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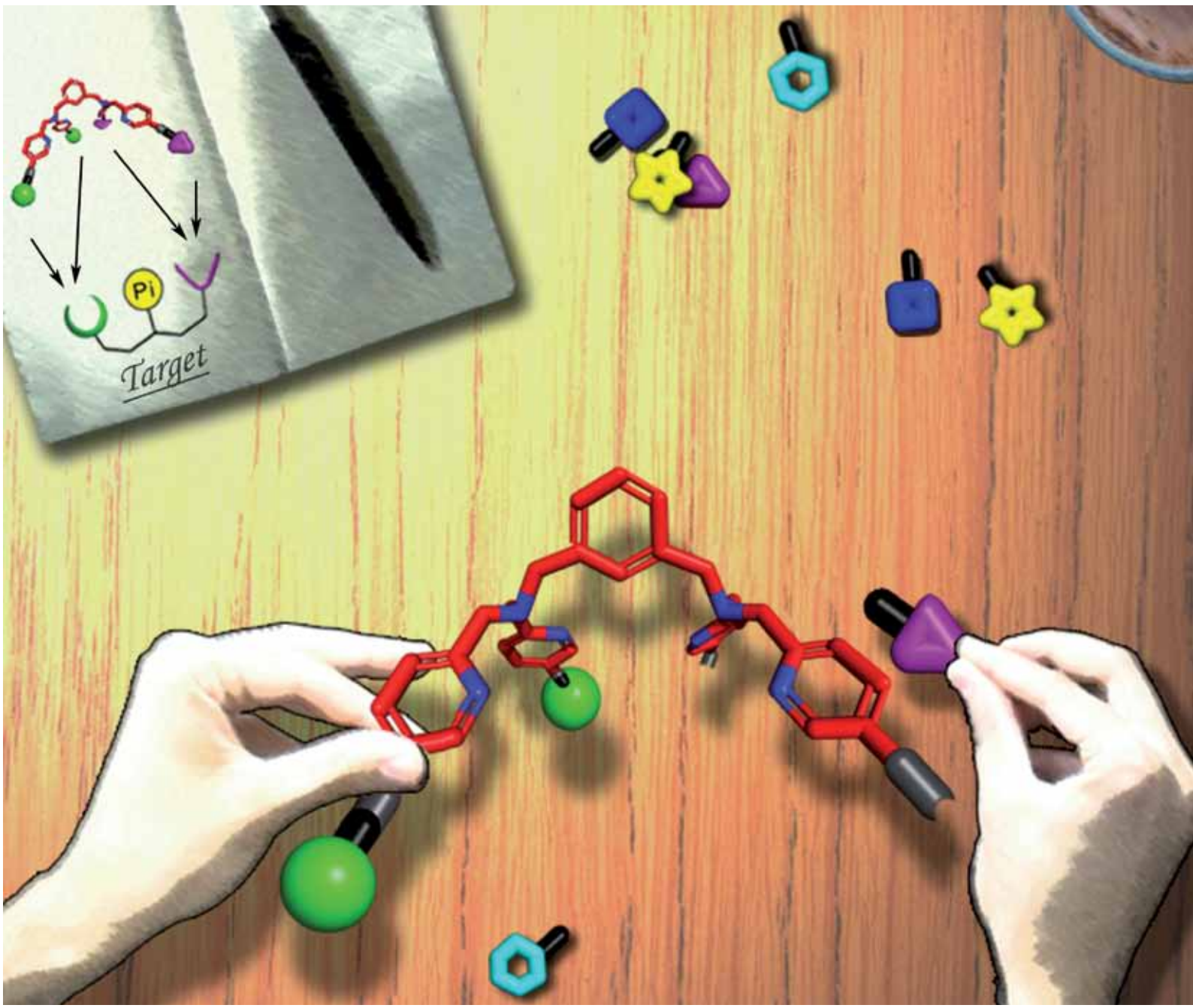

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Novel asymmetrically functionalized bis-dipicolylamine metal complexes: peripheral decoration of a potent anion recognition scaffold†

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We report the design and synthesis of a novel class of asymmetrically functionalized, ditopic bis-dipicolylamine (BDPA) ligands. A key feature of this research involved the controlled, sequential functional group decoration of a potent molecular recognition scaffold. Calorimetric screening identified a BDPA analogue as a highly potent ($K_a \sim 10^6$ M^{-1}) and selective sensor for inorganic phosphate.

Phosphate-containing anionic species are ubiquitous in biological systems and play important mediatory roles in signal transduction pathways, as well as in carrying genetic information. Despite a significant effort to develop potent binding motifs, the design and synthesis of receptors capable of phosphate anion recognition under physiological conditions remains a significant challenge.[1] Lewis acidic zinc(II)[2] and copper(II)[3] homo-dinuclear bis-dipicolylamine[4] (BDPA, Fig. 1) and polyazamacrocyclic ligand complexes[5] have been successfully employed as receptors of biologically relevant phosphates and phosphotyrosine-containing peptide sequences.[6] Homo-dinuclear BDPA scaffolds have been used extensively to distinguish between phosphorylated and non-phosphorylated species,[7] and more recently as non-covalent tags for phosphorylated proteins.[8] However, given the lack of binding functionality to bestow selectivity beyond metal–phosphate recognition, agent promiscuity for phosphorylated molecules is routinely reported.[9] Significantly, excepting the metal chelating nitrogen donor groups and the hydrophobic pyridine rings, BDPA constructs have no binding functionality and offer little to confer receptor–phosphate substrate selectivity.

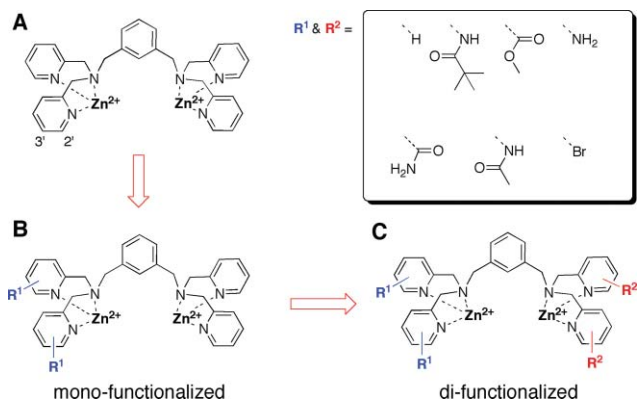
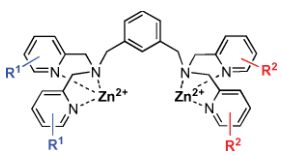


Fig. 1 Asymmetric functionalization of the BDPA receptor.

Our principal objective was to improve the substrate specificity of the bis-dipicolylamine motif by developing a facile, modular synthetic route to multi-functionalized BDPA receptors. In order to maximize the potential utility of the BDPA scaffold, we then wished to demonstrate the versatility of our synthetic route by constructing BDPA analogues containing functionality amenable to further covalent diversification, *e.g.* aryl halides that can participate in Suzuki couplings and amines that can form peptide bonds. Given the widespread application of BDPA constructs in molecular recognition, facile synthetic protocols that give access to customized receptors would be of significant benefit.

Scaffold designs focused primarily upon selectively substituting the 2′ and 3′ positions of the flanking pyridine groups, which, due to their proximities to the metal-chelating pyridine nitrogens, were considered the most relevant to achieving additional substrate-selective intermolecular interactions. Two novel sub-families of bis-dipicolylamine analogues were prepared: a *meta*-xylyl bridged, mono-functionalized bis-dipicolylamine (Fig. 1B), and a *meta*-xylyl bridged, di-functionalized bis-dipicolylamine (Fig. 1C). The structures of these novel BDPA-based molecular recognition architectures are given in Table 1, all of which were prepared *via* the

Table 1 Library of asymmetric substituted bis-dipicolylamine scaffolds[a]



Compound	R^1	R^2
1	H	H
2	3′-COOMe	H
3	2′- NH_2	H
4	2′-Br	H
5	2′-NH_2	2′-NH_2
6	2′-$NHCOCH_3$	2′-$NHCOCH_3$
7	3′-COOMe	2′-$NHCOC(CH_3)_3$
8	3′-COOMe	3′-COOMe
9	2′Br	2′-$NHCOC(CH_3)_3$

[a] Where the counter anion used was the non-coordinating triflate species.

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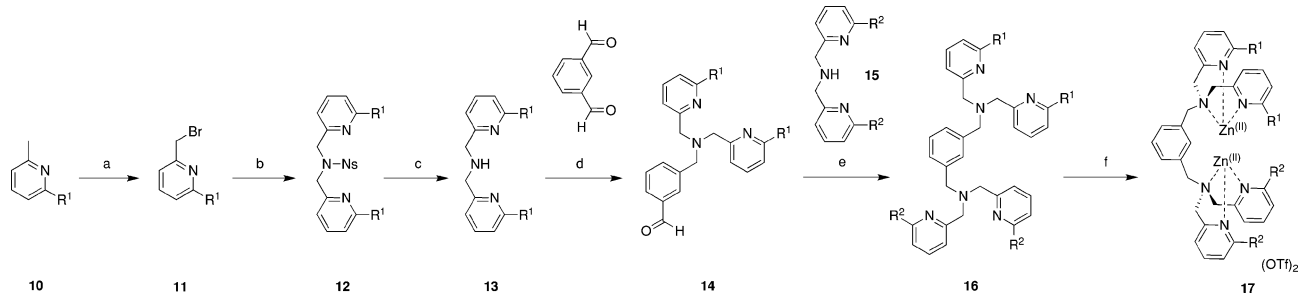
† Electronic supplementary information (ESI) available: ^1H and ^{13}C NMR, HRMS for compounds **1–9**, and representative ITC traces. See DOI: 10.1039/b917692f



Scheme 1 *Reagents and conditions:* (a) NBS, BPO, CCl_4, 70 °C, 12 h, 45–68%; (b) 2-$NsNH_2$, K_2CO_3, DMF, 60 °C, 8 h, 83–95%; (c) thiophenol, K_2CO_3, DMF, rt, 3 h, 88–93%; (d) benzene-1,3-dicarboxaldehyde, $NaBH(OAc)_3$, DCE, rt, 2 h, 84–92%; (e) $NaBH(OAc)_3$, DCE, rt, 2 h, 70–91%; (f) $Zn(OTf)_2$, MeOH, 4 h, 85–95%.

facile and modular synthetic route depicted in Scheme 1. A simple retrosynthetic analysis of BDPA and its analogues reveals that the target molecules may be accessed from double *N*,*N*-dialkylation of 1,3-bis(aminomethyl)benzene with 2-(bromomethyl)pyridines. Indeed, this is the most popular synthetic route to generating symmetrically functionalized BDPA structures.

However, such an approach is not compatible with the generation of asymmetrical BDPAs. To this end, we herein present the controlled, stepwise reductive amination of the alternative scaffold benzene-1,3-dicarboxaldehyde with synthetic secondary dipicolylamines (Scheme 1).

The desired secondary dipicolylamines **13** and **15** were generally furnished in three steps from commercially available starting materials employing 2-nitrobenzenesulfonamide (2-$NsNH_2$) as a latent secondary amino group.[10] Briefly, as a representative example, NBS-mediated aryl-methyl bromination of methyl 6-methylnicotinate **10** ($R^1 = CO_2Me$) with catalytic benzoyl peroxide (BPO) afforded **11** in 60% yield. Subsequently, *N*-dialkylation of 2-$NsNH_2$ with two equivalents of bromide **11** gave **12** in excellent yield. Mild nosyl deprotection was accomplished using thiophenol and K_2CO_3 in DMF at room temperature overnight to furnish the target secondary amine **13**. Under high dilution conditions, benzene-1,3-dicarboxaldehyde was mono-reductively aminated with the R^1-substituted dipicolylamine **13** using $NaBH(OAc)_3$ *in situ* at room temperature to give **14** in high yield. The remaining aldehyde group in **14** was then reductively aminated with the R^2-substituted dipicolylamine **15** to give the novel, asymmetrically functionalized *meta*-xylene-based scaffold **16**, exhibiting two different (R^1 and R^2) dipicolylamine moieties. Synthetic procedures and characterization for all derivatives in Table 1 are provided in the ESI.†

We considered that a BDPA scaffold containing a range of hydrogen bond donors, acceptors and hydrophobic substituents at either the 2′- or 3′-positions could interact effectively with a range of target substrates. Indeed, phosphate hydrolysis catalysts based on a 2′-substituted tetra-amino BDPA scaffold, prepared by *N*-dialkylation procedures, proved particularly potent against model RNA substrates.[11] Our goal was to exploit subtle differences in substrate composition with tailored receptor species.

First, we successfully prepared a small set of asymmetrically mono-functionalized BDPA analogues of the parent **1** ($R^1 = R^2 = H$), incorporating a range of substituents (compounds **2–4**, Table 1). Subsequently, we prepared both symmetrically and asymmetrically di-functionalized BDPA compounds **5–9** (Table 1), appending a similar selection of simple functional groups. As mentioned in the Introduction, we chose substituents amenable to further scaffold extension and diversification, including aryl halides, anilines and esters. To the best of our knowledge, this work represents the first controlled, sequential decoration of the BDPA scaffold. Given the excellent overall yields obtained and functional diversity incorporated, we believe our methodology lends itself well to rational receptor design and can be employed to construct numerous novel BDPA ligands.

Isothermal titration calorimetry (ITC) was used to screen receptors **1–9** for binding affinity against a range of phosphate species (Table 2). Control binding data was collected for receptor **1** ($R^1 = R^2 = H$), which we regarded as a 'naked' BDPA complex and from which all synthetic modifications were compared. Thus, ITC was used to accurately assess the relative effects of functionalization upon agent–substrate specificity and direct our synthetic modifications. The binding abilities of receptors **1–6** towards various phosphates were measured under identical experimental conditions (VP-ITC, 25 °C, pH 7.2, HEPES buffer (50 mM) in aqueous solution; 0.1 mM receptor (cell), 2 mM phosphate (syringe)). The resulting binding isotherms were fitted to a 1 : 1 non-linear regression model using the Microcal ORIGIN software package, affording the thermodynamic parameters of the host–guest interaction. From the data obtained, the most striking result was the highly potent binding of NaH_2PO_4 by the asymmetric mono-amino-functionalized ligand **3** (R^1 = 2′-NH_2, R^2 = H (entry 3)) relative to the other phosphorylated substrates and relative to the other ligands. For example, **3** binds to NaH_2PO_4 ($K_a = 1.50 \times 10^6$ M^{-1}) almost eleven times as potently as adenosine-5′-monophosphate (5′AMP; $K_a = 1.37 \times 10^5$ M^{-1}) and about 100 times more strongly than both β-glycerophosphate (β-GP; $K_a = 1.60 \times 10^4$ M^{-1}) and *p*-nitrophenyl phosphate (PNPP; $K_a = 1.32 \times 10^4$ M^{-1}). Of particular significance is the observation that ligand **3** binds NaH_2PO_4 approximately ten times as strongly as the control compound **1** (**3**, $K_a = 1.50 \times 10^6$ M^{-1} *cf.* **1**, $K_a = 1.93 \times 10^5$ M^{-1}, Fig. 2). Initially, it was presumed that the ten-fold increase in K_a was a direct result of increased hydrogen bond interactions between the two 2′-amino groups of **3** and the oxygen hydrogen bond acceptors of NaH_2PO_4. However, binding of **3** with NaH_2PO_4 incurred an unfavourable, two-fold increase in the enthalpy contribution (**3**, $\Delta H° = 4.38$ kcal mol^{-1} *cf.* **1**, $\Delta H° = 2.18$ kcal mol^{-1}) that was more than compensated for by a significant increase in favourable entropy (**3**, $T\Delta S° = 12.8$ kcal mol^{-1} *cf.* **1**, $T\Delta S° = 9.38$ kcal mol^{-1}), suggesting that tighter binding was entropically driven.

Table 2 Thermodynamic binding data (ITC) for various substrates to a range of receptors in 50 mM HEPES buffer, pH 7.2, 25 °C

Entry	Agent	n^a	Substrate	$K \times 10^4$ M^{-1}	$\Delta G°$ /kcal mol $^{-1}$ [b]	$\Delta H°$ /kcal mol^{-1}	$T\Delta S°$ /kcal mol^{-1}
1	**1**	1	$Na[H_2PO_4]$	19.3 ± 1.23	−7.20	2.18 ± 0.02	9.38
2	**2**	1	$Na[H_2PO_4]$[f]	15.5 ± 3.94	−7.08	−0.17 ± 0.02	6.91
3	**3**	1	$Na[H_2PO_4]$	150 ± 6.82	−8.42	4.38 ± 0.02	12.8
4	**4**	1	$Na[H_2PO_4]$[f]	10.6 ± 0.52	−6.82	3.58 ± 0.07	10.4
5	**5**	1	$Na[H_2PO_4]$	16.3 ± 0.42	−7.12	5.98 ± 0.04	13.6
6	**6**	1	$Na[H_2PO_4]$	1.70 ± 0.11	−5.75	4.31 ± 0.37	10.1
7	**1**	1	5′-AMP[c]	16.3 ± 0.71	−7.09	3.31 ± 0.27	10.4
8	**2**	1	5′-AMP[c,f]	12.4 ± 0.73	−6.94	2.83 ± 0.05	9.77
9	**3**	1	5′-AMP[c]	13.7 ± 0.59	−6.99	5.01 ± 0.05	12.0
10	**4**	nd[g]	5′-AMP[c,f]	nd[g]	nd[g]	nd[g]	nd[g]
11	**5**	nd[g]	5′-AMP[c,f]	nd[g]	nd[g]	nd[g]	nd[g]
12	**6**	1	5′-AMP[c,f]	7.17 ± 0.61	−6.62	0.98 ± 0.02	7.59
13	**1**	1	β-GP[d]	2.35 ± 0.09	−5.87	6.03 ± 0.17	11.9
14	**2**	1	β-GP[d,f]	2.93 ± 0.16	−6.08	6.12 ± 0.30	12.2
15	**3**	1	β-GP[d]	1.60 ± 0.04	−5.66	7.14 ± 0.22	12.8
16	**4**	nd[g]	β-GP[d,f]	nd[g]	nd[g]	nd[g]	nd[g]
17	**5**	1	β-GP[d]	1.00 ± 0.01	−5.48	0.89 ± 0.05	6.37
18	**6**	1	β-GP[d]	1.26 ± 0.01	−5.60	1.76± 0.09	7.36
19	**1**	1	PNPP[e]	1.47 ± 0.03	−5.66	7.54 ± 0.20	13.2
20	**2**	1	PNPP[e,f]	1.34 ± 0.06	−5.08	9.02 ± 1.11	14.6
21	**3**	1	PNPP[e]	1.32 ± 0.02	−5.55	7.25 ± 0.11	12.8
22	**4**	1	PNPP[e,f]	4.47 ± 0.61	−6.35	0.62 ± 0.02	6.97
23	**5**	1	PNPP[e,f]	4.53 ± 1.74	−6.34	0.25 ± 0.02	6.58
24	**6**	1	PNPP[e,f]	1.57 ± 0.01	−5.70	2.64 ± 0.12	8.34

[a] Binding stoichiometry. [b] Calculated from $\Delta G° = \Delta H° - T\Delta S°$. [c] Adenosine-5′-monophosphate. [d] β-Glycerophosphate; [e] *p*-Nitrophenyl phosphate. [f] Buffer included 5% DMSO (v/v). [g] Not determined: thermodynamic parameters were not obtained due to insufficient enthalpy of binding to accurately fit the data to a 1 : 1 binding model.

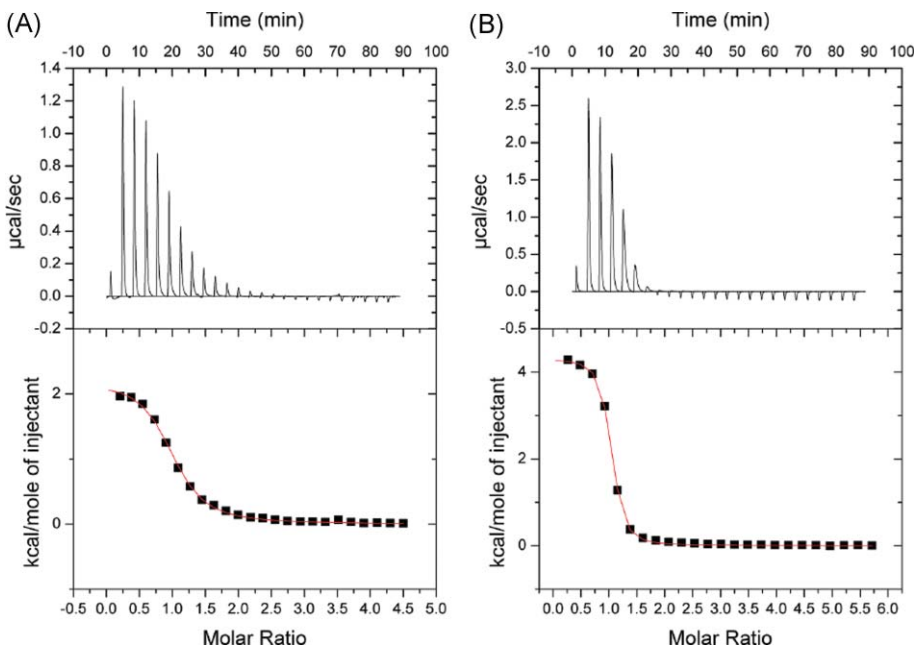


Fig. 2 Representative ITC binding isotherms for **1** (A) and **3** (B) binding to NaH_2PO_4 (30 × 10 µL injections), 50 mM HEPES buffer, pH 7.4, 25 °C.

In order to rationalize this thermodynamic data, we postulated that the increased enthalpic penalty was required to break the solvation sphere of the hydrophilic amino groups of **3**, but in which this cost was more than repaid by the associated liberation of several ordered water molecules to afford the observed positive entropic term. Our data does not preclude the fact that increased intermolecular hydrogen bonding interactions are taking place between **3** and NaH_2PO_4, only that overall there is an enthalpic penalty component involved in their formation—specifically, desolvation—for which the new bonds do not fully compensate. In turn, this would suggest that BDPA receptors equipped with more hydrogen bond donors may better compensate the apparent loss of enthalpy *via* increased host–guest interactions. Thus, receptor **5**, equipped with four aniline hydrogen bond donors (R^1 = 2′-NH_2, R^2 = 2′-NH_2) was prepared; however, this incurred a ten-fold decrease in binding potency with NaH_2PO_4 relative to the binding

of **3** with NaH_2PO_4, respectively (**5**, $K_a = 16.3 \times 10^4$ M^{-1} *cf.* **3**, $K_a = 150 \times 10^4$ M^{-1} (entries 3 and 5)). Unsurprisingly, binding **5** with NaH_2PO_4 resulted in a favourable increase in the entropy relative to **3** (**5**, $T\Delta S^\circ = 13.6$ kcal mol^{-1} *cf.* **3**, $T\Delta S^\circ = 12.8$ kcal mol^{-1}), presumably due to the desolvation of the additional aniline groups. However, the improved entropic component ($\Delta(T\Delta S^\circ)$ of 0.8 kcal mol^{-1}) was negated with a concomitant unfavourable increase in enthalpy contribution (**5**, $\Delta H^\circ = 5.98$ kcal mol^{-1}, *cf.* **3**, $\Delta H^\circ = 4.38$ kcal mol^{-1}), a likely result of the increased energy required to desolvate the additional aniline functional groups. Our study illustrates the delicate structural balance required to furnish potent anion receptors operating in an aqueous environment. As noted by Berger and Schmidtchen,[12] simply increasing the number of complementary binding groups on the receptor molecule does not necessarily confer increased binding affinity and ignores enthalpy–entropy compensation effects.[13] One must consider whether the favourable binding enthalpy achieved through a more extensive binding interface is sufficient to compensate for the resultant unfavourable enthalpic cost of increased receptor desolvation. Our modular synthetic approach facilitates rapid and iterative functional group modifications to the BDPA receptor–allowing facile access to 'solvation design' receptors.

More generally, the binding of all four phosphate substrates including NaH_2PO_4 to our host molecules **1–6** were driven by large positive entropic terms and were, with the exception of the binding of **2** to NaH_2PO_4 (entry 2), endothermic ($+\Delta H^\circ$). Several groups have reported similar entropy-driven endothermic binding interactions between organometallic complexes and anion guests.[6,14] In water, thermodynamically favourable increases in entropy are routinely observed in both artificial[15] and biological host–guest systems.[16] Better known as the hydrophobic effect, the gain in entropy is obtained by effective displacement of ordered water from the hydrophobic surfaces of the host–guest binding interfaces.[17] Therefore, desolvation of the predominantly hydrophobic DPA units *via* anion binding to the Lewis acidic zinc ion would explain the favourable entropic term.[18] Unsurprisingly, binding to the most hydrophobic substrate PNPP, with presumably the most ordered and largest solvation sphere of the four phosphates examined, resulted in the biggest entropic gain (where **1–3**, $T\Delta S^\circ > 12.8$ kcal mol^{-1} (entries 19–21, respectively)).

More generally, we noted the high affinities of receptors **1–6** for NaH_2PO_4 and 5′-AMP relative to β-GP and PNPP. The binding affinities for β-GP and PNPP were approximately ten-fold lower in potency in all four host compounds, *e.g.* **1** binds preferentially to 5′-AMP ($K_a = 1.63 \times 10^5$ M^{-1}) over PNPP ($K_a = 1.47 \times 10^4$ M^{-1}). However, receptor specificity for the organic phosphate substrates (5′-AMP, β-GP and PNPP) remained elusive, with the differences in binding affinities, within error, generally negligible. We attributed the lack of substrate specificity to a lack of complementary binding functionality present on the BDPA scaffold. We are currently developing BDPA scaffolds with increased diversity of substituents to probe further substrate specificity.

In conclusion, we report the development of a highly efficient, facile and modular synthetic route to orthogonally functionalized BDPA receptors. Our initial synthetic efforts have identified BDPA derivative **3** as an extremely potent and relatively selective receptor for NaH_2PO_4 ($K_a = 1.50 \times 10^{-6}$ M^{-1}). Given the modular nature of the synthetic protocols outlined here, there is great scope to develop novel multi-functionalized BDPA recognition architectures.

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