Synthesis and Biological Evaluation of Phorbol-Resiniferatoxin (RTX) Hybrids

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Phorboid 20-homovanillates modified on ring C to mimic the constitution and conformation of the ultrapotent vanilloid resiniferatoxin (RTX) and on the AB ring system to suppress the tumour-promoting potential of the terpenoid core were prepared. Several unexpected reactions were discovered, and the structure-activity relationships for this class of vanilloid ligands were expanded.

Introduction

The discovery that the phorboid resiniferatoxin (RTX, **1a**) acts as an ultrapotent vanilloid, and the discovery of a specific receptor (TRPV1), have spurred an intense research activity aimed at the discovery of new biological analogues of capsaicin (**2**), the pungent principle of hot pepper and the archetypal vanilloid.[1] As a result of these studies, the structural database of vanilloids has been considerably expanded, and now encompasses seven distinct chemotypes from the natural product pool,[2] two of which (anandamide[2f] and *N*-acyldopamides[2g]) also occur endogenously in mammal tissues. While undoubtedly providing new and exciting opportunities for discovery, none of these new ligands approaches RTX in terms of potency and pharmaceutical potential. RTX is currently in advanced clinical trials for treatment of diabetic neuropathy[3] and detrusor hyperreflexia,[4] and it is therefore surprising that its structure-activity relationships are still poorly understood. Furthermore, the recent discovery that the ultrapotent vanilloid activity of RTX can be reversed from agonist to antagonist by simple phenolic iodination[5] lends support to the view that this compound represents an especially advantageous structure with which to investigate vanilloid receptors. Since neither RTX nor its terpenoid core (resiniferonol, **1b**) are commercially available in amounts sufficient to sustain a significant structure-activity effort,[6,7] we have investigated the potential of phorbol (**3a**) as a resiniferonol surrogate for the synthesis of vanilloids. Phorbol can be obtained in multigram amounts from croton oil, a commercial commodity.[8] The structural differences between phorbol and resiniferonol are localised on ring C, and are limited to a shift of the hydroxy group from the secondary C-12 to the secondary C-14 and to the opening of the adjacent cyclopropane moiety. Our first efforts in this area resulted in the discovery of a series of phorbol-RTX hybrids endowed with vanilloid activity.[9] These compounds, exemplified by PPAHV (**3b**), raised interest because of their unique behaviour in binding and functional assays of vanilloid activity.[9,10] Since the vanilloid profile of phorboid homovanillates is distinct from those of both capsaicinoids and resiniferonoids,[10] it was interesting to expand their structure-activity relationships. Thus, taking PPAHV as a lead, we have focused on modifications of ring C to mimic the constitution and conformation of resiniferatoxin, and on changes of the AB ring system to provide phorboid platforms devoid of tumour-promoting potential. A significant drawback of resiniferatoxin and phorbol homovanillates is indeed the generation of tumour promoters through the loss of the 20-ester group, a reaction that takes place under mild acidic conditions[8] and the relevance of which in vivo is unclear.

While the chemistry of resiniferol is essentially uncharted territory, phorbol has long been fascinating organic chemists, and its reactivity has been extensively investigated.[8] However, a series of surprising reactions were discovered in

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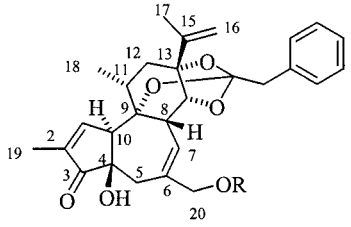
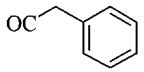
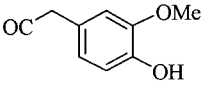
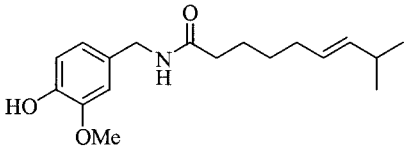


PhAc = 

HMV = 

Trit = CPh_3

	R
1a	HMV
1b	H



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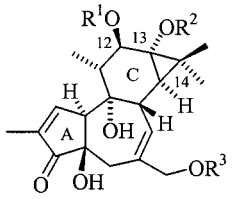


	R^1	R^2	R^3
3a	H	H	H
3b	PhAc	Ac	HMV
3c	H	Ac	Trit
3d	H	PhAc	Trit

the attempt to steer the reactivity of this polyol to the synthesis of our targets.

Results and Discussion

Ring C Modifications

The cyclopropane ring of phorbol can be opened under both acidic and basic conditions, providing the opportunity to change the constitution and conformation of ring C. This ring adopts a half-chair conformation in phorbol, but is constrained into a boat geometry by the orthoester group of RTX.[11] Constraint into a boat conformation can also be achieved by a shift of the methano bridge (C-15), a type of reaction documented for neophorbol (= 12-dehydrophorbol).[12] Upon mild basic treatment, 12-dehydrophorbol 13,20-diacetate (**4a**) undergoes an α-ketol rearrangement, affording a mixture of two cyclobutane derivatives [hydroxyphorbobutanone (**5a**) and hydroxyphorboisobutyl ketone (**6a**)].[12] The new location of the bridge forces ring C into a boat-like conformation, and the closure of a semiacetal ring with the 9-hydroxy group of **6a** further contributes to the overall mimicry of the conformation of RTX. A precursor for the α-ketol rearrangement (**4b**) was prepared from 13-acetylphorbol 20-trityl ether (**3c**)[9] by PCC oxidation of the secondary C-12 hydroxy group. Surprisingly, the rearrangement of **4b** took different courses in methanol and in ethanol. Both the "external" (C-13/C-15) and the "internal" (C-13/C-14) cyclopropane bonds can migrate to the C-12 carbonyl, giving a mixture of two different *abeo*-phorbols, as is indeed reported in the literature (Figure 1; pathways a and b).[12] However, when the reaction was carried out with sodium methoxide in methanol, the phorbobutanone derivative **5b** was formed as the only reaction product, the result of migration to the carbonyl of the external cyclopropane bond. Conversely, when the reaction was carried out in ethanol with sodium ethoxide, a mixture of two products was obtained in a reproducible way. The minor one (10%) was the same phorbobutanone as obtained from the rearrangement in methanol (**5b**), while the major one (78%) was the phorboisobutyl ketone derivative **6b**. The two compounds could be distinguished very easily from the chemical shift of C-13, which is a ketone in **5b** (δ = 214.7 ppm) and a semiacetal in **6b** (δ = 108.3 ppm). Similar behaviour was observed with **4c**, the 20-*tert*-butyldimethylsilyl (TBDMS) analogue of **4b**. The regiochemistry of cyclopropane ring-opening in expansion reactions is, in general, poorly understood,[13] but the steric and electronic similarity between methanol and ethanol makes it difficult to propose an explanation for the observed differences in product distribution in the rearrangement of **4b**. The *abeo*-phobols **5b** and **6b** were next esterified with excess phenylacetic acid, affording **5c** and **6c** and endowing ring C with the acyl appendage typical of PPAHV and RTX. The regiochemistry of esterification was verified by NOE experiments carried out on the final 20-homovanillates **5d** and **6d**, obtained by a previously established procedure (removal of the trityl group with $HClO_4$, esterification with Mem-protected homovanillic acid, and $SnCl_4$-mediated deprotection of the phenolic hydroxy group).[9] The NOE experiments showed that in both **5d** and **6d** the phenylacetyl group was bound to the hydroxy group at C-12 [NOEs between the

phenyl protons and the α-oriented methyl groups (C-18 and C-16)]. Alternative locations for the phenylacetyl residue, namely at the C-9 hydroxy group for the phorbobutanone homovanillate (**5d**) or at the semiacetal oxygen for the phorboisobutyl ketone homovanillate (**6d**), would have involved lack of correlation with the methyl group (C-16) (esterification of the 10-hydroxy group of **5d**) or with the methyl group (C-18) (esterification of the semiacetalic hydroxy group of **6d**).

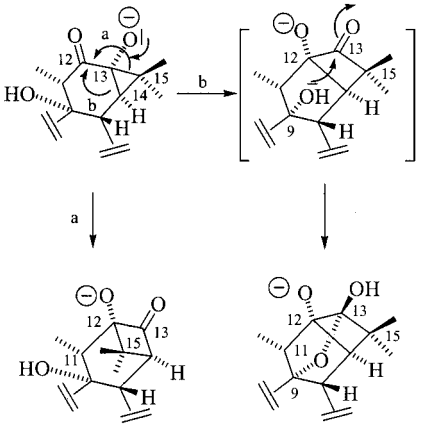
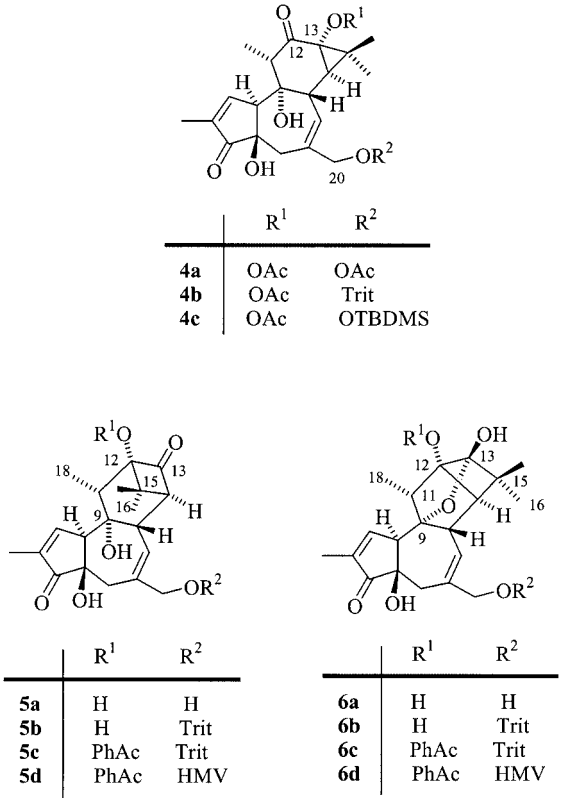


Figure 1. The α-ketol rearrangement of 12-dehydrophorbol derivatives

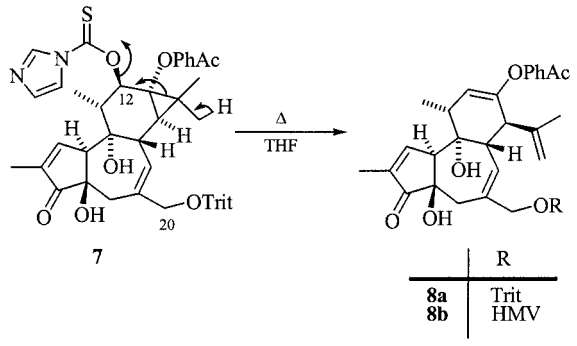


	R^1	R^2
4a	OAc	OAc
4b	OAc	Trit
4c	OAc	OTBDMS

	R^1	R^2
5a	H	H
5b	H	Trit
5c	PhAc	Trit
5d	PhAc	HMV

	R^1	R^2
6a	H	H
6b	H	Trit
6c	PhAc	Trit
6d	PhAc	HMV

Opening of the cyclopropane ring was next investigated. This reaction requires the harsh acidic conditions of the so-called Flaschenträger reaction,[14] and is incompatible with the functionalisation of advanced intermediates such as **3c**. After considerable experimentation, however, we discovered that heating at reflux of a THF solution of the 13-phenylacetyl-12-thioimidazolide **7** − formed in situ from **3d** by treatment with thiocarbonyldiimidazole − could trigger the cyclopropyl version of a β-elimination, affording the enolcrotophorbolone **8a** in almost quantitative yield and with preservation of the trityl protection of the primary C-20 hydroxy group and the enol ester moiety. (Scheme 1)[15] Removal of the trityl group and introduction of the homovanillyl moiety[9] eventually afforded the D-*seco*-phorbol (rhamnofolane) **8b**.

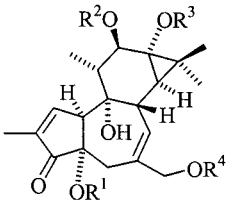


	R
8a	Trit
8b	HMV

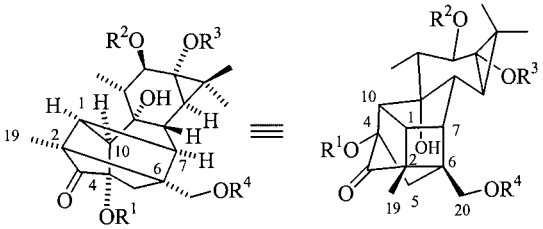
Scheme 1. Fragmentation of the thioimidazolide **7**

2. Rings A/B Modifications

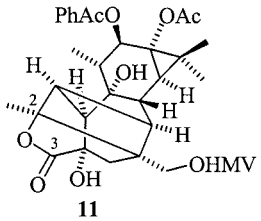
The PKC binding and tumour-promoting properties of phorboids are critically dependent on the configuration of the AB ring junction, and are abolished by the vinylogous retro-aldol epimerisation of the tertiary C-4 hydroxy group.[16] In order to suppress the tumour-promoting potential of the phorboid core, the synthesis of the 4α epimer of PPAHV and a series of derivatives was attempted. To this end, the primary and the tertiary C-13 hydroxy groups of 4α-phorbol (**9a**)[17] were acetylated, and the secondary C-12 hydroxy group was esterified with phenylacetic acid, affording the triester **9b**. The 20-hydroxy group was removed by transesterification, and the 20-alcohol **9c** was then treated with MEM-homovanillic acid and then deprotected to give a mixture of the homovanillate **9d** and the bis-homovanillate **9e** (ca. 1:0.8).[18] The critical 20-ester bond formation could be achieved in a selective way by switching from an acyl to an alkyl nucleophilic substitution, and capitalising on the Mitsunobu reaction.[19] Thus, treatment of **9c** with the triphenylphosphane/di-*tert*-butyl azodicarboxylate couple and homovanillic acid cleanly provided 4α-PPHV (**9d**) in a gratifying 91% yield. The high reactivity of the tertiary C-4 hydroxy group of 4α-phorbol in esterification reaction is reminiscent of the reactivity of the tertiary C-13 hydroxy group in both phorbol and 4α-phorbol.[8] Stabilisation of the tertiary ester by formation of strong in tramolecular hydrogen bonding with the 9-hydroxy group



	R^1	R^2	R^3	R^4
9a	H	H	H	H
9b	H	PhAc	Ac	Ac
9c	H	PhAc	Ac	H
9d	H	PhAc	Ac	HMV
9e	HMV	PhAc	Ac	HMV



	R^1	R^2	R^3	R^4
10a	H	PhAc	Ac	HMV
10b	H	H	Ac	Ac
10c	PhAc	PhAc	Ac	Ac
10d	H	PhAc	Ac	H
10e	MEM-HMV	PhAc	Ac	MEM-HMV
10f	H	PhAc	Ac	Ac



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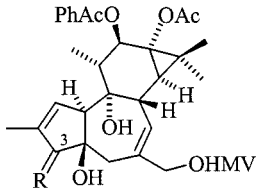

	R
12a	αH, β-OH
12b	βH, α-OH

presumably underlies these remarkable effects, which were also detected in lumiphorbol (vide infra). It remains unclear, however, why the parent polyol **9a** could be converted into the triester **9b** without involvement of the 4-hydroxy group, the reactivity of which seems to be magnified by the presence of a free 20-hydroxy group.

4α-Phorbol is cup-shaped, and easily undergoes a photochemical [$2\pi_s + 2\pi_s$] cycloaddition to afford lumiphorbol, a cage-like compound.[20] In the event, irradiation of PPAHV in degassed ethanol afforded a quantitative yield of the lumi derivative **10a**. Attempts to use lumiphorobol itself as a platform for the synthesis of specific esters were frustrated by inability to manipulate its various hydroxy groups selectively, an unfortunate event, given the potential of 4α-phorbol to act as a polyfunctionalized, cubane-like scaffold for iterative, orthogonal acylations. A set of lumiphorbol esters was therefore obtained by irradiation of the corresponding 4α-phorbol esters,[17] and then subjected to transesterification or acylation to change their acylation patterns. Treatment of lumiphorbol 13,20-diacetate (**10b**) with phenylacetic acid with EDC promotion {EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride} afforded the 4,12-bis(phenylacetate) **10c** as the only reaction product, while treatment of the 12,13-diester **10d** with MEM-homovanillic acid gave (after deprotection) the 4,20-bis(homovanillate) **10e** even with stoichiometric (1 molar equivalent) amounts of acylating reagent. The location of the tertiary homovanillate at the 4-oxygen function was evident from the detection of a strong NOE effect between one of the homovanillate methylene groups (δ = 3.61 ppm) and the two protons at C-5 (δ = 1.99 and 3.27 ppm), as well as with 10-H (δ = 2.61 ppm). On the other hand, transesterification of the 13,20-diacetate-12-phenyl acetate **10f** afforded a complex mixture, as did the Mitsunobu esterification of the 12,13-diester **10d**. These results make it clear that the rigid scaffold of lumiphorbol engenders the formation of a web of hydrogen bonds, which makes the tertiary C-4 hydroxy group as reactive in acylation reactions as the secondary C-12 hydroxy group and the primary C-20 hydroxy group.

Interestingly, when the irradiation of 4α-PPHAV was carried out under oxygen, the 2,3-*seco*-lactone **11** was obtained as the major reaction product (51%). The same compound could be obtained in similar yield (48%) by treatment of lumi-PPAHV (**10a**) with peracids in buffered medium. The photo-cleavage of **10a** is presumably initiated by a Norrish I α-cleavage of the cyclopentane ring, but the further steps of the reaction are unclear.[21]

We lastly investigated the reduction of the 3-keto group, stereoselectively preparing the epimeric 3-dihydro derivatives of PPAHV. Reduction with $NaBH_4$ in methanol is dominated by steric effects, and affords the 3βH-hydro derivative **12a** as the only reaction product.[9] Conversely, reduction with tetrabutylammonium triacetoxyborohydride followed a chelation-controlled pathway,[9,22] affording the 3βH-dihydro derivative **12b**.

Table 1. Biological evaluation of the vanilloid activities of phorboid homovanillates (n.d. = not detectable; n.a. = not assayed)

Compound	Rat TRPV1		Human TRPV1	
	EC_{50} (nM)	Maximal effect at 10 μM (% ionomycin)	EC_{50} (nM)	Maximal effect at 10 μM (% ionomycin)
Capsaicin (**2**)	40.0 ± 3.0	34.6 ± 1.9	40.2 ± 3.1	70.1 ± 3.7
PPAHV (**3b**)	85.2 ± 4.1	43.2 ± 3.1	3100 ± 230	65.2 ± 4.1
5d	n.d.	n.a.	n.a.	n.a.
6d	n.d.	n.a.	n.a.	n.a.
8b	91.0 ± 5.2	n.a.	n.a.	n.a.
4α-PPAHV (**9d**)	653 ± 61	25.6 ± 2.7	4180 ± 360	11.7 ± 2.9
Lumi-PPAHV (**10a**)	848 ± 72	34.8 ± 6.2	3090 ± 510	16.1 ± 3.3
Oxylumi-PPAHV (**11**)	648 ± 55	34.4 ± 6.0	N.D.	5.0 ± 3.0
3αH-DihydroPPAHV (**12a**)	2480 ± 280	79.8 ± 5.3	3050 ± 290	65.1 ± 3.9
3βH-DihydroPPAHV (**12b**)	n.d.	n.a.	n.a.	n.a.

3. Biological Evaluation

The activities of the phorboid homovanillates **3b**, **5d**, **6d**, **8b**, **9d**, **10a** **11**, **12a** and **12b** were evaluated by measuring the entry of Ca^{2+} into embryonic kidney cells transfected with the rat vanilloid TRPV1 receptor, with evaluation of potency (EC_{50}) and efficiency (maximal effect at 10 μM concentration).[23] Potency and efficiency reflect the affinity for the CPS binding site on TRPV1, and the capacity to induce a change eventually translating into a functional response (calcium efflux), respectively. Despite their increased structural similarity to RTX relative to PPAHV, the ring C-constrained phorboids **5d** and **6d** did not show any measurable vanilloid activity. Conversely, the crotophorbolone enol ester **8b** retained most of the vanilloid activity of PPAHV. These observations show that modifications on ring C have a profound effect on the vanilloid activity of phorbol homovanillates, and highlight the key role of this moiety for binding to TRPV1.

Changes on the AB ring system that suppress the tumour-promoting potential of phorboid esters also decreased their vanilloid potency, while their effect on efficacy was only minimal. While the 3α-dihydro derivative **12a** showed only marginal vanilloid potency, its epimer **12b** was devoid of detectable vanilloid activity. Surprisingly, **12a** showed an increased efficacy relative to capsaicin.

Remarkably, when the measurements of vanilloid activity were done on cells transfected with the human orthologue of the receptor, general decreases in both potency and efficacy were observed, and only two compounds (PPAHV and **12a**) retained efficacy similar to that of capsaicin. These findings confirm a previous report on the attenuation of the vanilloid activity of PPAHV when assayed on human TRPV1,[23] and show that, despite the close similarity between human and rat TRPV1, ligand discrimination is still possible. The remarkable attenuation of potency observed for phorbol homovanillates in the assays on human TRPV1 caution against the exclusive use of TRPV1-expressing systems from one only species in screening programs of vanilloid activity.

Conclusions

Ever since its isolation, interest in phorbol within the organic community has always been high, because of its challenging structure, remarkable bioactivity, and puzzling reactivity.[8] While preparing a series of phorbol-RTX hybrids useful as vanilloid probes, we made several unexpected observations regarding the reactivity of phorboids in terms of modification of functional groups and constitution. Since phorboids are remarkably versatile polyhydroxylic scaffolds for the induction of bioactivity, clarification of the mechanistic principles underlying our findings should be important to make phorboids amenable to predictable chemistry. The biological evaluation of the final homovanillates in cells transfected with the murine and human TRPV1 orthologues showed the existence of strict structure-activity relationships, but also evidenced remarkable differences within the two species, with a marked (100-fold) decrease in potency with use of human TRPV1 compared to rat TRPV1. These differences had already been noticed with PPAHV,[23] and have recently been interpreted in terms of the primary structures of the two orthologues.[24] This, and the discovery that a 4α-phorbol derivative can activate TRPV4, a thermo-, osmotic and mechanosensor insensitive to capsaicin and RTX,[25] highlight the relevance of phorboids as selective probes for neurosciences.

Experimental Section

Caution: 12,13-Diesters of phorbol are severe irritant to skin and mucous membranes, and several of them display tumour-promoting properties. Their handling should be carried out with 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 500 (500 MHz and 125 MHz for ^1H and ^{13}C, respectively). For ^1H NMR, $CDCl_3$ as solvent, $CHCl_3$ at δ = 7.26 ppm as reference. For ^{13}C NMR, $CDCl_3$ as solvent, $CDCl_3$ at δ = 77.0 ppm as reference. The ^1H and ^{13}C NMR spectra were fully assigned by a combination of 1D

and 2D (COSY, HMBC, HMQC) techniques. CH_2Cl_2 was dried by distillation from CaH_2, and THF by distillation from Na/benzophenone. Na_2SO_4 was used to dry solutions before the evaporation. Reactions were monitored by TLC on Merck 60 F_{254} (0.25 mm) plates, and spots were viewed by UV inspection and/or by staining with 5% H_2SO_4 in ethanol and heating. Phorbol was obtained from commercial Croton oil (Alexis Biochemicals).[8] To avoid undue manipulation of potentially tumour-promoting compounds, many intermediates with a free 20-hydroxy group were not fully characterised but directly converted into their corresponding and non-tumour-promoting 20-esters.

13-Acetyl-20-tritylneophorbol (4c): PCC (664 mg, 3.08 mmol, 2 mol equiv.) and activated powdered 4-Å molecular sieves (1.33 g) were added to a stirred solution of 13-acetyl-20-tritylphorbol (**3c**, 1.0 g, 1.54 mmol)[9] in dry CH_2Cl_2 (35 mL). After stirring overnight at room temperature, the reaction mixture was worked up by dilution with diethyl ether (20 mL) and filtration through Celite. After evaporation, the residue was purified by column chromatography on silica gel (10 g, petroleum ether/ EtOAc, 8:2 as eluent) to afford a white powder (738 mg, 74%). M.p. 125−127 °C. IR (KBr): $\tilde{\nu}$ = 3420, 1730, 1700, 1445, 1375, 1250, 760, 700 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 1.19 (d, *J* = 6.5 Hz, 18-H/3), 1.23 (s, 17-H/3), 1.37 (s, 16-H/3), 1.49 (d, *J* = 4.9 Hz, 14-H), 1.78 (br. s, 19-H/3), 2.17 (s, OAc), 2.43 (d, *J* = 19.0 Hz, 5a-H), 2.61 (d, *J* = 19.0 Hz, 5b-H), 2.94 (m. 11-H), 3.24 (br. s, 8-H and 10-H), 3.55 (br. s, 20-H/2), 5.40 (br. s, -OH), 5.76 (br. s, 7-H), ca. 7.30 (m, Trit-H/15), 7.56 (br. s, 1-H) ppm. HRMS (70 eV): *m*/*z* = 646.2917 (calcd. for $C_{41}H_{42}O_7$, 646.2931).

α-Ketol Rearrangement of 13-Acetyl-20-tritylneophorbol (4b):

A. With NaOMe/MeOH: Sodium methoxide (1 N, 1.08 mL, 1.08 mmol, 1 mol equiv.) was added dropwise, under nitrogen, to a solution of **4b** (697 mg, 1.08 mmol) in dry MeOH (12 mL), resulting in the development of a violet solution, which rapidly turned into a thick orange suspension. After 5 minutes, the reaction mixture was worked up by neutralisation with satd. NH_4Cl and extraction with EtOAc. Washing with brine and drying (Na_2SO_4) afforded a yellowish powder (660 mg), that, when analysed by ^1H NMR (300 MHz, $CDCl_3$) showed the presence only of **5b**. An analytical sample was obtained by washing the solid residue with diethyl ether, which afforded a white powder (565 mg, 88%).

B. With EtONa/EtOH: The reaction was carried out in the same way as described for the NaOMe/MeOH couple, but the violet colour observed upon addition of the bases developed into a brown limpid solution and not an orange paste. From 2.5 g **4b**, 2.4 g of a yellow powder was obtained after workup. When analysed by ^1H NMR (300 MHz, $CDCl_3$), the presence of a ca. 1:9 mixture of **6b** and **5b** was detected. The mixture was purified by column chromatography (150 g silica gel, petroleum ether/EtOAc, 5:5) to afford **6b** (1.87 g, 78%) and **5b** (267 mg, 10%).

20-Tritylphorbobutanone (5b): M.p. 76−78 °C. IR (KBr): $\tilde{\nu}$ = 3453, 3410, 1761, 1686, 1447, 1223, 1051, 754, 706 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 1.08 (s, 16-H/3), 1.10 (d, *J* = 6.5 Hz, 18-H/3), 1.37 (s, 17-H/3), 1.81 (br. s, 19-H/3), 2.35 (br. s, 14-H), 2.42 (q, *J* = 6.8 Hz, 11-H), 2.44 (d., *J* = 19 Hz, 5a-H), 2.53 (d, *J* = 19.0 Hz, 5b-H), 2.75 (br. s, 10-H), 3.61 (br. s, 20-H/2), 3.91 (m, 8-H), 5.67 (br. d, *J* = 6.2 Hz, 7-H), ca. 7.30 (m, Trit-H/15), 7.41 (br. s, 1-H) ppm. ^{13}C NMR ($CDCl_3$/[D_6]DMSO): δ = 8.7 (C-18), 10.4 (C-19), 25.7 (C-16), 32.6 (C-17), 38.2 (C-5), 41.6 (C-8), 45.8 (C-11), 55.1 (C-15), 55.3 (C-14), 55.8 (C-10), 69.0 (C-20), 74.3 (C-4), 87.2 (Trit), 88.5 (C-9), 97.2 (C-12), 124.3 (C-7), 127.0 (Trit), 127.8 (Trit), 128.6 (Trit), 136.7 (C-6), 138.3 (C-2), 154.1 (C-1), 207.0 (C-3), 214.7 (C-13) ppm. HRMS (70 eV): *m*/*z* = 604.2836 (calcd. for $C_{39}H_{40}O_6$, 604.2825).

20-Tritylphorboisobutyl Ketone (6b): M.p. 98−99 °C. IR (KBr): $\tilde{\nu}$ = 3389, 3047, 1697, 1628, 1491, 1448, 1248, 1047, 704 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 0.92 (d, *J* = 6.5 Hz, 18-H/3), 0.98 (s, 16-H/3), 1.30 (s, 17-H/3), 1.79 (br. s, 19-H/3), 1.94 (br. s, 14-H), 2.23 (d., *J* = 19 Hz, 5a-H), 2.57 (q, *J* = 6.8 Hz, 11-H), 2.60 (d, *J* = 19.0 Hz, 5b-H), 3.42 (br. s, 10-H), 3.44 (br. s, 20-H/2), 3.40 (m, 8-H), 4.85 (br. d, *J* = 6.1 Hz, 7-H), ca. 7.30 (m, Trit-H/15), 7.41 (br. s, 1-H) ppm. ^{13}C NMR ($CDCl_3$/[D_6]DMSO): δ = 10.2 (C-19), 11.7 (C-18), 18.3 (C-16), 22.1 (C-17), 39.0 (C-5), 40.2 (C-15), 44.9 (C-11), 45.8 (C-8), 50.6 (C-10), 53.2 (C-14), 69.3 (C-20), 73.2 (C-4), 81.5 (C-12), 84.4 (C-9), 87.0 (Trit), 108.3 (C-13), 121.2 (C-7), 127.0 (Trit), 127.8 (Trit), 128.6 (Trit), 134.8 (C-6), 135.9 (C-2), 159.1 (C-1), 208.5 (C-3) ppm. HRMS (70 eV): *m*/*z* = 604.2843 (calcd. for $C_{39}H_{40}O_6$, 604.2825).

20-Homovanillyl-12-(phenylacetyl)phorbobutanone (5d): Phenylacetic acid (excess, 873 mg, 6.4 mmol, 7 mol equiv.), EDC (1.23 g, 6.4 mmol, 7 mol equiv.) and DMAP (280 mg, 2.3 mmol, 2.5 mol equiv.) were added to a solution of **5b** (554 mg, 0.92 mmol) in dry CH_2Cl_2 (30 mL). After stirring at room temperature for 72 h, the reaction mixture was worked up by dilution with CH_2Cl_2 (20 mL), washing with Na_2CO_3 (5%, 3 × 10 mL) to remove excess phenylacetic acid, and drying (Na_2SO_4). Removal of the solvent left a yellowish residue of crude **5c** (570 mg), which was directly dissolved in methanolic $HClO_4$ (0.34%, 15 mL). After stirring at room temperature for 15 min, the reaction mixture was worked up by dilution with brine and extraction with EtOAc. The organic phase was washed with water and dried (Na_2SO_4), and the solvents were evaporated, to give a foam (270 mg). This was dissolved in dry CH_2Cl_2 and treated with MEM-homovanillic acid (244 mg, 0.90 mmol), EDC (174 mg, 0.90 mmol) and DMAP (55 mg, cat.). After stirring at room temperature for 45 min, the reaction mixture was worked up by washing with brine, drying (Na_2SO_4) and evaporation. The residue was dissolved in THF (10 mL) and treated with $SnCl_4$ (100 μL). After 4 h, the reaction mixture was worked up by dilution with CH_2Cl_2 and washing with brine. The organic phase was evaporated, and the residue was purified by column chromatography (10 g silica gel, petroleum ether/EtOAc, 6:4) to afford **5d** (196 mg, 33% from **5b**) as a foam. IR (KBr): $\tilde{\nu}$ = 3400, 1761, 1720, 1710, 1685, 1439, 1356, 1260, 809, 706 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 0.98 (s, 16-H/3), 0.88 (d, *J* = 6.5 Hz, 18-H/3), 1.24 (s, 17-H/3), 1.78 (br. s, 19-H/3), 2.27 (d, *J* = 19.0 Hz, 5a-H), 2.43 (d., *J* = 19 Hz, 5b-H), 2.65 (q, *J* = 6.8 Hz, 11-H), 2.88 (br. s, 14-H), 2.88 (br. s, 10-H), 3.51 (br. s, PhAc-H/2), 3.71 (br. s, HMV-H/2), 3.77 (m, 8-H), 3.86 (s, OMe), 4.45 (br. s, 20-H/2), 5.63 (br. d, *J* = 6.1 Hz, 7-H), 6.73 (br. d, *J* = 8.0 Hz, HMV-H) 6.80 (br. s, HMV-H), 6.82 (br. d, *J* = 8.0 Hz, HMV-H), ca. 7.33 (m, PhAc-H/5), 7.41 (br. s, 1-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 8.9 (C-18), 10.3 (C-19), 19.4 (C-16), 25.4 (C-17), 38.2 (C-5), 41.1 (HMV), 41.6 (PhAc), 44.3 (C-8), 46.2 (C-11), 51.0 (C-14), 53.7 (C-10), 55.9 (OMe), 58.3 (C-15), 69.0 (C-20), 74.3 (C-4), 87.0 (C-9), 100.9 (C-12), 111.7 (HMV), 114.3 (HMV), 122.1 (HMV), 125.7 (HOMV), 127.6 (PhAc), 127.8 (C-7), 128.8 (PhAc), 129.3 (PhAc), 132.7 (C-6), 134.1 (PhAc), 136.7 (C-2), 144.8 (HMV), 146.5 (HMV), 154.9 (C-1), 171.3 (HMV), 171.8 (PhAc), 207.0 (C-3), 209.4 (C-13) ppm. HRMS (70 eV): *m*/*z* = 644.2640 (calcd. for $C_{37}H_{40}O_{10}$, 644.2622).

20-Homovanillyl-12-phenylacetylphorboisobutyl Ketone (6d): Starting from **6b** (820 mg), **6d** was obtained as described for the synthesis of **5d** from **5b**, in an overall 39% yield. The compound was obtained as a white powder. M.p. 65−68 °C. IR (KBr): $\tilde{\nu}$ = 3452, 1736, 1717, 1516, 1271, 1236, 1132, 1046 cm^{-1}. ^1H NMR ($CDCl_3$):

δ = 0.87 (d, *J* = 6.5 Hz, 18-H/3), 1.04 (s, 17-H/3), 1.18 (s, 16-H/3), 1.78 (br. s, 19-H/3), 1.94 (br. s, 14-H), 2.17 (d., *J* = 19 Hz, 5a-H), 2.47 (d, *J* = 19.0 Hz, 5b-H), 2.80 (q, *J* = 6.8 Hz, 11-H), 3.35 (m, 8-H), 3.40 (br. s, 10-H), 3.49 (br. s, HMV-H/2), 3.65 (br. s, PhAc-H/2), 3.87 (OMe), 4.41 (br. s, 20-H/2), 5.37 (br. d, *J* = 6.0 Hz, 7-H), 6.70 (br. d, *J* = 8.0 Hz, HMV-H), 6.77 (s, HMV-H), 6.80 (br. d, *J* = 8.0 Hz, HMV-H), ca. 7.3 (m, PhAc-H/5), 7.41 (br. s, 1-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 10.2 (C-19), 12.1 (C-18), 22.2 (C-16 and C-17), 38.6 (C-5), 41.0 (C-15), 45.7 (C-8), 41.0 (PhAc), 41.6 (HMV), 45.7 (C-11), 50.5 (C-10), 56.0 (OMe), 69.6 (C-20), 73.0 (C-4), 82.9 (C-12), 84.6 (C-9), 108 (C-13), 111.9 (HMV), 114.8 (HMV), 122.6 (HMV), 125.9 (HMV), 127.8 (PhAc), 127.9 (C-7), 128.6 (PhAc), 129.5 (PhAc), 131.7 (C-7), 133.3 (C-6), 135.0 (C-2), 158.4 (C-1), 207.6 (C-3) ppm. HRMS (70 eV): *m*/*z* = 644.2639 (calcd. for $C_{37}H_{40}O_{10}$, 644.2622).

Fragmentation of 13-Phenylacetyl-20-tritylphorbol (3d): A solution of 13-phenylacetyl-20-tritylphorbol (**3d**, 180 mg, 0.25 mmol)[9] and thiocarbonyldiimidazole (88 mg, 0.50 mmol, 2 mol equiv.) in dry THF (2.0. mL), was heated at reflux for 8 h, and then worked up by dilution with EtOAc and washing with brine. After drying (Na_2SO_4), the organic phase was evaporated, and the residue was purified by column chromatography on silica gel (10 g, petroleum ether/EtOAc, 9:1 as eluent) to afford **8a** (174 mg, 97%) as a white powder. M.p. 72−74 °C. IR (KBr): $\tilde{\nu}$ = 3453, 1748, 1491, 1449, 1221, 1136, 700 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 1.03 (d, *J* = 6.5 Hz, 18-H/3), 1.58 (br. s, 17-H/3), 1.80 (br. s, 19-H/3), 2.13 (d, *J* = 19.0 Hz, 5a-H), 2.43 (d, *J* = 19.0 Hz, 5b-H), 3.17 (br. s, 10-H), 3.23 (m, 11-H), 3.40 (m, 14-H), 3.40 (br. s, 20-H/2), 3.41 (m, 8-H), 3.64 (s, PhAc-H/2), 4.85 (br. s, 16-H/2), 5.04 (br. s, 12-H), 5.63 (br. s, 7-H), ca. 7.30 (m, PhAc-H/5), ca. 7.30 (m, Trit-H/15), 7.53 (br. s, 1-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 10.1 (C-19), 16.7 (C-18), 17.8 (C-17), 37.1 (C-11), 39.4 (C-5), 42.8 (C-8), 41.0 (PhAc), 48.7 (C-14), 55.0 (C-10), 68.8 (C-20), 73.5 (C-4), 77.0 (C-9), 116.8 (C-12), 121.3 (C-16), 126.9 (Trit), 127.2 (Trit), 127.2 (PhAc), 127.7 (Trit) 128.5 (PhAc), 124.9 (C-7), 129.6 (PhAc), 134.0 (C-6), 134.6 (PhAc), 136.8 (C-2), 142.0 (C-15), 147.4 (C-13), 159.6 (C-1), 170.3 (PhAc), 208.9 (C-3) ppm. HRMS (70 eV): *m*/*z* = 706.3301. (calcd. for $C_{47}H_{46}O_6$, 706.3294).

Trityl to Homovanillate Exchange in 8a: A sample of **8a** (178 mg, 0.25 mmol) was dissolved in methanolic $HClO_4$ (0.34%). After stirring for 2 h at room temperature, the reaction mixture was diluted with satd. $NaHCO_3$ and extracted with EtOAc. After drying (Na_2SO_4) and removal of the solvent, the residue was dissolved in dry CH_2Cl_2 (4 mL) and treated with *O*-MEM homovanillic acid[9] (68 mg, 0.25 mmol, 1 mol equiv.), EDC (48 mg, 0.25 mmol, 1 mol equiv.) and DMAP (5 mg, cat.). After stirring for 1 h at room temp., the reaction mixture was worked up by dilution with CH_2Cl_2 (5 mL), washing with brine, and evaporation. The crude residue was dissolved in dry THF (5 mL) and treated with $SnCl_4$ (15 μL). After stirring overnight under a nitrogen atmosphere, the reaction mixture was diluted with EtOAc and washed with satd. $NaHCO_3$. The organic phase was dried (Na_2SO_4), and the solvents were evaporated. The residue was purified by column chromatography on silica gel (5 g; petroleum ether/EtOAc, 7:3 as eluent) to afford **8b** (83 mg, 52% from **8a**) as a white powder. M.p. 49−51 °C. IR (KBr): $\tilde{\nu}$ = 3453, 1736, 1713, 1516, 1497, 1454, 1273, 1236, 1136 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 1.02 (d, *J* = 6.5 Hz, 18-H/3), 1.48 (br. s, 17-H/3), 1.81 (br. s, 19-H/3), 2.17 (d, *J* = 19.0 Hz, 5a-H), 2.40 (d, *J* = 19.0 Hz, 5b-H), 3.07 (br. s, 10-H), 3.20 (m, 11-H), 3.27 (m, 14-H), 3.37 (m, 8-H), 3.48 (br. s, HMV-H/2), 3.63 (s, PhAc-H/2), 3.86 (s, HMV-H/3), 4.38 (d, *J* = 13.0 Hz, 20a-H), 4.46 (d, *J* = 13.0 Hz, 20b-H), 4.75 (br. s, 16a-H), 4.78 (br. s, 16b-H), 5.02 (br. s, 12-H), 5.64 (br. s, 7-H), 6.68 (br. d, *J* = 7.8 Hz, HMV-H), 6.75 (br. s, HMV-H), 6.80 (br. d, *J* = 7.8 Hz, HMV-H), 7.30 (m, PhAc-H/5), 7.52 (br. s, 1-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 10.1 (C-19), 16.8 (C-18), 17.7 (C-17), 37.1 (C-5), 39.1 (C-8), 41.0 (HMV and PhAc), 42.8 (C-11), 48.7 (C-14), 54.9 (C-10), 56.0 (OMe), 69.9 (C-20), 73.3 (C-4), 77.0 (C-9), 111.7 (HMV), 114.4 (HMV), 117.0 (C-16), 121.4 (C-12), 122.1 (HMV), 125.7 (HMV), 127.2 (PhAc), 128.5 (PhAc), 129.4 (C-7), 129.6 (PhAc), 133.0 (C-6), 134.4 (C-2), 134.6 (PhAc), 141.7 (C-15), 144.8 (HMV), 146.5 (HMV), 147.3 (C-13), 159.5 (C-1), 170.3 (PhAc), 171.4 (HMV), 208.5 (C-3) ppm. HRMS (70 eV): *m*/*z* = 628.2687 (calcd. for $C_{37}H_{40}O_9$, 628.2672).

13,20-Diacetyl-12-phenylacetyl-4α-phorbol (9b): TEA (30 mL, 20.9 g, 206 mmol, 16 mol equiv.) and Ac_2O (20 mL, 21.1 g, 206.4 mmol, 16 mol equiv.) were added to a solution of 4α-phorbol (4.7 g, 12.9 mmol) in dry THF/CH_2Cl_2 (1:1, overall 50 mL). After stirring for 1 h at room temperature, the reaction mixture was worked up by dilution with CH_2Cl_2 and washing with 2 N H_2SO_4 and satd. $NaHCO_3$. After drying (Na_2SO_4) and evaporation, the residue was purified by column chromatography on silica gel (50 g, petroleum ether/EtOAc, 4:6 as eluent) to afford 13,20-diacetyl-4α-phorbol (2.3 g, 49%).[17] A portion of this (700 mg, 1.56 mmol) was dissolved in dry CH_2Cl_2/THF (1:1, overall 80 mL) and treated with phenylacetic acid (638 mg, 4.69 mmol, 3 mol equiv.), EDC (894 mg, 4.69 mmol, 3 mol equiv.) and 286 mg DMAP (2.34 mmol, 1.5 mol equiv.). After stirring for 30 min at room temperature, the reaction mixture was worked up by dilution with CH_2Cl_2 and washing with brine. After drying (Na_2SO_4) and evaporation, the residue was purified by column chromatography on silica gel (15 g, eluent petroleum ether/EtOAc, 6:4) to afford **9b** (796 mg, 90%) as a white powder. M.p. 161 °C. IR (KBr): $\tilde{\nu}$ = 3414, 1736, 1717, 1637, 1455, 1381, 1246, 1120, 1040, 984 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 0.81 (d, *J* = 5.4 Hz, 14-H), 0.93 (s, 16-H/3), 0.97 (s, 17-H/3), 1.02 (d, *J* = 6.5 Hz, 18-H/3), 1.66 (m, 8-H), 1.83 (br. s, 19-H/3), 2.07 (s, Ac), 2.09 (s, Ac), 2.28 (d, *J* = 13.9 Hz, 5a-H), 2.88 (m, 11-H), 3.25 (s, 10-H), 3.69 (d, *J* = 13.9 Hz, 5b-H), 3.72 (br. s, PhAc-H/2), 4.26 (d, *J* = 11.8 Hz, 20a-H), 4.37 (d, *J* = 11.8 Hz, 20b-H), 5.25 (br. s, 7-H), 5.44 (d, *J* = 10.3 Hz, 12-H), 6.97 (s, 1-H), ca. 7.31 (m, PhAc-H/5) ppm. HRMS (70 eV): *m*/*z* = 566.2504 (calcd. for $C_{32}H_{38}O_9$, 566.2516).

13-Acetyl-20-homovanillyl-12-phenylacetyl-4α-phorbol (4α-PPAHV, 9d): A sample of **9b** (687 mg, 1.21 mmol) was dissolved in methanolic $HClO_4$ (0.35%, 15 mL), and the solution was stirred at room temperature for 24 h. After neutralisation with solid sodium acetate and concentration, brine and EtOAc were added. The organic phase was dried (Na_2SO_4), the solvents were evaporated, and the residue was washed with diethyl ether to afford **9c** (509 mg, 80% yield). This was dissolved in dry THF (25 mL) and, after the mixture had been cooled to 0 °C, triphenylphosphane (498 mg, 1.9 mmol, 2 mol equiv.), di-*tert*-butyl azodicarboxylate (446 mg, 1.94 mmol, 2 mol equiv.) and homovanillic acid (353 mg, 1.94 mmol, 2 mol equiv.) were added. After stirring at room temperature for 1 h, the reaction mixture was worked up by evaporation and the residue was taken up in toluene (10 mL). After the mixture had been kept at 4 °C overnight and filtered, the filtrate was evaporated, and the residue was purified by column chromatography on silica gel (15 g, petroleum ether-EtOAc, 6:4 as eluent) to afford a white powder (603 mg, 91%). M.p. 78 °C. IR (KBr): $\tilde{\nu}$ = 3414, 1719, 1605, 1516, 1433, 1375, 1271, 1250, 1148, 984 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 0.78 (d, *J* = 5.4 Hz, 14-H), 0.99 (s, 16-H/3), 1.01 (s, 17-H/3), 1.05 (d, *J* = 6.4 Hz, 18-H/3), 1.54 (m, 8-H), 1.76 (br. s, 19-H/3), 2.06 (Ac), 2.30 (d, *J* = 14.0 Hz, 5a-H), 3.26 (br. s, 10-H), 3.60 (br. s, HMV-H/2), 3.61 (d, *J* = 14.0 Hz, 5b-H),

3.69 (br. s, PhAc-H/2), 3.89 (s, OMe), 4.33 (br. s, 20-H/2), 5.22 (s, 7-H), 5.46 (d, J = 10.0 Hz, 12-H), 6.83 (m, HMV-H), 6.85 (d. J = 8.0 Hz, HMV-H), 6.87 (d, J = 8.0 Hz, HMV-H), 6.98 (s, 1-H), ca. 7.32 (m, PhA-H/5) ppm. ^{13}C NMR ($CDCl_3$/[D_6]DMSO): δ = 10.6 (C-19), 11.9 (C-18), 16.9 (C-17), 24.0 (C-16), 24.9 (C-15), 35.0 (C-5), 36.9 (C-14), 41.0 (HMV), 41.7 (PhAc), 41.9 (C-8), 43.4 (C-11), 55.9 (C-10), 56.7 (OMe), 65.2 (C-13), 70.7 (C-20), 75.9 (C-12), 77.0 (C-4), 77.3 (C-9), 111.2 (HMV), 114.8 (HMV), 122.0 (HMV), 124.9 (C-7), 125.2 (HMV), 127.6 (PhAc), 128.5 (PhAc), 129.6 (PhAc), 132.2 (C-6), 134.0 (PhAc), 140.9 (C-2), 144.3 (HMV), 146.2 (HMV, 155.6 (C-1), 170.5 (PhAc), 171.8 (HMV),208.0 (C-3) ppm. HRMS (70 eV): m/z = 688.2866 (calcd. for $C_{39}H_{44}O_{11}$, 688.2884).

13-Acetyl-20-homovanillyl-12-(phenylacetyl)lumiphorbol (LumiPPAHV, 10a): A degassed ethanolic solution of 4α-PPAHV (**9d**; 100 mg, 0.14 mmol in 50 mL) was irradiated at 254 nm in a quartz tube in a Rayonet apparatus. After two hours, the reaction mixture was worked up by evaporation to afford **10a** in quantitative (^{1}H NMR analysis) yield. Compound **10a** was obtained as a white powder, m.p. 62 °C. IR (KBr): ṽ = 3386, 1773, 1736, 1605, 1516, 1456, 1375, 1271, 1244, 1150, 1032, 982 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 0.98, 1.02, 1.04 (s, 16-H/3, 17-H/3, 19-H/3), 1.06 (d, J = 6.4 Hz, 18-H/3), 1.24 (d, J = 6.2 Hz, 14-H), 1.43 (m, 11-H), 1.59 (m, 8-H), 1.92 (d, J = 11.8 Hz, 5a-H), 2.09 (s, OAc), 2.34 (dd, J = 7.1, 5.2 Hz, 1-H), 2.64 (d, J = 7.1 Hz, 10-H), 2.75 (t, J = 5.2 Hz, 7-H), 3.17 (d, J = 11.8 Hz, 5b-H), 3.56 (br. s, HMV-H/2), 3.65 (br. s, PhAc-H/2), 3.89 (s, OMe), 4.00 (br. s, 20-H/2), 5.47 (d, J = 7.8 Hz, 12-H), 6.76 (m HMV-H/3), ca. 7.30 (m, PhAc-H/5) ppm. HRMS (70 eV): m/z = 688.2886 (calcd. for $C_{39}H_{44}O_{11}$, 688.2884).

Phenylacetylation of Lumiphorbol 13,20-Diacetate (10b): EDC (23.2 mg, 0.12 mmol, 1 mol equiv.), phenylacetic acid (16.5 mg, 0.12 mmol, 1 mol equiv.) and DMAP (5 mg, catalytic) were added to a solution of **10b** (54 mg, 0.12 mmol, prepared from 13,20-diacetyl-4α-phorbol [8] as described for **10a** from **9d**) in dry CH_2Cl_2 (2 mL). After stirring for 5 min at room temperature, the reaction mixture was worked up by dilution with CH_2Cl_2 (5 mL) and washed with brine. After drying (Na_2SO_4) and evaporation, the residue was purified by column chromatography on silica gel (2.5 g, petroleum ether/EtOAc, 8:2 as eluent) to give **10c** (39.2 mg, 47%) and recovered **10b** (15 mg). Compound **10c** was obtained as a white foam. IR (KBr): ṽ = 3397, 1782, 1740, 1456, 1377, 1240, 1156, 1032, 982 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 0.73 (d, J = 6.4 Hz, 18-H/3), 1.05, 1.14, 1.19 (s, 16-H/3, 17-H/3, 19-H/3), 1.35 (m, 11-H, 14-H), 1.60 (m, 8-H), 2.04, 2.09 (s, OAc), 2.28 (d, J = 11.8 Hz, 5a-H), 2.42 (t, J = 6.7 Hz, 1-H), 2.85 (t, J = 5.2 Hz, 7-H), 3.44 (d, J = 7.2 Hz, 10-H), 3.55 (d, J = 11.8 Hz, 5b-H), 3.65 (br. s,PhAc-H/2), 3.71 (br. s, PhAc-H/2), 3.87 (br. d, J = 11.6 Hz, 20a-H), 4.06 (br. d, J = 11.6 Hz, 20b-H), 5.45 (d, J = 7.5 Hz, 12-H), ca. 7.30 (m, 2 × PhAc-H/5). HRMS (70 eV): m/z = 684.2926 (calcd. for $C_{40}H_{44}O_{10}$, 684.2935).

Treatment of 13-Acetyl-12-(phenylacetyl)lumiphorbol (10d) with MEM-homovanillic Acid: MEM-homovanillic acid (25 mg, 0.097 mmol, 1 mol equiv.) and EDC (11.9 mg, 0.098 mmol, 1 mol equiv.) were added to a solution of **10d** (59 mg, 0.097 mmol) in dry CH_2Cl_2 (2 mL). After stirring at room temperature for 24 h, the reaction mixture was worked up by washing with brine. After drying (Na_2SO_4) and evaporation, the residue was dissolved in dry THF (2 mL) and treated with $SnCl_4$ (10 μL). After stirring for 4 h at room temp., the reaction mixture was worked up by dilution with diethyl ether and washing with satd. $NaHCO_3$. After washing with brine, the organic phase was evaporated, and the residue was purified by column chromatography (3 g silica gel, petroleum ether/EtOAc, 8:2 as eluent) to afford the bis(homovanillate) **10e** (34 mg, 40%) and recovered starting **10d** (16 mg). Compound **10e** was obtained as a colourless foam. IR (KBr): ṽ = 3397, 1800, 1731, 1725, 1609, 1524, 1412, 1275, 1251, 1264, 1100, 1096, 982 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 0.98, 1.02, 1.09 (s, 16-H/3, 17-H/3, 19-H/3), 1.10 (d, J = 6.4 Hz, 18-H/3), 1.26 (d, J = 6.2 Hz, 14-H), 1.42 (m, 11-H), 1.59 (m, 8-H), 1.99 (d, J = 11.8 Hz, 5a-H), 2.04 (s, OAc), 2.39 (t, J = 6.7 Hz, 1-H), 2.61 (d, J = 7.1 Hz, 10-H), 2.79 (t, J = 5.2 Hz, 7-H), 3.27 (d, J = 11.8 Hz, 5b-H), 3.51, 3.61 (br. s, 2 × HMV-H/2), 3.69 (br. s, PhAc-H/2), 3.89, 3.92 (s, 2 × OMe), 4.07 (br. s, 20-H/2), 5.41 (d, J = 7.8 Hz, 12-H), ca 6.70 (m, 2 × HMV-H/3), ca. 7.30 (m, PhAc-H/5) ppm. HRMS (70 eV): m/z = 852.3369 (calcd. for $C_{48}H_{52}O_{14}$, 862.3357).

Synthesis of the 2,3-*seco*-Lumiphorboid 11

A. By Irradiation of 4α-PPAHV under Oxygen: An ethanolic solution of 4α-PPAHV (**9d**; 74 mg, 0.11 mmol in 50 mL) in a quartz tube was flushed with oxygen for 5 min and then irradiated at 254 nm in a Rayonet apparatus. After two hours, the reaction mixture was worked up by flushing with nitrogen and evaporation, and the residue was purified by column chromatography to provide **11** (39 mg, 51%) as a foam. IR (KBr): ṽ = 3400, 1740, 1605, 1516, 1456, 1371, 1273, 1151, 1066, 1032, 982 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 0.95, 1.03, 1.14 (s, 16-H/3, 17-H/3, 19-H/3), 1.16 (d, J = 6.4 Hz, 18-H/3), 1.24 (m, 11-H and 14-H), 1.55 (m, 8-H), 1.76 (d, J = 12.8 Hz, 5a-H), 2.11 (s, Ac), 2.31 (t, J = 6.7 Hz, 1-H), 2.61 (m, 7-H and 10-H), 3.01 (d, J = 12.8 Hz, 5b-H), 3.49 (br. s, HMV-H/2), 3.65 (br. s, PhAc-H/2), 3.92 (s, OMe), 3.97 (br. s, 20-H/2), 5.41 (d, J = 7.8 Hz, 12-H), ca. 6.77 (m, HMV-H/3), ca. 7.30 (m, PhAc-H/5) ppm. HRMS (70 eV): m/z = 704.2822 (calcd. for $C_{39}H_{44}O_{12}$, 704.2833).

B. By Treatment of LumiPPAHV (10a) with Peracids: MCPBA (80%, 18 mg, 0.108 mmol, 1 mol equiv.) and NaOAc (20 mg) were added to a solution of lumiPPAHV (**10a**; 75 mg, 0.107 mmol) in dry CH_2Cl_2 (10 mL). After stirring for 90 min at room temperature, the reaction mixture was worked up by dilution with CH_2Cl_2, filtration and washing with NaOH (2 N). The organic phase was dried (Na_2SO_4), the solvents were evaporated, and the residue was purified by column chromatography on silica gel (petroleum ether/EtOAc, 5:5) to afford **11** (39 mg, 48%), identical (^{1}H NMR) to the product obtained from the irradiation of **10a** under oxygen.

3βH-Dihydro-13-acetyl-20-homovanillyl-12-(phenylacetyl)phorbol (12b): Tetramethylammonium triacetoxyborohydride (152 mg, 0.56 mmol, 4 mol equiv.) was added to a stirred solution of PPAHV (100 mg, 0.142 mmol) in acetonitrile/HOAc (1:1, overall 2 mL). After stirring at room temperature for 4 h, the reaction mixture was worked up by dilution with EtOAc and washing with satd. $NaHCO_3$. After drying, the organic phase was evaporated and purified by column chromatography (5 g silica gel, petroleum ether/EtOAc, 6:4 as eluent) to afford **12b** (48 mg, 48%) as a white powder. M.p. 75−77 °C. IR (KBr): ṽ = 3453, 1734, 1516, 1252, 1375, 1150, 1456, 1021 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 0.83 (d, J = 6.5 Hz, 18-H/3), 0.86 (d, J = 5.2 Hz, 14-H), 1.04 (s, 16-H/3), 1.06 (s, 17-H/3), 1.74 (br. s, 19-H/3), 1.96 (m, 11-H), 2.06 (s, Ac), 2.26 (d, J = 19.0 Hz, 5a-H), 2.48 (s, 8-H), 2.83 (d, J = 19.0 Hz, 5b-H), 2.96 (s, 10-H), 3.53 (s, HMV H/2), 3.62 (s, PhAc H/2), 3.87 (s, OMe), 4.47 (s, 20-H/2), 5.20 (br. s, OH), 5.38 (d, J = 10.2 Hz, 12-H), 5.53 (s, OMe), 5.53 (br. s, 3-H), 5.82 (br. s, 1-H), 6.73 (d, J = 8.0 Hz, HMV-H), 6.77 (s, HMV-H), 6.78 (d, J = 8.0 Hz, HMV-H, 7.24 (m, PhAc H/5) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.5 (C-19), 14.6 (C-18), 16.6 (C-16), 21.1 (OAc), 23.7 (C-17), 25.2 (C-15), 36.0 (C-14), 40.4 (C-8), 41.2 (HMV), 42.5 (PhAc), 43.5 (C-11), 43.4 (C-5), 58.0 (C-10), 65.4 (C-13), 67.7 (C-20), 75.4 (C-4), 76.6 (C-12), 81.3 (C-9), 86.5

(C-3), 111.7 (HMV), 114.0 (HMV), 122.1 (HMV), 125.7 (HMV), 126.1 (PhAc), 126.2 (PhAc), 131.0 (C-1), 132.2 (C-7), 132.7 (PhAc), 135.2 (PhAc), 134.7 (C-6), 137.7 (C-2), 144.8 (HMV), 146.5 (HMV), 171.5 (HMV), 172.3 (PhAc), 173.6 (OAc) ppm. HRMS (70 eV): m/z = 6903045 (calcd. for $C_{39}H_{46}O_{11}$, 690.3040).

Evaluation of Vanilloid Activity: The activities of compounds at TRPV1 receptors were evaluated by assessing their effects on cytosolic calcium concentrations ($[Ca^{2+}]_i$) in human embryonic kidney (HEK) 293 cells over-expressing either the human or rat TRPV1, a kind gift from John Davis (SmithKline Beecham, UK). Cells were grown as monolayers in minimum essential medium supplemented with non-essential amino acids, 10% fetal calf serum and 0.2 mM glutamine, and maintained under 95%/5% O_2/CO_2 at 37 °C. The effect of the substances on $[Ca^{2+}]_i$ was determined by use of Fluo-3, a selective intracellular fluorescent probe for Ca^{2+}. One day prior to experiments, cells were transferred into six-well dishes coated with poly-L-lysine (Sigma) and grown in the culture medium described above. On the day of the experiment, the cells (50−60,000 per well) were loaded for 2 h at 25 °C with 4 μM Fluo-3 methyl ester (Molecular Probes) in DMSO containing 0.04% Pluoronic. After the loading, cells were washed with Tyrode's pH = 7.4, trypsinized, resuspended in Tyrode's and transferred to the cuvette of the fluorescence detector (Perkin−Elmer LS50B) with continuous stirring. Experiments were carried out by measurement of cell fluorescence at 25 °C (λ_{EX} = 488 nm, λ_{EM} = 540 nm) before and after the addition of the test compounds at various concentrations. Potency is expressed as the concentration exerting a half-maximal effect (EC_{50}). The efficacy of the effect was determined by comparing it to the analogous effect observed with 4 μM ionomycin.

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