

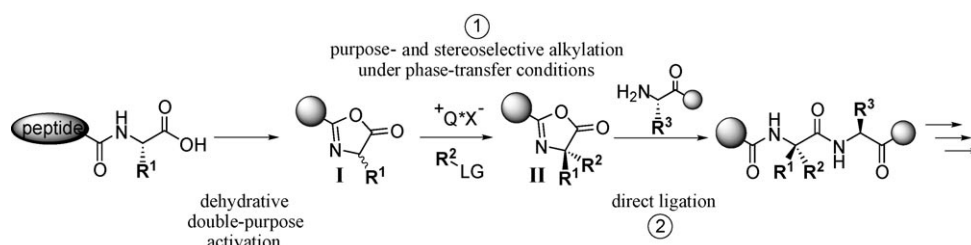
Site-Directed Asymmetric Quaternization of a Peptide Backbone at a C-Terminal Azlactone**

Daisuke Uraguchi, Yoshihiro Asai, and Takashi Ooi*

The incorporation of nonproteinogenic amino acids into peptides is a rational and indispensable approach to the elucidation of the diverse roles of peptides and can also impart new functions to the peptides to facilitate their use as pharmaceuticals.^[1] In particular, the appropriate introduction of α,α -dialkyl α -amino acids (quaternary α -amino acids) is known to induce significant conformational constraints and can thus be used to probe the molecular structure of receptors or enhance the biological activity of the peptides by helping to preorganize the optimum conformation for binding or by inhibiting metabolic degradation.^[2] For example, naturally occurring aminoisobutyric acid (Aib) and related achiral amino acids are often employed to study the relationship between the structure, stability, and function of bioactive peptides.^[3] In sharp contrast, however, the use of chiral, nonracemic quaternary α -amino acids has been very limited, despite a long-standing interest in the correlation of the chirality of these amino acids with peptide structure, such as the handedness of the helix, and peptide function.^[3c,d,4] This situation is primarily due to the lack of flexible synthetic strategies for incorporating a wide range of chiral quaternary α -amino acids into peptide strands at specific sites, which seems to be synonymous with the intricacy of peptide-bond construction with independently prepared, enantiomerically enriched quaternary α -amino acids.^[5–7] Herein, we report our own solution to this problem. This solution involves the direct and highly stereoselective construction of quaternary stereogenic carbon centers on C-terminal amino acid residues of growing peptides by alkylation under organic–aqueous biphasic conditions in the presence of a catalytic amount of an optically pure,

D_2 -symmetric tetraaminophosphonium salt as a chiral phase-transfer catalyst (PTC).^[8] In combination with a simple and direct ligation process,^[9] this site-directed quaternization method enables the customized asymmetric synthesis of nonnatural oligopeptides that contain quaternary α -amino acid residues at the requisite positions.

Our strategy is based on the dehydrative activation of the C terminus of a peptide as an oxazol-5-(4*H*)-one **I**, otherwise known as an azlactone,^[10] and its stereoselective alkylation under practical biphasic conditions in the presence of an appropriate PTC (Scheme 1).^[11,12] Since an azlactone is known to be an active methine compound as well as a



Scheme 1. Strategy for the incorporation of chiral quaternary α -amino acids at specific sites of a peptide strand. $^+Q^*X^-$ = chiral quaternary onium salt; LG = leaving group.

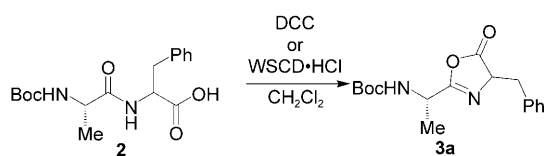
reactive acyl donor, **I** can be regarded as a doubly activated form of a peptide for two purposes, alkylation and subsequent ligation. Thus, if we are able to utilize **I** selectively as the key substrate for the first purpose, the purpose- and stereoselective introduction of an additional side chain R^2 (① in Scheme 1), the resulting azlactone **II** could be employed directly for the second purpose, the ligation of the peptide (②). A crucial factor in enabling the stereoselective double functionalization of the peptide C-terminal azlactone is the structure of the PTC. It would be ideal if the primary structure of the PTC was easy to synthesize and readily tunable so that the most suitable catalyst for a particular transformation could be identified promptly. With this aim in mind, we focused our attention on the structural features of tetraaminophosphonium salts,^[13,14] which can be assembled from a variety of amines and a commercial phosphorous source.

We first attempted the allylation of azlactone **3a**, which was formed from Boc-L-Ala-D,L-Phe by dehydration with a carbodiimide (Scheme 2), in the presence of a known achiral aminophosphonium salt (Table 1, entry 1). Thus, a mixture of **3a**, allyl bromide, and tetra(*N*-methylcyclohexylamino)phosphonium tetrafluoroborate^[13b,c] (5 mol %) in a mixture of toluene and saturated aqueous tripotassium phosphate was stirred vigorously at 0 °C. The starting material was consumed

[*] Dr. D. Uraguchi, Y. Asai, Prof. Dr. T. Ooi
Department of Applied Chemistry
Graduate School of Engineering, Nagoya University
Furo-cho B2-3(611), Chikusa, Nagoya 464-8603 (Japan)
Fax: (+81) 52-789-3338
E-mail: tooi@apchem.nagoya-u.ac.jp

[**] This research was partially supported by the Global COE Program in Chemistry of Nagoya University, a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology (Japan), and the Naito Science & Engineering Foundation.

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.200803661>.



Scheme 2. Dehydration to the azlactone. Boc = *tert*-butoxycarbonyl, DCC = *N,N'*-dicyclohexylcarbodiimide, WSCD·HCl = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride.

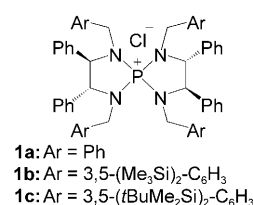
Table 1: Effect of the catalyst structure on the purpose selectivity and stereoselectivity under organic–aqueous biphasic conditions.^[a]

Entry	1	Solvent	<i>T</i> [°C]	<i>t</i> [h]	Yield ^[b] [%]	d.r. ^[c] <i>S,R</i> / <i>S,S</i>
1 ^[d]	(<i>c</i> -C ₆ H ₁₁ MeN) ₄ PBF ₄	toluene	0	2	21	52:48
2	1a		0	1.5	26	64:36
3	1b		0	2	65	78:22
4	1c		0	1	78	94:6
5	1c	Et ₂ O	0	2	60	92:8
6	1c	TBME ^[e]	0	1.5	75	94:6
7	1c	CPME ^[e]	0	0.75	79	95:5
8	1c		25	0.25	75	93:7
9	1c		−15	2	86	95:5
10 ^[f]	1c		−15	2	85	93:7
11 ^[g]	1c		0	0.75	69	9:91
12 ^[g]	1c		25	0.25	60	13:87
13 ^[g]	1c		−15	1.5	70	7:93
14	<i>ent</i> - 1c		−15	2	70	8:92

[a] Unless otherwise noted, reactions were carried out on a 0.1 mmol scale with 1.2 equivalents of allyl bromide. [b] Yield of the isolated product. [c] The diastereomeric ratio was determined by HPLC on a chiral stationary phase. [d] Catalyst: 5 mol %. [e] TBME = *tert*-butyl methyl ether; CPME = cyclopentyl methyl ether. [f] The azlactone **3b** derived from Cbz-L-Ala-D,L-Phe was used as the substrate instead of **3a** (Cbz = carbobenzyloxy). The absolute configuration of **4b** was assigned by analogy with **4a**. [g] Compound *ent*-**3a** was used instead of **3a**. The diastereomeric ratio (*R,S*)-**4a**/*(R,R)*-**4a** is given.

within 2 h; however, the allylated dipeptide derivative **4a** was obtained in just 21 % yield. The observed (*S,R*)-**4a**/*(S,S)*-**4a** ratio of 52:48 reveals the intrinsic stereoselectivity of the transformation under these conditions. The concomitant yet substantial formation of a diastereomeric mixture of an undesired self-acylation product (73 %) suggested the extreme difficulty of the purpose-selective utilization of a reactive azlactone moiety.^[15]

These initial observations led us to design a series of optically pure tetraaminophosphonium chlorides **1** as new, chiral PTCs and to evaluate the relationship between their structure and their selectivity in the allylation of **3a** under similar conditions.^[16] Although an improvement in diastereoselectivity was observed with the *N*-benzylated aminophosphonium salt **1a**, which was prepared from commercially available (*R,R*)-1,2-diphenylethylenediamine and phosphorus pentachloride in two steps, the chemical yield of **4a**



remained unsatisfactory (Table 1, entry 2). However, the introduction of 3,5-bis(trialkylsilyl)benzyl substituents on the nitrogen atoms of the catalyst led to a dramatic enhancement of both the purpose- and stereoselectivities (Table 1, entries 3 and 4): Product **4a** was isolated in 78 % yield with an excellent diastereomeric ratio of 94:6 when **1c**, which contains 3,5-bis(*tert*-butyldimethylsilyl)benzyl substituents, was used as the catalyst (Table 1, entry 4).

The structure of the chiral *D*₂-symmetric P-spiro amino-phosphonium salts with silyl substituents was verified by single-crystal X-ray diffraction analysis of **1b** (Figure 1). The absolute configuration of the newly created quaternary carbon center of the major isomer of **4a** was determined to be *R* by X-ray analysis of an amide derivative.^[17]

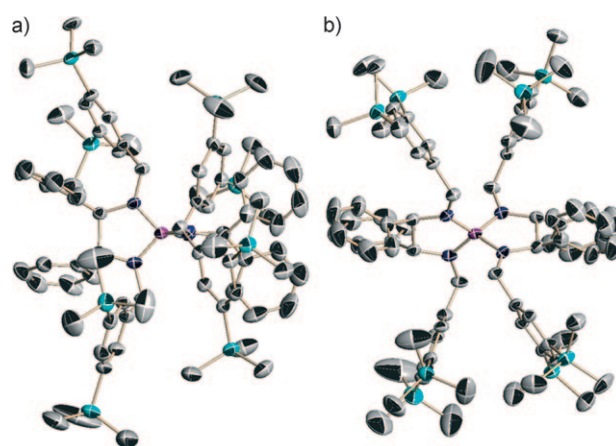


Figure 1. Molecular structure of the tetraaminophosphonium salt **1b**. The counterion (Cl[−]), hydrogen atoms, and solvent molecules are omitted for clarity. a) Perpendicular view of the 5,5-spirocycle; b) diagonal view. Purple: phosphorus, blue: nitrogen, light blue: silicon.

This biphasic alkylation system is compatible with ethereal solvents (Table 1, entries 5–7). Cyclopentyl methyl ether (CPME) was found to be the solvent of choice (Table 1, entry 7). The reaction temperature affected the stereoselectivity only subtly (Table 1, entries 7–9), but self-acylation was suppressed further (and thus the chemical yield was higher) when the reaction mixture was cooled in an ice–salt bath (−15 °C; Table 1, entry 9). The reaction of the *N*-carbobenzyloxy-protected dipeptide **3b** proceeded with comparable reactivity and selectivity under the optimized conditions (Table 1, entry 10).

To gain insight into the origin of the facial discrimination in the present inherently diastereoselective reaction, we carried out the allylation of the enantiomer of **3a**, *ent*-**3a**

(derived from Boc-D-Ala-D,L-Phe), in the presence of **1c** at 0 °C. The diastereomeric product (*R,R*)-**4a** was produced as the major isomer (69%, (*R,S*)-**4a**/*R,R*-**4a** 9:91; Table 1, entry 11). The yield and selectivity both increased when the temperature was lowered (Table 1, entries 11–13). Although the slightly lower stereoselectivity suggests that *ent*-**3a** has a mismatched stereocenter with respect to diastereofacial differentiation with **1c**, the establishment of the quaternary carbon center with high enantioselectivity and the absolute configuration of this center indicate clearly that the stereochemical outcome of the reaction is mainly determined by the chirality of the catalyst. The pivotal role of the chiral catalyst was also supported by the predominant formation of (*S,S*)-**4a** in the allylation of **3a** with *ent*-**1c** under similar conditions (Table 1, entry 14).

To explore the substrate generality of the phase-transfer allylation catalyzed by **1c**, various dipeptide-derived azlactones were treated with allyl bromide under the optimized conditions (Table 2). First, the reactions of five azlactones

Table 2: Asymmetric allylation of dipeptide-derived azlactones under phase-transfer conditions.^[a]

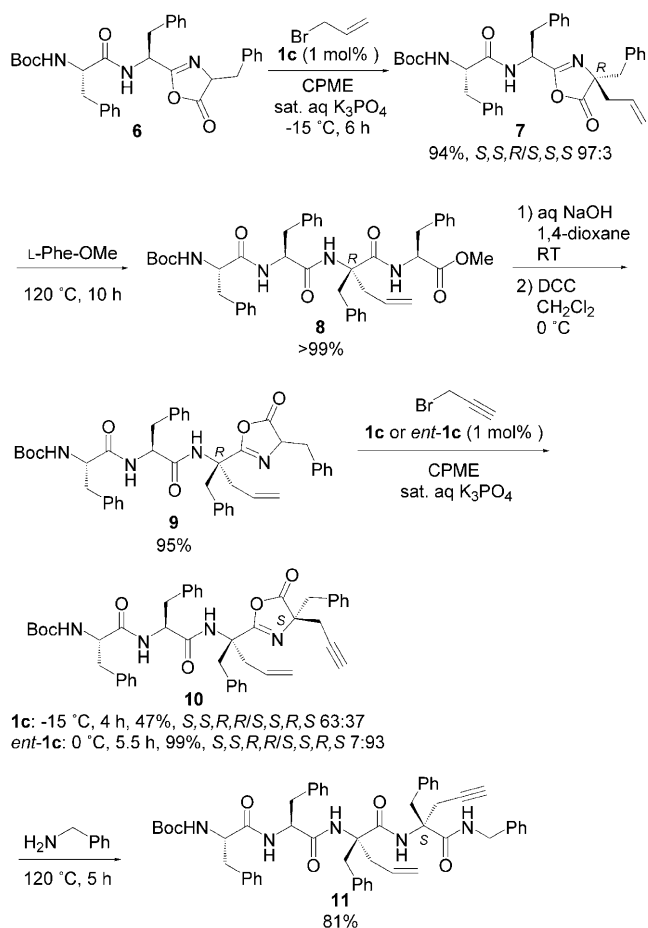
Entry	AA1	AA2	3	R-X	4	t [h]	Yield ^[b] [%]	d.r. ^[c]
1	Leu	Phe	3c	Br-CH ₂ CH=CH ₂	4c	7	89	97:3
2	Val		3d		4d	8	94	99:1
3	Pro		3e		4e	12	74	93:7
4 ^[d]	Gly		3f		4f	4.5	77	91:9
5	Phe		3g		4g	2.5	82	97:3
6		Trp	3h		4h	12	93	91:9
7		Leu	3i		4i	8	97	97:3
8 ^[d]	Leu	Leu	3j		4j	1.5	98	95:5
9 ^[d]				Br-CH ₂ Ph	4k	2.5	87	95:5
10 ^[e]	Phe	Phe	3g	Br-CH ₂ C≡CH	4l	7	97	98:2
11				Br-CH ₂ CN	4m	3	88	93:7
12				Br-CH ₂ CO ₂ Me	4n	7	82	93:7
13 ^[e]				Cl-CH ₂ OMe	4o	2	66	95:5

[a] Unless otherwise noted, reactions were carried out on a 0.1 mmol scale with 1.2 equivalents of alkyl halide. [b] Yield of the isolated product. [c] The diastereomeric ratio was determined by HPLC on a chiral stationary phase. The absolute configuration was assigned by analogy with **4a** and **10**. [d] The reaction was performed at 0 °C. [e] Alkyl halide: 5.0 equivalents.

with a different pendent amino acid (AA1) were examined, and excellent stereoselectivities were uniformly observed (Table 2, entries 1–5). Even **3f**, which contains a glycine unit, reacted with high stereoselectivity (Table 2, entry 4), and **3g** derived from the homodipeptide of phenylalanine proved to be a good substrate (Table 2, entry 5). Similarly, the amino acid of the azlactone moiety (AA2) can be varied (Table 2, entries 6–8). Notably, a tryptophan unit was used successfully without protection of the reactive indole nitrogen atom

(Table 2, entry 6).^[18] We also probed the scope of the reaction with respect to the alkyl halide component (Table 2, entries 9–13). A terminal triple bond, a potential functionality for use in click chemistry,^[19] was introduced with rigorous stereochemical control (Table 2, entry 10). Alkyl halides containing nitrile and ester functionalities also reacted efficiently (Table 2, entries 11 and 12). The use of such electrophiles enables the incorporation of asparagine and aspartic acid analogues at the C terminus of a peptide. Furthermore, a serine derivative was constructed in moderate yield with high selectivity (Table 2, entry 13).

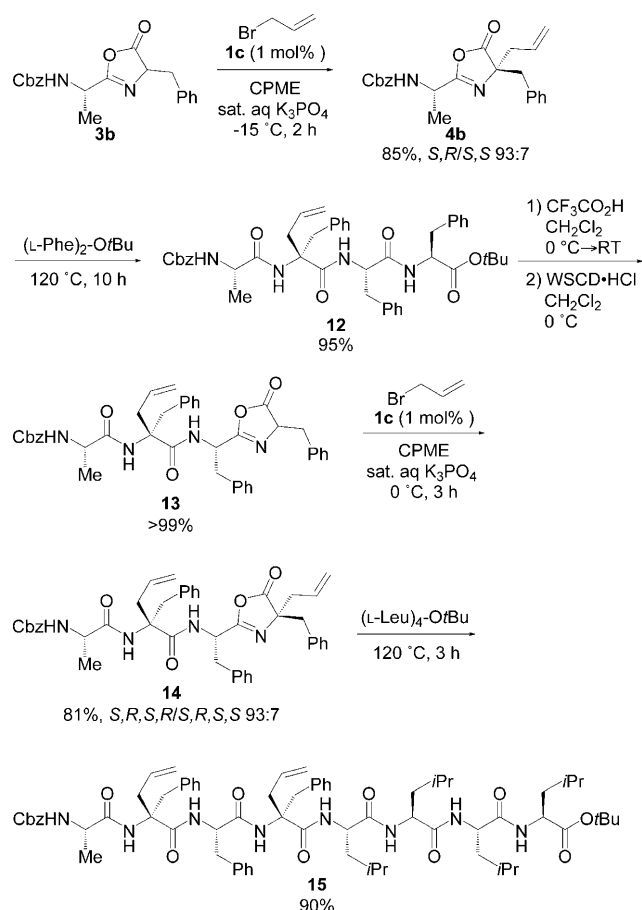
We next extended the asymmetric phase-transfer catalysis of **1c** to the C-terminal alkylation of tripeptides. The allylation of azlactone **6**, derived from the homotripeptide of phenylalanine, with **1c** under the standard conditions resulted in the formation of **7** in 94% yield with 97:3 diastereoselectivity (Scheme 3). The distinct advantage of this stereoselective alkylation strategy is that an alkylated, diastereomerically enriched azlactone, such as **7**, can be used directly as a reactive acyl donor for a subsequent ligation. For example, the simple treatment of **7** with a nearly equimolar amount of L-phenylalanine methyl ester at 120 °C for 10 h furnished **8** quantitatively without detectable epimerization of any stereocenter. The resulting *N*-Boc tetrapeptide



Scheme 3. Asymmetric allylation of a tripeptide-derived azlactone and construction of a tetrapeptide with two adjoining quaternary α-amino acid residues.

methyl ester **8** was converted readily into a new azlactone **9** through a saponification–dehydration sequence in preparation for another alkylation. However, when **9** was treated with propargyl bromide in the presence of **1c** under biphasic conditions, significantly diminished diastereoselectivity was observed. This result implied that the adjacent, sterically congested quaternary carbon stereocenter had a considerable influence on the stereochemical outcome of the reaction, and that **1c** may be a mismatched catalyst for the diastereofacial differentiation of (*S,S,R*)-**9**. Indeed, a high level of stereoselectivity was observed with catalyst *ent*-**1c**. Thus, the straightforward asymmetric construction of stereochemically dense tetrapeptides with two adjoining quaternary α -amino acid residues is possible. The absolute stereostructure of the major isomer of tetrapeptide **10** was determined to be *S,S,R,S* by X-ray diffraction analysis after conversion into the amide derivative **11**.

To further highlight the broad scope of our strategy, we undertook the stereoselective synthesis of a tetrapeptide with two non-adjacent chiral quaternary α -amino acid residues. The allylated azlactone **4b** was prepared as described earlier with the catalyst **1c** (Table 1, entry 10) and elongated by two amino acid residues through amide-bond formation with the *tert*-butyl ester of the homodipeptide of L-phenylalanine (Scheme 4). The protected tetrapeptide **12** thus obtained was



Scheme 4. Facile asymmetric synthesis of a tetrapeptide with two quaternary stereocenters on non-adjacent amino acid residues and its use as an intermediate in the synthesis of an octapeptide.

then transformed into the C-terminal azlactone **13**, the subsequent phase-transfer allylation of which in the presence of **1c** proceeded in high yield with high diastereoselectivity. The resulting tetrapeptide **14** can be used as a transient substrate for a further ligation–alkylation sequence or alternatively for the synthesis of oligopeptides with chiral quaternary α -amino acid residues incorporated at specific sites through ligation with a preexisting, different oligopeptide strand, as exemplified by the assembly of octapeptide **15**.

In conclusion, we have developed a practical method for the incorporation of a wide variety of chiral, nonracemic quaternary α -amino acids at specific sites of a peptide strand. This backbone modification of a growing peptide is based on the dehydrative, double-purpose activation of the C terminus as an azlactone and its highly selective quaternization under biphasic conditions with the chiral *D*₂-symmetric tetraamino-phosphonium chloride PTC **1c**. Since the alkylated azlactone can be employed directly for the second purpose, that is, peptide ligation, appropriate repetition of the operationally simple alkylation–ligation process provides a powerful basis for the synthesis of oligopeptides with quaternary α -amino acid residues of the desired configuration at the requisite positions. We hope that the results presented herein will stimulate and even facilitate research in diverse fields in which such nonnatural peptides serve as valuable tools.

Received: July 26, 2008

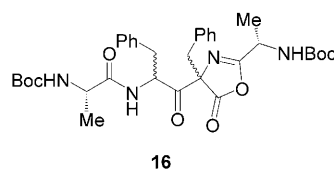
Published online: November 25, 2008

Keywords: alkylation · amino acids · peptide modification · phase-transfer catalysis · phosphonium salts

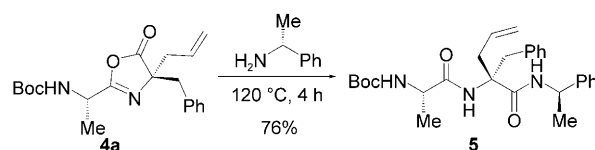
- [1] a) J. Gante, *Angew. Chem.* **1994**, *106*, 1780–1802; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1699–1720; b) J. Venkatraman, S. C. Shankaramma, P. Balaram, *Chem. Rev.* **2001**, *101*, 3131–3152; c) L. Wang, P. G. Schultz, *Angew. Chem.* **2005**, *117*, 34–68; *Angew. Chem. Int. Ed.* **2005**, *44*, 34–66.
- [2] A. Giannis, T. Kolter, *Angew. Chem.* **1993**, *105*, 1303–1326; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1244–1267.
- [3] a) S. D. Bryant, R. Guerrini, S. Salvadori, C. Bianchi, R. Tomatis, M. Attila, L. H. Lazarus, *J. Med. Chem.* **1997**, *40*, 2579–2587; b) I. Ségalas, Y. Prigent, D. Davoust, B. Bodo, S. Rebuffat, *Biopolymers* **1999**, *50*, 71–85; c) C. Toniolo, M. Crisma, F. Formaggio, C. Peggion, *Biopolymers* **2001**, *60*, 396–419; d) C. Toniolo, F. Formaggio, B. Kaptein, Q. B. Broxterman, *Synlett* **2006**, 1295–1310.
- [4] a) H. Heimgartner, *Angew. Chem.* **1991**, *103*, 271–297; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 238–264; b) R. T. Shuman, R. B. Rothenberger, C. S. Campbell, G. F. Smith, D. S. Gifford-Moore, J. W. Paschal, P. D. Gesellchen, *J. Med. Chem.* **1995**, *38*, 4446–4453; c) M. Tanaka, *Chem. Pharm. Bull.* **2007**, *55*, 349–358.
- [5] a) J. M. Humphrey, A. R. Chamberlin, *Chem. Rev.* **1997**, *97*, 2243–2266; b) Y. Fu, L. G. J. Hammarström, T. J. Miller, F. R. Fronczek, M. L. McLaughlin, R. P. Hammer, *J. Org. Chem.* **2001**, *66*, 7118–7124; c) S. Stamm, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2006**, *89*, 1–15; d) A. R. Katritzky, E. Todadze, P. Angrish, B. Draghici, *J. Org. Chem.* **2007**, *72*, 5794–5801.
- [6] a) S. Wenglowsky, L. S. Hegedus, *J. Am. Chem. Soc.* **1998**, *120*, 12468–12473; b) Y. Ohfuné, T. Shinada, *Bull. Chem. Soc. Jpn.* **2003**, *76*, 1115–1129; c) K. A. Brun, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2008**, *91*, 526–558.

- [7] a) C. Cativiela, M. D. Díaz-de-Villegas, *Tetrahedron: Asymmetry* **1998**, 9, 3517–3599; b) Y. Ohfuné, T. Shinada, *Eur. J. Org. Chem.* **2005**, 5127–5143; c) C. Cativiela, M. D. Díaz-de-Villegas, *Tetrahedron: Asymmetry* **2007**, 18, 569–623; d) H. Vogt, S. Bräse, *Org. Biomol. Chem.* **2007**, 5, 406–430.
- [8] a) *Handbook of Phase Transfer Catalysis* (Eds.: Y. Sasson, R. Neumann), Blackie Academic & Professional, London, **1997**; b) *Asymmetric Phase Transfer Catalysis* (Ed.: K. Maruoka), Wiley-VCH, New York, **2008**.
- [9] a) F. Formaggio, Q. B. Broxterman, C. Toniolo in *Houben-Weyl: Methods of Organic Chemistry, Vol. E22c* (Eds.: M. Goodman, A. Felix, L. Moroder, C. Toniolo), Thieme, Stuttgart, **2002**, chap. 10.3; b) D. Obrecht, U. Bohdal, C. Broger, D. Bur, C. Lehmann, R. Ruffieux, P. Schönholzer, C. Spiegler, K. Müller, *Helv. Chim. Acta* **1995**, 78, 563–580.
- [10] J. S. Fisk, R. A. Mosey, J. J. Tepe, *Chem. Soc. Rev.* **2007**, 36, 1432–1440.
- [11] M. L. Gelmi, D. Pocar, L. M. Rossi, *Synthesis* **1984**, 763–765.
- [12] a) T. Ooi, E. Tayama, K. Maruoka, *Angew. Chem.* **2003**, 115, 599–602; *Angew. Chem. Int. Ed.* **2003**, 42, 579–582; b) K. Maruoka, E. Tayama, T. Ooi, *Proc. Natl. Acad. Sci. USA* **2004**, 101, 5824–5829.
- [13] a) R. Schwesinger, J. Willaredt, H. Schlemper, M. Keller, D. Schmitt, H. Fritz, *Chem. Ber.* **1994**, 127, 2435–2454; b) R. Schwesinger, R. Link, P. Wenzl, S. Kossek, M. Keller, *Chem. Eur. J.* **2006**, 12, 429–437; c) R. Schwesinger, R. Link, P. Wenzl, S. Kossek, *Chem. Eur. J.* **2006**, 12, 438–445.
- [14] D. Uraguchi, S. Sakaki, T. Ooi, *J. Am. Chem. Soc.* **2007**, 129, 12392–12393.
- [15] As an azlactone is a reactive acyl donor, **3a** is prone to react with its enolate under the present conditions to give the self-acylation product **16**. The yield was calculated by considering a 100% yield to correspond to the complete dimerization of **3a**. For the

structural assignment of the dimer **16**, see the Supporting Information.



- [16] The results of the allylation with other representative chiral quaternary ammonium salts are reported in the Supporting Information.
- [17] A simple ring-opening reaction of **4a** with (*R*)-phenylethylamine without a solvent gave the amide **5** for X-ray crystallographic analysis.



- [18] Although we attempted to use histidine as a more hydrophilic amino acid in the azlactone moiety (AA2), the corresponding azlactone, such as that derived from Boc-L-Ala-D,L-His(π -Bom), was not stable enough under the present biphasic conditions (Bom = benzyloxymethyl).
- [19] Y. L. Angell, K. Burgess, *Chem. Soc. Rev.* **2007**, 36, 1674–1689.