New Synthetic Non-peptide Ligands for Classical Major Histocompatibility Complex Class I Molecules*

(Received for publication, May 13, 1998, and in revised form, August 6, 1998)

Alberto Bianco‡§, Carsten Brock†, Claus Zabel§, Tilmann Walk‡, Peter Walden¶, and Günther Jung‡

From the ‡Institut für Organische Chemie, Universität Tübingen, D-72076 Tübingen, Germany and the ¶Dermatologische Klinik, Charité, Humboldt-Universität, D-10099 Berlin, Germany

Poly-N-acylated amines, as a new class of synthetic non-peptide ligands for the murine major histocompatibility complex (MHC) class I molecule H-2Kb, were developed on the basis of the ovalbumin-derived peptide epitope SIINFEKL. Non-peptidic structural elements were introduced at the C-terminal part of the ligand and include the two dominant anchors at positions 5 and 8. Several oligomers and five different combinatorial libraries were synthesized and tested for their H-2Kb-binding capacities in an MHC stabilization assay. First, the optimal spacing and geometry of the side chains were determined using a series of oligomers with different main chain modifications. Then, based on the structure with the highest binding efficiency, randomized libraries were designed that contain 26 different aromatic, heteroaromatic, or pseudoaromatic side chains for the dominant anchor at position 5, which is deeply buried inside the MHC peptide-binding groove and is crucial for the conformational stability of the entire peptide-MHC complex. Similarly, a series of aliphatic side chains were tested for the second dominant anchor at position 8. MHC-binding and MHC-stabilizing oligomers with defined structures were derived from these libraries by deconvolution.

Major histocompatibility complex (MHC)1 class I (MHC-I) molecules are heterotrimers of a 45-kDa α-chain encoded by the polymorphic genes of the MHC, noncovalently associated in invariant 12-kDa β2-microglobulin, and a short peptide of 8–10 amino acids derived from usually cellular proteins by proteolytic degradation (1). The complex is presented at the cell surface for recognition by MHC-I-restricted cytotoxic T lymphocytes. The peptide-binding groove is formed by two α-helices on top of a β-pleated sheet (2). The peptide is bound in extended conformation, with most of its surface buried inside the groove. The interactions of the peptide with the MHC molecule can be classified as follows: first, compensation of the C- and N-terminal charges of the peptide by complementary MHC residues (3); second, extensive hydrogen bonding between the peptide main chain and MHC side chains that adds to sequence-unspecific binding (4); and third, polymorphic MHC side chains inside the binding pocket that determine MHC allele-specific peptide motifs corresponding to two dominant and several subdominant anchor positions with strong constraints for the peptide side chains (5).

The peptide ligand is essential for the conformational stability of the MHC molecule and thus can be seen as an integral part of the protein (6). The analysis of the requirements for peptide selection by MHC molecules has revealed rules that are reminiscent of the packing in the core of a typical globular protein rather than for typical receptor-ligand interactions (7, 8). The tight incorporation of the peptide into the MHC molecule as a third subunit makes it very challenging and tempting to search for non-peptide ligands for this peptide receptor. Such peptide analogues can be synthesized in large varieties with diverse structural features, and the MHC heterotrimers appear to be a suitable model for exploring the possibilities and requirements for the design of non-peptidic structural elements in proteins.

The complex of the ovalbumin-derived octapeptide SIINFEKL and the murine MHC-I molecule H-2Kb (9) has been extensively studied (10). Pool sequencing of peptides, naturally presented by H-2Kb, has revealed a strong preference for octamers with tyrosine or phenylalanine at position 5 and leucine or methionine at position 8 (11). This binding motif is implemented by the steric requirements as well as by specific interactions of the anchoring side chains with the binding pockets: hydrophobic effects and π-stacking in the case of the aromatic side chain at position 5 and hydrophobic interaction in the case of the aliphatic side chain at position 8. Alanine scans confirmed the importance of these anchor positions for H-2Kb stabilization, whereby positions 4, 6, and 7 were shown to be important for T cell recognition (12). In a more comprehensive study, the influence of each amino acid in each position was examined using randomized peptide libraries in a positional scanning format (7). In MHC stabilization experiments, positions 4, 6, and 7 were found to be relatively tolerant to amino acid variations, and positions 5 and 8 were found to be the most restrictive. Moreover, a preference for hydrophobic side chains was found for positions 1–3, 5, and 8. The crystal structures of H-2Kb in complex with three different peptides revealed detailed information about the interactions of peptides and MHC residues, confirming the above-mentioned functional studies (4, 8).

In recent years, strategies for the automated and combinatorial synthesis of molecular libraries of different classes of organic molecules have been developed (for review, see Ref. 13). In a search for new compounds with desired features, e.g.
therapeutic activity, such libraries allow for high throughput screening of a large number of different molecules. Library approaches are being applied to identify alternative ligands of various receptors. Combinatorial peptide libraries were used to elucidate the rules for peptide selection by MHC molecules and to determine synthetic T cell receptor epitopes (10, 14).

For a vigorous test of the indicated strategy, this work has focused on the structurally most constrained C-terminal part of H-2K\(^b\)-binding peptides (positions 5–8). The peptide amide backbone was replaced by poly-N-acylated amine (PAA) elements. Many efforts have been made to generate new classes of peptidomimetics (15, 16). In comparison with peptides, synthetic non-peptide oligomers display new physicochemical properties. Modifications of the amide backbone can solve the problems of rapid enzymatic degradation of peptides and low bioavailability. The design and synthesis of new peptidomimetics offer the possibility to choose building blocks different from natural amino acids. Depending on the chemical class of the monomers, protocols with efficient, high-yielding coupling reactions on solid support need to be developed. These strategies have been successfully applied, for example, to the preparation of oligocarbamates, oligosulfones, and peptoids (17, 18). Non-natural peptides involved in the stabilization of the MHC-I molecules can be considered as the starting point for the discovery of new immune modulators such as antitumor vaccines and T cell receptor antagonists (19–24).

Several oligomers and five types of randomized molecular libraries were synthesized and assayed. First, the optimal binding geometry was determined by testing a series of 12 defined structures with different main chain variations (PAA1–PAA12) (Fig. 1). Second, based on the structure with the highest binding efficiency, randomized libraries with 26 different aromatic side chains in position 5 (see Table I) and four different aliphatic side chains in position 8 were tested. Finally, one library was deconvoluted to identify the best H-2K\(^b\) ligand. The objective of this study was to establish a combinatorial strategy including oligomer screening, randomized libraries, and systematic deconvolution for the determination of non-peptide ligands for the highly specialized peptide receptor MHC molecules.

**EXPERIMENTAL PROCEDURES**

**Synthesis of Poly-N-acylated Amines (PAA1–PAA12)**—The synthesis of the poly-N-acylated amines was performed using two different strategies, which are illustrated in Schemes 1 and 2. All reagents, amino acids, and solvents were purchased from Fluka (Buchs, Switzerland), Aldrich, Novabiochem (Läufelfingen, Switzerland), and Merck (Darmstadt, Germany). Wang resin and trityl chloride resin were obtained from Rapp Polymere (Tübingen, Germany) and PepChem (Tübingen). Reagent K is a mixture of 82.5% trifluoroacetic acid, 5% (w/v) phenol, 5% (w/v) thioanisole, 2.5% (w/v) ethanedithiol, and 5% (w/v) water. Trityl-monoprotected diamines were prepared from triphenylmethyl chloride and 1,3-diaminopropane or 1,4-diaminobutane in dichloromethane (DCM). The ligands were synthesized manually in small syringes fitted with a frit up until the introduction of the amino acids at the N-terminal part; this was performed on a simultaneous multiple peptide synthesizer (SMPS 350, Zinsser Analytic, Frankfurt, Germany; Software Syro, MultiSynTech, Bochum, Germany) using Fmoc/tert-butyl strategy (25). After cleavage from the resin and precipitation with cold diethyl ether, the compounds were lyophilized from tert-butyl alcohol/water (4:1, v/v) and characterized by analytical reverse-phase HPLC and electrospray mass spectrometry.

**Synthesis and Deconvolution of the Libraries**—The library (SII-X–X) based on the molecular structure (Ser\(^1\)-Ile-Ile-spacer-Xaa\(^8\)) of the ligand PAA6 was synthesized following the strategy of Scheme 2. Four batches of trityl chloride resin were functionalized with Leu, Ile, Val, and Met, respectively. Equimolar amounts of each resin were combined, and the oligomers were elongated. For the acylation of the secondary amine, a mixture of aromatic, heteroaromatic, and pseudoaromatic carboxylic acids (see Table I), equimolar with the coupling sites on the resin, was preactivated with 1-hydroxybenzotriazole/diisopropylcarbodiimide (DIC) in N,N-dimethylformamide (DMF) for 30 min and subsequently added to the resin. After 90 min of coupling, the acylation was repeated using a 5-fold molar excess of the same mixture. The related PAA sublibraries, in which the C-terminal amino acids leucine (SII-X–L), isoleucine (SII-X–I), valine (SII-X–V), and methionine (SII-X–M) were
Non-peptide MHC ligands

RESULTS

Non-peptide elements were introduced at the C-terminal part of the SIINFEKL peptide, with the dominant anchor amino acids at positions 5 (phenylalanine) and 8 (leucine). Peptide positions 5–8 (FEKL), 4–8 (NFEKL), or 4–7 (NFKE) were replaced by different linear polyamines acylated with side chains mimicking the anchoring amino acids. First, the optimal geometry was determined using a series of 12 compounds with defined structures (Fig. 1). Then, based on the oligomer with the highest binding efficiency, randomized libraries with different side chains mimicking the anchor amino acids, phenylalanine and leucine, respectively, were tested. Finally, good binders were identified after deconvolution of the most potent sublibrary. All these compounds were tested for biological activity in MHC stabilization assays with H-2Kb-expressing peptide-deficient RMA-S cells.

Synthetic Strategies—The synthesis of novel non-natural polymers should be designed to be suitable for solid-phase methods. The efficiency of the coupling procedures is of fundamental importance to obtain oligomers with a satisfactory purity for biological tests without extensive purification.

Two different solid-phase strategies, illustrated in Schemes 1 and 2, have been designed and used in the synthesis of a new class of MHC-I ligands, PAAs. Initially, the oligomers PAA1–PAA3 were prepared on solid support using trityl-protected diamines (Scheme 1). Then, we modified the reaction procedures for the synthesis of PAA4–PAA12 (Scheme 2) using free diamines and (4,4-dimethyl-2,6-dicyclohexylidene)ethyl as the protecting group. This change in strategy was based on the following observations. First, the trityl group on the amines is less acid labile than Nα-trityl-amino acids; in fact, double treatment with 8% Reagent K in DCM for 20 min caused a partial cleavage of the oligomers from the resin. Second, solution synthesis of the monoprotected diamines can be avoided with the (4,4-dimethyl-2,6-dicyclohexylidene)ethyl protecting group for primary amines because it can be easily coupled directly on the resin and is selective in the presence of secondary amines (29, 30). Both methods of synthesis are straightforward, and the compounds were obtained in good purity. The second strategy (Scheme 2) was applied to the synthesis of randomized libraries. The design of poly-N-acylated amine libraries was based on the possibility of using commercially available building blocks, namely aromatic, heteroaromatic, and pseudoaromatic carboxylic acids (Table 1).

Determination of the Optimal Main Chain Geometry—The molecular structures of the 12 compounds designed, synthesized, and tested to determine the lead structure for the subsequent randomized libraries are given in Fig. 1. The main structures are linear polyamines with the side chains for the anchor positions introduced by condensation with organic acids that mimic the side chain functionalities of the anchor amino acids. The backbone length was varied. The main chain segment between the anchor positions contains one (PAA1–PAA4 and PAA9–PAA12) or two (PAA5–PAA8) amide bonds to promote hydrogen bonding between the ligand and MHC side chains. The spacers have a higher degree of conformational freedom compared with the peptide main chain. The length of the spacer, the position of the aromatic side chain, and the position of the additional amide bond were varied in order to determine the optimal spacing geometry. PAA1, PAA2, PAA6, PAA8, PAA10, and PAA12 have, in comparison with PAA3, PAA4, PAA5, PAA7, PAA9, and PAA11, an additional methylene group in the spacer between the carbonyl and the aromatic side chain. PAA1–PAA3 have, in comparison with PAA4, an additional methylene group in the C-terminal aliphatic side chain.
Eight of the 12 poly-N-acylated amines (PAA5–PAA12) bind to the MHC molecule and stabilize its conformation, namely all compounds with the original leucine in position 8 and a total main chain length of 24 atoms (Fig. 3). The concentration required for half-maximal H-2Kb stabilization ($C_{stab50}$) by the most potent compound PAA6 is $1 \times 10^{-2}$ M; the corresponding value for SIINFEKL is $1 \times 10^{-8}$ M. The oligomers with isovaleric (PAA1–PAA3) or isobutyric (PAA4) acid instead of leucine at the C terminus and a total main chain length of 25 (PAA3 and PAA4), 26 (PAA1), or 28 (PAA2) atoms do not stabilize H-2Kb. The compounds with an additional amide bond inside the spacers and the aromatic side chain at the same position as in the parent SIINFEKL peptide (PAA5–PAA8) display a higher MHC stabilization capacity than those without the additional amide bond and with the position of the aromatic side chain shifted by one atom (PAA9–PAA12). Variation of the main chain does not reveal a clear preference for the amide bond position (PAA5 versus PAA7, PAA6 versus PAA8). The compounds with the position of the aromatic side chain shifted one atom toward the N terminus (PAA9 and PAA10) have a higher binding efficiency than those with a shift toward the C terminus (PAA9 and PAA10). Most of the compounds with phenylacetic acid, with a higher side chain flexibility due to an additional methylene group, have a higher binding efficiency.
than those with benzoic acid as the acylating agent mimicking the phenylalanine side chain (PAA5 versus PAA6, PAA7 versus PAA8, PAA11 versus PAA12). The compound with the highest binding efficiency, PAA6, which was chosen as a lead structure for the subsequent studies, has a phenylacetyl-like side chain at the central anchor position and the original leucine at the C-terminal position. This oligomer is thus composed of 3 amino acids at the N terminus (serine, isoleucine, and isoleucine) and 2 amino acids (glycine and leucine) at the C terminus. The central part with the aromatic side chain has an acylated polyamine structure. Moreover, the internal amide bond that contributes to stabilize the MHC complex is in the same backbone position as in the natural SIINFEKL epitope (peptide bond between Glu6 and Lys7).

Randomization and Deconvolution of MHC-I-binding Poly-N-acylated Amines—Based on PAA6, libraries with randomized side chains were designed. For the central anchor position, 26 building blocks (Table I) were selected, mixed, and built in to obtain a randomized position. Similarly, random mixtures of leucine, isoleucine, methionine, and valine were introduced at the C-terminal position. The library randomized at both positions has the format SII-X-X and contains all possible permutations of the components, thus 104 compounds. The sublibraries SII-X-L, SII-X-I, SII-X-V, and SII-X-M each contain only 1 of the 4 C-terminal amino acids (and thus 26 compounds each). All five libraries are slightly less potent than PAA6 (Fig. 4). The sublibrary with the highest binding efficiency, SII-X-L, was deconvoluted to give the compounds PAA6 and PAA13–PAA37 (Fig. 5). The compounds PAA13, PAA15–PAA17, PAA19, PAA22–PAA26, PAA29, and PAA32–PAA35 have similar binding efficiencies as the parent library (SII-X-L) and the original compound (PAA6). The compounds PAA18, PAA20, PAA31, and PAA36 are more potent binders, whereas PAA14, PAA21, PAA27, PAA28, PAA30, and PAA37 are less potent. Consistent with the previous results, the inferior compounds have benzyol-like side chains, the aromatic ring being directly linked to the carbonyl group. Moreover, spacious polar para-substitutions at the aromatic ring confer reduced binding efficiencies. The superior binders have an additional methylene group in the side chain. para-Substitution in the phenyl ring by a methoxy group (PAA22) or a fluorine atom (PAA20, best binder) results in increased binding efficiency. The pocket in the MHC peptide-binding groove for the central anchor side chain can also accommodate bulkier aromatic moieties, such as naphthyl (PAA31, PAA34, PAA36, and PAA37), benzofurane (PAA21), quinoline (PAA25), indole (PAA26), and biphenyl (PAA32). Two of the good binders (PAA31 and PAA36) have a naphthyl group.

**DISCUSSION**

Combinatorial library approaches are applied increasingly in the development of new compounds with desired biological activities (13). The general principle is based on the generation of diversity by randomization to find a lead structure and subsequent selection of specific compounds active, for instance,
in receptor-ligand interaction. Randomization and subsequent deconvolution allow for systematic high throughput screening of a large number of different structures. The major steps in the generation of diverse libraries are as follows: first, the definition of a suitable parent structure and, second, the synthesis of randomized libraries. The selection of specific effector molecules is restricted on the one hand by the complexity of the randomized libraries and on the other hand by the sensitivity and the specificity of the readout system. Complex development problems might require iterative approaches with successive steps to define the parent structures scaffolds and to optimize the efficiency with different classes of molecular libraries. The success of this approach crucially depends on the right balance between the complexity of the libraries on one hand and the selectivity of the test system on the other. The number of steps for testing randomized and defined structures necessary to get clear results (and thus the optimal approach to a specific question) is dependent on the test system.

Randomized peptide libraries were successfully applied to examine different questions concerning the interaction of the MHC and peptide ligand (7). The very restrictive interaction of peptide and MHC molecules (31) makes it very challenging to search for new peptidomimetic ligands for this highly specialized peptide receptor. Replacing the peptide by polyamine structure results in more conformational freedom. The additional flexibility can aid the identification of new biologically active compounds. On the other hand, the increased numbers of conformational variants will reduce the efficiency of the screening system. This increased flexibility could be one reason for the lower binding efficiencies of the poly-N-acylated amines compared with SIINFEKL.

The following approach was chosen to solve this problem. Non-peptide ligands for MHC-I H-2Kb were developed by, first, tailoring the optimal main chain spacing and, second, determining the best side chain to mimic the dominant anchors by deconvolution of randomized libraries. This approach was previously successful for peptides (7). The aim of this study was to test its applicability also for non-peptide structures. This work includes, first, the definition of a suitable oligomeric parent structure scaffold; second, the demonstration that randomized peptidomimetic structures can exhibit biological activity; and third, results showing that defined structures can be derived from the randomized libraries. Poly-N-acylated amines were designed based on the structure of a model octapeptide. The nature of the polymer backbone and the side chain functionalities are of fundamental importance in the design of new ligands. The new framework was designed in order to exploit readily available building blocks and efficient coupling reactions. The protocol for the preparation of such poly-N-acylated amines can be fully automated. The developed synthetic strategies were successfully applied to the synthesis of randomized libraries.

The central dominant anchor amino acid as well as the backbone at the C-terminal or the central part of the ligand were replaced by non-peptide elements. The structural requirements were determined with two different classes of molecules: a series of defined oligomers with main chain variations and randomized libraries with side chain variations. MHC-binding and MHC-stabilizing compounds were successfully derived from the randomized libraries by deconvolution. The concept was then proven to be suitable for the development of a new class of MHC ligands.

The results presented here reveal new aspects of the molecular interaction between the MHC and ligand. Increasing the main chain length and replacing the C-terminal leucine by a polyamine structure result in a complete loss of MHC stabilization. This finding emphasizes the importance of the terminal amino acids for MHC binding. Poly-N-acylated amine binding is improved by including amide bonds in the main chain, supporting earlier reports on the significance of hydrogen bonding MHC side chains and the peptide backbone. The exact position of the amide bond appears to be less important for the binding capacity. This observation might be a reflection of the crystallographic finding that some of the hydrogen bonds are indirect, mediated by incorporated water molecules (4, 8). Testing different...
ferent aromatic, heteroaromatic, and pseudoaromatic side chains for the central anchor position revealed interesting new insights into the structural requirements for this position. First, a high flexibility of the side chains was found to be beneficial for the binding, as can be concluded from the comparison of the phenylacetic acid-like with the benzoic acid-like side chains. It is reasonable to assume that the steric constraints of the binding pocket can be fulfilled easier by more flexible side chains. The specific molecular interactions of the aromatic amino acid at the dominant central anchor position of H-2K\(^{b}\)-binding peptides with MHC side chains are of a hydrophobic and aromatic nature (4, 31). Interestingly, polyamine structures containing side chains with large aromatic systems are among the best binders (PAA31 and PAA36 with a naphthyl group). Employing an alternative strategy for designing and incorporating non-peptide elements into MHC-binding peptides, Weiss et al. (24) substituted 3–5 central amino acids of HLA A2.1-, HLA Aw68-, and HLA B27-binding nona- or decapeptides with the tricyclic aromatic compound phenanthridine. The phenanthridine was part of the main chain and incorporated into a central position of the peptide between the two dominant anchor amino acids at positions 2 and 9. These epitope variants bound to the MHC molecules and formed relatively stable crystallizable compounds. In our study, we specifically targeted one of the dominant anchor positions of H-2K\(^{b}\)-binding peptides, which had to fulfill much more stringent structural requirements for fitting into the corresponding pocket of the peptide-binding groove of the MHC molecule. Moreover, Weiss et al. incorporated the peptide variants into MHC molecules expressed in \textit{Escherichia coli} and reconstituted in the presence of the mixed oligomers. Binding of the compounds used in the present study was done with prefolded intact MHC molecules, leaving less room for artificial adaptation of the MHC molecules.

The peptide-MHC complex plays a central role in T cell immunity (1). Thus, MHC-binding oligomers may have diverse immunomodulatory functions, e.g. as T cell antagonists or MHC blockers in the treatment of autoimmune diseases (32). The increased stability of non-peptide structures to enzymatic degradation should enhance bioavailability for therapeutic applications. In our study, the amino acids that are most important for T cell recognition were replaced by simple linear hydrocarbon spacers. Introduction of additional side chains in these positions could possibly lead to structures with defined effects on T cells, such as enhanced activation (as vaccines) or induction of anergy (as T cell receptor antagonists). Hereby, it is not necessary that the molecules bind tightly to MHC molecules. Besides their potent roles for immunotherapy, poly-N-acylated amines could also be developed for other applications such as design of new hormone receptor antagonists or enzyme inhibitors.

REFERENCES

1. Barber, L. D., and Parham, P. (1993) \textit{Annu. Rev. Cell Biol.} 9, 163–206
2. Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L., and Wiley, D. C. (1987) \textit{Nature} 329, 506–512
3. Binz, T., and Wiley, D. C. (1994) \textit{Science} 265, 398–402
4. Fremont, D. H., Matsumura, M., Stura, E. A., Peterson, P. A., and Wilson, I. A. (1992) \textit{Science} 257, 919–927
5. Madden, D. R., Garbozzi, D. N., and Wiley, D. C. (1993) \textit{Cell} 75, 693–708
6. Fahnestock, M. L., Tamir, I., Narhi, L., and Bjorkman, P. J. (1992) \textit{Science} 258, 1668–1662
7. Udaka, K., Wiesmüller, R.-H., Kienle, S., Jung, G., and Walden, P. (1995) \textit{J. Biol. Chem.} 270, 24130–24134
8. Fremont, D. H., Stura, E. A., Matsumura, M., Peterson, P. A., and Wilson, I. A. (1995) \textit{Proc. Natl. Acad. Sci. U. S. A.} 92, 2479–2483
9. Carbone, F. R., and Bevan, M. J. (1989) \textit{J. Exp. Med.} 169, 603–612
10. Udaka, K., Wiesmüller, R.-H., Kienle, S., Jung, G., and Walden, P. (1995) \textit{J. Exp. Med.} 181, 2097–2108
11. Falk, K., Rötzsche, O., Stevanovic, S., Jung, G., and Rammensee, H. (1991) \textit{Nature} 351, 296–299
12. Jameson, S. C., and Bevan, M. J. (1992) \textit{J. Pept. Sci.} 8, 37, 41, 47, 51, 55
13. Jung, G. (1996) \textit{Combinatorial Peptide and Non-peptide Libraries}, VCH Verlagsgesellschaft mbH, Weinheim, Germany
14. Walden, P., Wiesmüller, R.-H., and Jung, G. (1995) \textit{Biochem. Soc. Trans.} 23, 678–681
15. Cho, C. Y., Moran, E. J., Cherry, S. R., Stephans, J. C., Fodor, S. P. A., Adams, C. L., Sundaram, A., Jacobs, J. W., and Schultz, P. G. (1993) \textit{Science} 261, 1303–1305
16. Zuckermann, R. N. (1993) \textit{Curr. Opin. Struct. Biol.} 3, 580–584
17. Moran, E. J., Wilson, T. E., Cho, C. Y., Cherry, S. R., and Schultz, P. G. (1995) \textit{Polyklyonides} 37, 213–219
18. Simon, R. J., Kania, R. S., Zuckermann, R. N., Huebner, V. D., Jewell, D. A., Banville, S., Ng, S., Wang, L., Rosenberg, S., Marlowe, C. K., Spellmeyer, D. C., Tan, R., Frankel, A. D., Santi, D. V., Cohen, F. E., and Bartlett, P. A. (1992) \textit{Proc. Natl. Acad. Sci. U. S. A.} 89, 3067–3071
19. Guichard, G., Calbo, S., Muller, S., Kourilsky, P., Briand J.-P., and Abastado, J.-P. (1995) \textit{J. Biol. Chem.} 270, 26057–26059
20. Guichard, G., Connon, F., Graff, R., Ostankovitch, M., Muller, S., Guillet, J.-G., Choppin, J., and Briand, J.-P. (1996) \textit{J. Med. Chem.} 39, 2030–2039
21. Durr, H., Goodman, M., and Jung, G. (1992) \textit{Angew. Chem. Int. Ed. Engl.} 31, 785–787
22. Rognan, D., Scapozza, L., Falkers, G., and Daser, A. (1995) \textit{Proc. Natl. Acad. Sci. U. S. A.} 92, 755–757
23. Bianco, A., Zehel, C., Walden, P., and Jung, G. (1998) \textit{J. Pept. Sci.}, in press
24. Weiss, G. A., Collins, E. J., Garbozzi, D. N., Wiley, D. C., and Schreiber, S. L. (1995) \textit{Chem. Biol.} 2, 401–407
25. Jung, G., and Beck-Sickinger, A. G. (1992) \textit{Angev. Chem. Int. Ed. Engl.} 31, 367–383
26. Metzger, J. W., Kempter, C., Wiesmüller, K.-H., and Jung, G. (1994) \textit{Anat. Biochem.} 219, 261–277
27. Anderson, G. W., and Callahan, F. M. (1960) \textit{Science} 89, 927–934
28. Franzén, H., Grehn, L., and Ragnarsson, U. (1984) \textit{Chem. Soc. Chem. Commun.} 1699–1700
29. Nash, I. A., Byroth, B. W., and Chan, W. C. (1996) \textit{Tetrahedron Lett.} 37, 2625–2628
30. Augustyns, K., Kraas, W., and Jung, G. (1998) \textit{J. Pept. Res.} 51, 127–133
31. Matsumura, M., Fremont, D. H., Peterson, P. A., and Wilson, I. A. (1992) \textit{Science} 257, 927–934
32. Franco, A., Southwood, S., Arhenius, T., Kuchroo, V., Grey, H. M., Sette, A., and Ishiioka, G. I. (1994) \textit{Eur. J. Immunol.} 24, 940–946