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Design and synthesis of prodrugs of the rat selective toxicant norbormide

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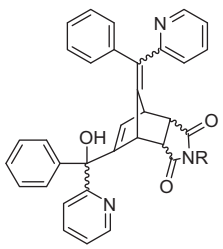
ABSTRACT

Norbormide [5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide] (NRB), an existing but infrequently used rodenticide, is known to be uniquely toxic to rats but relatively harmless to other rodents and mammals. However, one major drawback of NRB as a viable rodenticide relates to an evolutionary aversion developed by the rat leading to sub-lethal dosing due to either its unpleasant 'taste' or rapid onset of effects. A series of NRB prodrugs were prepared in an effort to 'mask' this acute response. Their synthesis and biological evaluation (in vitro vasoconstrictory activity, in vitro hydrolytic and enzymatic stability and lethality/palatability in vivo) is described. Compound **19** displayed the most promising profile with respect to a delay in the onset of symptoms and was subsequently demonstrated to be significantly more palatable to rats.

1. Introduction

Rats cause substantial damage each year to agricultural interests worldwide. The World Health Organization estimates that 20% of all human food is destroyed or contaminated by rodents each year,[1] while one US government report claims that each rat damages up to $10 worth of food and stored grains annually, and contaminates 5 to 10 times that amount.[2] More recently, the Food and Agriculture Organization of the United Nations reported that, worldwide, rats ruined more than 42 million tons of food, worth an estimated US$30 billion.[3] In addition to this vast economic loss, rats are responsible for a number of health problems, acting as vectors for both viral and bacterial diseases, transmitting more than 35 types of disease to humans such as leptospirosis, cholera, salmonella and the bubonic plague.[2] Furthermore, rats are known to be one of the most invasive species responsible for loss of biodiversity and native habitats, second only to humans, threatening both plant and animal survival through predation and habitat destruction.[4] Currently, there are a number of toxicants on the market that are effective in controlling rats, almost all being non-specific broad-spectrum rodenticides. To date, rodent control has been achieved through the use of sub-chronic poisons (e.g., cholecalciferol, bromethalin), acute poisons (e.g., zinc phosphide), first-generation anticoagulants (e.g., warfarin, coumatetralyl, diphacinone, chlorophacinone) and second-generation anticoagulants (e.g., brodifacoum, bromadiolone, bromethalin, difethialone, difenacoum) with varying degrees of success.[5] Annually, more than US$500 million is spent on rodent control products, with second-generation anticoagulants being the most preferred products. Most of these, however, have one common disadvantage in that they have secondary non-target risks associated with them and are dangerous not only to children, but also to domestic pets, wildlife, and livestock. Rodenticides rank second in the number of pesticide related poisonings recorded each year, with a recent study revealing just under 15,000 people were exposed to such toxicants in the US in 2008 alone, 86% being children under the age of six.[6] Additionally, these poisons also pose increasing risks through environmental contamination, accumulation of residues in the food chain and a general lack of humaneness.

Norbormide (NRB), a vasoactive also known under the trade names Shoxin® and Raticate,® was first introduced to the market as a rodenticide over forty years ago (Fig. 1). Discovered in the



NRB R = H; **1** R = Me

Figure 1. Norbormide (NRB) and *N*-methyl norbormide **1**.

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1960s NRB was found to be uniquely toxic to rats but relatively harmless to other rodents and mammals.[7,8] NRB displays unique species-specific constrictor activity that is restricted to the peripheral arteries of the rat. In arteries from all other species tested, as well as in rat aorta and extravascular smooth muscle tissue, NRB exhibits vasorelaxant properties at concentrations that induce vasoconstriction in the rat peripheral arteries.[9] Furthermore, detailed studies conducted upon the individual stereoisomers of NRB, isolated from the *endo* rich stereoisomeric mixture, found the parent compound's physiological effects to be strongly stereospecific. In rat peripheral arteries only the *endo* isomers of NRB retained the contractile activity elicited by the stereoisomeric mixture, with both the *endo* and *exo* isomers exhibiting vasodilatory activity in rat aorta.[10] In vivo evaluation established that only the *endo* isomers of NRB were toxic in rats.[11] The mechanisms involved in these divergent effects of NRB have yet to be clarified. Available evidence suggests that the vasoconstrictor effect may be mediated by the stimulation of a number of signal transduction pathways[12] that lead to modulation of calcium influx, presumably mediated by phospholipase C (PLC)-coupled receptors expressed in rat peripheral artery myocytes,[12] whereas the relaxant effect may be the result of a reduction of Ca^{2+} entry through L-type Ca^{2+} channels.[13]

To date, efforts to establish NRB as a viable rodenticide have been largely unsuccessful. Over time, rats as a species have developed an evolutionary trait relating to how they sample food, particularly novel food, such that they do not ingest a potentially toxic dose at the first encounter. This survival strategy is most likely linked to their lack of an emetic centre, and thus their incapacity, as a species, to vomit. As an acute poison, NRB has a rapid onset of action with toxic symptoms being recorded almost immediately, and evidence suggests rats develop a learnt aversion to this poison following the consumption of sub-lethal doses during sampling, a phenomenon referred to as bait-shyness.[14,15] NRB is also known to be relatively unpalatable to rats.[16–18]

While a pre-requisite for lethality in rats, it is this aforementioned intrinsic vasoconstrictory activity which is believed to be the most significant shortcoming of NRB as a viable rodenticide. Sub-lethal dosing due to the toxicants unpleasant 'taste' is believed to be a consequence of NRB-induced vasoconstriction of the blood vessels of the buccal cavity, a primary affect leading to bait-shyness. Although efforts to address this palatability problem using microencapsulation technologies have had varying degrees of success, the rapid release of the toxicant in vivo has not been sufficiently delayed and bait shyness has remained a major problem.[19,20] In an endeavour to circumnavigate these detrimental qualities we herein report the synthesis of a select series of NRB prodrugs, designed to both mask and delay such unfavourable 'side-effects' prior to systemic breakdown, whereby the active toxicant would then subsequently be released leading to death.

2. Results and discussion

2.1. Synthesis and in vitro evaluation for vasoconstrictory activity pre-cleavage

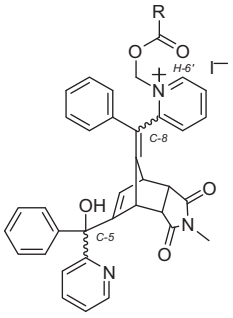
The synthesis of NRB has been reported previously.[7,10,21] Earlier studies have demonstrated that it is exclusively the *endo*-isomers of NRB which are responsible for both the unique vasoconstrictory activity and species-selective lethality observed in rats.[11] Purification by recrystallisation allowed the exclusive isolation of mixed *endo*-NRB, with all NRB prodrugs herein prepared as *endo*-specific stereoisomeric mixtures.[22]

The first series of prodrugs to be explored were based on *N*-(α-acyloxyalkyl)esters in the form of pyridinium salts of type **2**–**4**, using established methods similar to those reported by Davidsen et al. (Fig. 2, Table 1).[23] With the knowledge that *N*-methyl norbor-

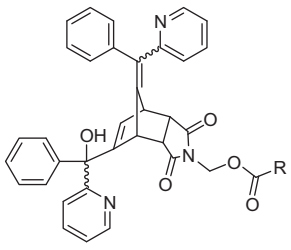
Table 1
In vitro evaluation for vasoconstrictory activity of **2**–**4**

Compd	R	Yield (%)	Vasoconstriction[a]
2	*t*-Bu	76	⩾132[b]
3	Ph	37	⩾132[b]
4	$CHPh_2$	51	⩾132[b]

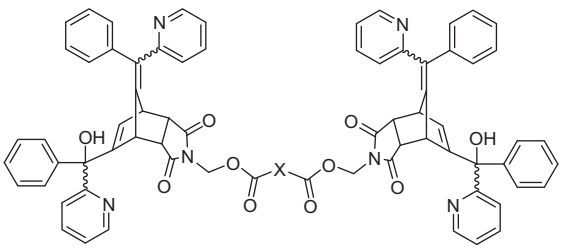
[a] Maximum contractile effect as a % of 90 mM KCl contraction (rat caudal artery); stereoisomeric mixture of NRB = 132%.
[b] lag time ca. 5–10 min.



2 R = *t*-Bu
3 R = Ph
4 R = $CHPh_2$

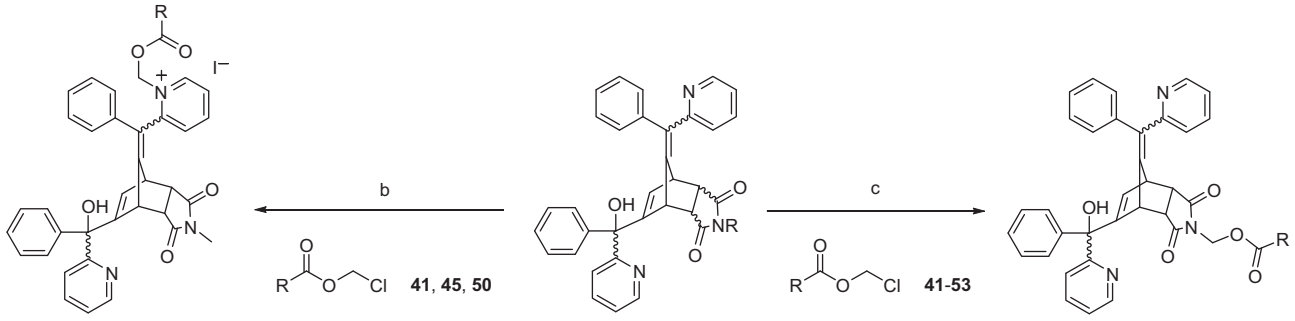


8 R = *t*-Bu
9 R = $(CH)_2CH_3$
10 R = $(CH)_6CH_3$
11 R = $(CH)_{10}CH_3$
12 R = Ph
13 R = *o*-OMePh
14 R = *m*-OMePh
15 R = *p*-OMePh
16 R = CH_2Ph
17 R = $CHPh_2$
18 R = CH_2CH_2Ph
19 R = CH=CHPh
20 R = 2-Naph



21 X = $(CH_2)_2$
22 X = $(CH_2)_4$
23 X = $(CH_2)_6$
24 X = $(CH_2)_8$
25 X = $(CH_2)_{10}$
26 X = *p*-(C_6H_4)
27 X = $(CH_2)_2C(O)O(CH_2)_2OC(O)(CH_2)_2$

Figure 2. *N*-(α-Acyloxymethyl)pyridinium iodide prodrugs **2**–**4**, *N*-(α-acyloxymethyl)dicarboximide prodrugs **8**–**20** and *N*-(α-acyloxymethyl)dicarboximide dimer prodrugs **21**–**27** (Tables 1–5).



2 R = *t*-Bu
3 R = Ph
4 R = $CHPh_2$

NRB R = H
1 R = Me

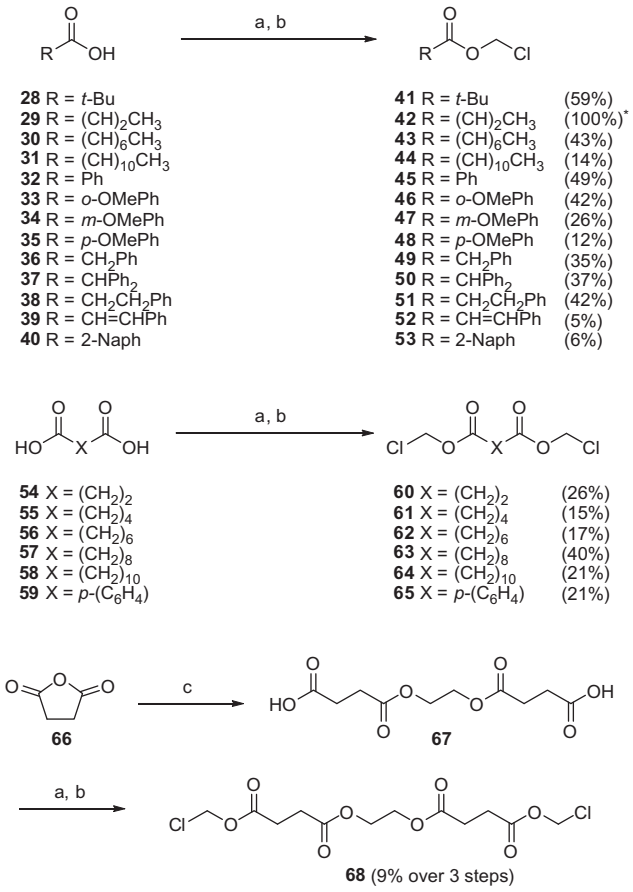
8 R = *t*-Bu
9 R = $(CH)_2CH_3$
10 R = $(CH)_6CH_3$
11 R = $(CH)_{10}CH_3$
12 R = Ph
13 R = *o*-OMePh
14 R = *m*-OMePh
15 R = *p*-OMePh
16 R = CH_2Ph
17 R = $CHPh_2$
18 R = CH_2CH_2Ph
19 R = CH=CHPh
20 R = 2-Naph

Scheme 1. Reagents and conditions: (a) MeI, K_2CO_3, DMF, rt, 16 h, 75% (to give **1**); (b) (i) **41**, **45** or **50**, NaI, acetone, rt, 3 h, (ii) then **1**, MeCN, 80 °C (see Table 1); (c) **41**–**53**, K_2CO_3, DMF, rt, 16 h (see Table 2).

mide **1** shares a similar pharmacological profile to NRB,[11] albeit of marginally lower potency, **1** was selected over NRB for this particular class of prodrug on synthetic grounds, on the basis that the dicarboximide nitrogen would not require protection within this series (Fig. 1). Methylation of the imide nitrogen[24] using iodomethane afforded **1** in 75% yield (Scheme 1). Initial attention focused on the synthesis of *N*-(α-acyloxymethyl)pyridinium iodide prodrugs **2**–**4**, our design rationale throughout being based on the concept that the introduction of such steric bulk into the toxicant by way of such a pro-moiety would impair its intrinsic capacity to elicit vasoconstriction pre-cleavage.

Chloromethyl esters **41**, **45** and **50** were prepared from the corresponding acid chlorides **28**, **32** and **37**, respectively, through treatment with paraformaldehyde in the presence of zinc chloride under heating (Scheme 2).[25] Conversion to the corresponding iodides in situ using sodium iodide,[26] and subsequent alkylation[23] of **1** at elevated temperature afforded *N*-(α-acyloxymethyl)pyridinium iodide salts **2**, **3** and **4** in 76%, 37% and 51% yields, respectively (Scheme 1, Table 1). Although the formation of *N*-pyridinium dialkylation products of **1** were initially anticipated, given that NRB contains two pyridine rings, the ^{1}H NMR spectra of the products formed indicated the formation of only a single monoalkylation product. A significant ^{1}H NMR resonance assigned to the more deshielded H-6′ proton on the C-8 pyridyl ring was observed in all three pyridinium salt prodrugs **2**–**4**, resonating in the range δ 9.00–9.50 ppm compared to δ 8.43–8.64 ppm in **1** (Fig. 2). It is known, from X-ray crystallographic data,[27] that a hydrogen bond exists between the hydroxyl group of the carbinol carbon and the nitrogen of the pyridyl ring at C-5. This is put forward as one potential hypothesis for the exclusive recovery of monoalkylation products only, due to the inability to effect alkylation at the C-5 pyridyl ring as a consequence of this hydrogen bonding.

In vitro evaluation of *N*-(α-acyloxymethyl)pyridinium iodides **2**–**4** for pre-cleavage vasoconstrictory activity revealed all three prodrugs to exhibit NRB-like vasoconstriction in rat peripheral arteries at levels comparable to the parent compound, albeit with a lag time of around 5–10 min (Fig. 2, Table 1). Subsequent hydrolytic stability studies later revealed compounds **2**–**4** to be susceptible to cleavage under bioassay conditions (Table 4), providing



28 R = *t*-Bu
29 R = $(CH)_2CH_3$
30 R = $(CH)_6CH_3$
31 R = $(CH)_{10}CH_3$
32 R = Ph
33 R = *o*-OMePh
34 R = *m*-OMePh
35 R = *p*-OMePh
36 R = CH_2Ph
37 R = $CHPh_2$
38 R = CH_2CH_2Ph
39 R = CH=CHPh
40 R = 2-Naph

41 R = *t*-Bu (59%)
42 R = $(CH)_2CH_3$ (100%)*
43 R = $(CH)_6CH_3$ (43%)
44 R = $(CH)_{10}CH_3$ (14%)
45 R = Ph (49%)
46 R = *o*-OMePh (42%)
47 R = *m*-OMePh (26%)
48 R = *p*-OMePh (12%)
49 R = CH_2Ph (35%)
50 R = $CHPh_2$ (37%)
51 R = CH_2CH_2Ph (42%)
52 R = CH=CHPh (5%)
53 R = 2-Naph (6%)

54 X = $(CH_2)_2$
55 X = $(CH_2)_4$
56 X = $(CH_2)_6$
57 X = $(CH_2)_8$
58 X = $(CH_2)_{10}$
59 X = *p*-(C_6H_4)

60 X = $(CH_2)_2$ (26%)
61 X = $(CH_2)_4$ (15%)
62 X = $(CH_2)_6$ (17%)
63 X = $(CH_2)_8$ (40%)
64 X = $(CH_2)_{10}$ (21%)
65 X = *p*-(C_6H_4) (21%)

66

67

68 (9% over 3 steps)

Scheme 2. Reagents and conditions: (a) $SOCl_2$, reflux, 1 h; (b) paraformaldehyde, $ZnCl_2$, 80 °C, 2 h or *$ClSO_3CH_2Cl$, $NaHCO_3$, TBABr, DCM, H_2O, rt, 2 h; (c) ethylene glycol, pyridine, reflux, 16 h, 95%.

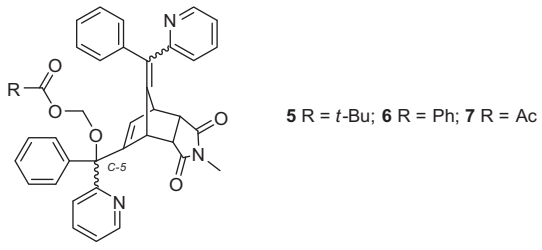
one possible explanation for the observed delayed vasoconstrictory activity in vitro. As a result *N*-(α-acyloxyalkyl)pyridinium salt prodrugs of NRB were abandoned. It should be stated however,

that although LC–MS was strongly supportive of *N*-(α-acyloxymethyl)pyridinium iodide formation, the failure to obtain full NMR characterization, due to solubility problems, means such data should be treated with a degree of caution. Attention now shifted to utilizing the hydroxyl group at C-5 as a potential 'handle' for prodrug formation, through a series of *O*-(α-acyloxymethyl)ethers of **1** (Fig. 3). Disappointingly, all efforts to prepare prodrugs of type **5**–**7** using standard literature procedures failed, with the recovery of starting materials in all instances. Consequently, *O*-(α-acyloxyalkyl)ether prodrugs of NRB were likewise abandoned.

Difficulties encountered in the preparation of both *N*-(α-acyloxymethyl)pyridinium salts and *O*-(α-acyloxymethyl)ether prodrugs of NRB led us to utilizing the dicarboximide nitrogen as a third and final potential alkylation site for the formation of prodrugs of NRB (Fig. 2, Table 2). Treatment of NRB with chloromethyl esters **41**–**53**, prepared analogously to those described previously (Scheme 2), in the presence of potassium carbonate at room temperature, afforded *N*-(α-acyloxymethyl)dicarboximide prodrugs **8**–**20** (Scheme 1, Table 2).

In vitro evaluation of prodrugs **8** and **12** for pre-cleavage vasoconstrictory activity in rat blood vessels revealed both the pivalate and benzoate pro-moieties, respectively, to be of insufficient steric bulk to hinder vasoconstriction at all; in both cases eliciting levels of efficacy similar to that observed for the parent compound. Within the aliphatic *N*-(α-acyloxymethyl)dicarboximide series, *n*-butanoate **9** was found to display partial vasoconstriction, while efforts to further increase the size of the pro-moiety, through alkyl chain homologues *n*-octanoate **10** and *n*-dodecanoate **11**, proved successful, in both examples being devoid of all vasoconstrictory activity pre-cleavage. A strategy to incorporate further steric bulk into benzoate **12**, through the introduction of ring substituents, proceeded via methoxybenzoate analogues **13** (*ortho*), **14** (*meta*) and **15** (*para*). In vitro evaluation revealed *o*-methoxybenzoate **13** to be a partial vasoconstrictor, while structural regioisomers **14** and **15** both failed to elicit any vasoconstrictory response. Concurrently, with full vasoconstrictory activity observed in the unsubstituted aromatic *N*-(α-acyloxymethyl)dicarboximide series, efforts were made to further increase the size of the benzoate derived pro-moiety through the insertion of a 'spacer' between the carbonyl carbon of the ester and the phenyl group, revealing alkylene chain homologues phenylacetate **16** and dihydrocinnamate **18**. Additional steric bulk was introduced into phenylacetate **16** through the incorporation of a second phenyl ring to give diphenylacetate **17**. Conformationally-restricted cinnamate **19**, along with naphthoate **20**, were also prepared to further expand the series. In vitro evaluation revealed diphenylacetate **17**, cinnamate **19** and naphthoate **20** to be devoid of all vasoconstrictory activity pre-cleavage. By contrast, phenylacetate **16** and dihydrocinnamate **18** remained partially active as vasoconstrictors, albeit displaying markedly reduced levels of efficacy, 6% and 13%, respectively. It is hypothesized that the marginal activity observed in phenylacetate **16** is a consequence of insufficient steric bulk, while conformationally flexible dihydrocinnamate **18**, versus conformationally restricted non-vasoconstricting cinnamate **19**, permits the pro-moiety to orientate itself so as to allow some degree of weak binding, thus permitting low level efficacy (Fig. 2, Table 2).

We now elected to explore a series of *N*-(α-acyloxyalkyl)dicarboximide dimer prodrugs of type **21**–**27** (Fig. 2, Table 3), making use of the considerable steric bulk of a second NRB molecule, introduced through a linker, to act as the pro-moiety; with the concept that two equivalents of NRB would now be released upon cleavage. As a more elaborate example of a NRB dimer prodrug, *N*-[α-(ethylene bis(hydrogensuccinoyloxy))methyl]dicarboximide tetraester **27** was proposed to provide a molecule incorporating a second independent site of cleavage, positioned further away from the sterically demanding NRB molecule, with a view to making it more susceptible to enzymatic breakdown. Commercially available diacid chlorides **54**–**59** were converted to the corresponding dichloromethyl esters **60**–**65** using conditions similar to those detailed previously.[28] Treatment of dichloromethyl esters **60**–**65** with two equivalents of NRB, in the presence of potassium carbonate, afforded dimers **21**–**26**, respectively, in yields ranging from 12% to 81%.[24] Ethylene bis(hydrogen succinate)[29] (**67**) was prepared in 95% yield via the addition of ethylene glycol to a solution of succinic anhydride (**66**) in pyridine, under heating. Diacid **67** was then sequentially taken through to the corresponding diacid chloride,[30] dichloromethyl ester **68**,[28] and finally, via the subsequent reaction of in situ generated iodide with two equivalents of NRB, to the desired dimer **27**, in 26% yield overall (Schemes 2 and 3, Table 3).[24,26] All dicarboximide dimers evaluated within this study were found to be devoid of all pre-cleavage vasoconstrictory activity (Table 3).



5 R = *t*-Bu; **6** R = Ph; **7** R = Ac

Figure 3. *O*-(α-Acyloxymethyl)ether prodrug candidates **5**–**7**.

Table 2
In vitro evaluation for vasoconstrictory activity of **8**–**20**

Compd	R	Yield (%)	Vasoconstriction[a]
8	*t*-Bu	48	⩾132
9	$(CH_2)_2CH_3$	62	86
10	$(CH_2)_6CH_3$	56	0
11	$(CH_2)_{10}CH_3$	32	0
12	Ph	71	⩾132
13	*o*-OMePh	65	90
14	*m*-OMePh	34	0
15	*p*-OMePh	65	0
16	CH_2Ph	19	6
17	$CHPh_2$	15	0
18	CH_2CH_2Ph	6	13
19	CH=CHPh	12	0
20	2-Naph	32	0

[a] Maximum contractile effect as a% of 90 mM KCl contraction (rat caudal artery); stereoisomeric mixture of NRB = 132%.

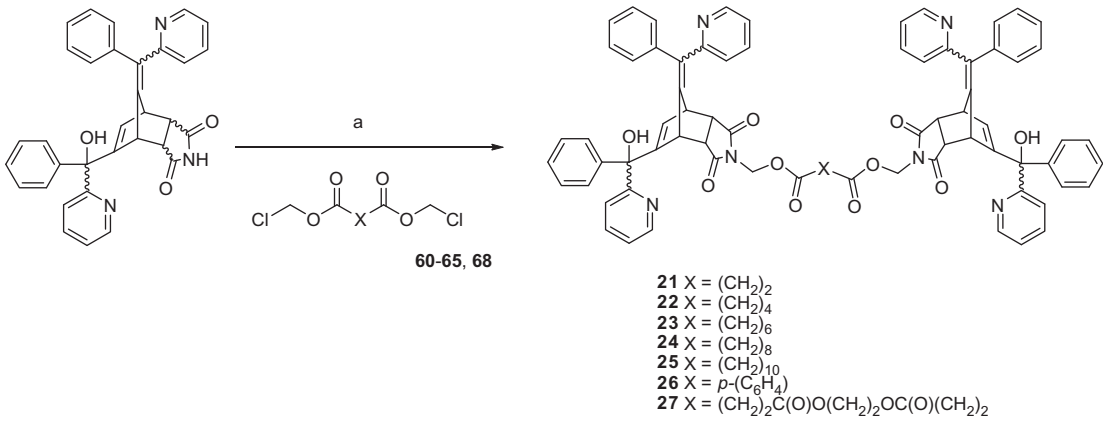
2.2. In vitro cleavage studies and in vivo evaluation

To evaluate the hydrolytic stability of lead *N*-(α-acyloxymethyl)dicarboximide prodrug candidates **10**–**11**, **14**–**17**, **19** and **20**

Table 3
In vitro evaluation for vasoconstrictory activity of **21**–**27**

Compd	X	Yield (%)	Vasoconstriction[a]
21	$(CH_2)_2$	17	0
22	$(CH_2)_4$	16	0
23	$(CH_2)_6$	6	0
24	$(CH_2)_8$	81	0
25	$(CH_2)_{10}$	12	0
26	*p*-(C_6H_4)	21	0
27	$(CH_2)_2C(O)O(CH_2)_2OC(O)(CH_2)_2$	26	0

[a] Maximum contractile effect as a% of 90 mM KCl contraction (rat caudal artery); stereoisomeric mixture of NRB = 132%.



Scheme 3. Reagents and conditions: (a) (i) **60**–**65**, **68**, NaI, acetone, rt, 3 h, (ii) then NRB, K_2CO_3, DMF, rt, 48 h (see Table 3).

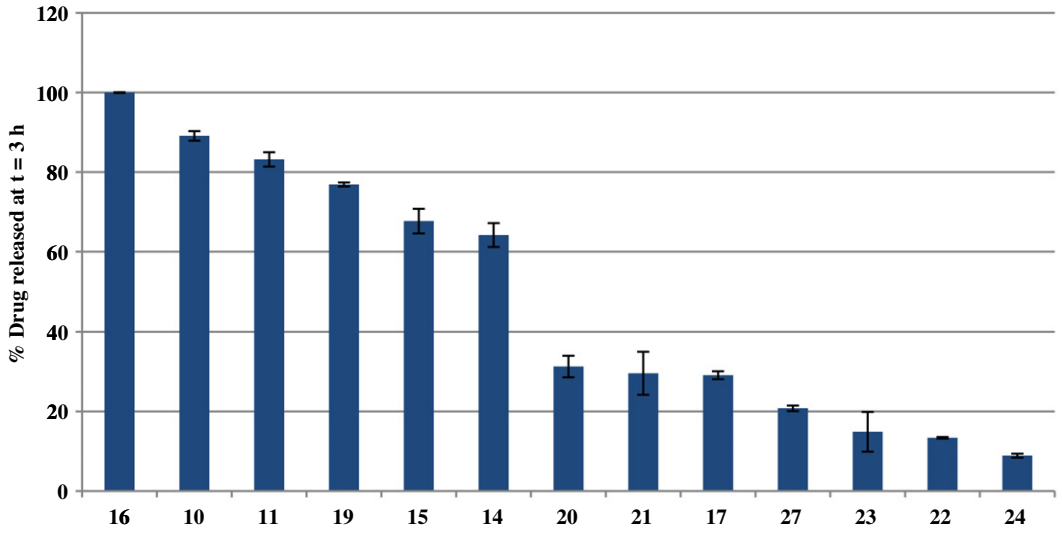


Figure 4. In vitro enzymatic cleavage studies on selected prodrugs using rat serum (37 °C, 3 h, $n = 3$).

(pre-cleavage vasoconstrictory activity <10%) in rat blood, in vitro enzymatic cleavage studies using rat serum were conducted in-house (Fig. 4, Table 4). Results showed full cleavage of phenylacetate **16** following the standard adopted incubation time of 3 h, revealing our most labile prodrug under such conditions within this study. Given that compound **16** incorporated the smallest pro-moiety of those prodrugs under evaluation this data was largely expected. Next most susceptible were alkyl chain homologues *n*-octanoate **10** and *n*-dodecanoate **11** (ca. 89% and 83% NRB release, respectively) while methoxybenzoate analogues **14** (*meta*) and **15** (*para*) both cleaved in the region of 65%. Cinnamate **19** was found to release around 77% NRB post exposure to rat serum, with related naphthoate **20** being considerably less prone to cleavage, displaying similar rates of enzymatic breakdown to diphenylacetate **17**, with toxicant release levels both in the region of 30%.

Surprisingly, and contrary to our design rationale with respect to the dimer concept, we discovered that it was in fact the diester prodrugs of increased chain length which were the least susceptible to enzymatic degradation. Within this series dodecanedioate **25** ($n = 10$, <5% NRB release) was observed to be practically inert towards cleavage while sebacate **24** ($n = 8$) was found to release around 10% toxicant following the 3 h exposure period. By contrast, succinate **21** ($n = 2$, ca. 30% NRB release) was observed to cleave the most readily in the presence of rat serum. The remaining alkyl chain diesters, adipate **22** and suberate **23** ($n = 4$, 6, respectively), cleaved in the region of 15%. Efforts to further increase the susceptibility of the dicarboximide diesters towards enzymatic degradation, through the incorporation of a second independent site of cleavage within the linker, were found to be moderately successful, with compound **27** (ca. 21% NRB release) revealing a higher degree of cleavage (vs un-functionalized chain length equivalent, dodecanedioate **25**, <5% NRB release). With regards to aromatic ester derived prodrug **26**, incorporating a terephthalate linker, relatively low levels of NRB release were observed (ca. 5%). As a consequence of their common relative resistance to hydrolytic cleavage (<30% NRB release, 3 h), and on synthetic grounds, dicarboximide dimer prodrugs **21**–**27** were taken no further; given that the rate of NRB clearance[31] in rats is known to be relatively high the aforementioned candidates were viewed to be of inadequate lability, if the goal of lethal systemic levels of toxicant were ultimately to be attained.

Having now established a relative order of hydrolytic stability against rat blood enzymes within our non-vasoconstricting prodrug series, six candidates (**10**, **14**, **16**, **17**, **19** and **20**; ranging from ca. 100% to 29% toxicant release, 3 h) were nominated for further evaluation (Fig. 4, Table 4). As an alternative source of carboxylesterase, and with the knowledge that the liver has the highest levels of carboxylesterase activity in the biological system of many

Table 4
Pre-cleavage vasoconstrictory activity, in vitro hydrolytic stability (low pH, rat blood and liver enzymes) and in vivo lethality of NRB and prodrugs **2**–**4**, **8**–**27** in rats

Compd	Vasoconstriction[a]	% NRB released			In vivo lethality (iv)	In vivo lethality (oral)
		Hydrolytic stability	Rat serum[d]	Rat liver S9[e]		
***endo*-NRB**	132	–	–	–	Yes[f,g]	Yes[h,i]
2	132	100[b]	–	–	–	–
3	132	100[b]	–	–	–	–
4	132	100[b]	–	–	–	–
8	132	0[b]	–	–	–	–
9	86	0[b]	–	–	–	–
10	0	20[c]	89.1 ± 1.2	73.0 ± 5.2	–	–
11	0	20[c]	83.2 ± 1.8	–	–	–
12	132	0[b]	–	–	–	–
13	90	0[b]	–	–	–	–
14	0	0[c]	64.2 ± 3.0	53.1 ± 3.3	–	–
15	0	0[c]	67.7 ± 3.1	–	–	–
16	6	0[b], <5[c]	100.0 ± 0.1	69.9 ± 0.8	Yes[f,g]	–
17	0	0[c]	29.1 ± 1.0	12.4 ± 0.3	nt[j]	No[h]
18	13	0[b]	–	–	–	–
19	0	0[c]	76.9 ± 0.5	55.4 ± 2.4	Yes[f,g]	Yes[h,i]
20	0	0[c]	31.3 ± 2.7	20.2 ± 0.8	Yes[f,g]	No[h]
21	0	0[c]	29.6 ± 5.4	–	–	–
22	0	0[c]	13.4 ± 0.2	–	–	–
23	0	0[c]	14.9 ± 5.0	–	–	–
24	0	0[c]	8.6 ± 0.5	–	–	–
25	0	0[c]	<5	–	–	–
26	0	0[c]	<5	–	–	–
27	0	0[c]	20.8 ± 0.7	–	–	–

[a] Maximum contractile effect as a% of 90 mM KCl contraction (rat caudal artery, $n = 3$), stereoisomeric mixture of NRB = 132%.
[b] For vasoconstricting prodrugs pre-cleavage, Tyrode solution (37 °C, 1 h, $n = 3$).
[c] For non-vasoconstricting prodrugs pre-cleavage, simulated gastric fluid without pepsin (pH 1.2, 37 °C, 1 h, $n = 3$).
[d] 80% rat serum (37 °C, 3 h, $n = 3$).
[e] 1 mg/mL rat liver S9 fraction (37 °C, 6 h, $n = 3$);
[f] 20 mg/Kg (rat, iv, $n = 3$).
[g] 10 mg/Kg (rat, iv, $n = 3$).
[h] 40 mg/Kg (rat, oral, $n = 3$).
[i] 20 mg/Kg (rat, oral, $n = 3$).
[j] Solubility problems encountered preventing injection by iv nt = not tested.

mammals, including rats,[32] further information was sought on the probable routes of cleavage of the aforementioned prodrugs through their incubation in the presence of rat liver S9 fraction. Compounds **10** and **16** were once again shown to cleave most readily (73% and 69%, respectively, 6 h), as revealed earlier in our serum based experiments, while prodrugs **19**, **14**, **20** and **17** (ca. 55%, 53%, 20% and 12% NRB release, 6 h, respectively) also appeared to roughly fit a similar trend to previous observations (Fig. 5, Table 4).

All prodrug candidates considered for further evaluation were now subject to stability appraisal under simulated gastric fluid (SGF) conditions (without pepsin)[33] prior to in vivo evaluation, to reveal any potential chemical instabilities that may occur in the acidic environment of the stomach prior to uptake (Table 4). Prodrugs **14**, **17**, **19** and **20** were found to be stable at low pH following the standard adopted incubation period of 1 h, with low levels of cleavage (<5%) observed for phenylacetate **16**. Compound **10** was found to be unstable (ca. 20% cleavage) under the aforementioned conditions and were thus taken no further.

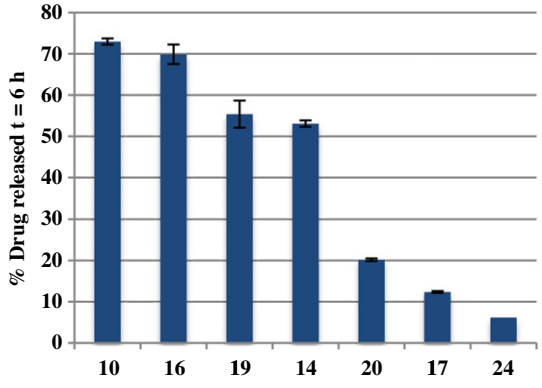


Figure 5. In vitro enzymatic cleavage studies on selected prodrugs using rat liver S9 fraction (37 °C, 6 h, $n = 3$).

As part of our preliminary in vivo assessment, and in an endeavour to ascertain proof-in-principle, prodrugs **16**, **19** and **20** were successfully administered intravenously to euthanized rats; formulation problems prevented the delivery of compound **17** by iv (Table 4). Doses of both 20 and 10 mg/Kg resulted in instant death, or death within minutes, in all cases, illustrating the rapid cleavage of the aforementioned prodrugs in vivo. Oral gavage experiments at an initial dose of 40 mg/Kg were next undertaken for prodrugs **14**, **17**, **19** and **20**, leading to a lethal end-point in the case of compounds **14** and **19**, while compounds **17** and **20** both failed to induce death; potentially a consequence of the cleavage (too slow) versus clearance phenomenon, thus leading to sub-lethal dosing (Table 4). At a reduced dose of 20 mg/Kg compound **19** was again found to be lethal in rats (mean time to death 90 min), with an all important lag time in the onset of symptoms of around 30 min. At this same dose, NRB (see positive control, *endo*-NRB) would on average result in death within 35 min, and more significantly with symptoms being evident within approximately 5 min of administration (Tables 4 and 5).

In order to provide further experimental evidence that NRB's unpleasant 'taste', a probable primary affect leading to bait-shyness, is indeed a consequence of the parent compound's inherent capacity to elicit constriction of the blood vessels of the buccal cavity, a palatability trial was commissioned to further evaluate prodrug **19** (Fig. 6, Table 5). Following 4 days 'pre-baiting' with a peanut butter formulation free of toxicant rats were fed with a bait containing 1% w/w prodrug in peanut butter. Upon exchanging the 'placebo' bait for one containing compound **19** consumption levels

Table 5
In vivo lethality and palatability trial observations for NRB and prodrug **19** in rats

Compd	In vivo lethality[a]		Palatability trial[d]	
	Onset of Symptoms[b] (min)	Time to Death (min)	Average Bait Consumed (g) (of 5 g)	No of deaths observed
endo-NRB	5[c]	35[c]	1.9 ± 0.5	3/6
19	30[c]	90[c]	4.3 ± 0.2	6/6
PB	—	—	5.0 ± 0.2	0/6

[a] 20 mg/Kg (rat, oral, $n = 3$).
[b] Visual signs of distress (laboured/irregular breathing, lethargy, tail twitching), consistent with NRB-like symptoms.
[c] Mean time.
[d] Rats fed with 1% w/w toxicant ($n = 6$) in a peanut butter bait following 4 days 'pre-baiting' with a peanut butter formulation free of toxicant. PB = Peanut butter only.

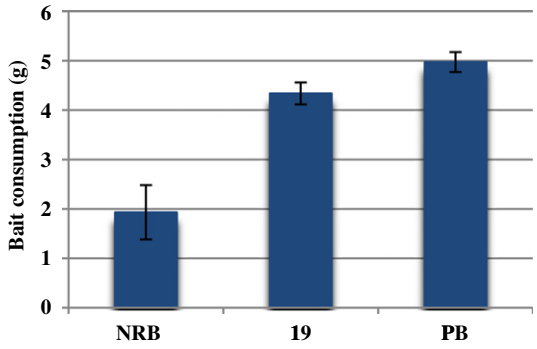


Figure 6. Palatability trial observations for NRB and prodrug **19**; rats fed with 1% w/w toxicant ($n = 6$) in a peanut butter bait following 4 days 'pre-baiting' with a peanut butter formulation free of toxicant. PB = Peanut butter only.

remained high (4.3 ± 0.2 g of 5.0 g, $n = 6$), versus the control bait (peanut butter only; complete consumption observed, 5 g, $n = 6$); and enhanced with respect to the average consumption of norbormide-containing baits (1.9 ± 0.5 g of 5.0 g, $n = 6$). Strikingly, a kill rate of six from six rats was observed for compound **19**, versus 3/6 for the parent toxicant in non-prodrug form.

3. Conclusion

In summary, in an endeavour to circumnavigate the well documented problem of NRB-associated bait shyness in rats, a select series of prodrugs in the form of *N*-(α-acyloxymethyl)dicarboximides were prepared. Having successfully demonstrated a number of these analogues to be devoid of any undesirable vasoconstrictory activity pre-cleavage, subsequent in vitro cleavage studies revealed a broad range of hydrolytic stabilities in the presence of both rat blood and liver carboxylesterases. Of the six candidates evaluated in vivo NRB prodrug **19** displayed the most promising profile with respect to a delay in the onset of symptoms and was subsequently demonstrated to be significantly more palatable to rats. On the basis of the findings of this study compound **19** has since been selected to undergo advanced appraisal in a two-choice bait trial, an undertaking which sits outside the scope of this manuscript.

4. Experimental (Chemistry)

4.1. General experimental methods

All reagents were used as supplied unless otherwise stated. Solvents were purified by standard methods.[34] Analytical thin layer chromatography (TLC) was carried out on pre-coated silica gel plates (Merck/UV_{254}) and products were visualized by UV fluorescence and/or staining. Potassium permanganate solution was the stain of choice. Flash chromatography was performed using silica gel (Riedel-de Haën, particle size 0.032–0.063 mm). Distillation was carried out using a Büchi GKR-51 Kugelrohr apparatus. Melting points in degrees Celsius (°C) were measured on an Electrothermal® melting point apparatus and are uncorrected. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker AVANCE DRX400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) or a Bruker AVANCE 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) spectrometer at 298 K. For ^{1}H NMR data, chemical shifts are described in parts per million (ppm) relative to tetramethylsilane (δ 0.00) and are reported consecutively as position (δ_H), relative integral, multiplicity (s = singlet, bs = broad singlet, d = doublet, bd = broad doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, qd = quartet of doublets, m = multiplet, bm = broad multiplet), coupling constant (*J*/Hz) and assignment. For ^{13}C NMR data, chemical shifts (ppm) are referenced internally to $CDCl_3$ (δ 77.0) and are reported consecutively as position (δ_C) and degree of hybridization. Assignments were aided by DEPT135 and HSQC experiments. Infrared spectra were recorded on a Perkin–Elmer Spectrum One Fourier Transform Absorption peaks are reported in wavenumbers (ν, cm^{-1}), with the major peaks assigned to the appropriate functional groups. Mass spectra were recorded on a VG-70SE mass spectrometer (EI, CI and FAB). High-resolution mass spectra were recorded at a nominal resolution of 5000. The purity of all target compounds was assigned using reverse-phase HPLC [Dionex P680 system using a Phenomenex Gemini C_{18}-Si column (50 mm × 2 mm, 5 μm)]—eluted using a gradient of 100:0% A/B to 5:95% A/B over 15 min at 0.2 mL/min; where solvent A was water (0.1% formic acid) and solvent B was CH_3CN (0.1% formic acid); with detection at 254 and 280 nm.

4.1.1. 5-(α-Hydroxy-α-2-pyridylbenzyl)-*N*-methyl-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide[11] (1)

Compound **1** was prepared by a procedure similar to that of Hursthouse et al.[24] To a solution of NRB (0.5 g, 0.98 mmol) in dimethylformamide (5 mL) was added dropwise a solution of iodomethane (0.15 mL, 2.44 mmol) in dimethylformamide (1 mL), followed by potassium carbonate (0.34 g, 2.44 mmol), and the reaction stirred at room temperature for 16 h. The mixture was taken up in chloroform/water (1:1) (30 mL) and the organic phase separated. The aqueous layer was further extracted with chloroform (3 × 10 mL) and the combined organic phases washed with water (2 × 20 mL), dried over anhydrous sodium sulfate and the solvent removed in vacuo to give crude **1** as a yellow residue. Purification by flash chromatography (hexane/ethyl acetate 1:2) afforded **1** as a colourless solid (150 mg, 0.28 mmol, 30%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.93 and 2.96 (3H, s, NMe), 3.29–3.40 (0.7H, m, 0.2H W/H-3 and 0.5H Y/H-3), 3.43–3.54 (0.4H, m, 0.2H U/H-2 and U/H-3, 0.2H V/H-2), 3.58–3.66 (1.1H, m, 0.2H V/H-3, 0.4H W/H-2 and W/H-4, 0.5H Y/H-2), 3.85–3.90 (0.3H, m, 0.1H U/H-1 and 0.2H V/H-1), 3.94 (0.5H, dt, $J = 4.5$ and 1.4 Hz, Y/H-4), 4.13–4.16 (0.1H, m, U/H-4), 4.28 (0.2H, dt, $J = 4.5$ and 1.4 Hz, V/H-4), 4.44–4.48 (0.7H, m, 0.2H W/H-1 and 0.5H Y/H-1), 5.48 (0.2H, dd, $J = 3.3$ and 1.3 Hz, V/H-6), 5.50–5.52 (0.7H, m, 0.5H Y/H-6 and 0.2H OH), 5.54 (0.5H, s, OH), 5.59 (0.3H, s, OH), 5.96–5.99 (0.3H, m, 0.1H U/H-6 and 0.2H W/H-6), 6.63–7.67 (16H, m, Ar), 8.43–8.45 (0.2H, m, 2U/αPyr),

8.46–8.50 (1.1H, m, 0.4H 2V/αPyr, 0.2H W/αPyr and 0.5H Y/αPyr), 8.61–8.64 (0.7H, m, 0.2H W/αPyr and 0.5H Y/αPyr).

4.1.2. 5-(α-Hydroxy-α-2-pyridylbenzyl)-*N*-methyl-7-[α-2-pyridyl-*N*-(pivaloyloxymethyl)benzylidene]-5-norbornene-2,3-dicarboximide iodide (2)

Compound **2** was prepared by a procedure similar to that of Davidsen and co-workers,[23] and Bodor and co-workers.[26] To a solution of chloromethyl pivalate (**41**) (345 mg, 2.28 mmol) in acetone (3 mL) was added a solution of sodium iodide (342 mg, 2.28 mmol) in acetone (2 mL), and the mixture stirred at room temperature for 3 h. The solvent was removed in vacuo and the crude residue taken up in acetonitrile (2 mL). A solution of **1** (300 mg, 0.60 mmol) in acetonitrile (1.5 mL) was added and the mixture stirred at 80 °C for 16 h. The solvent was removed in vacuo with purification by flash chromatography (dichloromethane/methanol 20:1) affording **2** as a yellow solid (348 mg, 0.45 mmol, 76%). mp 111–120, 150–168 °C; ν_{max}(NaCl)/cm^{-1} 1104 and 1277 (C–O ester), 1691 (C=O imide), 1751 (C=O ester); *m/z* (FAB+) 640 (M^+, 100%); (Found: M^+ 640.2803, $C_{40}H_{38}N_3O_5$ requires 640.2812).

4.1.3. 5-(α-Hydroxy-α-2-pyridylbenzyl)-*N*-methyl-7-[α-2-pyridyl-*N*-(benzoyloxymethyl)benzylidene]-5-norbornene-2,3-dicarboximide iodide (3)

A similar procedure[23,26] to that previously described for the preparation of **2** was followed using chloromethyl benzoate (**45**) (106 mg, 0.76 mmol) in acetone (0.2 mL), sodium iodide (339 mg, 2.28 mmol) in acetone (2.5 mL), and the mixture stirred at room temperature for 30 min. The solvent was removed in vacuo and the residue taken up in acetonitrile (1.5 mL). A solution of **1** (200 mg, 0.38 mmol) in acetonitrile (2 mL) was added and the mixture stirred at 80 °C for 16 h. The solvent was removed in vacuo with purification by flash chromatography (dichloromethane/methanol 20:1) affording **3** as a yellow solid (110 mg, 0.14 mmol, 37%). mp 165–169 °C; ν_{max}(NaCl)/cm^{-1} 1087 and 1261 (C–O ester), 1697 (C=O imide), 1733 (C=O ester); *m/z* (FAB+) 660 (M^+, 100%); (Found: M^+ 660.2490, $C_{42}H_{34}N_3O_5$ requires 660.2498).

4.1.4. 5-(α-Hydroxy-α-2-pyridylbenzyl)-*N*-methyl-7-[α-2-pyridyl-*N*-(diphenylacetoyloxymethyl)benzylidene]-5-norbornene-2,3-dicarboximide iodide (4)

A similar procedure[23,26] to that previously described for the preparation of **2** was followed using chloromethyl diphenylacetate (**50**) (197 mg, 0.76 mmol) in acetone (1 mL), sodium iodide (113 mg, 0.76 mmol) in acetone (0.5 mL), and the mixture stirred at room temperature for 3 h. The solvent was removed in vacuo and the crude residue taken up in acetonitrile (0.5 mL). A solution of **1** (199 mg, 0.38 mmol) in acetonitrile (1 mL) was added and the mixture stirred at 80 °C for 16 h. The solvent was removed in vacuo with purification by flash chromatography (dichloromethane/methanol 20:1) affording **4** as a yellow solid (171 mg, 0.20 mmol, 51%). mp 145–155 °C; ν_{max}(nujol)/cm^{-1} 1112 and 1277 (C–O ester), 1618 (C=O imide), 1693 (C=O ester); *m/z* (FAB+) 750 (M^+, 84%), 167 (100); (Found: M^+ 750.2963, $C_{49}H_{40}N_3O_5$ requires 750.2968).

4.1.5. 5-(α-Hydroxy-α-2-pyridylbenzyl)-*N*-pivaloyloxymethyl-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (8)

A similar procedure[24] to that previously described for the preparation of **1** was followed using NRB (200 mg, 0.39 mmol) in dimethylformamide (2 mL), chloromethyl pivalate (**41**) (59 mg, 0.39 mmol) in dimethylformamide (0.5 mL) and potassium carbonate (54 mg, 0.39 mmol). The mixture was stirred at room temperature for 16 h, taken up in chloroform (15 mL), washed with water (2 × 10 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (hexane/ethyl acetate 1:1) afforded **8** as a colourless solid (118 mg, 0.19 mmol, 48%). mp 95–98 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18–1.19 (8.4H, m, *t*Bu), 1.25–1.26 (0.6H, m, *t*Bu), 3.38 (0.2H, dd, *J* = 7.9 and 4.5 Hz, W/H-3), 3.45 (0.5H, dd, *J* = 7.9 and 4.5 Hz, Y/H-3), 3.51–3.59 (0.4H, m, 0.2H U/H-2 and U/H-3, 0.2H V/H-2), 3.63–3.64 (0.2H, m, W/H-4), 3.67–3.76 (0.9H, m, 0.2H V/H-3, 0.2H W/H-2 and 0.5H Y/H-2), 3.88–3.92 (0.3H, m, 0.1H U/H-1 and 0.2H V/H-1), 4.00–4.01 (0.5H, dt, *J* = 4.5 and 1.3 Hz, Y/H-4), 4.20–4.21 (0.1H, m, U/H-4), 4.35–4.36 (0.2H, dt, *J* = 4.5 and 1.3 Hz, V/H-4), 4.49–4.52 (0.7H, m, 0.2H W/H-1 and 0.5H Y/H-1), 5.26–5.29 (0.7H, m, CH_2), 5.31–5.62 (3H, m, 1.3H CH_2, 0.2H V/H-6, 0.5H Y/H-6 and 1H OH), 6.04–6.06 (0.3H, m, 0.1H U/H-6 and 0.2H W/H-6), 6.71–7.57 (16H, m, Ar), 8.42–8.50 (0.6H, m, 0.2H 2U/αPyr and 0.4H 2V/αPyr), 8.54–8.55 (0.7H, m, 0.2H W/αPyr and 0.5H Y/αPyr), 8.61–8.63 (0.7H, m, 0.2H W/αPyr and 0.5H Y/αPyr); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.9 (CH_3), 44.0–46.9 (CH), 49.0–49.6 (CH), 62.0–62.2 (CH_2), 77.7–77.8 (C), 121.7–121.8 (CH), 122.4–122.8 (CH), 123.7–124.3 (CH), 124.9 (CH), 126.5–130.2 (CH), 133.2 (CH), 133.9 (CH), 135.1 (CH), 135.8–135.9 (CH), 136.4–136.6 (CH), 138.3–138.5 (C), 141.8 (C), 142.2 (C), 142.8 (C), 143.1 (C), 147.7–148.1 (CH), 148.9–149.3 (CH), 152.6 (C), 154.0–155.5 (C), 157.9–158.3 (C), 160.6–161.0 (C), 174.6 (C), 175.0–175.5 (C), 177.3–177.4 (C); ν_{max}(nujol)/cm^{-1} 1140 and 1212 (C–O ester), 1586 (C=O imide), 1715 (C=O ester); *m/z* (FAB+) 626 (MH^+, 67%), 120 (100); (Found: MH^+ 626.2644, $C_{39}H_{36}N_3O_5$ requires 626.2655).

4.1.6. *N*-Butanoyloxymethyl-5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (9)

Compound **9** was prepared by a procedure similar to that of Hursthouse and co-workers,[24] and Binderup and co-workers.[35] To a mixture of butyric acid (426 μL, 4.6 mmol), water (5 mL), dichloromethane (5 mL), sodium hydrogen carbonate (1.46 g, 17.5 mmol) and tetrabutylammonium bromide (148 mg, 0.46 mmol) was added dropwise chloromethyl chlorosulfate (534 μL, 5.28 mmol) in dichloromethane (1.5 mL). The reaction was stirred at room temperature for 16 h and the organic layer separated, dried over anhydrous sodium sulfate and the solvent removed in vacuo. The crude residue was taken up in dimethylformamide (1 mL) and added to a solution of NRB (500 mg, 0.98 mmol) in dimethylformamide (2 mL), followed by potassium carbonate (135 mg, 0.98 mmol). The mixture was stirred at room temperature for 16 h, taken up in dichloromethane/water (1:1) (30 mL), washed with water (2 × 20 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (hexane/ethyl acetate 1:1) afforded **9** as a colourless solid (375 mg, 0.61 mmol, 62%). mp 68–79 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89–0.96 (3H, m, Me), 1.55–1.71 (2H, m, $OCOCH_2CH_2$), 2.25–2.35 (2H, m, $OCOCH_2CH_2$), 3.37–3.74 (2.2H, m, 0.2H U/H-2 and U/H-3, 0.6H V/H-2 and V/H-3, 0.6H W/H-2, W/H-3 and W/H-4, 0.8H Y/H-2 and Y/H-3), 3.86–3.93 (0.4H, m, 0.1H U/H-1 and 0.3H V/H-1), 3.96–4.02 (0.4H, m, Y/H-4), 4.19–4.24 (0.1H, m, U/H-1), 4.37–4.43 (0.3H, m, V/H-4), 4.50–4.59 (0.6H, m, 0.2H W/H-1 and 0.4H Y/H-1), 5.30–5.35 (0.7H, m, 0.3H V/H-6 and 0.4H Y/H-6), 5.44–5.64 (3H, m, 2H NCH_2O and 1H OH), 6.03–6.08 (0.3H, m, 0.1H U/H-6 and 0.2H W/H-6), 6.75–7.58 (16H, m, Ar), 8.37–8.44 (0.8H, m, 0.2H 2U/αPyr and 0.6H 2V/αPyr), 8.49–8.51 (0.6H, m, 0.2H W/αPyr and 0.4H Y/αPyr), 8.58–8.63 (0.6H, m, 0.2H W/αPyr and 0.4H Y/αPyr); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.2 (CH_3), 17.7 (CH_2), 35.2 (CH_2), 43.8–46.7 (CH), 48.8–49.3 (CH), 61.2–61.4 (CH_2), 77.2 (C), 121.3–122.4 (CH), 123.6–124.0 (CH), 126.4–130.0 (CH), 132.8–133.5 (CH), 135.6–136.4 (CH), 138.0–138.3 (C), 141.7–142.9 (C), 147.4–147.8 (CH), 148.7–148.9 (CH), 152.4 (C), 153.7 (C), 155.2 (C), 157.7–158.0 (C), 160.3–160.7 (C), 172.0–172.1 (C), 174.3–175.1 (C); ν_{max}/cm^{-1} 1041 and 1208 (C–O ester),

1585 (C=O imide), 1713 (C=O ester); *m/z* (ESI) 612 (MH^+, 100%); (Found: MH^+ 612.2489, $C_{38}H_{34}N_3O_5$ requires 612.2493).

4.1.7. 5-(α-Hydroxy-α-2-pyridylbenzyl)-*N*-octanoyloxymethyl-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (10)

A similar procedure[24] to that previously described for the preparation of **1** was followed using NRB (500 mg, 0.98 mmol) in dimethylformamide (2 mL), chloromethyl octanoate (**43**) (377 mg, 1.96 mmol) in dimethylformamide (0.5 mL) and potassium carbonate (135 mg, 0.98 mmol). The mixture was stirred at room temperature for 16 h, taken up in dichloromethane/water (1:1) (30 mL), washed with water (2 × 20 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (hexane/ethyl acetate 2:1) afforded **10** as an oily residue (367 mg, 0.55 mmol, 56%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85–0.89 (3H, m, Me), 1.21–1.38 (8H, m, $OCO(CH_2)_2(CH_2)_4$), 1.55–1.69 (2H, m, $OCOCH_2CH_2$), 2.29–2.37 (2H, m, $OCOCH_2$), 3.36 (0.2H, dd, *J* = 7.5 and 4.5 Hz, W/H-3), 3.42 (0.4H, dd, *J* = 7.5 and 4.5 Hz, Y/H-3), 3.48–3.74 (1.6H, m, 0.2H U/H-2 and U/H-3, 0.6H V/H-2 and V/H-3, 0.4H W/H-2 and W/H-4, 0.4H Y/H-2), 3.86–3.91 (0.4H, m, 0.1H U/H-1 and 0.3H V/H-1), 3.97–4.00 (0.4H, m, Y/H-4), 4.20–4.22 (0.1H, m, U/H-4), 4.36–4.39 (0.3H, m, V/H-4), 4.50–4.54 (0.6H, m, 0.2H W/H-1 and 0.4H Y/H-1), 5.28–5.32 (0.7H, m, NCH_2O), 5.46–5.60 (3H, m, 1.3H NCH_2O, 0.3H V/H-6 and 0.4H Y/H-6), 6.03–6.07 (0.3H, m, 0.1H U/H-6 and 0.2H W/H-6), 6.74–7.58 (16H, m, Ar), 8.40–8.48 (0.7H, m, 0.1H U/αPyr and 0.6H 2V/αPyr), 8.51–8.53 (0.7H, m, 0.1H U/αPyr, 0.2H W/αPyr and 0.4H Y/αPyr), 8.60–8.63 (0.6H, m, 0.2H W/αPyr and 0.4H Y/αPyr); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9 (CH_3), 22.4 (CH_2), 24.4 (CH_2), 28.7–28.8 (CH_2), 31.4 (CH_2), 33.6 (CH_2), 43.9–46.9 (CH), 49.0–49.5 (CH), 61.4–61.7 (CH_2), 77.2 (C), 77.6 (C), 77.7 (C), 121.5–122.6 (CH), 123.8–124.2 (CH), 126.6–130.2 (CH), 133.0–133.8 (CH), 135.7–136.5 (CH), 138.2–138.5 (C), 141.8–143.0 (C), 147.6–148.0 (CH), 149.0–149.2 (CH), 152.5 (C), 153.9–155.4 (C), 157.9–158.2 (C), 160.5–160.9 (C), 172.4–172.5 (C), 174.5–175.3 (C); ν_{max}/cm^{-1} 1040 and 1214 (C–O ester), 1585 (C=O imide), 1712 (C=O ester); *m/z* (ESI) 668 (MH^+, 1%), 650 (MH^+-H_2O, 100); (Found: MH^+ 668.3123, $C_{42}H_{42}N_3O_5$ requires 668.3119).

4.1.8. *N*-Dodecanoyloxymethyl-5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (11)

A similar procedure[24] to that previously described for the preparation of **1** was followed using NRB (500 mg, 0.98 mmol) in dimethylformamide (2 mL), chloromethyl dodecanoate (**44**) (488 mg, 1.96 mmol) in dimethylformamide (0.5 mL) and potassium carbonate (135 mg, 0.98 mmol). The mixture was stirred at room temperature for 16 h, taken up in dichloromethane/water (1:1) (30 mL), washed with water (2 × 20 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (hexane/ethyl acetate 2:1) afforded **11** as a colourless oil (231 mg, 0.32 mmol, 32%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85–0.89 (3H, m, Me), 1.20–1.36 (16H, m, $OCO(CH_2)_2(CH_2)_8$), 1.55–1.68 (2H, m, $OCOCH_2CH_2$), 2.29–2.36 (2H, m, $OCOCH_2$), 3.36 (0.2H, dd, *J* = 9.0 and 3.0 Hz, W/H-3), 3.42 (0.4H, dd, *J* = 9.0 and 3.0 Hz, Y/H-3), 3.48–3.74 (1.6H, m, 0.2H U/H-2 and U/H-3, 0.6H V/H-2 and V/H-3, 0.4H W/H-2 and W/H-4, 0.4H Y/H-2), 3.86–3.92 (0.4H, m, 0.1H U/H-1 and 0.3H V/H-1), 3.98–3.99 (0.4H, m, Y/H-4), 4.20–4.21 (0.1H, m, U/H-4), 4.37–4.38 (0.3H, m, V/H-4), 4.51–4.54 (0.6H, m, 0.2H W/H-1 and 0.4H Y/H-1), 5.29–5.33 (0.7H, m, NCH_2O), 5.43–5.60 (3H, m, 1.3H NCH_2O, 0.3H V/H-6, 0.4H Y/H-6 and 1H OH), 6.03–6.07 (0.3H, m, 0.1H U/H-6 and 0.2H W/H-6), 6.75–7.58 (16H, m, Ar), 8.39–8.46 (0.7H, m, 0.1H U/αPyr and 0.6H 2V/αPyr), 8.51–8.52 (0.7H, m, 0.1H U/αPyr, 0.2H W/αPyr and 0.4H Y/αPyr), 8.60–8.61 (0.6H, m, 0.2H W/αPyr and 0.4H Y/αPyr); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9 (CH_3), 22.4 (CH_2), 24.4 (CH_2), 28.8–29.2 (CH_2), 31.6 (CH_2), 33.5 (CH_2), 43.9–46.8 (CH), 48.9–49.4 (CH), 61.3–61.6 (CH_2), 77.1–77.6 (CH), 121.4–122.5 (CH), 123.7–124.1 (CH), 126.5–130.1 (CH), 132.9 (CH), 133.7 (CH), 135.6–136.4 (CH), 138.1–138.4 (C), 141.8–143.0 (C), 147.6–147.9 (CH), 148.9–149.1 (CH), 152.5 (C), 153.8–155.4 (C), 157.8–158.1 (C), 160.5–160.9 (C), 172.3–172.5 (C), 174.4–175.2 (C); ν_{max}/cm^{-1} 1041 and 1209 (C–O ester), 1585 (C=O imide), 1715 (C=O ester); *m/z* (ESI) 724 (MH^+, 1%), 706 (MH^+-H_2O, 100); (Found: MH^+ 724.3747, $C_{46}H_{50}N_3O_5$ requires 724.3745).

4.1.9. *N*-Benzoyloxymethyl-5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (12)

A similar procedure[24] to that previously described for the preparation of **1** was followed using NRB (200 mg, 0.39 mmol) in dimethylformamide (2 mL), chloromethyl benzoate (**45**) (66 mg, 0.39 mmol) in dimethylformamide (0.5 mL) and potassium carbonate (54 mg, 0.39 mmol). The mixture was stirred at room temperature for 16 h, taken up in chloroform (15 mL), washed with water (2 × 10 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (hexane/ethyl acetate 1:1) afforded **12** as a colourless solid (180 mg, 0.28 mmol, 71%). mp 94–96, 105–108 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.41–3.44 (0.2H, dd, *J* = 8.0 and 4.4 Hz, W/H-3), 3.48–3.52 (0.4H, dd, *J* = 7.9 and 4.6 Hz, Y/H-3), 3.54–3.63 (0.5H, m, 0.2H U/H-2 and U/H-3 and 0.3H V/H-2), 3.65–3.67 (0.2H, m, W/H-2), 3.70–3.79 (0.9H, m, 0.3H V/H-3, 0.3H W/H-2 and 0.4H Y/H-2), 3.91–3.95 (0.4H, m, 0.1H U/H-1 and 0.3H V/H-1), 4.02–4.03 (0.4H, m, Y/H-4), 4.23–4.24 (0.1H, m, U/H-4), 4.39–4.40 (0.3H, m, V/H-4), 4.54–4.56 (0.6H, m, 0.2H W/H-1 and 0.4H Y/H-1), 5.53–5.58 (1.9H, m, 0.3H V/H-6, 0.4H Y/H-6 and 1.2H CH_2), 5.64 (0.3H, s, OH), 5.65 (0.2H, s, OH), 5.68–5.75 (0.6H, m, CH_2 and OH), 5.82–5.86 (0.7H, m, CH_2 and OH), 6.11–6.12 (0.3H, m, 0.1H U/H-6 and 0.2H W/H-6), 6.74–7.59 (19.2H, m, Ar), 8.01–8.03 (1.8H, m, OCOPh), 8.40–8.50 (1.4H, m, 0.2H 2U/αPyr, 0.6H 2V/αPyr, 0.2H W/αPyr and 0.4H Y/αPyr), 8.61–8.62 (0.6H, m, 0.2H W/αPyr and 0.4H Y/αPyr); ^{13}C NMR (100 MHz, $CDCl_3$) δ 44.0–46.9 (CH), 49.0–49.6 (CH), 62.0–62.2 (CH_2), 77.7–77.8 (C), 121.7–122.6 (CH), 123.9–124.2 (CH), 126.5–130.2 (CH), 133.2 (CH), 133.9 (CH), 135.8–136.6 (CH), 138.2–138.5 (C), 141.8 (C), 142.2 (C), 142.8 (C), 147.8–148.0 (CH), 149.0–149.2 (CH), 152.6 (C), 154.0–154.2 (C), 154.7–154.9 (C), 155.5 (C), 157.8–158.2 (C), 160.5–160.9 (C), 165.2–165.4 (C), 174.6–175.4 (C); ν_{max}(NaCl)/cm^{-1} 1092 and 1265 (C–O ester), 1585 (C=O imide), 1720 (C=O ester); *m/z* (FAB+) 646 (MH^+, 8%), 120 (100); (Found: MH^+ 646.2349, $C_{41}H_{32}N_3O_5$ requires 646.2342).

4.1.10. 5-(α-Hydroxy-α-2-pyridylbenzyl)-*N*-*o*-methoxybenzoyl oxymethyl-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (13)

A similar procedure[24] to that previously described for the preparation of **1** was followed using NRB (500 mg, 0.98 mmol) in dimethylformamide (2 mL), chloromethyl o-methoxybenzoate (**46**) (393 mg, 1.96 mmol) in dimethylformamide (1 mL) and potassium carbonate (135 mg, 0.98 mmol). The mixture was stirred at room temperature for 16 h, taken up in dichloromethane/water (1:1) (30 mL), washed with water (2 × 20 mL), dried over anhydrous sodium sulfate and the solvent removed in vacuo. Purification by flash chromatography (hexane/ethyl acetate 1:1) afforded **13** as a colourless solid (432 mg, 0.64 mmol, 65%). mp 83–88 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.39–3.43 (0.2H, m, W/H-3), 3.45–3.49 (0.5H, m, Y/H-3), 3.53 (0.3H, dd, *J* = 9.0 and 6.0 Hz, V/H-2), 3.64–3.77 (1H, m, 0.3H V/H-3, 0.2H W/H-2 and 0.5H Y/H-2), 3.80 and 3.83 (3H, s, OMe), 3.90–3.94 (0.3H, m, V/H-1), 4.00–4.05

(0.5H, m, Y/H-4), 4.37–4.42 (0.3H, m, V/H-4), 4.50–4.57 (0.7H, m, 0.2H W/H-1 and 0.5H Y/H-1), 5.49–5.57 (2.3H, m, 2H CH_2, 0.3H V/H-6 or OH), 5.65–5.68, 5.79–5.84 (1.5H, m, 0.3H V/H-6 or OH, 0.5H Y/H-6 and 0.7H OH), 6.08–6.09 (0.2H, m, W/H-6), 6.72–7.78 (20H, m, Ar), 8.37–8.43 (1.3H, m, 0.6H 2V/αPyr, 0.2H W/αPyr and 0.5H Y/ααPyr), 8.58–8.60 (0.7H, m, 0.2H W/αPyr and 0.5H Y/αPyr); ^{13}C NMR (75 MHz, $CDCl_3$) δ 44.2–46.3 (CH), 48.8–49.3 (CH), 55.6 (CH_3), 61.8–62.1 (CH_2), 77.1 (C), 77.2 (CH), 77.4–77.5 (C), 111.7 (CH), 118.5–118.6 (CH), 119.7 (CH), 121.4–122.4 (CH), 123.8–124.0 (CH), 126.3 (CH), 126.9–128.0 (CH), 128.9–129.2 (CH), 129.9 (CH), 131.4 (CH), 133.5–133.7 (CH), 135.7–136.4 (CH), 138.0–138.3 (C), 141.6 (C), 142.1 (C), 142.6 (C), 147.5–147.8 (CH), 148.8–148.9 (CH), 152.4 (C), 153.7–155.3 (C), 157.6–158.1 (C), 159.1 (C), 160.3–160.6 (C), 164.4 (C), 174.4–175.1 (C); ν_{max}/cm^{-1} 1124 and 1235 (C–O ester), 1584 (C═O imide), 1713 (C═O ester); *m/z* (ESI) 676 (MH^+, 10%), 658 (MH^+-H_2O, 100); (Found: MH^+ 676.2448, $C_{42}H_{34}N_3O_6$ requires 676.2442).

4.1.11. 5-(α-Hydroxy-α-2-pyridylbenzyl)-*N*-*m*-methoxybenzoyl oxymethyl-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (14)

A similar procedure[24] to that previously described for the preparation of **1** was followed using NRB (500 mg, 0.98 mmol) in dimethylformamide (2 mL), chloromethyl m-methoxybenzoate (**47**) (393 mg, 1.96 mmol) in dimethylformamide (1 mL) and potassium carbonate (135 mg, 0.98 mmol). The mixture was stirred at room temperature for 16 h, taken up in dichloromethane/water (1:1) (30 mL), washed with water (2 × 20 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (hexane/ethyl acetate 1:1) afforded **14** as a colourless solid (224 mg, 0.33 mmol, 34%). mp 171–180 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.40 (0.2H, dd, *J* = 7.5 and 4.5 Hz, W/H-3), 3.47 (0.5H, dd, *J* = 9.0 and 6.0 Hz, Y/H-3), 3.56 (0.3H, dd, *J* = 7.5 and 4.5 Hz, V/H-2), 3.65–3.66 (0.2H, m, W/H-4), 3.70–3.79 (4H, m, 3H OMe, 0.3H V/H-3, 0.2H W/H-2 and 0.5H Y/H-2), 3.91–3.94 (0.3H, m, V/H-1), 4.00–4.03 (0.5H, m, Y/H-4), 4.41–4.42 (0.3H, m, V/H-4), 4.54–4.56 (0.7H, m, 0.2H W/H-1 and 0.5H Y/H-1), 5.54–5.76 and 5.82–5.87 (3.8H, m, 2H CH_2, 0.3H V/H-6, 0.5H Y/H-6 and 1H OH), 6.11–6.13 (0.2H, m, W/H-6), 6.75–7.62 (20H, m, Ar), 8.41–8.48 (1.3H, m, 0.6H 2V/αPyr, 0.2H W/αPyr and 0.5H Y/αPyr), 8.59–8.61 (0.7H, m, 0.2H W/αPyr and 0.5H Y/αPyr); ^{13}C NMR (75 MHz, $CDCl_3$) δ 44.3–46.4 (CH), 48.9–49.4 (CH), 55.1 (CH_3), 61.8–62.2 (CH_2), 77.0 (C), 77.2 (CH), 77.5–77.6 (C), 114.0 (CH), 119.5 (CH), 121.5–122.5 (CH), 123.8–124.0 (CH), 126.4–130.2 (CH), 133.7 (CH), 135.7–136.4 (CH), 138.1–138.4 (C), 141.6–142.6 (C), 147.5–147.8 (CH), 148.8–149.0 (CH), 152.4 (C), 153.8–155.3 (C), 157.7–158.0 (C), 159.2 (C), 160.3–160.8 (C), 164.9–165.0 (C), 174.4–175.3 (C); ν_{max}/cm^{-1} 1040 and 1218 (C–O ester), 1586 (C═O imide), 1712 (C═O ester); *m/z* (ESI) 676 (MH^+, 3%), 658 (MH^+-H_2O, 100); (Found: MH^+ 676.2452, $C_{42}H_{34}N_3O_6$ requires 676.2442).

4.1.12. 5-(α-Hydroxy-α-2-pyridylbenzyl)-*N*-*p*-methoxybenzoyl oxymethyl-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (15)

A similar procedure[24] to that previously described for the preparation of **1** was followed using NRB (200 mg, 0.39 mmol) in dimethylformamide (1 mL), chloromethyl p-methoxybenzoate (**48**) (157 mg, 0.78 mmol) in dimethylformamide (0.5 mL) and potassium carbonate (54 mg, 0.39 mmol). The mixture was stirred at room temperature for 16 h, taken up in dichloromethane/water (1:1) (15 mL), washed with water (2 × 10 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (hexane/ethyl acetate 1:1) afforded **15** as a colourless solid (172 mg, 0.25 mmol, 65%). mp 94–112 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.40 (0.2H, dd, *J* = 8.0 and 4.4 Hz, W/H-3), 3.47 (0.4H, dd, *J* = 8.0 and 4.8 Hz, Y/H-3), 3.53–3.62 (0.5H, m, 0.2H U/H-2 and U/H-3, 0.3H V/H-2), 3.65–3.67 (0.2H, m, W/H-4), 3.68–3.78 (0.9H, m, 0.3H V/H-3, 0.2H W/H-2 and 0.4H Y/H-2), 3.84 and 3.85 (3H, s, OMe), 3.91–3.95 (0.4H, m, 0.1H U/H-1 and 0.3H V/H-1), 4.00–4.04 (0.4H, m, Y/H-4), 4.22–4.24 (0.1H, m, U/H-4), 4.34–4.40 (0.3H, m, V/H-4), 4.51–4.56 (0.6H, m, 0.2H W/H-1 and 0.4H Y/H-1), 5.51–5.54, 5.59–5.60, 5.64–5.70 and 5.78–5.81 (3.7H, m, 2H CH_2, 0.3H V/H-6, 0.4H Y/H-6 and 1 OH), 6.10–6.12 (0.3H, m, 0.1H U/H-6 and 0.2H W/H-6), 6.71–7.61 and 7.95–8.02 (20H, m, Ar), 8.41–8.54 (1.4H, m, 0.2H 2U/αPyr, 0.6H 2V/αPyr, 0.2H W/αPyr and 0.4H Y/αPyr), 8.61–8.65 (0.6H, m, 0.2H W/αPyr and 0.4H Y/αPyr); ^{13}C NMR (100 MHz, $CDCl_3$) δ 44.2–47.1 (CH), 49.2–49.8 (CH), 55.5 (CH_3), 62.0–62.3 (CH_2), 77.4 (CH), 77.9–78.0 (C), 113.7 (CH), 121.5–122.8 (CH), 124.0–124.4 (CH), 126.8–130.4 (CH), 132.0 (CH), 133.2 (CH), 134.0 (CH), 136.0–136.9 (CH), 138.4–138.7 (C), 142.0–143.3 (C), 148.0–148.2 (CH), 149.2–149.4 (CH), 152.8 (C), 154.2–155.7 (C), 158.1–158.4 (C), 160.8–161.2 (C), 163.7 (C), 165.1–165.2 (C), 174.8–175.6 (C); ν_{max}/cm^{-1} 1079 and 1251 (C–O ester), 1584 (C═O imide), 1712 (C═O ester); *m/z* (ESI) 676 (MH^+, 1%), 658 (MH^+-H_2O, 100); (Found: MH^+ 676.2434, $C_{42}H_{34}N_3O_6$ requires 676.2442).

4.1.13. 5-(α-Hydroxy-α-2-pyridylbenzyl)-*N*-phenylacetyloxymethyl-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (16)

A similar procedure[24] to that previously described for the preparation of **1** was followed using NRB (200 mg, 0.39 mmol) in dimethylformamide (2 mL), chloromethyl phenylacetate (**49**) (144 mg, 0.78 mmol) in dimethylformamide (0.4 mL) and potassium carbonate (54 mg, 0.39 mmol). The mixture was stirred at room temperature for 16 h, taken up in chloroform (15 mL), washed with water (2 × 10 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (hexane/ethyl acetate 1:2) afforded **16** as a colourless solid (50 mg, 0.08 mmol, 19%). mp 71–78 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.35 (0.2H, dd, *J* = 7.9 and 4.5 Hz, W/H-3), 3.40 (0.4H, dd, *J* = 7.8 and 4.5 Hz, Y/H-3), 3.45 (0.3H, dd, *J* = 7.9 and 5.0 Hz, V/H-2), 3.62–3.71 (3.3H, m, 2H C*H*$_2$Ph, 0.2H U/H-2 and U/H-3, 0.3H V/H-3, 0.4H W/H-2 and W/H-4, 0.4H Y/H-2), 3.87–3.94 (0.4H, m, 0.1H U/H-1 and 0.3H V/H-1), 3.99–4.00 (0.4H, m, Y/H-4), 4.10–4.11 (0.1H, m, U/H-4), 4.27–4.29 (0.3H, m, V/H-4), 4.43–4.46 (0.6H, m, 0.2H W/H-1 and 0.4H Y/H-1), 5.30 (0.45H, s, H_a/NCH_2O), 5.31 (0.45H, s, H_b/NCH_2O), 5.48–5.52 (1.5H, m, 0.3H V/H-6, 0.4H Y/H-6, 0.8H NCH_2O and OH), 5.56–5.62 (1.3H, m, NCH_2O and OH), 6.06–6.07 (0.3H, m, 0.1H U/H-6 and 0.2H W/H-6), 6.74–7.59 (21H, m, Ar), 8.46–8.47 (0.2H, m, 2U/αPyr), 8.50–8.51 (1.2H, m, 0.6H 2V/αPyr, 0.2H W/αPyr and 0.4H Y/αPyr), 8.63–8.65 (0.6H, m, 0.2H W/αPyr and 0.4H Y/αPyr); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.6 (CH_2), 41.4 (CH_2), 44.4–46.6 (CH), 49.1–49.6 (CH), 62.0–62.3 (CH_2), 77.7–77.8 (C), 121.7–122.7 (CH), 124.3–124.4 (CH), 126.7–129.5 (CH), 130.3 (CH), 133.1 (C), 133.9 (C), 136.3–136.7 (CH), 138.2–138.3 (C), 141.9–142.2 (CH), 147.5–148.2 (C), 149.0 (C), 152.6–155.6 (C), 157.8–158.2 (C), 161.0 (C), 170.6 (C), 174.4–175.4 (C); ν_{max}/cm^{-1} 1138 and 1211 (C–O ester), 1585 (C═O imide), 1714 (C═O ester); *m/z* (FAB+) 660 (100%); (Found: MH^+ 660.2501, $C_{42}H_{34}N_3O_5$ requires 660.2498).

4.1.14. *N*-Diphenylacetyloxymethyl-5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (17)

A similar procedure[24] to that previously described for the preparation of **1** was followed using NRB (200 mg, 0.39 mmol) in dimethylformamide (2 mL), chloromethyl diphenylacetate (**50**)

(102 mg, 0.39 mmol) in dimethylformamide (0.5 mL) and potassium carbonate (54 mg, 0.39 mmol). The mixture was stirred at room temperature for 16 h, taken up in chloroform (15 mL), washed with water (2 × 10 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (hexane/ethyl acetate 2:1) afforded **17** as a colourless solid (42 mg, 0.06 mmol, 15%). mp 113–115 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.35 (0.7H, dd, J = 7.9 and 4.6 Hz, Y/H-3), 3.41 (0.3H, dd, J = 7.9 and 4.9 Hz, V/H-2), 3.58–3.67 (1H, m, V/H-3 and Y/H-2), 3.86–3.89 (0.3H, m, V/H-1), 3.96 (0.7H, dt, J = 4.6 and 1.4 Hz, Y/H-4), 4.29 (0.3H, dt, J = 4.5 and 1.5 Hz, V/H-4), 4.45–4.48 (0.7H, m, Y/H-1), 5.05 (0.7H, s, Y/C*H*Ph_2), 5.06 (0.3H, s, V/C*H*Ph_2), 5.36 (0.5H, s, H_a/CH_2), 5.39 (0.5H, s, H_b/CH_2), 5.46–5.49 (2H, m, 0.6H V/H-6 and V/OH, 1.4H Y/H-6 and Y/OH), 5.64–5.66 (1H, m, CH_2), 6.70–7.58 (26H, m, Ar), 8.47–8.50 (1.3H, m, 0.6H 2V/αPyr and 0.7H Y/αPyr), 8.62–8.63 (0.7H, m, Y/αPyr); ^{13}C NMR (100 MHz, $CDCl_3$) δ 44.5 (CH), 45.1 (CH), 45.9–46.5 (CH), 49.1–49.4 (CH), 56.5 (CH), 62.2–62.3 (CH_2), 77.8–77.9 (C), 121.8–121.9 (CH), 122.6–122.7 (CH), 124.1–124.3 (CH), 127.1–129.5 (CH), 130.2 (CH), 136.0–136.1 (CH), 136.5 (CH), 138.1–138.5 (C), 141.8 (C), 142.2 (C), 147.9–148.0 (CH), 149.1 (CH), 149.3 (CH), 154.1–154.3 (C), 154.8 (C), 155.6 (C), 157.9 (C), 158.3 (C), 161.2 (C), 171.6 (C), 175.0–175.4 (C); ν_{max}(NaCl)/cm^{-1} 1139 and 1215 (C–O ester), 1585 (C=O imide), 1715 (C=O ester); *m/z* (ESI+) 736 (MH^+, 27%), 120 (100); (Found: MH^+ 736.2814, $C_{48}H_{38}N_3O_5$ requires 736.2811).

4.1.15. *N*-Dihydrocinnamoyloxymethyl-5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (18)

Compound **18** was prepared by a procedure similar to that of Hursthouse and co-workers,[24] and Bodor and co-workers.[26] To a solution of chloromethyl dihydrocinnamate (**51**) (155 mg, 0.78 mmol) in acetone (1.5 mL) was added sodium iodide (117 mg, 0.78 mmol), and the mixture stirred at room temperature for 3 h. The solvent was removed in vacuo and the crude iodomethyl dihydrocinnamate was taken through to the next step without further purification. A solution of NRB (200 mg, 0.39 mmol), iodomethyl dihydrocinnamate and potassium carbonate (54 mg, 0.39 mmol) in dimethylformamide (1.5 mL) was stirred at room temperature for 16 h. The mixture was then taken up in chloroform (15 mL), washed with water (2 × 10 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (hexane/ethyl acetate 1:2) afforded **18** as a colourless solid (15 mg, 0.02 mmol, 6%). mp 89–94 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.66-2.71 (2H, m, $OCOCH_2$), 2.94-2.99 (2H, m, C*H*$_2$Ph), 3.36–3.40 (0.2H, m, W/H-3), 3.42–3.47 (0.3H, m, Y/H-3), 3.52–3.56 (0.7H, m, 0.4H U/H-2 and U/H-3, 0.3H V/H-2), 3.62–3.74 (1H, m, 0.3H V/H-3, 0.4H W/H-2 and W/H-4, 0.3H Y/H-2), 3.85-3.94 (0.5H, m, 0.2H U/H-1 and 0.3H V/H-1), 3.98-4.00 (0.3H, m, Y/H-4), 4.18–4.24 (0.2H, m, U/H-4), 4.35–4.40 (0.3H, m, V/H-4), 4.48–4.49 (0.5H, m, 0.2H W/H-1 and 0.3H Y/H-1), 5.29–5.33 (0.6H, m, 0.3H V/H-6 and 0.3H Y/H-6), 5.43–5.60 (3H, m, 2H NCH_2O and 1H OH), 6.03-6.07 (0.4H, m, 0.2H U/H-6 and 0.2H W/H-6), 6.74–7.63 (21H, m, Ar), 8.42–8.52 (1.5H, m, 0.4H 2U/αPyr, 0.4H 2V/αPyr, 0.2H W/αPyr and 0.3H Y/αPyr), 8.63-8.65 (0.5H, m, 0.2H W/αPyr and 0.3H Y/αPyr); ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.6 (CH_2), 35.4 (CH_2), 44.5–46.7 (CH), 49.2–49.7 (CH), 61.7–61.9 (CH_2), 79.2 (C), 121.8–122.7 (CH), 124.0–124.8 (CH), 126.3–130.3 (CH), 133.8–134.0 (CH), 136.0–136.8 (CH), 138.4–138.6 (C), 140.2 (C), 142.0 (C), 147.9–148.4 (CH), 149.2–149.4 (CH), 152.7 (C), 155.6 (C), 158.4 (C), 161.1 (C), 168.4 (C), 171.8 (C), 175.5 (C); ν_{max}(NaCl)/cm^{-1} 1141 and 1214 (C–O ester), 1582 (C=O imide), 1717 (C=O ester); *m/z* (ESI+) 674 (MH^+, 100%), 656 (MH^+-H_2O, 15); (Found: MH^+ 674.2649, $C_{43}H_{36}N_3O_5$ requires 674.2665).

4.1.16. *N*-Cinnamoyloxymethyl-5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (19)

A similar procedure[24] to that previously described for the preparation of **1** was followed using NRB (174 mg, 0.34 mmol) in dimethylformamide (1.5 mL), chloromethyl cinnamate (**52**) (68 mg, 0.34 mmol) in dimethylformamide (0.5 mL) and potassium carbonate (47 mg, 0.34 mmol). The mixture was stirred at room temperature for 16 h, taken up in chloroform (15 mL), washed with water (2 × 10 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (hexane/ethyl acetate 4:1, then 1:1) afforded **19** as a colourless solid (28 mg, 0.04 mmol, 12%). mp 108–113 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.46 (0.8H, dd, J = 8.0 and 4.6 Hz, Y/H-3), 3.55 (0.2H, dd, J = 8.0 and 4.6 Hz, V/H-2), 3.69–3.77 (1H, m, 0.2 V/H-3 and 0.8 Y/H-2), 3.90–3.94 (0.2H, m, V/H-1), 4.00 (0.8H, dt, J = 4.5 and 1.4 Hz, Y/H-4), 4.37 (0.2H, dt, J = 4.5 and 1.4 Hz, V/H-4), 4.51–4.54 (0.8H, m, Y/H-1), 5.43 (0.5H, s, H_a/CH_2), 5.46 (0.5H, s, H_b/CH_2), 5.53–5.56 (1.8H, m, 0.2H V/H-6, 0.8H Y/H-6 and 0.8H OH), 5.64 (0.2H, bs, OH), 5.69 (0.5H, s, H_a/CH_2), 5.70 (0.5H, s, H_b/CH_2), 6.43 (0.8H, d, J = 16.1 Hz, Y/OCOCH), 6.45 (0.2H, d, J = 16.1 Hz, V/OCOCH), 6.75–7.59 (21H, m, Ar), 7.70 (1H, d, J = 16.1 Hz, 0.2H V/C*H*Ph and 0.8H Y/C*H*Ph), 8.48–8.50 (0.2H, m, V/αPyr), 8.52–8.55 (1H, m, 0.2H V/αPyr and 0.8H Y/αPyr), 8.62–8.64 (0.8H, m, Y/αPyr); ^{13}C NMR (75 MHz, $CDCl_3$) δ 44.5 (CH), 45.2 (CH), 46.0–46.2 (CH), 46.8 (CH), 49.2–49.5 (CH), 62.0 (CH_2), 77.8–77.9 (C), 116.9 (CH), 121.8–122.0 (CH), 122.6–122.7 (CH), 124.1–124.4 (CH), 127.4 (CH), 127.4–129.7 (CH), 130.4–130.5 (CH), 134.2 (C), 136.0 (CH), 136.5 (CH), 138.4–138.6 (C), 142.0–142.4 (C), 146.1 (CH), 148.0 (CH), 149.2–149.4 (C), 154.1 (C), 154.9 (C), 158.4 (C), 161.1 (C), 165.7 (C), 175.5 (C); ν_{max}/cm^{-1} 1144 and 1202 (C–O ester), 1584 (C=O imide), 1712 (C=O ester); *m/z* (FAB+) 672 (MH^+, 100%); (Found: MH^+ 672.2486, $C_{43}H_{34}N_3O_5$ requires 672.2498).

4.1.17. 5-(α-Hydroxy-α-2-pyridylbenzyl)-2′-naphthoyloxymethyl-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (20)

A similar procedure[24] to that previously described for the preparation of **1** was followed using NRB (184 mg, 0.36 mmol) in dimethylformamide (1.5 mL), chloromethyl 2-naphthoate (**53**) (80 mg, 0.36 mmol) in dimethylformamide (0.5 mL) and potassium carbonate (47 mg, 0.34 mmol). The mixture was stirred at room temperature for 16 h, taken up in chloroform (15 mL), washed with water (2 × 10 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (hexane/ethyl acetate 4:1, then 1:1) afforded **20** as a colourless solid (80 mg, 0.11 mmol, 32%). mp 108–112 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.51 (0.8H, dd, J = 7.9 and 4.5 Hz, Y/H-3), 3.60 (0.2H, dd, J = 7.9 and 5.0 Hz, V/H-2), 3.74–3.81 (1H, m, 0.2H V/H-3 and 0.8H Y/H-2), 3.94–3.96 (0.2H, m, V/H-1), 4.03–4.04 (0.8H, m, Y/H-4), 4.39–4.40 (0.2H, m, V/H-4), 4.54–4.57 (0.8H, m, Y/H-1), 5.55–5.57 (1.8H, m, 0.2H V/H-6, 0.8H Y/H-6 and 0.8H OH), 5.61–5.63 (1.2H, m, CH_2 and OH), 5.87 (0.1H, s, H_a/CH_2), 5.88 (0.4H, s, H_a/CH_2), 5.89 (0.1H, s, H_b/CH_2), 5.90 (0.4H, s, H_b/CH_2), 6.73–7.61 (18H, m, Ar), 7.86–7.95 (3H, m, Ar), 8.02–8.05 (1H, m, Ar), 8.48–8.52 (1.2H, m, 0.4H 2V/αPyr and 0.8H Y/αPyr), 8.59–8.64 (1.8H, m, 0.8H Y/αPyr and 1H Ar); ^{13}C NMR (75 MHz, $CDCl_3$) δ 45.2 (CH), 46.1 (CH), 46.7 (CH), 49.5 (CH), 62.5 (CH_2), 77.8 (C), 121.7–121.8 (CH), 122.7 (CH), 124.3 (CH), 125.2 (CH), 126.4–128.4 (CH), 129.4 (CH), 130.3 (CH), 131.6 (CH), 132.3 (C), 135.7 (C), 136.0 (CH), 136.5 (CH), 138.4 (C), 141.9 (C), 148.0 (CH), 149.2 (CH), 154.1 (C), 154.8 (C), 158.4 (C), 161.1 (C), 165.7 (C), 175.6 (C); ν_{max}/cm^{-1} 1079 and 1263 (C–O ester), 1585 (C=O imide), 1712 (C=O ester); *m/z* (FAB+) 696 (MH^+, 9%), 120 (100); (Found: MH^+ 696.2504, $C_{45}H_{34}N_3O_5$ requires 696.2498).

4.1.18. 5-(α-Hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-*N*-succinoyloxymethyl-5-norbornene-2,3-dicarboximide dimer (21)

A similar procedure[24] to that previously described for the preparation of **1** was followed using NRB (200 mg, 0.39 mmol) in dimethylformamide (2 mL), dichloromethyl succinate (**60**) (42 mg, 0.20 mmol) in dimethylformamide (0.5 mL) and potassium carbonate (54 mg, 0.40 mmol). The mixture was stirred at room temperature for 16 h, taken up in chloroform (20 mL), washed with water (2 × 10 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (chloroform/methanol 100:1) afforded **21** as a colourless solid (40 mg, 0.03 mmol, 17%). mp 129–148 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.65–2.71 (4H, m, 2x $OCOCH_2$), 3.38–3.76 (4.3H, m, H-1, H-2, H-3 and H-4), 3.87–3.93 (0.4H, m, H-1, H-2, H-3 and H-4), 3.98–3.99 (1.1H, m, H-1, H-2, H-3 and H-4), 4.17–4.19 (0.4H, m, H-1, H-2, H-3 and H-4), 4.34–4.35 (0.4H, m, H-1, H-2, H-3 and H-4), 4.49–4.51 (1.4H, m, H-1, H-2, H-3 and H-4), 5.30–5.34 (1.5H, m, NCH_2O and OH), 5.48–5.64 (5.1H, m, NCH_2O, H-6 and OH), 5.79–5.80 (0.6H, m, NCH_2O, H-6 and OH), 6.04–6.07 (0.8H, m, H-6 and OH), 6.74–7.61 (32H, m, Ar), 8.42–8.47 (1.2H, m,αPyr), 8.55–8.56 (1.3H, m,αPyr), 8.61–8.62 (1.5H, m,αPyr); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.6 (CH_2), 44.7–46.6 (CH), 49.5 (CH), 49.7 (CH), 61.7–62.0 (CH_2), 77.7 (C), 121.7–122.7 (CH), 124.3 (CH), 126.6–130.3 (CH), 133.9 (CH), 136.0–136.6 (CH), 138.4–138.6 (C), 142.0 (C), 142.8 (C), 148.0–148.1 (CH), 149.2 (CH), 152.7 (C), 154.0–155.0 (C), 158.0–158.3 (C), 160.7–161.0 (C), 170.8–170.9 (C), 174.6 (C), 175.2–175.4 (C); ν_{max}(NaCl)/cm^{-1} 1144 and 1265 (C–O ester), 1585 (C═O imide), 1716 (C═O ester); *m/z* (FAB+) 1165 (MH^+, 14%), 397 (100); (Found: MH^+ 1165.4106, $C_{72}H_{57}N_6O_{10}$ requires 1165.4136).

4.1.19. *N*-Adipoyloxymethyl-5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide dimer (22)

A similar procedure[24] to that previously described for the preparation of **1** was followed using NRB (100 mg, 0.20 mmol) in dimethylformamide (1 mL), dichloromethyl adipate (**61**) (24 mg, 0.10 mmol) in dimethylformamide (0.5 mL) and potassium carbonate (27 mg, 0.20 mmol). The mixture was stirred at room temperature for 16 h, taken up in chloroform (10 mL), washed with water (2 × 5 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (chloroform/methanol 100:1) afforded **22** as a colourless solid (40 mg, 0.03 mmol, 16%). mp 117–124 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.56–1.80 (4H, m, 2× $OCOCH_2CH_2$), 2.26–2.48 (4H, m, 2× $OCOCH_2$), 3.37–3.78 (4.5H, m, H-1, H-2, H-3 and H-4), 3.84–3.92 (0.7H, m, H-1, H-2, H-3 and H-4), 3.97–3.99 (0.9H, bm, H-1, H-2, H-3 and H-4), 4.16–4.21 (0.2H, bm, H-1, H-2, H-3 and H-4), 4.32–4.39 (0.5H, bm, H-1, H-2, H-3 and H-4), 4.45–4.55 (1.2H, bm, H-1, H-2, H-3 and H-4), 5.28–5.36 (1.4H, m, NCH_2O and OH), 5.42–5.61 (5.4H, m, NCH_2O, H-6 and OH), 5.74, 5.75 (0.5H, bs, OH), 5.82 (0.1H, bs, OH), 6.03–6.06 (0.6H, m, H-6 and OH), 6.74–7.58 (32H, m, Ar), 8.43–8.54 (2.8H, m,αPyr), 8.61–8.62 (1.2H, m, αPyr); 13C NMR (75 MHz, $CDCl_3$) δ 23.8–23.9 (CH_2), 33.3–33.4 (CH_2), 44.1–47.0 (CH), 49.2–49.7 (CH), 61.6–61.8 (CH_2), 77.8 (C), 78.7 (C) 79.1 (C), 121.7–122.8 (CH), 123.9–124.3 (CH), 126.7–130.3 (CH), 133.9 (CH), 135.8–136.0 (CH), 136.5–136.6 (CH), 138.4–138.6 (C), 142.0 (C), 142.4 (C), 142.9 (C), 147.8–148.2 (C), 149.2–149.4 (C), 152.7 (C), 154.0–155.6 (C), 158.1–158.4 (C), 160.7–161.1 (C), 171.9–172.1 (C), 174.7–175.5 (C); ν_{max}(NaCl)/cm^{-1} 1139 and 1216 (C–O ester), 1585 (C═O imide), 1717 (C═O ester); *m/z* (FAB+) 1193 (MH^+ 33%), 397 (100); (Found: MH^+ 1193.4463, $C_{74}H_{61}N_6O_{10}$ requires 1193.4449).

4.1.20. 5-(α-Hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-*N*-suberoyloxymethyl-5-norbornene-2,3-dicarboximide dimer (23)

A similar procedure[24,26] to that previously described for the preparation of **18** was followed using dichloromethyl suberate (**62**) (44 mg, 0.16 mmol) in acetone (0.2 mL), and sodium iodide (49 mg, 0.32 mmol) in acetone (0.2 mL), at room temperature for 3 h. The solvent was removed in vacuo and the crude diiodomethyl suberoate was taken through to the next step without further purification. A solution of NRB (164 mg, 0.32 mmol), diiodomethyl suberoate and potassium carbonate (50 mg, 0.36 mmol) in dimethylformamide (1.5 mL) was stirred at room temperature for 48 hours. The mixture was taken up in chloroform (15 mL), washed with water (2 × 10 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (hexane/ethyl acetate 1:2) afforded **23** as a colourless solid (15 mg, 0.01 mmol, 6%). mp 112–116 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20–1.41 (4H, m, 2× $OCOCH_2CH_2CH_2$), 1.49–1.76 (4H, m, 2× $OCOCH_2CH_2$), 2.21–2.44 (4H, m, 2× $OCOCH_2$), 3.36 (0.6H, dd, *J* = 8.1 and 4.5 Hz, W/H-3), 3.43 (0.8H, dd, *J* = 7.8 and 4.5 Hz, Y/H-3), 3.52–3.74 (3.2H, m, 1.2H V/H-2 and V/H-3, 1.2H W/H-2 and W/H-4 and 0.8H Y/H-2), 3.86–3.92 (0.6H, m, V/H-1), 3.95–4.03 (0.8H, m, Y/H-4), 4.34–4.39 (0.6H, m, V/H-4), 4.48–4.59 (1.4H, m, 0.6H W/H-1 and 0.8H Y/H-1), 5.27 (0.85H, s, H_a/NCH_2O), 5.30 (0.85H, s, H_b/NCH_2O), 5.45–5.58 (5.3H, m, 2.3H NCH_2O, 0.6H V/H-6, 0.8H Y/H-6 and 1.6H OH), 5.74 (0.4H, s, OH), 6.05–6.06 (0.6H, m, W/H-6), 6.73–7.60 (32H, m, Ar), 8.48–8.54 (2.6H, m, 1.2H 2V/αPyr, 0.6H W/αPyr, 0.8H Y/αPyr), 8.62–8.64 (1.4H, m, 0.6H W/αPyr and 0.8H Y/αPyr); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.3–24.4 (CH_2), 28.6 (CH_2), 29.7 (CH_2), 33.7–33.8 (CH_2), 44.5–46.7 (CH), 49.2–49.7 (CH), 61.9 (CH_2), 77.8 (C), 121.7–121.9 (CH), 122.6–122.7 (CH), 124.3 (CH), 126.7–129.6 (CH), 130.3 (CH), 136.0–136.6 (CH), 138.4 (C), 142.0 (C), 148.0 (CH), 149.2–149.3 (CH), 154.1 (C), 154.9 (C), 158.4 (C), 161.1 (C), 172.6 (C), 175.4–175.5 (C); ν_{max}/cm^{-1} 1118 and 1211 (C–O ester), 1584 (C═O imide), 1715 (C═O ester); *m/z* (FAB+) 1221 (MH^+, 28%), 397 (100); (Found: MH^+ 1221.4771, $C_{76}H_{65}N_6O_{10}$ requires 1221.4762).

4.1.21. 5-(α-Hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-N-sebacoyloxymethyl-5-norbornene-2,3-dicarboximide dimer (24)

A similar procedure[24] to that previously described for the preparation of **1** was followed using NRB (100 mg, 0.20 mmol) in dimethylformamide (1 mL), dichloromethyl sebacate (**63**) (29 mg, 0.10 mmol) in dimethylformamide (0.2 mL) and potassium carbonate (27 mg, 0.20 mmol). The mixture was stirred at room temperature for 48 h, taken up in chloroform (10 mL), washed with water (2 × 5 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (chloroform/methanol 100:1) afforded **24** as a colourless solid (101 mg, 0.08 mmol, 81%). mp 113–121 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23–1.37 (8H, bm, 2x $OCO(CH_2)_2(CH_2)_2$), 1.55–1.67 (4H, bm, 2× $OCOCH_2CH_2$), 2.26–2.38 (4H, m, 2x $OCOCH_2$), 3.37 (0.4H, dd, *J* = 7.9 and 4.5 Hz, W/H-3), 3.43 (0.9H, dd, *J* = 7.9 and 4.5 Hz, Y/H-3), 3.50–3.59 (0.9H, m, 0.4H U/H-2 and U/H-3 and 0.5H V/H-2), 3.62–3.74 (2.2H, m, 0.5H V/H-3, 0.8H W/H-2 and W/H-4 and 0.9H Y/H-2), 3.88–3.92 (0.7H, m, 0.2H U/H-1 and 0.5H V/H-1), 3.98 (0.9H, dt, *J* = 4.4 and 1.3 Hz, Y/H-4), 4.19–4.20 (0.2H, m, U/H-4), 4.36–4.37 (0.5H, m, V/H-4), 4.50–4.53 (1.3H, m, 0.4H W/H-1 and 0.9H Y/H-1), 5.28–5.31 (1.4H, m, NCH_2O), 5.42–5.60 (4H, m, 2.6H NCH_2O, 0.5H V/H-6, 0.9H Y/H-6), 5.74 (2H, s, OH), 6.03 (0.2H, dd, *J* = 3.3 and 1.1 Hz, U/H-6), 6.05 (0.4H, dd, *J* = 3.3 and 1.1 Hz, W/H-6), 6.74–7.59 (32H, m, Ar), 8.41–8.48 (1.4H, m, 0.4H 2U/αPyr and 1H 2V/αPyr),

8.52–8.53 (1.3H, m, 0.4H W/αPyr and 0.9H Y/αPyr) 8.61–8.63 (1.3H, m, 0.4H W/αPyr and 0.9H Y/αPyr); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.5 (CH_2), 28.8–28.9 (CH_2), 33.7–33.8 (CH_2), 44.0–46.9 (CH), 49.1–49.6 (CH), 61.5–61.8 (CH_2), 77.5–77.7 (C), 79.0 (C), 121.6–122.2 (CH), 122.4–122.7 (CH), 123.9–124.2 (CH), 126.6–130.2 (CH), 133.1 (CH), 133.9 (CH), 135.8–136.6 (CH), 138.3–138.5 (C), 141.9–143.1 (C), 147.7–148.1 (CH), 149.1–149.3 (CH), 152.6 (C), 153.9–155.5 (C), 158.0–158.3 (C), 160.6–161.0 (C), 172.3–172.6 (C), 174.6–175.4 (C); ν_{max}(NaCl)/cm^{-1} 1152 and 1211 (C–O ester), 1585 (C═O imide), 1718 (C═O ester); *m/z* (FAB+) 1249 (MH^+, 19%), 397 (100); (Found: MH^+ 1249.5065, $C_{78}H_{69}N_6O_{10}$ requires 1249.5075).

4.1.22. *N*-Dodecanedioyloxymethyl-5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide dimer (25)

A similar procedure[24] to that previously described for the preparation of **1** was followed using NRB (200 mg, 0.39 mmol) in dimethylformamide (1 mL), dichloromethyl dodecanedioate (**64**) (66 mg, 0.20 mmol) in dimethylformamide (0.2 mL) and potassium carbonate (54 mg, 0.39 mmol). The mixture was stirred at room temperature for 48 h, taken up in chloroform (10 mL), washed with water (2 × 5 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (hexane/ethyl acetate 1:2) afforded **25** as a colourless solid (60 mg, 0.05 mmol, 12%). mp 105–110 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20–1.39 (12H, bm, 2× $OCO(CH_2)_2(CH_2)_3$), 1.54–1.73 (4H, m, 2× $OCOCH_2CH_2$), 2.28–2.40 (4H, m, 2× $OCOCH_2$), 3.43 (1.5H, dd, *J* = 7.8 and 4.5 Hz, Y/H-3), 3.52 (0.5H, dd, *J* = 8.1 and 5.0 Hz, V/H-2), 3.66–3.74 (2H, m, 0.5H V/H-3, 1.5H Y/H-2), 3.88–3.92 (0.5H, m, V/H-1), 3.98–4.00 (1.5H, m, Y/H-4), 4.35–4.37 (0.5H, m, V/H-4), 4.50–4.53 (1.5H, m, Y/H-1), 5.28–5.32 (2H, m, NCH_2O), 5.46–5.59 (5.5H, m, 2H NCH_2O, 0.5H V/H-6, 1.5H Y/H-6 and 1.6H OH), 5.74 (0.4H, s, OH), 6.74–7.59 (32H, m, Ar), 8.48–8.54 (2.5H, m, 1H 2V/αPyr and 1.5H Y/αPyr), 8.62–8.64 (1.5H, m, 1.5H Y/αPyr); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.6 (CH_2), 29.0–29.6 (CH_2), 33.8–33.9 (CH_2), 44.5–46.7 (CH), 49.2–49.4 (CH), 61.8 (CH_2), 77.8 (C), 79.0 (C), 121.7–122.0 (CH), 122.6–122.8 (CH), 124.1–124.3 (CH), 126.7–130.3 (CH), 136.0–136.5 (CH), 138.4–138.6 (C), 142.0–142.4 (C), 147.8–147.9 (CH), 149.2–149.4 (CH), 154.0 (C), 154.9 (C), 155.6 (C), 158.0–158.4 (C), 161.1 (C), 172.5–172.7 (C), 175.2–175.5 (C); ν_{max}/cm^{-1} 1149 and 1209 (C–O ester), 1585 (C═O imide), 1713 (C═O ester); *m/z* (ESI+) 1277 (MH^+, 28%), 627 (100); (Found: MH^+ 1277.5441, $C_{76}H_{65}N_6O_{10}$ requires 1277.5383).

4.1.23. 5-(α-Hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-*N*-terephthaloyloxymethyl-5-norbornene-2,3-dicarboximide dimer (26)

A similar procedure[24] to that previously described for the preparation of **1** was followed using NRB (200 mg, 0.39 mmol) in dimethylformamide (1 mL), dichloromethyl terephthalate (**65**) (53 mg, 0.20 mmol) in dimethylformamide (0.2 mL) and potassium carbonate (54 mg, 0.39 mmol). The mixture was stirred at room temperature for 48 h, taken up in chloroform (10 mL), washed with water (2 × 5 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (hexane/ethyl acetate 1:2) afforded **26** as a colourless solid (49 mg, 0.04 mmol, 21%). mp 150–157 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.42 (0.5H, dd, *J* = 8.0 and 4.4 Hz, W/H-3), 3.49 (0.8H, dd, *J* = 8.0 and 4.4 Hz, Y/H-3), 3.55–3.65 and 3.70–3.80 (3.2H, 0.4H U/H-2 and U/H-3, 1H V/H-2 and V/H-3, 1H W/H-1 and W/H-2, 0.8H Y/H-2), 3.90–3.95 (0.7H, m, 0.2H U/H-1 and 0.5H V/H-1), 3.99–4.05 (0.8H, m, Y/H-4), 4.19–4.23 (0.2H, m, U/H-4), 4.35–4.40 (0.5H, m, V/H-4), 4.48–4.56 (1.3H, bm, 0.5H W/H-1 and 0.8H Y/H-1), 5.53–5.57 (3.4H, m, NCH_2O and OH), 5.64–5.74 (2.3H, m, 1H NCH_2O and OH, 0.5H V/H-6, 0.8H Y/H-6), 5.82–5.86 (1.3H, m, NCH_2O and OH), 6.09–6.16 (0.7H, m, 0.2H U/H-6 and 0.5H W/H-6), 6.26 (0.3H, s, OH), 6.74–7.60 (32H, m, Ar), 8.04–8.16 (4H, m, Ar), 8.41–8.50 (2.7H, m, 0.4H 2U/αPyr, 1H 2U/αPyr, 0.5H W/αPyr and 0.8H Y/αPyr), 8.62–8.63 (1.3H, m, 0.5H W/αPyr and 0.8H/αPyr); ^{13}C NMR (100 MHz, $CDCl_3$) δ 44.1–47.0 (CH), 49.2–51.1 (CH), 62.3–62.6 (CH_2), 77.8 (C), 121.7–122.8 (CH), 124.0–124.3 (CH), 126.6–130.9 (CH), 133.2–134.0 (CH), 135.9–136.7 (CH), 138.3–138.5 (C), 141.8–143.1 (C), 147.8–148.0 (CH), 149.1–149.3 (CH), 152.6 (C), 154.0–155.6 (C), 157.9–158.3 (C), 160.6–161.0 (C), 164.3–164.6 (C), 174.6–175.5 (C); ν_{max}/cm^{-1} 1079 and 1241 (C–O ester), 1585 (C═O imide), 1713 (C═O ester); *m/z* (FAB+) 1213 (MH^+, 4%), 120 (100); (Found: MH^+ 1213.4124, $C_{76}H_{57}N_6O_{10}$ requires 1213.4136).

4.1.24. *N*-[α-(Ethylenebis(hydrogensuccinoyloxy))methyl]-5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide dimer (27)

A similar procedure[24,26] to that previously described for the preparation of **18** was followed using dichloromethyl ethylene bis(hydrogen succinate) (**68**) (56 mg, 0.2 mmol) in acetone (0.5 mL), and sodium iodide (30 mg, 0.2 mmol) in acetone (0.5 mL), at room temperature for 3 h. The solvent was removed in vacuo and the crude iodomethyl ethylene bis(hydrogen succinate) was taken through to the next step without further purification. A solution of NRB (200 mg, 0.39 mmol), diiodomethyl ethylene bis(hydrogen succinate) and potassium carbonate (54.0 mg, 0.40 mmol) in dimethylformamide (2 mL) was then stirred at room temperature for 16 h. The mixture was taken up in chloroform (20 mL), washed with water (2 × 10 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (chloroform/methanol 100:1, then hexane/ethyl acetate 4:1–3:2) afforded **27** as an oily colourless solid (68 mg, 0.05 mmol, 26%). mp 49 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.61–2.73 (8H, m, 2× $NCH_2OCO(CH_2)_2$), 3.37 (0.6H, dd, *J* = 7.9 and 4.4 Hz, W/H-3), 3.44 (1H, dd, *J* = 7.9 and 4.6 Hz, Y/H-3), 3.50–3.74 (3H, m, 0.4H U/H-2 and U/H-3, 0.4H V/H-2 and V/H-3, 1.2H W/H-2 and W/H-4 and 1H Y/H-2), 3.87–3.91 (0.4H, m, 0.2H U/H-1 and 0.2H V/H-1), 3.97 (1H, dt, *J* = 4.4 and 1.2 Hz, Y/H-4), 4.18–4.19 (0.2H, m, U/H-4), 4.26–4.36 (4.2H, m, 0.2H V/H-4, 4H $COO(CH_2)_2OCO$), 4.50–4.51 (1.6H, m, 0.6H W/H-1 and 1H Y/H-1), 5.30–5.34 (1.3H, m, NCH_2O and OH), 5.47–5.60 (5.9H, m, 4.7H NCH_2O and OH, 0.2H V/H-6, 1H Y/H-6), 6.04 (0.2H, dd, *J* = 3.3 and 1.2 Hz, U/H-6), 6.06 (0.6H, dd, *J* = 3.2 and 1.0 Hz, W/H-6), 6.75–7.59 (32H, m, Ar), 8.42–8.54 (2.4H, m, 0.4H 2U/αPyr, 0.4H 2V/αPyr, 0.6H W/αPyr and 1H Y/ααPyr), 8.62–8.63 (1.6H, m, 0.6H W/αPyr and 1H Y/αPyr); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.7 (CH_2), 29.6 (CH_2), 44.0–46.6 (CH), 49.1–49.6 (CH), 61.7–62.4 (CH_2), 77.7 (CH), 121.7–122.7 (CH), 123.9–124.3 (CH), 126.6–130.3 (CH), 133.2–134.0 (CH), 135.8–136.6 (CH), 138.3–138.6 (C), 141.9–142.8 (C), 147.8–148.1 (CH), 149.2–149.4 (CH), 152.6–155.5 (C), 158.0–158.3 (C), 160.6–161.0 (C), 171.0–171.7 (C), 174.6–175.4 (C); ν_{max}(NaCl)/cm^{-1} 1147 and 1214 (C–O ester), 1586 (C═O imide), 1714 (C═O ester); *m/z* (FAB+) 1309 (MH^+, 8%), 120 (100); (Found: MH^+ 1309.4546, $C_{78}H_{65}N_6O_{14}$ requires 1309.4559).

5. Experimental (Pharmacology)

5.1. Rat caudal artery and aortic ring isolation and recording of contractile force

Male Wistar rats (150–250 g) were obtained from Charles River Italia (Milano, Italy) and killed by decapitation. Ventral caudal artery was isolated, placed in Tyrode solution at room temperature

and cleaned of extraneous fatty and connective tissue under a dissection microscope. All vessels were cut into rings 2 mm long, mounted on a custom-built plexiglass support by means of two intraluminal tungsten wires and placed in 20 mL double-jacketed organ baths filled with Tyrode solution of the following composition (mM): NaCl 125, KCl 5, $CaCl_2$ 2.7, $MgSO_4$ 1, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11, maintained at 37 °C, pH 7.35, bubbled with 95% O_2 and CO_2. The endothelium was removed by gently rubbing the lumen of the rings with a very thin rough-surfaced tungsten wire (caudal artery). The mechanical activity of the rings was detected by means of an isometric force transducer (Ugo Basile, Comerio, Italy) coupled to a pen recorder (Ugo Basile, Comerio, Italy). Rings were passively stretched to impose a resting tension (2 g) and were allowed to equilibrate for 60 min. After equilibration, each ring was repeatedly simulated with both KCl (90 mM) and phenylephrine (10 μM) until reproducible responses were obtained. To verify the absence of the endothelium, rings contracted with 1 μM phenylephrine were exposed to 2 μm carbamylcholine. The absence of the endothelium was revealed by the lack of carbamylcholine-induced relaxation. Each compound was tested for the vasoconstrictor activity at a concentration of 50 μM, which has been previously reported to be the one evoking the maximal response to norbormide.[36] The contractile responses to the compounds were expressed as percent of the 90 mM KCl response.

5.2. Hydrolytic stability assay

All prodrugs were subject to a 1 h hydrolytic stability appraisal [200 μL total volume, at a final prodrug concentration of 200 μM, 2.5% DMSO overall, 37 °C, $n = 3$] using Tyrode solution for those candidates which displayed vasoconstrictory activity in the rat caudal artery contractile experiment, and phosphate buffer (0.1 M, pH 7.4) for those revealed to be non-vasoconstricting precleavage. Analysis was by RP-LCMS at an injection volume of 5 μL. Each prodrug and corresponding drug were subject to external calibration curves at 50, 100 and 200 μM to allow conversion of absorbance unit area into nmol, from which the percentage of drug released from each prodrug was calculated.

5.3. Rat serum assay

Rat serum was obtained from Sigma-Aldrich and stored at −78 °C. Similar assay conditions to those reported by Li Di and co-workers[37] were followed [200 μL total volume, 80% rat serum (diluting with phosphate buffer (0.1 M, pH 7.4)), at a final prodrug concentration of 200 μM, 2.5% DMSO overall, 37 °C, 3 h, $n = 3$] using an Eppendorf Thermomixer Compact. Reactions were quenched by transferring 150 μL of the incubation mixture to 450 μL of ice-cold acetonitrile, affording a final prodrug/drug concentration of 50 μM. Samples were centrifuged at 14,500×g for 15 min using an Eppendorf Mini Spin Plus centrifuge, at ambient temperature. 400 μL of the supernatant was removed and transferred to clean tubes. Analysis was by RP-LCMS at an injection volume of 20 μL. Each prodrug and corresponding drug were subject to external calibration curves at 50 μM, 100 and 200 μM to allow conversion of absorbance unit area into nmol, from which the percentage of drug released from each prodrug was calculated.

5.4. Rat liver S9 fraction assay

Rat liver S9 fraction (20 mg/mL), pooled from male rat (Sprague-Dawley), was obtained from Sigma–Aldrich, diluted to 1 mg/mL with phosphate buffer (0.1 M, pH 7.4) and stored at -78 °C. Similar assay conditions to those reported by Li Di and co-workers[37] were followed [200 μL total volume, 1 mg/mL rat liver S9 fraction (diluting with phosphate buffer (0.1 M, pH 7.4)), at a final prodrug concentration of 200 μM, 2.5% DMSO overall, 37 °C, 6 h, $n = 3$]. The assay protocol followed is as that described above for the rat serum assay, with an adjusted end-point of 6 h.

5.5. Simulated gastric fluid (SGF) assay

All in vivo prodrug candidates were subject to a 1 h hydrolytic stability appraisal in the presence of SGF, without pepsin[33] [200 μL total volume, sodium chloride solution (0.03 M) acidified to pH 1.2 with concentrated hydrochloric acid, at a final prodrug concentration of 200 μM, 2.5% DMSO overall, 37 °C, $n = 3$). The RP-LCMS analysis protocol followed is as that described above for the hydrolytic stability assay.

5.6. In vivo experiments and palatability trial

Wistar rats (150–200 g) were used to appraise the rodenticidal activity of the compounds put forward for in vivo evaluation. Briefly, prior to administration the prodrug candidates were dissolved in 0.2 M hydrochloric acid (5% DMSO overall), to the desired dose, and without delay either injected intravenously, via the tail, or orally gavaged. All treated animals were housed in a quiet room and monitored every minute (iv) or every 15 min (*oral*) to verify early signs of toxicity. Wistar rats (ca. 250 g) were presented with 1% w/w toxicant ($n = 6$) in a peanut butter bait, following 4 days 'pre-baiting' with a peanut butter formulation free of toxicant.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.05.014.

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