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Synthesis and preliminary biological evaluation of new phenolic and catecholic dehydroamino acid derivatives

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ABSTRACT

A library of *N*-phenolic and *N*-catecholic dehydroamino acid derivatives was prepared using an innovative synthetic strategy that involves mild reaction conditions and simple work-up procedures. The method comprises coupling of phenolic or catecholic acids with β-hydroxyamino acids followed by *tert*-butyloxycarbonylation of all hydroxyl groups using *tert*-butyldicarbonate and 4-dimethylaminopyridine as catalyst. Treatment of these amino acids with *N,N,N′,N′*-tetramethylguanidine affords the corresponding *O*-*tert*-butyloxycarbonyldehydroamino acid derivative. Deprotection of the aromatic hydroxyl groups is carried out with trifluoroacetic acid. This synthetic strategy can be applied in a one-pot procedure and yields compounds that can be easily inserted into peptides or other biomolecules after cleavage of the C-protecting group. Preliminary studies of cell viability show that these new compounds display very low or no toxicity. These dehydroamino acids with a phenolic or catecholic moiety can have intrinsic biological activity or used to prepare new hydrogels that mimic mussel adhesive proteins.

1. Introduction

Phenolic acids coupled with amino acids or amines can be obtained from natural sources or synthetically. It is assumed that these bioactive substances are involved in suppression of deleterious effects of oxidative stress and have a wide range of biological activities such as antioxidant,[1–5] anticancer[6] and antimicrobial.[7–12] In fact, the accumulation of hydroxycinnamic acid amides constitutes part of the defence system of plants that is activated as response to various environmental stimuli such as wounding, fungal infection or heavy metal ions.[13–16] Lee et al. prepared a series of cinnamic acid derivatives and evaluated their biological activities in lipoprotein metabolism.[17] The methyl esters of *N*-(4-hydroxycinnamoyl)-L-phenylalanine and the dibenzyl ester of *N*-caffeoyl-aspartic acid showed potent anti-atherogenic and antioxidant activities. Moreover, amides of cinnamic, ferulic and sinapic acids with natural and unnatural C-protected amino acids have been synthesized and also showed antimicrobial activity, radical scavenging activity against the free 2,2-diphenyl-1-picrylhydrazyl radical and antioxidant activity in bulk oil.[18,19]

Several studies suggest that a cocktail of antioxidants, endowed with different molecular structures and mechanisms of action, result more effective than a single antioxidant, due to the synergic effect between different types of molecules.[20–26] To highlight possible synergic mechanisms and to better understand mechanistic aspects, the design of modified and/or dualistic molecules is a valuable approach. Studies have confirmed that conjugation between different types of compounds such as amino acids with phenolic acids is useful, not only to investigate structure-activity relationships, but can also constitute a strategy to improve antioxidant efficiency and bioactivity.[27–29]

Another application of phenolic or catecholic amino acids is in the design of new peptide hydrogels that mimic mussel adhesive proteins.[30] The extraordinary ability of mussel adhesive proteins is mainly attributed to the reversible metal-catechol coordination between metals like Fe^{3+} and catechol groups from the amino acid 3,4-dihydroxyphenyl-L-alanine (DOPA), and also to other interactions such as cation-π interactions.[31] Recently, a new type of injectable hydrogel based on self-assembly of an *ABA* tri-block copolymer with rapid self-healing properties through mussel-

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inspired catechol-mediated hydrogen bonding interactions and aromatic interactions and with anti-biofouling capability was described.[32]

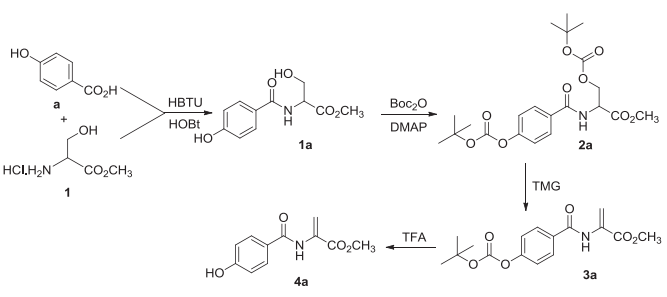
Non-coded amino acids can have a variety of applications such as in structure-activity relationship studies, as antiviral, antitumor, anti-inflammatory or immunosuppressor compounds or in the development of new biomaterials.[33–35] In particular, dehydroamino acids constitute an important class of non-proteinogenic amino acids with various biological activities including antioxidant.[36–48] In general, the presence of one or more α,β-dehydroamino acid in a polypeptide chain has strong impact, not only on the secondary structure adopted, but also on their biological behaviour, including antibacterial, antifungal and antitumor activities[36–38] and resistance to proteolysis. Recently, several low molecular weight dehydrodipeptide hydrogelators were prepared and studied as new drug delivery systems.[49,50] Thus, the combination of phenolic or catecholic moieties with dehydroamino acids can be a valuable approach to the development of new biologically active compounds.

In our laboratories we developed an efficient method for the synthesis of *N,N*-diacyl-α,β-dehydroamino acid derivatives by using two equivalents of *tert*-butyldicarbonate (Boc_2O) and 4-dimethylaminopyridine (DMAP) as catalyst in dry acetonitrile.[51] In order to allow the synthesis of *N*-monoprotected dehydroamino acid derivatives, a modification of this method was subsequently reported.[52] Thus, by reacting β-hydroxyamino acid derivatives with one equivalent of Boc_2O and DMAP it was possible to obtain the corresponding β-carbonates that undergo β-elimination by treatment with *N,N,N′,N′*-tetramethylguanidine (TMG).

In this work and in order to explore the effect of combining a dehydroamino acid moiety with phenolic or catecholic acids, an innovative strategy for the synthesis of these conjugates was developed. This new methodology involves the simultaneous *tert*-butyloxycarbonylation of all hydroxyl groups present in the N-protected β-hydroxyamino acids followed by a selective β-elimination reaction and cleavage of the aromatic *O*-*tert*-butyloxycarbonyl groups. The non-coded amino acids prepared can have biological activity or can be use in the design of new peptide hydrogels that mimic mussel adhesive proteins.

2. Results and discussion

The methyl ester of *N*-(4-hydroxybenzoyl) dehydroalanine was prepared from 4-hydroxybenzoic acid (**a**) and the methyl ester of serine (**1**). The coupling between **a** and **1** was carried out using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and 1-hydroxybenzotriazole (HOBt) to give the methyl ester of *N*-(4-hydroxybenzoyl)serine in 71% yield (Scheme 1, compound **1a**). This compound was treated with two equivalents of Boc_2O and DMAP as catalyst in acetonitrile to afford compound **2a** (Scheme 1). In the proton NMR spectrum of this compound it is possible to observe two singlets at 1.47 ppm and 1.57 ppm that correspond to the protons of the two *tert*-butyloxycarbonyl (Boc) groups. Compound **2a** was reacted with a solution of TMG in acetonitrile to give the dehydroalanine derivative **3a** in 31% yield (Scheme 1). The proton NMR spectrum of **3a** shows two singlets at 6.01 ppm and 6.79 ppm characteristic of the β-CH_2 protons of dehydroalanine. Cleavage of the aromatic *O*-Boc group from **3a** was accomplished using trifluoroacetic acid, giving compound **4a** in 47% yield (Scheme 1).



Scheme 1. Synthesis of the methyl ester of *N*-(4-hydroxybenzoyl) dehydroalanine.

In order to improve the low overall yield, a one-pot procedure was tested. Thus, compound **1a** was used in a sequential reaction with Boc_2O/DMAP, followed by TMG and finally TFA. Compound **4a** was isolated in 47% yield (Scheme 2, Table 1).

The same methodology was applied with the methyl ester of serine and other phenolic and catecholic acids to give the dehydroalanine derivatives **4c**, **4e** and **4f** (Scheme 2, Table 1). Although attempted, it was impossible to isolate the coupling products between serine and protocatechuic acid (**b**), 2-(3,4-dihydroxyphenyl) acetic acid (**d**), hydrocaffeic acid (**g**) and gallic acid (**h**). This may be due to the high hydrophilic character of compounds **1b**, **1d**, **1g** and **1h** when compared with the other serine derivatives (compounds **1a**, **1c**, **1e** and **1f**).

The same approach was applied to the synthesis of dehydrophenylalanine derivatives *N*-capped with phenolic or catecholic acids. Thus, the methyl ester of *N*-caffeoylphenylserine was prepared by coupling caffeic acid (**f**) with the methyl ester of phenylserine (**5**) to give compound **5f** (Scheme 3). Compound **5f** was treated with three equivalents of Boc_2O and DMAP to give compound **6f**. The three singlets corresponding to the 27 protons of the three Boc groups were observed in the proton NMR spectrum at 1.47, 1.56 and 1.57 ppm. Compound **6f** was reacted with TMG to give dehydrophenylalanine **7f** which gave **8f** after cleavage of the aromatic *O*-Boc groups. The dehydrophenylalanine derivative was obtained in 29% overall yield (Scheme 3).

As observed for the dehydroalanine derivatives the overall yield in compound **5f** was considerably improved using the one-pot procedure (Scheme 4, Table 2). Thus, all other phenolic and catecholic acids (**a**-**e**, **g**, **h**) were reacted with the methyl ester of phenylserine. The lower hidrophylicity of the phenylserine derivatives obtained, allowed the preparation of all N-protected phenylserine derivatives in good yields (Scheme 4, Table 2, compounds **5a**-**e**, **5g**, **5h**). The one-pot procedure was carried out with all N-protected phenylserine derivatives to give the Z-isomer of the corresponding dehydrophenylalanine derivative (Scheme 4, Table 2, compounds **8a**-**e**, **8g**). The stereochemistry of the dehydrophenylalanine moieties was confirmed using NOE difference experiments by irradiating the OMe protons and observing an NOE enhancement on the signal of the β-CH proton. The *N*-galloyl phenylserine derivative (compound **5h)** was treated in the same conditions but the only product isolated was *N*-deprotected dehydrophenylalanine (H-*Z*-ΔPhe-OMe). This probably resulted from the higher nucleophilic character of the amide nitrogen of the galloyl derivative, which led to the preferential *tert*-butyloxycarbonylation of the amide, making the galloyl group susceptible to cleavage by TMG. This was described by Ragnarsson and coworkers for cleavage of acyl groups from *N*-acyl-*N*-Boc-amines.[53] The subsequent treatment with TFA of the *N*-*tert*-butyloxycarbonyl dehydrophenylalanine formed led to the methyl ester of dehydrophenylalanine.

The preparation of *C*-deprotected dehydroalanine and dehydrophenylalanine derivatives requires the removal of the methyl esters. However, due to the relative ease with which catechol groups oxidize, are prone to nucleophilic attack and phenolic coupling reactions in basic media, it is not possible to remove the methyl esters from compounds **4a**, **4c**, **4e**, **4f** and **8a-g**.