Synthesis of a Trisubstituted 1,4-Diazepin-3-one-Based Dipeptidomimetic as a Novel Molecular Scaffold

Iris S. Weitz,[†,‡] Maria Pellegrini,[§] Dale F. Mierke,[§] and Michael Chorev*[,‡,||]

Department of Pharmaceutical Chemistry, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 91120, Israel, Carlson School of Chemistry, Clark University, 950 Main Street, Worcester, Massachusetts 01610, and Division of Bone & Mineral Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline Avenue (HIM 944), Boston, Massachusetts 02215

Received December 3, 1996[⊗]

We describe two routes for the synthesis of a trisubstituted 1,2,5-hexahydro-3-oxo-1*H*-1,4-diazepine ring (DAP), a novel, conformationally constrained, seven-membered dipeptidomimetic ring system. The linear precursor for the model DAPs, targeted for conformational analysis studies, was obtained by reductive alkylation of *tert*-butyl alaninate or phenylalaninate by *N*-Boc-α-amino-γ-oxo-*N*,*N*-dimethylbutyramide. Acetylation of the newly formed secondary amine followed by acidolytic deprotection of the amino and carboxyl terminal protecting groups and subsequent diphenylphosphorazidate-mediated ring formation yielded the blocked model DAPs. The synthesis of the DAP synthon started with 1-*tert*-butyl hydrogen *N*-(benzyloxycarbonyl)aspartate. The aldehyde obtained from the β-carboxyl was used to reductively alkylate benzyl phenylalaninate, generating a secondary amine. Hydrogenolytic deprotection of the end-groups yielded the linear precursor which was cyclized via lactam formation mediated by 1-hydroxy-7-azabenzotriazolyl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate. This route yielded the reversibly protected hexahydro-1*H*-3-oxo-2(*S*)-benzyl-5(*S*)-(*tert*-butyloxycarbonyl)-1,4-diazepine. This synthon unit can be subsequently elaborated by substituting the functional groups (secondary amine and carboxyl). Therefore, the DAPs may serve as novel molecular scaffolds to reproduce a biologically relevant topology or as a dipeptido-conformation-mimetic that can be incorporated into bioactive peptides. In addition, these synthetic routes will allow the introduction of different chiralities at positions 2 and 5 as well as the diversification of the side chains at position 2. Furthermore, the synthetic routes described here can be easily modified to obtain larger ring systems with variable degrees of conformational flexibility.

Introduction

Drugs based on peptides such as insulin, calcitonin, adrenocorticotropin, vasopressin, gonadotropin-releasing hormone, somatostatin, glucagon and thyrotropin-releasing hormone are used as prescription medications in treatment of various disease. Nevertheless, in search for increased specificity and improved pharmacokinetics and pharmacodynamics, great efforts in peptide-based drug design are being made in the development of a wide-range of nonpeptidic structural modifications. These include pseudopeptides,[1−3] peptidomimetics,[4−7] conformation-mimetics[8,9] and mass-screening-derived nonpeptides.[10−13]

In an effort to stabilize a putative bioactive conformation, conformational constraints are frequently employed. Global reduction in the conformational degrees of freedom of linear peptides is achieved by a variety of long- and medium-range homodetic and heterodetic cyclizations.[14−17] In addition, local constraints are accomplished by short-range cyclizations generated through bridging neighboring amino acid residues via small ring formation.[17−21] For example, γ-lactams[18] and piperazinone[19−21] rings are obtained by bridging either $C\alpha_i$-to-N_{i+1} or N_i-to-N_{i+1}, respectively.

A major objective in the development of low molecular weight, nonpeptidic peptidomimetics is the close reproduction of the so-called "bioactive topology" which is derived from the putative "bioactive conformation". Small,

* Author to whom correspondence should be addressed.
† This work is presented as partial fulfillment of the requirement toward a Ph.D. thesis.
‡ The Hebrew University of Jerusalem.
§ Clark University.
|| Harvard Medical School.
⊗ Abstract published in *Advance ACS Abstracts,* April 1, 1997.

(1) Spatola, A. F. In *Chemistry and Biochemistry of Amino acids, Peptides, and Proteins*; Weinstein, B. Ed.; Marcel Deker: New York, 1983; p 267.
(2) Spatola, A. F.; Darlak, K. *Tetrahedron* **1988**, *44*, 821.
(3) Spatola, A. F. In *Methods in Neurosciences*; Conn, P. M., Ed.; Academic Press: Orlando, FL, 1993; pp 119−143.
(4) Farmer, P. S. In *Drug Design*; Ariens, E. J., Ed.; Academic Press: New York, NY, 1980; Vol X, pp 19−43.
(5) Olson, G. L.; Bolin, D. R.; Pat Bonner, M.; Bos, M.; Cook, C. M.; Fry, D. C.; Graves, B. J.; Hatada, M.; Hill, D. E.; Kahn, M.; Madison, V. S.; Rusiecki, V. K.; Sarabu, R.; Sepinwall, J.; Vincent, G. P.; Voss, M. E. *J. Med. Chem.* **1993**, *36*, 3039.
(6) Sawyer, T. K. In *Peptide-Based Drug Design*; Taylor, M. D., Amidon G. L., Eds.; American Chemical Society Washington, DC, 1995; pp 287−422.
(7) Hirschmann, R. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1278.
(8) Holzemann, G, *Kontakte* (*Darmstadt*) **1991**, *1*, 3.
(9) Holzemann, G, *Kontakte* (*Darmstadt*) **1991**, *2*, 55.
(10) Morgan, B. A.; Gainor, J. A. *Annu. Rep. Med. Chem.* **1989**, *24*, 243.
(11) Freidinger, R. M. *Trends Pharmacol. Sci.* **1989**, *10*, 270.
(12) Rees, D. C. *Annu. Rep. Med. Chem.* **1993**, *28*, 59.
(13) Wiley, R. A.; Rich, D. H. *Med. Res. Rev.* **1993**, *13*, 327.
(14) Kopple, K. D. *J. Pharm. Sci.* **1972**, *61*, 1345.
(15) Gilon, C.; Halle, D.; Chorev, M.; Selinger, Z.; Byk. G. *Biopolymers* **1991**, *31*, 745.
(16) Byk, G.; Gilon, C. *J. Org. Chem.* **1992**, *57*, 5687.
(17) Toniolo, C. *Int. J. Pept. Protein Res.* **1990**, *35*, 287.
(18) Freidinger, R. M.; Perlow, D. S.; Veber, D. F. *J. Org. Chem.* **1982**, *47*, 104.
(19) DiMaio, J.; Belleau, B. *J. Chem. Soc., Perkin Trans 1* **1989**, 1687, and the references therein.
(20) Kojima, Y.; Ikeda, Y.; Kumata, E.; Maruo, J.; Okamoto, A.; Hirotsu, K.; Shibata, K.; Ohsuka, A. *Int. J. Pept. Protein Res.* **1991**, *37*, 468.
(21) Yamashita, T.; Kojima, Y.; Hirotsu, K.; Ohsuka, A. *Int. J. Pept. Protein Res.* **1989**, *33*, 110.

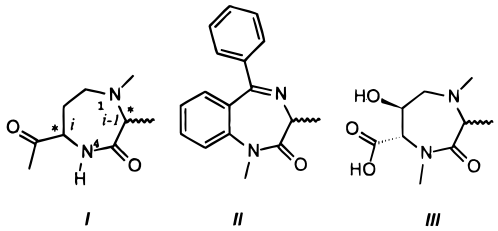
S0022-3263(96)02257-8 CCC: $14.00

polyfunctional, conformationally constrained, mono- or polycyclic "molecular scaffolds" which can be substituted by the essential pharmacophores are ideal for this transformation.[4,22−35] To date, the 1,4-benzodiazepin-3-one system has been the most widely used "molecular scaffold".[24−35] Changing the appropriate pharmacophores yielded potent and selective CCK-A receptor antagonists,[25−29] CCK-B and gastrin receptor antagonists,[28,29] a κ^1-opiate receptor agonist,[24] GP_{IIb}/III_a receptor antagonists,[30,31] a HIV Tat receptor antagonist,[32] a inhibitor of Ras farnesylation,[33,34] and inhibitors of HIV reverse transcriptase.[35]

We report here the synthesis of trisubstituted 1,2,5-hexahydro-3-oxo-1*H*-1,4-diazepine (DAP) (**I**) (Scheme 1) a novel "molecular scaffold" structurally related to the 1,4-benzodiazepin-2-one (**II**) (Scheme 1). It is a monoheterocycle lacking the fused benzene ring included in the 1,4-benzodiazepin-2-one system.

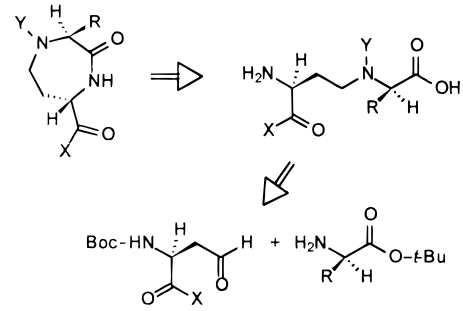
Interestingly, liposidomycin B and C are lipid-bearing nucleoside antibiotics which contain a structurally related 1,4-diazepan-3-one ring system (**III**) (Scheme 1).[36,37] Recently, the diazepanone ring was synthesized as part of the total synthesis of liposidomycins.[38−41]

Scheme 1. 1,2,5-Trisubstituted Hexahydro-3-oxo-1*H*-1,4-dizaepine (DAP) and Structurally Related Systems



I *II* *III*

Scheme 2. Retrosynthesis of a DAP System



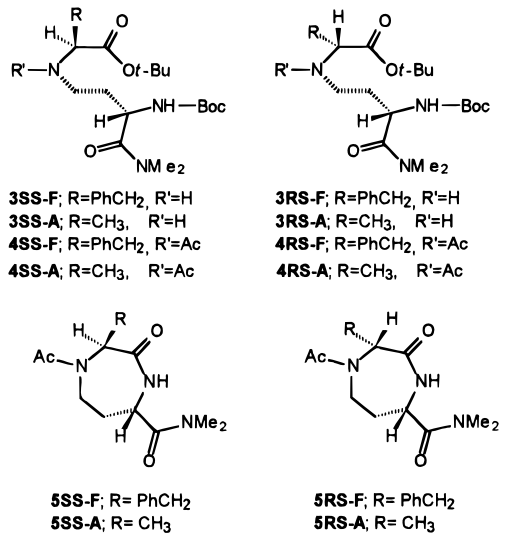
The DAP system is comprised of two chiral centers, C-2 and C-5, and three potential substitution sites: a side chain on C-2, the N-1 as a secondary amine, and the carboxyl group on C-5. This rigidified dipeptidomimetic system may function as a "conformation mimetic", introducing local conformational constraints, or as a "molecular scaffold" to maintain a defined topology. One can also realize the potential of this system as a novel "diversomer" in randomized combinatorial "analogous" libraries.[42] Therefore, the DAP system offers new opportunities in bridging the gap between bioactive peptides and peptidomimetic drugs[4] and is an important addition to the arsenal of tools available for rational drug design.

Results and Discussion

Synthesis of Model DAPs. The side-chain-to-backbone bridging between $C\alpha_i$ and N_{i-1} in a dipeptidic unit to generate a seven-membered ring (Scheme 1, **I**), detailed in this report, is a novel approach for introducing local conformational constraint. Our retrosynthetic analysis, outlined in Scheme 2, requires the construction of a linear precursor, generated by a reductive alkylating step, which will undergo cyclization to form the seven-membered lactam. The synthetic design is based on the utilization of the expansive range of commercially available protected, coded and noncoded, amino acid derivatives as building blocks for the assembly of the trisubstituted 1,2,5-hexahydro-3-oxo-1*H*-1,4-diazepine (DAP). In addition to the predefined chiralities of the Cαs, one can diversify the structure by introducing a wide range

(22) Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Salvino, J.; Leahy, E. M.; Sprengeler, P. A.; Furst, G.; Smith, A. B.; Strader, C. D.; Cascieri, M. A.; Candelore, M. R.; Donaldson, C.; Vale, W.; Maechler, L. *J. Am. Chem. Soc.* **1992**, *114*, 9217.
(23) Chorev, M.; Roubini, E.; Gilon, C.; Selinger, Z. *Biopolymers* **1991**, *31*, 725.
(24) Romer, D.; Buscher, H. H.; Hill, R. C.; Maurer, R.; Petcher, T. J.; Zeugner, H.; Benson, W.; Finner, E.; Milkowski, W.; Thies, P. W. *Nature (London)* **1982**, *298*, 759.
(25) Chang, R. S. L.; Lotti, V. J.; Monaghan, R. L.; Birnbaum, J.; Stapley, E. O.; Goetz, M. A.; Albers-Schonberg, G.; Patchett, A. A.; Liesch, J. M.; Hensens, O. D.; Springer, J. P. *Science* **1985**, *230*, 177.
(26) Evans, B. E.; Rittle, K. E.; Bock, M. G.; DiPardo, R. M.; Freidinger, R. M.; Whitter, W. L.; Gould, N. P.; Lundell, G. F.; Homnick, C. F.; Veber, D. F.; Anderson, P. S.; Chang, R. S. L.; Lotti, V. J.; Cerino, D. J.; Chen, T. B.; King, P. J.; Kunkel, K. A.; Springer, J. P.; Hirshfield, J. *J. Med. Chem.* **1987**, *30*, 1229.
(27) Evans, B. E.; Rittle, K. E.; Bock, M. G.; DiPardo, R. M.; Freidinger, R. M.; Whitter, W. L.; Lundell, G. F.; Veber, D. F.; Anderson, P. S.; Chang, R. S. L.; Lotti, V. J.; Cerino, D. J.; Chen, T. B.; Kling, P. J.; Kunkel, K. A.; Springer, J. P.; Hirshfield, J. *J. Med. Chem.* **1988**, *31*, 2235.
(28) Bock, M. G.; DiPardo, R. M.; Evans, B. E.; Rittle, K. E.; Whitter, W. L.; Veber, D. F.; Anderson, P. S.; Freidinger, R. M. *J. Med. Chem.* **1989**, *32*, 13.
(29) Bock, M. G.; DiPardo, R. M.; Newton, R. C.; Bergman, J. M.; Veber, D. F.; Freedman, S. B.; Smith, A. J.; Chapman, K. L.; Patel, S.; Kemp, J. A.; Marshall, G. R.; Freidinger, R. M. *Bioorg. Med. Chem.* **1994**, *2*, 987.
(30) Ku, T. W.; Ali, F. E.; Barton, L. S.; Bean, J. W.; Bondinell, W. E.; Burgess, J. L.; Callahan, J. F.; Calvo, R. R.; Chen, L.; Eggleston, D. S.; Gleason, J. G.; Huffman, W. F.; Hwang, S.-M.; Jakas, D. R.; Karash, C. B.; Keenan, R. M.; Kopple, K. D.; Miller, W. H.; Newlander, K. A.; Nichols, A.; Parker, M. F.; Peishoff, C. E.; Samanen, J. M.; Uzinskas, I.; Venslavsky, J. W. *J. Am. Chem. Soc.* **1993**, *115*, 8861.
(31) Bondinell, W. E.; Keenan, R. M.; Miller, W. H.; Ali, F. E.; Allen, A. C.; De Brosse, C. W.; Eggleston, D. S.; Erhard, K. F.; Haltiwanger, R. C.; Huffman, W. F.; Hwang, S.-M.; Jakas, D. R.; Koster, P. F.; Ku, T. W.; Lee, C. P.; Nichols, A. J.; Ross, S. T.; Samanen, J. M.; Valocik, R. E.; Vasko-moser, J. A.; Venslavsky, J. W.; Wong, A. S.; Yuan, C.-K. *Bioorg. Med. Chem.* **1994**, *2*, 897.
(32) Hsu, M.-C.; Schutt, A. D.; Holly, M.; Slice, L. W.; Sherman, M. I.; Richman, D. D.; Potash, M. J.; Volsky, D. J. *Science* **1991**, *254*, 1799.
(33) James, G. L.; Goldstein, J. L.; Brown, M. S.; Rawson, T. E.; Somers, T. C.; McDowell, R. S.; Crowley, C. W.; Lucas, B. K.; Levinson, A. D.; Marsters, J. C., Jr. *Science* **1993**, *260*, 1937.
(34) Marsters, J. C., Jr.; McDowell, R. S.; Reynolds, M. E.; Oare, D. A.; Somers, T. C.; Stanley, M. S.; Rawson, T. E.; Struble, M. E.; Burdick, D. J.; Chan, K. S.; Duarte, C. M.; Paris, K. J.; Tom, J. Y. K.; Wan, D. T.; Xue, Y.; Burnier, J. P. *Bioorg. Med. Chem.* **1994**, *2*, 949.
(35) Pauwels, R.; Andries, K.; Desmyter, J.; Schols, D.; Kukla, M. J.; Breslin, H. J.; Raeymaeckers, A.; Van Gelder, J.; Woestenborghs, R.; Heykants, J.; Schellekens, K.; Janssen, M. A. C.; De Clercq, E.; Janssen, P. A. J. *Nature* **1990**, *343*, 470.
(36) Isono, K.; Uramoto, M.; Kusakabe, H.; Kimura, K.-I.; Izaki, K.; Nelson, C. C.; McCloskey, J. A. *J. Antibiot.* **1985**, *38*, 1617.
(37) Ubukata, M.; Isono, K.; Kimura, K.-I.; Nelson, C. C.; McCloskey, J. A. *J. Am. Chem. Soc.* **1985**, *110*, 4416.
(38) Knapp, S.; Nandan, S.; Resnick, L. *Tetrahedron Lett.* **1992**, *33*, 5485.
(39) Spada, M. R.; Ubukata, M.; Isono, K. *Heterocycles* **1992**, *34*, 1147.
(40) Moore, W. J.; Luzzio, F. A. *Tetrahedron Lett.* **1995**, *36*, 6599.
(41) Kim, K. S.; Cho, I. H.; Ahn, Y. H.; Park, J. I. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1783.
(42) (a) Chen, C.; Ahlberg Randall, L. A.; Miller, R. B.; Jones, A. D.; Kurth, M. J. *J. Am. Chem. Soc.* **1994**, *116*, 2661. (b) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385. (c) DeWitt, S. H.; Kiely, J. S.; Stankovic, C. J.; Schroeder, M. C.; Reynolds Cody, D. M.; Pavia, M. R. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6909.

Scheme 3. Diastereomeric Pairs of DAP Model Compounds and Their Linear Precursors



of substituents on C-2, as well as expand the ring size by employing amino acid residues with longer side chains in position *i* (see **I**, Scheme 1).

The synthesis of the model DAP systems **5SS-** and **5RS-F**/**A** (Scheme 3) was based on the readily available N-protected homoserine lactone (**1S**) which functions as an *in situ*-activated precursor. Dimethylamine-mediated ring opening generates a primary alcohol which can be directly oxidized to the corresponding aldehyde (Scheme 4). Blocking the carboxyl of the homoserine as an *N*,*N*-dimethylcarboxamide eliminated the favored and spontaneous γ-lactonization. Acetylation of the newly formed secondary amine (N_{i-1}) prevented potential side reactions during the lactamization step. The conformational equivalence of enantiomers *RS* to *SR* and *SS* to *RR* reduced the synthetic effort to a single representative of each pair, namely, **5SS-F**/**A** and **5RS-F**/**A**. The choice of alanyl and phenylalanyl as the (i − 1) amino acid residues (see **I**, Scheme 1) incorporated into the model DAP compounds was aimed to compare the effect of a methyl versus a benzyl side chain on conformational preferences of this system.[43]

Oxidation of the methylol (**2S**) to the corresponding aldehyde was carried out by pyridinium chlorochromate (PCC) in the presence of silica gel.[44] Monitoring the progress of the oxidation by RP-HPLC reveals the formation of a more hydrophilic minor side product. Amino acid analysis and FAB-MS (not shown) suggest the formation of an ester generated by the condensation of the β-carboxyl in Boc-Asp-NMe_2 (formed by overoxidation of the aldehyde) with the methylol of Boc-Hse-NMe_2 (**2S**).

We preferred to carry out the more demanding reductive alkylation first. The preformed N-protected α-amino-γ-oxo-*N*,*N*-dimethylbutyramide, the (*i*) amino acid residue, was reductively alkylated[45] by the free α-amino function of the (i − 1) amino acid residue prior to the lactam formation (Scheme 4). Acetylation of the newly formed secondary amines (**3SS-F**, **3RS-F**, **3SS-A**, and **3RS-A**, Schemes 3 and 4) results in a mixture of isomers (in each of the **4SS-F**, **4RS-F**, **4SS-A**, and **4RS-A**, see Schemes 3 and 4), generated by the *cis*−*trans* isomerization around the acetylated tertiary amide bond, which reveals itself in the ^{1}H-NMR as two sets of extensively overlapping signals.

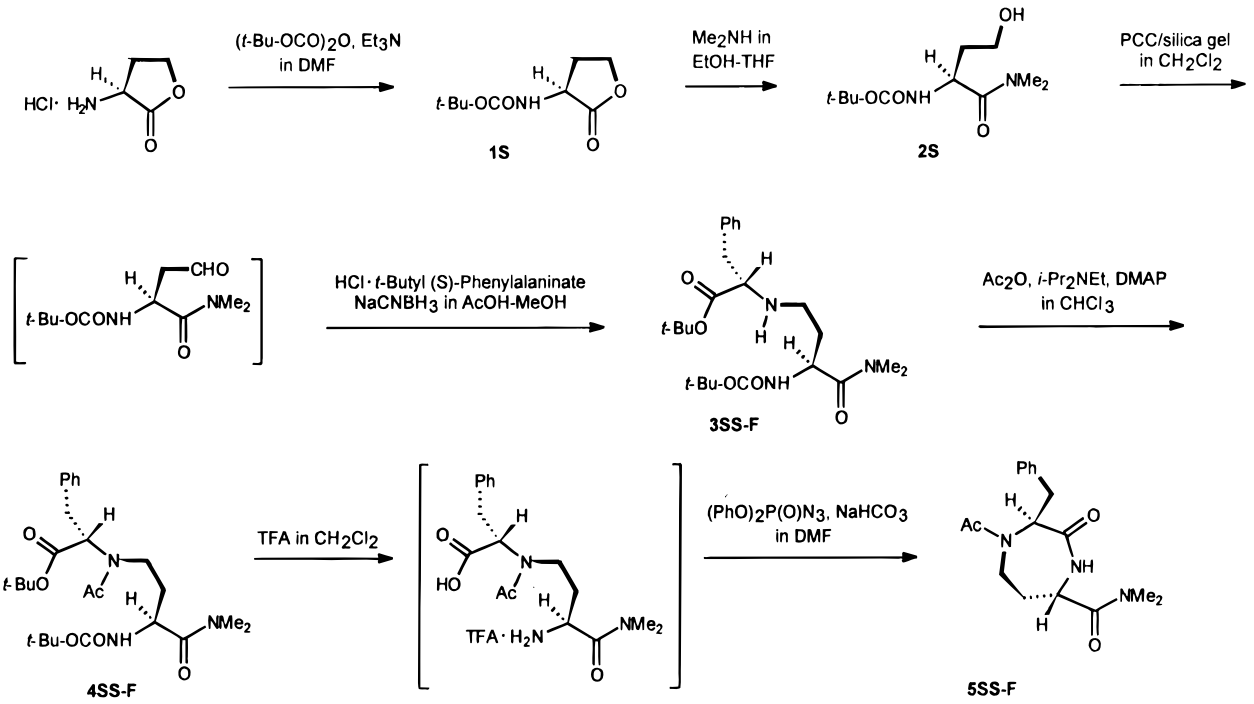
Cyclization of the deprotected linear precursors was carried out in DMF at high dilution and was mediated by diphenyl phosphorazidate (DPPA) in the presence of $NaHCO_3$.[46] This procedure resulted in better yields of the DAP model systems (**5SS-F**, **5RS-F**, **5SS-A** and **5RS-A**, see Scheme 3) than cyclization mediated by (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) in the presence of DIPEA.[46] In the latter case, monitoring the reaction by analytical RP-HPLC revealed presence of starting material even after three days.

NMR characterization of the four model DAP analogs, 5SS-F/A and 5RS-F/A. The protons of the DAP analogs were assigned through 2D, homonuclear, ^{1}H−^{1}H, DQF COSY, and TOCSY experiments, which display spin−spin coupling ($^3J_{H-H}$) of the protons, and ROESY and NOESY spectra, which illustrate the dipole−dipole interactions between pairs of protons closer than 5.0 Å from one another. The HMQC then allowed for the assignment of all the carbons with directly attached protons. The HMBC experiment, which illustrates long-range ^{1}H−^{13}C ($^3J_{H-C}$) couplings, allowed for the correlation of all the protons with carbon atoms three bonds removed. Therefore, the constitution of the molecule, the presence of all of the covalent bonds, was unambiguously proven. The ^{13}C assignments are an important confirmation of the ^{1}H alignments obtained from the homonuclear experiments described above. In addition, the HMBC experiment allowed for the diastereotopic assignment of the methylene protons of the ring system and the methylene of phenylalanine. In both diastereomeric pairs (**5SS** and **5RS**) *cis* isomer (around the Ac-N-1) is the predominant one. These assignments were important for the conformational investigation of the fully blocked DAP system (N-1 acetylated and C-5 *N*,*N*-dimethylcarboxy amidated) as described in detail elsewhere.[43]

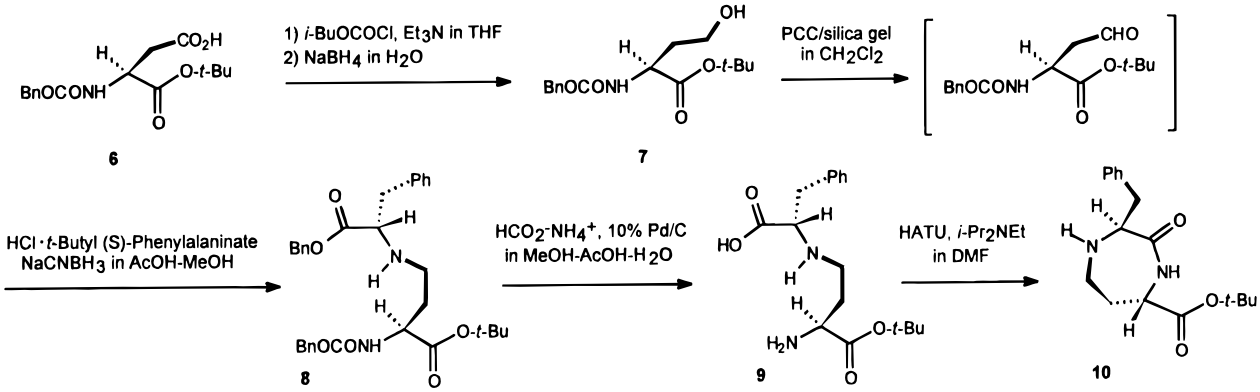
Synthesis of a DAP Synthon. To be widely used as a dipeptidomimetic unit or as a molecular scaffold, a protected DAP synthon that can be readily incorporated into peptides or substituted selectively by pharmacophores of choice is required. Therefore, an alternative synthetic route was developed to generate a protected DAP synthon that could serve as a building block (Scheme 5). Orthogonally protected and commercially available, 1-*tert*-butyl hydrogen *N*-(benzyloxycarbonyl)-L-aspartate (Z-L-Asp-O-*t*-Bu) (**6**) was used as the starting material. The bulky *tert*-butyl ester blocks the spontaneous lactonization of the N-protected L-homoserine ester **7**, obtained upon $NaBH_4$-mediated reduction of the mixed carbonic−carboxyl acids anhydride, generated *in situ*. Oxidation of the methylol moiety to the corresponding aldehyde and the subsequent reductive alkylation to yield the secondary amine **8** were carried out in an identical manner as previously described for the DAP model compounds (**5SS-F/A** and **5RS-F/A**). In an attempt to develop a more general synthesis of DAP synthons, we

(43) Pellegrini, M.; Weitz, I. S.; Chorev, M.; Mierke, D. F. J. *Am. Chem. Soc.* **1997**, *43*, 2430.
(44) Adams, L. L.; Luzzio, F. A. *J. Org. Chem.* **1989**, *54*, 5387.
(45) Guichard, G.; Benkirane, N.; Graff, R.; Muller, S.; Briand, J. *Pept. Res.* **1994**, *7*, 308.
(46) (a) Brady, S. F.; Varga, S. L.; Freidinger, R. M.; Schwenk, D. A.; Mendlowski, M.; Holly, F. W.; Veber, D. F. *J. Org. Chem.* **1979**, *44*, 3101. (b) Lyle, T. A.; Freidinger, R. M.; Nutt, R. F.; Honnick, C. F.; Saperstein, R.; Veber, D. F. *Int. J. Pept. Protein Res.* **1987**, *29*, 244. (c) Boger, D. L.; Yohannes, D. *J. Org. Chem.* **1990**, *55*, 6000. (d) Said-Nejad, O. E.; Felder, E. R.; Mierke, D. F.; Yamazaki, T.; Schiller, P. W.; Goodman, M. Int. *J. Pept. Protein Res.* **1992**, *39*, 145.

Scheme 4. Representative Synthesis of the Model DAP Compound 5SS-F



Scheme 5. Synthesis of the DAP Synthon 10



avoided the introduction of a third protecting group, on the secondary amino function, orthogonal to the *N*-benzyloxycarbonyl and the *O*-*tert*-butyl ester. This third orthogonal protecting group will be necessary for the cases in which the $i - 1$ amino acid residue possesses a side chain with a functional group that requires protection. Apparently, the new secondary amine is neither reactive enough[47] nor favorably positioned to compete with the cyclization that generates the targeted DAP system.

Catalytic transfer hydrogenation[48] removed the *N*-benzyloxycarbonyl and the benzyl ester from fully protected compound **8** and yielded the partially protected linear precursor **9** as an acetate salt. The latter was transformed into a trifluoroacetate by several lyophilization cycles of a diluted trifluroacetic acid solution. The cyclization of the trifluoroacetate **9** to obtain the *tert*-butyl ester of the DAP synthon **10** was achieved with 1-hydroxy-7-azabenzotriazolyl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU)[49] in highly diluted solution of DMF. Interestingly, DPPA was less effective than HATU as a cyclization reagent. The absence of a protecting group on the secondary amine and the presence of a bulky *tert*-butyl ester group in **9** may explain the lower yields of **10** as compared to the similar DPPA-mediated cyclization leading to **5**, the model DAPs. These structural differences may result in the very slow cyclization rate in the presence of DPPA and the appearance of several side products (as observed by analytical RP-HPLC, not shown).

Two of the syntheses of the closely related 1,4-diazepan-3-one system (**III**, Scheme 1) employed reductive cyclization as the last step.[38,39] A more recent synthetic route[41] utilized a similar approach to those reported by us. In their synthesis the secondary amine is formed by nucleophilic opening of an epoxide ring by sarcosine methyl ester. Deprotection followed by condensation cyclization yields the anticipated **III** in a significantly shorter and more efficient synthetic route than previously reported.[38,39]

Interestingly, one of our initial attempts to synthesize the model DAP compounds involved an intramolecular

(47) (a) Sasaki, Y.; Coy, D. H. *Peptides* **1987**, *8*, 119. (b) Ron, D.; Laufer, R.; Frey, J.; Gilon, C.; Selinger, Z.; Chorev, M. In *Substance P and Neurokinins*; Henry, J. L., Coutre, R., Cuello, A. C., Pelletier, G., Quirion, R., Regoli, D., Eds.; Springer-Verlag: New York, 1987; pp 144−145.

(48) Anwer, M. K.; Spatola, A. F. *Synthesis* **1980**, 929.

(49) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.

N-alkylation of a urethane NH by the sulfonium methiodide Met(S^+Me)I^- in Boc-Phe-Met[(S^+Me)I^-]-NMe_2; this scheme failed because of a favorable cyclopropyl ring formation (data not shown).[50] A similar approach when applied to the synthesis of the 1,4-diazepan-3-one system (**III**, Scheme 1) led to an azetidine ring formation.[41]

Conclusion

We report the synthesis of a trisubstituted 1,2,5-hexahydro-3-oxo-1*H*-1,4-diazepine (DAP) system, starting from readily available amino acid derivatives to obtain fully blocked model diastereomers (**5SS-F**/**A** and **5RS-F**/**A**) and a representative synthon **10**. The synthetic scheme developed for the synthon is general and could be conveniently applied to construct higher homologs of the DAP system in which the C_i-to-N_{i-1} bridging chain could be larger than the currently present ethylene moiety. The representative synthon is designed to be used as a novel molecular scaffold, presenting the substituting pharmacophores in a specific molecular topology, or as a novel dipeptido-conformation-mimetic unit, which can be readily incorporated into peptides. The potential chiral and substitutional diversification featured in the DAP system offers novel opportunities in peptidomimetic drug design.

Experimental Section

General Methods. Thin layer chromatography (TLC) was performed on plates coated with a 0.20 mm thickness of silica gel 60 F-254 (Merck). Visualization was accomplished by UV light, 4% (w/v) ninhydrin in ethanol, 0.1% (w/v) fluorescamine in acetone, or in the presence of iodine vapors. Gravity column chromatography was carried out on silica gel 60 (70−230 mesh) columns (2.5 × 35 cm) (Merck). Final organic solutions were dried over $MgSO_4$, filtered, and rotary evaporated *in vacuo*. Analytical reversed-phase high performance liquid chromatography (RP-HPLC) was carried out on a Lichrospher 100 RP-18 column (5 μm, 4 × 250 mm) (Merck) at a flow-rate of 1 mL/min. Semipreparative RP-HPLC was carried out on either μBondapak RP-18 column (10 μm, 19 × 150 mm) (Waters) or Vydac 300 Å C-18 (10 μm, 22 × 250 mm) at a flow-rate 6 mL/min. The solvent system used in RP-HPLC included the following: solvent A, 0.1% (v/v) TFA in CH_3CN; solvent B, 0.1% (v/v) TFA in H_2O. The effluent was monitored at 220 nm. ^{1}H- and ^{13}C-NMR spectra were collected at 300, 400, and 500 MHz instruments. Chemical shifts (δ) are reported in ppm. ^{1}H resonances were calibrated using the 7.24 ppm $CDCl_3$ or 4.8 ppm D_2O resonances of the solvents as internal standards. ^{13}C resonances were referenced using the $CDCl_3$ resonance of the solvent (77 ppm) as an internal standard unless noted otherwise. The coupling constants in hertz were measured from one-dimensional spectra unless otherwise stated. The chemical shift assignment was carried out by double-quantum filtered correlation (DQFCOSY) and heteronuclear correlation (HMQC, HMBC) experiments unless otherwise stated. Melting points were determined with a capillary melting point apparatus and are uncorrected. Elementary microchemical analysis was carried out by Dr. S. Blum at the Microanalytical Laboratory of Organic Chemistry Department in the Hebrew University. Analytical results were within 0.3% of the theoretical values. Mass spectroscopy was performed at the Mass Spectra Laboratory of Chemistry Department at the Harvard University (Cambridge, MA), using a FAB ion source.

N,*N*′-Dimethylformamide (DMF) was distilled under reduced pressure over ninhydrin. EtOAc was distilled over $CaCl_2$, and dichloromethane (DCM) was distilled over sodium wire. Other commercially available chemicals are of the best grade and were used as received. Catalytic hydrogenations were carried out in the presence of 10% Pd/C (Merck).

***N*-Boc-L-homoserine Lactone (1S).** An ice-cold suspension of L-homoserine lactone HCl (Sigma) (4.68 g, 34 mmol) in DMF (57 mL) was treated with triethylamine (TEA) (5.1 mL. 37 mmol) and di-*tert*-butyl dicarbonate (Boc_2O) (8.7 mL, 41 mmol), and allowed to warm-up to room temperature (rt). Upon completion of reaction (determined by negative fluorescamine test), the solvent was removed *in vacuo* and the residue was taken-up in DCM (200 mL) and washed consecutively with 1 N $KHSO_4$ (3 × 200 mL) and brine (1 × 200 mL). The organic phase was dried over $MgSO_4$ and the solvent removed *in vacuo*. Recrystallization of the solid residue from EtOAc/petroleum ether afforded 5.19 g of a white powder (76% yield): $R_f = 0.71$ (acetone−petroleum ether / 1:1); mp 125−126 °C (lit. 125−126.5 °C[51]); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.01 (1H, brs), 4.44 (1H, dt J = 0.9, 9.00 Hz), 4.35 (1H, brs), 4.24 (1H, ddd J = 11.4, 9.3, 5.7 Hz), 2.74 (1H, m), 2.19 (1H, m), 1.45 (9H, s). Anal. Calcd for $C_9H_{15}N_1O_4$: C, 53.72; H, 7.51; N, 6.96. Found: C, 53.52; H, 7.59; N, 7.10.

2(*S*)-[(Butyloxycarbonyl)amino]-4-hydroxy-*N*,*N*-dimethylbutyramide (2S). To a solution of *N*-(*tert*-butyloxycarbonyl)-L-homoserine lactone (**1S**) (3.22 g, 16 mmol) in dry THF (80 mL) (Aldrich) was added 33% (v/v) dimethylamine in ethanol (8.6 mL, 48 mmol). After stirring over night at rt the solvents were removed *in vacuo*, and the product was dried over P_2O_5 to give 3.94 g of a clear oil (100% yield): $R_f = 0.41$ (acetone − petroleum ether/1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.70 (1H, d, J = 9.0 Hz, N*H*), 4.72 (1H, ddd, J = 9.0, 3.0, 12.0 Hz, C*H*), 3.68 (3H, m, CH_2CH_2OH, O*H*), 3.05 (3H, s, CH_3N), 2.94 (3H, s, CH_3N), 1.89 (1H, m, CH_2CH_2OH), 1.45 (1H, m, CH_2CH_2OH), 1.42 (9H, s, CH_3 *t*-Bu). ^{13}C NMR (300 MHz, $CDCl_3$) δ 172.6 (CO), 157.5 (CO), 80.8 (*C*-*t*-Bu), 58.6 (CH_2CH_2-OH), 47.9 (*C*H), 37.7 (NCH_3), 37.0 ($CHCH_2$), 36.4 (NCH_3), 29.0 (CH_3*t*-Bu). Anal. Calcd for $C_{11}H_{22}N_2O_4$: C, 53.64; H, 9.00; N, 11.37. Found: C, 53.70; H, 9.14; N, 11.24.

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-4-[[2-phenyl-1(*S*)-(*tert*-butyloxycarbonyl)ethyl]amino]-*N*,*N*-dimethylbutyramide (3SS-F). A solution of the alcohol (**2S**) (0.99 g, 4 mmol) in dry DCM (10 mL) was added to a suspension of pyridinium chlorochromate (PCC) (3.45 g, 16 mmol) and silica gel 60 (70−230 mesh, 3.45 g) in dry DCM (10 mL). The reaction was monitored by TLC $R_f = 0.59$ (acetone−petroleum ether/1:1) and terminated after 2.5 h by the addition of diethyl ether (20 mL). The reaction mixture was filtered through a Celite bed, and the filtrate was concentrated *in vacuo* to afford the crude aldehyde which was carried to the next step without further purification.

To a mixture of a solution of the crude *N*,*N*-dimethyl-2(*S*)-[(butyloxycarbonyl)amino]-4-oxobutyramide in 1% (v/v) AcOH in MeOH (7 mL) and molecular sieves Type 3A (8−12 mesh) pellets (Aldrich) (∼2 g) was added a solution of *tert*-butyl L-phenylalaninate hydrochloride (0.36 g, 1.4 mmol) in MeOH (2 mL). After 5 min, $NaCNBH_3$ (0.19 g, 3.0 mmol) was added in two aliquots. After 3 h of stirring at rt the reaction mixture was filtered, and the filtrate was concentrated *in vacuo*. The residue was treated with saturated solution of $NaHCO_3$ (75 mL) and extracted with ether (4 × 75 mL). The combined ethereal extracts were dried over $MgSO_4$, and the solvent was removed *in vacuo*. Gravity column chromatography on silica gel 60 eluting with a step gradient of 20−33% (v/v) acetone in petroleum ether afforded 0.34 g of a clear oil as a product (54% yield, based on *tert*-butyl phenylalaninate hydrochloride): R_f = 0.35 (acetone − petroleum ether/1:3); k' = 5.3 (30−75% (v/v) A in B in 30 min); $[\alpha]^{25}_D = -1.2$ (c = 1.295, methanol); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29−7.19 (5H, m, Ar), 5.48 (1H, d, J = 8.4 Hz, N*H* carbamate), 4.70 (1H, dd, J = 8.4, 13.2 Hz, CH_2CH_2CH), 3.33 (1H, t, J = 7.2 Hz, $CHCH_2Ph$), 2.95 (3H, s, CH_3N), 2.93 (3H, s, CH_3N), 2.86 (2H, dd, J = 7.2, 2.0 Hz, $CHCH_2Ph$), 2.76 (1H, m, CH_2CH_2CH), 2.42 (1H, m, CH_2CH_2-CH), 1.72 (1H, m, CH_2CH_2CH), 1.61 (1H, m, CH_2CH_2CH), 1.46 (9H, s, CH_3 *t*-Bu), 1.36 (9H, s, CH_3 *t*-Bu). ^{13}C NMR (400 MHz, $CDCl_3$) δ 174.6 (CO), 173.0 (CO), 156.2 (CO), 138.4 (C Ar),

(50) Rich, D. H.; Tam, J. P. *Synthesis* **1978**, 46.

(51) Baldwin, J. E.; Flinn, A. *Tetrahedron. Lett.* **1987**, *28*, 3605.

130.1 (*C*H Ar), 128.9 (*C*H Ar), 127.1 (*C*H Ar), 81.8 (*C*-*t*-Bu), 80.1 (*C*-*t*-Bu), 64.4 (*C*HCH$_2$Ph), 49.0 (CH$_2$CH$_2$*C*H), 44.4 (*C*H$_2$-CH$_2$CH) 40.6 (CH*C*H$_2$Ph), 37.5 (*C*H$_3$N), 36.3 (*C*H$_3$N), 34.6 (CH$_2$*C*H$_2$CH), 29.1 (*C*H$_3$ *t*-Bu), 28.7 (*C*H$_3$ *t*-Bu); FAB-MS Calcd for $C_{24}H_{39}N_3O_5$: 449.592, found: m/z = 450 (M + H)$^+$. Anal. Calcd for $C_{24}H_{39}N_3O_5 \cdot H_2O$: C, 61.65; H, 8.84; N, 8.99. Found: C, 61.87; H, 9.00; N, 9.12.

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-4-[[2-phenyl-1(*R*)-(*tert*-butyloxycarbonyl)ethyl]amino]-*N*,*N*-dimethylbutyramide (3RS-F). Preparation of this diastereomer followed the procedure described for (**3SS-F**) except for employing *tert*-butyl D-phenylalaninate hydrochloride. The pure product was obtained as a clear oil (50% yield): R_f = 0.35 (acetone−petroleum ether/1:3); k' = 5.3 (30−75%(v/v) A in B in 30 min); $[\alpha]^{25}_D$ = −7.2 (c = 0.815, methanol); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.28−7.18 (5H, m, Ar), 5.54 (1H, d, J = 9.0 Hz, NH carbamate), 4.68 (1H, ddd, J = 9.0, 4.2, 9.6 Hz, CH$_2$CH$_2$C*H*), 3.41 (1H, t, J = 6.9 Hz, C*H*CH$_2$Ph), 3.03 (3H, s, C*H*$_3$N), 2.93 (3H, s, C*H*$_3$N), 2.88 (2H, m, CHC*H*$_2$Ph), 2.70 (1H, m, C*H*$_2$CH$_2$-CH), 2.57 (1H, m, C*H*$_2$CH$_2$CH), 1.68 (2H, m, CH$_2$C*H*$_2$CH), 1.42 (9H, s, C*H*$_3$ *t*-Bu), 1.32 (9H, s, C*H*$_3$ *t*-Bu). ^{13}C NMR (400 MHz, CDCl$_3$) δ 174.56 (CO), 172.9 (CO), 156.3 (CO), 138.1 (*C*-Ar), 138.1 (*C*H-Ar), 128.9 (*C*H-Ar), 127.2 (*C*H-Ar), 81.7 (*C*-*t*-Bu), 80.1 (*C*-*t*-Bu), 64.0 (*C*HCH$_2$Ph), 49.2 (CH$_2$CH$_2$*C*H), 44.6 (*C*H$_2$-CH$_2$CH), 40.5 (CH*C*H$_2$Ph), 37.7 (*C*H$_3$N), 36.4 (*C*H$_3$N), 34.6 (CH$_2$*C*H$_2$CH), 29.0 (*C*H$_3$ *t*-Bu), 28.7 (*C*H$_3$ *t*-Bu); FAB-MS calcd for $C_{24}H_{39}N_3O_5$: 449.592, found: m/z = 450 (M + H)$^+$. Anal. Calcd for $C_{24}H_{39}N_3O_5$: C, 64.12; H, 8.74; N, 9.35. Found: C, 64.38; H, 8.77; N, 9.63.

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-4-[[1(*S*)-(*tert*-butyloxycarbonyl)ethyl]amino]-*N*,*N*-dimethylbutyramide (3SS-A). Preparation followed the procedure described for **3SS-F** except for employing *tert*-butyl L-alaninate hydrochloride. Gravity chromatography on a silica gel 60 column using step gradient of 66−80% acetone in petroleum ether as eluent afforded a clear oil (47% yield): R_f = 0.55 (acetone−petroleum ether/4:1); k' = 6.1 (15−55% (v/v) A in B in 30 min); $[\alpha]^{25}_D$ = −23.4 (c = 0.937, methanol); ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.49 (1H, d, J = 8.4 Hz, NH carbamate), 4.70 (1H, ddd, J = 8.4, 4.8, 8.4 Hz, CH$_2$CH$_2$C*H*), 3.12 (1H, q, J = 6.9 Hz, C*H*CH$_3$), 3.06 (3H, s, C*H*$_3$N), 2.91 (3H, s, C*H*$_3$N), 2.71 (1H, m, C*H*$_2$CH$_2$CH), 2.41 (1H, m, C*H*$_2$CH$_2$CH), 1.76 (1H, m, CH$_2$C*H*$_2$CH), 1.62 (1H, m, CH$_2$C*H*$_2$CH), 1.41 (9H, s, C*H*$_3$ *t*-Bu), 1.38 (9H, s, C*H*$_3$ *t*-Bu), 1.18 (3H, d, J = 6.9 Hz, CHC*H*$_3$). ^{13}C NMR (300 MHz, CDCl$_3$) δ 175.6 (CO), 172.9 (CO), 156.2 (CO), 81.5 (C), 80.1 (C), 57.9 (*C*HCH$_3$), 49.1 (CH$_2$CH$_2$*C*H), 44.3 (*C*H$_2$-CH$_2$CH), 37.6 (*C*H$_3$N), 36.3 (*C*H$_3$N), 34.5 (CH$_2$*C*H$_2$CH), 29.0 (*C*H$_3$ *t*-Bu), 28.7 (*C*H$_3$ *t*-Bu), 19.7 (CH*C*H$_3$); FAB-MS calcd for $C_{18}H_{35}N_3O_5$: 373.494, found: m/z = 374 (M + H)$^+$. Anal. Calcd for $C_{18}H_{35}N_3O_5$: C, 57.89; H, 9.45; N, 11.25. Found: C, 57.79; H, 9.66; N, 11.45.

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-4-[[1(*R*)-(*tert*-butyloxycarbonyl)ethyl]amino]-*N*,*N*-dimethylbutyramide (3RS-A). This compound was prepared following the procedure described for **3SS-F** except for employing *tert*-butyl D-alaninate hydrochloride. Gravity chromatography was performed on a silica gel 60 column using step gradient of 66−80% (v/v) acetone in petroleum ether as eluent afforded a clear oil (43% yield): R_f = 0.55 (acetone− petroleum ether/4:1); k' = 6.1 (15−55%(v/v) A in B); $[\alpha]^{25}_D$ = +10.7 (c = 0.475, methanol); ^{1}H NMR (400 MHz, CDCl$_3$) δ 5.56 (1H, d, J = 8.4 Hz, NH carbamate), 4.71 (1H, ddd, J = 8.4, 4.4, 8.4 Hz, CH$_2$-CH$_2$C*H*), 3.20 (1H, q, J = 6.8 Hz, C*H*CH$_3$), 3.08 (3H, s, C*H*$_3$N), 2.94 (3H, s, C*H*$_3$N), 2.69−2.55 (2H, m, C*H*$_2$CH$_2$CH), 1.81 (1H, m, CH$_2$C*H*$_2$CH), 1.64 (1H, m, CH$_2$C*H*$_2$CH), 1.45 (9H, s, C*H*$_3$ *t*-Bu), 1.41 (9H, s, C*H*$_3$ *t*-Bu), 1.23 (3H, d, J = 6.8 Hz, CHC*H*$_3$). ^{13}C NMR (300 MHz, CDCl$_3$) δ 175.8 (CO), 173.0 (CO), 156.3 (CO), 81.5 (C), 80.1 (C), 58.2 (*C*HCH$_3$), 49.3 (CH$_2$CH$_2$*C*H), 44.5 (*C*H$_2$CH$_2$CH), 37.7 (*C*H$_3$N), 36.4 (*C*H$_3$N), 34.7 (CH$_2$*C*H$_2$CH), 29.1 (*C*H$_3$ *t*-Bu), 28.8 (*C*H$_3$ *t*-Bu), 19.8 (CH*C*H$_3$); FAB-MS calcd for $C_{18}H_{35}N_3O_5$: 373.494, found: m/z = 374 (M + H)$^+$. Anal. Calcd for $C_{18}H_{35}N_3O_5$: C, 57.89; H, 9.45; N, 11.25. Found: C, 57.69; H, 9.67; N, 11.02.

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-4-[*N*-[2-phenyl-1(*S*)-(*tert*-butyloxycarbonyl)ethyl]-*N*-acetylamino]-*N*,*N*-dimethylbutyramide (4SS-F). A solution of **3SS-F** (140 mg, 0.32 mmol) in $CHCl_3$ (1.35 mL) was treated with *N*,*N*-diisopropylethylamine (DIPEA) (56 μL, 0.32 mmol), Ac_2O (60 μL, 0.64 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) (78 mg, 0.32 mmol) and was left to stir overnight. The residue obtained following removal of solvent *in vacuo* was dissolved in EtOAc (70 mL), and washed successively with cold 1 N $KHSO_4$ (3 × 50 mL), 5%(w/v) $NaHCO_3$ (3 × 50 mL), and brine (2 × 50 mL), and dried over $MgSO_4$, and the solvent was removed *in vacuo* to afford a yellow oil: (130 mg, 83% yield); k' = 6.4 (30−75% (v/v) A in B in 30 min); FAB-MS calcd for $C_{26}H_{41}N_3O_6$: 491.629, found: m/z = 492 (M + H)$^+$. Anal. Calcd for $C_{26}H_{41}N_3O_6$: C, 63.52; H, 8.41; N, 8.55. Found: C, 63.38; H, 8.61; N, 8.34.

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-4-[*N*-[2-phenyl-1(*R*)-(*tert*-butyloxycarbonyl)ethyl]-*N*-acetylamino]-*N*,*N*-dimethylbutyramide (4RS-F). The N-acetylation of **3RS-F** was carried out in the same manner as that of **4SS-F**. The product was obtained as a yellow oil: 84% yield; k' = 6.6 (30−75% (v/v) A in B in 30 min); FAB-MS calcd for $C_{26}H_{41}N_3O_6$: 491.629, found: m/z = 492 (M + H)$^+$. Anal. Calcd for $C_{26}H_{41}N_3O_6$: C, 63.52; H, 8.41; N, 8.55. Found: C, 63.32; H, 8.62; N, 8.35.

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-4-[*N*-[1(*S*)-(*tert*-butyloxycarbonyl)ethyl]-*N*-acetylamino]-*N*,*N*-dimethylbutyramide (4SS-A). The N-acetylation of **3SS-A** followed the procedure detailed above for **4SS-F**. The product was obtained as a yellow oil: 83% yield; k' = 7.6 (15−55%(v/v) A in B in 30 min); FAB-MS calcd for $C_{20}H_{37}N_3O_6$: 415.531, found: m/z = 416 (M + H)$^+$. Anal. Calcd for $C_{20}H_{37}N_3O_6$: C, 57.81; H, 8.98; N, 10.11. Found: C, 57.59; H, 9.17; N, 9.73.

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-4-[*N*-[1(*R*)-(*tert*-butyloxycarbonyl)ethyl]-*N*-acetylamino]-*N*,*N*-dimethylbutyramide (4RS-A). The *N*-acetylation of **3RS-A** followed the procedure detailed above for **4SS-F**. The product was obtained as a yellow oil: 80% yield; k' = 7.6 (15−55%(v/v) A in B in 30 min); FAB-MS calcd for $C_{20}H_{37}N_3O_6$: 415.531, found: m/z = 416 (M + H)$^+$. Anal. Calcd for $C_{20}H_{37}N_3O_6$: C, 57.81; H, 8.98; N, 10.11. Found: C, 57.89; H, 8.76; N, 10.10.

Hexahydro-1*H*-3-oxo-1-acetyl-2(*S*)-benzyl-5(*S*)-(*N*,*N*-dimethylcarbamoyl)-1,4-diazepine (5SS-F). An ice-cooled solution of **4SS-F** (0.15 g, 0.31 mmol) in DCM (2 mL) was treated with trifluoroacetic acid (TFA) (2 mL) for 30 min followed by 4 h at rt. The oily residue obtained after removal of solvent and TFA under *vacuo* was dried over KOH pellets overnight and yielded 2(*S*)-amino-4-[*N*-[2-phenyl-1(*S*)-carboxyethyl]-*N*-acetylamino]-*N*,*N*-dimethylbutyramide trifluorocaetate as a white powder: k' = 3.0 (15−55%(v/v) A in B in 30 min); FAB-MS calcd for $C_{17}H_{25}N_3O_4$: 335.404, found: m/z = 336 (M + H)$^+$. This trifluoroacetate salt was used in the next step without further purification.

To an ice-cold solution of the trifluroacetate salt in dry DMF (200 mL) were added $NaHCO_3$ (130 mg, 1.55 mmol) and diphenyl phosphorazidate (DPPA) (0.1 mL, 0.47 mmol). The reaction mixture was stirred at 4 °C overnight under nitrogen and monitored by analytical RP-HPLC. The ionic components were removed by batch treatment for 3 h at 4 °C with 1.5 g of Biorad AG 501-X8, mixed-bed ion-exchange resin. The resin was removed by filtration and washed three-times with DMF. The solvent from the combined filtrate and washes was removed *in vacuo*. The residue obtained was purified by semipreparative RP-HPLC (0−15% (v/v) A in B in 60 min on a μBondapak column), affording 78 mg of white powder (79% yield): k' = 3.6 (15−55%(v/v) A in B in 30 min); mp 129 °C; $[\alpha]^{25}_D$ = +65.5 (c = 0.832, methanol); ^{1}H NMR (500 MHz, CDCl$_3$) δ *cis* isomer (86%) 7.17−7.25 (5H, Ar), 6.63 (1H, N*H*), 4.79 (1H, C*H*CH$_2$Ph), 4.43 (1H, C*H*CH$_2$CH$_2$), 4.40 and 3.04 (2H, C*H*$_2$CH$_2$CH), 3.46 and 3.12 (2H, C*H*$_2$Ph), 3.00 and 2.94 (6H, N(C*H*$_3$)$_2$), 2.24 and 2.08 (2H, CH$_2$C*H*$_2$CH), 1.65 (3H, C*H*$_3$-CO); *trans* isomer (14%) 7.17−7.25 (5H, Ar), 6.79 (1H, N*H*), and 5.55 (1H, C*H*CH$_2$Ph).^{13}C NMR (500 MHz, CDCl$_3$) δ *cis* isomer (86%) 174.6 (*C*OCH$_3$), 171.6 (*C*ONH), 170.4 (*C*ON-(CH$_3$)$_2$), 129.0−136.7 (C- Ar), 65.4 (*C*HCH$_2$Ph), 52.4 (*C*HNH), 38.6 (NCH$_2$), 36.4 and 36.8 (N(*C*H$_3$)$_2$), 35.6 (CH$_2$Ph), 30 (CH$_2$*C*H$_2$CH), 20.6 (*C*H$_3$CO); *trans* isomer (14%) 36.4 and 36.8 (N(*C*H$_3$)$_2$). Detailed NMR assignment and conformational analysis are published elsewhere.[43] FAB-MS calcd for

$C_{17}H_{23}N_3O_3$: 317.389, found: m/z = 318 $(M + H)^+$. Anal. Calcd for $C_{17}H_{23}N_3O_3 \cdot {}^7/_4H_2O$: C, 58.52; H, 7.66; N, 12.04. Found: C, 58.53; H, 7.31; N, 11.57.

Hexahydro-1*H*-3-oxo-1-acetyl-2(*R*)-benzyl-5(*S*)-(*N*,*N*-dimethylcarbamoyl)-1,4-diazepine (5RS-F). Deprotection of **4RS-F** followed the procedure described above for **5SS-F** and afforded 2(*S*)-amino-4-[*N*-[2-phenyl-1(*R*)-carboxyethyl]-*N*-acetylamino]-*N*,*N*-dimethylbutyramide trifluoroacetate: k' = 3.0 (15−25% A in B); FAB-MS calcd for $C_{17}H_{25}N_3O_4$: 335.404, found: m/z 336 $(M + H)^+$. Cyclization of the TFA salt in a similar manner as detailed for **5SS-F** and semipreparative RP-HPLC purification ((0−15% A in B, 60 min, μBondapak) afforded a white powder (82% yield): k' = 4.1 (15−55% A in B); mp 192 °C; $[\alpha]^{25}_D = -53.8$ (c = 0.550, methanol); ^{1}H NMR (500 MHz, $CDCl_3$) δ *cis* isomer (73%) 7.14−7.24 (5H, Ar), 7.12 (1H, N*H*), 4.61 (1H, C*H*CH_2Ph), 4.24 (1H, C*H*CH_2CH_2), 4.21 and 2.99 (2H, C*H*$_2$$CH_2$CH), 3.67 and 2.97 (2H, C*H*$_2$Ph), 2.98 (6H, N(C*H*$_3$)$_2$), 2.55 and 1.70 (2H, CH_2CH_2CH), 1.49 (3H, C*H*$_3$-CO); *trans* isomer (27%) 7.14−7.24 (5H, Ar), 6.85 (1H, N*H*), and 5.46 (1H, C*H*CH_2Ph), 4.42 (1H, C*H*CH_2CH_2), 3.43 and 2.71 (2H, C*H*$_2$$CH_2$CH), 3.42 and 3.23 (2H, C*H*$_2$Ph), 2.93 (6H, N(C*H*$_3$)$_2$), 2.14 (3H, C*H*$_3$CO), 2.03 and 1.63 (2H, CH_2C*H*$_2$CH). ^{13}C NMR (500 MHz, $CDCl_3$) δ *cis* isomer (73%) 168.8 (*C*OCH_3), 168.8 (*C*ONH), 168.7 (*C*ON$(CH_3)_2$), 129.4−136.7 (C Ar), 64.6 (*C*HCH_2Ph), 48.6 (*C*HNH), 38.8 (N*C*H_2), 35.8 (N(*C*H$_3$)$_2$), 36.4 (*C*H_2Ph), 28.3 (CH_2*C*H2CH), 19.5 (*C*H$_3$CO); *trans* isomer (27%) 168.6 (*C*ON$(CH_3)_2$), 168.4 (*C*ONH), 137.1−130.0 (C Ar), 60.2 (*C*HCH_2Ph), 48 (*C*HNH), 42.8 (N*C*H_2), 35.8 (N(*C*H$_3$)$_2$), 34.4 (*C*H_2Ph), 30.2 (CH_2*C*H2CH), 20.8 (*C*H$_3$CO). Detailed NMR assignment and conformational analysis are published elsewhere.[43] FAB-MS Calcd for $C_{17}H_{23}N_3O_3$: 317.389 Found: m/z = 318 $(M + H)^+$. Anal. Calcd for $C_{17}H_{23}N_3O_3 \cdot H_2O$: C, 60.88; H, 7.51; N,12.53. Found: C, 60.77; H, 7.37; N, 12.16.

Hexahydro-1*H*-3-oxo-1-acetyl-2(*S*)-methyl-5(*S*)-(*N*,*N*-dimethylcarbamoyl)-1,4-diazepine (5SS-A). Deprotection of **4SS-A** followed procedure described above for **5SS-F** and afforded 2(*S*)-amino-4-[*N*-[1(*S*)-carboxyethyl]-*N*-acetylamino]-*N*,*N*-dimethylbutyramide trifluoroacetate: k' = 3.6 (0−30% (v/v) A in B); FAB-MS calcd for $C_{11}H_{21}N_3O_4$: 259.309, found: m/z = 260 $(M + H)^+$. Cyclization of the TFA salt in a similar manner as described for **5SS-F** above and purification on a semipreparative RP-HPLC (0−25% (v/v) A in B, 100 min, Vydac C-18) afforded an oil (62% yield): k' = 4.3 (0−30% (v/v) A in B); $[\alpha]^{25}_D = +109.1°$ (c = 0.430, methanol); ^{1}H NMR (500 MHz, $CDCl_3$) δ *cis* isomer (88%) 6.55 (1H, N*H*), 4.7 (1H, C*H*CH_3), 4.4 (1H, C*H*NH), 4.52 and 3.02 (2H, NC*H*$_2$$CH_2$), 2.99 and 2.95 (6H, N(C*H*$_3$)$_2$), 1.98 (CH_2C*H*$_2$CH), 2.13 (3H, C*H*$_3$CO), 1.56 (3H, C*H*$_3$CH); *trans* isomer (12%) 6.55 (1H, N*H*), 5.2 (1H, C*H*CH_3), 4.5 (1H, C*H*NH), 3.77 and 3.58 (2H, NC*H*$_2$$CH_2$), 2.95 (6H, N(C*H*$_3$)$_2$), 1.98 ($CH_2$C*H*$_2$CH), 1.51 (3H, C*H*$_3$CH). ^{13}C NMR (500 MHz, $CDCl_3$) δ *cis* isomer (88%) 170.9 (CH_3*C*O), 174 (*C*ONH), 170.1 (*C*ON$(CH_3)_2$), 59 (*C*HCH_3), 52 (*C*HCH_2CH_2), 38.5 (*C*H_2CH_2CH), 37.1 (N(*C*H$_3$)$_2$), 31.25 (CH_2*C*H_2CH), 21.93 (*C*H$_3$CO), 15.47 (*C*H$_3$CH); *trans* isomer (12%), 174 (*C*ONH), 174.4 (*C*ON$(CH_3)_2$), 56 (*C*HCH_3), 51 (*C*HCH_2CH_2), 43.3 (*C*H$_2$-CH_2CH), 36.4 (N(*C*H$_3$)$_2$), 32.7 (CH_2*C*H_2CH). Detailed NMR assignment and conformational analysis are published elsewhere.[43] FAB-MS calcd for $C_{11}H_{19}N_3O_3$: 241.291, found: m/z = 242 $(M + H)^+$.

Hexahydro-1*H*-3-oxo-1-acetyl-2(*R*)-methyl-5(*S*)-(*N*,*N*-dimethylcarbamoyl)-1,4-diazepine (5RS-A). Deprotection of **4RS-A** followed the procedure described above for **5SS-F** and afforded (2(*S*)-amino-4-[*N*-[1(*R*)-carboxyethyl]-*N*-acetylamino]-*N*,*N*-dimethylbutyramide trifluoroacetate: k' = 3.7 (0−30% (v/v) A in B); FAB-MS calcd for $C_{11}H_{21}N_3O_4$: 259.309, found: m/z = 260 $(M + H)^+$. Cyclization of the TFA salt in a similar manner as described for **5SS-F** above and purification similar to that described for **5SS-A** afforded an oil (88% yield): k' = 4.7 (0−30% (v/v) A in B); $[\alpha]^{25}_D = -103.2°$ (c = 0.250, methanol); ^{1}H NMR (500 MHz, $CDCl_3$) δ *cis* isomer (62%) 6.98 (1H, N*H*), 4.6 (1H, C*H*CH_3), 4.3 (1H, C*H*NH), 4.22 and 2.95 (2H, NC*H*$_2$$CH_2$), 2.96 and 2.95 (6H, N(C*H*$_3$)$_2$), 2.47 and 1.65 (CH_2C*H*$_2$CH), 2.18 (3H, C*H*$_3$CO),1.51 (3H, C*H*$_3$CH); *trans* isomer (38%) 6.79 (1H, N*H*), 5.2 (1H, C*H*CH_3), 4.4 (1H, C*H*NH), 3.80 and 3.35 (2H, NC*H*$_2$$CH_2$), 2.99 (6H, N(C*H*$_3$)$_2$), 2.15 and 1.80 ($CH_2$C*H*$_2$CH), 2.16 (3H, C*H*$_3$CO),1.37 (3H, C*H*$_3$-CH). ^{13}C NMR (500 MHz, $CDCl_3$) δ *cis* isomer (62%) 170.3 (CH_3*C*O), 170 (*C*ONH), 169.2 (*C*ON$(CH_3)_2$), 58 (*C*HCH_3), 49 (*C*HCH_2CH_2), 38.3 (CH_2CH_2CH), 35.8 and 36.5 (N(*C*H$_3$)$_2$), 28.7 (CH_2*C*H_2CH), 20.57 (*C*H$_3$CO), 16.7 (*C*H$_3$CH); *trans* isomer (38%) 169.2 (CH_3*C*O), 171 (*C*ONH), 169 (*C*ON$(CH_3)_2$), 55 (*C*HCH_3), 48 (*C*HCH_2CH_2), 41.1 (*C*H_2CH_2CH), 35.8 and 36.5 (N(*C*H$_3$)$_2$), 30.7 (CH_2*C*H_2CH), 20.57 (*C*H$_3$CO), 15.3 (*C*H$_3$CH). Detailed NMR assignment and conformational analysis are published elsewhere.[43] FAB-MS calcd for $C_{11}H_{19}N_3O_3$: 241.291, found: m/z = 242 $(M + H)^+$.

***tert*-Butyl 2(*S*)-[(Benzyloxycarbonyl)amino]-4-hydroxybutyrate (7).** A solution of Z-L-Asp-OtBu (**6**) (8.08 g, 25 mmol) in dry THF (25 mL) and TEA (3.5 mL, 25 mmol) was cooled in an ice−salt bath and treated with of isobutyl chloroformate (IBCF) (3.3 mL, 25 mmol). After 30 min the triethylammonium chloride precipitate was filtered off, and the filtrate was added dropwise to a solution of $NaBH_4$ (1.89 g, 50 mmol) in water (10 mL) at 10 °C. After stirring at rt overnight, the reaction mixture was slowly acidified (pH ≈ 3) with 1 N HCl and extracted with EtOAc (4 × 100 mL). The combined organic phases were washed consecutively with 1 N NaOH (4 × 150 mL) and brine (2 × 150 mL), dried over $MgSO_4$, and concentrated *in vacuo*. Gravity chromatography on silica gel 60 column using step-gradient of 10−50% (v/v) EtOAc in petroleum ether afforded 5.18 g of a clear oil (67% yield): R_f = 0.51 (33% (v/v) acetone in petroleum ether); k' = 3.2 (35−95% (v/v) A in B); $[\alpha]^{25}_D = -14.0$ (c = 0.75, AcOEt);[52] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (5H, m), 5.61 (1H, d, J = 7.5 Hz), 5.11 (2H, s), 4.40 (1H, m), 3.68 (2H, m), 3.06 (1H, dd, J = 5.1, 7.8 Hz), 2.15 (1H, m), 1.58 (1H, m), 1.45 (9H, s). FAB-MS calcd for $C_{16}H_{23}N_1O_5$: 309.362, found: m/z = 332 $(M + Na)^+$. Anal. Calcd for $C_{16}H_{23}N_1O_5 \cdot 0.5H_2O$: C, 60.36; H, 7.60; N, 4.40. Found: C, 60.87; H, 7.48; N, 4.66.

***tert*-Butyl 2(*S*)-[(Benzyloxycarbonyl)amino]-4-[[2-phenyl-1(*S*)-(benzyloxycarbonyl)ethyl]amino]butyrate (8).** A solution of alcohol **7** (4.94 g, 16 mmol) in dry DCM (40 mL) was added to a suspension of PCC (13.8 g, 64 mmol) and silica gel 60 (13.8 g) in dry DCM (40 mL). The reaction was monitored by TLC R_f = 0.60 (33% (v/v) acetone in petroleum ether) and analyzed by ninhydrin. After 2.5 h, diethyl ether (100 mL) was added, and the mixture was filtered through Celite bed. The crude aldehyde obtained upon removal of solvent from the filtrate *in vacuo* was dissolved in 2% (v/v) AcOH in MeOH (20 mL) and mixed with a solution of L-H-Phe-OBz tosylate (3.55 g, 8.3 mmol) in methanol (22 mL) in the presence of molecular sieves Type 3A (8−12 mesh) pellets (∼5 g). After 5 min, $NaCNBH_3$ (1.15 g, 18.3 mmol) was added in two aliquots. After 1.5 h, the reaction mixture was filtered and the solvent removed *in vacuo*. The residue was treated with saturated $NaHCO_3$ (250 mL) and extracted with diethyl ether (3 × 400 mL). The combined ethereal phases were dried over $MgSO_4$ and concentrated *in vacuo*. Purification on silica gel 60 column employing a step-gradient of 20−25% (v/v) acetone/petroleum ether as eluent afforded 3.05 g of a clear oil (67% yield, based on the benzyl phenylalaninate salt): R_f = 0.45 (25% (v/v) acetone in petroleum ether); k' = 6.3 (35−95% (v/v) A in B); $[\alpha]^{25}_D = -6.8°$ (c = 0.558, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33−7.07 (15H, m, Ar), 5.93 (1H, d, J = 7.8 Hz, NH carbamate), 5.10 (2H, s, C*H*$_2$Ph), 5.05 (2H, s, C*H*$_2$Ph), 4.26 (1H, dd, J = 7.8, 12.0 Hz, CH_2CH_2C*H*), 3.52 (1H, t, J = 6.9 Hz, C*H*CH_2Ph), 2.92 (2H, m, CHC*H*$_2$Ph), 2.74 (1H, m, C*H*$_2CH_2$CH), 2.50 (1H, m, C*H*$_2CH_2$CH), 1.90 (1H, m, CH_2C*H*$_2$CH), 1.77 (1H, m, CH_2C*H*$_2$CH), 1.43 (9H, s, C*H*$_3$ *t*Bu). ^{13}C NMR (400 MHz, $CDCl_3$) δ 174.1 (CO), 171.3 (CO), 156.0 (CO), 136.8 (*C*-Ar), 136.5 (*C*-Ar), 135.4 (*C*-Ar), 129.2 (*C*H-Ar), 128.5 (*C*H-Ar), 128.5 (*C*H-Ar), 128.4 (*C*H-Ar), 128.4 (*C*H-Ar), 128.3 (*C*H-Ar), 128.1 (*C*H-Ar), 128.0 (*C*H-Ar), 126.7 (*C*H-Ar), 81.9 (*C*-*t*-Bu), 66.8 (*C*H_2Ph), 66.5 (*C*H_2Ph), 62.7 (*C*HCH_2Ph), 53.3 (CH_2CH_2*C*H), 44.2 (*C*H_2CH_2CH), 39.7 (CH*C*H_2Ph), 31.9 (CH_2CH_2CH), 28.0 (CH_3 *t*-Bu). FAB-MS calcd for $C_{32}H_{38}N_2O_6$: 546.664, found: m/z = 547 $(M + H)^+$. Anal.

(52) Valerio, R. M.; Alewood, P. E.; Hohns, R. B. *Synthesis* **1988**, 786.

Calcd for $C_{32}H_{38}N_2O_6$: C, 70.31; H, 7.01; N, 5.12. Found: C, 70.23; H, 6.90; N, 5.20.

***tert*-Butyl 2(*S*)-amino-4-[[2-phenyl-1(*S*)-carboxyethyl]-amino]butyrate Trifluoroacetate (9).** A nitrogen-flushed solution of **8** (1.42 g, 2.6 mmol) in MeOH (12 mL)/AcOH (2 mL)/H_2O (1 mL) was treated with 10% Pd/C (0.56 g) and ammonium formate (0.66 g, 10.4 mmol). The reaction was monitored by HPLC. Upon completion, the reaction mixture was filtered through a Celite bed, and the filtrate was concentrated *in vacuo*. The residue obtained was lyophilized three times from TFA/H_2O to afford a white powder (1.39 g) of the TFA salt (97% yield): $k' = 5.1$ (5−50% (v/v) A in B); ^{1}H NMR (400 MHz, D_2O) δ 7.46−7.33 (5H, m, Ar), 4.25(1H, t J = 6.4 Hz, C*H*CH_2Ph), 4.04 (1H, dd J = 4.8, 7.6 Hz, C*H*CH_2CH_2), 3.40−3.27 (4H, m, $CHCH_2CH_2$, $CHCH_2$Ph), 2,29 (2H, m, $CHCH_2CH_2$), 1.26 (9H, s, CH_3 t-Bu; FAB-MS calcd for $C_{17}H_{26}N_2O_4$: 322.405, found: $m/z = 323$ $(M + H)^+$.

Hexahydro-1*H*-3-oxo-2(*S*)-benzyl-5(*S*)-*tert*-butyloxy-carbonyl)-1,4-diazepine Trifluoroacetate (10). A solution of the TFA salt **9** (0.16 g. 0.29 mmol) in DMF (150 mL) was treated with DIPEA (0.1 mL, 0.58 mmol) and *O*-7-aza-1-hydroxybenzotriazolyl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) (0.22 g, 0.58 mmol). The mixture was stirred at rt under nitrogen overnight (complete disappearance of the characteristic yellow color and the starting material as monitored by analytical RP-HPLC). The residue obtained following concentration *in vacuo* was dissolved with EtOAc (100 mL), washed with saturated solution of $NaHCO_3$ (3 × 100 mL) and brine (30 mL), and dried over $MgSO_4$ and the solvent removed *in vacuo*. Purification by semipreparative RP-HPLC on Vydac C-18, 300 Å (0−10% (v/v) in 20 min followed by 10−40% in 90 min) afforded 49 mg of white powder (40% yield): $k' = 9.0$ (5−50% (v/v) A in B); mp 73 °C; $[\alpha]^{25}_D = +7.6$ (c = 0.275, methanol); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30−7.22 (5H, m, Ar), 6.80 (1H, d J = 5.2 Hz, NHCO), 4.30 (1H, ddd J = 4.8, 4.8, 9.6 Hz, CH_2CH_2CH), 4.21 (1H, t J = 6.8 Hz, C*H*CH_2Ph), 3.40−3.18 (3H overlapping $CHCH_2$Ph CH_2CH_2-CH), 3.03 (1H, m, CH_2CH_2CH), 2.38 (1H, m, CH_2CH_2CH), 2.08 (1H, m, CH_2CH_2CH),1.48 (9H, s, $(CH_3)_3C$). ^{13}C NMR (300 MHz, $CDCl_3$) δ 169.4 (CO), 169.2 (CO), 135.2 (C-Ar), 130.0 (CH-Ar), 129.6 (CH-Ar), 128.3 (CH-Ar), 85.0 (C t-Bu), 63.7 (CH_2CH_2CH), 51.9 ($CHCH_2$Ph), 43.7 (CH_2CH_2CH), 36.6 (CH_2-Ph), 30.1 (CH_2CH_2CH), 20.5 (CH_3 *t*-Bu). FAB-MS calcd for $C_{17}H_{24}N_2O_3$: 304.39, found: $m/z = 305$ $(M + H)^+$. Anal. Calcd for $C_{17}H_{24}N_2O_3 \cdot {}^3/_2$TFA: C, 50.53; H, 5.41; N, 5.89. Found: C, 50.86; H, 5.64; N, 6.36.

This research was supported, in part, by grant No. 89-00248 from the United States-Israel Binational Science Foundation (BSF), Jerusalem, Israel (M.C.). D.F.M. would like to thank the Research Corporation for partial support of this research in the form of a Cottrell Scholar Award.

JO962257E