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Phenyl-EDOTn derivatives as super acid labile carboxylic acid protecting groups for peptide synthesis

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Abstract

A series of new 3,4-ethylenedioxy-2-thenyl (EDOTn) derived alcohols have been synthesized and evaluated as super acid labile carboxylic acid protecting groups. All the derivatives are labile to very low concentrations of TFA (0.01–0.5%).

Keywords: Peptide synthesis; EDOTn; EDOT; Acid labile protecting groups; Carboxylic acid protection

1. Introduction

Nowadays, most peptide syntheses are carried out on solid phase in the C to N direction with the α-carboxylate of the C-terminal amino acid attached to the solid support.[1] Nevertheless, the synthesis of several important peptides involves side chain or backbone attachment to the resin or synthesis in solution.[2–4] In all these strategies, a suitable protecting group for the C-terminal α-carboxylate is mandatory. In addition to that, carboxylic acid protection is also necessary for the side chains of Asp and Glu and broadly for other carboxylic groups.

In the case of solid phase peptide synthesis, the most widely used strategy is the Fmoc/^{t}Bu strategy, which involves α-amino temporary protection by the base labile Fmoc group, side chain protection with trifluoroacetic acid (TFA) labile protecting groups, usually ^{t}Bu type ones; and cleavage from the resin also with TFA. Therefore, a suitable carboxyl protecting group for solid phase peptide synthesis should be resistant to the base treatments used to remove the Fmoc group and, for most of the applications, the conditions for its removal should leave ^{t}Bu type groups unaltered, allowing the obtention of ^{t}Bu protected peptides as well as other labile moieties present in the molecule such as sugars.

3,4-Ethylenedioxythiophene (EDOT) is a highly electron-rich compound. Thus, protecting groups based on 3,4-ethylenedioxy-2-thenyl (EDOTn) should be very acid labile due to the stabilization of the resulting thenyl carbocation. EDOTn derivatives have recently been described as very acid labile BAL-type linkers[5] as well as amide backbone protectors,[6] which can be removed by TFA–DCM (1:99) and (95:5), respectively.

New EDOTn-derived compounds (**1a**–**e**) as very acid labile carboxylic acid protecting groups are herein described (Fig. 1).

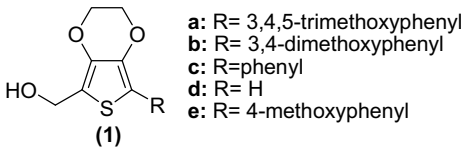

Fig. 1. EDOTn-derived alcohols prepared.

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In the previous works, the acid lability of EDOTn was slightly increased by adding electron-donating substituents via an inductive effect such as alkyl or alkylsulfide.[5]

In the present work, EDOTn has been functionalized with electron-rich phenyl rings in order to increase the acid lability by resonance effect. A series of esters of different phenyl-EDOTn derivatives have been synthesized and their lability to TFA evaluated, investigating also the effect of adding electron-donating substituents to the phenyl ring.

2. Synthesis of alcohols based on electron-rich EDOTn

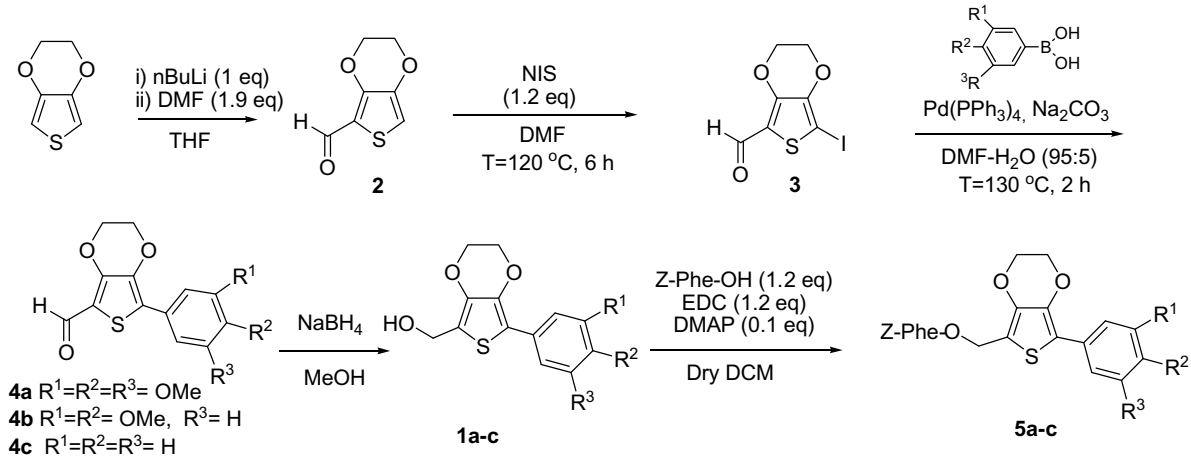
Two different synthetic methods were tested. In Method A (Scheme 1), the commercially available EDOT was formylated in high yield using *n*-BuLi and DMF as described in the previous works of the group[6] yielding **2**. This latter was iodinated by reaction with *N*-iodosuccinimide (NIS) in DMF to afford **3**. Suzuki–Miyaura reaction[7] of the iodinated aldehydes with different phenylboronic acids yielded furnished **4a–c**, which were reduced with $NaBH_4$ to the corresponding alcohols **1a–c**. In Method B, compound **2** was reduced with $NaBH_4$ to alcohol **1d**. Then, it was protected as acetate and iodinated with NIS in DMF. The iodide was converted to **1e** by reaction with 4-methoxyphenyl boronic acid (Scheme 2).

From the two methods, A is the best because although the iodination of **2** requires harsher conditions than the iodination of **1d**, the yields of the Suzuki reactions on compound **3** are much higher than those on compound **6**.[8]

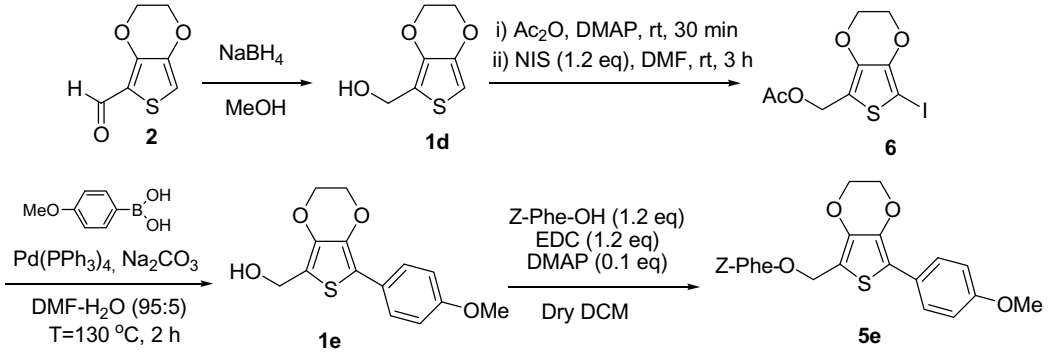
Finally, for evaluation purposes alcohols **1a–e** were esterified with *N*-benzyloxycarbonylphenylalanine (Z–Phe–OH) affording **5a–e** esters in high yields.[9]

3. Removal assays[10]

All the EDOTn derivatives prepared are labile to very low concentrations of TFA (0.01–0.5% TFA in DCM) (Table 1). Thus, they can be removed in the presence of ^{t}Bu type protecting groups. **1a–c** and **1e** are more acid labile than **1d** confirming that the conjugation provided by the aromatic ring stabilizes the resulting carbocation. Among the phenyl-EDOTn derivatives **1a–c** and **1e**, **1b** is more labile than **1e** and the latter more labile than **1c**. Interestingly, **1a** exhibits very similar lability to **1c**. A probable explanation is that the steric hindrance between the three methoxy substituents makes them go out of the aromatic ring plane and consequently their electron-donating effect decreases dramatically. Similar results have been found in a protecting group for Arg.[11] To corroborate this hypothesis, UV spectra of compounds **4a–c** were registered



Scheme 1. The synthesis of *N*-(benzyloxycarbonyl)phenylalanine (Z–Phe–OH) esters of EDOTn derivatives, Method A.



Scheme 2. The synthesis of *N*-(benzyloxycarbonyl)phenylalanine (Z–Phe–OH) esters of EDOTn derivatives, Method B.

Table 1
Acid labilities of the different EDOTn derivatives

	1a (%)	**1b** (%)	**1e** (%)	**1c** (%)	**1d** (%)
0.01% TFA, $t = 10$ min	5	35	20	3	0
0.01% TFA, $t = 1$ h	—	87	68	—	—
0.5% TFA, $t = 5$ min	100	100	100	96	59
0.5% TFA, $t = 1$ h	—	—	—	—	100

showing the following λ_{max}: 362.0, 373.8 and 339.4 nm, respectively. The decrease in λ_{max} (hypsochromic shift) of compound **4a** compared to compound **4b** is probably due to the fact that in compound **4a** the lone pairs of the methoxy groups are less delocalized into the aromatic nucleus.

4. Orthogonality to the Fmoc group

Nowadays, most peptides synthesized on solid phase are prepared using the Fmoc/^{t}Bu strategy. Therefore, it is interesting to check how resistant alcohols **1a–e** are used to the piperidine-mediated removal of the Fmoc group. 3,4-Ethylenedioxythenyl *N*-(benzyloxycarbonyl)phenylalaninate (**5d**) was chosen as a model because it contains the least electron-rich system and can, therefore, be the most base labile. **5d** was treated with piperidine-DMF (2:8) for 48 h. HPLC analysis revealed that only 5% of EDOTn removal took place, confirming that the EDOTn derivatives described are compatible and orthogonal with the Fmoc group.

5. Conclusions

In conclusion, the new PhEDOTn carboxyl protecting groups can be removed using very low concentrations of TFA, being rather stable to the standard protocols to remove the Fmoc group. These groups can be useful alternatives for the preparation of complex peptides. Furthermore, the unexpected stability of **1c** should be useful for the design of new protecting groups.

Acknowledgments

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8. *Method A*: *5-Iodo-3,4-ethylenedioxythiophene-2-carbaldehyde* (**3**). Compound **2** (510 mg, 3 mmol) and *N*-iodosuccinimide (NIS) (810 mg, 3.6 mmol) were dissolved in dry DMF (5 mL) and stirred at 120 °C until no starting material was detected by HPLC (usually 6–8 h). The reaction mixture was cooled to room temperature. Diethylether (60 mL) was added and the resulting solution was washed with H_2O (3 × 50 mL). The organic portion was dried over $MgSO_4$ and filtered; the solution was then stored at −20 °C and used within 24 h maximum. (The dry product is very unstable even at low temperatures). A small aliquot was characterized by ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.78$ (s, 1H), 4.36 (s, 4H) HPLC.
5-(3,4,5-Trimethoxyphenyl)-3,4-ethylenedioxythiophene-2-carbaldehyde (**4a**): DMF (25 mL) was added to the above obtained solution of **3** in diethyl ether (60 mL). The diethyl ether was evaporated and more DMF was added to reach a total volume of 85 mL, then 3,4,5-trimethoxyphenylboronic acid (859 mg, 4.05 mmol), $Pd(PPh_3)_4$ (212 mg, 0.184 mmol), and 2 M aqueous Na_2CO_3 (5.4 mL) were also added and the mixture was stirred at 135 °C for 4 h. The course of the reaction was followed by TLC (EtOAc–hexane; 1:1). The reaction mixture was evaporated to dryness, DCM (100 mL) was added and the solution was washed with H_2O (3 × 100 mL). The organic phase was dried over $MgSO_4$ and evaporated to dryness. The crude obtained was purified by silica gel chromatography (hexane, EtOAc) to render 620 mg of **4a** (62% yield). Mp 146.3–149.8 °C. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.93$ (s, 1H), 7.02 (s, 2H), 4.42 (m, 4H), 3.9 (s, 6H), 3.88 (s, 3H).
5-(3,4-Dimethoxyphenyl)-3,4-ethylenedioxythiophene-2-carbaldehyde (**4b**): 630 mg (69% yield, 90% purity). Mp 185.4–190.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.92$ (s, 1H), 7.40 (dd, 1H, $J = 8.4$ and 2.1 Hz), 7.31 (d, 1H, $J = 2.1$ Hz), 6.90 (d, 1H, $J = 8.4$ Hz), 4.40 (m, 4H), 3.93 (s, 3H), 3.92 (s, 3H).
5-Phenyl-3,4-ethylenedioxythiophene-2-carbaldehyde (**4c**): 474.1 mg (64% yield, 90% purity) mp 135.2–139.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.94$ (s, 1H), 7.80 (dd, 2H, $J = 7.2$ and 1.4 Hz), 7.37 (m, 3H), 4.41 (m, 4H).
5-(3,4,5-Trimethoxyphenyl)-3,4-ethylenedioxythenyl alcohol (**1a**): Compound **4a** (150 mg, 0.45 mmol) was dissolved in MeOH (5 mL) and the resulting suspension was cooled in an ice bath. $NaBH_4$ (135.1 mg, 3.57 mmol) was added. The evolution of H_2 was observed and the initial suspension became a solution which was stirred at room temperature for 1 h. After that, H_2O (20 mL) was added and the pH adjusted to 8 by adding NH_4Cl; DCM extractions were then carried out (3 × 20 mL). The organic extracts were dried with $MgSO_4$, evaporated to dryness, and dried in the vacuum desiccator to get rid of all the MeOH. 133.6 mg of a solid was obtained (89% yield, 90% purity).
^{1}H NMR (400 MHz, DMSO): $\delta = 6.86$ (s, 2H), 5.29 (t, 1H, $J = 5.6$ Hz), 4.46 (d, 2H, $J = 5.6$ Hz), 4.26 (m, 4H), 3.78 (s, 6H), 3.65 (s, 3H).
5-(3,4-Dimethoxyphenyl)-3,4-ethylenedioxythenyl alcohol (**1b**): 129.7 mg (86% yield, 95% purity) ^{1}H NMR (400 MHz, DMSO): $\delta = 7.16$ (d, 1H, $J = 2.1$ Hz), 7.13 (dd, 1H, $J = 8.4$ Hz, $J = 2.1$ Hz), 6.95 (d, 1H, $J = 8.4$ Hz), 5.25 (t, 1H, $J = 5.6$ Hz), 4.45 (d, 2H, $J = 5.6$ Hz), 4.25 (m, 4H), 3.76 (s, 3H), 3.75 (s, 3H).
5-Phenyl-3,4-ethylenedioxythenyl alcohol (**1c**): 92.5 mg (84% yield, 85% purity) ^{1}H NMR (400 MHz, DMSO): $\delta = 7.61$ (dd, 2H, $J = 8.4$ Hz and 1.1 Hz), 7.36 (dd, 2H, $J = 7.4$ and 7.4 Hz), 7.20 (dd, 1H, $J = 7.8$ and 7.8 Hz), 5.30 (t, 1H, $J = 5.6$ Hz), 4.47 (d, 2H, $J = 5.6$ Hz), 4.27 (m, 4H).

3,4-Ethylenedioxythenyl alcohol (**1d**): 2 g, (99% yield, 99% purity). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.29$ (s, 1H), 4.66 (s, 2H), 4.21 (m, 4H).
Method B: 5-Iodo-3,4-ethylenedioxythenyl acetate (**6**) *3,4-Ethylenedioxythenyl acetate:* **1d** (700 mg, 4.07 mmol) was dissolved in Ac_2O (3 mL) and DMAP (49 mg, 0.41 mmol) was added. The reaction mixture was stirred for 30 min at room temperature, poured into saturated aqueous Na_2CO_3 (25 mL), and extracted with EtOAc (20 mL). The organic layer was washed with saturated aqueous Na_2CO_3 (6 × 15 mL) and 0.1% HCl in H_2O (3 × 20 mL), dried with $MgSO_4$, and then evaporated to dryness to yield 760 mg of an oil (87% yield).
^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.35$ (s, 1H), 5.11 (s, 2H), 4.22 (m, 4H), 2.08 (s, 3H).
5-Iodo-3,4-ethylenedioxythenyl acetate (**6**) 3,4-Ethylenedioxythenyl acetate (760 mg, 3.56 mmol) was dissolved in dry DMF(5.5 mL), and NIS (958 mg, 4.27 mmol) was added. After 3 h of stirring at room temperature, Et_2O (75 mL) was added and the resulting solution was washed with H_2O (3 × 100 mL). The organic layer was dried with $MgSO_4$, filtered and a small aliquot was taken for ^{1}H NMR. DMF (10 mL) was added to the remaining solution before removing Et_2O (the dry product is unstable) and this solution was immediately used for the next reaction.
^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.07$ (s, 2H), 4.25 (4H), 2.07 (s, 3H).
5-(4-Methoxyphenyl)-3,4-ethylenedioxythenyl alcohol (**1e**): DMF (90 mL) was added to the above-mentioned DMF solution of **6**. 4-methoxyphenylboronic acid (810 mg, 5.33 mmol), $Pd(PPh_3)_4$ (278 mg, 0.241 mmol), and 3.5 M aqueous Na_2CO_3 (8 mL) were successively added and the mixture was stirred at 135 °C for 5 h. The course of the reaction was followed by TLC (AcOEt–hexane, 1:1). The reaction mixture was evaporated to dryness. Et_2O (100 mL) was added and the solution was washed with H_2O (3 × 100 mL). Et_2O (40 mL) was added to the organic phase and it was washed with brine (3 × 100 mL). Then it was dried with $MgSO_4$ and evaporated to dryness. The crude obtained was purified by column chromatography (hexane, AcOEt) and 97 mg of an orange solid was obtained (10% yield).
^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.61$ (d, 2H, $J = 8.9$ Hz), 6.90 (d, 2H, $J = 8.9$ Hz), 4.37 (d, 2H, $J = 4.4$ Hz), 4.27 (m, 4H), 3.82 (s, 3H).

9. *5-(3,4,5-Trimethoxyphenyl)-3,4-ethylenedioxythenyl-N-(benzyloxycarbonyl)phenylalaninate* (**5a**): Compound **1a** (130 mg, 0.39 mmol), Z–Phe–OH (140 mg, 0.47 mmol), EDC (145 mg, 0.47 mmol), and DMAP (4.8 mg, 0.04 mmol) were dissolved in dry DCM (1.5 mL). The reaction mixture was stirred for 90 min and checked by TLC (DCM-MeOH, 95:5). After that, EtOAc (25 mL) was added, and the organic layer was washed with saturated aqueous Na_2CO_3 (3 × 25 mL) and H_2O (6 × 25 mL). The organic phase was dried with $MgSO_4$ and evaporated to dryness to yield 245.1 mg of an oil (98% yield, 80% purity).
^{1}H NMR (400 MHz, DMSO): $\delta = 7.83$ (d, 1H, $J = 8.2$ Hz), 7.25 (m, 10H), 6.89 (s, 2H), 5.09 (s, 2H), 4.96 (m, 3H), 4.30 (m, 4H), 3.78 (s, 6H), 3.66 (s, 3H), 3.0 (dd, 1H, $J = 13$ and 5.0 Hz) 2.86 (dd, 1H, $J = 13.9$ Hz and 10.2 Hz).
5-(3,4-Dimethoxyphenyl)-3,4-ethylenedioxythenyl-N-(benzyloxycarbonyl)phenylalaninate (**5b**): 150.6 mg, (78% yield, 70% purity) ^{1}H NMR (400 MHz, DMSO): $\delta = 7.82$ (d, 1H, $J = 8.1$ Hz), 7.21 (m, 12H), 6.89 (d, 1H, $J = 8.1$ Hz), 5.09 (s, 2H), 4.96 (m, 3H), 4.29 (m, 4H), 3.76 (s, 3H, CH_3), 3.75 (s, 3H), 3.01 (dd, 1H, $J = 13.8$ and 5.2 Hz) 2.85 (dd, 1H, $J = 13.8$ Hz and 10.1 Hz).
5-Phenyl-3,4-ethylenedioxythenyl-N-(benzyloxycarbonyl)phenylalaninate (**5c**): 163.9 mg, (91% yield, 75% purity). ^{1}H NMR (400 MHz, DMSO): $\delta = 7.83$ (d, 1H, $J = 8.2$ Hz), 7.60 (m, 2H), 7.28 (m, 13H), 5.01 (m, 5 H), 4.29 (m, 4 H), 3.01 (dd, 1H, $J = 13.8$ Hz and 5.2 Hz), 2.85 (dd, 1H, $J = 13.8$ and 10.1 Hz).
3,4-Ethylenedioxythenyl-N-(benzyloxycarbonyl)phenylalaninate (**5d**): 592 mg, (87% yield, 90% purity) ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.27 (m, 8H), 7.03 (m, 2H), 6.37 (s, 1H, CH), 5.12 (m, 3H), 4.68 (m, 1H), 4.19 (m, 4H), 3.11 (m, 2H).
5-(4-Methoxyphenyl)-3,4-ethylenedioxythenyl-N-(benzyloxycarbonyl)-phenylalaninate (**5e**): 67.4 mg, (82% yield, 80% purity). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.62$ (d, 2H, $J = 8.9$ Hz), 7.33 (m, 6H), 7.19 (m, 3H), 7.05 (m, 2H), 6.91 (d, 2H, $J = 8.9$ Hz), 5.25–5.06 (m, 5H), 4.70 (m, 1H), 4.28 (m, 4H), 3.83 (s, 3H), 3.12 (m, 2H).

10. For each removal test, 1 mg of amino acid was treated with a solution of the corresponding concentration of TFA with 2% of triethylsilane as a scavenger. In all the solutions, a minimum of 2 equiv of TFA was used. The reactions were stopped by adding 2 equiv of pyridine per equivalent of TFA, evaporated, dissolved in H_2O–AcCN, and analyzed by HPLC. The removal percentage was calculated by comparing the areas at $\lambda = 220$ nm of the peaks corresponding to the free and protected amino acid.

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