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CH/π Interaction in the Conformation of Peptides. A Database Study†

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Abstract—A study was carried out, with use of the Cambridge Structural Database, to examine the role of the CH/π interaction in the conformation of peptides. A number of short intramolecular CH/π distances have been shown in the crystal structure of peptides bearing at least an aromatic residue in the sequence. The molecular structure in the crystal was inspected individually to know whether the conformation is merely a consequence of the so-called packing forces, or the CH/π interaction plays a role. It has been demonstrated that the CH/π interaction constitutes one of the key factors in controlling the conformation of peptides.

Introduction

Evidence has accumulated that the CH/π interaction[1] plays an important role in determining the conformation of organic compounds.[2,3] For instance, compounds bearing an aromatic group in the peptide sequence are often reported to prefer the folded conformation. A compact structure was suggested for the solution conformation of a cyclic heptapeptide antibiotic ilamycin B_1.[4] Thus a high-field ^{1}H NMR chemical shift observed for one of the leucyl isopropyl methyl resonances was attributed to the ring current effect of the indole ring of a tryptophane residue in the peptide. Iitaka et al. determined the crystal structure of ilamycin B_1 and showed that the isopropyl methyl groups in a leucine side-chain lie just above the aromatic ring (Ar) of a tryptophane residue.[5] The perpendicular distances of the geminal carbon atoms and the least-squares plane of the indole ring were 3.70 and 4.00 Å; the reason for this conformation remained unexplained. Here, we carried out a database study, with use of the Cambridge Structural Database (CSD),[6] to understand the significance of the CH/π interaction in the conformation of peptides.

Key words: CH/π interaction; peptide; conformation; database; CSD.
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† This paper is dedicated to the memory of the late Professor Sir Derek H. R. Barton, the founder of the concept of conformation. The authors sincerely regret the immeasurable loss of an outstanding figure in the world of science.

Method

The method of exploring CH/π interactions in the crystal structure was reported earlier.[7] Thus, we wrote a program, with use of the CSD software QUEST3D, to find contacts between CH groups and π systems. Several kinds of distance (D_{pln}, D_{atm}, D_{lin}) and angle (θ, ω) parameters were defined to cover every possibility (Fig. 1). In this study, D_{max} shorter than 3.05 Å (=2.9Å[8] (1.2 Å for C−H plus 1.7 Å[9] for a half thickness of the aromatic molecule)×1.05) was considered as relevant for the presence of CH/π interaction.[10]

Results

First, a sub-database was edited, from the entire database (CSD version 515: 181,309 entries), for peptides and peptide derivatives bearing at least a phenylalanine, tyrosine or tryptophane residue in the sequence. The number of compounds in the sub-database was 130.[11] Next, nonbonded atomic contact was sought between a CH[12] (alkyl or aromatic) and an sp^2 carbon in the six-membered aromatic ring in the Phe, Tyr or Trp side-chain. Table 1 summarizes the results.

The number of compounds bearing at least one intramolecular CH/π interaction was 55. The ratio of peptides with short CH/C(sp^2) contact in their molecular structure is 42% (55/130). The corresponding value for

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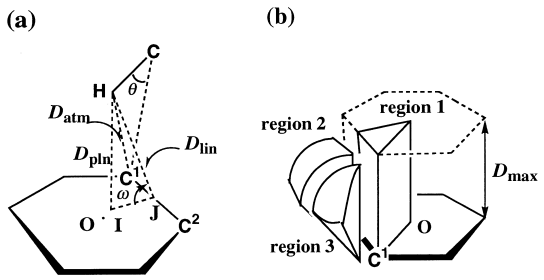

Figure 1. Method for exploring CH/π contacts in peptides. (a) O: centre of the plane. C^1 and C^2: nearest and second nearest sp^2-carbons, respectively, to H. ω: dihedral angle defined by C^1OC^2 and HC^1C^2 planes. θ: $\angle HCC^1$. D_{p1n}: H/π-plane distance (H/I). D_{atm}: interatomic distance (H/C^1). D_{lin}: distance between H and line C^1C^2 (H/J). (b) 1: region where H is above the aromatic ring. 2 and 3: regions where H is out of region 1 but may interact with π-orbitals. The program was run to search for H/π distance shorter than a cut-off value D_{max} in every region: $D_{p1n} < D_{max}$, $\theta < 60\%$, $|\omega| < 90°$ for region 1, $D_{lin} < D_{max}$, $\theta < 60\%$, $90° < |\omega| < 130°$ for region 2, and $D_{atm} < D_{max}$, $\theta < 60°$, $\omega = 180° - \phi$ (φ: HC^1I), $90° < \omega < 130°$ for region 3.

the intermolecular interaction is 94% (122/130). Figure 2(a) and (b) are histograms in the search. Note that there are many CH/π distances shorter than the accepted value of the sum of the van der Waals radii of the relevant nuclei.

A short interatomic distance may arise from the balance of a number of molecular forces. In other words, the crystal structure may accommodate the CH/π geometry within the overall framework of various effects. The structure of the above peptides was therefore inspected to know whether the crystal conformation is merely a consequence of the well-known packing forces such as the hydrogen bond, or the CH/π interaction plays a part.

Table 1. Intramolecular and intermolecular CH/π contacts disclosed in the crystal structure of peptides bearing at least one Phe, Tyr or Trp residue ($D_{max} = 3.05$Å)[a]

	Entries[b]	Distances[c]	D_{atm} (Å)[d]
Intramolecular			
CH/π	55 (45)	95 (69)	2.87 ± 0.15 (2.81 ± 0.11)
C(sp^3)H/π	49[e] (38)	78 (54)	2.88 ± 0.15 (2.81 ± 0.16)
C(Ar)H/π	10[f] (10)	17 (15)	2.82 ± 0.11 (2.80 ± 0.09)
Intermolecular			
CH/π	122 (109)	432 (259)	2.89 ± 0.13 (2.81 ± 0.11)
C(sp^3)H/π	109 (86)	295 (171)	2.89 ± 0.13 (2.81 ± 0.11)
C(Ar)H/π	61 (49)	134 (85)	2.88 ± 0.14 (2.81 ± 0.11)

[a] In the parentheses are data for a shorter cut-off value 2.9 Å.
[b] Number of entries with short CH/Ar contact.
[c] Number of short distances.
[d] CH/(Csp^2) interatomic distance.
[e] CSD Refcode: BAJNOP10, BAMGOL10, BIHTUH10, BIHXUL10, BIXPAZ10, BOLWEE, CACNOJ10, CAHWEN, CINYED, CITXEI10, COPHOE, COYRIR, DINROH10, DUPKEE, DUYTIA, DUZDUX, FEYZEO, FIFMEM, FIZWIU01, FILPLA, GERCEL, GLYTYR10, HEBHUR, HICJUY, HICKEJ, HICKIN, JADVAL, JUXHAL, LINCOA, LPTILL, PHLEGL10, ROHVEP, ROHVIT, ROHVOZ, SUPBOU, SUPBUA, VOMLEO, VUGMEP, WASPOV, WIPXUO, WIPYAV, WIPYEZ, YIJDIE, YOWDAP, ZUBWUO, ZUHNOF, ZUHNUL, ZUHPAT, ZYKRAY.
[f] CSD Refcode: DIRPUP, DUNLON, DUZDUX, FEYZEO, FIFMEM, FONNAX, GEWWAG, KUSHAH, SUPBOU, TALDEP.

Figure 3 is the structure[13] of L-phenylalanyl-glycyl-glycyl-D-phenylalanine trihydrate.[14] Short intramolecular CH/Ar distances are found between a glycine and an aromatic carbon of a phenylalanine (3.06 Å: Gly3/Phe1) and between aromatic rings of the phenylalanines (2.76 Å: Phe1/Phe4). NH/π contact is also shown between NH of Gly3 and the aromatic rings of Phe1. The molecule adopts the compact structure without any intramolecular hydrogen bonds.

Figure 4 gives the crystal structure of leucine-enkephalin (Tyr-Gly-Gly-Phe-Leu) trihydrate.[15] We see an aromatic CH/π bond (2.67 Å) between the side-chains of Tyr and Phe. The folded structure is assisted by two hydrogen bonds.

Figure 5 is the molecular structure of *t*-butoxycarbonyl-glycyl-glycyl-phenylalanyl-D-methionine methyl ester dihydrate.[16] CHs in the methionine side-chain are in CH/π contact (2.97, 3.03 Å) with the aromatic ring of the phenylalanine residue. Similar conformations were found for the D-leucine (ZUHNOF) and D-homoleucine analogues (ZHUPAT).

Figure 6 shows the crystal structure of *cyclo*(L-phenylalanyl-D-leucyl-glycyl-L-phenylalanyl-L-leucyl-glycyl) tetrahydrate.[17] Short CH/Ar distances (2.92, 2.96, 3.03 Å) are shown between the leucine and phenylalanine side-chains. A hydrogen bond is found in the cyclic structure. Similar conformations are also found for its analogues *cyclo*-(L-Phe-D-Leu-Gly-D-Phe-L-Leu-Gly) (CAHWEN) and *cyclo*-(L-Phe-L-Leu-Gly-D-Phe-L-Leu-Gly) (BIXPAZ10).

Discussion

Proportion of the crystal structures showing CH/π interaction has been found considerable in the CSD; we see many CH/π contacts shorter than the van der Waals distance, intramolecular as well as intermolecular. Inspection of the individual peptide structures has suggested that the crystal conformation is not necessarily the result of only well-known molecular forces. It is remarkable that in a number of cases the molecule adopts the compact structure without any intramolecular hydrogen bonds.

Conformation of cyclic dipeptides

Kopple et al.[18–20] reported on the preference of folded conformation for a series of cyclic dipeptides (diketopiperazines), *cyclo*(Gly-L-Tyr), *cyclo*(L-Ala-L-Tyr) and *cyclo*(L-His-L-Tyr). Later, the solid structure of *cyclo*(Gly-L-Tyr) was found to be similar as in solution.[21] They attributed the phenomenon to the presence of an attractive interaction occurring between the aromatic ring and the neighboring polar systems. Preference for the aromatic side-chain of a residue to fold over the diketopiperazine ring has since been shown in a number of solution[22–24] and crystal structures, *cyclo*(L-Pro-D-Phe),[25] *cyclo*(L-Ser-L-Tyr),[26] *cyclo*(*N*-Me-L-Phe-*N*-Me-L-Phe), *cyclo*(*N*-Me-L-Phe-*N*-Me-D-Phe),[27] and *cyclo*

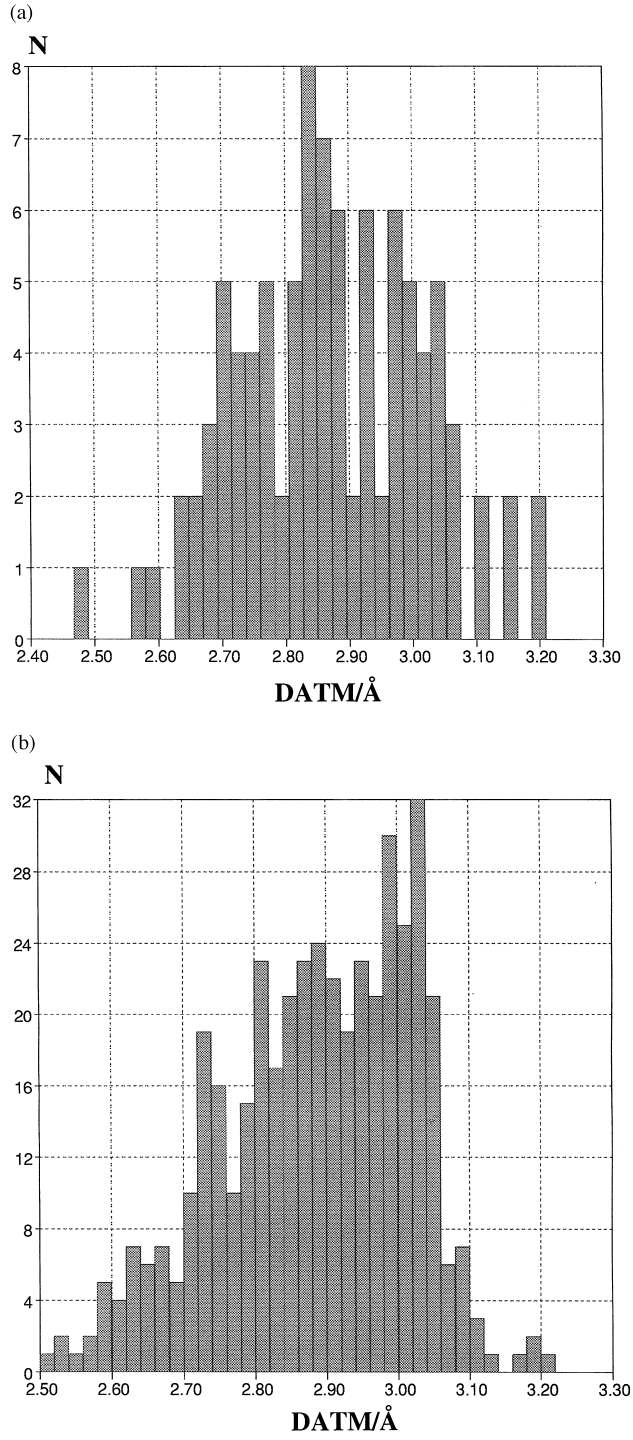

Figure 2. Number of observations plotted against the H/C interatomic distance D_{atm} for CH/Ar interaction ($D_{max} = 3.05$ Å): (a) intramolecular interaction; (b) intermolecular interaction.

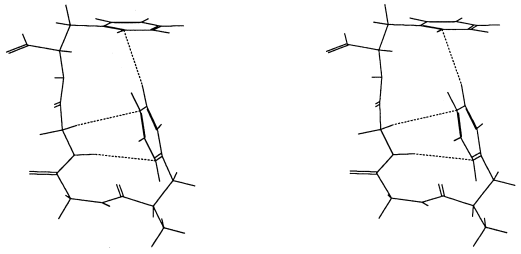

Figure 3. Crystal structure of L-Phe-Gly-Gly-D-Phe, $3H_2O$ (CSD Refcode FEYZEO). Dotted lines indicate CH/π and NH/π contacts. The water molecules are omitted for clarity.

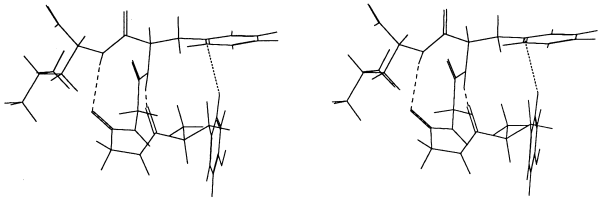

Figure 4. Crystal structure of leucine-enkephalin, $3H_2O$ (GEWWAG). Dotted and dashed lines indicate CH/π contacts and hydrogen bonds, respectively. The water molecules are omitted.

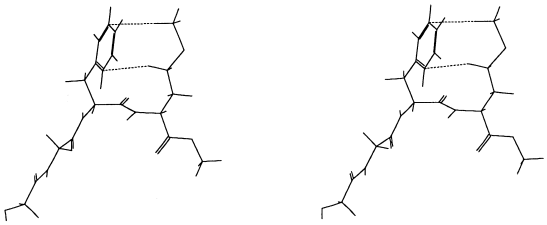

Figure 5. Crystal structure of *t*-butoxycarbonyl-Gly-Gly-L-Phe-D-Met methyl ester, $2H_2O$ (ZUHNUL). Dotted lines indicate CH/π contacts. The Boc group and water molecules are not shown.

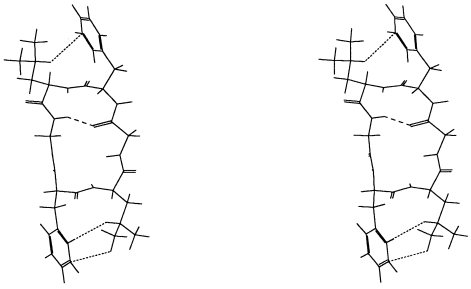

Figure 6. Crystal structure of *cyclo*-(L-Phe-D-Leu-Gly-L-Phe-L-Leu-Gly), $4H_2O$ (PHLEGL10). Dotted and dashed lines indicate CH/π contacts and hydrogen bonds, respectively. The water molecules are omitted.

(D-Phe-L-γ-thiaPro),[28] *cyclo*(L-Leu-L-Tyr),[29] *cyclo*(aminoisobutyryl-L-Phe),[30] *cyclo*(L-Phe-L-Phe),[31] *cyclo*(*N*-Me-L-Phe-L-Phe)[32] and *cyclo*(*N*-Me-L-α-aminobutyryl-L-Phe).[33] In Table 1, we find diketopiperazines showing short CH/π contacts between CH(s) and the aromatic ring. They are GLYTYR10: *cyclo*(Gly-L-Tyr), BAJNOP10: *cyclo*(*N*-Me-L-Aba-L-Phe) Aba = α-aminobutyric acid, BOLWEE: *cyclo*(D-Phe-L-γ-(*S*)Pro), COPHOE: *cyclo*(L-Leu-L-Tyr), COYRIR: *cyclo*(Aib-L-Phe) Aib = aminoisobutyric acid, DUZDUX: *cyclo*(L-Phe-L-Phe) and FIFMEM: *cyclo*(*N*-Me-L-Phe-L-Phe). It appears that the CH/π interaction is playing an important role in maintaining the compact structure of these compounds. To illustrate an example, Figure 7 gives the crystal structure of *cyclo*(glycyl-L-tyrosyl). The distance between an α-CH of glycine and an *o*-carbon of the tyrosine aromatic ring is 3.03 Å.

Figure 7. Crystal structure of *cyclo*(glycyl-L-tyrosyl), GLYTYR10. A dotted line indicates CH/π contact.

Figure 8 is the crystal structure of *cyclo*(L-Leu-L-Tyr) monohydrate. A CH (Hβ) in the leucine side-chain is in CH/π contact (2.85 Å) with the aromatic ring of the tyrosine residue.

Figure 9 shows the crystal structure of *cyclo*(L-Phe-L-Phe). We see two CH/π contacts (2.79, 2.87 Å) between the side-chain groups.

Crystal structure of acyclic peptides

Short CH/Ar distances have also been found in acyclic dipeptides L-Asp-L-Phe (2.71 Å, WASPOV) and L-Phe-L-Pro (2.58, 3.00 Å, JADVAL). Inspection of the crystal structures listed in Table 1 showed a number of CH/π-interacted conformations in higher analogues. Examples include tripeptides Gly-Phe-Phe (DIRPUP), Phe-Gly-Gly (FIZWIU), formyl-Met-Leu-Phe (FMLPLA), Lys-Phe-Phe (FONNAX), and Leu-Leu-Tyr (GERCEL), and higher peptides such as Pro-Tyr-Ile-Leu (LPTILL), Tyr-D-Thr-Gly-Phe-Leu-Thr (HICJUY), Val-Phe-Phe-Ala-Phe-Phe-Val-Phe-Gly (KUSHAH). Burley et al. reported on the Ar/Ar proximate[34,35] crystal structure for L-phenylalanyl benzyl ester and *N*-phenylacetyl-L-phenylalanine.[36,37] This type of interaction is often referred to as the 'edge-to-face Ar/Ar interaction.' The term 'aromatic CH/π interaction' is more adequate in view of its nature.

Solution conformation of acyclic peptides

With regard to the solution conformation of acyclic peptides, shielding of aliphatic side-chain protons has long been known for linear dipeptides with one residue aromatic and the other aliphatic.[38] Examples of the folded conformation include flavinyl peptides,[39] somatostatin,[40–42] bradykinin analogues[43,44] and a cyclic hexapeptide *cyclo*(D-Tyr-Arg-Gly-Asp-Phe-Gly).[45] Deber and Joshua, in particular, suggested the folded conformation for a series of dipeptides bearing phenylalanine (L-Phe-D-aa; aa = Asn, Asp, Gln, Glu, Arg, Lys, Aba, norvaline), on the basis of a systematic NMR study.[46] Significant upfield shifts were observed, in every case, for methylene protons of the residue aa, as compared to those of dipeptides with only aliphatic residues (L-Ala-D-aa). It is remarkable that more pronounced upfield shifts were observed for methylenes in polar residues (Asp, Glu, Arg, Lys). They discussed the results in view of the presence of attractive interaction between the phenylalanine aromatic ring and the side-chain group of the residue aa. Preference of the CH/Ar proximate conformation was suggested also for dipeptide derivatives D-aa-L-Phe-Bzl (aa = Leu, Val, Ala; Bzl = $CH_2C_6H_5$)[47] and D-Arg-L-Phe-NHBzl.[48] Shimohigashi et al. recently showed the preference of alkyl/Ar proximate geometry for a series of chymotrypsin inhibitors D-Leu-L-Phe-NHBzl[49] and D-Thr-L-Phe-NHBzl;[50] they interpreted the result in terms of the CH/π interaction. Kim et al.[51] reported on the folded conformation of a chymotrypsin inhibitory peptidomimetic 2-allyl-3-benzenepropanoate and attributed the result to the CH/π interaction. It is intriguing to speculate that the compact shape of the peptides correlates with their biological activity.[15,16,28,29,52–59]

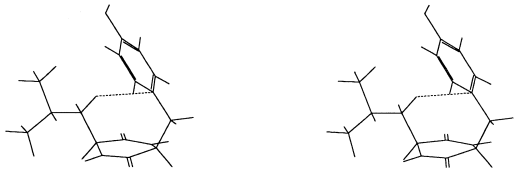

Figure 8. Crystal structure of *cyclo*(L-leucyl-L-tyrosyl), COPHOE. A dotted line indicates CH/π contact.

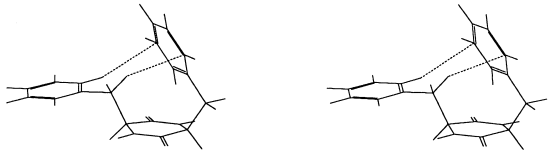

Figure 9. Crystal structure of *cyclo*(L-phenylalanyl-L-phenylalanyl), DUZDUX. Dotted lines indicate CH/π contacts.

Conclusion

In view of the above discussions, we conclude that the CH/π interaction is a dominant factor in maintaining the compact structure of the peptides. Recently, we suggested that the CH/π interaction is important in stabilizing the 3D structure of proteins such as enzymes,[60] G proteins, SH2 domains[61] and major histocompatibility complex antigens,[62] and their complexes with specific substrates. The suggestion has been made on the basis of the results obtained by analyzing the crystal structures in the Brookhaven Protein Data Bank (PDB). Coordinates of the hydrogen atoms (from neutron studies) are available for bovine pancreatic trypsin inhibitor (BPTI), ribonuclease A (RNase A), insulin and myoglobin. However, the data in the PDB do not ordinarily contain coordinates of hydrogen atoms. In these cases, hydrogens were generated on nonhydrogen atoms and the positions optimized. The CH/π distances within a cut-off value were then collected and the results compared with those obtained by use of the neutron diffraction data. Agreement of the two sets of data was satisfactory in the cases of BPTI[63] and RNase A,[64] at least in view of the purpose of surveying CH/π interactions in the protein structure. Discussions regarding the CH/π distances in the protein structures remained qualitative, however, according to the limitation of the methodology and the precision in the X-ray crystallographic determination. The present finding that CH/π interactions are often found in the crystal structure of peptides renders a strong support to the above method

of analyzing CH/π interactions in proteins. It is certain that the CH/π interaction, which is responsible for the peptide structure, also contributes to the stabilization of the protein structure. Implication of the concept in structural biology[60–72] and drug design is apparent.

References

1. Nishio, M.; Hirota, M.; Umezawa, Y. *The CH/π Interaction. Evidence, Nature, and Consequences*; Wiley–VCH: New York, 1998.
2. See ref 1, Chapter 5.
3. Nishio, M.; Hirota, M. *Tetrahedron* **1989**, *45*, 7201–7245.
4. Cary, L. W.; Takita, T.; Ohnishi, M. *FEBS Lett*. **1971**, *17*, 145–148.
5. Iitaka, Y.; Nakamura, H.; Takada, K.; Takita, T. *Acta Crystallogr., Sect. B* **1974**, *30*, 2817–2825.
6. Allen, F. H.; Davies, J. E.; Galloy, J. L; Johnson, O.; Kennard, O.; Macrae, C. F.; Mitchell, E. M.; Mitchell, G. F.; Smith, J. M.; Watson, D. G. *J. Chem. Inf. Comput. Sci.* **1991**, *31*, 187–204.
7. Umezawa, Y.; Tsuboyama, S.; Honda, K.; Uzawa, J.; Nishio, M. *Bull. Chem. Soc. Jpn*. **1998**, *71*, 1207–1213.
8. Pauling, L. *The Nature of the Chemical Bonds*; Cornell University Press: Ithaca, NY, 1960; p 260.
9. Bondi took 1.77 Å as the half width of a sp^2-carbon: Bondi, A. *J. Phys. Chem*. **1964**, *68*, 441-451.
10. Discrimination of the CH/π interaction from the attractive part of the van der Waals force is difficult. The CH/π interaction includes the dispersion force, however, contribution from the delocalization mechanism is also important (Takagi, T.; Tanaka, A.; Matsuo, S.; Maezaki, H.; Tani, M.; Fujiwara, H.; Sasaki, Y. *J. Chem. Soc., Perkin Trans. 2* **1987**, 1015–1018). The van der Waals force (hence the van der Waals distance) is an ambiguous concept representing a blend of a variety of non-specific interactions. For discussions regarding the nature of the CH/π interaction, see refs 1 and 7.
11. Only entries with hydrogen coordinates were collected.
12. The C−H bond distance was corrected to a standard value of 1.083 Å in the CSD software.
13. The dotted lines in Figures 3–9 were drawn by use of our CHPI program after optimizing the hydrogen coordinates.
14. Fujii, S.; Burley, S. K.; Wang, A. H.-J. *Acta Crystallogr., Sect. C* **1987**, *43*, 1008–1009.
15. Aubry, A.; Birlirakis, N.; Sakarellos-Daitsiotis, M.; Sakarellos, C.; Marraud, M. *J. Chem. Soc., Chem. Commun*. **1988**, 963-964.
16. Fenude, E.; Casalone, G. *Acta Crystallogr., Sect. C* **1996**, *52*, 973–978.
17. Barnes, C. L.; van der Helm, D. *Acta Crystallogr., Sect. B* **1982**, *38*, 2589–2595.
18. Kopple, K. D.; Marr, D. H. *J. Am. Chem. Soc*. **1967**, *89*, 6193–6200.
19. Kopple, K. D.; Ohnishi, M. *J. Am. Chem. Soc*. **1969**, *91*, 962–970.
20. Ziauddin; Kopple, K. D. *J. Org. Chem*. **1970**, *35*, 253–255.
21. Webb, L. E.; Lin, C.-F. *J. Am. Chem. Soc*. **1971**, *93*, 3818–3819.
22. Edelhoch, H.; Bernstein, R. S.; Wilchek, M. *J. Biol. Chem*. **1968**, *243*, 5985–5992.
23. Strickland, E. H.; Wilchek, M.; Horwitz, J.; Billups, C. *J. Biol. Chem*. **1970**, *245*, 4168–4177.
24. Deslauriers, R.; Grzonka, Z.; Schaumburg, K.; Shiba, T.; Walter, R. *J. Am. Chem. Soc*. **1975**, *97*, 5093–5100.
25. Ramani, R.; Venkatesan, K.; Marsh, R. E.; Hu Kung, W. J. *Acta Crystallogr., Sect. B* **1976**, *32*, 1051–1056.
26. Lin, C.-F.; Webb, L. E. *J. Am. Chem. Soc*. **1973**, *95*, 6803–6811.
27. Benedetti, E.; Marsh, R. E.; Goodman, M. *J. Am. Chem. Soc*. **1976**, *98*, 6676–6684.
28. van Poucke, M.; Geise, H. J.; Lenstra, A. T. H. *Acta Crystallogr., Sect. C* **1983**, *39*, 227–230.
29. Suguna, K.; Ramakumar, S.; Kopple, K. D. *Acta Crystallogr., Sect. C* **1984**, *40*, 2053–2056.
30. Suguna, K.; Ramakumar, S.; Nagaraj, R.; Balaram, P. *Acta Crystallogr., Sect. C* **1985**, *41*, 284–286.
31. Gdaniec, M.; Liberek, B. *Acta Crystallogr., Sect. C* **1986**, *42*, 1343–1345.
32. Gdaniec, M.; Liberek, B. *Acta Crystallogr., Sect. C* **1987**, *43*, 982–985.
33. Gdaniec, M. *Acta Crystallogr., Sect. C* **1988**, *44*, 1042–1044.
34. Burley, S. K.; Petsko, G. A. *Science* **1985**, *229*, 23–28.
35. Singh, J.; Thornton, J. M. *FEBS Lett*. **1985**, *191*, 1–6.
36. Wang, A. H.-J.; Burley, S. K. *Acta Crystallogr., Sect. C* **1987**, *43*, 1635–1636.
37. Burley, S. K.; Wang, A. H.-J.; Votano, J. R.; Rich, A. *Biochemistry* **1987**, *26*, 5091–5099.
38. Lemieux, R. U.; Barton, M. A. *Can. J. Chem*. **1971**, *49*, 767–776.
39. McKenzie, R. E.; Föry, W.; McCormick, D. B. *Biochemistry* **1969**, *8*, 1839–1844.
40. Holladay, L. A.; Puett, D. *Proc. Natl. Acad. Sci., USA* **1976**, *73*, 1199–1202.
41. Veber, D. F.; Holly, F. W.; Paleveda, W. J.; Nutt, R. F.; Bergstrand, S. J.; Torchiana, M.; Glitzer, M. S.; Saperstein, R.; Hirschmann, R. *Proc. Natl. Acad. Sci., USA* **1978**, *75*, 2636–2640.
42. Arison, B. H.; Hirschmann, R.; Paleveda, W. J.; Brady, S.E; Veber, D. F. *Biochem. Biophys. Res. Commun*. **1981**, *100*, 1148–1153.
43. London, R. E.; Stewart, J. M.; Williams, R.; Cann, J. R.; Matwiyoff, N. A. *J. Am. Chem. Soc*. **1979**, *101*, 2455–2462.
44. Anteunis, M. J. O.; Borremans, F. A. M.; Stewart, J. M.; London, R. E. *J. Am. Chem. Soc*. **1981**, *103*, 2187–2191.
45. McDowell, R. S.; Gadek, T. R.; Barker, P. L.; Burdick, D.L; Chan, K. S.; Quan, C. L.; Skelton, N.; Struble, M.; Thorsett, E. D.; Tischler, M.; Tom, J. Y. K.; Webb, T. R.; Burnier, J. P. *J. Am. Chem. Soc*. **1994**, *116*, 5069–5076.
46. Deber, C. M.; Joshua, H. *Biopolymers* **1972**, *11*, 2493–2503.
47. Sakamoto, H.; Shimohigashi, Y.; Maeda, I.; Nose, T.; Nakashima, K.; Nakamura, I.; Ogawa, T.; Kawano, K.; Ohno, M. *J. Molec. Recogn*. **1993**, *6*, 95–100.
48. Maeda, I.; Shimohigashi, Y.; Nakamura, I.; Sakamoto, H.; Kawano, K.; Ohno, M. *Biochem. Biophys. Res. Commun*. **1993**, *193*, 428–433.
49. Shimohigashi, Y.; Maeda, I.; Nose, T.; Ikesue, K.; Sakamoto, H.; Ogawa, T.; Ide, Y.; Kawahara, M.; New, T.; Terada, Y.; Kawano, K.; Ohno, M. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2479–2485.
50. Maeda, I.; Shimohigashi, Y.; Ikesue, K.; Nose, T.; Ide, Y.; Kawano, K.; Ohno, M. *J. Biochem*. **1996**, *119*, 870–877.
51. Kim, D. H.; Li, Z.-R; Soo, S. L.; Park, J.; Chung, S. J. *Bioorg. Med. Chem*. **1998**, *6*, 239–249.
52. Cotrait, M.; Geofre, S.; Hospital, M.; Pricigoux, G. *Acta Crystallogr., Sect. B* **1979**, *35*, 114–118.
53. Pricigoux, G.; Cotrait, M.; Geofre, S. *Acta Crystallogr., Sect. C* **1986**, *42*, 315–317 (neurotensin activity).
54. Refs 28 and 29 (bitterness).
55. Delettré, J.; Berthou, J.; Lifchitz, A. *Acta Crystallogr., Sect. C* **1988**, *44*, 902–904 (immunomodulating acivity).
56. Görbitz, C. H.; Etter, M. C. *Acta Crystallogr., Sect. C* **1993**, *49*, 1673–1676 (sweetness).
57. Ref. 34 (antisickling).
58. Nikiforovich, G. V.; Kövér, K. E.; Kolodziej, S. A.; Nock, B.; George, C; Deschamps, J. R.; Flippen-Anderson, J. L.; Marchall, G. R. *J. Am. Chem. Soc*. **1996**, *118*, 959–969 (opioid).

59. Refs 48–51 (enzyme inhibitory activity).
60. Nishio, M.; Umezawa, Y.; Hirota, M.; Takeuchi, Y. *Tetrahedron* **1995**, *51*, 8665–8701.
61. Umezawa, Y.; Nishio, M. *Bioorg. Med. Chem.* **1998**, *6*, 493–504.
62. Umezawa, Y.; Nishio, M. *Bioorg. Med. Chem.* **1998**, *6*, 2507–2515.
63. Ref 1, pp 178–181; Tables 11.1 and 11.2.
64. Ref. 61, Tables 1 and 2.
65. Yun, M.; Park, C.; Kim, S.; Nam, D.; Kim, S.C; Kim, D. H. *J. Am. Chem. Soc.* **1992**, *114*, 2281–2282.
66. Tanaka, T.; Hirama, M.; Fujita, K.; Imajo, S.; Ishiguro, M. *J. Chem. Soc., Chem. Commun.* **1993**, 1205–1207.
67. Chakrabarti, P.; Samanta, U. *J. Mol. Biol.* **1995**, *251*, 9–14.
68. Harata, K.; Muraki, M. *Acta Crystallogr., Sect. D* **1997**, *53*, 650–657.
69. Nishinaka, T.; Ito, Y.; Yokoyama, S.; Shibata, T. *Proc. Natl. Acad. Sci., USA* **1997**, *94*, 6623–6628.
70. Zographos, S. E.; Oikonomakos, N. G.; Tsitsanou, K. E.; Leonidas, D. D.; Chrysina, E. D.; Skamnaki, V. T.; Bischoff, H.; Goldmann, S.; Watson, K. A.; Johnson, L. N. *Structure* **1997**, *5*, 1413–1425.
71. Sato, T.; Kanai, Y.; Hoshino, T. *Biosci. Biotech. Biochem.* **1998**, *62*, 407–411.
72. Nishinaka, T.; Shinohara, A.; Ito, Y.; Yokoyama, S.; Shibata, T. *Proc. Nad. Acad. Sci., USA* **1998**, *95*, 11071–11076.