New Methoxy-Substituted Tritylamine Linkers for the Solid Phase Synthesis of Protected Peptide Amides

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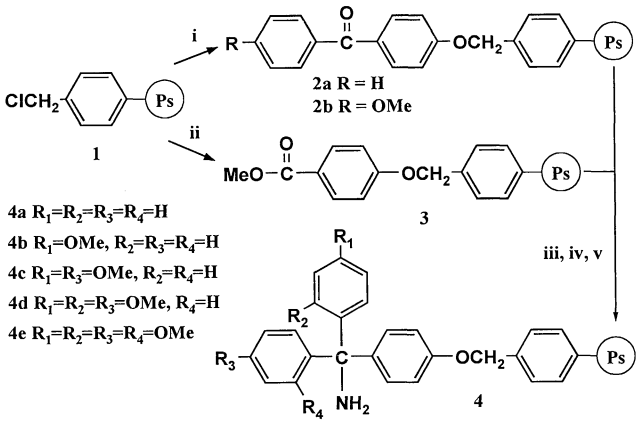
Syntheses of new methoxy-substituted tritylamine linkers in only four steps for the solid phase synthesis of peptide amides *via* the Fmoc/*t*Bu strategy are described and their properties compared. Peptide amides can be cleaved under mild acidic conditions in high purity in their protected or deprotected form.

The use of acid labile trityl linkers for the solid phase sythsis of protected peptides is well established.[1,2,3] However, for the synthesis of biologically active peptide amides *via* the Fmoc/*t*Bu strategy only a few useful solid supports are available,[4,5] but the high concentrations of TFA usually required for the final cleavage from these resins, make them unsuitable for the preparation of peptide amides in the protected form,[6] needed e.g. for the synthesis of large peptide sequences *via* convergent SPPS.

Therefore, we enhanced the acid lability of the trityl system by increasing the number of electron-donating methoxy functions and prepared the following Trt-derived linkers: the 4-benzyloxytritylamine (**4a**), 4-benzyloxy-4'-methoxytritylamine (**4b**), 4-benzyloxy-4',4''-dimethoxytritylamine (**4c**), 4-benzyl-oxy-2',4',4''-trimethoxytritylamine (**4d**) and 4-benzyloxy-2',2'', 4',4''-tetramethoxytritylamine (**4e**) on styrene-divinylbenzene co-polymers.



Scheme 1. Preparation of 4-benzyloxytritylamine (**4a**), 4-benzyloxy-4'-methoxytritylamine (**4b**), 4-benzyloxy-4',4''-dimethoxytritylamine (**4c**), 4-benzyloxy-2',4',4''-trimethoxytritylamine (**4d**) and 4-benzyloxy-2',2'',4',4''-tetramethoxytritylamine (**4e**) resin, Ps = polystyrene. Reagents and conditions: i, 3 equiv. MeONa, 3 equiv. 4-hydroxy-benzophenone (for **2a**) or 4-hydroxy-4'-methoxy-benzophenone (for **2b**), in DMA, 65 °C 15 h; ii, 3 equiv. MeONa and 3 equiv. methyl 4-hydroxybenzoate, in DMA, 65 °C 15 h; iii, 10 equiv. Mg and 10 equiv. bromobenzene (for **4a**), 4-bromoanisole (for **4b**, **4c**) or 4-bromoresorcin dimethyl ether (for **4d**, **4e**), respectively, in THF, 12 h, reflux; iv, 20 equiv. AcCl, in PhMe, 70 °C, 1 h; v, 50% NH_3 in CH_2Cl_2, -45 °C to room temp., 1 h.

The target supports were directly synthesized on polystyrene, starting with the Merrifield resin in only four steps. The chloromethylated polystyrene (1.7 mmol Cl × g^{-1}) was treated with 3 equiv. of 4-hydroxymethyl benzoate, 4-hydroxy-benzophenone or 4-hydroxy-4'-methoxy-benzophenone (synthesized according to a procedure of K. Nakazawa et. al.),[7] respectively and 3 equiv. MeONa in dimethylacetamide (DMA) at 65 °C for 15 h. The Beilstein tests and microanalyses showed that the resulting resins were free of chloride. The trityl alcohol resins were prepared *via* a Grignard reaction refluxing 10 equiv. of the corresponding bromo compounds and 10 equiv. magnesium in THF for 12 h.[8] The structures were confirmed by the disappearance of the CO band in the IR spectra at 1710 cm^{-1} (**4a, 4c, 4e**) and 1645 cm^{-1} (**4b, 4d**) and the appearance of a strong OH band at 3400 cm^{-1}, indicating that the reaction had gone to completion. The hydroxyl groups were transformed into the strongly coloured halide forms within 1 h with AcCl in toluene at 70 °C and the yellow amino resins were received by treatment with a cold (-45 °C) solution of ammonia in CH_2Cl_2 (50%), the temperature of which was increased to room temperature (1 h).

As a model sequence, LH/FSH-RH (pGlu-His(Trt)-Trp(Boc)-Ser(*t*Bu)-Tyr(*t*Bu)-Gly-Leu-Arg(Pmc)-Pro-Gly-NH_2) was chosen. Fmoc-Gly-OH was coupled with DCCI (3 equiv., 2 h) in CH_2Cl_2 to the resins and the remaining amino groups were capped with Ac_2O/DIEA (1:1) in CH_2Cl_2 within 20 min. Then, the solid phase syntheses of LH/FSH-RH were accomplished by standard Fmoc/*t*Bu batchwise procedures using an ECOSYN P peptide synthesizer (Eppendorf-Biotronik). The Fmoc amino acids (3 equiv.) were activated by 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) and *N*,*N*-diisopropylethylamine (DIEA) in DMF (coupling time: 0.5 h), except for pGlu-OH, which was coupled as a preformed pentachlorophenol active ester in the presence of catalytic amounts of HOBT in DMF/CH_2Cl_2 (1:1) (coupling time: 1 h).

After assembly of the peptide chains, the products were cleaved and simultaneously deprotected with TFA/ethanedithiol/thioanisole/H_2O (9.5:1.5:1.5:0.5) from the solid supports **4a**, **4b** and **4c**. To isolate the peptide amides from the resins **4d** and **4e**, the cleavage was performed with 10% TFA/CH_2Cl_2 in 10 min, as the use of too high concentrations of TFA tends to remove the linker from its support, resulting in slightly coloured products. Then, CH_2Cl_2 was removed in vacuum and the product treated with TFA/ethanedithiol/thioanisole/H_2O (9.5:1.5:1.5:0.5) to remove the side chain protecting groups, followed by ether precipitation and lyophilization. The products were obtained in excellent yields (86% for **4a**, 90% for **4b**, 94% for **4c**, 95% for **4d** and 96% for **4e**) and high purity (72% for **4a**, 93,5% for **4b**, 92,5% for **4c**, 90% for **4d** and 97% for **4e**) according to RP-HPLC. All synthetic peptides showed the expected results on MALDI-MS and IS-MS, respectively. These data demonstrate the stability of the peptidyl linker bond during synthesis under

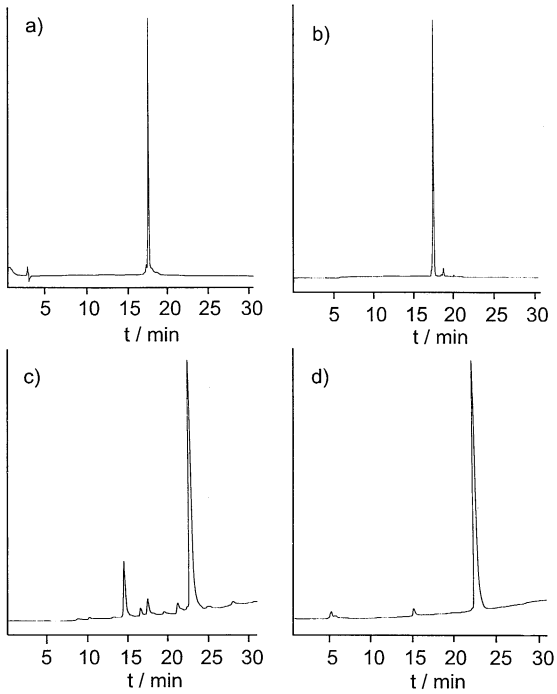


Figure 1. RP-HPLC profiles of synthetic LH/FSH-RH. a) crude product, cleaved from **4b**; b) crude product, cleaved from **4e**; c) crude protected LH/FSH-RH, cleaved from **4c**, peak at Rt = 14.9 min detritylated product (12%); d) crude protected LH/FSH-RH, cleaved from **4e**; conditions: column: Nucleosil® 5 C_{18} 4.6 × 250 mm; eluents: a) and b): A: H_2O/TFA (1000:0,25), B: MeCN/ H_2O/TFA (600/ 400/0,2); gradient: 0-1 min 95% A, 1-31 min 95-5% A; detection: λ = 214; c) and d): A: H_2O/TFA (1000:0,25), B: MeCN/TFA (1000/0,2); gradient: 0-1 min 50% A, 1-31 min 50-0% A, 31-36 min 0% A; flow rate: 1 ml/min; detection: λ = 254 nm; concentration: 1 mg/ml.

standard basic Fmoc/*t*Bu synthetic conditions.

For cleavage of the protected LH/FSH-RH, 100 mg of each peptidyl resin was brought into a sintered bottomed reaction vessel and allowed to swell in CH_2Cl_2 for 10 min and treated with 15 ml 1% TFA/CH_2Cl_2 (v:v). Then, the solutions were filtered off into a vessel, containing 3% pyridine in CH_2Cl_2 to neutralize the TFA. This process was repeated periodically several times. To follow the cleavage kinetics (Figure 2), the resulting mixtures were extracted separately three times with H_2O, evaporated for removal of CH_2Cl_2, and then *t*BuOH/H_2O (4:1) was added, before the products were lyophilized.

The protected peptide amides were cleaved within 30 min from resin **4c** in 88 % yield, within 15 min from resin **4d** in 83% yield and within 10 min from resin **4e** in 82% yield (crude products, based on the starting loading). The products gave the expected results on FAB-MS. Under these conditions no complete cleavage was achieved from the peptide resins **4a** and **4b**. Therefore, **4a** and **4b** can not be recommended for the SPPS of protected peptide amides.

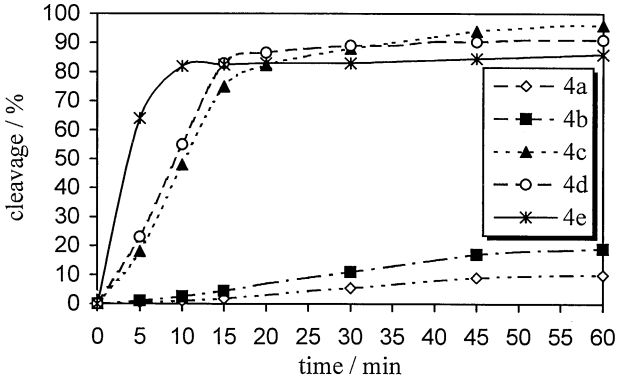


Figure 2. Cleavage of protected LH/FSH-RH with 1% TFA/ CH_2Cl_2.

Our results show that the introduction of additional methoxy ring substituents to the trityl-linker system increases the acid lability and reduces the cleavage time of the peptide amides drastically. This fact is of importance, as prolongation of TFA exposure over more than 15 min leads to substantial loss of acid labile side-chain protecting groups, e.g. after a period of 30 min 12% of the trityl protecting group from His is lost under these cleavage conditions from **4c** (Figure 1).

The high yields and the excellent purities of the synthetic peptides, produced with these new methoxy-functionalized tritylamine linkers show, that the peptidyl linker bond is stable under the basic Fmoc/*t*Bu synthetic conditions. Therefore, our easily accessible new trityl linkers can be recommended for the synthesis of peptide amides, almost free of side products. Especially, the 4-benzyloxy-2',2",4',4"-tetramethoxytritylamine resin, cleavable under very mild acidolytic conditions, is suitable for the synthesis of peptide amides in the protected form.

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