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# New phenylfluorenyl based linkers for solid phase synthesis

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**Abstract**

The synthesis of 9-(4-hydroxyphenyl)-9-H-fluoren-9-ol and 5-(4-(9-hydroxy-9H-fluoren-9-yl)-phenoxy)-pentanoic acid and their applications as new linkers for solid phase synthesis is reported. These supports show higher acid stability compared to standard trityl resins. Carboxylic acids and amines are immobilized and cleaved off after further modifications. TFA treatment releases the products in high yield and excellent purity. © 2000 Elsevier Science Ltd. All rights reserved.

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Solid phase organic synthesis (SPOS) has become a useful tool for the generation of diverse compound libraries in the drug discovery and lead optimization process.[1] In recent publications an increasing number of solution phase methodologies were transferred successfully to solid phase synthesis.[2] One of the key issues in SPOS is to find a proper linker system for the attachment of the first building block to the solid support. Trityl resins were described for the immobilization of various nucleophiles like carboxylic acids,[3] alcohols[4] and amines.[5] The convenient loading of amino acids and the mild cleaving conditions make these resins very attractive for the syntheses of peptides and protected peptide fragments.[3,6] For solid phase synthesis of small molecules the acid sensitivity of trityl resins often turns out to be a drawback, therefore they can only be used for a limited range of reactions. The 9-phenylfluoren-9-yl group (PhFl) has been described for the protection of primary and secondary amines[7] as a more acid stable alternative to the trityl group. This protecting group shows a 6000 times higher stability than trityl due to the antiaromatic character of the fluorenyl cation produced upon acidic treatment.[8] We have investigated the phenylfluorenyl protecting strategy as a linkage approach in SPOS for several nucleophiles as disclosed in earlier publications.[9] Henkel et al. describe an analogous approach for the immobilization of amino acids[10] and recently a publication appeared using the PhFl linker concept for soluble polymer supported chemistry.[11]

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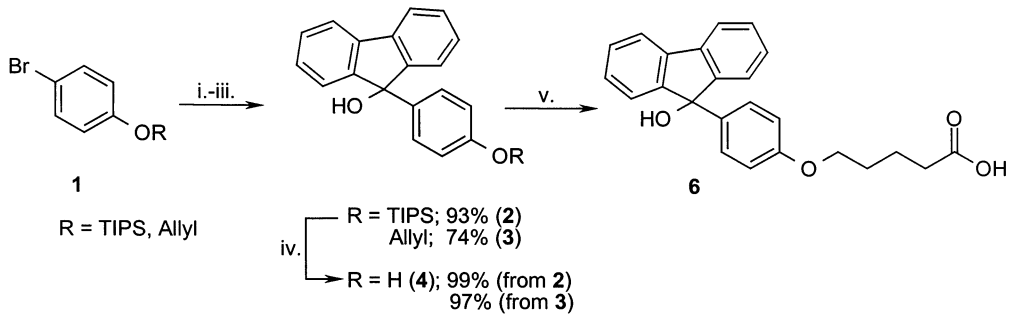
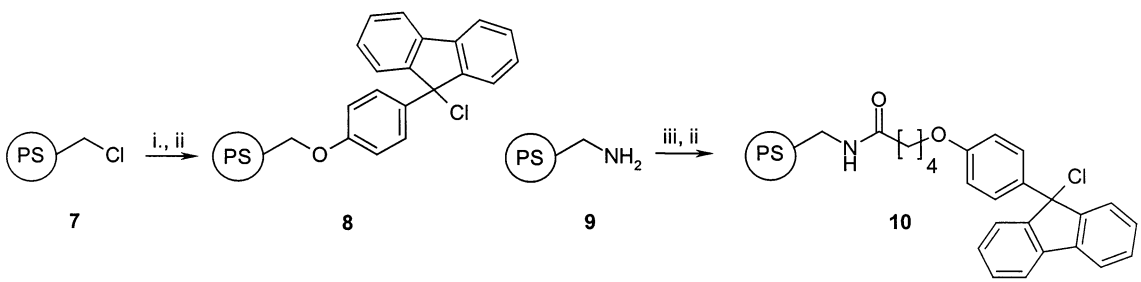
Herein we wish to report new phenylfluorenyl based linkers as interesting alternatives to the trityl- and PhFl-modified resins published so far. The syntheses of two derivatives are described that can be used for the modification of either chloromethyl polystyrene or any aminomethyl modified resins[12] (Scheme 1).



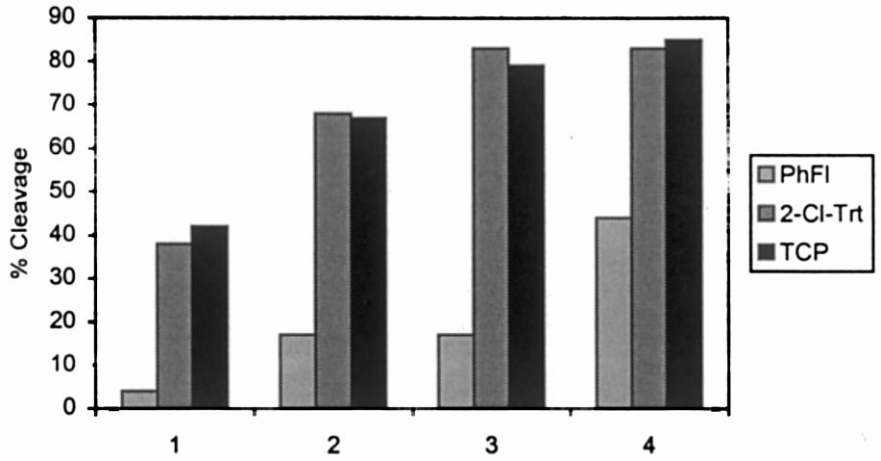

Scheme 1. (i) *n*-BuLi, THF −78°C; (ii) 9-fluorenone −78°C to rt; (iii) $NH_4Cl$ sat.; (iv) For R = TIPS: TBAF (1M) in THF; for R = allyl: 10 mol% $Pd(PPh_3)_4$, morpholine (5 equiv.); (v) ethyl 5-bromovalerate (**5**), $K_2CO_3$, acetone, reflux

Starting from either allyl- or triisopropylsilyl protected 4-bromophenol **1** protected phenylfluorenyl alcohols **2**, **3** are obtained in good yield after lithiation and 9-fluorenone quenching. Subsequent deprotection with either TBAF or $Pd(PPh_3)_4$/morpholine[13] results in deprotected alcohol **4** in excellent yields. Compound **6** is obtained by ether formation of **4** with ethyl 5-bromovalerate **5** followed by ester hydrolysis. Alternatively either 9-(4-hydroxyphenyl)-9H-fluoren-9-ol **4** or 5-(4-(9-hydroxy-9H-fluoren-9-yl)-phenoxy)-pentanoic acid **6** are linked to Merrifield resin and 1% cross linked aminomethyl polystyrene, respectively (Scheme 2).[14]




Scheme 2. (i) Cs–Salt of **4**, DMF; (ii) 20% AcCl, DCM; (iii) **6**, TBTU, NMM, DMF

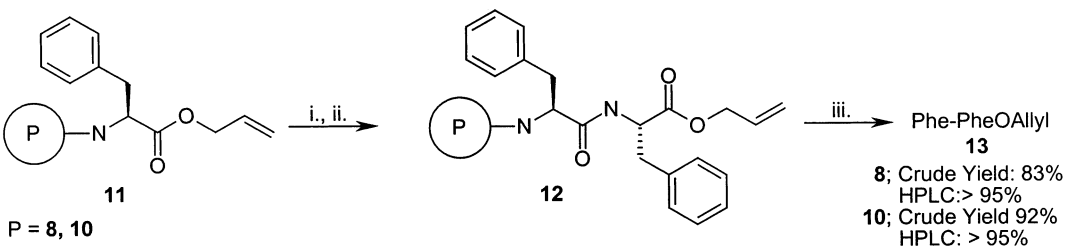
To investigate the acid stability of the oxygen-modified PhFl-linkage resin, **10** was loaded with Fmoc–Phe–OH[15] and treated under several acidic conditions. Fmoc–Phe–modified TCP[16] and 2-chlorotrityl[6] resins were also used for direct comparison. After 30 min the resins were filtered off and the solutions were evaporated and the white solids obtained were dried, weighed and analyzed by HPLC. Scheme 3 shows the amount of recovered Fmoc–Phe–OH as a percentage of the theoretical total recovery.




Scheme 3. Comparison of linker stability. The resins were treated under several acidic conditions for 30 min. (1) acetic acid/DCM (1:9); (2) acetic acid/DCM (2:8); (3) TFA/MeOH/DCM (1:10:89); (4) acetic acid/2,2,2-trifluoroethanol/DCM (1:1:8)

While carboxylic acids are cleaved quantitatively from trityl resins under very mild conditions (dilute AcOH or hexafluoroisopropanol)[6] less than 5% cleavage is observed for the PhFl derivative after treatment with 10% AcOH for 30 min. This resin shows reasonable stability up to 1% TFA. Quantitative cleavage of the compound is obtained by treatment with 10% TFA for 60 min (data not shown). To demonstrate the applicability of the resin for peptide synthesis a model compound (Fmoc–Ala–Val–Phe–OH) was synthesized using standard conditions.[17] The tripeptide was obtained with a crude yield of 83% and an HPLC purity of >95%.

To show the utility of these resins for the immobilization of *N*-nucleophiles an 'inverse' peptide synthesis was performed on both resins (**8** and **10**). Loading of phenylalanine allyl ester via the N-terminus, allyl ester cleavage and coupling of phenylalanine allyl ester gave the corresponding dipeptide ester.[18] The product **13** was obtained in good yield and high purity (Scheme 4).

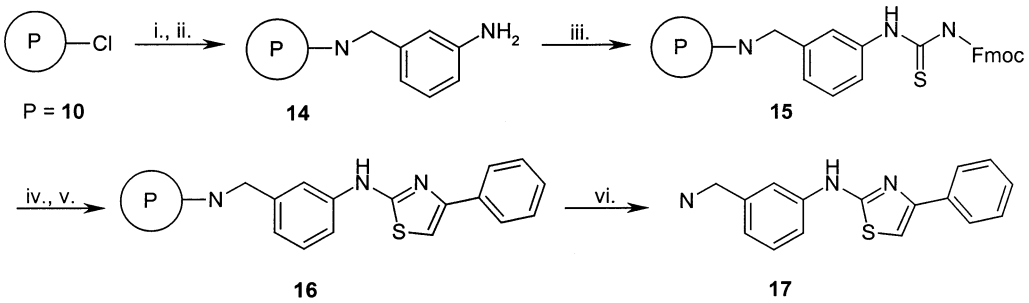



Scheme 4. (i) $Pd(PPh_3)_4$, morpholine; (ii) phenylalanine allyl ester, TBTU, DIPEA; (iii) 20% TFA, 2% $Et_3SiH$

For the original PhFl polystyrene resin[9] the linkage of more basic amines turned out to be too stable for reasonable product recovery after TFA treatment. To investigate the cleavage conditions for such basic amines from the oxygen modified PhFl resins described herein 9-(4-methoxyphenyl)-9-H-fluoren-9-ol was synthesized in analogy to compounds **2** and **3** (Scheme 1 $R = CH_3$). Conversion of the alcohol to the chloride and addition of benzylamine resulted in a model compound that allowed us to investigate the cleavage conditions for the

C–N bond in solution. Therefore the compound was treated under various acidic conditions and the cleavage rate was followed by HPLC analysis. A quantitative cleavage was observed with 40% TFA, 10% $Et_3SiH$ in DCM/MeOH (9:1) for 90 min. A decrease of the scavenging reagent led to an increase in reaction time. In our hands the most reasonable conditions for a solid phase approach turned out to be a 4 h treatment with 50% TFA, 2% $Et_3SiH$ in DCM/MeOH (9:1).

As an example for the immobilization and recovery of basic amines after a multi step reaction the synthesis of compound **17** (Scheme 5)[19] was carried out. Immobilization of 3-nitrobenzylamine followed by $SnCl_2$ reduction gave resin **14**. Thiourea **15** was cleanly formed by reaction with FmocNCS. Fmoc deprotection and cyclisation with α-bromo acetophenone resulted in the formation of resin bound 2-aminothiazole **16**.




Scheme 5. (i) 3-Nitrobenzylamine; (ii) $SnCl_2$; (iii) FmocNCS; (iv) 20% piperidine/DMF; (v) α-bromoacetophenone, (vi) 50% TFA, 2% $Et_3SiH$ in DCM/MeOH (9:1)

The cleavage of the benzylamine moiety from the resin was investigated under various conditions. $Et_3SiH$ as a scavenger turned out to be essential for the final cleavage step. Omission of triethylsilane resulted in decomposition of the products. Additionally for resin **8** a liberation of the linker moiety was observed. Too high concentrations of TFA tend to cleave the benzyl ether bond resulting in the cleavage of the linker moiety from the solid support. Similar side reactions have been reported for other linker systems fixed via a benzyl ether bond.[20] This side reaction is prevented when using resin **10**. Here the product is formed with a HPLC purity of >95% and a crude yield of 70% after six steps using a cleavage cocktail of 50% TFA, 2% $Et_3SiH$ in DCM/MeOH (9:1) for 4 h.[21] Since resin **8** is not stable under these cleavage conditions, resin **10** is recommended when higher TFA concentrations are needed for releasing the product from the solid support.

In conclusion, two new phenylfluorenyl based linkers for solid phase organic synthesis are introduced and their acid stability is compared to known trityl linker systems. We have demonstrated that oxygen and nitrogen nucleophiles can be immobilized, modified and cleaved off in good yield and high purity under appropriate cleavage conditions.

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12. (a) *Synthesis of* ***4***: A three neck flask with argon inlet, low temperature thermometer, stirring bar and septum was charged with **1** (either allyl or TIPS protected, 1.0 equiv., 1.5 M solution in THF). *n*-Butyllithium (1.2 equiv., 1.6 M solution in hexane) was added at −78°C. After 20 min a solution of 9-fluorenone (1.2 equiv. in THF) was added slowly and warmed to rt. After hydrolysis ($NH_4Cl$ sat.), TBME extraction and evaporation of the solvent the crude product was purified by Kieselgel chromatography using ethyl acetate/hexane. The products (**2**, **3**) were obtained in 93 and 74% yield, respectively. Compound **2** was deprotected with TBAF (1.2 equiv. in THF) at rt overnight. Compound **3** was treated with $Pd(PPh_3)_4$/morpholine as described.[13] (b) *Synthesis of* **6***:* A round bottomed flask with reflux condenser was charged with $K_2CO_3$ (5 equiv.), ethyl-5-bromovalerate (2 equiv.), sodium iodide (cat.) and **4** (1.0 equiv.) in acetone. The mixture was refluxed for 8 h, filtered and evaporated. The crude product was dissolved in dioxan, conc. NaOH (9:1) and warmed to 100°C for 2 h. After acidification (20% HCl solution, pH 1) the precipitated product was filtered off and dried under vacuum. 5-(4-(9-Hydroxy-9H-fluoren-9-yl)-phenoxy)-pentanoic acid (**6**) was obtained in 75% yield.
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14. (a) *Coupling of* ***4*** *to Merrifield resin*: 1.2 equiv. **4** (Cs–salt) and cat. KI were dissolved in DMF. Merrifield resin (0.7 mequiv./g) was added and the slurry was shaken for 2 days at 80°C and extensively washed with DMF/$H_2O$, MeOH, THF, DCM. A loading of 0.61 mequiv./g was obtained as determined by weight increase. (b) *Coupling of* ***6*** *to aminomethyl polystyrene*: 1.5 equiv. of **6**, 1.5 equiv. TBTU, 1.5 equiv. NMM were added in DMF to aminomethyl polystyrene (1% DVB, 1.19 mequiv./g) and the slurry was shaken for 16 h. After washing with DMF, MeOH, DCM (×2) Kaiser test showed 100% conversion resulting in a loading of 0.84 mequiv./g. (c) *Activation of resins* ***8*** *and* ***10***: The resins were treated with 20% acetyl chloride in DCM for 6 h. The resins were washed with DCM and dried under vacuum.
15. *Loading of Fmoc–Phe–OH*: The resin was treated with 2 equiv. Fmoc–Phe–OH, 4 equiv. DIPEA in DCM for 2 h. The reaction was quenched with MeOH and washed with DMF, MeOH, DCM (×2). A quantitative loading was obtained as determined by weight increase and elemental analysis.
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17. 100 mg of the resin obtained (see Ref. 15) was treated with 20% piperidine/DMF for 30 min for Fmoc deprotection. The resin was washed with DMF, DCM (×2). 2 equiv. of Fmoc–Val, TBTU and DIPEA in DMF were added. After 1 h the resin was washed with DMF, MeOH, DCM (×2). Fmoc cleavage and coupling of Fmoc–Ala were performed as described above. The tripeptide was cleaved with 10% TFA, 2% $Et_3SiH$ in DCM/MeOH (9:1) for 1 h. The solution was evaporated and the white solid obtained dried under vacuum.
18. (a) *Loading of phenylalanine allyl ester*: 2 equiv. of phenylalanine allyl ester toluenesulfonate were dissolved in DCM by adding 4 equiv. DIPEA. The solution was added to the resin and stirred for 2 h at rt. The reaction was quenched with MeOH and the resin washed with DMF, MeOH, THF, DCM (×2). A quantitative loading was obtained as determined by elemental analysis. (b) *Allyl ester cleavage*: 0.2 equiv. $Pd(PPh_3)_4$ and 10 equiv.

morpholine were dissolved in DCM and added to the resin. After 1 h the resin was rinsed with DCM, a solution of sodium diethyl dithiocarbamate/DIPEA (1:1) in DMF (20 mmol), DMF, MeOH, and DCM (×2). (c) *Coupling of phenylalanine allyl ester*: 2 equiv. of phenylalanine allyl ester toluenesulfonate, 2 equiv. TBTU, 2 equiv. DIPEA in DMF were added. After 1 h the resin was washed with DMF, MeOH, DCM (×2). The compound was cleaved with 20% TFA, 2% $Et_3SiH$ in DCM/MeOH (9:1) for 1 h. The cleavage procedure was repeated, the solutions combined and evaporated. The product was dried under vacuum overnight and obtained as a white solid.

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21. (a) *Loading of nitrobenzylamine*: Resin **10** was treated with 3-nitrobenzylamine×HCl (2.0 equiv.) and DIPEA (4.0 equiv.) in DCM/DMF (9:1). After 2 h the reaction was quenched with MeOH and the resin washed with DMF, MeOH, DCM (×2). A loading of 87% was obtained as determined by elemental analysis. No further optimization for increase of the loading (excess of reagent or increase of reaction time etc.) was undertaken. (b) *$SnCl_2$-reduction*: The resin was suspended in DMF and 5 equiv. $SnCl_2 \times 2\ H_2O$ were added. After 16 h the resin was washed with a solution of sodium diethyl dithiocarbamate/DIPEA (1:1) in DMF (20 mmol), DMF, MeOH, DCM (×2). (c) *Urea formation and deprotection*: FmocNCS (1.5 equiv.) in DCM was added for 4 h. After washing with DMF and DCM (×2) Fmoc was cleaved with piperidine (20% in DMF) for 30 min. (d) *Thiazole formation*: The α-bromoacetophenone (3 equiv.) was dissolved in dioxan and added to the resin. After 3 h the resin was washed with DMF, MeOH, DCM (×2) and the product cleaved off with 50% TFA, 2% $Et_3SiH$ in DCM/MeOH (9:1) for 4 h.