Tetrahedron Letters 56 (2015) 6565–6568

Contents lists available at ScienceDirect

Tetrahedron Letters

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

# Synthesis of D,L-amino acid derivatives bearing a thiol at the β-position and their enzymatic optical resolution

Yasuhito Morishita, Tomoka Kaino, Ryo Okamoto, Masayuki Izumi, Yasuhiro Kajihara *

*Department of Chemistry, Graduate School of Science, Osaka University, 1-1, Machikaneyama, Toyonaka, Osaka 560-0043 Japan*

ARTICLE INFO

*Article history:*
Received 7 July 2015
Revised 30 September 2015
Accepted 5 October 2015
Available online 9 October 2015

*Keywords:*
Protein chemical synthesis
Native chemical ligation
Desulfurization
Amino acid

ABSTRACT

Amino acids bearing a thiol group at the β-position are useful for native chemical ligation. Phenylalanine and tyrosine derivatives bearing a thiol group at the β-position were synthesized. Racemic phenylalanine and tyrosine were selected as starting materials and were introduced a bromo atom at the β-position by photoreaction. Subsequent substitution reaction of the bromo atom with *p*-methoxybenzylmercaptan yielded the corresponding amino acids bearing a thiol group at the β-position. Enzymatic optical resolution using L-aminoacylase and subsequent chemical conversion gave the corresponding optically pure L- and D-phenylalanine and tyrosine derivatives bearing a thiol group at the β-position.



Ever since native chemical ligation (NCL) was found in 1994, chemical syntheses of protein have gained momentum. This reaction has enabled us to easily couple a peptide-α-thioester with another peptide bearing a cysteine at the N-terminus in a neutral solution.[1] NCL utilizes a thiol exchange reaction and the subsequent S-to-N acyl migration results in a native amide bond. This powerful peptide coupling reaction allows us to synthesize not only the native proteins but also post-translationally modified proteins such as glycoproteins.

NCL requires a cysteine residue at the ligation site and therefore cannot be applied for the synthesis of some proteins that have no cysteine or unsuitable number of cysteine residues in the target polypeptide chains. In general, a number of suitable cysteine residues are essential, as chemical synthesis of protein requires repetitive NCL reactions employing 20–30 amino acid residue peptides to construct a target polypeptide chain.

In order to overcome these drawbacks, a recent strategy for protein synthesis employed a unique amino acid bearing a thiol group at the β-position for NCL as a cysteine surrogate. The thiol group was removed by desulfurization reaction resulting in the formation of a native amino acid at the ligation site after NCL.[2] Using this concept, syntheses of amino acids bearing a thiol at the β-position or γ-position that correspond to phenylalanine, valine, leucine, lysine, proline, glutamine, threonine, asparagines, aspartic acid, tryptophan, and arginine have been reported recently.[3–13] These amino acid derivatives incorporated at the N-terminal of peptide indeed showed suitable reactivity in NCL and desulfurization with reductive conditions such as radical initiator (VA044), *tert*-butylthiol, and (tris(2-carboxyethyl)phosphine) (TCEP).

However, mild reaction conditions were selected for the syntheses of these amino acid derivatives to avoid undesired racemization and these strategies resulted in long synthetic steps. These synthetic steps would hinder the preparation of corresponding amino acid derivatives bearing a thiol group in sufficient amounts for chemical protein synthesis.

In addition to the chemical syntheses of proteins and glycoproteins, recently chemical synthesis of D-protein using D-amino acids has also been demonstrated in order to examine racemic protein crystallization and quasi-racemic glycoprotein crystallization.[14,15] These emerging protein crystallization methodologies require both corresponding D- and L-proteins and therefore the practical chemical synthetic strategy of these derivatives requires D- and L-amino acids bearing a thiol at the β-position.

This Letter describes efficient syntheses of phenylalanine and tyrosine bearing a thiol group at the β-position in both D- and L-forms using a single synthetic procedure for the syntheses of D- and L-proteins which combine the NCL and desulfurization reaction strategies.

In order to synthesize corresponding amino acids bearing a thiol group at the β-position in both D- and L-forms, we designed a synthetic strategy using racemic amino acid as a starting material and optical resolution employing D- and L-aminoacylases. Because aminoacylase is known to be a cheap enzyme that hydrolyzes an α-amide group, this enzyme is suitable for organic synthesis and

* Corresponding author. Tel.: +81 60 6850 5380.
*E-mail address:* kajihara@chem.sci.osaka-u.ac.jp (Y. Kajihara).

http://dx.doi.org/10.1016/j.tetlet.2015.10.015
0040-4039/

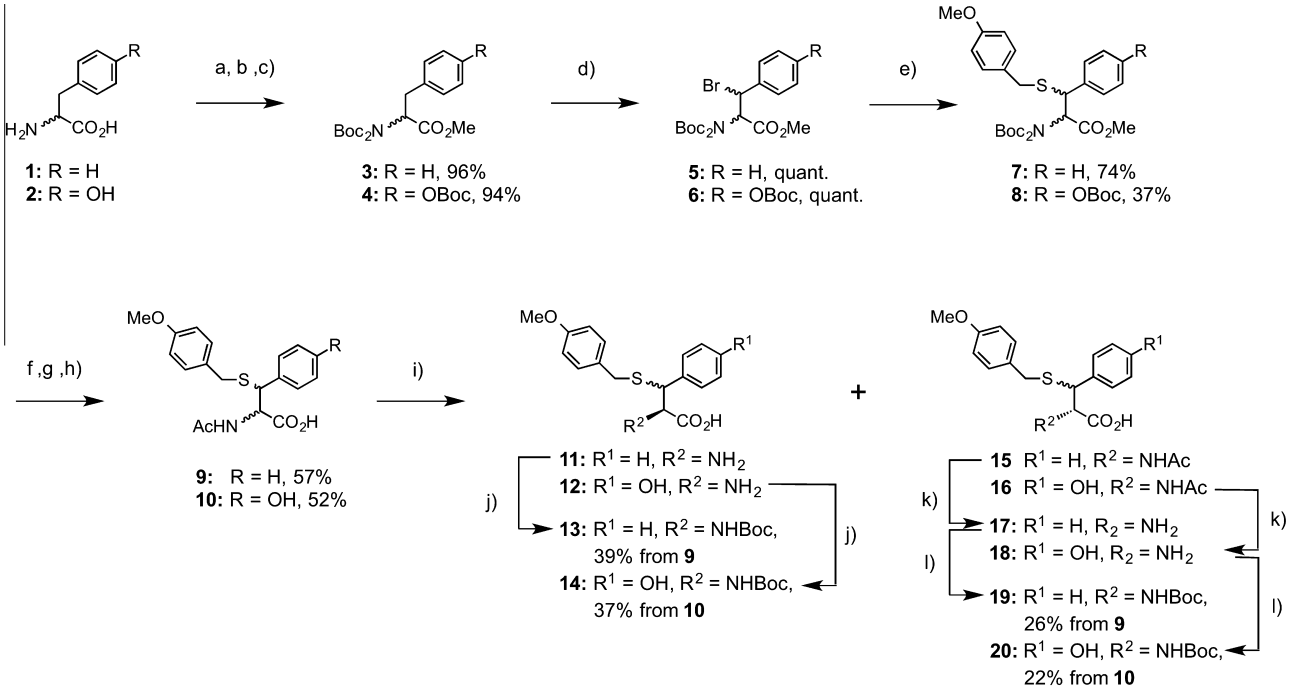


**Figure 1.** Synthesis of *N*-*t*-Boc-DL-phenylalanine and tyrosine derivatives bearing a thiol group at the β-position. (a) $SOCl_2$, MeOH, reflux, (b) $Boc_2O$, $Na_2CO_3$, ($THF/H_2O$ = 1:1), rt, (c) $Boc_2O$, 4-dimethylaminopyridine, MeCN, rt, (d) NBS, hν (200 W), $CCl_4$, (e) *p*-methoxybenzylmercaptan, DBU, DMF, rt, (f) TFA, DCM, rt, (g) $Ac_2O$, pyridine, DCM, rt, (h) 1 M NaOH aq, rt, THF, (i) L-aminoacylase, sodium phosphate buffer (20 mM, pH 8.0), 37 °C, (j) $Boc_2O$, THF, rt, (k) 2 M HCl aq, 100 °C, (l) $Boc_2O$, $Na_2CO_3$, THF, rt.

there were indeed many reports employing this enzyme for optical resolution.[16,17]

A racemic mixture of commercially available phenylalanine **1** and tyrosine **2** was converted into β-bromo derivatives **5**[3] and **6**, respectively, by photoreaction, followed by a substitution reaction with alkylthiol. Aromatic amino acids **1** and **2** were dissolved in $CCl_4$ and *N*-bromosuccinimide (NBS) was added to this solution. Irradiation of the reaction solution with 200 W incandescent lamp for 30 min under the reflux condition gave the corresponding brominated aromatic amino acids **5** and **6** quantitatively and these compounds were used in the next step without further purification. Diastereomer ratio (*RR*+*SS*/*RS*+*SR*) was estimated to be ~1/1 based on the integrals of corresponding $^1H$ NMR signals. Subsequent substitution reaction with *p*-methoxybenzylmercaptan yielded **7** and **8** in 74% and 37%, respectively, after silica gel column chromatography. Diastereomer ratios of isolated **7** and **8**

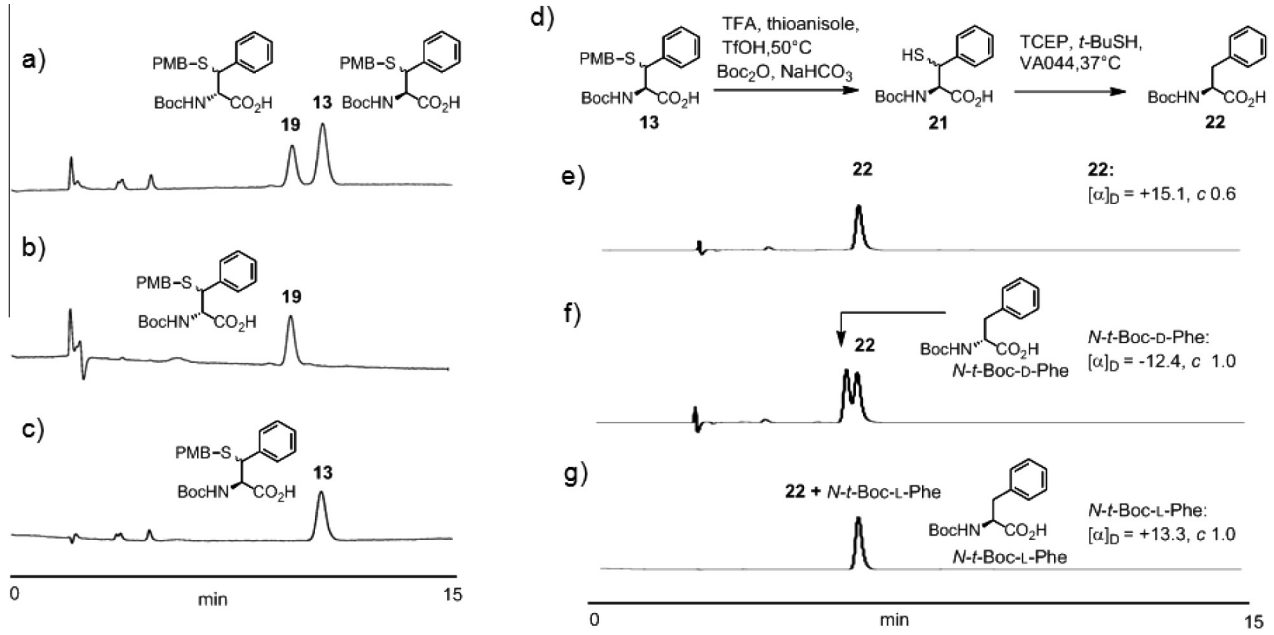


**Figure 2.** Confirmation of optical resolutions of D- and L-phenylalanine derivatives. HPLC analysis of phenylalanine derivatives bearing a thiol group at the β-position using chiral column (DAICEL, OJ-RH 4.6 × 150 mm) (a–c, e–g). HPLC analytical conditions: isocratic water/MeCN = 70:30 containing 0.1% formic acid at a flow rate of 0.8 mL/min and detection at 254 nm. (d) Conversion of L-phenylalanine derivative **13** to *N*-*t*-Boc-L-phenylalanine **22**.

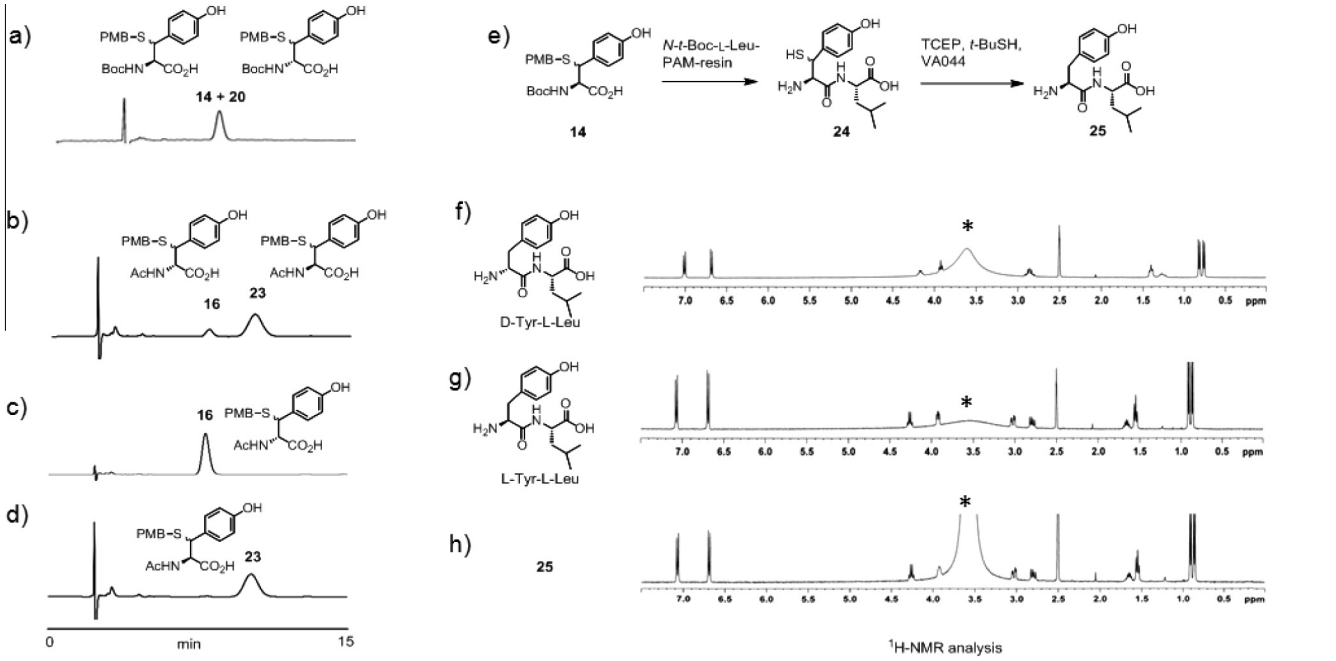


**Figure 3.** Confirmation of optical resolutions of tyrosine derivatives. (a–d) HPLC analysis of tyrosine derivatives bearing a thiol group at the β-position using chiral column (DAICEL, OJ-RH 4.6 × 150 mm). HPLC analytical conditions: isocratic water/MeCN = 75:25 containing 0.1% formic acid at a flow rate of 0.8 mL/min and detection at 254 nm. (e) Synthesis of dipeptide by *t*-Boc-solid phase peptide synthesis and desulfurization. $^1$H NMR spectra of (f) authentic D-Tyr-L-Leu, (g) authentic L-Tyr-L-Leu, and (h) compound **25**. Asterisk ∗ indicates water signal.

were estimated to be ~4/6 and ~1/4, respectively, although we did not determine which is *RR*+*SS* or *RS*+*SR*. In this reaction we found dehydroamino acid derivative as a byproduct. Formation of dehydroamino acid derivative may indicate involvement of the Michael addition reaction when racemic amino acids **7** and **8** bearing a *p*-methoxybenzylthio group at the β-position are formed, however we excluded such a possibility because treatment of dehydroamino acid derivatives with *p*-methoxybenzylmercaptan under the same condition did not give **7** and **8** (data not shown). Therefore we speculated that this substitution reaction proceeds via both $S_N2$ and $S_N1$ type mechanisms.

For subsequent optical resolution, corresponding *N*-di *t*-Boc-phenylalanine derivative **7** and tyrosine derivative **8** were converted into acetamide derivatives **9** and **10** through deprotection of *N*-di-*t*-Boc group followed by acetamidation in 57% and 52% yields (recrystallized as diastereomer mixtures) respectively. Enzymatic deacetylation with L-aminoacylase was then examined. Enzymatic optical resolution toward phenylalanine derivative **9** with *Aspergillus melleus* L-aminoacylase [EC3.5.1.14] was first examined. This reaction was monitored by reverse phase HPLC and this enzymatic reaction smoothly gave product **11** in reasonable ca. 50% yield (quantitative based on L-substrate, HPLC data not shown). The same L-aminoacylase also converted tyrosine derivative **10** to amino derivative **12** in ca. 50% yield (quantitative based on L-substrate). The resultant **11** and **12** were used in the next step without further purification. Amino derivatives **11** and **12** were converted into *N*-*t*-Boc-β-(*p*-methoxybenzylthio)-L-Phe-OH **13** and *N*-*t*-Boc-β-(*p*-methoxybenzylthio)-L-Tyr-OH **14** with $Boc_2O$ that are suitable monomers for the *t*-Boc solid phase peptide synthesis.

In terms of the optical resolution with *Escherichia coli* D-aminoacylase [EC3.5.1.81], each racemic mixture of phenylalanine derivative **9** or tyrosine derivative **10** was treated with D-aminoacylase, however these D-aminoacylase reactions did not give products in suitable yields. Despite our extensive optimization of reaction conditions with regard to temperature, pH and substrate concentration, we could not obtain H-D-Phe-OH derivative **17** and H-D-Tyr-OH derivative **18** in moderate yields.

We thus changed the strategy to employ the chemical conversion of D-acetamide derivatives **15** and **16** into corresponding D-amino derivatives **17** and **18** by acid hydrolysis after complete consumption of L-amino acid derivative in a mixture of racemic phenylalanine derivative **9** and tyrosine derivative **10** by L-aminoacylase. This chemical hydrolysis was performed with 2 M HCl solution at 100 °C followed by protection of amino group with *t*-Boc group was performed to give Boc-D-Phe-OH derivative **19** and Boc-D-Tyr-OH derivative **20** in 26% and 22% yields from racemic mixtures **9** and **10**, respectively.

Because we obtained presumably L- and D-amino acid derivatives by the optical resolution based on the L-aminoacylase strategy and the chemical conversion strategy, respectively, we then analyzed the optical purity of presumably individual D- and L-phenylalanine (Fig. 2) and tyrosine (Fig. 3) derivatives bearing a *p*-methoxylbenzylthio group at the β-position by a chiral column, however this analysis did not give suitable results. Figure 2b and c shows the isolated enantiomeric isomers of phenylalanine derivatives **19** and **13**, respectively, and Figure 2a shows the HPLC profile of an intentional mixture of both phenylalanine derivatives. As shown in Figure 2b and c, both HPLC analyses unfortunately showed only one peak each. As individual enantiomeric amino acid derivatives **13** and **19** have two chiral centers corresponding to the α- and β-positions, we thus expected to observe two peaks in both Figure 2b and c.

As we could not evaluate the absolute configuration at the α-carbon using chiral columns, we instead examined the desulfurization at the β-position to convert it into amino acid bearing a chiral center at the only α-carbon. Then we successfully evaluated optical purity of individual L- and D-phenylalanine derivatives. Deprotection of **13** gave thiol **21** and subsequent radical desulfurization reaction with VA-044, *tert*-butylthiol and TCEP gave **22** (Fig. 2d). The resultant compound **22** was compared with authentic *N*-*t*-Boc-L- and D-phenylalanine. Figure 2e shows the purity of the

product **22** ($[\alpha]_D^{25}$ +15.1, *c* 0.6, MeOH) by HPLC using a chiral column and Figure 2f shows an HPLC profile of a mixture of **22** with authentic *N*-*t*-Boc-D-phenylalanine (commercially available sample: $[\alpha]_D^{25}$ −12.4, *c* 1.0, MeOH). Figure 2g shows a mixture of **22** with authentic *N*-*t*-Boc-L-phenylalanine (commercially available sample: $[\alpha]_D^{25}$ +13.3, *c* 1.0, MeOH). Figure 2e–g clearly indicates the product of desulfurization reaction toward **13** to be homogeneous *N*-*t*-Boc-L-phenylalanine. This result also indicated that optical resolution with L-aminoacylase recognizing the α-carbon was successfully performed. Figure 2a–c show that the separation of **13** and **19** resulted from the difference of chirality at the α-carbon of the phenylalanine derivative. Through these chemical conversions and analyses using a chiral column, we could clearly confirm that we obtained the desired *N*-*t*-Boc-phenylalanine bearing a thiol group at the β-position in both D- and L-forms in sufficient yield.

We also analyzed L-tyrosine derivative **14** and D-tyrosine derivative **20** (Fig. 1) using a chiral column, however, the chiral column could not separate these derivatives (Fig. 3a). We already knew that racemic acetamide derivative **10** (Fig. 1), a precursor for the L-aminoacylase reaction, showed a well-separated HPLC profile on the chiral column (data not shown). We therefore converted **14**, which was prepared by the treatment of **10** with L-aminoacylase and subsequent protection with $Boc_2O$, into the acetamide derivative **23** (Fig. 3b: synthetic scheme is not shown). The resulting derivative was compared with *N*-Ac-D-tyrosine derivative **16** that had remained after the treatment of racemic mixture **10** by L-aminoacylase reaction (Fig. 3c).

As shown in Figure 3b–d, although both *N*-Ac-tyrosine derivatives **16** and **23** were well separated by the chiral column, we could not confirm whether this separation resulted from the chirality of the α-carbon or β-carbon. We therefore prepared a dipeptide by using *N*-*t*-Boc-L-tyrosine derivative **14** and L-leucine under the Boc-solid phase peptide synthetic conditions[18] to confirm the absolute configuration of the α-carbon. After purification, synthetic dipeptide **24** was treated with TCEP, *tert*-butylthiol and VA-044 for desulfurization to yield dipeptide **25**. We then compared **25** with authentic L-Tyr-L-Leu and D-Tyr-L-Leu using NMR. As shown in Figure 3f–h, individual NMR spectra clearly indicated that the dipeptide **25** derived from *N*-*t*-Boc-tyrosine derivative **14** was identical to L-Tyr-L-Leu. As shown in Figure 3b–d, the HPLC profiles showed well separated patterns of tyrosine derivative **23** (Fig. 3d), derived from L-aminoacylase treatment and followed by acetamidation, and **16,** which had remained after L-aminoacylase treatment of a racemic mixture of **10** (Fig. 3c). When both results were combined, as shown in Figure 3b–d and f–h, these data clearly indicated that the tyrosine derivatives bearing thiol groups at the β-position were L-derivative **14** and D-derivative **20**.

As shown in Figures 2 and 3, we confirmed the absolute configurations of the α-carbons of phenylalanine and tyrosine derivatives bearing a thiol at the β-position in both D- and L-forms. These data clearly prove that our synthetic strategy yielded the desired optically pure amino acid derivatives. Compounds **13**, **14**, **19**, and **20** were obtained as diastereomixtures at the β-positions, however we assume that both diastereomers would be usable in NCL even though there may be some differences in reaction velocities.[4]

In conclusion, we successfully synthesized *N*-*t*-Boc-D- and L-phenylalanine and new *N*-*t*-Boc-D- and L-tyrosine bearing a *p*-methoxybenzylthio group at the β-position in 10 steps and 11 steps, respectively. Although 10-step conversion for the synthesis of the L-phenylalanine derivative[3] is the same compared to that of a previous report, the conversion yield elevated from 9% to 16% (total yield of the previous synthesis in 11 steps). More importantly, our synthetic strategy makes it unnecessary to consider racemization. We can easily separate corresponding D- and L-amino acids, both of which are useful building blocks for racemic protein crystallization, via enzymatic optical resolution using L-aminoacylase and the chemical conversion strategy. Research to obtain other amino acids bearing a thiol group at the β-position is currently in progress.

## Acknowledgments

This work was supported in part by Grant-in-Aid for Scientific Research from the Ministry of Education, Sports, Science and Technology of Japan (No. 26248040 and 23245037).

## Supplementary data

Supplementary data (experimental procedures, and spectral/characterization data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.10.015.

## References and notes

1. Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776–779.
2. Yan, L. Z.; Dawson, P. E. *J. Am. Chem. Soc.* **2001**, *123*, 526–533.
3. Crich, D.; Banerjee, A. *J. Am. Chem. Soc.* **2007**, *129*, 10064–10065.
4. Chen, J.; Wan, Q.; Yuan, Y.; Zhu, J. L.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2008**, *47*, 8521–8524.
5. Harpaz, Z.; Siman, P.; Kumar, S. A.; Brik, A. *Chembiochem* **2010**, *11*, 1232–1235.
6. Yang, R. L.; Pasunooti, K. K.; Li, F. P.; Liu, X. W.; Liu, C. F. *J. Am. Chem. Soc.* **2009**, *131*, 13592–13593.
7. Chen, J.; Wang, P.; Zhu, J. L.; Wan, Q.; Danishefsky, S. J. *Tetrahedron* **2010**, *66*, 2277–2283.
8. Shang, S. Y.; Tan, Z. P.; Dong, S. W.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2011**, *133*, 10784–10786.
9. Siman, P.; Karthikeyan, S. V.; Brik, A. *Org. Lett.* **2012**, *14*, 1520–1523.
10. Malins, L. R.; Cergol, K. M.; Payne, R. J. *ChemBioChem* **2013**, *14*, 559–563.
11. Thompson, R. E.; Chan, B.; Random, L.; Jolliffe, K. A.; Payne, R. J. *Angew. Chem., Int. Ed.* **2013**, *52*, 9723–9727.
12. Guan, X.; Drake, M. R.; Tan, Z. *Org. Lett.* **2014**, *16*, 290–293.
13. Malins, L. R.; Cergol, K. M.; Payne, R. *J. Chem. Sci.* **2014**, *5*, 260–266.
14. Pentelute, B. L.; Gates, Z. P.; Tereshko, V.; Dashnau, J. L.; Vanderkooi, J. M.; Kossiakoff, A. A.; Kent, S. B. H. *J. Am. Chem. Soc.* **2008**, *130*, 9695–9701.
15. Okamoto, R.; Ma-ndal, K.; Ling, M.; Luster, A. D.; Kajihara, Y.; Kent, S. B. H. *Angew. Chem., Int. Ed.* **2014**, *53*, 5194–5198.
16. Baker, S. R.; Goldsworthy, J.; Harden, R. C.; Salhoff, C. R.; Schoepp, D. D. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 223–228.
17. Sugie, M.; Suzuki, H. *Agric. Biol. Chem.* **1980**, *44*, 1089–1095.
18. Murakami, M.; Okamoto, R.; Izumi, M.; Kajihara, Y. *Angew. Chem., Int. Ed.* **2012**, *51*, 3567–3572.