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Short and Highly Efficient Synthesis of Lipid Peroxidation Inhibitor Pyrrolostatin and Some Analogues Thereof

Cite this: DOI: 10.1039/xoxxoooooox

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

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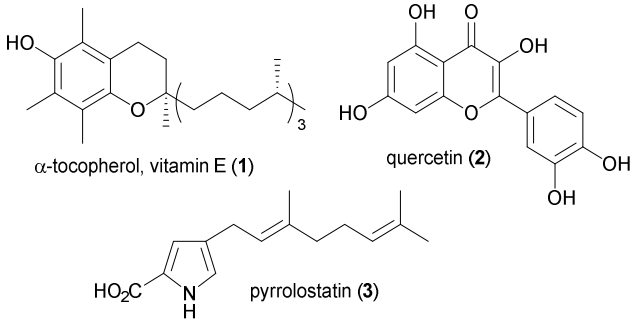
A highly efficient and scalable synthesis of potent lipid peroxidation inhibitor pyrrolostatin is reported (4 steps, 48%). In addition to the synthesis of the natural product, strategies for the preparation of analogues differing in the three structural subunits, the polar head group, the *N*-substituent and the lipophilic tail are described.

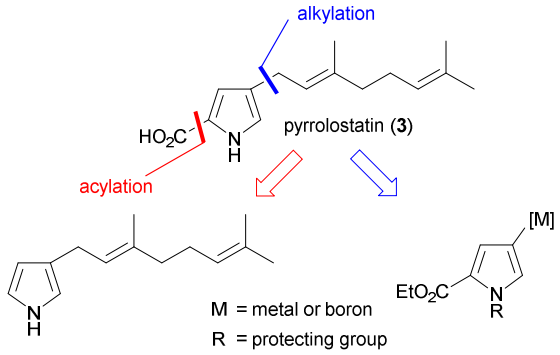
Lipid peroxidation has been shown to be a cause of the perturbation of membrane organization, damage of lipid function and modification of proteins and DNA. It has been connected to serious diseases such as cardiovascular diseases, neurological disorders and cancer.[1] Anti-oxidants usually inhibit lipid peroxidation and suppress formation of toxic oxidation products. Thus, the search for new anti-oxidants different from the commonly used tocopherols (e.g. vitamin E, **1** in Fig. 1) or polyphenols (e.g. quercetin, **2** in Fig. 1) represents a research topic of significant importance.

Fig. 1 Chemical structures of natural products with lipid peroxidation inhibitory activity.



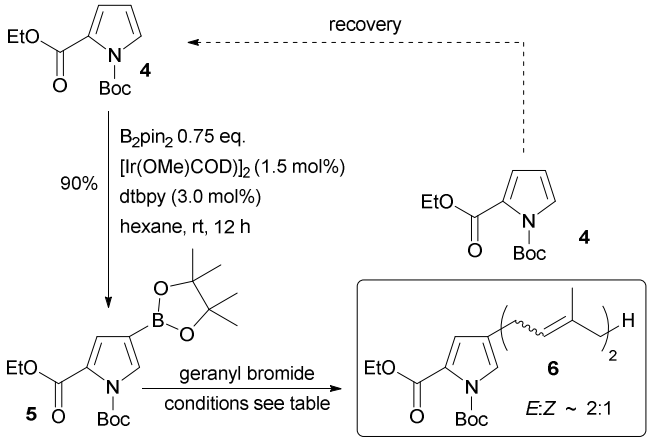
In 1993 Kato *et al.* reported the isolation and characterization of pyrrolostatin (**3**, Fig. 1) from *Streptomyces chrestomyceticus* EC 40, a strain derived from a soil sample collected in Brazil.[2] From an 800 litre culture 1.7 g of this novel metabolite could be isolated. Pyrrolostatin consists of a 2-carboxy-pyrrole to which a geranyl chain is attached in the 4 position of the aromatic core (Fig. 1). In addition to the structural elucidation of the natural product, Kato *et al.* were able to establish its inhibitory activity against lipid peroxidation and demonstrate a protective effect against acute hypoxia in mice.[2] In this study, pyrrolostatin was shown to be more effective than α-tocopherol.[2] Until to date only a single total synthesis of pyrrolostatin has been reported.[3] Ono and co-workers used a Barton-Zard reaction[4] as a key step of their approach and were able to prepare the natural product in 9 steps (starting from geraniol) with an overall yield of <10%. In addition, a mechanism of anti-oxidation different to that of vitamin E was suggested.

As part of our interest in the synthesis of bioactive meroterpenes[5] and amphiphilic natural products[6] and our commitment to all areas of oxidation chemistry[7], we decided to investigate a total synthesis of pyrrolostatin and some analogues thereof. As a fairly simple target molecule, there are two obvious disconnections. One can either start from a putatively easily accessible geranyl pyrrole and investigate a regioselective acylation (red reaction path in Scheme 1) or alkylate a metallated pyrrole derivative with the 2-carboxy substituent already present (blue pathway in Scheme 1).



Scheme 1 Key step of the pyrrolostatin retrosynthesis.

At the outset of our studies we focused on a Suzuki based cross-coupling approach (Scheme 2) according to the blue pathway in Scheme 1 (M = BR_2). To this end, a regioselective C-H-borylation of a 2-substituted pyrrole was envisaged. A sterically demanding *N*-protecting group was used to ensure a regioselective



Scheme 2 Pyrrole borylation and cross-coupling.

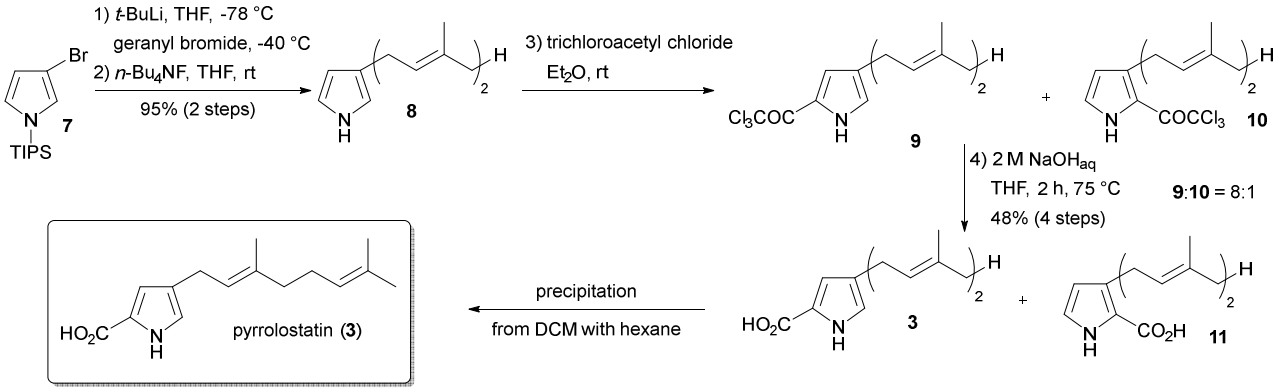
functionalization.[8] Thus, Boc-pyrrole **4**[9] was borylated with bis(pinacolato)diboron in the presence of 1.5 mol% of iridium catalyst at room temperature (Scheme 2).[8,9] The reaction is easily scalable under Schlenk conditions (no glovebox necessary) and boronic ester product **5** could be isolated in 90% yield as a single regioisomer. Various reagents and conditions (catalysts, bases, solvents and reaction temperatures) were then tested for the cross-coupling of pyrrole **5** with geranyl bromide. Full conversion could be achieved when $Pd(dba)_2$ was used as catalyst (e.g. Table 1, entry 5). However, the desired product was isolated as a mixture of double bond isomers (*E*/*Z*: 2:1) and in addition a significant amount of hydrodeborylation product (29%) was obtained. It turned out that the formation of the deborylation by-product **4** could be minimized at higher temperature (Table 1, entry 7 vs. entry 8) and with higher catalyst loading (Table 1, entry 12 vs. entry 13). Under optimized conditions with potassium phosphate as base in THF (Table 1, entry 13) cross-coupling product **6** could be isolated in high yield (83%) and easily separated from **4** (15%) by column chromatography. Nevertheless, as the main obstacle of this reaction, the *E*/*Z*-isomerization of the allylic double bond could not be suppressed in any of these experiments. Irrespective of the reaction conditions the diastereomeric ratio was determined as 2:1 by GC-MS analysis after *N*-deprotection (for details see Table 1). A simple separation of the two double bond isomers on a preparative scale was not efficient at this stage.

Table 1 Suzuki cross-coupling of pyrrole **5** with geranyl bromide.[a]

	Catalyst	Base	Solvent	T [°C]	t [min]	**6**[b,d]	**4**[b]
1	$Pd(PPh_3)_4$	K_2CO_3	toluene	120	300	no conv.	
2	$Pd(OAc)_2$	K_2CO_3	toluene	120	300	no conv.	
3	$PdCl_2$	K_2CO_3	toluene	120	300	no conv.	
4	$Pd(dba)_2$	K_2CO_3	toluene	120	300	slow conv.	
5	$Pd(dba)_2$	Cs_2CO_3	toluene	120	120	70	29
6	$Pd(dba)_2$	Cs_2CO_3	DMF	100	20	decomp.	
7	$Pd(dba)_2$	Cs_2CO_3	THF	80	30	82	17
8	$Pd(dba)_2$	Cs_2CO_3	THF	50	30	70	28
9	$Pd(dba)_2$	Cs_2CO_3	DMSO	120	300	no conv.	
10	$Pd(dba)_2$	Cs_2CO_3	dioxane	120	20	37	20
11	$Pd(dba)_2$	KOAc	THF	80	300	slow conv.	
12	**$Pd(dba)_2$**	**K_3PO_4**	**THF**	**80**	**30**	**83**	**15**
13	$Pd(dba)_2$[c]	K_3PO_4	THF	80	30	77	22

[a] Reaction conditions: **5** (0.5 mmol), solvent (20 mL, abs.), geranyl bromide (0.6 mmol) and base (4.5 mmol) were degassed. The catalyst (10 mol%) was added and the reaction mixture stirred for the time and at the temperature indicated. When no further reaction progress was detected, the mixture was filtered over silica, the solvent removed and the residue purified by flash chromatography (diethyl ether/hexanes 1/20). [b] In order to quickly determine the product ratio, compounds **4** and **6** were isolated as a mixture and not separated; yields were calculated based on the ratio determined by ^{1}H-NMR. [c] 2 mol% catalyst were used. [d] The diastereomeric ratio of **6** was determined as 2:1 after *N*-deprotection.

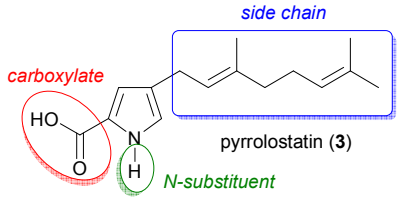
At this point and with the aim to provide reasonable quantities of the natural product as well as some analogues thereof, we turned our attention to a different synthetic approach according to the retrosynthetic pathway marked in red in Scheme 1. This synthesis commences with commercially available *N*-silylated 3-bromopyrrole **7** (Scheme 3). Halogen-lithium exchange and subsequent alkylation with geranyl bromide gave a regioselective S_N2-reaction without any *E*/*Z*-isomerization. The crude product was directly deprotected with tetra-*n*-butylammonium fluoride and yielded pyrrole **8** in 95% over two steps. Next, the electron rich heterocycle was acylated with trichloroacetyl chloride under Friedel-Crafts conditions.[10] No Lewis-acid catalyst was required to achieve this reaction.[11] The bulky acylation reagent gave a regioisomeric mixture of trichloromethyl ketones in favour of the wanted isomer (**9**:**10** = 8:1). The following nucleophilic displacement of the trichloromethyl group to furnish the carboxylate was carried out on the mixture of **9** and **10** under basic conditions (2M NaOH in THF) and yielded pyrrolostatin along with its regioisomer **11** in 48% yield over the whole four steps sequence. The target molecule was easily purified and separated from the minor isomer **11** by precipitation from dichloromethane and hexane. Thus, the natural product is accessible in high purity and sufficient quantities for derivatizations and further investigations.



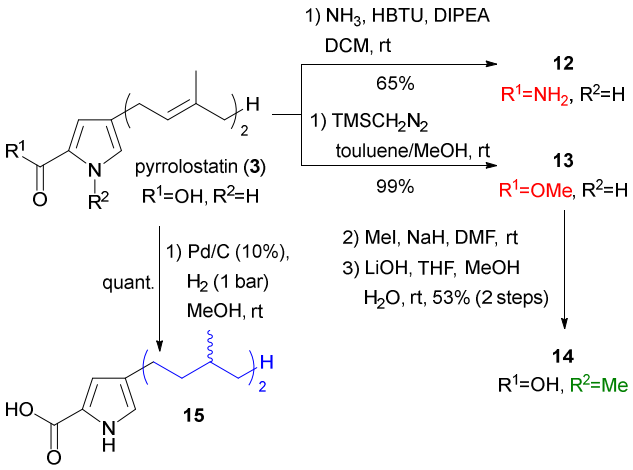
Scheme 3 Total synthesis of pyrrolostatin.

We next turned our attention to the synthesis of some pyrrolostatin analogues (Fig. 2). Therefore, we decided to modify the substituents around the pyrrole core: the side chain (blue), the carboxylate (red) and the *N*-substituent (green in Fig. 2).

Fig. 2 Possible sites of modification on lipid peroxidation inhibitor pyrrolostatin.

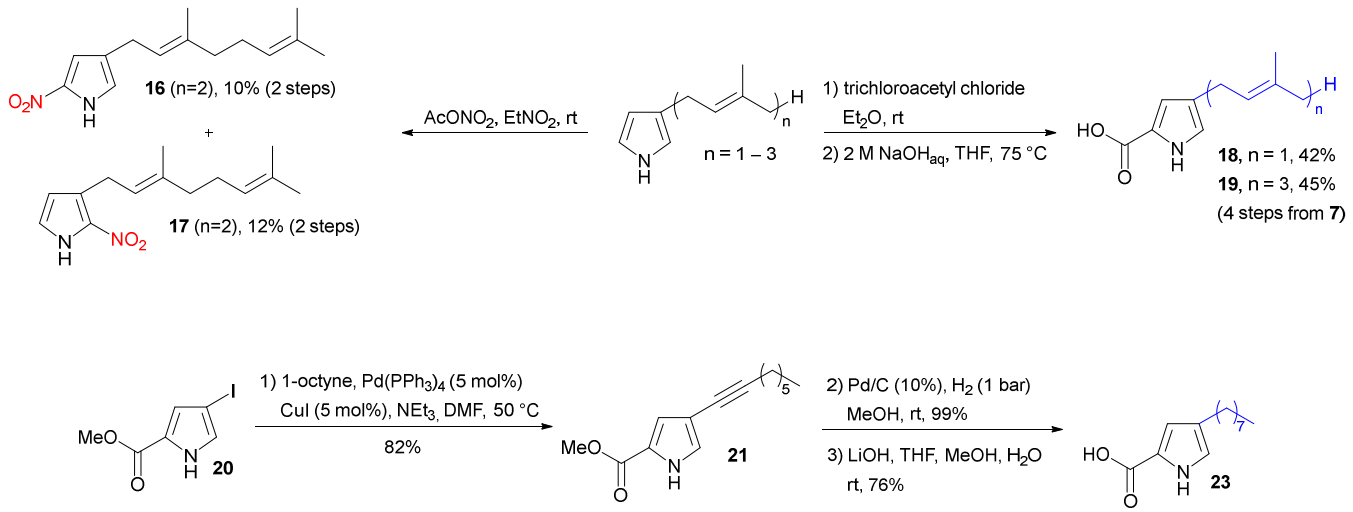


Amide **12** and ester **13** were directly synthesized from pyrrolostatin with (trimethylsilyl)diazomethane[12] and ammonia (using HBTU activation and Hünig's base), respectively (Scheme 4). Thus, the amide **12** was obtained in 65% and the ester in 99% yield. Starting from ester **13**, *N*-methylation followed by deprotection of the carboxylate (LiOH in methanol) gave *N*-methyl pyrrolostatin **14** (53% over two steps; Scheme 4). Hydrogenation (Pd/C, H_2) of pyrrolostatin (**3**) provided an analogue with a saturated terpenoid subunit in quantitative yield (**15**; Scheme 4).[13]



Scheme 4 Synthesis of pyrrolostatin-analogues.

Also, an analogue where the polar carboxylate terminus is replaced by a bioisosteric nitro function was prepared (Scheme 5). The respective nitration[14] of 3-geranylated pyrrole **8** (n=2) with nitronium acetate[15] gave a mixture of two regioisomers (**16** and **17** in Scheme 5) in a 1:1.2 ratio.[6b,16] These isomers were separable by standard column chromatography. Analogues with an altered length of the terpenoid side chain (**18** and **19**) were readily accessible using an approach closely resembling that of the pyrrolostatin synthesis. Thus, adaption of the four step sequence summarized in Scheme 3 using prenyl or farnesyl bromide as electrophiles in the first step furnished **18** and **19** in 42% and 45% yield, respectively (yields over four steps). Pyrroles with an unbranched side chain were synthesized by coupling commercially available iodide **20** to 1-octyne under sonogashira conditions[17] (Scheme 5). Hydrogenation of the triple bond and ester hydrolysis gave analogue **23** (75% yield over two steps; Scheme 5). The linear C_8-chain was chosen so to have an equal length but lacking the methyl branching as compared to the pyrrolostatin lipophilic tail.



Scheme 5 Preparation of analogues of pyrrolostatin differing in the acceptor on the aromatic core and the side chain.

In summary, we have developed a straightforward and easily scalable synthesis of lipid peroxidation inhibitor pyrrolostatin (48% yield over 4 steps). In addition to the total synthesis, it has been demonstrated that all relevant structural subunits, the hydrophilic head, the amino substituent and the lipophilic tail, can be modified synthetically in a *de novo* approach. Thus, a variety of analogues were synthesized. The anti-oxidant activity of all analogues is currently being investigated and will help to understand the mechanism of action.

Acknowledgements

Generous support of this research by the Fonds der Chemischen Industrie (FCI) is gratefully acknowledged.

Notes and references

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Electronic Supplementary Information (ESI) available: Full experimental details, analytical data and reproductions of NMR-spectra. See DOI: 10.1039/c000000x/

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