

Fast and Selective N-Alkylation on Solid Support for SAR Studies and Functionalization of Peptidic CXCR4 Antagonists

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Introduction

Peptidic antagonists of the chemokine receptor CXCR4 have already shown anti-metastatic, anti-HIV and anti-rheumatoid arthritic activity [1], which represents a promising novel approach for therapeutic intervention in these diseases. Therefore, development of potent and optimized CXCR4 antagonists is an important requirement for the therapeutic success of this approach.

We started our modifications from the pentapeptide *cyclo*-(D-Tyr1-Orn2-Arg3-Nal4-Gly5) (Orn = ornithine; Nal = L-3-(2-naphthylalanine) [2] which is a CXCR4 antagonist and has a high binding affinity (IC₅₀ = 9 nM). Our aim was to subject the ornithine side chain to structure-activity relationship (SAR) studies under retention of the positive charge of the amine with an additional focus on procedures that are on the one side convenient and allow on the other side to introduce a great diversity of different residues and functionalities. In this context convenient especially means mild conditions and fast reaction rates with good to excellent yields for readily available starting materials compatible with the existing protection group strategy on solid support. We investigated *N*-alkylation via the Mitsunobu reaction and direct alkylation to realize all of these goals at the same time.

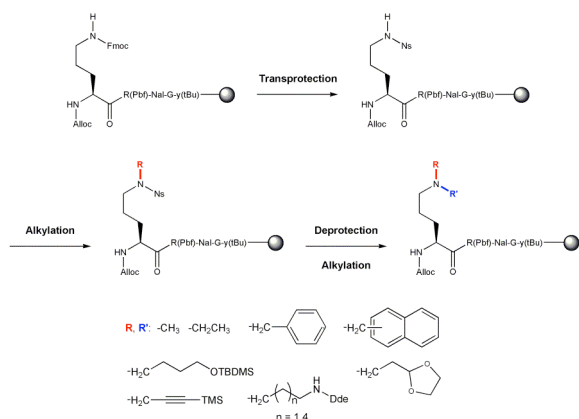


Fig. 1 Schematic mono- and di-*N*-alkylation for on-resin use in combination with standard Fmoc synthesis

Results and Discussion

Aliphatic and aromatic alcohols varying in size were used under Mitsunobu conditions to alkylate the 2-nitrobenzenesulfonamide (Ns) protected (Fukuyama

variant) ornithine side chain. The Ns group can be rapidly introduced to the amine on-resin in 15 minutes using collidine and 2-nitrobenzenesulfonylchloride [3] or the corresponding amino acid prepared prior to coupling in solution [4]. For subsequent alkylation under Mitsunobu conditions triphenylphosphine (PPh₃), diisopropylazodicarboxylate (DIAD) and the chosen alcohol are mixed in tetrahydrofuran (THF). Treatment of the alkylated, protected amine with 2-mercaptoethanol and 1,8-Diazabicyclo[5.4.0] undec-7-ene (DBU) selectively removes the Ns group by treating the resin two times for five minutes.

The course of the alkylation was monitored by RP-HPLC (Tab. 1) to give almost full conversion in ten minutes. A second alkyl residue was introduced in selected examples after removal of the Ns group but yielded less of the desired product due to partial formation of the quaternary ammonium salt. Starting from the sterically less demanding methyl amine compound gave a better yield in comparison with the benzyl derivative.

Tab. 1 Yield of *N*-alkylations after ten minutes monitored by HPLC

Amine	First Alkylation	Second Alkylation	
	-N ⁺ H(Ns)	-N ⁺ H(Me)	-N ⁺ H(Bz)
Alcohol			
Methanol	>99	*	n.t.
Benzylalcohol	>99	84	60
1-Naphthylmethanol	>99	82	n.t.
2-Naphthylmethanol	>99	73	n.t.

*a mixture of mono- di- and trimethylated compounds was obtained

n.t.: not tested

As example for the mono-*N*-alkylation of the linear peptide strand RP-HPLC spectra (water/acetonitrile 10 to 100% in 40 minutes) before and after the reaction with 2-naphthylmethanol under Mitsunobu conditions are shown (Fig. 2). After the alkylation virtually no starting material is present.

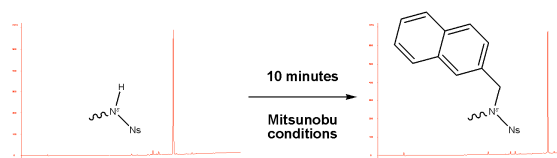


Fig. 2 HPLC spectra before and after *N*-alkylation

In comparison to the non-alkylated starting compound (IC₅₀: 9 nM) the binding affinities could not be improved and is in most cases four to five fold weaker. However,

rather voluminous residues like naphthylmethyl (Nap) do not impair the affinity more than a comparatively small ethyl group. This suggests that the binding pocket has available space that could be used for further enhancement in binding affinity.

Tab. 2 Affinities of alkylated peptides towards the CXCR4 receptor

N-Alkylation	Me	Et	Bz	2-Nap	1-Nap
IC ₅₀ [nM]	105 ± 7	38 ± 2	155 ± 63	49 ± 1	40 ± 3
N-Alkylation	Me, Bz	Me, 2-Nap	Me, 1-Nap	Bz, Bz	
IC ₅₀ [nM]	38 ± 8	34.2	39.7	131 ± 5	

The central problem when trying to introduce functional groups by *N*-alkylation is to find appropriate protecting groups that are both stable to the reaction conditions and further couplings and orthogonal to the residual protecting groups.

We chose to introduce aldehyde, alkyne, amine, and hydroxyl functions in a way that they can be deprotected orthogonally on-resin as well as in solution. The aldehyde is an exception and is deprotected together with the other acid labile groups (Fig. 3).

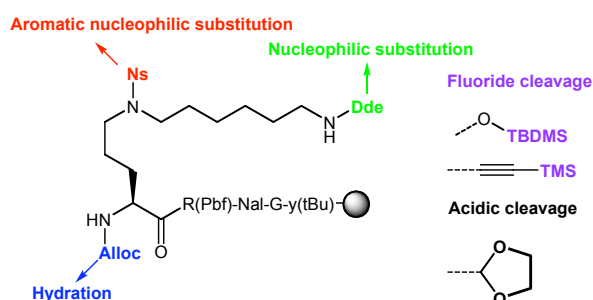


Fig. 3 Orthogonally protected functionalities introduced by *N*-alkylation and cleavage conditions

For example, after introducing the *N*-[1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl] (Dde) protected amine three identical functionalities can be deprotected independently and further selectively modified. This opens up a wide variety of possible alterations in combination with all the advantages solid phase chemistry has to offer.

In some cases a halide may be easier available than the corresponding alcohol. Thus direct alkylation using DBU as base is an alternative to the Mitsunobu reaction. However, the reaction time is considerably elongated or it has to be repeated more often to reach complete conversion, respectively.

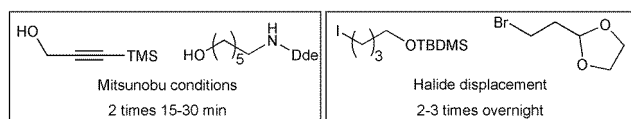


Fig. 4 Reaction time of different alkylation methods until complete turnover is achieved

In general introducing functional groups via *N*-alkylation is in contrast to incorporation of simple alkyl groups not complete after a single step. Therefore, repetitive treatment is needed to convert the remaining starting material.

In summary, we used an optimized protocol of the mild and selective Fukuyama-Mitsunobu reaction as *N*-alkylation procedure to quickly and quantitatively introduce non-functionalized residues into transiently protected amines of a linear peptide in combination with Fmoc SPPS. Conversion into cyclic pentapeptides produced compounds with nanomolar affinity towards the CXCR4 receptor. Beyond that, three functionalities were implemented with orthogonal protection to the rest of the peptide. A fourth functional group whose protection group is cleaved together with other acid labile groups could also be incorporated. This chemical versatility opens up a lot of possibilities for modifications like the direct synthesis of reduced amide bonds and peptoids on solid support. Furthermore, it is an alternative for commonly used acyl spacing units and an additional tool for SAR studies.

References

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