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Uncovering new structural insights for antimalarial activity from cost-effective aculeatin-like derivatives

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A series of new aculeatin-like analogues were synthesized in two steps by combining two sets of building blocks. Many compounds showed inhibitory activities *in vitro* against *Plasmodium falciparum* and have helped to gain more insight into structure-activity relationships around the spirocyclohexadienone pharmacophoric scaffold. *Plasmodium falciparum* thioredoxin reductase (PfTrxR) has been investigated as a putative cellular target. Moreover, a new aculeatin-like scaffold without Michael acceptor properties, efficient at 0.86 μM against *P. falciparum* 3D7, was identified and raises the prospect of developing a new antimalarial agent.

Introduction

Malaria is one of the most widespread parasitic infections in the world, infecting more than 207 million people and is responsible for an estimated 627 000 deaths in 2012,[1] mainly in tropical and subtropical regions and in Africa. WHO estimates that 77% of the deaths caused by malaria affect children under five years of age. Emergence of resistance to virtually all available antimalarial drugs has become a major concern for the control of *Plasmodium falciparum* infection.[2] Research and validation of new cellular targets in association with novel therapeutic agents are therefore urgently needed.[3] In this area, natural products extracted from plants used in traditional pharmacopoeia have been recognised for their long-standing contribution to the discovery of important antimalarial drugs like quinine or artemisinin.[4] Recent phenotypic screening efforts of large synthetic and natural products libraries have helped identify and explore new chemotypes to address this growing issue.[5] Within this context, we made accessible the synthesis of (–)-aculeatin A[6] (Figure 1), an antimalarial natural product extracted from the rhizomes of *Amomum aculeatum,* a plant used in Papua New Guinea pharmacopeia.[7] This scaffold is a new antimalarial chemotype. However, its chemical optimization and progression toward a safer drug is hampered by the lack of data on the cellular target(s). Another challenge in the fight against malaria is the need to develop low-cost and easily accessible drug, due to unequal access to medication for most of the concerned countries. Best candidates should be obtained quickly, simply and cost-effectively, thus proving the economic viability for their further development. This mainstream concept, extendable to the optimization and the manufacturing phases, could greatly accelerate the quest for the development of new affordable antimalarial drugs.

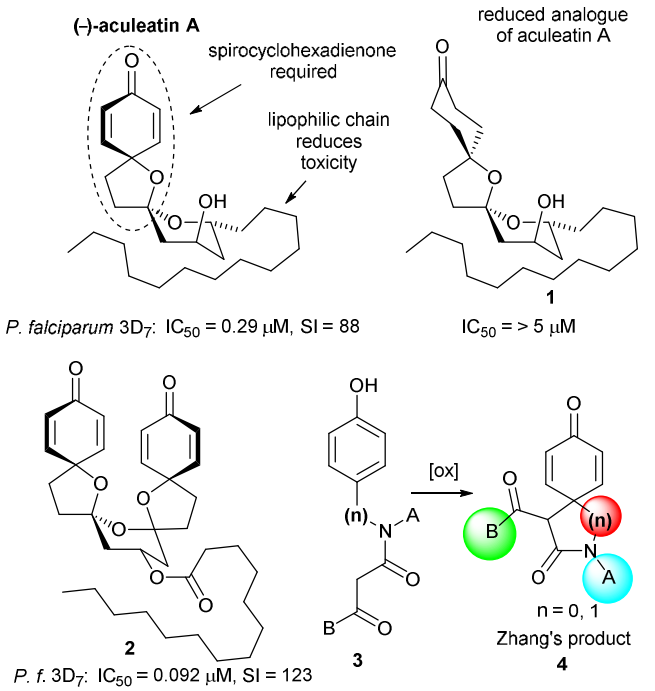


Figure 1. Structural features in aculeatin A for antimalarial activity. SI = selectivity index, human versus parasite cells

One apparent pharmacophoric group in aculeatin is the spirocyclohexadienone moiety which is prone to act as Michael acceptor by covalently interacting with key cellular targets in

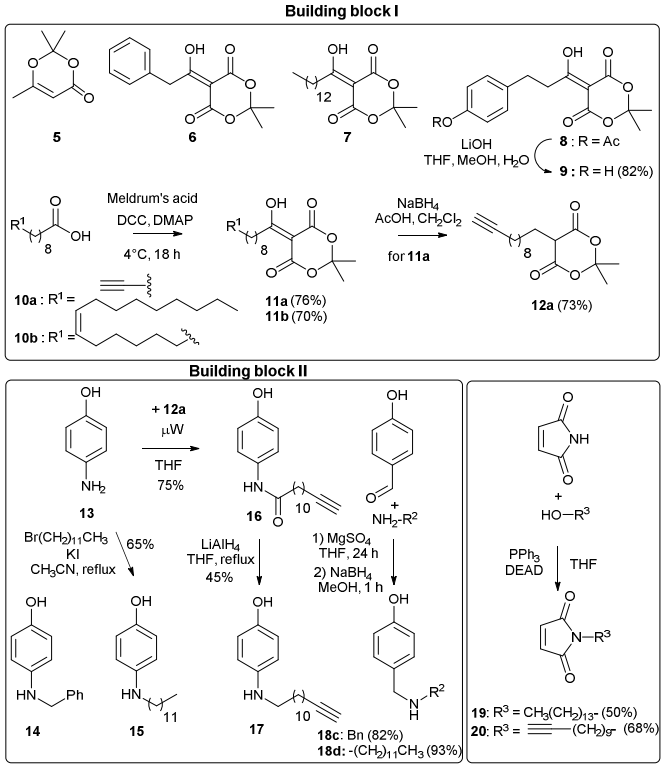
the parasite. Our previous investigations had showed that reduction of aculeatin, giving rise to the corresponding ketone analogue **1**, results in loss of antimalarial activity.[8] Building up aculeatin analogues having two spirocyclohexadienone units like **2** led in turn to improved antimalarial potency.

Starting from phenolic precursors **3** (Figure 1), Zhang and co-workers have reported an efficient access to new spirocyclohexadienone lactams **4** having four to five-membered ring.[9] We wondered whether the change of strain in the spirocyclic structure could influence the cyclohexadienone reactivity and alter antimalarial activity. In addition, changing oxygen by a nitrogen atom allows the introduction of an additional fragment (A) that brings a modular and steric hindrance nearby the pharmacophoric group. Using Zhang's phenolic oxidation as key step, we have developed an operationally simple and modular approach to make many different precursors in just a few steps. This has allowed the identification of cost-effective aculeatin-like derivatives having sub-micromolar antimalarial efficacy. In the course of our investigation, a new aculeatin-like scaffold without Michael acceptor moieties was established as being active against the parasite. Furthermore, on the assumption that *Plasmodium falciparum* thioredoxin reductase (PfTrxR) could be a potential cellular target, the inhibitory effects of representative bioactive analogues were evaluated on this enzyme.

Results and discussion

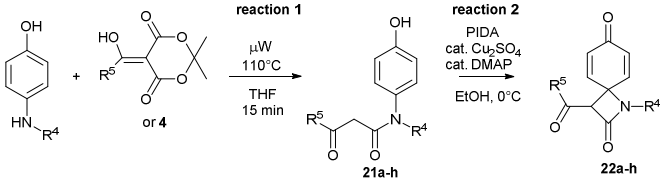
We made different phenolic precursors **3** by combining two building blocks I and II (Scheme 1) that simply react together under thermal condition without the need of protecting group and reagent. These precursors are conveniently obtained from simple starting materials. Phenol deprotection of **8**[8] gave the acylated Meldrum **9** in good yield. N-substituted aniline **15**[10] is synthesized by direct alkylation of 4-aminophenol **13** in 65% yield. Alternatively, **13** can react with the substituted Meldrum's acids **12a** to give the corresponding amide **16** which was reduced by $LiAlH_4$ to produce the N-substituted anilines **17**. Thus, the 2,2,6-trimethyl-1,3,-dioxin-4-one **5** and the acylated Meldrums **6**,[11] **7**,[8] **9** and **11a**,**b**[12], as precursors of the thermally inducible α-keto ketene species, cleanly condense under heating with the N-substituted anilines **14**,[13] **15**,[14] **17** and **18c-d** to give the corresponding β-keto amides **21a-h**, **23a-c**, **23e-g** and **25α**,**β** in moderate to good yields (Tables 1-3). Microwave irradiation allows the reaction to be complete within 15 min. We also prepared the maleimides **19** and **20** to evaluate whether another Michael acceptor group tethered with a lipophilic alkyl chain would exhibit antimalarial effect.

We first looked at the antimalarial activity for spirocyclohexadienones having four-membered ring **22** and examined the influence of their substituents R4 and R5 (Table 1). Conversion of **21a-h** into **22a-h** according to Zhang's oxidative procedure gave moderate to good yields (47-86%). Bioassays were conducted with *P. falciparum* 3D7 strain and have revealed that half of the compounds were indeed active. The localisation and the nature of the two appendages R4 and R5 influenced the bioactivity outcome. In either the absence of lipophilic alkyl chain (entries 4 and 5) or, in contrary, in the presence of too much lipophilicity (entries 3 and 9), activities are either reduced or lost. The best analogue **22f** (entry 6) is efficient with an IC_{50} = 1.35 μM. Its regioisomer **22b** reveals that the aliphatic long chain is best positioned at the R5 rather than R4 (entry 2). The two maleimides **19** and **20** were tested and turned out to be weakly active against *P. falciparum* 3D7 (IC_{50} = 27.5 μM and 37.9 μM, respectively) stressing the importance of the aculeatin-like scaffold for higher antimalarial activity.



Scheme 1

Table 1



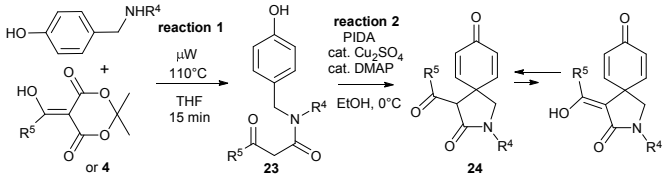
entry	**21** (%)[a]	**22** (%)[a]	R^4	R^5	IC_{50}[b] μM
1	**a** (91)	**a** (59)	$CH_3(CH_2)_{11}$	CH_3	3.46
2	**b** (68)	**b** (59)	$CH_3(CH_2)_{11}$	$PhCH_2$	>10
3	**c** (65)	**c** (61)	$CH_3(CH_2)_{11}$	$CH_3(CH_2)_{12}$	>10
4	**d** (82)	**d** (47)	$PhCH_2$	CH_3	9.78
5	**e** (82)	**e** (58)	$PhCH_2$	$PhCH_2$	>10
6	**f** (72)	**f** (73)	$PhCH_2$	$CH_3(CH_2)_{12}$	1.35± 0.36
8	**g** (55)	**g** (86)	$PhCH_2$	$CC(CH_2)_8$	7.7±0. 94
9	**h** (96)	**h** (50)	$PhCH_2$	$CH_3(CH_2)_7CH$ $CH(CH_2)_{11}$	>10

[a] yield; [b] IC_{50} on *P.f.* 3D7 strain for **22**; Values are the means of two independent experiments (each performed in duplicate) differing by <30%. Otherwise, values are the means of at least four independent experiments performed in duplicate (SEM is indicated).

We then turned our attention to spirocyclohexadienones having five-membered ring **24** (Table 2). The yields observed for the

conversion of **23** into **24** were lower (19-41%) compared to the synthesis of four-membered ring. Most of the products **24** displayed a keto-enol equilibrium as shown by their NMR spectra in $CDCl_3$. Again, the best R4 and R5 substituents combination was the long aliphatic chain associated with the benzyl group. The right proportion of lipophilic character seems to be essential for bioactivity (entries 3 and 4). The best compound **24f** (IC_{50} = 2.29 µM, entry 5) remains however two folds less potent than **22f**. Interestingly, both have the R4 and R5 substituents at the same position, the product **24f** being more effective than its regioisomer **24b** (entry 2).

Table 2

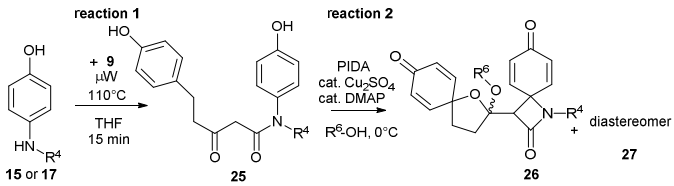


entry	**23** (%)[a]	**24** (%)[a]	R^4	R^5	IC_{50}[b] µM
1	**a** (83)	**a** (25)	$CH_3(CH_2)_{11}$	CH_3	3.98
2	**b** (98)	**b** (27)	$CH_3(CH_2)_{11}$	$PhCH_2$	7.09
3	**c** (91)	**c** (38)	$CH_3(CH_2)_{11}$	$CH_3(CH_2)_{12}$	>10
4	**e** (84)	**e** (33)	$PhCH_2$	$PhCH_2$	>10
5	**f** (98)	**f** (41)	$PhCH_2$	$CH_3(CH_2)_{12}$	2.29
6	**g** (89)	**g** (19)	$PhCH_2$	$CC(CH_2)_8$	5.74

[a] yield; [b] IC_{50} on *P.f.* 3D7 strain for **24**; values are the means of two independent experiments (each performed in duplicate) differing by <30%.

Due to its bioactivity, the absence of keto-enol equilibrium and the higher yielding syntheses, the spirocyclohexadienone with the four-membered ring core **22** was selected for further investigations. Starting from the initial set of building blocks, the double spirocyclohexadienone analogues were made in two steps. The idea was to mimic the previous double pharmacophore **2** by integrating the spirocyclohexadienone having the new four-membered spiro-ring.

Table 3



R^4, R^6	**25** (%)[a]	**26** (%)[a]	**27** (%)[a]	**26** (IC_{50})[b]	**27** (IC_{50})[b]
$R^4 = CH_3(CH_2)_{11}$ $R^6 = CH_3$	**α** (89)	**α** (15)	**α** (17)	**α** (1.68)	**α** (0.91)
$R^4 = CC(CH_2)_{11}$ $R^6 = CH_3CH_2$	**β** (74)	**β** (11)	**β** (25)	**β** (1.72)	**β** (1.41)

[a] yield; [b] IC_{50} (in µM) on *P.f.* 3D7 strain; Values are the means of two independent experiments (each performed in duplicate) differing by <30%.

Reacting **15** or **17** with the acylated Meldrum **9** under microwave heating gave **25α** or **25β**, respectively, in good yields (Table 3). The double phenolic oxidation step, in a presence of methanol or ethanol as solvent[8,15] results in the formation of two major diastereomers **26** and **27** separable by flash chromatography. Against *P. falciparum* 3D7 strain, these double pharmacophore analogues showed an average level of efficiency at micromolar concentration comparable to the simpler analogue **22f**.

We also evaluated the selectivity of a few representative analogues to inhibit human cell lines, such as lymphoblast Jurkat and erythroblast K562 strains (Table 4). All selected compounds showed inhibitor effects on these strains. Only compound **22f** was slightly more selective against *P. falciparum* (entry 1). Shorter lipophilic chain seems to reduce the efficiency and the selectivity gap (entry 2). The double-pharmacophore analogues exhibit a significant reversal of selectivity (entries 4-7).

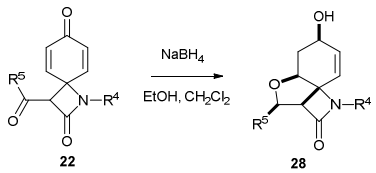
Table 4

entry	compound	IC_{50} *P.f.* 3D7 (µM)	IC_{50}[a] Jurkat (µM)	IC_{50}[b] K562 (µM)
1	**22f**	1.35±0.36	2.88	5.47
2	**22g**	7.7±0.94	7.40	15.30
3	**24f**	2.29	1.42	1.73
4	**26α**	1.68	0.35	0.62
5	**27α**	0.91	0.06	0.18
6	**26β**	1.72	0.14	0.32
7	**27β**	1.41	0.15	0.36

[a] lymphoblast Jurkat strain; [b] erythroblast K562 strain; values are the means of two independent experiments (each performed in duplicate) differing by <30%. Otherwise, values are the means of at least four independent experiments performed in duplicate (SEM is indicated).

Inspired by our previous investigations,[8] we wanted to check whether the removal of the Michael acceptor groups would result in the loss of the bioactivity. Product **22f** was reduced by $NaBH_4$[9] to form the polycyclic compound **28f** in moderate yield (Table 5). Unexpectedly, this reduced analogue **28f** remains active at sub-micromolar concentration against *P. falciparum* (IC_{50} = 0.86 µM). To further explore this new bioactive chemotype, **22g**, **22c** and **22h** were reduced in the same manner to afford **28g**, **28c** and **28h**, respectively. The shorter **28g** and longer unsaturated **28h** chain analogues were also active, albeit at a lesser extent (IC_{50} = 2.42 µM and 3.34 µM respectively). Too much lipophilicity was again detrimental to the activity as shown by **28c**. This new antimalarial scaffold has displayed some selectivity against the parasite. In particular, **28h** had a selectivity index (SI) over 30.

Table 5



28 (%)[a]	R^4	R^5	IC_{50}[b] *P.f.*	IC_{50}[c] Jurkat	IC_{50}[d] K562

			μM	μM	μM
f (43)	$PhCH_2$	$CH_3(CH_2)_{12}$	0.86± 0.12	6.14± 0.33	5.55± 0.82
g (30)	$PhCH_2$	$CC(CH_2)_8$	2.42	18.8	41.2
c (42)	$CH_3(CH_2)_{11}$	$CH_3(CH_2)_{12}$	>25	>25	>25
h (38)	$PhCH_2$	$CH_3(CH_2)_7CHCH(CH_2)_{11}$	3.34	>100[e]	>100[e]

[a] yield; [b] *P.f.* 3D7 strain; [c] lymphoblast Jurkat strain; [d] erythroblast K562 strain; [e] over 100 μM, the product shows solubility issue; values are the means of two independent experiments (each performed in duplicate) differing by <30%. Otherwise, values are the means of at least four independent experiments performed in duplicate (SEM is indicated).

At this stage of the project, we started looking for putative cellular targets that could be affected by our two distinct and most bioactive derivatives **22f** and **28f**. *P. falciparum* thioredoxin reductase is a promising antimalarial target due to its essential role for the survival of the parasite in the control of the redox homeostasis.[16] Inhibitors acting as a double Michael acceptor[17] or with a different kind of electrophilic group that was identified to be selective for PfTrxR[18] were reported to target the catalytic active site of the enzyme by covalently binding to crucial cysteine residues. Recently, the crystal structure of PfTrxR has been disclosed,[19] revealing new structural disparity between the human and the parasite TrxR located outside of the catalytic site.[19a] We wondered whether **22f** or **28f** could be inhibitor of PfTrxR by interfering with these two druggable sites.

Two kinds of enzymatic assays were performed to assess PfTrxR enzymatic activity: the 2-nitrobenzoic acid (DTNB) assay[20] that monitors the electron transfer activity from NADPH to the FAD active site, and the classic turbidimetric assay[21] that monitors the electron transfer from PfTrxR to the macromolecular target Trx. None of the compounds, including the natural product (–)-aculeatin A, which was the starting point of this research, were able to inhibit PfTrxR without pre-incubation. This is an indication of the fact that the enzyme must at least perform one productive cycle before a putative covalent inactivation can take place.[22] After a pre-incubation of at least 2 h, (–)-aculeatin A completely blocked the electron transfer from PfTrxR to the macromolecular acceptor Trx, and dramatically reduced the electron transfer from NADPH to the FAD active site by 68% (*i.e.* 32% residual activity, Table 6, entry 1). This is compatible with the formation of a covalent adduct on the C-terminal Cys-containing active site. Compounds **22f** and **28f**, on the other hand, were able to reduce the activity of the C-terminal active site (33% and 74% residual activity, respectively, entry 2), with only a marginal effect on the FAD active site (>80% residual activity, entry 1). These results suggest that the strong antiplasmodial activity of the newly synthesized compounds **28f** may also be the results of the involvement of other plasmodial target, thus widening the druggable spectrum over and above those accepting a Michael adduct.

Table 6. PfTrxR enzymatic activity after 2 h incubation (DTNB assay, entry 1) or 3 h incubation (Trx turbidimetric assay, entry 2). Percentage activities are referred with respect to the activity of the enzyme without any inhibitor, in the same conditions of incubation, i.e. after incubation with buffer only.

entry	assay	control (buffer)	**28f**	**22f**	(–)-aculeatin A
1	**DTNB** k (μM/sec)	0.32	0.27	0.30	0.102
	residual activity %	**100%**	**84%**	**93%**	**32%**
2	**Trx** Starting time (min)	20	30	50	>90
	Slope (ΔAbs/sec)	$5.43 \cdot 10^{-4}$	$4.01 \cdot 10^{-4}$	$1.78 \cdot 10^{-4}$	0
	residual activity %	**100%**	**74%**	**33%**	**0**

Conclusion

A series of new bioactive aculeatin analogues can be conveniently assembled in only two steps from the combination of two simple building blocks. These new spirocyclohexadienone skeletons having four or five-membered ring including a nitrogen atom exerts the desired antimalaria effect and confirms and prioritizes spirocyclohexadienone as a bioactive scaffold. Analogues **22f** and **24f** displaying the same R4 and R5 substitutions represent the most effective products from each series. Their aliphatic chain length is closely related to the natural product. However, in contrary to our previous results with the double pharmacophoric products,[8] inclusion of the spirocyclohexadienone unit with a four-membered ring does not increase the efficiency and has instead resulted in the reversal of selectivity. As for selectivity, results are mixed for the other analogues and more work would be needed to address this issue. During this investigation, we also took the opportunity to explore PfTrxR as a putative cellular target since various Michael acceptor inhibitors were previously described to covalently bind to this enzyme. (–)-Aculeatin A turned out to be a potent inhibitor of PfTrxR and the C-terminal Cys-containing active site can be a plausible target. Spirocyclohexadienone **22f** is less active on PfTrxR. This different profile suggests that its Michael acceptor group could be slightly different in reactivity. Steric, morphological interactions or spiro-ring strain could account for this effect. This result broadens perspectives that the putative covalent ability of spirocyclohexadienone could be tuned by proper modulation.

An unexpected result has emerged during this work. We identified a new antimalarial product **28f** (IC_{50} = 0.86 μM) that holds the essential bioactive structural features of aculeatin without having a Michael acceptor group. Its effect on PfTrxR is marginal and raises the question of an alternative new mode of action outside the covalent hypothesis. Other cellular targets could interact with this aculeatin-like scaffold leading to the antimalarial phenotype. In particular, the new scaffold **28** showed a broader selectivity compared to the analogues **22** and

represents a novel opportunity to discover novel antimalarial agent. Efforts are now ongoing to explore these hypotheses in order to understand the mode of action of these inhibitors inspired by natural products.

Acknowledgements

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Experimental section

Measurement of biological activity

In vitro antimalarial activities were performed using the chloroquine-sensitive *P. falciparum* strain (3D7) which was maintained in continuous culture in human erythrocytes according to the method of Trager and Jensen.[23] *In vitro* antimalarial activities were measured in microtiter plates according to Desjardins' procedure.[24] Suspensions of infected RBC at 1.5% final haematocrit and 0.6% parasitemia were cultured in complete medium (RPMI 1640, complemented with 25 mM Hepes, pH 7.4, and 10 % AB human serum) either in absence (controls) or presence of the compounds within a range of molecular concentrations. The final volume in each well was 200 µL, consisting of 50 µL of complete medium without (controls) or with drug and 150 µL of *P. falciparum*-infected erythrocyte suspension. The compounds were usually previously dissolved in DMSO and were diluted in culture medium so that the final DMSO concentration never exceeded 0.25%. After 48 h incubation at 37°C, 30 µL of complete medium containing 0.6 µCi [^{3}H]-hypoxanthine were added to each well. After a further 18 h incubation at 37°C, cells were lyzed using an automatic cell harvester and the parasite macromolecules, including radioactive nucleic acids, were retained onto glass fiber filters. The filters were counted for radioactivity, after adding scintillation cocktail, in a liquid scintillation spectrometer. Radioactivity background was obtained from incubation of non-infected erythrocytes under the same condition, and deduced. Drug effect were expressed as IC_{50} which is the drug concentration leading to 50% inhibition of parasite growth.

Human lymphoblasts (Jurkat), erythroblasts (K-562) were cultured in complete medium (RPMI 1640 complemented with 10% FCS in 96-well microplates (8 000 cells per well) and incubated 24 h at 37°C and 5% CO_2 in presence of various concentration of tested molecules as above. After 24 h incubation at 37°C, 30 µL of complete medium containing 0.7 µCi [^{3}H]-thymidine were added to each well. After a further 6 h incubation at 37°C, cells were lyzed using an automatic cell harvester and the cell macromolecules, including radioactive nucleic acids, were retained onto glass fiber filters and counted for radioactivity as indicated above.

PfTrxR was recombinantly produced in *E. coli* BL21-DE3 cells and purified as previously described.[19a] The electron transfer from NADPH to the FAD active site was performed with the 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) assay.[20] PfTrxR (200 nM) was added to a mixture of 20 mM Tris/HCl pH 7.4, 200 mM NaCl, 1 mM EDTA, 1mM DTNB, 300 µM NADPH and each compound (100 µM), at 20 °C; (–)-aculeatin A 100 µM was used as a control. The increase in absorbance at 412 nm due to the formation of TNB^- was monitored (ε_{412} = 13.6 mM^{-1} cm^{-1}). The interference with this electron transfer was checked by both adding the molecules directly to the mix and after pre-incubating the enzyme with each molecule for about 2 h. Each assay was performed in triplicate.

The electron transfer from PfTrxR to the macromolecular target Trx was tested by classical turbidimetric assay.[21] Here the precipitation of insulin at 650 nm was spectrophotometrically followed after addition of 300 nM PfTrxR to a reaction mixture composed of 100 mM potassium phosphate pH 7.0, 2 mM EDTA, 300 µM NADPH, 200 µM insulin, 10 µM thioredoxin from *Schistosoma mansoni*, and 100µM each of (–)-aculeatin A and compounds **22f** and **28f**. The starting time of precipitation and the slope of the reaction were monitored. Also in this case the measurements were carried out without and with 3 h pre-incubation of each compound with the enzyme. Each experiment was repeated twice.

Chemistry-general considerations

All the reagents were used without further purification or distillation. Air sensitive reactions were performed under argon condition. NMR spectra were performed on a Bruker Advance 400 MHz for ^{1}H-NMR spectra and 100 MHz for ^{13}C-NMR spectra. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane (TMS) as an internal standard or relative to the solvents: ^{13}C δ($CDCl_3$) = 77.23 ppm and ^{13}C δ(MeOD) = 49.13 ppm. Accurate mass spectra were recorded on a TOF spectrometer.

5-(3-(4-Hydroxyphenyl)-1-hydroxypropylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione [9]. Compound **8** (1.8 g, 5.4 mmol) was dissolved in 10 mL THF/MeOH (1/1) and was added to a solution of $LiOH.H_2O$ (387 mg, 9.2 mmol) in 5 mL H_2O at room temperature. The mixture was stirred for 2 h and then treated with aqueous 1N HCl solution (15 mL). The product was extracted with EtOAc (4×30 mL). The organic layers were combined, dried with $MgSO_4$ and evaporated. The product was then crystallized from EtOH to give **9** (1.3 g, 4.45 mmol, 82%) as a white solid. R_f = 0.1 in CH_2Cl_2/MeOH = 9.5/0.5; m.p. 201.8°C; IR υ_{max} (thin film, CH_2Cl_2) 3000, 2941, 1713, 1671, 1566, 1580, 1406, 1292, 1203, 924, 826 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.68 (s, 6H), 2.95 (dd, J = 6.8, 8.7 Hz, 2H), 3.37 (dd, J = 6.8, 8.7 Hz, 2H), 4.91 (s, 1H), 6.76 (d, J = 8.6 Hz, 2H), 7.12 (d, J = 8.6 Hz, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 26.9 (2×CH_3), 31.5 (CH_2), 37.7 (CH_2), 92.0 (C), 105.2 (C), 115.6 (2×CH), 129.9 (2×CH), 132.0 (C), 154.5 (C), 160.6 (C), 170.7 (C), 196.9 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{15}H_{17}O_6$) 293.1020, found 293.1019.

5-(1-Hydroxyundec-10-ynylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione [11a]. 10-undecynoic acid **10a** (1 g, 5.5 mmol), Meldrum's acid (815 mg, 5.7 mmol) and DMAP (1 g, 8.2 mmol) were diluted in anhydrous CH_2Cl_2 (20 mL) and stirred at –20°C. DCC (1.17 g, 5,7 mmol) was added in one portion and the mixture was stirred at 4°C overnight. The white precipitate (DCU) was removed by filtration and the organic layer was washed with an aqueous solution of 1 M $NaHSO_4$ (6×50 mL) and brine (2×50 mL). After evaporation in vacuo of

the organic solvent, the product **11a** (1.3 g, 4.2 mmol, 76%) was isolated as a yellow thick oil. An aliquot was purified by flash chromatography for spectral analyses; R_f = 0.3 in cyclohexane/EtOAc = 7/3; IR υ_{max} (thin film, CH_2Cl_2) 3296, 2933, 2857, 1741, 1694, 1577, 1409, 1295, 1205 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.31 (m, 4H), 1.41 (m, 4H), 1.52 (m, 2H), 1.68 (m, 2H), 1.73 (s, 6H), 1.94 (t, J = 2.6 Hz, 1H), 2.18 (dt, J = 2.6, 7.1 Hz, 2H), 3.07 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 18.5 (CH_2), 26.3 (CH_2), 27.0 (2×CH_3), 28.6 (CH_2), 28.8 (CH_2), 29.0 (CH_2), 29.2 (CH_2), 29.4 (CH_2), 35.9 (CH_2), 68.3 (CH), 84.8 (C), 91.4 (C), 104.9 (C), 160.4 (C), 170.7 (C), 198.4 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{17}H_{25}O_5$) 309.1697, found 309.1697.

(*Z*)-5-(1-Hydroxydocos-13-en-1-ylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione [11b]. According to the procedure described for **11a**, and starting from **10b** (500 mg, 1.48 mmol), the product **11b** (480 mg, 1.04 mmol, 70%) was obtained as a yellow solid. R_f = 0.35 in cyclohexane/EtOAc = 7/2; m.p. 93.8°C; IR υ_{max} (thin film, CH_2Cl_2): 2923, 2853, 1742, 1663, 1574, 1406, 1289, 1204, 1153 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, J = 6.9 Hz, 3H), 1.21-1.44 (m, 30H), 1.73 (s, 6H), 198-205 (m, 4H), 3.06 (m, 2H), 5.31-5.38 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (CH_3), 22.9 (CH_2), 26.4 (CH_2), 27.0 (CH_2), 27.4 (2×CH_2), 29.5 (3×CH_2), 29.6 (2×CH_2), 29.7 (2×CH_2), 29.7 (2×CH_2), 29.8 (2×CH_2), 30.0 (2×CH_2), 32.1 (CH_2), 36.0 (CH_2), 91.5 (C), 105.0 (C), 130.1 (2×CH_2), 160.4 (C), 170.9 (C), 198.6 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{28}H_{49}O_5$) 465.3574, found 465.3574.

2,2-Dimethyl-5-(undec-10-ynyl)-1,3-dioxane-4,6-dione [12a]. The acylated Meldrum's acid **11a** (848 mg, 2.75 mmol) was dissolved in CH_2Cl_2 (10 mL) and AcOH (1.65 mL), cooled to 0°C and $NaBH_4$ (412 mg, 10.8 mmol) was added portionwise over 1 h. The solution was stirred for 30 min at 0°C, quenched with water and extracted several times with DCM. The combined organic layers were dried over $MgSO_4$, the solvent was removed under reduced pressure and after a rapid flash column chromatography (cyclohexane/EtOAc = 7/3), the product **12a** (589 mg, 2.0 mmol, 73%) was isolated as white solid. R_f = 0.6 in cyclohexane/EtOAc = 6/4; m.p. 72.0°C; IR υ_{max} (thin film, CH_2Cl_2) 2920, 2850, 1772, 1733, 1381, 1318, 1068 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.29 (m, 8H), 1.42 (m, 4H), 1.52 (m, 2H), 1.76 (s, 3H), 1.79 (s, 3H), 1.94 (t, J = 2.6 Hz, 1H), 2.08 (m, 2H), 2.17 (dt, J = 2.6, 7.1 Hz, 2H), 3.53 (t, J = 5.0 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 18.3 (CH_2), 26.2 (CH_2), 26.6 (CH_2), 26.8 (CH_3), 28.4 (CH_3, CH_2), 28.6 (CH_2), 29.0 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 46.1 (CH), 68.2 (CH), 84.7 (C), 104.7 (C), 165.7 (2×C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{17}H_{27}O_4$) 295.1904, found 295.1906.

4-(Dodecylamino)phenol [15]. 4-aminophenol **13** (2.62 g, 24.0 mmol, 3 eq.), 1-bromododecane (2.0 g, 8.0 mmol) and potassium iodide (134 mg, 0.8 mmol) were dissolved in acetonitrile and the mixture was refluxed for 18 h. The solvent was removed with reduced pressure and after flash chromatography (cyclohexane/EtOAc = 8/2), the product (1.44 g, 5.2 mmol, 65%) is obtained as white solid. R_f = 0.15 in cyclohexane/EtOAc = 8/2; m.p. 76.6°C; IR υ_{max} (thin film, CH_2Cl_2) 3369, 2914, 2845, 1515, 1438, 1232, 1131 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, J = 6.9 Hz, 3H), 1.34 (m, 18H), 1.59 (m, 2H), 3.04 (m, 2H), 6.53 (m, 2H), 6.69 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (CH_3), 22.9 (CH_2), 27.5 (CH_2), 29.7 (2×CH_2), 29.8 (2×CH_2), 29.8 (2×CH_2), 29.9 (CH_2), 32.1 (CH_2), 45.8 (CH_2), 115.2 (2×CH_2), 116.5 (2×CH_2), 142.3 (C), 148.4 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{18}H_{32}NO$) 278.2478, found 278.2475.

***N*-(4-Hydroxyphenyl)tridec-12-ynamide [16].** 4-aminophenol **13** (50 mg, 0.46 mmol) and compound **12a** (148 mg, 0.5 mmol) were diluted in anhydrous THF (2 mL). The solution was refluxed in a micro wave reactor (300W) at 110 °C for 30 min. After cooling to room temperature, the solvent was removed with reduced pressure and the crude product purified with flash column chromatography (cyclohexane/EtOAc = 7/3) to give the amide 16 (104 mg, 0.34 mmol, 75%) as white solid. R_f = 0.2 in cyclohexane/EtOAc = 7/3; m.p. 39.4°C; IR υ_{max} (thin film, CH_2Cl_2) 3284, 2920, 2849, 1658, 1541, 1512, 1460, 1238, 829 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.33 (m, 12H), 1.52 (m, 2H), 1.72 (m, 2H), 1.94 (t, J = 2.7 Hz, 1H), 2.18 (dt, J = 2.6, 7.2 Hz, 2H), 2.33 (t, J = 7.6 Hz, 2H), 6.78 (m, 2H), 7.02 (s, 1H, *NH*), 7.33 (m, 2H); ^{13}C-NMR (100 MHz, MeOD): δ (ppm) = 19.1 (CH_2), 27.2 (CH_2), 29.8 (CH_2), 29.9 (CH_2), 30.3 (CH_2), 30.5 (CH_2), 30.6 (CH_2), 30.7 (2×CH_2), 38.0 (CH_2), 69.5 (CH), 85.2 (C), 116.3 (2×CH), 123.5 (2×CH), 131.9 (C), 155.5 (C), 174.6 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{19}H_{28}NO_2$) 302.2115, found 302.2114.

4-(Tridec-12-ynylamino)phenol [17]. Amide **16** (270 mg, 0.89 mmol) was dissolved in THF and 6 eq. $LiAlH_4$ (2 M in THF) were added. The solution was then refluxed for 24 h under argon, cooled to room temperature and treated with EtOAc, followed by a saturated NH_4Cl solution. The solution was extracted with EtOAc (3×), the combined organic layers dried with $MgSO_4$ and evaporated. The product was then purified by flash column chromatography (cyclohexane/EtOAc = 8/2) to give **17** (117.0 mg, 0.41 mmol, 45%) as white solide. R_f = 0.45 in cyclohexane/EtOAc = 7/3; m.p. 69.8°C; IR υ_{max} (thin film, CH_2Cl_2): 3369, 3288, 2913, 2848, 1518, 1461, 1435, 1230 cm^{-1}; ^{1}H-NMR (400 MHz, MeOD): δ (ppm) = 1.34 (m, 12H), 1.49 (m, 2H), 1.68 (m, 2H), 2.15 (m, 3H), 2.32 (t, J = 7.5 Hz, 2H), 3.57 (m, 2H), 6.73 (d, J = 8.9 Hz, 2H), 7.31 (d, J = 8.9 Hz, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 18.6 (CH_2), 27.4 (CH_2), 28.7 (CH_2), 29.0 (CH_2), 29.3 (CH_2), 29.6 (CH_2), 29.7 (2×CH_2), 29.8 (2×CH_2), 45.6 (CH_2), 68.3 (CH), 85.0 (C), 114.8 (2×CH_2), 116.4 (2×CH_2), 142.5 (C), 148.1 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{19}H_{30}NO$) 288.2322, found 288.2321.

Preparation of 4-((benzylamino)methyl)phenol [18c]. 4-Hydroxybenzaldehyde (350 mg, 2.86 mmol) and phenylamine (337 mg, 3.15 mmol) were dissolved in THF, $MgSO_4$ was added and the mixture stirred at room temperature for 24 h. $MgSO_4$ was filtered off and THF was removed with reduced pressure. The resulting solid was then dissolved in MeOH, $NaBH_4$ (217 mg, 5.72 mmol) were added and the solution stirred at room temperature for 30 min. Water was added and MeOH was removed under reduced pressure. After flash chromatography (CH_2Cl_2/MeOH 99/1), the product **18c** (503 mg, 2.26 mmol, 82 %) was obtained as a white solid. R_f = 0.2 in CH_2Cl_2/MeOH 99/1; m.p. 89.2°C; IR υ_{max} (thin film, CH_2Cl_2) 3262, 2926, 1510, 1468, 1454, 1394, 1245, 1106, 1049 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 3.71 (s, 2H), 3.82 (s, 2H), 6.65 (d, J = 8.5 Hz, 2H), 7.11 (d, J = 8.5 Hz, 2H), 7.26 (m, 1H), 7.30-7.34 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 52.5 (CH_2), 53.1 (CH_2), 116.1 (2×CH_2), 127.5 (CH), 128.7 (2×CH_2), 128.8 (2×CH_2), 130.0 (2×C), 130.1 (C), 139.1 (C), 156.2 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{14}H_{16}NO$) 214.1226, found 214.1227.

4-[(Dodecylamino)methyl]phenol [18d]. According to the procedure described for **18c**, product **18d** (769 mg, 2.64 mmol, 93%) was obtained as a white solid. R_f = 0.5 in CH_2Cl_2/MeOH = 9/1; m.p. 72.9°C; IR υ_{max} (thin film, CH_2Cl_2) 2917, 2845,

1516, 1463, 1370, 1251, 1091 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, J = 6.9 Hz, 3H), 1.20-1.32 (m, 18H), 1.53 (m, 2H), 2.67 (t, J = 7.6 Hz, 2H), 3.68 (s, 2H), 6.59 (d, J = 8.4 Hz, 2H), 7.06 (d, J = 8.4 Hz, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (CH_3), 22.9 (CH_2), 27.5 (CH_2), 29.6 (CH_2), 29.65 (CH_2), 29.7 (CH_2), 29.78 (CH_2), 29.8 (CH_2), 29.84 (CH_2), 29.86 (CH_2), 32.1 (CH_2), 49.6 (CH_2), 55.6 (CH_2), 116.1 (2×CH_2), 129.9 (2×CH_2), 130.1 (C), 156.5 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{19}H_{34}NO$) 292.2635, found 292.2637.

General procedure for 19 and 20:

2.3 mmol PPh_3 was dissolved in 50 mL THF and cooled to – 78 °C. 1 eq. DEAD was added slowly over 2 min and the reaction stirred for additional 5 min. Then 1.1 eq. of 1-tetradecanol or undec-10-yn-1-ol were added following the addition of 1 eq. maleimide after 5 min. The reaction was allowed to reach room temperature and was stirred for 48 h. The solvent was then removed under reduced pressure and the respective crude product purified by flash column chromatography.

1-Tetradecyl-1*H*-pyrrole-2,5-dione [19]. Yield: 114 mg (0.39 mmol, 50%). R_f = 0.5 in cyclohexane/EtOAc = 7/3; IR υ_{max} (thin film, CH_2Cl_2) 3075, 2917, 2847, 1695, 1400, 1346, 1132, 846 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, J = 6.9 Hz, 3H), 1.20-1.34 (s, 22H), 1.57 (m, 2H), 3.50 (m, 2H), 6.68 (s, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (CH_3), 22.9 (CH_2), 27.0 (CH_2), 28.8 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 29.8 (CH_2), 29.84 (CH_2), 29.9 (4×CH_2), 32.1 (CH_2), 38.2 (CH_2), 134.2 (2×CH), 171.1 (2×C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{18}H_{32}NO_2$) 294.2428, found 204.2426.

1-(Undec-10-ynyl)-1*H*-pyrrole-2,5-dione [20]. Yield: 351.5 mg (1.4 mmol, 68%). R_f = 0.6 in cyclohexane/EtOAc = 8/2; IR υ_{max} (thin film, CH_2Cl_2) 3459, 3277, 3099, 2928, 2853, 2115, 1709, 1445, 1413, 1374, 1132, 834 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.29 (s, 8H), 1.39 (m, 2H), 1.51 (m, 2H), 1.58 (m, 2H), 1.95 (t, J = 2.5 Hz, 1H), 2.17 (dt, J = 2.5, 7.0 Hz, 2H), 3.51 (t, J = 7.3 Hz, 2H), 6.70 (s, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 18.5 (CH_2), 26.8 (CH_2), 28.6 (2×CH_2), 28.8 (CH_2), 29.1 (CH_2), 29.2 (CH_2), 29.4 (CH_2), 38.0 (CH_2), 68.3 (CH), 84.8 (C), 134.2 (2×CH), 171.0 (2×C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{15}H_{22}NO_2$) 248.1651, found 248.1645.

General procedure for the synthesis of the β-keto amides 21, 23 and 25 by microwave heating:

The respective compounds were put together in a 1 (amine): 1.2 (5-substituted Meldrum's acid) ratio and dissolved with anhydrous THF (3 mL for 100 mg amine). All the compounds must be dissolved prior microwave heating. The solution was then refluxed in a micro wave reactor (300W) at 110 °C for 15 min. After cooling to room temperature, the solvent was removed with reduced pressure and the crude product purified with flash column chromatography. Procedure modification for compounds **21a** and **23a**: the respective amine and 1.2 eq. 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one **5** were dissolved in THF and refluxed in a micro wave oven (300W) at 110 °C for 2.5 h.

***N*-Dodecyl-*N*-(4-hydroxyphenyl)-3-oxobutanamide [21a]**. Yield: 59.3 mg (0.16 mmol, 91%). R_f = 0.1 in cyclohexane/EtOAc = 8/2; IR υ_{max} (thin film, CH_2Cl_2) 3160, 2910, 2846, 1720, 1612, 1588, 1508, 1450, 1273, 1226, 1166, 1147 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.87 (t, J = 6.9 Hz, 3H), 1.15-1.35 (m, 18H), 1.53 (m, 2H), 2.06 (s, 3H), 3.35 (s, 2H), 3.69 (m, 2H), 6.92 (d, J = 8.7 Hz, 2H), 6.99 (d, J = 8.7 Hz, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.2 (CH_3), 22.7 (CH_2), 26.8 (CH_2), 27.6 (CH_2), 29.4 (CH_2), 29.4 (CH_2), 29.6 (2×CH_2), 29.7 (2×CH_2), 30.4 (CH_3), 32.0 (CH_2), 49.9 (CH_2), 50.3 (CH_2), 116.8 (2×CH), 129.1 (2×CH), 133.5 (C), 157.3 (C), 168.0 (C), 202.9 (C); HRMS (ESI) *m/z* calcd for $[M+Na]^+$ ($C_{22}H_{35}NO_3Na$) 384.2515, found 384.2513.

***N*-Dodecyl-*N*-(4-hydroxyphenyl)-3-oxo-4-phenylbutanamide [21b]**. Yield: 106.7 mg (0.24 mmol, 68%). R_f = 0.2 in CH_2Cl_2/MeOH = 99/1; IR υ_{max} (thin film, CH_2Cl_2) 3236, 2917, 2848, 1720, 1626, 1511, 1454, 1270 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.87 (t, J = 6.9 Hz, 3H), 1.15-1.35 (m, 18H), 1.50 (m, 2H), 3.31 (s, 2H), 3.60 (s, 2H), 3.65 (m, 2H), 6.80-6.90 (m, 4H), 7.03 (m, 2H), 7.21-7.28 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (CH_3), 22.9 (2×CH_2), 26.9 (CH_2), 27.7 (CH_2), 29.5 (CH_2), 29.7 (CH_2), 29.8 (2×CH_2), 32.1 (2×CH_2), 48.7 (CH_2), 49.8 (CH_2), 50.3 (CH_2), 53.6 (CH_2), 116.7 (2×CH), 127.3 (CH), 128.9 (2×CH), 129.2 (2×CH), 129.7 (2×CH), 133.5 (C), 133.7 (C), 156.9 (C), 167.9 (C), 202.7 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{28}H_{40}NO_3$) 438.3003, found 438.3004.

***N*-Dodecyl-*N*-(4-hydroxyphenyl)-3-oxohexadecanamide [21c]**. Yield: 186 mg (0.35 mmol, 65%). R_f = 0.3 in CH_2Cl_2/EtOAc = 8/2; IR υ_{max} (thin film, CH_2Cl_2) 3162, 2917, 2844, 1712, 1618, 1511, 1462, 1266, 1234, 837 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, J = 6.8 Hz, 6H), 1.15-1.35 (m, 38H), 1.47 (m, 2H), 1.62 (m, 2H), 2.31 (t, J = 7.4 Hz, 2H), 3.25 (s, 2H), 3.65 (m, 2H), 6.85 (d, J = 8.7 Hz, 2H), 7.01 (d, J = 8.7 Hz, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (2×CH_3), 22.8 (2×CH_2), 23.7 (CH_2), 26.9 (CH_2), 27.8 (CH_2), 29.2 (CH_2), 29.5 (3×CH_2), 29.6 (CH_2), 29.7 (CH_2), 29.7 (CH_2), 29.8 (3×CH_2), 29.8 (3×CH_2), 29.8 (CH_2), 32.1 (3×CH_2), 43.4 (CH_2), 49.6 (CH_2), 116.6 (2×CH), 129.7 (2×CH), 134.9 (C), 156.0 (C), 167.4 (C), 205.2 (C); HRMS (ESI) *m/z* calcd for $[M+Na]^+$ ($C_{34}H_{59}NO_3Na$) 552.4393, found 552.4391.

***N*-Benzyl-*N*-(4-hydroxyphenyl)-3-oxobutanamide [21d]**. Yield: 465.0 mg (1.6 mmol, 82%). R_f = 0.1 in cyclohexane/EtOAC = 7/3; IR υ_{max} (thin film, CH_2Cl_2) 3271, 3030, 1721, 1633, 1514, 1446, 1274, 844 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 2.03 (s, 3H), 3.37 (s, 2H), 4.86 (s, 2H), 6.80 (m, 4H), 7.18-7.28 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 30.5 (CH_3), 50.1 (CH_2), 53.5 (CH_2), 116.7 (2×CH), 127.7 (CH), 128.6 (2×CH), 128.9 (2×CH), 129.4 (2×CH), 133.5 (C), 136.7 (C), 157.0 (C), 168.1 (C), 202.8 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{17}H_{18}NO_3$) 284.1281, found 284.1285.

***N*-Benzyl-*N*-(4-hydroxyphenyl)-3-oxo-4-phenylbutanamide [21e]**. Yield: 294.0 mg (0.82 mmol, 82%). R_f = 0.4 in cyclohexane/EtOAc = 6.5/3.5; IR υ_{max} (thin film, CH_2Cl_2) 3269, 3027, 2925, 1712, 1622, 1511, 1446, 1270, 837 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 3.33 (s, 2H), 3.62 (s, 2H), 4.84 (s, 2H), 6.68 (m, 3H), 6.78 (m, 1H), 7.03 (m, 2H), 7.15-7.28 (m, 8H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 48.6 (CH_2), 50.3 (CH_2), 53.5 (CH_2), 116.6 (2×CH), 127.4 (CH), 127.7 (CH), 128.6 (2×CH), 128.9 (4×CH), 129.4 (2×CH), 129.6 (2×CH), 133.4 (C), 133.5 (C), 136.7 (C), 156.8 (C), 168.2 (C), 202.7 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{23}H_{22}NO_3$) 360.1598, found 360.1594.

***N*-Benzyl-*N*-(4-hydroxyphenyl)-3-oxohexadecanamide [21f]**. Yield: 118.4 mg (0.26 mmol, 72%). R_f = 0.3 in CH_2Cl_2/MeOH = 99.5/0.5; IR υ_{max} (thin film, CH_2Cl_2) 3207, 2904, 2840, 1716, 1622, 1507, 1434, 1270, 1201, 833 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, J = 6.9 Hz, 3H), 1.16-1.28 (m, 22H), 1.43 (m, 2H), 2.27 (t, J = 7.4 Hz, 2H), 3.34 (s, 2H), 4.86 (s, 2H), 6.68-6.86 (m, 4H), 7.21-7.38 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (CH_3), 22.9 (CH_2), 23.6 (CH_2), 29.2 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.8 (CH_2), 29.9 (2×CH_2), 29.9 (CH_2), 32.1 (CH_2), 43.5 (CH_2), 49.4 (CH_2),

53.5 (CH_2), 116.6 (2×CH), 127.7 (CH), 128.6 (2×CH), 129.0 (2×CH), 129.6 (2×CH), 134.0 (C), 137.0 (C), 156.7 (C), 168.2 (C), 205.1 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{29}H_{42}NO_3$) 452.3165, found 452.3159.

***N*-Benzyl-*N*-(4-hydroxyphenyl)-3-oxotridec-12-ynamide [21g].** Yield: 54.5 mg (0.13 mmol, 55%). R_f = 0.1 in CH_2Cl_2/MeOH = 99/1; IR υ_{max} (thin film, CH_2Cl_2) 3285, 3217, 2908, 2846, 1719, 1645, 1508 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.22-1.45 (m, 8H), 1.47 (m, 4H), 1.94 (s, 1H), 2.16 (dt, J = 2.3 Hz, J = 6.9 Hz, 2H), 2.28 (t, J = 7.3 Hz, 2H), 3.34 (s, 2H), 4.86 (s, 2H), 6.73-6.83 (m, 4H), 7.18-7.28 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 18.6 (CH_2), 23.6 (CH_2), 28.6 (CH_2), 28.8 (CH_2), 29.1 (CH_2), 29.3 (CH_2), 29.9 (CH_2), 43.4 (CH_2), 49.4 (CH_2), 53.5 (CH_2), 68.4 (CH), 85.0 (C), 116.5 (2×CH), 127.7 (CH), 128.6 (2×CH), 128.9 (2×CH), 129.6 (2×CH), 134.0 (C), 137.0 (C), 156.6 (C), 168.0 (C), 205.0 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{26}H_{32}NO_3$) 406.2314, found 406.2309.

(Z)-*N*-Benzyl-*N*-(4-hydroxyphenyl)-3-oxotetracos-15-enamide [21h]. Yield: 404.8 mg (0.72 mmol, 96%). R_f = 0.1 in cyclohexane/EtOAc = 8/2; IR υ_{max} (thin film, CH_2Cl_2) 2923, 2853, 1719, 1629, 1514, 1451, 1274 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.87 (t, J = 6.8 Hz, 3H), 1.10-1.35 (m, 26H), 1.43 (m, 4H), 2.01 (m, 4H), 2.25 (t, J = 7.4 Hz, 2H), 3.36 (s, 2H), 4.86 (s, 2H), 5.35 (m, 2H), 6.74-7.82 (m, 4H), 7.17-7.27 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (CH_3), 22.9 (CH_2), 23.7 (CH_2), 27.4 (CH_2), 29.2 (CH_2), 29.5-30.0 (12×CH_2), 32.0 (CH_2), 43.5 (CH_2), 49.4 (CH_2), 53.4 (CH_2), 116.5 (2×CH), 127.7 (CH), 128.6 (2×CH), 129.0 (2×CH), 129.6 (2×CH), 130.1 (CH), 130.1 (CH), 134.2 (C), 137.0 (C), 156.4 (C), 168.0 (C), 205.1 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{37}H_{56}NO_3$) 562.4255, found 562.4255.

***N*-(4-Hydroxybenzyl)-*N*-dodecyl-3-oxobutanamide [23a]**. Yield: 214.0 mg (0.57 mmol, 83%). R_f = 0.2 in cyclohexane/EtOAc = 7/3; IR υ_{max} (thin film, CH_2Cl_2) 3289, 2917, 2848, 1720, 1630, 1516, 1454, 1356, 1230, 817 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): Mixture of rotamers, δ (ppm) = 0.88 (t, J = 6.7 Hz, 6H), 1.25 (s, 36H), 1.55 (m, 4H), 2.24 (d, J = 2.8 Hz, 2H, COCH_3), 2.29 (d, J = 3.2 Hz, 2.6H, COCH_3), 2.35 (d, J = 1.6 Hz, 1.4H, COCH_3), 3.14 (dd, J = 6.5, 15.3 Hz, 2.2H, NCH_2CH$_2$), 3.36 (dd, J = 6.7, 14.3 Hz, 1.8H, NCH_2CH$_2$), 3.53 (d, J = 7.1 Hz, 1.3H, COCH_2CO), 3.61 (d, J = 8.1 Hz, 1.6H, COCH_2CO), 3.70 (d, J = 5.7 Hz, 1.1H, COCH_2CO), 4.39 (s, 1.2H, NCH_2Ph), 4.52 (m, 1.9H, NCH_2Ph), 4.60 (d, J = 9.5 Hz, 0.9H, NCH_2Ph), 6.81 (m, 2H), 7.08 (m, 4H), 7.30 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): Mixture of rotamers, δ (ppm) = 14.2 (CH_3), 22.8 (CH_2), 26.9 (CH_2), 27.0 (CH_2), 27.5 (CH_2), 28.6 (CH_2), 29.5-29.7 (CH_2), 30.5 (CH_3), 32.0 (CH_2), 46.6-51.2 (3×CH_2), 115.7 (CH), 116.1 (CH), 121.7 (CH), 122.2 (CH), 127.5 (CH), 129.1 (CH), 129.5 (CH), 134.5 (C), 135.3 (C), 149.8 (C), 150.0 (C), 165.8 (C), 167.1 (C), 200.1 (C), 202.5 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{23}H_{38}NO_3$) 376.2846, found 376.2850.

***N*-(4-Hydroxybenzyl)-*N*-dodecyl-3-oxo-4-phenylbutanamide [23b]**. Yield: 150.2 mg (0.33 mmol, 98%). R_f = 0.1 in cyclohexane/EtOAc = 8/2; IR υ_{max} (thin film, CH_2Cl_2) 3297, 2917, 2852, 1720, 1622, 1511, 1450, 1225 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): Mixture of 2 rotamers, δ (ppm) = 0.88 (m, 6H), 1.25 (m, 36H), 1.39 (m, 4H), 2.98 (m, 2.3H), 3.33 (m, 1.7H), 3.51 (s, 1.7H), 3.58 (s, 2.3H), 3.84 (s, 1.7H), 3.87 (s, 2.3H), 4.28 (s, 2.7H), 4.51 (s, 2.3H), 6.77 (m, 4H), 6.91 (m, 2H), 7.12 (m, 2H), 7.21 (m, 4H), 7.31 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (CH_3), 22.9 (2×CH_2), 26.9 (CH_2), 28.5 (CH_2), 29.6 (CH_2), 29.8 (CH_2), 29.8 (2×CH_2), 32.1 (2×CH_2), 47.9 (CH_2), 48.3 (CH_2), 50.4 (CH_2), 51.4 (CH_2), 115.8 (CH), 116.1 (CH), 127.5 (CH), 127.5 (CH), 127.9 (CH), 129.0 (2×CH), 129.5 (CH), 129.8 (CH), 133.7 (C), 133.8 (C), 136.5 (C), 155.6 (C), 155.9 (C), 202.6 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{29}H_{42}NO_3$) 452.3159, found 452.3163.

***N*-(4-Hydroxybenzyl)-*N*-dodecyl-3-oxohexadecanamide [23c]**. Yield: 169.7 mg (0.31 mmol, 91%). R_f = 0.45 in CH_2Cl_2/MeOH 95/5; IR υ_{max} (thin film, CH_2Cl_2) 3227, 2913, 2848, 1708, 1626, 1511, 1466, 1266, 1225, 812 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): Mixture of 2 rotamers, δ (ppm) = 0.88 (t, J = 6.8 Hz, 6H), 1.26 (m, 38H), 1.56 (m, 2H), 2.57 (t, 2H, J = 8.0 Hz), 3.13 (m, 1H), 3.34 (m, 1H), 3.51 (s, 1H), 3.58 (s, 1H), 4.41 (s, 1H), 4.53 (s, 1H), 6.99 (m, 2H), 7.11 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (2×CH_3), 22.9 (3×CH_2), 23.7 (CH_2), 23.8 (CH_2), 27.0 (CH_2), 27.1 (CH_2), 28.7 (CH_2), 29.5-29.9 (12×CH_2), 32.1 (3×CH_2), 43.5 (CH_2), 48.0 (2×CH_2), 49.2 (CH_2), 49.6 (CH_2), 51.4 (CH_2), 115.7 (CH), 116.1 (CH), 128.9 (C), 129.5 (2×CH), 155.6 (C), 156.0 (C), 167.3 (C), 167.6 (C), 205.2 (C); HRMS (ESI) *m/z* calcd for $[M+Na]^+$ ($C_{35}H_{61}NO_3Na$) 566.4549, found 566.4553.

***N*-(4-Hydroxybenzyl)-*N*-benzyl-3-oxo-4-phenylbutanamide [23e]**. Yield: 147.1 mg (0.39 mmol, 84%). R_f = 0.1 in CH_2Cl_2/MeOH 99/1; IR υ_{max} (thin film, CH_2Cl_2) 3305, 3027, 2925, 1716, 1618, 1511, 1450, 1225 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): Mixture of 2 rotamers, δ (ppm) = 3.63 (s, 1H), 3.66 (s, 1H), 3.82 (s, 1H), 3.84 (s, 1H), 4.18 (s, 1H), 4.27 (s, 1H), 4.54 (s, 1H), 4.60 (s, 1H), 6.78 (m, 2H), 6.85 (m, 1H), 7.02 (m, 1H), 7.08 (m, 1H), 7.16 (m, 2H), 7.20-7.35 (m, 7H); ^{13}C-NMR (100 MHz, $CDCl_3$): Mixture of rotamers, δ (ppm) = 48.1 (CH_2), 48.3 (CH_2), 48.5 (CH_2), 50.4 (CH_2), 50.4 (CH_2), 50.7 (CH_2), 115.9 (CH), 116.2 (CH), 126.5 (CH), 127.6 (CH), 127.7 (CH), 128.0 (CH), 128.3 (CH), 128.9 (CH), 129.1 (2×CH), 129.2 (CH), 129.7 (4×CH), 133.4 (C), 133.5 (C), 135.6 (C), 136.7 (C), 155.9 (C), 156.2 (C), 168.1 (C), 202.6 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{24}H_{24}NO_3$) 374.1751, found 374.1752.

***N*-(4-Hydroxybenzyl)-*N*-benzyl-3-oxohexadecanamide [23f]**. Yield: 430.1 mg (0.92 mmol, 98%). R_f = 0.2 in CH_2Cl_2/MeOH = 99/1; IR υ_{max} (thin film, CH_2Cl_2) 3262, 2921, 2844, 1716, 1610, 1511, 1450, 1221, 817 cm^{-1}; No purification necessary. ^{1}H-NMR (400 MHz, $CDCl_3$): Mixture of 2 rotamers, δ (ppm) = 0.88 (t, J = 6.8 Hz, 3H), 1.25 (m, 20H), 1.58 (m, 2H), 2.55 (m, 2H), 3.60 (s, 1H), 3.64 (s, 1H), 4.33 (s, 1H), 4.40 (s, 1H), 4.55 (s, 1H), 4.60 (s, 1H), 6.81 (m, 2H), 7.00 (m, 1H), 7.14 (m, 2H), 7.25 (m, 1H), 7.35 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (CH_3), 22.9 (2×CH_2), 23.7 (CH_2), 29.3-29.9 (6×CH_2), 32.1 (2×CH_2), 43.6 (CH_2), 48.2 (CH_2), 49.2 (CH_2), 50.4 (CH_2), 50.8 (CH_2), 115.8 (CH), 116.2 (CH), 126.6 (CH), 128.1 (2×CH), 128.3 (CH), 128.9 (CH), 129.3 (CH), 129.7 (CH), 136.1 (C), 136.8 (C), 155.8 (C), 168.1 (C), 205.1 (C); HRMS (ESI) *m/z* calcd for $[M+Na]^+$ ($C_{30}H_{43}NO_3Na$) 488.3141, found 488.3144.

***N*-(4-Hydroxybenzyl)-*N*-benzyl-3-oxotridec-12-ynamide [23g]**. Yield: 119.4 mg (0.28 mmol, 89%). R_f = 0.35 in cyclohexane/EtOAc = 7/3; IR υ_{max} (thin film, CH_2Cl_2) 3293, 2938, 2852, 1716, 1610, 1511, 1442, 1225 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): Mixture of 2 rotamers, δ (ppm) = 1.25 (m, 6H), 1.36 (m, 2H), 1.53 (m, 4H), 1.93 (t, 1H, J = 2.6 Hz), 2.17 (m, 2H), 2.54 (dd, J = 7.2, 15.6 Hz, 2H), 3.63 (s, 1H), 3.67 (s, 1H), 4.32 (s, 1H), 4.41 (s, 1H), 4.55 (s, 1H), 4.61 (s, 1H), 6.80 (d, J = 8.1 Hz, 1H), 6.83 (d, J = 8.0 Hz, 1H), 6.96 (d, J = 7.8 Hz, 1H), 7.13 (m, 2H), 7.33 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$): Mixture of rotamers, δ (ppm) = 18.5 (CH_2), 23.6 (CH_2), 28.8 (CH_2), 29.0 (CH_2), 29.1 (CH_2), 29.1 (CH_2), 29.3 (CH_2), 43.5 (CH_2), 43.6 (CH_2), 48.3 (CH_2), 48.4 (CH_2), 49.1 (CH_2), 49.2

(CH_2), 50.5 (CH_2), 50.8 (CH_2), 68.4 (CH), 84.9 (C), 115.9 (CH), 116.2 (CH), 126.6 (CH), 128.0 (CH), 128.2 (CH), 128.9 (CH), 129.3 (CH), 129.7 (CH), 135.9 (C), 136.6 (C), 156.1 (C), 156.5 (C), 168.3 (C), 205.1 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{27}H_{34}NO_3$) 420.2533, found 420.2534.

***N*-Dodecyl-*N*-5-bis(4-hydroxyphenyl)-3-oxopentanamide [25α]**. Yield: 150.5 mg (0.32 mmol, 89%). R_f = 0.5 in cyclohexane/EtOAc = 1/1; IR υ_{max} (thin film, CH_2Cl_2) 3316, 2924, 2853, 1716, 1629, 1514, 1452, 1220, 841 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.87 (t, *J* = 6.9 Hz, 3H), 1.17-1.31 (m, 18H), 1.49 (m, 2H), 2.55 (t, *J* = 7.0 Hz, 2H), 2.69 (t, *J* = 7.0 Hz, 2H), 3.27 (s, 2H), 3.65 (m, 2H), 6.71 (d, *J* = 8.5, 2H,), 6.79 (d, *J* = 8.5, 2H), 6.84-6.90 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (CH_3), 22.9 (CH_2), 26.9 (CH_2), 27.7 (CH_2), 28.6 (CH_2), 29.5 (CH_2), 29.7 (4×CH_2), 32.1 (2×CH_2), 44.9 (CH_2), 49.8 (CH_2), 50.0 (CH_2), 115.7 (2×CH), 116.8 (2×CH), 129.1 (2×CH), 129.5 (2×CH), 132.0 (C), 133.6 (C), 154.5 (C), 156.9 (C), 168.3 (C), 205.1 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{29}H_{42}NO_4$) 468.3108, found 468.3107.

***N*-(Dec-9-yn-1-yl)-*N*-5-bis(4-hydroxyphenyl)-3-oxopentanamide [25β]**. Yield: 134.8 mg (0.28 mmol, 74%). R_f = 0.6 in cyclohexane/EtOAc = 6/4; IR υ_{max} (thin film, CH_2Cl_2) 3287, 2926, 2853, 1711, 1615, 1515, 1447, 1230, 825 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.18-1.30 (m, 12H), 1.36 (td, *J* = 7.1, 13.8 Hz, 2H), 1.46-1.54 (m, 4H), 1.93 (t, *J* = 2.6 Hz, 1H), 2.16 (dt, *J* = 2.6, 7.1 Hz, 2H), 2.54 (t, *J* = 7.1 Hz, 2H), 2.68 (t, *J* = 7.0 Hz, 2H), 3.27 (s, 2H), 3.65 (m, 2H), 6.71 (d, *J* = 8.3 Hz, 2H), 6.80 (d, *J* = 8.3 Hz, 2H), 6.83-6.90 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 18.6 (CH_2), 26.9 (CH_2), 27.7 (CH_2), 28.7 (2×CH_2), 28.9 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (3×CH_2), 44.9 (CH_2), 49.9 (CH_2), 50.0 (CH_2), 68.3 (C), 85.0 (CH), 115.7 (2×CH), 116.8 (2×CH), 129.2 (2×CH), 129.5 (2×CH), 132.1 (C), 133.8 (C), 154.6 (C), 156.9 (C), 168.1 (C), 204.8 (C) cm^{-1}; HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{30}H_{40}NO_4$) 478.2952, found 478.2952.

Generale procedure for the synthesis of spirocyclohexadienones

To a solution of β-keto amide (0.1 mmol) in anhydrous EtOH (10 mL) was added $CuSO_4$×$5H_2O$ (1 mg) and DMAP (1 mg) at 0°C. The solution was stirred for 30 min at 0°C. PIDA (35.5 mg, 0.11 mmol) was then added in one portion (for the products **26** and **27**, 3 equivalents of PIDA was used). After stirring for 1 h at 0°C, the solvent was removed *in vacuo* and EtOAc was added. The organic layer was washed with water, brine and dried over $MgSO_4$. The solvent was evaporated and the crude product was purified on silica gel to give the spirocyclohexadienone.

3-Acetyl-1-dodecyl-1-azaspiro[3.5]nona-5,8-diene-2,7-dione [22a]. Yield: 29.3 mg (0.08 mmol, 59%). R_f = 0.45 in CH_2Cl_2/MeOH = 99/1; IR υ_{max} (thin film, CH_2Cl_2) 2915, 2856, 1759, 1666, 1357, 1234 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, *J* = 6.9 Hz, 3H), 1.21-1.30 (m, 18H), 1.47 (m, 2H), 2.28 (s, 3H), 3.10 (m, 2H), 4.30 (s, 1H), 6.45 (ddd, *J* = 2.0, 10.1, 12.1 Hz, 2H), 6.78 (dd, *J* = 3.0, 10.1 Hz, 1H), 6.97 (dd, *J* = 3.0, 10.1 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (CH_3), 22.9 (CH_2), 27.1 (CH_2), 29.0 (CH_2), 29.2 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 29.8 (2×CH_2), 31.0 (CH_3), 32.1 (CH_2), 42.1 (CH_2), 58.2 (C), 70.9 (CH), 132.3 (CH), 132.5 (CH), 145.4 (CH), 146.5 (CH), 162.0 (C), 184.5 (C), 198.2 (C); HRMS (ESI) *m/z* calcd for $[M+Na]^+$ ($C_{22}H_{33}NO_3Na$) 382.2358, found 382.2362.

1-Dodecyl-3-(2-phenylacetyl)-1-azaspiro[3.5]nona-5,8-diene-2,7-dione [22b]. Yield: 29.2 mg (0.07 mmol, 59%). R_f = 0.45 in CH_2Cl_2/MeOH = 99/1; IR υ_{max} (thin film, CH_2Cl_2) 2915, 2845, 1765, 1666, 1625, 1392, 878 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, *J* = 6.9 Hz, 3H), 1.20-1.32 (m, 18H), 1.45 (m, 2H), 3.06 (t, *J* = 7.3 Hz, 2H), 3.79 (s, 2H), 4.36 (s, 1H), 6.27 (dd, *J* = 2.0, 10.0 Hz, 1H), 6.33 (dd, *J* = 2.0, 10.2 Hz, 1H), 6.62 (dd, *J* = 3.0, 10.0 Hz, 1H), 6.88 (dd, *J* = 3.0, 10.2 Hz, 1H), 7.14 (m, 2H), 7.27 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (CH_3), 22.9 (CH_2), 27.0 (CH_2), 28.9 (CH_2), 29.2 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 29.8 (2×CH_2), 32.1 (CH_2), 42.1 (CH_2), 51.0 (CH_2), 57.9 (C), 68.9 (CH), 127.9 (CH), 129.2 (2×CH), 129.9 (2×CH), 132.0 (C), 132.4 (CH), 132.5 (CH), 144.9 (CH), 146.3 (CH), 162.0 (C), 184.3 (C), 198.3 (C); HRMS (ESI) *m/z* calcd for $[M+Na]^+$ ($C_{28}H_{37}NO_3Na$) 458.2671, found 458.2675.

1-Dodecyl-3-tetradecanoyl-1-azaspiro[3.5]nona-5,8-diene-2,7-dione [22c]. Yield: 32.0 mg (0.06 mmol, 61%). R_f = 0.05 in CH_2Cl_2/MeOH = 99.5/0.5; IR υ_{max} (thin film, CH_2Cl_2) 2921, 2845, 1759, 1666, 1462, 878 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, *J* = 6.8 Hz, 6H), 1.15-1.30 (m, 38H), 1.62 (m, 2H), 1.51 (m, 2H), 2.52 (m, 2H), 3.10 (dd, *J* = 6.6, 8.1 Hz, 2H), 4.28 (s, 1H), 6.43 (dd, *J* = 1.9, 6.5 Hz, 1H), 6.45 (dd, *J* = 1.9, 6.7 Hz, 1H), 6.79 (dd, *J* = 3.0, 10.2 Hz, 1H), 6.98 (dd, *J* = 3.0, 10.4 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (CH_3), 22.9 (3×CH_2), 23.1 (CH_2), 27.0 (CH_2), 29.0 (CH_2), 29.2 (2×CH_2), 29.5 (4×CH_2), 29.7 (6×CH_2), 29.8 (2×CH_2), 32.1 (2×CH_2), 42.1 (CH_2), 43.9 (CH_2), 58.1 (C), 70.2 (CH), 132.3 (CH), 132.4 (CH), 145.6 (CH), 146.8 (CH), 162.1 (C), 184.6 (C), 200.8 (C); HRMS (ESI) *m/z* calcd for $[M+Na]^+$ ($C_{34}H_{57}NO_3Na$) 550.4236, found 550.4238.

3-Acetyl-1-benzyl-1-azaspiro[3.5]nona-5,8-diene-2,7-dione [22d]. Yield: 97.2 mg (0.35 mmol, 47%). R_f = 0.3 in cyclohexane/EtOAc = 7/3; IR υ_{max} (thin film, CH_2Cl_2) 3058, 2920, 1767, 1670, 1630, 1358, 1239, 1180 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 2.26 (s, 3H), 4.31 (m, 3H), 6.22 (dd, *J* = 2.0, 10.0 Hz, 1H), 6.28 (dd, *J* = 2.0, 10.2 Hz, 1H), 6.55 (dd, *J* = 3.0, 10.0 Hz, 1H), 6.74 (dd, *J* = 3.0, 10.2 Hz, 1H), 7.17 (m, 2H), 7.26-7.30 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 31.0 (CH_3), 45.8 (CH_2), 58.4 (C), 71.0 (CH), 128.6 (CH), 129.0 (2×CH), 129.1 (2×CH), 131.9 (CH), 132.0 (CH), 135.1 (C), 144.8 (CH), 146.1 (CH), 161.6 (C), 184.5 (C), 198.0 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{17}H_{16}NO_3$) 282.1125, found 282.1124.

1-Benzyl-3-(2-phenylacetyl)-1-azaspiro[3.5]nona-5,8-diene-2,7-dione [22e]. Yield: 115.8 mg (0.32 mmol, 58%). R_f = 0.15 in CH_2Cl_2/MeOH = 99/1; IR υ_{max} (thin film, CH_2Cl_2) 3025, 2909, 1765, 1666, 1631, 1491, 1450, 1386, 1339, 878 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 3.77 (s, 2H), 4.20-4.32 (m, 2H), 4.42 (s, 1H), 6.06 (dd, *J* = 2.0, 10.0 Hz, 1H), 6.14 (dd, *J* = 2.0, 10.2 Hz, 1H), 6.40 (dd, *J* = 3.0, 10.0 Hz, 1H), 6.65 (dd, *J* = 3.0, 10.2 Hz, 1H), 7.12 (m, 4H), 7.26 (m, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 45.7 (CH_2), 51.0 (CH_2), 58.1 (2×C), 68.9 (CH), 127.8 (CH), 128.5 (CH), 128.9 (2×CH), 129.0 (2×CH), 129.1 (2×CH), 129.8 (2×CH), 131.7 (CH), 132.0 (CH), 135.0 (C), 144.3 (CH), 145.9 (CH), 161.5 (CH), 184.3 (C), 198.3 (C); HRMS (ESI) *m/z* calcd for $[M+Na]^+$ ($C_{23}H_{19}NO_3Na$) 380.1262, found 380.1262.

1-Benzyl-3-tetradecanoyl-1-azaspiro[3.5]nona-5,8-diene-2,7-dione [22f]. Yield: 80.2 mg (0.18 mmol, 73%). R_f = 0.1 in CH_2Cl_2/MeOH = 99/1; IR υ_{max} (thin film, CH_2Cl_2) 2921, 2851, 1765, 1666, 1625, 1450 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, *J* = 6.8 Hz, 3H), 1.15-1.30 (m, 20H), 1.53 (t, *J* = 6.6 Hz, 2H), 2.49 (m, 2H), 4.29 (s, 2H), 4.31 (s, 1H), 6.22 (dd, *J* = 2.0, 10.0 Hz, 1H), 6.26 (dd, *J* = 2.0, 10.2 Hz, 1H), 6.55 (dd, *J* = 2.9, 10.0 Hz, 1H), 6.76 (dd, *J* = 2.9, 10.2 Hz, 1H), 7.14-7.20 (m, 3H), 7.26-7.32 (m, 2H); ^{13}C-NMR (100 MHz,

$CDCl_3$): δ (ppm) = 14.3 (CH_3), 22.9 (2×CH_2), 23.1 (CH_2), 29.1 (CH_2), 29.5 (CH_2), 29.7 (CH_2), 29.8 (2×CH_2), 32.1 (2×CH_2), 44.0 (CH_2), 45.9 (CH_2), 55.1 (C), 58.4 (C), 77.3 (CH_2), 128.6 (CH), 129.1 (2×CH), 129.2 (2×CH), 131.9 (CH), 131.9 (CH), 135.1 (C), 145.0 (CH), 146.3 (CH), 161.8 (C), 184.6 (C), 200.7 (C); HRMS (ESI) *m/z* calcd for $[M+Na]^+$ ($C_{29}H_{39}NO_3Na$) 472.2828, found 472.2825.

1-Benzyl-3-(undec-10-ynoyl)-1-azaspiro[3.5]nona-5,8-diene-2,7-dione [22g]. Yield: 38.9 mg (0.10 mmol, 86%). R_f = 0.3 in CH_2Cl_2/MeOH 99/1; IR υ_{max} (thin film, CH_2Cl_2) 3277, 2921, 2851, 1754, 1707, 1666 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.26 (s, 6H), 1.36 (m, 2H), 1.51 (m, 4H), 1.93 (s, 1H), 2.17 (dt, *J* = 2.7, 7.0 Hz, 2H), 2.51 (m, 2H), 4.30 (s, 2H), 4.32 (s, 1H), 6.22 (dd, *J* = 2.0, 10.0 Hz, 1H), 6.26 (dd, *J* = 2.0, 10.2 Hz, 1H), 6.55 (dd, *J* = 3.0, 10.0 Hz, 1H), 6.75 (dd, *J* = 3.0, 10.2 Hz, 1H), 7.15-7.20 (m, 2H), 7.26-7.31 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 18.5 (CH_2), 23.0 (CH_2), 28.6 (CH_2), 28.8 (CH_2), 29.0 (CH_2), 29.1 (CH_2), 29.3 (CH_2), 43.9 (CH_2), 45.8 (CH_2), 58.4 (C), 68.3 (CH), 70.3 (CH), 84.9 (C), 128.6 (CH), 129.0 (2×CH), 129.1 (2×CH), 131.9 (CH), 132.0 (CH), 135.1 (C), 144.9 (CH), 146.3 (CH), 161.7 (C), 184.5 (C), 200.6 (C); HRMS (ESI) *m/z* calcd for $[M+Na]^+$ ($C_{26}H_{29}NO_3Na$) 426.2045, found 426.2044.

1-Benzyl-3-[(13Z)-docos-13-enoyl]-1-azaspiro[3.5]nona-5,8-diene-2,7-dione [22h]. Yield: 51.0 mg (0.09 mmol, 50%). R_f = 0.3 in cyclohexane/EtOAc = 8/2; IR υ_{max} (thin film, CH_2Cl_2) 2924, 2853, 1770, 1671, 1456 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, *J* = 6.9 Hz, 3H), 1.18-1.38 (m, 26H), 1.53 (m, 2H), 1.68 (m, 2H), 2.01 (m, 4H), 2.38-2.62 (m, 2H), 4.30 (m, 3H), 5.34 (m, 2H), 6.22 (dd, *J* = 2.0, 10.0 Hz, 1H), 6.26 (dd, *J* = 2.0, 10.2 Hz, 1H), 6.54 (dd, *J* = 2.9, 10.0 Hz, 1H), 6.75 (dd, *J* = 2.9, 10.2 Hz, 1H), 7.17 (m, 2H), 7.28 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (CH_3), 22.9 (CH_2), 23.0 (CH_2), 27.4 (CH_2), 29.1 (CH_2), 29.5-30.0 (12×CH_2), 32.1 (CH_2), 44.0 (CH_2), 45.8 (CH_2), 58.3 (C), 70.3 (CH_2), 128.6 (CH), 129.0 (2×CH), 129.1 (2×CH), 130.1 (2×CH), 131.9 (CH), 131.9 (CH), 135.1 (C), 144.9 (CH), 146.3 (CH), 161.7 (C), 184.5 (C), 200.6 (C); HRMS (ESI) m/z calcd for $[M+H]^+$ ($C_{37}H_{54}NO_3$) 560.4098, found 560.4097.

4-Acetyl-2-dodecyl-2-azaspiro[4.5]deca-6,9-diene-3,8-dione [24a]. Tautomers with a ratio of Enol/Keto = 1/0.3 in $CDCl_3$. Yield: 29.7 mg (0.08 mmol, 25%). R_f = 0.20 in cyclohexane/EtOAc = 8/2. Due to the low Keto-portion, only NMR data of the Enol form are shown; IR υ_{max} (thin film, CH_2Cl_2) 2921, 2848, 1732, 1642, 1454, 1197, 1115 cm^{-1}; ^{1}H-NMR_{Enol} (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, *J* = 6.8 Hz, 3H), 1.18-1.36 (m, 18H), 1.54 (m, 2H), 2.30 (s, 3H), 3.33 (m, 2H), 3.42 (s, 2H), 6.33 (d, *J* = 10.0 Hz, 2H), 6.87 (d, *J* = 10.0 Hz, 2H), 12.46 (s, 1H). ^{13}C-NMR_{Enol} (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (CH_3), 17.6 (CH_3), 22.9 (CH_2), 27.0 (CH_2), 27.3 (CH_2), 29.4-29.9 (6×CH_2), 32.0 (CH_2), 42.6 (CH_2), 42.9 (C), 54.2 (CH_2), 100.1 (C), 128.3 (2×CH), 150.5 (2×CH), 167.2 (C), 171.9 (C), 185.3 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{23}H_{36}NO_3$) 374.2695, found 374.2690.

2-Dodecyl-4-(2-phenylacetyl)-2-azaspiro[4.5]deca-6,9-diene-3,8-dione [24b]. Tautomers with a ratio of Enol/Keto = 1/0.8 in $CDCl_3$. Yield: 37.1 mg (0.08 mmol, 27%). R_f = 0.1 in cyclohexane/EtOAc = 8/2; IR υ_{max} (thin film, CH_2Cl_2) 2915, 2839, 1666, 1485, 1456, 1258, 855 cm^{-1}; ^{1}H-NMR_{Keto} (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, *J* = 6.8 Hz, 3H), 1.18-1.36 (m, 20H), 1.54 (m, 2H), 3.29-3.35 (m, 3H), 3.71 (s, 1H), 3.88 (q, *J* = 16.0 Hz, 2H), 6.25 (ddd, *J* = 1.9, 5.1, 10.2 Hz, 1H), 6.73 (dd, *J* = 3.0, 10.2 Hz, 1H), 7.03 (dd, *J* = 3.0, 10.4 Hz, 1H), 7.13 (m, 2H), 7.20-7-34 (m, 3H); ^{1}H-NMR_{Enol} (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, *J* = 6.8 Hz, 3H), 1.18-1.36 (m, 18H), 1.55 (m, 2H), 3.22 (d, *J* = 9.9 Hz, 1H), 3.34 (m, 2H), 3.42 (s, 2H), 3.66 (d, *J* = 9.9 Hz, 1H), 6.32 (d, *J* = 10.0 Hz, 2H), 6.90 (d, *J* = 10.0 Hz, 2H), 7.14 (m, 2H), 7.20-7.34 (m, 3H), 12.67 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (2×CH_3), 22.9 (2×CH_2), 26.9 (CH_2), 27.0 (CH_2), 27.3 (CH_2), 27.4 (CH_2), 29.4 (CH_2), 29.4 (CH_2), 29.6 (2×CH_2), 29.7 (2×CH_2), 29.8 (2×CH_2), 29.8 (4×CH_2), 32.1 (2×CH_2), 37.5 (CH_2), 42.7 (CH_2), 43.0 (C), 43.4 (CH_2), 44.4 (C), 52.1 (CH_2), 54.0 (CH_2), 54.4 (CH_2), 61.8 (CH_{Keto}), 101.0 (C_{Enol}), 127.0 (CH), 127.8 (CH), 128.4 (2×CH), 128.7 (2×CH), 129.1 (2×CH), 129.2 (2×CH), 129.7 (CH), 130.0 (2×CH), 130.9 (CH), 135.6 (C), 146.8 (CH_{Keto}), 148.5 (CH_{Keto}), 150.8 (2×CH), 168.2 (C), 168.4 (C), 172.1 (C), 184.8 (C), 185.1 (C), 202.3 (C_{Keto}); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{29}H_{40}NO_3$) 450.3003, found 450.3006.

2-Dodecyl-4-tetradecanoyl-2-azaspiro[4.5]deca-6,9-diene-3,8-dione [24c]. Tautomers with a ratio of Enol/Keto = 1/0.8 in $CDCl_3$. Yield: 75.0 mg (0.14 mmol, 38%). R_f = 0.25 in CH_2Cl_2/MeOH 99/1; IR υ_{max} (thin film, CH_2Cl_2) 3277, 2921, 2845, 1654, 1491, 1392, 1374, 861 cm^{-1}; ^{1}H-NMR_{Keto} (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, *J* = 6.8 Hz, 6H), 1.15-1.35 (m, 38H), 1.42-1.60 (m, 4H), 2.45 (m, 1H), 2.69 (m, 1H), 3.23 (d, *J* = 9.8 Hz, 1H), 3.32 (m, 2H), 3.57 (s, 1H), 3.76 (d, *J* = 9.8 Hz, 1H), 6.36 (m, 2H), 6.88 (m, 1H), 7.08 (dd, *J* = 3.0, 10.1 Hz, 1H); ^{1}H-NMR_{Enol} (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, *J* = 6.8 Hz, 6H) 1.15-1.35 (m, 38H), 1.42-1.60 (m, 4H), 1.91 (m, 2H), 3.33 (m, 2H), 3.40 (s, 2H), 6.31 (d, *J* = 10.0 Hz, 2H), 6.88 (d, *J* = 10.0 Hz, 2H), 12.53 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (4×CH_3), 22.9 (4×CH_2), 22.9 (CH_2), 26.6 (CH_2), 26.9 (CH_2), 27.0 (CH_2), 27.3 (CH_2), 27.3 (CH_2), 29.1 (CH_2), 29.4 (2×CH_2), 29.5-29.8 (25×CH_2), 31.4 (CH_{2Enol}), 32.1 (4×CH_2), 42.6 (CH_2), 43.0 (C), 43.3 (CH_2), 44.4 (C_{Keto}), 45.6 (CH_2), 53.9 (CH_2), 54.2 (CH_2), 62.7 (CH_{Keto}), 99.7 (C_{Enol}), 128.0 (2×CH_{Enol}), 129.4 (CH_{Keto}), 131.4 (CH_{Keto}), 146.8 (CH_{Keto}), 149.1 (CH_{Keto}), 151.1 (2×CH_{Enol}), 168.6 (C_{Enol}), 171.1 (C_{Keto}), 172.2 (C_{Enol}), 184.9 (C), 185.2 (C), 205.2 (C_{Keto}); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{35}H_{60}NO_3$) 542.4568, found 542.4565.

2-Benzyl-4-(2-phenylacetyl)-2-azaspiro[4.5]deca-6,9-diene-3,8-dione [24e]. Tautomers with a ratio of Enol/Keto = 1/0.8 in $CDCl_3$. Yield: 39.7 mg (0.11 mmol, 33%). R_f = 0.20 in cyclohexane/EtOAc = 7/3; IR υ_{max} (thin film, CH_2Cl_2) 3020, 2926, 1684, 1660, 1619, 1444, 1264, 1176, 861 cm^{-1}; ^{1}H-NMR_{Keto} (400 MHz, $CDCl_3$): δ (ppm) = 3.30 (s, 2H), 3.76 (s, 1H), 3.88 (q, *J* = 15.9 Hz, 2H), 4.52 (m, 2H), 6.17 (ddd, *J* = 1.8, 6.5, 10.2 Hz, 2H), 6.62 (dd, *J* = 3.0, 10.2 Hz, 1H), 6.92 (dd, *J* = 3.0, 10.4 Hz, 1H), 7.18-7.37 (m, 10H); ^{1}H-NMR_{Enol} (400 MHz, $CDCl_3$): δ (ppm) = 3.09 (d, *J* = 10.0 Hz, 1H), 3.28 (s, 2H), 3.49 (d, *J* = 10.0 Hz, 2H), 4.51 (s, 2H), 6.25 (d, *J* = 10.0 Hz, 2H), 6.82 (d, *J* = 10.0 Hz, 2H), 7.18-7.37 (m, 10H), 12.66 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 37.4 (CH_2), 42.8 (CH_2), 44.1 (CH_2), 46.7 (CH_2), 47.3 (CH_2), 52.1 (CH_2), 53.2 (CH_2), 53.8 (CH_2), 61.5 (CH_{Keto}), 100.8 (C_{Enol}), 127.1 (CH), 127.8 (CH), 128.4 (8×CH), 128.7 (2×CH), 129.1 (2×CH), 129.1 (2×CH), 129.2 (4×CH), 129.6 (CH_{Keto}), 130.0 (2×CH_{Enol}), 130.7 (CH_{Keto}), 132.1 (C), 135.2 (C), 135.4 (C), 135.5 (C), 146.6 (CH_{Keto}), 148.3 (CH_{Keto}), 150.7 (2×CH_{Enol}), 168.6 (C), 168.8 (C), 172.0 (C), 184.6 (C), 184.9 (C), 202.0 (C_{Keto}); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{24}H_{22}NO_3$) 372.1594, found 372.1596.

2-Benzyl-4-tetradecanoyl-2-azaspiro[4.5]deca-6,9-diene-3,8-dione [24f]. Tautomers with a ratio of Enol/Keto = 1/0.6 in $CDCl_3$. Yield: 41.7 mg (0.09 mmol, 41%). R_f = 0.20 in cyclohexane/EtOAc = 7/3; IR υ_{max} (thin film, DCM) 2921,

2851, 1654, 1450, 1264, 867 cm^{-1}; ^{1}H-NMR$_{Keto}$ (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, 3H, J = 6.8 Hz), 1.14-1.36 (m, 20H), 1.44-1.58 (m, 2H), 2.46 (m, 1H), 2.70 (m, 1H), 3.11 (d, 1H, J = 9.9 Hz), 3.61 (d, 1H, J = 9.9 Hz), 3.62 (s, 1H), 4.54 (d, 2H, J = 7.9 Hz), 6.31 (m, 2H), 6.79 (dd, 1H, J = 3.0, 10.0 Hz), 6.99 (dd, 1H, J = 3.0, 10.0 Hz), 7.25 (m, 2H), 7.34 (m, 3H); ^{1}H-NMR$_{Enol}$ (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, 3H, J = 6.8 Hz), 1.14-1.36 (m, 20H), 1.44-1.58 (m, 2H), 1.92 (m, 2H), 3.28 (s, 2H), 4.51 (s, 2H), 6.26 (d, 2H, J = 10.0 Hz), 6.83 (d, 2H, J = 10.0 Hz), 7.25 (m, 2H), 7.35 (m, 3H), 12.53 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (4×CH_3), 22.9 (2×CH_2), 23.0 (2×CH_2), 26.6 (CH_2), 29.1 (CH_2), 29.6 (6×CH_2), 29.7 (2×CH_2), 29.8 (5×CH_2), 31.4 (CH_2), 32.1 (3×CH_2), 42.8 (C), 44.2 (C), 45.7 (CH_2), 46.7 (CH_2), 47.3 (CH_2), 53.3 (CH_2), 53.7 (CH_2), 62.5 (CH_{Keto}), 99.5 (C_{Enol}), 128.0 (2×CH), 128.3 (2×CH), 128.4 (5×CH), 129.2 (2×CH), 129.5 (CH), 131.3 (CH), 135.2 (C), 135.5 (C), 146.7 (CH), 148.8 (CH), 151.0 (2×CH), 168.8 (C), 171.8 (C_{Keto}), 172.1 (C_{Enol}), 184.8 (C), 185.1 (C), 205,1 (C_{Keto}); HRMS (ESI) *m/z* calcd for $[M+Na]^+$ ($C_{30}H_{41}NO_3Na$) 486.2984, found 486.2985.

2-Benzyl-4-(undec-10-ynoyl)-2-azaspiro[4.5]deca-6,9-diene-3,8-dione [24g]. Tautomers with a ratio of Enol/Keto = 1/1 in $CDCl_3$. Yield: 19.4 mg (0.05 mmol, 19%). R_f = 0.20 in cyclohexane/EtOAc = 8/2; IR υ_{max} (thin film, CH_2Cl_2) 3296, 2928, 2855, 1665, 1450, 1266, 860 cm^{-1}; ^{1}H-NMR$_{Keto}$ (400 MHz, $CDCl_3$): δ (ppm) = 1.21 (m, 2H), 1.28 (m, 4H), 1.36 (m, 2H), 1.51 (m, 4H), 1.94 (t, J = 2.6 Hz, 1H), 2.17 (m, 2H), 2.46 (m, 1H), 2.71 (m, 1H), 3.12 (d, J = 10.0 Hz, 1H), 3.60 (d, J = 10.0 Hz, 1H), 3.62 (s, 1H), 4.54 (d, J = 7.3 Hz, 2H), 6.31 (m, 2H), 6.79 (dd, J = 3.0 Hz, J = 9.9 Hz, 1H), 6.99 (dd, J = 3.0, 9.9 Hz, 1H), 7.26 (m, 2H), 7.35 (m, 3H); ^{1}H-NMR$_{Enol}$ (400 MHz, $CDCl_3$): δ (ppm) = 1.21 (m, 2H), 1.28 (m, 4H), 1.36 (m, 2H), 1.51 (m, 4H), 1.91 (m, 2H), 1.94 (t, J = 2.6 Hz, 1H), 2.17 (m, 2H), 3.28 (s, 2H), 4.51 (s, 2H), 6.26 (d, J = 10.0 Hz, 2H), 6.84 (d, J = 10.0 Hz, 2H), 7.26 (m, 2H), 7.35 (m, 3H), 12.53 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 18.6 (2×CH_2), 22.9 (CH_2), 26.6 (CH_2), 28.6 (CH_2), 28.6 (CH_2), 28.8 (2×CH_2), 29.0 (4×CH_2), 29.4 (2×CH_2), 29.5 (2×CH_2), 31.4 (CH_2), 42.8 (CH_2), 44.2 (CH_2), 45.7 (CH_2), 46.7 (CH_2), 47.3 (CH_2), 53.3 (CH_2), 53.7 (CH_2), 62.6 (CH_{Keto}), 68.3 (C), 68.3 (C), 84.9 (CH), 84.9 (CH), 99.6 (C_{Enol}), 128.1 (2×CH), 128.3 (CH), 128.4 (2×CH), 129.2 (2×CH_{Enol}), 129.5 (CH_{Keto}), 131.3 (CH_{Keto}), 135.2 (C), 135.5 (C), 146.6 (CH_{Keto}), 148.7 (CH_{Keto}), 150.9 (2×CH_{Enol}), 168.7 (C), 171.7 (2×C), 172.2 (C), 184.7 (C), 185.1 (C), 205.0 (C_{Keto}); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{27}H_{32}NO_3$) 418.2377, found 418.2378.

1-Dodecyl-3-{2-methoxy-8-oxo-1-oxaspiro[4.5]deca-6,9-dien-2-yl}-1-azaspiro[3.5]nona-5,8-diene-2,7-dione [26α]. Yield: 13.7 mg (0.03 mmol, 15%). R_f = 0.15 in cyclohexane/EtOAc = 7/3; IR υ_{max} (thin film, CH_2Cl_2) 2924, 2854, 1760, 1671, 1631, 1456, 1393, 1078, 1015, 856 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, J = 6.8 Hz, 3H), 1.16-1.36 (s, 18H), 1.49 (m, 2H), 2.23 (m, 2H), 2.43 (m, 2H), 3.08 (s, 3H), 3.17 (m, 2H), 4.04 (s, 1H), 6.15 (m, 2H), 6.45 (dd, J = 1.9, 4.8 Hz, 1H), 6.47 (dd, J = 1.9, 5.1 Hz, 1H), 6.78 (m, 1H), 6.84 (dd, J = 3.0, 10.1 Hz, 1H), 6.97 (dd, J = 3.0, 10.4 Hz, 1H), 7.07 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (CH_3), 22.9 (CH_2), 27.1 (CH_2), 29.2 (2×CH_2), 29.5 (CH_2), 29.7 (CH_2), 29.8 (3×CH_2), 32.1 (CH_2), 36.0 (CH_2), 36.2 (CH_2), 41.8 (CH_2), 49.7 (CH_3), 58.1 (C), 64.7 (CH), 79.0 (C), 108.1 (C), 127.5 (CH), 128.2 (CH), 132.3 (CH), 132.5 (CH), 145.4 (CH), 148.2 (CH), 149.2 (CH), 149.9 (CH), 165.4 (C), 184.5 (C), 185.5 (C); HRMS (ESI) *m/z* calcd for $[M+NH_4]^+$ ($C_{30}H_{45}N_2O_5$) 513.3323, found 513.3319.

1-Dodecyl-3-{2-methoxy-8-oxo-1-oxaspiro[4.5]deca-6,9-dien-2-yl}-1-azaspiro[3.5]nona-5,8-diene-2,7-dione [27α]. Yield: 15.7 mg (0.03 mmol, 17%). R_f = 0.10 in cyclohexane/EtOAc = 7/3; IR υ_{max} (thin film, CH_2Cl_2) 2924, 2853, 1756, 1670, 1463, 1014, 857 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, J = 6.8 Hz, 3H), 1.16-1.36 (m, 18H), 1.44 (m, 2H), 2.13 (m, 1H), 7.45 (m, 1H), 2.27 (m, 2H), 2.68 (m, 1H), 3.00 (m, 1H), 3.10 (s, 3H), 3.17 (m, 1H), 3.76 (s, 1H), 6.12 (dd, J = 2.0, 10.1 Hz, 1H), 6.18 (dd, J = 2.0, 10.1 Hz, 1H), 6.41 (m, 2H), 6.78 (m, 1H), 6.89 (dd, J = 3.0, 10.1 Hz, 1H), 7.04 (dd, J = 3.0, 10.1 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.4 (CH_3), 22.9 (CH_2), 27.0 (CH_2), 29.1 (CH_2), 29.2 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 29.8 (3×CH_2), 32.1 (CH_2), 34.7 (CH_2), 34.8 (CH_2), 41.7 (CH_2), 51.3 (CH_3), 59.3 (C), 65.9 (CH), 80.7 (C), 109.5 (C), 127.3 (CH), 128.2 (CH), 131.4 (CH), 132.3 (CH), 147.1 (CH), 148.0 (CH), 148.8 (CH), 150.1 (CH), 164.8 (C), 185.1 (C), 185.5 (C); HRMS (ESI) *m/z* calcd for $[M+Na]^+$ ($C_{30}H_{41}NO_5Na$) 518.2877, found 518.2873.

3-{2-Ethoxy-8-oxo-1-oxaspiro[4.5]deca-6,9-dien-2-yl}-1-(tridec-12-yn-1-yl)-1-azaspiro[3.5]nona-5,8-diene-2,7-dione [26β]. Yield: 11.3 mg (0.02 mmol, 11%). R_f = 0.15 in cyclohexane/EtOAc = 8/2; IR υ_{max} (thin film, CH_2Cl_2) 2925, 2854, 1758, 1671, 1630, 1393, 1013, 857 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.04 (t, J = 7.0 Hz, 3H), 1.14-1.32 (m, 14H), 1.34-1.42 (m, 2H), 1.46-1.56 (m, 4H), 1.94 (t, J = 2.6 Hz, 1H), 2.18 (dt, J = 2.6, 7.1 Hz, 2H), 2.21 (m, 1H), 2.39 (m, 2H), 3.10 (m, 2H), 3.60 (m, 1H), 4.05 (s, 1H), 6.15 (m, 2H), 6.46 (ddd, J = 2.0, 7.7, 10.2 Hz, 2H), 6.77 (m, 1H), 6.83 (dd, J = 3.0, 10.2 Hz, 1H), 7.00 (dd, J = 3.0, 10.5 Hz, 1H), 7.08 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 15.2 (CH_3), 18.6 (CH_2), 27.1 (CH_2), 28.7 (CH_2), 28.9 (CH_2), 29.2 (CH_2), 29.3 (2×CH_2), 29.6 (3×CH_2), 36.2 (CH_2), 36.3 (CH_2), 41.8 (CH_2), 57.9 (CH_2), 58.2 (CH_2), 65.2 (CH), 68.3 (C), 78.9 (C), 85.0 (C), 107.9 (C), 127.5 (CH), 128.2 (CH), 132.2 (CH), 132.4 (CH), 145.6 (CH), 148.3 (CH), 149.3 (CH), 150.0 (CH), 165.5 (C), 184.5 (C), 185.5 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{32}H_{42}NO_5$) 520.3058, found 520.3053.

3-{2-Ethoxy-8-oxo-1-oxaspiro[4.5]deca-6,9-dien-2-yl}-1-(tridec-12-yn-1-yl)-1-azaspiro[3.5]nona-5,8-diene-2,7-dione [27β]. Yield: 26.0 mg (0.05 mmol, 25%). R_f = 0.1 in cyclohexane/EtOAc = 8/2; IR υ_{max} (thin film, CH_2Cl_2) 2926, 2854, 1755, 1670, 1630, 1008, 857 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.06 (t, J = 7.0 Hz, 3H), 1.16-1.56 (m, 20H), 1.94 (t, J = 2.7 Hz, 1H), 2.19 (m, 2H), 2.28 (m, 1H), 2.65 (ddd, J = 8.7, 10.5, 11.6 Hz, 1H), 3.01 (m, 1H), 3.24 (m, 2H), 3.77 (s, 1H), 4.07 (m, 1H), 6.11 (dd, J = 2.0, 10.1 Hz, 1H), 6.18 (dd, J = 2.0, 10.1 Hz, 1H), 6.40 (ddd, J = 2.0, 8.3, 10.4 Hz, 2H), 6.77 (dd, J = 2.9, 10.2 Hz, 1H), 6.91 (dd, J = 3.0, 10.1 Hz, 1H), 7.05 (dd, 1H, J = 3.0, 10.1 Hz, 1H), 7.47 (dd, J = 2.9, 10.2 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 15.4 (CH_3), 18.6 (CH_2), 26.9 (CH_2), 28.7 (CH_2), 28.9 (CH_2), 29.1 (CH_2), 29.2 (CH_2), 29.3 (2×CH_2), 29.6 (CH_2), 29.7 (CH_2), 34.8 (CH_2), 35.1 (CH_2), 41.7 (CH_2), 59.2 (2×CH_2), 66.2 (CH), 68.3 (C), 80.5 (C), 85.0 (C), 109.1 (C), 127.2 (CH), 128.1 (CH), 131.3 (CH), 132.2 (CH), 147.4 (CH), 148.2 (CH), 149.0 (CH), 150.3 (CH), 165.0 (C), 185.1 (C), 185.5 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{32}H_{42}NO_5$) 520.3058, found 520.3053.

General procedure for the synthesis of 28.

To a solution of spirocyclohexadienone (0.1 mmol) in anhydrous ethanol/CH_2Cl_2 (1:1, 20 mL) at 0°C was added in one portion $NaBH_4$ (3.8 mg, 0.1 mmol). The mixture was stirred for 2 h at 0°C. A saturated NH_4Cl solution was added and the mixture was stirred for 10 min. After concentration *in vacuo*, EtOAc was added. The organic layer was washed with

brine and dried over $MgSO_4$. After removal of solvent *in vacuo*, the crude product was purified by silica gel to give the compound **28**.

(2a*S,3*R**,4a*S**,6*R**)-1-benzyl-6-hydroxy-3-tridecyl-2a,3,5,6-tetrahydro-1*H*-benzofuro[3a,3-*b*]azet-2(4a*H*)-one [28f]**. Yield: 24.2 mg (0.05 mmol, 43%). R_f = 0.1 in cyclohexane/EtOAc = 8/2; IR υ_{max} (thin film, CH_2Cl_2) 2923, 2853, 1738, 1455, 1068 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, J = 6.8 Hz, 3H), 1.20-1.34 (s, 20H), 1.45 (m, 2H), 1.60 (m, 2H), 1.81 (m, 2H), 3.28 (d, J = 3.4 Hz, 1H), 3.81 (dd, J = 5.2, 8.4 Hz, 1H), 4.11 (m, 1H), 4.11 (d, J = 15.0 Hz, 1H), 4.23 (ddd, J = 3.4, 6.5, 8.0 Hz, 1H), 4.49 (d, J = 15.0 Hz, 1H), 5.67 (dd, J = 1.5, 10.1 Hz, 1H), 6.11 (dd, J = 3.1, 10.1 Hz, 1H), 7.24 (m, 2H), 7.27-7.34 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) =14.3 (CH_3), 22.9 (CH_2), 26.1 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 29.8 (CH_2), 29.9 (CH_2), 29.9 (CH_2), 29.9 (CH_2), 29.9 (CH_2), 32.1 (CH_2), 37.1 (CH_2), 37.3 (CH_2), 44.8 (CH_2), 64.6 (CH), 68.2 (CH), 68.6 (C), 76.5 (CH), 78.6 (CH), 126.0 (CH), 128.2 (CH), 128.9 (2×CH), 129.0 (2×CH), 135.8 (C), 138.2 (CH), 167.4 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{29}H_{44}NO_3$) 454.3316, found 454.3316.

(2a*S,3*R**,4a*S**,6*R**)-1-benzyl-3-(dec-9-yn-1-yl)-6-hydroxy-2a,3,5,6-tetrahydro-1*H*-benzofuro[3a,3-*b*]azet-2(4a*H*)-one [28g]**. Yield: 10.5 mg (0.03 mmol, 30%). R_f = 0.15 in CH_2Cl_2/MeOH 98/2; IR υ_{max} (thin film, CH_2Cl_2) 2929, 2856, 1736, 1643, 1455, 1272, 1069 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.24-1.42 (m, 14H), 1.51 (m, 2H), 1.94 (t, J = 2.6 Hz, 1H), 2.17 (dt, J = 2.6, 7.1 Hz, 2H), 3.28 (d, J = 3.3 Hz, 1H), 3.81 (dd, J = 5.0, 8.6 Hz, 1H), 4.11 (d, J = 15.0 Hz, 2H), 4.23 (m, 1H), 4.49 (d, J = 15.0 Hz, 1H), 5.67 (dd, J = 1.5, 10.1 Hz, 1H), 6.11 (dd, J = 3.0, 10.1 Hz, 1H), 7.24 (m, 2H), 7.27-7.35 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 18.6 (CH_2), 26.0 (CH_2), 28.6 (CH_2), 28.9 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 37.0 (CH_2), 37.4 (CH_2), 44.8 (CH_2), 64.7 (CH), 68.2 (C), 68.3 (CH), 68.6 (CH), 76.4 (CH), 78.5 (CH), 85.0 (C), 125.9 (CH), 128.2 (CH), 128.9 (2×CH), 129.0 (2×CH), 135.8 (C), 138.3 (CH), 167.3 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{26}H_{34}NO_3$) 408.2533, found 408.2534.

(2a*S,3*R**,4a*S**,6*R**)-6-hydroxy-3-tridecyl-1-undecyl-2a,3,5,6-tetrahydro-1*H*-benzofuro[3a,3-*b*]azet-2(4a*H*)-one [28c]**. Yield: 17.1 mg (0.03 mmol, 42%). R_f = 0.15 in CH_2Cl_2/MeOH 98/2; IR υ_{max} (thin film, CH_2Cl_2) 2914, 2849, 1732, 1469, 1067 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, J = 6.8 Hz, 6H), 1.18-1.36 (m, 38H), 1.38–1.52 (m, 3H), 1.53–1.75 (m, 3H), 1.99 (ddd, J = 7.6, 8.9, 13.1 Hz, 1H), 2.22 (dt, J = 4.6, 13.0 Hz, 1H), 3.01 (m, 1H), 3.12 (m, 1H), 3.23 (d, J = 3.2 Hz, 1H), 4.14–4.25 (m, 2H), 4.31 (dt, J = 3.2, 6.6 Hz, 1H), 5.78 (dd, J = 1.6, 10.0 Hz, 1H), 6.20 (dd, J = 3.1, 10.0 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (2×CH_3), 22.9 (2×CH_2), 26.1 (CH_2), 27.2 (CH_2), 28.9 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (2×CH_2), 29.7 (2×CH_2), 29.8 (CH_2), 29.9 (8×CH_2), 32.2 (CH_2), 37.1 (CH_2), 37.8 (CH_2), 41.0 (CH_2), 64.8 (CH), 67.7 (CH), 68.1 (C), 76.5 (CH), 78.9 (CH), 126.6 (CH), 138.1 (CH), 167.8 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{34}H_{62}NO_3$) 532.4724, found 532.4724.

(2a*S,3*R**,4a*S**,6*R**)-1-benzyl-3-((*Z*)-henicos-12-en-1-yl)-6-hydroxy-2a,3,5,6-tetrahydro-1H-benzofuro[3a,3-*b*]azet-2(4a*H*)-one [28i]**. Yield: 28.0 mg (0.05 mmol, 38%). R_f = 0.15 in cyclohexane/EtOAc = 8/2; IR υ_{max} (thin film, CH_2Cl_2) 2924, 2853, 1739, 1456, 1069 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (t, J = 6.9 Hz, 3H), 1.16-1.38 (m, 28H), 1.55-1.73 (m, 4H), 1.82 (m, 2H), 2.00 (m, 4H), 3.28 (d, J = 3.3 Hz, 1H), 3.81 (dd, J = 5.1, 8.6 Hz, 1H), 4.11 (m, 2H), 4.23 (m, 1H), 4.49 (d, J = 15.0 Hz, 1H), 5.34 (m, 2H), 5.67 (dd, J = 1.5, 10.1 Hz, 1H), 6.11 (dd, J = 3.0, 10.1 Hz, 1H), 7.24 (m, 2H), 7.28-7.34 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.3 (CH_3), 22.9 (CH_2), 26.1 (CH_2), 27.1 (CH_2), 27.4 (CH_2), 29.4-30.0 (12×CH_2), 30.4 (CH_2), 32.1 (CH_2), 37.1 (CH_2), 37.3 (CH_2), 44.8 (CH_2), 64.7 (CH), 68.2 (CH), 68.6 (C), 76.4 (CH), 78.6 (CH), 125.9 (CH), 128.2 (CH), 128.9 (2×CH), 129.0 (2×CH), 130.1 (C), 135.8 (C), 138.2 (CH), 167.4 (C); HRMS (ESI) *m/z* calcd for $[M+H]^+$ ($C_{37}H_{58}NO_3$) 564.4411, found 564.4410.

Notes and references

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† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

Electronic Supplementary Information (ESI) available: ^{1}H and ^{13}C spectra. See DOI: 10.1039/b000000x/

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