Total Synthesis of (−)-Caprazamycin A**

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Abstract: *Caprazamycin A has significant antibacterial activity against Mycobacterium tuberculosis (TB). The first total synthesis is herein reported featuring (i) the scalable preparation of the syn-β-hydroxy amino acid with a thiourea-catalyzed diastereoselective aldol reaction, (ii) construction of a diazepanone with an unstable fatty acid side chain, and (iii) global deprotection with hydrogenation. This report provides a route for the synthesis of related liponucleoside antibiotics with fatty acid side chains.*

Caprazamycin A (**1**) was isolated from *Streptomyces sp.* MK730 62F2 and is a liponucleoside characterized by a seven-membered diazepanone core with an amino ribose, a uridine and a fatty acid side chain (Figure 1).[1] Several analogs isolated by Igarashi *et al.* in 2003 share these features. Caprazamycins have antibacterial activity against *Mycobacterium tuberculosis* (TB), including multidrug-resistant TB (MDR-TB). Biological studies showed that it is an inhibitor of the peptidoglycan biosynthetic enzyme MraY.[2] MraY is essential for bacterial cell growth and is biosynthetically located upstream of an enzyme targeted by β-lactam and glycopeptide antibiotics (e.g. vancomycin). New antimicrobial agents targeting MraY are expected to be active against vancomycin- and methicillin-resistant *Staphylococcus aureus* (VRSA and MRSA).[3] Recently, CPZEN-45, which exhibits more potent activity against TB—including extensively multidrug-resistant TB (XDR-TB), has been developed based on caprazamycins.[2b,4]

The complex structure and significant biological activities of caprazamycins have drawn much attention from synthetic chemists.[5,6] Matsuda and Ichikawa accomplished the first total synthesis of palmitoyl caprazol and caprazol (**2**), which does not possess a fatty acid side chain.[7] Shibasaki and Watanabe recently reported the synthesis of **2** and the fatty acid side chain.[8] However, a total synthesis of the caprazamycins has not yet been reported, because of the difficulty in introducing an unstable fatty acid side chain. This has also hampered the total synthesis of related liponucleoside antibiotics, such as liposidomycin C (**3**).[9] Therefore, we initiated a caprazamycin A (**1**) synthetic project, which would also be applicable to related natural products.

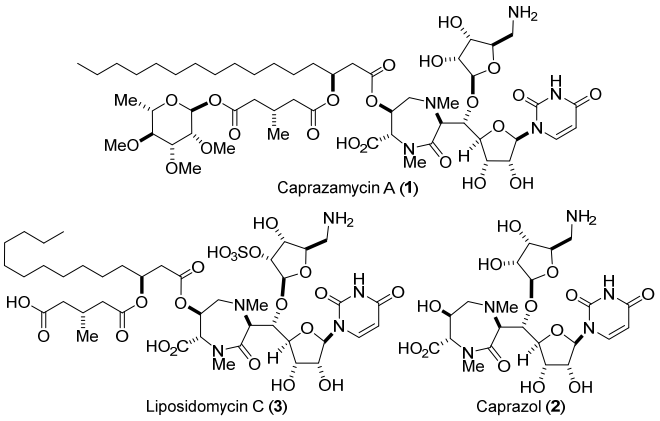
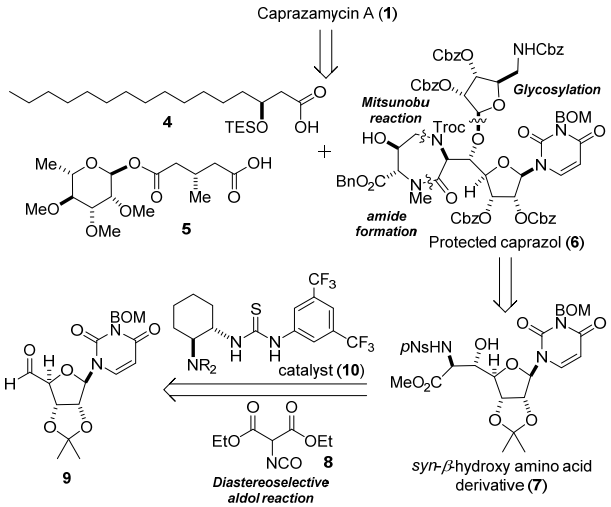


Figure 1. Caprazamycin A (**1**), caprazol (**2**) and liposidomycin C (**3**).

It is challenging to introduce the side chain containing unstable structures. To access caprazamycin A (**1**), it was envisioned that unstable side chains **4** and **5** could be introduced to protected caprazol **6** as the final step. This would be followed by global deprotection without adversely affecting any functional groups (Scheme 1). Benzyl (Bn), carboxybenzyl (Cbz) and benzyloxymethyl (BOM) protecting groups were selected and are readily removed by Pd-catalyzed hydrogenation. Protected **6** was prepared using (i) the Mitsunobu reaction to construct the seven-membered diazepanone, and (ii) a diastereoselective aldol reaction of isocyanate **8** and aldehyde **9** with thiourea catalyst **10** to obtain *syn*-β-hydroxy amino acid derivative **7**.[10]



Scheme 1. Retrosynthesis of caprazamycin A (**1**).

Fatty acid side chains **4** and **5** were first prepared. β-Siloxy carboxylic acid **4** was synthesized from acid chloride **11** via the modified Noyori asymmetric reduction[11]

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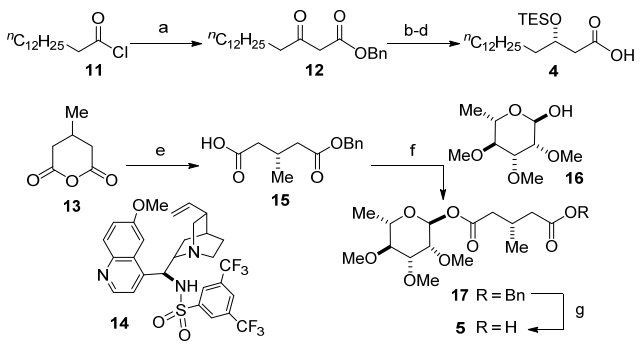
of *β*-ketoester **12**[12] (Scheme 2). Enantioselective desymmetrization of 3-methyl glutaric anhydride **13** using cinchona alkaloid catalyst **14** with Song's procedure[13] gave carboxylic acid **15** with high enantioselectivity (92% ee). Condensation of **15** with L-rhamnose derivative **16**,[14] followed by removal of the benzyl group of ester **17** with hydrogenolysis gave carboxylic acid **5**.



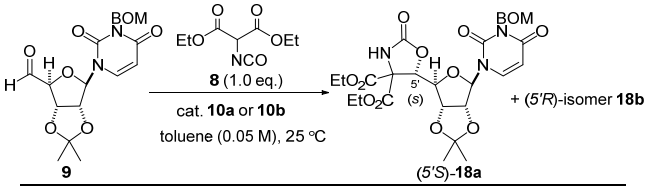
Scheme 2. Synthesis of fatty acid side chains **5** and **6**. Reagents and conditions: a) BnOAc, LDA, THF, −78 °C, 51%; b) H_2, (*S*)-BINAP-$RuBr_2$ (4.0 mol%), MeOH, 50 °C, 48%, 94%ee for two steps; c) TESOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 94%; d) H_2, 10% Pd/C, EtOAc, 25 °C, 92%; e) BnOH, catalyst **14** (10 mol%), CPME, 86%, 92% ee.; f) Ghosez reagent, CH_2Cl_2, 0 °C, then ^{n}BuLi, THF, 48%; g) H_2, 10% Pd/C, EtOAc, 25 °C, 92%. LDA = lithium diisopropylamide, CPME = cyclopentyl methyl ether, Ghosez reagent = 1-chloro- *N,N*,2- trimethylpropenylamine.

Construction of the *syn*-*β*-hydroxy amino acid moiety with *S* configuration at C5' was then investigated. Several strategies have been employed for this in the past,[5d,e,6g,i-k,7a,8a] two of which were for the total synthesis of caprazol. One is Sharpless' asymmetric aminohydroxylation of the *α,β*-unsaturated ester[7a] and the other is the diastereoselective isocyanoacetate aldol reaction.[8a] We anticipated that the stereochemistry at C5' could be controlled with a novel diastereoselective aldol reaction using isocyanate **8** in the presence of an organocatalyst.[10]

Initially, aldehyde **9**[15] was treated with **8** and Et_3N (10 mol%) in toluene to give a mixture of aldol adducts **18a** and **18b** in 50% yield with poor diastereoselectivity (1:1.8) (Table 1, Entry 1). In contrast, treatment with (*S,S*)-thiourea catalyst **10a** (10 mol%) in toluene gave desired aldol adduct **18a** as the major product in 64% yield (3.1:1), along with a small amount of byproduct **19** (Entry 2). The selectivity was improved to 6.5:1 by changing to (*S,S*)-thiourea catalyst **10b** (10 mol%) (Entry 3). Formation of byproduct **19** was suppressed by reducing the amount of catalyst (7 mol%) (Entry 4). Use of (*R,R*)-thiourea catalyst **10b** (10 mol%) gave undesired diastereomer **18b** in 80% yield with high selectivity (>20:1) (Entry 5). This protocol was also applied to the large scale synthesis of aldol adduct **18a**.

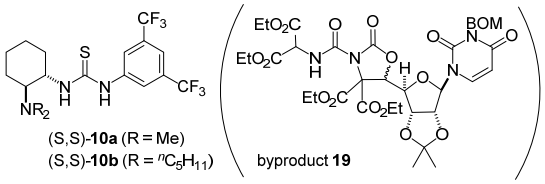
Aldol adduct **18a** was converted to *syn*-*β*-hydroxy amino acid derivative **7** in good yield by regioselective decarboxylation and transesterification of the resultant thermodynamically stable *trans*-oxazolidinone in the presence of zinc cluster $Zn_4(OCOCF_3)_6O$ (Scheme 3).[16] The minor isomer was removed during these transformations. Following Matsuda and Ichikawa's procedure,[7] fluoride **21** underwent *β*-selective glycosylation, reduction of the azido group, Cbz protection and hydrolysis under basic conditions to give **22**. Carboxylic acid **22** was treated with Ghosez reagent[17] and coupled with *anti*-*β*-hydroxy amino acid derivative **23**.[18] The TBS group was selectively removed

Table 1. Optimization of diastereoselective aldol reaction.



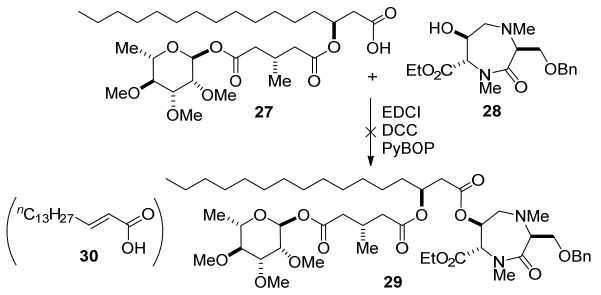
Entry	Catalyst	Results[a]	By-product
1	Et_3N (10 mol%)	50% (dr = 1:1.8)	10%
2	(*S,S*)-**10a** (10 mol%)	64% (dr = 3.1:1)	7%
3	(*S,S*)-**10b** (10 mol%)	77% (dr = 6.5:1)	9%
4	(*S,S*)-**10b** (7 mol%)	80% (dr = 5.0:1)	0%
5	(*R,R*)-**10b** (10 mol%)	80% (dr = 1:>20)	10%

[a] Diastereomeric ratio (dr) was determined by ^{1}H NMR.



and construction of the diazepanone core was extensively investigated. The Mitsunobu reaction of **25** using PPh_3 and di-*tert*-butyl azodicarboxylate (DBAD) proceeded to give the seven-membered ring without epimerization or other side reactions. Finally, protecting group manipulation of **26** gave protected caprazol **6** and the structure was confirmed through conversion to caprazol (**2**).[1,7a,8a]

With side chain fragments **4** and **5** and protected caprazol **6** in hand, we focused on the introduction of the fatty acid side chain. This side chain readily decomposes through *β*-elimination of the *β*-acyloxy carbonyl under basic conditions and cleavage of the *O*-acylglycoside under acidic conditions. In fact, attempts to introduce the fatty acid side chain **27**[19] to model diazepanone **28** using EDCI caused *β*-elimination to give unsaturated carboxylic acid **30** instead of desired **29** (Scheme 4). DCC and PyBOP were also ineffective. The *β*-hydroxy ester of diazepanone may also decompose through *β*-elimination and a retro-aldol reaction. Thus, fragments **4** and **5** were introduced in a stepwise manner.

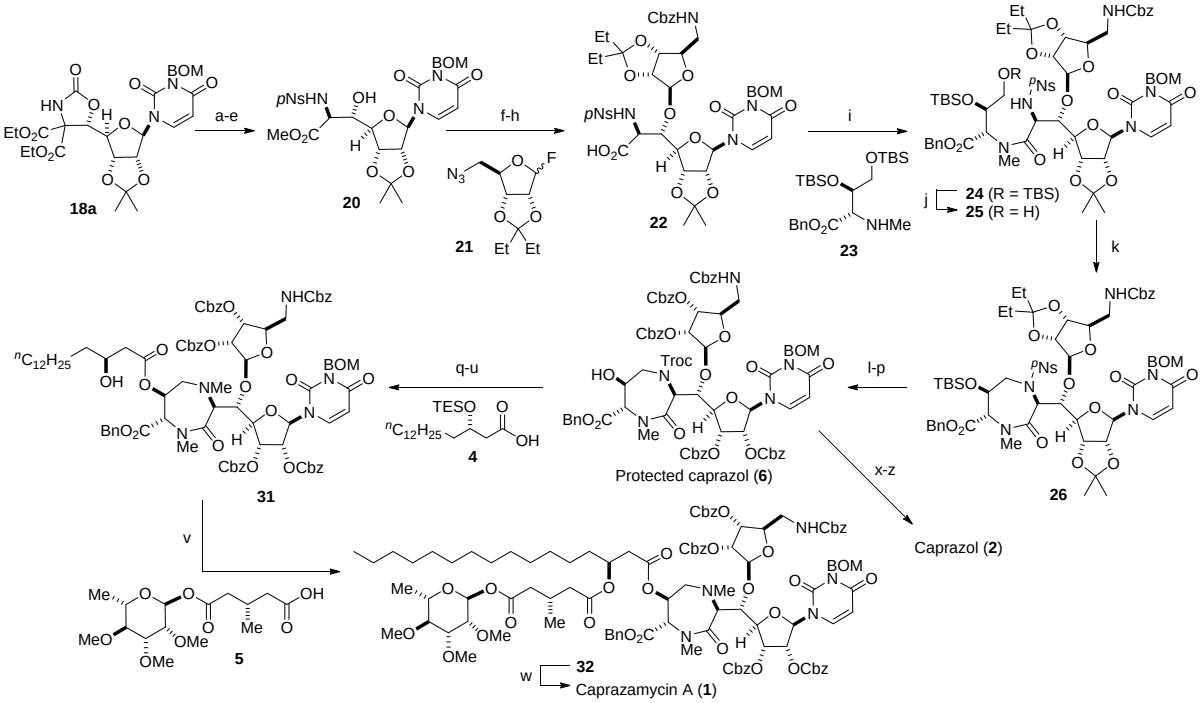


Scheme 4. Initial attempts to introduce fatty acid side chain (**27**).

The final stage of this synthesis began with coupling *β*-siloxy carboxylic acid **4** with protected caprazol **6** without epimerization (Scheme 3). The Troc group was removed under mild conditions without touching the unstable *β*-acyloxy moiety. This was followed by reductive amination. After removal of the TES group, carboxylic acid **5** was introduced to resultant alcohol **31** using Yamaguchi

conditions to give protected caprazamycin A (**32**).[8b] Finally, global deprotection with hydrogenation in the presence of Pd black was successful without side-chain decomposition. This completed the first total synthesis of caprazamycin A (**1**). The ^{1}H and ^{13}C NMR, IR and HRMS for this matched those of the natural product.[20]

In summary, we have accomplished the first total synthesis of caprazamycin A in 23 steps (longest linear sequence from aldehyde **9**). The key points are (i) scalable synthesis of the *syn*-β-hydroxy amino acid moiety with a thiourea-catalyzed diastereoselective aldol reaction, (ii) maintaining the structural integrity of the diazepanone core during introduction of the fatty acid side chain, and (iii) global deprotection with hydrogenation. This is the first report detailing the introduction of the unstable fatty acid side chain. This should allow the synthesis of related liponucleoside antibiotics.



Scheme 3. Total synthesis of caprazamycin A (**1**). Reagents and conditions: a) aq. KOH, THF, 0 to 25 °C; b) DBU, THF, 70 °C, 86% (2 steps); c) $Zn_4(OCOCF_3)_6O$ (3.2 mol%), MeOH, 50 °C, quant.; d) NaH, *p*NsCl, DMF, 0 to 25 °C; e) NaOMe, MeOH, 65% (2 steps); f) **21**, $BF_3{\cdot}Et_2O$, MS4Å, CH_2Cl_2, −30 °C, 71%; g) PPh_3, THF/PhH = 1:1 then CbzCl, aq $NaHCO_3$, 0 to 25 °C; h) $Ba(OH)_2{\cdot}8H_2O$, THF/H_2O = 4:1, 0 to 25 °C; i) Ghosez reagent, CH_2Cl_2, 0 °C then **23**, aq. $NaHCO_3$, 0 °C, 46% (3 steps); j) CSA, MeOH/CH_2Cl_2 = 1:1, 0 °C, 68% (17% for recovered **24**, b.r.s.m. 82%); k) PPh_3, DBAD, toluene, 0 °C, 75%; l) K_2CO_3, PhSH, MeCN, 0 to 25 °C, 73%; m) TrocCl, DMAP, pyridine, CH_2Cl_2, 0 to 25 °C, 79%; n) TsOH·H_2O, MeOH, 60 °C, 41% and diol having penthylidene acetal (21%); o) CbzCl, DMAP, CH_2Cl_2, 0 to 25 °C; p) *p*TsOH·H_2O, MeOH, 25 to 60 °C, 71% (2 steps); q) **4**, EDCI, DMAP, CH_2Cl_2, 0 to 25 °C; r) Zn, AcOH/THF, 25 °C; s) AcOH, $ClCH_2CH_2Cl$, 25 °C; t) $(CH_2O)_n$, $NaBH(OAc)_3$, AcOH/$ClCH_2CH_2Cl$, 25 °C; u) HF·py, THF, 0 °C to 25 °C, 43% (5 steps); v) **5**, 2,4,6- trichlorobenzoyl chloride, DMAP, Et_3N, 0 to 25 °C, 64%; w) Pd black, EtOH/HCO_2H = 20:1, 25 °C, 98%; x) Zn, AcOH/THF, 25 to 50 °C; y) $(CH_2O)_n$, $NaBH(OAc)_3$, AcOH/CH_2Cl_2, 25 °C, quant. (2 steps); z) Pd black, EtOH/HCO_2H = 10:1, 25 °C, 46%. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, *p*Ns = 4-nitrobenzenesulfonyl, Ghosez reagent = 1-chloro-*N,N*,2-trimethylpropenylamine, CSA = 10-camphor sulfonic acid, DBAD = di-tert-butyl azodicarboxylate, TrocCl = 2,2,2-trichloroethyl chloroformate, DMAP = N,N-dimethyl-4-aminopyridine, Cbz = benzyloxycarbonyl, *p*Ts = *p*-toluenesulfonyl, EDCI = 1-Ethyl-3- (3-dimethylaminopropyl) carbodiimide

Keywords: antibiotics · natural products · total synthesis · caprazamycin · liponucleoside

[1] a) M. Igarashi, N. Nakagawa, N. Doi, S. Hattori, H. Naganawa, M. Hamada, *J. Antibiot.* **2003**, *56*, 580; b) T. Takeuchi, M. Igarashi, H. Naganawa, JP P2003-12687A, 2003; c) M. Igarashi, Y. Takahashi, T. Shitara, H. Nakamura, H. Naganawa, T. Miyake, Y. Akamatsu, *J. Antibiot.* **2005**, *58*, 327; d) C. Dini, *Curr. Top. Med. Chem*. **2005**, *5*, 1221; e) T. D. H. Bugg, A. J. Lloyd, D. I. Roper, *Infect. Disord. Drug Targets*, **2006**, *6*, 85.

[2] a) C. Dini, P. Collette, N. Drochon, J. C. Guillot, G. Lemoine, P. Mauvais, J. Aszodi, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1839; b) Y. Ishizaki, C. Hayashi, K. Inoue, M. Igarashi, Y. Takahashi, V. Pujari, D. C. Crick, P. J. Brennan, A. Nomoto, *J. Biol. Chem.* **2013**, *288*, 30309.

[3] K. Kimura, T. D. H. Bugg, *Nat. Prod. Rep*. **2003**, *20*, 252.

[4] a) Y. Takahashi, M. Igarashi, T. Miyake, H. Soutome, K. Ishikawa, Y. Komatsuki, Y. Koyama, N. Nakagawa, S. Hattori, K. Inoue, N. Doi, Y. Akamatsu, *J. Antibiot.* **2013**, *66*, 171; b) S. N. M. Hanif, A. J. Hickey, L. Garcia-Contreras, *J. Pharm. Biomed. Anal.* **2014**, *88*, 370.

[5] Synthetic studies toward caprazamycins: a) S. Hirano, S. Ichikawa, A. Matsuda, *Bioorg. Med. Chem.* **2008**, *16*, 5123; b) S. Ichikawa, *Chem. Pharm. Bull.* **2008**, *56*, 1059; c) K. Ii, S. Ichikawa, B. Al-Dabbagh, A. Bouhss, A. Matsuda, *J. Med. Chem.* **2010**, *53*, 3793; d) A. P. Spork, S. Koppermann, B. Dittrich, R. Herbst-Irmer, C. Ducho, *Tetrahedron: Asymmetry* **2010**, *21*, 763; e) F. Sarabia, C. Vivar-García, C. García-Ruiz, L. Martín-Ortiz, A. Romero-Carrasco, *J. Org. Chem.* **2012**, *77*, 1328; f) C. Tsukano, S. Yokouchi, A. L. Girard, T. Kuribayashi, S. Sakamoto, T. Enomoto, Y. Takemoto, *Org. Biomol. Chem.* **2012**, *10*, 6074; g) H. Miyaoka, J. Wada, E. Kawashima, *Heterocycles* **2014**, *88*, 719.

[6] Synthetic studies towards related liponucleoside antibiotics: a) M. R. Spada, M. Ubukata, K. Isono, *Heterocycles* **1992**, *34*, 1147; b) S. Knapp, S. Nandan, L. Resnick, *Tetrahedron Lett.* **1992**, *33*, 5485; c) K. S. Kim, I. H. Cho, Y. H. Ahn, J. I. Park, *J. Chem. Soc., Perkin Trans 1* **1995**, 1783; d) W. J. Moore, F. A. Luzzio, *Tetrahedron Lett.* **1995**, *36*, 6599; e) K. S. Kim, C. S. Cheong, J. S. Hahn, J. I. Park, *Bull. Korean Chem. Soc.* **1997**, *18*, 465; f) Y. L. Merrer, C. Gravier-Pelletier, M. Gerrouache, J. C. Depezay, *Tetrahedron Lett.* **1998**, *39*, 385; g) K. S. Kim, Y. H. Ahn, *Tetrahedron: Asymmetry* **1998**, *9*, 3601; h) S. Knapp, G. J. Morriello, S. R. Nandan, T. J. Emge, G. A. Doss, R. T. Mosley, L. Chen, *J. Org. Chem.* **2001**, *66*, 5822; i) C. Gravier- Pelletier, M. Milla, Y. L.

Merrer, J. C. Depezay, *Eur. J. Org. Chem*. **2001**, *16*, 3089; j) S. Knapp, G. J. Morriello, G. A. Doss, *Org. Lett*. **2002**, *4*, 603; k) B. Drouillat, O. Poupardin, Y. Bourdreux, C. Greck, *Tetrahedron Lett.* **2003**, *31*, 2781; l) A. Yamashita, E. B. Norton, R. T. Williamson, D. M. Ho, S. Sinishtaj, T. S. Mansour, *Org. Lett*. **2003**, *5*, 3305; m) F. Sarabia, L. Martin-Ortiz, F. J. Lopez-Herrera, *Org. Lett*. **2003**, *5*, 3927; n) N. Nakajima, T. Isobe, S. Irisa, M. Ubukata, *Heterocycles* **2003**, *59*, 107; o) S. Fukunishi, M. Ubukata, N. Nakajima, *Heterocycles* **2005**, *66*, 129; p) Y. Bourdreux, B. Drouillat, C. Greck, *Lett. Org. Chem.* **2006**, *3*, 368; q) S. Fukunishi, M. Ubukata, N. Nakajima, *Heterocycles* **2007**, *73*, 627; r) O. Monasson, M. Ginisty, G. Bertho, C. Gravier-Pelletier, Y. L. Merrer, *Tetrahedron Lett.* **2007**, *48*, 8149; s) X. H. Xu, A. E. Trunkfield, T. D. H. Bugg, F. L. Qing, *Org. Biomol. Chem.* **2008**, *6*, 157; t) O. Monasson, M. Ginisty, J. Mravljak, G. Bertho, C. Gravier-Pelletier, Y. L. Merrer, *Tetrahedron: Asymmetry* **2009**, *20*, 2320; u) M. J. Fer, P. Doan, T. Prangé, S. Calvet-Vitale, C. Gravier-Pelletier, *J. Org. Chem.* **2014**, *79*, 7758; v) A. P. Spork, M. Büschleb, O. Ries, D. Wiegmann, S. Boettcher, A. Mihalyi, T. D. H. Bugg, C. Ducho, *Chem. Eur. J.* **2014**, *20*, 15292.

[7] a) S. Hirano, S. Ichikawa, A. Matsuda, *Angew. Chem. Int. Ed*. **2005**, *44*, 1854; b) S. Hirano, S. Ichikawa, A. Matsuda, *J. Org. Chem*. **2007**, *72*, 9936; c) S. Hirano, S. Ichikawa, A. Matsuda, *J. Org. Chem.* **2008**, *73*, 569.

[8] a) P. Gopinath, L. Wang, H. Abe, G. Ravi, T. Masuda, T. Watanabe, M. Shibasaki, *Org. Lett.* **2014**, *16*, 3364; b) P. Gopinath, T. Watanabe, M. Shibasaki, *J. Org. Chem.* **2012**, *77*, 9260.

[9] a) K. Isono, M. Uramoto, H. Kusakabe, K. Kimura, K. Izaki, C. C. Nelson, J. A. McCloskey, *J. Antibiot*. **1985**, *38*, 1617; b) K. Kimura, Y. Ikeda, S. Kagami, M. Yoshihama, K. Suzuki, H. Osada, K. Isono, *J. Antibiot*. **1998**, *51*, 1099; c) M. Ubukata, K. Kimura, K. Isono, C. C. Nelson, J. M. Gregson, J. A. McCloskey, *J. Org. Chem.* **1992**, *57*, 6392; d) K. Kimura, Y. Ikeda, S. Kagami, M. Yoshihama, M. Ubukata, Y. Esumi, H. Osada, K. Isono, *J. Antibiot*. **1998**, *51*, 647; e) K. Kimura, S. Kagami, Y. Ikeda, H. Takahashi, M. Yoshihama, H. Kusakabe, H. Osada, K. Isono, *J. Antibiot*. **1998**, *51*, 640.

[10] S. Sakamoto, N. Kazumi, Y. Kobayashi, C. Tsukano, Y. Takemoto, *Org. Lett*. **2014**, *16*, 4758.

[11] a) R. Noyori, H. Takaya, *Acc. Chem. Res.* **1990**, *23*, 345; b) V. Ratovelomanana-Vidal, C. Girard, R. Touati, J. P. Tranchier, B. Ben Hassine, J. P. Genêt, *Adv. Synth. Catal.* **2003**, *345*, 261.

[12] D. F. Taber, P. B. Deker, H. M. Fales, T. H. Jones, H. A. Lloyd, *J. Org. Chem.* **1988**, *53*, 2968.

[13] a) S. H. Oh, H. S. Rho, J. W. Lee, J. E. Lee, S. H. Youk, J. Chin, C. E. Song, *Angew. Chem. Int. Ed.* **2008**, *47*, 7872; b) T. Honjo, T. Tsumura, S. Sano, Y. Nagao, K. Yamaguchi, Y. Sei, *Synlett*, **2009**, *20*, 3279.

[14] a) M. S. Arias-Pérez, M. S. López, M. J. Santos, *J. Chem. Soc., Perkin Trans. 2*, **2002**, 1549; b) D. A. Evans, W. C. Black, *J. Am. Chem. Soc.* **1993**, *115*, 4497.

[15] Aldehyde **9** was prepared from commercially available uridine in three steps. See Supporting Information.

[16] T. Ohshima, T. Iwasaki, Y. Maegawa, A. Yoshiyama, K. Mashima, *J. Am. Chem. Soc.* **2008**, *130*, 2944.

[17] A. Devos, J. Remion, A.-M. Frisque-Hesbain, A. Colens, L. Ghosez, *J. Chem. Soc., Chem. Commun.* **1979**, 1180.

[18] Amine **23** was synthesized from L-(+)-diethyl tartrate in ten steps. See Supporting Information.

[19] Carboxylic acid **27** was prepared from the precursor of **4** and **5** as described by Shibasaki and Watanabe *et al* (ref 8b).

[20] The NMR spectrum of caprazamycin A was dependent on concentration and pKa. Thus, the NMR was measured in DMSO-d_6/D_2O/DCO_2D (20:1:1 for comparison. Also see Supporting Information.