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Complestatin synthetic studies: the effect of the amino acid configuration on peptide backbone conformation in the common western BCD macrocycle

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Abstract—The synthesis and structural analysis, involving X-ray crystallographic, nuclear magnetic resonance, and computational studies of four diastereomers of the common western BCD diarylether macrocycle of the complestatins, a family of HIV entry inhibitors, has been achieved exploiting a ruthenium-promoted intramolecular S_NAr reaction. The stereogenicity of the individual phenylglycines (residues C and D) results in remarkable effects on the backbone conformation.
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The initial event in cell entry of the human immunodeficiency virus (HIV) entails the binding of the 120 kDa viral-envelope glycoprotein (gp120) to the human lymphocyte cell-surface cluster determinant 4 (CD4).[1] Subsequent conformational change in the viral glycoprotein generates a secondary binding epitope specific for the lymphocyte cell-surface chemokine receptors CCR5 and/or CXCR4. Once bound to both cell-surface proteins, the virus is poised for membrane fusion. Recognition of this cascade of events has led to a world-wide campaign to identify compounds that abrogate CD4–gp120 binding interactions, and thereby viral entry and HIV infection.[2] The complestatins, a small family of bismacrocycle heptapeptides isolated from various soil Streptomyces species, typified by chloropeptin I [(−)-**1**],[3] represent a particularly intriguing class of CD4–gp120 binding inhibitors. Chloropeptin I [(−)-**1**], the most soluble member of the complestatin family, was shown by Ōmura et al. to inhibit the binding of CD4 to gp120 in the presence of bovine serum albumin (BSA) with an IC_{50} value of 2.0 μM; in the absence of BSA the potency is 32 nM.

Given our interest in the development of novel gp120–CD4 agonists and antagonists,[4] we initiated a program directed toward the synthesis of members of the complestatin family. Early in this venture, in collaboration with Ōmura and Tanaka,[5] we determined the absolute stereochemistry and solution conformation of (−)-chloropeptin I [(−)-**1**, Figure 1], exploiting a combination of computational and spectroscopic methods. Subsequently, the synthetic community has shown considerable interest in the complestatins,[6] culminating recently in the total synthesis of chloropeptin I.[6h]

In this paper, we report the synthesis of four diastereomers of the complestatin western BCD macrocycle, including the isostructural congener. The stereogenicity of the two phenylglycines within the macrocyclic diarylether tripeptide were found to effect dramatically the overall conformation of the peptide backbone, a result expected to play a significant role in the future design of complestatin based CD4–gp120 inhibitors.

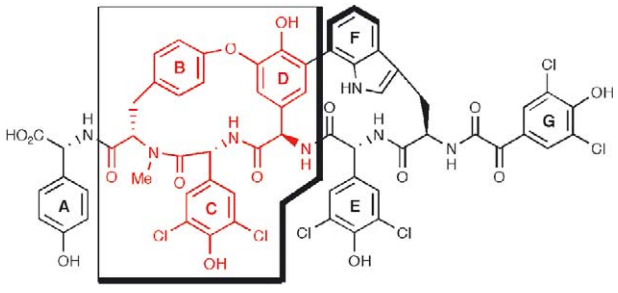

Figure 1. Chloropeptin I (−)-**1**.

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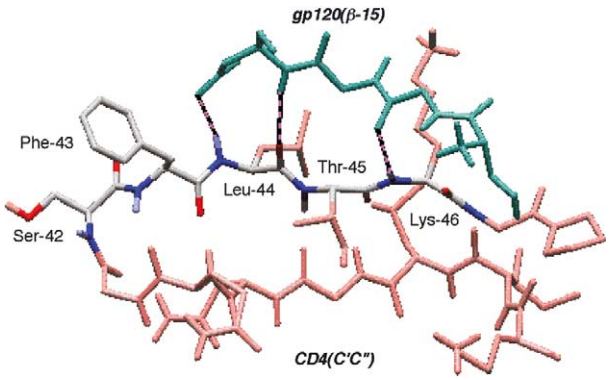


Figure 2. C′C″ loop of CD4 (mostly pink) in relationship to the β-15 strand of gp120 (cyan). Critical residues Ser-42 and Phe-43, as well as the backbone of Leu-44, Thr-45, and Lys-46 are presented in standard atom colors, highlighting strand-to-strand interactions with β-15 of gp120.

The crystal structure of a ternary complex composed of laboratory-adapted gp120 core (HxBC2), the D1D2 portion of CD4, and 17b, a mouse antibody known to bind to the chemokine receptor-binding epitope on gp120 was reported in 1998 by Hendrickson et al.[7] The nine contiguous amino acid span of CD4, comprising residues 40–48 (C′C″ strands), was suggested to be responsible for 63% of all interfacial contacts, of which 23% were covered by the previously solvent-exposed side-chain of Phe-43, which caps a hydrophobic pocket in gp120. Half of the interacting CD4 residues, however, make interfacial contributions only through their backbone atoms. The C′ strand of CD4 is the dominant contributor of the main chain interactions participating in the β-pleated sheet alignment with the β15 strand of gp120 (Fig. 2). These observations strongly suggest that interfering with the ability of gp120 to form a β-sheet with CD4 would greatly reduce binding affinity.

Chloropeptin I [(−)-**1**] exhibits a high degree of structural and electrostatic homology to the CD4-binding domain of gp120 (e.g., a hydrophobic pocket and a rigid peptide β-strand crowned by a hemisphere of acidic sites). This structural homology suggests that (−)-**1** and gp120 may compete for a common binding site on CD4. In support of this hypothesis, we constructed a computational docking model in which (−)-**1** was bound to the gp120 binding domain of CD4. The model indicates that (−)-**1** can form a β-sheet with the C′C″ region of CD4, similar to the β15 strand of gp120 (Fig. 3a). Comparable β-sheet interactions have been reported for the related conformationally-constrained peptide antibacterial agent vancomycin with the bacterial cell-wall precursor L-Lys-D-Ala-D-Ala.[8] Interestingly, in contrast with chloropeptin I, 100 μM vancomycin does not inhibit gp120/CD4 binding; the complestatins are equally inactive against bacteria. In addition to possible β-sheet interactions, multiple ionic hydrogen-bonding opportunities between the deprotonated phenolic oxygens of chloropeptin I with protonated side-chains of CD4 would increase the binding potential (Fig. 3b). Rotation of the Phe-43 side-chain, which is most likely freely rotating in solvent, into the diaryl ether hydrophobic pocket of (−)-**1**, would result in additional binding affinity.

Having determined the stereostructure of chloropeptin I and developed a plausible hypothesis for the inhibition of gp120 binding to CD4, we initiated a stereodivergent synthesis of the complestatins and related diastereomers. Of particular interest was how the configuration of the phenylglycine stereocenters affects the peptide backbone conformation in the rigid diphenylether and biaryl macrocyclic constructs. Our initial efforts focused on the construction of the common western BCD macrocycle. Given the commonality of this structural unit throughout the complestatin family of peptides and their potential biological importance, an efficient route to the BCD macrocycle and congeners thereof was viewed as central both to our unified synthetic strategy for the complestatins and for the development of novel agonists/antagonists of HIV-1 gp120-CD4 binding.

To construct the common complestatin western macrocycle, we selected the elegant arene-ruthenium chemistry pioneered by Pearson[9] and Rich.[10] The requisite individual amino acid residues (−)-**3**, (−)-**5**, and (−)-**7** were prepared as outlined in Scheme 1. Residue B [(−)-**3**] was

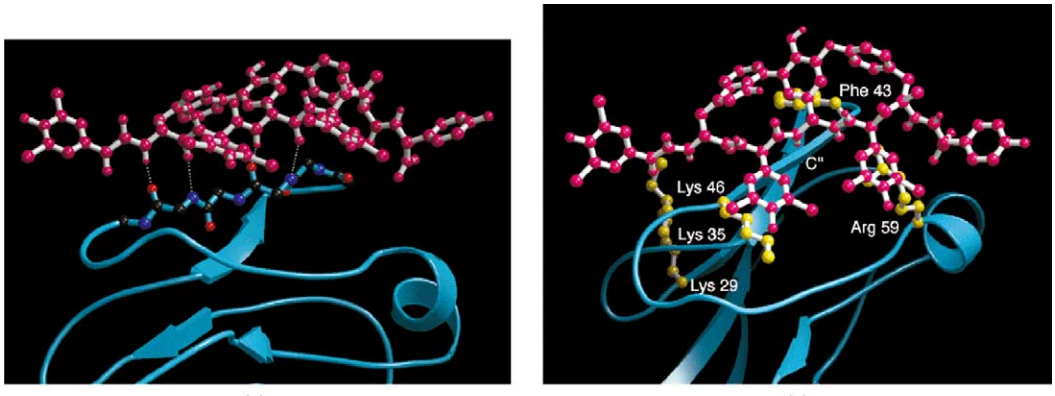
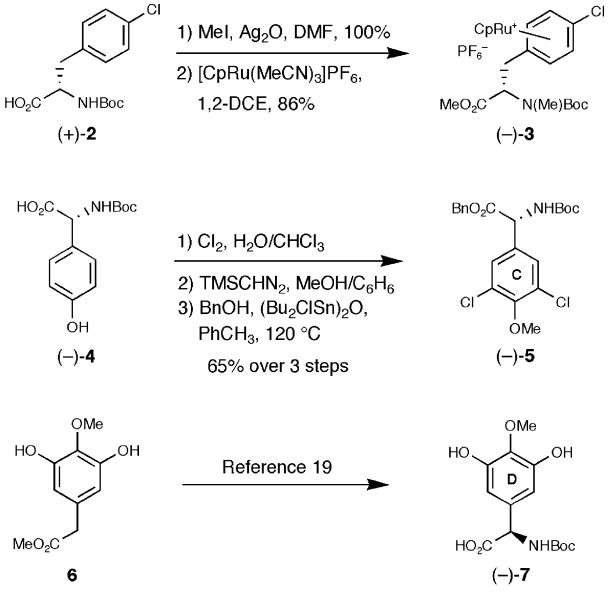


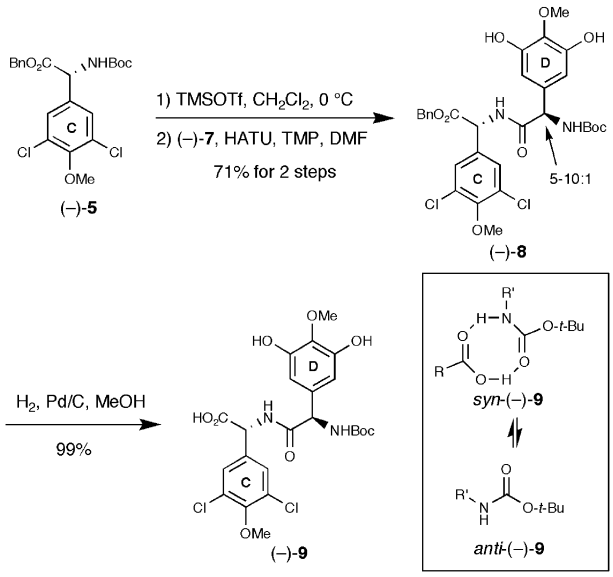
Figure 3. Computational Model of (−)-**1** Docked to CD4. (a) Potential β-sheet interaction between (−)-**1** (pink) and the C′C″ subregion of CD4 (cyan) [amide N = blue, α-C = black, carbonyl O = red], (b) Hydrogen bonding opportunities between (−)-**1** (pink) and CD4 (cyan): the positively charged amino acid side chains and Phe-43 of CD4, key to CD4-gp120 binding (yellow) are highlighted. [Figures created using MOLSCRIPT (Kraulis, P. J. *J. Appl. Crystallogr.* **1991**, *24*, 946.)].

obtained in 86% yield[11] upon *N*,*O*-bis-methylation of Boc-protected (4-chlorophenyl)alanine (+)-**2**,[12] followed by ruthenium-arene complexation. For residue C [(−)-**5**], bis-chlorination of *N*-Boc-D-(4-hydroxyphenyl)-glycine [(−)-**4**][13] with Cl_2 in 3:5H_2O/$CHCl_3$, followed in turn by *O*,*O*-bismethylation with $TMSCHN_2$[14,15] and catalytic *trans*-esterification to the benzyl ester exploiting the Otera protocol[16] proceeded in good overall yield and with high enantioselectivity (65% and ≥97% e.e.; 3 steps).[17,18] The final amino acid, residue D [(−)-**7**], was prepared in 7 steps and 31% overall yield (≥90% e.e.) from methyl (3,5-dihydroxy-4-methoxyphenyl)acetate (**6**) following literature precedent.[19]

With the individual amino acids in hand, we turned to the construction of the linear BCD tripeptide. The initial route, involving a C-to-N protocol (residues B-to-D), was quickly abandoned due both to our inability to purify adequately the arene-ruthenium salt intermediates, and to the near quantitative hydrolysis of the newly formed *N*-methyl amide bond upon Boc-deprotection. We instead employed an N-to-C coupling strategy (residues D-to-B) to form the linear tripeptide (Scheme 2). Accordingly, *N*-Boc-phenylglycinate (−)-**5** was deprotected with TMSOTf in dichloromethane, and the resulting free amine was coupled to phenylglycine (−)-**7** employing HATU to afford dipeptide (−)-**8** in 71% overall yield, with variable diastereomeric purity (5–10:1 d.r.).[20] Palladium-catalyzed hydrogenolysis of benzyl ester (−)-**8** next proceeded in near quantitative yield to provide the CD dipeptide acid (−)-**9**. In $CDCl_3$, the *N*-Boc-protected acid (−)-**9** exists as a concentration dependent mixture of rotamers, as determined by ^{1}H NMR. In this regard, Marcovici-Mizrahi et al. reported that the sterically unfavored *syn*-carbamate rotamer of *N*-Boc-protected mono-phenylglycines is stabilized in apolar solvents by a hydrogen-bond dimer between the free carboxylic acid and the carbamate carbonyl and amine (see insert, Scheme 2).[21] Presumably, this stabilization also occurs, though to a lesser extent, in *N*-Boc



Scheme 1. Synthesis of amino acids (−)-**3**, (−)-**5**, and (−)-**7**.

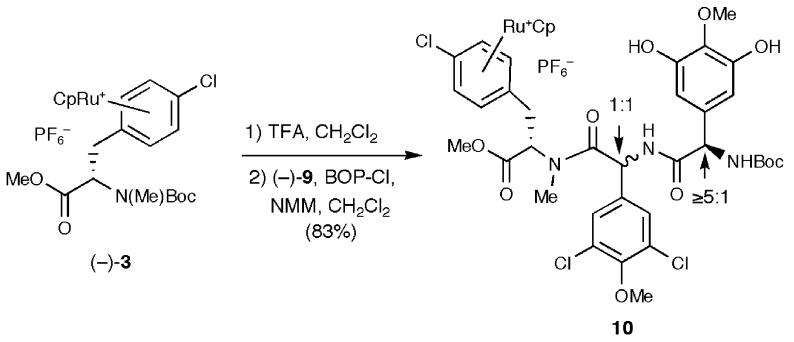


Scheme 2. synthesis of CD-dipeptide (−)-**9**.

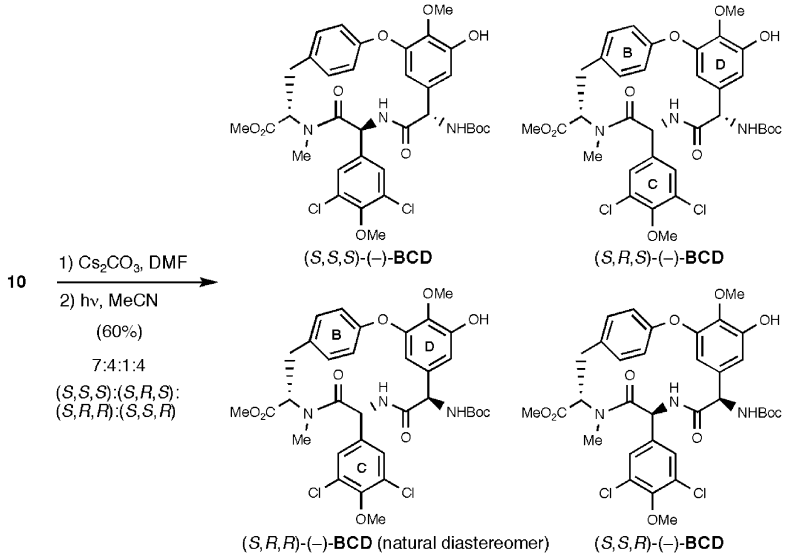
protected diphenylglycine (−)-**9**. Polar solvents would be expected to disrupt the hydrogen-bonding network that stabilizes the *syn*-rotamer; accordingly, in CD_3CN only one conformation was observed in the ^{1}H NMR spectra of (−)-**9**.

We next explored a variety of coupling protocols to form the *N*-methyl amide linkage. Not unexpectedly, near or complete epimerization of the C residue α-center resulted (Scheme 3).[22] From the perspective of yield, the best results were obtained by removing the Boc-moiety from the phenylalanine (−)-**3** with TFA and then coupling to the CD dipeptide (−)-**9** using BOP-Cl in dichloromethane;[23] acyclic tripeptide **10** was obtained in 83% yield as a mixture of diastereomers. Treatment of a dilute solution (1–5 mM) of **10** in DMF with Cs_2CO_3, followed by photolytic demetalation of the macrocycle in acetonitrile led to a mixture of tripeptide macrocycle diastereomers and rotamers in 60% combined yield (Scheme 4). Separation of the individual diastereomers was accomplished by normal-phase HPLC.[24] Two of the four diastereomers (vide infra) existed as separable mixtures of *N*-methyl amide rotational isomers; the remaining two showed no evidence for rotational isomerism. In accord with literature precedents, the rotameric ratios and rates of equilibration for the two rotational isomers of both diastereomers were solvent dependent.[22,6a,h] As noted by us and others,[5,6a,h,25] the C-terminal residue of chloropeptin I (residue A) locks the BCD-macrocycle of the natural product in the *cis* *N*-methyl amide conformation. Presumably, this increased conformational rigidity protects the residue C chiral center of (−)-**1** from the base induced-epimerization suffered by (*S*,*R*,*R*)-**BCD**.

Interestingly, the ratio of diastereomers was significantly different from that of the starting acyclic tripeptide **10**, with the isostructural natural (*S*,*R*,*R*)-diastereomer being the least favored product. Attempts to epimerize the (*S*,*S*,*S*), (*S*,*R*,*S*), and (*S*,*S*,*R*)-**BCD**



Scheme 3. Synthesis of acyclic BCD-tripeptide **10**.



Scheme 4. Formation of BCD macrocycle diastereomers.

macrocycles (DBU, CD_3CN, 50 °C, 18 h; monitored by ^1H NMR) proved unsuccesful.[26] However, when the (*S,R,R*)-diastereomer was submitted to similar conditions, 75% of the material was converted into the (*S,S,R*) congener within 3 h.

Determination of the complete stereostructures of the individual **BCD** macrocycle diastereomers was particularly challenging. Gratifyingly, X-ray quality crystals of the two diastereomers that did not show evidence of *N*-methyl amide rotational isomerism were obtained. The X-ray analysis confirmed these to be the (*S,S,R*) and (*S,S,S*)-**BCD** tripeptide macrocycles. The unit cell of the (*S,S,R*) diastereomer displayed two distinct conformers, while three different conformers were observed for the (*S,S,S*) diastereomer, each representing, for the most part, different torsional orientations about the methyl ester carbonyl-phenylalanine α-carbon bond or the dichlorohydroxy-phenylglycine sidechain-α-carbon linkage[27]

Despite considerable efforts, X-ray quality crystals of the two remaining diastereomers, which both displayed *N*-methyl amide rotational isomerism, could not be obtained. Their absolute configurations and resulting conformations were deduced from extensive NMR analysis (TOCSY, HMBC, HMQC, and NOESY) followed by NOE-constrained computational analyses, similar to those employed to elucidate the stereostructure of (−)-chloropeptin I (**1**).[5] In short, distance constraints were generated from experimental NOE values for both the *cis* and *trans* rotamers of (*S,R,S*)-**BCD** and (*S,R,R*)-**BCD**; these constraints were then employed in a Monte Carlo conformation search (AMBER* force field, chloroform solvation) and the resulting 75 lowest-energy conformations were selected, superimposed and shown to represent, except for (*S,R,S*)-*trans*-**BCD** (vide infra), one major conformational group as shown in Figure 4.[28] Although the *cis*:*trans* ratio of the *N*-methyl amide moiety for both the (*S,R,S*) and (*S,R,R*) diastereomers was solvent dependent, the *cis* rotamer was consistently the dominant conformational isomer. The *cis* rotamer possessing the *R* configuration at residue C was easily distinguished by a strong NOE crosspeak between the α-CHs of residues B and C.[29]

Structural analysis of the individual **BCD** stereoisomers revealed that the stereogenicities of the amino acids produce a diverse array of peptide backbone conformations. Shown in Figure 4 is a comparison of the conformations of the four **BCD** diastereomers, including where appropriate the *cis* and *trans* rotamers, with the previously determined stereostructure of chloropeptin I

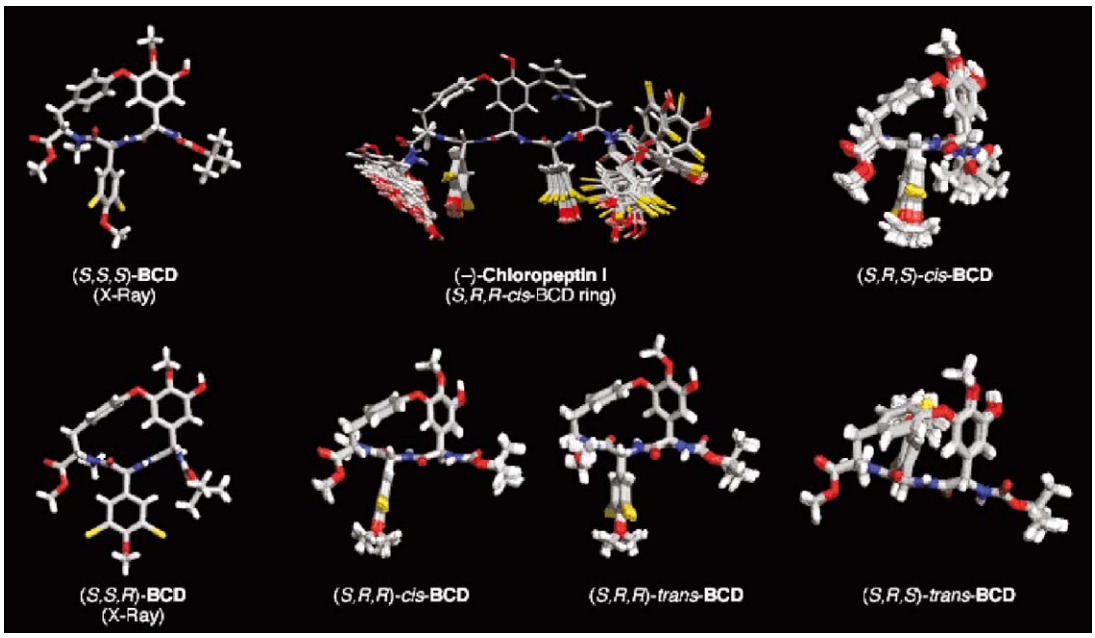


Figure 4. Conformational comparison of chloropeptin I [(−)-**1**] with the four synthesized BCD diastereomers. The structures for the (*S,S,S*) and (*S,S,R*) diastereomers were determined by X-ray crystallography.[27] The four other conformations were determined by NOE-constrained computational methods, as described earlier, and represent overlays of the 75 lowest-energy conformations, except for (*S,R,S*)-*trans*-**BCD** (30 lowest-energy structures).[28]

[(−)-**1**].[5] Unlike the western macrocycle of (−)-**1** which has the (*S,R,R*) configuration, the *N*-methyl amides of (*S,S,R*) and (*S,S,S*)-**BCD** are clearly in the *trans* conformation about the *N*-methyl amide linkage. Other structural distinctions between the (*S,S,R*)/(*S,S,S*) diastereomers and (−)-**1** include the opposite orientation of the NH and carbonyl moieties in the macrocyclic peptide backbone. These remarkable conformational differences would be expected to elicit pronounced changes in biological potency, based on our proposed model for inhibition of CD4-gp120 binding by (−)-chloropeptin I.

The *cis* amide rotamer of (*S,R,R*)-**BCD**, not surprisingly, is nearly isostructural with the conformation of the **BCD** macrocycle of chloropeptin I. Similarly, the *trans* rotamer of (*S,R,R*)-**BCD** possesses the same peptide backbone directionality between residues C and D to (−)-**1**. The *trans* rotamer of (*S,R,S*)-**BCD** was found to be conformationally the most different member of the four diastereomers. Inspection of the 75 lowest- energy conformations of *trans*-(*S,R,S*)-**BCD** revealed three unique structural groups, of which the lowest energy grouping (30 conformations) is shown in Figure 4. The other two (not shown) were similar to the (*S,R,S*)-*cis*-**BCD** conformation, save the conformation about the *N*-methyl amide linkage. The directionality of the peptide backbone between residues C and D in the lowest energy conformation is opposite to that of chloropeptin I. A more striking feature in the *S,R,S*-*trans*-congener is placement of the residue C aromatic ring, with one edge directly underneath the biaryl linkage. This unique orientation seems reasonable since the residue C aryl hydrogens are split into two different peaks in the ^{1}H NMR spectra of (*S,R,S*)-**BCD**. Finally, unlike the *trans* rotamer, the intracyclic region of the peptide backbone of the *cis*-rotamer of (*S,R,S*)-**BCD** displays a directionality similar to the (*S,R,R*) diastereomer as well as to (−)-chloropeptin I (**1**).

In summary, a stereodivergent synthesis of four of the possible diastereomers of the complestatin western (BCD) macrocycle, including the isostructural natural configuration, has been achieved, employing a ruthenium-activated intramolecular S_NAr cyclization tactic. Pleasingly, the route provides direct access to useful quantities of advanced intermediates to explore the role of the stereogenicity of the C and D residues on peptide backbone conformation. The observed complex relationships between phenylglycine configuration and peptide backbone conformation/directionality in the rigid macrocyclic constructs provides potentially important information for the design of small molecule inhibitors of CD4–gp120 binding. The results of these studies will be reported in due course.

Acknowledgements

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References and notes

1. (a) McDougal, J. S.; Kennedy, M. S.; Sligh, J. M.; Cort, S. P.; Mawle, A.; Nicholson, J. K. A. *Science* **1986**, *231*, 382. (b) Bour, S.; Geleziunas, R.; Wainberg, M. A. *Microbiol. Rev*. **1995**, *59*, 63.
2. (a) Ōmura, S.; Tanaka, H.; Matsuzaki, K.; Ikeda, H.; Masuma, R. *J. Antibiotics* **1993**, *46*, 1908. (b) Patil, A. D.; Kumar, N. V.; Kokke, W. C.; Bean, M. F.; Freyer, A. J.; De Brosse, C.; Mai, S.; Truneh, A.; Faulkner, D. J.; Carte, B.; Breen, A. L.; Hertzberg, R. P.; Johnson, R. K.; Westley, J. W.; Potts, B. C. M. *J. Org. Chem*. **1995**, *60*, 1182. (c) Nara, P. L.; Hwang, K. M.; Rausch, D. M.; Lifson, J. D.; Eiden, L. E. *Proc. Natl. Acad. Sci. U.S.A*. **1989**, *86*, 7139. (d) Martin, L.; Strichner, F.; Missé, D.; Sironi, F.; Pugnière, M.; Barthe, P.; Prado-Gotor, R.; Freulon, X.; Roumestand, C.; Ménez, A.; Lusso, P.; Veas, F.; Vita, C. *Nature-Biotech*. **2003**, *21*, 71. (e) Ferrer, M.; Harrison, S. C. *J. Virol*. **1999**, *73*, 5795.
3. Tanaka, H.; Matsuzaki, K.; Nakashima, H.; Ogino, T.; Matsumoto, A.; Ikeda, H.; Woodruff, H. B.; Tanaka, H.; Ōmura, S. *J. Antibiot*. **1997**, *50*, 58 and references cited therein..
4. Smith, A. B., III; Savinov, S. N.; Manjapparra, U. V.; Chaiken, I. M. *Org. Lett*. **2002**, *4*, 4041.
5. Gouda, H.; Matsuzaki, K.; Tanaka, H.; Hirono, S.; Ōmura, S.; McCauley, J. A.; Sprengler, P. A.; Furst, G. T.; Smith, A. B. *J. Am. Chem. Soc*. **1996**, *118*, 13087.
6. (a) Kai, T.; Kajimoto, N.; Konda, Y.; Harigaya, Y.; Takayanagi, H. *Tetrahedron Lett*. **1999**, *40*, 6289. (b) Elder, A. M.; Rich, D. H. *Org. Lett*. **1999**, *1*, 1443. (c) Roussi, G.; Zamora, E. G.; Carbonnelle, A. C.; Beugelmans, R. *Heterocycles* **1999**, *51*, 2041. (d) Carbonelle, A. C.; Zamora, E. G.; Beugelmans, R.; Roussi, G. *Tetrahedron Lett*. **1998**, *39*, 4467. (e) Carbonelle, A. C.; Zamora, E. G.; Beugelmans, R.; Roussi, G. *Tetrahedron Lett*. **1998**, *39*, 4471. (f) Roussi, G.; Zamora, E. G.; Carbonelle, A. C.; Beugelmans, R. *Tetrahedron Lett*. **1997**, *38*, 4401. (g) Gurjar, M. K.; Tripathy, N. K. *Tetrahedron Lett*. **1997**, *38*, 2163. (h) Deng, H.; Jung, J.-K.; Liu, T.; Kuntz, K. W.; Snapper, M. L.; Hoveyda, A. H. *J. Am. Chem. Soc*. **2003**, *123*, 9032.
7. Kwong, P. D.; Wyatt, R.; Robinson, J.; Sweet, R. W.; Sodroski, J.; Hendrickson, W. A. *Nature* **1998**, *393*, 648.
8. (a) Sheldrick, G. M.; Jones, P. G.; Kennard, O.; Williams, D. H.; Smith, G. A. *Nature* **1978**, *271*, 223. (b) Knox, J. R.; Pratt, R. F. *Antimicrob. Agents Chemother*. **1990**, *34*, 1342. See also: Williams, D. H. *Acc. Chem. Res*. **1984**, *17*, 364.
9. (a) Pearson, A. J.; Park, J. G. *J. Org. Chem*. **1992**, *57*, 1744. (b) Pearson, A. J.; Zigmantas, S. *Tetrahedron Lett*. **2001**, *42*, 8765 and references cited therein..
10. Rich, D. H.; Janetka, J. W. *J. Am. Chem. Soc*. **1995**, *117*, 10585.
11. Reported yields are averages of at least three runs and refer to chromatographically and spectroscopically pure compounds, except as otherwise indicated.
12. RSP Amino Acid Analogues, Inc., Worcester, MA.
13. Miller, M. J.; Mattingly, P. G. *Tetrahedron* **1983**, *39*, 2563.
14. (a) Hashimoto, N.; Aoyama, T.; Shioiri, T. *Chem. Pharm. Bull*. **1981**, *29*, 1475. (b) Aoyama, T.; Terasawa, S.; Sudo, K.; Shioiri, T. *Chem. Pharm. Bull*. **1984**, *32*, 3759.
15. *O*,*O*-Bismethylation with MeI and K_2CO_3 in DMF afforded the desired methyl ester in 83% yield, but with highly variable enantiopurity (0–95% e.e.), as determined by chiral HPLC analysis.
16. (a) Otera, J.; Yano, T.; Kawabata, A.; Nozaki, H. *Tetrahedron Lett*. **1986**, *27*, 2383. (b) Otera, J.; Dan-oh, N.; Nozaki, H. *J. Org. Chem*. **1991**, *56*, 5307.
17. Despite previous reports (ref 6c,f), ester hydrolysis of methyl *N*-Boc-D-(3,5-dichloro-4-methoxyphenyl)glycinate under various conditions, including K_2CO_3 in MeOH, resulted in immediate and complete racemization, as determined by chiral HPLC analysis.
18. Enantiomeric excess determined by chiral HPLC analysis: Chiralpak AD® (Chiral Technologies) 4.6×250 mm column, 3:97 *i*-PrOH/Hexanes, 1 mL/min, $\lambda = 220$ nm, $k_1' = 2.19$, $\alpha = 1.24$, $R_s = 1.04$.
19. (a) Pearson, A. J.; Chelliah, M. V.; Bignan, G. C. *Synthesis* **1997**, 536. (b) Pearson, A. J.; Zhang, P. *J. Org. Chem*. **1996**, *61*, 9603.
20. Carpino, L. A. *J. Am. Chem. Soc*. **1993**, *115*, 4397.
21. Marcovici-Mizrahi, D.; Gottlieb, H. E.; Marks, V.; Nudelman, A. *J. Org. Chem*. **1996**, *61*, 8402.
22. Attempts to couple the free amine of (−)-3 to *N*-Boc-(3,5-dichloro-4-hydroxyphenyl)glycine with DEPBT (ref 6h) led to competitive formation of phenolic esters.
23. Diago-Messeguer, J.; Palomo-Coll, A. L.; Fernández-Lizarbe, J. R.; Zugaza-Bilbao, A. *Synthesis* **1980**, 547.
24. HPLC conditions: Waters Novapak Si 19×300 mm column, 45:50 EtOAc/Hexanes, 5 mL/min then 40:2:58 EtOAc/*i*-PrOH/Hexanes, 10 mL/min.
25. (a) Singh, S. B.; Jayasuriya, H.; Hazuda, D. L.; Felock, P.; Homnick, C. F.; Sardana, M.; Patane, M. A. *Tetrahedron Lett*. **1998**, *39*, 8769. (b) Singh, S. B.; Jayasuriya, H.; Salitruo, G. M.; Zink, D. L.; Shafiee, A.; Heimbuch, B.; Silverman, K. C.; Lingham, R. B.; Genilloud, O.; Teran, A.; Vilella, D.; Felock, P.; Hazuda, D. *J. Nat. Prod*. **2001**, *64*, 874.
26. In all cases, starting material was recovered unaltered.
27. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 223088 and 223089. Copies of data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223-336033 or email: deposit@ccdc.cam.as.uk]
28. For all Monte Carlo conformational searches, the program Maestro® Molecular Modeling Interface version 4.1.012 (Schrodinger, Inc.) was utilized and the following parameters were employed: AMBER* force field with a $CHCl_3$ solvation model, PCRG method, maximum number of iterations = 99,999, converge on gradient with a convergence threshold of 0.05, 10,000 steps, 50.0 energy window. NOE values were translated into distance constraints as follows: strong (s) = 1.5–2.5 Å, medium (m) = 1.5–3.5 Å, weak (w) = 1.5–5.0 Å, all with a force constant of 1,000.
29. For a similar *N*-methyl diarylether tripeptide macrocycle Kai proposed that the *trans* rotamer was dominant based on an NOE signal between the *N*-Me group and the residue C α-CH (ref 6a and X-ray: Kai, T.; Kajimoto, N.; Yamada, Y.; Harigaya, Y.; Takayanagi, H. *Anal. Sci*. **2002**, *18*, 369) Our computational studies (this paper and ref. 5) however, suggests that the strength of the NOE between α-CHs of residues B and C is a more accurate method for distinguishing between the *cis* and *trans* amide rotamers of complestatin-like diarylether macrocycles.