Towards the stereochemical assignment of natural lydiamycin A†

Bo Chen,[a] Lu Dai,[a] Hui Zhang,[a] Wenfei Tan,[a] Zhengshuang Xu*[ab] and Tao Ye*[ab]

Received (in College Park, MD, USA) 28th October 2009, Accepted 23rd November 2009
First published as an Advance Article on the web 14th December 2009
DOI: 10.1039/b922544g

A convergent approach leading to the stereoselective synthesis of four diastereomers of lydiamycin A has been established and verified.

Tuberculosis (TB) is among the most devastating infectious diseases in the world.[1] There were 9.27 million new cases of TB in 2007, 1.78 million deaths from TB and 13.7 million prevalent cases.[2] The general treatment of TB requires a cocktail of three or four different drugs comprising isoniazid, rifampin, pyrazinamide, and/or ethambutol for a minimum of six months.[3] While these first-line agents remain useful in treating susceptible *Mycobacterium tuberculosis* strains, the increasing occurrence of potentially incurable multiple-drug-resistant (MDR) TB strains demands the discovery of *anti*-TB agents with novel structures.

Lydiamycin A was isolated from the fermentation broth of *Streptomyces lydicus* (strain HKI0343) by Sattler and co-workers in 2008. It displayed potent antimycobacterial activity against slow-growing and pathogenic mycobacteria, including the *M. tuberculosis* standard strain (H37Rv) and a multiresistant clinical isolate.[4] Furthermore, lydiamycin A is devoid of obviously reactive functional groups, and it is not cytotoxic to various tumor cell lines, suggesting a selective antibacterial target.[4,5] The proposed structure of lydiamycin A was established by a combination of CID ESIMS/MS data and extensive NMR spectroscopic analysis. However, the relative and absolute stereochemistry of C19 and C25 stereogenic centers were not assigned. The core of lydiamycin A is a 13-membered macrocycle composed of four amino acid residues with an ester linkage between the hydroxyl group of serine and the carboxyl group of piperazic acid residue (PBB1). A 2,3,4,5-tetrahydro-3-pyridazine-carboxylic-acid-containing side chain is appended to the N-terminus of the core depsipeptide. In order to obtain sufficient material for more extensive biological evaluation, as well as to determine the absolute stereochemistry of lydiamycin A, we hereby report our synthetic approaches leading to the construction of four diastereomers of lydiamycin A.

Since the stereochemistry of the side chain was not established, we planned a convergent synthetic approach (Fig. 1, route A) including the attachment of four possible diastereomers of the side chain to the cyclodepsipeptide core (**3**) in the late stage. Accordingly, **3** can be constructed from the acyclic precursor **6** *via* a macrolactonization reaction. Tetrapeptide **6** was envisaged to be synthesized from dipeptide **7**. Furthermore, side chain moiety **5** was synthetically disconnected to give two subunits, **8** and **9**. The advantage of this strategy lies in the facile installation of the four possible stereoisomers of side chain or other amino-acid-derived fragments to the macrocycle. While this strategy is appealing, intermediate **4** might be poised to undergo *O*,*N*-acyl migration.[6,7] Further inspection of the literature revealed that some depsipeptide macrocycles may be able to suppress the propensity for *O*,*N*-acyl migration upon generation of a free N-terminal amino group.[8] So, we decided to carry out an alternative unambiguous approach (route B of Fig. 1), including the macrolactonization of seco acid **2** that would serve as an excellent proof of structure for our convergent route. The acyclic precursor **2** could be obtained from building blocks **5** and **6**, which are advanced intermediates to be employed in route A.

The synthesis of macrocycle **3** commenced with the hydrogenolysis of bis-Cbz protecting groups in (*R*)-tetrahydropyridazine **10**[9] as described in Scheme 1. Regioselectively protection of the sterically less-hindered nitrogen of the resulting cyclic hydrazine with Cbz-Cl provided **11** in 86% yield. Silver cyanide-mediated coupling reaction between Fmoc-Ala-Cl and **11** afforded peptide **7** in excellent yield.[7d,10] Subsequent removal of Fmoc in **7** with diethylamine and coupling with L-Cbz-Ser-(OTBDPS)-D-Leu-OH yielded the desired tetrapeptide **12** in 95% yield. Selective cleavage of the primary TBS-ether of **12** was followed by sequential Dess–Martin[11] and Pinnick[12] oxidations to afford the corresponding acid **6** in 66% yield. After removal of the TBDPS ether of **6**, the resulting hydroxy acid was subjected to macrolactonization by carboxylic acid activation under a variety of conditions, including those of Yamaguchi,[13] Keck[14] and Mukaiyama,[15] but only recovered starting material or undesired side products were isolated. The failure of the aforementioned macrolactonizations might be attributed to the sterically hindered nature of the carboxylic acid. To our delight, an esterification strategy involving hydroxyl activation under the Mitsunobu conditions[16] produced macrocycle **3** in 60% yield along with some unidentified side products.

The preparation of the side-chain-derived fragment **5** began with the stereospecific alkylation of imide **13**[17] with *tert*-butyl bromoacetate.[18] Subsequent hydrolysis of the chiral auxiliary followed by protection of the corresponding acid with allyl bromide, produced **8** in 53% yield over three steps. Cleavage of the *tert*-butyl ester of **8**, and activation of the resultant acid as its acyl chloride allowed condensation with the known monoprotected hydrazine **9**,[19] affording fragment **5** in 60% overall yield (Scheme 2).

[a] *Laboratory of Chemical Genomics, Peking University Shenzhen Graduate School, University Town of Shenzhen, Xili, Nanshan District, 518055, Shenzhen, China E-mail: yet@szpku.edu.cn; xuzs@szpku.edu.cn*
[b] *Department of Applied Biology & Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong, China. E-mail: bctaoye@inet.polyu.edu.hk; Tel: +852 34008722*

† Electronic supplementary information (ESI) available: Full details for experimental procedures for compounds **1**, **1a**, **1b**, **1c**, **3**, **5**, **5a**, **5b**, **5c**, **7**, **8**, **11**, **12**, **14**, **15**, **15a**, **15b**, **15c** and ^{1}H and ^{13}C NMR spectra for compounds **1**, **1a**, **1b**, **1c**, **3**, **5**, **5a**, **6′**, **8**, **11**, **12**, **13′**, **14**, **15**, **15a**, **15b**, **15c**. See DOI: 10.1039/b922544g

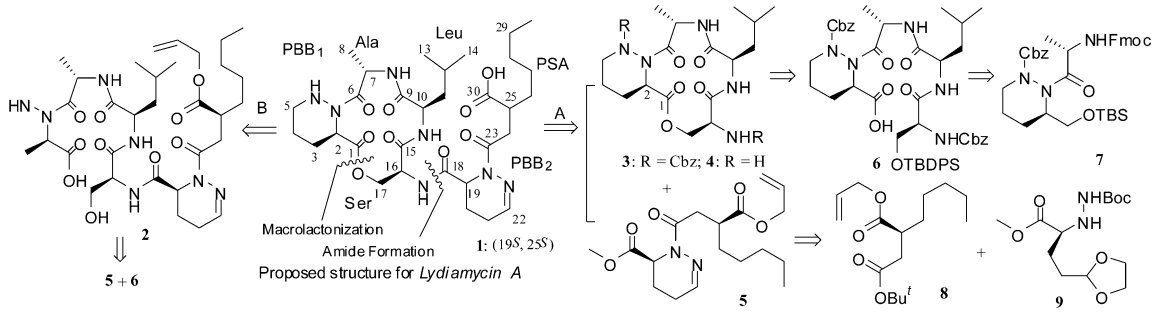
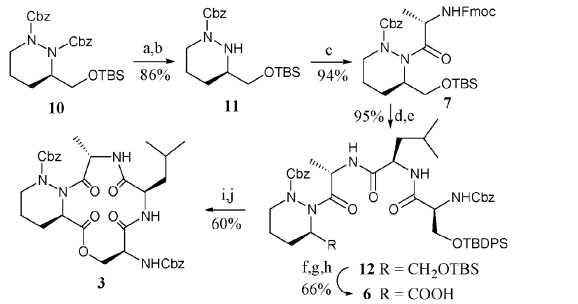
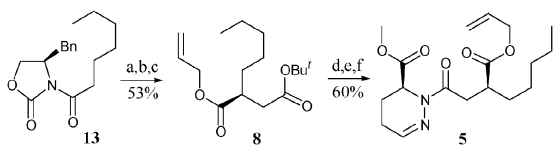


Fig. 1 Structure and retrosynthetic analysis of the proposed structure of lydiamycin A.



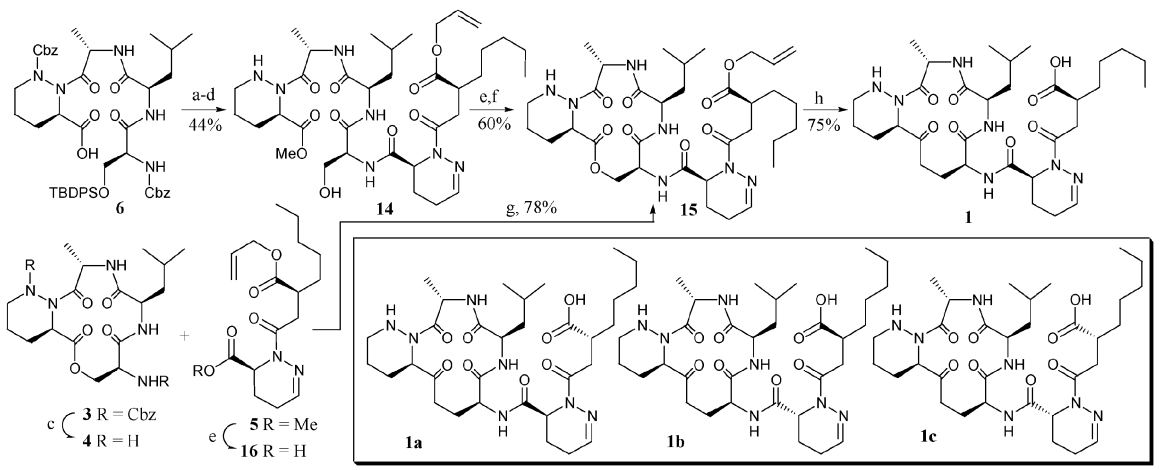
Scheme 1 *Reagents and conditions*: (a) H_2, Pd/C, 72 h; (b) Cbz-Cl, Et_3N, MeOH; (c) Fmoc-L-Ala, $(COCl)_2$, DMF (cat.), CH_2Cl_2, then **11**, AgCN, benzene, 80 °C; (d) Et_2NH, MeCN; (e) L-Cbz-Ser(OTBDPS)-D-Leu-OH, EDCI, HOBt, DIPEA, 20 h; (f) PPTS, MeOH, 3 h; (g) DMP, $NaHCO_3$, CH_2Cl_2; (h) $NaClO_2$, NaH_2PO_4, *t*-BuOH–H_2O; (i) TBAF, THF; (j) DEAD, Ph_3P, THF, 24 h.



Scheme 2 *Reagents and conditions*: (a) LiHMDS, $BrCH_2CO_2Bu^t$, THF, −78 °C; (b) LiOH, H_2O_2, THF–H_2O; (c) allyl bromide, DIPEA, MeCN, 24 h; (d) TFA, CH_2Cl_2, 3 h; (e) $(COCl)_2$, DMF, CH_2Cl_2, then **9**, collidine; (f) TFA–H_2O, 0.5 h.

With the requisite subunits in hand, we embarked on the elaboration of both routes toward the allyl ester of the proposed structure of lydiamycin A (Scheme 3). The linear precursor **14** was prepared from **6** in four steps involving the treatment of acid **6** with diazomethane, followed by removal of the silicon protecting group, hydrogenolysis of the bis-Cbz groups and condensation of the crude free amine with acid **16** through the action of HATU/HOAt,[20] Hydrolysis of the methyl ester of **14** with LiOH afforded the corresponding acid **2**, which then underwent Mitsunobu esterification under identical conditions as for **3**, to afford cyclodepsipeptide **15** in 60% yield. Next, we turned to explore whether the convergent route would also produce cyclodepsipeptide **15**, so as to pave the way for the preparation of diastereomers. Gratifyingly, the HATU/HOAt-promoted coupling reaction between amine **4** and acid **16** provided **15** in 78% yield, which was identical in all respects with the product derived from **14** *via* the linear route as described above. This result indicated that amine **4**, derived from macrocycle **3**, did not undergo *O*,*N*-acyl migration during the peptide coupling process. Finally, the allyl ester of **15** was efficiently removed by tetrakis(triphenylphosphine)palladium generated *in situ* in the presence of diethylamine to afford the (2*R*,7*S*,10*R*,16*S*,19*S*,25*S*)-diastereomer (**1**) in 75% yield.[21] The convergent synthetic strategy with a key late-stage divergent introduction of the side chain detailed herein is flexible, thereby allowing for the synthesis of multiple diastereomers that might facilitate the stereochemistry determination of lydiamycin A.

As mentioned previously, both relative stereochemistry and absolute configuration of the side chain were unknown. Having one diastereomer (**1**), and a practical route to lydiamycin A in hand, we set out to pursue total syntheses of the other three possible (2*R*,7*S*,10*R*,16*S*)-diastereomers. In the event,



Scheme 3 *Reagents and conditions*: (a) CH_2N_2, Et_2O; (b) TBAF, THF, 3 h; (c) H_2, Pd/C, MeOH; (d) **16**, HATU, HOAt, DIPEA, DMF, 20 h; (e) LiOH, THF–MeOH–H_2O; (f) DEAD, PPh_3, THF, 24 h; (g) HATU, HOAt, DIPEA, DMF, 24 h; (h) Pd_2dba_3, Ph_3P, Et_2NH, 2 h.

three additional diastereomers of the side chain were synthesized from imide **13** or its enantiomer *ent*-**13**, and hydrazine **9** or its enantiomer *ent*-**9** under identical conditions as for **5**. An attachment of individual diastereomers of the side chain to the macrocycle, *via* amine **4** was carried out according to the established convergent route, in the presence of HATU/HOAt, to afford (2*R*,7*S*,10*R*,16*S*,19*S*,25*R*)-diastereomer **1a**, (2*R*,7*S*,10*R*,16*S*,19*R*,25*S*)-diastereomer **1b** and (2*R*,7*S*,10*R*,16*S*,19*R*,25*R*)-diastereomer **1c** (Scheme 3). To our disappointment, the ^{1}H and ^{13}C NMR spectroscopic properties of each diastereomer **1**, **1a**, **1b** and **1c** were different from those of lydiamycin A (see ESI†). Significant discrepancies are most apparent in the region 4–5 ppm of ^{1}H NMR spectra. In addition, the specific rotation value of each diastereomer (**1**, **1a**, **1b** and **1c**) was far different from that reported for the natural lydiamycin A. Both the magnitude and sign of the optical rotation measured for the four diastereomers did not match the value reported for the natural product. It would appear that the error in the original assignment of the stereochemistry of lydiamycin A lies somewhere in the macrocycle. Since both the stereochemical analysis of the serine residue by the Marfey method and tentative stereochemical assignment of the piperazic acid moiety (PBB1) by means of molecular modeling are not very reliable, we suspected that the proposed stereochemical assignment of the serine residue or/and the piperazic acid residue (PBB1) might be incorrect. In order to resolve the true structure of natural lydiamycin A, further synthetic studies towards all the possible isomers with respect to C2 and C16 stereogenic centers are required.

In summary, we have developed both linear and convergent routes for the synthesis of the proposed structure of lydiamycin A. Efforts toward the total synthesis of additional diastereomers are currently underway in our laboratory. A more detailed account of this work and further studies toward the establishment of the stereochemistry of lydiamycin A and biological evaluation of diastereomers will be disclosed in due course.

We acknowledge financial support from the Hong Kong Research Grants Council (Project: PolyU 5407/06M) and financial support from the Shenzhen Bureau of Science, Technology and Information (08systs-01, JC200903160367A). Z. S. X. would like to thank the support from Shenzhen Foundation for R&D (SZKJ-2007011, SY2008063 00179A), Nanshan Science & Technology (NANKEYUAN2007019) and NSF of GuangDong Province (8451805704000656).

Notes and references

1 (*a*) C. Dye, *Nat. Rev. Microbiol.*, 2009, **7**, 81; (*b*) D. G. Russell, *Nat. Rev. Microbiol.*, 2007, **5**, 39; (*c*) Y. A. W. Skeiky and J. C. Sadoff, *Nat. Rev. Microbiol.*, 2006, **4**, 469; (*d*) H. I. Boshoff and C. E. Barry, *Nat. Rev. Microbiol.*, 2005, **3**, 70; (*e*) A. Koul, T. Herget, B. Klebl and A. Ullrich, *Nat. Rev. Microbiol.*, 2004, **2**, 189; (*f*) K. Duncan and C. E. Barry, *Curr. Opin. Microbiol.*, 2004, **7**, 460.

2 Global tuberculosis control-epidemiology, strategy, financing http://www.who.int/tb/publications/global_report/2009/en/.

3 World Health Organization: *Tuberculosis handbook*, WHO, Geneva, 1998 (WHO/TB/98.253).

4 X. Huang, E. Roemer, I. Sattler, U. A. Christner and S. Grabley, *Angew. Chem., Int. Ed.*, 2006, **45**, 3067.

5 B. R. Copp, Antimycobacterial natural products, *Nat. Prod. Rep.*, 2003, **20**, 535.

6 (*a*) A. Taniguchi, Y. Sohma, M. Kimura, T. Okada, K. Ikeda, Y. Hayashi, T. Kimura, S. Hirota, K. Matsuzaki and Y. Kiso, *J. Am. Chem. Soc.*, 2006, **128**, 696; (*b*) I. Coin, R. Dölling, E. Krause, M. Bienert, M. Beyermann, C. D. Sferdean and L. A. Carpino, *J. Org. Chem.*, 2006, **71**, 6171; (*c*) T. Yoshiya, Y. Sohma, T. Kimura, Y. Hayashi and Y. Kiso, *Tetrahedron Lett.*, 2006, **47**, 7905; (*d*) Y. Sohma, A. Taniguchi, M. Skwarczynski, T. Yoshiya, F. Fukao, T. Kimura, Y. Hayashi and Y. Kiso, *Tetrahedron Lett.*, 2006, **47**, 3013; (*e*) Y. Sohma, Y. Hayashi, M. Kimura, Y. Chiyomori, A. Taniguchi, M. Sasaki, T. Kimura and Y. Kiso, *J. Pept. Sci.*, 2005, **11**, 441; (*f*) Y. Sohma, M. Sasaki, Y. Hayashi, T. Kimura and Y. Kiso, *Chem. Commun.*, 2004, 124; (*g*) L. Mouls, G. Subra, C. Enjalbal, J. Martinez and J.-L. Aubagnac, *Tetrahedron Lett.*, 2004, **45**, 1173; (*h*) M. Horikawa, Y. Shigeri, N. Yumoto, S. Yoshikawa, T. Nakajima and Y. Ohfune, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 2027.

7 (*a*) H. Seo and D. Lim, *J. Org. Chem.*, 2009, **74**, 906; (*b*) J. Lécaillon, P. Gilles, G. Subra, J. Martinez and M. Amblard, *Tetrahedron Lett.*, 2008, **49**, 4674; (*c*) M. Stawikowski and P. Cudic, *Tetrahedron Lett.*, 2006, **47**, 8587; (*d*) P. L. Durette, F. Baker, P. L. Barker, J. Boger, S. S. Bandy, M. L. Hammond, T. J. Lanza, A. A. Pcssolano and C. G. Caldwell, *Tetrahedron Lett.*, 1990, **31**, 1237; (*e*) A. B. Mauger and O. A. Stuart, *Int. J. Pept. Protein Res.*, 1987, **30**, 481.

8 (*a*) J. Adrio, C. Cuevas, I. Manzanares and M. M. Joullie, *J. Org. Chem.*, 2007, **72**, 5129; (*b*) M. Gutiérrez-Rodriguez, M. Martin-Martinez, M. T. Garcia-Lopez, R. Herranz, C. Polanco, I. Rodriguez-Campos, I. Manzanares, F. Cardenas, M. Feliz, P. Lloyd-Williams and E. Giralt, *J. Med. Chem.*, 2004, **47**, 5700; (*c*) J. E. Tarver, Jr., A. J. Pfizenmayer and M. M. Joullie, *J. Org. Chem.*, 2001, **66**, 7575; (*d*) D. L. Boger and J. H. Chen, *J. Am. Chem. Soc.*, 1993, **115**, 11624; (*e*) P. Jouin, J. Poncet, M. N. Dufour, A. Pantaloni and B. Castro, *J. Org. Chem.*, 1989, **54**, 617.

9 For a landmark review on piperazic acids and dehydropiperazic acid, see: M. A. Ciufolini and N. Xi, *Chem. Soc. Rev.*, 1998, **27**, 437; for the synthesis of tetrahydropyridazine **10**, see: Y. Henmi, K. Makino, Y. Yoshitomi, O. Hara and Y. Hamad, *Tetrahedron: Asymmetry*, 2004, **15**, 3477.

10 (*a*) K. J. Hale, S. Manaviazar, J. H. George, M. A. Walters and S. A. Dalby, *Org. Lett.*, 2009, **11**, 733; (*b*) K. J. Hale, S. Manaviazar, L. Lazarides, J. George, M. A. Walters, J. Cai, V. M. Delisser, G. S. Bhatia, S. A. Peak, S. M. Dalby, A. Lefranc, Y.-N. P. Chen, A. W. Wood, P. Crowe, P. Erwin and M. El-Tanani, *Org. Lett.*, 2009, **11**, 737; (*c*) K. J. Hale and L. Lazarides, *Org. Lett.*, 2002, **4**, 1903; (*d*) K. J. Hale and L. Lazarides, *Chem. Commun.*, 2002, 1832; (*e*) K. J. Hale and J. Cai, *Chem. Commun.*, 1997, 2319.

11 D. B. Dess and J. C. Martin, *J. Am. Chem. Soc.*, 1991, **113**, 7277.

12 B. S. Bal, W. E. Childers, Jr and H. W. Pinnick, *Tetrahedron*, 1981, **37**, 2091.

13 (*a*) J. Inanaga, K. Hirata, H. Saeki, T. Katsuki and M. Yamaguchi, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1989; (*b*) M. Hikota, Y. Sakurai, K. Horita and O. Yonemitsu, *Tetrahedron Lett.*, 1990, **31**, 6367.

14 E. P. Boden and G. E. Keck, *J. Org. Chem.*, 1985, **50**, 2394.

15 T. Mukaiyama, M. Usui and K. Saigo, *Chem. Lett.*, 1976, 49.

16 O. Mitsunobu, *Synthesis*, 1981, 1.

17 S. Monma, T. Sunazuka, K. Nagai, T. Arai, K. Shiomi, R. Matsui and S. Ōmura, *Org. Lett.*, 2006, **8**, 5601.

18 For a related approach to the synthesis of a chiral pentylsuccininate ester, see: (*a*) K. Tamaki, T. Ogita, K. Tanzawa and Y. Sugimura, *Tetrahedron Lett.*, 1993, **34**, 683; (*b*) K. Tamaki, S. Kurihara, T. Oikawa, K. Tanzawa and Y. Sugimura, *J. Antibiot.*, 1994, **47**, 1481.

19 For the application of related *ab initio* methodology for the asymmetric construction of dehydropiperazic acid derivatives see the following papers: (*a*) Y. Nakamura and C.-G. Shin, *Chem. Lett.*, 1991, 1953; (*b*) I. H. Aspinall, P. M. Cowley, G. Mitchell and R. J. Stoodley, *J. Chem. Soc., Chem. Commun.*, 1993, 1179; (*c*) U. Schmidt and B. Riedl, *J. Chem. Soc., Chem. Commun.*, 1992, 1186; (*d*) Y. Nakamura, A. Ito and C.-G. Shin, *Bull. Chem. Soc. Jpn.*, 1994, **67**, 2151; (*e*) D. Valognes, P. Belmont, N. Xi and M. A. Ciufolini, *Tetrahedron Lett.*, 2001, **42**, 1907; (*f*) D. L. Boger, M. W. Ledeboer and M. Kume, *J. Am. Chem. Soc.*, 1999, **121**, 1098; (*g*) M. A. Ciufolini, D. Valognes and N. Xi, *Angew. Chem., Int. Ed.*, 2000, **39**, 2493.

20 For the synthesis of monoprotected hydrazine **9**, see: N. Xi, L. B. Alemany and M. Ciufolini, *J. Am. Chem. Soc.*, 1998, **120**, 80; L. A. Carpino, *J. Am. Chem. Soc.*, 1993, **115**, 4397.

21 H. Kogen, T. Kiho, M. Nakayama, Y. Furukawa, T. Kinoshita and M. Inukai, *J. Am. Chem. Soc.*, 2000, **122**, 10214.

