Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 849–853

Facile peptide thioester synthesis via solution-phase tosylamide preparation

Shino Manabe,[a,b,*] Tomoyuki Sugioka[b] and Yukishige Ito[a,*]

[a]*RIKEN (The Institute of Physical and Chemical Research), Hirosawa, Wako, Saitama 351-0198, Japan*
[b]*PRESTO, Japan Science and Technology Agency (JST), Kawaguchi, Saitama 332-1102, Japan*

Received 9 October 2006; revised 20 November 2006; accepted 24 November 2006
Available online 13 December 2006

Abstract—Preparation of peptide thioester is essential for native chemical ligation and block condensation. Our novel methodology involves conversion of the carboxylic acid of a peptide into a thioester using *p*-toluenesulfonyl isocyanate, followed by alkylation, then thiol substitution. Our methodology can also be used for the preparation of glycopeptide thioesters. Furthermore, it is possible to carry out the reaction as a sequential peptide chemical ligation.

Recently, peptide thioesters have gained attention as intermediates in peptide synthesis because of their utility towards thioester peptide block coupling and native chemical ligation methods.[1] Although peptide thioesters are essential for native chemical ligation or block coupling methodology, the chemical synthesis of C-terminal thioester peptides via traditional Fmoc-based solid-phase strategy has been hampered by the poor stability of the thioester bond under basic conditions, which are required for the deprotection of the *N*-Fmoc group. It is highly desirable to develop a synthetic scheme that is compatible with Fmoc solid-phase peptide synthesis.

Recent examples of peptide thioester preparations can be classified into three synthetic approaches: the first scheme attempts to attenuate the basicity during cleavage of the Fmoc group with direct attachment of the thioester to the solid-phase resin. For example, Aimoto synthesized a 25-mer thioester peptide using a combination of 1-methylpyrrolidine–hexamethyleneimine–HOBt as the Fmoc cleavage reagent to prevent cleavage of the thioester from the resin.[2] Unfortunately, the thioester racemized more readily than other esters under basic conditions.[3] The second approach for peptide thioester preparation is based on Kenner's safety-catch linker, which is particular by stable under basic and acidic conditions and nucleophiles.[4] In the case of the peptide thioester formation using Kenner's linker, as reported by Pessi and co-workers, upon completion of the elongation process, the peptide was cleaved by a thiolate attack as the corresponding thioester.[5] Subsequently, Bertozzi and co-workers applied this methodology for the synthesis of a glycopeptide.[6] Although the safety-catch type linker methodology is conceptually elegant, difficulties have been recently reported. For example, the attachment of the first amino acid to the sulfonamide Kenner's linker resulted in poor coupling yields and racemization.[7] In regards to glycopeptide synthesis, Unverzagt reported on the limitations of the Kenner's sulfonamide linker during the capping step,[8] and Bertozzi and co-workers reported on the unsuccessful glycopeptide synthesis using a combination of the safety-catch linker and solid-phase Fmoc peptide synthesis.[9] As a note, Camarero has recently introduced an aryl hydrazine linker as a safety-catch linker.[10] The third approach for peptide thioester preparation involved the creation of the thioester group after cleaving the peptide from resin. This approach appears to be most practical because conventional peptide synthetic methodology is fully utilized.[11] However, there are some reports involving this approach, for which a satisfactory methodology is needed. Sewing and Hilvert has reported on the use of Me_3Al–thiophenol as a releasing reagent from the resin; unfortunately, concomitant formation of aspartimide at the aspargine residue proved to be problematic.[12] A novel method for the preparation of a thioester using a trithioorthoester was reported by Brask et al.[13]

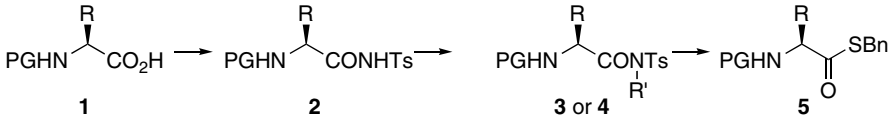
Keywords: Thioester; Glycopeptide; Sequential peptide ligation.
* Corresponding author. Tel.: +81 48 467 9432; fax: +81 48 462 4680; e-mail: smanabe@riken.jp

0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.11.143

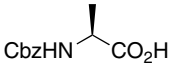
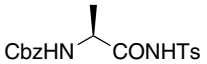
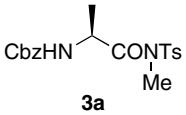
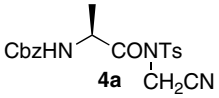
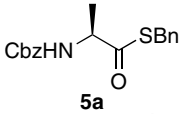
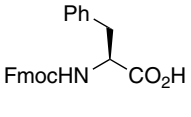
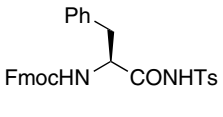
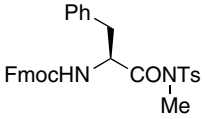
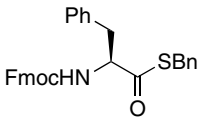
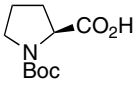
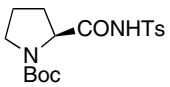
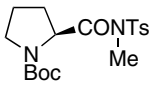
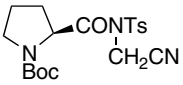
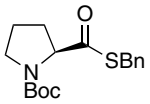
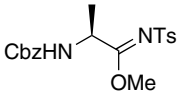
However, its utility is restricted mainly to the preparation of glycine thioesters. Botti and Danishefsky have reported the peptide thioester formation by *O–S* acyl shift and its application to the synthesis of a large peptide.[14] As well as *O–S* shift, *N–S* acyl shift strategy was also reported recently.[15] We report here the facile preparation of sulfonamides from carboxylic acids using *p*-toluenesulfonyl isocyanate.

N-Protected amino acids **1a–c** were converted to the corresponding tosylamide in quantitative yields (Table 1). Typical *N*-protecting groups such as Boc, Fmoc and Cbz have been shown to be stable under these conditions. Subsequent to the establishment of the facile preparation of tosylamides, conversion to the corresponding thioester was carried out according to a reported procedure based on Kenner's linker on solid-phase resin. When tosylamide **2a** was treated with $TMSCHN_2$, N-methylated **3a** and O-methylated **6** were obtained in 63% and 18% yields, because of the nature of delocalized *N*-acylsulfonamide.[16] In constant, methylation using MeI in the presence of K_2CO_3 resulted in the desired N-methylated **3a** in a high yield. Similarly, alkylation using iodoacetonitrile was also successful giving N-alkylated **4a** in a good yield, but requiring a longer reaction time. It was found that microwave irradiation enhanced the reaction rate dramatically. The alkylation reaction was completed within 10 min under microwave

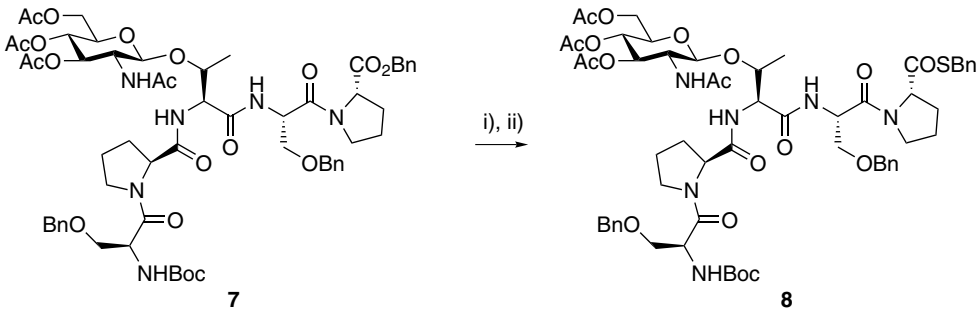
Table 1. The preparation of amino acid thioesters



1 **2** **3** or **4** **5**

Entry	Acid	Tosylamide yield (%)	Alkyl tosylamide yield (%)	Thioester yield (%)
1	 **1a**	 **2a** quant.	 **3a** 63 (method A) 83 (method B)  **4a** 70 (method C) 76 (method D)	 **5a** 90 (from **3a**) 71 (from **4a**)
2	 **1b**	 **2b** quant.	 **3b** 68 (method A) 95 (method E)	 **5b** 89 (from **3b**)
3	 **1c**	 **2c** quant.	 **3c** 84 (method B)  **4c** 90 (method C)	 **5c** 90 (from **3c**) 82 (from **4c**)
			 **6**	

Method A: $TMSCHN_2$, MeOH, PhH, room temperature, Method B: MeI, K_2CO_3, DMF, 60 °C, overnight, Method C: ICH_2CN, K_2CO_3, DMF, 60 °C, overnight, Method D: ICH_2CN, K_2CO_3, DMF, microwave irradiation, 300 W, 1 bar, <10 min, Method E: trimethyloxonium tetrafluoroborate, *i*-Pr_2NEt, DMF.



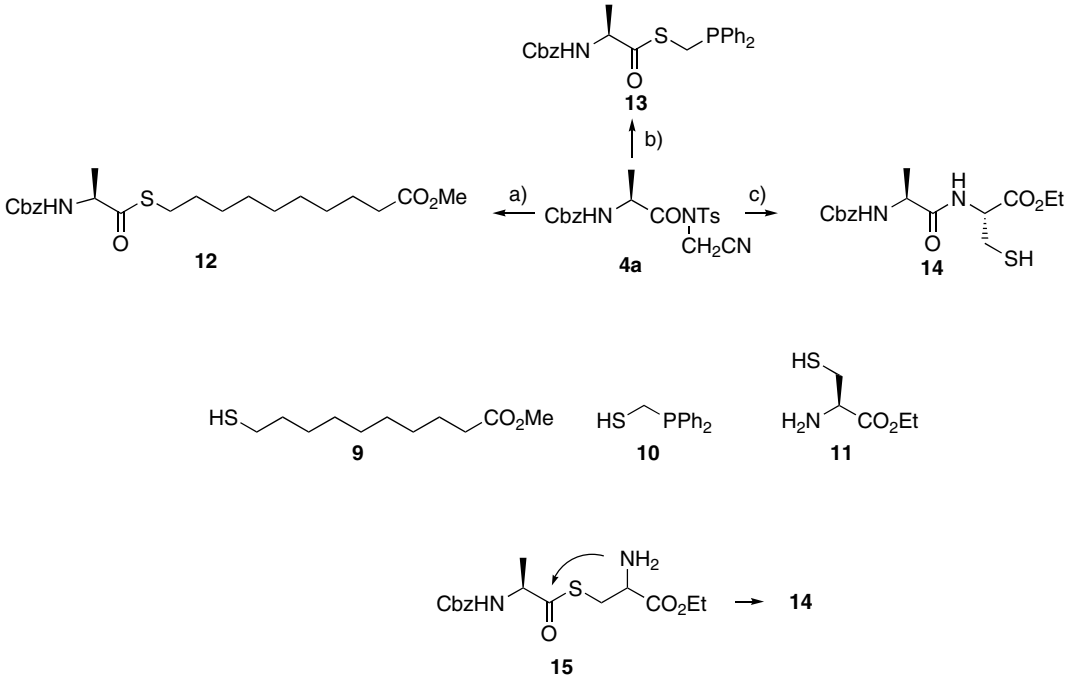
Scheme 1. The synthesis of glycopeptide thioester. Reagents and conditions: (i) H_2, Pd/C (en), MeOH, then *p*-toluenesulfonyl isocyanate, Et_3N, THF, 87%; (ii) $TMSCHN_2$, MeOH, PhH, then benzyl mercaptane, *i*-Pr_2NEt, DMF, 57% (two steps).

irradiation. Compound **3a** reacted with benzyl mercaptan in the presence of *i*-Pr_2NEt in DMF to give thioester **5a** in a high yield very smoothly. Similarly, the reaction of Fmoc-protected amino acid **3b** was also successful. The yield of **3b** was increased by a combination of trimethyloxonium tetrafluoroborate–*i*Pr_2NEt at room temperature for 5 min to 95%. Comparatively, cyanomethylated compounds **4** were more reactive to nucleophilic attacks than methylated compounds **3**. Thus, the reaction of **4a** and 1.5 equiv of benzyl mercaptan was completed within 20 min and afforded the corresponding thioester in 71% yield, while in the case of **3a**, the reaction was not completed within 20 min and afforded the corresponding thioester in 90% yield. The O-alkylated product **6** was not a substrate for substitution reaction of benzyl mercaptan and was recovered unchanged quantitatively. Based on the above results, it appears that the safety-catch linker may be attributable to the alkylation step—in other words, *N*-selective alkylation is required.[17]

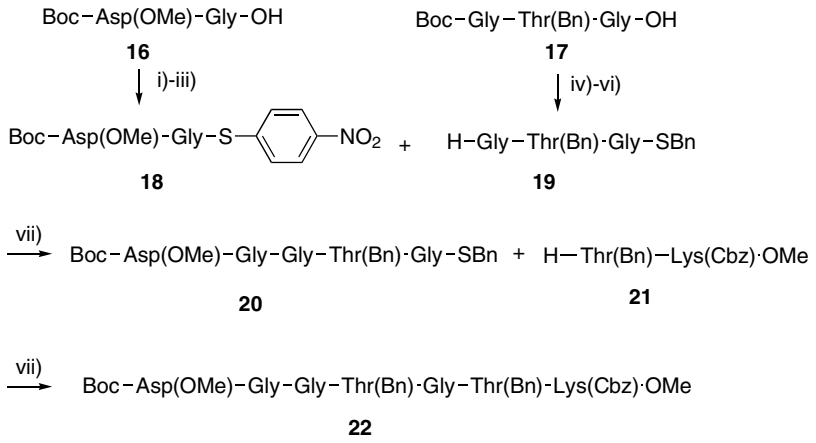
Because the utility of glycopeptide thioester is obvious, we then set out to apply our methodology to glycopeptide synthesis.[18] After selective removal of benzyl ester of **7** by Pd/C(en) under hydrogen atmosphere,[19] carboxylic acid was converted to sulfonamide in 87% yield (Scheme 1). After subsequent activation of sulfonamide by $TMSCHN_2$ in MeOH–PhH, the corresponding benzyl thioester **8** was synthesized in 57% yield in two steps. The glycopeptide sequence is a part of repeating unit at the C-terminus of RNA polymerase II, and *O*-GlcNAc is suggested to play an important role in transcription process.[20]

Next, to demonstrate the advantage of our methodology, various substituents were introduced into the thiol moiety (Scheme 2). Besides benzyl mercaptan, an ester group with a long alkyl thiol **9** and $HSCH_2PPh_2$ **10**[21] gave the corresponding thioesters in high yields under similar conditions. Thiol unprotected cysteine **11** afforded a dipeptide in 85% yield in 5 min; however, it remains unclear whether the amino group reacted directly or thioester **15** was an intermediate.

Since thioester derivatives are now readily available, sequential ligation approach can be achieved in a



Scheme 2. Reagents and conditions: (a) **9**, *i*-Pr_2NEt, DMF, 70%; (b) **10**, *i*-Pr_2NEt, DMF, 87%; (c) **11**, *i*-Pr_2NEt, DMF, 81%.



Scheme 3. Reagents and conditions: (i) H_2, 10% Pd/C, MeOH; then *p*-toluenesulfonyl isocyanate, Et_3N, THF, 91% (two steps); (ii) ICH_2CN, K_2CO_3, DMF, 75%; (iii) *p*-nitrothiophenol, *i*-Pr_2NEt, DMF, quant.; (iv) H_2, 10% Pd/C, MeOH, then *p*-toluenesulfonyl isocyanate, Et_3N, THF 68% (two steps); (v) MeI, K_2CO_3, DMF, 92%; (vi) BnSH, *i*-Pr_2NEt, DMF, quant.; (vii) *i*-Pr_2NEt, DMF, 64%; (viii) **20**, **21**, HOOBt, AgCl, *i*-Pr_2NEt, DMF, 64%.

chemoselective manner (Scheme 3).[22] The first peptide segment **18** has a highly selective *p*-nitrophenyl thioester, whereas the middle segment **19** was benzyl thioester moiety. Both thioesters **18** and **19** were prepared from carboxylic acids **16** and **17** using our methodology in high yields. The first ligation between *p*-nitrophenyl thioester **18** and amino group carrying benzyl thioester **19** was carried out in DMF, which was complete within 30 min at room temperature.[23] The final product **22**, which is a part of a glycosaminoglycan-binding protein Midkine,[24] was obtained in 64% yield by the subsequent reaction between amine **21** and terminal thioester **20** by AgCl–HOOBt (3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine) activation.[25–27]

In conclusion, the novel methodology for peptide thioester preparation was demonstrated. The sequential peptide ligation was also demonstrated. Our peptide thioester synthesis can readily accommodate to the standard Fmoc as well as Boc-based peptide synthetic strategy. Our methodology is operationally simple, and we believe that it will find practical use and be highly utilized for preparing thioesters of various chemical groups such as glycoside, phosphates, biotins and fluorescent chromophores.

Acknowledgements

We thank Professor R. T. Raines for helpful discussions. We thank Dr. Teiji Chihara and his staff for elemental analyses and Ms. Ikuko Kagawa at the Research Resource Center of RIKEN Brain Science Institute for the preparation of the peptides. We also thank Ms. Akemi Takahashi for her technical assistance.

References and notes

1. (a) Coltart, D. M. *Tetrahedron* **2000**, *56*, 349–3491; (b) Yeo, D. S. Y.; Srinivasa, R.; Chen, G. Y. J.; Yao, S. Q. *Chem. Eur. J.* **2004**, *10*, 664–4672; (c) Kimmerlin, T.; Seebach, D. *J. Peptide Res.* **2005**, *65*, 229–260; (d) Nilsson, B. L.; Soellner, M. B.; Raines, R. T. *Annu. Rev. Biophys. Biomol. Struct.* **2005**, *34*, 91–118; (e) Muralidharan, V.; Muir, T. W. *Nat. Methods* **2006**, *3*, 429–438.
2. (a) Li, X.; Kawakami, T.; Aimoto, S. *Tetrahedron Lett.* **1998**, *39*, 8669–8672; (b) Clippingdale, A. B.; Barrow, C. J.; Wade, J. D. *J. Pept. Sci.* **2000**, *6*, 225–234; (c) Bu, X.; Xie, G.; Law, C. W.; Guo, Z. *Tetrahedron Lett.* **2002**, *43*, 2419–2422.
3. Hasegawa, K.; Sha, Y. L.; Bang, J. K.; Kawakami, T.; Akaji, K.; Aimoto, S. *Lett. Pept. Sci.* **2002**, *8*, 277–284.
4. (a) Kenner, G. W.; Mcdermott, J. R.; Sheppard, R. C. *J. Chem. Commun.* **1971**, 636–637; (b) Backes, B. J.; Ellman, J. A. *J. Org. Chem.* **1999**, *64*, 2322–2330.
5. (a) Ingenito, R.; Bianchi, E.; Fattori, D.; Pessi, A. *J. Am. Chem. Soc.* **1999**, *121*, 11369–11374; (b) Ollivier, N.; Behr, J.-B.; El-Mahdi, O.; Blanpain, A.; Melnyk, O. *Org. Lett.* **2005**, *7*, 2647–2650.
6. 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.
7. Ingenito, R.; Dreznjak, D.; Guffler, S.; Wenschuh, H. *Org. Lett.* **2002**, *4*, 1187–1188.
8. Mezzato, S.; Schaffrath, M.; Unverzagt, C. *Angew. Chem., Int. Ed.* **2005**, *44*, 1650–1654.
9. Marcaurelle, L. A.; Mizoue, L. S.; Wilken, J.; Oldham, L.; Kent, S. B. H.; Handel, T. M.; Bertozzi, C. R. *Chem. Eur. J.* **2001**, *7*, 1129–1132.
10. Camarero, J. A.; Hackel, B. J.; De Yoreo, J. J.; Mitchell, A. R. *J. Org. Chem.* **2004**, *69*, 4145–4151.
11. von Eggelkraft-Gottanka, R.; Klose, A.; Beck-Sickinger, A. G.; Beyermann, M. *Tetrahedron Lett.* **2003**, *44*, 3551–3554.
12. Sewing, A.; Hilvert, D. *Angew. Chem., Int. Ed.* **2001**, *40*, 3395–3396.
13. Brask, J.; Albericio, F.; Jensen, K. J. *Org. Lett.* **2003**, *5*, 2951–2953.
14. (a) Botti, P.; Villain, M.; Manganiello, S.; Gaertner, H. *Org. Lett.* **2004**, *6*, 4861–4864; (b) Warren, J. D.; Miller, J. S.; Keding, S. J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 6576–6578; (c) Chen, G.; Warren, J. D.; Chen, J.; Wu, B.; Wan, Q.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2006**, *128*, 7460–7462.
15. (a) Kawakami, T.; Sumida, M.; Nakamura, K.; Vorherr, T.; Aimoto, S. *Tetrahedron Lett.* **2005**, *46*, 8805–8807; (b)

Ohta, Y.; Itoh, S.; Shigenaga, A.; Shintaku, S.; Fujii, N.; Otaka, A. *Org. Lett.* **2006**, *8*, 467–470; (c) Nagaike, F.; Onura, Y.; Kanazawa, C.; Hojo, H.; Ueki, A.; Nakahara, Y.; Nakahara, Y. *Org. Lett.* **2006**, *8*, 4465–4468.
16. (a) Ho, T.-L. *Chem. Rev.* **1975**, *75*, 1–20; (b) He, Y.; Wilkins, J. P.; Kiessling, L. L. *Org. Lett.* **2006**, *8*, 2483–2485.
17. Merkx, R.; Brouwer, A. J.; Rijkers, D. T. S.; Liskamp, R. M. J. *Org. Lett.* **2005**, *7*, 1125–1128. They prepared sulfonamides from thio acids. The difficulty of the alkylation is also mentioned.
18. (a) Warren, J. D.; Miller, J. S.; Keding, S. J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 6576–6578; (b) Hojo, H.; Matsumoto, Y.; Nakahara, Y.; Ito, E.; Suzuki, Y.; Suzuki, M.; Suzuki, A.; Nakahara, Y. *J. Am. Chem. Soc.* **2005**, *127*, 13720–13725; (c) Kajihara, Y.; Yoshihara, A.; Hirano, K.; Yamamoto, N. *Carbohydr. Res.* **2006**, *341*, 1333–1340.
19. Sajiki, H.; Hattori, K.; Hirota, K. *J. Org. Chem.* **1998**, *63*, 7990–7992.
20. Comer, F. I.; Hart, G. W. *Biochemistry* **2001**, *40*, 7845–7852.
21. Nilsson, B. L.; Kiessling, L. L.; Rains, R. T. *Org. Lett.* **2001**, *3*, 9–12.
22. (a) Bang, D.; Pentelute, B. L.; Gates, Z. P.; Kent, S. B. H. *Org. Lett.* **2006**, *8*, 1049–1052; (b) Johnson, E. C. B.; Kent, S. B. H. *J. Am. Chem. Soc.* **2006**, *128*, 6640–6646.
23. Gly was chosen at the C-termini as an initial investigation to avoid the racemization possibility.
24. (a) Tsutsui, J. I.; Uehara, K.; Kadomatsu, K.; Matsubara, S.; Muramatsu, T. *Biochem., Biophys. Res. Commun.* **1991**, *176*, 792–797; (b) Kojima, T.; Katsumi, A.; Yamazaki, T.; Muramatsu, T.; Nagasaka, T.; Ohsumi, K.; Saito, H. *J. Biol. Chem.* **1996**, *271*, 5914–5920; (c) Kinnunen, T.; Kaksonen, M.; Saarinen, J.; Kalkkinen, N.; Peng, H. B.; Rauvala, H. *J. Biol. Chem.* **1998**, *273*, 10702–10708.
25. The sequential ligation approaches using Staudinger methodology and quinolium thioester methodology are now under investigation: (a) Nilsson, B. L.; Hondal, R. J.; Soellner, M. B.; Raines, R. T. *J. Am. Chem. Soc.* **2003**, *125*, 5267–5268; (b) Leleu, S.; Penhoat, M.; Bouet, A.; Dupas, G.; Papamicaël, C.; Marsais, F.; Levacher, V. *J. Am. Chem. Soc.* **2005**, *127*, 15668–15669.
26. Kawakami, T.; Kogure, S.; Aimoto, S. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 3331–3338.
27. The corresponding cyanomethylated tosylamide of **16** can be also used directly in sequential ligation protocol instead of **18** to give **20** in 55% yield.