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THAL, a Sterically Unhindered Linker for the Solid-Phase Synthesis of Acid-Sensitive Protected Peptide Acids

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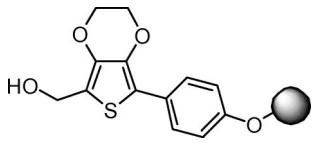

The 5-(4-hydroxyphenyl)-3,4-ethylenedioxythienyl alcohol (THAL, **Th**iophene **A**cid **L**abile) is described as a new linker for the solid-phase synthesis of peptide carboxylic acids. It is based on the electron-rich 3,4-ethylenedioxythenyl (EDOTn) moiety and allows the obtention of free and *tert*-butyl-protected peptides by cleavage with 90% and 0.5% TFA, respectively. This very high acid lability makes it useful for the synthesis of sensitive peptides. Free and *tert*-butyl-protected Leu-enkephalins have been synthesized as models to demonstrate the utility of the linker.

At present most solid-phase peptide syntheses are performed using the Fmoc/^{t}Bu orthogonal strategy.[1−5] The obtention of biologically interesting sensitive or complex peptides requires the use of mild reagents for deprotection and cleavage operations. In this aspect, super acid-labile resins are of vital importance because they allow the cleavage of very acid-sensitive peptidic moieties or *tert*-butyl-protected peptide fragments, which will be further modified after the cleavage. The 2-chlorotrityl chloride resin (2-CTC)[6] has been the resin of choice for most of these cases, and although it has given very good results in a large number of complicated synthesis,[7,8] it has the drawback of its steric hindrance due to the bulkiness of the 2-chlorotrityl moiety.

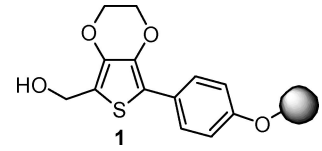

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FIGURE 1. THAL-resin.

In previous work we demonstrated the utility of EDOTn derivatives as super acid-labile protecting groups.[9−11] In the present work we propose 5-(4-hydroxyphenyl)-3,4-ethylenedioxythenyl alcohol (THAL) (Figure 1) as a sterically nonhindered super acid-labile linker for the solid-phase synthesis of peptide carboxylic acids, in particular as the C-terminal acid.

5-(4-Hydroxyphenyl)-3,4-ethylenedioxythiophene-2-carbaldehyde (**5**) was prepared by formylation of 3,4-ethylenedioxythiophene (**2**),[9] followed by iodination and a Suzuki coupling with *p*-hydroxyphenylboronic acid (Scheme 1), and it was coupled to a conventional hydroxymethyl Merrifield polystyrene resin. From the different coupling methods and resins tried, the best approach was the coupling via formation of a trichloroacetimidate on the hydroxymethyl polystyrene resin. After the coupling of the phenol, the resin was treated with DMF−H_2O to hydrolyze the remaining trichloroacetimidate. Then, it was acetylated with Ac_2O and DMAP, and the aldehyde was reduced

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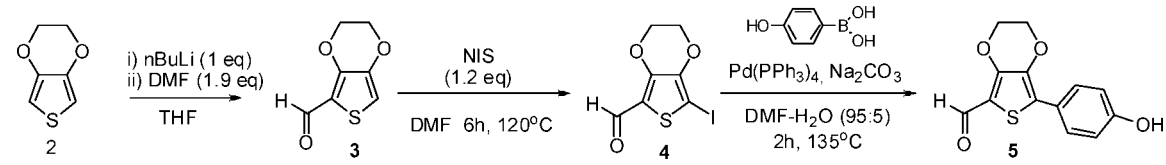
SCHEME 1. Synthesis of 5-(4-Hydroxyphenyl)-3,4-ethylenedioxythiophene Carbaldehyde



SCHEME 2. Obtention of THAL-Resin

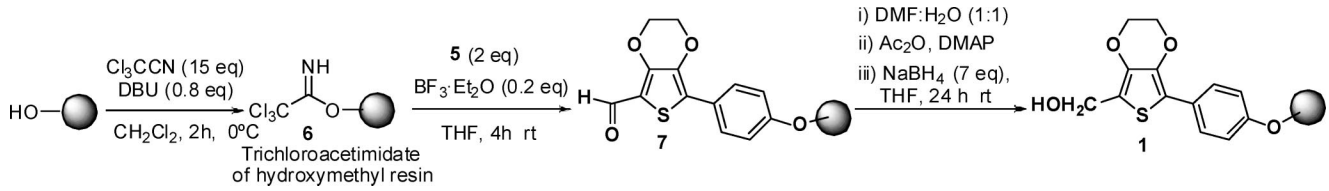


TABLE 1. Percentages of Cleavage of Fmoc-Gly-Phe-Leu-OH from THAL-Resin

	cleavage (%)
TFA-TES-CH_2Cl_2 (0.1:2:97.9), 10 min	80
TFA-TES-CH_2Cl_2 (0.5:2:97.5), 10 min	100

using $NaBH_4$ (Scheme 2). Fmoc-Leu was coupled via formation of the symmetric anhydride with DIC and DMAP. The loading of the resin was 0.29 mmol/g (calculated by Fmoc UV titration at $\lambda = 290$ nm).

Acidolytic Release Studies. In order to test the acid lability of the new linker, the tripeptide Fmoc-Gly-Phe-Leu-OH was prepared on the resin. Aliquots of the peptide bonded to the resin were treated with solutions containing 0.1% TFA, 0.5% TFA, and 90% TFA.

It has been observed that phenyl-EDOTn derivatives are completely removed using concentrations of 90% TFA.[9] The percentage of cleavage was calculated by HPLC analysis of the crude cleavage mixtures using *p*-nitrobenzyloxycarbonyl-Ala-OH (*p*NZ-Ala) as a standard. To determine the cleavage rates, the same amount of a 1 mg/mL standard solution of *p*NZ-Ala was added to the cleavage crude of the three aliquots. Then, they were analyzed by HPLC, and the ratio of the areas from the peaks corresponding to *p*NZ-Ala and Fmoc-Gly-Phe-Leu-OH of the 0.1% and 0.5% TFA samples were compared with the 90% of TFA sample to calculate the percentage of cleavage in each case (Table 1).

Synthesis of Protected and Unprotected Leu-Enkephalin: H-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-OH (8) and H-Tyr-Gly-Gly-Phe-Leu-OH (9) using THAL-Resin. The Leu-enkephalin was chosen as a model peptide to demonstrate the utility of the new derivatized resin. The coupling of Fmoc-Leu was performed as indicated above. All remaining couplings were performed using DIC and HOBt as coupling reagents. After the last Fmoc removal, the peptide was cleaved from the resin using two different conditions: 0.5% TFA for 10 min and 90% TFA for 1 h in order to obtain the *tert*-butyl-protected and free peptide, respectively. In the former case the crude showed an excellent purity by HPLC (Figure 2, left), whereas in the case of the latter to obtain an acceptable purity washings with CH_2Cl_2 should be performed (Figure 2, right). This lower purity at high concentrations of TFA was also observed for the case of the 2-CTC resin and suggests that the best method to obtain free peptides using the THAL-resin is performing the cleavage of the protected peptide with low concentrations of TFA and after filtering the resin global deprotection by increasing the TFA concentration to remove the *tert*-butyl-type protecting groups.

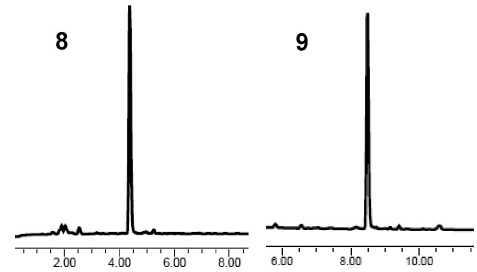


FIGURE 2. HPLC ($\lambda = 220$ nm) of **8** and **9**. Linear gradients of CH_3CN (+0.036% TFA) into H_2O (+0.045% TFA), from 30% to 100% and from 0% to 80% in 15 min, respectively

The present THAL-resin allows release of peptide acids at low concentrations of TFA (0.5% TFA within 10 min). Thus *tert*-butyl-protected peptides and possibly very acid-sensitive peptidic structures can easily be obtained. In addition, cleavage with high concentrations of TFA provides unprotected peptides with acceptable purities. As the new resin is based on a primary alcohol, it is likely to cause little steric hindrance (Figure 3).

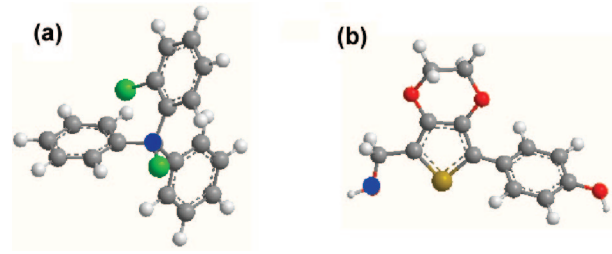


FIGURE 3. Comparison of the steric hindrance of (a) 2-chlorotrityl chloride resin, (b) THAL-resin. The attachment of the peptide to the resin is depicted in blue.

Experimental Section

(5-Iodo-3,4-ethylenedioxythiophene-2-carbaldehyde (4). Compound **3** (1 g, 5.88 mmol) and *N*-iodosuccinimide (NIS) (1.59 g, 7.05 mmol) were dissolved in dry DMF (10 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, Et_2O (100 mL) was added, and the resulting solution was washed with H_2O (3 × 100 mL). The organic portion was dried with $MgSO_4$ and filtered, and the solution was stored at −20 °C and used within 24 h (dry product from this procedure was very unstable even at low temperature).[12]

5-(4-Hydroxyphenyl)-3,4-ethylenedioxythiophene-2-carbaldehyde (5). DMF (50 mL) was added to a solution of **4** in diethyl ether. The Et_2O was evaporated, and more DMF was added to a

total volume of 150 mL. 4-Hydroxyphenylboronic acid (1.10 g, 7.94 mmol), $Pd(PPh_3)_4$ (416 mg, 0.360 mmol), and 10 mL of 2 M aqueous Na_2CO_3 were added, and the mixture was stirred at 135 °C for 2 h. The course of the reaction was followed by TLC (CH_2Cl_2). The reaction mixture was evaporated to dryness, an aqueous solution of saturated NH_4Cl (100 mL) was added, and the mixture was extracted with AcOEt (3 × 125 mL). The organic phase was dried with $MgSO_4$, filtered, and evaporated to dryness. MeOH (10 mL) was added to the crude obtained. The mixture was filtered, and the light brown solid was dried under vacuum to yield 666 mg (43% total yield from **3**) of the desired product: mp = 265.1−269.3 °C. ^{1}H NMR (400 MHz, DMSO): δ 9.92 (s, 1H, CHO), 9.81 (s, 1H, OH), 7.61 (d, 2H, 2CH arom, J = 8.8 Hz), 6.83 (d, 2H, 2CH arom, J = 8.8 Hz), 4.42 (m, 4H, CH_2CH_2). ^{13}C NMR (100 MHz, DMSO): δ 179.49 (CHO), 158.97 (C), 150.52 (C), 137.52 (C), 128.86 (CH), 122.87 (C), 116.58 (CH), 113.88 (C), 65.98 (CH_2), 65.21 (CH_2). HRMS (CI): m/z calcd for $C_{13}H_9O_4S$ $[M - H]^-$ 261.0227, found 261.0225.

Resin Preparation. Trichloroacetimidate of the Hydroxymethyl Merrifield Resin (6). Hydroxymethylpolystyrene resin (300 mg, 0.98 mmol/g) was placed in a 5-mL polypropylene syringe fitted with a polyethylene filter disk. The resin was then washed with dry CH_2Cl_2 (5 × 0.5 min), swollen with CH_2Cl_2 (3 mL) for 20 min, and transferred to a tube equipped with a septum and under nitrogen atmosphere. CCl_3CN (427.4 μL, 14.5 equiv) was added, and the tube was cooled in an ice bath. After 10 min of stirring DBU (35.6 μL, 0.81 equiv) was added, and the resin was stirred for 2 h at 0 °C. Then, the resin was transferred to the 5-mL syringe and washed with CH_2Cl_2 (5 × 1 min) and Et_2O (5 × 1min). An aliquot of the resin was dried, and IR spectrometry was performed showing the formation of the trichloroacetimidate. IR (KBr): ν = 3341, 1663 cm^{-1}.

5-(4-Hydroxyphenyl)-3,4-ethylenedioxythiophene-2-carbaldehyde Resin (7). The above obtained resin **6** was swelled in dry CH_2Cl_2 for 20 min under nitrogen atmosphere, washed with dry THF (3 × 1 min), and transferred to a tube equipped with a septum and under nitrogen atmosphere. A suspension of **5** (154 mg, 2 equiv) in dry THF was added, and the resin was stirred for 5 min. After that $BF_3 \cdot Et_2O$ (7.5 μL, 0.2 equiv) was added. The resin was gently stirred for 4 h at room temperature in a shaker. The green resin obtained was washed with THF, DMF, and CH_2Cl_2; treated with DMF−H_2O (2 × 15 min) in order to hydrolyze the remaining trichloroacetimidate; and then washed with DMF (5 × 1 min), THF anhydrous (5 × 1 min), and Et_2O (5 × 1 min). An aliquot of the resin was dried, and IR spectrometry was performed showing the disappearance of the trichloroacetimidate and the formation of the aldehyde. IR (KBr): ν = 1649 cm^{-1}.

The resin was swollen in CH_2Cl_2 (15 min), washed with DMF (5 × 30 s), and acetylated by treatment with Ac_2O (554.8 μL, 20 equiv) and DMAP (2 equiv) in DMF (2 mL) for 45 min.

5-(4-Hydroxyphenyl)-2-hydroxymethyl-3,4-ethylenedioxythiophene Resin, THAL-Resin (1). Resin **7** was swollen with THF for 15 min in a tube placed in an ice bath. $NaBH_4$ (77.9 mg, 7 equiv) was added, and the suspension was stirred 15 min in the ice bath and 24 h at room temperature. The yellow resin obtained was cooled in an ice bath, and saturated aqueous NH_4Cl was slowly added; hydrogen evolution was observed. After 10 min, the NH_4Cl was removed, new NH_4Cl was added, and the resin was left 5 min at 0 °C and 15 min more at room temperature. The resin was placed to a 5-mL polypropylene syringe fitted with a polyethylene filter disk and washed with H_2O, MeOH, THF−H_2O, THF, MeOH, and Et_2O. IR spectrometry was performed showing the absence of the aldehyde absorption band.

General Solid-Phase Peptide Synthesis. Fmoc-Leu-OH (10 equiv) was coupled to **1** by forming the symmetric anhydride with DIC (5 equiv) and DMAP (0.1 equiv) in CH_2Cl_2 (3 h).The loading of the resin was determined by Fmoc UV titration (Fmoc group was removed using piperidine−DMF (2:8) (1 × 1 min, 2 × 10 min). The remaining amino acids were coupled as Fmoc derivatives (4 equiv), using DIC (4 equiv) and HOBt (4 equiv) as coupling agents in DMF (1 h).

H-Tyr(^{t}Bu)-Gly-Gly-Phe-Leu-OH (**8**) and H-Tyr-Gly-Gly-Phe-Leu-OH (**9**) were obtained by treating the resin with TFA-TES-CH_2Cl_2 (0.5:2:97.5) for 10 min and TFA-TES-CH_2Cl_2 (90:2:8) for 1 h, respectively. The cleavage solution for **8** was collected in H_2O, the TFA was evaporated, and after addition of CH_3CN, the product was characterized by HPLC (λ = 220 nm), 95% purity. HRMS: m/z calcd for $C_{32}H_{46}N_5O_7$ $[M + Hl]^+$ 612.3397, found 612.3384. In the case of the cleavage solution for **9**, it was filtered, evaporated to dryness, and dissolved in 0.1 N aqueous HCl (1 mL). This aqueous phase was washed with CH_2Cl_2 (6 × 1 mL) to remove apolar impurities and characterized by HPLC (λ = 220 nm) and LC-MS. The final product was obtained with 90% purity. HRMS: m/z calcd for $C_{28}H_{38}N_5O_7$ $[M + Hl]^+$ 556.2771, found 556.2762.

Cleavage Assays (Table 1). Aliquots of resin (10 mg) were taken for cleavage assays. The first aliquot was treated with TFA−TES−CH_2Cl_2 (90:2:8) (0.5 mL) for 1 h, the second aliquot was treated with TFA−TES−CH_2Cl_2 (0.5:2:97.5) (1 mL) for 10 min, and the third with TFA−TES−CH_2Cl_2 (0.1:2:97.9) (0.5 mL) for 10 min. The resulting cleavage solutions were separated from the resin by filtration, evaporated to dryness, and dissolved in H_2O−CH_3CN. Then, 0.8 mL of a 1 mg/mL standard solution of *p*NZ-L-Ala-OH in CH_3CN was added, and HPLC of the resulting solution was performed.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of the prepared compounds and HPLC cleavage assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Alternatively, compound **2** can be prepared by a more laborious protocol[10] that provides a more stable product.