The Bull-James assembly: efficient iminoboronate complex formation for chiral derivatization and supramolecular assembly.

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Abstract: Chiral molecules are widely used in many fields of research and so practically simple, accurate methods to measure their enantiopurities are required. This review's initial focus is on one such method, the Bull-James assembly, which employs a three-component protocol combining 2-formylphenyl boronic acid, an amine, and a diol to self-assemble diastereomeric iminoboronate ester (IBE) complexes whose ratio can be used to measure the *ee's* of amine and diol analytes using ^{1}H and ^{19}F NMR spectroscopic analysis. Examples where this supramolecular IBE assembly approach has been adapted to determine the *ee* of a range of analytes using other analytical techniques such as circular dichroism, fluorescence, and electrochemistry that are potentially applicable to high-throughput *ee* analysis are also discussed. Selected examples where this orthogonal self-assembly process has been used as a platform technology to construct boracyles, chiral auxiliaries/ligands, synthesise intelligent polymers/hydrogels, and prepare labelled peptides/proteins/biomolecules are also be discussed.

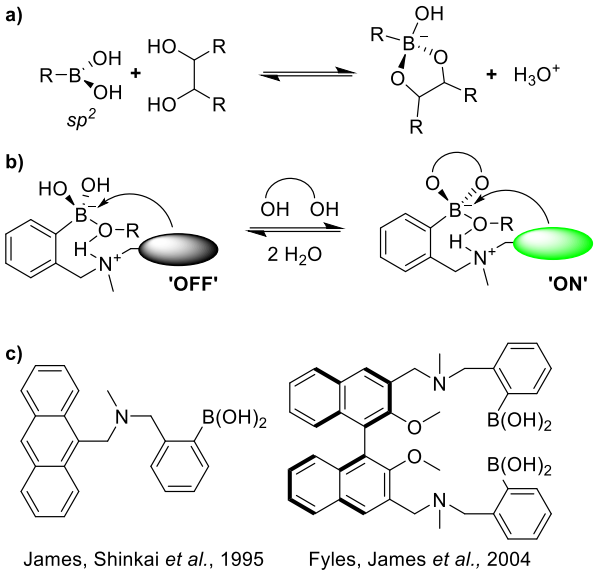
Abbreviations: 2-FPBA: 2-formylphenyl boronic acid; IBE: iminoboronate ester; IB: iminoboronate; CDA: chiral derivatizing agent; 2-APBA: 2-acetlyphenylboronic acid

1. Introduction

This review describes the many applications of a three-component self-assembly reaction that occurs when an amine, a diol, and a 2-formyl-phenyl boronic acid (2-FPBA) template are mixed together to afford stable iminoboronate ester (IBE) complexes. Development of this versatile supramolecular methodology has been pioneered in the Bull and James groups at the University of Bath (UK) over the last two decades, with its widespread use by numerous research groups for different supramolecular applications resulting in this type of reaction now being termed the "Bull-James assembly". To date, this self-assembly methodology has found a wide range of applications, including: use as chiral derivatization agents (CDAs) for determining the enantiomeric excess (*ee*) of a range of chiral analytes using NMR, optical and electrochemical techniques; as a supramolecular self-assembly reaction to produce boracycles, chiral auxiliaries and ligands for stereoselective synthesis, and new types of polymers and stimuli-responsive materials; and as the basis of "click" chemistry methodology for modifying/functionalising peptides and proteins.

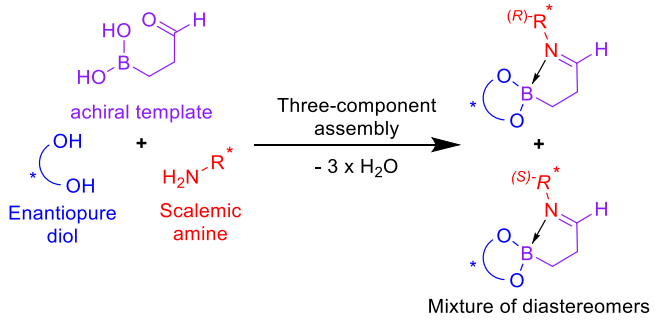
The Bull group have had an interest in the development of asymmetric methodologies for the synthesis of chiral amines for many years, and have often needed to determine the *ee* of new types of chiral amines containing single stereocenters.[1–6] One approach that they have commonly employed involves reaction of a scalemic amine with a CDA such as Mosher's acid chloride (expensive, moisture sensitive, multiple steps) to afford diastereomeric amide derivatives whose diastereomeric ratio (*dr*) can then be determined by NMR spectroscopic analysis.[7,8] Alternatively, the *ee*'s of these chiral amines (or their derivatives) have been determined using chiral HPLC analysis. The range of structures and functional groups present in the chiral amines meant that different CDAs or multiple expensive chiral HPLC columns often needed to be screened before a suitable system was identified for each different class of amine.[9,10] Therefore, the Bull group were interested in identifying a practically simple, cheap, and rapid CDA approach that could be used to rapidly analyse the *ee* values of a wide range of chiral amines using NMR spectroscopic analysis.

The James group have been interested in chemical sensing and supramolecular chemistry for many years, having developed a wide range of self-assembled fluorescent sensors that employ reversible binding of boronic acids (planar sp^2 boron) to diol fragments to produce boronate ester complexes (tetrahedral sp^3 boron) to induce a change in fluorescence response (Scheme 1a).[11–16] They have described that *ortho*-aminomethylphenylboronic acid sensors are particularly effective for the fluorescence, optical, and electrochemical sensing of sugars, with this class of sensors finding recent commercial application for continuous monitoring of glucose levels in critical care patients.[17,18] Diol complexation in this class of sensors is favoured by the presence of the proximal Lewis-basic tertiary amino group,[19] which bind to the boron centre to produce stable intramolecular amino-boronate ester complexes. Orthogonal binding of both the diol analyte and the amine to the boron centre occurs in a cooperative manner, with complexation of the diol producing a boronate ester with a more Lewis acidic sp^2 boron centre, and the intramolecular N→B interaction increasing the overall stability of the complex. Complexation of these types of aminoboronic acid sensors to diols in aqueous/alcoholic media has been shown to produce solvent-inserted aminoboronate complexes, whose formation results in fluorescence "turn-on" through elimination of "loose-bolt" internal conversion quenching of the fluorescence of the parent boronic acid probe (Scheme 1b).[20,21] The versatility and strength of this type of aminoboronic acid complexation process has been exploited to produce many sensors for the fluorescence detection of a wide range of diols and sugars, as well as sensors for pH, anion and reactive oxygen species sensing (Scheme 1c).[11] The added stability of this type of aminoboronate ester complexes has also been used as the basis of supramolecular assemblies for the generation of a wide range of hydrogels, boronic acid appended porphyrins, amphiphiles, polymers and covalent organic frameworks.[14,22,23]



Scheme 1: (a) Rapid complexation of a boronic acid with a vicinal diol reversibly affords a cyclic boronate ester. (b) Complexation of a diol to a non-fluorescent *o*-aminomethylphenylboronic acid sensor in water or an alcohol solvent results in formation of a solvent-inserted fluorescent boronic ester complex. Diol binding results in fluorescence "turn-on" due to elimination of a "loose-bolt" effect that causes internal conversion quenching of the fluorescence of the uncomplexed boronic acid probe. (c) Representative *o*-aminomethylphenylboronic acid glucose/diol sensors developed by the James group.

Nomikai-inspired[24] conversations during a research trip to Japan in 2002[25] led James and Bull (and Arimori – PDRA in the groups) to realise that this type of boronate ester complexation chemistry could be exploited to develop a simple three-component protocol for determining the enantiopurity of chiral amines (and diols). Our simple idea was to react an achiral bifunctional template that contained a boronic acid and a proximal aldehyde group (purple) with a chiral 1,2-diol (blue) and a scalemic amine (red) to selectively afford a pair of diastereomeric IBE complexes, whose *dr* could then be determined through integration of pairs of diastereomeric signals in their ^{1}H NMR spectrum. So long as no kinetic resolution occurred during the derivatisation process, this *dr* value would be an accurate reflection of the *ee* of the parent scalemic amine. Moreover, the orthogonal three-component nature of the protocol meant that it would be easy to adapt this derivatisation approach to determine the *ee* of chiral diols (and other chiral analytes) (Scheme 2).

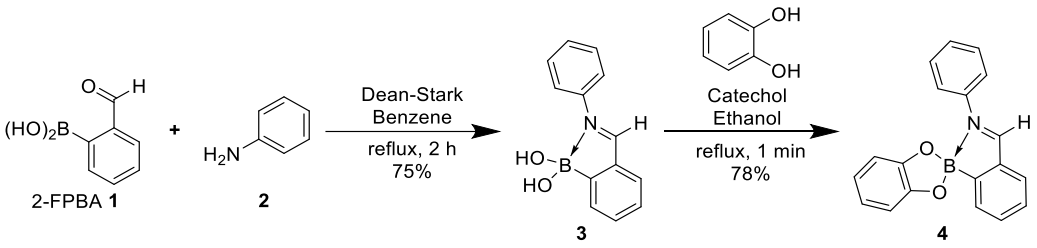


Scheme 2: Design principles for a three-component derivatisation protocol to produce an IBE-based CDA for determining the *ee* of a scalemic amine.

2. Discovery and structural features of the Bull-James assembly

2.1. *Discovery of the Bull-James assembly CDA for determining the* ee *of amines*

A review of the literature revealed a promising report by Dunn *et al.,* [26] who had described the stepwise synthesis of stable IBEs based on imine condensation of 2-FPBA **1**[27] with aniline **2** to afford an iminoboronic acid **3** intermediate that was then reacted with catechol to afford iminoboronate ester **4** (Scheme 3). This precedent indicated that reaction of 2-FPBA **1** with a chiral diol and a scalemic amine could be used as the basis of a three-component derivatisation protocol for determining the *ee* of chiral amines, as outlined in Scheme 2.



Scheme 3: Stepwise three-component self-assembly of an achiral IBE complex **4** by Dunn *et al*.

This three-component assembly concept was initially investigated by mixing 2-FPBA **1**, (*S*)-BINOL **5** and (*rac*)-4-methoxy-α-methylbenzylamine **6a** in $CDCl_3$ with 4 Å molecular sieves to drive the condensation reactions to completion. To our delight, this reaction led to quantitative formation of a 50:50 mixture of the diastereomeric IBE complexes (α-*S*,*S*)-**7aa** and (α-*R*,*S*)-**7ba** within 5 min (Figure 1a),[28] with complexation reactions of scalemic 4-methoxy-α-methylbenzylamine **6a** of known *ee* indicating that no kinetic resolution was occurring. Examination of the ^{1}H NMR spectra revealed that the *ee*'s of scalemic amines could be easily determined by integration of corresponding pairs of ^{1}H NMR resonances originating from each of the IBE diastereomers that were formed. Resonances for the imine (black), α-methine (red), *p*-methoxy (green), and α-methyl (blue) proton resonances of each diastereomer were fully baseline-resolved, exhibiting relatively large chemical shift differences $\Delta\delta_H$ values of 0.11-0.21 ppm (Figure 1b). The presence of multiple well-resolved diastereomeric peaks in these ^{1}H NMR spectra enabled the integral ratios of multiple pairs of diastereomeric resonances to be used to accurately measure high *ee* values (>95 % *ee*), thus minimising any risk of inaccuracy caused by baseline noise or the presence of impurities (Figure 1b).

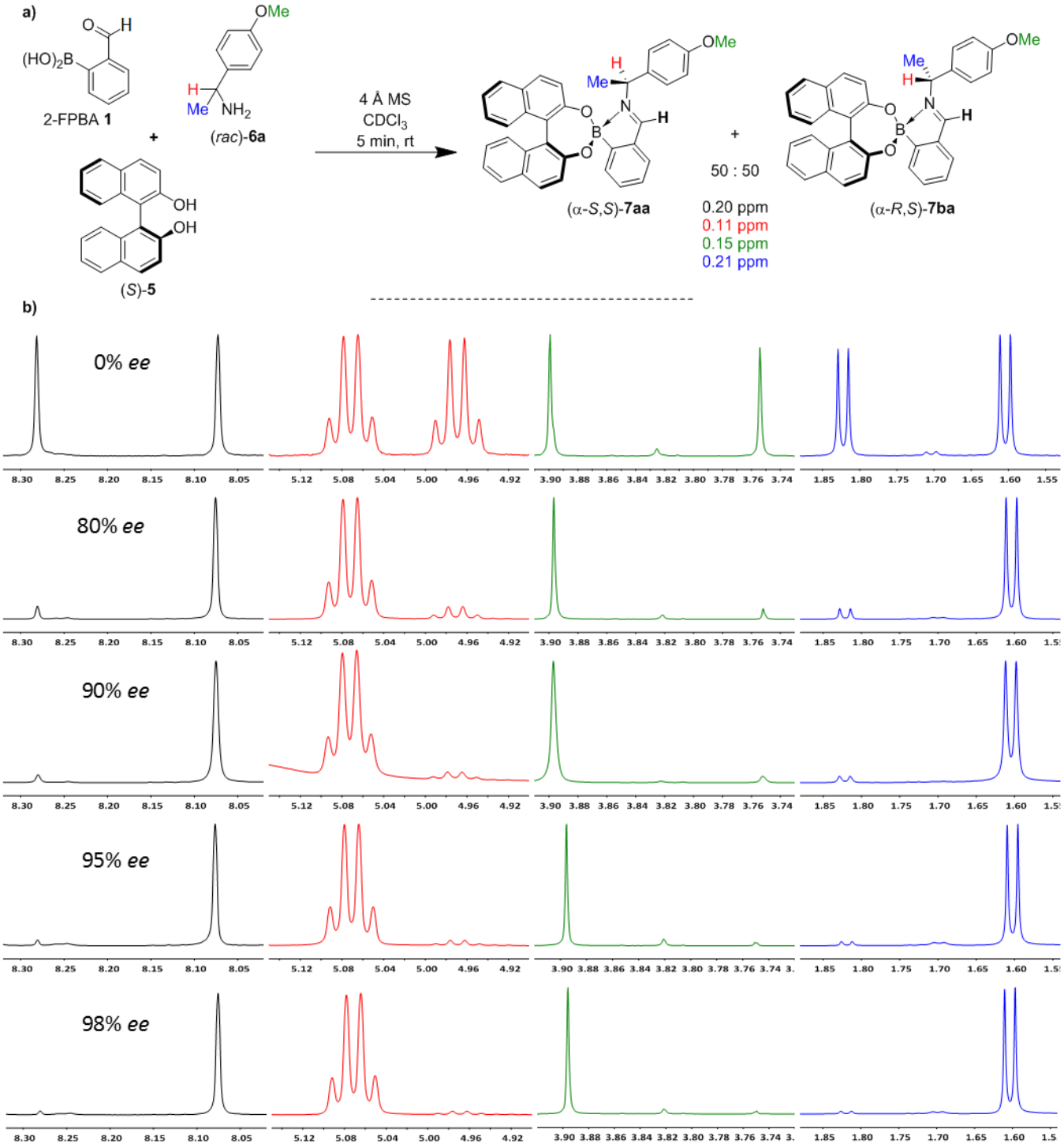


Figure 1: (a) Three-component assembly of 2-FPBA **1**, (*S*)-BINOL **5** and (*rac*)-4-methoxy-α-methylbenzylamine **6a** and observed $\Delta\delta_H$'s. (b) Expanded ^{1}H NMR (500 MHz, $CDCl_3$) spectra of diastereomeric complexes produced from reaction of 2-FPBA **1** with (*S*)-BINOL **5** and (*S*)-**6a** of 0, 80, 90, 95 and 98% *ee*.

This three-component derivatisation reaction was attractive from a practical standpoint, as it was moisture tolerant, employed cheap, commercially available, bench-stable reagents, and proceeded rapidly at room temperature (5 min) in an NMR solvent with no need for reaction workup or purification. Moreover, it produced diastereomeric IBEs whose ^{1}H NMR spectra exhibited multiple pairs of baseline-resolved diastereomeric proton resonances with a large $\Delta\delta_H$, which meant that their *dr* could be analysed using low field NMR spectrometers (e.g. 250 MHz). Furthermore, the imine signals appeared in a region of the ^{1}H NMR spectrum that was well removed

from any other resonances, thus limiting the risk of overlapping peaks resulting in inaccurate integration values. These initial results indicated that this self-assembling CDA stood a strong chance of being applicable for determining the *ee* of a wide range of chiral amines, with its combinatorial three-component nature affording the opportunity to change the chiral diol component used for derivatisation to maximise the signal resolution of pairs of diastereomeric peaks as required (*vide infra*). The modular nature of this CDA also afforded the opportunity to use an enantiopure amine as a chiral reporter to analyse the *ee* of chiral diols or any other chiral analyte that might show orthogonal reactivity for either the boronic acid or formyl groups of the 2-FPBA template.[22]

2.2. *Structural and mechanistic features of IBE complex formation*

Since our initial report describing the use of this three-component method to determine the *ee*'s of amines, significant structural and mechanistic work has been carried out to understand the efficiency of the self-assembling pathways leading to formation of these stable IBE complexes. X-ray crystallographic analysis of the diastereomeric IBEs (α-*S*,*S*)-**7ab** and (α-*R*,*S*)-**7bb**[29] produced in the three-component assembly reaction of (*S*)-BINOL **5**, 2-FPBA **1** and enantiopure α-methylbenzylamine **6b** (Figure 2) revealed N-B distances of 1.656 Å and 1.642 Å respectively, clearly indicating the presence of strong N→B coordination bonds that confer structural rigidity. This was further confirmed by ^{11}B NMR spectroscopy which revealed upfield 'tetrahedral boron' signals for both complexes. This rigidity leads to the benzylic C-H bonds being positioned directly above the boronate centres to minimise steric interaction with the BINOL ligand. Differences in the ^{1}H NMR chemical shifts of the α-methyl protons of the diastereomers can be explained by the homochiral complex (α-S,S)-**7ab** experiencing anisotropic shielding effects from the BINOL naphthyl moiety that are not present in the heterochiral (α-*S*,*R*)-**7bb** complex. Similar variations in local anisotropic shielding effects between diastereomeric complexes are responsible for the different chemical shifts of multiple pairs of diastereomeric proton resonances observed in the ^{1}H NMR spectra. The ease of crystallisation of Bull-James-assembled IBEs also provides the opportunity to determine the absolute configuration of a chiral amine (or diol) analyte through X-ray crystal analysis of a diastereomerically pure IBE complex prepared from a chiral diol (or amine) of known absolute configuration.

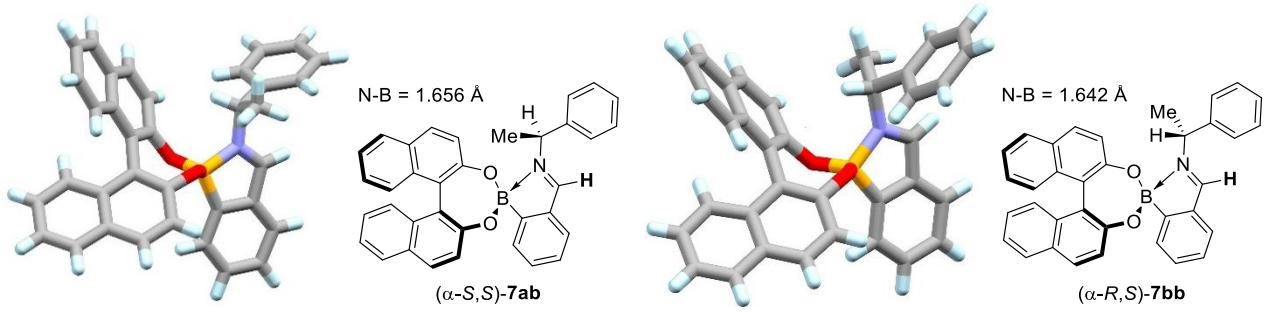
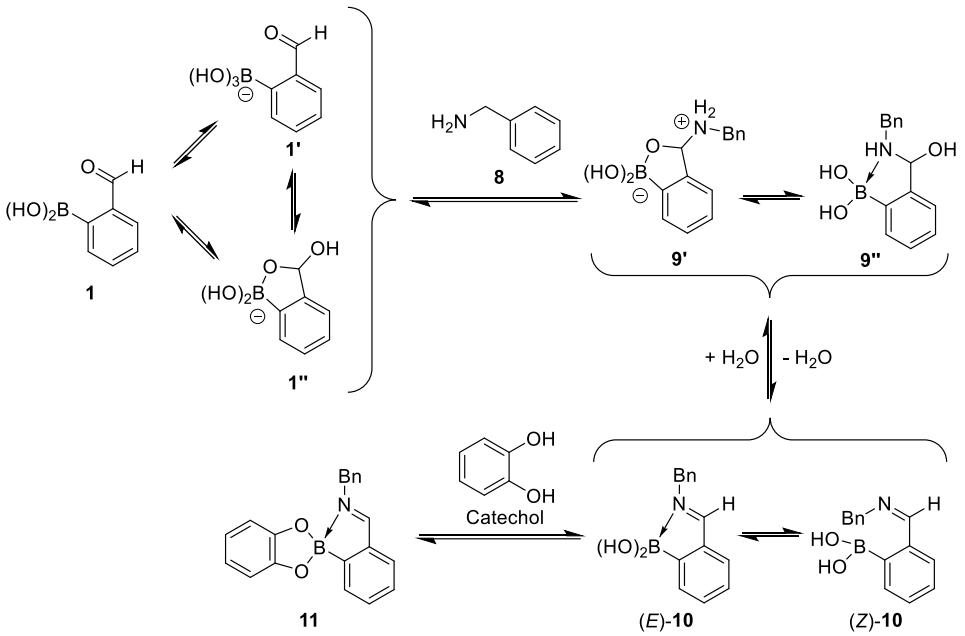


Figure 2: X-ray crystal structures of IBEs (α-*S*,*S*)-**7ab** and (α-*R*,*S*)-**7bb**.

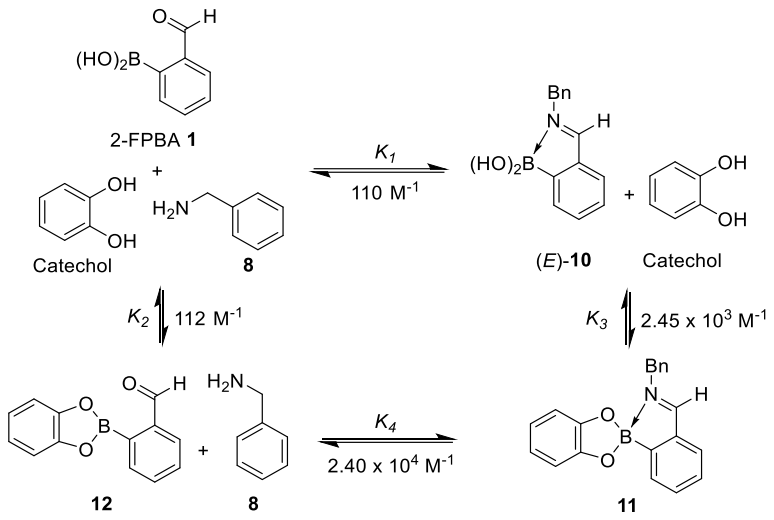
A simplified achiral three-component system using 2-FPBA **1**, catechol, and benzylamine **8** was used to explore the mechanism and kinetics of the stepwise formation of these self-assembled IBE complexes.[30] ^{1}H and ^{11}B NMR spectroscopic analysis of two- and three-component reactions in acetonitrile-d_3 (improved solubility of reagents/products) revealed the presence of a multistep reaction pathway leading to complex formation (Scheme 4). These studies revealed that the 2-FPBA **1** template exists in equilibrium with its corresponding borate **1'** and benzoxaborole **1''** species, with strong intramolecular binding of a lone-pair of its aldehyde group to the boron centre, activating the aldehyde towards nucleophilic attack.[31,32] Reaction of the aldehyde with an amine produces hemi-aminals **9'** and **9''** that then eliminate water to produce iminoboronic acid **10**. Subsequent addition of catechol then leads to formation of the desired achiral iminoboronate complex **11**. Interestingly, a small amount of the (*Z*)-imine (*Z*)-**10** (no intramolecular N→B coordination) was observed in the two-component complexation

reaction, which is consumed through equilibration to (*E*)-IBE **10** upon addition of catechol. Similar reaction pathways and intermediates have been suggested and observed by others, including important works by Sporzyński and Yatsimirisky.[33–35]



Scheme 4: Stepwise mechanism of the three-component assembly of 2-FPBA **1**, benzylamine **8** and catechol in CD_3CN.

In order to further evaluate the nature of the self-assembly processes operating in these complexation reactions, the observed binding constants for each individual two- and three-component assembly step in methanol were calculated (Scheme 5). These data clearly revealed that guest binding of the diol and amine to the 2-FPBA host is a cooperative process, as demonstrated by the dramatic increase in binding affinities when moving from two- to three-component assemblies. This difference in reactivity was observed when catechol binds to the boron centre, as equimolar mixtures of the diol and 2-FPBA **1** did not lead to quantitative formation of formyl boronate ester **12** (K_2 = 112 M^{-1}), whereas addition of catechol to iminoboronic acid **10** strongly favoured formation of iminoboronate ester **11** (K_3 = 2.45 x 10^3 M^{-1}). Similarly, addition of benzylamine to boronate ester **12** to give iminoboronate ester **11** (K_4 = 2.40 x 10^4 M^{-1}) was more favoured than addition of benzylamine to 2-FPBA **1** to afford imine **10** (K_1 = 1100 M^{-1}) by an order of magnitude. This further confirms that the strength of binding of the diol to the boron centre to produce a boronate ester complex is increased by the presence of a proximal imine functionality (and *vice versa*). These complexation results are consistent with results reported by Gillingham *et al*. to explain the efficiency of bioorthogonal iminoboronate complexation reactions (*vide infra*), as well as explanations provided to explain the reaction pathways present in analogues of *o*-aminomethylphenylboronic acid complexes.[36–38]

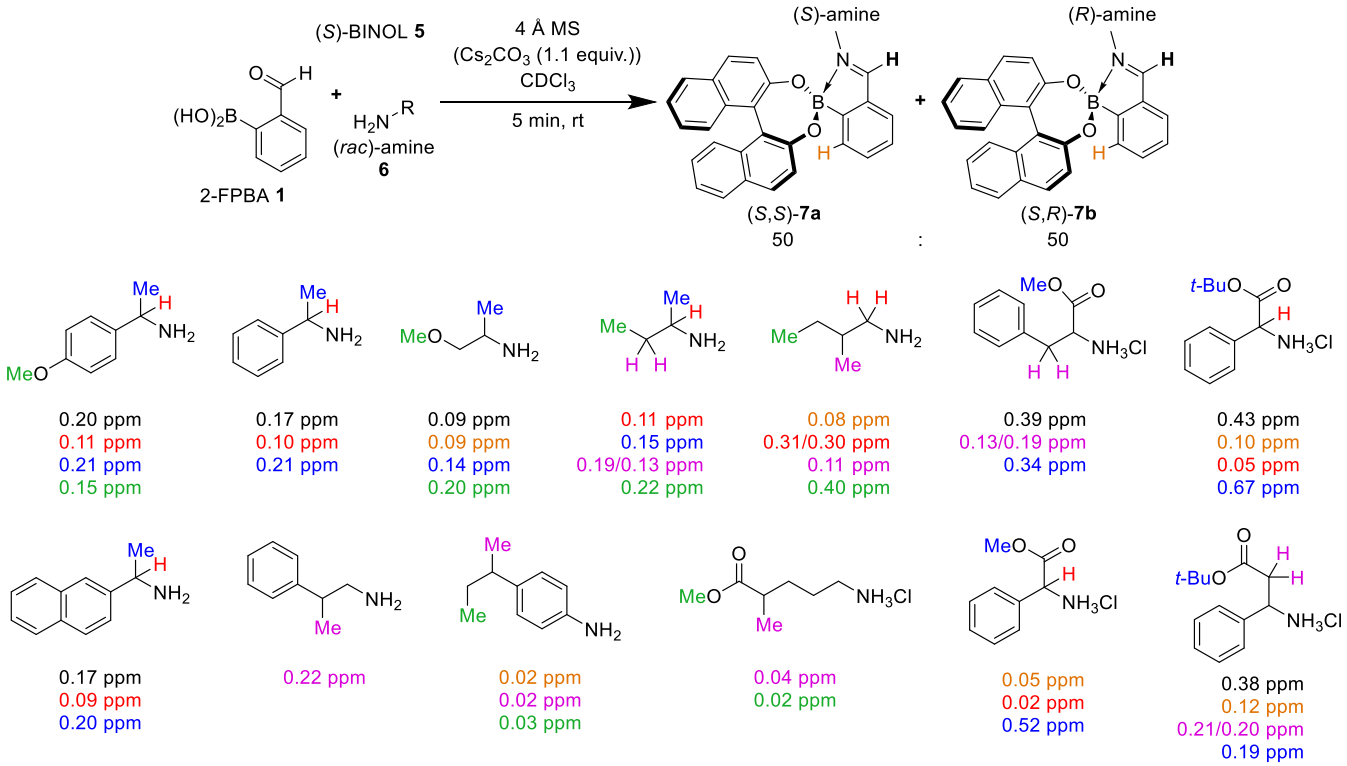


Scheme 5:Observed binding constants for intermediates generated in the three-component assembly reaction of 2-FPBA **1**, benzylamine **8** and catechol in CD_3OD.

3. Three-component assembly for determining *ee* by NMR spectroscopic analysis

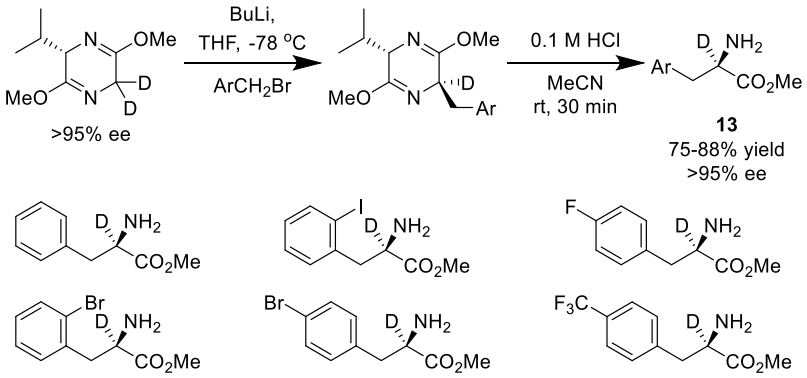
3.1. *Primary amines*

The optimal conditions (enantiopure BINOL, $CDCl_3$, 4 Å molecular sieves, 5 min) that were established to determine the *ee* of 4-methoxy-α-methylbenzene **6a** have been applied to determine the enantiopurities of a wide array of primary chiral amine analytes (Scheme 6).[28] This derivatisation approach shows good scope, affording a series of diastereomeric IBEs **7** whose ^{1}H NMR spectra all exhibited at least one pair of well-resolved diastereomeric signals that could be integrated to determine their *dr*'s. Complexation using scalemic samples confirmed that none of these chiral amines underwent any kinetic resolution (or epimerisation) during the derivatisation process, thus allowing this new CDA to be used to accurately measure the *ee*'s of a wide range of chiral amine analytes. Interestingly, this derivatisation method was found to be effective for analysing the *ee* of primary amines containing remote stereocenters up to 5 carbon atoms removed from the complexed amino group, whilst direct analysis of chiral ammonium salts could be achieved through incorporation of Cs_2CO_3 (1.1 equiv.) as a base for neutralisation. A subsequent report by Urriolabeitia and co-workers described that derivatisation of enantiopure phenylglycine methyl ester salts (more labile α-stereocenter) resulted in formation of mixtures of diastereoisomeric IBEs when derivatisation reactions were left for extended periods of time (> 1 h).[39] We subsequently solved this racemisation issue by switching the base used for amine salt neutralisation from Cs_2CO_3 to less-soluble K_2CO_3, which allowed racemisation-free derivatisation of chiral amine salts containing potentially labile stereocenters to be carried out.[40]

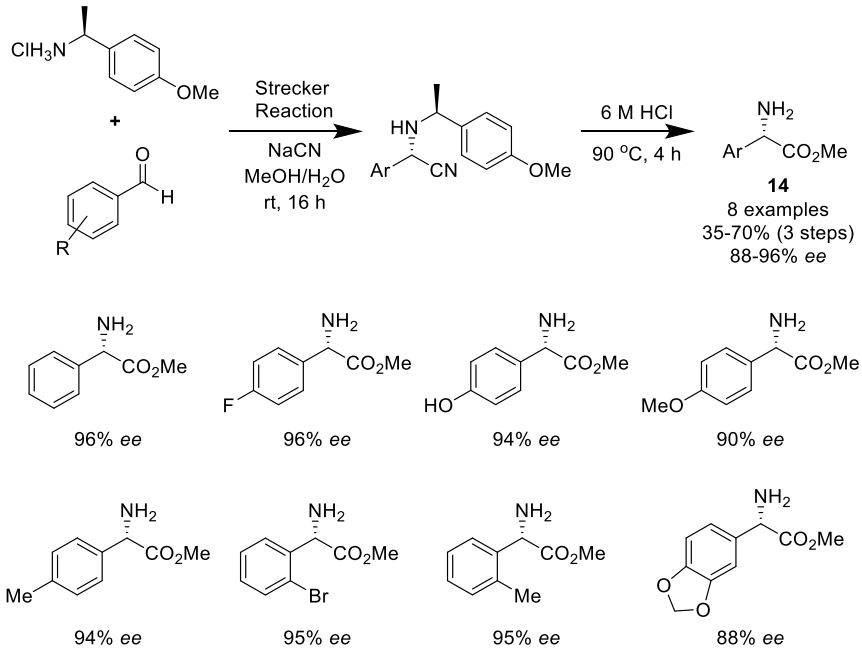


Scheme 6: Three-component assembly reaction of 2-FPBA **1**, (*S*)-BINOL **5** and (*rac*)-amines **6** to afford diastereomeric IBEs with ^{1}H NMR (300 MHz, $CDCl_3$) $\Delta\delta_H$ values quoted for selected pairs of diastereomeric resonances.

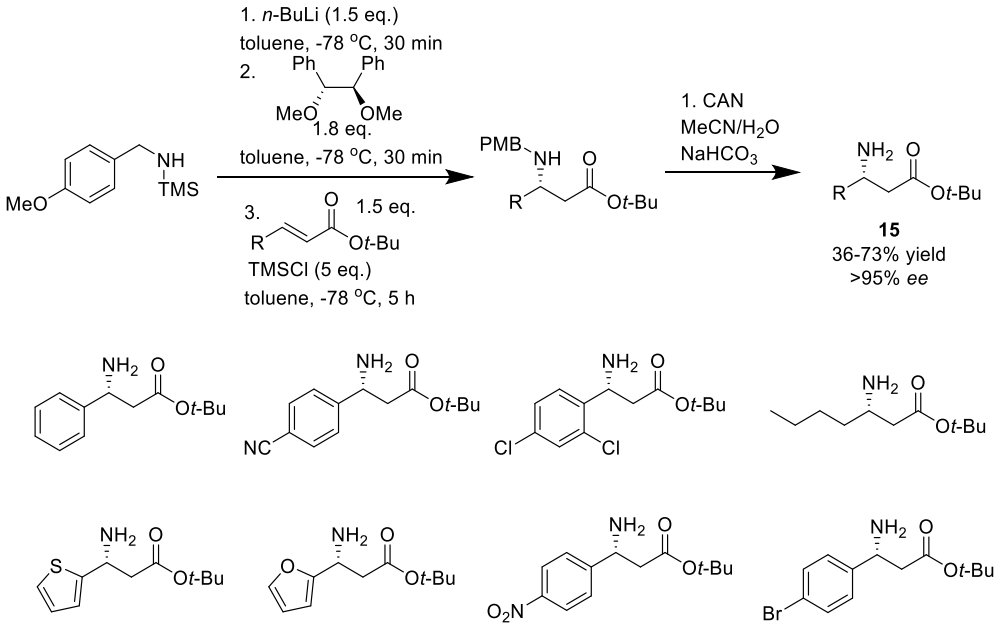
Since our initial report, this CDA method has been published as a general procedure in *Nature Protocols*,[41] and been used by the Bull group to validate the enantioselectivities of a number of new asymmetric methods for the production of chiral amines. Their first application was to confirm the enantiopurities of (*R*)-[α-^{2}H]-phenylalanine methyl esters generated by alkylation of the *aza*-enolate of deuterated Schöllkopf's *bis*-lactim ether **13** (Scheme 7).[42] This CDA method has also been used to confirm the enantiopurities of α- and β-amino esters **14** and **15** prepared using asymmetric Strecker (Scheme 8) and enantioselective *aza*-conjugate addition reactions, respectively (Scheme 9).[40,43] It has also been used to confirm the enantiopurity of a chiral α-methylbenzyl-amine intermediate (*R*)-**16** that was used for the synthesis of a chiral ligand for the preparation of a pseudo-C_3-symmetric titanium alkoxide propeller-like complex (Scheme 10).[44]



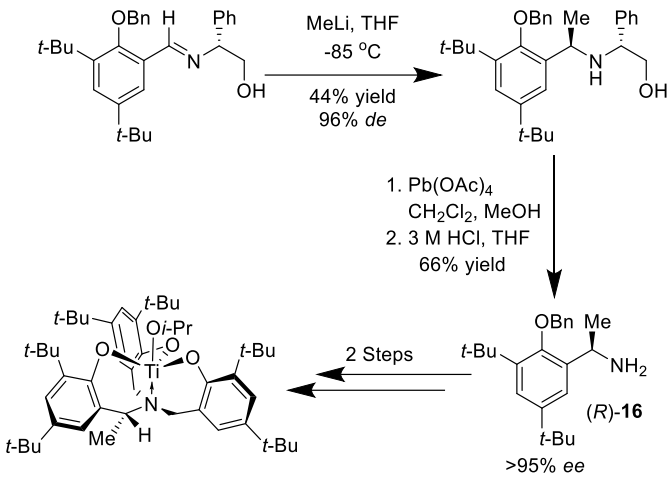
Scheme 7: Three-component CDA method (using enantiopure (*R*)-BINOL) used to determine the *ee*'s of α-deuterated-α-amino esters **13** produced in asymmetric enolate alkylation reactions.



Scheme 8: Three-component CDA (using enantiopure (*S*)-BINOL) used to determine the *ee*'s of α-arylglycines **14** produced in asymmetric Strecker reactions.

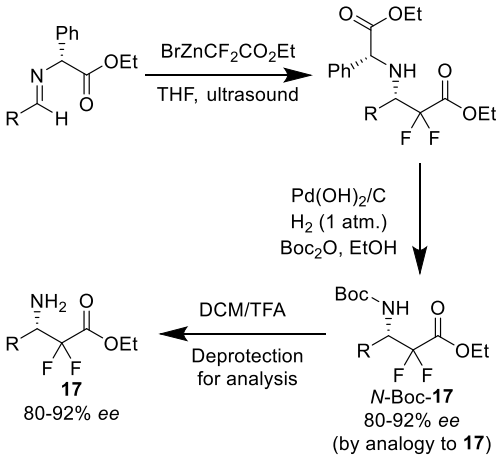


Scheme 9: Three-component CDA (using enantiopure (*R*)-BINOL) used to determine the *ee*'s of *tert*-butyl β-amino esters **15** produced in enantioselective *aza*-conjugate addition reactions.

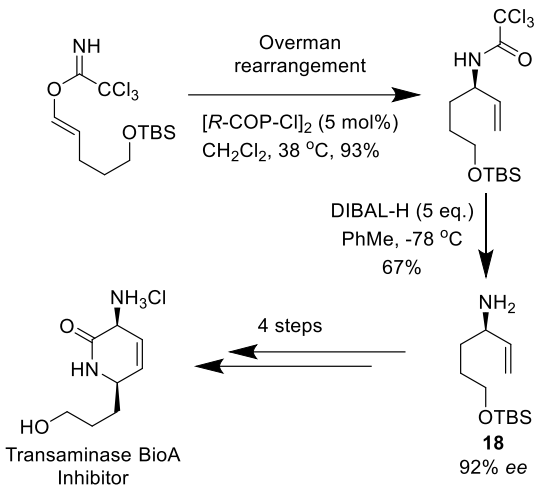


Scheme 10: Three-component CDA (using enantiopure BINOL) used to determine the *ee* of a tetradentate amine ligand (*R*)-**16** used to prepare an enantiopure 'propeller-like' pseudo-C_3-symmetric titanium alkoxide.

Other research groups have also used the Bull-James assembly to determine the *ee* of amines produced in various stereoselective protocols. Duggan *et al*., for instance, reported a novel synthesis of aliphatic α,α-difluoro-β^3-amino esters **17** through addition of zinc enolates to chiral phenylglycine-derived imines (Scheme 11),[45] with the three-component CDA approach then used to demonstrate that the *N*-Boc-deprotected amine products had *ee*'s of 80-92%. The *ee* of a chiral allyl amine intermediate **18,** produced in an enantioselective Overman-rearrangement that was used to synthesise a transaminase BioA inhibitor (potential antitubercular agent), was also measured in this manner (Scheme 12).[46]

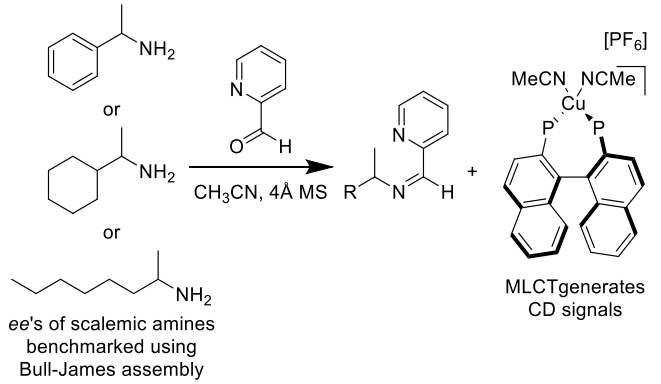


Scheme 11: Three-component CDA method (using enantiopure (*S*)-BINOL) used to determine the *ee* of an α,α-difluoro-β^3-amino esters **17** prepared using a sonocatalyzed Reformatsky reactions.



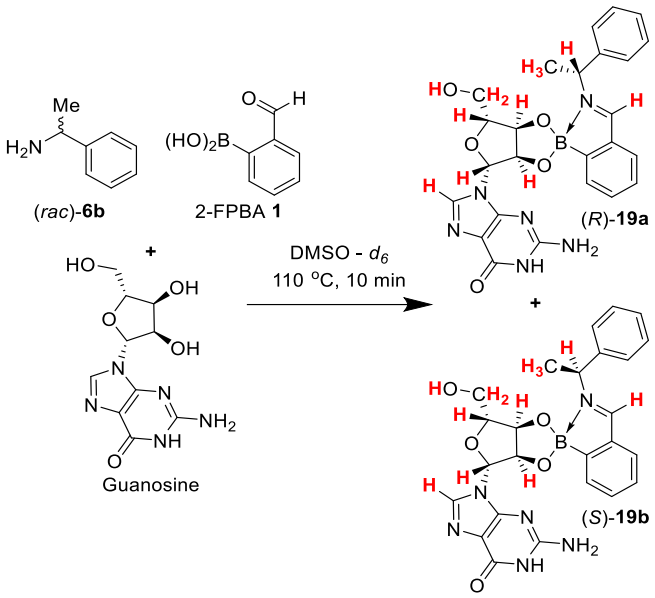
Scheme 12: Three-component CDA method (using enantiopure BINOL) used to determine the *ee* of a chiral allylamine **18** produced in an enantioselective Overman rearrangement reaction.

The Anslyn group have also employed NMR spectroscopic analysis of three-component IBE assemblies to benchmark the *ee's* of amine analytes. These amines were subsequently used to develop a new CD method for high-throughput *ee* determination based on formation of diastereomeric chiral copper complexes that produce different metal-to-ligand charge transfer (MLCT) bands in the visible region of the CD spectrum (Scheme 13).[47]



Scheme 13: Three-component analysis used to benchmark the *ee*'s of chiral amines used to develop a MLCT CD assay for high-throughput determination of the *ee's* of primary amines (using (*S*)-BINOL).

Suryaprakash *et al*. have reported the use of the chiral diol fragments of RNA nucleosides as chiral selectors for determining the *ee* of a small range of amines,[48] as shown for the complexation reaction of guanosine, 2-FPBA **1** and α-methyl-benzylamine **6b** to produce the diastereomeric complexes **19a** and **19b** shown in Scheme 14. These complexation reactions required more forcing reaction conditions (DMSO, 110 °C) to proceed to completion, and whilst the structural complexity of these diastereomeric IBEs afforded multiple resolved resonance pairs, 800 MHz ^{1}H NMR spectra were required to fully resolve them all.

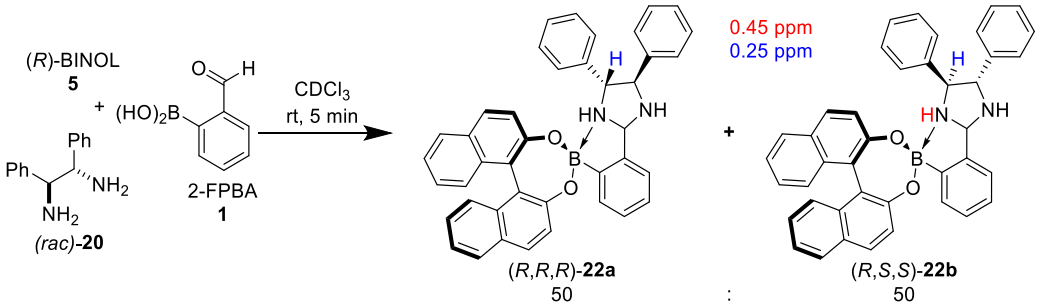


Scheme 14: Three-component assembly of 2-FPBA **1**, guanosine, and (*rac*)-α-methylbenzylamine **6b**. Pairs of diasteromeric protons that exhibited resolved resonances in a 800 MHz ^{1}H NMR spectrum are shown in red.

Fossey and co-workers have exemplified the experimental simplicity and reproducibility of this NMR derivatisation protocol by successfully using it as the basis of a research-informed undergraduate teaching class that was used to train a cohort of > 100 2nd year undergraduate students at the University of Birmingham (UK).[49] An optimised iminoboronate protocol using 2-FPBA **1**, (*R*)-BINOL **5**, and α-methylbenzylamine **6b** was used as an educational tool to introduce the students to the principles of dynamic covalent supramolecular chemistry and methods of determining the enantiopurities of chiral molecules, whilst reinforcing their knowledge of carbonyl condensation chemistry and fundamental Lewis acid/base coordination processes.

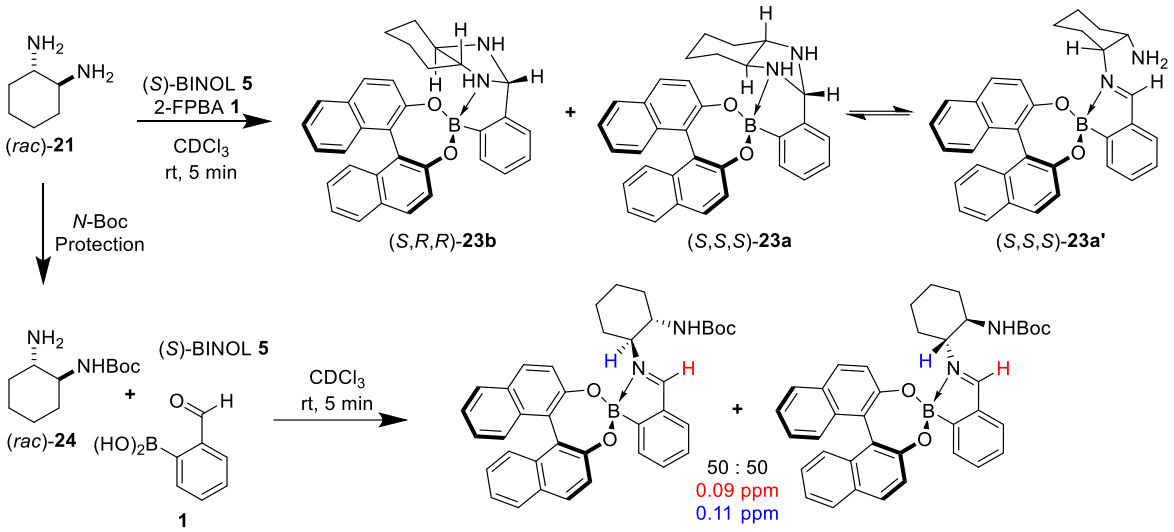
3.2. *Diamines*

The Bull-James CDA protocol was then applied to determine the *ee's* of two widely used *trans*-diamines: 1,2-diphenylethane-1,2-diamine **20** and *trans*-cyclohexane-1,2-diamine **21**.[50] Reaction of diamine (*rac*)-**20** with (*R*)-BINOL **5** and 2-FPBA **1** resulted in the formation of a pair of diastereomeric imidazolidines (*R*,*R*,*R*)-**22a** and (*R*,*S*,*S*)-**22b** ,[51–53] which exhibited well-resolved pairs of diastereomeric signals for the amino (red) and benzylic (blue) protons proximal to their BINOL fragments being observed in their ^{1}H NMR spectra (Scheme 15).[50]. Furthermore, these diastereomeric IBE complexes were found to be stable enough for N-H deuteration by addition of D_2O, which resulted in simplified ^{1}H NMR spectra that enabled more accurate determination of *dr*'s.



Scheme 15: Three-component assembly of 2-FPBA **1**, (*R*)-BINOL **5** and (*rac*)-*trans*-diphenylethylenediamine **20** to produce a pair of diastereomeric imidazolidine boronate esters **22** with ^{1}H NMR (500 MHz, $CDCl_3$) $\Delta\delta_H$ of selected resonances.

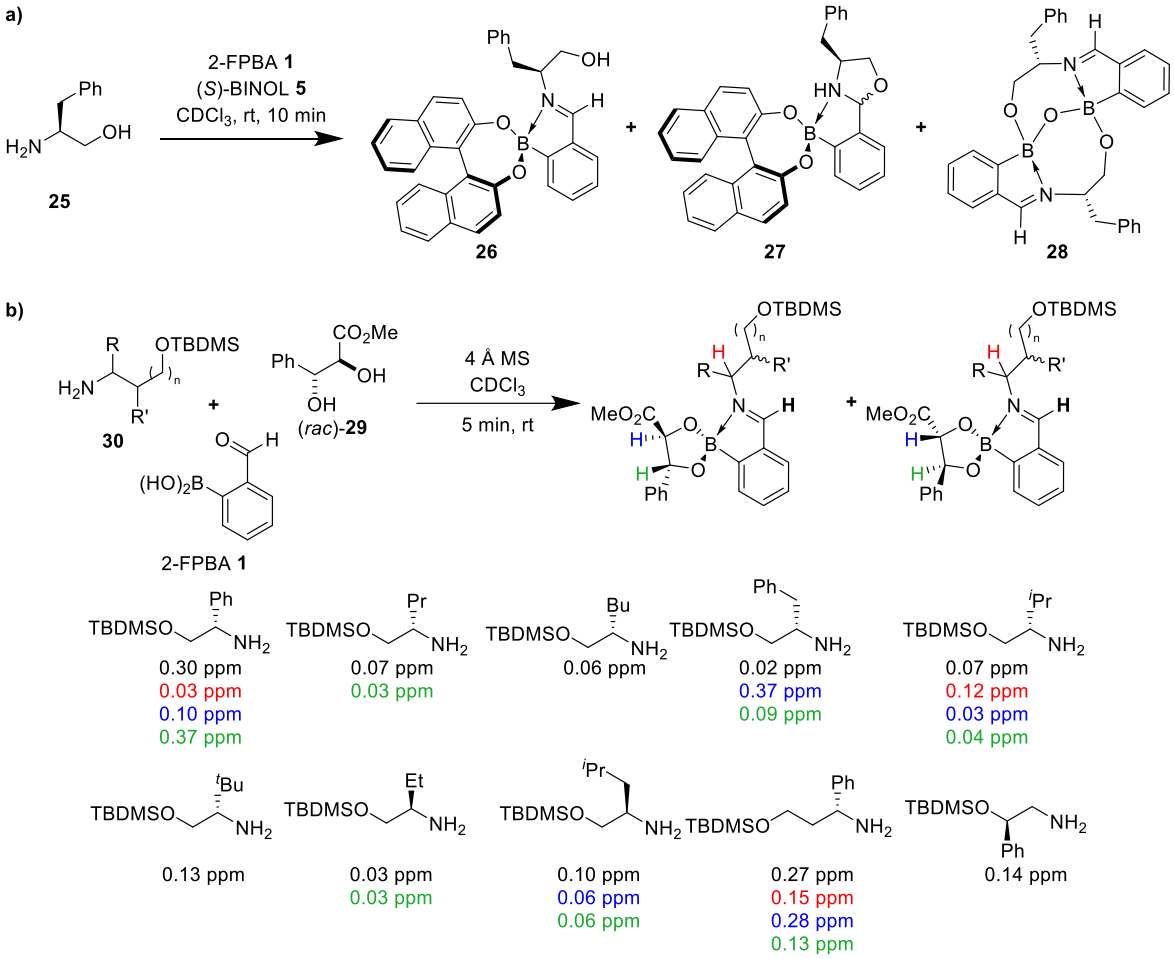
Unfortunately, applying this CDA approach to *trans*-cyclohexane-1,2-diamine **21** proved unsuccessful, with its derivatisation with (*S*)-BINOL **5** and 2-FPBA **1** producing a mixture of products (Scheme 16). Although the heterochiral imidazolidine complex (*S*,*R*,*R*)-**23b** proved stable, increased steric demands within the homochiral complex resulted in formation of a dynamically equilibrating mixture of imidazolidine (*S*,*S*,*S*)-**23a** and its corresponding imine (*S*,*S*,S)-**23a'**. A simple solution to this problem was achieved, through *N*-Boc-protection of the parent diamine **21** to afford mono-*N*-Boc-diamine **24**, which then underwent IBE derivatisation to afford the desired mixture of IBE diastereomers in the usual manner.



Scheme 16: Three-component derivatisation of 2-FPBA **1**, (*S*)-BINOL **5** with (*rac*)-*trans*-cyclohexane-1,2-diamine **21** and (*rac*)-*N*-Boc-*trans*-cyclohexane-1,2-diamine **24** with 1H NMR (400 MHz, $CDCl_3$) $\Delta\delta_H$ of selected resonances.

3.3. *Amino alcohols*

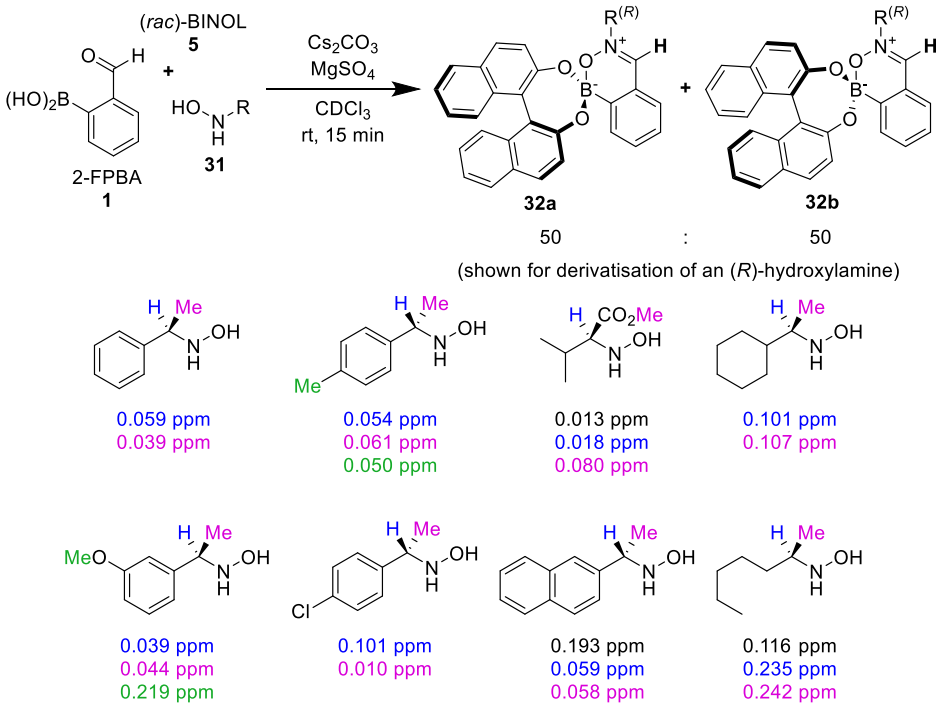
Attempts to apply the CDA methodology to 1,2-amino-alcohols proved similarly problematic, with assembly of (*S*)-phenylglycinol **25**, 2-FPBA **1** and (*S*)-BINOL **5** producing complex equilibrating mixtures of products (Scheme 17), including the desired IBE **26**, oxazolidine boronate ester **27** and a larger polyboracycle **28.**[54] Once again, the problems caused by these competing complexations could be solved using a protection strategy, with *O*-silylation of the problematic alcohol functionality prior to assembly resulting in the three-component complexation proceeding smoothly to give the desired diastereomeric IBEs. A simple diol screen revealed that the best results were obtained when BINOL was substituted by (*rac*)-(*syn*)-methyl 2,3-dihydroxy-3-phenylpropionate **29,** which was subsequently employed for the successful three-component derivatization of ten enantiopure *O*-silyl amino alcohol analytes **30**.



Scheme 17: (a) Problematic three-component assembly of (*S*)-phenylglycinol **25**, 2-FPBA **1** and (*S*)-BINOL **5**. (b) Three-component derivatisation of 2-FPBA **1**, (*rac*)-**29** and *O*-silylated 1,2-amino alcohols **30** with ^{1}H NMR (400 MHz, $CDCl_3$) $\Delta\delta_H$ of selected resonances.

3.4. *Hydroxylamines*

Bull-James assembly of hydroxylamines **31** with 2-FPBA **1** and (*rac*)-BINOL **5** in the presence of Cs_2CO_3 as base gave mixtures of diastereomeric nitrono-boronate esters **32** (Scheme 18).[55] Unlike amines, which form five-membered IBEs containing a relatively labile intramolecular N→B bond, hydroxylamines gave more stable diastereomeric six-membered nitrono-boronate ester complexes whose formation was favoured by both strong N-O and O-B bonds.[26,56] These structures were confirmed by X-ray crystallography of (α-*S*, *R*)-**32bf,** which revealed a bicyclic assembly containing a coplanar zwitterionic -C=N$^+$-O-B$^-$ arrangement (Figure 3), which produces a rigid ring system that produces relatively large chemical shift differences for selected pairs of diastereomer resonances (up to 0.242 ppm) in their ^{1}H NMR spectra.



Scheme 18: Three-component assembly of 2-FPBA **1**, (*rac*)-BINOL 5, and hydroxylamines **31** to form diastereomeric nitrono-boronate ester complexes **32a** and **32b** with ^{1}H NMR (500 MHz, $CDCl_3$) $\Delta\delta_H$ of selected resonances.

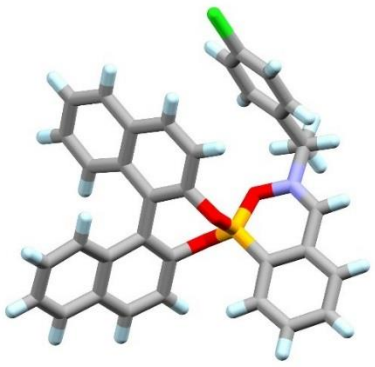
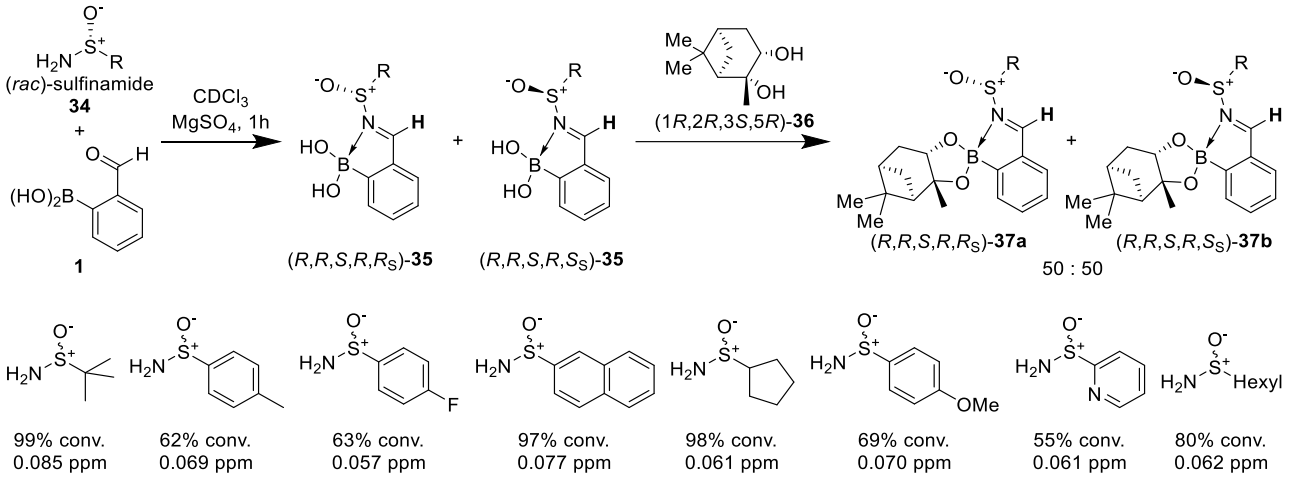


Figure 3: X-ray crystal structure of (α-*S*, *R*)-**32bf**, from (*S*)-4-chloro-α-methylbenzylamine **31f**.

3.5. *Sulfinamides*

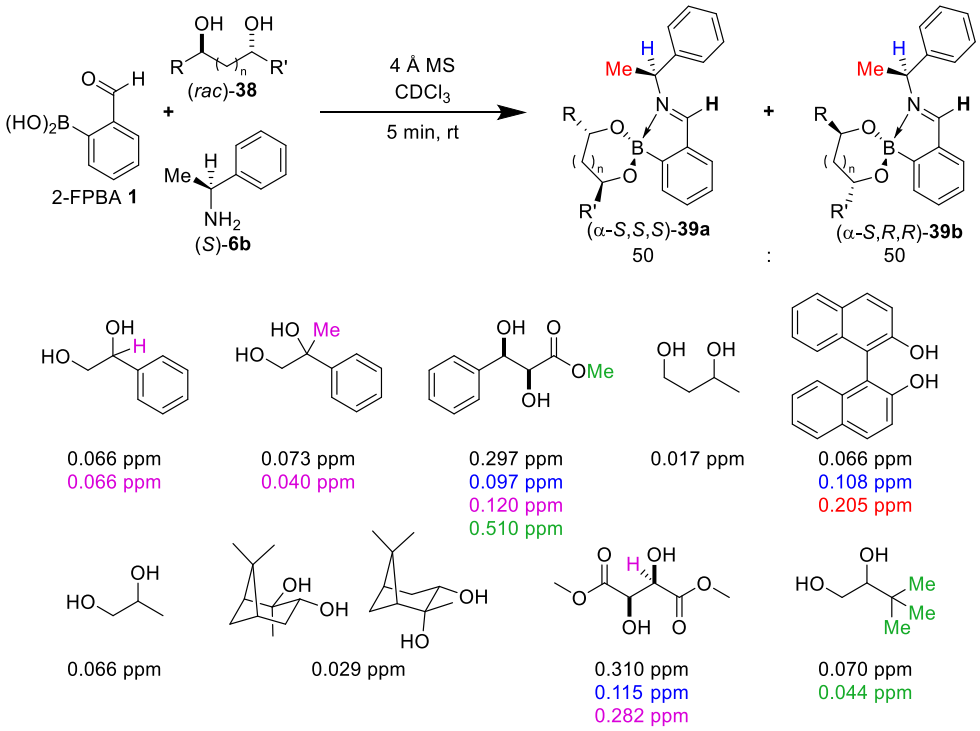
Chiral sulfinamides (primarily Ellman's and Davis') are widely used as chiral auxiliaries and ligands to control stereoselectivities in a wide range of asymmetric reactions. These sulfinamides are normally prepared in enantiopure form *via* either classical resolution of their corresponding racemates or stereoselective synthesis, which means that robust methods are required to accurately determine their enantiopurities. Application of standard Bull-James complexation conditions to these sulfinamides proved unsuccessful, with their less nucleophilic nitrogen atoms only affording small amounts of the desired sulfiniminoboronates, regardless of reaction conditions or additives employed. Consequently, a stepwise 'one-pot' two-component protocol was developed based on initial reaction of 2-FPBA **1** with a sulfinamide **34** to afford a sulfiniminoboronic acid intermediate **35,** whose boronic acid fragment was then reacted with pinanediol **36** to afford the desired sulfiniminoboronate ester complexes **37** (Scheme 19).[57] This stepwise protocol was successfully applied to 8 racemic sulfinamides, which resulted in baseline-resolved imine signals for their diastereomeric IBEs in their ^{1}H NMR spectra in all instances, with no evidence of kinetic resolution.



Scheme 19: Stepwise three-component assembly of 2-FPBA **1**, (1*R*,2*R*,3*S*,5*R*)-pinanediol **36** and (*rac*)-sulfinamides **34** with ^{1}H NMR (500 MHz, $CDCl_3$) $\Delta\delta_H$ of selected resonances.

3.6. *Diols*

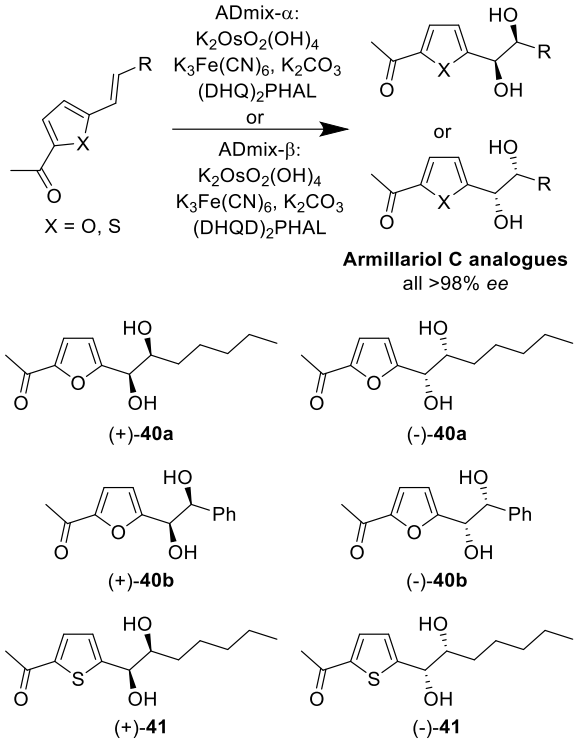
The role of analyte and chiral reporter in the three-component CDA are broadly interchangeable, and so the Bull-James assembly has also been adapted to determine the *ee*'s of chiral 1,2- and 1,3-diol analytes through use of an enantiopure amine chiral reporter (Scheme 20).[58] α-Methylbenzylamine (*S*)-**6b** was chosen as a cheap readily available chiral amine reporter for reaction with 2-FPBA **1** and a range of racemic chiral diols **38**, which produced diastereomeric complexes (α-*S*,*S*,*S*)-**39a** and (α-*S*,*R*,*R*)-**39b**, which exhibited one or more baseline-resolved pairs of signals for their IBE diastereomers in their ^{1}H NMR spectra.



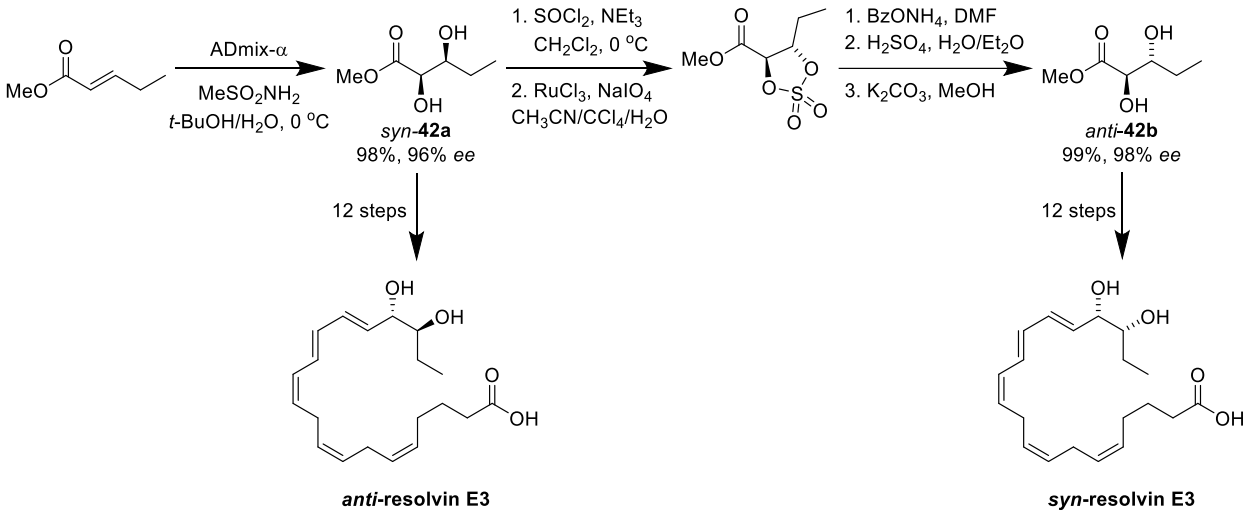
Scheme 20: Three-component assembly using 2-FPBA **1**, (*S*)-α-methyl benzylamine **6b** and (*rac*)-diols **38** with ^{1}H NMR (300 MHz, $CDCl_3$) $\Delta\delta_H$ of selected resonances.

This method has also been published as a detailed general procedure in *Nature Protocols,*[59] and has subsequently been applied to determine the *ee* of a range of chiral 1,2-diols by a number of research groups. One

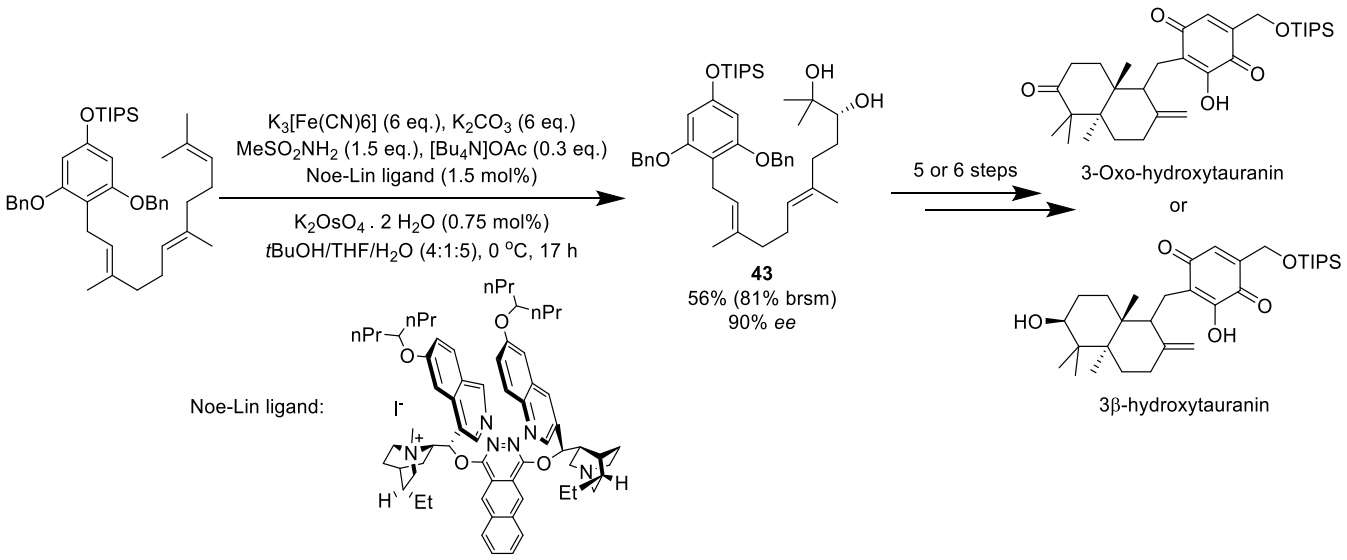
elegant example is the work by Watkins *et al*., who employed the CDA (using (*S*)-α-methylbenzylamine **6b**) to determine the *ee*'s of a range of chiral furan and thiophene diols (**40** and **41**, respectively) prepared using Sharpless enantioselective ADmix dihydroxylation methodology, that were used for the first stereoselective synthesis of (+)-armillariol C **40a** (Scheme 21).[60] Inoue *et al*. used enantioselective dihydroxylation reactions of α,β-unsaturated esters to prepare both enantiomers of *syn*-diol **42** (shown for ADmix-α), whose β-stereocenters were then inverted in two steps *via* cyclic organosulfate intermediates to afford their corresponding *anti*-diols. The enantiopurities of all four diol stereoisomers were determined as 96-99% *ee* using three-component chiral derivatization (using α-methylbenzylamine **6b**), with these stereoisomers then transformed into the four corresponding stereoisomers of resolvin E3 (Scheme 22).[61] Similarly, this CDA approach has been used to determine the enantiopurity of diol **43** (90% *ee*, single stereocenter, using both (*R*)-and (*S*)-**6b**) that was also produced in an enantioselective dihydroxylation reaction and subsequently used to prepare 3-oxo and 3β-hydroxytauranin (Scheme 23).[62]



Scheme 21: Three-component CDA method (using enantiopure (*S*)-α-methylbenzylamine) used to determine the *ee* of both enantiomers of armillariol C and analogues **41** that were produced using a Sharpless asymmetric dihydroxylation reaction.

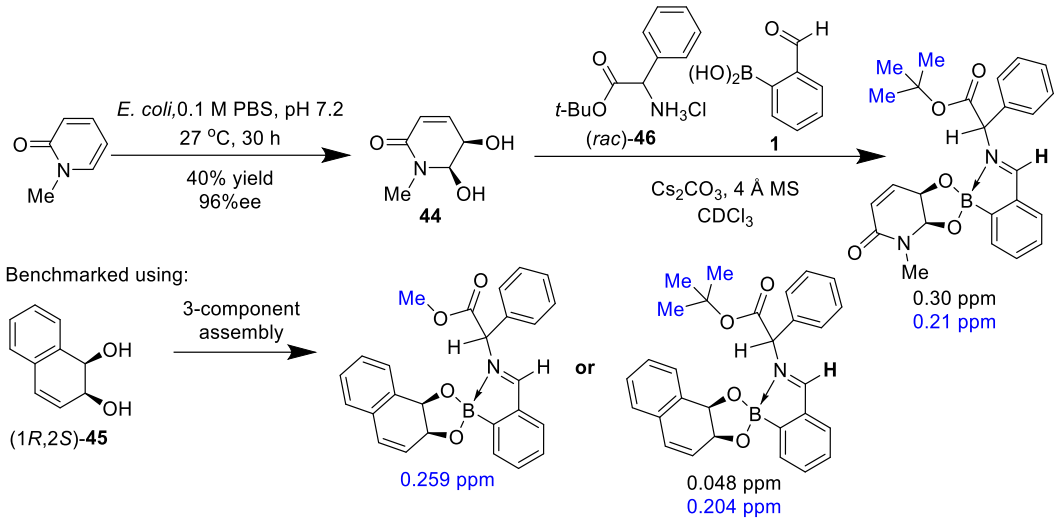


Scheme 22: Three-component CDA method (using enantiopure α-methylbenzylamine) used to determine the *ee*'s of *syn*- and *anti*-diols **42a** & **42b** that were subsequently used to synthesis all four possible stereoisomers of resolvin E3 (shown for ADmix-α).



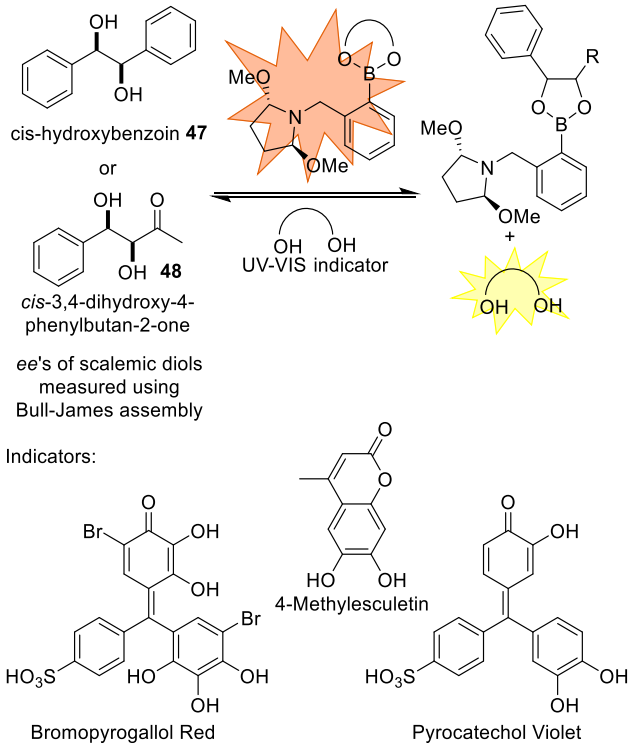
Scheme 23: Three-component CDA method (using enantiopure (*R*)- and (*S*)-α-methylbenzylamine) used to determine the *ee* of diol **43** that was subsequently used for total syntheses of 3-oxo- and 3β-hydroxytauranin.

Chopard *et al*. have used the three-component CDA to determine the enantiopurities of *cis*-diols **44** and **45**, produced from the microbial *cis*-dihydroxylation of naphthalenes and pyridinones. In this instance, the chiral amine reporter used for derivatisation was optimised, which identified phenylglycine *tert*-butyl ester **46** as the chiral reporter that gave diastereomeric IBEs with the best $\Delta\delta_H$ values (Scheme 24).[63]



Scheme 24: Three-component assembly for determining the enantiopurity of a *cis*-diol arene using phenylglycine *tert*-butyl ester **46** and 2-FPBA **1** with ^{1}H NMR (250 MHz, $CDCl_3$) $\Delta\delta_H$ of selected resonances.

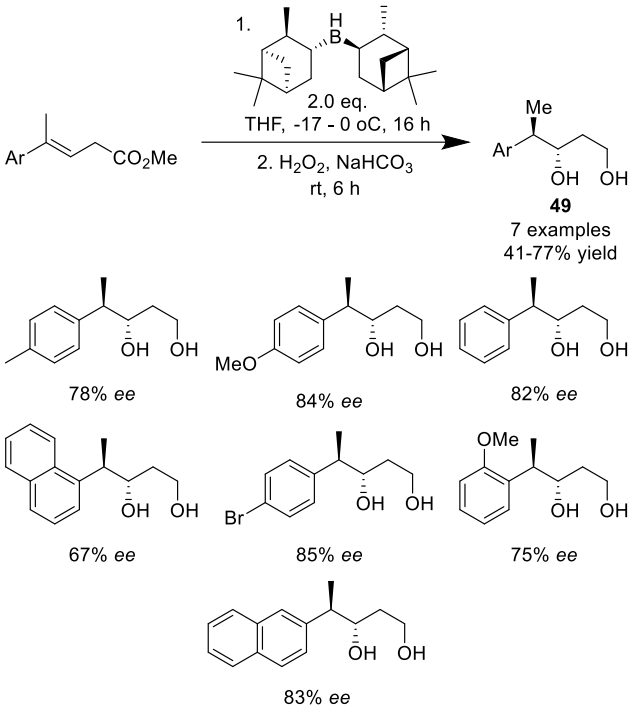
The three-component CDA was also used to measure the *ee*'s of *cis*-diols **47** and **48** produced in Sharpless dihydroxylations reactions by Anslyn *et al*. (Scheme 25). The *ee*'s of these diols were then used to benchmark indicator displacement UV-Vis assays for the high-throughput determination of yields and enantioselectivities of Sharpless dihydroxylation reactions, using reversible host/guest assemblies based on an *o*-aminomethylphenylboronic acid sensor, in which the UV-VIS signal intensity is directly determined by the *ee* and concentration of the analyte.[64,65]



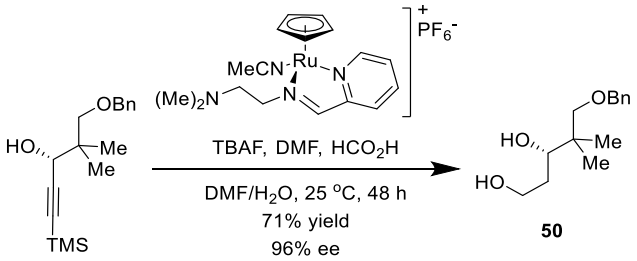
Scheme 25: Indicator displacement assay used for UV-Vis and colorimetric determination of enantioselectivity and yield of *cis*-diols **47** and **48** produced in Sharpless dihydroxylation reactions.

The Bull group have applied the CDA method to determine the *ee* of a range of chiral 1,3-diols **49** synthesised in moderate to good *ee* by tandem hydroboration/reduction of β,γ-unsaturated esters (Scheme 26).[66] The three-component assembly CDA has also been used by Herzon *et al*. to determine the *ee* of 1,3-diol **50** (92%) that was

synthesised by catalytic reductive hydration of a chiral alkynylsilane by sequential hydration/hydrogenation using a novel half-sandwich ruthenium complex and formic acid (Scheme 27).[67]



Scheme 26: Three-component CDA method (using enantiopure (*S*)-α-methylbenzylamine) used to measure the *ee*'s of chiral 1,3-diols **49** formed in tandem chiral borane-mediated asymmetric hydroboration/reduction reactions of β,γ-unsaturated esters.



Scheme 27: Three-component CDA (using enantiopure α-methylbenzylamine) to measure the *ee* of a 1,3-diol **50** formed in a stereoselective reductive hydration reaction of an alkynyl alcohol catalysed by a half-sandwich ruthenium complex.

The three-component CDA has also been used to assess the enantiopurity of polymers containing diol fragments, with Kressler *et al*. reporting its application to determine the enantiopurities of poly(glycerol methacrylate)s (PGMAs, **51**) that were prepared from enantiopure solketal methacrylate monomers using atom transfer radical polymerization (ATRP) reactions.[68] Enantiopure and racemic polymer chains were derivatised with α-methylbenzylamine **6b** and 2-FPBA **1** in DMSO-d_6, to afford mixtures of iminoboronates (α-*S*,*S*)-**52a** and (α-*S*,*R*)-**52b** that exhibited several pairs of distinct diastereomeric resonances in their ^{1}H NMR spectra (Figure 4). Peak broadening caused by the polymeric backbone meant that baseline resolution was not observed, however the $\Delta\delta_H$'s of the polymer's methine, *exo* methylene and *endo* methylene proton signals (*a*H, *b*H, *c*H, respectively) were sufficiently different to enable qualitative assessment of the enantiopurity and absolute configurations of the PGMA side-chains of these polymers.

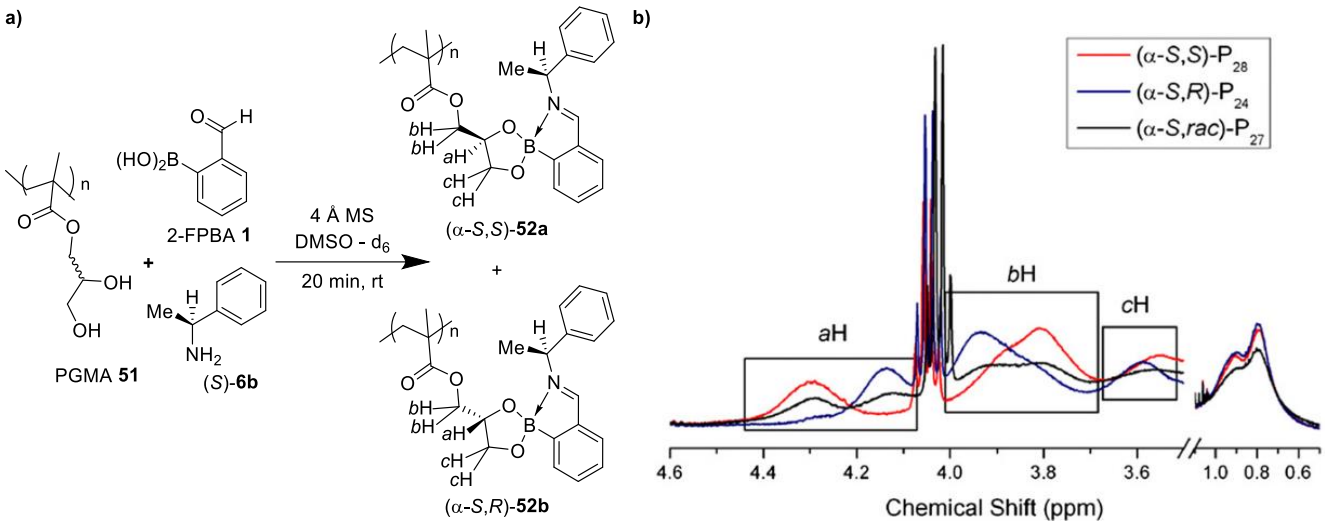
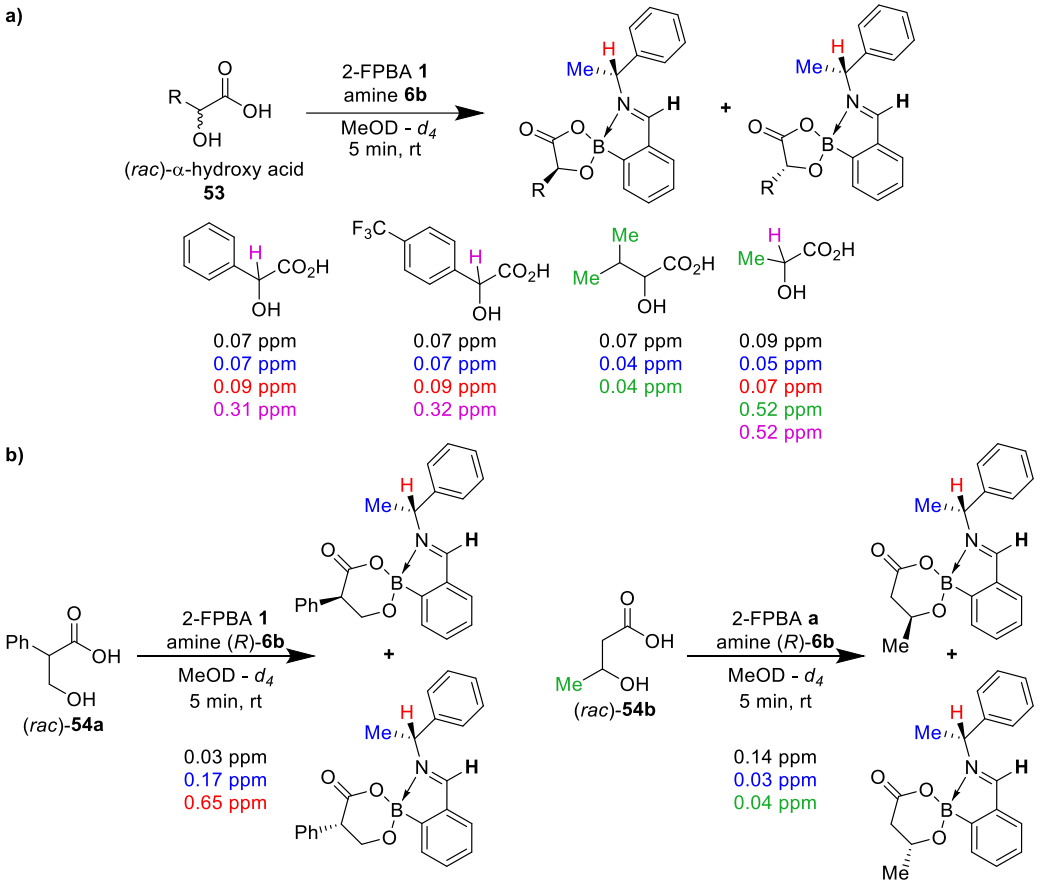


Figure 4: (a) Bull-James assembly used for derivatisation of the diol side-chain of poly(glycerol methacrylate)s **51**. (b) Inset of ^{1}H NMR (400 MHz, DMSO – d_6) spectra showing chemical shift variation of *a*H, *b*H and *c*H resonances of complexes of (*S*)-PGMA (red), (*R*)-PGMA (blue) and (*rac*)-PGMA (black). Reproduced from ref. [68] with permission from Elsevier.

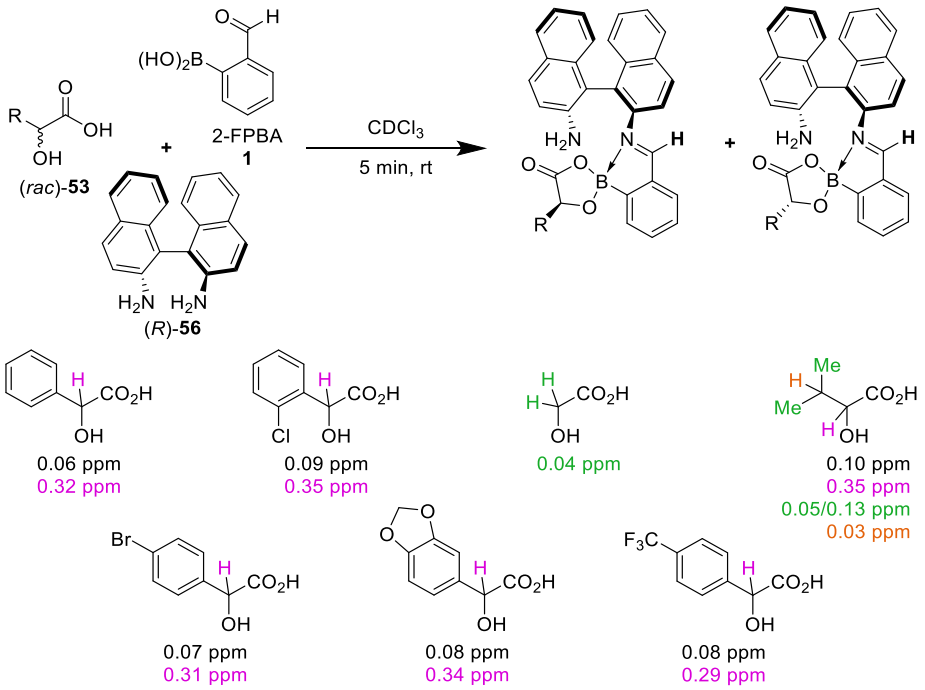
3.7. *Hydroxyacids and diacids*

The groups of Chaudhari and Suryaprakash have also expanded the scope of the Bull-James assembly CDA by demonstrating that it could be used to determine the enantiopurities of hydroxyacids **53**/**54** and 1,4-diacids **55**.[69–71] Treatment of (*rac*)-α-hydroxyacids (Scheme 28a) and (*rac*)-β-hydroxyacids (Scheme 28b) with 2-FPBA **1** and α-methylbenzylamine **6b** in MeOD–d_4 resulted in mixtures of diastereomeric iminoboronate esters which showed modest to excellent $\Delta\delta_H$ (0.04-0.65 ppm) values in their ^{1}H NMR spectra. As in previous reports, the role of analyte and reporter in these IBE complexes was found to be interchangeable, and so corresponding use of an enantiopure hydroxyacid could be used to determine the *ee* of scalemic amines.



Scheme 28: Three-component CDA for determining the enantiopurities of (a) α-hydroxyacids **53**; and (b) β-hydroxyacids **54** with ^{1}H NMR (400 MHZ, MeOD – d_4) $\Delta\delta_H$ of selected resonances.

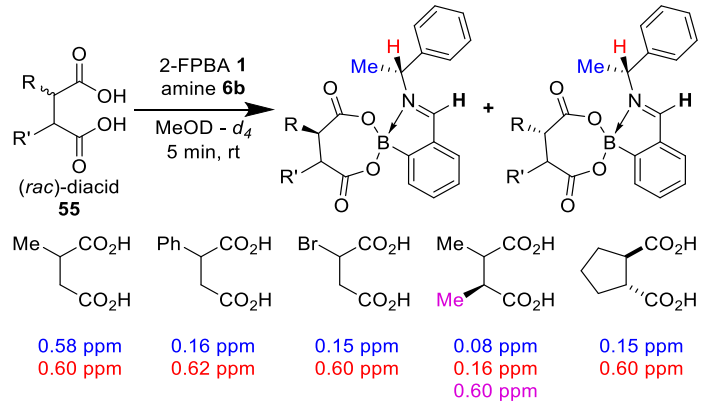
This methodology was optimised further to improve resolution and sensitivity, with the chiral amine reporter used for IBE complex formation changed from α-methylbenzylamine **6b** to axially chiral diamine BINAM **56**.[71] Three-component assembly of α-hydroxyacids **53**, 2-FPBA **1** and BINAM **56** produced diastereomeric IBEs which exhibited excellent chemical shift differences for pairs of diastereomeric resonances in their ^{1}H,^{13}C{^{1}H} and ^{11}B NMR spectra (Scheme 29). Interestingly, the excellent chiral discrimination produced in this self-assembled system resulted in chemical shift differences being observed in an IBE complex derived from achiral substrate glyconic acid, which exhibited a $\Delta\delta_H$ = 0.04 ppm value for the prochiral α-protons of its IBE complex.



Scheme 29: Three-component CDA for determining the enantiopurities of hydroxyacids **53** using 2-FPBA **1** and BINAM **56** with selected ^{1}H NMR (400 MHz, $CDCl_3$) $\Delta\delta_H$ of selected resonances.

Simple conformational models of the IBE complexes formed in these systems were developed, allowing the absolute configuration of hydroxyacids to be predicted using either BINAM **56** or α-methylbenzylamine **53** as a chiral reporter.[72,73] Following benchmarking, analysis of the relative signs of the $\Delta\delta_H$ values, broadness of signals and 2D nOe interactions enabled the absolute configuration of a range of hydroxyacids and primary amines to be assigned using BINAM **56** as a chiral reporter. In those cases where assignment was hampered by significant signal overlap in the ^{1}H NMR spectra, these resonances could be successfully deconvoluted using simple 2D RES-TOCSY ^{1}H NMR experiments.[74]

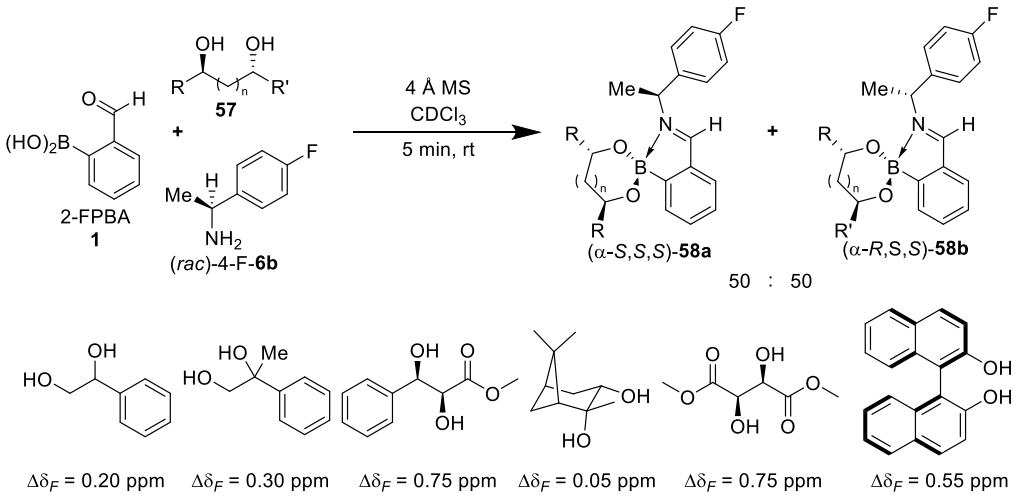
These three-component assembly protocols were also used to determine the *ee*'s of chiral 1,4-diacids **55** (Scheme 30), resulting in moderate to excellent chemical shift differences ($\Delta\delta_H$ = 0.08-0.62 ppm) in the ^{1}H NMR spectra of the diastereomeric IBEs of five diacid analytes.[70] Once again, the components of this assembly could be switched, enabling chiral diacids to be used to produce diastereomeric IBE complexes to determine the *ee's* of chiral primary amines. In some instances, the large chemical shift differences observed in these diacid/amine-derived IBE complexes led to full resolution of certain ^{13}C{^{1}H} NMR signals.



Scheme 30: Three-component CDA for determining the enantiopurity of 1,4-diacids **55** with ^{1}H NMR (400 MHz, MeOD – d_4) $\Delta\delta_H$ of selected resonances.

3.8. *^{19}F NMR spectroscopic analysis*

Fluorine was the first NMR-active heteronucleus to be studied for compatibility with the Bull-James assembly, due to the strength of its signal, its broad range of chemical shifts and the simplicity of ^{19}F NMR spectra, making it an excellent and widely-used NMR-active reporter. Bull and James first demonstrated incorporation of fluorine into their three-component assembly in 2009,[75,76] with initial work focusing on using a fluorinated chiral amine reporter in the three-component protocol (Scheme 31). A range of diols **57**, 4-fluoro-α-methylbenzylamine 4-F-**6b** and 2-FPBA **1** were derivatized to form ^{19}F NMR-active diastereomeric complexes (α-*S*,*S*,*S*)-**58a** and (α-*R*,*S*,*S*)-**58b**, which exhibited a $\Delta\delta_F$ range of 0.05-0.75 ppm. A similar approach was subsequently employed by Suryaprakash *et al*. for analysis of hydroxyacid and diacid protocols, with CF_3-appended chiral reporters and analytes affording diastereomeric complexes with non-equivalent ^{19}F NMR signals that could be integrated to determine their *dr*.[69,70]



Scheme 31: Three-component protocol using 2-FPBA **1**, 4-fluoro-α-methylbenzylamine 4-F-**6b** and chiral diols **57** to produce fluorinated diastereomeric complexes with good ^{19}F NMR (400 MHz, $CDCl_3$) $\Delta\delta_F$ values.

A significant improvement to this fluorous approach was achieved by incorporating the fluorine reporter atom into the achiral 2-FPBA template to produce a generally applicable method for determining the *ee* of different classes of chiral analytes. 4-fluoro-2-formylphenylboronic acid (4-F-2-FPBA, 4-F-**1**) was synthesised and used in the three-component assembly protocol, producing fluorinated diastereomeric complexes **60** which afforded baseline-resolved signals in their ^{19}F NMR spectra, allowing for *ee* determination of diols by both ^{19}F and ^{1}H NMR spectroscopic analysis (Figure 5). Similar results were reported by Suryaprakash *et al*. during their later work on applying this CDA to determine the enantiopurity of diacids.[70]

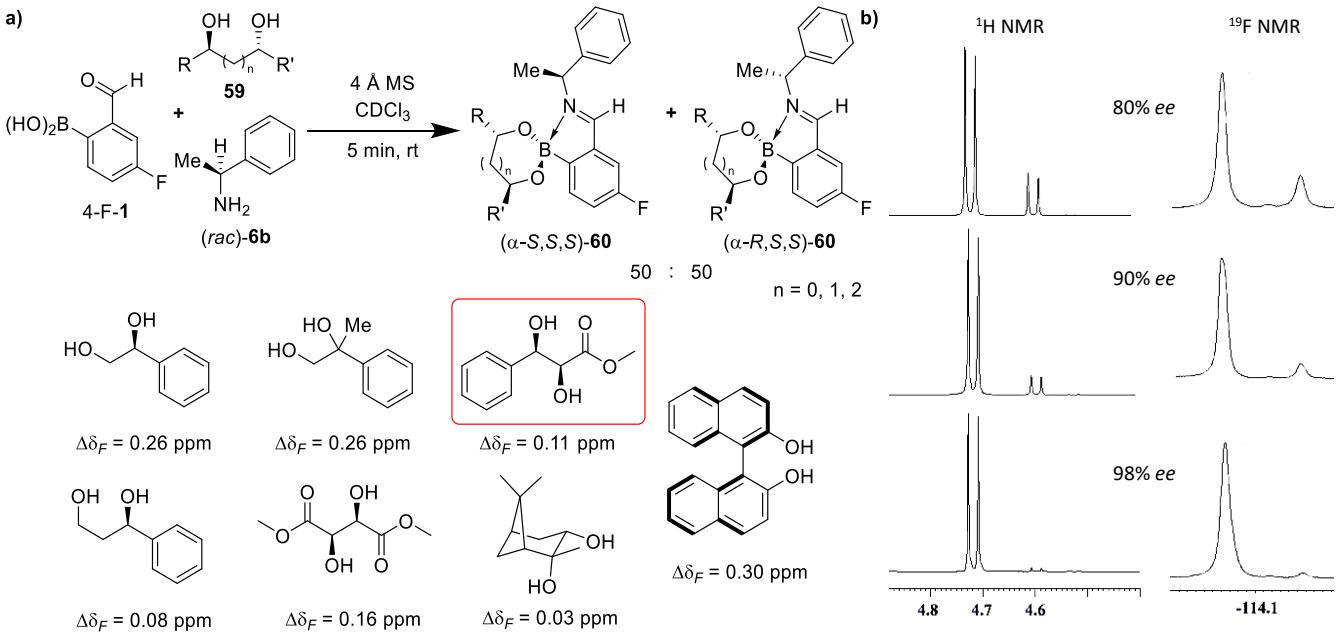
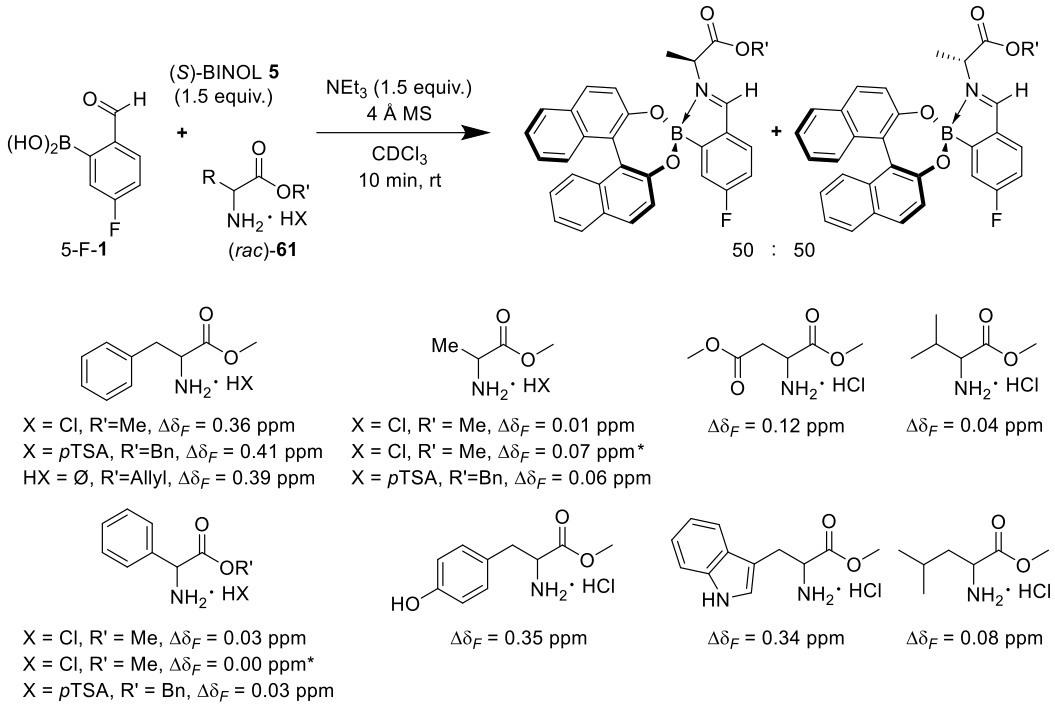


Figure 5: (a) Three-component protocol using 4-F-2-FPBA 4-F-**1**, (*rac*)-α-methylbenzylamine **6b** and chiral diols **59**. (b) Expansion of ^{1}H (500 MHz, $CDCl_3$)and ^{19}F (470 MHz, $CDCl_3$) NMR spectra of three-component assembly of 4-F-**1**, (*R*)-**6b** and a scalemic diol (red) at 80%, 90% and 98% *ee*. Adapted from ref. [75] with permission from the American Chemical Society.

Recently, Oe *et al*. have also reported the three-component assemblies of fluorinated 2-FPBA derivatives 3-F-**1**, 4-F-**1** and 5-F-**1** with (*S*)-BINOL **5** and α-methylbenzylamine **6b** with the aim of identifying diastereomeric IBEs with the greatest $\Delta\delta_F$ values (Scheme 32).[77] After establishing that 5-F-**1** was the best fluorinated template (93% conversion, $\Delta\delta_F = 0.10$ ppm for their model system), this system was optimised using excess BINOL and triethylamine (1.5 equiv. each) to minimize kinetic resolution and/or epimerisation of α-amino ester salts **61**.



Scheme 32: Modified Bull-James assembly of amino ester salts **61** with 5-F-**1** and (*S*)-BINOL **5** with ^{19}F NMR (376 MHz, $CDCl_3$) $\Delta\delta_F$ of selected resonances. * CD_2Cl_2 used as solvent.

Finally, a recent study on all four regioisomers of fluoro-2-FPBA as bifunctional templates for analysis of the *ee's* of sulfinamides revealed that 3-fluoro-2-FPBA 3-F-**1** was the optimal template (Figure 6),[57] producing an impressive chemical shift difference of $\Delta\delta_F$ = -2.328 ppm between the IBE diastereomers produced from Ellman's sulfinamide (Figure 6b). A stepwise approach was used to derivatise a small range of sulfinamides **34**, 3-F-**1** and (1*R*,2*R*,3*S*,5*R*)-pinanediol **36** which gave large chemical shift differences and full baseline resolution of the imine and fluorine peaks of their diastereomeric IBE complexes.

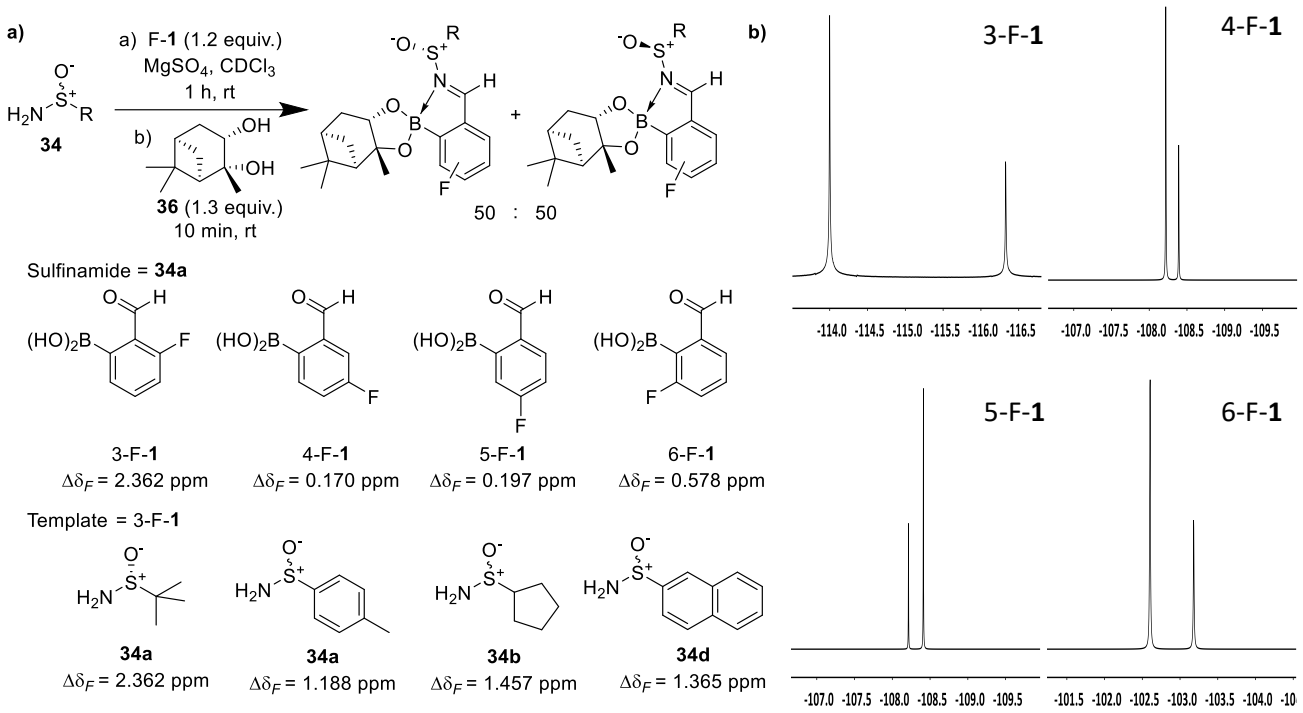
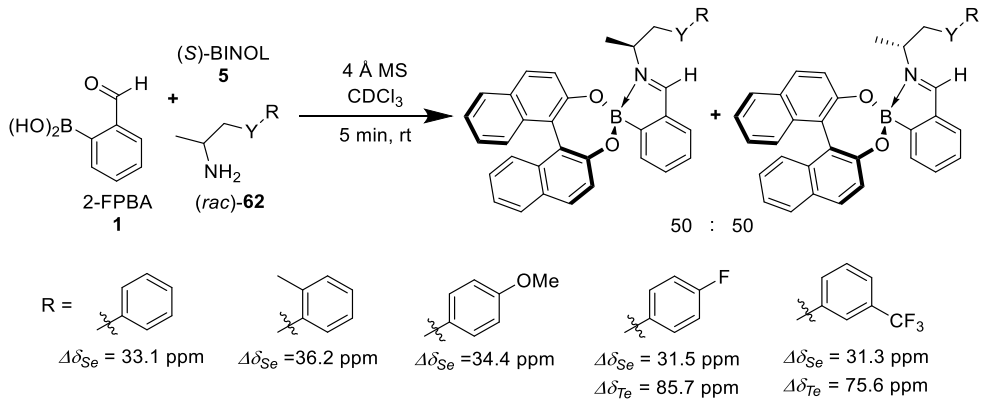


Figure 6: (a) Stepwise three-component assembly of fluorinated 2-FPBA templates, (1*R*,2*R*,3*S*,5*R*)-pinanediol **36** and sulfinamides **34**. (b) Chemical shift differences in the ^{19}F NMR (470 MHz, $CDCl_3$) spectra of IBEs of the three-component assembly of Ellman's sulfinamide (*R*)-**34a** (33%), (1*R*,2*R*,3*S*,5*R*)-pinanediol **36** and four fluorinated 2-FPBA isomers (same scale).

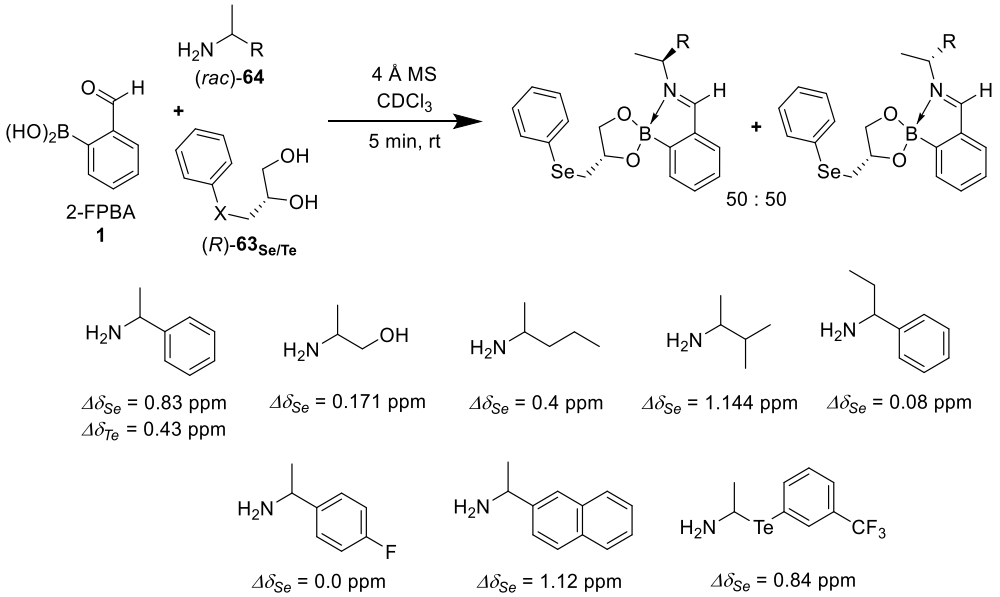
3.9. *Chalcogen NMR spectroscopic analysis*

Silva *et al*. have shown that incorporation of NMR-active chalcogens ^{77}Se and ^{125}Te into the analyte or chiral reporting unit can also be used to determine *ee* using three-component assembly protocols.[78,79] Their initial report focused on derivatising racemic chalcogen-containing amines **62** (Scheme 33) with 2-FPBA **1** and (*S*)-BINOL **5** to afford pairs of iminoboronate complexes. ^{77}Se{^{1}H} and ^{125}Te{^{1}H} NMR spectroscopy of these complexes showed excellent chemical shift anisochrony for the diastereomeric IBE complexes formed, with $\Delta\delta_{Se}$ values ranging from 26.2 – 34.4 ppm and $\Delta\delta_{Te}$ values ranging from 75.6 – 85.7 ppm. Although only racemic samples were employed in this work, the magnitude of chemical shift differences observed indicates that these systems would be useful for determining the *ee* of diol analytes.



Scheme 33: Three-component assembly of 2-FPBA **1**, (*S*)-BINOL **5** and chalcogen containing amines **62**, and the $\Delta\delta_{Se}$ (99 MHz, $CDCl_3$) and $\Delta\delta_{Te}$ (132 MHZ, $CDCl_3$) values of their diastereomeric IBE complexes.

Subsequently, Silva *et al*. synthesised selenium-containing 3-phenylchalcogen-1,2-propanediol **63** for use as a chiral reporter with 2-FPBA **1** and chiral amines **64** which gave pairs of diastereomeric IBEs, the majority of which exhibited baseline-resolved diastereomeric signals in their NMR spectra with chemical shift differences for $\Delta\delta_{Se}$ and $\Delta\delta_{Te}$ of 0-1.144 ppm and 0.43 ppm, respectively (Scheme 34).[78] Interestingly, the chemical shift differences observed in this instance were 100-fold smaller than for their previous examples, implying that the chalcogen atoms occupy positions in space that are relatively remote from the amine stereocenters and so only experience small anisotropic shielding effects. Nevertheless, integration of diastereomeric ^{77}Se NMR signals could be used to produce accurate measurements of the *ee*'s of scalemic samples of known enantiopurites (±4%).



Scheme 34: Three-component assembly of 2-FPBA **1**, chalcogen containing diols (*R*)-**63$_{Se/Te}$** and racemic amines **64** with $\Delta\delta_{Se}$ (99 MHz, $CDCl_3$) and $\Delta\delta_{Te}$ (132 MHz, $CDCl_3$).

4. Three-component assembly for determining *ee* by optical methods

The Bull-James assembly has also been applied to the optical sensing of *ee* using methods that rely on CD, UV-Vis, or fluorescence spectroscopic analysis, with the aim of developing methods potentially applicable for high-throughput analysis.[80,81] All of these approaches rely on exploiting differences in the spectroscopic response of diastereomeric IBE complexes, whose *dr*'s correspond to the *ee* of the parent chiral analyte used for the IBE complexation.

4.1. *Determining the* ee *of amines and diols using circular dichroism*

A collaboration between the Anslyn, Bull and James groups in 2012 reported the use of circular dichroism spectroscopy to analyse diastereomeric IBE complexes formed from the three-component self-assembly of chiral amines **66**, chiral BINOL derivatives **67**/**68**, and 2-FPBA **1** (Figure 7a).[82] As with many multicomponent host-guest assemblies, a strong CD signal was observed between 250 and 270 nm (Figure 7b), with a maximum difference in signal response between diastereomeric complexes produced from the enantiomers of α-methylbenzylamine **6b** observed at 253 nm (98,941 deg.cm^2/dmol). This enabled BINOL and two brominated derivatives to be employed as chiral reporters in an array of sensing ensembles, whose CD signals were processed using Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) to produce chemometric statistical models that were capable of differentiating between different α-chiral amine analytes and determining their *ee*'s with an average

error of ± 5.8% (Figure 7c, d). The use of PCA and LDA is widespread in the field of differential sensing as multivariate statistical tools which recognise and amplify patterns from large datasets.[83]

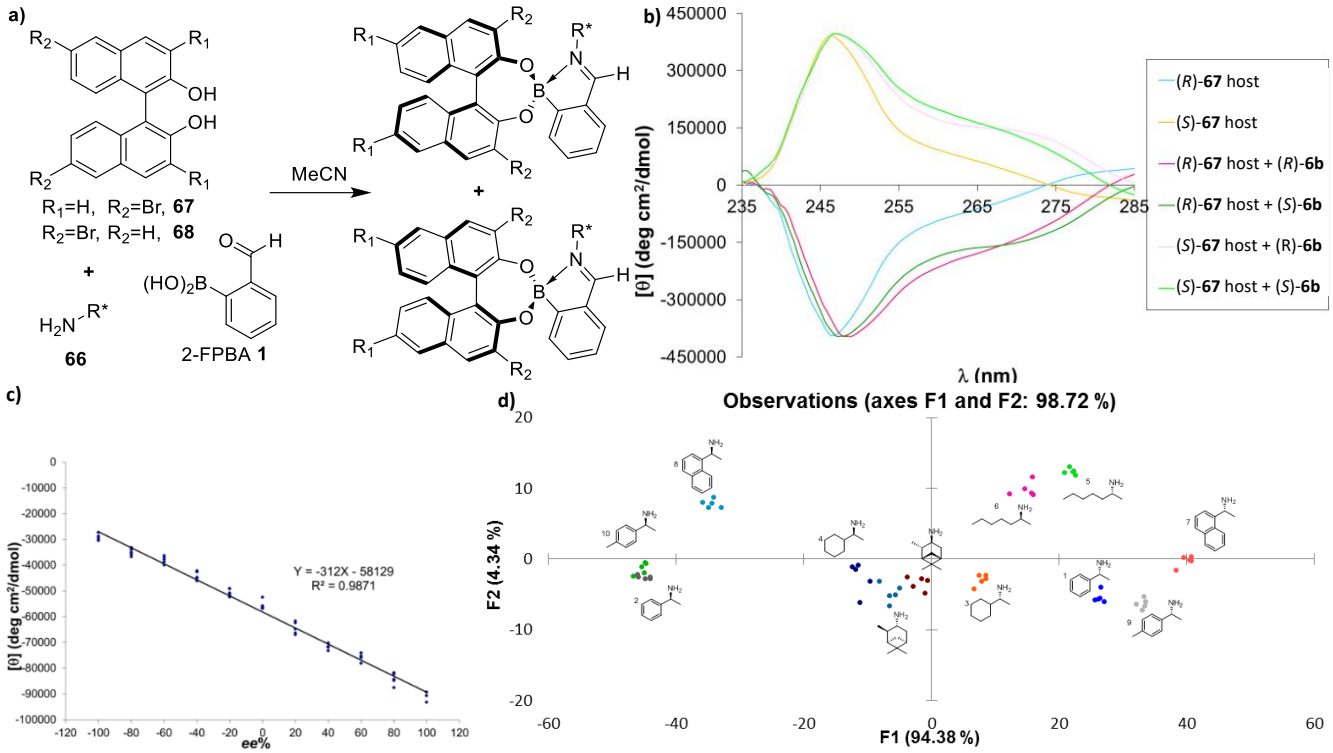


Figure 7: (a) Three-component assembly of 2-FPBA **1**, BINOL-derivatives and a chiral amine. (b) CD spectra of diastereomeric IBE complexes obtained from 2-FPBA **1**, 6,6-dibromoBINOL **68** and α-methylbenzylamine **6b**. (c) Calibration curve for CD outputs of complexes produced from mixing (*R*)-BINOL **5**, 2-FPBA **1** and scalemic **6b** of known *ee*. (d) LDA plot of chiral amine analytes. b, c, d Adapted from ref. [82] with permission from the Royal Society of Chemistry.

Subsequent to this report, Wolf *et al*. described a self-assembling system based on host complexes derived from 4-methoxy-2-FPBA (4-OMe-**1**) and non-chiral 2,2'-binaphthol **69** (Figure 8a).[84] Two-component assembly of chiral amines (1-cyclohexylethylamine **70** and 1-aminoindane **71**) with 4-OMe-**1** gave iminoboronic acid complexes with only weak CD signals (dashed lines). However, addition of achiral BINOL-derivative **69** resulted in a large increase in the Cotton signals of the resultant IBEs, consistent with the self-assembly process controlling the helicity of its BINOL fragment (solid lines, Figure 8b). Although this system was not used for *ee* determination, the amplitude of signal change indicates this type of assembly is likely to be suitable for this purpose.

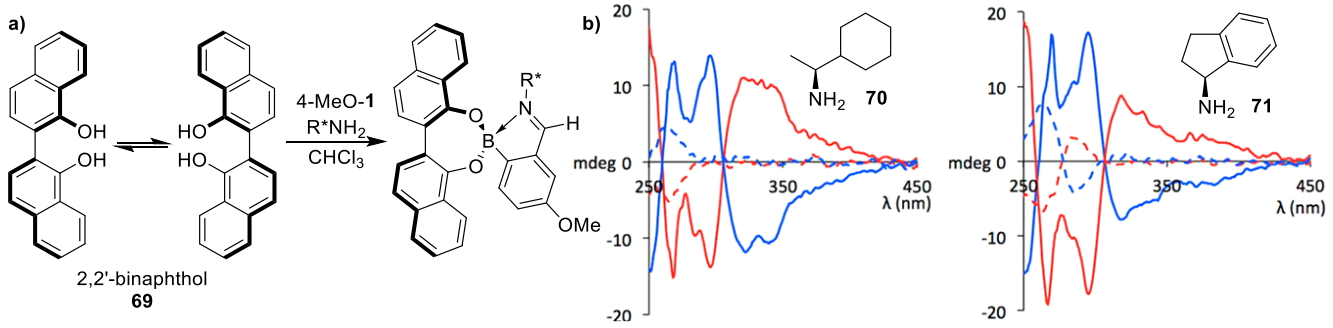


Figure 8: (a) Three-component assembly of 2,2'-binaphthol **69**, 4-OMe-**1** and a chiral amine to afford complexes for CD spectroscopic analysis. (b) CDA spectra produced from complexes derived from amines **70** (left) or **71** (right). Blue and red lines correspond to complexes produced from the (*R*)- or (*S*)- enantiomers of the amines, respectively. Dashed lines correspond to two-component complexes formed from 4-MeO-**1** and the enantiomers of the amines **70** and **71**. C = 37.5 μM. Adapted from ref. [81] with permission from the American Chemical Society.

4.2. *Determining the* ee *of amines, amino-alcohols and diols using fluorescence*

Collaborations with Anzenbacher have led to the development of multiple Bull-James assembly-derived fluorescence assays,[85–88] with the practicality and versatility of this methodology leading to a *Nature Protocols,* validating its use as an effective method for the high-throughput analysis of the *ee* of chiral diols, amino alcohols and amines produced in stereoselective reactions.[89] Initial reports focused on the development of "turn-off" fluorescence-based assemblies using fluorescent host systems comprised of 2-FPBA **1** and 3,3'-diphenyl-2,2'-bi-1-naphthol (VANOL) or 2,2'-diphenyl-(4-biphenanthrol) (VAPOL) as chiral reporter diols for determining the *ee*'s of scalemic amines (Figure 9a).[85–87] Interestingly, these extended aryl systems exhibited the same NMR chiral shift behaviour as seen in previous BINOL-based systems, with several sets of baseline-resolved signals observed for each pair of diastereomeric complexes in their ^{1}H NMR spectra. This host system (2-FPBA + chiral fluorescent diol) was found to be suitable for *ee* determination of both amines and amino alcohols. In the case of amines (and amino acids/esters), IBE formation resulted in PeT quenching, leading to a "turn-off" fluorescence response (Figure 9b). As shown in Figure 9c, fluorescence intensity (FI) was dependent on the chirality of the amine analyte, which enabled *ee* values of amine samples to be correlated to changes in fluorescence intensity with good levels of accuracy (± 1-2%) (Figure 9d).

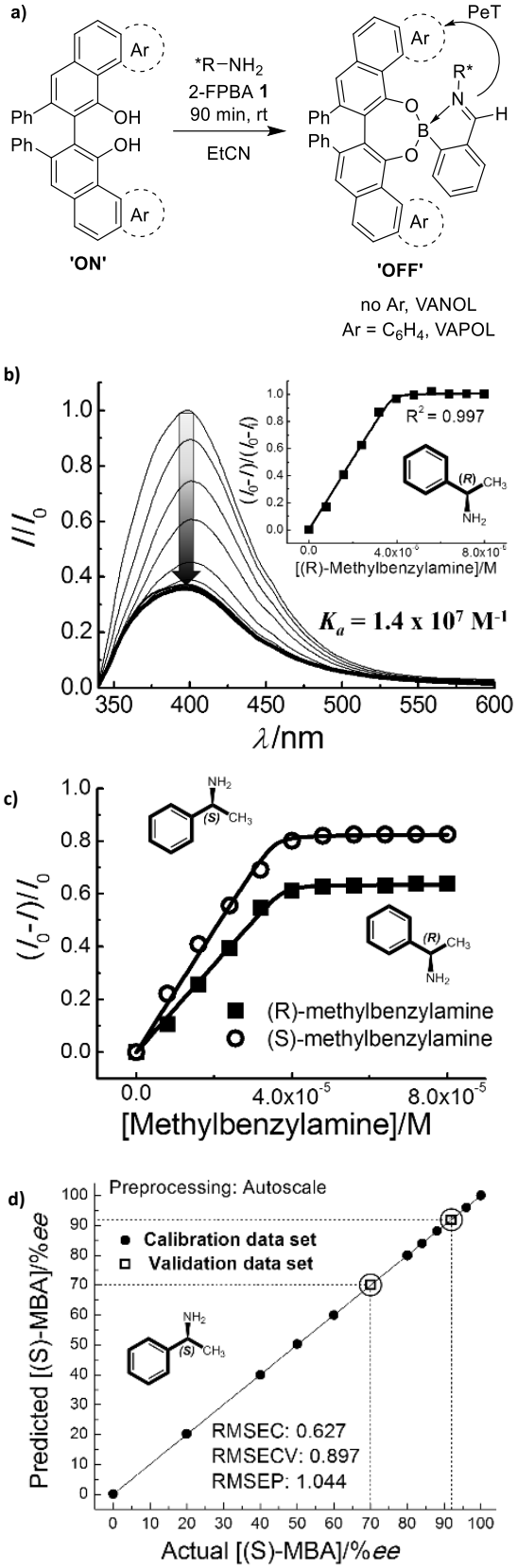


Figure 9: (a) Three-component assembly of 2-FPBA **1**, a chiral primary amine and a fluorescent diol. (b) Fluorescence (λ_{ex}=335 nm) of a mixture of (*S*)-VANOL (40 μM) and 2-FPBA **1** (40 μM) in dry EtCN decreases on addition of (*R*)-α-methylbenzylamine **6b** (0-80 μM). (c) Binding isotherms of (*S*)- and (*R*)-α-methylbenzylamine **6b** to (*S*)-VANOL-2-FPBA host. (d) Qualitative LDA of amine, amino alcohol and amino acid enantiomers in EtCN. b, c, d reproduced from ref. [85] with permission from John Wiley and Sons.

This type of fluorescence-based three-component self-assembly platform was also applied to the analysis of the *ee*'s of amino alcohols, with formation of oxazolidine intermediates resulting in a red-shift of the fluorescence signal rather than PeT quenching (Figure 10a). Differential changes in fluorescence intensities were once again observed between the diastereomeric oxazolidine products produced (*vide supra*), thus allowing for the measurement of the enantiopurity of the parent amino-alcohol analyte. This enabled ratiometric changes in fluorescence to be used to determine the *ee*'s of amino alcohols, as well as providing the ability to distinguish between amino-alcohol and amine analytes. This is seen clearly in Figure 10b, with LDA revealing large distances between clusters of enantiomers and functional groups of the parent analytes. Interestingly, these studies found that addition of polar/protic additives (water, citric acid, ethylene glycol, sucrose, glycerol) had a more pronounced effect on the equilibrium constants for formation of the heterochiral complexes over the homochiral complexes, thus indicating that the heterochiral complexes were less stable. This led to the discovery that these types of additives could be used to further discriminate between analyte enantiomers in these complexation reactions.

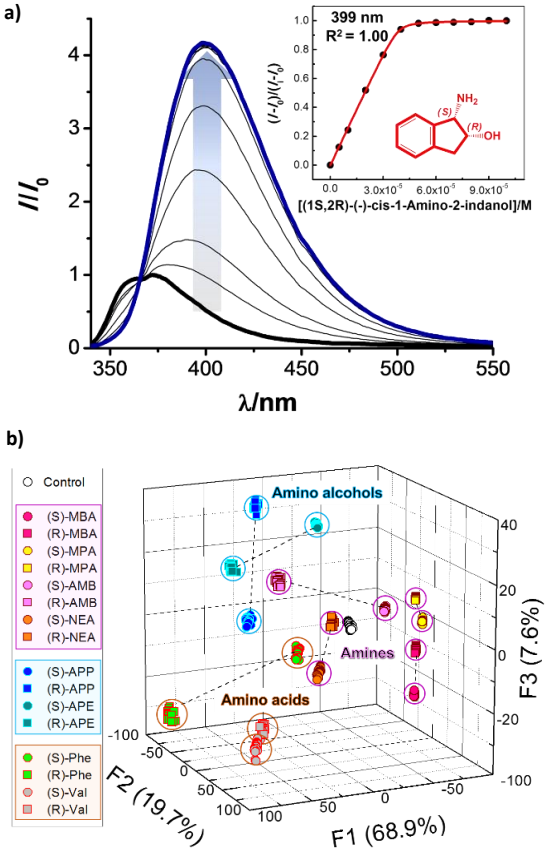


Figure 10: (a) Fluorescence spectra of the three-component assembly of 2-FPBA **1**, (*S*)-VANOL and [(1*S*,2*R*)-(-)-*cis*-1-amino-2-indanol (0-100 μM). (b) Qualitative LDA of chiral amine, amino-alcohol and amino acid analytes. Reproduced from refs. [85,86] with permission from John Wiley and Sons.

Use of enantiopure L-tryptophan derivatives as fluorescent reporters for three-component complexation meant that these types of fluorescence assays could be adapted to determine the *ee*'s of scalemic diols (Figure 11).[87] to within a 2% error limit. As for amines and amino-alcohols, the fluorescence profiles of the diastereomeric homochiral and heterochiral complexes produced from various classes of diols were sufficiently different to enable LDA to be used to accurately determine both their structures and *ee* values (Figure 11).

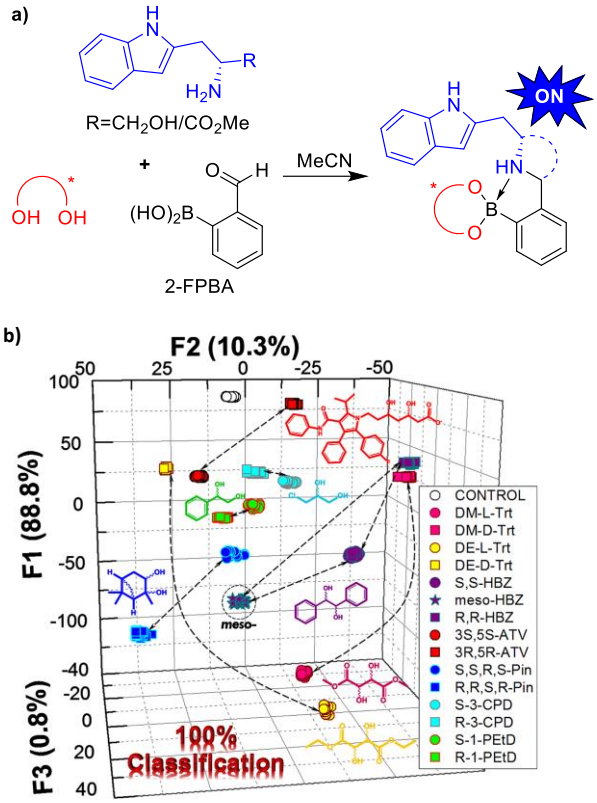


Figure 11: (a) Three-component assembly of 2-FPBA **1**, a chiral diol and a fluorescent tryptophanol derivative. (b) Qualitative LDA of 16 chiral diols showing 100% correct structural classification. Reproduced from ref. [87] with permission from John Wiley and Sons.

The practicality of this fluorescence methodology for high-throughput screening was demonstrated by measuring the enantiopurities of 14 samples of Atorvastatin (a hypercholesterolemia drug) of unknown *ee*'s using a high-throughput assay (Figure 12a), with quantitative linear regression analysis revealing accurate enantiopurity determination in all cases (R^2=0.999). This type of fluorescence assay was also employed to analyse the *ee* of diols produced in Noyori asymmetric transfer hydrogenation reactions of benzil to hydrobenzoin (diol). In this case, an artificial neural network was developed that was used to correctly determine the absolute configuration, *ee* and concentration of hydrobenzoin products (both crude and recrystallized) with high levels of accuracy (Figure 12b, c).

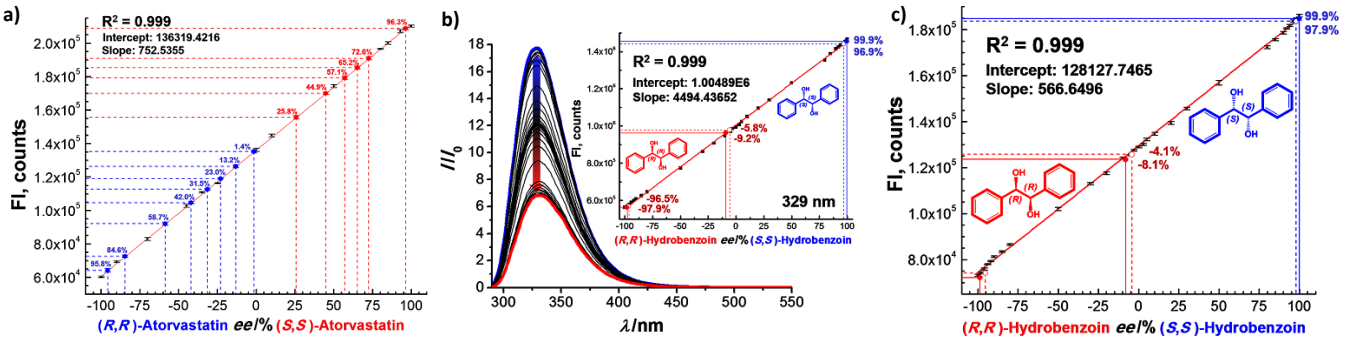
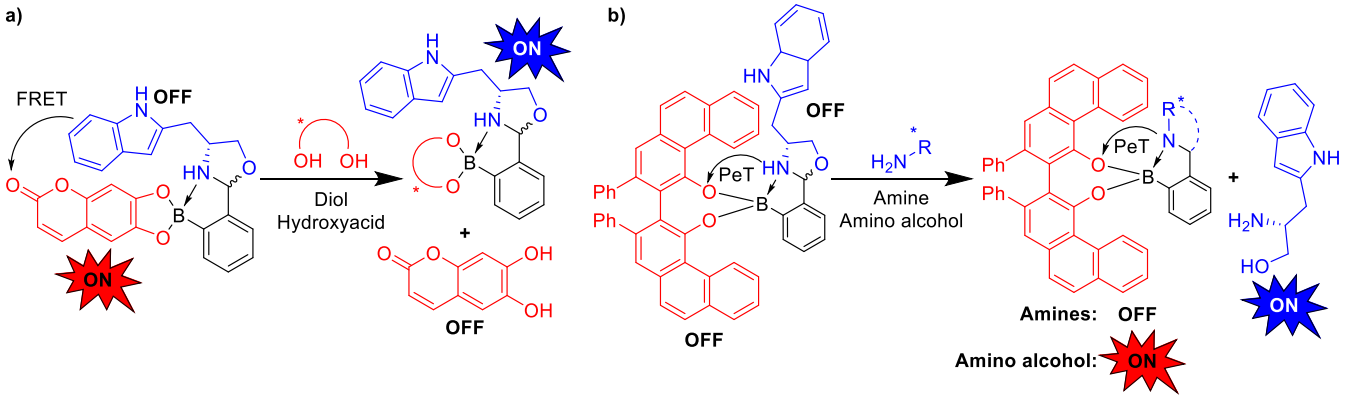


Figure 12: (a) Standard graph of FI *vs. ee* of L-tryptophanol and 2-FPBA **1** assemblies (1:1, 40 mm) of atorvastatin of known (black) and unknown (blue and red) *ee* values. (b) Fluorescence titration profile of L-tryptophanol–2-FPBA (1:1, 40 mm) complexes with hydrobenzoin standards (inset: Standard curve of FI *vs. ee*). (c) HT fluorescence assay standard curves for FI readings from mixtures of hydrobenzoin of known *ee* in comparison with six hydrobenzoin samples of unknown *ee* (red, blue circles). Reproduced from ref. [87] with permission from John Wiley and Sons.

Most recently, Anzenbacher *et al*. have reported a dual chromophore indicator displacement assay which proved to be more sensitive for determining *ee* than their previously developed "turn-off" systems.[88] This approach employed a combination of two fluorescent dyes capable of orthogonal binding to the aldehyde and boronic acid fragments of the 2-FPBA template (Scheme 35). Initial assembly of L-tryptophanol and 6,7-dihydroxycoumarin produced a bichromophoric oxazolidine-boronate complex, with intramolecular fluorescence resonance energy transfer (FRET) processes leading to weak fluorescence of its tryptophanol moiety and enhanced fluorescence of its coumarin fragment. Addition of a scalemic diol (or hydroxyacid) analyte results in displacement of the coumarin dye and separation of the FRET pair, which leads to fluorescence "turn on" of the tryptophanol fluorophore, and "turn off" of the dihydroxycoumarin (Scheme 35a). Since assembly of each enantiomer of the parent analyte proceeds diastereoselectively, each enantiomer leads to a different fluorescence response which can be used to determine the *ee's* of a scalemic analyte.

Alternatively, use of (*S*)-VAPOL as a chiral reporter produced an IBE system suitable for determining the enantiopurity of amines and amino alcohols (Scheme 35b). In this case, the fluorescence of both fragments of the enantiopure oxazolidine sensor is likely to be quenched through PeT donation of the nitrogen lone-pair of the oxazolidine fragment to the VAPOL fragment, although the exact mechanism of fluorescence and quenching was not determined. Addition of a scalemic amine analyte results in displacement of the L-tryptophanol unit producing an IBE complex that results in a fluorescence "turn-on" response, with the fluorescence of the VAPOL remaining "turned off". Use of an amino-alcohol analyte to afford an imidazoline-boronate ester complex also results in displacement and "turn-on" of tryptophanol, however the ensuing PeT process leads to amplification of the (*S*)-VAPOL fluorescence signal which is also "turned-on". Since addition of the enantiomers of amine, amino ester, diol and hydroxyacid analytes to these chiral indicator displacement sensors result in different fluorescence responses, this bichromophoric Bull-James sensing system could be used to successfully classify the structures of 26 different analytes and accurately determine their absolute configurations and enantiopurities (Figure 13).



Scheme 35: Displacement assays using bichromophoric three-component assemblies for determining the enantiopurities of a range of scalemic analytes: (a) Use of 2-FPBA, L-tryptophanol and 6,7-dihydroxycoumarin for the detection and *ee* analysis of diols and hydroxyacids. (b) Use of 2-FPBA, L-tryptophanol and (*S*)-VAPOL for the detection and *ee* analysis of amines and amino alcohols.

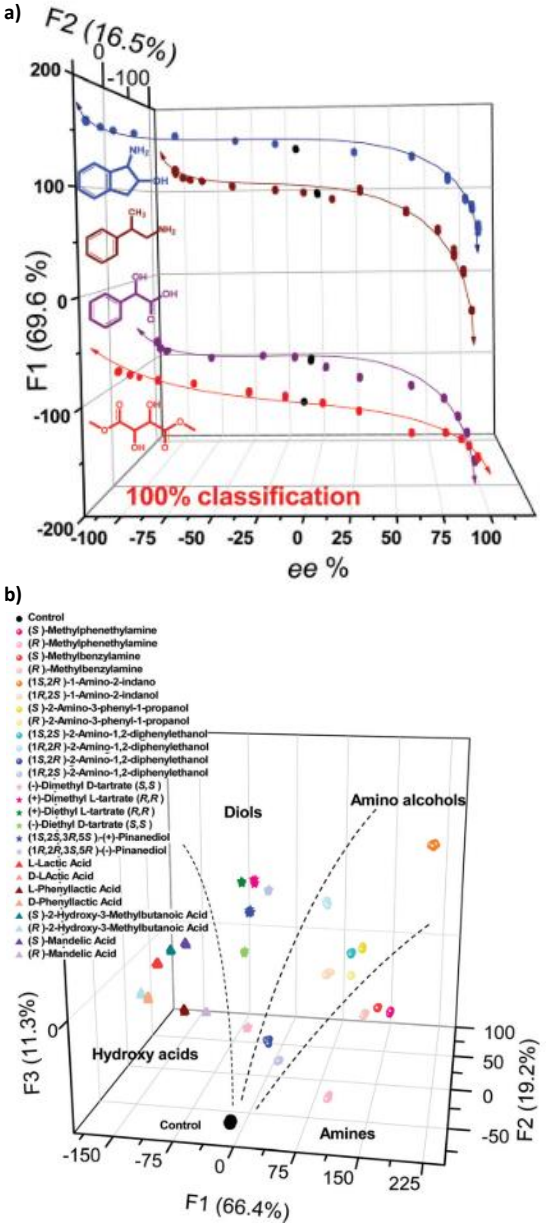


Figure 13: (a) Semi-quantitative LDA of fluorescence response data from displacement assays enable simultaneous determination of the *ee* values of four different types of amine, amino alcohol, α-hydroxy acid and diol analytes. (b) Qualitative LDA of the fluorescence response of 26 chiral amines, amino alcohols, diols and hydroxyacids (+ controls) in the displacement assay enabled their structures to be predicted with a 100% success rate. Reproduced from ref. [88] with permission from the Royal Society of Chemistry.

5. Three-component assembly for electrochemical determination of the *ee* of BINOL

Finally, a collaboration with the Tucker group demonstrated that the *ee* of BINOL could be measured electrochemically through derivatisation with a redox-active two-component iminoboronic acid complex derived from a ferrocene amine and 2-FPBA **1** (Figure 14a).[90] It was found that the resultant diastereomeric complexes (α-*R*,*R*)-**72a** and (α-*R*,*S*)-**72b** exhibited significantly different electropotentials of 614 mV and 665 mV, respectively (Figure 14b). This difference allowed the *ee* of BINOL **5** to be determined with an error of ± 3%, thus enabling minor enantiomers (<5%) to be detected, even at low concentrations. Crystallographic and ^{1}H and ^{11}B NMR spectroscopic analysis showed that whilst the homochiral diastereomeric complex (α-*R*,*R*)-**72a** formed an

intramolecular iminoboronate N→B bond, the more sterically hindered heterochiral complex (α-*R*,*S*)-**72b** did not, once again indicating that heterochiral IBE complexes are generally less stable (*vide supra*).[86] This structural difference is responsible for the differences in their electrochemical behaviour, with the N→B bond of the homochiral complex resulting in (*R*)-BINOL **5** being more tightly bound, with a ratio of binding strengths $K_{(\alpha\text{-}R,R)}/K_{(\alpha\text{-}R,S)}$ of ≈ 19. Electrochemical oxidation of these IBEs results in the binding strength ratio $K_{(\alpha\text{-}R,R)}{}^{+}/K_{(\alpha\text{-}S,S)}{}^{+}$ dropping to only 2.5, thus indicating a much larger decrease in stability of the homochiral complex (α-*R*,*R*)-**72a**. This difference is proposed to be due to weakening of the N→B coordination bond of complex (α-*R*,*R*)-**72a** caused by the proximal positive charge of its oxidised ferrocene fragment. Evidence for weakening of the N→B coordination bond of the homochiral (α-*R*,*R*)-**72a** complex was also provided by the larger positive shift in redox potential upon addition of (*R*)- or (*S*)-BINOL **5** to iminoboronic acid (*R*)-**73** (+ 95 mV for (α-*R*,*R*)-**72a** *vs.* +44 mV for (α-*R*,*S*)-**72b**)). This indicates that the ferrocene unit of complex (α-*R*,*R*)-**72a** is harder to oxidise than (α-*R*,*S*)-**72b**, in line with its imine-boron coordination bond withdrawing electron density from the ferrocene redox system.

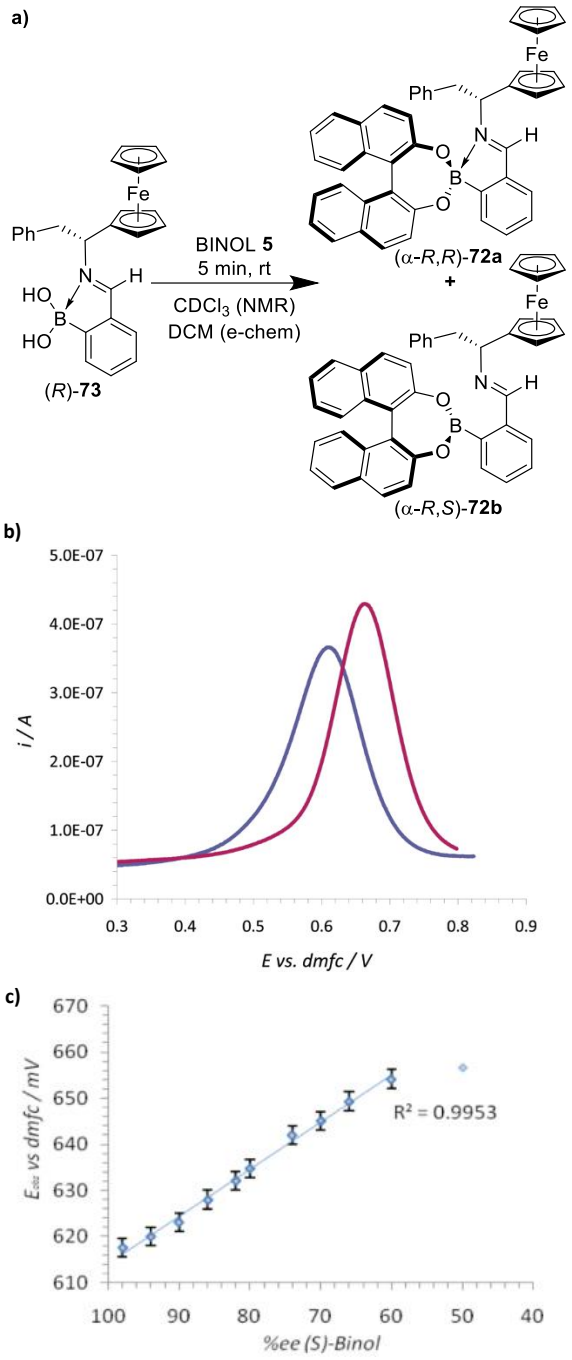


Figure 14: (a) Three-component assembly of 2-FPBA **1**, redox-active ferrocene amine (*R*)-**73** (pre-assembled) and BINOL **5**. (b) Square wave voltamograms of three-component ferrocene IBEs acquired in CH_2Cl_2 (0.1 M TBA · PF_6); ((α-*R*,*S*)-**72b** shown in blue) and (α-*R*,*R*)-**72a** shown in purple). (c) Plot of E_{obs} against % *ee* for IBE complexes produced from (*S*)-BINOL **5** showing a linear dependence between 60% and 98% *ee*. b, c Reproduced from ref. [90] with permission from the American Chemical Society.

6. IBE assemblies as synthetic tools

The use of the Bull-James three-component assembly for determining enantiopurity is often credited as one of the first examples where orthogonal dynamic covalent bond formation was used to construct functional supramolecular assemblies.[14,91–93] The power of these chiral iminoboronate systems for self-assembly has led to supramolecular constructs of this type being used to prepare new types of boron-containing materials and as a mechanism to control reactivity and stereoselectivity.[94–97]

6.1. *Self-assembled synthesis of polyheteroatomic boracycles*

The three-component assembly reaction of 2-FPBA **1** with (*S*)-BINOL **5** and (*S*)-leucinol **74** resulted in a mixture of imine and oxazolidine boronate products (*vide supra*),[54] however oxazolidine boronate ester (*S*, 2*R*, 4*S*)-**75** fractionally crystallized out of solution after the crude reaction mixture was allowed to stand overnight (Figure 15a).[98] Carrying out a two-component assembly using (*R*)-valinol **74b** and 2-FPBA **1** produced bridged iminoboronate (*R*,*R*)-**76b**, comprised of two fused boracycle rings containing two tetrahedral boron centres and a bridging oxygen atom linker (Figure 15b), in the same manner as related systems reported by Westcott *et al*.[99,100] Five additional chiral amino alcohols **74a-f** were used as substrates in this two-component self-assembly reaction in combination with either 2-FPBA **1** or 2-formyl furanylboronic acid **77**, which gave their respective boracycles in excellent 84-96% isolated yields. Achiral aromatic amino alcohols **74g** and **74h** were also shown to form boracycles in quantitative yields, although their decreased reactivity required heating under Dean-Stark conditions for complexation reactions to proceed to completion.

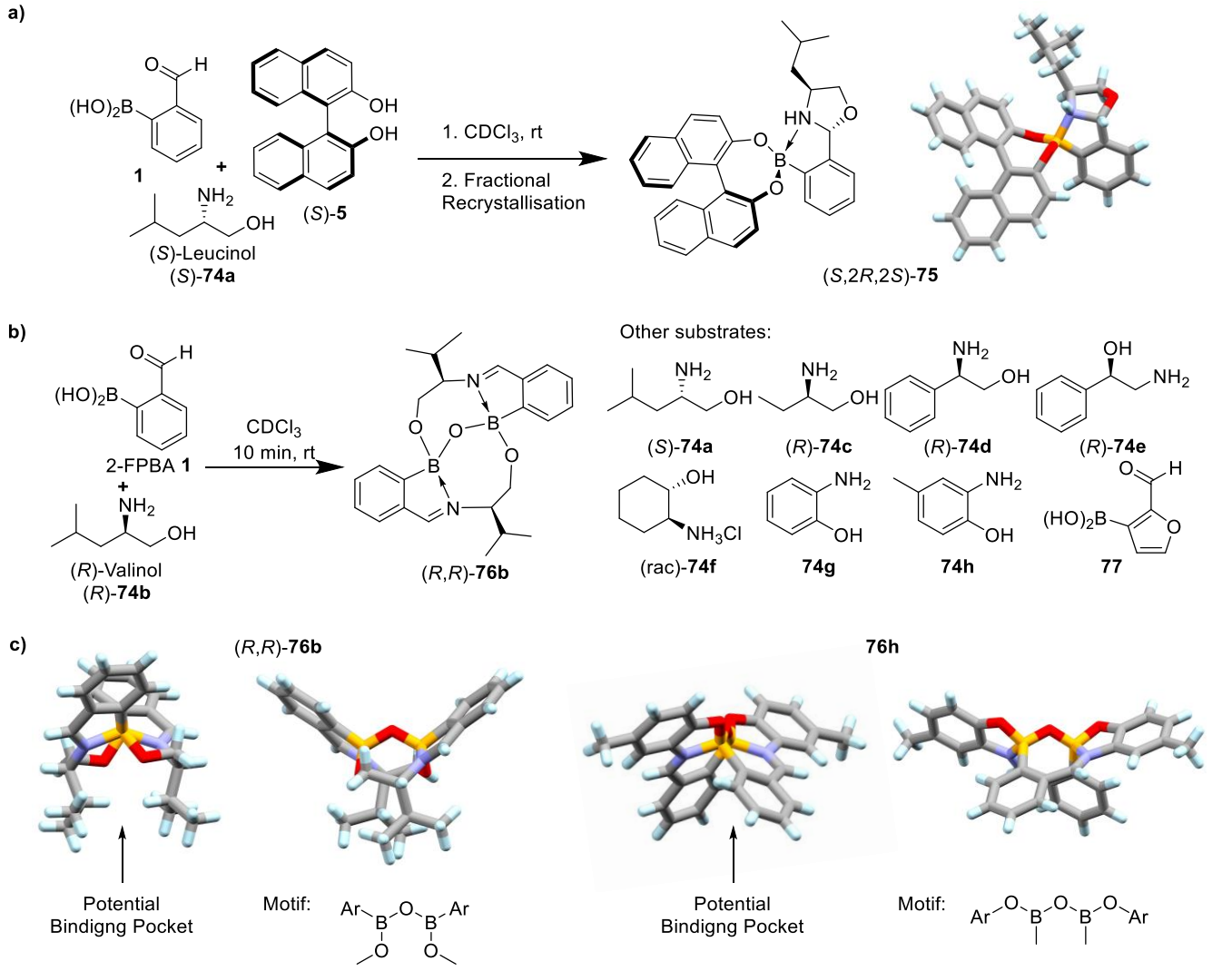


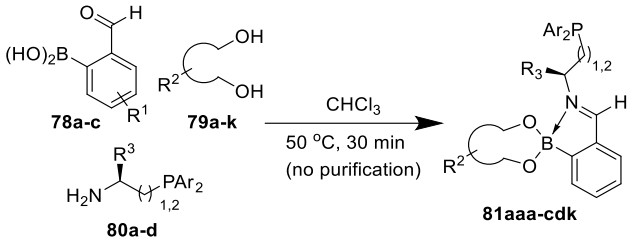
Figure 15: (a) X-ray crystal structure of three-component assembly of (*S*,2*R*,2*S*)-**75** formed from reaction of (*S*)-leucinol **74a**, BINOL **5** and 2-FPBA **1**. (b) Two-component assembly of formyl aryl boronic acids and 1,2-amino alcohols **74**. (c) X-ray crystal structures of (*R*,*R*)-**76b** and **76h** viewed along and perpendicular to the boron-boron axis (left and right respectively).

Both types of fused bridged bicycles were characterised using X-Ray crystallography (Figure 15c), which revealed interesting structural variation between the two-component products produced from chiral or achiral amino alcohols. In the case of (*R*,*R*)-**76b**, the B-O-B linkage is positioned on the opposite face to the two non-bridging oxo-substituents, which creates a binding pocket walled by the non-bridging oxygens and side-chains that is

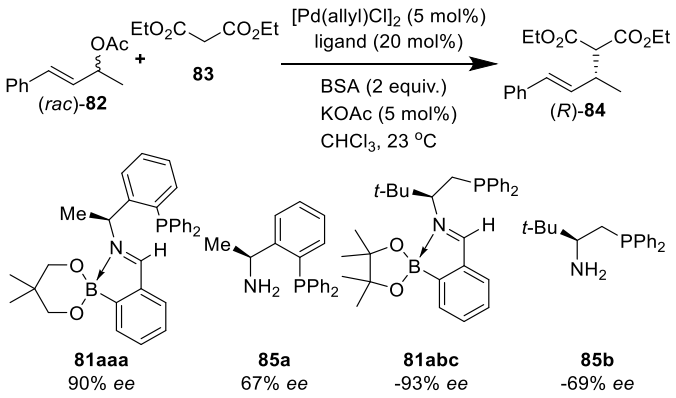
capped by a bridging B-O-B bond. Alternatively, all of the atoms of the O-B-O-B-O motif are present in the same plane for complex **76h**, with all three oxygen atoms sitting on the same side of the complex. These structural differences result in the pocket of the chiral complexes containing two potentially coordinating oxygen atoms, whilst the pocket of the achiral complexes are purely hydrophobic in nature.

6.2. *Chiral IBE ligands for asymmetric catalysis*

Three-component assemblies have also been used by the Taylor group, who employed IBE bond forming reactions for combinatorial synthesis of a library of chiral phosphine ligands for enantioselective palladium-catalysed allylic acetate substitution reactions.[101] They permed three achiral formyl boronic acid templates **78a-c**, eleven diol ligands **79a-k** (both chiral and achiral), and four chiral aminophosphines **80a-d** to create a library of 100 phosphinoiminoboronate ligands **81** (Scheme 36) that were individually screened as chiral ligands in palladium-catalysed allylic substitution reactions of (*rac*)-**82** with diethyl malonate **83** (Scheme 37). A wide range of enantioselectivities were observed, with the best results obtained for ligands **81aaa** and **81abc** which respectively produced (*R*)-**84** in 90% *ee* and (*S*)-**84** in 93% *ee*, which was a significant improvement on the 67% and 69% *ee* values obtained using non-iminoboronate aminophosphine ligands **85a** and **85b**. The sheer volume of data acquired using this combinatorial approach enabled Taylor and co-workers to rapidly assign trends that would not have been evident from a conventional stepwise ligand optimisation strategy. For instance, they were able to show that aliphatic diol ligands gave better stereocontrol as they decreased the Lewis acidity of the boron centre, which weakened the intramolecular N→B bond, thus facilitating stronger bidentate *P*,*N*-coordination of the ligand to the metal.



Scheme 36: Combinatorial IBE reactions used for the combinatorial synthesis of 100 chiral phosphine ligands.

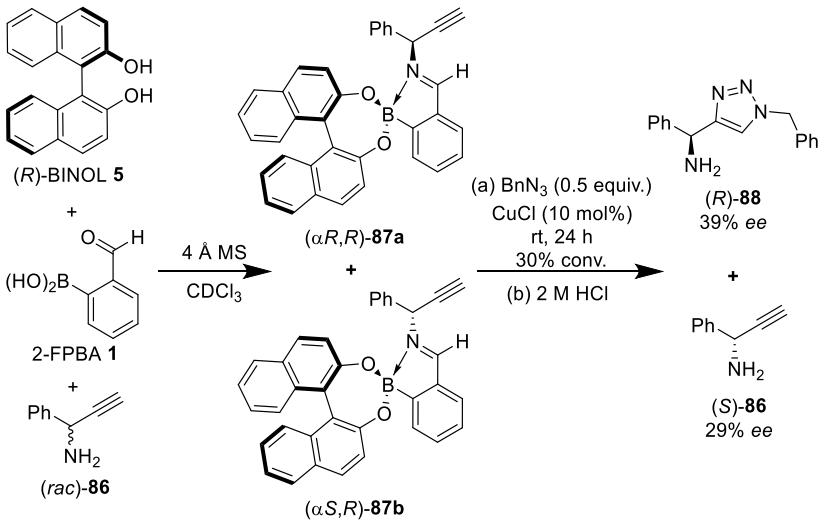


Scheme 37: Chiral phosphine-iminoboronate ligands afford enhanced enantioselectivities in palladium-catalysed allylic alkylation reactions.

6.3. *IBE-derived chiral auxiliaries in CuAAc click reactions*

Fossey and co-workers have reported use of the Bull-James assembly for asymmetric synthesis, employing it to construct a chiral auxiliary for the kinetic resolution of alkyne amines using a copper(I)-catalysed azide-alkyne

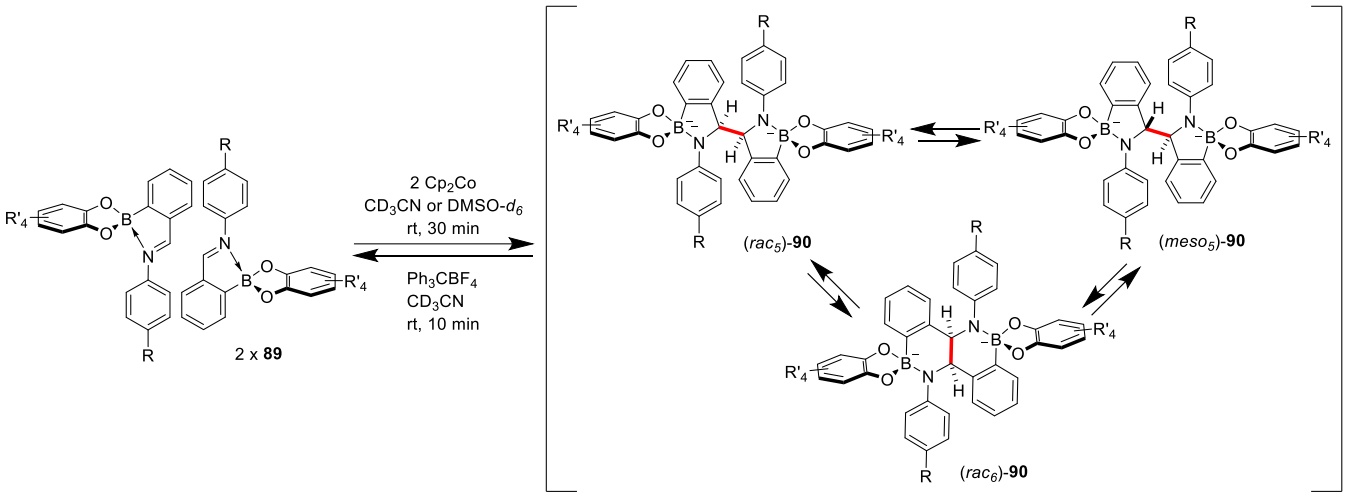
cycloaddition (CuAAc) reaction (Scheme 38).[102] In this system, a racemic alkyne-containing primary amine **86** was self-assembled with 2-FPBA **1** and (*R*)-BINOL **5** to form a mixture of diastereomeric iminoboronate complexes **87** that were subjected to CuAAc conditions using 0.5 equivalents of benzyl azide. This resulted in the alkyne fragment of the (α-*R*,*R*)-**87** diastereomer preferentially undergoing a stereoselective click reaction with a selectivity value of *S* = 4.1. Subsequent acid-catalysed hydrolysis of the IBE ester complexes then afforded amino-azide (*R*)-**88** in 39% *ee* and recovered amine (*S*)-**86** in 29% *ee*. Although only moderate stereocontrol was achieved in this unoptimized 'one-pot' kinetic resolution reaction, the simplicity of installing and removing the chiral auxiliary (e.g. BINOL) in this type of system is noteworthy, particularly if more stereoselective transformations of these types of IBE complexes can be identified.



Scheme 38: Formation of diastereomeric IBE complexes from alkyne (*rac*)-**86** enables a CuAAc-catalysed click reaction to be used for their kinetic resolution.

6.4. *Reversible radical coupling of iminoboronates*

McConnell *et al.* found that treatment of a pre-assembled *N*-aryl iminoboronate catechol ester **89** with the single electron reductant Cp_2Co resulted in radical homocoupling of its imino benzylic groups to afford amido-boronates (*rac*$_5$)-**90**, (*meso*$_5$)-**90** and (*rac*$_6$)-**90** (Scheme 39).[103] Kinetic analyses and structural studies revealed that 5-membered (*rac*$_5$)-**90** and (*meso*$_5$)-**90** were formed as kinetic products which then rearranged to 6-membered (*rac*$_6$)-**90** under thermodynamic control, leading to mixed time-, temperature- and substrate-dependent ratios of product **90**. These dimeric homo-coupled products were found to be significantly less stable than their IBE precursors, with their treatment with trityl cation $(Ph_3C)^+$ as an electron acceptor resulting in regeneration of the original IBE monomers.

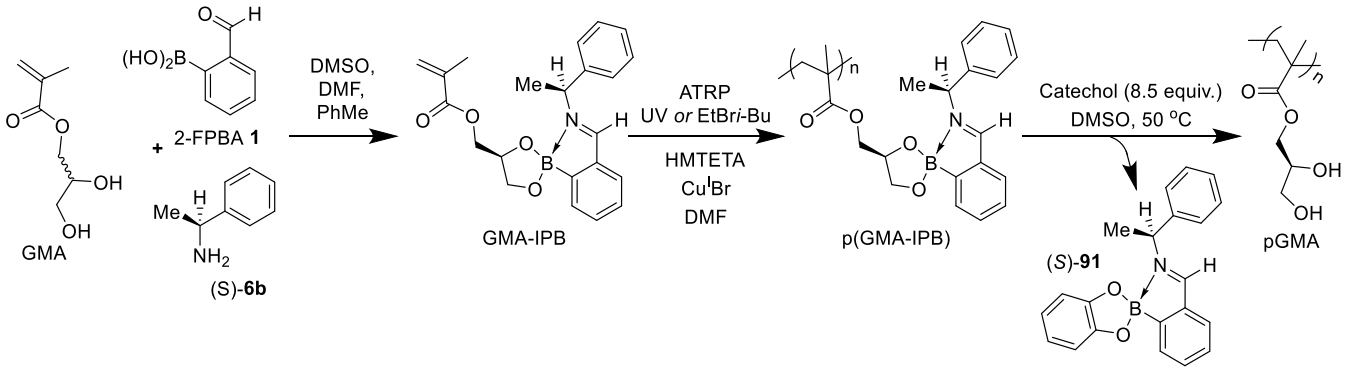


Scheme 39: Reversible radical coupling of iminoboronates **89** to afford amidoboronates **90** (radical-coupled bond in red) under thermodynamic control.

7. Iminoboronate complexes for the formation of polymers and hydrogels

7.1. *Iminoboronate polymers and hydrogels*

Following their demonstration that the Bull-James assembly could be used to assess the chirality of polymers (*vide supra*), Kressler and co-workers have reported that derivatisation of GMA monomers with 2-FPBA **1** and (*S*)-α-methylbenzylamine **6b** gave iminoboronate GMA-IPB monomers that underwent radical or UV-initiated low-temperature ATRP polymerisation to afford iminoboronate ester polymers in one pot (Scheme 40).[104] These polymers could then be decomplexed *via* treatment with a large excess of catechol to afford simple p(GMA)s containing free diol units caused by elimination of catechol-iminoboronate (*S*)-**91**. A similar process could also be used to polymerise iminoboronate ester monomers containing two equivalents of 2-hydroxyethyl-methacrylate (HEMA), affording highly syndiotactic polymers (*rr* = 70.7−75.5% for pGMAs and 74.9−79.7% for pHEMAs).



Scheme 40: One-pot complexation and polymerisation of 2-FPBA **1**, (*S*)-**6b**, and GMA to afford iminoboronate ester functionalised polymers that could be decomplexed by treatment with catechol to afford pGMAs.

7.2. *Dynamic, self-healing and stimuli-responsive polymers and hydrogels*

Iminoboronates have also been incorporated into polymeric systems as a structural element to facilitate cross-linking of polymer and hydrogel materials.[105] For example, Raquez *et al*. have developed self-assembled imine-coordinated boroxine polymeric systems that are produced from reaction of a diamine, a polyether-containing

terminal bis-cyclic carbonate unit and a 2-FPBA boroxine trimer **92** (Figure 16a). Ring opening of the terminal cyclic anhydride groups by one of the diamine amines results in a urethane bond, with the other amino group then reacting to form a highly cross-linked iminoboroxine complex.[106–108] This self-assembly approach produces polymers with a high degree of stiffness (Young's modulus = 551 MPa) and tensile strength (11 MPa) despite the labile nature of iminoboronates. These dynamic iminoboronate covalent bonds were found to confer self-healing properties to these materials, with heating/cooling and wetting/drying enabling broken imine or boroxine bonds to be reformed (Figure 16b). Similarly, changes in temperature and humidity can be used as stimuli to make or break the bonds used to construct the iminoboronate-boroxine hubs, thus creating stimuli-responsive materials which are remoldable under mild treatment conditions. This provides a simple alternative to common isocyanate-derived polyurethane self-healing and stimuli-responsive polymers, which have been shown to have potential applications as solid polymer electrolytes.[109] Following these initial reports, functional variants of this core motif have been developed, based on substitution of the iminoboronate moieties with similar amino- and acrylamido-boronate motifs.[110,111]

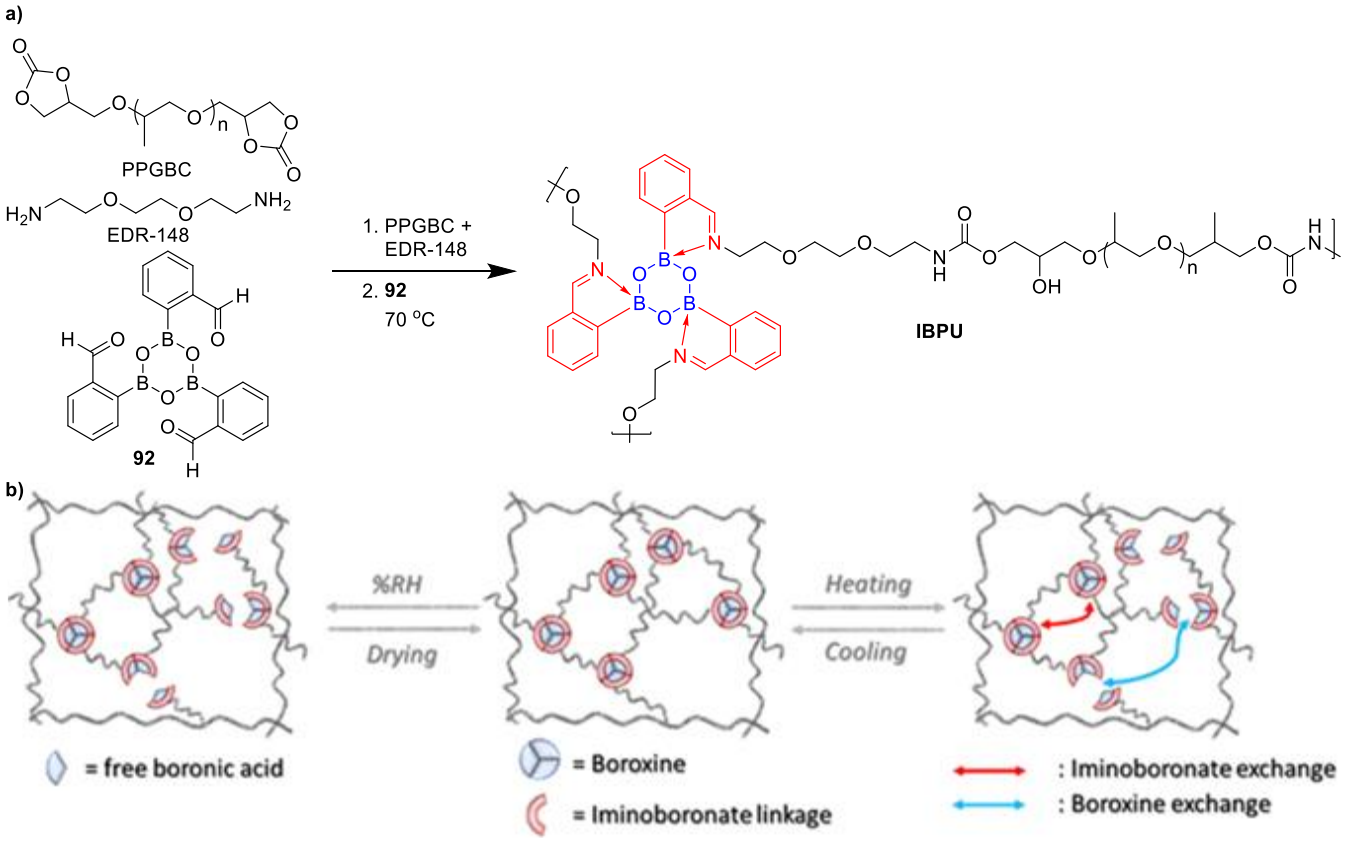


Figure 16: Three-component self-assembly of iminoboroxine-containing self-healing polymers and hydrogels. (a) Synthesis of an iminoboroxine polyurethane network polymer. (b) Self-healing and modular behaviour of iminoboroxine-polyurethane polymers. Reproduced from ref. [106] with permission from the American Chemical Society.

This concept has been expanded further for the design of self-assembled IBE-containing polymers that are prepared from supramolecular assembly of 2-FPBA, guanosine (G), aminoglycosides and potassium chloride (Figure 17). These stimuli-responsive hydrogels contain a large network of hydrogen-bonded K^+-centred guanosine tetramers, whose diol units are crosslinked through formation of iminoboronate ester groups with the amino groups of aminoglycoside units.[112–116] These hydrogels were found to be responsive to multiple stimuli, with an increase in temperature or addition of potassium-chelating crown ethers resulting in disruption of the G-quadruplex arrays and release of the aminoglycoside di-iminoboronate guanosine units. The iminoboronate bonds of these complexes are also responsive to disruption by other stimuli, with addition of aqueous acid leading to their hydrolysis to afford 2-FPBA, amine and diol components. Alternatively, the addition of glucose results in

transesterification of the boronate ester, releasing a guanosine fragment and the production of new glucose-iminoboronate-aminoglycoside species. Finally, the reactivity of boronates towards reactive oxygen and nitrogen species (ROS/RNS)[117–119] may be exploited, with addition of hydrogen peroxide triggering oxidative deborylation to produce boric acid and release of the guanosine fragment. This multi-responsive behaviour has been exploited for drug delivery for selective release of antibacterial aminoglycosides and the anticancer drug Doxorubicin.[112,116] CO_2-responsive iminoboronate poly(oligo(ethylene glycol)) polymers have also been reported by Jiang and co-workers, with bubbling of CO_2 reversibly producing carbonic acid that triggers IBE bond hydrolysis to trigger depolymerisation processes that can be reversed by purging with N_2 gas.[120] This CO_2-dependent behaviour has been demonstrated in multiple systems (*vide infra*) using both ^{1}H NMR and fluorescence assays to measure the fragmentation/re-complexation of IBE systems upon sequential CO_2/N_2 bubbling.

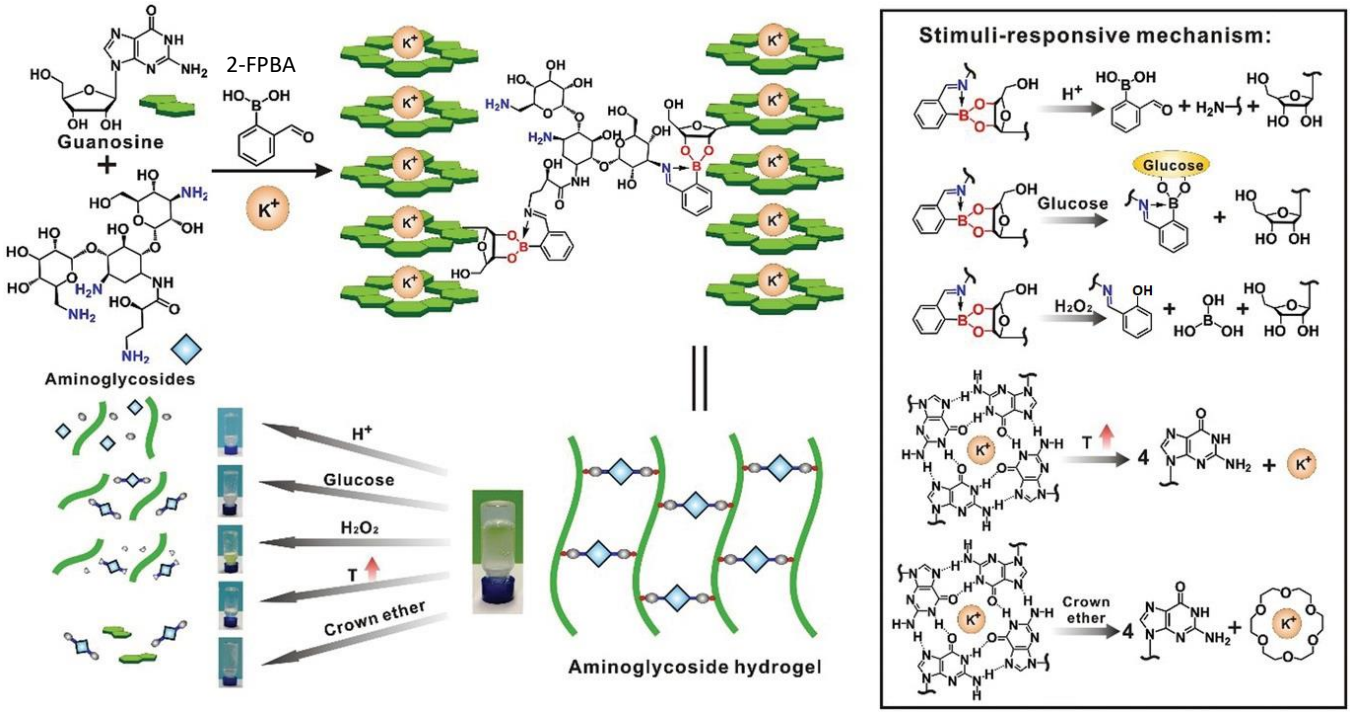


Figure 17: An aminoglycoside iminoboronate hydrogel assembled from guanosine, K^+, an aminoglycoside and 2-FPBA. These materials are responsive to multiple external stimuli such as acids, glucose, H_2O_2, heat and crown ethers, all of which act on different structural elements of the hydrogel network. Reproduced from ref. [112] with permission from John Wiley and Sons.

7.3. *Stimuli-responsive aggregates and micelles*

The Bull-James multicomponent approach has also been used to produce stimuli-responsive iminoboronate-containing nano-aggregates, micellar assemblies and polymersomes that are stable in aqueous systems. Jiang and co-workers, for example, have reported the three-component assembly of poly(ethylene glycol) amine with 2-FPBA **1** and a nitrophenyl ethanediol (PEG-INEC) to produce amphipathic IBE complexes that self-assemble into nano-aggregates in aqueous systems (Figure 18).[121] These nano-aggregates were found to be responsive to three common stimuli: light - which results in release of a nitrosoaryl α-hydroxy-ketone and an iminoboronic acid fragment ; acid - which hydrolyses both the boronate ester and imine bonds to regenerate the original three components; and hydrogen peroxide which oxidatively cleaves the boronate ester to give boric acid, o-hydroxy-benzaldehyde and nitrophenyl ethanediol. Therefore, different external stimuli can be used to trigger controlled decomposition of these aggregates, which is potentially useful for the selective release of encapsulated hydrophobic guest molecules.

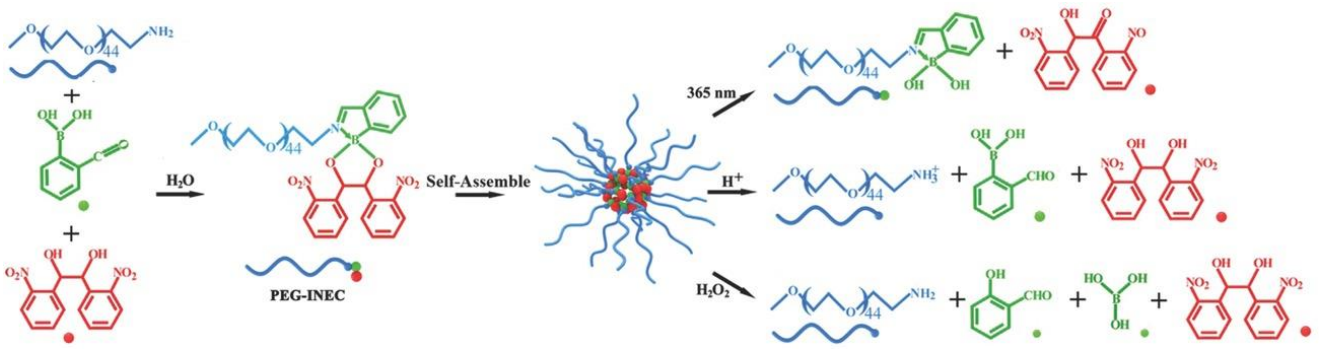


Figure 18: Self-assembled PEG-iminoboronate polymeric nano-aggregates and their stimuli-responsive degradation by light, acid and H_2O_2. Reproduced from ref. [121] with permission from John Wiley and Sons.

The same group have also reported the development of different iminoboronate aggregate systems, whose disassembly is triggered by the action of nucleophilic ROS or CO_2-induced solvent acidification.[122,123] For example, CO_2 responsive N_3-(OEG-IBCAPE)$_4$ polymersomes are stable at physiological pH 7.4, however protonation of their tris-amine cores results in nano-aggregate disassembly at mildly acidic pH levels. This enabled iminoboronate ester linkers to be used to generate polymersomes attached to the diol unit of caffeic acid phenethyl ester (CAPE, anti-cancer drug, red) as a CO_2-responsive drug delivery system (Figure 19). These polymersomes exhibited improved transport properties that enabled their delivery to CO_2-rich HL-60 leukaemia cells that exhibit a mildly acidic environment. This acidity results in intracellular hydrolysis of the iminoboronate bonds of the polymersome aggregates, which leads to their disassembly and release of CAPE as a cytotoxic agent within the target cancer cells. The same transport principles have also been employed by Shi and co-workers for pH/GSH-responsive delivery of encapsulated capecitabine to HepG2 liver cancer cells.[124]

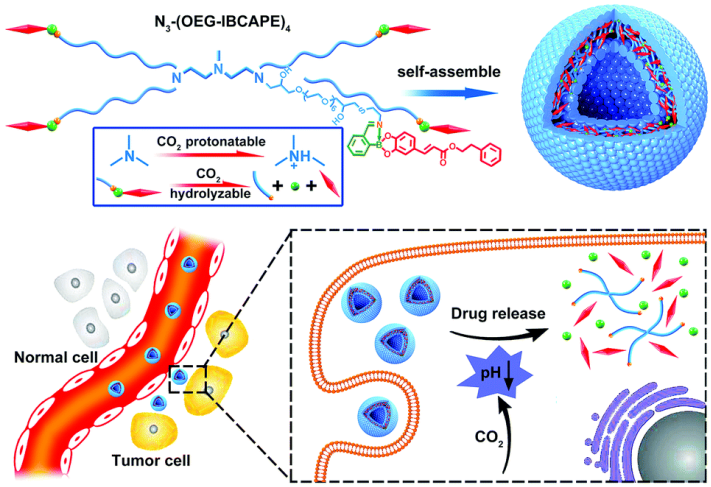
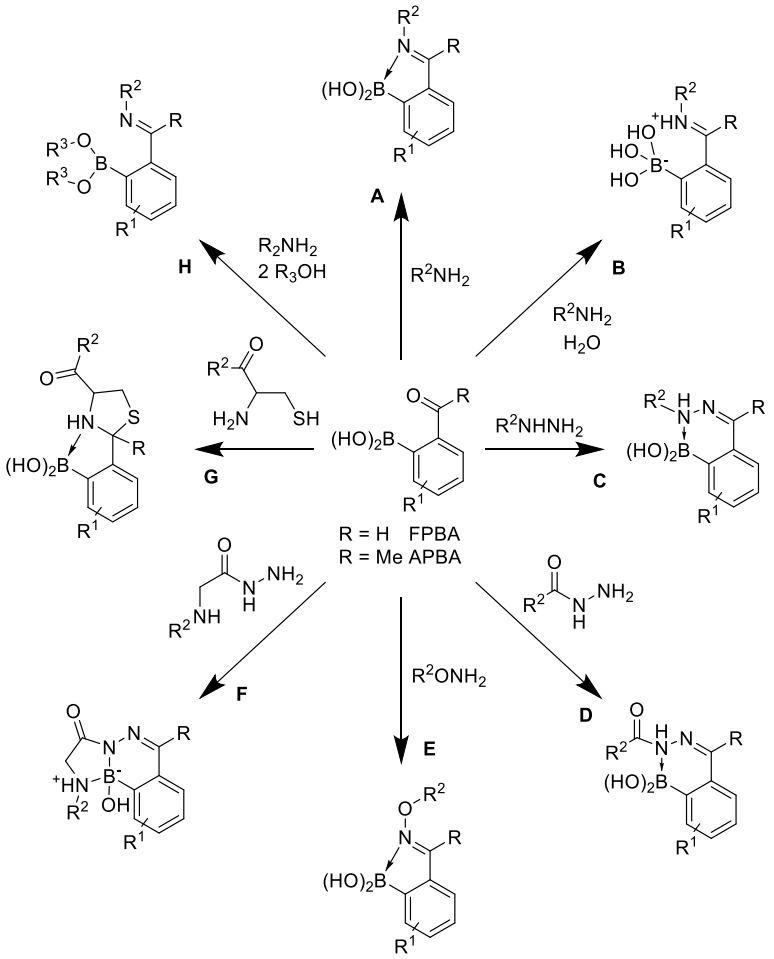


Figure 19: Self assembled prodrug N_3-(OEG-IBCAPE)$_4$ polymersomes and the stimuli-responsive CO_2-triggered release of CAPE in cancer cells. Reproduced from ref. [123] with permission from the Royal Society of Chemistry.

8. Iminoboronate derivatives for biological targeting and tagging

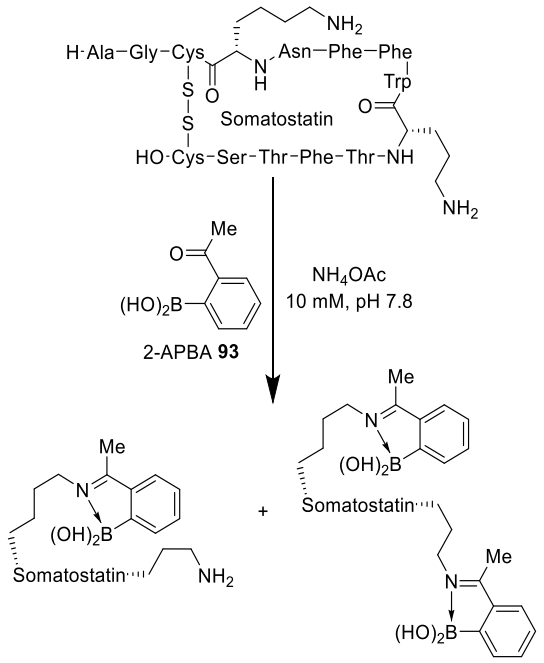
IB-type assemblies have also been employed for the functionalisation and tagging of the amino groups of peptides and proteins, with several recent specialised reviews having covered this topic in detail,[125–127] with only a general overview of this area provided herein. The majority of bioorthogonal labelling reactions that have been reported to date are two-component in nature, involving reaction of 2-FPBA (or 2-acetylphenylformyl boronic acid, 2-APBA) with amine or aminothiol residues of peptides or proteins to form imine/thioxazolidine bonds that are stabilised by the presence of a proximal boron centre (Scheme 41). These condensation reactions have been

found to proceed with rate constants of over 10^2-10^3 M^{-1} s^{-1},[128] which is orders of magnitude faster than traditional alkyne-azide 'click' coupling reactions. Gois, Gillingham and Anslyn have carried out binding studies that clearly demonstrate that the proximal boron centre accelerates imine condensation reactions and stabilises imine complex formation, with additives or external stimuli (e.g. changes in pH, ROS, nucleophiles…) normally required to achieve hydrolysis, degradation, or decomplexation.[36,37,129,130] For example, computational studies on the condensation of *n*-butylamine and 2-APBA **93** have shown that the adjacent boronic acid reduces the activation enthalpy for imine condensation drastically by 35-36 kcal/mol.[129]



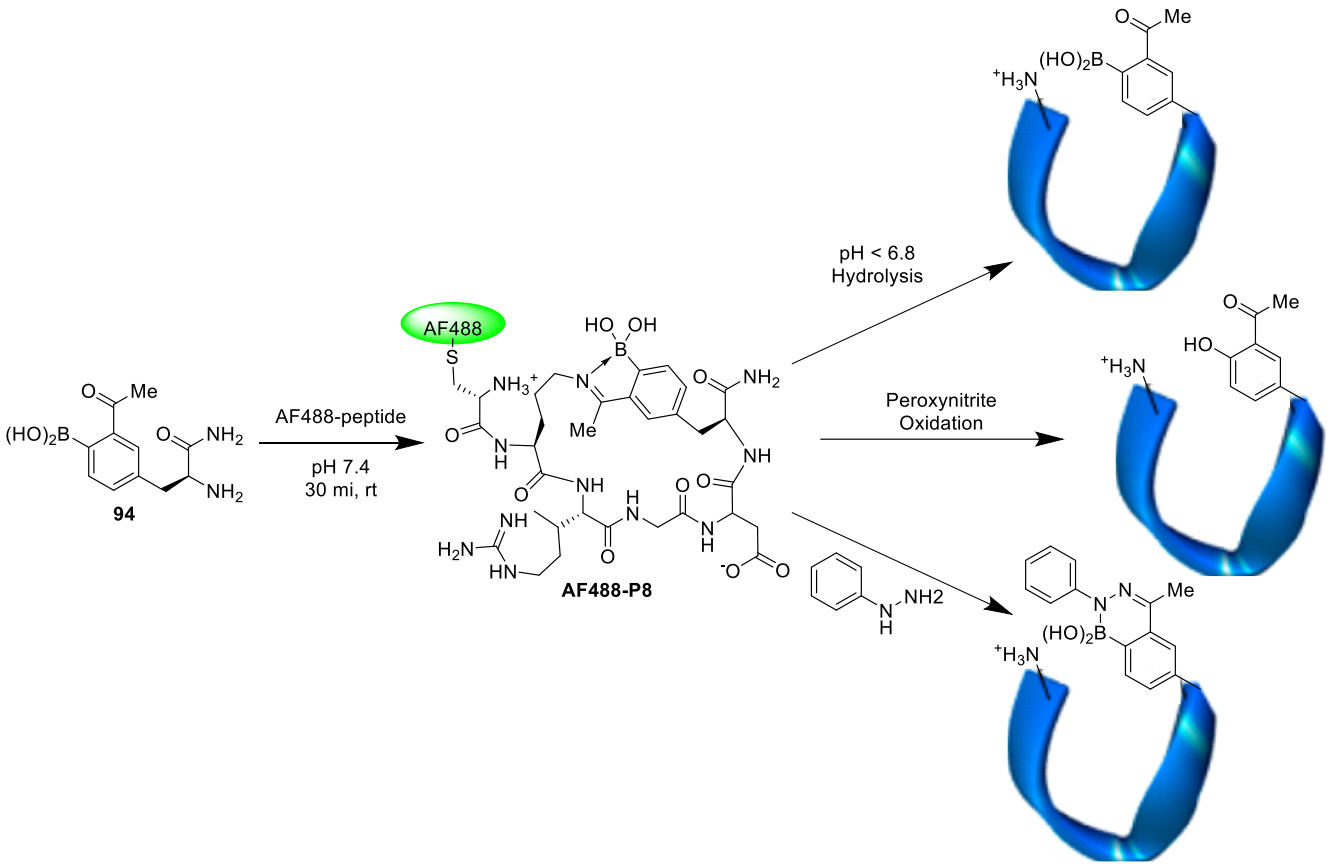
Scheme 41: Diverse biorthogonal IB conjugation chemistries of 2-FPBA- and 2-APBA-derived linkers.

The most commonly employed amine tagging systems involve generation of the two component iminoboronic acid assemblies **A** and **B** (pH interconvertible), both of which have been widely used to label the free ε-amine groups of lysine residues in peptides and proteins. This approach was first pioneered in 2012 by Gois *et al*. who reported formation of an iminoboronic acid complex between the hormonal neuropeptide Somatostatin and 2-APBA **93** in ammonium acetate buffer (20 mM, pH 5.0 - 7.0) (Scheme 42).[129] Following this success, they demonstrated that 2-APBA could be used to successfully tag lysine groups present in lysozyme, cytochrome C, ribonuclease A and myoglobin with a range of 2-formylaryl boronic acids. Improvements to this tagging approach have subsequently been reported based on the use of peptides/proteins containing α-nucleophiles such as hydrazides, acylhydrazides and alkoxyamines which react more rapidly to afford hydrazone and oxime linkers (**C**, **D**, **E**, Scheme 41) that are more hydrolytically stable.[128,131–134] Similarly, multidentate coordination of bifunctional nucleophiles such as α-amino hydrazides or 1,2-aminothiols to 2-FPBA/2-APBA templates have proved popular for producing stable bioconjugates containing tricyclic azadiborolidine boracycles (**F**, Scheme 41) and stabilised thioxazolidine linkers (**G**, Scheme 41).[131,133,135–137].



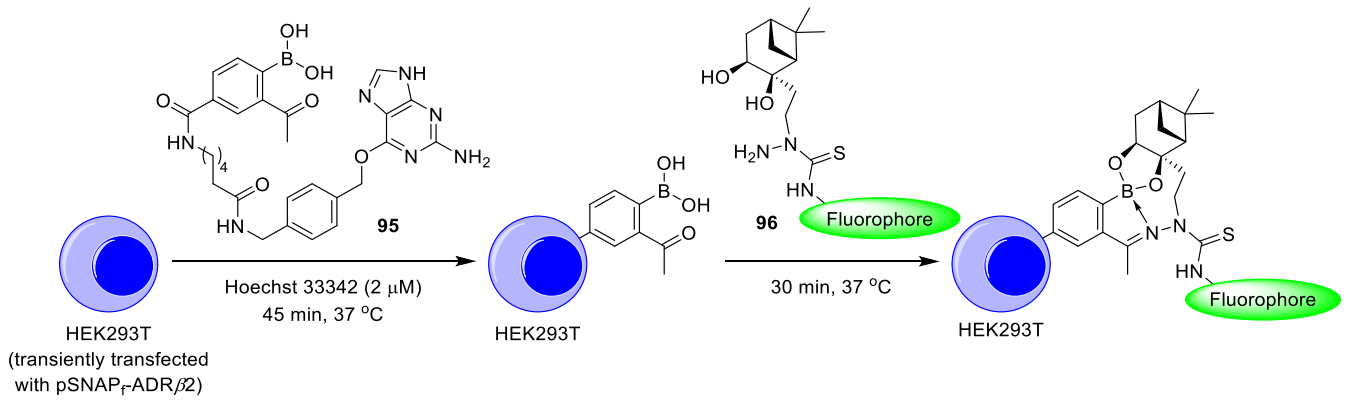
Scheme 42: Reaction of lysine groups in Somatostatin with 2-APBA **93**.

Proof of concept studies have shown that stimulus-triggered decomplexation of this type of protein-boracycle conjugates can be achieved through treatment with fructose, dopamine, glutathione, aqueous acid, ROS/RNS, *etc.*, with this reversibility exploited to induce partial or complete hydrolysis of intramolecular imine bonds to control ring-opening of cyclic peptides (Scheme 43). Since their inception, these types of stimuli-responsive two-component IB assemblies have been used to derivatise peptides, proteins, aminoglycosides, biological polyamines and amine-rich membrane lipids for fluorescent tagging, targeted fluorophore, biomolecule and therapeutic delivery, covalent protein inhibition, and reversible biomolecule functionalization.[138–144]



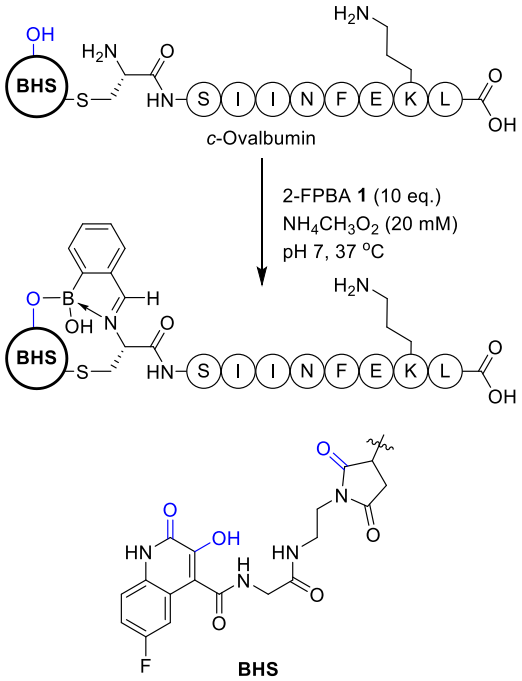
Scheme 43: A stimuli-responsive intramolecular iminoboronic acid bond can be used to control the cyclisation of an AF488 fluorophore-appended peptide.

The use of three-component strategies for tagging the amino groups of biomolecules has been less well explored (e.g. **H**, Scheme 42), although three recent reports demonstrate the potential of this approach for producing stable bioconjugates. In 2017, Hall and co-workers reported the bioorthogonal tagging of live cells using a fluorescein fluorophore attached to a "click" boronate/thiosemicarbazone warhead, where the thiosemicarbazide unit underwent rapid imine condensation to afford a complex that was stabilised by the presence of a pendant pinanediol that formed an intramolecular boronate ester bond. This system was employed for live cell imaging using fluorescence microscopy using a SNAP-tag approach, in which HEK293T cancer cells were transiently transfected with the pSNAP$_f$-ADRβ2 plasmid, allowing 2-APBA-derivative **95** to be secured on the cell membrane, enabling 'click' fluorescent tagging of these cells with **96** for visualisation using fluorescence microscopy at concentrations as low as 10 µM (Scheme 44).[145]



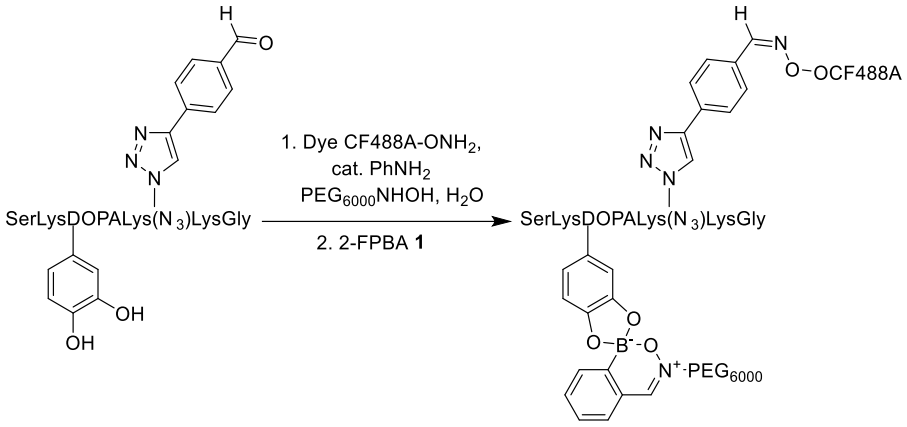
Scheme 44: 2-APBA modification of HEK293T cancer cells and subsequent three-component "click" boronate/thiosemicarbazone fluorescent labelling.

Most recently, Gois *et al*. have reported a "boron hot spot" (BHS) approach to selectively target the amino groups of *N*-terminal cysteine residues, which was developed to address some of the promiscuity and reversibility issues that are often observed when two-component iminoboronate acid complexation reactions are used to functionalise biomolecules (Scheme 45)[146]. They found that attachment of 3-hydroxyquinolin-2(1H)-one (3HQ)/succinimide groups to the thiol units of *N*-terminal cysteine residues resulted in selective imine condensation of the *N*-terminal amino group with 2-FPBA **1**. This was proposed to be due to the IB complex being stabilised by formation of an intramolecular B-O bond between the boronic acid and the α-hydroxy-amide fragment of the *S*-appended 3HQ fragment, with further hydrogen bonding stabilisation from the succinimide (blue, Scheme 45). This boron hot spot approach was used to selectively tag 2-FPBA-modified *c*-ovalbumin with an impressive K_a value of 58,128 ±2 M^{-1}, thus allowing for site-selective labelling of its free *N*-terminal amino groups in the presence of other lysine residues despite a large excess of 2-FPBA **1**. This tagging approach was used to prepare glutathione-labile boron hot spot fluorescent labelled protein conjugates that were capable of delivering their fluorescent payloads to HT29 cancer cells.



Scheme 45: Site-selective iminoboronate complexation of an *N*-terminal boron hot spot-modified *c*-ovalbumin.

Finally, a collaboration with Anslyn has reported the use of 2-FPBA **1** and hydroxylamine to irreversibly functionalise the catechol fragment of an L-Dopa-containing peptide derivative. Fluorescent tagging of the peptide containing a Cu(I) Sharpless-Huisgen 'click' appended benzaldehyde group was achieved through imine bond formation between the *O*-functionalized hydroxylamine residue of the CF488A dye. Subsequent addition of 2-FPBA **1** then templated irreversible three-component formation of a highly stable nitrono-boronate linker (*vide supra*) that was formed from incorporation of the catechol unit of the L-Dopa residue and the *N*-functionalised hydroxylamine group of the solubilising PEG side-chain (Scheme 46).[147]



Scheme 46: Dual one-pot labelling of L-Dopa-containing peptide with a fluorescent dye and a solubilising PEG side-chain.

9. Conclusions and outlook

The body of work presented in this review clearly highlights the versatility and practicality of iminoboronate assemblies, with potential applications across many fields of chemistry and chemical biology. From its initial discovery as a CDA for determining the *ee*'s of chiral amines and diols, the Bull-James three-component assembly has now been developed into a wide-ranging method for the chiral analysis of other analytes using NMR, CD, fluorescence, and electrochemical methods. Beyond analytical applications, iminoboronate assemblies have also proven popular as an orthogonal self-assembly tool for preparing boracycles, polymers, hydrogels and aggregates that exhibit stimuli-responsive properties. Similarly, bioconjugation applications have also been demonstrated, with ongoing development of two- and three-component dynamic labelling methodologies showing great promise as a versatile tool for "click" modification of the free amino groups (or diols) of biomolecules. Although the original application of these IBE assemblies as analytical tools for determining enantiopurities continues to grow both in scope and popularity, the potential applications of these IB systems are far wider ranging than was originally anticipated. Although we expect additional analytical IBE methods to be developed, it is clear that the future of these three-component iminoboronate ester assemblies lies in their innate ability to act as reversible yet highly rigidified linkers. The prospect of expanding the use of these IBEs as chiral auxiliaries for asymmetric synthesis is also an exciting one, and should lead to highly versatile and practically simple methodologies. We also anticipate that the "click" and stimuli-responsive capabilities of these boron-coordination complexes will lead to further development of wide-ranging bioorthogonal and materials-based systems, with increasingly wide-ranging sensing, tagging, theranostic, and logic-based applications.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We would like to thank the many past members of the Bull and James groups who have contributed to the development of the Bull-James assembly (including Drs. Susumu Arimori, Andrew Kelly, Yolanda Perez-Fuertes,

Sonia Lozano-Yeste, Magdalena Powell, Ewan Galbraith, David Tickell and Prof. John Fossey), as well as the many local and international collaborators who have also worked on this project. The contributions of the Material and Chemical Characterisation (MC^2) facility at the University of Bath must also be acknowledged, with particular mention of our crystallographers Drs Gabriel Kociok-Köhn and Mary Mahon.

We would like to thank the University of Bath, the Royal Society and EPSRC for their generous support over the past 20 years (EPSRC Grants: EP/D075351/1, GR/S70289/01). RRG would like to thank the EPSRC Centre for Doctoral Training in Catalysis for funding and support (EP/L016443/1). TDJ would like to thank the Royal Society for a Wolfson Research Merit Award. SDB would like to thank the Royal Society for an International Travel Award (IEC\R3|183068) that provided much needed time to write this review.

References

[1] S.D. Bull, S.G. Davies, S.W. Epstein, J.V.A. Ouzman, Chiral relay auxiliary for the synthesis of enantiomerically pure α-amino acids, Chem. Commun. (1998) 659–660. https://doi.org/10.1039/a800407b.

[2] S.D. Bull, S.G. Davies, S. Delgado-Ballester, P.M. Kelly, L.J. Kotchie, M. Gianotti, M. Laderas, A.D. Smith, Asymmetric synthesis of β-haloaryl β-amino acid derivatives, J. Chem. Soc. Perkin 1. 1 (2001) 3112–3121. https://doi.org/10.1039/b108573p.

[3] S.D. Bull, S.G. Davies, D.J. Fox, M. Gianotti, P.M. Kelly, C. Pierres, E.D. Savory, A.D. Smith, Asymmetric synthesis of β-pyridyl-β-amino acid derivatives, J. Chem. Soc. Perkin 1. 2 (2002) 1858–1868. https://doi.org/10.1039/b204653a.

[4] D.J. Aitken, S.D. Bull, I.R. Davies, L. Drouin, J. Ollivier, J. Peed, An expedient asymmetric synthesis of N-protected (*S*,*S*)-2-aminomethyl-1-cyclopropanecarboxylic acid, Synlett. 2010 (2010) 2729–2732. https://doi.org/10.1055/s-0030-1258814.

[5] C.D. Evans, M.F. Mahon, P.C. Andrews, J. Muir, S.D. Bull, Intramolecular ester enolate-imine cyclization reactions for the asymmetric synthesis of polycyclic β-lactams and cyclic β-amino acid derivatives, Org. Lett. 13 (2011) 6276–6279. https://doi.org/10.1021/ol202750u.

[6] T. Boddaert, J.E. Taylor, S.D. Bull, D.J. Aitken, A Selective Deprotection Strategy for the Construction of *trans*-2-Aminocyclopropanecarboxylic Acid Derived Peptides, Org. Lett. 21 (2019) 100–103. https://doi.org/10.1021/acs.orglett.8b03533.

[7] M. Raban, K. Mislow, The determination of optical purity by nuclear magnetic resonance spectroscopy, Tetrahedron Lett. 48 (1965) 4249–4253. https://doi.org/10.1016/S0040-4039(00)71080-X.

[8] J.A. Dale, D.L. Dull, H.S. Mosher, α-Methoxy-α-trifluoromethylphenylacetic Acid, a Versatile Reagent for the Determination of Enantiomeric Composition of Alcohols and Amines, J. Org. Chem. 34 (1969) 2543–2549. https://doi.org/10.1021/jo01261a013.

[9] T.J. Ward, K.D. Ward, Chiral separations: A review of current topics and trends, Anal. Chem. 84 (2012) 626–635. https://doi.org/10.1021/ac202892w.

[10] Y. Zhang, D.R. Wu, D.B. Wang-Iverson, A.A. Tymiak, Enantioselective chromatography in drug discovery, Drug Discov. Today. 10 (2005) 571–577. https://doi.org/10.1016/S1359-6446(05)03407-0.

[11] E. Galbraith, T.D. James, Boron based anion receptors as sensors, Chem. Soc. Rev. 39 (2010) 3831–3842. https://doi.org/10.1039/b926165f.

[12] T.D. James, K.R.A. Samankumara Sandanayake, S. Shinkai, Chiral discrimination of monosaccharides using a fluorescent molecular sensor, Nature. 374 (1995) 345–347. https://doi.org/10.1038/374345a0.

[13] T.D. James, K.R.A. Samankumara Sandanayake, S. Shinkai, Saccharide sensing with molecular receptors based on boronic acid, Angew. Chemie (International Ed. English). 35 (1996) 1910–1922. https://doi.org/10.1002/anie.199619101.

[14] S.D. Bull, M.G. Davidson, J.M.H. Van Den Elsen, J.S. Fossey, A.T.A. Jenkins, Y.-B. Jiang, Y. Kubo, F. Marken, K. Sakurai, J. Zhao, T.D. James, Exploiting the Reversible Covalent Bonding of Boronic Acids: Recognition, Sensing, and Assembly Reversible Covalent Bonding of Boronic Acids, Acc. Chem. Res. 46 (2012) 312–326. https://doi.org/10.1021/ar300130w.

[15] D. Wu, A.C. Sedgwick, T. Gunnlaugsson, E.U. Akkaya, J. Yoon, T.D. James, Fluorescent chemosensors: The past, present and future, Chem. Soc. Rev. 46 (2017) 7105–7123. https://doi.org/10.1039/c7cs00240h.

[16] T.D. James, K.R.A.S. Sandanayake, R. Iguchi, S. Shinkai, Novel Saccharide-Photoinduced Electron Transfer Sensors Based on the Interaction of Boronic Acid and Amine, J. Am. Chem. Soc. 117 (1995) 8982–8987. https://doi.org/10.1021/ja00140a013.

[17] M. Mortellaro, A. DeHennis, Performance characterization of an abiotic and fluorescent-based continuous glucose monitoring system in patients with type 1 diabetes, Biosens. Bioelectron. 61 (2014) 227–231. https://doi.org/10.1016/j.bios.2014.05.022.

[18] B.C. Crane, N.P. Barwell, P. Gopal, M. Gopichand, T. Higgs, T.D. James, C.M. Jones, A. Mackenzie, K.P. Mulavisala, W. Paterson, The Development of a Continuous Intravascular Glucose Monitoring Sensor., J. Diabetes Sci. Technol. 9 (2015) 751–61. https://doi.org/10.1177/1932296815587937.

[19] M. Lauer, H. Böhnke, R. Grotstollen, M. Salehnia, G. Wulff, Zur Chemie von Haftgruppen, IV. Über eine außerordentliche Erhöhung der Reaktivität von Arylboronsäuren durch Nachbargruppen, Chem. Ber. 118 (1985) 246–260. https://doi.org/10.1002/cber.19851180124.

[20] X. Sun, T.D. James, E. V. Anslyn, Arresting "loose Bolt" Internal Conversion from -$B(OH)_2$ Groups is the Mechanism for Emission Turn-On in ortho-Aminomethylphenylboronic Acid-Based Saccharide Sensors, J. Am. Chem. Soc. 140 (2018) 2348–2354. https://doi.org/10.1021/jacs.7b12877.

[21] X. Sun, B.M. Chapin, P. Metola, B. Collins, B. Wang, T.D. James, E. V. Anslyn, The mechanisms of boronate ester formation and fluorescent turn-on in *ortho*-aminomethylphenylboronic acids, Nat. Chem. 11 (2019) 768–778. https://doi.org/10.1038/s41557-019-0314-x.

[22] N. Hiller, N. Silva, T. Tavares, R. Faria, M. Eberlin, D. Martins, Arylboronic acids and their myriad of applications beyond organic synthesis, European J. Org. Chem. 2020 (2020) 4841–4877. https://doi.org/10.1002/ejoc.202000396.

[23] Y. Kanekiyo, S. Shinkai, CHAPTER 1: Supramolecular chemistry of boronic acids, in: Monogr. Supramol. Chem., Royal Society of Chemistry, 2016: pp. 1–43. https://doi.org/10.1039/9781782622123-00001.

[24] Wikipedia, Nomikai, (n.d.). https://en.wikipedia.org/wiki/Nomikai (accessed July 31, 2020).

[25] T.D. James, Self and directed assembly: people and molecules, Beilstein J. Org. Chem. 12 (2016) 391–405. https://doi.org/10.3762/bjoc.12.42.

[26] H.E. Dunn, J.C. Gatlin, H.R. Snyder, Arylboronic Acids. Imino Derivatives from *o*-Formylbenzeneboronic Acid, J. Org. Chem. 33 (1968) 4483–4486. https://doi.org/10.1021/jo01276a040.

[27] G.A. Molander, D.L. Sandrock, 2-Formylphenylboronic Acid, in: Encycl. Reagents Org. Synth., John Wiley & Sons, Ltd, 2009. https://doi.org/10.1002/047084289x.rn00931.

[28] Y. Pérez-Fuertes, A.M. Kelly, A.L. Johnson, S. Arimori, S.D. Bull, T.D. James, Simple Protocol for NMR Analysis of the Enantiomeric Purity of Primary Amines, Org. Lett. 8 (2006) 609–612. https://doi.org/10.1021/ol052776g.

[29] Y. Pérez-Fuertes, New strategies using BINOL-boron compounds for organic synthesis, University of Bath, 2005.

[30] B.M. Chapin, P. Metola, V.M. Lynch, J.F. Stanton, T.D. James, E. V Anslyn, Structural and Thermodynamic Analysis of a Three-Component Assembly Forming *ortho*-Iminophenylboronate Esters, J. Org. Chem. 81 (2016) 8319–8330. https://doi.org/10.1021/acs.joc.6b01495.

[31] S.L. Lulinski, I. Madura, J. Serwatowski, H. Szatyłowicz, J. Zachara, A tautomeric equilibrium between functionalized 2-formylphenylboronic acids and corresponding 1,3-dihydro-1,3-dihydroxybenzo [*c*][2,1]oxaboroles, New J. Chem. 31 (2007) 144–154. https://doi.org/10.1039/b611195e.

[32] K. Kowalska, A. Adamczyk-Woźniak, P. Gajowiec, B. Gierczyk, E. Kaczorowska, Ł. Popenda, G. Schroeder, A. Sikorski, A. Sporzyński, Fluoro-substituted 2-formylphenylboronic acids: Structures, properties and tautomeric equilibria, J. Fluor. Chem. 187 (2016) 1–8. https://doi.org/10.1016/j.jfluchem.2016.05.001.

[33] A. Adamczyk-Woźniak, I. Madura, A.H. Velders, A. Sporzyński, Diverse reactivity of 2-formylphenylboronic acid with secondary amines: Synthesis of 3-amino-substituted benzoxaboroles, Tetrahedron Lett. 51 (2010) 6181–6185. https://doi.org/10.1016/j.tetlet.2010.09.091.

[34] A. Adamczyk-Woźniak, M.K. Cyrański, B.T. Frczak, A. Lewandowska, I.D. Madura, A. Sporzyński, Imino- and aminomethylphenylboronic acids: Stabilizing effect of hydrogen bonds, Tetrahedron. 68 (2012) 3761–3767. https://doi.org/10.1016/j.tet.2012.02.072.

[35] N.J. Gutiérrez-Moreno, F. Medrano, A.K. Yatsimirsky, Schiff base formation and recognition of amino sugars, aminoglycosides and

biological polyamines by 2-formyl phenylboronic acid in aqueous solution, Org. Biomol. Chem. 10 (2012) 6960–6972. https://doi.org/10.1039/c2ob26290h.

[36] P. Schmidt, C. Stress, D. Gillingham, Boronic acids facilitate rapid oxime condensations at neutral pH, Chem. Sci. 6 (2015) 3329–3333. https://doi.org/10.1039/c5sc00921a.

[37] D. Gillingham, The role of boronic acids in accelerating condensation reactions of α-effect amines with carbonyls, Org. Biomol. Chem. 14 (2016) 7606–7609. https://doi.org/10.1039/c6ob01193d.

[38] C.J. Stress, P.J. Schmidt, D.G. Gillingham, Comparison of boron-assisted oxime and hydrazone formations leads to the discovery of a fluorogenic variant, Org. Biomol. Chem. 14 (2016) 5529–5533. https://doi.org/10.1039/c6ob00168h.

[39] S. Nieto, P. Arnau, E. Serrano, R. Navarro, T. Soler, C. Cativiela, E.P. Urriolabeitia, Functionalization of Methyl (*R*)-Phenylglycinate Through Orthopalladation: C-Hal, C-O, C-N, and C-C Bond Coupling, Inorg. Chem. 48 (2009) 11963–11975. https://doi.org/10.1021/ic901941s.

[40] Y. Pérez-Fuertes, J.E. Taylor, D.A. Tickell, M.F. Mahon, S.D. Bull, T.D. James, Asymmetric Strecker Synthesis of α-Arylglycines, J. Org. Chem. 76 (2011) 6038–6047. https://doi.org/10.1021/jo200528s.

[41] Y. Pérez-Fuertes, A.M. Kelly, J.S. Fossey, M.E. Powell, S.D. Bull, T.D. James, Simple protocols for NMR analysis of the enantiomeric purity of chiral primary amines, Nat. Protoc. 3 (2008) 210–214. https://doi.org/10.1038/nprot.2007.524.

[42] P.J.M. Taylor, S.D. Bull, An improved synthesis of deuterated Schöllkopf's bis-lactim ether and its use for the asymmetric synthesis of (*R*)-[α-^{2}H]-phenylalanine methyl esters, Tetrahedron: Asymmetry. 17 (2006) 1170–1178. https://doi.org/10.1016/j.tetasy.2006.04.005.

[43] R.M. Archer, M. Hutchby, C.L. Winn, J.S. Fossey, S.D. Bull, A chiral ligand mediated *aza*-conjugate addition strategy for the enantioselective synthesis of β-amino esters that contain hydrogenolytically sensitive functionality, Tetrahedron. 71 (2015) 8838–8847. https://doi.org/10.1016/j.tet.2015.08.018.

[44] P. Axe, S.D. Bull, M.G. Davidson, C.J. Gilfillan, M.D. Jones, D.E.J.E. Robinson, L.E. Turner, W.L. Mitchell, Enantiopure Pseudo-C_3-Symmetric Titanium Alkoxide with Propeller-Like Chirality, Org. Lett. 9 (2007) 223–226. https://doi.org/10.1021/ol062655w.

[45] T.L. March, M.R. Johnston, P.J. Duggan, Diastereoselective Synthesis of Aliphatic α,α-Difluoro-β^{3}-Amino Esters via a Sonocatalyzed Reformatsky Reaction, Org. Lett. 14 (2012) 182–185. https://doi.org/10.1021/ol202969w.

[46] C. Shi, T.W. Geders, S. Woong Park, D.J. Wilson, H.I. Boshoff, O. Abayomi, C.E. Barry, D. Schnappinger, B.C. Finzel, C.C. Aldrich, Mechanism-based Inactivation by Aromatization of the Transaminase BioA Involved in Biotin Biosynthesis in Mycobaterium tuberculosis, J. Am. Chem. Soc. 133 (2011) 18194–18201. https://doi.org/10.1021/ja204036t.

[47] S. Nieto, J.M. Dragna, E. V. Anslyn, A Facile Circular Dichroism Protocol for Rapid Determination of Enantiomeric Excess and Concentration of Chiral Primary Amines, Chem. a Eur. J. 16 (2010) 227–232. https://doi.org/10.1002/chem.200902650.

[48] N. Lokesh, S.L. Sachin, L. V. Narendra, K. Arun, N. Suryaprakash, RNA nucleosides as chiral sensing agents in NMR spectroscopy, Org. Biomol. Chem. 13 (2015) 7230–7235. https://doi.org/10.1039/c5ob00513b.

[49] J.S. Fossey, E. V Anslyn, W.D.G. Brittain, S.D. Bull, B.M. Chapin, C. Cécile, S. Le Duff, T.D. James, G. Lees, S. Lim, J.A.C. Lloyd, C. V Manville, D.T. Payne, K.A. Roper, Rapid Determination of Enantiomeric Excess via NMR Spectroscopy: A Research-Informed Experiment, J. Chem. Educ. 94 (2017) 79–84. https://doi.org/10.1021/acs.jchemed.6b00355.

[50] A.M. Kelly, S.D. Bull, T.D. James, Simple chiral derivatisation protocols for NMR analysis of the enantiopurity of 1,2-diphenylethane-1,2-diamine and *N*-Boc-cyclohexane-1,2-diamine, Tetrahedron Asymmetry. 19 (2008) 489–494. https://doi.org/10.1016/j.tetasy.2008.01.026.

[51] P. Mangeney, A. Alexakis, J.F. Normant, Resolution and determination of enantiomeric excesses of chiral aldehydes *via* chiral imidazolidines, Tetrahedron Lett. 29 (1988) 2677–2680. https://doi.org/10.1016/0040-4039(88)85258-4.

[52] D. Cuvinot, A. Alexakis, J.-F. Normant, J.-P. Lellouch, Chiral Trifluoro Diamines as Convenient Reagents for Determining the Enantiomeric Purity of Aldehydes by Use of ^{19}F NMR Spectroscopy, J. Org. Chem. 54 (1989) 2420–2425. https://doi.org/10.1021/jo00271a034.

[53] A. Alexakis, J.C. Frutos, P. Mangeney, An easy and fast way to determine the enantiomeric purity of substituted cyclanones, Tetrahedron: Asymmetry. 4 (1993) 2431–2434. https://doi.org/10.1016/S0957-4166(00)82217-X.

[54] M.E. Powell, A.M. Kelly, S.D. Bull, T.D. James, A simple chiral derivatisation protocol for 1H NMR spectroscopic analysis of the

enantiopurity of *O*-silyl-1,2-amino alcohols, Tetrahedron Lett. 50 (2008) 876–879. https://doi.org/10.1016/j.tetlet.2008.12.013.

[55] D.A. Tickell, M.F. Mahon, S.D. Bull, T.D. James, A Simple Protocol for NMR Analysis of the Enantiomeric Purity of Chiral Hydroxylamines, Org. Lett. 15 (2013) 860–863. https://doi.org/10.1021/ol303566k.

[56] W. Kliegel, D. Nanninga, Nitrone von 2-formylphenylboronsäureestern, J. Organomet. Chem. 247 (1983) 247–252. https://doi.org/10.1016/0022-328X(83)85166-3.

[57] R.R. Groleau, R.S.L. Chapman, H. Ley-Smith, L. Liu, T.D. James, S.D. Bull, A Three-Component Derivatization Protocol for Determining the Enantiopurity of Sulfinamides by ^{1}H and ^{19}F NMR Spectroscopy, J. Org. Chem. 85 (2020) 1208–1215. https://doi.org/10.1021/acs.joc.9b02473.

[58] A.M. Kelly, Y. Pérez-Fuertes, S. Arimori, S.D. Bull, T.D. James, Simple Protocol for NMR Analysis of the Enantiomeric Purity of Diols, Org. Lett. 8 (2006) 1971–1974. https://doi.org/10.1021/ol0602351.

[59] A.M. Kelly, Y. Pérez-Fuertes, J.S. Fossey, S. Lozano Yeste, S.D. Bull, T.D. James, Simple protocols for NMR analysis of the enantiomeric purity of chiral diols, Nat. Protoc. 3 (2008) 215–219. https://doi.org/10.1038/nprot.2007.523.

[60] M.D. Reddy, H. Kobori, T. Mori, J. Wu, H. Kawagishi, E.B. Watkins, Gram-Scale, Stereoselective Synthesis and Biological Evaluation of (+)-Armillariol C, J. Nat. Prod. 80 (2017) 2561–2565. https://doi.org/10.1021/acs.jnatprod.7b00484.

[61] D. Urabe, H. Todoroki, K. Masuda, M. Inoue, Total syntheses of four possible stereoisomers of resolvin E3, Tetrahedron. 68 (2012) 3210–3219. https://doi.org/10.1016/j.tet.2012.02.045.

[62] M. Göhl, K. Seifert, Total Synthesis of 3-Oxo- and 3β-Hydroxytauranin via Negishi Coupling of a Bis(*ortho*-oxy)-Functionalized Benzyl Chloride, European J. Org. Chem. 2015 (2015) 6249–6258. https://doi.org/10.1002/ejoc.201500815.

[63] C. Chopard, R. Azerad, T. Prangé, Naphthalene-dioxygenase-catalysed *cis*-dihydroxylation of azaarene derivatives: Part 1: 2-Pyridones and 2-quinolones, J. Mol. Catal. B Enzym. 50 (2008) 53–60. https://doi.org/10.1016/j.molcatb.2007.09.013.

[64] S.H. Shabbir, C.J. Regan, E. V Anslyn, J. Rebek, A general protocol for creating high-throughput screening assays for reaction yield and enantiomeric excess applied to hydrobenzoin, Proc. Natl. Acad. Sci. 106 (2009) 10487–10492. https://doi.org//10.1073/pnas.0809530106.

[65] S.H. Shabbir, L.A. Joyce, G.M. Da Cruz, V.M. Lynch, S. Sorey, E. V. Anslyn, Pattern-based recognition for the rapid determination of identity, concentration, and enantiomeric excess of subtly different threo diols, J. Am. Chem. Soc. 131 (2009) 13125–13131. https://doi.org/10.1021/ja904545d.

[66] P.S. Fordred, S.D. Bull, Tandem hydroboration/reduction of trisubstituted β,γ-unsaturated esters for the asymmetric synthesis of chiral 1,3-diols, Tetrahedron Lett. 54 (2013) 27–31. https://doi.org/10.1016/j.tetlet.2012.10.047.

[67] M. Zeng, S.B. Herzon, Synthesis of 1,3-Amino Alcohols, 1,3-Diols, Amines, and Carboxylic Acids from Terminal Alkynes, J. Org. Chem. 80 (2015) 8604–8618. https://doi.org/10.1021/acs.joc.5b01220.

[68] Z. Li, C. Chen, S. Gröger, J. Kressler, Detection of chirality of poly(glycerol methacrylate)s after derivatization by ^{1}H NMR spectroscopy, Polymer (Guildf). 53 (2012) 2613–2618. https://doi.org/10.1016/j.polymer.2012.04.019.

[69] S. Rama Chaudhari, N. Suryaprakash, Three-Component Chiral Derivatizing Protocols for NMR Spectroscopic Enantiodiscrimination of Hydroxy Acids and Primary Amines, J. Org. Chem. 77 (2012) 648–651. https://doi.org/10.1021/jo202334d.

[70] S.R. Chaudhari, N. Suryaprakash, Simple and efficient methods for discrimination of chiral diacids and chiral alpha-methyl amines, Org. Biomol. Chem. 10 (2012) 6410–6419. https://doi.org/10.1039/c2ob25599e.

[71] S.K. Mishra, N. Suryaprakash, A simple and rapid approach for testing enantiopurity of hydroxy acids and their derivatives using ^{1}H NMR spectroscopy, RSC Adv. 5 (2015) 67277–67283. https://doi.org/10.1039/c5ra11919g.

[72] S.R. Chaudhari, N.R. Suryaprakash, Facile protocols for the configurational assignment of primary amines and hydroxy acids by NMR, New J. Chem. 38 (2014) 790–794. https://doi.org/10.1039/c3nj01273e.

[73] S.K. Mishra, N. Suryaprakash, Assignment of the absolute configuration of hydroxy acids using ^{1}H NMR spectroscopy: a simple and rapid approach, Tetrahedron: Asymmetry. 28 (2017) 250–256. https://doi.org/10.1016/J.TETASY.2017.01.004.

[74] N. Lokesh, S.R. Chaudhari, N. Suryaprakash, RES-TOCSY: A simple approach to resolve overlapped ^{1}H NMR spectra of enantiomers, Org. Biomol. Chem. 12 (2014) 993–997. https://doi.org/10.1039/c3ob42087f.

[75] S.L. Yeste, M.E. Powell, S.D. Bull, T.D. James, Simple Chiral Derivatization Protocols for ^{1}H NMR and ^{19}F NMR Spectroscopic Analysis of the Enantiopurity of Chiral Diols, J. Org. Chem. 74 (2009) 427–430. https://doi.org/10.1021/jo8019187.

[76] S. Lozano Yeste, M.E. Powell, S.D. Bull, T.D. James, Correction: Simple Chiral Derivatization Protocols for ^{1}H NMR and ^{19}F NMR Spectroscopic Analysis of the Enantiopurity of Chiral Diols, J. Org. Chem. 84 (2019) 9395–9396. https://doi.org/10.1021/acs.joc.9b01735.

[77] N. Hamaguchi, Y. Okuno, Y. Oe, T. Ohta, A simple quantitative chiral analysis of amino acid esters by fluorine-19 nuclear magnetic resonance using the modified James–Bull method, Chirality. 31 (2019) 34–40. https://doi.org/10.1002/chir.23028.

[78] Y.R. Lima, T.J. Peglow, P.C. Nobre, P.T. Campos, G. Perin, E.J. Lenardão, M.S. Silva, Chalcogen-Containing Diols: A Novel Chiral Derivatizing Agent for ^{77}Se and ^{125}Te NMR Chiral Recognition of Primary Amines, ChemistrySelect. 4 (2019) 4797–4803. https://doi.org/10.1002/slct.201900097.

[79] S.S. Oliveira, R.L.O.R. Cunha, M.S. Silva, ^{77}Se and ^{125}Te NMR spectroscopy for enantiopurity determination of chalcogen amines, Tetrahedron Lett. 57 (2016) 4556–4559. https://doi.org/10.1016/j.tetlet.2016.08.091.

[80] D. Leung, S.O. Kang, E. V. Anslyn, Rapid determination of enantiomeric excess: A focus on optical approaches, Chem. Soc. Rev. 41 (2012) 448–479. https://doi.org/10.1039/c1cs15135e.

[81] B.T. Herrera, S.L. Pilicer, E. V Anslyn, L.A. Joyce, C. Wolf, Optical Analysis of Reaction Yield and Enantiomeric Excess: A New Paradigm Ready for Prime Time, J. Am. Chem. Soc. 140 (2018) 10385–10401. https://doi.org/10.1021/jacs.8b06607.

[82] P. Metola, E. V Anslyn, T.D. James, S.D. Bull, Circular dichroism of multi-component assemblies for chiral amine recognition and rapid *ee* determination, Chem. Sci. 3 (2012) 156–161. https://doi.org/10.1039/c1sc00496d.

[83] S. Stewart, M.A. Ivy, E. V. Anslyn, The use of principal component analysis and discriminant analysis in differential sensing routines, Chem. Soc. Rev. 43 (2014) 70–84. https://doi.org/10.1039/c3cs60183h.

[84] K.W. Bentley, Y.G. Nam, J.M. Murphy, C. Wolf, Chirality Sensing of Amines, Diamines, Amino Acids, Amino Alcohols, and α-Hydroxy Acids with a Single Probe, J. Am. Chem. Soc. 135 (2013) 18052–18055. https://doi.org/10.1021/ja410428b.

[85] E.G. Shcherbakova, T. Minami, V. Brega, T.D. James, P. Anzenbacher, Determination of Enantiomeric Excess in Amine Derivatives with Molecular Self-Assemblies, Angew. Chemie Int. Ed. 54 (2015) 7130–7133. https://doi.org/10.1002/anie.201501736.

[86] E.G. Shcherbakova, V. Brega, T. Minami, S. Sheykhi, T.D. James, P. Anzenbacher, Toward Fluorescence-Based High-Throughput Screening for Enantiomeric Excess in Amines and Amino Acid Derivatives, Chem. - A Eur. J. 22 (2016) 10074–10080. https://doi.org/10.1002/chem.201601614.

[87] E.G. Shcherbakova, V. Brega, V.M. Lynch, T.D. James, P. Anzenbacher, High-Throughput Assay for Enantiomeric Excess Determination in 1,2- and 1,3-Diols and Direct Asymmetric Reaction Screening, Chem. - A Eur. J. 23 (2017) 10222–10229. https://doi.org/10.1002/chem.201701923.

[88] M. Pushina, S. Farshbaf, E.G. Shcherbakova, P. Anzenbacher, A dual chromophore sensor for the detection of amines, diols, hydroxy acids, and amino alcohols, Chem. Commun. 55 (2019) 4495–4498. https://doi.org/10.1039/c9cc01051c.

[89] E.G. Shcherbakova, T.D. James, P. Anzenbacher, High-throughput assay for determining enantiomeric excess of chiral diols, amino alcohols, and amines and for direct asymmetric reaction screening, Nat. Protoc. (2020) 1–27. https://doi.org/10.1038/s41596-020-0329-1.

[90] G. Mirri, S.D. Bull, P.N. Horton, T.D. James, L. Male, J.H.R. Tucker, Electrochemical Method for the Determination of Enantiomeric Excess of Binol Using Redox-Active Boronic Acids as Chiral Sensors, J. Am. Chem. Soc. 132 (2010) 8903–8905. https://doi.org/10.1021/ja103462x.

[91] A. Wilson, G. Gasparini, S. Matile, Functional systems with orthogonal dynamic covalent bonds, Chem. Soc. Rev. 43 (2014) 1948–1962. https://doi.org/10.1039/c3cs60342c.

[92] J.F. Reuther, S.D. Dahlhauser, E. V Anslyn, Tunable Orthogonal Reversible Covalent (TORC) Bonds: Dynamic Chemical Control over Molecular Assembly, Angew. Chemie Int. Ed. 58 (2019) 74–85. https://doi.org/10.1002/ange.201808371.

[93] H.M. Seifert, K.R. Trejo, E. V Anslyn, Four Simultaneously Dynamic Covalent Reactions. Experimental Proof of Orthogonality, J. Am. Chem. Soc. 138 (2016) 10916–10924. https://doi.org/10.1021/jacs.6b04532.

[94] V.E. Campbell, J.R. Nitschke, Complex Systems from Simple Building Blocks via Subcomponent Self-Assembly, Synlett. 20 (2008) 3077–3090. https://doi.org/10.1055/s-0028-1087361.

[95] M. Hutin, G. Bernardinelli, J.R. Nitschke, An Iminoboronate Construction Set for Subcomponent Self-Assembly, Chem. - A Eur. J. 14 (2008) 4585–4593. https://doi.org/10.1002/chem.200800074.

[96] R. Nishiyabu, Y. Kubo, T.D. James, J.S. Fossey, Boronic acid building blocks: tools for self assembly, Chem. Commun. 47 (2011) 1124–1150. https://doi.org/10.1039/c0cc02921a.

[97] Y. Kubo, R. Nishiyabu, T.D. James, Hierarchical supramolecules and organization using boronic acid building blocks, Chem. Commun. 51 (2015) 2005–2020. https://doi.org/10.1039/c4cc07712a.

[98] E. Galbraith, A.M. Kelly, J.S. Fossey, G. Kociok-Köhn, M.G. Davidson, S.D. Bull, T.D. James, Dynamic covalent self-assembled macrocycles prepared from 2-formyl-aryl-boronic acids and 1,2-amino alcohols, New J. Chem. 33 (2009) 181–185. https://doi.org/10.1039/b815138e.

[99] D.W. Norman, J.P. Edwards, C.M. Vogels, A. Decken, S.A. Westcott, Synthesis and reactivity of novel Schiff bases containing boronate esters, Can. J. Chem. 80 (2002) 31–40. https://doi.org/10.1139/v01-188.

[100] F.A. Appoh, S.S. Barnes, M.J. Manning, C.S. Turner, C.M. Vogels, S.A. Westcott, A. Decken, Synthesis of boron macrocycles from 1,2-aminoalcohols and 2-formylphenylboronic acid, J. Heterocycl. Chem. 45 (2008) 1415–1418. https://doi.org/10.1002/jhet.5570450526.

[101] H.Y. Su, D. Gorelik, M.S. Taylor, Chiral phosphine ligand libraries based on the Bull-James three-component supramolecular assembly, Supramol. Chem. 31 (2019) 190–202. https://doi.org/10.1080/10610278.2018.1564829.

[102] W.D. G Brittain, B.M. Chapin, W. Zhai, V.M. Lynch, B.R. Buckley, E. V Anslyn, J.S. Fossey, The Bull–James assembly as a chiral auxiliary and shift reagent in kinetic resolution of alkyne amines by the CuAAC reaction, Org. Biomol. Chem. 14 (2016) 10778–10782. https://doi.org/10.1039/c6ob01623e.

[103] E.N. Keyzer, A. Sava, T.K. Ronson, J.R. Nitschke, A.J. McConnell, Post-Assembly Reactivity of N-Aryl Iminoboronates: Reversible Radical Coupling and Unusual B−N Dynamic Covalent Chemistry, Chem. - A Eur. J. 24 (2018) 12000–12005. https://doi.org/10.1002/chem.201802790.

[104] E. Amado, J. Rg Kressler, Reversible Complexation of Iminophenylboronates with Mono-and Dihydroxy Methacrylate Monomers and Their Polymerization at Low Temperature by Photoinduced ATRP in One Pot, Macromolecules. 49 (2016) 1532–1544. https://doi.org/10.1021/acs.macromol.5b02771.

[105] D. Ailincai, L. Marin, S. Morariu, M. Mares, A.C. Bostanaru, M. Pinteala, B.C. Simionescu, M. Barboiu, Dual crosslinked iminoboronate-chitosan hydrogels with strong antifungal activity against Candida planktonic yeasts and biofilms, Carbohydr. Polym. 152 (2016) 306–316. https://doi.org/10.1016/j.carbpol.2016.07.007.

[106] S.S. Delpierre, B. Willocq, G. Manini, V. Lemaur, J. Goole, P. Gerbaux, J. Jérô Me Cornil, P. Dubois, J.-M. Raquez, Simple Approach for a Self-Healable and Stiff Polymer Network from Iminoboronate-Based Boroxine Chemistry, Chem. Mater. 31 (2019) 3736–3744. https://doi.org/10.1021/acs.chemmater.9b00750.

[107] S. Delpierre, B. Willocq, J. De Winter, P. Dubois, P. Gerbaux, J.-M. Raquez, Dynamic Iminoboronate-Based Boroxine Chemistry for the Design of Ambient Humidity-Sensitive Self-Healing Polymers, Chem. - A Eur. J. 23 (2017) 6730–6735. https://doi.org/10.1002/chem.201700333.

[108] Y. Yang, L. Huang, R. Wu, W. Fan, Q. Dai, J. He, C. Bai, Assembling of reprocessable polybutadiene-based vitrimers with high-strength and shape-memory via catalyst-free imine-coordinated boroxine, ACS Appl. Mater. Interfaces. 12 (2020) 33305–33314. https://doi.org/10.1021/acsami.0c09712.

[109] S. Li, C. Zuo, Y.H. Jo, S. Li, K. Jiang, L. Yu, Y. Zhang, J. Wang, L. Li, Z. Xue, Enhanced ionic conductivity and mechanical properties *via* dynamic-covalent boroxine bonds in solid polymer electrolytes, J. Memb. Sci. 608 (2020) 118218. https://doi.org/10.1016/j.memsci.2020.118218.

[110] X. Zhang, J. Gao, X. Zhao, Z. Liu, Z. Liu, K. Wang, G. Li, J. Jiang, Hyperbranched polymer micelles with triple-stimuli backbone-breakable iminoboronate ester linkages, Chinese Chem. Lett. 31 (2020) 1822–1826. https://doi.org/10.1016/j.cclet.2020.03.018.

[111] C.C. Deng, W.L.A. Brooks, K.A. Abboud, B.S. Sumerlin, Boronic acid-based hydrogels undergo self-healing at neutral and acidic pH, ACS Macro Lett. 4 (2015) 220–224. https://doi.org/10.1021/acsmacrolett.5b00018.

[112] J. Hu, Q. Hu, X. He, C. Liu, Y. Kong, Y. Cheng, Y. Zhang, Stimuli-Responsive Hydrogels with Antibacterial Activity Assembled from Guanosine, Aminoglycoside, and a Bifunctional Anchor, Adv. Healthc. Mater. 9 (2020) 1901329. https://doi.org/10.1002/adhm.201901329.

[113] Y. Li, Y. Liu, R. Ma, Y. Xu, Y. Zhang, B. Li, Y. An, L. Shi, A G-Quadruplex Hydrogel via Multicomponent Self-Assembly: Formation and Zero-Order Controlled Release, ACS Appl. Mater. Interfaces. 9 (2017) 13056–13067. https://doi.org/10.1021/acsami.7b00957.

[114] C. Arnal-Hérault, A. Pasc, M. Michau, D. Cot, E. Petit, M. Barboiu, Functional G-Quartet Macroscopic Membrane Films, Angew. Chemie Int. Ed. 46 (2007) 8409–8413. https://doi.org/10.1002/anie.200702605.

[115] S. Mihai, Y. Le Duc, D. Cot, M. Barboiu, Sol-gel selection of hybrid G-quadruplex architectures from dynamic supramolecular guanosine libraries, J. Mater. Chem. 20 (2010) 9443–9448. https://doi.org/10.1039/c0jm01248c.

[116] A. Biswas, T. Ghosh, P.K. Gavel, A.K. Das, PEG Functionalized Stimuli Responsive Self-Healable Injectable Dynamic Imino-boronate G-quadruplex Hydrogel for the Delivery of Doxorubicin, ACS Appl. Bio Mater. 3 (2020) 1052–1060. https://doi.org/10.1021/acsabm.9b01034.

[117] A. Sikora, J. Zielonka, M. Lopez, J. Joseph, B. Kalyanaraman, Direct oxidation of boronates by peroxynitrite: Mechanism and implications in fluorescence imaging of peroxynitrite, Free Radic. Biol. Med. 47 (2009) 1401–1407. https://doi.org/10.1016/j.freeradbiomed.2009.08.006.

[118] K. Debowska, D. Debski, M. Hardy, M. Jakubowska, B. Kalyanaraman, A. Marcinek, R. Michalski, B. Michalowski, O. Ouari, A. Sikora, R. Smulik, J. Zielonka, Toward selective detection of reactive oxygen and nitrogen species with the use of fluorogenic probes - Limitations, progress, and perspectives, Pharmacol. Reports. 67 (2015) 756–764. https://doi.org/10.1016/j.pharep.2015.03.016.

[119] L. Wu, A.C. Sedgwick, X. Sun, S.D. Bull, X.P. He, T.D. James, Reaction-Based Fluorescent Probes for the Detection and Imaging of Reactive Oxygen, Nitrogen, and Sulfur Species, Acc. Chem. Res. 52 (2019) 2582–2597. https://doi.org/10.1021/acs.accounts.9b00302.

[120] J. Yu, H. Chao, G. Li, R. Tang, Z. Liu, Z. Liu, J. Jiang, Backbone-Based LCST-Type Hyperbranched Poly(oligo(ethylene glycol)) with CO_2-Reversible Iminoboronate Linkers, Macromol. Chem. Phys. 219 (2018) 1800346. https://doi.org/10.1002/macp.201800346.

[121] Y. Liu, G. Li, J. Chen, Z. Liu, Z. Liu, J. Jiang, Fabricating Triple-Sensitive Polymer Nano-Aggregates via an Aqueous Iminoboronate Multicomponent Reaction, Macromol. Rapid Commun. 38 (2017) 1600805. https://doi.org/10.1002/marc.201600805.

[122] X. Zhang, G. Li, Z. Liu, Z. Liu, J. Jiang, Iminoboronate Backbone-Based Hyperbranched Polymeric Micelles with Fenton-Like Enhanced ROS Response, Macromol. Chem. Phys. 221 (2020) 2000022. https://doi.org/10.1002/macp.202000022.

[123] R. Cheng, G. Li, L. Fan, Z. Liu, Z. Liu, J. Jiang, CO_2-Acidolysis of iminoboronate ester based polymersomes, J. Mater. Chem. B. 6 (2018) 7800–7804. https://doi.org/10.1039/c8tb02496k.

[124] R. Ma, C. Zhang, Y. Liu, C. Li, Y. Xu, B. Li, Y. Zhang, Y. An, L. Shi, Iminoboronate-based dual-responsive micelles via subcomponent self-assembly for hydrophilic 1,2-diol-containing drug delivery, RSC Adv. 7 (2017) 21328–21335. https://doi.org/10.1039/C7RA01742A.

[125] B. Akgun, D.G. Hall, Boronic Acids as Bioorthogonal Probes for Site-Selective Labeling of Proteins, Angew. Chemie Int. Ed. 57 (2018) 13028–13044. https://doi.org/10.1002/anie.201712611.

[126] J.P.M. António, R. Russo, C.P. Carvalho, P.M.S.D. Cal, P.M.P. Gois, Boronic acids as building blocks for the construction of therapeutically useful bioconjugates, Chem. Soc. Rev. 48 (2019) 3513–3536. https://doi.org/10.1039/C9CS00184K.

[127] S. Cambray, J. Gao, Versatile Bioconjugation Chemistries of *ortho*-Boronyl Aryl Ketones and Aldehydes, Acc. Chem. Res. 51 (2018) 2198–2206. https://doi.org/10.1021/acs.accounts.8b00154.

[128] A. Bandyopadhyay, J. Gao, Iminoboronate Formation Leads to Fast and Reversible Conjugation Chemistry of α-Nucleophiles at Neutral pH, Chem. - A Eur. J. 21 (2015) 14748–14752. https://doi.org/10.1002/chem.201502077.

[129] P.M.S.D. Cal, J.B. Vicente, E. Pires, A. V. Coelho, L.F. Veiros, C. Cordeiro, P.M.P. Gois, Iminoboronates: A new strategy for reversible protein modification, J. Am. Chem. Soc. 134 (2012) 10299–10305. https://doi.org/10.1021/ja303436y.

[130] X. Liu, Z. Li, H. Xu, Y. Zhan, P. Ma, H. Chen, B. Jiang, Tris(2-carboxyethyl)phosphine promotes hydrolysis of iminoboronates, Tetrahedron Lett. 58 (2017) 3101–3106. https://doi.org/10.1016/j.tetlet.2017.06.069.

[131] H. Gu, T.I. Chio, Z. Lei, R.J. Staples, J.S. Hirschi, S. Bane, Formation of hydrazones and stabilized boron-nitrogen heterocycles in aqueous solution from carbohydrazides and *ortho*-formylphenylboronic acids, Org. Biomol. Chem. 15 (2017) 7543–7548. https://doi.org/10.1039/c7ob01708a.

[132] O. Dilek, Z. Lei, K. Mukherjee, S. Bane, Rapid formation of a stable boron-nitrogen heterocycle in dilute, neutral aqueous solution for bioorthogonal coupling reactions, Chem. Commun. 51 (2015) 16992–16995. https://doi.org/10.1039/c5cc07453c.

[133] H. Gu, S. Ghosh, R.J. Staples, S.L. Bane, β-Hydroxy-Stabilized Boron-Nitrogen Heterocycles Enable Rapid and Efficient C-Terminal Protein Modification, Bioconjug. Chem. 30 (2019) 2604–2613. https://doi.org/10.1021/acs.bioconjchem.9b00534.

[134] A. Bandyopadhyay, S. Cambray, J. Gao, Fast diazaborine formation of semicarbazide enables facile labeling of bacterial pathogens, J. Am. Chem. Soc. 139 (2017) 871–878. https://doi.org/10.1021/jacs.6b11115.

[135] H. Faustino, M.J.S.A. Silva, L.F. Veiros, G.J.L. Bernardes, P.M.P. Gois, Iminoboronates are efficient intermediates for selective, rapid and reversible *N*-terminal cysteine functionalisation, Chem. Sci. 7 (2016) 5052–5058. https://doi.org/10.1039/c6sc01520d.

[136] T.S.L. Tang, D. Cardella, A.J. Lander, X. Li, J.S. Escudero, Y.-H. Tsai, L.Y.P. Luk, Use of an Asparaginyl Endopeptidase for Chemo-enzymatic Peptide and Protein Labeling, Chem. Sci. 11 (2020) 5881–5888. https://doi.org/10.1039/D0SC02023K.

[137] T.I. Chio, H. Gu, K. Mukherjee, L.N. Tumey, S.L. Bane, Site-specific bioconjugation and multi-bioorthogonal labeling via rapid formation of a boron-nitrogen heterocycle, Bioconjug. Chem. 30 (2019) 1554–1564. https://doi.org/10.1021/acs.bioconjchem.9b00246.

[138] A. Bandyopadhyay, J. Gao, Iminoboronate-Based Peptide Cyclization That Responds to pH, Oxidation, and Small Molecule Modulators, J. Am. Chem. Soc. 138 (2016) 2098–2101. https://doi.org/10.1021/jacs.5b12301.

[139] K. Li, J. Gao, Iminoboronate-Mediated Peptide Cyclization with Lysine Homologues, Synlett. 28 (2017) 1913–1916. https://doi.org/10.1055/s-0036-1590795.

[140] S. Borsley, S.L. Cockroft, *In Situ* Synthetic Functionalization of a Transmembrane Protein Nanopore, ACS Nano. 12 (2018) 786–794. https://doi.org/10.1021/acsnano.7b08105.

[141] P.M.S.D. Cal, R.F.M. Frade, V. Chudasama, C. Cordeiro, S. Caddick, P.M.P. Gois, Targeting cancer cells with folic acid-iminoboronate fluorescent conjugates, Chem. Commun. 50 (2014) 5261–5263. https://doi.org/10.1039/c3cc47534d.

[142] P.M.S.D. Cal, R.F.M. Frade, C. Cordeiro, P.M.P. Gois, Reversible Lysine Modification on Proteins by Using Functionalized Boronic Acids, Chem. - A Eur. J. 21 (2015) 8182–8187. https://doi.org/10.1002/chem.201500127.

[143] G. Akçay, M.A. Belmonte, B. Aquila, C. Chuaqui, A.W. Hird, M.L. Lamb, P.B. Rawlins, N. Su, S. Tentarelli, N.P. Grimster, Q. Su, Inhibition of Mcl-1 through covalent modification of a noncatalytic lysine side chain, Nat. Chem. Biol. 12 (2016) 931–936. https://doi.org/10.1038/nCHeMBIO.2174.

[144] A. Bandyopadhyay, K.A. Mccarthy, M.A. Kelly, J. Gao, Targeting bacteria via iminoboronate chemistry of amine-presenting lipids, Nat. Commun. 6 (2015) 6561. https://doi.org/10.1038/ncomms7561.

[145] B. Akgun, C. Li, Y. Hao, G. Lambkin, R. Derda, D.G. Hall, Synergic “click” Boronate/Thiosemicarbazone System for Fast and Irreversible Bioorthogonal Conjugation in Live Cells, J. Am. Chem. Soc. 139 (2017) 14285–14291. https://doi.org/10.1021/jacs.7b08693.

[146] R. Russo, R. Padanha, F. Fernandes, L. Veiros, F. Corzana, P.M.P. Gois, Engineering Boron Hot Spots for the Site-Selective Installation of Iminoboronates on Peptide Chains, Chem. – A Eur. J. 26 (2020) 15226–15231. https://doi.org/10.1002/chem.202002675.

[147] M.K. Meadows, E.K. Roesner, V.M. Lynch, T.D. James, E. V Anslyn, Boronic Acid Mediated Coupling of Catechols and *N*-Hydroxylamines: A Bioorthogonal Reaction to Label Peptides, Org. Lett. 19 (2017) 3179–3182. https://doi.org/10.1021/acs.orglett.7b01198.