Pergamon

Tetrahedron Letters 41 (2000) 9451–9454

TETRAHEDRON LETTERS

Preparation of thioesters for the ligation of peptides with non-native substrates†

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Received 31 August 2000; revised 12 September 2000; accepted 14 September 2000

Abstract

A one-pot procedure for the synthesis of thioesters from primary amines is reported. Polyamides containing one or more primary amines were prepared by solid-phase synthesis and reacted with thiolane-2,5-dione followed by alkylation with benzyl bromide to produce the target thioesters in good yield. The thioesters thus prepared were conjugated to peptides to produce polyamide–peptide chimeras using the 'native chemical ligation' method. This flexible synthetic procedure provides a ready route to both natural and unnatural substrates for chemical ligation reactions. © 2000 Elsevier Science Ltd. All rights reserved.

In the course of preparing artificial transcription factors based upon small, DNA-binding molecules called polyamides, we found it necessary to synthesize a wide variety of polyamide–peptide conjugates.[1,2] Of the many coupling techniques developed to prepare peptide conjugates, the most versatile from our standpoint was the procedure termed 'native chemical ligation' originally developed for the synthesis of proteins too large to be accessed by standard solid-phase synthesis approaches.[3] In this reaction, a peptide containing a carboxy-terminal thioester and a peptide with an amino-terminal cysteine are combined in denaturing buffer. Upon transesterification of the thioester with the cysteine thiol, an S→N acyl shift takes place to generate a ligated product in which the two halves are now connected by an amide bond. The product recovery of this sequence is generally good, and the facility of the reaction appears sequence independent. Total syntheses of many natural and modified proteins have been reported using this method.[4]

To adapt this powerful reaction to our purposes, it was necessary to prepare polyamides containing the requisite thioester functional group. Available methods for the preparation of suitable thioesters range from biochemical approaches[5] to solid-phase synthesis using modified

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† Dedicated to Professor Harry H. Wasserman on the occasion of his 80th birthday.

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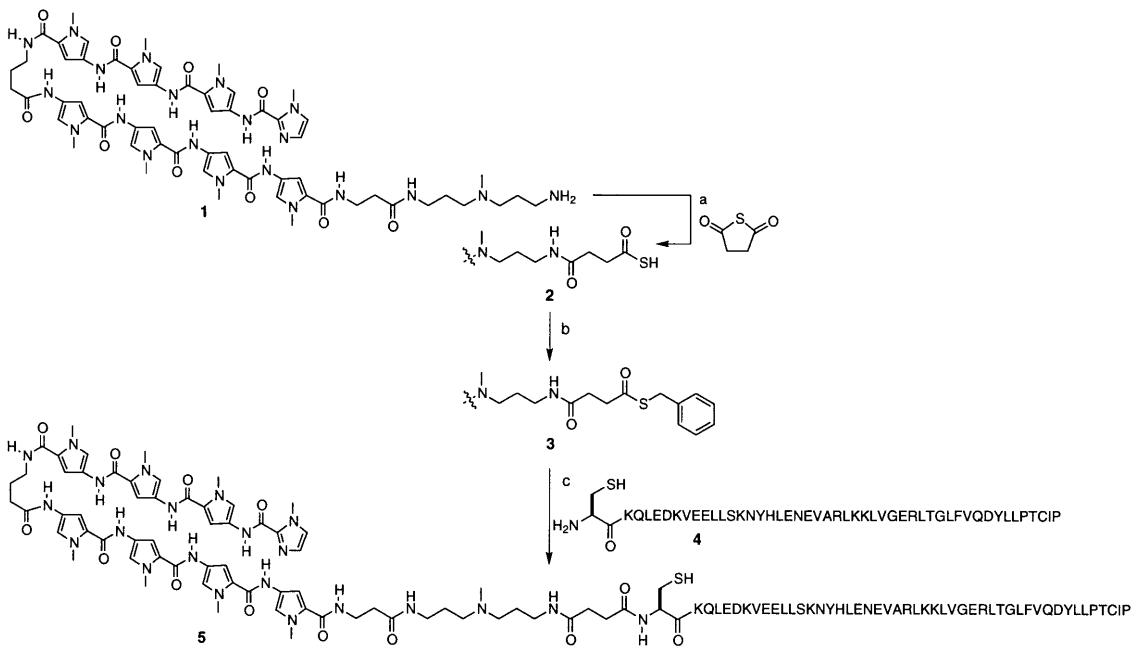
PII: S0040-4039(00)01600-2

resins.[6] However, all methods provide products with thioesters at the carboxy-terminal position, and this severely restricted our efforts to prepare a variety of structurally diverse polyamide–peptide conjugates. In particular, we desired to prepare conjugates with multiple peptides attached to one polyamide, and it was thus necessary to develop a synthetic approach that would allow such flexibility. Polyamides are prepared by solid-phase synthesis,[7] and a functional group that is straightforward to introduce at one or more positions is a primary amine. The final requirement was therefore an electrophile that would produce a thioester or thioacid when reacted with an amine, and an excellent candidate was thiolane-2,5-dione, readily available from succinic anhydride by reaction with sodium sulfide.[8]

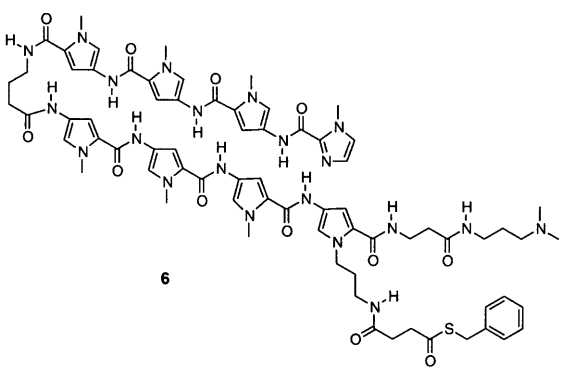
To test this approach, hairpin polyamide **1**, containing a primary amine, was prepared by solid-phase synthesis.[7,9] Polyamide **1** and thiolane-2,5-dione were combined in *N*-methylpyrrolidinone (NMP) with *N*,*N*-diisopropylethylamine (DIPEA) at ambient temperature and reaction progress monitored by analytical reversed-phase HPLC (Scheme 1). Polyamide **1** was rapidly consumed and thioacid **2** isolated by ether precipitation. Alternatively, thioester **3** is produced in a 'one-pot' conversion by lowering the pH of the initial reaction mixture with pH 5.2 NaOAc buffer and adding benzyl bromide. As monitored by analytical HPLC, the conversion from **1** to **3** is virtually quantitative. A typical reaction procedure is as follows: to a solution of 18.6 μmol (23.5 mg) polyamide **1** in 0.600 mL NMP was added thiolane-2,5-dione (25.0 μL of a 1.00 M solution, 25.0 μmol), followed by 9.70 μL (55.6 μmol) of DIPEA. After 10 minutes, conversion to thioacid **2** appeared complete. The reaction mixture was diluted with 900 μL 100 mM NaOAc buffer (pH 5.2) and cooled to 4°C, necessary to prevent the formation of dialkylation products in the subsequent step. Benzyl bromide (55.8 μmol, 6.70 μL) was added with thorough mixing and after an additional 10 minutes, the product **3** was isolated by semi-preparatory reversed-phase HPLC as a pale yellow powder in 52% overall yield (14.2 mg, 9.66 μmol).[10] Thioester **3**



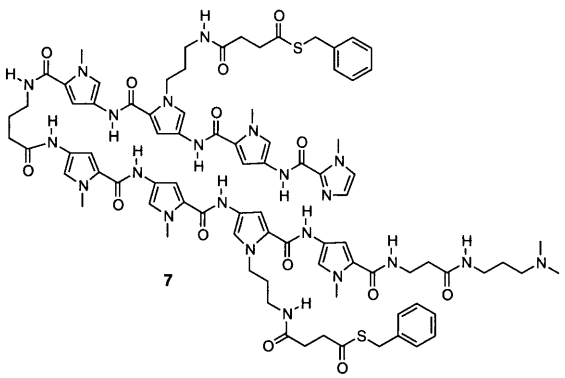
Scheme 1. *Conditions*: (a) DIPEA, NMP, rt. (b) 100 mM NaOAc (pH 5.2), benzyl bromide, 0°C (52%, two steps). (c) 100 mM potassium phosphate buffer (pH 7.3), 6 M Gn·HCl, 5% NMP, 5% PhSH, 4 days, rt (29%)

underwent reaction under standard 'native chemical ligation' conditions[3] with peptides having an amino-terminal cysteine such as **4** to produce conjugate **5**. Due to the hydrophobicity of **3**, it is necessary to dissolve the thioester in NMP (5–10% of reaction volume) prior to addition of aqueous denaturing buffer. Conjugate **5** was recovered in 29% yield under these reaction conditions.[11]

The next goal was to gauge the generality of this method for preparing polyamides with multiple thioesters and at diverse positions within the structure. Polyamides containing one or more internal primary amine(s) provided the requisite functional group handles, and the reaction sequence was applied to afford thioesters such as **6** and **7**.[12] Polyamide **6** was isolated in 55% yield after HPLC purification. Incorporation of two thioesters was as facile as one, with conversion to product nearly quantitative as determined by analytical HPLC to furnish thioester **7** in 30% yield. In all cases, subsequent conjugation via 'native chemical ligation' to peptides containing amino-terminal cysteine residues proceeded readily (5–45% yields of isolated conjugate).[2]



6



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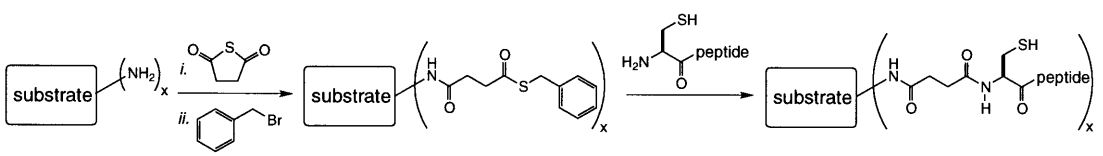
In summary, we have presented a one-pot reaction sequence which allows rapid access to thioester-modified products suitable for 'native chemical ligation' reactions (Scheme 2). Most notably, this procedure requires only a primary amine and can be used to install multiple functional groups. This sequence works equally well for the functionalization of α-amino acids.[13] While we have used this method to prepare polyamide–peptide conjugates, it should be readily extendable to the preparation of other chimeric target molecules. Finally, although the thioester products function well in the native chemical ligation reaction, other ligation approaches are equally applicable such as the Staudinger ligation[14] or alkylation of thioacids.[15]



substrate = any 1° amine-containing natural or unnatural molecule
x is greater than or equal to 1

Scheme 2.

Acknowledgements

We are grateful to the National Institutes of Health for support of this work (GM-27681) and a National Research Service Award to A.K.M.

References

1. For a review see: Dervan, P. B.; Burli, R. W. *Curr. Opin. Chem. Biol.* **1999**, *3*, 688–693.
2. Mapp, A. K.; Ansari, A. Z.; Ptashne, M.; Dervan, P. B. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 3930–3935.
3. Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776–779.
4. Cotton, G. J.; Muir, T. W. *Chem. Biol.* **1999**, *6*, R247–R256.
5. (a) Muir, T. W.; Sondhi, D.; Cole, P. A. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 6705–6710. (b) Welker, E.; Scheraga, H. A. *Biochem. Biophys. Res. Commun.* **1999**, *254*, 141–151.
6. For example see: (a) Camarero, J. A.; Adeva, A.; Muir, T. W. *Lett. Peptide Sci.* **2000**, *7*, 17–21. (b) Shin, Y.; Winans, K. A.; Backes, B. J.; Kent, S. B. H.; Ellman, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **1999**, *121*, 11684–11689. (c) Ingenito, R.; Bianchi, E.; Fattori, D.; Pessi, A. *Tetrahedron Lett.* **1999**, *121*, 11369–11374. (d) Schwabacher, A. W.; Maynard, T. L. *Tetrahedron Lett.* **1993**, *34*, 1269–1270. (e) Clippingdale, A. B.; Barrow, C. J.; Wade, J. D. *J. Pept. Sci.* **2000**, *6*, 225–234. (f) Hojo, H.; Aimoto, S. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 111–117. (g) Yamashiro, D.; Li, C. H. *Int. J. Peptide Protein Res.* **1988**, *31*, 322–334. (h) Yamashiro, D.; Blake, J. *Int. J. Peptide Protein Res.* **1981**, *18*, 385–392.
7. Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1996**, *118*, 6141–6146.
8. Kates, M. J.; Schauble, J. H. *J. Heterocyclic Chem.* **1995**, *32*, 971–978.
9. Trauger, J. W.; Baird, E. E.; Dervan, P. B. *Nature* **1996**, *382*, 559–561.
10. Data for **3**: ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.58–1.68 (m, 2H), 1.72–1.82 (m, 4H), 2.28–2.36 (m, 4H), 2.41 (t, 2H, $J=6.6$ Hz), 2.72 (d, 2H, $J=4.9$ Hz), 2.84 (t, 2H, $J=6.8$ Hz), 2.94–3.0 (m, 2H), 3.04–3.10 (m, 4H), 3.16 (tt, 2H, $J=6.9$, 13.9 Hz), 3.21 (m, 2H), 3.80 (s, 3H), 3.81 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 3.84 (s, 3H), 3.85 (br s, 5H), 3.91 (d, 3H, $J=2.0$ Hz), 3.99 (s, 3H), 4.10 (s, 3H), 6.84 (d, 1H, $J=1.7$ Hz), 6.88 (d, 1H, $J=2.0$ Hz), 6.91 (d, 1H, $J=2.0$ Hz), 7.05 (br s, 3H), 7.16–7.18 (m, 3H), 7.21–7.30 (m, 7H), 7.39 (s, 1H), 7.86 (t, 1H, $J=5.6$ Hz,), 7.99–8.10 (m, 6H), 9.09 (br s, 1H), 9.84 (s, 1H), 9.89 (s, 1H), 9.90 (s, 1H), 9.91 (s, 1H), 9.93 (s, 1H), 9.96 (s, 1H), 10.46 (s, 1H). MALDI-TOF MS [M+H] (monoisotopic) calcd 1470.7, obsd 1470.7.
11. Yield is for conjugate **5** isolated after reversed-phase HPLC purification. MALDI-TOF MS [M+H] (average mass) calcd 6935.0, obsd 6934.8.
12. MALDI-TOF MS [M+H] (monoisotopic) **6**: calcd 1470.7, obsd 1470.7; **7**: calcd 1719.7, obsd 1719.8.
13. Jäger, R.; Dervan, P. B., unpublished results.
14. Nillson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett.* **2000**, *2*, 1939–1941.
15. Schnölzer, M.; Kent, S. B. H. *Science* **1992**, *256*, 221–225.