Peptoid – Peptide Hybrids That Bind Syk SH2 Domains Involved in Signal Transduction

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Peptoid – peptide hybrids are oligomeric peptidomimetics that contain one or more N*-substituted glycine residues. In these hybrids, the side chains of one or several amino acids are "shifted" from the* α*-carbon atom to the amide nitrogen atom. A library of phosphorylated peptoid – peptide hybrids derived from the sequence pTyr-Glu-Thr-Leu was synthesized and tested for binding to the tandem SH2 domain of the protein tyrosine kinase Syk. A considerable influence of the side chain position was observed. Compounds* ***19*** *–* ***21***, ***24***, *and* ***25*** *comprising a peptoid NpTyr and/or NGlu residue did not show any binding. Compounds* ***22***, ***23***, *and* ***26*** *containing an NhThr (hThr = homothreonine) and/or NLeu peptoid residue showed binding with* IC_{50} *values that were only five to eight times higher than that of the tetrapeptide lead compound* ***18****. These data show that side chain shifting is possible with retention of binding capacity, but only at the two C-terminal residues of the tetramer. This method of a peptoid scan using peptoid – peptide hybrids appears to be very useful to explore to what extent a peptide sequence can be transformed into a peptoid while retaining its affinity.*

KEYWORDS:

peptides · peptidomimetics · peptoids · SH2 domains · signal transduction

Introduction

Peptoids are oligomeric peptidomimetics that consist of *N*-substituted glycine residues. In recent years peptoids have been explored to mimic peptides structurally and biologically.[1–9] An improved stability[3, 10, 11] was found, for example, against a series of proteases.[12] Furthermore, peptoids are more hydrophobic than the corresponding peptides,[11] which makes them suitable candidates in the search for peptidomimetics that target intracellular receptors. For instance, it has been demonstrated that methylation of amide groups in the peptide backbone increased their ability to permeate into Caco (colon carcinoma) cells.[13]

In peptoid – peptide hybrids one or several amino acid residues are substituted by *N*-substituted glycine residues. These compounds can be used to explore to what extent a peptide sequence can be transformed into a peptoid without loss of its binding capacity. This is designated as a "peptoid scan", which is defined as a systematic replacement of one to several amino acid residues by the corresponding peptoid residues, that is *N*-substituted glycine derivatives having *N*-substituents that are virtually identical to the amino acid side chains. As a consequence, it seems that in the structure of peptoids and peptoid – peptide hybrids the side chain of an amino acid residue has now "shifted" in the backbone from the α-carbon atom to the amide nitrogen atom in the peptoid residue. In this study we have investigated the effect of side chain shifting in the phosphotyrosine-containing tetramer Ac-pTyr-Glu-Thr-Leu-NH_2 on binding to the protein tyrosine kinase (PTK) Syk.

The protein Syk ($p72^{Syk}$) is a protein tyrosine kinase that is closely related to the 70-kDa zeta-associated protein ZAP-70, a tyrosine kinase that plays an essential role in the activation of T-cell receptors.[14] This family of PTKs possesses two Src homology 2 (SH2) domains. SH2 domains are protein domains that are found in cytosolic tyrosine kinases, phosphotyrosine phosphatases, and several other proteins that play crucial roles in signal transduction.[15] SH2 domains contain approximately 100 amino acids and are capable of binding to specific tyrosine-phosphorylated protein sequences.

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Syk plays a crucial role in the signal transduction of the high-affinity receptor for IgE (FcεRI), present on mast cells and basophils.[16–18] FcεRI is an important initiator of type-I allergic reactions. This multisubunit receptor consists of an α, β, and two disulfide-linked γ chains. The cytosolic tails of both the β and the γ chains contain sequences known as immunoreceptor tyrosine-based activation motifs (ITAMs) that feature the consensus sequence (D/E)X_2YXXLX_{6-7}YXX(L/I). In general, ITAMs play an important role in signal transduction cascades of immunoreceptors functioning as docking sites for signal-propagating enzymes or other effector proteins that contain SH2 domains. In FcεRI signaling Syk binds to the diphosphorylated γITAM motif (KADAVpYTGLNTRSQETpYETLKHEK).[18, 19] This tandem SH2-domain-mediated association localizes Syk to the plasma membrane and stimulates Syk kinase activity.[18, 20, 21] Activation of Syk plays a key role in initiating a cascade of events which results in mast cell degranulation, leading to the release of inflammatory mediators.[20, 22–25] It is hypothesized that prevention of Syk recruitment to the phosphorylated receptor by SH2 domain blockers will inhibit mast cell activation.

Both tyrosines of the FcεRI γITAM need to be phosphorylated to enable the high-affinity interaction with the tandem SH2 domain of Syk.[18, 19, 26] However, monophosphorylated peptides are also able to compete for binding although affinities are reduced by three to four orders of magnitude to the micromolar range.[26] The lower affinity of monodentate compounds in comparison to bidentate compounds is expected to be compensated in vivo by improved absorption as only one charged phosphate group is present.

Here we demonstrate that the tetrapeptide Ac-pYETL-NH_2 (part of the γITAM) is capable of inhibiting the high-affinity binding of the Syk tandem SH2 domain to the diphosphorylated γITAM peptide. The syntheses of a phosphorylated peptoid and a library of peptoid – peptide hybrids derived from this tetrapeptide are described. The effect of side chain shifting on binding, which is the result of the peptide-to-peptoid transformations, is reported.

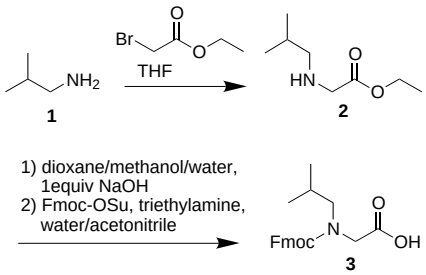
[*] *Members of the Editorial Advisory Board will be introduced to the readers with their first manuscript.*

Results and Discussion

Synthesis of peptoid monomers

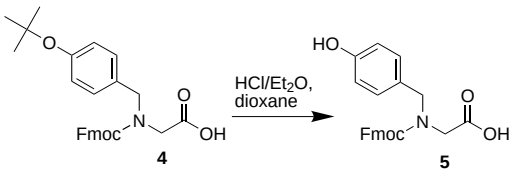
Two methods for the synthesis of peptoids by organic solid-phase chemistry have been described in the literature. In the "submonomer method"[3, 27, 28] the oligomer is prepared on the resin by repeated coupling of bromoacetic acid followed by a substitution reaction with an appropriate amine that comprises the side chain functionality. In the "monomer approach"[3, 11] 9-fluorenylmethoxycarbonyl(Fmoc)-protected *N*-substituted glycines are coupled. Recently we described a monomer approach for the synthesis of peptoids,[11] and this Fmoc-based approach is employed here for the synthesis of the peptoid – peptide hybrids.

The required *N*-substituted glycine derivatives, denoted as peptoid monomers, for the solid-phase synthesis of the peptoid – peptide hybrids were those derived from the amino acids Leu, Tyr, Glu, and Thr: Fmoc-NLeu-OH (**3**), Fmoc-NTyr-OH (**5**), Fmoc-NGlu(O*t*Bu)-OH (**11**), and Fmoc-NhThr(*t*Bu)-OH (**17**; hThr = homothreonine) (Schemes 1, 2, 3, and 4, respectively). The general strategy for peptoid monomer synthesis, exemplified by the preparation of peptoid monomer Fmoc-NLeu-OH (**3**),[11] is shown in Scheme 1. The appropriate amine **1** is alkylated

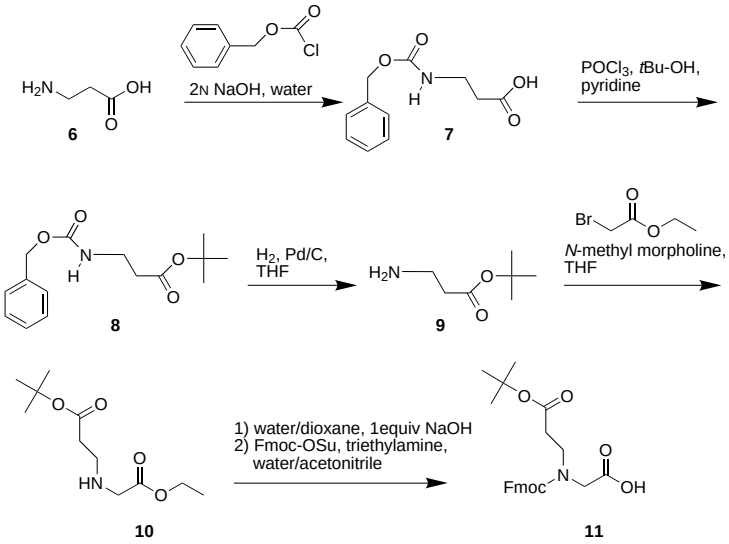


Scheme 1. *General strategy for the synthesis of peptoid monomers, exemplified by the synthesis of Fmoc-NLeu-OH[11] (**3**). Fmoc = 9-fluorenylmethoxycarbonyl; Su = succinimidyl.*

with ethyl bromoacetate to afford the *N*-substituted glycine ethyl ester **2**. Saponification and Fmoc-group attachment gives the peptoid monomer **3**. The other monomers were synthesized analogously. Fmoc-NTyr-OH (**5**) was obtained by synthesizing Fmoc-NTyr(*t*Bu)-OH (**4**) as described previously,[11] followed by removal of the *tert*-butyl protecting group by treatment with HCl/diethyl ether (Scheme 2). As shown in Scheme 3 the NGlu monomer **11** was synthesized starting from β-alanine. The primary amino group of β-alanine was protected with a benzyloxycarbonyl group by using benzyl chloroformate, after which the carboxylic acid function was protected as a *tert*-butyl ester by using phosphorus oxychloride and *tert*-butanol in pyridine. The amine functionality was deprotected by hydro-



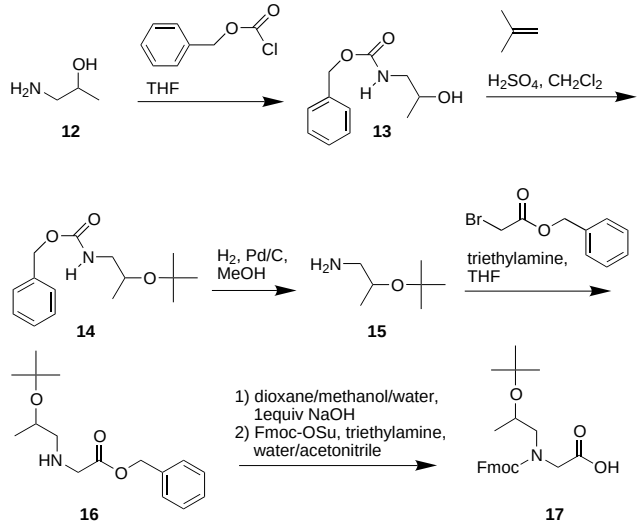
Scheme 2. *Synthesis of Fmoc-NTyr-OH (**5**).*



Scheme 3. *Synthesis of Fmoc-NGlu(OtBu)-OH (**11**).*

genolysis. The resulting β-alanine *tert*-butyl ester was used in the alkylating reaction with ethyl bromoacetate. Saponification and Fmoc-group attachment gave the NGlu peptoid monomer **11**.[29]

The corresponding peptoid analogue of threonine is a glycine residue containing a hemiaminal moiety. Since this derivative cannot be prepared and would be instable, the homothreonine peptoid monomer was used as a suitable analogue. For its preparation a racemic mixture of 1-amino-2-propanol (**12**) was used as starting material (Scheme 4). The amine was protected



Scheme 4. *Synthesis of Fmoc-NhThr(tBu)-OH (**17**).*

with a benzyloxycarbonyl group, after which the hydroxy function had to be protected as a *tert*-butyl ether. Introduction of a *tert*-butyl group on this secondary alcohol **13** was troublesome. A reaction with *tert*-butyl 2,2,2-trichloroacetamidate in the presence of boron trifluoride ethyl etherate gave poor yields (30%). The second alternative method for creation of the *tert*-butyl carbenium ion by using *tert*-butanol, magnesium sulfate, and sulfuric acid[30] afforded slightly higher but still poor yields (40%). The method with isobutylene and sulfuric acid, which was finally used, resulted in 60% yield. The amine functionality was deprotected by hydrogenolysis in the presence of palladium on carbon. The resulting amine was alkylated with benzyl bromoacetate. Saponification and attachment of the Fmoc group gave the NhThr peptoid monomer **17**. Since we started from a racemic mixture of 1-amino-2-propanol, the NhThr peptoid monomer was also obtained as a racemate.

Synthesis of peptoid – peptide hybrids

Peptoid **19** and peptoid – peptide hybrids **20** – **26** (Tables 1 and 2) were synthesized manually on Argogel resin, containing the acid-labile Rink linker.[31, 32] As a coupling reagent bromotrispyrrolidinophosphonium hexafluorophosphate (PyBroP) was chosen, which is especially suited for the synthesis of peptides containing hindered amino acids or *N*-methyl amino acids.[11, 33] All coupling reactions were followed by using the Kaiser or chloranil[60] test and lasted 45 min on average.

The phosphotyrosine amino acid was incorporated as a monobenzyl-protected Fmoc-phosphotyrosine (Fmoc-Tyr(PO(OBzl)OH)-OH). When a phosphotyrosine peptoid monomer was required, the Fmoc-NTyr-OH monomer was introduced without protection of the phenolic hydroxy group, which was

Table 1. *IC_{50} and K_d values of peptoid – peptide hybrids comprising one shifted side chain.*

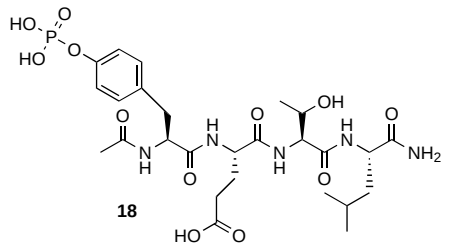
Compd	Sequence	IC_{50} [μM]	K_d [μM]	Rel. IC_{50}[a]
18	Ac-pTyr-Glu-Thr-Leu-NH_2	117	27	1
20	Ac-*NpTyr*-Glu-Thr-Leu-NH_2	$\gg 1000$[b]	$\gg 250$	$\gg 10$
21	Ac-pTyr-*NGlu*-Thr-Leu-NH_2	$\gg 1000$[b]	$\gg 250$	$\gg 10$
22	Ac-pTyr-Glu-*NhThr*-Leu-NH_2	545	119	5
23	Ac-pTyr-Glu-Thr-*NLeu*-NH_2	986	239	8

[a] IC_{50} values relative to that of reference compound **18** (= 1). [b] No inhibition detected at 1000 μM.

Table 2. *IC_{50} and K_d values of peptoid – peptide hybrids comprising two shifted side chains.*

Compd	Sequence	IC_{50} [μM]	K_d [μM]	Rel. IC_{50}[a]
18	Ac-pTyr-Glu-Thr-Leu-NH_2	117	27	1
24	Ac-*NpTyr*-*NGlu*-Thr-Leu-NH_2	$\gg 1000$[b]	$\gg 250$	$\gg 10$
25	Ac-pTyr-*NGlu*-*NhThr*-Leu-NH_2	$\gg 1000$[b]	$\gg 250$	$\gg 10$
26	Ac-pTyr-Glu-*NhThr*-*NLeu*-NH_2	954	233	8

[a] IC_{50} values relative to that of reference compound **18** (= 1). [b] No inhibition detected at 1000 μM.



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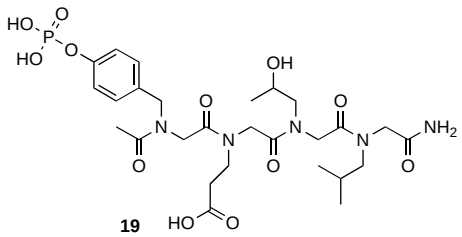

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subsequently phosphorylated on the solid support by using di-*tert*-butyl-*N*,*N*-diethylphosphoramidite.[34–36] Oxidation by using *meta*-chloroperbenzoic acid (MCPBA) afforded the peptoid–peptide hybrid containing the phosphorylated tyrosine residue. After Fmoc deprotection and acetylation of the N terminus the peptides were deprotected and cleaved from the resin. In the case of compounds **25** and **26**—containing the racemic NhThr peptoid residue—it was possible to separate the epimers by reversed-phase HPLC.

Surface plasmon resonance binding studies

To examine the effect of side chain shifting in a phosphopeptide on binding to the Syk tandem SH2 domain, the endogenous bidentate interaction with the γ-chain of the high-affinity receptor for IgE was chosen as a model platform. A glutathione *S*-transferase (GST) fusion protein containing both SH2 domains of murine Syk[37] (Syk GST-tdSH2[38]) was cloned, expressed, and purified (Figure 1a) as described in the Experimental Section. The affinity of this protein for a peptide encoding the γITAM motif of the high-affinity receptor for IgE (biotin-Ahx-KADAVpYTGLNTRS-QETpYETLKHEK-NH_2; Ahx = aminohexanoic acid) was measured by surface plasmon resonance (SPR) spectroscopy at equilibrium

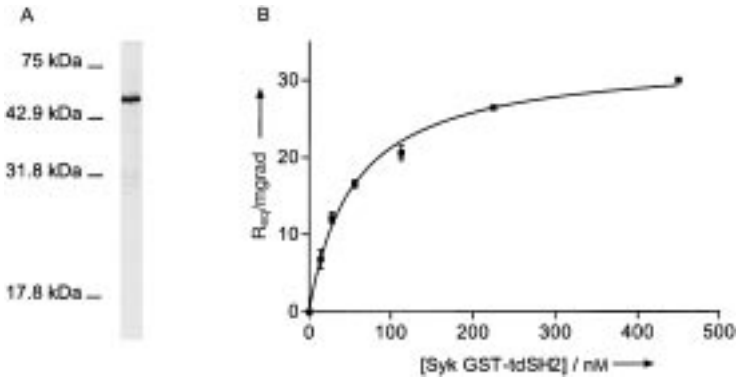

Figure 1. *A: SDS-PAGE analysis (Coomassie Blue staining) of murine Syk GST-tdSH2 expressed in* E. coli. *B: Binding of recombinant Syk GST-tdSH2 to the biotinylated and diphosphorylated γITAM peptide that was immobilized on an avidin-coated SPR chip. The data in the binding isotherm represent the mean equilibrium SPR values (R_{eq}, expressed in milligrad (mgrad)) of triplicate determinations.*

(Figure 1b). The binding constant for the interaction on the chip (K_d) was 33 ± 4 nM ($n = 5$).

In the competition experiments with the peptoid–peptide hybrids, peptide **18** was taken as a reference giving an IC_{50} value of 117 μM and a K_d of 27 μM. These values are of the same order of magnitude as the IC_{50} value reported by Narula et al.[39] for the competition of the single C-terminal SH2 domain of human Syk binding to a monophosphorylated γITAM peptide. Chen et al.[26] used a scintillation proximity-based assay (SPA) to determine IC_{50} values by immobilizing the tdSH2 domain on a solid support and monitoring the binding of the γITAM peptide. When these authors used a monophosphorylated γITAM peptide comprising the pYETL sequence, an IC_{50} value of 22 μM was found. Although the competing peptide they used is much more C- and N-terminally extended than compound **18**, the IC_{50} value is of the same order of magnitude.

Peptoid **19**, in which all side chains present in the corresponding peptide **18** are shifted to the backbone nitrogen atom, did not show any inhibitory activity up to a concentration of 1.5 mM. This result raised the question to what extent the Ac-pTyr-Glu-Thr-Leu-NH_2 peptide can be transformed into a peptoid while retaining its affinity for the Syk tandem SH2 domain, that is, which side chains are allowed to be shifted on the backbone and which are not.

First, a set of peptoid–peptide hybrids (**20**–**23**) containing one peptoid residue was evaluated for binding in a preliminary screening at a concentration of 1 mM. Subsequently, IC_{50} and K_d values were determined for the compounds that showed inhibition. As shown in Table 1, the hybrids comprising an NpTyr or NGlu peptoid residue (**20** and **21**, respectively) do not bind tandem SH2, but the ones with an NhThr or NLeu peptoid residue (**22** and **23**, respectively) were active. Apparently, the shifting of one of either of the two side chains at the pY and pY + 1 positions resulted in a loss of binding capacity. On the other hand, comparable modifications at the pY + 2 or pY + 3 positions had a less dramatic influence on binding. The binding capacity of the NLeu compound **23** was eight times lower as compared to that of the reference peptide **18**. The influence of transforming the Thr residue into an NhThr peptoid residue was even less: a fivefold reduction in the IC_{50} value.

To investigate if more peptoid residues can be incorporated, compounds **24**–**26** containing two adjacent peptoid residues were also tested. As shown in Table 2, compounds **24** and **25** showed no binding. Both compounds contain a shifted pTyr and/or Glu side chain, which—similar to compounds **19**–**21**—led to a greater than tenfold decrease in binding affinity as compared to that of the reference peptide **18**. However, binding was observed with compound **26** in which the side chains of both Thr and Leu residues near the C terminus were shifted. As stated above, both compound **25** and **26** were obtained as a mixture of epimers, but in both cases the epimers could be separated by HPLC. In the initial SPR screening experiment both epimers of compound **25** were examined separately and both showed no inhibition. The separated epimers of compound **26** showed the same degree of inhibition in the screening.

In summary, the tetrapeptide Ac-pTyr-Glu-Thr-Leu-NH_2 was capable of competing in the high-affinity bidentate interaction of a Syk tdSH2 protein with a diphosphorylated γITAM peptide. After translation of this peptide sequence into the peptoid **19**, in which all side chains are shifted, the activity in the binding assay was lost. However, some peptoid – peptide hybrids, which are only partly translated, were still capable of competing. Interestingly, shifting of the Thr and/or Leu side chains to the backbone nitrogen atom is possible, although with some loss of affinity, whereas the same shift of the pTyr or Glu side chains was deleterious. A reason for the generally reduced binding potency of the peptoid – peptide hybrids can be found in the increased flexibility of the backbone. This is indicated by NMR spectroscopic experiments with other peptoids, which show the presence of many conformations owing to rotations about the tertiary amide bonds.[3, 11, 40] However, this increased flexibility does not explain the differences in binding affinities of the peptoid – peptide hybrids.

We think the following two factors should be considered in explaining the differences in binding affinities of the peptoid – peptide hybrids. First, the relative distances between side chains with respect to their position on the backbone, and second the altered hydrogen bond characteristics of the backbone. For example, the relative distance between the two important side chains of the phosphotyrosine and leucine residues is increased if only the phophotyrosine residue is substituted by a peptoid phosphotyrosine residue. But this distance will be decreased if only the leucine residue is transformed into a peptoid residue. It will not be changed if both residues are transformed. Binding was still observed when the leucine side chain was shifted to the nitrogen atom, but no binding was observed when the phosphotyrosine side chain was shifted. This would suggest that a decrease in relative distance is allowed, but not an increase. However, as the relative distance is not altered in the peptoid **19**, which did not bind, this indicates that the altered relative distance is not the only factor to be considered.

In explaining the differences in affinities, especially the highly reduced binding potency of all hybrids containing an NpTyr and/or NGlu peptoid residue, the altered hydrogen bond characteristics of the backbone should be considered. Fütterer et al.[41] published a crystal structure of the Syk tandem SH2 domain complexed with a diphosphorylated CD3ε ITAM peptide (PDpYEPIRKGQRDLpYSGLNQR), derived from the CD3ε chain of the T-cell receptor. Comparable to other SH2 domain structures,[42, 43] in essence this structure shows (Figure 2) that the peptide conformation is fixed by hydrogen bonds between Arg-αA2 and the backbone carbonyl oxygen atom of pY-1, and between the carbonyl oxygen atom of His-βD4 and the backbone amide hydrogen atom of pY + 1 (in both SH2 domains[44, 45]). In case our peptoid – peptide hybrids comprise an NpTyr peptoid residue, the Arg-αA2 interaction with the peptide backbone can possibly be impaired as the relative positions of the phosphate and the carbonyl group are altered by placing the pTyr side chain on the adjacent nitrogen atom. Furthermore, this may influence the hydrogen bond acceptor property of this carbonyl group. With respect to a Glu → NGlu transformation, formation of the hydrogen bond between His-βD4 and the backbone amide group is impossible as the amide hydrogen atom is substituted by the glutamate side chain.

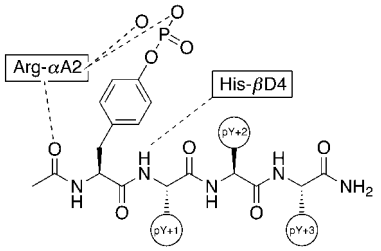

Figure 2. *Schematic representation of hydrogen-bonding interactions (dashed lines) of the Syk tandem SH2 domain with the peptide backbone (found in both C- and N-terminal SH2 domains). This figure is based on the X-ray crystal structure of the Syk tandem SH2 domain complexed with the diphosphorylated CD3ε ITAM peptide published by Fütterer et al.[41] The interactions with the peptide backbone are possibly impaired in case the pY or the pY + 1 residues are transformed into a peptoid residue. Hydrogen-bonding interactions were assigned by the program LIGPLOT[59] and were found in all of the six complexes in the asymmetric unit.*

An ever increasing number of papers report the finding of low molecular weight inhibitors of SH2 domains of the Src family protein tyrosine kinases (reviewed in ref. [46, 47]). This family of kinases contain only one SH2 domain, which in general can bind with high specificity and up to nanomolar affinity to monotyrosine-phosphorylated peptides or peptidomimetics. A much more limited number of papers have reported inhibitors of tandem SH2 domains as are found in PTKs Zap-70 and Syk.[48–51] Among these is the only report of SH2-domain-binding peptoids by Revesz et al.[49] They synthesized a minilibrary of tetrapeptoids containing a phosphotyrosine mimic. The other three peptoid residues contained lipophilic and nonfunctionalized substituents. These compounds were screened for inhibition of the bidentate interaction of the Zap-70 tandem SH2 domain with a diphosphorylated ITAM motif, which is comparable to the interaction studied here. The IC_{50} values were in the micromolar range. The decreased affinities of monophosphorylated (monodentate) compounds as compared to the nanomolar affinities of the bidentate interaction is probably due to the lack of the entropically favorable bidentate effect. This anticipated lower affinity of monophosphorylated inhibitors is expected[49] to be compensated in vivo by an improved metabolic stability,[3, 10–12] which is due to a reduced proteolytic degradation as well as an improved absorption of the peptoid compound having only one charged phosphate group.

Conclusion

We have synthesized a set of phosphorylated peptoid – peptide hybrids derived from the pTyr-Glu-Thr-Leu sequence of the γITAM motif of the high-affinity receptor for IgE. The compounds were tested in a surface plasmon resonance competition assay, in which selected compounds can compete with the high-affinity binding of the Syk tandem SH2 domain to an immobilized γITAM peptide. A considerable influence of the side chain position was observed. Compounds **22**, **23**, and **26** containing an NhThr and/or NLeu peptoid residue showed binding with IC_{50} values that were only five to eight times higher than that for the

tetrapeptide lead compound **18**. These data show that in the Syk tdSH2 domain binding sequence pTyr-Glu-Thr-Leu side chain shifting is possible with retention of binding capacity, but only at the Thr and/or Leu residues of the tetrapeptide. The importance of hydrogen-bonding interactions between the SH2 domains and the peptide backbone, which has been suggested based on structural studies, is in agreement with these findings. In perspective of the improved stability of peptoids as compared to the corresponding peptides, our peptoid–peptide hybrids appear to be very useful in order to explore to what extent a peptide sequence can be transformed into a peptoid while retaining its affinity.

Experimental Section

General: All reagents were purchased from Fluka, Aldrich, or Sigma. Amino acid derivative Fmoc-Tyr(PO(OBzl)OH)-OH was purchased from Nova Biochem, Fmoc-Glu(O*t*Bu)-OH, Fmoc-Thr(*t*Bu)-OH, and Fmoc-Leu-OH were obtained from Advanced Chemtech, and Argogel Fmoc Rink Resin from Argonaut.

Electrospray ionization (ESI) mass spectrometry was carried out with a Shimadzu LCMS QP-8000 single quadrupole benchtop mass spectrometer, coupled with a QP-8000 data system. Exact masses were obtained by nanoelectrospray quadrupole time-of-flight mass spectrometry (nanoES-Q-TOF-MS) which was performed by using a Micromass Q-TOF hybrid mass spectrometer equipped with a Z-spray sample introduction system and gold-coated glass capillaries in a nanospray ionization source. The capillary was loaded with 2 μL of the sample and a potential of +1500 V was applied to it. The source temperature was 80 °C. The quadrupole was used in the RF-only mode. Exact masses were measured by scanning the TOF at a resolution of 6500 FWHM (full width at half-maximum) and using pentaphenylalanine as reference mass. The MS–MS spectra of the tetrapeptides were obtained on the same apparatus. ^{1}H NMR data were obtained on a Varian G-300 spectrometer (300 MHz) and are reported in ppm (δ) relative to TMS. ^{13}C NMR spectra were obtained on the same apparatus operating at 75.5 MHz and the chemical shifts are given in ppm (δ) relative to $CDCl_3$ ($\delta = 77.0$) or $(CD_3)_2SO$ ($\delta = 39.7$) as internal standard. ^{13}C NMR spectra were monitored by the attached proton test (APT) technique. Solvents were removed by rotary evaporation under reduced pressure, and silica gel chromatography was performed with Merck silica gel 60 with a particle size of 40–63 μm. Reactions were monitored and R_f values were determined by thin-layer chromatography (TLC) on Merck pre-coated silicagel 60 F-254 (0.25 mm) plates. Spots were visualized with UV light, ninhydrin, or Cl_2/TDM (TDM = *N*,*N*,*N*′,*N*′-tetramethyl-4,4′-diaminodiphenylmethane).[52] Anhydrous solvents were obtained commercially and used without further drying. HPLC was conducted on two HPLC systems: a Gilson automated HPLC system 205 with UV detector system operating at 220 nm and 254 nm, or a Shimadzu automated HPLC system with a UV detector system operating at the same wavelenghts. Preparative HPLC was conducted by using an Alltech Adsorbosphere C8 (10 μm, 250 × 22 mm) or C18 (10 μm, 250 × 10 mm) column at a flow rate of 11.5 or 5.0 mL min^{-1}, respectively. Analytical HPLC was conducted by using an Alltech Adsorbosphere C18 (5 μm, 250 × 4.6 mm) column at a flow rate of 1.0 mL min^{-1}. Elution was effected with a gradient from 0.1% trifluoroacetic acid (TFA) in water to 0.085% TFA in acetonitrile/water (95:5) over 50 min (preparative) or 20 min (analytical).

Chemical syntheses

Glycine derivative 2 and Fmoc-NLeu-OH (3): Compounds **2** and **3** were prepared as described previously.[11]

Fmoc-NTyr-OH (5): A solution of HCl in diethyl ether (6.0 M, 50 mL) was added to a solution of Fmoc-NTyr(*t*Bu)-OH (**4**)[11] (1.38 g, 3.00 mmol) in dioxane (50 mL). The mixture was stirred at room temperature for 1.5 h. Subsequently, the solvent was removed by co-evaporation with chloroform to afford **5** as a clear oil (1.15 g, 95%). $R_f = 0.30$ (CH_2Cl_2/MeOH/AcOH, 90:10:0.5). MS (ESI): *m*/*z*: 426.1 [*M* + Na]$^+$; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 3.75$, 3.92 (two s, 2H), 4.25 (two t, 1H), 4.32, 4.45 (two s, 2H), 4.50, 4.60 (two d, 2H, $J = 5.8$ Hz), 6.72 (m, 2H), 6.83 (d, 1H, $J = 7.9$ Hz), 7.00 (d, 1H, $J = 7.9$ Hz), 7.2 (m, 2H), 7.35 (m, 2H), 7.55 (m, 2H), 7.77 (t, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 47.1$, 47.5, 50.8, 51.0, 67.9, 68.0, 115.6, 120.0, 124.8, 127.5, 127.7, 129.4, 129.9, 141.3, 143.6, 155.7, 156.4, 156.8, 174.3, 174.4.

Cbz-β-Ala-OH (7): Benzyl chloroformate (77.5 mL, 550 mmol) and 2 N NaOH (275 mL) were added simultaneously to a cooled (0 °C) solution of H-β-Ala-OH (**6**) (44.5 g, 500 mmol) in 2 N NaOH (250 mL) over a period of 75 min. During an additional hour of vigorous stirring the pH value was kept between 8 and 10 by adding 2 N NaOH. The mixture was washed with diethyl ether (4 × 250 mL). The pH value of the water layer was adjusted to 2.0 by adding conc aq HCl, and this layer was extracted with EtOAc (3 × 200 mL). The combined EtOAc layers were washed with water and brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by crystallization from EtOAc/hexanes to afford **7** as a white solid (107.5 g, 96%). $R_f = 0.47$ (CH_2Cl_2/MeOH/AcOH, 90:10:0.5). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.58$ (t, 2H, $J = 5.7$ Hz), 3.46 (m, 2H), 5.09 (s, 2H), 5.33 (br. s, 1H), 7.34 (br. s, 5H); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 34.1$, 36.2, 66.9, 128.2, 128.3, 128.6, 136.4, 156.5, 177.7.

Cbz-β-Ala-O*t*Bu (8): $POCl_3$ (10.1 mL, 110 mmol) was added to a cooled (−10 °C) solution of **7** (22.3 g, 100 mmol) in pyridine (150 mL) and *tert*-butyl alcohol (250 mL). The mixture was stirred for 15 min at −10 °C and 16 h at room temperature. The solvent was removed and water was added. The mixture was extracted with EtOAc (3 × 250 mL). The collected organic layers were washed with water (3 × 300 mL), sat. Na_2CO_3 (3 × 300 mL), water (3 × 300 mL), 2 N $KHSO_4$ (3 × 300 mL), and brine. The EtOAc solution was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (eluted with CH_2Cl_2/MeOH, 99:1) to afford **8** as a clear oil (18.79 g, 67%). $R_f = 0.59$ (CH_2Cl_2/MeOH, 95:5). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.44$ (s, 9H), 2.44 (t, 2H, $J = 5.7$ Hz), 3.42 (q, 2H), 5.10 (s, 2H), 5.34 (br. s, 1H), 7.33 (m, 5H); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 27.9$, 35.4, 36.6, 66.5, 80.9, 128.1, 128.5, 136.6, 156.3, 171.7.

H-β-Ala-O*t*Bu (9): A solution of **8** (20.9 g, 75 mmol) in THF (150 mL) was hydrogenated in the presence of 10% Pd/C overnight at room temperature in a Parr hydrogenation apparatus. The reaction mixture was filtered, washed with EtOAc, and concentrated under reduced pressure to give **9** as a clear oil (10.90 g, 99%). ^{1}H NMR (300 MHz, $(CD_3)_2SO$): $\delta = 1.39$ (s, 9H), 1.93 (br. s, 2H), 2.27 (t, 2H, $J = 6.6$ Hz), 2.73 (t, 2H, $J = 6.6$ Hz); ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$): $\delta = 27.9$, 37.9, 38.7, 79.6, 171.8.

H-NGlu(O*t*Bu)-OEt (10): A solution of ethylbromo acetate (4.44 mL, 40 mmol) in THF (200 mL) was slowly added to a mixture of amine **9** (5.8 g, 40 mmol) and *N*-methyl morpholine (NMM) (4.84 mL, 44 mmol) in THF (40 mL). The mixture was stirred overnight at room temperature. THF was removed, diethyl ether (200 mL) was added, and the mixture was filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (eluted with diethyl ether/hexanes, 4:1) to afford **10** as a clear oil (3.49 g, 38%). $R_f = 0.20$ (diethyl ether/hexanes, 4:1). ^{1}H NMR

(300 MHz, $CDCl_3$): $\delta = 1.28$ (t, 3H, $J = 7.0$ Hz), 1.46 (s, 9H), 1.86 (br. s, 1H), 2.43 (t, 2H, $J = 6.6$ Hz), 2.86 (t, 2H, $J = 6.6$ Hz), 3.41 (s, 2H), 4.19 (q, 2H, $J = 7.0$ Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 14.1$, 28.0, 35.9, 44.9, 50.9, 60.6, 80.5, 171.9, 172.4.

Fmoc-NGlu(O*t*Bu)-OH (11): 1 N NaOH (10 mL) was added to a solution of **10** (2.31 g, 10 mmol) in a mixture of dioxane (20 mL) and water (20 mL). The mixture was stirred at room temperature for 20 min. Solvent was removed by co-evaporation with dioxane and the resulting white solid was dissolved in water (10 mL). The pH was adjusted to 9.0 – 9.5 with conc aq HCl. A solution of Fmoc-OSu (3.37 g, 10 mmol) in acetonitrile (20 mL) was added to this mixture. Stirring was continued for 4 h, and the pH was kept between 8.5 and 9.0 by adding triethylamine. Acetonitrile was removed and the residue was dissolved in 20% citric acid (100 mL). The mixture was extracted with EtOAc (3 × 150 mL), and the combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (eluted with CH_2Cl_2/MeOH, 50:1) to afford **11** as a clear oil (2.77 g, 65%). $R_f =$ 0.45 (CH_2Cl_2/MeOH/AcOH, 90:10:0.5). MS (ESI): *m/z*: 448.2 $[M + Na]^+$; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.41$, 1.43 (two s, 9H), 2.29, 2.55 (two t, 2H, $J = 6.6$ Hz), 3.42, 3.55 (two t, 2H, $J = 6.6$ Hz), 4.02, 4.09 (two s, 2H), 4.18, 4.26 (two t, 1H), 4.42, 4.51 (two d, 2H, $J = 6.2$ Hz), 7.25 – 7.77 (m, 8H); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 27.9$, 34.7, 44.5, 47.1, 50.1, 67.7, 81.0, 120.0, 124.9, 127.2, 127.8, 141.4, 143.9, 171.6, 175.1.

1-(Cbz-amino)propan-2-ol (13): A solution of Cbz-Cl (14.3 mL, 100 mmol) in THF (50 mL) was slowly added to a cooled (0 °C) solution of racemic aminopropan-2-ol (**12**) (19.3 mL, 250 mmol) in THF (200 mL) and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was concentrated and 1 N $KHSO_4$ (300 mL) was added. The mixture was extracted with EtOAc (3 × 150 mL), washed with water (100 mL) and brine (100 mL), dried (Na_2SO_4), and concentrated under reduced pressure to give **13** as a clear oil (20.80 g, 99%). $R_f = 0.75$ (CH_2Cl_2/MeOH, 4:1). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.14$ (d, 3H, $J = 6.6$ Hz), 2.86 (br. s, 1H), 3.02, 3.30 (two m, 2H), 3.86 (m, 1H), 5.08 (s, 2H), 5.42 (br. s, 1H), 7.33 (d, 5H); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 20.4$, 48.2, 66.8, 67.1, 128.1, 128.2, 128.5, 136.5, 157.2.

Benzyl 2-(*tert*-butoxypropyl)carbamate (14): A solution of alcohol **13** (15.7 g, 75 mmol) in CH_2Cl_2 (100 mL) was poured into a sealed flask, cooled to − 8 °C. 2-Methylpropene (isobutylene) (200 mL) was added (condensed) followed by sulfuric acid (5 mL), and the mixture was stirred at room temperature for 4 d. The reaction mixture was cooled to − 20 °C and CH_2Cl_2 (150 mL) was added. After washing with 0.4 N NaOH (0 °C) the mixture was extracted with CH_2Cl_2 (2 × 50 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (eluted with EtOAc/hexanes, 1:4) to afford **14** as a clear oil (11.94 g, 60%). $R_f = 0.34$ (EtOAc/hexanes, 1:3). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.09$ (d, 3H, $J = 6.2$ Hz), 1.16 (s, 9H), 2.99 (m, 1H) 3.24, 3.75 (two m, 2H), 5.09 (s, 2H), 5.14 (br. s, 1H), 7.26 – 7.36 (m, 5H); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 20.5$, 28.3, 47.3, 66.0, 66.5, 73.6, 128.0, 128.1, 128.5, 136.7, 156.6.

1-Aminoprop-2-yl *tert*-butyl ether (15): A solution of **14** (10.58 g, 40 mmol) in methanol (100 mL) was hydrogenated in the presence of 10% Pd/C overnight at room temperature in a Parr hydrogenation apparatus. The reaction mixture was filtered, washed with EtOAc, and concentrated under reduced pressure by co-evaporation with chloroform to give **15** as a clear oil (4.50 g, 86%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.22$ (d, 3H, $J = 6.2$ Hz), 1.29 (s, 9H), 2.81 (m, 1H), 3.00, 4.01 (two m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 20.9$, 28.5, 45.4, 63.6, 74.9.

H-NhThr(*t*Bu)-OBzl (16): A solution of benzyl bromoacetate (3.66 mL, 23.3 mmol) in THF (50 mL) was slowly added to a mixture of amine **15** (3.05 g, 23.3 mmol) and triethylamine (TEA) (6.5 mL, 46.6 mmol) in THF (100 mL) and the mixture was strirred overnight at room temperature. The solvent was removed and the residue was washed with ether (3 × 100 mL) and filtrated. The filtrate was concentrated under reduced pressure and purified by column chromatography on silica gel (eluted with CH_2Cl_2/MeOH, 99:1) to afford **16** as a clear oil (3.58 g, 55%). $R_f = 0.70$ (CH_2Cl_2/MeOH, 9/1). ^{1}H NMR (300 MHz, $CDCl_3$). $\delta = 1.12$ (d, 3H, $J = 6.2$ Hz), 1.20 (s, 9H), 2.40 (br. s, 1H), 2.54 (d, 2H, $J = 5.9$ Hz), 3.45 (d, 2H, $J = 2.6$ Hz), 3.76 (m, 1H), 5.16 (s, 2H), 7.35 (s, 5H); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 21.2$, 28.5, 51.2, 56.3, 66.4, 66.8, 73.5, 128.4, 128.6, 135.8, 172.3.

Fmoc-NhThr(*t*Bu)-OH (17): 4 N NaOH (3.2 mL) was added to a solution of **16** (3.54 g, 12.7 mmol) in a mixture of dioxane (45 mL) and methanol (16 mL). The mixture was strirred at room temperature for 1 h. Solvent was removed by co-evaporation with dioxane and the resulting white solid was dissolved in water (30 mL). The pH was adjusted to 9.0 – 9.5 with 1 N $KHSO_4$. To this mixture a solution of Fmoc-O-Su (4.28 g, 12.7 mmol) in acetonitrile (60 mL) was added followed by vigorous stirring for 2 h. During the reaction the pH was kept between 8.5 and 9.0 by adding triethylamine. The reaction mixture was concentrated to a white suspension and 20% citric acid (100 mL) was added. The mixture was extracted with EtOAc (3 × 100 mL) and the combined organic layers were washed with water (100 mL) and brine (100 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (eluted with CH_2Cl_2/MeOH, 20:1) to afford **17** as a clear oil (2.80 g, 54%). $R_f = 0.42$ (CH_2Cl_2/MeOH/AcOH, 90:10:0.5). MS (ESI): *m/z*: 434.2 $[M + Na]^+$; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.72$, 1.09 (two d, 3H, $J = 6.2$ Hz), 0.92, 1.14 (two s, 9H), 2.59, 3.11 (two m, 2H), 2.92, 2.96, 3.41, 3.46 (four d, 1H), 3.95, 4.43 (two d, 2H), 4.21 (m, 1H), 4.65, 4.74 (two m), 7.32 (m, 4H), 7.54 (m, 2H), 7.75 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 20.3$, 20.5, 28.0, 28.2, 50.7, 54.4, 55.6, 66.6, 67.7, 66.8, 73.9, 74.3, 119.9, 124.5, 124.6, 124.8, 124.9, 127.1, 127.3, 127.7, 127.8, 141.4, 141.6, 143.9, 144.0, 144.1, 156.6, 174.5.

Peptide 18, peptoid 19, and peptoid – peptide hybrids 20 – 26: These compounds were synthesized by standard solid-phase peptide methods in a reaction vessel through which nitrogen was bubbled for mixing. Before coupling of the first monomer, Fmoc-Rink amide resin (125 μmol, Argogel) was washed with CH_2Cl_2 (5 min) and *N*-methyl pyrrolidone (NMP) (3 × 5 min). A typical cycle for the coupling of an individual monomer by the Fmoc strategy was: i) Fmoc deprotection with 20% piperidine in NMP (4 mL) for 20 min; ii) washing with NMP (5 mL; 5 × 5 min); iii) coupling for 45 min of the Fmoc monomer by addition of a freshly prepared mixture of a peptoid monomer or an amino acid (0.5 mmol), PyBroP (0.5 mmol), and *N*,*N*-diisopropylethylamine (DiPEA) (1.0 mmol) in NMP (4 mL). After coupling of the last monomer the final Fmoc deprotection was carried out and the N terminus was acetylated by reaction with a mixture of acetic acid anhydride (0.5 M), DiPEA (0.125 M), and 1-hydroxy-1*H*-benzotriazole (HOBt) (0.015 M) in NMP (5 mL) for 30 min. The peptidyl resin was washed with NMP (5 mL; 5 × 5 min) and CH_2Cl_2 (5 mL; 5 × 5 min), and dried (P_2O_5, in vacuo). Prior to capping, NTyr-containing oligomers were phosphorylated on the resin by using the global phosphorylation method according to Hoffmann et al. [36] with 20 equiv of di-*tert*-butyl-*N*,*N*-diethylphosphoramidite[34] and 20 equiv of 1*H*-tetrazole in acetonitrile. Oxidation of the phosphite triester to the corresponding phosphate was carried out using 10 equiv of MCPBA. Oligomers were deprotected and cleaved from the resin by treatment with a mixture of TFA, water, and triisopropyl silane (95:2.5:2.5) for 3 h, followed by precipitation in a mixture of methyl *tert*-butyl ether (MTBE) and hexanes (1:2), washed with the same solvent mixture (3 ×), and dried. Every product was purified by preparative HPLC. Purity of all oligomers was verified by reversed-phase analytical HPLC. All oligomers gave

accurate ESI mass spectra and high-resolution mass spectra. HR-MS: calcd for $C_{26}H_{41}N_5O_{12}P$ 646.2489 $[M+H]^+$ and $C_{26}H_{40}N_5NaO_{12}P$ 668.2309 $[M+Na]^+$, found 646.2513 and 668.2368 (**18**), 646.2521 and 668.2339 (**20**), 646.2531 and 668.2342 (**21**), 646.2513 and 668.2313 (**23**), 646.2513 and 668.2324 (**24**); calcd for $C_{27}H_{43}N_5O_{12}P$ 660.2645 $[M+H]^+$ and $C_{27}H_{42}N_5NaO_{12}P$ 682.2465 $[M+Na]^+$, found 660.2692 and 682.2510 (**19**), 660.2700 and 682.2490 (**22**), 660.2679 and 682.2495 (**25**), 660.2699 and 682.2490 (**25'**, the other epimer), 660.2661 and 682.2473 (**26**), 660.2679 and 682.2475 (**26'**, the other epimer).

Mass spectrometric studies: Mass spectrometric studies are essential in the characterization of peptoids, since interpretation of NMR spectra is severely hampered by the presence of many conformers arising from rotation about the tertiary amide bonds.[3, 11, 40] Q-TOF-MS – MS spectra of the $[M+H]^+$ ion of peptide **2**, peptoid **19**, and the corresponding peptoid – peptide hybrids **20** – **26** exhibit both B- and Y''-type sequence ions at identical *m/z* values. However, differences in the relative abundance of these sequence ions can be related to the structural characteristic of each compound.[53–55] The characteristic Q-TOF-MS – MS spectral data of the peptoid – peptide hybrids are available in the Supporting Information.

Construction of pGEX-3X-Syk-TDSH2 expression plasmids and purification of recombinant fusion proteins: A plasmid containing full-length murine *syk* gene was kindly provided by Dr. A. Ziemiecki.[37] From this plasmid the cDNA fragment corresponding to amino acid residues 2 – 259, that is, the tandem SH2 domain, was amplified by standard PCR methodology using the primer pair 5'-cgggatccTGGCGGGAAGTGCTGT-3' and 5'-ggaattcCTATTGGCATGG-TACCGTGAGG-3'. The PCR fragment was cleaved with *Bam* HI and *Eco* RI, and then ligated with the *Bam* HI/*Eco* RI-cut fragment of pGEX-3X (Pharmacia). Standard recombinant DNA techniques were employed, and the correct construction of the recombinant plasmid was confirmed by dideoxy nucleotide sequencing.[56] The protein was expressed in *Escherichia coli* (BL21(DE3)pLysS) and shown to have the predicted molecular mass. The Syk GST-tdSH2 protein was purified by affinity chromatography on glutathione – Sepharose (Pharmacia), in accordance with the instructions provided by the manufacturer. Protein concentration was determined by a protein assay (Bio-Rad) using bovine serum albumin (BSA) as a standard.

Analysis of inhibition of SH2 domain binding with surface plasmon resonance (SPR) spectroscopy: The IC_{50} and K_d values were determined using a SPR protocol[57] for a double-channeled IBIS II SPR instrument.[58] In our competition experiments the immobilized phase was generated by using a biotinylated FceRI γITAM phosphopeptide, biotin-Ahx-KADAVpYTGLNTRSQETpYETL-KHEK-NH2 (> 95% purity by C8 HPLC and of the appropriate molecular weight as determined by mass spectrometry). This peptide was attached to an avidin-coated CM5 chip (Biacore). Binding of Syk GST-tdSH2 was conducted at 100 nM in HBS buffer (10 mM HEPES, 150 mM NaCl, 4 mM EDTA, 1 mM DTT, 0.005% (w/v) Tween-20, pH 7.4) at continuous pipet mixing. After each measurement, the surface was regenerated with a solution of 0.2% (w/v) SDS. Compounds tested as inhibitors were premixed with Syk GST-tdSH2 30 min prior to introduction onto the immobilized chip. The amount of binding at equilibrium was determined. Equilibrium values were plotted in a competition curve and IC_{50} and K_d values were determined by using the "Graphpad Prism" software (GraphPad Software Inc., San Diego, USA). IC_{50} and K_d values are reported as means of at least three determinations, which were conducted using at least two separate sensor chips.

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