Tetrahedron 65 (2009) 844–848

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis and application in SPPS of a stable amino acid isostere of palmitoyl cysteine

Elena Cini [a], Lucia Raffaella Lampariello [b], Manuela Rodriquez [c], Maurizio Taddei [a,*]

[a] Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Siena, Via A. Moro 2, 53100 Siena, Italy
[b] Dipartimento di Chimica, Università degli Studi di Siena, Via A. Moro 2, 53100 Siena, Italy
[c] Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, Via Ponte don Melillo, 84084 Fisciano, Salerno, Italy

ARTICLE INFO

Article history:
Received 5 September 2008
Received in revised form 30 October 2008
Accepted 13 November 2008
Available online 18 November 2008

Keywords:
Amino acid
Microwaves
Hydrogenation
Peptidomimetics
Solid-phase synthesis

ABSTRACT

(*S*)-2-Amino-4-(2-pentadecyl-1,3-dioxolan-2-yl)-butanoic acid, **11**, Pdiob, a synthetic analogue *C*-isostere of palmitoylated cysteine, has been prepared starting from tetrabenzyl glutamic acid. The less hindered benzyl carboxylate ester was transformed into the corresponding β-ketophosphonate and subjected to a Horner–Wadsworth–Emmons reaction followed by hydrogenation/hydrogenolysis. This product was used for the preparation on solid phase and under microwave dielectric heating of a highly lipidated peptide that can be considered as an acid stable analogue of the C-terminus of the *H*-Ras heptapeptides 180–186.

1. Introduction

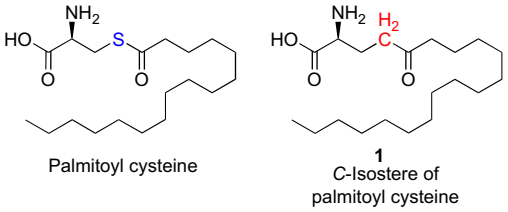
Covalent lipid modified proteins play a strategic role in many signal transduction processes involving cell membrane-anchorage mechanism. The high hydrophobicity, required for a proper subcellular localization of such proteins, allows favourable interactions with membranes, controls the active conformation of proteins within a membrane layer and is involved in the switch on/off of effective signal transduction.[1] Post-translational linkage of long fatty acyl chains on target proteins occurs through a thioetherification and thioesterification processes on specific cysteine residues (S-palmitoylation and S-farnesylation)[2] or amidation of lysine residues (N-myristoylation).[3] A variety of transmembrane protein and heterodimeric G-proteins and many other important signalling proteins are lipidated[4,5] and, based on structural elements adjacent to the palmitoylation site, they can be divided into N-terminal myristoyl class and C-terminal farnesyl class, which includes *N*-, *H*- and *K*-Ras proteins. As the Ras pathway controls cell growth and proliferation, when deregulation happens, uncontrolled cellular growth and cancer may occur. S-Farnesylation of Ras cysteines provides an irreversible modification that drives the lipidated proteins to the plasma membrane inducing the proteolysis of the three last amino acids followed by the carbomethylation of the new C-terminal Cys. The Ras carboxyl-terminal CAAX box (C is cysteine, A is an aliphatic amino acid and X is usually Met or Ser) is recognized by another transferase enzyme (palmitoyl acyltransferase (PAT) in H-Ras, for example), which brings about S-palmitoylation of cysteine. The latter is a reversible process involved in the steering of biological events like regulated membrane trapping mechanism, suggesting its importance in regulating protein role.[6]

Although many attempts have been made to investigate the interactions of these proteins with the enzyme, the demand of new peptide analogues is still high to enhance our understanding of these phenomena.[7] Moreover, depending on the nature of the lipid motif, several problems have been encountered in some synthetic approaches. The solid-phase synthesis of characteristic lipidated Ras peptides containing both farnesylated and palmitoylated cysteine may be complicated by the acid and base sensitivity of these groups.[8] Consequently, several methods that employ particular protections or synthetic strategies different from standard solid-phase peptide synthesis have been successfully developed.[9] Most of these syntheses follow an Fmoc protocol adapted in order to introduce the palmitoylated amino acid (or carry out palmitolylation) in one of the final steps. However, the instability of the final peptide remains a potential issue with the risk of lipid loss during purification work-up or during biological evaluation. In continuation of

* Corresponding author. Tel.: +39 0577234280; fax: +39 0577234275.
E-mail address: taddei.m@unisi.it (M. Taddei).

0040-4020/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2008.11.033

our work in peptidomimetics and modified or not-coded α-amino acids,[10] we considered that the *C*-isostere of palmitoylated cysteine **1** (Scheme 1) could behave as an effective and stable surrogate of the original post-translational modified amino acid.

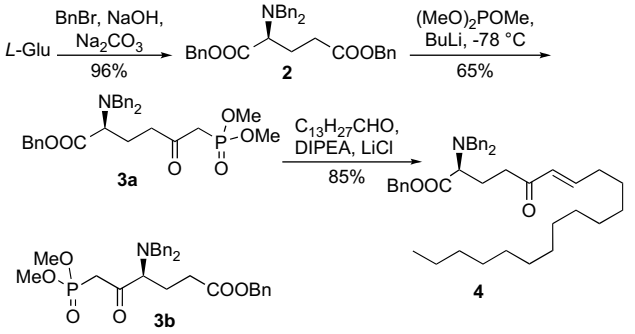


Scheme 1. Comparison between palmitoyl cysteine and *C*-isostere **1**.

Moreover, due to the presence of the carbonyl group, **1** could be considered as a transition state analogue of natural *S*-palmitoylated cysteine for potential inhibition of acyl-protein thioesterase enzyme. The *N*-Fmoc protected derivative of **1** could be also suitably employed in standard solid-phase synthesis of stable lipidated peptides as the final C-terminus of *N*-Ras protein.

2. Results and discussion

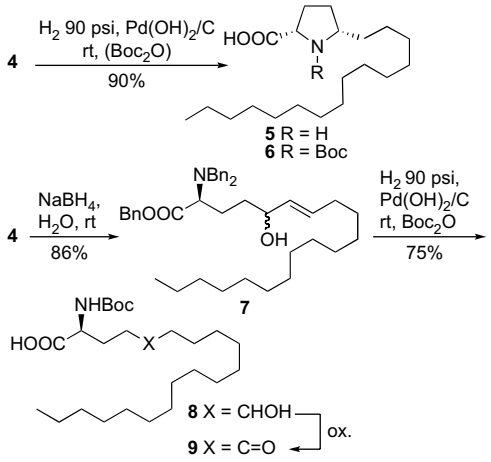
The synthesis of **1** was approached starting from L-glutamic acid that was fully protected as the tetrabenzyl derivative **2** as recently reported in the literature.[11] This product was then treated with dimethyl methylphosphonate in the presence of BuLi at −78 °C in THF. We hypothesized that the hindrance around the α-carboxylic group should direct the nucleophile towards the other carboxylic group to produce compound **3** (Scheme 2).



Scheme 2. Synthesis of **4**.

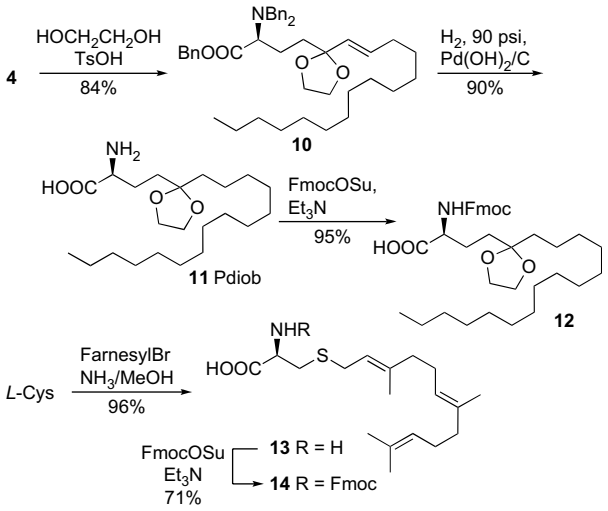
First attempt carried out with $(MeO)_2POMe$ and BuLi in THF gave a mixture of the two regioisomers **3a** and **3b** in a 1:1 ratio together with some starting material. Changing the base (LDA, $KN(SiMe_3)_2$, *i*-PrMgBr) did not improve the formation of the required isomer. The best result was achieved by slow addition of the tetrabenzyl glutamic acid **2** to a solution of the lithium phosphonate in THF at −78 °C and doing the aqueous work-up at this temperature after 4 h of stirring. Thus **3a** was obtained in 65% isolated yields after flash chromatography that is required to separate it from 20% of **3b**.

Horner–Wadsworth–Emmons reaction of **3a** under anhydrous conditions in MeCN using LiCl and DIPEA with tetradecanal[12] gave unsaturated ketone **4** in 85% yield. Compound **4** was then subjected to H_2 under Pd catalysis in order to get simultaneous removal of the benzyl protections and reduction of the double bond. During this step, 5-pentadecyl proline **5** was obtained in good yield (Scheme 3). The free NH_2 (or the intermediate NHBn group) reacted with the carbonyl forming an imine that was further reduced to **5**. Cyclization also occurred under neutral or alkaline conditions (MeOH/Na_2CO_3) or in the presence of Boc_2O introduced with the aim to protect the NH_2 before imine formation. In this last case, only compound **6** was obtained as a single *cis* diastereoisomers as revealed by ^1H NMR and NOE spectroscopic analysis.



Scheme 3. Attempts to obtain *N*-Fmoc *C*-isostere of palmitoyl cysteine directly from **4**.

The carbonyl group of **4** was then reduced to the alcohol with $NaBH_4$ in H_2O to give compound **7** in good yield. Further hydrogenolysis (H_2 90 psi, rt, MeOH, 24 h) in the presence of Boc_2O gave alcohol **8** on which oxidation to **9** was immediately attempted. However, Dess–Martin periodinane, Swern oxidation or Cr(VI) reagents did not give acceptable results. As the carbonyl protection could be useful also during peptide synthesis, **4** was transformed into dioxolane **10** (Scheme 4) using ethylene glycol and TsOH (toluene, Dean–Stark apparatus, 84%).



Scheme 4. Synthesis of **12** and **14**.

Amine **10** was deprotected (H_2 90 psi, $Pd(OH)_2/C$, rt, MeOH, 24 h) to give the free amino acid, (*S*)-2-amino-4-(2-pentadecyl-1,3-dioxolan-2-yl)-butanoic acid, **11** (Pdiob). Introduction of the Fmoc gave **12** in 72% overall yield.[13] At the same time, the *N*-Fmoc farnesyl cysteine **14** was also prepared through a direct alkylation of free cysteine with farnesyl bromide in $NH_3/MeOH$[14] followed by standard introduction of the Fmoc protection with FmocOSu. Following this procedure, **14** was isolated in 68% overall yield.

With compounds **12** and **14** in hand, we approached the solid-phase synthesis of an isosteric C-terminus (residues 180–186) of

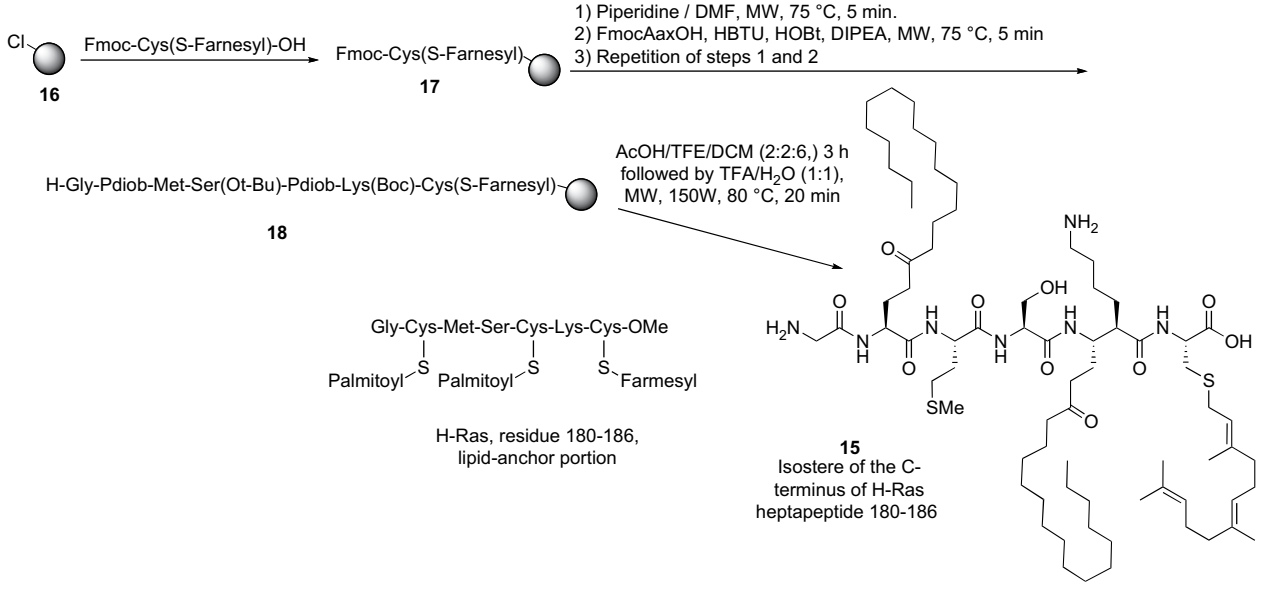
the lipidated human *H*-Ras peptide.[15] *H*-Ras is a small protein that is active when the binding to membranes is achieved by farnesylation at Cys186 followed by reversible palmitoylation at Cys184 and Cys18.[16] The synthetic availability of mimetic peptides, which are palmitoylated as well as farnesylated, should be useful to better characterize the activities of key enzymes such as farnesyl protein transferase or PAT and their biochemical regulation.[17] Moreover, it has been demonstrated that inhibition of farnesyl protein transferase by Ras carboxyl-terminal CAAX box analogues results in anticancer effect.[18]

The synthesis of **15** was attempted by following typical solid-phase peptide chemistry Fmoc protocol using a 2-chlorotrityl chloride-PS resin as the support and HBTU/HOBT as the coupling agent. Unfortunately, after cleavage from the resin with AcOH, peptide **15** was obtained in low yields as a mixture with several other compounds as shown by HPLC/MS analysis. In order to increase the efficacy of the synthesis, we tried to carry out the protocol under microwave dielectric heating.[19] Using a microwave oven dedicated to peptide synthesis (Liberty from CEM)[20] Fmoc-Cys(*S*-Farnesyl)-OH **14** was loaded on the resin in DMF with a double coupling protocol at 23 W and 75 °C. Fmoc deprotection and HBTU/HOBT mediated coupling with Fmoc-Lys(Boc)-OH were carried out in 5 min at 23 W and 75 °C. Further couplings were carried out with Fmoc-Pdiob-OH **12**, Fmoc-Ser(O-*t*-Bu)-OH, Fmoc-Met-OH and FmocGly-OH (staggered by piperidine/DMF deprotection) under the same reaction conditions.

This procedure yielded the highly lipidated peptide **18** on the resin without any particular difficulty, as pointed out by the Kaiser tests. The fully protected precursor **18** was then cleaved from the resin using AcOH/TFE/DCM for 3 h. With this standard cleavage protocol, we obtained a mixture of **15** together with other species carrying some of the side protections employed during the synthesis. In order to get a complete removal of the protection, the residue obtained from the resin cleavage was treated with TFA/DMSO/H_2O 1:1:1 for 48 h. Alternatively, a more rapid approach was realized reacting the product coming from the cleavage with TFA/H_2O 1:1 under microwave dielectric heating for 20 min at 80 °C (150 W of power). Pure compound **15** was obtained in 30% overall yield (Scheme 5). The analytical data registered on the synthetic sample confirmed the formation of the expected isostere of the *H*-Ras lipid-anchor portion with the unprotected carbonyl groups (Fig. 1). Moreover, the formation of a single compound at HPLC analysis pointed out that Fmoc-amino acids **12** and **14** were obtained without racemization.

3. Conclusions

In conclusion, we have developed the synthesis of a new kind of a lipophilic amino acid that is a structural analogue to palmitoyl cysteine and that can be used for the synthesis of highly lipidated peptides without particular precautions. This synthetic approach can be extended in principle to the synthesis of different other long



Scheme 5. Solid-phase synthesis of the *H*-Ras heptapeptide isostere.

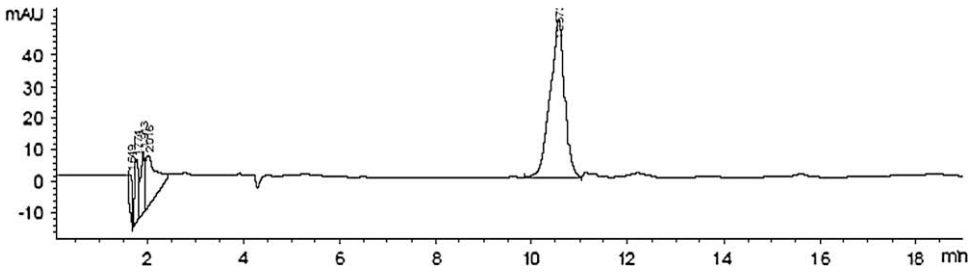


Figure 1. HPLC graph of compound **15** prepared under microwave dielectric heating (for HPLC conditions see Section 4).

chain chiral α-amino acids that can be considered as useful tools in drug delivery.[21]

4. Experimental

4.1. (*S*)-2-Dibenzylamino-6-(dimethoxy-phosphoryl)-5-oxo-hexanoic acid benzyl ester (3a)

To a solution of dimethyl methylphoshonate (0.191 g, 1.54 mmol) in dry THF (17 mL) cooled at −78 °C, BuLi (0.62 mL of a 2.5 M solution in hexane) was added dropwise. After 40 min, a solution of tetrabenzyl glutamic acid (0.390 g, 7.7 mmol) in dry THF (14 mL) was added. After stirring for 4 h at −78 °C, a saturated aqueous solution of NH_4Cl (6 mL) was added followed by ethyl acetate (30 mL). After warming to rt, the organic phase was separated. The aqueous phase was extracted with EtOAc, all the organic fractions collected and dried over Na_2SO_4. The solvent was removed in vacuo and the crude mixture was purified by column chromatography (petroleum ether/EtOAc 1:3) to give compound **3a** as colourless oil (0.26 g, 65% yield). ν_{max} (Neat) 3012, 2990, 2980, 1745, 1710. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=1.95–2.02 (m, 2H, H-3), 2.41–2.78 (m, 2H, H-4), 2.79–2.97 (m, 2H, H-6), 3.29 (t, J=6.8, 1H, H-2), 3.67 (AB system, J=7.0, 4H, NCH_2Ph), 3.68 (d, J=2.8, 3H, OCH_3), 3.71 (d, J=2.8, 3H, OCH_3), 5.19 (AB system, J=7.9, 2H, OCH_2Ph) 7.19–7.36 (m, 15H, Ar–H). ^{13}C NMR (50 MHz, $CDCl_3$): δ=22.6, 40.0, 40.4, 42.5, 52.8, 53.0, 54.4, 59.8, 66.1, 127.1 54.5, 59.8, 66.2, 127.1, 128.2, 128.3, 128.5, 128.6, 128.9, 135.9(2C), 139.21, 172.13, 200.7, 200.8. HRMS (ES) calcd for $C_{29}H_{35}NO_6P$ $(M+H)^+$: 524.2202, found: 524.2180.

4.2. (*S*,*E*)-2-Dibenzylamino-5-oxo-6-icosenoic acid benzyl ester (4)

To a solution of phosphonate **3a** (0.900 g, 1.7 mmol) in dry MeCN (18 mL), dry LiCl (72.0 mg, 1.7 mmol) was added followed by freshly distilled DIPEA (180 mg, 239 μL, 1.4 mmol). After stirring for 2 h at rt, tetradecanal (0.276 g, 1.3 mmol) in MeCN was added and the mixture was stirred at rt for 72 h. A saturated aqueous solution of NaCl was used for quenching and the organic layer was separated. The aqueous phase was extracted with EtOAc, all the organic fractions collected and dried over Na_2SO_4. The solvent was removed in vacuo and the crude mixture was purified by column chromatography (petroleum ether/EtOAc 5:1) to give compound **4** as colourless oil (0.673 g, 85% yield). ν_{max} (Neat) 3113, 3081, 2982, 2975, 1742, 1710–1690. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=0.91 (t, J=6.6, 3H, CH_3), 1.24–1.47 (m, 20H, 10-H, 11-H, 12-H, 13-H, 14-H, 15-H, 16-H, 17-H, 18-H, 19-H), 1.45 (m, 2H, 9-H), 2.07–2.19 (m, 4H, 8-H, 3-H), 2.41–2.69 (m, 2H, 4-H), 3.39 (dd, J=8.4, 6.2, 1H, 2-H), 3.73 (AB system, J=8, 4H, NCH_2Ph), 5.23 (AB system, J=8, 2H, OCH_2Ph), 6.00 (d, J=15.6, 1H, H-7), 6.71 (dt, J=15.6, 7.0, 1H, H-6), 7.22–7.43 (m, 15H, Ar–H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=14.0, 20.8, 22.6, 23.3, 28.0, 29.1, 29.2, 29.3, 29.4, 29.5, 31.8, 32.3, 36.3, 54.4, 60.1, 65.9, 126.9, 128.1, 128.2, 128.4, 128.4, 128.7, 130.1, 135.9, 139.2, 147.2, 172.2, 199.3. HRMS (ES) calcd for $C_{41}H_{55}NO_3Na$ $(M+Na)^+$: 632.4080, found: 632.4062.

4.3. (*S*,*E*)-2-Dibenzylamino-4-(2-pentadec-1-enyl-[1,3]-dioxolan-2-yl)-butanoic acid benzyl ester (10)

To a solution of **4** (0.500 g, 0.82 mmol) in benzene (10 mL), ethylene glycol (112 mg, 100 μL, 1.80 mmol) and *p*-TsOH (6 mg, 0.03 mmol) were added and the reaction mixture was heated at reflux for 12 h with a Dean–Stark apparatus for azeotropic water removal. Water was added and the organic layer was separated. The aqueous phase was extracted with Et_2O, all the organic fractions collected and dried over Na_2SO_4. The solvent was removed in vacuo and the crude mixture was purified by column chromatography (petroleum ether/EtOAc 20:1) to give compound **10** as a colourless oil (0.450 g, 84% yield). ν_{max} (Neat) 3080, 2983, 2865, 1732, 1643. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=0.91 (t, J=6.4, 3H, CH_3), 1.24–1.57 (m, 22H, 9-H, 10-H, 11-H, 12-H, 13-H, 14-H, 15-H, 16-H, 17-H, 18-H, 19-H), 1.84–1.99 (m, 4H, 3-H, 8-H), 2.18–2.34 (m, 2H, 4-H), 3.37 (t, J=6.4, 1H, 2-H), 3.72 (AB system, J=8, 4H, NCH_2Ph), 3.78–3.93 (m, 4H, OCH_2CH_2O), 5.22 (AB system, J=8, 2H, OCH_2Ph), 5.27–5.53 (m, 3H, 2-H, 6-H, 7-H), 7.22–7.41 (m, 15H, Ar–H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=14.1, 22.7, 22.5, 29.3, 29.4, 29.7, 32.0, 32.7, 33.5, 40.8, 54.4, 60.9, 64.9, 65.9, 111.1, 124.3, 127.1, 128.4, 128.8, 129.0, 134.4, 136.2, 139.6, 172.7. HRMS (ES) calcd for $C_{43}H_{59}NO_4Na$ $(M+H)^+$: 676.4342, found: 676.4310.

4.4. (*S*)-2-Amino-4-(2-pentadecyl-[1,3]dioxolan-2-yl)-butenoic acid (11)

To a solution of **10** (0.400 g, 0.61 mmol) in CH_2Cl_2/MeOH (1:9, 20 mL) in a bottle connected to a Parr apparatus for medium pressure hydrogenation, $Pd(OH)_2$ on C (20%) (17 mg) was added. The bottle was filled with H_2 at 6 atm and shaken at rt for 12 h. The bottle was degassed, the catalyst filtered (*attention: the residue Pd may be pyrophoric*) and washed several times with MeOH. The solvent was removed in vacuo to give compound **11** as a white gel (0.212 g, 90% yield). ν_{max} (Nujol) 3067–2901, 1660. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=0.79 (t, J=7.2, 3H, CH_3), 1.19–1.43 (m, 26H, 7-H, 8-H, 9-H, 10-H, 11-H, 12-H, 13-H, 14-H, 15-H, 16-H, 17-H, 18-H, 19-H), 1.46–1.54 (m, 2H, 3-H), 1.63–1.81 (m, 2H, 6-H), 1.82–2.04 (m, 2H, 4-H), 3.41–3.69 (m, 1H, H-2), 3.81–3.97 (m, 4H, OCH_2CH_2O). ^{13}C NMR (100 MHz, $CDCl_3$): δ=13.9, 22.6, 23.7, 24.4, 29.6, 31.8, 32.1, 37.0, 64.9, 110.9, 172.7. HRMS (ES) calcd for $C_{22}H_{44}NO_4$ $(M+H)^+$: 386.3270, found: 386.3264.

4.5. (*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-4-(2-pentadecyl-[1,3]dioxolan-2-yl)-butanoic acid (12)

To a solution of **11** (0.210 g, 0.54 mmol) in H_2O (6 mL), Et_3N (203 μL, 147 mg, 1.46 mmol) was added followed by FmocOSu (0.376 g, 0.70 mmol) in CH_3CN (6 mL). The mixture was stirred at rt for 30 min. HCl (0.5 M) was added until the mixture reached pH 3–4. The organic layer was separated and the aqueous phase was extracted with EtOAc, all the organic fractions collected and dried over Na_2SO_4. The solvent was removed in vacuo. The crude mixture was purified by column chromatography (CH_2Cl_2/MeOH 99:1) to give compound **12** as a colourless oil (0.314 g, 95% yield). HPLC analysis (Chiralpack IB, 0.46×15 cm, eleuent isocratic *i*-PrOH/hexane 25:75, adsorbance 254 nm): t_R=16.43 min, MS/ES $(M+H)^+$ 631, >95%; t_R=17.93 min, MS/ES $(M+H)^+$ 631, <5%. ν_{max} (Neat) 360, 3417, 3342, 1720, 1703, 1684. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=0.88 (t, J=7.2, 3H, CH_3), 1.15–1.48 (m, 26H, 7-H, 8-H, 9-H, 10-H, 11-H, 12-H, 13-H, 14-H, 15-H, 16-H, 17-H, 18-H, 19-H), 1.51–1.58 (m, 2H, 3-H), 1.59–2.43 (m, 4H, 4-H, 6-H), 3.90 (s, 4H, OCH_2CH_2O), 4.21 (t, J=6.8, 1H, 2-H), 4.38–4.49 (m, 3H, $OCOCH_2CH$, $OCOCH_2CH$), 5.79 (d, J=6.8, 1H, NH), 7.23–7.41 (m, 4H, Ar–H), 7.50–7.59 (m, 2H, Ar–H), 7.73 (d, J=7.2, 2H, Ar–H), 10.31 (br s, 1H, COOH). ^{13}C NMR (100 MHz, $CDCl_3$): δ=14.2, 22.7, 23.9, 26.3, 29.4, 29.7, 30.0, 32.0, 32.5, 37.4, 47.1, 53.8, 65.0, 67.2, 111.3, 120.0, 125.1, 127.1, 127.7, 141.3, 143.7, 176. HRMS (ES) calcd for $C_{37}H_{53}NO_6Na$ $(M+Na)^+$: 630.3771, found: 630.3762.

4.6. L-Farnesyl cysteine (13)

To a solution of cysteine (0.194 g, 1.60 mmol) in MeOH (5.2 mL) cooled to 0 °C, a solution of ammonia in MeOH (6.8 mL of a 7 M solution) was added followed by farnesyl bromide (0.228 g, 1.60 mmol). The mixture was stirred at 0 °C for 3 h and at rt for 1 h. The solvent was removed at reduced pressure and the residue was partitioned between 1-butanol and water. The butanol layer was

dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue was dissolved in MeOH and washed with hexane. The MeOH was removed under reduced pressure to give **13** as a white solid (0.500 g, 96%). Mp 145–146 °C (lit.[14] 148–150 °C). ν_{max} (KBr) 3428–2640, 1620–1542.^{1}H NMR ($CDCl_3$, 200 MHz): δ=1.57 (s, 6H, CH_3CCH_3), 1.64 (s, 3H, CH_3CCH_2), 1.72 (d, 2H, J=7.1, 3H, CH_3CCH_2), 1.89–2.20 (m, 8H, CH_2), 2.91–3.31 (m, 2H, 3-H), 3.49–3.88 (m, 3H, H-2, 1′-H), 4.71 (br s, 2H, NH_2), 4.99–5.11 (m, 2H, 6′-H, 10′-H), 5.12–5.34 (m, 1H, 2′-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=16.2, 16.3, 17.8, 26.0, 27.5, 27.8, 30.3, 33.6, 40.8, 40.9, 55.2, 121.0, 125.0, 125.3, 132.0, 136.2, 140.9, 172.5. ES-MS: m/z 324 $(M-H)^-$.

4.7. L-Fmoc farnesyl cysteine (14)

To a solution of **13** (100 mg, 0.31 mmol) in CH_2Cl_2 (1.2 mL), FmocOSu (114 mg, 0.34 mmol) was added followed by Et_3N (34 mg, 47 μL, 0.34 mmol). The mixture was stirred at rt for 2 h. HCl (1 M) was added until the mixture reached pH=1. The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2, all the organic fractions collected and dried over Na_2SO_4. The solvent was removed in vacuo and the crude mixture was purified by column chromatography ($CHCl_3$/MeOH 9:1) to give compound **14** as colourless oil (119 mg, 71%). ν_{max} (Neat) 3391, 3367, 2925, 1738, 1691. ^{1}H NMR ($CDCl_3$, 200 MHz): δ=1.57 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 1.61 (s, 3H, CH_3), 1.66 (s, 3H, CH_3), 1.90–2.18 (m, 8H, CH_2), 2.75–3.04 (m, 2H, 1-H), 3.21 (d, J=6.9, 2H, 1′-H), 4.19 (t, J=6.2, 1H, CH–Fmoc), 4.40 (d, J=6.2, 2H, CH_2–Fmoc), 4.49–4.74 (m, 1H, 2-H), 5.01–5.31 (m, 2H, 6′-H, 10′-H), 5.21 (t, J=6.9, 1H, 2′-H), 5.75 (br s, 1H, NH), 7.23–7.41 (m, 4H, Ar–H), 7.50–7.63 (m, 2H, Ar–H), 7.73 (d, J=7.2, 2H, Ar–H). ^{13}C NMR (50 MHz, $CDCl_3$): δ=15.9, 16.1, 17.6, 25.6, 26.4, 26.7, 30.1, 33.4, 39.5, 39.6, 47.1, 67.3, 119.5, 119.9, 123.7, 124.3, 125.1, 127.0, 127.6, 131.2, 135.3, 140.0, 141.2, 143.7, 143.8, 156.1, 172.5. MS (ES) m/z 546 $(M-H)^-$. HRMS (ES) calcd for $C_{33}H_{41}NO_4SNa$ $(M+Na)^+$: 570.2654, found: 570.2664.

4.8. Peptide (15)

The synthesis was carried out using an automatic microwave peptide synthesizer. **(a) Loading of the resin (2-ClTrt-Cl)**. 2-ClTrt-Cl resin (25 mg) was loaded with a solution of Fmoc-Farnesyl-Cys **14** (102 mg, 0.19 mmol) and DIPEA (32 μL, 0.19 mmol) in dry DMF (3 mL) with a double coupling protocol at 23 W and 75 °C. **(b) Fmoc deprotection**. Removal of the Fmoc protecting group was carried out using 20% piperidine in DMF (3 mL) at 23 W for 3 min at 75 °C. **(c) Peptide coupling conditions**. Fmoc-amino acid (5 equiv), HOBt (5 equiv), HBTU (5 equiv) and DIPEA (5 equiv) were stirred under N_2 in DMF (3 mL) for 5 min at 23 W and 75 °C. **(d) Cleavege**. The dried resin was treated for 3 h with AcOH/TFE/DCM (2:2:6, 3 mL), and then the resin was filtered off and washed with neat cleavage mixture (3×1 mL). A solution of TFA/H_2O (1:1, 200 μL) was added and the solution stirred under microwave heating (150 W and 80 °C) for 20 min to complete the deprotection of amino acids side chains. After addition of hexane (15 mL), the product was concentrated and lyophilized to give compound **15** (38 mg, 61%). The product was analyzed by analytic LC–MS (EC 125/4.6 NUCLEODUR 100–5C18ec) using the following gradient: from 0% B to 15% B over 15 min, then 15% B for 5 min at flow rate of 1 mL/min. The binary solvent system (A/B) was as follows: 0.1% TFA in water (A) and acetonitrile (B). The adsorbance was detected at 254 nm. The HPLC analysis showed one main peak at t_R=10.573 min that was identified as pure **15** on the basis of HRMS (ES) calcd for $C_{73}H_{133}N_7S_2O_{10}Na$ $(M+Na)^+$: 1354.9453, found: 1354.9274.

Acknowledgements

This work was financially supported by MIUR (Rome) within the PRIN project 2006. The authors thank also Mr. Luigi Verrengia for the skilful technical help.

References and notes

1. Resh, M. D. *Science STKE* **2006**, *359*, 14–19.
2. Clarke, S. *Annu. Rev. Biochem.* **1992**, *61*, 355–386.
3. Towler, D.; Gordon, J. I.; Adams, S. P.; Glaser, L. *Annu. Rev. Biochem.* **1988**, *57*, 69–73.
4. Mumby, S. M. *Curr. Opin. Cell Biol.* **1997**, *9*, 148–154.
5. Resh, M. D. *Cell Signalling* **1996**, *8*, 403–412.
6. (a) Milligan, G.; Parenti, M.; Magee, A. I. *Trends Biochem. Sci.* **1995**, *20*, 181–184; (b) Veit, M.; Schmidt, M. F. G. *Methods Mol. Biol.* **1998**, *88*, 227–239.
7. Stradley, S. J.; Rizo, J.; Gierasch, L. M. *Biochemistry* **1993**, *32*, 12586–12591; (b) Schroeder, H.; Leventis, R.; Rex, S.; Schelhaas, M.; Nägele, E.; Waldmann, H.; Silvius, J. R. *Biochemistry* **1997**, *36*, 13102–13121; (c) Xia, Z. D.; Smith, C. D. *J. Org. Chem.* **2001**, *66*, 5241–5244.
8. Lumbierres, M.; Palomo, J. M.; Kragol, G.; Waldmann, H. *Tetrahedron Lett.* **2006**, *47*, 2671–2674.
9. (a) Kuhn, K.; Owen, D. J.; Bader, B.; Wittinghofer, A.; Kuhlmann, J.; Waldmann, H. *J. Am. Chem. Soc.* **2001**, *123*, 1023–1035; (b) Kragol, G.; Lumbierres, M.; Palomo, J. M.; Waldmann, H. *Angew. Chem., Int. Ed.* **2004**, *43*, 5839–5842; (c) Rijkers, D. T. S.; Kruijtzer, J. A. W.; Killian, J. A.; Liskamp, R. M. J. *Tetrahedron Lett.* **2005**, *46*, 3341–3345; (d) Lumbierries, M.; Palomo, J. M.; Kragol, G.; Roehrs, S.; Müller, O.; Waldmann, H. *Chem.—Eur. J.* **2005**, *11*, 7405–7415.
10. Lampariello, L. R.; Piras, D.; Rodriquez, M.; Taddei, M. *J. Org. Chem.* **2003**, *68*, 7893–7895; Rodriquez, M.; Bruno, I.; Cini, E.; Marchetti, M.; Taddei, M.; Gomez-Paloma, L. *J. Org. Chem.* **2006**, *71*, 103–107; See also: Ciapetti, P.; Falorni, M.; Taddei, M. *Tetrahedron* **1996**, *52*, 7379–7380.
11. Rodriquez, M.; Taddei, M. *Synthesis* **2005**, 493–496.
12. Tetradecanal was obtained by Swern oxidation of commercially available 1-tetradecanol. Chandrasekhar, S.; Shameen, S. S.; Kiranmai, K.; Narsihmulu, Ch. *Tetrahderon Lett.* **2007**, *48*, 2373–2375.
13. The optical integrity of **12** was determined by HPLC analysis using a column Chiralpack IB 0.46×15 cm.
14. Brown, M. J.; Milano, P. D.; Lever, D. C.; Epstein, W. W.; Poulter, C. D. *J. Am. Chem. Soc.* **1991**, *113*, 3176–3177.
15. Brunsveld, L.; Kuhlmann, J.; Alexandrov, K.; Wittinghofer, A.; Goody, R. S.; Waldmann, H. *Angew. Chem., Int. Ed.* **2006**, *45*, 6622–6646; (b) Rotblat, B.; Prior, I. A.; Muncke, C.; Parton, R. G.; Kloog, Y.; Henis, Y. I.; Hancock, J. F. *Mol. Cell. Biol.* **2004**, *24*, 6799–6810; (c) Jaumot, M.; Yan, J.; Clyde-Smith, J.; Sluimer, J.; Hancock, J. F. *J. Biol. Chem.* **2002**, *277*, 272–278.
16. Gorfe, A. A.; Babakhani, A.; McCammon, J. A. *J. Am. Chem. Soc.* **2007**, *129*, 12280–12286.
17. Draper, J. M.; Xia, Z.; Smith, C. D. *J. Lipid Res.* **2007**, *48*, 1873–1884.
18. (a) Whyte, D. B.; Kirschmeier, P.; Hockenberry, T. N.; Nunez-Oliva, I.; James, L.; Catino, J. J.; Bishop, W. R.; Pai, J.-K. *J. Biol. Chem.* **1997**, *272*, 14559–14569; (b) Deck, P.; Pendzialek, D.; Biel, M.; Wagner, M.; Popkirova, B.; Ludolph, B.; Kragol, G.; Kuhlman, J.; Giannis, A.; Waldmann, H. *Angew. Chem., Int. Ed.* **2005**, *44*, 4975–4979.
19. For some examples on MW assisted synthesis of peptides see: Chighine, A.; Sechi, G.; Bradley, M. *Drug Discov. Today* **2007**, *12*, 459–464; Collins, J. M.; Cox, Z.; Singh, S. K. *Biopolymers* **2007**, *88*, 641–642; Cezamar, M.; Craig, D. K. *J. Pept. Sci.* **2008**, *14*, 683–689; Grieco, P.; Cai, M.; Liu, L.; Mayrov, A.; Chandler, K.; Trivedi, K.; Lin, G.; Campiglia, P.; Novellino, E.; Hruby, V. J. *J. Med. Chem.* **2008**, *51*, 2701–2707.
20. For information and leading references see: http://www.cem.com/biosciences/autopep.asp.
21. Yaghmur, A.; Laggner, P.; Zhang, S.; Rappolt, M. *PLoS ONE* **2007**, *2*. doi:10.1371/journal.pone.0000479 e479; Toth, I.; Horvath, A.; Olive, C.; Wong, A.; Clair, T.; Yarwood, P.; Good, M. *J. Med. Chem.* **2002**, *45*, 1387–1390 and references therein.