[2] R. L. Wadlinger, G. T. Kerr, E. J. Rosinski (Mobil), US-A 3308069, **1967**.
[3] J. M. Newsam, M. M. J. Treacy, W. T. Koetsier, C. B. de Gruyter, *Proc. R. Soc. London A* **1988**, *420*, 375 – 405.
[4] a) M. A. Camblor, A. Corma, P. Lightfoot, L. A. Villaescusa, P. A. Wright, *Angew. Chem.* **1997**, *109*, 2774 – 2776; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2659 – 2661; b) M. A. Camblor, A. Corma, L. A. Villaescusa, *Chem. Commun.* **1997**, 749 – 750; c) M. J. Díaz-Cabañas, P. A. Barrett, M. A. Camblor, *Chem. Commun.* **1998**, 1881 – 1882; d) L. A. Villaescusa, P. A. Barrett, M. A. Camblor, *Chem. Commun.* **1998**, 2329 – 2330; e) P. A. Barrett, M. J. Díaz-Cabañas, M. A. Camblor, R. H. Jones, *J. Chem. Soc. Faraday Trans.* **1998**, *94*, 2475 – 2481; f) M. A. Camblor, M. J. Díaz-Cabañas, J. Pérez-Pariente, S. J. Teat, W. Clegg, I. J. Shannon, P. Lightfoot, P. A. Wright, R. E. Morris, *Angew. Chem.* **1998**, *110*, 2234 – 2239; *Angew. Chem. Int. Ed.* **1998**, *37*, 2122 – 2126.
[5] M. A. Camblor, A. Corma, S. Valencia, *Chem. Commun.* **1996**, 2365 – 2366.
[6] P. A. Barrett, E. T. Boix, M. A. Camblor, A. Corma, M. J. Díaz-Cabañas, S. Valencia, L. A. Villaescusa in *Proc. 12th Int. Zeolite Conference* (Eds.: M. M. J. Treacy, B. Marcus, J. B. Higgins, M. E. Bisher), Materials Research Society, Warrendale, **1999**, pp. 1495 – 1502.
[7] R. F. Lobo, S. I. Zones, M. E. Davis, *J. Inclusion Phenom. Mol. Recognit. Chem.* **1995**, *21*, 47 – 78.
[8] J. M. Thomas, J. Klinowski, S. Ramdas, B. K. Hunter, D. T. B. Tennakoon, *Chem. Phys. Lett.* **1983**, *102*, 158 – 161.
[9] A change in the reference system is required to keep the symmetry axis of higher order along the *c* direction.
[10] C. Baerlocher, A. Hepp, W. M. Meier, *DLS-76*, *a Program for the Simulation of Crystal Structures by Geometric Refinement*, Institute of Crystallography and Petrography, ETH, Zürich (Switzerland), **1977**.
[11] a) P. Caullet, J. L. Guth, J. Hazm, J. M. Lamblin, H. Gies, *Eur. J. Solid State Inorg. Chem.* **1991**, *28*, 345 – 361; b) L. A. Villaescusa, P. A. Barrett, M. A. Camblor, *Chem. Mater.* **1998**, *10*, 3966 – 3973.
[12] J. D. Gale, *J. Chem. Soc. Faraday Trans.* **1997**, *93*, 629 – 637.
[13] M. Sanders, M. Leslie, C. R. A. Catlow, *J. Chem. Soc. Chem. Commun.* **1984**, 1271 – 1273.
[14] J. L. Guth, H. Kessler, P. Caullet, J. Hazm, A. Merrouche, J. Patarin in *Proc. 9th Int. Zeolite Conference* (Eds.: R. von Ballmoos, J. B. Higgins, M. M. J. Treacy), Butterworth-Heinemann, London, **1993**, pp. 215 – 222.
[15] H. M. Rietveld, *J. Appl. Crystallogr.* **1969**, *2*, 65.
[16] A. Larson, R. B. Von Dreele, GSAS Manual, Los Alamos Report **1986**, LA-UR-86-748.
[17] J. B. Hastings, W. Thomlinson, D. E. Cox, *J. Appl. Crystallogr.* **1984**, *17*, 85.
[18] Note added in proof (May 25, 1999): The structure of ITQ-7 has been given the code ISV by the International Zeolite Association.

Interaction of a PDZ Protein Domain with a Synthetic Library of All Human Protein C Termini**

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Through increasingly efficient sequencing of genes and whole genomes, the amino acid sequences of unknown proteins are being deduced at an accelerating rate. This means that questions about the function of these identified proteins in the corresponding organisms are becoming increasingly important. Alongside the "classical" approaches to analyzing the roles of these proteins by molecular biological/biochemical methods, through the use of bioinformatics it is possible to gain information about the new protein by sequence comparisons with proteins of known function. At the same time it has been demonstrated that large globular proteins are usually built up of individual domains that show independent folding and functions. Many of these domains are protein modules which occur in a number of, often unrelated, proteins.[1] Protein sequence comparison not only reveals known protein domains, but also may identify novel, previously undetected stretches of homologous sequence in the new proteins.[2] While known domains potentially point to the role of a protein, the function of new domains must be derived experimentally. Generally, protein domains can be produced by recombinant technology and folding in vitro. Many of them play a role in signal transduction and regulation processes where the identification of binding partners is a basic step towards discovering their function. To accomplish this, usually biologically generated protein libraries are enlisted to screen for the potential binding partner of the newly discovered domain.[3]

We report here the chemical synthesis of a new kind of library, designed to discover binding partners for a PDZ

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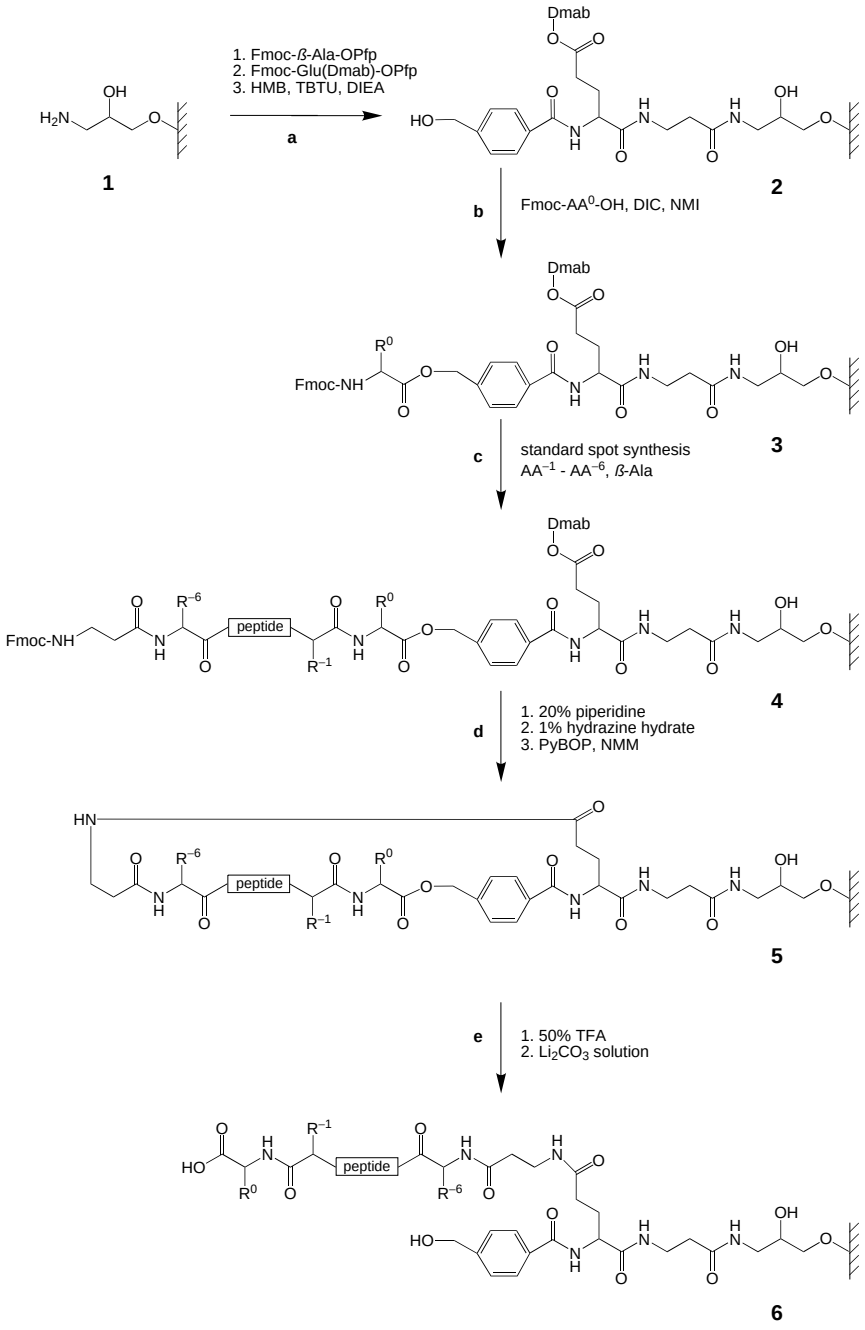
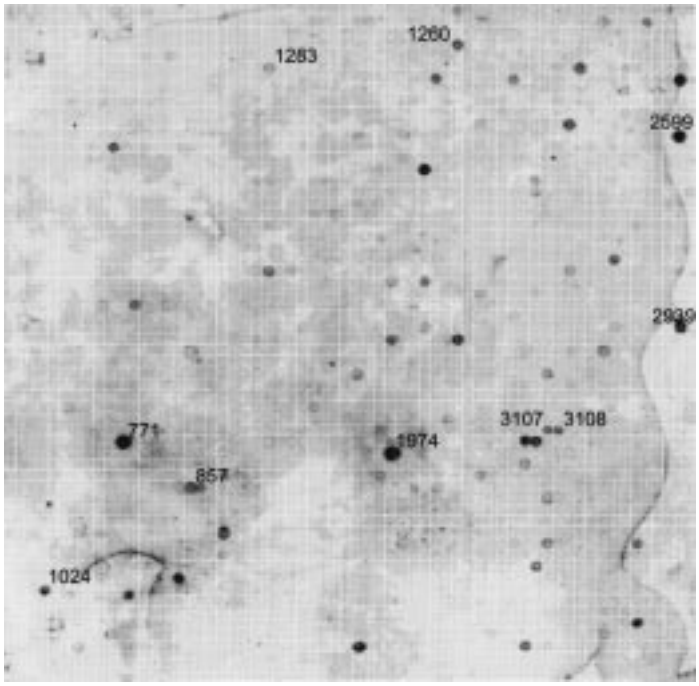
domain comprising cellulose membrane-bound peptides with free carboxy termini (C termini). PDZ domains, the name being derived from the first letter of the proteins in which they were first discovered (*p*ost synaptic density, *d*isc large, *z*onula occludens), are protein modules about 100 amino acids long which mainly occur in proteins of the cytoskeleton and play a role in signal transduction.[4] Besides the observed heterodimerization[5] PDZ domains interact predominantly with the C termini of their binding partners, the terminal carboxyl group being essential for this binding.[6]

To be able to investigate these interactions using cellulose-bound peptide libraries, a method was developed for positionally directed generation of membrane-bound, inverted peptides. The peptides were synthesized by spot synthesis,[7] cyclized, and finally linearized again to generate a free C terminus[8] (Scheme 1). In this way the C termini of all known human proteins (SWISSPROT databank, release 34, 3514 sequences) were synthesized and tested for binding to a syntrophin – PDZ domain[9] (Figure 1). A total of 38 clearly binding C termini were identified, and from chosen peptides the dissociation constants of the peptide – PDZ complexes were determined (Table 1).

In addition to a voltage-gated sodium channel already identified as a PDZ binding partner by two different approaches,[10, 11] the strongest binding C termini belong to the α_2 subunit of soluble guanylate cyclase and the α-1A adrenergic receptor. An interaction with the α-1A adrenergic receptor is quite plausible since this membrane protein, like syntrophin, exists in heart muscle.[12] The potential interaction of soluble guanylate cyclase[13] is particularly interesting since as yet no binding partner for this enzyme is known; its gene was identified by cDNA sequencing. The binding of the native cyclase with the syntrophin – PDZ domain was confirmed using coprecipitation and subsequent Western blots (Figure 2 A). The cyclase is activated by nitric oxide (NO) and produces the signal molecule cyclic guanosine monophosphate (cGMP). Therefore, functionally this interaction is plausible and also very interesting (Figure 2 C) since NO synthase also interacts with syntrophin[5] and, moreover, the cGMP-dependent protein kinase has been identified on the cytoplasmic side of the cell membrane.[14] This spatial proximity of the three components of the NO/cGMP-mediated signal transduction cascade could facilitate higher local concentrations of the enzyme activators NO and cGMP and therefore efficient transfer of the signal.



Scheme 1. Synthesis of inverted peptides on cellulose membranes. Aminopropyl ether cellulose[17] (**1**) was used as the matrix. a) β-Alanine serves both as a spacer and to define the synthesis position.[7] Dmab-glutamic acid is coupled as a bivalent linker followed by hydroxymethylbenzoic acid (HMB) as a base-labile cleavage site (→**2**). b) The intended C-terminal amino acid is coupled through an ester bond (→**3**). c) Remaining amino acids and β-alanine as spacer are coupled (→**4**). d) The Fmoc and Dmab protecting groups on the N terminus and the side chains of the glutamic acids are cleaved off and the construct cyclized (→**5**). e) After removal of the side chain protecting groups, hydrolysis of the ester bond linearizes the construct and generates a free C terminus (→**6**). Dmab=α-4-[*N*-{1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl}amino]benzyl, Fmoc = 9-fluorenylmethoxycarbonyl, HMB = 4-hydroxymethylbenzoic acid, TBTU = 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, DIEA = *N*,*N*-diisopropylethylamine, DIC = diisopropylcarbodiimide, NMI = *N*-methylimidazole, PyBOP = benzotriazole-1-yloxytripyrrolidinophosphonium hexafluorophosphate, NMM = *N*-methylmorpholine, TFA = trifluoroacetic acid.



Investigations into the binding specificity of this syntrophin – PDZ domain was made possible by the novel method of synthesizing membrane-bound peptide libraries with free C termini.[8] A selection of binding peptides in noninverted form on cellulose showed no, or severely reduced, binding (not shown). For a more universal application of this method to identify linear binding sites which do not lie on the N or C terminus, the synthesis and testing of considerably greater numbers of peptides would be necessary. However, through selection of sequences which, for example, are found on the surface of known protein structures, the number of peptides can be reduced to a range that in the near future will be synthetically feasible.

Figure 1. Library of carboxy-terminal peptides from all known human proteins. The cellulose-bound library contains the C termini (heptamers) of all human proteins from the SWISSPROT databank (release 34). The figure shows the chemiluminescence intensity measured following incubation with horseradish peroxidase labeled syntrophin – PDZ domain. The numbers correspond to the peptides for which the dissociation constant was measured. The 38 strongest binders are listed in Table 1.

Table 1. Syntrophin – PDZ-binding peptides from human C termini.[a]

Spot	Sequence	K_D	Acc. no.	Protein description
95	AKRCGCI		P39905	glial cell line-derived neurotrophic factor precursor
250	CHSETVV		P01135	transforming growth factor alpha precursor
771	FLRETSL	6 μM	P33402	guanylate cyclase soluble, alpha-2 chain (EC 4.6.1.2)
857	GDRFFTI	1.3 mM	P18440	arylamine N-acetyltransferase, monomorphic (EC 2.3.1.5)
940	GLNQRRI		P07766	T-cell surface glycoprotein CD3 epsilon chain precursor
1016	GTRLTVV		P01733	T-cell receptor beta chain precursor V region (YT35)
1024	GVKESLV	76 μM	P35499	sodium channel protein, skeletal muscle alpha-subunit
1260	IRTVVKI	804 μM	P11766	alcohol dehydrogenase class III chi chain (EC 1.1.1.1)
1283	ITTKKYI	500 μM	P51587	breast cancer type 2 susceptibility protein
1318	KAVETDV		P22459	potassium channel protein KV1.4
1477	KRISKRI		P07148	fatty acid-binding protein, liver (L-FABP)
1643	LKFRTEI		P49888	estrogen sulfotransferase (EC 2.8.2.4)
1677	LLRHERI		P16415	zinc finger protein ZFP-36 (fragment)
1774	LRHWLKV		Q01959	sodium-dependent dopamine transporter
1780	LRRESEI		P48049	inward rectifier potassium channel 2
1974	NLRETDI	3 μM	P25100	alpha-1A adrenergic receptor.
2331	PSQGHQP		P28329	choline O-acetyltransferase (EC 2.3.1.6)
2396	QSLETSV		P48050	calcium-transporting ATPase plasma membrane, isoform 4
2470	RGMRGKI		P15248	interleukin-9 precursor (IL-9)
2484	RIRHFKV		P02765	alpha-2-HS-glycoprotein precursor (FETUIN)
2499	RKSRRTI	–	P01589	interleukin-2 receptor alpha chain precursor
2569	RRSAGFI		P48645	neuromedin U-25 precursor (contains: NMU-25)
2599	RTRITFV	45 μM	P33993	DNA replication licensing factor CDC47 homolog
2666	SERISSV		P28335	5-hydroxytryptamine 2C receptor
2813	SQKETSI		P53618	coatomer beta subunit (beta coat protein) (beta-COP) (fragment)
2939	TCRTSII		P47872	secretin receptor precursor(SCR-R)
2972	THFLPRI		P10767	fibroblast growth factor-6 precursor (FGF-6) (HBGF-6)
3007	TKNRFVV		P06730	eukar. initiation factor 4 E (EIF-4E)
3107	TRVTVLG	246 μM	P01702	Ig lambda chain V-I region (NIG-64)
3108	TRVTVLS	354 μM	P01705	Ig lambda chain V-II region (NEI)
3125	TSRETDL		P22460	potassium channel protein KV1.5 (HK2) (HPCN1)
3126	TSRITTL		P30872	somatostatin receptor type 1 (SS1R) (SRIF-2)
3165	VAAFNKI		P24534	elongation factor 1-beta (EF-1-BETA)
3181	VCYQCKI		P26371	keratin, ultra high-sulfur matrix protein (UHS keratin)
3307	VQDKRFI		Q07001	acetylchonline receptor protein, delta chain precursor
3346	VTVETVV		P04201	MAS proto-oncogene
3455	YLRRSDV		P35080	profilin II
3485	YRRESAI		P48050	inward rectifier potassium channel 4

[a] The table shows the spot number corresponding to the position in the library (Figure 1), the amino acid sequence, the databank number (Acc. no.) of the appropriate SWISSPROT entry, and the protein description of the 38 identified C termini. For chosen binders the dissociation constant (K_D) was determined.

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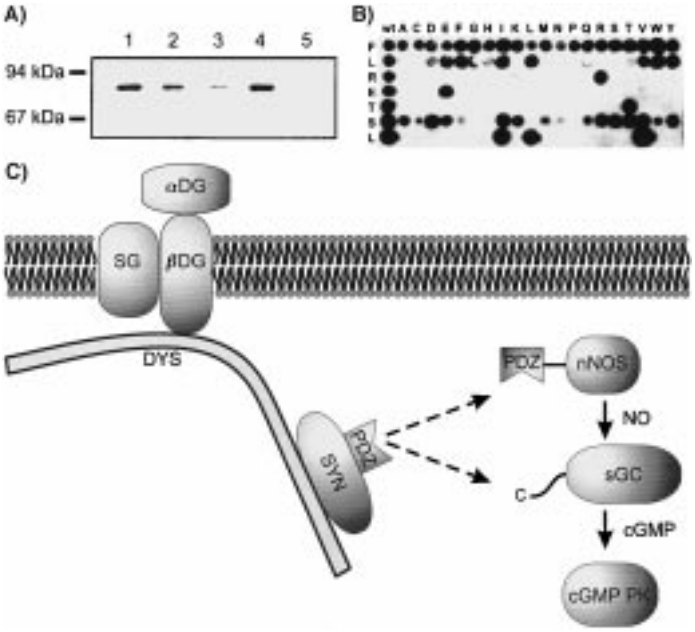

Figure 2. A) Interaction between soluble guanylate cyclase and syntrophin. Cell lysate containing guanylate cyclase[20] was incubated with sepharose-bound syntrophin–PDZ domain.[10] The PDZ domain was precipitated by sedimentation, and the whereabouts of cyclase analyzed by Western blotting.[22] In a control experiment the C-terminal peptide FLRETSL-COOH was added to the cyclase. Lane 1: cell lysate, lane 2: supernatant, lane 3: pellet, lane 4: supernatant of the control experiment, lane 5: pellet of the control experiment. B) Substitutional analysis of the C terminus of cyclase. The peptide FLRETSL and its single-exchange analogues were synthesized on the cellulose membrane and tested for binding to the syntrophin–PDZ domain. The first column (wt) contains identical peptides with the sequence FLRETSL as controls. At every position the corresponding amino acid (rows) was substituted by all 20 L-amino acids (columns). This led to the identification of amino acids arginine (R), glutamic acid (G), and threonine (T) as key residues which cannot be exchanged without loss of binding activity. At the C-terminal position, in addition to leucine (L), isoleucine (I) or valine (V) was also tolerated. C) Syntrophin in the cell (model). Dystrophin (DYS) is anchored to the sarcolemma at the neuromuscular junction through α- and β-dystroglycan (αDG, βDG) and sarcoglycan (SG).[23] Syntrophin (SYN) attaches to dystrophin[24] and brings the neuronal NO synthase (nNOS) and the soluble guanylate cyclase (sGC) within proximity of the dystrophin–glycoprotein complex. nNOS, sGC, and the cGMP-dependent protein kinase (cGMP PK), which is also associated with the sarcolemma, build a signal transduction cascade with the help of the mediators NO and cGMP.

With the newly developed method, the door is opened to the future identification of binding partners for other PDZ domains in addition to derivation of a detailed description of their binding specificity by substitutional analyses (Figure 2B). Further protein–peptide interactions where terminal carboxyl groups play a role—for example, as described for some monoclonal antibodies[15]—will similarly be open to investigation. Moreover, variations of this method will allow the synthesis of peptides with modified C termini,[16] facilitating ways of studying the biological function of C-terminal modifications using peptide libraries.

Experimental Section

Library synthesis (Scheme 1): Library peptides with a chain length of seven were chosen since in the PDZ–peptide complexes identified to date the last seven C-terminal amino acids are sufficient for the interaction. Whatman 50 filter paper (20×30 cm) which was amino functionalized through the anchor molecule 3-amino-2-hydroxy-propyl ether was used as the matrix.[17] The peptide combinations were synthesized by the spot technique.[7] a) 0.3 M Fmoc-β-alanine-OPfp in DMSO, double coupling, $2 \times$ 15 min; Fmoc cleavage, 20% piperidine in DMF, 2×5 min; 0.3 M Dmab-glutamic acid-OH, 0.6 M DIEA, 0.3 M DIC, 0.3 M Pfp in *N*-methylpyrrolidine (NMP), double coupling, 2×15 min; Fmoc cleavage, 20% piperidine in DMF, 2×5 min; 0.3 M HMB, 0.6 M DIEA, 0.3 M TBTU, fourfold coupling, 4×15 min. b) 0.3 M Fmoc-AS-OH, 0.3 M DIC, 0.6 M NMI, fourfold coupling, 4×15 min. c) 0.3 M Fmoc-AS-OPfp, standard spot synthesis.[7] d) Fmoc cleavage, 20% piperidine in DMF, 2×5 min; Dmab cleavage, 1% hydrazine hydrate in DMF, max. 2×3 min (to prevent ester hydrazinolysis); cyclization with 300 mg of PyBOP and 100 mL of NMM in 40 mL of DMF, 1×4 h, 1×16 h. e) Cleavage of the side chains, 50% TFA, 1% phenol, 2% water, 3% triisobutylsilane (TIBS) in CH_2Cl_2, 2.5 h; ester hydrolysis, saturated Li_2CO_3 solution in H_2O, 16 h. Due to the essential stable ether bridges with the cellulose the peptides are not amenable to analysis. Therefore, to confirm the procedure, analogous synthesis steps were carried out on Tentagel S RAM resin (Rapp Polymere, Tübingen, Germany). Usually syntheses on cellulose and on resin results in peptides of comparable purity.[7, 17] Following cleavage from the resin the products were analyzed by RP-HPLC and MALDI-TOF mass spectrometry. Compound **6** with the peptide sequence NYKQTSV was obtained with 87% purity, and the mass spectrum showed the calculated mass of this combination (not shown).

Detection of PDZ domain interactions with the membrane-bound peptide library: The PDZ domain as a glutathione-S-transferase (GST) fusion protein[10] was attached through the sugar group to horseradish peroxidase.[18] The library was washed with ethanol, then with TBS (50 mM Tris buffer, 100 mM NaCl, pH 8.0), blocked for 2 h with blocking buffer (CRB, Northwich, UK), and finally incubated with 1 mg mL^{-1} labeled domain in blocking buffer at 4 °C for 16 h. After threefold washing of the library with TBS, the bound domain was detected following addition of the chemiluminescent substrate Lumi-Light Plus Western Blotting substrate in a Lumi-Imager (Boehringer, Mannheim, Germany).

ELISA measurements of the dissociation constants: Microtiter plates were coated with streptavidin (125 ng per well in 0.1 M carbonate buffer, pH 9.6), washed three times with PBS (10 mM phosphate buffer, 150 mM NaCl, pH 7.3)/0.1% Tween 20 (Merck, Darmstadt, Germany), and 50 mL of peptide biotin-βA-βA-FLRETSL-OH (βA: β-alanine) (100 mM) in water were added. After threefold washing with PBS/0.1% Tween 20, horseradish peroxidase-labeled syntrophin–PDZ domain in the presence of various concentrations of the peptides (0.5–250 mM) in PBS/0.1% Tween 20/6% Gelifundol (Biotest, Dreieich, Germany) was added to the wells. Following three washes with PBS/0.1% Tween 20 the enzyme activity was measured using 5.5 mM *o*-phenylendiamine dihydrochloride (Fluka, Buchs, Switzerland)/8.5 mM H_2O_2 in 0.1% citrate buffer (pH 5.0). The reaction was stopped by the addition of 1 M sulphuric acid/0.05 M sodium sulphite. The absorption was measured at 492 nm and 620 nm (reference). The inhibition constants were calculated according to Friguet et al.,[19] and the values obtained by ELISA confirmed by grating coupler measurements (F. Kleinjung, not shown).

Expression of soluble guanylate cyclase and precipitation experiments: The $\alpha_2\beta_1$ isoform of soluble guanylate cyclase was expressed in Sf9 insect cells.[20] The cells were collected by centrifugation at 800 *g* for 10 min, resuspended in buffer (2 mM dithiothreitol, 2 mM EDTA, 0.2 mM benzamidine, 0.5 mM PMSF, 1 mM pepstatin A, and 50 mM triethanolamine/HCl, pH 7.4) and lysed by sonication; the cytoplasm was extracted by ultracentrifugation (200 000 *g* for 30 min at 4 °C). The syntrophin–PDZ–GST fusion protein was coupled to glutathione sepharose CL-4B (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions. Protein concentrations were determined following the method of Bradford.[21] Precipitation of the guanylate cyclase was carried out with 2 mg of Sf9 cytoplasmic cell protein and 1 mg of sepharose-bound PDZ domain in a volume of 500 mL (75 mM NaCl, 1 mM EDTA, 2 mM DL-dithiothreitol, 50 mM TEA, pH 7.4) for 10 min at room temperature. Sedimentation of the sepharose was carried out on ice (5 min), and separation of the proteins from the relevant fractions achieved by 7.5% SDS-PAGE. To detect the cyclase an immunoblot was carried out as described by

Harteneck et al.[22] The antibodies used are directed against the C-terminal peptide of the α_2 subunit (KKVSYNIGTTMFLRETSL).

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[1] P. Bork, J. Schultz, C. P. Ponting, *Trends Biochem. Sci.* **1997**, *22*, 296 – 298.
[2] P. Bork, E. V. Koonin, *Curr. Opin. Struct. Biol.* **1996**, *6*, 366 – 376.
[3] a) J. K. Scott, G. P. Smith, *Science* **1990**, *249*, 386 – 390; b) S. Fields, O. Song, *Nature* **1989**, *340*, 245 – 246.
[4] a) K. O. Cho, C. A. Hunt, M. B. Kennedy, *Neuron* **1992**, *9*, 929 – 942; b) C. P. Ponting, C. Phillips, *Trends Biochem. Sci.* **1995**, *20*, 102 – 103; c) C. P. Ponting, C. Phillips, K. E. Davies, D. J. Blake, *BioEssays* **1997**, *19*, 469 – 479.
[5] J. E. Brenman, S. S. Chao, S. H. Gee, A. W. McGee, S. E. Craven, D. R. Santillano, Z. Wu, F. Huang, H. Xia, M. F. Peters, S. C. Froehner, D. S. Brendt, *Cell* **1996**, *84*, 757 – 767.
[6] J. Saras, C. Heldin, *Trends Biochem. Sci.* **1996**, *21*, 455 – 458.
[7] a) R. Frank, *Tetrahedron* **1992**, *48*, 9217 – 9232; b) R. Frank, H. Overwin, *Methods Mol. Biol.* **1996**, *66*, 149 – 169; c) A. Kramer, J. Schneider-Mergener, *Methods Mol. Biol.* **1998**, *87*, 25 – 39.
[8] a) R. S. Kania, R. N. Zuckermann, C. K. Marlowe, *J. Am. Chem. Soc.* **1994**, *116*, 8835 – 8836; b) M. Lebl, V. Krchnak, N. F. Sepetov, V. Nikolaev, A. Stierradova, P. Safar, B. Seligmann, P. Stop, P. Thorpse, S. Felder, D. F. Lake, K. S. Lam, S. E. Salmon in *Innovation and Perspectives in Solid Phase Synthesis* (Ed.: R. Epton), Mayflower Worldwide, Oxford, **1994**, p. 233.
[9] M. E. Adams, T. M. Dwyer, L. L. Dowler, R. A. White, S. C. Froehner, *J. Biol. Chem.* **1995**, *270*, 25859 – 25865.
[10] J. Schultz, U. Hoffmüller, G. Krause, J. Ashurst, M. Macias, P. Schmieder, J. Schneider-Mergener, H. Oschkinat, *Nat. Struct. Biol.* **1998**, *5*, 19 – 24.
[11] S. H. Gee, R. Madhavan, S. R. Levinson, J. H. Caldwell, R. Sealock, S. C. Froehner, *J. Neurosci.* **1998**, *18*, 128 – 137.
[12] a) L. Fu, G. Wallukat, A. Hjalmarson, J. Hoebeke, *Clin. Exp. Immunol.* **1994**, *97*, 146 – 151; b) A. H. Ahn, C. A. Freener, E. Gussoni, M. Yoshida, E. Ozawa, L. M. Kunkel, *J. Biol. Chem.* **1996**, *271*, 2724 – 2730.
[13] D. Koesling, J. Herz, H. Gausepohl, F. Niroomand, K. D. Hinsch, A. Mulsch, E. Böhme, G. Schultz, R. Frank, *FEBS Lett.* **1988**, *239*, 29 – 34.
[14] D. S. Chao, F. Silvagno, H. Xia, T. L. Corwell, T. M., Lincoln, D. S. Bredt, *Neuroscience* **1997**, *76*, 665 – 672.
[15] T. C. Liang, W. Luo, J. T. Hsieh, S. H. Lin, *Arch. Biochem. Biophys.* **1996**, *329*, 208 – 14
[16] M. Davies, M. Bradley, *Angew. Chem.* **1997**, *109*, 1135 – 1138; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1097 – 1099.
[17] R. Volkmer-Engert, B. Hoffmann, J. Schneider-Mergener, *Tetrahedron Lett.* **1997**, *38*, 1029 – 1032.
[18] M. B. Wilson, P. K. Nakane in *Immunofluorescence Related Staining Techniques* (Eds.: W. Knapp, K. Hlubar, G. Wick), Elsevier, Amsterdam, **1978**, pp. 215 – 224.
[19] B. Friguet, A. F. Chaffotte, L. Djavadi-Ohaniance, M. E. Goldberg, *J. Immunol. Methods* **1985**, *77*, 305 – 319.
[20] M. Russwurm, S. Behrends, C. Harteneck, D. Koesling, *Biochem. J.* **1998**, *335*, 125 – 130.
[21] M. M. Bradford, *Anal. Biochem.* **1976**, *72*, 248 – 254.
[22] C. Harteneck, B. Wedel, D. Koesling, J. Malkewitz, E. Böhme, G. Schultz, *FEBS Lett.* **1991**, *292*, 217 – 222.
[23] M. Michalak, M. Opas, *Curr. Opin. Neurol.* **1997**, *10*, 436 – 42.
[24] A. Castello, V. Brocheriou, P. Chafey, A. Kahn, H. Gilgenkrantz, *FEBS Lett.* **1996**, *383*, 124 – 128.

N_5^+: A Novel Homoleptic Polynitrogen Ion as a High Energy Density Material**

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Dedicated to Professor George Olah

Polynitrogen compounds are of significant interest as high energy density materials (HEDM) for propulsion or explosive applications.[1–3] In spite of numerous theoretical studies predicting that certain all-nitrogen compounds might be stable, only a few unsuccessful experimental studies aimed at their actual synthesis have been undertaken.[4] Presently, only two homoleptic polynitrogen species are known that can be prepared on a macroscopic scale: dinitrogen, N_2, which was independently isolated in pure form from air in 1772 by Rutherford, Scheele, and Cavendish, and the azide anion, N_3^-, discovered in 1890 by Curtius.[5] Other species such as $N_3^{\bullet}$, N_3^+, and N_4^+ have been observed only as free gaseous or matrix-isolated ions or radicals.[6–8] In view of the extensive theoretical studies indicating that molecules such as N_4, N_8, $N(N_3)_2^-$, $N(N_3)_3$, and $N(N_3)_4^+$ are vibrationally stable,[4] the lack of a single successful synthesis of a new species on a macroscopic scale is surprising and may be a testament to the great experimental difficulties resulting from their high endothermicities, which give rise to instability and unpredictable explosiveness.

The high energy content of polynitrogen candidates stems from the N–N single and double bonds they possess. The average bond energies of 160 and 418 kJ mol^{-1}, respectively, are much less than one-third or two-thirds the N_2 triple bond energy of 954 kJ mol^{-1}.[9] Therefore, any transformation of a polynitrogen compound to N_2 molecules is accompanied by a very large energy release, and any new metastable polynitrogen compound will be isolable and manageable only if it possesses a sufficiently large energy barrier to decomposition.

In view of the dearth of potential synthetic pathways for the construction of homoleptic polynitrogen rings or polycycles, and because many chain- or branch-type polynitrogen compounds are calculated to be lower in energy than their cyclic or polycyclic isomers,[3] our efforts are focused on the synthesis of catenated polynitrogen species, which may be more readily accessible. The weakest link in a chain always

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