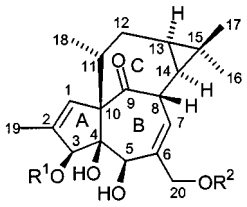
# Synthesis of Modified Ingenol Esters

**Giovanni Appendino,*[a] Gian Cesare Tron,[a] Giancarlo Cravotto,[a] Giovanni Palmisano,[b] Rita Annunziata,[c] Germano Baj,[d,e] and Nicola Surico[d]**

**Keywords:** Natural products / Diterpenes / Ingenol / Antitumor agents / Isosters

Synthetic protocols for the manipulation of the polyhydroxylated southern region of ingenol (**1a**) were developed, and a series of isosteres of the anticancer compound ingenol 3,20-dibenzoate (**1b**) was prepared. The biological evaluation of these compounds showed that cytotoxicity was relatively tolerant to changes at C-20, while PKC activation was markedly affected by these modifications. These data suggest that chemical manipulation can effectively dissect cytotoxicity and tumour-promoting activity (or potential) of ingenoids, affording more optimal candidates for development, like 20-deoxy-20-fluoroingenol 3,20-dibenzoate (**5b**). In mild acidic medium, an unexpected vinylogous retro-pinacol rearrangement of ingenol to a tigliane derivative was observed.

Ingenol (**1a**) is a diterpenoid polyol first characterised in the course of studies aimed at the identification of the irritant and co-cancerogenic constituents of plants from the spurge family (*Euphorbiaceae*).[1] Paradoxically, following a seminal report by Kupchan on the anticancer activity of ingenol-3,20-dibenzoate (**1b**),[2] various derivatives of ingenol were also identified as the active constituents of antitumour plant extracts,[3] and certain ingenol mono- and diesters were even reported as powerful inhibitors of the enzyme protein kinase C (PKC),[4] the target of tumor-promoting phorboid esters.[5] Dual co-cancerogenic and antitumour activity is not unprecedented for PKC activators,[6] but, at least for ingenol esters, a limited overlapping seems to exist between these contrasting actions, since many cytotoxic ingenol esters lack a free hydroxyl at C-20. This group is required for binding of phorboids to PKC by a twofold hydrogen bonding interaction, where the 20-hydroxyl acts both as a donor and as an acceptor.[7] Esterification of the 20-hydroxyl has been suggested to abolish PKC activation while keeping the anticancer activity,[3] but the matter is complicated by the possibility of enzymatic hydrolysis of the 20-ester group, as in the case of ingenol 3,20-dibenzoate itself (**1b**). This compound shows a low affinity for PKC,[8] but was found to be very active in in vivo irritancy and tumour-promotion assays,[9] presumably because of the enzymatic loss of the 20-ester group and the formation the corresponding 3-monoester (**1c**), a powerful PKC activator.[8] Since certain ingenol-3-monoesters are cytotoxic,[10] the hydrolysis at C-20 might also affect the evaluation of the antitumour activity. The potential of ingenol as a scaffold for bioactivity was further fuelled by the discovery of the outstanding anti-HIV activity of some of its derivatives, which are able to downregulate CD4 expression in host cells.[11] As with cytotoxicity, the anti-HIV activity was not seemingly directly correlated to PKC activation.[11]



| | $R^1$ | $R^2$ |
|---|---|---|
| **1a** | H | H |
| **1b** | Bz | Bz |
| **1c** | Bz | H |
| **1d** | H | Bz |

The structural complexity and the spectacular biological activity of ingenol derivatives has sparked great interest, and generated an intense synthetic activity.[12] However, systematic investigations on the structure-activity relationships and information on the cellular targets underlying the cytotoxic and anti-HIV properties of these compounds are still lacking. Furthermore, interference in the biological measurements by PKC activation from 20-deacylderivatives formed in the assay conditions remains an open issue, further compounded by the in vivo tumour-promoting activity of these compounds.[13] A solution to this problem is obviously needed before any ingenoid lead can qualify for medicinal development.

To address this point, we investigated the possibility of replacing the 20-ester linkage of ingenol 3,20-dibenzoate, the first ingenoid for which anticancer activity was reported,[2] with more stable bonds in hydrolytic terms (am-

[a] Dipartimento di Scienza e Tecnologia del Farmaco, Via Giuria 9, I-10125 Torino, Italy
E-mail: appendin@pharm.unito.it
[b] Dipartimento di Scienze Mediche, Facoltà di Farmacia, Viale Ferrucci 33, I-28100 Novara, Italy
[c] Dipartimento di Chimica Organica e Industriale, Via Venezian 21, I-20133 Milano, Italy
[d] Dipartimento di Scienze Mediche, Clinica Ginecologica e Ostetricia, Via Solaroli 17, I-28100 Novara, Italy
[e] Laboratorio di Biologia Cellulare, via Santena 19, I-10126 Torino, Italy

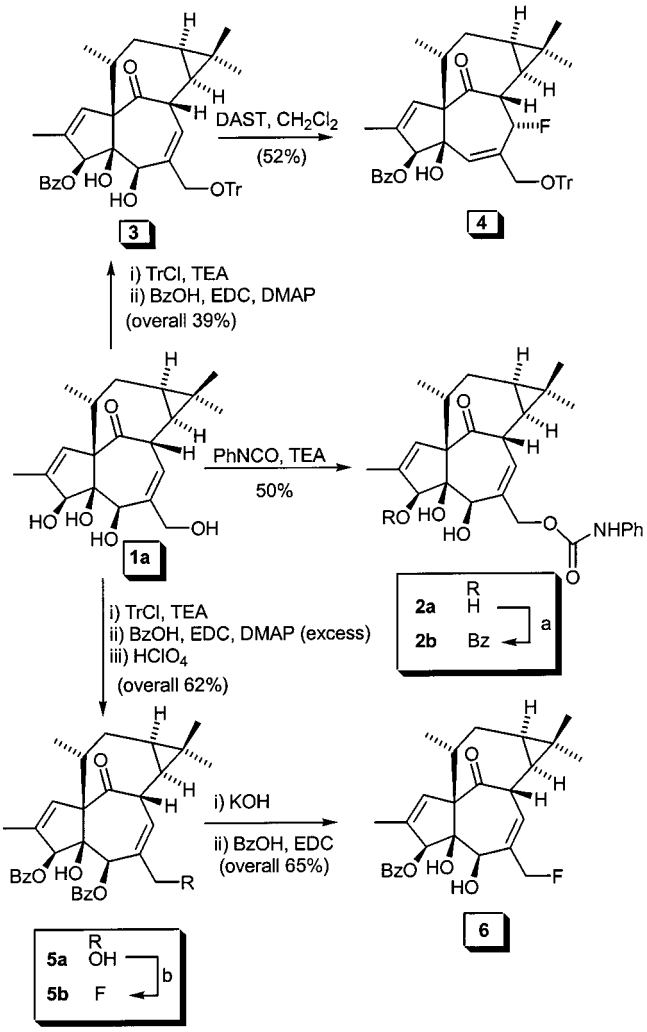
  1434–193X/99/1212–3413 $ 17.50+.50/0 

ide, carbamate, urea). The amide-type N−H bond and the adjacent oxygen carbonyl can offer alternative options of hydrogen bonding, but the lipophilic phenyl group should preclude fitting into the hydrophylic pocket of PKC which binds the 20-hydroxyl of phorboids.[7] Replacement of the 20-hydroxyl with a fluorine, an electronegative group unable to form hydrogen bondings,[14] was also considered, as well as the removal of the other structural element involved in PKC binding,[15] namely the 3-ester carbonyl group. A series of analogues of ingenol 3,20-dibenzoate, where these changes are independently featured, became the target of our investigation. Since these modifications are expected to render the resulting ingenoids unable of functioning as PKC activators,[15] their cytotoxicity, or lack thereof, should shed light on the relationships between PKC binding and antitumour activity.

The selective modification of the sugar-like polyhydroxylated southern region of ingenol represents a daunting synthetic challenge, further compounded by the propensity of the system to undergo rearrangements (vide infra). There was also no clue in the literature on how to reduce our line of thinking to practice, since previous studies had focused on the preparation of ingenol 3-monoesters, which are interesting for the study of PKC activation. Their synthesis was accomplished with a protocol unsuitable for the preparation of C-20 modified analogues (protection of the 5- and 20-hydroxyls as an acetonide, esterification of the 3-hydroxyl and deprotection).[16] The observation that acyl migration from the 3-hydroxyl to the 20-hydroxyl took place during the final deprotection[16] suggests an inherent thermodynamic bias for the formation of the 20-monoesters, and thus the possibility of selective modification of the 20-hydroxyl without protecting manoeuvres.

Preliminary experiments showed that no selectivity exists between the primary 20-hydroxyl and the secondary 3-hydroxyl under standard esterification conditions (benzoyl chloride, pyridine, room temperature or 0 °C). Thus, a mixture of the 3- and 20-monobenzoates **1c** and **1d** (ca. 1:1, 28%) along with starting material (12%) and the 3,20-dibenzoate **1b** (9%) was obtained. The steric factors favouring acylation of the primary 20-hydroxyl are seemingly overridden by hydrogen-bonding network effects,[17] a situation reminiscent of glucose and many hexose, where the secondary 4-hydroxyl is more reactive than the primary 6-hydroxyl in acylation reactions when pyridine bases are present.[17] Attempts to prepare ingenol 20-benzoate (**1d**) with a Mitsunobu reaction (BzOH, DEAD, TPP)[18] gave a complex mixture, but a certain selectivity in favour of the primary hydroxyl could eventually be accomplished with the EDC-DMAP protocol [EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride] at low temperature (42% yield). In a similar way, the reaction of ingenol with phenyl isocyanate in the presence of triethylamine afforded the 20-phenylcarbamate **2a**, which was then selectively mono-benzoylated to give the 3-benzoyl-20-carbamate isostere **2b** (Scheme 1) in overall 23% yield from **1a**.

Replacement of the 20-hydroxyl with a fluorine required a more complex protocol, since the reaction of ingenol and

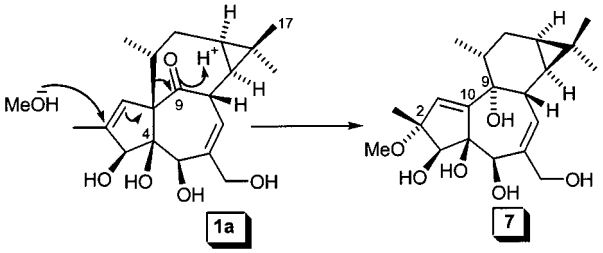



Scheme 1. Synthesis of the carbamate isostere **2b** and the fluorinated analogue **6**. a) BzOH, EDC, DMAP, 56%. − b) DAST, $CH_2Cl_2$, −78 °C, 67%

ingenol 3-benzoate with DAST [= (diethylamino)sulfur trifluoride] gave complex mixtures, which did not lend themselves to separation. The smooth conversion of the 3-benzoate-20-trityl ether **3** to the rearranged fluoride **4** upon treatment with DAST (Scheme 1) suggested that no discrimination between the various hydroxyls could be achieved with this reagent. The α-orientation of the 7-fluorine was indicated by the observed through-space coupling between the fluorine and 14-H ($J_{F,H}$ = 14.0 Hz), C-13 ($J_{F,C}$ = 13.0 Hz), and C-14 ($J_{F,C}$ = 27.0 Hz).[19] Protection of the 3- and 5-hydroxyls was obviously requested before the deoxyfluorination step. To implement this strategy, the orthogonally protected 3,5-diester-20-ether **5a** was prepared by sequential reaction of ingenol with trityl chloride and an excess benzoic acid (Scheme 1). Protection with an aromatic acid limited acyl rearrangement during the successive removal of the trityl group, affording ingenol 3,5-dibenzoate (**5a**) in an overall 62% yield from the parent polyol. Compound **5a** reacted smoothly with DAST to give the fluoride **5b**. Removal of the benzoyl protecting groups and selective

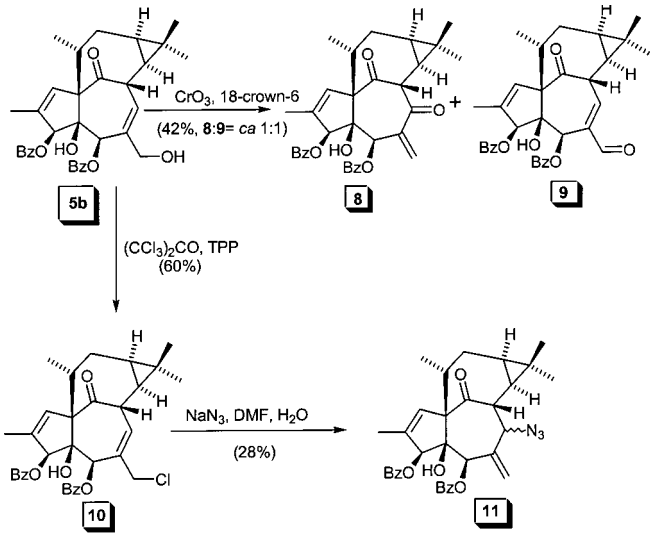
re-esterification of the 3-hydroxyl eventually afforded 20-deoxy-20-fluoroingenol 3-benzoate (**6**).

Attempts to prepare the key intermediate ingenol 3,5-dibenzoate by acid-catalysed transesterification failed. This reaction has been extensively used for the selective removal of the 20-acyl group in phorboid polyesters,[20] but with ingenol esters the completely deacylated tigliane **7** was obtained as the major reaction product (Scheme 2). Compound **7** could also be prepared in 49% yield (84% on conversion) by overnight treatment of ingenol with $10^{-2}$ N methanolic $HClO_4$. Compared to ingenol, the $^{13}$C-NMR spectrum of **7** showed the disappearance of the carbonyl resonance ($\delta$ = 207.7), apparently replaced by one downfield quaternary carbon ($\delta$ = 86.5, s), while an extra methoxyl ($\delta$ = 51.1, q) was also present. The major differences between the $^{1}$H-NMR spectra of ingenol and **7** were the upfield shift of the 19-methyl ($\Delta\delta$ − 0.48 ppm), and its lack of coupling with the vinyl proton 1-H. The quaternary signal at $\delta$ = 86.5 showed HMBC correlations with the methoxyl, the 19-methyl, 1-H, and 3-H, and was thus identified as C-2. The detection of diagnostic long-range couplings between the carbon at $\delta$ 7 = 6.6 (C-9) and 11-H, 12-H, 8-H, and 7-H, and between the carbon at $\delta$ = 151.0 (C-10) and 1-H, 3-H, and the 4-hydroxyl, suggested a shift of the double bond from C-1, C-2 to C-1, C-10. A change of carbon-carbon connectivity was requested to rationalise these observations, and the tigliane structure **7** could nicely fit all the spectroscopic data. 2D-NOESY experiments confirmed the structural assignment (correlation 19-H/OMe), and revealed the α-orientation of the methoxyl (correlation 3-H/OMe and 5-H/OMe) and the 9-hydroxyl (lack of correlation between 11-H and 1-H, and 11-H and 3-H). The rearrangement of **1a** to **7** is triggered by the protonation of the 9-carbonyl. After the anionotropic migration of C-11, the reaction is terminated in a "vinylogous" fashion by the attack of methanol to C-2 (Scheme 2). The overall rearrangement is thus as a vinylogous *retro*-pinacol process. The rearrangement of **1a** to **7** is the first skeletal rearrangement reported for ingenol, and is of great biogenetic relevance, being opposite to the one involved in the generation of the ingenane skeleton from a tigliane precursor. The mild conditions and the good yield makes it surprising that this rearrangement had escaped detection for a long time.[21]




Scheme 2. Acidic degradation of ingenol (**1a**)

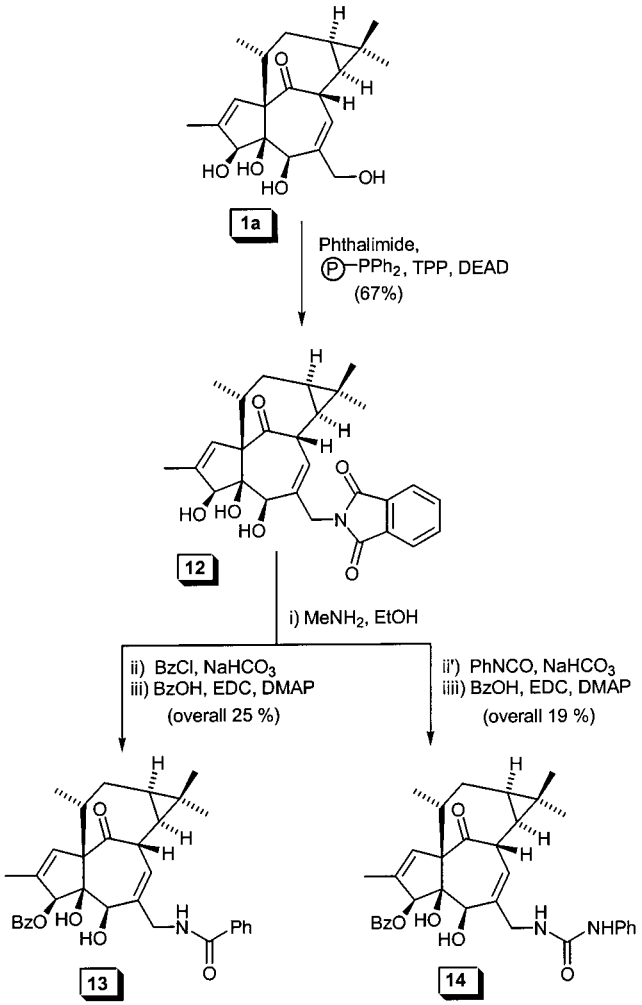
The synthesis of the 20-amide- and the 20-carbamate isosteres requires the replacement of the 20-hydroxyl with an amino group. Attempts to prepare the benzamide derivative **13** from ingenol 3,5-dibenzoate (**5a**) by a reductive amination or via the corresponding azide failed. Oxidation of **5a** with $MnO_2$, PCC, or PDC gave degraded products, while the reaction with $CrO_3$ in the presence of 18-crown-6[22] afforded an unseparable mixture of the enal **9** and the enone **8** (Scheme 3). These were completely degraded during the attempted reductive amination of the mixture ($NaBH_3CN$, $NH_4OAc$). The enone **8** is the result of a 3.3-sigmatropic rearrangement of the 20-chromic ester, and a similar reaction was presumably responsible for the obtaining of the allyl azide **11** when the chloride **10**, prepared from **5b** using the Magid protocol,[23] was treated with $NaN_3$.




Scheme 3. Attempted synthesis of key intermediates to 20-amino-20-deoxyingenol

We were eventually pleased to discover that, despite the failure to selectively esterify ingenol with benzoic acid using a Mitsunobu protocol, this chemistry could be successfully applied to the preparation of the phthalimide derivative **12** (Scheme 4). The increased bulkiness of the phthalimide anion compared to a carboxylate anion is presumably responsible for the selective outcome of the reaction, which required a somewhat modified experimental protocol to secure reproducibility (see Experimental Section), and the use of a polymer-supported phosphane to avoid contamination of the phthalimido derivative with triphenylphosphane oxide. Aminolysis of the phthalimide group, reaction with benzoyl chloride or phenyl isocyanate under Schotten−Baumann conditions, and selective esterification at C-3 complete the synthesis of the amide isostere **13** and the phenylureido isoster **14**. The overall yield from ingenol was modest (17% and 13%, respectively), but the strategy was straightforward, and did not involve any protection-deprotection steps.

The library of isosteres of ingenol-3,20-dibenzoate was assayed for cytotoxicity (MCF7 breast cancer cells)[24] and for PKC activation (Jurkat T cells, inhibition of receptor-mediated phosphatidyl inositol metabolism after CD3 or CD38 signalling).[25] The PKC-assay was run at high concentration of substrates (10 μM) in order to detect also weak activity.[11] Some intermediates and by-products obtained




Scheme 4. Synthesis of the benzamido (**13**) and the phenylureido (**14**) isosteres

en route to the targets were also tested. The results, summarised in Table 1, showed that structure-activity relationships within cytotoxic ingenoids are multifaceted, and in sharp contrast to the data obtained on PKC, go beyond the presence or the absence of an acyl group at a specific hydroxyl.

Table 1. In vitro evaluation of ingenol 3,20-dibenzoate (**1b**) and its analogues

| Compound | $IC_{50}$ (μM)[a][24] | PKC Activation degree [b][25c] |
|---|---|---|
| **1b** | 4.0 | 9.50 |
| **2a** | 4.0 | 8.34 |
| **2b** | 4.0 | 0.34 |
| **5b** | 3.3 | −1.00 |
| **6** | 2.3 | 11.34 |
| **12** | 7.5 | 7.47 |
| **13** | 8.2 | 11.00 |
| **14** | 6.9 | 0.13 |

[a] MCF7 breast cancer cells. − [b] % of the activity of PMA (phorbol 12-myristate 13-acetate), $c$ = 10 μM.

Thus, compared to ingenol 3,20-dibenzoate (**1b**), cytotoxicity was maintained (in **2b**) or only slightly decreased (in **13** and **14**) by isosteric changes of the 20-ester linkage, a change affording compounds that are per se only weak PKC-activators and much more stable to hydrolysis than the lead compound. Furthermore, the 20-deoxy-20-fluoro-3,5-dibenzoate **5b** showed cytotoxicity compared to that of **1b**, but failed completely to activate PKC, behaving instead as a weak inhibitor. Though surprising, inhibition of PKC by ingenol esters is not unprecedented.[4]

In summary, synthetic protocols for the manipulation of the polyhydroxylated southern region of ingenol have been developed, paving the way to the preparation of C-20 modified analogues. Their evaluation for antitumour activity and PKC activation showed that chemical manipulation can effectively dissect cytotoxicity and tumour-promoting activity (or potential), affording more optimal candidates for further development.

## Experimental Section

***Caution:*** Ingenol 3-monoesters are highly irritant to skin and mucous membranes, and display tumour-promoting activity. Their handling should be carried out wearing latex gloves and face protection, and avoiding contact with the skin.

**General:** Column chromatography: Merck Silica gel; − IR: Shimadzu DR 8001 spectrophotometer. − NMR: Bruker AM 300 (300 MHz and 75 MHz for $^1H$ and $^{13}C$, respectively). For $^1H$ NMR, $CDCl_3$ as solvent, $CHCl_3$ at δ = 7.26 as reference. For $^{13}C$ NMR, $CDCl_3$ as solvent, $CDCl_3$ at δ = 77.0 as reference. The $^1H$- and $^{13}C$-NMR spectra of all final products and the key intermediates were fully assigned using a combination of 1D- and 2D (COSY, HMBC, HMQC, ROESY) techniques. − $CH_2Cl_2$ was dried by distillation from $CaH_2$, and THF from distillation from Na/benzophenone. $Na_2SO_4$ was used to dry solutions before the evaporation. Ingenol was obtained from the seeds of *Euphorbia lathyris* L. as previously described.[26] − Cytotoxicity and PKC-assays were carried out according to established literature protocols.[24,25c]

**Ingenol 20-Benzoate (1c):** To a cooled (0°C) solution of ingenol (**1a**) (20 mg, 0.057 mmol) in dry $CH_2Cl_2$ (0.2 mL), EDC (21.8 mg, 0.11 mmol, 2 mol.-equiv.), DMAP (13.9 mg, 0.11 mmol, 2 mol.-equiv.), and a solution of benzoic acid (13.9 mg, 0.11 mmol, 2 mol.-equiv.) in $CH_2Cl_2$ (0.2 mL) were sequentially added. The reaction mixture was stirred at −18°C until TLC (hexane/EtOAc, 6:4, $R_f$ **1a** = 0.1; $R_f$ **1c** = 0.27; $R_f$ **1b** = 0.72) showed the complete disappearance of the starting material (72 h). The reaction was then worked up by dilution with $CH_2Cl_2$ (ca. 1 mL) and washing with 5% NaOH (× 2) and brine. After drying and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 9:1 as eluant) to give 5.9 mg **1b** (18%) and 10.6 mg **1c** (42%) as a white powder, m.p. 65−70°C. − IR (KBr): $\tilde{\nu}$ = 3453 $cm^{-1}$ (OH), 1720 (C=O), 1452, 1381, 1273, 1115, 713. − $^1H$ NMR ($CDCl_3$): δ = 5.97 (d, $J$ = 1.5 Hz, 1-H), 4.47 (s, 3-H), 3.73 (br. s, 5-H), 6.21 (d, $J$ = 4.5 Hz, 7-H), 4.13 (br. dd, $J$ = 12.5, 4.5 Hz, 8-H), 2.33 (m, 11-H), 2.33 (m, 12a-H), 1.80 (m, 12b-H), 0.73 (ddd, $J$ = 8.5, 8.5, 6), 0.98 (dd, $J$ = 12.5, 8.5 Hz, 14-H), 1.14 (s, 16-H), 1.08 (s, 17-H), 0.99 (d, $J$ = Hz, 18-H), 1.86 (d, $J$ = 1.5 Hz, 19-H), 4.98 (br. d, $J$ = 12 Hz, 20a-H), 4.82 (br. d, $J$ = 12 Hz, 20b-H), 3.07 (br. s, O5-H), 2.67 (br. s, O3-H), 8.05 (br. d, $J$ = 8 Hz, *o*-Ph), 7.45 (br. t, $J$ = 8 Hz, *m*-Ph), 7.60 (br. d, $J$ = 8 Hz, *p*-Ph). − $^{13}C$ NMR ($CDCl_3$): δ = 130 (d, C-1), 138.8 (s, C-2), 80.6 (d, C-3), 84.4 (s, C-4), 74.0 (d, C-5), 136.7 (s, C-6), 128.3 (d, C-7), 44.1 (d, C-8), 207.5

(s, C-9), 73.0 (s, C-10), 39.7 (d, C-11), 31.0 (t, C-12), 23.0 (d, C-13), 23.2 (d, C-14), 23.9 (s, C-15), 15.4 (q, C-16), 28.5 (q, C-17), 17.4 (q, C-18), 15.3 (q, C-19), 66.8 (q, C-20), 167.5 (s, $Bz_{C=O}$), 130.0 (s, *i*-Bz), 129.7 (d, *o*-Bz), 128.4 (d, *m*-Bz), 133 (d, *p*-Bz). − HRMS (70 eV); *m*/*z* 452.2191 (calcd. for $C_{27}H_{32}O_6$, 452.2199).

**Ingenol 20-Phenylcarbamate (2a):** To a solution of ingenol (**1a**) (300 mg, 0.86 mmol) in dry $CH_2Cl_2$ (3 mL), triethylamine (120 µL, 87.8 mg, 0.86 mmol, 1 mol.-equiv), and phenylisocyanate (93.7 µL, 103 mg, 0.86 mmol, 1 mol.-equiv.) were added, and the solution was stirred at room temp. for 2 h and then worked up by evaporation of the solvent. The residue was taken up in $CH_2Cl_2$ (5 mL), washed with brine, evaporated, and purified by column chromatography (hexane/EtOAc, 8:2 to remove unchanged phenylisocyanate; 6:4 to elute **2a**). Compound **2a** (203 mg, 51%) was obtained as a white powder, m.p. 100−103°C. − IR (KBr): $\tilde{\nu}$ = 3410 $cm^{-1}$ (OH), 1718 (C=O), 1601, 1545, 1446, 1315, 1223, 1053, 754. − $^1$H NMR ($CDCl_3$): δ = 5.93 (d, *J* = 1.5 Hz, 1-H), 4.45 (s, 3-H), 3.73 (br. s, 5-H), 6.16 (d, *J* = 4.5 Hz, 7-H), 4.15 (br. dd, *J* = 12.5, 4.5 Hz, 8-H), 2.33 (m, 11-H), 2.26 (m, 12a-H), 1.73 (m, 12b-H), 0.70 (ddd, *J* = 8.5, 8.5, 6), 0.95 (dd, *J* = 12.5, 8.5 Hz, 14-H), 1.13 (s, 16-H), 1.08 (s, 17-H), 0.98 (d, *J* = Hz, 18-H), 1.86 (d, *J* = 1.5 Hz, 19-H), 4.86 (br. d, *J* = 12 Hz, 20a-H), 4.60 (br. d, *J* = 12 Hz, 20b-H), 6.81 (br. s, NH), 7.38 (br. d, *J* = 8 Hz, *o*-Ph), 7.32 (br. t, *J* = 8 Hz, *m*-Ph), 7.06 (br. d, *J* = 8 Hz, *p*-Ph). − $^{13}$C NMR ($CDCl_3$): δ = 129.2 (d, C-1), 139.0 (s, C-2), 80.5 (d, C-3), 84.3 (s, C-4), 73.6 (d, C-5), 136.9 (s, C-6), 129.6 (d, C-7), 44.1 (d, C-8), 208.0 (s, C-9), 72.7 (s, C-10), 39.5 (d, C-11), 31.0 (t, C-12), 23.0 (d, C-13), 23.3 (d, C-14), 23.6 (s, C-15), 28.5 (q, C-16), 15.4 (q, C-17), 17.4 (q, C-18), 15.3 (q, C-19), 67.4 (q, C-20), 153.5 (s, isocyanate. C=O), 153.5 (s, *i*-Ph), 118.3 (d, *o*-Ph), 129.0 (d, *m*-Ph), 123.5 (d, *p*-Ph). − HRMS (70 eV); *m*/*z* 467.2315 (calcd. for $C_{27}H_{33}NO_6$, 467.2308).

**Ingenol 3-Benzoate-20-phenylcarbamate (2b):** To a cooled (−18°C) solution of ingenol 20-phenylcarbamate (**2a**) (202.6 mg, 0.43 mmol) in dry $CH_2Cl_2$ (3 mL), EDC (100 mg, 0.521 mmol, 1.2 mol.-equiv.), DMAP (64 mg, 0.52 mmol, 1.2 mol.-equiv.), and benzoic acid (63.6 mg, 0.52 mmol, 1.2 mol.-equiv.) were sequentially added. The reaction mixture was stirred at −18°C for 24 h, and worked up by dilution with $CH_2Cl_2$ (5 mL) and washing with 5% NaOH and brine. Evaporation of the solvent and column chromatography (hexane/EtOAc, 8:2) gave 83.2 mg **2b** (56% based on conversion) and 80.3 mg of recovered starting material (hexane/EtOAc, 4:6). **2b** is a colourless amorphous gum. − IR (liquid film): $\tilde{\nu}$ = 3453 $cm^{-1}$ (OH), 1709 (C=O), 1655, 1601, 1541, 1447, 1315, 1217, 1069, 754. − $^1$H NMR ($CDCl_3$): δ = 6.21 (d, *J* = 1.5 Hz, 1-H), 5.82 (s, 3-H), 4.00 (br. s, 5-H), 6.21 (d, *J* = 4.5 Hz, 7-H), 4.21 (br. dd, *J* = 12.5, 4.5 Hz, 8-H), 2.66 (m, 11-H), 2.26 (m, 12a-H), 1.80 (m, 12b-H), 0.75 (ddd, *J* = 8.5, 8.5, 6), 1.01 (dd, *J* = 12.5, 8.5 Hz, 14-H), 106 (s, 16-H), 1.07 (s, 17-H), 1.08 (d, *J* = Hz, 18-H), 1.85 (d, *J* = 1.5 Hz, 19-H), 4.96 (br. d, *J* = 12 Hz, 20a-H), 4.55 (br. d, *J* = 12 Hz, 20b-H), 6.76 (br. s, NH), 8.06 (Bz AA′), 7.63 (Bz BB′), 7.39 (br. d, *J* = 8 Hz, *o*-Ph), 7.33 (br. t, *J* = 8 Hz, *m*-Ph), 7.09 (br. d, *J* = 8 Hz, *p*-Ph). − $^{13}$C NMR ($CDCl_3$): δ = 132.6 (d, C-1), 136.7 (s, C-2), 83.8 (d, C-3), 86.0 (s, C-4), 74.8 (d, C-5), 136.0 (s, C-6), 131.0 (d, C-7), 44.1 (d, C-8), 207.0 (s, C-9), 72.5 (s, C-10), 39.0 (d, C-11), 31.7 (t, C-12), 23.9 (d, C-13), 23.5 (d, C-14), 24.4 (s, C-15), 15.9 (q, C-16), 28.9 (q, C-17), 17.9 (q, C-18), 15.9 (q, C-19), 68.0 (q, C-20), 154.0 (s, isocyan. C=O), 139.0 (s, *i*-Ph), 119.2 (d, *o*-Ph), 129.5 (d, *m*-Ph), 124.0 (d, *p*-Ph), 168.0 (s, $Bz_{C=O}$), 129.8 (s, *i*-Bz), 130.2 (*o*-Bz), 129.0 (d, *m*-Bz), 133.8 (d, *p*-Bz). − HRMS (70 eV); *m*/*z* 571.2580 (calcd. for $C_{34}H_{37}NO_7$, 571.2570).

**Ingenol 3-Benzoate-20-trityl Ether (3):** To a solution of ingenol (**1a**) (100 mg, 0.29 mmol) in dry pyridine (2 mL) trityl chloride (787 mg, 28.7 mmol, 10 mol.-equiv.), and DMAP (10 mg) were added. After a few hours, a copious white precipitate started to form, and after 16 h the reaction was worked up by dilution with $CHCl_3$ (ca. 5 mL) and washing with dil. HCl and brine. After drying and removal of the solvent, the residue was purified by column chromatography (8 g silica gel), using as eluant hexane/EtOAc (9:1) to elute the excess trityl alcohol, and hexane/EtOAc (5:5) to recover ingenol 20-trityl ether (150 mg, 86%) as a white powder [m.p. 212−215°C. − IR (KBr): $\tilde{\nu}$ = 3431 $cm^{-1}$ (OH), 1710 (C=O), 1448, 1381, 1018, 762, 702. − $^1$H NMR ($CDCl_3$): δ = 5.88 (s, 1-H), 3.91 (br. s, 3-H), 4.18 (br. s, 5-H), 6.07 (d, *J* = 5 Hz, 7-H), 1.16 (s, 16-H), 1.08 (s, 17-H), 0.99 (d, *J* = 6 Hz, 18-H), 2.82 (s, 19-H), 3.69 (br. s, 20a,b-H), 7.32 (m, 15 H, Ph). − CIMS (70 eV); *m*/*z*%: 591 (100) [M + $H^+$] [$C_{39}H_{31}O_5$ + $H^+$]. To a cooled solution of ingenol 20-trityl ether (690 mg, 1.17 mmol) in dry $CH_2Cl_2$, EDC (127 mg, 1.29 mmol, 1.1 mol.-equiv.), DMAP (157 mg, 1.29 mmol, 1.1 mol.-equiv.), and a solution of benzoic acid (157.2 mg, 1.29 mmol, 1.1 mol.-equiv.) were added. After stirring at −18°C overnight, the reaction was worked up by dilution with $CH_2Cl_2$, washing with 5% NaOH and brine. After drying and evaporation of the solvent, the residue was purified by column chromatography (15 g silica gel) to obtain 61 mg unchanged ingenol 20-trityl ether and 343 mg **3** (45%, hexane/EtOAc, 9:1) as a white powder, m.p. 100−105°C. − IR (KBr): $\tilde{\nu}$ = 3474 $cm^{-1}$ (OH), 1720 (C=O), 1448, 1273, 1068, 708, 632. − $^1$H NMR ($CDCl_3$): δ = 8.05 (AA′ Bz), 7.35 (m, 3 × Ph and BB′ Bz), 6.09 (d, *J* = 5 Hz, 7-H), 5.88 (s, 1-H), 4.18 (br. s, 5-H), 3.91 (br. s, 3-H), 3.69 (br. s, 20a,b-H), 2.82 (s, 19-H), 1.16 (s, 16-H), 1.08 (s, 17-H), 0.99 (d, J = 6 Hz, 18-H). − CIMS (70 eV); *m*/*z*%: 695 (100) [M + $H^+$] [$C_{46}H_{46}O_6$ + $H^+$]

**5-Deoxy-7-fluoro-6,7-dihydro-5,6-dehydroingenol 3-Benzoate 20-Trityl Ether (4):** To a cooled (−78°C) and stirred solution of ingenol 3-benzoate-20-trityl ether (**3**) (150 mg, 0.22 mmol) in dry $CH_2Cl_2$ (1.5 mL), DAST was added dropwise (57 µL, 70 mg, 0.43 mmol, 2 mol.-equiv.). After 2 h the reaction was worked up by dilution with $CH_2Cl_2$ (3 mL), removal from the cooling bath and quenching with sat. aq. $NaHCO_3$. After washing with brine, drying and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 8:2) to give 63 mg **4** (52%) as a white powder, m.p. 115−120°C. − IR (KBr): $\tilde{\nu}$ = 3433 $cm^{-1}$ (OH), 1699 (C=O), 1601, 1448, 1282, 1244, 898, 709. − $^1$H NMR ($CDCl_3$): δ = $^1$H NMR ($CDCl_3$): δ = 6.00 (d, *J* = 1.5 Hz, 1-H), 5.55 (s, 3-H), 6.35 (br. s, 5-H), 5.00, 4.83 (t, *J* = 8.0 Hz, 7-H), 2.92 (br. d, *J* = 8 Hz, 8-H), 2.36 (m, 11-H), 2.36 (m, 12a-H), 1.61 (m, 12b-H), 0.70 (m, 13-H and 14-H), 1.11 (s, 16-H), 1.07 (s, 17-H), 1.22 (d, *J* = Hz, 18-H), 1.49 (d, *J* = 1.5 Hz, 19-H), 4.00 (br. d, *J* = 12 Hz, 20a-H), 3.70 (br. d, *J* = 12 Hz, 20b-H), 2.93 (br. s, 4-OH), 7.51 (br. d, *J* = 8 Hz, *o*-Ph), 7.31 (br. t, *J* = 8 Hz, *m*-Ph), 7.25 (br. d, *J* = 8 Hz, *p*-Ph), 8.07 (Bz, AA′) 7.51 (Bz C), 7.60 (Bz. BB′). − $^{13}$C NMR ($CDCl_3$): δ = 139.4 (d, C-1), 133.8 (s, C-2), 87.1 (d, C-3), 79.9 (s, C-4), 127.9 (d, C-5), 142.0 (s, C-6), 95.9, 93.5 (d, C-7), 59.5, 59.2 (s, C-8), 206.4 (s, C-9), 65.6 (s, C-10), 43.1 (d, C-11), 29.3 (t, C-12), 24.3, 24.2 (d, C-13), 31.2, 30.8 (d, C-14), 22.0 (s, C-15), 28.4 (q, C-16), 14.7 (q, C-17), 16.0 (q, C-18), 15.0 (q, C-19), 65.6 (t, C-20), 87.3 (s, *C*$Ph_3$), 143.9 (*i*-Ph), 128.6 (d, *o*-Ph), 128.0 (d, *m*-Ph), 127.1 (d, *p*-Ph), 167.6 (s, $Bz_{C=O}$), 130.0 (s, *i*-Bz), 129.7 (d, *o*-Bz), 128.6 (d, *m*-Bz), 133.3 (d, *p*-Bz). − HRMS (70 eV); *m*/*z* 684.3254 (calcd. for $C_{45}H_{45}FO_5$, 684.3251).

**Ingenol 3,5-Dibenzoate (5a):** To a stirred solution of ingenol-20-trityl ether (644 mg, 1.1 mmol) in dry $CH_2Cl_2$ (6 mL), EDC (626 mg, 3.3 mmol, 3 mol.-equiv.), DMAP (399 mg, 3.3 mmol, 3 mol.-equiv.), and benzoic acid (400 mg, 3.3 mmol, 3 mol.-equiv.) were added. After stirring at room temp. for 18 h, the reaction was worked up by dilution with $CH_2Cl_2$ (5 mL) and washing with 5%

aq. NaOH and brine. After drying and removal of the solvent, the residue was dissolved in methanolic 0.01 N $HClO_4$ (7 mL). A white precipitate formed, which was dissolved with a few drops of $CH_2Cl_2$. After stirring at room temp. for 8 h, the reaction was worked up by neutralization with solid NaOAc and extraction with $CH_2Cl_2$. The organic phase was washed with brine, dried, and evaporated. The residue was purified by column chromatography (hexane/EtOAc, 9:1 to remove trityl alcohol, then hexane/EtOAc, 7:3) to give, along with 20 mg **1b**, 440 mg **5a** (72%) as a white powder, m.p. 122−125°C. − IR (KBr): $\tilde{\nu}$ = 3367 $cm^{-1}$ (OH), 1718 (C=O), 1425, 1271, 1095, 1026, 711. − $^1H$ NMR ($CDCl_3$): δ = 6.21 (br. s, 1-H), 5.34 (br. s, 3-H), 5.78 (br. s, 5-H), 5.26 (d, *J* = 4 Hz, 7-H), 4.43 (dd, *J* =, 8-H), 1.57 (s, 16-H), 1.26 (s, 17-H), 1.18 (d, *J* = 6.8 Hz, 18-H. ), 1.84 (s, 19-H), 3.94 (d, *J* = 10 Hz, 20a-H), 3.88 (d, *J* = 10 Hz, 20b-H), 8.21 (AA′-Bz), 7.96 (AA′-Bz), ca. 7.58 (m, 6 H, 2 × Bz). − HRMS (70 eV); *m*/*z* 556.2470 (calcd. for $C_{34}H_{36}O_7$, 556.2461).

**20-Deoxy-20-fluoroingenol 3,5-Dibenzoate (5b):** To a cooled (−78°C) and stirred solution of ingenol 3,20-dibenzoate (**5a**) (207 mg, 0.37 mmol) in dry $CH_2Cl_2$ (3 mL), DAST was added dropwise (99 μL, 120 mg, 0.75 mmol, 2 mol.-equiv.). After 2 h the reaction was worked up by dilution with $CH_2Cl_2$ (5 mL), removal of the cooling bath, and quenching with sat. aq. $NaHCO_3$. After washing with brine, drying, and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 8:2) to give 139 mg **5b** (67%) and 25 mg (12%) **1b**. **5b** is a white powder, m.p. 179−182°C. − IR (KBr): $\tilde{\nu}$ = 3431 $cm^{-1}$ (OH), 1718 (C=O), 1452, 1383, 1273, 1093, 709. − $^1H$ NMR ($CDCl_3$): δ = 6.22 (d, *J* = 1.5 Hz, 1-H), 5.39 (s, 3-H), 5.81 (br. s, 5-H), 6.35 (d, *J* = 4.5 Hz, 7-H), 4.40 (br. dd, *J* = 12.5, 4.5 Hz, 8-H), 2.69 (m, 11-H), 2.33 (m, 12a-H), 1.80 (m, 12b-H), 1.06 (ddd, *J* = 8.5, 8.5, 6, 13-H), 0.76 (dd, *J* = 12.5, 8.5 Hz, 14-H), 1.13 (s, 16-H), 1.06 (s, 17-H), 1.06 (d, *J* = Hz, 18-H), 1.86 (d, *J* = 1.5 Hz, 19-H), 4.70 (dd, *J* = 47, 12 Hz, 20a-H), 4.62 (dd, *J* = 47, 12 Hz, 20b-H), 8.21 (Bz AA′), 7.96 (Bz AA′), ca. 7.50 (m, 6 H, Bz). − $^{13}C$ NMR ($CDCl_3$): δ = 132.5 (d, C-1), 136.4 (s, C-2), 83.0 (d, C-3), 86.2 (s, C-4), 76.0 (d, C-5), 134.7, 134.5 (s, C-6), 132.3, 132.1 (d, C-7), 43.9 (d, C-8), 205.8 (s, C-9), 72.4 (s, C-10),38.7 (d, C-11), 31.1 (t, C-12), 22.9 (d, C-13), 23.2 (d, C-14), 24.3 (s, C-15), 15.4 (q, C-16), 28.3 (q, C-17), 17.1 (q, C-18), 15.5 (q, C-19), 85.5, 83.2 (d, C-20), 168.1, 166.8 (s, $Bz_{C=O}$), 128.4 (s, 2 × *i*-Bz), 129.7, 130.2 (d, *o*-Bz), 128.5, 128.4 (d, *m*-Bz), 133.4, 133.2 (d, *p*-Bz). − HRMS (70 eV); *m*/*z* 558.2408 (calcd. for $C_{34}H_{35}FO_6$, 558.2418).

**20-Deoxy-20-fluoroingenol 3-Benzoate (6):** 20-Deoxy-20-fluoroingenol 3,20-dibenzoate (**5b**) (140 mg, 0.25 mmol) was dissolved in 5% methanolic KOH (5 mL). After stirring at room temp. for 1 h, the reaction was worked up by evaporation of methanol. The residue was taken up in $CH_2Cl_2$ and washed with brine, dried and evaporated. The residue was dissolved in dry $CH_2Cl_2$ and cooled (−18°C). Benzoic acid (61 mg, 0.25 mmol, 1 mol.-equiv.), EDC (96 mg, 0.25 mmol, 1 mol.-equiv.), and DMAP (61.4 mg, 0.25 mmol, 1 mol.-equiv.) were added. After standing at −18°C for 96 h, the reaction was worked up by dilution with water,washing with 5% NaOH and with brine. After drying and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 8:2, as eluant) to give 73 mg **6** as a white powder (65% from **5b**), m.p. 83−86°C. − IR (KBr): $\tilde{\nu}$ = 3474 $cm^{-1}$ (OH), 1722 (C=O), 1452, 1275, 1111, 972, 711. − $^1H$ NMR ($CDCl_3$): δ = 6.14 (d, *J* = 1.5 Hz, 1-H), 5.77 (s, 3-H), 4.07 (br. s, 5-H), 6.22 (d, *J* = 4.5 Hz, 7-H), 4.12 (br. dd, *J* = 12.5, 4.5 Hz, 8-H), 2.60 (m, 11-H), 2.26 (m, 12a-H), 1.83 (m, 12b-H), 0.75 (ddd, *J* = 8.5, 8.5, 6), 1.00 (dd, *J* = 12.5, 8.5 Hz, 14-H), 1.27 (s, 16-H), 1.08 (s, 17-H), 1.06 (d, *J* = Hz, 18-H), 1.85 (d, *J* = 1.5 Hz, 19-H), 5.05 (br. d, *J* = 12 Hz, 20a-H), 4.86 (br. d, *J* = 12 Hz, 20b-H), 8.06 (Bz AA′), 7.50 (Bz C), 7.63 (Bz BB′). − $^{13}C$ NMR ($CDCl_3$): δ = 132.6 (d, C-1), 135.6 (s, C-2), 83.5 (d, C-3), 85.0 (s, C-4), 74.8 (d, C-5), 137.0 (s, C-6), 130.0 (d, C-7), 43.7 (d, C-8), 206.0 (s, C-9), 72.1 (s, C-10), 38.8 (d, C-11), 31.2 (t, C-12), 23.1 (d, C-13), 22.9 (d, C-14), 24.0 (s, C-15), 15.3 (q, C-16), 28.5 (q, C-17), 17.3 (q, C-18), 15.0 (q, C-19), 86.6, 84.4 (d, C-20), 168.1, (s, $Bz_{C=O}$), 130.7 (s, *i*-Bz), 129.7, 129.8 (d, *o*-Bz), 128.6 (d, *m*-Bz), 133.5 (d, *p*-Bz). − HRMS (70 eV); *m*/*z* 454.2150 (calcd. for $C_{27}H_{31}FO_5$, 454.2156).

**Acidic Degradation of Ingenol:** A sample of ingenol (200 mg, 0.57 mmol) was dissolved in $10^{-2}$ N methanolic $HClO_4$ (4 mL). After stirring at room temperature for 16 h, the reaction was worked up by neutralisation with solid NaOAc, and evaporation of the solvent. The residue was taken up in EtOAc and washed sequentially with water and brine. After drying and evaporation, the residue was purified by column chromatography (hexane/EtOAc, 5:5 to elute unchanged ingenol, hexane/EtOAc, 3:7 to elute **7**) to give 83.5 mg unchanged ingenol and 102 mg of **7** (49%; 84% on conversion) as a white powder, m.p. 137−140°C. − $[\alpha]_D^{25}$ = −89 (*c* = 0.6, MeOH). − IR (KBr): $\tilde{\nu}$ = 3387 $cm^{-1}$ (OH), 1284, 1088, 987, 879, 626. − $^1H$ NMR ($CDCl_3$): δ = 5.88 (br. s, 1-H), 3.80 (s, 3-H), 4.47 (br. s, 5-H), 5.75 (d, *J* = 6.0 Hz, 7-H), 3.06 (br. dd, *J* = 12.5, 4.5 Hz, 8-H), 1.39 (m, 11-H), 1.75 (m, 12a-H), 1.53 (m, 12b-H), 0.77 (ddd, *J* = 8.5, 8.5, 6), 0.56 (dd, *J* = 12.5, 8.5 Hz, 14-H), 1.08 (s, 16-H), 0.66 (s, 17-H), 0.76 (d, *J* = Hz, 18-H), 1.37 (d, *J* = 1.5 Hz, 19-H), 4.17 (br. s, 20a,b-H), 3.23 (s, OMe). − $^{13}C$ NMR ($CDCl_3$): δ = 135.0 (d, C-1), 86.5 (s, C-2), 80.6 (d, C-3), 83.1 (s, C-4), 73.3 (d, C-5), 139.0 (s, C-6), 137.0 (d, C-7), 36.5 (d, C-8), 76.6 (s, C-9), 131.1 (s, C-10), 36.8 (d, C-11), 25.2 (t, C-12), 19.7 (d, C-13), 24.9 (d, C-14), 25.0 (s, C-15), 29.2 (q, C-16), 15.6 (q, C-17), 17.8 (q, C-18), 18.4 (q, C-19), 69.0 (t, C-20), 51.1 (q, OMe). − HRMS (70 eV); *m*/*z* 362.2085 [calcd. for $C_{21}H_{30}O_5$ (M − $H_2O$), 362.2093].

Standard acetylation of crude **7** ($Ac_2O$, pyridine) afforded a triacetate (yield 41%) as an amorphous gum. − $[\alpha]_D^{25}$ = −137 (*c* = 0.6, $CHCl_3$). − $^1H$ NMR ($CDCl_3$): δ = 5.99 (br. s, 1-H), 5.17 (s, 3-H), 5.69 (br. s, 5-H), 5.99 (d, *J* = 6.0 Hz, 7-H), 3.06 (br. dd, *J* = 12.5, 4.5 Hz, 8-H), 1.40 (m, 11-H), 1.73 (m, 12a-H), 1.56 (m, 12b-H), 0.78 (ddd, *J* = 8.5, 8.5, 6), 0.60 (dd, *J* = 12.5, 8.5 Hz, 14-H), 1.09 (s, 16-H), 1.01 (s, 17-H), 0.77 (d, *J* = Hz, 18-H), 1.34 (d, *J* = 1.5 Hz, 19-H), 4.63 (br. d, *J* = 12 Hz, 20a-H), 4.40 (br. d, *J* = 12 Hz, 20b-H), 3.28 (s, OMe), 2.17 (s, OAc), 2.15 (s, OAc), 2.17 (s, OAc). − $^{13}C$ NMR ($CDCl_3$): δ = 136.4 (d, C-1), 132.0 (s, C-2), 79.1 (d, C-3), 83.0 (s, C-4), 73.1 (d, C-5), 148.0 (s, C-6), 144.5 (d, C-7), 37.0 (d, C-8), 74.0 (s, C-9), 84.3 (s, C-10), 36.5 (d, C-11), 24.8 (t, C-12), 19.3 (d, C-13), 24.2 (d, C-14), 24.2 (s, C-15), 23.7 (q, C-16), 15.2 (q, C-17), 17.3 (q, C-18), 19.3 (q, C-19), 67.6 (t, C-20), 51.0 (q, OMe), 171.0 (s, 3 × OAc), 20.9 (q, 3 × OAc).

**20-Chloro-20-deoxyingenol 3,5-Dibenzoate (10):** To a cooled (0°C) solution of ingenol 3,5-dibenzoate (**5a**) (278 mg, 0.50 mmol) in hexachloroacetone (1.5 mL, 2.4 g, 10 mmol, 20 mol.-equiv.) and dry $CH_2Cl_2$ (0.5 mL), a solution of triphenylphosphane (144 mg, 0.55 mmol, 1.1 mol.-equiv.) in dry $CH_2Cl_2$ (1 mL) was added dropwise. After 1 h, the reaction was worked up by dilution with ether and washing with sat. $NaHCO_3$ and brine. After drying and removal of the solvent, the oily residue was purified by column chromatography using as eluant petroleum ether to remove the excess hexachloroacetone, and hexane/EtOAc (7:3) to elute **10** (173 mg, 60%), obtained as a white powder, m.p. 175°C. − IR (KBr): $\tilde{\nu}$ = 3431 $cm^{-1}$ (OH), 1718 (C=O), 1381, 1273, 1177, 1066, 1026. − $^1H$ NMR (200 MHz, $CDCl_3$): δ = 6.22 (br. s, 1-H), 5.40 (br. s, 3-H), 5.82 (br. s, 5-H), 6.39 (d, *J* = 3.4 Hz, 7-H), 4.36 (m, 8-H), 1.57 (s,

16-H and 17-H), 1.08 (d, $J$ = 6.8 Hz, 18-H), 1.87 (s, 19-H), 4.01 (d, $J$ = 12 Hz, 20a-H), 3.90 (d, $J$ = 12 Hz, 20b-H),8.22 (Bz-AA′), 7.95 (Bz-BB′), ca. 7.52 (m, 6 H, 2 × Bz). − CIMS (70 eV); *m*/*z*%: 577, 575 [M + $H^+$] [$C_{34}H_{35}ClO_6$ + $H^+$].

**7-Azido-6,7-dihydro-20-deoxy-6,20-dehydroingenol 3,5-Dibenzoate (11):** To a solution of **10** (195 mg, 0.34 mmol) in DMF (2 mL), a solution of $NaN_3$ (66 mg, 1.0 mmol, 3 mol.-equiv.) in 20% aqueous DMF was added dropwise. After stirring 24 h at room temperature, the reaction mixture was worked up by pouring into a suspension of Celite (ca. 300 mg) in water (10 mL). The suspension was filtered and the cake was washed with water to remove DMF, and then with EtOAc to recover the reaction product. After washing with brine, the EtOAc phase was dried and evaporated. Purification of the residue by column chromatography (hexane/EtOAc, 9:1) gave 55 mg (28%) **11** as the major reaction product as a white powder, m.p. 170−175°C. − IR (KBr): $\tilde{\nu}$ = 3553 $cm^{-1}$ (OH), 1718 (C=O), 1452, 1265, 1097, 1070, 715. − $^1$H NMR (200 MHz, $CDCl_3$): δ = 5.82 (br. s, 1-H), 5.92 (s, 3-H), 5.56 (br. s, 5-H), 4.41 (br. d, J =, 8-H), 3.62 (m, 7-H), 1.26, 1.23 (s, 16-H), 1.17 (s, 17-H), 1.22 (d, $J$ = 6.8 Hz, 18-H), 1.58 (s, 19-H), 5.57 (br. d, $J$ = 12 Hz, 20a-H), 5.50 (br. d, $J$ = 12 Hz, 20b-H), 8.21 (Bz-AA′), 8.10 (Bz-BB′), ca. 7.52 (m, 6 H, 2 × Bz),. − HRMS (70 eV); *m*/*z* 581.2519 (calcd. for $C_{34}H_{35}N_3O_6$, 581.2526).

**20-Deoxy-20-phthalimidoingenol (12):** To a cooled (0°C) solution of ingenol (**1a**) (876 mg, 2.52 mmol) in dry THF (8 mL), phtalimide (371 mg, 2.52 mmol, 1 mol.-equiv.), polymer-supported triphenylphosphane (TPP) (842 mg, 2.52 mmol, 1 mol.-equiv.) were added. A solution of DEAD (379 μL, 439 mg, 2.52 mmol, 1 mol.-equiv.) in dry THF (17 mL) was then added dropwise, and the solution was stirred at 0°C for two hours. The cooling bath was then removed and 2 extra mol.-equivalents of phtalimide, polymer-supported triphenylphosphane, and DEAD were sequentially added. After two hours, the reaction was worked up by evaporation, and the residue was purified by column chromatography. Elution with hexane/EtOAc (8:2) removed unchanged phtalimide. The solvent was then changed to hexane/EtOAc (5:5) to elute **12** (718 mg, 67% based on conversion) and finally changed to pure EtOAc to recover unchanged ingenol (95 mg). For reasons which are not obvious, addition of 4 equivalents of all reagents in one step gave erratic yields, both with normal and with polymer-supported TPP. Compound **12** was obtained as a white powder, m.p. 127−130°C. − IR (KBr): $\tilde{\nu}$ = 3453 $cm^{-1}$ (OH), 1701 (C=O), 1396, 1240, 1018, 953, 731. − $^1$H NMR ($CDCl_3$): δ = 5.90 (d, $J$ = 1.5 Hz, 1-H), 4.46 (s, 3-H), 3.70 (br. s, 5-H), 6.00 (d, $J$ = 4.5 Hz, 7-H), 4.13 (br. dd, $J$ = 12.5, 4.5 Hz, 8-H), 2.40 (m, 11-H), 2.26 (m, 12a-H), 1.76 (m, 12b-H), 0.66 (ddd, $J$ = 8.5, 8.5, 6), 0.90 (dd, $J$ = 12.5, 8.5 Hz, 14-H), 1.10 (s, 16-H), 1.03 (s, 17-H), 0.95 (d, $J$ = Hz, 18-H), 1.85 (d, $J$ = 1.5 Hz, 19-H), 4.36 (br. s, 20a,b-H), 7.86 (Pthal. AA′), 7.40 (Phtal. BB′). − $^{13}$C NMR ($CDCl_3$): δ = 129.6 (d, C-1), 138.9 (s, C-2), 80.6 (d, C-3), 84.4 (s, C-4), 74.2 (d, C-5), 136.3 (s, C-6), 127.3 (d, C-7), 44.0 (d, C-8), 206.7 (s, C-9), 72.6 (s, C-10), 39.5 (d, C-11), 31.0 (t, C-12), 23.0 (d, C-13), 23.2 (d, C-14), 23.8 (s, C-15), 18.3 (q, C-16), 28.4 (q, C-17), 17.4 (q, C-18), 15.3 (q, C-19), 41.0 (t, C-20), 168.5 (Phtal. C=O), 132.0 (s. Ph), 123.5 (d, Ph), 134.1 (d, Ph). − HRMS (70 eV); *m*/*z* 477.2159 (calcd. for $C_{28}H_{31}NO_6$, 477.2151).

**20-Benzamido-20-deoxyingenol 3-Benzoate (13) and 20-Deoxy-20-phenylureidoingenol 3-Benzoate (14):** To a solution of 20-deoxy-20-phtalimidoingenol (648 mg, 1.36 mmol) in EtOH (10 mL), an excess methylamine was added (4 mL of a 40% aqueous solution). After stirring at room temperature for 6 h, the reaction was worked up by removal of the solvent, dilution with EtOAc, and extraction with 2N $H_2SO_4$. The acidic phase was washed with EtOAc (× 2), neutralized with conc. $NH_3$, and then extracted with EtOAc. After drying and removal of the solvent, 328 mg (70%) 20-deoxy-20-aminoingenol was obtained as an unstable glass, which was directly used for the following steps. The compound was dissolved in EtOAc (10 mL), and the solution was divided into two 5 mL aliquots. The aliquots were treated with. aq. $NaHCO_3$ (5 mL) and with benzoyl chloride (93 μL, 113 mg, 0.81 mmol, 1.2 mol.-equiv.) in one case, and phenyl isocyanate in the other. After stirring at room temperature for 30 min, the reactions were worked up by separation of the phases and washing of the organic phase with brine. After drying and removal of the solvent, the residues were purified by a short filtration on silica gel (hexane/EtOAc, 4:6), and the crude 20-benzoate and the 20-phenylurea were next benzoylated with the the EDC-DMAP protocol, as described for the synthesis of **1c** from **1a** and **2b** from **2a** (2 mol.-equiv, −18°C, 18 h). The final products were purified by column chromatography using hexane/EtOAc (7:3) as eluant, to give 94 mg **13** and 73 mg **14** (25% and 19% from **12**, respectively).

**Compound 13** was obtained as a white powder, m.p. 104°C. − IR (KBr): $\tilde{\nu}$ = 3410 $cm^{-1}$ (OH), 1763 (C=O), 1718 (C=O), 1275, 1151, 1109, 1026, 711. − $^1$H NMR ($CDCl_3$): δ = 6.07 (d, $J$ = 1.5 Hz, 1-H), 5.90 (s, 3-H), 3.83 (br. s, 5-H), 6.07 (d, $J$ = 4.5 Hz, 7-H), 4.23 (br. dd, $J$ = 12.5, 4.5 Hz, 8-H), 2.68 (m, 11-H), 2.33 (m, 12a-H), 1.73 (m, 12b-H), 0.73 (ddd, $J$ = 8.5, 8.5, 6), 0.95 (dd, $J$ = 12.5, 8.5 Hz, 14-H), 1.07 (s, 16-H), 1.06 (s, 17-H), 1.06 (d, $J$ = Hz, 18-H), 1.80 (d, $J$ = 1.5 Hz, 19-H), 4.66 (dd, $J$ = 12, 6.0 Hz, 20a-H), 3.70 (dd, $J$ = 12, 6.0 Hz, 20b-H), 6.61 (t, $J$ = 6.0 Hz, NH), 7.60 (NHBz AA′), 7.48 (NHBz BB′), 8.07 (Bz AA′), 7.78 (Bz BB′). − $^{13}$C NMR ($CDCl_3$): δ = 131.9 (d, C-1), 136.3 (s, C-2), 83.8 (d, C-3), 85.3 (s, C-4), 73.4 (d, C-5), 138.6 (s, C-6), 128.1 (d, C-7), 43.5 (d, C-8), 207.0 (s, C-9), 71.6 (s, C-10), 38.6 (d, C-11), 31.2 (t, C-12), 23.5 (d, C-13), 23.1 (d, C-14), 24.0 (s, C-15), 15.5 (q, C-16), 28.5 (q, C-17), 17.3 (q, C-18), 15.5 (q, C-19), 44.7 (t, C-20), 168.4 (amide C=O), 130.1 (s. *i*-NHBz), 126.8 (d, *o*-NHBz), 128.5 (d, *m*-NHBz), 132.1 (d, p-NHBz), 166.8 (s, ester C=O), 133.3 (s, *i*-OBz), 129.8 (d, *o*-OBz), 128.7 (d, *m*-OBz), 133.1 (d, *p*-OBz). − HRMS (70 eV); *m*/*z* 555.2625 (calcd. for $C_{34}H_{37}NO_6$, 555.2621).

**Compound 14** was obtained as a white powder, m.p. 90°C. − IR (KBr): $\tilde{\nu}$ = 3410 $cm^{-1}$ (OH), 1719 (C=O), 1599, 1315, 1261, 1026, 802, 711. − $^1$H NMR ($CDCl_3$): δ = 6.08 (d, $J$ = 1.5 Hz, 1-H), 5.94 (s, 3-H), 3.91 (br. s, 5-H), 5.94 (d, $J$ = 4.5 Hz, 7-H), 4.25 (br. dd, $J$ = 12.5, 4.5 Hz, 8-H), 2.71 (m, 11-H), 2.33 (m, 12a-H), 2.13 (m, 12b-H), 0.70 (ddd, $J$ = 8.5, 8.5, 6), 0.88 (dd, $J$ = 12.5, 8.5 Hz, 14-H), 1.07 (s, 16-H), 1.08 (s, 17-H), 1.07 (d, $J$ = Hz, 18-H), 1.83 (d, $J$ = 1.5 Hz, 19-H), 4.46 (br. dd, $J$ = 12, 6.0 Hz, 20a-H), 3.73 (br. dd, $J$ = 12, 6.0 Hz, 20b-H), 5.33 (br. t, $J$ = 6 Hz, NH), 8.07 (Bz AA′), 7.60 (Bz BB′), 7.30 (NHBz AA′), 7.10 (NHBz. BB′). − $^{13}$C NMR ($CDCl_3$): δ = 132.0 (d, C-1), 136.5 (s, C-2), 83.8 (d, C-3), 85.5 (s, C-4), 73.2 (d, C-5), 140.0 (s, C-6), 126.8 (d, C-7), 43.5 (d, C-8), 207.0 (s, C-9), 72.0 (s, C-10), 38.8 (d, C-11), 31.2 (t, C-12), 23.4 (d, C-13), 23.0 (d, C-14), 24.0 (s, C-15), 15.5 (q, C-16), 28.5 (q, C-17), 17.3 (q, C-18), 15.5 (q, C-19), 45.2 (t, C-20), 158.0 (urea. C=O), 138.0 (s, *i*-NHPh), 121.0 (d, *o*-NHPh), 129.3 (d, *m*-NHPh), 124.1 (d, *p*-NHPh), 167.0 (s, Bz C=O), 130.0 (s, *i*-Bz), 129.8 (d, *o*-Bz), 128.5 (d, *m*-Bz), 133.1 (d, *p*-Bz). − HRMS (70 eV); *m*/*z* 477.2159 (calcd. for $C_{28}H_{31}NO_6$, 477.2151. − HRMS (70 eV); *m*/*z* 570.2735 (calcd. for $C_{34}H_{38}N_2O_6$, 570.2730).

## Acknowledgments

This work was supported by the EU (Contract FAIRCT961781), and by MURST (Fondi ex-40%, project: Chemistry of Bioactive

Organic Compounds) as regards the chemical part, and by Telethon (Roma, Italy), AIRC (Milano, Italy), MURST ex 60%, Special Project AIDS (Istituto Superiore di Sanità), CRT, Compagnia Sanpaolo, Cariverona Foundations, and Regione Piemonte as regards the biological evaluation of the compounds. We are grateful to F. Malavasi, M.D., for supplying the CBT3G (anti-CD3) mAb used in the PKC assay, and for his valuable scientific advice. We also thank A. Arnulfo, M. D. for helpful discussions.

[1] K. Zeichmeister, F. Brandl, W. Hoppe, E. Hecker, H. J. Opferkuch, W. Adolf, *Tetrahedron Lett.* **1970**, 4075−4078.
[2] S. M. Kupchan, I. Uchida, A. R. Branfman, R. G. Dailey Jr., B. Yu Fei, *Science* **1976**, *191*, 571−572.
[3] T.-S. Wu, Y.-M. Lin, M. Haruna, D.-J. Pan, T. Shingu, Y.-P. Chen, H.-Y. Hsu, T. Nakano, K.-H. Lee, *J. Nat. Prod.* **1991**, *54*, 823−829.
[4] Z. H. Mbwambo, S. K. Lee, E. N. Mshiu, J. M. Pezzuto, A. D. Kinghorn, *J. Nat. Prod*. **1996**, *59*, 1051−1055.
[5] Y. Nishizuka, *Nature* **1984**, *308*, 693−697.
[6] Other pre-eminent examples within phorboids are mezerein (G. Fürstenberger, E. Hecker, *Planta Med.* **1972**, *22*, 241−266 ), and phorbol 12-tigliate 13-decanoate (ref.[2]).
[7] G. C. Zhang, M. G. Kazanietz, P. M. Blumberg, J. H. Hurley, *Cell* **1995**, *81*, 917−924.
[8] J. D. Winkler, B.-C. Hong, S. Kim, N. E. Lewin, P. M. Blumberg, *Synlett* **1995**, 533−535.
[9] E. H. Seip, E. Hecker, *Planta Med.* **1982**, *46*, 215−218
[10] H. Hitokawa, Y. Ichihara, K. Watanabe, K. Takeya, *Planta Med.* **1989**, *55*, 271−272.
[11] M. Fujiwara, K. Ijichi, K. Tokuhisa, K. Katsuura, G.-Y.-S. Wang, D. Umemura, S. Shigeta, K. Konno, T. Yokota, M. Baba, *Antiviral Chem. Chemother.* **1996**, *7*, 230−236.
[12] For a review on the various approaches attempted, see: S. Kim, J. D. Winkler, *Chem. Soc. Rev.* **1997**, *26*, 387−399. For recent advances, see: J. D. Winkler, S. Kim, S. Harrison, N. E. Lewin, P. M. Blumberg, *J. Am. Chem. Soc*. **1999**, *121*, 296−300.
[13] E. Hecker in: *Cancerogenesis: A Comprehensive Survey, Mechanisms of Tumor Promotion and Cocancerogenesys* (Ed: T. J. Slaga, A. Sivak, R. K. Boutwell), Raven, New York, **1987**, Vol. 2, pp 11−48.
[14] S. K. Holmgren, K. M. Taylor, L. E. Bretwcher, R. T. Raines, *Nature* **1998**, *392*, 666.
[15] G. Krauter, C.-W Von der Lieth, R. Schmidt, E. Hecker, *Eur. J. Biochem*. **1996**, *242*, 417−427.
[16] B. Sorg, E. Hecker, *Z. Naturforschung* **1982**, *37B*, 748−756.
[17] T. Kurahashi, T. Mizutani, J. Yoshida, *J. Chem. Soc., Perkin Trans.1* **1999**, 465−473.
[18] O. Mitsunobu, *Synthesis* **1981**, 1−28.
[19] This coupling is due to a direct orbital interaction between the fluorine and the hydrogen and/or carbon nucleus. See: S. Berger, S. Braun, H. O. Kalinowski, in: *NMR Spectroscopy of the Nonmetallic Elements*, Wiley, New York, **1997**, pp. 610−617.
[20] E. Hecker, R. Schmidt, in: *Progress in the Chemistry of Organic Natural Products*, Ed: W. Herz, H. Grisebach, G. W. Kirby, Springer, Wien, **1974**, Vol. 31, pp. 377−467.
[21] A similar rearrangement was reported on a fully protected 9-dihydroingenol derivative upon treatment with mesyl chloride: E. Hecker, *Pure and Appl. Chem*. **1977**, *49*, 1423−31.
[22] I. Ganboa, J. M. Aizpurua, C. Palomo, *J. Chem. Res.* **1984**, 92.
[23] R. M. Magid, O. S. Fruchery, W. L. Johnson, *Tetrahedron Lett.* **1977**, 2999−3002.
[24] M. Hall, A. M. Z. Hoemann, J. Aubé, L. A. Mitscher, G. I. Georg, R. McCall, L. R. Jayasinghe, *J. Med. Chem*. **1995**, *38*, 2821−2828.
[25] [25a] M. Morra, M. Zaubiaur, C. Terhorst, J. Sancho, F. Malavasi, *Faseb J.* **1998**, *12*, 581−592. − [25b] D. A. Cantrell, S. C. Lucas, S. Ward, J. Westwick, M. Gullberg, *J. Immunol.* **1989**, *143*, 3653−3658. − [25c] S. Deaglio, M. Morra, R. Mallone, C. M. Ausiello, E. Prager, G. Garbarino, U. Dianzani, H. Stockinger, F. Malavasi, *J. Immunol.* **1998**, *160*, 395−402.
[26] G. Appendino, G. C. Tron, G. Cravotto, G. Palmisano, J. Jakupovic, *J. Nat. Prod.* **1999**, *62*, 76−79.

Received April 22, 1999
[O99239]