Deoxyhydantocidin: Synthesis by Base-Catalyzed Spiro Cyclization and Interconversion with the 1′-Epimer

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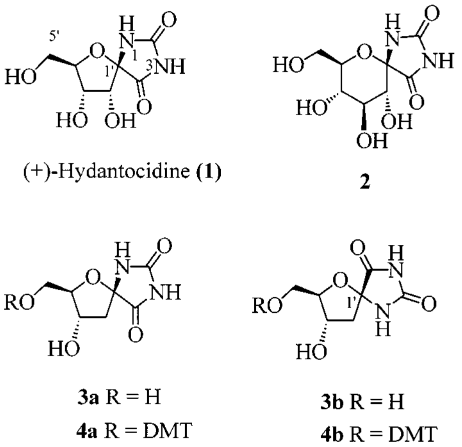
Syntheses of the spiro nucleosides 2′-deoxyhydantocidin **3a** and its 1′-epimer **3b** are described. The newly developed route involves a Horner-Wadsworth-Emmons condensation of the phosphonate **16** with the erythrose derivative **15** affording a mixture of six isomers which was fully assigned by NMR spectroscopy. The mixture was directly converted into the final compounds in an efficient base-catalyzed cyclization reaction. A base-catalyzed interconversion between the two isomers was observed.

Introduction

(+)-Hydantocidin **1** (Scheme 1) is the first natural spiro nucleoside that was isolated from the culture broth of *Streptomyces hygroscopicus* SANK 63584,[1a–1d] Tu-2474[1e] and A1491.[1f] It exhibits potent herbicidal activity with high selective toxicity between plants and animals.[1a,1b] A combined analysis of MS and ^{1}H NMR spectra revealed its intriguing structural features containing a hydantoin ring fused with a ribose moiety at the anomeric position to form a spiro system.[2] Recent biochemical studies have shown that hydantocidin is a proherbicide that, after phosphorylation at 5′-OH, inhibits adenylosuccinate synthase, an enzyme involved in de novo purine synthesis.[3] The crystal structure of the enzyme-inhibitor complex has been determined.[3d,3e] The unique structure of hydantocidin with its remarkable biological activity has stimulated many synthetic studies towards total synthesis of the parent compound[4] and its related structural analogues.[5–12] In particular, the D-glucopyranose analogue of hydantocidin **2** was found to be the most potent inhibitor of glycogen phosphorylase known to date.[8a,8b,8f,8g,13]

We were interested in the highly constrained structural feature of these spiro nucleosides which may be used as promising building units for modified oligonucleotide synthesis in the antisense and/or the antigene strategy.[14] Using molecular modeling studies, 2′-deoxyhydantocidin **3a** and its 1′-epimer **3b** were chosen as synthetic targets.[15] We also anticipated that these compounds would be devoid of the known epimeric instability of hydantocidin in acidic medium.[4d–4h,5c–5e] In a previous communication, we outlined our newly developed short synthesis of the trityl derivatives **4a** and **4b**.[7c] Here we give a full account of this synthesis. We also present a full understanding of the complex mixtures used as intermediates in the synthesis by high field NMR spectroscopic analysis with modern decoupling techniques. We also describe another feature concerning the hitherto unknown base-catalyzed interconversion of deoxyhydantocidin epimers in basic medium.



Scheme 1. Spiro hydantoins

Results and Discussion

Stereoselective Synthesis of Deoxyhydantocidin 3a

The synthesis of deoxyhydantocidin **3a** (Scheme 2) has been mostly documented in a series of reports by the Sankyo laboratory.[1b,7a,7b] The synthesis essentially involves the building up of the hydantocidin skeleton by condensation of a protected hydantoin derivative with an aldehydic chiral moiety,[4c] followed by cyclization into a spiro nucleoside and inversion of the hydroxyl configuration at C-3′. In our first attempts to obtain sufficient amounts of **3a**, we repeated the reported synthesis and increased significantly the overall yield by introducing modifications at several stages.[4c,7a]

The condensation of *N*-acetyl *N*-methoxybenzylhydantoin **(6)**[4c] with Mukaiyama′s aldehyde **5**[16] in THF in the presence of potassium *tert*-butoxide afforded the *Z*-olefin **7** in 89% yield. Compound **7** was subsequently treated in

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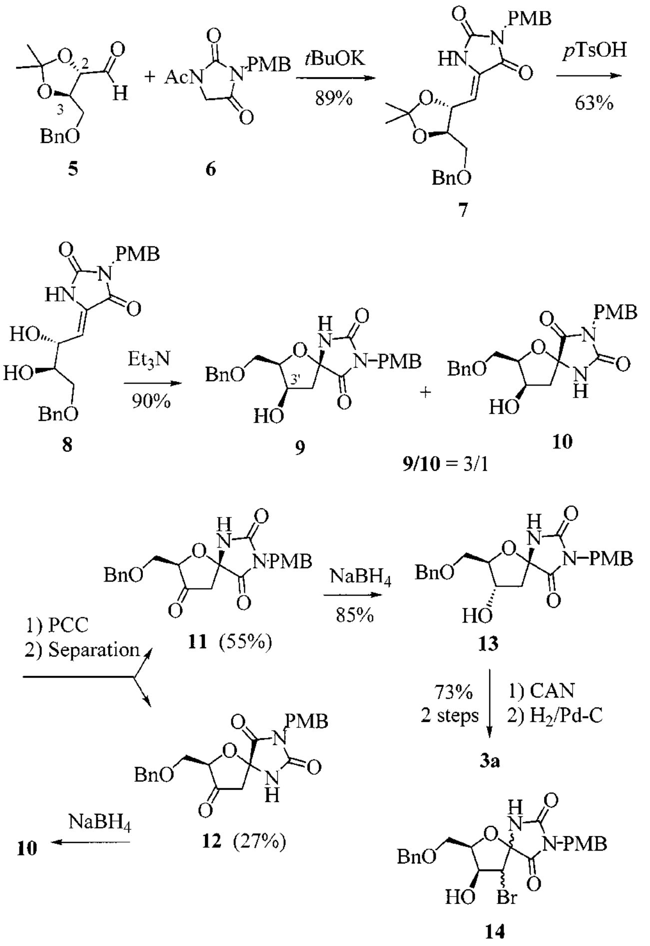
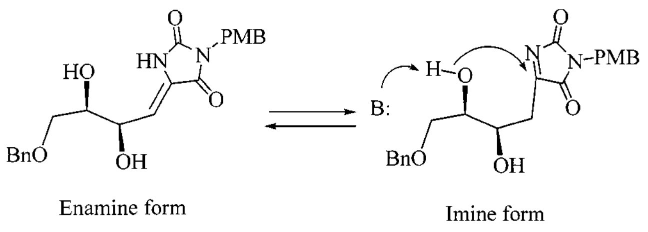


Scheme 2. Deoxyhydantocidin synthesis by Sankyo route

acidic conditions, to remove the acetonide protection, giving diol **8**.

The next step was the key intramolecular cyclization to the spiro hydantoins **9** and **10**. While this was previously carried out in two steps with 33% yield,[7a] we found that this spiro cyclization could be achieved in one step under basic conditions in 90% yield. Indeed, in the previous method, compound **8** was first treated with NBS to afford a mixture of four bromospirohydantoin isomers **14** which, upon reductive debromination, gave the mixture **9/10** (1:4.4). In this method, the stereoselectivity of the spiro cyclization was in favor of the α diastereoisomer. We found that treatment of diol **8** in a mixture of refluxing Et$_3$N/EtOH, yielded the same **9/10** mixture (3:1) in which the β epimer **9** was the major compound. This method is far more advantageous in the synthesis as only the diastereoisomer **9** can be used in the following steps. This efficient base-catalyzed spiro cyclization most probably proceeds via initial equilibration between the exocyclic enamine and the endocyclic imine forms (Scheme 3), followed by intramolecular nucleophilic addition of the alkoxide on the imino function.

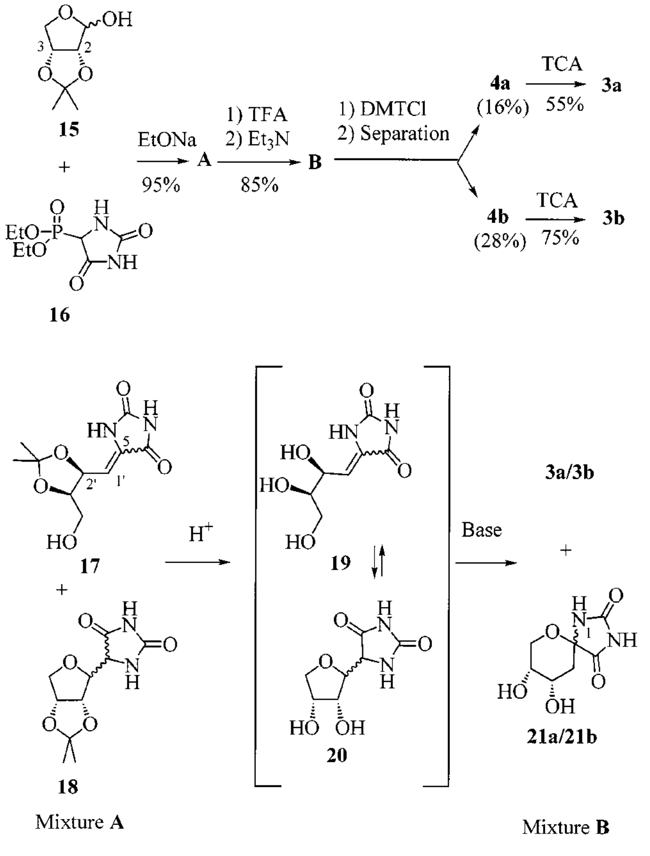


Scheme 3. Spiro cyclization of **8**

We next focused on the inversion of the C-3′ configuration in **9** using the described oxidation-reduction sequence. Since **9** and **10** are not easily separated at this level, we directly treated the **9/10** mixture with PCC and obtained the mixture of the keto spiro hydantoins **11/12**. The major ketone **11** and the minor ketone **12** were easily isolated by flash chromatography in 55% and 27% yield, respectively. The stereoselective reduction of **11** with NaBH$_4$ afforded deoxyhydantocidin **13** in 85% yield while ketone **12**, treated under the same conditions, gave the starting alcohol **10**. Only the alcohol **13** possessing the correct β-configuration at C-3′ was then converted into **3a**. Removal of the *p*-methoxybenzyl group by treatment with ceric ammonium nitrate followed by hydrogenolysis of the benzyl protection afforded **3a** in 73% yield (from **13**). Compound **3a** was thus obtained from compound **8** in five steps with an improved overall yield of 28% as compared to the 7% yield previously reported for the conversion of **3a** into **8** in seven steps.

New Short Synthesis of 3a and 3b

The synthetic route described above (Scheme 4) requires inversion of the configuration at C-3′ in the spiro nucleoside **9**, as the precursor **5** possesses the (2*S*,3*R*) configuration. However, erythronolactol **15**, readily available from er-



Scheme 4. New short synthesis of deoxyhydantocidin

ythronolactone, is a synthetic isomeric equivalent of **5** that possesses the required (2*R*,3*R*) configuration at the critical centers. This precursor was thus prepared from ascorbic acid by successive H_2O_2 oxidation into erythronolactone, in situ protection of the secondary hydroxyls by acetonide, and DIBAL reduction.[17] However, the hemiacetal **15** failed to react with the protected hydantoin **6** in the conditions that were successful for condensation of aldehyde **5**. We thus turned to different condensation strategies making use of the Wittig−Horner reaction. The cyclic hemiacetal **15** has indeed been reported to condense with simple phosphonates.[18] Furthermore, the hydantoin phosphonate **16** has been shown to react with aliphatic and aromatic aldehydes under the Horner-Wadsworth-Emmons conditions to produce the alkylidene derivatives generally as a mixture of *E* and *Z* isomers.[19]

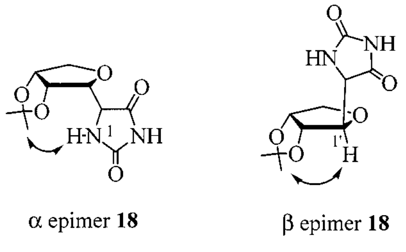
We thus treated a stoichiometric amount of lactol **15** with phosphonate **16** in the presence of sodium ethoxide. The reaction afforded a complex mixture **A** of compounds corresponding to a 95% condensation of the two stating materials. A high field NMR spectroscopic analysis of the mixture (vide infra) indicated the presence of the desired isomeric hydroxyolefins **17** together with the erythrosyl hydantoins **18** resulting from intramolecular Michael addition of **17**. Integration of the various signals revealed the composition to be as follows: 50% accounted for the hydroxyolefins **17** in the ratio *Z*/*E* = 70:30. The other 50% corresponded to the four diastereoisomers of erythrosylhydantoin **18** in the proportions βa/βb/αa/αb = 42:31:20:7. Without any separation of the constituents, the reaction mixture was treated successively with trifluoroacetic acid to remove the acetonide protection, then with refluxing triethylamine to catalyze the spiro cyclization of **19** into the 2′-deoxyhydantocidin **3**, assuming that the intermediate hydantoins **20** undergo retro Michael ring-opening to triols **19** under these conditions. Indeed, high field NMR spectroscopic analysis (vide infra) indicated that the resulting mixture **B** was made up of four spiro hydantoin derivatives: the desired furanosyl 2′-deoxyhydantocidins **3a** and **3b** as major components (≈ 75%) and the isomeric pyranosyl hydantoins **21a** and **21b** resulting from spiro cyclization involving the free primary hydroxyl group in **19**. The relative ratio between these four compounds was determined by NMR spectroscopy (**3b**/**3a**/**21b**/**21a** = 50:25:18:7).[20] From this mixture, we could isolate the deoxyhydantocidins **3a** and **3b** as their 5′-*O* tritylated derivatives **4a** and **4b** by direct treatment of mixture **B** with stoichiometric amounts of DMTCl, under the standard tritylation conditions that allow selective derivatization of primary alcohols. The resulting compounds **4a** and **4b** were easily separated by flash chromatography, while the non-tritylated derivatives **21a** and **21b** remained adsorbed on the silica column. Starting from mixture **A**, the tritylated epimers of deoxyhydantocidins **4a** and **4b** were thus obtained in 16% and 28% yield, respectively. Subsequent acid-catalyzed detritylation of **4a** and **4b** afforded **3a** and **3b** in 55% and 75% yield, respectively. All analytical data for **3b** were identical to those of authentic samples obtained in the first synthesis. In summary, this new short synthesis allowed the isolation of the two epimers **3a** and **3b** in four steps from mixture **A**, with only one intermediate separation, thus improving significantly the previous methods.

High Field NMR Analysis of the Intermediate Mixtures A and B

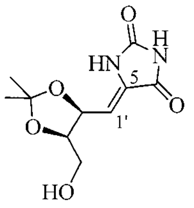
In order to determine the nature of their components, the intermediate mixtures **A** and **B** (Scheme 4) were examined by high field NMR spectroscopy using a combination of TOCSY, COSY, NOESY and HMQC experiments.

Mixture A: The six components, i.e. the two olefins **17** ***Z*** and **17** ***E*** and the four furanoside diastereoisomers **18** αa, **18** αb, **18** βa and **18** βb were unambiguously identified through a complete assignment of the ^{1}H and ^{13}C NMR spectra Figure 1, Table 1).

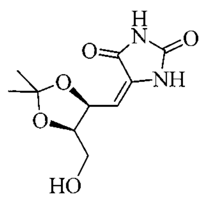
In the ^{1}H NMR spectrum of mixture **A**, two doublets (5.31 and 5.38 ppm) and two doublets of doublets (at 4.95 and 5.70 ppm) were assigned to H-1′ and H-2′ of the olefinic structures **17** ***Z*** and **17** ***E***. To discriminate between the ***Z*** and ***E*** isomers, the chemical shift of the resonance corresponding to H-2′ was analyzed. In fact the proximity of the hydantoin carbonyl C-4 function is responsible for the chemical shift difference of 0.79 ppm. The 1D-TOCSY spectra performed by selective excitation of the H-1′ olefinic protons then allowed assignment of the H-3′ and H-4′ corresponding to both **17** ***Z*** and **17** ***E***. On the other hand, several singlets at 1.22 ppm and 1.40 ppm, a complex signal pattern between 3.4 and 4.9 ppm characteristic of the sugar acetonide group and several broad signals between 7−8 ppm and 10−11.1 ppm corresponding to the N−H imide and amide protons of the hydantoin ring are the signature of the presence of four erythrosyl hydantoin derivatives **18**. A series of 1D-TOCSY, -COSY and -NOESY experiments allowed complete assignment of each signal. A 2D-COSY spectrum of mixture **A** (not shown) allowed us to locate four sets of well-separated H-5 proton resonances belonging to each of the erythrosyl hydantoin through a cross peak between the N1−H region (7.2−8.0 ppm) and the H-5 region (4.3 ppm) of the spectrum. The 1D-TOCSY spectra obtained by selective excitation of each of the four H-5 signals (4.22, 4.24, 4.28, 4.34 ppm) then allowed us to assign and separate the spin systems corresponding to H-1′, H-2′, H-3′ and H-4′ in each of the four diastereoisomers **18 αa**, **18 αb**, **18 βa** and **18 βb**. The α or β stereochemistry at the anomeric carbon in diastereoisomers **18** (Scheme 5) was subsequently determined from the NOESY spectrum (not shown).



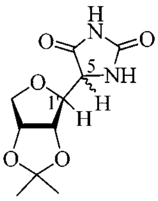
Scheme 5. NOE in isomers **18**



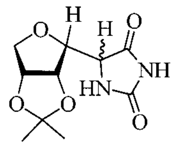
17 *Z*



17 *E*



18 β



18 α

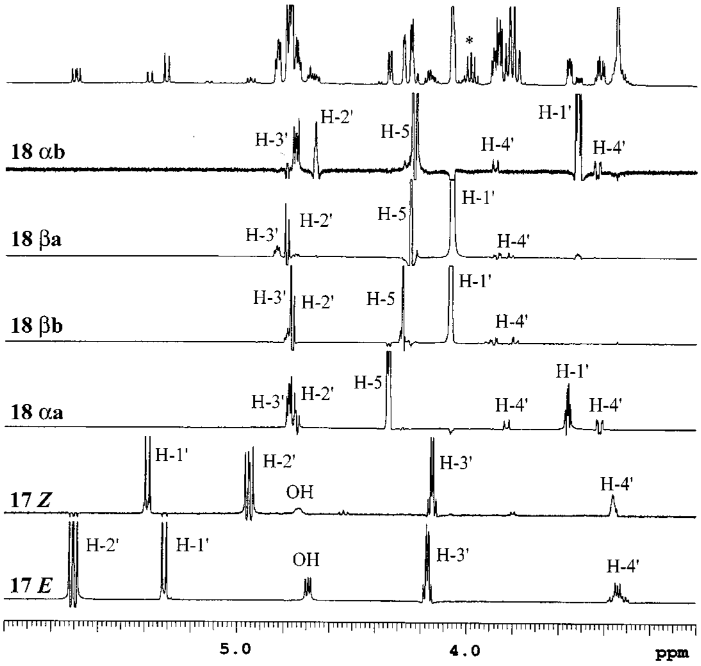


Figure 1. Mixture **A**: NMR spectra 1D-TOCSY − mixing time: 110 msec − selective irradiation of 2′-H for **17** and H-5 for **18**; * impurity [PO(OEt)$_3$]

As a matter of fact, a cross peak between the N1−H proton and one CH$_3$ of the isopropylidene group (1.35 ppm and 7.76 ppm; 1.38 ppm and 7.19 ppm) was observed exclusively in the case of the α epimers, which is in agreement with the hydantoin ring and the acetonide group being on the same side of the furanose ring. On the contrary a cross peak between the H-1′ proton and one CH$_3$ (4.06 ppm and 1.38 ppm) indicated the β stereochemistry in the other two stereoisomers. Nevertheless the stereochemistry at C-5 could not be determined for compounds **18**. The resonances in the ^{13}C NMR spectra were assigned using DEPT and GHMQC experiments and taking into account the relative ratios between the components.

Mixture B: Using similar experiments, mixture **B** was examined in D$_2$O (Figure 2). The same procedure allowed identification of the four spiro hydantoins **3a**, **3b**, **21a** and **21b** constituting mixture **B** (Table 2).

We first identified the signals of **3a** based on the results of the analysis of the NMR spectrum of **3a**. In the 1D ^{1}H NMR spectrum, we observed four signals between 1.70 and 2.50 ppm corresponding to the AB part of an ABX system that could be assigned to the H-2′ protons of the spiro hydantoin compounds. From these well-separated signals, four 1D-TOCSY experiments were performed that allowed us to assign the remaining protons H-3′, H-4′, and H-5′. The relative ratios between the different spiro hydantoins were determined from the H-2′ integral values (**3b/3a/21b/21a** = 50: 25:17:8). The furanose structure of the major isomer **3b** was confirmed by the C-4′ chemical shift at 88.1 ppm as compared to the 65.0 and 66.9 ppm shift in the pyranose form.

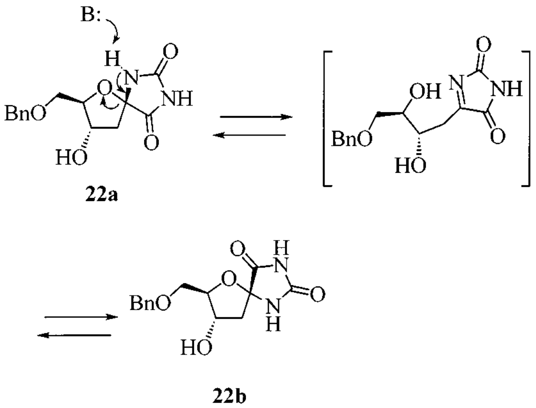
Base-Catalyzed Epimerization of Deoxyhydantocidin

Deoxyhydantocidin turned out not to be stable in basic conditions, being slowly transformed into its epimer. The kinetics of the epimerization process were most conveniently studied on the 5′-benzyl derivatives **22a** (Scheme 6).

Pure **22a** was treated in refluxing triethylamine for 16 h affording a mixture of the two epimers that could be separated and purified on silica. The α-epimer **22b** was obtained in pure form in 36% yield while the β-epimer **22a** was recovered in 32% yield. We next investigated by ^{1}H NMR spectroscopy the behavior of each isolated epimer in basic aqueous solution and determined the rate of interconversion at different pH′s. The ratios **22a/22b** were determined at differ-

Table 1. NMR spectroscopic data of mixture **A**

	^{13}C	^{1}H	Product	^{13}C	^{1}H	Product	^{13}C	^{1}H	Product
	δ	δ		δ	δ		δ	δ	
CH_3	25.4	1.38	**17 *Z***	24.4	1.38	**18 αa**	24.8	1.38	**18 βa**
	25.3	1.38	**17 *E***	24.7	1.35	**18 αb**	24.7	1.38	**18 βb**
CH_3	27.8	1.28	**17 *Z***	25.7	1.25	**18 αa**	26.5	1.25	**18 βa**
	28.0	1.27	**17 *E***	25.7	1.22	**18 αb**	26.6	1.25	**18 βb**
1′	105.5	5.38	**17 *Z***	79.7	3.56	**18 αa**	83.5	4.06	**18 βa**
	111.4	5.31	**17 *E***	79.8	3.52	**18 αb**	84.2	4.07	**18 βb**
2′	72.1	4.94	**17 *Z***	80.3	4.74	**18 αa**	82.0	4.79	**18 βa**
	70.8	5.70	**17 *E***	80.2	4.66	**18 αb**	80.9	4.76	**18 βb**
3′	78.5	4.15	**17 *Z***	80.5	4.77	**18 αa**	80.7	4.83	**18 βa**
	78.7	4.17	**17 *E***	80.4	4.75	**18 αb**	80.8	4.78	**18 βb**
4′	60.2	3.36	**17 *Z***	72.0	3.42	**18 αa**	73.7	3.81	**18 βa**
	60.1	3.32	**17 *E***	72.1	3.43	**18 αb**	73.4	3.79	**18 βb**
4′′	–	3.36	**17 *Z***	–	3.83	**18 αa**	–	3.87	**18 βa**
	–	3.36	**17 *E***	–	3.88	**18 αb**	–	3.88	**18 βb**
5	131.9	–	**17 *Z***	57.2	4.34	**18 αa**	59.6	4.24	**18 βa**
	131.0	–	**17 *E***	56.5	4.22	**18 αb**	59.7	4.28	**18 βb**
$C(CH_3)_2$	108.3	–	**17 *Z***	111.9	–	**18 αa**	111.8	–	**18 βa**
	107.9	–	**17 *E***	111.3	–	**18 αb**	111.8	–	**18 βb**
C-2	154.8	–	**17 *Z***	157.5	–	**18 αa**	157.6	–	**18 βa**
	154.1	–	**17 *E***	157.5	–	**18 αb**	157.7	–	**18 βb**
C-4	164.3	–	**17 *Z***	173.4	–	**18 αa**	173.7	–	**18 βa**
	164.4	–	**17 *E***	173.7	–	**18 αb**	173.8	–	**18 βb**
NH-1	–	10.28	**17 *Z***	–	7.19	**18 αa**	–	7.97	**18 βa**
	–	10.07	**17 *E***	–	7.76	**18 αb**	–	7.97	**18 βb**
NH-3	–	11.1	**17 *Z***	–	10.69	**18 αa**	–	10.69	**18 βa**
	–	11.1	**17 *E***	–	10.56	**18 αb**	–	10.77	**18 βb**
OH-4′	–	4.7	**17 *Z***	–	–	**18 αa**	–	–	**18 βa**
	–	4.69	**17 *E***	–	–	**18 αb**	–	–	**18 βb**



Scheme 6. Epimerization between **22a** and **22b**

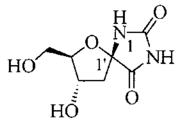
ent periods of time from the integral values of the well-separated H-2′ signals of the two epimers. These experiments showed that **22a** and **22b** were in equilibrium in basic solution to reach the ratio **22a/22b** = 45:55. The plot

$$\ln([\mathbf{22a}] - [\mathbf{22a}]_{eq}) = \ln([\mathbf{22a}]_o - [\mathbf{22a}]_{eq}) - kt$$

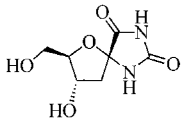
determined for epimer **22a** was a straight line at each pH indicating pseudo first-order kinetics. An identical plot was obtained for epimer **22a**. Calculated apparent rate constants are reported in Table 3. The increase of k_{obs} with pH is consistent with the following mechanism: interconversion of the epimers in basic media shouldproceed by deprotonation of N1–H leading to the opening and reclosure of the furanose ring.[21] A similar epimerization process has been described in related spiro structures[10b] but has never been reported for deoxyhydantocidin until now.

Conclusion

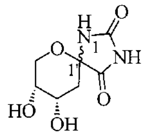
In summary, the described routes constitute a rapid and convenient access to deoxyhydantocidin and its C-1′ epimer. The efficiency of the cyclization steps offers a new route to the new spiro nucleosides. The present results also



3a



3b



21a/21b

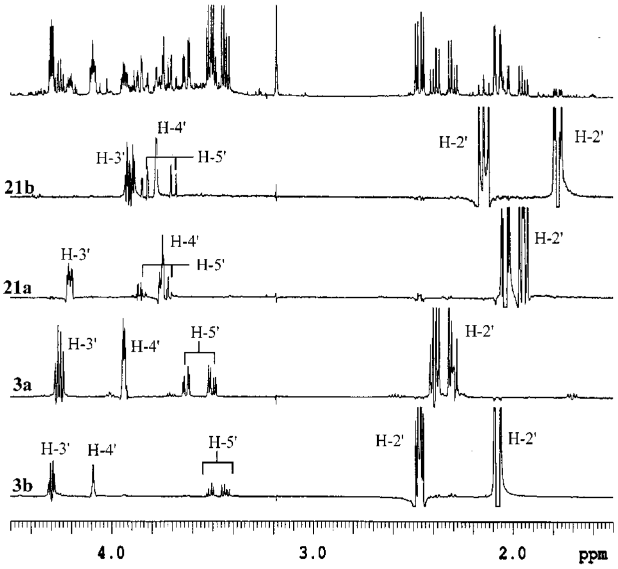


Figure 2. Mixture **B**: NMR spectra 1D-TOCSY – mixing time: 110 msec – selective irradiation of 2'-H

Table 2. NMR spectroscopic data of mixture **B**

	^{13}C	^{1}H	Product	^{13}C	^{1}H	Product
	δ	δ		δ	δ	
2'	40.9	2.08	**3b**	33.3	1.94	**21a**
	40.6	2.30	**3a**	31.7	1.78	**21b**
2''	–	2.47	**3b**	–	2.04	**21a**
	–	2.39	**3a**	–	2.15	**21b**
3'	71.3	4.30	**3b**	65.7	3.74	**21a**
	70.2	4.25	**3a**	64.3	3.91	**21b**
4'	88.1	4.09	**3b**	65.0	4.21	**21a**
	87.1	3.94	**3a**	66.9	3.78	**21b**
5'	61.9	3.43	**3b**	67.2	3.75	**21a**
	61.2	3.50	**3a**	63.6	3.70	**21b**
5''	–	3.51	**3b**	–	3.87	**21a**
	–	3.63	**3a**	–	3.84	**21b**
1'	92.8	–	**3b**	85.1	–	**21a**
	92.0	–	**3a**	85.5	–	**21b**
2	157.3	–	**3b**	156.4	–	**21a**
	157.4	–	**3a**	157.8	–	**21b**
4	175.2	–	**3b**	173.3	–	**21a**
	175.7	–	**3a**	174.0	–	**21b**

indicate fairly good stability of deoxyhydantocidin in neutral media. However, as previously documented for structurally related spiro derivatives, epimerization occurs rapidly in basic media.

Table 3. Rate constants for the isomerization between **22a** and **22b**

pH[a]	$k \times 10^4$ s^{-1}[b]
11.0	1.40 ± 0.14
11.5	3.45 ± 0.34
12.0	7.68 ± 0.77

[a] pH values are not corrected for the isotope effect. – [b] Observed pseudo first-order rate constant for isomerization.

Experimental Section

General Remarks: All chemicals and solvents were purchased from Fluka, Aldrich, Merck, SDS, Carlo-Elba, they were of analytical or HPLC grade or were distilled before use. – TLC: Merck Kieselgel 60 F_{254}, layer thickness 0.25 mm. Visualization by UV light (254 nm), H_2SO_4 solution and/or phosphomolybdic acid solution. – Preparative column chromatography: Merck Kieselgel, 230–400 mesh. – m.p.: Reichert Thermovar (uncorrected). – Optical rotations: Perkin–Elmer polarimeter 341. – IR: Nicolet Impact 400. – UV/Vis: Perkin–Elmer lambda 5. – NMR: Bruker WP80, AM200, WP250, AM300 and Varian U+500 spectrometers. NMR Spectra were referenced to the residual solvent peak; chemical shifts δ in ppm; apparent scalar coupling constants *J* in Hz. – MS: Delsi-Nermag R10–10. – Elemental analysis were performed by the "Service central de microanalyse du CNRS". The compounds **5**,[16] **6**,[4c] **15**[17] and **16**[19] were prepared according to known procedures.

(2'*R*,3'*R*,5*Z*)-5-(4'-Benzyloxy-2',3'-isopropylidenedioxybutylidene)-3-(*p*-methoxybenzyl)hydantoin (7): To a mixture of **5** (6.80 g, 27.2 mmol) and **6** (6.80 g, 25.9 mmol) in THF (220 mL) at 0 °C was added *t*BuOK (2.96 g, 26.3 mmol) in several portions. The mixture was stirred for 30 min at 0 °C then allowed to warm to room temp. during 2 h. After removal of THF under reduced pressure (<40 °C), the residue was diluted with water (300 mL) and extracted with AcOEt (1 × 150 mL and 3 × 50 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$ and evaporated. The crude solid was recrystallized from ethanol to afford **7** (8.8 g, 75%) as a white solid. From the filtrate, an additional amount of **7** (1.6 g, 14%) was obtained by chromatography (acetone/CH_2Cl_2 3:97). m.p. 119–121 °C. (ref.[4c] 119–120 °C) – TLC (acetone/CH_2Cl_2 2:98): R_f = 0.28. – IR (KBr): $\tilde{\nu}$ = 3246, 2996, 2880, 1773, 1720, 1682, 1515, 1456, 1418, 1353, 1293, 1253, 1180, 1072, 1032, 876, 781, 736, 625 cm^{-1}. – UV (CH_2Cl_2): λ_{max} (ε) = 13100 (273), 19120 (229). – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.44 (s, 6 H, CH_3), 3.64 (m, 2 H, 4'-H), 3.76 (s, 3 H, CH_3O), 3.95 (m, 1 H, 3'-H), 4.57 (s, 2 H, $ArCH_2O$), 4.62 (s, 2 H, $ArCH_2N$), 4.63 (m, 1 H, 2'-H), 5.75 (d, *J* = 3.7 Hz, 1 H, 1'-H), 6.80 (d, *J* = 8.9 Hz, 2 H, aromatic H), 7.2–7.4 (m, 7 H, aromatic H), 7.85 (br. s., 1 H, NH). – ^{13}C NMR (50 MHz, $CDCl_3$): δ = 26.4 (CH_3), 26.7 (CH_3), 41.5 (CH_2N), 55.2 (CH_3O), 69.2 (C-4'), 73.7 (CH_2O), 76.5, 79.5 (C-2', C-3'), 108.1 (C-1'), 110.3 [$C(CH_3)_2$], 113.9 (aromatic C), 127.8, 127.9, 128.5, 128.7, 130.0 (5 aromatic C), 137.2, 153.5 (aromatic C), 159.2 (C=O of C-2), 162.6 (C=O of C-4).

(2'*R*,3'*R*,5*Z*)-5-(4'-Benzyloxy-2',3'-dihydroxybutylidene)-3-(*p*-methoxy-benzyl)hydantoin (8): A mixture of **7** (9.17 g, 20.3 mmol), *p*TsOH·H_2O (1.60 g, 8.80 mmol), ethylene glycol (35 mL) and MeOH (35 mL) in CH_2Cl_2 (170 mL) was stirred under argon at room temp. for 48 h. The reaction mixture was diluted with CH_2Cl_2 (180 mL) then washed with $NaHCO_3$ (1 N, 90 mL). The aqueous layer was extracted three times with CH_2Cl_2 and the combined organic phase was washed with brine, then dried over $MgSO_4$. Solvents were removed under vacuum to give a colorless oil which solidified on standing. The diol **8** was thus obtained as a powder and used without purification (6.37 g, 75%). m.p. 114−116 °C. − TLC (AcOEt/hexane 2:1): R_f = 0.40. − $[\alpha]_D^{25}$ = +27.3 (*c* = 0.52, $CHCl_3$). − IR (KBr): $\tilde{\nu}$ = 3516, 3429, 3302, 3068, 3042, 3007, 2900, 2864, 2834, 1778, 1723, 1688, 1615, 1515, 1443, 1413, 1373, 1359, 1294, 1253, 1219, 1202, 1181, 1169, 1033, 997, 970, 932, 907, 877, 823, 800, 782, 751, 703, 648, 628, 613 cm^{-1}. − ^1H NMR (200 MHz, $CDCl_3$): δ = 2.64 (d, *J* = 6.2 Hz, 1 H, OH), 3.3 (br. s., 1 H, OH), 3.60−3.75 (m, 3 H, 3′-H and 4′-H), 3.76 (s, 3 H, CH_3O), 4.53 (s, 2 H, CH_2N), 4.55−4.61 (m, 3 H, CH_2O, 2′-H), 5.62 (d, *J* = 4.0 Hz, 1 H, 1′-H), 6.80 (d, *J* = 8.9 Hz, 2 H, aromatic H), 7.2−7.4 (m, 7 H, aromatic H), 8.0 (br. s., 1 H, NH). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 41.4 (CH_2N), 55.2 (CH_3O), 71.3, 73.7 (CH_2O, C-4′), 71.4, 72.7 (C-3′, C-2′), 110.1 (C-2′), 113.9 (aromatic C), 127.8, 128.0, 128.1, 128.5, 129.9, 130.0 (5 aromatic C, C-1′), 137.0 (aromatic C), 153.9, 159.2, (aromatic C, C=O of C-2), 162.8 (C=O of C-4).

5′-*O*-Benzyl-2′-deoxy-3-*N*-(*p*-methoxybenzyl)-3′-epihydantocidin (9) and its 1′-Epimer (10): A mixture of **8** (2.86 g, 6.90 mmol) and Et_3N (3 mL) in 1,2-dichloroethane (150 mL) was refluxed for 6 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography (AcOEt/cyclohexane 1:2) to give a mixture **9/10** (in 3:1 ratio estimated by ^1H NMR spectroscopy) as a white solid (2.51 g, 90%). The mixture of the two isomers was used without separation in the following step.

A pure sample of **9** was obtained by two successive recrystallizations of the crude product first in AcOEt/cyclohexane (1:2) then in EtOH/H_2O (1:1) (39% yield). No attempt was made to isolate the epimer **10**.

9: m.p. 116−118 °C. − TLC (AcOEt/hexane 4:1): R_f = 0.41. − $[\alpha]_D^{25}$ = −33.2 (*c* = 1.10, $CHCl_3$), {ref.[7a] $[\alpha]_D^{25}$ = −26.7 (*c* = 0.33, $CHCl_3$)}. − IR (KBr): $\tilde{\nu}$ = 3399, 3307, 3063, 3002, 2966, 2951, 2935, 2895, 2844, 1789, 1728, 1613, 1588, 1516, 1448, 1416, 1361, 1346, 1294, 1250, 1203, 1185, 1119, 1094, 1063, 1047, 1036, 1002, 982, 943, 934, 904, 855, 812, 775, 751, 735, 708, 640, 625, 608 cm^{-1}. − ^1H NMR (200 MHz, $CDCl_3$): δ = 2.13 (d, *J* =13.6 Hz, 1 H, 2′-H), 2.56 (dd, *J* = 13.6, 4.4 Hz, 1 H, 2′-H), 3.3 (br.s., 1 H, OH), 3.76 (s, 3 H, CH_3O), 3.75−3.89 (m, 2 H, 5′-H), 4.19 (m, 1 H, 4′-H), 4.53−4.61 (m, 5 H, 3′-H, CH_2N, CH_2O), 6.1 (br. s., 1 H, NH), 6.82 (d, *J* = 8.9 Hz, 2 H, aromatic H), 7.30−7.40 (m, 7 H, aromatic H). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 41.6, 42.5 (CH_2N, C-2′), 55.2 (CH_3O), 68.2 (C-5′), 72.4 (C-3′), 73.9 (CH_2O), 82.7 (C-4′), 91.0 (C-1′), 113.9 (aromatic C), 127.8, 128.1, 128.6, 130.0 (4 aromatic C), 137.0 (aromatic C), 154.9, 159.2 (aromatic C, C=O of C-2), 171.9 (C=O of C-4).

5′-*O*-Benzyl-2′-deoxy-3-*N*-(*p*-methoxybenzyl)-3′-oxohydantocidin (11) and its 1′-Epimer (12): A mixture of 3′-epihydantocidin **9/10** (1.0 g, 2.5 mmol), molecular sieves 3Å (4.0 g), sodium acetate (1.0 g) and PCC (1.8 g, 8.3 mmol) in CH_2Cl_2 (26 mL) was stirred under argon at room temp. for 15 min. The mixture was diluted with AcOEt (10 mL) and the resulting suspension was directly loaded onto silica gel column. Chromatographic separation (AcOEt/cyclohexane 1:2) gave **11** (550 mg, 55%) and **12** (275 mg, 27%) as oils.

11: TLC (AcOEt/hexane 1:1): R_f = 0.43. − $[\alpha]_D^{25}$ = −84.0 (*c* = 0.75, $CHCl_3$). − IR (KBr): $\tilde{\nu}$ = 3511, 3378, 3007, 2925, 2864, 2844, 1774, 1752, 1735, 1618, 1552, 1520, 1445, 1415, 1351, 1314, 1250, 1184, 1118, 1072, 1040, 954, 892, 848, 818, 782, 752, 731, 692 cm^{-1}. − ^1H NMR (200 MHz, $CDCl_3$): δ = 2.50 (dd, *J* = 1.0, 18.9 Hz, 1 H, 2′-H), 3.13 (d, *J* =18.9 Hz, 1 H, 2′-H), 3.70−3.85 (m, 2 H, 5′-H), 3.76 (s, 3 H, CH_3O), 4.35 (m, 1 H, 4′-H), 4.51 (m, 2 H, CH_2N), 4.59 (m, 2 H, CH_2O), 6.40 (br. s., 1 H, NH), 6.82 (d, *J* = 8.9 Hz, 2 H, aromatic H), 7.4−7.2 (m, 7 H, aromatic H). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 41.9, 43.2 (NCH_2, C-2′), 55.2 (CH_3O), 70.0 (C-5′), 74.2 (CH_2O), 81.1 (C-4′), 88.8 (C-1′), 114.1 (aromatic C), 127.6, 128.1, 128.4, 128.8 (aromatic C), 130.0 (aromatic C), 136.1 (aromatic C), 154.8, 159.4 (aromatic C, C=O of C-2), 170.5 (C-4), 209.3 (C=O of C-3′).

12: TLC (AcOEt/hexane 1:1): R_f = 0.27. − $[\alpha]_D^{25}$ = +26.2 (*c* = 0.74, $CHCl_3$). − IR (KBr): $\tilde{\nu}$ = 3515, 3318, 2984, 2939, 2868, 2843, 1780, 1768, 1747, 1616, 1587, 1520, 1446, 1412, 1351, 1305, 1252, 1175, 1106, 1084, 1050, 957, 922, 860, 843, 784, 752, 743, 711 cm^{-1}. − ^1H NMR (200 MHz, $CDCl_3$): δ = 2.62 (d, *J* = 18.9 Hz, 1 H, 2′-H), 3.10 (d, *J* = 18.9 Hz, 1 H, 2′-H), 3.70−3.85 (m, 2 H, 5′-H), 3.76 (s,CH_3O), 4.38 (t, *J* = 3.2 Hz, 1 H, 4′-H), 4.60 (s, 2 H, CH_2N), 4.70 (s, 2 H, CH_2O), 6.18 (br. s., NH), 6.82 (d, *J* = 8.9 Hz, 2 H, aromatic H), 7.22−7.35 (m, 7 H, aromatic H).

5′-*O*-Benzyl-2′-deoxy-3-*N*-(*p*-methoxybenzyl)hydantocidin (13): To a solution of **11** (470 mg, 1.14 mmol) in MeOH (45 mL) was added $NaBH_4$ (47 mg, 1.1 mmol) at −10 °C. This solution was stirred for 2 min then poured into NH_4Cl (10 mM). The aqueous phase was extracted three times with Et_2O. The combined organic phase was washed with brine, dried over $MgSO_4$ and then concentrated under reduced pressure to give a colorless oil. Chromatographic separation (AcOEt/hexane 1:1) afforded **13** as a white solid (400 mg, 85%). − m.p. 55−59 °C. − TLC (AcOEt/hexane 2:1): R_f = 0.37. − $[\alpha]_D^{25}$ = −24.0 (*c* = 0.55, $CHCl_3$) {ref.[7a] $[\alpha]_D^{25}$ = −25.7 (*c* = 1.1, $CHCl_3$)}. − IR (KBr): $\tilde{\nu}$ = 3389, 3358, 2920, 2833, 1780, 1723, 1613, 1520, 1502, 1454, 1420, 1402, 1359, 1352,1300, 1269, 1250, 1185, 1178, 1102, 1081, 1035, 968, 943, 911, 848, 809, 771, 746, 705 cm^{-1}. − UV (CH_2Cl_2): λ_{max} (ε) = 274 (1660), 242 (1146). − ^1H NMR (200 MHz,$CDCl_3$): δ = 2.19 (d, *J* = 14.2 Hz, 1 H, 2′-H), 2.47 (dd, *J* = 5.3, 14.2 Hz, 1 H, 2′-H), 3.54−3.60 (m, 2 H, 5′-H), 3.76 (s, CH_3O), 4.09 (d, *J* = 12.0 Hz, 1 H, OH), 4.24−4.35 (m, 2 H, 4′-H, 3′-H), 4.49 (s, 2 H, CH_2N), 4.55 (s, 2 H, CH_2O), 5.70 (br. s., 1 H, NH), 6.81 (d, *J* = 8.9 Hz, 2 H, aromatic H), 7.22−7.45 (m, 7 H, aromatic H). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 41.6, 41.8 (CH_2N, C-2′), 55.2 (CH_3O), 70.6 (C-5′), 73.6 (CH_2O), 74.3 (C-3′), 88.4 (C-4′), 92.6 (C-1′), 114.0 (aromatic C), 127.6, 128.1, 128.6, 128.8, 129.8, 130.8 (aromatic C), 137.2 (aromatic C), 154.8, 159.2 (aromatic C, C=O of C-2), 174.0 (C=O of C-4).

5′-*O*-Benzyl-2′-deoxyhydantocidin (22a): To a solution of **13** (1.55 g, 3.76 mmol) in CH_3CN (100 mL) was rapidly added a solution of CAN (20.3 g, 37.0 mmol) in H_2O (50 mL) at room temp. under argon. The reaction mixture was stirred for 30 min then poured into a solution of $NaHCO_3$ (1 N, 300 mL). The resulting suspension was extracted with AcOEt (3 × 100 mL). Solid Na_2CO_3 was added to the aqueous phase until its pH reached 12. It was then extracted three more times with AcOEt. The combined organic phase was washed with brine, dried over $MgSO_4$ and concentrated to give an orange oil. This residue was triturated in Et_2O, filtered and washed with Et_2O to afford **22a** as a solid (500 mg, 50%). The filtrate was concentrated under reduced pressure. The residue was passed through a silica gel column (acetone/CH_2Cl_2 1:2) to yield an additional amount of **22a** (250 mg, 25%). − m.p. 132−141 °C. − TLC (acetone/CH_2Cl_2 1:2): R_f = 0.41. − $[\alpha]_D^{25}$ = + 7.1 (*c* = 0.78, CH_3OH) {ref.[7a] $[\alpha]_D^{25}$ = + 5.4 (*c* = 0.43, CH_3OH)}. − IR (KBr): $\tilde{\nu}$ = 3306, 3236, 3152, 3005, 2934, 2902, 2858, 2774, 1758, 1732, 1715, 1455, 1415, 1398, 1381, 1364, 1310, 1261, 1212, 1201, 1150, 1124, 1093, 1050, 1024, 953, 816, 765, 742, 719, 697, 642, 514, 465, 437 cm^{-1}. − UV (H_2O): λ_{max} (ε) = 193 (19800). − ^1H NMR (200 MHz, D_2O): δ = 2.44 (dd, J = 7.0, 14.0 Hz, 1 H, 2′-H), 2.55 (dd, J = 7.0, 14.0 Hz, 1 H, 2′-H), 3.64 (dd, J = 6.0, 11.3 Hz, 1 H, 5′-H), 3.79 (dd, J = 3.0, 11.3 Hz, 1 H, 5′-H), 4.19 (m, 1 H, 4′-H), 4.41 (m, 1 H, 3′-H), 4.65 (s, 2 H, CH_2O), 7.45 (s, 5 H, aromatic H). − ^{13}C NMR (50 MHz, [D_6]DMSO): δ = 32.0 (C-2′), 61.2 (C-5′), 62.2 (C-3′), 64.4 (CH_2O), 77.2 (C-4′), 83.2 (C-1′), 118.8, 119.0, 119.4 (aromatic C), 129.3 (aromatic C), 147.9 (C=O of C-2), 166.6 (C=O of C-4). − MS (FAB+, glycerol): m/z = 293 [M + H]$^+$. − $C_{14}H_{16}N_2O_5$ (292.3): calcd. C 57.53, H 5.52, N 9.58; found: C 57.36, H 5.42, N 9.58.

1′-Epi-5′-*O*-benzyl-2′-deoxyhydantocidin (22b): A solution of **22a** (200 mg, 0.66 mmol) in EtOH/Et_3N 1:1 was refluxed for 16 h. After removal of solvents under vacuum, the crude residue was purified by chromatography on silica gel (acetone/CH_2Cl_2 1:2) to afford **22a** (65 mg; 32%) and **22b** (72 mg; 36%) as white solids with an isomeric purity >95% as determined by ^1H NMR spectroscopy.

22b: m.p. 132−134 °C. − TLC (acetone/CH_2Cl_2 1:2): R_f = 0.32. $[\alpha]_D^{25}$ = + 12.6 (c = 0.47, CH_3OH). − IR (KBr): $\tilde{\nu}$ = 3429, 3326, 3269, 3178, 3033, 2921, 2860, 2778, 1780, 1768, 1744, 1500, 1436, 1401, 1364, 1354, 1316, 1291, 1210, 1138, 1100, 1076, 1056, 1023, 996, 897, 885, 811, 768, 752, 742, 728, 718, 697 cm^{-1}. − UV (H_2O): λ_{max} (ε) = 193 (25100). − ^1H NMR (200 MHz, D_2O): δ = 2.22 (dd, J = 3.0, 14.3 Hz, 1 H, 2′-H), 2.62 (dd, J = 5.3, 14.3 Hz, 1 H, 2′-H), 3.50−3.78 (m, 1 H, 5′-H), 4.32−4.49 (m, 2 H, 4′-H, 3′-H), 4.63 (s, 2 H, CH_2O), 7.44 (s, 5 H, aromatic H). − ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 29.6 (C-2′), 70.6 (C-5′), 70.8 (C-3′), 72.3 (CH_2O), 84.7 (C-4′), 91.7 (C-1′), 127.4, 127.5, 128.2 (aromatic C), 138.3 (aromatic C), 155.6 (C=O of C-2), 174.2 (C=O of C-4). − MS (FAB+, glycerol): m/z = 293 [M + H]$^+$. − $C_{14}H_{16}N_2O_5$ (292.3): calcd. C 57.53, H 5.52, N 9.58; found: C 57.36, H 5.42, N 9.58.

2′-Deoxyhydantocidin (3a from 22a): A mixture of 10% Pd/C (1.5 g) and compound **22a** (750 mg, 2.56 mmol) in MeOH (150 mL) was stirred at room temp. under an H_2 atmosphere for 8 h. The suspension was filtered through celite and the solid residue was washed with MeOH. The filtrate was evaporated under reduced pressure to give the deoxyhydantocidin **3a** as a foam (510 mg, 98%). (spectroscopic data: vide infra).

Mixture A: (see Scheme 4) To a stirred solution of EtONa prepared from Na (0.13 g, 5.5 mmol) in absolute EtOH (8 mL) was added successively the phosphonate **16** (1.42 g, 6.00 mmol) and a solution of the lactol **15** (794 mg, 4.96 mmol) in EtOH (2 mL) at 50 °C under argon. The mixture was stirred at 60 °C for 5 h. The solvent was then removed under reduced pressure. Chromatography on silica gel (AcOEt) gave mixture **A** as a white foam (1.14 g, 95%). − NMR spectroscopic data: see Table 1. − MS (DCI, Isobutane, NH_3): m/z = 243 [M + H]$^+$. − $C_{10}H_{14}N_5O_2$ (242.2): calcd. C 49.58, H 5.83, N 11.56; found C 49.82, H 5.93, N 11.42.

Mixture B: A solution of the above mixture **A** (1.00 g, 4.16 mmol) in TFA/H_2O 1:2 (15 mL) was stirred at room temp. for 20 min. The reaction mixture was then concentrated and coevaporated four times with H_2O. The resulting residue was dissolved in MeOH/Et_3N (10 mL, 1:1, v/v) and this solution was refluxed for 14 h. The solvents were then removed under reduced pressure. The residue was dissolved in H_2O (≈10 mL) and passed through a column of Amberlyst 15 (10 g). The resin was washed with H_2O (400 mL) and the aqueous fractions were concentrated under vacuum to gave the mixture **B** as a foam. The residue was triturated in Et_2O and decanted repeatedly to give mixture **B** as a solid (0.70 g, 85%). This mixture was used without further purification in the following step. − NMR spectroscopic data see Table 2. − MS (FAB+, glycerol): m/z = 203 [M + H]$^+$. − $C_7H_{10}N_2O_5 \cdot 0.25H_2O$ (206.7): calcd. C 40.73, H 5.01, N 13.57; found C 40.41, H 5.28, N 13.15.

5′-*O*-Dimethoxytrityl-2′-deoxyhydantocidin (4a) and (4b): Mixture **B** (0.20 g, 1.0 mmol) was coevaporated three times with freshly distilled pyridine. The residue was dissolved in dry pyridine (1.5 mL) then DMTCl (0.34 g, 1.0 mmol) was added in small portions at 4 °C (ice bath) under argon. The mixture was stirred at room temp. for 3 h. MeOH (0.5 mL) was then added to the solution to quench the excess of DMTCl. After 10 min stirring, solvents were removed under reduced pressure and the resulting foam was coevaporated three times with toluene. The residue was dissolved in a minimum amount of acetone/MeOH (9:1) and was passed through a silica gel column (acetone/CH_2Cl_2 1:3) to give two fractions, one enriched in **4a** and the other enriched in **4b**. Each fraction was chromatographed a second time to give **4a** (90 mg, 18%) and **4b** (410 mg; 42%) as foams. Compound **4a** was precipitated from CH_2Cl_2/acetone by addition of pentane to afford a solid (80 mg; 16%) with an isomeric purity > 99% (estimated by NMR spectroscopy). Compound **4b** was obtained after recrystallization from CH_2Cl_2 as a white powder (140 mg; 28%) with an isomeric purity > 99%.

For the spectroscopic data for **4a** and **4b**, see ref.[7c]

2′-Deoxyhydantocidin (3a from 4a): To a stirred solution of **4a** (101 mg, 0.198 mmol) in CH_2Cl_2/MeOH (97:3, v/v) was added a solution of TCA (3% in CH_2Cl_2, 0.4 mL). The solution was stirred at room temp. for 40 min. The resulting suspension was directly loaded onto silica gel in a chromatography column and eluted with CH_2Cl_2 (20 mL), acetone/CH_2Cl_2 (1:3, 20 mL) then acetone/CH_2Cl_2 (4:1, 50 mL). The fractions containing **3a** were gathered then concentrated under reduced pressure. The resulting residue was triturated in Et_2O to afford **3a** as a white solid (19 mg, 50%). − TLC (acetone/CH_2Cl_2 4:1): R_f = 0.45. − $[\alpha]_D^{25}$ = + 14.7 (c = 0.98, CH_3OH) {ref.[7a] $[\alpha]_D^{25}$ = + 14.8 (c = 1.17, CH_3OH)}. − IR (KBr): $\tilde{\nu}$ = 3460, 3388, 3321, 3224, 3079, 2933, 2745, 1794, 1768, 1715, 1458, 1448, 1400, 1369, 1321, 1298, 1260, 1224, 1187, 1163, 1109, 1081, 1039, 1014, 999, 979, 942, 906, 891, 869, 839, 814, 799, 757, 719 cm^{-1}. − ^1H NMR (200 MHz, D_2O): δ = 2.49 (dd, J = 6.6, 13.9 Hz, 1 H, H-2′), 2.60 (dd, J = 6.6, 13.9 Hz, 1 H, H-2′), 3.64 (dd, J = 4.9, 14.6 Hz, 1 H, 5′-H), 3.83 (dd, J = 2.9, 10.6 Hz, 1 H, 5′-H), 4.14 (td, J = 3.3, 5.6 Hz, 1 H, 4′-H), 4.45 (m, 1 H, 3′-H). − ^{13}C NMR (75 MHz, CD_3OD): δ = 41.7 (C-2′), 62.5 (C-5′), 71.5 (C-3′), 88.4 (C-4′), 92.9 (C-1′), 157.7 (C-2), 176.6 (C-4). − MS (FAB+, nba): m/z = 203 [M + H]$^+$. − $C_7H_{10}N_2O_5$ (202.2): calcd. C 41.59, H 4.99, N 13.86; found C 41.85, H 5.15, N 13.88.

1′-Epi-2′-deoxyhydantocidin (3b): Starting from **4b** (101 mg, 0.198 mmol), **3b** was obtained in the same manner as **3a** (30 mg, 75%). − ^1H NMR (200 MHz, D_2O): δ = 2.25 (dd, J = 3.3, 14.6 Hz, 1 H, 2′-H), 2.65 (dd, J = 5.6, 14.3 Hz, 1 H, 2′-H), 3.61 and 3.70 (ABX, J_{AX} = 4.6, J_{BX} = 6.3, J_{AB} = 12.3 Hz, 2 H, 5′-H and 5′′-H), 4.27 (m, 1 H, 3′-H), 4.47 (m, 1 H, 4′-H). − ^{13}C NMR (50 MHz, D_2O): δ = 40.8 (C-2′), 61.6 (C-5′), 71.2 (C-3′), 88.0 (C-4′), 92.9 (C-1′), 158.0 (C=O of C-2), 175.7 (C=O of C-4). − MS (FAB+, glycerol): m/z = 201 [M + H]$^+$.

NMR Spectroscopic Study of Mixture A and Mixture B: NMR experiments were conducted at 25 °C on a Unity+500 Varian spectrometer equipped with a 5 mm indirect probe, two radio frequency channels with waveform generator. COSY spectra were recorded using a 2048 × 1024 acquisition matrix and processed using a 4096 × 4096 transformed matrix with zero filling in both dimensions; a sine squared function was applied prior to Fourier transformation. − 1D-TOCSY was recorded using a selective pulse "e-burp" shaped, 132 msec, with arrayed mixing time from 10 to 140 ms - NOESY spectra were obtained in phase sensitive mode with 600 ms mixing time 4096×1024 acquisition matrix and 4096×2048 transformed matrix for processing. − HMQC spectra without proton decoupling were recorded using a 2048×256 acquisition matrix and processed using a 4096×2048 transformed matrix with zero filling. Sine multiplication was performed prior to Fourier tranformation. − HMBC spectra were recorded in a similar way. The delay was optimized for coupling constants value ^{n}J (HC) = 5 Hz.

Interconversion of 22a and 22b: Epimerization of **22a** and **22b** was followed at pH 11.5 and 12.0 by NMR spectroscopy. Kinetic experiments were carried out at 298 K, at constant ionic strength (addition of NaCl 2 N/D_2O to produce $\mu = 0.7$). MeOH was used as internal reference. In a standard experiment, the NMR sample was prepared as followed: to a suspension of compound **22a** or **22b** (3.0 mg, 10 µmol) in D_2O (230 µL at pH 12 or 217 µL at pH 11.5) was added a solution of NaCl 2 N/D_2O (120 µL at pH 12, 133 µL at pH 11.5) and a solution of MeOH/D_2O 25 mM (210 µL). The sample temperature was stabilized at 298 K for 30 min. The phosphate buffer 0.5 M (140 µL) in D_2O was then added to obtain a final concentration of 0.1 M. ^{1}H NMR spectra (300 MHz) were recorded every five min during the first hour. Integration of the 2′-H α and β areas gave the relative epimeric ratio α/β. Both experiments were duplicated.

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