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Introduction of Functional Groups into Peptides via *N*-Alkylation

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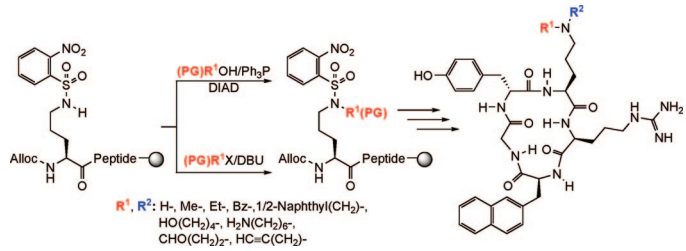
ABSTRACT



An optimized protocol for the mild and selective Fukuyama−Mitsunobu reaction was used for mono- and di-*N*-alkylation on solid support. Thereby, nonfunctionalized aliphatic and aromatic residues are quickly introduced into transiently protected, primary amines of a linear peptide. *N*-Alkylation can also be used to implement alkyl chains carrying (protected) functionalities suited for subsequent modification. Applicability of this method is demonstrated by various *N*-alkylated analogues of a cyclic CXCR4 receptor antagonist originally developed by Fujii et. al.

Many different ways to alkylate amines have been developed,[1] including reductive methods for imines,[2] amides,[3] direct alkylation by halide,[4] sulfonate[5] or tosylate[6] displacement, or epoxide opening.[7] However, in most cases when used on solid support, small organic molecules are modified that contain only little to no functionalities other than the reaction center.

Our method of choice to modify more complex molecules such as peptides is the Fukuyama sulfonamide alkylation,[8] as it is especially mild and efficient and fits well with Fmoc solid phase chemistry.[9] Protecting the amine as a sulfonamide

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(1) Olsen, C. A.; Franzyk, H.; Jaroszewski, J. W. *Synthesis* **2005**, *263*, 1–2653, and references cited therein.

(2) (a) Sasaki, Y.; Murphy, W. A.; Heiman, M. L.; Lance, V. A.; Coy, D. H. *J. Med. Chem.* **1987**, *30*, 1162–1166. (b) Sasaki, Y.; Coy, D. H. *Peptides* **1987**, *8*, 119–121. (c) Meyer, J. P.; Davis, P.; Lee, K. B.; Porreca, F.; Yamamura, H. I.; Hruby, V. J. *J. Med. Chem.* **1995**, *38*, 3462–3468. (d) Gazal, S.; Gellerman, G.; Gilon, C. *Peptides* **2003**, *24*, 1847–1852.

(3) (a) Dankwardt, S. M.; Smith, D. B.; Porco, J. A.; Nguyen, C. H. *Synlett* **1997**, 854–856. (b) Kan, T.; Kobayashi, H.; Fukuyama, T. *Synlett* **2002**, 1338–1340.

(4) (a) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646–10647. (b) Barn, D. R.; Morphy, J. R.; Rees, D. C. *Tetrahedron Lett.* **1996**, *37*, 3213–3216. (c) Byk, G.; Frederic, M.; Scherman, D. *Tetrahedron Lett.* **1997**, *38*, 3219–3222.

(5) (a) Virgilio, A. A.; Schürer, S. C.; Ellman, J. A. *Tetrahedron Lett.* **1996**, *37*, 6961–6964. (b) Souers, A. J.; Virgilio, A. A.; Schürer, S. S.; Ellman, J. A.; Kogan, T. P.; West, H. E.; Ankener, W.; Vanderslice, P. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2297–2302. (c) Souers, A. J.; Virgilio, A. A.; Rosenquist, A.; Fenuik, W.; Ellman, J. A *J. Am. Chem. Soc.* **1999**, *121*, 1817–1825.

(6) Sajjadi, Z.; Lubell, W. D. J. *Pept. Res.* **2005**, *65*, 298–310.

(7) (a) Rossé, G.; Ouertani, F.; Schröder, H. *J. Comb. Chem.* **1999**, *1*, 397–401. (b) Bryan, W. M.; Huffman, W. F.; Bhatnagar, P. K. *Tetrahedron Lett.* **2000**, *41*, 6997–7000. (c) Wong, J. C.; Sternson, S. M.; Louca, J. B.; Hong, R.; Schreiber, S. L. *Chem. Biol.* **2004**, *11*, 1279–1291. (d) Tremblay, M. R.; Wentworth, P.; Lee, G. E.; Janda, K. D. *J. Comb. Chem.* **2000**, *2*, 698–709.

(8) (a) Rew, Y.; Goodman, M. *J. Org. Chem.* **2002**, *67*, 8820–8826. (b) Andersen, T. F.; Strømgaard, K. *Tetrahedron Lett.* **2004**, *45*, 7929–7933. (c) Beaver, K. A.; Siegmund, A. C.; Spear, K. L. *Tetrahedron Lett.* **1996**, *37*, 1145–1148. (d) Boeijen, A.; Kruijtzer, J. A. W.; Liskamp, R. M. J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2375–2380. (e) Fukuyama, T.; Jow, C. K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373–6374. (f) Fukuyama, T.; Cheung, M.; Jow, C. K.; Hidai, Y.; Kan, T. *Tetrahedron Lett.* **1997**, *38*, 5831–5834.

(9) Biron, E.; Chatterjee, J.; Kessler, H. *J. Pept. Sci.* **2006**, *12*, 213–219.

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allows alkylation either by using alcohols under Mitsunobu conditions or by using halides, giving access to a vast amount of alkylating agents. The Fukuyama procedure was slightly modified, leading to an efficient and fast procedure for *N*-methylation.[9]

In the present work, we explored this optimized procedure toward a wider range of alkylating agents and toward the synthesis of not only secondary but also tertiary amines. Our aim was to use *N*-alkylation to introduce additional functional groups to peptides. These functionalities need orthogonal protection or protecting groups, which are cleaved simultaneously during final acidic deprotection of the peptide. They were chosen such as to allow easy subsequent on-resin modifications like amide and esther bond synthesis. Furthermore, functionalities needed for the so-called "click-chemistry" (oxime ligations with aldehydes or 1,3-dipolar addition of azides to alkynes) were also included.

To demonstrate the practical applicability of this *N*-alkylation procedure, the CXCR4 chemokine receptor antagonist cyclo(-D-Tyr[1]-Orn[2]-Arg[3]-Nal[4]-Gly[5]) (Orn = L-ornithine, Nal = L-3-(2-naphthyl)alanine) was chosen as a scaffold for the synthesis of various *N*-alkylated CXCR4 ligands.[10]

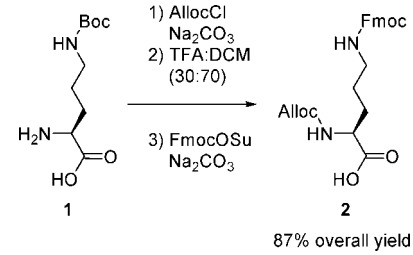
The CXCR4 receptor and its ligands are interesting for different medical applications as they are involved in a variety of diseases, such as HIV-1, rheumatoid arthritis, and at least 23 different types of cancer.[11] Peptidic CXCR4 antagonists have already shown activity against these diseases and therefore represent a novel approach for therapeutic intervention.[12]

To probe which substituents are tolerated at the Orn side chain of the modified Fujii peptide, we investigated several mono- and dialkylated analogues of cyclo(-D-Tyr[1]-Orn[2]-Arg[3]-Nal[4]-Gly[5]).

The linear peptide N^{α}-Alloc-Orn-N^{δ}-Fmoc-Arg(Pbf)-Nal-Gly-D-Tyr(tBu), which was subjected to the *N*-alkylation procedure, was assembled on trityl chloride resin by the Fmoc strategy using TBTU/HOBt as coupling reagents.

The terminal amino acid N^{α}-Alloc-Orn-N^{δ}-Fmoc-OH **2** was synthesized starting from H-L-Orn-N^{δ}-Boc-OH **1** via protection of N^{α} with allylchloroformate (AllocCl), followed by Boc deprotection with 30% TFA in DCM and N^{δ} reprotection using 9-fluorenylmethyloxycarbonyl-*N*-hydroxysuccinimide (FmocOSu). **2** was obtained in 87% overall yield and was sufficiently pure for direct use on solid support (Scheme 1). Alternatively, the amine can be protected as *o*-nitrobenzenesulfonamide (Ns).[13] However, as the Ns group can only be cleaved after alkylation of the sulfonamide, it is not as versatile as the aforementioned approach.[9]

Scheme 1. Synthesis of the Ornithine Derivative



87% overall yield

On-resin alkylation of the ornithines N^{δ} was achieved by transitional Ns protection[14] of the primary amines, followed by *N*-alkylation under Mitsunobu conditions[15] or direct alkylation with halides.[16] Final deprotection of the Ns group[17] yielded the secondary amine. Tertiary amines were synthesized by an additional alkylation step. They were built up by first alkylating with the smaller alcohol and then using the sterically more demanding alcohol in a second step.

Cleavage of peptides from the resin was performed after Alloc deprotection with retention of side chain protecting groups using hexafluoroisopropanol (HFIP) in DCM. Diphenylphosphoryl azide (DPPA) was used as a cyclization reagent with $NaHCO_3$ as a solid base in DMF.[18] Ns deprotection of secondary amines was carried out in DMF using β-mercaptoethanol and DBU prior to the acidic deprotection step. TMS and TBDMS groups were cleaved with TBAF in DMF and *N*-[1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl] (Dde) groups with hydrazine. The final peptides were obtained after deprotection in TFA/H_2O/triisopropyl silane (TIPS) and subsequent RP-HPLC purification.

One goal of this work was to explore the CXCR4 receptor binding pocket at the Orn side chain of its ligands. Emphasis was put on modifications with various aromatic compounds as these have been shown to contribute beneficially to the CXCR4 binding affinity.[19]

The alkylation reactions were carried out using Mitsunobu conditions because they are milder than alkylation with halides.[20] Starting from the Ns-protected amine, the course of the alkylation reaction was monitored via RP-HPLC. After 10 min, almost full conversion could be observed for most small and large mono-*N*-alkylated compounds.

After removal of the Ns group of the *N*-methylated compound, a second alkylation step led to the tertiary amines

(10) (a) Fujii, N.; Oishi, S.; Hiramatsu, K.; Araki, T.; Ueda, S.; Tamamura, H.; Otaka, A.; Kusano, S.; Terakubo, S.; Nakashima, H.; Broach, J. A.; Trent, J. O.; Wang, Z. X.; Peiper, S. C. *Angew. Chem., Int. Ed. Engl.* **2003**, *42*, 3251–3253. (b) Tamamura, H.; Araki, T.; Ueda, S.; Wang, Z. X.; Oishi, S.; Esaka, A.; Trent, J. O.; Nakashima, H.; Yamamoto, N.; Peiper, S. C.; Otaka, A.; Fujii, N. *J. Med. Chem.* **2005**, *48*, 3280–3289.

(11) (a) Feng, Y.; Broder, C. C.; Kennedy, P. E.; Berger, E. A. *Science* **1996**, *272*, 872–877. (b) Nanki, T.; Hayashida, K.; El-Gabalawy, H. S.; Suson, S.; Shi, K. R.; Girschick, H. J.; Yavuz, S.; Lipsky, P. E. *J. Immunol.* **2000**, *165*, 6590–6598. (c) Balkwill, F. *Nat. Rev. Cancer* **2004**, *4*, 540–550.

(12) Tamamura, H.; Fujii, N. *Expert Opin. Ther. Targets* **2005**, *9*, 1267–1282, and references cited therein.

(13) Biron, E.; Kessler, H. *J. Org. Chem.* **2005**, *70*, 5183–5189.

(14) 5 equiv of NsCl, 10 equiv of collidine, NMP, 15 min, RT.

(15) 10 equiv of ROH, 5 equiv of diisopropylazodicarboxylate (DIAD), 5 equiv of PPh_3, THF, RT.

(16) 4 equiv of halide, 6 equiv of DBU, NMP, RT.

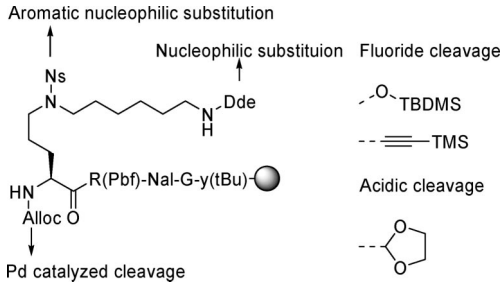
(17) 16 equiv of DBU, 20 equiv of β-mercaptoethanol, NMP, 5 min, RT, two times.

(18) Brady, S. F. P. W. J.; Arison, B. H.; Freidinger, R. M.; Nutt, R. F.; Veber, D. F. *In 8th Annual Peptide Symposium*; 1983; pp 127−130.

(19) Demmer, O.; Koglin, N.; Kessler, H.; Wester, H. J. *J. Pept. Sci.* **2006**, *12*, 172.

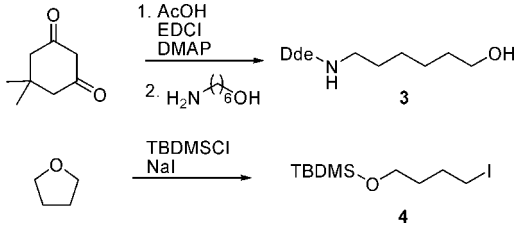
(20) The side chain of histidine is methylated under loss of the trityl protecting group by alkylation with halides, but not under Mitsunobu conditions. See ref 9.

Scheme 2. Orthogonally Protected Functionalities Introduced by *N*-Alkylation



within 10 min. However, in addition to the tertiary amine, the quaternary amine was also observed as a side product along with traces of unreacted primary amine. For the dibenzylated amine, the reaction time was extended to 20 min to raise yields. Compared to the other tertiary amines, a higher amount of unreacted reactant and quaternary ammonium salt was observed in the latter case. Dibenzylation of an amine has already been shown to be feasible using a simpler system with virtually equal purity.[21]

Scheme 3. Synthesis of Starting Compounds for *N*-Alkylation



To elucidate the influence of additional functionalities on CXCR4 affinity, different protecting groups were chosen that are stable under alkylation conditions. Emphasis was put on orthogonal protection compatible with Fmoc solid phase peptide synthesis (Scheme 2).

An aldehyde functionality was introduced, protected as dioxolan, which is cleaved together with the other acid labile

Table 1. Purity of (Ns Protected) Amines in Relation to the Starting Compound after Alkylation via Mitsunobu Reaction

	purity (%)		
ROH	$N^{\delta}R$(Ns)	$N^{\delta}R$(Me)	$N^{\delta}R$(Bz)
MeOH	>99	[a]	n.t.[b]
EtOH	84	n.t.[b]	n.t.[b]
BzOH	>99	84	60
1-naphthyl(CH_2)OH	92	82	n.t.[b]
2-naphthyl(CH_2)OH	>99	73	n.t.[b]

[a] An inseparable mixture of mono-, di-, and trialkylated amines was obtained. [b] n.t.: not tested.

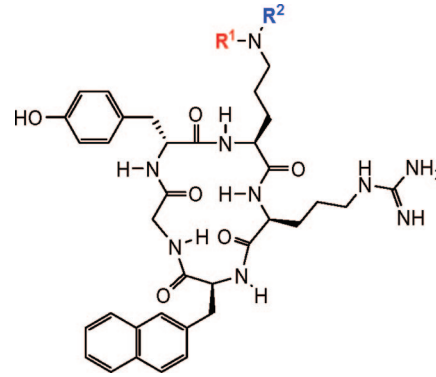
Table 2. Comparison of Mitsunobu Reactions and Direct Alkylation by Halide Displacement

	compound	reaction time	purity (%)
Mitsunobu conditions	**3**	2 * 30 min	74
	6	2 * 15 min	96
halide displacement	**4**	3 * 1 day	72
	5	3 * 1 day	98

groups in the final deprotection step. Silyl protecting groups were chosen for the alcohol and the alkyne groups, as they can be cleaved orthogonally by fluorides on resin and in solution. The hydrazine labile Dde amine protection group is ideally suited for *N*-alkylation, as it is orthogonal,[22] easy to synthesize and to cleave,[23] and stable under Mitsunobu reaction conditions.[23c,24] The protecting group DdeOH itself was synthesized after optimizing an existing protocol[23b] by using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) as the coupling reagent and by modifying the workup conditions. Subsequent stirring with 6-aminohexanol in DCM at RT gave **3** in 93% yield (Scheme 3).

To compare different *N*-alkylation procedures, two protected functionalities were introduced under Mitsunobu conditions and two by halide displacement. While 2-(2-bromoethyl)-1,3-dioxolane **5** and 3-(trimethylsilyl)prop-2-yn-1-ol **6** were commercially available, **4** was obtained by acidic ether cleavage of THF[25] (Scheme 3).

Table 3. Affinities of Cyclopeptides Towards the CXCR4 Receptor



R^1	R^2	IC_{50} [nM]
Me	H	105 ± 7
Et	H	38 ± 2
Bz	H	155 ± 63
1-naphthyl(CH_2)−	H	40 ± 3
2-naphthyl(CH_2)−	H	49 ± 1
Bz	Me	38 ± 8
1-naphthyl(CH_2)−	Me	39 ± 2
2-naphthyl(CH_2)−	Me	38 ± 5
Bz	Bz	131 ± 5
HO(CH_2)$_4$−	H	57 ± 16
$H_2N(CH_2)_6$−	H	15 ± 3
CHO(CH_2)$_2$−	H	29 ± 21
HC≡CCH_2−	H	109 ± 47

In comparison to direct alkylation by halide displacement, faster quantitative conversion is observed using Mitsunobu conditions. Although both reaction types showed little to no formation of byproducts when analyzed by ESI-MS, control by RP-HPLC exhibited reduced purities in relation to nonfunctionalized residues (Table 2).

Evaluation of the *N*-alkylated peptide affinities toward CXCR4 gave mixed results (Table 3). In comparison to the starting compound cyclo(-D-Tyr[1]-Orn[2]-Arg[3]-Nal[4]-Gly[5]), the IC_{50} values could not be improved by alkylating the Orn side chain. Interestingly, large aromatic substituents like naphthylmethyl are in most cases better tolerated than smaller ones. This aromatic moiety is found in many peptidic and nonpeptidic CXCR4 antagonists.[12,19,26]

The mild and selective *N*-alkylation method presented in this study offers an efficient way to functionalize resin bound peptides with a variety of functional moieties which allow subsequent introduction of fluorescence labels, lipids, biopolymers, or others.

Additional applications include the synthesis of peptidomimetics like so-called "reduced amides" or peptoids.

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Supporting Information Available: Experimental procedures and compound characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) Zaragoza, F.; Stephensen, H. *Tetrahedron Lett.* **2000**, *41*, 1841–1844.

(22) (a) Augustyns, K.; Kraas, W.; Jung, G. *J. Pept. Res.* **1998**, *51*, 127–133. (b) Rohwedder, B.; Mutti, Y.; Dumy, P.; Mutter, M. *Tetrahedron Lett.* **1998**, *39*, 1175–1178.

(23) (a) Nash, I. A.; Bycroft, B. W.; Chan, W. C. *Tetrahedron Lett.* **1996**, *37*, 2625–2628. (b) Portal, C.; Launay, D.; Merritt, A.; Bradley, M. *J. Comb. Chem.* **2005**, *7*, 554–560. (c) Murray, P. J.; Kranz, M.; Ladlow, M.; Taylor, S.; Berst, F.; Holmes, A. B.; Keavey, K. N.; Jaxa-Chamiec, A.; Seale, P. W.; Stead, P.; Upton, R. J.; Croft, S. L.; Clegg, W.; Elsegood, M. R. J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 773–776.

(24) Chhabra, S. R.; Khan, A. N.; Bycroft, B. W. *Tetrahedron Lett.* **2000**, *41*, 1099–1102.

(25) Nyström, J. E.; McCanna, T. D.; Helquist, P.; Amouroux, R. *Synthesis* **1988**, 56–58.

(26) (a) Zhan, W. Q.; Liang, Z. X.; Zhu, A. Z.; Kurtkaya, S.; Shim, H.; Snyder, J. P.; Liotta, D. C. *J. Med. Chem.* **2007**, *50*, 5655–5664. (b) Tamamura, H.; Ojida, A.; Ogawa, T.; Tsutsumi, H.; Masuno, H.; Nakashima, H.; Yamamoto, N.; Hamachi, I.; Fujii, N. *J. Med. Chem.* **2006**, *49*, 3412–3415.