Structure Elucidation of the First Inhibitors of Human Papillomavirus Type 11 E1–E2 Protein–Protein Interaction

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A novel series of inhibitors of the HPV11 E1–E2 protein–protein interaction was identified. These inhibitors, which were discovered as a result of high-throughput screening, feature an indandione system spiro-fused onto an appropriately substituted tetrahydrofuran ring. Early stability studies indicated, surprisingly, that this particular series of compounds were readily converted, in binding assay buffer, to the corresponding carboxylates. NMR and mass spectrometry techniques were used to elucidate the structures of these products and the mechanism by which they are produced.

Introduction. – There are more than 100 human papillomavirus (HPV) types, associated with a broad spectrum of disease ranging from cutaneous and genital warts to anogenital cancer. Each of these different HPV types infects specific tissues and is responsible for particular pathologies. For example, HPV1 infections cause plantar warts on the feet, HPV6 and 11 cause genital warts, and HPV16 is the major cause of cervical cancer [1].

The HPV genome encodes eight well-characterized proteins, only one of which, the E1 helicase, has enzymatic activity. The others either serve structural roles or are involved in interactions with host-cell factors [2]. In addition to E1, only one other viral protein, E2, is required for replication of the genome [3][4]. Viral-DNA replication is initiated by the cooperative binding of E1 and E2 to the HPV origin. Assembly of this E1–E2–origin (= E1–E2–ori) complex is dependent on the binding of E2 to high-affinity sites in the origin as well as on a critical protein–protein interaction between E1 and E2 [5][6]. E1 and E2 also recruit to the origin host-cell proteins required for replication, including the polymerase α primase [7].

Currently, genital warts are treated by a variety of ablative and cytodestructive therapies including the use of liquid nitrogen or trichloroacetic acid or by topical administration of the immunostimulator imiquimod [8]. These treatments are far from ideal, and there clearly remains a need for specific inhibitors of viral replication. The essential proteins E1 and E2 are attractive targets for such antiviral chemotherapy [9].

Recently, we described a series of compounds that inhibit the cooperative binding of E1 and E2 to the viral origin of replication [10][11]. These inhibitors, which were discovered as a result of high-throughput screening, feature an indandione system

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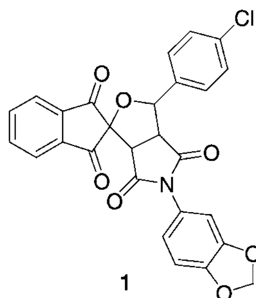
spirofused onto an appropriately substituted tetrahydrofuran ring. The initial hit, compound **1**, had an activity of 11 μM in our *in vitro* E1–E2–ori-complex-formation assay [12]. Modest structural modifications gave materials that showed sub-micromolar activity in the *in vitro* binding assay and that were capable of inhibiting HPV-DNA replication in a surrogate cellular assay [12]. Additional studies also provided evidence that this class of inhibitors specifically antagonizes the E1–E2 protein–protein interaction by binding reversibly to the E2 transactivation domain, the region of E2 that interacts with E1 [12].

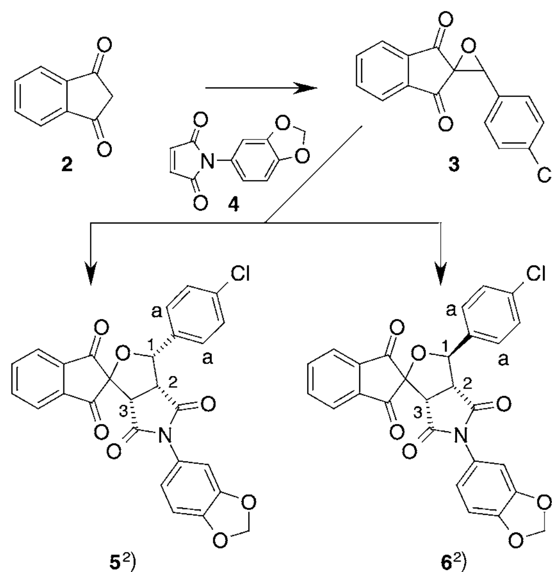


Early stability studies indicated that compound **1** and some structurally related analogs were readily converted in assay buffer to other products. In this paper, we describe the elucidation of the structures of these products and the mechanism by which they are produced. We also disclose experiments that address the structural species which is responsible for the activity of these compounds.

Results and Discussion. – Compound **1** was resynthesized from indan-1,3-dione (=1*H*-indene-1,3(2*H*)-dione; **2**) as illustrated in *Scheme 1*. *Knoevenagel* condensation with 4-chlorobenzaldehyde in the presence of piperidine [13] gave the corresponding benzylidene derivative, which was treated with peroxide under basic conditions [14] to give spirooxiranedione **3**. Dipolar cycloaddition [15] with maleimide **4** in refluxing toluene gave **1** as a 1:1 mixture of the '*syn*/*syn*' adduct **5** and the '*syn*/*anti*' compound **6**, which were readily separated by flash chromatography. The structures and relative configurations of **5** and **6** were confirmed by a variety of NMR-spectroscopic techniques, including HMQC, HMBC, and 1D NOE. The ^{1}H-NMR signal of H–C(1) of **5** gave a stronger NOE enhancement (5%) than that of H–C(a) (0.8%) upon irradiation of the H–C(2) resonance[2]), consistent with the depicted '*syn*/*syn*' relationship (*Scheme 1*). In contrast, compound **6** showed a stronger NOE enhancement for H–C(a) (5%) than for H–C(1) (2%) upon irradiation of the H–C(2) signal, in agreement with the depicted '*syn*/*anti*' relative configuration. The inhibitory activity of these compounds was found to reside in the '*syn*/*syn*' isomer **5**, which showed an IC_{50} of 8.3 μM in the *in vitro* E1–E2–ori-complex-formation assay [12]. Compound **6** was inactive under the same assay conditions.

[2]) Arbitrary numbering; for systematic names, see *Exper. Part*.

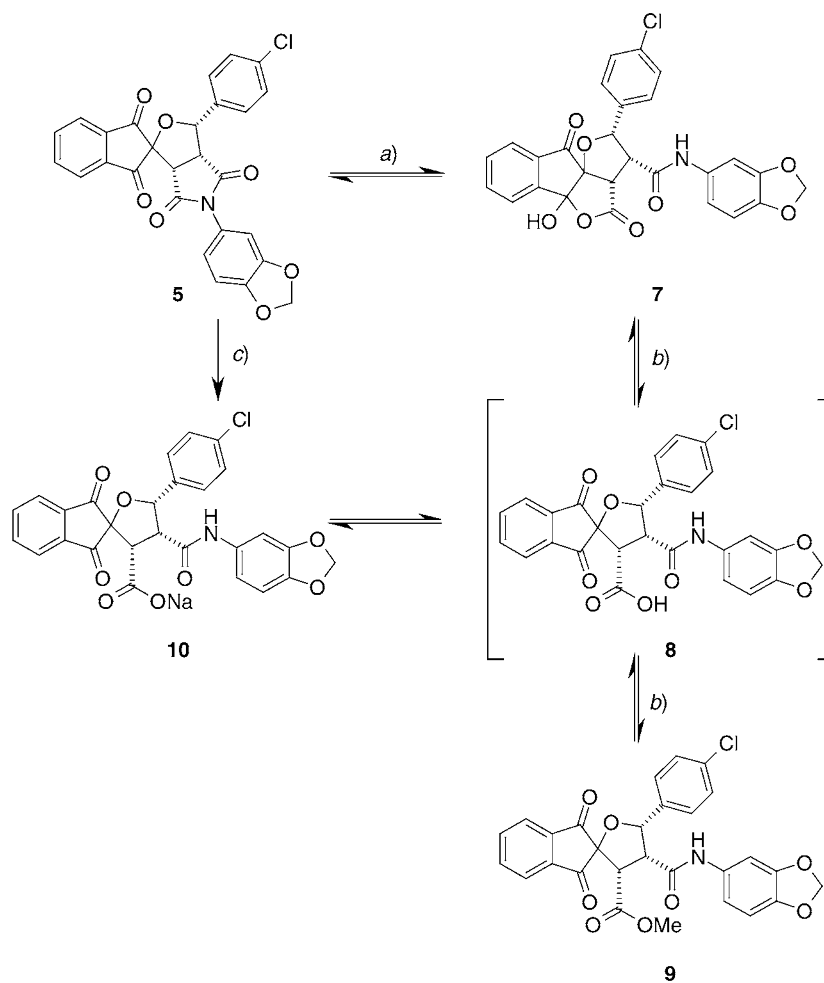
Scheme 1. *Synthesis*



Compound **5** was rapidly converted to another product in the assay buffer. As indicated by HPLC experiments, solutions of **5** in MeCN and phosphate buffer at pH 7 or 8 were transformed into 6:4 mixtures of **5** and this new product after incubation times of *ca.* 2 h. The 6:4 ratio was maintained for up to 24 h, suggesting that the products were in equilibrium. This process was found to happen very slowly at lower pH (13% conversion after 24 h at pH 5). To identify the product present in the assay buffer, a larger quantity of **5** was dissolved in DMSO and the resulting solution added to phosphate buffer prepared at pH 7. After 2 h, the more-polar product was isolated by flash chromatography. The NMR spectra (*Table 1*) of this product suggested that the indandione, 4-chlorophenyl, and 1,3-benzodioxol-5-yl moieties were all present in the product and that the tetrahydrofuran was intact. Additional NMR experiments established structure **7** for this compound (*Scheme 2*).

Considerable asymmetry was apparent in the ^{1}H-NMR resonances of **7** for the indandione benzene moiety, a phenomenon not observed for the parent compound **5**. The resonance at δ 7.58, attributed to an OH group, exchanged very rapidly upon the addition of D_2O. The signal δ 10.15, attributed to an amide NH, exchanged at a slower rate, requiring more than 48 h for complete attenuation of the peak. The ^{13}C-NMR spectrum of **7** contained 25 resonances, indicating that no C-atoms had been lost; however, only three C=O signals were found in the carbonyl region (δ 160–220). Assignment of all C-atoms of **7** was achieved by a combination of HMQC and HMBC experiments (*Table 1*). Thus, the exchangeable NH at δ 10.15 as well as H–C(1), H–C(2), and H–C(3) gave cross-peaks with the C(18)=O signal at δ 168.4 in the HMBC plot, suggesting an opening of the maleimide moiety of **5** to generate an amide.[2]) This was confirmed by correlations between NH and C(19), C(20), and C(25) in the HMBC spectrum. The new ^{13}C-NMR signal at δ(C) 105.6 (C(5)) was correlated with H–C(3), H–C(7), and OH (δ(H) 7.58), and the signals of H–C(2), H–C(3), and OH correlated with C(13). In addition, the OH signal at δ(H) 7.58 gave an HMBC cross-peak with C(6). Both H–C(10) and H–C(3) correlated with C(12)=O (δ(C) 200.0). These correlations were consistent with the hydroxy lactone structure of **7**.

Scheme 2. *Maleimide Hydrolysis*

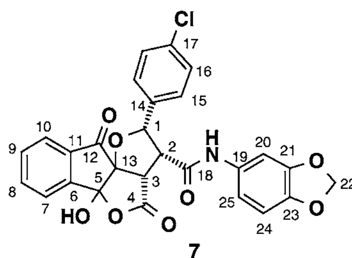


a) pH 7.1 phosphate buffer. *b*) CH_2N_2, THF. *c*) MeCN, NaOH

In THF, compound **7** was found to be in a rapid equilibrium with acid **8** (*Scheme 2*). The latter was not directly observed, but its existence was inferred by the formation of ester **9** upon treatment with CH_2N_2. This suggested that **7** might also be in equilibrium with the carboxylate of **8** in aqueous media. To test this hypothesis, compound **5** was converted to sodium salt **10** by the slow addition of aqueous NaOH solution. Slow addition conditions, so as to maintain a neutral pH, were necessary for the clean conversion to **10**. The structure of compound **10** was confirmed by NMR experiments (*Table 2*) and by the X-ray crystallographic analysis of the related compound **11**.

In the ^{13}C-NMR spectrum of **10**, 4 C=O resonances were apparent. Assignment of all C-atoms was accomplished by HMQC and HMBC experiments, H−C(1), H−C(2), H−C(3), and NH (*δ*(H) 9.91) gave

Table 1. *¹H- and ¹³C-NMR Data* (27°, (D_6)DMSO) *of Compound* **7**[2]). δ in ppm, *J* in Hz

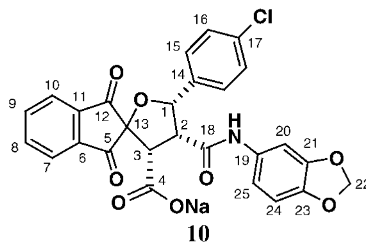


	δ(H)	δ(C)	COSY	HMBC
H−C(1)	6.02 (*d*, *J* = 5.4)	88.0	H−C(2)	C(2), C(3), C(14), C(15), C(18)
H−C(2)	3.98 (*dd*, *J* = 5.4, 8.8)	53.0	H−C(1), H−C(3)	C(1), C(3), C(13), C(18)
H−C(3)	4.32 (*d*, *J* = 8.8)	52.7	H−C(2)	C(2), C(4), C(5), C(12), C(13), C(18)
C(4)	–	171.4		
C(5)	–	105.6		
C(6)	–	148.2		
H−C(7)	7.94 (*d*, *J* = 7.4)	124.8	H−C(8)	C(5), C(9), C(11)
H−C(8)	7.98 (*t*, *J* = 7.4)	137.4	H−C(7), H−C(9)	C(6), C(10)
H−C(9)	7.77 (*t*, *J* = 7.4)	131.8	H−C(8), H−C(10)	C(7), C(11)
H−C(10)	7.87 (*d*, *J* = 7.4)	123.6	H−C(9)	C(6), C(8), C(12)
C(11)	–	133.8		
C(12)	–	200.0		
C(13)	–	88.4		
C(14)	–	134.4		
H−C(15)	7.49 (*d*, *J* = 8.4)	127.9	H−C(15)	C(1), C(14), C(16), C(17)
H−C(16)	7.39 (*d*, *J* = 8.4)	127.9	H−C(14)	C(14), C(15), C(17)
C(17)	–	132.6		
C(18)	–	168.4		
C(19)	–	131.2		
H−C(20)	6.71 (*d*, *J* = 1.7)	102.4	H−C(25)	C(19), C(21), C(23), C(25)
C(21)	–	146.9		
CH_2(22)	5.96 (*s*)	101.1		C(21), C(23)
C(23)	–	144.0		
H−C(24)	6.76 (*d*, *J* = 8.3)	107.8	H−C(25)	C(19), C(21), C(23)
H−C(25)	6.51 (*dd*, *J* = 8.3, 1.7)	113.6	H−C(24), H−C(20)	C(19), C(20), C(23), C(24)
NH	10.15 (*s*)			C(18), C(19), C(20), C(25)
OH	7.58 (*s*)			C(5), C(6), C(13)

HMBC cross-peaks with C(18)=O[2]). The NH signal also correlated with C(19), C(20), and C(25). These observations again suggested that the maleimide moiety was replaced by a single amide group. H−C(2) and H−C(3) coupled with a C=O (C(4)), which did not correlate with H−C(1). In addition, H−C(3) coupled with two other C=O groups, C(5) at δ(C) 199.8 and C(12) at δ(C) 202.0, both of which gave cross-peaks with different aromatic protons.

The conversion of **5** to **7** and (presumably) **10** in buffer at pH 7 was at first glance surprising. Indeed, maleimides are normally difficult to hydrolyze, yet this moiety was opened rapidly under mild conditions. In addition, this reaction was highly regioselective, no products resulting from cleavage of the maleimide at the other amide

Table 2. *^{1}H- and ^{13}C-NMR Data* (27°, (D$_6$)DMSO) *of Compound* **10**[2]). δ in ppm, J in Hz



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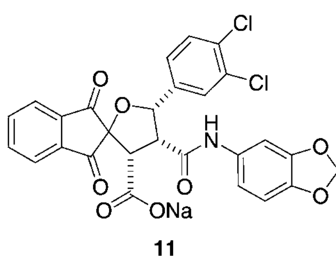
	δ(H)	δ(C)	COSY	HMBC
H–C(1)	5.71 (*d*, *J* = 5.9)	85.1	H–C(2)	C(2), C(3), C(14), C(15), C(18)
H–C(2)	3.65 (*dd*, *J* = 5.9, 8.7)	56.7	H–C(1), H–C(3)	C(3), C(13), C(18)
H–C(3)	4.15 (*d*, *J* = 8.7)	62.0	H–C(2)	C(2), C(4), C(5), C(12), C(13), C(18)
C(4)	–	169.1		
C(5)	–	199.8[a])		
C(6)	–	143.8[b])		
H–C(7)	8.04–8.10 (*m*)	123.4[c])	[e])	C(5), C(9), C(11)
H–C(8)	8.04–8.10 (*m*)	137.2[d])	H–C(9)	C(6), C(10)
H–C(9)	7.96–8.04 (*m*)	135.8[d])	H–C(8)	C(7), C(11)
H–C(10)	7.96–8.04 (*m*)	123.4[c])	[e])	C(6), C(8), C(12)
C(11)	–	138.8[b])		
C(12)	–	202.0[a])		
C(13)	–	83.0		
C(14)	–	136.1		
H–C(15)	7.38 (*d*, *J* = 8.5)	127.7	H–C(16)	C(1), C(16), C(17)
H–C(16)	7.31 (*d*, *J* = 8.5)	127.7	H–C(15)	C(14), C(15), C(17)
C(17)	–	131.8		
C(18)	–	167.5		
C(19)	–	133.5		
H–C(20)	7.09 (*d*, *J* = 1.6)	102.0	H–C(25)	C(19), C(21), C(23), C(25)
C(21)	–	146.8		
CH_2(22)	5.96 (*s*)	100.8		C(21), C(23)
C(23)	–	142.7		
H–C(24)	6.83 (*d*, *J* = 8.4)	107.8	H–C(25)	C(19), C(21), C(23)
H–C(25)	6.89 (*dd*, *J* = 8.4, 1.6)	112.5	H–C(25), H–C(20)	C(19), C(20), C(23)
NH	9.91 (*s*)			C(18), C(19), C(20), C(25)

[a])–[d]) These assignments may be interchanged. [e]) H–C(7) and H–C(8) as well as H–C(9) and H–C(10) are overlapping.



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linkage could be detected. Therefore, we investigated this regioselective hydrolysis process more thoroughly to better understand the behavior of our inhibitor series and to gain some insight into their mode of inhibition.

The hydrolysis mechanism of **5** was elucidated by means of ^{18}O labeling. Reactions were carried out in 80% $H_2{}^{18}O$ with mass-spectrometric (MS) detection [16] under electrospray-ionization (ES) or atmospheric-pressure chemical-ionization (APCI) conditions. To avoid back exchange of ^{18}O during injection, samples were prepared in 50% aqueous MeCN immediately prior to infusion. Over the course of 30 min, no observable change in peak ratios was detected. The isotope distribution of ions was measured by MS, while the site of isotope incorporation was elucidated by MS/MS. Sufficient resolution in the MS/MS experiments was used to isolate single isotopes.

Compound **5** ionized in negative electrospray mode and gave a moderately intense $[M-H]^-$ peak at m/z 500, a strong peak at m/z 518 corresponding to a hydrated ion $[M+H_2O-H]^-$, and a signal at m/z 474 resulting from loss of CO_2 from the hydrated ion. Compound **7** also produced ions at m/z 518, $[M-H]^-$ in this case, and lost CO_2, whereas the sodium salt **10** generated the carboxylate ion at m/z 518 and gave signals in the positive-ion mode. However, the MS/MS fragmentation patterns for the m/z 518 ions from **7** and **10** shared common features, *e.g.*, loss of CO_2, that were useful for the determination of the site of labeling (*Fig. 1*). A key fragment for the subsequent analysis was found at m/z 173. As shown in *Fig. 1*, this ion could arise from two possible fragmentations. This ion was subsequently shown to result from fragmentation B.

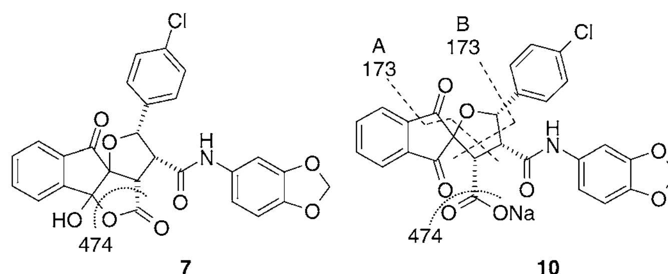
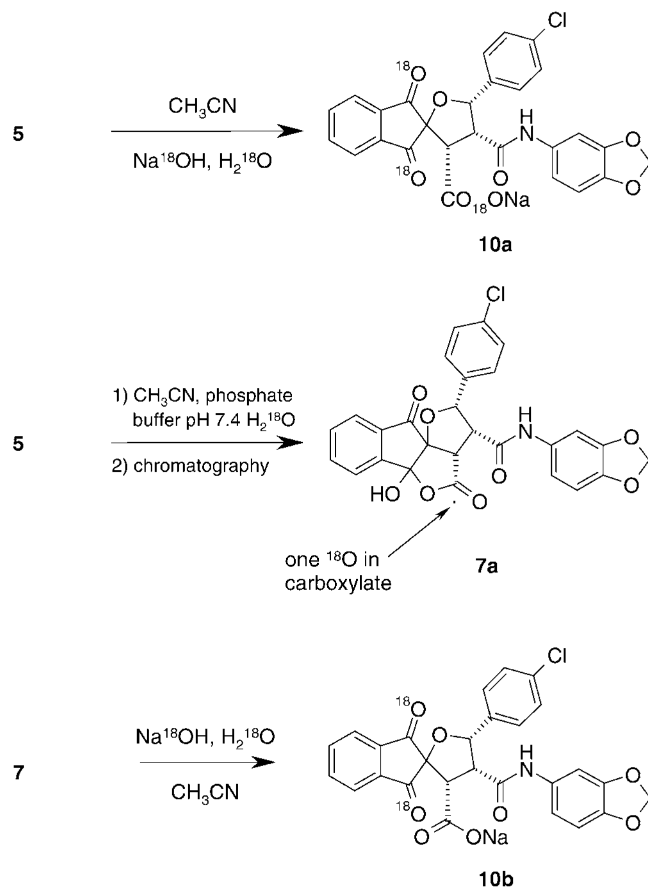


Fig. 1. *MS/MS fragmentation features*

Upon treatment of **5** in MeCN with $Na^{18}OH$ (0.02N) in $H_2{}^{18}O$, **10a** was obtained and was found to contain three isotopic labels. As shown in *Scheme 3*, the two indandione C=O groups had exchanged as well as one of the O-atoms of the carboxylate group. This experiment suggested that the proximal ketone of the indandione was implicated in the ring-opening reaction.

A solution of **5** in MeCN was added to phosphate buffer, prepared from 80% $H_2{}^{18}O$, at pH 7.1. After 2 h, the mixture was flash chromatographed (silica gel) to afford a mixture of compounds **5** and **7a** (*Scheme 3*). MS Analysis of the recovered **5** did not show the presence of ^{18}O. The sample of **7a** from this experiment contained one ^{18}O label only which was found to reside on the lactone carboxylate moiety. The lack of ^{18}O in the ketone C=O groups was surprising; however, the result would be consistent with hydration, where back exchange of the ketones had occurred on exposure to silica gel.

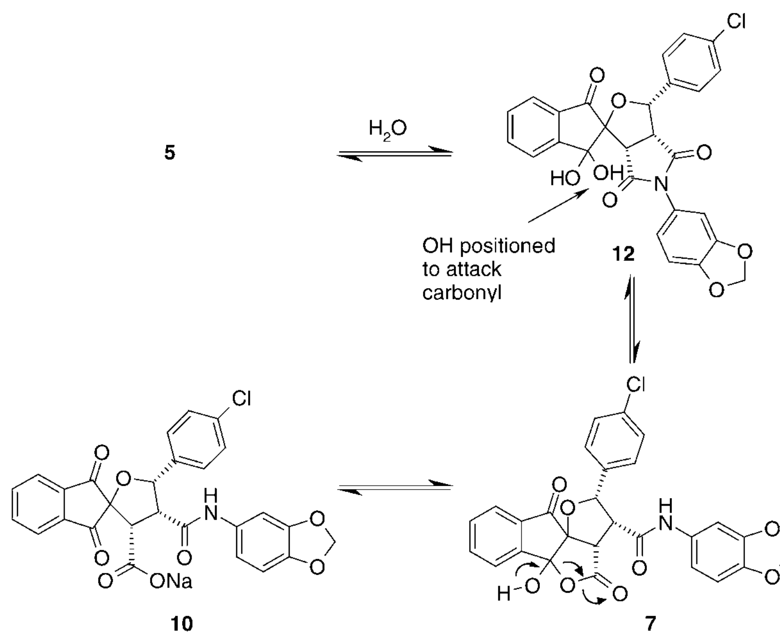
Scheme 3. *^{18}O-Labeling Experiments*



Finally, hydrolysis of lactone **7** with $Na^{18}OH$ gave **10b**, incorporating two labeled atoms (*Scheme 3*). The MS of this material showed the presence of two ^{18}O atoms, neither of which was located at the carboxylate (loss of 44 not 46 from *m/z* 518). In the MS an ion at *m/z* 177 was noted. This could arise only by fragmentation B (see *Fig. 1*).

Based on these and other results (*vide infra*), the mechanism for the formation of **7** and **10** from **5** must involve an assisted hydrolysis of the imide moiety (*Scheme 4*). When placed in buffer at pH > 5, the C=O groups undergo rapid equilibrium between the carbonyl and hydrate forms. The formation of hydrates is not unusual; this type of equilibrium is well-documented [17] for all types of ketones and has been shown to be very rapid and very much shifted towards the ketone form in most cases. Because of the spiro geometry of the present compounds, the formation of a hydrate such as **12** places an OH group near the imide C=O in a geometry in which nucleophilic attack at that C=O is highly favored. This results in facile opening of the imide moiety to generate

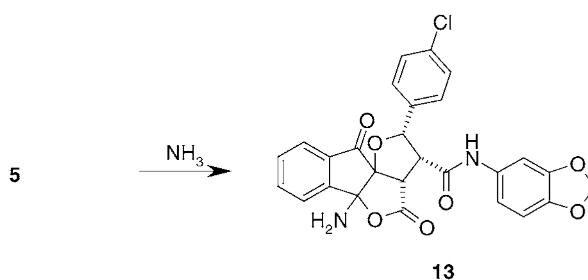
Scheme 4. *Mechanism of Hydrolysis*



the intermediate hydroxy lactone **7**. That this was the initial step in opening is supported by the observations that: 1) imide opening was very rapid and highly regioselective; 2) hydrolysis of **5** to **7** or **10** resulted in ^{18}O exchange at the carboxylate, but the conversion of **7** to **10** did not; 3) treatment of **5** with ammonia in wet MeCN resulted in the rapid formation of **13** and not of a primary amide (*Scheme 5*).

The transformation of **7** to **10** did not involve nucleophilic attack of OH^- at the lactone C=O but instead removal of the OH H-atom resulting in collapse of the hydroxy lactone structure as shown in *Scheme 4*. This is nicely illustrated by the fact that basic hydrolysis of **7** with labeled hydroxide gave **10b** in which there is no ^{18}O incorporation at the carboxylate moiety (*Scheme 3*). This is also consistent with the fact

Scheme 5. *Aminolysis Reaction*



that conversion of **10** to **7** with acid involved no isotope exchange (microscopic reversibility). The fact that these processes are in equilibrium is evidenced by the observations that *a*) compound **7** was readily titrated to **9** with CH_2N_2 (*Scheme 2*); *b*) placing **5** in buffer at pH 7 gave a steady 6 : 4 mixture of **5** and **7** after 2 h, as shown by HPLC experiments; *c*) when **7** or **10** were dissolved in pH 7.4 buffer at higher concentrations, a precipitate of **5** slowly deposited. In addition, compounds **5**, **7**, and **10** all had similar activity in our binding assay (IC_{50} 8.3, 6.6, and 7.8 μM, resp.).

In our initial HPLC experiments, we had detected the presence of **5** and **7** in buffer at pH 7. The presence of **10** in this solution was surmised by the observation that **7** was rapidly converted to **9** by CH_2N_2 titration. The fact that **10** was not observed in the initial HPLC experiments could be attributed to the rapid conversion of **10** to **7** under the acidic conditions used to perform the chromatography. Indeed, the HPLC retention times t_R of **7** and **10** were identical. To directly observe the presence of carboxylates such as **10** in buffer, we had to resort to NMR methods.

The relatively low solubility of **5** in buffer at concentrations required for NMR work precluded the use of **5**, **7**, or **10** in this study. Instead the experiment was conducted with the related derivatives **14**, **15**, and **16** (*Fig. 2*). When each of these compounds was placed in phosphate buffer, the NMR spectra of the resulting solution showed the same single product. This indicated that there was, indeed, an equilibrium between **14**, **15**, and **16** and also suggested that the equilibrium was shifted towards one of the species, at least to the limit of detection by the NMR method. Unfortunately, the spectra obtained in buffer did not allow for unequivocal identification of this compound. Rather than perform a detailed structure determination in assay buffer, the solutions were lyophilized and the spectra of the residues recorded in (D_6)DMSO. Using this method, we were able to confirm by simple comparison of spectra that the equilibrium was shifted in favor of the carboxylate **16** (*Fig. 3*).

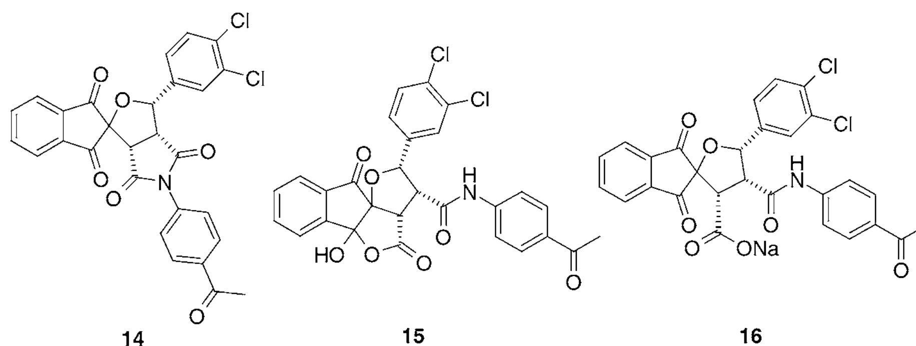


Fig. 2. *Structure of compounds used in the NMR studies*

Our original investigation of the behavior of these compounds was motivated by a desire to understand which structural species was required to interact with its molecular target E2. The fact that **5** was found to exist in equilibrium with **7** and **10** raised the question as to which of these was, in fact, the bioactive form. Given the mechanism of formation of **7** from **5**, it was also possible that these compounds were irreversible covalent inhibitors. This would happen if a nucleophilic group at the E2 target protein

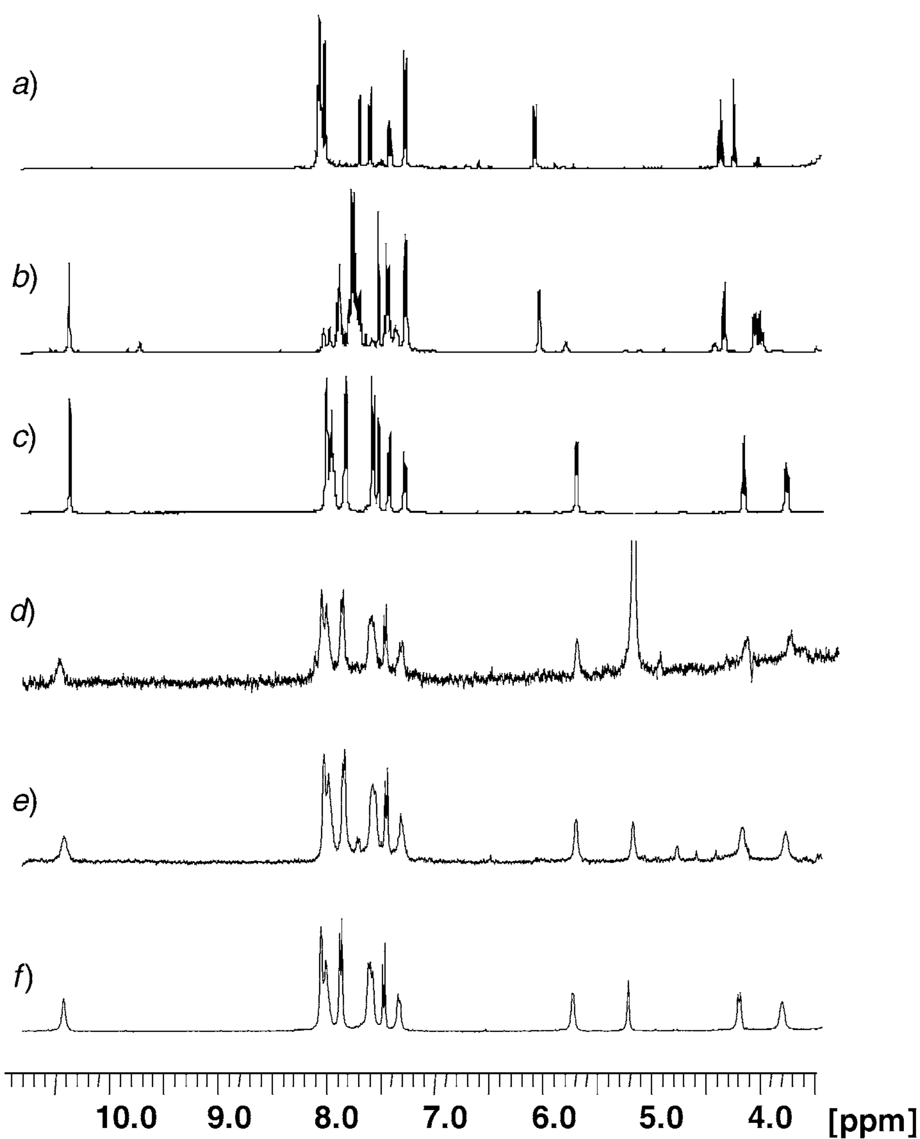
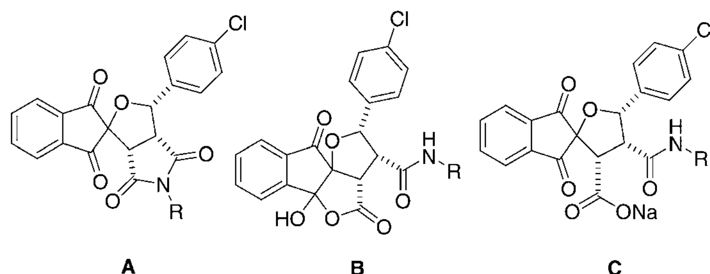


Fig. 3. *^{1}H-NMR Spectra* ((D$_6$)DMSO) of a) **14**, b*)* **15**, *and* c*)* **16**, *and ^{1}H-NMR Spectra* ((D$_6$)DMSO) *resulting from* d*)* **14**, e*)* **15**, *and* f*)* **16** *after incubation of separate samples in phosphate buffer and lyophilization showing the presence of* **16** *only* (*cf.* c)

were to attack the 'bottom' ketone group of **5**, resulting in opening of the imide moiety and formation of a reasonably stable complex. However, this last possibility was ruled out, as was recently described [11], by experiments which demonstrated that these compounds were inhibiting E1–E2 protein–protein interaction by a reversible mechanism.

The possibility that the original imide form, as exemplified by **5**, could be the active species was ruled out by early structure–activity-relationship (SAR) studies. Since we did not know what the active species was in early SAR work, all imide compounds (*i.e.*, **5**) were converted to the corresponding sodium salts or lactones (*i.e.*, **10** or **7**) and all forms were tested in the *in vitro* E1–E2–ori-complex-formation assay [12]. In most cases, the IC_{50} values of all three entities (imide, hydroxy lactone, or carboxylate) for a given set of substituents were virtually the same (*Table 3*, *Entries 1–3*). A few examples, however, were active in the assay when prepared as the hydroxy lactone or carboxylate forms but were less active when submitted as an imide (*Entries 4–9*). Conversion from the imide form to the hydroxy lactone or carboxylate forms was perhaps somewhat slow in these cases. The fact that these imides were much less active than the lactones or carboxylates suggested that the imide form (*i.e.*, **5**) is not the active species. This left two possibilities, hydroxy lactones such as **7** or carboxylates such as **10**.

Table 3. *Biological Activities in the* in vitro *E1–E2–ori-Complex-Formation Assay* [12]

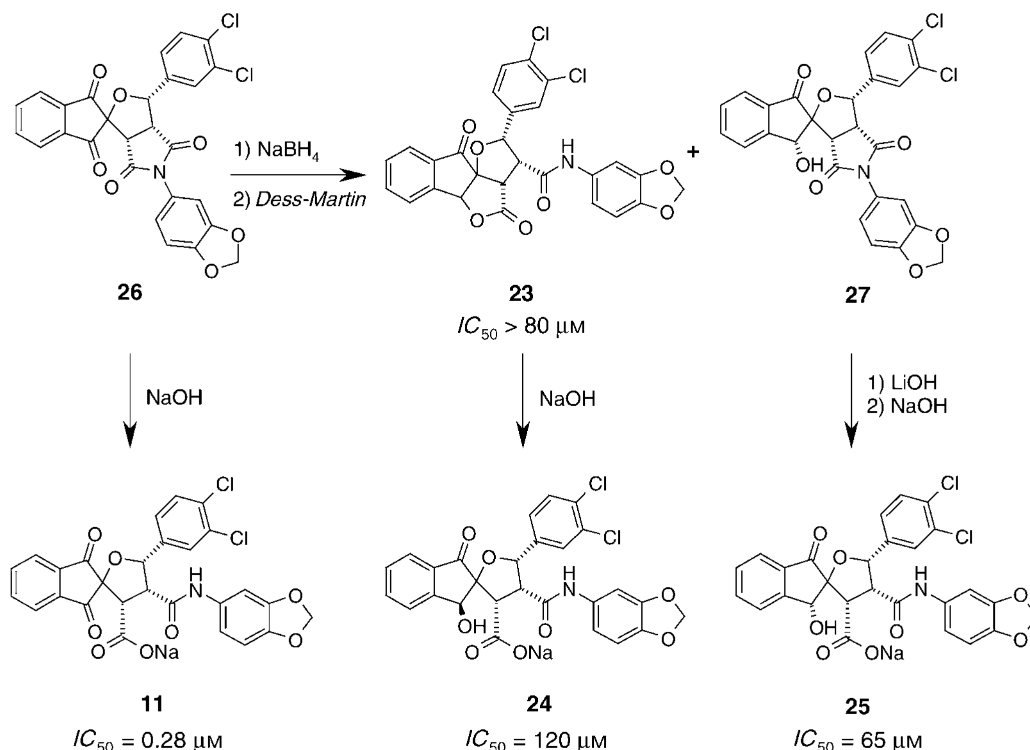


Entry	Compound	R	Form	IC_{50} [μM]
1	**5**	1,3-Benzodioxol-5-yl	**A**	8.3
2	**7**	1,3-Benzodioxol-5-yl	**B**	6.6
3	**10**	1,3-Benzodioxol-5-yl	**C**	7.8
4	**17**	Ph	**A**	64
5	**18**	Ph	**C**	16
6	**19**	Bn	**A**	> 160
7	**20**	Bn	**B**	9.9
8	**21**	C_6H_{11}	**A**	> 80
9	**22**	C_6H_{11}	**B**	9.3

To differentiate these species in our assay, we prepared several derivatives in which the overall structure was locked into either a carboxylate or lactone. As shown in *Scheme 6*, these derivatives were all prepared from imide **26**. Reduction of both C=O functions of **26** with $NaBH_4$ followed by reoxidation of the less hindered alcohol function with *Dess-Martin* periodinane [18] gave a mixture of **23** and **27**. Hydrolysis of **23** with NaOH delivered **24**. Imide **27** proved to be resistant to hydrolysis but succumbed to LiOH. Cation exchange with NaOH gave **25**. Careful hydrolysis of **26** with NaOH gave **11**. Lactone **23** when tested in the E1–E2–ori-complex-formation assay [12] showed no activity up to the maximum concentration tested (> 80 μM). Carboxylates **24** and **25** showed marginal activity (IC_{50} = 120 and 65 μM, resp.) in the same assay. Although the inhibition seen with these compounds was weak, the fact that

they showed some activity perhaps suggested that the carboxylate (as exemplified by **10**) was the form preferred by the protein. It is interesting to note that the major form in buffer solution as observed by NMR was also the carboxylate form (*Fig. 3*). These results need to be treated with caution, however, since both **24** and **25** were more than two orders of magnitude less active than reference compound **11**. It is apparent also from these experiments that the inhibitory activity of this series of compounds was sensitive towards modification of the indandione moiety (compare **11** with **24** and **25**), as has been confirmed by subsequent SAR explorations [19].

Scheme 6. *Ketone-Reduction Experiments*



Conclusions. – The behavior of inhibitors of the HPV11 E1–E2–ori-complex formation in assay buffer was elucidated as described. Dissolution of compounds such as **1** in aqueous solutions at pH 7 resulted in the rapid conversion of these maleimides into carboxylates such as **10**. This process was found to involve neighboring-group participation by the indandione group *via* hydroxy lactones such as **7**. The key experiments in this elucidation were hydrolyses conducted in $H_2^{18}O$ by mass-spectrometric detection. SAR Work comparing the activity of maleimide *vs.* hydroxy lactones and carboxylates indicated that the active species is not the maleimide form. Experiments are underway to try to determine whether the active species is the

carboxylate or hydroxy lactone. NMR Techniques established, however, that the dominant form in pH 7 buffer is the carboxylate.

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Experimental Part

General. All reactions were carried out under Ar. Solvents and reagents were used as purchased. Column flash chromatography (FC): silica gel (10–40 µm or 230–400 mesh ASTM, *Merck*). Prep. HPLC: *Partisil-10-ODS-3 C18* prep. column (50 cm × 22 mn). Anal. HPLC: *Vydac-C$_{18}$* column (10 µm, 4.6 × 250 mm); MeCN/H_2O gradient containing 0.06% CF_3COOH. IR Spectra: $\tilde{\nu}$ in cm^{-1}. ^{1}H-NMR Spectra: *Bruker-AMX-400* spectrometer, δ in ppm rel. to $SiMe_4$ as internal standard, J in Hz. ES-MS: *Micromass-Quattro-II* mass spectrometer.

*3′-(4-Chlorophenyl)spiro[2*H*-indene-2,2′-oxirane]-1,3-dione* (**3**) [20]. To a soln. of 1*H*-indene-1,3(2*H*)-dione (**2**; 6.0 g, 41.1 mmol) in EtOH (50 ml) was added 4-chlorobenzaldehyde (6.3 g, 45.2 mmol) followed by piperidine (5 drops). The mixture was heated at reflux for 30 min. After cooling, the mixture was diluted with EtOH (150 ml) and the precipitate filtered. The resulting solid was triturated twice with EtOH and dried under high vacuum to give 2-(4-chlorobenzylidene)-1*H*-indene-1,3(2*H*)-dione (10.0 g, 91%). ^{1}H-NMR ((D_6)DMSO): 8.43 (*d*, J = 8.6, 2 H); 8.03–8.01 (*m*, 2 H); 7.84–7.82 (*m*, 3 H); 7.44 (*d*, J = 8.6, 2 H).

To a suspension of 2-(4-chlorobenzylidene)-1*H*-indene-1,3(2*H*)-dione (5.0 g, 18.7 mmol) in MeOH (45 ml) was added 30% hydrogen peroxide soln. (3 ml). The mixture was cooled to 0°, and 1N NaOH (0.5 ml) was added dropwise. After addition was completed, stirring was continued at r.t. for 1 h. The mixture was then poured into H_2O (200 ml), and the resulting solid was collected by filtration and washed with H_2O. Drying under high vacuum gave **3** (5.0 g, 95%). ^{1}H-NMR ((D_6)DMSO): 8.05–8.03 (*m*, 1 H); 7.43–7.87 (*m*, 3 H); 7.55 (*d*, J = 8.6, 2 H); 7.37 (*d*, J = 8.6, 2 H); 4.70 (*s*, 1 H).

rel-*(3*R*,3a*S*,6a*R*)- and* rel-*(3*R*,3a*R*,6a*S*)-5-(1,3-Benzodioxol-5-yl)-3-(4-chlorophenyl)-3a,6a-dihydrospiro[1*H*-furo[3,4-*c*]pyrrole-1,2′-[2*H*]indene]-1′,3′,4,6(3*H*,5*H*)-tetrone* (**5** and **6**, resp.). A mixture of **3** (284 mg, 1 mmol) and 1-(1,3-benzodioxol-5-yl)-1*H*-pyrrol-2,5-dione (**4**; 227 mg, 1.05 mmol) in toluene (10 ml) was heated to reflux for 16 h. After cooling and concentration, the residue was purified by FC (AcOEt/hexane 1:1 → 7:3): **5** (225 mg, 45%) and **6** (161 mg, 32%).

Data of **5**: IR (KBr): 1788, 1713. ^{1}H-NMR ((D_6)DMSO): 8.13–8.10 (*m*, 4 H); 7.49 (*d*, J = 8.5, 2 H); 7.43 (*d*, J = 8.5, 2 H); 7.03 (*d*, J = 8.1, 1 H); 6.67 (*d*, J = 1.9, 1 H); 6.65 (*dd*, J = 8.1, 1.9, 1 H); 6.09 (*s*, 2 H); 6.08 (*d*, J = 8.0, 2 H); 4.26 (*dd*, J = 7.9, 7.9, 1 H); 4.16 (*d*, J = 8.0, 1 H). ^{13}C-NMR ((D_6)DMSO): 197.8; 194.6; 173.4; 172.6; 147.3; 140.7; 139.0; 137.7; 137.6; 135.3; 132.5; 128.1; 128.0; 125.2; 124.5; 123.8; 120.6; 108.2; 107.5; 101.8; 84.1; 83.0; 52.1; 51.2. ES-MS (neg.): 500.0 ([M − H]$^-$), 518.1 ([M + 18 − H]$^-$), 474.1 (see text). Anal. calc. for $C_{27}H_{16}ClNO_7$: C 64.62, H 3.21, N 2.79, found: C 64.50, H 3.20, N 2.88.

Data of **6**: IR (KBr): 1743, 1708, 1687. ^{1}H-NMR ((D_6)DMSO): 8.18–8.09 (*m*, 4 H); 7.58 (*d*, J = 8.0, 2 H); 7.55 (*d*, J = 8.0, 2 H); 7.07 (*d*, J = 8.3, 1 H); 6.80 (*d*, J = 1.9, 1 H); 6.74 (*dd*, J = 8.3, 1.9, 1 H); 6.12 (*s*, 2 H); 5.73 (*d*, J = 7.2, 1 H); 4.27 (*d*, J = 9.8, 1 H); 3.96 (*dd*, J = 9.8, 7.2, 1 H). ^{13}C-NMR ($CDCl_3$): 198.0; 194.2; 174.1; 173.1; 148.2; 141.3; 141.1; 137.3; 137.2; 137.0; 134.5; 129.0; 127.3; 124.7; 124.6; 124.3; 120.7; 108.5; 107.9; 101.9; 84.2; 83.0; 55.0; 51.1. ES-MS (neg.): 500.0 ([M − H]$^-$), 518.0 ([M + 18 − H]$^-$), 474.1 ([M + 18 − 44]$^-$). Anal. calc. for $C_{27}H_{16}ClNO_7$: C 64.62, H 3.21, N 2.79; found: C 64.46, H 3.27, N 2.82.

rel-*(2*R*,3*S*,3a*R*)-*N*-(1,3-Benzodioxol-5-yl)-2-(4-chlorophenyl)-2,3,3a,4,5a,10-hexahydro-5a-hydroxy-4,10-dioxofuro[2,3-*c*]indeno[1,2-*b*]furan-3-carboxamide* (**7**). To a soln. of **5** in MeCN (5 ml) was added phosphate buffer (pH 8.0; 5 ml). After 48 h, the mixture was evaporated and the resulting residue purified by FC (AcOH/hexane 1:1 → AcOEt): **7** (86 mg, 43%). White solid. M.p. 157.6°. IR (KBr): 3296–2900, 1746, 1710, 1655. ^{1}H-NMR ((D_6)DMSO): 10.15 (*s*, 1 H); 7.98 (*dd*, J = 7.4, 7.4, 1 H); 7.94 (*d*, J = 7.4, 1 H); 7.87 (*d*, J = 7.4, 1 H); 7.77 (*dd*, J = 7.4, 7.4, 1 H); 7.58 (*s*, 1 H); 7.49 (*d*, J = 8.4, 2 H); 7.39 (*d*, J = 8.4, 2 H); 6.76 (*d*, J = 8.43, 1 H); 6.71 (*d*, J = 1.7, 1 H); 6.51 (*dd*, J = 8.3, 1.7, 1 H); 6.02 (*d*, J = 5.4, 1 H); 5.96 (*s*, 2 H); 4.32 (*d*, J = 8.8, 1 H); 3.98 (*dd*, J = 8.8, 5.4, 1 H). ^{13}C-NMR ((D_6)DMSO): 200.0; 171.4; 168.4; 148.2; 146.9; 144; 137.4; 134.4; 133.8; 132.6; 131.8; 131.1; 127.9; 124.8; 123.6; 113.6; 107.8; 105.6; 102.4; 101.1; 88.4; 88.0; 53.0; 52.7. ES-MS (neg.): 517.9 ([M − H]$^-$), 473.9 ([M − CO_2]$^-$).

rel-*(3*R*,4*S*,5*R*)-4-[(1,3-Benzodioxol-5-ylamino)carbonyl]-5-(4-chlorophenyl)-1′,3′,4,5-tetrahydro-1′,3′-dioxospiro[furan-2(3*H*),2′-[2*H*]indene]-3-carboxylic Acid Methyl Ester* (**9**). To a soln. of **7** (70 mg, 0.13 mmol) in

CH_2Cl_2 (2 ml) was added excess CH_2N_2 as a soln. in Et_2O. The mixture was stirred briefly and then evaporated. The resulting residue was purified by FC (AcOEt/hexane 1:1): **9** (70 mg, 99%). M.p. 175.2°. IR (KBr): 1742, 1710, 1686, 1637. ^{1}H-NMR ((D_6)DMSO): 9.37 (*s*, 1 H); 8.22–8.15 (*m*, 4 H); 7.50 (2*d*, *J* = 8.6, 4 H); 6.91 (*d*, *J* = 1.9, 1 H); 6.85 (*d*, *J* = 8.3, 1 H); 6.63 (*dd*, *J* = 8.3, 1.9, 1 H); 6.04 (2*s*, 2 H); 5.90 (*d*, *J* = 6.0, 1 H); 4.60 (*d*, *J* = 8.3, 1 H); 4.05 (*dd*, *J* = 8.3, 6.0, 1 H); 3.46 (*s*, 3 H). ES-MS (neg.): 532.1 ($[M - H]^-$). ES-MS (pos.): 534.0 ($[M + H]^+$), 556.0 ($[M + Na]^+$). Anal. calc. for $C_{28}H_{20}ClNO_8$: C 62.99, H 3.78, N 2.62; found: C 62.83, H 3.86, N 2.61.

rel-*(3*R*,4*S*,5*R*)-4-[(1,3-Benzodioxol-5-ylamino)carbonyl]-5-(4-chlorophenyl)-1′,3′,4,5-tetrahydro-1′,3′-dioxospiro[furan-2(3*H*),2′-[2*H*]indene]-3-carboxylic Acid Sodium Salt* (**10**). To a soln. of **5** (143 mg, 0.27 mmol) in MeCN (27 ml) was added 0.02N NaOH (135 ml, 0.27 mmol) with a syringe pump over 1 h. After the addition was completed, the mixture was stirred for an extra 2 h. The resulting soln. was concentrated and lyophilized: **10** (161 mg, 100%). White solid. M.p. 204.2°. IR (KBr): 1744, 1707, 1660. ^{1}H-NMR ((D_6)DMSO): 9.91 (*s*, 1 H); 8.04–7.96 (*m*, 4 H); 7.38 (*d*, *J* = 8.5, 2 H); 7.31 (*d*, *J* = 8.5, 2 H); 7.09 (*d*, *J* = 1.6, 1 H); 6.89 (*dd*, *J* = 8.4, 1.6, 1 H); 6.83 (*d*, *J* = 8.4, 1 H); 5.96 (*s*, 2 H); 5.71 (*d*, *J* = 5.9, 1 H); 4.15 (*d*, *J* = 8.7, 1 H); 3.65 (*dd*, *J* = 8.7, 5.9, 1 H). ^{13}C-NMR ((D_6)DMSO): 202.0; 199.8; 169.1; 167.5; 146.8; 143.8; 142.7; 138.8; 137.2; 136.1; 135.8; 133.5; 131.8; 127.7; 123.4; 112.5; 107.8; 102.0; 100.8; 85.1; 83.0; 62.0; 56.7. ES-MS (neg.): 518.1 ($[M - H]^-$). ES-MS (pos.): 520.0 ($[M + H]^+$), 542.0 ($[M + Na]^+$), 502.0 ($[M - Na + 2 H]^+$).

rel-*(3*R*,4*S*,5*R*)-4-[(1,3-Benzodioxol-5-ylamino)carbonyl]-5-(3,4-dichlorophenyl-1′,3′,4,5-tetrahydro-1′,3′-dioxospiro[fluran-2(3*H*),2′-[2*H*]indene]-3-carboxylic Acid Sodium Salt* (**11**). As described for **10** with **26** (143 mg; 0.27 mmol): **11** (161 mg, 100%). White solid. IR (KBr): 3616–3100, 1745, 1708, 1661. ^{1}H-NMR ((D_6)DMSO): 10.02 (*s*, 1 H); 8.07–7.96 (*m*, 4 H); 7.59 (*d*, *J* = 1.6, 1 H); 7.52 (*d*, *J* = 8.6, 1 H); 7.35 (*dd*, *J* = 8.3, 1.6, 1 H); 7.06 (*d*, *J* = 1.9, 1 H); 6.83 (*d*, *J* = 8.3, 1 H); 6.73 (*dd*, *J* = 8.6, 1.9, 1 H); 5.96 (*s*, 2 H); 5.70 (*d*, *J* = 5.7, 1 H); 4.13 (*d*, *J* = 8.6, 1 H); 3.66 (*dd*, *J* = 8.9, 6.0, 1 H). ^{13}C-NMR ((D_6)DMSO): 201.7; 199.5; 168.7; 167.3; 146.7; 143.7; 142.7; 138.8; 138.5; 137.0; 135.7; 133.3; 130.4; 139.8; 129.7; 127.8; 126.2; 123.4; 123.3; 112.6; 107.7; 102.0; 100.7; 84.3; 83.0; 61.9; 56.2. ES-MS (neg.): 552.1 ($[M - H]^-$).

rel-*(2*R*,3*S*,3a*R*)-5a-Amino-*N*-(1,3-benzodioxol-5-yl)-2-(4-chlorophenyl)-2,3,3a,4,5a,10-hexahydro-4,10-dioxofuro[2,3-*c*]indeno[1,2-*b*]furan-3-carboxamide* (**13**). To a soln. of **5** (150 mg, 0.29 mmol) in MeCN (5 ml) was added aq. NH_4OH soln. (0.5 ml). After 2 h, the mixture was evaporated and co-evaporated twice with benzene. The resulting solid was triturated with AcOEt to give **13** (57 mg, 37%). White solid.(IR (KBr): 3068, 3013, 1723, 1679. ^{1}H-NMR ((D_6)DMSO): 10.02 (*s*, 1 H); 8.24 (*d*, *J* = 7.9, 1 H); 8.15 (*t*, *J* = 7.3, 1 H); 8.01 (*d*, *J* = 7.9, 1 H); 7.90 (*t*, *J* = 7.3, 1 H); 7.68 (*d*, *J* = 8.0, 2 H); 7.57 (*d*, *J* = 8.0, 2 H); 6.93 (*d*, *J* = 7.9, 2 H); 6.70 (*d*, *J* = 8.6, 1 H); 6.18 (*d*, *J* = 5.8, 1 H); 6.14 (*s*, 2 H); 4.42 (*d*, *J* = 9.2, 1 H); 4.28 (br. *s*, 2 H); 4.08 (*dd*, *J* = 9.2, 5.8, 1 H). ES-MS (neg.): 517.9 ($[M - H]^-$).

rel-*(3*R*,3a*S*,6a*R*)-5-(4-Acetylphenyl)-3-(3,4-dichlorophenyl)-3a,6a-dihydrospiro[1*H*-furo[3,4-*c*]pyrrole-1,2′-[2*H*]indene]-1′,3′,4,6(3*H*,5*H*)-tetrone* (**14**). As described for **5**, **14** was obtained in 40% yield. White solid. ^{1}H-NMR ((D_6)DMSO): 8.17–8.07 (*m*, 6 H); 7.76–7.75 (*m*, 1 H); 7.65 (*d*, *J* = 8.6, 1 H); 7.47 (*dd*, *J* = 8.2, 1.9, 1 H); 7.33 (*d*, *J* = 8.3, 2 H); 6.11 (*d*, *J* = 7.6, 1 H); 4.38 (*t*, *J* = 7.9, 1 H); 4.24 (*d*, *J* = 8.3, 1 H); 2.60 (*s*, 3 H). ES-MS (neg.): 532.1 ($[M - H]^-$), 550.1 ($[M + H_2O - H]^-$). ES-MS (pos.): 552.2 ($[M + H_2O + H]^+$), 534.1 ($[M + H]^+$).

rel-*(2*R*,3*S*,3a*R*)-*N*-(4-Acetylphenyl)-2-(3,4-dichlorophenyl)-2,3,3a,4,5a,10-hexahydro-5a-hydroxy-4,10-dioxofuro[2,3-*c*]indeno[1,2-*b*]furan-3-carboxamide* (**15**). As described for **10**, with **14** (100 mg, 0.19 mmol), MeCN, and 0.02N NaOH (9.4 ml, 0.19 mmol). The lyophilizate was purified by reversed-phase semi-prep. HPLC (gradient MeCN/H_2O containing CH_3COOH (0.06%)). The desired fractions were lyophilized: **15** (43 mg, 42%). White solid. IR (KBr): 3241, 1764, 1726, 1673. ^{1}H-NMR ((D_6)DMSO): 8.82 (*s*, 1 H); 7.99–7.93 (*m*, 2 H); 7.88–7.84 (*m*, 3 H); 7.77–7.75 (*m*, 1 H); 7.64 (*s*, 1 H); 7.43 (*s*, 2 H); 7.34 (*s*, 2 H); 7.34 (*d*, *J* = 8.3, 2 H); 6.83 (*s*, 1 H); 6.08 (*d*, *J* = 4.8, 1 H); 4.16 (*d*, *J* = 8.6, 1 H); 3.99 (*dd*, *J* = 8.5, 5.2, 1 H); 2.5 (*s*, 3 H). ES-MS (neg.): 549.9 ($[M - H]^-$). ES-MS (pos.): 552.0 ($[M + H]^+$).

rel-*(3*R*,4*S*,5*R*)-4-{[(4-Acetylphenyl)amino]carbonyl}-5-(3,4-dichlorophenyl)-1′,3′,4,5-tetrahydro-1′,3′-dioxospiro[furan-2(3*H*),2′-[2*H*]indene]-3-carboxylic Acid Sodium Salt* (**16**): As described for **10**, with **14** (43 mg, 0.08 mmol), MeCN, and 0.02N NaOH (4 ml, 0.08 mmol). The lyophilizate was triturated with MeCN: **16** (28 mg, 62%). White solid. IR (KBr): 2900, 1710, 1591. ^{1}H-NMR ((D_6)DMSO): 10.53 (*s*, 1 H); 8.10–7.97 (*m*, 4 H); 7.89 (*d*, *J* = 8.6, 2 H); 7.63 (*d*, *J* = 8.9, 2 H); 7.58–7.57 (*m*, 1 H); 7.48 (*d*, *J* = 8.3, 1 H); 7.35–7.33 (*m*, 1 H); 5.72 (*d*, *J* = 5.7, 1 H); 4.16 (*d*, *J* = 8.6, 1 H); 3.89 (*dd*, *J* = 8.6, 5.7, 1 H); 2.45 (*s*, 3 H). ES-MS (neg.): 550.1 ($[M - H]^-$). ES-MS (pos.): 552.1 ($[M + H]^+$).

rel-*(3*R*,3a*S*,6a*R*)-3-(4-Chlorophenyl)-3a,6a-dihydro-5-phenylspiro[1*H*-furo[3,4-*c*]pyrrol-1,2′-[2*H*-indene]-1′,3′,4,6(3*H*,5*H*)-tetrone* (**17**). As described for **5**, **17** was obtained in 61% yield. White solid. M.p. 209°. IR (KBr): 1774, 1750, 1717. ^{1}H-NMR ((D_6)DMSO): 8.15–8.08 (*m*, 4 H); 7.40–7.27 (*m*, 7 H); 7.22 (*d*, *J* = 8.3, 2 H); 6.04 (*dd*, *J* = 7.6, 1.6, 1 H); 4.55 (*d*, *J* = 14.9, 1 H); 4.35 (*d*, *J* = 14.9, 1 H); 4.18–4.13 (*m*, 2 H). ^{13}C-NMR

((D_6)DMSO): 198.3; 193.2; 173.0; 172.7; 141.8; 139.0; 137.0; 135.1; 134.3; 133.5; 129.2; 128.6; 128.5; 128.1; 127.7; 124.4; 124.3; 83.5; 83.4; 51.7; 51.1; 43.0. ES-MS (neg.): 456.0 ($[M-H]^-$), 474.0 ($[M+H_2O-H]$).

rel-*(3*R*,4*S*,5*R*)-5-(4-Chlorophenyl)-1′,3′,4,5-tetrahydro-1′,3′-dioxo-4-[(phenylamino)carbonyl]spiro[furan-2(3*H*),2′-[2*H*]indene]-3-carboxylic Acid Sodium Salt* (**18**). Similarly as described for **10**, **18** was isolated as a white solid. M.p. 132°. IR (KBr): 1774, 1763, 1719. ¹H-NMR ((D_6)DMSO): 8.94 (br. *s*, 1 H); 7.94–7.74 (*m*, 5 H); 7.44–7.39 (*m*, 4 H); 7.18–7.16 (*m*, 3 H); 6.63–6.62 (*m*, 2 H); 5.91 (*d*, *J* = 5.1, 1 H); 4.26 (*d*, *J* = 8.6, 1 H); 4.15 (*dd*, *J* = 15.3, 14.9, 1 H); 3.93–3.86 (*m*, 2 H). ¹³C-NMR ($CDCl_3$): 200.3; 171.1; 169.2; 148.7; 137.6; 136.1; 134.7; 133.9; 133.3; 131.8; 128.8; 128.7; 127.8; 127.6; 127.3; 125.4; 124.0; 107.2; 88.5; 88.4; 54.3; 53.0; 43.9. ES-MS (neg.): 474.1 ($[M-Na]^-$). ES-MS (pos.): 478.1 ($[M+H]^+$), 520.1 ($[M+Na]^+$).

rel-*(3*R*,3a*S*,6a*R*)-3-(4-Chlorophenyl)-3a,6a-dihydro-5-(phenylmethyl)spiro[1*H*-furo[3,4-*c*]pyrrole-1,2′-[2*H*]-indene]-1′,3′,4,6(3*H*,5*H*)-tetrone* (**19**). Similarly as described for **5**, **19** was isolated as a white solid. M.p. 209°. IR (KBr): 1774, 1750, 1717. ¹H-NMR ((D_6)DMSO): 8.15–8.08 (*m*, 4 H); 7.40–7.27 (*m*, 7 H); 7.22 (*d*, *J* = 8.3, 2 H); 6.04 (*dd*, *J* = 7.6, 1.6, 1 H); 4.55 (*d*, J_{AB} = 14.9, 1 H); 4.35 (*d*, J_{AB} = 14.9, 1 H); 4.18–4.13 (*m*, 2 H). ¹³C-NMR ($CDCl_3$): 198.3; 193.2; 173.1; 172.7; 141.8; 139.0; 137.3; 137.0; 135.1; 134.3; 133.5; 129.2; 128.6; 128.5; 128.1; 127.7; 124.4; 124.3; 83.5; 83.4; 51.7; 51.1; 43.0. ES-MS (neg.): 488.0 ($[M+H_2O-H]^-$). ES-MS (pos.): 494.0 ($[M+Na]^+$, 472 ($[M+H]^+$).

rel-*(2*R*,3*S*,3a*R*)-2-(4-Chlorophenyl)-2,3,3a,4,5a,10-hexahydro-5a-hydroxy-4,10-dioxo-*N*-(phenylmethyl)-furo[2,3-*c*]indeno[1,2-*b*]furan-3-carboxamide* (**20**). As described for **7**, **20** was obtained as a white solid in 58% yield. M.p. 231.6°. IR (KBr): 1756, 1719, 1711. ¹H-NMR ((D_6)DMSO): 8.26–8.20 (*m*, 4 H); 7.56 (*s*, 4 H); 6.12 (*d*, *J* = 7.7, 1 H); 4.17 (*t*, *J* = 8.2, 1 H); 4.11 (*d*, *J* = 8.3, 1 H); 3.77 (*m*, 1 H); 2.11–1.16 (*m*, 10 H). ¹³C-NMR ($CDCl_3$): 198.6; 193.8; 173.7; 173.0; 141.9; 139.3; 137.4; 137.1; 134.5; 133.9; 128.6; 127.8; 124.4; 83.8; 83.7; 52.7; 51.5; 50.8; 29.8; 28.8; 28.7; 25.9; 25.8; 25.0. ES-MS (neg.): 488.2 ($[M-H]^-$). ES-MS (pos.): 490.1 ($[M+H]^+$). Anal. calc. for $C_{26}H_{22}ClNO_5$: C 67.32, H 4.78, N 3.02; found: C 67.02, H 4.87, N 2.89.

rel-*(3*R*,3a*S*,6a*S*)-3-(4-Chlorophenyl)-5-cyclohoxyl-3a,6a-dihydrospiro[1*H*-furo[3,4-*c*]pyrrole-1,2′-[2*H*]indene]-1′,3′,4,6(3*H*,5*H*)-tetrone* (**21**). As described for **5**, **21** was isolated as a white solid. M.p. 175.7°. IR (KBr): 1777, 1726, 1643. ¹H-NMR ((D_6)DMSO): 8.29 (*m*, 1 H); 8.09–7.76 (*m*, 5 H); 7.45–7.41 (*m*, 4 H); 5.90 (*d*, *J* = 5.1, 1 H); 4.21 (*d*, *J* = 8.9, 1 H); 3.83 (*dd*, *J* = 8.9, 5.1, 1 H); 3.23 (*m*, 1 H); 1.56–0.49 (*m*, 10 H). ES-MS (neg.): 480.1 ($[M+H_2O-H]^-$), 462.1 ($[M-H]^-$). ES-MS (pos.): 486.0 ($[M+Na]^+$), 464.0 ($[M+H]^+$).

rel-*(2*R*,3*S*,3a*R*)-2-(4-Chlorophenyl)-*N*-cyclohexyl-2,3,3a,4,5a,10-hexahydro-5a-hydroxy-4,10-dioxofuro-[2,3-*c*]indeno[1,2-*b*]furan-3-carboxamide* (**22**). As described for **7**, **22** was isolated as a white solid. IR (KBr): 1777, 1726, 1643. ¹H-NMR ((D_6)DMSO): 8.29 (*d*, *J* = 7.9, 1 H); 8.09 (br. *s*, 1 H); 8.00–7.93 (*m*, 2 H); 7.87 (*d*, *J* = 7.6, 1 H); 7.80–7.76 (*m*, 1 H); 7.44–7.42 (*m*, 4 H); 5.91 (*d*, *J* = 5.1, 1 H); 4.21 (*d*, *J* = 8.9, 1 H); 3.82 (*dd*, *J* = 8.9, 5.1, 1 H); 3.23 (br. *m*, 1 H); 1.57–1.35 (*m*, 4 H); 1.20–0.90 (*m*, 4 H); 0.83 (*m*, 1 H); 0.50 (*m*, 1 H). ES-MS (neg.): 480.2 ($[M-H]^-$). ES-MS (pos.): 482.2 ($[M+H]^+$).

rel-*(3*R*,3a*S*,6a*R*)-5-(1,3-Benzodioxol-5-yl)-3-(3,4-dichlorophenyl)-3a,6a-dihydrospiro[1*H*-furo[3,4-*c*]pyrrole-1,2′-[2*H*]indene]-1′,3′,4,6(3*H*,5*H*)-tetrone* (**26**). As described for **5**, with 3′-(3,4-dichlorophenyl)spiro[2*H*-indene-2,2′-oxirane]-1,3-dione (11 g, 33.4 mmol), **4** (7.3 g, 33.4 mmol), and toluene (167 ml): **26** (17.9 g, 50%). IR (KBr): 1785, 1717. ¹H-NMR ((D_6)DMSO): 8.14–8.10 (*m*, 4 H); 7.74 (*d*, *J* = 1.6, 1 H); 7.66 (*d*, *J* = 8.3, 1 H); 7.46 (*dd*, *J* = 8.3, 1.9, 1 H); 7.03 (*d*, *J* = 7.9, 1 H); 6.66–6.63 (*m*, 2 H); 6.09 (*d*, *J* = 7.3, 1 H); 6.09 (*s*, 2 H); 4.31 (*t*, *J* = 8.3, 1 H); 4.18 (*d*, *J* = 8.3, 1 H). ¹³C-NMR ((D_6)DMSO): 197.5; 194.6; 173.3; 172.7; 147.4; 147.3; 140.7; 139.0; 137.7; 137.6; 137.5; 130.6; 130.4; 130.3; 128.2; 126.4; 125.1; 124.5; 123.8; 120.5; 108.2; 107.4; 101.8; 84.2; 82.1; 51.9; 51.2. ES-MS (neg.): 552.0 ($[M+H_2O-H]^-$), 534.0 ($[M-H]^-$).

rel-*(2*R*,3*S*,3a*R*)-*N*-(1,3-Benzodioxol-5-yl)-2-(3,4-dichlorophenyl)-2,3,3a,4,5a,10-hexahydro-4,10-dioxo-furo[2,3-*c*]indeno[1,2-*b*]furan-3-carboxamide* (**23**) *and* rel-*(3*R*,3′*S*,3a*S*,6a*R*)-5-(1,3-Benzodioxol-5-yl)-3-(3,4-dichlorophenyl)-3a,6a-dihydro-3′-hydroxyspiro[1*H*-furo[3,4-*c*]pyrrole-1,2′[2*H*]indene]-1′,4,6(3′*H*,3*H*,5*H*)-trione* (**27**). To a soln. of **26** (500 mg, 0.9 mmol) in THF/MeOH 4 : 1 (20 ml) was added $NaBH_4$ (70 mg, 1.96 mmol) at 0°. After 30 min, the mixture was quenched with 10% aq. citric acid soln. and extracted with AcOEt. The org. phase was washed with H_2O and brine, dried ($MgSO_4$), and filtered through a short silica gel pad. Concentration of the soln. provided a complex mixture (257 mg). To the mixture in CH_2Cl_2 (2 ml) was added *Dess-Martin* periodinane (310 mg, 0.7 mmol) at r.t. After 20 min, the reaction was quenched with $NaHCO_3$ and $Na_2S_2O_3$ solns. and stirred vigorously until a clear soln. was obtained. The mixture was diluted with AcOEt, washed with $NaHCO_3$ soln., H_2O, and brine, dried ($MgSO_4$), and evaporated. The resulting residue was purified by prep. HPLC: **23** (110 mg, 22%) and **27** (97 mg, 20%).

Data of **23**: M.p. 239.8°. IR (KBr): 1788, 1724, 1686, 1648. ¹H-NMR ((D_6)DMSO): 9.83 (*s*, 1 H); 8.14–7.45 (*m*, 7 H); 6.82 (*s*, 1 H); 6.78 (*d*, *J* = 8.7, 1 H); 6.55 (*d*, *J* = 8.7, 1 H); 6.18 (*s*, 1 H); 6.05 (*m*, 1 H); 5.99 (*s*, 2 H); 4.25 (*m*, 1 H); 3.98 (*m*, 1 H). ES-MS (neg.): 536.0 ($[M-H]^-$).

Data of **27**: M.p. 161.3°. IR (KBr): 3500, 1710, 1503. ^{1}H-NMR ((D_6)DMSO): 7.84–7.46 (*m*, 7 H); 7.01 (*d*, *J* = 8.3, 1 H); 6.70 (*d*, *J* = 1.9, 1 H); 6.65 (*dd*, *J* = 8.3, 1.9, 1 H); 6.08 (*s*, 2 H); 6.06 (*m*, 1 H); 5.69 (*d*, *J* = 9.5, 1 H); 5.44 (*d*, *J* = 7.4, 1 H); 4.16 (*dd*, *J* = 7.4, 7.9, 1 H); 3.83 (*d*, *J* = 7.9, 1 H). ES-MS (neg.): 536.0 ([*M* – H]$^-$).

rel-*(1′R,3R,4S,5R)-4-[(1,3-Benzodioxol-5-ylamino)carbonyl]-5-(3,4-dichlorophenyl)-1′,3′,4,5-tetrahydro-1′-hydroxy-3′-oxospiro[furan-2(3H),2′-[2H]indene]-3-carboxylic Acid Sodium Salt* (**24**). To a soln. of **23** (30 mg, 0.06 mmol) in MeCN (5 ml) was added dropwise 0.02N NaOH (2.8 ml). The resulting soln. was stirred for 48 h. The mixture was evaporated and the residue purified by prep. HPLC: **24** (14 mg, 47%). White solid. M.p. 261.5°. IR (KBr): 3300, 1721, 1653, 1638. ^{1}H-NMR ((D_6)DMSO): 9.97 (*s*, 1 H); 7.70–7.31 (*m*, 7 H); 6.71–6.67 (*m*, 2 H); 6.50–6.46 (*m*, 2 H); 5.88 (*d*, *J* = 1.5, 1 H); 5.58 (*d*, *J* = 5.7, 1 H); 5.43 (*s*, 1 H); 3.93 (*d*, *J* = 6.8, 1 H); 3.73 (*dd*, *J* = 6.8, 6.1, 1 H). ES-MS (neg.): 554.1 ([*M* – H]$^-$). Anal. calc. for $C_{27}H_{19}Cl_2NO_8$: C 58.29, H 3.44, N 2.52; found: C 58.15, H 3.30, N 2.52.

rel-*(1′R,3S,4R,5S)-4-[(1,3-Benzodioxol-5-ylamino)carbonyl]-5-(3,4-dichlorophenyl)-1′,3′,4,5-tetrahydro-1′-hydroxy-3′-oxospiro[furan-2(3H),2′-[2H]indene]-3-carboxylic Acid Sodium Salt* (**25**). To a soln. of **27** (60 mg, 0.11 mmol) in THF/MeOH 4:1 (5 ml), was added aqueous 1.0N LiOH (117 µl). After 1 h, the mixture was evaporated and the resulting residue purified by prep. HPLC to provide a white solid. The solid was dissolved in MeCN (5 ml), and 0.02N NaOH (1.5 ml) was added: lyophilization yielded **25** (18 mg, 28%). M.p. 239.6°. IR (KBr): 3216, 1717, 1686, 1653. ^{1}H-NMR ((D_6)DMSO): 9.89 (*s*, 1 H); 7.79–7.65 (*m*, 4 H); 7.50 (*t*, *J* = 7.2, 1 H); 7.47 (*d*, *J* = 8.4, 1 H); 7.36 (*d*, *J* = 8.4, 1 H); 6.83 (*d*, *J* = 1.6, 1 H); 6.71 (*d*, *J* = 8.3, 1 H); 6.57 (*dd*, *J* = 8.3, 1.6, 1 H); 6.22 (*s*, 1 H); 5.92 (*s*, 2 H); 5.79 (*s*, 1 H); 5.40 (*d*, *J* = 5.4, (1 H); 4.02 (*d*, *J* = 7.2, 1 H); 3.68 (*t*, *J* = 6.2, 1 H). ES-MS (neg.): 554.1 ([*M* – H]$^-$).

NMR Experiments in Assay Buffer [22]. ^{1}H- and ^{13}C-NMR spectra were recorded at 300 K with a *Bruker DRX-400* spectrometer at 400 and 100 MHz, resp., equipped with a gradient triple resonance probe. All spectra were recorded in (D_6)DMSO (δ rel. to internal DMSO set at δ(H) 2.50 and δ(C) 39.51). 1D NOE and 2D COSY, ROESY, HMQC, and HMBC experiments were recorded by using the standard pulse sequences provided by the manufacturer. 2D HMQC and HMBC experiments were optimized for ^1J(C,H) = 145 Hz and ^nJ(C,H) = 5 Hz and/or 8 Hz, resp. Each compound **14**–**16** was first dissolved in (D_6)DMSO. Three sample tubes (5 mm) containing each *ca.* 0.5 mM of inhibitor (**14**–**16**) were prepared by adding 50 µl of the previous conc. (D_6)DMSO soln. to 550 µl of an aq. buffer composed of 25 mM Na_2PO_4, 300 mM NaCl, 1 mM (D_{10})DTT, 0.1 mM (D_{10})EDTA, and 10% (*v*/*v*) D_2O at pH 7.3. Following the preparation of these samples, 1D ^{1}H-NMR spectra were immediately recorded at 300 K. Suppression of the H_2O signal was achieved by inserting a 3-9-19 WATERGATE module prior to data acquisition [21]. The three samples were then lyophilized in the NMR tubes and dissolved back in 600 µl of (D_6)DMSO. A second series of 1D ^{1}H-NMR spectra were then recorded.

REFERENCES

[1] K. V. Shah, P. M. Howley, in 'Fields Virology', 3rd edn., Ed. B N. Fields, Lippincott-Raven Publishers, Philadadelphia, 1996, p. 2077.

[2] P. M. Howley, in 'Fields Virology', 3rd edn., Ed. B. N. Fields, Lippincott-Raven Publishers, Philadadelphia, 1996, p. 2045.

[3] M. Ustav, *EMBO J.* **1991**, *10*, 449.

[4] L. Lang, R. Li, I. J. Mohr, R. Clark, M. R. Botchan, *Nature (London)* **1991**, *353*, 628.

[5] I. L. Blitz, L. A. Laimins, *J. Virol.* **1991**, *65*, 649.

[6] M. Lusky, E. Fontane, *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6363.

[7] C. Bonne-Andrea, S. Santucci, P. Clertant, F. Tillier, *J. Virol.* **1995**, *69*, 2341.

[8] K. R. Beutner, A. Ferenczy, *Am. J. Med.* **1997**, *102 (5A)*, 28.

[9] W. C. Phelps, J. A. Barnes, D. C. Lobe, *Antiviral Chem. Chemother.* **1998**, *9*, 359.

[10] C. Yoakim, W. W. Ogilvie, N. Goudreau, J. Naud, B. Haché, J. A. O'Meara, M. G. Cordingley, J. Archambault, P. W. White, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2539.

[11] P. W. White, S. Titolo, K. Brault, L. Thauvette, A. Pelletier, E. Welchner, L. Bourgon, L. Doyon, W. W. Ogilvie, C. Yoakim, M. G. Cordingley, J. Archambault, *J. Biol. Chem.* **2003**, *278*, 26765.

[12] P. W. White, A. Pelletier, K. Brault, S. Titolo, L. Thauvette, M. Fazekas, M. G. Cordingley, J. Archambault, *J. Biol. Chem.* **2001**, *276*, 22426.

[13] M. V. Ionescu, *Bull. Soc. Chim. Fr.* **1930**, *47*, 210.

[14] M. Weigele, J. P. Tengi, S. De Bernardo, R. Czajkowski, W. Leimgruber, *J. Org. Chem.* **1976**, *41*, 388.

[15] M. Y. Krysin, I. K. Anohina, L. P. Zalukaev, *Khimiya Geterotsiklicheskikh Soedinenii* **1987**, *11*, 1463.

[16] K. L. Busch, G. L. Glish, S. A. McLuckey, 'Mass Spectrometry/Mass Spectrometry', VCH, New York, 1988.
[17] W. P. Jencks, *Chem. Rev.* **1972**, *72*, 705.
[18] D. B. Dess, J. C. Martin, *J. Org. Chem.* **1983**, *48*, 4155; S. D. Meyer, S. L. Schreiber, *J. Org. Chem.* **1994**, *59*, 7549.
[19] C. Yoakim, R. Deziel, N. Goudreau, I. Guse, B. Haché, E. Malenfant, J. Naud, W. W. Ogilvie, J. O'Meara, R. Plante, B. Thavonekham, P. White, unpublished results.
[20] M. Y. Krysin, I. K. Anokhina, L. P. Zalukaev, N. V. Zyk, A. M. Boganov, *Zh. Org. Khim.* **1989**, *25*, 2094.
[21] M. Piotto, V. Saudek, V. Sklenar, *J. Biomol. NMR* **1992**, *2*, 661.

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