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SYNTHESIS OF ORTHOGONALLY PROTECTED ACTINOIDIC ACID TRIMETHYL ETHER

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We dedicate this manuscript to Professor Tohru Fukuyama on the occasion of his 70th birthday and his retirement from Nagoya University.

Abstract – We describe the asymmetric synthesis of actinoidic acid trimethyl ether with orthogonal protecting groups on the respective amino and carboxyl groups. The Stille biaryl coupling reaction of suitably functionalized aryl bromide and (trimethylstannyl)benzaldehyde gave the key intermediate biaryl aldehyde. Synthesis was accomplished by applying the asymmetric Strecker reaction to this aldehyde, followed by selectively removing the chiral auxiliary. By comparing its ^{1}H-NMR spectra with those obtained from vancomycin, the stereochemistry of the synthesized diastereoisomer of actinoidic acid trimethyl ether was confirmed as an atropisomer of the (αS,$\alpha' R$)-isomer.

Antibiotics in the glycopeptide group, for example, vancomycin (**1**), have attracted multidisciplinary interest because of their clinical significance (Figure 1).[1] These dauntingly complex molecules pose significant synthetic and stereochemical challenges. The total synthesis of vancomycin aglycone was accomplished by three research groups in the late 1990s.[2–4] Of particular interest are issues associated with: (i) constructing the racemization-prone arylglycine units; (ii) the correct axial chirality of the chlorine-substituted biaryls; (iii) the congested macrocycle construction and; (iv) the unique biaryl *bis*-arylglycine segment A/B ring system, that is also known as actinoidic acid (**2**, Figure 1). Several efforts to synthesize actinoidic acid trimethyl ether (**3**) have been reported.[5–10] One problem associated with the synthesis of **3** is the fact that the α-stereogenic centers are of different absolute configurations. In

addition, it comprises eight stereoisomers owing to its asymmetric centers at Cα, Cα′ and its axial chirality. Methodologies must be devised in order to determine the correct absolute stereochemistry as well as address the congested biaryl coupling. Moreover, it is necessary to synthesize **2** or **3** with different protecting groups on two amino and two carboxyl groups, that is, orthogonally protected *bis*-amino acid, as the practical synthetic unit for the total synthesis of the vancomycin family.

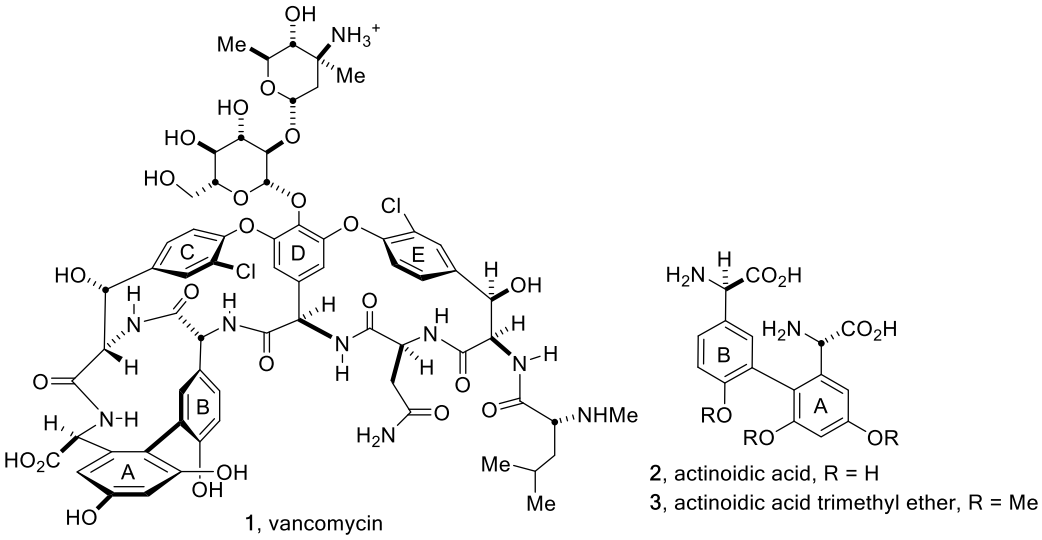
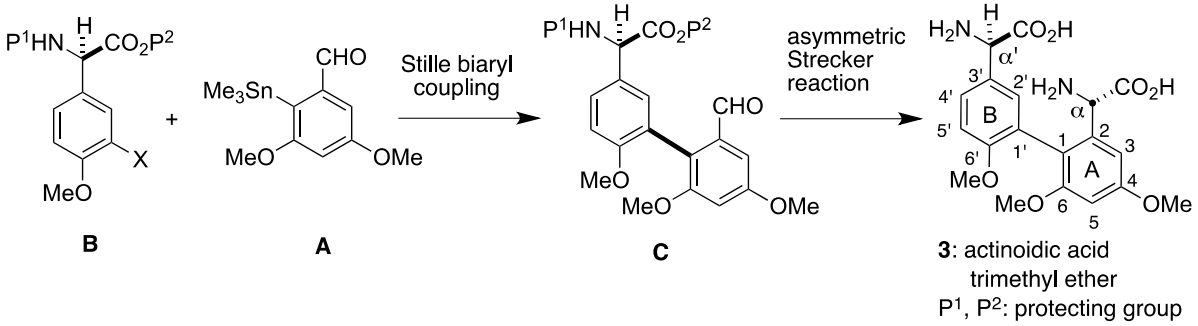


Figure 1. Structures of vancomycin (**1**) and actinoidic acid (**2**) and actinoidic acid trimethyl ether (**3**)

After examining a range of strategies, including asymmetric arylglycine synthesis,[11,12] we realized the successful combination of the Stille biaryl coupling reaction[13] of the protected arylglycine fragment **B** with (3,5-dimethoxy-2-trimethylstannyl)benzaldehyde **A**, providing key intermediate **C**, and asymmetric Strecker homologation[14,15] to achieve the synthesis of suitably protected **3**, as summarized in Scheme 1.

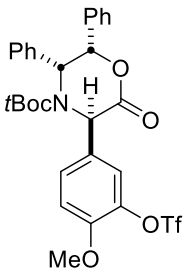
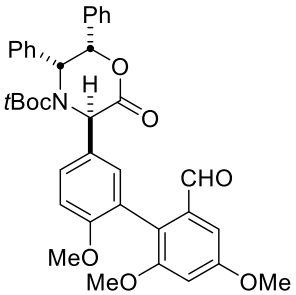
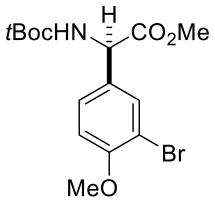
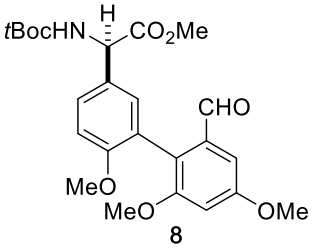
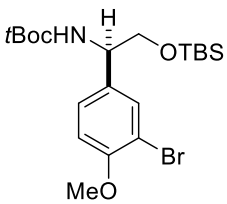
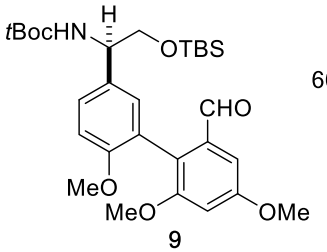


Scheme 1. Synthetic strategies for actinoidic acid trimethyl ether (**3**)

Starting materials were prepared as follows. (3,5-Dimethoxy-2-trimethylsilylstannyl)benzaldehyde (**A**, Scheme 1) was prepared from 2-bromo-3,5-dimethoxybenzaldehyde[16] by treatment with hexamethylditin

and a catalytic amount of $Pd(PPh_3)_4$. An arylglycine derivative (**4**) was prepared using our previously reported arylation of electrophilic glycinate with good optical purity,[11,12] followed by deprotection of the *tert*-butyldimethylsilyl group[17] and treatment of the resulting phenol with triflic anhydride in the presence of *N*,*N*-diisopropylethylamine. The commercially available 4-hydroxy-D-phenylglycine was transformed to a brominated amino acid according to the method of Brown et al.[18] The carboxylic acid and amino groups of the brominated amino acid were protected by treatment with $SOCl_2$ in MeOH, followed by di-*tert*-butyl dicarbonate ($(Boc)_2O$), $NaHCO_3$, and NaCl in aqueous $CHCl_3$. The protected amino acid was transformed to methyl ether (**5**) in an excellent yield by treatment with dimethyl sulfate, K_2CO_3 in DMF. Compound **5** was reduced with LiCl and $NaBH_4$ in tetrahydrofuran-ethanol (THF-EtOH)[19] to afford the corresponding alcohol. It was protected by treatment with *tert*-butyldimethylsilyl chloride (TBDMSCl), imidazole, and *N*,*N*-dimethyl-4-aminopyridine (DMAP) in DMF to afford the protected amino alcohol (**6**).

Table 1. The Stille coupling reaction of (3,5-dimethoxy-2-trimethylstannyl)benzaldehyde (**A**) with aryl triflate (**4**) or aryl bromide (**5** and **6**)

Entry	Substrate	Product	Yield (%)
1	 4 (>90%ee)	 7	44
2	 5 (90%ee)	 8	62
3	 6 (90%ee)	 9	66

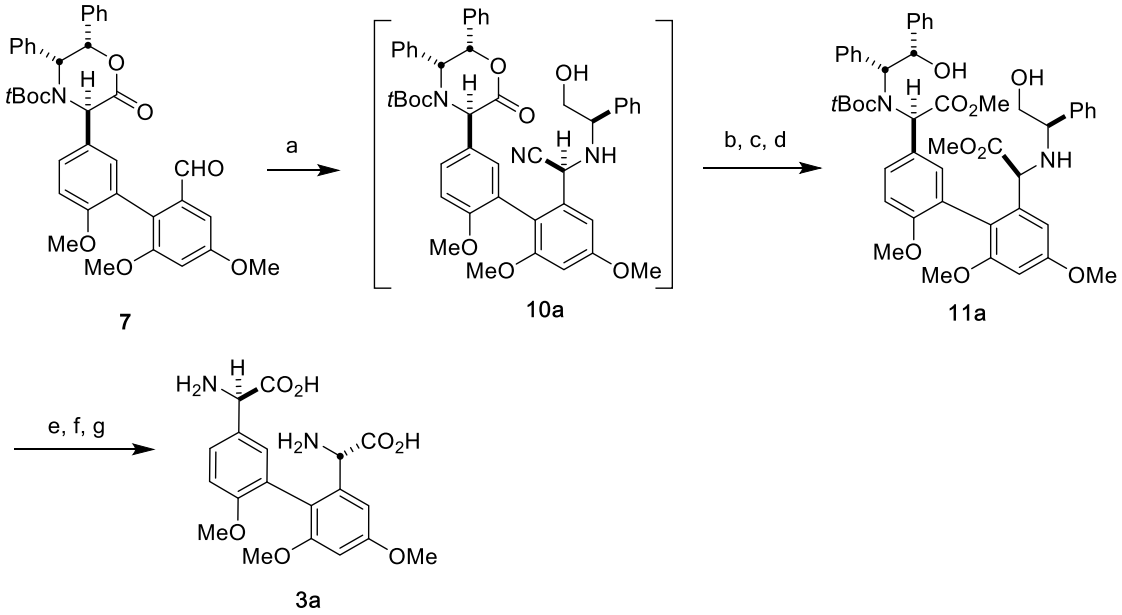
Reaction conditions: $Pd(PPh_3)_4$ (0.4 eq.), CuBr (0.4 eq.), dioxane, reflux, 16 h

The result of the biaryl coupling reaction of (3,5-dimethoxy-2-trimethylstannyl)benzaldehyde (**A**) with aryl triflates (**4**) or aryl bromides (**5** and **6**) is shown in Table 1. The previously reported standard Stille biaryl coupling conditions[13] (0.05 equiv. $Pd(PPh_3)_4$, 3.0 equiv. LiCl, refluxing dioxane) were unsuccessful in producing the desired cross-coupling products of these sterically hindered and electron-rich substrates (~0% yield for **4**). The yield of the desired biaryl (**8**) from aryl bromide (**5**) was improved (~20% yield) when the amount of $Pd(PPh_3)_4$ was increased to 0.4 equiv. The beneficial effect of adding Cu(I) salts in Pd-catalyzed cross-coupling has been previously reported.[20] In the present case, the addition of CuBr was also effective in increasing the yield. Thus, when the reaction was performed with 0.4 equiv. $Pd(PPh_3)_4$ and 0.4 equiv. CuBr in refluxing dioxane for 16 h, satisfactory yields of the desired biaryls (**7**, **8**, and **9**) were consistently observed. However, when the amount of catalyst was further increased, the yield was not improved. It is considered that the added copper facilitated the transmetalation step, resulting in rate acceleration and an increase of the yield in the coupling reaction. Since two singlets of aldehyde proton signals were observed in the ^{1}H-NMR spectra of **8** and **9**, the biaryl products are considered to be a 1:1 mixture of atropisomers. Although Prieto et al. observed a significant loss of optical purity in compound **5** in Suzuki's biaryl coupling,[21] the epimerization of α-asymmetric stereogenic centers appears to be negligible in the Stille biaryl coupling of **5**.

The asymmetric Strecker reaction was investigated for the introduction of the second amino acid unit. The first attempt to obtain the Strecker adduct of biaryl aldehyde (**7**) and (*R*)-phenylglycinol (according to the procedure of Inaba et al.[14]) failed owing to the poor solubility of compound **7** in the reaction solvent (MeOH-H_2O) or the instability of **7** under basic conditions. However, using the procedure of Chakraborty et al.,[15] the desired Strecker adduct (**10**) was obtained, and this was successfully transformed to compound **11** without purification (Scheme 2).

Condensation of **7** (>90%ee, 1:1 mixture of atropisomers) with (*R*)-phenylglycinol for 2 h at room temperature (rt) to give the imine intermediate, followed by treatment with 2 equiv. trimethylsilyl cyanide in MeOH at rt for 4 h, furnished the α-amino nitrile (**10a**) and its diastereoisomers in a quantitative yield. The structure of **10** was confirmed by fast atom bombardment mass spectrometry (FABMS) (*m*/*z*: 770.0 $(M+H)^+$). The stereoselectivity of this reaction was considered to be approximately 7:3 to 8:2, and the configuration of the predominantly constructed asymmetric center (*S*) was estimated from the literature.[15] This compound was sequentially hydrolyzed to the corresponding diacid compound under acidic conditions and esterified with CH_2N_2 to give the *bis*-amino acid dimethyl ester (**11**). Diastereoisomers of compound **11** were separated into two fractions, **11a** and **11b**, using preparative thin-layer chromatography (PTLC, eluted with hexane/AcOEt = 1:2) at a 68:32 ratio by weight in 53% overall yield over four steps. Using ^{1}H-NMR, these fractions were shown to be 5:1 and 2:1 mixtures, respectively, of

diastereoisomers. Therefore, the ratio of the diastereoisomers of compound **11**, as determined by ^{1}H-NMR, was 57:11:21:11.



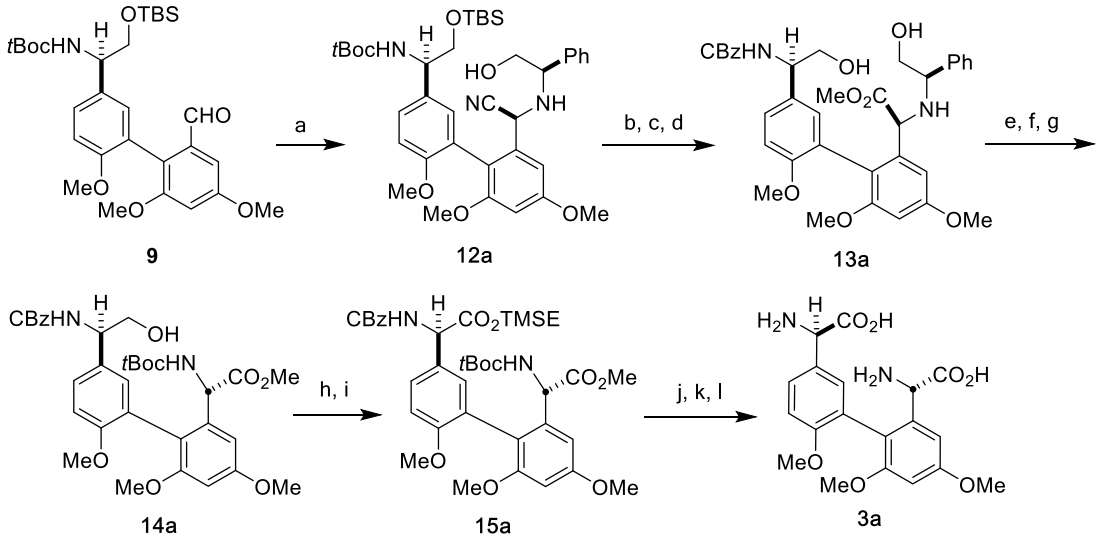
Scheme 2. Synthesis of actinoidic acid trimethyl ether (**3**) from **7**

Reagents and conditions: (a) (*R*)-phenylglycinol (1.15 eq.), rt, 2 h, TMSCN (trimethylsilyl cyanide) (2.0 eq.), MeOH, rt, 4 h; (b) conc. HCl, dioxane, rt, 4 h; (c) NH_4OH(aq); (d) CH_2N_2, MeOH-Et_2O, 1 h, 53%, four steps (diastereomer ratio, 57 : 11 : 21 : 11); (e) $Pb(OAc)_4$ (2.4 eq.), CH_2Cl_2-MeOH, 0 °C, 5 min; (f) 2 N HCl, dioxane, reflux, 16 h; (g) Dowex 50W (H^+) (3% NH_4OH), Sep-Pak C18 (H_2O), 60%, three steps, for **11a**.

In order to remove the two chiral auxiliaries of the major component **11a** in a single step, **11a** was subjected to oxidative cleavage by treatment with $Pb(OAc)_4$ in CH_2Cl_2-MeOH, followed by washing with aqueous $NaHCO_3$ to give benzaldehyde diimine. This material was hydrolyzed *via* treatment with refluxing 2 N HCl-dioxane to give *bis*-amino acid **3a**·2HCl, which was purified by passage through a Dowex 50W (H^+) ion exchange resin and filtration through a Sep-Pak C18 cartridge to afford Actinoidic acid trimethyl ether (**3**) as a 5:1 mixture of diastereoisomers in a 60% yield over three steps.

Next, an amino alcohol derivative (**9**) was used for the synthesis of **3** and its orthogonally protected derivative (**15a**) (Scheme 4). Condensation of **9** with (*R*)-phenylglycinol followed by treatment with 2 equiv. trimethylsilyl cyanide in MeOH furnished α-amino nitrile (**12a**) and its diastereoisomers, which were quickly separated by PTLC (hexane/AcOEt = 1:1) into three fractions with a 11:54:35 ratio by weight in a 59% yield. The structure of **12** was confirmed by FABMS (*m*/*z*: 692 $(M+H)^+$). The most predominant fraction of adduct (**12**) was sequentially hydrolyzed to the corresponding acid (conc.

HCl-dioxane), esterified ($SOCl_2$, MeOH), and selectively protected by benzyloxycarbonyl (Cbz) group (CbzCl, $NaHCO_3$, and CH_2Cl_2-H_2O) to give compound **13** (80%, three steps). Compound **13** was carefully separated again using PTLC (hexane/AcOEt = 1:5) to give two diastereomers, **13a** and **13b**, in a 69:31 ratio by weight. Selective cleavage of the β-phenylamino alcohol unit in the major diastereomer (**13a**) was successfully achieved by treatment of **13a** with 2 equiv. $Pb(OAc)_4$ in MeOH-$CHCl_3$, followed by hydrolysis of the resulting benzaldehyde imine by treatment with 2 N HCl in MeOH. It is noteworthy that the Cbz-protected amino alcohol unit remained intact under these conditions. This material was esterified by treatment with $SOCl_2$ in MeOH and then protected by treatment with $(Boc)_2O$, $NaHCO_3$, and NaCl in $CHCl_3$-H_2O to give compound **14a** (68%, three steps).



Scheme 3. Synthesis of the orthogonally protected form of actinoidic acid trimethyl ether (**15a**)

Reagents and conditions: (a) (*R*)-phenylglycinol (1.15 eq.), rt, 3 h, TMSCN (trimethylsilyl cyanide) (2.0 eq.), MeOH, rt, 14 h; (b) conc. HCl, dioxane, rt, 16 h; (c) $SOCl_2$ (4.0 eq.), MeOH, rt, 16 h; (d) CbzCl (1.1 eq.), $NaHCO_3$ (3.5 eq.), CH_2Cl_2-H_2O, rt, 6 h, 47%, four steps; (e) $Pb(OAc)_4$ (2.0 eq.), CH_2Cl_2-MeOH, 0 °C, 5 min; (f) 2 N HCl, MeOH, rt, 16 h; (g) $(Boc)_2O$ (2.0 eq.), $NaHCO_3$ (2.5 eq.), NaCl, $CHCl_3$-H_2O, 68%, three steps, for **13a**; (h) $RuCl_3 \cdot 3H_2O$ (0.04 eq.), $NaIO_4$ (4.0 eq.), MeCN-H_2O, 0 °C, 1 h; (i) (trimethylsilyl)ethanol (1.5 eq.), EDCI·HCl (1.5 eq.), DMAP (0.5 eq.), CH_2Cl_2, rt, 16 h, 29%, two steps, for **14a**; (j) H_2, 5% Pd-C, 1 N HCl-THF, rt, 4 h; (k) 2 N HCl, reflux, 16 h; (l) Dowex 50W (H^+) (3% NH_4OH), Sep-Pak C18 (H_2O), 64%, three steps, for **15a**.

Most attempts to reoxidize the hydroxymethyl moiety of compound **14** were unsuccessful (pyridinium dichromate (PDC) in DMF; PDC in CH_2Cl_2 and then $KMnO_4$ in buffer; and SO_3·pyridine in DMSO and then $NaClO_2$ in buffer). Ultimately, it was successfully transformed to a trimethylsilylethyloxycarbonyl (TMSE) group by sequential treatment with $RuCl_3 \cdot 3H_2O$; $NaIO_4$ in $CHCl_3$-H_2O;[13] and (trimethylsilyl)ethanol, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl), and DMAP in CH_2Cl_2 to give orthogonally protected actinoidic acid trimethyl ether (**15a**)[22] (29% yield, two steps).

In order to confirm the stereochemistry of compound **15a**, it was treated with Pd/C (5%) under a H_2 atmosphere in 2 N HCl-THF and then refluxed with 2 N HCl to give the hydrochloride salt of actinoidic acid trimethyl ether (**3a**). This substance was then desalted by passage through a Dowex 50W (H^+) ion exchange resin and filtration through a Sep-Pak C18 cartridge to afford the free form of actinoidic acid trimethyl ether (**3a**)[23] (64%, three steps).

The ^{1}H-NMR aromatic region signals of the obtained **3a** are shown in Figure 2(a); these were identical to the major signals of **3a** obtained according to the above synthesis procedure starting from compound **7**. They were also similar to the aromatic region signals of the minor diastereoisomer of **3** isolated from vancomycin (**1**) [Figure 2(b)], as described in the following section. However, the absolute axial chirality of biaryl of the synthesized **3a** is unclear. In addition, partial epimerization at one or two stereogenic centers occurred during the transformation.

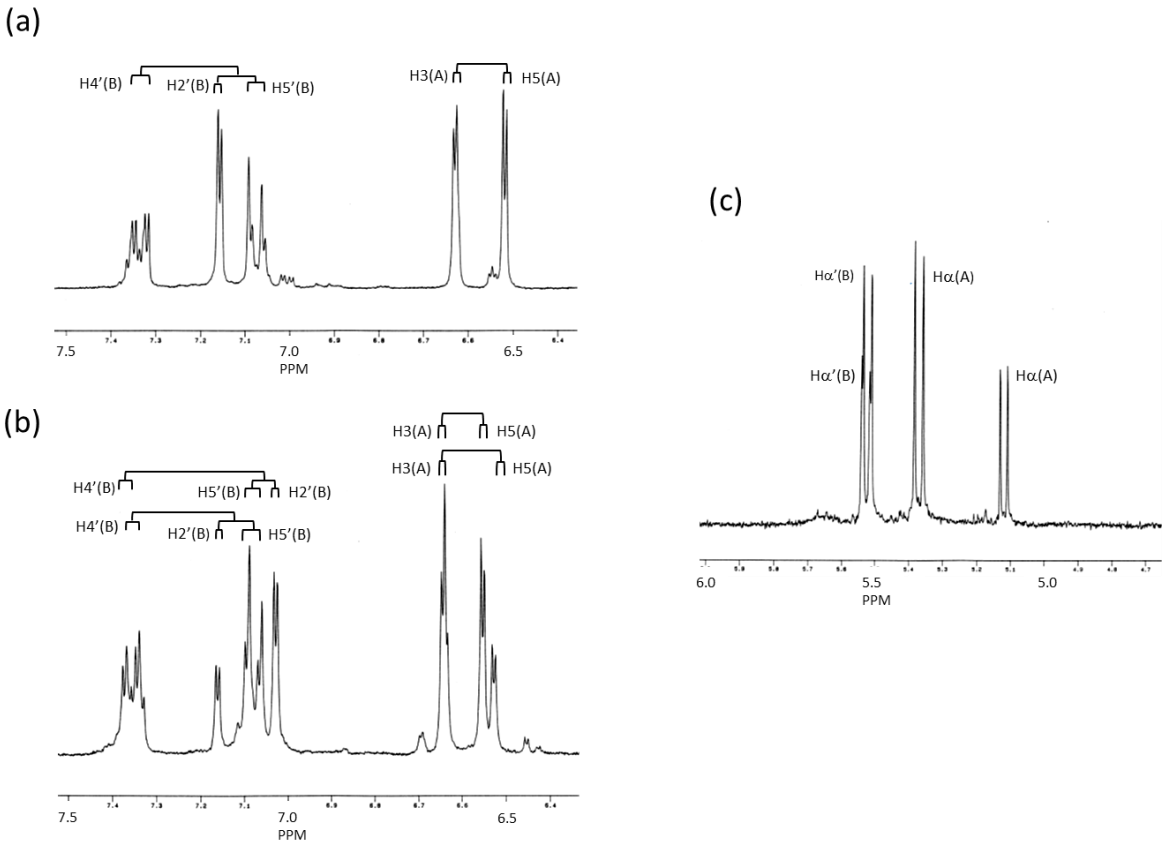
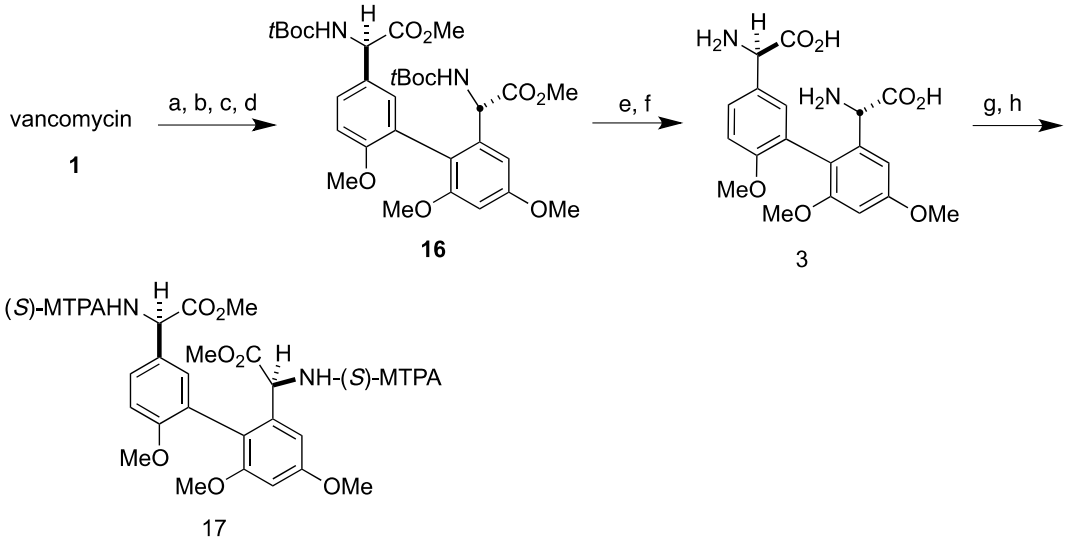


Figure 2. ^{1}H-NMR signals of actinoidic acid trimethyl ether (**3**) and its derivative (**17**)

Notes: (a) Aromatic region signals of actinoidic acid trimethyl ether (**3a**) synthesized from compound **9**; (b) Aromatic region signals of natural actinoidic acid trimethyl ether (**3**) isolated from vancomycin (**1**); (c) α-CH signals of *bis*-*N*-*(S)*-MTPA amide *bis*-methyl ester of actinoidic acid trimethyl ether (**17**) derived from natural actinoidic acid trimethyl ether (**3**).

The isolation procedure of authentic actinoidic acid trimethyl ether (**3**) is shown in Scheme 4. According to the procedure of Jeffs et al.,[24] vancomycin (**1**) was hydrolyzed in refluxing 6 N HCl for 8 h. The hydrolyzed material was then partially purified by passage through a Dowex 50W (H^+) ion exchange resin. The obtained amino acid mixture was treated sequentially with $(Boc)_2O$, 1 N NaOH in dioxane at rt, MeI, K_2CO_3, and *n*-Bu_4NI in refluxing acetone. The protected amino acid (**16**) was purified using PTLC. The ^{1}H-NMR spectrum showed reasonable agreement with that reported by Boisnard et al.[8] Compound **16** was hydrolyzed and purified as described above to afford an authentic sample of actinoidic acid trimethyl ether (**3**) as a 2:1 mixture of two diastereoisomers (atropisomers), as determined by ^{1}H-NMR [Figure 2(b)].[25] The ^{1}H-NMR signal pattern of the major isomer was almost identical to that reported by Gause et al.[26] The ^{1}H-NMR spectrum of the *bis*-*N*-(*S*)-methoxy(trifluoromethyl)phenylacetyl (MTPA) amide *bis*-methyl ester of **3** (**16**) also indicated the presence of only two diastereoisomers in a 2:1 ratio [Figure 2(c)]. Therefore, these two diastereoisomers are expected to be atropisomers of the (α*S*,α′*R*)-isomer of **3**.



Scheme 4. Isolation of actinoidic acid trimethyl ether (**3**) from vancomycin (**1**)

Reagents and conditions: (a) 6 N HCl, reflux, 8 h; (b) Dowex 50W (H^+) (3% NH_4OH); (c) $(Boc)_2O$ (excess), 1 N NaOH-dioxane, rt, 4 h; (d) MeI, K_2CO_3, *n*-Bu_4NF, acetone, reflux, 15 h; (e) 2 N HCl, reflux, 13 h; (f) Dowex 50W (H^+) (3% NH_4OH), Sep-Pak C18 (H_2O); (g) (*S*)-(+)-Mosher's acid chloride (2.3 eq.), propylene oxide (7.85 eq.), reflux, 30 min; (h) CH_2N_2 (excess), Et_2O, rt, 1 h, 84%, two steps, for **3**.

In summary, the asymmetric synthesis of actinoidic acid trimethyl ether with different protecting groups on the respective two amino groups and two carboxyl groups has been achieved. In addition, the reaction conditions for the Stille biaryl coupling of a sterically hindered and electron-rich substrate were investigated and elucidated in order to construct the key highly substituted biaryl intermediate using CuBr as an additive. Identification of the synthetic material was carried out by comparison of its ^{1}H-NMR

spectrum with that of an authentic specimen isolated from the hydrolytic degradation of vancomycin (**1**). The remaining challenge of this approach is to devise a method to control the absolute axial stereochemistry.

ACKNOWLEDGEMENTS

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REFERENCES AND NOTES

1. D. H. Williams and B. Bardsley, *Angew. Chem. Int. Ed.*, 1999, **38**, 1173.
2. D. A. Evans, M. R. Wood, B. W. Trotter, T. I. Richardson, J. C. Barrow, and J. L. Katz, *Angew. Chem. Int. Ed.*, 1998, **37**, 2700.
3. K. C. Nicolaou, S. Natarajan, H. Li, N. F. Jain, R. Hughes, M. E. Solomon, J. M. Ramanjulu, C. N. C. Boddy, and M. Takayanagi, *Angew. Chem. Int. Ed.*, 1998, **37**, 2708.
4. D. L. Boger, S. Miyazaki, S. H. Kim, J. H. Wu, O. Loiseleur, and S. L. Castle, *J. Am. Chem. Soc.*, 1999, **121**, 3226.
5. A. V. Rama Rao, T. K. Chakraborty, and S. P. Joshi, *Tetrahedron Lett.*, 1992, **33**, 4045.
6. J. Zhu, R. Beugelmans, A. Bigot, G. P. Singh, and M. Bois-Choussy, *Tetrahedron Lett.*, 1993, **34**, 7401.
7. A. V. Rama Rao, K. L. Reddy, and M. M. Reddy, *Tetrahedron Lett.*, 1994, **35**, 5039.
8. S. Boisnard, L. Neuville, M. Bois-Choussy, and J. Zhu, *Org. Lett.*, 2000, **2**, 2459.
9. K. Kamikawa, A. Tachibana, S. Sugimoto, and M. Uemura, *Org. Lett.*, 2001, **3**, 2033.
10. R. Wilhelm and D. A. Widdowson, *Org. Lett.*, 2001, **3**, 3079.
11. R. M. Williams and J. A. Hendrix, *J. Org. Chem.*, 1990, **55**, 3723.
12. R. M. Williams and J. A. Hendrix, *Chem. Rev.*, 1992, **92**, 889.
13. A. M. Echavarren and J. K. Stille, *J. Am. Chem. Soc.*, 1987, **109**, 5478.
14. T. Inaba, I. Kozono, M. Fujita, and K. Ogura, *Bull. Chem. Soc. Jpn.*, 1992, **65**, 2359.
15. T. K. Chakraborty, K. A. Hussain, and G. V. Reddy, *Tetrahedron*, 1995, **51**, 9179.
16. A. K. Sinhabab and R. T. Borchardt, *J. Org. Chem.*, 1983, **48**, 2356.
17. P. M. Kendall, J. V. Johnson, and C. E. Cook, *J. Org. Chem.*, 1979, **44**, 1421.
18. A. G. Brown, M. J. Crimmin, and P. D. Edwards, *J. Chem. Soc., Perkin Trans. 1*, 1992, 123.
19. Y. Hamada and T. Shiori, *Tetrahedron Lett.*, 1982, **23**, 1193.
20. N. Tamayo, A. M. Echavarren, and M. C. Paredes, *J. Org. Chem.*, 1991, **56**, 6488.
21. M. Prieto, S. Mayor, K. Rodriguez, P. Lioyd-Williams, and E. Giralt, *J. Org. Chem.*, 2007, **72**, 1047.

22. **15a**: a pale-yellow foam. ^{1}H-NMR ($CDCl_3$) δ: −0.20 (9/2H, s), 0.14 (9/2H, s), 0.97 (2H, brt, *J* = 8.3 Hz), 1.36 (9H, s), 3.63 (3H, s), 3.68 (3H, s), 3.80 (6H, s), 4.11−4.30 (2H, m), 4.92 (1H, brd, *J* = 7.1 Hz), 5.09 (2H, s), 5.17 (1H, brs), 5.27 (1H, brt, *J* = 8.6 Hz), 5.70 (1H, brd, *J* = 7.2 Hz), 6.46 (1H, d, *J* = 2.3 Hz), 6.48 (1H, d, *J* = 2.2 Hz), 6.89 (1H, d, *J* = 8.6 Hz), 7.14 (1H, d, *J* = 2.1 Hz), 7.23–7.36 (6H, m); IR (neat): 3364, 2954, 2839, 1718, 1606, 1584, 1506, 1465, 1367, 1329, 1251, 1203, 1161, 1152, 990, 938, 860, 838, 755, 698 cm^{-1}; $[\alpha]_D^{25}$ +52.1 (C 1.0, $CHCl_3$); FABMS *m*/*z*: 739.0 $(M+H)^+$; exact mass (FABMS): 739.3226 $(M+H)^+$ (Calcd. for $C_{38}H_{51}N_2O_{11}Si$: 739.3262).
23. **3a**: a colorless solid. ^{1}H-NMR (D_2O) δ: 3.58 (3H, s), 3.63 (3H, s), 3.74 (3H, s), 4.27 (1H, s), 4.67 (1H, s), 6.53 (1H, d, *J* = 2.2 Hz), 6.63 (1H, d, *J* = 2.2 Hz), 7.08 (1H, d, *J* = 8.6 Hz), 7.16 (1H, d, *J* = 2.3 Hz), 7.37 (1H, dd, *J* = 2.3 and 8.6 Hz); $[\alpha]_D^{25}$ +58.6 (C 1.0, H_2O); FABMS *m*/*z*: 391.0 $(M+H)^+$; exact mass (FABMS): 391.1505 $(M+H)^+$ (Calcd. for $C_{19}H_{23}N_2O_7$: 391.1518).
24. P. W. Jeffs, G. Chan, L. Mueller, C. Debrosse, L. Webb, and R. Sitrin, *J. Org. Chem.*, 1986, **51**, 4272.
25. **3**: a white solid. ^{1}H-NMR (D_2O) δ: major isomer: 3.57 (3H, s), 3.62 (3H, s), 3.73 (3H, s), 4.34 (1H, s), (4.60, (3H, s)), 6.55 (1H, d, *J* = 2.3 Hz), 6.64 (1H, d, *J* = 2.2 Hz), 7.03 (1H, d, *J* = 2.3 Hz), 7.07 (1H, d, *J* = 8.6 Hz), 7.36 (1H, dd, *J* = 2.5 and 8.7 Hz); main isomer: 3.58 (3H, s), 3.64 (3H, s), 3.73 (3H, s), 4.28 (1H, s), 4.71 (1H, s), 6.53 (1H, d, *J* = 2.3 Hz), 6.64 (1H, d, *J* = 2.2 Hz), 7.08 (1H, d, *J* = 8.7 Hz), 7.16 (1H, d, *J* = 2.3 Hz), 7.35 (1H, dd, *J* = 2.3 and 8.6 Hz); IR (KBr): 3433, 2944, 1609, 1508, 1491, 1466, 1388, 1347, 1326, 1273, 1204, 1160, 1062, 1023, 946, 809, 554 cm^{-1}; $[\alpha]_D^{25}$ +5.0 (C 1.0, H_2O); mp 187-188 °C; FABMS *m*/*z*: 391.0 $(M+H)^+$.
26. G. F. Gause, M. G. Brazhnikova, N. N. Lomakina, T. F. Berdnikova, G. B. Fedorova, N. L. Tokareva, V. N. Borisova, and G. Y. Batta, *J. Antibiot.*, 1989, **42**, 1790.