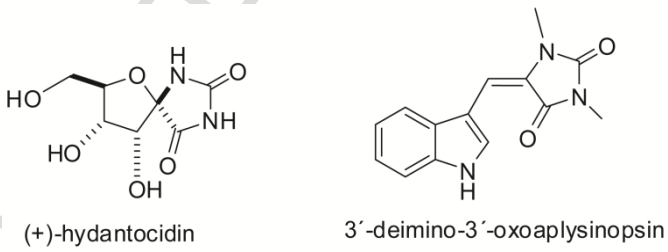
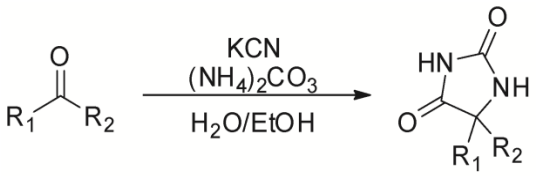


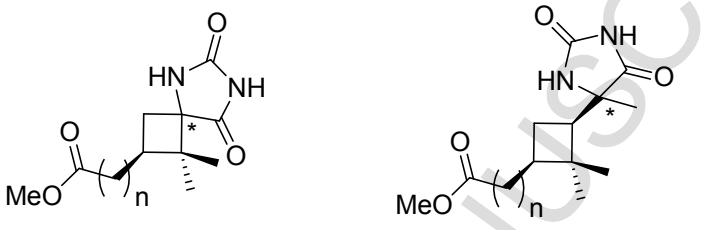
Figure 2. Natural products bearing the hydantoin moiety

One of the methods of choice for the synthesis of 5,5-disubstituted hydantoins is a multicomponent reaction proposed by Bucherer and Berg (BB) in 1934 [5]. This reaction involves the heterocyclization of either an aldehyde or a ketone with ammonium carbonate and potassium cyanide under strong basic conditions (Scheme 1). Other methods of synthesis, as well as the reactivity of this group of heterocyclic compounds, have been reviewed by Meusel and Gutschow in 2004 and by Colacino et al, in 2017 [6].The behavior of these compounds in solid phase has been recently described by Nogueira et al (2017) [7].



Scheme 1. General procedure to obtain hydantoins from carbonyl compounds

In this work we describe the synthesis and characterization of two hydantoine classes developed in our laboratory, namely, class I hydantoins, obtained from cyclobutanones, and class II hydantoins obtained from methylcyclobutyl ketones. The synthesis of these compounds was achieved through the BB reaction on homochiral ketones derived from terpenes(Figure 3).

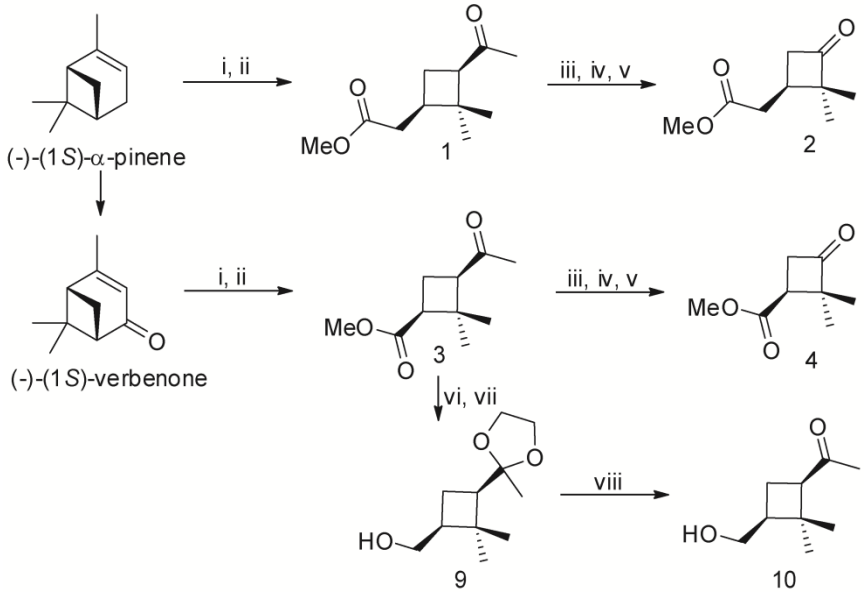


type I type II

Figure 3. General structure for the hydantoins synthesized, with n = 0 or 1.

Results and Discussion

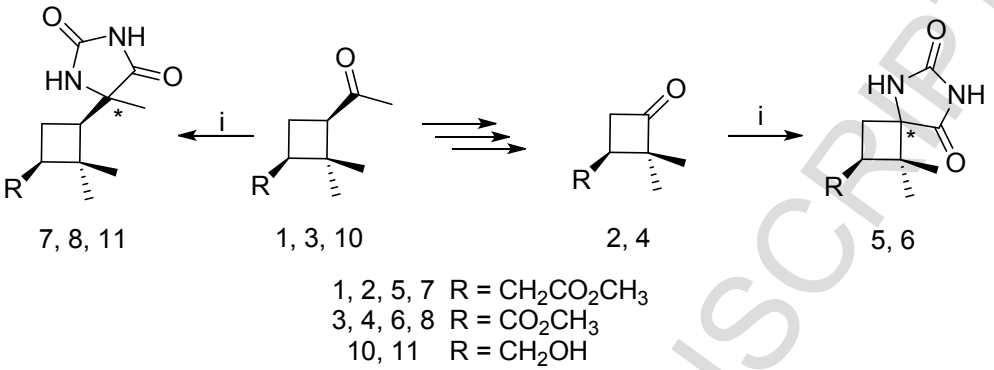
The cyclobutyl precursors were prepared through the cleavage of commercially available bicyclic enantiomerically enriched (-)-(1*S*)-α-pinene and (-)-(1*S*)-verbenone, according the pathway showed in the Scheme 2.



Scheme 2. Synthesis of ketones **1**-**4** and **10**. *Reagents and conditions*: i) $NaIO_4$, $RuCl_3$, $H_2O/CH_3CN/CCl_4$, rt; ii) CH_2N_2, Et_2O, 50 ºC; iii) *m*-CPBA, CH_2Cl_2, rt; iv) K_2CO_3, H_2O/MeOH/THF, rt; v) DMP, CH_2Cl_2, rt; vi) $(CH_2OH)_2$, PPTS, PhH, reflux; vii) $LiBH_4$, THF, rt; viii) PPTS, $(CH_3)_2CO$, 56 ºC.

The synthetic pathways outlined in Scheme 2 allowed obtaining methylcyclobutyl ketones **1** [8] and **3** [8] and cyclobutanones **2** [9] and **4** [9] through the ruthenium-catalysed oxidative cleavage of the double bond present in (-)-(1*S*)-α-pinene and (-)-(1*S*)-verbenone.

Ketones **1**-**4** were then treated with ammonium carbonate and potassium cyanide, under BB conditions, to form hydantoins **5**-**8** (Scheme 3).

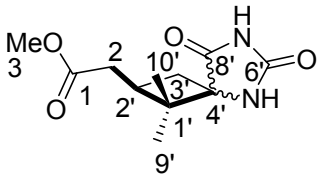


Scheme 3. Synthesis of hydantoins **5**-**8** and **11**.*Reagents and conditions*: i) $(NH_4)_2CO_3$, KCN, NH_4Cl, H_2O/EtOH, 60ºC.

Under BB conditions, to obtain class I hydantoins, cyclobutanones **2** and **4** were extremely reactive, obtaining only one hydantoin compound in the case of cyclobutanone **2**. The expected hydantoine **6** could not be obtained. Considering that the starting material has one stereogenic center, with a second one being generated during the reaction; only two diastereoisomeric products were possible. Only compound **5** was identified as one hydantoinic product, which could be isolated by preparative thin layer chromatography from the complex reaction mixture containing ketone **2**. The NMR (Cl_3CD) spectra analysis of compound **5** is shown in Table 1.

The NOESY and nOe experiments carried out with compound **5** in DMSO-*d*6, a solvent that, in this case, allowed a greater separation of the signals corresponding to the methyl groups, demonstrated that the hydrogen atom signals corresponding to the methyl group appearing at a higher field (δ 0.96 *ppm*) presented a nOe effect with the methine hydrogen atom at δ 2.48 ppm. On the other hand, the hydrogen atoms corresponding to the side chain methylene group presented a nOe effect with the methyl group at δ 0.99 ppm, and with the endocyclic methylene hydrogen atom (3'-Ha). These phenomena allowed concluding that these three atomic groups are on the same face of the ring (Figure 4). Although different NMR experiments allowed assigning each hydrogen and carbon atoms of compound **5**, the configuration of the spiro-carbon could not be determined.

Table 1. Chemical shifts (ppm) and hetero- and homonuclear correlations for compound **5** determined inCl_3CD.



Position	**δ ^{1}H**	**δ ^{13}C (HSQC)**	**δ ^{1}H (HMBC)**
1	-	172.4	3.69 and 2.35
2	$H_{a/b}$ 2.35 (dd, *J*= 7.8, 15.5 Hz, 1H)	34.7	
	$H_{a/b}$ 2.42 (dd, *J*= 7.6, 15.4 Hz, 1H)		
3	3.69 (s, 3H)	51.7	
1'	-	46.9	2.35; 2.65
2'	2.80 (ddd, *J*= 7.8, 8.0, 8.0 Hz, 1H)	34.3	2.35; 2.42; 1.98 and 1.15
3'	$H_{a/b}$2.65 (dd, *J*= 8.5, 11.4 Hz, 1H)	35.0	
	$H_{a/b}$ 1.98 (dd, *J*= 11.2, 11.2 Hz, 1H)		
4'	-	66.0	1.15
5'	5.76 (br, 1H)	-	
6'	-	155.4	
7'	7.51 (br, 1H)	-	
8'	-	175.2	
9'	1.16 (s, 3H)	22.6	
10'	1.15 (s, 3H)	19.0	

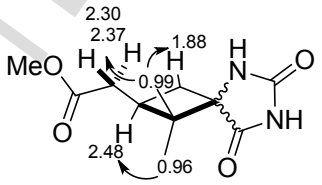
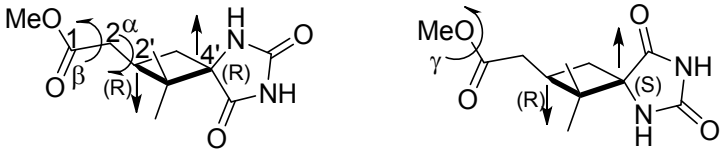


Figure 4. nOe effect observed for compound **5**

Chemical shifts in ^{1}HNMR spectra were determined in DMSO-*d6*.

Given that only one diastereomer of compound 5 was obtained, both possible stereoisomers were considered for structural assignment. This was carried out, taking into account that the closure of the hydantoin ring can generate the compound 5a, with the nitrogen atom located in relation *syn* to the chain in position 2' of the original ring, and its diastereomer 5b of *anti* ratio (Figure 5).



5a **5b**

Figure 5. Possible stereoisomers of compound **5**

In order to determine the absolute configuration of the new chiral center, computational chemistry methods, such as DP4 and ANN were employed to distinguish both diastereoisomers, allowing the assignment of one of them to the compound obtained. Possible structures were subjected to conformational searching using the "conformational search" module of the Hyperchem 8.0.92 software [10]. These conformational searches were performed starting with the structures previously optimized with the MM+ force field corresponding to each diastereoisomer. For the α and β angles (Figure 5), the rotation was studied between 0° and ± 180°, rotating every 10°, and allowing the simultaneous folding of the cyclobutanic ring.

As described by Pawar *et al.*, the γ angle was fixed at 180° (conformation *Z*) [11]. The convergence criterion in each case was defined at 1000 cycles or a gradient less than 0.01 kcal/mol, leaving the other parameters unchanged. At least 10 conformers for each diastereoisomer, whose energy values were ≤ 5kcal/mol in relation to the lowest energy conformer, were selected. These 20 conformers were then re-optimized using the density functional theory (DFT) module///tool of the Gaussian 03 software [12], using the Becke, 3-parameter, Lee Yang-Parr (B3LYP) functional combined with the 6-31+g(d,p) base. Those conformers displaying energy values within 2kcal/mol in relation to the lowest energy conformer were selected (Table 2).

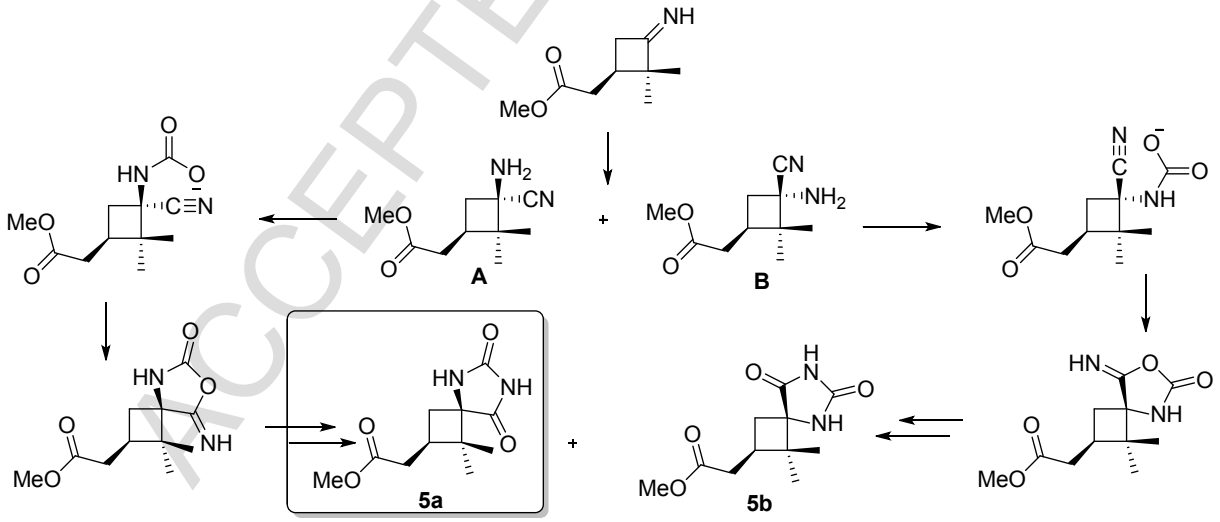
Table 2. Relative energy values obtained by the quantum mechanical method for conformers, calculated with DFT B3LYP/6-31+g(d,p).

	5a (2'*R*, 4'*R*)	**5b** (2'*R*, 4'*S*)
Conformer	relative energy (kcal/mol)	relative energy (kcal/mol)
1	0.863699	0.171445
2	0.799112	0.173021
3	0.000000	8.017752
4	0.000069	8.150602
5	2.357174	8.021953
6	2.356911	0.174333
7	0.375184	0.171971
8	0.375184	0.172496
9	0.960933	0.000000
10	0.859761	0.000263

Finally, the Gauge Independent Atomic Orbitals (GIAO) method [13] was applied for each selected conformer, using the mPW1PW91 functional to obtain the values of the shielding constant (σ) for each atom. This set of values was subjected to weighting through the Boltzmann analysis on all selected conformers. The DP4 method (http://www-jmg.ch.cam.ac.uk/tools/nmr/DP4/), developed by Smith and Goodman (2010) [14] was applied to these data. Taking together the chemical shifts of ^{1}H and ^{13}C atoms (data not shown), the highest probability value obtained was 99.7% for the stereoisomer **5a**, with the absolute configuration *2'R,4'R*. This configuration was assigned to compound **5**.

These values were also loaded, together with the experimental chemical shifts of the ^{13}C and ^{1}H atoms, in the spread sheet containing the neural network provided by Zanardi et al. [14b]. The correlation analysis performed between the experimental and the calculated data for the*2'R,4'R* isomer (**5a**) and isomer *2'R,4'S* (**5b**) indicated that the structure **5a** was the correct one in the ANN-TMSvac, in accordance with the results obtained applying the methodology previously described by Goodman et al. [14a]. Therefore, we decided to apply DP4 and CP3, for the rest of the compounds described in this work.

The absolute configuration of the newly formed stereogenic center in compound **5** can be explained on the basis of the mechanistic considerations raised by other authors [5]. In the intermediate A, the hydrogen bond formation between the amino group and the carbonyl group of the ester in the 1,3-*cis* relative position, would further favor this reaction pathway (Scheme 4). The presence of the methyl ester chain is a determining factor in the course of the reaction, as we have previously observed in other heterocyclization reactions[15]. Besides, such methyl ester group is responsible for the observed diastereoselectivity.

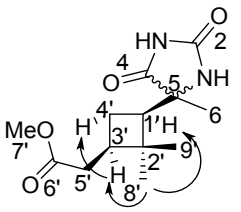


Scheme 4. Possible reaction pathway to obtain spirohydantoins **5a** and **5b**

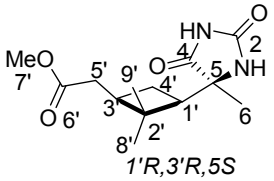
From ketones **1** and **3**, a complex mixture of products was obtained. Taking into account that a new chiral center was generated during the reaction, and the potential isomerization from a *cis* to a *trans* configuration in the starting materials [8], we decided to determine whether the stereogenic centers had been modified during the heterocyclization reaction. To that end, a complete determination of the conformation of all diastereoisomers was determined by mono- and bi-dimensional NMR methodologies (HSQC, HMBC and NOESY).

The analysis of the complex reaction mixture resulting from ketone **1** shows a main product together with other compounds, including saponification derivatives of the methyl ester group. Mono- and bi-dimensional NMR spectra allowed determining that the main product, isolated from the reaction mixture, corresponded to hydantoin **7**. The complete assignments of compound **7** are presented in Table 3. The nOe-effects observed in the NOESY spectrum of this compound (see figure in Table 3) allowed concluding that the substituents at the 1,3-positions in the cyclobutane ring retained the *cis* relative configuration. However, the configuration assignment of the new stereocenter (*C5*) created in the hydantoin ring could not be determined. In order to assign such configuration, the conformational minima for each of the possible diastereoisomers of compound **7** (**7a** and **7b**) were calculated. For this purpose, a geometry optimization was performed to obtain the σ values for each H and C atom. The chemical shifts of H atoms for **7a** and **7b** are shown in Table 3.

Table 3. Chemical shifts (ppm) assigned from hetero- and homonuclear correlations spectra for compound **7a** and **7b** (COSY and HSQC spectra) and calculated chemical shifts for H of compound **7**

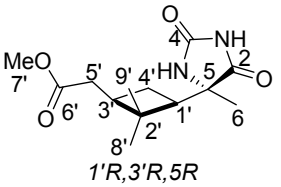


7



1'R,3'R,5S

7a



1'R,3'R,5R

7b

Position	$\delta^1H_{exp.}$	**7a** $\delta^1H_{calc.}$	**7b** $\delta^1H_{calc.}$	$\delta^{13}C_{exp.}$
6'	-			173.1
5'	2.27 (m, 1H)	2.18	2.11	34.7
	2.34 (ddd, *J*= 7.7, 7.8, 8.2 Hz, 1H)	2.29	2.47	
7'	3.67 (s, 3H)	3.71	3.62	51.5
1'	2.27 (m, 1H)	1.89	2.30	38.4
2'	-			42.7
3'	2.32 (m, 1H)	2.25	2.13	48.3
4'	1.63 (dd, *J*= 10.8, 10.9 Hz, 1H)	2.13	1.22	24.3
	1.92 (m, 1H)	2.29	2.32	
8'	1.22 (s, 3H)	1.15	1.20	30.9
9'	1.08 (s, 3H)	0.94	0.90	17.1
1	8.68	-	-	-
2	-	-	-	157.0
3	6.18	-	-	-
4	-	-	-	176.9
5	-	-	-	64.7
6	1.44 (s, 3H)	1.25	1.12	22.4

The energy difference between both diastereoisomers of **7** is small (ΔE=1.99 kcal/mol), being the stereoisomer of configuration *1'R,3'R,5S* (**7a**) slightly more stable. Taking into account that only one of the two posible diastereoisomers of compound **7**was characterized, and that such structure could correspond to one of the two stereoisomers calculated, the DP4 method applied. The same procedure was followed for compound **5**. In this case, the highest probability value (93.9%), obtained with data calculated for ^1H atoms, was assigned to stereoisomer **7b**, with the absolute configuration *1'R,3'R,5R*. Then, the structure **7b** was assigned to the isolated compound **7**.The DP4 method applied to ^1H and ^{13}C chemical shifts (data not shown) supported this assignment.

From ketone **3**, a mixture of diastereoisomers could be isolated by preparative TLC. The bi-dimensional HMBC and HSQC NMR spectra of compounds **8**, allowed determining the position of atoms in **8a** and **8b**. Table 4 shows the complete assignment of each stereoisomer. The nOe effects observed for compounds **8a** and **8b** (Figure 6) indicated that the *cis* relative configuration present in the starting material was maintained.

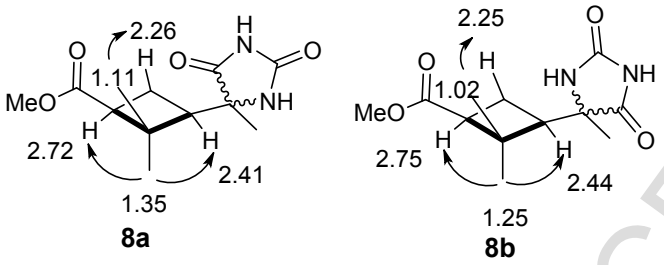
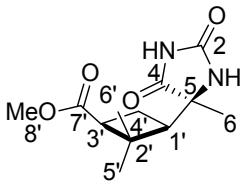


Figure 6. nOe effect observed for compounds **8a** and **8b**

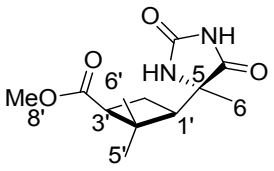
In order to define the configuration of the new chiral center generated in each one of the diastereoisomers of compound **8**, the conformational minima were calculated as performed for compound **5**. The calculated energy difference between both diastereoisomers is small (ΔE=1.80kcal/mol) with a slight difference in favor of the *1'R,3'S,5S* configuration.

Considering that the two possible diastereoisomers of **8** were characterized and that each structure may correspond to one of the two stereoisomers calculated, the CP3 method (http://www-jmg.ch.cam.ac.uk/tools/nmr/CP3.html) was applied as described by Smith and Goodman [14].By the CP3 method a 100% probability was obtained between the **8a** isomer values and the calculated values for the *1'R,3'S,5R* absolute configuration and between the **8b** isomer values and the calculated values for the *1'R,3'S,5S* absolute configuration. No changes in this probability value were obtained when the CP3 method was applied to the combination of ^{1}H and ^{13}C chemical shift values (data not shown).

Table 4.Chemical shifts (ppm) and hetero- and homonuclear correlations for compounds **8a** and **8b** and calculated chemical shifts (ppm) for ^{1}H of compounds **8**



1'R,3'S,5S



1'R,3'S,5R

Position	8a δ $^{1}H_{exp.}$	8a δ $^{13}C_{exp.}$ (HSQC)	8b δ $^{1}H_{exp.}$	8b δ $^{13}C_{exp.}$ (HSQC)	δ $^{1}H_{calc.}$ for 1'*R*,3'*S*,5*S*	δ $^{1}H_{calc.}$ for 1'R,3'S,5R
1'	2.72 (m, 1H)[a]	45.5	2.75 (m, 1H)[a]	46.1	2.58	2.54
2'	-	44.7	-	44.3	-	-
3'	2.41 (m, 1H)[a]	47.5	2.44 (m, 1H)[a]	46.1	2.36	2.48
4'	$H_{a/b}$1.85 (ddd, *J*= 8.3, 8.7, 11.1 Hz, 1H)	19.7	$H_{a/b}$2.15 (ca, 1H)	20.5	2.24	1.64
	$H_{a/b}$2.26 (m, 1H)[a]		$H_{a/b}$2.25 (m, 1H)[a]		2.07	2.15
5'	1.35(s, 3H)	31.3	1.25(s, 3H)	31.3	1.26	1.31
6'	1.11 (s, 3H)	17.8	1.02 (s, 3H)	17.3	0.91	1.09
7'	-	172.4	-	173.1	-	-
8'	3.68 (s, 3H)	51.6	3.69 (s, 3H)	51.4	3.75	3.70
1	6.86	-	6.28	-	-	-
2	-	156.3/156.0	-	156.3/156.0	-	-
3	8.68/8.80	-	8.68/8.80	-	-	-
4	-	176.8	-	176.4	-	-
5	-	64.9	-	64.3	-	-
6	1.45(s, 3H)	22.4	1.38(s, 3H)	24.3	1.17	1.25

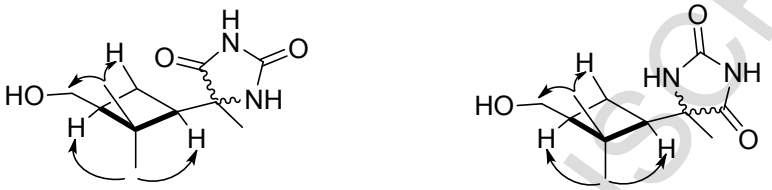
[a]*These values were observed overlapped on the spectrum. An approximate assignment of signals was done according to the best adjustment with respect to the calculated values. ca: complex absorption.*

Taking into account that the hydantoinic compounds obtained could be useful intermediates to prepare nucleoside analogs, the direct reduction of the ester group present in compounds **7** and **8**, to the hydroxymethyl group was performed. Even though lithium aluminum hydride is known to render a complex mixture of reduced heterocycles, even generating the diamine [16], this compound was employed, since under the mild experimental conditions employed in this work, it proved to render compound **9** [8]. To date, the reduction of hydantoinic compounds using this reagent has not been reported.

The complex mixture obtained lacked the 3'-carbinol hydantoins, thus it was concluded that this reducing agent also affects the heterocycle, rendering a wide range of products that are in different

states of reduction. Therefore, compound **10** [15] was synthetized through a three-step process obtaining 47% global yield. The application of BB´s conditions reaction on compound **10** rendered two hydantoic products (**11a** and **11b**). This behavior was similar to that obtained when the BB's reaction is applied to its parent compound **6**. The isolation of **11a** and **11b** was not possible by any chromatographic method, including HPLC. Therefore, a complete assignment of this mixture was attempted through mono- and bidimentional NMR studies. NOESY experiments confirmed that the 1,3-*cis* relative configuration remained unaffected, confirming that the products were a diastereoisomeric 1:1 mixture of hydantoins (Figure 7).

The HMBC and HSQC spectra, allowed determining the position of atoms in each of these molecules. Table 5 shows the complete assignment for each diastereoisomer of **11**.



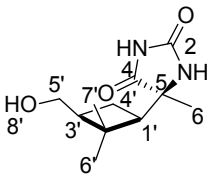
11a **11b**

Figure 7. nOe effect observed for compounds **11a** and **11b**

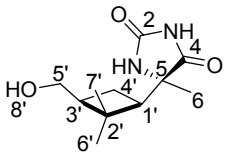
In order to determine the configuration of both diastereoisomers of **11** (**a** and **b**), the conformational minima were calculated, as previously described for compounds **5** and **6**. Table 5 also shows the δ values calculated by GIAO for ^{1}H and ^{13}C atoms of **11a** and **11b**. The calculated energy difference between both diastereoisomers was notsignificant (ΔE=0.06 kcal/mol), with a slight difference in favor of the *1'R,3'S,5S* configuration. This result is in line with those obtained experimentally.

Given that the two possible diastereoisomers of **11** were characterized, and knowing that each structure could correspond to one of the calculated stereoisomers, the CP3 method was applied. However, the calculation of probabilities employing the ^{1}H and ^{13}C chemical shifts independently led to contradictory results. An approximate assignment was attained by the combination of both values, obtaining a probability value of 98.8% between the **11a** isomer values and the calculated values for the *1'R,3'S,5S* absolute configuration, and between the **11b** isomer values and the calculated values for the *1'R,3'S,5R* absolute configuration.

Table 5. Experimental and calculated chemical shifts (ppm), and hetero- and homonuclear correlations for compounds **11a** and **11b**



1'*R*,3'*S*,5 *S*



1'*R*,3'*S*,5 *R*

Position	11a		11b		1'R,3'S,5R		1'R,3'S,5S	
	δ ^{1}H	δ ^{13}C (HSQC)	δ ^{1}H	δ ^{13}C (HSQC)	δ ^{13}C $_{calc.}$	δ ^{1}H$_{calc.}$	δ ^{13}C$_{calc.}$	δ ^{1}H$_{calc.}$
1	*	-	*	-		-		-
2	-	156.9/157.3	-	156.9/157.3	151.7	-	150.4	-
3	*	-	*	-		-		-
4	-	178.7	-	178.9	173.2	-	174.6	-
5	-	63.3	-	63.4	69.9	-	68.7	-
6	1.18 (s, 3H)	22.8	1.10 (s, 3H)	24.2	27.4	1.57	21.8	1.57
1'	2.02 (m, 1H)[a]	48.5	2.04 (m, 1H)[a]	46.2	50.0	2.39	55.6	2.33
2'	-	41.7	-	41.0	48.3	-	47.5	-
3'	1.84 (m, 1H)[a]	43.7	1.86 (m, 1H)[a]	44.6	49.2	2.69	47.7	2.27
4'	$H_{a/b}$1.48 (m, 1H)[a] $H_{a/b}$1.57 (m, 1H)	21.5	$H_{a/b}$1.50 (m, 1H)[a] $H_{a/b}$1.83 (m, 1H)[a]	22.5	28.8	2.03	30.3	2.81
5'	$H_{a/b}$3.26 (m, 1H)	61.7	$H_{a/b}$3.34 (m, 1H)	61.9	68.3	3.84	67.6	3.90
6'	$H_{a/b}$3.33 (m, 1H)		$H_{a/b}$3.50 (m, 1H)			4.00		4.10
7'	1.11 (s, 3H)	32.6	0.98 (s, 3H)	31.9	33.6	1.43	34.5	1.49
8'	0.97 (s, 3H)	16.7	0.93 (s, 3H)	15.6	20.0	1.33	19.6	1.24

[a]*Values were observed overlapped on the spectrum. An approximate assignment was done, according to the best adjustment with respect to the calculated values.*Since the spectrum was performed in D_2O,the assignment was not possible.*

Since compounds **11** are nucleoside analogs, and their isolation was not possible, a preliminary screening of their biological activity was undertaken. The antiproliferative activity of these compounds was evaluated to guide future studies evaluating either the antiviral or the anticancer activity. The MTS reduction assay was performed to evaluate the inhibitory activity of **11** against U937human promyelocytic leukemia cells to find that the mixture of **11** did not display any antiproliferative activity between 4.40 and 100 μM.

Conclusions

• The reactivity of enantiopure methylcyclobutyl ketones and cyclobutanones was studied through the BB reaction. Complex mixtures of products were obtained in all cases, including decomposition ones.

• Product **5** (class I hydantoin) was obtained as one of the two possible diastereoisomers. Its configuration could be explained on the basis of the influence of the ester group on the chiral center present on the starting material **2**.

• An exhaustive structural analysis of the new compounds **7**, **8** and **11**(class II hydantoins), employing mono- and bi-dimensional NMR spectroscopy methods, allowed confirming the retention of the 1,3-*cis* relative stereochemistry of the cyclobutanic precursors.

• The use of mono- and bi-dimentional NMR methods, together with theoretical calculations, was useful to assign the absolute configuration of compounds **5**, **7, 8** and **11**.

Experimental Section

General

Reagents were purchased from Sigma-Aldrich, and used without further purification. Solvents were distilled before use by standard purification methods [17,18]. (-)-(1*S*)-α-pinene (98 %) and (-)-(1*S*)-verbenone (94%), were purchased from Acros Organics and employed without purification. Thin layer chromatography (TLC) and preparative TLC were performed on Silica Gel F254 (Merck). The visualization reagents employed were 1.6% $KMnO_4$ in H_2SO_4 conc.; 2.2% $CH_3OC_6H_4CHO$ in EtOH or iodine. Column chromatography was performed on Silica Gel 60 240-400 mesh (Merck) with the solvent mixture indicated in each case. Melting points were measured in a Thomas-Hoover equipment. ^1H and ^{13}C NMR spectra, homo- and heteronuclear correlation spectroscopy and NOESY experiments were recorded in a Bruker 300 MHz, Bruker 500 MHz or Bruker UltraShield 600 MHz spectrometers. Unless otherwise specified, all experiments were performed at ambient temperature in the following solvents: $CDCl_3$, DMSO-*d*6 or D_2O as stated in each case. Chemical shifts are expressed in ppm (relative to the solvent). Coupling constants (J) are in Hz. FT-IR spectra were recorded as a film from acetone with a Nicolet 380 Thermo spectrometer. High resolution mass spectra (HRMS) were determined in a Bruker micrOTOF-Q II™ spectrometer.

Conformational searches were performed using MM+ force field with the Hyperchem program [10]. All conformers within 5 kcal/mol from the minimal energy one, were re-optimized using B3LYP/6-31+g (d,p) level with Gaussian G03 program package [12]. Conformers, within 2kcal/mol from the global minimum, were used in NMR calculations. A single point using mPW1PW91/6-31+g(d,p) functional and the GIAO method [13] were employed to calculate the shielding constants (σ).The tetramethylsilane (TMS) molecule has also optimized to determine the chemical shifts as a function

of this reference compound. These values, showing a Boltzmann distribution, were analyzed through different methods, as appropriate: CP3, DP4 or ANN-TMSvac [14].

Procedures and analytical data

Synthesis of hydantoins. General procedure.

To a solution of ketone (1 equiv) in a 1:1 EtOH/H_2O mixture (26 mL), $(NH_4)_2CO_3$ (1.83 g, 22.07 mmol, 10 equiv) and NH_4Cl (410 mg, 7.67 mmol, 3.6 equiv) were added followed by, KCN (560 mg, 8.63 mmol, 4 equiv) after 15 min. The solution was stirred at 60 °C for 72 h. The solvent was reduced *in vacuo*, and the solid obtained was filtered and washed with cold water. The product was purified by preparative TLC, eluting with either $PhCH_3$/EtOH 6:1 or hexane/EtOAc/MeOH 7:2:1 as stated in each case.

Methyl (2'R,4'R)-(1',1'-dimethyl-6',8'-dioxo-5',7'-diaza-spiro[3.4]oct-2'-yl)-acetate ***5***

Compound **5** was obtained as a white solid and purified by preparative TLC ($PhCH_3$/EtOH 6:1, Rf: 0.31) (101 mg, 20%) mp: 88-99 °C (decomposition), IR νmax (cm^{-1}) (film) 3292, 2970, 2931, 1720, 1367, 1205. ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$): see Table 1. HRMS: Calcd for $C_{11}H_{16}N_2NaO_4$ $[M+Na]^+$ 263.10078. Found 263.10152.

(5RS)-(1'R,3'R)-5-(3'-methylacetate-2',2'-dimethylcyclobutyl)-imidazolidin-2,4-dione ***7***

Compound **7** was obtained as a white solid. The purification of each diastereoisomer by preparative TLC (hexane/EtOAc/MeOH 7:2:1) was not successful. Therefore, they were analyzed as a mixture (425 mg, 52%) mp: 219-220 °C IR νmax (cm^{-1}) (film) (as a 4:1 diastereoisomeric mixture) 3261, 3055, 3041, 2962, 2873, 1753, 1727, 1431, 1265, 1171, 771. ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$): see Table 3. HRMS: Calcd for $C_{13}H_{20}N_2O_4Na$ $[M+Na]^+$ 291.1321. Found 291.1334.

(5RS)-(3S,3'R)-5-(3'-methoxycarbonyl-2',2'-dimethylcyclobutyl)-5-methyl-imidazolidin-2,4-dione ***8***

Compound **8** was obtained as a white solid. The purification of each diastereoisomer by preparative TLC (hexane/EtOAc/MeOH7:2:1) was not successful. Therefore, they were analyzed as a mixture (229 mg, 44%) IR νmax (cm^{-1}) (film) (as a 1:1 diastereoisomeric mixture) 2958, 2924, 1724, 1464, 1456, 1375, 1230, 1203.^1H NMR (600 MHz, CDCl3) and ^{13}C NMR (150 MHz, $CDCl_3$): see Table 4. HRMS: Calcd for $C_{12}H_{18}N_2O_4Na$ $[M+Na]^+$ 277.1164. Found 277.1165.

(5RS)-(1'R,3'S)-5-(3'-hydroxymethyl-2',2'-dimethyl-cyclobutyl)-5-methyl-imidazolidin-2,4dione ***11***

Compound **11** was obtained as a white solid. The purification of each diastereoisomer by preparative TLC (hexane/EtOAc/MeOH 7:2:1) was not successful. Therefore, they were analyzed as a mixture (133 mg, 45%) IR νmax (cm^{-1}) (film) (as a mixture) 3294, 2954, 2929, 2877, 2748,

1759, 1410, 1367. ^{1}H NMR (600 MHz, D_2O) and ^{13}C NMR (150 MHz, D_2O): see Table 5. HRMS: Calcd for $C_{12}H_{18}N_2O_4Na$ $[M-H]^-$ 225.12392. Found 225.12444.

Reduction using $LiBH_4$. General procedure

To a solution of hydantoin (1 equiv) in dry THF (9.6 mL), a 2M solution of $LiBH_4$ in THF was added dropwise (9.60mmol, 3.4 equiv). The solution was kept under a nitrogen atmosphere at reflux temperature with stirring for 24 h. The mixture was then diluted with MeOH (3 mL) and H_2O (1 mL). The solvent was reduced, and the resulting solution was extracted with EtOAc (4x30 mL). The organic phase was separated, washed with brine and dried over Na_2SO_4until dryness.

Cell proliferation assay

The *in vitro* cell proliferation was determined by a colorimetric assay (CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay, Promega, USA) according to the manufacturer's instructions. For the MTS assay, U937 human promyelocytic leukemia cells (American Type Culture Collection, Rockville, MD, USA) growing in exponential phase ($2.0x10^6$cells/mL) were seeded in a 96-well plate and incubated in an atmosphere of 5% CO_2 at 37°C. Cells were incubated with serial dilutions of the mixture of compounds **11** (4.4µM to 100 µM), 0.05% (v/v) DMSO (vehicle control group) or 400 µM db-AMPc (positive inhibition control). After incubation for 48h, 20 µl of MTS were added to each well and further incubated for 2h at 37°C. The absorbance was measured at 490 nm using the FlexStation 3 microplate reader (Molecular Devices Inc., USA). Assays were carried out in triplicate and at least three independent experiments were conducted.

Acknowledgements

This work was financially supported by grant PICT 2012-0032 from Agencia Nacional de Promoción Científica y Tecnológica, grant B033 from Universidad de Buenos Aires and grant PIP 11220120100074CO from Consejo Nacional de Investigaciones Científicas y Tecnológicas (Argentina).

References:

[1] P. J. Hajduk and J. Greer, Nat. Rev. Drug Discov. 6 (2007) 211-219.

[2] a) M. Nakajima, K. Itoi, Y. Takamatsu, H. Okazaki, T. Kinoshita, M. Shindou, K. Kawakubo, T. Honma, M. Toujigamori, T. Haneishi, J. Antibiot. 44 (1991) 293-300. b) M. S.Luer, Neurol. Res. 20 1998 178-182, c) D. Bialonska and J.K. Zjawiony, Marine Drugs 7 (2009) 166-183.

[3] H. Prevet, M. Flipo, P. Roussel, B. Deprez and N. Willand, Tetrahedron Lett.57 (2016) 2888-2894.

[4] a) Y. Chen, L. Su, X. Yang, W. Pan and H. Fang, Tetrahedron 71 (2015) 9234-9239; b) M. A. Khanfar, B. A. Asal, M. Mudit, A. Kaddoumi and K. A. El Sayed, Bioorg. Med. Chem.17(2009) 6032; c) M. Jansen, H. Potschka, C. Brandt, W. Löscher and G. Dannhardt, J. Med. Chem. 46 (2003) 64-73.

[5] J. T. Edward and C. Jitrangsri, Can. J. Chem. 53 (1975) 3339-3350.

[6] a) M. Meusel and M. Gütschow, Org. Prep. Proced. Int. 36 (2004) 391-443; b) L. Konnert, F. Lamaty, J. Martinez and E. Colacino, Chem. Rev.117 (2017) 13757−13809.

[7] a) B. A. Nogueira, G. O. Ildiz, J. Canotilho, M. E. S. Eusébio, M. S. C. Henriques, J. A. Paixão and R. Fausto, J. Phys. Chem. A 121(2017)5267-5279; b) B. A. Nogueira, G. O. Ildiz, M. S. C. Henriques, J. A. Paixão and R. Fausto, J. Mol. Struct. 1148 (2017) 111-118.

[8] A. G. Moglioni, E. García-Expósito, G. P. Aguado, T. Parella, V. Branchadell, G. Y. Moltrasio and R. M. Ortuño, J. Org. Chem. 65 (2000) 3934-3940.

[9] J. A. Caturelli Kuran and A. Moglioni, Synthetic Commun. 44 (2014) 2393-2400.

[10] HyperChem® Professional 7.51, Hypercube, Inc. 1115NW 4th Street, Gainesville, FL 32601, US.

[11] D. M. Pawar, A. A. Khalil, D. R. Hooks, K. Collins, T. Elliott, J. Stafford, L. Smith and E. A. Noe, J. Am. Chem. Soc. 120 (1998) 2108-2112.

[12] Gaussian 03, Revision C.02, M. J. Frisch, G. W Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D.Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez and J. A. Pople, Gaussian, Inc., Wallingford CT, 2004.

[13] K. Wolinski, J. F. Hilton and P. Pulay, J. Am. Chem. Soc. 112 (1990) 8251-8260.

[14] a) S. G. Smith and J. M. Goodman, J. Org. Chem. 74 (2009) 4597-4607, b) S. G. Smith and J. M. Goodman, J. Am. Chem. Soc. 132 (2010)12946-12959, c) A. M. Sarotti, Org. Biomol. Chem.11(2013) 4847-4859

[15] G.P. Sarmiento, P.D. Rouge, L. Fabian, D. Vega, R. M. Ortuño, G. Moltrasio and A. G. Moglioni,Tetrahedron Asymmetry 22 (2011) 1924-1929

[16] S. Cortes and H. Kohn, J. Org. Chem. 48 (1983) 2246-2254.

[17] B. S. Furniss, A.J. Hannaford, P.W.G. Smith and A.R. Tatchell, Vogel's Textbook of Practical Organic Chemistry; fifth ed.; Longman, England,1989.

[18] W. L. F. Armarego, D.D. Perrin, Purification of Laboratory Chemicals; fourthed ed., Butterworth-Heinemann, 1997.

Highlights

A new series of hydantoin compounds derived from cyclobutyl ketones and cyclobutanone were synthesized.

Class I and class II were fully characterized by mono and bi-dimensional NMR spectroscopy.

Theoretical calculations were assayed for stereochemical assignment of the hydantoin compounds.