Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron Letters
Tetrahedron Letters 47 (2006) 2671–2674

Solid-phase synthesis of palmitoylated and farnesylated lipopeptides employing the fluoride-labile PTMSEL linker

Maria Lumbierres, Jose M. Palomo, Goran Kragol and Herbert Waldmann*

Max-Planck-Institut für Molekulare Physiologie, Abteilung Chemische Biologie, Otto-Hahn-Str. 11, 44227 Dortmund, Germany
Universität Dortmund, Fachbereich 3, Chemische Biologie, 44221 Dortmund, Germany

Received 13 January 2006; revised 16 February 2006; accepted 17 February 2006

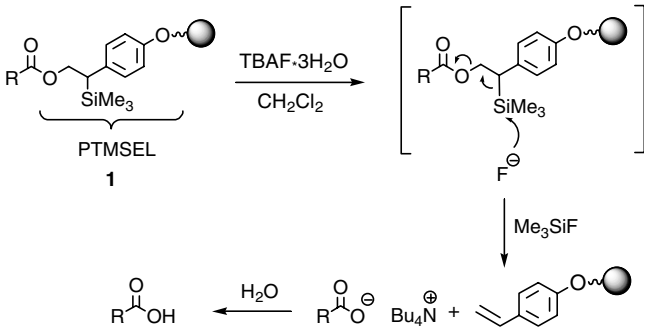
Abstract—Acid- and base-labile S-palmitoylated and S-farnesylated lipopeptides can be synthesized in high overall yield employing the (2-phenyl-2-trimethylsilyl) ethyl (PTMSEL) linker for anchoring to and release under almost neutral conditions from the solid support.

Lipid modified proteins fulfil important roles in various cellular processes like cell-signalling, vesicular transport, growth and differentiation.[1] Tailor-made lipidated peptides embodying the characteristic lipidated peptide sequences of their parent proteins, for example, sequences incorporating S-palmitoylated and S-farnesylated cysteines, as well as reporter groups and additional tags, in particular, for coupling to expressed proteins are efficient tools for the study of the biophysical, biochemical, structural and biological properties of lipidated proteins.[2] Therefore, the availability of flexible and high-yielding methods for the solid-phase synthesis of such lipidated peptides is of substantial interest.

Since S-palmitoylated and S-farnesylated peptides are both acid- and base-labile[3] the choice of the linker to the solid support is crucial. It must be cleavable under almost neutral conditions and without any harm to the thioesters and the isoprenyl-thioethers. Currently, only the hydrazide linker[4] and the 4-sulfonamidobutyryl linker[5] are available for this purpose. However, the hydrazide linker often yields the desired lipidated peptides only with moderate yield so that further viable alternatives to these methods are in demand.

Here we report that acid- and base-labile doubly lipidated peptides can be synthesized efficiently on the solid phase if the (2-phenyl-2-trimethylsilyl)ethyl (PTMSEL) linker is employed for anchoring to the polymeric carrier. The PTMSEL linker was recently introduced by Kunz and co-workers[6] for the synthesis of glycopeptides. In contrast to other silyl-based linkers which are cleaved under basic conditions with tetrabutylammonium fluoride (TBAF) in DMF,[7] the benzylic C–Si bond in the PTMSEL linker can be cleaved with TBAF in CH_2Cl_2, that is, under nearly neutral conditions (Scheme 1).

As a model sequence guiding the development of the synthesis method, the C-terminal heptapeptide sequence of the human N-Ras protein GlyCys(Pal)MetGlyLeuProCys(Far) (Fig. 1) was chosen. The Ras proteins are critically involved in cellular signal transduction and belong to the most important human oncogene products.[1–3]



Scheme 1. Structure and fluoride-induced cleavage of the PTMSEL linker.

Keywords: Lipopeptides; Solid-phase synthesis; Linker groups; Ras protein.
* Corresponding author. Tel.: +49 231 133 2400; e-mail: herbert.waldmann@mpi-dortmund.mpg.de

0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.02.109

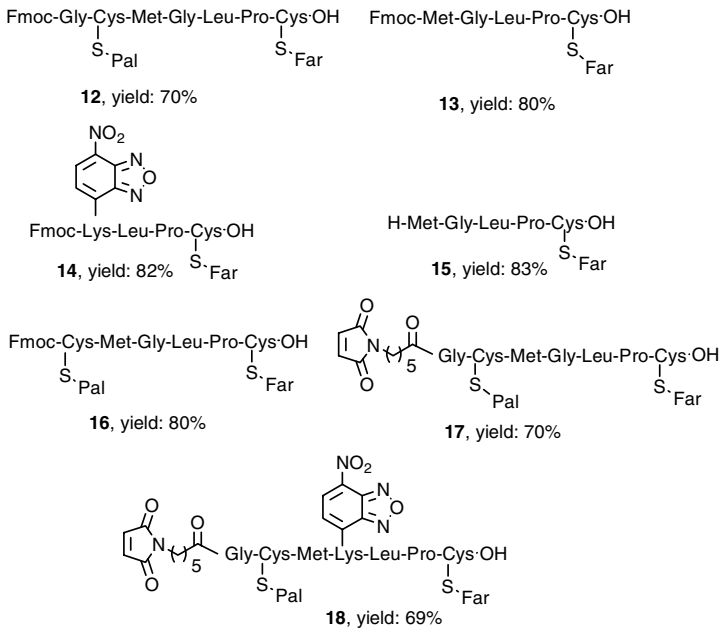
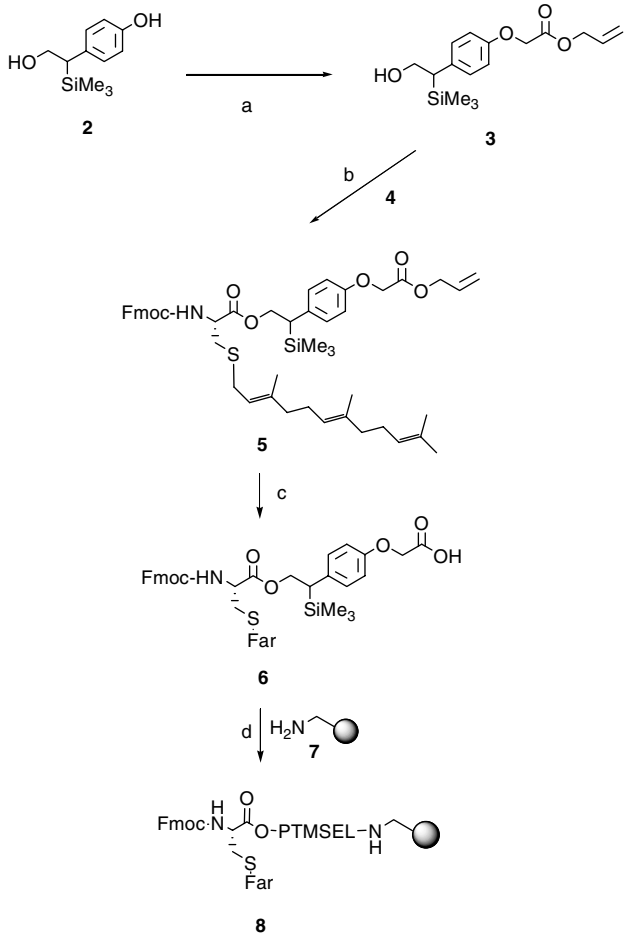


Figure 1. Lipidated peptides synthesized employing the PTMSEL linker.

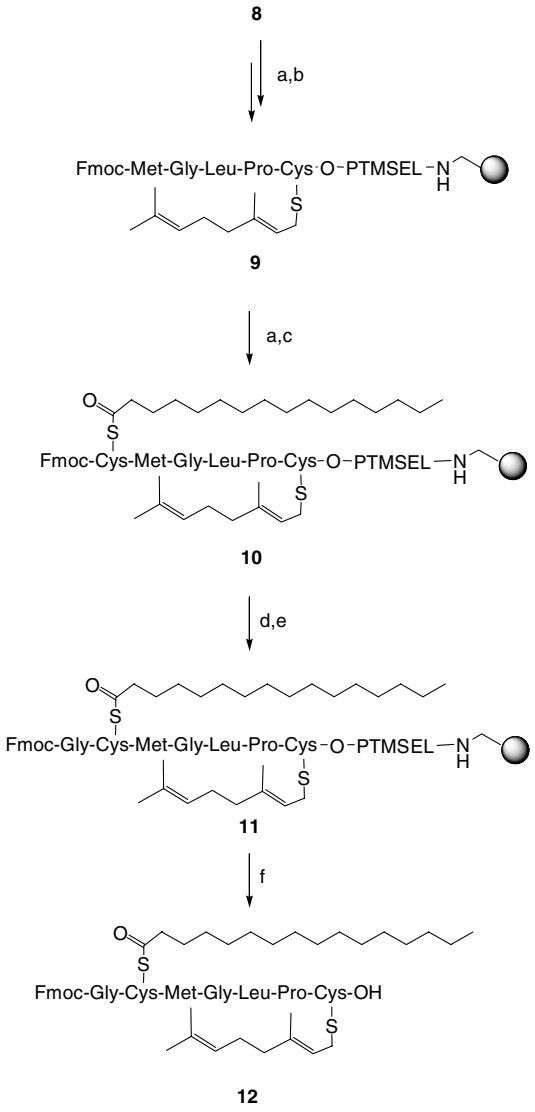
The PTMSEL linker **2** was synthesized as described[6] and the phenol was alkylated with allyl chloroacetate to give alcohol **3** (Scheme 2). In order to establish an efficient and flexible synthesis it was planned to develop a method that allows for sequential introduction of prelipidated building blocks instead of the less efficient alternative on-resin-lipidation.[4] To this end, Fmoc-protected S-farnesylated cysteine **4** was coupled to alcohol **3** and after Pd(0)-catalyzed cleavage of the allyl ester[8] **5**, carboxylic acid building block **6** embodying the first lipidated amino acid was attached to the solid support. The carboxylic acid **6** was coupled to the aminomethylpolystyrene resin **7** by activation with *N*-[(dimethylamino)-1-*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium (HATU) and diisopropylethylamine (DIPEA) in DMF (Scheme 2) with 98% yield.

Elongation of the peptide chain was carried out using Fmoc chemistry (Scheme 3). The Fmoc group was removed from **8** with 20% piperidine in DMF. Couplings were performed with 5 equiv of amino acid/HBTU/HOBt and 10 equiv of diisopropylethylamine in DMF for 2 h. Palmitoylated cysteine was coupled using *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methyl methanaminium hexafluorophosphate N-oxide (HBTU)/1-hydroxybenzotriazole (HOBt)/trimethylpyridine in CH_2Cl_2/DMF (1:1) in order to avoid racemization[9] to give immobilized lipidated peptide **10**.

In the presence of the base sensitive palmitoyl moiety, piperidine cannot be employed any more for deprotection of the Fmoc-group. Furthermore, after removal of the Fmoc group from the N-terminal S-palmitoylated cysteine a rapid S→N acyl shift can occur.[10] Therefore, the stronger but nonnucleophilic hindered base 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU) was used. It allows a fast Fmoc-deprotection (2×30 s) which, followed by immediate addition of a preactivated coupling cocktail



Scheme 2. Synthesis of farnesylated cysteine building block **6** and attachment to the solid support. Reagents and conditions: (a) allylchloroacetate K_2CO_3, KI, acetone; (b) FmocCys(Far)OH (**4**), DCC, DMAP, CH_2Cl_2; (c) $Pd(PPh_3)_4$ (5 mol %), $PhSiH_3$, THF/CH_3OH; (d) HATU (1.2 equiv), DIPEA (2.4 equiv), DMF, 24 h.



Scheme 3. Solid phase synthesis of the farnesylated and palmitoylated peptide **12**. Reagents and conditions: (a) 20% piperidine in DMF; (b) 5 equiv Fmoc-AA-OH, 5 equiv HBTU/HOBt, 10 equiv DIPEA, DMF, 2 h (repeat for assembly of the peptide); (c) 4 equiv Fmoc-Cys(Pal)-OH, 4 equiv HBTU/HOBt/TMP, CH_2Cl_2/DMF (1:1), 4 h; (d) 1% DBU in DMF, 30 s (×2); (e) 5 equiv Fmoc-Gly-OH, 5 equiv HATU, 20 equiv DIPEA, CH_2Cl_2/DMF (7:1), 2 h; (f) 2 equiv TBAF·$3H_2O$, CH_2Cl_2, 25 min (×2); DCC: *N*,*N*′-dicyclohexylcarbodiimide; DMAP: 4-dimethylaminopyridine.

consisting of 5 equiv Fmoc-Gly-OH/HATU and 20 equiv DIPEA in the nonpolar mixture CH_2Cl_2/DMF (7:1), avoids the *S*,*N*-shift of the palmitoyl group.[4c,d]

TBAF·$3H_2O$ is a strong base in polar, aprotic solvents such as DMF, NMP or THF but is an only weak, almost neutral base in CH_2Cl_2. This property had already secured its applicability to glycopeptide synthesis[6] and it also enables its use in the presence of the base-sensitive palmitoyl thioester. The doubly lipidated peptide **12** was released from the solid support by treatment with 2 equiv TBAF·$3H_2O$ in CH_2Cl_2 twice for 25 min each. The CH_2Cl_2 solution was extracted with water to remove the remaining fluoride, dried over $MgSO_4$ and concentrated in vacuo and the heptapeptide **12** was obtained in 70% yield without further purification.

In order to demonstrate the scope of the method, different lipidated peptides were synthesized (Fig. 1). In all the cases, the mono- or dilipidated peptides were obtained in excellent yields. The successful synthesis of peptide **15** with a free amino group at the N-terminus is remarkable. Application of the hydrazide linker allowed only deprotection of N-protected peptides, due to the complexation of the copper salts used for its oxidative cleavage with free amino functions.

The new methodology also gives access to labeled peptides carrying a fluorescent group (**14** and **18**) and/or a maleimido group for further ligation to proteins at the N-terminus (**17** and **18**).

In conclusion the PTMSEL linker offers new and advantageous opportunities for the solid-phase synthesis of differently lipidated peptides. The release of the peptide from the solid support is efficient in the presence of TBAF·$3H_2O$ in CH_2Cl_2, that is, under almost neutral conditions. The cleavage conditions are so mild that acid- and base-labile lipid moieties are not attacked.

Acknowledgements

This research was supported by the Max-Planck-Gesellschaft and the Fonds der Chemischen Industrie. Jose M. Palomo would like to thank the European Molecular Biology Organization (EMBO), for a long term Fellowship and G. Kragol is grateful to the Humboldt Foundation for a Research Fellowship.

References and notes

1. (a) Hinterding, K.; Alonso-Diaz, D.; Waldmann, H. *Angew. Chem.* **1998**, *110*, 716; *Angew. Chem., Int. Ed.* **1998**, *37*, 688; (b) Wittinghofer, A.; Waldmann, H. *Angew. Chem.* **2000**, *112*, 4360; *Angew. Chem., Int. Ed.* **2000**, *39*, 4193.
2. (a) Bader, B.; Kuhn, K.; Owen, D. J.; Waldmann, H.; Wittinghofer, A.; Kuhlmann, J. *Nature* **2000**, *403*, 223; (b) Kuhlmann, J.; Tebbe, A.; Völkert, M.; Wagner, M.; Uwai, K.; Waldmann, H. *Angew. Chem.* **2002**, *114*, 2655; *Angew. Chem., Int. Ed.* **2002**, *41*, 2546; (c) Rak, A.; Pylypenko, O.; Durek, T.; Watzke, A.; Kushnir, S.; Brunsveld, L.; Waldmann, H.; Goody, R. S.; Alexandrov, K. *Science* **2003**, *302*, 646; (d) Rocks, O.; Peyker, A.; Kahms, M.; Verveer, P. J.; Koerner, C.; Lumbierres, M.; Kuhlmann, J.; Waldmann, H.; Wittinghofer, A.; Bastiaens, P. I. H. *Science* **2005**, *307*, 1746–1752.
3. Kadereit, D.; Kuhlmann, J.; Waldmann, H. *ChemBioChem* **2000**, *1*, 144.
4. (a) Ludolph, B.; Eisele, F.; Waldmann, H. *J. Am. Chem. Soc.* **2002**, *124*, 5954; (b) Ludolph, B.; Waldmann, H. *Chem. Eur. J.* **2003**, *9*, 3683; (c) Kragol, G.; Lumbierres, M.; Palomo, J. M.; Waldmann, H. *Angew. Chem.* **2004**, *116*, 5963; *Angew. Chem., Int. Ed.* **2004**, *43*, 5839; (d) Lumbierres, M.; Palomo, J. M.; Kragol, G.; Roehrs, S.; Müller, O.; Waldmann, H. *Chem. Eur. J.* **2005**, online.

5. Palomo, J. M.; Lumbierres, M.; Kragol, G.; Waldmann, H. *Angew. Chem., Int. Ed.*, in press.
6. (a) Wagner, M.; Kunz, H. *Angew. Chem.* **2002**, *114*, 315; *Angew. Chem., Int. Ed.* **2002**, *41*, 317; (b) Wagner, M.; Dziadek, S.; Horst, H. *Chem. Eur. J.* **2003**, *9*, 6018.
7. (a) Ramage, R.; Barron, C. A.; Bielecki, S.; Thomas, D. W. *Tetrahedron Lett.* **1987**, *28*, 4105; (b) Mullen, D. G.; Barany, G. *J. Org. Chem.* **1988**, *53*, 5240; (c) Chao, H.-G.; Bernatowicz, M. S.; Reiss, P. D.; Klimas, C. E.; Matsueda, G. R. *J. Am. Chem. Soc.* **1994**, *116*, 1746.
8. (a) Friedrich-Bochnitschek, S.; Waldmann, H.; Kunz, H. *J. Org. Chem.* **1989**, *54*, 751–756; Reviews: (b) Guibé, F. *Tetrahedron* **1997**, *53*, 13509–13556; Guibé, F. *Tetrahedron* **1998**, *54*, 2967–3042; (c) Schmittberger, T.; Waldmann, H. *Bioorg. Med. Chem.* **1999**, *7*, 749–762.
9. Angell, Y. M.; Alsina, J.; Albericio, F.; Barany, G. *J. Pept. Res.* **2002**, *60*, 292–299.
10. Trudelle, Y.; Caille, A. *Int. J. Pept. Prot. Res.* **1977**, *10*, 291–298.