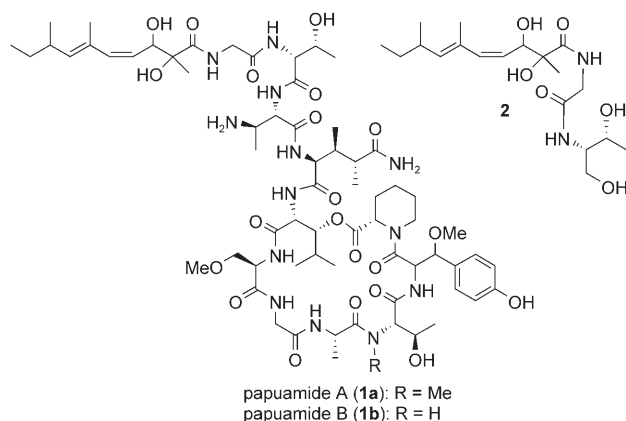


Total Synthesis and Structure Assignment of Papuamide B, A Potent Marine Cyclodepsipeptide with Anti-HIV Properties**

Weiying Xie, Derong Ding, Weiwei Zi, Guangyu Li, and Dawei Ma*

Papuamides A (**1a**) and B (**1b**) (Scheme 1) are two novel cyclic depsipeptides that were isolated from Papua New



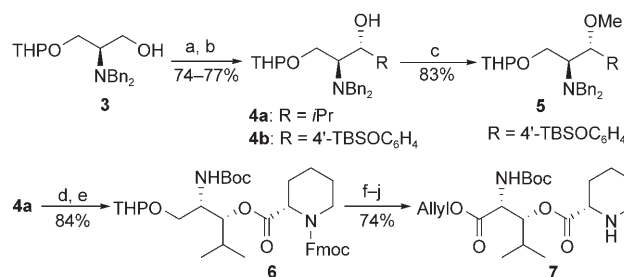
Scheme 1. Structures of papuamides A and B, as well as their chemical degradation product **2**.

Guinea collections of the marine sponges *Theonella mirabilis* and *Theonella swinhoei*.^[1] Both compounds exhibit a strong inhibitory effect on the infection of human T-lymphoblastoid cells by HIV-1_{RF} in vitro, with an EC₅₀ value of about 4 ng mL⁻¹. Similar biological activities were also observed for other cyclic depsipeptides isolated from different marine sponges, namely: callipeltin A,^[2] neamphamide A,^[3] and mirabamides A–D.^[4] Given this background, it is not surprising that recent intensive efforts have been directed towards gaining synthetic access to these natural products.^[5–7] These efforts have led to the successful establishment of the stereochemistry of some stereogenic centers within this class of compounds.^[5] However, none of these molecules have yet been assembled. Herein, we report our synthetic strategy towards papuamide B.^[8]

Structurally, papuamide B comprises a 22-membered macrocycle, which is connected to a complex linear tetrapep-

tide through an amide bond. Several nonproteinogenic α -amino acid residues are present in the molecule, namely (2*S*,3*S*,4*R*)-3,4-dimethylglutamine, (2*R*,3*R*)-3-hydroxyleucine, (2*S*,3*R*)-2,3-diaminobutanoic acid, and β -methoxytyrosine. As the configuration of the β -methoxytyrosine unit was assigned as 2*R*,3*R* by synthetic preparation,^[5a,b] the remaining stereochemical uncertainty of papuamides concerns the configuration of the three chiral centers in the 2,3-dihydroxy-2,6,8-trimethyldeca-(4*Z*,6*E*)-dienoic acid unit. As the ¹H NMR spectroscopic data of alcohol **2**, a degradation product of papuamide A, is known,^[1] we planned to use this information to determine the stereochemistry of the papuamide side chain.

The required (2*R*,3*R*)-3-hydroxyleucine and (2*R*,3*R*)- β -methoxytyrosine units, **7** and **5**, respectively, were elaborated from the same L-serine derivative **3** (Scheme 2).^[9] Alcohol **3**



Scheme 2. Reagents and conditions: a) Swern oxidation; b) RMgBr, THF; c) NaH, MeI, THF; d) Pd(OH)₂/C, (Boc)₂O, MeOH; e) Fmoc-(*S*)-pipecolic acid, TCBC, *i*Pr₂NEt, DMAP; f) I₂, MeOH; g) Dess–Martin oxidation; h) NaClO₂, NaH₂PO₄; i) allyl bromide, KHCO₃; j) Et₃NH, MeCN. Bn = benzyl, Boc = *tert*-butoxycarbonyl, DMAP = 4-dimethylaminopyridine, Fmoc = 9-fluorenylmethyloxycarbonyl, TBS = *tert*-butyldimethylsilyl, TCBC = trichlorobenzoyl chloride, THP = tetrahydropyran.

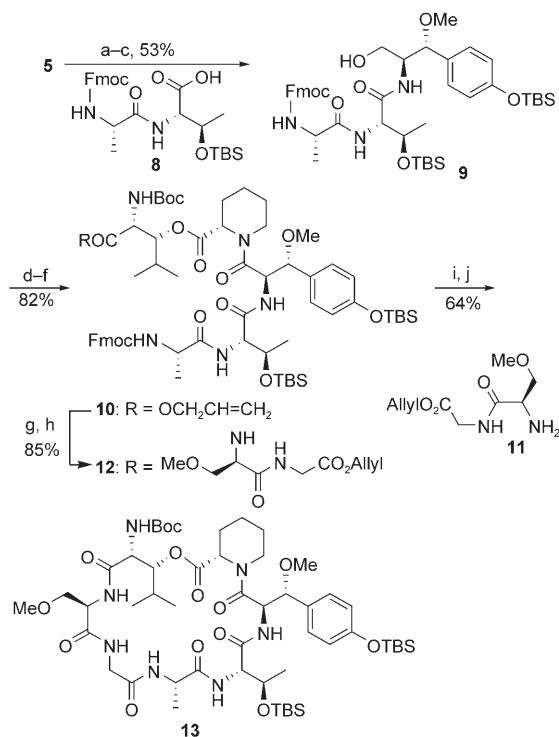
was oxidized to the aldehyde, and the desired *anti* amino alcohols **4** were prepared by a Grignard reaction. Alkylation of **4b** with iodomethane and NaH provided amino ether **5**. In a parallel procedure, esterification of Boc-protected **4a** with Fmoc-(*S*)-pipecolic acid in the presence of TCBC^[10] and DMAP furnished ester **6**, which was subsequently deprotected by using I₂/MeOH, oxidized stepwise to an acid, and converted into an allyl ester. Removal of the Fmoc protecting group afforded amino ester **7** in 68% overall yield.

Assembly of the macrocycle of papuamide B is outlined in Scheme 3. Treatment of **5** with TsOH/MeOH selectively removed the THP group, and subsequent hydrogenolysis over Pd/C in methanol produced the free amino alcohol, which was then coupled to dipeptide **8** to afford amide **9**. Stepwise

[*] Dr. W. Xie, Dr. D. Ding, W. Zi, Dr. G. Li, Prof. Dr. D. Ma
State Key Laboratory of Bioorganic & Natural Products Chemistry,
Shanghai Institute of Organic Chemistry
Chinese Academy of Sciences
354 Fenglin Lu, Shanghai 200032 (China)
Fax: (+86) 21-6416-6128
E-mail: madw@mail.sioc.ac.cn

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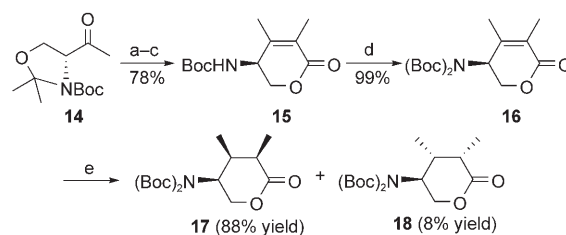
Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.



Scheme 3. Reagents and conditions: a) TsOH, MeOH; b) Pd/C, H₂; c) **8**, EDC, HOAt, *i*Pr₂NEt; d) Dess–Martin oxidation; e) NaClO₂, NaH₂PO₄; f) **7**, DEPBT, *i*Pr₂NEt; g) [Pd(PPh₃)₄], PhNHMe; h) **11**, HATU, *i*Pr₂NEt; i) 1. [Pd(PPh₃)₄], PhNHMe; 2. Et₂NH; j) HATU, *i*Pr₂NEt, CH₂Cl₂, RT. EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, DEPBT = 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one, HATU = *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, HOAt = 1-hydroxyazobenzotriazole, Ts = *para*-toluenesulfonyl.

oxidation of the primary hydroxy group in **9** provided an acid, which was then condensed with the amino ester **7** to yield ester **10**. In this case, DEPBT^[11] was employed because other coupling reagents such as HATU and EDCI/HOAt failed to give satisfactory yields. After cleavage of the allyl ester in **10** with Pd⁰/*N*-methylaniline,^[12] the free acid was coupled to dipeptide **11** to give linear peptide **12**. Subsequent deprotection of **12** with [Pd(PPh₃)₄] and diethylamine provided a macrocyclization precursor, which was treated with HATU and DIPEA in dilute methylene chloride solution (0.002 M) to furnish lactam **13**.

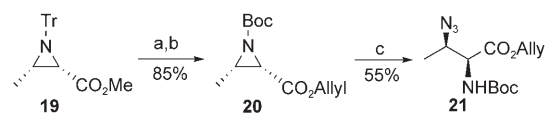
As (2*S*,3*S*,4*R*)-3,4-dimethylglutamine is a common fragment of papuamides A and B, callipeltin A, and neamphamide A,^[1–3] considerable effort has been directed toward its synthesis.^[7] However, the reported synthetic routes suffer from laborious and inconvenient operations or poor diastereoselectivity. We assumed that the key problem with the current protocols is the difficulty of opening the pyrrolidinone rings with ammonia. We therefore envisaged using the more reactive δ -valerolactone **17** to build the required amide part of this glutamine fragment. The synthesis of **17** is illustrated in Scheme 4. By employing a known procedure, ketone **14** was prepared from D-serine in seven steps with an overall yield of 61%.^[13] Condensation of **14** with the lithium enolate generated from ethyl propionate, followed by HCl-mediated



Scheme 4. Reagents and conditions: a) CH₃CH₂CO₂Et, LDA, THF, –78 °C; b) HCl, THF, H₂O; c) Ac₂O, Et₃N, DMAP, CH₂Cl₂, reflux; d) (Boc)₂O, DMAP; e) Pd(OH)₂/C, NaHCO₃, H₂O, *t*BuOH, H₂. LDA = lithium diisopropylamide.

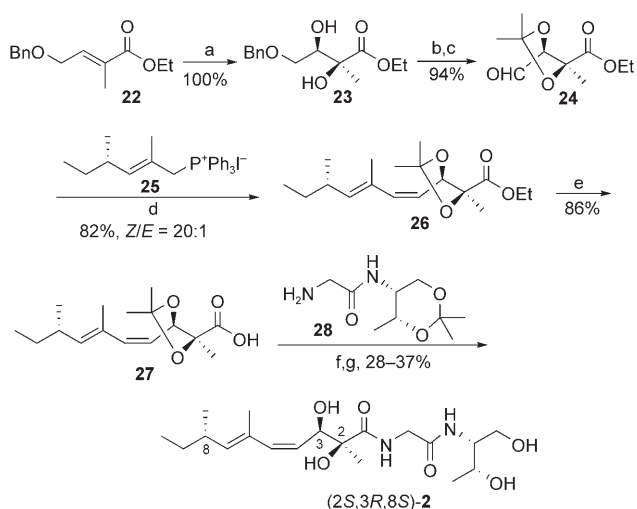
cyclization and dehydration with Ac₂O/Et₃N/DMAP, afforded the unsaturated lactone **15** in 78% yield. Hydrogenation of **15** under various conditions resulted in an inseparable mixture of two diastereomers in an approximate ratio of 2:1. Fortunately, we found that when a second Boc protecting group was introduced, the resultant substrate **16** could be hydrogenated with Pd(OH)₂/C/H₂ in *t*BuOH to give the desired product **17** in 91% yield, together with its diastereomer **18** in 9% yield. The stereochemistry of **17** was confirmed by X-ray structural analysis (see the Supporting Information).

For the construction of the (2*S*,3*R*)-2,3-diaminobutanoic acid fragment (see **21**), aziridine **19** was assembled based on the method of van der Donk (Scheme 5).^[14] Switching the protecting groups in **19** provided aziridine **20**, which was treated with trimethylsilyl azide in methanol to afford the required allyl ester **21**.



Scheme 5. Reagents and conditions: a) LiOH, then allyl bromide, KHCO₃; b) CF₃CO₂H, then (Boc)₂O, Et₃N; c) TMSN₃, MeOH, 70 °C. TMS = trimethylsilyl, Tr = trityl = triphenylmethyl.

The most complex fragment in the papuamide framework is the 2,3-dihydroxy-2,6,8-trimethyldeca-(4*Z*,6*E*)-dienoic acid moiety. Our efforts on its elaboration and the determination of the stereochemistry are summarized in Scheme 6. Sharpless asymmetric dihydroxylation of olefin **22** produced diol **23** in quantitative yield and 97% *ee*, as determined by chiral HPLC. Hydrogenolysis of **23** and subsequent Swern oxidation led to aldehyde **24**, which was treated with the ylide generated from **25** to afford diene **26** in 82% yield and 20:1 *Z/E* selectivity. The reaction conditions for this step proved to be very critical, as low yields, poor *Z/E* selectivity, and racemization were encountered when other bases such as KO^{*t*}Bu, as well as other solvent systems such as THF or diethyl ether were used. Hydrolysis of **26** provided acid **27**, which was condensed with amine **28**, generated from L-threonine and glycine, to furnish (2*S*,3*R*,8*S*)-**2**, following removal of the acetone unit with 60% HOAc. By adopting the same



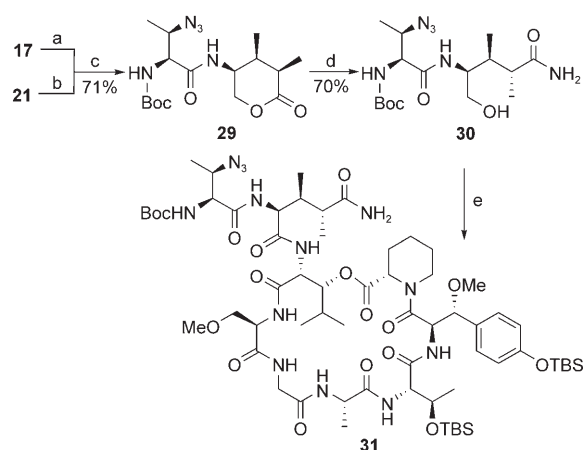
Scheme 6. Reagents and conditions: a) AD-mix- α , $\text{H}_2\text{NSO}_2\text{Me}$, $t\text{BuOH}$, H_2O ; b) TsOH , DMP , then $\text{Pd}(\text{OH})_2/\text{C}$, H_2 ; c) Swern oxidation; d) **25**, NaHMS , THF , HMPA , -78°C to RT ; e) NaOH , MeOH , H_2O ; f) **28**, HATU ; g) 60% HOAc , 50°C . HMS = hexamethyldisilazane, HMPA = hexamethyl phosphoramide.

procedure, and employing AD-mix- α as well as the *Z* isomer of olefin **22**, we obtained three other isomers, namely: (2*R*,3*S*,8*S*)-**2**, (2*S*,3*S*,8*S*)-**2**, and (2*R*,3*R*,8*S*)-**2** (see the Supporting Information for structures). Additionally, use of the enantiomer of **28** as the coupling substrate afforded enantiomers of (2*R*,3*S*,8*R*)-**2**, (2*S*,3*R*,8*R*)-**2**, (2*R*,3*R*,8*R*)-**2**, and (2*S*,3*S*,8*R*)-**2**, respectively.

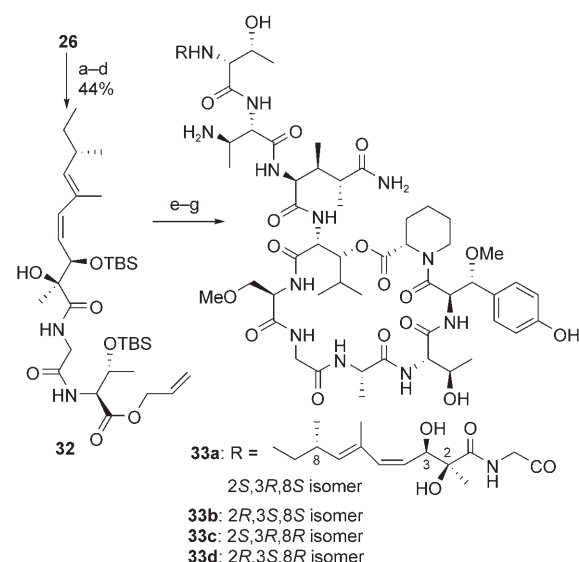
With this selection of stereoisomers at hand, we set out to establish the stereochemistry of the side chain in papuamides by comparison of the ^1H and ^{13}C NMR spectroscopic data with that reported for the degradation product **2**. Unfortunately, although (2*S*,3*S*,8*S*)-**2** and (2*R*,3*R*,8*S*)-**2**, which are the enantiomers of (2*R*,3*R*,8*R*)-**2** and (2*S*,3*S*,8*R*)-**2**, gave spectroscopic signals which are different to those of **2**, the chemical shift data of the other four isomers were indistinguishable from the literature data. Thus, we could only rule out the *anti* relationship of the 2,3-diol in the side chain.

With all the required fragments in hand, assembly of the target molecules became our next task. Treatment of lactone **17** with $\text{CF}_3\text{CO}_2\text{H}$ afforded an amine, which was coupled to the acid released from allyl ester **21** to furnish amide **29** in 71% yield (Scheme 7). As expected, ring opening of **29** with ammonia in methanol proceeded smoothly to provide amide **30** in 70% yield. After oxidation of **30** with $\text{RuCl}_3\cdot\text{H}_2\text{O}/\text{NaIO}_4$ to give the carboxylic acid, coupling with the deprotected amine of the macrocycle **13** was carried out, resulting in the key intermediate **31**.

The final stages of the total synthesis of papuamide B and its isomers are depicted in Scheme 8. Removal of the acetonide moiety of diene **26** with $(\text{NH}_4)_2\text{Ce}(\text{NO}_3)_6$ (ceric ammonium nitrate, CAN)^[15] provided the deprotected diol in satisfactory yield. Notably, alternative deprotection conditions such as TsOH/MeOH and 60% HOAc gave considerably lower yields owing to the poor stability of the resulting diol. After selective protection of the secondary alcohol with TBSCl and subsequent ester hydrolysis with aqueous NaOH



Scheme 7. Reagents and conditions: a) $\text{CF}_3\text{CO}_2\text{H}$, CH_2Cl_2 ; b) $[\text{Pd}(\text{PPh}_3)_4]$, PhNHMe ; c) HATU , $i\text{Pr}_2\text{NEt}$; d) NH_3 , MeOH ; e) 1. oxidation of **30**: **30**, $\text{RuCl}_3\cdot\text{H}_2\text{O}$, NaIO_4 , MeCN , CCl_4 , H_2O , 69%; 2. deprotection of **13**: **13**, $\text{CF}_3\text{CO}_2\text{H}$, CH_2Cl_2 , then 3. Coupling of intermediates from steps 1. and 2.: DEPBT , $i\text{Pr}_2\text{NEt}$, 89%.

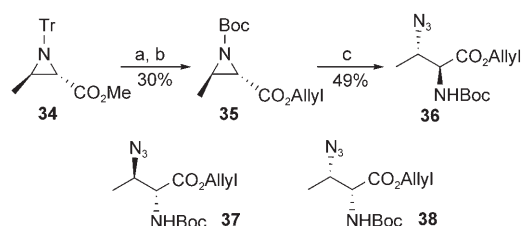


Scheme 8. Reagents and conditions: a) $(\text{NH}_4)_2\text{Ce}(\text{NO}_3)_6$, borate buffer, MeCN , THF , 60°C ; b) TBSCl , imidazole, DMF ; c) aq. NaOH , MeOH ; d) $\text{H-Gly-L-Thr}(\text{TBS})\text{-O-allyl}$, BOP , $i\text{Pr}_2\text{NEt}$, MeCN ; e) 1. Deprotection of **32**: $[\text{Pd}(\text{PPh}_3)_4]$, PhNHMe , 2. Deprotection of **31**: **31**, AlCl_3 , CH_2Cl_2 , then 3. Coupling of intermediates from steps 1. and 2.: DEPBT , $i\text{Pr}_2\text{NEt}$; f) TAS-F ; g) Me_3P , H_2O , THF . BOP = benzotriazol-1-yloxytris-(dimethylamino)phosphonium, TAS-F = tris(dimethylamino)sulfur(trimethylsilyl)difluoride.

in methanol, coupling to $\text{H-Gly-L-Thr}(\text{TBS})\text{-O-allyl}$ then afforded ester **32**. After cleavage of the allyl ester of **32** under palladium catalysis, the resulting acid was coupled to the amine liberated from **31** to furnish an amide, which was subjected to deprotection with TAS-F and reduction with trimethylphosphine/water to afford **33a** in 22% overall yield. By using the same procedure described above, we elaborated three other isomers (**33b–33d**) from the corresponding stereoisomers of **26**. Much to our surprise, none of these

isomers had ^1H NMR spectroscopic data identical to that previously reported for papuamide B. This finding indicated that a structural revision of this natural product was required.

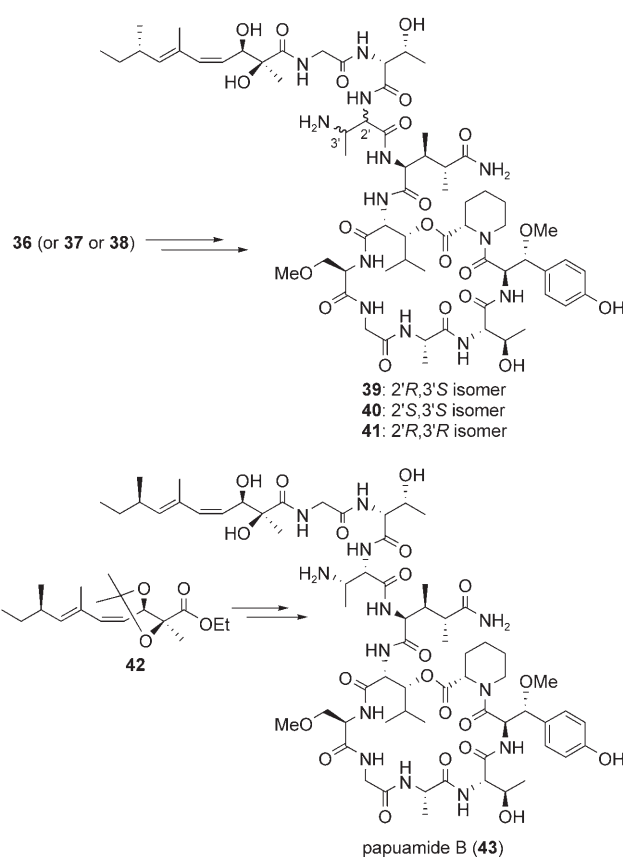
As the ^1H NMR spectra of **33a** and **33c** are very similar to that of natural papuamide B (see Supporting Information), and major differences arose only from the signals of two protons adjacent to the two amino groups of the (2*S*,3*R*)-2,3-diaminobutanoic acid segment (assigned by TOCSY studies), we concluded that the stereochemistry of this part of the molecule might have been misassigned. Thus, we decided to elaborate the other three isomers of **33a** by varying the stereochemistry of the 2,3-diaminobutanoic acid part. The required building block **38** was prepared from the enantiomer of **19** by the procedure depicted in Scheme 5, while the two *anti* isomers **36** and **37** were obtained through a slightly different reaction sequence (Scheme 9), because direct hydrolysis of **34** led to decomposition.



Scheme 9. Reagents and conditions: a) $\text{CF}_3\text{CO}_2\text{H}$, then $(\text{Boc})_2\text{O}$, Et_3N ; b) LiOH , MeOH , H_2O , then allyl alcohol, DCC, DMAP; c) TMSN_3 , MeOH , 70°C . DCC = 1,3-dicyclohexylcarbodiimide.

Starting from the azides **36–38**, three isomers of **33a** were assembled following the same procedure reported for the transformation of azide **21** to **33a** (Scheme 10). Fortunately, by comparison, macrolactam **40** gave ^1H NMR spectroscopic data very similar to that of natural papuamide B, thus suggesting that the diaminobutanoic acid segment might indeed have a 2*S*,3*S* configuration. In order to unambiguously confirm the stereochemistry of this natural product, we next introduced different side chains to the revised core structure. Accordingly, when the same sequence was used as that for the elaboration of **26** to lactam **33**, product **43** was obtained from 2*S*,3*R*,8*R* ester **42** in about 7% overall yield. Much to our delight, ^1H NMR spectra obtained for synthetic **43** were found to be identical to the literature data of papuamide B.^[16] Although we found some difference in optical rotation ($[\alpha]_{\text{D}}^{21} = +23.8$ ($c = 0.37$, MeOH) (lit. $[\alpha]_{\text{D}}^{21} = +12.9$ ($c = 0.13$, MeOH)) and ^{13}C NMR spectroscopic data,^[16] we nevertheless propose that our synthetic material **43** is natural papuamide B and that this natural product has a side chain with 2*S*,3*R*,8*R* configuration; therefore the originally assigned 2*S*,3*R* stereochemistry in the 2,3-diaminobutanoic acid segment should be revised to 2*S*,3*S*. This conclusion is further supported by the fact that the 2*R*,3*S*,8*S* isomer of **43** generated from the 2*R*,3*S*,8*S* isomer of **42** gave ^1H NMR spectroscopic data remarkably different from that of natural papuamide B.

In conclusion, a convergent route to natural papuamide B has been developed, which features stereoselective assembly



Scheme 10. Synthesis of papuamide B and its three isomers.

of its dienolic acid fragment and an efficient elaboration of the (2*S*,3*S*,4*R*)-3,4-dimethylglutamine residue through a 3,4,5-trisubstituted δ -valerolactone. The latter approach will be of great benefit for the total synthesis of other anti-HIV cyclic depsipeptides such as callipeltin A and neamphamide A. Accompanying the total synthesis, the configuration of three stereogenic centers of its 2,3-dihydroxy-2,6,8-trimethyldeca-(4*Z*,6*E*)-dienolic acid unit was established, and the stereochemistry of its 2,3-diaminobutanoic acid segment was revised. This information should be of benefit for fully establishing the structures of related cyclodepsipeptides like papuamide A^[1] and mirabamides A–D.^[4] This synthesis opens the door for elaboration of related natural products and their analogues, which would prompt the structure–activity relationship (SAR) studies of these potent anti-HIV agents. Further investigations in this area are being conducted within our research group and will be reported in due course.

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[1] P. W. Ford, K. R. Gustafson, T. C. McKee, N. Shigematsu, L. K. Maurizi, L. K. Pannell, D. E. Williams, E. D. Silva, P. Lassota,

- T. M. Allen, R. V. Soest, R. J. Andersen, M. R. Boyd, *J. Am. Chem. Soc.* **1999**, *121*, 5899.
- [2] A. Zampella, M. V. D'Auria, L. G. Paloma, A. Casapullo, L. Minale, C. Debitus, Y. Henin, *J. Am. Chem. Soc.* **1996**, *118*, 6202.
- [3] N. Oku, K. R. Gustafson, L. K. Cartner, J. A. Wilson, N. Shigematsu, S. Hess, L. K. Pannell, M. R. Boyd, J. B. McMahon, *J. Nat. Prod.* **2004**, *67*, 1407.
- [4] A. Plaza, E. Gustchina, H. L. Baker, M. Kelly, C. A. Bewley, *J. Nat. Prod.* **2007**, *70*, 1753.
- [5] a) K. Makino, E. Nagata, Y. Hamada, *Tetrahedron Lett.* **2005**, *46*, 6827; b) N. Oku, R. Krishnamoorthy, A. G. Benson, R. L. Ferguson, M. A. Lipton, L. R. Phillips, K. R. Gustafson, J. B. McMahon, *J. Org. Chem.* **2005**, *70*, 6842; c) A. Zampella, R. D'Orsi, V. Sepe, A. Casapullo, M. C. Monti, M. V. D'Auria, *Org. Lett.* **2005**, *7*, 3585; d) Y. Hamada, T. Shioiri, *Chem. Rev.* **2005**, *105*, 4441; e) S. Calimsiz, Á. I. Morales Romos, M. A. Lipton, *J. Org. Chem.* **2006**, *71*, 6351; f) R. Krishnamoorthy, L. D. Vazquez-Serrano, J. A. Turk, J. A. Kowalski, A. G. Benson, N. T. Breaux, M. A. Lipton, *J. Am. Chem. Soc.* **2006**, *128*, 15392.
- [6] a) J. C. Thoen, Á. I. Morales Romos, M. A. Lipton, *Org. Lett.* **2002**, *4*, 4455; b) D. C. Cranfill, Á. I. Morales Romos, M. A. Lipton, *Org. Lett.* **2005**, *7*, 5881; c) J. Jeon, S.-K. Hong, J. S. Oh, Y. G. Kim, *J. Org. Chem.* **2006**, *71*, 3310; d) S. Chandrasekhar, M. S. Reddy, G. S. Kiranbabu, A. S. K. Murthy, *Tetrahedron Lett.* **2006**, *47*, 7307.
- [7] For other selected references, see: a) B. Liang, P. J. Carroll, M. M. Joullie, *Org. Lett.* **2000**, *2*, 4157; b) C. M. Acevedo, E. F. Kogut, M. A. Lipton, *Tetrahedron* **2001**, *57*, 6353; c) N. Okamoto, O. Hara, K. Makino, Y. Hamada, *Tetrahedron: Asymmetry* **2001**, *12*, 1353; d) S. Calimsiz, M. M. Lipton, *J. Org. Chem.* **2005**, *70*, 6218.
- [8] For other efforts on the total synthesis of marine cyclic peptides by our research group, see: a) J. Zhu, D. Ma, *Angew. Chem.* **2003**, *115*, 5506; *Angew. Chem. Int. Ed.* **2003**, *42*, 5348; b) B. Zou, J. Wei, G. Cai, D. Ma, *Org. Lett.* **2003**, *5*, 3503; c) S. Yu, X. Pan, X. Lin, D. Ma, *Angew. Chem.* **2005**, *117*, 137; *Angew. Chem. Int. Ed.* **2005**, *44*, 135; d) W. Xie, B. Zou, D. Pei, D. Ma, *Org. Lett.* **2005**, *7*, 2775; e) B. Zou, K. Long, D. Ma, *Org. Lett.* **2005**, *7*, 4237; f) S. Yu, X. Pan, D. Ma, *Chem. Eur. J.* **2006**, *12*, 6572; g) D. Ma, B. Zou, G. Cai, X. Hu, J. O. Liu, *Chem. Eur. J.* **2006**, *12*, 7615.
- [9] T. Laïb, J. Chastanet, J. Zhu, *J. Org. Chem.* **1998**, *63*, 1709.
- [10] J. Inanaga, K. Hirata, H. Saeki, T. Katsuki, M. Yamaguchi, *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989.
- [11] H. Li, X. Jiang, Y. Ye, C. Fan, T. Romoff, M. Goodman, *Org. Lett.* **1999**, *1*, 91.
- [12] M. Ciommer, H. Kunz, *Synlett* **1991**, 593.
- [13] X. E. Hu, N. K. Kim, B. Ledoussal, *Org. Lett.* **2002**, *4*, 4499.
- [14] H. Zhou, W. A. van der Donk, *Org. Lett.* **2002**, *4*, 1335.
- [15] W. R. Dichtel, S. Hecht, J. M. J. Fréchet, *Org. Lett.* **2005**, *7*, 4451.
- [16] See the Supporting Information for detailed analytical data. Notably, our ^1H NMR spectroscopic data were all identical with those reported except for a less than $\delta = 0.03$ ppm difference at $\delta = 4.82$ and 0.59 ppm. However, for ^{13}C NMR spectroscopic data we found a $\delta = 1.36$ ppm difference for the signal at $\delta = 173.06$, while the other data are indistinguishable from those reported. Considering the appearance of many additional peaks in the original ^{13}C NMR spectra of natural papuamide B (see the Supporting Information), we reasoned that these differences might result from impurities in the original sample of natural product analyzed.