End-to-end conformational communication through a synthetic purinergic receptor by ligand-induced helicity switching

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Abstract

The long-range communication of information, exemplified by signal transduction through membrane-bound receptors, is a central biochemical function. Reversible binding of a messenger ligand induces a local conformational change that is relayed through the receptor, inducing a chemical effect typically several nanometres from the binding site. We report a synthetic receptor mimic that transmits structural information from a boron-based ligand binding site to a spectroscopic reporter located > 2 nm away. Reversible binding of a diol ligand to the N-terminal binding site induces a screw-sense preference in a helical oligo(aminoisobutyric acid) foldamer, which is relayed to a reporter group at the remote C-terminus, communicating information about the structure and stereochemistry of the ligand. The reversible nature of boronate esterification was exploited to switch the receptor sequentially between left- and right-handed helices, while the exquisite conformational sensitivity of the helical relay allowed the reporter to differentiate even between purine and pyrimidine nucleosides as ligands.

Introduction

The close relationship between local molecular conformation and global structure is apparent in the complex architecture of biopolymers, where subtle changes in the arrangement of functional groups are translated and amplified into gross changes in biomolecule morphology. However, the functions of biomolecules are dictated not only by their static structure, but also their dynamic properties. Allostery in enzymes, cooperative binding to transport proteins such as haemoglobin, and communication through transmembrane receptors are examples of key biological processes that depend on long-range conformational changes initiated by binding of a ligand or substrate.^{1,2} Synthetic molecules designed to mimic the architecture and binding properties of biopolymers are now commonplace, with examples including peptoids,³ peptide-nucleic acids,⁴ amide foldamers,⁵ and anti-sense nucleotides.⁶ However synthetic mimics of dynamic, switchable structures, capable of translating information about ligand binding into remote conformational consequences, are much rarer.⁷⁻⁹

We therefore set out to create functional mimics of biological receptors, which use conformational change to effect a remote response. These biomimetic receptors will carry a terminal binding site capable of strong, but reversible, interactions with selected ligands. Ligand binding will lead to a conformational change that propagates along the receptor, carrying information to a reporter terminus several nanometres distant from the binding site.

To allow unidirectional information transfer by conformational relay in the artificial receptor mimic we chose to make use of a helical foldamer^{10,11} in the form of an oligomer constructed from achiral monomers. Configurationally achiral helical foldamers necessarily populate equally left- and right-handed screw-sense conformers, but the equilibrium between these conformers is sensitive to subtle chiral influences. The screw-sense of helical polymers^{12,13} can be controlled by relatively

few chiral monomers among many achiral ones (the 'sergeants and soldiers' principle)^{14,15} or by coordination to chiral counterions.^{9,16,17} It is well established that a single chiral residue sited at the terminus of an otherwise achiral helical oligomer is sufficient to bias the screw-sense of the oligomer,¹⁸⁻²² and remote spectroscopic reporters have been used to detect the resulting conformational preference.^{9,21-27} Helical oligomers may consequently behave as communication devices, since relayed conformational change allows stereochemistry at one end of the oligomer to influence a reporter several nanometres away.²⁸ We have shown that achiral helical peptide oligomers made from the quaternary amino acid Aib may communicate information about configurational inversion at a terminal chiral residue to a remote NMR reporter group.²³

In principle, such conformational changes may be induced in a biomimetic fashion, using a reversible interaction between a chiral ligand and an achiral, but helical, oligomer bearing a binding site. For example, the screw-sense of an amino-terminated oligomer may be influenced by weak binding to a large excess of a chiral amino acid.⁹ The much stronger, yet reversible complexation of a boronic acid with a vicinal diol has been widely exploited for the development of selective carbohydrate sensors.²⁹⁻³¹ The well-studied complexes and defined geometries that result seemed to us an ideal basis for the design of the selective binding site needed to build a functional, switchable synthetic receptor.

In this paper we show that dynamic esterification at a boron-containing binding site can be used to transmit structural information about diol ligands along the multi-nanometre length of a helical oligoamide receptor. Ligand complexation is translated into a conformational preference, which is communicated to a reporter group at the far terminus through a helical relay. The reversible nature of boronate esterification allows dynamic switching between left- and right-handed helices.

Figure 1 | Remote detection of ligand binding to a synthetic receptor by NMR spectroscopy. **a**, Diastereotopic ‘reporter’ nuclei A and B appear as a single (isochronous) signal due to fast exchange between equally populated *M* and *P* conformers of the unbound receptor. **b**, On binding, a chiral ligand induces a preference for one screw-sense over the other, biasing the helical equilibrium and resulting in separate (anisochronous) signals for nuclei A and B.

Results and discussion

Design and synthesis of artificial receptors. The proposed functioning of the synthetic receptor is illustrated in Figure 1. Binding to a chiral ligand will perturb the foldamer’s rapidly interconverting conformational equilibrium of *M* and *P* helices. The strong tendency of the foldamer (an oligomer of 2-aminoisobutyric acid, or Aib) to maintain a 3_{10} helical conformation^{18,32,33} means that this perturbation propagates along the entire length of the oligomer, and is detectable spectroscopically at some distance from the binding site. In the system we present here, NMR is used to report the resulting unequal populations of *M* and *P* helices. Because interconversion between the screw-sense conformers of the oligo-Aib in question is fast on the NMR timescale,³⁴ any imbalance between the populations of *M* and *P* screw-senses becomes evident in the anisochronicity of a pair of groups A and B, which are rendered diastereotopic by the chiral environment provided by the helix.³⁵ Thus a rapidly interconverting, equally populated mixture of screw-sense conformers will give rise to a single NMR signal for A and B, while an interconverting, unequally populated mixture of screw-sense conformers will give rise to anisochronous signals for A and B separated by a chemical shift difference ($\Delta\delta$) that is proportional to the

excess of one screw-sense conformer over the other. In the first part of this paper, A and B are ^1H nuclei; in the latter part of the paper, A and B are carbon nuclei each with a different $^{12}\text{C}/^{13}\text{C}$ ratio.

Helical oligomers of Aib were synthesised in a stepwise manner (Supplementary Fig. 1) as N-terminal azido derivatives **1a-c** each bearing one of two different C-terminal NMR reporters: a diastereotopic CH_2 group in the case of **1a**;³⁶ a pair of diastereotopic methyl groups that are enantioselectively labelled with ^{13}C in **1b** and **1c**.³⁷ We decided to use a 2-(aminomethyl)phenylboronic acid derivative³⁸ as an N-terminal binding site, since this motif shows high affinity for vicinal diols,³⁰ which condense with the boronic acid to form cyclic boronate esters. Coordination between boron and a nearby amino group stabilises tetrahedral geometry at boron and can increase the rate of esterification by a factor of up to 10^4 .³⁹ We reasoned that boronate ester formation between a diol ligand and a boronic acid binding site at the N-terminus of a helical peptide foldamer would allow stereochemical information to be translated from the ligand into the conformational screw-sense preference of the helix.

Figure 2 | Synthesis of boronate-capped receptors used in this study. Me* = $^{13}\text{CH}_3$. Enantiomeric ratio = 70:30 (*R/S*) in **1b**, **1c**, **4** and **5**. ^aIsolated after chromatography on SiO_2 using methanol-containing eluent.

The diol recognition site was successfully constructed by a two-step procedure, starting with hydrogenation of the azido-capped Aib oligomers **1** (Fig. 2). We found that protection of 2-formylphenylboronic acid as its neopentyl glycol ester **2** was crucial to the success of the subsequent one-pot reductive amination. Methanolysis of the protecting group during purification by chromatography gave receptors **3-5** as their dimethyl boronate esters.

Establishing ligand-induced conformational control. To assess both the strength of binding to chiral diols by the receptors and the extent of any helical screw-sense preference induced as a result, we devised a titration assay using ^{11}B and ^1H NMR spectroscopy. Receptor **3**, in which the N-terminal boronate binding site is separated from a C-terminal GlyNH₂ NMR reporter by an Aib tetramer, was first dissolved in deuterated methanol. The ^{11}B NMR spectrum of **3** (Fig. 3a, 0 equiv) revealed a single resonance at 11.1 ppm, consistent with the expected N-B coordinated tetrahedral geometry at the boron centre.⁴⁰ In parallel, structural information about the 3_{10} helical domain of the receptor was revealed by the ^1H NMR spectrum. The racemic nature of the rapidly interconverting helix in **3** produces singlets (see Fig. 1a) for the diastereotopic methylene groups both at the binding site and at the C-terminal GlyNH₂ reporter, at 4.07 and 3.82 ppm respectively. Monitoring these diagnostic signals while increasing diol concentration allowed us to observe structural and conformational changes at both termini during binding.

We began by titrating receptor **3** with the C₂-symmetric vicinal diol (+)-hydrobenzoin **6a**. Binding of **6a** to form the ester **3a** was confirmed by a shift in the ^{11}B NMR signal from 11.1 to 8.4 ppm (Fig. 3a).⁴⁰ The 5-membered cyclic boronate esters possess increased Lewis acidity at the boron centre that can strengthen a dative N-B interaction,³⁰ but if steric bulk impedes direct N-B bonding, a solvent-inserted

complex may result.⁴¹ The upfield shift of the ^{11}B NMR signal is consistent with methanol insertion across the N-B bond to give zwitterionic species **3a**,⁴⁰ with tetrahedral geometry at the resulting anionic boron center stabilised by the adjacent ammonium cation.

Conformational changes in the helix of **3** are clearly visible in the ^1H NMR spectrum as the concentration of **6a** increases. Firstly, the previously isochronous benzylic protons of unbound receptor **3** (4.07 ppm) split into separate signals in the now asymmetric environment of diol-receptor complex **3a**. This new AB system also experiences a downfield shift due to protonation of the adjacent amine during solvent insertion.⁴¹ Crucially, the geminal protons of the C-terminal GlyNH_2 NMR reporter also now appear anisochronous ($\Delta\delta = 84$ ppb), despite being separated from the nearest chiral centre by more than one full turn of the helix.³³ This spatial separation precludes any change in chemical shift (the new signals remain centered around 3.82 ppm) but the anisochronicity in the reporter CH_2 group provides clear evidence that binding of the chiral diol ligand has induced a screw-sense preference in the otherwise achiral helix.

Figure 3 | Probing ligand-induced conformational control by NMR. ^{11}B NMR (160 MHz) and ^1H NMR (500 MHz) spectra tracking binding and conformational induction in ligand-receptor complexes with increasing [diol] in CD_3OD at 298 K. Initial $[3] = 0.03\text{ M}$. **a**, Addition of (+)-hydrobenzoin **6a** to receptor **3**. **b**, Addition of *meso*-hydrobenzoin **6b** to receptor **3**. **c**, Addition of (+)-hydrobenzoin **6a** to an equimolar mixture of receptor **3** and control peptide **1a**.

By contrast, adding the *meso* diastereoisomer of hydrobenzoin **6b** to **3** to form complex **3b** indicated significant differences. The ^{11}B NMR spectrum revealed a similar upfield shift on coordination (Fig. 3b), but quantitative binding was not achieved even after adding 5 equivalents of the diol. We attribute the receptor's lower affinity for **6b** to the production of a less stable *cis*-substituted cyclic ester in **3b**.³⁰ The ^1H NMR spectrum shows chemical shift differences for both CH_2 resonances in **3b** that are analogous to those in **3a**, but crucially these signals remain isochronous due to the symmetry of the diol ligand **6b**.

The NMR spectra indicate that all aspects of the conformational dynamics of ligand-receptor complex **3a** are in fast exchange on the NMR timescale. The simplicity of the NMR signals for the bound diol indicates rapid exchange of the inserted CD_3OD and stereochemical inversion at the boron centre.⁴² Nonetheless, the anisochronicity

of the reporter signals shows that the bound ligand exerts an effect on the conformation of the receptor, which we interpret as the induction of a thermodynamic bias towards a prevailing helical screw-sense (see Fig. 1b), that has propagated along the receptor. To support this conclusion, and rule out the possibility that anisochronicity arises as a result of direct intermolecular interactions between the chiral additive and the reporter group, we repeated the formation of complex **3a** from an equimolar mixture of **3** and azido-capped peptide N₃Aib₄GlyNH₂ **1a**. This control peptide contains a C-terminal GlyNH₂ NMR reporter, but lacks the boronate motif required for diol complexation. As before, addition of (+)-hydrobenzoin **6a** resulted in the formation of complex **3a** from **3** but no changes could be detected in the ¹H NMR spectrum of peptide **1a** (Fig. 3c). This fact, along with the lack of changes on mixing the control peptide **1a** and diol **6a** alone (Supplementary Information, page S41), and the concentration independence of the anisochronicity of the NMR reporter signals in complex **3a**, together rule out the possibility that anisochronicity arises from direct intermolecular interactions between the NMR reporter and the chiral diol or boronate. Dilution of a methanolic solution of complex **3a** with hexafluoroisopropanol (Supplementary Information, page S42), which disrupts 3₁₀ helices,⁴³ does however collapse the anisochronous CH₂ peaks of the C-terminal GlyNH₂ reporter without the analogous change in the N-terminal CH₂ peaks, indicating that a stable helix is required for intramolecular conformational communication from the binding site to the reporter group.

Following the successful use of (+)-hydrobenzoin **6a** as a simple ligand to induce a screw-sense bias, we screened structurally related diols to optimise both binding affinity and the degree of conformational control. Equilibrium constants for the binding of diol ligands **6a-m** to receptor **3** were estimated and the anisochronicity ($\Delta\delta$) in the CH₂ protons of the GlyNH₂ probe measured in the resulting complexes **3a-m** (Table 1).

Table 1 | Complexation of diol ligands 6a-m with receptor 3: binding constants and induced conformational preferences in boronate esters 3a-m.

	diol	R	$K^{a,b} / M^{-1}$	$\Delta\delta^{a,c} / \text{ppb}$
(+)-6a,c-l	6a ((+)-hydrobenzoin)	Ph	1500 ± 100	84
	6b (<i>meso</i> -hydrobenzoin)	-	40 ± 3	0
	6c^d	2-MeO-C ₆ H ₄	600 ± 50	80
	6d	2-Cl-C ₆ H ₄	1100 ± 300	100
	6e^e	2-Br-C ₆ H ₄	1200 ± 300	99
meso-6b	6f^e	2-Me-C ₆ H ₄	1500 ± 200	125
	6g^d	1-naphthyl	>2000	126
	6h	CO ₂ Me	240 ± 30	75
	6i	CO ₂ Et	220 ± 80	64
	6j^d	CO ₂ <i>i</i> -Pr	300 ± 100	59
6m	6k^d	CO ₂ Bn	180 ± 60	57
	6l	CON(Me) ₂	300 ± 50	89
	6m (pinanediol)	-	>>2000	0

^a From ¹H NMR experiments in CD₃OD at 298 K. ^b Binding constant K estimated by integrating spectra of mixtures (of **3**, **3a-m** and **6a-m**) arising from addition to **3** of 1, 2 and 5 equiv **6a-m** (see Supplementary Information, page S24). ^c Anisochronicity, in parts per billion (ppb), of the AB system arising from the Gly CH₂ group of **3a-m**, given by $v_0\Delta\delta = [(f_1 - f_3)^2 - J_{AB}^2]^{1/2} = [(f_2 - f_4)^2 - J_{AB}^2]^{1/2} = [(f_1 - f_4)(f_2 - f_3)]^{1/2}$ where $f_{1,2,3,4}$ are the observed resonance frequencies in order of the four lines comprising the AB multiplet, J_{AB} is the coupling constant, and v_0 is the spectrometer frequency (500 MHz). ^d(-)-Enantiomer used. ^eRacemic mixture used.

Increased bulk at the *ortho*-position of the diol's phenyl rings (Table 1, **6c-f**) led to increasing conformational bias in **3c-f**, without significant diminution in affinity relative to (+)-hydrobenzoin **6a**. This general increase in chemical shift separation with increasing size culminated in a value of $\Delta\delta = 126$ ppb for the 1-naphthyl-substituted diol **6g**. We also tested a series of C₂-symmetric tartaric acid derivatives, and found that they too induced a screw-sense preference, albeit with significantly weaker binding to the receptor. In contrast to the hydrobenzoin derivatives, increasing substituent size across tartrates **6h-k** decreased the diol's ability to control the helical screw-sense. All of the complexes **3a-l** exhibit a solvent-inserted N–B interaction,

with ^{11}B NMR chemical shifts between 8.4 and 9.8 ppm.⁴⁰ In contrast, (–)-pinanediol **6m**, known for its strong interaction with boronic acids, formed complex **3m** that displayed a broad ^{11}B NMR signal centered at 28.2 ppm, indicative of trigonal geometry at boron.⁴⁰ The corresponding ^1H NMR spectrum of complex **3m** revealed that (–)-pinanediol, despite its chirality, failed to induce a detectable screw-sense preference, perhaps in part due to the lack of N–B interaction required to bring the chiral boronate ester into proximity with the helix.

Switching helicity by competitive ligand binding. To function effectively, biological receptors must be able to switch between discrete states after changes in external stimuli, and that switching must induce a conformational change remote from the site of ligand binding. Determining the binding affinities for different diols (Table 1) allowed an experiment to be designed that would demonstrate dynamic stimulus-induced switching in our receptor mimic. Diols with low affinity for the boronate binding site should be easily displaced by more strongly coordinating ligands,⁴⁴ and we expected that such an exchange using diols of opposite stereochemical configuration would result in reversal of the screw-sense preference, from *M* to *P* or vice versa.

To detect switching between *M* and *P* helix conformers, they must be rendered diastereoisomeric by the inclusion of an NMR reporter that is chiral, but nonetheless unable to exert any controlling influence of its own. We consequently replaced the GlyNH₂ ^1H NMR probe used in earlier experiments with an isotopically desymmetrised chiral mono- ^{13}C -labelled Aib*OMe ^{13}C NMR probe,²³ which was ligated to the C-terminus of the otherwise identical receptor **4**. The enantioselective isotopic labeling method used to synthesise this probe from L-Ala³⁷ gave (*R*)-Aib*OMe and (*S*)-Aib*OMe in a ca. 70:30 ratio. Once located within the chiral

environment of the Aib helix, the ^{13}C nuclei of this labelled residue appear as a pair of diastereotopic signals as before. However, the relative intensity of these signals in the ^{13}C NMR spectrum is now dictated by the 70:30 *R:S* enantiomeric ratio, while their relative position is dictated by the screw-sense (*M* or *P*) of the helix. This Aib*OMe NMR reporter thus allows unambiguous assignment of the absolute sense of induced helicity, based on whether the major signal (arising from (*R*)-Aib*) appears upfield or downfield of its minor partner (arising from (*S*)-Aib*).⁴⁵

Artificial receptor **4** was dissolved in CD_3OD , and (–)-diisopropyl tartrate **6j** ($K = 300 \text{ M}^{-1}$) was added in 0.5 equivalent increments. Complexation was confirmed by an upfield shift in the ^{11}B resonance (Fig. 4a) and the gradual disappearance of the singlet at 25.2 ppm in the ^{13}C NMR spectrum (Fig. 4b), belonging to the labelled methyl groups of the unbound receptor. In its place emerged a pair of signals in the expected 70:30 ratio, the major peak upfield of the minor, indicating that the chiral ligand **6j** induces predominantly left-handed (*M*) helicity in **4j** (Fig 4b, blue signals). After the addition of 2.0 equivalents of this diol, the more strongly coordinating (+)-hydrobenzoin **6a** ($K = 1500 \text{ M}^{-1}$) was added in 0.5 equivalent increments, which displaced the tartrate ligand to form complex **4a** (Fig. 4b, red signals). Exchange of ligands in the receptor was indicated by the switching of the probe's major and minor signals; the major signal relocated to the downfield position indicating that the left handed (*M*) helicity induced by **6j** had switched to right-handed (*P*) helicity induced by **6a**. Finally, the hydrobenzoin was displaced from the receptor by addition of (–)-pinanediol **6m** ($K \gg 2000 \text{ M}^{-1}$), which switched the receptor helix back to its original, racemic state with a single ^{13}C NMR signal from the ^{13}C -labelled reporter (Fig. 4b, green signals). A downfield shift in the ^{11}B NMR spectrum (Fig. 4a) was diagnostic of the severance of the N-B interaction during formation of **4m**.

Figure 4 | Dynamic switching of screw-sense preference. NMR spectra tracking the screw-sense preference of artificial receptors on successive addition of (–)-diisopropyl tartrate **6j**, (+)-hydrobenzoin **6a**, and (–)-pinanediol **6m** in CD_3OD at 298 K. Initial [receptor] = 0.03 M. **a**, ^{11}B NMR (160 MHz) spectra of receptor **4** ($n=1$). Non-interfering impurity at 18 ppm (grey) corresponds to boric acid, later esterified by excess **6m** and shifted downfield to 22 ppm. **b**, ^{13}C NMR (125 MHz) spectra of receptor **4** ($n=1$). **c**, ^{13}C NMR (125 MHz) spectra of receptor **5** ($n=3$).

Transesterification of the boronate ester, with (+)-hydrobenzoin **6a** displacing (–)-diisopropyl tartrate **6j**, leads to screw-sense inversion as complex **4j** becomes **4a**, despite both diols possessing the same absolute configuration (Fig. 4). This switch from *M* to *P* suggests that the tartrate and hydrobenzoin derivatives exert control over the conformation of the helix by differing mechanisms. The most likely origin of this difference is a hydrogen bond between a carbonyl group on the tartrate ligand and one of the two N-terminal amide protons that do not participate in the hydrogen bond network of the 3_{10} helix.³³ For the tartrate series, this attractive interaction appears to overcome the underlying steric repulsion that governs screw-sense induction in the hydrobenzoin series, and consequently inverts the screw-sense preference of the helix (see Supplementary Information, page S52). This interpretation is consistent with earlier observations that the ability of tartrate esters

to bias helical sense decreases as the size of their substituents increases (Table 1, **6h-l**). It is also consistent with the greater screw-sense induction is achieved with the stronger hydrogen bond acceptor in tartramide **6l** evident in the value of $\Delta\delta$ (Table 1), which is larger for **6l** than for **6h-6k**.³⁵

Biological receptors use conformational changes to transmit information over multi-nanometre distances.² Given that the fidelity of information encoded in the screw-sense preference of helical oligomers of Aib decays only slowly as the length of the helix increases,^{18,35} we reasoned that it should be possible for our ligand-switchable receptor structures to communicate information over distances commensurate with, for example, the thickness of a phospholipid bilayer (ca. 2-4 nm).⁴⁶ We therefore synthesised receptor **5** (Fig. 2). This nanoscale foldamer contains an Aib₁₀ unit separating the binding site from the ¹³C NMR reporter, a linear distance of over 2 nm.³³ We subjected the synthetic receptor **5** to the same ligand-switching regime used for **4**. The ¹³C NMR spectrum of the probe (Fig. 4c) revealed the same successful switching of helical sense, becoming left handed on binding to **6j**, switching to right handed on addition of **6a**, and finally becoming racemic with **6m**. Information about ligand configuration was thus communicated from the binding site to the probe through more than three full turns of the 3₁₀ helix. As anticipated, a slight attenuation of the signal over the longer 2 nm distance was evident from the decrease in chemical shift separation of the reporter signals, e.g. between tartrate complexes **4j** ($\Delta\delta$ = 290 ppb) and **5j** ($\Delta\delta$ = 212 ppb).^{18,35}

Ribonucleoside-induced conformational communication: a purinergic receptor.

A biological example of conformational change induced by a compound containing a vicinal diol can be found in the purinergic receptor family of membrane-bound signalling proteins, which are activated primarily by the purine nucleoside

adenosine.^{47,48} Since the 2' and 3' hydroxyl groups of ribonucleosides can form stable cyclic boronates,⁴⁹⁻⁵² we explored the possibility that ribonucleosides might also be competent ligands for our own receptor mimic.

Mixing receptor **4** with 2.0 equivalents of adenosine resulted in an equilibrium mixture favouring complex **4A** with $K \sim 625 \text{ M}^{-1}$. The ^1H NMR spectrum (Fig. 5a) reveals downfield shifts for protons *a-d* of the ribose unit upon binding, but no such change in the signals of its C5 methylene protons, consistent with exclusively formation of the 2',3'- (rather than the six-membered 3',5'-) cyclic boronate.⁵³ In the ^{13}C NMR spectrum (Fig. 5b), the appearance of characteristic major and minor signals of the labelled reporter flanking that of residual unbound receptor **4** confirms the induction of left-handed (*M*) helicity in nucleoside-receptor complex **4A**. The receptor **4** showed similar affinity for guanosine ($K \sim 600 \text{ M}^{-1}$), giving complex **4G**, which also adopted *M* helicity (Fig. 5b).

The pyrimidine ribonucleosides uridine and cytidine likewise bound to receptor **4**, albeit somewhat more tightly ($K > 2000 \text{ M}^{-1}$), to give nucleoside-receptor complexes **4U** and **4C**. As observed for purine complexes **4A** and **4G**, the pyrimidine complexes **4U** and **4C** displayed ^{11}B NMR chemical shifts consistent with solvated N-B interactions.⁴⁰ However the corresponding ^{13}C NMR spectra revealed an unexpected result. While uridine failed to induce a detectable screw-sense bias, cytidine induces right-handed (*P*) helicity in the receptor (Fig. 5b). By analogy with the earlier tartrate ligands, it seems possible that this intriguing ability of receptor **4** to communicate subtle differences in ligand structure may arise from hydrogen bonding to the 2-position of the pyrimidine nucleobase (see Supplementary Information, page S52).

Figure 5 | Screw-sense control by ribonucleosides. NMR spectra of equilibrated mixtures of receptor in CD₃OD saturated with nucleoside (2.0 equiv) at 298 K. Initial [receptor] = 0.03 M (See Supplementary Information, page S23). **a**, ¹H NMR (500 MHz) spectra showing the chemical shift changes of adenosine (top) on binding to receptor **4**. **b**, ¹³C NMR (125 MHz) spectra after addition of adenosine, guanosine, uridine and cytidine to receptor **4** (n = 1) to form complexes **4A**, **4G**, **4U** and **4C** respectively. Signals coloured green arise from unbound receptor. **c**, ¹³C NMR (125 MHz) spectrum after addition of adenosine, guanosine, uridine and cytidine to receptor **5** (n = 3) to form complexes **5A**, **5G**, **5U** and **5C** respectively. Signals coloured green arise from unbound receptor.

Extended receptor **5** gave a unique opportunity to demonstrate remote signalling over distances comparable to those in natural purinergic receptors, where the extracellular binding site is 4.2 nm from the intracellular active site.⁴⁸ As with **4**, addition of adenosine to **5** induced an *M* helical conformational preference, which now propagated through three full turns of the helical receptor. The ¹³C NMR

spectrum (Fig. 5c) confirmed the successful communication of this conformational purinergic signal, which was reported by the NMR probe located more than 2 nm from the binding site. Furthermore, guanosine, uridine and cytidine also bound to receptor **5**, but each induced a different conformational preference (*M*, *rac* and *P* helices respectively), illustrating how subtle changes in ligand structure can be detected and effectively communicated over distances commensurate with biological signal transduction.

Conclusions

We have demonstrated that a synthetic mimic of a biological receptor is able to recognize and reversibly bind a ligand, to communicate information about the binding event through distances of several nanometers, and to generate from a remote reporter a spectroscopic output that is sensitive to fine structural details of the ligand. The exquisite structural sensitivity of the helical relay in response to relatively small structural changes in the diol-boronate complex, as seen with cytidine and adenosine, provides an unexpected insight into how minor changes in molecular structure can be amplified to become far-reaching conformational changes within large biopolymers. Since boronate complexes are structurally well defined, the induction of conformational preference can be rationalised using a three point interaction model, with the solvent inserted into the dative B-N bond playing a key role in rigidifying the receptor-ligand complex and feeding induced asymmetry into the poly(Aib) foldamer. The third interaction point can be a steric block (as in hydrobenzoin **6a**), but we suggest secondary hydrogen bonding interactions between diol and helix can also play a crucial role counteracting steric effects. The net result is a finely balanced *M*↔*P* helical equilibrium that is highly sensitive to structural features in the diol. To transmit this structural information over the multinanometre distances commonly

seen in biopolymers, efficient information transfer is crucial. This is provided in our receptors by cooperative changes in the hydrogen-bonding backbone of the poly(Aib)₃₁₀ helix, with a fidelity of >97% per residue.^{18,35}

Changing the environment around this receptor would be expected to provide further insights. Diol-boronic acid recognition in water is well established, for example with sugars,²⁹⁻³¹ and would allow the role of hydrophobicity and competitive hydrogen bonding with the solvent to be measured. Similarly, the effect of a membrane environment on conformational change is poorly understood, despite being central to the function of many membrane-bound receptors. Natural Aib-containing peptides are known to embed into cellular membranes and form ion channels,⁵⁴ and the thickness of the membrane is commensurate with the length of Aib₁₁ receptor **5**.^{33,46} Not only would dynamic conformational switching of membrane-embedded poly(Aib) foldamers in response to biological polyols like sugars or nucleosides inform us about the efficiency of relaying conformational change through phospholipid bilayers, it may also lead to antibiotics with switchable activity or provide functional mimics of G-protein coupled receptors.

Methods

For full experimental procedures, characterization data and complete NMR titration spectra see Supplementary Information.

General information relating to NMR experiments. All NMR experiments were performed in Norell™ quartz NMR tubes on a Bruker Ultrashield 500 MHz spectrometer at 298 K. Stock solutions of diols **6a-m** (0.15 M) and receptors **3-5** (0.03 M) in CD₃OD were prepared and left to stand for 48 h before use to allow complete exchange of the GlyNH₂ (in **3**) or Aib*OMe (in **4** and **5**) amide NH proton for

deuterium, to avoid spin-spin coupling (to Gly CH₂) or small isotope effects on chemical shifts (for Aib*OMe). In all cases, the tube was shaken and left for 5 min to reach equilibrium after adding an aliquot of diol solution.

Procedure for diol assays with 3. Receptor stock solution (500 µL, 0.015 mmol) was added to an NMR tube and the ¹H and ¹¹B NMR spectra recorded. The spectra were acquired following addition of: 100 µL of the appropriate diol solution (0.015 mmol); another 100 µL (0.015 mmol), and finally a further 300 µL (0.045 mmol). These volumes corresponded to 1.0, 2.0 and 5.0 total equivalents of diol respectively.

Procedure for dynamic switching experiments on 4 and 5. Receptor stock solution (500 µL, 0.015 mmol) was added to an NMR tube and the ¹H, ¹³C and ¹¹B NMR spectra recorded. The spectra were reacquired following each successive addition of diols **6j**, **6a** and finally **6m** in 50 µL increments (each containing 0.0075 mmol, 0.5 equivalents) up to 2.0 total equivalents each.

Procedure for formation of ribonucleoside-receptor complexes from 4 and 5. Receptor stock solution (500 µL) was combined with 2.0 equivalents of the appropriate nucleoside in a vial. The suspension was shaken vigorously and left to stand for 1 h, before being filtered (without dilution) to remove excess undissolved nucleoside. The filtrate was added to an NMR tube and the ¹H, ¹³C and ¹¹B NMR spectra of the resulting equilibrium mixture were recorded.

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Acknowledgements

This work was funded by the BBSRC through a Research Grant (I007962) and a Doctoral Training Grant studentship. We thank Dr Timothy Wallace for donating

samples of **6c**, **6e** and **6f**. We are grateful to the EPSRC National Mass Spectrometry Centre for performing HRMS measurements.

Author Contributions

RAB, SJW and JC conceived and designed the project. RAB and VD designed and executed the synthesis of the receptors. RAB performed the analytical studies. RAB, SJW and JC wrote the paper.

Figure Legends

Figure 1 | Remote detection of ligand binding to a synthetic receptor by NMR spectroscopy. **a**, Diastereotopic ‘reporter’ nuclei A and B appear as a single (isochronous) signal due to fast exchange between equally populated *M* and *P* conformers of the unbound receptor. **b**, On binding, a chiral ligand induces a preference for one screw-sense over the other, biasing the helical equilibrium and resulting in separate (anisochronous) signals for nuclei A and B.

Figure 2 | Synthesis of boronate-capped receptors used in this study. Me* = $^{13}\text{CH}_3$. Enantiomeric ratio = 70:30 (*R/S*) in **1b**, **1c**, **4** and **5**. ^aIsolated after chromatography on SiO₂ using methanol-containing eluent.

Figure 3 | Probing ligand-induced conformational control by NMR. ^{11}B NMR (160 MHz) and ^1H NMR (500 MHz) spectra tracking binding and conformational induction in ligand-receptor complexes with increasing [diol] in CD₃OD at 298 K. Initial [**3**] = 0.03 M. **a**, Addition of (+)-hydrobenzoin **6a** to receptor **3**. **b**, Addition of *meso*-hydrobenzoin **6b** to receptor **3**. **c**, Addition of (+)-hydrobenzoin **6a** to an equimolar mixture of receptor **3** and control peptide **1a**.

Table 1 | Complexation of diol ligands 6a-m with receptor 3: binding constants and induced conformational preferences in boronate esters 3a-m. ^a From ¹H NMR experiments in CD₃OD at 298 K. ^b Binding constant *K* estimated by integrating spectra of mixtures (of **3**, **3a-m** and **6a-m**) arising from addition to **3** of 1, 2 and 5 equiv **6a-m** (see Supplementary Information, page S23). ^c Anisochronicity, in parts per billion (ppb), of the AB system arising from the Gly CH₂ group of **3a-m**, given by $\nu_0 \Delta\delta = [(f_1 - f_3)^2 - J_{AB}^2]^{1/2} = [(f_2 - f_4)^2 - J_{AB}^2]^{1/2} = [(f_1 - f_4)(f_2 - f_3)]^{1/2}$ where $f_{1,2,3,4}$ are the observed resonance frequencies in order of the four lines comprising the AB multiplet, J_{AB} is the coupling constant, and ν_0 is the spectrometer frequency (500 MHz). ^d(-)-Enantiomer used. ^eRacemic mixture used.

Figure 4 | Dynamic switching of screw-sense preference. NMR spectra tracking the screw-sense preference of artificial receptors on successive addition of (-)-diisopropyl tartrate **6j**, (+)-hydrobenzoin **6a**, and (-)-pinanediol **6m** in CD₃OD at 298 K. Initial [receptor] = 0.03 M. **a**, ¹¹B NMR (160 MHz) spectra of receptor **4** (*n* = 1). Non-interfering impurity at 18 ppm (grey) corresponds to boric acid, later esterified by excess **6m** and shifted downfield to 22 ppm. **b**, ¹³C NMR (125 MHz) spectra of receptor **4** (*n* = 1). **c**, ¹³C NMR (125 MHz) spectra of receptor **5** (*n* = 3).

Figure 5 | Screw-sense control by ribonucleosides. NMR spectra of equilibrated mixtures of receptor in CD₃OD saturated with nucleoside (2.0 equiv) at 298 K. Initial [receptor] = 0.03 M (See Supplementary Information, page S23). **a**, ¹H NMR (500 MHz) spectra showing the chemical shift changes of adenosine (top) on binding to receptor **4**. **b**, ¹³C NMR (125 MHz) spectra after addition of adenosine, guanosine, uridine and cytidine to receptor **4** (*n* = 1) to form complexes **4A**, **4G**, **4U** and **4C** respectively. Signals coloured green arise from unbound receptor. **c**, ¹³C NMR (125 MHz) spectrum after addition of adenosine, guanosine, uridine and cytidine to receptor **5** (*n* = 3) to form complexes **5A**, **5G**, **5U** and **5C** respectively. Signals coloured green arise from unbound receptor.