Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 45 (2004) 491–494
Tetrahedron Letters

Synthesis of L-α-amino-ω-bromoalkanoic acid for side chain modification

Louis A. Watanabe,[a] Binoy Jose,[b] Tamaki Kato,[c] Norikazu Nishino[b,c,*] and Minoru Yoshida[b,d]

[a]*Faculty of Engineering, Kyushu Institute of Technology, Kitakyushu 804-8550, Japan*
[b]*CREST Research Project, Japan Science and Technology Agency, Saitama 332-001, Japan*
[c]*Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, Kitakyushu 808-0196, Japan*
[d]*RIKEN, Saitama 351-0198, Japan*

Received 18 October 2003; revised 30 October 2003; accepted 4 November 2003

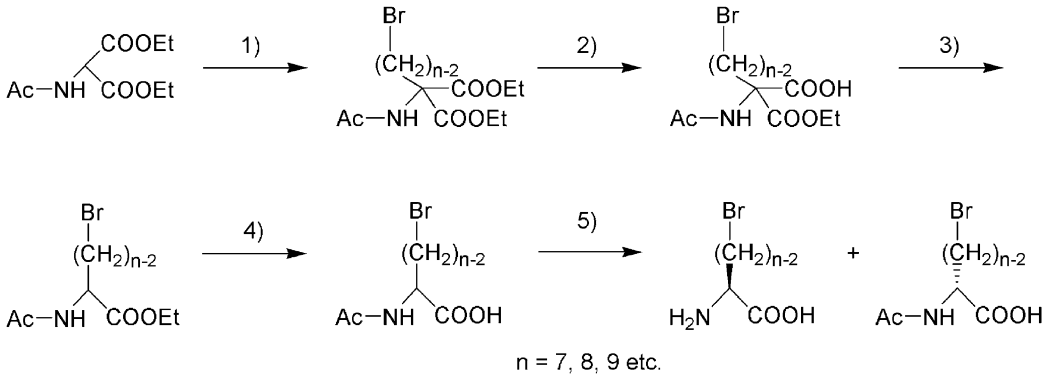
Abstract—L-α-Amino-ω-bromoalkanoic acids with side chain lengths varying from 4 to 10 methylene units have been conveniently synthesized as useful intermediates for the synthesis of functionalized non-natural amino acids.

The synthesis of non-natural amino acids always attracted attention of synthetic chemists due to the improvement in the binding potency, chemical and biological stability and pharmacokinetic characteristics upon introduction into peptide based compounds. This has resulted in the development of vast number of methods for the synthesis of non-natural amino acids.[1] They include at least 100 derivatives of phenylalanine, in which the phenyl group is widely modified, while aliphatic amino acids have been paid less attention. Optically pure aliphatic amino acids having easily replaceable functional groups at the ω-positions are highly interesting synthetic targets, as the functional group at the ω-position can modify to the required group, which provide a series of non-natural amino acids. We are interested in the synthesis of optically pure non-natural aliphatic amino acid containing a bromide in the side chain that serves as a precursor of many non-natural amino acid. Few methods for the synthesis of ω-halo-α-amino acids are available.[2–5] However, the scope of the reported methods for the synthesis of ω-halo-α-amino acids are limited due to many reasons such as, the formation of racemic products with difficulty in purification,[2] involve tedious procedures and resulting in low yields,[3] and could not use 9-fluorenylmethoxycarbonyl (Fmoc) protection strategy. The use of natural amino acids[4,5] as the starting material would not provide α-amino-ω-bromoalkanoic acids with varying side-chain lengths. Here we report a convenient method for the synthesis of α-amino-ω-bromoalkanoic acids (Ab*n*, $n = 6,7,8,9,12$) from readily available reagents.

The general method for the synthesis of α-amino-ω-bromoalkanoic acids (Ab*n*) starting from commercially available diethyl acetamidomalonate with dibromo alkanes is illustrated in Scheme 1. The synthesis of amino acids starting from diethyl acetamidomalonate and bromide derivatives is a widely used method especially for the synthesis of phenylalanine derivatives.[6] However, to our knowledge, there was no report on the synthesis of an amino acid containing a long alkyl chain with a replaceable bromide at the ω-position. Excess amount of dibromoalkanes were reacted with diethyl acetamidomalonate in the presence of sodium ethoxide in abs ethanol.[7] One equivalent of 1 N NaOH was added at 0–5 °C to the reaction mixture and obtained the corresponding monoacid monoester as white solid after work up. It was then decarboxylated by refluxing in toluene. After evaporation of toluene the resulting Ac-DL-Ab*n*-OEt as an oil was dissolved in ethanol and hydrolysed using NaOH at 0–5 °C to yield Ac-DL-Ab*n* as white powder in quantitative yield after work up. Finally, *Aspergillus genus* aminoacylase (TCI) was

Keywords: Amino acid; Non-natural; Functional group; Side chain modification.
* Corresponding author. Tel./fax: +81-93-695-6061; e-mail: nishino@life.kyutech.ac.jp

0040-4039/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2003.11.007



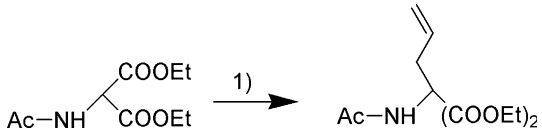
Scheme 1. Reagents and conditions: (1) (i) EtOH, sodium ethoxide, reflux, 30 min; (ii) $Br(CH_2)_{n-2}Br$, reflux, 3 h, 80–87%; (2) NaOH aq, EtOH, 0 °C, 5 h, 70–79%; (3) toluene, reflux, 3 h, 96–100%; (4) NaOH aq, EtOH, 0 °C, 3 h, 70–90%; (5) aminoacylase, H_2O, pH 7, 38 °C, 24 h, 66–80%.

applied to resolve the racemic mixture.[8] The precipitated L-Ab*n*s were obtained as crystalline powder by the filtration of the aqueous solution.[9] The unreacted Ac-D-Ab*n*s were recovered from the acidified aqueous solutions by extraction.

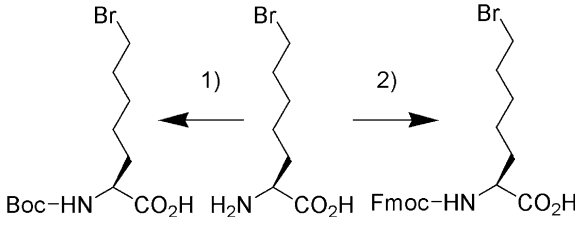
Eventhough, the method is a traditional one, working well for the synthesis of Ab*n*s with $n \geqslant 7$. On the other hand, the amino acids with lower chain length encountered some difficulty during the synthesis. In the case of Ab6, we could not obtain any L-Ab6 after resolution step due to the cyclization of L-Ab6 to pipecolic acid during the enzymatic separation by aminoacylase.[10] To overcome the problem of cylization, we prepared Boc-DL-Ab6-OEt using the same procedure, with diethyl (Boc-amino)malonate as one of the starting material instead of diethyl acetamidomalonate as shown in Scheme 2. Boc-DL-Ab6-OEt was subjected to the action of subtilisin Carlsberg from *Bacillus licheniformis* (Sigma) in a mixture of DMF and water (1/1, v/v). Boc-L-Ab6 was isolated as a colourless oil, which was deprotected using 4 N HCl/dioxane for the characterization of L-Ab6 hydrochloride.[11]

The syntheses of Ab*n*s ($n \leqslant 5$) with short side chain lengths were failed in our hands. During the first step of the reaction the expected bromopropyl group was changed to allyl group as shown in Scheme 3 and the simultaneous treatments afforded L-allylglycine.

The Ab*n*s synthesized can be used for both Boc and Fmoc methods for the synthesis of peptides. To verify this we protected the N-terminal of the new amino acids using Boc and Fmoc groups. The Boc protection of L-Ab7 yielded Boc-L-Ab7 as colourless oil in quantitative yield. Similarly the Fmoc protection of L-Ab7 also yield the Fmoc-L-Ab7 in quantitative yield as white solid (Scheme 4).

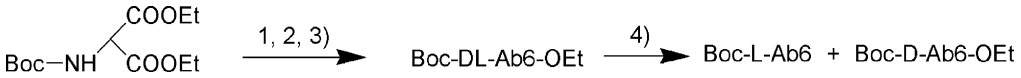


Scheme 3. Reagents and conditions: (i) EtOH, sodium ethoxide, reflux, 30 min; (ii) $Br(CH_2)_3Br$, reflux, 3 h, 78%.

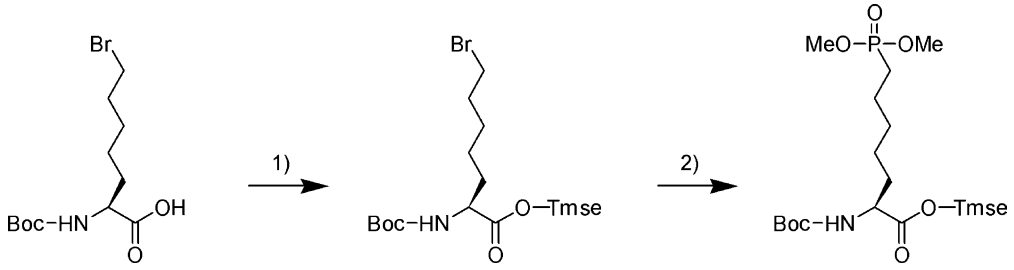


Scheme 4. Reagents and conditions: (1) $(Boc)_2O$, dioxane, water, 8 h, 0 °C, 100%; (2) Fmoc-OSu, Na_2CO_3, water, dioxane, 8 h, 98%.

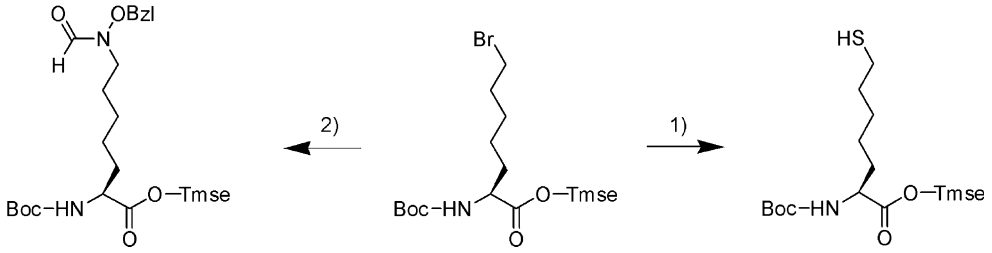
The bromide at the ω-position is labile, and therefore a wide variety of functional group transformation is possible with Ab*n*s to synthesize several interesting non-natural amino acids. For example, we illustrate here the synthesis of 2-amino-7-phosphonatoheptanoic acid starting from Boc-Ab7. The C-terminal of Boc-Ab7 was protected with 2-(trimethylsilyl)ethanol and the Boc-Ab7-OTmse was treated with $P(OMe)_3$ in the presence of KI in THF at 70 °C to yield the desired compound in almost quantitative yield (Scheme 5). α-Amino phosphonate derivatives have been found to be highly potent inhibitors of a number of enzymes such as protein-tyrosine phosphatases,[12] metalloproteases[13] and serine proteases.[14] Also amino phosphonopeptides are known for their significant antibacterial activity.[15] Due to the reasons, our method for the synthesis of amino acid



Scheme 2. Reagents and conditions: (1) (i) EtOH, sodium ethoxide, reflux, 30 min; (ii) $Br(CH_2)_4Br$, reflux, 3 h, 78%; (2) NaOH aq, EtOH, 0 °C, 5 h, 83%; (3) toluene, reflux, 3 h, 95%; (4) subtilisin, H_2O/DMF = 1/1, pH 8, 38 °C, 3 h, 96%.



Scheme 5. Reagents and conditions: (1) TmseOH, DCC, DMAP, DCM, 0 °C, 90%; (2) KI, $P(OMe)_3$, 70 °C, THF, 98%.



Scheme 6. Reagents and conditions: (1) (i) AcSK, DMF, 100%; (ii) NH_3/MeOH, 90%; (2) *n*-benzyloxyformamide, KI, K_2CO_3, acetone, reflux, 90%.

containing a phosphonate side chain is useful for the discovery of novel enzyme inhibitors.

To exemplify the application of the L-Ab*n*s, we synthesized cysteine homologues by replacing the bromide at the side chain with thiol group. This was achieved by the reaction of fully protected ω-bromo-α-amino acid with potassium thioacetate and subsequent treatment with ammonia or methylamine in methanol as shown in Scheme 6. These cysteine homologues have immense potential as enzyme inhibitors and as peptidomimetics.[16] Further, we synthesized *N*-formylhydroxylamine (retrohydroxamate) derivatives in one step starting from L-Ab*n*s. Retrohydroxamates are reported as inhibitors of metalloproteinases.[17] In extension of this work, we have synthesized lysine homologues by the reaction of fully protected L-Ab*n*s using phthalimide reaction and subsequent reaction with hydrazine hydrate. The Ab*n*s may be reacted with NaN_3 to the corresponding azides, which is useful for Staudinger ligation.[18]

In conclusion, we have synthesized optically pure L-α-amino-ω-bromoalkanoic acids (Ab*n*s with $n \geqslant 6$) for side chain modification. The N-terminals of these amino acids can be easily protected with Boc or Fmoc groups quantitatively. The bromide group at the ω-position can be transformed to several other functionalities to synthesize a wide variety of non-natural amino acids.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research (no. 13450380) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References and Notes

1. For lead references, see: *Asymmetric Synthesis of Novel Sterically Constrained Amino Acids*; Symposium-in-print, Hruby, V. J.; Soloshonok, V. A. Eds.; *Tetrahedron*, **2001**, *57*, 6329–6650.
2. Nollet, A. J. H.; Huting, C. M.; Pandit, U. K. *Tetrahedron* **1969**, *25*, 5971–5981.
3. (a) Lundquist, J. T., IV; Dix, T. A. *Tetrahedron Lett.* **1998**, *39*, 775–778; (b) Lundquist, J. T., IV; Büllesbach, E. E.; Dix, T. A. *Tetrahedron: Asymmetry* **1998**, *9*, 2739–2743.
4. (a) Yamaguchi, J.; Ueki, M. *Chem. Lett.* **1996**, *25*, 621–622; (b) Ciapetti, P.; Mann, A.; Shoenfelder, A.; Taddei, M. *Tetrahedron Lett.* **1998**, *39*, 3843–3846.
5. (a) Adamczyk, M.; Johnson, D. D.; Reddy, R. E. *Tetrahedron: Asymmetry* **1999**, *10*, 775–781; (b) Adamczyk, M.; Johnson, D. D.; Reddy, R. E. *Tetrahedron* **1999**, *55*, 63–88; (c) Adamczyk, M.; Akireddy, S. R.; Reddy, R. E. *Tetrahedron* **2000**, *56*, 2379–2390; (d) Sugiyama, H.; Yokokawa, F.; Shioiri, T.; Katagiri, N.; Oda, O.; Ogawa, H. *Tetrahedron Lett.* **1999**, *40*, 2569–2572.
6. (a) Redman, J. E.; Ghadiri, M. R. *Org. Lett.* **2002**, *4*, 4467–4469; (b) Fugita, T.; Nose, T.; Matsuhima, A.; Okada, K.; Asai, D.; Yamauchi, Y.; Shirasu, N.; Honda, T.; Shigehiro, D.; Shimohigashi, Y. *Tetrahedron Lett.* **2000**, *41*, 923–927; (c) Stirling, I. R.; Freer, I. K. A.; Robins, D. J. *J. Chem. Soc., Perkin Trans. 1* **1997**, 5, 677–680; (d) Rogers, L. M.-A.; Rouden, J.; Lecomte, L.; Lasne, M.-C. *Tetrahedron Lett.* **2003**, *44*, 3047–3050.
7. For the characterization and to determine the yield the product was purified by column chromatography after the evaporation of ethanol.
8. Nishino, N.; Arai, T.; Ueno, Y.; Ohba, M. *Chem. Pharm. Bull.* **1996**, *44*, 212–214.
9. Typical experimental procedure: Metallic sodium (0.19 mol, 0.95 equiv) was dissolved in abs EtOH (200 mL) and diethyl acetamidomalonate (0.2 mol) was added to the solution. The mixture was refluxed for 30 min for the complete dissolution and then dibromoalkane (3 equiv) was added and refluxed for 3 h. The reaction mixture was cooled on an ice bath and hydrolysis was carried out by the addition of 1 N NaOH (aq, 1 equiv in five

portions) at intervals of 15 min. After selective hydrolysis, ethanol was evaporated and the unreacted dibromoalkane was removed by extraction with diethyl ether under basic condition. Then the aqueous solution was extracted with ethyl acetate (at pH = 3–4, by adding citric acid) and washed with brine, dried and evaporated to obtain white solid of monoester monoacid (60–80%). It was then dissolved in toluene (100 mL) and refluxed for 3 h and toluene was evaporated to a colourless oil (96–100%). The resulting oil was dissolved in ethanol (100 mL) and hydrolysis and work up was carried out as described above to get Ac-DL-Ab*n* as white solid (70–90%). The acid (0.1 mol) was dissolved in water (400 mL) at pH = 7 by the addition of 1 N NaOH (aq). Then $CoCl_2 \cdot 6H_2O$ (120 mg) and *Aspergillus genus* aminoacylase (3 g) were added and incubated at 38 °C for 24 h. L-Ab*n* was solidified from the aqueous solution and was filtered after cooling the reaction mixture and washed with water and ethanol (68–80%). The aqueous solution was extracted with ethyl acetate at pH = 3 to recover Ac-D-Ab*n* (80–90%). Characterization data for: L-Ab7 $[\alpha]_D^{25}$ +25, HRMS $(M+H)^+$ found 224.0286 (calcd for $C_7H_{15}O_2N^{79}Br$ 224.0286); L-Ab8 $[\alpha]_D^{25}$ +24, HRMS $(M+H)^+$ found 238.0466 (calcd for $C_8H_{17}O_2N^{79}Br$ 238.0442); L-Ab12 $[\alpha]_D^{25}$ +23, HRMS $(M+H)^+$ found 294.1104 (calcd for $C_{12}H_{25}O_2N^{79}Br$ 294.1069). ^1H NMR of the Boc-L-Ab*n*s gave acceptable data. $[\alpha]_D$ Values were measured in Cl solution of L-Ab*n*s in 4 N HCl/dioxane.

10. The synthesis of pipecolic acid and related compounds from Ab6 will be described elsewhere.
11. L-Ab6·HCl $[\alpha]_D^{25}$ +24, HRMS $(M+H)^+$ found 210.0144 (calcd for $C_6H_{13}O_2N^{79}Br$ 210.0130).
12. Burke, T. R., Jr.; Zhang, Z.-Y. *Biopolym. (Peptide Sci.)* **1998**, *47*, 225–241.
13. De Lombaert, S.; Blachard, L.; Stamford, L. B.; Tan, J.; Wallace, E. M.; Satoh, Y.; Fitt, J.; Hoyer, D.; Simonsbergen, D.; Moliterni, J.; Marcopoulos, N.; Savage, P.; Chou, M.; Trapani, A. J.; Jeng, A. Y. *J. Med. Chem.* **2000**, *43*, 488–504.
14. Jackson, D. S.; Frase, S. A.; Ni, L.-M.; Kam, Ch.-M.; Winkler, U.; Johnson, D. A.; Froelich, C. J.; Hudig, D.; Powers, J. C. *J. Med. Chem.* **1998**, *41*, 2289–2301.
15. Atherton, F. R.; Hassal, C. H.; Lambert, R. W. *J. Med. Chem.* **1986**, *29*, 29–40.
16. (a) Inguimbert, N.; Poras, H.; Teffo, F.; Beslot, F.; Selkti, M.; Tomas, A.; Scalbert, E.; Bennejean, C.; Renard, P.; Fournié-Zaluski, M.-C.; Roques, B.-P. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2001–2005; (b) Hofland, J.; van Koetsveld, P. M.; Waaijers, M.; Zuyderwijk, J.; Lamberts, S. W. *J. Endocrinol.* **1994**, *134*, 301–306.
17. Michaelides, M. R.; Dellaria, J. F.; Gong, J.; Holms, J. H.; Bouska, J. J.; Stacey, J.; Wada, C. K.; Heyman, H. R.; Curtin, M. L.; Guo, Y.; Goodfellow, C. L.; Elmore, I. B.; Albert, D. H.; Magoc, T. J.; Marcotte, P. A.; Morgan, D. W.; Davidsen, S. K. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1553–1556.
18. (a) Staudinger, H.; Meyer, J. *Helv. Chim. Acta* **1919**, *2*, 635–646; (b) Soellner, M. B.; Dickson, K. A.; Nilsson, B. L.; Raines, R. T. *J. Am. Chem. Soc.* **2003**, *125*, 11790–11791.