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Prodrug and Covalent Linker Strategies for the Solubilization of Dual-Action Antioxidants/Iron Chelators

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Abstract—Water soluble prodrugs of hybrid free radical scavenger/iron chelating molecules, based on 3,5-disubstituted-4-hydroxyphenyl derivatives and 3-hydroxy-2-methyl-4*(1H)*-pyridinone (deferiprone), have been prepared. Related hybrid molecules containing a covalent poly(ethylene)glycol or an amine linker were also synthesized.

The role that oxidative stress plays in the onset of neurodegenerative disorders is now well established.[1] The brain is particularly susceptible to oxidative stress since it has a high consumption of both energy and oxygen, yet has a relatively limited antioxidant capacity, and contains high levels of iron and polyunsaturated fatty acids in its cell membranes. Both antioxidants and iron chelating molecules have shown neuroprotective efficacy in animal models of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and stroke.[2]

Recently, we disclosed novel, dual action, neuroprotective, hybrid molecules such as **1** and **2**, which synergistically combine an iron chelating molecule, such as deferiprone, with an antioxidant, e.g., BHT or a hydroxybenzo-furan or -pyran derivative (see Fig. 1).[3]

Hybrid molecules **1** and **2** were shown to offer protection against oxidative stress in a model of iodoacetate-induced cell toxicity in cerebellar granule cells, and in a model of lipid peroxidation in rat brain homogenates.[3,4] For example **1** ($n=0$) inhibited lipid peroxidation with an IC_{50} of 0.3 μM, which represents at the very least a ca. 10-fold improvement over its separate components, that is 5.9 μM for BHT and 3.9 μM for deferiprone.

In an extension to this study we wished to prepare analogues of **1** and **2** with improved water solubility, both for systemic administration and to eliminate toxic effects associated with the use of a non-aqueous vehicle, as **1** ($n=1$) and **2** ($n=0$) show a water solubility of less than 0.1 mg/mL.

Our strategy, summarized in Figure 2, was aimed at targeting three specific regions of molecules **1** and **2**. Approach (i) would necessitate the synthesis of further analogues of **1** by the development of a new solubilizing linker to bridge the iron chelating and radical scavenging entities,[5] whereas approaches (ii) and (iii) would involve the derivatization of either the phenolic or hydroxy pyridone groups of **1** or **2** respectively via a prodrug approach.[6]

Results and Discussion

Poly(ethyleneglycol) (PEG) derivatives have been successfully employed in medicinal chemistry, as esters or ethers, to improve the aqueous solubility and administration of drugs.[7] To this end we decided to devise synthetic routes to new analogues of **1** containing a covalently-attached PEG ether linkage, that is deferiprone and BHT attached with z=PEG linker (Approach (i), Fig. 2). One of the key steps in the synthesis of compounds such as **1** involves the formation of a BHT derivative bearing a primary amine. The amine is subsequently condensed with benzyl maltol,[8] to afford, after deprotection, the final hybrid BHT-deferiprone molecule. Using this approach, we synthesized

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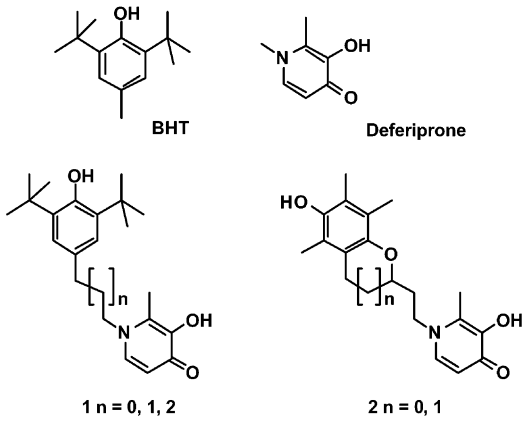


Figure 1. Hybrid iron chelator radical scavengers.

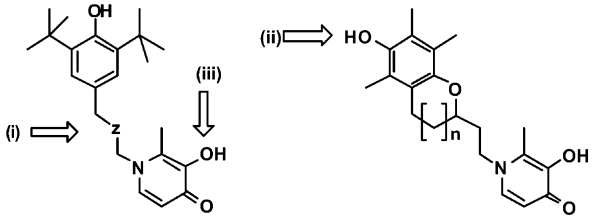
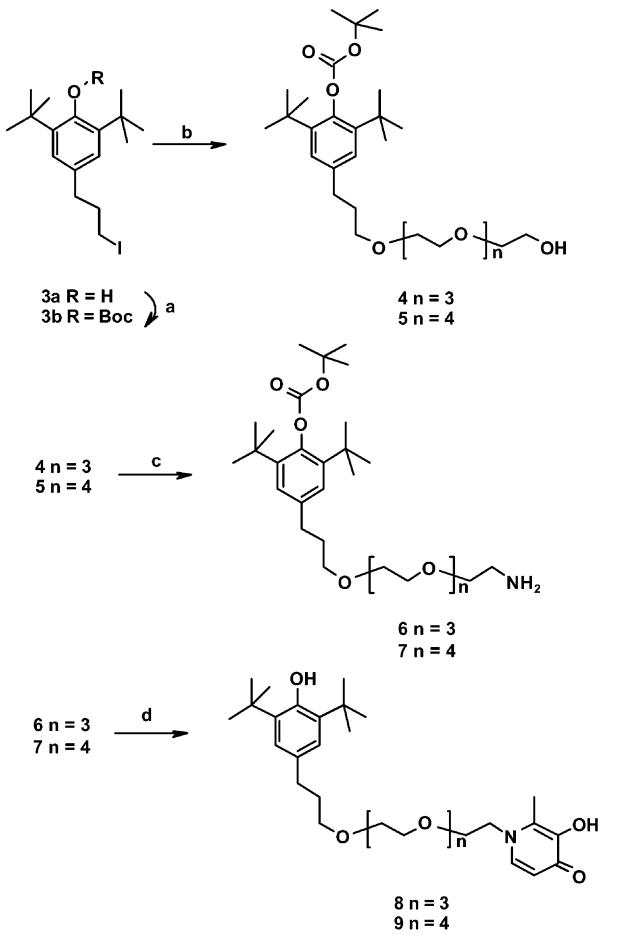


Figure 2. Derivatization of **1** and **2** using prodrug and new linker strategies.



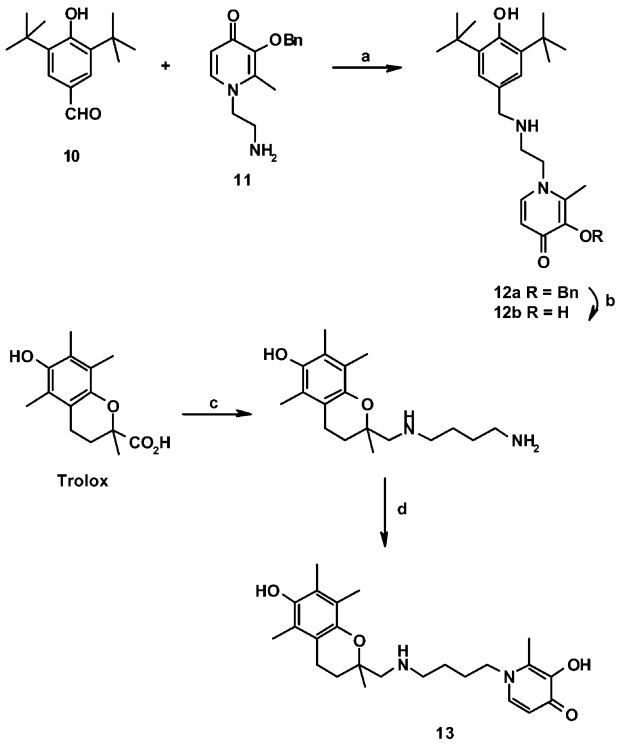
Scheme 1. Reagents and conditions: (a) $(Boc)_2O$, DMAP, heptane, 87%; (b) NaH, DMF, $HO(CH_2CH_2O)_nCH_2CH_2OH$, 0 °C then 90 °C, 37% ($n=3$), 41% ($n=4$); (c) (i) NEt_3, MsCl, CH_2Cl_2; (ii) NaN_3, DMF, 90 °C; (iii) PtO_2, EtOH, H_2, 1 atm, 86% ($n=3$), 68% ($n=4$); (d) (i) benzyl maltol, EtOH, H_2O, reflux; (ii) Pd/C, EtOH, H_2, 1 atm; (iii) TFA, 7% ($n=3$), 9% ($n=4$) for last 3 steps.

the PEG-containing amines **6** and **7** (see Scheme 1). This protocol necessitated suitable *O*-protection of **3a**, as the free phenol itself did not react cleanly with poly (ethyleneglycols). Gratifyingly, the use of a recently disclosed procedure for the protection of hindered phenols with a *tert*-butoxycarbonyl (Boc) protecting group was successful,[9] and the carbonate **3b** was formed in 87% yield.

The ensuing reactions were straightforward: displacement of the iodide from **3b** by the sodium salts of either tetraethylene or pentaethylene glycol yielded the expected alcohols **4** and **5** respectively, along with some elimination (-HI) product. Mesylation of alcohols **4** and **5**, followed by displacement with sodium azide and then reduction furnished amines **6** and **7**. These amines underwent condensation reactions with benzyl maltol, which after deprotection gave the requisite hybrid molecules **8** and **9**. Although the yields for the final maltol condensation reactions were low (less than 10%), ca. 100 mg samples of **8** and **9** were readily prepared.[10] However, **8** and **9** were found to be largely insoluble in water, despite the presence of up to five oxygens in the linker group, so this led us to consider shorter linkers with basic centres.

The alternative system **12b**, with a simple amine linker, was synthesized by a low-yielding reductive amination reaction of benzaldehyde **10** and 1-(2-aminoethyl) deferiprone **11**, followed by debenzylation (Scheme 2). The related amine linked derivative **13** was synthesized by a five-step process: a coupling reaction between Trolox® and a *N*-Boc protected diamine, deprotection, amide reduction followed by condensation with benzyl maltol and finally debenzylation. Compounds **12b** and **13** showed excellent activities for lipid peroxidation (IC_{50} of 0.2 and 0.1 μM for **12b** and **13** respectively), but limited aqueous solubilities, although the former was soluble in a DMSO/H_2O (1:9) mixture.

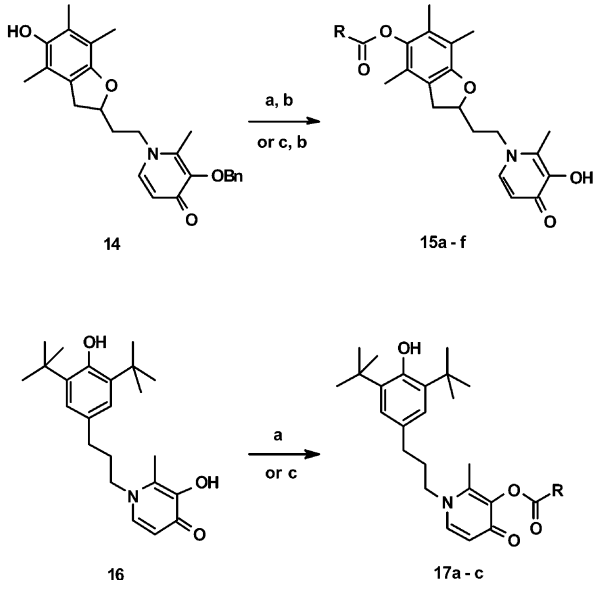
Our next approach required the formation of ester prodrugs by functionalising a hydroxyl group of **1** or **2**, that is strategies (ii) and (iii) as outlined in Figure 2. The ester prodrug approach is well established, as exemplified by the formation of water soluble prodrugs of Vitamin E.[11] Thus, the phenolic hydroxyl of hybrid molecules **2** was esterified to prepare aminoalkyl- and aminoaryl-carboxylic acid esters. Initial attempts to functionalise the phenolic hydroxyl of **14** (Scheme 3), using DCC and *N*-Boc β-alanine in pyridine, as described for the preparation of Vitamin E prodrugs, were unsuccessful. However, treatment of **14** with potassium carbonate in acetone followed by the addition of *N*-hydroxysuccinimide (HOSu) esters of various *N*-Boc protected amino acids resulted in esterification in yields of greater than 76%. Following deprotection compounds **15a**–**e** were isolated. Compound **15f** was prepared by treating a solution of **14** in pyridine at reflux



Scheme 2. Reagents and conditions: (a) $NaCNBH_3$, MeOH, 13%; (b) Pd/C, EtOH, H_2, 1 atm, 96%; (c) (i) EDCI, HOBt, DIPEA, $BocHN(CH_2)_4NH_2$, DMF, 72%; (ii) TFA, CH_2Cl_2, 60%; (iii) $LiAlH_4$, THF, reflux, 57%; (d) (i) benzyl maltol, EtOH, H_2O, reflux, 21%; (ii) Pd/C, EtOH, H_2, 1 atm, 96%.

with *N*,*N*-dimethylglycinyl chloride HCl and in a yield of 54%. The water solubility of the hydrochloride salts of **15a**–**f** was determined.[12]

In the final approach, method (iii) in Figure 2, hybrid derivatives **16** were functionalized by the chemoselective acylation of the pyridone 3-hydroxyl group to prepare esters **17** (Scheme 3). Esterification was also achieved by



Scheme 3. Reagents and conditions: (a) K_2CO_3, acetone, acylating agent (see Table 1), reflux; (b) Pd/C, EtOH, H_2, 1 atm; (c) acylating agent (see Table 1), pyridine, reflux, HCl treatment.

reaction of **16** with HOSu esters of *N*-Boc protected amino acids, without the need for protection of the phenolic group,[13] except for **17c** which was prepared by treating **16** with acetic anhydride in pyridine.

The water solubilities of the prodrugs and the hetero-atom linked hybrids are summarized in Table 1. All of the hydrochloride salts of the aminoalkyl carboxylic acid ester prodrugs (**15a**, **15c**–**f** and **17a**) showed good solubility in water, that is at least 10 mg/mL. The two aminoaryl carboxylic acid esters (**15b** and **17b**) showed little water solubility, as did the PEG linked hybrid molecules **8** and **9**. The simple acetate prodrug **17c** was also very sparingly soluble.

Having achieved our primary objective of synthesizing water soluble, hybrid molecules, it was then necessary to explore the usefulness of the prodrugs by measuring their ease of hydrolysis under physiological conditions, and their stability as an aqueous formulation. Preliminary experiments have shown that **15c** underwent almost complete hydrolysis to **2** ($n=0$) on incubation in rat plasma at 37 °C for 3 h, and yet during this time remained unchanged as a solution in phosphate buffer (pH 7.4). Under the same conditions **15a** and **15d** were hydrolysed to a lesser extent in rat plasma (approximately 20–30%), and were also stable to the phosphate buffer.

Conclusions

Three approaches to improve the water solubilization of dual action, hybrid molecules **1** and **2** have been described. Covalently-attached PEG and amine linkers offered little improvement towards increasing the water solubility. However, the prodrug strategy, as exemplified by attaching amino acids via ester linkages, led to substantial improvements in the water solubility of the hybrid molecules. Early experiments in vitro have demonstrated that the ester can be hydrolysed relatively efficiently, and thus the prodrug approach is a viable

Table 1. Water solubility

Compd	Acylating agent[c]	R	Solubility in water (mg/mL)
8[a]	—	—	<0.1
9[a]	—	—	<0.1
12b[b]	—	—	10–20[d]
13[b]	—	—	<0.1
15a[b]	Boc-β-Ala-OSu	$CH_2CH_2NH_2$	10–20
15b[a]	$BocHNpC_6H_4C(O)OSu$	p-$C_6H_4NH_2$	<0.1
15c[b]	Boc-Sar-OSu	CH_2NHCH_3	>20
15d[b]	Boc-Gaba-OSu	$CH_2CH_2CH_2NH_2$	10–20
15e[b]	(*S*)-Boc-Val-OSu	$CH(i\text{-}Pr)NH_2$	>20
15f[b]	DMG-Cl.HCl	$CH_2N(CH_3)_2$	>20
17a[b]	Boc-β-Ala-OSu	$CH_2CH_2NH_2$	>20
17b[a]	$BocHNpC_6H_4C(O)OSu$	p-$C_6H_4NH_2$	<0.1
17c[a]	Ac_2O	CH_3	<0.1

[a]mono.HCl salt. Boc removal by HCl treatments.
[b]bis.HCl salt. Boc removal by HCl treatments.
[c]Ala, alanine; Sar, sarcosine; Gaba, γ-aminobutyric acid; Val, valine; DMG-Cl, *N*,*N*-dimethylglycinyl chloride.
[d]In DMSO/H_2O (1:9).

strategy as these molecules could be formulated as 10 mg/mL aqueous solutions suitable for administration in vivo.[14]

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