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PhFl Acetic Acid: A New Linker for Solid Phase Organic Synthesis

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***Abstract**: A 9-phenylfluoren-9-yl based linker for the immobilization of nitrogen and oxygen nucleophiles is described. Improved acid stability compared to the common trityl linker is demonstrated by a quantitative method for analysis of loading. This new linker is used for the synthesis of a peptide alcohol in the 'inverse' direction via reduction of the corresponding N linked peptide methyl ester. Several other nucleophiles are immobilized and further modified. TFA treatment releases the corresponding products in high purity.*

The synthesis of small organic molecules on solid supports has become widespread in the field of drug discovery.[1] The variety of impressive examples published during the last few years illustrates the importance of this technology in pharmaceutical research.[2] A major issue in selecting a synthetic approach to a target structure is the choice of linker for the immobilization of the first building block. Easy loading, stability during synthesis and relatively mild and specific cleavage conditions are some of the requirements. Most currently available linker systems originate from solid phase peptide synthesis, lead to either acid or amide functional residues in the products, and are only applicable for restricted chemistry. It would be desirable to immobilize building blocks with different functionalities onto a single linker molecule. Trityl resins have been described for the immobilization of various nucleophiles but acid sensitivity makes them only useful for a limited range of reactions.

We were interested in a linker system that shows the broad applicability of the trityl group but with improved acid stability and can also be coupled to any amino modified resin or crown/pin based solid support. We first evaluated the Bayer and Goldammer α,α-diphenyl hydroxymethyl benzoic acid handle used for the synthesis of peptides in both C and N terminal directions,[3] but its acid lability, comparable to the standard 2-chlorotrityl resin, turned out to be unsuitable for many of our synthesis approaches.

In the preceding publication in this issue we introduce a new resin based on the 9-phenylfluoren-9-yl protecting group.[4] Herein we describe a phenylfluorenyl linker system that can be easily coupled onto various aminomodified supports. Its synthesis and utility as a linker for nitrogen and oxygen nucleophiles is discussed.

The PhFl linker **1** was synthesized as described in Scheme 1. Starting from 9-fluorenone and p-tolylmagnesium bromide the methyl group of the resulting Grignard product was brominated via AIBN/NBS. Nucleophilic substitution with KCN and subsequent hydrolysis of the nitrile produces the corresponding linker compound 9-(4-carboxy methyl) fluoren-9-ol **1**.[5] Coupling of the carboxylic acid derivative onto several solid supports (aminomethyl polystyrene, TentaGel®-S-NH_2 and Gly-HMD-MA/DMA-crowns) was carried out using standard coupling conditions. For the activation of the immobilized PhFl-linker the solid supports were treated with acetyl chloride.[6] The resulting chloride was then substituted by adding the corresponding nucleophile.

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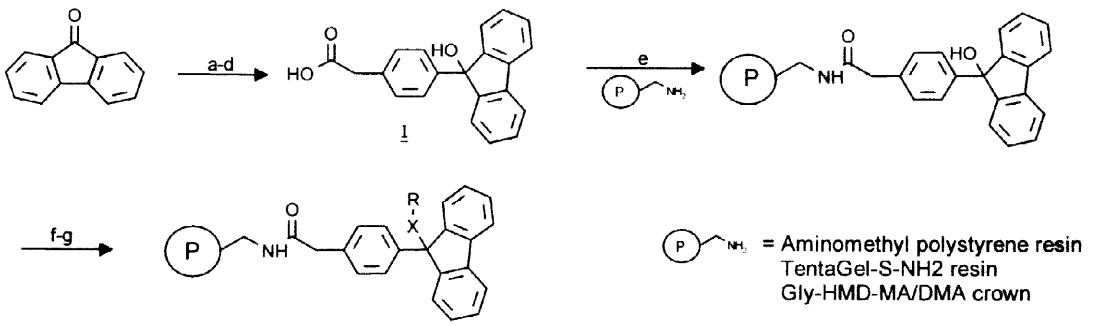

Scheme 1: *Linker synthesis, coupling and immobilization of nucleophiles. a) MePhMgBr b) 2,2'-Azobisisobutyronitrile (AIBN), N-bromo succinimide (NBS) c) KCN d) NaOH e) 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), N-Methylmorpholine (NMM) f) 20% AcCl/CH_2Cl_2 g) Nucleophile (RXII)*

In order to quantitate the acid stability of this linker system a method was devised using amino acid analysis.[7] The PhFl linker attached to glycine modified HMD-MA/DMA-crowns[8] was reacted with the primary amine nitrogen of phenyl alanine allyl ester. This positions glycine on the resin side of the linker and attachment of a second amino acid to the cleavable side of the linker allows the ratio of the two amino acids, obtained by amino acid analysis, to quantitate the material on the cleavable side of the linker. The pins were treated under five different trifluoroacetic acid (TFA) concentrations (1-95% TFA see Figure 1) for 30 min. After quenching and washing the pins were subjected to amino acid analysis and the ratio of Gly to Phe determined. For comparison, the standard trityl linker (α,α-diphenyl hydroxymethyl benzoic acid) was similarly attached to glycine modified HMD-MA/DMA crowns and reacted with the primary amine nitrogen of phenyl alanine allyl ester. The same investigations were carried out as for the PhFl linker. Figure 1 shows a comparison of the results for both linker systems. The amino acid ester is cleaved virtually quantitatively from the trityl linker with 5% TFA after 30 min. The PhFl linker shows no cleavage after 30 minutes with 5% TFA and even treatment with 95% TFA cleaved only about 20% of the compound. Treatment with 95% TFA overnight gave quantitative cleavage (determined by the same method-data not shown).

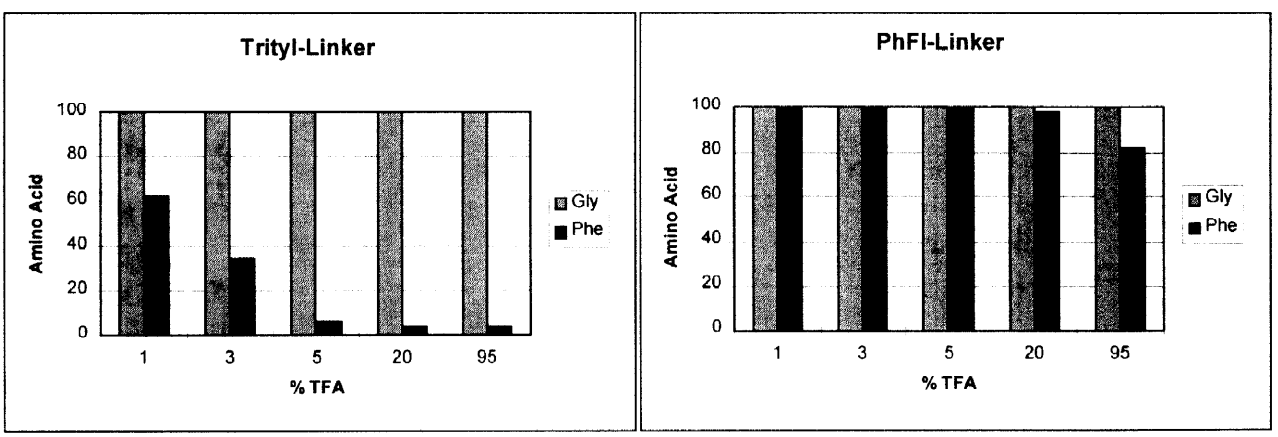

Figure 1: *Comparison of linker acid stability using amino acid analysis. Glycine is used as internal standard and normalized to 100. The samples were treated under several acidic conditions for 30 min. The remaining Phe is measured and compared to Gly.*

Five different amino acid esters (PheOAll, MetOAll, LeuOAll, AlaOAll and ProOMe) were coupled via their N-terminus onto PhFl-modified crowns[9] and the loading determined by amino acid analysis. The lowest loading, 75% of theory, was obtained for the secondary amine proline. All the other amino acid esters coupled quantitatively. To demonstrate the utility of the PhFl linker, a dipeptide and the corresponding peptide alcohol were synthesized in the 'inverse' direction on both polystyrene and TentaGel® resins. PheOAll was immobilized onto both PhFl modified resins.[9] Palladium catalyzed allyl ester cleavage and 'inverse' coupling of PheOMe gave the dipeptide methyl ester in 66% isolated yield after 95% TFA cleavage from polystyrene resin. Reduction of the methyl ester to the alcohol on solid support gave the Phe-Phe-ol in 68% isolated yield, this time after cleavage from PhFl-modified TentaGel® resin.[10] Figure 2 shows the HPLC chromatogram of the Phe-Phe-ol crude product. Structure confirmation was obtained by ^{1}H-NMR. The small second peak is identified by LC/MS as a trifluoroacetate derivative of the product formed during the TFA cleavage.

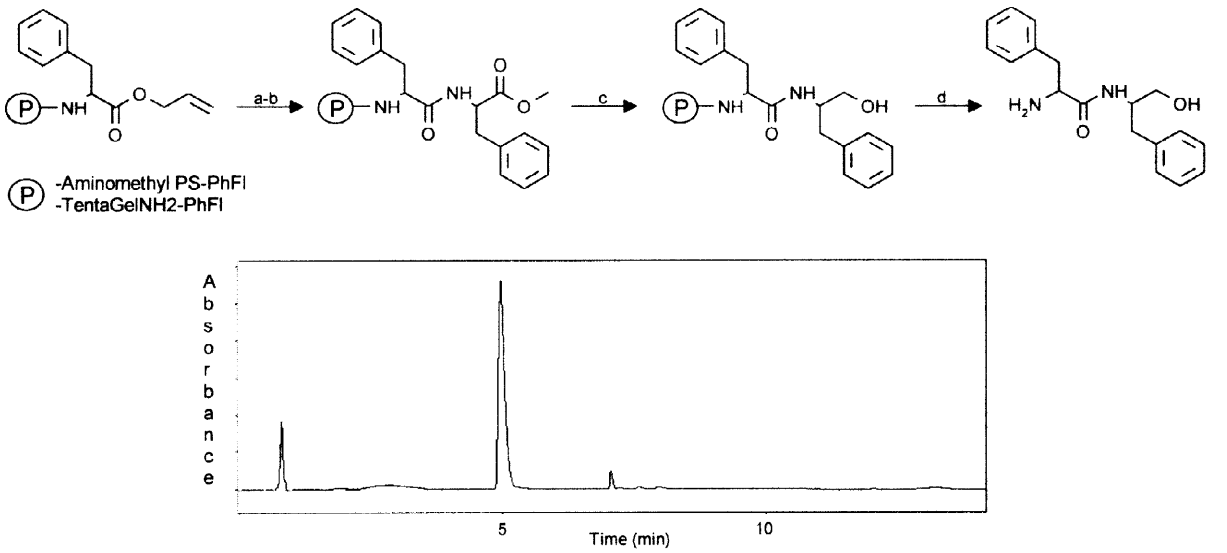

Figure 2: *Synthesis and RP-HPLC of Phe-Phe-ol crude product. a) PdP(Ph3)4, Morpholine b)TBTU, PheOMe c) NaBH4 d) TFA*

As an example for the immobilization of anilines 4-aminoacetophenone was loaded onto the PhFl-modified TentaGel® resin.[11] Claisen condensation and cyclization with hydrazine as described by Marzinzik and Felder[12] gave the corresponding pyrazole derivative after cleavage with 20% TFA in CH_2Cl_2/MeOH (9:1) for 2h. An HPLC crude product purity of > 95 % and a final yield after Kieselgel chromatography of 74% was obtained.

As an oxygen nucleophile example 4-bromobenzoic acid was reacted with PhFl-modified aminomethyl polystyrene. The immobilized compound was modified via Suzuki coupling to lead to the corresponding biphenyl derivative.[13] The resulting 4-phenyl benzoic acid was cleaved with 20% TFA in CH_2Cl_2/MeOH (9:1) for 2h. The HPLC analysis showed > 95% purity of the crude product with a yield of 72%. After additional Kieselgel chromatography a 67% yield of the pure compound was obtained. Identification of the product was confirmed by MS and HPLC co-injection of commercially available 4-phenyl benzoic acid.

In summary, a new linker for solid phase organic synthesis is introduced and improved acid stability relative to trityl is quantitated by amino acid analysis. The linker is attached to aminomethyl polystyrene, TentaGel®-S-NH2 and Gly-HMD-MA/DMA-crowns. (Amino acid) amines, anilines and carboxylic acid building blocks are immobilized and some simple reactions demonstrated. Further investigations on immobilizing other nucleophiles are in progress and will be reported in due course.

Acknowledgment

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References and Notes

(All wash steps use an appropriate volume of the indicated solvent to create a resin slurry)

1. Hermkens, P.H.H.; Ottenheijm, H.C.J; Rees D. *Tetrahedron*, **1997**, *53*, 5643, Hermkens, P.H.H.; Ottenheijm, H.C.J; Rees D. *Tetrahedron*, **1996**, 52, 4527
2. Balkenhohl, F.; von dem Bussche-Hünnefeld, C.; Lansky, A.; Zechel, C. *Angew. Chem. Int. Ed. Engl.*, **1996**, *35*, 2288
3. Bayer, E.; Goldammer, C. *German Pat.* DE 43 06 839 A1 **1994**, Bayer, E.; Henkel, B.; Zhang, L.; Clausen, N.; Goldammer C.; Panhaus, G. *Peptides 1994*, Proceedings of the 23rd European Peptide Symposium (Ed.: H.L.S. Maia) Escom, Leiden, **1995**, p.201
4. Gerspacher, M.; Rapoport, H. *J. Org. Chem.* **1991**, *56*, 3700
5. 100 mmol of 9-fluorenone was treated with p-tolylmagnesium bromide in THF. After standard work up of the product a crystalline compound was obtained after Kieselgel chromatography in 88% yield. Treatment with 1 eq N-bromo succinimide and catalytic 1.3 mmol AIBN in chloroform under reflux for 2h resulted in a yellow oil. A colorless resin was obtained after Kieselgel chromatography in 69% yield. The resin was dissolved in EtOH and 2.5 eq KCN (ethanol/water 1:1) were added. The solution was refluxed for 2.5h. A colorless resin was obtained after chromatography in 73% yield. The nitrile was hydrolyzed with 20% NaOH in EtOH/H_2O under reflux for 16h. A crystalline solid was obtained in 86% yield. The final product and all intermediates were analyzed by TLC, MS and NMR.
6. Activation and coupling of the linker onto aminomodified TentaGel and PS-resin was performed with TBTU and NMM in DMF for 4h in a twofold excess. The crowns were treated with an 0.1M solution of the PhFl-linker, TBTU and NMM for 2h. After rinsing with DMF, MeOH and CH_2Cl_2 the solid supports were treated with 20% acetyl chloride in CH_2Cl_2 for 6h at RT. After washing with dry CH_2Cl_2 the supports were dried under vacuum (overnight).
7. Albericio, F.; Barany, G. *Int. J. Peptide Protein Res.*, **1987**, *30*, 206
8. Maeji, N.J.; Valerio, R.M.; Bray A.M.; Campbell, R.A.; Geysen, H.M. *Reactive Polymers*, **1994**, *22*, 203
9. *Loading of amino acid esters:* 5 eq of the corresponding amino acid allyl ester toluenesulonate salt was dissolved in DMF by adding 5 eq of NMM. This solution was added to the activated resin and stirred for 16h at 80°C. The crowns were treated with 0.2M solutions of amino acid esters and 0.4M NMM in CH_2Cl_2 for 16h at RT.
10. *Allyl ester cleavage:* 0.2 eq $Pd(PPh_3)_4$, 10 eq Morpholine in CH_2Cl_2 for 2h. The resin was rinsed with CH_2Cl_2, a solution of sodium diethyldithiocarbamate/DIPEA (1:1) in DMF (20 mmol), DMF, MeOH and CH_2Cl_2 (3 x each). *Activation and coupling:* 5 eq TBTU, 5 eq NMM in DMF for 30 min. The resin was drained and the amino acid ester added. Quantitative coupling was achieved within 4h. The resin was washed with DMF, MeOH and CH_2Cl_2 (3 x each). *Reduction of the methyl ester:* 3.0M $NaBH_4$ in EtOH/Water (1:1) for 3h. After rinsing with MeOH, H_2O, DMF, MeOH, CH_2Cl_2 (x3) the compound was cleaved with 95% TFA for 16h.
11. *Loading of 4-aminoacetophenone:* 5 eq of 4-aminoacetophenone were dissolved in DMF and 5 eq of NMM were added. This solution was added to the activated resin and stirred for 20h at 80°C. The reaction was quenched with methanol and the resin washed with DMF, MeOH and CH_2Cl_2 (3 x each).
12. Marzinzik, A.L.; Felder, E.R. *Tetrahedron Lett.*, **1996**, *37*, 1003
13. *Loading of 4-bromobenzoic acid:* A 10 fold excess of 4-bromobenzoic acid in DMF and a 10-fold excess of NMM were added to the activated resin for 4d at 80°C. The reaction was quenched with methanol and the resin was rinsed with DMF, MeOH and CH_2Cl_2. *Suzuki cross-coupling:* 2 eq of phenyl boronic acid, 2.5 eq of Na_2CO_3 (2M aq solution) and 0.05 eq tetrakis (triphenylphosphine) palladium were added in DME for 16h at 80°C. After rinsing with DME/water, sodium diethyldithiocarbamate/DIPEA (1:1) in DMF (20 mmol), DMF, MeOH and CH_2Cl_2 (3 x each) the compound was cleaved with 20% TFA in CH_2Cl_2/MeOH (9:1) for 2h. For Suzuki coupling on solid support see also: Frenette, R.; Friesen, R.W. *Tetrahedron Lett.*, **1994**, *35*, 9177