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Solid-phase synthesis: a linker for side-chain anchoring of arginine

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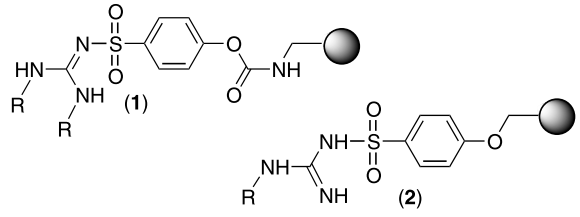
Abstract—A new linker based on a chroman system is described for the side-chain anchoring of Arg and other guanidine-containing molecules. The system is compatible with the Fmoc/*t*Bu solid-phase strategy, because the release of the final product is achieved by treatment with TFA in the presence of scavengers. © 2003 Published by Elsevier Science Ltd.

1. Introduction

A key aspect in all solid-phase programmes is to specify the mode of attachment of the first building block to the solid support.[1] This is usually accomplished through the use of bifunctional spacer molecules known as handles or linkers.[2] Such handles become attached permanently to a functionalised resin at one end, often through a stable amide bond, and are linked temporarily to the growing molecule.[3] At the end of the solid-phase synthetic process, cleavage of the temporary building block–handle bond results in release of the molecule from the solid support. In this regard, handles can be considered as temporary protecting groups. Hundreds of handles have been described in the literature and the choice is such that they are compatible with the majority of organic functional groups.[1–3] Treatment with acid is considered a very convenient cleavage method, but there are handles that are susceptible to cleavage by other reagents/chemical mechanisms such as electrophiles, nucleophiles, photolysis, metals, oxidative and reductive conditions, and cycloadditions/cycloreversions.[1c] Despite this myriad of handles, there is a niche to be filled in terms of systems for anchoring guanidine groups. Besides being present in natural building blocks such as arginine or guanidine, the guanidine group is an important motif in a broad range of therapeutic programmes. The protection of the guanidine group can be accomplished using arylsulfonyl- or bis(alkoxycarbonyl)-based groups.[4,5] The latter system involves a double protection and so only arysulfonyl systems represent a real alternative to be converted into a handle. However, only two *p*-alkoxybenzenesulfonyl linkers have been described in the literature.



Linker **1** was used to prepare a guanidinium-based 'tweezer' receptor[6] and **2** was used to anchor Arg through the side-chain to prepare small peptides.[7] Both of these examples required the use of strong acids such as CF_3SO_3H or HF and are not therefore the best choice for synthetic schemes that require mild conditions, such as the Fmoc/*t*Bu[8] strategy used in peptide chemistry. Finally, Bernhardt et al.[9] have reported the anchoring with low yields of Arg residue via its side-chain to a Barlos resin, but acidolytical cleavage from the resin is problematic and led to complex mixture of products. So, as a consequence, they anchored Orn residue and after cleavage guanidination was performed.

Keywords: benzofuran; benzopyran; combinatorial chemistry; handle; linker; protecting group; solid phase.

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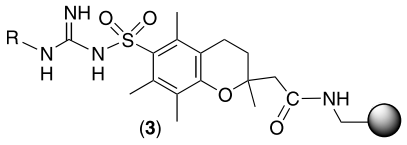
The work described here concerns a new linker strategy (**3**) based on a chroman structure.



(**3**)

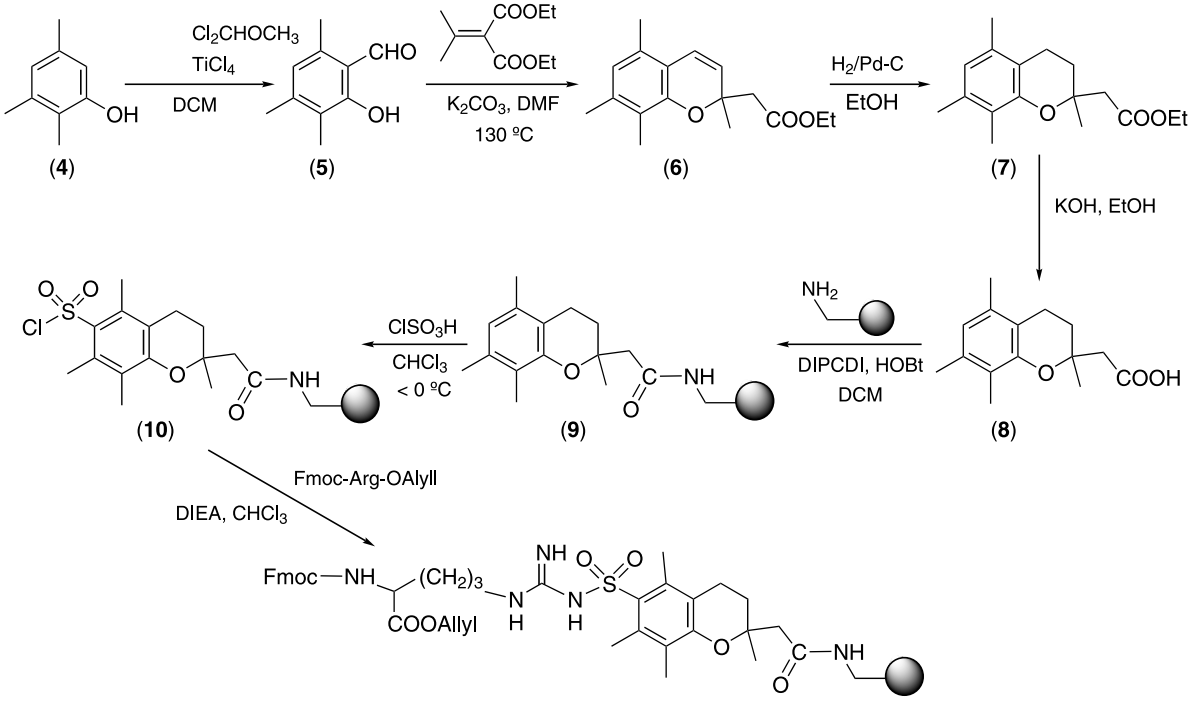
2. Results and discussion

Among the thirteen proteinogenic amino acids whose side-chain requires protection, Arg is the most problematic case. A system that satifies all the characteristics of an ideal protecting group has not been found to date for this residue.[4] For peptides containing a single Arg residue, 2,2,7,7,8-pentamethylchroman-6-sulfonyl (Pmc)[10] and 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf)[11] are the protecting groups of choice. By considering the Pmc group as a model, the new linker was prepared from 2,3,4-trimethylphenol (**4**) according the synthetic pathway outlined in Scheme 1.

Reaction of **4** with $TiCl_4$ (2.2 equiv.) followed by addition of dichloromethyl methyl ether led after 4 h to a mixture of 2-hydroxy-3,4,6-trimethylbenzaldehyde (**5**) and 4-hydroxy-2,3,6-trimethylbenzaldehyde (93%). Separation of the two isomers was achieved easily by crystallisation from ethanol/water to give **5** with 71% overall yield (4-hydroxy-2,3,6-trimethylbenzaldehyde derivative was obtained in 15% overall yield; ratio 5:1).[12] Formation of the benzopyran structure (**6**, 75%) was achieved by reaction of **5** with diethyl isopropylidenemalonate following a modified version of the method first proposed by Yamaguchi et al.[13] for similar molecules.[14] Practically quantitative yields were obtained in the catalytic reduction of the double bond and subsequent hydrolysis of the ethyl ester. The handle (3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)acetic acid (**8**) was obtained with an overall yield of 47% after four steps. Incorporation of **8** to Val (internal reference amino acid)[15] containing *p*-methylbenzhydrylamine was carried out with DIPCDI/HOBt in DCM.

A key step in this strategy is the chlorosulfonation of the resin **9**. Chlorosulfonation of polystyrene resins is usually carried out with $ClSO_3H$ in $CHCl_3$ at reflux.[16] However, these strong conditions are not necessary in this case because the aromatic ring of the linker in **9** is activated by the electron-donating substituents. Furthermore, forcing conditions will also lead to chlorosulfonation of the aromatic rings of polystyrene itself. After several trials, the chlorosulfonation was carried out with 4 equiv. of freshly distilled $ClSO_3H$ in anhydrous $CHCl_3$ under a nitrogen atmosphere for 30 min at −10°C, then 30 min at 0°C, and 90 min at 25°C. The resin was washed with cold H_2O for 5 min, followed by dioxane/H_2O, dioxane, and DCM.

Incorporation of Fmoc-Arg-OAllyl (5 equiv.)[17] was carried out in $CHCl_3$ in the presence of DIEA (10 equiv.) for 8 h at 25°C.[5] The resin was washed with DMF and DCM. Acid hydrolysis and amino acid analysis (AAA) of a sample of resin-bound peptide showed that the incorporation of Fmoc-Arg-OAllyl took place to give a 79% yield. Cleavage of the Fmoc-Arg-OAllyl [97% purity; MALDI-TOF MS (DHB): m/z: $[M+H]^+$ 437. 78 (calcd 437.53); $[M+K]^+$ 475.78 (calcd 476.62)] from the resin was carried out with TFA/H_2O(10:1) for 3 h at



Scheme 1.

25°C. The crude compound was analyzed by HPLC and had a purity of 97%.

Unreacted chlorosulfonyl groups could be capped with either Et_2NH/DMF (1:19) or piperidine/DMF (2:8), which can also be used to remove the Fmoc group. After removal of the Fmoc, incorporation of Fmoc-Phe-OH was carried with DIPCDI/HOBt in DCM. The protected dipeptide was obtained with excellent purity, as shown by HPLC after treatment with TFA in the manner described above [97% purity; MALDI-TOF MS (DHB): m/z: $[M+H]^+$ 585.14 (calcd 584.53)].

3. Conclusions

A new handle, (3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)acetic acid, is easily synthesised and can be used, after attachment to an amino resin and subsequent chlorosulfonation, for anchoring arginine derivatives (through their side-chain) as well as other guanidine-containing molecules. Compounds are released from the solid support by treatment with TFA in the presence of scavengers. This strategy, which is compatible with the Fmoc/*t*Bu approach for peptide synthesis, is currently being used in our laboratory for the solid-phase preparation of *C*-terminal Arg *p*-nitroanilide[18] and cyclic peptides through side-chain anchoring of Arg.[19]

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