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Peptide–peptoid hybrids based on (1–11)-parathyroid hormone analogs

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A series of peptide–peptoid hybrids, containing *N*-substituted glycines, were synthesized based on the H-Aib-Val-Aib-Glu-Ile-Gln-Leu-Nle-His-Gln-Har-NH_2 (Har = Homoarginine) as the parent parathyroid hormone (1–11) analog. The compounds were pharmacologically characterized in their agonistic activity at the parathyroid hormone 1 receptor. Copyright © 2010 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: *N*-substituted glycine; peptidomimetics; PTH analogs; conformation; bioactivities

Introduction

Although bioactive peptides are known to play decisive roles in many physiological processes via both inter- and intracellular communication and signal transduction mediated by various classes of receptors, the development of peptides into drugs for human medicine still represents a difficult task because of their poor bioavailability and fast clearance rates. Nonetheless, synthetic replicates of naturally occurring bioactive peptides and related analogs are excellent tools to characterize biochemically and biologically receptor systems for their further development into diagnostic or therapeutic drugs by improving their pharmacological properties often applying peptidomimetic strategies [1–4].

Among peptide mimicries peptoids consisting of *N*-substituted glycines (NSGs) represent an interesting class of synthetic molecules suitable for drug discovery because of their potential biological activities and high-proteolytic stability [5]. Indeed, peptoids were found to act as efficient receptor ligands [6–9] and to retain the biological activity of the parent natural peptides despite the shift of the amino acid side chains to the nitrogen atom [10–15].

The versatility and efficiency of the synthetic methods developed over the past years including the use of microwaves allow for efficient preparation of peptoid oligomers containing the most diversified side chains for proper mimicry of their peptide counterparts [16–20]. A special class of these molecules is peptoid–peptide hybrids (peptomers) in which only one or several amino acid residues are substituted by NSGs [21]. Such peptomers allow to explore the peptide tolerance toward transformation into peptoids in terms of potential bioactive structures and thus receptor binding affinities [16].

Parathyroid hormone (PTH) is an 84-amino acid peptide hormone secreted by the parathyroid glands. It acts primarily on bones and kidneys to maintain extracellular calcium levels within normal limits. The study of reduced-size PTH(1–34) agonist and antagonist analogs has been the subject of extensive research for the development of safer and non-parental bone anabolic drugs [22–24].

The interaction of (1–34)-PTH with its parathyroid hormone 1 receptor (PTH1R), a receptor of the G-protein coupled receptor (GPCR) family, has been postulated to follow a 'two-domain' model. The *N*-terminal (1–14) segment interacts with the 7-transmembrane (7-TM) helical domain embedded in the membrane and the *C*-terminal (19–34) segment with the *N*-terminal extracellular domain of the receptor [24,25]. These two domains, which are structured in α-helices, are separated by two hinge-like motifs located around positions 12 and 19 [26,27]. Studies on reduced-size (1–34)-PTH molecules [28,29] have shown that analogs of the (1–11)-PTH fragment containing residues capable of enhancing its helicity yielded potent agonists capable of reproducing the biological responses characteristic of the native intact PTH [23,30,31]. The lack of high-resolution structural data for GPCRs led to the design of mimetic molecules to gain more detailed information on the receptor/ligand complex. In this work, we have addressed the role and orientation of the Val^2, Nle^8 and Arg^{11} side chains in the interaction of short PTH fragments with the PTH1R receptor using three peptomers (Table 1), in which only one amino acid residue at the time is replaced by the corresponding peptoid residue. Their agonist activity is compared with the *N*-terminally modified (1–11)-PTH, peptide **1** (Table 1) as reference 32,33.

Materials and Methods

Materials

All solvents were purchased from commercial sources and used without purification. Reagent grade materials were obtained from GL Biochem (Shangai, China) and Inalco-Novabiochem (Milano, Italy). Molecular weights of final peptides were determined by ESI-MS-TOF on a Perseptive Biosystems MARINER™ API-TOF spectrometer (Foster City, CA, USA). RP purification was routinely performed on a Shimadzu LC-8A equipped with a Shimadzu SPD-6A UV detector on a Deltapak Waters C_{18}-100 Å silica HPLC column (19 mm × 30 cm, part no. 11799) at a flow rate of 17 ml/min by elution with a linear gradient 20–45% (v/v) of solvent B in solvent

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Table 1. Activity of analogs *versus* parent peptide

Entry	Peptide	Chemical formula	MW_{calc} [a]	$[M+H]^+$ found	R_t (min) [b]	EC_{50} (nM) [c]
1	H-Aib-Val-Aib-Glu-Ile-Gln-Leu-Nle-His-Gln-Har-NH_2 (parent peptide)	$C_{59}H_{103}N_{19}O_{15}$	1317.74	1318.73	12.07	1.1 ± 0.1
2	H-Aib-Val-Aib-Glu-Ile-Gln-Leu-Nle-His-Gln-NArg-NH_2 (NArg^{11}-PTH(1–11))	$C_{58}H_{101}N_{19}O_{15}$	1303.74	1304.56	13.85	2130 ± 160
3	H-Ala-NVal-Aib-Glu-Ile-Gln-Leu-Nle-His-Gln-Har-NH_2 (NVal^2-PTH(1–11))	$C_{58}H_{101}N_{19}O_{15}$	1303.74	1304.46	12.31	Not active
4	H-Aib-Val-Aib-Glu-Ile-Gln-Leu-NNle-His-Gln-Har-NH_2	$C_{59}H_{103}N_{19}O_{15}$	1317.74	1318.62	15.38	Not active

[a] Monoisotopic (m.i.) molecular weight calculated for chemical formula.
[b] R_t was determined with a linear gradient of 20–45% (v/v) B over 20 min (A: water + 0.1% TFA; B: 90% acetonitrile + 0.1% TFA).
[c] EC_{50} (effective concentration 50) is the result of the average on at least three values. EC_{50} is defined as the half maximal effective concentration and is referred to the concentration of peptide that induces a response halfway between the baseline and the maximum.

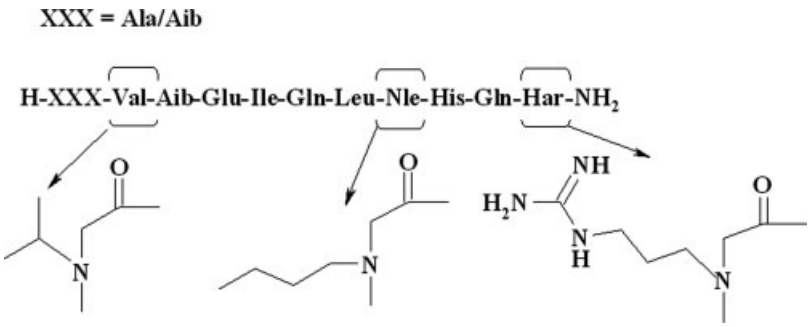

Figure 1. Scheme of strategic substitution of amino acids with NSG residues to generate peptomers.

A over 20 min. Homogeneity of the products was assessed by analytical RP-HPLC using a Vydac C18 column (218TP510), UV detection at 214 nm, flow rate 1 ml/min with a linear gradient 20–45% (v/v) of solvent B in solvent A over 20 min (solvent A: water + 0.1% TFA; solvent B: 90% acetonitrile + 0.1% TFA in water).

Peptide Synthesis

Peptomers were prepared by Fmoc solid-phase peptide synthesis for both proteinogenic amino acid residues and NSG residues on 0.1 mmol of Rink-amide-MBHA resin (0.73 mmol/g substitution grade) from Inalco-Novabiochem. The NSG residues, NVal, NNle and NArg, were synthesized according to the literature procedures [8,34,35]. The coupling of the suitably protected amino acids was performed with HOBt, HBTU and DIPEA, except for Aib, Val and the amino acid residues *N*-terminal to NSG where HOAt, HATU and collidine were used [36]. Fmoc cleavage was performed with 20% piperidine (v/v) in DMF. The resin-bound peptides were cleaved/deprotected with TFA/TIS/water (95 : 2.5 : 2.5 v/v/v) at room temperature for 2 h. After filtration, the filtrate was concentrated under nitrogen stream and precipitated with methyl *tert*-butyl ether. All crude peptide–peptoid hybrid analogs were purified and analytically characterized using RP C_{18} HPLC and molecular weights were determined by ESI-MS. The average yield of a complete synthesis after purification and lyophilization was 10%.

Biological Tests

Adenylyl cyclase assays for agonistic activities were performed with C20 HEK293 cells stably expressing the PTH1R and seeded at 10^6 cells/well in collagen-coated 24-well plates as previously reported [23]. After 24 h, the cells were treated with FuGENE 6 Transfection Reagent and CRE-luc plasmid, according to the manufacturer's procedure; 18 h after transfection, the cells were incubated for 4 h at 37 °C yielding maximal response to luciferase. Each peptide concentration was tested in triplicate. Luciferase activity was measured on a Lumat LB 9507 luminometer (EG&G Berthold, Bad Wildbad, Germany). The mean read-out from three wells with identical peptide concentration was used to present the data.

Circular Dichroism

CD measurements were carried out on a JASCO J-715 spectropolarimeter interfaced with a PC. The CD spectra were acquired and processed using the J-700 program for Windows. All experiments were carried out at room temperature using HELLMA quartz cells with Suprasil windows and optical path lengths of 0.01 and 0.1 cm. All spectra were recorded using a bandwidth of 2 nm and a time constant of 8 s at a scan speed of 20 nm/min. The signal to noise ratio was improved by accumulating eight scans. Measurements were carried out in the 190–250 nm wavelength range and the peptide concentration was in the range of 0.07–1.07 mM. The peptides were analyzed in aqueous solutions containing 20% TFE (v/v). The spectra are reported in terms of mean residue molar ellipticity (deg · cm^2 · d/mol). The helical content for each peptide was estimated according to the literature [37].

NMR Measurements

NMR spectra were recorded at 298 K on a BRUKER AVANCE DMX-600 spectrometer. The experiments were carried out in H_2O/TFE-d_3 (4 : 1, v/v). The sample concentration was approximately 1 mM in 600 µl solution. The water signal was suppressed by pre-saturation during the relaxation delay. The spin systems of all

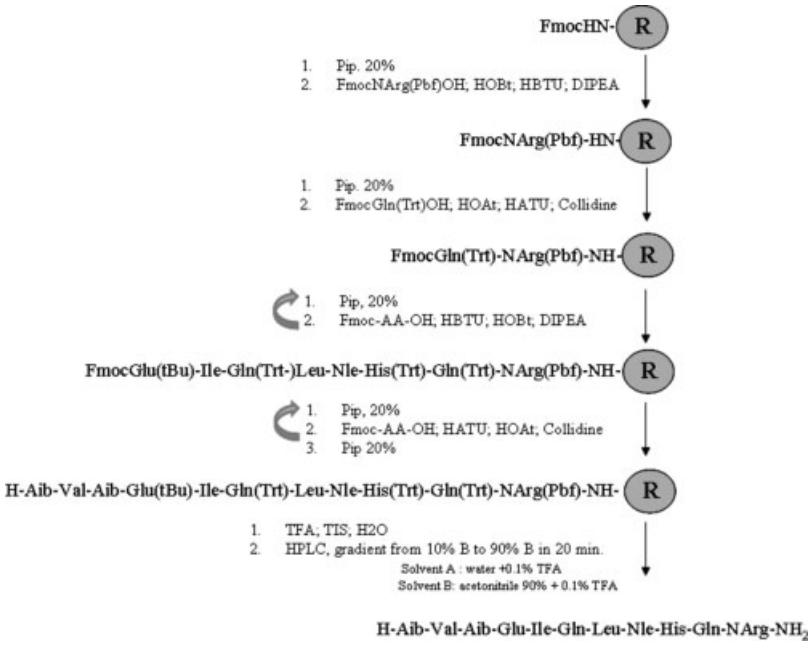

Figure 2. Synthetic scheme for analogue **2**. All analogues were synthesized in a similar way.

amino acid residues were identified using standard DQF (double-quantum-filtered)-COSY [38] and clear TOCSY [39] spectra. In the latter case, the spin-lock pulse sequence was 70 ms long. The sequence-specific assignment was accomplished using the ROESY experiment, with a mixing time of 150 ms. In all experiments, the spectra were acquired by collecting 400–512 experiments, each one consisting of 32–256 scans and 4K data points.

Spectral processing was carried out using XWINNMR. Spectra were calibrated against the TMS signal.

Results and Discussion

The peptoid–peptide hybrids were designed as outlined in Figure 1 and the resulting primary structures are reported in Table 1. As reference, the most active (1–11)-PTH analog peptide **1** [22,31] was used in accordance with our previous studies [32,33,40], although for a very similar peptide no activity was observed [23]. The most plausible explanation for this surprising finding may be the presence of the homoarginine residue in *C*-terminal position. Indeed, very recently we could demonstrate that the distance of the positive charge may play a decisive role in receptor binding affinity [40]. Moreover, Gardella *et al*. [28,29] have recently reported how modifications of (1–11)-PTH are very often additive, such as the replacement of Asn^{10} by Gln^{10} that leads to enhanced potency.

The syntheses were performed by a suitable combination of standard Fmoc solid-phase peptide synthesis for proteinogenic amino acid residues [36] and of the submonomer approach for NSG residues [8,16,34,35]. The synthesis of peptide **2** is reported in Figure 2 as an example. For peptide **3,** Aib in position 1 has been replaced by Ala to avoid steric hindrance caused by NVal. The analytical data are reported in Table 1.

Peptides **3** and **4**, with NVal and NNle in positions 2 and 8, respectively, showed no biological activity on the PTH receptor. The substitution of Arg in position 11 with the corresponding NSG maintained a low activity with an EC_{50} value of 2.1×10^{-6} M, according to a previous observation [40]. Specifically, the observed reduced potency supports the strategic role of arginine or homoarginine, which replaces leucine of native PTH, in anchoring (1–11)-PTH to the receptor [41] either by stabilizing the bioactive conformation [28] or by specific interactions with the receptor binding pocket [29]. Our previous work on molecular modeling suggests that this role might involve the insertion of the guanidinium side-chain group between the extracellular ends of TM1 and TM7 [27].

A CD study of the synthesized peptides was performed to compare their conformation with the results of the biological tests. The conformational properties of the new analogs were investigated first in 20% TFE/water [23,32] and then in the presence of increasing TFE concentrations [42]. The CD spectra of the (1–11)-PTH analogs are reported in Figure 3. Peptomer **2** shows a typical α-helical CD spectrum, similar to that of the parent peptide (Figure 3a), and the helix content increases at TFE concentrations higher than 10% (Figure 4). This is consistent with the presence of biological activity for **2** (Table 1), albeit three orders of magnitude lower than that of the parent peptide. By increasing the TFE concentration, the CD spectra become compatible with the presence of some 3_{10}-helix or β-turn, according to the low-intensity ratio between the bands at 222 and 208 nm [43]. Anyway, peptides **2** and **3** have the identical helix-propensity, as demonstrated in Figure 4, although the hybrid **3** (Figure 3b) is not biologically active (Table 1). The introduction of $NVal^2$ at the *N*-terminal position might disrupt the nucleation of the helix, or the side chain of NVal assumes an inadequate orientation for interaction with the receptor.

The peptide–peptoid hybrid **4** exhibits a CD spectrum typical of a flexible and extended structure even at high percentages of TFE (not shown). The absence of any structural effect of TFE (Figure 4) indicates that the introduction of an NSG in position 8 disrupts the stability of the helix, which leads to the loss of bioactivity (Table 1).

To prove CD results, NMR analysis of secondary chemical shifts of the C^{α} protons was performed on all analogs (Figure 5). The secondary chemical shifts of peptide **4** are very close to zero or positive, which confirms the absence of ordered structure as

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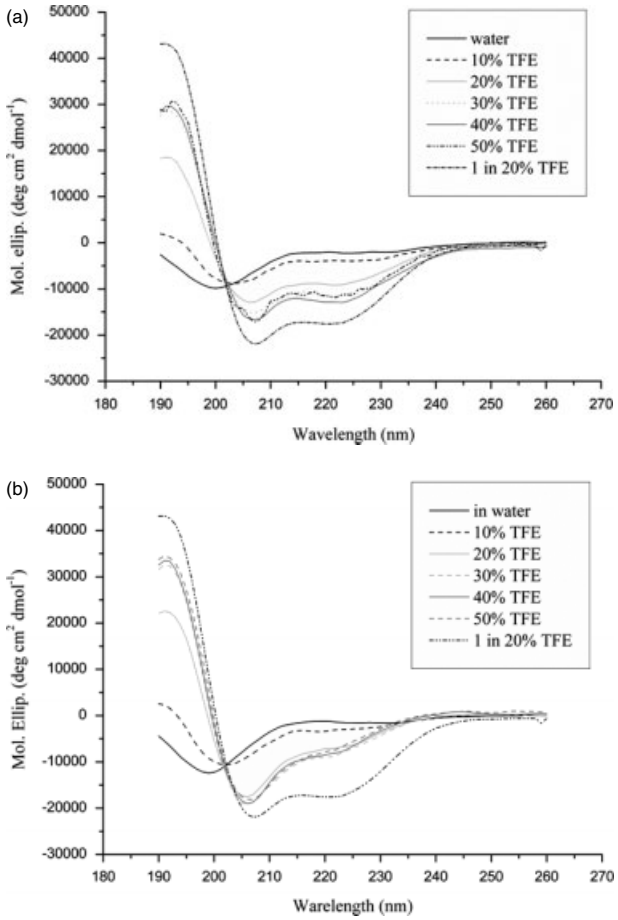

Figure 3. CD spectra of **2** (a) and **3** (b) at different concentrations of TFE in water (from 0 to 50%). In both panels, the CD spectrum of the parent peptide in 20% TFE is reported as a reference.

determined by CD analysis. The crucial role of Nle in position 8 in stabilizing the α-helix from the *N*-terminal to the *C*-terminal residue has just been confirmed in our preceding study [32]. Both hybrid peptides **2** and **3** show partially ordered structures. In the *C*-terminus, the helical structure is better maintained in compound **2** than in **3**. The secondary chemical shifts in the *N*-terminal region

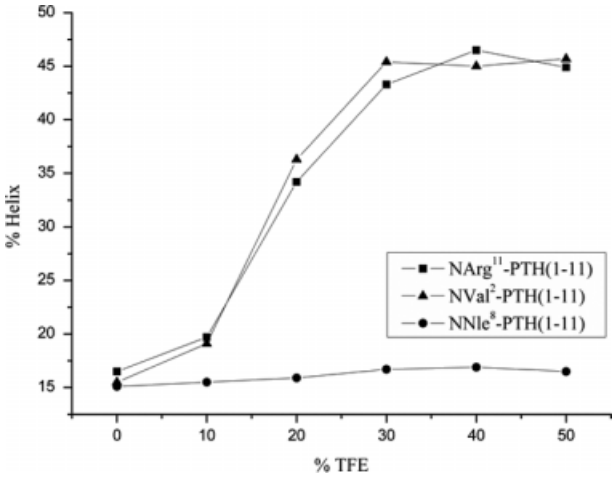

Figure 4. % Helix *versus* % TFE calculated according to Greenfield and Fasman [44].

are not very informative because random coil chemical shifts for NSG are not available in the literature. Nevertheless, the effect in the central part of the molecule is similar for these two analogs.

In these peptomers, the shift of the side chains from the α-carbon to the amide nitrogen creates tertiary amides, thus favoring *cis–trans* isomerism and preventing intramolecular H-bonds, destabilizing the secondary structure [38] or placing the side chain in an unfavorable topological position [45,46].

Conclusions

The hybrid compounds **2**–**4** demonstrate that replacing Val^2, Nle^8 and Har^{11} with peptoid residues leads to decreased potency at the PTH1 receptor, although the magnitude of such decrease depends on the site of substitution. Compound **2** (substitution of Har) was the only hybrid that retained functional activity, although the potency was reduced when compared with the reference peptide **1**. The residual activity of NArg^{11} suggests that the positive charge at the end of the peptide is important for binding, but its distance from the peptide backbone is significantly affecting recognition by the receptor [40]. The structural role of residues 2 and 8 was

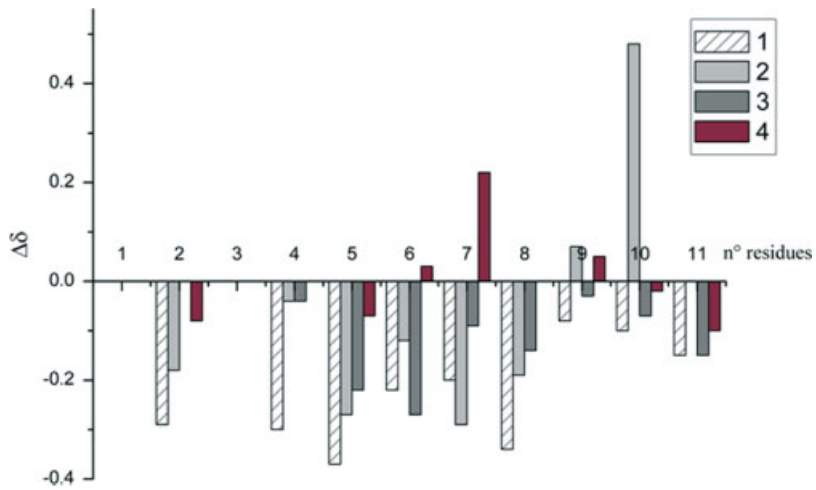

Figure 5. Secondary chemical shifts ($\Delta\delta = \delta_{measured} - \delta_{random\ coil}$) of peptomers. Numbering is according to Table 1.

again confirmed [24]. Indeed, disruption of helicity is possibly the main cause for the inactivity of the peptomer containing NNle[8], whereas the side-chain orientation of NVal[2] is most probably the main cause of the inactivity of the corresponding hybrid. A significant reduction in the α-helical content was found in all the analogs studied, showing that a stable helix is necessary, although not sufficient, for PTH-like ligand interaction with the receptor.

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References

1 Adessi C, Soto C. Converting a peptide into a drug: strategies to improve stability and bioavailability. *Curr. Med. Chem.* 2002; **9**: 963–978.
2 Vagner J, Qu H, Hruby VJ. Peptidomimetics, a synthetic tool of drug discovery. *Curr. Opin. Chem. Biol.* 2008; **12**: 292–296.
3 Hruby VJ. Designing peptide receptor agonists and antagonists. *Nat. Rev. Drug Discov.* 2002; **1**: 847–858.
4 Holder JR, Bauzo RM, Xiang Z, Scott J, Haskell-Luevano C. Design and pharmacology of peptoids and peptide–peptoid hybrids based on the melanocortin agonists core tetrapeptide sequence. *Bioorg. Med. Chem. Lett.* 2003; **13**: 4505–4509.
5 Vercillo OE, Andrade CKZ, Wessjohann LA. Design and synthesis of cyclic RGD pentapeptoids by consecutive Ugi reactions. *Org. Lett.* 2008; **10**: 205–208.
6 Zuckermann RNK, Kerr JM, Kent SBH, Moos WH. Efficient method for the preparation of peptoids [oligo(N-substituted glycines)] by submonomer solid-phase synthesis. *J. Am. Chem. Soc.* 1992; **114**: 10646–10647.
7 Simon RJ, Kania RS, Zuckermann RN, Huebner VD, Jewell DA, Banville S, Ng S, Wang L, Rosenberg S, Marlowe CK, Spellmeyer DC, Tan R, Frankel AD, Santi DV, Cohen FE, Bartlett PA. Peptoids: a modular approach to drug discovery. *Proc. Natl. Acad. Sci. U. S. A.* 1992; **89**: 9367–9371.
8 Schröder T, Niemeier N, Afonin S, Ulrich AS, Krug HF, Bräse S. Peptoidic amino- and guanidinium-carrier systems: targeted drug delivery into the cell cytosol or the nucleus. *J. Med. Chem.* 2008; **51**: 376–379.
9 Kessler H. Peptoids: a new approach to the development of pharmaceuticals. *Angew. Chem. Int. Ed. Engl.* 1993; **32**: 543–544.
10 Messeguer J, Cortes N, Garcja-Sanz N, Navarro-Vendrell G, Ferrer-Montiel A, Messeguer A. Synthesis of positional scanning library of pentamers of N-alkylglycines assisted by microwave activation and validation via the identification of trypsin inhibitors. *J. Comb. Chem.* 2008; **10**: 974–980 and references therein.
11 Edwards P. Peptoid positional scanning libraries for identification of multidrug resistance reversal agents. *Drug Discov. Today* 2006; **11**: 669–670.
12 Goodman M, Bhumralkar M, Jefferson EA, Kwak J, Locardi E. Collagen mimetics. *Biopolymers* 1998; **47**: 127–142.
13 Heizmann G, Hildebrand P, Tanner H, Ketterer S, Pansky A, Froidevaux S, Beglinger C, Eberle AN. A combinatorial peptoid library for the identification of novel MSH and GRP/bombesin receptor ligands. *J. Recept. Signal Transduct. Res.* 1999; **19**: 449–466.
14 Zuckermann RN, Martin EJ, Spellmeyer DC, Stauber GB, Shoemaker KR, Kerr JM, Figliozzi GM, Goff DA, Siani MA, Simon RJ, Banville SC, Brown EG, Wang L, Richter LS, Moos WH. Discovery of nanomolar ligands for 7-transmembrane G-protein-coupled receptors from a diverse N-(substituted)glycine peptoid library. *J. Med. Chem.* 1994; **37**: 2678–2685.
15 Kruijtzer JAW, Nijenhuis WAJ, Wanders N, Gispen WH, Liskamp RMJ, Adan RAH. Peptoid-peptide hybrids as potent novel melanocortin receptor ligands. *J. Med. Chem.* 2005; **48**: 4224–4230.
16 Humet M, Carbonell T, Masip I, Sánchez-Baeza F, Mora P, Cantón E, Gobernado M, Abad C, Pérez-Payá E, Messeguer A. A positional scanning combinatorial library of peptoids as a source of biological active molecules: identification of antimicrobials. *J. Comb. Chem.* 2003; **5**: 597–605.
17 Udugamasooriya DG, Dineen SP, Brekken RA, Kodadek T. A peptoid "antibody surrogate" that antagonizes VEGF receptor 2 activity. *J. Am. Chem. Soc.* 2008; **130**: 5744–5752.
18 Roy O, Faure S, Thery V, Didierjean C, Taillefumier C. Cyclic β-peptoids. *Org. Lett.* 2008; **10**: 921–924.
19 Diaz-Mochon JJ, Fara MA, Sanchez-Martin RM, Bradley M. Peptoid dendrimers – microwave-assisted solid-phase synthesis and transfection agent evaluation. *Tetrahedron Lett.* 2008; **49**: 923–926.
20 Ruijtenbeek R, Kruijtzer JAW, van de Wiel W, Fischer MJE, Flück M, Redegeld FAM, Liskamp RMJ, Nijkamp FP. Peptoid-peptide hybrids that bind Syk SH2 domains involved in signal transduction. *ChemBioChem* 2001; **2**: 171–179.
21 Fowler SA, Stacy DM, Blackwell HE. Design and synthesis of macrocyclic peptomers as mimics of quorum sensing signal from Staphylococcus. *Org. Lett.* 2008; **10**: 2329–2332.
22 Shimizu N, Guo J, Gardella TJ. Parathyroid hormone (PTH)-(1–14) and -(1–11) analogs conformationally constrained by alpha-aminoisobutyric acid mediate full agonist responses via the juxtamembrane region of the PTH-1 receptor. *J. Biol. Chem.* 2001; **276**: 49003–49012.
23 Barazza A, Wittelsberger A, Fiori N, Schievano E, Mammi S, Toniolo C, Alexander JM, Rosenblatt M, Peggion E, Chorev M. Bioactive N-terminal undecapeptides derived from parathyroid hormone: the role of alpha-helicity. *J. Pept. Res.* 2005; **65**: 23–35.
24 Potts JT, Gardella TJ. Progress, paradox, and potential parathyroid hormone research over five decades. *Ann. N. Y. Acad. Sci.* 2007; **1117**: 196–208.
25 Gardella TJ, Jüppner H. Molecular properties of the PTH/PTHrP receptor. *Trends Endocrinol. Metab.* 2001; **5**: 210–217.
26 Scian M, Marin M, Bellanda M, Tou L, Alexander JM, Rosenblatt M, Chorev M, Peggion E, Mammi S. Backbone dynamics of human parathyroid hormone (1–34): flexibility of the central region under different environmental conditions. *Biopolymers* 2006; **84**: 147–160.
27 Monticelli L, Mammi S, Mierke DF. Molecular characterization of a ligand-tethered parathyroid hormone receptor. *Biophys. Chem.* 2002; **95**: 165–172.
28 Shimizu M, Potts JT Jr, Gardella TJ. Minimization of parathyroid hormone. Novel amino-terminal parathyroid hormone fragments with enhanced potency in activating the type-1 parathyroid hormone receptor. *J. Biol. Chem.* 2000; **275**: 21836–21843.
29 Shimizu M, Carter PH, Khatri A, Potts JT Jr, Gardella TJ. Enhanced activity in parathyroid hormone-(1–14) and -(1–11): novel peptides for probing ligand-receptor interactions. *Endocrinology* 2001; **142**: 3068–3074.
30 Tsomaia N, Pellegrini M, Hyde K Gardella TJ, Mierke DF. Toward parathyroid hormone minimization: conformational studies of cyclic PTH(1–14) analogues. *Biochemistry* 2004; **43**: 690–699.
31 Shimizu M, Petroni BD, Khatri A, Gardella TJ. Functional evidence for an intramolecular side chain interaction between residues 6 and 10 of receptor-bound parathyroid hormone analogues. *Biochemistry* 2003; **42**: 2282–2290.
32 Caporale A, Biondi B, Schievano E, Wittelsberger A, Mammi S, Peggion E. Structure-function relationship studies of PTH(1–11) analogues containing D-amino acids. *Eur. J. Pharmacol.* 2009; **611**: 1–7.
33 Fiori N, Caporale A, Schievano E, Mammi S, Geyer A, Tremmel P, Wittelsberger A, Woznica I, Chorev M, Peggion E. Structure-function relationship studies of PTH(1–11) analogues containing sterically hindered dipeptide mimetics. *J. Pept. Sci.* 2007; **13**: 504–512.
34 Kruijtzer JAW, Hofmeyer LJF, Heerma W, Versluis C, Liskamp RMJ. Solid-phase syntheses of peptoids using Fmoc-protected N-substituted glycines: the synthesis of (retro)peptoids of Leu-enkephalin and substance P. *Chem. Eur. J.* 1998; **4**: 1570–1580.
35 Uno T, Beausoleil E, Goldsmith RA, Levine BH, Zuckermann RN. New submonomers for poly N-substituted glycines (peptoids). *Tetrahedron Lett.* 1999; **40**: 1475–1478.

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36 Carpino LA, El-Faham A, Albericio F. Efficiency in peptide coupling: 1-hydroxy-7-azabenzotriazole vs 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine. *J. Org. Chem.* 1995; **60**: 3561–3564; Carpino LA, El-Faham A. Effect of tertiary bases on O-benzotriazolyluronium salt-induced peptide segment coupling. *J. Org. Chem.* 1994; **59**: 695–698; Carpino LA. 1-Hydroxy-7-azabenzotriazole. An efficient peptide coupling additive. *J. Am. Chem. Soc.* 1993; **115**: 4397–4398.

37 Yang JT, Wu CS, Martinez HM. Calculation of protein conformation from circular dichroism. *Methods Enzymol.* 1986; **130**: 208–269.

38 Rance M, Sørensen OW, Bodenhausen G, Wagner G, Ernst RR, Wüthrich K. Improved spectral resolution in cosy 1H NMR spectra of proteins via double quantum filtering. *Biochem. Biophys. Res. Commun.* 1983; **117**: 479–485.

39 Griesinger C, Otting G, Wüthrich K, Ernst RR. Clean TOCSY for proton spin system identification in macromolecules. *J. Am. Chem. Soc.* 1988; **110**: 7870–7872.

40 Caporale A, Woznica I, Schievano E, Mammi S, Peggion E. Role of the Guanidine group in the N-terminal fragment of PTH(1–11). *Amino Acids* 2010; **4**: 1269–1275.

41 Lim S-K, Lee EJ, Kim H-Y, Lee W. The 10th and 11th residues of short length N-terminal PTH(1–12) analogue are important for its optimum potency. *J. Pept. Res.* 2004; **64**: 25–32.

42 Buck M. Trifluoroethanol and colleagues: cosolvents come of age. Recent studies with peptides and proteins. *Q. Rev. Biophys.* 1998; **31**: 297–355.

43 Moretto A, Formaggio F, Kaptein B, Broxterman QB, Wu L, Keiderling TA, Toniolo C. First homo-peptides undergoing a reversible 3_{10}-helix/α-helix transition: critical main-chain length. *Biopolymers* 2008; **90**: 567–574.

44 Greenfield N, Fasman GD. Computed circular dichroism spectra for the evaluation of protein conformation. *Biochemistry* 1969; **8**: 4108–4116.

45 Richardson JS. The anatomy and taxonomy of protein structure. *Adv. Protein Chem.* 1981; **34**: 167–339.

46 de Haan EC, Wauben MHM, Grosfeld-Stulemeyer MC, Kruijtzer JAW, Liskamp RMJ, Moret EE. Major histocompatibility complex class II binding characteristics of peptoid–peptide hybrids. *Bioorg. Med. Chem.* 2002; **10**: 1939–1945.

