Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 179–182

TETRAHEDRON LETTERS

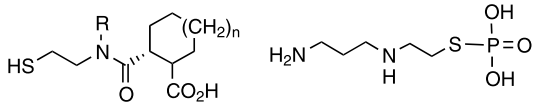
Resin-bound aminothiols: synthesis and application

Spyros Mourtas, Christina Katakalou, Andriana Nicolettou, Chryssoula Tzavara, Dimitrios Gatos and Kleomenis Barlos*

Department of Chemistry, University of Patras, Patras, Greece

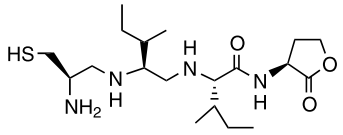
Received 22 June 2002; revised 15 October 2002; accepted 24 October 2002

Abstract—Aminothiols were attached through their thiol group onto the 4,4′-dimethoxytrityl (Dmt)-, 4-methoxytrityl (Mmt)-, 4-methyltrityl (Mtt)-, trityl (Trt)- and 2-chlorotrityl (Clt)-resins. The new resins were used in the solid-phase synthesis of aminothiol containing peptides utilizing *N*-Fmoc amino acids. The synthesized peptides were cleaved from the resins by treatment with trifluoroacetic acid (TFA) solutions using triethylsilane (TES) or ethanedithiol (EDT) as scavengers. © 2002 Published by Elsevier Science Ltd.



1

2



3

Aminothiols are constituents of important biologically active compounds such as coenzyme A. In addition, they are contained in the antihypertensives **1**,[1] the wide range cytoprotectant amifostine (ethyol, **2**)[2–4] and the antitumor activity revealing Ras protein farnesyltransferase inhibitors, such as **3**.[5,6] For the development of new lead structures using solid-phase combinatorial methods, suitable resin-bound aminothiol derivatives are required. For their synthesis we applied commercially available resins of the trityl-type.[7–9]

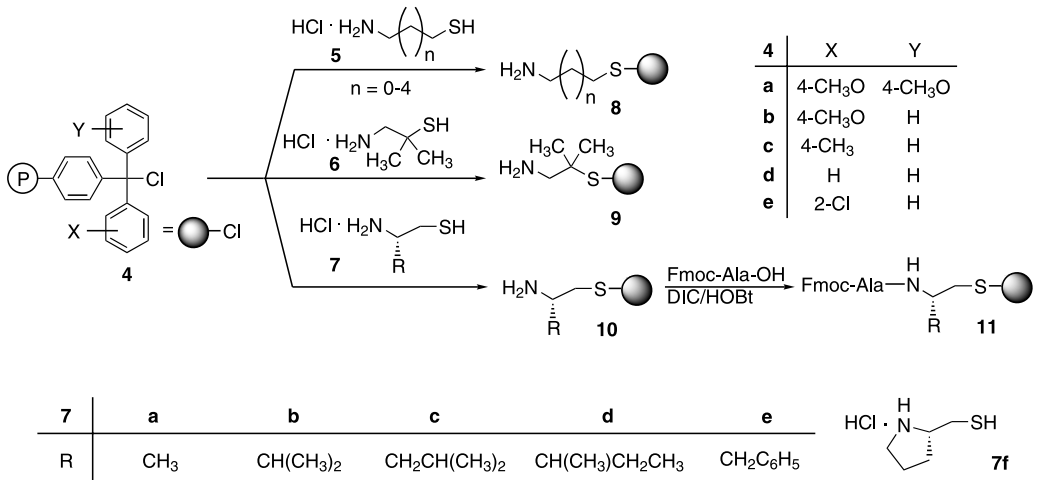
In fact, the reaction of the trityl resins **4** (polystyrene based polymers, 100–200 and 200–400 mesh, crosslinked with 1% divinylbenzene and loaded as follows: **4a** = 1.63; **4b** = 1.92; **4c** = 1.48; **4d** = 1.81; **4e** = 1.74 mmol Cl^-/g resin) with a two-fold molar excess of the linear aminothiols **5** or aminothiols derived from naturally occurring amino acids **7**[10] (Scheme 1) and diisopropylethylamine (DIPEA) in dichloromethane/dimethylformamide (DCM/DMF) (1:1) for 1–5 h at rt, led to resins loaded with 0.5–1.0 mmol aminothiol/g resin. Highest reaction rates were observed in the case of the Dmt- and Mmt-resins **4a**,**b** and lowest for **4e**. The increased reactivity of the electron rich Dmt- and Mmt-resins was important for the attachment of the steric hindered penicillamine-derived thiol **6**. In this case only **9a**,**b** were obtained with a loading of >0.2 mmol thiol/g resin. To ensure that **5**–**7** were not attached to the resins through their amino group, resins **8**–**10** were treated with a mixture of acetic acid (AcOH)/trifluoroethanol (TFE)/DCM (1:2:7) for 30 min at rt. This mixture cleaves the amino–trityl bond effectively. Unreacted remaining trityl chlorides were converted to the corresponding inert tritylmethyl ethers by washing resins **8**–**10** with DCM/methanol (MeOH)/DIPEA (80:15:5).

Under the conditions used for the attachment of the free aminothiols **5**–**7**, the phthalimidothiols **12**, *N*-Fmoc-aminothiols **13**, *N*-Trt-aminothiols **14** and the derived from naturally occurring L-amino acids (Ala, Val, Leu, Ile, Pro, Phe) *N*-Trt-aminothiols **18**,[10] were also attached in 75–85% yield to the resins **4** (Scheme 2).

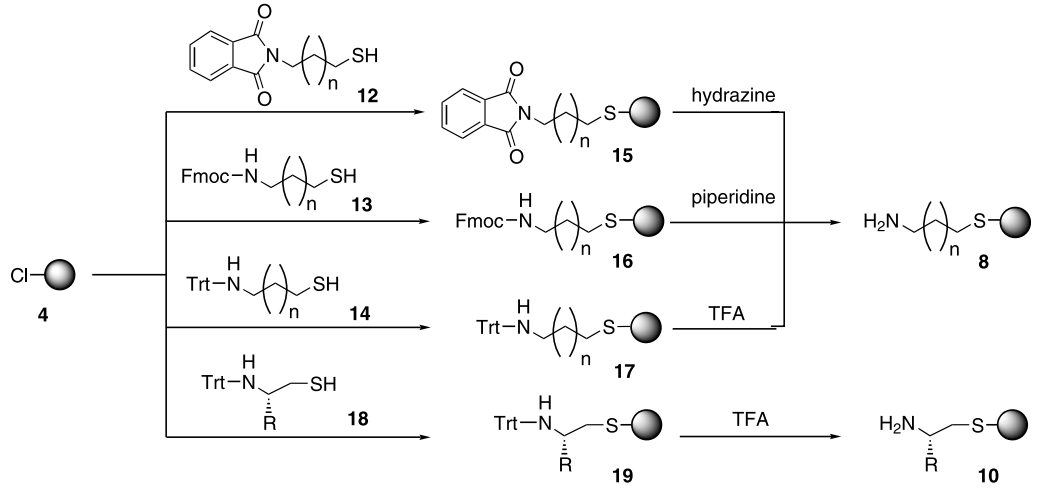
The removal of the phthaloyl group in **15** was performed with 15% hydrazine in DMF/MeOH (8:2) for 0.5–1 h. *N*-Fmoc deprotection of **16** was effected by a 2×15 min treatment with 25% piperidine in DMF. Complete and selective *N*-detritylation of the resin-bound aminothiols **17** and **19** was achieved by treating the Dmt- and Mmt-derivatives with AcOH/TFE/DCM

* Corresponding author.

0040-4039/03/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0040-4039(02)02445-0



Scheme 1.



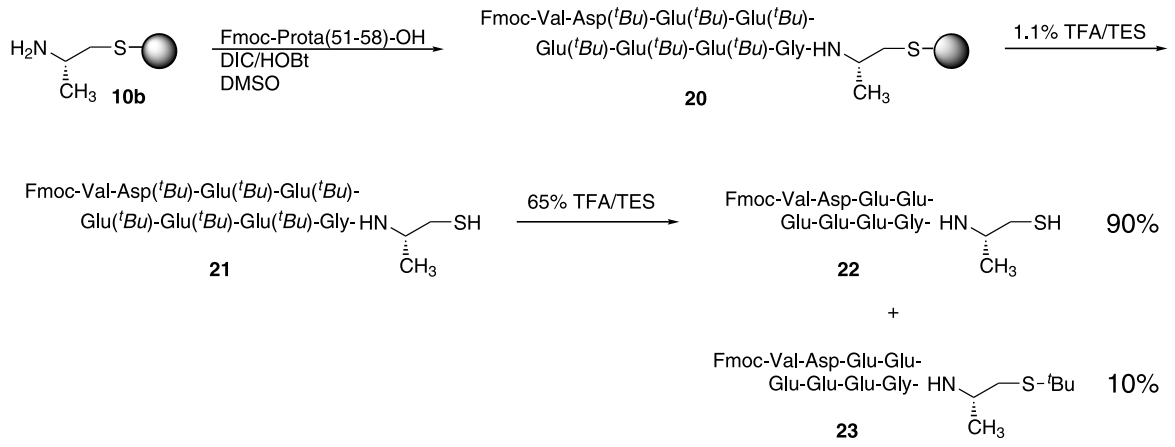
Scheme 2. n = 0–4.

(1:2:7) for 4×15 min at rt, while the Trt-, Mtt- and Clt-derivatives were treated with 1% TFA in DCM for 3×4 min. The obtained resin-bound aminothiols were then converted to their free amino form by washing the resins with DCM/MeOH/DIPEA (80:15:5).

To determine the acid sensitivity of the thioether bond of aminothiols to the various resins, **10** were coupled with Fmoc-alanine, using diisopropylcarbodiimide (DIC)/1-hydroxybenzotriazole (HOBt) as condensing agent (Scheme 1). The obtained resins **11** were then treated with solutions of various concentrations of TFA in DCM. As expected, the acid sensitivity of the aminothiol-resin bond increases in the order Clt-<Trt-<Mtt-<Mmt-<Dmt-resin. Complete cleavage of the aminothiols from the resins **11a–e** was effected by a 4×3 min treatment with 0.5, 1.1, 10, 30 and 65% TFA, respectively, in a 95:5 mixture of DCM/TES or DCM/EDT.

To examine the utility of the various resin-bound aminothiols in solid-phase synthesis, we coupled them with *N*-Fmoc amino acids using DIC/HOBt for their activation. The *N*-Fmoc protecting group was removed by treatment with 20% piperidine in DMF for 2×15 min at rt. The aminothiol peptides were cleaved from the resins **4c**,**e** with simultaneous removal of the *tert*-butyl-type protecting groups, or in the protected form using resins **4a**,**b**. As an example, resin-bound alaninothiol **10b** was coupled in DMSO, with an *N*-Fmoc protected fragment (51–58) of prothimosine α (ProTα) activated with DIC/HOBt (Scheme 3). Peptide **21** was obtained in 92% yield and 98% purity, according to HPLC analysis (Fig. 1a) after a 4×3 min treatment of **20** with 1.1% TFA in DCM/TES (95:5) and precipitation from ether. Treatment of **21** with 65% TFA in DCM/TES (95:5) for 2 h at rt, gave the fully deprotected alaninothiol peptide **22** (A; Fig. 1b) in 94% yield. As a by-product the *S*-^{t}Bu-alaninothiol peptide **23** (B; Fig. 1b) was detected in 10% ratio, due to attack of the ^{t}Bu cations formed during the deprotection.[7] The exact molecular weight of **21**, **22** and of the *S*-^{t}Bu peptide **23** (Fig. 1c) were determined by ES-MS.

Using resins **4a**,**b** and *N*-Fmoc amino acids side-chain protected with very acid-labile groups, such as: Cys-



Scheme 3.

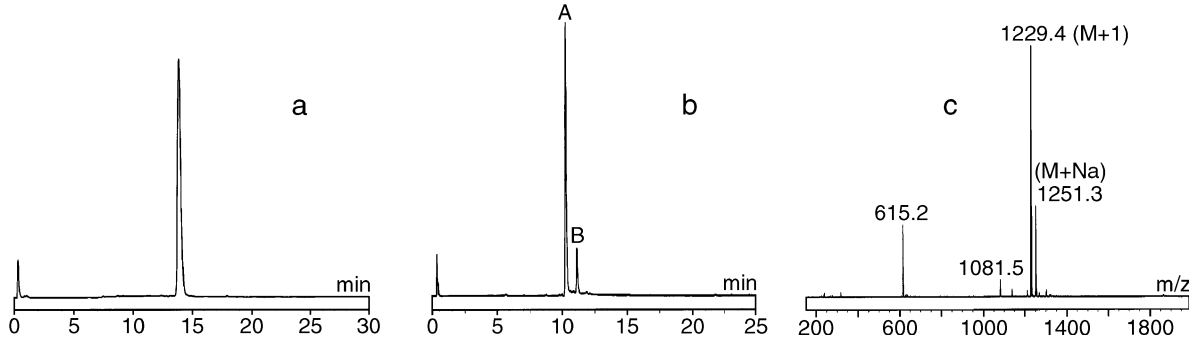
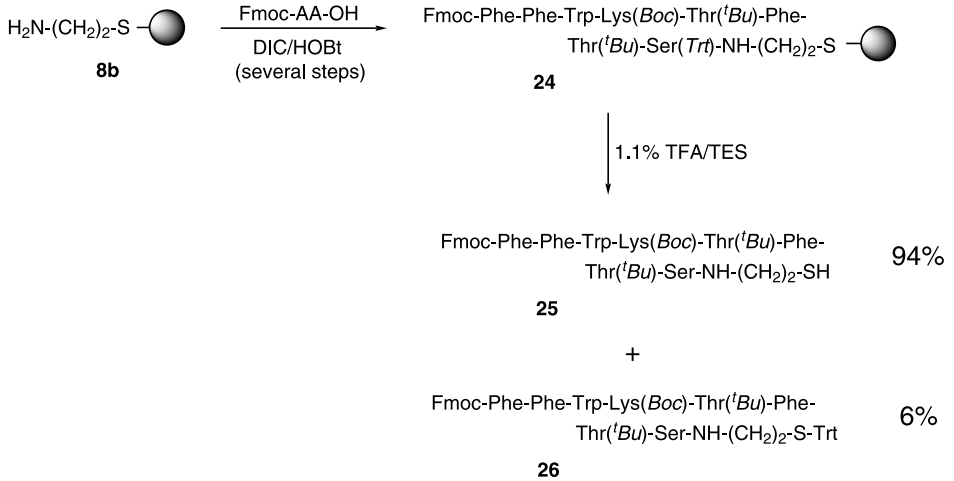


Figure 1. (a) Analytical HPLC of **21**; Column: Zorbax SB-C18, 3.5 μm, 2.1×30 mm; flow rate: 0.4 ml/min; gradient: from 50 to 100% acetonitrile in water within 20 min; detection at 265 nm. (b) Analytical HPLC of the mixture obtained after treatment of 21 with 65% TFA/TES; Column: Zorbax SB-C18, 3.5 μm, 2.1×30 mm; flow rate: 0.4 ml/min; gradient: from 0 to 100% acetonitrile in water within 20 min; detection at 265 nm. (c) ES-MS of purified ^{t}Bu-alaninothiol derivative **23**.

(Mmt)-, Ser(Trt)-, Thr(Trt)-, Tyr(Clt)-, His(Mmt)- and Lys(Mtt)-, the cleavage of the aminothiol peptides from the resins occurs concurrently with the removal of these protecting groups. In all cases tested the crude peptides were obtained in high purity according to HPLC analysis and were identified by ES-MS. In one example, we synthesized the cysteamine nona-peptide **24**, which contains a Ser(Trt)- and several ^{t}Bu-protected amino acids (Scheme 4). The peptide chain of **24** was assembled on resin **8b**, using DIC/HOBt for the activation of the applied *N*-Fmoc amino acids. Treatment of resin **24** with 1.1% TFA in DCM/TES for 4×3 min at rt, afforded the partially deprotected peptide **25** in 88% yield. Besides **25** (A; Fig. 2a), HPLC analysis also



Scheme 4.

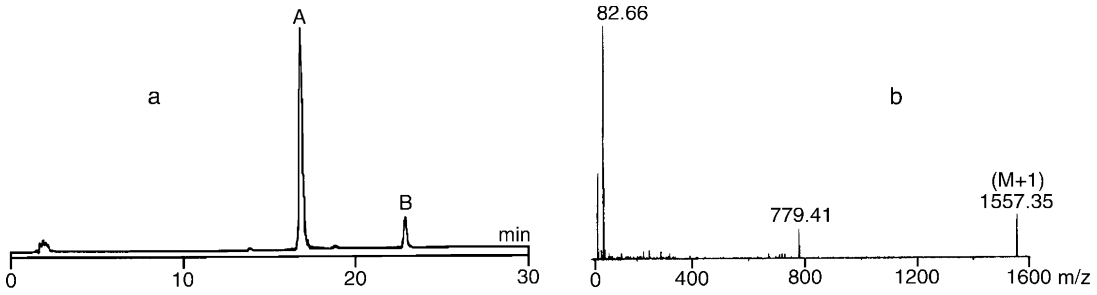


Figure 2. (a) Analytical HPLC of the mixture obtained after treatment of 24 with 1.1% TFA/TES; Column: Lichrospher RP-8, 5 μm, 4×150 mm; flow rate: 1 ml/min; gradient: from 50 to 100% acetonitrile in water within 30 min; detection at 265 nm. (b) ES-MS of purified *S-Trt*-cysteamine derivative **26**.

showed the formation of the *S*-Trt-cysteamine peptide **26** (B; Fig. 2a), in a 6% ratio, due to migration of the Trt-group[11] during the acidic treatment of **24**. Peptide **25** and the corresponding *S*-Trt-cysteamine derivative **26** were identified by ES-MS analysis (Fig. 2b). No reduction of Trp was observed under these cleavage conditions.

Acknowledgements

The authors acknowledge CBL Patras S.A. for financial support.

References

1. Turbanti, L.; Cerbai, G.; Di Bugno, C.; Giorgi, R.; Garzelli, G.; Criscuoli, M.; Renzetti, A. R.; Subissi, A.; Bramante, G. *J. Med. Chem.* **1993**, *36*, 699–707.
2. Griggs, J. J. *Leuk. Res.* **1998**, *22*, 27–33 (Review).
3. Kurbacher, C. M.; Mallmann, P. K. *Anticancer Res.* **1998**, *18*, 2203–2210 (Review).
4. Douay, L. *Bone Marrow Transplant.* **1998**, *22*, 423–430 (Review).
5. Graham, S. L.; deSolms, S. J.; Giuliani, E. A.; Kohl, N. E.; Mosser, S. D.; Oliff, A. I.; Pompliano, D. L.; Rands, E.; Breslin, M. J.; Deana, A. A.; Garsky, W. M.; Scholz, T. H.; Gibbs, J. B.; Smith, R. L. *J. Med. Chem.* **1994**, *37*, 725–732.
6. deSolms, S. J.; Giuliani, E. A.; Graham, S. L.; Koblan, K. S.; Kohl, N. E.; Mosser, S. D.; Oliff, A. I.; Pompliano, D. L.; Rands, E.; Scholz, T. H.; Wiscount, C. M.; Gibbs, J. B.; Smith, R. L. *J. Med. Chem.* **1998**, *41*, 2651–2656.
7. Barlos, K.; Gatos, D.; Hatzi, O.; Koch, N.; Koutsogianni, S. *Int. J. Pept. Protein Res.* **1996**, *47*, 148–153.
8. Barlos, K.; Gatos, D.; Kallitsis, J.; Papaphotiou, G.; Sotiriou, P.; Wenqing, Y.; Schäfer, W. *Tetrahedron Lett.* **1989**, *30*, 3943–3946.
9. Barlos, K.; Gatos, D.; Chatzi, O. In *Peptides 1992*, (Proceedings of the 22nd European Peptide Symposium), Schneider, C. H.; Eberle, A. N., Eds.; ESCOM, Leiden, 1993, pp. 281–282.
10. Mourtas, S.; Karavoltsos, M.; Kalaitzi, V.; Katacalou, C.; Gatos, D.; Barlos, K. In *Peptides 2000*, (Proceedings of the 26th European Peptide Symposium), Martinez, J.; Fehrentz, J.-A., Eds.; EDK, Paris, France, 2001; pp. 257–258.
11. Barlos, K.; Gatos, D.; Mourtas, S.; Nicolettou, A.; Karavoltsos, M.; Bali, D. In *Peptides for the New Millenium*, (Proceedings of the 16th American Peptide Symposium), Fields, G. B.; Tam, J. P.; Barany, G., Eds.; Kluwer Academic Publishers: Dordrecht, 2000; pp. 198–199.