Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 1143–1147

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A Practical Approach to the Synthesis of Hairpin Polyamide–Peptide Conjugates Through the Use of a Safety-Catch Linker

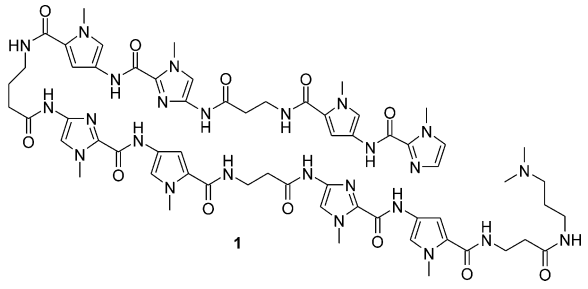
Daniela Fattori,† Olaf Kinzel, Paolo Ingallinella, Elisabetta Bianchi and Antonello Pessi*

Istituto di Ricerche di Biologia Molecolare P. Angeletti (IRBM), Via Pontina Km 30.600, 00040 Pomezia, Rome, Italy

Received 12 November 2001; revised 31 January 2002; accepted 18 February 2002

Abstract—Hairpin polyamides are high-affinity, sequence selective DNA binders. The use of a safety-catch linker for the solid phase synthesis of hairpin polyamides allows for easy preparation of derivatives ready for chemoselective ligation with unprotected peptides. Examples of ligations reported include thioether bond formation and thioester-mediated amide bond formation ('Native Chemical Ligation').

Non viral gene delivery systems represent a promising approach for gene therapy and for vaccination, but the efficiency of DNA transfection needs to be improved. Therefore increasing attention is given to methodologies which allow the functionalization of plasmid DNA with full control of stoichiometry and point of attachment.[1] We are focusing our efforts on the production of synthetic molecules consisting of two moieties: a DNA-binding moiety, connected, through a flexible arm, to another moiety providing a desired property. Examples of the latter are ligands for receptor targeting, nuclear localization signal peptides, and so on (see also Table 1). For the DNA-binding moiety, our choice fell on the minor groove-binding hairpin polyamides developed by Dervan and co-workers,[2] because of their high affinity for a given DNA sequence, high on-rate of complex formation, and good solubility and cell permeability properties.



1

For our experiments, we selected polyamide **1**, which is reported to have an extremely high affinity ($K_d = 0.05$ nM) and selectivity for the DNA sequence TGCTGCA.[3]

The final goal, that is to endow plasmid DNA with the functional moieties which best improve transfection efficiency, calls for a flexible strategy to prepare several peptide conjugates with *the same* DNA-binding moiety. Direct assembly of the peptide–polyamide conjugate on solid phase is possible,[4] but a separate synthesis is required for each derivative. A better alternative is therefore the chemoselective condensation of the two (unprotected) moieties. An example of this approach, using thioester-mediated ligation ('Native Chemical Ligation')[5] has been described recently.[6] The reactions to attach the thioester group to the polyamide are performed in solution, with intermediate purification steps.

Thiol-Containing Polyamides with Connecting Arms of Variable Length and Structure

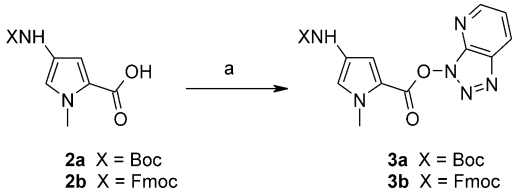
To readily produce a range of polyamides, incorporating a chemoselective function at the end of variable-length connecting arms, we decided to take advantage of the properties of Kenner's safety-catch linker[7] (as modified by Backes and Ellman).[8] Since cleavage by nucleophilic displacement is effective with near-stoichiometric amounts of reagent, we could economically use functionalized amines of increasing complexity. In particular, amines containing a trityl protected thiol moiety were suitable for the well-known thioether ligation.[9]

*Corresponding author. Tel.: +39-06-9109-3445; fax: +39-06-9109-3482; e-mail: antonello_pessi@merck.com

†Present address: Menarini Ricerche, Via Tito Speri 10, 00040 Pomezia, Roma, Italy

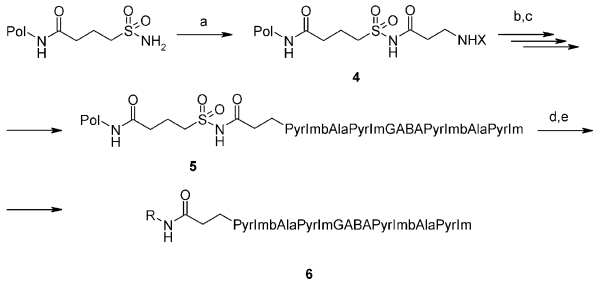
0960-894X/02/$ - see front matter
PII: S0960-894X(02)00122-1

Polyamide synthesis was carried out on commercially available 4-sulfonamidobutyryl polystyrene resin (NovaBiochem). This resin allows the use of both Boc and Fmoc chemistry.[10] Commercially not available monomers for Boc chemistry were prepared as reported in the literature[11] with the exception of pyrroles, which were used as the more reactive HOAt esters **3**[12] instead of the published HOBt derivatives.[11] The HOAt esters **3** gave better coupling yields with shorter reaction times (Scheme 1).



Scheme 1. Reagents and conditions: (a) HOAt, DMAP, EDCA, CH_2Cl_2, 12 h.

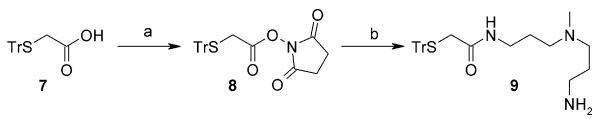
Fmoc-pyrrole was prepared according to Vázquez et al.[13] In our hands, Boc- and Fmoc-based chemistry performed equally well in terms of yield and product quality (Scheme 2).



Scheme 2. Reagents and conditions: (a) XβAla (where X = Boc or Fmoc), DIPC, DMAP, CH_2Cl_2; (b) *Fmoc chemistry*: 20% piperidine in DMF; *Boc chemistry*: TFA, thiophenol, CH_2Cl_2, (c) *activated Pyr*: DIPEA, DMF; *other acids*: EDCA, HOAt, DMAP, DMF; (d) iodoacetonitrile, DIPEA, DMF, 4 h; (e) RNH_2 (2 equiv), DIPEA, DMF, 12 h, then isocyanate resin.

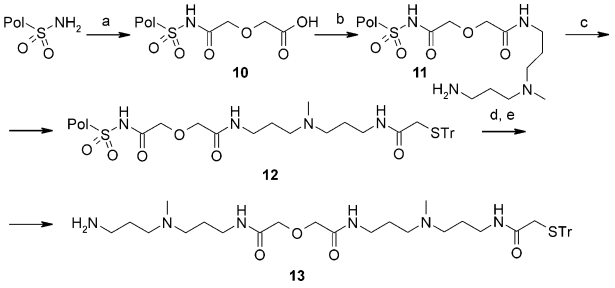
Linker activation with trimethylsilyl-diazomethane[8] proceeded smoothly, but the subsequent cleavage was slow, worsening with increasing size of the cleaving amine. Activation by iodoacetonitrile[8] gave better results, and by maintaining reaction times less than 4 h we could avoid polyamide alkylation.[14]

Thiol amine **9** was chosen as the shortest amine joining the 3-methylaminopropyl motif and a thiol group for peptide ligation.[15] Trityl mercaptoacetic acid was activated as *N*-hydroxysuccinimide ester and reacted with 3,3′-diamino-*N*-methyldipropylamine (Scheme 3).



Scheme 3. Reagents and conditions: (a) *N*-hydroxysuccinimide, DCC, THF; (b) 3,3′-diamino-*N*-methyldipropylamine (20 equiv), CH_2Cl_2.

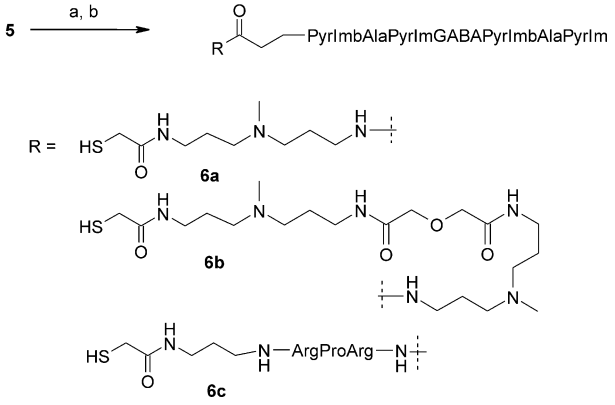
For ligations involving larger peptide moieties, we also wanted a longer connecting arm, and we prepared the thiol amine **13** (Scheme 4).[16] We used a solid-phase approach based on the safety-catch linker, which is suitable for the rapid preparation of a connecting arm of any length, by alternating diglycolic acid and 3,3′-diamino-*N*-methyldipropylamine units.[17,18]



Scheme 4. Reagents and conditions: (a) diglycolic acid anhydride, DMAP, DIPEA, DMF; (b) CDI, HOBt, 3,3′-diamino-*N*-methyldipropylamine, DMF; (c) DIC, HOBt, **7**, DMF; (d) $TMSCHN_2$, THF; (e) 3,3′-diamino-*N*-methyldipropylamine, DMF.

Displacement of the polyamide from the activated resin[19] proceeded smoothly at room temperature. Following displacement, the small excess of unreacted amine was trapped with a 4-fold excess of thiocyanate resin, and the crude product was directly treated with TFA/TIPS in CH_2Cl_2 (Scheme 5).

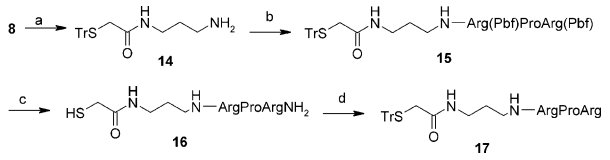
Chromatographic purification yielded the polyamide thiols **6a** and **6b**, ready for ligation with bromoacetyl-derivatized peptides.[20]



Scheme 5. Reagents and conditions: (a) amines **9**, **13** or **17**, DIPEA, DMF; (b) TFA, TIPS, CH_2Cl_2.

In 1998, Dervan et al.[4] proposed, as an alternative to 3,3-dimethylaminopropylamine as C-terminal polyamide tail, the tripeptide ArgProArg, which added an interaction with the phosphate groups of DNA, while maintaining selectivity and affinity. We prepared the protected thiol derivative (**15**) of this peptide according to Scheme 6.

By contrast with the ready displacement observed with amines **9** and **13**, amine **15** did not react with the activated linker. Heating the reaction solution to 80 °C (as suggested in ref 23) resulted in part in the formation of



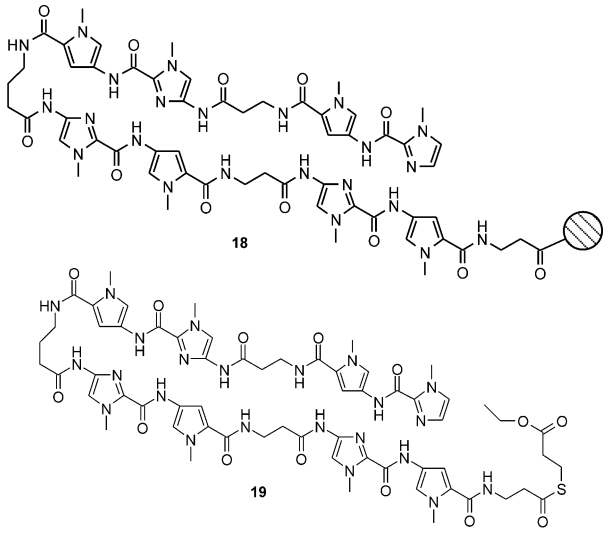
Scheme 6. Reagents and conditions: (a) 1,3-diaminopropane, 20 equiv; (b) (i) Fmoc aminoacid, EDCA, CH_2Cl_2; (ii) 4-aminomethylpiperidine, CH_2Cl_2, flash chromatography on silica gel; (c) TFA, CH_2Cl_2, anisole; (d) $BF_3{\cdot}2Et_2O$, triphenylmethanol, HOAc, CH_2Cl_2.

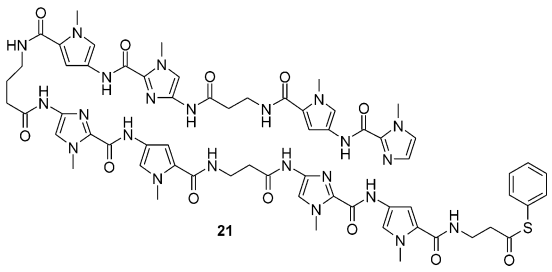
the unwanted polyamide thioester, derived from thiol deprotection and subsequent nucleophilic attack on the alkylated acylsulfonamide linker moiety.[22] Having attributed the lack of reactivity to the steric bulk of the arginine protecting groups, from **15** we proceeded to thiol amine **17**,[21] containing the unprotected tripeptide. Indeed, **17** was able to cleave polyamide **5** from the resin at room temperature, yielding **6c**.[20]

The above findings highlight two features of general relevance: (i) direct displacement from the resin can be efficiently performed with unprotected peptides, provided no free amines/free thiols are present in the sequence (these would compete with the N-terminal amino group); (ii) thiol displacement from the safety-catch linker readily forms the thioester of the hairpin polyamide, which can be used for conjugation to N-terminal cysteine peptides via Native Chemical Ligation. This method is simpler than the solution-phase derivatization of Mapp and Dervan,[6] and we decided to explore it further.

C-Terminal Thioester Hairpin Polyamides and Native Chemical Ligation

Starting from resin bound polyamide **18** (precursor of **1**), we prepared thioester **19**, essentially as previously described for standard peptides.[22] The safety-catch linker was activated through alkylation with iodoacetonitrile, and the resin treated with a 2:1 solution of DMF and ethyl 3-mercaptopropionate together with a catalytic amount of sodium thiophenoxide for 12 h.[24] The resulting polyamide thioester **19** was obtained as a crude





material in 41% yield, and used as such for ligation with the peptide CMVEYPYRV (**20**, C-terminal carboxyamide). The reaction however proceeded slowly. HPLC analysis showed that the ethyl 3-mercaptopropionate thioester was not appreciably converting into the thiophenol thioester (**21**). We attributed this unexpected failure of thiol exchange to the lack of an electron-withdrawing amido group in alpha position of the amino acid, and prepared directly the more activated species **21**.[25] Indeed, equivalent amounts of peptide **20** and thioester **21** underwent complete ligation in 12 h.[26]

Chemoselective Thioether Ligation

The majority of conjugates for our investigation were prepared by thioether ligation. The bromoacetyl-peptide precursors were synthesized by standard solid-phase Fmoc chemistry, and the sequences are shown in Table 1.

Table 1. Bromoacetyl-peptide derivatives

Bromoacetyl-peptides[a]
GPGSDDEAAADAQHAAPPKKKRKVGY (**23**[28])
GPGNEWTLELLEELKNEAVRHF (**24**[29])
GGGGGYGRKKRRQRRR (**25**[30])
GPGRQIKIWFQNRRMKWKK (**26**[30])

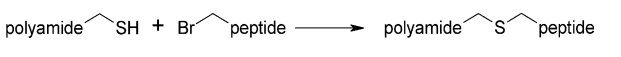
[a]All peptides are N-terminal bromoacetyl ($BrCH_2CO$–), C-terminal carboxyamides.

Peptides **23** and **24** belong to the family of nuclear localization signals,[27–29] while **25** and **26** are known to mediate cell penetration.[30]

Because of the low solubility of polyamides **6a–c** in aqueous media, the ligations were performed in DMF in the presence of 1% DIPEA, using a 2-fold excess of the bromoacetylated peptides.[31] The reactions were monitored by HPLC. When the polyamide was completely consumed, the solvent was distilled off in vacuo and the crude material was purified by preparative HPLC and characterized by mass spectrometry.[32] Overall yields varied between 17 and 45% (Table 2).

In conclusion, we have shown here that the use of an acylsulfonamide safety-catch linker allows for the easy preparation of hairpin polyamide–peptide conjugates, with intervening arms of various lengths, by readily yielding the necessary precursors for chemoselective

Table 2. Ligation reactions of peptides 23–27 with polyamide thiols **6a–c**



Peptide	Polyamide	Product	Yield[a]
23	**6b**	**27**	26
23	**6c**	**28**	45
24	**6a**	**29**	17
25	**6a**	**30**	23
26	**6a**	**31**	32

[a]Calculated with respect to the starting polyamides.

ligation. Evaluation of the ability of these compounds to increase gene expression is in progress, and will be reported separately.

Acknowledgements

The authors would like to thank S. Pesci for analytical NMR, A. Alfieri and F. Bonelli for mass spectrometry, R. Ingenito for helpful discussions throughout the work and Gennaro Ciliberto for critical reading of the manuscript.

References and Notes

1. Zelphati, O.; Liang, X.; Hobart, P.; Felgner, P. L. *Human Gene Ther*. **1999**, *10*, 15.
2. (a) Pilch, D. S.; Poklar, N.; Baird, E. E.; Dervan, P. B.; Breslauer, K. J. *Biochemistry* **1999**, *38*, 2143. (b) Pilch, D. S.; Poklar, N.; Gelfand, C. A.; Law, S. M.; Breslauer, K. J.; Baird, E. E.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A*. **1996**, *93*, 8306. (c) Parks, M. E.; Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc*. **1996**, *118*, 6147.
3. Dickinson, L. A.; Gulizia, R. J.; Trauger, J. W.; Baird, E. E.; Mosier, D. E.; Gottesfeld, J. M.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A*. **1998**, *95*, 12890.
4. Bremer, R. E.; Baird, E. E.; Dervan, P. B. *Chem. Biol*. **1998**, *5*, 119.
5. Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776.
6. (a) Mapp, A. K.; Dervan, P. B. *Tetrahedron Lett*. **2000**, *41*, 9451. (b) Mapp, A. K.; Ansari, A. Z.; Ptashne, M.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A*. **2000**, *97*, 3930.
7. Kenner, G. W.; McDermott, J. R.; Sheppard, R. C. *J. Chem. Soc., Chem. Commun*. **1971**, 636.
8. (a) Backes, B. J.; Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc*. **1996**, *118*, 3055. (b) Backes, B. J.; Ellman, J. A. *J. Org. Chem*. **1999**, *64*, 2322.
9. Schelté, P.; Boeckler, C.; Frisch, B.; Schuber, F. *Bioconj. Chem*. **2000**, *11*, 118.
10. Bodanszky, M. In *Principles of Peptide Synthesis*; Springer: Berlin, Heidelberg, 1993; p 215. To evaluate the starting resin substitution via dibenzofulvene release quantitation,[8] the first amino acid was always introduced as Fmoc-derivative.
11. Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc*. **1996**, *118*, 6141.
12. **^{1}H NMR and analytical details for:** (**3a**), white solid, mp 189–190 °C, ^{1}H NMR (DMSO-d_6) δ 9.40 (s, 1H), 8.85 (d, J=4.0 Hz, 1H), 8.70 (d, J=7.5 Hz, 1H), 7.65 (dd, J=4.9, 7.5 Hz, 1H), 7.55 (s, 1H), 7.20 (s, 1H), 3.80 (s, 3H), 1.45 (s, 9H). Ion spray mass spectrometry: calculated for $C_{16}H_{18}N_6O_4$, 358.36 Da, found 358.9 Da. (**3b**), white solid, mp 192–193 °C, ^{1}H NMR (DMSO-d_6) δ 9.74 (s, 1H), 8.83 (d, J=4.4 Hz, 1H), 8.72 (d, J=7.2 Hz, 1H), 7.95 (d, J=7.6 Hz, 2H), 7.73 (d, J=7.2 Hz, 2H), 7.65 (d, J=4.8, 8.4 Hz, 1H), 7.54 (s, 1H), 7.43 (dd, J=7.2, 7.2 Hz, 2H), 7.31 (dd, J=7.6, 7.6 Hz, 2H), 7.25 (s, 1H), 4.53 (d, J=6.4 Hz, 2H), 4.30 (t, J=6.4 Hz, 1H), 3.85 (s, 3H). Ion spray mass spectrometry: calculated for $C_{26}H_{20}N_6O_4$, 480.49 Da, found 480.2 Da.
13. Vázquez, E.; Caamaño, A. M.; Castedo, L.; Gramberg, D.; Mascareñas, J. L. *Tetrahedron Lett*. **1999**, *40*, 3625.
14. In a typical experiment 154 mg resin (19 μmol product), preswollen in NMP, were treated with a solution of 0.72 mL (13 mmol) iodoacetonitrile and 0.42 mL (2.4 mmol) DIPEA in 2 mL NMP for 4 h, then washed with NMP, CH_2Cl_2 and dried under nitrogen.
15. **^{1}H NMR and analytical details for 9 (TFA salt):** ^{1}H NMR (DMSO-d_6): 8.05 (t, J=3.0 Hz, 1H), 7.30 (m, 15H), 3.20–2.80 (m, 8H), 2.80 (s, 2H), 2.70 (s, 3H), 1.90 (m, 2H), 1.70 (m, 2H). Ion spray mass spectrometry: calculated for $C_{28}H_{35}N_3OS$ 461.67 Da, found 461.4 Da.
16. **^{1}H NMR and analytical details for 13 (TFA salt): 13:** ^{1}H NMR (DMSO-d_6): 8.23 (m, 2H), 8.03 (t, J=5.7 Hz, 1H), 7.99 (s, br, 2H), 7.33 (m, 15H), 3.96 (s, 4H), 3.18 (m, 4H), 3.02 (m, 10H), 2.88 (s, br, 2H), 2.80 (s, 2H), 2.75 (s, 3H), 2.71 (s, 3H), 1.94 (m, 2H), 1.82 (m, 4H), 1.70 (m, 2H). Ion spray mass spectrometry: calculated for $C_{39}H_{56}N_6O_4S$, 704.98 Da, found 704.6 Da.
17. Bell, R. A.; Hughes, D. W.; Lock, C. J. L.; Valliant, J. F. *Can. J. Chem*. **1996**, *74*, 1503.
18. Rose, K.; Vizzavona, J. *J. Am. Chem. Soc*. **1999**, *121*, 7034.
19. **Displacement of polyamide from activated resin:** In a typical experiment a batch of resin corresponding to 39 μmol of product was swollen in DMF. A solution of 2 equiv amine and 6 equiv DIPEA in 3 mL DMF was added and the resulting mixture shaken overnight. The resin was filtered off and the remaining solution shaken for 1 h with isocyanate polystyrene resin (4 equiv). Filtration and concentration under reduced pressure left a yellow oil, which was treated for 30 min with 10 mL of a (1:1) TFA/CH_2Cl_2 solution, containing 10% TIPS. Concentration under reduced pressure and trituration with diethyl ether left the crude product as a yellow solid. The crude product was purified by RP-HPLC on a Symmetry C-18 column, 100×19 mm, 5 μm, using water (0.1% TFA) and acetonitrile (0.1% TFA) as eluents. Final yields ranged from 14 to 34% (based on initial resin loading).
20. **Analytical data for:** (**6a**), ^{1}H NMR (DMSO-d_6) δ 10.36 (s, 1H), 10.27 (s, 1H), 10.25 (s, 1H), 10.23 (s, 1H), 9.90 (s, 1H), 9.88 (s, 1H), 9.10 (s, br, 1H), 8.13 (m, 6H), 7.45 (s, 2H), 7.43 (s, 1H), 7.37 (s, 1H), 7.23 (s, 1H), 7.20 (s_{br}, 3H), 7.02 (s, 1H), 6.97 (s, 1H), 6.96 (s, 1H), 6.94 (s, 1H), 3.97 (s, 3H), 3.94 (s, 6H), 3.93 (s, 3H), 3.94 (m, 12H), 3.50–3.00 (m, 15H), 2.99 (m, 2H), 2.72 (m, 2H), 2.58 (m, 4H), 2.35 (m, 4H), 1.76 (m, 6H). Ion spray mass spectrometry: calculated for $C_{66}H_{86}N_{26}O_{13}S$, 1483.66 Da, found 1483.2 Da; (**6b**), ion spray mass spectrometry: calculated for $C_{77}H_{107}N_{29}O_{16}S$, 1726.96 Da, found 1727.6 Da; (**6c**), ion spray mass spectrometry: calculated for $C_{79}H_{108}N_{34}O_{16}S$, 1822.02 Da, found 1822.4 Da.
21. **^{1}H NMR and analytical details for 17 (TFA salt):** ^{1}H NMR (DMSO-d_6): 8.17 (m, 3H), 7.85 (m, 2H), 7.76 (t, J=5.2 Hz, 1H), 7.66 (t, J=5.2 Hz, 1H), 7.76–7.05 (m, 23H), 4.41 (m, 1H), 4.12 (m, 2H), 3.65 (m, 1H), 3.45 (m, 1H), 3.15–2.90 (m, 8H), 2.77 (s, 2H), 2.49 (m, 1H), 2.00–1.40 (m, 13H). Ion spray mass spectrometry: calculated for $C_{41}H_{57}N_{11}O_4S$, 800.05 Da, found 799.6 Da.
22. Ingenito, R.; Bianchi, E.; Fattori, D.; Pessi, A. *J. Am. Chem. Soc*. **1999**, *121*, 11369.
23. Backes, B. J.; Harris, J. L.; Leonetti, F.; Craik, C. S.; Ellman, J. A. *Nature Biotechnol*. **2000**, *18*, 187.

24. **Polyamide 19:** A batch of iodoacetonitrile activated resin (17 μmol of product) was swollen in a mixture of 0.5 mL DMF and 0.2 mL ethyl-3-mercaptopropionate. A catalytic amount of sodium thiophenoxide was added and the mixture was shaken overnight. The solvents were then distilled off under reduced pressure and the residual oil was triturated with diethyl ether to obtain a yellow solid (10 mg, 41%). This crude material was used as such in the subsequent native chemical ligation. Ion spray mass spectrometry: calculated for $C_{62}H_{75}N_{23}O_{14}S$, 1398.50 Da, found 1398.6 Da.
25. **Polyamide 21:** A batch of iodoacetonitrile activated resin (6.8 μmol of product) was swollen in a mixture of 1.0 mL DMF and 0.4 mL thiophenol and shaken overnight. Solvents were then distilled off in vacuo and the residual oil triturated with diethyl ether to obtain a white solid. This crude material was used as such in the subsequent native chemical ligation. Ion spray mass spectrometry: calculated for $C_{63}H_{71}N_{23}O_{12}S$, 1374.48 Da, found 1373.8 Da.
26. **Native chemical ligation:** Peptide **20** (1.3 mg, 1.0 μmol) was dissolved in 400 μL water and the pH adjusted to 7.0 with diluted NaOH. The solution was then added, together with 16 μL thiophenol, to a solution of 5.3 mg of crude thioester **21** in 400 uL DMF. Some additional drops of DMF were necessary to obtain a clear solution. The mixture was shaken overnight. By HPLC analysis complete disappearence of peptide **20** and almost complete disappearance of polyamide **21** was found. The crude product was purified by RP-HPLC on a Beckman C-18 column, 25 cm×4.6 mm, 5 μm, using water (0.1% TFA) and acetonitrile (0.1% TFA) as eluents, affording 0.45 mg of conjugate as a fluffy pale yellow material (21% yield, based on starting peptide). Ion spray mass spectrometry: calculated for $C_{109}H_{144}N_{36}O_{25}S_2$, 2422.72 Da, found 2422.4 Da.
27. Christophe, D.; Christophe-Hobertus, C.; Pichon, B. *Cell. Signal*. **2000**, *12*, 337.
28. Hübner, S.; Xiao, C.-Y.; Jans, D. A. *J. Biol. Chem*. **1997**, 17191.
29. Karni, O.; Friedler, A.; Zakai, N.; Gilon, C.; Loyter, A. *FEBS Lett*. **1998**, *429*, 421.
30. Lindgren, M.; Hallbrink, M.; Prochiantz, A.; Langel, U. *TiPS* **2000**, *21*, 99.
31. **Thioether ligation, general procedure**: 1 μmol of polyamide and 2 equiv of bromoacetyl peptide were dissolved in 400 μL DMF, containing 0.05% DIPEA. The solution was shaken at room temperature and the reaction was monitored by analytical RP-HPLC (column symmetry RP-C18, 10×4.6 mm, 5 μm) using water (0.1% TFA) and acetonitrile (0.1% TFA) as eluents.
32. **Analytical characterization for peptide–polyamide conjugates:** Ion spray mass spectrometry: (**27**) calculated for $C_{193}H_{294}N_{69}O_{51}S$, 4430.94 Da, found 4430.4 Da; (**28**) calculated for $C_{195}H_{291}N_{71}O_{54}S$, 4526.00 Da, found 4525.6 Da; (**29**) calculated for $C_{184}H_{264}N_{58}O_{49}S$, 4104.58 Da, found 4104.8 Da; (**30**) calculated for $C_{142}H_{220}N_{64}O_{32}S$, 3367.81 Da, found 3367.8 Da; (**31**) calculated for $C_{181}H_{268}N_{64}O_{36}S_2$, 3980.69 Da, found 3981.0 Da.